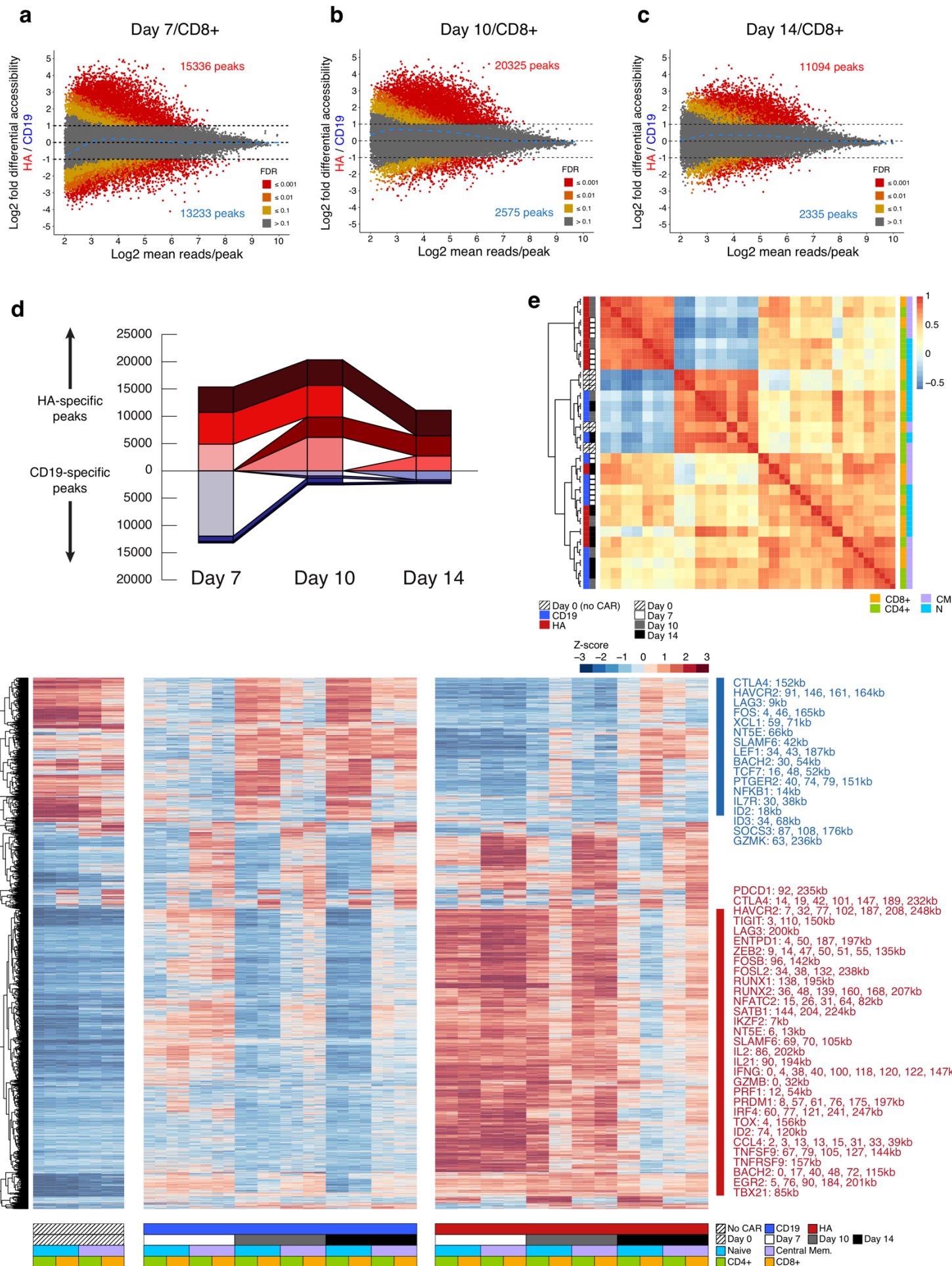
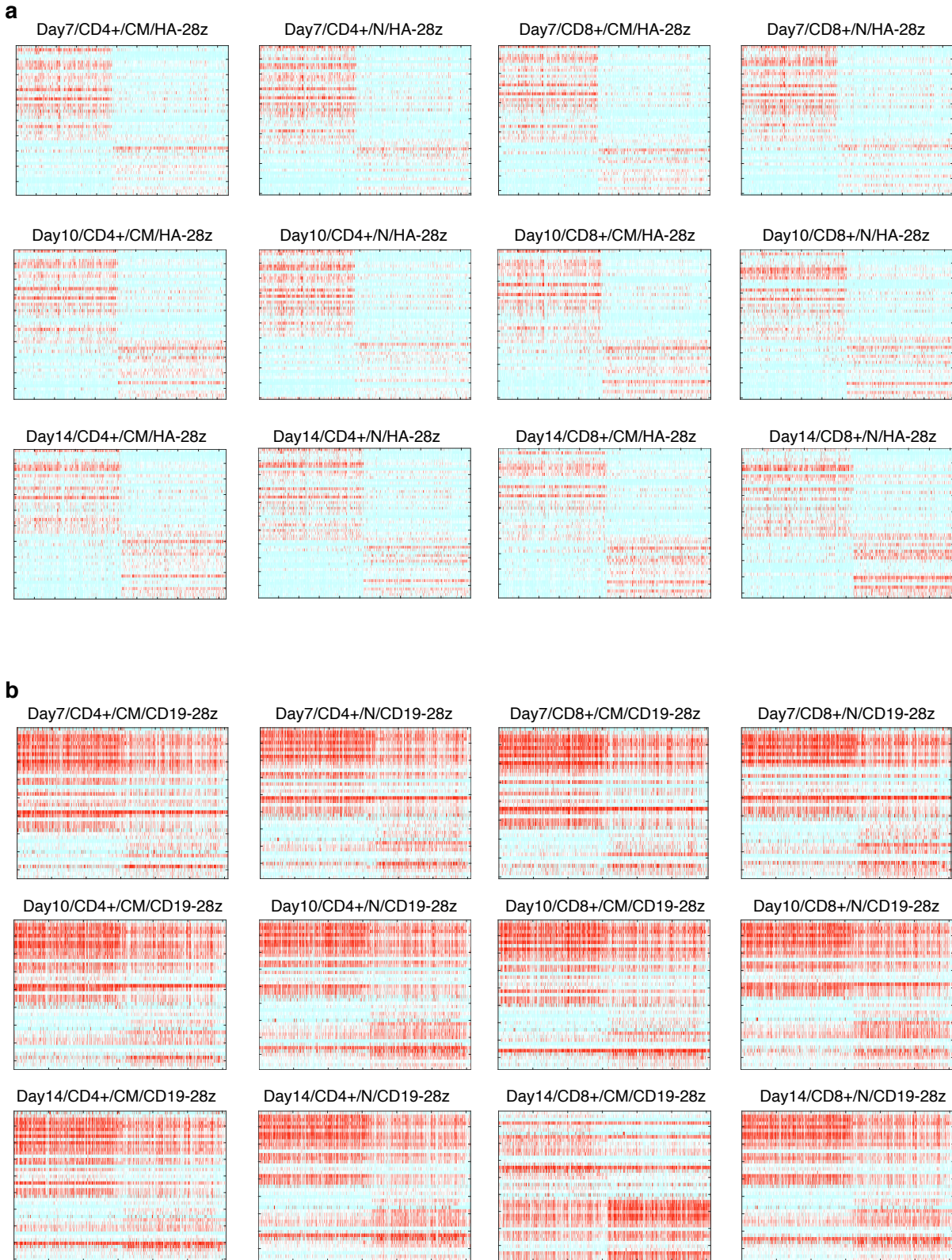


Supplemental Figure 1. Donor-derived CAR T cell phenotyping and Omni-ATAC-seq quality control metrics. (A-B) Surface expression and cytokine secretion of markers of T cell exhaustion throughout the CAR T cell maturation time course. (C) All samples demonstrate high replicate concordance. (D) All samples demonstrate high enrichment of genome alignment to transcription start sites.

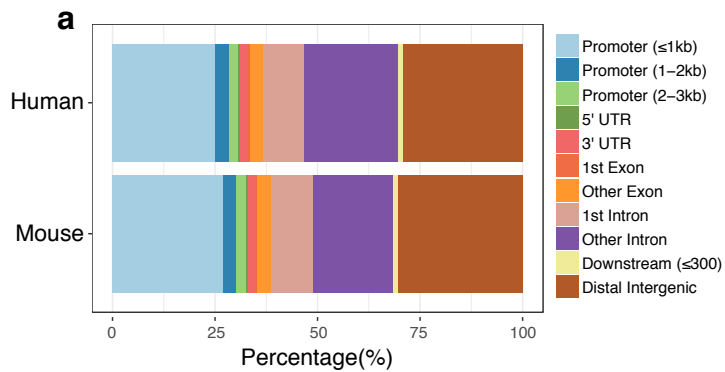


Supplemental Figure 2. The dynamics of accessible chromatin loci over time identify loci with varying temporal control. (A-C) Differentially accessible chromatin loci (peaks) in CD8+ CAR T cells at 7, 10, and 14 days following CAR transfection. (D) Differentially accessible loci across time points, colored by which timepoints and the number of timepoints in which they are differentially accessible. Loci differentially accessible in HA-28z CAR T cells (red) are more numerous persist through multiple time points to a greater degree and are more numerous than the loci differentially accessible in CD19-28z CAR T cells (blue). (E) Sample clustering by Pearson correlation of global chromatin accessibility profiles. (F) Top 5000 most variable chromatin accessibility peaks across all samples colored by z-score of peak accessibility in each sample. Many of the peaks differentially accessible in either the CD19-28z or HA-28z CAR T cells appear proximal to known exhaustion-associated and T cell effector genes. Distances between peaks and select gene promoters are listed.

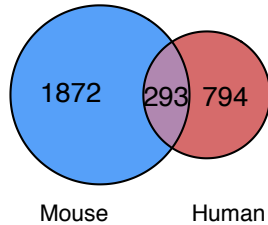


Supplemental Figure 3. Transcription factor-gene dynamics demonstrate coregulated modules in exhausted CAR T cells. PECA2 coregulation score heatmaps for all (A) HA-28z

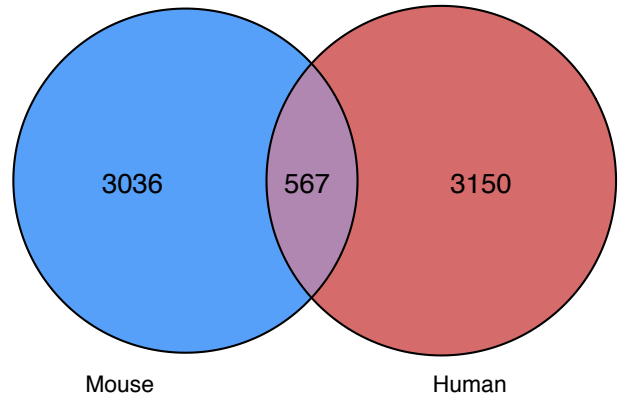
and (B) CD19-28z CAR T cell samples, merging replicates, indicating the strength of correlation between transcription and accessibility dynamics across samples.



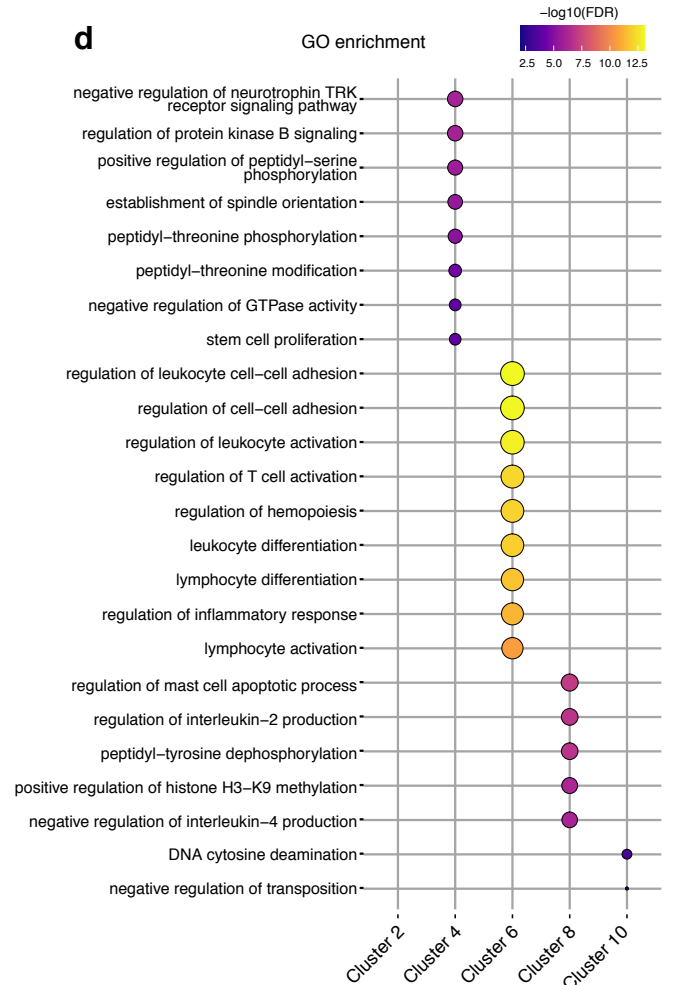
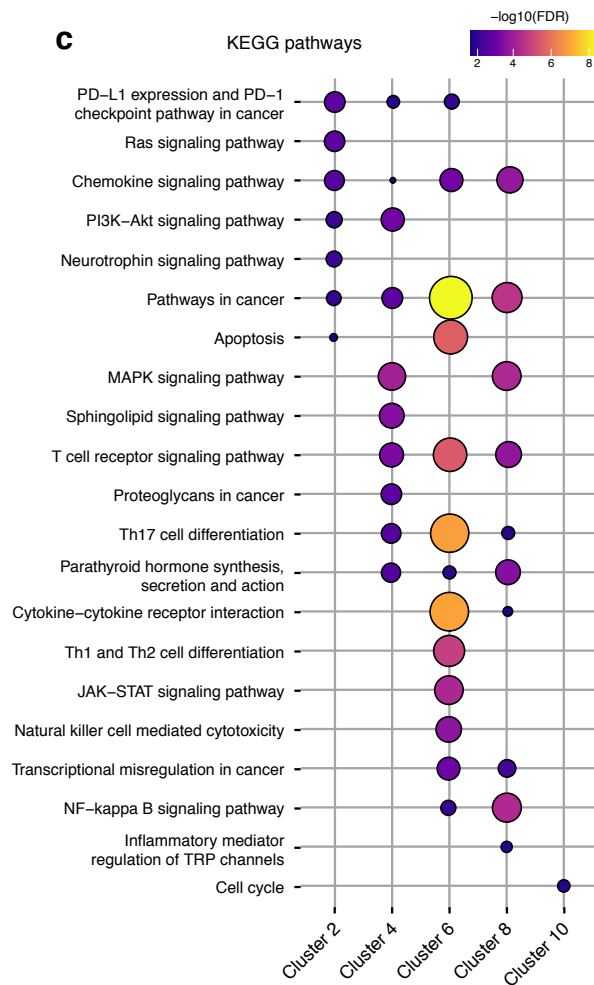
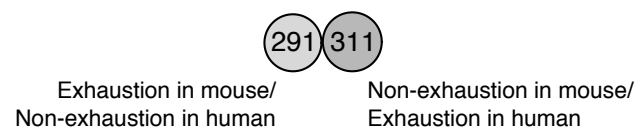
Loci differentially accessible in non-exhausted T cells



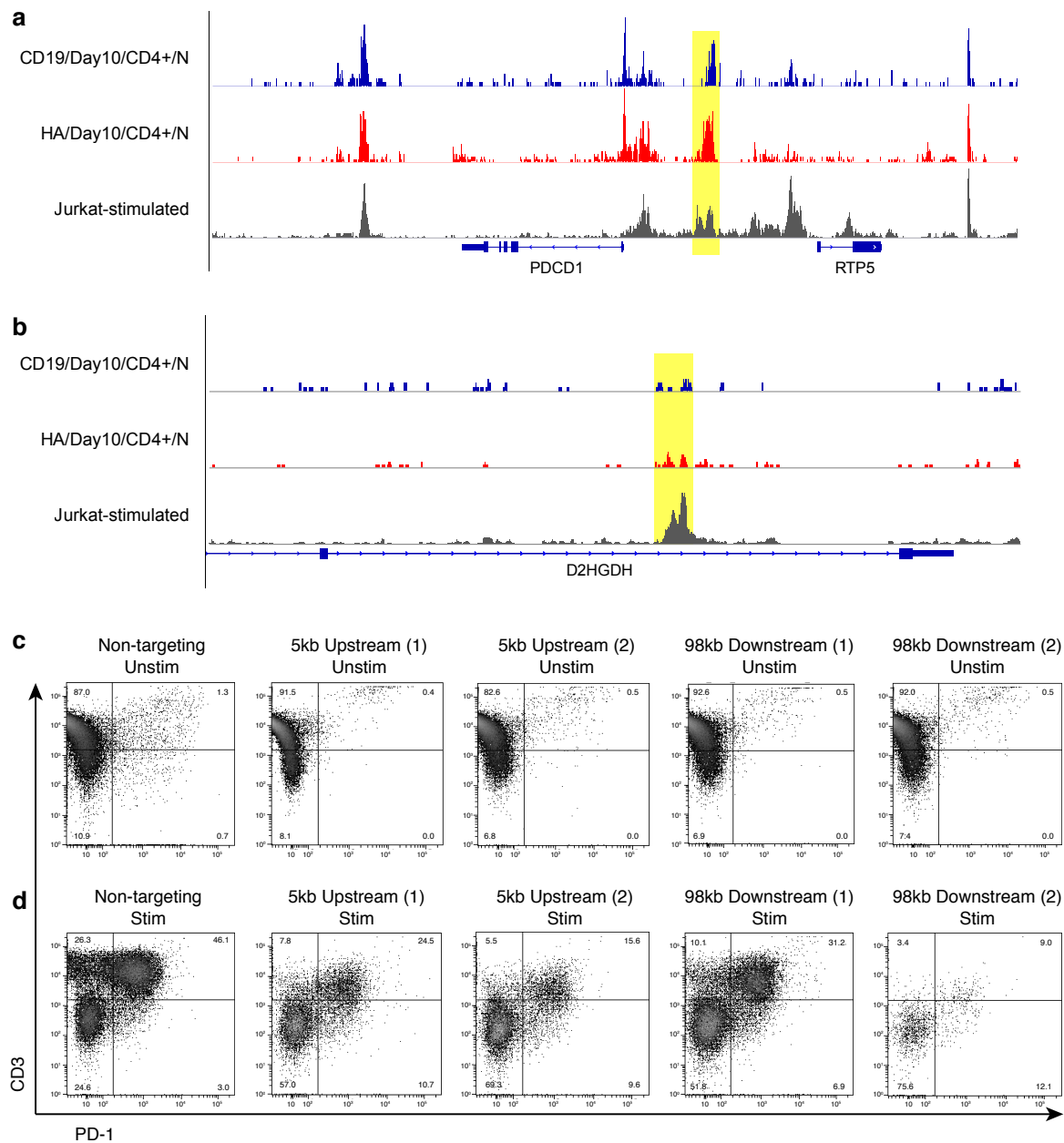
b Loci differentially accessible in exhausted T cells



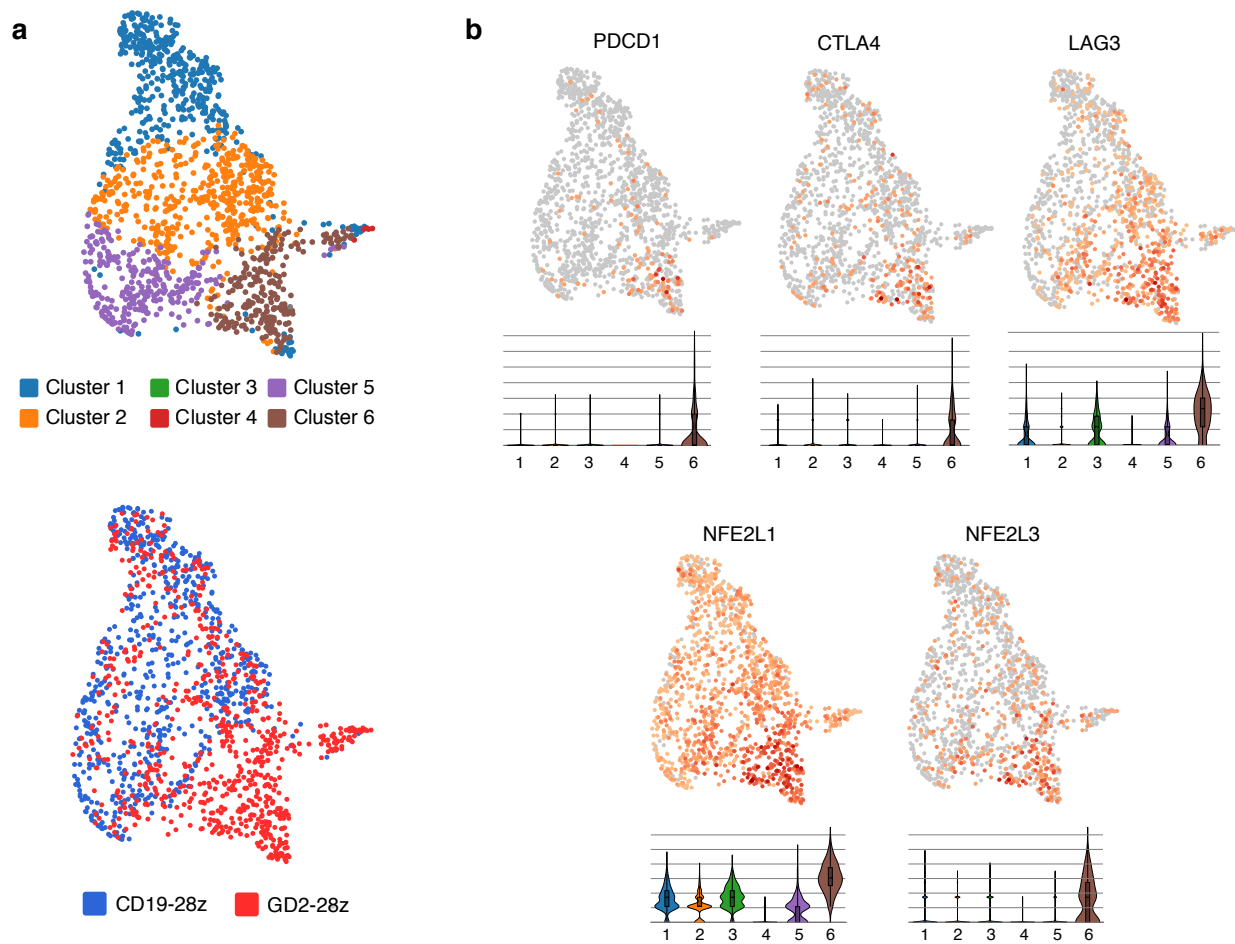
Loci with varying accessibility in exhausted T cells by species



Supplemental Figure 4. Features of human and mouse dysfunctional T cell accessible chromatin. (A) Genomic features of the accessible chromatin loci in exhausted human HA-28z CAR T cells and chronically stimulated mouse T cells (from Sen et al., 2016). (B) Quantification of the ATAC-seq peaks in the T cells of each species associated with (1) exhausted T cells, (2) non-exhausted T cells, or (3) both exhausted and non-exhausted T cells with varying accessibility by species. (C) KEGG pathway enrichment and (D) Gene Ontology (GO) term enrichment for genes associated with the ATAC-seq peaks within clusters of exhaustion-accessible ATAC-seq peaks in mouse and/or human T cells.



Supplemental Figure 5. The Jurkat T cell line shares regulatory loci with stimulated and exhausted CAR T cells. (A) Accessible chromatin sequencing alignment tracks of Day 10 CD19-28z, Day 10 HA-28z CAR T cells, and stimulated Jurkat cells (from Brignall et al., 2017) at the PDCD1 promoter locus and (B) 98 kb downstream from the PDCD1 transcription start site. (C) Cell surface expression of CD3 and PD-1 in unstimulated and (D) IL-2/CD3/CD28-stimulated Jurkat cells treated with non-targeting CRISPR/Cas9 RNPs, RNP pairs targeting the exhaustion-associated accessible chromatin locus 5 kb upstream of the PDCD1 promoter, or RNP pairs targeting 98 kb downstream of PDCD1 following 14 days of *in vitro* culture.



Supplemental Figure 6. NFE2L1/NFE2L3 are co-expressed with markers of exhaustion.

(A) UMAP dimensionality reduction of single-cell RNA-seq on non-exhausted CD19-28z and exhausted HA-28z CAR T cells (from Lynn et al., 2019) identifies a cluster of cells unique to the exhausted CAR T cell population. (B) Inhibitory receptor markers of exhaustion (PD-1, CTLA4, LAG3) and NFE2L1/3 are expressed in the exhaustion-specific cell cluster.