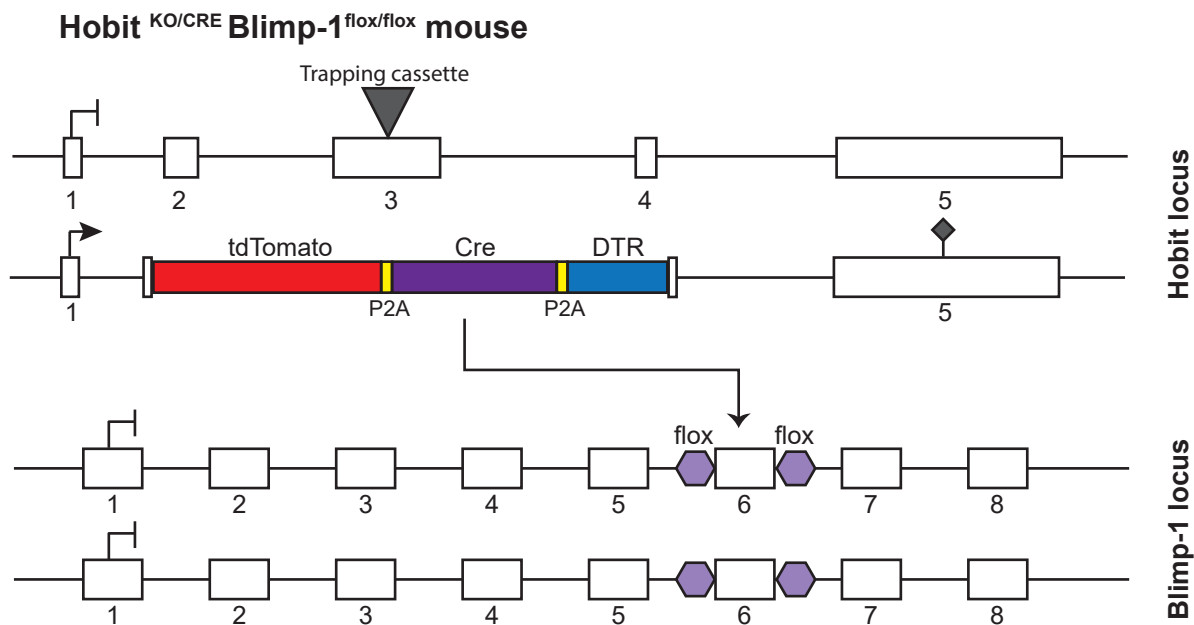
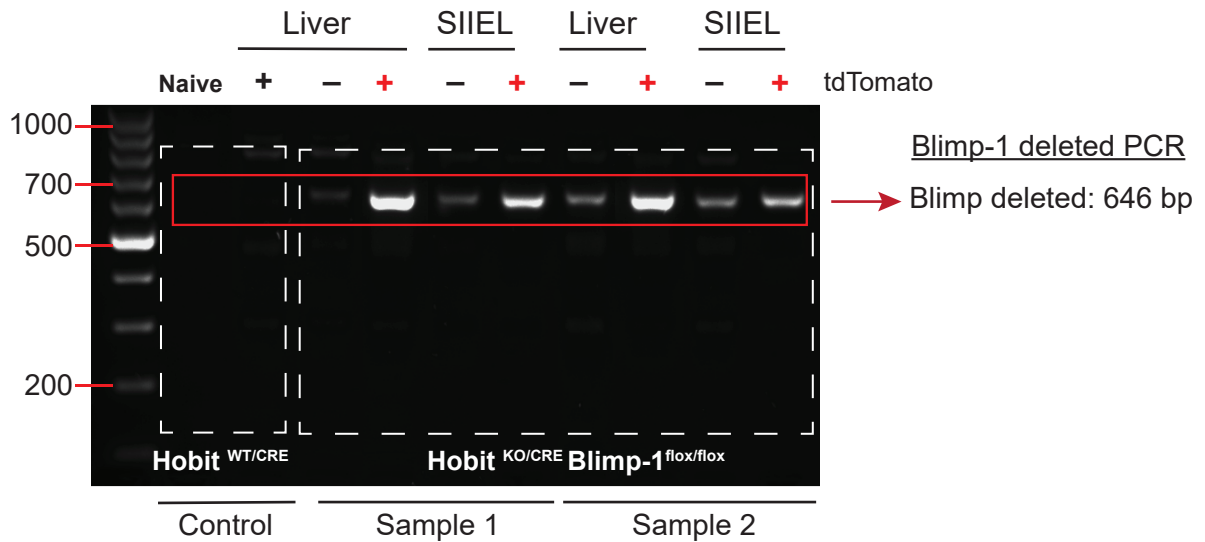
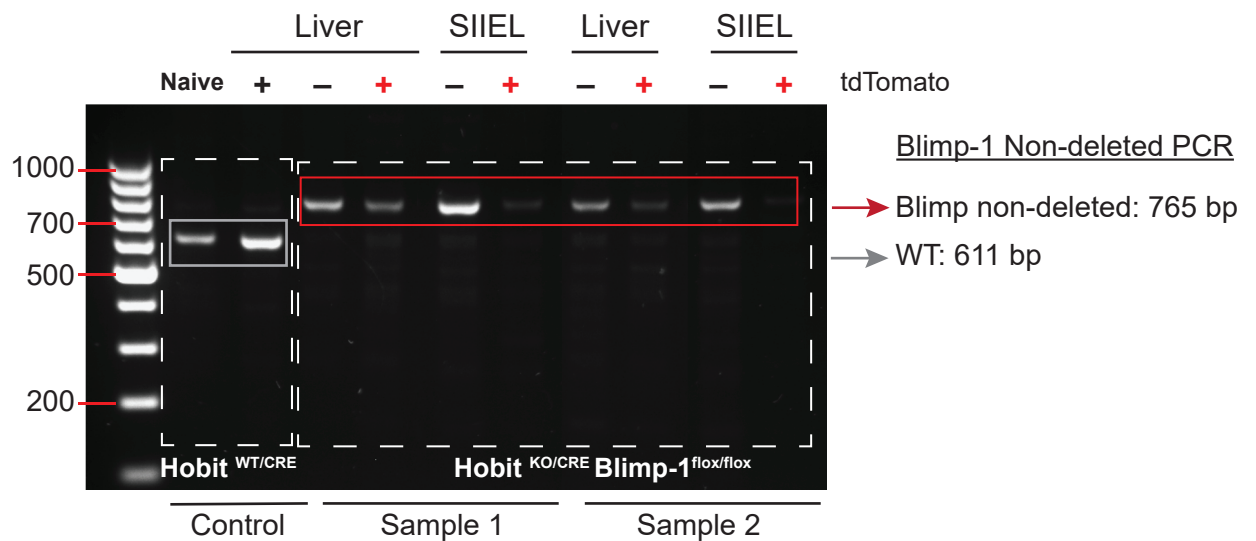


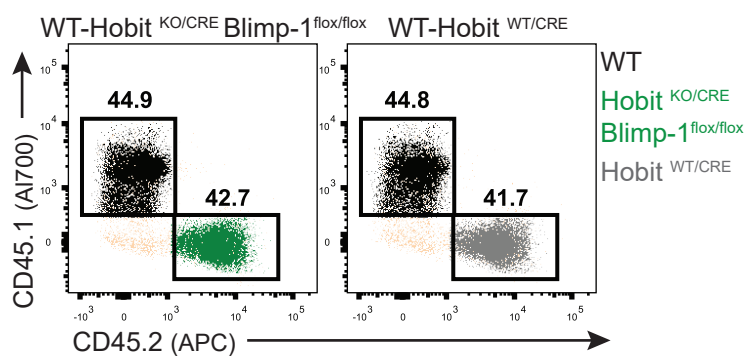
**Figure S1. Hobit is restricted to the TRM lineage and Blimp-1 is widely expressed in antigen-experienced CD8+ T cells.** (A) Representative flow cytometry plot shows CD62L and CD69 expression which identifies T<sub>CM</sub> (CD62L+ CD69-), T<sub>EM</sub> (CD62L- CD69-) and T<sub>RM</sub> (CD62L- CD69+) cells, as indicated. (B) Expression (cpm) of Prdm1 encoding Blimp-1 was determined in naïve and Listeria-OVA-specific memory CD8+ T cells by RNA sequencing. (C) Representative flow cytometry plot shows KLRG1 and CD127 expression to identify SLECs (KLRG1+ CD127-) and MPECs (KLRG1- CD127+) in the spleen of Hobit<sup>tdTomato/WT</sup> × Blimp-1<sup>GFP/WT</sup> mice. (D) Representative histogram shows expression of Blimp-1 (GFP) in the indicated cell subsets. (E) The geo MFI of GFP expression was quantified in SLECs and MPECs. (F) Representative flow cytometry plot displays the gating of tdTomato+ and tdTomato- virus-specific CD8+ T cells at day 8 after LCMV infection. (G) Expression (cpm) of Prdm1 in naïve and tdTomato+ and tdTomato- LCMV-specific effector CD8+ T cells isolated at day 8 p.i. was determined by RNA sequencing. Symbols represent individual mice. Error bars represent mean ± SEM. Dotted lines connect paired samples. (A) Representative data of one (n=4) out of two independent experiments. (B, G) Data from one experiment (n = 3 pooled samples). (C-F) Combined data from two independent experiments (n=6). Paired t test. \*\*P < 0.01.

**A****B****C**

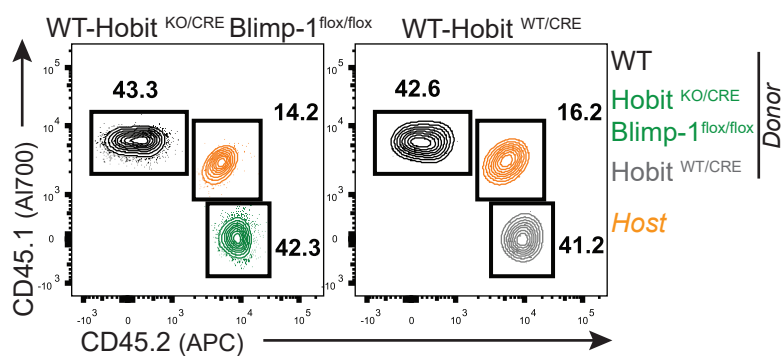
**Figure S2. Blimp-1 is efficiently deleted in Hobit+ CD8+ T cells of Hobit<sup>KO/CRE</sup> x Blimp-1<sup>flx/flx</sup> mice.** (A) Schematic representation of the Hobit<sup>KO/CRE</sup> x Blimp-1<sup>flx/flx</sup> mouse is shown. (B, C) The presence of (B) deleted Blimp-1 and (C) non-deleted Blimp-1 was analyzed by semi-quantitative PCR in CD8+ T cells isolated from the liver and SI IEL of Hobit<sup>KO/CRE</sup> x Blimp-1<sup>flx/flx</sup> and control Hobit<sup>WT/CRE</sup> mice at day >30 after LCMV infection. Data display 2 individual samples from two independent experiments.

**A**

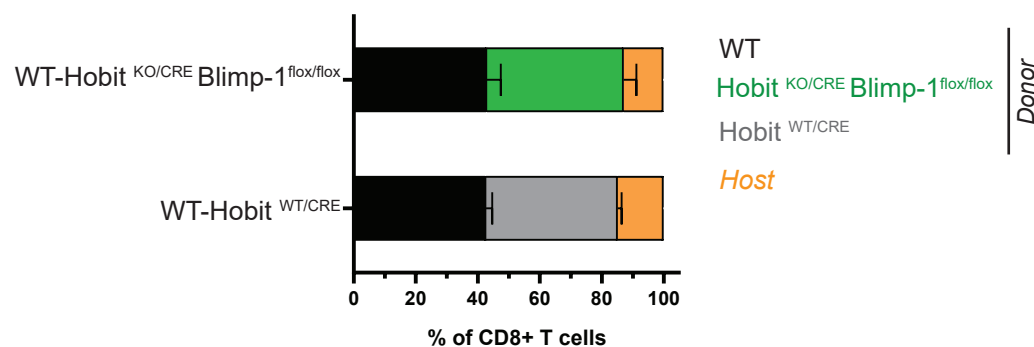
Bone marrow mix (Pre-transfer)

**B**

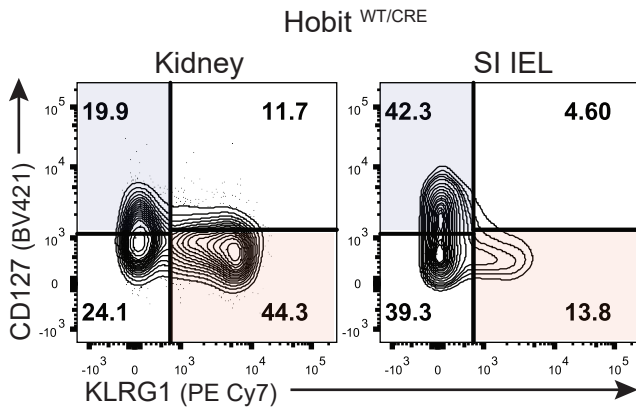
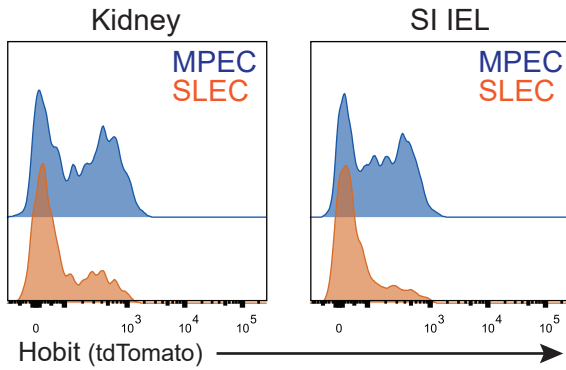
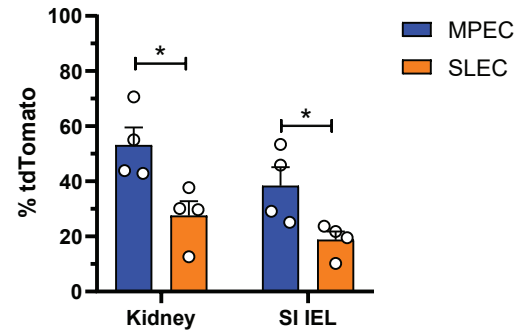
Day 0 (Steady-state)

**C**

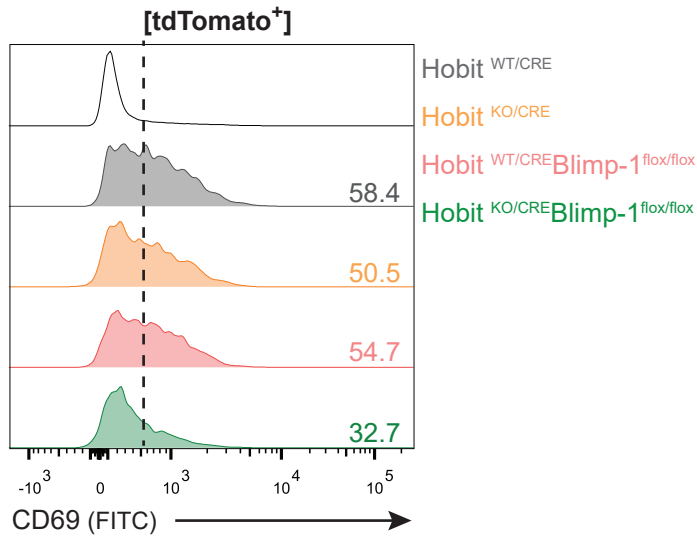
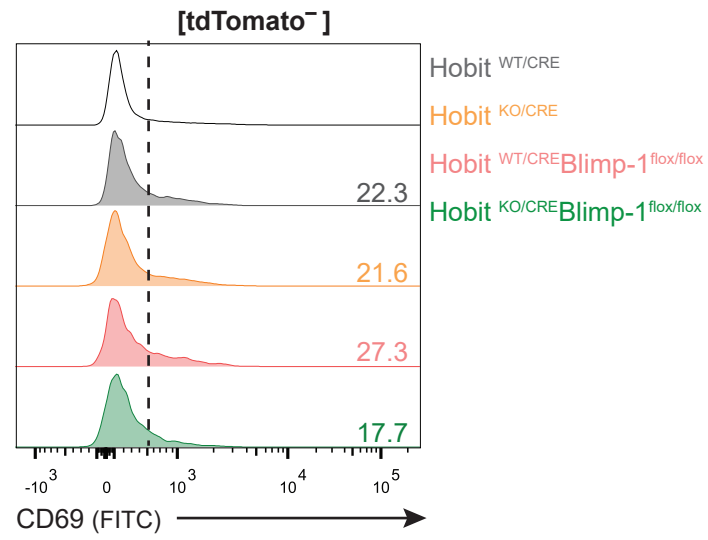
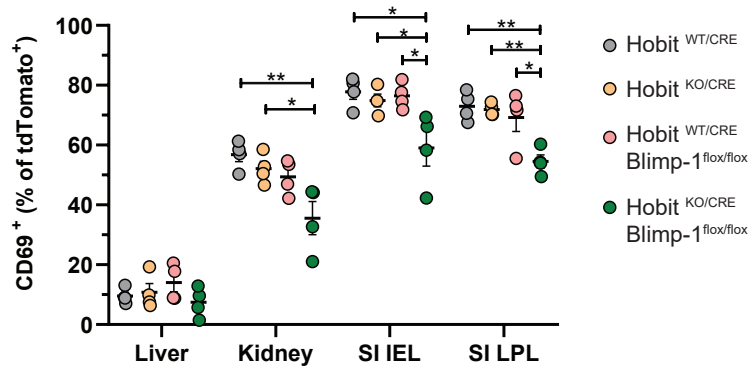
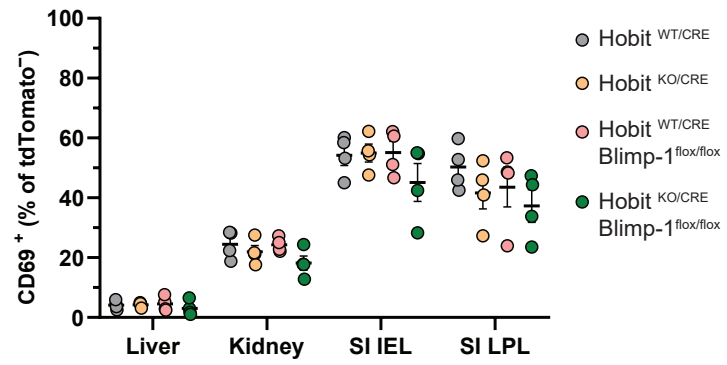
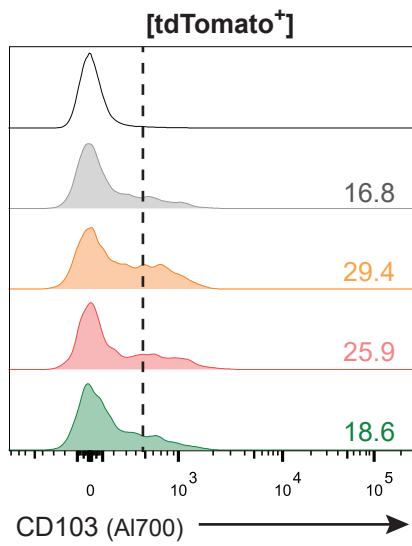
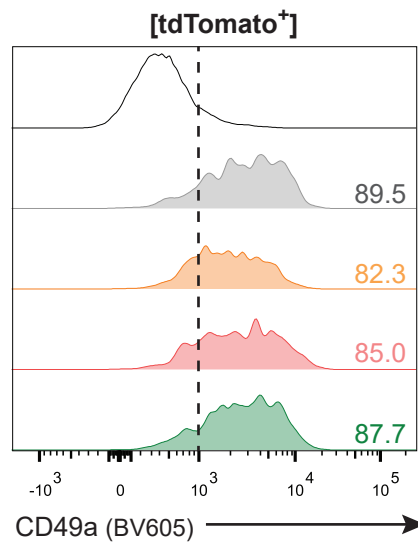
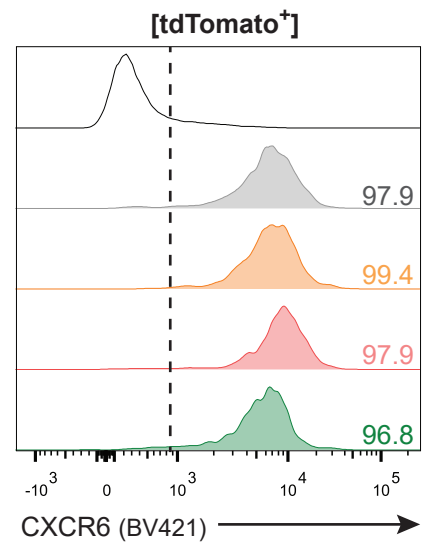
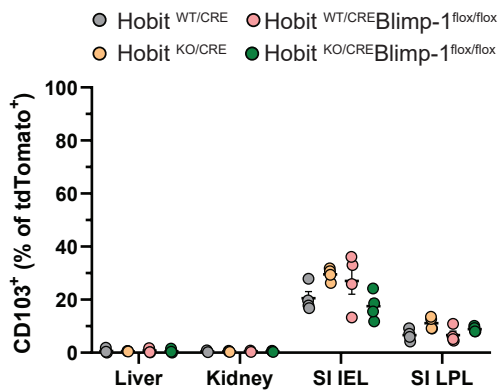
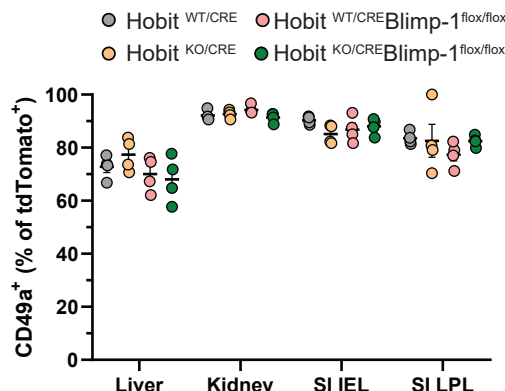
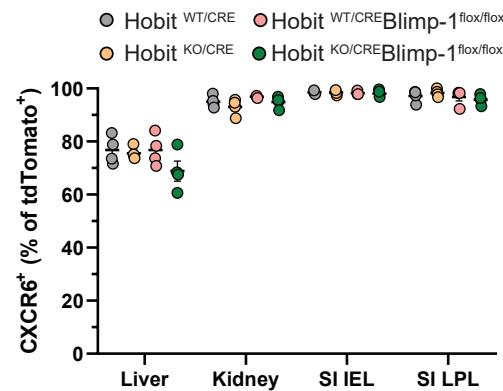
Blood



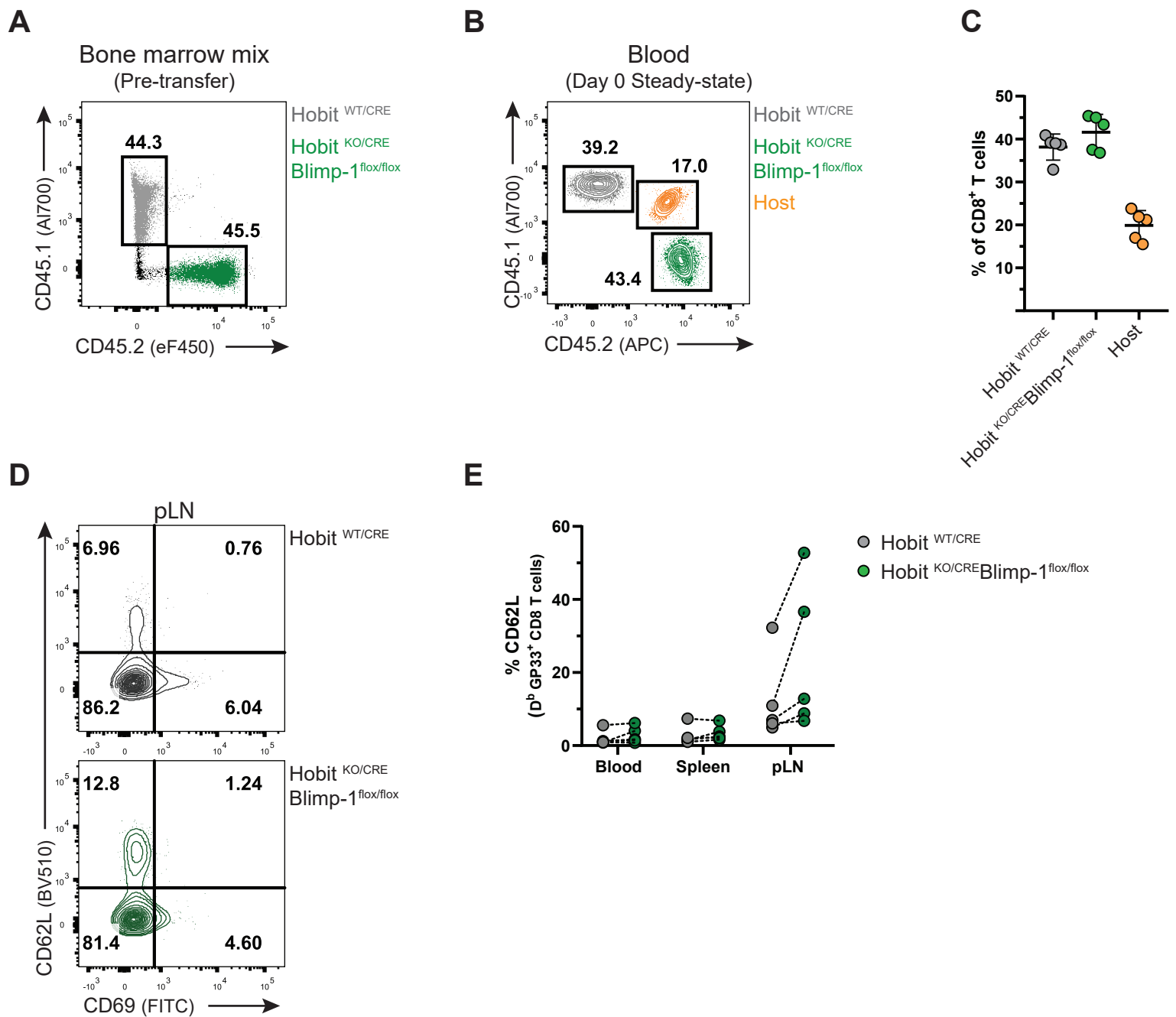
**Figure S3. Contribution of different cell compartments in mixed bone marrow (BM) chimeras.** (A, B) Representative flow cytometry plots display CD45.1 and CD45.2 to identify the contribution of WT (CD45.1<sup>+</sup>) and Hobit<sup>WT/CRE</sup> (CD45.2<sup>+</sup>) or Hobit<sup>KO/CRE</sup> x Blimp-1<sup>flox/flox</sup> (CD45.2<sup>+</sup>) compartments to the (A) pre-transfer BM cell mix and (B) to the total CD8<sup>+</sup> T cell fraction in the blood of chimeric mice 60 days after reconstitution and just prior to infection with LCMV. (C) The contribution of WT and transgenic CD8<sup>+</sup> T cells to the total CD8<sup>+</sup> T cell fraction was quantified. Representative data of one (n=3-4) out of two independent experiments.

**A****B****C**

**Figure S4. Hobit<sup>+</sup> effector CD8<sup>+</sup> T cells are enriched for an MPEC phenotype.** (A) Representative flow cytometry plots show expression of CD127 and KLRG1 within GP33<sup>+</sup> CD8<sup>+</sup> T cells in the indicated tissues of Hobit<sup>WT/CRE</sup> mice. (B) Representative histograms display the expression of tdTomato within MPECs (KLRG1<sup>-</sup> CD127<sup>+</sup>) and SLECs (KLRG1<sup>+</sup> CD127<sup>-</sup>). (C) The percentage of tdTomato expression was quantified in MPECs and SLECs in the indicated tissues. Combined data from two independent experiments (n=4). Unpaired t test. \*P < 0.05.

**A****C****B****D****E****F****G****H****I****J**

**Figure S5. Hobit and Blimp-1 cooperatively instruct upregulation of CD69 on TRM precursors.** (A-D) Representative histograms display CD69 expression in (A) tdTomato+ and (C) tdTomato- virus-specific CD8+ T cells isolated from the kidney of Hobit<sup>WT/CRE</sup>, Hobit<sup>KO/CRE</sup>, Hobit<sup>WT/CRE</sup> x Blimp-1<sup>flox/flox</sup> and Hobit<sup>KO/CRE</sup> x Blimp-1<sup>flox/flox</sup> mice at day 8 after LCMV infection. The percentage of CD69 expression was quantified in (B) tdTomato+ and (D) tdTomato- virus-specific CD8+ T cells. (E-G) Representative histograms display expression of (E) CD103, (F) CD49a and (G) CXCR6 on tdTomato+ virus-specific CD8+ T cells isolated from the SI IEL of Hobit<sup>WT/CRE</sup>, Hobit<sup>KO/CRE</sup>, Hobit<sup>WT/CRE</sup> x Blimp-1<sup>flox/flox</sup> and Hobit<sup>KO/CRE</sup> x Blimp-1<sup>flox/flox</sup> mice at day 8 p.i. Control sample gated on CD62L+ T cells from the spleen. (H-J) The percentage of (H) CD103, (I) CD49a and (J) CXCR6 expression was quantified in tdTomato+ virus-specific CD8+ T cells of Hobit<sup>WT/CRE</sup>, Hobit<sup>KO/CRE</sup>, Hobit<sup>WT/CRE</sup> x Blimp-1<sup>flox/flox</sup> and Hobit<sup>KO/CRE</sup> x Blimp-1<sup>flox/flox</sup> mice. Symbols represent individual mice. Error bars represent mean ± SEM. (A-J) Combined data from two independent experiments (n=4). One-way ANOVA. \*P < 0.05; \*\*P < 0.01.



**Figure S6. Hobit and Blimp-1 promote CD69 expression on TRM precursors in mixed bone marrow (BM) chimeras.** Representative flow cytometry plots display CD45.1 and CD45.2 expression to identify the contribution of Hobit<sup>WT/CRE</sup> (CD45.1+) and Hobit<sup>KO/CRE</sup> x Blimp-1<sup>flox/flox</sup> (CD45.2+) compartments to the **(A)** pre-transfer BM cell mix and **(B)** to the total CD8<sup>+</sup> T cell fraction in the blood of chimeric mice 60 days after reconstitution and just prior to infection with LCMV. **(C)** The percentage of CD8<sup>+</sup> T cells originating from the host, and the donor Hobit<sup>WT/CRE</sup> and Hobit<sup>KO/CRE</sup> x Blimp-1<sup>flox/flox</sup> compartments was determined. **(D)** Representative flow cytometry plots display CD62L and CD69 expression in GP33<sup>+</sup> CD8<sup>+</sup> T cells from the Hobit<sup>WT/CRE</sup> and Hobit<sup>KO/CRE</sup> x Blimp-1<sup>flox/flox</sup> compartment at day 8 p.i. with LCMV. **(E)** The percentage of CD62L expression was quantified in the Hobit<sup>WT/CRE</sup> and Hobit<sup>KO/CRE</sup> x Blimp-1<sup>flox/flox</sup> compartment of GP33<sup>+</sup> CD8<sup>+</sup> T cells in the indicated tissues of the chimeric mice. Symbols represent individual mice. Representative data of one (n=5) out of two independent experiments. **(C)** Error bars represent mean  $\pm$  SEM. **(E)** Dotted lines connect paired samples.