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Supplementary figures and tables

Effect of hydralazine on angiotensin II-induced abdominal aortic aneurysm in apolipoprotein E-deficient mice

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Table S1: List of mouse primer sequences.

Name	Forward primer	Reverse primer
Caspase 3	5’-CGTGGTTCATCCAGTCCCTTT-3’	5’-ATTCCGTTGCCACCTTCCT-3’
Caspase 8	5’-TCTACCCTCCAGCTCTCTGT-3’	5’-AGAGGTAGAAGAGCTGTAACCTTAT-3’
Caspase 9	5’-ACCTTCCCAGGTTGCCAATG-3’	5’-CCCCGAGCCTCATGAAGTTTT-3’
EEF2	5’-ACATGTCAGTGATCGCCCAT-3’	5’-GAGATGGTGGTGGATTTGATTG-3’
IFNγ	5’-TGGCTGTTTCTGGCTGTTACT-3’	5’-TCATGTCACCATCCTTTTGCCA-3’
IL-1	5’-GCCACCTTTTGACAGTGATGAA-3’	5’-GACAGCCCAGGTCAAAGGTT-3’
IL-6	5’-CGGCCTTCCCTACTTCACAA-3’	5’-GCCATTGCACAACTCTTTCTCA-3’
TNF-α	5’-TAGCCCACGTCGTAGCAAAC-3’	5’-ACAAGGTACAACCCATCGGC-3’

Abbreviations: EEF2, eukaryotic translation elongation factor 2; IFNγ, interferon-gamma; IL, interleukin; T*NF-α,* tumor necrosis factor-alpha.

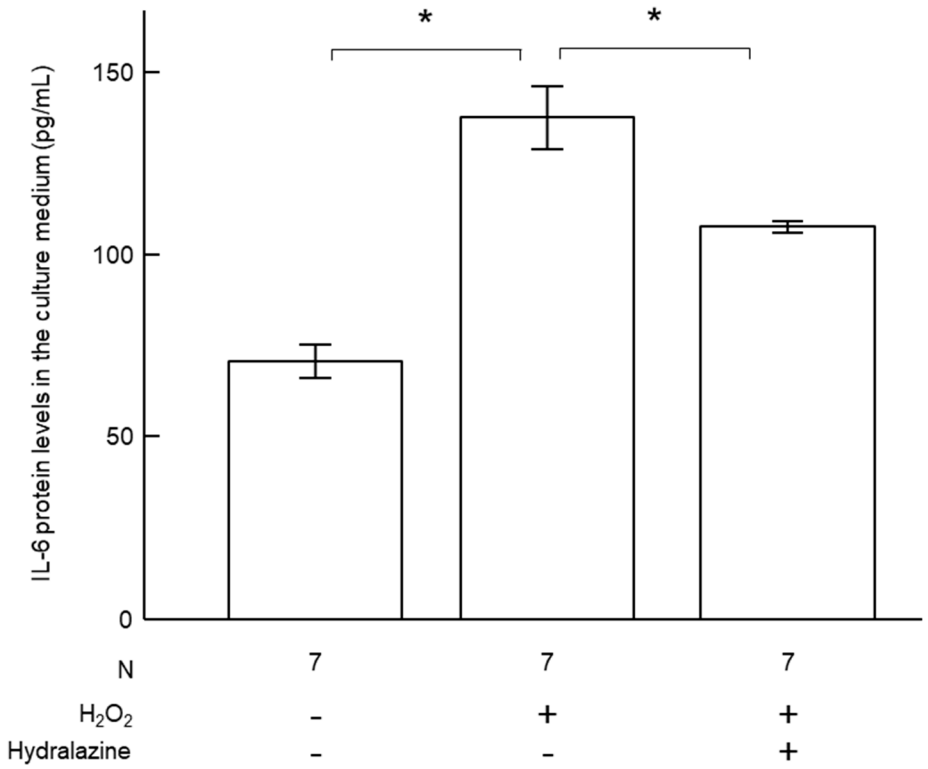

Figure S1: Effect of hydralazine on IL-6 protein levels in culture media. VSMCs were treated with H_2O_2 (200 μM) or co-treated with H_2O_2 and hydralazine (100 μM) for 24 h. IL-6 protein in the culture media was then quantified using ELISA. Error bars = standard error. Data were analyzed using Kruscal Wallis one-way ANOVA. * $P<0.05$. ELISA, enzyme-linked immunosorbent assay; H_2O_2, hydrogen peroxide; IL-6, interleukin 6; VSMCs, vascular smooth muscle cells.

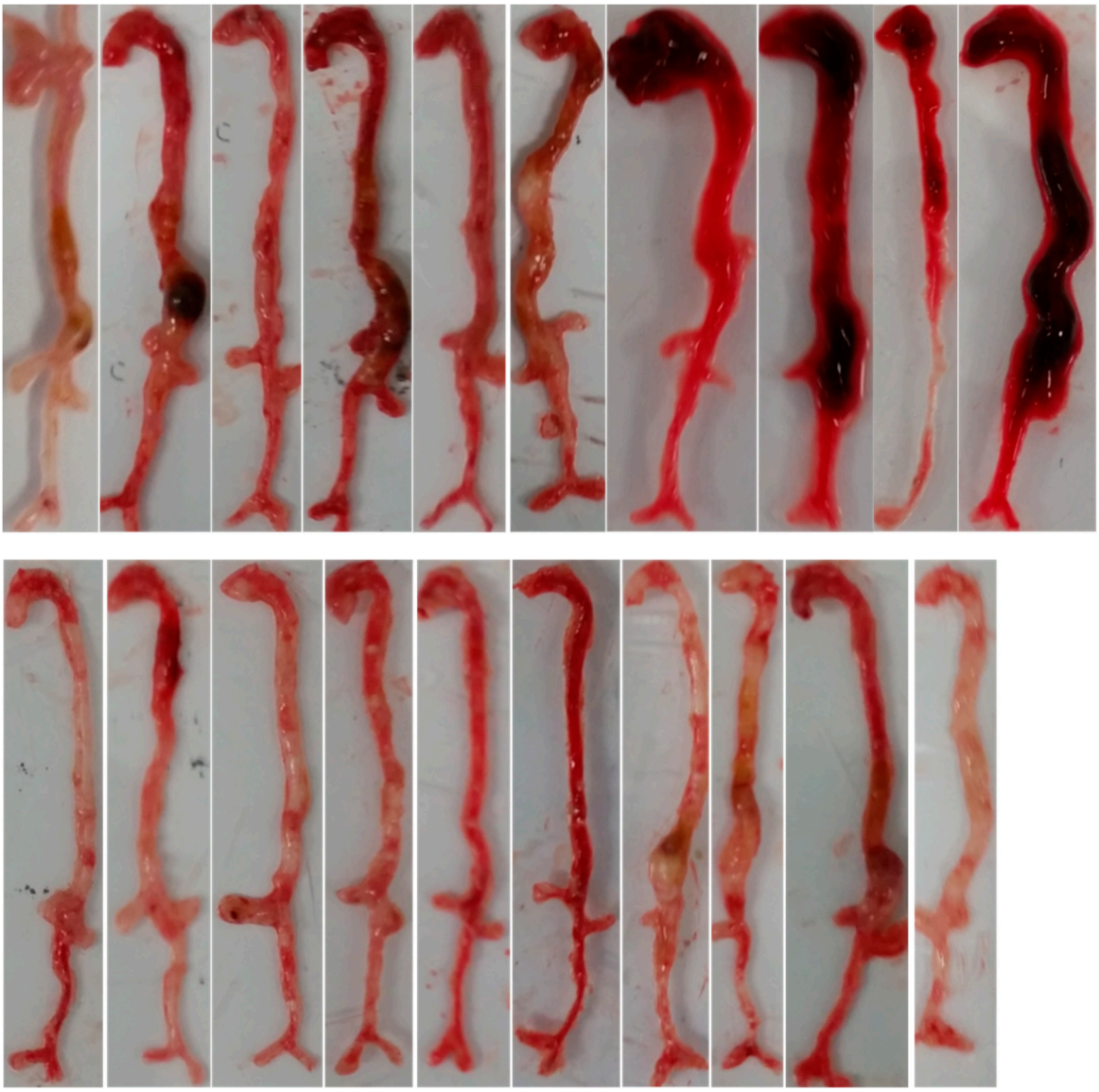

Figure S2: Aortic images. The top row images were the aortas of the control mice and the bottom row images were the aortas of the mice treated with hydralazine.

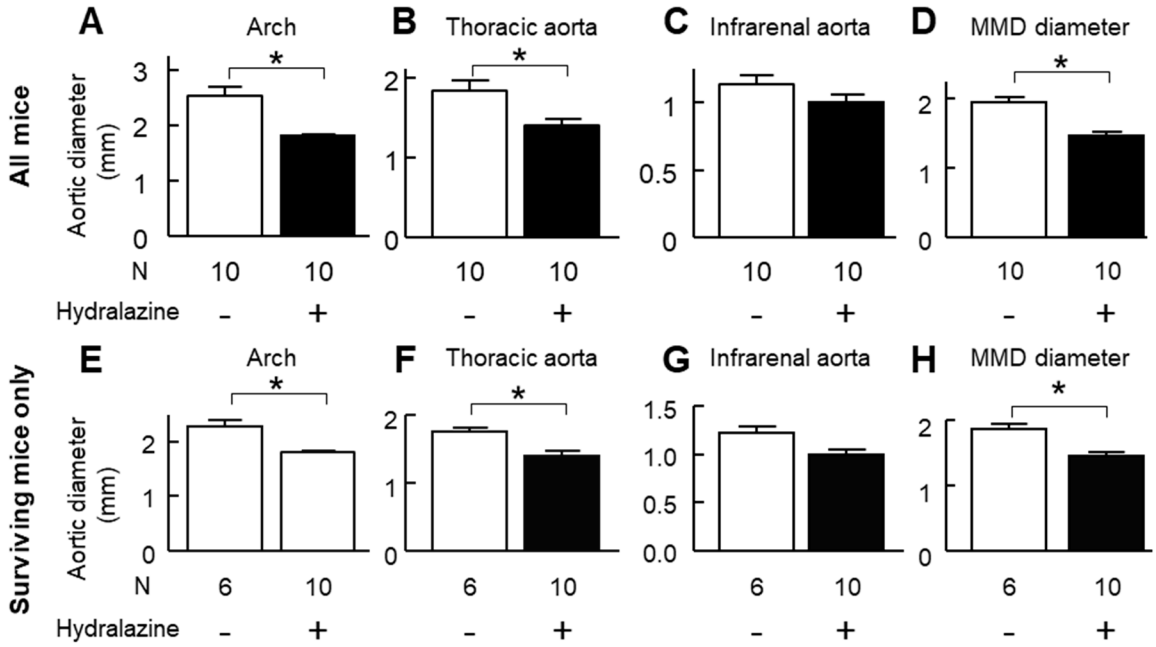

Figure S3: Effect of hydralazine treatment on aortic diameters of the aortic arch, thoracic aorta, infrarenal aorta, and mean maximum aortic diameter (MMD). **A-D**, All mice (surviving mice plus dead mice due to aortic rupture). **E-H**, Surviving mice. MMD was the mean of the maximum diameter of the following four aortic segments: aortic arch, descending thoracic aorta, suprarenal aorta, and infrarenal aorta. Error bars = standard error. Data were analyzed using the Mann-Whitney U test. * $P<0.05$.

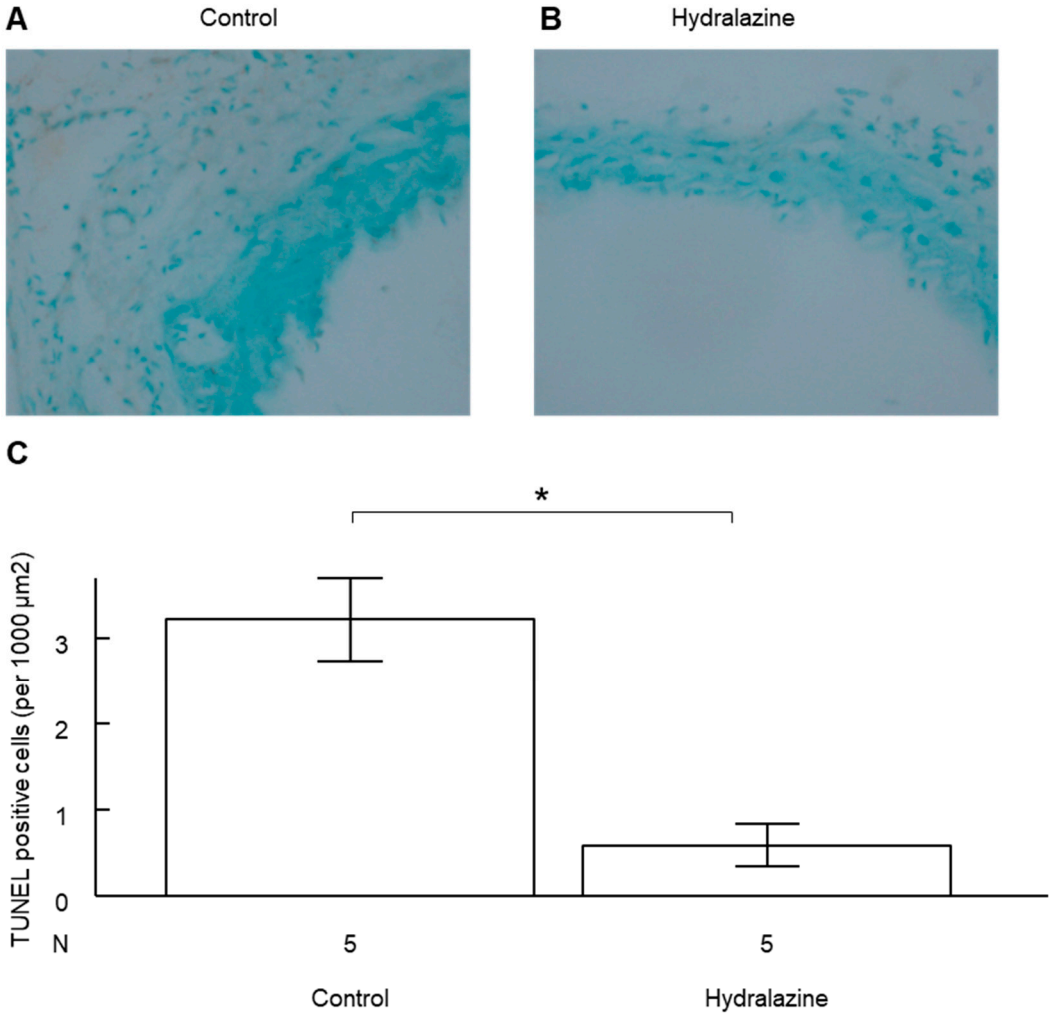

Figure S4: Effect of hydralazine treatment on apoptosis. Mice, treated with or without hydralazine (24 mg/kg/day), were sacrificed at the end of the 28-day subcutaneous infusion of angiotensin II (1 µg/kg body/min). The suprarenal aortas were harvested, fixed, sectioned, and stained for apoptosis using the terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) assay. Nuclei were counterstained with methyl green. **A-B**, Representative images of TUNEL stained aortic sections (magnification, 40X) from the control (untreated, **A**) and hydralazine-treated mice (**B**). **C**, The mean TUNEL-positive cells per 1000 μm^2. Error bars = standard error. Data were analyzed using the Mann-Whitney U test. * $P<0.05$.

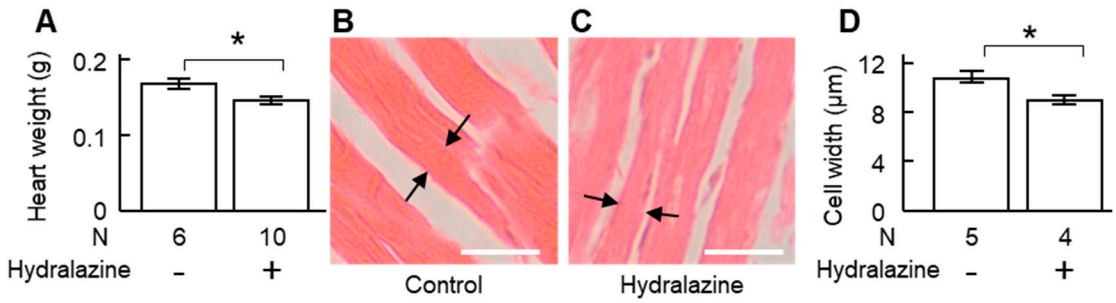

Figure S5: Effect of hydralazine treatment on heart weight and cardiomyocyte width. Hearts were harvested from the mice treated with or without hydralazine (24 mg/kg/day) at the end of the 28-day subcutaneous infusion of angiotensin II (1 μg/kg body/min). **A**, Heart weight of the surviving mice. **B-C**, Representative images of cardiomyocyte fibers of the control (untreated, **B**) and hydralazine-treated mice (**C**). Scale bar =20 μM. Between the arrows: cardiomyocyte fibers. **D**, The mean width of cardiomyocytes. Error bars = standard error. Data were analyzed using the Mann-Whitney U test. * P<0.05.