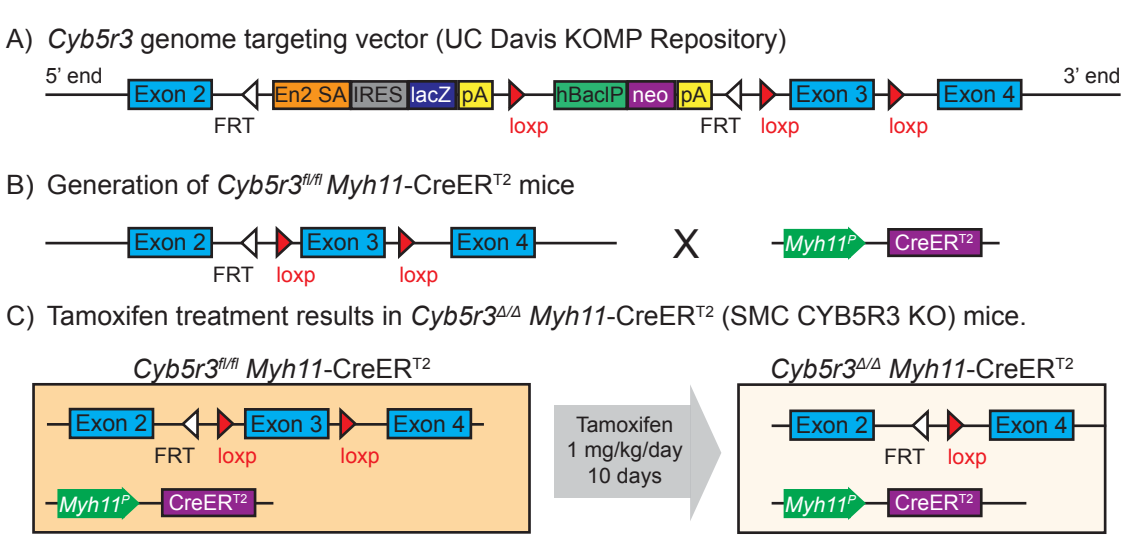
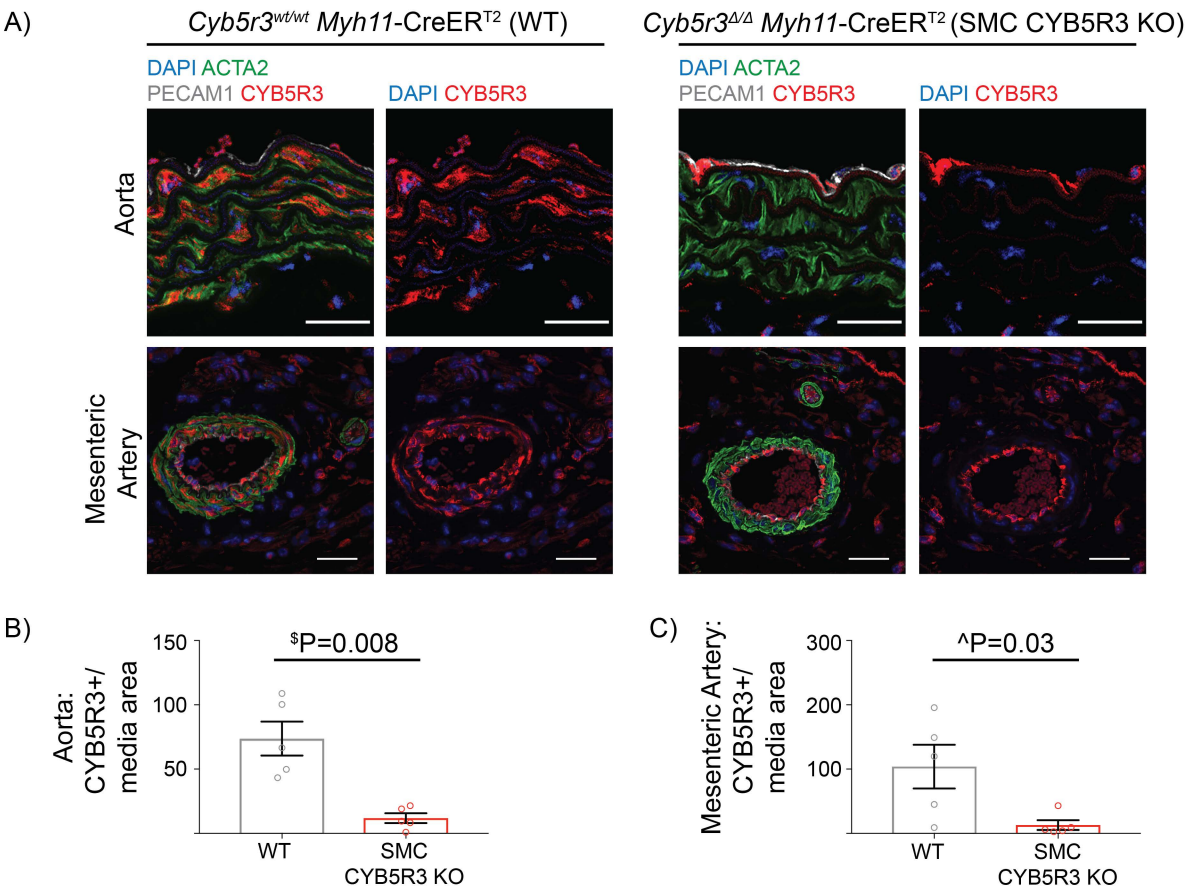
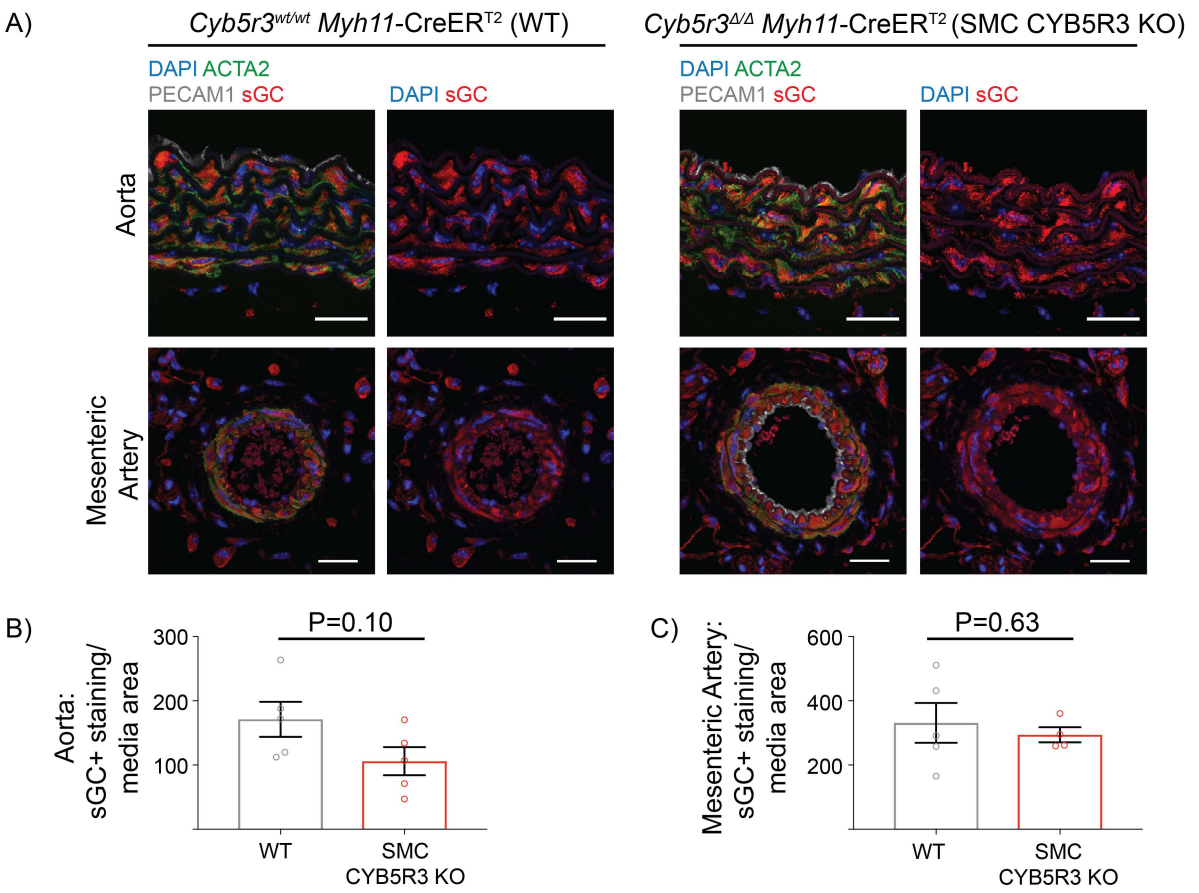


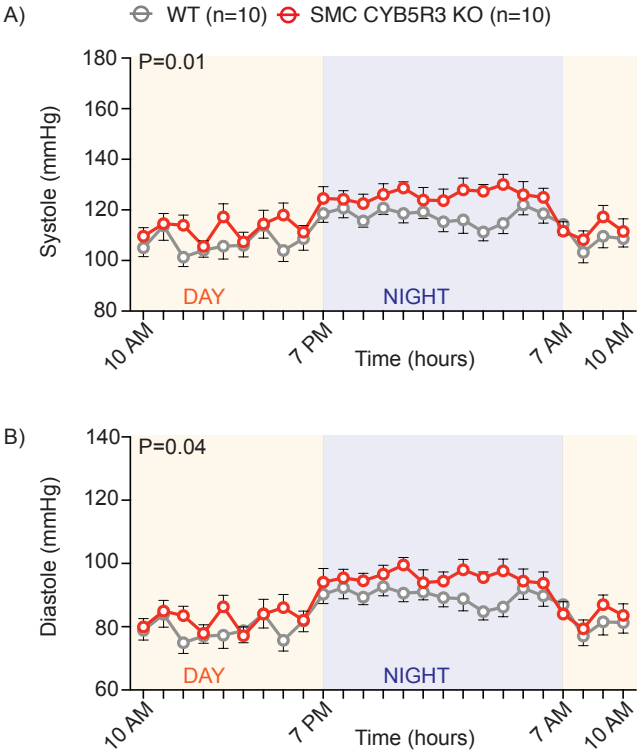
Supplemental Figure 1: Generation of *Cyb5r3*$^{fl/fl}$ *Myh11*-CreER^{T2} (SMC CYB5R3 KO) mice. A) Schematic of genome targeting vector for *Cyb5r3* generated by the University of California (UC) Davis Knockout Mouse Project Repository (KOMP). Chimeras containing this targeting vector were crossed with FLPe recombinase mice to form *Cyb5r3*$^{fl/fl}$ mice. B) Schematic of *Cyb5r3*$^{fl/fl}$ mice which were crossed with *Myh11*-CreER^{T2} mice to create *Cyb5r3*$^{fl/fl}$ *Myh11*-CreER^{T2} mice. C) Tamoxifen treatment of *Cyb5r3*$^{fl/fl}$ *Myh11*-CreER^{T2} mice results in excision of exon 3 of *Cyb5r3* solely in MYH11 expressing cells resulting in SMC-specific *Cyb5r3* knockout.



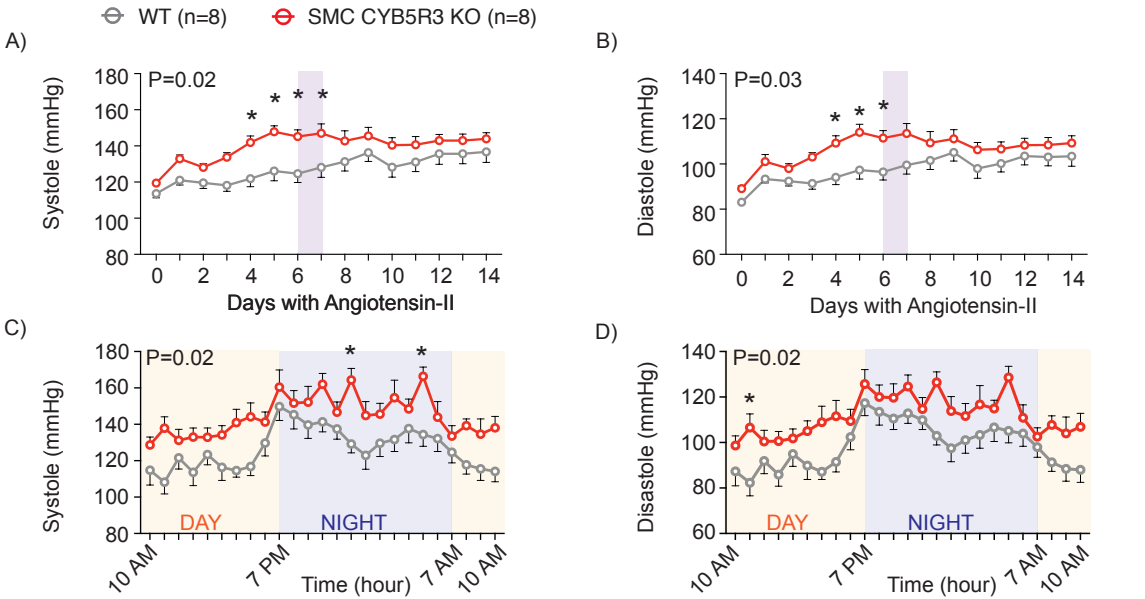
Supplemental Figure 2: Validation of SMC-specific CYB5R3 knockout. A) Representative Images of aorta and mesenteric arteries of non-diseased *Cyb5r3*$^{wt/wt}$*Myh11*-CreER^{T2} (WT, n=5) and *Cyb5r3*$^{\Delta/\Delta}$ *Myh11*-CreER^{T2} (SMC CYB5R3 KO, n=5) mice post-tamoxifen treatment stained for CYB5R3 (red), PECAM1 (endothelial cells, gray), ACTA2 (SMC, green), and DAPI (nuclei, blue). B-C) SMC CYB5R3 KO (n=5) mice show a significant loss of CYB5R3 expression the media of the aorta and mesenteric arteries compared to WT. $P value via unpaired two-tailed *t*-test with Welch's correction and ^P value via Mann-Whitney *U* test. Error bars represent SEM. Scale bars represent 25 μM.



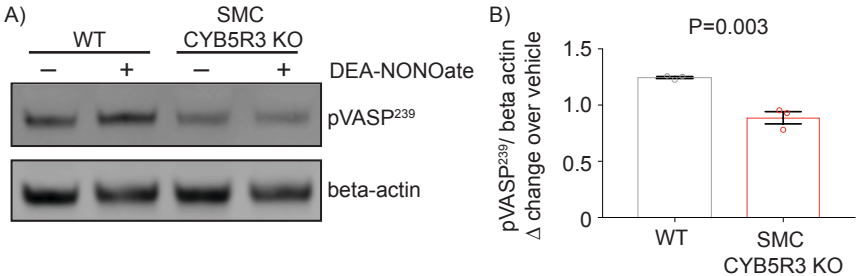
Supplemental Figure 3: Soluble guanylyl cyclase - beta (sGC- β) levels in SMC do not change between untreated WT and SMC CYB5R3 KO mice. A) *Cyb5r3*$^{wt/wt}$*Myh11*-CreER^{T2} (WT, n=5) and *Cyb5r3*$^{\Delta/\Delta}$ *Myh11*-CreER^{T2} (SMC CYB5R3 KO, n=4) mice arteries were stained for soluble guanylyl cyclase - beta (sGC-β, red), PECAM1 (endothelial cells, gray), ACTA2 (SMC, green) and DAPI (nuclei, blue). B-C) No differences were seen in sGC levels within the media of WT and SMC CYB5R3 KO mice in either the aorta or mesenteric arteries. P values determined by unpaired, two tailed *t*-test. Error bars represent SEM. Scale bars represent 25 μM.



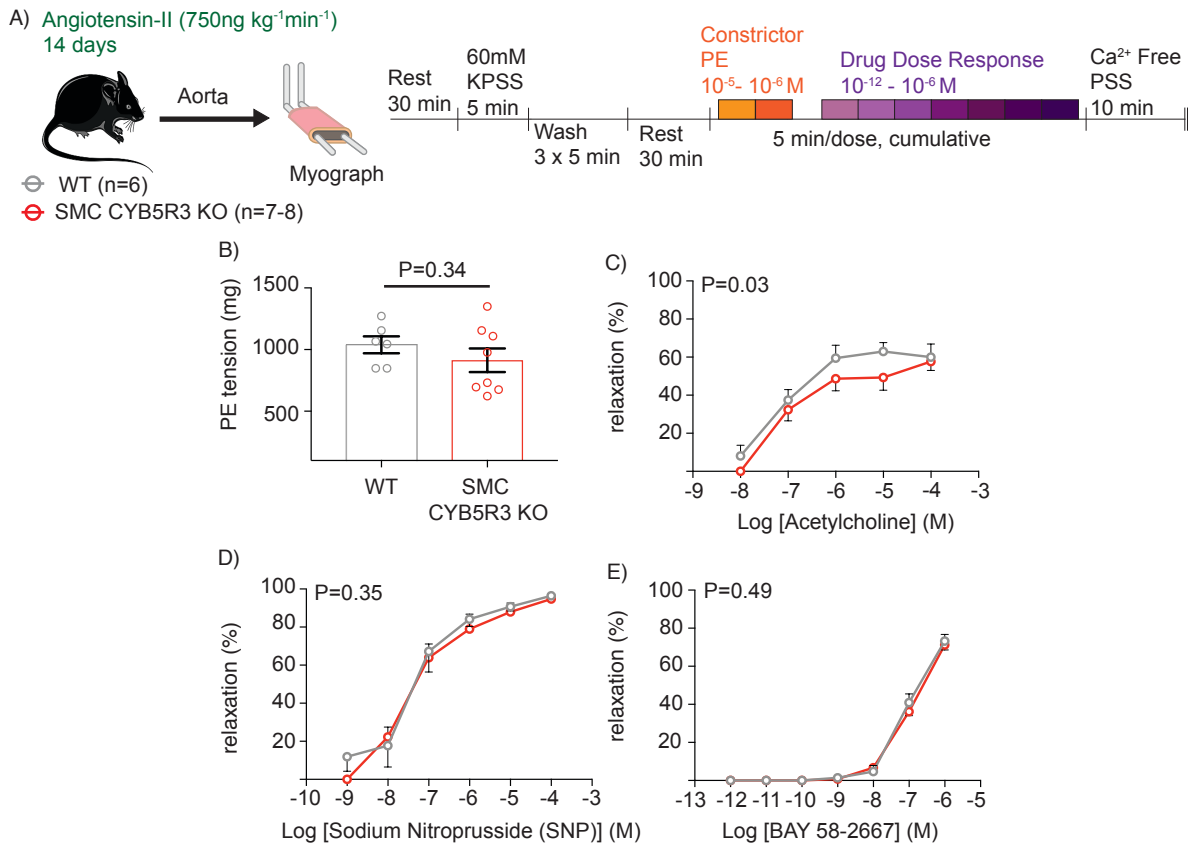
Supplemental Figure 4: SMC CYB5R3 KO mice have elevated systolic and diastolic pressures compared to WT mice. A) The systolic pressure (mmHg) reading for SMC CYB5R3 KO mice (n=10, red) was significantly higher than for WT mice (n=10, gray). B) SMC CYB5R3 KO mice averaged a significantly increased diastolic pressure than WT mice. P values were determined by two-way repeated measures ANOVA with * representing $P<0.05$ by post hoc Sidak multiple comparisons test. Error bars are SEM.



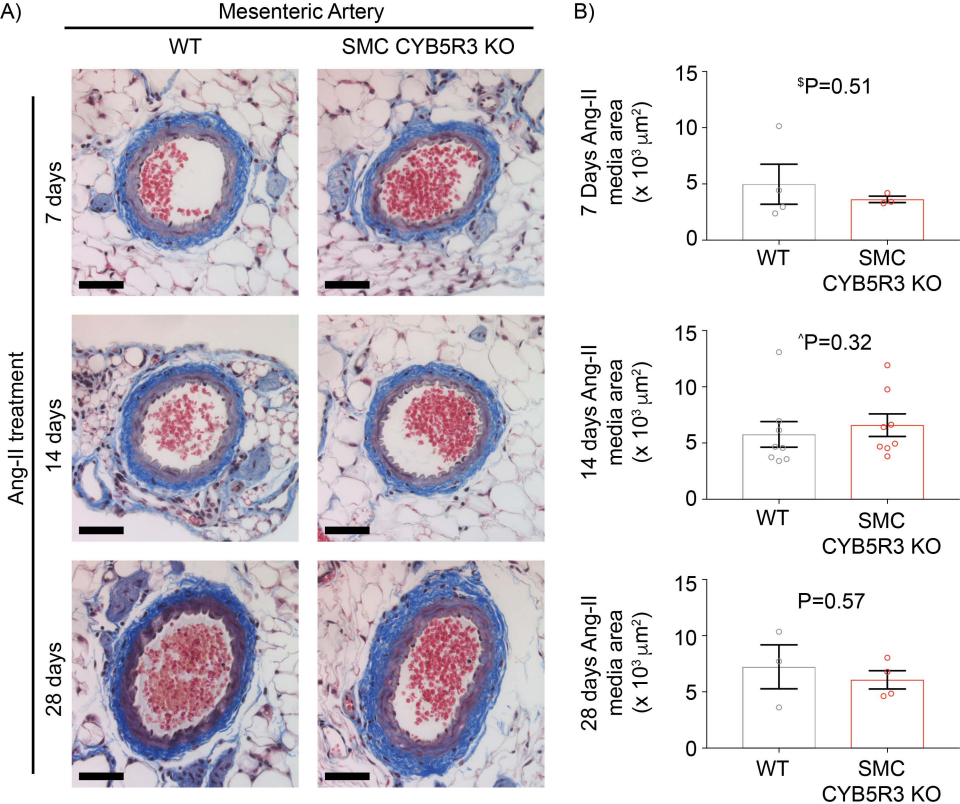
Supplemental Figure 5: Ang-II treated SMC CYB5R3 KO mice have significantly increased systolic and diastolic blood pressures compared to WT mice. A-B) SMC CYB5R3 KO mice (n= 8, red) averaged significantly elevated systolic and diastolic blood pressure per day for the first week of Ang-II treatment compared to WT mice (n=8, gray). Shaded regions highlight Days 6-7 of Ang-II where peak differences were observed between WT and SMC CYB5R3 KO mice. C-D) The 24-hour recording of systolic and diastolic blood pressure of Days 6-7 of Ang-II treatment that is shaded in A-B. A-D) P value represents significance by two-way repeated measures ANOVA across genotype with * showing P< 0.05 as determined by post-hoc Sidak multiple comparison tests. Error bars are SEM.



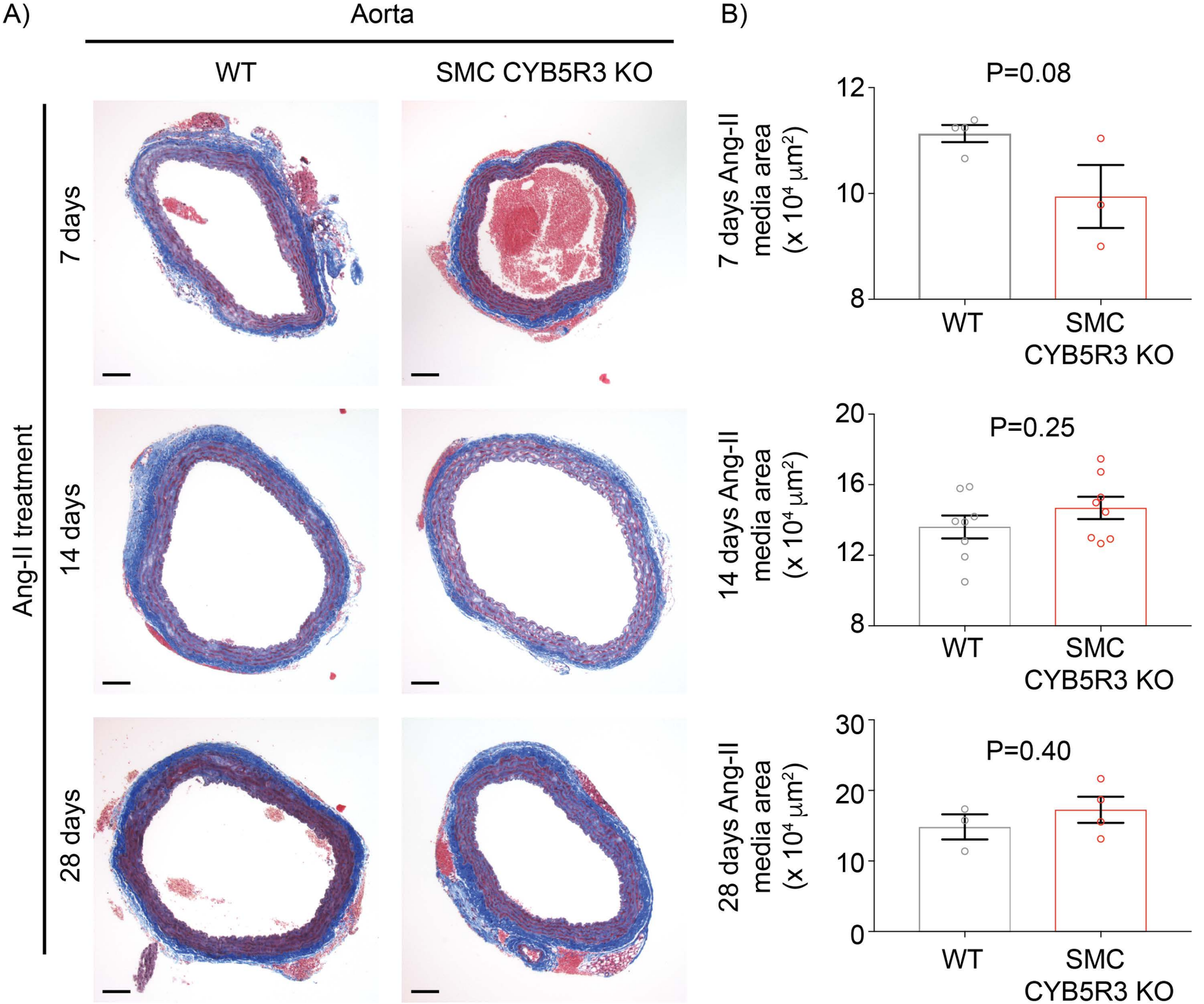
Supplemental Figure 6: Mesenteric arteries from 14 days Ang-II treated SMC CYB5R3 KO mice have less NO- induced pVASP^{239} compared to WT mice. A) Representative Western blot image of 14 days Ang-II WT and SMC CYB5R3 KO mesenteric arteries treated with or without 100 μM DEA-NONOate. B) Western blot quantification of pVASP^{239} per actin normalized to untreated control (n=3/ group). P value determined by unpaired two-tailed *t*-test. Error bars are SEM.



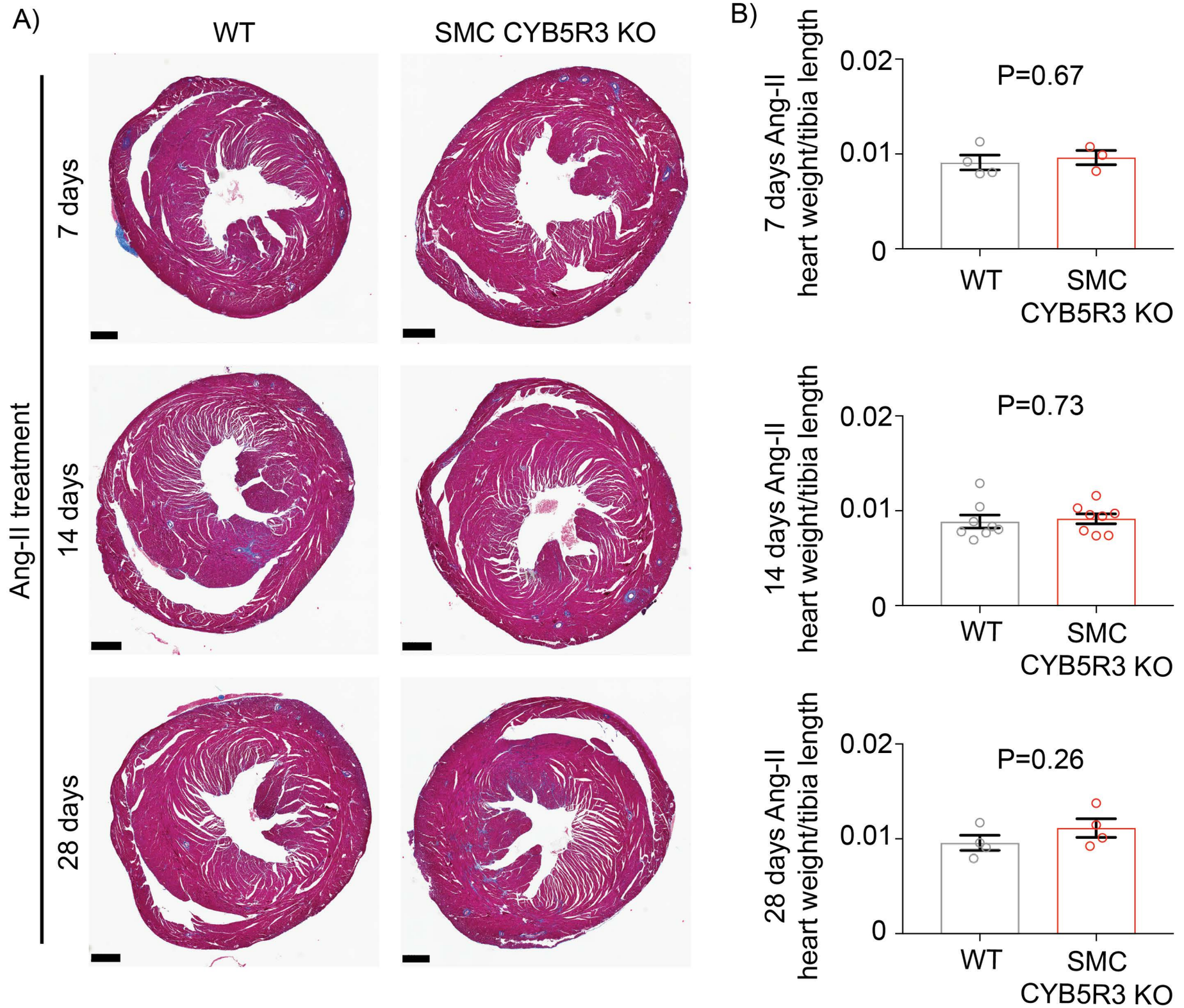
Supplemental Figure 7: Ang-II treated SMC CYB5R3 KO aortas have a blunted response to acetylcholine-induced vasodilation compared to WT aortas. A) Experimental design showing that aortas from WT (n=6) and SMC CYB5R3 KO (n=7-8) mice were subjected to ex vivo wire myography to assess vasoreactivity after 14 days Ang-II treatment. B) There are no differences in WT and SMC CYB5R3 KO response to phenylephrine (PE)-mediated vasoconstriction. P values determined by unpaired two-tailed *t*-test. Error bars are SEM. C) SMC CYB5R3 KO mice aortas are less responsive to acetylcholine induced vasodilation as compared to WT mice aortas. D-E) No significant differences were seen between WT and SMC CYB5R3 KO responsiveness to vasodilators SNP or BAY 58-2667. C-E) P values represent statistical differences between WT and SMC CYB5R3 KO by two-way ANOVA with * representing P<0.05 by post-hoc Sidak multiple comparisons test. Error bars are SEM.



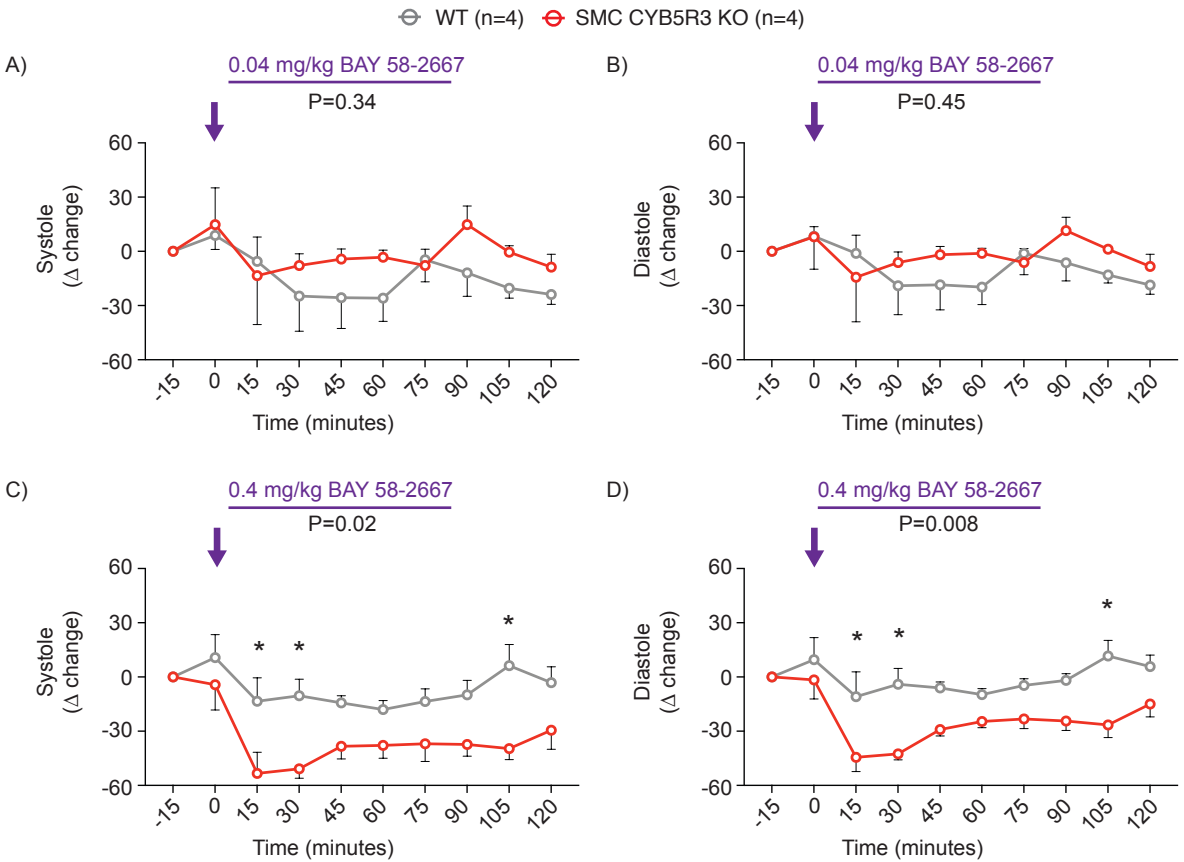
Supplemental Figure 8: No gross differences in mesenteric artery remodeling or fibrosis observed between Ang-II treated WT and SMC CYB5R3 KO mice treated with Ang-II. A) Representative images of WT and SMC CYB5R3 KO mesenteric arteries stained with Masson's trichrome after 7 days (n=3-4/ group), 14 days (n=8/ group), and 28 days (n=3-4/ group) Ang-II treatment show no gross differences in fibrosis between groups at any point of treatment. B) No significant differences were observed in remodeling measured by media area between WT and SMC CYB5R3 KO irrespective of length of Ang-II treatment. P values represent unpaired two-tailed *t*-test. $P values represent unpaired two-tailed *t*-test with Welch's correction. ^P values represent Mann Whitney *U* test. Error bars are SEM. Scale bars are 100 μm.



Supplemental Figure 9: No gross differences in fibrosis or remodeling between 14 day Ang-II treated WT and SMC CYB5R3 KO mice aorta. A) Representative images of WT and SMC CYB5R3 KO aortas stained with Masson's trichrome after 7 days (n=3-4/ group), 14 days (n=8/ group), and 28 days (n=3-4/ group) Ang-II treatment show no gross differences in fibrosis. B) In addition, there were no significant differences in media area between WT and SMC CYB5R3 KO mice at any point of Ang-II treatment. P values represent unpaired two-tailed *t*-test. Error bars are SEM. Scale bars are 100 μm.



Supplemental Figure 10: No gross differences in cardiac fibrosis or hypertrophy were observed between WT and SMC CYB5R3 KO mice treated with Ang-II. A) Representative images of WT and SMC CYB5R3 KO hearts stained with Masson's trichrome after 7 days (n=3-4/ group), 14 days (n=8/ group), and 28 days (n=4/group) Ang-II treatment show no gross differences in intra-cardiomyocyte fibrosis. B) No significant differences were seen in cardiac hypertrophy (heart weight /tibia length) between WT and SMC CYB5R3 KO mice treated with Ang-II for any length of time. P values represent unpaired two-tailed *t*-test. Error bars are SEM. Scale bars are 500 μm.



Supplemental Figure 11: Acute BAY 58-2667 injection in vivo shows SMC CYB5R3 KO mice are more sensitive than WT mice to BAY 58-2667 blood pressure lowering effects at the 0.4 mg/kg dose. 0.04mg/kg BAY 58-2667 intraperitoneal injection (time 0 minutes, purple arrow) showed no significant difference in change in A) systolic pressure or B) diastolic pressure between WT (n=4) and SMC CYB5R3 KO (n-4) mice. The higher 0.4 mg/kg BAY 58-2667 injection (time 0 minutes, purple arrow) resulted in a significant decrease in change in C) systolic pressure and D) diastolic pressure in SMC CYB5R3 KO mice as compared to WT mice. This indicates SMC CYB5R3 KO mice are more sensitive than WT to BAY 58-2667 blood pressure lowering effects at the 0.4 mg/kg dose. A-D) P value represents significance between WT and SMC CYB5R3 KO by two-way repeated measures ANOVA with * representing P value < 0.05 by post-hoc Sidak multiple comparison tests. Error bars are SEM.