Cell Reports Medicine, Volume *4*

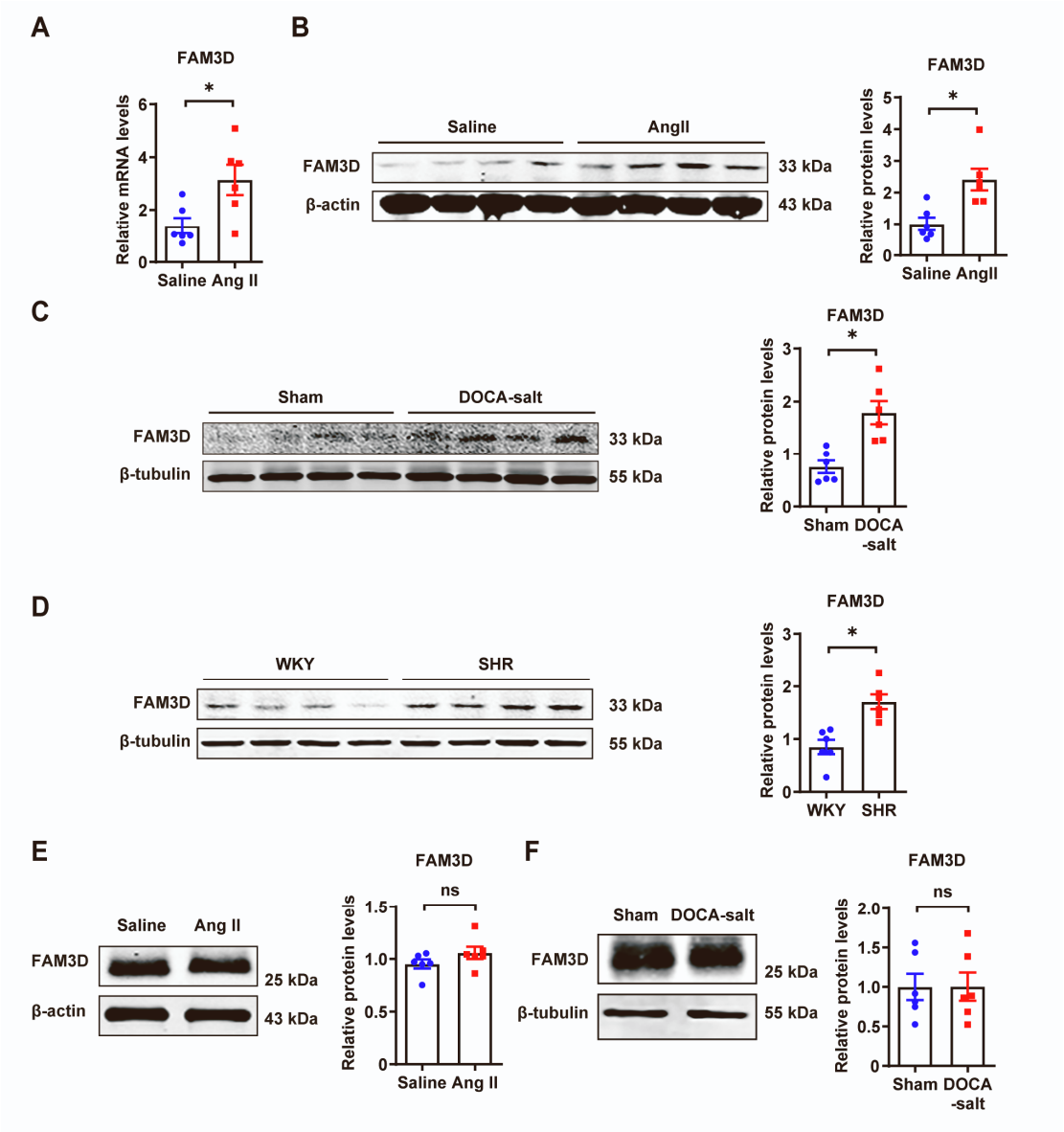
Supplemental information

Targeting cytokine-like protein FAM3D lowers blood pressure in hypertension

Yicong Shen, Zhigang Dong, Fangfang Fan, Kaiyin Li, Shirong Zhu, Rongbo Dai, Jiaqi Huang, Nan Xie, Li He, Ze Gong, Xueyuan Yang, Jiaai Tan, Limei Liu, Fang Yu, Yida Tang, Zhen You, Jianzhong Xi, Ying Wang, Wei Kong, Yan Zhang, and Yi Fu

Supplemental information

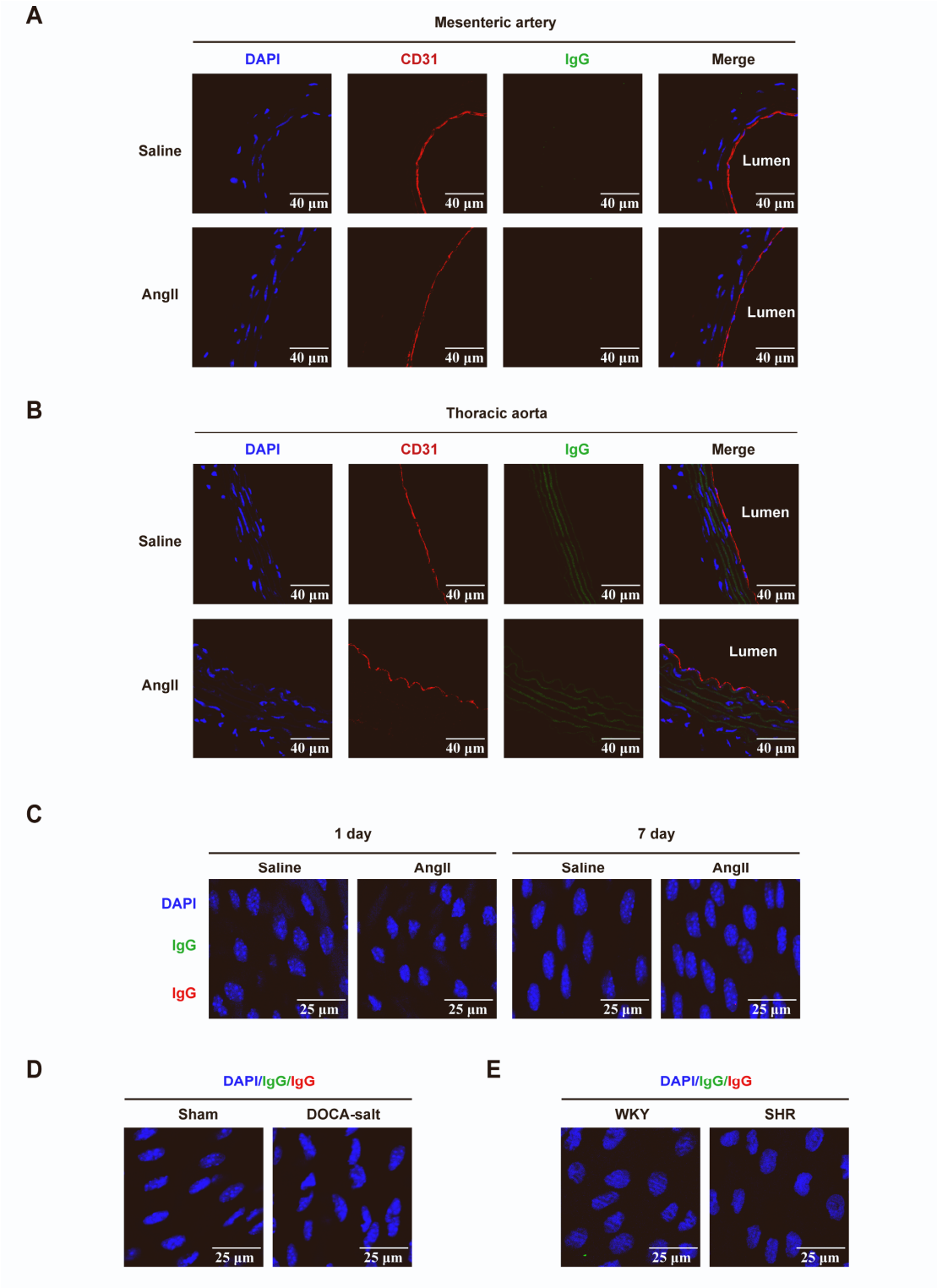
Supplemental Figure 1



Supplemental Figure 1. FAM3D is upregulated in arteries from hypertensive mice or rats, Related to Figure 1. A, Real-time PCR analysis of FAM3D expression in the aortas of C57BL/6 mice treated with saline or AngII for 7 days. N = 6. Data are represented as mean ± SEM. Unpaired Student's *t*-test, $*P < 0.05$. **B-D,** Western blot analysis of FAM3D expression in the aortas from C57BL/6 mice treated with saline or AngII for 7 days (B), from sham-operated or DOCA-salt-induce

C57BL/6 mice (C) and from Wistar-Kyoto (WKY) rats or spontaneously hypertensive rats (SHR) (D). N = 6. Data are represented as mean ± SEM. Unpaired Student's *t*-test, **P* < 0.05. **E-F**, Representative western blot and quantification of FAM3D expression in the colons from C57BL/6 mice with saline or AngII infusion for 7 days (E) and from C57BL/6 mice with sham or DOCA-salt treatment (F) N = 6. Data are represented as mean ± SEM. Unpaired Student's *t*-test.

Supplemental Figure 2

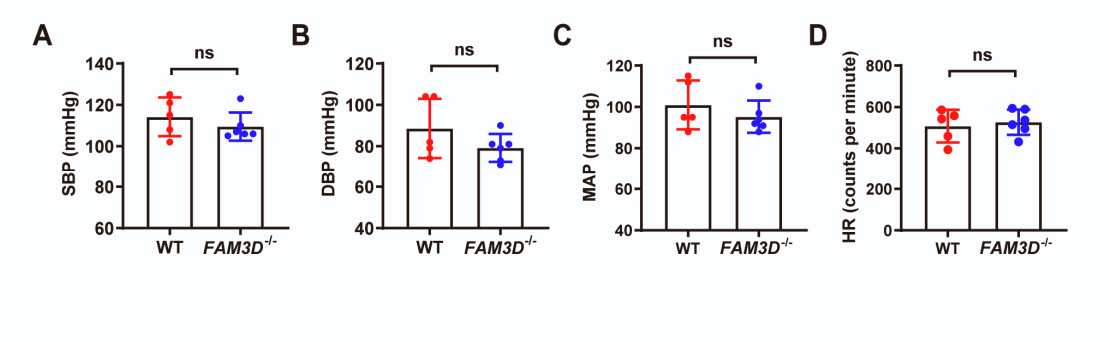


Supplemental Figure 2. Negative controls of immunofluorescent staining in arteries, Related to

Figure 1. A-B, Immunofluorescent staining using goat IgG corresponding to anti-FAM3D antibodies in

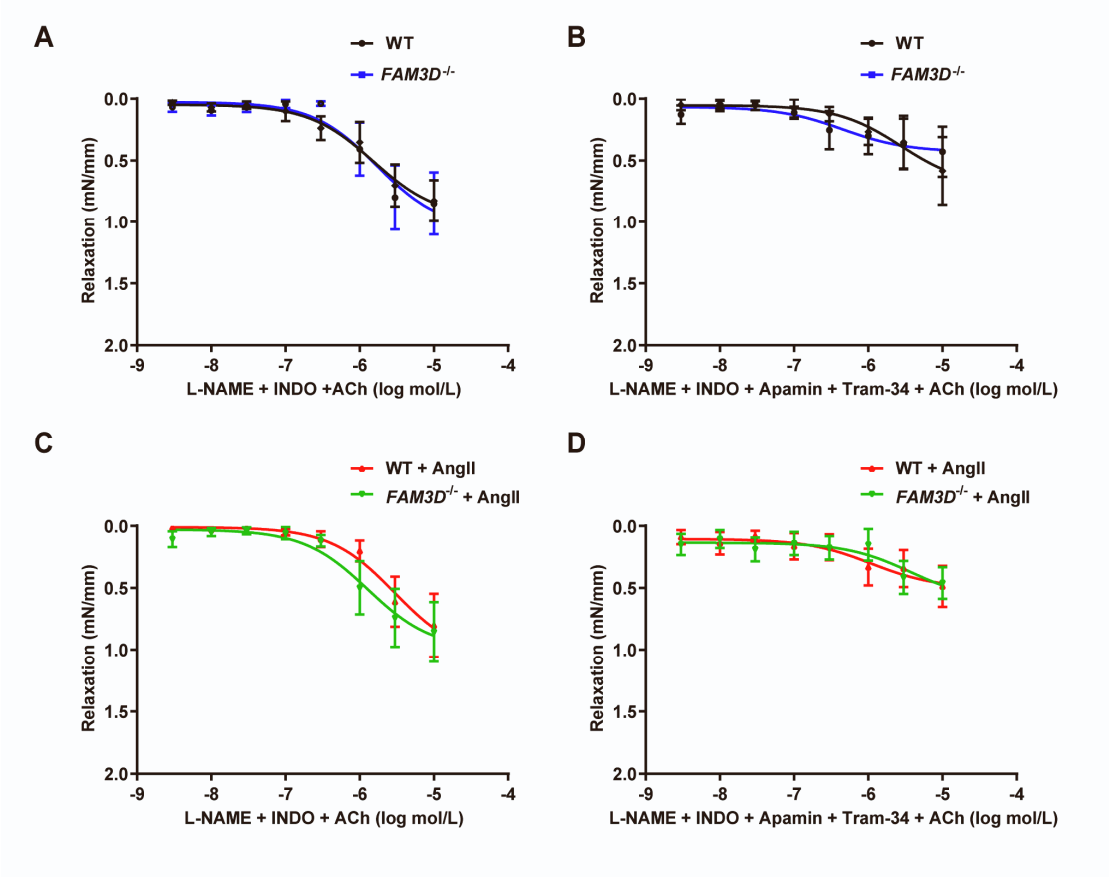
mesenteric arteries (A) and thoracic aortas (B) from C57BL/6 mice after saline or AngII infusion for 7 days. Scale bar, 40 μm. **C**, Immunofluorescent *en face* staining using goat and mouse IgGs respectively corresponding to anti-FAM3D and anti-VE-cadherin antibodies in the endothelial layer of thoracic aortas of C57BL/6 mice after saline or AngII infusion for 1 day or 7 days. Scale bar, 25 μm. **D**, Immunofluorescent *en face* staining using goat and mouse IgGs respectively corresponding to anti-FAM3D and anti-VE-cadherin antibodies in the endothelial layer of thoracic aortas of sham-treated or DOCA-salt-induced mice. Scale bar, 25 μm. **E**, Immunofluorescent *en face* staining using goat and mouse IgGs respectively corresponding to anti-FAM3D and anti-CD31 antibodies in the endothelial layer of thoracic aortas of WKY or SHR. Scale bar, 25 μm.

Supplemental Figure 3



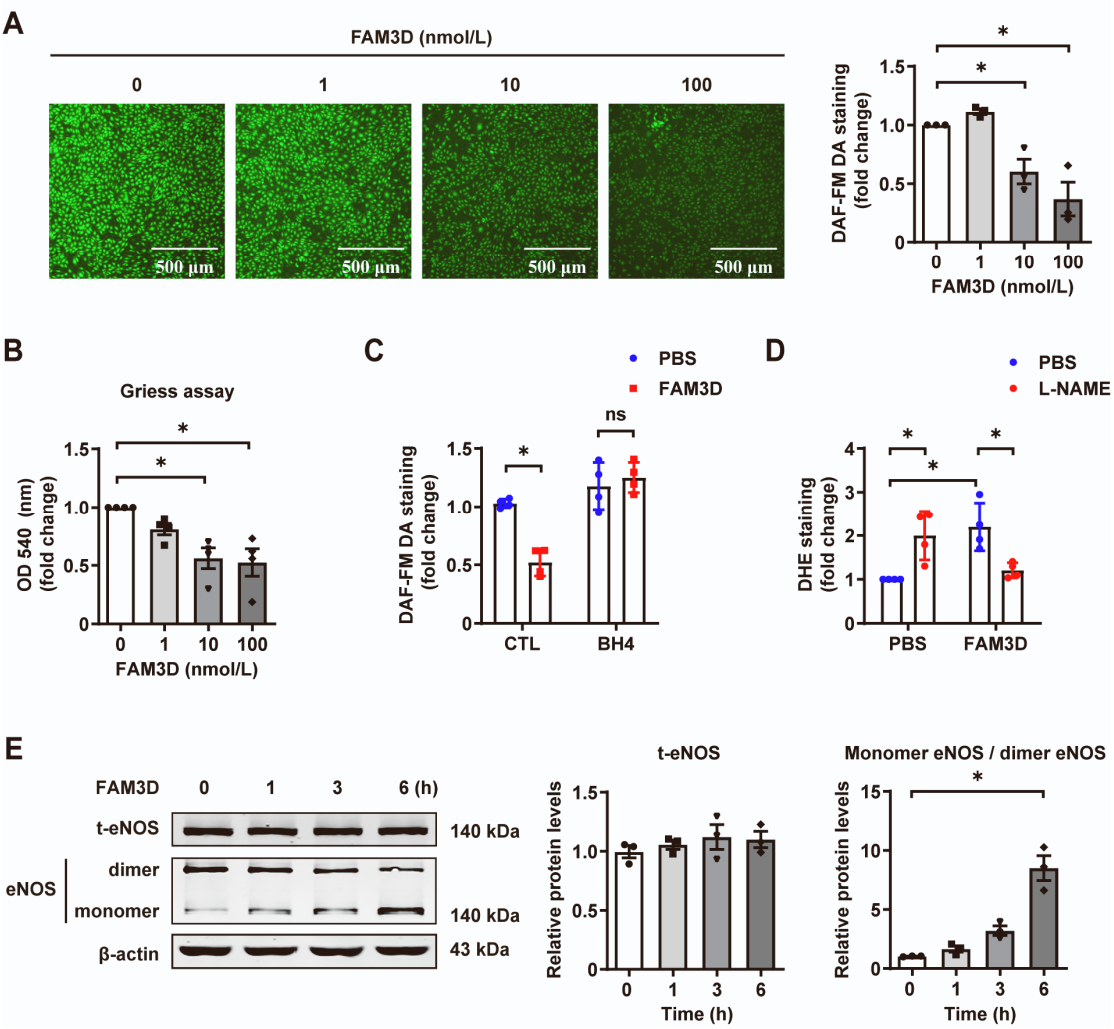
Supplemental Figure 3. Basel blood pressures and heart rates of WT and *FAM3D*$^{-/-}$ mice, Related to Figure 2. A-D, Telemetry measurements of systolic blood pressure (SBP) (A), diastolic blood pressure (DBP) (B), mean artery pressure (MAP) (C) and heart rate (HR) (D) of WT and *FAM3D*$^{-/-}$ mice. N=5-6. Data are represented as mean ± SEM. Unpaired Student's *t*-test.

Supplemental Figure 4



Supplemental Figure 4. Deficiency of FAM3D does not affect EDHF-induced vasodilation, Related to Figure 3. A-B, Endothelial hyperpolarizing factor (EDHF)-induced endothelial-dependent relaxation in response to Ach with or without EDHF inhibitor (Apamin and Tram-34) in the mesenteric arteries from WT and *FAM3D*$^{-/-}$ mice. N = 6. Data are represented as mean ± SEM. Two-way ANOVA by Tukey's multiple comparisons test. **C-D**, EDHF-induced endothelial-dependent relaxation in response to Ach with or without EDHF inhibitor (Apamin and Tram-34) in the mesenteric arteries from WT and *FAM3D*$^{-/-}$ mice infused with AngII for 14 days. N = 6. Data are represented as mean ± SEM. Two-way ANOVA by Tukey's multiple comparisons test.

Supplemental Figure 5



Supplemental Figure 5. FAM3D causes eNOS uncoupling in endothelial cells, Related to Figure 4.

A, Representative DAF-FM DA staining and quantification of intracellular nitric oxide (NO) in HUVECs treated with increasing amounts of FAM3D for 6 hours. N = 3. Data are represented as mean ± SEM. One-way ANOVA followed by Dunnett's multiple comparisons test, **P* < 0.05. **B**, Griess assay of NO release in the conditioned media of HUVECs treated with increasing amounts of FAM3D for 24 hours. N = 4. Data are represented as mean ± SEM. One-way ANOVA followed by Dunnett's multiple comparisons test, **P* < 0.05. **C**, Quantification of intracellular NO by DAF-FM DA staining in HUVECs treated with FAM3D (10 nmol/L) in the absence or presence of BH4 (50 μmol/L) for 6 hours.

N = 4. Data are represented as mean ± SEM. Two-way ANOVA followed by Sidak's multiple comparisons test, *$P < 0.05$. **D**, Quantification of O_2^- by DHE staining in HUVECs treated with FAM3D (10 nmol/L) for 6 hours and followed in the presence or absence of L-NAME (200 μmol/L) for another 30 minutes was measured by flow cytometry. N = 4. Data are represented as mean ± SEM. Two-way ANOVA followed by Tukey's multiple comparisons test, *$P < 0.05$. **E**, Representative western blot analysis and quantification of eNOS expression and monomerization (as the ratios of monomer to dimer eNOS) in HUVECs stimulated by FAM3D (10 nmol/L). N = 3. Data are represented as mean ± SEM. One-way ANOVA followed by Dunnett's multiple comparisons test, *$P < 0.05$.

Supplemental Table 1. Baseline characteristics of 80 pairs of cases and controls, Related to Figure 1.

Variables	Control	Case	*P* value
	n = 80	n = 80	
Age (years)	52.40 ± 6.71	53.00 ± 6.71	0.573
Male, n (%)	35 (43.75%)	35 (43.75%)	1.000
Female, n (%)	45 (56.25%)	45 (56.25%)	1.000
BMI (kg/m^2)	25.42 ± 2.81	25.70 ± 2.87	0.538
SBP (mmHg)	115.17 (110.33-117.67)	160.17 (149.92-166.67)	<0.001
DBP (mmHg)	68.05 ± 5.93	91.49 ± 6.78	<0.001
HR (counts per minute)	75.83 (70.33-82.75)	81.00 (70.58-88.33)	0.017
FAM3D (ng/mL)	5.54 (4.51-7.15)	7.27 (5.33-10.70)	<0.001
TC (mmol/L)	4.98 (4.45-5.74)	5.26 (4.87-5.75)	0.091
TG (mmol/L)	1.12 (0.81-1.62)	1.29 (0.97-1.77)	0.099
FBG (mmol/L)	5.38 (5.02-5.71)	5.52 (5.24-5.81)	0.027

Data are presented as mean ± standard deviation for normally distributed continuous variables, median (interquartile range) for non-normally distributed continuous variables, and number (percentage) for dichotomous variables. BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; FAM3D, family with sequence similarity 3, member D; TC, total cholesterol; TG, triglyceride; FBG, fasting blood-glucose.

Supplemental Table 2. Univariable and multivariable conditional logistic regression analyses for the association between FAM3D level and hypertension, Related to Figure 1.

Variables	Non-adjusted model		Multivariate-adjusted model	
	OR (95%*CI*)	*P* value	*OR* (95%*CI*)	*P* value
plasma FAM3D, ng/mL	1.06 (1.00, 1.13)	0.0403	1.09 (1.01, 1.17)	0.0226
FAM3D Tertiles				
<5.20	1.0		1.0	
5.20-7.89	1.86 (0.76, 4.57)	0.1732	1.23 (0.45, 3.36)	0.6924
⩾7.89	4.78 (1.88, 12.19)	0.0010	7.34 (2.27, 23.70)	0.0009

Multivariable-Adjusted Model adjusted for total cholesterol, triglycerides, fasting glucose and heart rate. OR indicates odd ratio; CI, confidence interval; FAM3D, family with sequence similarity 3, member D.

Supplemental Table 3. Characteristics of AngII-induced hypertensive mice after 7 day, Related to Figure 1.

Group	Saline	AngII
Number	6	6
Age (week)	12	12
Weight (g)	26 ± 0.6	26 ± 0.5
SBP (mmHg)	97 ± 3	144 ± 4*

Unpaired Student's *t*-test, *$P < 0.05$. SBP, systolic blood pressure.

Supplemental Table 4. Characteristics of DOCA-salt hypertensive mice after 7 day, Related to Figure 1.

Group	Sham	DOCA salt
Number	6	6
Age (week)	7	7
Weight (g)	20 ± 0.2	20 ± 0.1
SBP (mmHg)	91 ± 1	116 ± 1*

Unpaired Student's *t*-test, *$P < 0.05$. SBP, systolic blood pressure.

Supplemental Table 5. Characteristics of WKY rats and SHR, Related to Figure 1.

Group	WKY rats	SHR
Number	6	6
Age (week)	8	8
Weight (g)	222 ± 4	237 ± 4
SBP (mmHg)	149 ± 2	195 ± 4*

Unpaired Student's *t*-test, *$P < 0.05$. WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rats; SBP, systolic blood pressure.

Supplemental table 6. Real-time qPCR primers, Related to STAR Methods.

Genes	Forward primers	Reverse primers
mFAM3D	GACAGCTTTGACATGTACTCTGGA	CACCAGGGTGCTATCTGGA
mβ-actin	GTGACGTTGACATCCGTAAAGA	GCCGGACTCATCGTACTCC