Supplementary information

Supplementary figures

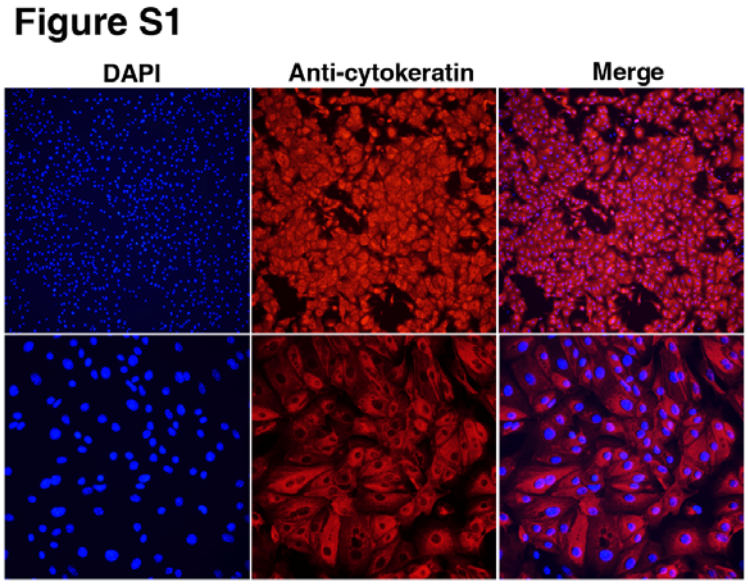

Figure S1. Purity of primary cultures of renal proximal tubular epithelial culture ascertained by cytokeratin staining.

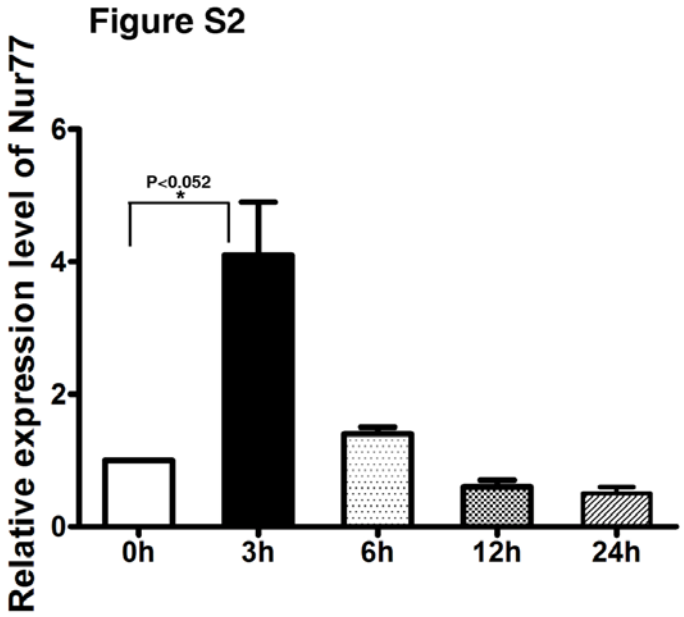

Figure S2. Induction of Nur77 in immortalized human RPTEC line, HK2. Nur77 expression is triggered by ischemia reperfusion injury induced by a cocktail of Antimycin (10 uM), 2-Deoxy Glucose (10 mM) and A23187 (2 uM) for 1 hour and reperfused for indicated periods of time.

Figure S3

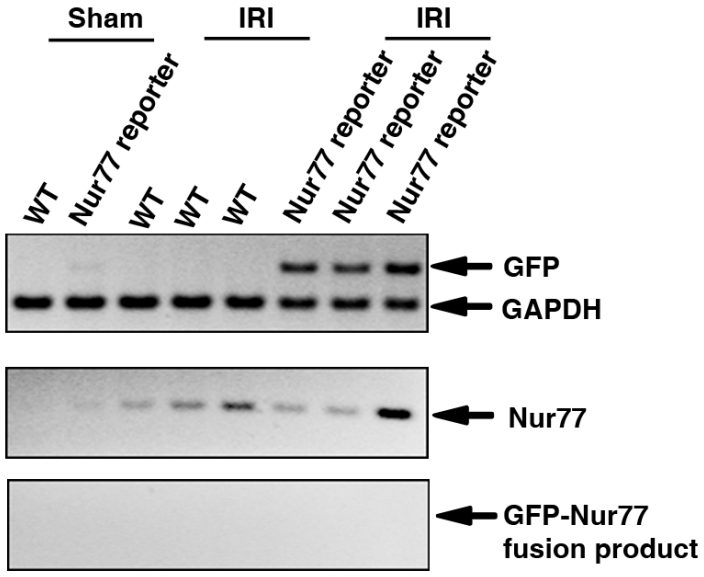

Figure S3. Nur77-GFP mice express GFP under the control of Nur77 promoter and GFP is not a fusion with the Nur77 protein. Semi-quantitative RT-PCR showing the expression of GFP and Nur77 transcripts independently (Top and middle panels) and not forming a fusion of GFP and Nur77 (Bottom panel).

Figure S4

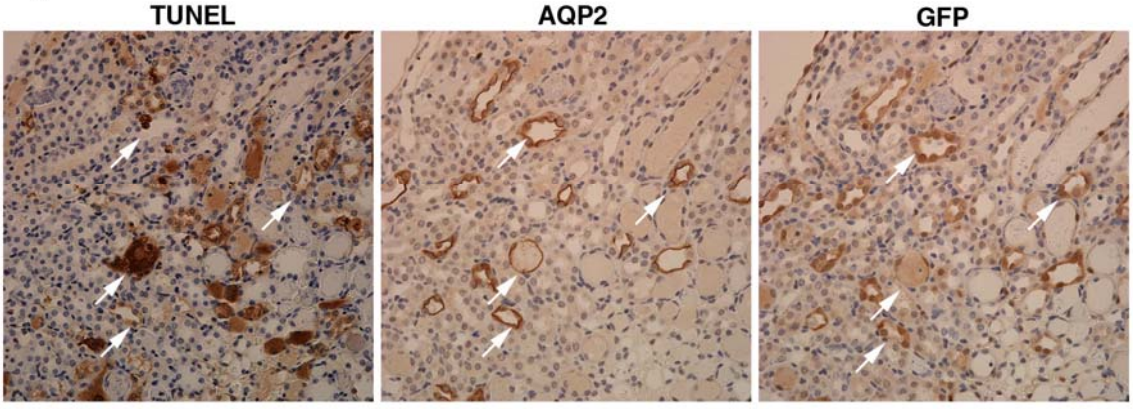

Figure S4. Nur77 expressing collecting ducts do undergo apoptosis. Staining of serial sections of kidney tissues from GFP-Nur77 reporter mice subjected to IRI for TUNEL, AQP2 and GFP (Nur77 reporter), demonstrates overlap of TUNEL, AQP2 and GFP staining indicated by arrows.

Figure S5

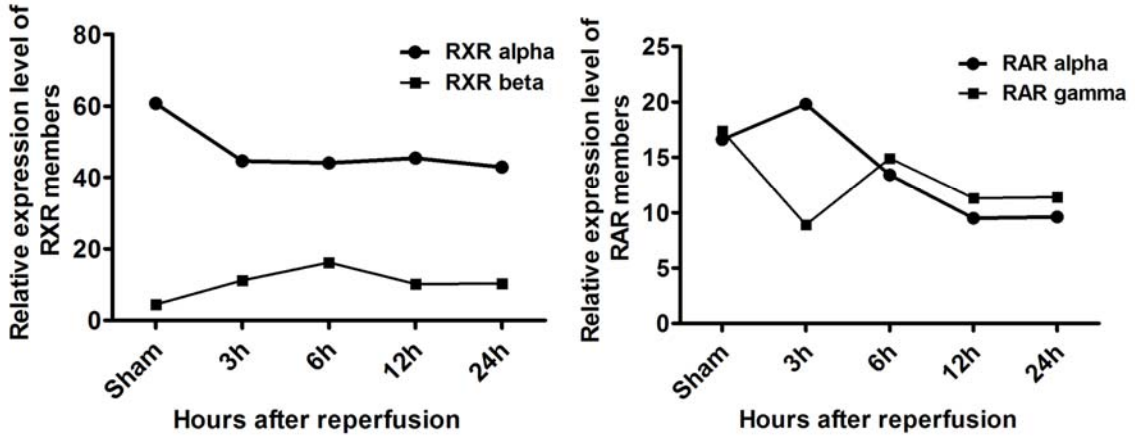

Figure S5. Expression kinetics of Retinoid X Receptor (RXR) and Retinoic Acid Receptor (RAR) family members during IRI.

Figure S6

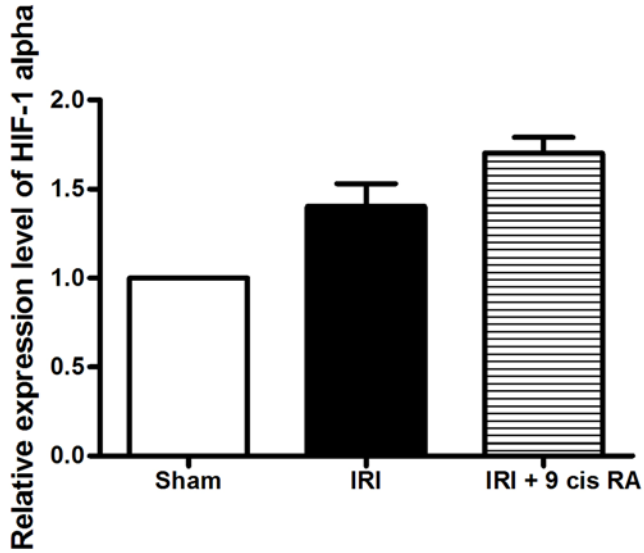

Figure S6. Transcript level of HIF-1 alpha is unchanged upon treatment with 9 cis RA, prior to the induction of IRI.

Table 1. Summary of primers used in the quantitative real time PCR analyses.

Table 1. Primers used in the qPCR analysis

Mouse		
Nur77	Sense	5'-GGT GTT GAT GTT CCC GCC TT-3'
	Anti-sense	5'-GAC AGC TAG CAA TGC GAT TCT-3'
Nurr1	Sense	5'-GAT GAG TGG AGA TGA TAC CC-3'
	Anti-sense	5'-CAG GTC AGC AAA GCC AGG GA-3'
Nor-1	Sense	5'-GAA CTC AAG CCC TCC TGC CT-3'
	Anti-sense	5'-CTG CTG TTG CTG GTG GTG GT-3'
IL-6	Sense	5'-TGATGCACTTGCAGAAAACA-3'
	Anti-sense	5'-ACCAGAGGAAATTTTCAATAGGC-3'
Cxcl2	Sense	5′-GCCCCCAGGACCCCA-3′
	Anti-sense	5′-CTTTTTGACCGCCCTTGAGA-3
Icam-1	Sense	5'-AGATCACATTCACGGTGCTG-3'
	Anti-sense	5'-CTTCAGAGGCAGGAAACAGG-3'
Csf-1	Sense	5'-CTCATGAGCAGGAGTATTGCCA-3'
	Anti-sense	5'-ATTTGACTGTCGATCAACTGCTG-3'
Gapdh	Sense	5'-CATGGCCTTCCGTGTTCCTA-3'
	Anti-sense	5'-CCTGCTTCACCACCTTCTTGAT-3'
Cxcl10	Sense	5'-CTCATCCTGCTGGGTCTGAG-3'
	Anti-sense	5'-CCTATGGCCCTCATTCTCAC-3'
Il1b	Sense	5'-GAAAGACGGCACACCCACC-3'
	Anti-sense	5'-AGACAAACCGCTTTTCCATCTTC-3'
Ccl20	Sense	5'-TCTGCTCTTCCTTGCTTTGG-3'
	Anti-sense	5'-TGTACGAGAGGCAACAGTCG-3'
Tgf beta	Sense	5'-GGAGAGCCCTGGATACCAAC-3'
	Anti-sense	5'-CAACCCAGGTCCTTCCTAAA-3'
Human		
NUR77	Sense	5'-CGCACAGTGCAGAAAAACG-3'
	Anti-sense	5'-TGTCTGTTCGGACAACTTCCTT-3'

Supplementary methods

Renal histopathology, immunohistochemistry and immunofluroscence staining

Kidneys from animals subjected to IRI were harvested. Transverse sections of the kidneys were either snap frozen in OCT or fixed in 10% formalin and processed for paraffin embedded sections. Renal morphology was assessed by PAS staining (Periodic acid-Schiff reagent).

Neutrophil infiltration was assessed by staining formalin fixed paraffin embedded (FFPE) sections with anti-Ly6G (BD biosciences). Infiltrating neutrophils were counted in

3 independent HPFs (400X) per section per animal and represented as mean (Ave ± SEM).

Nur77 staining was carried out on FFPE sections after antigen retrieval using anti-mouse Nur77 antibody (Ebioscience). Endogenous mouse Ig was blocked using M.O.M kit from vector labs.

Staining of FFPE or frozen sections were carried out to visualize GFP using anti-GFP antibody (Cell signaling technology) and staining of serial sections were performed to study coexpression of GFP and kidney and endothelial markers viz., AQP2 (Abcam), SGLT1 (Abcam), CD31 (Santa Cruz).

Kidney cell apoptosis was assessed by TUNEL staining using ApopTag® Peroxidase In Situ Apoptosis Detection Kit (Millipore). The apoptotic index was calculated as percentage of dead cells in 3 independent 400X HPF per section per animal by counting both stained nuclei and total nuclei.

Staining for Cxcl2 and cleaved caspase3 was carried out in primary cultures of RPTECs that were subjected to 1h of hypoxia by mineral oil overlay followed by reoxygenation for 6h. The reoxygenation medium was supplemented with monensin to block the secretion of Cxcl2. Double staining was carried out on paraforamldehyde fixed cells using anti-mouse Cxcl2 (R&D) and anti-cleaved caspase 3 (Cell signaling technology). Staining for Bcl2-BH3 domain (Abgent Inc) and Bcl2 (Cell signaling technology) were performed on FFPE sections.

Mouse primary renal proximal tubule culture

Kidney cortex was separated and minced into ~1mm^3 pieces and digested using collagenase and soybean trypsin inhibitor for 30 min and 37°C. The digested mix was spun down and filtered through 200 uM filter followed by a 70 uM filter. The tubules that are retained on the top of the 70 uM filter were collected and cultured in Renal Life cell growth medium (Lifeline cell technology) on collagen coated plates. After 7 days of culturing, the cells were used for experiments. The purity of the culture was ascertained by staining for cytokeratin (Anti-cytokeratin antibody-Sigma). To induce IRI / hypoxia-reoxygenation, confluent monolayer of RPTECs was overlayed with mineral oil for 1h, followed by reperfusion with complete medium.

Chemical induced IRI

To perform chemical induced IRI, cells were washed with Hank's Buffered Salt Solution (HBSS) and incubated with HBSS containing Antimycin (10 uM), 2-Deoxy Glucose (10 mM) and A23187 (2 uM) for 1 hour at 37° C, 5% CO_2. Subsequently the cells were washed with HBSS and cultured in complete culture medium for defined period of time.

Real time PCR quantification

Kidney tissues were tissues were homogenized and total RNA was prepared using RNeasy kit (Qiagen). DNA contamination was eliminated by DNase digestion. The reverse transcribed cDNA was subjected to qPCR using a Sybr green based detection system (SA Bioscience). Relative levels of mRNA was normalized to GAPDH levels and quantified based on 2e-deltaCT method.

Semi quantitative RT-PCR

Semi quantitative RT-PCR was carried out using cDNA as template in a reaction mix containing both GAPDH and gene-specific primers and amplified for 25 cycles. A reaction to detect the formation of GFP-Nur77 transcripts was performed using forward primer spanning GFP and reverse primer spanning Nur77.

Western blotting analysis

Kidney tissue lysates were prepared by homogenizing the tissue in lysis buffer (50mM Tris pH7.5, 150mM NaCl, 5mM EDTA, 10% Glycerol, 0.5% Triton X-100, 1% NP-40 and protease inhibitor cocktail). Protein concentrations were quantified using Bio-rad DC kit and equal amounts of protein samples were separated on 4-12% polyacrylamide gel (Invitrogen) and blotted onto PVDF membrane (Millipore). The membrane was probed with anti-Bcl-xL antibody (cross-reacts with Bcl-xS) (Cell signaling technology), followed by stripping and probing with anti-GAPDH antibody (abcam).