Supplemental Figure 1 Kidney morphology is normal in NOX4 KO mice. (A-B) Representative images of Haematoxylin/Eosin (A1-2 and B1-2) and Masson trichrome staining (B3-4) analysis of kidney morphology and structure performed in contralateral (CTL) kidney sections from WT and NOX4 KO mice. (C) Representative images of AQP2 (C1-2) and NaPi2a (C3-4) immunostaining performed in contralateral (CTL) kidney sections from WT and NOX4 KO mice showing a globally preserved tubular architecture in NOX4 deficient kidneys. Scale bars= 200 μm.
Supplemental Fig. 2

**Tubulo-interstitial fibrosis is enhanced in obstructed kidneys of NOX4 KO mice** (A-B) Representative images of Argentic and Masson trichrome staining performed in contralateral (CTL) and obstructed (UOO7d) kidneys from WT and NOX4 KO mice after 7 days of UOO. (C) Representative images of polarized Sirus red staining performed in obstructed kidneys (UOO7d) from WT and NOX4 KO mice after 7 days of UOO. (D) Renal cortex quantification of polarized Sirus red staining performed in obstructed kidneys (UOO7d) from WT and NOX4 KO mice after 7 days of UOO (WT n=6, NOX4 KO n=6). Results are expressed as the mean ratio of stained area to the total tissue area over the mean value obtained in WT± SEM. ns p>0.05, * p<0.05.
Supplemental Fig. 3

Supplemental Figure 3: **Coomassie staining** Coomassie blue staining coupled to BCA assay used as a loading control for Western blot analysis of 7 (UUO7d and CTL kidneys) or 14 day UUO protein samples.
Supplemental Figure 4 **Tubular cell apoptosis occurs in the collecting duct**

Representative images of TUNEL (A), merged TUNEL/DAPI (B), DAPI (C) and AQP2 (D) immunostaining performed in obstructed kidneys (UO7d) serial sections from WT mice.
Supplemental Figure 5  VEGF and HIF-1α expression are unchanged in contralateral kidneys, whereas NRF2 expression is decreased (A-C) VEGF, HIF1α and NRF2 Western blot analysis and respective densitometric quantification performed in contralateral kidneys (Control) from WT and NOX4 KO mice after 7 days of UUO. Results are expressed as the mean ratio of individual values over the mean value obtained in WT ± SEM. ns p>0.05, * p<0.05
Supplemental Figure 6 Representative images of Nitrotyrosine immunostaining performed in contralateral (CTL) and obstructed (UUO) kidney sections from WT and NOX4 KO mice showing enhanced nitrotyrosine labeling in NOX4 KO mice subjected to UUO.
Supplemental Figure 7 Other NOX isoforms expression is not increased in NOX4 KO mice

Relative NOX2, DUOX2 and NOX1 mRNA expression performed in obstructed kidneys (UO7d) from WT and NOX4 KO mice after 7 days of UUO (WT n=3, NOX4 KO n=3). Results are expressed as a ratio of the expression measured in UO7d to the relative expression measured in control kidneys reported to the expression of p0 as a house-keeping gene.
Supplemental Methods

Primary antibodies:
The antibodies used in this study for IHC included Mouse anti-8-Hydroxy-2'-deoxyguanosine (Abcys, dilution 1:100), Biotinylated anti-isoelectin B4 (Vector Laboratories, 1:5000), Rat anti-mouse F4/80 (abcam, 1:100), Mouse anti-PCNA (Santa Cruz, 1:100), Biotinylated anti-α-SMA (a kind gift from D ML Piallat, Université de Genève ; 1:200), Rabbit polyclonal anti-AQP2 (7661AP, a kind gift from Pr S Nielsen, University of Aarhus, Denmark 1:10000), Rabbit anti-NaPi2a (a kind gift from Dr Jurg Biber, Zurich ; 1:100), Rabbit polyclonal anti-VEGF (Abcam; 1:4000), Rabbit anti-NRF2 (Santa Cruz, 1:100), and rabbit anti-nitrotyrosine (Millipore 1:5000).

The following primary antibodies were used for western blotting: Rabbit anti-collagen-I (Mdbioproducts, Mdbiosciences), Rabbit polyclonal anti-VEGF (ab46154, abcam), Rabbit anti-fibronectin, Mouse anti-αSMA (a kind gift from Dr ML Piallat, Université de Genève), Mouse anti-E-cadherin (BD Biosciences), Rabbit polyclonal anti-PARP, Rabbit polyclonal anti-VEGF (Abcam; 1:1000), Rabbit anti-NRF2 (Santa Cruz, 1:500), Rabbit anti-HIF-1α (Novus biological, 1:500) Mouse anti-GAPDH (Milipore), rabbit anti Na-K ATPase34, Mouse anti-β-actin (Sigma, 1:10000).

Real-time PCR primers:
mouse NOX4 5’-CTGCTCATTTTGCTGCCTCTA-3’ and 5’-CGGCTACATGCACACCTGAGAA-3’, mouse NOX2 5’-TCAACTACTATAAGGTTATGATGATCG-3’ and 5’-CAGATATCTAAATTATGCTCTTCCAAA-3’, mouse NOX1 5’-GAAATTCTTGCTGCCCGCT-3’ and 5’-GCTGGAGAGAACAGAAGCGAGA-3’, mouse Duox1 5’-AGAGGCTCATTGCCACCTAC-3’ and 5’-TACTCCGAGGAGATTTGCTT-3’, mouse Duox2 5’-CTGCTGGCTGACAAAGATGATGATCG-3’ and 5’-AACATCAGGCGGACTTATC-3’, mouse GAPDH 5’-GTCGTGGATCTGACCTGCC-3’ and 5’-GATGCTTGCTGCC-3’, mouse GSTa2 5’-GCTTGATGCCGCACCTCTTCTG-3’ and 5’-GCTTGCTGATTCTGCTCTTTGCA-3’, mouse Txnrd1 5’-GATGCAACCAGCGACTTATC-3’ and 5’-TCTGCGCTTTCCAGCATGATCTC-3’, mouse NQO1 5’-GCCGCATGCAGATCTC-3’ and 5’-GCTGCTCTCACCAGCGGT-3’, mouse TGF-β1 5’-CGGAGAGCCCGGATACCA-3’ and 5’-GCCGCACACAGCAGTTCTT-3’, mouse p0 5’-ATCTCCAGAGGCCCACCT GTG-3’ and 5’-GTTCAGCATGTTCCAGCAGTG-3’.