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Supplemental Information

Qa-1-Restricted CD8$^+$ T Cells Can Compensate for the Absence of Conventional T Cells during Viral Infection

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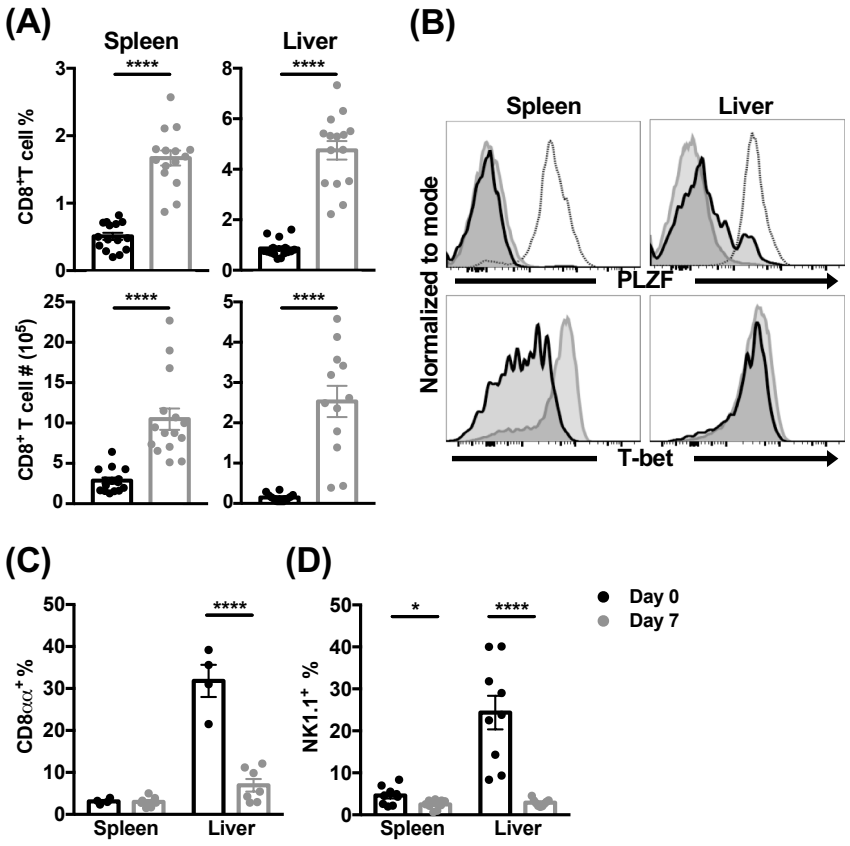
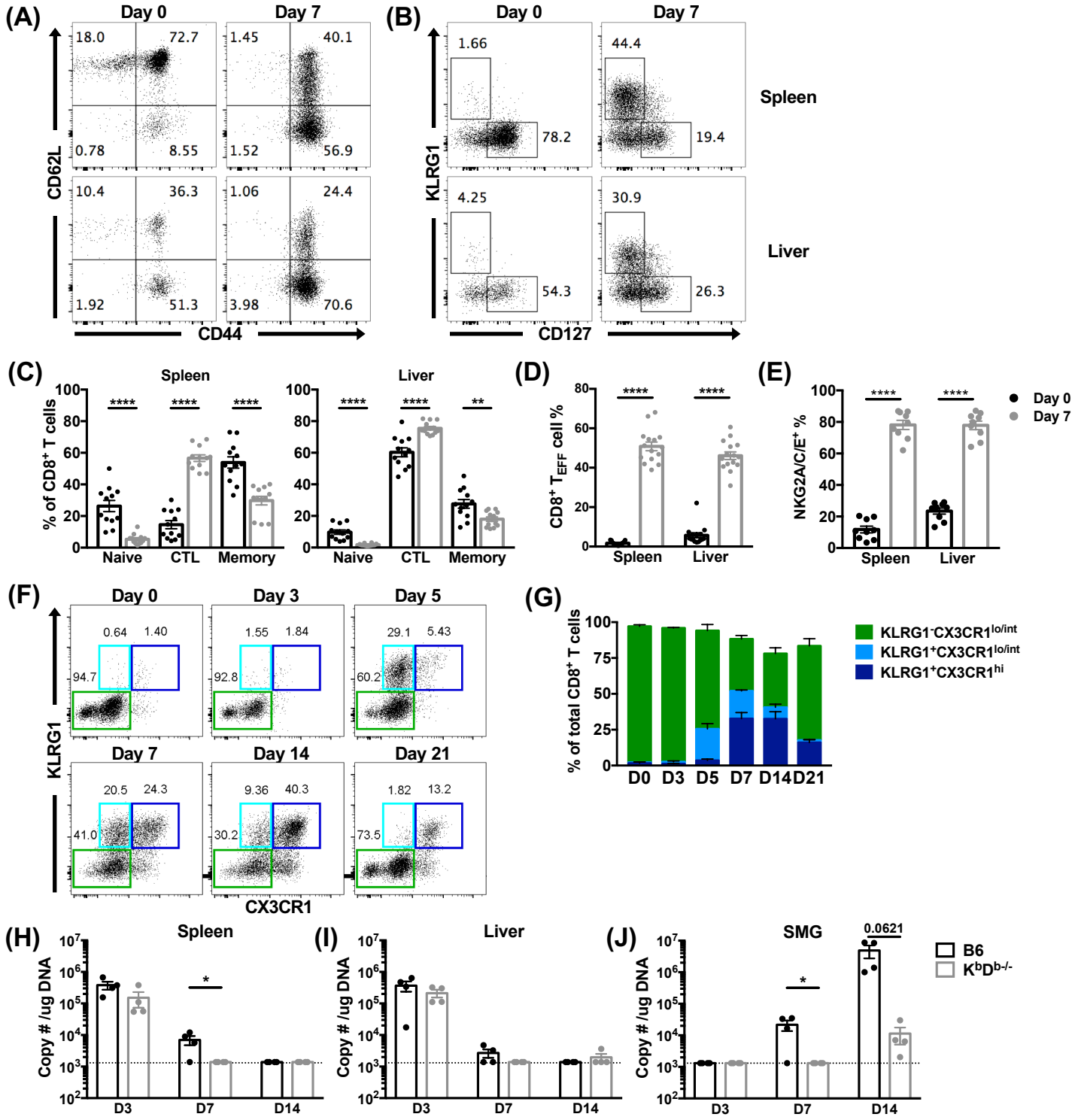
Supplementary Figure 1



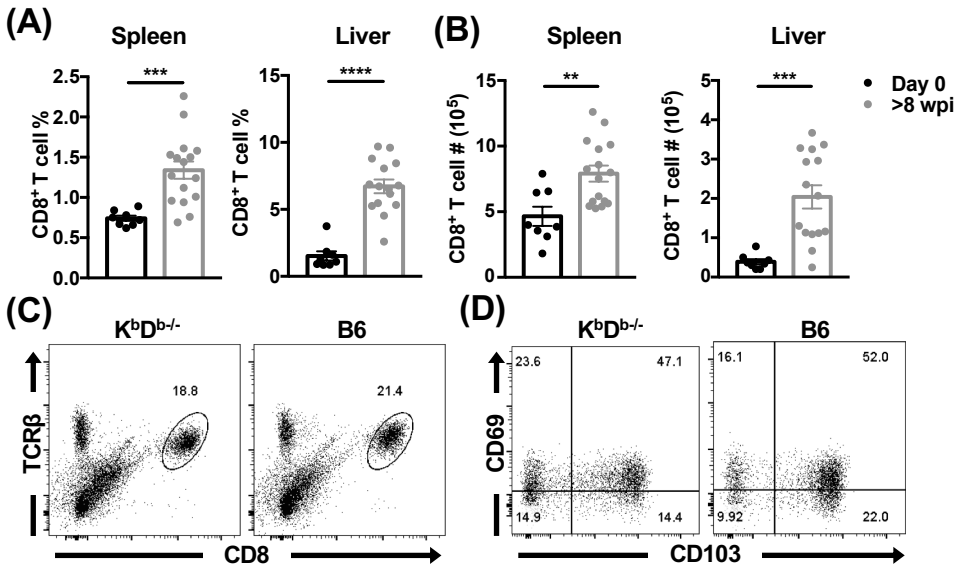
Supplementary Figure 1. MCMV-expanded non-classical CD8$^+$ T cells are distinct from innate-like T cells. Related to Figure 1. (A) Frequency and number of CD8$^+$ T cells in the spleen and liver of K^{b}D$^{b-/-}$ mice on day 0 and day 7 post-MCMV infection (n=12-15). (B) Representative histograms of PLZF and T-bet expression of CD8$^+$ T cells from the spleen and liver of K^{b}D$^{b-/-}$ mice on day 0 (black line) and 7 (grey line) post-MCMV infection. iNKT cells were used as positive control for PLZF (dotted line). Frequency of CD8$^+$ T cells that are (C) CD8αα$^+$ (n=4-7) or (D) NK1.1$^+$ (n=9) from the spleen and liver of K^{b}D$^{b-/-}$on day 0 and 7 post-MCMV infection. Data are pooled from at least three (A, D) or two (C) independent experiments. Data represent mean ± SEM. *p<0.05 and ****p<0.0001.

Supplementary Figure 2



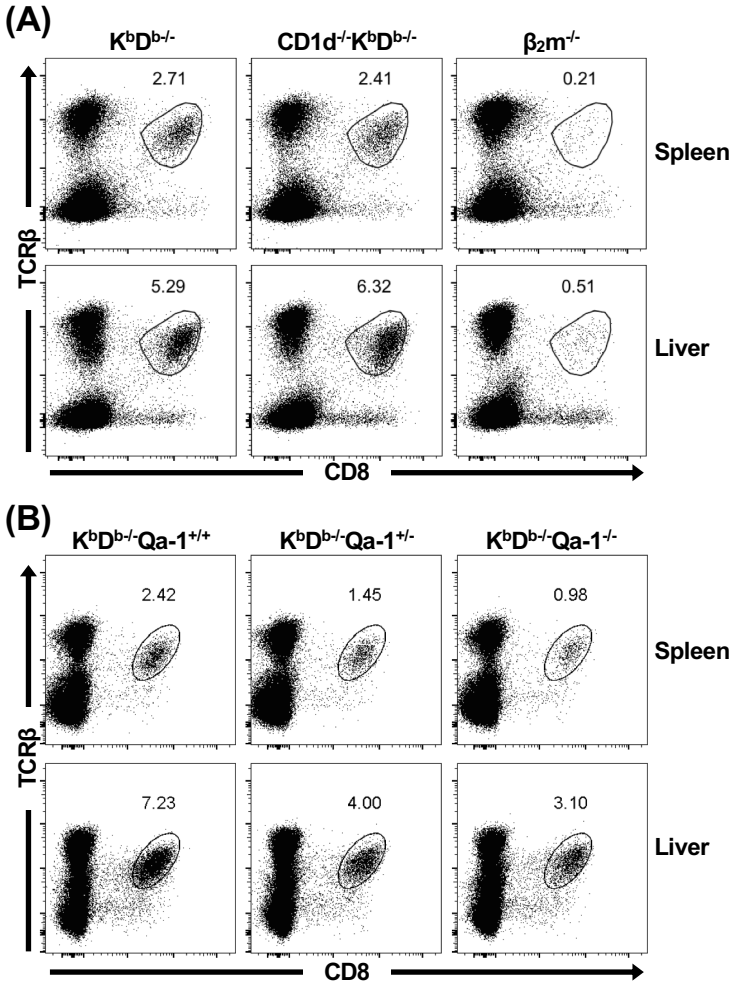
Supplementary Figure 2. Non-classical CD8⁺ T cells acquire an effector phenotype following acute MCMV infection. Related to Figure 1. (A) CD44 and CD62L expression and (B) CD127 and KLRG1 expression of CD8⁺ T cells from the spleen and liver of $K^bD^{b-/-}$ mice on day 0 and 7 post-MCMV infection. (C) Frequency of naïve ($CD62L^+CD44^-$), CTL ($CD62L^-CD44^+$), and memory ($CD62L^+CD44^+$) CD8⁺ T cells in the spleen and liver of $K^bD^{b-/-}$ mice on day 0 and day 7 post-infection (n=12). (D) Frequency of CD8⁺ T_{EFF} cells ($KLRG1^+CD127^-$) in the spleen and liver of $K^bD^{b-/-}$ mice on day 0 and day 7 post infection (n=15). (E) Frequency of NKG2A/C/E⁺ CD8⁺ T cells in the spleen and liver of $K^bD^{b-/-}$ mice on day 0 and day 7 post-infection (n=9). (F) Expression and (G) frequency of CD8⁺ T cells that are $CX3CR1^{hi}KLRG1^+$ from the spleen and liver of $K^bD^{b-/-}$ mice on indicated day post-MCMV infection (n=3). Viral quantification of the MCMV gene *IE1* by qPCR in the (H) spleen, (I) liver, and (J) SMG of $K^bD^{b-/-}$ and C57BL/6 mice on indicated days post-infection (n=4). Dotted line is limit of detection. Data are pooled or representative of at least three (A-E) or one (F-J) independent experiment. Data represent mean ± SEM . *p<0.05, **p<0.01 and ****p<0.0001.

Supplementary Figure 3



Supplemental Figure 3. Non-classical CD8$^+$ T cells persist in long-term MCMV-infected K^{b}D$^{b-/-}$ mice and form memory populations. Related to Figure 4. (A) Frequency and (B) number of CD8$^+$ T cells in the spleen and liver from naïve (n=8) and long-term infected K^{b}D$^{b-/-}$ mice (n=15-16). (C) Representative CD8$^+$ T cells in the SMG of long-term infected K^{b}D$^{b-/-}$ and C57BL/6 mice. (D) CD103 and CD69 expression of SMG CD8$^+$ T cells from long-term infected K^{b}D$^{b-/-}$ and C57BL/6 mice. Data are representative of two (C-D) or pooled from three (A, B) independent experiments and represent mean ± SEM. **p<0.01, ***p<0.001 and ****p<0.0001.

Supplementary Figure 4



Supplementary Figure 4. Non-classical CD8$^+$ T cell expansion following MCMV infection is Qa-1- and β_2m-dependent. Related to Figures 6 and 7. (A) Representative staining of non-classical CD8$^+$ T cells in the spleen and liver of $K^bD^{b-/-}$, $CD1d^{-/-}K^bD^{b-/-}$, and $\beta_2m^{-/-}$ mice on day 7 post-MCMV infection. (B) Representative staining of non-classical CD8$^+$ T cells in the spleen and liver on day 7 post-MCMV infection from $K^bD^{b-/-}$Qa-$1^{+/+}$, $K^bD^{b-/-}$Qa-$1^{+/-}$, and $K^bD^{b-/-}$Qa-$1^{-/-}$ mice. Data are representative of at least two independent experiments.