Supplementary Figure 1. SIRT3 negatively regulates fibrotic responses in skin fibroblasts. Normal human skin fibroblasts were infected with SIRT3 adenovirus (10 MOI or as indicated), and incubated with TGF-β2 (10 ng/ml) for 24 h. A. qRT-PCR. Results normalized with GAPDH are means ± SD of triplicate determinations of an experiment representative of three. *p<0.05. B. Western immunoblot of whole cell lysates. Images representative of duplicate experiments. -fold change in band intensities normalized for tubulin in each lane shown below. MOI, multiplicity of infection. Cgn I, Type I collagen.
Supplementary Figure 2. Hexafluoro abrogates basal and stimulated fibrotic gene expression in skin fibroblasts. Human skin fibroblasts were preincubated with hexafluoro (10 µM) for 30 min prior to TGF-β (10 ng/ml) for 24 h, or hexafluoro was added to the cultures 24 h after TGF-β (E). A. Western analysis. Representative images. -fold change in protein levels shown below. B, E. Real-time qPCR. Results are means + SD of triplicate determinations from an experiment representative of three; *p<0.05. C. Left panel, real-time qPCR results normalized with GAPDH. Means ± SD of triplicate determinations. *p<0.05. Right panels, Western analysis of whole cell lysates. Representative images. Cgn I, Type I collagen. D. Immunofluorescence confocal microscopy with antibodies to αSMA. Nuclei identified with DAPI. Representative images; bar =10 µm.
Supplementary Figure 3. Isotype controls in skin and lung biopsies. Isotype control IgG was used to replace primary Ac-MnSOD antibody; left, representative IF images in skin biopsies; right, IHC images of lung biopsies.