Supplemental Information

Conditional Deletion of Smooth Muscle Cullin-3 Causes Severe Progressive Hypertension

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SUPPLEMENTAL FIGURES AND LEGENDS

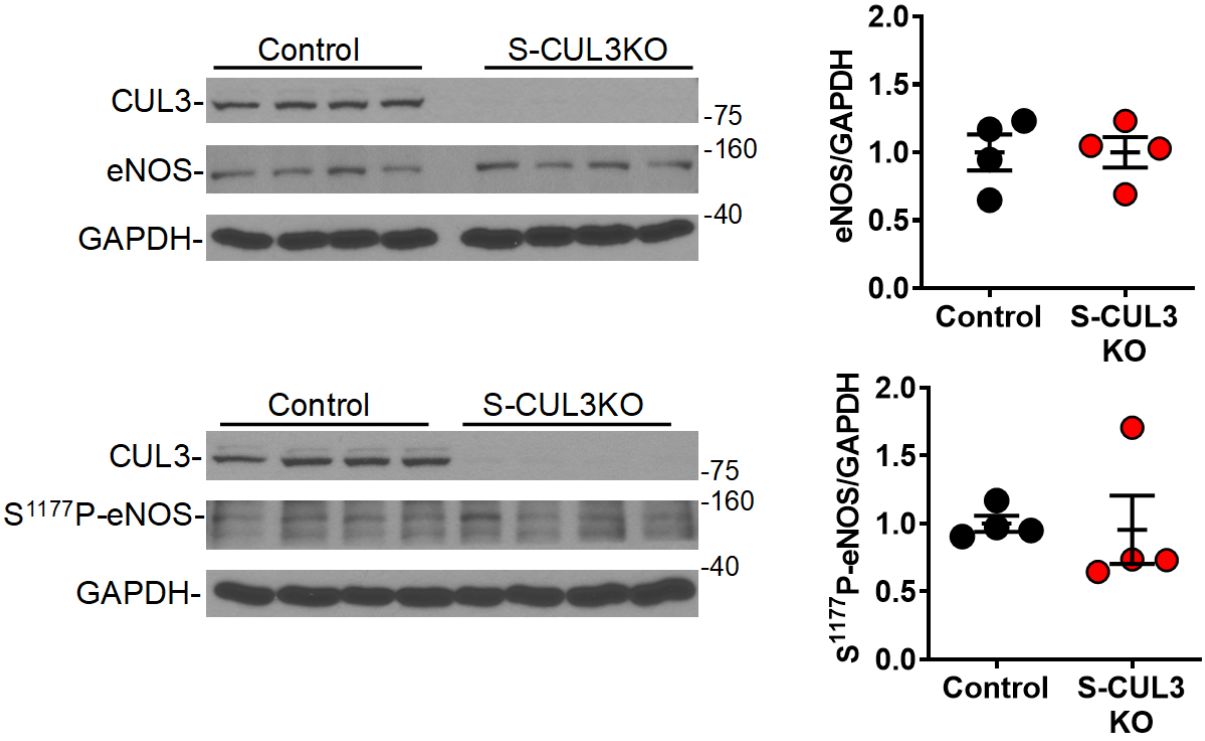

Figure S1: *Expression of eNOS and Ser1177-P-eNOS*.
Western blots showing expression of the indicated proteins in aorta which were cleaned of perivascular adipose tissue and adventitia. Quantification of total eNOS and Ser1177-P-eNOS is shown (n=4/genotype). Expression and quantification of phospho-Ser117-eNOS (active form) in aorta.

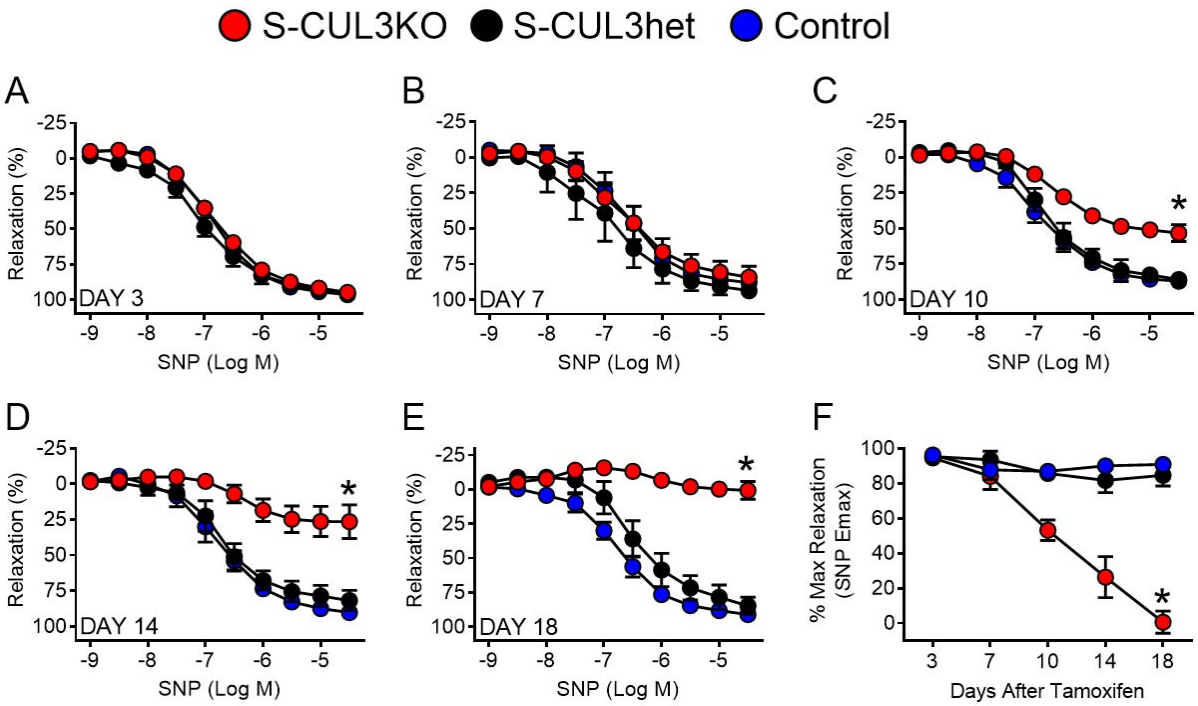

Figure S2: *Time Course of SNP Response*.
Isometric tension was measured on aortic rings. Dose-dependent vasorelaxation to sodium nitroprusside (SNP) was measured (following pre-contraction with $PGF_{2\alpha}$ to 45-50% of maximal at days 3 (A, n=4-6/genotype), 7 (B, n=4-6/genotype), 10 (C, n=4-6/genotype), 14 (D, n=4-7/genotype), and 18 (E, n=5-9/genotype) following the last day of tamoxifen administration. F) Percent of maximum relaxation was determined for all genotypes. Error bars represent the mean±SEM. *$P<0.05$ by 2-way repeated-measures ANOVA.

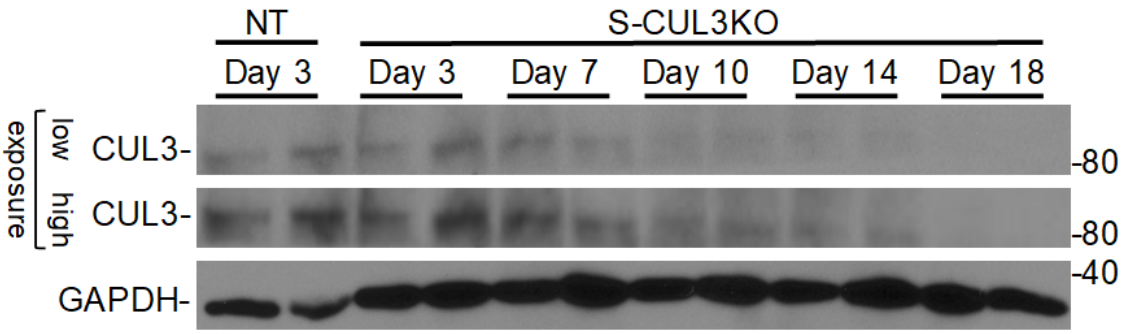

Figure S3: *Time-dependent Ablation of CUL3 Protein in Aorta.*
Representative Western Blots showing expression of the indicated proteins. Mice were administered tamoxifen (75 mg/kg) for 5 consecutive days and aortas were collected at different time points following the last dose of tamoxifen as Day 1. Aortas were cleaned of perivascular adipose tissue and probed by western blotting. A low and high exposure of the same CUL3 blot is shown. The blot was cut and separately probed for CUL3 and GAPDH.

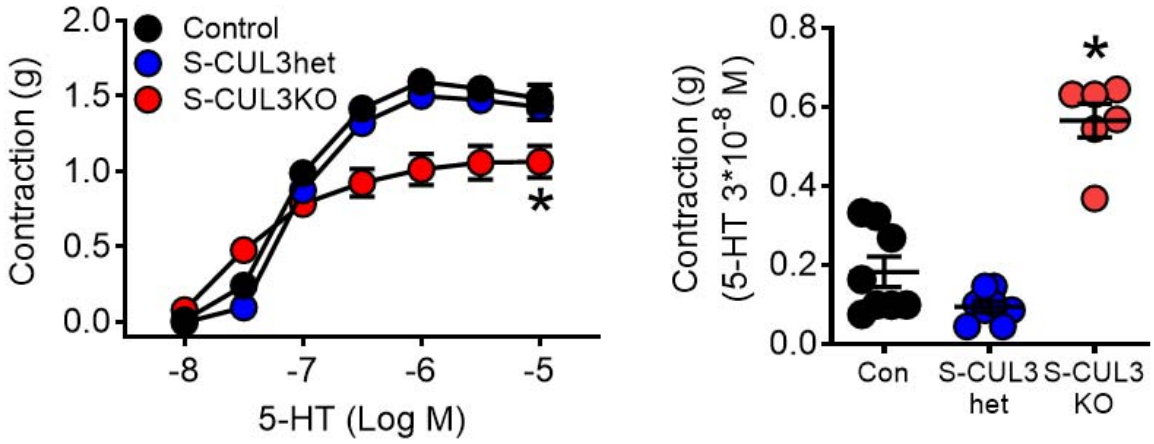

Figure S4: *Vasocontraction of Thoracic Aorta*.
Vasocontraction of aorta from the indicated mice. Aorta rings were isolated and cleaned of perivascular adipose fat and were equilibrated for 45 min at 0.5 g tension and vasocontraction responses to 5-HT (n=6-7/genotype) was assessed. Error bars represent the mean±SEM. **P<0.05* by 1-way (right) or 2-way repeated measures ANOVA (left).

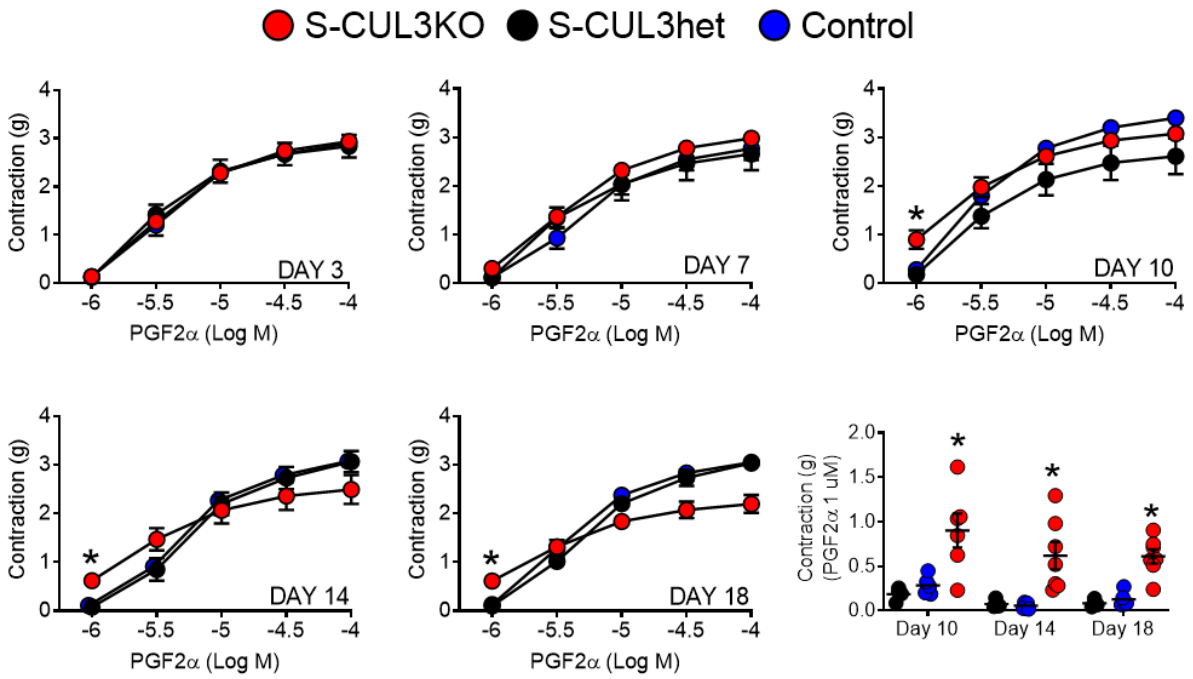

Figure S5: *Time Course of the Contractile Response.*
Dose-dependent vasocontraction to $PGF_{2\alpha}$ was measured (following 45 min equilibration at 0.5 g) on days 3 (A, n=4-6/genotype), 7 (B, n=4-6/genotype), 10 (C, n=4-6/genotype), 14 (D, n=4-7/genotype), and 18 (E, n=5-7/genotype) following the last day of tamoxifen administration. Contraction to 1 μM $PGF_{2\alpha}$ was determined. Note that the S-CUL3het graph (Day 14) was shifted 0.05 units in the X and Y directions to clearly indicate the presence of three data sets on the graph because the control and S-CUL3 were essentially superimposed. Error bars represent the mean±SEM. *$P<0.05$ by 2-way repeated-measure ANOVA.

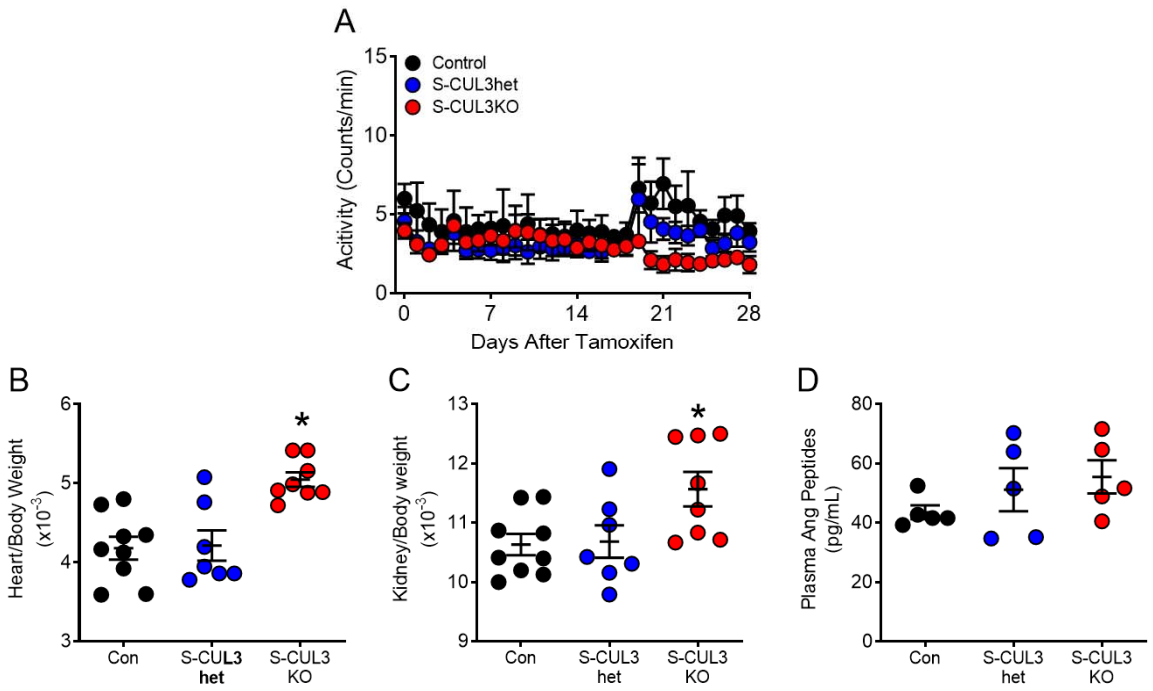

Figure S6: *Activity, Organ Weight, Angiotensin Peptides.*
A) Activity was measured continuously every 5 minutes for 10 seconds by radiotelemetry. B-C) Heart (B) and kidney (C) weights (normalized to body weight) were measured two weeks following the last day of tamoxifen administration. D) Total angiotensin peptides were measured in plasma 2 weeks after tamoxifen by ELISA. Error bars represent the mean±SEM. *$P<0.05$ by 1-way ANOVA.

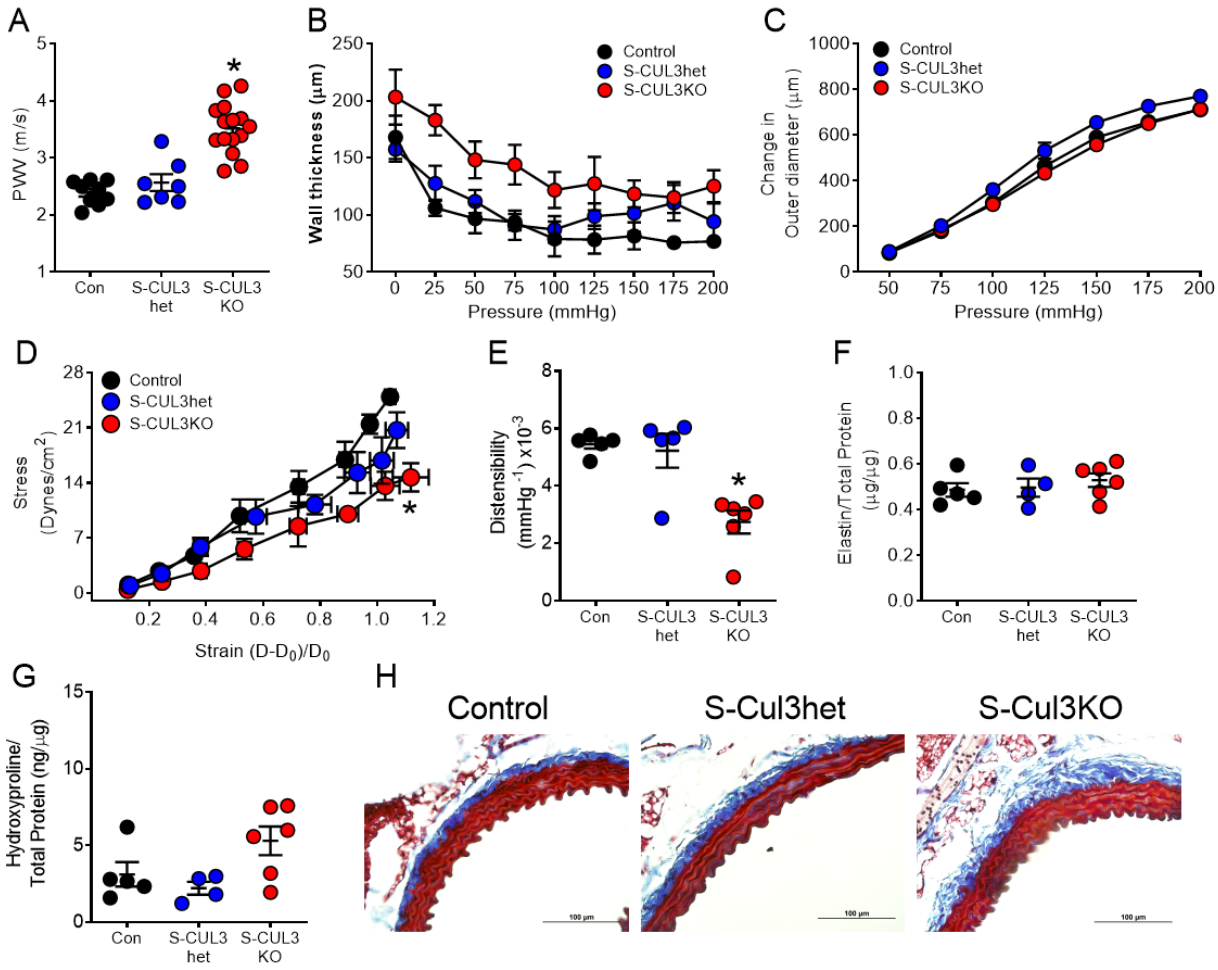

Figure S7: *Arterial Stiffness*.
A) Pulse wave velocity (PWV) measurements were determined 2 weeks after tamoxifen administration by Doppler ultrasound. n=7-15/genotype; **P<0.05* S-CUL3KO vs controls by 1-way ANOVA. B-D) Wall thickness (B, n=4-6/genotype), pressure-diameter curves (C, n=4-6/genotype), and stress–strain relationships (D, n=4-6/genotype). **P<0.05* calculated by 2-way ANOVA. E-G) Aortic distensibility (E, n=5-6/genotype), elastin (F, n=4-6/genotype), and aortic collagen content (G, n=4-6/genotype) as measured by hydroxyproline assay. **P<0.05* calculated by 1-way ANOVA. H) Adventitial collagen was determined by Masson Trichrome staining. Images are 40X magnification and scale bars are 100 μm. Error bars represent the mean±SEM.

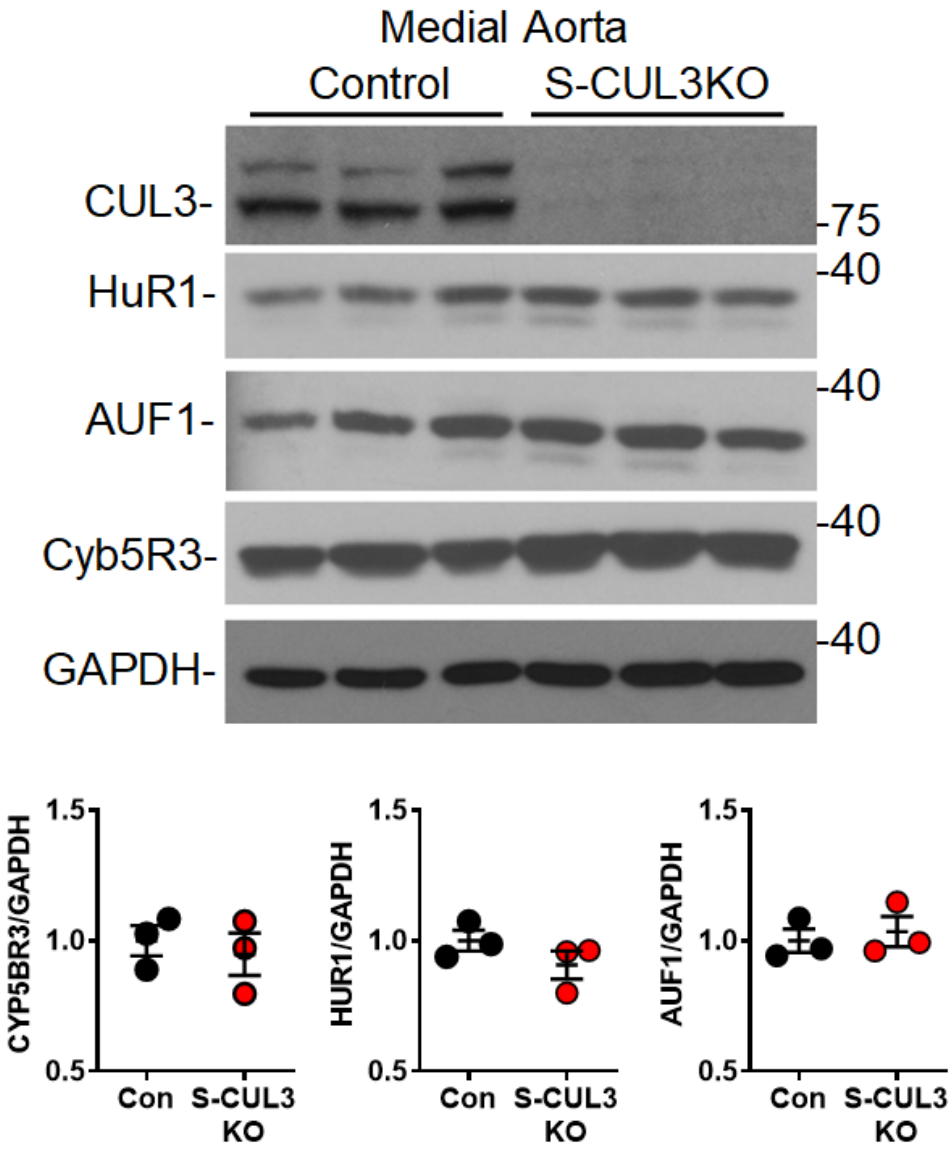

Figure S8: *Regulation of Guanylate Cyclase Expression*.
Western blot showing expression levels of indicated proteins in aortas cleaned of perivascular fat and adventitia. Protein quantification is shown (n=3).