High efficiency CRISPR knock-in demonstrates that TCF1 is insufficient to reverse T cell exhaustion

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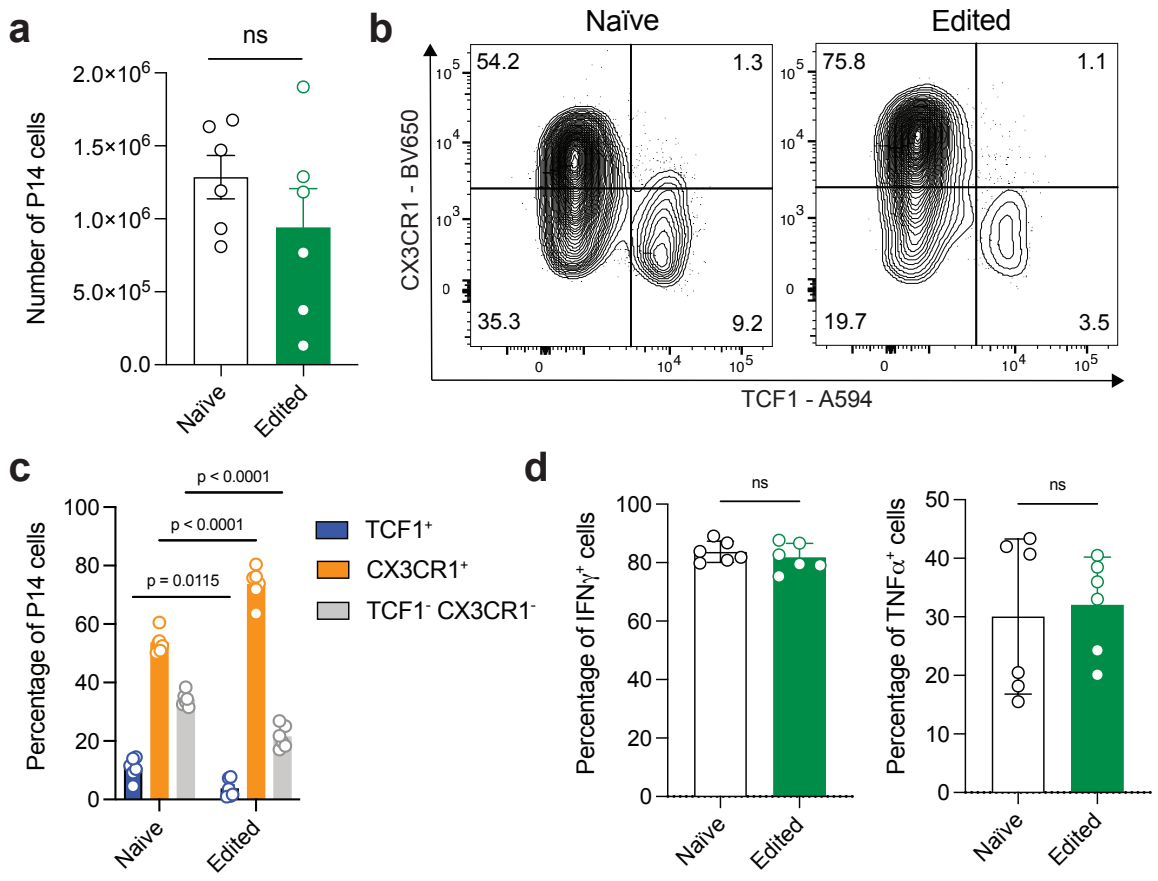
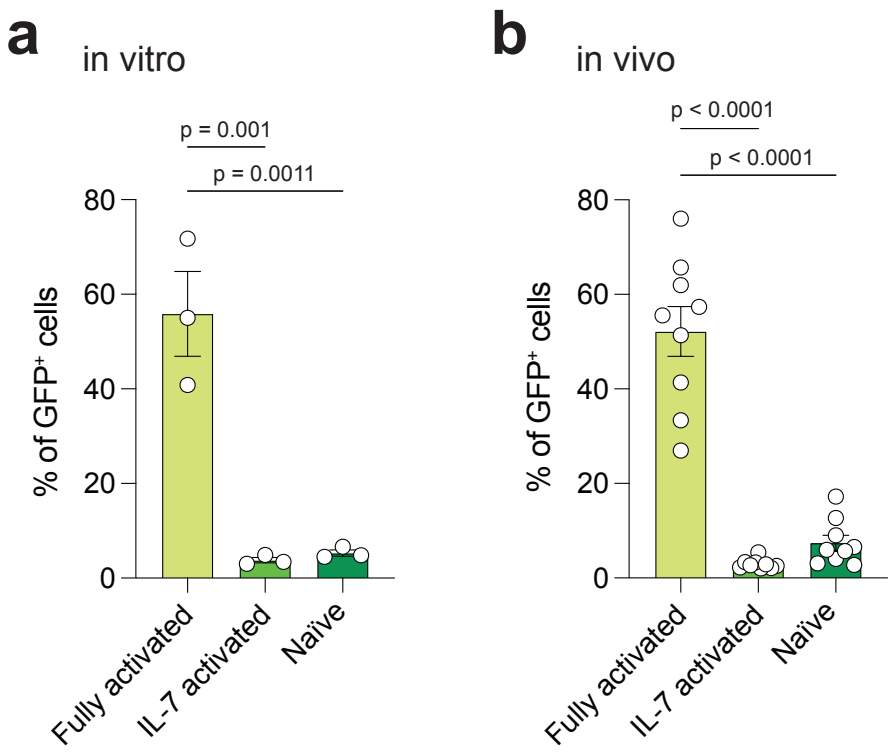
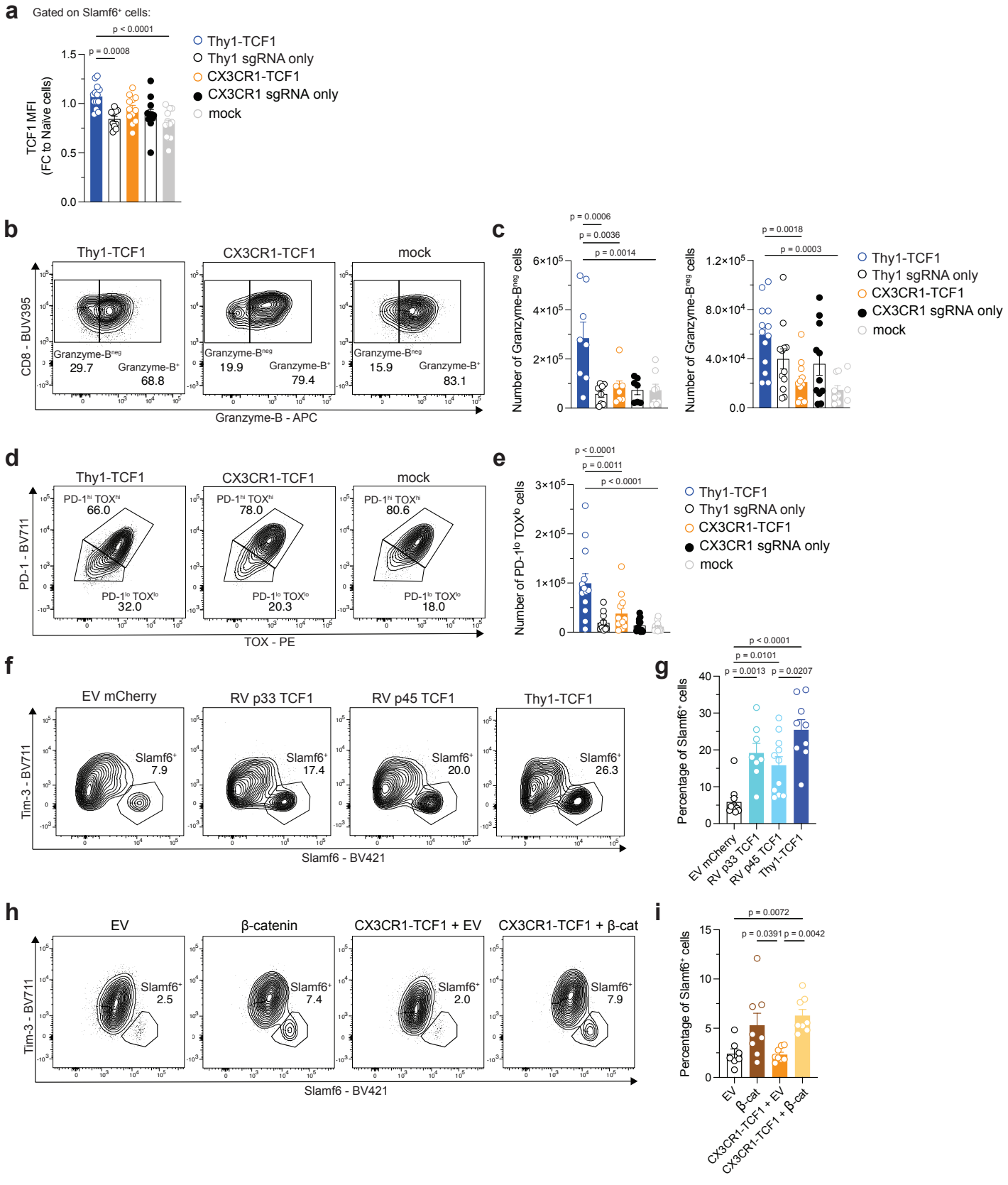
Supplementary Information



Supplementary Fig. 1 | High efficiency CRISPR HDR protocol does not impact P14 differentiation in response to LCMV chronic infection. (a-d) P14 cells were either CRISPR edited or left naïve before transfer into mice. (a) Pooled data showing number of splenic P14 cells recovered at day 9 p.i. Representative (b) and pooled (c-d) data showing expression of TCF1 and CX3CR1 (b-c) and cytokine production after peptide restimulation (d) in P14 cells at day 9 p.i. Pooled data from 2 independent experiments (n=6 mice per group). Error bars show ± SEM. P values were calculated by ordinary two-way ANOVA (c). ns = not-significant.

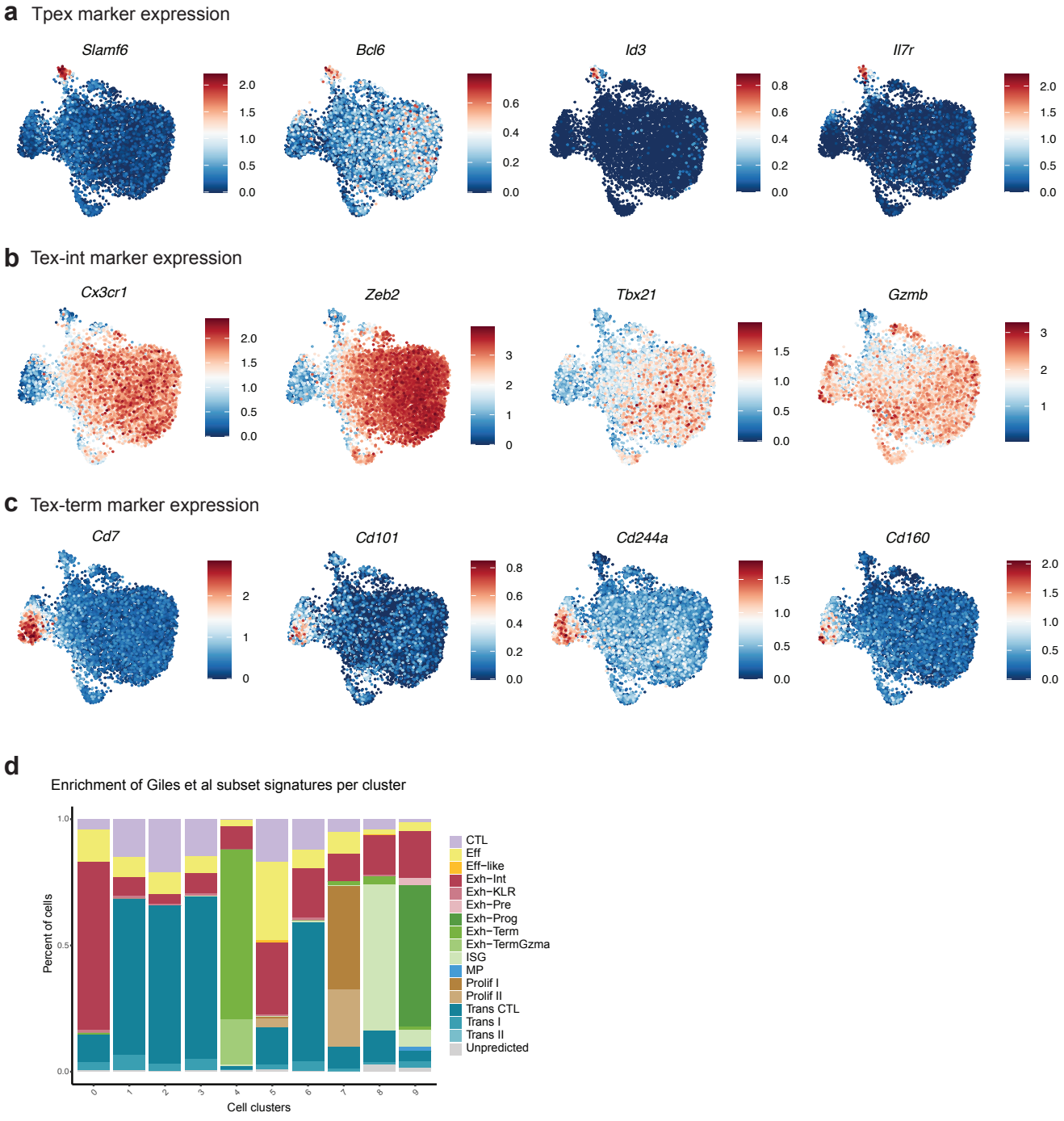


Supplementary Fig. 2 | High efficiency CRISPR-HDR editing is dependent on T cell activation. (a and b) P14 cells were either activated or kept in IL-7 for 24 h or left as naïve before CRISPR HDR and then cultured or transferred to mice as described in Figure 1. Pooled data showing GFP expression in CRISPR edited P14 cells after 72 h of in vitro culture (a) or at day 8 post-infection (p.i.) (a) Pooled data from 3 independent experiments (n=3 biological replicates). (b) Pooled data from 3 independent experiments (n=9 mice per group). Error bars show ± SEM. P values were calculated by ordinary one-way ANOVA with Tukey's multiple comparison test.

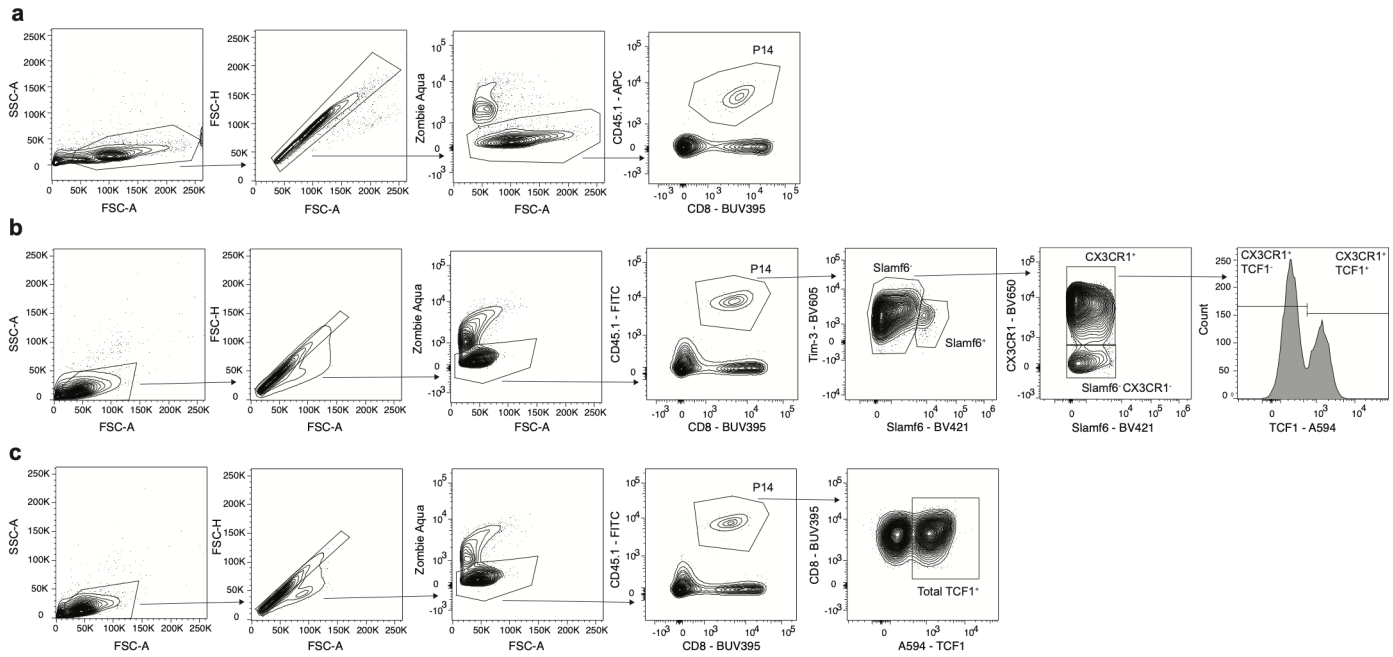


Supplementary Fig. 3 | Constitutive, but not conditional, TCF1 over-expression increases Tpex. (a-e) P14 cells were CRISPR edited using the AAV6-Thy1-TCF1 or AAV6-CX3CR1-TCF1 and transferred into mice as described in Figure 1. (a) Pooled data showing fold change (FC) of mean fluorescence intensity (MFI) of TCF1

expression in Tpex CRISPR edited P14 cells after 20 days p.i.. Representative (b and d) and pooled (c and e) data showing expression of Granzyme-B or PD-1/TOX in P14 cells after 8 (c) or 20 days p.i. (c and e). (a, c and e) Data from 2 independent experiments for day 8 (n=8 mice per group for Thy1-TCF1, Thy1 sgRNA and mock groups and n=7 mice per group for CX3CR1-TCF1 and CX3CR1 sgRNA groups) and data from 3 independent experiments for day 20 data (n=13 mice for Thy1-TCF1 group and n=12 mice per group for Thy1 sgRNA, CX3CR1-TCF1, CX3CR1 sgRNA and mock groups). (f-g) Representative (f) and pooled (g) Tpex frequency in P14 cells at day 20 p.i. either retrovirally (RV) over-expressing different TCF1 isoforms or CRISPR edited using AAV6-Thy1-TCF1. EV = Empty vector. Data from 3 independent experiments (n=11 mice per group for EV mCherry and p45 TCF1 groups, n=9 mice for Thy1-TCF1 group and n=8 mice for p33 TCF1 group). (h-i) Representative (h) and pooled (i) Tpex frequency in P14 cells at day 8 p.i. after retroviral over-expression of β-catenin (β-cat) combined with CRISPR editing using AAV6-CX3CR1-TCF1. Data from 2 independent experiments (n=8 mice per group for β-cat, CX3CR1-TCF1 + β-cat and CX3CR1 + EV groups and n=7 mice for EV mCherry group). β-cat = β-catenin. Error bars show ± SEM. P values calculated by ordinary one-way ANOVA with Tukey's multiple comparison test.



Supplementary Fig. 4 | Multi-ome marker expression and cluster annotation. Total P14 cells were sorted 20 days p.i. and analyzed by combined whole cell scRNA-seq and scATAC-seq (multi-ome) analysis as described in Figure 6. (a-c) Expression heatmaps on the Combined UMAP for Tpex (a), Tex-int (b) and Tex-term (c) marker genes. (d) Cluster identity as assessed by relative enrichment of the exhausted cluster signatures from Giles et al[1].



Supplementary Fig. 5 | Flow cytometry gating strategy. (a) Gating strategy used for analysis and cell sorting of total P14 cells as presented in Figures 1, 2, 3, 4g-j, 5b-c, f-g and 6. (b) Gating strategy for analysis of CX3CR1^{+} P14 cells for data presented in Figures 4b-f and 6h-k. (c) Gating strategy used for analysis of TCF1^{+} P14 cells for data presented in Figures 5d-e, h.

Supplementary References

1 Giles, J. R. *et al.* Shared and distinct biological circuits in effector, memory and exhausted CD8(+) T cells revealed by temporal single-cell transcriptomics and epigenetics. *Nat Immunol* **23**, 1600-1613, doi:10.1038/s41590-022-01338-4 (2022).