In order to verify that the sclerotic phenotype previously described in the ROP-Os mice is still expressed in our animal colony, we phenotyped the mice using biochemical and histological measures. Figure 1s shows a representative image of PAS stained kidney section from ROP-Os and C57-Os mice, there is increased PAS positive material in the ROP-Os compared to C57-Os.

**Urinary albumin measurement.** Urinary albumin concentration was determined using a mouse albumin ELISA kit (Bethyl Laboratories, Montgomery, TX) according to the manufacturer’s protocol. In brief, microtiter ELISA plates were coated with an affinity-purified goat anti-mouse albumin antibody, then washed and blocked. Subsequently, protein standards and unknown test samples were added to the plates and incubated for 1 hour. The plates were vigorously washed multiple times and then incubated with a different goat anti-mouse albumin antibody conjugated to HRP. After the incubation step, the colorimetric reaction was initiated using the TMB color substrate (Kirkegaard & Perry Labs, Gaithersburg, MD 20879). The color reaction was stopped by adding 2 M sulfuric acid and the color depth determination was measured at a wavelength of 450 nm. The standard curve was plotted using a semi-logarithmic scale and sample concentration was determined by comparison to the standard curve. Samples with concentration outside the range of the standard curve were diluted and re-assayed.

**Urinary and serum creatinine measurements:** Urine and serum creatinine were assayed by the enzymatic creatininase method using CREA-Plus reagent according to the manufacturer’s protocol (Roche diagnostics; Indianapolis, IN). This assay has been shown to be more accurate in mice and correlates with results obtained by HPLC [1,2]. The ROP-Os/+ mice had higher albumin to creatinine ratio as early as 6 weeks.

**Sclerosis score:** Mesangial sclerosis was scored by three blinded observers and averaged using a previously described method. [3,4]. ROP-Os mice displayed a higher sclerosis score as previously described.
Confirmation of SAGE tag differential expression. To validate differential gene expression, we confirmed the relative mRNA concentrations of a selected number of genes using kidney RNA derived from different animals (average, n=3). We used multiple methods for confirmation based on abundance of the expression of the target cDNA, the presence of different isofroms or similar cDNAs, and the possibility of designing accurate real-time PCR primers.

RNase protection assays were carried out using the Direct Protect and Max-Script kit (Ambion, Austin, TX). Briefly, cDNA fragments were PCR-generated using intron-spanning, exon-specific primers. The PCR products were cloned into pCR-II-topo (Invitrogen, Carlsbad, CA). The recombinant plasmids were sequenced to ensure identity. $[^{32}P]$ labeled cRNA probes were generated using the Max-Script kit and the probes were gel-purified according to manufacturer recommendation. The labeled probes were then annealed to the mRNA, followed by RNAse digestion. Protected probe fragments were resolved by electrophoresis and subjected to autoradiography using a phosphorimager, followed by quantification of the protected fragment intensity using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). The signal from the protected probe is directly proportional to the abundance of the corresponding mRNA in the assayed pool. A Gapdh probe was used as a control to ensure equal input of mRNA across samples and normalize expression level.

Real-time quantitative RT-PCR was done using a Roche LightCycler PCR machine in combination with SYBR green reagent supplied by Roche (Roche, Indianapolis, IN) according to the manufacturer’s protocol. The data obtained were normalized to either the Gapdh housekeeping gene or 18S rRNA. Northern Blot analysis: Northern blots were performed as previously described [5]. Briefly, $[^{32}P]$ labeled cDNA probes generated by PCR were used to probe Northern blots using RNA obtained from both ROP-Os/+ and C57-Os/+ kidney cortices. Equal loading of RNA was ascertained by hybridization with a Gapdh cDNA probe. Signal intensity was quantified using densitometry in combination with ImageQuant software (Molecular Dynamics).

We selected the genes for confirmatory experiments based on the possible pathophysiologic and/or statistical significance. Among the genes we chose to confirm were Gpx3 (glutathione peroxidase 3), Nrp2 (neuropilin), and Itch (itchy E3 ubiquitin protein ligase homolog). All confirmatory approaches revealed relative expression levels in agreement with SAGE analysis.
Primers used in Real-time quantitative RT-PCR are depicted in table 1s. Table 2s contains a listing of tags that are differentially expressed between ROP-Os and C57-Os mouse kidneys.
| Gene | Upper Primer | Lower Primer |
|------|--------------|--------------|
| **Nrp2** | AGGACACGAAGTGAGAAGCC | GTGAGGGTTGAAGTTGAGAACA |
| **Itch** | CATGCCATCTACCGCCACTAC | TCGGCAGGTTCCAGTAACAAA |
| **Siah1a** | GGCCATCTTTGTTGTAGCAAC | TTTCGGTGTGTGGCAGAGTTATT |
| **Pcbp1** | AGACGCTACTCGATTCAAGG | CGTCATCATGGCAAAGTGA |

**Table 1s.** Sequence of primers used in real-time PCR reactions
| TAG       | ROP-Os | C57-Os | P-Value       | Gene Symbol |
|-----------|--------|--------|---------------|-------------|
| CTATCCTCTC | 873    | 613    | 1.47E-10      | Gpx3        |
| ATTAACCTGG | 54     | 14     | 2.75E-06      | Glud1       |
| TGGTTGCTGG | 8      | 42     | 2.53E-05      | Nrp2b       |
| TCAAAAAAA  | 15     | 0      | 0.00011587    | Pea15       |
| ACAAAAAA   | 20     | 2      | 0.000138392   | Pde6c       |
| AAACCTGATTA| 14     | 0      | 0.000237608   | Ndufa12     |
| GTTACTTTTC | 27     | 6      | 0.000390333   | Atp5e       |
| CTGGCTGTAAT| 15     | 1      | 0.000577048   | Aspm        |
| TGGTTGTTT  | 0      | 15     | 0.000858214   | Lman2l      |
| GTGAGCCCAT | 0      | 14     | 0.001280824   | Hsp90ab1    |
| GCCACGCCC  | 24     | 6      | 0.001296626   | Hpd         |
| ATTCTCCAGT | 14     | 39     | 0.0017137     | Rpl23       |
| GTCTTGAGAG | 9      | 0      | 0.002106134   | Vamp8       |
| ATAAAAAAA  | 9      | 0      | 0.002106134   | Bag4        |
| ATTCTAACAT | 15     | 2      | 0.002151531   | Acadm       |
| TGAGTGCCCT | 1      | 16     | 0.002228166   | Adh1        |
| GATTCCGTGA | 19     | 4      | 0.002494576   | Rpl37       |
| GCTTTGAATG | 19     | 4      | 0.002494576   | Atpif1      |
| TGCTGCTCCC | 0      | 12     | 0.00287027    | Gyk         |
| GCTGGCCTCC | 1      | 15     | 0.003314916   | Rhoq        |
| GCCAGTTGGA | 22     | 6      | 0.0041826     | Eef2        |
| GTTTGTAAAA | 22     | 6      | 0.0041826     | Acsm3       |
| AGATAACACA | 8      | 0      | 0.004417209   | Rere (atrophin-2) |
| AAGACCTATG | 39     | 17     | 0.004902036   | Dbi         |
| ATCCGATTCC | 11     | 31     | 0.005368014   | Miox        |
| GTCATGACG  | 1      | 13     | 0.007371123   | Aqp1        |
| TCAAGGGTGCC| 180    | 130    | 0.008291421   | Fth1        |
| TTGTATTGTC | 36     | 66     | 0.008331722   | Mdh1        |
| CTAGCTTTTG | 22     | 7      | 0.008750438   | Rps29       |
| CTGCTGTGGA | 22     | 7      | 0.008750438   | Hmgcs2      |
| AGAGAAGAG  | 46     | 23     | 0.008962126   | Ndrq1       |
| GATCAGAAAA | 7      | 0      | 0.009358827   | Prdm16      |
| GTGGCATAC  | 7      | 0      | 0.009358827   | Pccb        |
| CAGTTGGGTTC | 7     | 0      | 0.009358827   | Mm.399814   |
| CAGTAAAAAA | 7      | 0      | 0.009358827   | Map3k7ip1   |
| AACTTTTAAA | 7      | 0      | 0.009358827   | Hp1bp3      |
| GCTGTATTCA | 7      | 0      | 0.009358827   | Folh1       |
| AATAAAAACT | 7      | 0      | 0.009358827   | FBXL12      |
| TTGTGACTG  | 7      | 0      | 0.009358827   | Ctbp1       |
| AGATCTGGCC | 7      | 0      | 0.009358827   | Atp6v1g1    |
| CTGGGGGTC  | 7      | 0      | 0.009358827   | Angptl7     |
| GACCGTCTCA | 0      | 9      | 0.0098287     | Slc4a4      |
| TTGAGCTGAG | 0      | 9      | 0.0098287     | Gabarap12   |
| TGATTGAA   | 9      | 1      | 0.010110742   | Por         |
| GAGACTAGCA | 3      | 15     | 0.010247643   | Tspan3      |
| GTGAGCCCAT | 14     | 33     | 0.010481768   | Nudt19      |
| TGAGGGGAGC | 1      | 12     | 0.011021395   | Flrt2       |
| TGGCCCCCTCC | 1    | 12     | 0.011021395   | Gcn1        |
| TAGCTTTAAA | 74     | 45     | 0.011671747   | Igfbp7      |
| CAGCTCTGGA | 14     | 3      | 0.012161366   | Gstt2       |
| TTGTGTGACC | 14     | 3      | 0.012161366   | 8430408G22Rik |
| AATAGTGTTG | 39     | 19     | 0.012221381   | Cox6c       |
| CTAATAAAG  | 26     | 10     | 0.012316032   | Cox4i1      |
| GCCTTCCAAT  | 27 | 11 | 0.013319653 | Ddx5 |
| TGGGCASTCCA | 5  | 19 | 0.014666055 | Rpl26 |
| CGTGCAACGTT | 0  | 8  | 0.014928188 | Siah1a |
| GTGACTGCACT | 4  | 16 | 0.018307961 | Lifr |
| AACTGAGGGG  | 53 | 30 | 0.018772506 | Psap |
| TCTTACAGATA | 27 | 11 | 0.019019108 | Atp1b1 |
| GGACTGAGAG  | 5  | 0  | 0.020110086 | Uqcrh |
| CGATGACTGCA | 5  | 0  | 0.020110086 | Uhmk1 |
| TATGAAACCA  | 8  | 1  | 0.020870958 | Hint3 or 1700015F17Rik |
| TCTAATGTTG  | 5  | 0  | 0.020110086 | Prnp |
| TCGCTTCTAG  | 5  | 0  | 0.020110086 | Psap |
| GACAATGTTG  | 5  | 0  | 0.020110086 | Slc13a1 |
| TTTTATTG    | 5  | 0  | 0.020110086 | Atp6v1a |
| GACCTATAAG  | 5  | 0  | 0.020110086 | Mlf2 |
| CTGCATTATG  | 5  | 0  | 0.020110086 | Psma3 |
| GAATCTTCTG  | 5  | 0  | 0.020110086 | Uqcrh |
| TCTTACGTTT  | 5  | 0  | 0.020110086 | Prtn |
| TGCCTGACTG  | 5  | 0  | 0.020110086 | Tmem37 |
| TATGAAACCA  | 8  | 1  | 0.020870958 | Slc6a15 |
| GACACTCTGG  | 5  | 0  | 0.020110086 | Csrp2 |
| TATCTGTGGT  | 5  | 0  | 0.020110086 | Clec2h |
| CTGCATTAGT  | 5  | 0  | 0.020110086 | Agps |
| TCTGTTGGA   | 5  | 0  | 0.020110086 | Mdh2 |
| CTATGAAAAT  | 8  | 1  | 0.020870958 | Tmem37 |
| GAACTCCTGG  | 8  | 1  | 0.020870958 | Tmemb4m |
| TCTGTGGTTG  | 5  | 0  | 0.020110086 | Tmem176a |
| TGCACTGGCA  | 5  | 0  | 0.020110086 | Atp6v1a |
| TGAGCTGACC  | 7  | 20 | 0.022573009 | Pdzk1p1 |
| ACTGCGGATT  | 0  | 7  | 0.022793216 | Slc22a6 |
| AAATATAGCT  | 0  | 7  | 0.022793216 | Serc1c3 |
| TATGTCAGCT  | 0  | 7  | 0.022793216 | Rps12 |
| AAATAACTCA  | 0  | 7  | 0.022793216 | Mrpl32 |
| TAAACCAACAA | 0  | 7  | 0.022793216 | Cff |
| AGAAACAAGA  | 12 | 3  | 0.022949534 | Sod1 |
| TCTGGAGAAC  | 12 | 3  | 0.022949534 | Mdh2 |
| TACCGGGTGC  | 8  | 22 | 0.023778988 | Pdzk1p1 |
| ATCTGCTCAC  | 1  | 10 | 0.02480443 | Mmb |
| GCATAGAAT  | 12 | 28 | 0.02492426 | Tmem27 |
| TGTGAGAGCA  | 30 | 52 | 0.025647948 | Acsm2 |
| GGCAGACCT  | 23 | 43 | 0.025882638 | Sord |
| CAACGTATT  | 20 | 8  | 0.027820704 | Aco2 |
| Sequence          | Length | ID  | Value         | Description     |
|-------------------|--------|-----|---------------|-----------------|
| TGTGAACGAA        | 22     | 9   | 0.02997984    | Cpt1a           |
| AAAAAGTACC       | 23     | 10  | 0.031830932   | ambiguous       |
| GGGAGCGAAAA      | 3      | 12  | 0.032439845   | ld2             |
| GGAAGAAAAA       | 9      | 2   | 0.032510072   | Tspan33         |
| GCACCGAACA       | 9      | 2   | 0.032510072   | Dstn            |
| TACACACACA       | 0      | 6   | 0.035034341   | Tubb4           |
| GCCCAGTTT        | 0      | 6   | 0.035034341   | Sdf4            |
| CTAGATTGGC       | 0      | 6   | 0.035034341   | Entpd5          |
| AGAGTCAGCG       | 0      | 6   | 0.035034341   | Atp6v0e         |
| AAGTAAGCGG       | 1      | 9   | 0.037359878   | Sec61g          |
| AGACATACGTG      | 4      | 14  | 0.037827945   | Tns1            |
| AATTCGCGGA       | 16     | 6   | 0.038419946   | Tr              |
| TTAGGAGAC        | 16     | 6   | 0.038419946   | Herpud1         |
| ATGTTGTTGG       | 5      | 16  | 0.041379494   | Prdx1           |
| CCGACGGGCCG      | 5      | 16  | 0.041379494   | ambiguous       |
| GATGACACCA       | 11     | 3   | 0.042872521   | Rps28           |
| GCCAACAAGTCG     | 11     | 3   | 0.042872521   | Calm2           |
| GACTGAACTCT      | 14     | 28  | 0.042939254   | Slc25a3         |
| GTGATGGTTTC      | 14     | 28  | 0.042939254   | Cyb5            |
| TTAGACACTG       | 7      | 1   | 0.043285935   | Tmem111         |
| TCATTATTTG       | 7      | 1   | 0.043285935   | Tgoln1          |
| ACCCACTCTGC      | 7      | 1   | 0.043285935   | Ptms            |
| TGACTATAGGG      | 7      | 1   | 0.043285935   | Ost3            |
| CTACAAAATG       | 7      | 1   | 0.043285935   | Gng12           |
| TTCCAAGAGT       | 7      | 1   | 0.043285935   | Fech            |
| TAATCTAGTG       | 7      | 1   | 0.043285935   | Ankhd1          |
| GGCAATGTGG       | 7      | 1   | 0.043285935   | 231003F16Rik    |
| GTTCCTGCAAG      | 7      | 1   | 0.043285935   | 1700020O03Rik   |
| CCCACACTAC       | 7      | 18  | 0.043521115   | Gnb2            |
| AGTGTTTTGC       | 7      | 18  | 0.043521115   | 2310043N10Rik   |
| ACAATAAACA       | 4      | 0   | 0.044128269   | Zfp623          |
| GCAGCGTCCG       | 4      | 0   | 0.044128269   | Zdhhc8          |
| TTGTATTTAA       | 4      | 0   | 0.044128269   | Wdr75           |
| TGGAAGTGTC       | 4      | 0   | 0.044128269   | UBE3A,FBP2      |
| CACAAAAGTC       | 4      | 0   | 0.044128269   | Trim2           |
| CATATAATGG       | 4      | 0   | 0.044128269   | Tmem53          |
| AAACATTTGGG      | 4      | 0   | 0.044128269   | Tm9sf3          |
| TATTTTATA        | 4      | 0   | 0.044128269   | Tloc1           |
| ACCCAATTAT       | 4      | 0   | 0.044128269   | 213,Tpm4        |
| AATTAGAGAG       | 4      | 0   | 0.044128269   | Spp1            |
| TAATAAAATT       | 4      | 0   | 0.044128269   | Slc7a8,Atf2     |
| AAAATAACTT       | 4      | 0   | 0.044128269   | Sirt3           |
| AGCTAGGGCC       | 4      | 0   | 0.044128269   | Sipa111         |
| AGTGTAAGAC       | 4      | 0   | 0.044128269   | Sfrs6           |
| ACCCGCATCT       | 4      | 0   | 0.044128269   | Scpep1          |
| GTACAGCCCC       | 4      | 0   | 0.044128269   | Sbf1            |
| GCCGAGGAAG       | 4      | 0   | 0.044128269   | Rps12           |
| TAGCCCTTGTC      | 4      | 0   | 0.044128269   | Rg9mtd1         |
| AGAGGGGTTA       | 4      | 0   | 0.044128269   | Rab24           |
| ATGCTTTAAT       | 4      | 0   | 0.044128269   | Ptp4a1          |
| CAAAGAAAAA       | 4      | 0   | 0.044128269   | Ptger4          |
| Sequence       | Start | End | Score         | Gene  |
|---------------|-------|-----|---------------|-------|
| GAAAACATTA     | 4     | 0   | 0.044128269   | Psat1 |
| TCAGCAGAGG     | 4     | 0   | 0.044128269   | Prkab1|
| AGGATGCTTG     | 4     | 0   | 0.044128269   | Ppp2ca|
| GGAGTTGTGC     | 4     | 0   | 0.044128269   | Pmm1  |
| CAGTACAAAT     | 4     | 0   | 0.044128269   | Pigx  |
| TGTATGCTCT     | 4     | 0   | 0.044128269   | Pfkp  |
| AAGAAAGGAG     | 4     | 0   | 0.044128269   | Pcolce|
| AAAAAATGGA     | 4     | 0   | 0.044128269   | Pccb  |
| GCCCCAGGAG     | 4     | 0   | 0.044128269   | Nucb1 |
| GAGTTTCAC      | 4     | 0   | 0.044128269   | Nadk  |
| TCCTCCAGTC     | 4     | 0   | 0.044128269   | Mucdh1|
| TCTTACTCTT     | 4     | 0   | 0.044128269   | Mpv17l|
| GTTTTAAATTG     | 4     | 0   | 0.044128269   | Mpu1  |
| CAAGCACTTT     | 4     | 0   | 0.044128269   | Mknk2 |
| GGAAAAATGA     | 4     | 0   | 0.044128269   | Mcl1  |
| CGCTGATAAGG    | 4     | 0   | 0.044128269   | Kctd2 |
| TTATTAAAT      | 4     | 0   | 0.044128269   | Jam2,Pfdn4|
| TGCAGGTGCA     | 4     | 0   | 0.044128269   | Itgb1 |
| AATAAAGGCC     | 4     | 0   | 0.044128269   | Imp4  |
| AACATTCTCT     | 4     | 0   | 0.044128269   | Ikbg  |
| ATCACAGGTTG    | 4     | 0   | 0.044128269   | Igfbp4|
| AGCTGCTGCT     | 4     | 0   | 0.044128269   | Igfals|
| TATATGCACT     | 4     | 0   | 0.044128269   | HNMT  |
| ACCTTACAC      | 4     | 0   | 0.044128269   | Hnf4a |
| CTAATATGGG     | 4     | 0   | 0.044128269   | H2-Ke6|
| AAGCTGCTTG     | 4     | 0   | 0.044128269   | Grpeli1|
| GGAAAGATGAA    | 4     | 0   | 0.044128269   | Grin2a|
| CTCTTGTATT     | 4     | 0   | 0.044128269   | Gats  |
| CACTACACGG     | 4     | 0   | 0.044128269   | Fkbp2 |
| GTGCAAAACTC    | 4     | 0   | 0.044128269   | Fgg   |
| AGCCAAGAGA     | 4     | 0   | 0.044128269   | Fads2 |
| TCTTAATAT      | 4     | 0   | 0.044128269   | Dld   |
| TTTGCCACCT     | 4     | 0   | 0.044128269   | Ctgf  |
| CTGCCAGCTC     | 4     | 0   | 0.044128269   | Creld1|
| TGTTCTGTCA     | 4     | 0   | 0.044128269   | Clnk2 |
| AAAAGATACT     | 4     | 0   | 0.044128269   | Cited2|
| GGAAAAAAT      | 4     | 0   | 0.044128269   | Chpt1 |
| CCCAAGGAGA     | 4     | 0   | 0.044128269   | Cct4  |
| CGATCCCCCT     | 4     | 0   | 0.044128269   | B3gat3|
| AACTTGGAGGT    | 4     | 0   | 0.044128269   | Atp6ap1|
| GCTGCTGTTA     | 4     | 0   | 0.044128269   | Aspa  |
| GCAGAGACTA     | 4     | 0   | 0.044128269   | Asl   |
| ATACTGACTT     | 4     | 0   | 0.044128269   | ambiguous|
| TTCCAGCTCT     | 4     | 0   | 0.044128269   | ambiguous|
| GTTTTGGATT     | 4     | 0   | 0.044128269   | ambiguous|
| CAATCGTGAC     | 4     | 0   | 0.044128269   | Aig1  |
| Sequence         | Count | Unique Count | P-value      | Gene(s)                                      |
|------------------|-------|--------------|--------------|----------------------------------------------|
| TTTTTTTAAG       | 4     | 0            | 0.044128269  | Acadl,Ncbp2                                  |
| ACCGTGAGGG       | 4     | 0            | 0.044128269  | Acad9                                       |
| CTTATTTTTA       | 4     | 0            | 0.044128269  | Abhd3                                       |
| ATTTGATATT       | 4     | 0            | 0.044128269  | 2810407C02Rik                               |
| ATAAACAGATT      | 4     | 0            | 0.044128269  | 2610204K14Rik                               |
| AAGCAGCAGC       | 4     | 0            | 0.044128269  | Omg                                         |
| AATTAGTTGT       | 19    | 8            | 0.045348967  | Atp5j ATP                                   |
| AGAAGACAGA       | 19    | 8            | 0.045348967  | Abcb6 ATP                                   |
| CAGGCCACAC       | 53    | 33           | 0.045918906  | Atp5b                                       |
| GTTCAGTCAA       | 3     | 11           | 0.047594628  | Sult1d1                                     |
| ATGCTTTTCA       | 3     | 11           | 0.047594628  | Kap                                         |
| TGGTGTAAGGA      | 3     | 11           | 0.047594628  | Hspa5                                       |
| TGGTGTAAGC       | 3     | 11           | 0.047594628  | Adh1                                        |
| TGCAGTATT        | 19    | 35           | 0.047766609  | G6pc,                                       |
| CAAAAAAAAA       | 20    | 9            | 0.04794967    | Psmb3                                       |

**Table 2s.** Table showing statistically significant and annotated SAGE tags. Ambiguous tags are those corresponding to more than one gene and tags not corresponding to a known gene are excluded.
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