Supplementary Materials and Methods

Histopathology and electron microscopy. The slides were then scanned digitally by the Advanced Optical Microscope Facility (Princess Margaret Hospital, Toronto, Canada) and mean cross sectional areas were calculated using Aperio ImageScope software (Aperio Technologies Inc., Vista, CA). Glomerular volume ($\bar{V}_G$) was calculated from the mean cross sectional area ($\bar{A}_G$) of 33 glomerular profiles on each animal using the following equation: $\bar{V}_G = \beta/k \cdot (\bar{A}_G)^{3/2}$, where β = 1.38 is the shape coefficient for spheres (the idealized shape of glomeruli) and k = 1.1 is the a size distribution coefficient as previously described.(1) For electron microscopy, tissue was fixed in buffered 1% glutaraldehyde-4% formaldehyde, post-fixed in 1% osmium tetroxide, embedded in epon-araldite and then processed for GBM thickness measurements as previously described.(1) The mesangial matrix expansion (MME) score was calculated based on a random and blinded assessment by our expert renal pathologist (AMH) of 50 glomeruli per group from PAS-stained sections. Each glomerulus was scored from 0 to 3 with 0=no mesangial expansion, 1=mesangial expansion greater than the width of 1 mesangial cell nucleus, 2=mesangial expansion greater than 2 mesangial cell nuclei width and 3=mesangial expansion greater than 3 mesangial cell nuclei width. Immunoperoxidase staining and relative quantification for alpha-smooth muscle actin and collagen III staining was performed as previously described.(2-4) Neutrophils were stained using rat anti-neutrophil antibody (AbD Serotec, Raleigh, NC), and macrophages were stained using F4/80 staining as previously described.(4; 5)

NADPH Oxidase Activity and Dihydroethidium Fluorescence (DHF). The cells were then treated with placebo or hrACE2 (25 ng/ml) and then exposed to 100 nM of Ang II for 18 hours with and without 1 hr pretreatment with the specific ACE2 inhibitor, DX600 (1 μM).(6) In a separate set of experiments, cells were pretreated with 10μM Losartan for 30 minutes, or 250ng/ml $hrACE_2$ for 1 hour, or both and then exposed to high glucose (25 mM) for 24 hrs. In the third series of experiments, cells were pretreated with 100 nM Ang-(1-7) for 1 hour, 250ng/ml $hrACE_2$ alone for 1 hour, or 10μM D-Ala^7-Ang 1-7 (A-779) for 2 hours before $hrACE_2$ was added and then exposed to high glucose (25 mM) for 24 hrs. Cells were washed twice with ice-cold PBS, collected, sonicated and centrifuged at 3000rpm for 20 minutes at 4^{o}C. NADPH oxidase activity in cultured cells and renal cortical tissue was measured using lucigenin (5 uM) and NADPH (1 mM) at 37^{o}C in an FB12 luminometer (Berthold Detection Systems, Pforzheim, Germany) as previously reported.(5) For DHE fluorescence, Sprague-Dawley rat mesangial cells (MC) were cultured on autoclaved glass coverslips and grown to 80% confluence. Cells were serum-starved and then treated as described above. DHE (4 uM) was applied to the coverslips and incubated in light-protected and humidified chamber at 37^{0}C for 1 hour. In situ fluorescence was assessed using a Zeiss confocal laser-scanning microscope (LSM 510, Dusseldorf, Germany).

Supplementary Table 1. Real time PCR Taqman Primers, Probes and TaqMan Assays

Gene	Forward Primer	Reverse Primer	Probe
Ace	5'-TGA GAA AAG CAC GGA GGT ATC C-3'	5'-AGA GTT TTG AAA GTT GCT CAC ATC A-3'	5'-FAM-ACC CTG AAA TAT GGC ACC CGG GC-TAMRA-3'
Ace2	5'-GGA TAC CTA CCC TTC CTA CAT CAGC-3'	5'-CTA CCC CAC ATA TCA CCA AGC A-3'	5'-FAM-CCA CTG GAT GCC TCC CTG CCC-TAMRA-3'
AT1R	5'-CCA TTG TCC ACC CGA TGA AG-3'	5'-TGC AGG TGA CTT TGG CCAC-3'	5'-FAM-CTC GCC TCC GCC GCA CGA-TAMRA-3'
AT2R	5'-CAG CAG CCG TCC TTT TGA TAA-3'	5'-TTA TCT GAT GGT TTG TGT GAG CAA-3'	5'-FAM-CAA CTG GCA CCA ATG AGT CCG CCT-TAMRA-3'
B2R	5'-ATG TTC AAC GTC ACC ACA CAAGT-3'	5'-GGC AGT TGT CCT TCG AAA GG-3'	5'-FAM-CTC GGG TCT GCT CTT AAC GGG A-TAMRA-3'
Pro-coll III-α1	5'- TGT CCT TTG CGA TGA CAT AAT CTG-3'	5'- AAT GGG ATC TCT GGG TTG GG-3'	5'-FAM- ATG AGG AGC CAC TAG ACT-TAMRA-3'
TNFα	5'- ACA AGG CTG CCC CGA CTA C-3'	5'- TTT CTC CTG GTA TGA GAT AGC AAA TC-3'	5'-FAM-TGC TCC TCA CCC ACA CCG TCA GC-TAMRA-3'
IL-1β	5'-AAC CTG CTG GTG TGT GAC GTT C-3'	5'-CAG CAC GAG GCT TTT TTG TTG T-3'	5'- FAM-TTA GAC AGC TGC ACT ACA GGC TCC GAG ATG-TAMRA-3'
IL-6	5'-ACA ACC ACG GCC TTC CCT ACT T-3'	5'-CAC GAT TTC CCA GAG AAC ATG TG-3'	5'-FAM-TTC ACA GAG GAT ACC ACT CCC AAC AGA CCT-TAMRA-3'
MCP-1	5'- GTT GGC TCA GCC AGA TGC A-3'	5'-AGC CTA CTC ATT GGG ATC ATC TTG-3'	5'-FAM-TTA ACG CCC CAC TCA CCT GCT GCT ACT-TAMRA3'
	Applied Biosystems Assay ID		
MasR	Mm01313004_m1		
Fibronectin-1	Mm01256742_m1		
p47^{phox}	Mm00447920_g1		
NOX1	Mm00549169_g1		
NOX2	Mm01287743_m1		
NOX4	Mm01317083_m1		
p22^{phox}	Mm00514478_m1		
p40^{phox}	Mm00476299_g1		
p67^{phox}	Mm00726636_s1		

Ace=angiotensin converting enzyme; Ace2=angiotensin converting enzyme 2; AT1R=angiotensin II type 1 receptor; AT2R=angiotesnin II type 2 receptor; B2R=bradykinin receptor-B2; MasR=Mas Receptor (Ang 1-7 receptor); TNFα=tumor necrosis factor alpha; IL-1β=interleukin-1beta; IL-6=interleukin-6; MCP-1=monocyte chemoattractant protein-1.

Supplementary Table 2. Expression Profile of the Mas Receptor, ProInflammatory Cytokines and the Chemokine, MCP-1

	WT+Placebo	WT+hrACE2	Akita+Placebo	Akita+hrACE2
N	6	6	8	8
MasR/18S	0.51±0.09	0.49±0.07	0.46±0.08	0.55±0.09
TNFα/18S	0.47±0.051	0.53±0.059	0.41±0.06	0.61±0.073
Interleukin-1β/18S	1.46±0.15	1.51±0.12	1.25±0.16	1.19±0.17
Interleukin-6/18S	0.072±0.009	0.076±0.008	0.081±0.011	0.069±0.008
MCP-1/18S	3.02±0.15	3.87±0.23	3.11±0.17	3.42±0.21

MasR=Mas Receptor (Angiotensin 1-7 Receptor); TNFα=tumor necrosis factor alpha; MCP-1= monocyte chemoattractant protein-1 (also known as CCL2); p=NS using one-way ANOVA with multiple comparison testing.

Supplementary Table 3. Expression Profile of the NADPH oxidase subunits

	WT+Placebo	WT+hrACE2	Akita+Placebo	Akita+hrACE2
N	6	6	8	8
NOX1/18S	0.23±0.031	0.25±0.022	0.28±0.034	0.31±0.036
NOX4/18S	219±11.5	187±14	231±22	239±23.7
$p22^{phox}$/18S	45.8±4.1	40.2±5.9	49.6±6.3	42.4±4.8
$p40^{phox}$/18S	0.71±0.04	0.67±0.05	0.79±0.07	0.81±0.08
$p67^{phox}$/18S	0.49±0.05	0.54±0.08	0.63±0.09	0.52±0.07

p=NS using one-way ANOVA with multiple comparison testing.

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