Lactic acid is elevated in IPF and induces myofibroblast differentiation via pH dependent activation of TGF- ß.

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ONLINE SUPPLEMENT

Supplemental Figure Legends

Suppl. Figure 1: LDH5 expression was measured by Western blot on protein lysates from fibroblasts isolated from healthy patients and patients with IPF (Figure 1A) and on whole lung lysates from patients with IPF and healthy controls (Figure 1B). Representative western blot's (n=3 each) are shown and correlate to the analysis shown in Figure 2A & B.

Suppl. Figure 2: As shown in Figure 3 A &B, primary human lung fibroblasts were cultured with 1, 10 and 20 mM lactic acid for 72 hours. Representative Western blot analysis of protein lysates performed for markers of myofibroblast differentiation, (A) αSMA and (B) calponin are shown here.

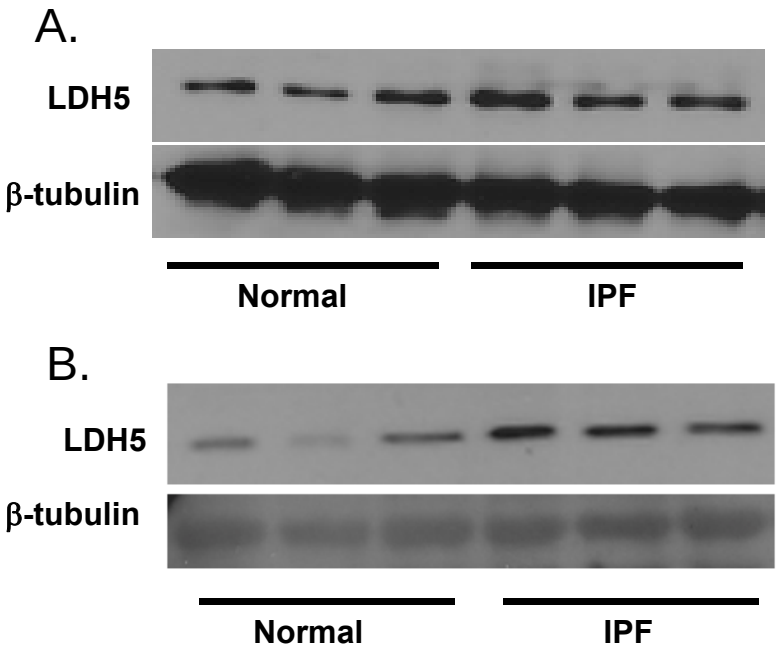
Suppl. Figure 3: As shown in Figure 4 A, human lung fibroblasts were cultured with TGF-β, media containing lactic acid that pH was adjusted after the addition of lactic acid, or media containing lactic acid that was pH adjusted prior to its addition to the media (n=3 each). Myofibroblast differentiation was assessed by Western blot for expression of αSMA. As shown in Figure 4B, human lung fibroblasts were cultured in serum free media (SF) and media containing 1%, 5% and 10% fetal bovine serum (n=3 each). Protein lysates were analyzed by Western blot for αSMA (* ANOVA $p < 0.05$ compared to untreated). Representative Western blots for Figure 4A and B are shown here.

Suppl. Figure 4: As shown in Figure 7, primary human lung fibroblasts were treated with either a HIF1α overexpressing plasmid or a dominant negative HIF1a plasmid.

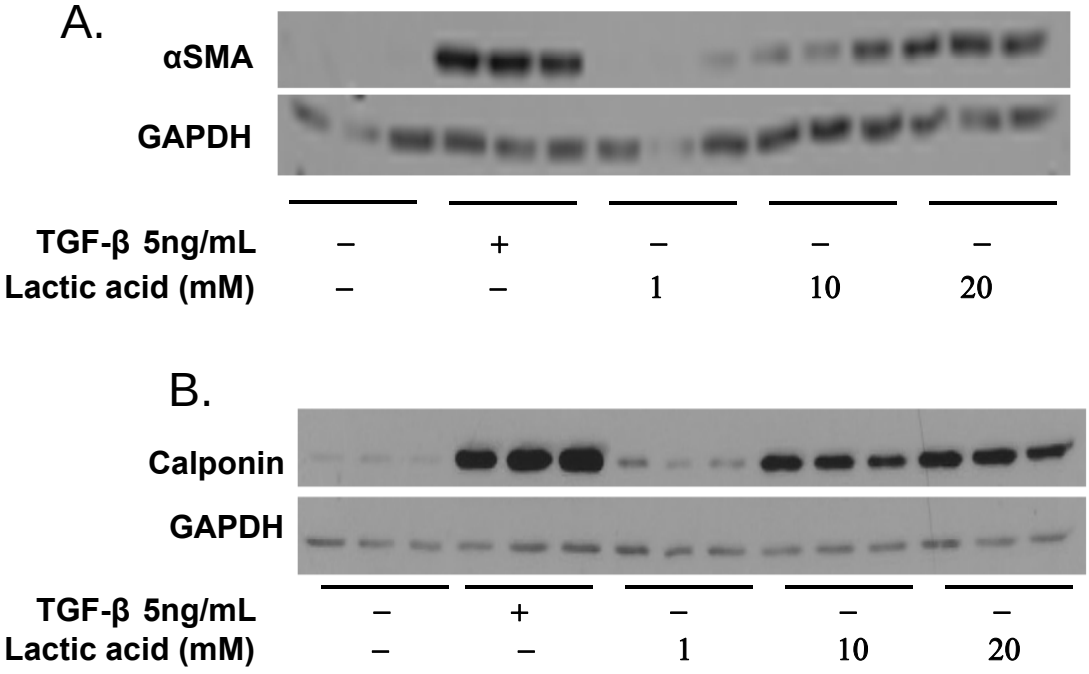
Expression of HIF1α was assessed by Western blot. Representative Western blot data demonstrating (A) HIF1α over-expression and (B) DN-HIF1α overexpression are shown here. Subsequently, human lung fibroblasts were transfected with either the HIF1α over-expressing plasmid or the DN-HIF1α plasmid and treated with or without 1ng/mL of TGF-β. Protein lysates were run, transferred, probed and developed simultaneously onWestern blots for expression of LDH5 and αSMA. Representative Western blots are shown here (C & D).

Supplemental Figures

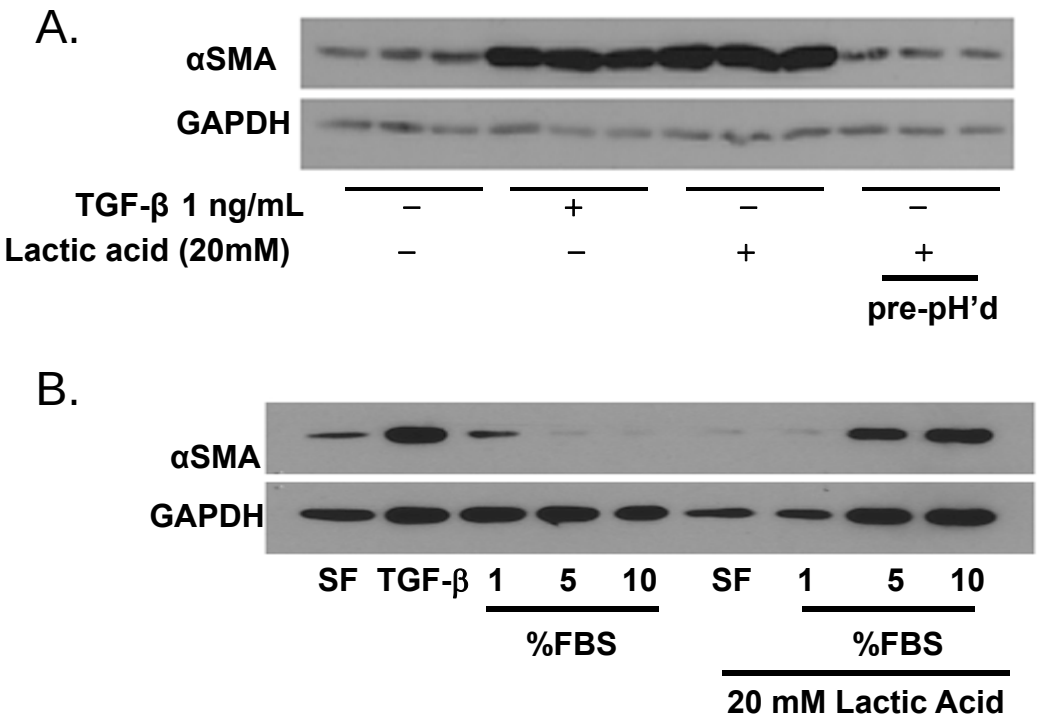
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4