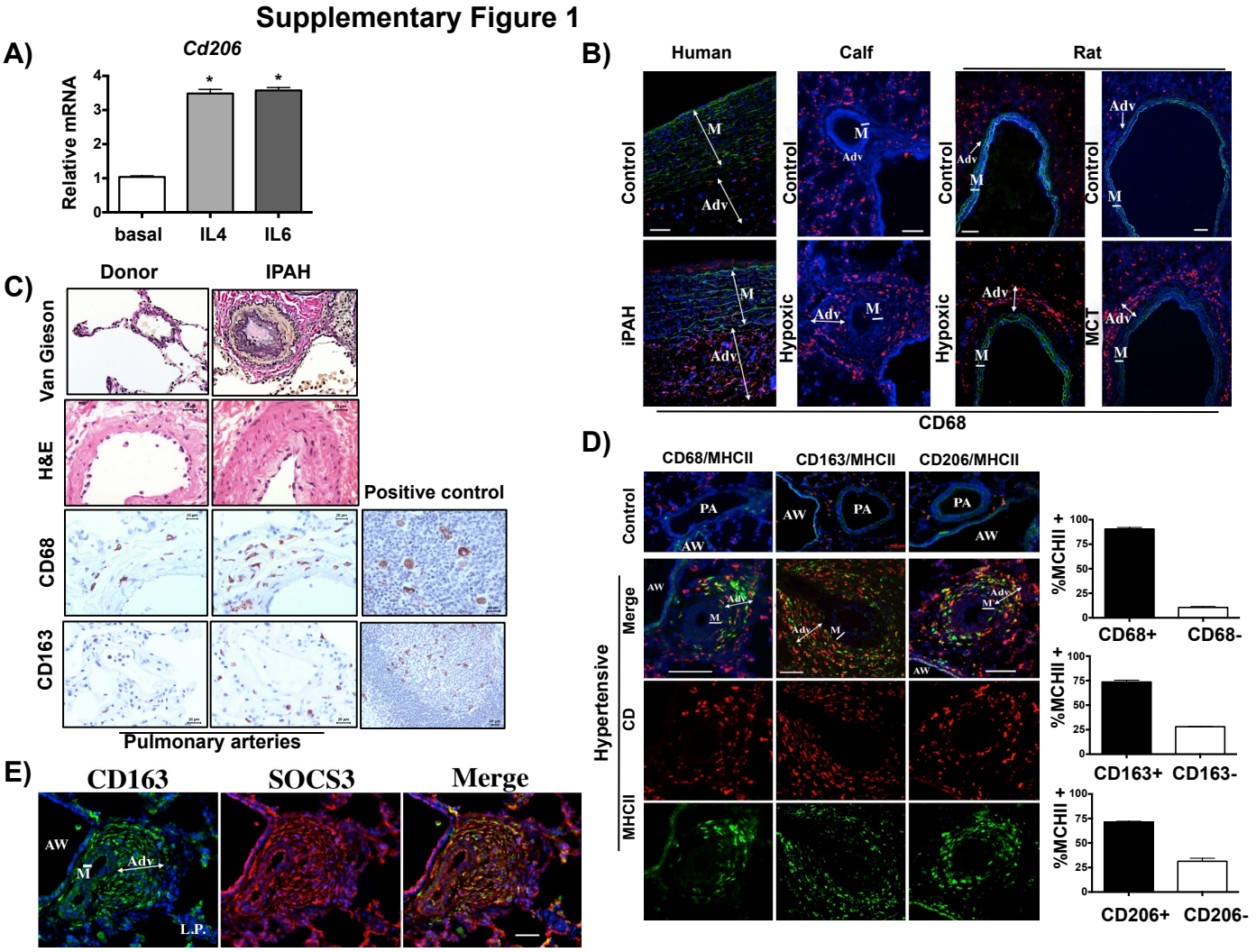
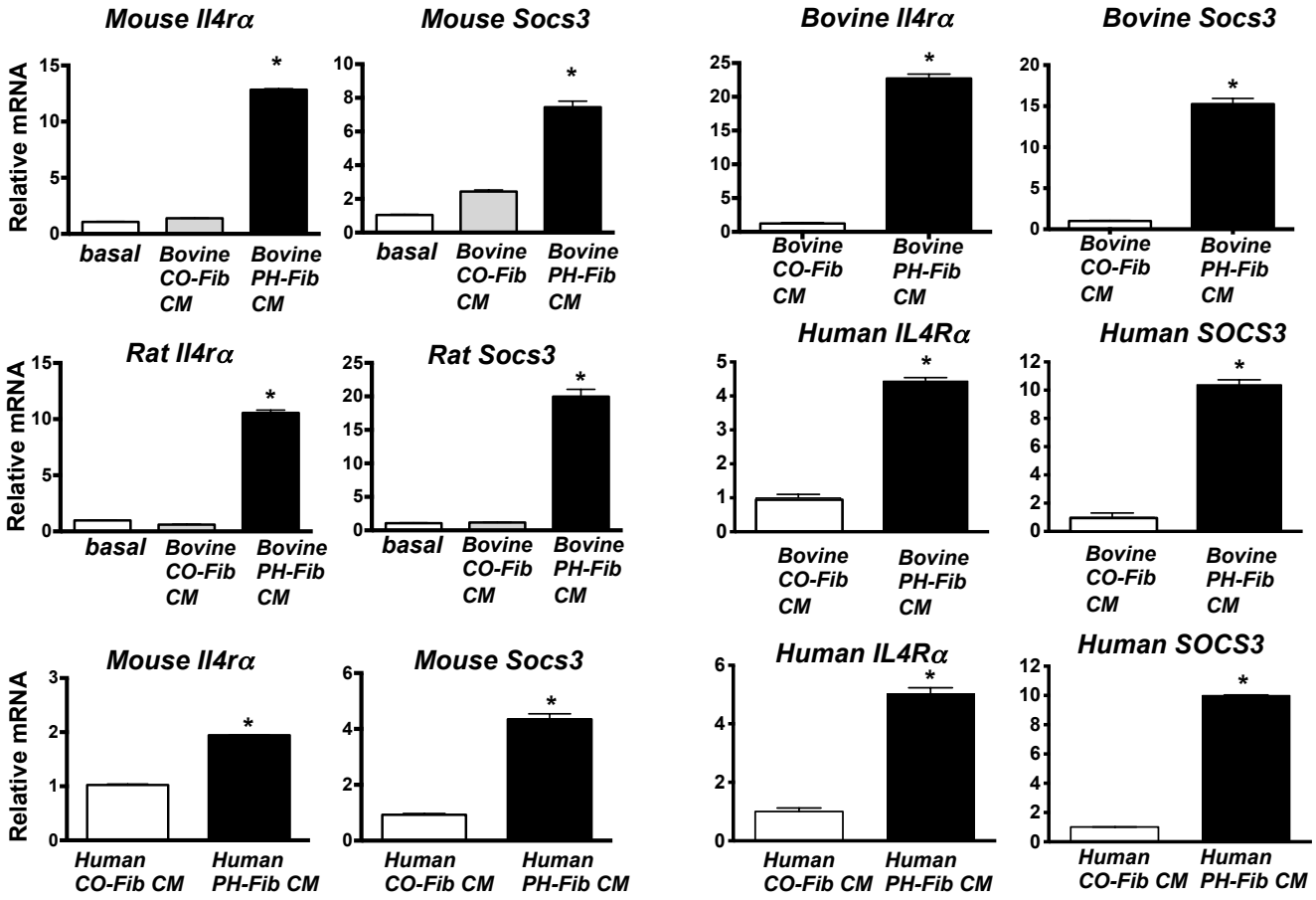


Supplementary Fig. 1. Adventitial macrophages express CD163, CD206, and SOCS3. (A) Gene expression of *Cd206* in response to recombinant mouse IL4 or IL6 (both at 10ng/ml) in mouse BMDMS after 16hrs of exposure compared to untreated (basal). Data are obtained from PCR triplicates and representative of results from two separate experiments obtained with cytokine stimulation using BMDMs from different animals. Gene expression was normalized to *Hprt1* and data are relative to gene expression in untreated samples. *$P < 0.05$ by One-Way ANOVA **(B)** CD68-positive cells (macrophages) in the PA of humans with PH (iPAH), in calves with hypoxia-induced PH, and in rats with hypoxia- and monocrotaline (MCT)-induced PH, as detected by immunofluorescence in cross-sectioned fresh-frozen tissue. Control indicates tissue from donors or normoxic animals. **(C)**

CD68- and CD163-positive cells in formalin-fixed PA tissue of humans with iPAH (idiopathic pulmonary arterial hypertension). Note localization of CD68+ and CD163+ cells to the perivascular area of the thickened remodeled PA vasculature in the patient with iPAH compared to the control (Donor); tonsil (CD163) and spleen (CD68) are positive controls. Images are representative of 8 patients and 5 donors. **(D)** Confocal images of bovine PA tissue from control (normoxic, top panels) or hypertensive (chronic hypoxia, lower panels) calves indicate that the majority of MHCII-positive (green) cells are macrophages ($CD68^+$, red, left panel) and co-express CD163 or CD206 (Merge, yellow). Quantitation of macrophage marker co-expression is presented as filled bars for dual positive (MHCII and CD68, CD163, or CD206) and open bars for MHCII positive only. **(E)** Co-localization (Merge, yellow) of SOCS3 (red) and CD163 (green) in dPA from calf with hypoxia-induced PH. All immunofluorescence panels show DAPI counterstain (cell nuclei, blue) and autofluorescence of vascular elastic lamellae (green) defining borders of vascular media. Scale bars = 100 μm. PA = pulmonary artery; AW = airway; M = PA media; Adv = PA adventitia.

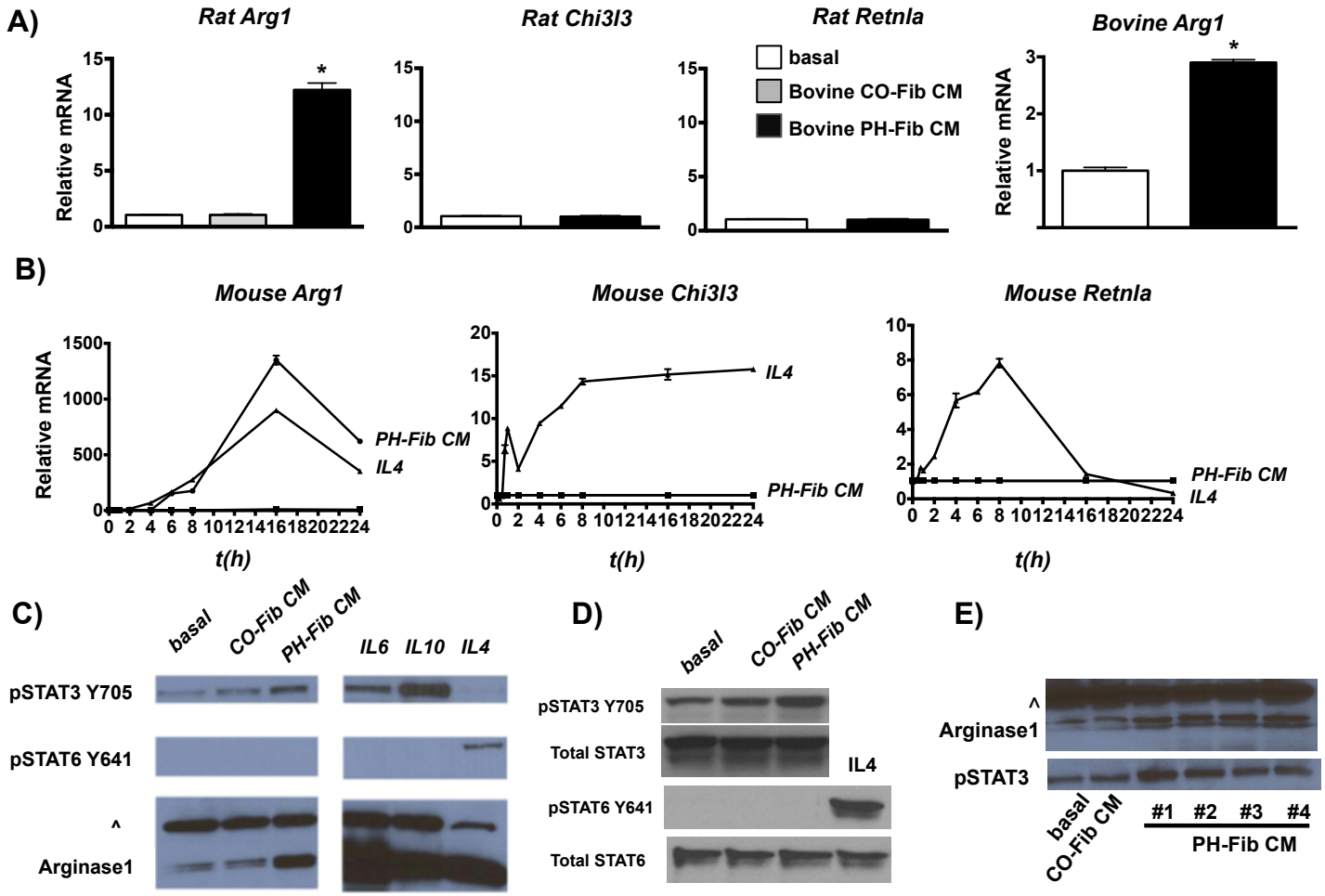
Supplementary Figure 2.



Supplementary Figure 2. Fibroblast activated macrophages express *Il4ra* and *Socs3*.

Gene expression of *Il4ra* and *Socs3* in mouse, rat, and bovine BMDMs as well as human THP1 monocytes in response to bovine or human PH- or CO-Fib CM after stimulation for 16 hrs. Mean±SEM of PCR triplicates after normalization to expression of Hprt1 and relative to gene expression in untreated macrophages/monocytes (basal, not shown in all graphs) is presented. These data are representative of experiments with CM from PH-Fibs and CO-Fibs isolated from 3 different cell populations repeated on BMDMs from 3 different animals. $*P < 0.05$ by one-way ANOVA or Student's *t*-test.

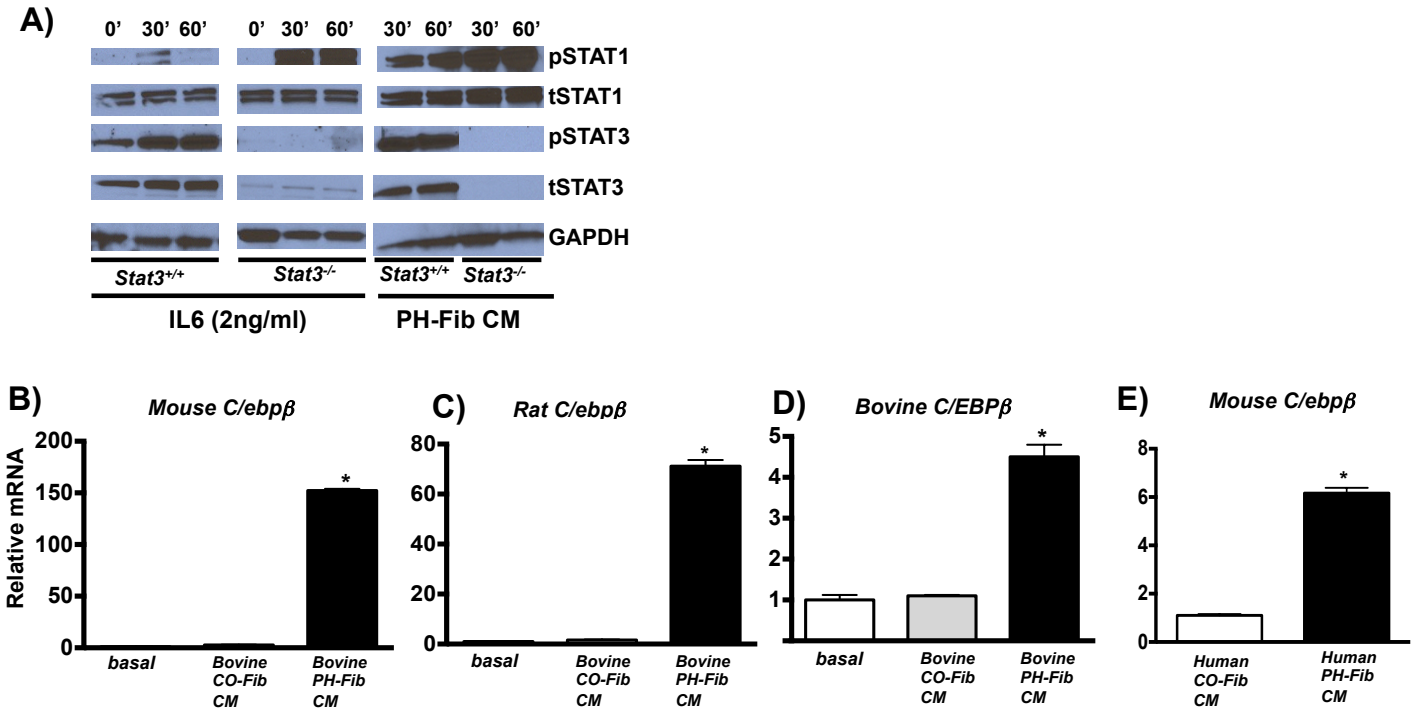
Supplementary Figure 3



Supplementary Figure 3. Fibroblast activated macrophages express Arginase1 independently of STAT6 but in association with STAT3. (A) Expression of *Arg1*, *Chi3l3,* and *Rentla* in response to bovine PH-Fib CM in rat BMDMs, bovine BMDMs, as well as mouse BMDM (16 hr time point). **P* < 0.05 by Student's *t*-test compared to gene induction by CO-Fib CM. **(B)** Gene expression of *Arg1*, *Chi3l3,* and *Rentla* in response to recombinant mouse IL4. In A, B the relative mRNA expression (ΔΔCT, mean ± SEM) of PCR triplicates representative of experiments with CM from 3 different PH-Fib and CO-Fib cell populations (different animals) tested on BMDMs from 2 different animals are presented. The data are normalized to expression of Hprt1 and relative to gene expression in untreated macrophages. **(C, D, E)** Immunoblot of phosphorylated STAT3 (Y705), phosphorylated STAT6 (Y641), and Arginae1 using protein lysates from mouse (**C**), bovine (**D**) and rat **(E)** BMDMs either unstimulated (basal) or after 24-h exposure to bovine CO-Fib CM, PH-Fib CM, recombinant IL6,

IL10, or IL4 (10 ng/ml each). Note absence of STAT6 phosphorylation in response to PH-Fib CM. Phosphorylated STAT6 in response to IL4 served as positive control. STAT3 phosphorylation was increased in response to PH-Fib CM and positive controls IL6 and IL10, while *Arg1* expression was associated with STAT3 but not STAT6 phosphorylation. Note PH-Fib CM from four different cell populations obtained from four different animals induce Arginase1 in rat BMDMs. ^ denotes non-specific binding of anti-Arginase1 antibody used to verify equal loading in **C** and **E**.

Supplementary Figure 4



Supplementary Figure 4. STAT3 and C/EBPβ are critical regulators of fibroblast-mediated macrophage activation.

(A) Immunoblot of phosphorylated (Y705) and total STAT3, phosphorylated (Y701) and total STAT1, and GAPDH on *Stat3-/-* and *Stat3+/+* BMDMs either unstimulated (0 min) or stimulated for 30 and 60 min with 2 ng/ml recombinant murine IL6 or bovine PH-Fib CM. Note decreased phosphorylation of STAT3 in *Stat3*$^{-/-}$ but not in *Stat3*$^{+/+}$ BMDMs in response to PH-Fib CM, and increased and prolonged

STAT1 phosphorylation in *Stat3*$^{-/-}$ compared to *Stat3*$^{+/+}$ BMDMs. The same samples were run on replicate gels and probed after stripping for either phosphorylated STAT1/3 or total STAT1/3 protein. Results are representative of 2 experiments. **(B-D)** Expression of *C/ebpb* in response to bovine PH-Fib CM or CO-Fib CM in mouse, rat and bovine BMDMs. **(E)** Expression of *C/ebpb* in response to human PH-Fib CM or CO-Fib CM in mouse BMDMs $*P < 0.05$ by unpaired two-tailed Student's *t*-test. 16 hr time point shown for all experiments. Data are mean±SEM of PCR triplicates after normalization to expression of *Hprt1* and relative to gene expression in untreated macrophages and representative of at least n=2 experiments with CM from PH-Fibs and CO-Fibs isolated from at least 2 different patients/animals and BMDMs from at least 2 different animals. $*P < 0.05$ by Student's *t*-test.