

# Heterogeneity and transcriptome changes of human CD8<sup>+</sup> T cells across nine decades of life

Jian Lu<sup>1\*</sup>, Raheel Ahmad<sup>1\*</sup>, Thomas Nguyen<sup>1</sup>, Jeffrey Cifello<sup>1</sup>, Humza Hemani<sup>1</sup>, Jiangyuan Li<sup>1</sup>, Jinguo Chen<sup>2</sup>, Siyi Li<sup>1</sup>, Jing Wang<sup>1</sup>, Achouak Achour<sup>1</sup>, Joseph Chen<sup>1</sup>, Meagan Colie<sup>1</sup>, Ana Lustig<sup>1</sup>, Christopher Dunn<sup>1</sup>, Linda Zukley<sup>3</sup>, Chee W. Chia<sup>4</sup>, Irina Burd<sup>5</sup>, Jun Zhu<sup>6</sup>, Luigi Ferrucci<sup>3</sup>, and Nan-ping Weng<sup>1\*\*</sup>

<sup>1</sup> Laboratory of Molecular Biology and Immunology, National Institute on Aging, National Institutes of Health

<sup>2</sup> Center for Human Immunology, Autoimmunity and Inflammation (CHI), National Institutes of Health

<sup>3</sup> Translational Gerontology Branch, National Institute on Aging, National Institutes of Health

<sup>4</sup> Laboratory of Clinical Investigation, National Institute on Aging, National Institutes of Health

<sup>5</sup> Department of Obstetrics and Gynecology, Johns Hopkins University School of Medicine

<sup>6</sup> National Heart, Lung, and Blood Institute, National Institutes of Health

\* These two authors contribute equally.

\*\*Correspondence to Nan-ping Weng (Wengn@mail.nih.gov)

Supplementary materials:

Supplementary Figure 1 Analysis of human CD8<sup>+</sup> T cell subpopulations by scRNAseq and multi-color flow cytometry.

Supplementary Figure 2 CD8<sup>+</sup> T cell subpopulations in participants and their age changes.

Supplementary Figure 3 Gene Set Enrichment Analysis (GSEA) of differentially expressed genes in ten subpopulations of CD8<sup>+</sup> T cells

Supplementary Figure 4 Age-related increased and decreased genes in nine subpopulations of CD8<sup>+</sup> T cells

Supplementary Figure 5 Predicting cell age by two machine learning models

Supplementary Figure 6 Mutational analysis of CD8<sup>+</sup> T cell subpopulations and their change with age

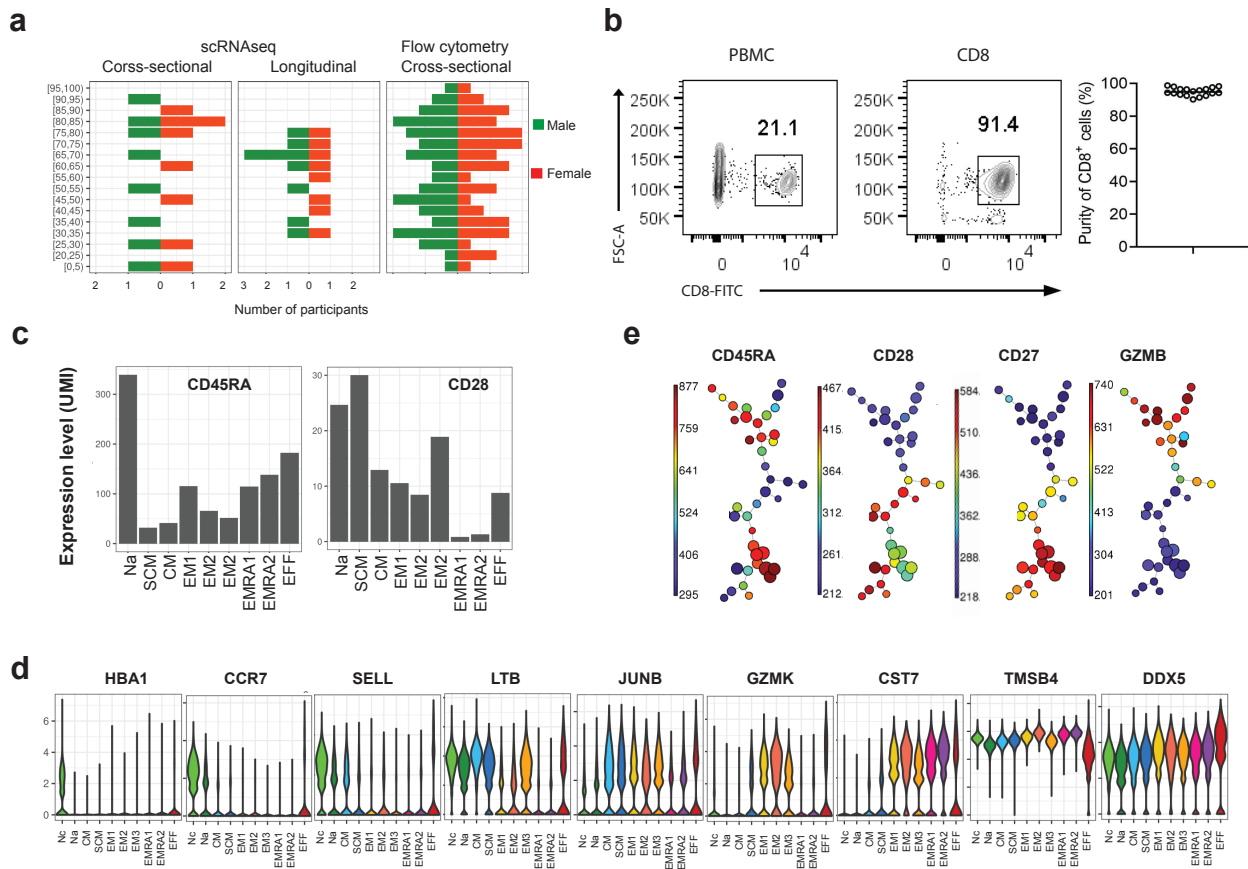
Supplementary Figure 7 Overlap age-changed genes identified by MELR and MERF model

Supplementary Table 1 Information of participants' age, gender, number of CD8 T cells, composition of CD8 T cell subpopulations by scRNAseq and coverage by exome sequencing

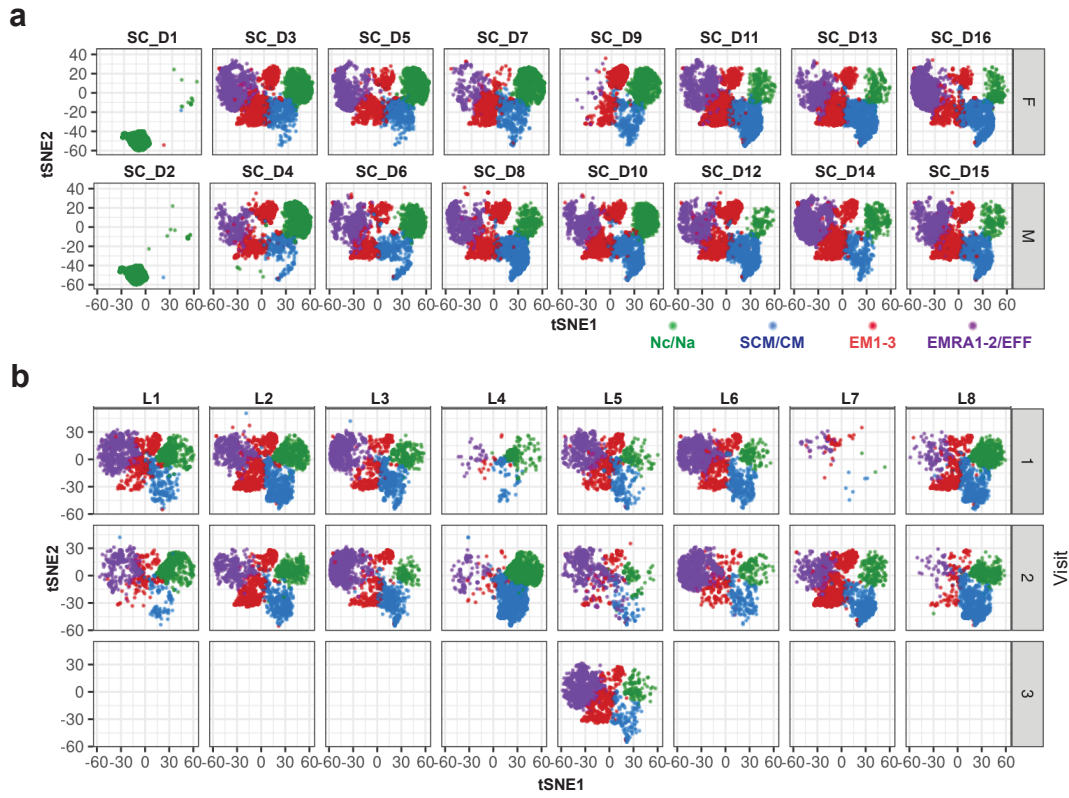
Supplementary Table 2 Selected enriched gene expression function in CD8 T cell subpopulations

Supplementary Table 3 Antibodies used in this study

Supplementary Table 4 Sequences of primers used to construct antibody-derived tag (ADT) libraries

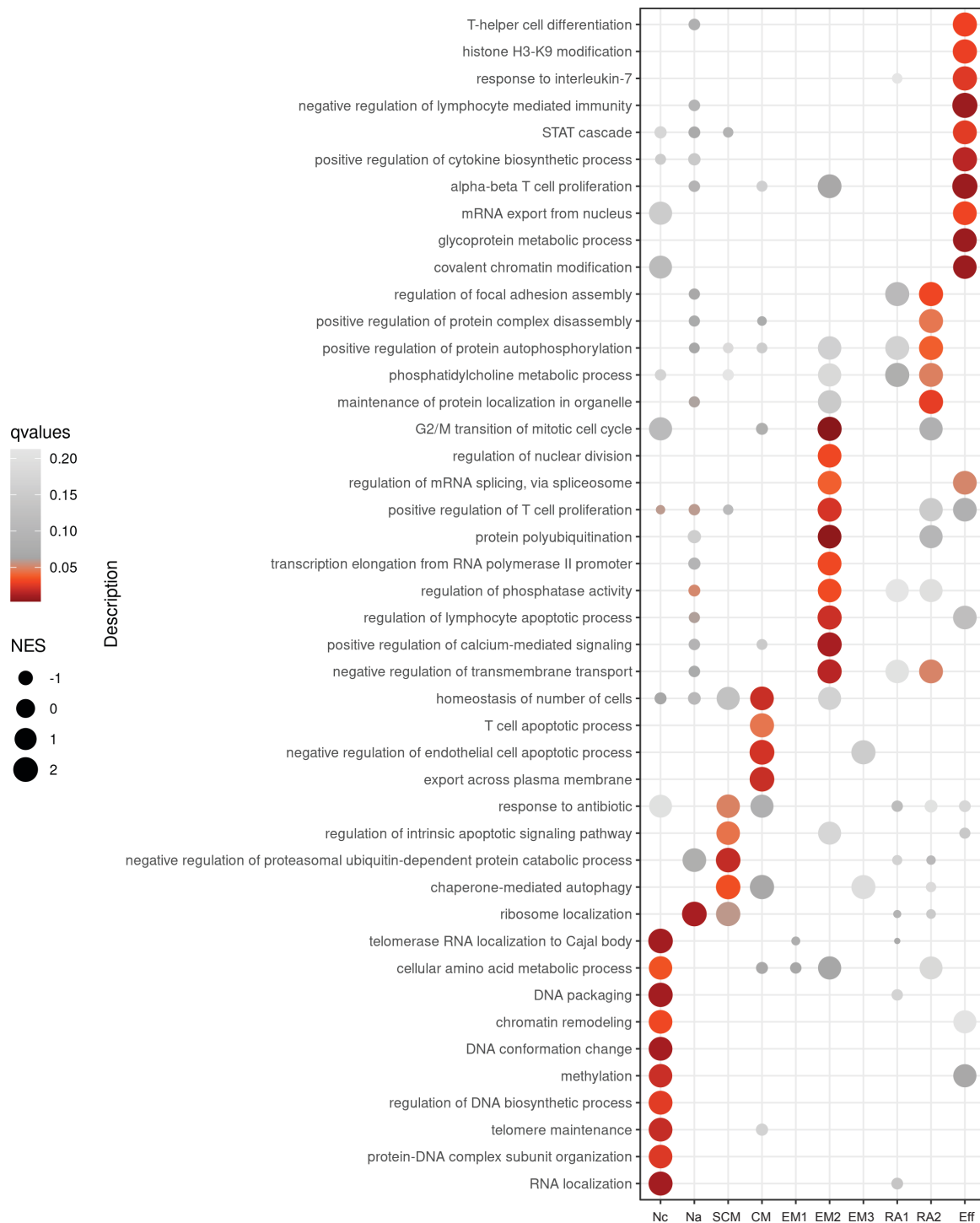


**Supplementary Figure 1:** Analysis of human CD8<sup>+</sup> T cell subpopulations by scRNAseq and multi-color flow cytometry. **a.** Distribution of Age and Sex of donors used in three cohorts used in study (Female – Orange, Male – Green). Donor age is binned for every 5 years. **b.** Left - FACS gating strategy for CD8<sup>+</sup> T cell sorting from a representative donor. Right – dot plot showing the distribution of CD8<sup>+</sup> T cell purity. **c.** Bar graphs showing the average expression of ADT libraries for CD45RA and CD28 antibodies in each cluster identified by scRNA-seq. **d.** DGEs of CD8<sup>+</sup> T cell subpopulations. The percentage of cells expressing each gene and average level of gene expression in each cluster shown. **e.** FlowSOM plots of selected markers from flow cytometry cohort.

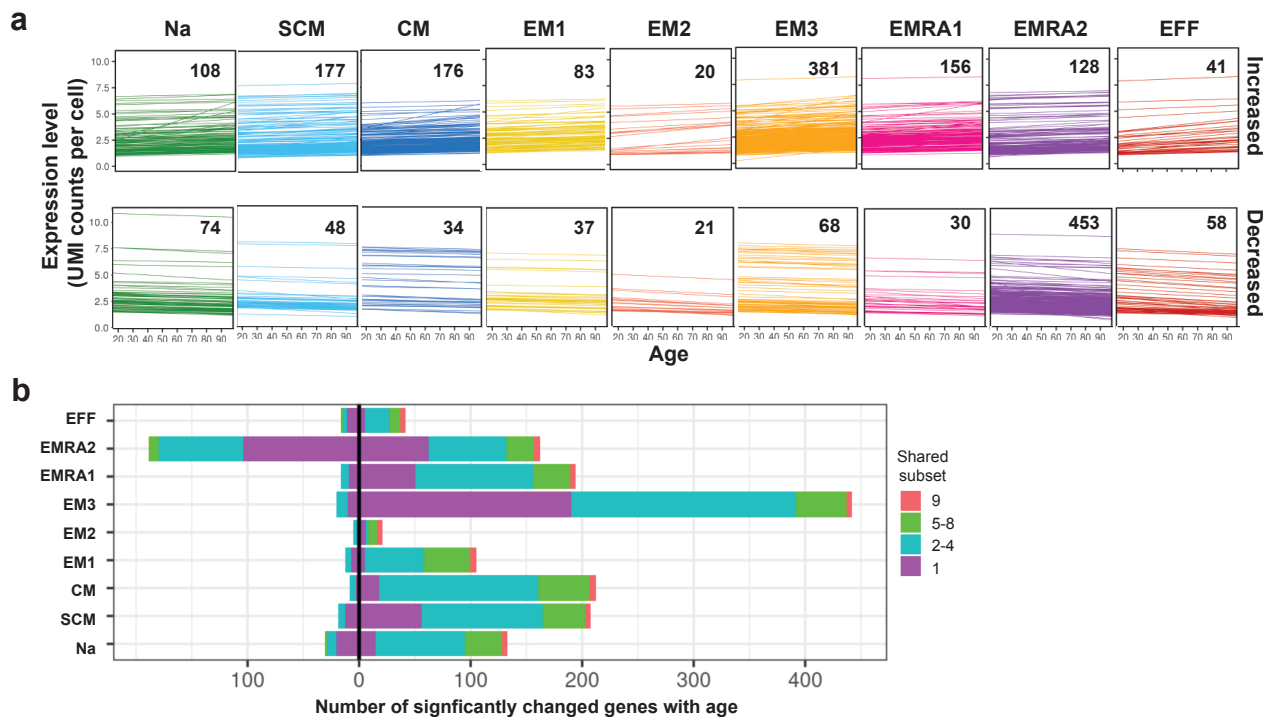


**Supplementary Figure 2: CD8<sup>+</sup> T cell subpopulations in participants and their age changes.**

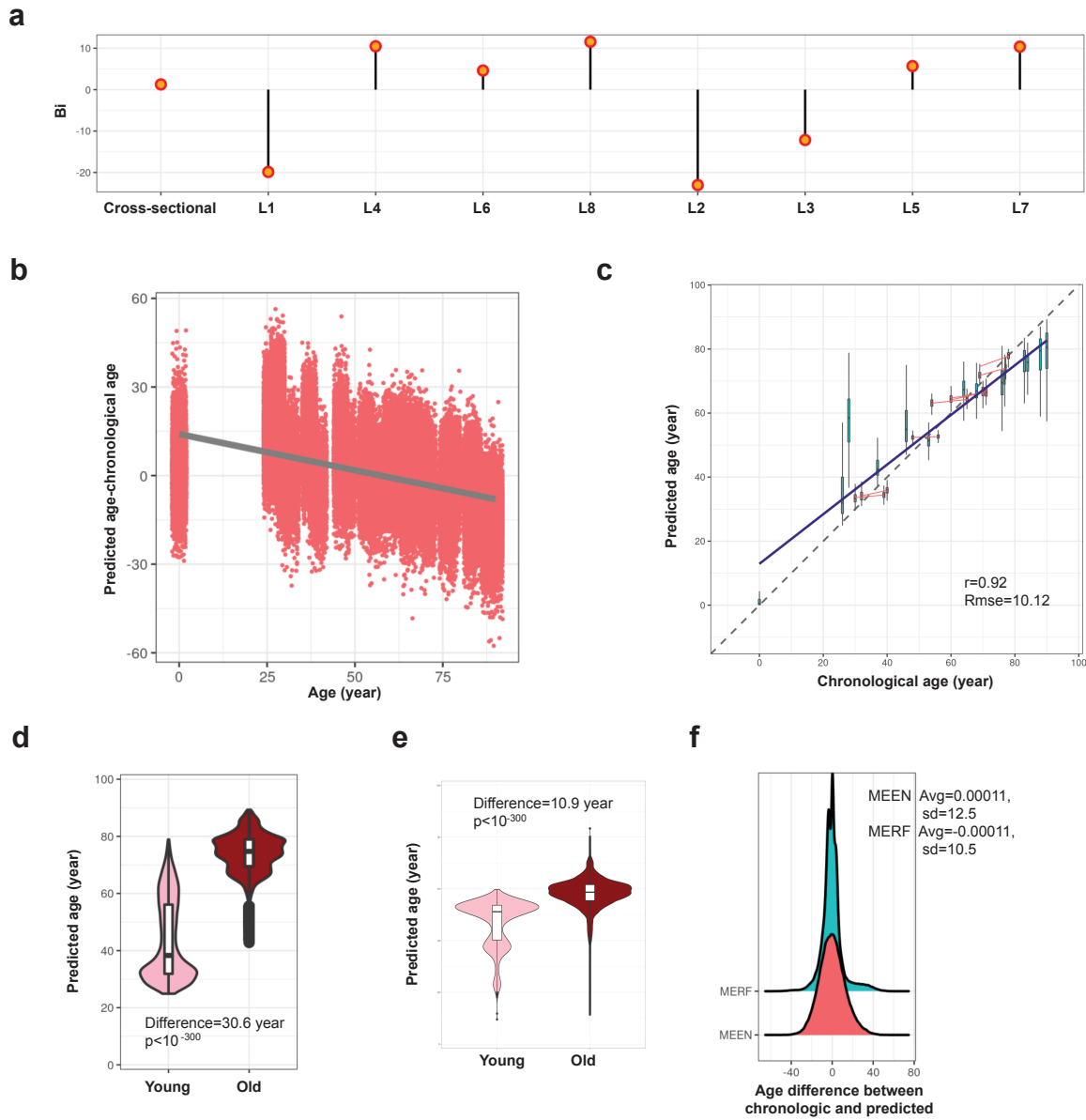
**a.** TSNE plots of single cells from each donor in the cross-sectional scRNA-seq cohort. Donors are ordered by age and stratified by sex (above – female; below - male). Plots are colored by major CD8<sup>+</sup> T cell subset identities. **b.** TSNE plots of single cells from each donor in the longitudinal scRNA-seq cohort. Donors are ordered by age and stratified by visit number. Plots are colored by major CD8<sup>+</sup> T cell subset identities.



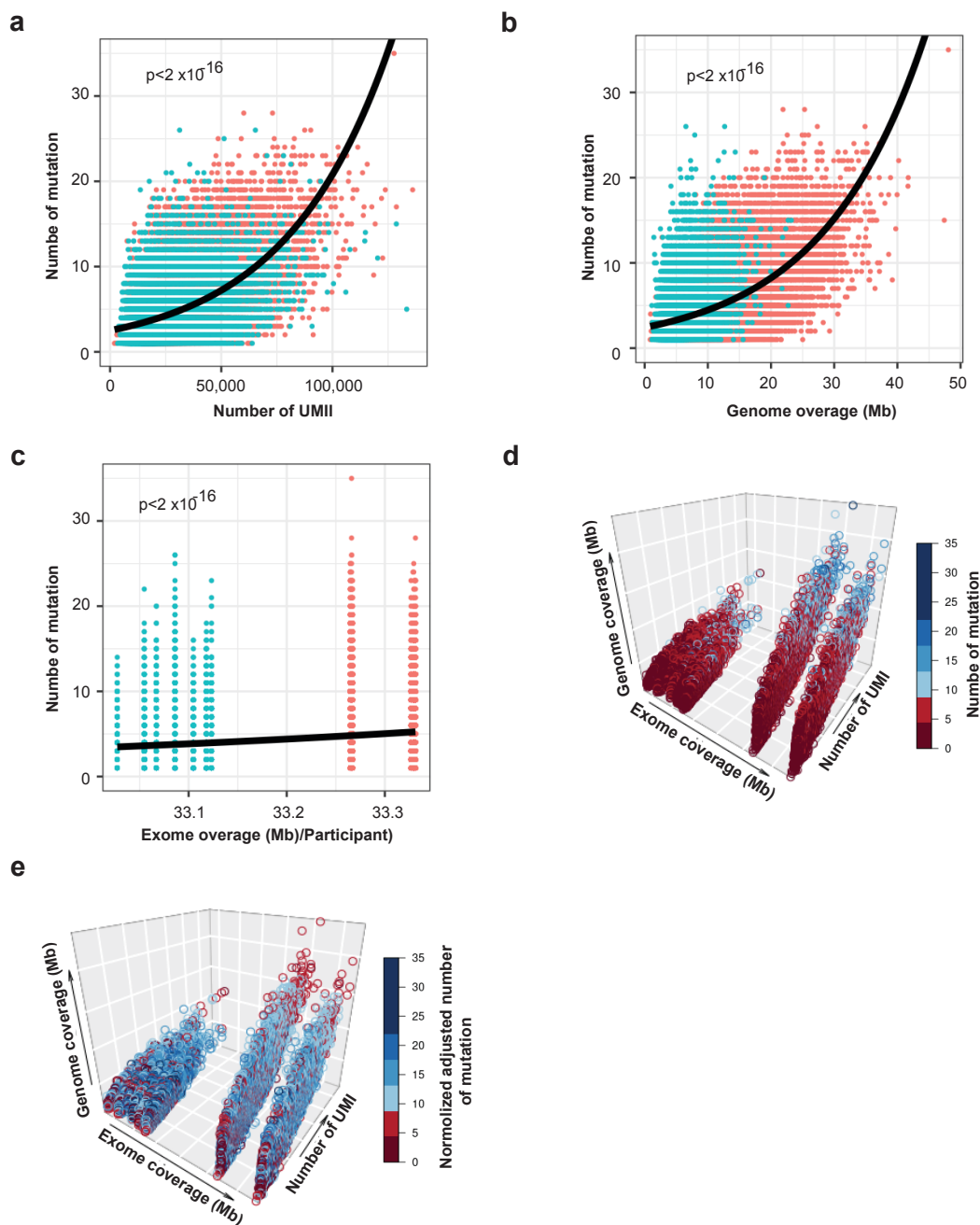
**Supplementary Figure 3:** Gene Set Enrichment Analysis (GSEA) of differentially expressed genes in ten subpopulations of CD8<sup>+</sup> T cells. Bubble plot showing selected functional gene sets enriched in each cluster identified by scRNA-seq. Gene Set Enrichment Analysis (GSEA) was performed in the clusterprofiler R package with gene sets from the ‘Biological Processes’ groups. Size of bubbles represents normalized enrichment score (NES). The color of each bubble represents the significance of enrichment (qvalues).



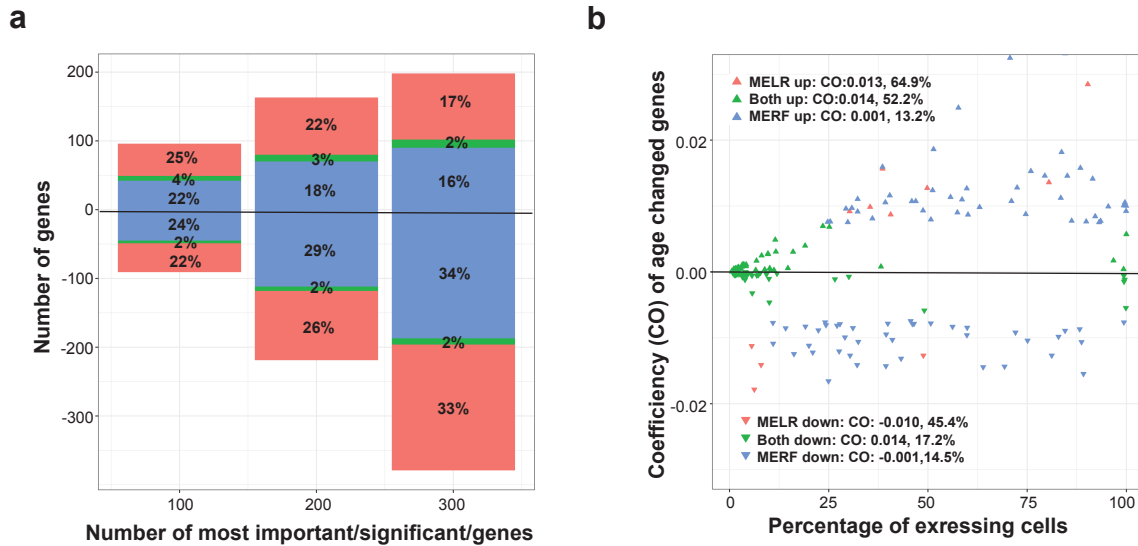
**Supplementary Figure 4:** Age-related increased and decreased genes in nine subpopulations of CD8<sup>+</sup> T cells. **a.** Line graphs showing the change in the average expression of significantly up-regulated age-related genes. Genes were identified based on a linear mixed effect regression model with a random intercept. Cells were grouped by donor in the longitudinal cohort; cells from the cross-sectional cohort were treated as one group. **b.** Line graphs showing the change in the average expression of significantly down-regulated age-related genes. Genes were identified based on a linear mixed effect regression model with a random intercept. Cells were grouped by donor in the longitudinal cohort; cells from the cross-sectional cohort were treated as one group. The number of significant genes is displayed in each graph. **c)** Bar graphs showing the shared and unique significant age-related gene expression changes in each cluster identified by scRNA-seq (left – downregulated; right – upregulated).



**Supplementary Figure 5:** Predicting cell age by two machine learning models. **a.** Lollipop plot showing the random effect coefficient for each longitudinal donor in the mixed effect elastic net model used to estimate single cell age. The random effect coefficient for cross-sectional donors was calculated as one group. **b.** Scatter plot showing the residuals from the mixed effect elastic net model ordered by chronological age. A least squares line was fit to identify the trend in residual value across age. **c.** Box plots showing the cross-validation predictions from the mixed effect random forest model ordered by chronological age. A least squares line was fitted to show the relationship between chronological age and predicted age of cells in the dataset. **d.** Violin plots showing predictions generated from the mixed effect random forest model on a test set of CD8<sup>+</sup> T cells from the validation dataset. Data are grouped into grouped into old (>70) and young (<30). **e.** Test predictions for an independent dataset from healthy donors older than 70 years (Old) and younger than 30 years (Young) using publicly available scRNA-seq datasets of CD8<sup>+</sup> T cells. The box-whisker plots in **d** and **e**, the center line is the median, the box is from the 25<sup>th</sup> to the 75<sup>th</sup> percentile. The upper or lower whisker extends from the hinge to the 1.5 \* IQR from the hinge (where IQR is distance between the first and third quartiles) for up and low, respectively. P values were calculated by 2-sided T-test. **f.** Ridge plots showing the distribution of residuals from both mixed effect models used in the study.



**Supplementary Figure 6:** Mutational analysis of CD8<sup>+</sup> T cell subpopulations and their change with age. **a.** Scatter plot showing the relationship between umi number and number of raw mutations in single cells. Fitted negative binomial regression line shown. Red color represents cross-sectional and blue represents longitudinal samples. **b.** Scatter plot showing the relationship between coverage and number of raw mutations in single cells. Fitted negative binomial regression line shown. **c.** Scatter plot showing the relationship between 10X coverage for exome sequencing for each donor and number of raw mutations in single cells. Fitted negative binomial regression line shown. **d.** 3D scatter plot showing the relationship between three variables and number of mutations in single cells. **e.** 3D scatter plot showing the relationship between three variables and number of scaled mutations in single cells after adjustment using the residuals from negative binomial regression.



**Supplementary Figure 7:** a. Overlap of age-associated genes in CD8<sup>+</sup> T cells between MELR and MERF models. The percentages of overlaps were incremented by the top 100, 200, and 300 genes identified by MELR based on FDR and coefficient of age. Percentages are grouped by if the genes were up or downregulated across aging as identified by the MELR method. b. Scatter plot of the coefficient of age (CO) of age-associated genes identified by MELR and the percentages of cells expressing each gene. The average change (CO) and percentage of each six groups are presented.

Supplementary Table 1 Information of participants' age, gender, number of CD8 T cells, composition of CD8 T cell subpopulations by scRNAseq and coverage by exome sequencing

| Donor ID | Age | Sex | CD8 T cell <sup>a</sup> | Cluster Percentages |      |      |      |      |      |      |       |       |      | Reads (M) <sup>b</sup> |         | Coverage (Mb) <sup>c</sup> |        |
|----------|-----|-----|-------------------------|---------------------|------|------|------|------|------|------|-------|-------|------|------------------------|---------|----------------------------|--------|
|          |     |     |                         | Nc                  | Na   | SCM  | CM   | EM1  | EM2  | EM3  | EMRA1 | EMRA2 | EFF  | Exome                  | Genome  | Exome                      | Genome |
| sc_d1    | 0   | M   | 5185                    | 0.96                | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00  | 0.00  | 0.04 | 109,496                | 151,321 | 33                         | 117    |
| sc_d2    | 0   | F   | 3836                    | 0.95                | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00  | 0.00  | 0.05 | 107,497                | 148,993 | 33                         | 118    |
| sc_d3    | 26  | M   | 5525                    | 0.00                | 0.64 | 0.02 | 0.05 | 0.06 | 0.01 | 0.15 | 0.04  | 0.03  | 0.01 | 115,366                | 156,665 | 33                         | 116    |
| sc_d4    | 28  | F   | 4704                    | 0.00                | 0.25 | 0.02 | 0.12 | 0.22 | 0.01 | 0.05 | 0.27  | 0.04  | 0.01 | 111,847                | 154,610 | 33                         | 117    |
| sc_d5    | 37  | F   | 5591                    | 0.00                | 0.32 | 0.03 | 0.17 | 0.12 | 0.01 | 0.05 | 0.29  | 0.01  | 0.01 | 104,399                | 142,859 | 33                         | 115    |
| sc_d6    | 46  | M   | 6635                    | 0.00                | 0.27 | 0.02 | 0.09 | 0.13 | 0.04 | 0.03 | 0.09  | 0.25  | 0.09 | 117,520                | 160,829 | 33                         | 117    |
| sc_d7    | 53  | F   | 4351                    | 0.00                | 0.39 | 0.04 | 0.12 | 0.36 | 0.01 | 0.01 | 0.04  | 0.02  | 0.01 | 108,834                | 150,416 | 33                         | 117    |
| sc_d8    | 64  | M   | 4944                    | 0.00                | 0.08 | 0.29 | 0.13 | 0.11 | 0.02 | 0.16 | 0.16  | 0.03  | 0.03 | 97,985                 | 134,322 | 33                         | 114    |
| sc_d9    | 68  | F   | 4323                    | 0.00                | 0.32 | 0.06 | 0.05 | 0.07 | 0.01 | 0.45 | 0.00  | 0.00  | 0.03 | 112,257                | 153,600 | 33                         | 117    |
| sc_d10   | 76  | F   | 5322                    | 0.00                | 0.04 | 0.36 | 0.07 | 0.19 | 0.02 | 0.03 | 0.08  | 0.20  | 0.01 | 109,832                | 150,430 | 33                         | 117    |
| sc_d11   | 77  | M   | 4899                    | 0.00                | 0.31 | 0.10 | 0.13 | 0.14 | 0.02 | 0.06 | 0.10  | 0.11  | 0.02 | 114,630                | 156,209 | 33                         | 116    |
| sc_d12   | 83  | M   | 4908                    | 0.00                | 0.03 | 0.16 | 0.14 | 0.11 | 0.02 | 0.32 | 0.12  | 0.06  | 0.04 | 104,323                | 143,523 | 33                         | 114    |
| sc_d13   | 83  | F   | 5839                    | 0.00                | 0.04 | 0.12 | 0.23 | 0.29 | 0.02 | 0.03 | 0.08  | 0.11  | 0.09 | 103,256                | 141,517 | 33                         | 113    |
| sc_d14   | 84  | M   | 4956                    | 0.00                | 0.06 | 0.03 | 0.05 | 0.18 | 0.01 | 0.05 | 0.54  | 0.07  | 0.01 | 103,805                | 142,393 | 33                         | 114    |
| sc_d15   | 88  | M   | 5217                    | 0.00                | 0.04 | 0.09 | 0.11 | 0.30 | 0.02 | 0.07 | 0.09  | 0.27  | 0.01 | 116,460                | 159,003 | 33                         | 117    |
| sc_d16   | 90  | F   | 6865                    | 0.00                | 0.04 | 0.12 | 0.17 | 0.08 | 0.02 | 0.02 | 0.51  | 0.01  | 0.02 | 107,770                | 146,975 | 33                         | 115    |
| L1-1     | 30  | F   | 2329                    | 0.00                | 0.33 | 0.06 | 0.06 | 0.11 | 0.01 | 0.08 | 0.20  | 0.15  | 0.00 | 92,495                 | 138,104 | 33                         | 122    |
| L1-2     | 39  | F   | 1832                    | 0.00                | 0.63 | 0.04 | 0.02 | 0.07 | 0.00 | 0.02 | 0.07  | 0.13  | 0.00 |                        |         |                            |        |
| L2-1     | 32  | M   | 3878                    | 0.00                | 0.25 | 0.14 | 0.07 | 0.19 | 0.04 | 0.07 | 0.10  | 0.14  | 0.00 | 120,847                | 175,192 | 33                         | 132    |
| L2-2     | 40  | M   | 2030                    | 0.00                | 0.23 | 0.13 | 0.08 | 0.24 | 0.03 | 0.09 | 0.13  | 0.07  | 0.00 |                        |         |                            |        |
| L3-1     | 48  | M   | 2386                    | 0.00                | 0.11 | 0.08 | 0.16 | 0.09 | 0.01 | 0.05 | 0.45  | 0.05  | 0.00 | 98,196                 | 144,562 | 33                         | 128    |
| L3-2     | 56  | M   | 3141                    | 0.00                | 0.04 | 0.04 | 0.12 | 0.06 | 0.01 | 0.05 | 0.59  | 0.09  | 0.01 |                        |         |                            |        |
| L4-1     | 54  | F   | 450                     | 0.00                | 0.50 | 0.36 | 0.03 | 0.03 | 0.00 | 0.00 | 0.01  | 0.06  | 0.00 | 101,174                | 148,152 | 33                         | 129    |
| L4-2     | 65  | F   | 5187                    | 0.00                | 0.35 | 0.51 | 0.10 | 0.01 | 0.00 | 0.00 | 0.01  | 0.01  | 0.00 |                        |         |                            |        |
| L5-1     | 60  | F   | 1414                    | 0.00                | 0.09 | 0.08 | 0.10 | 0.19 | 0.03 | 0.08 | 0.17  | 0.25  | 0.01 | 102,113                | 170,473 | 33                         | 143    |
| L5-2     | 65  | F   | 1536                    | 0.00                | 0.12 | 0.04 | 0.03 | 0.04 | 0.01 | 0.04 | 0.06  | 0.66  | 0.01 |                        |         |                            |        |
| L5-3     | 70  | F   | 2017                    | 0.00                | 0.06 | 0.04 | 0.04 | 0.17 | 0.02 | 0.05 | 0.39  | 0.23  | 0.01 | 102,005                | 151,478 | 33                         | 128    |
| L6-1     | 60  | M   | 2217                    | 0.00                | 0.06 | 0.07 | 0.11 | 0.12 | 0.02 | 0.05 | 0.34  | 0.23  | 0.00 |                        |         |                            |        |
| L6-2     | 71  | M   | 2091                    | 0.00                | 0.08 | 0.04 | 0.05 | 0.07 | 0.01 | 0.01 | 0.38  | 0.35  | 0.00 | 91,381                 | 135,938 | 33                         | 130    |
| L7-1     | 69  | F   | 118                     | 0.00                | 0.03 | 0.04 | 0.01 | 0.03 | 0.03 | 0.40 | 0.11  | 0.26  | 0.08 |                        |         |                            |        |
| L7-2     | 78  | F   | 2632                    | 0.00                | 0.08 | 0.18 | 0.04 | 0.26 | 0.12 | 0.11 | 0.06  | 0.14  | 0.00 | 104,046                | 154,062 | 33                         | 122    |
| L8-1     | 69  | M   | 2108                    | 0.00                | 0.40 | 0.19 | 0.11 | 0.17 | 0.02 | 0.04 | 0.03  | 0.04  | 0.00 |                        |         |                            |        |
| L8-2     | 77  | M   | 1952                    | 0.00                | 0.25 | 0.48 | 0.18 | 0.04 | 0.01 | 0.02 | 0.01  | 0.02  | 0.00 |                        |         |                            |        |

<sup>a</sup> Cell number was determined by scRNAseq data<sup>b</sup> Number of sequencing reads in each donor exome sequencing file<sup>c</sup> Calculated as the number of basepairs that were covered by at least 10 sequencing reads (10X depth)

Supplementary Table 2 Selected enriched gene expression function in CD8 T cell subpopulations

| Description                                                                      | ID         | setSize | NES  | qvalues | Enriched genes (selected)                                               | Number of enriched gene | CD8 T sobpopulation |
|----------------------------------------------------------------------------------|------------|---------|------|---------|-------------------------------------------------------------------------|-------------------------|---------------------|
| RNA localization                                                                 | GO:0006403 | 203     | 1.71 | 0.010   | TGFB2/CCT4/CCT3/TPR/CCT5/CCT6A/CCT2/UFP3A/RBM26/ATM/NFM1/NOP10/ZFP36L1  | 15                      | Nc                  |
| protein-DNA complex subunit organization                                         | GO:0071824 | 165     | 1.55 | 0.025   | PTMA/PARP1/NAP1L1/CAND1/MYCNFM1/HMGA1/ANP32B/SET/RBX1/NAP1L4/RPL23/UBTF | 13                      | Nc                  |
| telomere maintenance                                                             | GO:0000723 | 123     | 1.61 | 0.019   | CCT4/CCT3/PARP1/CCT5/CCT6A/APEX1/CCT2/MYCATM/POLD4/HSP90AB1/NOP10       | 12                      | Nc                  |
| regulation of DNA biosynthetic process                                           | GO:2000278 | 76      | 1.64 | 0.026   | CCT4/CCT3/CCT5/CCT6A/RGCC/CCT2/MYCATM/HSP90AB1/ARRB2                    | 10                      | Nc                  |
| methylation                                                                      | GO:0032259 | 261     | 1.49 | 0.020   | SATB1/MTERF4/DPY30/PARP1/PRMT2/MA22B/MYCDPH5/PRMT1/GSTO1                | 10                      | Nc                  |
| DNA conformation change                                                          | GO:0071103 | 183     | 1.61 | 0.010   | TPR/PARP1/NAP1L1/SMC4/NFM1/HMGA1/ANP32B/SET/RBX1/NAP1L4                 | 10                      | Nc                  |
| chromatin remodeling                                                             | GO:0006338 | 126     | 1.57 | 0.032   | SATB1/PTMA/TPR/CBX3/MYCNFM1/HMGA1/HDAC1                                 | 8                       | Nc                  |
| DNA packaging                                                                    | GO:0006323 | 91      | 1.82 | 0.010   | TPR/NAP1L1/SMC4/NFM1/HMGA1/ANP32B/SET/NAP1L4                            | 8                       | Nc                  |
| cellular amino acid metabolic process                                            | GO:0006520 | 200     | 1.46 | 0.035   | PSMB4/PSMA5/SERINC5/PRM1K/EGLN2/PPA1/PSMA4                              | 7                       | Nc                  |
| telomerase RNA localization to Cajal body                                        | GO:0090671 | 19      | 1.92 | 0.010   | CCT4/CCT3/CCT5/CCT6A/CCT2/NOP10                                         | 6                       | Nc                  |
| ribosome localization                                                            | GO:0033750 | 13      | 1.96 | 0.011   | NPM1/RPS15                                                              | 2                       | Na                  |
| chaperone-mediated autophagy                                                     | GO:0061684 | 12      | 1.88 | 0.034   | HSPA8                                                                   | 1                       | SCM                 |
| negative regulation of proteasomal ubiquitin-dependent protein catabolic process | GO:0032435 | 29      | 1.92 | 0.018   | RPL11                                                                   | 1                       | SCM                 |
| regulation of intrinsic apoptotic signaling pathway                              | GO:2001242 | 117     | 1.53 | 0.044   | SOD1/TPP1/RPL11/MIF/CYLD                                                | 5                       | SCM                 |
| response to antibiotic                                                           | GO:0046677 | 168     | 1.49 | 0.049   | TXNIP/SOD1/TNFAIP3/CDKN1B/KLF2/GATA3/ANXA1/LDHA/CRP1/EEF2               | 10                      | SCM                 |
| export across plasma membrane                                                    | GO:0140115 | 12      | 1.95 | 0.020   | FXR2                                                                    | 1                       | CM                  |
| negative regulation of endothelial cell apoptotic process                        | GO:2000352 | 10      | 2.00 | 0.022   | TNFAIP3                                                                 | 1                       | CM                  |
| T cell apoptotic process                                                         | GO:0070231 | 32      | 1.76 | 0.045   | IL7R/TSC22D3                                                            | 2                       | CM                  |
| homeostasis of number of cells                                                   | GO:0048872 | 164     | 1.57 | 0.020   | IL7R/TNFAIP3/LYAR/FLT3LG/TSC22D3/BCL2                                   | 6                       | CM                  |
| negative regulation of transmembrane transport                                   | GO:0034763 | 50      | 1.75 | 0.015   | ARL6IP5/YWHAO/BN1/CALM2/SRRTGFB1/CALM3/PRKCB/CALM1/OAZ1/SLC9A3R1        | 11                      | BM2                 |
| positive regulation of calcium-mediated signaling                                | GO:0050850 | 22      | 1.78 | 0.011   | CALM2/CALM3/CIB1/CCL4/CALM1                                             | 5                       | BM2                 |
| regulation of lymphocyte apoptotic process                                       | GO:0070228 | 34      | 1.75 | 0.020   | CD74/CD27/CD3G/PRELI1/BAV/CCL5                                          | 6                       | BM2                 |
| regulation of phosphatase activity                                               | GO:0010921 | 109     | 1.53 | 0.033   | GNAI2/PPP1R7/PLEK/CALM2/PTPRC/ENSA/FKBP1A/PPP1R12A/MNKK1/CALM3/CALM1    | 12                      | BM2                 |
| transcription elongation from RNA polymerase II promoter                         | GO:0006368 | 77      | 1.61 | 0.032   | NELFCD/CNHPOLR2J/ELOC/POLR2F/ELOB/POLR2G/RTF1/EAPP                      | 9                       | BM2                 |
| protein polyubiquitination                                                       | GO:0000209 | 245     | 1.56 | 0.004   | PSMA5/PSMD4/PSMB4/PSMA7/RBCK1/PSMB1/PSMA2/TNFAIP3/UBC/UBE2N/RNF19A      | 30                      | BM2                 |
| positive regulation of T cell proliferation                                      | GO:0042102 | 53      | 1.68 | 0.023   | PTPRCHMGB1/HLA-DPA1/HLA-DPB1/HLA-E/ANXA1/CORO1A/PYCARD/CD81/CCL5        | 10                      | BM2                 |
| regulation of mRNA splicing, via spliceosome                                     | GO:0048024 | 75      | 1.59 | 0.038   | SRSF7/RBM8A/SON/SRSF9/RBM25/DDX5                                        | 6                       | BM2                 |
| regulation of nuclear division                                                   | GO:0051783 | 101     | 1.56 | 0.032   | TPR/CHMP2A/TGFB1/CALR/PIN1/FBXW5/BUB3                                   | 7                       | BM2                 |
| G2/M transition of mitotic cell cycle                                            | GO:0000086 | 183     | 1.73 | 0.003   | TUBA4A/PSMA5/PSMD4/ENSA/PSMB4/PSMA7/CDC25B/CCNH/PSMB1/PSMA2             | 31                      | BM2                 |
| maintenance of protein localization in organelle                                 | GO:0072595 | 34      | 1.73 | 0.027   | SYNE1/PARK7/TXNHSPA5/SUN2                                               | 5                       | BMRA2               |
| phosphatidylcholine metabolic process                                            | GO:0046470 | 38      | 1.65 | 0.049   | DBI/CAPN2/PLAAT4                                                        | 3                       | BMRA2               |
| positive regulation of protein autophosphorylation                               | GO:0031954 | 13      | 1.73 | 0.038   | IQGAP1/CALM1                                                            | 2                       | BMRA2               |
| positive regulation of protein complex disassembly                               | GO:0043243 | 23      | 1.70 | 0.046   | PLEK/DSTN/WDR1/CFL1                                                     | 4                       | BMRA2               |
| regulation of focal adhesion assembly                                            | GO:0051893 | 33      | 1.73 | 0.031   | RHOA/ITGB1BP1/RAC1/MACF1/IQGAP1/ROCK1                                   | 6                       | BMRA2               |
| covalent chromatin modification                                                  | GO:0016569 | 357     | 1.52 | 0.007   | MECP2/ARID4B/ASH1L/USP16/KMT2E/USP15/ATM/RSP1/KMT2A/SFPQ/EPC1/ATRX      | 15                      | EFF                 |
| glycoprotein metabolic process                                                   | GO:0009100 | 233     | 1.60 | 0.007   | RPN2/CHST12/ITM2B/TMEM165/TMEM59/SPOCK2/ITM2A/OGA/CCR7                  | 9                       | EFF                 |
| mRNA export from nucleus                                                         | GO:0006406 | 102     | 1.60 | 0.031   | SRSF7/TPRH/NRNPA2B1/DDX39B/SRSF11/UFP2/SRSF5/SRSF2                      | 8                       | EFF                 |
| alpha-beta T cell proliferation                                                  | GO:0046633 | 22      | 2.16 | 0.007   | ZAP70/PTPRC/IRF1/CD3E/HLA-E/RASAL3/VSIR                                 | 7                       | EFF                 |
| positive regulation of cytokine biosynthetic process                             | GO:0042108 | 33      | 1.81 | 0.016   | PTPRC/CD74/IRF1/CD3E/SPN/STAT3                                          | 6                       | EFF                 |
| STAT cascade                                                                     | GO:0097696 | 70      | 1.66 | 0.026   | STAT4/PTPRC/IL7R/IL10RA/STAT3/PTPN2                                     | 6                       | EFF                 |
| negative regulation of lymphocyte mediated immunity                              | GO:0002707 | 21      | 2.13 | 0.007   | PTPRC/IL7R/CD98/HLA-E/HLA-F                                             | 5                       | EFF                 |
| response to interleukin-7                                                        | GO:0098760 | 22      | 1.79 | 0.025   | IL7R/IL2RG/STAT3/FDIA3/P4HB                                             | 5                       | EFF                 |
| histone H3-K9 modification                                                       | GO:0061647 | 32      | 1.78 | 0.028   | MECP2/ARID4B/ASH1L/KMT2A/ATRX                                           | 5                       | EFF                 |
| T-helper cell differentiation                                                    | GO:0042093 | 40      | 1.74 | 0.030   | FOXPI/ANXA1/SPN/STAT3/RORA                                              | 5                       | EFF                 |

**Supplementary Table 3. Antibodies used in this study**

| Antibodies            | fluorochrome | Clone     | Dilution | Company     | Catalog#   | Used in                                |
|-----------------------|--------------|-----------|----------|-------------|------------|----------------------------------------|
| CD8                   | FITC         | HIT8a     | 1:20     | BioLegend   | 300906     | Longitudinal cell labeling and sorting |
| CD14                  | Pacific Blue | HCD14     | 1:20     | BioLegend   | 325616     |                                        |
| TotalSeq™-A0386 CD28  | N/A          | CD28.2    | 1:100    | BioLegend   | 302955     |                                        |
| TotalSeq™-A0063CD45RA | N/A          | HI100     | 1:100    | BioLegend   | 304157     |                                        |
| CD3                   | BV510        | OKT3      | 1:20     | BioLegend   | 317332     | Cross sectional Cell sorting           |
| CD19                  | BV421        | HIB19     | 1:20     | BioLegend   | 302234     |                                        |
| CD4                   | PE           | OKT4      | 1:20     | BioLegend   | 317410     |                                        |
| CD8                   | FITC         | HIT8a     | 1:20     | BioLegend   | 300906     |                                        |
| B2M                   | BB515        | 2M2       | 1:100    | BioLegend   | 316304     | Multi-color phenotyping                |
| CD127                 | BV711        | A019D5    | 1:50     | BioLegend   | 351328     |                                        |
| CD18                  | AF700        | TS1/18    | 1:100    | BioLegend   | 302124     |                                        |
| CD184                 | BUV737       | 12G5      | 1:1000   | BD          | 741862     |                                        |
| CD2                   | BV605        | RPA-2.10  | 1:100    | BioLegend   | 300224     |                                        |
| CD27                  | BUV395       | L128      | 1:20     | BD          | 563815     |                                        |
| CD28                  | BV785        | CD28.2    | 1:50     | BioLegend   | 302950     |                                        |
| CD28                  | BV711        | CD28.2    | 1:100    | BD          | 563131     |                                        |
| CD3                   | BV570        | UCHT1     | 1:50     | BioLegend   | 300436     |                                        |
| CD4                   | BB700        | SK3       | 1:1000   | BD          | 566393     |                                        |
| CD45RA                | PE-Cy7       | HI100     | 1:100    | BioLegend   | 304126     |                                        |
| CD45RO                | BV605        | UCHL1     | 1:100    | BD          | 562791     |                                        |
| CD62L                 | APC          | DREG-56   | 1:100    | BioLegend   | 304809     |                                        |
| CD62L                 | BV786        | DREG-56   | 1:100    | BioLegend   | 304830     |                                        |
| CD69                  | BV650        | FN50      | 1:20     | BioLegend   | 310934     |                                        |
| CD8                   | BUV496       | RPA-T8    | 1:50     | BD          | 564804     |                                        |
| CD95                  | PE/Cy5       | DX2       | 1:100    | BioLegend   | 305610     |                                        |
| EOMES                 | eF710        | WD1928    | 1:100    | eBioscience | 44-4877-42 |                                        |
| GZMA                  | APC          | CB9       | 1:20     | BioLegend   | 507220     |                                        |
| GZMB                  | AF700        | GB11      | 1:100    | BD          | 560213     |                                        |
| KLRG1                 | PE           | 2F1/KLRG1 | 1:20     | BioLegend   | 138408     |                                        |
| PD-1                  | BUV737       | EH12.1    | 1:20     | BD          | 565299     |                                        |
| Perforin              | PE-CF594     | 8G9       | 1:100    | BD          | 563763     |                                        |

| Supplementary Table 4 Sequences of primers used to construct antibody-derived tag (ADT) libraries |                                                                |             |
|---------------------------------------------------------------------------------------------------|----------------------------------------------------------------|-------------|
| Primer name                                                                                       | Primer sequences                                               | Source      |
| R2-PCR handle primer for 1st round                                                                | 5'-GTGACTGGAGTTCAGACGTGTCCTTGGCA CCCGAGAA TTCC-3'              | IDT         |
| Reverse primer for 1st round                                                                      | 5'-AATGATACGGCGACCA CCGA-GA TCTACACTCTTCCCTACACGACGCTC-3'      | IDT         |
| P5 forward primer for 2nd round                                                                   | 5'-AATGATACGGCGACCA CCGAGA-TCTACACTCTTCCCTACACGACGCTC-3'       | IDT         |
| Individual sample index primer for 2nd round                                                      | 5'-CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN-GTGA CTGGAGTTCAGACGTGT-3' | 10xGenomics |