

SUPPLEMENTAL MATERIAL

Janas et al., <http://www.jem.org/cgi/content/full/jem.20091430/DC1>

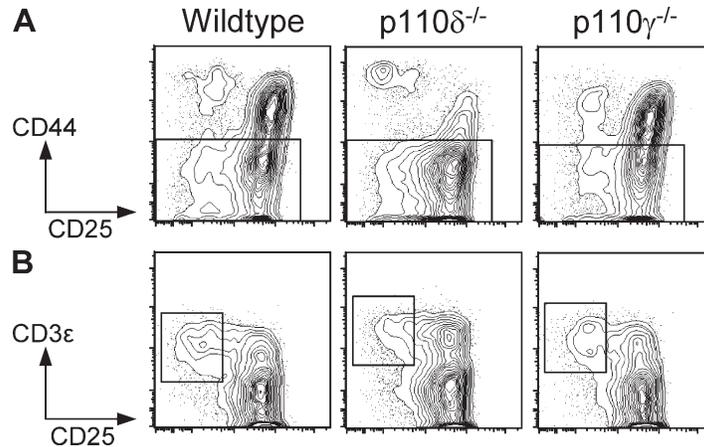


Figure S1. Surface profile of DN3/DN4 cells derived from long-term BM HSC cultures. Isolated HSCs were cultured on OP9-DL1 in the presence of 10 ng/ml IL-7 and 5 ng/ml Flt3L for 21 d as described in Materials and methods. (A) The expression of CD25 and CD44 on CD4⁻CD8⁻ cells. The gate indicates the population defined at the DN3/DN4 stage of development. (B) Surface CD3ε staining. Gates indicate the DN4 population used to measure the response to CD3ε stimulation in Fig. 1.

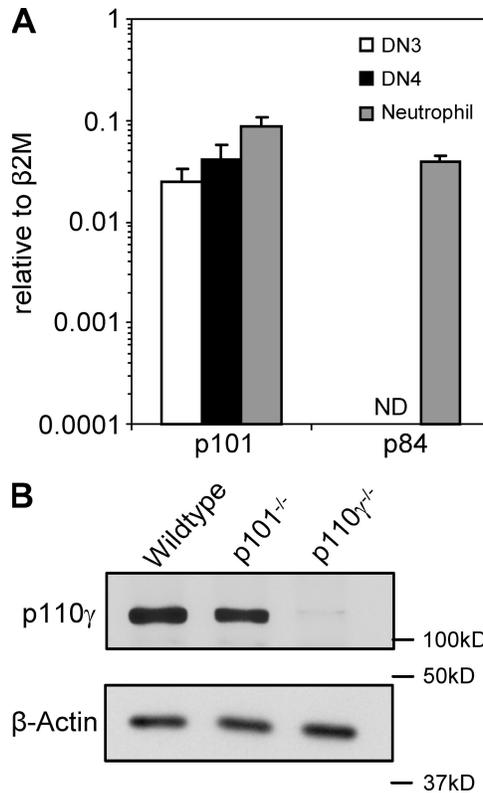


Figure S2. p101 expression in developing thymocytes. (A) Expression of mRNA encoding the class IB PI3K regulatory subunits in WT DN3 thymocytes, DN4 thymocytes, and BM-derived neutrophils (■) as measured by real-time PCR. Graphs show the mean and SD of three to five biological replicates. ND = not detected. (B) Western blot analysis of p110γ expression in whole thymic lysates from WT, p101^{-/-}, or p110γ^{-/-} mice.

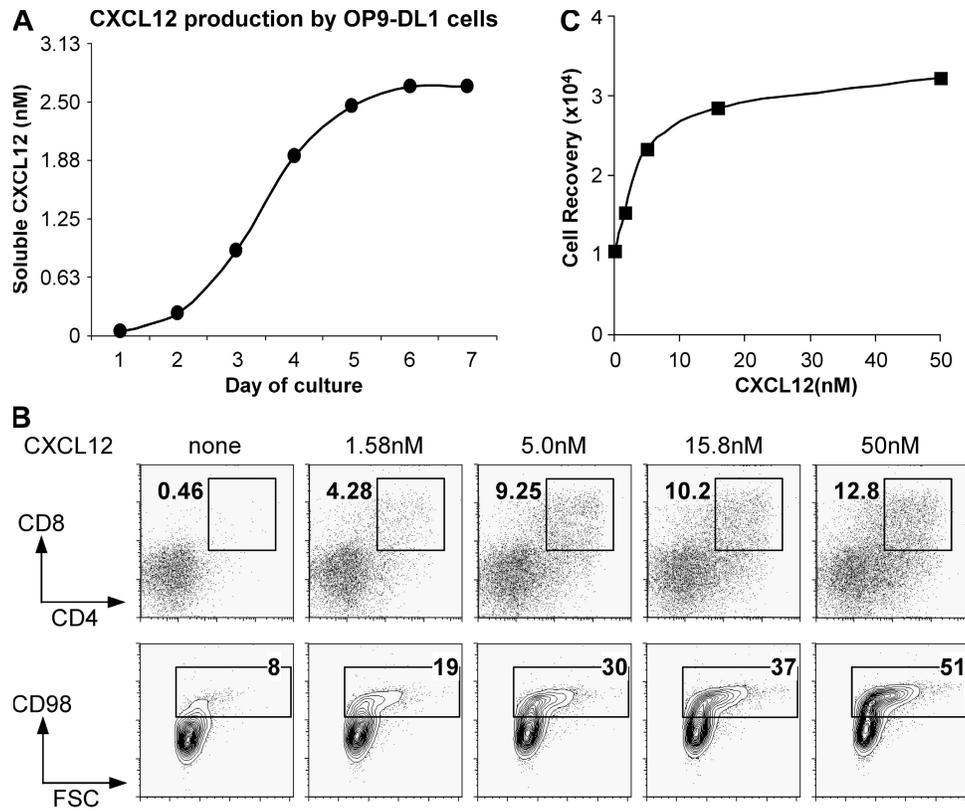


Figure S3. Cellular responses to CXCL12 are dose dependent. (A) Secretion of CXCL12 by OP9-DL1 stromal cells. OP9-DL1 stromal cells were cultured at 2×10^4 cells/ml and supernatant was harvested every 24 h. Soluble CXCL12 was quantified by immunoassay (R&D Systems). CXCL12 was undetectable in culture medium alone (α -MEM supplemented with 20% FCS). (B) WT DN3 cells were cultured on plates coated with 10 mg/ml of rDL4 together with increasing concentrations of CXCL12. The percentage of CD4⁺CD8⁺ and CD98⁺ cells after 3 d are shown. (C) The total number of cells harvested in B.