

Supplementary material for:

# A tight control of non-canonical TGF- $\beta$ pathways and microRNAs downregulates nephrin in podocytes

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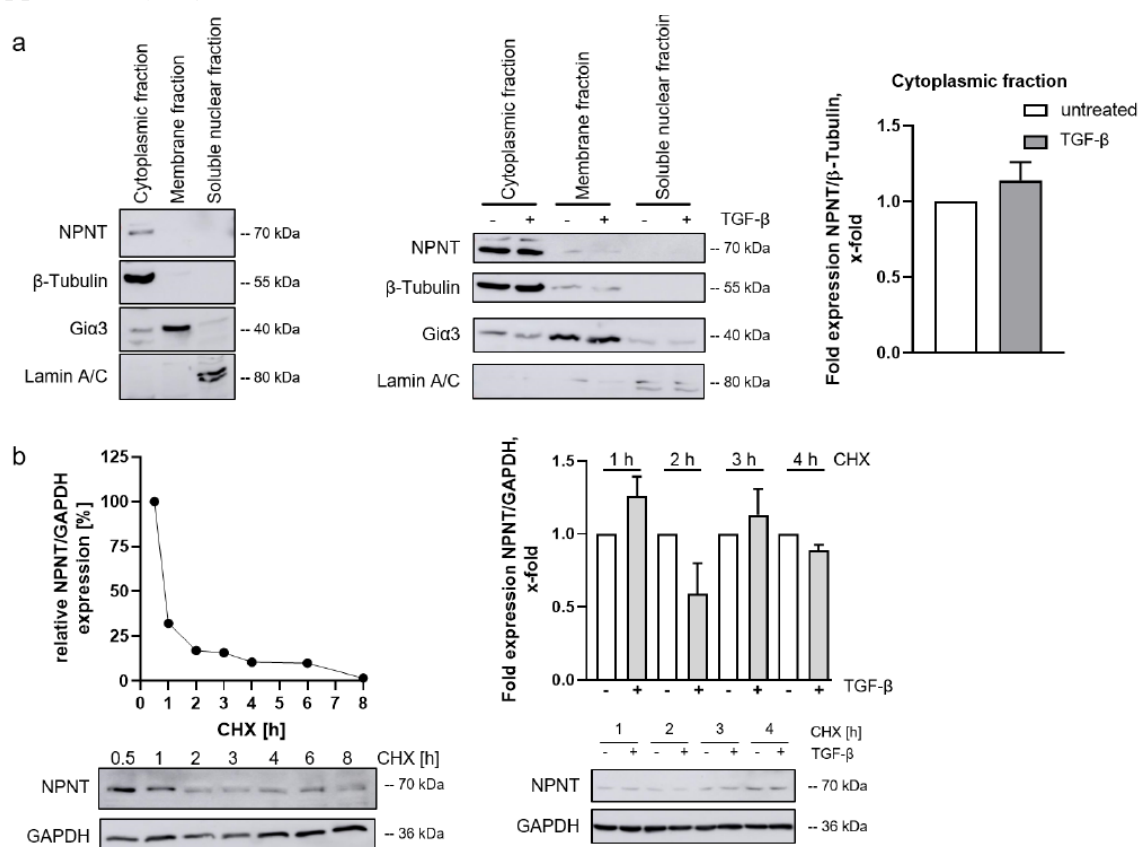
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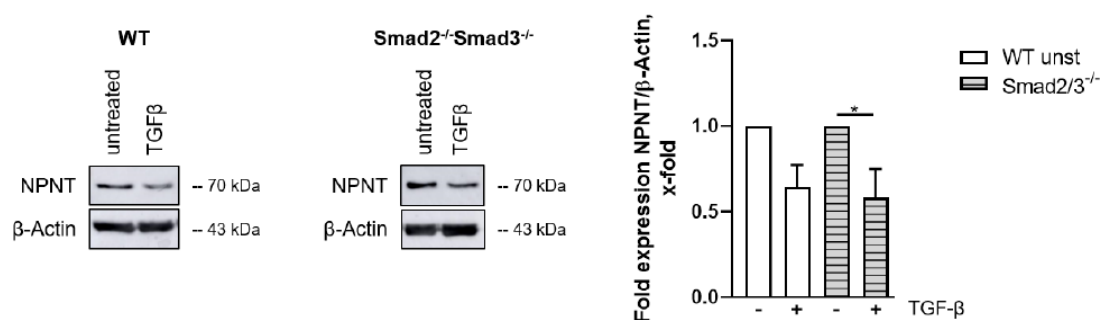
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## Supplementary Figures:



**Figure S1.** NPNT expression in podocyte cell fractions and protein half-life. a: NPNT protein expression in cytosol, membrane and nucleus of cultured human podocytes, as determined by markers for the respective fractions:  $\beta$ -tubulin,  $\text{G}\alpha 3$  and lamin A/C. The left panel depicts a representative Western Blot of untreated human podocytes, the right panel a comparison of untreated and TGF- $\beta$  treated human podocytes. A representative blot is shown. Densitometric analysis shows NPNT/ $\beta$ -Tubulin expression in the cytoplasmic fraction. n=2-3 independent experiments. b: Cycloheximide chase assay to determine baseline

protein turnover of NPNT in cultured human podocytes. Human podocytes were treated with cycloheximide (CHX) for the indicated time-points and NPNT protein was analyzed by Western Blot. Right panel: Protein turnover rate at each time point was illustrated as the percentage of NPNT/GAPDH at  $t = 0.5$  of each time point. Left panel: Densitometric analysis of NPNT/GAPDH expression after 1 to 4 hours of CHX treatment of human podocytes in the presence or absence of TGF- $\beta$ .  $n=2$  independent experiments, representative experiments are shown.



**Figure S2.** NPNT protein expression in murine wildtype (WT) and SMAD2- and SMAD3-deficient (Smad2/3<sup>-/-</sup>) podocytes. Western Blot analysis of NPNT protein expression in murine podocytes in the presence or absence of SMAD2/3 signaling and under baseline conditions or after treatment with TGF- $\beta$ . NPNT expression is normalized to  $\beta$ -Actin and given as fold change compared to unstimulated cells, respectively. Representative western blots are shown for each treatment.  $n=3$  independent experiments.