Arsenic trioxide inhibits the functions of lung fibroblasts derived from patients with
idiopathic pulmonary fibrosis.

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Supplemental Figure 1: ATO does not modulate the viability of IPF and control HLFs stimulated with PDGF-BB. IPF and control HLFs were treated with 0.01-1 μ M ATO for 72 h (A-B) or with 0.01-1 μ M ATO for 24 h and then stimulated with 50 ng/ml PDGF-BB for 72 h (C-D). Cell viability was determined by fluorescent acridine orange/propidium iodide staining and automated fluorescence cell counting. Results are expressed as means ± SEM of 6 (A, B, D) and 8 (C) independent experiments. Statistical analysis was determined ANOVA followed by Dunnett's multiple comparison *t*-test. No significant differences were detected.



27	Supplemental Figure 2: ATO alters cell cycle distribution in control HLFs. Control HLFs
28	were treated with 1 μM ATO for 24 h and then stimulated with 50 ng/ml PDGF-BB for 24 h
29	(C-D) or 72 h (A-B). Cells were washed, fixed and stained with propidium iodide. A.
30	Representative graphs saved during flow cytometry analysis. Values for each phase of the cell
31	cycle are expressed as percentages of total control HLFs (B). C. Representative Western blot
32	showing the cell cycle-related proteins cyclin D1, CDK4, cyclin A, CDK2, cyclin B and
33	CDK1 in control HLFs. Proteins were quantified by densitometry and then normalized to the
34	GAPDH (D). The fold change in protein expression measured in treated and stimulated cells
35	was determined by arbitrarily setting at 1 the protein expression measured in untreated cells.
36	B , D . Results are expressed as means \pm SEM of 4 (B) and 5-6 (D) independent experiments.
37	Significant differences were determined by Student's <i>t</i> -test (\$ p<0.05).



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Supplemental Figure 3: ATO decreases TGF-*β*1-induced ACTA2 and COL1A1 mRNA in 42 IPF and control HLFs. IPF (A, B) and control HLFs (C,D) were treated with 0.01-1 µM 43 ATO for 24 h and then stimulated with 1 ng/ml TGF-B1 for an additional 24 h. Relative 44 ACTA2 and COL1A1 mRNA levels were measured by quantitative RT-PCR and normalized 45 to that of endogenous ribosomal 18S RNA. The fold change in mRNA concentrations 46 47 measured in treated and/or stimulated HLFs was determined by arbitrarily setting at 1 mRNA concentrations measured in unstimulated HLFs. Results are expressed as means \pm SEM of 5 48 independent experiments. Significant differences were determined by Student's t-test (\$ 49 p<0.05; \$\$ p<0.01), or by ANOVA followed by Dunnett's Multiple comparison *t*-test ** 50 p<0.01; *** p<0.001). 51





Supplemental Figure 4: Effects of ATO on basal expression of Collagen-1 (COL1) and 53 α -SMA in IPF control HLFs. A, C. Representative Western blot of COL1 and α -SMA in 54 IPF (A) and control (C) HLFs treated with 0.01-1 µM ATO for 48 h. B, D. Proteins were 55 quantified by densitometry and then normalized to the GAPDH. The fold change in protein 56 57 expression measured in treated cells was determined by arbitrarily setting at 1 the protein expression measured in untreated cells. Results are expressed as means ± SEM of 5 58 independent experiments. Significant differences were determined by ANOVA followed by 59 Dunnett's multiple comparison *t*-test (* p<0.05; *** p<0.001). 60



Supplemental Figure 5: ATO induces the expression of NRF2 and antioxidant proteins 62 in control HLFs. A, C, E. Representative Western blot of NRF2, HO-1 or NQO-1 in control 63 HLFs treated with 0.1-0.5 µM ATO for the indicated time (A) or for 24 h (C, E) and then 64 stimulated with 1 ng/ml TGF-\beta1 for an additional 24 h (E). **B**, **D**, **F**. Proteins were quantified 65 by densitometry and then normalized to GAPDH. The fold change in protein expression 66 measured in treated and stimulated cells was determined by arbitrarily setting at 1 the protein 67 expression measured in untreated cells. Results are expressed as means ± SEM of 3 (B), 5-6 68 69 (D) and 5-6 (E) independent experiments. Significant differences were determined by ANOVA followed by Dunnett's multiple comparison t-test (* p<0.05; ** p<0.01). 70