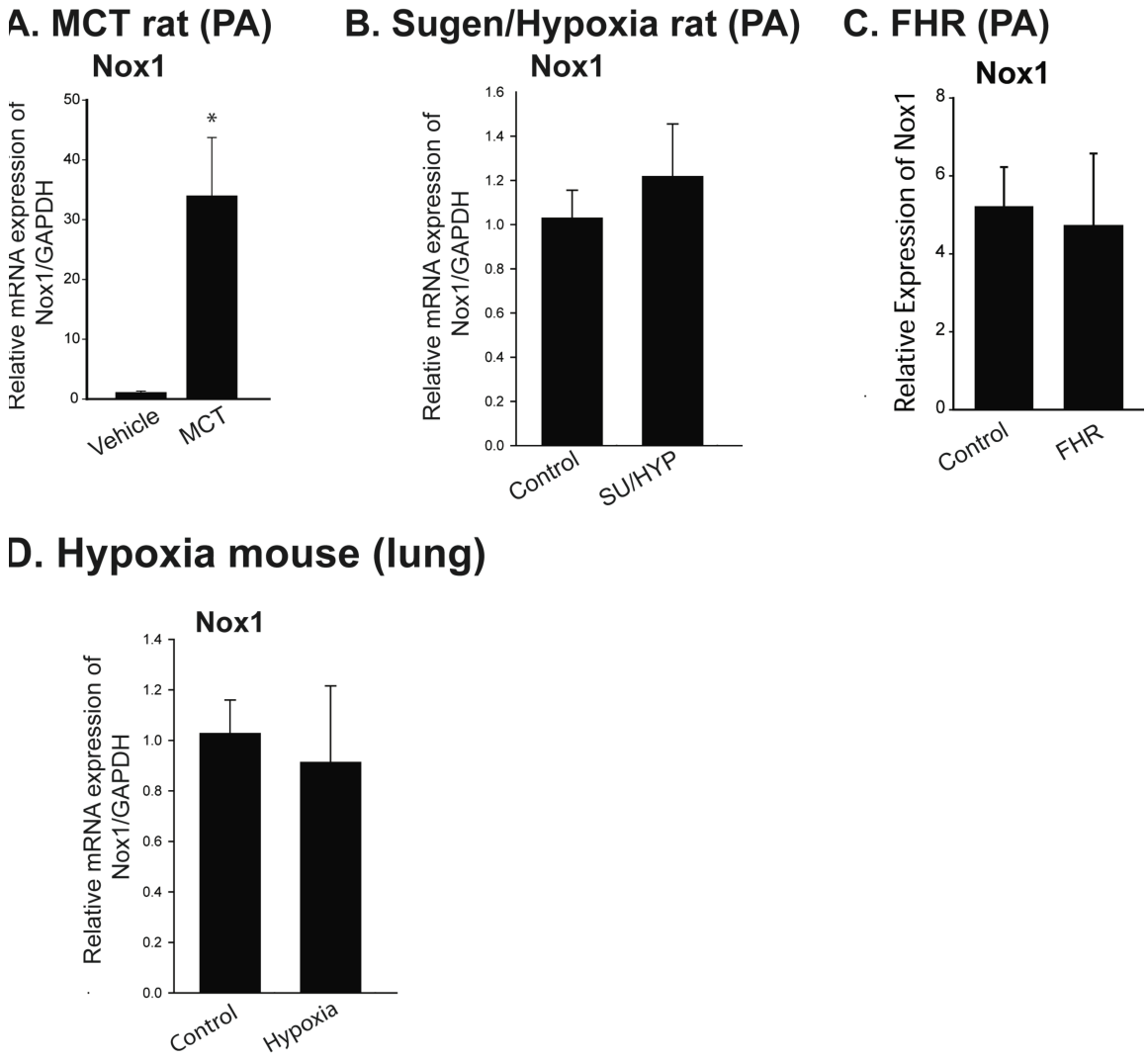
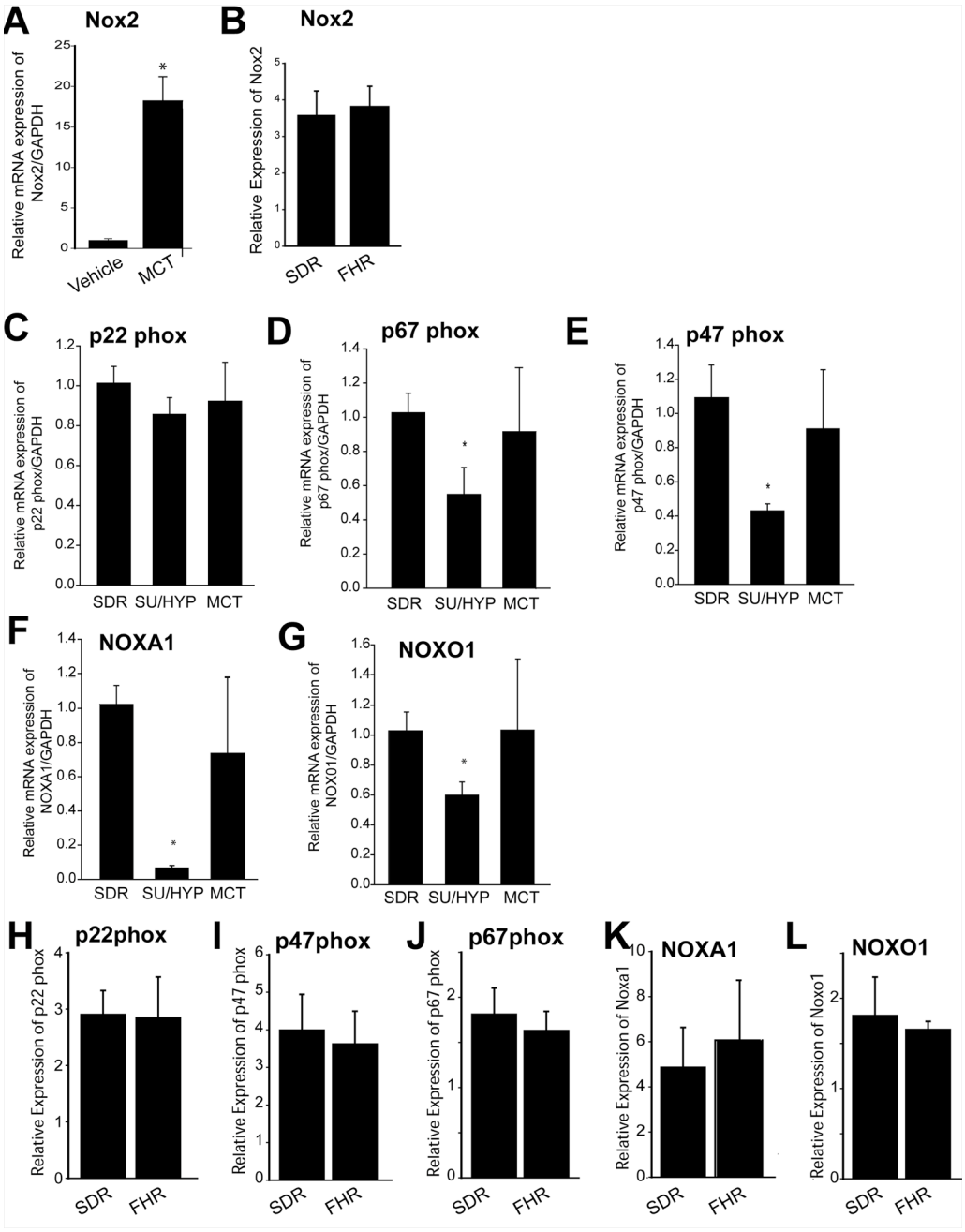
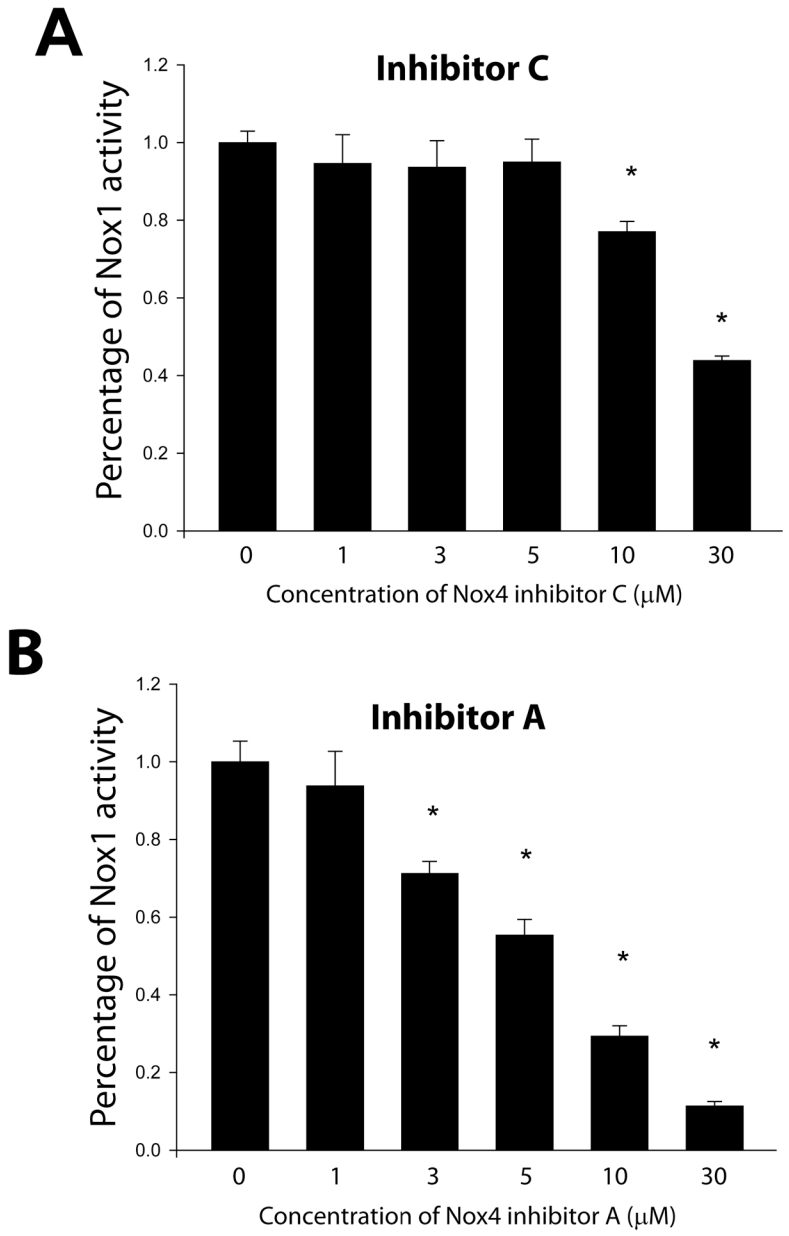
SUPPLEMENTAL MATERIAL



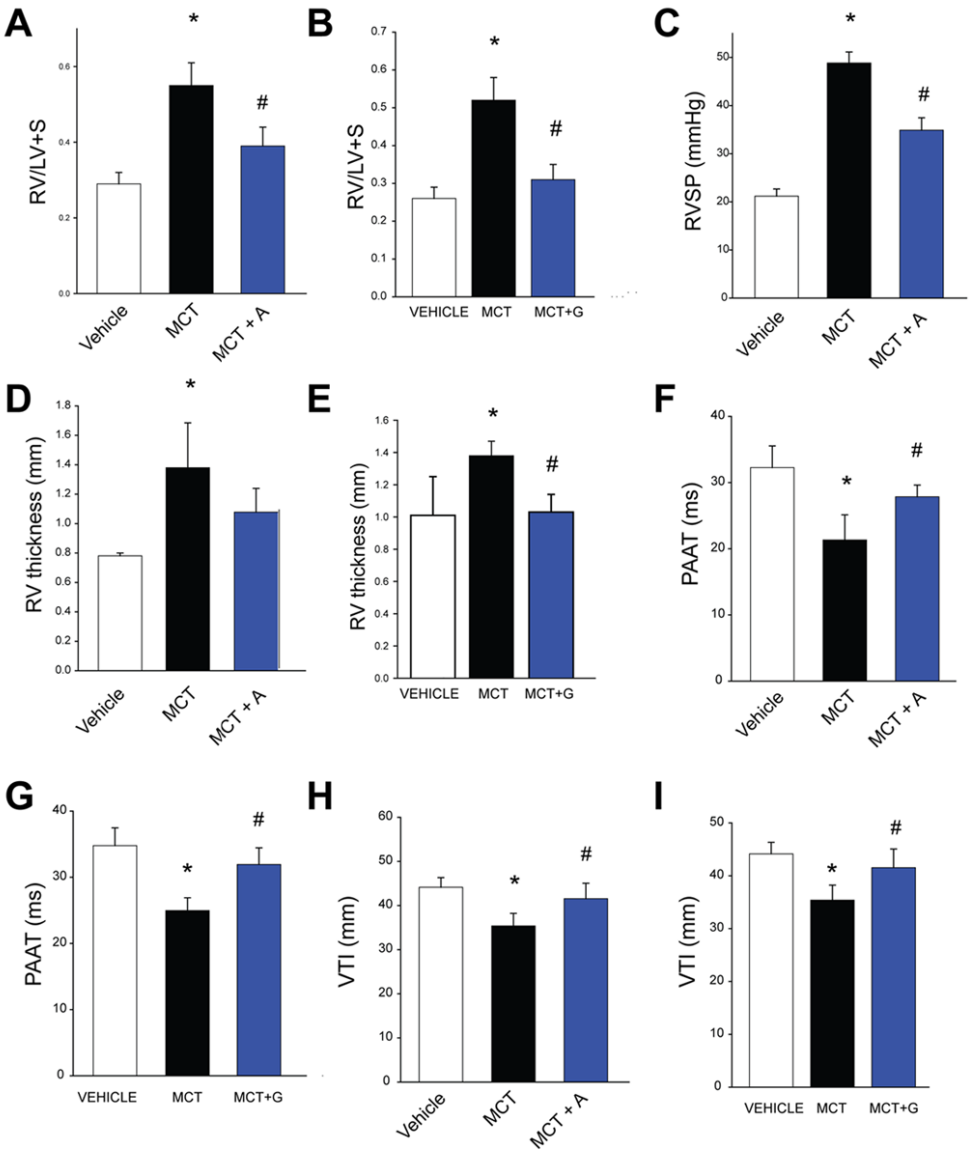
Supplemental Figure I. Nox1 expression is increased only in MCT-treated rat hypertensive pulmonary arteries (PA). Nox1 mRNA (measured by qRT-PCR ($\Delta\Delta$Ct) normalized to GAPDH) is significantly increased in PA from 4week MCT-treated rats (**A**). In contrast, Nox1 expression is not increased in PA from SU/HYP treated rats (14week; **B**) or FHR (24week; **C**) relative to control or in hypoxic mouse lung (3week; **D**). * Significantly different from Vehicle, $p < 0.05$ (n = 4-5).



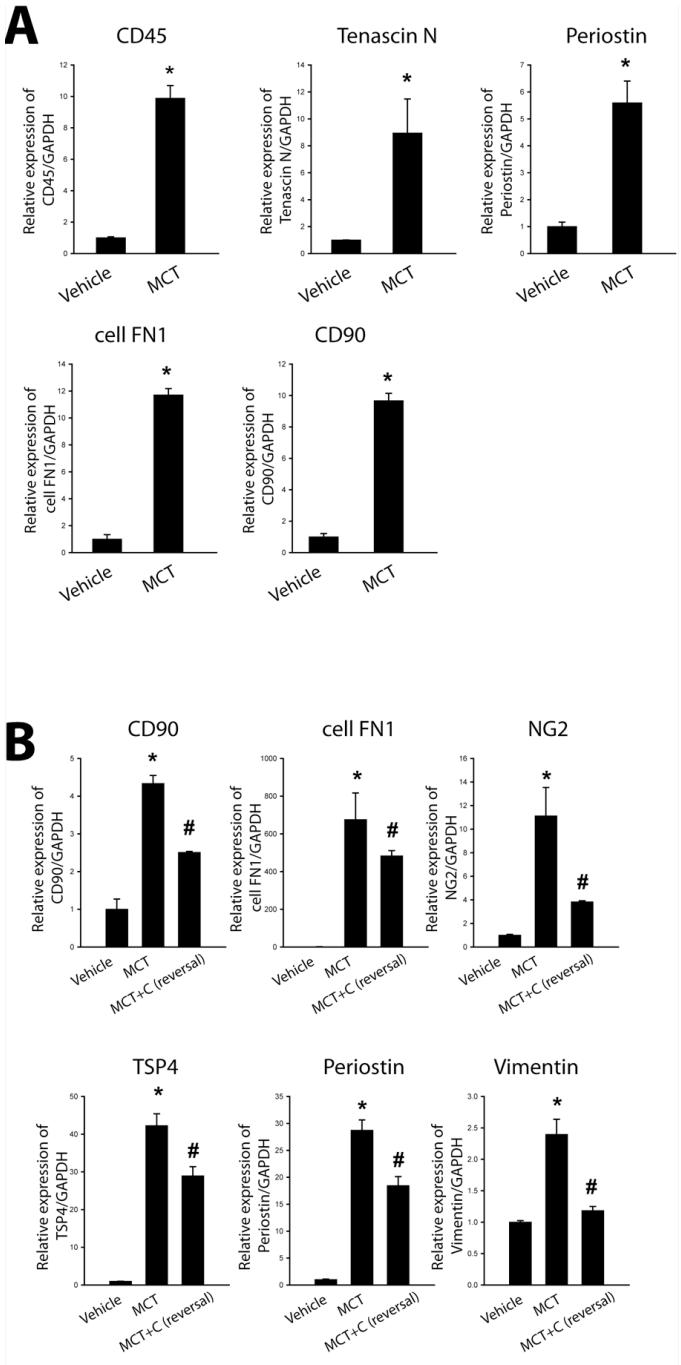
Supplemental Figure II. Relative expression of Nox2 and Nox subunits in isolated pulmonary arteries (PA) from rats with pulmonary hypertension. Nox2 mRNA (measured by qRT-PCR ($\Delta\Delta$Ct) normalized to GAPDH) is significantly increased in PA from MCT-treated rats (4week; **A**) and unchanged in FHR rats (24 week; **B**). Expression levels of p22phox (**C**), p67phox (**D**), and p67phox (**E**) and NOXA1 (**F**) and NOXO1 (**G**) in PA from normotensive (SDR), SUGEN/hypoxia (SU/HYP; 14week) and MCT-treated (4week) hypertensive rats. Expression levels of p22phox (**H**), p47phox (**I**), and p67phox (**J**) and NOXA1 (**K**) and NOXO1 (**L**) in PA from normotensive (SDR) and 24 week FHR. * Significantly different from SDR/ vehicle controls, $p < 0.05$ (n = 5-6).



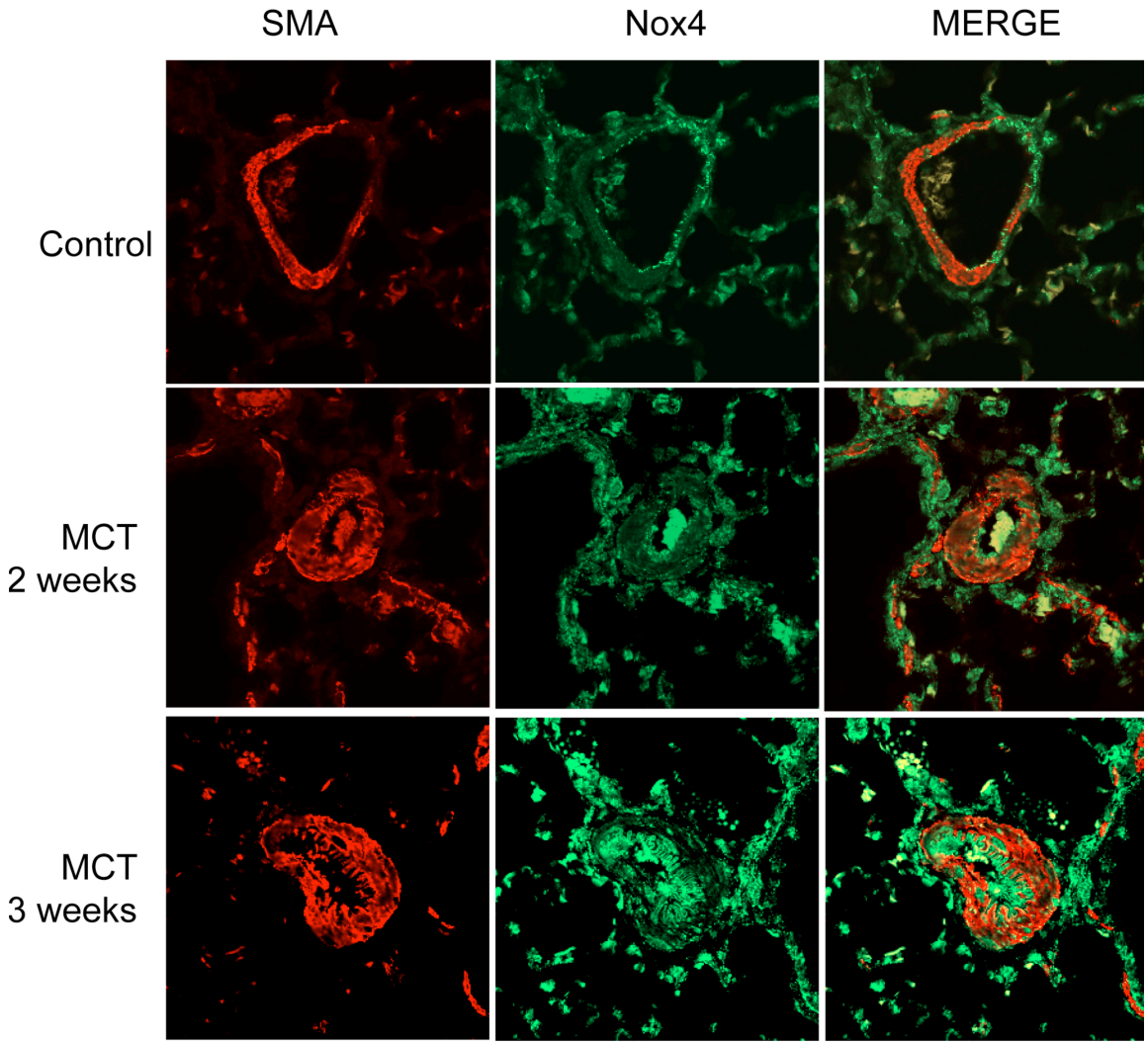
Supplemental Figure III. Relative potency of Nox4 inhibitor A (VCC588646) and C (VCC202273) against ROS production in cells expression Nox1. HEK293 cells expressing Nox1, NOXO1, NOXA1 were exposed to the indicated concentrations of inhibitor VCC202273 (C) and VCC588646 (A) (**A-B**) for 1h and the Nox1-dependent production of reactive oxygen species determined by L-012 chemiluminescence. * Significantly different from Vehicle $p < 0.05$ ($n = 7$).



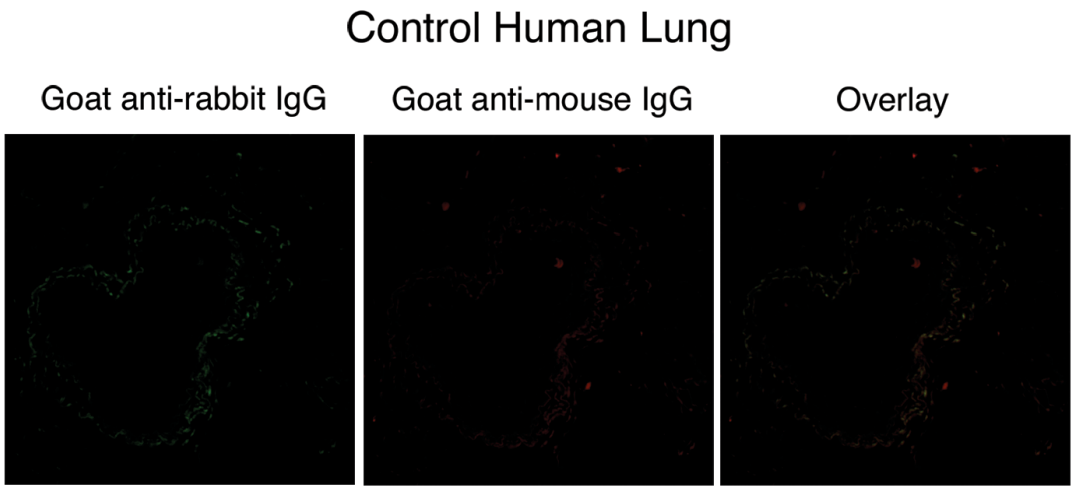
Supplemental Figure IV. Effect of Nox4 inhibition on cardiac remodeling and indices of cardiopulmonary function in MCT-treated rats. MCT-treatment significantly increases right ventricular (RV) hypertrophy as measured by the increased Fulton Index (RV/LV+S; **A-B**), right ventricular systolic pressure (RVSP; **C**) and right ventricle (RV) thickness (**D-E**). These variables are decreased in rats treated with the Nox4 inhibitors VCC588646 (A) (**A, C, D**) and GKT136901 (G) (**B, E**). MCT-treatment significantly decreased pulmonary artery acceleration time (PAAT; **F, G**), velocity time integral (VTI; **H, I**) and these were increased with Nox4 inhibitor VCC588646 (A) and also with GKT136901 (G) (**F-I**). * Significantly different from Vehicle, # significantly different from MCT, $p < 0.05$ (n = 5-6).



Supplemental Figure V. Densitometric analysis of changes in gene expression in hypertensive PA and reversal by Nox4 inhibition. Analysis of selected markers of inflammatory cells and fibroblasts in PA isolated from control and hypertensive (MCT-treated) rats (CD45, CD90, cellFN1, periostin, tenascin N, and vimentin) **(A)**. Expression of markers of fibroblasts in PA isolated from MCT-treated rats in the presence and absence of the Nox4 inhibitor VCC202273 (C) in a reversal protocol (see Methods) (**B**). (cell FN1 = cellular fibronectin, TSP4 = thrombospondin-4; NG2 = chondroitin sulfate proteoglycan/ Cspg4) * Significantly different from Vehicle, # significantly different from MCT, $p < 0.05$ (n = 3-4).



Supplemental Figure VI. Time course of Nox4 expression in PA from MCT-treated rats. Confocal images of lung sections from control, and experimental PH (2-week; 3-week MCT). Sections were stained with Nox4 and α-actin antibodies (SMA).



Supplemental Figure VII. Confocal imaging of IgG staining of human lung sections. Sections were stained first with non-immune rabbit IgG and non-immune mouse IgG and then with goat anti-rabbit IgG and goat anti-mouse IgG.