SUPPLEMENTAL MATERIAL

Gilfillan et al., http://www.jem.org/cgi/content/full/jem.20081752/DC1

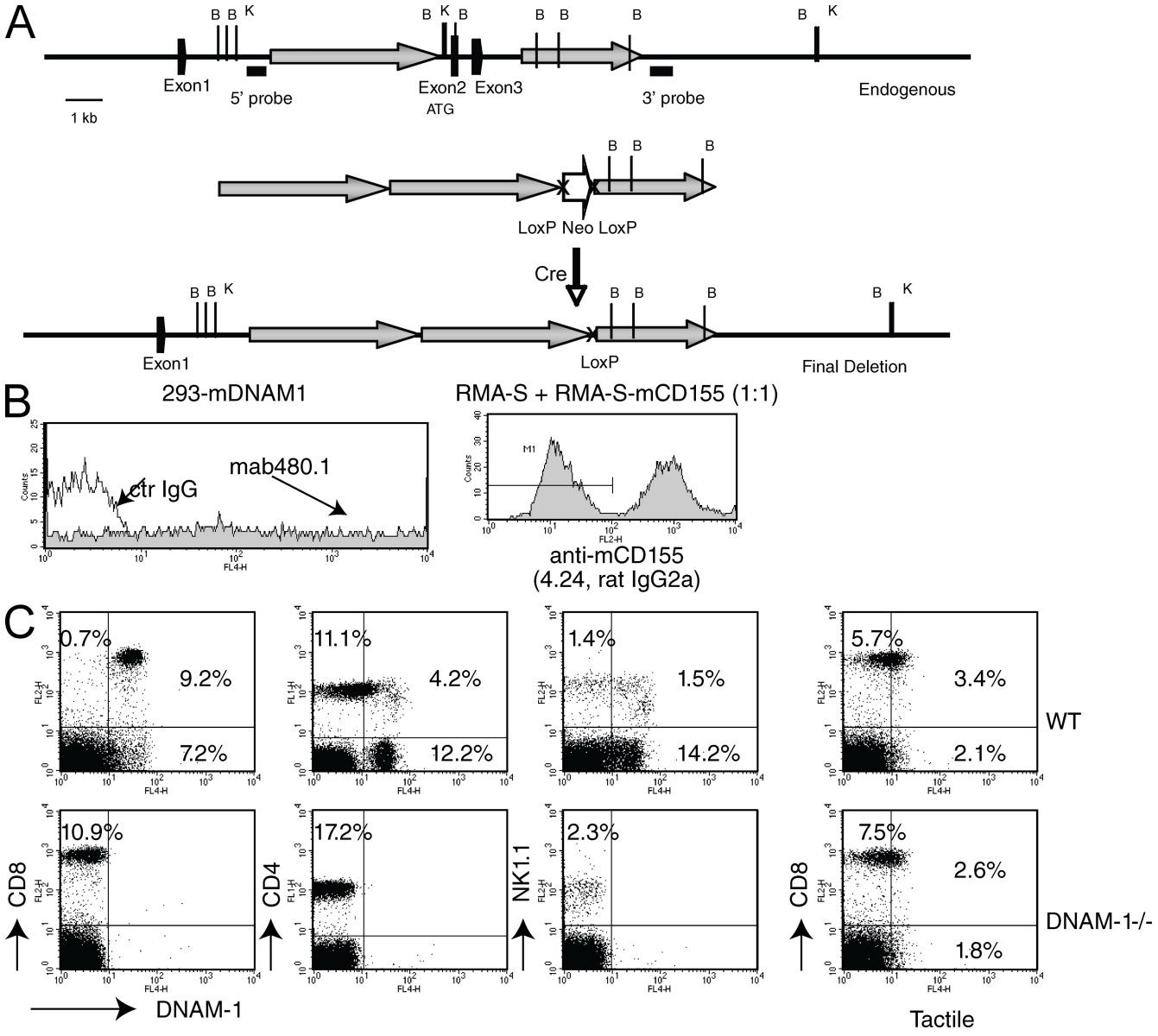

Figure S1. DNAM-1$^{-/-}$ mice. (A) DNAM-1 targeting strategy. (B, left) mAb480.1 detects mDNAM-1. (right) mAb4.24 detects mCD155. 293T cells were transiently transfected with mDNAM-1 cDNA cloned in the expression vector pEF6. After 48 h, cells were stained with mab480.1 or irrelevant rat IgG2a. Untransfected 293T cells did not react with mAb 480.1 (not depicted). RMA-S cells or RMA-S transfected with mCD155 were mixed at a 1:1 ratio and were stained with mab4.24. Approximately 50% of the cells were stained by mab4.24. The marker M1 indicates staining of the whole cell population by an irrelevant rat IgG2a. (C) Staining of WT and DNAM-1$^{-/-}$ spleen cells with the anti–mDNAM-1 mAb 480.1. In a naive WT mouse, DNAM-1 is expressed on all CD8 T cells, a small subset of CD4 T cells, ~40–50% of NK1.1^{high} NK cells, and all NK1.1^{low} (CD3^{+}) NKT cells. The staining is completely abrogated in DNAM-1$^{-/-}$ mice, whereas percentages of the indicated cell subsets do not differ significantly between WT and DNAM-1$^{-/-}$ mutants. Splenocytes were stained with the anti–DNAM-1 mAb 480.1 and were counterstained with CD4 and CD8 or NK1.1 and CD3.

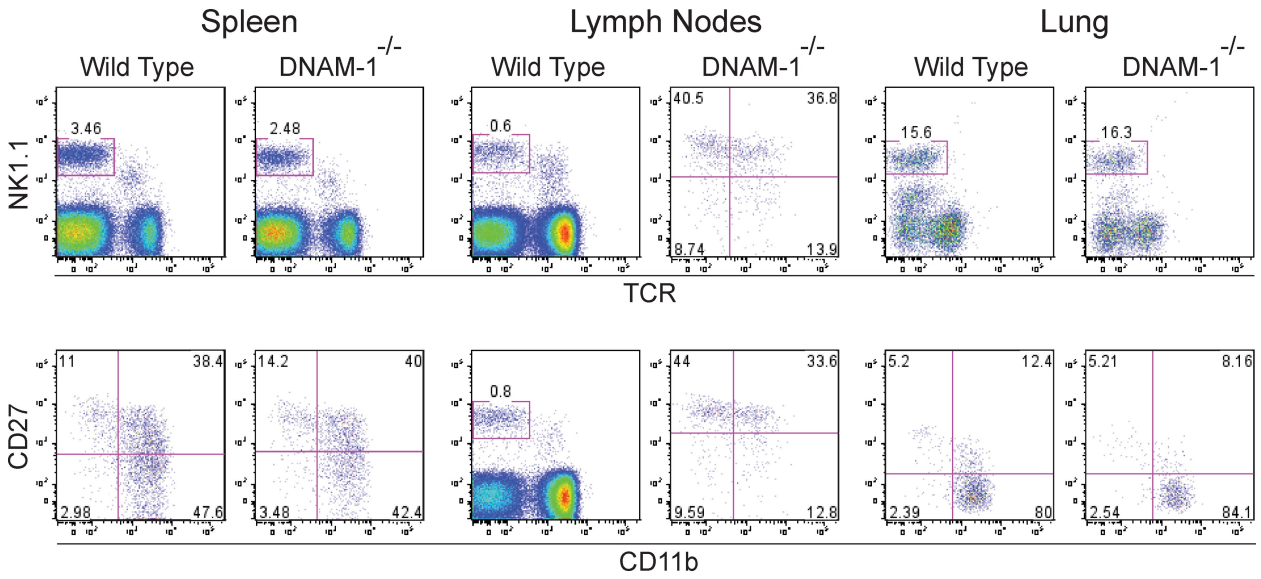

Figure S2. Normal NK cell homeostasis in DNAM-1$^{-/-}$ mice and DNAM-1 expression on different NK cell compartments. Spleen, lymph node, and lungs from B6 WT and DNAM-1$^{-/-}$ mice were processed for FACS analysis of NK cell subsets, as described in Materials and methods. NK cells (NK1.1^{+} TCR$\alpha\beta^{-}$) are present in similar percentages in wild-type and DNAM-1$^{-/-}$ mice and exhibit almost identical NK cell subsets (as determined by CD27 and CD11b) in the spleen, lymph node, and lung. Data shown are representative of three independent experiments each consisting of three to five mice per group.

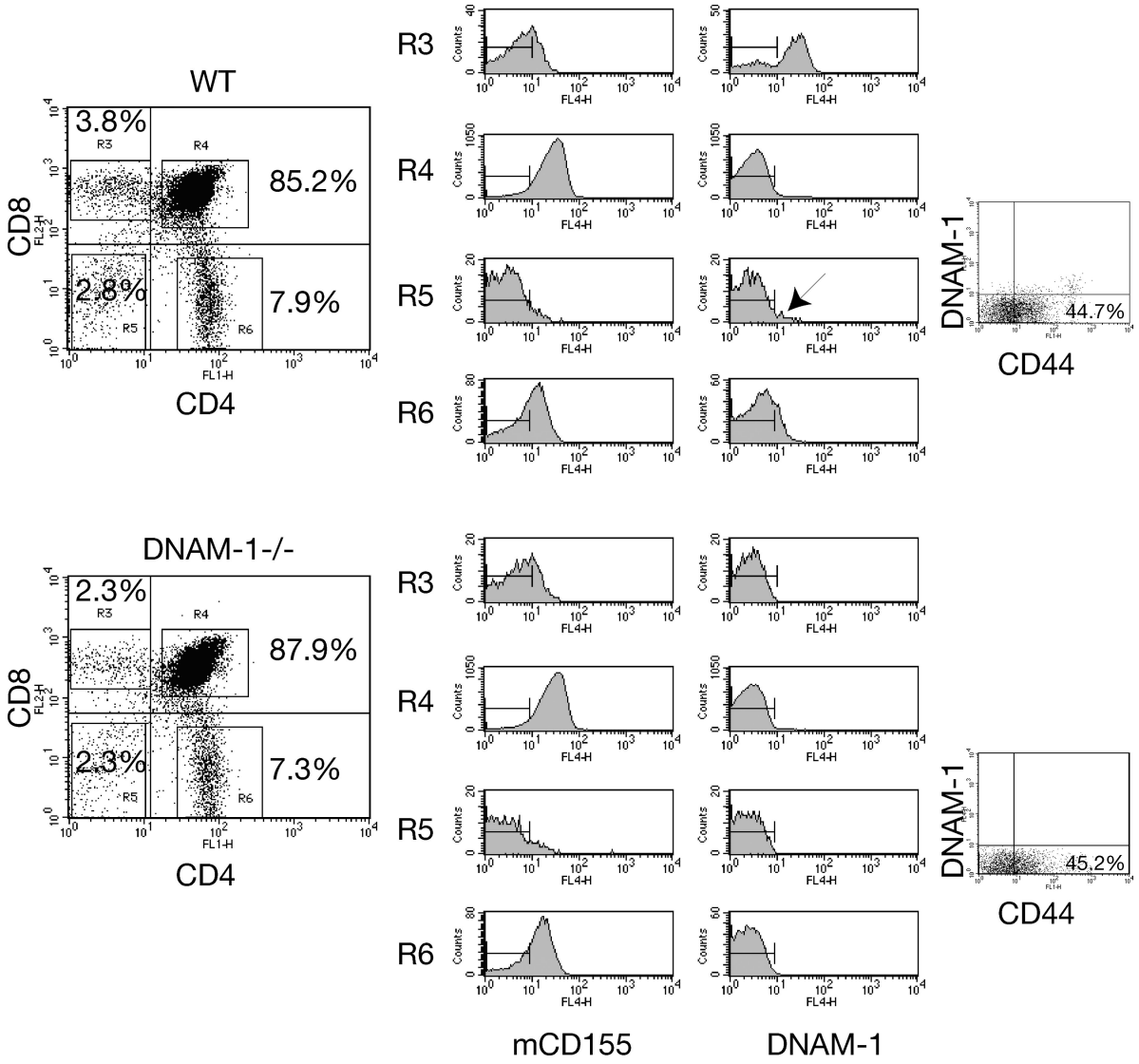

Figure S3. Thymic T cell compartments in WT and DNAM-1$^{-/-}$ mice. WT and DNAM-1$^{-/-}$ thymocytes were stained for CD4, CD8, DNAM-1, and CD44 or CD25. Gates were drawn to examine DNAM-1 and CD155 expression on CD8^{+} single-positive (SP; R3), CD4^{+} SP (R6), CD4^{+} CD8^{+} double-positive (DP; R4), and CD4^{-}CD8^{-} double-negative (DN; R5) cells. DNAM-1 is highly expressed on CD8^{+} SP cells and weakly on CD4^{+} SP cells. Low levels of DNAM-1 were detected within the DN cells on a small fraction of CD44^{+}CD25^{-} early T cell progenitors (arrow). CD155 is highly expressed on CD4^{+}CD8^{+} DP T cells. No significant differences in thymic cell development were observed between WT and DNAM-1$^{-/-}$ mice.