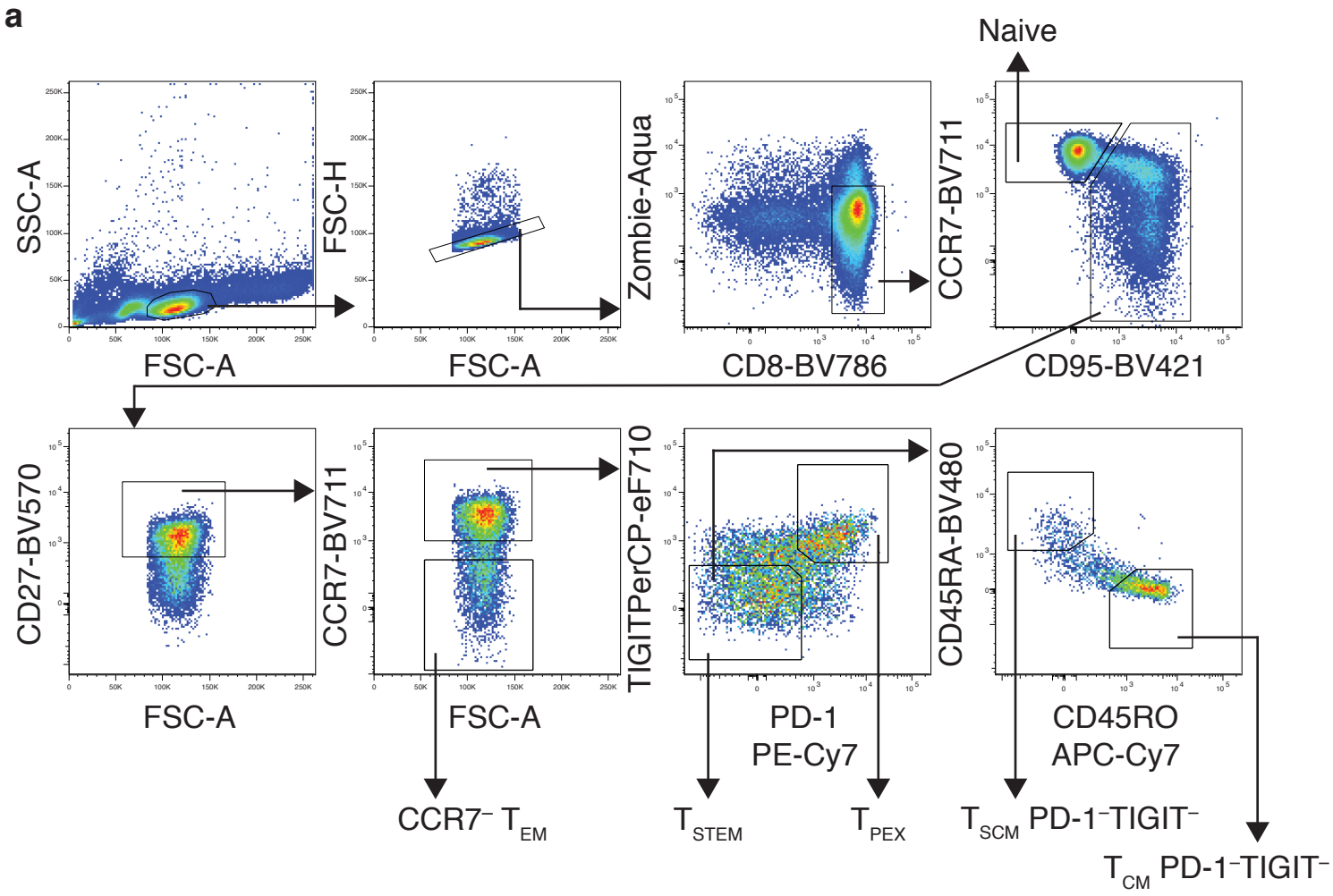
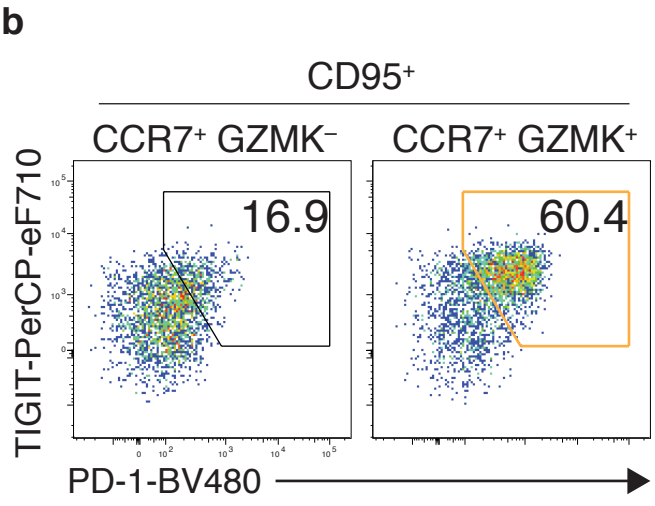
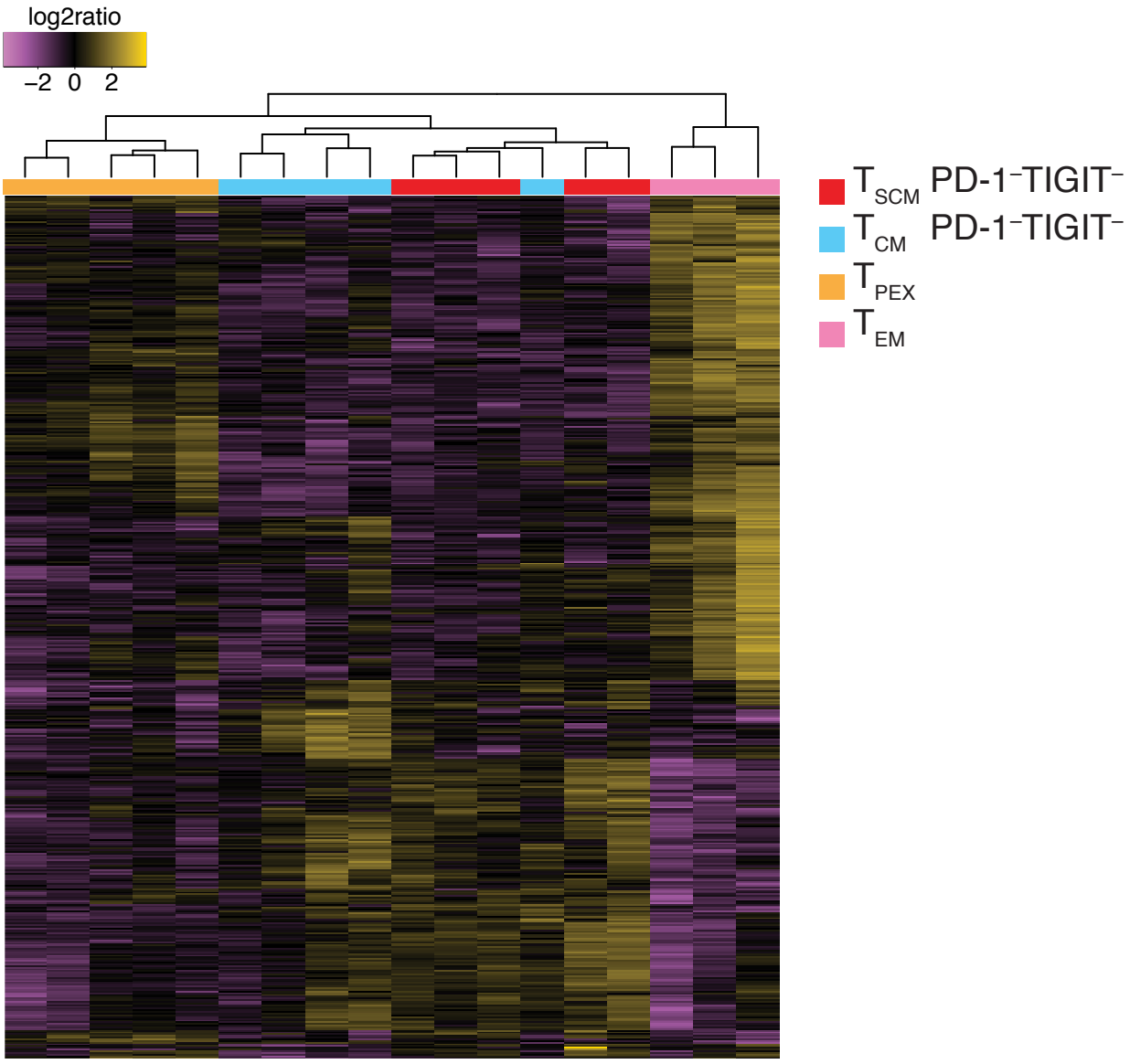
Supplementary Fig. 1





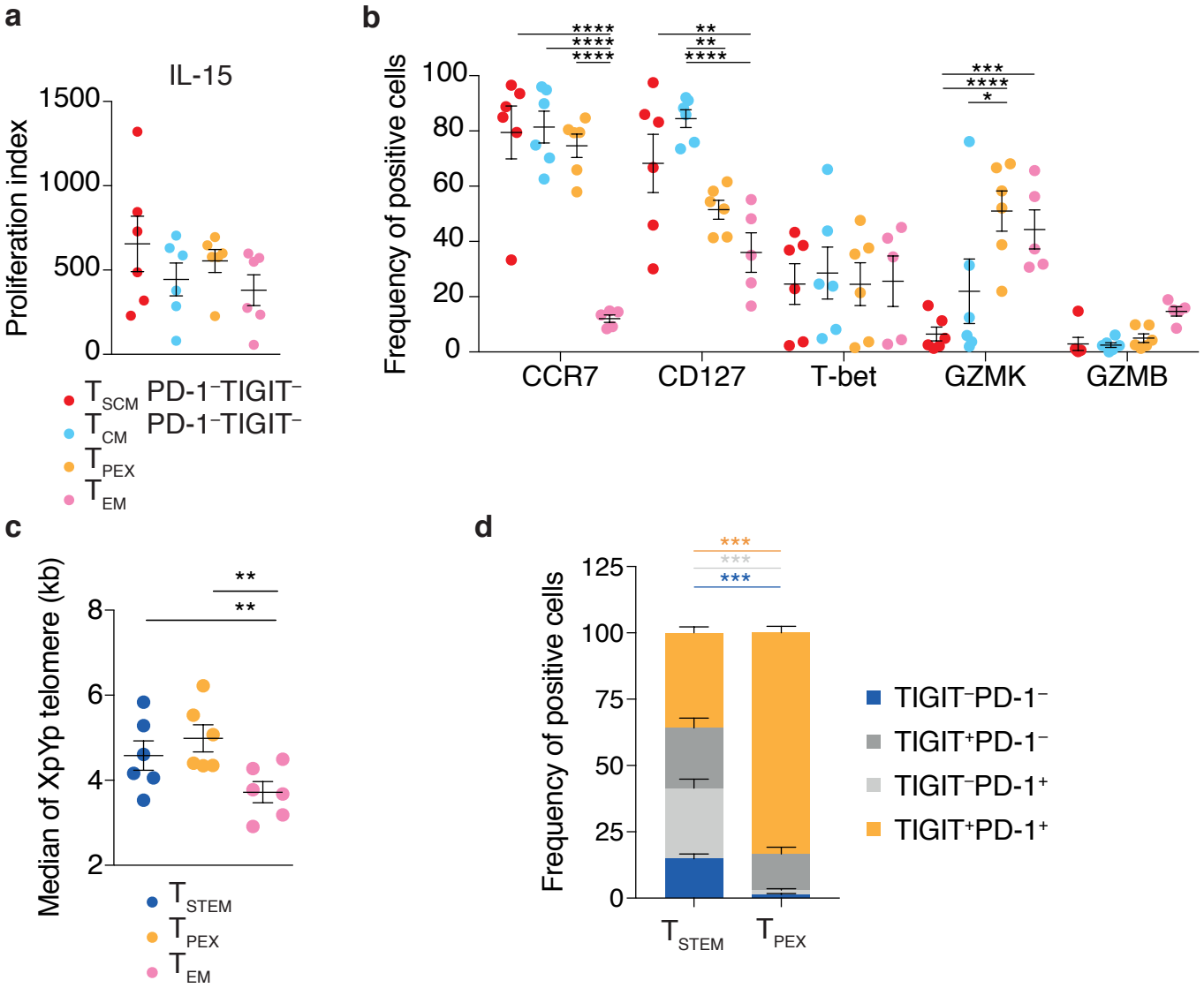
Supplementary Fig. 1. Strategy for the isolation of T cell subsets via FACS. a, Flow cytometric gating strategy for the isolation of $CD8^+$ naive, T_{STEM}, T_{SCM} PD-1^- $TIGIT^-$, T_{CM} PD-1^- $TIGIT^-$, T_{PEX}, and T_{EM} cells. **b**, Representative flow cytometric analysis of early differentiated $CD8^+$ memory T cells showing the expression of PD-1 and TIGIT.

Supplementary Fig. 2



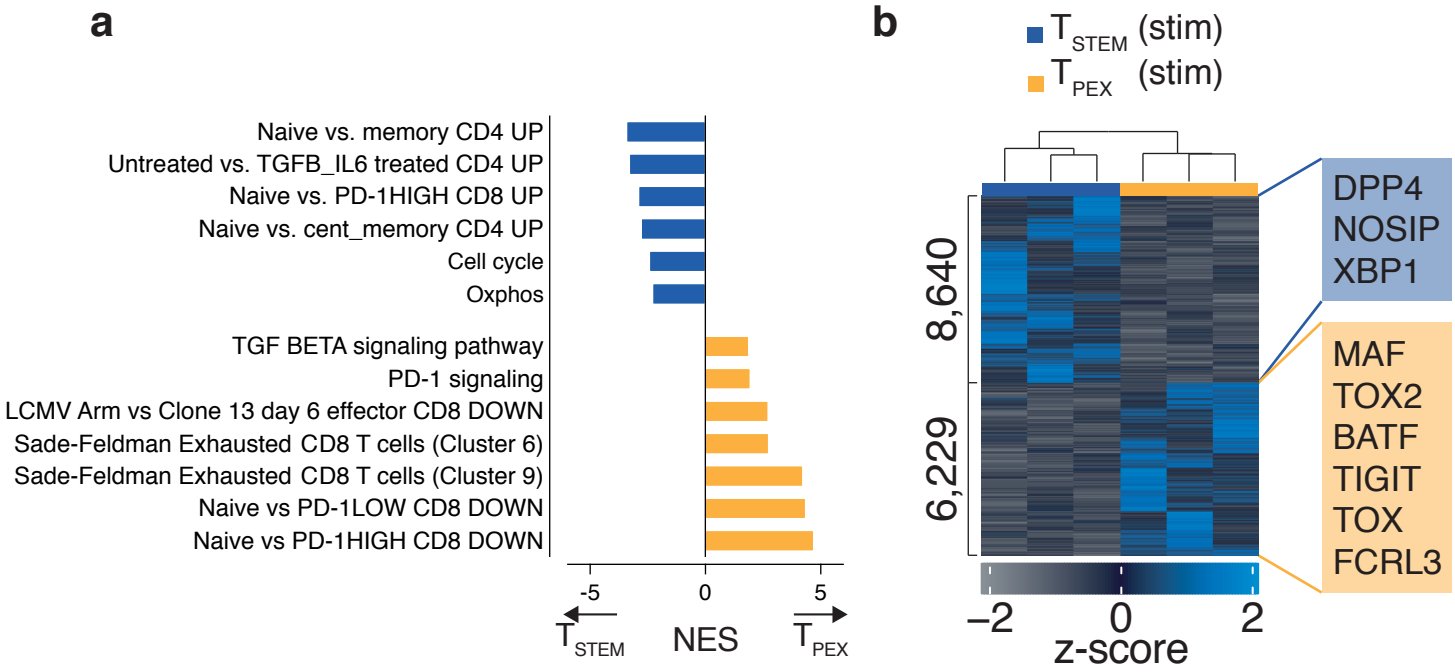
Supplementary Fig. 2. Transcriptomic comparison of T_{SCM} and T_{CM} cells after depletion of T_{PEX} cells. Heatmap showing DEGs (adjusted *P* value < 0.01) for the indicated $CD8^+$ memory T cell subsets (n = 3 donors for T_{EM}, n = 5 donors for T_{SCM}, T_{CM}, and T_{PEX}). Significance was evaluated using edgeR analysis with glmQLFTest and Benjamini-Hochberg correction.

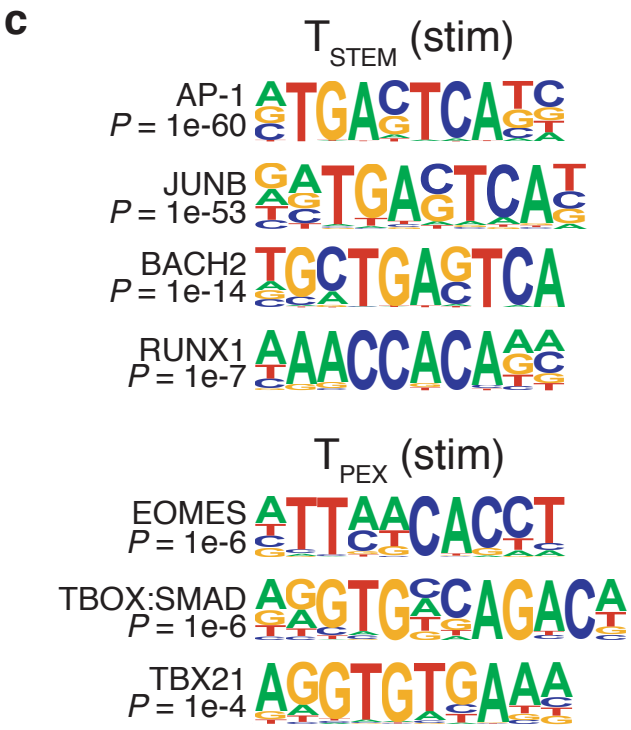
Supplementary Fig. 3



Supplementary Fig. 3. Proliferation and self-renewal capabilities of $CD8^+$ T cell subsets. a, Dot plot showing proliferation indices for the indicated FACS-purified $CD8^+$ T cell subsets after stimulation for 10 d with IL-15. Each dot represents one donor (n = 6 from four independent experiments). Bars indicate mean ± SEM. **b**, Dot plot showing the expression of selected markers among the indicated $CFSE^{dim}$ $CD8^+$ T cell subsets after stimulation as in **a**. Each dot represents one donor (n = 5 from four independent experiments for T_{EM}, n = 6 from four independent experiments for all other subsets). Bars indicate mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ (two-way ANOVA). **c**, Dot plot showing median telomere lengths for T_{STEM}, T_{PEX}, and T_{EM} cells. Each dot represents one donor (n = 6). Bars indicate mean ± SEM. $**P < 0.01$ (one-way repeated measures ANOVA). **d**, Bar graph summarizing the expression of PD-1 and TIGIT among FACS-purified T_{STEM} and T_{PEX} cells after stimulation with anti-CD3 plus CD28 for 4 d in the presence of IL-7 and IL-15 (n = 5 donors from three independent experiments). Bars indicate mean ± SEM. $***P < 0.001$ (two-tailed Mann-Whitney U test).

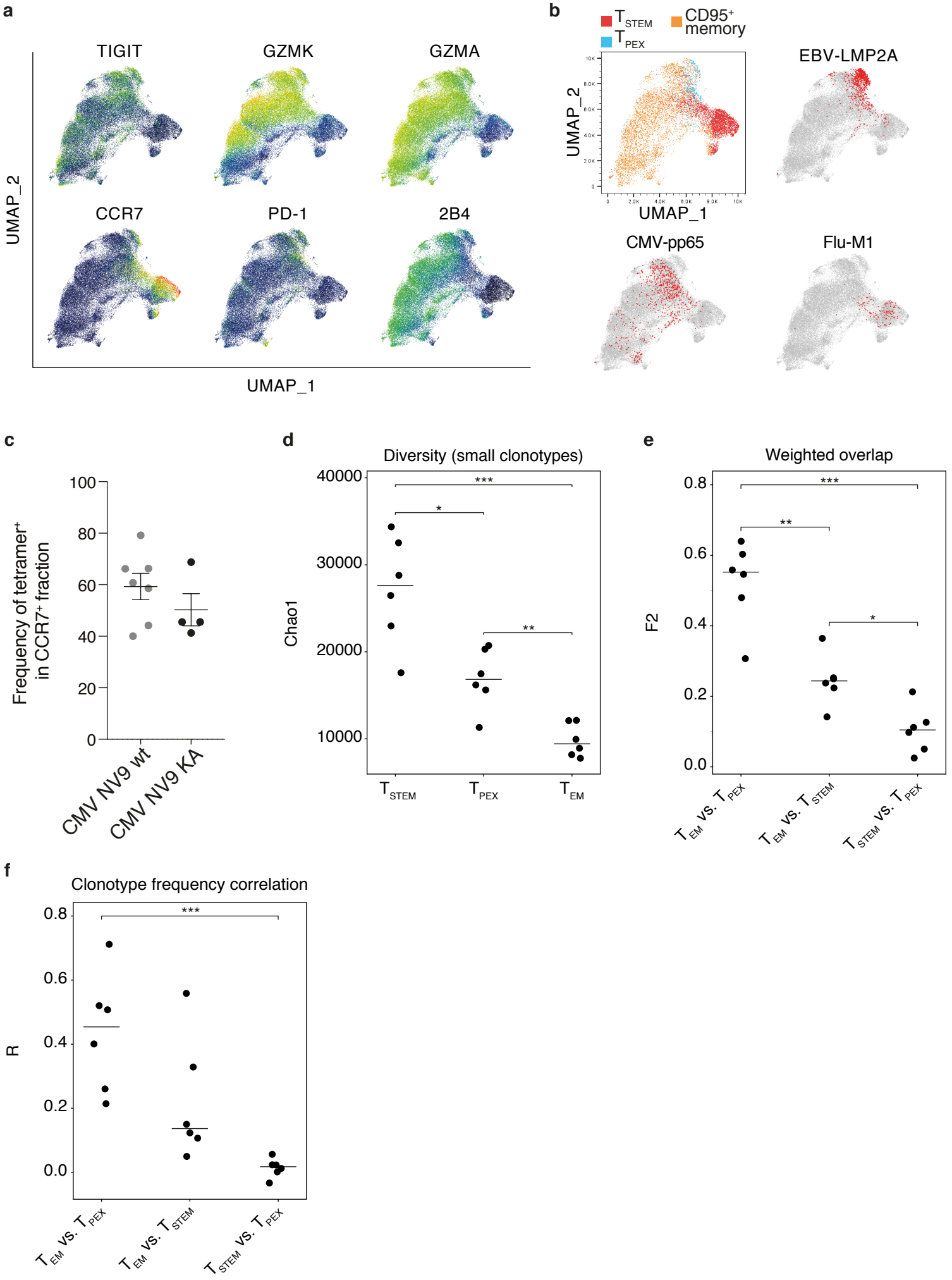
Supplementary Fig. 4





Supplementary Fig. 4. Epigenetic and transcriptomic comparison of activated T_{STEM} and T_{PEX} cells. FACS-purified T_{STEM} and T_{PEX} cells were stimulated with anti-CD3 plus CD28 for 4 d in the presence of IL-2 and IL-12. Cells were then processed for ATAC-seq (n = 3 donors) or RNA-seq (n = 4 donors). **a**, Normalized enrichment score (NES) of selected gene sets obtained from GSEA of the RNA-seq data in **Fig. 4h** (adjusted *P* value < 0.05 based on 1,000 permutations). **b**, Heatmap showing DARs related to the experiment in **Fig. 4i**. Labels highlight accessible genes associated with memory or effector differentiation or exhaustion. **c**, TFBMs enriched among the DARs identified between activated T_{STEM} and T_{PEX} cells in **Fig. 4i**. Enrichment was assessed using a one-sided hypergeometric test in HOMER with correction for FDR. Stim: stimulated.

Supplementary Fig. 5



Supplementary Fig. 5. Antigen specificity and repertoire characteristics of T_{STEM} and T_{PEX} cells. a, UMAP plot showing the expression of selected markers as determined by CyTOF. Similar data were obtained from other healthy donors (n = 4). **b,** UMAP plots showing the distribution of T_{STEM} and T_{PEX} cells (top left) and antigen-specific $CD8^+$ memory T cells as determined by CyTOF. Similar data were obtained from other healthy donors (n = 4). **c**, Dot plot showing the matched frequencies of all (wt) or high-avidity (KA) CMV NV9-specific $CCR7^+$ $CD8^+$ T cells expressing the T_{PEX} signature marker GZMK. Data were obtained using flow cytometry. Each dot represents one donor (n = 7 from three independent experiments for wt, n = 4 from three independent experiments for KA). Bars indicate mean ± SEM. **d**, Dot plot showing the Chao1 estimator of clonal diversity for TCRβ repertoires obtained from the T_{STEM}, T_{PEX}, and T_{EM} subsets. Each dot represents one donor (n = 6). Bars indicate median values. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (two-tailed paired t-test with Bonferroni correction). **e**, Dot plot showing pairwise comparisons of weighted overlap (F2 metric) for the TCRβ repertoires obtained in **d**. Each dot represents one donor (n = 6). Bars indicate median values. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (two-tailed paired t-test with Bonferroni correction). **f**, Dot plot showing clonotype frequency correlations (R metric) for the TCRβ repertoires obtained in **d**. Higher values indicate stronger correlations. Each dot represents one donor (n = 6). Bars indicate median values. $***P = 0.0007$ (two-tailed paired t-test with Bonferroni correction).