

## Supplemental Information

### Complete Materials and Methods

#### ***SOCS3 mRNA expression analysis***

Total RNA was isolated from MDCK cells using the RNeasy kit (Qiagen) according to the manufacturers protocol. Semi-quantitative RT-PCR of SOCS3 was carried out with normalized RNA levels using the ImProm-II Reverse Transcription System (Promega). Microarray and qPCR from normal and ADPKD human tissues were performed as previously described <sup>27</sup>. Renal cysts of different sizes were obtained from five polycystic kidneys removed for medical reasons. Small cysts (SC) were defined as less than 1 ml, medium cysts (MC) between 10 and 25 ml and large cysts (LC) greater than 50 ml. Minimally cystic tissues (MCT), which might have contained a few microscopic cysts from the renal cortex, was obtained as PKD control tissue from the same kidneys. Additionally, non-cancerous renal cortical tissue from three nephrectomized kidneys with isolated renal cell carcinoma was used as normal control tissue. As described previously <sup>27</sup>, using the top 200, 500, 1000 or 2000 most variable genes across all samples in an unsupervised hierarchical cluster analysis, all cyst samples consistently clustered as a single group, while the MCT and normal renal cortical samples clustered as a second group. These results suggest that the gene expression pattern is very similar between renal cysts, albeit of different sizes, and between MCT and normal renal cortical tissue.

#### ***Luciferase transcriptional reporter assays***

HEK293T cells were cultured overnight on 12-well plates and transfected with plasmids containing the luciferase reporter (0.25 µg), β-galactosidase (10 ng), and the gene of interest (0.25 µg). Plasmids amounts were balanced with pEGFP plasmid (Clontech). Cells were transfected with Lipofectamine2000 in OptiMEM (Invitrogen) according to manufacturer's protocol, and cultured in normal medium. Growth factors/cytokines were added post-transfection for at least 16 hrs prior to lysis. Approximately 24 hrs post-transfection cell lysates were assayed for luciferase and β-galactosidase activity. Luciferase units were normalized using β-galactosidase. Experimental conditions were assayed in triplicate, each bar represents average mean fold induction with respect to control. Error bars represent standard error of the mean. All experiments were repeated at least three times and representative experiments are shown.

#### ***Co-precipitation binding assay***

HEK293T cells were cotransfected with Src and glutathione-S-transferase (GST) or a fusion protein of GST and PC1-p30. Cells were lysed in 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% TX-100, 5 mM DTT, PMSF, and protease inhibitor cocktail (Sigma). Lysates were agitated at 4°C for 30 min and precleared with CL-2B Sepharose. Cleared lysates were precipitated with glutathione Sepharose. After extensive washing precipitates were analyzed by immuno-blot with the indicated antibodies.

#### ***Immuno blotting***

Cells were lysed on ice with SDS containing lysis buffer, boiled and used immediately or stored at -80°C. Tissues were chopped on dry ice, transferred to SDS buffer containing protease and phosphatase inhibitors (Sigma), sonicated, boiled and centrifuged. Protein concentration was measured by reading absorbance at 280 nm. Protein concentrations were normalized by addition of SDS lysis buffer containing glycerol, bromophenol blue and DTT. Proteins were separated by SDS-PAGE, transferred to PVDF membranes and probed with the indicated antibodies.

## Plasmids

cDNAs of membrane-anchored and soluble PC1 constructs cloned into pCDNA4/TO (Invitrogen) have been described previously <sup>4, 5, 71</sup>. Additional deletion and point mutations of PC1 constructs were made by PCR and/or site directed mutagenesis (Stratagene). Full-length wild-type PC1 was a gift from Gregory Germino (NIDDK, Bethesda). The STAT1/3 luciferase reporter containing 4 gamma interferon activation site (GAS) elements upstream of the luciferase gene <sup>4</sup> was a gift from Tom Hamilton (Cleveland Clinic). Native human STAT3 and SOCS3 promoter regions fused upstream of a luciferase reporter gene <sup>72</sup> were gifts from George Stark (Cleveland Clinic). EGFR plasmid was a gift from Deric Wheeler (University of Wisconsin). Wild type c-Src plasmid (Addgene plasmid 13663) was obtained from Joan Brugge (Harvard Medical School). Kinase dead (K297M), dominant negative (K297M Y529F), constitutively active (Y529F), SH2-binding (R175K), and SH3-binding (D99N) mutants of Src were all made on the same pCMV5 backbone using site directed mutagenesis. The SOCS3 plasmid (<sup>73</sup>Addgene plasmid 11486) was obtained from Ronald Kahn (Harvard Medical School).

## Antibodies and reagents

Src, total STAT3, pY-STAT3 (Tyr<sup>705</sup>), total JAK2 and pY-JAK2 (Tyr<sup>1007/1008</sup>) antibodies were from Cell Signaling Technology;  $\beta$ -actin antibody and PP2 from Sigma; Pan-14-3-3 antibody (K19) from Santa Cruz. IL6 and EGF were purchased from R&D Systems. Forskolin and pan Jak inhibitor (Pyridone 6) were purchased from Calbiochem. Forskolin was used at 10 $\mu$ M unless otherwise indicated. dDAVP was purchased from Bachem, Switzerland or Sigma. OPC31260 was provided by Otsuka.

## Cell culture

MDCK cells were cultured as described <sup>4, 74</sup>. mpCkCD-cl11 cells were seeded on Transwell membranes and cultured as previously described with some modifications <sup>75</sup>. After reaching confluency, cells were withdrawn from all hormone supplements and exposed to dDAVP (10<sup>-9</sup> M) added to the basolateral medium for 16h. dDAVP was withdrawn for 8h. PP2 (20  $\mu$ M, diluted in DMSO) or vehicle was added both to the apical and basal medium for 12h. Cells were exposed to dDAVP (5min, 10<sup>-9</sup>M) added to the basolateral side of the cells. Appropriate vehicle controls were performed. Cells were lysed and analyzed by immunoblotting.

## Rodent models

Animal studies were approved by the Institutional Animal Care and Use Committees and adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The bpk and Pkd1<sup>cond/cond</sup>:Nestin<sup>Cre</sup> mouse models have been described previously <sup>4, 71, 74, 76, 77</sup>. Bpk mice and littermate controls were euthanized at postnatal day 21. Pkd1<sup>cond/cond</sup>:Nestin<sup>Cre</sup> mice and littermate controls were euthanized at postnatal day 180. PCK/Brattleboro rats were generated by breeding F1 rats resulting from PCK (Pkh1<sup>-/-</sup>) and Brattleboro (AVP<sup>-/-</sup>) crosses. PCK rats are homozygous for a splicing mutation (IVS35-2A $\rightarrow$ T) that skips exon 36 and leads to a frame shift in Pkh1. Brattleboro rats lack circulating AVP and are homozygous for a 1-bp deletion of a guanine nucleotide in the second exon of the AVP gene that results in a frame shift of the coding sequence for the carrier neurophysin II. OPC31260 was administered to rats or mice as previously described <sup>22, 24</sup> by adding to ground chow (Purina labdiet 5053) at a concentration of 0.1% between three and ten weeks of age for rats, or 0.05% between 3 and 16 weeks of age for mice. dDAVP was administered to rats via osmotic minipumps (Alzet, CA) at a dose of 10ng/hr/100g body weight between 3 and 10 weeks of age.

## Immunohistochemistry

Specimens were fixed in 10% formalin, embedded in paraffin, and 5 $\mu$ m sections were cut. Paraffin slides were deparaffinized with xylene and rehydrated with ethanol followed by antigen

retrieval in Target Retrieval Solution (DAKO) using a pressure cooker (Biocare Medical, Concord, Calif.). Sections were incubated with 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase activity followed by 10% normal goat serum (Vector Laboratories) for 1 hour at room temperature. Primary antibodies were applied at 4°C overnight and then incubated with biotinylated secondary antibodies (Vector Laboratories) for 1 hour at room temperature. Avidin/streptavidin based detection system (Vectastain Elite ABC kit; Vector Laboratories) was used. The slides were developed using diaminobenzidine (Vector Laboratories) as substrate and counterstained with Mayer's hematoxylin.

## **Supplemental Figure Legends**

### **Supplemental Fig. 1**

MDCK cells were cultured under minimal serum conditions (0.5% FBS) for the indicated periods of time resulting in increasing confluence, full confluence and further compacting post-confluence. The phase-contrast images correspond to representative fields of cells used in Fig. 1A.

### **Supplemental Fig. 2**

Diagram of PC1 expression constructs used in this study. Relevant tyrosine residues and the PXXP motif are indicated.

### **Supplemental Fig. 3**

Luciferase assay using HEK293T cells transfected with the STAT1/3 luciferase reporter and indicated genes. While PC1-p30 strongly increases Src-dependent activation of STAT3, the N- or C-terminal halves (NTp15 or CTp15, respectively) of PC1-P30 independently do not activate Src.

### **Supplemental Fig. 4**

Luciferase assay using HEK293T cells transfected with the STAT1/3 luciferase reporter and indicated genes. ADPKD patient mutations within PC1-p30 do not disrupt the ability of the soluble PC1 tail to activate Src/STAT3.

### **Supplemental Fig. 5**

Model of Src highlighting its important regulatory residues. Src can be activated by relieving two separate auto-inhibitory mechanisms<sup>38</sup>: phosphorylation of Tyr<sub>529</sub> leads to an intramolecular interaction with Src's SH2 domain, and the adoption of a closed, inactive conformation. Phosphatases such as CSK1 dephosphorylate Tyr<sub>529</sub> and initiate the Src activation process. Similarly, Src's SH3 domain can undergo an intramolecular interaction that suppresses its enzymatic activity. Binding of proteins containing PXXP consensus motifs to the SH3 domain of Src can open the intramolecular interaction leading to kinase activation. The Src(R175K) mutant which has a significantly reduced ability to bind phosphotyrosyl-containing proteins to the Src SH2 domain<sup>40</sup>.

### **Supplemental Fig. 6**

A-B, native STAT3 (A) or native SOCS3 promoters (B) fused to a luciferase reporter gene transfected into HEK293T cells in combination with Src and PC1-p30. While Src and PC1-P30 do not independently induce the the STAT3 or SOCS3 promoters, PC1-p30 combined with Src does.

**Day 1**

**Day 2**

**Day 3**

**Day 4**

**Day 5**













