

#### Suppl. Fig. 1: T cell development in $K^b D^{b-/-}$ and $K^b D^{b-/-} \gamma c^{Tg}$ mice

**A.** CD4 versus CD8 profiles of total thymocytes of the indicated mouse strains. Results are representative of 6 independent experiments.

**B.** Frequencies (left) and cell numbers (right) of mature CD4SP and CD8SP thymocytes in WT and  $K^b D^{b-/-}$  mice. Results are the summary of 6 independent experiments with a total of 9 WT and 11  $K^b D^{b-/-}$  mice.



Suppl. Fig. 2: Unconventional innate-like CD8 T cells and conventional innate CD8 T cells Frequencies of CD69<sup>+</sup>NK1.1<sup>+</sup> innate-like CD8 T cells (bottom) was assessed in CD8 $\alpha\alpha$  and CD8 $\alpha\beta$  T cells among CD4-negative TCR $\beta^{hi}$  liver T cells (left) and CD44<sup>+</sup>CXCR3<sup>+</sup> CD8SP thymocytes (right) of the indicated mice (top). Results are representative of 2 independent experiments with a total of 4 WT, 3  $K^b D^{b-/-}$ , and 4  $K^b D^{b-/-} \gamma c^{Tg}$  mice.



#### Suppl. Fig. 3: *i*NKT and innate-like T cells in *Qa-1<sup>-/-</sup>* mice

**A.** Frequencies of CD8 $\alpha\alpha$  innate-like CD8 T cells among CD4-negative TCR $\beta^{hi}$  mononuclear lymphocytes in the liver of the indicated mice. Results are representative of 2 independent experiments with a total of 4 WT and 4 *Qa-1<sup>-/-</sup>* mice.

**B.** Frequencies of CXCR3<sup>+</sup>CD44<sup>+</sup> innate CD8 T cells (middle) were determined in TCR $\beta^{hi}$  mature CD8SP thymocytes of the indicated mice (top), and then assessed for CD8 $\alpha\beta$  versus CD8 $\alpha\alpha$  expression among innate CD8 T cells (bottom). Results are representative of 2 independent experiments with a total of 4 WT and 4 *Qa-1<sup>-/-</sup>* mice.

**C.** Frequencies of *i*NKT cells among total thymocytes of WT and  $Qa-1^{-/-}$  mice. Results are representative of 2 independent experiments with a total of 4 WT and 4  $Qa-1^{-/-}$  mice.



### Suppl. Fig. 4: Characterization of CD8SP thymocytes in $K^b D^{b-/-} \gamma c^{Tg}$ mice

**A.** Frequency of CD8SP thymocytes in  $K^b D^{b-/-}$  and  $K^b D^{b-/-} \gamma c^{Tg}$  mice. Results are the summary of 8 independent experiments.

**B.** Frequencies of CXCR3<sup>+</sup>CD44<sup>+</sup> CD8SP thymocytes in WT and  $K^b D^{b-/-} \gamma c^{Tg}$  mice. Results are the summary of 6 independent experiments with a total of 6 WT and 10  $K^b D^{b-/-} \gamma c^{Tg}$  mice.



#### Suppl. Fig. 5: Thymic *i*NKT cell development in *K<sup>b</sup>D<sup>b-/-</sup>*γc<sup>Tg</sup> mice

**A.** Frequencies (top) and cell numbers (bottom) of CD24<sup>lo</sup> mature *i*NKT cells among thymocytes of the indicated mouse strains. Results show the summary of 6 independent experiments with a total of 8 WT, 8  $K^b D^{b-/-}$ , and 9  $K^b D^{b-/-} \gamma c^{Tg}$  mice.

**B.** Frequencies and cell numbers of T-bet<sup>+</sup> NKT1 cells of the indicated mice. Results show the summary of 3 independent experiments with a total of 3 WT, 4  $K^b D^{b-/-}$ , and 6  $K^b D^{b-/-} \gamma c^{Tg}$  mice. **C.** Frequencies of NKT2 cells of the indicated mice. Results show the summary of 3 independent experiments with a total of 3 WT, 4  $K^b D^{b-/-}$ , and 6  $K^b D^{b-/-} \gamma c^{Tg}$  mice.

**D.** Frequencies and cell numbers of IL-4-producing *i*NKT cells of the indicated mice. Results show the summary of 3 independent experiments with a total of 6 WT, 4  $K^b D^{b-/-}$ , and 4  $K^b D^{b-/-} \gamma c^{Tg}$  mice.



#### Suppl. Fig. 6: Innate-like CD8αα T cells in *K<sup>b</sup>D<sup>b-/-</sup>*γc<sup>Tg</sup> mice

**A.** Contour plots show the CD8 $\beta$  versus Eomes profiles of TCR $\beta^{hi}$  mature CD8SP T cells in WT,  $K^b D^{b-/-}$ , and  $K^b D^{b-/-} \gamma c^{Tg}$  mice and CD8 $\alpha \alpha$  T cells of liver mononuclear cells in WT mice. Results are representative of 2 independent experiments.

**B.** Frequencies of CXCR3<sup>+</sup>CD44<sup>+</sup> innate CD8 T cells (top) was determined in TCR $\beta^{hi}$  mature CD8SP thymocytes of the indicated mice, and then assessed for CD8 $\alpha\beta$  versus CD8 $\alpha\alpha$  T cells among innate CD8 T cells (bottom). Results are representative of 2 independent experiments with a total of 4 WT, 3 *K*<sup>b</sup>*D*<sup>b-/-</sup>, and 4 *K*<sup>b</sup>*D*<sup>b-/-</sup> $\gamma c^{Tg}$  mice.



### Suppl. Fig. 7: Thymocyte development in *K<sup>b</sup>D<sup>b-/-</sup>Zbtb16<sup>-/-</sup>* mice

**A.** Contour plots show the CD4 versus CD8 profiles of TCR $\beta^{hi}$  mature thymocytes in WT,  $K^b D^{b-/-}$ , and  $K^b D^{b-/-} Zbtb16^{-/-}$  mice. Results show the summary of 3 independent experiments.

**B.** Frequency and number of mature *i*NKT cells that were identified as CD24<sup>lo</sup>CD1dTet<sup>+</sup> cells in the thymus of  $K^b D^{b-/-}$  and  $K^b D^{b-/-} Zbtb16^{-/-}$  mice. Results show the summary of 3 independent experiments with a total of 5  $K^b D^{b-/-}$  and 4  $K^b D^{b-/-} Zbtb16^{-/-}$  mice.

**C.** Frequency and number of thymic CXCR3<sup>+</sup>CD44<sup>+</sup> innate CD8 T cells from the indicated mice. Results show the summary of 3 independent experiments with a total of 5  $K^bD^{b-/-}$  and 4  $K^bD^{b-/-}$  Zbtb16<sup>-/-</sup> mice.



#### Suppl. Fig. 8: *i*NKT and innate CD8 T cells in *CD1d*<sup>-/-</sup>γc<sup>Tg</sup> mice

**A.** Frequencies of *i*NKT cells among total thymocytes of the indicated mouse strains. Results are representative of 6 independent experiments with a total of 12 WT, 7  $\gamma c^{Tg}$ , and 16 *Cd1d<sup>-/-</sup>*  $\gamma c^{Tg}$  mice.

**B.** Frequencies of CXCR3<sup>+</sup>CD44<sup>+</sup> innate CD8 T cells among mature CD8SP thymocytes in the indicated mice. Results are representative of 6 independent experiments with a total of 12 WT, 7  $\gamma c^{Tg}$ , and 16 *Cd1d<sup>-/-</sup>* $\gamma c^{Tg}$  mice.