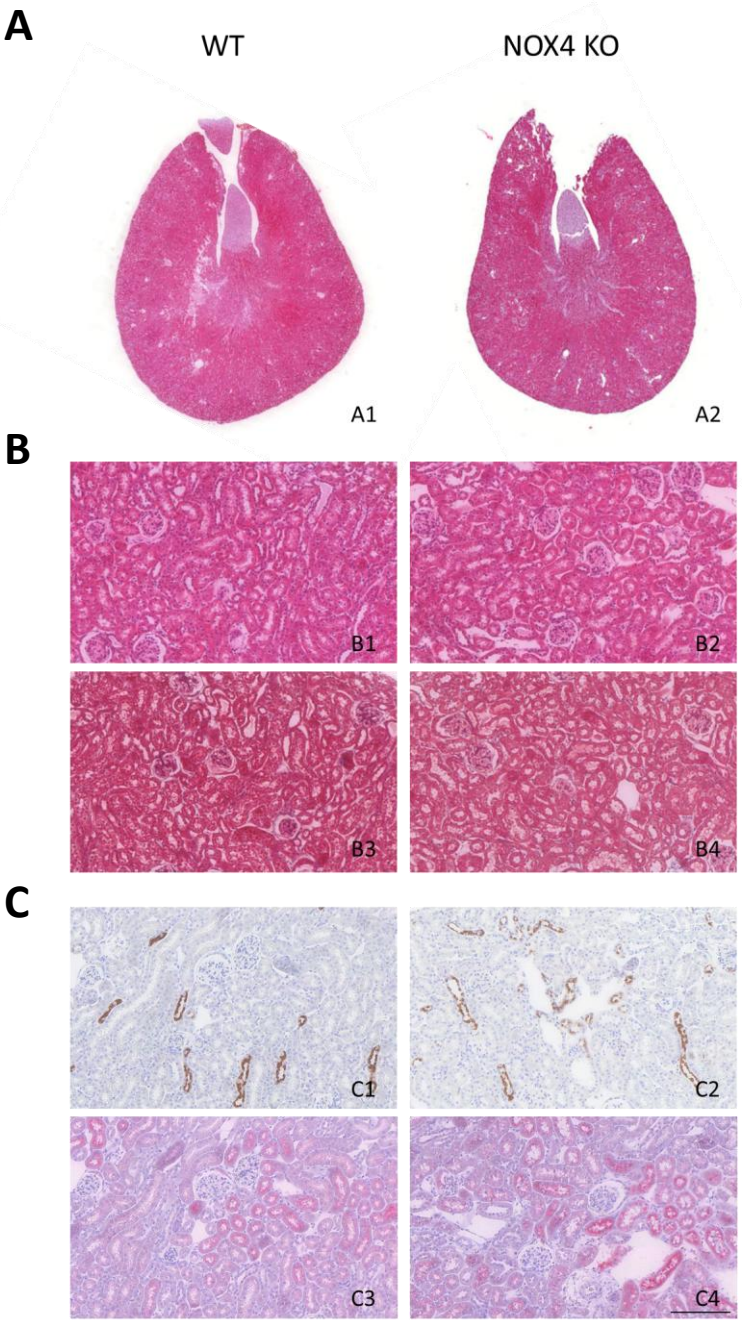
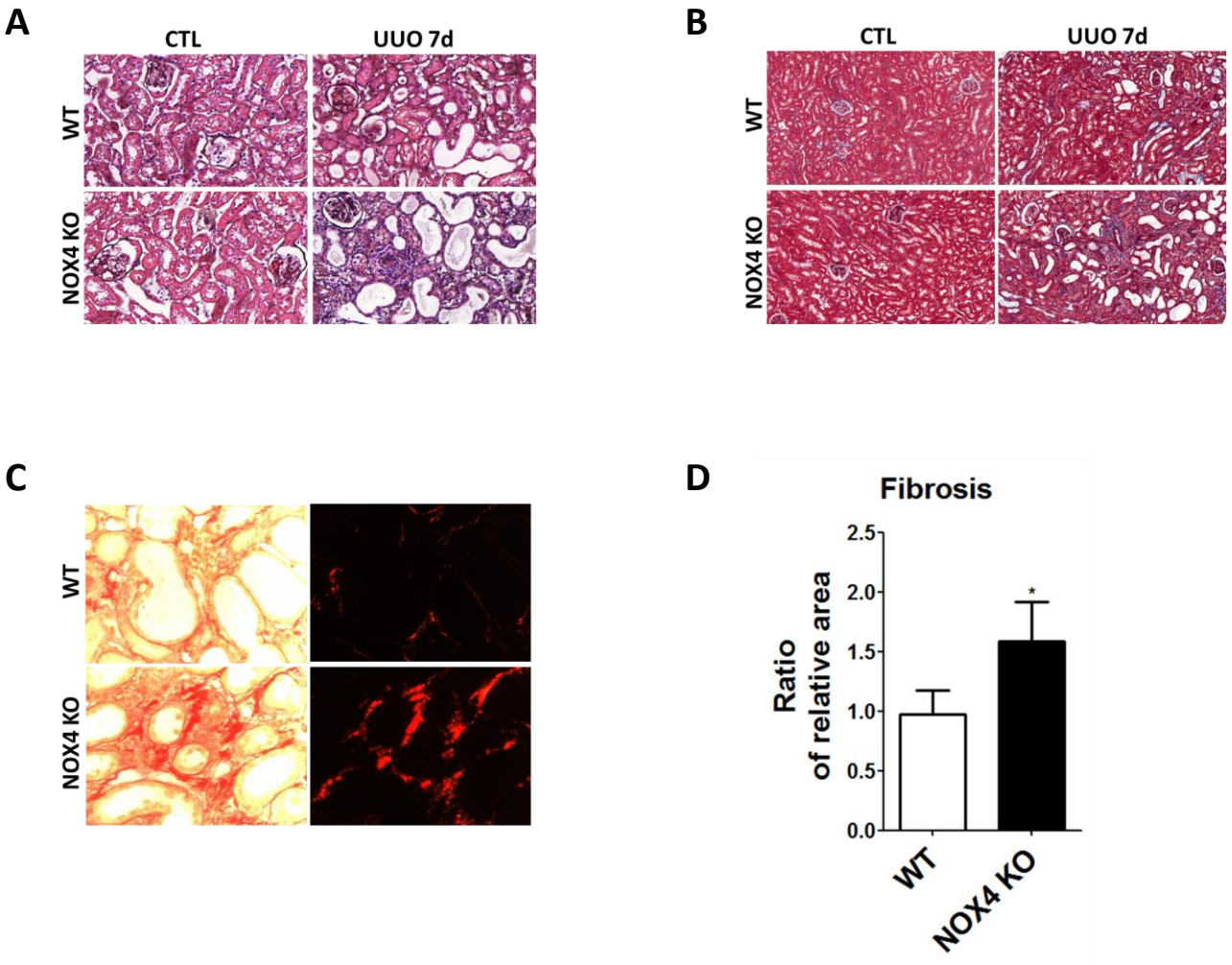
# Supplemental Fig. 1



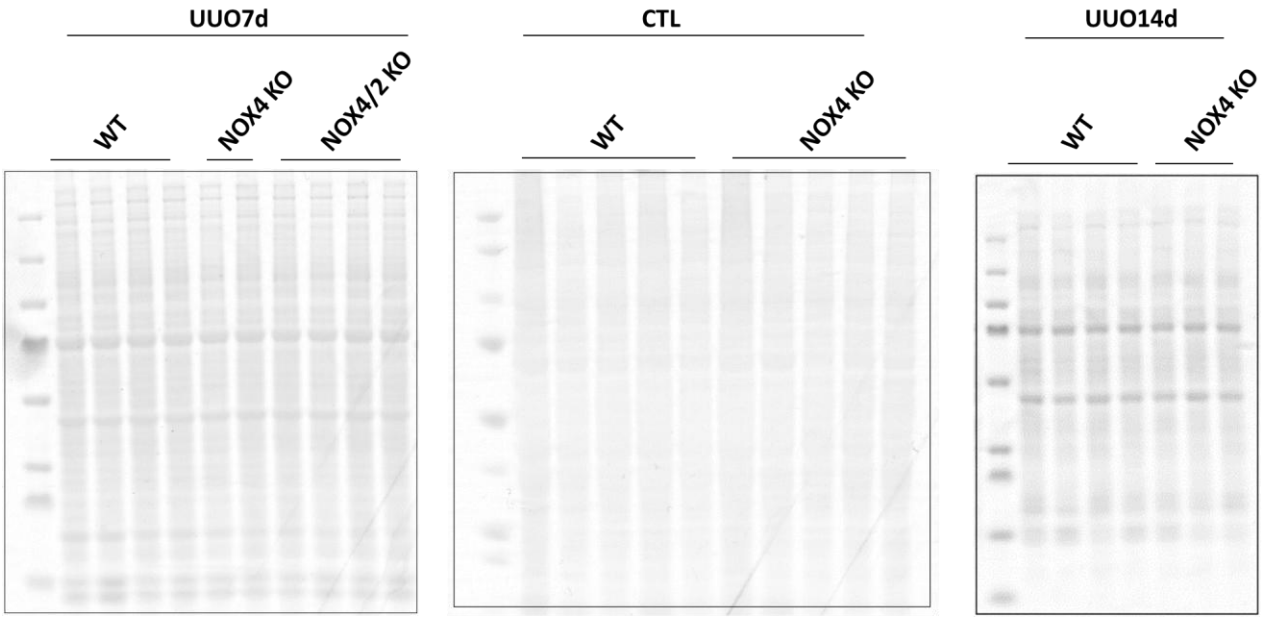

Supplemental Figure 1 **Kidney morphology is normal in NOX4 KO mice**. (A-B) Representative images of Haematoxylin/Eosin (A1-2 and B1-2) and Masson trichrome staining (B3-4) analysis of kidney morphology and structure performed in contralateral (CTL) kidney sections from WT and NOX4 KO mice. (C) Representative 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



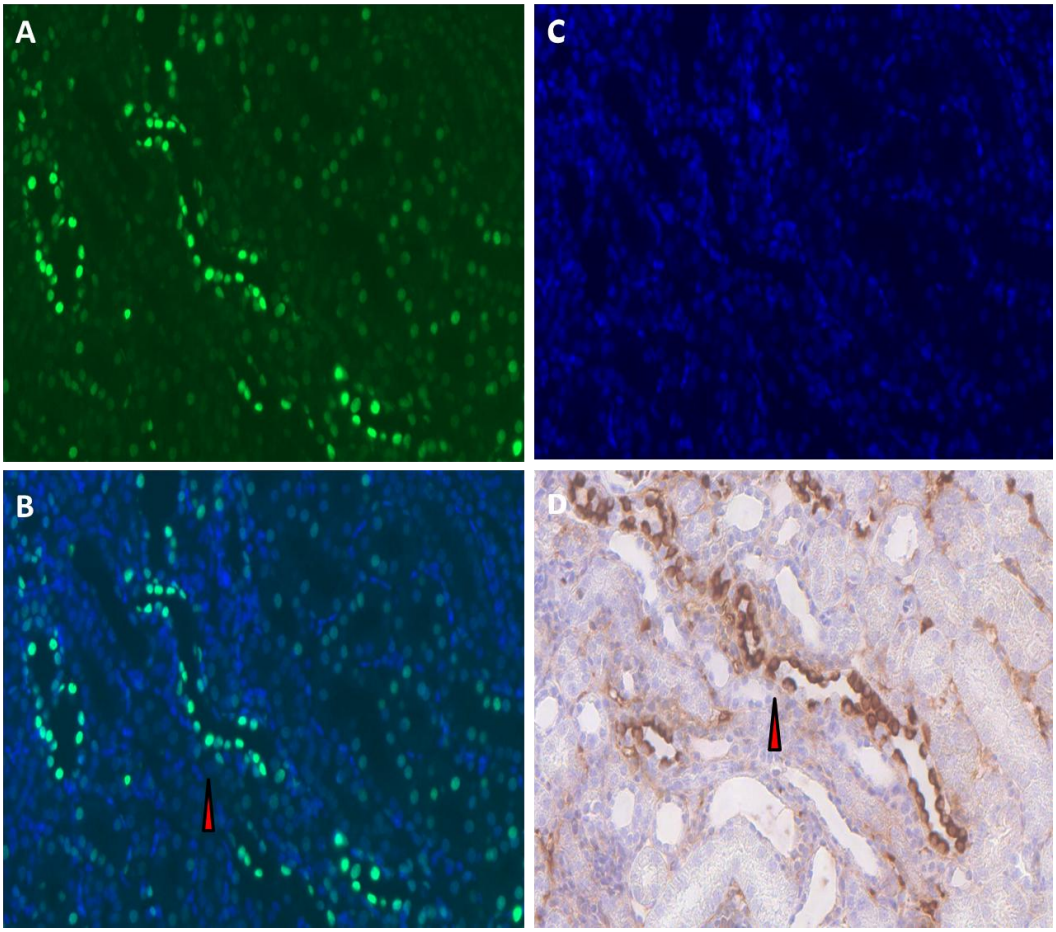

Supplemental Figure 2 **Tubulo-interstitial fibrosis is enhanced in obstructed kidneys of NOX4 KO mice** (A-B) Representative images of Argentic and Masson trichrome staining performed in contralateral (CTL) and obstructed (UUO7d) kidneys from WT and NOX4 KO mice after 7 days of UUO. (C) Representative images of polarized Sirus red staining performed in obstructed kidneys (UUO7d) from WT and NOX4 KO mice after 7 days of UUO. (D) Renal cortex quantification of polarized Sirius red staining performed in obstructed kidneys (UUO7d) from WT and NOX4 KO mice after 7 days of UUO (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



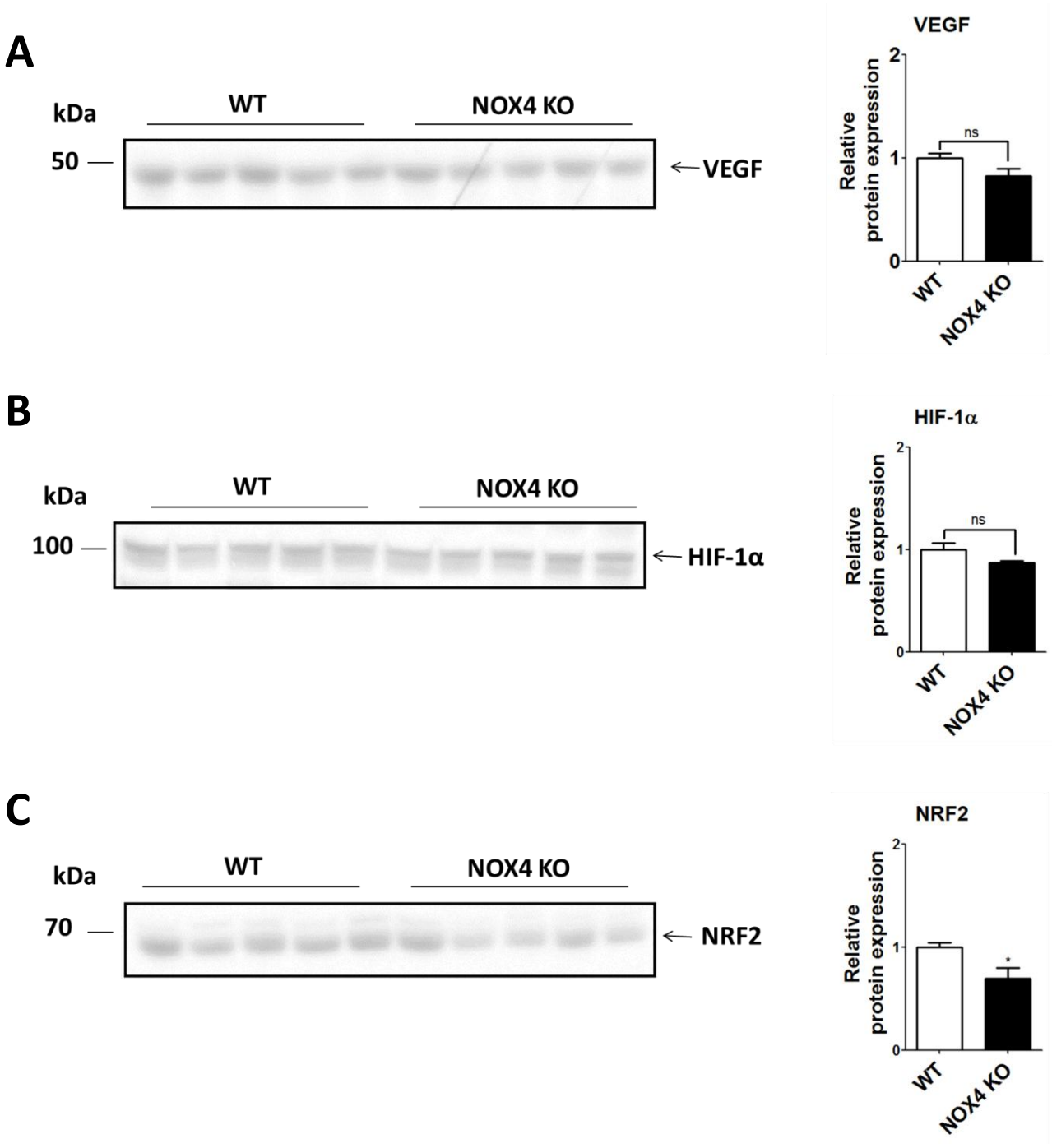

Supplemental Figure 3**: Coomassie staining** Coomassie blue staining coupled to BCA assay used as a loading control for Western blot analysis of 7 (UUO7d and CTL kidneys) or 14 day UUO protein samples.

# Supplemental Fig.4



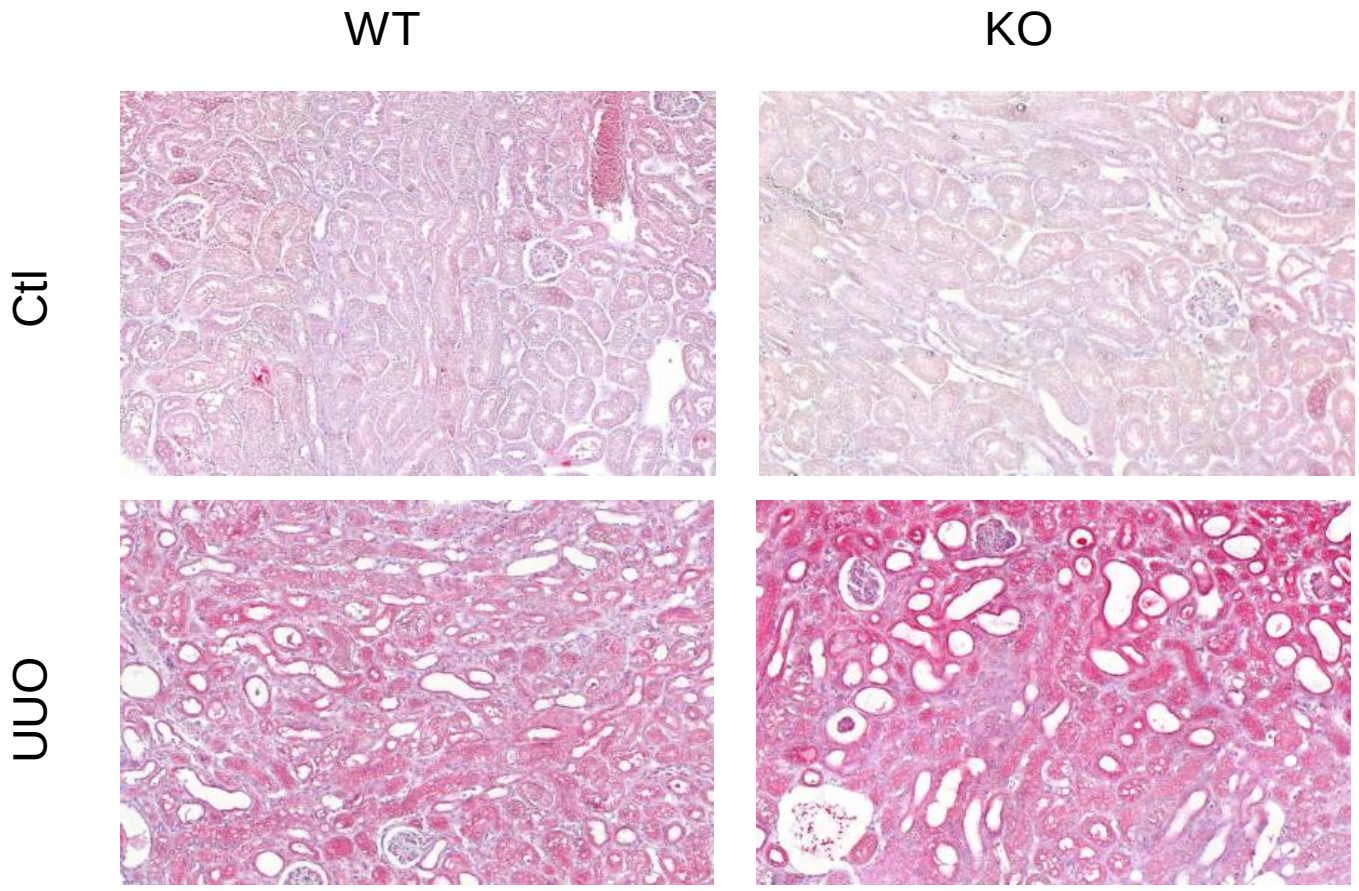

Supplemental Figure 4 **Tubular cell apoptosis occurs in the collecting duct** Representative images of TUNEL (A), merged TUNEL/DAPI (B), DAPI (C) and AQP2 (D) immunostaining performed in obstructed kidneys (UUO7d) serial sections from WT mice.

# Supplemental Fig. 5



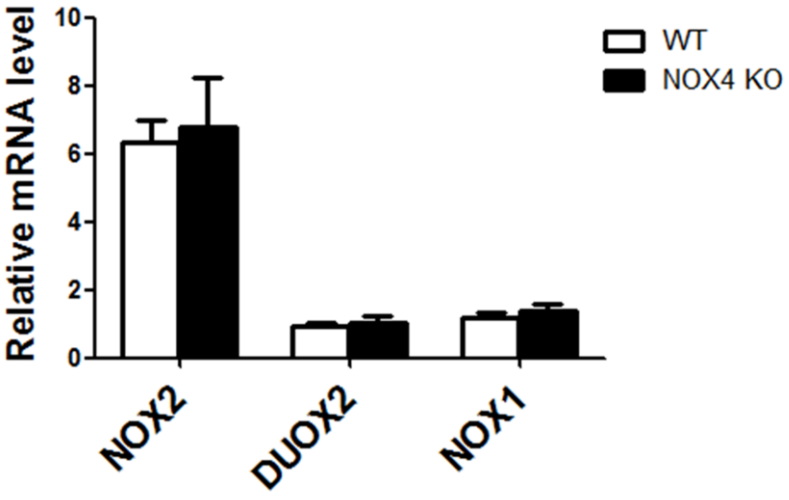

Supplemental Figure 5 **VEGF and HIF-1α expression are unchanged in contralateral kidneys, whereas NRF2 expression is decreased** (A-C) VEGF, HIF1α and NRF2 Western blot analysis and respective densitometric quantification performed in contralateral kidneys (Control) from WT and NOX4 KO mice after 7 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 fig. 6**




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 Fig. 7




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 (UUO7d) 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 UUO7d 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-isolectin 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 ATPase[34], Mouse anti-β-actin (Sigma, 1:10000).

**Real-time PCR primers**:

mouse NOX4 5'-CCTGCTCATTTGGCTGTCCCTA-3' and 5'-CGGCTACATGCACAC CTGAGAA-3', mouse NOX2 5'-TCAACTACTATAAGGTTTATGATGATGG-3' and 5'-CAGATATCTAAATTATGCTCTTCCAAA-3', mouse NOX1 5'-GAAATTCTTGGGACT GCCTTGG-3' and 5'-GCTGGAGAGAACAGAAGCGAGA-3', mouse Duox1 5'-AGAGGG TCATTGCCACCTAC-3' and 5'-TACTCCGGAGGAGTTTGCTT-3', mouse Duox2 5'-TTCTCTGGCTGACAA GGATG-3' and 5'-AACATCAGGCGGGACTTATC-3',mouse GAPDH 5'-GTCGTGGATCTGACGTGCC- 3' and 5'-GATGCCTGCTTCACCACCTT-3', mouse GSTa2 5'-GCTTGATGCCAGCCTTCTG-3' and 5'-GGCTGCTGATTCTGCTCTTGA-3', mouse Txnrd1 5'-GATGCACCAGGCAGCTTTG-3' and 5'-TCTTCGACTTTCCAGCCATAGT-3', mouse NQO1 5'-GCCCGCATGCAGATCCT-3' and 5'-GGTCTCCTCCCAGACGGTTT-3', mouse TGF-β1 5'-CGGAGAGCCCTGGATACCA-3' and 5'-GCCGCACACAGCAGTTCTT-3', mouse p0 5'-AATCTCCAGAGGCACCAT TG-3' and 5'-GTTCAGCATGTTCAGCAGTG-3'.