

Supplementary tables:

Table 1. TaqMan primers and probes for Real-time Quantitative PCR analysis.

Gene	TaqMan primers and probes
18S	Forward: CGGCTACCACATCCAAGGA
	Reverse: CCAATTACAGGGCTCGAAA
	Probe: CGCGCAAATTACCCACTCCCGA
COL1A1	Forward: GGCCAGAAAGAACTGGTACATC
	Reverse: CCGCCATACTCGAACTGGAA
	Probes: CCCCAAGGACAAGAGGCATGTCTG
ACTA2	Forward: GGGACGACATGGAAAAGATCTG
	Reverse: CAGGGTGGGATGCTCTTCA
	Probe: CACTCTTCTACAATGAGCTTCGTGTGCC

Table 2. SYBR green primer sequences used for Real-time Quantitative PCR Analysis.

Gene	SYBR green primers
<u>COL3A1</u>	Forward: CTGGACCCAGGGTCTTC
	Reverse: CATCTGATCCAGGGTTCCA
<u>COL4A1</u>	Forward: AGGAGAGAAGGGCGCTGT
	Reverse: TCCAGGTAAGCCGTCAACA
<u>COL6A1</u>	Forward: GAAGAGAAGGCCCCGTTG
	Reverse: CGGTAGCCTTATAGGTCGGATA
<u>FN1</u>	Forward: CTGGCCGAAAATACATGTAAA
	Reverse: CCACAGTCGGGTCAGGAG
<u>ELN</u>	Forward: CGGGAGTAGTTGGTGTCCC
	Reverse: AGCTGCTTCTGGTGACACAA

Table 3. Antibodies catalog numbers and dilutions.

Antibody	Cat no. (dilution)	Company
GAPDH	CB1001 (1:1000)	Calbiochem
Phospho-mTOR	2971 (1:1000)	Cell Signaling
mTOR	2983T (1:1000)	Cell Signaling
Phospho-p70S6K Thr389	9205 (1:1000)	Cell Signaling
p70S6K	9202S (1:1000)	Cell Signaling
Phospho-4E-BP1 Ser65	9451 (1:1000)	Cell Signaling
4E-BP1	9644T (1:1000)	Cell Signaling
<u>Phospho-Smad2 (S465/467)/3 (S423/425)</u>	<u>8828 (1:1000)</u>	<u>Cell Signaling</u>
<u>Smad2/3</u>	<u>3120 (1:1000)</u>	<u>Cell Signaling</u>
<u>Phospho-p38 MAPK</u>	<u>9211 (1:1000)</u>	<u>Cell Signaling</u>
<u>p38 MAPK</u>	<u>9212 (1:1000)</u>	<u>Cell Signaling</u>
Polyclonal Rabbit Anti-Mouse Immunoglobulins	P0260 (1:2000)	DAKO
Polyclonal Rabbit Anti-Goat Immunoglobulins	P0160 (1:2000)	DAKO
Polyclonal Goat Anti-Rabbit Immunoglobulins	P0448 (1:2000)	DAKO

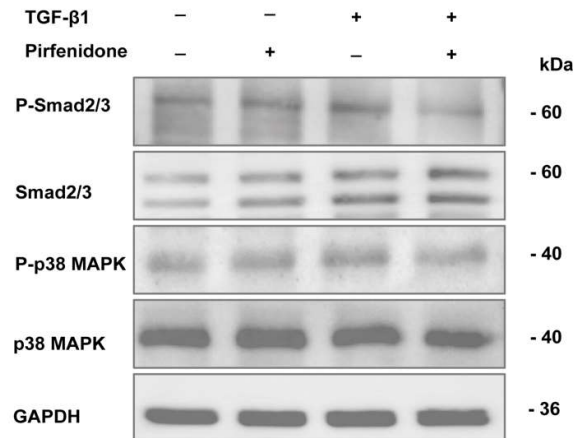
Supplementary Figure:

Figure 1. Pirfenidone does not suppress Smad2/3 and p38 MAPK phosphorylation in p-hIFs. (A) p-hIFs were exposed to pirfenidone (1 mg/ml) and/or, TGF- β 1 (2.5 ng/ml) for 6 h. Total and phosphorylated Smad2/3 and p38 MAPK were assessed by Western blot analysis. Compared with the control group, TGF- β 1 or pirfenidone did not pronouncedly change levels of phosphorylated Smad2/3 or p38 MAPK in p-hIFs. GAPDH is included as protein loading control.

Supplementary References for Figure 6:

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3. Li G.; Ren J.; Hu Q.; et al. Oral pirfenidone protects against fibrosis by inhibiting fibroblast proliferation and TGF-beta signaling in a murine colitis model. *Biochem. Pharmacol.* **2016**;117, 57–67.
4. Sun Y.W.; Zhang Y.Y.; Ke X.J.; Wu X.J.; Chen Z.F.; Chi P. Pirfenidone prevents radiation-induced intestinal fibrosis in rats by inhibiting fibroblast proliferation and differentiation and suppressing the TGF-beta1/Smad/CTGF signaling pathway. *Eur. J. Pharmacol.* **2018**, *822*, 199–206.
5. Li Z.; Liu X.; Wang B.; et al. Pirfenidone suppresses MAPK signalling pathway to reverse epithelial-mesenchymal transition and renal fibrosis. *Nephrology* **2017**;22, 589–597.
6. Ji X.; Naito Y.; Weng H.; et al. Renoprotective mechanisms of pirfenidone in hypertension-induced renal injury: Through anti-fibrotic and anti-oxidative stress pathways. *Biomed. Res.* **2013**, *34*, 309–319.
7. Conte E.; Gili E.; Fagone E.; Fruciano M.; Iemmolo M.; Vancheri C. Effect of pirfenidone on proliferation, TGF-beta-induced myofibroblast differentiation and fibrogenic activity of primary human lung fibroblasts. *Eur. J. Pharm. Sci.* **2014**, *58*, 13–19.
8. Boehme S.A.; Franz-Bacon K.; DiTirro D.N.; Ly T.W.; Bacon K.B. MAP3K19 Is a Novel Regulator of TGF-beta Signaling That Impacts Bleomycin-Induced Lung Injury and Pulmonary Fibrosis. *PLoS One* **2016**, *11*, doi:10.1371/journal.pone.0154874
9. Kurita Y.; Araya J.; Minagawa S.; et al. Pirfenidone inhibits myofibroblast differentiation and lung fibrosis development during insufficient mitophagy. *Respir. Res.* **2017**, *18*, 114.
10. Molina-Molina M.; Machahua-Huamani C.; Vicens-Zygmunt V.; et al. Anti-fibrotic effects of pirfenidone and rapamycin in primary IPF fibroblasts and human alveolar epithelial cells. *BMC Pulm. Med.* **2018**, *18*, 63.