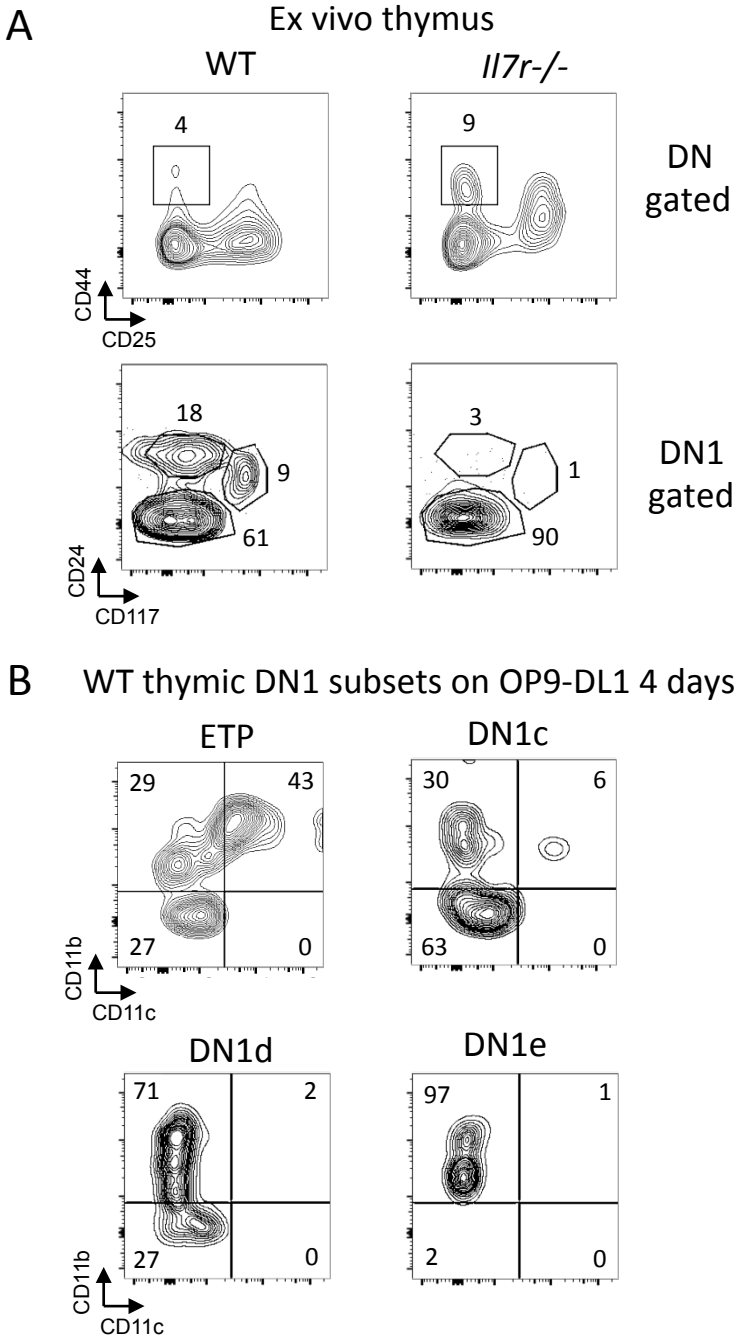

Figure S1. The *Il7r*$^{-/-}$ thymus lacks DN1 subsets that have DC potential in the presence of the DL1 Notch ligand. **A**. Flow cytometry analysis of WT and *Il7r*$^{-/-}$ thymocytes gated on the CD45^{+}CD4^{-}CD8^{-} population to show the distribution of subsets DN1 (CD44^{+}CD25^{-}), DN2 (CD44^{+}CD25^{+}), DN3 (CD44^{-}CD25^{+}), and DN4 (CD44^{-}CD25^{-}). The DN1 subset was then gated on and the distribution of ETPs (cKit^{hi} CD24$^{-/int}$), DN1c/d (cKit$^{int/low}$ CD24^{hi}) and DN1e (cKit^{lo} CD24^{-}) were assessed by flow cytometry. Numbers within the quadrants represent percentages. **B**. DN1 subsets were sorted from WT thymus and co-cultured with OP9-DL1 cells for four days to assess DC lineage potential. The cells were sorted on two successive gates: first a DN gate as in (**A**) and then into DN1 subsets based on CD24 and CD117 expression. Lineage potential was assessed by analysis of CD11c versus CD11b expression by flow cytometry.

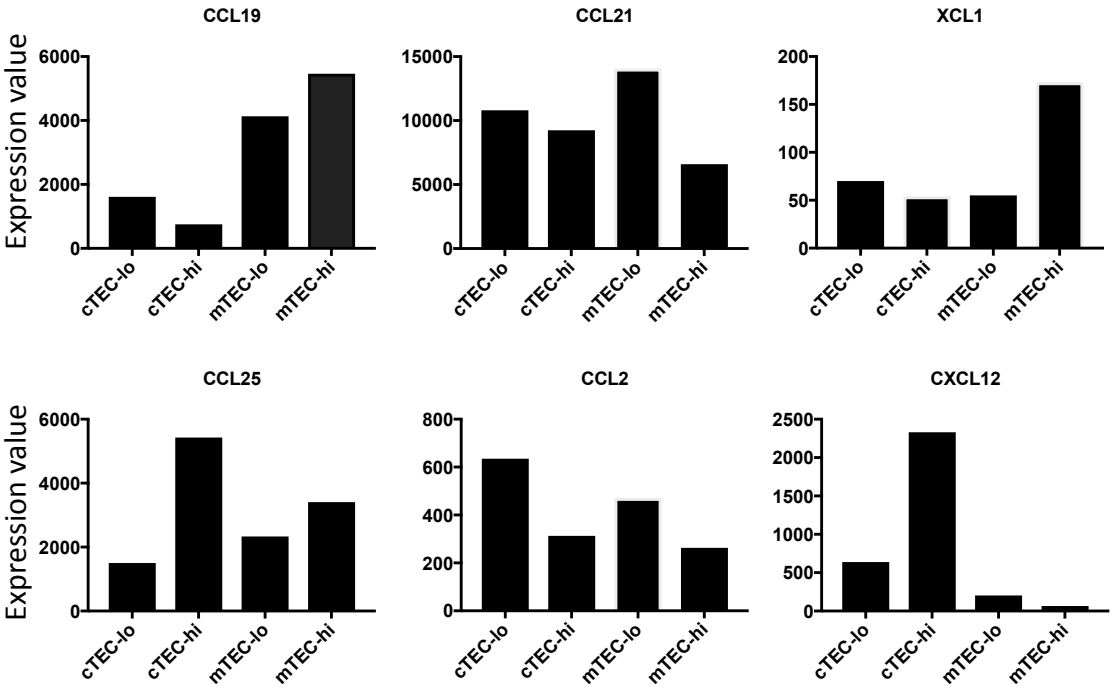

Figure S2. Expression of chemokines in WT TEC subsets as determined by the ImmGen project. Subsets were sorted from thymuses of 8 week old C57Bl/6J mice as PI^- $CD45^-$ $EpCAM^+$ cells and subdivided further as follows: cTEC-lo=$Ly51^+$ UAE^- MHC II^{lo} $Sca\text{-}1^+$; cTEC-hi=$Ly51^+$ UAE^- MHC II^{hi} $Sca\text{-}1^-$; mTEC-lo=$Ly51^-$ UAE^+ MHC II^{lo} $Sca\text{-}1^+$; mTEC-hi=$Ly51^-$ UAE^+ MHC II^{hi} $Sca\text{-}1^-$. Expression values are derived from the “My GeneSet” interface, based on hybridization of cDNA to the mouse Affymetrix microarray Gene1.0ST. This expression data complements the data depicted in Figure 4, with additional subdivision into MHC II high and low subsets.

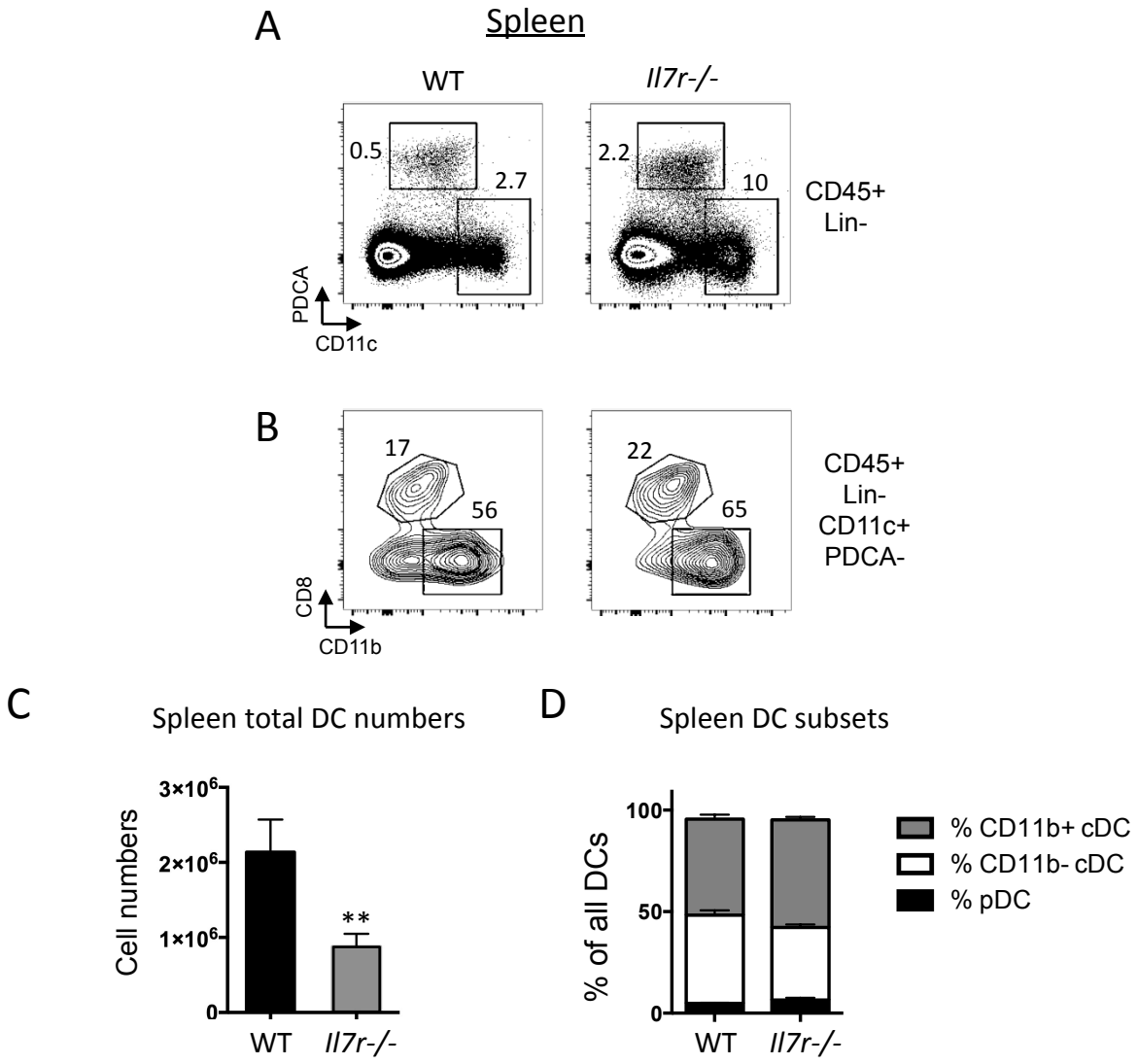

Figure S3. Defects in splenic DC numbers in *Il7r*$^{-/-}$ mice. WT and *Il7r*$^{-/-}$ mice were sacrificed at 4.5-5.5 weeks of age. **A**. Gates used to analyze pDCs and cDCs. Cells were first gated on the lineage (Lin)-negative, DAPI^{-}, CD45^{+} populations, where Lin=Dx5, NK1.1, F4/80, CD3e, TCR$\gamma\delta$, TCRβ, CD19, Ter119. Within this gate, two populations were analyzed: pDCs (CD11c^{int}, PDCA-1^{+}) and cDCs (CD11c^{high}, PDCA-1^{-}). **B**. To further differentiate between cDC subsets, cells within the cDC gate were analyzed for expression of CD11b and CD8α. Numbers in the quadrants indicate percentages. **C**. Total numbers of DCs per spleen, as calculated from manual counting from single cell suspensions multiplied by the percentages of CD45^{+}Lin^{-}CD11c^{+} cells as determined by Flow Jo. **D**. Ratios of the three DC subsets out of all DCs were calculated by taking the numbers of splenic pDCs, CD11b^{+} cDCs, and CD11b^{-} cDCs per thymus and dividing by the total number of DCs. n=3, data is representative of three separate experiments **p<0.01.