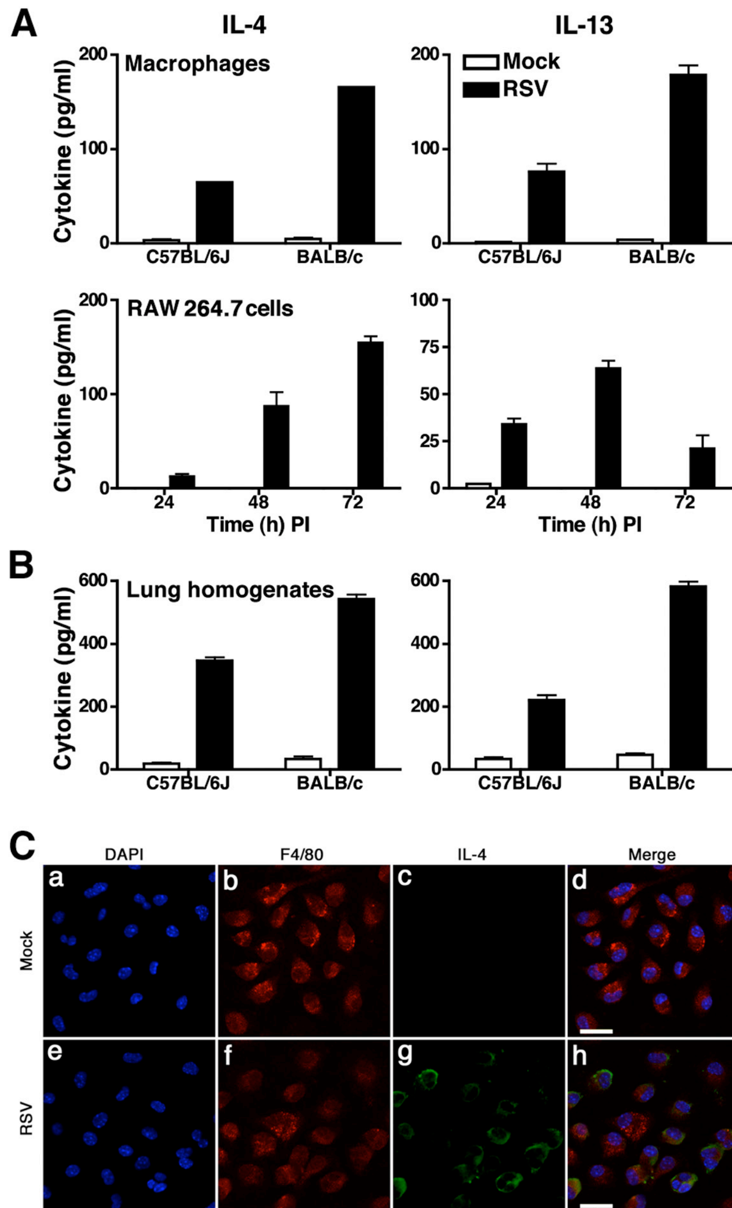
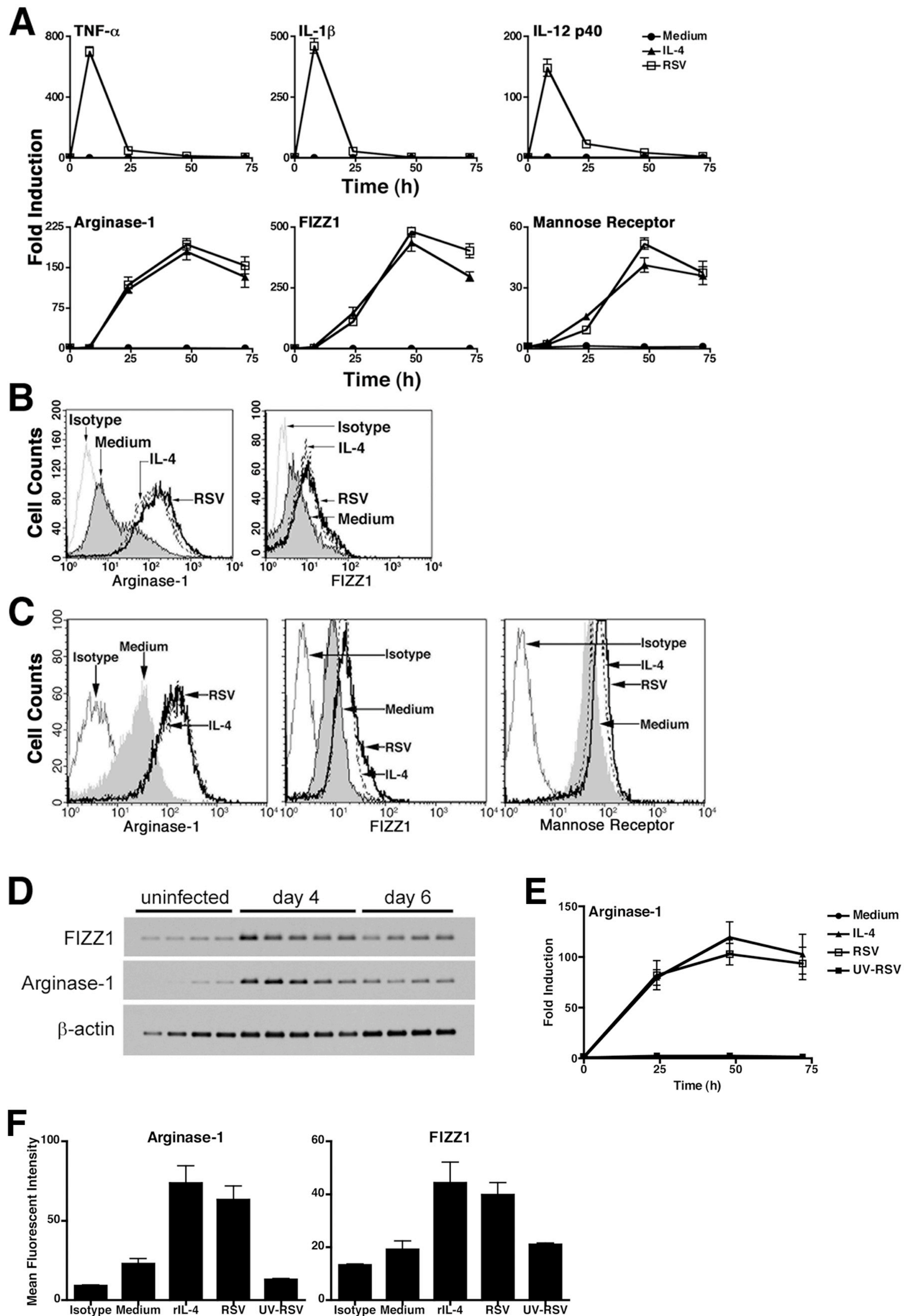
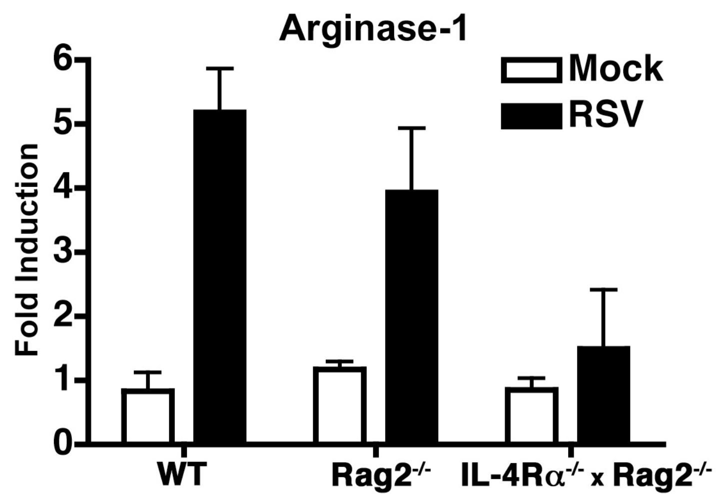


Supplemental Fig. S1. RSV induces macrophage-derived IL-4 and IL-13. (A) Peritoneal macrophages from WT C57BL/6J or BALB/cByJ mice were treated with medium only or infected with RSV (MOI = 2) for 48 h. Supernatants were collected and analyzed for IL-4 and IL-13 secretion by ELISA. RAW 264.7 cells were similarly treated. Supernatants were collected at the indicated times and analyzed by ELISA for the secretion of IL-4 and IL-13. (B) WT C57BL/6J and BALB/cByJ mice were treated i.n. with PBS (mock) or

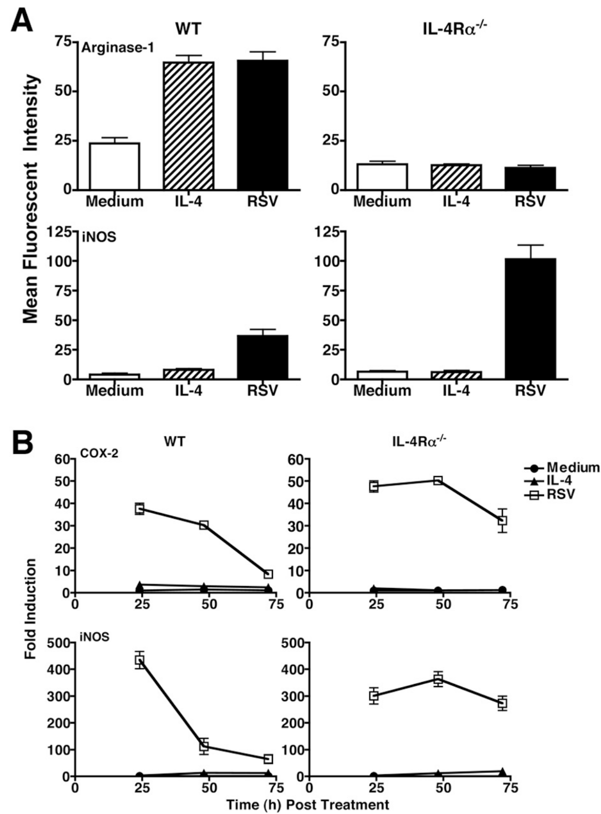
infected with RSV (10^6 pfu/animal). Lungs were harvested at 4 d p.i. and IL-4 and IL-13 protein levels were measured by ELISA from lung homogenates. Data represents the mean $\pm$ SEM of 3 independent experiments. (C) WT C57BL/6 mice were mock- or RSV-infected as described. BAL cells were collected 4 d p.i., cultured for 2 h, fixed, and stained for DAPI (a nuclear marker; blue), F4/80 (a macrophage marker; red), and IL-4 (green), then viewed by confocal microscopy. Bar represents 20 μm.


A
TNF-α
IL-1β
IL-12 p40
Medium
IL-4
RSV
Fold Induction
Time (h)
Arginase-1
FIZZ1
Mannose Receptor
Time (h)
B
Cell Counts
Isotype
Medium
IL-4
RSV
Arginase-1
Isotype
IL-4
RSV
Medium
FIZZ1
C
Cell Counts
Isotype
Medium
RSV
IL-4
Arginase-1
Isotype
Medium
RSV
IL-4
FIZZ1
Isotype
IL-4
RSV
Medium
Mannose Receptor
D
uninfected
day 4
day 6
FIZZ1
Arginase-1
β-actin
E
Arginase-1
Fold Induction
Time (h)
Medium
IL-4
RSV
UV-RSV
F
Arginase-1
FIZZ1
Mean Fluorescent Intensity
Isotype
Medium
rIL-4
RSV
UV-RSV

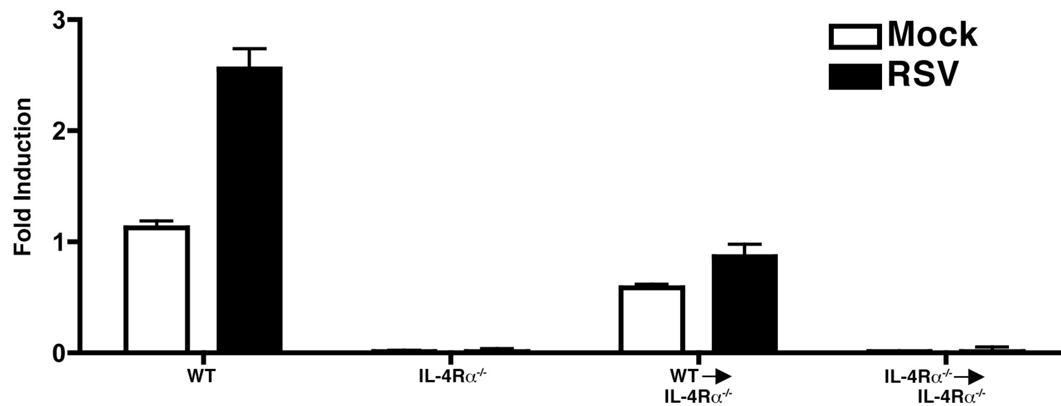
Supplemental Fig. S2. RSV infection induces differentiation of AA-Mϕ. (A) WT C57BL/6 peritoneal macrophages were treated with medium alone, rIL-4, or RSV. Expression of genes associated with CA-Mϕ and AA-Mϕ were analyzed by quantitative real-time PCR. Data are presented as means ± SEM and are derived from a single representative experiment (N = 4). (B) WT C57BL/6 macrophages were treated as in (A) for 48 h. Arginase-1 and FIZZ1 protein was analyzed by FACS. Data are from a single representative experiment (N = 4). Arrows indicate isotype control (gray line), medium (gray histogram), rIL-4 (dashed line), and RSV (black line). (C) Cotton rat peritoneal macrophages were treated as in (B) and analyzed for arginase-1, FIZZ1, and MR by FACS. Arrow indications are as described in (B). (D) Cotton rats were mock- or RSV-infected (10^6 pfu/animal). BALs were harvested 4 d and 6 d p.i. and analyzed for gene expression by RT-PCR and Southern blotting. (E) WT C57BL/6 macrophages were treated with medium alone, rIL-4, or infected with viable RSV or UV-inactivated RSV. mRNA gene expression of arginase-1 was analyzed by real time PCR. (F) WT C57BL/6 macrophages were treated as in (E) for 48 h. Arginase-1 and FIZZ1 protein was analyzed by FACS by staining with either isotype control IgG or anti-arginase-1 or anti-FIZZ1 antibodies.



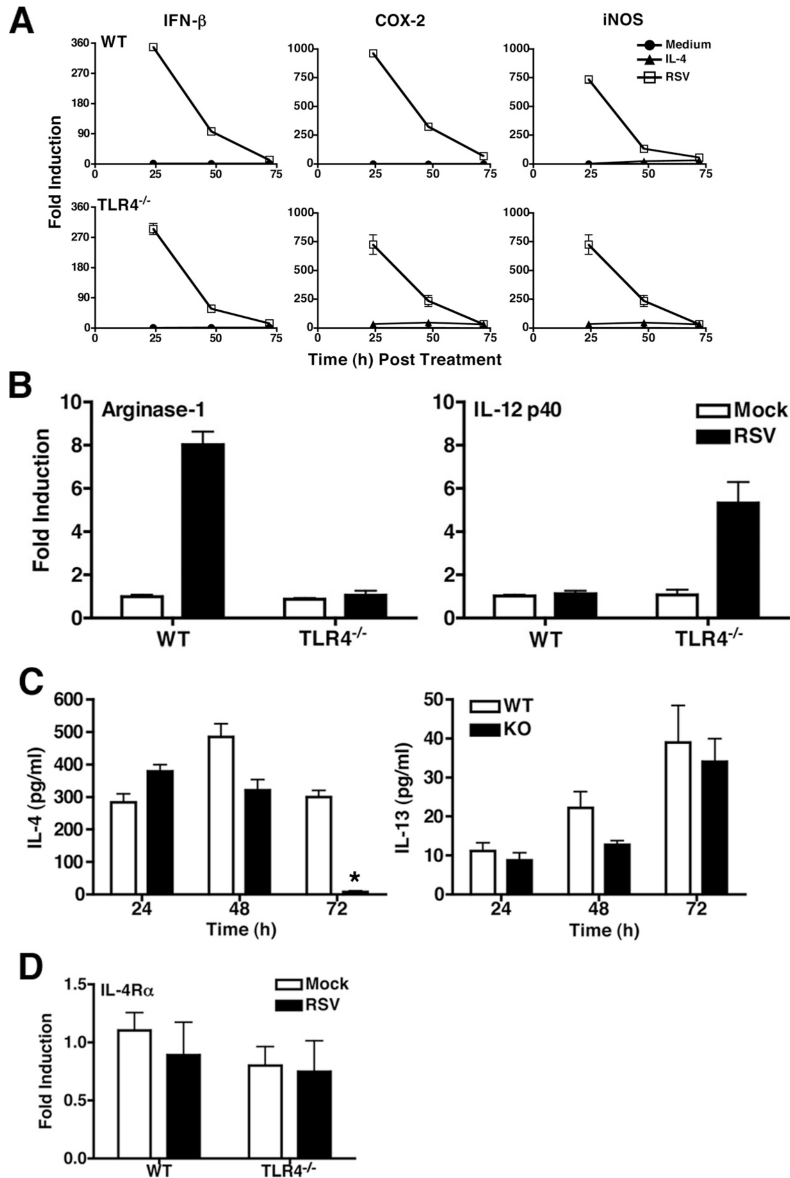
Supplemental Fig. S3. RSV-induced AA-Mϕ induction in B and T cell-deficient Rag2$^{-/-}$ mice. WT BALB/c, Rag2$^{-/-}$, and IL-4Rα$^{-/-}$ x Rag2$^{-/-}$ mice were treated i.n. with PBS (mock-) or RSV (10^6 pfu/animal)-infected . Lungs were harvested at 4 d p.i. and analyzed for arginase-1 mRNA by real time PCR.



Supplemental Fig. S4. Failure to induce AA-Mϕ prolongs CA-Mϕ phenotype. (A) WT BALB/c and IL-4R$\alpha^{-/-}$ peritoneal macrophages were treated as previously described. After 48 h, cells were analyzed by FACS for arginase-1 (R&D, Inc.) and iNOS (BD Biosciences). Treatments were performed in triplicate and data are presented as a single representative experiment (N = 3). (B). WT BALB/c and IL-4R$\alpha^{-/-}$ macrophages were treated as previously described and harvested at the indicated times. Expression of COX-2 (top graphs) and iNOS (bottom graphs) were analyzed by real-time PCR. Data are presented as means ± SEM and are derived from a single representative experiment (N = 3).

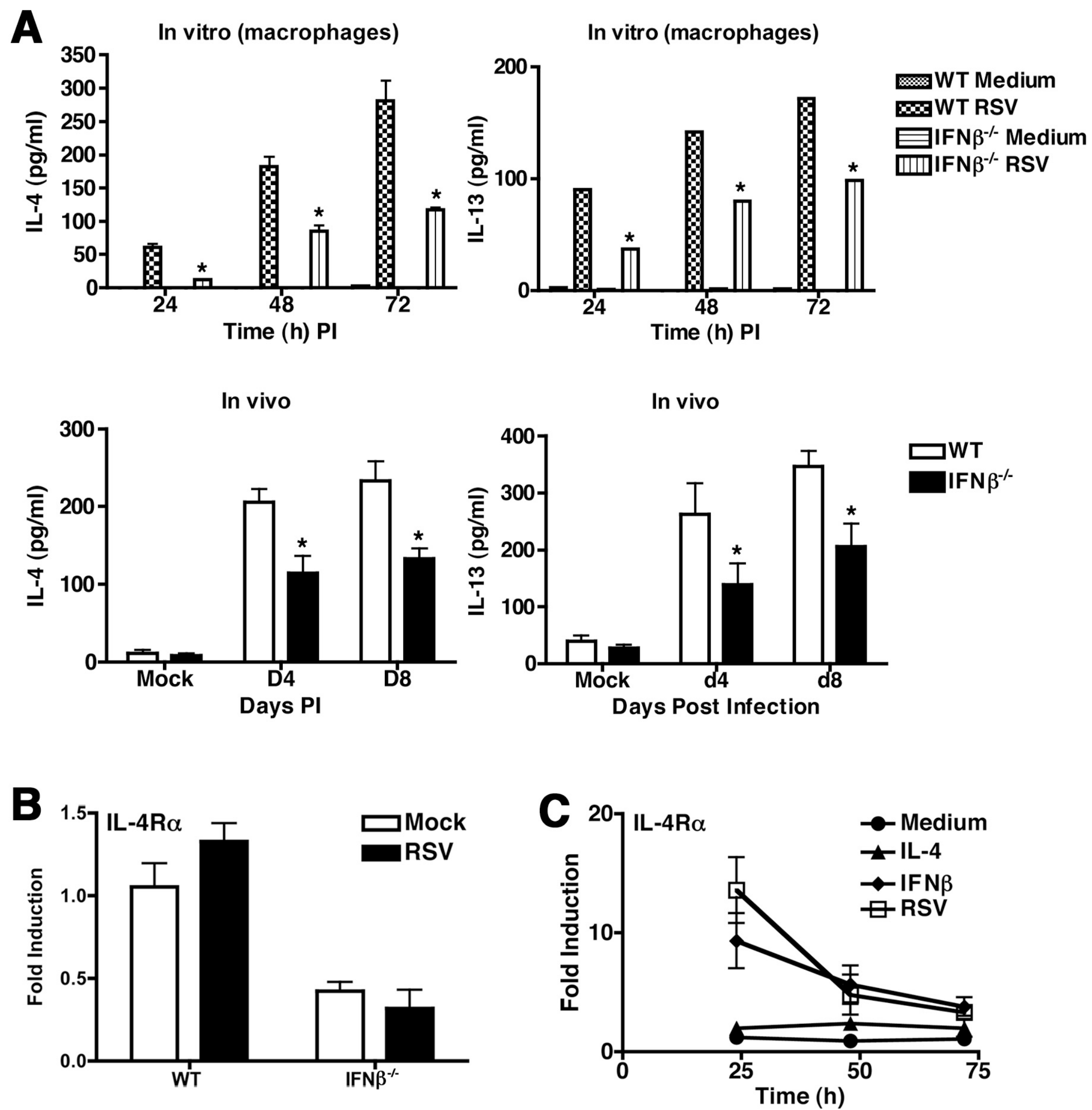


Supplemental Fig. S5. IL-4Rα expression in lungs of IL-4Rα$^{-/-}$ mice adoptively transfered with WT macrophages. BALB/c WT and IL-4Rα$^{-/-}$ mice, as well as IL-4Rα$^{-/-}$ mice that received either 1.5 x 10^7 WT or IL-4Rα$^{-/-}$ macrophages by i.p., were mock- or RSV-infected 5 d later. Lungs were harvested 4 d p.i. and analyzed for IL-4Rα gene expression by real-time PCR.



Supplemental Fig. S6. Effect of TLR4 deficiency on RSV-induced cytokine expression. (A) WT C57BL/6 and TLR4$^{-/-}$ peritoneal macrophages were treated with medium, rIL-4, or infected with RSV for up to 72 h and analyzed for IFN-β, COX-2, and iNOS mRNA by real-time PCR. *p<0.05 (B) WT and TLR4$^{-/-}$ mice were mock- or RSV-infected. Lungs were harvested 4 d p.i. and arginase-1 and IL-12 p40 mRNA measured by real-time PCR. (C) WT C57BL/6 and TLR4$^{-/-}$ macrophages were treated as indicated and

analyzed for IL-4 and IL-13 protein secretion by ELISA (N = 3). (D) WT and $TLR4^{-/-}$ mice were mock- or RSV-infected. Lungs were harvested 4 d p.i. and IL-4Rα mRNA measured by real-time PCR.



Supplemental Fig. S7. Effect of IFN-β deficiency on IL-4 and IL-4Rα mRNA expression in vitro and in vivo. (A) Top graphs: Peritoneal macrophages from WT C57BL/6J or IFN-$\beta^{-/-}$ mice were treated with medium only or infected with RSV for up to 72 h. (A; bottom graphs). WT and IFN-$\beta^{-/-}$ mice were mock- or RSV-infected. Lungs were harvested 4 d and 8 d p.i. and homogenized. Supernatants were collected and analyzed for IL-4 and IL-13 protein by ELISA. (B) WT C57BL/6J and IFN-$\beta^{-/-}$ mice

were mock- or RSV-infected. Lungs were harvested 4 d p.i. and analyzed for IL-4Rα mRNA by real-time PCR. Data represents the mean ± SEM of 3 independent experiments. (C) WT C57BL/6 peritoneal macrophages were treated with medium, rIL-4 (40 ng/ml), IFN-β (100 U/ml), or infected with RSV (MOI = 2) and analyzed for mRNA gene expression by real-time PCR (N = 2).

SUPPLEMENTAL METHODS

Harvest and culture of BAL and peritoneal thioglycollate-elicited peritoneal macrophages.

BAL cells were obtained from 6-8 week old mice. Mice were sacrificed and the thoracic cavity opened to expose the lungs. After cannulating the trachea, the lungs were instilled with 1 ml of warmed RPMI 5 times and the lavages pooled. Cells were centrifuged and washed with saline before re-suspension in RPMI supplemented with 2% FCS, 1% penicillin/streptomycin, and 1% L-glutamate and incubated for 2 h at 37º C. Cells were washed with PBS to remove non-adherent or loosely adherent cells and re-cultured in fresh RPMI. Two more additional washes were carried out after 2 h and the cells were incubated overnight in fresh RPMI at 37º C. FACS analysis was performed on a sample of cells to access the purity of the cells by staining for F4/80, a macrophage cell marker.

For peritoneal macrophages, 6-8 week old mice were injected with 2.5 ml of thioglycollate medium (Remel) i.p. Four d later, mice were sacrificed and peritoneal exudate cells were obtained by lavage and pooled. Cells were cultured and analyzed as described for BALs.

Cotton rat primer sequences:

Cotton rat IL-13 (S): 5'-AGTCTTCAGTTTAAGCCAGCTTAC-3'

Cotton rat IL-13 (AS): 5'-TTTTCAATGGAAGGTACCACAGCGG-3'

Cotton rat arginase-1 (S): 5'-AGCATCTCTGGCCAYGCCAG-3'

Cotton rat arginase-1 (AS): 5'-ATTKCTTCTGTGRATGTAGA-3'

Cotton rat Mannose receptor (S): 5'-GTGAATTTCAGAAATGGGAGTG-3'

Cotton rat Mannose receptor (AS): 5'-GTACCACTTGTTTTCAAACTTG-3'.

Flow cytometry analysis

Macrophages from mice or cotton rats were treated with medium alone, rIL-4, or infected with RSV for 48 h. Cells were harvested, washed with PBS, and then fixed with 4% PFA in 0.1 M phosphate buffer (PB) for staining of FIZZ1, arginase-1, mannose receptor, or iNOS as described (Shirey et al., 2008).

BAL macrophage intracellular staining

Detection of IL-4 and arginase-1-positive macrophages in the lung was performed at day 4 p.i. (≥4 mice/treatment). Mice were anesthetized with isoflurane and treated i.n with 50 μl PBS (mock) or RSV (10^6 pfu/animal). On day 4 p.i., mice were sacrificed, and BAL harvested. Cells were cultured in 2-well chamber slides for 2 h at 37º C, 5% CO_2, washed with PBS, fixed with 4% PFA, and analyzed by confocal microscopy for expression of IL-4. Fixed BAL macrophages from mock- or RSV-infected mice were incubated for 30 min with PBST at RT. F4/80, a macrophage marker, was visualized by immunofluorescence using rat MAb antibody against mouse F4/80 (Ab Cam), followed by Cy3-conjugated donkey anti-rat IgG. IL-4 was detected using anti-mouse MAb against mouse IL-4 (R&D, Inc.), followed by secondary and tertiary staining with biotin-conjugated donkey anti-mouse IgG and Cy2-conjugated streptavidin. Nucleic acid was labeled using DAPI stain.