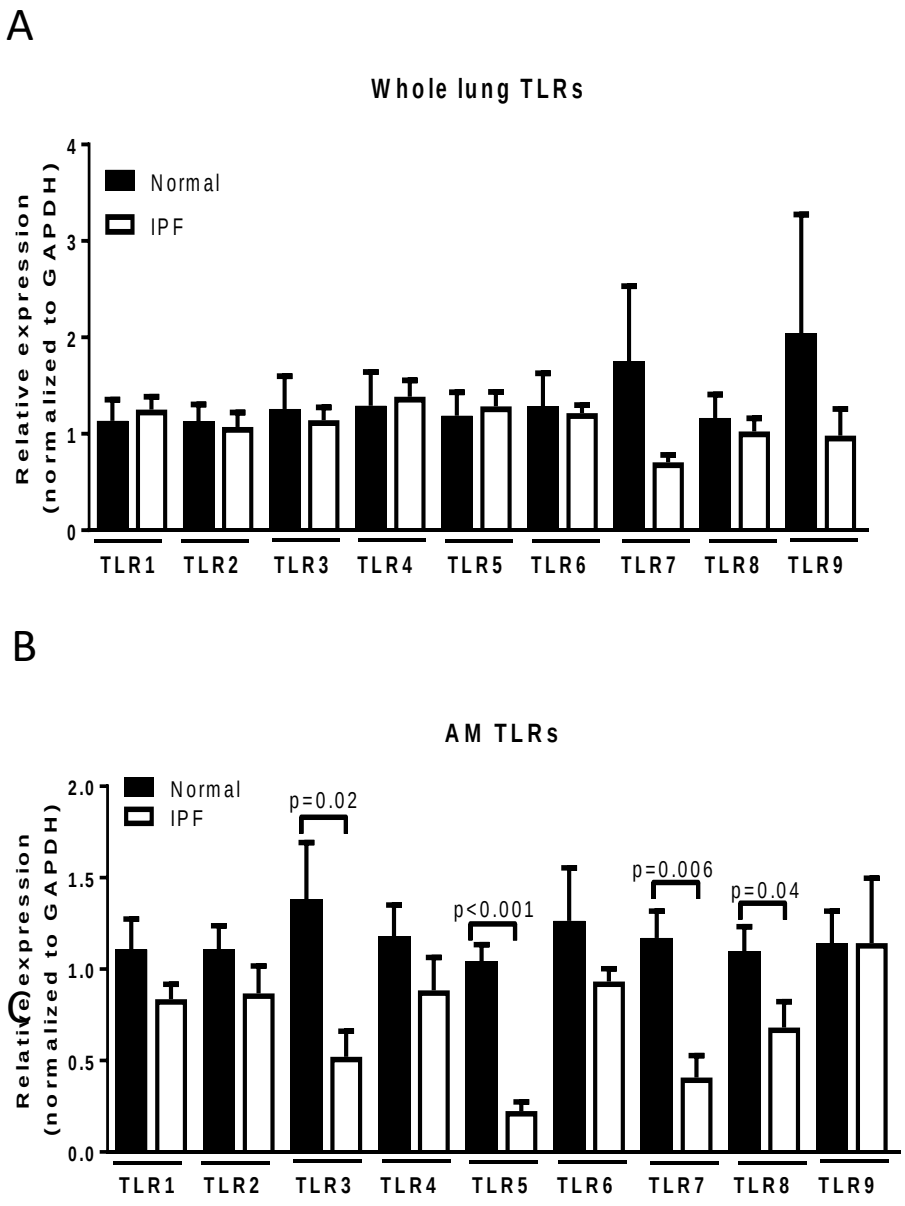
Figures for Thesis



C

Figure 1: Altered TLR expression IPF cells compared to normal donor controls.
Whole lung tissue, alveolar macrophages, and interstitial macrophages from either healthy donor controls or IPF patients were cultured for 24 hours in 10% FBS media (A-C). Expression of TLRs 1-9 was assessed by qPCR (n=5-7/sample) *p<0.05, **p<0.01, ***p<0.001

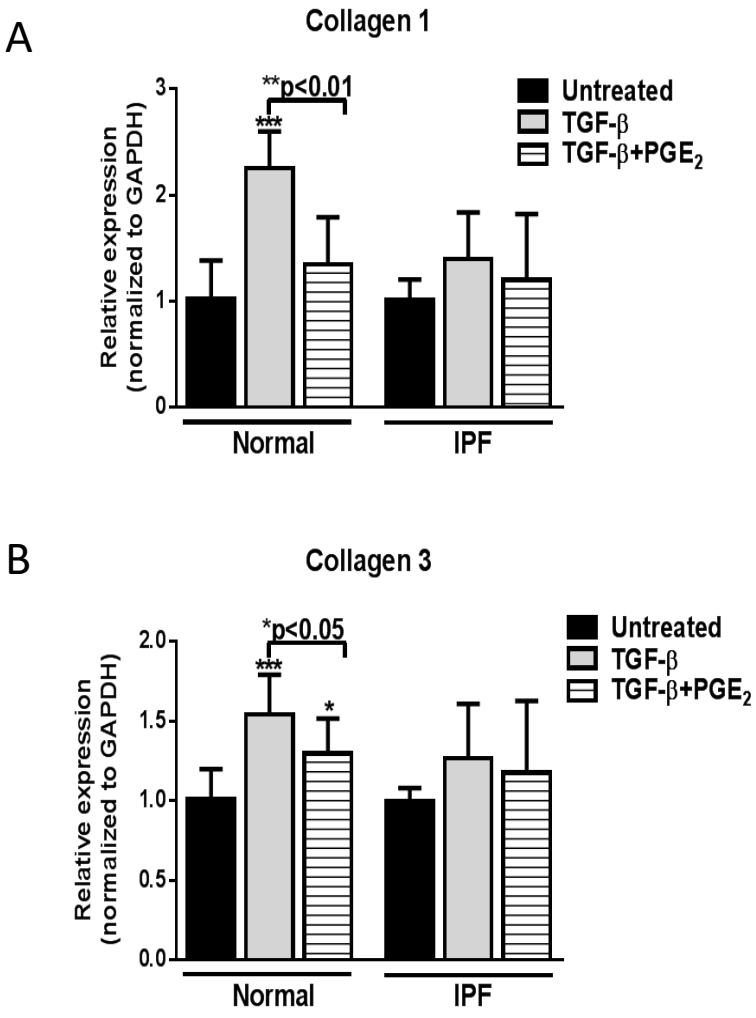


Figure 2: Treatment of lung fibroblasts with TGF-β and PGE_2 alters fibrotic genes in normal but not IPF samples.
Human lung fibroblasts were isolated from healthy donor controls or IPF patients. Cells were cultured in serum free media overnight and then stimulated with a profibrotic cytokine, TGF-β (2 ng/mL) alone, or in combination with an anti-fibrotic mediator PGE_2 (10 nM) for 18 hrs. mRNA was harvested from the cells and gene expression was determined by qPCR. (n=3/sample) *p<0.05, **p<0.01, ***p<0.001

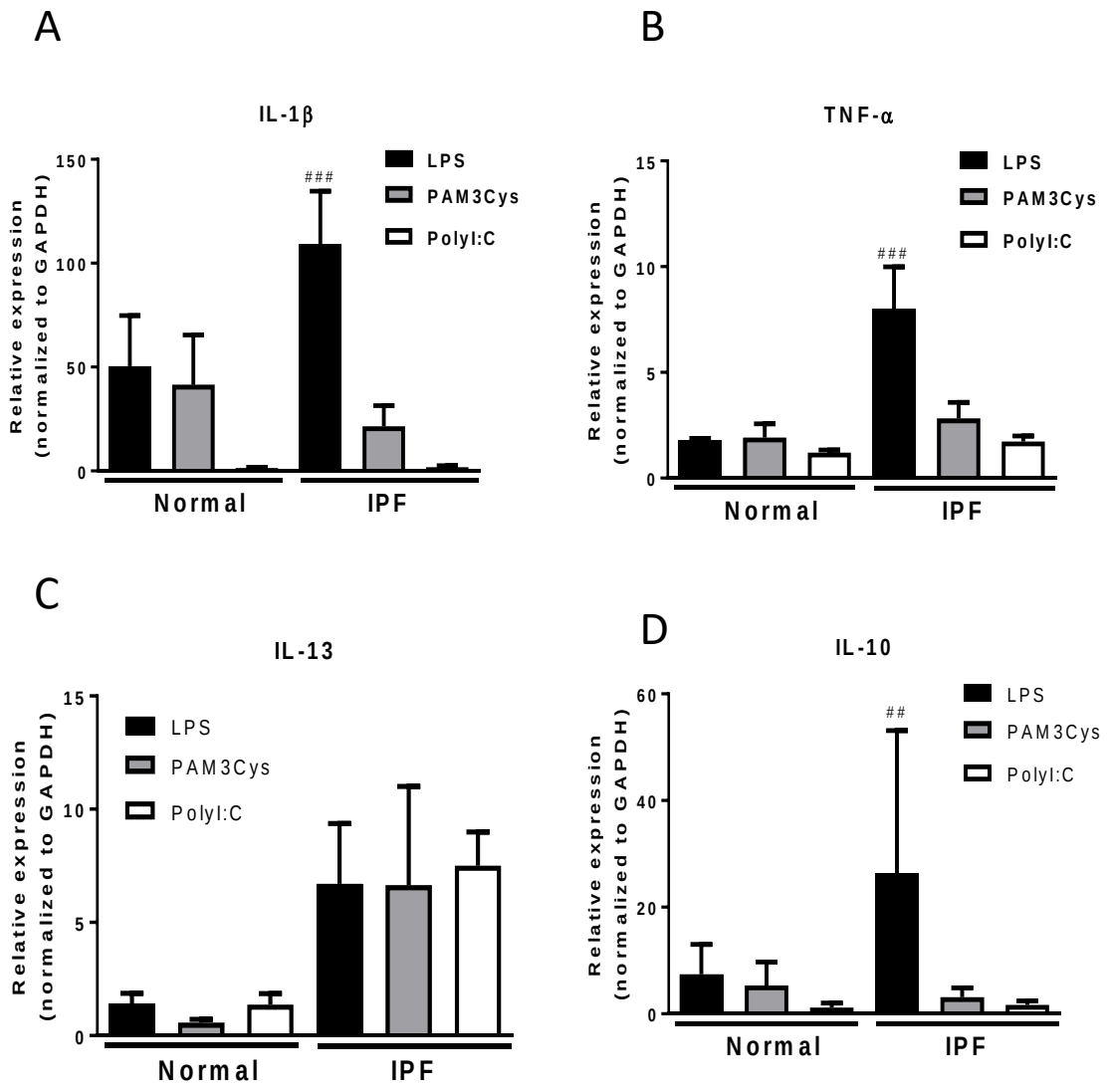


Figure 3: Stimulation of AMs from IPF patients with TLR ligands resulted in elevated expression of pro and anti-inflammatory cytokines

Alveolar macrophages were isolated from IPF patients and normal, donor controls and stimulated with LPS (TLR4 agonist), Pam3Cys (TLR 3 agonist) and PolyI:C (TLR1/2 agonist) for 24 hrs. mRNA was isolated from the cells after stimulation and expression of cytokines was determined by qPCR. A-D. #p<0.05, ##p<0.01, ###p<0.001 when compared to disease specific unstimulated

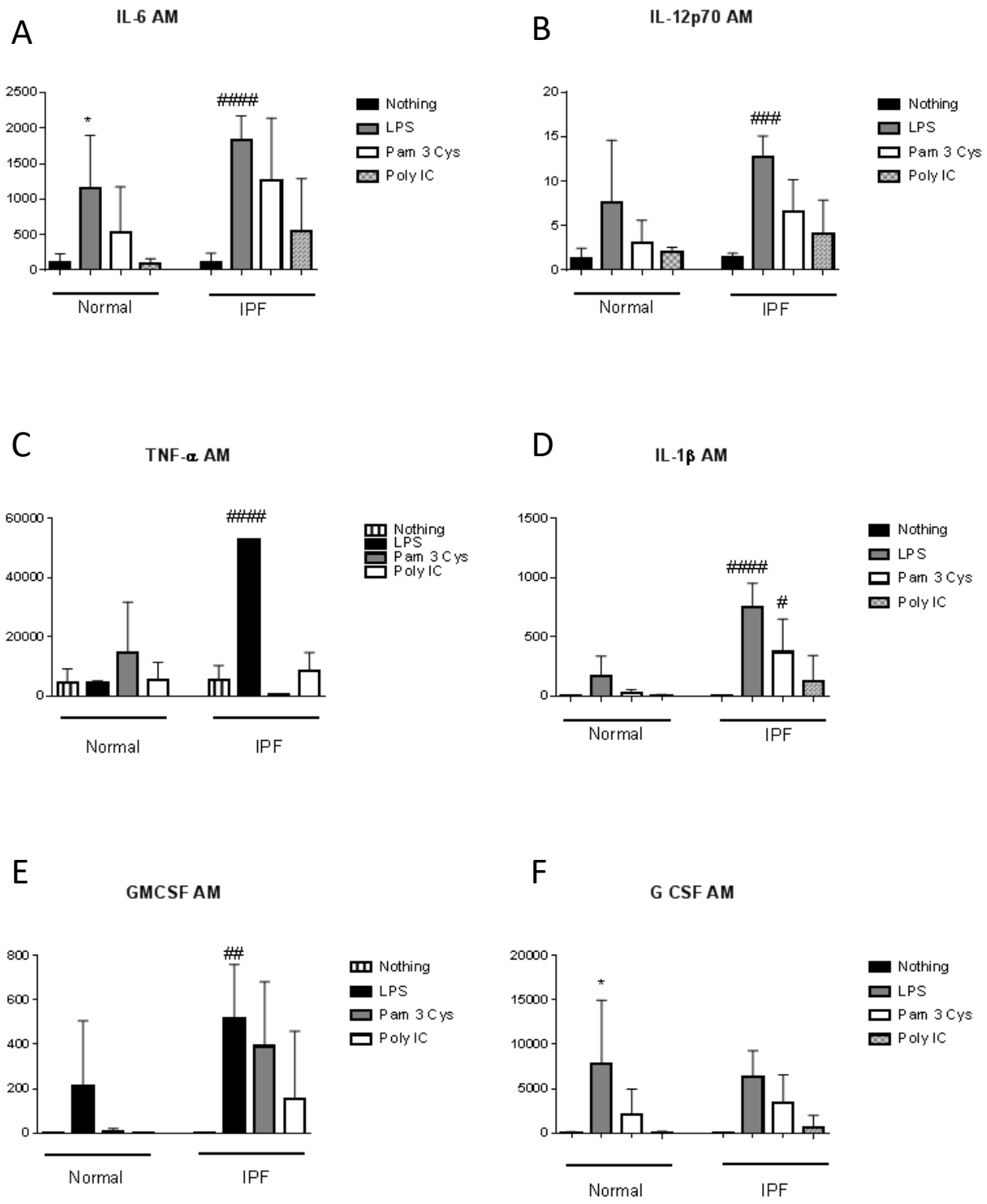


Figure 4: Luminex with both IPF and normal fibroblasts displays differences in cytokine production

Supernatants from human lung fibroblasts from both normal donor controls as well as IPF samples were collected from cells and amount of protein was measured using Luminex. A-F. *p<0.05, **p<0.01, ***p<0.001 when compared to disease specific unstimulated

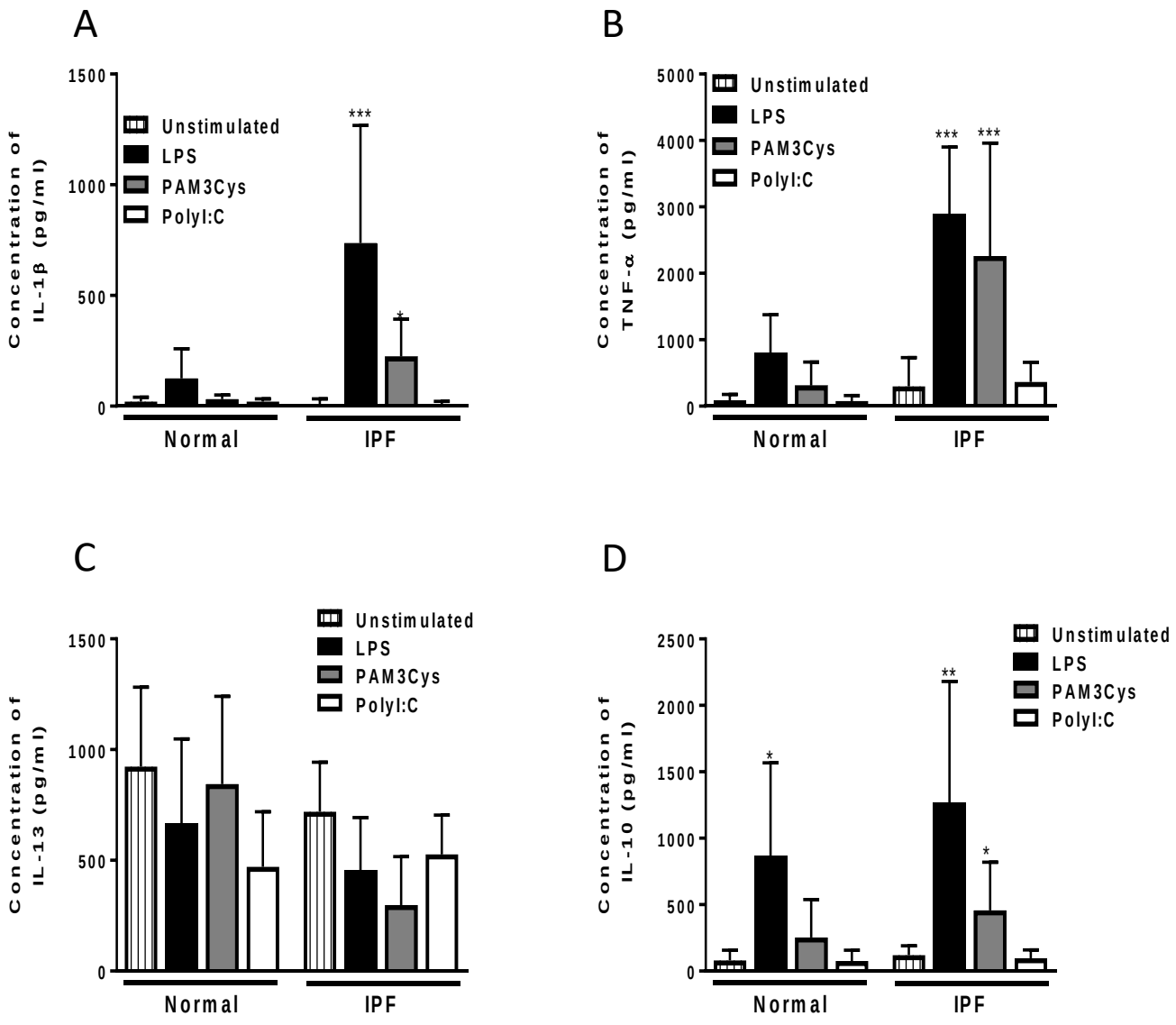


Figure 5: Stimulation of AMs from IPF patients with TLR ligands resulted in elevated expression of pro and anti-inflammatory cytokines

Alveolar macrophages were isolated from IPF patients and normal, donor controls and stimulated with LPS (TLR4 agonist), Pam3Cys (TLR 3 agonist) and PolyI:C (TLR1/2 agonist) for 24 hrs. Supernatants were collected from the cells and amount of protein was determined by specific ELISA. A-D. *p<0.05, **p<0.01, ***p<0.001 when compared to disease specific unstimulated

A

B

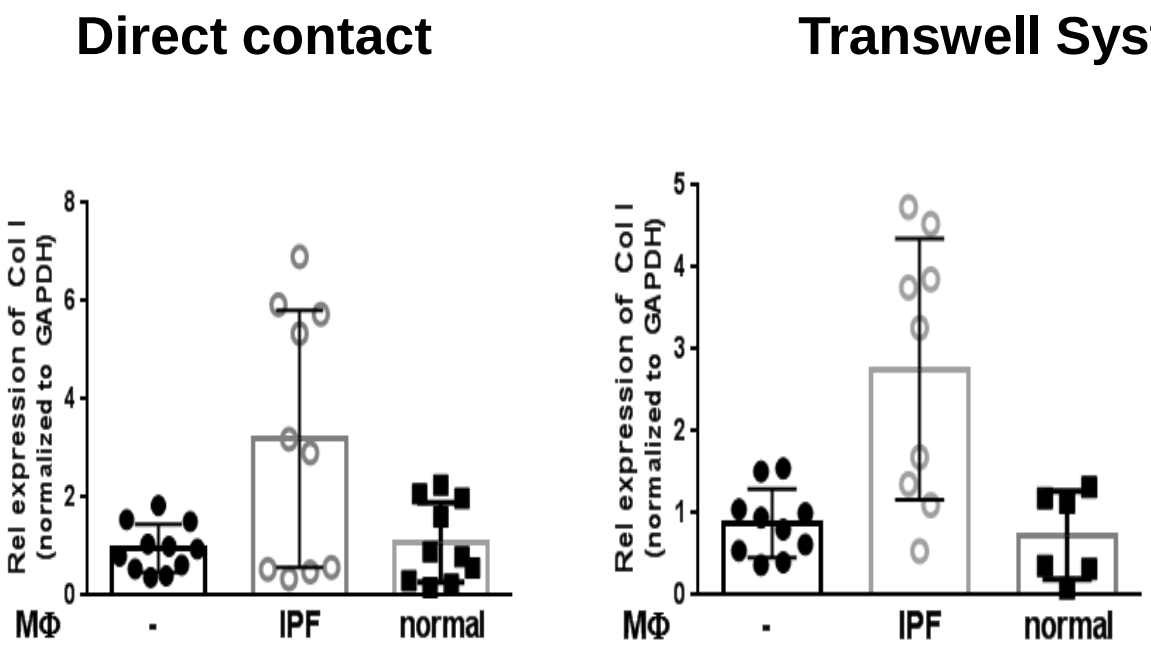


Figure 6: Co-culturing fibroblasts with healthy and IPF human alveolar macrophages results in decreased Collagen I
Human lung fibroblasts from both IPF patients and normal donor controls were cultured with human alveolar macrophages isolated from IPF patients and normal donor controls either directly on top of each other, or in trans-well plates. mRNA harvested from cells was assessed by qPCR. Expression is shown compared to fibroblast alone culture conditions. (n=2-4/sample) *p<0.05**p<0.01, ***p<0.001