Supplemental Material

Renal Fibrosis, Immune Cell Infiltration and Changes of TRPC Channel Expression after Unilateral Ureteral Obstruction in *Trpc6^{-/-}* Mice

Weiying Kong^{a,b,c,d} Timo Nicolas Haschler^{a,d,e} Bernd Nürnberg^{b,c} Stephanie Krämer^{d,f} Maik Gollasch^{a,g} Lajos Markó^a

^aExperimental and Clinical Research Center (ECRC), Charité Medical Faculty and Max-Delbrück Center for Molecular Medicine, Berlin, Germany, ^bDepartment of Pharmacology and Experimental Therapy, Institute of Experimental and Clinical Pharmacology and Toxicology, Eberhard Karls University Hospitals and Clinics, Tübingen, Germany, ^cInterfaculty Center of Pharmacogenomics and Drug Research, University of Tübingen, Tübingen, Germany, ^dMax-Rubner-Laboratory, German Institute of Human Nutrition Potsdam-Rehbrücke, Nuthetal, Germany, ^eCentre for Nephrology, UCL Medical School, Royal Free Campus, London, United Kingdom, ^fDepartment of Laboratory Animal Science and Animal Welfare, Justus-Liebig University Giessen, Germany, ^gCharité Campus Virchow, Nephrology/Intensive Care, Berlin, Germany

Figure S1



Fig. S1. (A) Average CT values of two housekeeping genes Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and Eukaryotic Translation Elongation Factor 1 alpha 1 (eEF1 α 1)]. **(B)** Average CT values of housekeeping genes GAPDH and **(C)** eEF1 α 1. Data are *means* ± *SD*; ns (*not significant*) *p*>0.05, **p*<0.05.

Figure S2



Fig. S2. Macroscopic analysis of the kidneys. Images of the left and right kidneys of **(A)** Wild type (WT) mice, **(B)** $Trpc6^{-/-}$ mice and **(C)** NZO mice. The left UUO kidneys of both WT, $Trpc6^{-/-}$ and NZO mice increased in size (hydronephrosis) compared to sham kidney. Scale bar: 1cm.



Fig. S3. Expression of *Trpc5* and *Trpc7* mRNA in brain and kidney samples in wildtype (WT) mice. (A) *Trpc5* mRNA expression and (B) *Trpc7* mRNA expression (detected by three primer pairs with different sequences). At mRNA level, *Trpc5* and *Trpc7* are highly expressed in brain but not in kidneys from WT mice. The $\Delta\Delta$ CT method was used for relative quantification. n.d., *not detected*.

Table S1: Details of specific primers used in real-time PCR experiments.

Gene	Forward	Probe	Reverse
GAPDH	5'-TGT GTC CGT CGT GGA TCT GA-3'	5'-6-FAM-TGC CGC CTG GAG AAA CCT GCC- TAMRA -3'	5'-CCT GCT TCA CCA CCT TCT TGA-3'
eEF1α1	5'-TCG TCG TAA TCG GAC ACG TA-3'	-	5'-CAG CAG CCT CCT TCT CAA AC-3'
Trpc1	5'-TGG GCC CAC TGC AGA TTT CAA-3'	-	5'-AAG ATG GCC ACG TGC GCT AAG GAG-3'
Trpc2	5'-TTG CCT CCC TCA TCT TCC TCA CCA-3'	-	5'-CCG CAA GCC CTC GAT CCA CAC CT-3'
Trpc3	5'-AGC CGA GCC CCT GGA AAG ACA C-3'	-	5'-CCG ATG GCG AGG AAT GGA AGA C-3'
Trpc4	5'-GGG CGG CGT GCT GCT GAT-3'	-	5'-CCG CGT TGG CTG ACT GTA TTG TAG-3'
Trpc5	5'-AAC TCC CTC TAC CTG GCA ACT A-3'	-	5'-GGA TAT GAG ACG CAA CGA ACT T-3'
Trpc6	5'-GAC CGT TCA TGA AGT TTG TAG CAC-3'	-	5'-AGT ATT CTT TGG GGC CTT GAG TCC-3'
Trpc7(primer1)	5'-GTG GGC GTG CTG GAC CTG-3'	-	5'-AGA CTG TTG CCG TAA GCC TGA GAG-3'
Trpc7(primer2)	5'-GCG GCC CCA TGA CTA CTT C-3'	-	5'-TGG ATA GGG ACA GGT AGG CG-3'
Trpc7(primer3)	5'-CGT CCA AGT CTG AGC CGA AT-3'	-	5'-GGT TTG TCC TAG CTT GCT GC-3'
Col1α1	5'-CAT GTT CAG CTT TGT GGA CCT-3'	-	5'-GCA GCT GAC TTC AGG GAT GT-3'
Col3a1	5'-CTC ACC CTT CTT CAT CCC ACT CTT A-3'	_	5'-ACA TGG TTC TGG CTT CCA GAC AT-3'

Col4a1	5'-TTA AAG G ACT CCA GGG ACC AC-3'	-	5'-CCC ACT GAG CCT GTC ACA C-3'
αSMA (ACTA2)	5'-ACT GGG ACG ACA TGG AAA AG-3'	-	5'-CAT CTC CAG AGT CCA GCA CA-3'
TGFβ1	5'-TGG AGC AAC ATG TGG AAC TC-3'	-	5'-GTC AGC AGC CGG TTA CCA-3'
VCAM1	5'-CTG GGA AGC TGG AAC GAA GT-3'	-	5'-GCC AAA CAC TTG ACC GTG AC-3'
ICAM1	5'-CTG GGC TTG GAG ACT CAG TG-3'	-	5'-CCA CAC TCT CCG GAA ACG AA-3'
MCP1 (CCL2)	5'-TTA AAA ACC TGG ATC GGA ACC AA-3'	-	5'-GCA TTA GCT TCA GAT TTA CGG GT-3'
IL1β	5'-GAA ATG CCA CCT TTT GAC AGT G-3'	-	5'-TGG ATG CTC TCA TCA GGA CAG-3'
IL6	5'-ATC CTC TGG AAC CCC ACA C-3'	-	5'-GAA CTT TCG TAC TGA TCC TCG TG-3'
ΤΝFα	5'-CTG AAC TTC GGG GTG ATC GG-3'	-	5'-GGC TTG TCA CTC GAA TTT TGA GA-3'