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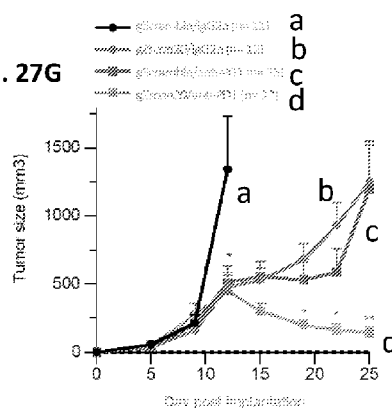
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(54) Title: TRANSCRIPTION FACTORS CONTROLLING T CELL DIFFERENTIATION AND DISRUPTION FOR TUMOR AND VIRUS CONTROL

FIG. 27G



(57) Abstract: Epigenomic and transcriptomic data were used to identify transcription factors (TFs) that define different CD8⁺ T cell states. T cells can be programmed to avoid a terminal exhaustion state (Tex_{Term}), a dysfunctional T cell state often found in tumors or chronic infections, and to favor a cytotoxic effector state. Tex_{Term} exhibits high similarity with the beneficial tissue-resident memory T states (T_{RM}) in terms of their locations and transcription profiles. *Zscan20*, a novel TF, was found to be active in Tex_{Term}. Its perturbation thwarted the differentiation of Tex_{Term} *in vivo*, but not that of T_{RM}. Perturbation of *Zscan20* programs T cells into an effector-like state that confers tumor and virus control and generates synergy with immune checkpoint therapy. *Jdp2*, *Nfil3*, and *Znf324* were also identified as Tex_{Term} drivers.

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- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
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TRANSCRIPTION FACTORS CONTROLLING T CELL DIFFERENTIATION AND DISRUPTION FOR TUMOR AND VIRUS CONTROL

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Application No. 63/436,403, filed December 30, 2022, which is incorporated by reference in its entirety.

INCORPORATION OF ELETRONIC SEQUENCE LISTING

10 The Sequence Listing is submitted as an XML file in the form of the file named “7158-109407-02_ST26.xml” (~61,942 bytes), which was created on December 27, 2023 which is incorporated by reference herein.

FIELD

15 This relates to immunotherapies, compositions and methods of preventing or reducing immune cell exhaustion and uses thereof for treating cancer or viral infections.

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BACKGROUND

25 Cell state can be described as the range of cellular phenotypes arising from the interaction of a defined cell type with its environment. Advanced genomics and proteomics technology enable not only precise characterization of cell state, but also provide a stunningly high-resolution view of transitions between states. T cells serve as an example: these cells exist in different activation states, which arise in response to different stimuli, yet they maintain their T-cell identity. Cell state is directly associated with the function of the given cell type. Cells in the same state will share the pathways that are active, the genes that are expressed, and the functions that are being performed.

30 Transcription factors (TFs) are fundamental regulators of both cell type and cell state differentiation.

Naive CD8⁺ T cells differentiate into heterogeneous states to offer immune defense against intracellular pathogens and cancer. In acute infection, T cells assume diverse effector and memory states. After the virus control, highly plastic memory precursor (MP) cells develop into memory T

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cells that mediate long-term protective immunity at different locations (T_{CM} cells at secondary lymphoid, T_{EM} in blood, T_{RM} at peripheral tissues). On the other hand, T cells in chronic virus infections or tumors progressively become dysfunctional T cells and lose memory potential, a phenomenon known as T cell exhaustion. Similar to acute infection, the $CD8^+$ T cells in the exhaustion trajectory include multiple cell states that serve different roles. Akin to MP cells and T_{CM} , cells in progenitor exhausted (Tex_{Prog}) state display plasticity and proliferative capacity and can differentiate into transitory-intermediate “effector-like” states $Tex_{eff-like}$. T cells in the chronic antigen presentation further develop into terminally exhausted T cells (Tex_{term}) that express even higher inhibitory receptors and lack effector and proliferation capacity.

SUMMARY

A multiomics approach was used to systematically identify transcription factors (TFs) that define different $CD8^+$ T cell states and that are utilized to program better antiviral and anti-tumor T cell immunity. Understanding the underlying regulatory TF mechanisms of T cell differentiation in multiple contexts can be helpful to programming T cells into a beneficial state for given therapeutic applications (13, 14). For example, for chronic virus and tumor treatment, the TF program can be harnessed to prevent terminal exhaustion. Tex_{term} cells are epigenetically imprinted to become dysfunctional and do not respond to immune checkpoint therapy such as anti-PD-1. Tex_{term} prevention without compromising T_{RM} is challenging because Tex_{term} cells display striking similarities to T_{RM} cells in transcriptional profiles and tissue locations (FIG. 2, (1)). Tumor-infiltrating lymphocytes (TILs) with T_{RM} characteristics are correlated with better patient survival and tumor control (15–19). Therefore, engineering T cells for robust and exclusive prevention of Tex_{term} without compromising T_{RM} formation is an effective strategy toward adoptive T cell therapy for cancer.

While knowledge of individual T cell states has increased and comparative analysis between exhaustion versus memory has been done (20), systems-level understanding of T cell state landscape and cell-state specifying TFs network remains minimal. Previously a global analysis of TF specification was performed for hard-wired cell types in embryonic development (21, 22) and hematopoietic lineage (23). As different cell states share a large number of same cell type-defining TFs (24), an advanced and precise bioinformatics approach is needed to identify cell states that specify TFs.

Using a systems biology approach, a comprehensive catalog of candidate TFs in different states was identified within the same cell. Specifically, an epigenetic and transcription atlas of

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heterogeneous CD8⁺ T cells was generated and a global analysis of TF activity and their interactions performed. *In vivo* data herein demonstrate that this approach identified novel TFs (*Zscan20*, *Jdp2*, *Nfil3*, and *Znf324*) that program unwanted Tex_{term}. Among the TFs, *Zscan20* is predicted to be active exclusively in Tex_{term} and *Zscan20* perturbation drives effector states without compromising the capacity of forming memory cell state. As a result, the T cells that are *Zscan20*-deficient offer greater virus and tumor control. Furthermore, the comprehensive TF analysis herein reveals observations about context-specific networks of TFs, cooperation between TF communities, and several new and reported pathways associated with Tex_{term} which provide insights to understand underlying molecular mechanism and therapeutic manipulation of T cell differentiation states. This precise pipeline of identification of cell state-specifying TFs can be adapted in cell state programming of other types of cells to improve the effectiveness of cell therapy.

Disclosed herein is a modified peripheral blood mononuclear cell (PBMC), which can have any combination of (a) an agent that reduces *Zscan20* expression or a non-naturally occurring genetic modification that reduces an amount of functional ZSCAN20; (b) an agent that reduces *Jdp2* expression or a non-naturally occurring genetic modification that reduces an amount of functional JDP2; (c) an agent that reduces *Nfil3* expression or a non-naturally occurring genetic modification that reduces an amount of functional NFIL3; and/or (d) an agent that reduces *Znf324* expression or a non-naturally occurring genetic modification that reduces an amount of functional ZNF324. In some examples the modified PBMC is a CD8⁺ T cell, or a chimeric antigen receptor (CAR) T cell. In some examples the modified PBMC is a tumor infiltrating lymphocyte (TIL) or tissue resident memory (T_{RM}) cell. In specific examples, the PBMC's reduced expression of *Zscan20*, reduced activity of ZSCAN20, reduced expression of *Jdp2*, reduced activity of JDP2, reduced expression of *Nfil3*, reduced activity of NFIL3, reduced expression of *Znf324*, and/or reduced activity of ZNF324, increases effector function of the T cell. In some examples, the PBMC's reduced expression of *Zscan20*, reduced activity of ZSCAN20, reduced expression of *Jdp2*, reduced activity of JDP2, reduced expression of *Nfil3*, reduced activity of NFIL3, reduced expression of *Znf324*, and/or reduced activity of ZNF324, reduces exhaustion of the T cell. In additional examples, the modified PBMC is administered to a patient with cancer or a viral infection.

The foregoing and other features of this disclosure will become more apparent from the following detailed description of several aspects which proceeds with reference to the accompanying figures.

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BRIEF DESCRIPTION OF THE FIGURES

FIGs. 1A-1H depict a transcription and epigenomic atlas of CD8+ T cell differentiation states and cell-state specific TFs identification pipeline. (A) Pipeline of integrative analysis. The matched RNA-seq and ATAC-seq data were used as input for Taiji algorithm to construct a regulatory network and output PageRank scores matrix representing the activity of transcription factors (TFs). The downstream analysis included the identification of cell-type-specific TFs, differentiation-specific TFs, and the construction of temporal transcriptional waves. (B) PageRank scores of 151 bona fide cell-state-specific TFs. TFs in rows (z-normalized), samples in columns, and the color of the cell in the matrix indicates the normalized PageRank scores. (C) 124 multi-tasker TFs. Multi-taskers are active in more than one T cell state. Color of the left bars represents the cell types of multitaskers are associated with. (D) UpsetR graph describes the intersection size of the multi-taskers. "A" bars represent multi-taskers of TE and MP, "B" bars are that of TRM and TexTerm. "C" bar is that of Naïve, MP, TCM, TexTerm multi-tasker (TCF7). (E) Bubble plots of TFs that are active in TexTerm, TRM and both. Color represents the normalized PageRank score and circle size represents the logarithm of gene expression. TFs are ordered based on the p-value. (F) Transcriptional waves associated with exhaustion. Circles represent specific cell state. Color indicates normalized PageRank scores with red displaying high values. (G) The expression score of TF members of exhaustion wave are calculated in the single-cell RNA sequencing cell clusters of CD8+ T cells from LCMV acute or chronic infection from GSE199565. (H) Biological pathways enriched in exhaustion transcriptional wave.

FIGs 2A-2E depict parallel differentiation of TRM and TexTerm and their similarity in the transaction. (A) CD8+ T cell state trajectories in acute and chronic infection/tumor where various memory and exhaustion states are assumed. (B) Transcription of virus specific T cell states from LCMV acute and chronic infection. (C) TexProg and TexTerm transcriptome were tested for the enrichment of TRM signature genes. (D) UMAP from Single-cell RNA sequencing data of T cells from blood, tumor, normal tissues of CRC, NSCLC, and HCC patients. Unbiased clustering demonstrate multiple T cell states that are previously reported in LCMV infection and tumor. (E) Module score of exhaustion signature gene in each cluster of cell state. Exhaustion signature genes are upregulated in both TRM and TexTerm.

FIGs. 3A-3C show how 121 experiments from multiple data sets are utilized to generate an epigenetic and transcriptional atlas of CD8+ T cells in chronic and acute antigen exposure. (A)

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Dataset summary. (B, C) Batch effect-corrected ATAC-seq data and top10000 most variable peak regions were selected for visualization. (B) Heatmap with rows clustered using kmeans by k=50 and columns arranged by cell state and study. (C) PCA shows the ATAC-seq samples tend to group together by cell states rather than study.

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FIG 4 depicts chromatin accessibility of *Ifng* locus from ATAC-seq data used in this study.

FIGs. 5A-5B depict a catalog of TFs in 9 different CD8+ T cell states. (A) Summary table of identified TFs in 9 cell states. (B) UpSetR plot shows the intersection size between multi-taskers along with single-taskers size.

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FIG. 6 depicts bubble plots for the naive cell state. Circle size represents the logarithm of gene expression, and the color represents the normalized PageRank score.

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FIG. 7 depicts bubble plots for the MP cell state. Circle size represents the logarithm of gene expression, and the color represents the normalized PageRank score.

FIG. 8 depicts bubble plots for the TE cell state. Circle size represents the logarithm of gene expression, and the color represents the normalized PageRank score.

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FIGs. 9A-B depict bubble plots for the (A) TCM and (B) TEM cell states. Circle size represents the logarithm of gene expression, and the color represents the normalized PageRank score.

FIG. 10 depicts bubble plots for the TRM cell state. Circle size represents the logarithm of gene expression, and the color represents the normalized PageRank score.

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FIG. 11 depicts bubble plots for the TexProg cell state. Circle size represents the logarithm of gene expression, and the color represents the normalized PageRank score.

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FIG. 12 depicts bubble plots for the TexEff-like cell state. Circle size represents the logarithm of gene expression, and the color represents the normalized PageRank score.

FIG. 13 depicts bubble plots for the TexTerm cell state. Circle size represents the logarithm of

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gene expression, and the color represents the normalized PageRank score.

FIGs. 14A-14B depict systemic analysis of driving TFs in each differentiation step. (A) Differentiation-step-specific TFs are identified for the cell state transition from naïve to MP and MP to TRM in the acute infection. Dots represent the labeled source and target cell state data. Circle size represents the logarithm of gene expression, and the color represents normalized PageRank score in each state. (B) The parallel differentiation step in chronic infection. Up and downregulation of TFs for the cell state transition from naïve to TexProg transition and TexProg to TexProg transition are shown.

FIGs. 15A-15C depict transcription factor wave analysis (A) Analysis pipeline. (B, C) Selecting algorithms and parameters for clustering analysis for transcription factor wave. (B) Plotting the cumulative proportion of variance explained against the number of principal components (PCs). The first 20 PCs were kept for the following K-means clustering, which explained ~70% variance. (C) Selecting the best distance metric and number of clusters according to the Silhouette metric. The Pearson correlation was chosen and k=7 was the ideal cluster number.

FIGs. 16A-16C depict transcription wave analysis for (A) TRM, (B) TCM, and (C) TEM. Circles represent specific cell state. Color indicates normalized PageRank scores with darker displaying high values. The gene expression score of the all TF members of each wave are calculated in the associated single-cell RNA sequencing cell clusters of CD8+ T cells from LCMV acute or chronic infection. Scores of TCM, TEM, and TRM are calculated from GSE181785 (40).

FIGs. 17A-17C depict transcription wave analysis for (A) MP, (B) TE & TEM, and (C) TE. Circles represent specific cell state. Color indicates normalized PageRank scores with darker displaying high values. The gene expression score of the all TF members of each wave are calculated in the associated single-cell RNA sequencing cell clusters of CD8+ T cells from LCMV acute or chronic infection. Scores of MP, TE, acute, chronic clusters are calculated from GSE199565 (4) and scores of TCM, TEM, and TRM are calculated from GSE181785 (40).

FIG. 18 depicts a heat map of biological pathways enriched in each transcription wave. Color represents p value.

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FIGs. 19A-19G depict global analysis of TF networks in terminal exhaustion (A) Pipeline of TF interaction network analysis. (B) Communities of TFs in TexTerm. Communities consist of TexTerm TFs and their interaction partners. Dots represent each TF and TexTerm TFs with high interaction scores are labeled. Lines represent the interactions between TexTerm-specific TFs. (C) Intersection size of the regulatees of communities. Top1000 regulatees for each community were selected by the average edge weight. Community (Cm.) Prdm1 shares the most regulatees with other communities (orange highlight). Cm.Pbx3, Cm.Bhlhe40, and Cm.Nfil3 also have a large number of common regulatees (red highlight). (D, E, F) Heat map of biological pathways enriched in each community. (G) Table of TF communities in TexTerm with regulatees of each community and associated biological pathways.

FIG. 20 depicts network analysis of TFs for TRM formation. Communities of TFs in TRM. Communities consist of TRM TFs and their interaction partners. Dots represent each TF. Dot size represents the node degree and color represents each community. TRM-specific TFs are labeled. Lines represent the interactions between TRM-specific TFs.

FIGs. 21A-21F depict TF-TF interaction analysis of multi-taskers for TRM and TexTerm differentiation. *Prdm1* and interaction partner TFs in (A) TexTerm or (B) TRM state are visualized. *Nfil3* and partner TF interactions of (C) TexTerm and (D) TRM are shown. (E) Venn Diagrams show *Prdm1* interacting partner TFs overlapping in TexTerm and TRM. (F) Shared or distinct *Nfil3* interaction TFs in TexTerm and TRM. Each color of the dots represents cluster where interaction partner belongs to and dot size represents the node degree.

FIG. 22 Depicts TF-TF interaction analysis of *Zscan20*. *Zscan20* and partner TF interactions in TexTerm are predicted and visualized.

FIGs. 23A-23C depict regulatee analysis – *Zscan20*. (A) Top 100 regulatees of *Zscan20* ranked by the mean gene expression difference between TexTerm and TE samples. (B) Regulatees of *Zscan20* are enriched in immunological genesets associated to downregulated genes in KRLG1 high effector or activated T cells. (C) Enriched GO terms (left) and KEGG pathways (right) of *Zscan20* regulatees.

FIGs. 24A-24C depict regulatee analysis – *Jdp2*. (A) Top 100 regulatees of *Jdp2*. (B) Regulatees

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of *Jdp2* are enriched in immunological genesets associated to downregulated genes in effector states. (C) Enriched GO terms (left) and KEGG pathways (right) of *Jdp2* regulatees.

FIGs. 25A-25I show how disruption of TexTerm single-tasker TFs prevents terminal exhaustion without perturbation of memory formation. (A) Three novel TFs (*Zscan20*, *Jdp2*, and *Nfil3*) and one previously reported TF (*Prdm1*) to validate in vivo. *Zscan20* and *Jdp2* are highly specific to T cells in exhaustion trajectory and have low activity in TRM. *Nfil3* and *Prdm1* are multitaskers that have high TF activity in both TexTerm and TRM. (B) LCMV-specific TCR expressing T cells (P14 cells) from Cas9 transgenic mice were transduced with gRNA targeting TFs and adoptively transferred to Day 1 LCMV chronic strain infected animals. (C) Representative flow plot of PD1 positive P14 T cells gated with SLAMF6 and CX3CR1. Quantification of (D) TexTerm PD1+ SLAMF6- CX3CR1- and (E) inhibitory receptors positive cells. CRISPR knock-outs of all four TFs fail to adopt TexTerm states. Deletion of TexTerm exclusive TF genes (*Zscan20*, *Jdp2*) does not compromise memory formation while deletion of TexTerm & TRM multitasker TF genes (*Nfil3*, *Prdm1*) significantly deters the process. (F) TF KO mice were tested in the acute LCMV infection model to profile memory formation. Percentage of (G) TCM (CD62L+ KLRG1-), (H) TEM (CD62L- KLRG1+) among gRNA positive P14 cells in the spleen. (I) The ratio of gRNA containing P14 cell number of small intestines (SI) and that of spleens. Perturbation of multitasker TFs (*Nfil3* & *Prdm1*) significantly compromises TRM formation. Statistic summary on each bar is from unpaired t-test between gScramble vs TF gRNA. Data include more than three biological replicates, $n \geq 5$.

FIGs. 26A-26C show how TexTerm driver TF KO mice express lower terminal exhaustion markers and inhibitory receptors. (A) Another set of representative image of Fig. 3A. CD8+ Tex subsets are defined below. TexProg: PD1+ CX3CR1- SLAMF6+, TexEff-like: PD1+ CX3CR1+, TexTerm: PD1+ CX3CR1- SLAMF6-. (B) Percentage of PD1 negative/TexProg/TexEff-like/TexTerm of gRNA marker positive donor P14 CD8 T cells. (C) Percentage of CD38+ CD39+ double positive cells of gRNA+ P14 donor cells. Statistic summary on each bar is from unpaired t-test between gScramble vs TF gRNA. Data include more than three biological replicates, $n \geq 5$.

FIGs. 27A-27L demonstrate how disruption of transcription factor *Zscan20* provides enhanced virus and tumor control. In LCMV chronic strain-infected mice (A-E), (A) Experiment timeline. Cas9+ P14 T cells are transduced with gRNA *Zscan20* or scramble gRNA. (B) g*Zscan20* RV group

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shows lower serum virus level. (C) gZscan20 RV group shows higher effector-associated markers (CX3CR1, KLRG1). (D) Quantification of (C). (E) T cells with gRNA *Zscan20* are more polyfunctional and release both IFN γ and TNF α . (F) *Zscan20* KO or control P14 cells were adoptively transferred to melanoma-bearing mice followed by anti-PD1 or isotype IgG2a control treatment. (J) P14 cells from Isotype control-treated mice show that *Zscan20* perturbation reduces exhaustion. Flow plot of PD1 positive P14 cells gated with SLAMF6 (TexProg marker) and TIM3 (TexTerm marker in cancer). (K) TexProg Quantification of (J). (L) TexTerm Quantification of (J). (G) Tumor growth (H) survival curve and (I) tumor weight show that *Zscan20* KO reduces tumor burden and elongates survival with both IgG2a and anti-PD1 treatment groups. With a combination of anti-PD1, disruption of *Zscan20* significantly suppress the tumor growth. All data include more than three biological replicates, $n \geq 9$. Data are expressed as mean $\pm$ SEM. Statistical analysis was performed using Student's t test (two-tailed) comparing TF gRNA vs scramble gRNA within no anti-PD1 treatment groups (black) or anti-PD1 group (gray).

FIGs. 28A-28D demonstrate that Jdp2 disrupted CD8+ T cell offers better virus control in LCMV chronic strain-infected mice (A-D). (A) Experiment timeline. Cas9+ P14 T cells are transduced with gRNA Jdp2 or scramble gRNA. (B) gJdp2 RV group shows lower serum virus level. (C) gJdp2 RV group shows higher effector-associated markers (CX3CR1, KLRG1). (D) T cells with gRNA *Zscan20* are more polyfunctional and release both IFN γ and TNF α . All data include more than three biological replicates, $n \geq 5$. Data are expressed as mean $\pm$ SEM. Statistical analysis was performed using Student's t test (two-tailed) comparing TF gRNA vs scramble gRNA.

FIGs. 29A-29C show tumor control by T cells with *Jdp2* deficiency. (A) Jdp2 KO or control P14 cells were adoptively transferred to melanoma-bearing mice that are implanted with 5×10^5 B16-gp33 cells) followed by anti-PD1 or isotype IgG2a control treatment. (B) Tumor growth (C) survival curve and show improved tumor control only when *Jdp2* KO is combined with anti-PD1 treatment. Data are expressed as mean $\pm$ SEM. Statistical analysis was performed using Student's t test (two-tailed) comparing TF gRNA vs scramble gRNA within anti-PD1 group.

FIGs. 30A-30B show tumor control by T cells with TRM and TexTerm multitasker, *Nfil3* deficiency. (A) *Nfil3* KO, *Zscan20* KO, or control P14 cells were adoptively transferred to melanoma-bearing mice that are implanted with 3×10^5 B16-gp33 cells. (B) Tumor growth show that only mice with *Zscan20* KO T cells show tumor reduction but *Nfil3* KO does not improve

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tumor control. Data are expressed as mean $\pm$ SEM. Statistical analysis was performed using Student's t test (two-tailed) comparing TF gRNA vs scramble gRNA.

FIGs. 31A-31I show that *in vivo* CRISPR screening coupled with scRNA-Seq systematically validates TEX-Driving TFs. (A) Experiment pipeline for *in vivo* CRISPR screening coupled with scRNA-Seq. The retroviral transcription factor (TF) gRNA library was generated based on predicted TF activity (described in FIG. 1). Four gRNAs were generated for two common T cell TFs (*Ikzf3*, *Stat3*), TRM and TEX dual-specific TFs (*Prdm1*, *Hic1*, *Gfi1*, *Nfil3*, *Nr4a2*), and TEX-specific TFs. Each gene was targeted with four gRNAs, expressed by two retrovirus gRNA vectors, each expressing dual gRNAs with a GFP expression marker. Cas9-expressing LCMV gp33 peptide-specific TCR T cells (Cas9+ P14) were transduced with the retroviral gRNA library at an MOI of 0.3 and adoptively transferred to mice infected with the chronic LCMV virus (Clone 13 strain) on post-day 1. On day 23 post-transfer, mice spleens were isolated, and GFP-positive P14 T cells were sorted. Additionally, library-transduced P14 cells were cultured *in vitro* for three days and sequenced to normalize gRNA distribution from *in vivo*. scRNA-seq was done to obtain gRNA barcodes and mRNA expression. (B) UMAP of gRNA-positive P14 T cells. Clusters of different cell states are shown: Progenitors of TEX (TEX Prog), cell-cycle, Effector-like TEX (TEX eff-like), and terminal TEX (TEX). (C) Differentiation markers of TEX Prog (*Tcf7*, *Slamf6*), TEX eff-like (*Cx3cr1*, *Klrd1*), cell cycle state (*Birc5*), and TEX (*Cd101*, *Cd38*, *Cd7*). (D) gRNA density on UMAP. (E) Individual *in vivo* validation of CRISPR KO of TFs in chronically LCMV (Clone 13 strain) infected mice. Flow cytometry analysis was performed using SLAMF6 and CX3CR1 markers to identify the TEX Prog, TEX eff-like, and TEX. Representative plots are shown. (F-H) Quantification of (E). (I) Quantification of exhaustion markers, PD1+ CD101+.

FIGs. 32A-32F show differential specificity of perturbation of exhaustion-inducing transcription factors in tissue-resident memory (TRM) formation. (A) Experiment Pipeline: scRNA-Seq of TF gRNA-positive cells was conducted on spleens and small intestines from mice acutely infected with LCMV Armstrong (memory-forming condition). (B) UMAP of gRNA-positive T cells displays TEM, TCM, TEM-like TRM, and TRM. Overall, CRISPR-mediated knockout of TEX-specific TFs does not compromise TRM formation (C, D). (C) Individual *in vivo* validation and profiling of the memory state in Armstrong-infected mice. Quantifications of TEM (D), TCM (E), and TRM (F) are presented. Knockout of TEX-specific TFs (*Zscan20*, *Jdp2*, highlighted in red) does not alter TEM, TCM, and TRM populations, but knockout of the TRM and TEX dual-driving TF, *Prdm1*,

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reduces both TRM and TEM.

FIGs. 33A-33M show that disruption of T_{EX} single-tasker TFs rewire terminal exhaustion and enhance virus and tumor control. (A) Gene set enrichment analysis (GSEA) of predicted targets and scRNA-seq obtained gene expressions from TF knockouts indicates that perturbation of TEX-driving TFs enhances effector function and skews differentiation towards effectors. (B) Test the improved effector function in a chronic virus infection and exhaustion environment. (C) TF knockouts show higher expression of cytotoxic cytokines IFN γ and TNF α . (D) Chronic LCMV-infected mice with *Zscan20* and *Jdp2* knockout T cells show reduced viremia. (E) *Zscan20* and *Jdp2* knockouts display higher levels of effector markers, CX3CR1 and KLRG1. (F) The enhanced function of T cells by knockout of TEX-selective TF (*Zscan20*) versus TRM and TEX dual-driving TF (*Hic1*) was tested in a tumor model. (G) TEX-selective TF, *Zscan20* knockout improved tumor control, while mice transferred with TRM/TEX-driving *Hic1* knockout T cells exhibited slightly faster tumor growth. (H) TEX-selective *Zscan20* knockout was tested for synergy with immune checkpoint anti-PD1 antibody therapy. (I) The tumor growth curve shows that *Zscan20* knockout combined with anti-PD1 antibody treatment significantly reduces tumor burden. (J) Survival curve corresponding to (H). (K) Similar to (I), TEX-selective TF, *Jdp2* knockout improves tumor control and shows synergy with anti-PD1 therapy. (L) Tumor weight data from (I) and (K). (M) Another novel TEX-selective TF, *Zfp324*, improves tumor control and synergizes with anti-PD1 therapy.

SEQUENCES

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and single letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand.

In the accompanying sequence listing:

SEQ ID NO: 1 is an exemplary amino acid sequence of human JDP2, from NCBI reference sequence NP_001128520.1.

mmpgqipdpsvttgslpglgpltgtpssaltveelkyadirnlgamiaaplhflevklgkrpqpvs
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nrhrptcivrttdsvktpesegnplleqlekk

SEQ ID NO: 2 is an exemplary nucleotide sequence of human *Jdp2*, from NCBI reference

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sequence NM_001135048.2. It encodes cDNA of transcript variant 3.

gcccgcgccacagcctgcgggagggacgctcggcgccgcgacggggggcgctggcggc
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25 **SEQ ID NO: 3** is an exemplary amino acid sequence of human NFIL3, from NCBI
 reference sequence NP_005375.2

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SEQ ID NO: 4 is an exemplary nucleotide sequence of human *Nfil3*, from NCBI reference sequence NM_005384.3. It encodes cDNA of transcript variant 3.

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5 **SEQ ID NO: 5** is an exemplary amino acid sequence of human ZSCAN20, from NCBI
reference sequence NP_001364305.1. It encodes isoform 1.
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25 **SEQ ID NO: 6** is an exemplary nucleotide sequence of human *Zscan20*, from NCBI
reference sequence NM_001377376.1. It encodes transcript cDNA of transcript variant 1.
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- 18 -

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taggcctccattcctcagacccccagaggcagcagacccaaaggtaggggcagggaaag
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gagccctaacttgggtggcaggtgacaaatgtggcacaaaggagaaaggagtcttctcctt
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ccctcttagggactgtgaaattaaaatggattaaatgttccatcaggagcctcagggga
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tttgtgctgttatgtacatctcttaaagctggtcaaatacatattttgggatggaatggcc
tgataagttaataaattatattaaactgccaaaaaa

15 **SEQ ID NO: 7** is an exemplary gRNA sequence for mouse *Zscan20*.

AGGAUUUGAGUUGAAGCGUG

SEQ ID NO: 8 is an exemplary gRNA sequence for mouse *Zscan20*.

CCCCUGAAGAGACUCCUCGG

20

SEQ ID NO: 9 is an exemplary gRNA sequence for mouse *Zscan20*.

GAAGGUUCUUGAAUCGGUAG

SEQ ID NO: 10 is an exemplary gRNA sequence for mouse *Zscan20*.

25 CAGGCACGACACCCCGAGAG

SEQ ID NO: 11 is an exemplary gRNA sequence for mouse *Jdp2*.

UGUGCCCUCACAGCUAGACG

30 **SEQ ID NO: 12** is an exemplary gRNA sequence for mouse *Jdp2*.

CCAGACCCUUCAGUGACCGC

SEQ ID NO: 13 is an exemplary gRNA sequence for mouse *Jdp2*.

CGCUGACAUCCGCAACAUUG

- 22 -

SEQ ID NO: 14 is an exemplary gRNA sequence for mouse *Jdp2*.

AACGCACAGAGUUUCUGCAG

5 **SEQ ID NO: 15** is an exemplary gRNA sequence for mouse *Nfil3*.

GAAGAUUUGCUCCUGAACGA

SEQ ID NO: 16 is an exemplary gRNA sequence for mouse *Nfil3*.

UUCCUGAGUGUGCUCCACCG

10

SEQ ID NO: 17 is an exemplary gRNA sequence for mouse *Nfil3*.

UCUCCAAAACCAGGUCAUUG

SEQ ID NO: 18 is an exemplary gRNA sequence for mouse *Nfil3*.

15 GAUUUCUUGGGCAUACGCCG

SEQ ID NO: 19 is an exemplary gRNA sequence for human *Zscan20*.

AGCGCUGGCGGGAGGCCUCU

20 **SEQ ID NO: 20** is an exemplary gRNA sequence for human *Zscan20*.

UUAGACAAUGGCUAUGGCCC

SEQ ID NO: 21 is an exemplary gRNA sequence for human *Zscan20*.

GAAACUGGAAGAGGACUCUU

25

SEQ ID NO: 22 is an exemplary gRNA sequence for human *Jdp2*.

UGGGCAGAUCCCGGACCCUU

SEQ ID NO: 23 is an exemplary gRNA sequence for human *Jdp2*.

30 CCGAGCUGGGGAGCCCGGUC

SEQ ID NO: 24 is an exemplary gRNA sequence for human *Jdp2*.

CAAUCAUGGCCCGAGGUUG

- 23 -

SEQ ID NO: 25 is an exemplary gRNA sequence for human *Nfil3*.

UGGACGCGUGUGUAAGAGCCU

SEQ ID NO: 26 is an exemplary gRNA sequence for human *Nfil3*.

5 GAUUAUCAAGCAAGAGCCGA

SEQ ID NO: 27 is an exemplary gRNA sequence for human *Nfil3*.

CUCCUGCGUGUGUUCUACUG

10 **SEQ ID NO: 28** is an exemplary amino acid sequence of human ZNF324.

MAFEDVAVYFSQEEWGLLDTAQRALYRRVMLDNFALVASLGLSTSRPRVVIQLERGEEPWVPSGTD
TTLSRTTYRRRNPGSWSLTEDRDVSGEWPRAFPDTPPGMTTSVFPVAGACHSVKSLQRQRGASPSR
ERKPTGVSVIYWERLLLGSQASVSLRLTSPLRPPEGVRLREKTLTEHALLGRQPRTPERQKPC
AQEVPGRFTGSAQDLEAAGGRGHHRMGAVWQEPHRLLLGGQEPSTWDELGEALHAGEKSFECRACSK
15 VFVKSSDLLKHLRTHTGERPYECAQCCKAFSQTSHLTQHQRHSGETPYACPVCGKAFRHSSSLVR
HQRHTAEKSFRCSECGKAFSHGSNLSQHRKIHAGGRPYACAQCGRRCRNSHLIQHERHTHTEKP
FVCALCGAAFSQGSLLFKHQRVHTGEKPFACPCGGRFSSNLTQHQLLHTGERPFRCVDCGKAF
AKGAVLLSHRRIHTGEKPFVCTQCGRAFRRERPALFHHQRIHTGEKTVRRSRASLHPQARSVAGASS
EGAPAKETEPTPASGPAAVSQPAEV

20

SEQ ID NO: 29 is an exemplary cDNA sequence of human *Znf324*. It encodes.

ATGGCCTTTGAGGATGTGGCTGTGTACTTCTCCCAGGAGGAGTGGGGGCTCCTGGACACAGCCCAG
AGGGCCCTGTACCGCCGCGTGATGCTAGACAACCTTCGCACTTGTGGCCTCGCTGGGACTCTCCACC
TCTCGACCTCGTGTGGTCATCCAACCTGGAGCGTGGCGAGGAGCCCTGGGTTCCTAGTGGAAACGGAC
25 ACAACCCTGTCCAGGACCACCTACAGGAGGCGCAACCCTGGTTCCTGGAGTTTGACAGAGGATAGA
GATGTTTCTGGAGAATGGCCACGAGCTTTCCAGATACCCACCTGGGATGACTACTAGCGTCTTC
CCTGTTGCCGGTGCTGCCACAGTGTAAGGAGCTGCAGAGACAACGGGGTGCTCCCCATCTCGG
GAGAGAAAACCCACGGGGGTGTCGGTGATCTACTGGGAGAGGCTCCTGCTAGGCTCAGGCAGTGGG
CAAGCCAGCGTCAGCTGCGACTGACCTCCCCGCTTAGGCCTCCCGAGGGCGTCCGGCTTAGAGAA
30 AAGACACTCACAGAGCATGCGTTGCTGGGGAGGAGCCAGGACGCCTGAGCGGCAGAAACCATGT
GCACAGGAGGTCCCTGGGAGAACCTTTGGGAGCGCCCAGGACCTGGAGGCTGCCGGCGGTTCGGGA
CATCACCGAATGGGTGCAGTTTGGCAGGAGCCTCATAGACTCCTCGGTGGCCAGGAGCCCTCGACC
TGGGACGAGCTGGGCGAGGCTCTTCACGCTGGGGAGAAGTCCTTCGAATGCAGGGCGTGCAGCAAA
GTGTTCTGTAAGAGCTCCGACCTCCTCAAGCACCTACGCACCCACACCGGGGAGCGGCCCTACGAG

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TGCGCCAGTGCGGCAAGGCCTTCAGCCAGACGTGCGACTTGACGCAGCACCAGCGCATCCACAGC
GGCGAGACGCCCTACGCGTGCCCCGTGTGCGGCAAGGCCTTCCGGCATAGCTCCTCGCTGGTGCGG
CACCAGCGCATCCACACGGCCGAGAAGTCCTTCCGCTGCTCCGAGTGCGGCAAGGCCTTCAGCCAC
GGCTCCAACCTCAGCCAGCACCGCAAGATCCACGCGGGTGGGCGTCCTTATGCTTGCGCACAGTGT
5 GGCCGCCGCTTCTGCCGCAACTCGCACCTGATCCAGCACGAGCGTACGCACACAGGCGAGAAGCCC
TTCGTGTGCGCGCTCTGCGGTGCTGCCTTCAGCCAGGGCTCCTCGCTCTTTAAGCACCAGCGCGTG
CACACAGGCGAGAAGCCCTTCGCCTGCCCACAGTGCGGCCGCGCCTTTAGCCACAGCTCCAACCTC
ACCCAGCACCAGCTCCTGCACACGGGCGAGCGGCCCTTCCGCTGCGTGGACTGTGGCAAGGCCTTC
GCCAAGGGCGCCGTGCTGCTCAGCCACCGGCGCATTCACACGGGCGAGAAGCCCTTCGTGTGTACG
10 CAGTGTGGCCGCGCCTTCCGTGAGCGCCCGGCCCTCTTCCACCACCAGAGGATCCATACCGGCGAG
AAGACCGTCCGGCGATCCAGGGCCAGCCTGCACCCCAGGCCAGGTCTGTTGCCGGGGCATCATCA
GAAGGTGCGCCAGCGAAGGAAACCGAGCCCCTCCCGCCTCGGGCCAGCCGCCGTCTCGCAGCCA
GCGGAGGTCTGA

15 **SEQ ID NO: 30** is an exemplary gRNA sequence for mouse ZFP324.

UGGAGUUGGAUGAUCACACG

SEQ ID NO: 31 is an exemplary gRNA sequence for mouse ZFP324.

GGGAGACCCCAAUGUUACAG

20

SEQ ID NO: 32 is an exemplary gRNA sequence for mouse ZFP324.

AGGGGUAUCAGUGAUUUACU

SEQ ID NO: 33 is an exemplary gRNA sequence for mouse ZFP324.

25 UAUGAGUACGCAGAUUCUUG

SEQ ID NO: 34 is an exemplary gRNA sequence for human ZNF324.

GCUCCUGGACACAGCCCAGA

30 **SEQ ID NO: 35** is an exemplary gRNA sequence for human ZNF324.

GCGGCGGUACAGGGCCCUCU

SEQ ID NO: 36 is an exemplary gRNA sequence for human ZNF324.

AGUUGUCUAGCAUCACGCGG

35

- 25 -

SEQ ID NO: 37 is an exemplary gRNA sequence for human ZNF324.

AAAAGCCUGCAGAGACAACG

5 **SEQ ID NO: 38** is an exemplary gRNA sequence for human ZNF324.

AGUUGUCUAGCAUCACGCGG

SEQ ID NO: 39 is an exemplary gRNA sequence for human ZNF324.

GCCCUCGGGAGGCCUAAGCG

10

SEQ ID NO: 40 is an exemplary gRNA sequence for human ZNF324.

GCGGCAGAAACCAUGUGCAC

SEQ ID NO: 41 is an exemplary gRNA sequence for human ZNF324.

15 GCUCCUCGCCACGCUCCAGU

SEQ ID NO: 42 is an exemplary gRNA sequence for human ZNF324.

GCUAGACAACUUCGCACUUG

20 **SEQ ID NO: 43** is an exemplary gRNA sequence for human ZNF324.

CAGGGUUGUGUCCGUUCCAC

SEQ ID NO: 44 is an exemplary gRNA sequence for human ZNF324.

UCGCCGGACGGUCUUCUCGC

25

SEQ ID NO: 45 is an exemplary gRNA sequence for human ZNF324.

GAGAACCUUUGGAGCGCCC

SEQ ID NO: 46 is an exemplary gRNA sequence for human ZNF324.

30 CGAAGUUGUCUAGCAUCACG

DETAILED DESCRIPTION

I. Summary of Terms

Unless otherwise noted, technical terms are used according to conventional usage.

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Definitions of many common terms in molecular biology may be found in Krebs et al. (eds.), Lewin's genes XII, published by Jones & Bartlett Learning, 2017; The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd., 1994; and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc.,
5 1995; and other similar references.

As used herein, the singular forms "a," "an," and "the," refer to both the singular as well as plural, unless the context clearly indicates otherwise. As used herein, the term "comprises" means "includes." Thus, "comprising a nucleic acid molecule" means "including a nucleic acid molecule" without excluding other elements. It is further to be understood that any and all base sizes given
10 for nucleic acids are approximate, and are provided for descriptive purposes, unless otherwise indicated. Although many methods and materials similar or equivalent to those described herein can be used, particular suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

15 In order to facilitate review of the various aspects of the disclosure, the following explanations of specific terms are provided:

About: Unless context indicated otherwise, "about" refers to plus or minus 5% of a reference value. For example, "about" 100 refers to 95 to 105.

Administration: To provide or give a subject an agent, such as a modified PBMC
20 described herein, by any effective route. Administration can be local or systemic. Exemplary routes of administration include, but are not limited to, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, intratumoral, intraprostatic, intrathecal, intraosseous, and intravenous), oral, sublingual, rectal, transdermal, intranasal, vaginal and inhalation routes. In some examples, modified PBMCs provided herein (such as T cells) are administered by
25 intravenous injection.

Adoptive Cell Transfer (ACT) Therapy: A type of immunotherapy in which a T cell that has been modified (e.g., modified to recognize a tumor antigen) and/or expanded in vitro (or ex vivo) is administered to a patient in need thereof. T cells for ACT therapy can be a patient's own T cells or T cells from a donor. ACT therapies include, for example, Chimeric Antigen Receptor T cell (CAR-T), Engineered T Cell Receptor (TCR), or Tumor-Infiltrating Lymphocyte (TIL)
30 therapies. ACT therapy is also sometimes referred to as adoptive cell therapy, cellular adoptive immunotherapy, or T-cell transfer therapy.

Agent: Any substance or any combination of substances (small molecules, proteins,

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peptides, nucleic acid molecules, antisense molecules etc.) that is useful for achieving an end or result; for example, a substance or combination of substances useful for inhibiting ZSCAN20 activity.

Cancer: A malignant tumor characterized by abnormal or uncontrolled cell growth. Other features often associated with cancer include metastasis, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels and suppression or aggravation of inflammatory or immunological response, invasion of surrounding or distant tissues or organs, such as lymph nodes, etc. “Metastatic disease” refers to cancer cells that have left the original tumor site and migrate to other parts of the body for example via the bloodstream or lymph system.

Cas9: An RNA-guided DNA endonuclease enzyme that that participates in the CRISPR-Cas immune defense against prokaryotic viruses. Cas9 has two active cutting sites (HNH and RuvC), one for each strand of the double helix. Thus, Cas9 proteins can be used to edit DNA in combination with an appropriate guide RNA. Cas9 sequences are publicly available. For example, GenBank® Accession Nos. nucleotides 796693..800799 of CP012045.1 and nucleotides 1100046..1104152 of CP014139.1 disclose exemplary Cas9 nucleic acids, and GenBank® Accession Nos. NP_269215.1, AMA70685.1, and AKP81606.1 disclose exemplary Cas9 proteins.

Catalytically inactive (deactivated or dead) Cas9 (dCas9) proteins, which have reduced or abolished endonuclease activity but still binds to dsDNA, are also encompassed by this disclosure.

In some examples, a dCas9 includes one or more mutations in the RuvC and HNH nuclease domains, such as one or more of the following point mutations: D10A, E762A, D839A, H840A, N854A, N863A, and D986A. An exemplary dCas9 sequence includes both a D10A and H840A substitutions. In one example, the dCas9 protein has mutations D10A, H840A, D839A, and N863A (see, e.g., Esvelt et al., Nat. Meth. 10:1116-21, 2013). Exemplary dCas9 sequences are provided in GenBank® Accession Nos. AKA60242.1 and KR011748.1.

Chimeric antigen receptor (CAR): Artificial, engineered T cell receptors, which graft an arbitrary specificity onto an immune effector cell. These receptors can be used to graft the specificity of a monoclonal antibody onto a T cell; with transfer of their coding sequence facilitated by vectors. Thus, a CAR that “specifically binds” or is “specific” for an antigen is a CAR that binds the antigen with high affinity and does not significantly bind other unrelated antigens. Using adoptive cell transfer, CARs can be useful to treat cancer. For example, T cells (obtained from the patient or from a donor) are modified such that they express receptors specific to the patient's particular cancer. The modified T cells, which can then recognize and kill the cancer cells, are

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introduced into the patient. In some examples, the modified PBMC disclosed herein express a CAR.

First generation CARs typically included the intracellular domain from the CD3 ζ - chain, which is the primary transmitter of signals from endogenous TCRs. Second generation CARs added intracellular signaling domains from various costimulatory protein receptors (e.g., CD28, 41BB, ICOS) to the cytoplasmic tail of the CAR to provide additional signals to the T cell. Third generation CARs combine multiple signaling domains, such as CD3 ζ -CD28-41BB or CD3 ζ -CD28-OX40, to augment potency. A multispecific CAR is a single CAR molecule comprised of at least two antigen-binding domains (such as scFvs and/or single-domain antibodies) that each bind a different antigen or a different epitope on the same antigen (see, for example, US 2018/0230225). For example, a bispecific CAR refers to a single CAR molecule having two antigen-binding domains that each bind a different antigen. A bicistronic CAR refers to two complete CAR molecules, each containing an antigen-binding moiety that binds a different antigen. In some cases, a bicistronic CAR construct expresses two complete CAR molecules that are linked by a cleavage linker. T cells expressing a bispecific or bicistronic CAR can bind cells that express both of the antigens to which the binding moieties are directed (see, for example, Qin et al., Blood 130:810, 2017; and WO/2018/213337). Any of these CARs can be used with the methods described herein.

Complementarity: The ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick base pairing or other non-traditional types. A percent complementarity indicates the percentage of residues in a nucleic acid molecule which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, and 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary, respectively).

In some aspects, a disclosed nucleic acid molecule (such as a disclosed gRNA or RNAi) hybridizes to a target nucleic acid, thus the nucleic acid molecule is complementary to the target sequence. For example, in some examples, a RNAi or gRNA specific for *Zscan20* gene or transcript is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% complementary to a unique portion of a target gene, such as *Zscan20*. In some examples, the target sequence is at least 10 contiguous nucleotides, for example, at least 12, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 contiguous nucleotides. In further examples, the target sequence is 10-50 contiguous nucleotides, for example, 12-40, 12-30, 12-20, 12-15, 15-30, 15-20, 20-30, or 20-40 contiguous nucleotides. In some examples, the targeting sequence is at least 80%, at least 85%, at

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least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% complementary to a sequence about 20 nucleotides long in a *Zscan20* gene or transcript.

Control: A reference standard. In some aspects, the control is a negative control. In other aspects, the control is a positive control. In some examples, a suitable control is a historical control or standard reference value or range of values (such as a previously tested control sample, such as a group of patients diagnosed with a disease or condition, for example cancer or viral infection, that have a known prognosis or outcome, or a group of samples that represent baseline or normal values). In some examples, the control may be a subject not receiving treatment with an agent (e.g., the disclosed modified PBMCs) or receiving an alternative treatment, or a baseline reading of the subject prior to treatment with an agent.

A difference between a test sample and a control can be an increase or conversely a decrease. The difference can be a qualitative difference or a quantitative difference, for example a statistically significant difference. In some examples, a difference is an increase relative to a control, for example, an increase of at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 350%, at least about 400%, or at least about 500%. In other examples, a difference is a decrease relative to a control, for example, a decrease of at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or at least about 100%.

CRISPR/Cas system: A prokaryotic immune system that confers resistance to foreign genetic elements, such as plasmids and phages, and provides a form of acquired immunity. In the endogenous system, a trans-activating crRNA (tracrRNA) hybridizes with the repeat sequence of another RNA molecule known as CRISPR RNA (crRNA) to form a unique dual-RNA hybrid structure that binds Cas endonuclease, forming a ribonucleoprotein (RNP) complex. The crRNA contains a targeting sequence complementary to a target gene, which guides the CRISPR/Cas RNP complex to the target.

In some examples, the Cas endonuclease is Cas9, which induces a double stranded DNA break in the target gene. The CRISPR/Cas9 system can be used to decrease gene expression, for example, by targeting and inducing double-stranded DNA breaks in a target gene, such as *Zscan20*. Similarly, a CRISPR/Cas13 system can be used to cut RNA. Cas endonucleases (or cas nucleases)

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include, but are not limited to, Cas3, dCas3, Cas9, dCas9, Cas12, dCas12, Cas13a, dCas13a, Cas13b, dCas13b, Cas13d, or dCas13d. Cas endonucleases can cleave RNA or DNA depending on the specific endonuclease chosen.

Down Regulation and Knock-Out: When used in reference to the expression of a molecule, such as a target, “down regulation” refers to any process which results in a decrease in production of an RNA of interest. In one example, downregulation decreases detectable RNA expression or RNA activity. A “knock-out” is the removal or inactivation of one or more genes (such as *Zscan20*, *Jdp2*, and/or *Nfil3*) from an organism, which can result in a decrease in detectable RNA expression or RNA activity.

Downregulation includes any detectable decrease in the RNA. In certain examples, detectable RNA in a cell or cell free system decreases by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% (such as a decrease of 40% to 90%, 40% to 80% or 50% to 95%) as compared to a control (such an amount of *Zscan20*, *Jdp2*, and/or *Nfil3* RNA detected in a corresponding non-treated cell or sample).

Effective amount: The amount of an agent (such as the modified PBMC, RNAi, gRNA, or other composition disclosed herein) that is sufficient to effect beneficial or desired results. An effective amount (also referred to as a therapeutically effective amount) may vary depending upon one or more of: the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like. The beneficial therapeutic effect can include enablement of diagnostic determinations; amelioration of a disease, symptom, disorder, or pathological condition; reducing or preventing the onset of a disease, symptom, disorder or condition; and generally counteracting a disease, symptom, disorder or pathological condition.

In one aspect, an “effective amount” of a therapeutic agent (e.g., a modified PBMC disclosed herein) is an amount sufficient to reduce the volume/size of a tumor, the weight of a tumor, the number of metastases, reduce the volume/size of a metastasis, the weight of a metastasis, or combinations thereof, for example by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, or at least 99% (as compared to a suitable control, such as no administration of the therapeutic agent). In one aspect, an “effective amount” of a therapeutic agent (e.g., a modified PBMC disclosed herein) is an amount sufficient to reduce signs or symptoms of the viral infection in a subject, reduce the viral load in a subject, reduce infectivity of

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a virus, reduce cytopathic effect in the subject's cells, or combinations thereof, for example by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, or at least 99% (as compared to a suitable control, such as no administration of the therapeutic agent). In one aspect, an "effective amount" of a therapeutic agent (e.g., a gRNA or siRNA disclosed herein) is an amount sufficient to reduce activity or expression of a target (e.g., ZSCAN20), for example by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, at least 98%, or even 100% (as compared to a suitable control, such as expression or activity prior to administering the therapeutic agent). In some examples, combinations of these effects are achieved.

Guide RNA (gRNA): An RNA component of a CRISPR/Cas system that targets the CRISPR/Cas ribonucleoprotein (RNP) complex to a target nucleic acid sequence, such as a target DNA (e.g., genomic sequence) or target RNA sequence. gRNA molecules include (1) a portion with sequence complementarity to the target nucleic acid (such as at least 80%, at least 90%, at least 95%, or 100% sequence complementarity), and (2) a portion with secondary structure that binds to the Cas nuclease. Such portions can be part of the same molecule (e.g., sgRNA: a synthetic chimera that combines a crRNA and tracrRNA into a single RNA transcript), or divided over two or more separate molecules (e.g., 2 part gRNA wherein the crRNA and tracrRNA are separate RNA transcripts). For simplicity, both types of molecules are referred to herein as gRNA.

Many techniques for genome editing using the CRISPR/Cas system have been described. In brief, gRNA directs a Cas DNA nuclease (such as Cas9) to a target gene (DNA). Cas9 then introduces a double stranded break at the target site. Disruptive mutations can be introduced through non-homologous end joining of the cut DNA. Cas9 can also be used to delete larger DNA fragments, for example, by using two gRNAs targeting separate sites, thus causing a deletion of the intervening sequence between the two cut sites. A DNA template with homology to the target site can also be added to introduce insertions using homology directed DNA repair mechanisms.

In RNA editing, the gRNA directs a Cas RNA nuclease (such as Cas13d) to a target RNA. In one such example, the gRNA includes from 5' to 3' (1) a crRNA containing a direct repeat (DR) region and (2) a spacer, for example for Cas13a, Cas13c, and Cas 13d nucleases. In one example includes about 36nt of DR followed by about 28-32nt of spacer sequence. In another such example, the gRNA includes from 5' to 3' (1) a spacer and (2) a crRNA containing a DR region, for example for Cas13b nuclease. In some examples, the gRNA is processed (truncated/modified)

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by a Cas RNA nuclease or other RNases into the shorter “mature” form. The DR is the constant portion of the sgRNA, containing secondary structure which facilitates interaction between the Cas RNA nuclease protein and the gRNA. The spacer portion is the variable portion of the gRNA, and includes a sequence designed to hybridize to a target RNA sequence (and in some examples edit the target RNA sequence). In some examples, the full length spacer is about 28-32nt (such as 30-32 nt) long while the mature (processed) spacer is about 14-30nt.

The targeting portion of the gRNA can be modified to facilitate targeting of any DNA or RNA sequence of interest. (See CRISPR–Cas9 Structures and Mechanisms. Fuguo Jiang and Jennifer A. Doudna, Annual Review of Biophysics, 46:1, 505-529 (2017)). A gRNA that is “specific” for a target has sufficient complementarity to the target sequence that it binds the target and does not significantly hybridize with other unrelated sequences. The targeting sequence of the gRNA is typically about 20 nucleotides, for example, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, or about 25 nucleotides. The degree of complementarity between a targeting sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, about 60%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97.5%, about 98%, about 99%, or more. In some aspects, the degree of complementarity is 100%. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting examples of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g., the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). In some examples, the targeting sequence is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% complementary to a contiguous amino acid sequence about 20 nucleotides long in a *Zscan20*, *Jdp2*, and/or *Nfil3* gene or transcript.

Heterologous: A heterologous protein or polypeptide refers to a protein or polypeptide derived from a different source or species. A heterologous nucleic acid molecule refers to a nucleic acid molecule derived from a different source or species. Thus, a heterologous protein, polypeptide, or nucleic acid molecule in a cell, refers to a protein, polypeptide, or nucleic acid molecule not naturally found in the cell in nature (e.g., an exogenous protein, polypeptide, or nucleic acid molecule). In some examples, a cell expressing a heterologous protein, polypeptide, or nucleic acid molecule is transgenic.

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Immune Checkpoint Blockade (ICB) (or Checkpoint Inhibitor or Checkpoint Blockade):

A therapeutic that targets a checkpoint protein. Checkpoint proteins help prevent over-active immune responses or autoimmunity, and in some examples reduce the ability of T cells to reduce/eliminate cancerous cells. When checkpoints are blocked (e.g., PD-1 blockade) T cells can better target and kill cancerous cells. Examples of checkpoint proteins found on T cells or cancerous cells include PD-1/PD-L1/PD-L2, and CTLA-4/B7-1/B7-2.

Exemplary ICB agents include ipilimumab (Yervoy®), nivolumab (Opdivo®), pembrolizumab (Keytruda®), atezolizumab (Tencentriq®), avelumab (Bavencio®), durvalumab (Imfinzi®), cemiplimab (Libtayo®), palbociclib (Ibrance®), ribociclib (Kisquali®), pidilizumab, avelumab, and abemaciclib (Verzenio®). Further examples are provided in Qiu et al., Journal of the European Society for Therapeutic Radiology and Oncology, 126(3):450-464, 2018; Visconti et al., J Exp Clin Cancer Res. 35(1): 153, 2016; and Mills et al. Cancer Res. 77(23): 6489-6498, 2017. Further exemplary ICB agents include anti-PD-1, anti-PD-L1, anti-CTLA-4, anti-LAG3 anti-GITR, anti-4-1BB, anti-CD40, and anti-OX40, anti-TIGIT, anti-VISTA, anti-CD73, anti-CD39, anti-HVEM, anti-BTLA, and anti-CD27.

Exemplary anti-PD-1 mAbs include nivolumab, pembrolizumab, pidilizumab, and cemiplimab. Exemplary anti-PD-L1 mAbs include atezolizumab, avelumab, durvalumab, cosibelimab, KN035 (envafolimab), BMS-936559, BMS935559, MEDI-4736, MPDL-3280A, and MEDI-4737. Exemplary anti-CTLA-4 mAbs include ipilimumab and tremelimumab

Immunostimulatory antibody: Antibodies that function to enhance an immune response.

Immunotherapy: A therapy that uses an agent to stimulate or suppress the immune system to treat a disease, such as cancer. Some examples of cancer immunotherapy include immune checkpoint inhibitors, adoptive cell transfer (ACT) immunotherapy, antibodies, vaccines, and immune system modulators. Specific, non-limiting examples include nivolumab, pembrolizumab, pidilizumab, atezolizumab, durvalumab, avelumab, and ipilimumab.

Increase or Decrease: A positive or negative change, respectively, in quantity from a control value (such as a value representing no therapeutic agent). An increase is a positive change, such as an increase at least 25%, at least 50%, at least 100%, at least 200%, at least 300%, at least 400% or at least 500%, as compared to the control value. A decrease is a negative change, such as a decrease of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 100% decrease as compared to a control value. In some examples, the increase or decrease is statistically significant relative to a suitable control.

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An agent (e.g., the RNAi or a gRNA specific for *Zscan20* disclosed herein) that decreases expression or activity of a gene (e.g., *Zscan20*) or gene product (e.g., ZSCAN20) is a compound that reduces the level of the mRNA or a functional product encoded by the gene in a cell or tissue (e.g., a PBMC), or reduces (including eliminates or inhibits) one or more activities of the gene product. In some aspects, expression of *Zscan20* is reduced at least 10%, at least 25%, at least 50%, at least 75%, at least 90%, at least 95%, at least 99%, at least 99.9%, or even 100% relative to a control, such as an untreated subject or cells. Conversely, an agent that increases expression or activity of a gene or gene product is a compound that increases the level of the mRNA or protein product encoded by the gene in a cell or tissue, or increases one or more activities of the gene product.

In some aspects, an agent (e.g., the RNAi or a gRNA specific for *Zscan20* disclosed herein) or non-naturally occurring genetic modification can increase or decrease an activity of a PBMC (e.g., a T cell) when it is present in the PBMC. For example, in some aspects the PBMC is a T cell and the agent (e.g., the RNAi or a gRNA specific for *Zscan20* disclosed herein) or genetic modification (a point mutation, a partial deletion, full deletion, or insertion that reduces expression of *Zscan20*, as disclosed herein) reduces T cell exhaustion, for example, by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% relative to a suitable control (e.g., measurements prior to treatment or comparison to an untreated control group). A decrease in T cell exhaustion can be measured, for example, by a decrease in CD101, CD39, or CD38, and/or by an increase in cytotoxic activity (e.g., increased tumor control, IFN- γ TNF- α production) and/or by increased CX3CR1 and/or KLRG1 expression, or by measuring another indicator of T cell effector activity, relative to a suitable control. In some examples, combinations of these effects are achieved.

In some aspects the agent (e.g., the RNAi or a gRNA specific for *Zscan20* disclosed herein) or genetic modification (a point mutation, a partial deletion, full deletion, or insertion that reduces expression of *Zscan20*, as disclosed herein) increases the activity or function of the PBMC. For example, in some examples the PBMC is a T cell and the agent can increase the activity or effector function of a T cell by at least 10%, at least 25%, at least 50%, at least 75%, at least 90%, at least 95%, at least 100%, at least 200%, at least 300%, at least 400%, or at least 500% relative to a suitable control (e.g., measurements prior to treatment or comparison to an untreated control group). An increase in effector function of a cytotoxic T cell can be measured, for example, by increased TNF- α and/or IFN- γ production, or by CX3CR1 and/or KLRG1 expression or by another indicator of effector function, relative to a suitable control. In some examples, combinations of

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these effects are achieved.

Isolated: An “isolated” biological component (e.g., a cell, PBMC, nucleic acid, protein) has been substantially separated, produced apart from, or purified away from other biological components in the cell or tissue of an organism in which the component occurs, such as other cells (e.g., RBCs), chromosomal and extrachromosomal DNA and RNA, and proteins. Nucleic acids and proteins that have been “isolated” include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids and proteins. For example, PBMCs or TILs isolated from patient blood, tumor, or other sample, are at least 50% pure, such as at least 60%, such as at least 75%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or more, pure.

Jun dimerization protein 2 (*Jdp2*): (e.g., OMIM 608657) a TF suggested to control transcription via direct regulation of the modification of histones and the assembly of chromatin or heterodimerization with a component of the AP-1 complex. Aronheim et al. Mol. And Cell. Bio. (1997) 17:3094-3102. *Jdp2* sequences are publicly available, and exemplary reference sequences include Amino Acid NCBI Sequence: NP_001128520.1 (human), NP_112149.2 (*Mus musculus*), XP_020955192.1 (*Sus scrofa*); Nucleotide NCBI Sequences: NC_000014.9 (human), NM_001135048.2 (human), NM_030887.3 (*Mus musculus*), XM_021099533.1 (*Sus scrofa*) each of which is herein incorporated by reference in their entirety. NCBI Gene ID: 122953. In this disclosure reference to *Jdp2* or JDP2 includes the corresponding gene or protein in any species: human, mouse, or otherwise, such as any mammalian *Jdp2*. Also known as JUNDM2.

Modification: A change in the sequence of a nucleic acid (a “genetic modification”) or protein molecule. For example, amino acid or nucleic acid sequence modifications include mutations thereof, for example, substitutions, insertions, and deletions, or combinations thereof. Insertions include 3’ or 5’ end fusions or amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues or nucleotides. Deletions are characterized by the removal of one or more amino acid residues from a protein sequence or nucleotides from a nucleic acid sequence. In some aspects herein, the modification (such as a substitution, insertion, or deletion) results in a change in function, such as a reduction or enhancement of a particular activity of a protein. Substitutional modifications are those in which at least one residue or nucleotide has been removed and a different residue or nucleotide inserted in its place. Substitutions, deletions, insertions, or any combination thereof may be combined to arrive at a final mutant sequence. Amino acid modifications can be prepared by modification of nucleotides

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in the DNA encoding the protein, thereby producing DNA encoding the modification. In particular examples, the presence of one or more modifications in a gene can significantly inactivate that gene. A “modified” protein, nucleic acid, or organism is one that has one or more modifications as outlined above. Genetic modifications can include point mutations, partial deletions, full deletions, or insertions.

Nuclear factor, interleukin 3 regulated (*Nfil3*): (e.g., OMIM 605327) a TF regulator in both innate and adaptive immune cells. Previously, *Nfil3* had no reported role in CD8+ T cell differentiation. *Nfil3* sequences are publicly available, and exemplary reference sequences include Amino Acid NCBI Reference Sequence: NP_005375.2 (human), NP_059069.1 (*Mus musculus*), XP_003359068.1 (*Sus scrofa*); Nucleotide NCBI Reference Sequence: NC_000009.12 (human), NM_005384.3 (human), NM_017373.3 (*Mus musculus*) XM_003359020.4 (*Sus scrofa*) each of which is herein incorporated by reference in their entirety. NCBI Gene ID: 4783. In this disclosure reference to *Nfil3* or NFIL3 includes the corresponding gene or protein in any species: human, mouse, or otherwise, such as any mammalian *Nfil3*. Also known as E4BP4; IL3BP1; NFIL3A; and NF-IL3A.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence (for example, a promoter that drives expression of the heterologous nucleic acid sequence encoding the siRNA or gRNA disclosed herein). Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, are in the same reading frame.

Peripheral Blood Mononuclear Cell (PBMC): Cells that have one round nucleus. This term includes cells found in the blood. In this disclosure, this term is not limited to cells found in peripheral blood. In this disclosure PBMC is inclusive of tissue resident populations of cells, such as tissue resident memory T cells or tumor infiltrating lymphocytes, which are not commonly found in the peripheral blood. Examples include mast cells, macrophages, natural killer cells, monocytes, T cells, B cells, plasma cells, and dendritic cells. PBMCs do not include neutrophils, eosinophils or basophils. In one example, PBMCs are substantially isolated from other blood cells prior to use. In another example, PBMCs includes CD8+ TRM and TILs isolated from a solid tumor. In a further example, PBMCs includes immune cells with one round nucleus which are isolated from a solid tumor, or a non-peripheral blood tissue.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers useful in

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this disclosure are conventional. Remington's Pharmaceutical Sciences, 23rd Edition, Academic Press, Elsevier, (2020), describes compositions and formulations suitable for pharmaceutical delivery of a therapeutic agent, such as modified PBMCs disclosed herein.

In general, the nature of the carrier depends on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, 5% human serum albumin, glycerol, or the like as a vehicle. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate. Supplementary active compounds can also be incorporated into the compositions.

Programmed cell death protein 1 (PD-1): A cell surface receptor that belongs to the immunoglobulin superfamily and is expressed on T cells and pro-B cells. PD-1 binds two ligands, PD-L1 and PD-L2. The human form is a 268 amino acid type 1 transmembrane protein. PD-1 is an inhibitory receptor that suppresses T cell activity and mediates T-cell exhaustion. PD-1 sequences are publicly available, for example from the GenBank® sequence database (e.g., Accession Nos. NP_005009.2 (mature peptide is aa 21-288), CAA48113.1, NP_001301026.1 (mature peptide is aa 25-288), and CAA48113.1 (mature peptide is aa 21-288) provide exemplary PD-1 protein sequences, while Accession Nos. L27440.1, NM_005018.2, X67914.1, AB898677.1 and EU295528.2 provide exemplary PD-1 nucleic acid sequences).

Promoter: An array of nucleic acid control sequences which direct transcription of a nucleic acid. A promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription.

Examples of promoters include, but are not limited to the SV40 promoter, the CMV enhancer-promoter, the CMV enhancer/ β -actin promoter, EF1a promoter, or PGK promoter. In one example, expression of a gRNA is driven by a polymerase III promoter, such as U6 or H1, such as human or mouse U6 or H1 promoter. Both constitutive and inducible promoters are included (see e.g., Bitter et al., Methods in Enzymology 153:516-544, 1987). Also included are those promoter elements that are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in

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the 5' or 3' regions of the gene. Promoters produced by recombinant DNA or synthetic techniques can also be used to provide for transcription of the nucleic acid sequences.

Prevent: Preventing a condition refers to reducing, delaying, or inhibiting the full development of a condition, for example preventing, reducing, or slowing the progression of a T cell to an exhausted T cell. In one example an agent that reduces *Zscan20* expression, or a non-naturally occurring genetic modification that reduces an amount of functional ZSCAN20, when present in a PBMC, such as a T cell, such as a CAR or TCR, prevents or reduces the likelihood that the cell will become exhausted (e.g., prevents or reduces the likelihood a T cell will overexpress programmed cell death 1 (PD1^{hi}), become positive for T cell immunoglobulin and mucin domain-containing protein 3 (TIM3⁺), express CTLA-4, express lymphocyte-activation gene 3 (LAG-3), express TIGIT, and/or express CD160) or may slow the progression of the cell to an exhausted state. In some examples, the disclosed modified PBMCs, such as modified T cells, do not become exhausted. In some examples, the disclosed modified PBMCs, such as modified T cells, show a reduction in exhaustion, such as a reduction of least 10%, at least 25%, at least 50%, at least 75%, at least 90%, at least 95%, at least 99%, or at least 99.9%, relative to an unmodified PBMC or T cell. In some examples, the disclosed modified PBMCs, such as modified T cells, show a slower progression to exhaustion, such as an increase in the number of days to exhaustion of least 10%, at least 25%, at least 50%, at least 75%, at least 90%, at least 95%, at least 99%, slower relative to an unmodified PBMC or T cell.

RNA interference or interfering RNA (RNAi): A cellular process that inhibits expression of genes, including cellular and viral genes. RNAi is a form of antisense-mediated gene silencing involving the introduction of double stranded RNA-like oligonucleotides leading to the sequence-specific reduction of RNA transcripts. RNA molecules that inhibit gene expression through the RNAi pathway can include siRNAs, miRNAs, gRNAs, and shRNAs. In one example, an RNAi is specific for *Zscan20*, and can specifically hybridize to a *Zscan20* nucleic acid molecule.

Sequence identity: The similarity between amino acid or nucleotide sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are. Homologs of a polypeptide (or nucleotide sequence) will possess a relatively high degree of sequence identity when aligned using standard methods.

Methods of alignment of sequences for comparison have been described. Various programs and alignment algorithms are described in: Smith and Waterman, Adv. Appl. Math. 2:482, 1981;

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Needleman and Wunsch, J. Mol. Biol. 48:443, 1970; Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85:2444, 1988; Higgins and Sharp, Gene 73:237, 1988; Higgins and Sharp, CABIOS 5:151, 1989; Corpet et al., Nucleic Acids Research 16:10881, 1988; and Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85:2444, 1988. Altschul et al., Nature Genet. 6:119, 1994, presents a
5 detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., J. Mol. Biol. 215:403, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. A description of how to determine
10 sequence identity using this program is available on the NCBI website on the internet.

Short hairpin RNA (shRNA): A sequence of RNA that makes a tight hairpin turn and can be used to silence gene expression via the RNAi pathway. The shRNA hairpin structure is cleaved by cellular machinery into siRNA. A shRNA that is “specific” for a target sequence (such as *Zscan20*) has sufficient complementarity to the target sequence that it binds the target and does not
15 significantly hybridize with other unrelated sequences.

Small interfering RNA (siRNA): A double-stranded nucleic acid molecule that modulates gene expression through the RNAi pathway. siRNA molecules are generally 15 to 40 nucleotides in length, such as 20-30 or 20-25 nucleotides in length, with 0 to 5 (such as 2)-nucleotide overhangs on each 3' end. However, siRNAs can also be blunt ended. Generally, one strand of a
20 siRNA molecule is at least partially complementary to a target nucleic acid, such as a target mRNA. siRNAs are also referred to as “small inhibitory RNAs.” A siRNA that is “specific” for a target sequence (such as *Zscan20*) has sufficient complementarity to the target sequence that it binds the target and does not significantly hybridize with other unrelated sequences.

Subject: A vertebrate, such as a mammal, for example a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. In one aspect, the
25 subject is a non-human mammalian subject, such as a monkey or other non-human primate, mouse, rat, rabbit, pig, goat, sheep, dog, cat, horse, or cow. In some examples, the subject has cancer (or a tumor), that can be treated using the modified PBMCs disclosed herein. In some examples, the subject is a laboratory animal/organism, such as a mouse, rabbit, or rat.

T cells: A white blood cell (lymphocyte) that is an important mediator of the immune response. T cells include, but are not limited to, CD3+ T cells, CD4+ T cells and CD8+ T cells. A
30 CD4+ T cell is an immune cell that carries a marker on its surface known as “cluster of differentiation 4” (CD4). These cells, also known as helper T cells, help orchestrate the immune

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response, including antibody responses as well as killer T cell responses. CD8+ T cells carry the “cluster of differentiation 8” (CD8) marker. In some examples, a CD8+ T cell is a cytotoxic T lymphocyte (CTL). CD3+ T cells carry the “cluster of differentiation 3” (CD3) marker, a multimeric protein complex historically known as the T3 complex.

Activated T cells can be detected by an increase in cell proliferation and/or expression of or secretion of one or more cytokines (such as IL-2, IL-4, IL-6, IFN- γ , or TNF α). Activation of CD8+ T cells can also be detected by an increase in cytolytic activity in response to an antigen.

“Exhausted T cells” are dysfunctional T cells (hyporesponsive) commonly found in cancer environments. T cell exhaustion is characterized by a progressive loss of effector function (for example, loss of IL-2, TNF- α , and IFN- γ production) and sustained expression of inhibitory receptors such as PD-1, T cell immunoglobulin domain and mucin domain-containing protein 3 (Tim-3), CTLA-4, lymphocyte-activation gene 3 (LAG-3), and CD160. In some examples, the exhausted T cell is a CD3+ T cell or CD8+ T cell. In some examples, the exhausted T cell is a terminally exhausted T cell (a terminally differentiated T cell that is exhausted). In some examples the exhausted cell is an exhausted progenitor (Tex_{Prog}) or an exhausted effector-like (Tex_{Eff-like}) cell.

“Terminally Exhausted T cells” (Tex_{Term}) may express PD1, and may lack expression of SLAMF6 and/or CX3CR1 relative to other T cells (PD1+, SLAMF6-, CX3CR1-). T cells that are PD1+, SLAMF6- and/or CX3CR1- can be determined by FACs analysis, for example, by FACs analysis of a population of T cells. In some examples, terminally exhausted T cells express ZSCAN20. A possible cause of T cell exhaustion is chronic activation or prolonged antigen stimulation. In some examples, the modified PBMC is an exhausted T cell (including a terminally exhausted T cell). In some examples terminally exhausted cells are defined as PD1+, SLAMF6-, CX3CR1- cells. In other examples terminally exhausted cells are defined as PD1+, CD101+ cells. In further examples terminally exhausted cells are CD39+, CD38+. In some examples, terminally exhausted T cells are defined as PD1+ LAG3+ CTLA4+ and CD45RA^{low}.

A **“Therapeutic T Cell”** is a T cell that is used for therapy, such as immunotherapy (e.g., cancer immunotherapy). Therapeutic T cells are administered to a subject for treatment of a particular disease, for example, cancer or an immune disease. In some examples, the therapeutic T cell recognizes and kill target cells, for example, cancerous cells, thereby treating a disease, such as cancer. Therapeutic T cells may be autologous or allogeneic to the subject. In some examples, the therapeutic T cell is a T cell to be used for Adoptive Cell Transfer (ACT) immunotherapy. In further examples, the therapeutic T cell expresses a Chimeric Antigen Receptor (CAR) or Engineered T Cell Receptor (TCR), and/or is a Tumor-Infiltrating Lymphocyte (TIL). In other

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examples the T cell is an exhausted T cell or a tissue resident memory (TRM) T cell.

T cell receptor (TCR): A receptor found on the surface of T lymphocytes (or T cells) responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules. The TCR is composed of two different protein chains. In humans, in
 5 95% of T cells the TCR consists of an alpha (α) and beta (β) chain, whereas in 5% of T cells the TCR consists of gamma and delta (γ/δ) chains. This ratio changes during ontogeny and in diseased states as well as in different species. When the TCR engages with antigenic peptide and MHC (peptide/MHC), the T lymphocyte is activated through signal transduction, that is, a series of biochemical events mediated by associated enzymes, co-receptors, specialized adaptor molecules,
 10 and activated or released transcription factors. In one example, a TCR is a recombinant TCR, such as one used in TCR-engineered T cells for ACT therapy.

Tissue Resident Memory (TRM) Cells: Immune memory subset cells that reside *in situ*, typically in nonlymphoid tissues, rather than recirculating. In some examples, the TRM are CD8+ TRM cells. In some examples, the TRM are CD4+ TRM cells. Exemplary TRM genetic markers
 15 include one or more of *Itgae*, *Itga1*, *Runx3*, *Cxcr3*, *Prdm1*, *Notch2*, *Il7r*, *Id3*, or *Cd69* and/or reduced expression of one or more of *Slpr1*, *Klf2*, *Klf3*, *Tox*, *Entpd1*, *Eomes*, *Tbx21*, *Tigit*, *Cd38*, *Lag3*, *Cx3cr1*, *Cd101*, and/or *Havcr2*. CD8+ TRM in some examples include CD69 and/or CD103 on the cell surface.

Transformed: A transformed cell is a cell (such as a PBMC, such as a T cell) into which a
 20 nucleic acid molecule has been introduced by molecular biology techniques. As used herein, the term transformed and the like (e.g., transformation, transfection, transduction, etc.) encompass all techniques by which a nucleic acid molecule might be introduced into such a cell, including viral vectors, plasmid vectors, nucleic acid-protein complexes (e.g., ribonucleoprotein), or naked nucleic acids (e.g., oligonucleotides).

25 Exemplary methods of transformation include chemical methods (e.g., calcium-phosphate transfection), physical methods (e.g., electroporation, microinjection, particle bombardment), fusion (e.g., liposomes), lipofection, nucleofection, receptor-mediated endocytosis (e.g., DNA-protein complexes, viral envelope/capsid-DNA complexes), particle gun accelerator (gene gun), and by biological infection by viruses such as recombinant viruses (Wolff, J. A., ed, Gene
 30 Therapeutics, Birkhauser, Boston, USA (1994)). In the case of infection by retroviruses, the infecting retrovirus particles are absorbed by the target cells, resulting in reverse transcription of the retroviral RNA genome and integration of the resulting provirus into the cellular DNA.

Treating, Treatment, and Therapy: Any success or indicia of success in the attenuation

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or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement, remission, diminishing of symptoms or making the condition more tolerable to the patient, slowing in the rate of degeneration or decline, making the final point of degeneration less debilitating, improving a subject's physical or mental well-being, or prolonging the length of survival. The treatment may be assessed by objective or subjective parameters; including the results of a physical examination, blood and other clinical tests, and the like. In some examples, treatment with the disclosed methods results in a decrease in the number, volume, and/or weight of a tumor and/or metastases. In some examples, treatment with the disclosed methods results in a decrease in signs or symptoms of the viral infection in a subject, and/or reduces viral load in a subject, and/or reduces infectivity of a virus, and/or reduce cytopathic effect in the subject's cells.

Tumor-Infiltrating Lymphocyte (TIL): lymphocytes that invade tumor tissue. For example, T cells found within a tumor sample. In ACT therapy, TIL therapy generally involves isolating TILs from a patient tumor, activating and expanding the TILs in culture, and then re-infusing into the patient. In some examples, the modified PBMC disclosed herein is a TIL. In some examples TILs express PD1, CD25, OX40, CD69, CD44 and/or CTLA4.

Tumor, neoplasia, or malignancy: A neoplasm is an abnormal growth of tissue or cells which results from excessive cell division. Neoplastic growth can produce a tumor. The amount of a tumor in an individual is the "tumor burden" which can be measured as the number, volume, or weight of the tumor. A "non-cancerous tissue" is a tissue from the same organ wherein the malignant neoplasm formed, but does not have the characteristic pathology of the neoplasm. Generally, noncancerous tissue appears histologically normal. A "normal tissue" is tissue from an organ, wherein the organ is not affected by cancer or another disease or disorder of that organ. A "cancer-free" subject has not been diagnosed with a cancer of that organ and does not have detectable cancer.

Exemplary tumors, such as cancers, that can be treated using the disclosed modified PBMCs include solid tumors, such as breast carcinomas (e.g. lobular and duct carcinomas, such as a triple negative breast cancer), sarcomas, carcinomas of the lung (e.g., non small cell carcinoma, large cell carcinoma, squamous carcinoma, and adenocarcinoma), mesothelioma of the lung, colorectal adenocarcinoma, stomach carcinoma, prostatic adenocarcinoma, ovarian carcinoma (such as serous cystadenocarcinoma and mucinous cystadenocarcinoma), ovarian germ cell tumors, testicular carcinomas and germ cell tumors, pancreatic adenocarcinoma, biliary adenocarcinoma, hepatocellular carcinoma, bladder carcinoma (including, for instance, transitional cell carcinoma,

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adenocarcinoma, and squamous carcinoma), renal cell adenocarcinoma, endometrial carcinomas (including, e.g., adenocarcinomas and mixed Mullerian tumors (carcinosarcomas)), carcinomas of the endocervix, ectocervix, and vagina (such as adenocarcinoma and squamous carcinoma of each of same), tumors of the skin (e.g., squamous cell carcinoma, basal cell carcinoma, malignant melanoma, skin appendage tumors, Kaposi sarcoma, cutaneous lymphoma, skin adnexal tumors and various types of sarcomas and Merkel cell carcinoma), esophageal carcinoma, carcinomas of the nasopharynx and oropharynx (including squamous carcinoma and adenocarcinomas of same), salivary gland carcinomas, brain and central nervous system tumors (including, for example, tumors of glial, neuronal, and meningeal origin), tumors of peripheral nerve, soft tissue sarcomas and sarcomas of bone and cartilage, head and neck squamous cell carcinoma, and lymphatic tumors (including B-cell and T- cell malignant lymphoma). In one example, the tumor is a melanoma.

The disclosed modified PBMCs can also be used to treat liquid tumors, such as a lymphatic, white blood cell, or other type of leukemia. In a specific example, the tumor treated is a tumor of the blood, such as a leukemia (for example acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), hairy cell leukemia (HCL), T-cell prolymphocytic leukemia (T-PLL), large granular lymphocytic leukemia, and adult T-cell leukemia), a lymphoma (such as Hodgkin's lymphoma or non-Hodgkin's lymphoma), or a myeloma.

Tumor-Specific Antigen: antigens unique to cancer cells or much more abundant on them, as compared to other cells, such as normal cells. Example tumor-specific antigens include, but are not limited to, CD19, CD20, BCMA, MUC1, PSA, CEA, HER1, HER2, TRP-2, EpCAM, GPC3, mesothelin 1(MSLN), and EGFR.

Vector: A nucleic acid molecule that can be introduced into a host cell (for example, by transfection or transformation), thereby producing a transformed host cell (such as a transformed PBMC). Recombinant DNA vectors are vectors having recombinant DNA. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements. Viral vectors (such as AAV and lentiviral vectors) are recombinant nucleic acid vectors having at least some nucleic acid sequences derived from one or more viruses. A replication deficient viral vector is a vector that requires complementation of one or more regions of the viral genome required for replication due to a deficiency in at least one replication-essential gene function.

Virus (or viral infection): infective agents typically including a nucleic acid molecule surrounded by a protein coat. Viruses that can be treated with the disclosed methods include

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positive-strand RNA viruses and negative-strand RNA viruses. Exemplary positive-strand RNA viruses include, but are not limited to: Picornaviruses (such as Aphthoviridae [for example foot-and-mouth-disease virus (FMDV)]), Cardioviridae; Enteroviridae (such as Cocksackie viruses, Echoviruses, Enteroviruses, and Polioviruses); Rhinoviridae (Rhinoviruses)); Hepataviridae (Hepatitis A viruses); Togaviruses (examples of which include rubella; alphaviruses (such as Western equine encephalitis virus, Eastern equine encephalitis virus, and Venezuelan equine encephalitis virus)); Flaviviruses (examples of which include Dengue virus, West Nile virus, and Japanese encephalitis virus); Calciviridae (which includes Norovirus and Sapovirus); hepaciviruses, (such as Hepatitis C virus), and Coronaviruses (examples of which include SARS coronaviruses, such as the Urbani strain, SARS-CoV and SARS-CoV-2). Exemplary negative-strand RNA viruses include, but are not limited to: Orthomyxoviruses (such as the influenza virus), Rhabdoviruses (such as Rabies virus), vesiculoviruses (such as VSV) and Paramyxoviruses (examples of which include measles virus, respiratory syncytial virus, and parainfluenza viruses).

Viruses that can be treated with the disclosed methods also include DNA viruses. DNA viruses include, but are not limited to: Hepadnaviridae (Hepatitis B virus) Herpesviruses (such as Varicella-zoster virus (VZV), for example the Oka strain; cytomegalovirus (CMV); Epstein-Barr virus (EBV), and Herpes simplex virus (HSV) types 1 and 2), Adenoviruses (such as Adenovirus type 1 and Adenovirus type 41), Poxviruses (such as Vaccinia virus), papillomaviridae (such as human papillomavirus (HPV)), (and Parvoviruses (such as Parvovirus B19).

Another group of viruses that can be treated with the disclosed methods includes Retroviruses. Examples of retroviruses include, but are not limited to: human immunodeficiency virus type 1 (HIV-1), such as subtype C; HIV-2; equine infectious anemia virus; feline immunodeficiency virus (FIV); feline leukemia viruses (FeLV); simian immunodeficiency virus (SIV); and avian sarcoma virus.

Viral infections of long duration or which recur over a long period of time are sometimes referred to as **chronic viral infections**. Exemplary viruses which can establish a chronic infection include adenovirus (Ad), a herpes simplex virus (HSV), a hepatitis B virus (HBV), a hepatitis C virus (HCV), a vesicular stomatitis virus (VSV), a human immunodeficiency virus (HIV), an influenza virus, a varicella zoster virus (VZV), a human papillomavirus (HPV), an Epstein-Barr virus (EBV), a cytomegalovirus (CMV), an enterovirus, a togavirus, a SARS-CoV virus, a SARS-CoV-2 virus, or a flavivirus.

The disclosed methods can be used in combination with one or more **antiviral agents**. Antiviral agents can work by a variety of mechanisms, including inhibiting any or all of:

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attachment, entry, uncoating, protease activity, polymerase activity, nucleoside and/or nucleotide reverse transcriptase activity, nonnucleoside reverse transcriptase activity, and integrase activity. Antiviral agents might also physically disrupt a virion. Exemplary antiviral agents that can be used with the methods provided herein include Lopinavir (for HIV), remdesivir (for SARS-CoV-2),
 5 acyclovir (for Herpes viruses), ribavirin (for viral hemorrhagic fevers), emtricitabine/tenofovir (for HIV), and bamlanivimab/etesevimab (for SARS-CoV-2), ZMapp (for ebolavirus). Antiviral agents can be given in combination, for example to prevent the target virus from developing resistance to the therapy.

Zinc Finger Protein 324 (Znf324): (e.g., OMIM 617477) a transcription factor with RNA
 10 polymerase II-specific and RNA polymerase II cis-regulatory region sequence-specific DNA binding activity. A human ortholog of Zinc Finger Protein 324 (*zfp324*) (mouse). NCBI Ref. Seq.: NC_000019.10 (human). NCBI Gene ID: 25799 (human); 243834 (*Mus musculus*). In this disclosure reference to *Zfp324*, *Zfp324*, *Znf324*, *ZNF324*, or *ZNF324* includes the corresponding gene or protein in any species: human, mouse, or otherwise, such as any mammalian *Znf324*. It is
 15 also envisioned that methods and compositions incorporating *ZNF324* (*ZNF324*) or *Zfp324* (*Zfp324*) may be adapted to the appropriate species by incorporating a species appropriate gene ortholog, for example, a composition incorporating a gRNA specific for *Zfp324* might be adapted for use in humans by incorporating a gRNA specific for *ZNF324*. Also known as ZF5128, ZNF324A.

Zinc Finger and SCAN Domain-Containing Protein 20 (Zscan20): (e.g., OMIM 611315) first reported by Thiesen, *Multiple genes encoding zinc finger domains are expressed in human T cells*, New Biol. (1990) 2:363-74, who speculated that 30 newly identified zinc finger domain-containing proteins might bind to DNA or RNA based on sequence similarity to other zinc finger containing proteins. *Zscan20*'s role as a TF was not previously studied. *Zscan20* sequences are
 25 publicly available, and exemplary sequences include Amino Acid NCBI Reference Sequences: NP_001364305.1 (human), NP_808426.2 (*Mus musculus*), XP_003127824.1 (*Sus scrofa*); Nucleotide NCBI Reference Sequence: NC_000001.11 (human), NM_001377376.1 (human), NM_177758.4 (*Mus musculus*), XM_003127776.4 (*Sus scrofa*), each of which is herein incorporated by reference in their entirety. NCBI Gene ID: 7579. In this disclosure reference to
 30 *Zscan20* or ZSCAN20 includes the corresponding gene or protein in any species: human, mouse, or otherwise, such as any mammalian *Zscan20*. Also known as KOX29, ZNF31, ZFP-31, and ZNF360.

II. Overview

The same types of cells can assume diverse states with varying functionalities. Single-cell genomics and proteomics enable not only precise characterization of cell state, but also provide a stunningly high-resolution view of transitions between states. As the cell state share the same gene expression pattern, migration pattern, and functionality, programming cells into a desirable cell state can achieve effective cell therapy. Cell state differentiation can be regulated by TFs that relay environmental signals through control of gene expression (5, 6). Therefore investigation of TFs of each cell state enables efficient and precise regulation of cell programming. Here an epigenomic and transcriptomic atlas was generated to systematically identify TFs that define different CD8⁺ T cell states. Novel TFs were discovered, which can improve T cell therapy.

Comprehensive analysis of the multiomics atlas enables the underlying molecular mechanism of the landscape of heterogeneous T cell states. First, cell-type bona fide specific regulators and multitaskers were systematically identified. It was observed that 48% of highly active TFs across the cell states are active in more than one cell states. This high reuse of TFs implies context-dependency of the TF regulatory network. Indeed, differential connectivity of TFs and their associated biological pathways along with the differentiation steps was discovered. These results illustrate a complex and dynamic network of TFs. Second, the integrative analysis of TF network in Tex_{Term} cell state uncovers cooperation between group of TFs and revealed biological circuits that have not been appreciated such as cellular catabolic process, GTPase activity, hypoxia and oxidative stress, which provide interesting pathways for future study. Furthermore, this systemic analysis indicated that the same sets of TFs are used in the transition of both Naive $\rightarrow$ MP $\rightarrow$ T_{RM} and Naive $\rightarrow$ Tex_{Prog} $\rightarrow$ Tex_{Term} , parallel differentiation trajectories from acute and chronic infection. Lastly, four different TFs (*Zscan20*, *Jdp2*, *Nfil3*, and *Znf324*) were discovered, *in vivo* validated, and are now reported as having roles in Tex_{Term} . The precise global analysis pipeline overcome the fact that TF activity is not proportional to the expression level of TF and identified TFs that have not yet been reported.

Singletasker TFs can be useful in designing therapeutic cell state. Effective T cell therapy can be achieved by programming T cells to avoid dysfunctional Tex_{Term} , to favor afunctional effector state, and without compromising immunological memory potential. To this end, this disclosure identifies TFs to perturb that are specifically active in Tex_{Term} and possibly regulate genes suppress effector state transition. Even though many studies have modulated the expression of TFs to improve anti-tumor immunity, they did not propose blocking Tex_{Term} or consider compromising T_{RM} formation by doing so. This disclosure prevents the dysfunctional and

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terminally differentiated Tex_{TcrM} state. For example, Tex_{TcrM} specific TF, *Zscan20* and *Jdp2*-deficiency in CD8^+ T cells confers enhanced virus and tumor control in adoptive therapy model of melanoma. This KO does not have impairment in T_{RM} or other memory formation but has higher tendency to become effector cells even in the chronic antigen encounter. Particularly with the combination of immune checkpoint therapy the disruption of *Zscan20* and *Jdp2* creates synergy and removes the tumor. Compared to a multi-tasker, *Nfil3*, single tasker *Zscan20* offer superior tumor control indicating the importance of identifying and modulating single taskers for cell programming.

This disclosure reports a rational approach to improve cell therapy by specific cell state engineering assisted by multiomics atlas. It provides a pipeline for unbiased identification of TF programs and associated biological circuits can be applied to many other cell types and utilized for therapeutic cell state programming.

Disclosed herein is a modified peripheral blood mononuclear cell (PBMC), which can have any combination of (a) an agent that reduces *Zscan20* expression or a non-naturally occurring genetic modification that reduces an amount of functional ZSCAN20; (b) an agent that reduces *Jdp2* expression or a non-naturally occurring genetic modification that reduces an amount of functional JDP2; (c) an agent that reduces *Nfil3* expression or a non-naturally occurring genetic modification that reduces an amount of functional NFIL3 and/or (d) an agent that reduces *Znf324* expression or a non-naturally occurring genetic modification that reduces an amount of functional ZNF324. In some aspects, the modified PBMC includes (a) the agent that reduces *Zscan20* expression or the non-naturally occurring genetic modification that reduces an amount of functional ZSCAN20 and either one or the other of (b) an agent that reduces *Jdp2* expression or a non-naturally occurring genetic modification that reduces an amount of functional JDP2; (c) an agent that reduces *Nfil3* expression or a non-naturally occurring genetic modification that reduces an amount of functional NFIL3.

In some examples, the agent that reduces *Zscan20* expression includes an inhibitory RNA (RNAi) specific for *Zscan20* or a guide RNA (gRNA) specific for *Zscan20*. In further examples the agent that reduces *Jdp2* expression includes an RNAi specific for *Jdp2* or a gRNA specific for *Jdp2*. In more examples the agent that reduces *Nfil3* expression includes an RNAi specific for *Nfil3* or a gRNA specific for *Nfil3*. In some examples, the agent that reduces *Znf324* expression includes an RNAi specific for *Znf324* or a gRNA specific for *Znf324*. In some examples the RNAi specific for *Zscan20* is a short hairpin RNA (shRNA) molecule, short interfering RNA (siRNA) molecule, or antisense RNA molecule. In some more examples the RNAi specific for *Jdp2* is a

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shRNA molecule, siRNA molecule, or antisense RNA molecule. In even more examples the RNAi specific for *Nfil3* is a shRNA molecule, siRNA molecule, or antisense RNA molecule. In some examples the RNAi specific for *Znf324* is a shRNA molecule, siRNA molecule, or antisense RNA molecule.

5 In further examples the agent that reduces *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* expression, includes a heterologous nucleic acid molecule encoding any of (i) an RNAi specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript, wherein the RNAi specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* comprises at least 90% complementarity to a portion of the *Zscan20*, *Jdp2*, and/or *Nfil3* gene or transcript, (ii) a gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript, wherein the gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* comprises at least 90% sequence identity to a portion of the *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript; and/or (iii) a gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript, wherein the gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* comprises at least 90% sequence identity to a portion of the *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript and a Cas nuclease. In other examples, the agent that reduces *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* expression includes (a) a gRNA specific for *Zscan20*, including SEQ ID NOs: 7, 8, 9, 10, 19, 20, or 21; (b) a gRNA specific for *Jdp2*, including SEQ ID NOs: 11, 12, 13, 14, 22, 23, or 24; (c) the gRNA specific for *Nfil3*, including SEQ ID NOs: 15, 16, 17, 18, 25, 26, or 27, and/or (d) the gRNA specific for *Znf324*, including SEQ ID NOs: 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, or 46. In further examples the modified PBMC includes an expression vector encoding a heterologous nucleic acid.

In additional examples, the modified PBMC includes a Cas nuclease. In some aspects, the Cas nuclease is a Cas3, dCas3, Cas9, dCas9, Cas12, dCas12, Cas13a, dCas13a, Cas13b, dCas13b, Cas13d, or dCas13d nuclease.

In one set of examples, the modified PBMC has a genetic modification that reduces ZSCAN20. The mutation may be a point mutation, a partial deletion, full deletion, or insertion of *Zscan20* that reduces expression of *Zscan20* and/or reduces activity of ZSCAN20. In a further set of examples, the modified PBMC has a genetic modification that reduces JDP2. The mutation may be a point mutation, a partial deletion, full deletion, or insertion of *Jdp2* that reduces expression of *Jdp2* and/or reduces activity of JDP2. In one more set of examples, the modified PBMC has a genetic modification that reduces NFIL3. The mutation may be a point mutation, a partial deletion, full deletion, or insertion of *Nfil3* that reduces expression of *Nfil3* and/or reduces activity of NFIL3. In some examples, the modified PBMC has a genetic modification that reduces ZNF324. The mutation may be a point mutation, a partial deletion, full deletion, or insertion of *Znf324* that

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reduces expression of *Znf324* and/or reduces activity of ZNF324.

In some examples the modified PBMC is a T cell. In further examples, the T cell can be a CD3+ T cell, a CD4+ T cell, and/or a CD8+ T cell. In some examples the PBMC is a therapeutic T cell, an exhausted T cell, tissue resident memory T cell (TRM), a chimeric antigen receptor (CAR) T cell, an engineered T cell receptor (TCR) T cell, a tumor-infiltrating lymphocyte (TIL), and/or a T cell comprising an antigen receptor reactive to a tumor-specific antigen. In some aspects, the tumor-specific antigen is one or more of CD19, CD20, BCMA, MUC1, PSA, CEA, HER1, HER2, TRP-2, EpCAM, GPC3, mesothelin 1(MSLN), or EGFR. In more examples, the modified PBMC is generated by any one of: (a) introducing an agent that reduces *Zscan20* expression or non-naturally occurring genetic modification that reduces functional ZSCAN20 into a PBMC, thereby generating the modified PBMC with reduced expression of *Zscan20*, reduced activity of ZSCAN20, or both; (b) introducing an agent that reduces *Jdp2* expression or non-naturally occurring genetic modification that reduces functional JDP2 into a PBMC, thereby generating the modified PBMC with reduced expression of *Jdp2*, reduced activity of JDP2, or both; (c) introducing an agent that reduces *Nfil3* expression or non-naturally occurring genetic modification that reduces functional NFIL3 into a PBMC, thereby generating the modified PBMC with reduced expression of *Nfil3*, reduced activity of NFIL3, or both; and/or (d) introducing an agent that reduces *Znf324* expression or non-naturally occurring genetic modification that reduces functional ZNF324 into a PBMC, thereby generating the modified PBMC with reduced expression of *Znf324*, reduced activity of ZNF324, or both. In some examples of this method, the PBMC is a T cell. In further examples of this method, the method includes incubating the modified PBMC with interleukin 2, (IL-2), interleukin 7 (IL-7), interleukin 15 (IL-15), or a combination thereof. In more examples of this method, the modified PBMC is reactive to a tumor-specific antigen, such as CD19, CD20, BCMA, MUC1, PSA, CEA, HER1, HER2, TRP-2, EpCAM, GPC3, mesothelin 1(MSLN), and EGFR. In further examples reduced expression of *Zscan20*, reduced activity of ZSCAN20, reduced expression of *Jdp2*, reduced activity of JDP2, reduced expression of *Nfil3*, reduced activity of NFIL3, reduced expression of *Znf324*, and/or reduced activity of ZNF324 increases effector function of the T cell; and/or reduced expression of *Zscan20*, reduced activity of ZSCAN20, reduced expression of *Jdp2*, reduced activity of JDP2, reduced expression of *Nfil3*, reduced activity of NFIL3, reduced expression of *Znf324*, and/or reduced activity of ZNF324 reduces exhaustion of the T cell. In other examples the method includes selecting the modified PBMC with reduced expression of *Zscan20*, reduced activity of ZSCAN20, or both; selecting the modified PBMC with reduced expression of *Jdp2*, reduced activity of JDP2, or both; selecting the modified PBMC with

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reduced expression of *Nfil3*, reduced activity of NFIL3, or both; selecting the modified PBMC with reduced expression of *Znf324*, reduced activity of ZNF324, or both; and/or introducing the selected modified PBMC into a subject. In some examples the selecting step includes the use of flow cytometry, panning, or magnetic separation. In further examples the subject has cancer, and in
5 additional examples the method includes the step of selecting a subject who has cancer.

Additionally disclosed is a pharmaceutical composition including the modified PBMC generated by the previous examples, and optionally a pharmaceutically acceptable carrier. In some examples the pharmaceutical composition is in an intravenous formulation. In more examples, the pharmaceutical composition further includes comprising one or more immune checkpoint blockade
10 (ICB) agents. In some examples, the pharmaceutical composition further includes one or more antiviral agents. In some examples, the pharmaceutical composition further includes one or more anti-tumor agents, such as a therapeutic monoclonal antibody.

Further disclosed is a method for treating cancer or a tumor in a subject, including administering a therapeutically effective amount of a modified PBMC as described by the previous
15 examples; or a therapeutically effective amount of the pharmaceutical composition described by the previous examples, to a subject having cancer or a tumor, treating the cancer or the tumor. In some examples the modified PBMC is autologous or allogenic to the subject. In further examples the method includes administering a therapeutically effective amount of IL-2, IL-7, and/or IL-15 to the subject, and/or treating the subject with one or more of surgery, radiation, chemotherapy, biologic
20 therapy, or immunotherapy. In more examples, the method includes administering to the subject a therapeutically effective amount of one or more of: a T cell agonist antibody, an oncolytic virus, or an adoptive cell transfer (ACT) immunotherapy. In even more examples the method includes administering to the subject a therapeutically effective amount of immune checkpoint blockade (ICB) agent or immunostimulatory antibody. In additional examples, the ICB agent comprises
25 anti-PD-1, anti-PD-L1, anti-CTLA-4, anti-LAG3 anti-GITR, anti-4-1BB, anti-CD40, and anti-OX40, anti-TIGIT, anti-VISTA, anti-CD73, anti-CD39, anti-HVEM, anti-BTLA, anti-CD27, or a combination of two or more thereof. In some examples the anti-PD-1 is nivolumab, pembrolizumab, pidilizumab, or cemiplimab. In more examples the anti-PD-L1 atezolizumab, avelumab, durvalumab, cosibelimab, KN035 (envafolimab), BMS-936559, BMS935559, MEDI-
30 4736, MPDL-3280A, or MEDI-4737. In even more examples the anti-CTLA-4 is ipilimumab or tremelimumab. In some examples, the modified PBMC is administered simultaneously with the ICB agent or the immunostimulatory antibody. In other examples the modified PBMC is administered before the ICB agent or the immunostimulatory antibody. In still other examples the

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modified PBMC is administered after the ICB agent or the immunostimulatory antibody. In still more examples non-modified lymphocytes are depleted in the subject prior to administering the modified PBMC. In some examples the cancer or tumor is a leukemia, colorectal cancer, melanoma, cervical cancer, lung cancer, ovarian cancer, bladder cancer, breast cancer, pancreatic
5 cancer, renal cell carcinoma, prostate cancer, or head and neck cancer.

Additionally disclosed is a method for treating a viral infection including steps of administering a therapeutically effective amount of the modified PBMC of the previous examples, and/or a therapeutically effective amount of the pharmaceutical composition of the previous examples to the subject having the viral infection, thereby treating the viral infection. In some
10 examples the method also includes treating the subject with an antiviral agent. In more examples the method includes administering to the subject a therapeutically effective amount of immune checkpoint blockade (ICB) agent or immunostimulatory antibody. In some examples the ICB agent includes anti-PD-1, anti-PD-L1, anti-CTLA-4, anti-LAG3 anti-GITR, anti-4-1BB, anti-CD40, and anti-OX40, anti-TIGIT, anti-VISTA, anti-CD73, anti-CD39, anti-HVEM, anti-BTLA, anti-CD27
15 and/or a combination of two or more of these ICB agents. In even more examples, the viral infection is caused by an adenovirus (Ad), a herpes simplex virus (HSV), a hepatitis B virus (HBV), a hepatitis C virus (HCV), a vesicular stomatitis virus (VSV), a human immunodeficiency virus (HIV), an influenza virus, a varicella zoster virus (VZV), a human papillomavirus (HPV), an Epstein-Barr virus (EBV), a cytomegalovirus (CMV), an enterovirus, a togavirus, a SARS-CoV, a
20 SARS-CoV-2, or a flavivirus.

III. RNAi and gRNA

Disclosed herein are interfering RNAs (RNAi) or guide nucleic acids (gRNA) specific for *Zscan20* (ZSCAN20) *Jdp2* (JDP2), *Nfil3* (NFIL3), and/or *Znf324* (ZNF324). The RNAi or gRNA
25 targets a *Zscan20*, *Jdp2*, *Nfil3* and/or *Znf324* nucleic acid molecule, such as a gene or transcript, to reduce expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, such as a reduction in expression of at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 75%, at least 90%, at least 95%, at least 99%, at least 99.9% or even 100% (for example relative to an amount prior to introduction/expression of the RNAi or gRNA). In some examples, the RNAi or gRNA are
30 introduced into a cell, for example, a PBMC, T cell, or exhausted T cell (including a terminally exhausted T cell). In other examples the RNAi or gRNA are introduced into a CD8+ T cell, a CD8+ TRM cell, a chimeric antigen receptor T cell, an engineered T cell receptor T cell, a tumor-infiltrating lymphocyte, or a CD8+ tumor-infiltrating tissue resident memory T cell, optionally,

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wherein the antigen receptor is reactive to a tumor-specific antigen. In further examples, the RNAi or gRNA target an antigen presenting cell, such as a dendritic cell, a natural killer (NK) cell, a monocyte/macrophage, or a B cell. In some examples, the RNAi or gRNA molecules are directly introduced into the cell, for example, as oligonucleotides. In some examples, RNAi of gRNA molecules are expressed from a vector that is introduced into the cell. In examples where a guide RNA is expressed (*e.g.*, from an expression cassette or vector) the guide RNA may be encoded as DNA.

In some aspects, an RNAi specific for a *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript is used to reduce or inhibit expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*. The specificity of the RNAi for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* allows hybridization of the RNAi molecule to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* DNA or RNA, thereby reducing or inhibiting *Zscan20*, *JDP2*, *Nfil3*, and/or *Znf324* expression. RNAi generically refers to a cellular process that inhibits expression of genes by inhibiting transcription and/or translation. Molecules that inhibit gene expression through the RNAi pathway include siRNAs, miRNAs, antisense RNAs, and shRNAs. In some examples, the RNAi specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* includes a sequence at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100% complementary to a unique (*e.g.*, not found elsewhere in the genome of the cell or organism into which the RNAi is introduced) contiguous portion of a *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript (such as a portion of SEQ ID NOs: 2, 4, 6, or 29). In some examples, the RNAi specific for *Zscan20*, *JDP2*, *Nfil3*, and/or *Znf324* consists of a sequence at least 90% complementary (*e.g.*, at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% complementary) to a unique contiguous portion of *Zscan20*, *JDP2*, *Nfil3*, and/or *Znf324* gene or transcript (such as a portion of SEQ ID NOs: 2, 4, 6, or 29).

In a specific, non-limiting example, the RNAi is a shRNA specific for a *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript. Methods of designing shRNA have been described, for example, *see Moore et al. (2010) Short Hairpin RNA (shRNA): Design, Delivery, and Assessment of Gene Knockdown, Methods Mol. Biol.*, 629:141–158. In some examples, the shRNA is specific to a unique contiguous portion of a sequence with at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to one of SEQ ID NOs: 2, 4, 6, or 29. In some examples, the shRNA is specific to a unique contiguous portion of a sequence with at least 90% sequence identity to one of SEQ ID NOs: 2, 4, 6, or 29.

In other examples, the RNAi is a siRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript, for example the siRNA is specific for a sequence with at least 70%, at least 80%,

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at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to a unique contiguous portion (such as a contiguous portion of at least 8, at least 9, at least 10, at least 12, at least 15, or at least 20 contiguous nucleotides) of one of SEQ ID NOs: 2, 4, 6, or 29. For example, the siRNA can be specific to a sequence with at least 95% sequence identity to a unique contiguous
 5 portion of SEQ ID NO: 6.

In some aspects, a gRNA specific for a *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript is used to reduce or inhibit expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*. For example, CRISPR/Cas methods can be used with a gRNA specific for a *Zscan20* gene or transcript to reduce or inhibit expression of *Zscan20*. In another example, CRISPR/Cas methods can be used
 10 with a gRNA specific for a *Jdp2* or *Nfil3* gene or transcript to reduce or inhibit expression of *Jdp2* or *Nfil3*, respectively. In a further example, CRISPR/Cas methods can be used with a gRNAs specific for *Zscan20* and *Jdp2* to reduce or inhibit expression of *Zscan20* and *Jdp2*. In another example, CRISPR/Cas methods can be used with a gRNAs specific for *Zscan20* and *Nfil3* to reduce or inhibit expression of *Zscan20* and *Nfil3*.

The specificity of the gRNA for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* in combination with a
 15 Cas nuclease or dead nuclease (such as Cas3, dCas3, Cas9, dCas9, Cas12, dCas12, Cas13a, dCas13a, Cas13b, dCas13b, Cas13d, or dCas13d) allows hybridization of the gRNA molecule to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* DNA and/or RNA, thereby editing the *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* (for example mutating it, such as knocking it down or knocking it out) to reduce or
 20 inhibit its expression. In some examples, the Cas nuclease (or a dead Cas nuclease) sequence is codon optimized for expression in a host cell. In some examples, gRNA molecules and Cas nucleases are expressed from a vector introduced into a host cell (*e.g.*, PBMC, antigen presenting cell, B cell, dendritic cell, monocyte/macrophage, NK cell, T cell, CD8+ TRM T cell, tumor infiltrating lymphocyte, CAR T cell, exhausted T cell, terminally exhausted T cell, or a cell with an
 25 antigen receptor reactive to a tumor-specific antigen). In some examples, an RNP complex containing gRNA molecules and Cas nucleases are introduced into a cell (*e.g.*, PBMC, antigen presenting cell, B cell, dendritic cell, monocyte/macrophage, NK cell, T cell, CD8+ TRM T cell, tumor infiltrating lymphocyte, CAR T cell, exhausted T cell, terminally exhausted T cell, or a cell with an antigen receptor reactive to a tumor-specific antigen). In some examples, the gRNAs are
 30 introduced into a cell, for example, as oligonucleotides.

In some examples, the gRNA is specific for *Zscan20* (ZSCAN20), *Jdp2* (JDP2), *Nfil3* (NFIL3), or *Znf324* (ZNF324) gene or transcript. For example, the gRNA is specific for a sequence with at 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%

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sequence identity to a unique contiguous portion (such as a contiguous portion of at least 8, at least 9, at least 10, at least 12, at least 15, at least 20, or at least 25 contiguous nucleotides) of one of SEQ ID NOs: 2, 4, 6, or 29. In some examples, the gRNA is specific to a sequence with at least 90% sequence identity to a unique contiguous portion of one of SEQ ID NOs: 2, 4, 6, or 29. In some examples, the gRNA comprises a targeting sequence (sometimes referred to as a spacer) specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* genes or transcripts, for example, by having a targeting sequence that is at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% complementary to a unique contiguous portion of one of SEQ ID NOs: 2, 4, 6, or 29. The targeting sequence of the gRNA is typically about 20 nucleotides, for example, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, or about 25 nucleotides. In a specific non-limiting example, the targeting sequence is about 20 nucleotides. The degree of complementarity between a targeting sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, about 60%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97.5%, about 98%, about 99%, or more. In some aspects, the degree of complementarity is about 100%. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting examples of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (*e.g.*, the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). In some examples, the targeting sequence is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% complementary to a unique, contiguous amino acid sequence about 20 nucleotides long in a *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript.

In some examples, the gRNA specific for a *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript includes a contiguous sequence at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to one of SEQ ID NOs: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, or 46. In some examples, the gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript includes one of SEQ ID NOs: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, or 46. In some examples, the gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript includes one having at least 90%, at least 95%, or at least 98% sequence identity to SEQ ID NOs: 7, 8, 9, 10, 11, 12, 13, 14, 15,

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16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, or 46 (such as substitution of 1, 2, 3, 4, 5, or 6 nt).

In some examples the gRNA is a sgRNA specific for a *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript. In some examples, the sgRNA includes a contiguous sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to one of SEQ ID NOs: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, or 46. In some examples, the sgRNA includes one of SEQ ID NOs: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, or 46 (such as substitution of 1, 2, 3, 4, 5, or 6 nt).

Methods of designing gRNA and determining appropriate targeting sequences have been described (*see e.g.*, Hanna and Doench (2020) *Design and analysis of CRISPR–Cas experiments, Nature Biotechnology*, 38:813–823(2020)). Software tools can be used to design and analyze of CRISPR–Cas experiments, including resources to design optimal gRNAs for various modes of manipulation and to analyze the results of such experiments. Online databases of validated gRNAs are also readily available (*see* addgene.org/crispr/reference/grna-sequence/ and genscript.com/gRNA-database.html).

IV. Nucleic Acids and Expression Vectors

Nucleic acids (*e.g.*, heterologous nucleic acids or isolated nucleic acid molecules, such as DNA, cDNA, RNA (*e.g.*, mRNA)) encoding the RNAi, gRNAs, and/or Cas protein are also provided herein. Nucleic acids can readily be produced using the disclosed sequences provided herein, sequences available in the art, and the genetic code. In one example, nucleic acids are DNA. In one example, nucleic acids are RNA.

Degenerate variants of the disclosed nucleic acid sequences are also disclosed. Silent mutations in the coding sequence result from the degeneracy (*i.e.*, redundancy) of the genetic code, whereby more than one codon can encode the same amino acid residue. Thus, for example, leucine can be encoded by CTT, CTC, CTA, CTG, TTA, or TTG; serine can be encoded by TCT, TCC, TCA, TCG, AGT, or AGC; asparagine can be encoded by AAT or AAC; aspartic acid can be encoded by GAT or GAC; cysteine can be encoded by TGT or TGC; alanine can be encoded by GCT, GCC, GCA, or GCG; glutamine can be encoded by CAA or CAG; tyrosine can be encoded by TAT or TAC; and isoleucine can be encoded by ATT, ATC, or ATA.

Codon preferences and codon usage tables for a particular species can be used to engineer isolated nucleic acid molecules encoding protein products, such as Cas9, that take advantage of the

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codon usage preferences of that particular species. For example, the nucleic acid can be designed to have codons that are preferentially used by a particular organism of interest (*e.g.*, the organism of origin for a PBMC to be modified, or an organism to be administered the nucleic acid). In some examples, the nucleic acids are codon optimized for expression in human. Thus, in some examples
5 a Cas nuclease (or dead nuclease) sequence is codon optimized for expression in a human PBMC (PBMC, antigen presenting cell, B cell, dendritic cell, monocyte/macrophage, NK cell, T cell, CD8+ TRM T cell, tumor infiltrating lymphocyte, CAR T cell, exhausted T cell, or a cell with an antigen receptor reactive to a tumor-specific antigen).

The disclosed nucleic acids can be prepared by any suitable method including, for example,
10 cloning of appropriate sequences or by direct chemical synthesis by standard methods. Chemical synthesis produces a single stranded oligonucleotide. This can be converted into double stranded DNA by hybridization with a complementary sequence or by polymerization with a DNA polymerase using the single strand as a template.

Nucleic acid sequences can be prepared using any suitable method, including, for example,
15 cloning of appropriate sequences or by direct chemical synthesis by methods such as the phosphotriester method of Narang *et al.*, *Meth. Enzymol.* 68:90-99, 1979; the phosphodiester method of Brown *et al.*, *Meth. Enzymol.* 68:109-151, 1979; the diethylphosphoramidite method of Beaucage *et al.*, *Tetra. Lett.* 22:1859-1862, 1981; the solid phase phosphoramidite triester method described by Beaucage & Caruthers, *Tetra. Letts.* 22(20):1859-1862, 1981, for example, using an
20 automated synthesizer as described in, for example, Needham-VanDevanter *et al.*, *Nucl. Acids Res.* 12:6159-6168, 1984; and, the solid support method of U.S. Patent No. 4,458,066. Chemical synthesis produces a single stranded oligonucleotide. This can be converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. While chemical synthesis of DNA is generally
25 limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

The disclosed nucleic acids can be prepared by cloning techniques. Examples of appropriate cloning and sequencing techniques can be found, for example, in Green and Sambrook (*Molecular Cloning: A Laboratory Manual*, 4th ed., New York: Cold Spring Harbor Laboratory
30 Press, 2012) and Ausubel *et al.* (Eds.) (*Current Protocols in Molecular Biology*, New York: John Wiley and Sons, including supplements). The nucleic acids can also be prepared by amplification methods. Amplification methods include the polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS), the self-sustained sequence

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replication system (3SR), and the Q β replicase amplification system (QB). A wide variety of cloning and *in vitro* amplification methodologies exist.

In some aspects, the disclosed nucleic acids are included in an expression vector (*e.g.*, viral vector, plasmid, or other vehicle) for expression in a host, or specifically in a target cell (*e.g.*,

5 PBMC, antigen presenting cell, B cell, dendritic cell, monocyte/macrophage, NK cell, T cell, CD8+ TRM T cell, tumor infiltrating lymphocyte, CAR T cell, exhausted T cell, terminally exhausted T cell, or a cell with an antigen receptor reactive to a tumor-specific antigen) In some examples, the expression vector includes a promoter operably linked to a disclosed nucleic acid molecule. For example, a promoter can be operably linked to an RNAi, gRNA, or Cas nuclease (or dead nuclease)
10 to drive its expression. In some examples, a vector encodes both a Cas nuclease (or dead nuclease) and a gRNA. Additional expression control sequences, such as one or more enhancers, transcription and/or translation terminators, and initiation sequences can also be included in the expression vector. In some aspects, the disclosed nucleic acids are included in a viral vector. Exemplary viral vectors that can be used include, but are not limited to, polyoma, SV40,
15 adenovirus, vaccinia virus, adeno-associated virus (AAV), herpes viruses including HSV and EBV, Sindbis viruses, alphaviruses and retroviruses of avian, murine, and human origin. Baculovirus (*Autographa californica* multinuclear polyhedrosis virus; AcMNPV) vectors can also be used. Other suitable vectors include orthopox vectors, avipox vectors, fowlpox vectors, capripox vectors, suipox vectors, lentiviral vectors, alpha virus vectors, and poliovirus vectors. Specific exemplary
20 vectors are poxvirus vectors such as vaccinia virus, fowlpox virus and a highly attenuated vaccinia virus (MVA), adenovirus, baculovirus and the like. Pox viruses of use include orthopox, suipox, avipox, and capripox virus. Orthopox include vaccinia, ectromelia, and raccoon pox. One example of an orthopox of use is vaccinia. Avipox includes fowlpox, canary pox and pigeon pox. Capripox include goatpox and sheeppox. In one example, the suipox is swinepox. Other viral vectors that
25 can be used include other DNA viruses such as herpes virus and adenoviruses, and RNA viruses such as retroviruses and polio. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a cell. In some examples, the vector includes a selectable marker (such as an antibiotic resistance gene (*e.g.*, puromycin) or a reporter gene (*e.g.*, green fluorescent protein (GFP))). In other examples, a selectable marker and/or reporter is not included in the vector.

30 The disclosed nucleic acids can be introduced into a host cell by DNA transfer (*e.g.*, oligonucleotides), or introduced and expressed in a suitable host cell (*e.g.*, expression cassette or vector). In some examples, the expressed product is an RNA (*e.g.*, siRNA or gRNA), in other examples, the expressed product is a protein (*e.g.*, Cas9). The cell may be prokaryotic or

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eukaryotic. In some aspects, the host cell is a PBMC (*e.g.*, B cell, monocyte/macrophage, dendritic cell, T cell). Methods of transient or stable transfer can be used. Transient transfer indicates that the foreign nucleic acid is only present transiently (*e.g.*, degraded after a period of time, cleared by the host cell, or otherwise not stably replicated). Stable transfer indicates that the foreign nucleic acid is continuously maintained in the host.

To obtain optimal expression of the disclosed nucleic acids, expression cassettes can contain, for example, a strong promoter to direct transcription, a ribosome binding site for translational initiation (*e.g.*, internal ribosomal binding sequences), and a transcription/translation terminator can be used. For expression in *E. coli*, a promoter, such as the T7, trp, lac, or lamda promoters, a ribosome binding site, and preferably a transcription termination signal can be used. For eukaryotic cells, such as a PBMC, the control sequences can include a promoter and/or an enhancer derived from, for example, an immunoglobulin gene, HTLV, SV40 or cytomegalovirus, and a polyadenylation sequence, and can further include splice donor and/or acceptor sequences (for example, CMV and/or HTLV splice acceptor and donor sequences). Additional operational elements include, but are not limited to, leader sequence, termination codons, polyadenylation signals and any other sequences necessary for the appropriate transcription and subsequent translation of the nucleic acid sequence.

The disclosed nucleic acids or vectors can be introduced into the host cell by any suitable method (*e.g.*, transformation). Numerous methods of transformation can be used, such as: chemical methods (*e.g.*, calcium-phosphate transfection), physical methods (*e.g.*, electroporation, microinjection, particle bombardment), fusion (*e.g.*, liposomes), lipofection, nucleofection, receptor-mediated endocytosis (*e.g.*, DNA-protein complexes, viral envelope/capsid-DNA complexes), particle gun accelerator (gene gun), and by biological infection by viruses such as recombinant viruses (Wolff, J. A., ed, Gene Therapeutics, Birkhauser, Boston, USA (1994)). In the case of infection by retroviruses, the infecting retrovirus particles are absorbed by the target cells, resulting in reverse transcription of the retroviral RNA genome and integration of the resulting provirus into the cellular DNA. Successfully transformed cells can be selected by resistance to antibiotics conferred by genes contained in the vector, such as the amp, gpt, neo and hyg genes. In some examples, a disclosed nucleic acid (*e.g.*, gRNA) is incorporated in a ribonucleoprotein (RNP) complex (*e.g.*, a gRNA-Cas complex). RNPs can be introduced into a host cell by transformation, for example, by nucleofection.

Modifications can be made to the disclosed nucleic acids without diminishing biological activity of the encoded product. For example, modifications can be made to facilitate the cloning,

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expression, or incorporation of the targeting molecule into a fusion protein. Such modifications include, for example, termination codons, sequences to create conveniently located restriction sites, and sequences to add a methionine at the amino terminus to provide an initiation site, or additional amino acids (such as poly His) to aid in purification steps.

5

V. Modified PBMCs

Provided herein are modified peripheral blood mononuclear cells (PBMCs) with reduced expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, reduced activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324, or any combination thereof. In some examples, modified PBMCs have reduced expression of *Zscan20* and/or reduced activity of ZSCAN20. In some examples, modified PBMCs have reduced expression of *Zscan20* (and/or reduced activity of ZSCAN20) and reduced expression of *Jdp2* (and/or reduced activity of JDP2). In some examples, modified PBMCs have reduced expression of *Zscan20* (and/or reduced activity of ZSCAN20) and reduced expression of *Nfil3* (and/or reduced activity of NFIL3). In some examples, modified PBMCs have reduced expression of *Zscan20* (and/or reduced activity of ZSCAN20), reduced expression of *Jdp2* (and/or reduced activity of JDP2), and reduced expression of *Nfil3* (and/or reduced activity of NFIL3). In some examples, modified PBMCs have reduced expression of *Nfil3* (and/or reduced activity of NFIL3). In some examples, modified PBMCs have reduced expression of *Jdp2* (and/or reduced activity of JDP2). In some examples, modified PBMCs have reduced expression of *Nfil3* (and/or reduced activity of NFIL3) and reduced expression of *Jdp2* (and/or reduced activity of JDP2). In some examples, modified PBMCs have reduced expression of *Znf324* (and/or reduced activity of ZNF324).

In some examples, expression of *Zscan20*, *Jdp2*, and/or *Nfil3* is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 100% relative to a suitable control (e.g., a PBMC prior to modification). In some examples, activity of ZSCAN20, JDP2, and/or NFIL3 is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or 100% relative to a suitable control (e.g., a PBMC prior to modification). Reducing activity includes reducing any measurable biological function of ZSCAN20, JDP2, and/or NFIL3, for example, reduced interaction by these TFs with the PBMC's genome. In some examples, the modified PBMC with reduced expression of *Zscan20*, *Jdp2*, and/or *Nfil3*, reduced activity of ZSCAN20, JDP2, and/or NFIL3, or any combination thereof, has increased effector activity (e.g., anti-tumor) relative to a suitable control (e.g., unmodified PBMC).

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In some examples, the modified PBMC is a T cell, and the T cell has increased resistance to T cell exhaustion relative to a suitable control (*e.g.*, unmodified PBMC).

The modified PBMC can further include additional modifications, for example, the PBMC can express or otherwise contain a chimeric antigen receptor (CAR) or engineered T cell receptor (TCR).

In some examples, the modified PBMC is a T cell, for example, a CD4+, a CD8+ or a CD3+ T cell. The T cell can be reactive to a tumor-specific antigen, for example, CD19, CD20, BCMA, MUC1, PSA, CEA, HER1, HER2, TRP-2, EpCAM, GPC3, mesothelin 1(MSLN), or EGFR. In some examples, the T cell is a tumor-infiltrating lymphocyte (TIL). In some examples, the T cell is a therapeutic T cell, or will be used as a therapeutic T cell, for example, as an ACT therapy. In some examples, the T cell is an exhausted T cell (including terminally exhausted T cells). Exhausted T cells are dysfunctional T cells characterized by a progressive loss of effector function (for example, loss of IL-2, TNF- α , and IFN- γ production) and sustained expression of inhibitory receptors such as PD-1, T cell immunoglobulin domain and mucin domain-containing protein 3 (Tim-3), CTLA-4, lymphocyte-activation gene 3 (LAG-3), and CD160. In some examples, the exhausted T cell is a terminally exhausted T cell, which have high and persistent expression of programmed cell death 1 (PD1^{hi}) and are positive for T cell immunoglobulin and mucin domain-containing protein 3 (TIM3⁺). In some examples the T cell is a tissue resident memory T cell.

In some aspects, the modified PBMC includes an agent that reduces *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* expression, for example, one or more of the disclosed inhibitory RNA (RNAi) specific for gene or transcript, or one or more guide RNA (gRNAs) specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript (for example in combination with a Cas nuclease or dead Cas nuclease, such as an RNP).

In some examples, the agent that reduces *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* expression is one or more of the disclosed inhibitory RNA (RNAi), for example, a short hairpin RNA (shRNA), short interfering RNA (siRNA), microRNA (miRNA), or an antisense RNA specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*. In specific, non-limiting examples, the RNAi is a shRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript, for example, the siRNA is specific to a sequence comprising at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NOs: 2, 4, 6, and/or 29. In some examples, the shRNA is specific to a sequence with at least 90% sequence identity to a unique, contiguous portion of SEQ ID NOs: 2, 4, 6, and/or 29.

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In some examples, the agent is a disclosed gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript, for example, the gRNA is specific for a sequence with at 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NOs: 2, 4, 6, and/or 29. For example, the gRNA can be specific to a sequence with at least 90%
 5 sequence identity to SEQ ID NOs: 2, 4, 6, and/or 29. In some examples, the gRNA comprises a targeting sequence specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript, for example, by having a targeting sequence that is at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% complementary to a unique, contiguous portion of SEQ ID NOs: 2, 4, 6, and/or 29.

10 In some examples, the gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript includes a contiguous sequence at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NOs: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, and/or 46. In some examples, the gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or
 15 transcript includes SEQ ID NOs: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, and/or 46. In some examples, the modified PBMC includes a RNP complex that includes the disclosed gRNA and a Cas nuclease, such as Cas3, dCas3, Cas9, dCas9, Cas12, dCas12, Cas13a, dCas13a, Cas13b, dCas13b, Cas13d, or dCas13d.

20 In some examples, the modified PBMC includes a heterologous nucleic acid molecule encoding one or more of the disclosed nucleic acids encoding the RNAi (*e.g.*, shRNA, siRNA, antisense RNA) or gRNA. RNAi or gRNA may be encoded as DNA (for example, encoded on a DNA vector), but expressed as RNA. In some examples, the heterologous nucleic acid molecule encodes the disclosed gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* and a Cas nuclease
 25 (or a dead Cas nuclease). In specific examples, the Cas nuclease is a Cas9 nuclease. In other examples, the Cas nuclease is a Cas13d nuclease, or a Cas12 nuclease.

In some examples, the modified PBMC includes the disclosed vector encoding the RNAi or gRNA. Thus, in some examples, the modified PBMC expresses the RNAi or gRNA. If gRNA is used, a Cas nuclease (*e.g.*, Cas3, dCas3, Cas9, dCas9, Cas12, dCas12, Cas13a, dCas13a, Cas13b, dCas13b, Cas13d, or dCas13d) can also be encoded on the same or different vector, for example, to
 30 co-express a Cas nuclease (or dead Cas nuclease) and one or more gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* in the modified PBMC. In some examples, the gRNA includes a spacer sequence and DR sequence (such as DR-spacer-DR-spacer) and the Cas nuclease is Cas13d, and

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Zscan20, *Jdp2*, *Nfil3*, and/or *Znf324* RNA is edited. In some examples, the gRNA includes a crRNA and tracrRNA (expressed either as two separate molecules, or as one fusion molecule, such as a sgRNA) and the Cas nuclease is Cas9. In some examples, the vector includes a cassette including two or more gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* wherein the two or
 5 more gRNAs have the same or different targeting sequences (*e.g.*, may target two different regions of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*). In some examples the vector includes gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*. In some examples the vector includes multiple gRNA specific for any combination of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* (*e.g.*, a vector with a first gRNA specific for *Zscan20* and a second gRNA specific for *Nfil3*).

10 Nucleic acids or vectors can be transiently or stably introduced into a PBMC (*e.g.*, T cell). In a specific, non-limiting example, the vector is stably introduced into the modified PBMC, thereby resulting in stable expression of the RNAi or gRNA in the modified PBMC. In some examples, the nucleic acid encoding the RNAi or gRNA is operably linked to a cell specific promoter (*e.g.*, a T cell specific promoter such as GzmB promoter or CD4 promoter) in the vector.
 15 Expression of the RNAi or gRNA can be constitutive or inducible. Exemplary promoters include NFAT, EF1a, PGK, U6, or H1. In one example, gRNA is expressed from a U6 or H1 promoter. In one example, a Cas nuclease (or dead Cas nuclease) is expressed from a CMV promoter.

In some aspects, the modified PBMC includes a non-naturally occurring genetic modification that reduces an amount of functional ZSCAN20, JDP2, NFIL3, and/or ZNF324.

20 Reducing functional ZSCAN20, JDP2, NFIL3, and/or ZNF324 includes genetic modifications that decrease *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* expression (*e.g.*, decreasing transcription or translation of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene or transcript) in the modified PBMC. In some examples, the genetic modification is a non-naturally occurring genetic modification of a *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene. In other examples, the genetic modification is a non-
 25 naturally occurring genetic modification of a regulatory element of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* (*e.g.*, a promoter, response element, enhancer, transcription factor, or other regulator that affects expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*). The regulatory element can be cis-acting or trans-acting.

In some examples, the non-naturally occurring genetic modification is a modification that
 30 reduces an amount of functional ZSCAN20, JDP2, NFIL3, and/or ZNF324 in the modified PBMC. For example, the genetic modification can result in the production of dysfunctional ZSCAN20, JDP2, NFIL3, and/or ZNF324. In some examples, the genetic modification results in the production of unstable ZSCAN20, JDP2, NFIL3, and/or ZNF324, such that the accumulation of

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functional ZSCAN20, JDP2, NFIL3, and/or ZNF324 is reduced. The genetic modification can be any non-naturally occurring modification that results in a decreased amount of ZSCAN20, JDP2, NFIL3, and/or ZNF324. Non-limiting examples of genetic modifications include a point mutation, partial deletion, full deletion, or insertion.

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Methods of Generating Modified PBMCs

Also provided herein are methods of generating the disclosed modified PBMCs by introducing the non-naturally occurring genetic modification into a PBMC, thereby generating the modified PBMC with reduced expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* and/or reduced activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324. In some aspects, the PBMC is obtained from a subject before introducing the non-naturally occurring genetic modification. PBMCs can be harvested or isolated, for example, from a blood sample, such as a venous blood sample, from the subject. Several techniques for isolating PBMCs can be used, for example, density centrifugation (the Ficoll approach), isolation by cell preparation tubes (CPTs), or isolation by SepMate™ tubes.

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In some examples, apheresis or leukapheresis is used to harvest PBMCs. Erythrocyte contamination can be evaluated, for example, by microscopic analysis of the sample. Flow cytometry techniques (e.g., FACS) can be used to assess the composition of the isolated PBMC populations, for example, to identify monocytes (e.g., CD14), T cells (e.g., CD3, CD8, CD4), B cells (e.g., CD20), or NK cells (e.g., CD56). FACS techniques can also be used to enrich or deplete a particular cell type from a PBMC (for example, enrich or deplete CD14, CD3, CD8, CD4, CD28, CD20, CD56, or combinations thereof).

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In some aspects, the PBMC is harvested or isolated from a solid tissue sample, for example from a tumor. Tumor samples can be surgically resected, enzymatically digested, and PBMCs can subsequently be isolated, for example via the methods of Donia et al., *Characterization and Comparison of 'Standard' and 'Young' Tumour-Infiltrating Lymphocytes for Adoptive Cell Therapy at a Danish Translational Research Institution*, Scand. J. Immuno. (2011) 75:157-67, incorporated by reference herein.

25

In some examples, T cells are isolated from a PBMC sample, or the PBMC sample is enriched for T cells, for example, isolated or enriched for CD3⁺ or CD8⁺ T cells. In some examples, a sample is enriched by negative selection, for example, by selecting and removing unwanted cell types from a sample (e.g., cell types other than T cells, and/or naïve or memory T cells). In some examples, FACS is used to enrich for a particular PBMC, for example, to enrich for T cells (e.g., CD3 or CD8 positive T cells). FACS can also be used to assess whether exhausted T

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cells, or specifically terminally exhausted T cells (PD-1^{hi}, TIM3⁺ or PD-1^{hi} LAG3⁺ or PD-1^{hi} CD39⁺), are present in a PBMC sample, or sort a PBMC sample to enrich for exhausted T cells (including terminally exhausted T cells), or conversely remove exhausted T cells. Antigen responsiveness of the PBMCs can be assessed, for example, by measuring release of cytokines,
 5 *e.g.*, IFN γ , IL-1 β , IL-6, IL-8 and TNF α .

In some examples, the PBMCs are obtained from a subject to be treated, such as a subject having cancer or one having a chronic viral infection. In other examples, the PBMCs are obtained from a donor subject, such as a subject who does not have cancer. In some examples, exhausted T cells are obtained from a tumor biopsy or sample (*e.g.*, tumor infiltrating lymphocytes).

10 In some examples, the agent, non-naturally occurring genetic modification, or inhibitor is introduced into a PBMC *ex vivo*. In such examples, such methods can further include selecting modified PBMCs having reduced expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, reduced activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324, or any combination of the aforementioned (such as purifying or isolating such cells away from cells not having reduced expression of
 15 *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, and not having reduced activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324). Such methods can also further include selecting modified PBMCs that are T cells, for example, T cells that are CD3⁺ or CD8⁺. Exemplary selection methods include using flow cytometry, panning or magnetic separation. The disclosed methods in some examples further include introducing the selected modified PBMCs having reduced expression of *Zscan20*, *Jdp2*,
 20 *Nfil3*, and/or *Znf324*, reduced activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324, or both, into a subject, such as a subject with a cancer to be treated with the selected modified PBMCs having reduced expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, reduced activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324, or any combination the aforementioned.

In some examples, the agent, non-naturally occurring genetic modification, or inhibitor is
 25 administered to the subject, and the agent, non-naturally occurring genetic modification, or inhibitor is introduced into a PBMC (*e.g.*, T cells, CD8⁺ TRM, tumor infiltrating lymphocytes, CAR T cells, or exhausted T cells (including terminally exhausted T cells)) *in vivo*.

In some aspects, the method of generating the modified PBMC further includes selecting a PBMC or cell type (*e.g.*, T cells, CD8⁺ TRM, tumor infiltrating lymphocytes, CAR T cells, or
 30 exhausted T cells (including terminally exhausted T cells)), for example, from a sample (*e.g.*, tumor biopsy, blood, population of T cells) before introducing the inhibitor, agent, or non-naturally occurring genetic modification. In some examples, the selected PBMC is reactive to a tumor-specific antigen, for examples, one or more of: CD19, CD20, BCMA, MUC1, PSA, CEA, HER1,

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HER2, TRP-2, EpCAM, GPC3, mesothelin 1(MSLN), or EGFR. In some examples the selected PBMC is a T cell. In some examples, the T cell is CD8+ or CD3+. In some examples, the T cell is an adoptive cell transfer (ACT) therapy T cell, for example, the selected exhausted T cell can include a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR) specific for a tumor antigen. In further examples, the selected PBMC is a tumor-infiltrating lymphocyte (TIL). In some examples, the T cell is an exhausted T cell, such as a terminally exhausted T cell, which highly expresses programmed cell death 1 (PD1^{hi}) and is positive for T cell immunoglobulin and mucin domain-containing protein 3 (TIM3⁺).

In some examples, the agent (RNAi or gRNA) is introduced, for example, by contacting a PBMC with the agent, thereby generating the modified PBMC. In other examples, the agent is introduced by transfecting or transforming a PBMC with the disclosed nucleic acid molecule encoding the inhibitor or agent or the vector encoding a disclosed nucleic acid molecule, thereby generating the modified PBMC. Methods of transforming or transfecting a host cell are described herein, and can include: chemical methods (*e.g.*, calcium-phosphate transfection), physical methods (*e.g.*, electroporation, microinjection, particle bombardment), fusion (*e.g.*, liposomes), nucleofection, receptor-mediated endocytosis (*e.g.*, DNA-protein complexes, viral envelope/capsid-DNA complexes) and by biological infection by viruses, such as recombinant viruses. In the case of infection by retroviruses, the infecting retrovirus particles are absorbed by the target cells, resulting in reverse transcription of the retroviral RNA genome and integration of the resulting provirus into the cellular DNA. In some examples, a ribonucleoprotein (RNP) complex including the gRNA and a Cas nuclease or dead nuclease (*e.g.*, Cas3, Cas9, Cas12, or Cas13d) is directly introduced into the PBMC. Methods of introducing a RNP complex into a host cell have been described. For example, the PBMC can be nucleofected with the RNP. In some examples, the PBMC is transfected with the RNP by electroporation (*see e.g.*, Seki and Rutz, (2018) *J Exp Med.* 215(3): 985–997). In some examples, lipid-containing oligoaminoamides (lipo-OAAs) are used to as a carrier for intracellular delivery of the RNP complex (*see e.g.*, Kuhn et al. (2020) *Bioconjugate Chem.* 31(3):729–742).

In specific, non-limiting examples, the introduced agent is shRNA, and the shRNA is introduced into the PBMC through infection with a viral vector encoding the shRNA. Introduction by a viral vector allows for stable integration of shRNA and long-term knockdown of the targeted gene. In another specific, non-limiting example, the introduced agent is siRNA, and siRNA is introduced cytosolically into a host cell capable of transfection.

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In some examples, the non-naturally occurring genetic modification is introduced into the PBMC. The genetic modification can be any non-naturally occurring modification that results in decreased expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* or reduced activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324. Non-limiting examples of genetic modifications include a point mutation, partial deletion, full deletion, or insertion. In some examples, the genetic modification is induced by a targeted genome editing technique, such as CRISPR/Cas, zinc finger nuclease, or TALEN modification of a *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* gene. Methods of genome editing have been previously described, for example, in Nemudryi *et al.* (2014) *Acta Naturae*; 6(3): 19–40, herein incorporated by reference in its entirety. In some examples, the genetic modification reduces *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* expression, for example, by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or 100%. In some examples, the genetic modification reduces ZSCAN20, JDP2, NFIL3, and/or ZNF324 activity, for example, by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or 100%.

In some examples the agent that reduces *Zscan20* expression or a non-naturally occurring genetic modification that reduces an amount of functional ZSCAN20 comprises a zinc finger nuclease (ZFN) or transcription activator-like effector nuclease (TALEN) specific for *Zscan20*. In further examples the agent that reduces *Jdp2* expression or a non-naturally occurring genetic modification that reduces an amount of functional JDP2 comprises a zinc finger nuclease (ZFN) or transcription activator-like effector nuclease (TALEN) specific for *Jdp2*. In more examples the agent that reduces *Nfil3* expression or a non-naturally occurring genetic modification that reduces an amount of functional NFIL3 comprises a zinc finger nuclease (ZFN) or transcription activator-like effector nuclease (TALEN) specific for *Nfil3*. In some examples the agent that reduces *Znf324* expression or a non-naturally occurring genetic modification that reduces an amount of functional ZNF324 comprises a zinc finger nuclease (ZFN) or transcription activator-like effector nuclease (TALEN) specific for *Znf324*.

In some examples, the modified PBMC is incubated with at least one cytokine selected from the group consisting of interleukin 2 (IL-2), interleukin 7 (IL-7), interleukin 15 (IL-15), TGF- β , and retinoic acid TGF- β , and retinoic acid.

In some examples, introducing the non-naturally occurring genetic modification reduces activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324 in the modified PBMC by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% relative to a suitable control (*e.g.*, an unmodified PBMC). In some

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examples, introducing the non-naturally occurring genetic modification protein levels of ZSCAN20, JDP2, NFIL3, and/or ZNF324 by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100% or more relative to a suitable control. In some examples, introducing the non-naturally occurring genetic modification
 5 reduces activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324 by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100% or more relative to a suitable control. Reducing activity includes reducing any measurable biological function of ZSCAN20, JDP2, and/or NFIL3, and/or ZNF324, for example, reducing the interaction between ZSCAN20, JDP2, and/or NFIL3, and/or ZNF324 and the genome.

10 In some examples, decreasing expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* or activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324 in a PBMC increases effector function, reduces exhaustion, increases resistance to exhaustion, or combinations thereof. In some examples, the PBMC is a T cell, and decreasing expression of *Zscan20*, *Jdp2*, *Nfil3*, *Znf324* or activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324 in the PBMC increases effector function of the T cell,
 15 reduces exhaustion of the T cell, or causes the T cell to express at least one of *Itgae*, *Itga1*, *Runx3*, *Cxcr3*, *Prdm1*, *Notch2*, *Tcf7*, *Cxcr5*, *Il7r*, *Id3*, or *Cd69* and/or causes reduced expression of *Slpr1*, *Klf2*, *Klf3*, *Pdcd1*, *Tox*, *Entpd1*, *Cxcr6*, *Eomes*, *Tbx21*, *Tigit*, *Cd38*, *Lag3*, *Cx3cr1*, *Cd101*, or *Havcr2* by the T cell. In some examples decreasing expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* or activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324 in a PBMC causes CD69 and/or
 20 CD103 to be present on the cell's surface.

In further examples, the PBMC is a T cell and decreasing expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* or activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324 in the PBMC increases resistance to T cell exhaustion. In some examples, the disclosed modified PBMCs, such as modified T cells, do not become exhausted (*e.g.*, do not become PD1^{hi} and TIM3⁺). In some
 25 examples, the disclosed modified PBMCs, such as modified T cells, become exhausted at a slower rate, for example the number of days to progress to an exhausted cell (*e.g.*, PD1^{hi} and TIM3⁺) is increased by at least 10%, at least 25%, at least 50%, at least 75%, at least 90%, at least 95%, or at least 99%, for example relative to a PBMC/T cell with native ZSCAN20, JDP2, NFIL3, and/or ZNF324 expression/activity. In some examples, the disclosed modified PBMCs, such as modified
 30 T cells, results in a population of modified PBMCs, such as modified T cells, with fewer exhausted cells (*e.g.*, PD1^{hi} and TIM3⁺), such as a reduction of at least 10%, at least 25%, at least 50%, at least 75%, at least 90%, at least 95%, or at least 99%, for example relative to a PBMC/T cell with native ZSCAN20, JDP2, NFIL3 and/or ZNF324 expression/activity.

VI. Pharmaceutical Compositions

Also disclosed herein are pharmaceutical compositions useful for treating cancer, increasing response to immunotherapy, and/or treating a viral infection (such as a chronic infection). In some examples, the pharmaceutical composition includes (1) one or more of: the disclosed RNAi specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, one or more gRNAs specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, the nucleic acid or vector encoding the RNAi or gRNA, the inhibitor (e.g., ZSCAN20, JDP2, NFIL3, and/or ZNF324 inhibitor), or the modified PBMC; and (2) a pharmaceutically acceptable carrier. In specific, non-limiting examples, the pharmaceutical composition includes a modified PBMC and a pharmaceutically acceptable carrier, such as water or saline.

In some examples, the pharmaceutical composition includes (1) one or more of: the RNAi specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, the gRNAs specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, the nucleic acid or vector encoding the RNAi or gRNA, the *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* inhibitor, or the modified PBMC; (2) a cancer immunotherapy; and (3) a pharmaceutically acceptable carrier. In some examples, the cancer immunotherapy is an ACT therapy (e.g., CAR-T, TCR, TIL), a monoclonal antibody (e.g., anti-PD-1, anti-EGFR, anti-CTLA4), a T cell agonist antibody, or an oncolytic virus. In one example, the cancer immunotherapy includes one or more ICB agents. In a specific, non-limiting example, the pharmaceutical composition includes the modified PBMC, an antibody cancer immunotherapy, and a pharmaceutically acceptable carrier. In another, non-limiting example, the pharmaceutical composition includes: one or more of the RNAi specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, the gRNAs specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, the nucleic acid or vector encoding the RNAi or gRNA; an ACT immunotherapy (e.g., CAR-T, TCR, TIL); and a pharmaceutically acceptable carrier.

In one example, the pharmaceutical composition includes: one or more of the RNAi specific to *Zscan20*, the gRNAs specific for *Zscan20*, the nucleic acid or vector encoding the RNAi or gRNA; one or more ICB agents (e.g., anti-PD1, anti-PD-L1, anti-CTLA4); and a pharmaceutically acceptable carrier. In one example, the pharmaceutical composition includes: one or more of the RNAi specific to *Jdp2*, the gRNAs specific for *Jdp2*, the nucleic acid or vector encoding the RNAi or gRNA; one or more ICB agents (e.g., anti-PD1, anti-PD-L1, anti-CTLA4); and a pharmaceutically acceptable carrier. In one example, the pharmaceutical composition includes: one or more of the RNAi specific to *Nfil3*, the gRNAs specific for *Nfil3*, the nucleic acid or vector

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encoding the RNAi or gRNA; one or more ICB agents (*e.g.*, anti-PD1, anti-PD-L1, anti-CTLA4); and a pharmaceutically acceptable carrier. In one example, the pharmaceutical composition includes: one or more of the RNAi specific to *Znf324*, the gRNAs specific for *Znf324*, the nucleic acid or vector encoding the RNAi or gRNA; one or more ICB agents (*e.g.*, anti-PD1, anti-PD-L1, anti-CTLA4); and a pharmaceutically acceptable carrier.

In further examples, the pharmaceutical composition includes (1) one or more of: the RNAi specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, the gRNAs specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, the nucleic acid or vector encoding the RNAi or gRNA, the *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* inhibitor, or the modified PBMC; (2) an antiviral agent; and (3) a pharmaceutically acceptable carrier. In some examples, the antiviral agent is aciclovir, ganciclovir, zidovudine, interferon alpha, or another direct acting antiviral agents.

A “pharmaceutically acceptable carrier” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration (see, *e.g.*, *Remington's Pharmaceutical Sciences*, 23rd Edition, Academic Press, Elsevier, (2020)). Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, balanced salt solutions, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. Supplementary active compounds can also be incorporated into the compositions. Methods for preparing administrable compositions include those provided in *Remington's Pharmaceutical Sciences*, 23rd Edition, Academic Press, Elsevier, (2020). In some examples, the pharmaceutical composition is formulated for intravenous administration.

VII. Methods of Treating Cancer and/or Viruses

Also disclosed herein are methods of treating cancer, a tumor, and/or a viral infection (such as a chronic viral infection) in a subject by administering an effective amount of a disclosed composition (the RNAi specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, the gRNA specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* and a Cas nuclease or dead Cas nuclease (which may be administered as an RNP complex), a nucleic acid or vector encoding the RNAi or gRNA (wherein in some examples the vector also expresses and a Cas nuclease or Cas dead nuclease), the modified PBMC, or the pharmaceutical composition disclosed herein (hereinafter collectively referred to as “composition”)), to the subject, thereby treating the cancer, tumor, or virus. In a specific, non-limiting example, the administered composition is an effective amount of the modified PBMCs disclosed herein. In some examples, PBMCs are removed from the subject and modified as

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disclosed herein *ex vivo*, then the modified cells are introduced into the subject. In some examples, PBMCs are modified *in vivo*, for example by introducing a therapeutic molecule provided herein (e.g., RNAi specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*) into the subject. In some examples, the administered composition includes one or more antiviral agents, such as aciclovir, ganciclovir, zidovudine, interferon alpha, and/or direct acting antiviral agents. In some examples, the administered composition includes one or more ICB agents.

Also disclosed herein are methods of increasing a response to immunotherapy in a subject by administering an effective amount of the disclosed composition, thereby increasing a response to immunotherapy. In a specific, non-limiting example, the method is a method of increasing a response to immunotherapy in a subject and the composition is the disclosed vector encoding the RNAi or gRNA.

In some examples, the subject has a tumor or cancer. In some examples, the subject has a solid tumor or cancer, such as breast carcinomas (e.g. lobular and duct carcinomas, such as a triple negative breast cancer), sarcomas, carcinomas of the lung (e.g., non-small cell carcinoma, large cell carcinoma, squamous carcinoma, and adenocarcinoma), mesothelioma of the lung, colorectal adenocarcinoma, stomach carcinoma, prostatic adenocarcinoma, ovarian carcinoma (such as serous cystadenocarcinoma and mucinous cystadenocarcinoma), ovarian germ cell tumors, testicular carcinomas and germ cell tumors, pancreatic adenocarcinoma, biliary adenocarcinoma, hepatocellular carcinoma, bladder carcinoma (including, for instance, transitional cell carcinoma, adenocarcinoma, and squamous carcinoma), renal cell adenocarcinoma, endometrial carcinomas (including, e.g., adenocarcinomas and mixed Mullerian tumors (carcinosarcomas)), carcinomas of the endocervix, ectocervix, and vagina (such as adenocarcinoma and squamous carcinoma of each of same), tumors of the skin (e.g., squamous cell carcinoma, basal cell carcinoma, malignant melanoma, skin appendage tumors, Kaposi sarcoma, cutaneous lymphoma, skin adnexal tumors and various types of sarcomas and Merkel cell carcinoma), esophageal carcinoma, carcinomas of the nasopharynx and oropharynx (including squamous carcinoma and adenocarcinomas of same), salivary gland carcinomas, brain and central nervous system tumors (including, for example, tumors of glial, neuronal, and meningeal origin), tumors of peripheral nerve, soft tissue sarcomas and sarcomas of bone and cartilage, head and neck squamous cell carcinoma (such as an HPV-positive HNSCC), and lymphatic tumors (including B-cell and T- cell malignant lymphoma).

In some examples, the subject has a liquid tumor or cancer, such as a lymphatic, white blood cell, or other type of leukemia. In a specific example, the tumor treated is a tumor of the

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blood, such as a leukemia (for example acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), hairy cell leukemia (HCL), T-cell prolymphocytic leukemia (T-PLL), large granular lymphocytic leukemia, and adult T-cell leukemia), a lymphoma (such as Hodgkin's lymphoma or non-

5 Hodgkin's lymphoma), or a myeloma.

In a non-limiting examples, the subject has leukemia, colorectal cancer, cervical cancer, lung cancer, bladder cancer, head and neck cancer, pancreatic cancer, glioblastoma, head and neck squamous cell carcinoma, ovarian cancer, uterine cancer, prostate cancer, breast cancer, melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma, sarcomas, or adrenal carcinoma. In

10 another non-limiting example, the subject has melanoma.

In further-non limiting examples, the subject has an acute or chronic leukemia, Hodgkin or Non-Hodgkin lymphoma, myeloma, gastric cancer, esophageal cancer, colorectal cancer, hepatocellular carcinoma or other liver cancer, cholangiocellular carcinoma, melanoma, cervical cancer, uterine cancer, lung cancer, ovarian cancer, bladder cancer, urothelial cancer, breast cancer, pancreatic cancer, renal cell carcinoma, prostate cancer, testicular cancer, glioblastoma, nephroblastoma, neuroblastoma, neuroendocrine cancer, pheochromocytoma, sarcoma, thyroid cancer, laryngeal cancer or head and neck cancer.

In some examples the subject has a viral infection, such as a chronic viral infection, such as an infection caused by: adenovirus (Ad), a herpes simplex virus (HSV, type 1 and 2), a hepatitis B virus (HBV), a hepatitis C virus (HCV), a hepatitis D virus (HDV), a hepatitis E virus (HEV), a vesicular stomatitis virus (VSV), a human immunodeficiency virus (HIV), an influenza virus, a varicella zoster virus (VZV), a human papillomavirus (HPV), an Epstein-Barr virus (EBV), a cytomegalovirus (CMV), a human herpesvirus (HHV-6, HHV-7), a human T-cell leukemia virus (HTLV-1, HTLV-2), JC virus, BK virus, an enterovirus, a parvovirus, a paramyxovirus (e.g. measles), a togavirus, SARS-CoV, SARS-CoV2, or a flavivirus.

In some examples, the subject is receiving, has received, or will receive immunotherapy, for example, one or more ICB agent targeting PD-1, PD-L1, CTLA-4, LAG3 GITR, 4-1BB, CD40, CD40L, and OX40, TIGIT, VISTA, CD73, CD39, HVEM, BTLA, CD27, CDK4, CDK6, or any combination of two or more of thereof. Exemplary checkpoint inhibitors include ipilimumab, nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, cemiplimab, palbociclib, ribociclib, abemaciclib, pidilizumab, cosibelimab, envafolelimab, BMS-936559, BMS935559, MEDI-4736, MPDL-3280A, MEDI-4737, and tremelimumab. In some examples, the effective amount of the composition is an amount that increases a response of the subject to an

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immunotherapy (*e.g.*, a checkpoint inhibitor or ACT); for example, an amount that when administered with the immunotherapy, is more effective at treating cancer or a tumor relative to administration of the immunotherapy (or composition) alone. In some examples, the effective amount is an amount that is synergistic when administered with an immunotherapy, for example, an amount that synergistically prevents, treats, reduces, and/or ameliorates one or more sign or symptom of cancer.

In some examples, the effective amount of the composition is an amount sufficient to prevent, treat, reduce, and/or ameliorate one or more signs or symptoms of cancer in the subject. For example, an amount sufficient to reduce tumor size or tumor load in the subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100%, as compared to a baseline measurement for the same subject, or a suitable control. In some examples, the effective amount is an amount sufficient to inhibit or slow metastasis in the subject. For example, by decreasing tumor spread in the subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% as compared to a baseline measurement for the same subject, or a suitable control. In some examples, the effective amount is an amount that increases life expectancy of the subject, for example, by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 200%, at least 400%, or more. In other examples, the effective amount is an amount sufficient to reduce tumor density in the subject, for example, by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% as compared to a baseline measurement for the same subject or other suitable control. Non-limiting examples of suitable controls include untreated subjects or subjects not receiving the composition (*e.g.*, subjects receiving other agents or alternative therapies). In further examples, the effective amount is an amount sufficient to target and eliminate tumor cells, for example, eliminate at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or even 100%, relative to a suitable control.

In some examples, the effective amount of the composition is an amount sufficient to prevent, treat, reduce, and/or ameliorate one or more signs or symptoms of viral infection in the subject, for example, an amount sufficient to reduce viral load by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100%, as compared to a baseline measurement for the same subject, or a suitable control. In some examples, the effective amount is an amount sufficient to inhibit or slow viral replication in

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the subject for example, an amount sufficient to inhibit or slow viral replication by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100%, as compared to a baseline measurement for the same subject, or a suitable control. In some examples, the effective amount is an amount that increases life

5 expectancy of the subject, for example, by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 200%, at least 400%, or more. In some one specific non-limiting example, an effective amount is an amount sufficient to increase T cell counts in an HIV infected subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%,
10 or 100%, as compared to a baseline measurement for the same subject, or a suitable control.

In some examples, the method reduces expression of *Zscan20*, *Jdp2*, and/or *Nfil3*, and/or *Znf324* or activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324 in a target tissue or cell in the subject, for example, in a PBMC, T cell, or exhausted T cell (including terminally exhausted T cells)). In some examples, expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* or activity of
15 ZSCAN20, JDP2, NFIL3, and/or ZNF324 is decreased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% relative to a suitable control (*e.g.*, an untreated subject or a baseline reading of the same subject prior to treatment). In some examples, the method reduces protein levels of ZSCAN20, JDP2, NFIL3, and/or ZNF324 (or functional ZSCAN20, JDP2, NFIL3, and/or ZNF324), for
20 example, by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% relative to a suitable control (*e.g.*, an untreated subject or a baseline reading of the same subject prior to treatment). In some examples, the method reduces expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* or accumulation of mRNA transcripts by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at
25 least 70%, at least 80%, at least 90%, at least 95%, or 100% relative to a suitable control (*e.g.*, an untreated subject or a baseline reading of the same subject prior to treatment).

In some examples, decreasing expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* or activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324 increases T cell effector function or decreases T cell exhaustion. In some examples, decreasing expression of *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* or
30 activity of ZSCAN20, JDP2, NFIL3, and/or ZNF324 reduces (including prevents or inhibits) T cell exhaustion or increases resistance to (including prevents or inhibits) T cell exhaustion. In some examples, increasing T cell response or reducing T cell exhaustion in a subject increases response to an immunotherapy in the subject.

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In a specific, non-limiting example, the method includes administering to the subject the modified PBMC and a pharmaceutically acceptable carrier. When the disclosed PBMC is administered, the composition includes about 10^4 to 10^{12} of the modified PBMCs (for example, about 10^4 - 10^8 cells, about 10^6 - 10^8 cells, about 10^6 - 10^{12} cells, about 10^8 - 10^{12} cells, or about 10^9 - 10^{10} cell). For example, the composition may be prepared such that about 10^4 to 10^{10} modified PBMCs (e.g., about 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , or 10^{10} cells/kg) are administered to a subject. In some examples, about 10^{10} cells/kg are administered to the subject. In specific examples, the composition includes at least 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , or 10^{10} modified PBMCs. In a specific, non-limiting example, about 10^8 - 10^{10} modified PBMCs are administered to the subject. An appropriate dose can be determined by a treating clinician based on factors such as the subject, the cancer being treated, treatment history, tumor load and type, clinical stage and grade of the disease, viral load, overall health of the subject, and other factors.

In some examples, non-modified lymphocytes are depleted in the subject prior to administering the disclosed composition. In some examples, the subject is also administered one or more cytokine(s) (such as IL-2, IL-7, IL-15, IL-21, and/or IL-12), for example, to support survival and/or growth of the disclosed modified PBMCs and/or an additional ACT therapy administered in combination, in the subject. In a specific, non-limiting example, at least one of IL-2, IL-7, and IL-15 is also administered to the subject. The cytokine(s) are administered before, after, or substantially simultaneously with the composition. In specific examples, at least one cytokine (e.g., IL-2, IL-7, and/or IL-15) is administered simultaneously, for example, with the composition. In some examples, the modified PBMC is reactive to a tumor-specific antigen in the subject having cancer. In some examples, the antigen is one or more of: CD19, CD20, BCMA, MUC1, PSA, CEA, HER1, HER2, TRP-2, EpCAM, GPC3, mesothelin 1(MSLN), or EGFR.

Administration of any of the disclosed compositions can be local or systemic. Exemplary routes of administration include, but are not limited to, oral, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, intravenous), sublingual, rectal, transdermal (for example, topical), intranasal, vaginal, and inhalation routes. In some examples, the agent is injected or infused into a tumor, or close to a tumor (local administration), or administered to the peritoneal cavity. Appropriate routes of administration can be determined by a treating clinician based on factors such as the subject, the condition being treated, and other factors.

Multiple doses of the composition can be administered to a subject. For example, the compositions can be administered daily, every other day, twice per week, weekly, every other week, every three weeks, monthly, or less frequently. A treating clinician can select an

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administration schedule based on the subject, the condition being treated, the previous treatment history, and other factors.

In some examples, the subject having cancer receives a treatment in addition to the composition, such as one or more of surgery, radiation, chemotherapy, biologic therapy, immunotherapy, or other therapeutic. Exemplary chemotherapeutic agents include (but are not limited to) alkylating agents, such as nitrogen mustards (such as mechlorethamine, cyclophosphamide, melphalan, uracil mustard or chlorambucil), alkyl sulfonates (such as busulfan), nitrosoureas (such as carmustine, lomustine, semustine, streptozocin, or dacarbazine); antimetabolites such as folic acid analogs (such as methotrexate), pyrimidine analogs (such as 5-FU or cytarabine), and purine analogs, such as mercaptopurine or thioguanine; or natural products, for example vinca alkaloids (such as vinblastine, vincristine, or vindesine), epipodophyllotoxins (such as etoposide or teniposide), antibiotics (such as dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, or mitocycin C), and enzymes (such as L-asparaginase). Additional agents include platinum coordination complexes (such as cis-diamine-dichloroplatinum II, also known as cisplatin), substituted ureas (such as hydroxyurea), methyl hydrazine derivatives (such as procarbazine), and adrenocortical suppressants (such as mitotane and aminoglutethimide); hormones and antagonists, such as adrenocorticosteroids (such as prednisone), progestins (such as hydroxyprogesterone caproate, medroxyprogesterone acetate, and magesrol acetate), estrogens (such as diethylstilbestrol and ethinyl estradiol), antiestrogens (such as tamoxifen), and androgens (such as testosterone propionate and fluoxymesterone). Examples of the most commonly used chemotherapy drugs include adriamycin, melphalan (Akeran®) Ara-C (cytarabine), carmustine, busulfan, lomustine, carboplatin, cisplatin, cyclophosphamide (Cytosan®), daunorubicin, dacarbazine, 5-fluorouracil, fludarabine, hydroxyurea, idarubicin, ifosfamide, methotrexate, mithramycin, mitomycin, mitoxantrone, nitrogen mustard, paclitaxel (or other taxanes, such as docetaxel), vinblastine, vincristine, VP-16, while newer drugs include gemcitabine (Gemzar®), trastuzumab (Herceptin®), irinotecan (CPT-11), leustatin, navelbine, rituximab (Rituxan®) imatinib (STI-571), Topotecan (Hycamtin®), capecitabine, ibritumomab (Zevalin®), and calcitriol.

A treating clinician can select appropriate additional therapies (from those listed here or other current therapies) for the subject, depending on factors such as the subject, the cancer being treated, treatment history, and other factors.

In some examples, the subject treated is administered an additional therapeutic, such as a monoclonal antibody cancer immunotherapy (*e.g.*, anti-CTLA-4, anti-PD1, or anti-PDL1), a T cell agonist antibody, an oncolytic virus, an adoptive cell transfer (ACT) therapy, or any combination of

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two or more thereof. The administration of an additional therapeutic may be before, after, or substantially simultaneously with the administration of the disclosed composition. In some examples, the additional therapeutic is a cell cycle or checkpoint inhibitor. In some examples, the checkpoint inhibitor targets PD-1, PD-L1, CTLA-4, CDK4, and/or CDK6. Exemplary inhibitors
5 include ipilimumab, nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, cemiplimab, palbociclib, ribociclib, and abemaciclib.

In some examples, the subject treated is also administered an ACT therapy, for example, a chimeric antigen receptor (CAR)-expressing T cell, engineered TCR T cell, or a tumor-infiltrating lymphocyte (TIL). In some examples, the subject is administered an effective amount of the
10 composition and the ACT therapy, and an effective amount of the composition is an amount that increases effectiveness of the ACT (*e.g.*, increases elimination of cancerous cells relative to ACT therapy alone).

The additional therapeutic may be administered substantially simultaneously with the disclosed composition. In some examples, the additional therapeutic is administered prior to
15 administering the composition, for example, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 12 days, at least 14 days, at least three weeks, at least four weeks, at least one month, or more prior. Multiple doses of the additional therapeutic can be administered to a subject, for example,
administered twice daily, once daily, every other day, twice per week, weekly, every other week,
20 every three weeks, monthly, or less frequently. A treating clinician can select an administration schedule based on the subject, the condition being treated, the previous treatment history, tumor load and type, clinical stage and grade of the disease and overall health of the subject, and other factors.

25 **VIII. Kits**

Also provided are compositions and kits that can be used with the disclosed methods. In some examples, the composition or kit includes one or more of the RNAi specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, the gRNA specific to *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, a nucleic acid or vector encoding the RNAi or gRNA, and the modified PBMC, for example with a
30 pharmaceutically acceptable carrier. In some examples, the kit includes one or more gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324* and a Cas nuclease or Cas dead nuclease (which may be an RNP complex). In a specific, non-limiting example, the kit includes a vector encoding one or more gRNA specific for *Zscan20*, *Jdp2*, *Nfil3*, and/or *Znf324*, which can further encode a

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Cas nuclease or Cas dead nuclease. In further examples, the kit includes the disclosed modified PBMCs.

In some examples, the kit includes ICB agents, optionally in a separate container. In some examples, the ICB agents target PD-1, PD-L1, CTLA-4, LAG3 GITR, 4-1BB, CD40, CD40L, and
5 OX40, TIGIT, VISTA, CD73, CD39, HVEM, BTLA, CD27, CDK4, and/or CDK6. In some examples the kit includes other anti-tumor agents, such as a chemotherapeutic agent, optionally in a separate container. In some examples, the kit includes anti-viral agents, optionally in a separate container. In some aspects, the anti-viral agents could include small molecules that inhibit virus replication, and/or antibodies which neutralize a virus.

10 The kit can include additional reagents, such as one or more of anti-CD3, anti-CD28, IL-2, and IL-15. In some examples, the reagents are present in separate containers. In one example, anti-CD3 and anti-CD28 are in the same container, and may be present, for example, on a bead. In some examples, the kit further includes one or more of a transfection reagent, culture medium, antibiotic, cytokines (*e.g.*, IL-2, IL-15, and IL-7), optionally wherein such reagents are present in
15 separate containers. In some examples the kit or composition includes media in which the PBMCs can be cultured or expanded *ex vivo*, such as AIM V® media.

EXAMPLES

The following examples are provided to illustrate particular features of certain aspects of
20 the disclosure, but the scope of the claims should not be limited to those features exemplified.

Example 1

Materials and Methods

This example illustrates the materials and methods of the following examples.

25 Dataset acquisition

The analyzed 42 CD8+ T cell samples were collected from ten datasets including this study (FIG. 3A). In total, 139 experiments including 64 ATAC-seq and 75 RNA-seq were harnessed to generate the paired samples and used as input of the Taiji pipeline. The CD8+ T cell samples span nine subtypes: naive cells (Naive), terminal effector cells (TE), memory precursor cells (MP),
30 tissue-resident memory cells (TRM), effector memory cells (TEM), central memory cells (TCM), progenitor exhausted cells (TexProg), intermediate exhausted cells (TexEff-like) and terminal exhausted cells (TexTerm).

TF regulatory networks construction and visualization

Taiji v1.1.0 with default parameters was used for the integrative analysis of RNA-seq and ATAC-seq data (Find the page for Taiji at the github website, under Taiji-pipeline / Taiji). The motif file was downloaded directly from the CIS-BP database (33). There were 871 mouse motifs
5 in the analysis.

Identification of cell-state-specific TFs

To identify cell-state-specific TFs, samples were divided into two groups: target group and background group. Target group included all the samples belonging to the cell type of interest and
10 the background group comprised the remaining samples. The normality test using Shapiro-Wilk's method was used to determine whether the two groups were normally distributed and it was found that the PageRank scores of most samples (90%) follow log-normal distribution. Based on log-normality assumption, an unpaired t-test was used to calculate the P-value. A P-value cutoff of 0.05 and log2 fold change cutoff of 0.5 were used for calling lineage-specific TFs.

Identification of differentiation-step-specific TFs

Taiji-reprogram framework was used(22) to identify differentiation-step-specific TFs, first PageRank ratios were calculated between target and source cell types. Ratio.abs was defined as the reciprocal of ratio if the ratio was smaller than 1, or otherwise, as the ratio itself. A higher
20 PageRank ratio.abs represents that the TF behaves quite differently between target and source. The top 30 TFs were selected based on PageRank score ratio.abs as candidate TFs. Then, the product of PageRank score ratio.abs of three candidate TFs for all combinations was calculated. All the products were transformed to z-scores and p-value of 0.001 was used as cut-off to select candidate recipes. Finally the TFs were ranked based on the frequency of candidate TFs in all the candidate
25 recipes.

Identification of transcriptional waves

To identify the TFs which show similar temporal activity patterns, the TFs were clustered based on the normalized PageRank scores across samples. First of all, principal component analysis
30 (PCA) was performed for dimension reduction of TF score matrix. The first 10 principal components were retained for further clustering analysis, which explained more than 70% variance (FIG. 15A). K-means algorithm was used for clustering analysis. To find the optimal number of clusters and similarity metric, Silhouette analysis was performed to evaluate the clustering quality

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using five distance metrics: Euclidean distance, Manhattan distance, Kendall correlation, Pearson correlation, and Spearman correlation (FIG. 15C). Pearson correlation was the most appropriate distance metric since the average Silhouette width was the highest among all five distance metrics. Based on these analyses, 7 distinct dynamic patterns of TF activity during immune cell
 5 development were identified. further performed functional enrichment analysis was performed to identify GO terms for these clusters.

TF community construction and visualization

Inspired by DBPNet (49), which is a framework to identify cooperations between DNA-
 10 binding proteins using Chromatin immunoprecipitation followed by sequencing (ChIP-seq) and Hi-C data, the TF interaction network was constructed based on Taiji's output, which is TF-regulatee network. First, Tex_{Term} samples' network was combined by taking the mean value of edge weight for each TF-regulatee pair. Next, regulatees with low variation across TFs (standard deviation ≤ 1) were removed. Then $n \times n$ correlation matrix (n is the total TF number) was calculated by taking
 15 account of the Spearman's correlation of edge weight for each TF-regulatee pair. R package "huge" (50) was used to build graphical model and to construct the graph. The Graphical lasso algorithm and the shrunken ECDF (empirical cumulative distribution function) estimator were employed, using a lasso penalty λ equal to 0.2 to control the regularization. This value was chosen because around 5% of TF-TF pairs have a correlation score > 0.2 . To estimate the false discovery rate, a
 20 null model was generated by random shuffling the edge weight of TF-regulatee pair across TFs. When the same algorithm is applied to this dataset, the chosen cutoff identifies zero interaction, suggesting that the method with cutoff equal to 0.2 has a very low false discovery rate. Communities were detected using Leiden algorithm (51) with modularity as objective function and resolution as 1.8. Network visualization was performed by igraph with Fruchterman-Reingold
 25 layout algorithm (52).

GO and KEGG pathway enrichment analysis

The enriched functional terms in this study were analyzed by R package
 clusterProfiler_4.0.5. For gene set enrichment analysis (GSEA) in fig. S9-11, the genes were first
 30 ranked by the mean log normalized expression values in Tex_{Term} samples and then CD8_TCELL related pathways were plotted using running scores. A cutoff of P-value < 0.05 was used to select the significantly enriched GO terms and KEGG pathways.

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Mice and Infections

C57BL/6/J mice were purchased from Jackson Laboratories. P14 mice (Pircher et al., 1987) mice have been previously described. Cas9 P14 mice were generated by crossing P14 mice with B6(C)-Gt(ROSA)26Soreml.1(CAG-cas9*, -EGFP)Rsky/J (Jackson Laboratories). Animals were housed in specific-pathogen-free facilities at the Salk Institute and all experimental studies were approved and performed in accordance with guidelines and regulations implemented by the Salk Institute Animal Care and Use Committee. Mice were infected with 2×10^5 PFU LCMV-Armstrong by intraperitoneal injection or 2×10^6 PFU LCMV-Clone13 by retro-orbital injection under anesthesia.

Cell Isolation

Spleens were mechanically dissociated with 1mL syringe plungers over a 70um nylon strainer. Spleens were incubated in ammonium chloride potassium (ACK) buffer for 5 minutes. For isolation of small intestinal IEL, Peyer's patches were first removed by dissection. Intestines were longitudinally cut and then cut into 1cm pieces and washed in PBS. Pieces were incubated in 30mL HBSS with 10% FBS, 10mM HEPES, and 1mM dithioerythritol with vigorous shaking at 37C for 30 minutes. Supernatants were collected, washed, and further isolated using a 40/67% discontinuous percoll density centrifugation for 20 minutes at room temp with no brakes.

Cell lines and in vitro cultures

B16-gp33 melanoma cell line was cultured in DMEM (Invitrogen) with 10% fetal bovine serum, 1% penicillin-streptomycin and 250 µg/ml G418 (Invitrogen #10131027). All the tumor cell lines were used for experiments when in exponential growth phase. For in vitro T cell culture, P14 splenocytes were activated in RPMI 1640 medium (Invitrogen) containing 10% fetal bovine serum and 1% penicillin-streptomycin, 2mM L-glutamine, 0.1 mg/ml gp33, BME and 10 U/ml IL-2.

Tumor engraftment and treatment of tumor-bearing mice

For tumor engraftment, 5×10^5 B16-gp33 tumor cells (Fig. 4; fig. S13) and 3×10^5 B16-gp33 tumor cells were injected subcutaneously in 100µl PBS. Tumors were measured every 2-3 days post tumor engraftment or indicated treatments and calculated. Tumor volume was calculated by $\text{volume} = (\text{length} \times \text{width}^2)/2$. For antibody-based treatment, tumor-bearing mice were treated with anti-PD1 antibody (200 µg per injection, clone GK1.5, BioXcell) twice per week from day 7 post

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tumor implantation. All experiments were conducted according to the Salk Institute Animal Care and Use Committee.

Tumor digestion and cell isolation

5 Tumors were minced into small pieces in RPMI containing 2% FBS, DNase I (0.5 µg/ml, Sigma-Aldrich), and collagenase (0.5 mg/ml, Sigma-Aldrich) and kept for digestion for 30 min at 37°C, followed by filtration with 70 µm cell strainers (VWR). Filtered cells were incubated with ACK lysis buffer (Invitrogen) to lyse red blood cells, mixed with excessive RPMI 1640 medium (Invitrogen) containing 10% fetal bovine serum and 1% penicillin-streptomycin, and centrifuged at
10 400g for 5 min to obtain single-cell suspension.

Retrovirus transduction and adoptive transfer

For gRNA retrovirus vector over-expression, 293T cells were transfected with Eco-helper and MSCV gRNA vectors. 48 hr and 72 hr later, supernatant containing retroviral particles was
15 ready for transduction. P14 donor splenocytes were in vitro activated by 0.1 mg/ml gp33 and 10 U/ml IL-2 at 37°C for 24h, then spin-transduced (1500 g) with fresh RV supernatant from 293T cells for 90 min at 30°C in the presence of 5 µg/ml polybrene. Right after viral transduction 0.5-106 Cas9 expressing gp33-specific P14 TCR transgenic CD8+ T cells were transferred into C57BL/6 mice (retro orbital) that were implanted with 5×10^5 B16-gp33 cells 7-8 days ago or were infected
20 with either LCMV clone 13 or Armstrong 1 day ago.

Flow cytometry, cell sorting and antibodies

Both single cell suspensions were incubated with Fc receptor-blocking anti-CD16/32 (BioLegend) on ice for 10 min before staining. Cell suspensions were first stained with Red Dead
25 Cell Stain Kit (ThermoFisher) for 10 min on ice. Surface proteins were then stained in FACS buffer (PBS containing 2% FBS and 0.1% sodium azide) for 30 min at 4°C. To detect cytokine production ex-vivo, cell suspensions were re-suspended in RPMI 1640 containing 10% FBS, stimulated by 50 ng/ml PMA and 3 µM Ionomycin in the presence 2.5 µg/ml Brefeldin A (BioLegend #420601) for 4 h at 37°C. Cells were processed for surface marker staining as described above. For intracellular
30 cytokine staining, cells were fixed in BD Cytofix/Cytoperm (BD #554714) for 30 min at 4 °C, then washed with 1× Permeabilization buffer (Invitrogen #00-8333-56). For transcription factor staining, cells were fixed in Foxp3 / Transcription Factor Fixation/Permeabilization buffer (Invitrogen #00-5521-00) for 30 min at 4 °C, then washed with 1× Permeabilization buffer. Cells were then stained

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with intraceulluar antibodies for 30 min at 4 °C. Samples were processed on LSR-II flow cytometer (BD Biosciences) and data were analyzed with FlowJo V10 (TreeStar). Cells were sorted either on FACS Aria™ III sorter or Fusion sorter (BD Biosciences).

The following antibodies against mouse proteins were used: anti-CD8a (53-6.7), anti-PD-1 (29F.1A12), anti-CX3CR1 (SA011F11), anti-SLAMF6 (13G3), anti-CD38 (90), anti-CD39 (24DMS1), anti-CD101 (Moushi101), anti-KRLG1 (2F1), anti-CD69 (H1.2F3), anti-CD103 (M290), anti-CD62L(MEL-14), anti-Tim3 (RMT3-23), anti-Ly5.1 (A20), anti-Ly5.2 (104), anti-IFN- γ (XMG1.2), anti-TNF- α (MP6-XT22), anti-GZMB (GB11). These antibodies were purchased from Invitrogen, Biolegend, Cell Signaling, or eBiosciences.

ATAC-Seq library preparation and sequencing

ATAC-seq was performed as previously described (Corces et al., 2017). Briefly, 5,000-50,000 viable cells were washed with cold PBS, collected by centrifugation, then lysed in resuspension buffer (RSB) (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂) supplemented with 0.1% NP40, 0.1% Tween-20, and 0.01% digitonin. Samples were incubated on ice for 3 min, then washed out with 1 ml RSB containing 0.1% Tween-20. Nuclei were pelleted by centrifugation at 500g for 10 min at 4°C then resuspended in 50 μ l transposition mix (25 μ l 2x TD buffer, 2.5 μ l transposase (100 nM final), 16.5 μ l PBS, 0.5 μ l 1% digitonin, 0.5 μ l 10% Tween-20, 5 μ l H₂O) and incubated at 37°C for 30 min in a thermomixer with 1000 RPM mixing. DNA was purified using a Qiagen MinElute PCR cleanup kit, then PCR amplified using indexed oligos. The optimal number of amplification cycles for each sample was determined by qPCR. Libraries were size selected using AmpureXP beads and sequenced using an Illumina NextSeq500 for 75bp paired-end reads.

ATAC-Seq analysis

Paired-end 42-bp, or paired-end 75-bp reads were aligned to the *M. musculus* mm10 genome using BWA (53, 54) with parameters “bwa mem -M -k 32”. ATAC-seq peaks were called using MACS2 (55) program using parameters “callpeaks -qvalue 5.0e-2 -shift -100 -extsize 200”. Differentially accessible regions were identified using DESeq2 (56). Batch effect was removed using limma (57). Heatmap visualization of ATAC-seq data was performed using pheatmap.

Single-cell RNA seq analysis Data analysis

Analysis was primarily performed in R (v 3.6.1) using the package Seurat (v 3.1) (58, 59), with the package tidyverse (v 1.2.1) (60) used to organize data and the package ggplot2 (v 3.2.1)

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used to generate figures. scRNA-seq data from GSE10898, GSE99254, GSE98638, GSE199565 and GSE181785 were filtered to keep cells with a low percentage of mitochondrial genes in the transcriptome (< 5%) and between 200 and 3000 unique genes to exclude poor quality reads and doublets. Cell cycle scores were regressed when scaling gene expression values and T cell receptor genes were regressed during the clustering process, which was performed with the Louvain algorithm within Seurat and visualized with UMAP. To quantify the gene expression patterns (FIGs. 2D, 2F, 16, and 17), Seurat's module score feature was used to score each cluster based on its per cell expression of TFs.

10 Statistical analyses

Statistical tests for flow cytometry data were performed using Graphpad Prism 7. p-values were calculated using either two-tailed unpaired Student's t-tests or one-way ANOVA while correcting for multiple comparisons via the Tukey method. Linear regressions were performed using the ordinary least squares method in R (v 3.6.1). Boxplots and violin plots were compared pairwise in R using the Wilcoxon test with Holm-Sidak correction. All data were presented as the mean $\pm$ SEM. The P values were represented as follows: ***P < 0.001, **P < 0.01 and *P < 0.05.

Example 2

Identification of TFs in T cells

This example illustrates identification of transcription factors (TFs) by dissecting the transcriptional networks and assessing the genome-wide influences of TFs.

The global influence of a regulator in the cell is conveyed through its regulatory effect on the target genes, which is propagated over the genetic network. A given regulator's activity is affected by its own expression level and post-translational modification as well as other mechanisms such as the presence of collaborative co-factors and target accessibility. Therefore, the expression level of a regulator such as a TF is not always correlated with its activity (25). In light of this, many methods have been proposed to infer the activity of regulators using statistical or machine-learning approaches. For instance, Schacht et al. (25) developed a statistical model to estimate the regulatory activity of TFs using their cumulative effects on their target genes. Arrieta-Ortiz et al. (26) used a linear model to infer the TF activity (TFA) by predicting target genes' expression levels. SCENIC (27) constructs the genetic network by predicting each gene's expression using the TF's expression levels and finding the most predictive TFs. Maslova et al. introduced AI-TAC (28) to predict ATAC-seq signals and identified the most enriched motifs when

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evaluating the TF importance. Although these methods were able to predict the local activity of a TF, i.e., the expression level of their direct target genes, measuring the system-wide influence of a given TF is not their focus. As genes rarely function alone, and can significantly crosstalk amongst each other to form complex regulatory logic, the global influence of a regulator can, in principle, better predict the cell state change upon perturbing the regulator than its local influence (29, 30).

Therefore, a recently developed method called Taiji (21) was utilized to uncover potentially important TFs in each CD8⁺ T cell subset. Taiji builds a genetic network by integrating transcriptomic and open chromatin data, upon which it assesses the global rather than local importance of regulators using the Personalized PageRank algorithm. This feature makes Taiji robust and suitable for integrating multiomics data from noisy genomic measurements. In fact, Taiji clearly outperforms the motif enrichment analysis and the TFA approach (15, 21, 31).

Example 3

Use of Transcription and Epigenetic Atlas

This example illustrates the use of a transcription and epigenetic atlas to generate a transcription factor catalog that specifies heterogeneous T cell state.

To perform an integrated TF activity analysis using the Taiji pipeline (21, 32), ATAC-seq and RNA-seq data were generated and collected in thirty-two CD8⁺ T cell populations spanning nine states from well-defined LCMV acute and chronic infection (FIG. 1A, FIGs. 2-3). Taiji integrates aforementioned multiomics data to build gene regulatory networks in which each node is a gene and an edge represents a regulatory interaction. Taiji first scans each open chromatin region, presumably active promoter or enhancer, to identify putative TF binding sites using motifs documented in the CIS-BP database (33). These TFs are then linked to their target genes predicted by EpiTensor (34). All the regulatory interactions are assembled into a genetic network where gene and interactions are defined between genes as “node” and “edge” respectively. Lastly, the personalized PageRank algorithm (21) was used to assess the global influences of TFs. The node weights were determined by the z scores of gene expression levels, allocating higher ranks to the TFs that regulate more differentially expressed genes. The edge weights were set to be proportional to expression levels and binding strength of TFs and open chromatin intensity (FIG. 1A). The average number of nodes and edges of the genetic networks of the landscape of the CD8⁺ T cell states were 18,041 and 1,613,381, respectively, including 871 (4.83%) TF nodes. On average, each TF regulated 1852 genes, and each gene was regulated by 20 TFs.

TFs were identified that have high PageRank scores suggesting high activity in nine states

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of CD8⁺ T cell (FIG. 1B, C). Interestingly 124 out of the 273 TFs are identified as potentially important TFs for more than one cell type (FIG. 1C, D). These TFs, called “multi-taskers”, include many well-known regulators. For instance, TCF7, widely known as a proliferation-related protein (3, 10), is identified as Naive-, MP-, and Tex_{Prog}-driving TF. All three cell states are multipotent and have high proliferative capacity. T-bet (encoded by *Tbx21*) is predicted to be highly active in both MP, and TE. T-bet is reported to program cytotoxic T cell terminal differentiation in response to LCMV viral infection in cooperation with another TF ZEB2 (35). MP and TE shared, not only T-bet, but an additional 21 TFs that program early activation (30% of TE or MP TFs) (FIG. 1D). Another large number of multi-taskers are observed in exhaustion-associated states, Tex_{Prog}, Tex_{Eff}-like, and Tex_{Term} such as *Vax2*, *Batf*, *Irf8*, *Stat1*, *Nfatc1*, and *Jdp2* (Fig. 1D). Tex_{Term} also shared 11 multi-taskers with T_{RM}. These include *Nr4a2*(18), *Bhlhe40*(36), *Prdm1*(37), and *Hic1*(38) (FIG 1E). This observation aligns well with the location and transcription similarity between T_{RM} and exhausted T cells (FIG. 2). Finding of a high portion of multi-taskers in each cell state extends this notion of reuse of TFs between different cell states (20, 39). This calls for the importance of state defining TFs that are specific to each cell state (“single-taskers”) of which information can be harnessed for T cell state program with specificity. Taiji analysis successfully identified novel single-tasker TFs (FIG. 1B; FIGs. 5-13). For example, FIG. 1E describes single-tasker TFs in T_{RM} and Tex_{Term} (FIG. 1E). These single-taskers include a multitude of TFs such as *Zscan20*, *Zbtb49*, *Arid3a*, and *Bhlhe41* that have not yet been reported before. Their study should improve the understanding of TF mechanisms underlying T cell state differentiation.

Example 4

Identification of Core TF Networks

This example illustrates how global analysis of the atlas identifies core TF networks that program context-dependent T cell differentiation.

How TF requirements and combinations are change depending on the differentiation steps was investigated. It was observed that in both acute and chronic infection, naive CD8⁺ T cells differentiate to early effector cells followed by the differentiation into various CD8⁺ T cell states in a parallel manner (1). In acute infection, naïve cells become either TE or MP. MP can further become diverse types of memory cells including T_{RM} (FIG. 14A). In chronic infection, naïve cells generate a counterpart of MP, Tex_{Prog}, which further differentiate into Tex_{Term} (FIG. 14B). To identify differentiation-step-specific TFs, PageRank ratios between target and source cell types were calculated. Interestingly, both Naïve → MP and Naïve → Tex_{Prog} transition share similar TF

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upregulation (*Zbtb32*, *Bhlhe40*, *Batf*) and $MP \rightarrow T_{RM}$ and $Tex_{Prog} \rightarrow Tex_{Term}$ share the downregulation of *Tcf7* and upregulation of *Nr4a2* (FIG 14). The shared TF programs suggest that T cell trajectories of CD8⁺ T cells in acute and chronic infection show not only structural similarity but also their TF regulatory circuits.

High degree of preservation of TFs in different cell states (FIG. 1C,D) and cell state transition (FIG 14) implies their context-dependent roles in T cell differentiation. It was hypothesized that distinct TF networks enable differential outcomes of the multi-taskers. To identify TF connectivity underlying cell state differentiation, unbiased TF clusters based on the normalized PageRank scores were generated across samples (FIG. 15A-C, FIG 1F). Those combinations of TFs activate in a spatiotemporal manner, which behave like transcriptional waves to orchestrate the developmental progress depending on the immunological context. For example, the transcriptional wave that specifically regulates exhaustion formation (FIG. 1F) was curated. The prediction was validated by analyzing published scRNAseq data (4, 40). The TF members of the cluster show higher expression in CD8⁺ T cells from chronic LCMV infection compared to acute LCMV infection (FIG. 1G). The TFs are enriched in gene sets associated with cell fate commitment, PD-1 pathway, chronic inflammation/infection (FIG. 1H). This transcriptional wave analysis also reports distinct clusters TFs bifurcating T cell differentiation in acute vs chronic, resident vs circulating, and MP vs TE and their associated biological pathways (FIGs 16-18). These analysis implicate the importance of differential combinations of TFs and connectivity to single taskers in specifically program each cell state.

Example 5

Integrative Analysis of TF Networks

This example illustrates how integrative analysis of TF networks reveals core biological circuits to drive given cell states.

It was hypothesized that effective T cell therapy can be achieved by programming T cells to avoid dysfunctional Tex_{Term} to favor functional effector state without compromising immunological memory potential. It was sought to identify TFs to perturb that are not only highly active in Tex_{Term} but also the core member of TF communities that cooperate with many other TFs to control biological circuits driving terminal exhaustion and/or suppressing effector function. The intricate network of TFs in Tex_{Term} states was constructed (FIG. 19A) and 11 communities were discovered (FIG. 19B). Next, a list of genes was curated (hereafter referred to as regulatees) controlled by TF members of each community. Different communities have distinct and shared regulatee pools (FIG.

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19C). Regulatees of Tex_{Term} TFs communities are commonly associated with biological circuits such as: negative regulation of immune system process, covalent chromatin modification, histone modification, dephosphorylation, FoxO signaling pathway, HBV, PD1-PDL1 pathway, and mitochondria organization (FIG. 19D, F) of which the associations to T cell exhaustion have previously been reported (7, 8, 41, 42). The network analysis also reveals connection between Tex_{Term} TFs and pathways that might otherwise have been unappreciated such as cellular catabolic process, GTPase activities, and response to hypoxia/oxidative stress, which provide interesting circuits for future study.

In accordance with the interaction intensities (proximity between communities) between TF communities (FIG. 19B), there are two big groups of communities that shares a large number of regulatees within each group (FIG. 19C). The first group has community (Cm.)Bhlhe40, Cm.Pbx3, Cm.Nfil3. The regulatees of this group are highly enriched in biological pathways associated with epigenetic regulation such as chromatin assembly, methylation, and demethylation (FIG. 2D, F). The second community group has Cm.Prdm1, Cm.Nfatc1, Cm.Hic1, and Cm.Nr4a2 of which regulatees are enriched in calcium ion transport, calcium homeostasis, TNF signaling, regulation of oxidative stress, and phosphates activities (Fig. 2D,F). Particularly, Cm.Prdm1 shares 86.7% of regulatees and large amount of pathways with other communities (FIG. 19C) implicating importance of Cm.Prdm1 in orchestrating Tex_{Term} biological circuits.

Next, the context-dependent nature of multi-taskers of Tex_{Term} and T_{RM} was investigated. The connection of well-known multi-tasker, *Prdm1*, a member of the central community in Tex_{Term} , and *Nfil3* which has not yet been reported on its role in CD8^+ T cell differentiation was dissected. Both *Prdm1* and *Nfil3* show clearly different networks in Tex_{Term} and T_{RM} states (FIG. 19B; fig. S6; fig. S7). For example, *Prdm1* is found in the community of *Zscan20*, *Irf8*, and *Gfi1* in Tex_{Term} state but *Prdm1* is neighboring with *Nr4a1*, *Irf4*, and *Hsf2* in T_{RM} state (FIGs. 19B, 20, and 21). The TF-TF interaction analysis of *Prdm1* and *Nfil3* reveals that ~ 50 % of interaction partners are distinct between two states (FIG. 21). This result indicates that even multi-taskers can be connected to distinct TF neighbors in different states.

Example 6

New TFs Programming Tex_{Term}

This example illustrates use of regulatee analysis and in vivo validation to reveal new TFs programming Tex_{Term} .

From the Tex_{Term} TF network analysis (FIG. 19), Cm. *Prdm1* is the hub of regulating

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biological circuits for overall Tex_{Term} . Cm. Bhlhe40 is the center of group 1 and particularly associated with cell cycle, demethylation, and chromatin reassembly. Among the members of these two groups, it was decided to examine *in vivo* the perturbation TFs regulating terminal exhaustion that prevents terminal differentiation to dysfunctional exhaustion states at the same time promotes effector function without compromising memory formation. *Zscan20* was selected from Cm. *Prdm1* and *Jdp2* from Cm. Bhlhe40 that have the most significant p-value activity score within the community and no activity in T_{RM} (FIG. 1E). As for the multi-tasker control, new TF, *Nfil3* and well-studied *Prdm1* were tested.

The expression of *Zscan20* in human T cells was first reported by Thiesen in 1990 (43). However, the role of the TFs has not yet been studied. According to the Taiji analysis, *Zscan20* is exclusively active in terminal exhaustion. The analysis of *Zscan20* TF network (FIG. 22) implies its interaction with exhaustion-associated TFs that are already published such as *Nfatc1*, *Nr4a1*, *Nr4a2*, and *Irf8* (44–47). The predicted regulatees are specifically upregulated in Tex_{term} state and associated with gene sets that are downregulated in KLRG1 high effector and stimulated T cells, DNA repair, T cell activation, lymphocyte differentiation, PDL-1 expression and PD-1 checkpoint pathway in cancer and covalent chromatin modification (FIG. 23). These data suggest that blockage of *Zscan20* prevents T cell exhaustion differentiation and promote effector state differentiation.

Pan-exhaustion specific TF, *Jdp2* is suggested to control transcription via direct regulation of the modification of histones and the assembly of chromatin or heterodimerization with a component of the AP-1 complex (48). The fact that the balance between AP-1 and NFAT activation is reported as one regulatory mechanism of T cell exhaustion (44) indicates a role of *Jdp2* in T cell exhaustion. Taiji analysis indicates that *Jdp2* regulatees suppress the effector-associated genes as they are enriched in naive cells compared to effector cells and biological pathways such as negative regulation of phosphorylation and dephosphorylation (FIG. 24). Therefore, depletion of *Jdp2* possibly prevents dysfunctional exhaustion states and restores effector function.

To interrogate the new regulators of Tex_{Term} (*Zscan20*, *Jdp2*, and *Nfil3*) *in vivo* CRISPR-mediated knockout (KO) (FIG. 25B) was used. On day 20+ of chronic LCMV infection, perturbation of these TFs and *Prdm1* altered the differentiation pattern of exhaustion subsets. All KOs are skewed away from Tex_{Term} (FIGs. 25C, D, and 26A) and show a reduction in inhibitory receptors such as CD38, CD39, and CD101 (FIG. 25E, 26B). In acute infection (FIG. 25F), KO of exhaustion-specific TFs (*Zscan20* and *Jdp2*) does not alter the properties of memory formation but KO of T_{RM} & Tex_{Term} multi-tasker (*Nfil3* and *Prdm1*) significantly reduces T_{RM} formation (FIG. 25F-I). This *in vivo* experiment demonstrates that the disclosed systemic approach can identify TFs

that specifically drive each cell state.

Example 7

Loss of *Jdp2* and *Zscan20* Contributes to Improved Effector Function

5 This example illustrates how loss of new Tex_{Term} regulators *Jdp2* and *Zscan20* contribute to better effector function.

Regulatee analysis indicates that top *Jdp2* regulatees are negatively enriched in effector-related genes and dephosphorylation (FIG. 24B,C). TF network analysis of *Zscan20* suggests that it has tight interaction with *Nfatc1*, *Nr4a1*, *Irf8* (FIG. 23A) and possibly regulates T cell activation and tolerance induction (FIG. 23C). KOs contain chronic LCMV infection better than control (FIG. 27A, B), express higher levels of KLRG1 and CX3CR1, effector-associated differentiation marker (FIG. 27C, D), and release more cytotoxic cytokines (FIG. 27E). Along with the bioinformatics analysis, loss of *Jdp2* or *Zscan20* programs the effector state differentiation of CD8^+ T cells in chronic infection where effector state are not found otherwise. This demonstrates the accuracy and implication of the disclosed bioinformatics approach in cell state engineering.

Example 8

Disruption of *Zscan20* Improves Tumor Treatment

20 This example illustrates disruption of *Zscan20* to improve tumor control and the synergy of disrupting *Zscan20* with ICB.

Similar to chronic virus control, it was hypothesized that Tex_{Term} -specific TF-deficiency confers enhanced tumor control. As Tex_{Prog} cells respond to ICB and become more effector-like, states which confer anti-tumor immunity, loss of *Zscan20*, exclusively deterring Tex_{Term} differentiation, will produce more beneficial cell states and generate synergy with immune checkpoint therapy. Utilizing an adoptive therapy model, g*Zscan20* RV transduced Cas9 P14 cells were transferred into mice with established melanoma tumors expressing GP33-41 (FIG. 27F).

Zscan20-deficiency enhanced tumor control in both anti-PD1 and control treatment conditions (FIG. 27G-I). This differential tumor growth is associated with the prevention of terminal exhaustion and promotion of Tex_{Prog} population with the loss of *Zscan20* (FIG. 27J-L). *Jdp2* KO also improves tumor suppression when it is combined with ICB (FIG. 29A). As *Jdp2* is likely to be less specific to Tex_{Term} , KO can alter the properties of Tex_{Prog} and $\text{Tex}_{\text{Eff-like}}$ in the tumor, possibly making the beneficial effect of KO moderate. Interestingly, T_{RM} and Tex_{Term} multi-tasker, *Nfil3*-deficiency does not improve tumor control (FIG. 30B) and reduce T_{RM} number (FIG.

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25I). This can be explained by the impaired properties of T_{RM} a positive prognosis has been correlated with TILs that present qualities of T_{RM} . Thus, depleting TFs selectively program $T_{EX-Term}$ such as *Zscan20* which does not perturb memory cells including T_{RM} will be a great strategy to enhance adoptive T cell therapy for tumors.

5 This precise pipeline of identification of cell state-specifying TFs can be easily adapted in cell state programming of other types of cells to improve the effectiveness of cell therapy.

Example 9

In Vivo CRISPR Screening Coupled with scRNA-Seq Systematically Validates TEX-Driving TFs

10 FIG. 31 showcases the systematic validation of cell state selectivity for transcription factors (TFs) using in vivo CRISPR-associated single-cell RNA sequencing. Retroviral tandem gRNA vectors were designed to express two gRNAs. A library of gRNAs was designed to target various TFs, and the resulting perturbations were analyzed for their impact on T cell exhaustion. The results validate bioinformatics-powered TF activity prediction and some TF knockouts lead to the upregulation of genes associated with effector and memory functions.

Example 10

In Vivo CRISPR Screening Coupled with scRNA-Seq Systematically Validates TEX-Driving TFs

20 An *in vivo* CRISPR screen coupled with scRNA-Seq was used to investigate the role of transcription factors (TFs) in T cell differentiation during acute viral infection. FIG. 32 shows that that knockout of TEX-specific TFs did not hinder the formation of tissue-resident memory T cells (TRMs), indicating selective roles for these TFs in T cell state regulation and demonstrating the accuracy of TF activity prediction.

Example 11

Disruption of T_{EX} single-tasker TFs rewire terminal exhaustion and enhance virus and tumor control

30 Gene set enrichment analysis (GSEA) of TEX-driving transcription factor (TF) knockouts suggests that such perturbations reduce cellular exhaustion and enhance effector functions. In chronic LCMV-infected mice, knockouts of specific TFs like *Zscan20* and *Jdp2* improve cytokine production and decrease viral load. Furthermore, in tumor studies, knockouts of TFs such as

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Zscan20, Jdp2, and Zfp324 lead to better tumor control, with the effects of these knockouts being amplified when used in conjunction with anti-PD1 antibody therapy, highlighting their therapeutic potential in tumor management and immunotherapy enhancement.

Mice and Infections: C57BL/6/J mice were purchased from Jackson Laboratories. P14 mice (Pircher et al., 1987) mice have been previously described. Cas9 P14 mice were generated by crossing P14 mice with B6(C)-Gt(ROSA)26Soreml.1(CAG-cas9*,-EGFP)Rsky/J (Jackson Laboratories). Animals were housed in specific-pathogen-free facilities. Mice were infected with 2×10^5 PFU LCMV-Armstrong by intraperitoneal injection or 2×10^6 PFU LCMV-Clone13 by retro-orbital injection under anesthesia.

Retrovirus transduction and adoptive transfer: For gRNA retrovirus vector over-expression, 293T cells are transfected with Eco-helper and MSCV gRNA vectors. 48 hr and 72 hr later, the supernatant containing retroviral particles was ready for transduction. P14 donor CD8+ T cells are in vitro activated in anti-CD3 and anti-CD28 antibody-coated plate with 100 U/ml hIL-2 at 37°C for 24h, then spin-transduced (1500 g) with fresh RV supernatant from 293T cells for 90 min at 30°C in the presence of 4 µg/ml polybrene. For FIGs. 33C-33E, right after viral transduction 2.5×10^4 Cas9 expressing gp33-specific P14 TCR transgenic CD8+ T cells were transferred into C57BL/6 mice (retro orbital) that were infected with LCMV one day prior. For FIGs. 33F-33M, right after viral transduction 1×10^6 Cas9 expressing gp33-specific P14 TCR transgenic CD8+ T cells were transferred into C57BL/6 mice (retroorbital) that were implanted with 5×10^5 B16-gp33 cells 7-8 days prior.

Cell lines and *in vitro* cultures: B16-gp33 melanoma cell line was cultured in DMEM (Invitrogen) with 10% fetal bovine serum, 1% penicillin-streptomycin and 250 µg/ml G418 (Invitrogen #10131027). All the tumor cell lines were used for experiments when in exponential growth phase. For *in vitro* T cell culture, P14 splenocytes were activated in RPMI 1640 medium (Invitrogen) containing 10% fetal bovine serum and 1% penicillin-streptomycin, 2mM L-glutamine, 0.1 mg/ml gp33, BME and 10 U/ml IL-2.

Tumor engraftment and treatment of tumor-bearing mice: For tumor engraftment, 5×10^5 B16-gp33 tumor cells (FIGs 33F-33L) and 3×10^5 B16-gp33 tumor cells (FIG. 33M) were injected subcutaneously in 100µl PBS. Tumors were measured every 2-3 days post-tumor engraftment or indicated treatments and calculated. Tumor volume was calculated by volume = (length × width²)/2. For antibody-based treatment, tumor-bearing mice were treated with anti-PD1 antibody (200 µg per injection, clone RMP1-14, BioXcell) twice per week from day 7 post tumor implantation.

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- 5 It will be apparent that the precise details of the methods or compositions described may be varied or modified without departing from the spirit of the described aspects of the disclosure. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

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Claims:

1. A modified peripheral blood mononuclear cell (PBMC), comprising:
 - a) an agent that reduces *Zscan20* expression or a non-naturally occurring genetic modification that reduces an amount of functional ZSCAN20;
 - b) an agent that reduces *Jdp2* expression or a non-naturally occurring genetic modification that reduces an amount of functional JDP2;
 - c) an agent that reduces *Nfil3* expression or a non-naturally occurring genetic modification that reduces an amount of functional NFIL3
 - d) an agent that reduces *Znf324* expression or a non-naturally occurring genetic modification that reduces an amount of functional ZNF324; or
 - e) any combination of a), b), c), and d).
2. The modified PBMC of claim 1, comprising:
 - a) the agent that reduces *Zscan20* expression or the non-naturally occurring genetic modification that reduces an amount of functional ZSCAN20; and:
either one of b) an agent that reduces *Jdp2* expression or a non-naturally occurring genetic modification that reduces an amount of functional JDP2; or c) an agent that reduces *Nfil3* expression or a non-naturally occurring genetic modification that reduces an amount of functional NFIL3.
3. The modified PBMC of claim 1 or claim 2, wherein:
 - a) the agent that reduces *Zscan20* expression comprises an inhibitory RNA (RNAi) specific for *Zscan20* or a guide RNA (gRNA) specific for *Zscan20*;
 - b) the agent that reduces *Jdp2* expression comprises an RNAi specific for *Jdp2* or a gRNA specific for *Jdp2*;
 - c) the agent that reduces *Nfil3* expression comprises an RNAi specific for *Nfil3* or a gRNA specific for *Nfil3*; or
 - d) the agent that reduces *Znf324* expression comprises an RNAi specific for *Znf324* or a gRNA specific for *Znf324*.
4. The modified PBMC of claim 3, wherein:
 - a) the RNAi specific for *Zscan20* is a short hairpin RNA (shRNA) molecule, short interfering RNA (siRNA) molecule, or antisense RNA molecule;

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b) the RNAi specific for *Jdp2* is a shRNA molecule, siRNA molecule, or antisense RNA molecule;

c) the RNAi specific for *Nfil3* is a shRNA molecule, siRNA molecule, or antisense RNA molecule; or

d) the RNAi specific for *Znf324* is a shRNA molecule, siRNA molecule, or antisense RNA molecule.

5. The modified PBMC of claim 3 or 4, wherein the agent that reduces *Zscan20* expression, *Jdp2* expression, *Nfil3* expression, or *Znf324* expression comprises a heterologous nucleic acid molecule; and

a) wherein the heterologous nucleic acid molecule encodes:

i) the RNAi specific for *Zscan20* gene or transcript, wherein the RNAi specific for *Zscan20* comprises at least 90% complementarity to a portion of the *Zscan20* gene or transcript;

ii) the gRNA specific for *Zscan20* gene or transcript, wherein the gRNA specific for *Zscan20* comprises at least 90% sequence identity to a portion of the *Zscan20* gene or transcript; or

iii) the gRNA specific for *Zscan20* gene or transcript, wherein the gRNA specific for *Zscan20* comprises at least 90% sequence identity to a portion of the *Zscan20* gene or transcript and a *Cas* nuclease;

b) wherein the heterologous nucleic acid molecule encodes:

i) the RNAi specific for *Jdp2* gene or transcript, wherein the RNAi specific for *Jdp2* comprises at least 90% complementarity to a portion of the *Jdp2* gene or transcript;

ii) the gRNA specific for *Jdp2* gene or transcript, wherein the gRNA specific for *Jdp2* comprises at least 90% sequence identity to a portion of the *Jdp2* gene or transcript; or

iii) the gRNA specific for *Jdp2* gene or transcript, wherein the gRNA specific for *Jdp2* comprises at least 90% sequence identity to a portion of the *Jdp2* gene or transcript and a *Cas* nuclease;

c) wherein the heterologous nucleic acid molecule encodes:

i) the RNAi specific for *Nfil3* gene or transcript, wherein the RNAi specific for *Nfil3* comprises at least 90% complementarity to a portion of the *Nfil3* gene or transcript;

ii) the gRNA specific for *Nfil3* gene or transcript, wherein the gRNA specific for *Nfil3* comprises at least 90% sequence identity to a portion of the *Nfil3* gene or transcript; or

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iii) the gRNA specific for *Nfil3* gene or transcript, wherein the gRNA specific for *Nfil3* comprises at least 90% sequence identity to a portion of the *Nfil3* gene or transcript and a *Cas* nuclease; or

d) wherein the heterologous nucleic acid molecule encodes:

i) the RNAi specific for *Znf324* gene or transcript, wherein the RNAi specific for *Znf324* comprises at least 90% complementarity to a portion of the *Znf324* gene or transcript;

ii) the gRNA specific for *Znf324* gene or transcript, wherein the gRNA specific for *Znf324* comprises at least 90% sequence identity to a portion of the *Znf324* gene or transcript; or

iii) the gRNA specific for *Znf324* gene or transcript, wherein the gRNA specific for *Znf324* comprises at least 90% sequence identity to a portion of the *Znf324* gene or transcript and a *Cas* nuclease.

6. The modified PBMC of claim 3, wherein the agent that reduces *Zscan20* expression, *Jdp2* expression, *Nfil3* expression, or *Znf324* expression comprises a heterologous nucleic acid molecule encoding:

- a) the gRNA specific for *Zscan20*, comprising SEQ ID NOs: 7, 8, 9, 10, 19, 20, or 21;
- b) the gRNA specific for *Jdp2*, comprising SEQ ID NOs: 11, 12, 13, 14, 22, 23, or 24;
- c) the gRNA specific for *Nfil3*, comprising SEQ ID NOs: 15, 16, 17, 18, 25, 26, or 27; or
- d) the gRNA specific for *Znf324* comprising SEQ ID NOs: 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, or 46.

7. The modified PBMC of claim 5 or 6, wherein the modified PBMC comprises an expression vector encoding the heterologous nucleic acid.

8. The modified PBMC of any one of claims 5 to 7, comprising the gRNA specific for the *Zscan20*, *Jdp2*, *Nfil3*, or *Znf324* gene or transcript and a *Cas* nuclease, wherein the *Cas* nuclease is a *Cas3*, *dCas3*, *Cas9*, *dCas9*, *Cas12*, *dCas12*, *Cas13a*, *dCas13a*, *Cas13b*, *dCas13b*, *Cas13d*, or *dCas13d* nuclease.

9. The modified PBMC of claim 1 or claim 2, comprising the genetic modification that reduces *ZSCAN20*, *JDP2*, *NFIL3*, or *ZNF324* wherein:

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a) wherein the genetic modification that reduces ZSCAN20 is a point mutation, a partial deletion, full deletion, or insertion of *Zscan20* that reduces expression of *Zscan20* and/or reduces activity of ZSCAN20;

b) wherein the genetic modification that reduces JDP2 is a point mutation, a partial deletion, full deletion, or insertion of *Jdp2* that reduces expression of *Jdp2* and/or reduces activity of JDP2;

c) wherein the genetic modification that reduces NFIL3 is a point mutation, a partial deletion, full deletion, or insertion of *Nfil3* that reduces expression of *Nfil3* and/or reduces activity of NFIL3; or

d) wherein the genetic modification that reduces ZNF324 is a point mutation, a partial deletion, full deletion, or insertion of *Znf324* that reduces expression of *Znf324* and/or reduces activity of ZNF324.

10. The modified PBMC of any one of claims 1-9, wherein the modified PBMC is a T cell.

11. The modified PBMC of claim 10 wherein the T cell is a CD8+ T cell or a CD4+ T cell.

12. The modified PBMC of claim 10 or 11, wherein the T cell is a therapeutic T cell.

13. The modified PBMC of any one of claims 10 to 12, wherein the T cell is an exhausted T cell, tissue resident memory T cell (TRM), a chimeric antigen receptor (CAR) T cell, an engineered T cell receptor (TCR) T cell, or a tumor-infiltrating lymphocyte (TIL).

14. The modified PBMC of any one of claims 10 to 13, wherein the T cell comprises an antigen receptor reactive to a tumor-specific antigen.

15. The modified PBMC of claim 14, wherein the tumor-specific antigen is one or more of CD19, CD20, BCMA, MUC1, PSA, CEA, HER1, HER2, TRP-2, EpCAM, GPC3, mesothelin 1(MSLN), or EGFR.

16. A method of generating the modified PBMC of any one of claims 1 to 15, comprising:

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a) introducing the agent that reduces *Zscan20* expression or non-naturally occurring genetic modification that reduces functional ZSCAN20 into a PBMC, thereby generating the modified PBMC with reduced expression of *Zscan20*, reduced activity of ZSCAN20, or both;

b) introducing the agent that reduces *Jdp2* expression or non-naturally occurring genetic modification that reduces functional JDP2 into a PBMC, thereby generating the modified PBMC with reduced expression of *Jdp2*, reduced activity of JDP2, or both;

c) introducing the agent that reduces *Nfil3* expression or non-naturally occurring genetic modification that reduces functional NFIL3 into a PBMC, thereby generating the modified PBMC with reduced expression of *Nfil3*, reduced activity of NFIL3, or both; and/or

d) introducing the agent that reduces *Znf324* expression or non-naturally occurring genetic modification that reduces functional ZNF324 into a PBMC, thereby generating the modified PBMC with reduced expression of *Znf324*, reduced activity of ZNF324, or both.

17. The method of claim 16, wherein the PBMC is a T cell.

18. The method of claim 16 or 17, wherein the method further comprises incubating the modified PBMC with interleukin 2, (IL-2), interleukin 7 (IL-7), interleukin 15 (IL-15), or a combination thereof.

19. The method of any one of claims 16 to 18, wherein the modified PBMC is reactive to a tumor-specific antigen.

20. The method of claim 19, wherein the tumor-specific antigen is one or more of CD19, CD20, BCMA, MUC1, PSA, CEA, HER1, HER2, TRP-2, EpCAM, GPC3, mesothelin 1(MSLN), and EGFR.

21. The method of any one of claims 16 to 20, wherein:

i) reduced expression of *Zscan20*, reduced activity of ZSCAN20, reduced expression of *Jdp2*, reduced activity of JDP2, reduced expression of *Nfil3*, reduced activity of NFIL3, reduced expression of *Znf324*, or reduced activity of ZNF324 increases effector function of the T cell; and/or

ii) reduced expression of *Zscan20*, reduced activity of ZSCAN20, reduced expression of *Jdp2*, reduced activity of JDP2, reduced expression of *Nfil3*, reduced activity of NFIL3, reduced

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expression of *Znf324*, or reduced activity of ZNF324 reduces exhaustion of the T cell.

22. The method of any one of claims 16 to 21, further comprising:

- a) selecting the modified PBMC with reduced expression of *Zscan20*, reduced activity of ZSCAN20, or both;
 - b) selecting the modified PBMC with reduced expression of *Jdp2*, reduced activity of JDP2, or both;
 - c) selecting the modified PBMC with reduced expression of *Nfil3*, reduced activity of NFIL3, or both; or
 - d) selecting the modified PBMC with reduced expression of *Znf324*, reduced activity of ZNF324, or both; and
- optionally introducing the selected modified PBMC into a subject.

23. The method of claim 22, wherein the selecting comprises use of flow cytometry, panning, or magnetic separation.

24. The method of claim 22 or 23, wherein the subject has cancer.

25. The method of claim 24, further comprising selecting the subject who has cancer.

26. A pharmaceutical composition comprising: the modified PBMC of any one of claims 1 to 15, or the modified PBMC generated by the method of any one of claims 16 to 25; and a pharmaceutically acceptable carrier.

27. The pharmaceutical composition of claim 26, wherein the composition is in an intravenous formulation.

28. The pharmaceutical composition of claim 26 or 27, further comprising one or more immune checkpoint blockade (ICB) agents.

29. A method for treating cancer or a tumor in a subject, comprising:
administering a therapeutically effective amount of the modified PBMC of any one of

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claims 1 to 15, a therapeutically effective amount of the modified PBMC generated by the method of any one of claims 16 to 25, or a therapeutically effective amount of the pharmaceutical composition of any one of claims 26 to 28, to the subject having cancer or the tumor, thereby treating the cancer or the tumor.

30. The method of claim 29, wherein the modified PBMC is autologous to the subject.

31. The method of claim 29, wherein the modified PBMC is allogenic to the subject.

32. The method of any one of claims 22 to 26 or 29 to 31, further comprising administering a therapeutically effective amount of IL-2, IL-7, and/or IL-15 to the subject.

33. The method of any one of claims 22 to 26 or 29 to 32, further comprising treating the subject with one or more of surgery, radiation, chemotherapy, biologic therapy, or immunotherapy.

34. The method of any one of claims 22 to 26 or 29 to 33, further comprising administering to the subject a therapeutically effective amount of one or more of: a T cell agonist antibody, an oncolytic virus, or an adoptive cell transfer (ACT) immunotherapy.

35. The method of any one of claims 22 to 26 or 29 to 34, further comprising administering to the subject a therapeutically effective amount of immune checkpoint blockade (ICB) agent or immunostimulatory antibody.

36. The method of claim 35, wherein the ICB agent comprises anti-PD-1, anti-PD-L1, anti-CTLA-4, anti-LAG3 anti-GITR, anti-4-1BB, anti-CD40, and anti-OX40, anti-TIGIT, anti-VISTA, anti-CD73, anti-CD39, anti-HVEM, anti-BTLA, anti-CD27, or a combination of two or more thereof.

37. The method of claim 36, wherein the anti-PD-1 is nivolumab, pembrolizumab, pidilizumab, or cemiplimab.

38. The method of claim 36, wherein the anti-PD-L1 atezolizumab, avelumab,

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durvalumab, cosibelimab, KN035 (envafolimab), BMS-936559, BMS935559, MEDI-4736, MPDL-3280A, or MEDI-4737.

39. The method of claim 36, wherein the anti-CTLA-4 is ipilimumab or tremelimumab.

40. The method of any one of claims 35 to 39, wherein the modified PBMC is administered simultaneously with the ICB agent or the immunostimulatory antibody.

41. The method of any one of claims 35 to 39, wherein the modified PBMC is administered before the ICB agent or the immunostimulatory antibody.

42. The method of any one of claims 35 to 39, wherein the modified PBMC is administered after the ICB agent or the immunostimulatory antibody.

43. The method of any one of claims 22 to 26 or 29 to 42, wherein non-modified lymphocytes are depleted in the subject prior to administering the modified PBMC.

44. The method of any one of claims 22 to 26 or 29 to 42, wherein the cancer or tumor is a leukemia, colorectal cancer, melanoma, cervical cancer, lung cancer, ovarian cancer, bladder cancer, breast cancer, pancreatic cancer, renal cell carcinoma, prostate cancer, or head and neck cancer.

45. A method for treating a viral infection in a subject, comprising:
administering a therapeutically effective amount of the modified PBMC of any one of claims 1 to 13, a therapeutically effective amount of the modified PBMC generated by the method of any one of claims 16 to 18 or 21 to 23, or a therapeutically effective amount of the pharmaceutical composition of claims 26 or 28, to the subject having the viral infection, thereby treating the viral infection.

46. The method of claim 45, further comprising treating the subject with an antiviral agent.

47. The method of any one of claims 45 to 46, further comprising administering to the

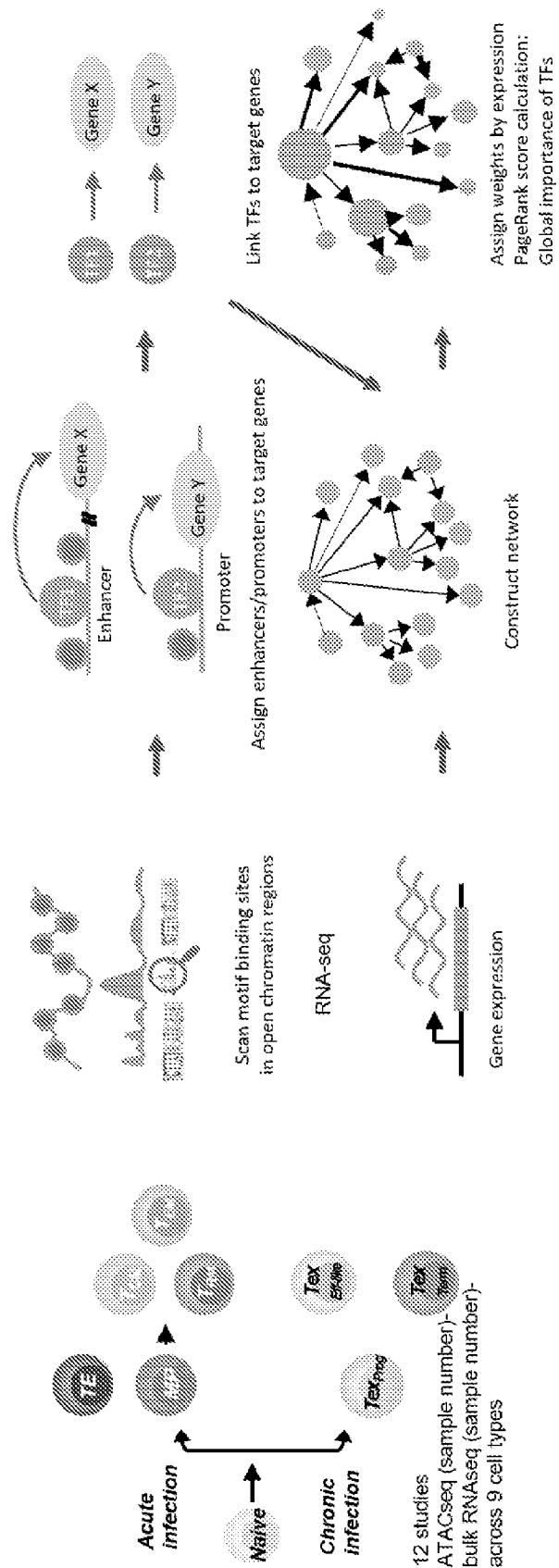
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subject a therapeutically effective amount of immune checkpoint blockade (ICB) agent or immunostimulatory antibody.

48. The method of claim 47, wherein the ICB agent comprises anti-PD-1, anti-PD-L1, anti-CTLA-4, anti-LAG3 anti-GITR, anti-4-1BB, anti-CD40, and anti-OX40, anti-TIGIT, anti-VISTA, anti-CD73, anti-CD39, anti-HVEM, anti-BTLA, anti-CD27, or a combination of two or more thereof.

49. The method of claims 45 to 48, wherein the viral infection is caused by an adenovirus (Ad), a herpes simplex virus (HSV), a hepatitis B virus (HBV), a hepatitis C virus (HCV), a vesicular stomatitis virus (VSV), a human immunodeficiency virus (HIV), an influenza virus, a varicella zoster virus (VZV), a human papillomavirus (HPV), an Epstein-Barr virus (EBV), a cytomegalovirus (CMV), an enterovirus, a togavirus, a SARS-CoV, SARS-CoV-2 virus, or a flavivirus.

FIG. 1A



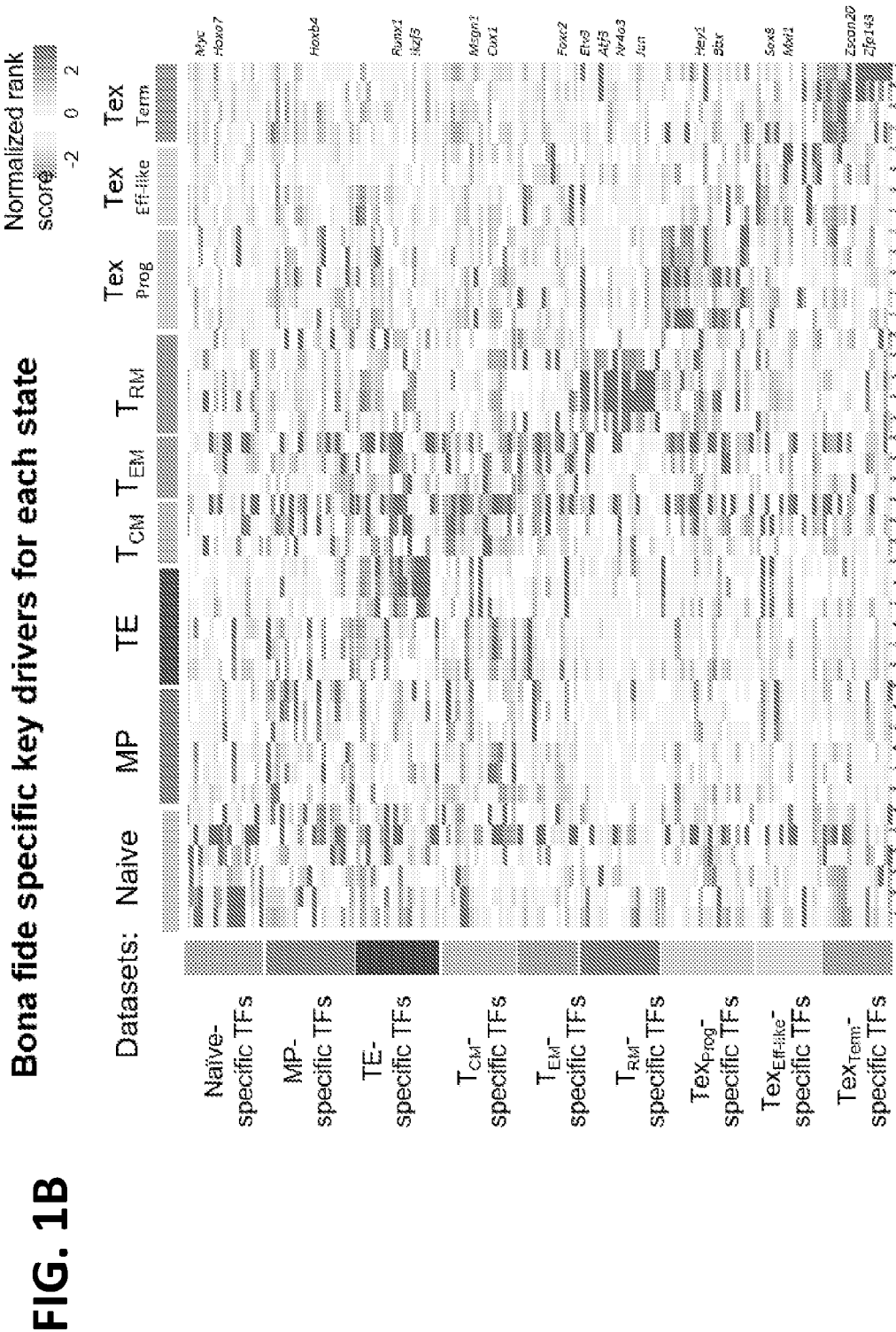


FIG. 1C

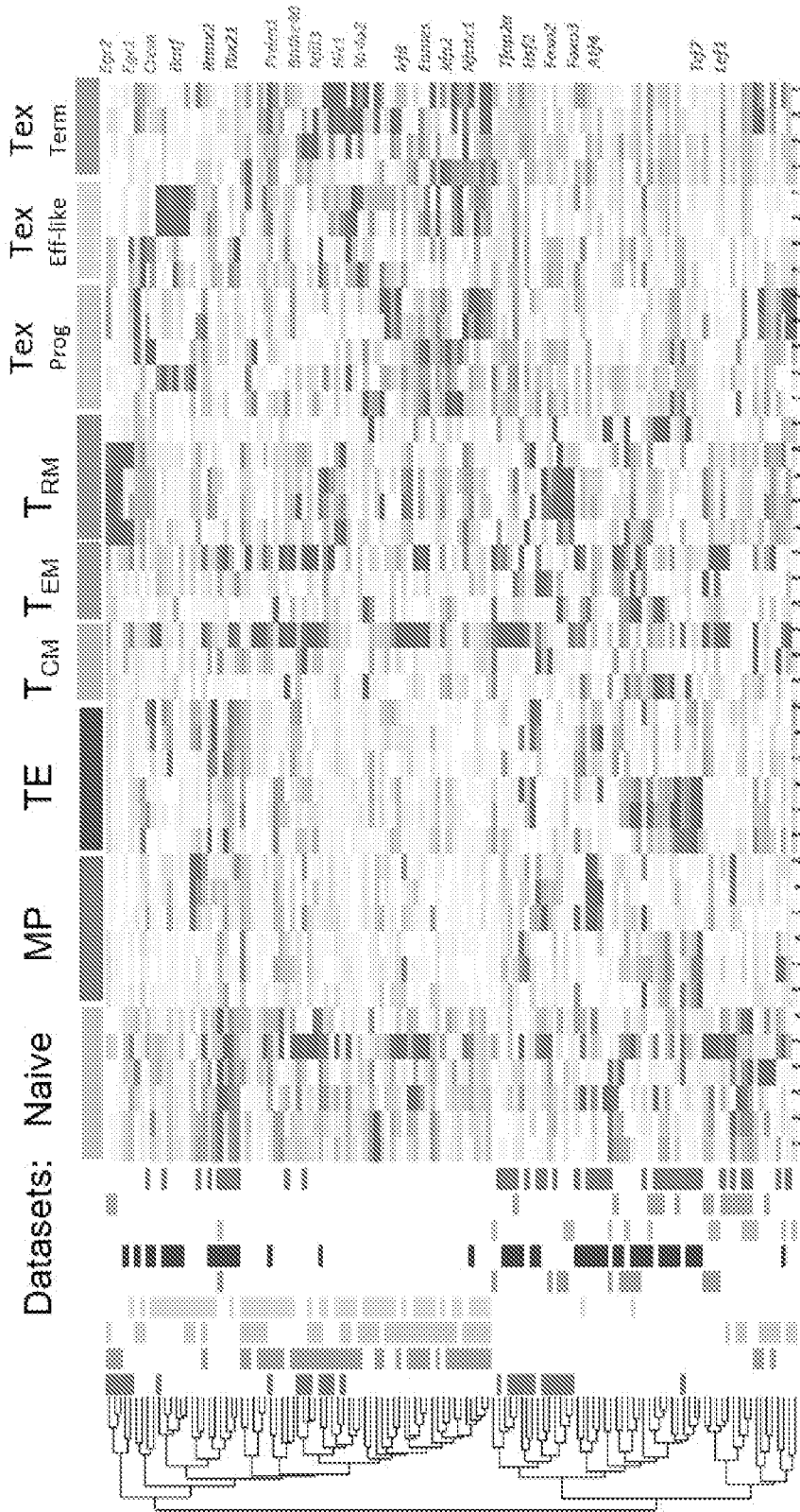


FIG. 1E

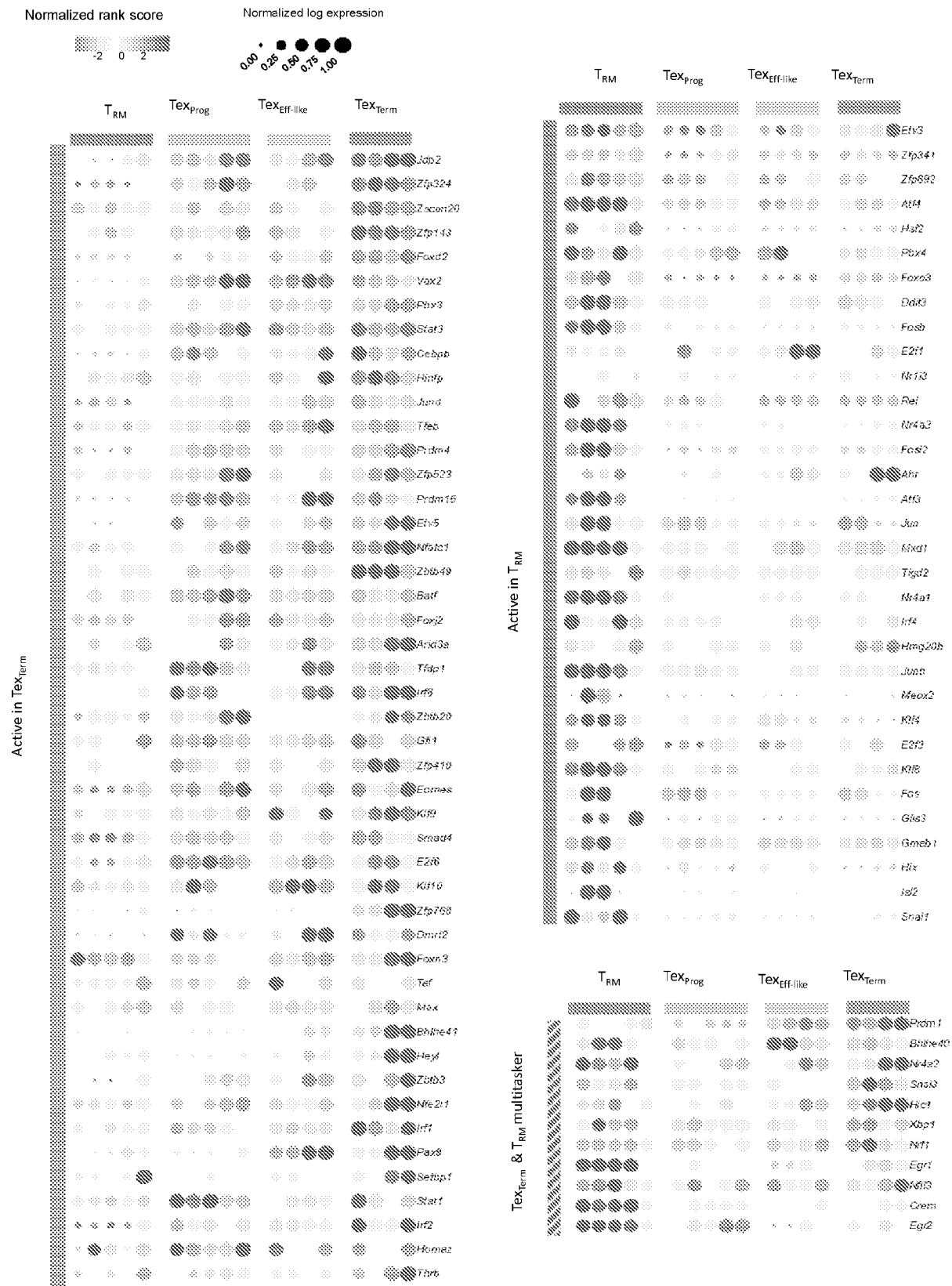


FIG. 1F

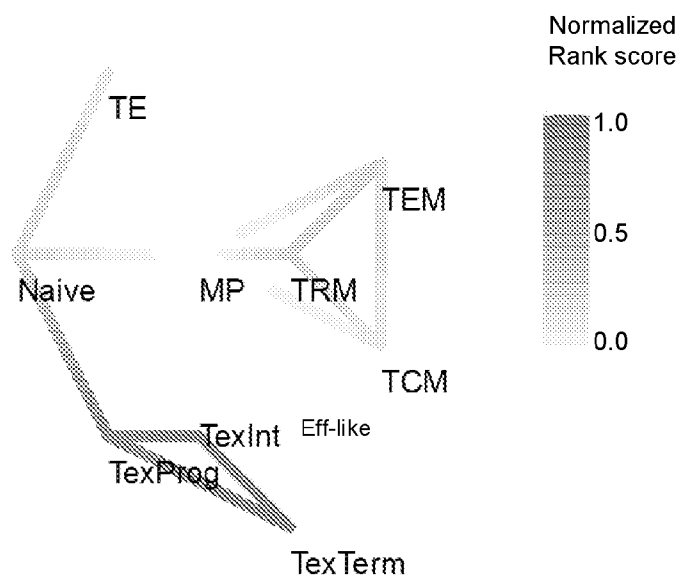


FIG. 1G

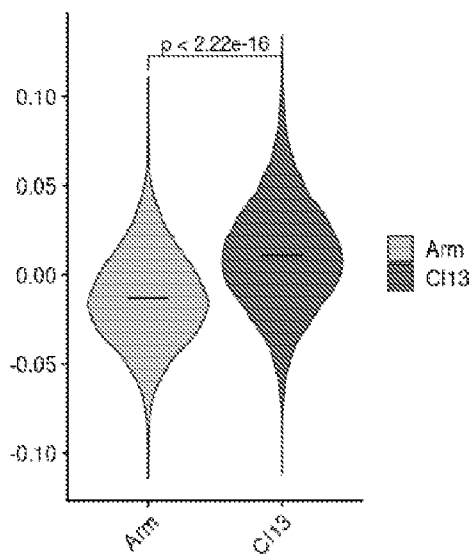


FIG. 1H

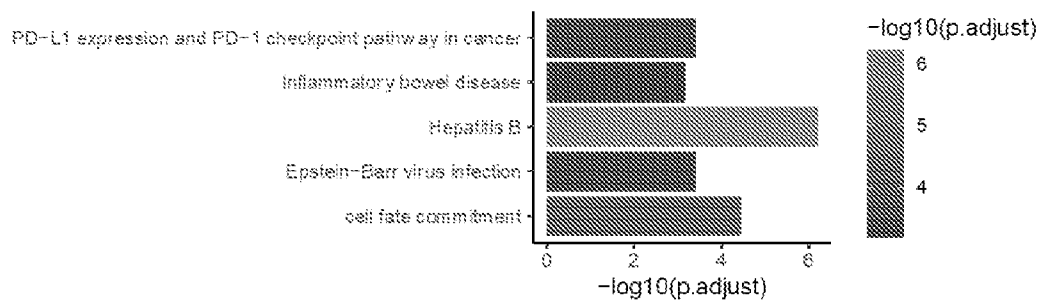


FIG. 2A

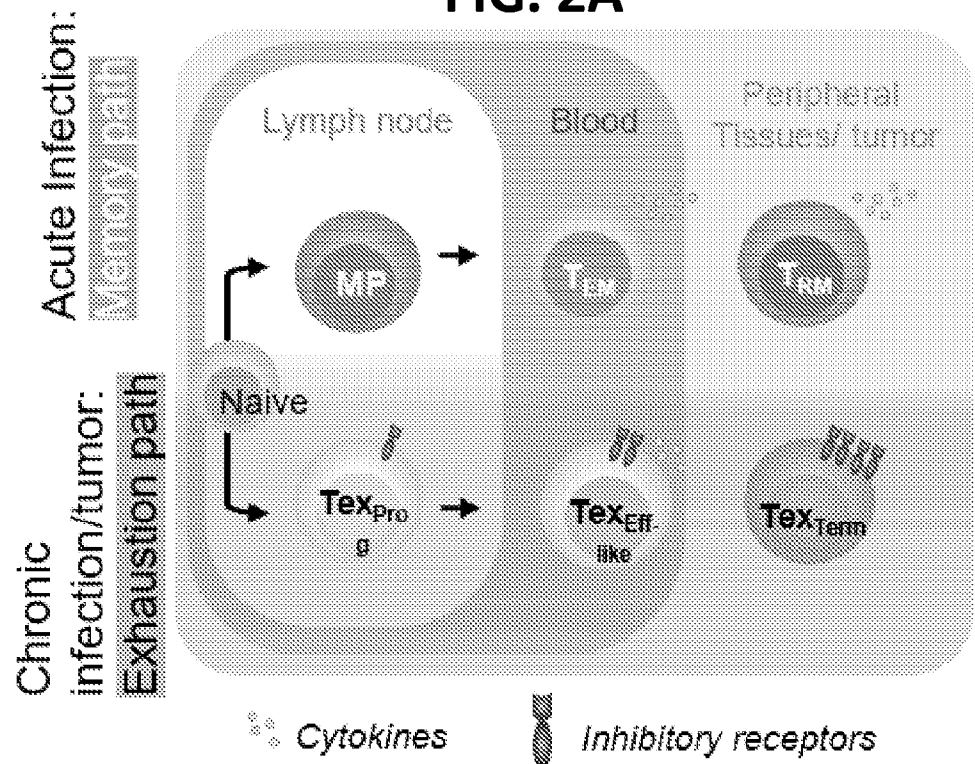


FIG. 2B

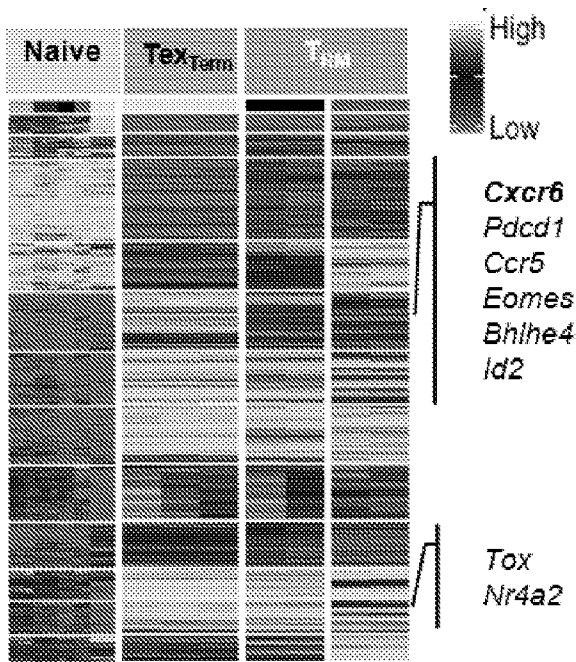


FIG. 2C

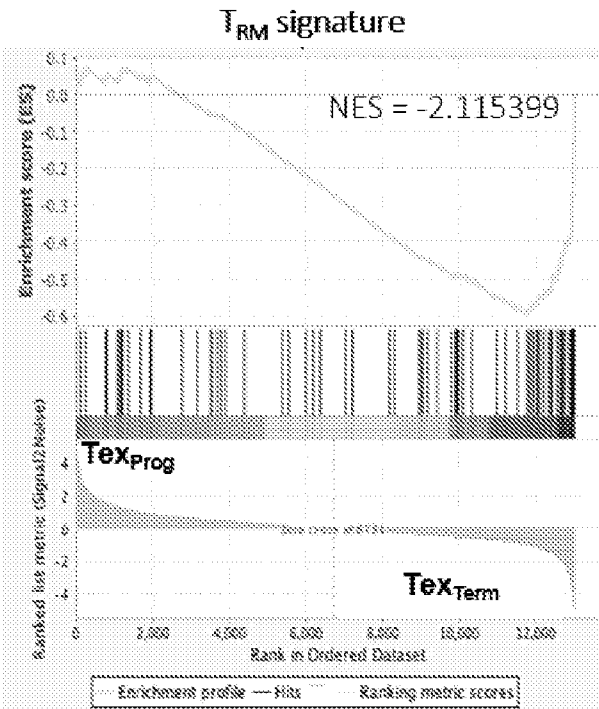


FIG. 2D

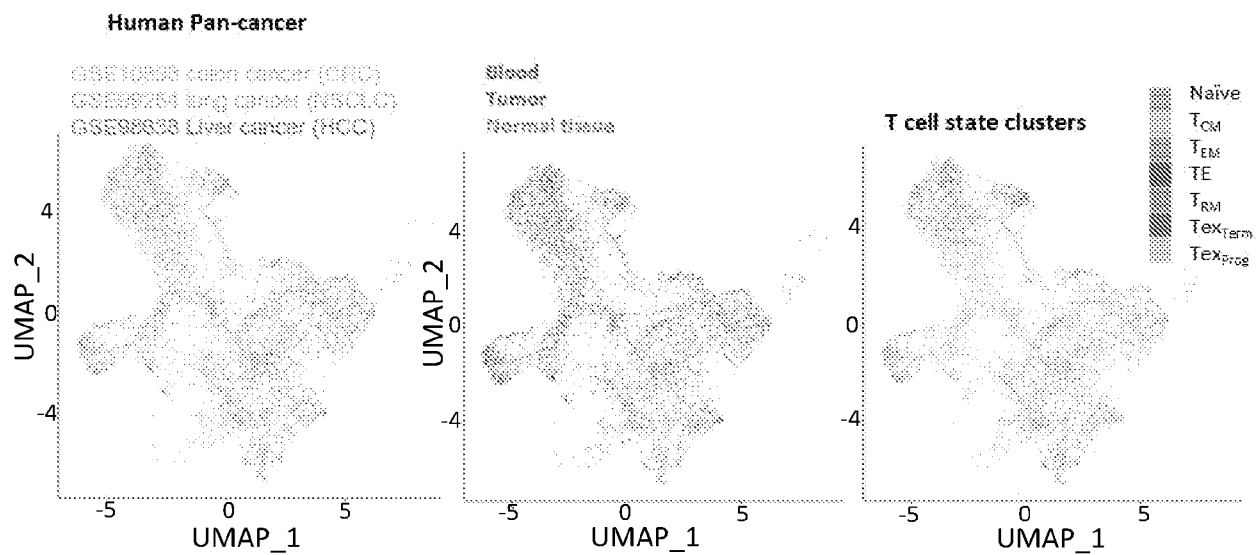


FIG. 2E

T_{EX} signature gene set module score

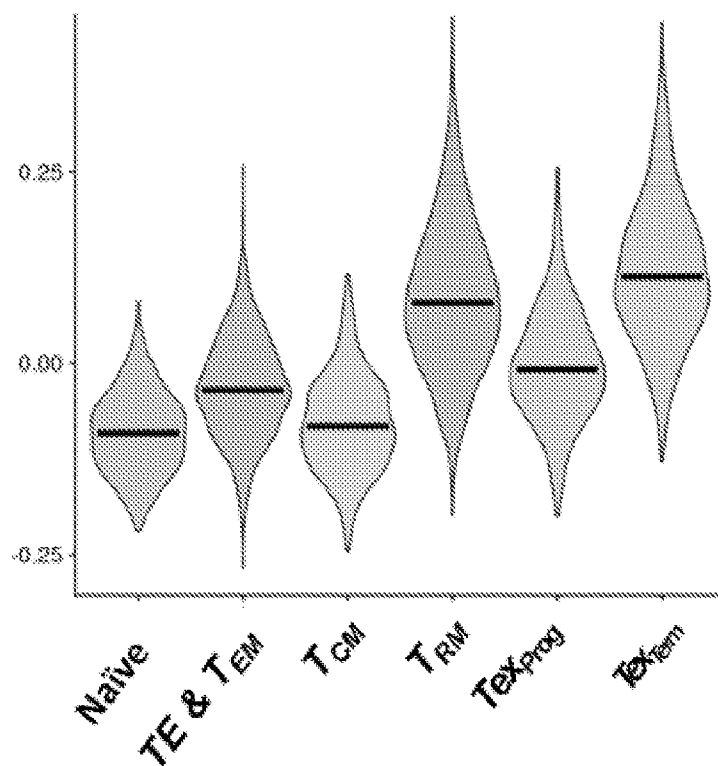


FIG. 3A

10 datasets 9 subsets 121 experiments 52 ATAC-seq 75 RNA-seq			
Dataset	Accession	# of experiments	Subsets
Milner(2017)	GSE107281	2 RNA + 2 ATAC	T _{CM}
Milner(2020.Apr)	GSE147080	2 RNA	T _{RM}
Milner(2020.Aug)	GSE157072	12 RNA	Naïve, MP, TE, T _{CM} , T _{EM}
Kaeht(2018)	GSE111902	9 RNA + 9 ATAC	Naïve, TE, MP
Scott(2016)	GSE88987	4 RNA + 11 ATAC	Naïve, TE, MP
Mackay(2016)	GSE70813	10 RNA	Naïve, T _{CM} , T _{EM} , T _{RM}
Renkema(2020)	GSE152841	9 RNA	Naïve, T _{CM} , T _{EM}
Hudson(2019)	PRJNA497086	9 RNA	T _{EM} _{progr} , T _{EM} _{Eff-like} , T _{EM} _{Term}
Beltra(2020)	GSE149876	12 RNA + 11 ATAC	T _{EM} _{progr} , T _{EM} _{Eff-like} , T _{EM} _{Term}
Chung(2022)	This study	19 ATAC	Naïve, TE, MP, T _{CM} , T _{EM} , T _{RM} , T _{EM} _{progr} , T _{EM} _{Eff-like} , T _{EM} _{Term}

FIG. 3B
ATAC-seq (with batch effect correction)

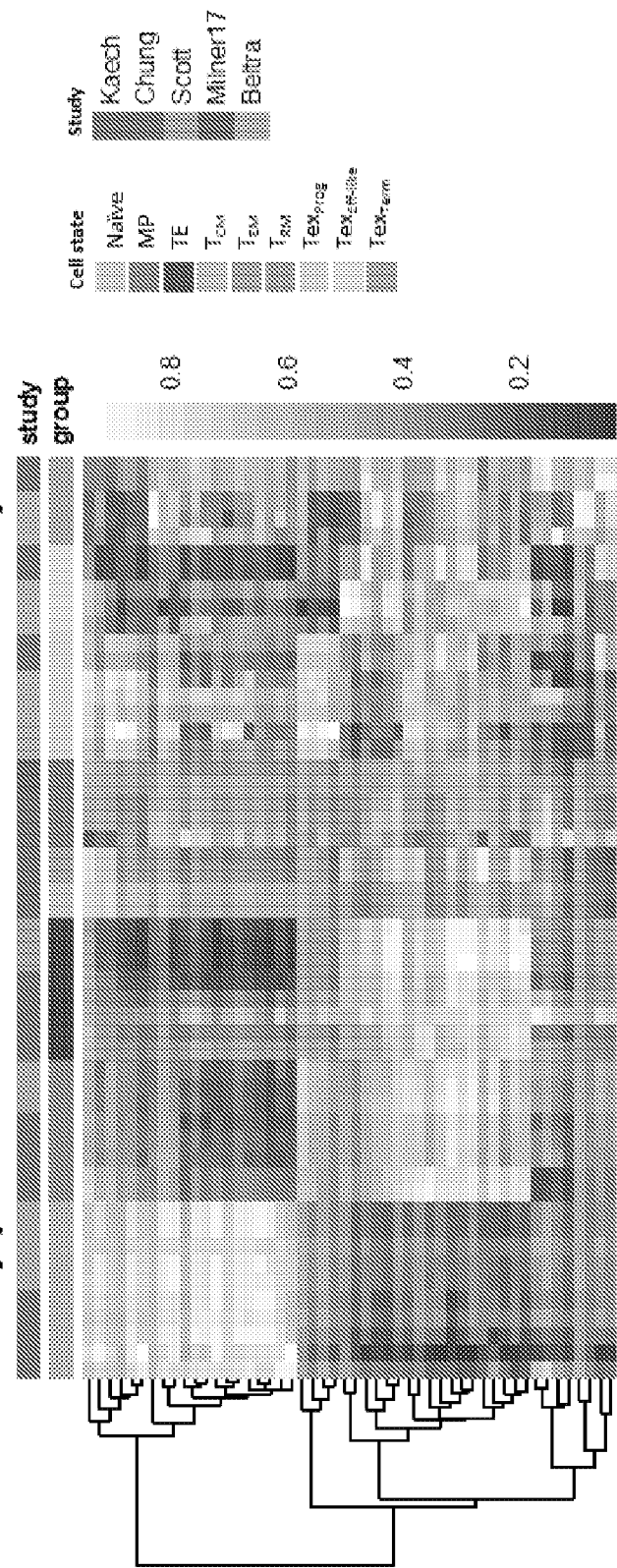


FIG. 3C

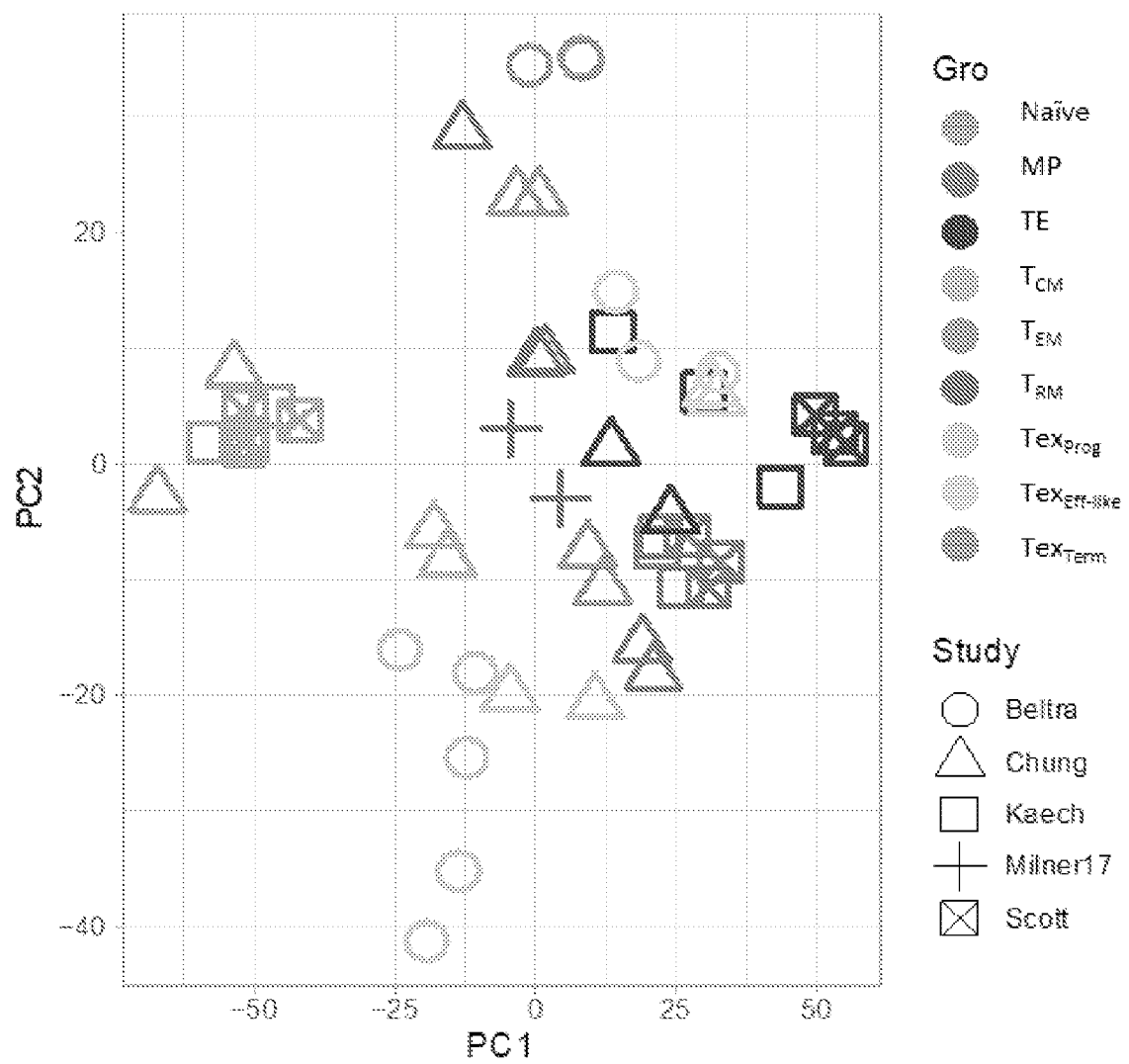


FIG. 4

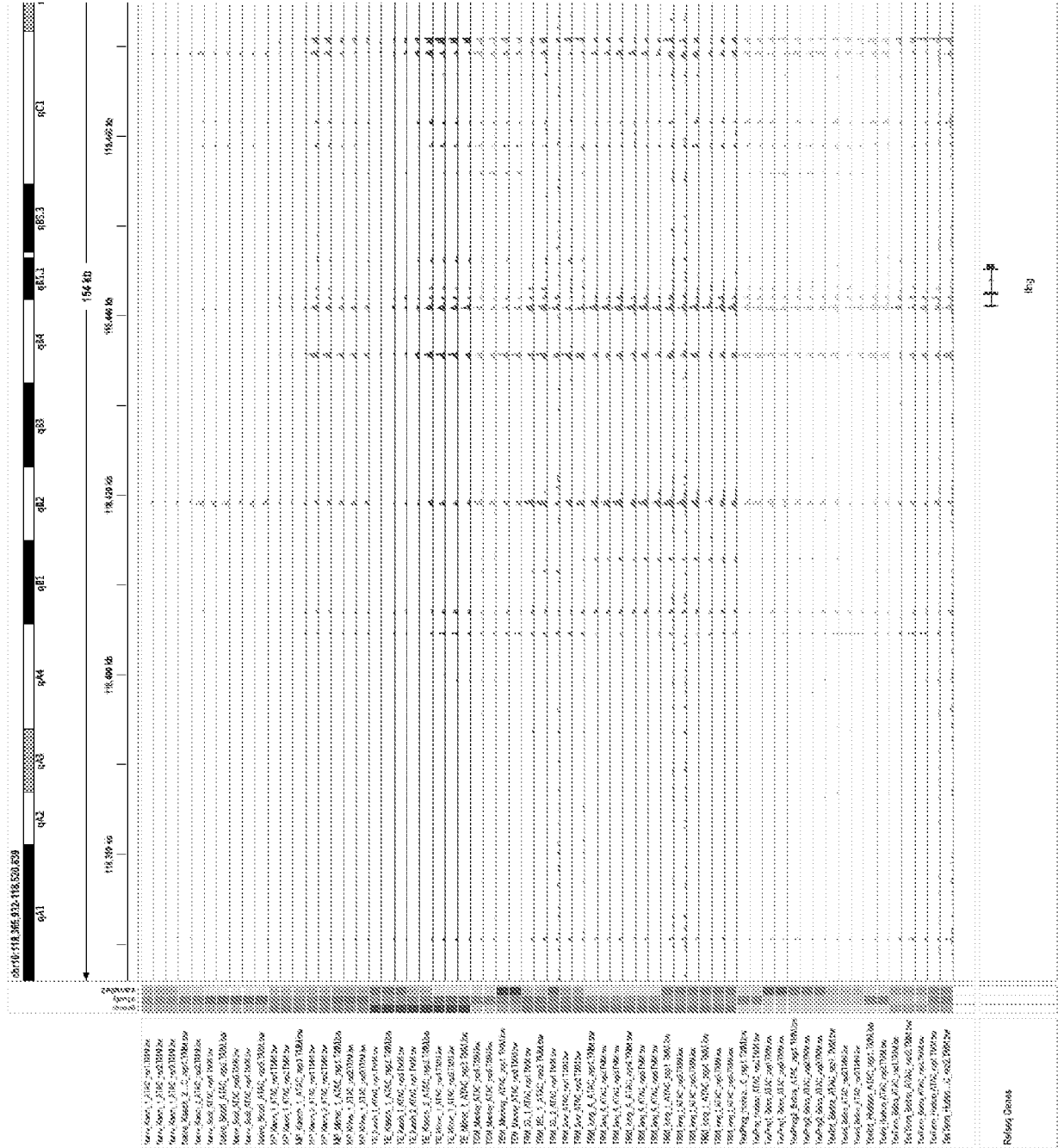


FIG. 5A

	Naive	MP	TE	T _{CM}	T _{EM}	T _{IM}	TeX _{prog}	TeX _{int}	TeX _{term}
# of specific TFs	34	59	61	30	26	44	67	66	58
# of unique TFs	17	19	18	16	13	17	20	14	15
Previously reported TFs examples	Tcf7 Lef1	Tcf7 Myb Runx2	Tbx21	Lef1	Prdm1 Runx2	Prdm1	Tcf7	Nfatc1	Nfatc1
			Irf4	Tcf7		Bhlhe40	Eomes	Prdm1	Prdm1
			Runx2	Runx2		Nr4a2	Nr4a2	Nr4a2	Nr4a2
			Runx1	Fosb		Hic1	Myb	Batf	Batf
New TFs examples	Rarg	E2f2 Gils3	Prdm1	Fosb	Fosb	Junb	Batf	Bhlhe40	Bhlhe40
						Fos	Jun	Jun	Jun
						Fosl2	Jdp2	Jdp2	Hic1
			E2f2	Fosl2		Nfil3	Irf9	Irf9	Jdp2
New, exclusive TFs examples	Hoxa7	Hoxb4	Atf6	Rara	Atf4	Atf4	Vax2	Vax2	Nfil3
						Vax2			Vax2
									Zscan20
			Ikzf3	Cux1		Snai1	Hey1	Sox8	Zfp143
								Arid3c	Stat3
									Arid3a

FIG. 6

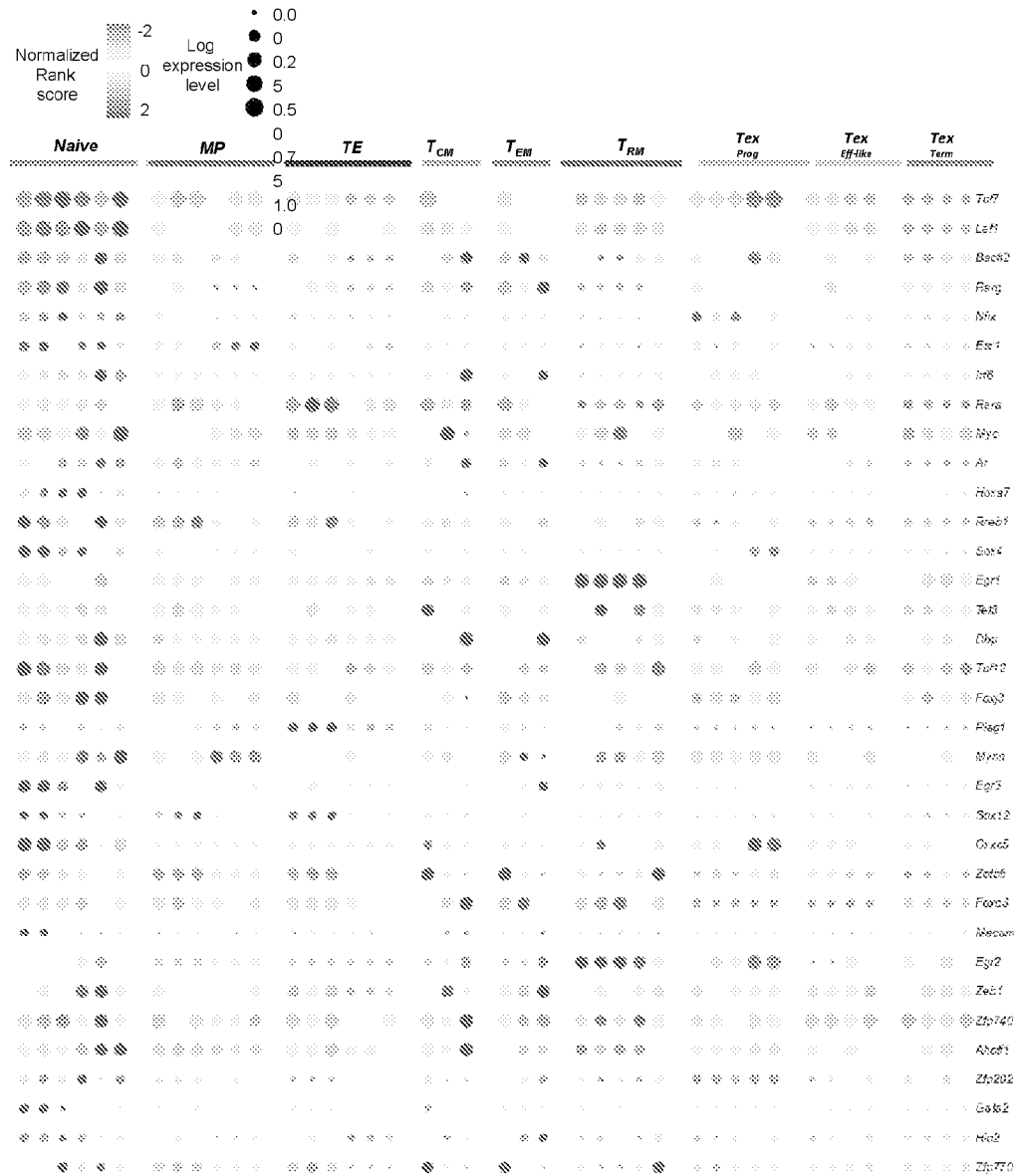


FIG. 7

[illegible]

FIG. 8

Naive	MP	TE	T_{CM}	T_{EM}	T_{RM}	Tex_{prog}	$Tex_{eff-like}$	Tex_{term}
***	***	***	*	*	*	***	***	E292
***	***	***	*	*	*	***	***	JN8
***	***	***	*	*	*	***	***	AP6
***	***	***	*	*	*	***	***	Rum1
***	***	***	*	*	*	***	***	Tax27
***	***	***	*	*	*	***	***	Plag1
***	***	***	*	*	*	***	***	Eras1
***	***	***	*	*	*	***	***	Arnt
***	***	***	*	*	*	***	***	Foxa1
***	***	***	*	*	*	***	***	Rbp1
***	***	***	*	*	*	***	***	Scx2
***	***	***	*	*	*	***	***	Prdm1
***	***	***	*	*	*	***	***	Sp4
***	***	***	*	*	*	***	***	Klf11
***	***	***	*	*	*	***	***	Zfp324
***	***	***	*	*	*	***	***	Vezf1
***	***	***	*	*	*	***	***	Rfx12
***	***	***	*	*	*	***	***	Naf1
***	***	***	*	*	*	***	***	Nr31
***	***	***	*	*	*	***	***	Npa
***	***	***	*	*	*	***	***	Smad3
***	***	***	*	*	*	***	***	Irf2
***	***	***	*	*	*	***	***	Rara
***	***	***	*	*	*	***	***	Irf3
***	***	***	*	*	*	***	***	Sp146
***	***	***	*	*	*	***	***	E2f8
***	***	***	*	*	*	***	***	Rfx3
***	***	***	*	*	*	***	***	Zfp109
***	***	***	*	*	*	***	***	Rarb
***	***	***	*	*	*	***	***	Zfp206
***	***	***	*	*	*	***	***	Hlx
***	***	***	*	*	*	***	***	Irf4
***	***	***	*	*	*	***	***	Zfp69
***	***	***	*	*	*	***	***	Qsox1
***	***	***	*	*	*	***	***	Shc1b
***	***	***	*	*	*	***	***	Qsox2
***	***	***	*	*	*	***	***	Nr1h2
***	***	***	*	*	*	***	***	Lmo1
***	***	***	*	*	*	***	***	Rorb2
***	***	***	*	*	*	***	***	Rorb2
***	***	***	*	*	*	***	***	Nfyc
***	***	***	*	*	*	***	***	Zfp2
***	***	***	*	*	*	***	***	Zfp200
***	***	***	*	*	*	***	***	Hes1
***	***	***	*	*	*	***	***	Lmo2
***	***	***	*	*	*	***	***	E2f7
***	***	***	*	*	*	***	***	Hic1
***	***	***	*	*	*	***	***	Eomes
***	***	***	*	*	*	***	***	Zfp5
***	***	***	*	*	*	***	***	Zfp51
***	***	***	*	*	*	***	***	Zfp7b
***	***	***	*	*	*	***	***	Lmo2
***	***	***	*	*	*	***	***	Nec
***	***	***	*	*	*	***	***	Sp100
***	***	***	*	*	*	***	***	Hmg20b
***	***	***	*	*	*	***	***	Ctcf
***	***	***	*	*	*	***	***	Scx1
***	***	***	*	*	*	***	***	Gata2
***	***	***	*	*	*	***	***	Zfp234
***	***	***	*	*	*	***	***	Rarb
***	***	***	*	*	*	***	***	Pbx2

FIG. 9A

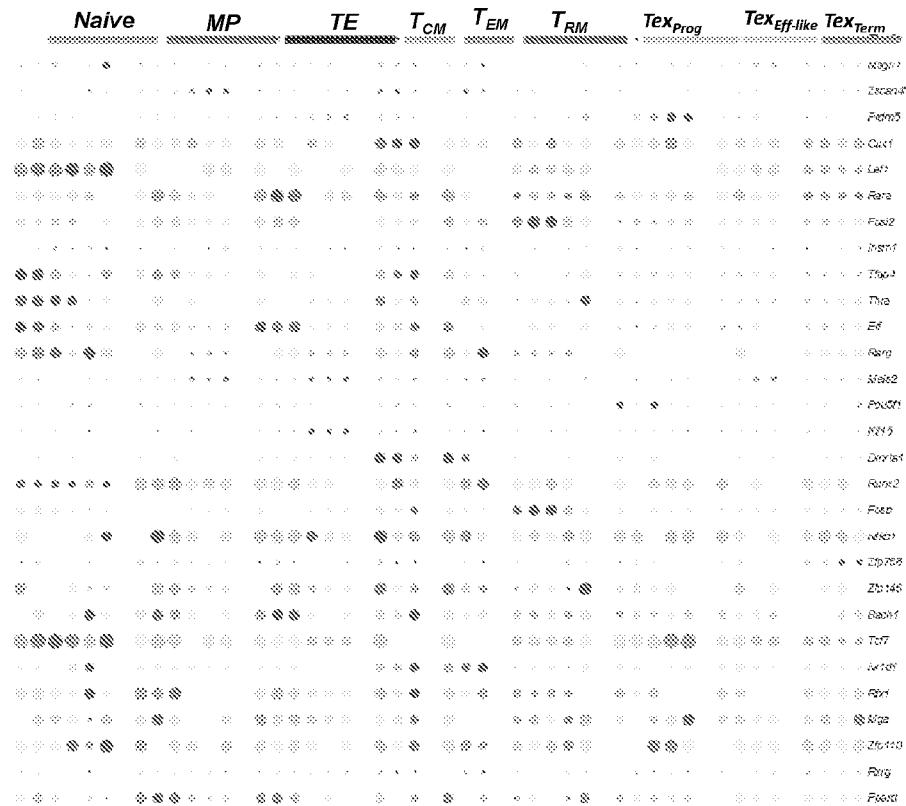


FIG. 9B

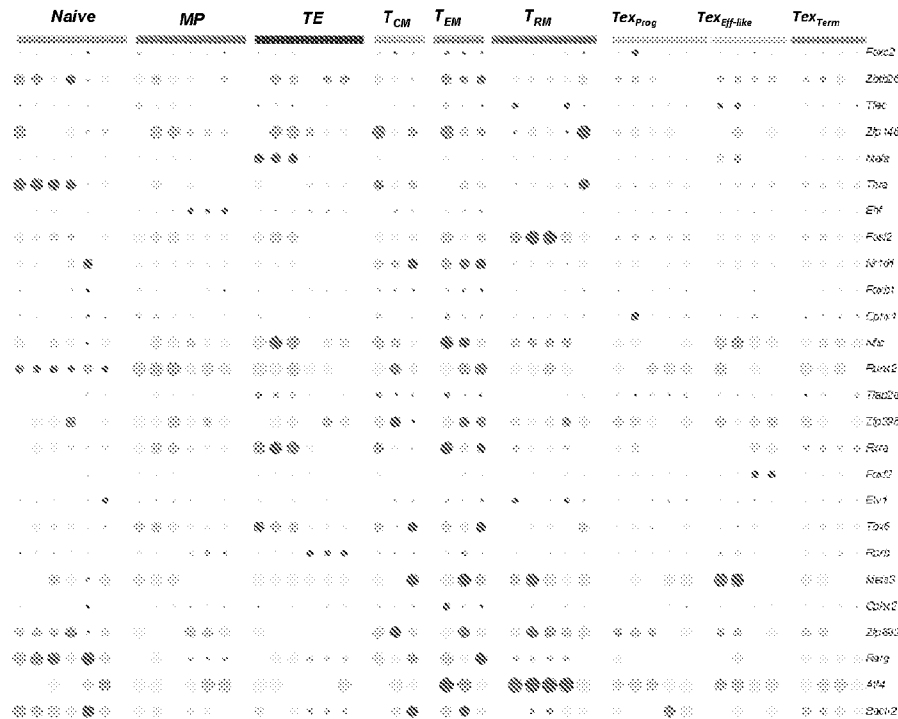


FIG. 10

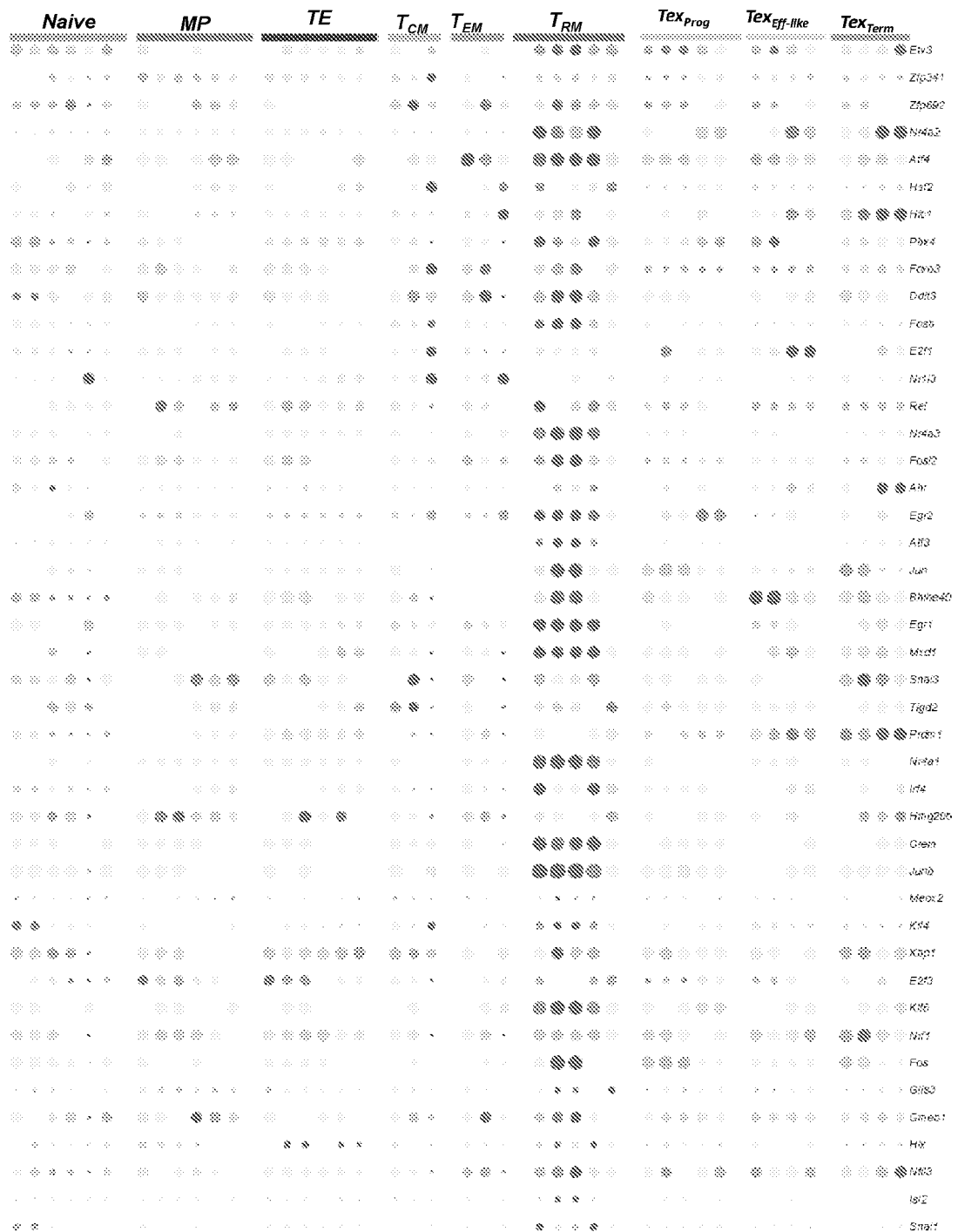


FIG. 12

<i>Naive</i>	<i>MP</i>	<i>TE</i>	T_{CM}	T_{EM}	T_{RM}	<i>Tex</i> <i>Prog</i>	<i>Tex</i> <i>Eff-like</i>	<i>Tex</i> <i>Term</i>	
...	...	...	...	...	...	...	...	...	v4t2
...	...	...	...	...	...	...	...	...	Chr65t1
...	...	...	...	...	...	...	...	...	Pax7
...	...	...	...	...	...	...	...	...	Lut34
...	...	...	...	...	...	...	...	...	Zbtb22
...	...	...	...	...	...	...	...	...	Ppox1
...	...	...	...	...	...	...	...	...	Sat3
...	...	...	...	...	...	...	...	...	Krt6
...	...	...	...	...	...	...	...	...	Zfp41
...	...	...	...	...	...	...	...	...	Map
...	...	...	...	...	...	...	...	...	Spic
...	...	...	...	...	...	...	...	...	mtf4
...	...	...	...	...	...	...	...	...	Foxn3
...	...	...	...	...	...	...	...	...	Teb
...	...	...	...	...	...	...	...	...	Tbx21
...	...	...	...	...	...	...	...	...	Foxp9
...	...	...	...	...	...	...	...	...	Meis
...	...	...	...	...	...	...	...	...	Batf
...	...	...	...	...	...	...	...	...	Foxp2
...	...	...	...	...	...	...	...	...	Wnt
...	...	...	...	...	...	...	...	...	E2f2
...	...	...	...	...	...	...	...	...	Zfp567
...	...	...	...	...	...	...	...	...	Gli1
...	...	...	...	...	...	...	...	...	Klf2c
...	...	...	...	...	...	...	...	...	Zfp104
...	...	...	...	...	...	...	...	...	Zfp32
...	...	...	...	...	...	...	...	...	v5
...	...	...	...	...	...	...	...	...	Enohe40
...	...	...	...	...	...	...	...	...	Rarb
...	...	...	...	...	...	...	...	...	Jmy2
...	...	...	...	...	...	...	...	...	Klf
...	...	...	...	...	...	...	...	...	Wnt3
...	...	...	...	...	...	...	...	...	E2f8
...	...	...	...	...	...	...	...	...	Wnt
...	...	...	...	...	...	...	...	...	E2f3
...	...	...	...	...	...	...	...	...	Jmy2
...	...	...	...	...	...	...	...	...	Ldhr1
...	...	...	...	...	...	...	...	...	Wnt
...	...	...	...	...	...	...	...	...	Enohe
...	...	...	...	...	...	...	...	...	Foxp2
...	...	...	...	...	...	...	...	...	Hes2
...	...	...	...	...	...	...	...	...	Klf1
...	...	...	...	...	...	...	...	...	Dn75
...	...	...	...	...	...	...	...	...	Zfp528
...	...	...	...	...	...	...	...	...	Zfp128
...	...	...	...	...	...	...	...	...	Hmg30a
...	...	...	...	...	...	...	...	...	M276
...	...	...	...	...	...	...	...	...	Aref2c
...	...	...	...	...	...	...	...	...	Klf2
...	...	...	...	...	...	...	...	...	Ctcf2
...	...	...	...	...	...	...	...	...	v5
...	...	...	...	...	...	...	...	...	Foxp1
...	...	...	...	...	...	...	...	...	E2f8
...	...	...	...	...	...	...	...	...	E2f7
...	...	...	...	...	...	...	...	...	Enohe2
...	...	...	...	...	...	...	...	...	Pf1
...	...	...	...	...	...	...	...	...	E2f1
...	...	...	...	...	...	...	...	...	Lap2
...	...	...	...	...	...	...	...	...	Zfp309
...	...	...	...	...	...	...	...	...	Klf2
...	...	...	...	...	...	...	...	...	Klf1
...	...	...	...	...	...	...	...	...	Klf2
...	...	...	...	...	...	...	...	...	Klf
...	...	...	...	...	...	...	...	...	Ctcf2
...	...	...	...	...	...	...	...	...	Pax4
...	...	...	...	...	...	...	...	...	Tsp1

FIG. 13

Naive	MP	TE	T_{CM}	T_{EM}	T_{RM}	Tex Prg	Tex Eff-like	Tex Term

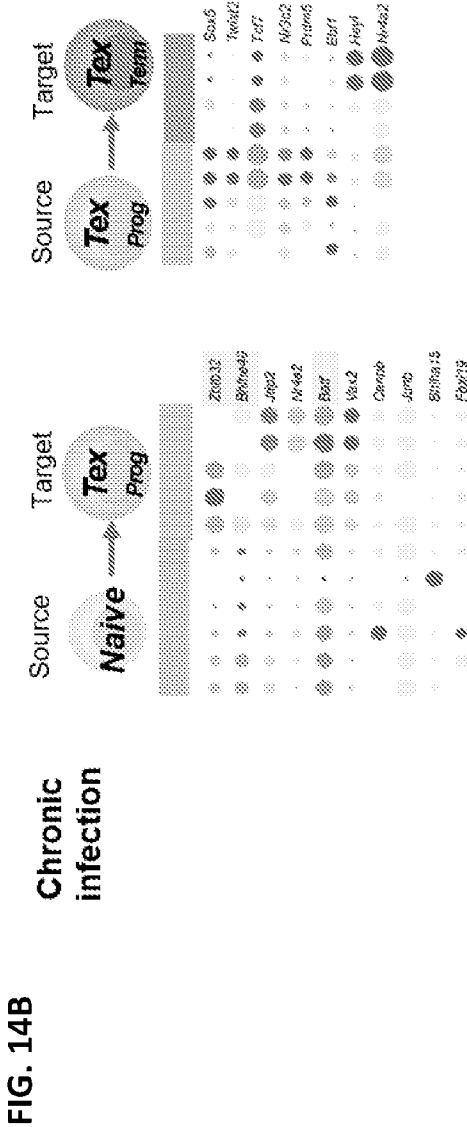
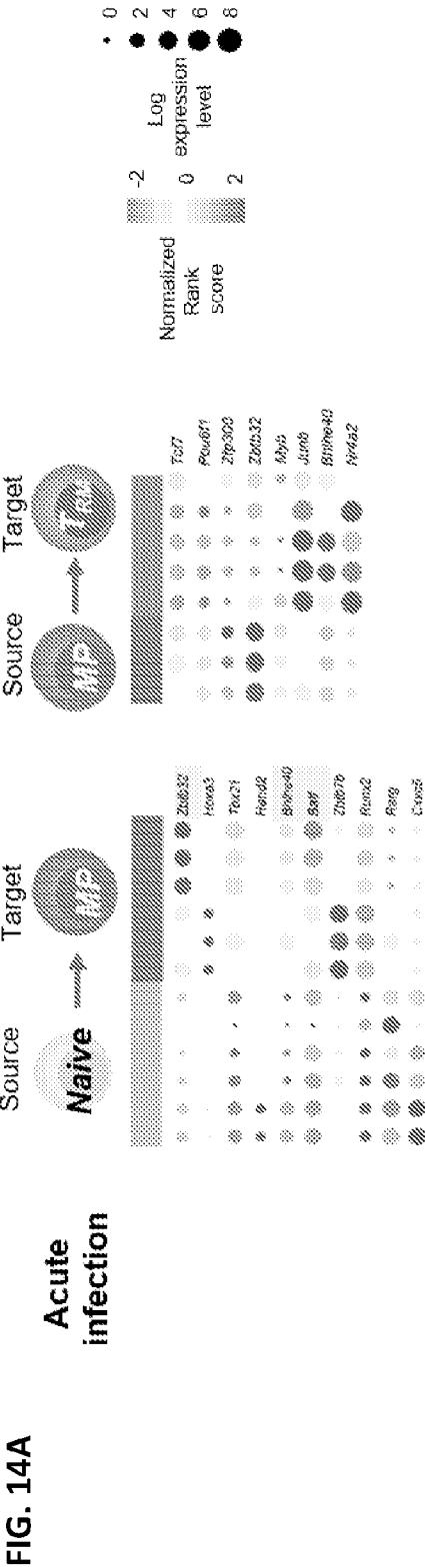


FIG. 15A

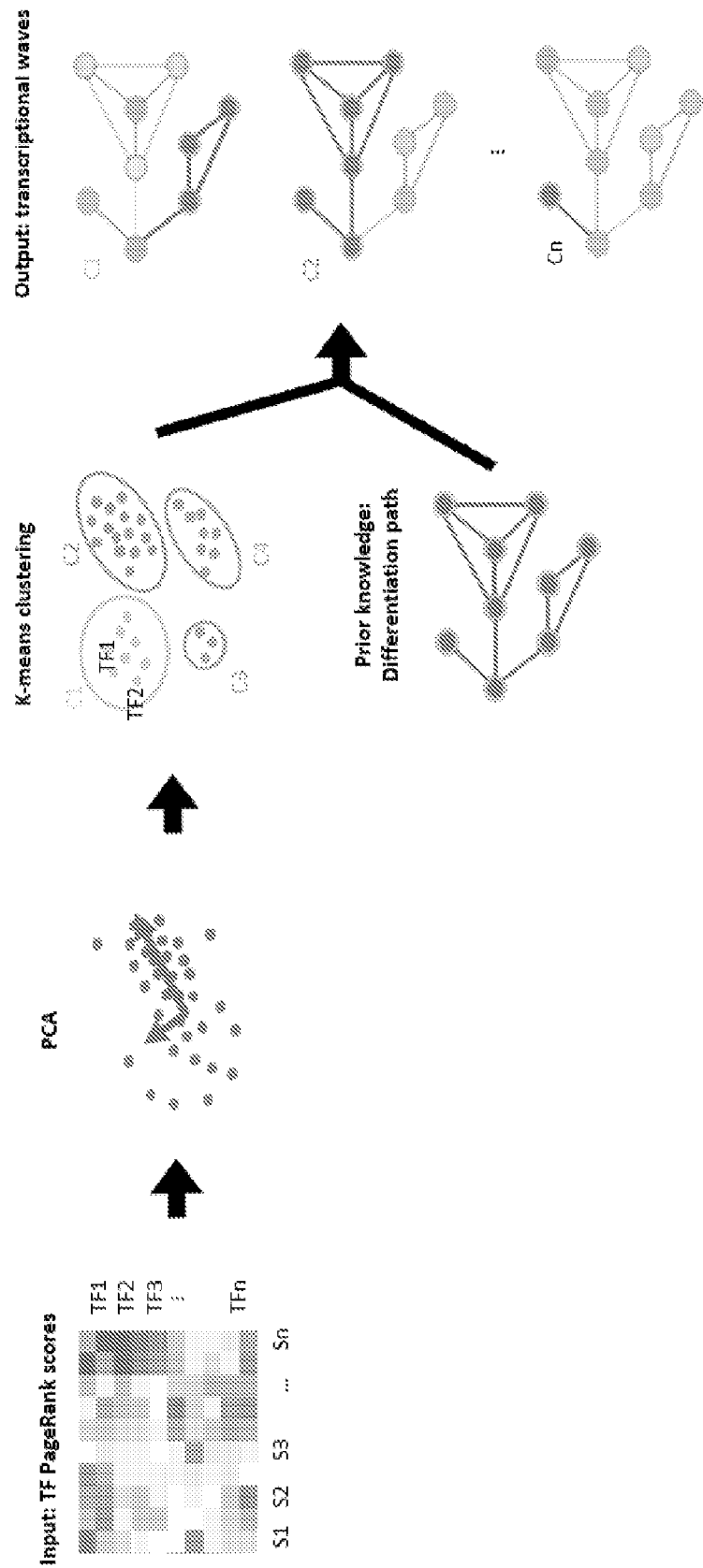


FIG. 15B

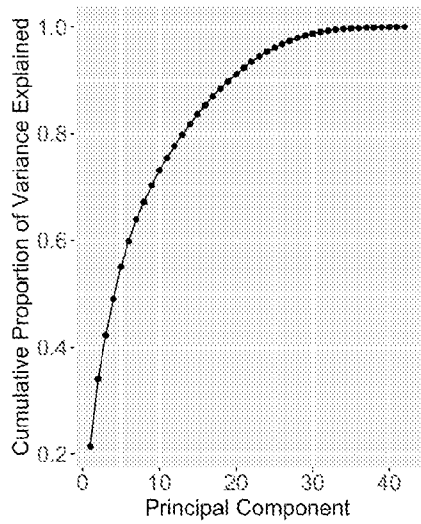
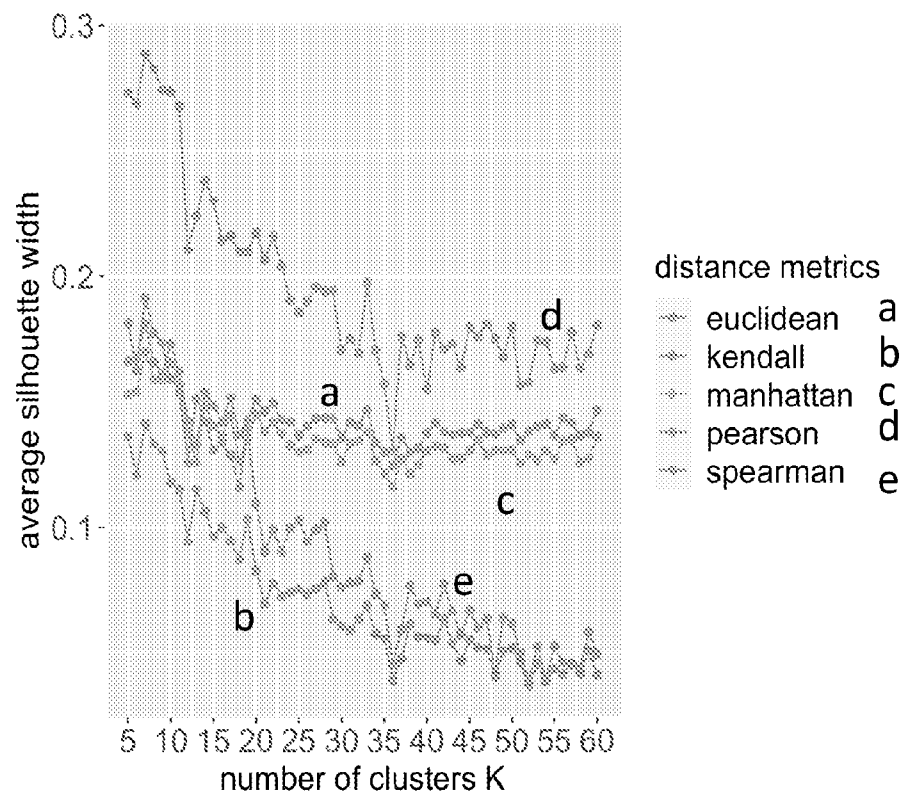


FIG. 15C



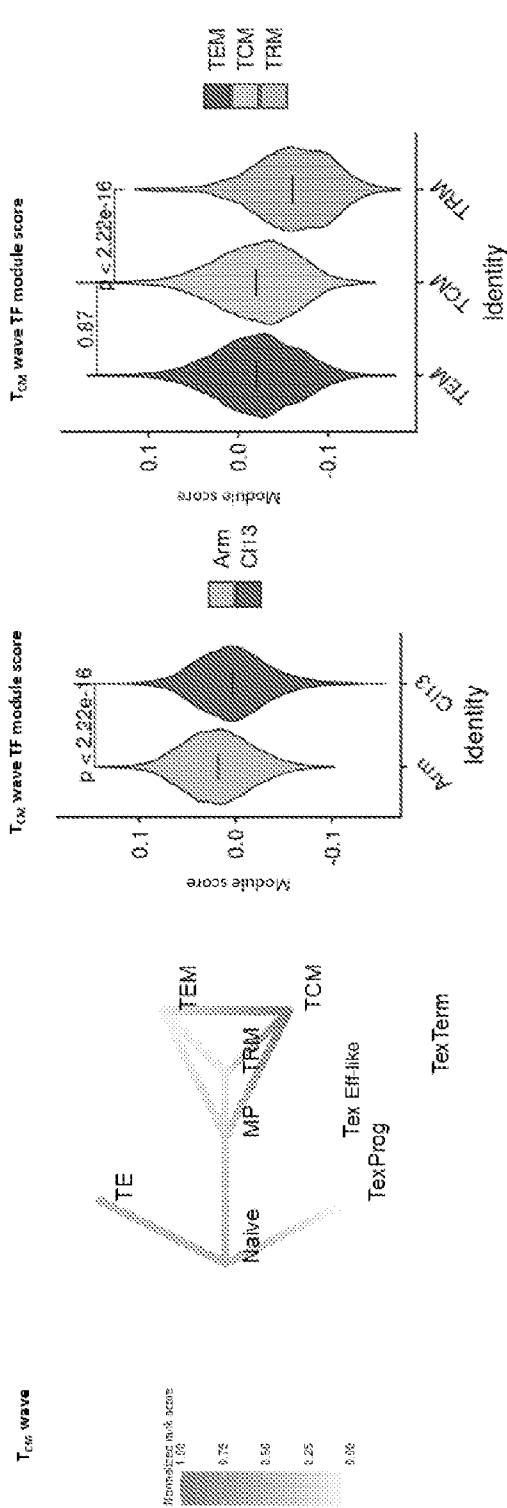
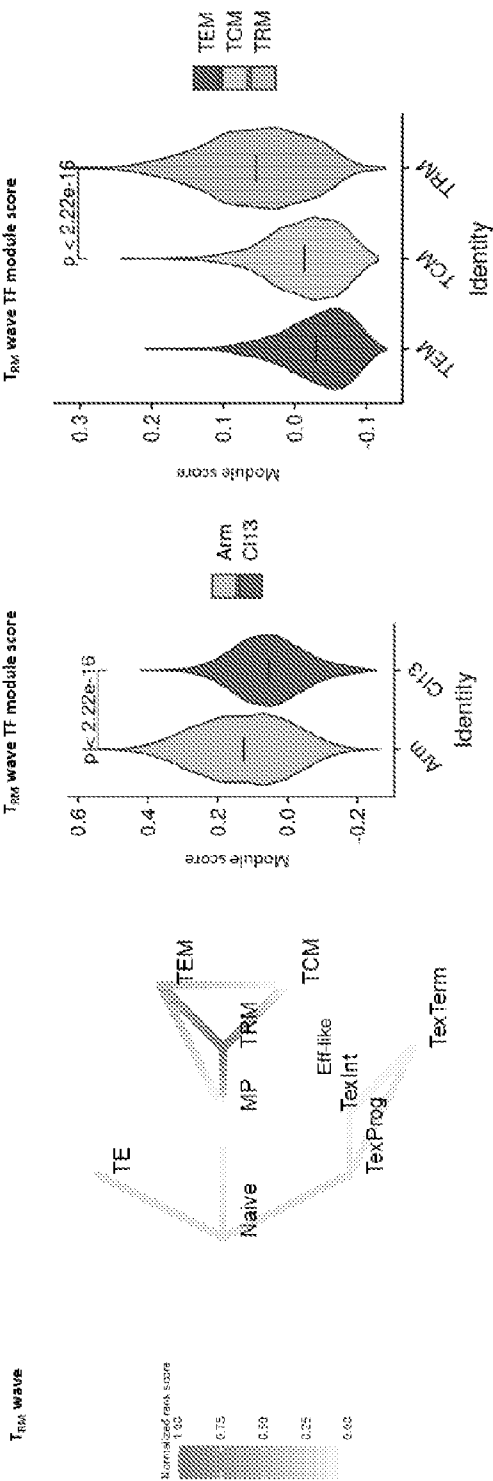


FIG. 16C

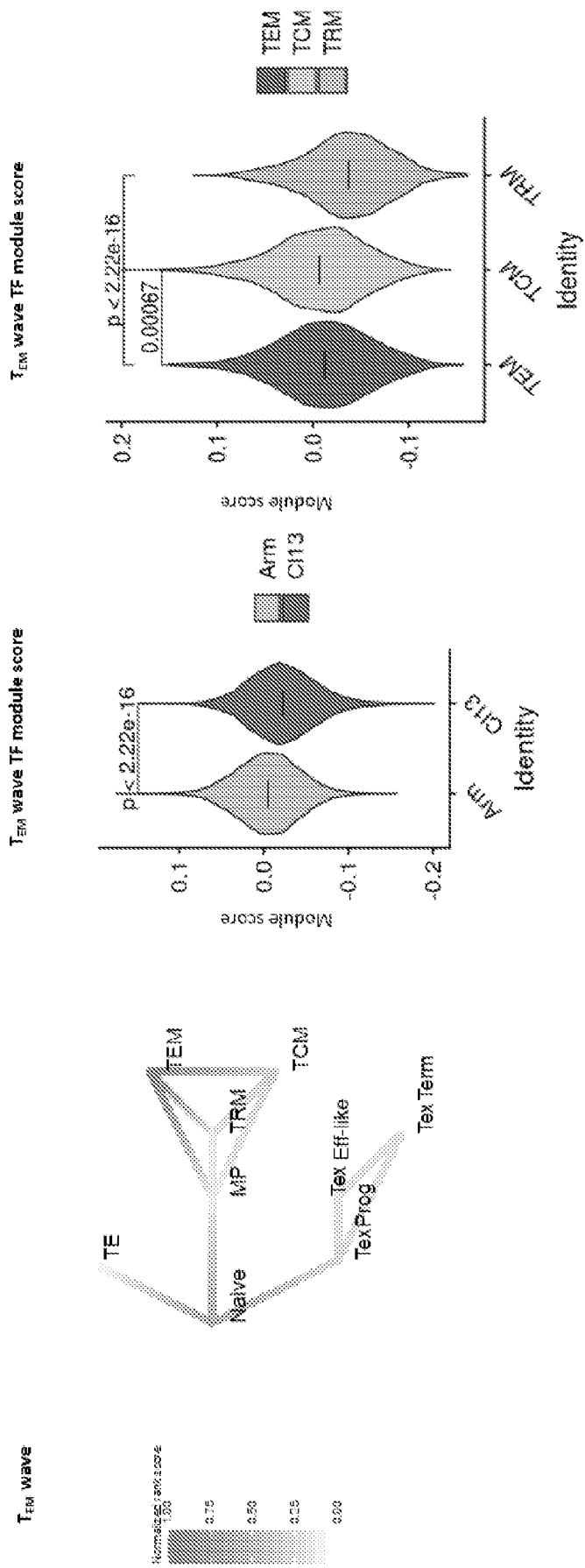


FIG. 17A

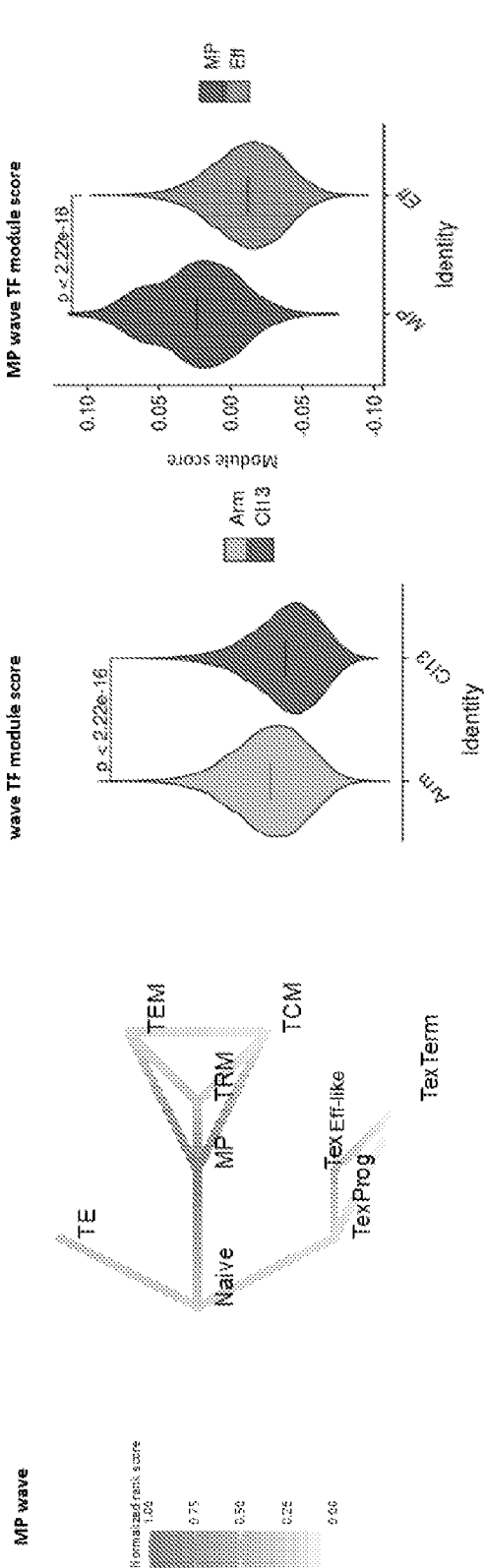


FIG. 17B

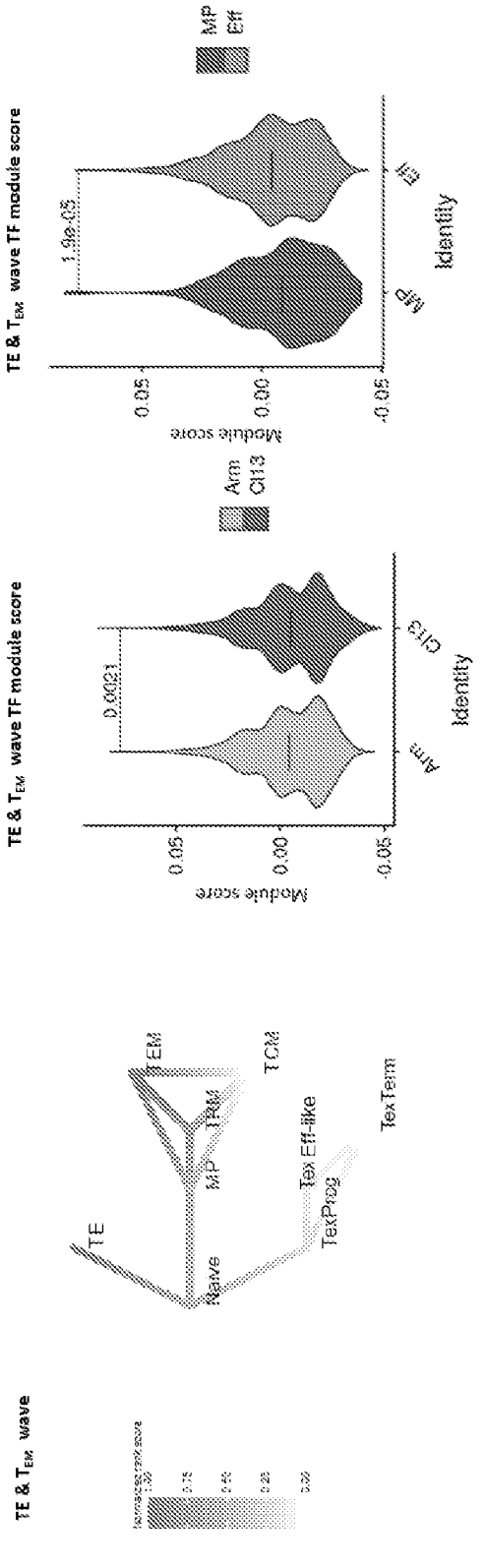


FIG. 17C

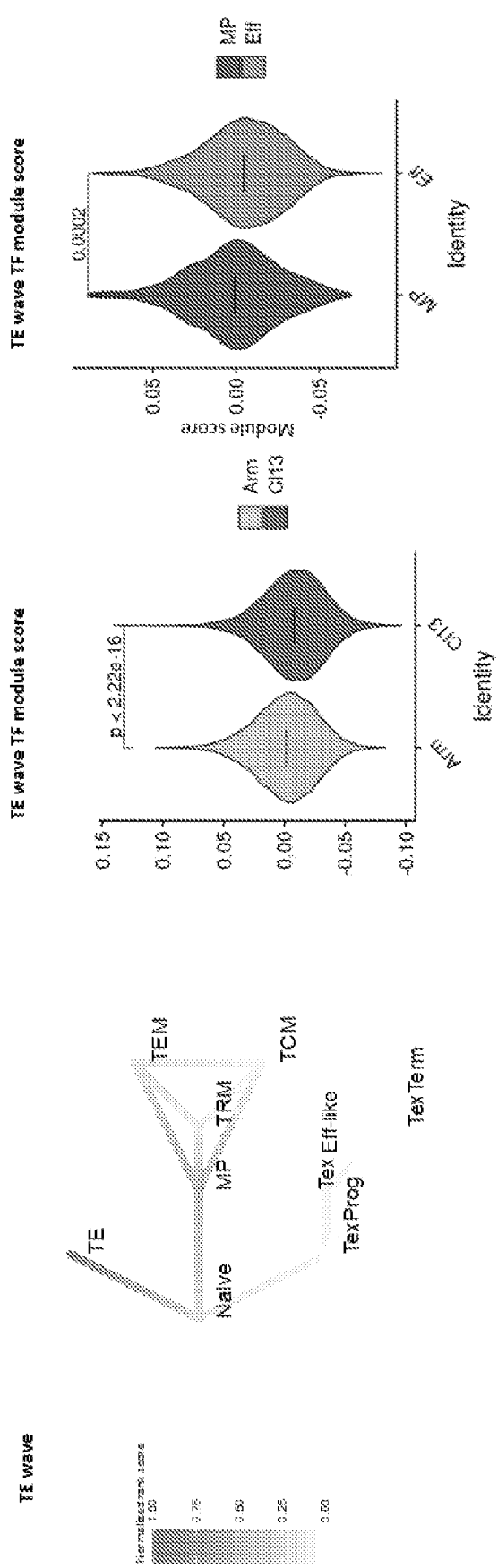


FIG. 18

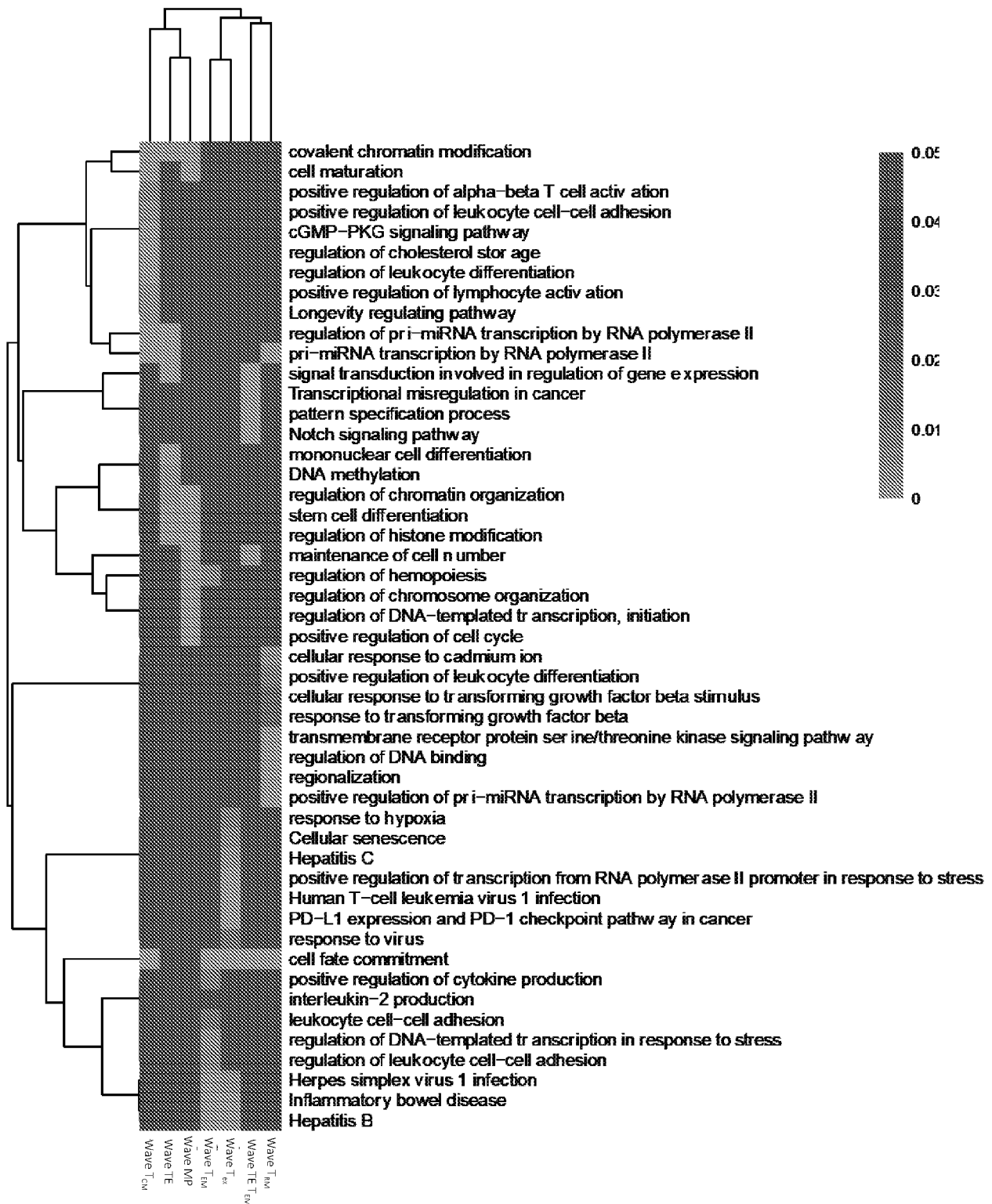


FIG. 19A

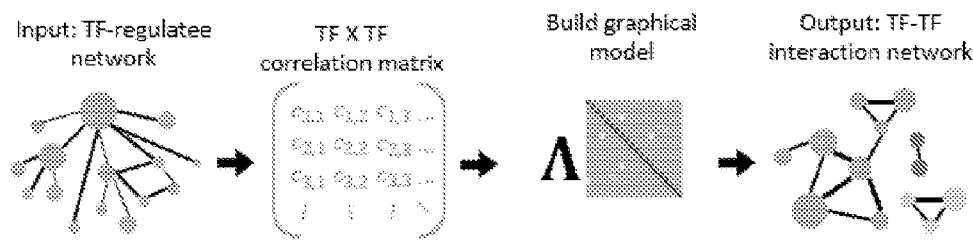


FIG. 19B

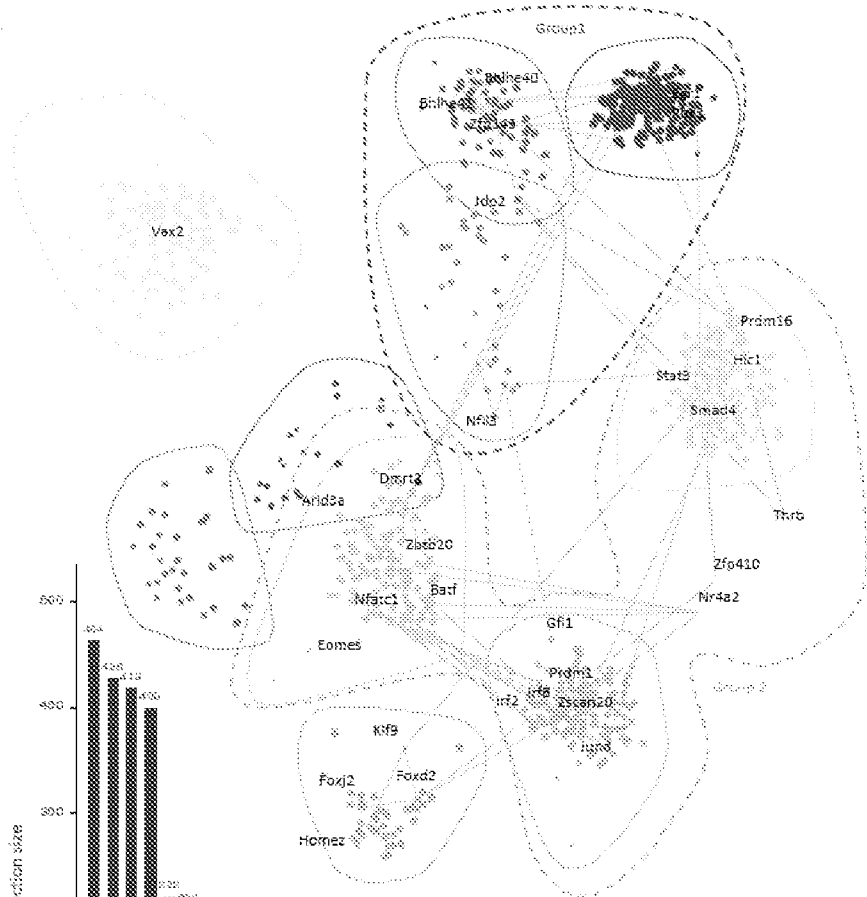


FIG. 19C

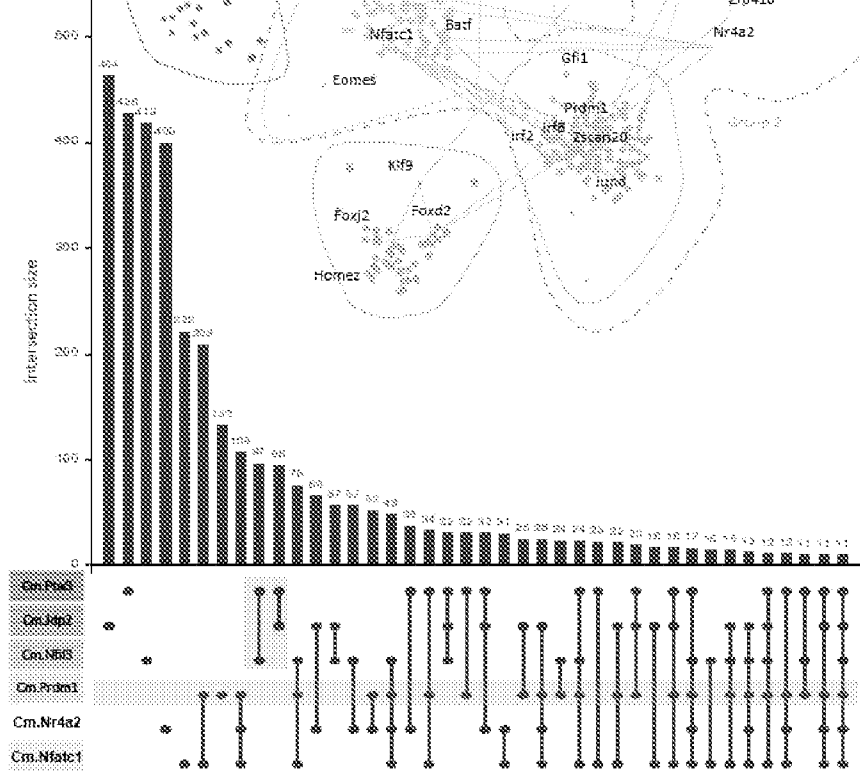


FIG. 19E

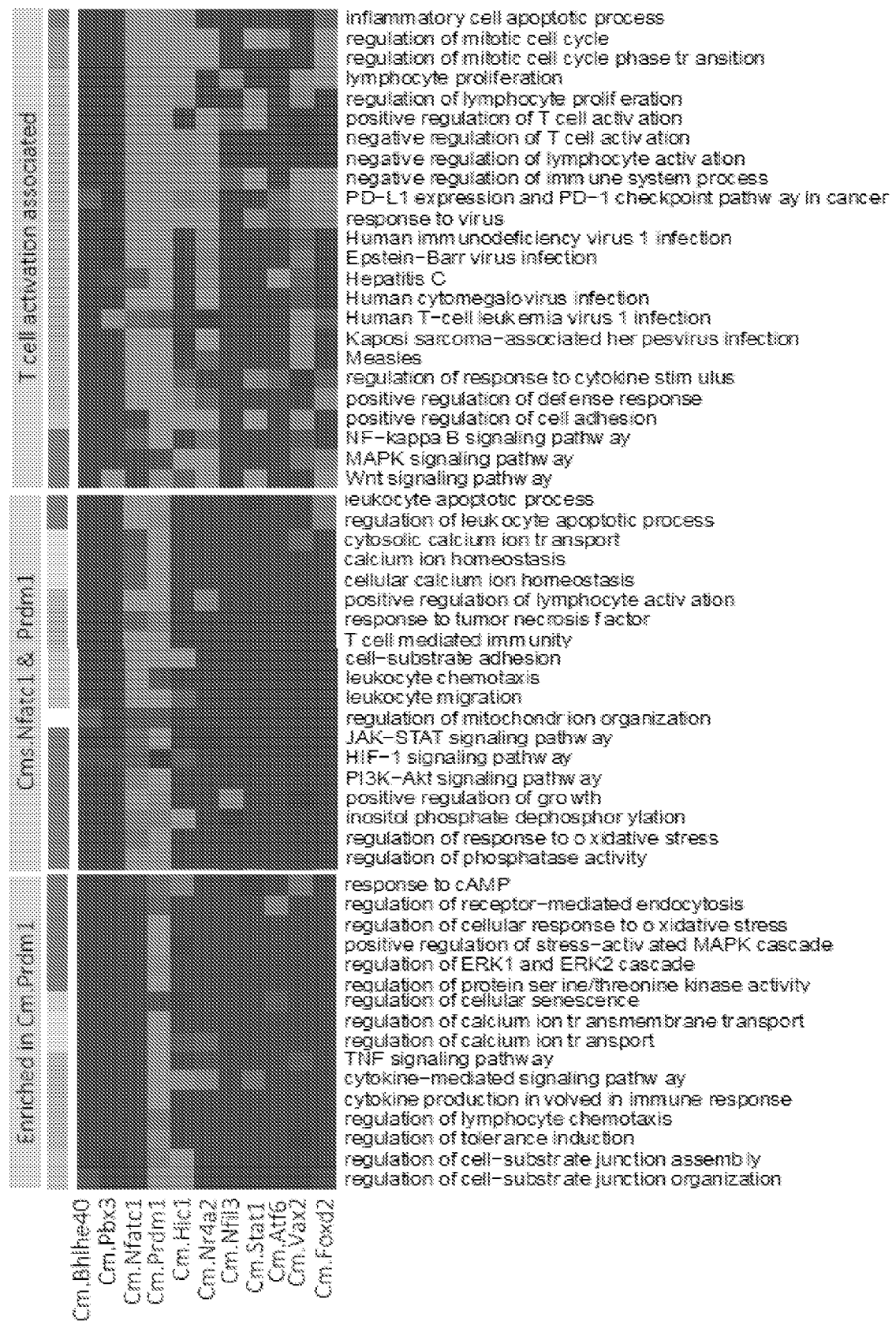


FIG. 19F

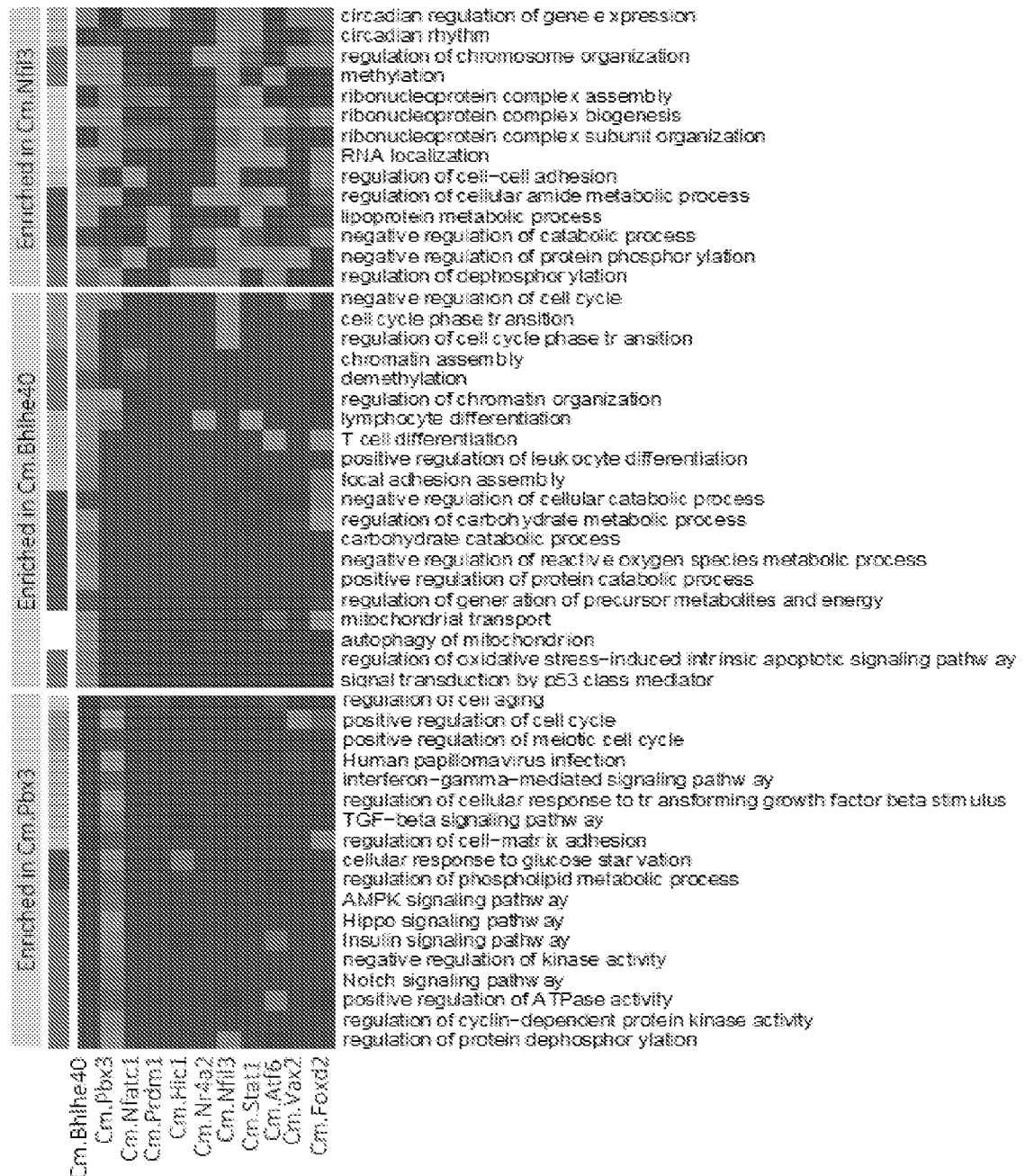


FIG. 19G

Communities	TF members	Shared pathways	Common or pathways shared by major communities		
Cm. Pbx3	Pbx3 Egr1 Hnf1a Zfp523 E2f4 Tfdp1 Nr1f1 E2f6 Etv5	Chromatin modification	Previously reported: - Chromatin remodeling - Histon modification - Dephosphorylation - Negative regulation of phosphorylation	New pathways: - Catabolic process - Cellular carbohydrate metabolic process	
Cm. Bhlhe40	Bhlhe40 Jdp2 Zfp143 Xbp1 Tfeb Mxd1	Chromosome organization			
Cm. Nfil3	Nfil3 Cebp b Atf4 Etv3	Chromatin organization			
Cm. Hic1	Hic1 Stat3 Prdm4 Snai3 Zfp324 Trp73 Prdm15 Smad4	Calcium ion homeostasis			
Cm. Prdm1	Zscan20 Prdm1 Gfi1 Jun d Irf8	Calcium ion transfer	PD-L1-PD-1 pathway Response to TGF beta FoxO signaling pathway Cell cycle regulation	Response to hypoxia	
Cm. Nr4a2	Nr4a2 Zfp410 Nr13	Response to TNF or TNF signaling			Response to oxidative stress
Cm. Nfat1	Nfatc1 Batf Eomes Arid3a				Hepatitis B Herpes simplex virus 1

FIG. 20

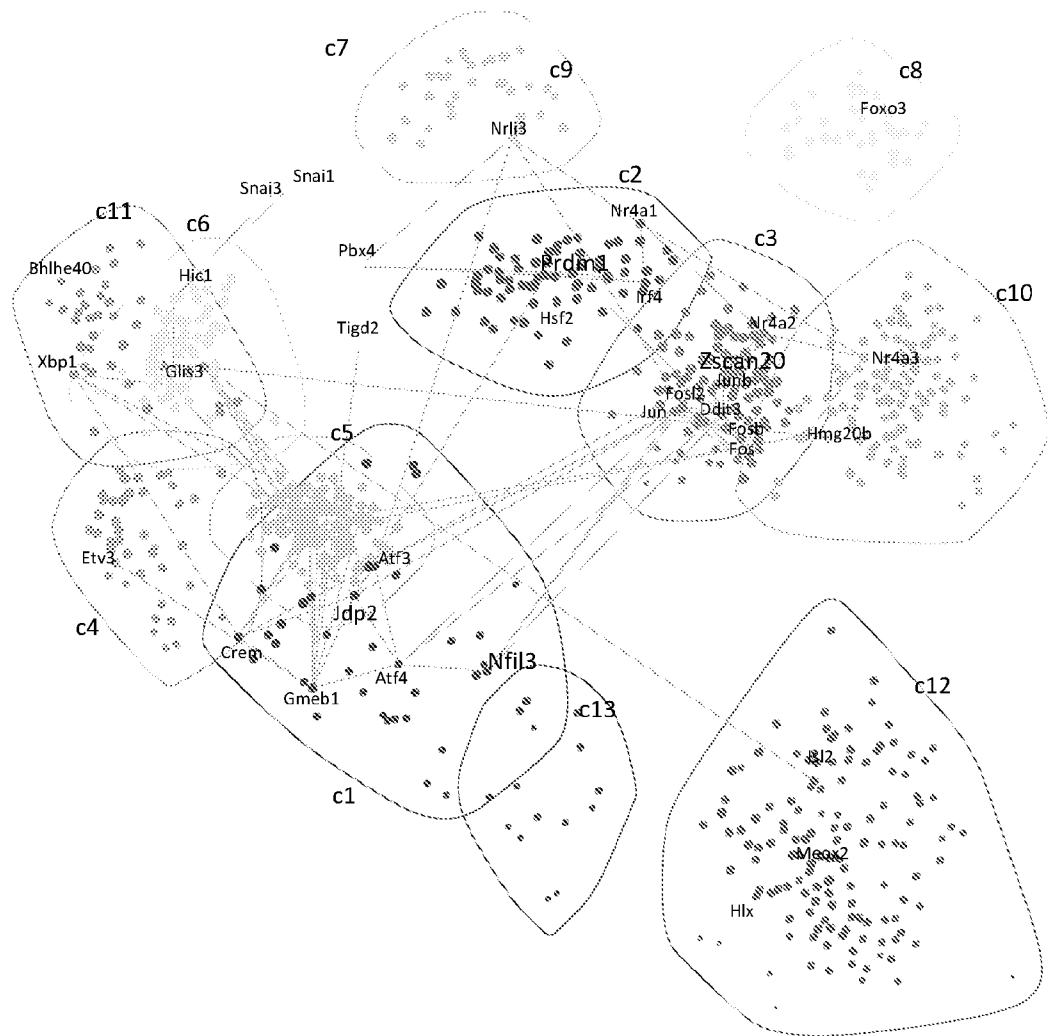


FIG. 21A *Prdm1* TF-TF interaction in *Tex*_{Term}

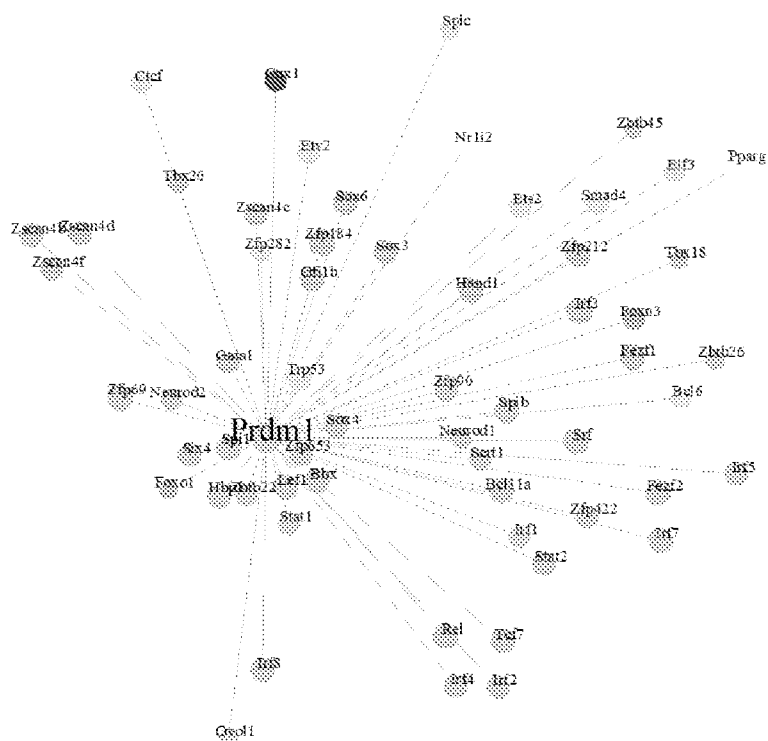
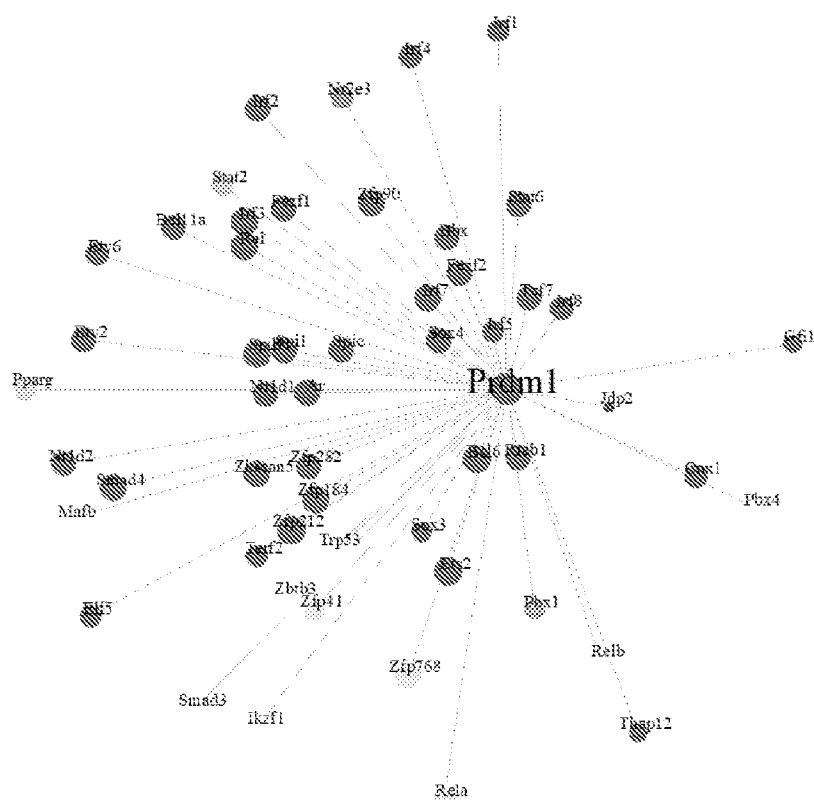
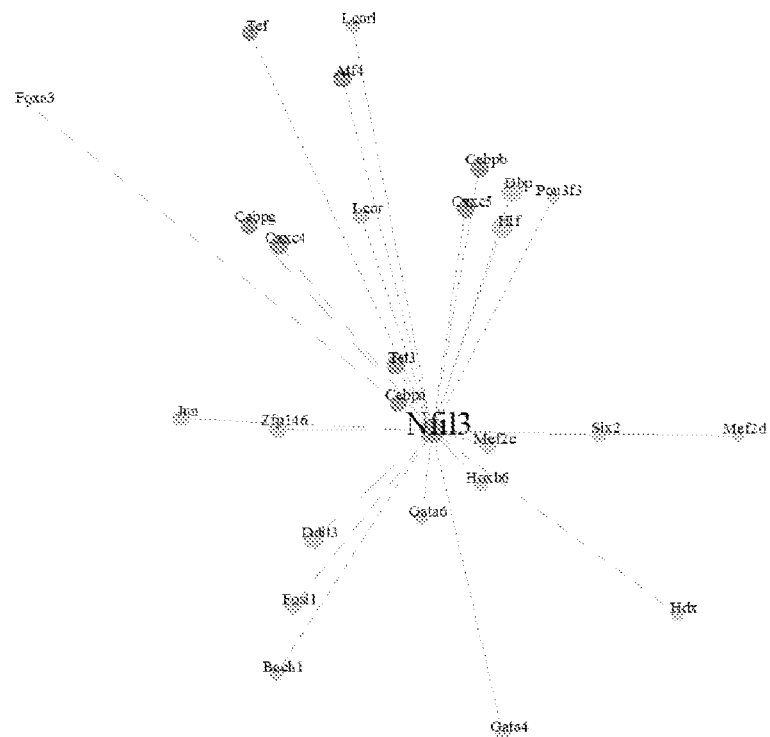


FIG. 21B *Prdm1* TF-TF interaction in T_{RM}



Nfil3 TF-TF interaction in Tex_{Term}



Nfil3 TF-TF interaction in T_{RM}

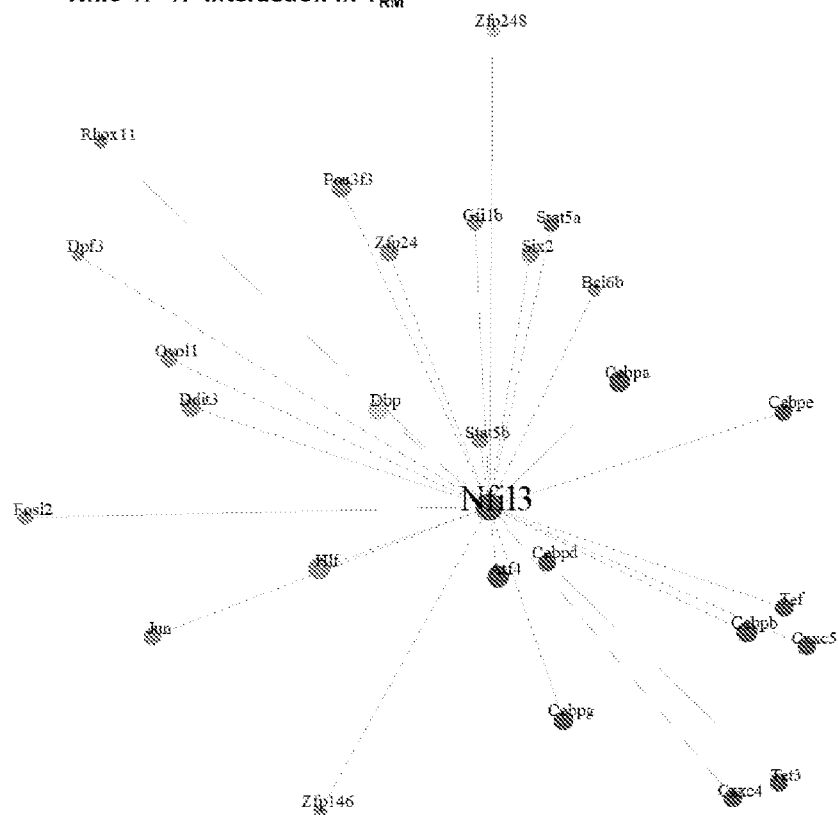


FIG. 21E

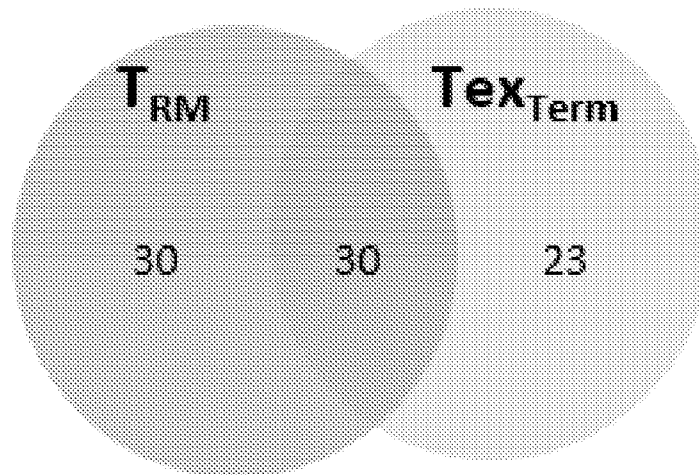
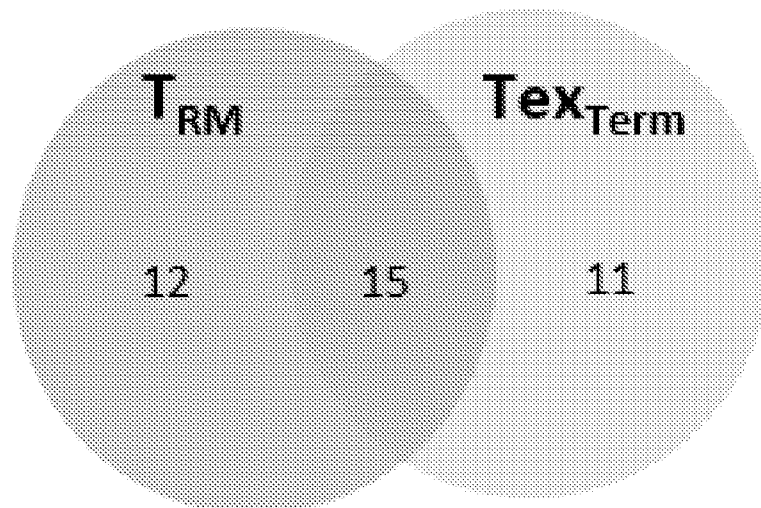
Prdm1 neighbor interaction partners

FIG. 21f

Nfil3 neighbor interaction partners

[illegible]

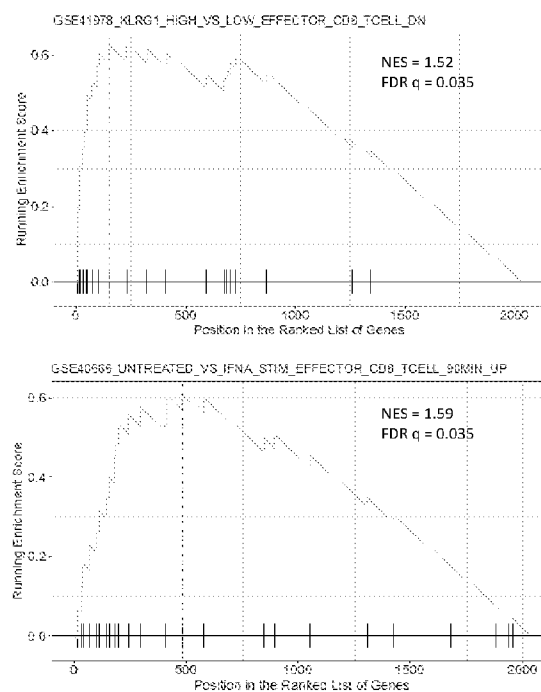
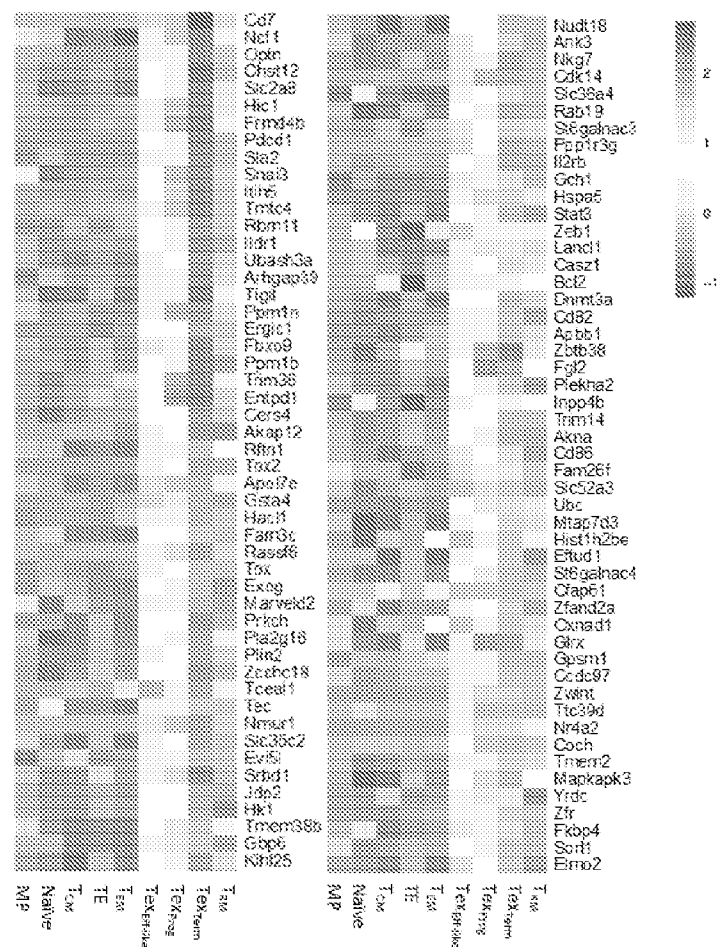


FIG. 23C

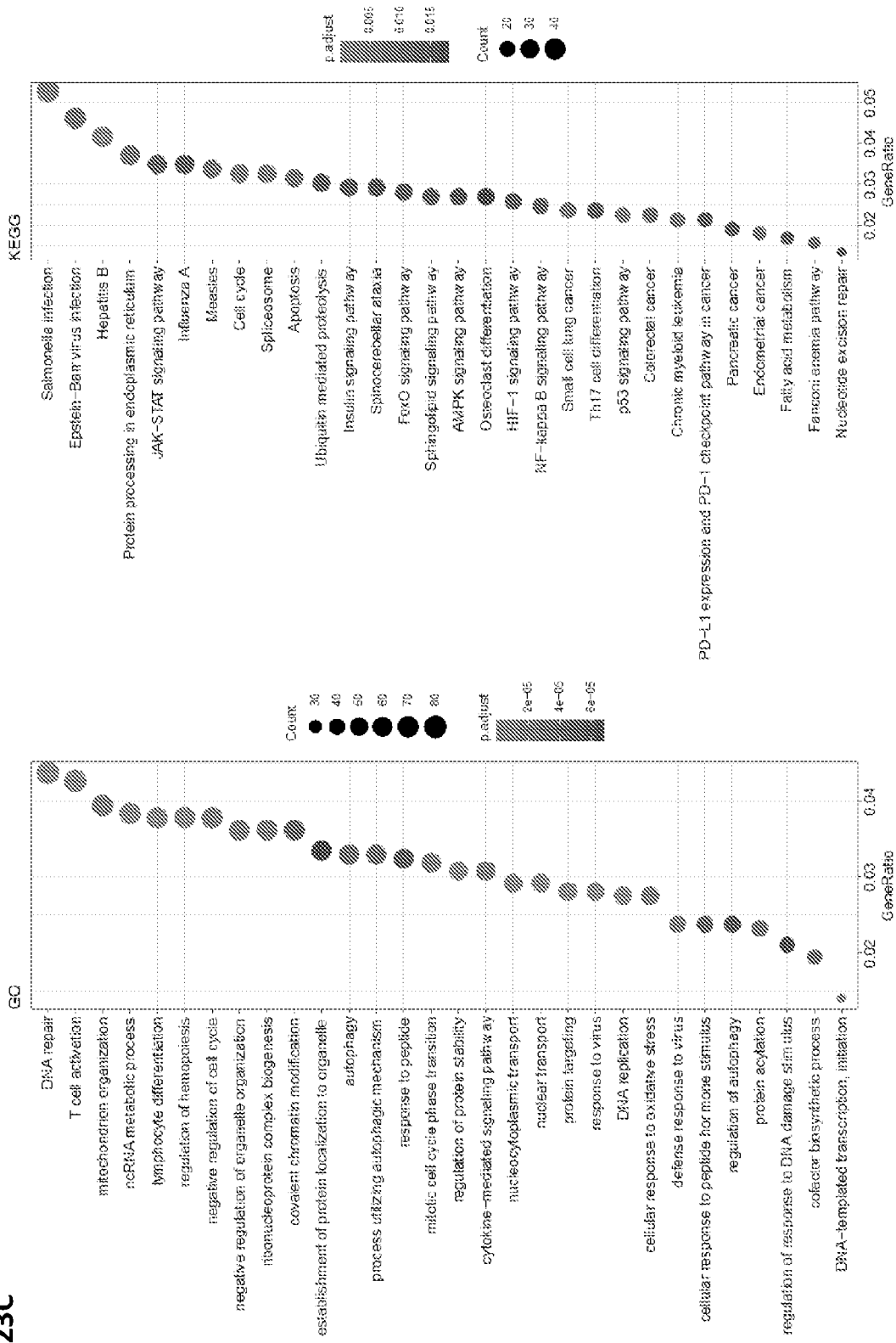


FIG. 24A

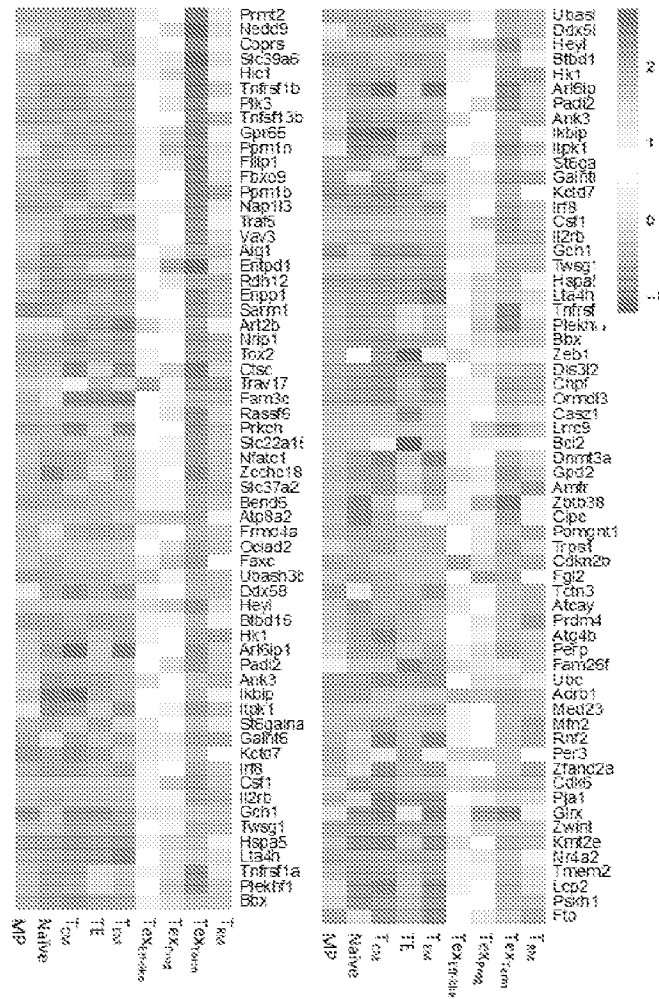


FIG. 24B

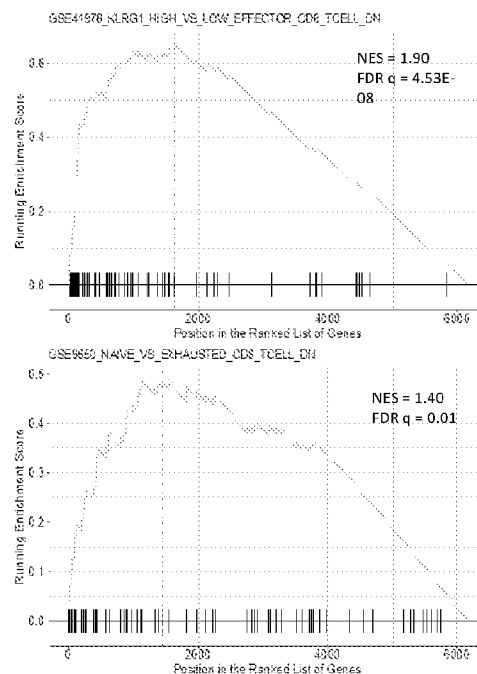


FIG. 24C

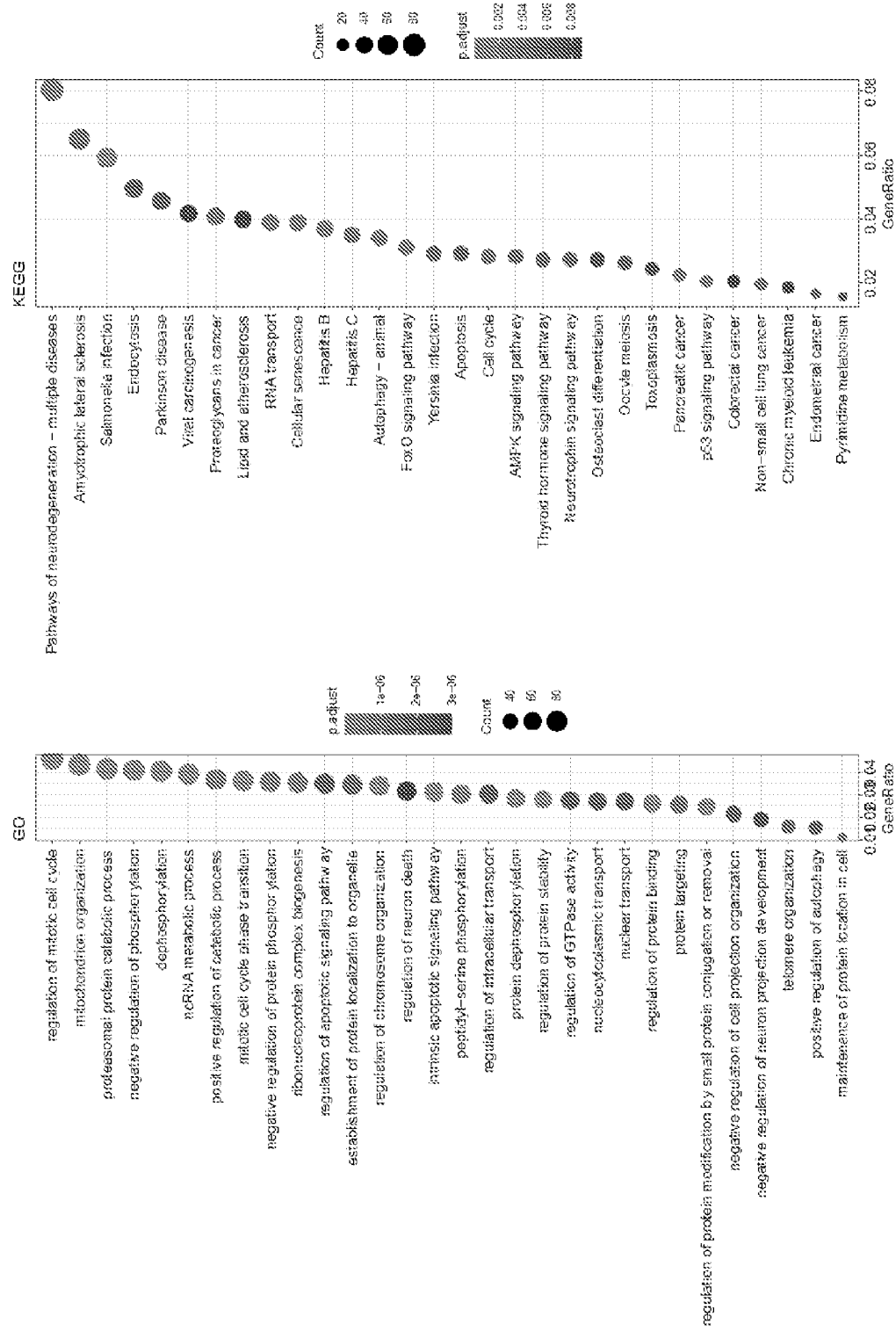


FIG. 25A

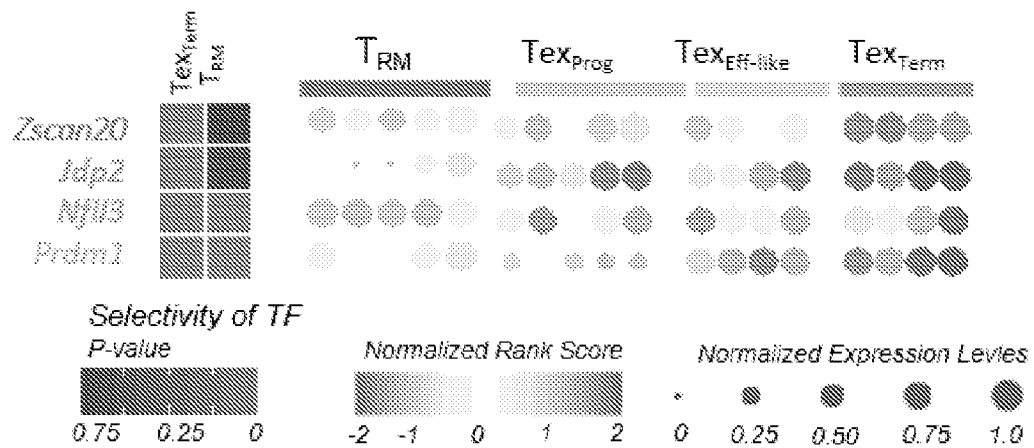


FIG. 25B

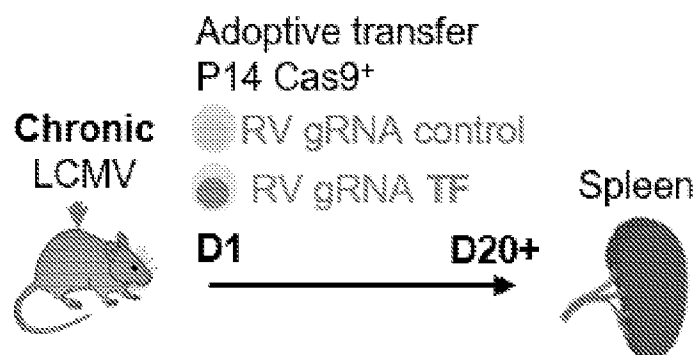


FIG. 25C

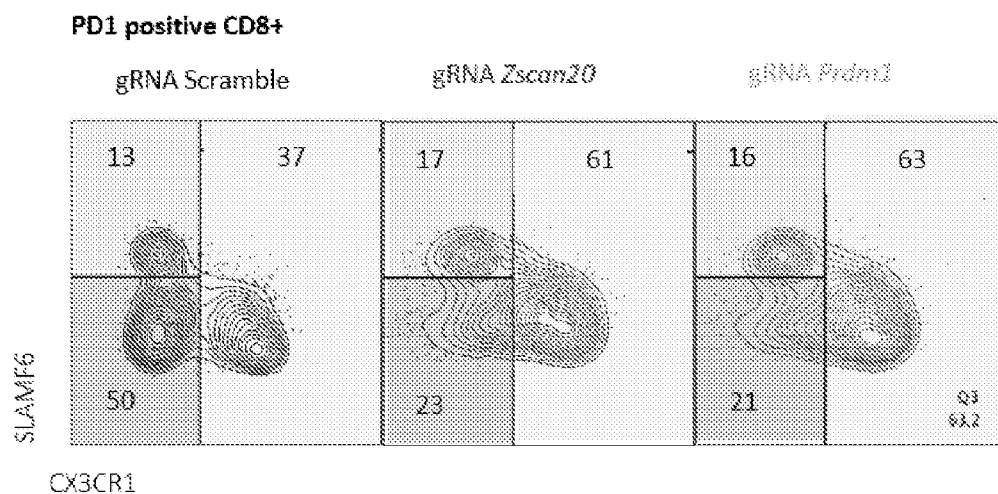


FIG. 25D

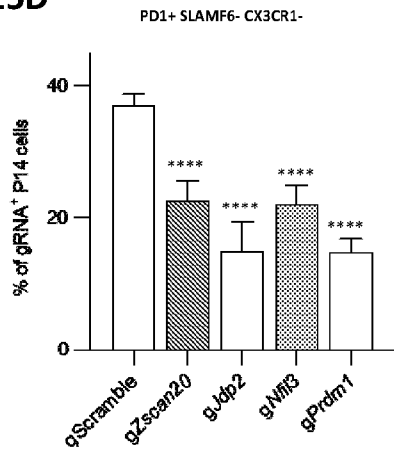


FIG. 25E

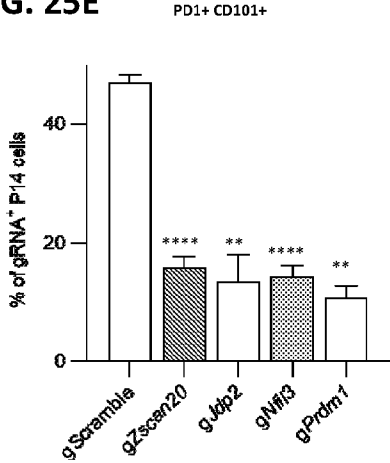


FIG. 25F

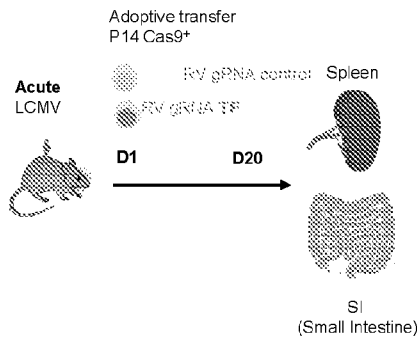


FIG. 25G

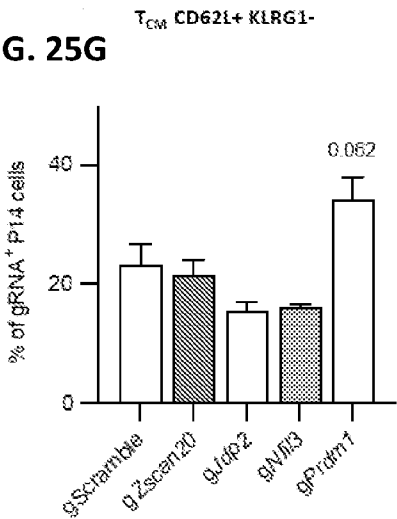


FIG. 25H

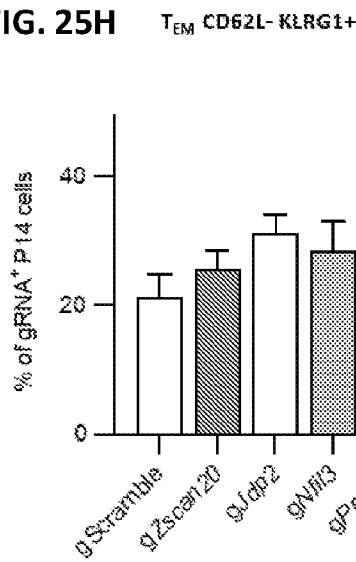


FIG. 25I

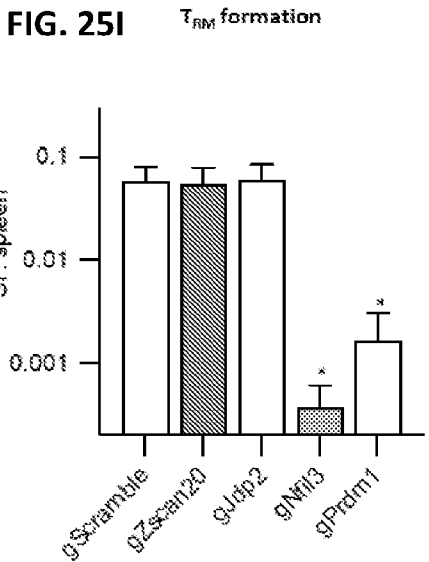


FIG. 26A

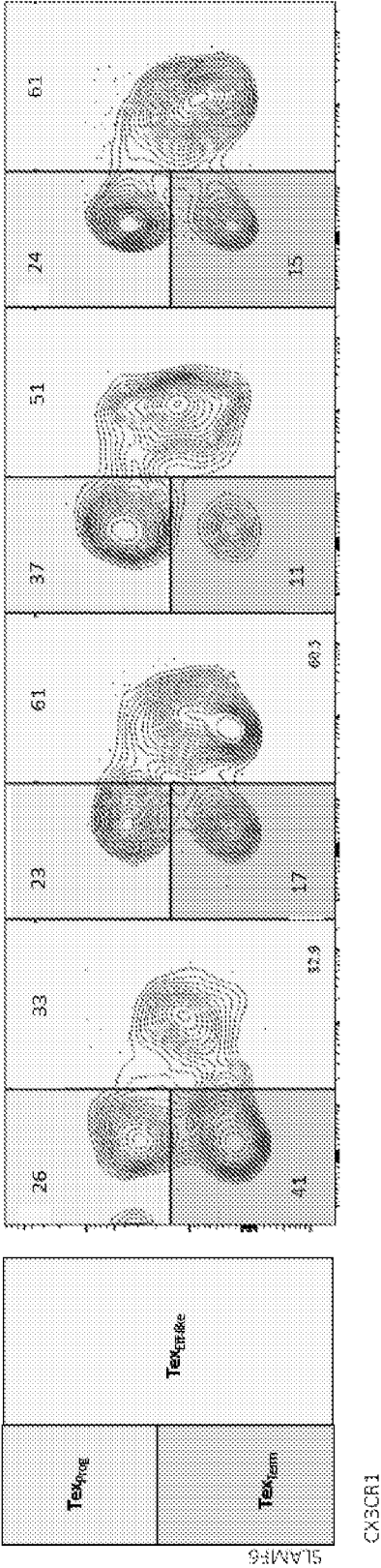


FIG. 26B

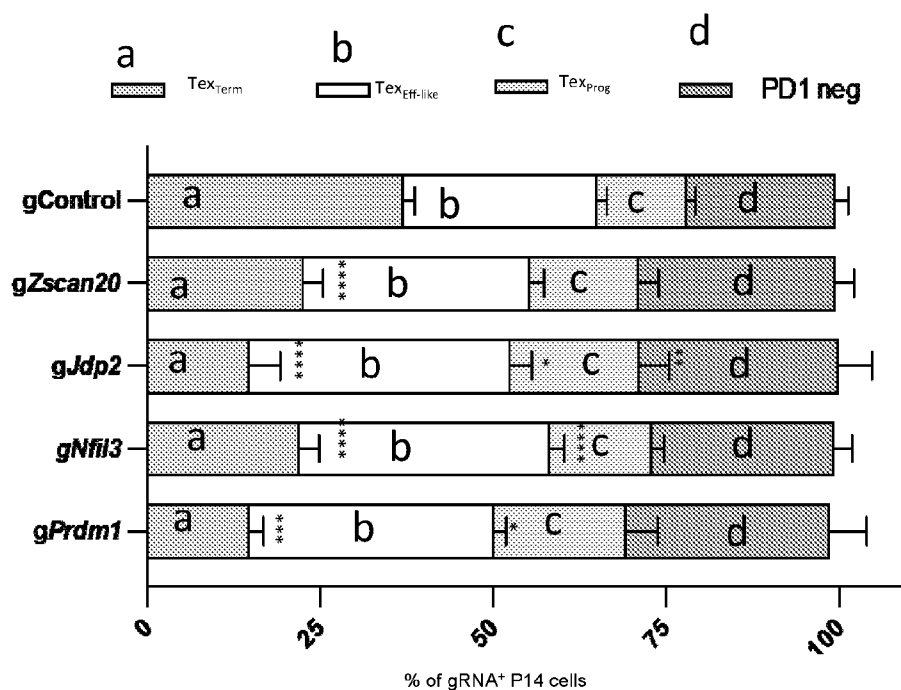
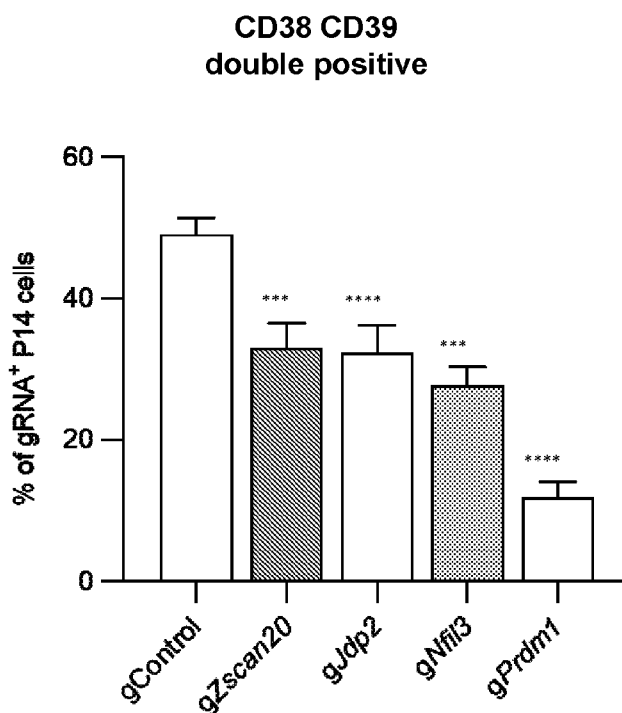


FIG. 26C



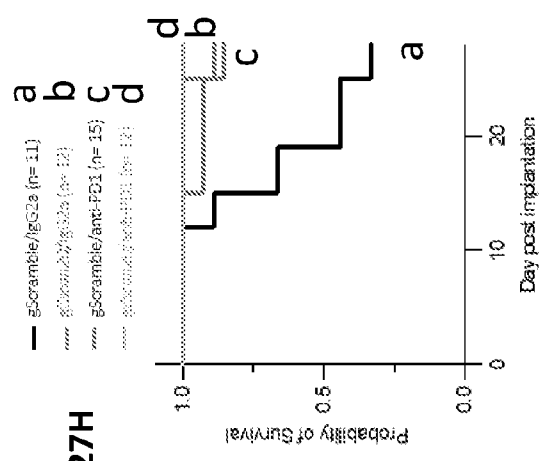
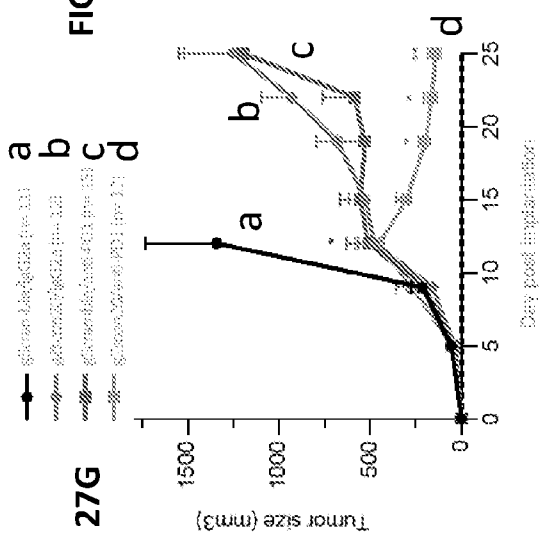
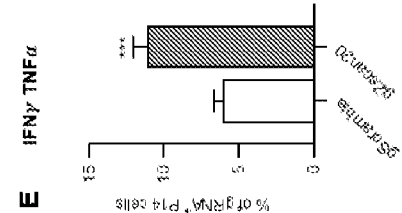
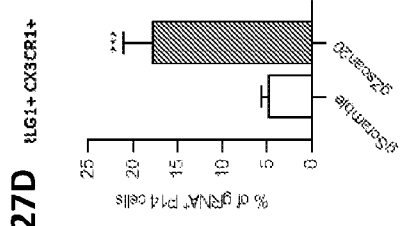
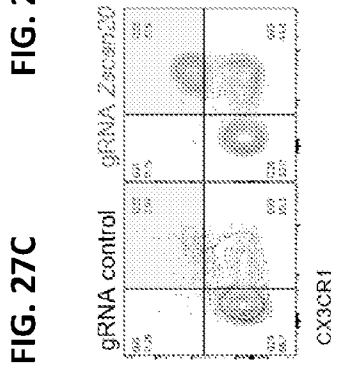
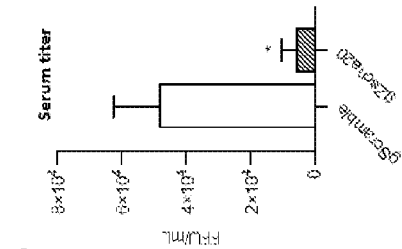
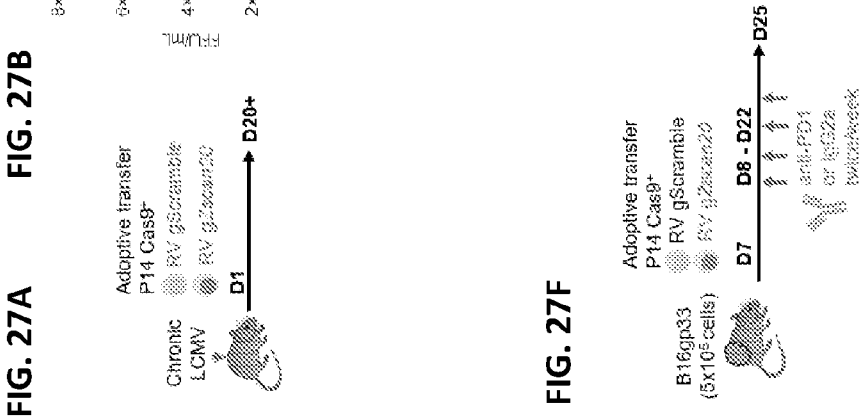


FIG. 27I

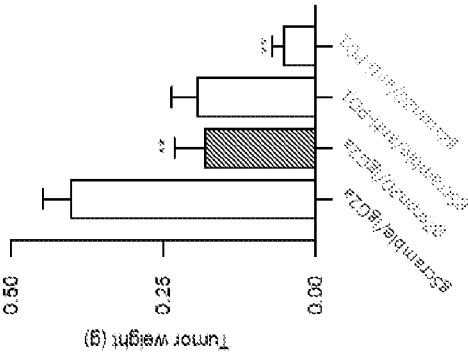


FIG. 27J

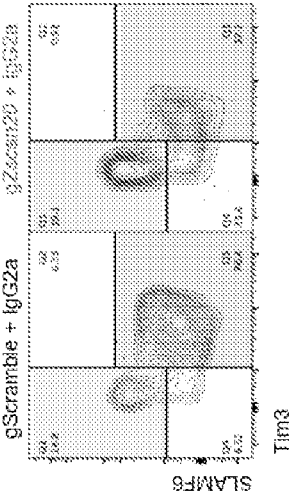


FIG. 27K

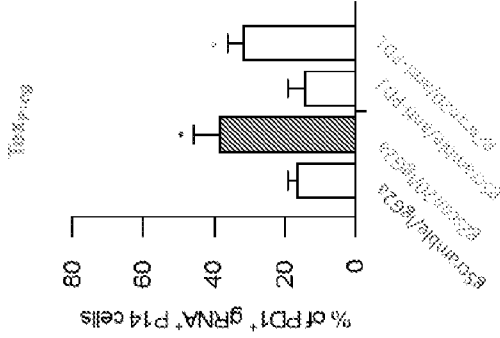


FIG. 27L

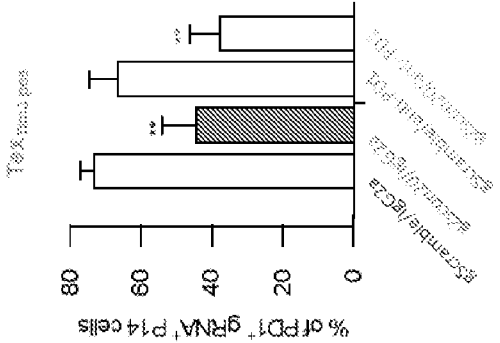


FIG. 28A

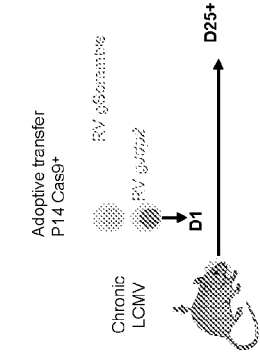


FIG. 28B

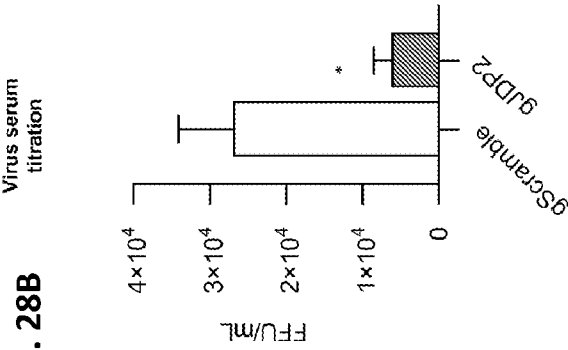


FIG. 28C

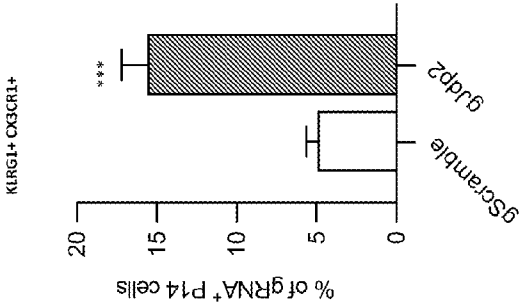


FIG. 28D

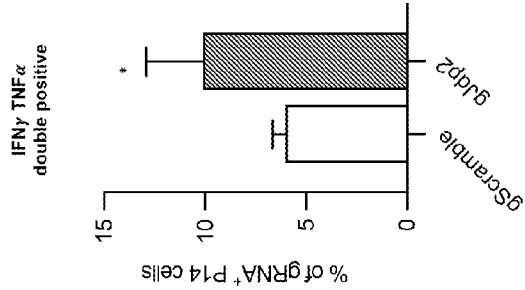


FIG. 29A

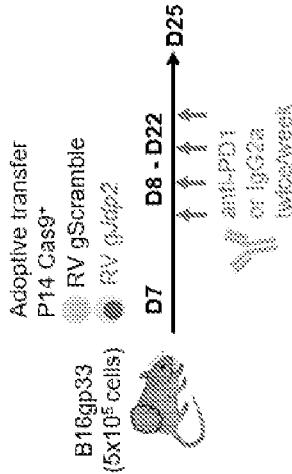


FIG. 29B

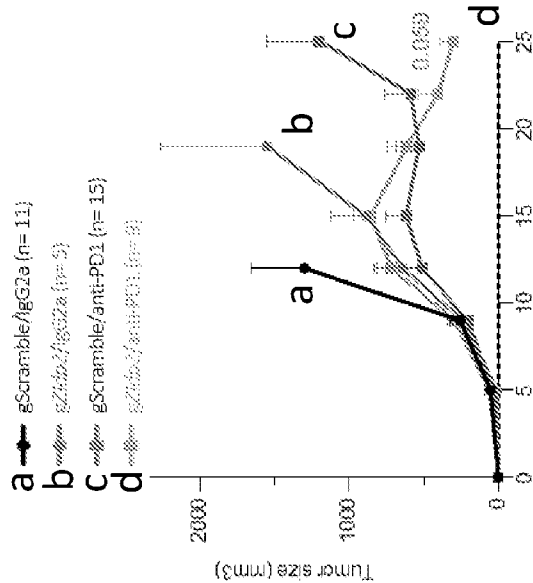


FIG. 29C

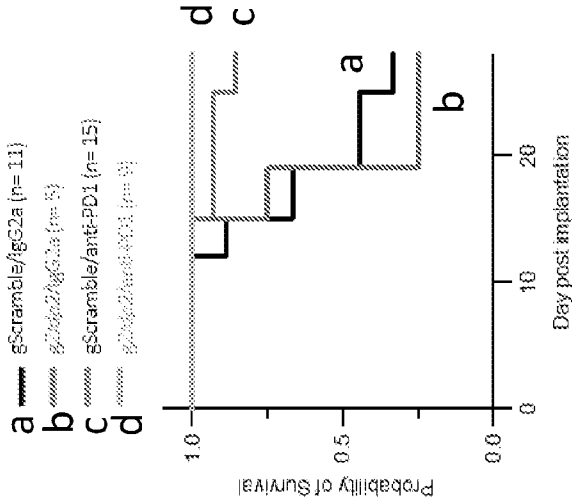


FIG. 30A

FIG. 30B

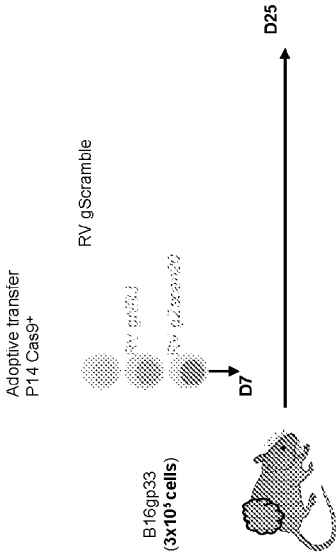
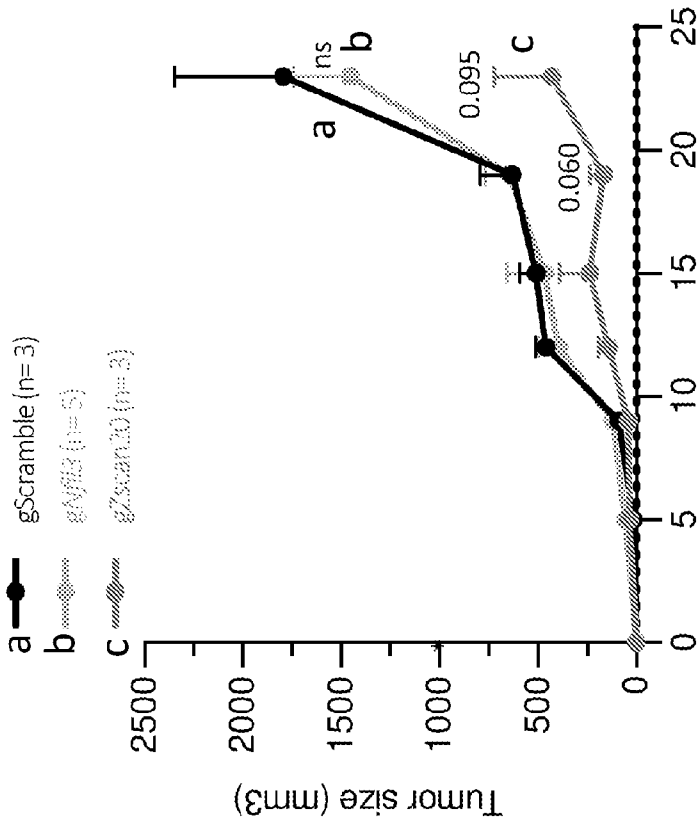


FIG. 31A

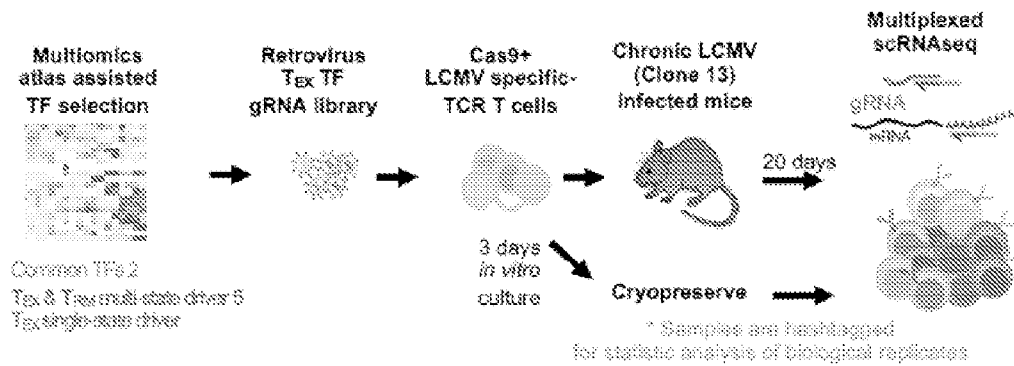


FIG. 31B

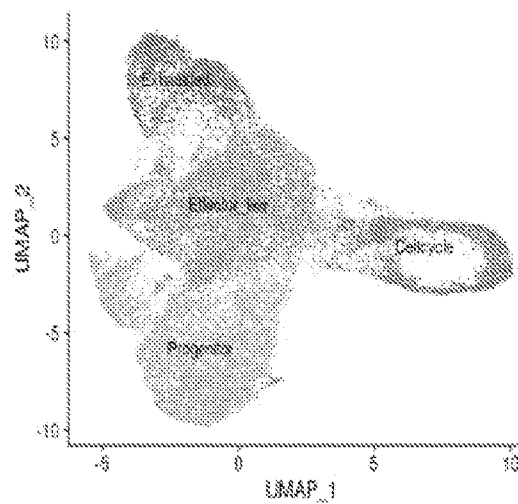


FIG. 31C

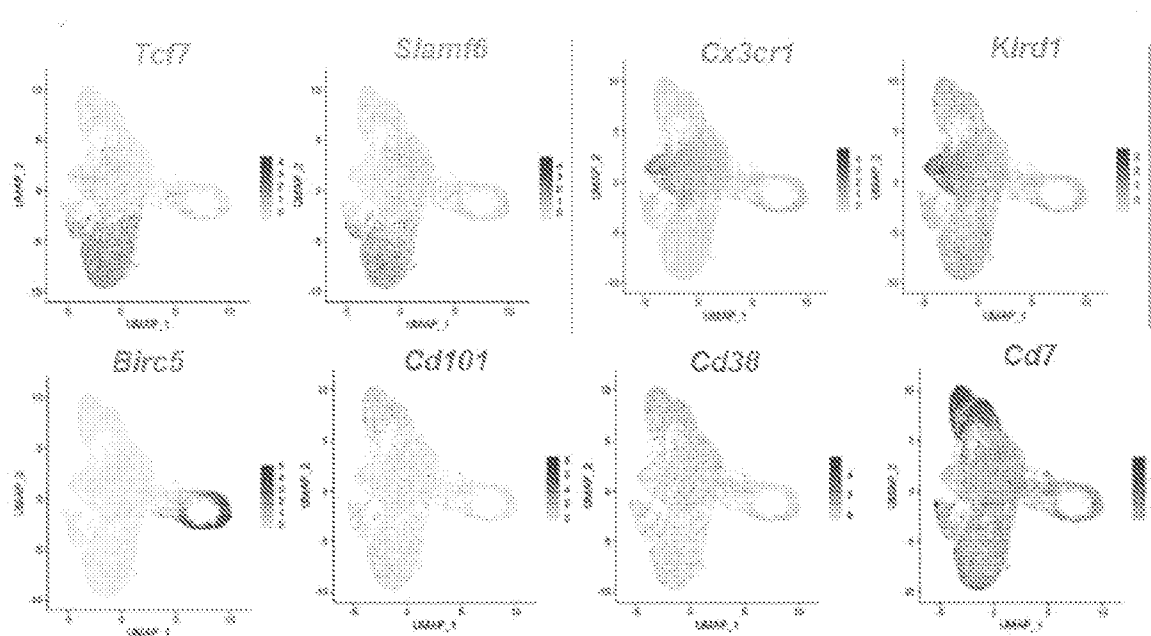


FIG. 31D

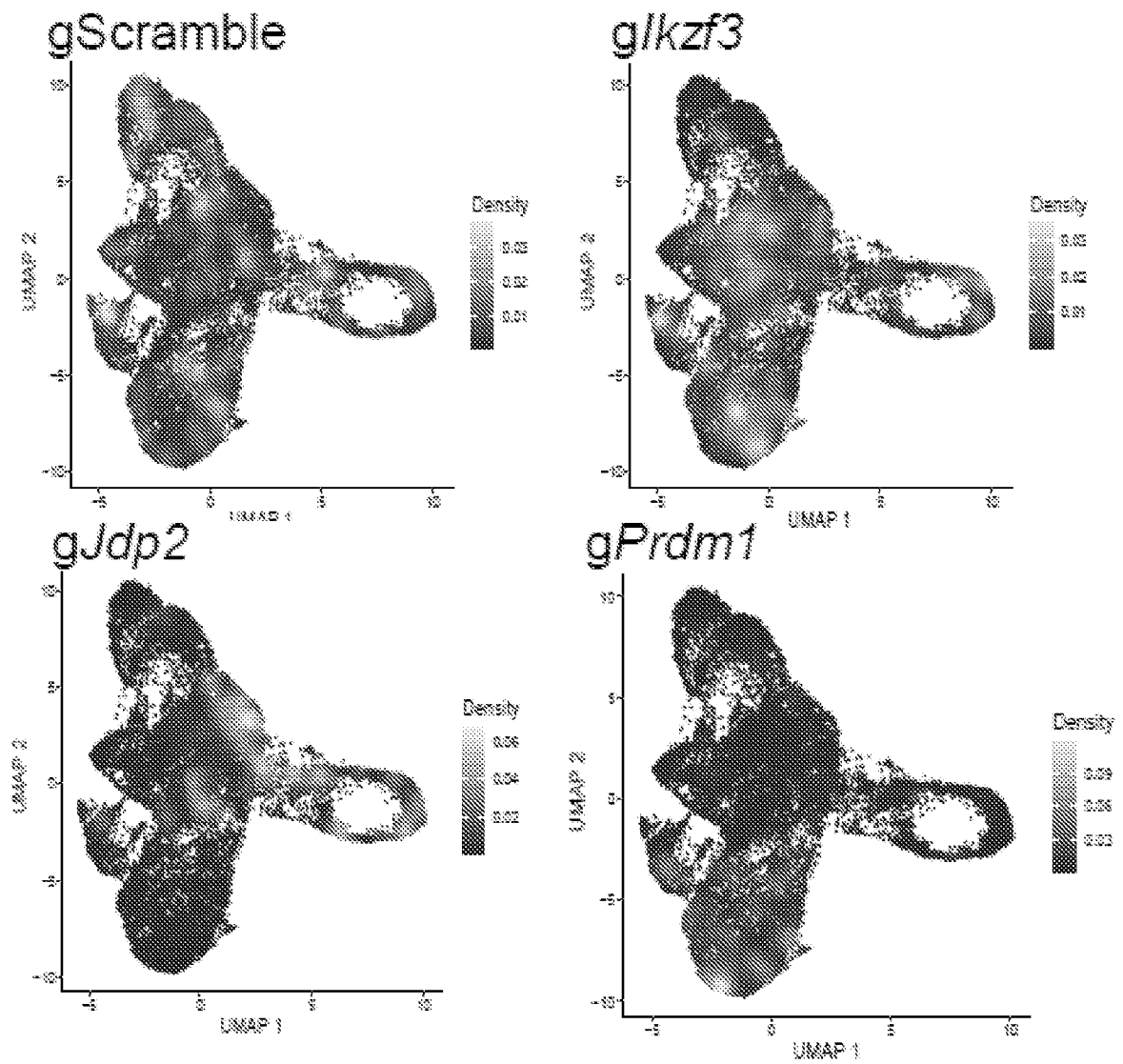


FIG. 31E

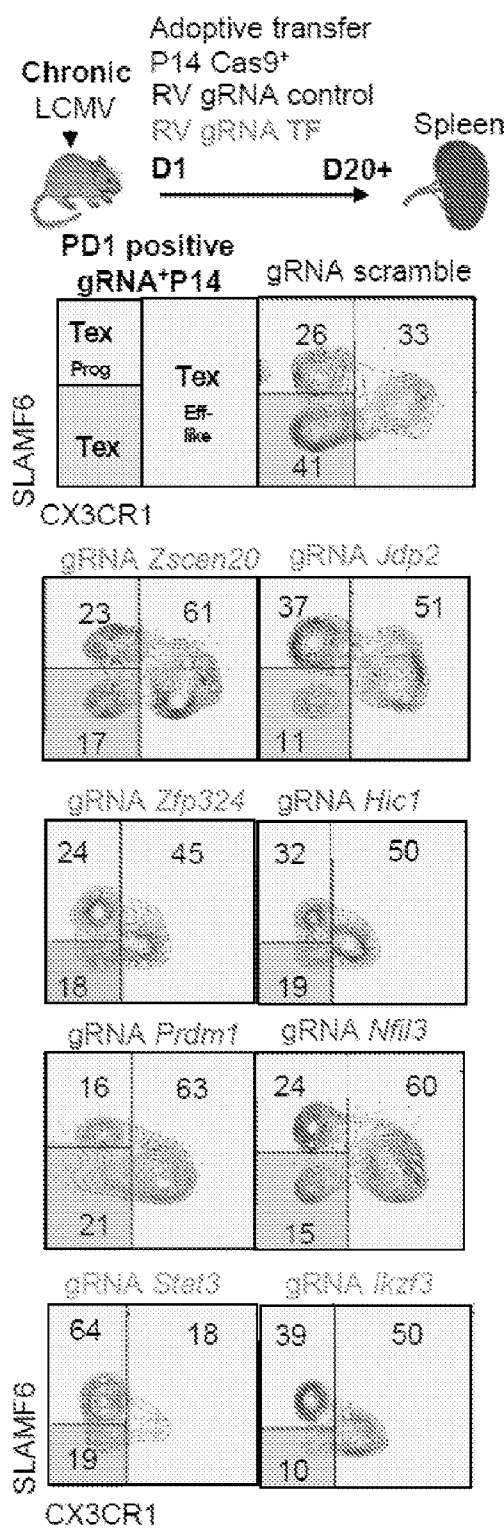


FIG. 31F

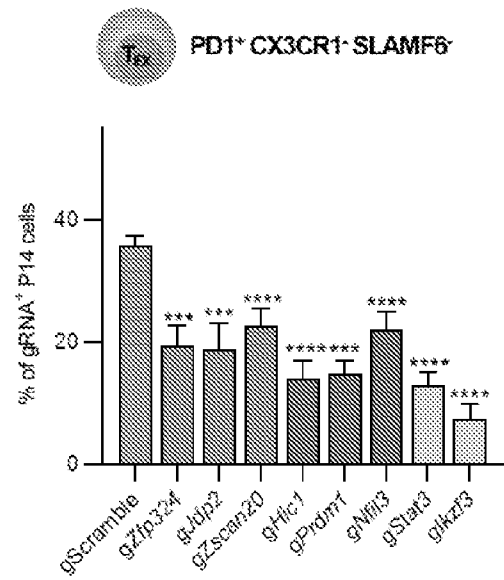


FIG. 31G

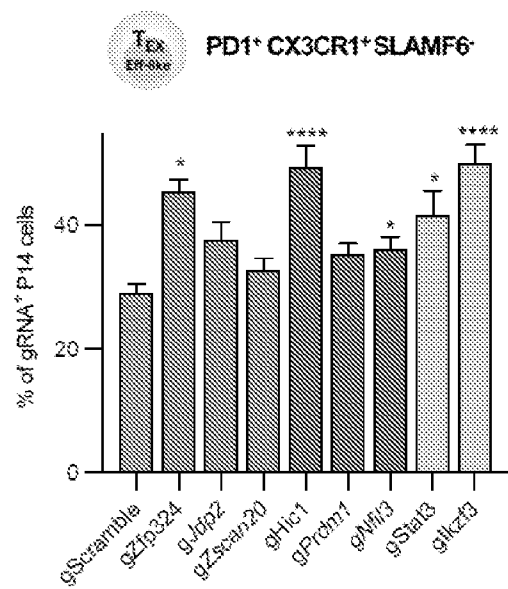


FIG. 31I

PD1⁺ CD101⁺

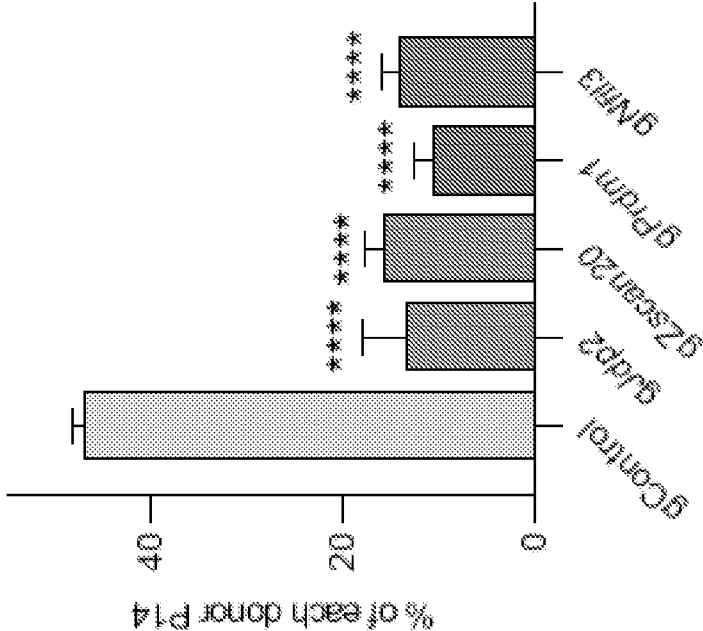


FIG. 31H

PD1⁺ CX3CR1⁺ SLAMF6⁺

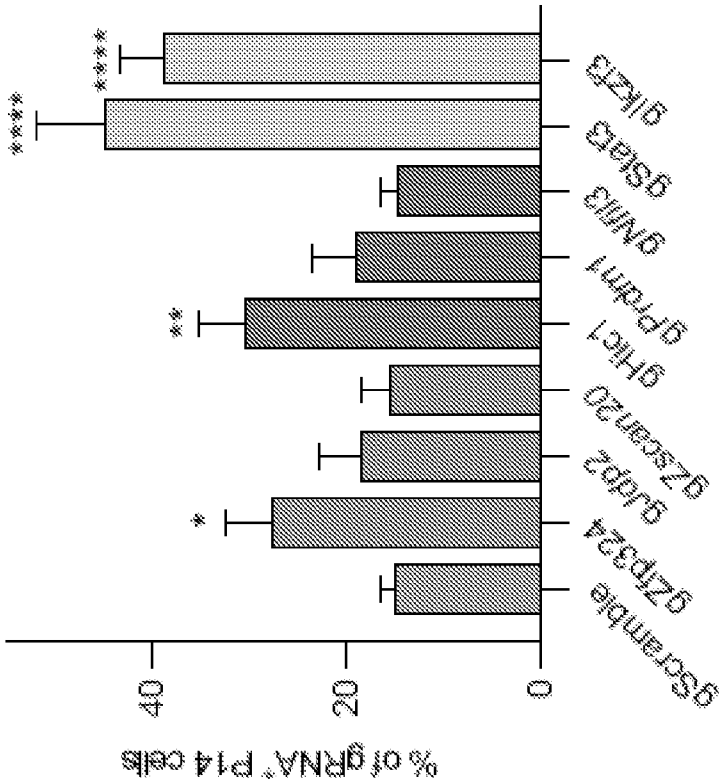


FIG. 32A

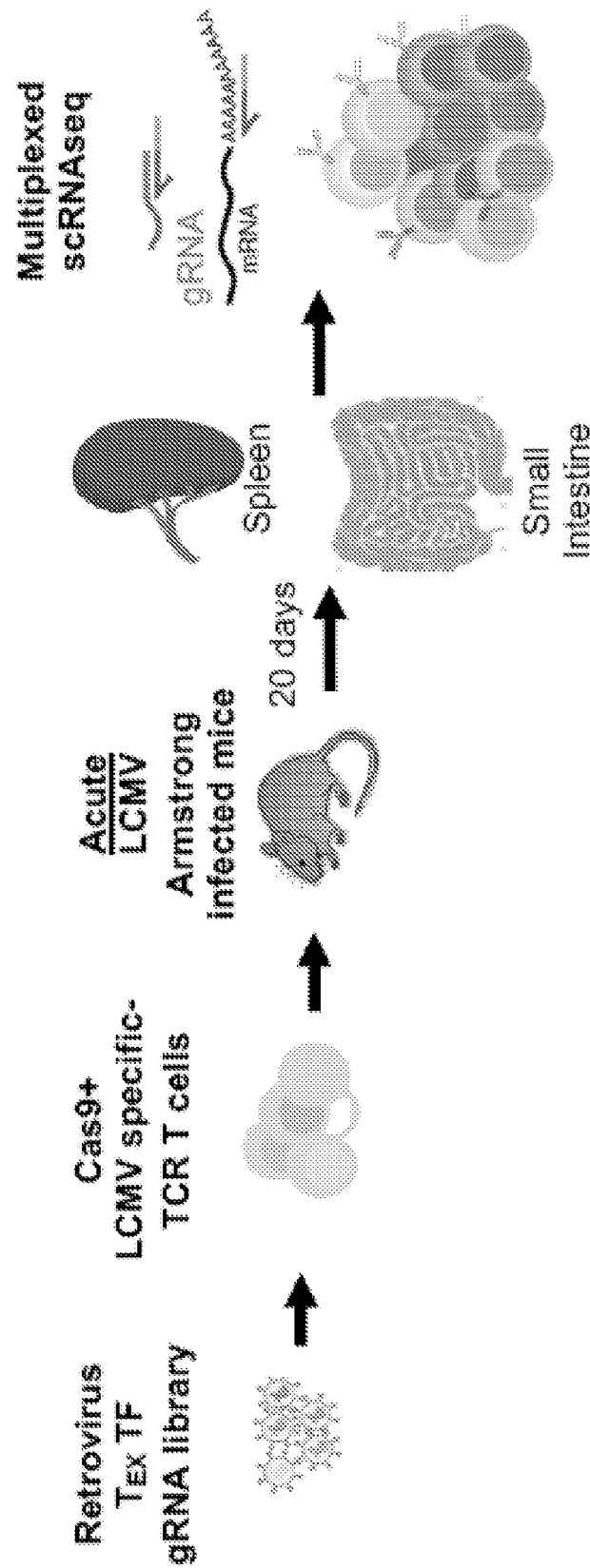


FIG. 32B

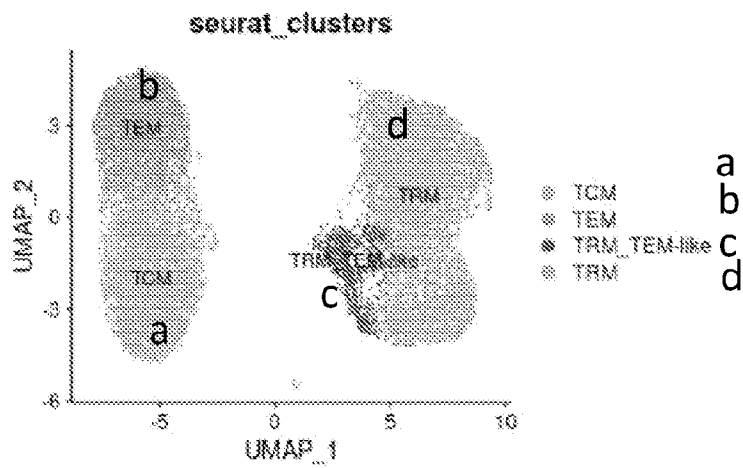


FIG. 32C

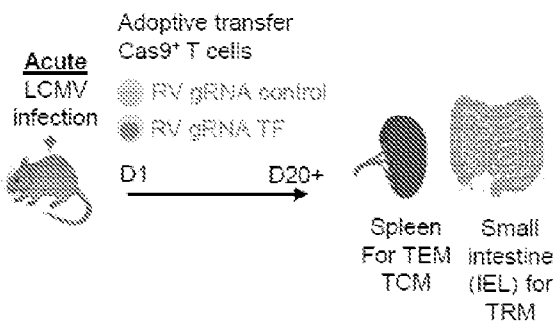


FIG. 32D

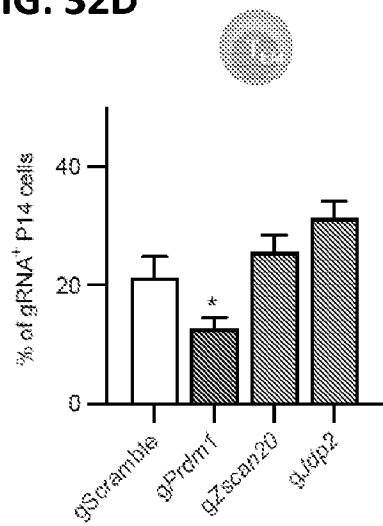


FIG. 32F

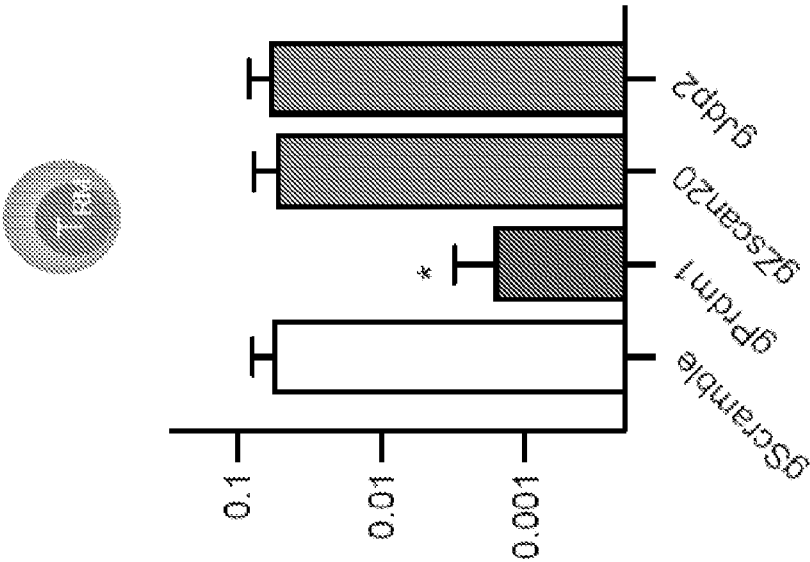


FIG. 32E

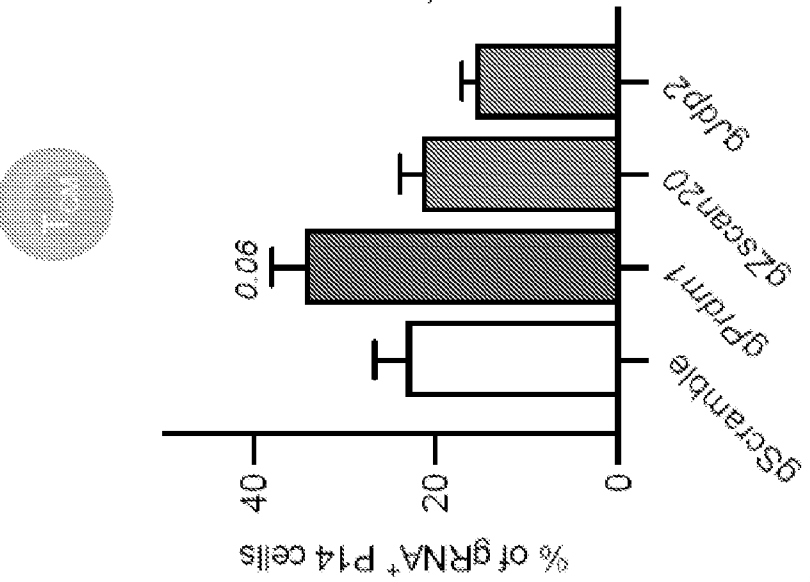


FIG. 33A

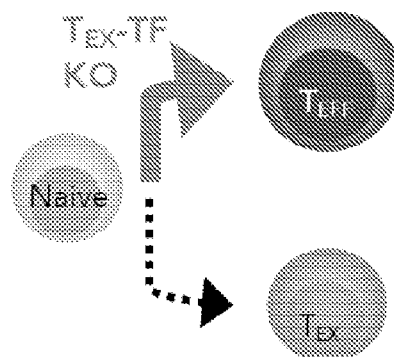


FIG. 33B

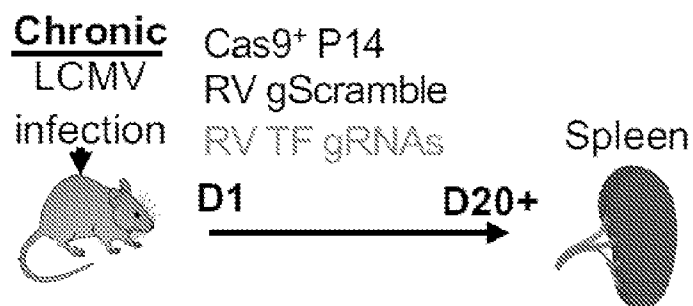


FIG. 33C

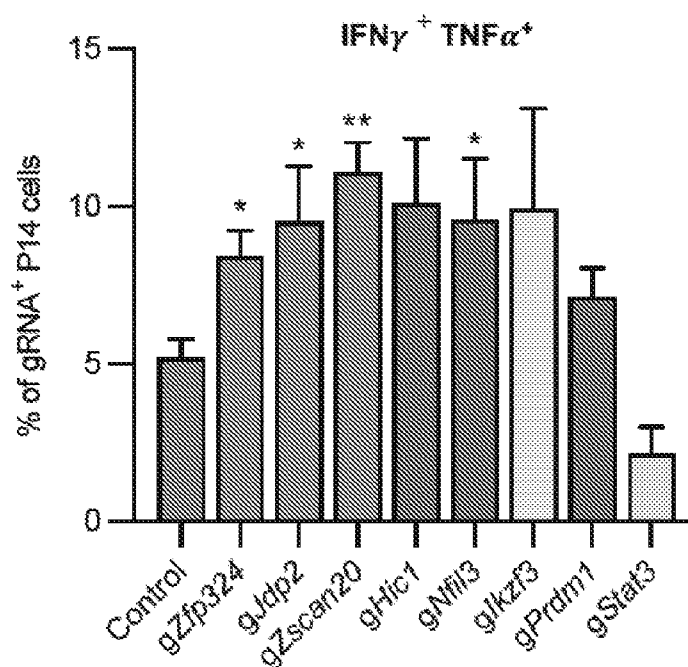


FIG. 33D
Serum titer

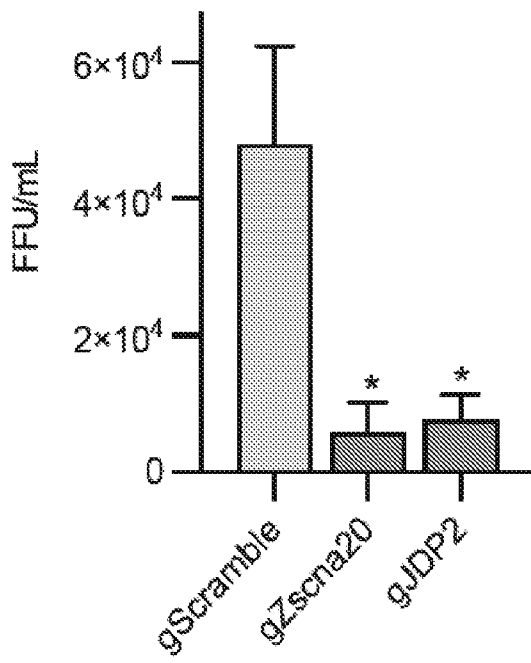


FIG. 33E
KRLG1⁺ CX3CR1⁺

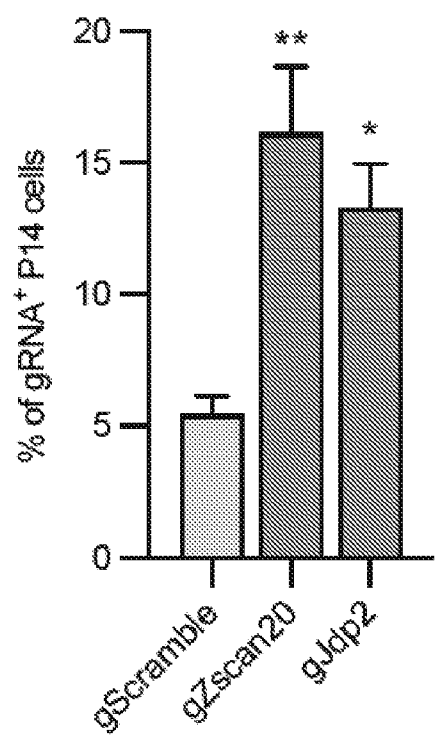


FIG. 33F

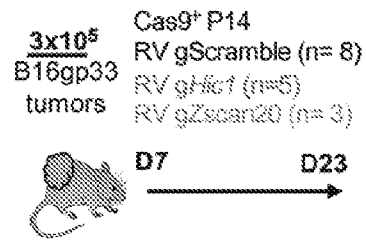


FIG. 33G

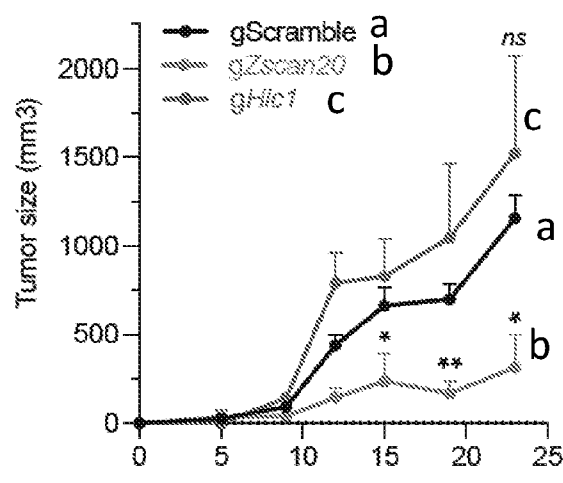


FIG. 33H

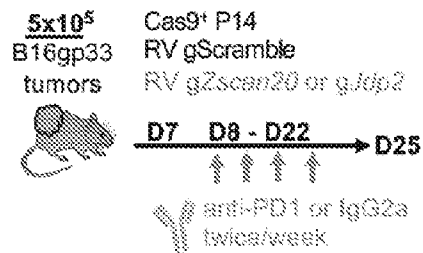


FIG. 33I

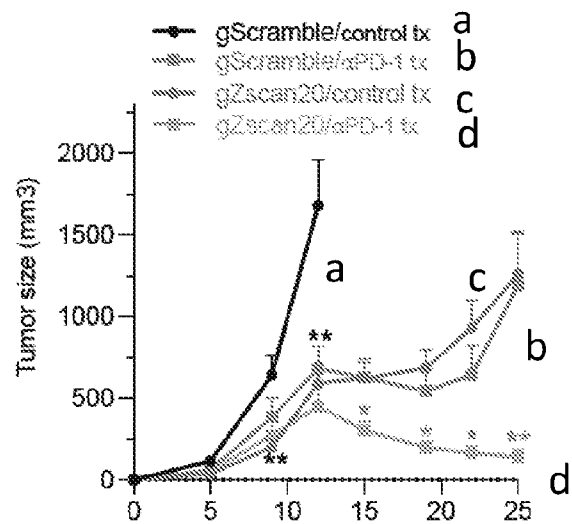


FIG. 33J

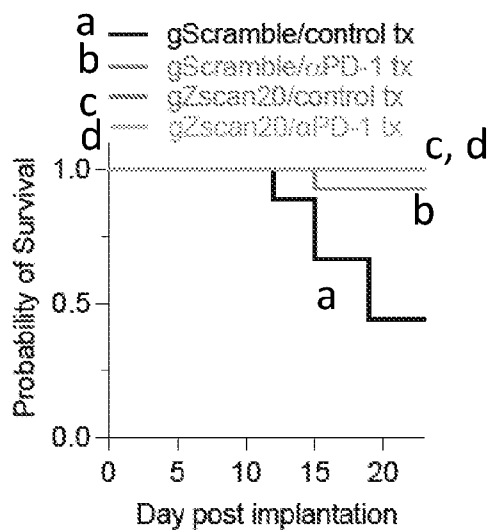


FIG. 33K

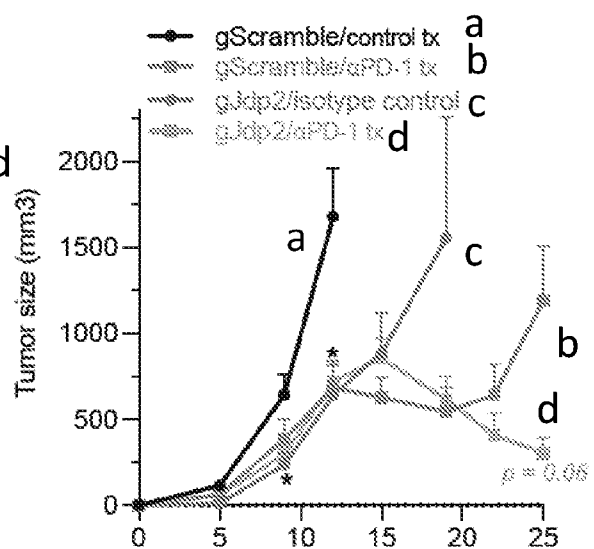


FIG. 33M

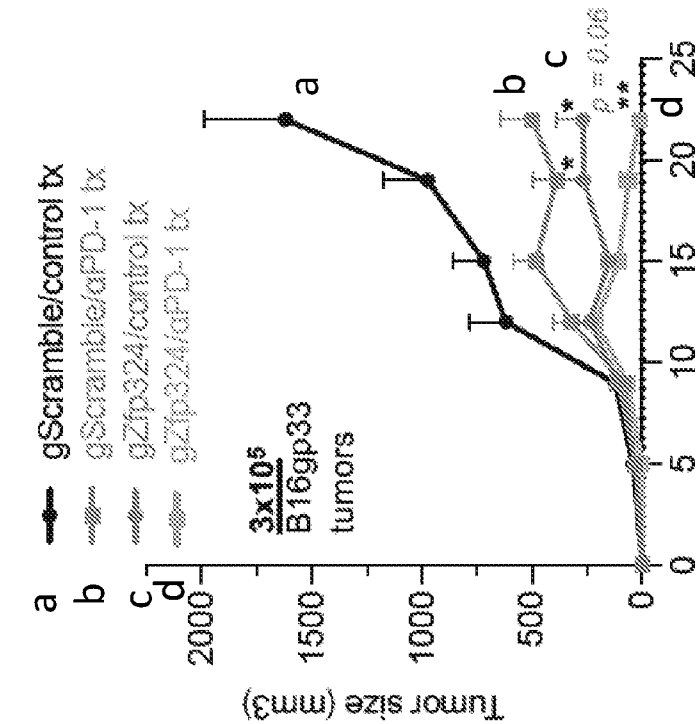
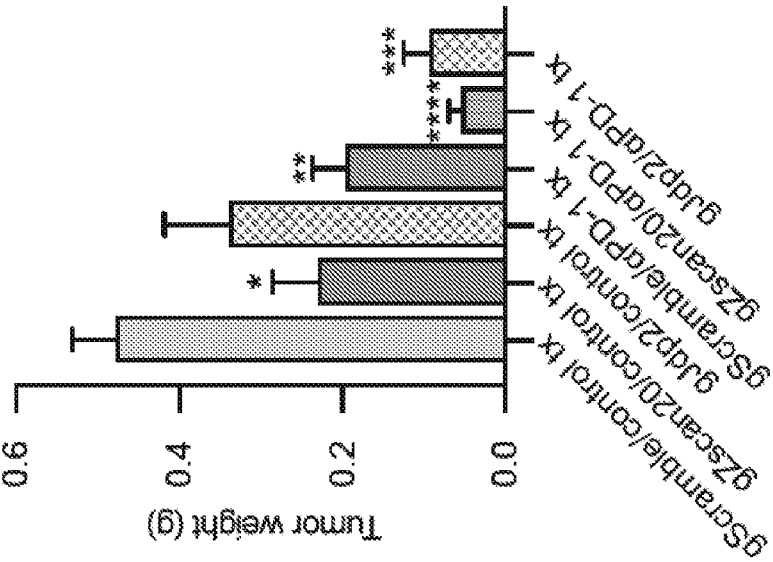


FIG. 33L



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/086236

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13^{ter}.1(a)).
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

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

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

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 3-49 (insofar as the claims relate to Zscan20)

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/086236

A. CLASSIFICATION OF SUBJECT MATTER

**C12N 5/0783 (2010.01) A61K 35/17 (2015.01) A61K 39/00 (2006.01) A61K 39/395 (2006.01) A61P 35/00 (2006.01)
C07K 16/28 (2006.01) C12N 15/11 (2006.01) C12N 15/113 (2010.01)**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

STN (MEDLINE, CAPLUS, BIOSIS, EMBASE databases): Zscan20 and like terms; **EPOQUE** (PATENW English language databases): A61K39/4611, A61K35/17, C12N5/0636, C12N5/0783, Zscan20, reduce, interfere, knockdown, PBMC, lymphocyte, T-cell, cancer, melanoma, virus, LCMV and like terms; **GenomeQuest**: SEQ ID NOs: 6-10, 19-21; **Google Scholar**: inventors names, Zscan20; **Google**: Zscan20, T cell, therapy, cancer, knockout; IP Australia's internal databases: applicant and inventors names.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

* "A" "D" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance document cited by the applicant in the international application earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
29 April 2024

Date of mailing of the international search report
29 April 2024

Name and mailing address of the ISA/AU

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Authorised officer

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(ISO 9001 Quality Certified Service)
Telephone No. +61 2 6283 2671

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/US2023/086236
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
L	Seo H, et al. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8+ T cell exhaustion. Proceedings of the National Academy of Sciences. 2019 Jun 18;116(25):12410-5 Lack of unity	
L	Gumber D et al. Improving CAR-T immunotherapy: overcoming the challenges of T cell exhaustion. EBioMedicine. 2022 Mar 1;77 Lack of unity	
X	WO 2019173636 A1 (Poseida Therapeutics, Inc.) 12 September 2019 [036, 069, 0185, 0218, 0422, 0587, 0663-0665, 0674, 0701, 0707, 0713]; Examples 1, 4; Table 3, page 290	1, 3-49
X	WO 2006063252 A2 (Alnylam Pharmaceuticals, Inc., et al) 15 June 2006 Table 8, page 69; Summary, page 5; Detailed description, page 12; Formulation, pages 25, 32; Methods of modulating an immune response via IFN production, pages 32, 34; Example 1; Figures 5-6	1, 3-49

INTERNATIONAL SEARCH REPORT	International application No. PCT/US2023/086236
Supplemental Box	
Continuation of: Box III This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept. This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups: • Group 1- Claims 1-49 (in part): a modified peripheral blood mononuclear cell (PBMC) comprising an agent (e.g. inhibitory RNA or guide RNA) or a genetic modification that reduces Zscan20 expression; methods of generating said PBMCs; pharmaceutical compositions thereof, and use in methods for treating cancer or viral infection; • Group 2- Claims 1-49 (in part): a modified PBMC comprising an agent or genetic modification that reduces Jdp2 expression; methods, compositions and treatment methods as for group 1; • Group 3- Claims 1-49 (in part): a modified PBMC comprising an agent or genetic modification that reduces Nfil3 expression; methods, compositions and treatment methods as for group 1; and • Group 4- Claims 1, 3-49 (in part): a modified PBMC comprising an agent or genetic modification that reduces Znf324 expression; methods, compositions and treatment methods as for group 1. PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art. When there is no special technical feature common to all the claimed inventions there is no unity of invention. In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is modified PBMC comprising an agent or genetic modification for reducing expression or function of a transcription factor. However this feature does not make a contribution over the prior art because it is disclosed in at least the following documents: • Seo H, et al. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8+ T cell exhaustion. Proceedings of the National Academy of Sciences. 2019 Jun 18;116(25):12410-5; and • Gumber D et al. Improving CAR-T immunotherapy: overcoming the challenges of T cell exhaustion. EBioMedicine. 2022 Mar 1;77. Seo et al (2019) and Gumber et al (2022) each disclose targeting of the transcription factors TOX and NR4A with knockout or knockdown, TCF1 for overcoming CAR T cell exhaustion (Seo et al (2019): Abstract; Gumber et al (2022): Figure 2; TOX and NR4A, paragraph bridging pages 4-5). Therefore in the light of these documents this common feature cannot be a special technical feature. As there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied <i>a posteriori</i>. As no additional search fees were paid, this International Search Authority has searched and examined Invention 1 (Claims 1, 3-49) with regard to modified PBMCs comprising an agent which reduces Zscan20 expression/function and use of said PBMCs in methods for treating cancer or viral infections. The claims have been searched and examined with respect to Zscan20 only. Claim 2 is directed to a combination of Zscan20 with other factors for which additional fees have not been paid. Since no search has been carried out on those other factors, opinion on claim 2 is therefore reserved.	
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INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/US2023/086236	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2019173636 A1	12 September 2019		
WO 2006063252 A2	15 June 2006	WO 2006063252 A2	15 Jun 2006
		AU 2005313883 A1	15 Jun 2006
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		CA 2589406 A1	15 Jun 2006
		EP 1819365 A2	22 Aug 2007
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		JP 2008523094 A	03 Jul 2008
		JP 4981681 B2	25 Jul 2012
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		US 2006217330 A1	28 Sep 2006
		US 8003619 B2	23 Aug 2011
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