Supplement material

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Supplementary figure 1

Urinary miR-378a-3p and miR-192-5p is upregulated in patients with iMGN independent from urine concentration and PLA2R titer.

A: Fold change in miR-378a-3p and miR-192-5p expression in pooled urines from patients with different glomerular diseases and healthy controls. MiRs were detected with the help of a TaqMan Array based miR-screening.
ANCA: ANCA associated vasculitis, CTRL: healthy control; DN: diabetic nephropathy; FSGS: focal segmental glomerulosclerosis; HUS: haemolytic uraemic syndrome; IgA-GN: IgA-glomerulonephritis; MCD: minimal change disease; MGN: membranous glomerulonephritis; miR: microRNA, PRAE: preeclampsia.
B: Correlation of fold change in urinary miR-378a-3p (a) and miR-192-5p (b) with urinary creatinine in 10 control and 20 patients with iMGN. There was no significant correlation. Correlation of fold change in urinary miR-378a-3p (c) and miR-192-5p (d) with PLA2R-ab titer in 20 patients with iMGN. There was no significant correlation.

Supplementary figure 2

A/B: In situ hybridization for U6 (A, positive control) and CTRL-miR (B, negative control) on human kidney biopsies. Scale bar = 50 µm.

C: Immunofluorescence staining with secondary antibody only and DAPI nuclei staining. Scale bar = 50 µm.

Supplementary figure 3

miR-378a-3p-NPNT and miR-192-5p-NPNT binding sites.

Illustration of miR-378a-3p-NPNT and miR-192-5p-NPNT binding sites with position of the binding site, loop score, delta G of the minimal free energy (MFE) of the binding. A computer based prediction tool calculated the mean free energy (MFE) of these bindings to be -15,9 kcal/mol, -8,24 kcal/mol and -7,4 kcal/mol, respectively. MiR-378a-3p has one binding side in the 3'UTR region of NPNT mRNA at position 2127-2149 with an MFE of -10.5 kcal/mol (supplementary Fig. 3A, B predicted by miRanda (http://mirtarbase.mbc.nctu.edu.tw/php/detail.php?mirtid=MIRT026404#target; 15th of june 2021).

Supplementary figure 4

Cross section at the pronephros level and TEM pictures of the zebrafish pronephros.

Cross section at the pronephros level (a'-e', scale bar = 200 µm) and TEM pictures of the zebrafish pronephros (a''-e'', scale bar = 10 µm) at 120 hpf. Zebrafish larvae were injected with CTRL-MO, npnt-MO, miR-CTRL mimic, miR-378a-3p mimic and miR-192-5p mimic. Stars in b', d', e' indicate whole body edema. Stars in b'', d'', e'' show widening and ratification of the capillary loops of the glomerulus.

Supplementary figure 5

Ultrastructural changes of the GFB in human iMGN.

TEM pictures of the GFB of iMGN. White arrow shows podocyte effacement, black arrows illustrate increased lucidity of the endothelial side of the GBM, stars illustrate thickening of the GBM. Scale bar: 500 µm.

Supplementary figure 6

Histology of podocyte-specific Npnt knock out mice.

A: HE staining of kidney tissue sections of WT, Npnt fl/fl, Nphs2 cre+, Npnt fl/+; Nphs2 cre+ mice and Npnt fl/fl; Nphs2 cre+ mice at 15 and 35 weeks of age. Scale bar 25 µm.

B: Immunofluorescence staining for Npnt in WT, Npnt fl/fl mice, Nphs2 cre+, Npnt fl/+; Nphs2 cre+ and Npnt fl/fl; Nphs2 cre+ mice at 35 weeks of age. Scale bar 25 µm.

Supplementary figure 7

A: Serum creatinine in podocyte-specific Npnt knockout mice.

Serum creatinine in mg/dl in WT, Npnt fl/fl, Nphs2 Cre+, Npnt fl/+; Nphs2 cre+ and Npnt fl/fl; Nphs2 cre+ mice at ~15 and ~35 weeks of age. n.s. not significant.

B: Autoimmunity against podocyte antigens in podocyte-specific Npnt knockout mice

Western blot of immortalized murine podocyte lysates incubated with serum from Npnt fl/fl; Nphs cre2+mice, Nphs cre2+ mice and WT mice. Secondary antibody was anti-mouse IgG. Anti-moues IgG only was used as negative control.

Supplementary video 1, 2

3-dimensional reconstruction of zebrafish larvae with the help of SBF-SEM.

Supplementary video 3

Time-lapse of co-culture of GFP-labelled human GECs (green) with podocytes labeled with eBioscience™ Cell Proliferation Dye eFluor™ 450 (blue). GECs were transfected with Alexa555-tagged miR-192-5p mimic (red) prior to co-culture. GEC-derived exosomes carry miR-192-5p that is taken up by podocytes.

Supplementary methods

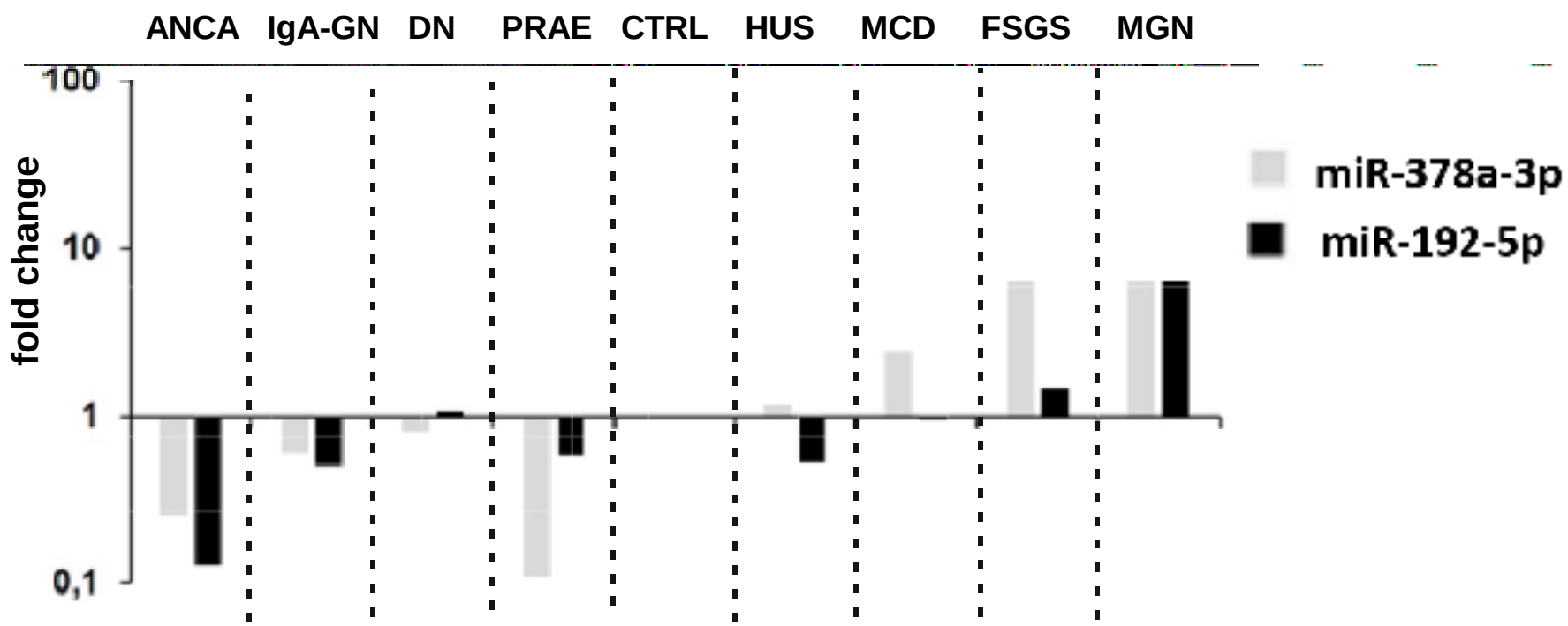
Urine sample preparation for miR-screening

Morning urine was collected from healthy volunteers and from patients with biopsy-proven glomerular diseases: FSGS, membranous glomerulonephritis (MGN), membrano-proliferative glomerulonephritis (MPGN), diabetic nephropathy (DN), minimal change dis-ease (MCD), preeclampsia (PREEC), haemolytic uraemic syndrome (HUS) and IgA-glomerulonephritis (IgA-GN). Ethical approval was obtained from Ethics Committee of the Hanover Medical School (#1709-2013). In total 36 patients were included in the study. Urine samples (50 mL) were centrifuged at 75455 g for 15 minutes to pel-let the cells and cellular debris. The cell-free urine supernatant was stored at −80°C until miR-screening analysis. Pooled urine samples from four patients per disease were used in the miR-screening.

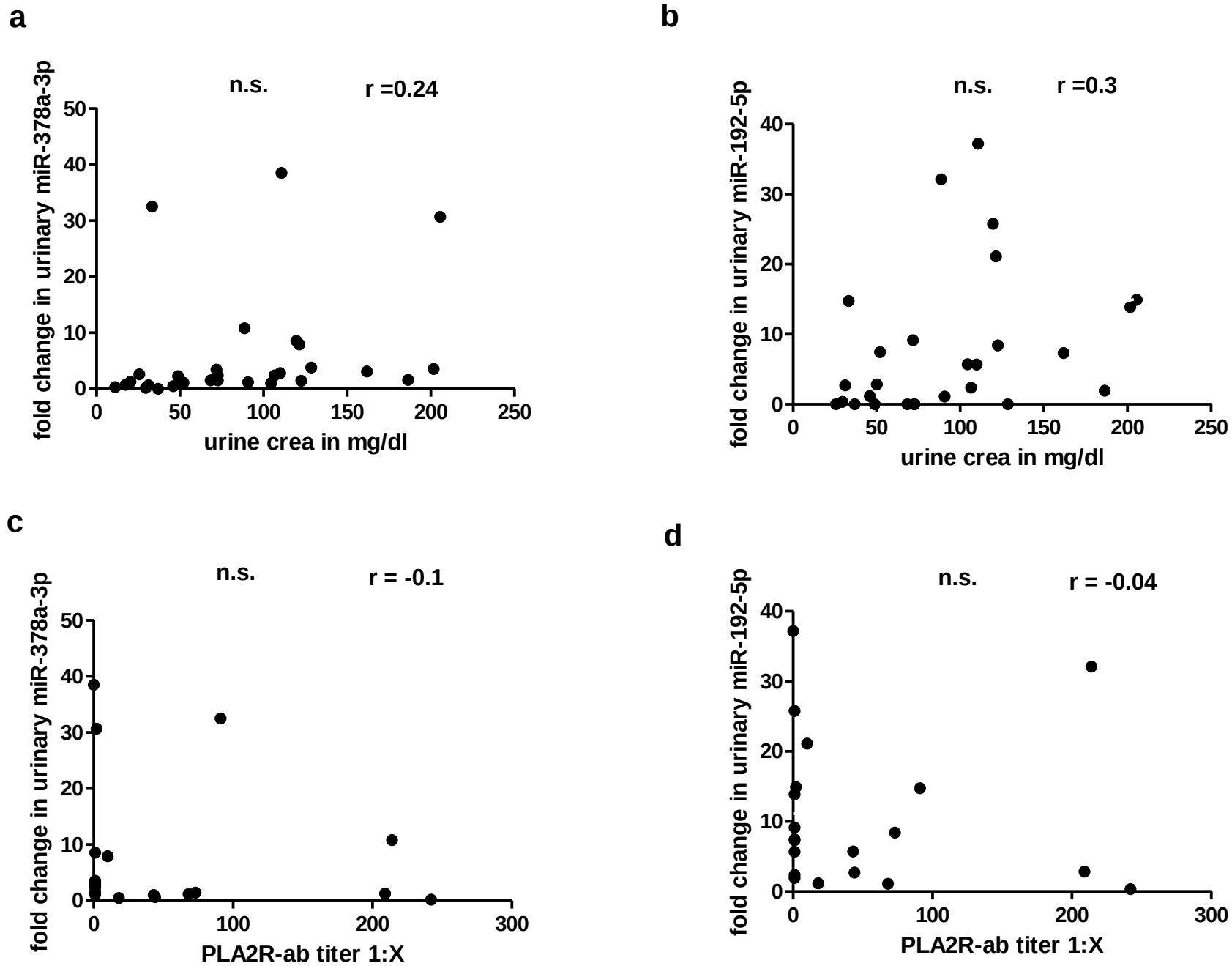
Transmission electron microscopy of human kidney

Human kidney biopsy samples were fixed overnight in 4% formalin, post-fixed with 1% OsO4 (90 min) and stained for 1 h with 1% UranyLess (Science services GmbH, Munich, Germany). After dehydration, tissue blocks were embedded in Araldite Renlam M1 resin (Serva Electrophoresis GmbH, Heidelberg, Germany). 80nm ultrathin sections were cut on an UC7 ultramicrotome (Leica, Wetzlar, Germany) and rinsed in lead citrate buffer for contrasting before analysis using an Leo912 transmission electron microscope (Zeiss, Oberkochen, Germany).

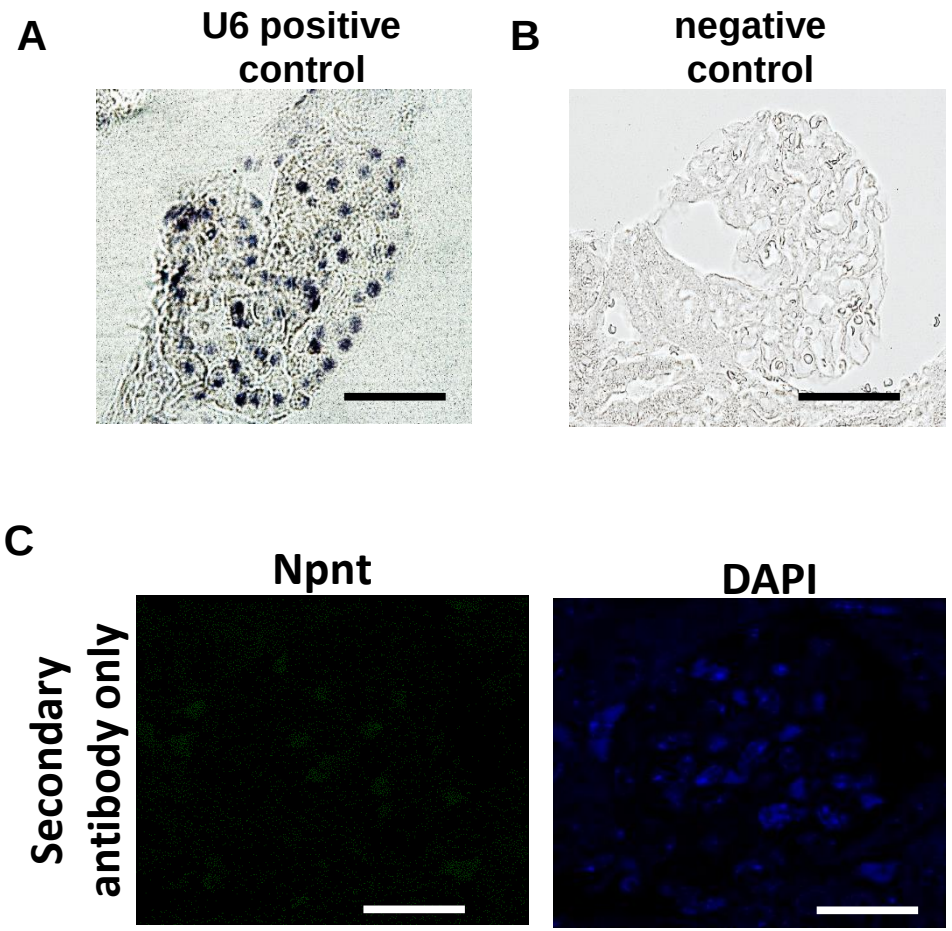
supplementary Fig. 1A



supplementary Fig. 1B

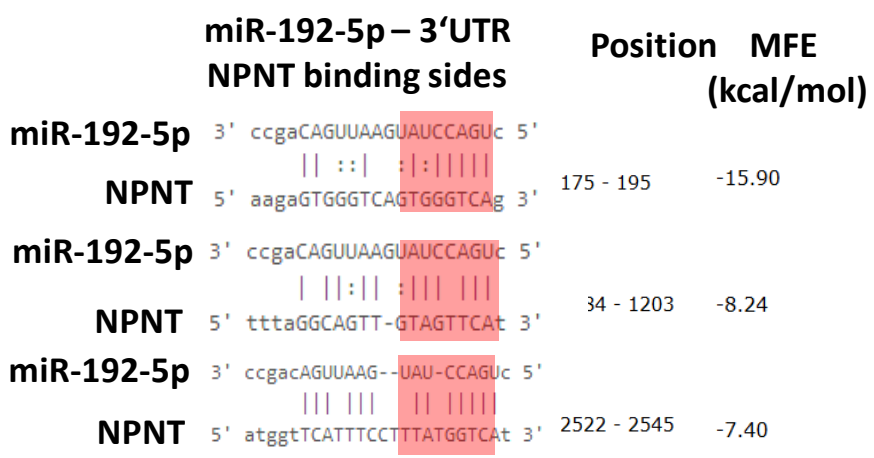


supplementary Fig. 2



supplementary Fig. 3

A



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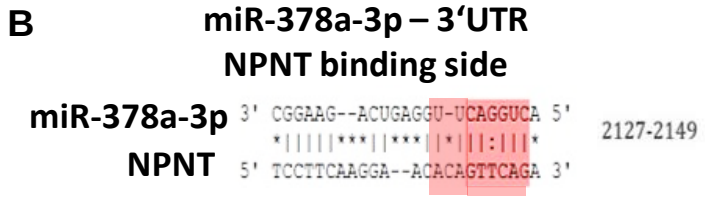
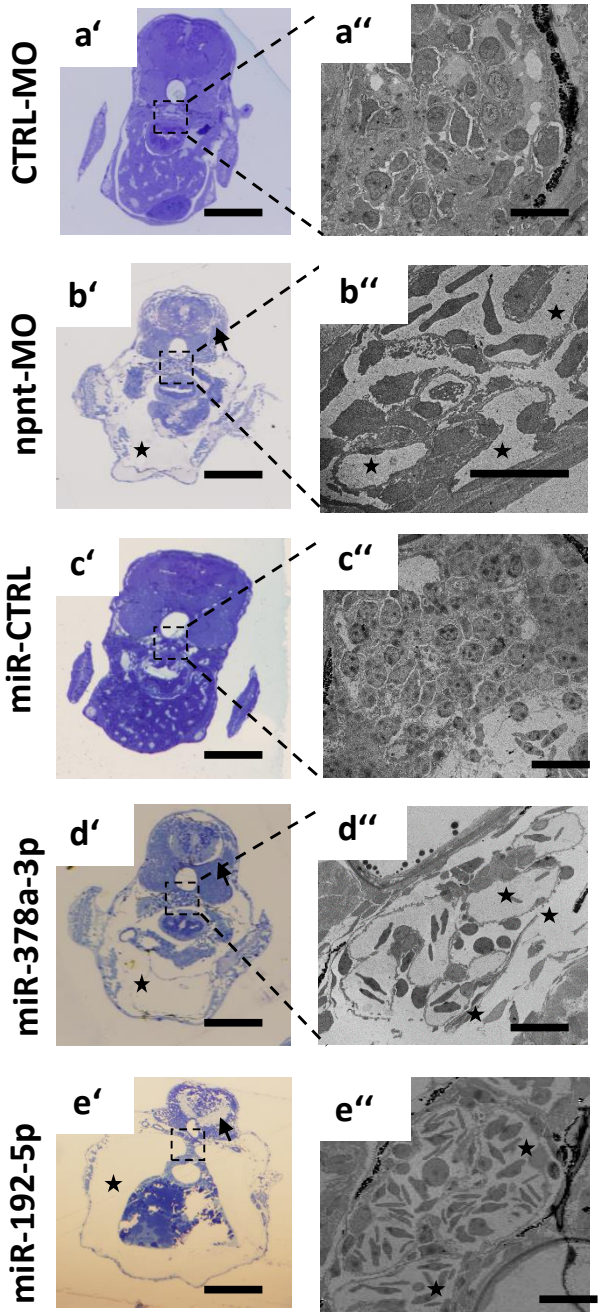
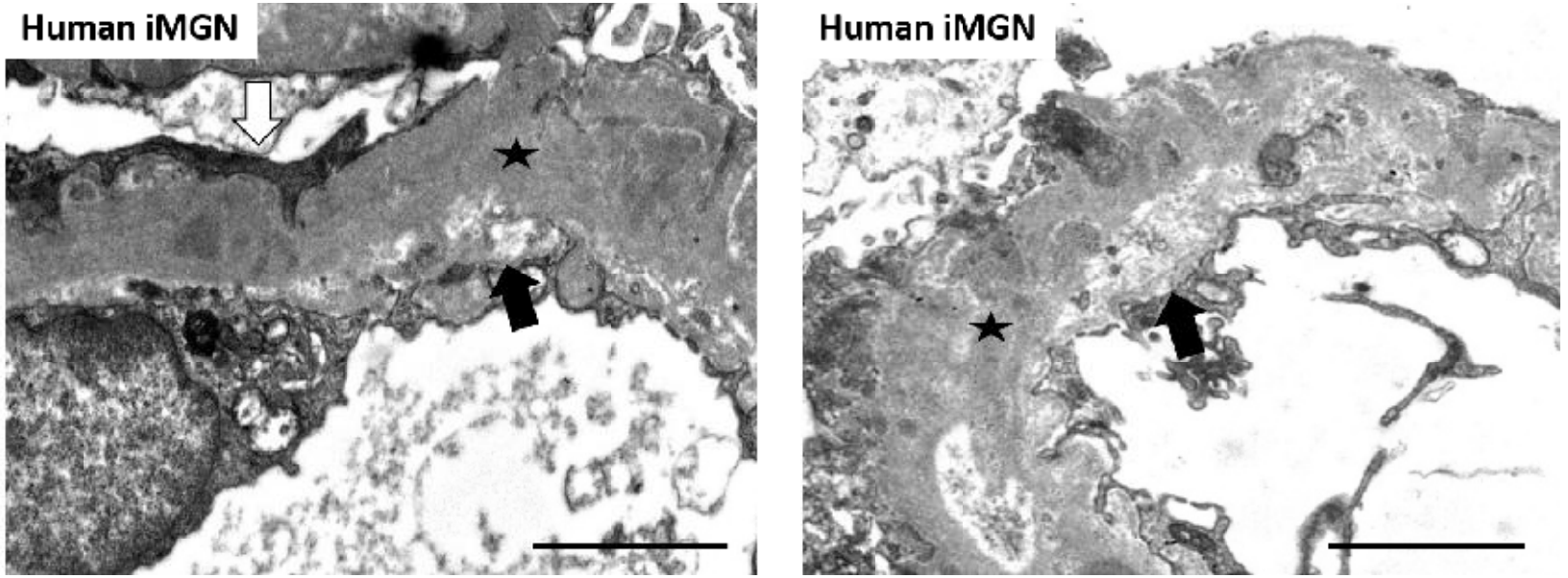


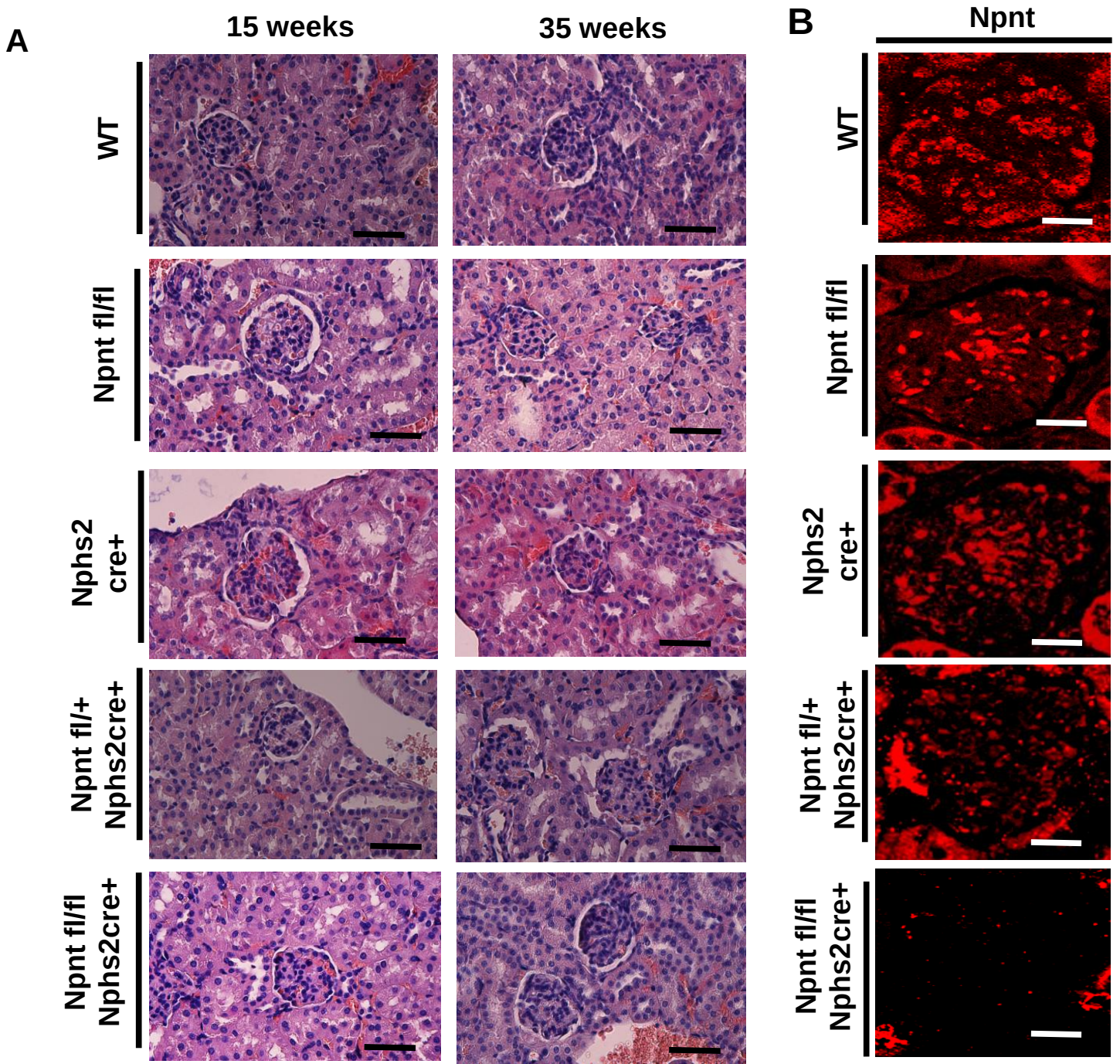
supplementary Fig. 4



supplementary Fig. 5



supplementary Fig. 6



supplementary Fig. 7