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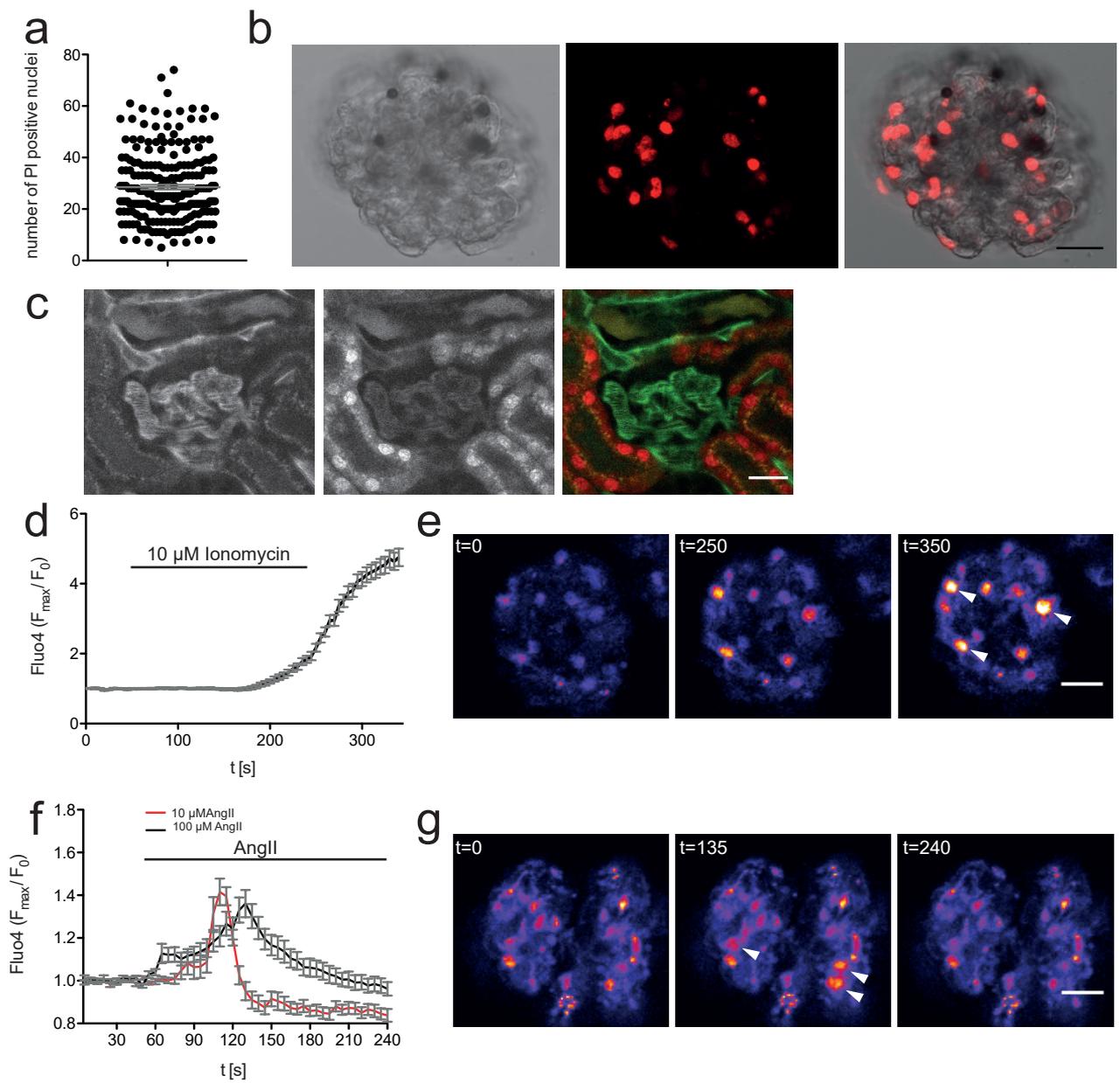
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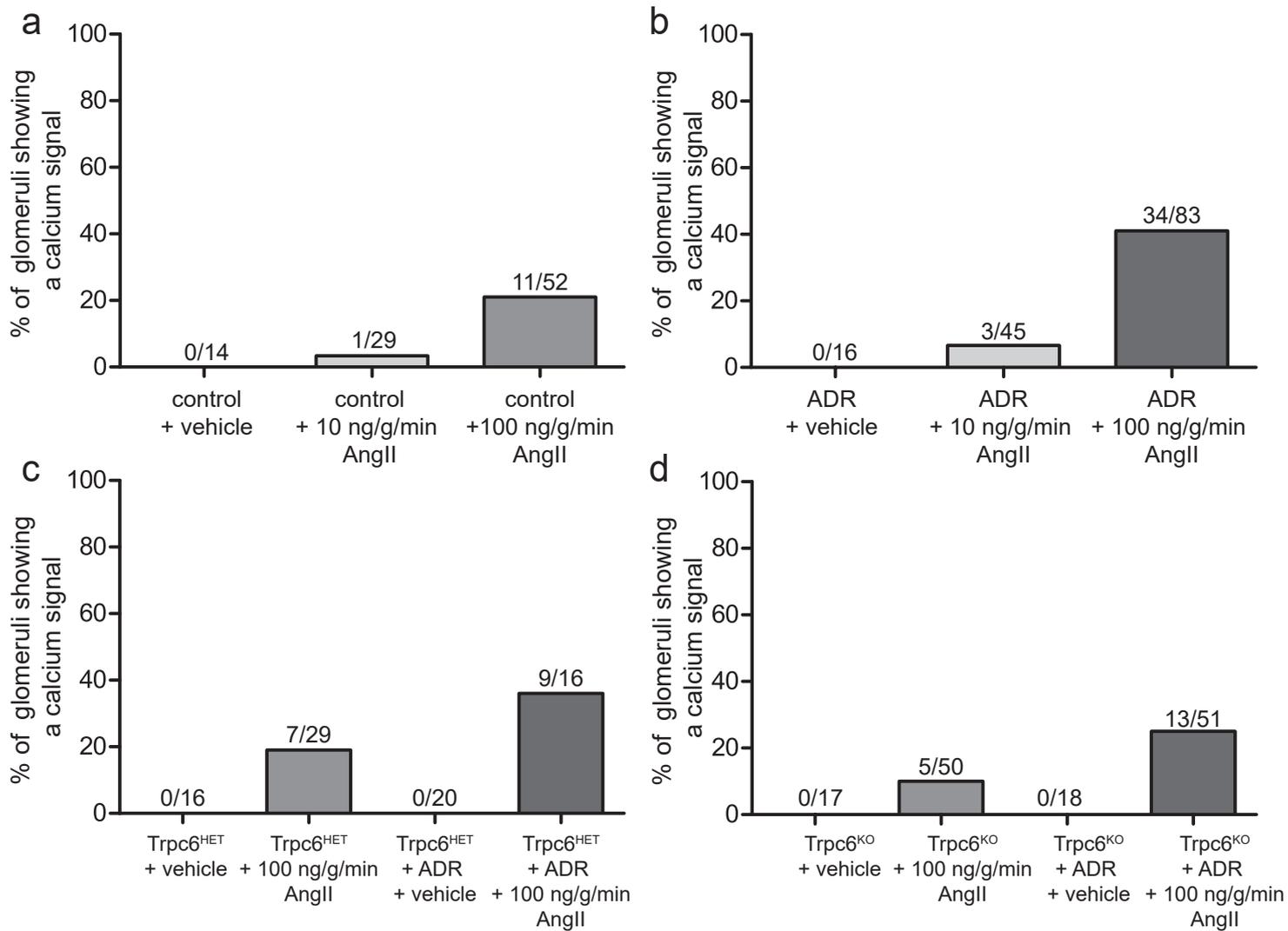
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Supplementary Figure 1



Supplementary Figure 1: Isolated glomeruli show a high number of propidium iodide positive cells and an attenuated podocyte calcium response to AngII *ex vivo*. **a**, Propidium iodide (PI) positive cells after isolation with a mean of 28.1 nuclei per glomerulus (n=225). **b**, Representative image of an isolated glomerulus stained with PI. Left: bright field image, middle: z-projection of PI positive nuclei, right: merge. Scale bar: 20 μ m. **c**, *In vivo* imaging of a glomerulus after the injection of PI (50 μ g), Lectin-FITC (15 μ g) and a green fluorescent 70kDa dextrane (15 μ g) were injected to label the vasculature. The left panel shows the green channel, the middle panel the red channel and the right panel the merged image. **d**, Relative changes (F_{max}/F_0) in Fluo4 fluorescence measured in isolated glomeruli perfused (1ml/min) with 10 μ M Ionomycin (m=75). Complete exchange of the buffer in the well was achieved within 1-2 minutes. **e**, Representative images of a Fluo4 loaded glomerulus showing a calcium signal upon stimulation with 10 μ M Ionomycin. Fluo4 fluorescence is shown in false colors. **f**, Relative changes (F_{max}/F_0) in Fluo4 fluorescence measured in isolated glomeruli perfused (1ml/min) with 10 μ M AngII (m=31) or 100 μ M AngII (m=66). Complete exchange of the buffer in the well was achieved within 1-2 minutes. **g**, Representative images of a Fluo4 loaded glomerulus showing a calcium signal upon stimulation with 100 μ M AngII. Fluo4 fluorescence is shown in false colors. Arrows indicate cells with increased fluorescence. Scale Bar: 20 μ m. n=glomeruli analyzed, m=cells analyzed.

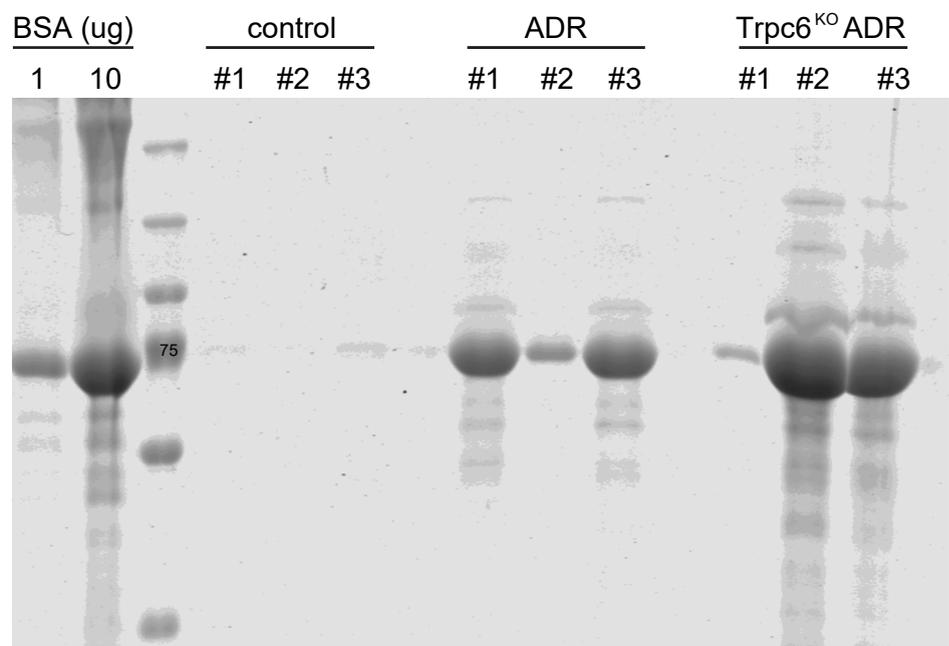
Supplementary Figure 2



Supplementary Figure 2: Injured podocytes show an increased calcium response upon AngII stimulation in vivo (Data from Figure 1 a and 1 c supplemented with the respective negative controls). **a**, Pod:cre GCaMP3^{fl/fl} (control) animals showing a calcium signal in podocytes upon infusion of vehicle (normal saline) (n=3), AngII (10 ng/g/min, n=5) or AngII (100 ng/g/min, n=13). **b**, Pod:cre GCaMP3^{fl/fl} animals pre-treated with Adriamycin (ADR) infused with vehicle (n=3), AngII (10 ng/g/min, n=6) or AngII (100 ng/g/min, n=24). **c**, Pod:cre GCaMP3^{fl/fl} Trpc6^{KO/WT} (Trpc6^{HET}) animals infused with vehicle (n=6), Trpc6^{HET} animals stimulated with AngII (100 ng/g/min, n=6), Trpc6^{HET} pre-treated with ADR and infused with vehicle (n=6) or AngII (100 ng/g/min, n=5). **d**, Pod:cre GCaMP3^{fl/fl} Trpc6^{KO} (Trpc6^{KO}) animals infused with vehicle (n=4), AngII (100 ng/g/min, n=9) and Trpc6^{KO} animals pre-treated with Adriamycin (ADR) and infused with vehicle (n=4) or stimulated with AngII (100 ng/g/min, n=16). Numbers above bars indicate: glomeruli showing a calcium signal / total number of glomeruli imaged. n= number of mice.

Supplementary Figure 3: Number of podocytes showing a calcium signal upon stimulation with AngII. Pod:cre GCaMP3^{fl/fl} (control) (mean=17, n=20), Pod:cre GCaMP3^{fl/fl} Trpc6^{KO/KO} (Trpc6^{KO}) (mean=11, n=3), Pod:cre GCaMP3^{fl/fl} treated with Adriamycin (ADR) (mean=15, n=81), TRPC6^{KO} ADR (mean=13, n=52), Pod:cre GCaMP3^{fl/fl} Podocin^{fl/fl} (Pod^{pKO}) (mean=15, n=41), Pod:cre GCaMP3^{fl/fl} Podocin^{fl/wt} (Pod^{pHet}) (mean=19, n=35), laser injury (mean=19, n=45) and Pod:cre GCaMP3^{fl/wt} R26hM3D^{tg/wt} (DREADD) stimulated with CNO (mean=32, n=16). n=number of glomeruli, mean=s.e.m. statistics: 1way-ANOVA Tukey's Multiple Comparison Test: #p=0.01.

Supplementary Figure 4



Supplementary Figure 4: Proteinuria analysis of spot urine. Urine samples (2 μ l) of Pod:cre GCaMP3^{fl/fl} (control), Pod:cre GCaMP3^{fl/fl} animals treated with Adriamycin (ADR), and Pod:cre GCaMP3^{fl/fl} Trpc6^{KO/KO} animals treated with ADR (Trpc6^{KO} ADR) were loaded on a 10 % poly-acrylamid gel and after electrophoresis stained with Coomassie blue. As control 1 and 10 μ g bovine serum albumin (BSA) was used.

Supplementary Table 1

Experimental Group: NaCl Infusion	Background	Number of mice	Number of Glomeruli	Percentage of Glomeruli showing a calcium signal in at least one podocyte
Control	C57Bl6	3	14	0
ADR	C57Bl6	3	16	0
Trpc6 ^{Het}	C57Bl6	6	16	0
Trpc6 ^{Het} ADR	C57Bl6	6	20	0
Trpc6 ^{KO}	C57Bl6	4	17	0
Trpc6 ^{KO} ADR	C57Bl6	4	18	0
Experimental Group: AngII Infusion	Background	Number of mice	Number of Glomeruli	Percentage of Glomeruli showing a calcium signal in at least one podocyte
Control	C57Bl6	13	52	21
ADR	C57Bl6	24	83	41
Trpc6 ^{Het}	C57Bl6	6	36	22
Trpc6 ^{Het} ADR	C57Bl6	5	25	36
Trpc6 ^{KO}	C57Bl6	9	50	10
Trpc6 ^{KO} ADR	C57Bl6	16	51	25
Pod ^{pHet}	Sv129/C57BL6	31	88	36
Pod ^{pKO}	Sv129/C57BL6	10	44	59
Experimental Group: Losartan/PD123319	Background	Number of mice	Number of Glomeruli	
ADR +AngII + Losartan	C57Bl6	3	6	
ADR + AngII + PD123319	C57Bl6	3	6	
Pod ^{pHet} + AngII + Losartan	Sv129/C57BL6	2	4	
Pod ^{pHet} + AngII + PD123319	Sv129/C57BL6	4	6	