Supplemental Figures



Fig. S1. DGKζ-deficient mice have altered CD8⁺ and CD8⁺CD122⁺ cell numbers

A. Analysis of the total number of CD44^{hi}CD122^{hi} CD8⁺ T cells in WT (n=6), DGK α - (n=5) and DGK ζ -deficient (n=6) mouse spleens. Mean \pm SEM, unpaired Student's t-test with Welch's correction. **B.** Analysis of CD122^{hi} CD8⁺ T cells in spleens of WT and DGKζ-deficient mice at different ages. Left, flow cytometry of CD8 and CD122 markers (gated on CD8⁺ cells). Right, CD122^{hi} percentages gated on CD8⁺. Mean ± SEM, two-way ANOVA and Bonferroni post-test $(n = 3/\text{genotype and age, except DGK}\zeta^{-/-} 50 \text{ weeks, } n = 2)$. C. Analysis of CD8⁺ population in spleens of WT and DGK ζ -deficient mice at different ages. Mean \pm SEM, two-way ANOVA and Bonferroni post-test (n = 3/genotype and age, except DGK^{-/-} 50 weeks, n = 2). **D.** Analysis of CD8⁺CD44^{hi}CD122^{hi} population in spleens of WT and DGK²-deficient mice at different ages. Mean \pm SEM, statistical analyses and groups as above. E. Analysis of the CD44^{hi}CD122^{hi}CD8 SP population in thymuses of WT and DGKζ-deficient mice at different ages. Percentages of $CD44^{hi}CD122^{hi}$ were analyzed in $CD8^+CD4^-$ cells. Mean \pm SEM, Bonferroni post-test (n = 3/genotype and age, except DGK ζ -/- 50 weeks, n = 2). F. Quantification of the $\gamma\delta$ T cell population (TCR δ^+ TCR β^-) in thymus and spleen of WT and DGK ζ -deficient mice. Data were acquired in two independent experiments. Mean \pm SEM, n = 6/genotype. Unpaired t test. G. Analysis of NK cells (CD3^{$^{-}}NKP46^{+}$) in spleens of WT and DGK ξ -deficient mice. Data were</sup> acquired in three independent experiments. Mean \pm SEM, n = 9 per genotype. Unpaired t test.



Fig S2. DGKζ-deficient CD8⁺ CD44^{hi} cells show similar STAT5 and S6 phosphorylation intensity than WT cells. The geometric mean of pSTAT5 (A) or pS6 (B) was quantified in the CD8⁺ CD44^{hi} pSTAT⁺/pS6⁺ population for the experiments described in figure 3. (Mean \pm SEM; n = 3/genotype, data were acquired in three independent experiments).



Fig. S3. Analysis of mRNA levels for NKG2D ligands and cytokines in A20 cells

mRNA levels of indicated molecules were analyzed by real-time qRT-PCR in A20 cells. Mean ± SEM.