Supplemental Data

The Transcription Factor PLZF Directs the Effector Program of the NKT Cell Lineage

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Supplemental Experimental Procedures

Th1 and Th2 Differentiation

TCRβ$^+$ CD1d-αGalCer tetramer-negative CD4$^+$ splenocytes were sorted from mixed bone marrow chimeras and cultured on anti-CD3-coated (10μg/ml) 96-well round-bottom plates in the presence of huIL-2 (10U/ml), and Th1 (20ng/ml IL-12, 20μg/ml anti-IL-4) or Th2 (4ng/ml IL-4, 20μg/ml anti-IL-12, 20μg/ml anti-IFNγ) differentiating conditions. Cultures were split in two after 4 days and rested for 3 more days prior to restimulation by anti-CD3 coated plates for 24 hours. The cells were then assessed by intracellular flow cytometry for the presence of IL-4 and IFNγ with Brefeldin A (10μg/ml) for the last 6 hours.

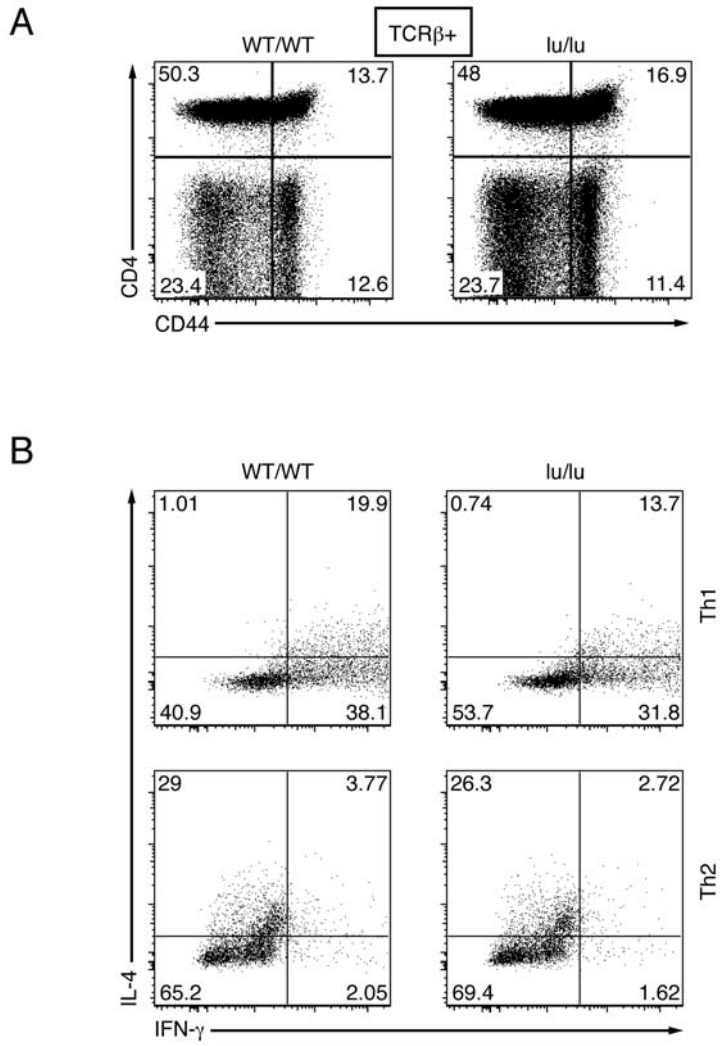

Figure S1. Memory and Effector Cell Formation in lu/lu T Cells

(A) Wt/WT and lu/lu CD44^{hi} memory and CD44^{lo} naïve CD4^{+} and CD4^{-} T cells among TCRβ^{+} gated splenocytes of mixed bone marrow chimeras. Data representative of 6 individual chimeras.

(B) In vitro generation of Th1 and Th2 polarized CD4 cells from fresh splenic CD4 T cells of mixed bone marrow chimeras. Dot plots show intracellular IL4 and IFNγ in gated CD45.1 (WT) and CD45.2 (lu/lu) cells after ionomycin/PMA restimulation, as indicated. Data representative of 3 individual chimeras.