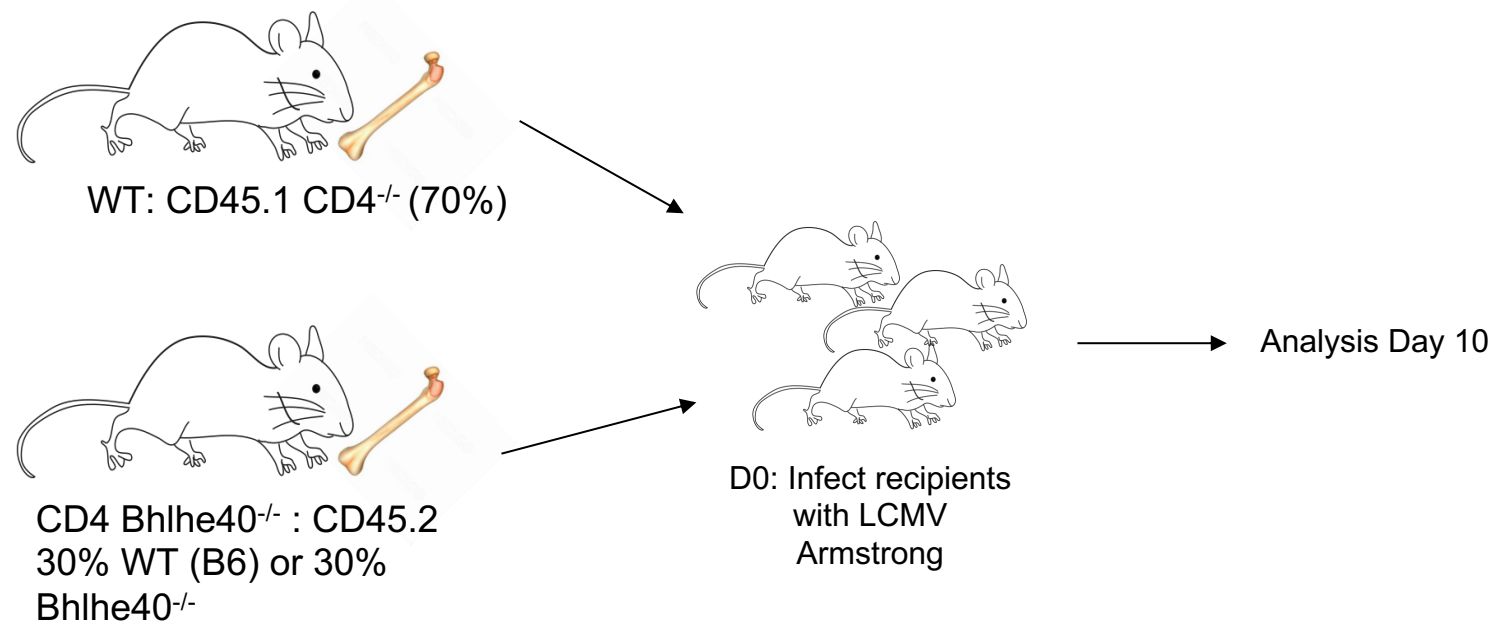
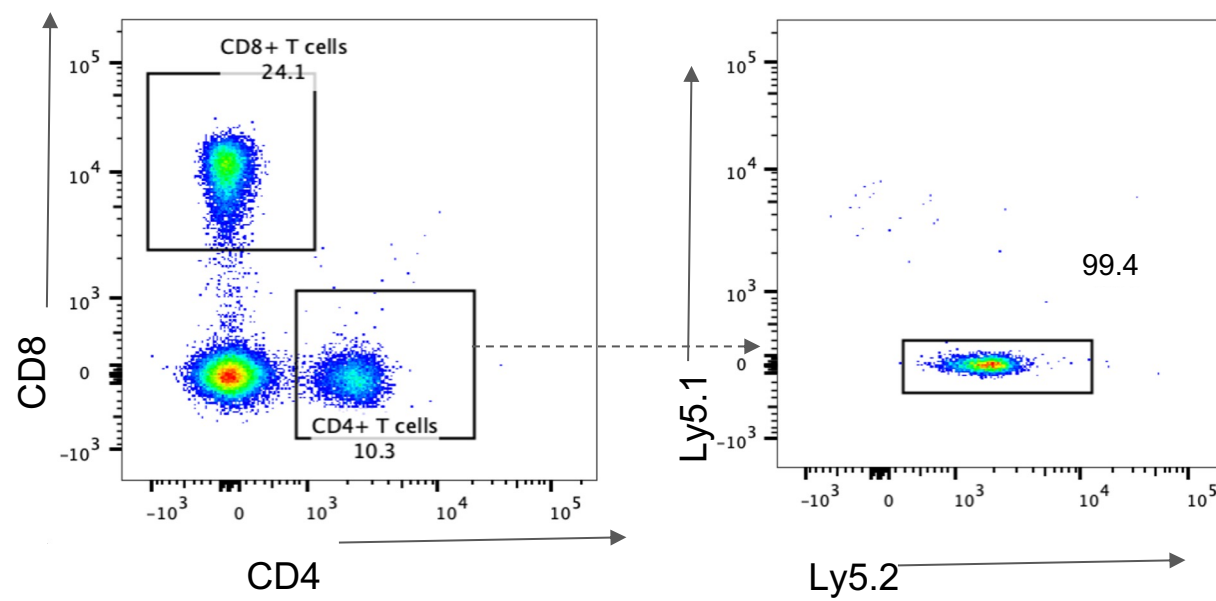


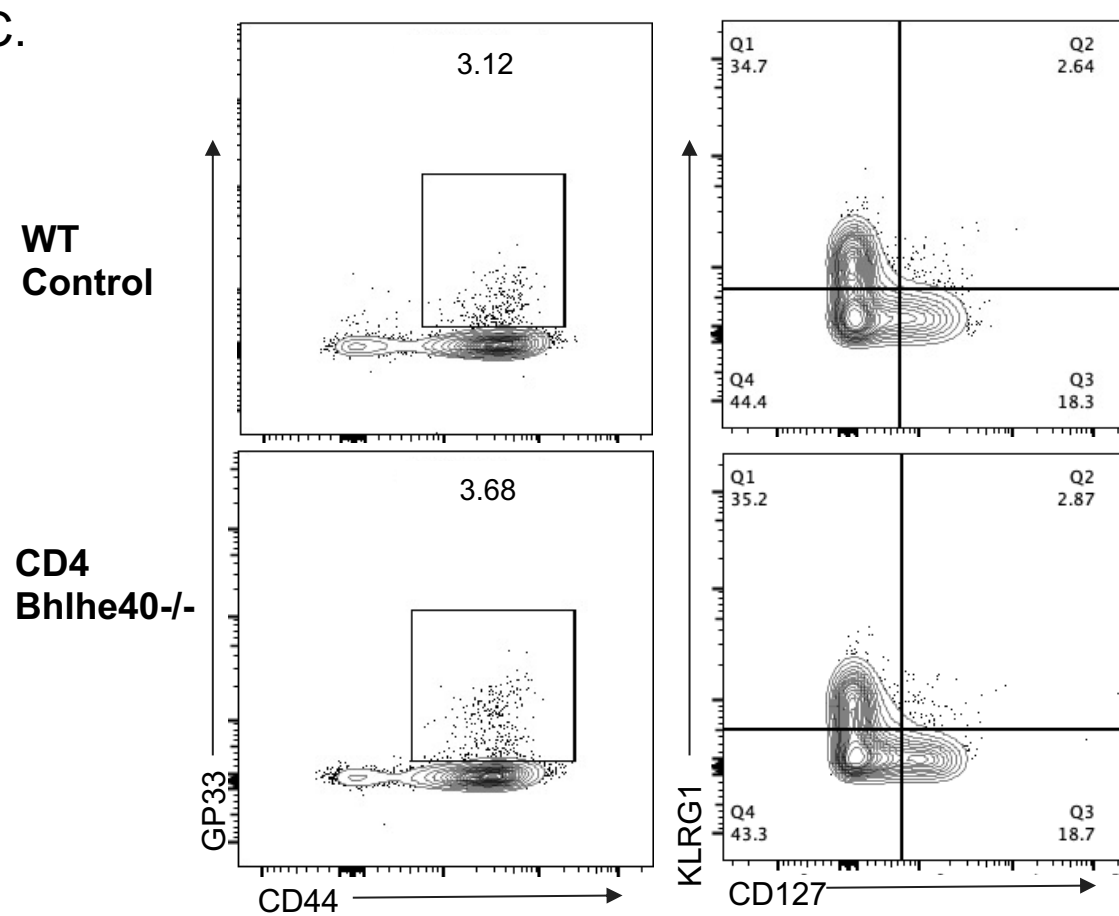
A.



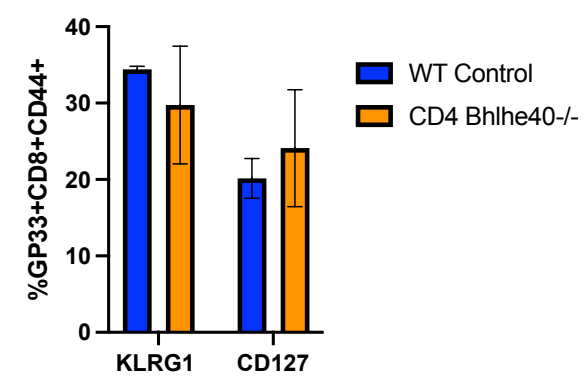
B.



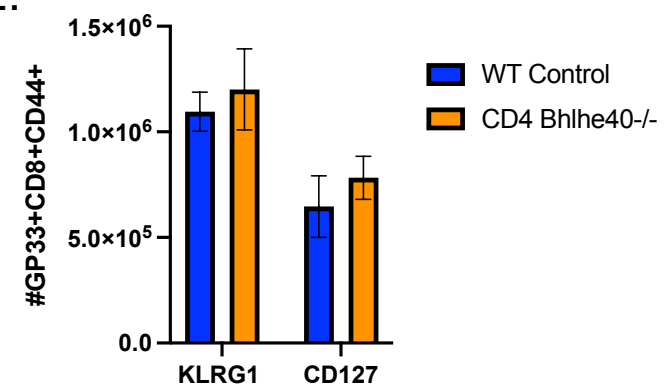
C.



D.



E.



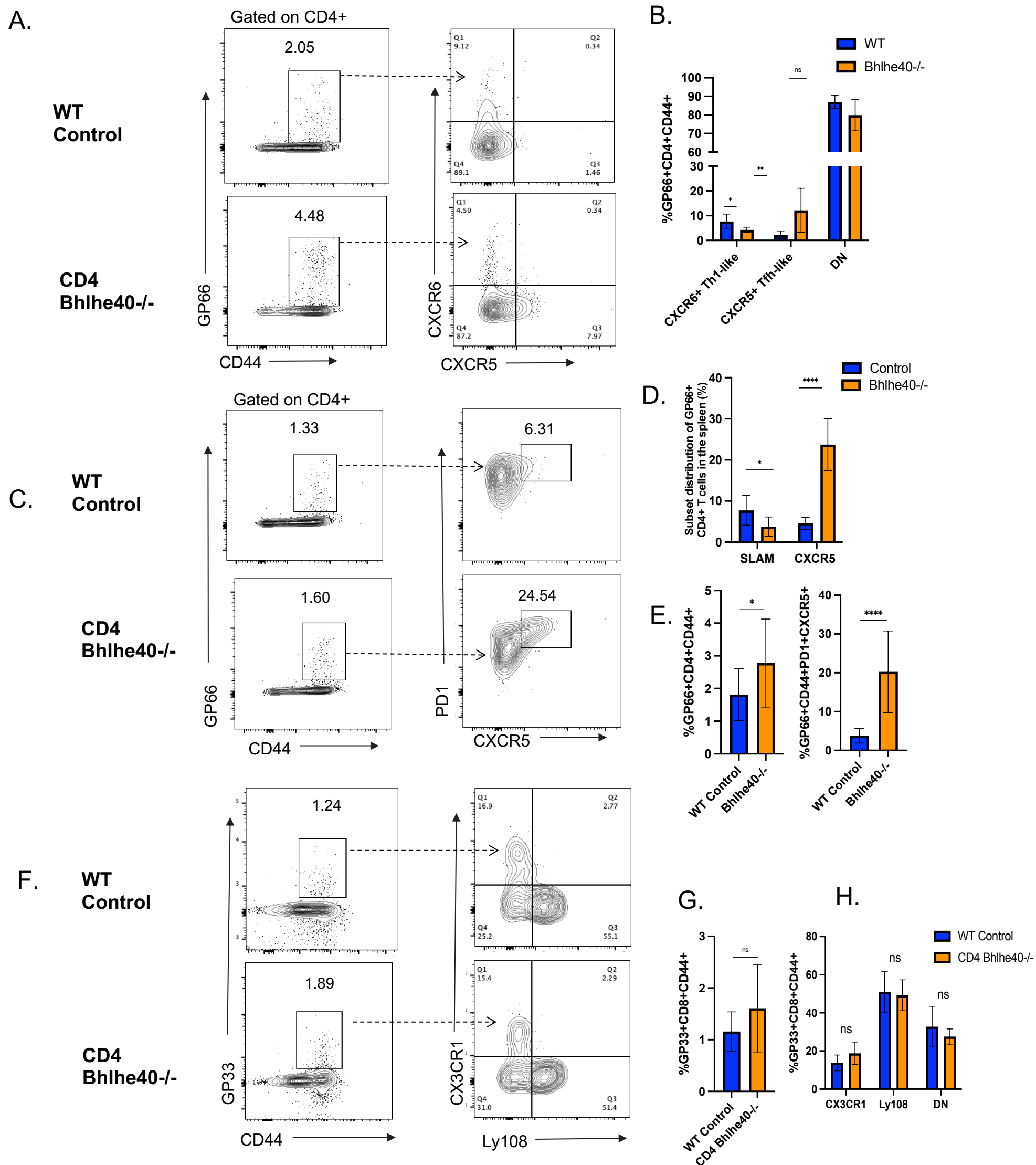
Supplemental Figure 1. Bhlhe40-deficient MBM chimera mice do not have altered levels of GP33+CD8+CD44+ T cells

A. Experimental Design

B. Representative plots showing reconstitution of MBM chimera mice

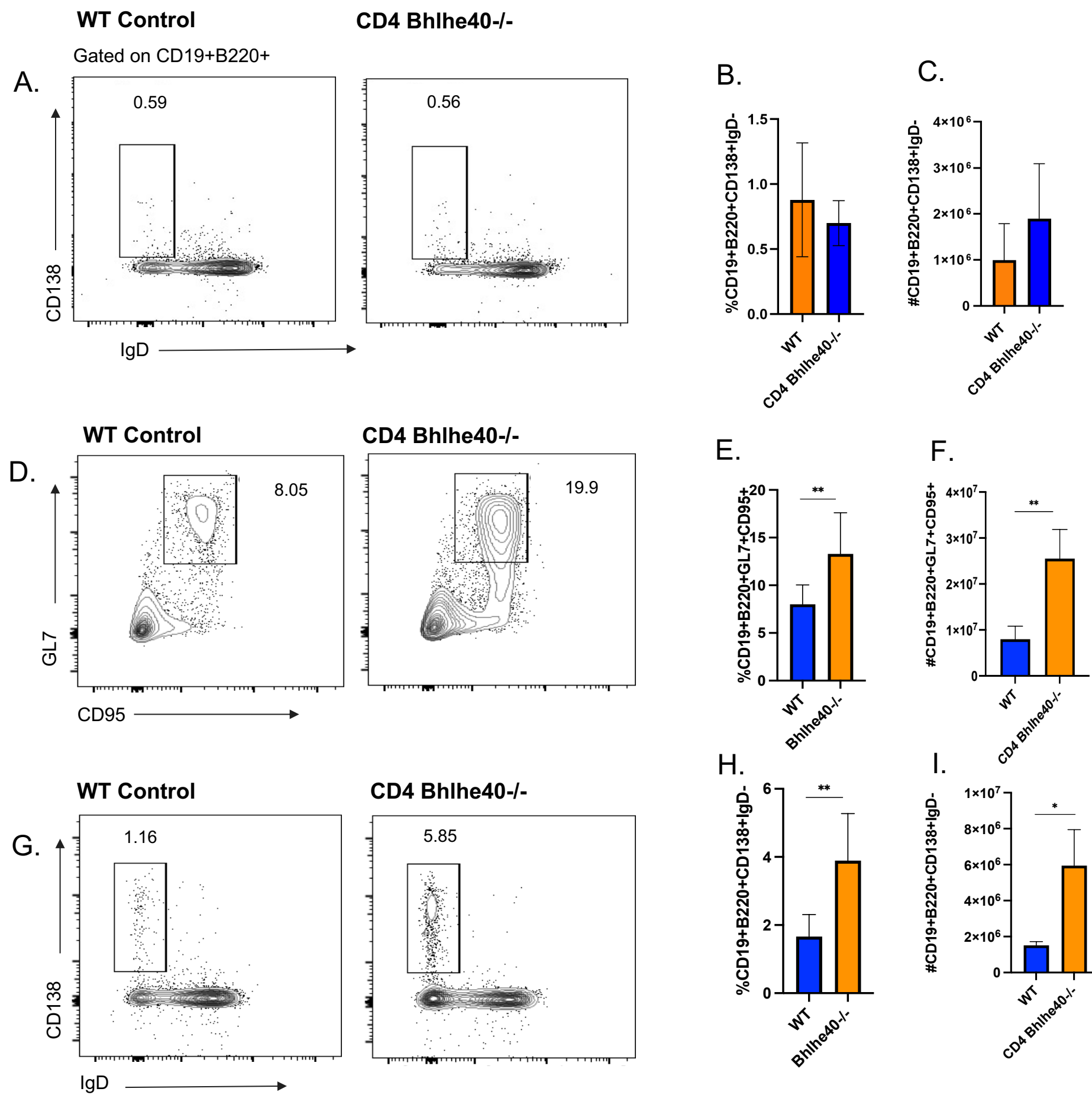
C. Representative plots showing subset distribution of GP33+CD8+CD44+ cells into KLRG1+ and CD127+ T cells on day 7 post Armstrong infection

D and E. Summary plots showing subset distribution (E) frequency and (F) total cell number of GP33+CD8+CD44+KLRG1+ or GP33+CD8+CD44+ CD127+ T cells



Supplemental Figure 2. Bhlhe40 regulation of CD4⁺ T cell fate decision is similar in CI 13 infection

- A. Experimental Design
- B. Representative plots displaying subset distribution of GP66+CD4⁺ T cells then gated on CXCR6⁺, CXCR5⁺, and DN T cell subsets in the spleen 21-32 days (pooled) post CI 13 infection
- C. Summary data showing the proportion, and subset distribution of each subset gated on CXCR6⁺, CXCR5⁺, and DN T cells
- D. Representative plots displaying subset distribution of GP66+CD4⁺PD1+CXCR5⁺ T cells subsets in the spleen 21-32 days (pooled) post CI 13 infection
- E. Summary data showing the proportion, and distribution of GP66+CD4⁺PD1+CXCR5⁺ T cells
- F. Representative flow plots
- G. G and H. (G) Representative plots showing subset distribution and (H) summary of GP33+CD8+CD44⁺ cells into CX3CR1⁺, Ly108⁺, and DN T cell subsets on day 21-32 (pooled) post CI 13 infection

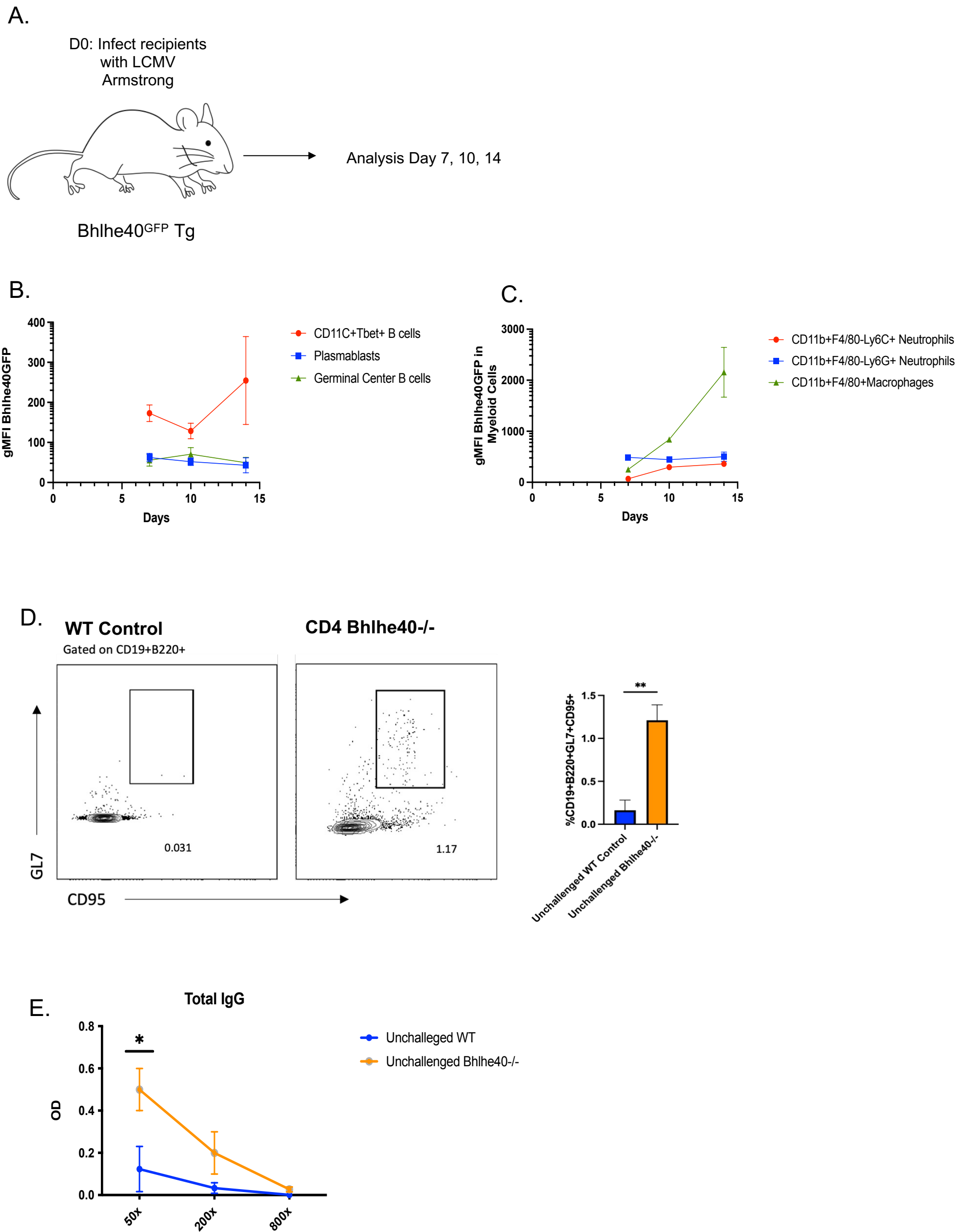


Supplemental Figure 3. Bhlhe40 regulates the GC response during CI 13 infection

A-C. D-F. Representative flow plots (A) and summary data (B and C) showing the proportion and total number of plasmablasts 7 days post Armstrong infection.

D-F. Representative flow plots (D) and summary data (E and F) showing the proportion and total number of Germinal center B cells 21-32 days post CI13 infection.

G-I. Representative flow plots (D) and summary data (E and F) showing the proportion and total number of plasmablasts 21-32 days post CI13 infection.



Supplemental Figure 4. Bhlhe40 expression is ubiquitous in other cell subsets during Armstrong infection

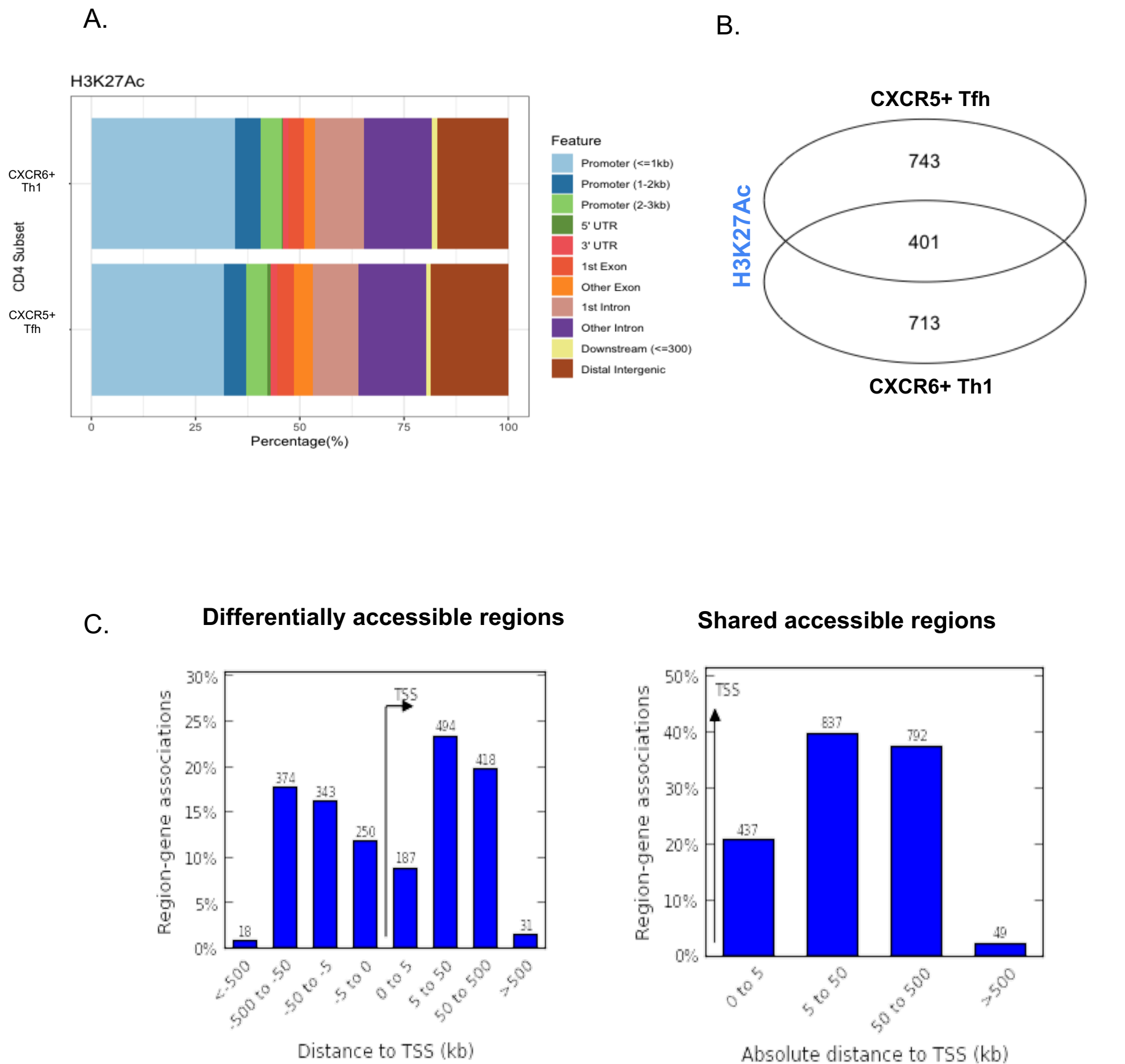
A. Experimental design

B. Summary graphs showing the kinetics of B cell subsets. Summary data is displaying 5-6 mice per time point.

C. Summary graphs showing the kinetics of Myeloid cell subsets. Summary data is displaying 5-6 mice per time point.

D. Representative flow plots and summary data showing the proportion and total number of Germinal center B cells from unchallenged mixed bone marrow chimera mice.

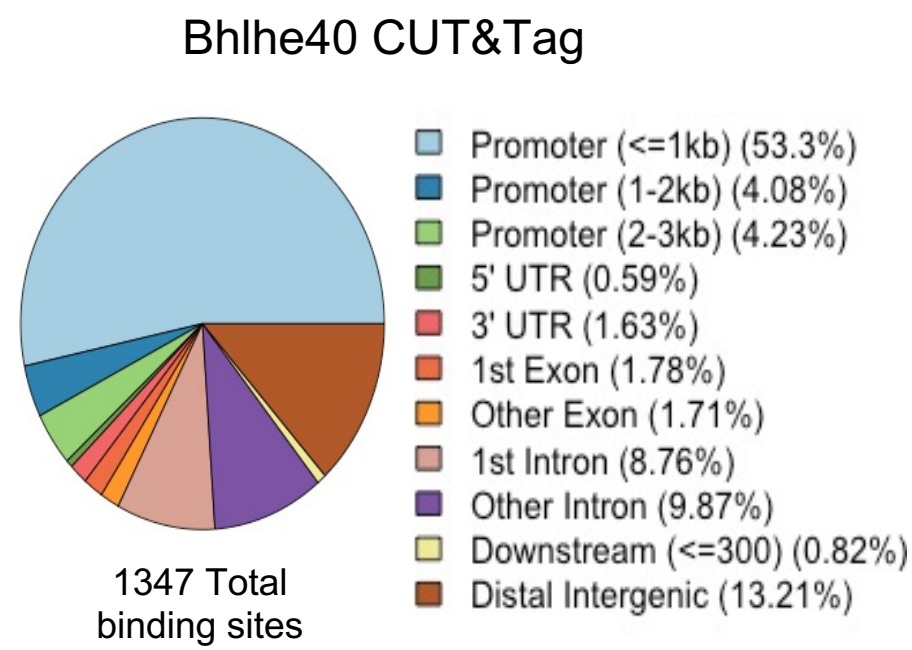
E. Serum titers from unchallenged mixed bone marrow chimera experimental mice (n=5).



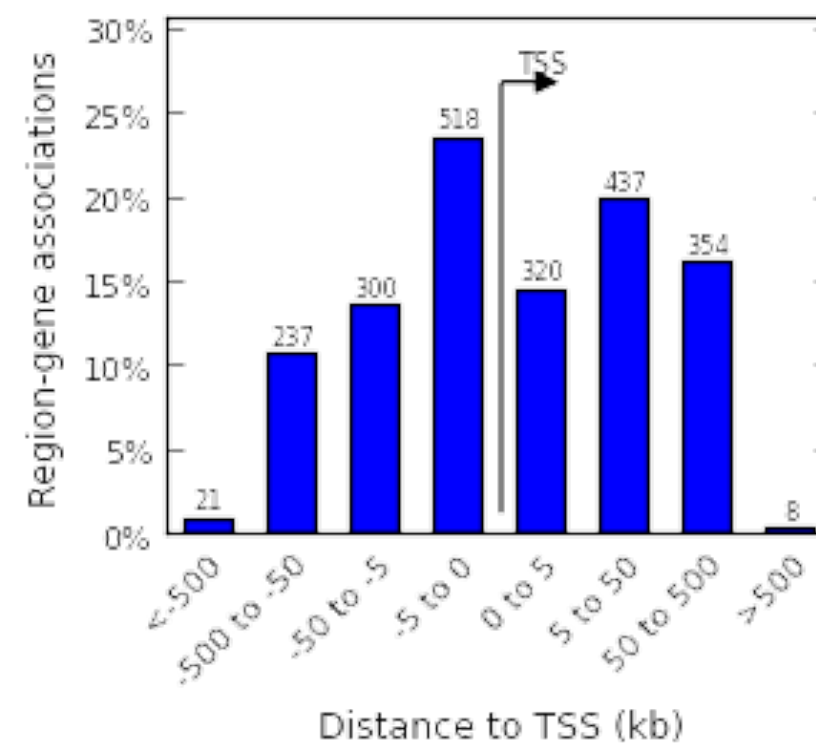
Supplemental Figure 5. Unique enhancer repertoires define characteristics of CD4⁺ T cells subsets

- A. Bar plot showing percentage of genome wide H3K27Ac enrichment at the at promoter regions, 5'UTR, 3'UTR, exons, introns, and intergenic regions in CD4 subsets.
- B. Venn diagram plots showing differential H3K27Ac signals at intersected gene promoters through pair-wise comparisons of CD4 subsets.
- C. Bar plot displaying the distance of chromatin accessible regions to TSS. Left plot is showing the chromatin accessible regions that are differentially accessible between CXCR6⁺ Th1 and CXCR5⁺ Tfh. Right plot is showing the shared accessible regions shared between the two CD4 T cells subsets.

A.

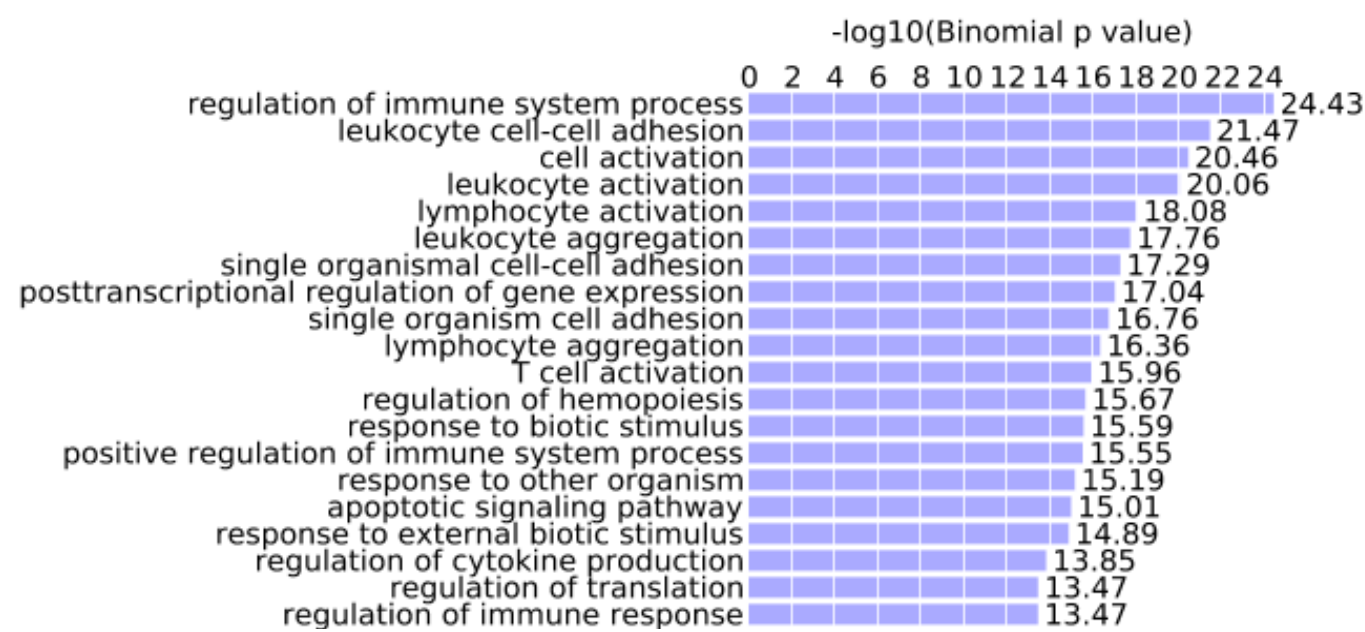


B.



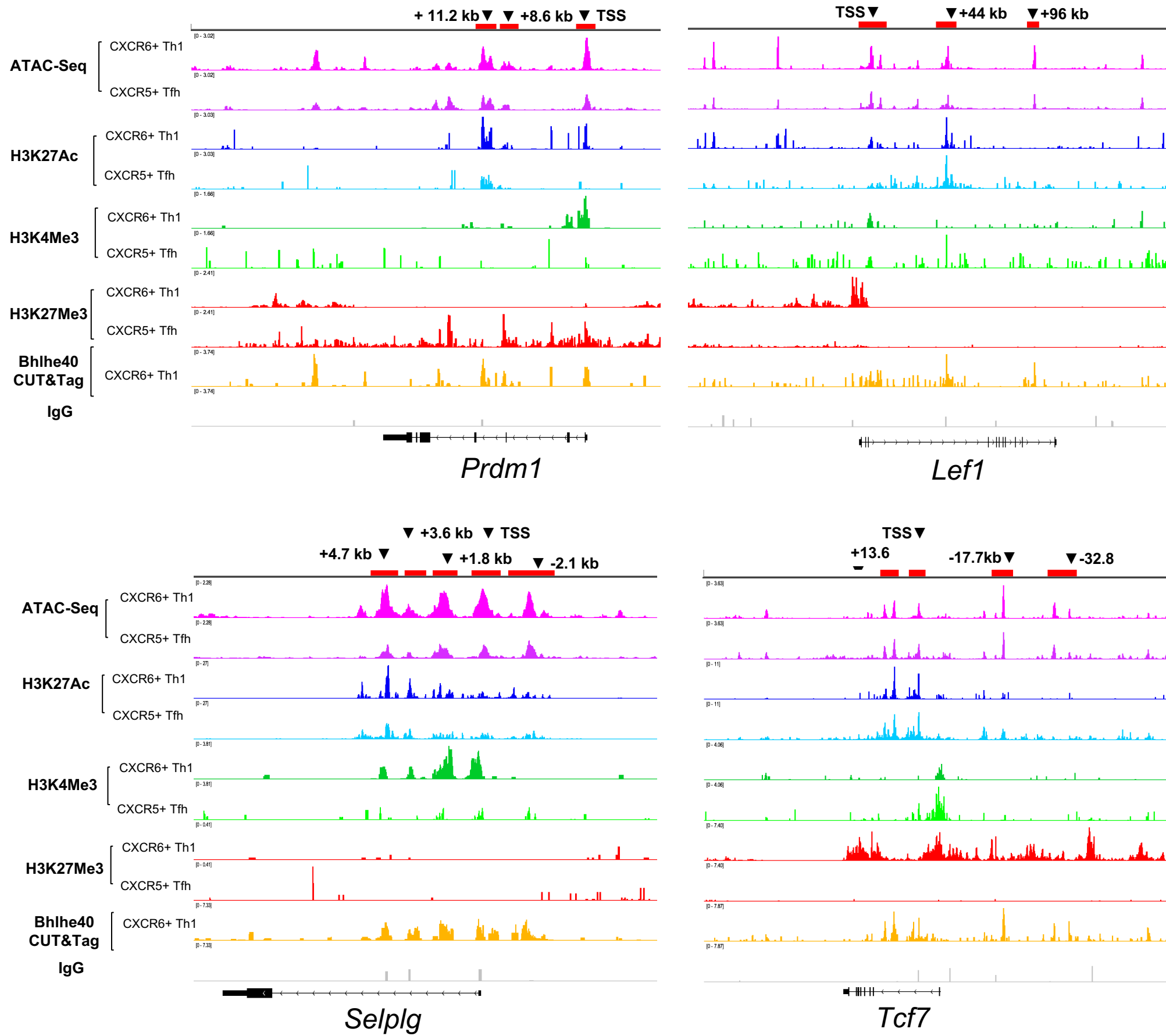
C.

Category name: user-provided data

GO Biological Process**Supplemental Figure 6. Genome-wide assessment of Bhlhe40 binding sites**

- A. Pie chart showing percentage of genome wide Bhlhe40 enrichment at the at promoter regions, 5'UTR, 3'UTR, exons, introns, and intergenic regions in CXCR6+ Th1 cells.
- B. Bar plot showing the distance of Bhlhe40-binding to TSS
- C. Gene ontology (GO) analysis showing the top enriched pathways Bhlhe40-bound genes are associated with.

A.



Supplemental Figure 7. States of Bhlhe40-bound enhancer regions that regulate gene expression in Th1 and Tfh subsets

A. Genome track plots showing ATAC-Seq, H3K27Ac, H3K4Me3, H3K27Me3, Bhlhe40 CUT&Tag-seq, and IgG peaks at various gene loci in CXCR6+ Th1 and CXCR5+ Tfh cells.