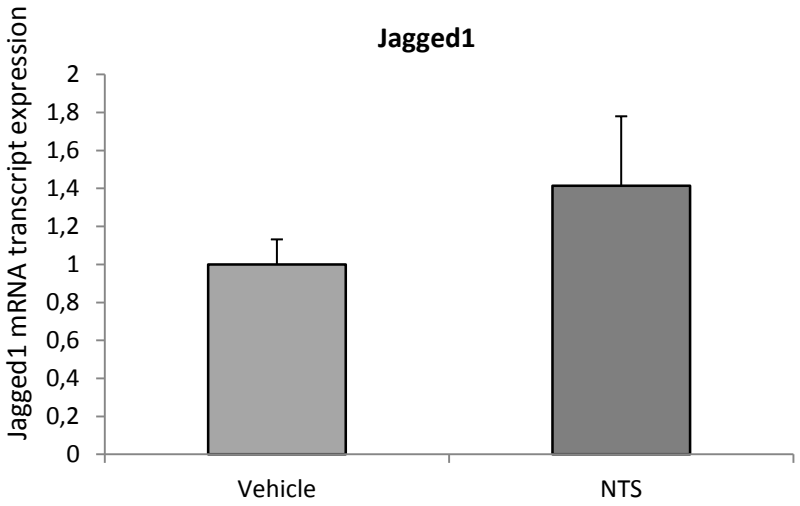
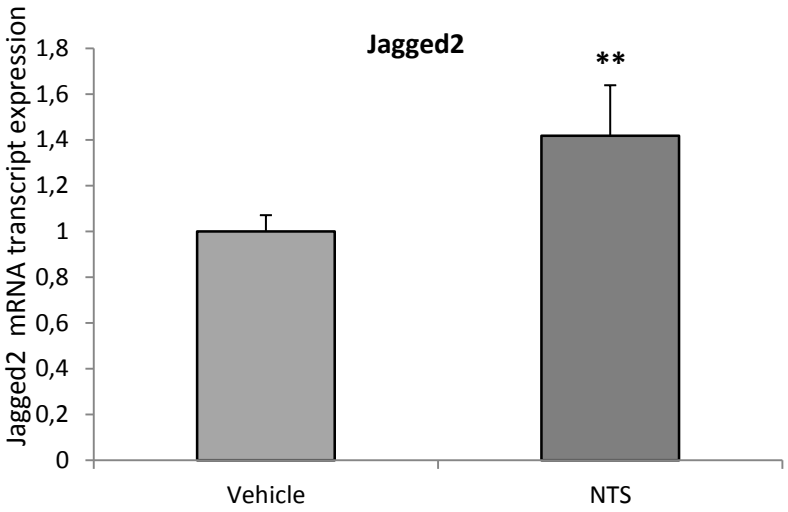
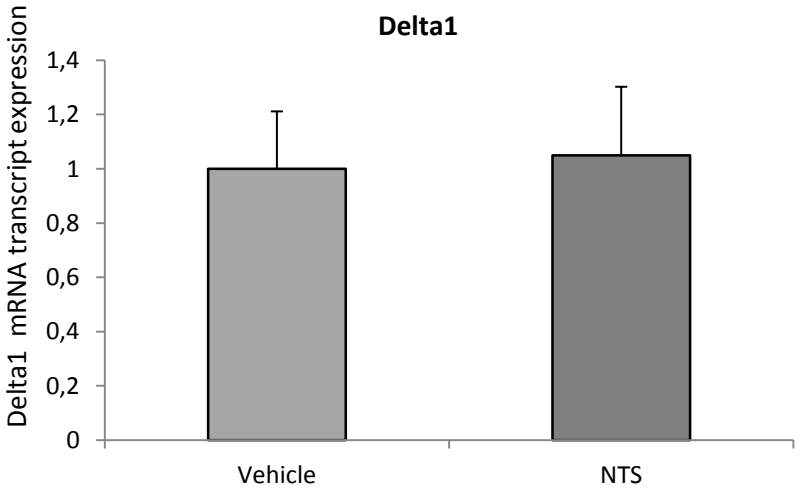
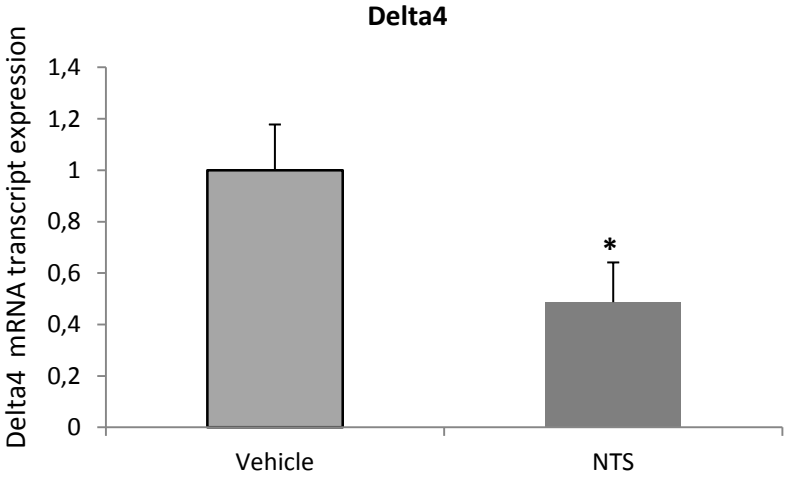
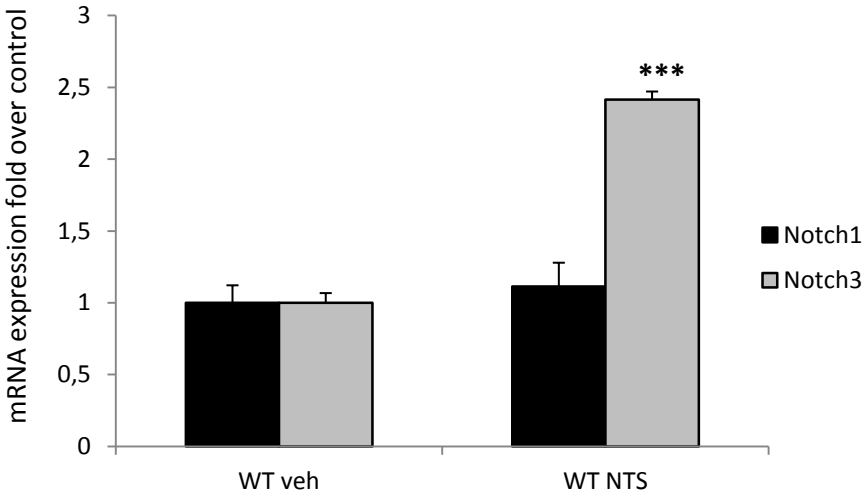


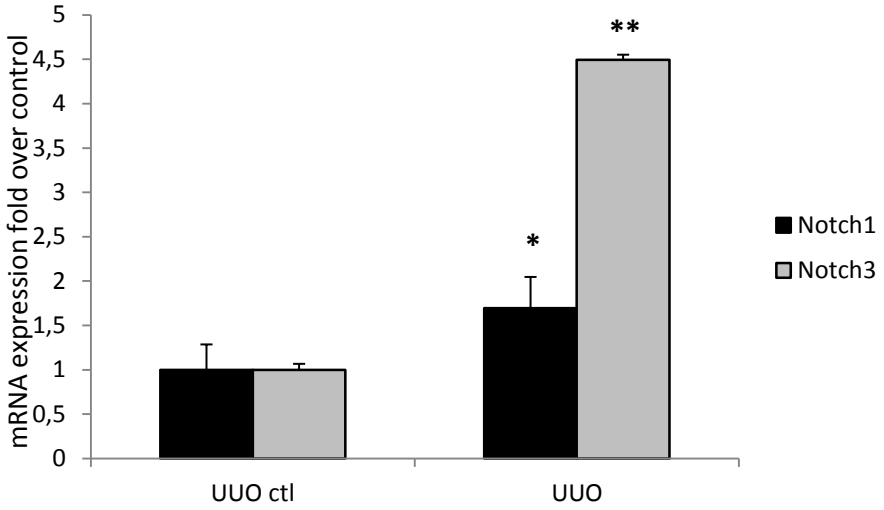


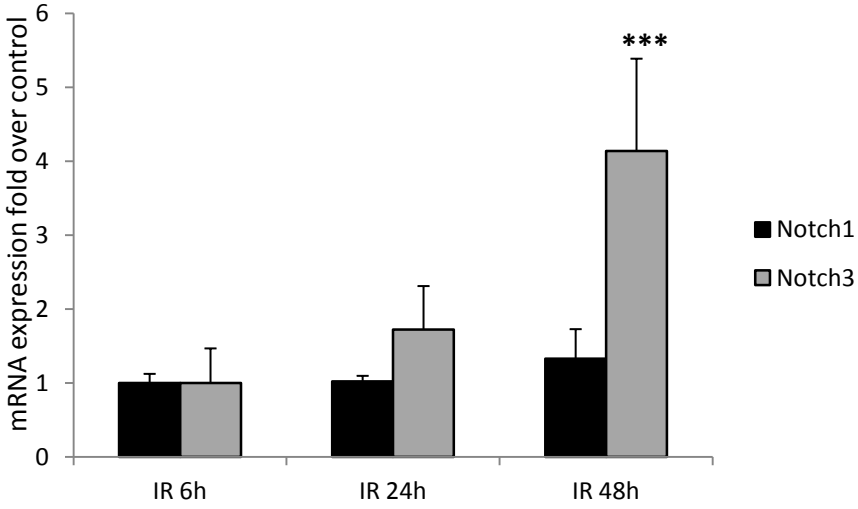


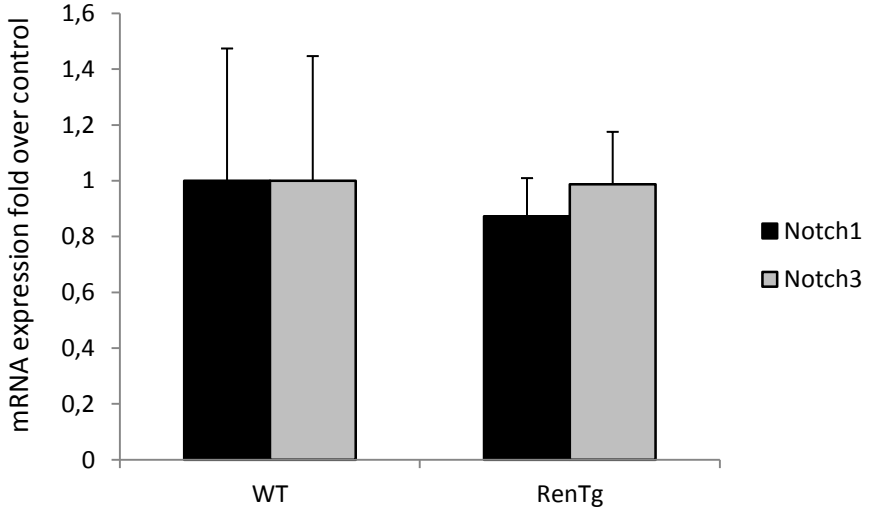


Supplementary Figure 1. Notch ligands expression in the NTS model.

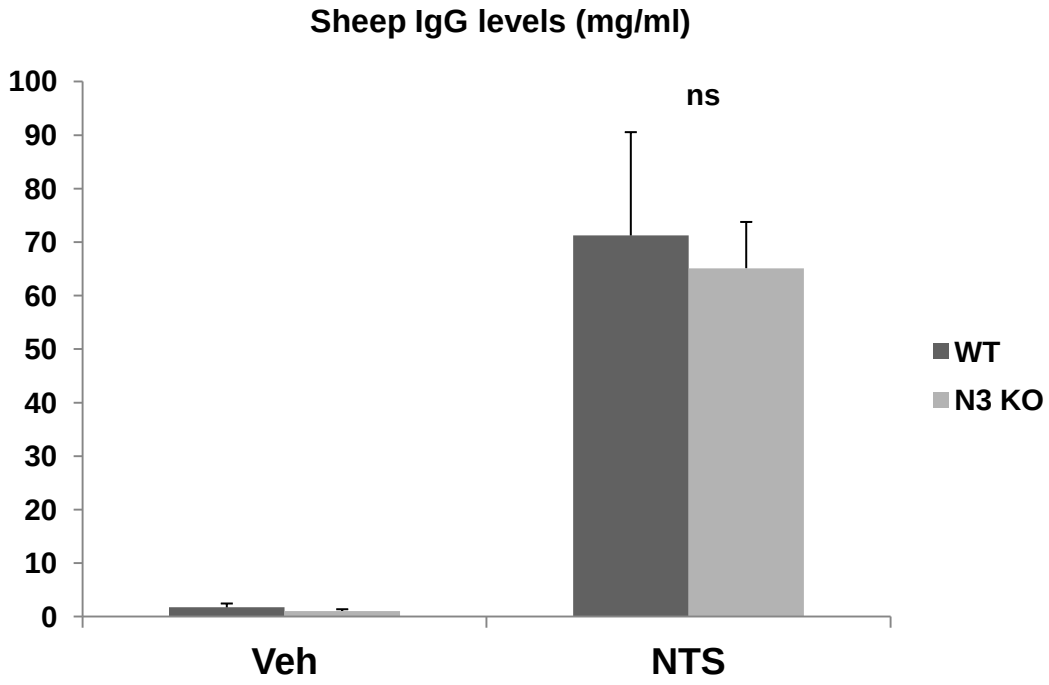




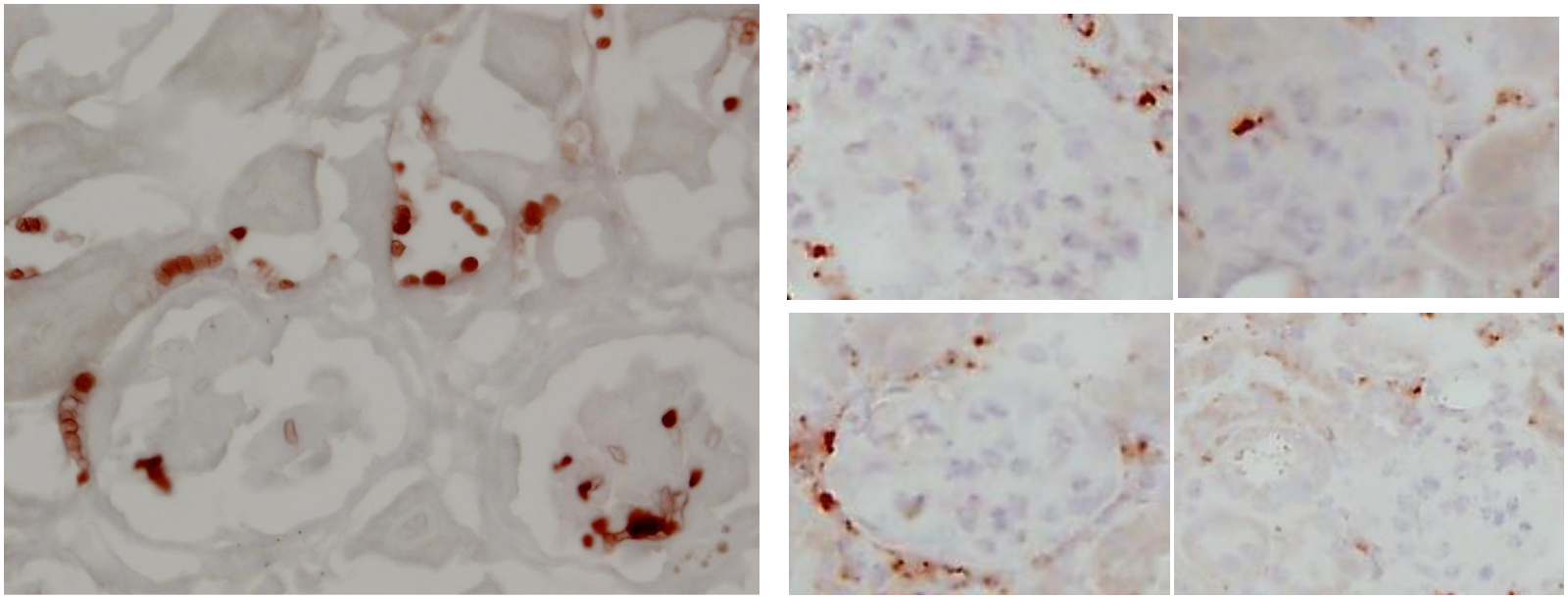




Supplementary Figure 2. Notch1 and Notch3 expressions in models of kidney injury.



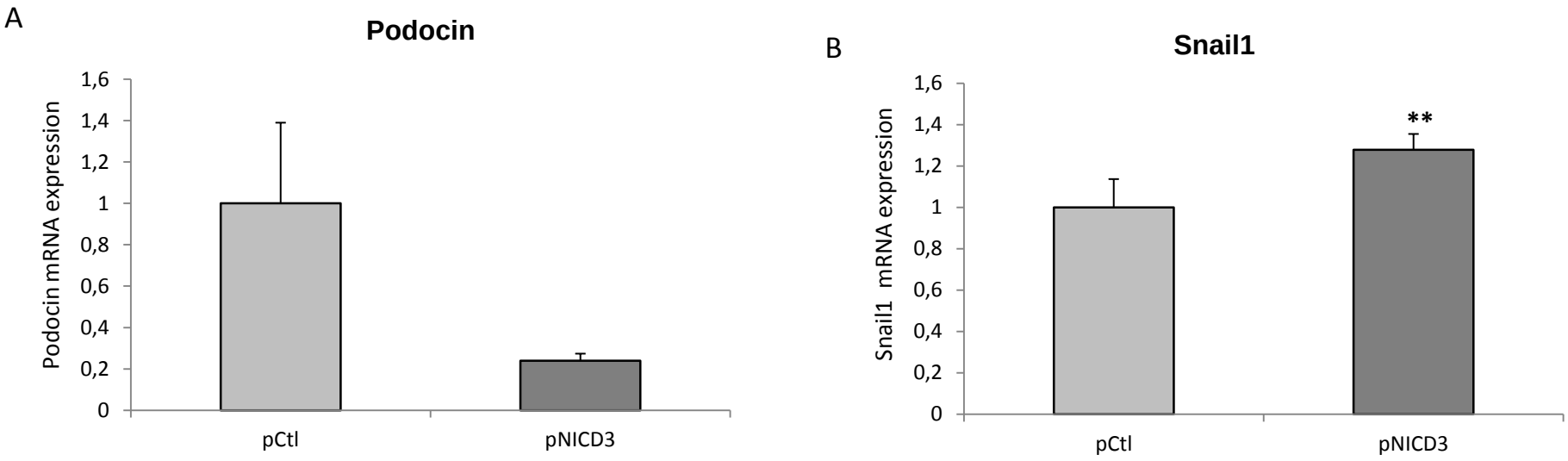
Supplementary Figure 3. Anti sheep IgG levels in NTS treated WT and Notch3 KO mice.



WT + NTS

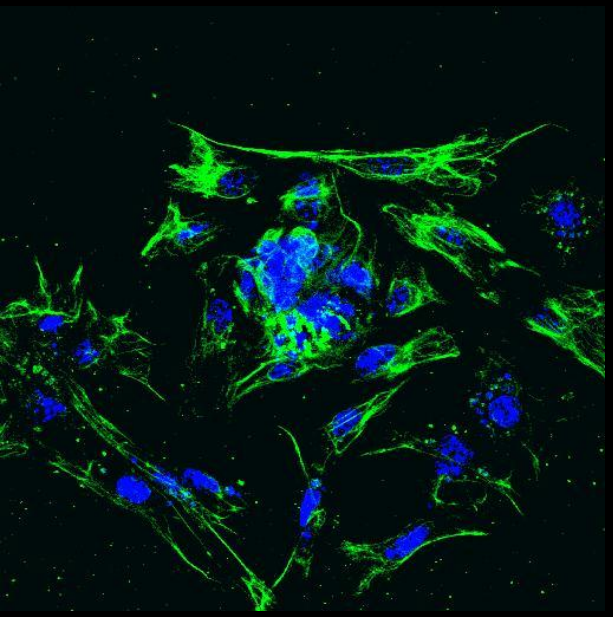
Notch3 KO + NTS

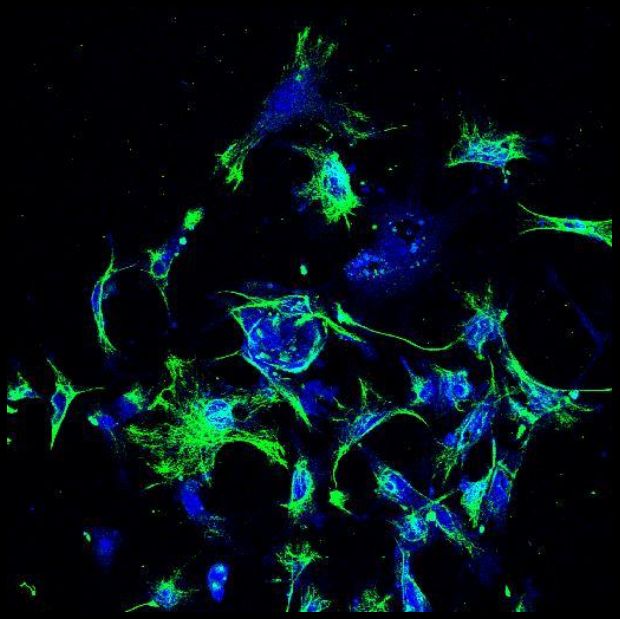
Supplementary Figure 4. Anti CD68 staining in renal cortex of NTS-treated mice showing the presence of macrophages into the glomeruli of WT mice (left). Macrophages were substantially decreased in the glomeruli of Notch3 KO mice (right panels).

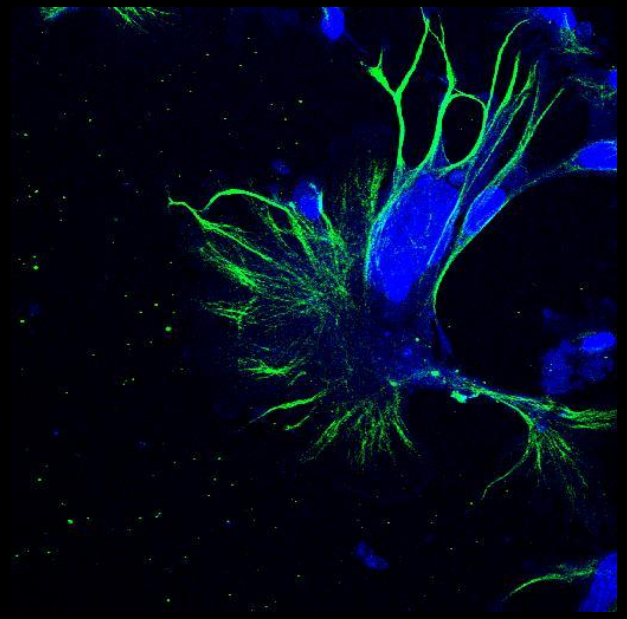


Supplementary Figure 5. Changes in marker expression in podocytes overexpressing N3ICD.

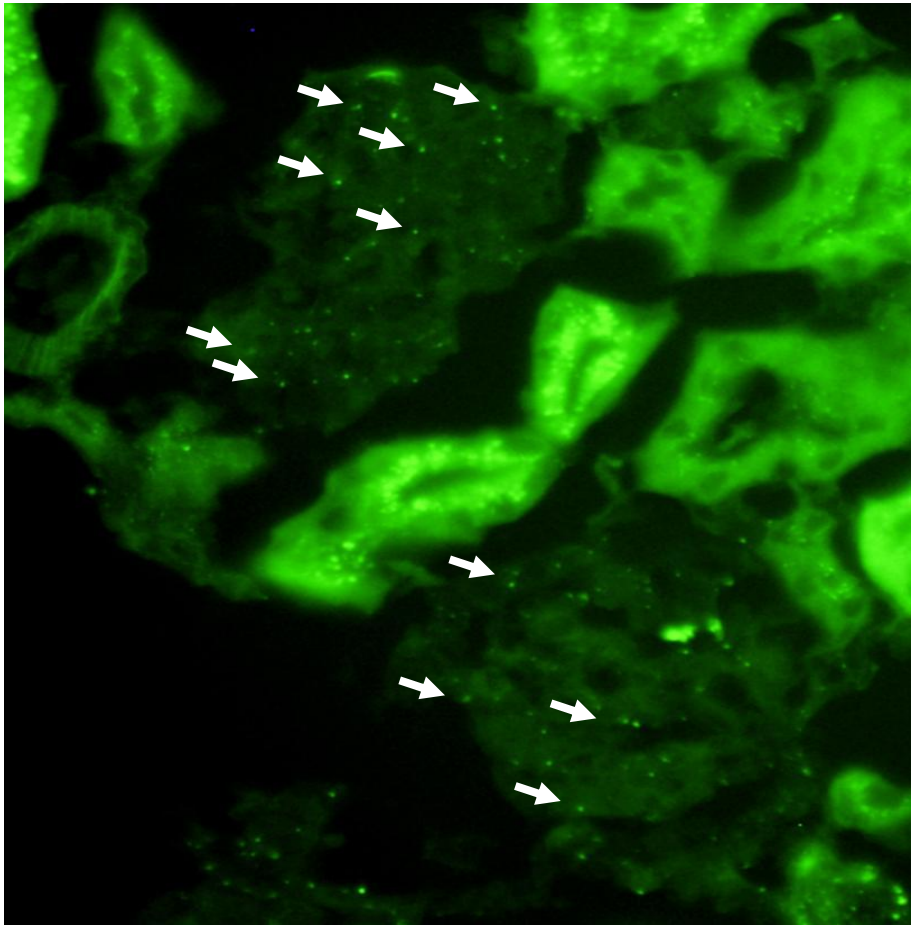
Nephrin



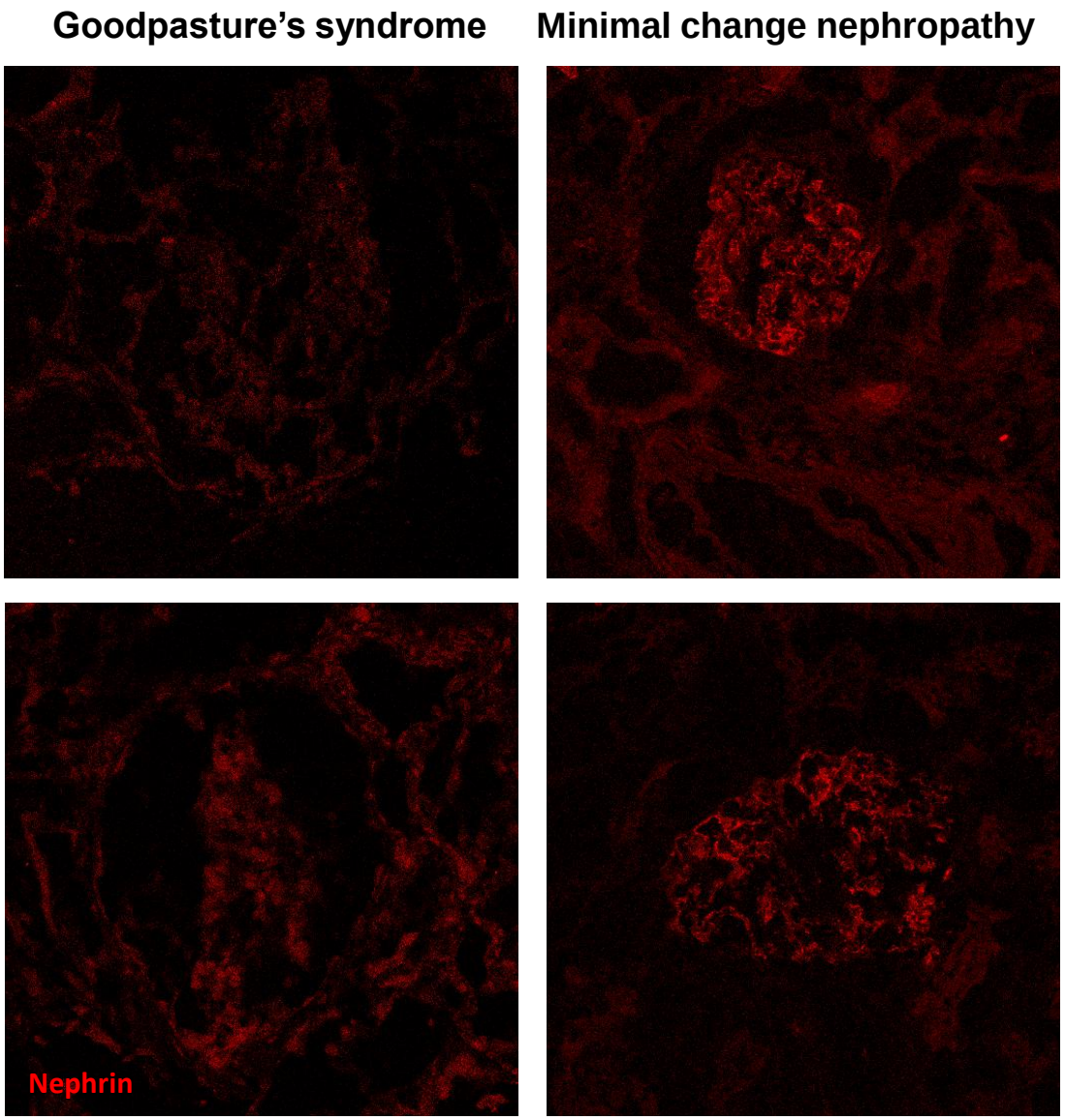




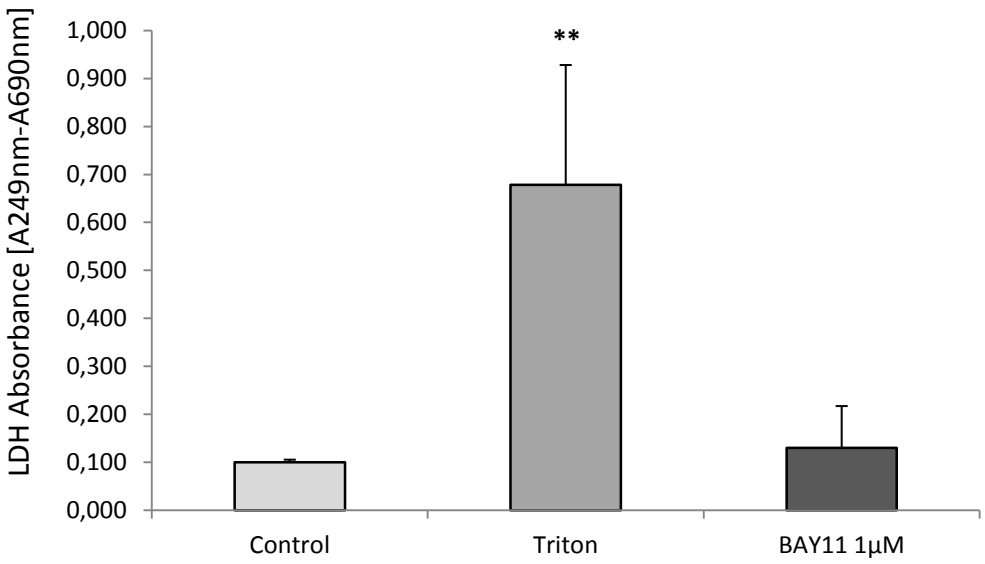
Supplementary Figure 6. Additional figures showing nephrin staining in the outgrowth experiment (at higher magnifications from the Figure 6E).



Supplementary Figure 7. Antisense ODN presence (spots) in the glomeruli of NTS-treated mice.



Supplementary Figure 8. Nephrin staining in biopsies of Goodpasture's (left) and minimal change nephropathy (right) patients.



Supplementary Figure 9. Cytotoxicity test of the NFκB inhibitor BAY11.

SUPPLEMENTARY FIGURE LEGENDS

Suppl Figure 1. Expression of Notch ligands in the NTS model.
The mRNA transcript expressions of Notch ligands were evaluated in mice sacrificed 9 days after NTS injections. Kidneys were collected, decapsulated, and the cortex was dissected from the medulla. The cortical tissue was then used for RNA extraction and subsequent RTqPCR analysis of Jagged1, Jagged2, Delta1 and Delta4.
*, $p < 0.05$; **, $p < 0.01$.

Suppl Figure 2. Notch1 and Notch3 mRNA transcript expression in models of kidney injury.
Notch1 and Notch3 mRNA expressions were analyzed in different models of kidney injury including NTS (A), Unilateral Ureteral Obstruction (UUO; B), Ischemia Reperfusion (IR; C) and mice over-expressing renin (RenTg (D). Mice were sacrificed at various time points detailed in the materials and methods. Kidneys were collected, decapsulated and snap frozen. Total mRNA was extracted and reverse transcriptase was performed. Notch1 and Notch3 expression levels were analyzed using RTqPCR. Results are expressed as a fold-induction of the mRNA level of WT control mice and represent the mean and standard deviation of 4-5 mice per group. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Suppl Figure 3. Anti sheep IgG levels in NTS-treated WT and Notch3 KO mice.
There is no difference in anti-sheep IgG levels between NTS wild type and NTS Notch3 KO mice.

Suppl Figure 4. Anti-CD68 staining in glomeruli of NTS-treated mice.
Note the presence of active macrophages within glomeruli of WT mice (left panel). Their presence was reduced in Notch3 KO mice (right panels).

Suppl Figure 5. Changes in marker expression in podocytes overexpressing N3ICD.

Suppl Figure 6. Additional figures at higher magnification showing that cells migrating from glomeruli in the outgrowth experiments express nephrin.

Suppl Figure 7. Detection of Notch3 antisense oligonucleotides in the glomeruli of NTS-treated mice.

Suppl Figure 8. Nephrin immunostaining disappears in patients with Goodpasture's syndrome.
Nephrin expression was tracked using immunofluorescence in patients with Goodpasture's syndrome (left images) and in patients with minimal change nephropathy (right images).
Nephrin staining can be easily detected in the minimal change nephropathy, but is negligible in the Goodpasture's patients indicating that the podocytes of these biopsies have lost the expression of characteristic proteins.
Representative images of two patients per disease are shown.

Suppl Figure 9. Cytotoxicity test of the NFκB inhibitor BAY11 is podocytes.
Triton used as a positive control, increased LDH levels, whereas BAY11 had similar to untreated control levels.

MATERIALS AND METHODS

Unilateral Ureteral Obstruction

After induction of general anesthesia (intraperitoneal injection of 50 mg/kg of pentobarbital) the left ureters of 3 month-old WT mice were ligated at two separate points through a left flank incision. Non-obstructed sham kidneys were used as controls. Four mice from each group were sacrificed after 7 days of obstructive nephropathy. Kidneys were collected, decapsulated, and the cortex was dissected from the medulla. The cortical tissue was then used for RNA extraction and subsequent RTqPCR analysis.

Renin Transgenic Mice

Experiments were performed using RenTg mice backcrossed in the genetic background Sv129 as already described (1). These mice express renin ectopically at a constant high level in the liver leading to elevated mRNA (2.7±0.3 renin/18S versus 0 in RenTg and WT mice respectively) and protein levels of active renin into the blood stream (1, 2). Thus, RenTg mice are hypertensive as endogenous synthesis of angiotenin II is increased. At 12 months of age 4 mice from each group were sacrificed. Kidneys were collected, decapsulated, and the cortex was dissected from the medulla. The cortical tissue was then used for RNA extraction and subsequent RTqPCR analysis.

Ischemia Reperfusion

After induction of general anesthesia (intraperitoneal injection of 50 mg/kg of pentobarbital) a posterior subcostal incision was made on the left side, and the renal pedicle was dissected and occluded with a small vascular clamp. After 45 min, the clamp was removed. Mice were sacrificed 6, 24 and 48 hours after clamp removal. Kidneys were collected, decapsulated, and the cortex was dissected from the medulla. The cortical tissue was then used for RNA extraction and subsequent RTqPCR analysis.

Assessment of anti-sheep IgG titers in mice sera

Anti-sheep IgG titers were measured in mice sera by ELISA assay (Alpha Diagnostic International, San Antonio, TX, USA). Plates were coated with 20µg/mL of sheep IgG overnight at 4°C and then blocked using a 5% albumin solution. Serum was added at various dilutions according to the manufacturer's instructions.

Antisense oligonucleotide detection

WT mice were injected with two different sequences of oligodeoxynucleotides (ODN) specifically targeting Notch3 mRNA, designed using IDT DNA (Integrated DNA Technologies). Sequences were modified with phosphorothioate to prevent their *in vivo* hydrolysis by exonucleases (Sigma-Aldrich, St. Quentin Fallavier, France) and included a fluorescein tag to allow detection by epi-fluorescence microscopy. The ODNs were diluted in saline sodium chloride and administrated by intraperitoneal injections of 100 pmol/ODN per mouse. Three mice were used and sacrificed 3 hours after injection. Kidneys were collected, snap-frozen and immediately sectioned. Cryosections were fixed in PFA4% and analyzed using epi-fluorescence microscopy.

Transfection of E11 podocytes

Cells were plated at 80% confluence and maintained in RPMI 1640 supplement with 10% fetal bovine serum, 100U/ml penicillin, and 0.1mg/ml streptomycin at 37°C. At 2 weeks of incubation at 37°C, an empty control vector pCDNA3 and the pUC57 vector containing the mouse Notch intracellular domain were transfected using the JetPei transfection reagent

(Polypus Transfection) according to the manufacturer's instructions. Cells were harvested 24 hours after transfection and total RNA was extracted for subsequent analysis by RTqPCR.

Cytotoxicity test

Cells were treated with 1μM NFκB inhibitor BAY11 in serum-free medium for 24hours. Podocytes were treated with 1% Triton as a positive control. Supernatants were collected and lactate dehydrogenase (LDH) activity was detected with the LDH Cytotoxicity Detection Kit (Roche) according to the manufacturer's instructions.

Reverse transcriptase and RTqPCR

RNA was extracted from podocytes using EZ Spin columns (Fermentas, Saint Léon-Rot, Germany) and from renal cortex using TRI Reagent (Euromedex, Mundolsheim, France). cDNA was synthesized from 1μg RNA using the Fermentas H minus First Strand cDNA synthesis kit according to the manufacturer's instructions. Real time PCR was performed with the Roche Light Cycler 480 sequence detection system using SYBR Green PCR master mix (Qiagen). Specific primers for target mRNAs were designed using the Universal Probe Library Roche website (Table 1) under the following program: 95°C for 5 min, 45 cycles at 95°C for 15 s and 60°C for 15 s, and 72°C for 15s was used. Results are expressed as 2-ΔCt, where Ct is the cycle threshold number normalized to the mean 2-ΔCt for each corresponding control group. Dissociation curves were analyzed after each run for each amplicon in order to determine the specificity of quantification when using SYBR Green. HPRT was used as the housekeeping reference gene.

Glomerular isolation and immunofluorescence

Glomeruli were isolated from WT mice by two-step sieving of renal cortices 4 days after NTS injection in WT and KO groups (3 mice per strain). Kidneys were decapsulated, cut into small pieces using a scalpel and digested in 1mg/mL collagenase for 3 minutes at 37°C. The tissue was then passed through a 100μm filter fitted on a 50mL tube. The filter was flushed with complete medium (RPMI 1640, supplemented with 10% fetal bovine serum, 100 U/ml penicillin/streptomycin), then thrown away. Next, the 50mL tube containing tubules and glomeruli was gently shaken several times and passed through a 40μM filter. The filter was flushed with medium using a 20cc syringe and 18G needle. The filter, now containing glomeruli, was turned upside down on a clean 50mL tube and flushed with complete medium using a 20cc syringe fitted with a 26G needle in order to remove glomeruli. The remaining solution containing the glomeruli was centrifuged at 1000rpm for 2 minutes. Complete medium was added to the pellet and glomeruli distributed on glass cover slips in cell culture plates. Isolated glomeruli were maintained in RPMI medium for 4 days. At four days glomeruli were fixed with 4% PFA for 15 minutes, permeabilized with 0.1% Triton then blocked with 2% BSA for 30 minutes. Glomeruli were then incubated with an anti-Nephrin antibody (R and D systems, AF3159) for 2 hours at 37°C. Alexa fluor 488 secondary antibody was used for detection, followed by staining of nuclei by ToPro3 iodide (Life Technologies, diluted 1000x, 15min at room temperature). Images were obtained at the Tenon hospital's confocal microscopy platform (Zeiss inverted confocal microscope).

Immunohistochemistry in mice

Cryostat sections (4 μm thick) of renal cortex from WT controls and NTS-treated mice were fixed with paraformaldehyde 4% for 5 min and incubated with 10% FBS/ 0.1% triton in PBS to block unspecific staining. Macrophages were detected by immunoperoxidase staining using an anti-CD68 antibody (1/100, Serotec).

Immunofluorescence of human biopsies

Renal biopsies from patients with minimal change nephropathy and Goodpasture's syndrome were analyzed for Nephrin staining. Sections were fixed in acetone for 10min., permeabilized with 0.1% Triton for 45min., blocked with BSA 2% for 45min., and stained with an anti-Nephrin antibody (R and D systems, AF3159). Alexa fluor 546 secondary antibody was used for detection, followed by staining of nuclei with TOPRO3 iodide (life technologies, D1000, 15min., RT). Images were obtained at the Tenon hospital's confocal microscopy platform (Zeiss inverted confocal microscope). A total of 3 patient's biopsies from each group were used for this set of experiments.

Table 1: Primer sequences used for RT-PCR

mRNA	Forward	Reverse
Notch1	ACTATCTCGGCGGCTTTTC	GGCACTCGTTGATCTCCTCT
Notch3	TCAACACCTAGCCCAGCAAC	GAGTGTCACTTCAGCACCCC
Snail1	GTCTGCACGACCTGTGGAA	CAGGAGAATGGCTTCTCACC
Podocin	CCATCTGGTTCTGCATAAAGG	CCAGGACCTTTGGCTCTTC
Jagged 1	GAGGCGTCCTCTGAAAAACA	ACCCAAGCCACTGTTAAGACA
Jagged 2	TCCTCCTGCTGCTTTGTGAT	TTGCAGGGCTGAAAGACAC
Delta1	GGGACAGAGGGGAGAAGATG	TCCATGTTGGTCATCACACC
Delta4	AAATGGTGGCAGCTGTAAGG	AGTCCGCACAGGTCAAGGTA
HPRT	GGAGCGGTAGCACCTCCT	CTGGTTCATCATCGCTAATCA

1. Caron KM et al. (2002) A genetically clamped renin transgene for the induction of hypertension. *Proc Natl Acad Sci U S A* 99 (12): 8248-8252.

2. Azibani F et al. (2012) Aldosterone inhibits antifibrotic factors in mouse hypertensive heart *Hypertension* 59 (6):1179-1187.