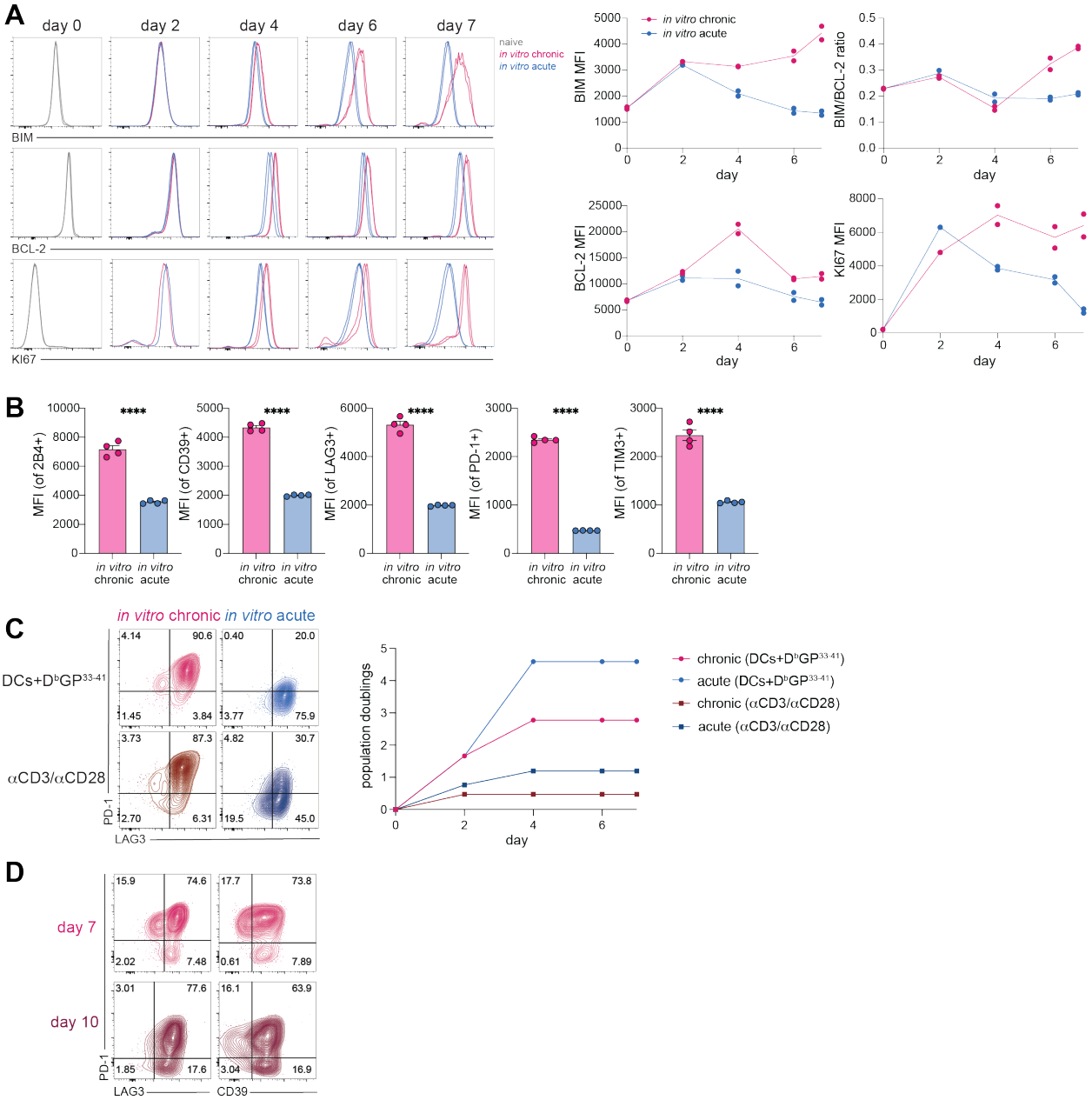

Fig. S1. Chronic antigenic stimulation *in vitro* induces high per-cell IR expression
(A) [left] Histograms and [right] summary data of longitudinal expression of BIM, BCL-2, and KI67 by *in vitro* chronically and acutely stimulated P14 cells. **(B)** Summary data indicating MFI (gated on IR+ population of CD44^{hi} CD8+ live singlets) of IRs after chronic and acute stimulation of P14 cells *in vitro*. Significance calculated by unpaired two-tailed t test; ****p< 0.0001. **(C)** [left] Representative flow cytometry data of IR expression (gated on CD44^{hi} CD8+ live singlets) and [right] cell expansion after chronic and acute stimulation, either via DCs+D^{b}GP$^{33-41}$ or αCD3/αCD28. **(D)** Representative flow cytometry data of IR expression (gated on CD44^{hi} CD8+ live singlets) after chronic stimulation for either 7 or 10 days.

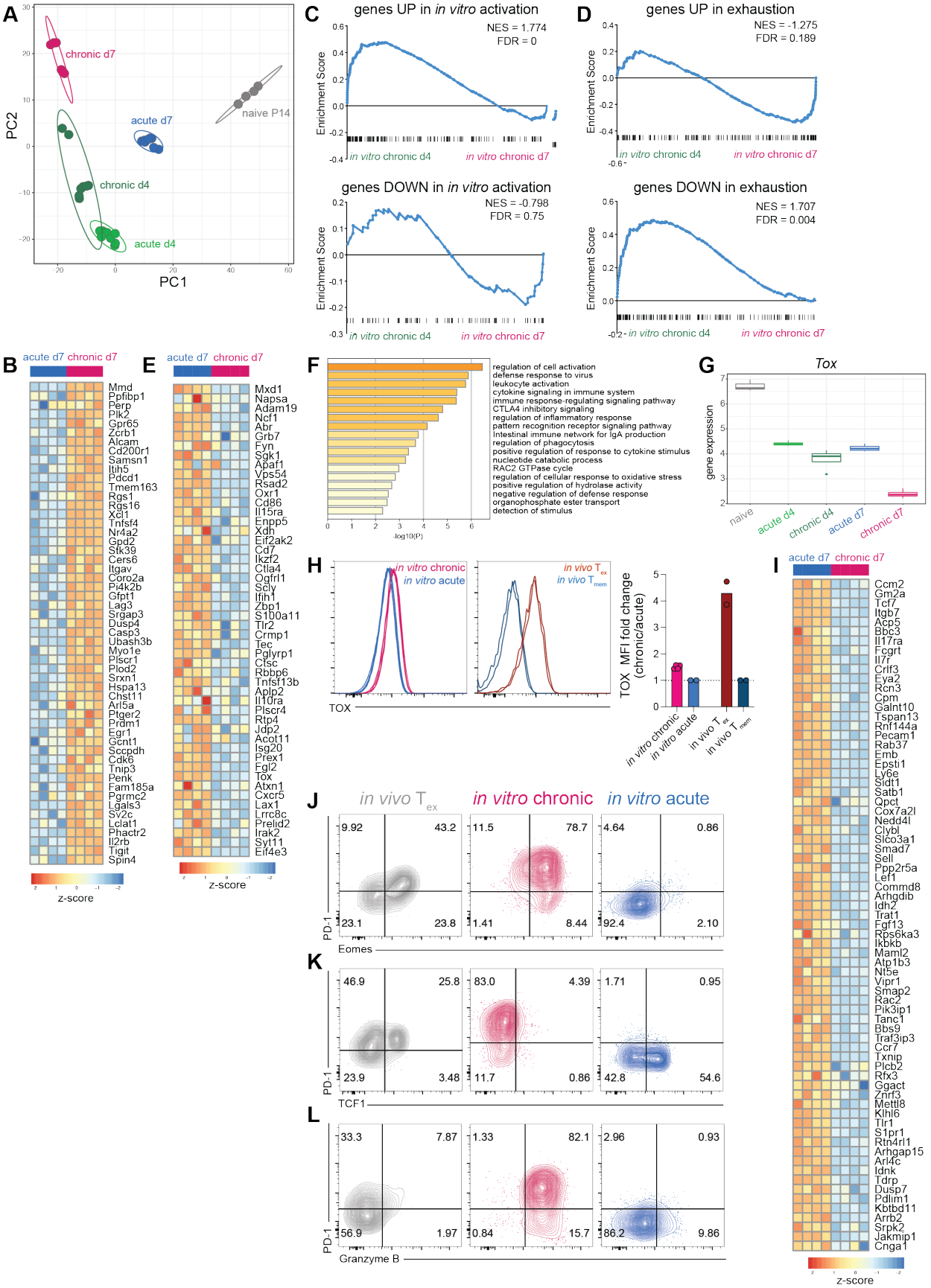

Fig. S2. *In vitro* chronically stimulated P14 cells develop a transcriptional signature of T_{ex}

(A) PCA of RNA-seq data from *in vitro* chronically and acutely stimulated P14 cells. **(B)** Gene expression of leading edge genes upregulated in *in vivo* T_{ex} enriched after chronic stimulation *in vitro*. **(C-D)** GSEA of gene sets for **(C)** *in vitro* activation and **(D)** exhaustion (*41*) in *in vitro* chronically stimulated cells at d4 and d7. **(E)** Heatmap and **(F)** GO analysis of genes upregulated in *in vivo* T_{ex} that were not enriched after chronic stimulation *in vitro*. **(G)** Gene expression of *Tox* in *in vitro* chronically and acutely stimulated P14 cells. **(H)** [left] TOX protein expression in *in vitro* chronically and acutely stimulated P14 cells and *in vivo* T_{ex} and T_{mem}. [right] Fold change of TOX MFI in *in vitro* chronic over acute and *in vivo* T_{ex} over T_{mem}. **(I)** Gene expression of leading edge of genes downregulated in *in vivo* T_{ex} enriched after chronic stimulation *in vitro*. **(J-L)** Representative flow cytometry data detailing co-expression of PD-1 and **(J)** Eomes, **(K)** TCF1, and **(L)** Granzyme B on *in vivo* T_{ex} (LCMV-Cl13 30dpi), *in vitro* chronically stimulated P14 cells, and *in vitro* acutely stimulated P14 cells (all gated on $CD44^{hi}$ CD8+ live singlets). Numbers in flow cytometry plots indicate percentage of parent population within each gate.

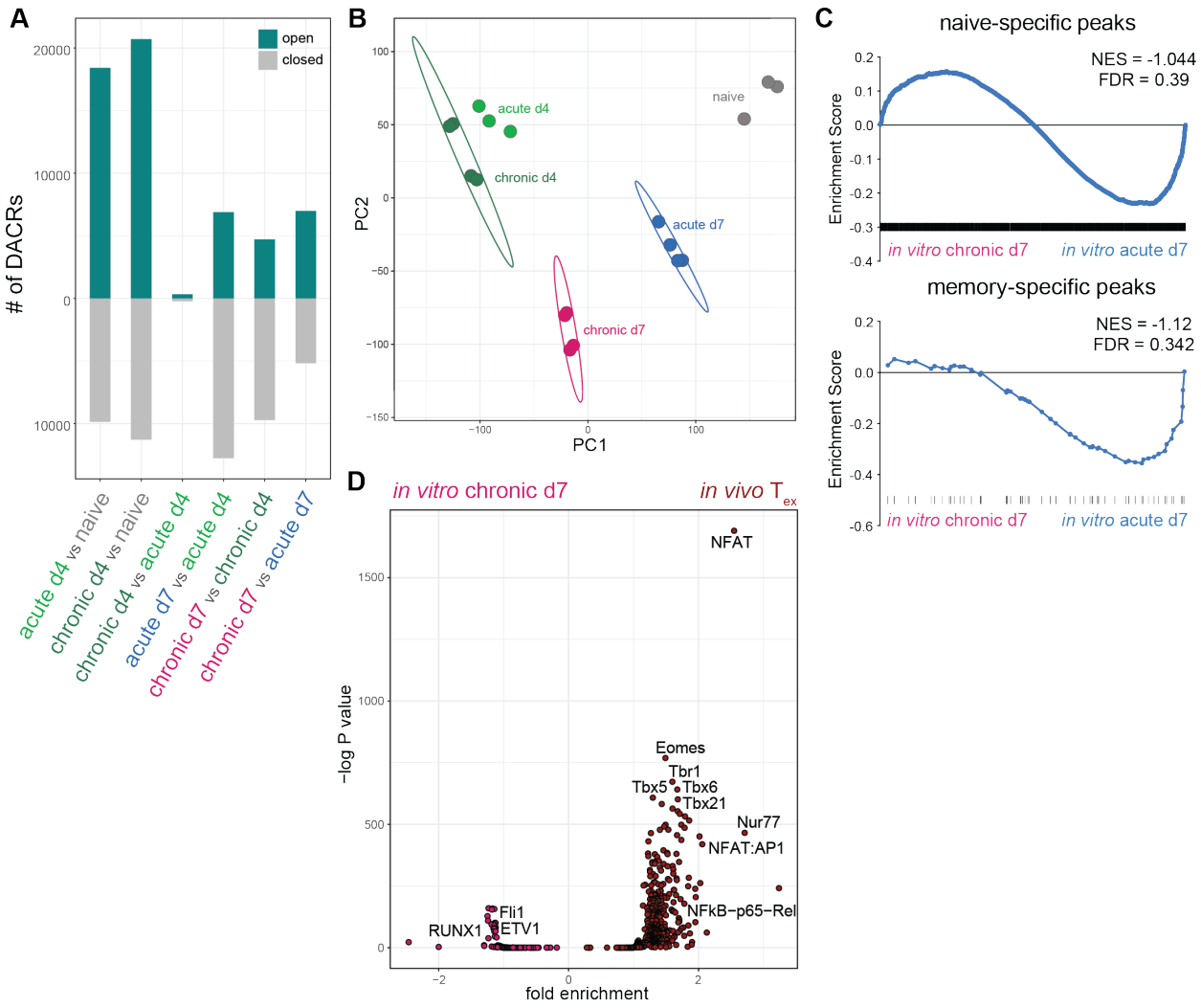

Fig. S3. *In vitro* chronically stimulated P14 cells develop epigenetic signatures of T_{eff} and T_{ex}

(A) Number of DACRs (filtered on lfc>1 and p<0.05) between pairwise comparisons as indicated. **(B)** PCA of ATAC-seq data from *in vitro* chronically and acutely stimulated P14 cells. **(C)** PSEA of naive- and memory-specific ACRs in *in vitro* chronically and acutely stimulated P14 cells. **(D)** TF motif accessibility in DACRs between *in vitro* chronically stimulated P14 cells and *in vivo* T_{ex}.

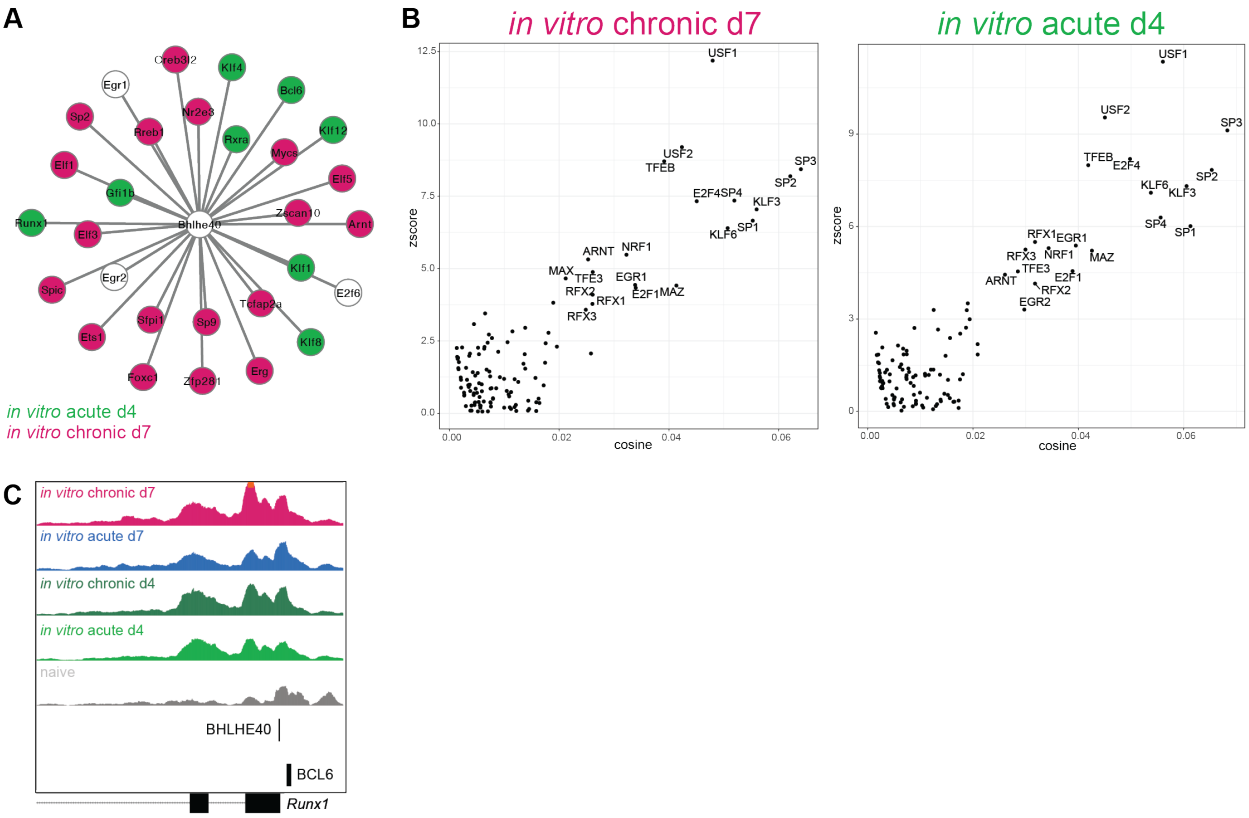

Fig. S4. BHLHE40 is predicted to regulate known transcriptional networks associated with CD8 T cell exhaustion

(A) Taiji analysis of differential transcriptional networks downstream of BHLHE40 at d7 of chronic stimulation *in vitro* and d4 of acute stimulation *in vitro*. Shared downstream TFs indicated in white. **(B)** TF motifs that co-occur with BHLHE40 motifs. **(C)** ATAC-seq signal tracks for *in vitro* chronically and acutely stimulated P14 cells for the *Runx1* locus; binding motifs for BHLHE40 and BCL6 shown below.

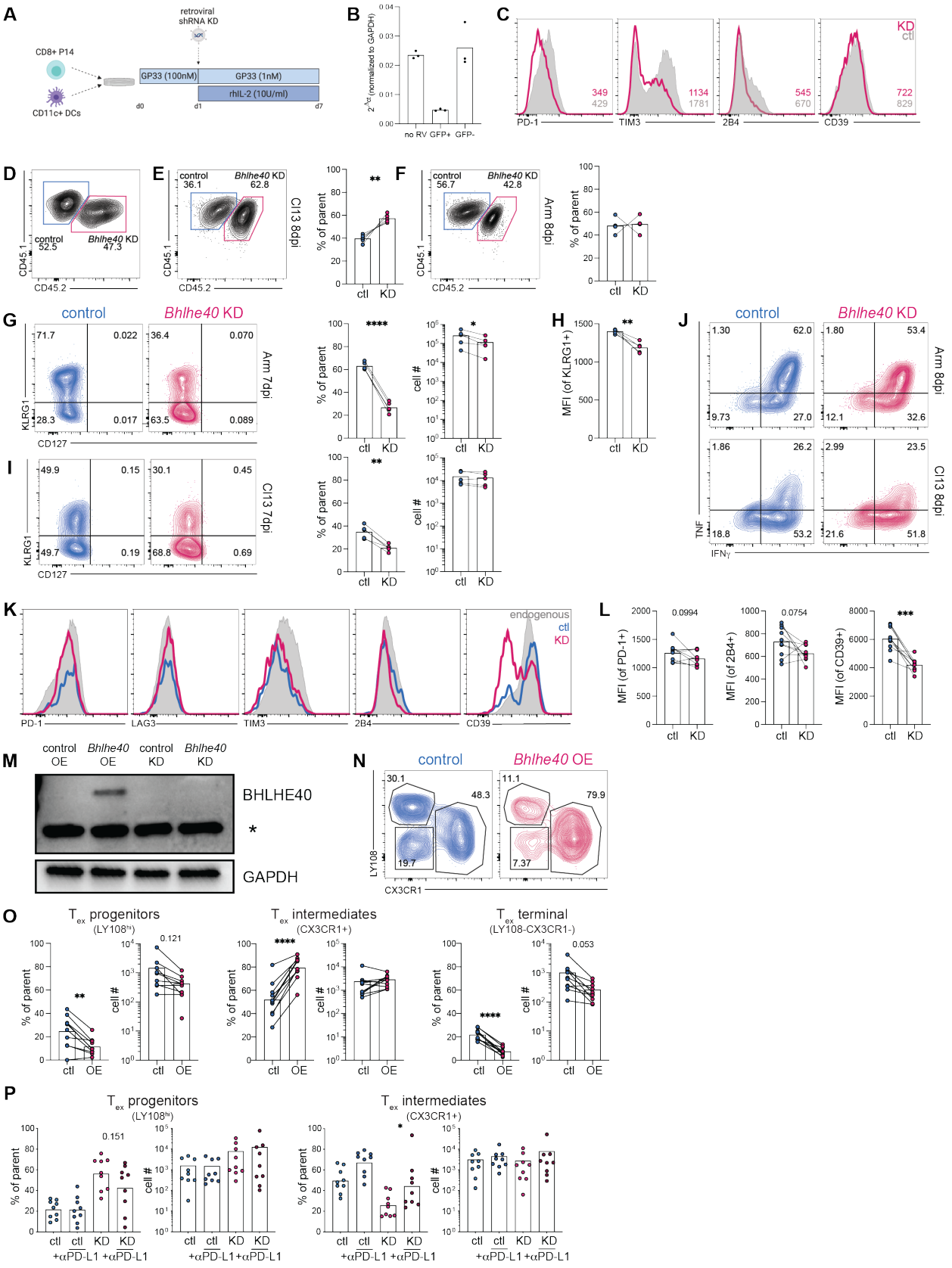

Fig. S5. BHLHE40 is a novel transcriptional regulator of CD8 T cell differentiation
(A) Experiment schematic of shRNA KD of *Bhlhe40* in *in vitro* chronically stimulated P14 cells. **(B)** Relative mRNA expression after *in vitro Bhlhe40* KD. **(C)** Representative flow cytometry histograms detailing IR expression by control and *Bhlhe40* KD P14 cells (gated on GFP+ CD44^{hi} CD8+ live singlets) after chronic stimulation *in vitro*. Mean fluorescence intensity (MFI) of each population indicated in lower right; representative of 2 experiments. **(D)** Representative flow cytometry plot of control and *Bhlhe40* KD input P14 cells (gated on GFP+ CD44^{hi} CD8+ live singlets) before transfer. **(E,F)** [left] Concatenated flow cytometry plot and [right] summary data of frequency of control and *Bhlhe40* KD P14 cells at 8dpi of **(E)** LCMV-Cl13 and **(F)** LCMV-Arm. **(G,I)** [left] Concatenated flow cytometry plots and [right] summary data of frequency and total number of KLRG1+ control and *Bhlhe40* KD P14 cells at 7dpi of **(G)** LCMV-Arm or **(I)** LCMV-Cl13. **(H)** MFI of KLRG1 (gated on KLRG1+ population) in control and *Bhlhe40* KD P14 cells at 7dpi of LCMV-Arm. **(J)** Concatenated flow cytometry plots of effector cytokine production in control and *Bhlhe40* KD P14 cells at 8dpi of [top] LCMV-Arm and [bottom] -Cl13. **(K)** Concatenated flow cytometry histograms detailing IR expression on endogenous (gated on GFP- gp33+ CD44^{hi} CD8+ live singlets), control, and *Bhlhe40* KD P14 cells at 31dpi of LCMV-Cl13. **(L)** Summary data indicating MFI (gated on IR+ population) of IRs on control and *Bhlhe40* KD P14 cells at 31dpi of LCMV-Cl13. **(M)** Protein expression of BHLHE40 after *in vitro* overexpression (OE) and KD of *Bhlhe40*; * indicates non-specific binding. **(N)** Concatenated flow cytometry plots and **(O)** summary data of frequency of T_{ex} subsets in control and *Bhlhe40* OE P14 cells at 31dpi of LCMV-Cl13. **(P)** Frequency and total number of progenitor and intermediate T_{ex} in control and *Bhlhe40* KD P14 cells at 37dpi of LCMV-Cl13, after treatment with vehicle control or αPD-L1 from 22-35dpi. **(E-L,N-P)** n=5 mice (Arm), n=10 mice (Cl13), representative of 3 experiments. Significance calculated by paired two-tailed t test; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **(D-J,N)** Numbers in flow cytometry plots indicate percentage of parent population within each gate.