SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

IFN-γ production assay. Pulmonary leukocytes (5 x 10^6 cells/ml) were re-stimulated in vitro with anti-CD3 (1 μg/ml) BD Pharmingen), (PPD (5 μg/ml), or the peptides Ag85baa240-254: FQDAYNAAGGHNAVF (1 μg/ml) and ESAT-6aa1-20: MTEQQWNFAGIEAAASAIQG (1 μg/ml) in complete RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 μM glutamine, 10 mM HEPES, and 50 μM 2-ME. IFN-γ levels in culture supernatants were then determined after 72 h by ELISA.

In vitro assay of responses of bone marrow derived macrophages infected with M. tuberculosis *to cytokine treatment*. BMM∅ were obtained as previously described with modifications (1). Briefly, cells were cultured in complete RPMI supplemented with 20% L929 cell-conditioned medium, as a source of M-CSF, 100 μg/ml streptomycin, and 100 U/ml penicillin. 2 x 10^6 cells/ml cells were incubated in six-well plates for 7 days at 37°C, 5% CO2. BMM∅ culture plates were then washed to remove non-adherent cells, and adherent cells were detached with ice-cold 5 μm EDTA PBS and plated at 1 x 10^6 cells per well in 24 well plates. BMM∅ were exposed to *M. tuberculosis* (multiplicities of infection - MOI = 1) in complete media for 6 h. Cells were then washed to remove any non-phagocytosed mycobacteria and 100 U/ml of IFN-γ (Peprotech), and 10 to 10.000 U/ml of IFN-α4 and IFN-β (PBL Biomedical Laboratories) were added to the cultures in

low cluster plates and left overnight. BMM∅ were tShen harvested, stained with mAb to CD11b and MHC-II, and analyzed by flow cytometry.

SUPPLEMENTAL REFERENCE

1. Rothfuchs AG, Gigliotti D, Palmblad K, Andersson U, Wigzell H, Rottenberg, ME. IFN-alpha beta-dependent, IFN-gamma secretion by bone marrow-derived macrophages controls an intracellular bacterial infection. *J Immunol.* 2001;167:6453-6461.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. FACS-sorting and purity of myeloid subpopulations. Representative dot plots showing sorting strategy used for isolating $CD11b^{+}Gr1^{int}$ cells (upper gate) and $CD11b^{+}Gr1^{-}$ cells (lower gate) from PBS- (**A**) or Poly-ICLC- (**B**), treated *M. tuberculosis*-infected mice. Numbers in post-sort analysis indicate the purity of isolated populations.

Supplemental Figure 2. Poly-ICLC treatment does not inhibit IFN-γ production in infected animals. Pulmonary leucocytes from *M. tuberculosis* infected animals (open bars), naïve mice treated with Poly-ICLC (gray bars), and *M. tuberculosis*-infected animals treated with Poly-ICLC (closed bars) were cultured for 72 hours in the presence of medium, PPD, ESAT-6 and Ag85B peptides and IFN-γ levels were determined by

ELISA. Bar graphs represent the mean (± SEM) of cytokine levels determined in 4 to 5 mice. The data shown are representative of two independent experiments.

Supplemental Figure 3. Poly-ICLC treatment changes the frequency of apoptotic $CD11b^{+}Gr1^{-}$ cell. (**A**) Frequencies of annexin-V expressing $CD11b^{+}Gr1^{int}$ (upper panel) and $CD11b^{+}Gr1^{-}$ (lower panel) pulmonary leukocytes isolated from PBS- (open symbols) or Poly-ICLC- (closed symbols) treated *M. tuberculosis*-infected mice. Circles indicate individual mice. (**B**) Each dot plot shows concatenated files of 5 individual mice.

Supplemental Figure 4. Endogenous and exogenous type I interferon downregulate MHC class II expression triggered by IFN-gamma treatment. (**A**) BMM∅ were generated from WT mice, infected with *M. tuberculosis* at a MOI of 1 and cultured with IFN-γ, in the presence of increasing concentration of recombinant IFN-α4 and IFN-β. Frequencies of MHC class II expressing cells were measured after overnight incubation by flow cytometry. Bars indicate mean (± SEM) of three replicates. (**B** and **C**) BMM∅ were generated from WT and *Ifnαβr-/-* mice, infected (**C**) or not (**B**) with *M. tuberculosis* at a MOI of 1, and cultured overnight with or without IFN-γ and recombinant IFN-α4 and IFN-β. Frequencies of MHC class II expressing cells were measured by flow cytometry after overnight incubation. The experiment shown is representative of 3 performed.

Supplemental Figure 5. While *Il13* is induced following Poly-ICLC treatment, the recruitment of $CD11b^{+}Gr1^{int}$ cells and exacerbation of *M. tuberculosis* infection are not dependent on IL-13 signaling. (**A**) *Il13* mRNA expression levels determined by real-time

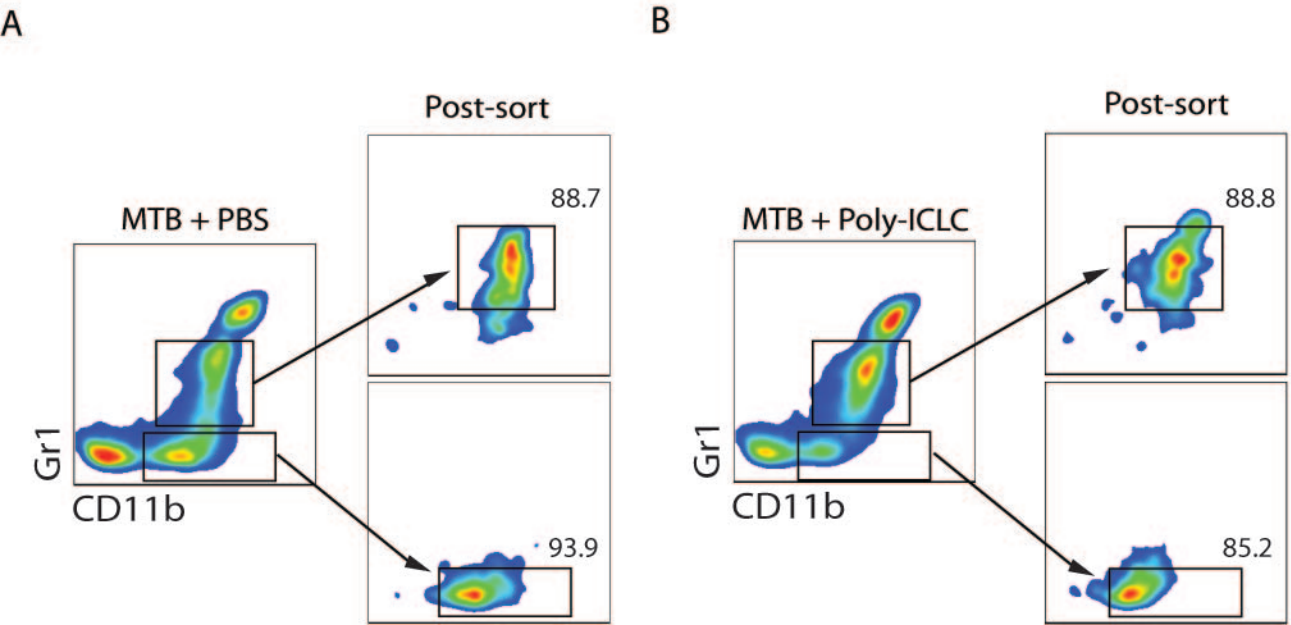
PCR in the lungs of *M. tuberculosis*-infected animals treated with PBS (open circles) or Poly-ICLC (closed circles). The results shown represent the fold increase relative to that observed in untreated, naïve mice with circles representing individual mice. Data were pooled from three independent experiments with similar results. (**B**) Pulmonary mycobacterial loads in PBS- (open circles) or Poly-ICLC- (closed circles) treated WT and *Il13rα2*$^{-/-}$ mice. Circles represent individual animals. (**C**) Representative flow cytometry dot plots of CD11b^{+}Gr1^{-}, CD11b^{+}Gr1^{int} and CD11b^{+}Gr1^{high} pulmonary leukocytes isolated from PBS- or Poly-ICLC- treated *M. tuberculosis*-infected WT mice (upper panel) or *Il13rα2*$^{-/-}$ animals (lower panel).

Supplemental Figure 6. Poly-ICLC treatment alters IFN-γ receptor expression on CD11b^{+}Gr1^{int} and CD11b^{+}Gr1^{-} pulmonary cells in *M. tuberculosis*-infected animals. (**A**) IFN-γ receptor median fluorescence intensity (MFI) on CD11b^{+}Gr1^{-} and CD11b^{+}Gr1^{int} pulmonary leukocytes isolated from PBS- (open circles) or Poly-ICLC-treated (closed circles) *M. tuberculosis*-infected WT mice. Circles represent individual animals. (**B**) Representative flow cytometry histograms of IFN-γ receptor MFI. Each line shows concatenated files of 5 individual mice.

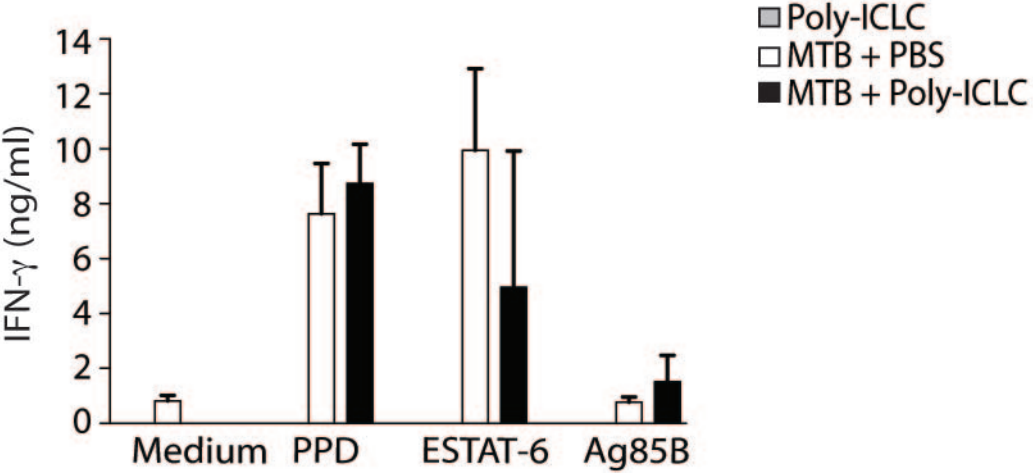
Supplemental Figure 7. Poly-ICLC treatment does not alter NOS2 levels in infected animals. (**A**) *Nos2* expression levels determined by real-time PCR in the lungs of *M. tuberculosis* infected WT mice treated with PBS (open symbols) or Poly-ICLC (closed symbols) for 4 weeks starting 1 day after pathogen exposure. Results are presented as the fold increase relative to transcript levels in PBS-treated, naïve mice. Circles indicate

individual animals. Data are pooled from 2 independent experiments with similar results. (**B**) Flow cytometry dot plots of F4/80^{+}iNOS^{+} pulmonary leukocytes isolated from PBS- (right panel) or Poly-ICLC-treated (left panel) *M. tuberculosis*-infected WT mice. The analysis was done after gating on CD11b^{+}Gr1^{int} cells and each dot plot shows concatenated files of 4 individual mice. The data shown are representative of two independent experiments.

Supplemental Figure 1



Supplemental Figure 2



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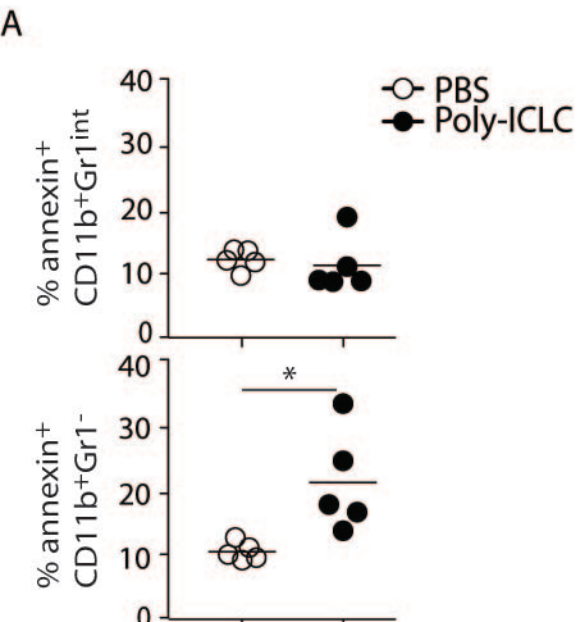
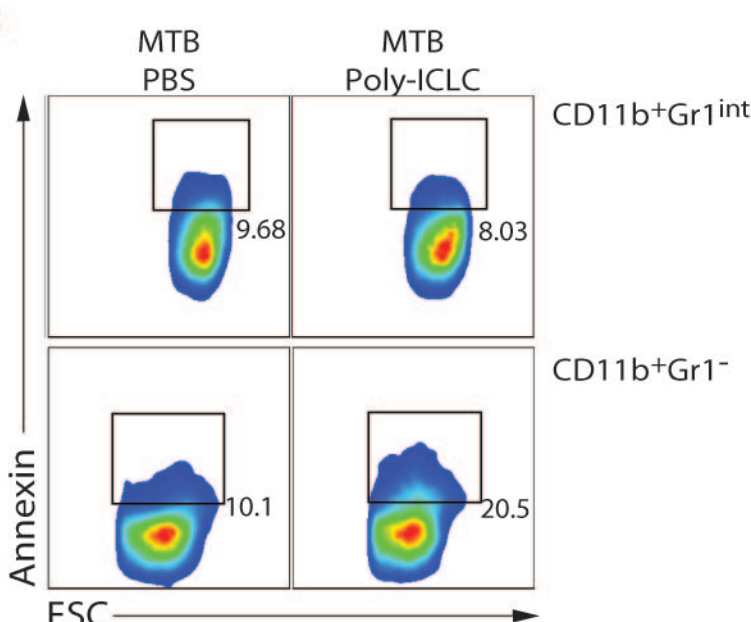
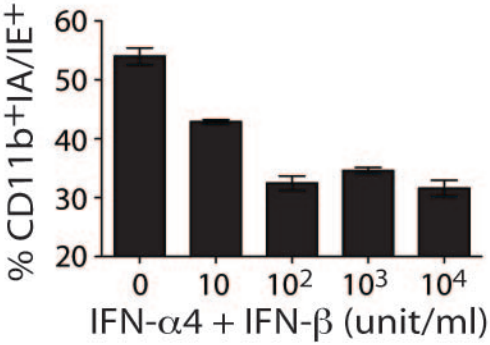




Supplemental Figure 4

A



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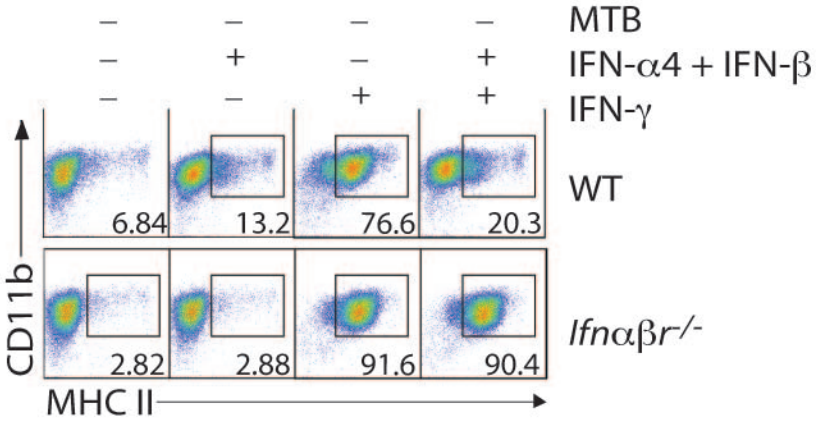

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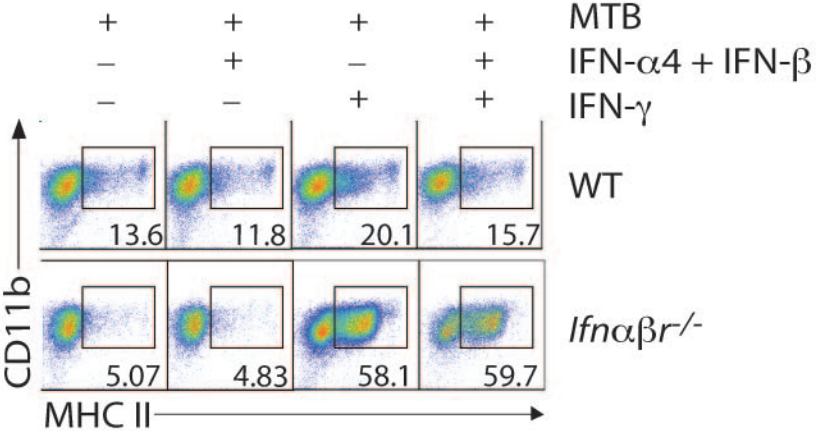

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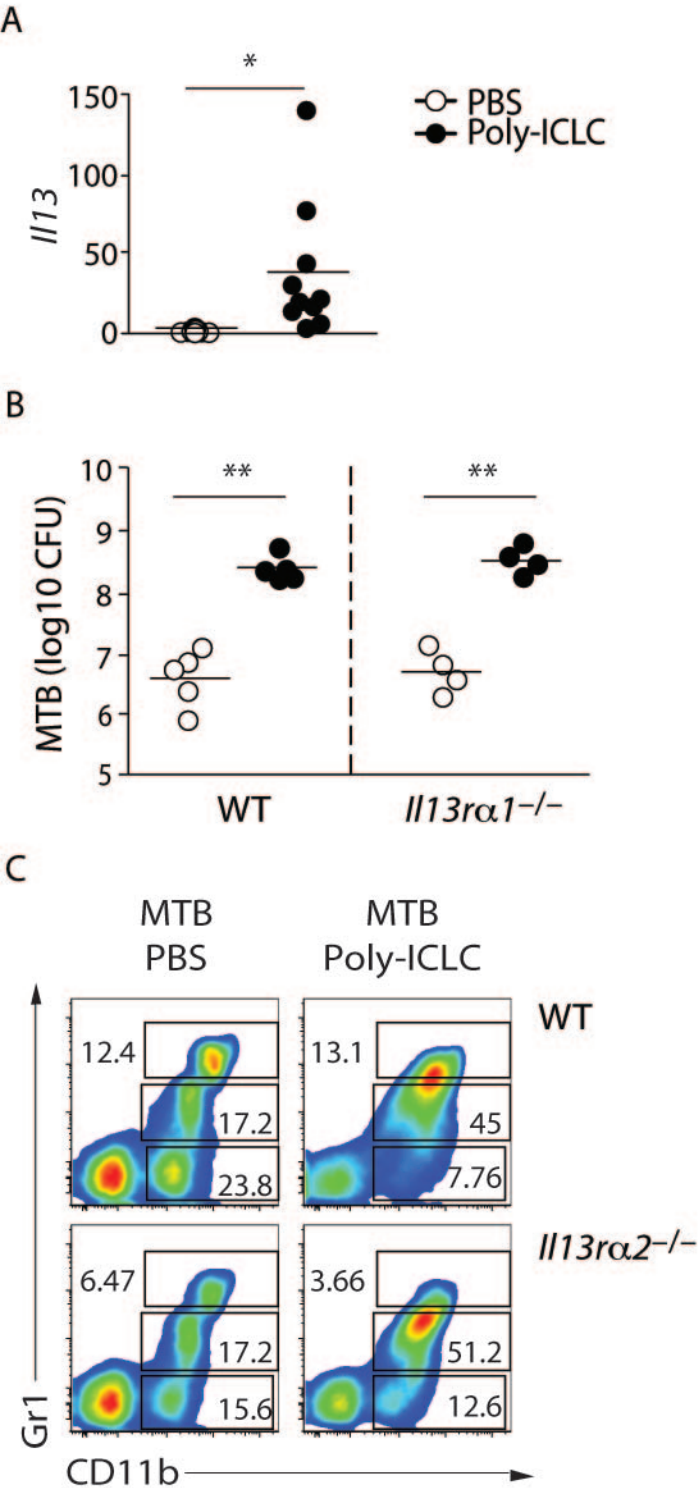
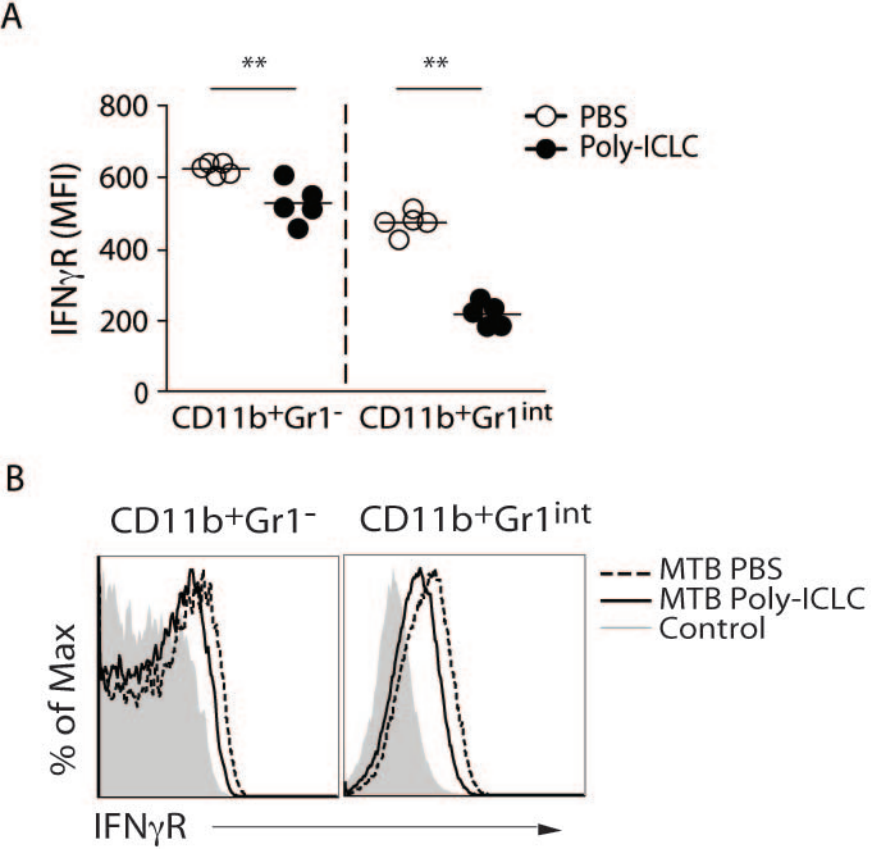


Supplemental Figure 6



Supplemental Figure 7