Supplemental Table 1. Body and kidney weights and blood parameters in vehicle- or aldosterone-infused wild-type and GC-A knockout mice

	WT veh	WT aldo	KO veh	KO aldo	KO aldo + hyd	KO aldo +spi	KO aldo +olm	KO aldo +temp
BW at -2 wk, g	28.2 ± 0.6	28.4 ± 0.6	26.8 ± 0.5	28.7 ± 1.5	29.2 ± 1.0	29.1 ± 0.7	28.7 ± 0.9	28.6 ± 0.5
BW at 4 wk, g	30.0 ± 0.6	28.9 ± 0.6	29.7 ± 0.4	27.8 ± 0.8	27.6 ± 1.0	32.0 ± 0.9††	30.4 ± 0.8†	29.1 ± 1.2
LKW at 4 wk, mg	221 ± 2††	317 ± 15##, ††	273 ± 14††,¶	383 ± 19**	375 ± 20	362 ± 11	389 ± 20	385 ± 10
LKW/BW at 4 wk, mg/g	7.36 ± 0.13††	10.95 ± 0.37##,††	9.16 ± 0.40††,¶	13.60 ± 0.32**	13.62 ± 0.32	11.32 ± 0.17††	12.79 ± 0.72	13.33 ± 0.83
Serum aldosterone, pg/mL	71 ± 6††	5120 ± 670##	65 ± 10††	4540 ± 734**	4620 ± 797	5002 ± 346	5483 ± 1583	4674 ± 1167
Serum potassium, mEq/L	4.38 ± 0.08††	3.14 ± 0.24##	5.08 ± 0.25††,¶¶	2.64 ± 0.12**	2.45 ± 0.12	2.80 ± 0.15	2.58 ± 0.16	2.58 ± 0.10
Serum UN, mg/dL	28.8 ± 0.9†	22.1 ±1.0##	24.6 ± 1.5†	17.5 ± 0.9*	17.9 ± 2.5	19.6 ± 1.3	20.3 ± 1.3	20.8 ± 1.5
Serum Cr, mg/dL	0.132 ± 0.006†	0.134 ± 0.008	0.106 ± 0.006¶	0.103 ± 0.005	0.098 ± 0.010	0.120 ± 0.011	0.116 ± 0.009	0.120 ± 0.004
Urinary sodium, mEq/day	1.99 ± 0.32	2.82 ± 0.21	2.03 ± 0.34	2.00 ± 0.28	2.26 ± 0.37	3.30 ± 0.28††	3.38 ± 0.37††	2.54 ± 0.15
Urinary potassium, mEq/day	0.363 ± 0.040	0.451 ± 0.033†	0.358 ± 0.052	0.369 ± 0.040	0.329 ± 0.049	0.544 ± 0.038††	0.631 ± 0.054††	0.406 ± 0.023
Urinary K/Na	0.188 ± 0.009	0.165 ± 0.013	0.181 ± 0.013	0.203 ± 0.027	0.147 ± 0.005†	0.166 ± 0.005	0.190 ± 0.008	0.160 ± 0.002

BW, body weight; LKW, left kidney weight; UN, urea nitrogen; Cr creatinine.

Values are expressed as the mean ± SEM for vehicle-infused wild-type mice (WT veh; n = 5), aldosterone-infused wild-type mice (WT aldo; n = 8), vehicle-infused GC-A knockout mice (KO veh: n = 5), aldosterone-infused GC-A knockout mice (KO aldo; n = 8), aldosterone-infused GC-A knockout mice with hydralazine (KO aldo+hyd; n = 6), aldosterone-infused GC-A knockout mice with spironolactone (KO aldo+spi; n = 5), aldosterone-infused GC-A knockout mice with olmesartan (KO aldo+olm; n = 5), and aldosterone-infused GC-A knockout mice with tempol (KO aldo+temp; n =5).

#$p < 0.05$, ##$p < 0.01$, WT veh vs. WT aldo

*$p < 0.05$, **$p < 0.01$, KO veh vs. KO aldo

†$p < 0.05$, ††$p < 0.01$, vs. KO aldo

¶$p < 0.05$, ¶¶$p < 0.01$, WT veh vs. KO veh

Supplemental Methods

Cell culture transfected with siRNA for GC-A

For GC-A knockdown experiment, differentiated podocytes were transfected with 10 nM siRNA for mouse GC-A (SI02668127; Qiagen, Germantown, MD) or 10 nM control (Qiagen) by Nucleofector (Lonza, Basel, Switzerland) and then cells were incubated with charcoal-treated 10% FBS for 48 hours. Cells were preincubated with 10 μM tempol, 100 nM olmesartan, 10 μM spironolactone or 10 μM 8-bromo-cGMP for 30 min and stimulated by 1 μM of aldosterone or vehicle. Cells were harvested with AllPrep DNA/RNA/protein Mini kit (Qiagen) at 15 minutes or 3 hours for ERK and p38 MAPK analysis, respectively. GC-A (Npr1) mRNA expression was evaluated by quantitative real-time RT-PCR. Npr1 forward primer, 5'-acagtaaatcaccaggagttcgtc-3'; Npr1 reverse primer, 5'-agggccaaaagcatcagattcc-3'; and NPR1 probe, 5'-FAM-cggaccactacaccaagctactgcgg-TAMRA-3'.

Supplemental Figure 1. The profiles of systolic blood pressure (SBP). (**A**) Time-dependent SBP profiles are shown in vehicle-infused wild-type mice (WT veh, open triangles), aldosterone-infused wild-type mice (WT aldo, filled triangles), vehicle-infused GC-A knockout mice (KO veh, open squares), and aldosterone-infused GC-A knockout mice (KO aldo, filled squares). Uninephrectomy was performed at -2 weeks. Administration of aldosterone and high sodium diet (6%) began at 0 week. GC-A knockout mice showed elevated SBP compared with wild-type mice at 0 week. Aldosterone-infused wild-type mice showed marginally higher SBP. Aldosterone infused-GC-A knockout mice exhibited accelerated hypertension. (**B**) SBP profiles in aldosterone-infused GC-A knockout mice (KO aldo, filled squares), aldosterone-infused GC-A knockout mice treated with hydralazine (KO aldo+hyd, open diamonds), those treated with spironolactone (KO aldo+spi, open circles), those treated with olmesartan (KO aldo+olm, filled circles), and those treated with tempol (KO aldo+temp, filled diamonds) are shown. Administration of hydralazine, spironolactone or olmesartan in aldosterone-infused GC-A knockout mice resulted in effective reduction in SBP throughout the course, to a comparable level. There was no significant SBP change after the administration of tempol. WT veh, n = 5; WT aldo, n = 8; KO veh, n = 5; KO aldo, n = 8; KO aldo + hyd, n = 6; KO aldo + spi, n = 5; KO aldo + olm, n = 5; and KO aldo + temp, n = 5. Mean ± SEM. #$p < 0.05$, WT aldo vs. WT veh; **$p < 0.01$, KO aldo vs. KO veh; †$p < 0.05$, ††$p < 0.01$, vs. KO aldo; ¶$p < 0.05$, KO veh vs. WT veh.

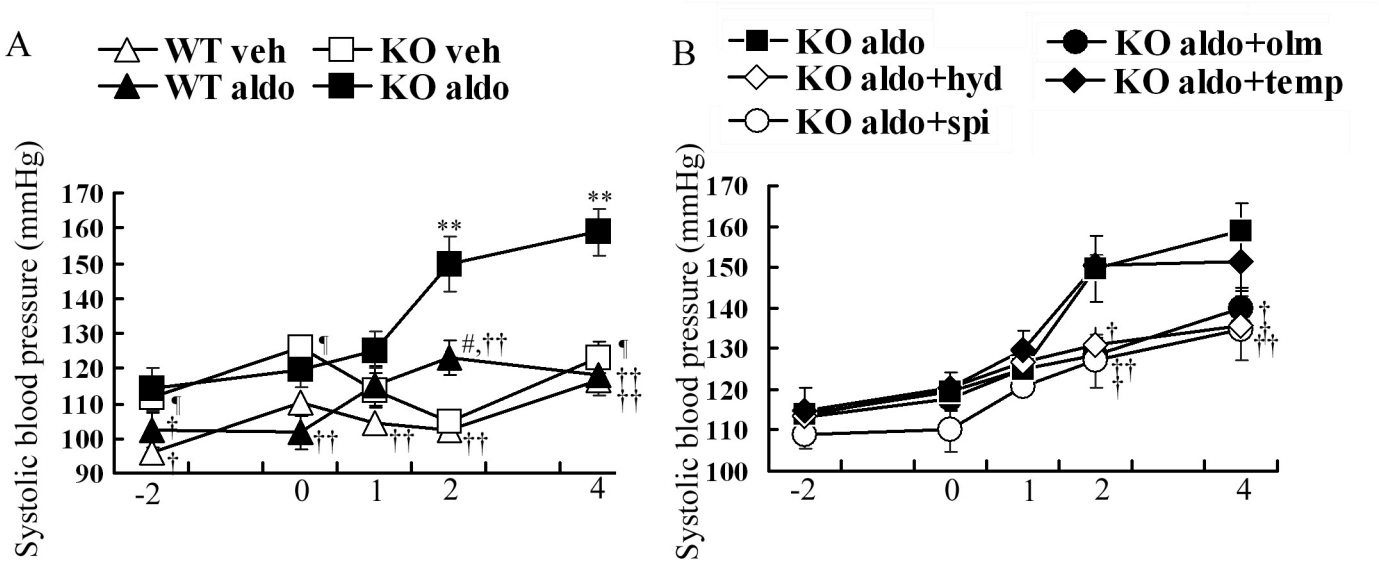
Supplemental Figure 2. Glomerular cross-sectional area in superficial (A) and juxtamedullary (B) glomeruli at 4 weeks. WT, wild-type mice; GC-A KO, GC-A knockout mice; aldo, aldosterone; hyd, hydralazine; spi, spironolactone; olm, olmesartan; and temp, tempol. WT veh, n = 5; WT aldo, n = 8; KO veh, n = 5; KO aldo, n = 8; KO aldo + hyd, n = 6; KO aldo + spi, n = 5; KO aldo + olm, n = 5; and KO aldo + temp, n = 5. Mean ± SEM. #$p < 0.05$, WT veh vs. WT aldo, *$p < 0.05$, KO veh vs. KO aldo, ††$p < 0.01$, vs. KO aldo, ¶¶$p < 0.01$, WT veh vs. KO veh.

Supplemental Figure 3. Glomerular mRNA expression at 4 weeks after aldosterone administration. Real-time RT-PCR analyses of α1(I) collagen (Col1a1), α3(IV) collagen (Col4a3), p22phox (Cyba) and NADPH oxidase-4 (Nox4) are shown. GAPDH mRNA expression is used as control. WT veh, n = 5; WT aldo, n = 8; KO veh, n = 5;

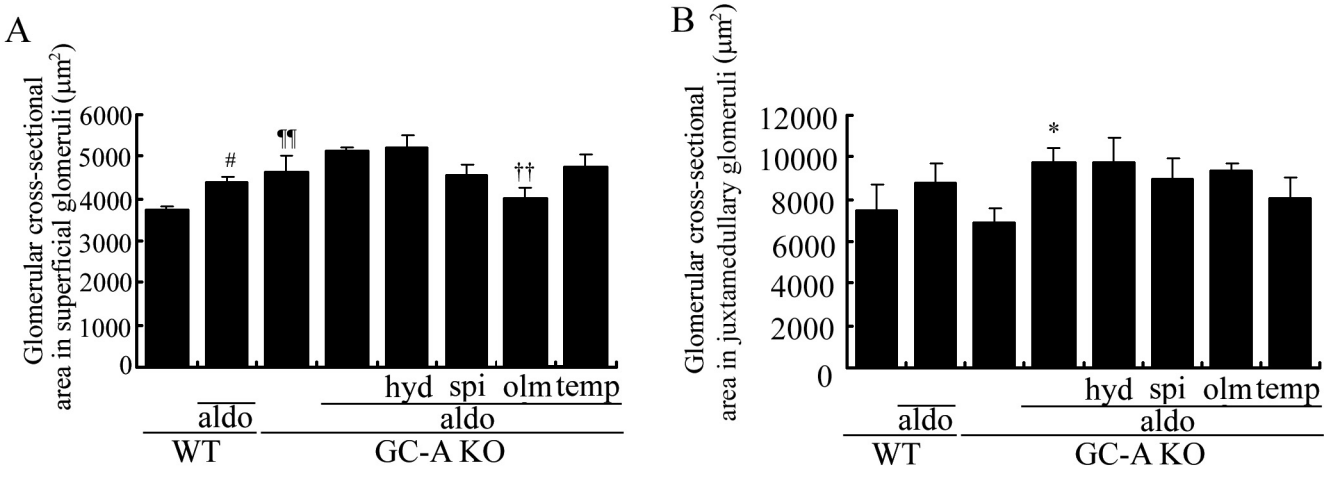
KO aldo, n = 8; KO aldo + hyd, n = 6; KO aldo + spi, n = 5; KO aldo + olm, n = 5; and KO aldo + temp, n = 5. Mean ± SEM. ##$p < 0.01$ WT veh vs. WT aldo, *$p < 0.05$ KO veh vs. KO aldo, †$p < 0.05$, ††$p < 0.01$, vs. KO aldo.

Supplemental Figure 4. (**A**) Effects of knockdown of GC-A on phosphorylation of ERK and p38 MAPK in cultured podocytes. Cells transfected with siRNA for GC-A (10 nM) or control (10 nM) were incubated for 48 hours with 10% charcoal-treated FBS. Cells were pretreated with tempol (temp; 10 μM), olmesartan (olm; 100 nM), spironolactone (spi; 10 μM), or 8-bromo-cGMP (cGMP; 10 μM) for 30 min and then were stimulated by 1 μM aldosterone or vehicle. (**B**) Expression of Npr1 (GC-A) in si-control or si-GC-A-transfected podocytes at 15 minutes after aldosterone stimulation. Transfection of si-GC-A reduced Npr1 mRNA by 42 percent in vehicle-treated cells. (**C**) Quantitative analysis for phospho-ERK and total ERK in siRNA-transfected podocytes at 15 minutes after stimulation. (**D**) Expression of Npr1 in si-control or si-GC-A-transfected podocytes at 3 hours after aldosterone stimulation. Transfection of si-GC-A reduced Npr1 mRNA by 41 percent in vehicle-treated cells and administration of aldosterone reduced Npr1 mRNA by 23 percent in si-control transfected cells. (**E**) Quantitative analysis for phospho-p38 MAPK and total p38 MAPK in podocytes at 3 hours after stimulation. #p <0.05, ##p < 0.01, vs. siRNA for control with vehicle, †$p < 0.05$, ††$p < 0.01$, vs. siRNA for GC-A with aldosterone, mRNA levels are normalized with Gapdh. * $p < 0.05$. n = 3, each.

Supplemental Figure 5. Proposed mechanism of the role of GC-A in aldosterone-induced renal injury. The aldosterone (Aldo)-mineralocorticoid receptor (MR) pathway activates p38 MAPK and ERK, which can lead to podocyte injury and glomerulosclerosis. Aldo/MR possibly activates the angiotensin II type 1 receptor (AT1R), and also stimulates reactive oxygen species (ROS) production, the inhibition of which ameliorates ERK and p38 MAPK activation. GC-A signaling inhibits the phosphorylation of ERK and p38 MAPK in podocytes and counteracts the AT1R pathway.



Supplemental figure 1. Ogawa *et al.*



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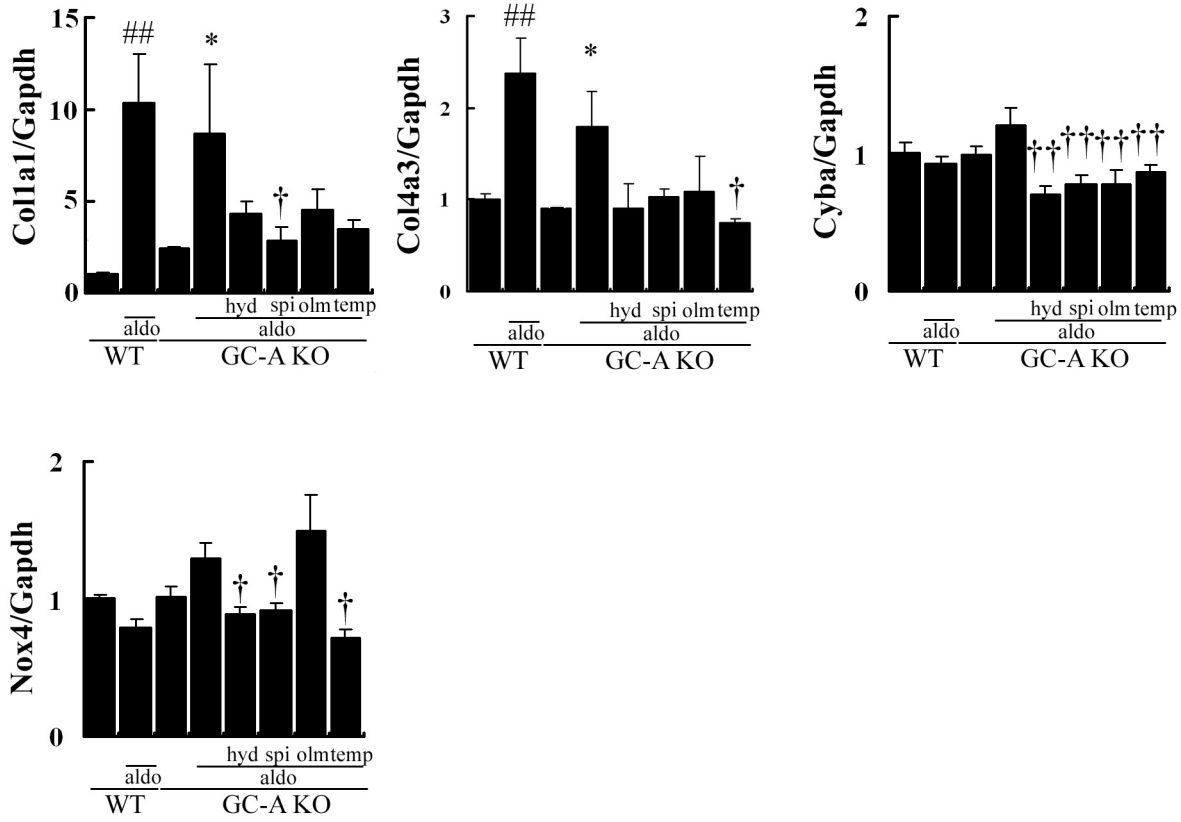
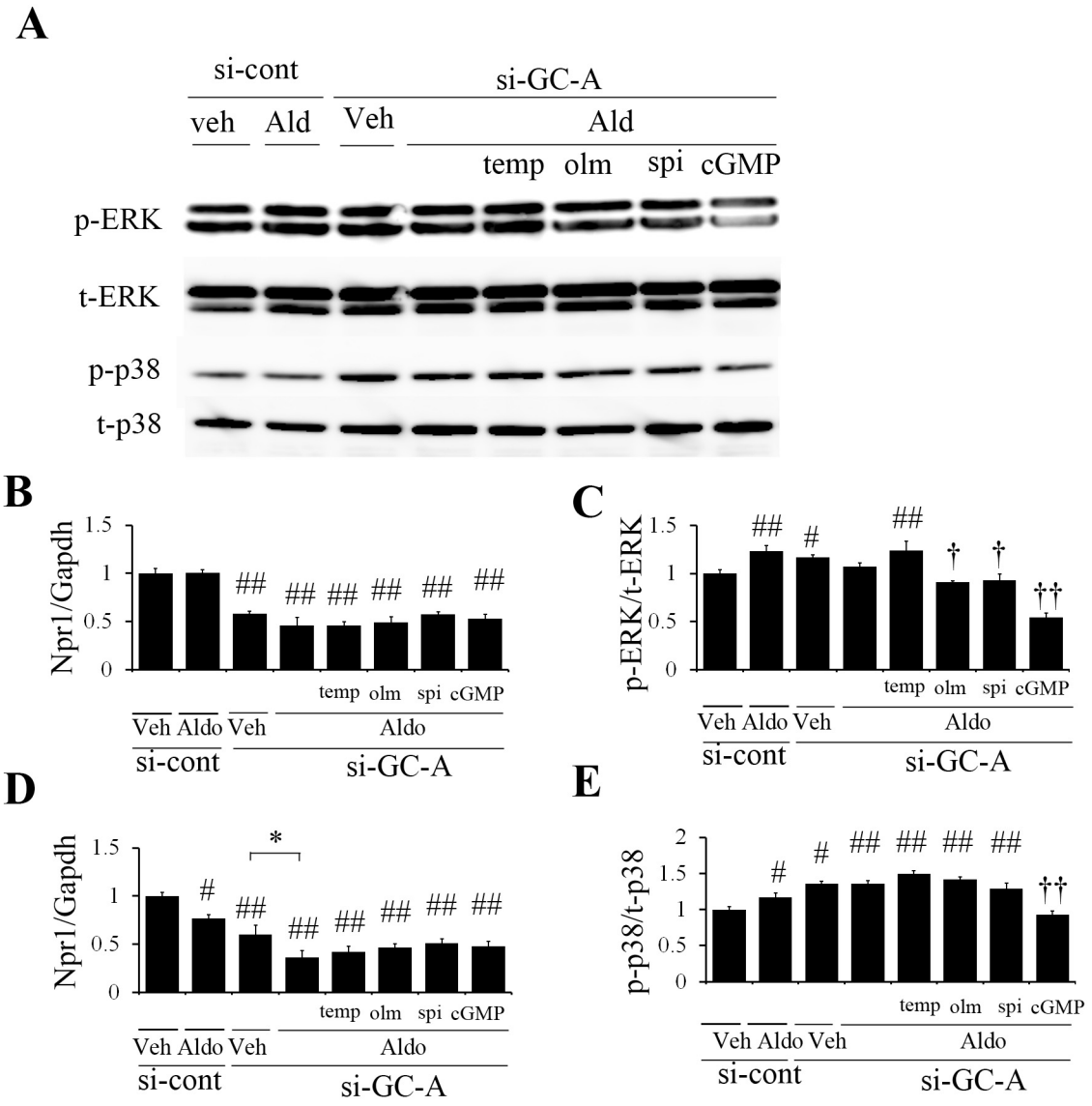
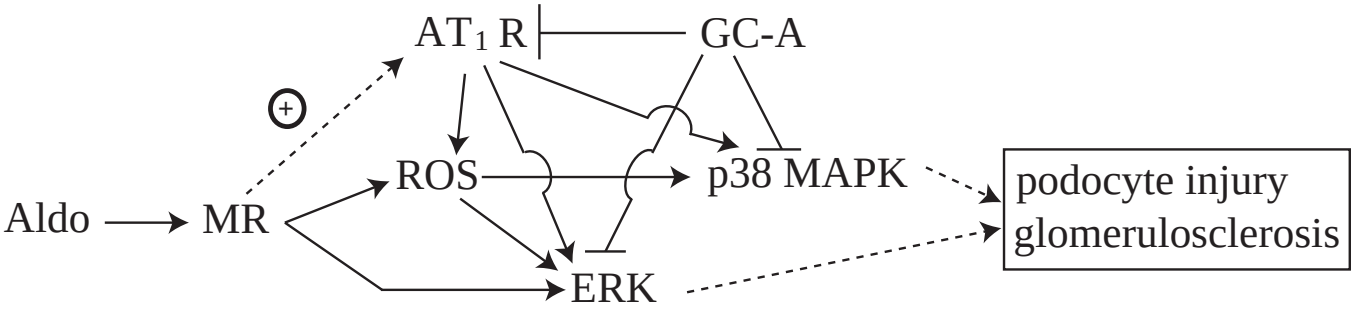


Supplemental figure 3. Ogawa *et al*.



Supplemental Figure 4. Ogawa *et al.*



Supplemental Figure 5. Ogawa *et al.*