Figure S1, related to main Figure 3: Gating strategy for sorting of CD101 and Tim3 subsets for RNA-sequencing at day 15 and 45 post-infection. A) Gating strategy for isolation of CD4⁻CD19⁻ lymphocytes. A representative presort sample is shown. B) Presort frequencies of PD-1⁺CD8⁺ CD4⁻CD19⁻ lymphocytes and expression of CD101 and Tim3 on PD-1⁺CD8⁺ cells. C-E) Postsort frequencies and Tim3/CD101 expression of CD101⁻Tim3⁻ cells (C), CD101⁻Tim3⁺ cells (E), and CD101⁺Tim3⁺ cells (D). The same representative sample is shown after cell sorting.
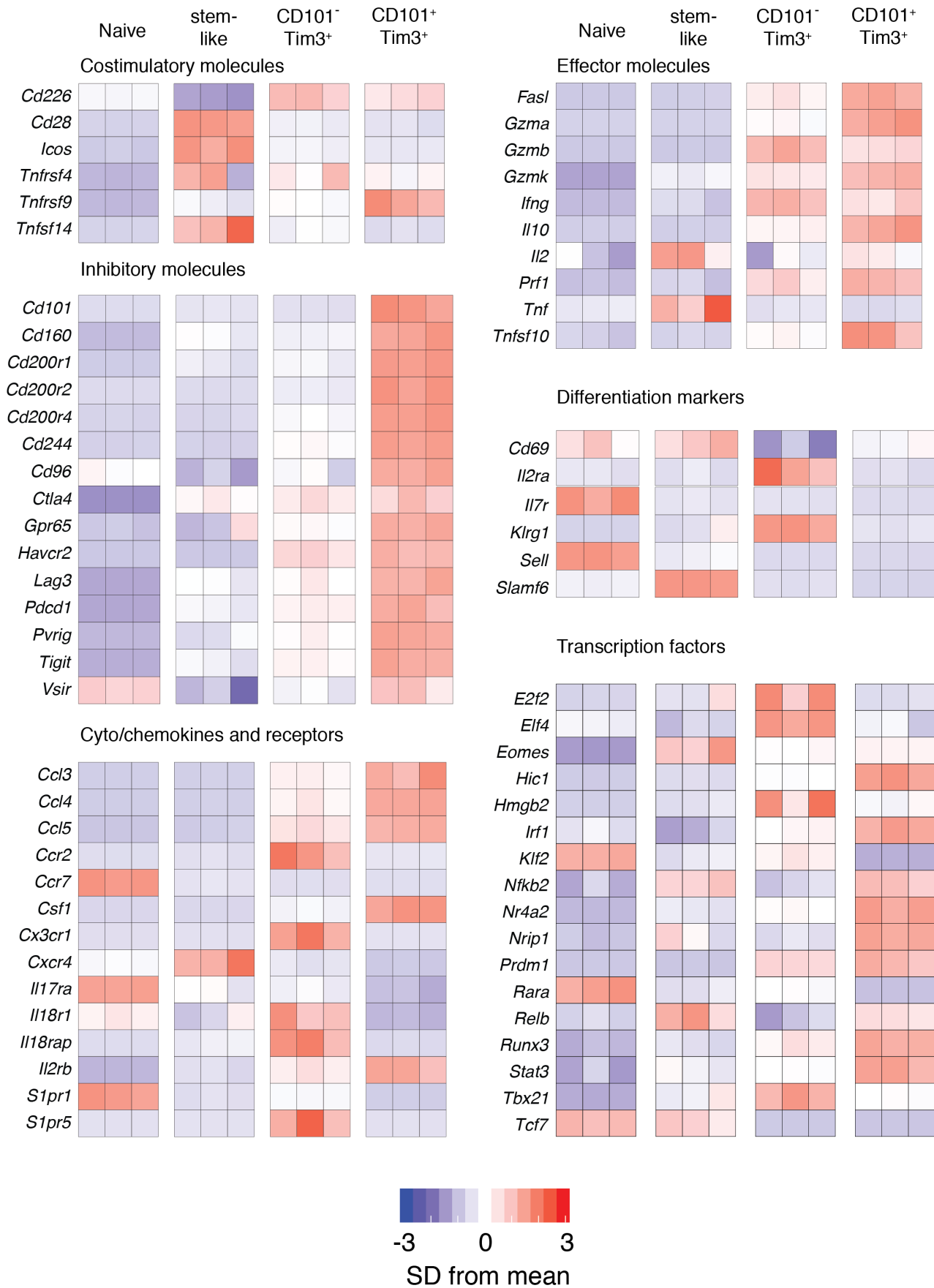
Figure S2, related to main Figure 3: Relative expression of selected genes in stem-like, transitory CD101⁻ Tim3⁺ cells, and exhausted CD101⁺Tim3⁺ cells at day 45 post-infection. Expression shown is standard deviation from mean (z-score) for all samples sequenced; absolute expression is shown in Table S2.
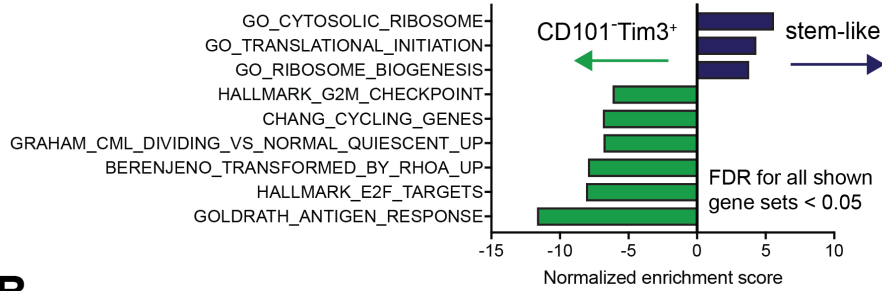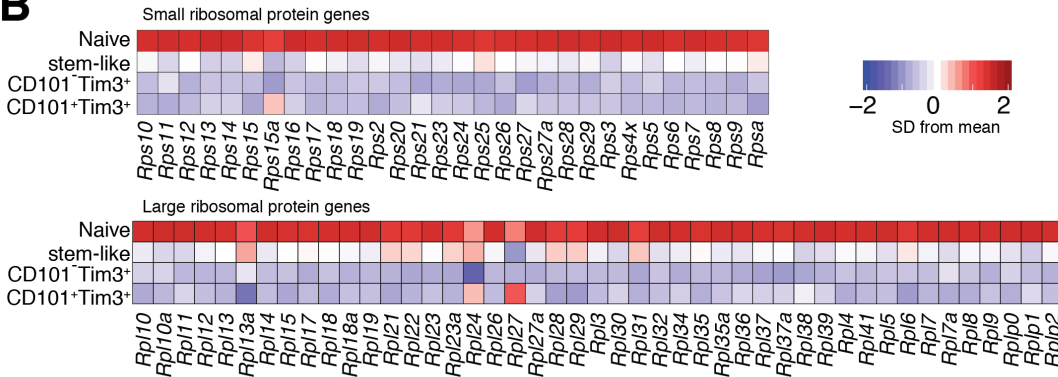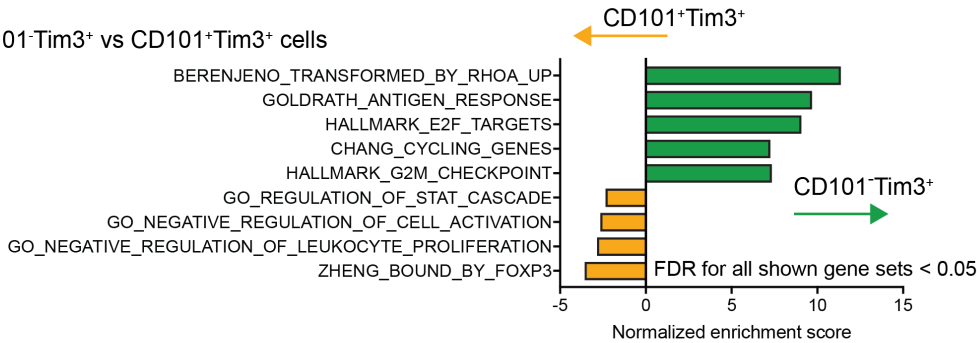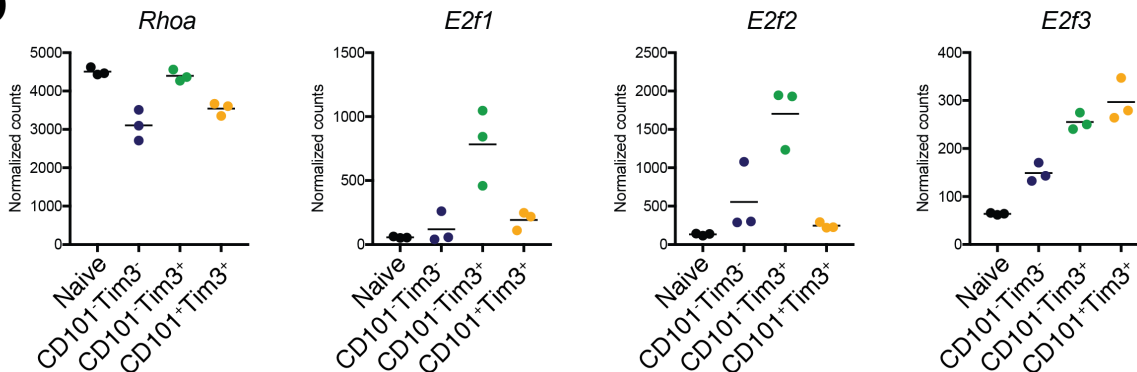
Figure S3, related to main Figure 3: GSEA reveals divergent transcriptional pathways among stem-like, CD101⁻Tim3⁺, and CD101⁺Tim3⁺ CD8⁺ T cells from chronic infection. A) Selected gene sets enriched in stem-like cells when compared to CD101⁻Tim3⁺ cells. Ribosomal gene sets were notably enriched in stem-like cells. B) Relative expression of small and large ribosomal subunit genes among all cell types sequenced. C) Selected enriched gene sets when comparing CD101⁻Tim3⁺ CD8⁺ T cells to CD101⁻Tim3⁺ cells. Proliferative gene sets were enriched in CD101⁻Tim3⁺ cells, including genes regulated by RhoA and E2F. D) Expression of *Rhoa* and E2F-family genes in CD8⁺ T cell subsets in chronic infection. Complete GSEA results from day 45 post-infection are shown in **Table S3**.

A) Gene list: genes highly expressed in CD101⁻Tim3⁺ cells ;
Expression levels: CD8⁺ T cells from acute LCMV Armstrong infection
(Hudson et al., *Nat Commun* 2019)

B) Gene list: genes highly expressed in CD101⁺Tim3⁺ cells ;
Expression levels: CD8⁺ T cells from acute LCMV Armstrong infection
(Hudson et al., *Nat Commun* 2019)

SD from mean
(LCMV Armstrong)

C)

chronic CD101⁻Tim3⁺ — LCMV clone 13

125 gene sets shared — 791 gene sets shared

d8 CD127⁺Klrg1⁻ — d8 CD127⁺Klrg1⁻ — LCMV Armstrong

GO_CELLULAR_RESPONSE_TO_CYTOKINE_STIMULUS
GO_CYTOKINE_MEDIATED_SIGNALING_PATHWAY
HALLMARK_INFLAMMATORY_RESPONSE

HALLMARK_E2F_TARGETS
CHANG_CYCLING_GENES
GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_UP
GOLDRATH_ANTIGEN_RESPONSE
GO_LEUKOCYTE_MIGRATION
GO_LEUKOCYTE_CELL_CELL_ADHESION

chronic CD101⁺Tim3⁺ — LCMV clone 13

7 gene sets shared — 5 gene sets shared

d8 CD127⁺Klrg1⁻ — d8 CD127⁺Klrg1⁻ — LCMV Armstrong

ZHENG_BOUND_BY_FOXP3
LEE_BMP2_TARGETS_UP

GO_REGULATION_OF_STAT_CASCADE
KARLSSON_TGFB1_TARGETS_DN

Figure S4, related to main Figure 3: CD101⁻Tim3⁺ cells from chronic LCMV infection express genes similar to Klrg1ʰⁱ effectors from acute LCMV Armstrong infection.

A) Heatmap of genes expressed predominately in CD101⁻Tim3⁺ CD8⁺ T cells during chronic infection (day >45). Color indicates relative expression of each gene in LCMV-specific CD8⁺ T cells isolated after LCMV Armstrong infection(Hudson et al., 2019). B) Heatmap of genes expressed predominately in CD101⁺Tim3⁺ during chronic infection (day >45). C) Number of gene sets shared between those enriched in CD101⁺Tim3⁺ cells (versus CD101⁻Tim3⁺ cells) and those enriched in day 8 CD127ʰⁱ (left) or Klrg1ʰⁱ (right) cells. Sample gene set names are shown. In parts (A) and (B), genes were selected by being highly differentially expressed (≥ 2-fold and p < 0.05) in the indicated subset versus both other subsets from chronic infection.
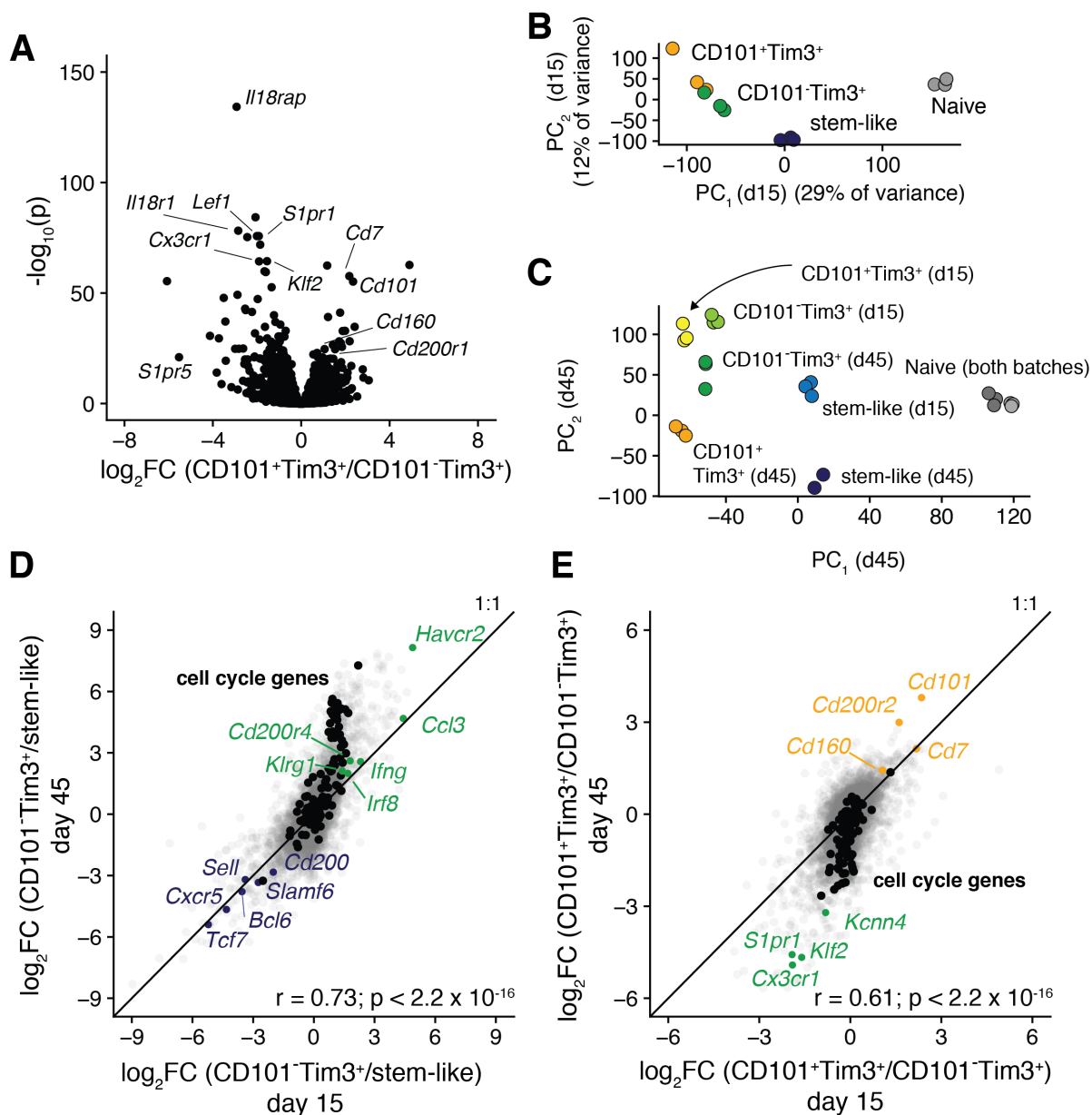
Figure S5, related to main Figure 3: Stem-like, CD101⁻Tim3⁺, and CD101⁺Tim3⁺ CD8⁺ T cells express similar transcriptional profiles at day 15 and day 45. RNA was isolated and sequenced from PD-1⁺CD8⁺ T cells of LCMV clone 13-infected mice (day 15). See **Figure S1** for gating strategy. A) Volcano plot showing differential gene expression between CD101⁺ Tim3⁺ and CD101⁻Tim3⁺ cells; 1,994 genes were differentially expressed between these two subsets at day 15 post-infection. B) PCA performed on day 15 RNA-sequencing data shows similar clustering of stem-like, CD101⁺ Tim3⁺ and CD101⁻Tim3⁺ cells as at day 45 (compare with **Figure 3B**). C) Day 15 samples were projected onto principal components from day 45 RNA-sequencing data (see **Figure 3B**). D) Plot showing fold changes of individual genes between CD101⁻Tim3⁺ cells and stem-like cells at day 45 (y-axis) and between the same subsets day 15 (x-axis). Differential expression of genes between these two subsets was very similar (Pearson's r = 0.73) at both time points. Notably, cell division associated genes are largely similar at day 15 but not day 45. However, expression of lineage-defining stem-like cell genes such as *Tcf7* (encoding Tcf-1) are firmly etched by day 15 post-infection. E) Plot showing fold changes of individual genes between CD101⁺Tim3⁺ cells and CD101⁻Tim3⁺ cells at day 45 (y-axis) and day 15 (x-axis). Differential expression of genes between these two subsets was also very similar (Pearson's r = 0.61) at both time points. Notably, cell division associated genes are largely similar at day 15 but not day 45. In panels D and E, cell cycle genes are from the "MITOTIC_CELL_CYCLE" gene set (http://amigo.geneontology.org/amigo/term/GO:0000278).