Supplement Material

Extended Materials and Methods Section

Sample collection and western blots. Tissue samples were collected and frozen immediately in liquid nitrogen. For Western blot, tissue homogenates were separated on SDS-polyacrylamide gels and transferred to PVDF nitrocellulose membranes. DDAH1 antibody was generated as we previously described [1]. DDAH2 antibody was obtained from Abcam. The secondary antibodies were from Bio-Rad Laboratories. Antibodies against PRMT1 (protein arginine methyltransferase 1), PRMT3 (protein arginine methyltransferase 3) are from Sigma. eNOS antibody is from BD Biosciences. CAT (cationic amino acid transporter) and GAPDH antibodies are from Santa Cruz Biotechnology Inc.

RT quantitative PCR. 2μg of total RNA was used for reverse transcription reaction (Applied Biosystems) followed by quantitative PCR using SYBR® Green PCR Master Mix (Applied Biosystems). Primer pairs 5'-CAA TAG GGT CCA GCG AAT CTG C-3' and 5'-GGG TAC AGT GAG CTT GTC ATA ACG-3' were used to amplify DDAH1. Primer pairs 5'- GAG CTG AGA TCG TGG CAG ACA-3'/5'- GGG AGG GTC AGA GAG GCG TAG-3' were used to amplify DDAH2.

Measurement of NO production in vessel rings. Cross sections of aorta (~2mm) were isolated and incubated in endothelial basal medium-2 (EBM-2, Cambrex) supplemented with 100 μM L-arginine (Sigma), and stained with 10μM NO-specific fluorescent probe 4, 5-diaminofluoresceine diacetate (DAF-2 DA) dye (Sigma) at 37°C for 30 minutes. Some vessel sections were incubated in EBM-2 media without L-arginine, and 100 μM L-NAME was added during the last 20 minutes of DAF-2 DA staining. After staining, vessel rings were washed with

DPBS and fluorescence intensity was recorded every 10 seconds for 5 minutes using an Olympus FluoView 1000 confocal microscope [1].

ADMA content and NOx production in small mesenteric vessels. Mesenteric microvessels were collected and stored in liquid nitrogen. ADMA content of small mesenteric vessels was determined using the ELISA method [2]. NOx production by these microvessels was also determined using the colorimetric assay kit from Cayman Chemical Company.

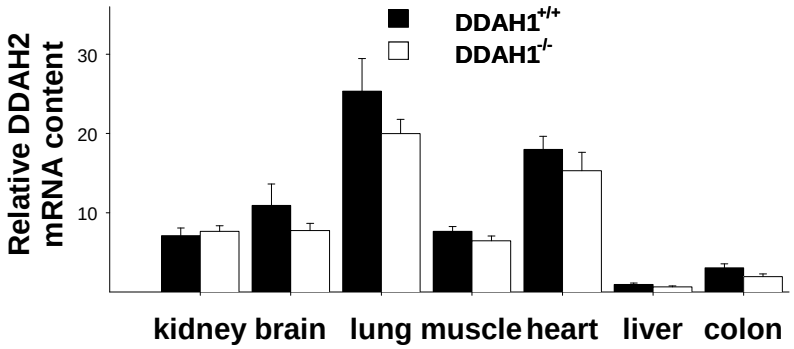
Supplemental Data

Supplementary Table I. Anatomic and functional data of DDAH1 KO mice and wild type controls under control conditions

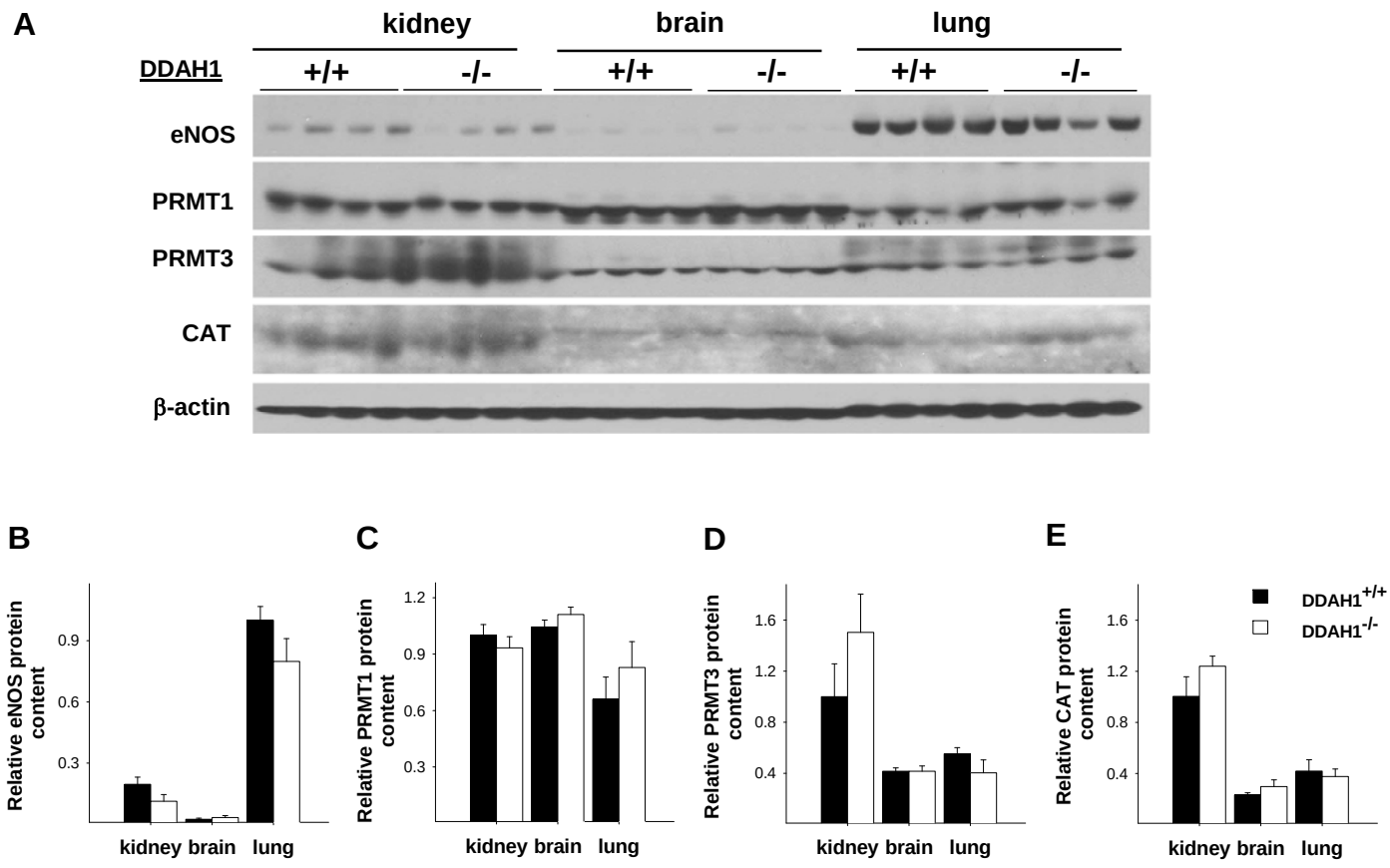
	Parameters	Wild type cont	DDAH1 KO
Anatomic data			
	Number of mice	8	8
	Body weight (g)	30.2 ± 0.81	29.8 ± 1.11
	Heart weight (mg)	127± 5.43	133 ± 6.63
	Lung weight (mg)	159 ± 3.19	159 ± 5.16
	Kidney weight (mg)	385 ± 11.2	372 ± 12.2
	Ratio of heart weight to bodyweight (mg/g)	4.2 ± 0.11	4.5 ± 0.09
	Ratio of lung weight to bodyweight (mg/g)	5.3 ± 0.17	5.4 ± 0.08
	Ratio of kidney weight to bodyweight (mg/g)	12.8 ± 0.19	12.5 ± 0.31
Cardiac functional data			
	Number of mice	8	8
	Heart rate (beat/min)	543 ± 16.1	529 ± 25.6
	LV end diastolic diameter (mm)	3.68 ± 0.20	3.83 ± 0.21
	LV end systolic diameter (mm)	2.20 ± 0.16	2.48 ± 0.24
	LV ejection fraction (%)	78.7 ± 1.98	72.7 ± 3.62
	LV wall thickness at end systole (mm)	1.20 ± 0.02	1.21 ± 0.01
	LV wall thickness at end diastole (mm)	0.75 ± 0.02	0.77 ± 0.02

Data are mean ± SE.

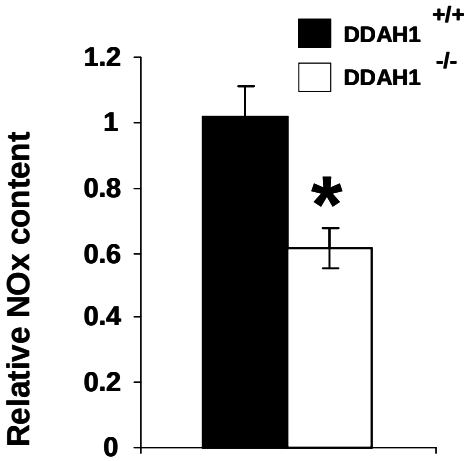
Supplementary Figure I. Global-DDAH1$^{-/-}$ had no effect on DDAH2 mRNA expression in tissues tested.



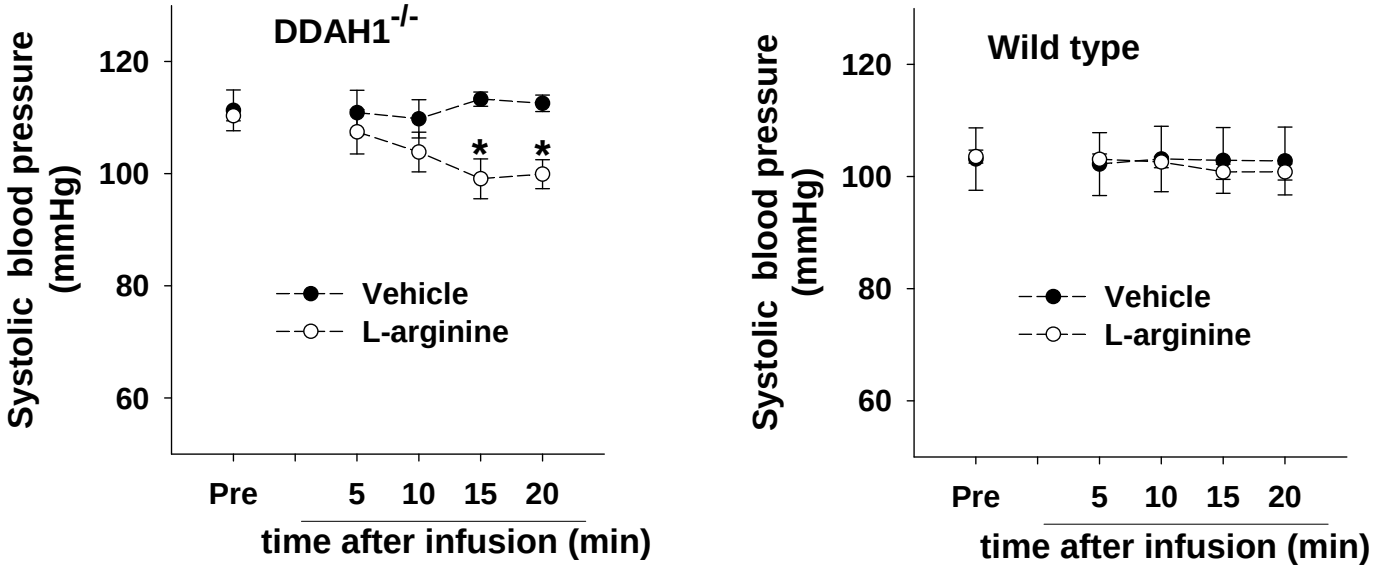
Supplementary Figure II. Global-DDAH1$^{-/-}$ had no significant effect on protein expression of eNOS, PRMT1, PRMT3, and CAT in all tissues tested.



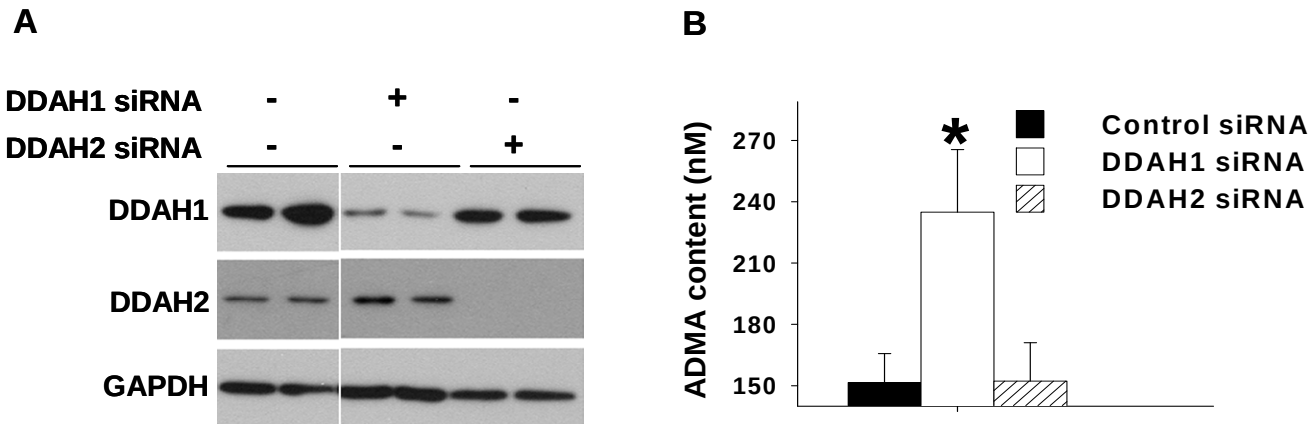
Supplementary Figure III. Total NOx production in mesenteric microvessels was significantly decreased in DDAH1$^{-/-}$ mice * p<0.05.



Supplementary Figure IV. L-arginine (400mg/kg, iv) infusion normalized blood pressure in DDAH1$^{-/-}$ mice.



Supplementary Figure V. Selective gene knockdown of DDAH1 but not DDAH2 caused ADMA accumulation in cultured HUVEC.



References

1. Hu X, Xu X, Zhu G, Atzler D, Kimoto M, Chen J, Schwedhelm E, Luneburg N, Boger RH, Zhang P, Chen Y. Vascular Endothelial-Specific Dimethylarginine Dimethylaminohydrolase-1-Deficient Mice Reveal That Vascular Endothelium Plays an Important Role in Removing Asymmetric Dimethylarginine. *Circulation.* 2009;120:2222-2229.
2. Schulze F, Wesemann R, Schwedhelm E, Sydow K, Albsmeier J, Cooke JP, Böger RH. Determination of asymmetric dimethylarginine (ADMA) using a novel ELISA assay. *Clinical Chemistry and Laboratory Medicine.* 2004;42:1377-1383.