Supplemental Methods

Targeted disruption of the rgs4 gene

A targeting construct was produced that contained three loxP sites and a neomycin resistance cassette (PGK Neo) (Supplmental Fig. 1a). Exons 2-5 of the *rgs4* gene encode the RGS box and were flanked by two of the loxP sites in the targeting construct. Linearized construct DNA was electroporated into embryonic stem cells derived from 129/SvJ mice. ES cells were screened for incorporation of the construct DNA by their ability to grow in the presence of G418 and by PCR and Southern blotting. Targeted disaggregated ES cells were electroporated with a mammalian expression vector encoding *cre* recombinase (pTurbo-*cre*) and individual clones were screened for deletion of both the neomycin resistance cassette and exons 2-5 of the *rgs4* gene by PCR and Southern blotting (Supplemental Fig. 1a).

To generate chimeric mice, targeted and *cre* recombinase-deleted ES cells were thawed and passed for 6 days in ES cell medium in the absence of G418. Blastocysts were flushed from pregnant C57BL/6 females (Jackson Laboratory), expanded for 1-2 hours in ES cell medium, transferred to a hanging drop chamber, and cooled to 4°C. ES cells were added to the hanging drops, and the blastocysts were injected with enough cells (20 or more) to fill the blastocoele. Injected blastocysts were then transferred to pseudopregnant recipient females (10-15 blastocysts/uterine horn). Chimeric offspring were bred with C57Bl/6J

mice, obtained from The Jackson Laboratory, and agouti offspring were repeatedly crossed with C57Bl/6J mice (>5X).

Magnetic resonance imaging of renal blood flow in mice

A single-slice, coronal image was acquired every 1.3 sec with the following parameters: flip angle, α=15°; repetition time, TR = 0.005 s; echo time, TE = 0.0023 s; FOV = 2.8 $\times$ 2.8 cm; slice thickness = 0.1 cm; data matrix = 64 × 64; number of transients, nt=4. The field of view was placed just distal of the renal pelvis. After one minute of scanning (46 baseline images), a 60 μl bolus of 10 mM OptiMARK (Covidien AG; Hazelwood, MO) was injected over 1.8 sec using a Harvard 2 dual syringe pump (Harvard Clinical Technology, Inc.; South Natick, MA), via a 28G jugular catheter (Strategic Applications Inc.; Chicago, IL; PN: MTV-01).

Signal intensity ($S_{Gd}(t)$) was converted to adjusted signal intensity ($S_{adj}(t)$) using the following equation:

$$S_{adj}(t) = \frac{S_{Gd}(t) - S_0}{S_0} \propto [Gd(t)],$$

where S_0 is the mean signal intensity prior to injection of contrast agent. The adjusted signal intensity is proportional to the concentration of gadolinium.Assuming the fast exchange limit[32, 59]. Regions of interest (ROI) over the cortex and medulla were drawn on a high-resolution T1 weighted image (data matrix= 128 x 128) and applied to the DCE data (see figure 7b). The initial area under the gadolinium curve for the first 60 sec post injection of contrast agent ($IAUC_{60}$) was calculated for each ROI.

Supplemental Figure 1. Generation of *rgs4*$^{-/-}$ mice. (**a**) Targeted disruption of the *rgs4* locus. Diagram of the targeting strategy. (**b**) Lower left panel, Southern blot analysis demonstrating successful gene targeting by use of tail genomic DNA; wild type (WT), heterozygote (HET). (**c,d**) Lower right panel, RT-PCR demonstration that *rgs4* mRNA is not present *rgs4*$^{-/-}$ (KO) cardiac tissue.

Supplemental Table 1. Phenotypic comparison of congenic control (WT) (n=10) and *rgs4*$^{-/-}$ mice (RKO) (n=10). Normalized kidney size to body weight (K/BW), glomerular number (GN), blood pressure (BP), serum creatinine, lean body mass (LBM), hemoglobin (Hb), and left ventricular mass index (LVMI).

Supplemental Figure 2. Analysis of gene expression in *rgs4*$^{-/-}$ mice compared to congenic wild type controls by gene expression microarray technique. (**a**) Hybridizing olignonucleotide signals derived from a GeneChip® microarray image are displayed by a color-coded white (high)- blue (low) intensity scale in an *rgs4*$^{-/-}$ mouse (**b**) Scatter plot of ratios of signal intensity (SI) detection above background (DABG) for 23,230 genes identified in an *rgs4*$^{-/-}$ mouse (RKO) and congenic wild type control. Data were normalized using 100 probe set controls and the robust multiarray average (RMA) method. SI•= signal intensity from *rgs4*$^{-/-}$ microarray. SI = signal intensity from congenic wild type control microarray. Pearson's correlation coefficient = 0.9832, $p=1x10^{-11}$.

Supplemental Table 2. Expression of Regulators of G-protein Signaling (RGS) proteins in *rgs4*$^{-/-}$ mice compared to controls. RGS2, RGS5, RGS10, RGS12, and RGS16 were expressed in each microarray analyzed. Mean signal intensity of up-regulated genes were defined as genes expressed with more than a twofold increase in the mean signal intensity DABG of three *rgs4*$^{-/-}$ mice compared to three congenic wild type controls. Down-regulated genes were defined as genes decreased by 50% or more.

Supplemental Figure 3. Cyclosporine blood levels after 7 days of daily administration is equivalent in RGS4$^{-/-}$ (n=10) and wild type control mice (n=10).

Supplemental Figure 4 . RGS4$^{-/-}$ mice metabolize cyclosporine at rates equal to congenic wild type control mice. RGS4$^{-/-}$ mice given 20mg/kg/d of cyclosporine had equivalent blood levels compared to congenic controls given the same dose. Cyclosporine blood levels were also equivalent between groups at a dose of 250mg/kg/d.

Supplemental Figure 5. Renal artery (A) blood flow is reduced in the kidneys (K) of mice treated with cyclosporine for one week. (**a)** Absolute systolic renal artery velocities were obtained noninvasively using a VEVO-770TTM Micro-Imaging System, (**b**) After one week of cyclosporine treatment the renal artery systolic velocity was decreased in both congenic wild type control mice (WT+C) (n=5) and RKO+C (n=4). (One-way independent ANOVA, $F(3,15) = 27.24$, composite $p < 0.0001$)

Supplemental Figure 6. Renal artery (RA) blood flow in *rgs4*$^{-/-}$ (RKO+C) (n=4) decreased after one week of daily cyclosporine A treatment compared to WT+C (n=5). The interrogated organ's blood supply was maintained throughout the procedure. (One-way independent ANOVA, $F(3,12) = 9.88$, composite $p=0.001$)

Supplemental Figure 7. Cyclosporine blood levels correspond with increasing dose of daily cyclosporine injection in the presence or absence of bosentan. *rgs4*$^{-/-}$ mice given 20mg/kg/d of cyclosprorine (n=3 for each group) alone or in combination with bosentan 100mg/kg/d had lower blood levels of cyclosporine than *rgs4*$^{-/-}$ mice given 250mg/kg/d of cyclosporine or in combination with cyclosporine. (One-way independent ANOVA, $F(5,12) = 1.33$, composite $p=0.31$)

Supplemental Table 1

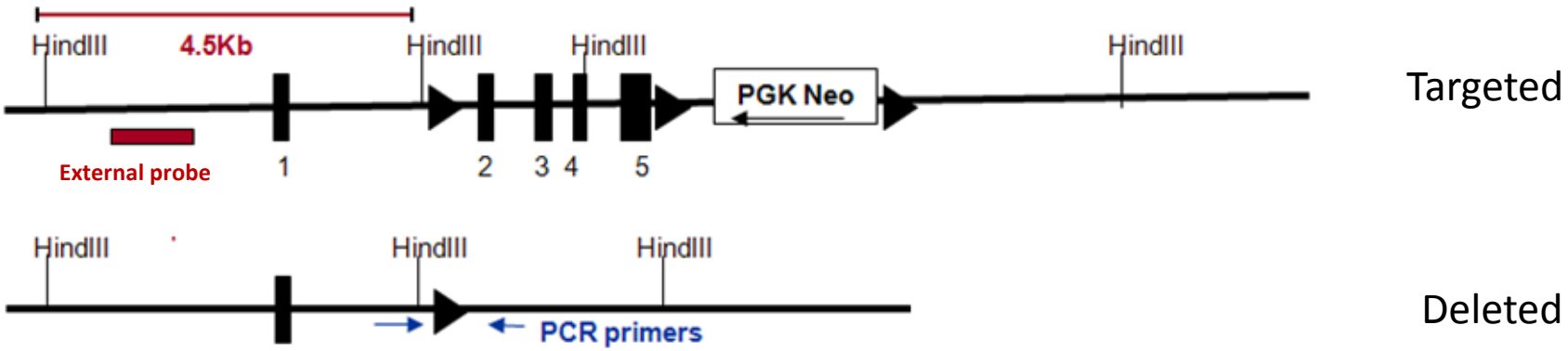
Physiologic Parameters of RGS4 $^{-/-}$ Mice Compared to Wild-Type Congenic Controls.

	WT (n=10)	RKO (n=10)	p
R kidney (g)	0.165 ± 0.005	0.166 ± 0.007	0.89
L kidney (g)	0.149 ± 0.009	0.153 ± 0.004	0.32
K/BW	0.0136 ± 0.0005	0.0133 ± 0.0004	0.63
GN (1/mm^2)	8.3 ± 0.3	8.1 ± 0.2	0.67
Systolic BP (mm/Hg)	93 ± 3	96 ± 2	0.29
LBM (g)	20.8 ± 0.6	20.3 ± 1.0	0.62
LVMI (mg/g)	3.01 ± 0.16	2.98 ± 0.19	0.92

Analysis of *rgs4*$^{-/-}$ mice in the absence of provocative stimulation demonstrated that these animals had comparable physiologic parameters. Normalized kidney weight was equal with no evidence of hypertension by noninvasive testing. These findings were correlated with equivalent glomerular number, glomerular filtration fraction as measured by serum creatinine , (0.106 ± 0.002 mg/dL vs 0.105 ± 0.007mg/dL, p=0.89), lean body mass (LBM). Cardiac ventricular mass was also equal compared to wild type congenic mice by transthoracic echocardiography (**Table 1**).

Supplemental Figure 1

a



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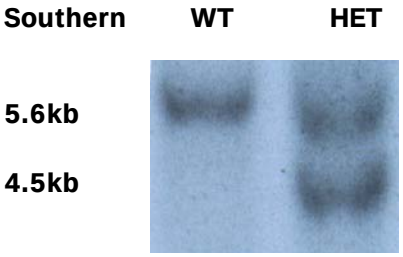
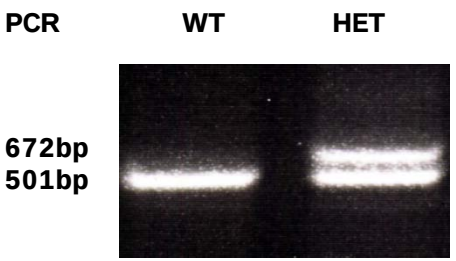
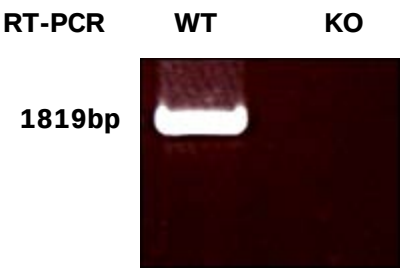


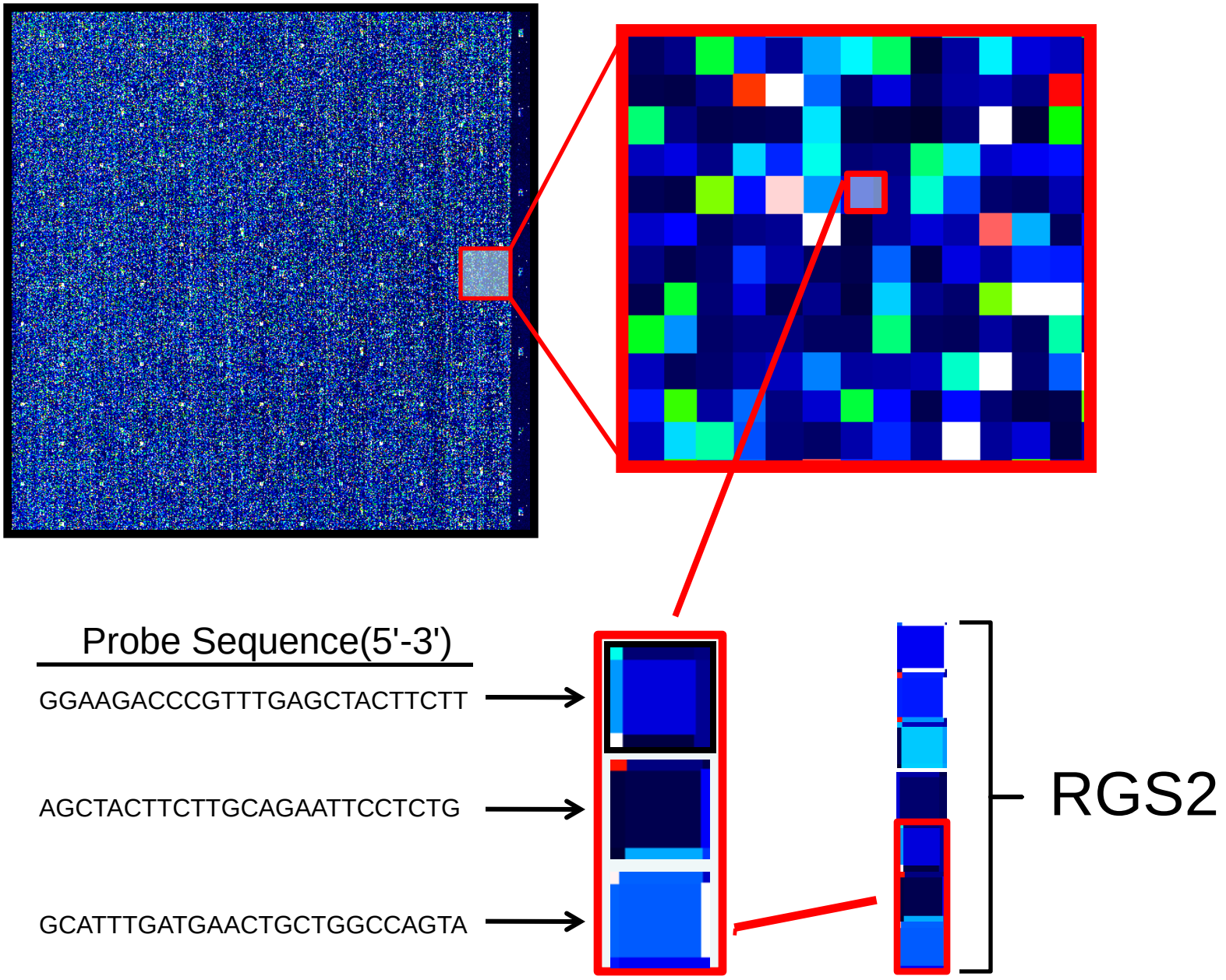
c



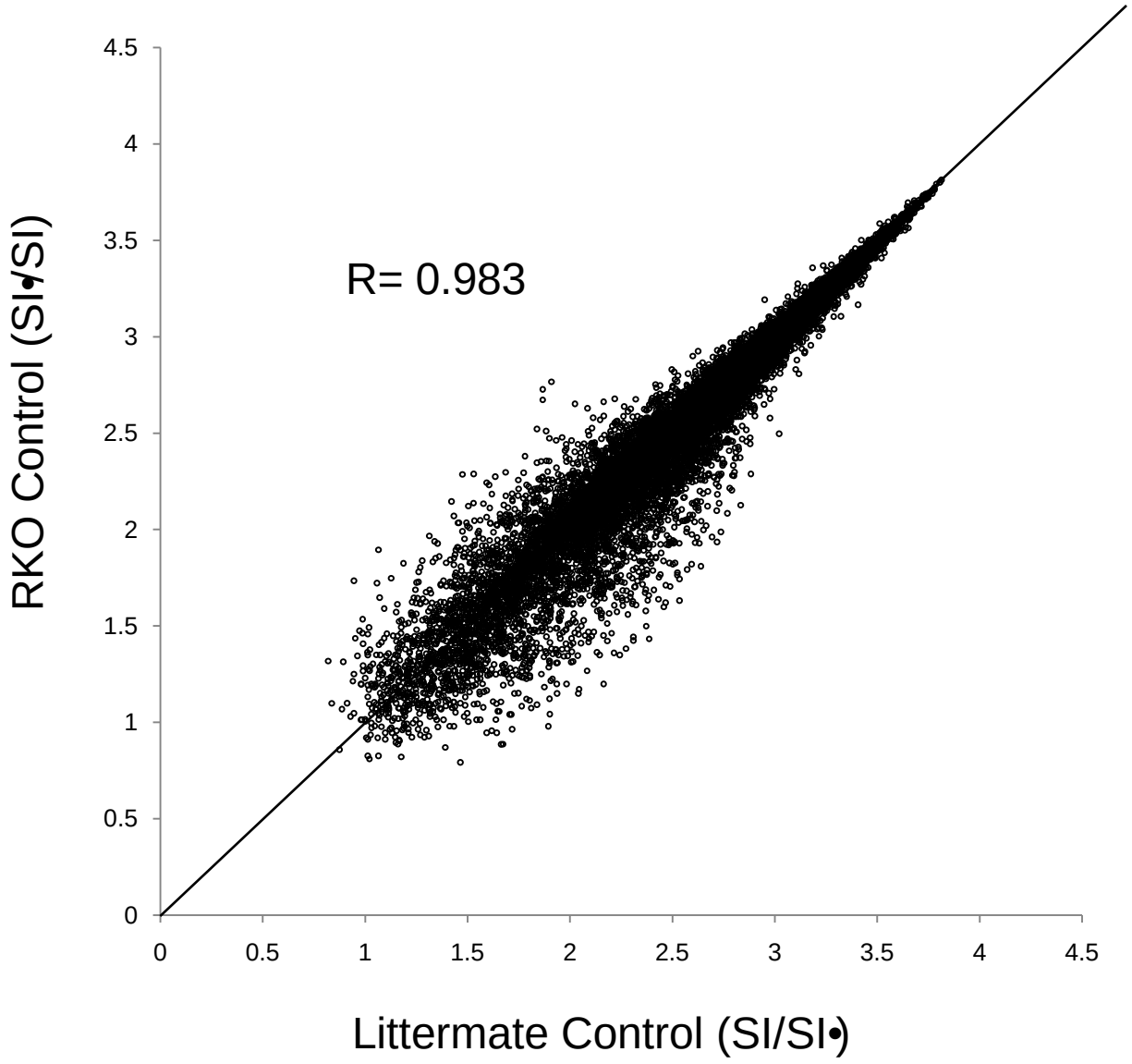
d



Supplemental Figure 2a



Supplemental Figure 2b



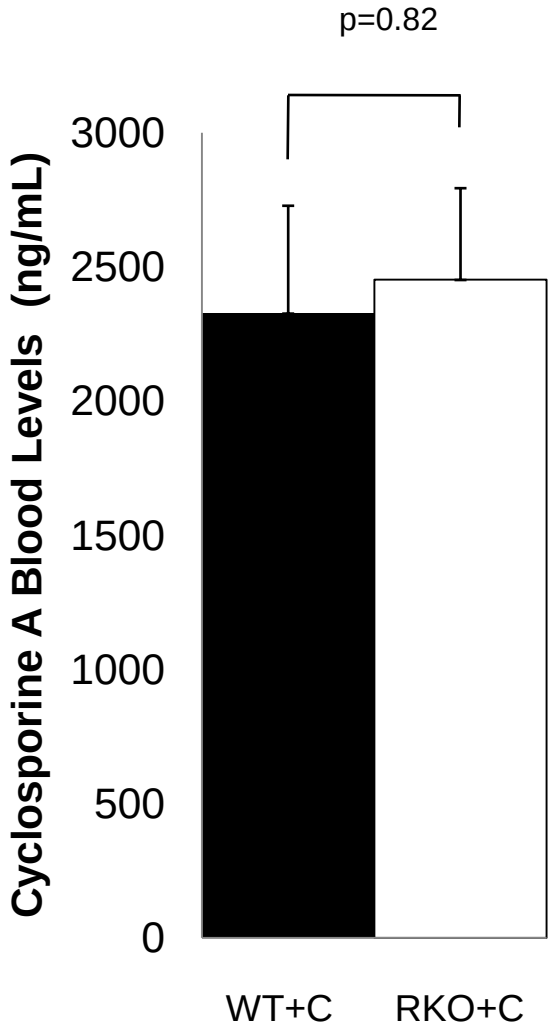
Supplemental Table 2

Baseline mRNA expression of RGS proteins in *rgs4*$^{-/-}$ mice

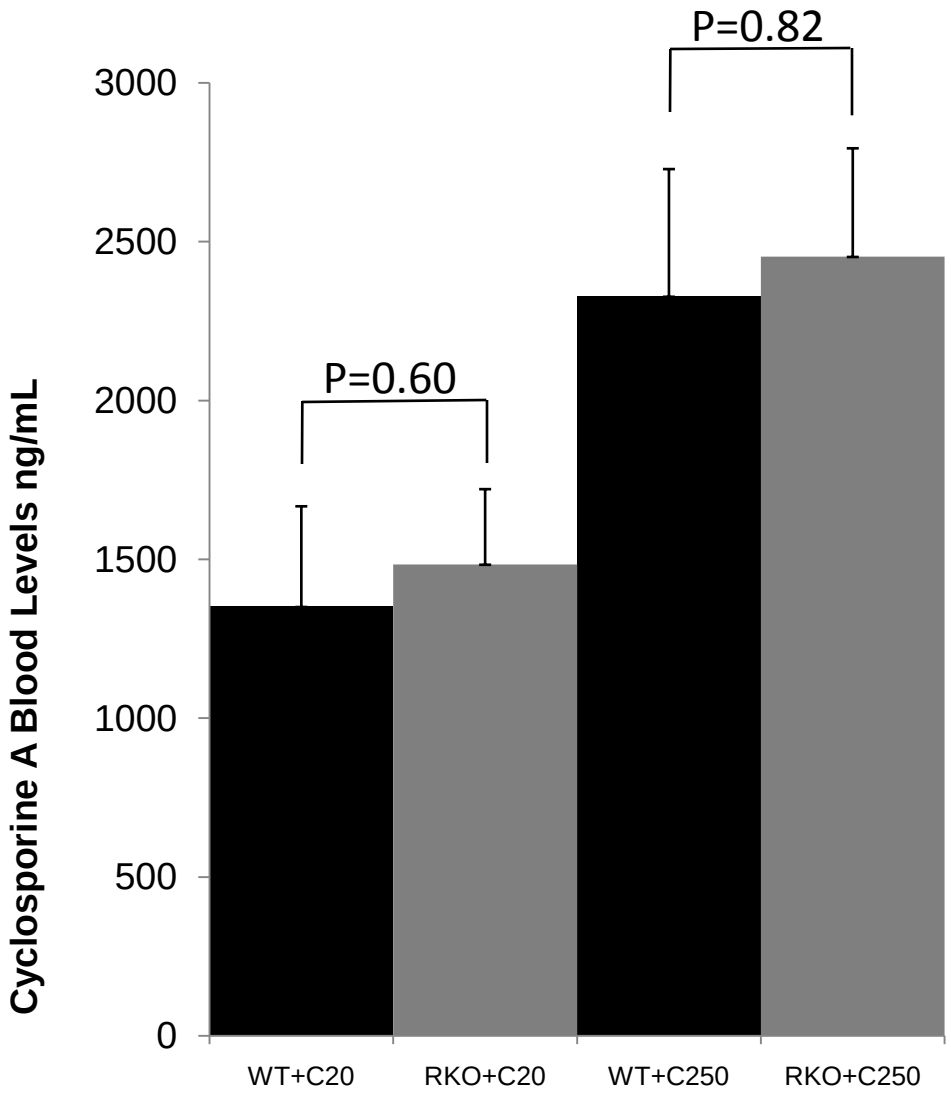
GenBank accession number	Gene symbol	SI DABG′ (n=3)	SI DABG (n=3)	SI DABG′/ SI DABG
NM_009061	RGS2	641± 261.8	613 ± 251.7	1.053 ± 0.087
NM_009063	RGS5	1558 ± 1288	3528 ± 3301	0.758 ± 0.581
NM_026418	RGS10	379.7 ± 149.9	333.3 ± 94.77	0.988 ± 0.132
NM_173402	RGS12	156 ± 6.9	162 ± 16.6	0.932 ± 0.0686
NM_019580	RGS16	375.7 ± 270.7	391.3 ± 270.3	0.880 ± 0.0450

Expression of the family of Regulators of G-protein Signaling (RGS) in *rgs4*$^{-/-}$ mice compared to controls. RGS2, RGS5, RGS10, RGS12, and RGS16 were expressed in each mouse analyzed. Mean signal intensity detection above background (SI DABG) of up-regulated genes were defined as genes expressed with more than twofold increase in the mean ratio of *rgs4*$^{-/-}$ mice (SI DABG′) compared to controls (DABG). Down-regulated genes were defined as genes decreased by 50%.

Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5a

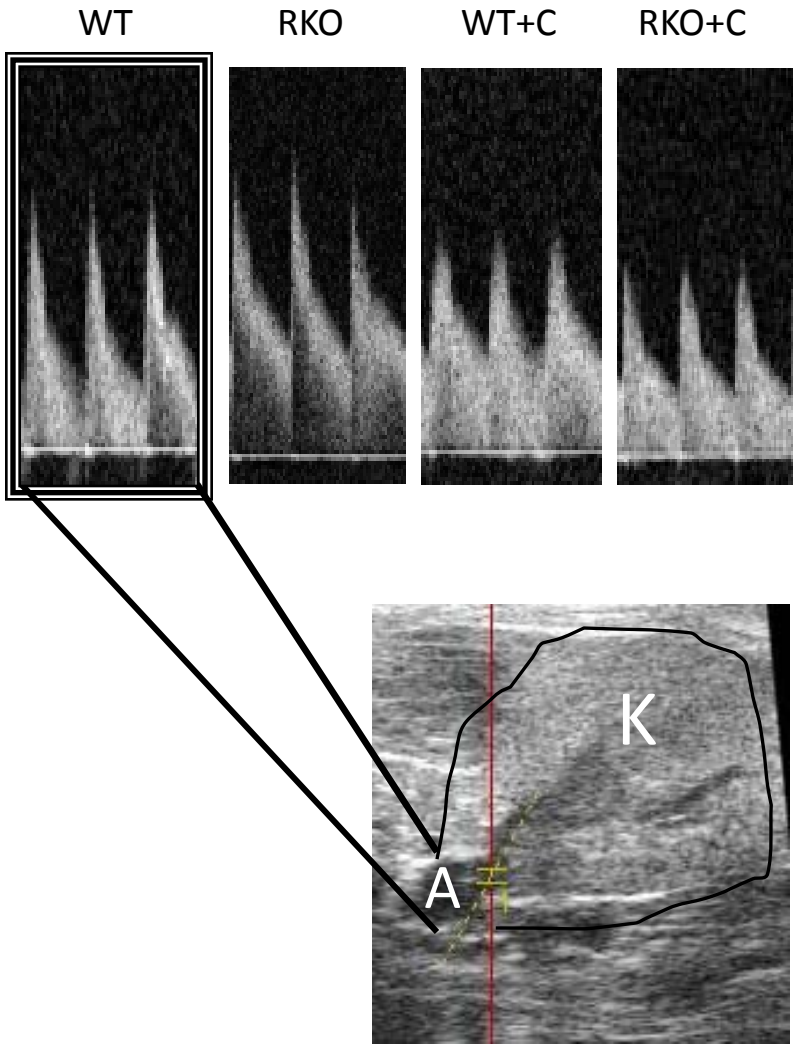


Figure 4b

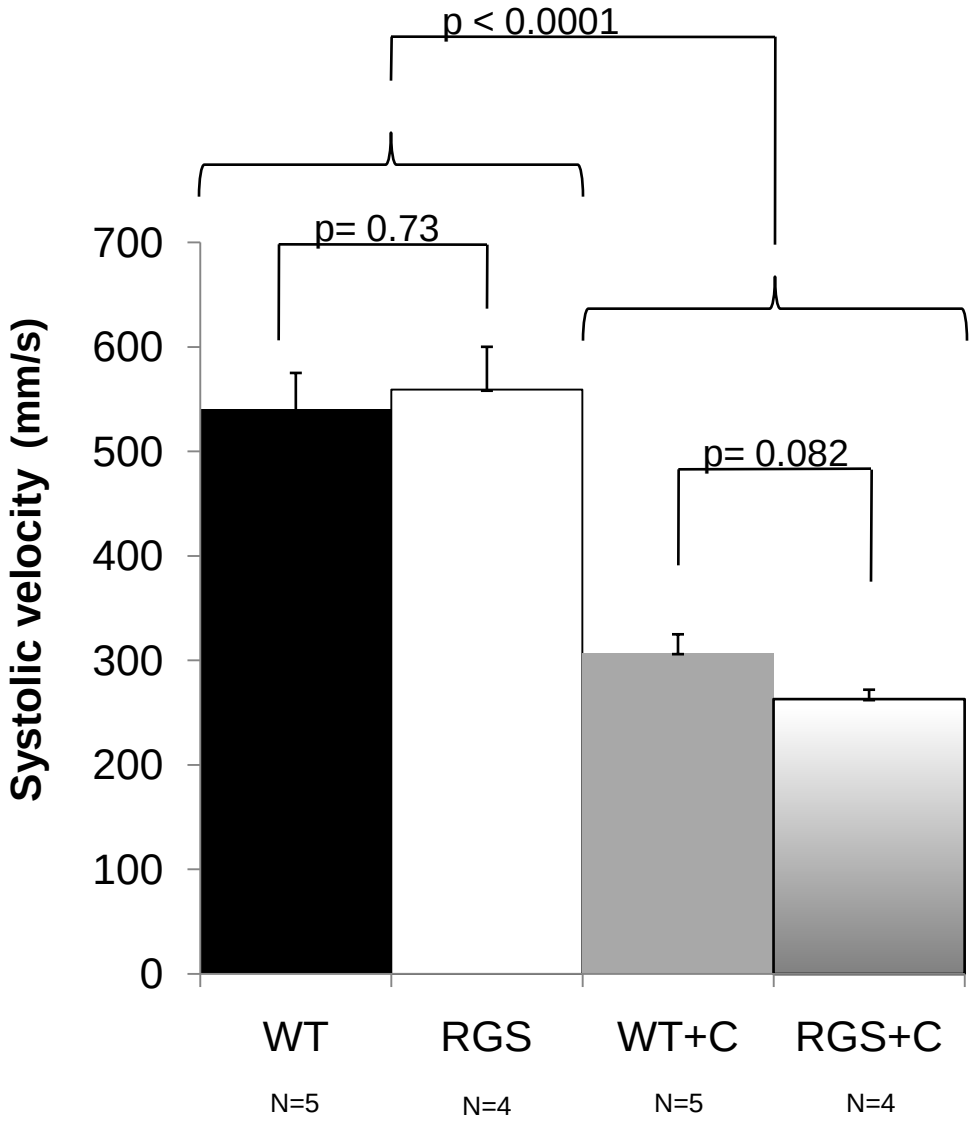
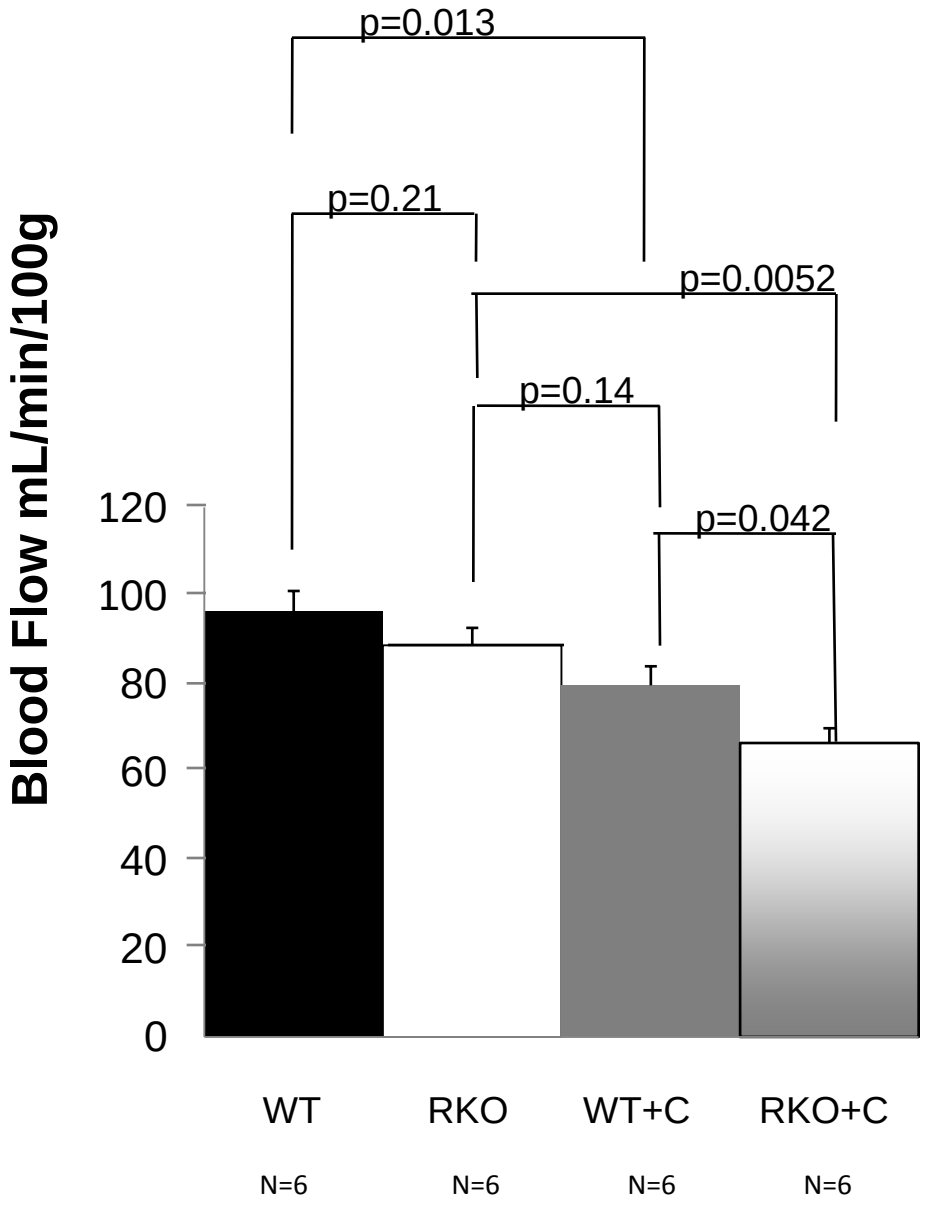
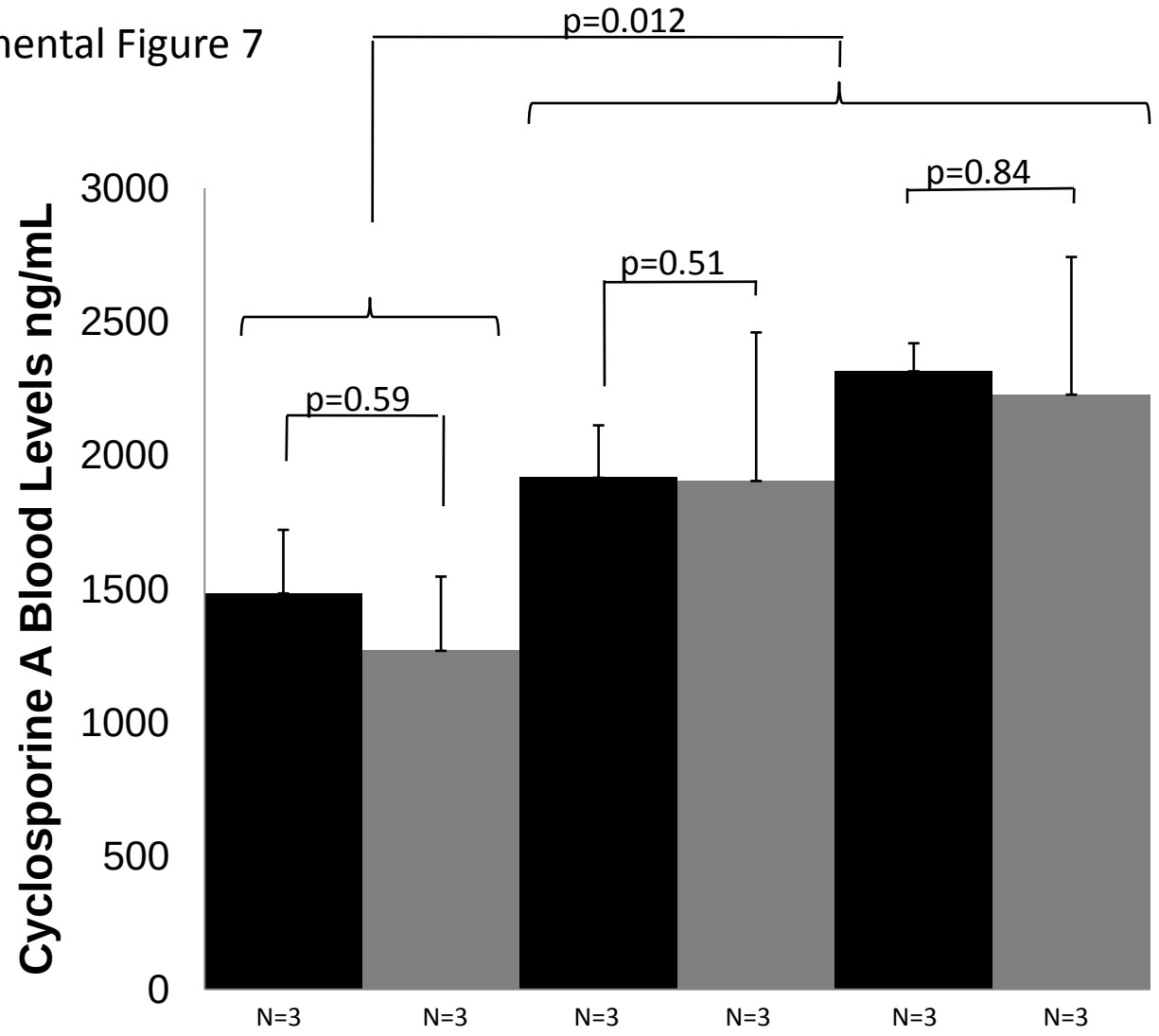

p < 0.0001
p= 0.73
p= 0.082
Systolic velocity (mm/s)
700
600
500
400
300
200
100
0
WT
RGS
WT+C
RGS+C
N=5
N=4
N=5
N=4

Figure 6


p=0.013
p=0.21
p=0.0052
p=0.14
p=0.042
Blood Flow mL/min/100g
120
100
80
60
40
20
0
WT
RKO
WT+C
RKO+C
N=6
N=6
N=6
N=6

Supplemental Figure 7



RKO	+	+	+	+	+	+
cyclosporine 20mg/kg/d	+	+				
cyclosporine 100mg/kg/d			+	+		
cyclosporine 250mg/kg/d					+	+
bosentan		+		+		+