Synaptopodin is dispensable for normal podocyte homeostasis but is protective in the

context of acute podocyte injury

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Supplemental Figure 1. Schematic illustration of *Synpo* genomic structure and indications of guide RNAs and primers used for genotyping.

Supplemental Figure 2. The absence of Synpo did not change the localization and expression level of α -actinin-4 in glomeruli.

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Supplemental Figure 4. Increased number and size of FAs are associated with less organized, shortened and discontinued stress fiber.

Supplemental Figure 5. Synpo2/myopodin is not found in *Synpo*^{+/-} or *Synpo*^{-/-} kidneys.



	Forward primer 5'-3'	Reverse primer 5'-3'
Primer pair 1: F1R1	CTGCGGCATCTGGAGAAGGTTGCCAGTGAG	GACTGCCCATCATCAGCACACAGAGTGCCG
Primer pair 2: F2R2	GGAGAATGCAGCCCTGCTGACAGCCAATGG	TCAAGAGAATGCTGGATCTCACCTCCTCTG
Primer pair 3: F3R3	CAGGCGCTTCTGGCGCGCAACATCATCAAC	CTCGGAGTCGAGAGACACGTCGGAGTCTGT
Primer Pair 4: F4R4	CGGCCC TTTTCCCCGC CGCA	CAGATGTTGTAGCCGAGGATGCCAGGCGAC
Primer pair 5: F1R4	CTGCGGCATCTGGAGAAGGTTGCCAGTGAG	CAGATGTTGTAGCCGAGGATGCCAGGCGAC

Supplemental Figure 1. The locations of gRNAs in *Synpo*'s two coding exons and the PCR primer pairs used for identification of the ~8 kb deletions (F1R4, pair 5) and for analysis of single site indels (pairs 1-4). Nucleotide numbering is based on GenBank Sequence ID: AC149216.5



actinin4/β-actin -0.0 -0.0 -0.0 -0.0 -0.0 -0.0 -0.0 -0.0 -0.0 -0.0

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Supplemental Figure 2. The absence of Synpo did not change the expression of α -Actinin-4 in glomeruli. (A) Representative confocal images of double staining for α -Actinin-4 (red) and Nidogen (green). Scale bar: 10 µm. (B) Western blot analysis of cytosolic extracts from glomeruli. Equal protein loading was confirmed by reprobing for β -actin. (C) Bar graph shows that the levels of α - Actinin-4 are not different between Synpo+/- and Synpo-/- mice.



Supplemental Figure 3. Within glomeruli, myosin IIA was localized to podocyte cell bodies and mesangial cells. There were no differences in the localization and expression level between *Synpo*^{+/-} and *Synpo*^{-/-} glomeruli.



Supplemental Figure 4. To study how the changes in the size and localization of FAs relates to the reorganization of actin stress fibers, primary podocytes were stained with phalloidin and anti-vinculin. *Synpo*^{-/-} podocytes showed less organized actin stress fibers, which were associated with increased number and size of FAs.



Supplemental Figure 5. The normal phenotype observed in unchallenged *Synpo^{-/-}* mice is not due to compensation by myopodin/Synpo2, which was not detected in either control or mutant glomeruli. Myopodin was easily detected in skeletal muscle fibers.