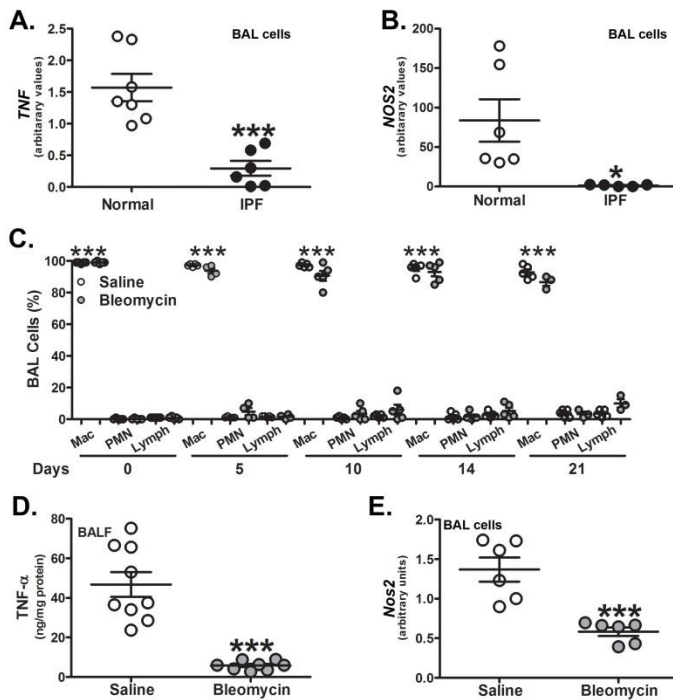


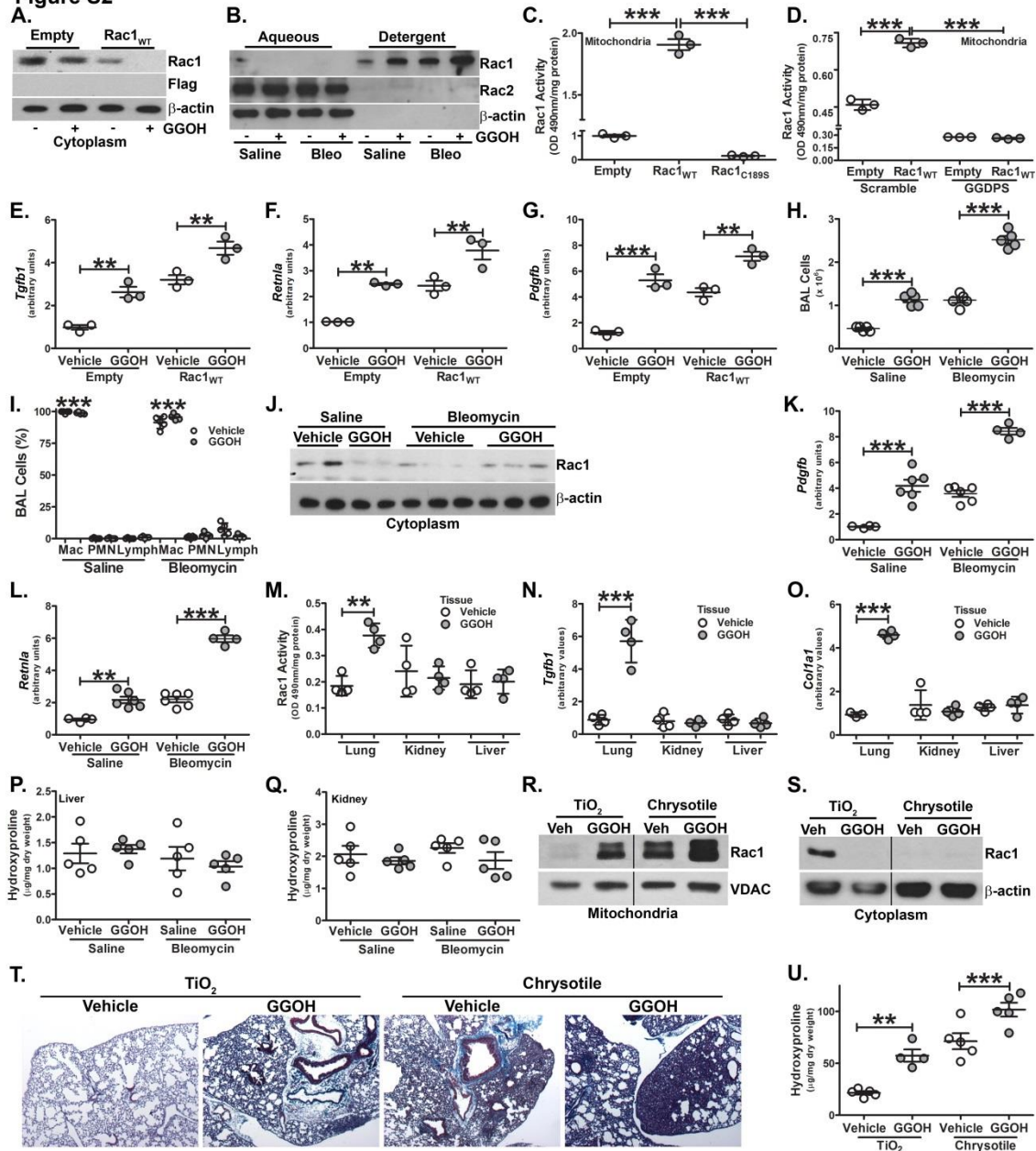
Supplemental Information:

Figure S1



Supplemental Figure 1. Classically activated macrophages are down-regulated in IPF BAL cells. (A) *TNF* (B) and *NOS2* mRNA expression in BAL cells from normal ($n = 6-7$) or IPF subjects ($n = 5-6$). (C) Cell differential from saline and bleomycin-exposed WT mice at indicated time points ($n = 3-5$ per time point). (D) *TNF-α* was measured in BALF from saline ($n = 9$) or bleomycin-exposed mice ($n = 8$). (E) *Nos2* mRNA expression in BAL cells from saline ($n = 6$) or bleomycin-exposed mice ($n = 6$). *, $p < 0.05$; ***, $p < 0.0001$. Values shown as mean \pm S.E.M. Two-tailed *t*-test statistical analysis was utilized for a, b, d, e. One-way ANOVA followed by Tukey's multiple comparison test was utilized for c.

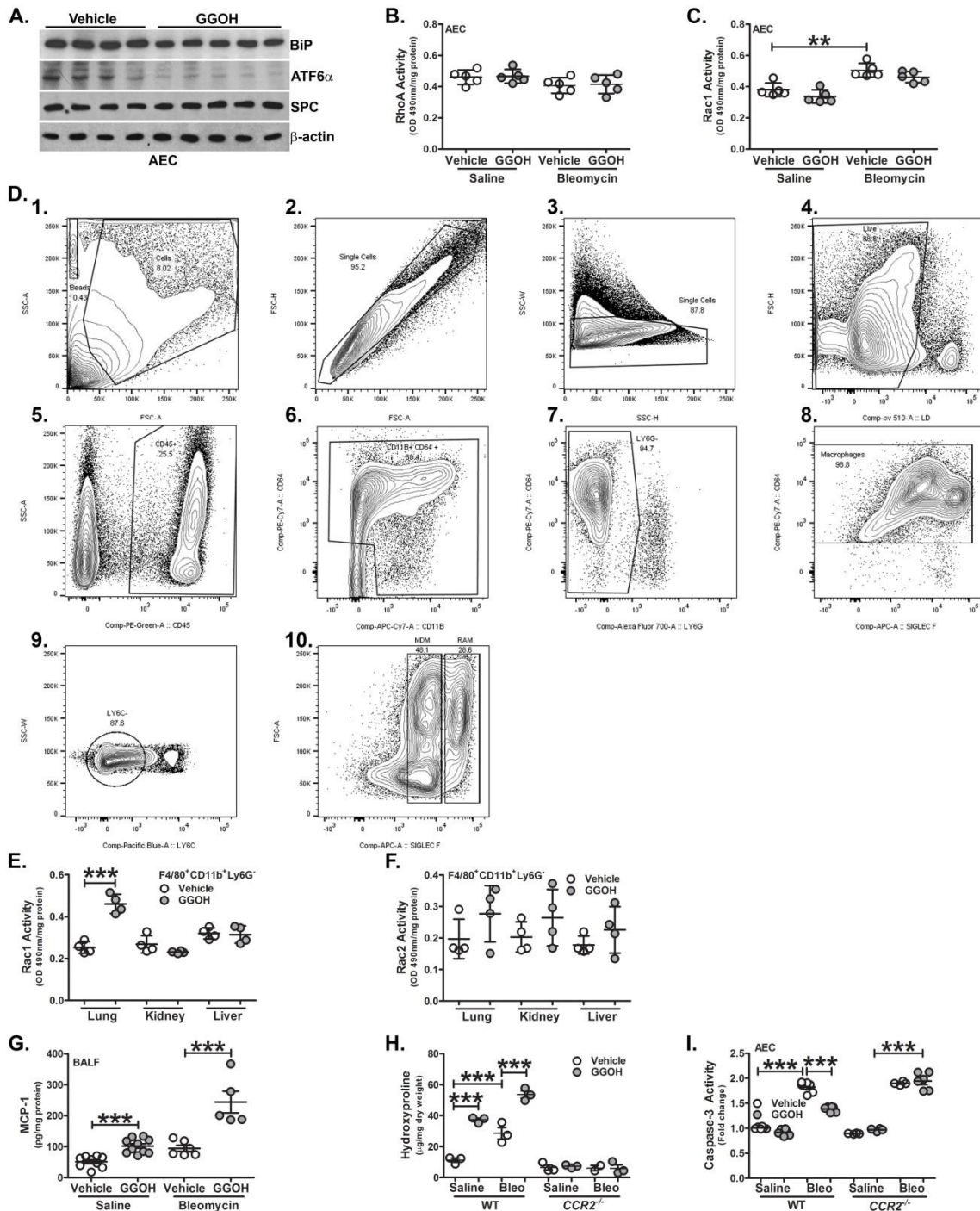
Figure S2



Supplemental Figure 2. GGOH promotes lung fibrosis in the absence of injury. (A) Immunoblot analysis in the cytoplasmic fraction from macrophages expressing empty or Rac1_{WT} treated with vehicle or GGOH (50 μM). (B) Transfected THP-1 cells were treated with vehicle or GGOH and separated into aqueous (unprenylated) or detergent (prenylated) fractions. Mitochondrial Rac1 activity in transfected THP-1 cells expressing (C) empty, Rac1_{WT}, or Rac1_{C189S} and (D) scramble or GGDPS siRNA and empty or Rac1_{WT}. mRNA expression for (E) *Tgfb1* ($n = 3$), (F) *Retnla* ($n = 3$) and (G) *Pdgfb* ($n = 3$) in MH-S cells expressing empty or Rac1_{WT} treated with vehicle or GGOH. Ten days after exposure of WT mice to saline or bleomycin, pumps containing vehicle or GGOH were implanted subcutaneously; mice were sacrificed 11 days later. (H) Total number of cells and (I) cell differential from BAL ($n = 5$ per

group). **(J)** Cytoplasmic Rac1 immunoblot analysis in isolated monocyte-derived macrophages. mRNA expression for **(K)** *Pdgfb* and **(L)** *Retnla* (saline, vehicle $n = 4$; saline, GGOH $n = 6$; bleomycin, vehicle $n = 6$; bleomycin, GGOH $n = 4$). **(M)** Rac1 activity and **(N)** *Tgfb1* and **(O)** *Colla1* mRNA expression in tissue isolated from saline-exposed WT mice treated with GGOH via osmotic pumps ($n = 4$ per group). Hydroxyproline analysis of **(P)** liver and **(Q)** kidney ($n = 5$ per group). WT mice were exposed to TiO_2 or chrysotile asbestos, pumps containing vehicle or GGOH were implanted subcutaneously 10 days after exposure; mice were sacrificed 11 days later. Rac1 immunoblot analysis in isolated monocyte-derived macrophages from **(R)** mitochondrial and **(S)** cytoplasmic fractions. **(T)** Representative lung histology with Masson's Trichrome staining ($n = 5$ per group) with 2.5x magnification and **(U)** hydroxyproline content ($n = 4-5$ per group). **, $p < 0.001$; ***, $p < 0.0001$. Values shown as mean \pm S.E.M. One-way ANOVA followed by Tukey's multiple comparison test was utilized.

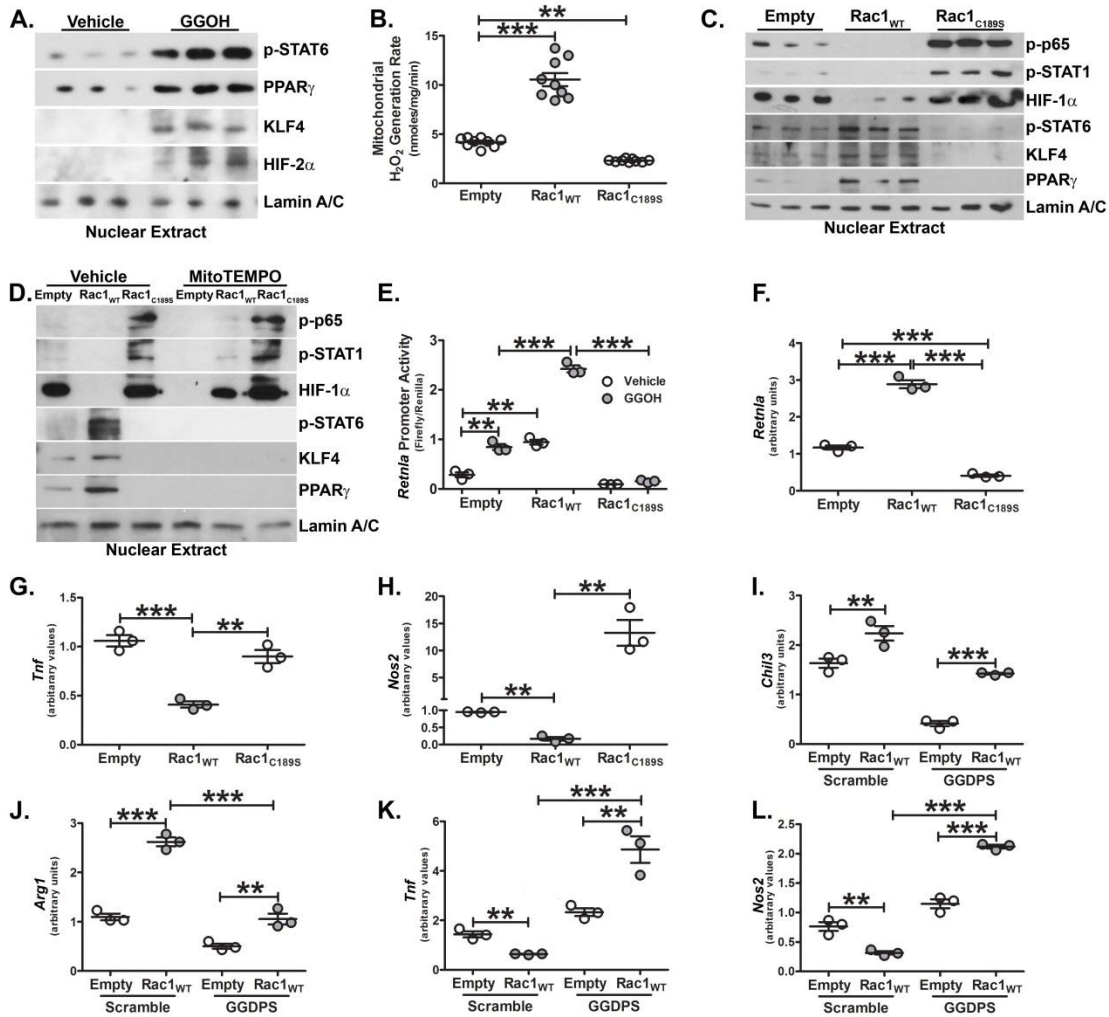
Figure S3



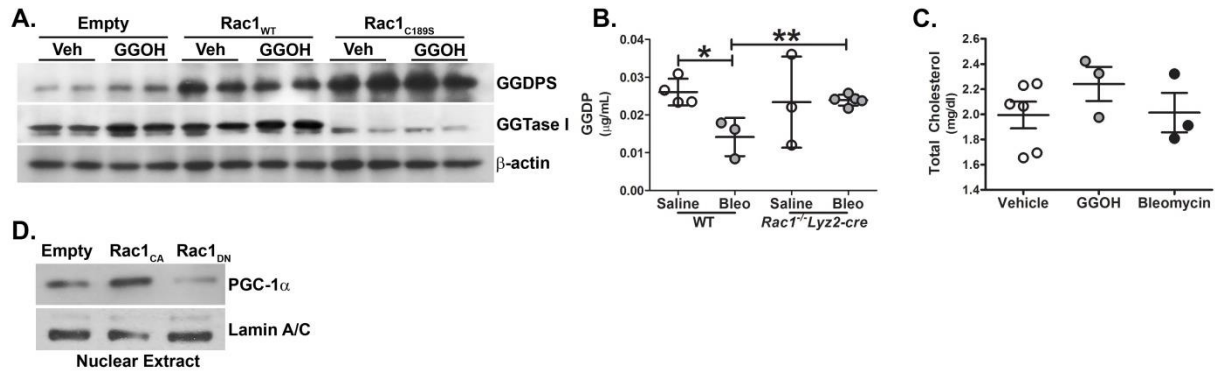
Supplemental Figure 3. GGOH-mediated lung fibrosis requires monocyte-derived macrophages. (A) Immunoblot analysis in alveolar epithelial cells (AECs) isolated from vehicle or GGOH treated mice (vehicle, $n = 4$; GGOH, $n = 5$). (B) RhoA and (C) Rac1 activity in AECs isolated from WT mice exposed to saline or bleomycin, 10 days after exposure pumps containing vehicle or GGOH were subcutaneously implanted; mice were sacrificed 11 days later. Ten days after exposure of WT mice to saline or bleomycin, pumps containing vehicle or GGOH were subcutaneously implanted; mice were sacrificed 11 days later. (D) Gating strategy used for flow

cytometry to sort monocyte populations in bleomycin-exposed WT mice treated with GGOH. (E) Rac1 and (F) Rac2 activity from F4/80⁺, CD11b⁺, and Ly6G⁻ sorted cells from saline-exposed WT mice treated with GGOH via osmotic pump ($n = 4$ per group). (G) MCP-1 measured in BALF ($n = 5-10$ per group). WT and *CCR2*^{-/-} mice were exposed and treated as described above. (H) Hydroxyproline in lung tissue ($n = 3$ per group). (I) Caspase-3 activity in isolated AECs ($n = 4-6$ per group). **, $p < 0.001$; ***, $p < 0.0001$. Values shown as mean \pm S.E.M. One-way ANOVA followed by Tukey's multiple comparison test was utilized.

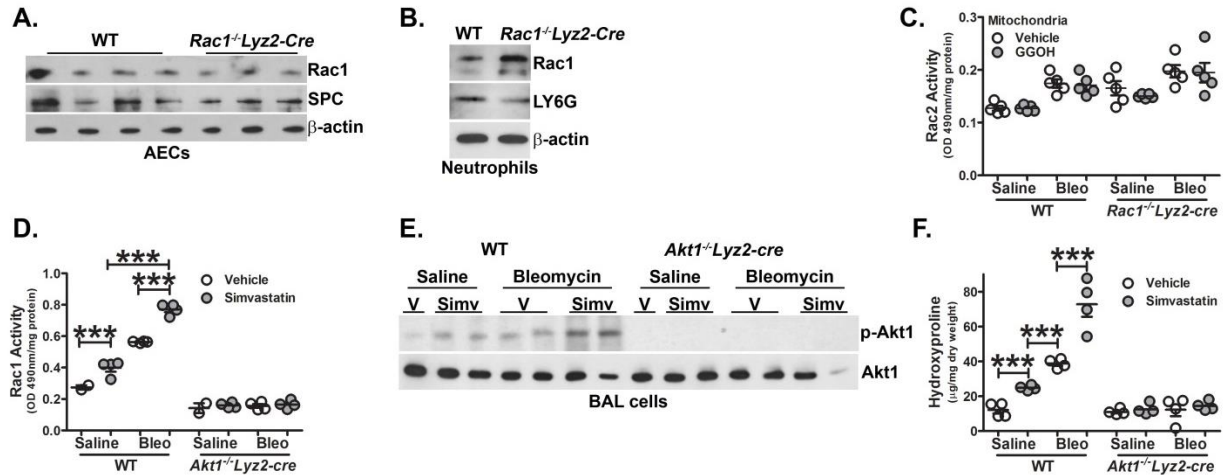
Figure S4



Supplemental Figure 4. GGOH promotes profibrotic polarization of macrophages. (A) Immunoblot analysis in the nuclear fraction from macrophages treated with vehicle or GGOH (50 μ M). (B) MtROS generation in transfected THP-1 cells treated with vehicle or GGOH ($n = 3$). (C) Immunoblot analysis in transfected MH-S cells. (D) Immunoblot analysis in transfected MH-S cells treated with vehicle or mitoTEMPO (10 μ M). (E) *Retnla* promoter activity in transfected MH-S cells treated with vehicle or GGOH. mRNA expression for (F) *Retnla* ($n = 3$), (G) *Tnf* ($n = 3$) and (H) *Nos2* ($n = 3$) in transfected MH-S cells ($n = 3$). mRNA expression for (I) *Chil3* ($n = 3$), (J) *Arg1* ($n = 3$), (K) *Tnf* ($n = 3$) and (L) *Nos2* ($n = 3$) in THP-1 cells expressing scramble or GGDPs siRNA and empty or Rac1 $_{WT}$. **, $p < 0.001$; ***, $p < 0.0001$. Values shown as mean \pm S.E.M. One-way ANOVA followed by Tukey's multiple comparison test was utilized.



Supplemental Figure 5. Inhibition of Rac1 geranylgeranylation increases GGDP levels in BAL cells. (A) Immunoblot analysis in transfected MH-S cells treated with vehicle or GGOH. (B) GGDP levels in WT and *Rac1*^{-/-}*Lyz2-cre* mice exposed to saline or bleomycin (WT, saline *n* = 4; WT, bleomycin *n* = 3; *Rac1*^{-/-}*Lyz2-cre*, saline *n* = 3; *Rac1*^{-/-}*Lyz2-cre*, bleomycin *n* = 5). (C) Total cholesterol levels in MH-S cells treated with vehicle, GGOH, or bleomycin (*n* = 3). (D) Immunoblot analysis in nuclear extract of THP-1 cells expressing constitutively active Rac1 (Rac1_{CA}) or dominant negative Rac1 (Rac1_{DN}). *, *p* < 0.05; **, *p* < 0.001. Values shown as mean ± S.E.M. One-way ANOVA followed by Tukey's multiple comparison test was utilized.



Supplemental Figure 6. Rac1 is expressed in AECs from *Rac1^{-/-}Lyz2-cre* mice. Rac1 immunoblot analysis in (A) alveolar epithelial cells (AECs) and (B) neutrophils isolated from WT and *Rac1^{-/-}Lyz2-cre* mice. (C) Ten days after exposure of WT and *Rac1^{-/-}Lyz2-cre* mice to saline or bleomycin, pumps containing vehicle or GGOH were subcutaneously implanted; mice were sacrificed 11 days later. Rac2 activity in BAL cells ($n = 5$). Ten days after exposure of WT or *Akt1^{-/-}Lyz2-cre* mice to saline or bleomycin, daily i.p. injections of simvastatin (20 mg/kg/day) were performed. Mice were sacrificed 11 days later. (D) Rac1 activity and (E) immunoblot analysis was performed in isolated BAL cells ($n = 2-4$ per group). (F) Hydroxyproline analysis ($n = 4$ per group). ***, $p < 0.0001$. Values shown as mean \pm S.E.M. One-way ANOVA followed by Tukey's multiple comparison test was utilized.