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Supplementary Materials for

Distinct Signal Codes Generate Dendritic Cell Functional Plasticity

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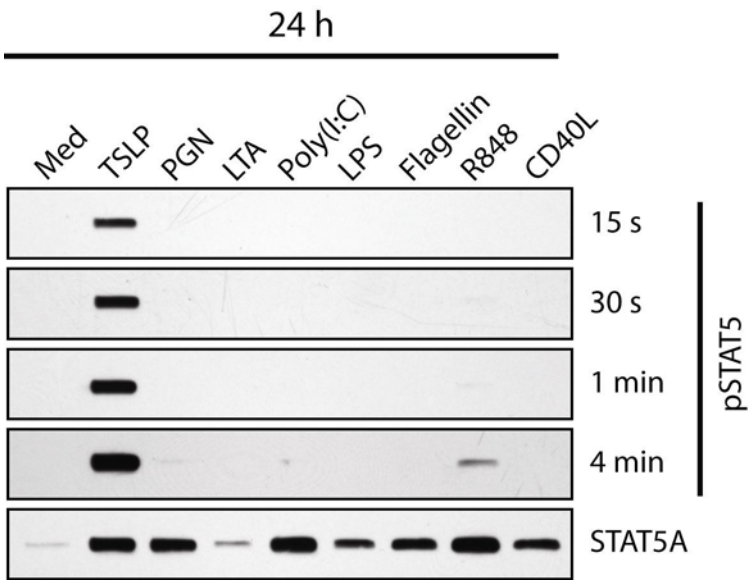

Fig. S1. R848 is a weaker inducer of STAT5 phosphorylation than TSLP. Four Western blots analyzed for the presence of phosphorylated STAT5 (pSTAT5) with the indicated exposure times are shown to demonstrate the specificity of STAT5 phosphorylation by TSLP, compared to that by R848. The 4 min-exposed blots are the data that appeared in Fig. 1A. The STAT5A blot is shown to demonstrate the similar abundance of STAT5 protein in TSLP- and R848-stimulated mDCs. Similar data were obtained from more than five independent experiments.

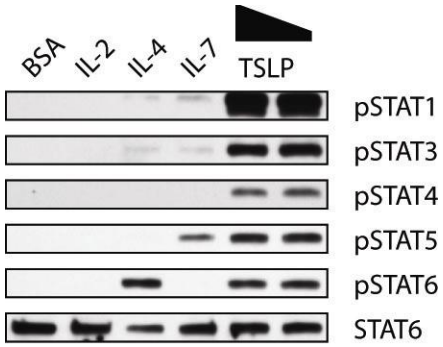

Fig. S2. TSLP, but not IL-7 or IL-4, is capable of inducing the activation of a range of STAT proteins in mDCs. Western blotting analysis was performed to compare differential STAT activation in mDCs by TSLP and the IL-2Rγ chain (γ_c)-sharing cytokines IL-2, IL-4, and IL-7. mDCs were stimulated with IL-2 (100 IU/ml), IL-4 (50 ng/ml), IL-7 (50 ng/ml), or TSLP (50 or 5 ng/ml) for 20 min at 37°C. Cell lysates were analyzed by Western blotting to examine the phosphorylation states of the indicated STAT proteins. The STAT6 blot is shown as a loading control. Data are representative of three independent experiments.

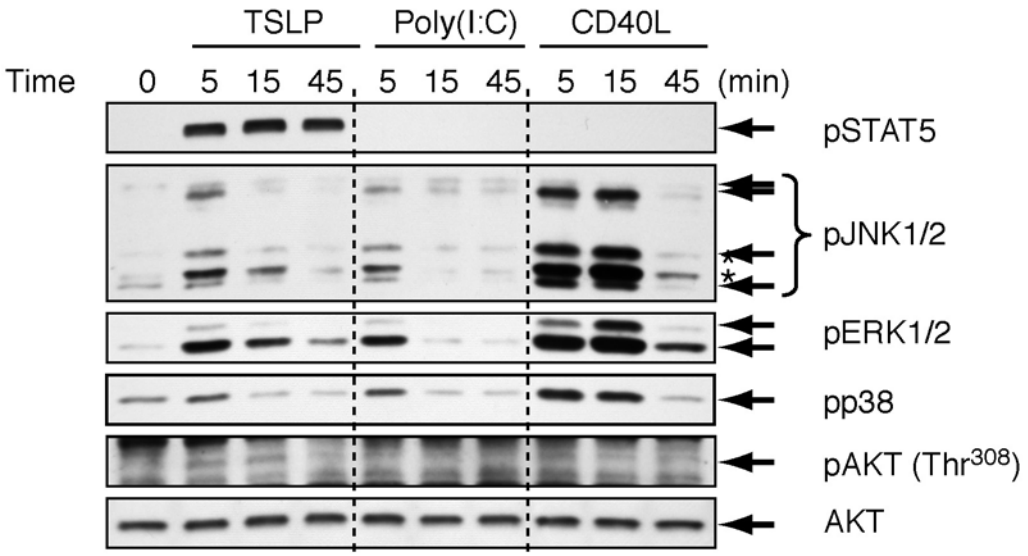

Fig. S3. TSLP induces the phosphorylation of ERK, JNK, and Akt in mDCs. Western blotting analysis was performed to compare TSLP-, poly(I:C)-, and CD40L-mediated phosphorylation of STAT5, JNK1/2, ERK1/2, p38, and Akt (at Thr^{308}) in mDCs. To determine equal loading of each lane, blots were analyzed with an antibody against total Akt protein. Bands marked with asterisks (*) show phosphorylated ERK species that were detected with an antibody against pJNK. Data are representative of three independent experiments.

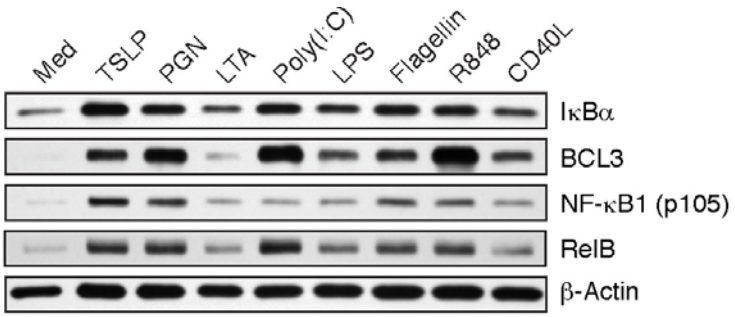

Fig. S4. TSLP increases the abundance of NF-κB target molecules in mDCs. Western blotting analysis was performed to compare the abundance of IκBα, BCL3, NF-κB1 (p105), and RelB in mDCs treated for 24 hours with the indicated stimuli (as in Fig. 1A). β-actin blots are shown as loading controls. Data are representative of three independent experiments.

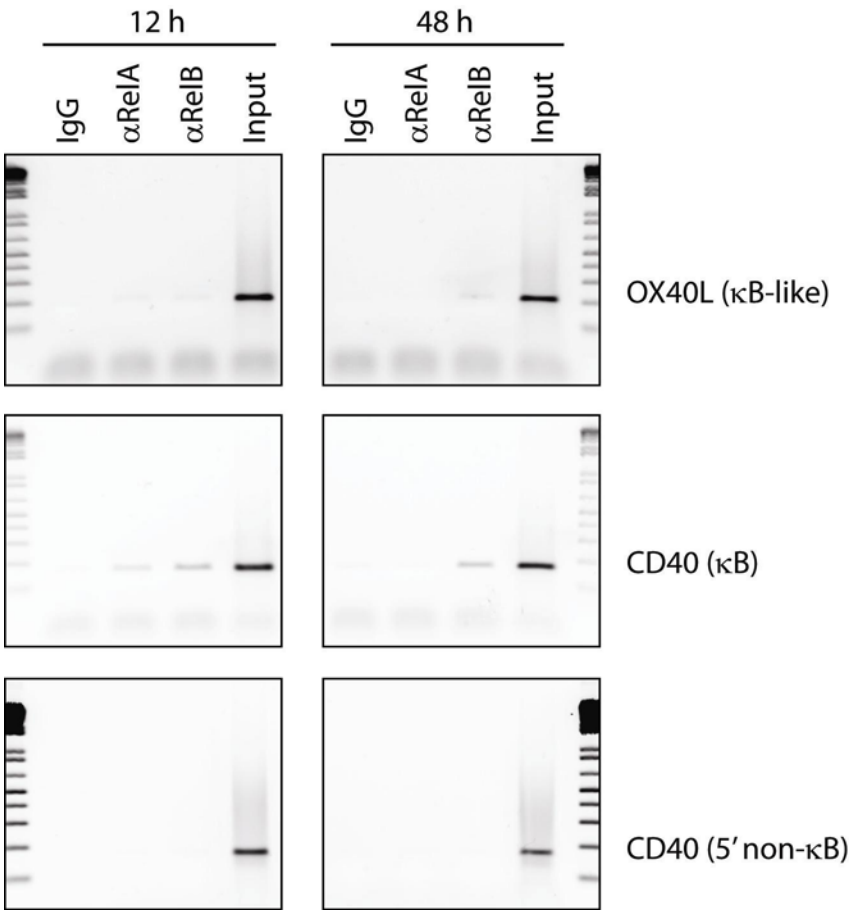

Fig. S5. PCR gel electrophoresis data for the ChIP assays shown in Fig. 3E. ChIP assays demonstrating the recruitment of RelA and RelB to the *OX40L* and *CD40* promoters in mDCs treated with TSLP for 12 hours and 48 hours. The bands in the electrophoresis gels are displayed as inverted images for easier visibility. Data are representative of three independent experiments.

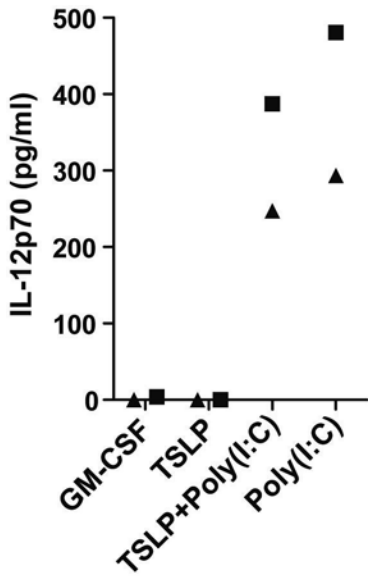

Fig. S6. TSLP is not a dominant-negative regulator of the production of IL-12. The production of IL-12p70 by mDCs cultured with GM-CSF, TSLP, poly(I:C), or TSLP and poly(I:C) for 24 hours was determined by ELISA. The square and triangle each represent one of two donors.