Vascular ADAM17 as a Novel Therapeutic Target in Mediating Cardiovascular Hypertrophy and Perivascular Fibrosis Induced by Angiotensin II

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Short title: Vascular ADAM17 mediates organ damage by AngII

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Supplementary Tables S1

S1A. M-mode echocardiography at 2 weeks after AngII infusion

	A17^{ff}smCre-/-		A17^{ff}smCre+/-	
	saline	AngII	saline	AngII
IVSd (mm)	0.620±0.050	0.781±0.097*	0.590±0.049	0.581±0.040†
LVIDd (mm)	3.72±0.15	3.02±0.12*	3.75±0.07	3.51±0.29
LVPWd (mm)	0.750±0.017	0.963±0.049*	0.638±0.060	0.755±0.070†
LVIDs (mm)	2.95±0.20	2.09±0.15*	2.88±0.11	2.70±0.21†
FS (%)	29.4±4.1	29.5±1.4	33.7±1.9	34.4±5.9

Mean±SD (n=8), $p<0.001$ compared with saline* or AngII† infusion.
IVSd: interventricular septum thickness in diastole; LVIDd: LV internal diameter in diastole; LVPWd: LV posterior wall thickness in diastole; LVIDs: LV internal diameter in systole; FS: fractional shortening.

S1B. Effects of VSMC ADAM17 deletion on characteristics of mice infused with AngII

Parameters		A17^{ff}Cre-/- saline	A17^{ff}Cre-/- AngII	A17^{ff}Cre+/- saline	A17^{ff}Cre+/- AngII
BW	(g)	25.4±4.2	19.9±3.5	24.5±2.4	21.8±3.7
SBP/DBP	(mmHg)	121±5/88±13	185±19*/142±19*	117±11/96±10	182±26*/131±18*
HR	(beats/min)	496±159	570±100	463±146	585±58

Mean±SD (n=8), *$p<0.001$ compared with saline infusion. BW: body weight; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate. No significance was detected among the parameters between Cre+/- and Cre-/- animals regardless of the treatment.

Supplementary Table S2

S2A. M-mode echocardiography at 2 weeks

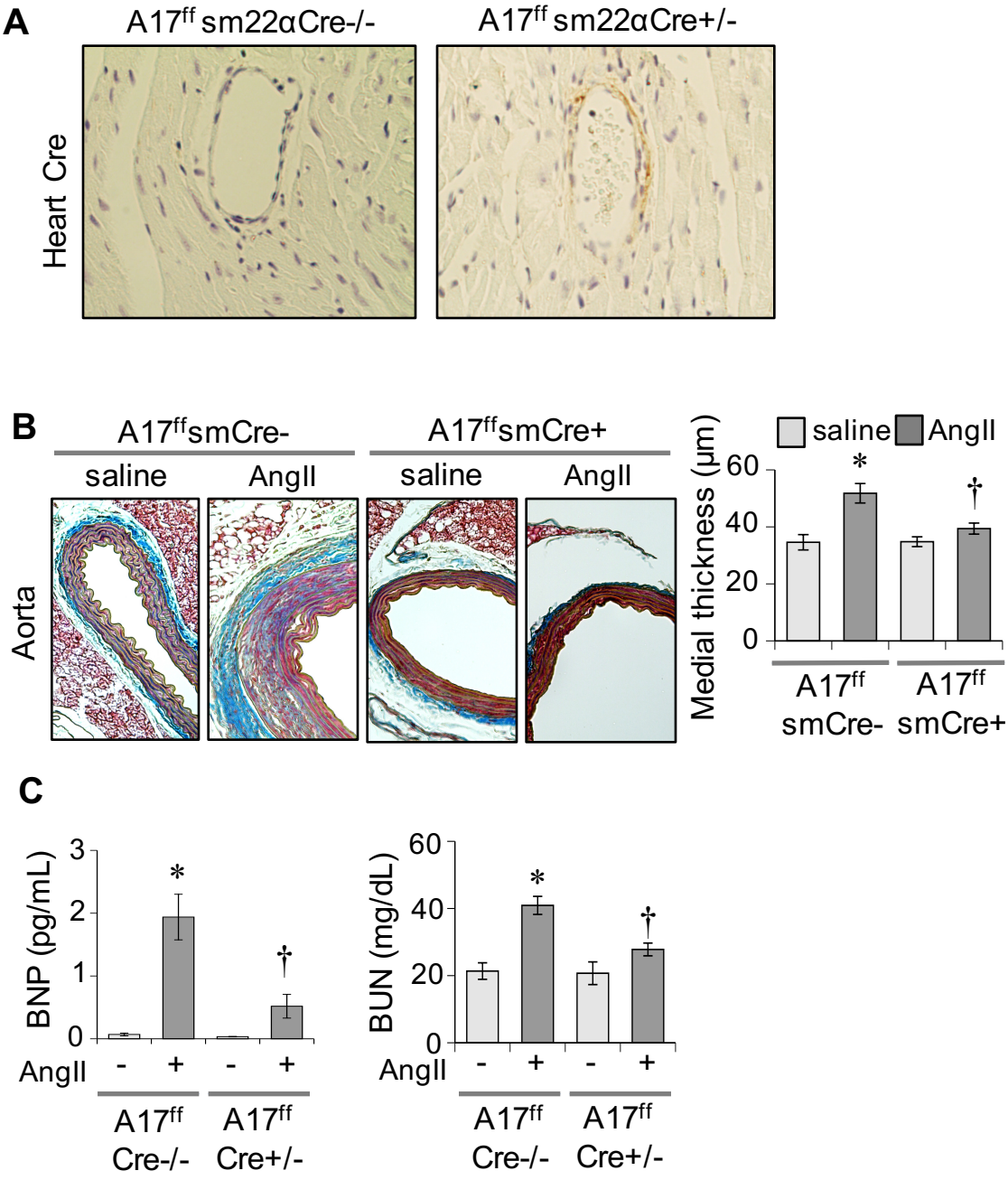
	saline	AngII+IgG2	AnGII+A9B8
IVSd (mm)	0.679±0.020	0.902±0.029*	0.774±0.025†
LVIDd (mm)	3.84±0.07	3.58±0.10*	3.65±0.12
LVPWd (mm)	0.707±0.020	0.847±0.020*	0.798±0.036†
LVIDs (mm)	3.11±0.07	2.634±0.08*	2.67±0.15
FS (%)	28.7±0.7	30.2±0.7	29.7±1.0

Mean±SD (n=8), $p<0.001$ compared with saline* or AngII† infusion.
IVSd: interventricular septum thickness in diastole; LVIDd: LV internal diameter in diastole; LVPWd: LV posterior wall thickness in diastole; LVIDs: LV internal diameter in systole; FS: fractional shortening.

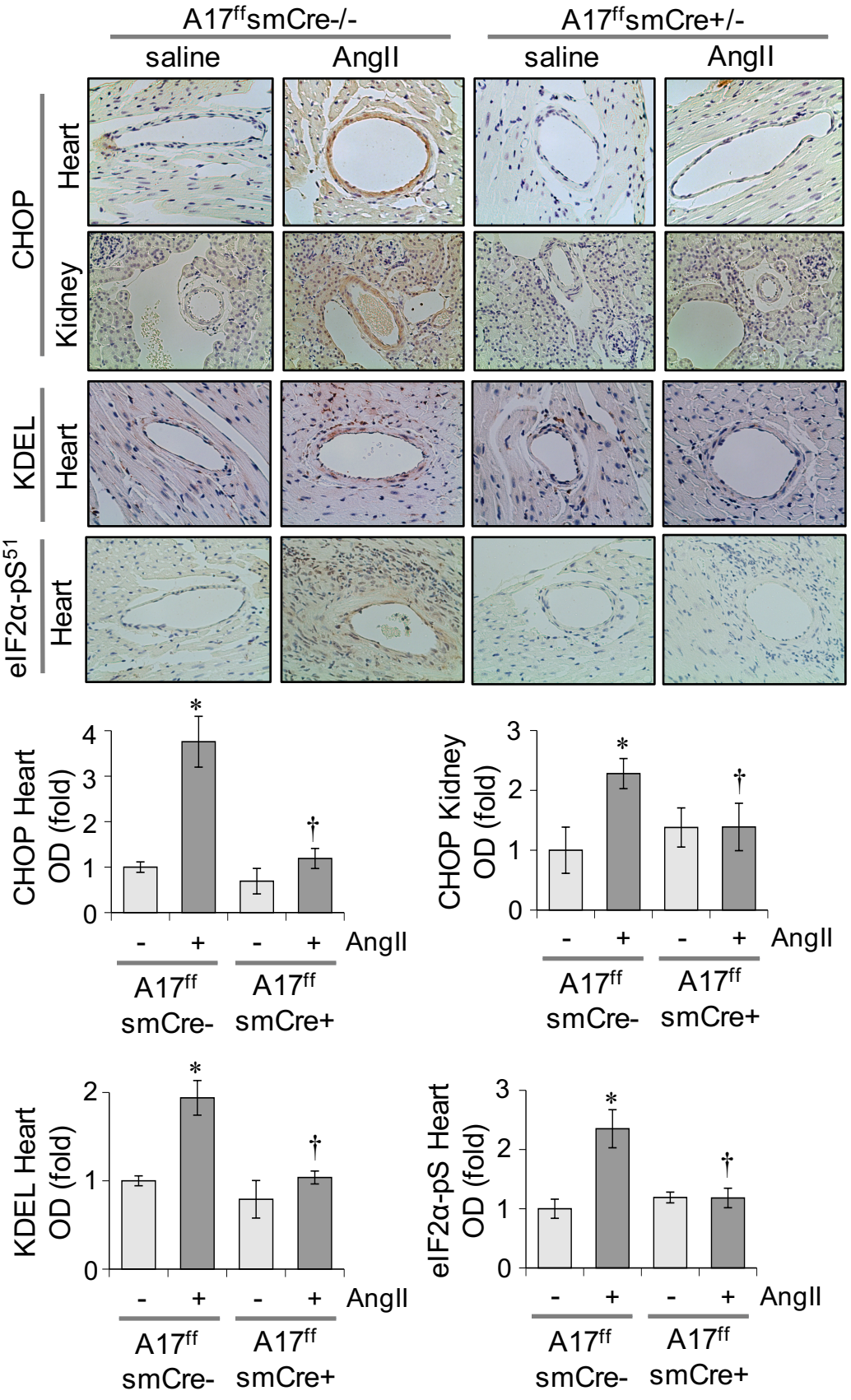
S2B. Effects of A9B8 on characteristics of mice infused with AngII

Parameters		saline	AngII+IgG2	AngII+A9B8
BW	(g)	23.3±2.2	25.0±2.0	24.6±2.1
SBP/DBP	(mmHg)	119±15/84±12	172±9*/137±17*	170±7*/141±6*
HR	(beats/min)	634±85	586±87	583±90

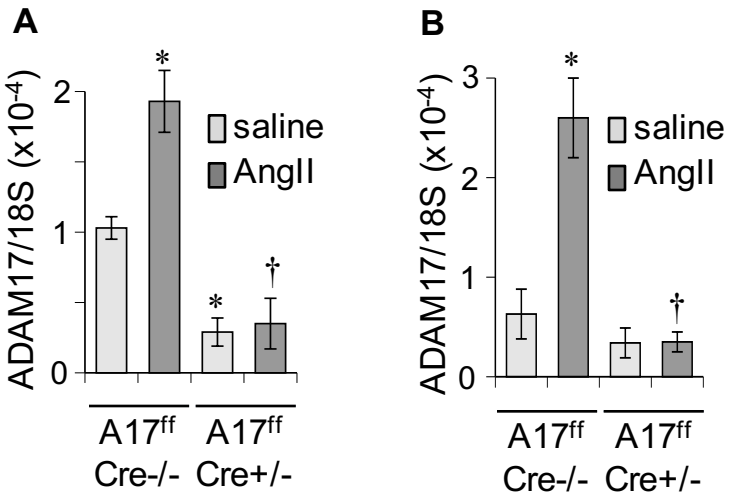
Mean±SD (n=6), *$p<0.001$ compared with saline infusion. BW: body weight; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate.



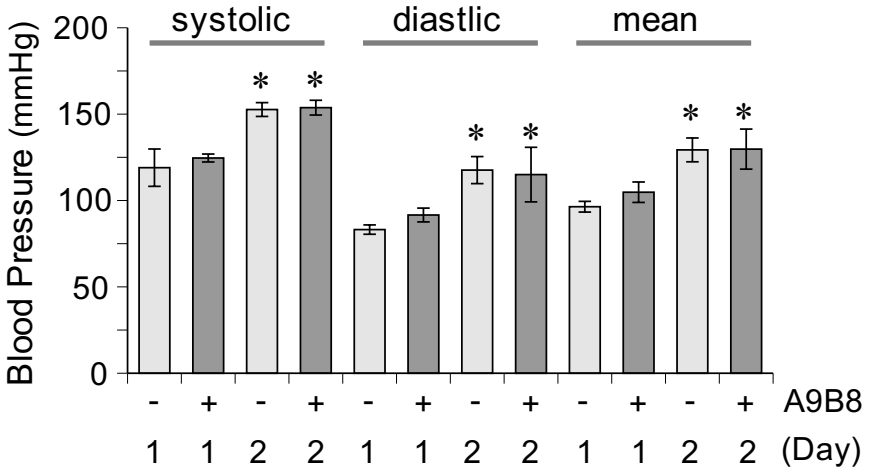
Supplementary Figure S1. **A**. Expression of Cre in VSMC ADAM17 deficient mice. Cre expression was analyzed by immunohistochemistry with anti-Cre antibody. **B**. VSMC ADAM17 deficient mice and control mice were infused with AngII or saline as in Figure 1. Aortas were stained with Masson's trichrome. Representative images are shown. Data are mean±SEM (n=6). **C**. Plasma BNP concentration. Plasma BUN concentration. Mean±SEM (n=6). *p<0.05 compared with saline control. †p<0.05 compared with AngII control.



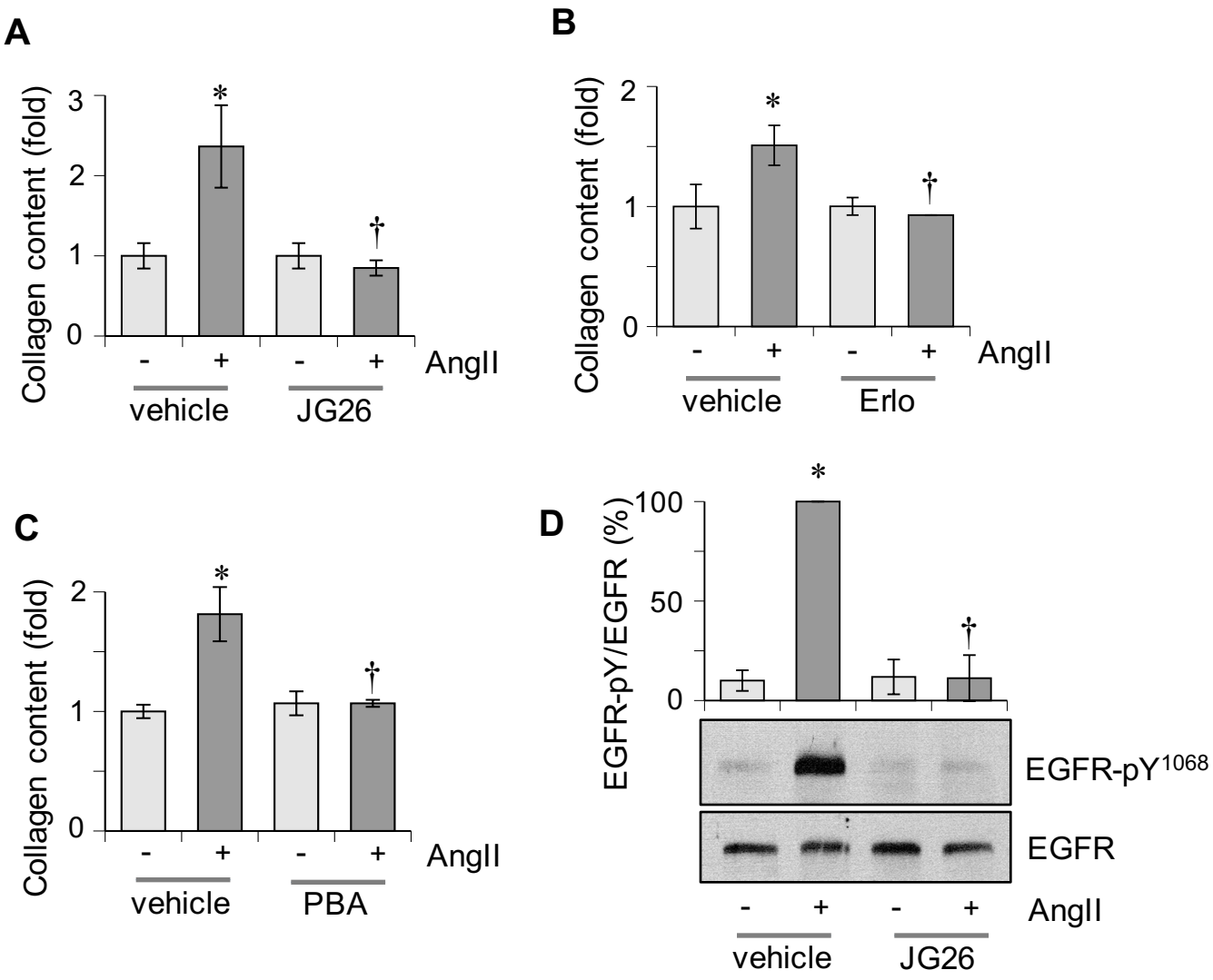
Supplementary Figure S2. **a.** Suppression of vascular ER stress in VSMC ADAM17 deficient mice. VSMC ADAM17 deficient mice and control mice were infused with AngII or saline as in Fig 1. Tissues were immuno-stained with the antibodies indicated. Representative images are presented (n=4). Data are mean±SEM (n=4). *p<0.05 compared with saline control. †p<0.05 compared with AngII control.



Supplementary Figure S3. The aorta (**A**) and heart (**B**) samples were evaluated for ADAM17 mRNA expression by qPCR. Mean±SEM (n=6). *p<0.05 compared with saline control. †p<0.05 compared with AngII control.



Supplementary Figure S4. Effects of ADAM17 inhibitory antibody, A9B8, on hypertension development induced by AngII. **A**: C57Bl/6 mice were infused with AngII from Day 0 with or without treatment of A9B8 on Day 1. Arterial pressure was evaluated by telemetry on Day 1 and Day 2 (Mean±SEM, n=3). Significant blood pressure elevation in response to AngII infusion was observed at Day 2 compared with Day 1 regardless of the antibody treatment. *$p<0.05$ compared with corresponding Day1 values.



Supplementary Figure S5. **A**: Rat aortic VSMCs pretreated with ADAM17 inhibitor JG26 (1 μmol/L) or vehicle (DMSO final concentration 0.1%) for 30 min were stimulated with 100 nmol/L AngII for 48 hours and extracellular collagen accumulation was quantified. Mean±SD (n=4). **B**: VSMCs pretreated with EGFR inhibitor erlotinib (Erlo) or vehicle (DMSO final concentration 0.1%) for 30 min were stimulated with 100 nmol/L AngII for 48 hours and extracellular collagen accumulation was quantified. Mean±SD (n=4). **C**: VSMCs pretreated with or without PBA (10 mmol/L in DMEM) were stimulated with 100 nmol/L AngII for 48 h and extracellular collagen accumulation was quantified. Mean±SD (n=4). **D**: VSMCs pretreated with JG26 (1 μmol/L) or vehicle (DMSO final concentration 0.1%) for 30 min were stimulated with 100 nmol/L AngII for 2 min and immonoblottings were performed with antibodies as indicated. Mean±SD (n=4). *p<0.05 compared with vehicle control. †p<0.05 compared with AngII control.