Supplementary Methods

Materials. Chemicals were obtained from the following sources: Ponasterone A (Invitrogen-Life Technologies), Y27632 (Calbiochem, La Jolla, CA). Antibodies were obtained from the following sources: rabbit or mouse anti-flag antibody (Sigma-Aldrich Canada, Mississauga, ON), mouse anti-synaptopodin antibody (PROGEN Biotechnik GmbH, Heidelberg, Germany), mouse anti-fibronectin antibody (BD Biosciences, San Jose, CA), mouse anti-phospho-myosin light chain 2 (Ser19) and rabbit-anti myosin light chain 2 antibodies (Cell Signaling Technology Inc., Danvers, MA), goat anti-podocalyxin antibody (R&D Systems, Inc., Minneapolis, MN), rabbit anti-goat FITC, donkey anti-rabbit FITC, donkey anti-goat TRITC, goat anti-mouse TRITC and donkey anti-mouse Cy3 (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Rabbit antiserum to nephrin was described previously.[1] GFP-CA-RhoA and GFP-DN-RhoA were described previously.[2] pGLF1900 FN-luc (rat fibronectin promoter-reporter construct) was a gift from Dr. Kim.[3]

Light and electron microscopy. For light microscopy, kidneys were fixed in buffered formalin and were processed and embedded in paraffin according to conventional techniques. Periodic acid Schiff (PAS) staining was performed by the Core facility of the Rosalind and Morris Goodman Cancer Research Centre, McGill University.

Electron microscopy was carried out at the Facility for Electron Microscopy Research, McGill University. Briefly, kidney tissue was fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 0.1% $CaCl_2$, pH 7.4. Samples were washed and post-fixed with 1% aqueous OsO_4 and 1.5% aqueous potassium ferrocyanide. Samples were dehydrated with serially-increasing concentrations of acetone (30-100%), and infiltrated with epon/acetone. Sections of 90-100 nm were placed onto grids, and stained with uranyl acetate and Reynold's lead. Sections were viewed with a FEI Tecnai 12 transmission electron microscope operating at an accelerating voltage of 120 kV, and equipped with a Gatan Bioscan CCD camera, model 792.

Real-time PCR. Glomeruli were isolated from mouse kidneys by differential sieving.[4] The purity of the glomeruli was ~65%. RNA was extracted by TriZol reagent (Invitrogen, Burlington, ON). cDNA was produced by Reverse transcriptase-PCR using QuantiTect Reverse Transcription kit (Qiagen, Mississauga, ON). Twenty µl of cDNA was amplified and quantified using iTaq SYBR Green Supermix with ROX (Bio-Rad laboratories, Hercules, CA). The primers were used at a final concentration of 100 µM. The primer sequences are provided in Supplementary Table 2. The PCR reaction was carried out for 40 cycles of the following: 95 ^{o}C for 15 Sec, 60 ^{o}C for 15 Sec and 72 ^{o}C for 30 Sec. Relative quantification was determined using the ΔΔCt method. Data were analyzed using ABI Prisms 7500 Sequence Detection System software (version 1.7) (ABI, Carlsbad, CA). Samples were run in duplicate.

REFERENCES

1. Li, H, Lemay, S, Aoudjit, L, Kawachi, H, Takano, T: SRC-family kinase Fyn phosphorylates the cytoplasmic domain of nephrin and modulates its interaction with podocin. *J Am Soc Nephrol* 15**:** 3006-3015, 2004
2. Attias, O, Jiang, R, Aoudjit, L, Kawachi, H, Takano, T: Rac1 contributes to actin organization in glomerular podocytes. *Nephron Exp Nephrol* 114**:** e93-e106, 2010
3. Lee, BH, Park, SY, Kang, KB, Park, RW, Kim, IS: NF-kappaB activates fibronectin gene expression in rat hepatocytes. *Biochem Biophys Res Commun* 297**:** 1218-1224, 2002
4. Cybulsky, AV, Takano, T, Papillon, J, Bijian, K, Guillemette, J, Kennedy, CR: Glomerular epithelial cell injury associated with mutant alpha-actinin-4. *Am J Physiol Renal Physiol* 297**:** F987-995, 2009

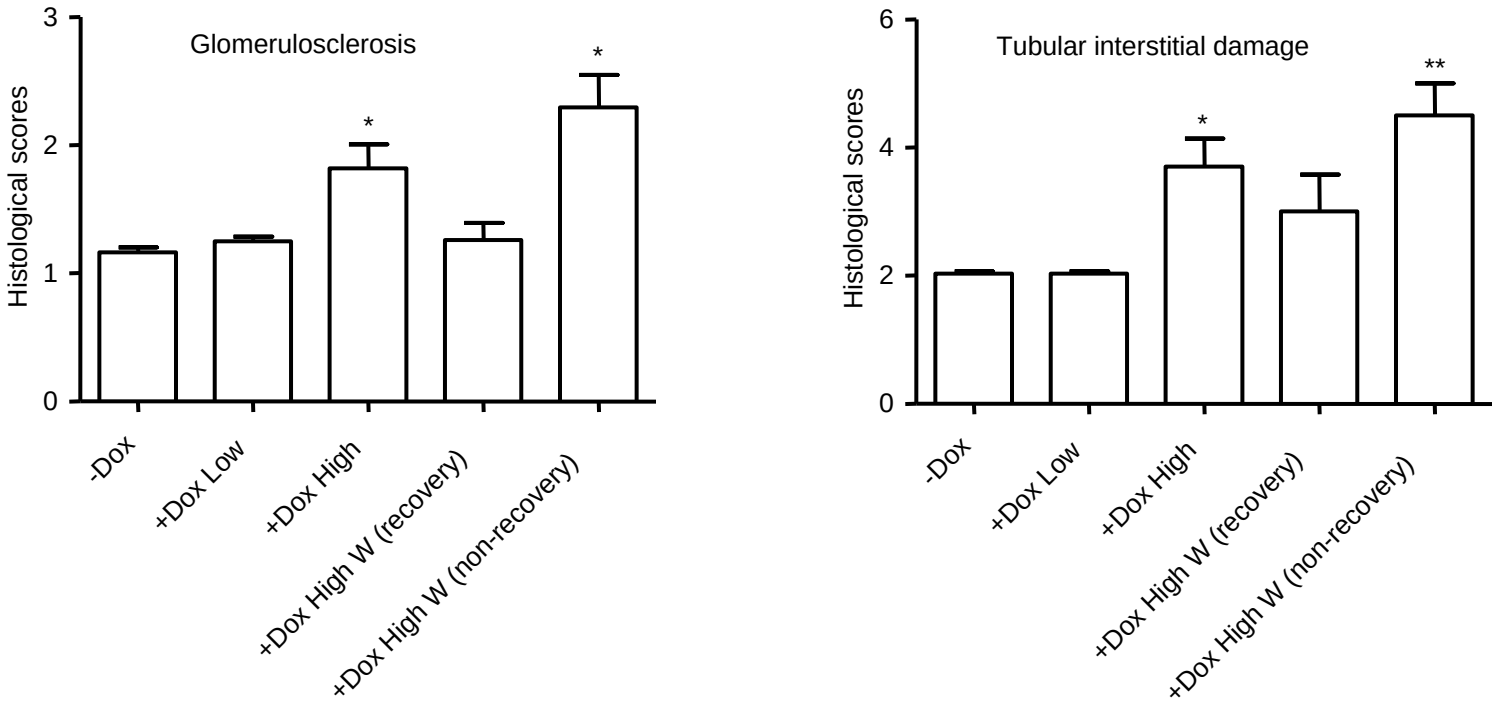
Supplementary Table 1. Addition of Flag epitope tag does not affect the activity of CA-RhoA. HEK293T cells were transiently transfected with empty vector (control), CA-RhoA, or Flag-CA-RhoA. Cell lysates were subjected to RhoA G-LISA assay (Cytoskeleton, Inc.). Results are the mean of two independent experiments.

	RhoA activity (arbitrary units)
Control	0.178
CA-RhoA	1.901
Flag-CA-RhoA	1.561

Supplementary Table 2: Real-time PCR primers

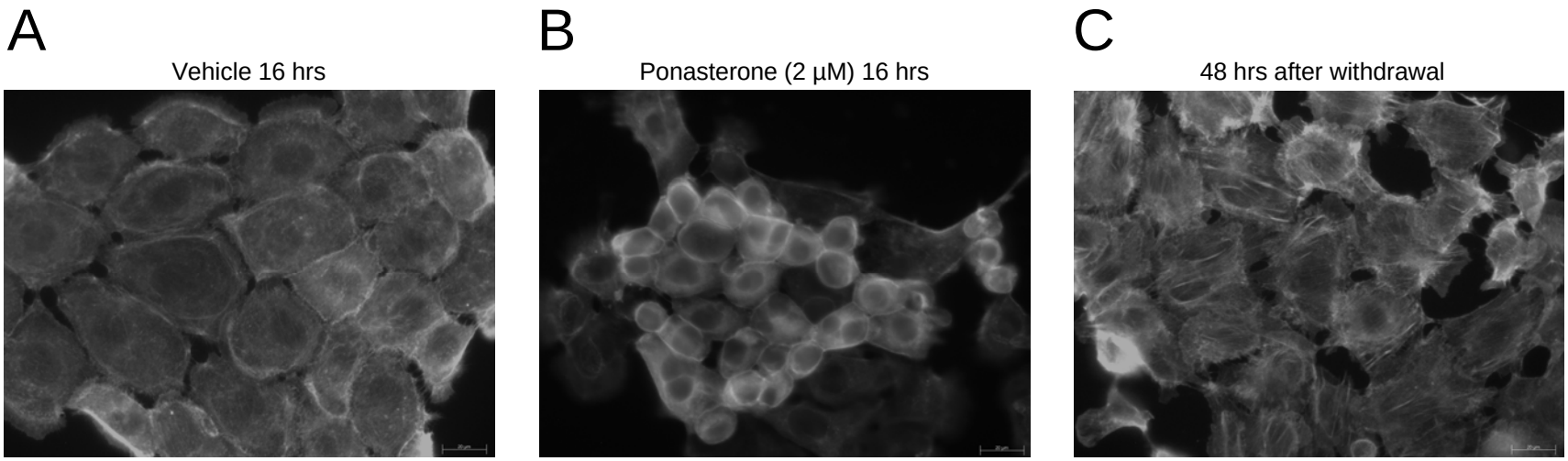
actinin alpha 4-Forward	5’ ATGGTCTCCGACATCAACAA 3’
actinin alpha 4-Reverse	5’ TCATTCAGCAGCCATTCTTC 3’
Collagen IA1-Forward	5’ TAAGGGTACCGCTGGAGAAC 3’
Collagen IA1-Reverse	5’ GTTCACCTCTCTCACCAGCA 3’
Fibronectin-Forward	Qiagen, Cat.no.: QT00135758
Fibronectin-Reverse	Qiagen, Cat.no.: QT00135758
Flag-RhoA-Forward	5’ TCCGGAAGAAACTGGTGATT 3’
Flag-RhoA-Reverse	5’ CACTGTGGGCACATACACCT 3’
Laminin alpha 1-Forward	5’ CTGATGGAGAGCGACTGTGT 3’
Laminin alpha 1-Reverse	5’ CCGTGAGGTTCAGAGACAGA 3’
Nephrin-Forward	Qiagen, Cat.no.: QT01053276
Nephrin-Reverse	Qiagen, Cat.no.: QT01053276
Podocin-Forward	5’ TCCATGAGGTGGTAACCAAA 3’
Podocin-Reverse	5’ CTTTGGACACATGGGCTAGA 3’
Synaptopodin-Forward	5’ TTCCCTTTCCTTCCATTCAC 3’
Synaptopodin-Reverse	5’ CATTTGCCAGGCTGTAGAAA 3’
Wilms tumor 1 homolog (Wt1)-Forward	5’ ACGTCCTTTCATGTGTGCAT 3’
Wilms tumor 1 homolog (Wt1)-Reverse	5’ TTTCTCACCAGTGTGCTTCC 3'
GAPDH-Forward	5’ TAAGAGCAACTGGGGGTTTG 3’
GAPDH-Reverse	5’ CCCTGTTGCTGTAGCCGTAT 3'

Supplementary Figure 1



Supplementary Figure 1. Quantification of histological changes in double transgenic mice. Histological changes were quantified by blinded scoring of glomerulosclerosis (1=none/trace, 2=mild/segmental, 3=moderate/global, 4=severe sclerosis) and tubular interstitial damage (composite score of leukocyte infiltration and tubular damage: 1=none/trace, 2=mild/patchy, 3=severe/prominent, respectively). Tubular damage includes atrophy, dilatation, and vacuolar change. Low and High responders (columns 2 and 3) were defined as in Fig. 3. Withdrawal (W) group was further divided in two groups; recovery (clear trend for recovery, column 4) and non-recovery (sustained albuminuria, column 5). N=3-5 mice per group, *$p<0.05$ and **$p<0.001$ vs –Dox.

Supplementary Figure 2



Supplementary Figure 2. Cell contraction induced by CA-RhoA is reversible *in vitro*. Rat visceral glomerular epithelial cells (GEC), which express CA-RhoA in a ponasterone-inducible manner (as in Fig. 9), were stimulated with ponasterone A (2 µM, B) or vehicle (ethanol, A) for 16 hrs. In C, cells were washed after 16 hrs of ponasterone stimulation and cultured for additional 48 hrs in the regular medium. Cells were fixed, permeabilized and stained for phalloidin. Ponasterone induced cell contraction with intense cortical F-actin (B) but these changes were no longer observed 48 hrs after ponasterone was removed. Magnification, 400x. Scale bars = 20 µm.