ONLINE SUPPLEMENT

LTB_4 activates pulmonary artery adventitial fibroblasts in pulmonary hypertension.

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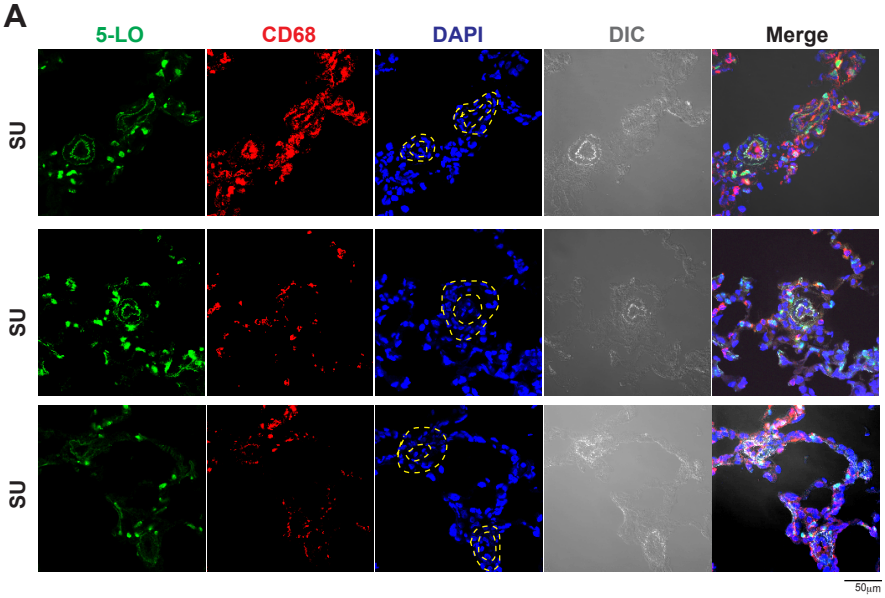
Supplemental Figure

A



B

	5-LO+ CD68+ (%)
Around the Adventitia	70±10
Outside the Adventitia	5±2

Figure S1. 5-LO positive macrophages are concentrated around the adventitial compartment.
A, Representative immunofluorescence images of lung sections stained with 5-LO (green) and CD68 (red) from SU treated animals. B, 5-LO+ and CD68+ double positive cells were counted and grouped as cell around the adventitia or cells outside of the adventitia. n= 5; DAPI (blue) stains nuclei. Data are expressed as means ± SEM.

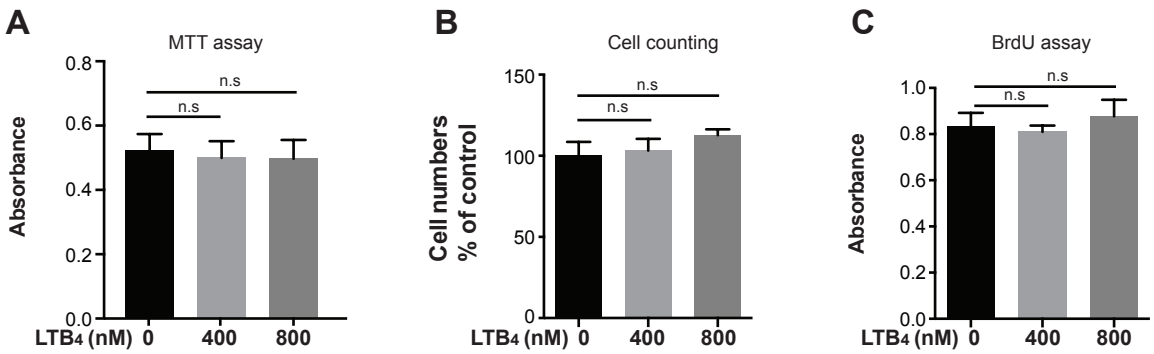


Figure S2. LTB4 has no effect on proliferation in human lung fibroblasts (HLF).
HLF were treated with LTB_4 for 72hrs. Proliferation of HLF was measured by A. MTT assay, B, cell counting, C, BrdU assay. Data are presented as mean ± SEM. (n.s = non-significant) The experiments were repeated three times.

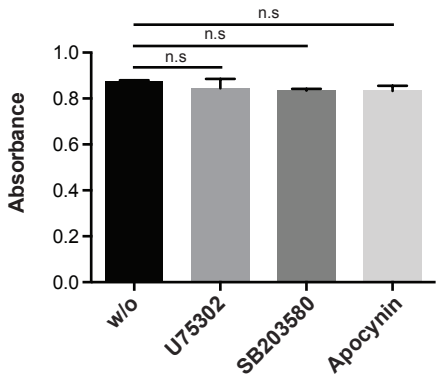


Figure S3. Inhibitors have no toxicity on HPAAF.
MTT assay were performed after 72 hrs with treatment of U75302 (1μM), SB203580 (10μM) or apocynin (300μM) in HPAAF. Data are presented as mean ± SEM. (n.s = non-significant) The experiments were repeated three times.

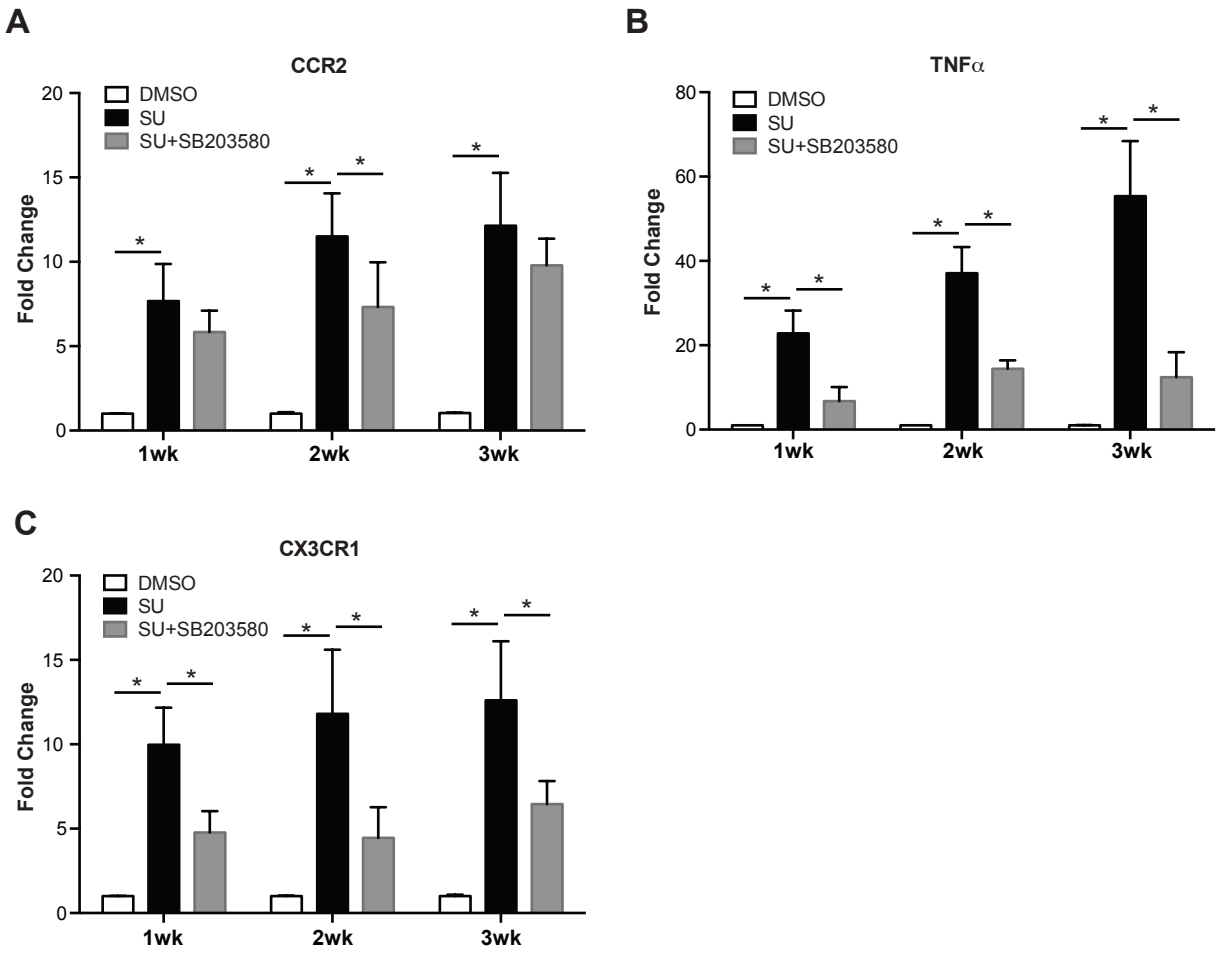


Figure S4. p38 MAPK inhibition treatment with SB203580 reduces lung inflammation in experimental PH.
The macrophage-associated cytokine TNF-α and chemokines, CXCR1 and CCR2 in PH were evaluated by RT-PCR of lung tissues (n=3 experiments per group). Data are expressed as means ± SEM. (*: $p<0.05$)

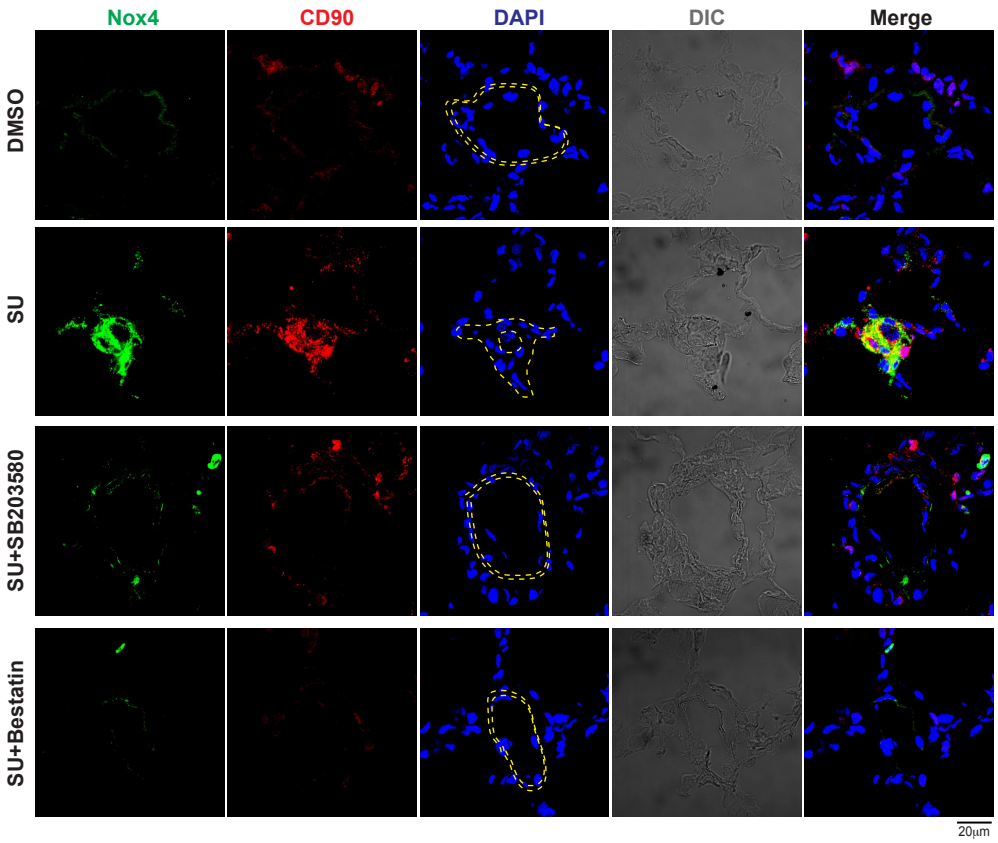


Figure S5. p38 MAPK inhibition therapy reduced Nox4 activation around the pulmonary vascular adventitia in experimental PH.
Confocal images of rat lung tissues stained with Nox4 (green), CD90 (fibroblast,red) and 5-LO (magenta) from SB203580 treatment group; n=5. DAPI (blue) stains nuclei; DIC highlights alveolar and vascular structures.