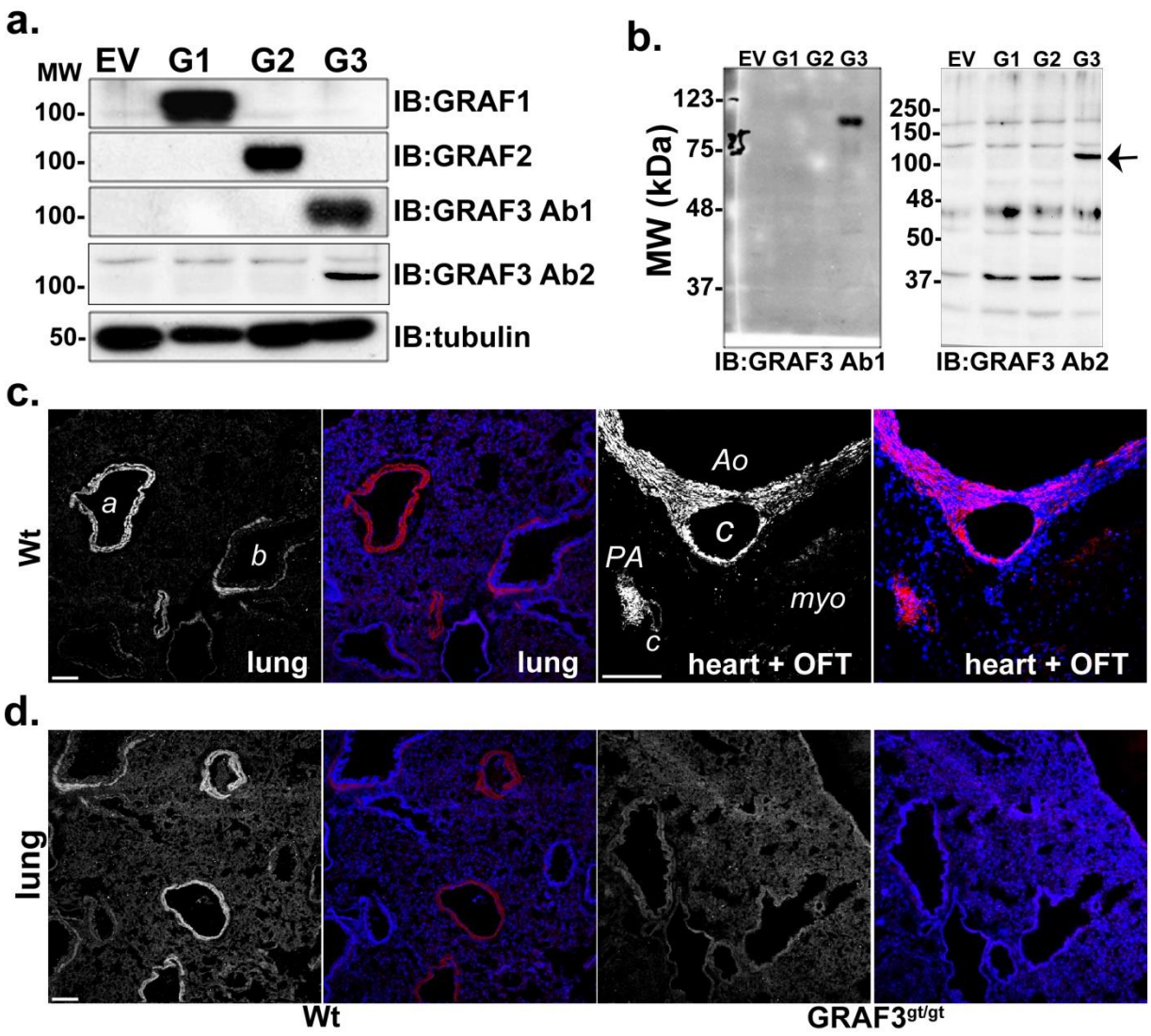
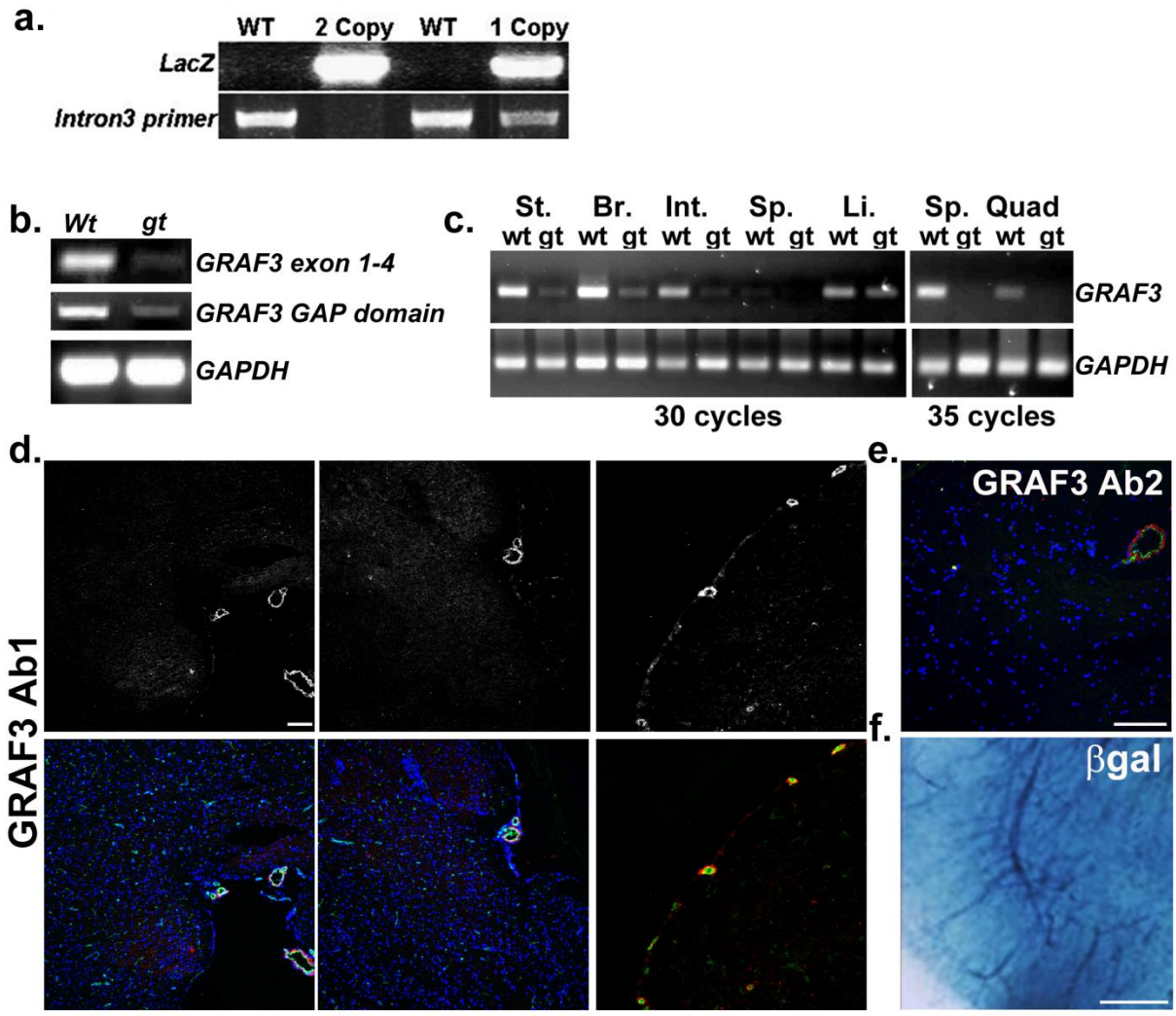
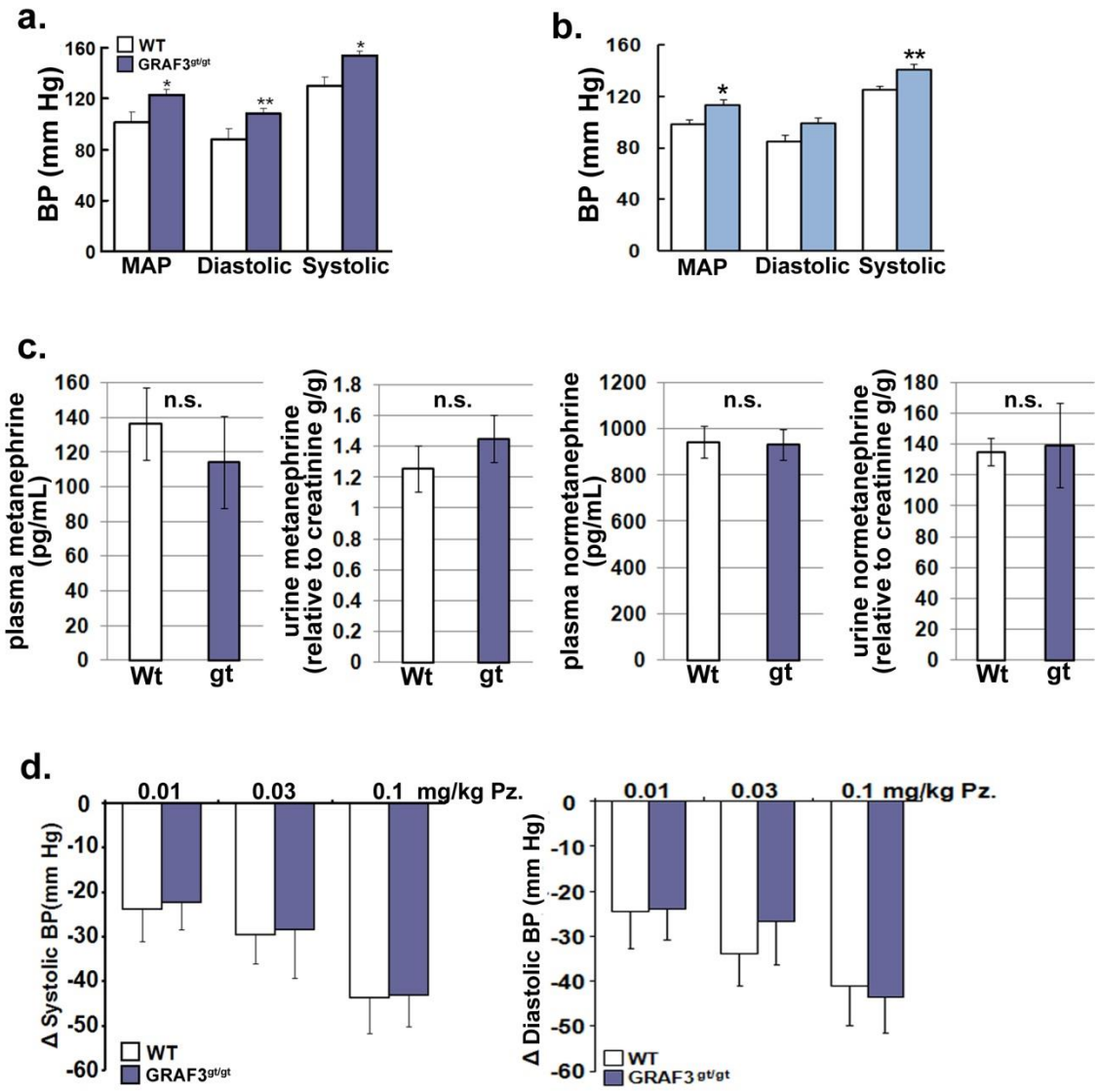


Supplementary Figure S1. Specificity of GRAF3 antibodies. a, b COS cells were transfected with GRAF (G)1, 2, and 3 cDNAs or empty vector (EV) for 24 h. Cell lysates were subjected to SDS-PAGE and immuno-blotting was performed with the indicated antibody. **a,** cropped blots

b, full length immunoblots. **c, d** Tissues from Wt or GRAF3$^{gt/gt}$ mice (2-3 wk old) were frozen in OCT, sectioned and stained with GRAF3 Ab2 (white, left panels or red, right panels;) DAPI is shown in blue). *a*, arteriole/artery; *b*, bronchi; Ao, aorta; PA, pulmonary artery; *c*, coronary artery; myo, myocardium; OFT, outflow tract. Scale bars are 100 microns

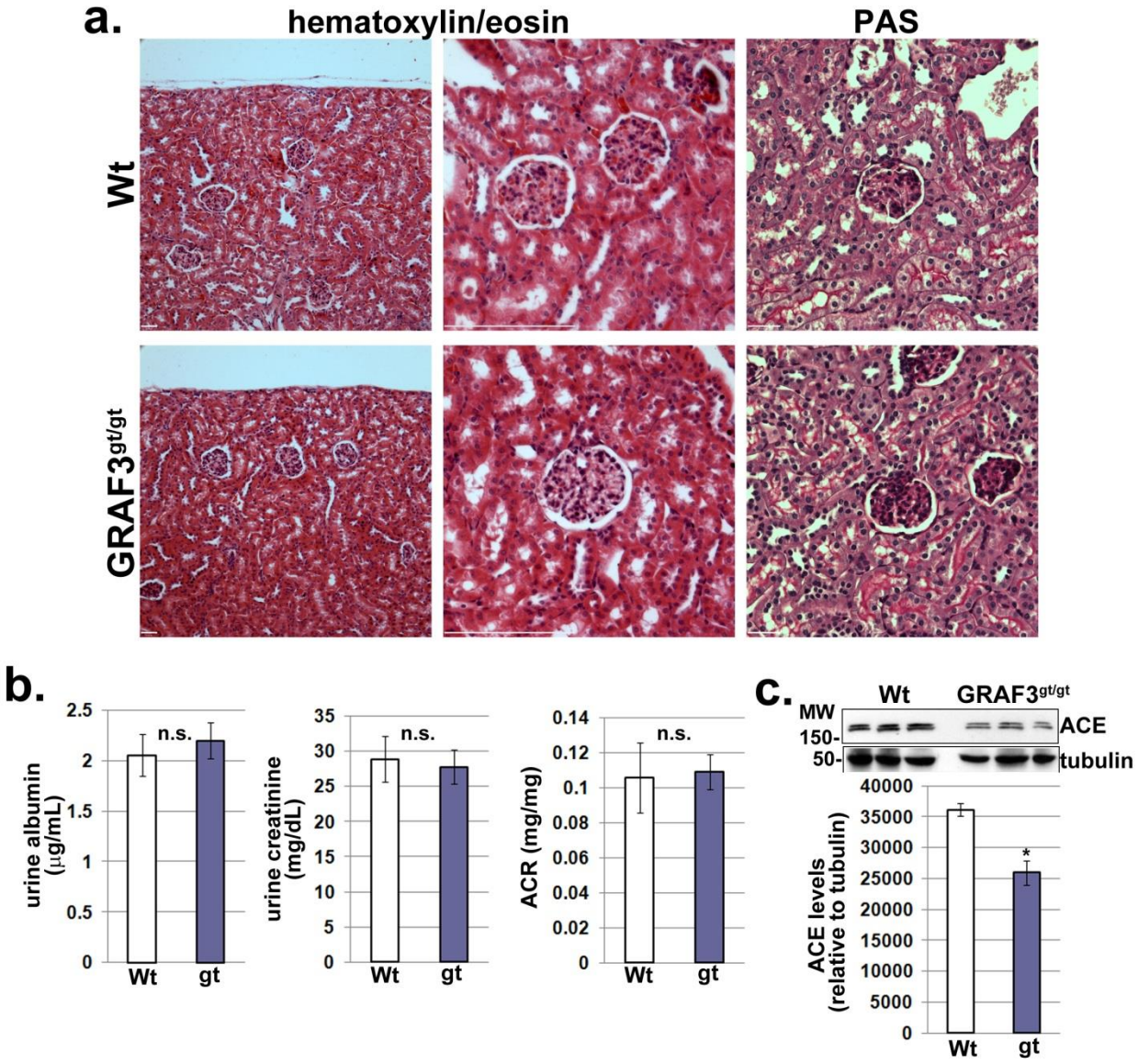


Supplementary Figure S2. Characterization of GRAF3$^{gt/gt}$ mice. **a,** Genotyping method: LacZ
primers verify presence of Gene trap insert. Intron3 primers directed toward sequences disrupted by gene trap integration only amplify a product from Wt allele (identifies 1 or 2 copies of inhibitory gene trap). **b,** RT-PCR analysis of GRAF3 message in SMC isolated from Wt or GRAF3$^{gt/gt}$ (gt) aortae using indicated primers. **c,** RT-PCR analysis of GRAF3 message in various tissues from wt and GRAF$^{gt/gt}$ mice using exon 1-4 primers. Note elevated levels of expression in tissues that contain a high SMC content. Data are representative of 3 mice/genotype. **d,e** Frozen Sections from Wt brains were stained with indicated GRAF3 antibodies. Note selective expression of GRAF3 in the smooth muscle layer of vessels within the cerebellum and lobes of the cerebrum. Scale bars are 100 microns **f,** Whole mount LacZ-stained brain from GRAF3$^{gt/gt}$ mouse reveals selective expression in microvasculature. Scale bar is 0.5 mm.

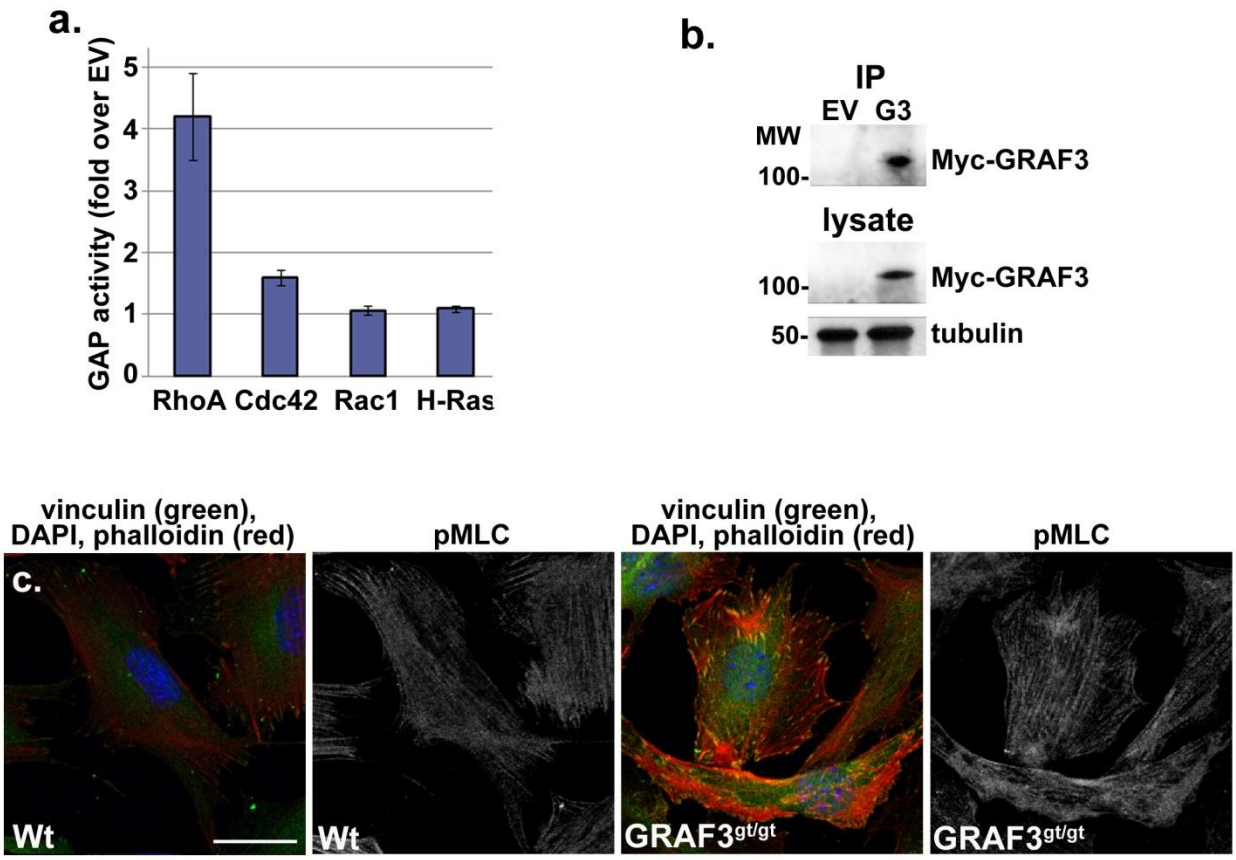


Supplementary Figure S3. Enhanced sympathetic signaling does not account for elevated

blood pressure in GRAF3$^{gt/gt}$ mice. **a,** Basal blood pressures of Wt (n=7) and GRAF3$^{gt/gt}$ (n=8) mice were measured by tail cuff for 6 days. Average recordings from the final day are shown **b,** Basal blood pressures of Wt (white bars, n=10) and GRAF3$^{gt/t}$ (light blue bars, n=13) mice were measured by tail cuff for 6 days. Average recordings from the final day are shown. **c,** Catecholamine metabolite concentration in plasma and urine from Wt (n=6) and GRAF3$^{gt/gt}$ (n=6) mice. **d,** Acute effects of prazosin (Pz) on blood pressure were monitored by tail-cuff following a 6 day acclimation. Shown is the maximal blood pressure change in mm Hg after injection. Mean values +/- s.e.m. for all panels and p values (Student's t-test) are expressed as : * $p<0.05$; ** $p<0.01$



Supplementary Figure S4. Kidney structure and function is normal in GRAF3$^{gt/gt}$ mice. a, Kidneys from 3 mo old Wt and GRAF3$^{gt/gt}$ mice were paraffin-embedded, sectioned and stained as indicated. Scale bars are 35 microns (right, left) and 140 microns (middle). Data are representative of 4 mice/genotype **b,** urine albumin and creatinine levels were analyzed in urine from Wt (n=9) and GRAF3$^{gt/gt}$ (n=8) mice. ACR is the albumin/creatinine ratio. **c,** Lungs from Wt and GRAF3$^{gt/gt}$ mice were harvested, lysed and analyzed by Western blotting for ACE and tubulin (loading control). bottom, Densitometric quantification of ACE levels relative to total tubulin (n=5/genotype). Mean values +/- s.e.m. are shown for panels **b** and **c** and p values (Student's t-test) are expressed as * $p<0.05$.



Supplementary Figure S5. GRAF3 functions as a RhoA-selective GTPase. a, GAP activity of GRAF3 towards Rho family members RhoA, Cdc42, Rac1 and Ras. Anti-Myc immunoprecipitates from COS cells expressing vehicle control or Myc-GRAF3 were incubated with purified His-tagged GTPases and GTPase activation was assessed using a standard kit (BK105, Cytoskeleton; n=3). **b,** One-half of the amount used in the GAP assay was removed and analyzed by immunoblotting to verify the presence of GRAF3 in the experimental but not the negative control samples. **c,** Primary mouse AoSMC isolated from GRAF3$^{gt/gt}$ mice exhibited a more contractile morphology (more abundant vinculin-labeled focal adhesions and phalloidin-labeled stress fibers) and elevated phospho-MLC (pMLC) when compared with Wt mouse AoSMC. Scale bar is 25 microns

Supplementary Table S1: 5' RACE sequence from CE0477ES clone. BLASTn alignment was identical to Arhgap42 (syn 9030420J04Rik) Chr. 9 Exons 1-3.

CCATGCGCCCGCNCCCCNnGnnGACnnnnTCTGnnnTCGnCnANNCCGGAGATCTGCGATAA
CTCCTCCTTGCGCCCCCCCCCCGAAGTTATCGATGCGATCTGCAAACTTCTTCTTTGGTTT
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CTTTCAGTGACTGAGCAATACTAATTTCATCATCAGTTTCTGCATCTCCACCACCCCCCCCC
CGCAAGTCTTGGAGAGACAGGnnnAAnNCTGTACAGCCATGGACAGATTTCTCAGCGCCCC
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ATCGCAACATAACACACCCTCCTCCCCCCCCCCCCCAATTGAAGGGATAAAAGTTCGCTCAA
NTCCAGAGTGGGCTGCCCCATGGCCCAGGCGC

Supplementary Table S2. Intercrossing GRAF$^{t/gt}$ mice yields appropriate Mendelian ratios.

genotype	Probability	Actual Ratio
GRAF3$^{+/+}$ (Wt)	25%	23.2% (69/297)
GRAF3$^{+/gt}$	50%	53.9% (160/297)
GRAF3$^{gt/gt}$	25%	22.9% (68/297)

Supplementary Table S3. Blood profile of kidney function-related parameters

genotype	Glu mg/dL	BUN mg/dL	Cre mg/dL	Alb g/dL	Ca^{2+} mg/dL	PO_4 mg/dL	Na^+ mmol/L	K^+ mmol/L	CL^- mmol/L	tCO_2 mmol/L
Wt	184 +/- 5.3	22 +/- 2.5	0.2 +/- 0	3.0 +/-0.7	9.6 +/- 0.2	6.0 +/- 0.3	149.5+/- 0.7	6.1 +/- 0.3	104+/- 0.6	22.5 +/- 0.5
$GRAF^{gt/gt}$	194.5 +/-12.5	26.3 +/- 1.1	0.2 +/- 0	3.1 +/-1.3	9.6 +/- 1.2	5.8 +/- 0.6	149 +/- 1.2	5.7 +/- 1.0	104.8+/- 1.3	21.8+/- 1.3

Values represent mean +/- s.d.; n=4 mice/genotype; p values (Student's t-test) are p>0.1 for all parameters. Glu, glucose; BUN, blood urea nitrogen; Cre, creatinine, Alb,albumin.