SUPPLEMENTAL MATERIAL

Robinson et al., http://www.jem.org/cgi/content/full/jem.20091085/DC1

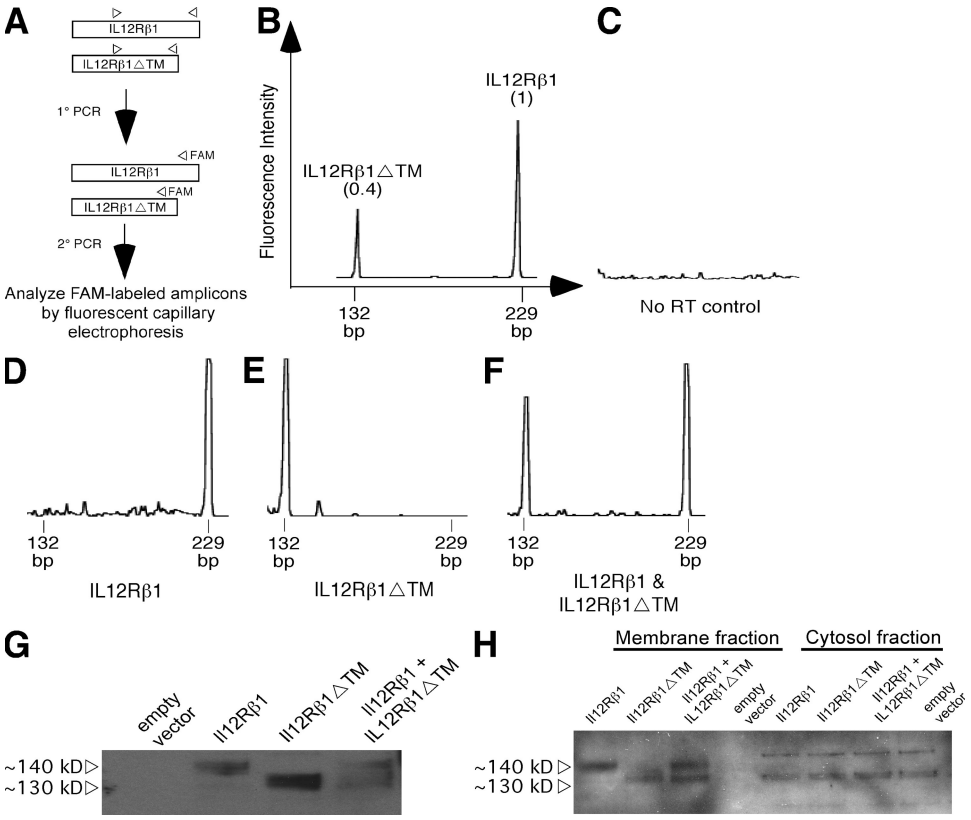

Figure S1. IL-12Rβ1 spectratype analysis. IL-12Rβ1 spectratype analysis is akin to TCR-CDR3 Spectratype analysis and is illustrated here. (A) cDNA is first amplified with primers that flank the transmembrane-encoding region to amplify both IL-12Rβ1 and IL-12Rβ1ΔTM; the resultant amplicons are then fluorescently (FAM)-labeled via a run-off PCR reaction with a single FAM-conjugated primer. Given the published sequence of IL-12Rβ1 and IL-12Rβ1ΔTM(Chua et al., 1995), the FAM-labeled amplicons of these transcripts have a predicted size of 229 bp and 132 bp, respectively. (B) Analyzing the samples by fluorescent capillary electrophoresis allows the FAM-labeled products to be separated by size and their relative abundance to one another quantified. To demonstrate this, two peaks of the anticipated sizes are observed using cDNA of concanavalin-A activated splenocytes; neither are observed in no-reverse-transcriptase controls, ruling out genomic DNA amplification (C). Using the area under the larger, transmembrane containing fluorescent peak as a unit reference, the relative abundance of IL-12Rβ1ΔTM can be determined. The numbers adjacent to peaks of an individual IL-12Rβ1 spectra indicate the relative ratio of that peak's area (the smaller peak representing IL-12Rβ1ΔTM) to the area of the larger peak that represents IL-12Rβ1. In concanavalin A–activated splenocytes, the ratio of IL-12Rβ1ΔTM to IL-12Rβ1 was observed to be 0.4:1 (B). To further test the fidelity of this assay to distinguish between IL-12Rβ1 and IL-12Rβ1ΔTM, we transfected NIH/3T3 cells with mammalian expression vectors containing each respective cDNA. IL-12Rβ1 spectratype analysis of single- (D and E) and double-transfectants (F) revealed that the 229 bp and 132 bp peaks observed via this assay do in fact represent IL-12Rβ1 and IL-12Rβ1ΔTM, respectively. Importantly, Western blot analysis with polyclonal anti-IL-12Rβ1 confirmed IL-12Rβ1ΔTM could be translated into a protein product as first demonstrated by Chua, A.O., V.L. Wilkinson, D.H. Presky, and U. Gubler. 1995. *J. Immunol.* 155:4286–4294 (G). Subcellular fractionation of cell membrane and cell cytosol confirmed IL-12Rβ1ΔTM to be membrane associated as first predicted by Chua et al. (Chua et al., 1995; H).

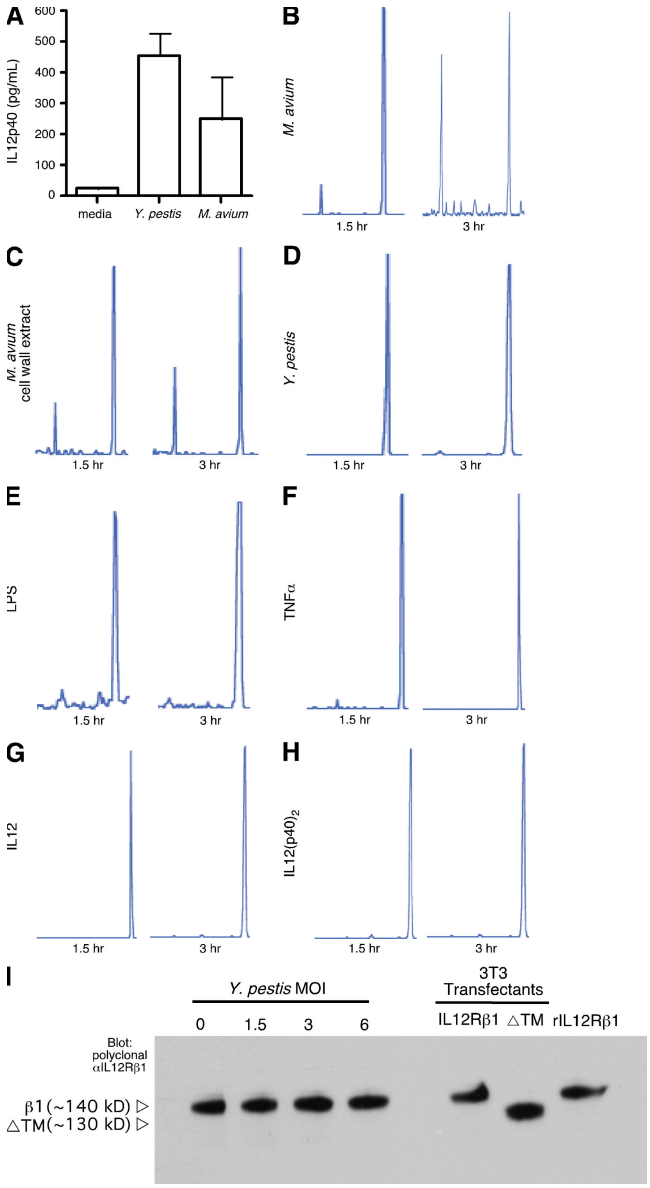

Figure S2. Preliminary experiments demonstrating that L12Rβ1ΔTM is expressed by BMDCs AFTER exposure to *M. avium* and *M. avium* cell wall extract, but not *Y. pestis*, LPS, TNF, IL-12 or IL-12(p40)$_2$. DCs prepared from C57BL/6 BM were exposed in vitro to either media alone, *Y. pestis* (5 MOI), *M. avium* (5 MOI), *M. avium* cell wall extract, and *E. coli* LPS or to cytokines TNF, IL-12, and IL-12(p40)$_2$ for a 3-h period. At the end of 1.5- and 3-h periods DC RNA was collected for IL-12Rβ1 spectratype analysis. (A) Measurement of IL-12p40 in the DC supernatant by ELISA served as a positive control that both *Y. pestis* and *M. avium* were capable of stimulating DCs. (B-H) Representative IL-12Rβ1 spectra from 1.5- and 3-h after exposure to (B) *M. avium*, (C) *M. avium* cell wall extract, (D) *Y. pestis* KIMD27, (E) LPS, (F) TNF, (G) IL-12, or (H) IL-12(p40)$_2$. These results are from a single experiment performed with three separate BMDC preparations per group. (I) Western blot analysis of total cell lysates from DCs exposed to increasing MOI of *Y. pestis*. Lysates were denatured, run on a 4–12% Bis-Tris SDS-PAGE gel, transferred to membrane, and probed with polyclonal anti-IL-12Rβ1. NIH/3T3 cells transfected with either IL-12Rβ1 or IL-12Rβ1ΔTM plasmids and recombinant IL-12Rβ1 served as positive controls.

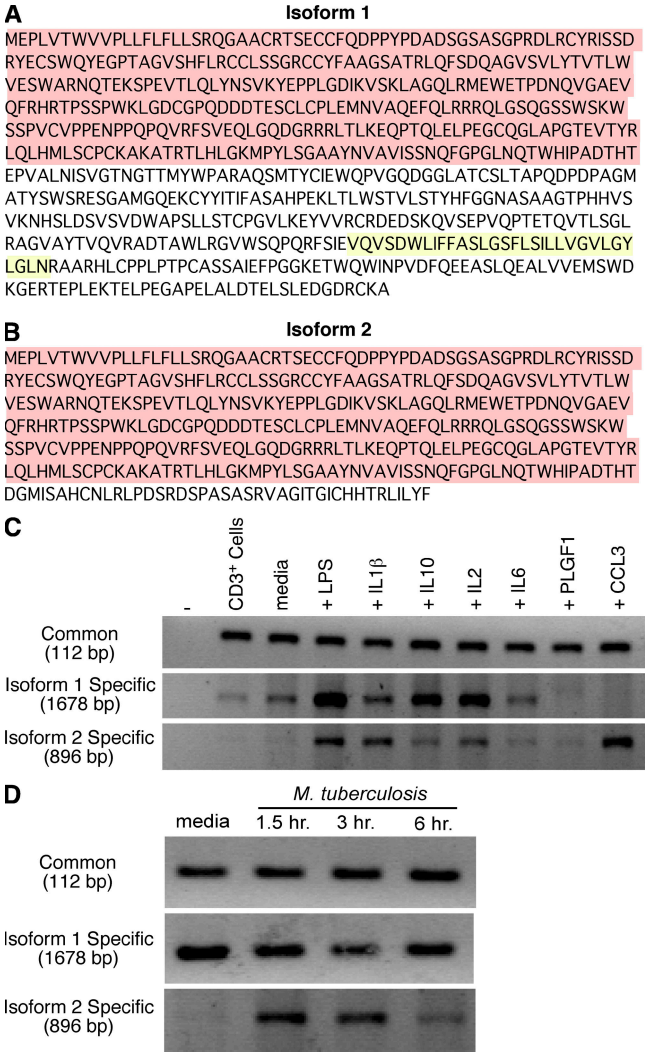

Figure S3. Preliminary experiments demonstrating that two isoforms of IL-12Rβ1 are expressed by human DCs AFTER exposure to *M. tuberculosis* and other specific stimuli. (A and B) Two isoforms of the human IL-12RB1 transcript are reported in publicly available databases: full-length IL-12RB1 (isoform 1; Swiss-Prot ID P42701-1) and a shorter isoform that is the product of alternative splicing (isoform 2; Swiss-Prot P42701-3). The amino acid sequences of (A) isoform 1 and (B) isoform 2 share the majority of the extracellular domain, but isoform 2 lacks the transmembrane domain and has an altered C terminal sequence. The pink highlighted portion indicates those amino acids that are shared between the two while the yellow highlight indicates the transmembrane domain. (C and D) Monocyte-derived DCs were generated by incubating magnetically purified $CD14^+$ monocytes from apheresis samples for 7 d with GMCSF and IL-4. (C) DCs were then incubated for 3 d with either media alone, IL-1β, IL-10, IL-2, IL-6, PLGF1, CCL3, or for 24 h with LPS. (D) Alternatively, DCs were stimulated with *M. tuberculosis* over a 6-h period. Subsequently, cDNA generated from both (C and D) was then amplified with primer pairs that either amplified both isoforms 1 and 2 (Common), only isoform 1 (Isoform 1 Specific), or only isoform 2 (Isoform 2 Specific). cDNA from $CD3^+$ PBMCs was used as a positive control for IL-12RB1 expression. These results are from two experiments performed with two separate monocyte-derived DC preparations.

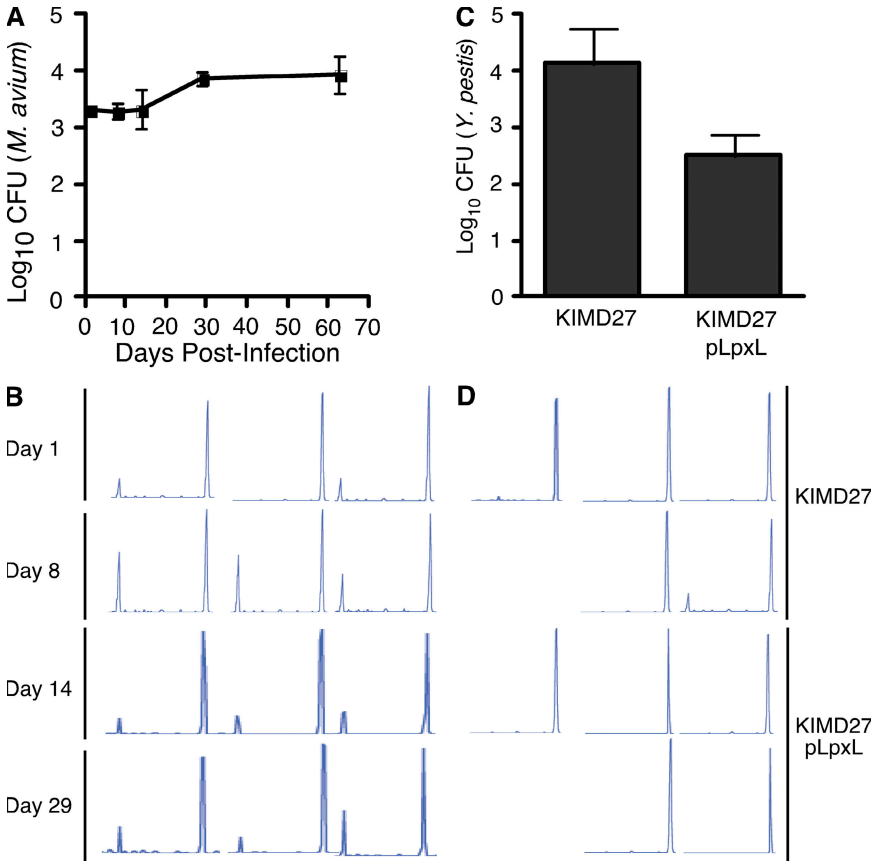

Figure S4. Preliminary experiments demonstrating that IL-12Rβ1ΔTM is expressed in the lung after infection with *M. avium*, but not *Y. pestis*. (A and B) C57BL/6 mice were aerogenically infected with 10^3 CFU of *M. avium* strain 2447. (A) The *M. avium* CFU per lung at various times after infection. (B) On days 1, 8, 14, and 29 after infection total lung RNA was harvested for IL-12Rβ1 Spectratype analysis. Shown are representative spectra from three individual *M. avium*-infected mice at each time point, with the smaller peak representing IL-12Rβ1ΔTM and the larger peak representing IL-12Rβ1. (C and D) C57BL/6 were intranasally infected with 10^5 CFU of *Y. pestis* strain KIMD27 or 10^6 CFU of *Y. pestis* strain KIMD27 pLpxL. 4 d after infection the lungs were harvested to both (C) determine the total CFU per lung and (D) assess total lung expression of IL-12Rβ1ΔTM. Shown are the spectra from five individual *Y. pestis* KIMD27 or KIMD27 pLpxL-infected mice at this time. Data points in (A and C) represent the mean number and SD of bacterial CFU present in the lungs of four to five individual mice per time point.