SUPPLEMENTAL MATERIAL

ACE2 Decreases the Formation and Severity of Angiotensin II-induced Abdominal Aortic Aneurysms

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Table I. Systolic blood pressures, plasma renin concentrations, serum ACE and triglyceride concentrations in AngII-infused mice from each study

	Whole Body		BMT			*Ace2*$^{+/y}$	*Ace2*$^{-/y}$
	Ace2$^{+/y}$	***Ace2***$^{-/y}$	***Ace2***$^{+/y}$	***Ace2***$^{-/y}$	**VEH**	**DIZE**	**DIZE**
Baseline Blood Pressure (mmHg)	114 ± 2	120 ± 2	105 ± 3	104 ± 4	114 ± 3	110 ± 4	111 ± 4
Endpoint Blood Pressure (mmHg)	138 ± 4*	150 ± 3*,†	130 ± 6*	123 ± 4*	139 ± 5*	137 ± 8*	140 ± 10*
Plasma AngII (pg/mL)	5 ± 4	62 ± 22†	ND	ND	214 ± 91	150 ± 9	140 ± 21
PRC (ng/mL)	0.2 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Serum ACE activity (nmoles His-Leu/mL/min)	886 ± 30	999 ± 137	1224 ± 82	1191 ± 61	ND	ND	ND
Serum Triglycerides (mg/dl)	532 ± 21	477 ± 19	1066 ± 86	896 ± 82	613 ± 68	480 ± 38	429 ± 74

BMT – Bone marrow transplantation

VEH, Vehicle (antipyrine in 0.9% saline); DIZE, Diminazine aceturate (30 mg/kg/day)

PRC = plasma renin concentration

ND = not determined

*P<0.05 compared to baseline blood pressure

†P<0.05 compared to *Ace2*$^{+/y}$ mice

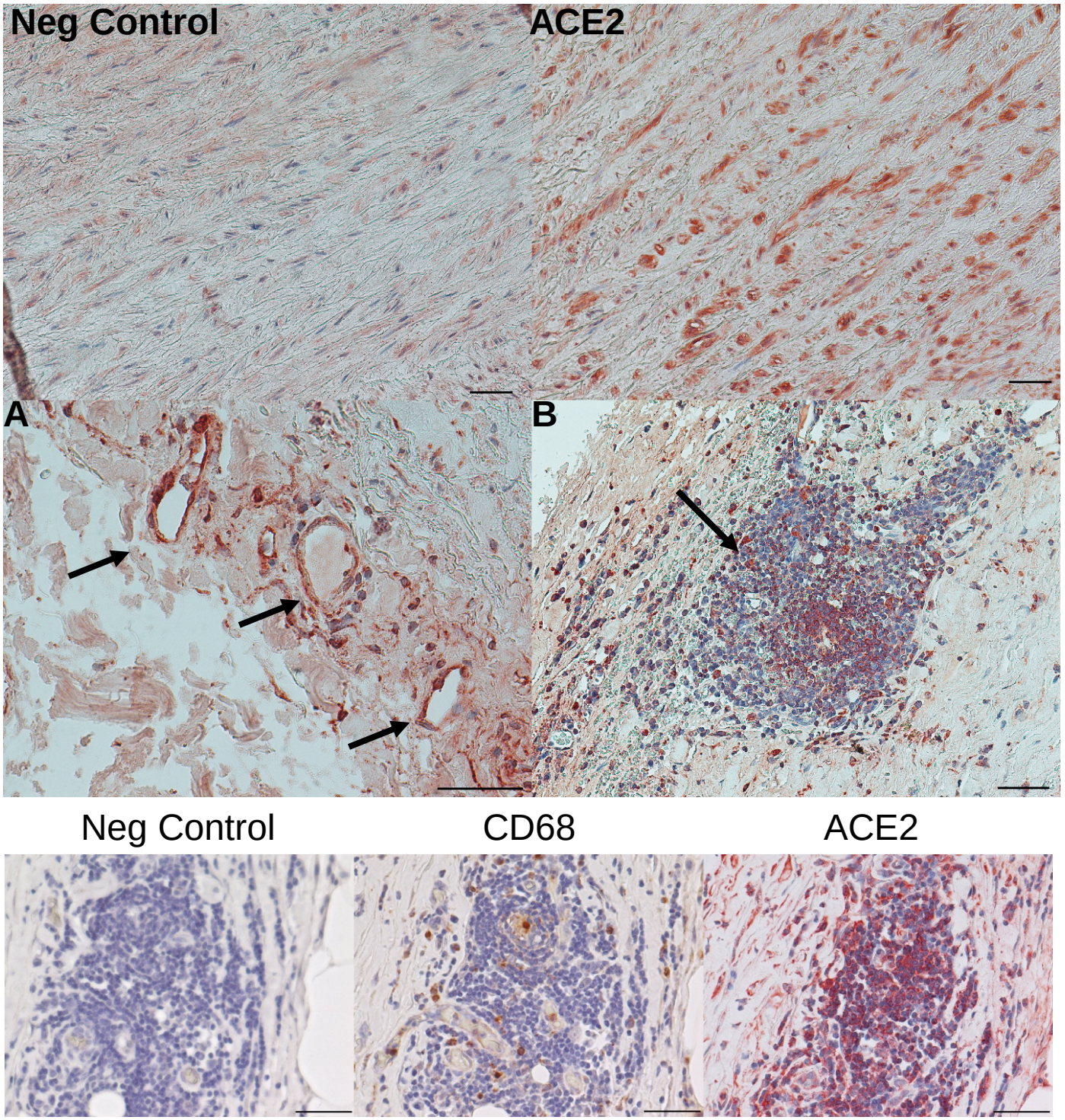
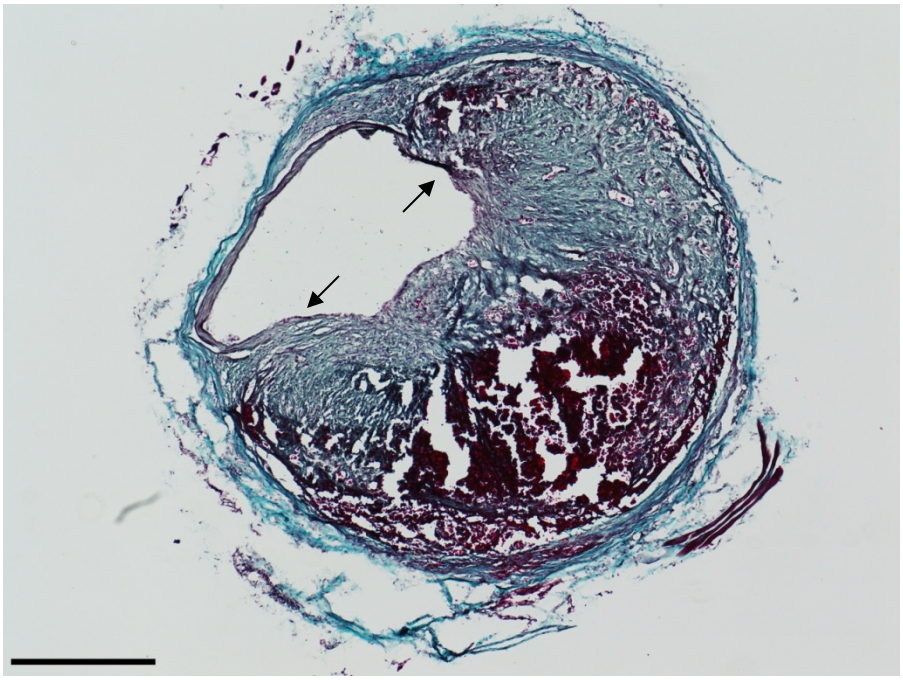


Figure I. Top, Non-immune IgG and ACE2 immunostaining in human abdominal aortas (upper panels), and CD68 immunostaining in inflammatory foci of human AAAs (lower panels). (A), Represents ACE2 staining in the vaso vasorum of a human non-AAA (arrows). (B), Represents ACE2 staining in an inflammatory foci of a human AAA (arrow). Lower panels: Non-immune IgG (Neg Control), CD68, and ACE2 immunostaining in inflammatory foci of human AAAs. Scale bars represent 50 µm.

***Ace2*$^{+/y}$**



***Ace2*$^{-/y}$**

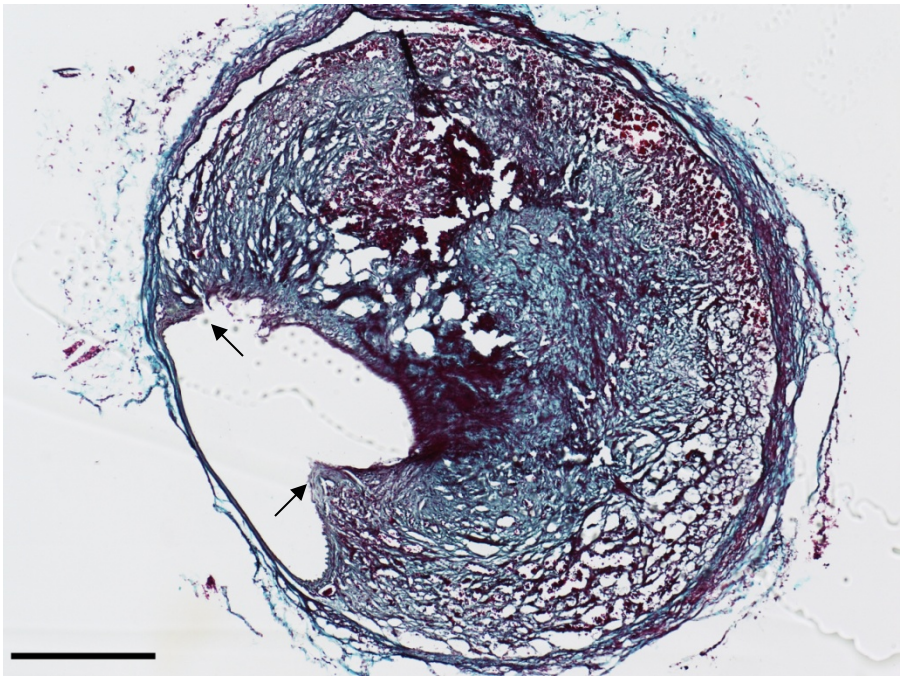


Figure II. Gomori trichrome stain of medial break (arrows) in *Ace2*$^{+/y}$ and $^{-/y}$ mice. Scale bar represents 500 μm.

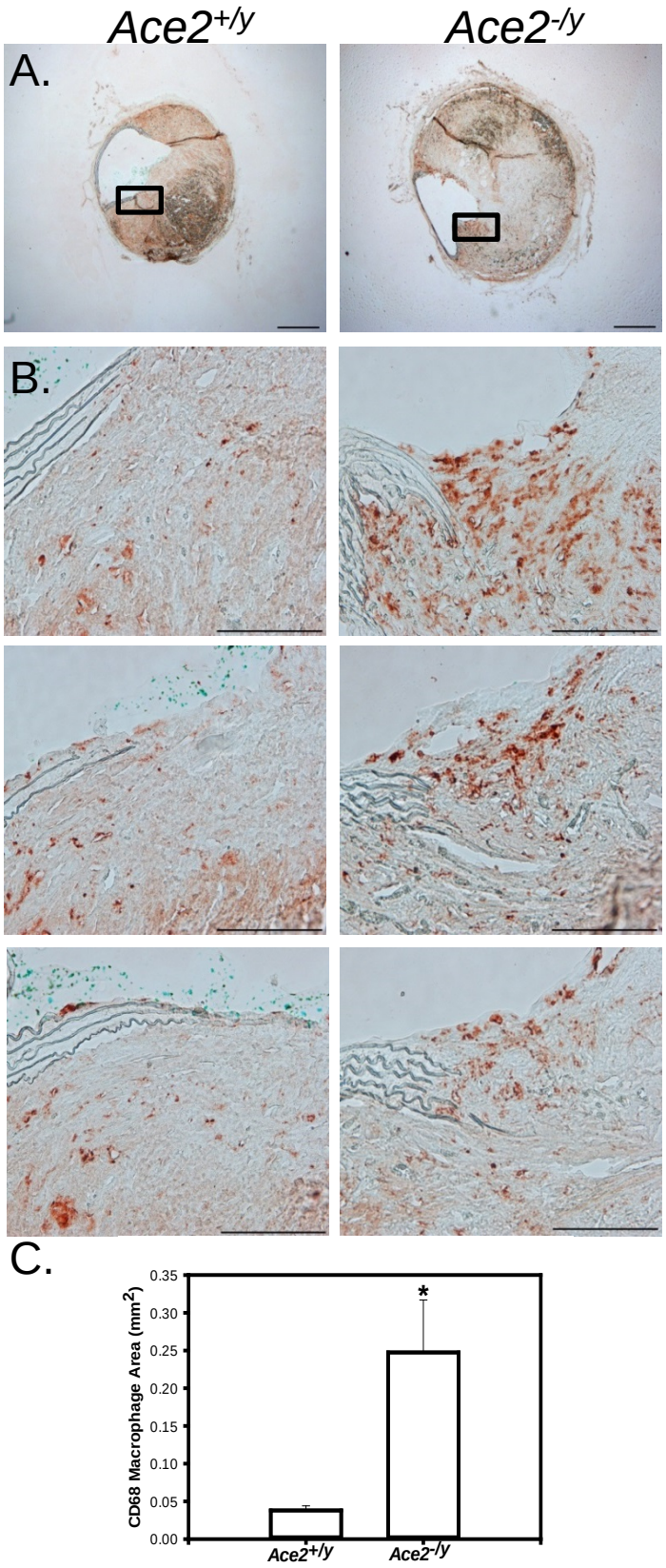


Figure III. CD68 staining in *Ace2*$^{+/y}$ and $^{-/y}$ AAA sections. (A) represents CD68 staining of sections at the medial break. Scale bar is 500 µm (B) represents increased magnification (200X) and images used for quantification of macrophages. Scale bar is 50 µm. (C) represents the average macrophage area (mm^2) in sections taken from *Ace2*$^{+/y}$ (N=7) and *Ace2*$^{-/y}$ (N=8) *Ldlr*$^{-/-}$ mice (*P<0.05)

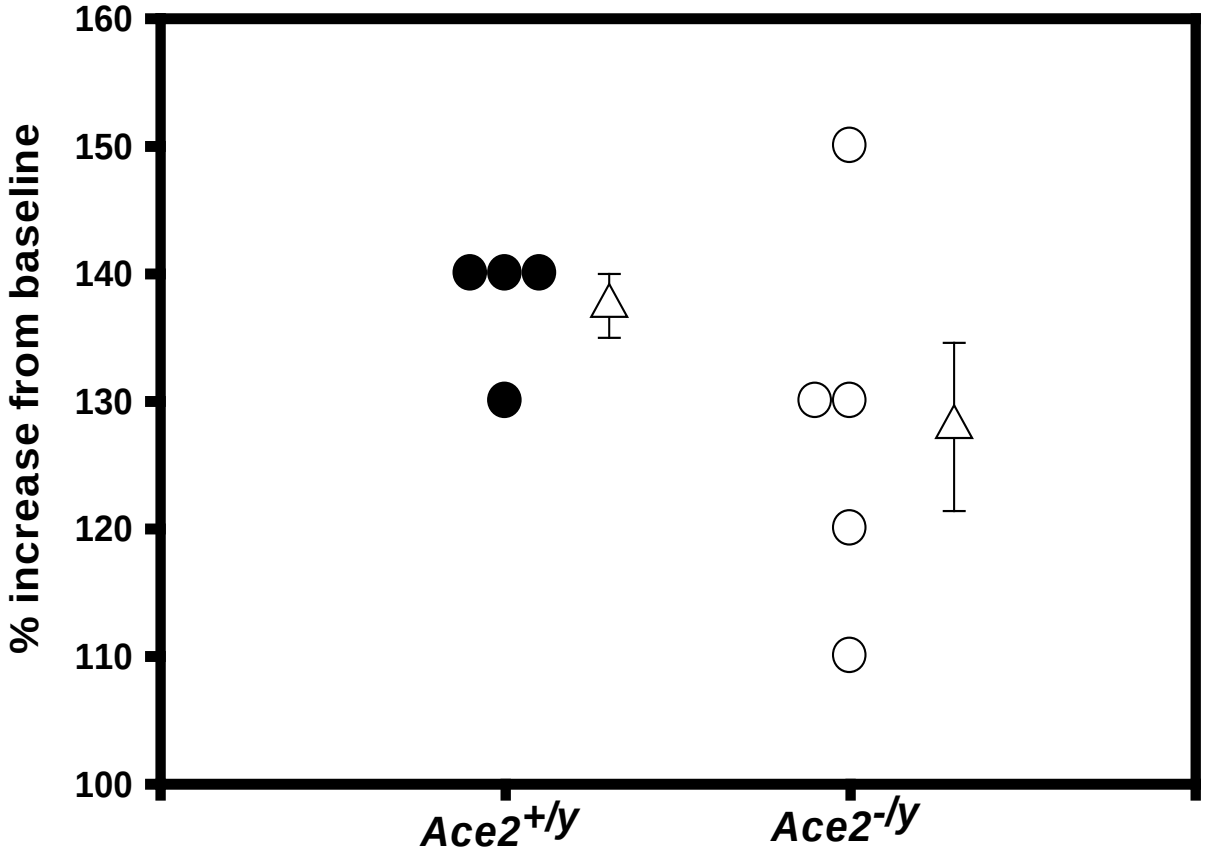


Figure IV. Percent increase from baseline for elastase-induced AAA model. *Ace2*$^{+/y}$ and $^{-/y}$ mice were infused with elastase and infrarenal aortas were examined 14 days later (N=4-5).

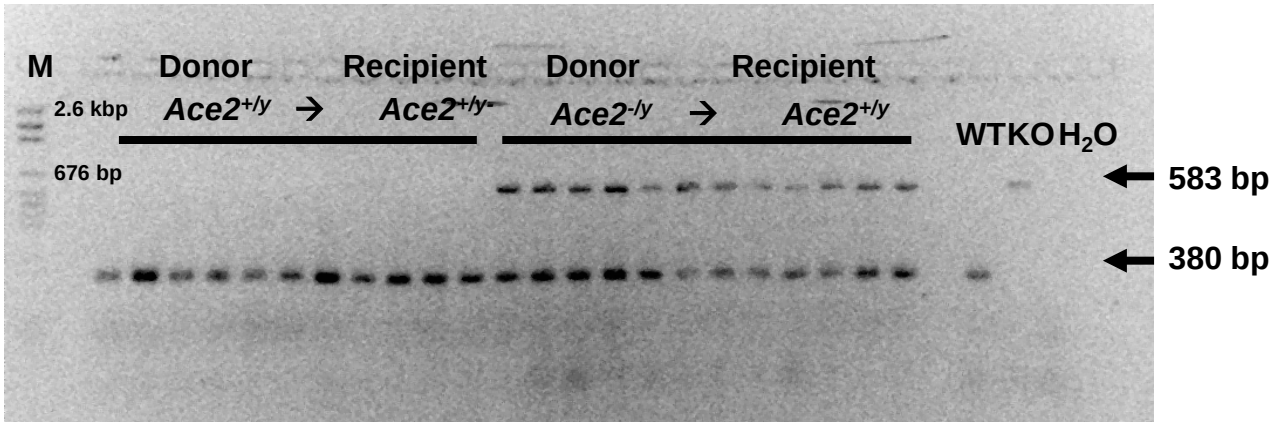


Figure V. PCR analysis of *Ldlr*$^{-/-}$ recipients that were transplanted with bone marrow-derived stem cells either from *Ace2*$^{+/y}$ or $^{-/y}$ mice. DNA bands of bone marrow from mice transplanted with cells from *Ace2*$^{+/y}$ (380 base pairs (bp)) or $^{-/y}$ mice (580 bp).

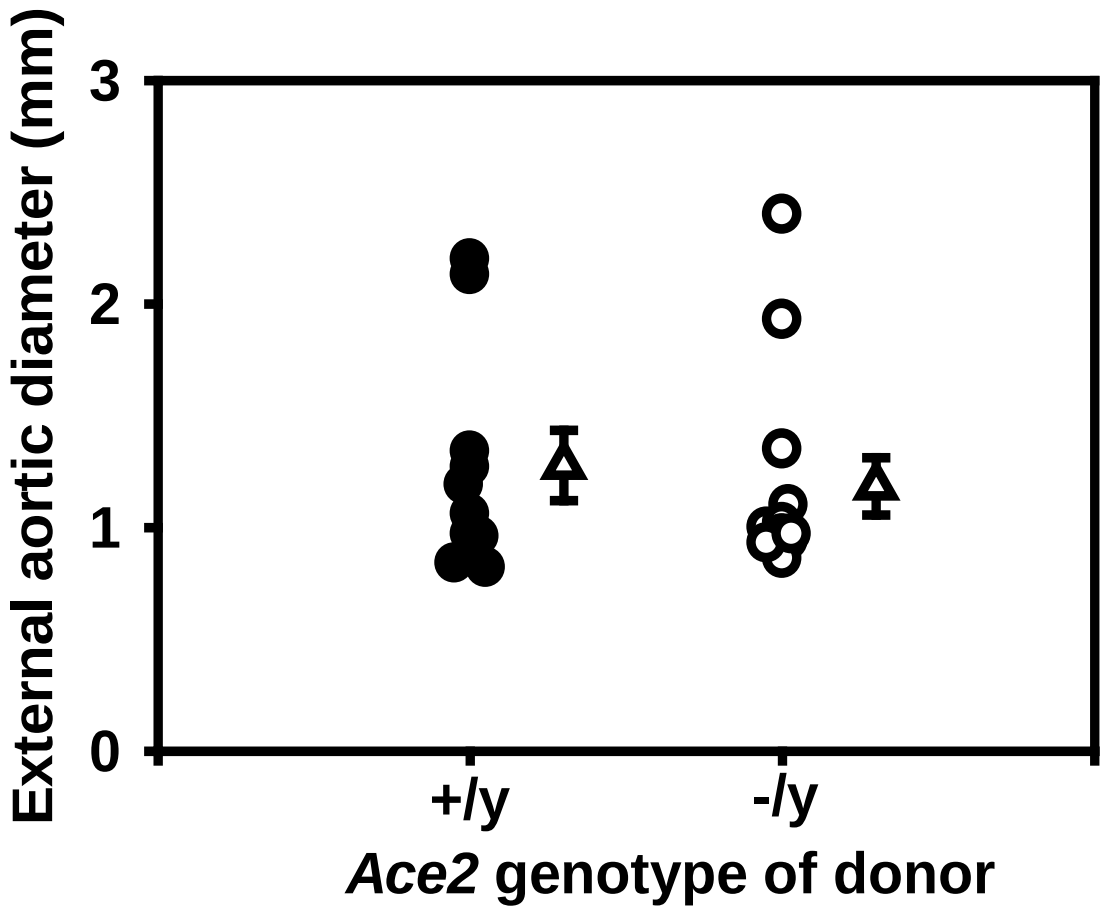


Figure VI. External aortic diameters of *Ace2*$^{+/y}$ mice that were transplanted with either *Ace2*$^{+/y}$ or $^{-/y}$ bone marrow and infused with AngII for 28 days (1,000 ng/kg/min). Closed or open circles represent individual mice and triangles represent the average of each group.

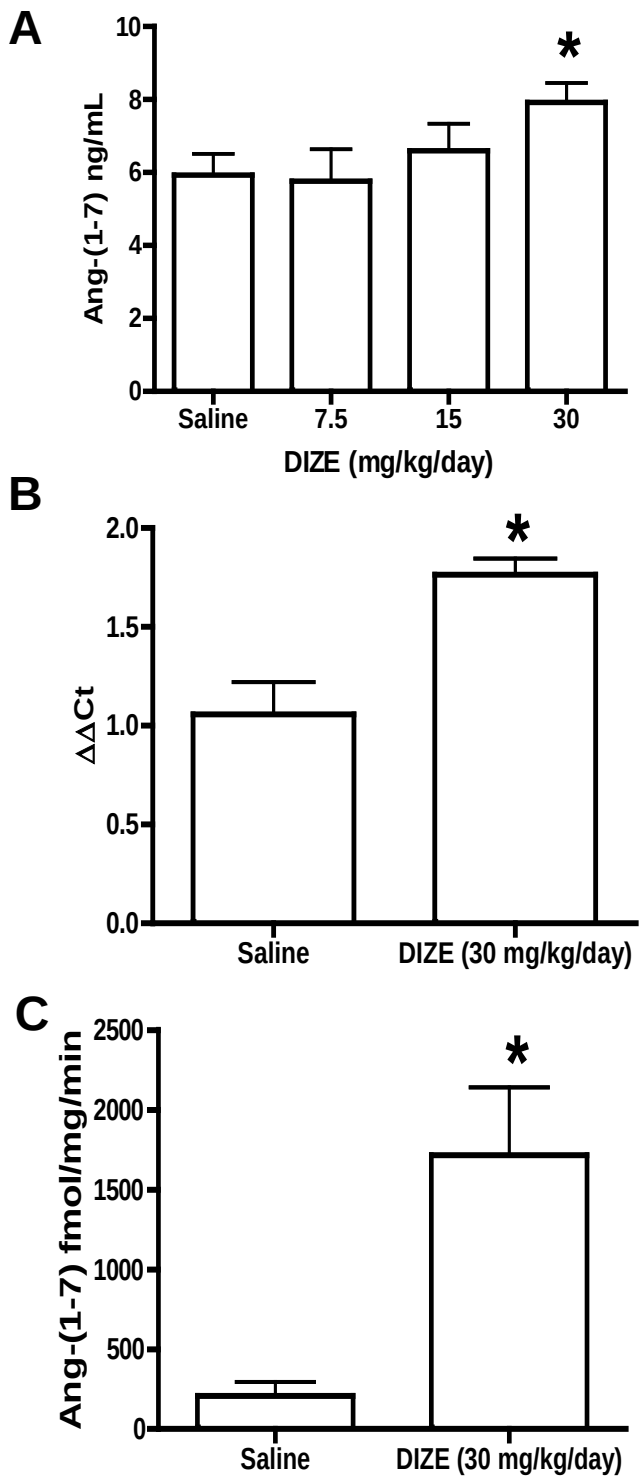


Figure VII. (A) Plasma Ang-(1-7) concentrations in mice infused with selected doses of DIZE for 7 days by osmotic minipump (N = 5/dose; P=0.04 for 30 mg/kg/day DIZE-treated mice compared to saline). ACE2 mRNA abundance (B) and activity (C) in kidneys from mice administered either saline or DIZE (30 mg/kg/day; N = 5; *P<0.05 compared to saline).

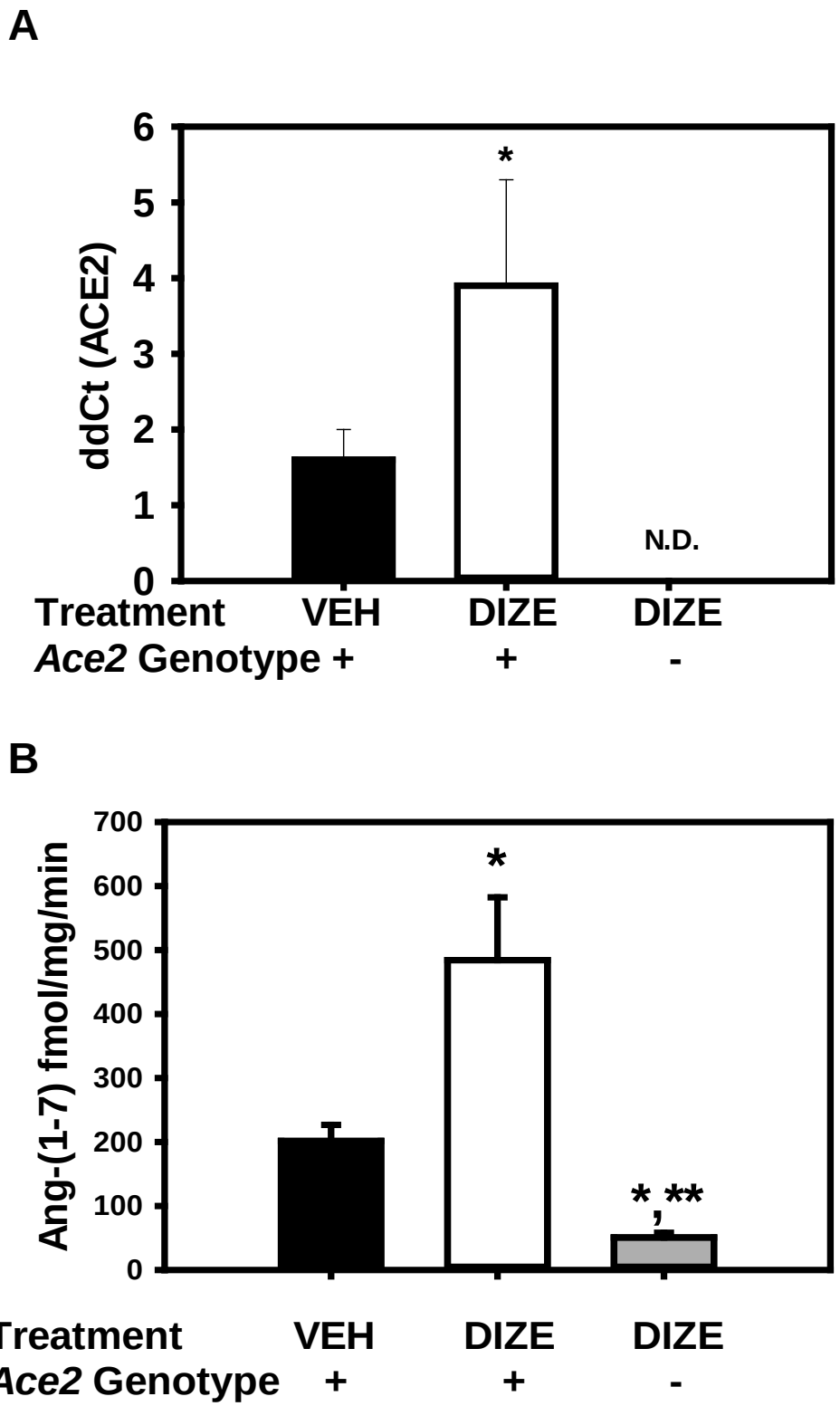


Figure VIII. ACE2 mRNA abundance (A) and activity (B) in kidneys from AngII-infused *Ace2*$^{+/y}$ or $^{-/y}$ mice; N = 5-8 mice/group) administered either vehicle (VEH) or DIZE (30 mg/kg/day by intramuscular injection). ND = not detected. *, P<0.05 compared to vehicle *Ace2*$^{+/y}$; **, P<0.05 compared to DIZE *Ace2*$^{+/y}$.

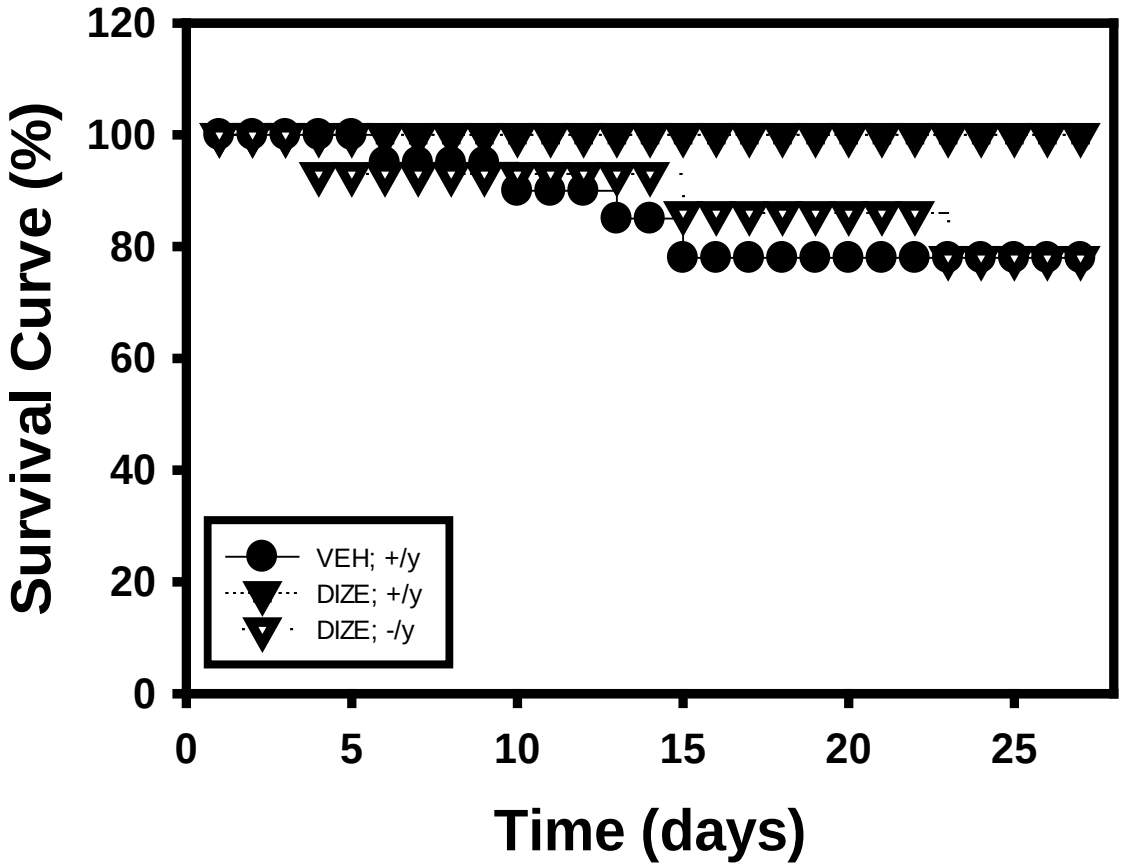


Figure IX. Kaplan-Meier survival curves for death due to AAA ruptures in AngII-infused *Ace2*$^{+/y}$ or $^{-/y}$ mice administered either vehicle (VEH) or DIZE (30 mg/kg/day). DIZE treatment prevented AAA ruptures in *Ace2*$^{+/y}$ male mice (*P<0.05 compared to vehicle and DIZE-treated *Ace2*$^{-/y}$ mice).