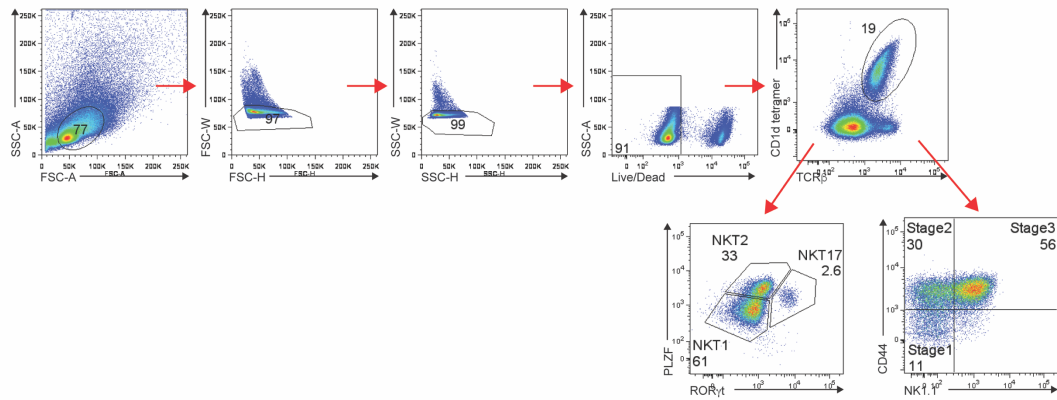
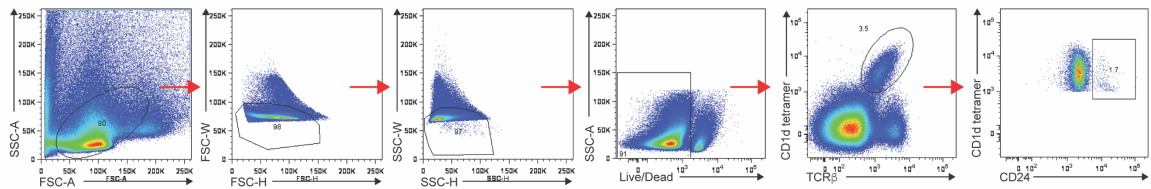


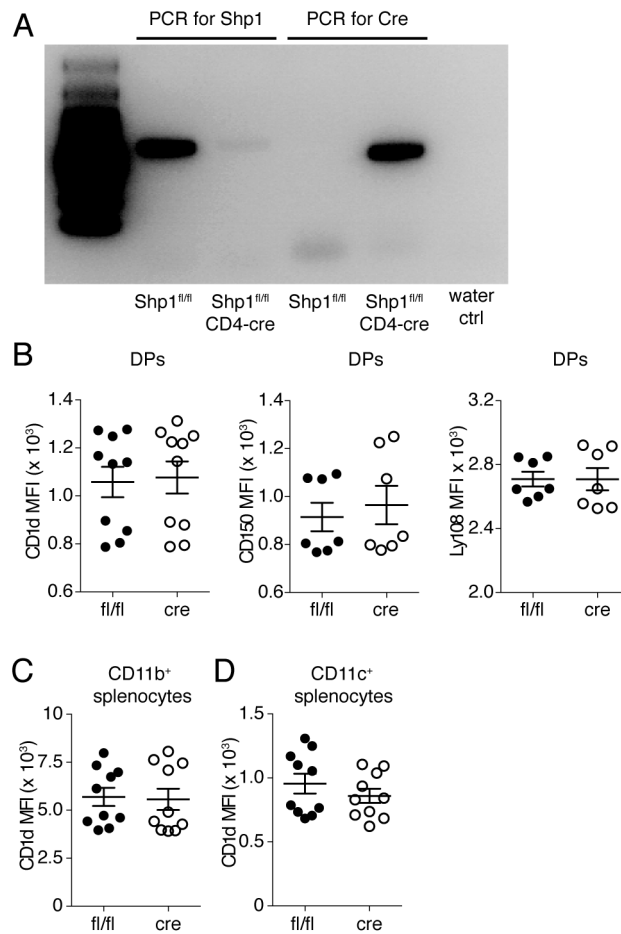
Gating Example 1



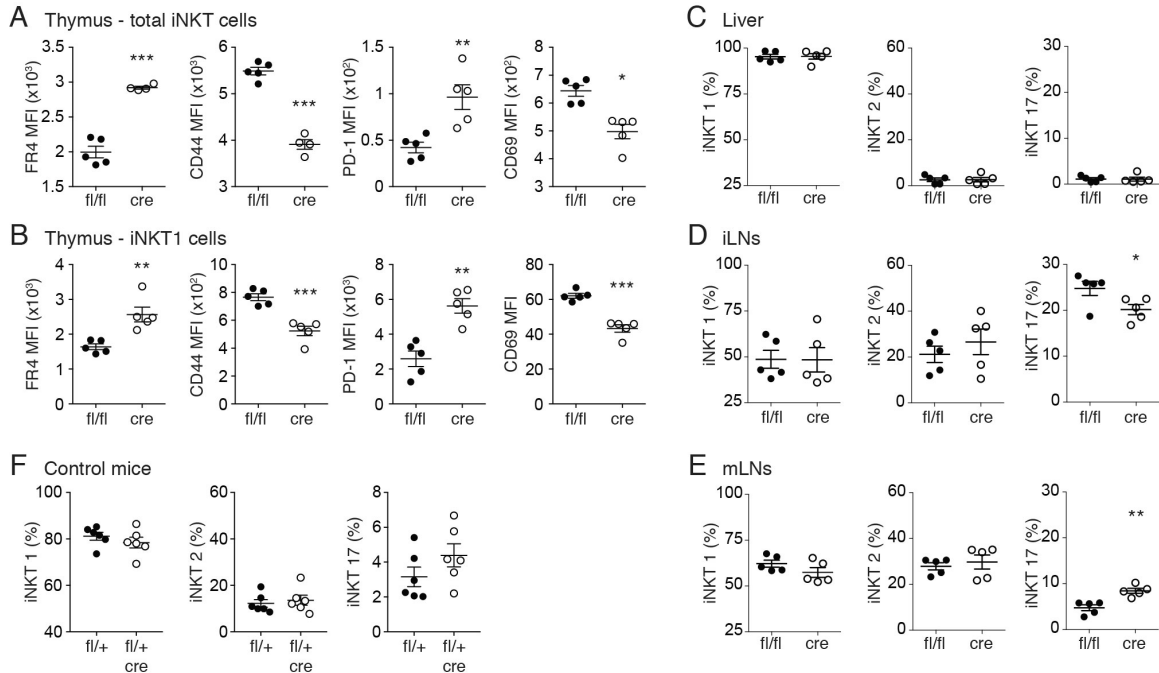
Gating Example 2



Supplementary Figure 1. Examples of gating strategies.

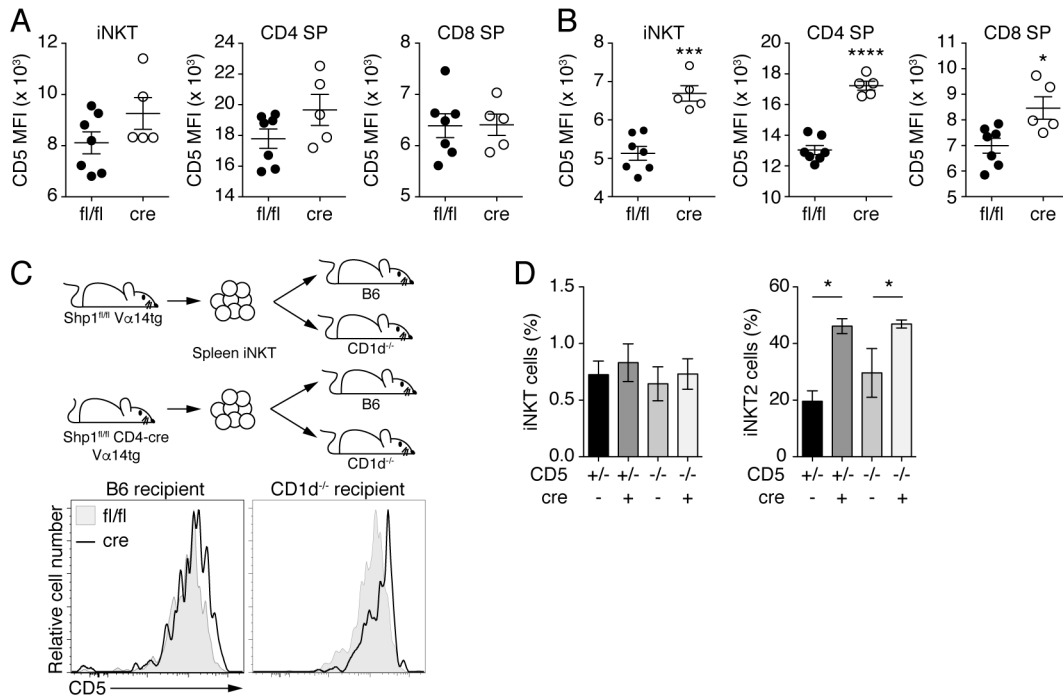


**Supplementary Figure 2.** Phenotype of Shp1<sup>fl/fl</sup> CD4-cre mice. **(A)** PCR of *Ptpn6* or *Cre* using DNA extracted from iNKT cells sorted from the thymus of Shp1<sup>fl/fl</sup> and Shp1<sup>fl/fl</sup> CD4-cre mice. **(B)** Expression of CD1d, SLAMF1 and SLAMF6 on thymic double positive (DP) thymocytes of Shp1<sup>fl/fl</sup> (fl/fl) and Shp1<sup>fl/fl</sup> CD4-cre (cre) mice. **(C, D)** Expression of CD1d on CD11b<sup>+</sup> (C) and CD11c<sup>+</sup> (D) splenocytes of Shp1<sup>fl/fl</sup> (fl/fl) and Shp1<sup>fl/fl</sup> CD4-cre (cre) mice. Data represents individual mice and the mean values +/- s.e.m.



**Supplementary Figure 3. Shp1 deletion biases iNKT cell effector differentiation.**

Expression of FR4, CD44, PD-1 and CD69 by total iNKT cells (**A**) or iNKT1 cells (**B**) from the thymus of Shp1<sup>fl/fl</sup> (fl/fl) and Shp1<sup>fl/fl</sup> CD4-cre (cre) mice. Frequency of iNKT cell subsets in the liver (**C**), inguinal lymph nodes (**D**) and mesenteric lymph nodes (**E**) of Shp1<sup>fl/fl</sup> (fl/fl) and Shp1<sup>fl/fl</sup> CD4-cre (cre) mice. (**F**) Frequency of iNKT cell subsets in the thymus of Shp1<sup>fl/+</sup> (fl/+) and Shp1<sup>fl/+</sup> CD4-cre (fl/+ cre) mice. Data represents individual mice and the mean values +/- s.e.m. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , two-tailed unpaired Student  $t$  test.



**Supplementary Figure 4. (A, B)** Expression of CD5 on thymic (A) and splenic (B) iNKT, CD4 and CD8 T cells from Shp1<sup>fl/fl</sup> and Shp1<sup>fl/fl</sup> CD4-cre mice. **(C)** Expression of CD5 on iNKT cells from Vα14<sup>Tg</sup> Shp1<sup>fl/fl</sup> and Vα14<sup>Tg</sup> Shp1<sup>fl/fl</sup> CD4-cre mice transferred into WT or CD1d<sup>-/-</sup> recipients. Data shows representative histogram plots from 3 independent experiments (n = 3 mice per group). **(D)** Frequency of total iNKT cells and iNKT2 cells in the thymus of CD5<sup>+/-</sup> Shp1<sup>fl/fl</sup>, CD5<sup>+/-</sup> Shp1<sup>fl/fl</sup> CD4-cre, CD5<sup>-/-</sup> Shp1<sup>fl/fl</sup> and CD5<sup>-/-</sup> Shp1<sup>fl/fl</sup> CD4-cre. Data shows the mean values +/- s.e.m. (n = 3 to 6 mice per group). \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001, \*\*\*\**p* < 0.0001, two-tailed unpaired Student *t* test.