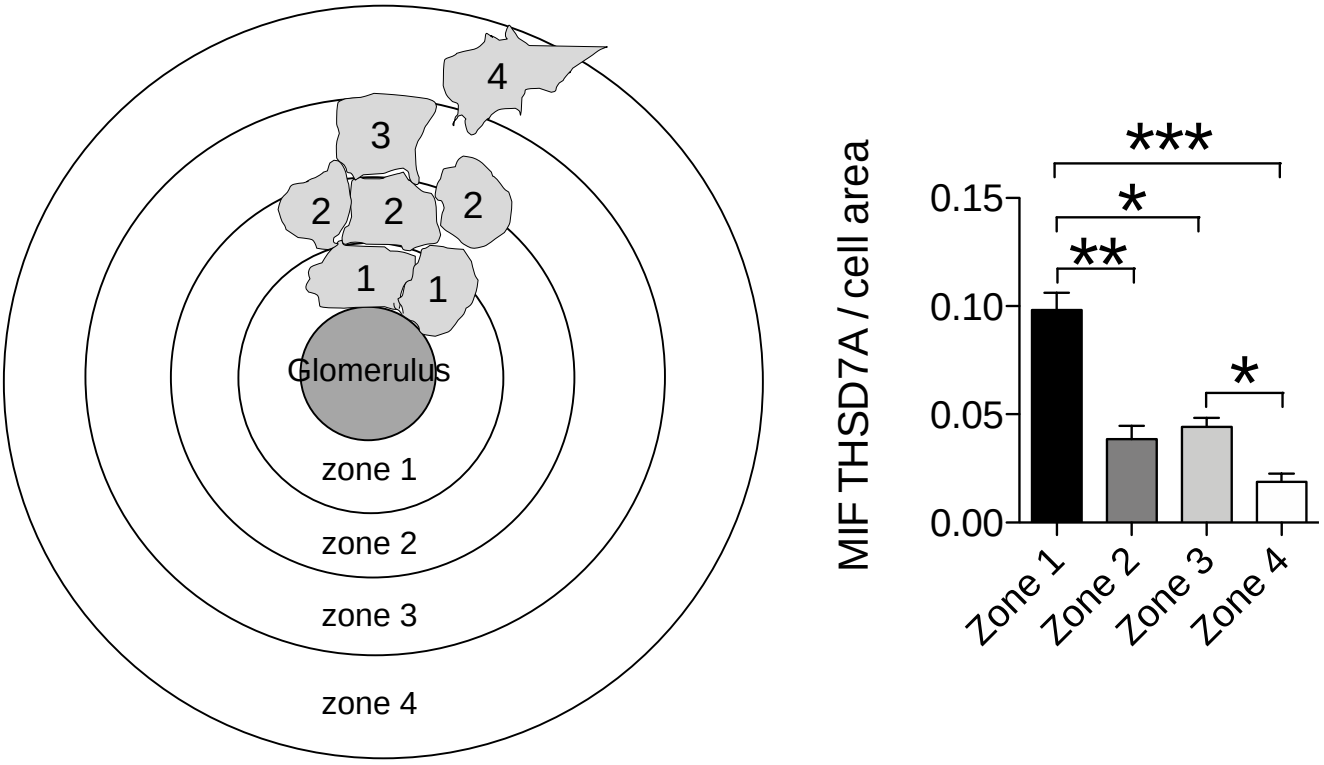
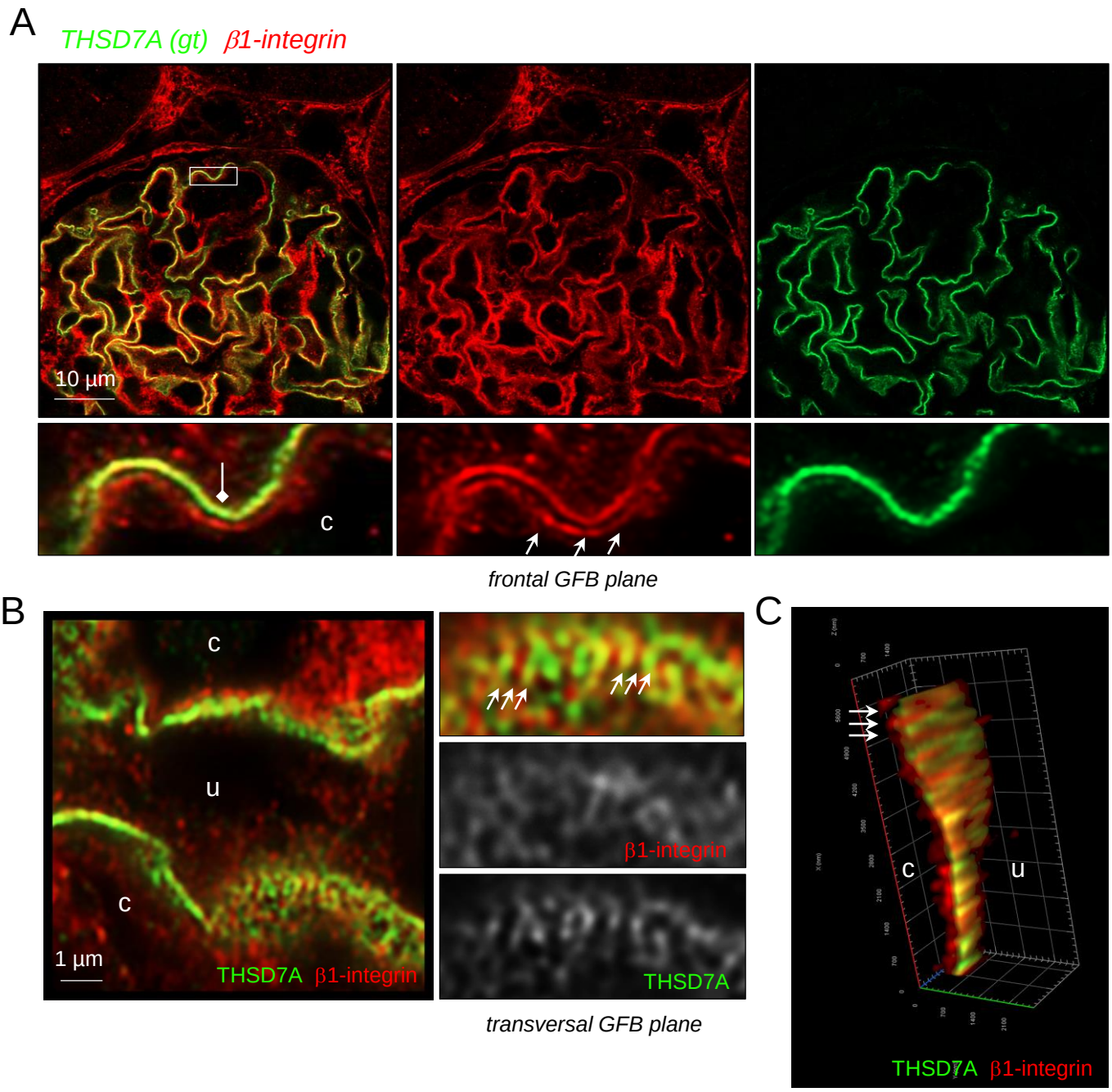
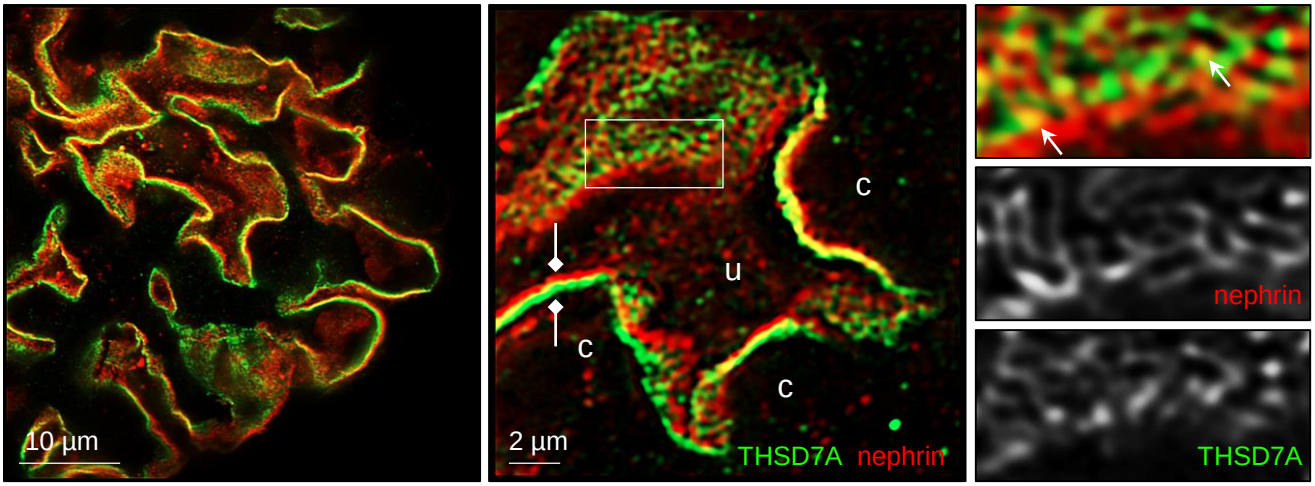


Supplemental Figure 1: THSD7A expression decreases upon outgrowth from isolated glomeruli. Isolated decapsulated glomeruli of C57Bl/6 mice were cultured on collagen type 4 coated plates for 4 days and stained for THSD7A expression. The mean intensity of fluorescence (MIF) was measured using FIJI in relation to the respective cell area. Cells adjacent to the isolated glomerulus were classified as being in zone 1. The layer of cells adjacent to the cells of zone 1 was classified as being in zone 2. The graph exhibits the mean intensity of fluorescence in relation to the zone the cells were localized in. Values are expressed as mean +/- SEM, N=5 micrographs of 2 independent experiments; *p<0.05, **p<0.01, ***p<0.001, One Way ANOVA, Dunn's Multiple Comparison test.

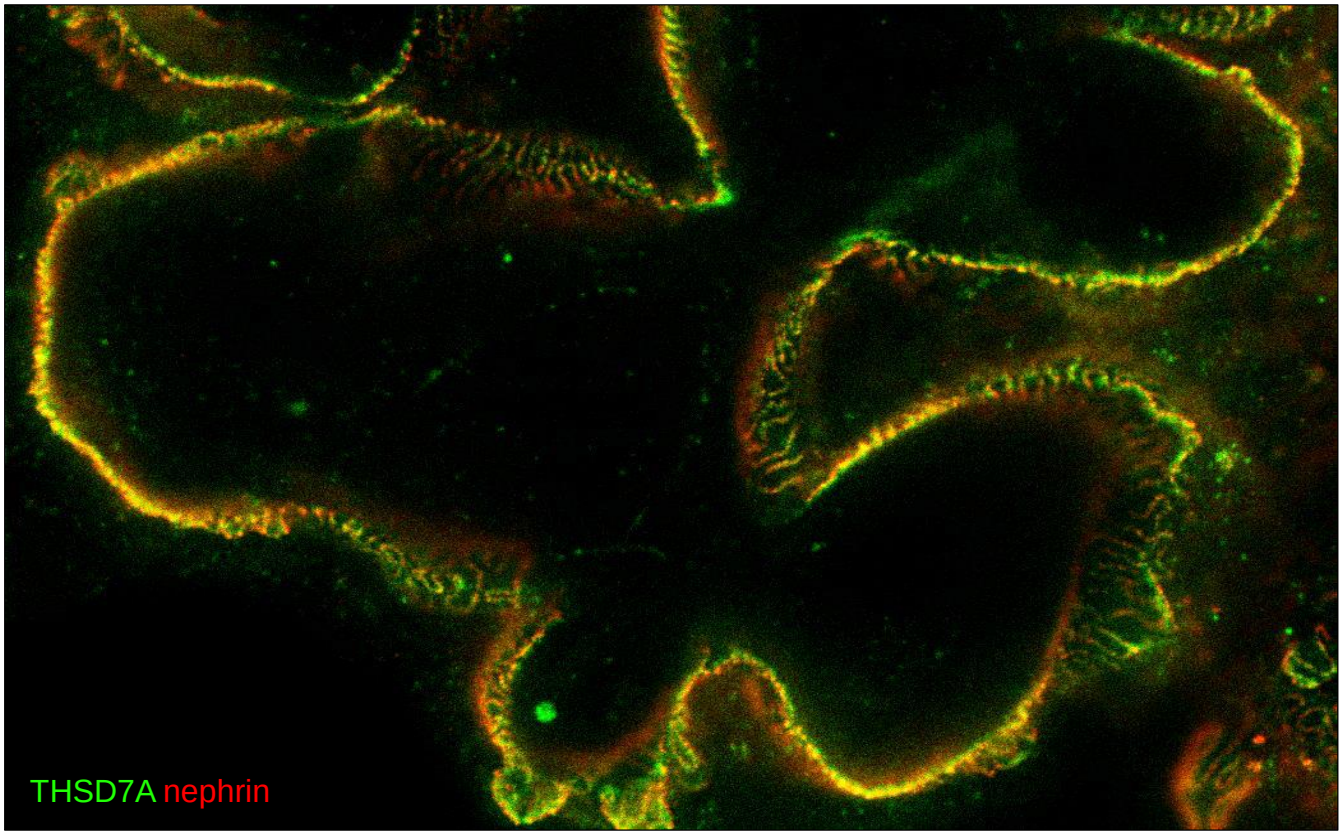


Supplemental figure 2: THSD7A lies in the confocal plane of podocyte β1-integrin but does not follow the β1-integrin staining pattern at the base of foot processes.
3 µm thick paraffin sections of kidneys from naïve C57BL/6 mice were stained for THSD7A (gt-anti-THSD7A, green) and β1-integrin (rb-anti-β1-integrin, red). **A:** Overview of glomerulus with double-layered β-integrin staining localizing to podocyte foot processes in conjunction with THSD7A and to endothelial cells without THSD7A expression (white arrows). THSD7A is found in the same plane as β1-integrin (white diamond arrow). **B:** Close-up of a capillary loop in a deeper confocal section. Note the distinct side-by-side expression of THSD7A with β1-integrin (white arrows) with few areas of overlap. **C:** 3D reconstruction of a confocal Z-stack. Arrows point towards side by side localized β1-integrin and THSD7A "fingers" in the Z-plane. Micrographs were taken with a LSM800 confocal microscope with airyscan. C = capillary lumen, u = urinary space

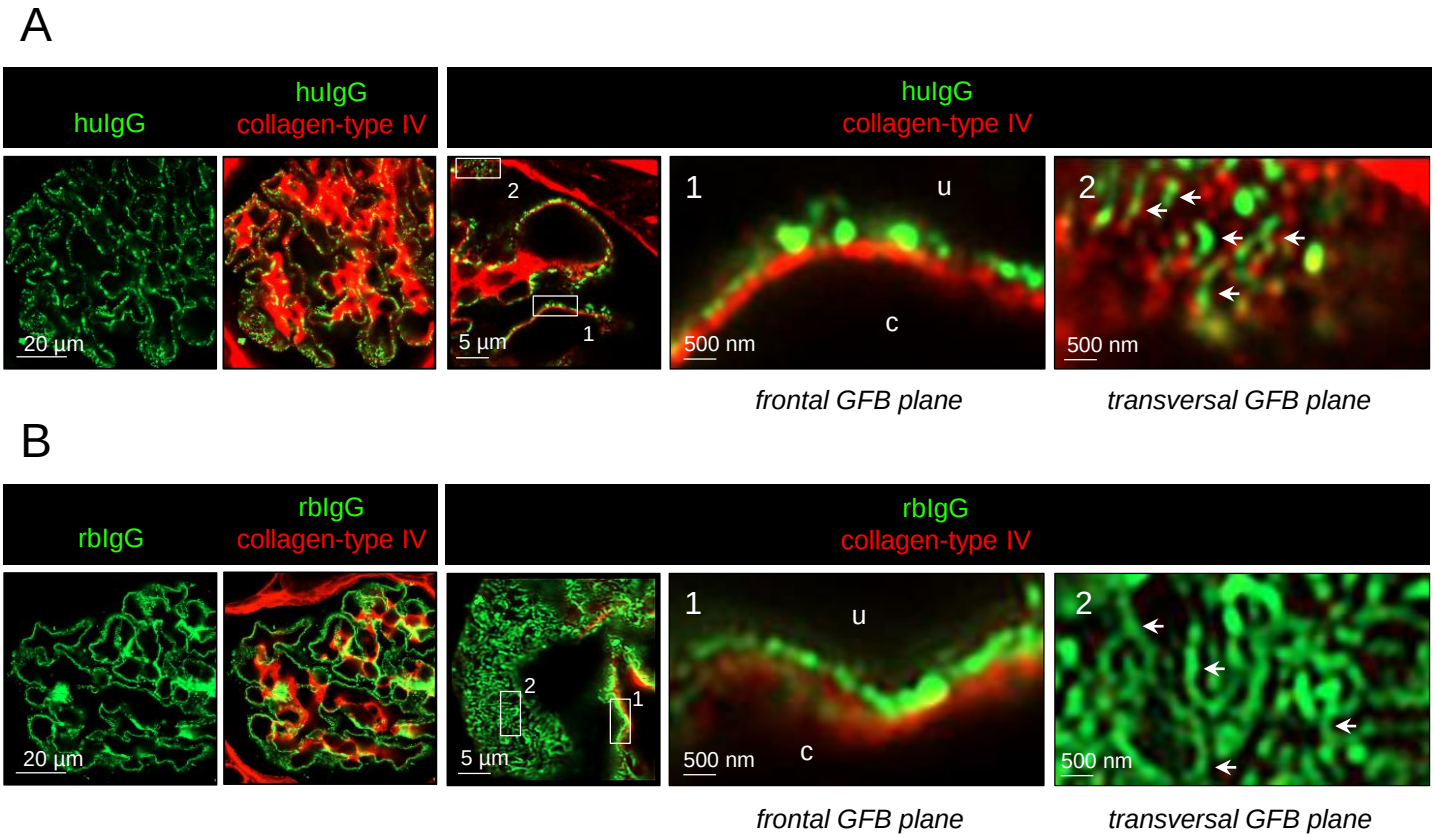
A



Supplemental figure 3: THSD7A is localized basally from nephrin partially following the slit diaphragm meanders.
3 µm thick paraffin sections of kidneys from naïve C57BL/6 mice were stained for THSD7A (gt-anti-THSD7A, green) and nephrin (gp-anti nephrin, red). Thereby THSD7A expression is basal to nephrin in the frontal confocal plain (diamond arrows) and partially localized within the slit diaphragm in the transversal confocal plane (white arrows). Micrographs were taken with a LSM800 confocal microscope with airyscan. C = capillary lumen, u = urinary space.

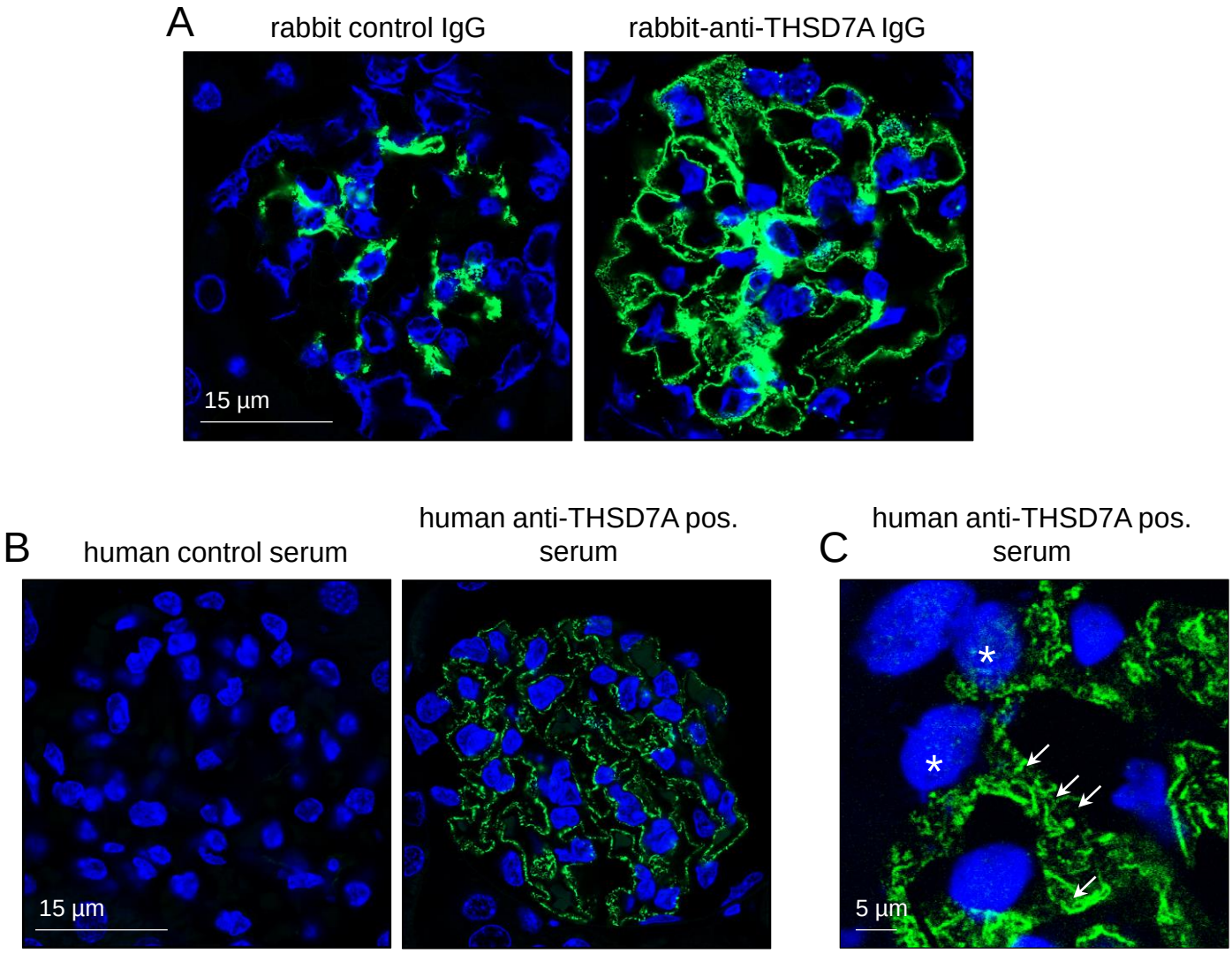


Supplemental figure 4: Overview of a capillary loop imaged for THSD7A (green) and nephrin (red) by STED microscopy.



Supplemental figure 5: Patterns of anti-THSD7A IgG deposition differ in frontal and transversal high resolution confocal planes.

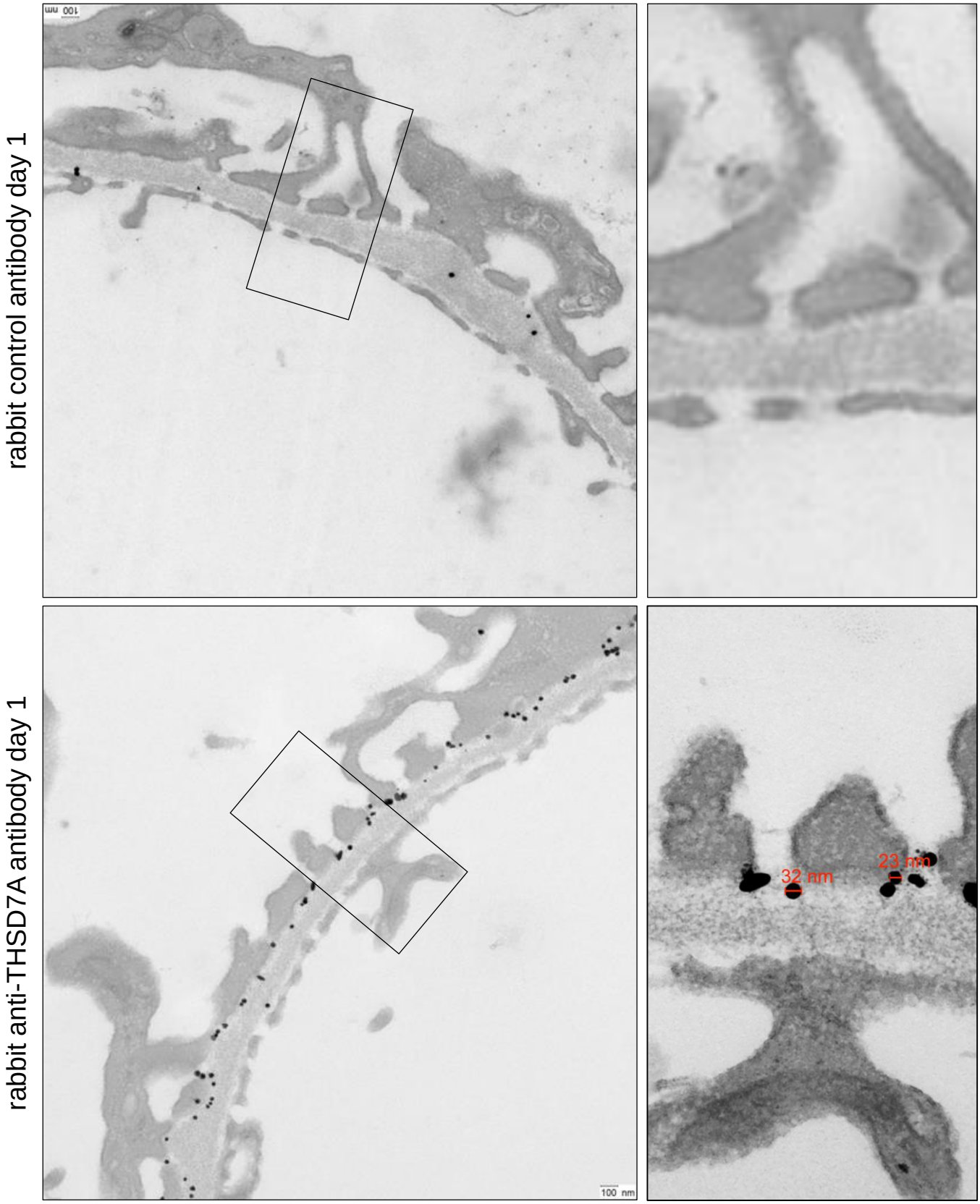
A: Balb/C mice were injected once with 180 µl purified rabbit control IgG or anti-THSD7A containing purified rabbit IgG. After 14 days, kidneys were harvested. **B:** Balb/C mice were injected once with 600 µl anti-THSD7A autoantibody containing patient serum or control human serum. After 18 weeks, kidneys were harvested. **A&B:** Paraffin sections were stained for bound rabbit IgG (green) and collagen type IV (red) to demarcate the glomerular basement membrane of the glomerular filtration barrier (GFB). Arrows point towards “finger-like” deposition of IgG visible in the transversal optical plane. C = capillary side, u = urinary side of the GFB. Micrographs were taken with a LSM800 confocal microscope with airyscan.



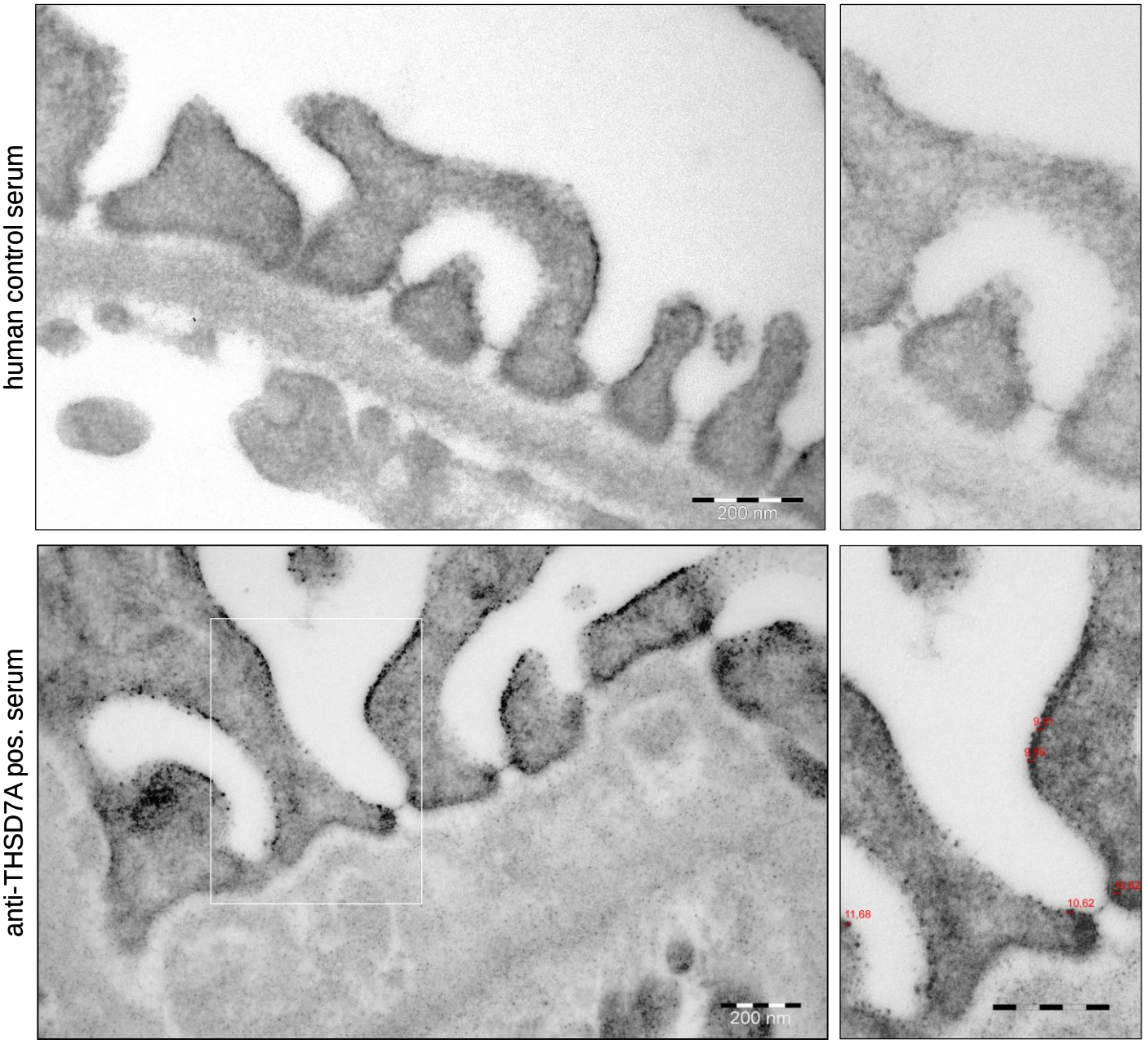
Supplemental figure 6: Anti-THSD7A IgG binding in murine glomeruli

A: Balb/C mice were injected once with 180 µl purified rabbit control IgG or anti-THSD7A containing purified rabbit IgG. After 14 days, kidneys were harvested. Paraffin sections were stained for bound rabbit IgG (green) and DNA (blue).

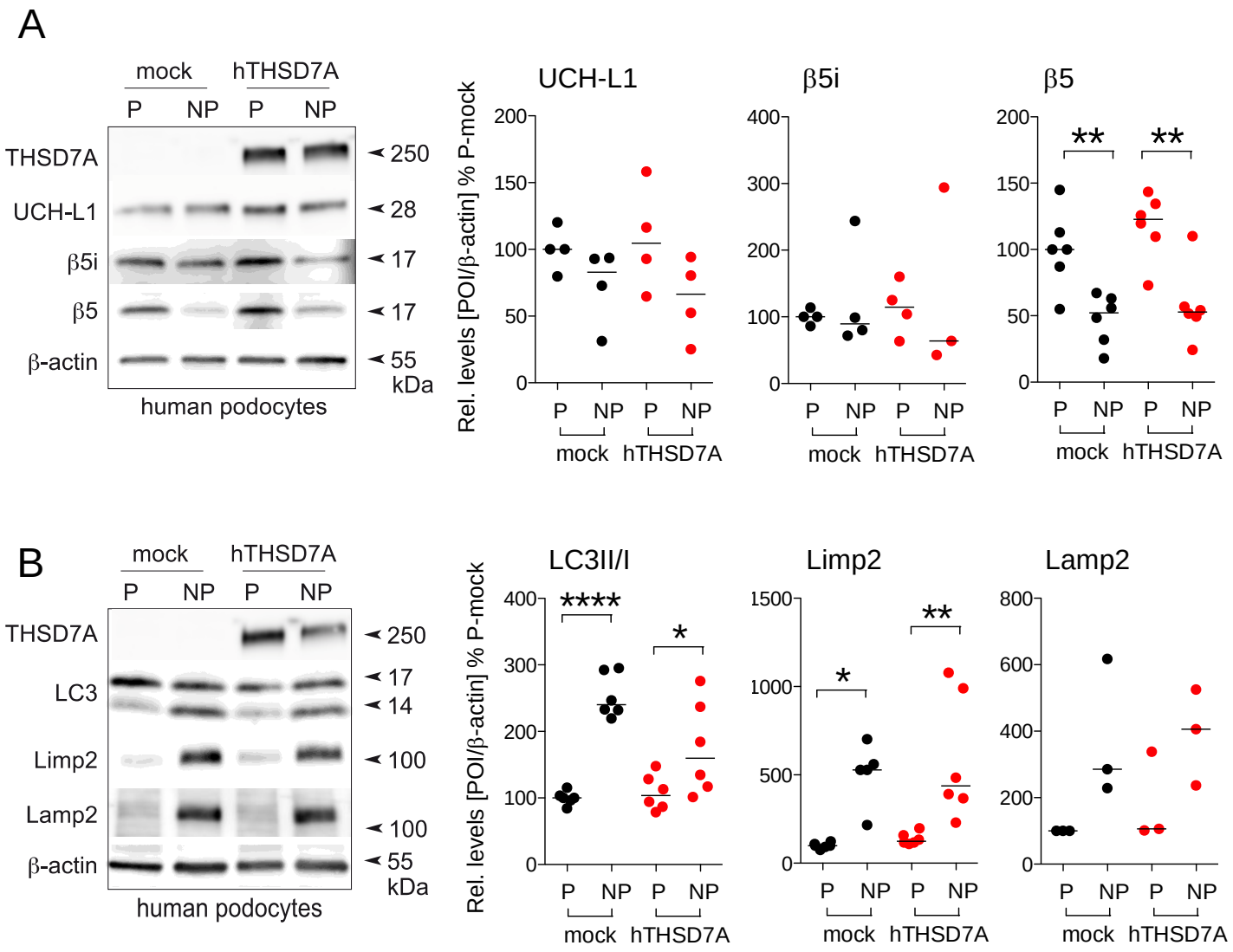
B&C: Balb/C mice were injected once with 600 µl anti-THSD7A autoantibody containing patient serum or control human serum. After 10 weeks, kidneys were harvested. **B:** Paraffin sections were stained for bound human IgG (green) and DNA (blue). **C:** Frozen sections were stained for bound human IgG (huIgG, green) and DNA (blue). Note the “finger-like” deposition of huIgG in the anti-THSD7A autoantibody injected mouse (arrows). In the control human serum injected mouse, huIgG is only found within the mesangial space. Podocyte nuclei are highlighted by the asterisks. Micrographs were taken with a LSM510 meta confocal microscope.



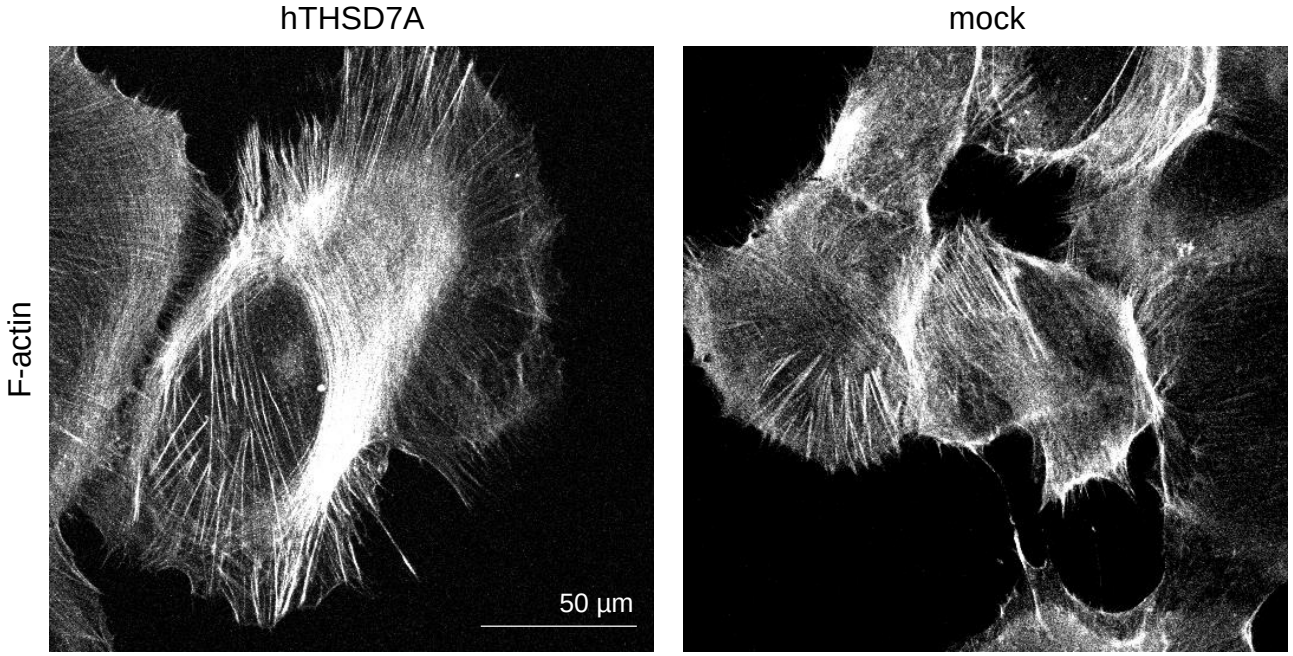
Supplemental figure 7: Balb/c mice were injected once with 180 µl purified rabbit IgG with and without specific anti-THSD7A antibodies. One day later bound rabbit IgG was specifically localized to podocyte foot processes by immunogold electron microscopy only in the mouse treated with anti-THSD7A positive serum. The red numbers indicate the size of the silver enhanced gold particles.



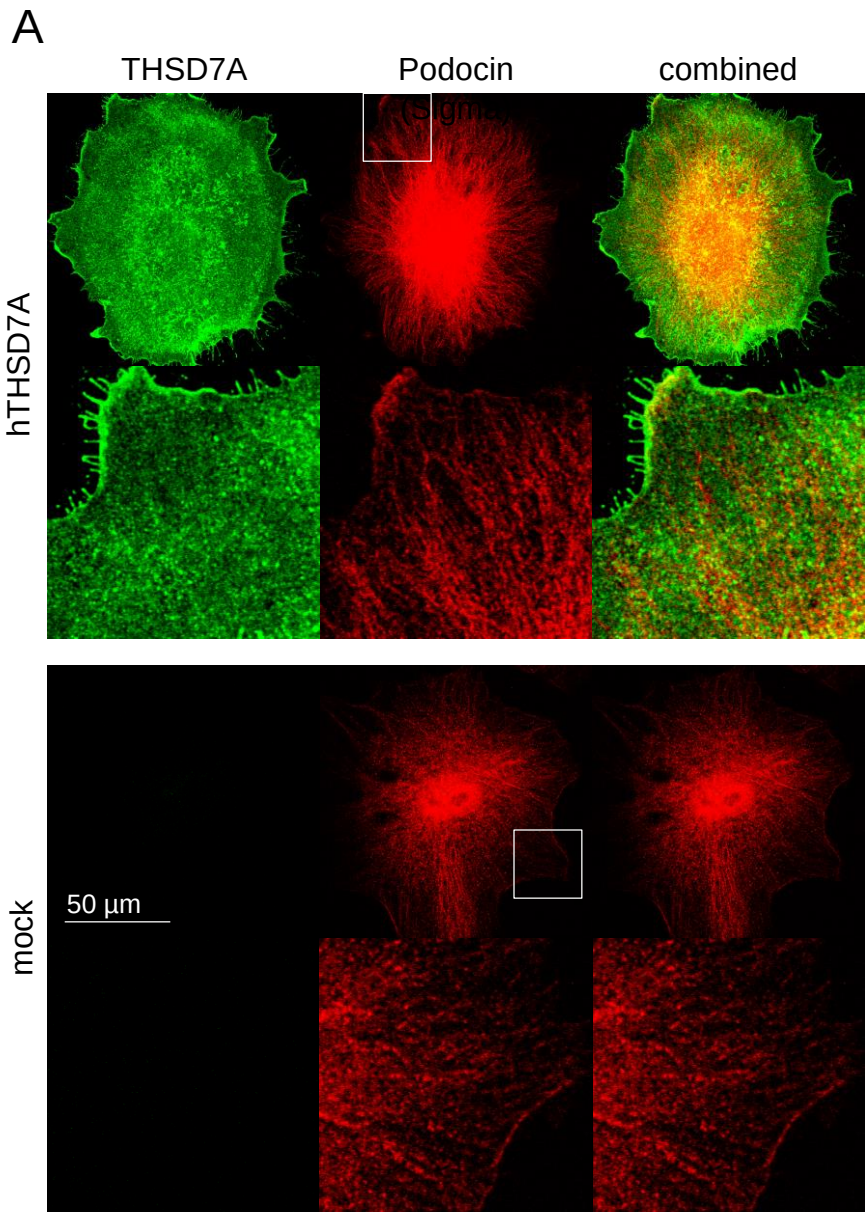
Supplemental figure 8: Balb/C mice were injected once with 600 µl human serum of a control patient or of a patient with THSD7A-associated membranous nephropathy. 18 weeks later, bound human IgG was localized to podocyte foot processes by immunogold electron microscopy only in the mouse treated with anti-THSD7A positive serum. The red numbers in the insert of the anti-THSD7A pos. serum injected mouse indicate the size of representative gold particles.

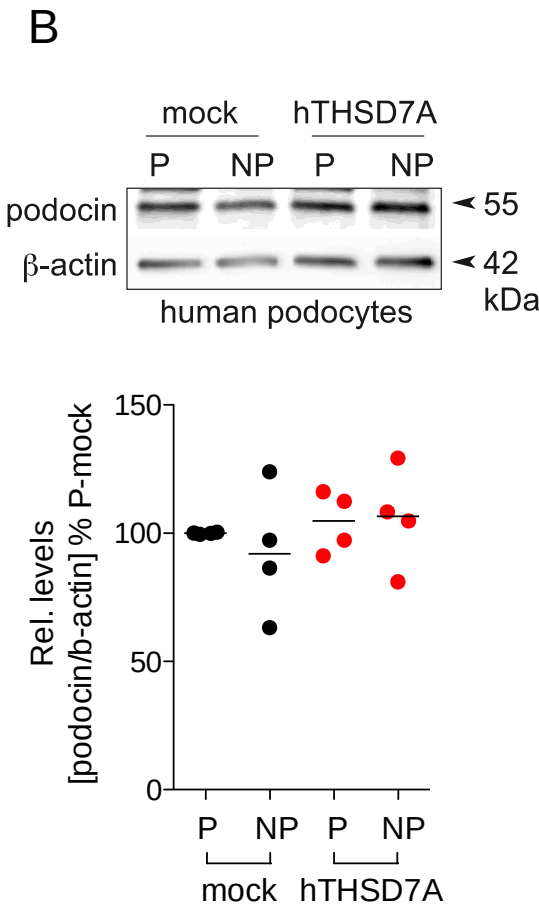


Supplemental figure 9: Overexpression of human THSD7A (hTHSD7A) in cultured immortalized human podocytes does not affect protein degradation pathways differently in comparison to mock transfected control cells. Western blot analyses of human podocytes cultured under permissive (P, 32°C) and non-permissive (NP, 38°C) conditions against the **A:** Ubiquitin proteasome and **B:** autophagosomal/lysosomal pathway proteins. UCH-L1 = Ubiquitin C-terminal hydrolase L1; β5i = immunoproteasome subunit β5i; β5 = standard proteasome subunit β5; LC3 = microtubule-associated protein 1A/1B-light chain; Limp2 = lysosomal integral membrane protein 2; Lamp2 = lysosomal associated membrane protein 2. β-actin was used to control for equal loading. Graphs exhibit densitometric analyses of 3-4 independent experiments with 1-2 biological replicates, values are expressed as mean +/- SEM and are calculated as % change to permissive mock levels. **p*<0.05, ***p*<0.01, ****p*<0.001, One-way Anova, Bonferroni's multiple comparisons.

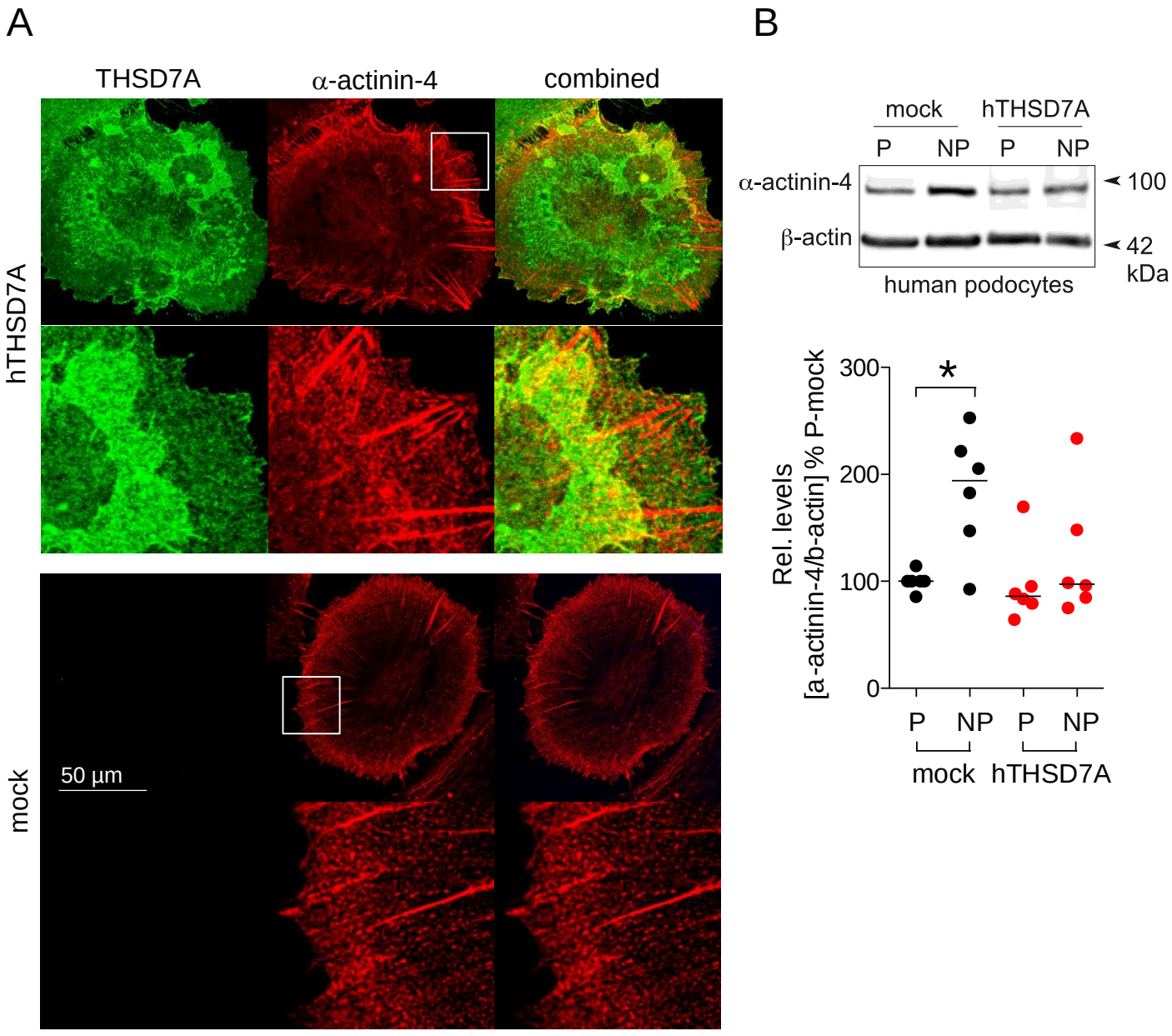


Supplemental figure 10: Overexpression of human THSD7A (hTHSD7A) in cultured immortalized human podocytes affects cell size and morphology. Representative confocal analyses of filamentous actin (F-actin). Note the increased cell size and elaborate morphology of hTHSD7A overexpressing podocytes. Micrographs were taken with a LSM510 meta confocal microscope.

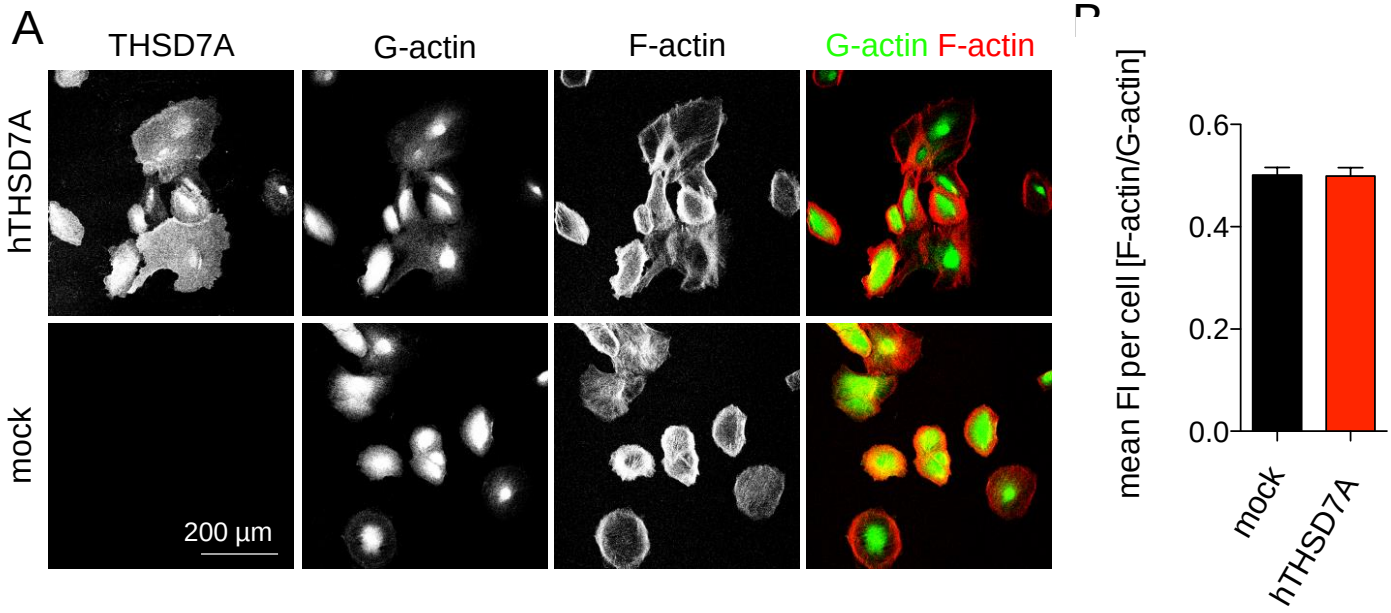




Supplemental figure 11: Overexpression of human THSD7A (hTHSD7A) in cultured immortalized human podocytes does not affect podocin expression (**A**) and levels (**B**).
A: Representative confocal analyses of podocin (red) in relation to THSD7A (green) expression. Lower row panels exhibit the enlarged boxed area of the upper row panels. Micrographs were taken with a LSM510 meta confocal microscope.
B: Western blot analyses of human podocytes cultured under permissive (P, 32°C) and non-permissive (NP, 38°C) conditions against podocin. β-actin of the same membrane was used to control for equal loading. Graph exhibits densitometric analyses of 2 independent experiments with 2 biological replicates, values are expressed as median and are calculated as % change to permissive mock levels (P-mock). Not significant, One-way Anova, Dunnett's multiple comparison test.



Supplemental figure 12: Overexpression of human THSD7A (hTHSD7A) in cultured immortalized human podocytes does not affect α-actinin-4 expression (**A**) and levels (**B**). **A:** Representative confocal analyses of α-actinin-4 (red) in relation to THSD7A (green) expression. Lower row panels exhibit the enlarged boxed area of the upper row panels. Micrographs were taken with a LSM510 meta confocal microscope.
B: Western blot analyses of human podocytes cultured under permissive (P, 32°C) and non-permissive (NP, 38°C) conditions against podocin. β-actin was used to control for equal loading. Graph exhibits densitometric analyses of 5 independent experiments with 1-2 biological replicates, values are expressed as median and are calculated as % change to permissive mock levels (P-mock). *p<0.05, One-way Anova, Dunnett's multiple comparison test.



Supplemental figure 13: Overexpression of human THSD7A (hTHSD7A) in cultured immortalized human podocytes does not affect actin polymerization.
A: Representative confocal analyses of THSD7A, G-actin (green) and F-actin (red) in mock and hTHSD7A podocytes differentiated for 10 days on collagen type IV coated silicon plates. Micrographs were taken with a LSM510 meta confocal microscope.
B: Graph exhibits quantification of F-actin/G-actin ratio by ImageJ from 4 independent experiments, values are expressed as mean +/-SEM. Not significant, Two-tailed Student t-test.