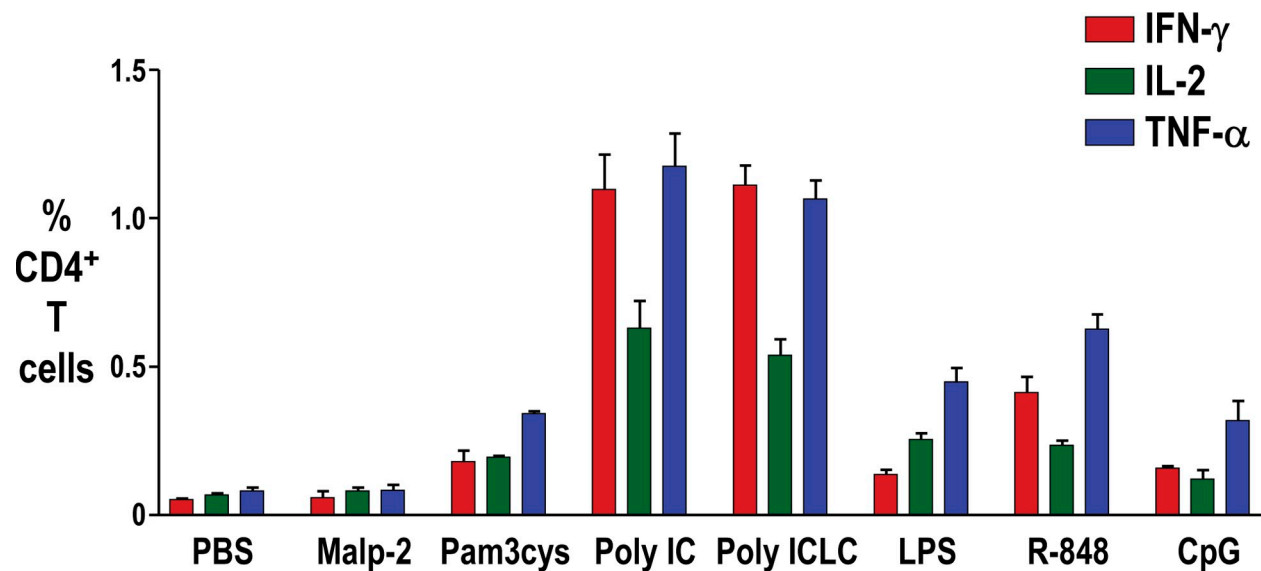
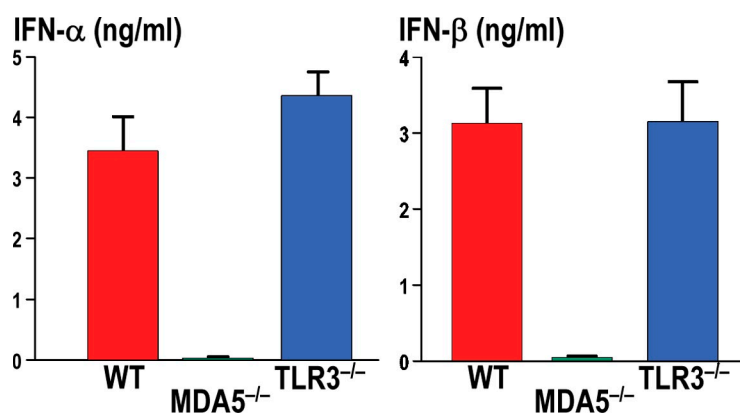


## SUPPLEMENTAL MATERIAL

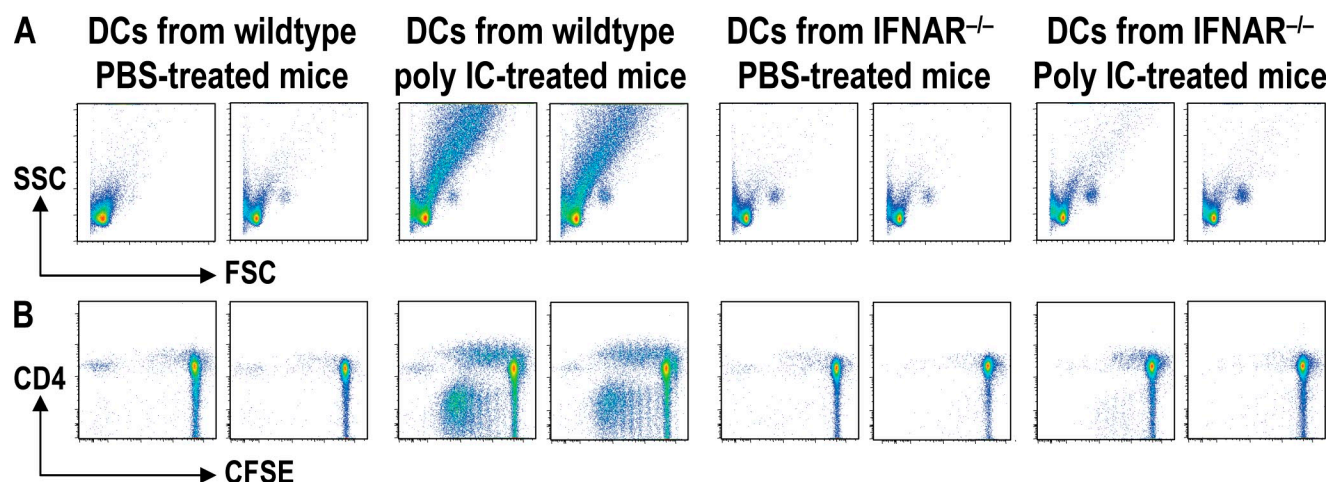
Longhi et al., <http://www.jem.org/cgi/content/full/jem.20090247/DC1>



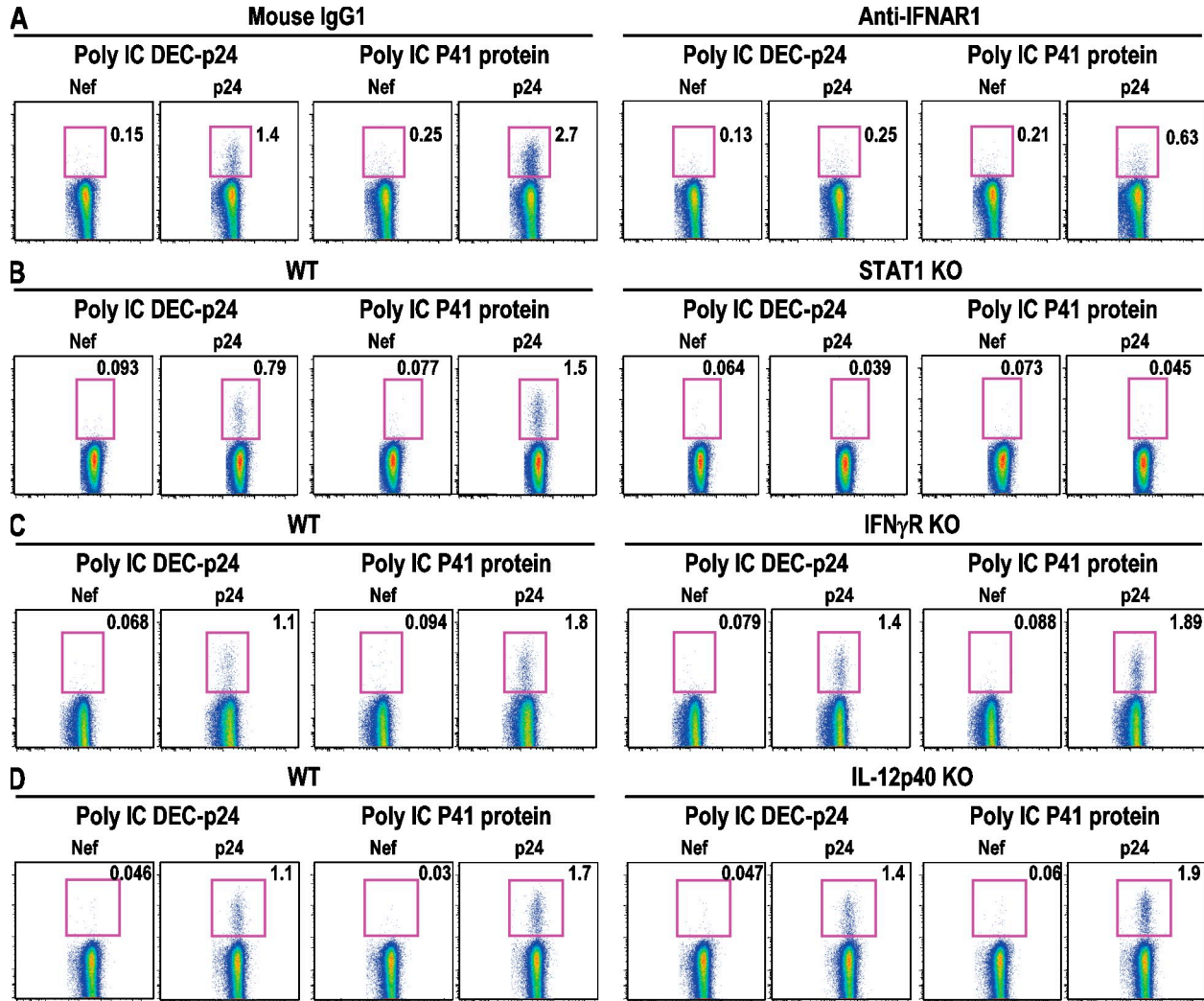
**Figure S1.** Poly IC is a superior adjuvant for the induction of IL-2 and TNF- $\alpha$ -producing CD4<sup>+</sup> T cells. As in Fig. 1 B, but the frequency of cells producing IL-2 and TNF- $\alpha$  were enumerated. Error bars represent the mean  $\pm$  SD from two independent experiments.



**Figure S2.** High levels of serum type I IFNs are dependent on Mda-5 not TLR3. WT, Mda5<sup>-/-</sup>, and TLR3<sup>-/-</sup> mice were treated i.p. with 50  $\mu$ g poly IC, and serum IFN- $\alpha$  and IFN- $\beta$  were analyzed by ELISA 6 h after injection. Error bars represent the means of four mice from two independent experiments.



**Figure S3. DCs need to respond directly to type I interferon to become immunostimulatory cells in the primary MLR.** C57BL/6 bone marrow chimeras were prepared with a 50:50 mixture of bone marrow from WT CD45.1<sup>+</sup> and IFN-AR<sup>-/-</sup> CD45.2<sup>+</sup> mice. 4–6 wk later, blood from the mice were validated to be 50:50 chimeras using antibodies to CD45.1 and CD45.2. The mice were then injected i.p. with PBS or 50 mg poly IC. 12 h later, CD11c<sup>+</sup> cells were enriched from the spleens of groups of five mice with magnetic beads coupled with anti-CD11c antibody, and the selected cells were stained with antibody to CD45.2. CD11c<sup>high</sup>, CD45.2<sup>+</sup>, and CD45.2<sup>-</sup> cells (each ~30% of the CD11c-selected spleen cells) were sorted as in Fig. 4 A. The sorted cells were lightly fixed in 1% paraformaldehyde, washed, and 20,000 were added in duplicate to cultures of  $2 \times 10^5$  T cells from BALB/c spleens to generate an MLR. Proliferation in the MLR requires that the DCs had undergone maturation in vivo from the poly IC, as previously described (Fujii et al. 2003. *J. Exp. Med.* doi:10.1084/jem.20030324). The T cells were labeled with CFSE to monitor their proliferation. At day 5 of the MLR, which was previously determined to be optimal for detecting a proliferative response involving two to six cell cycles, the cultures were harvested and analyzed by FACS. First, dead cells were gated out by live/dead staining. Second, the live cells were analyzed for light scattering (SSC vs. FSC) as shown in A. Then the live cells were analyzed for CFSE levels and CD4 expression, as shown in B, where proliferation induced by mature DCs is more vigorous in the CD4<sup>-</sup>CD8<sup>+</sup> T cells than the CD4<sup>+</sup> T cells. One of two similar experiments is shown in which it is evident that poly IC only matures the DCs from WT and not from IFN-AR<sup>-/-</sup> mice, even though both types of DCs coexist in the same spleens.



**Figure S4. Type I IFNs are required for Th1 priming and IFN- $\gamma$ .** Mice were immunized twice with poly IC and 5  $\mu$ g  $\alpha$ -DEC-p24 or 10  $\mu$ g gag-p41 at a 4-wk interval. Conditions are as in Fig. 6, but with representative dot plots of IFN- $\gamma$  secretion by CD4<sup>+</sup> T cells in response to HIV gag p24 or control HIV nef peptides. 1 wk after boost, p24-specific responses were analyzed by intracellular staining for IFN- $\gamma$ . (A) Anti-IFNAR1 antibody blocks the induction of Th1 immunity. (B–D) Genetic deletion of STAT1, but not IFN- $\gamma$ R or IL-12p40, blocks induction of Th1 immunity. Data are representative of two at least similar independent experiments.