

Supplementary Materials for

Mechanical activation of TRPV4 channels controls albumin reabsorption by proximal tubule cells

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Published 13 October 2020, *Sci. Signal.* **13**, eabc6967 (2020)
DOI: 10.1126/scisignal.abc6967

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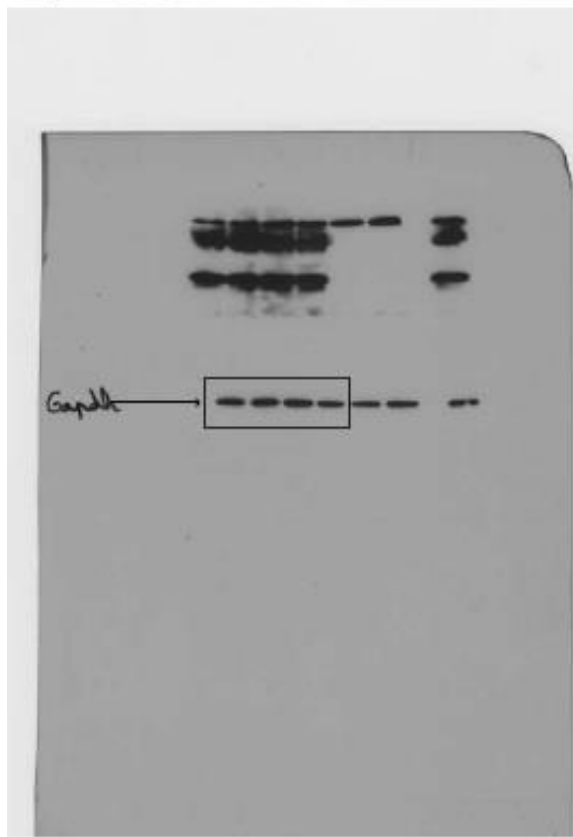
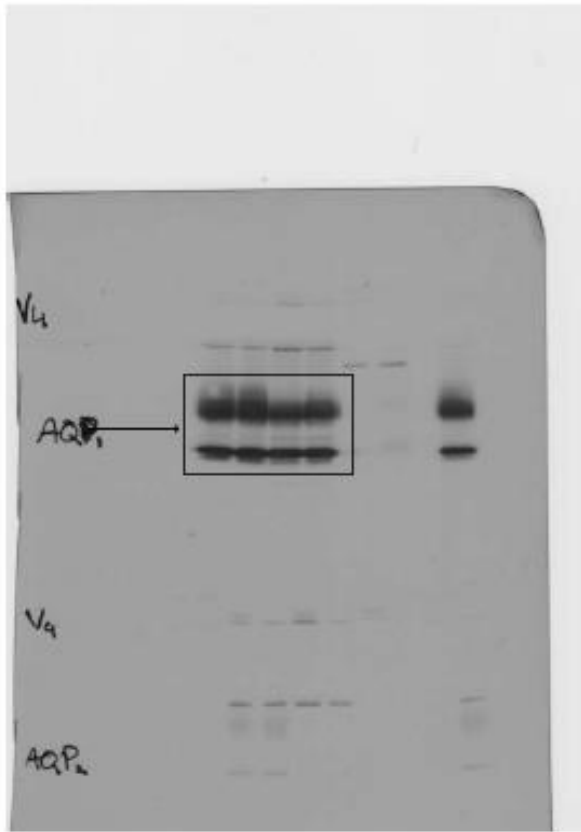
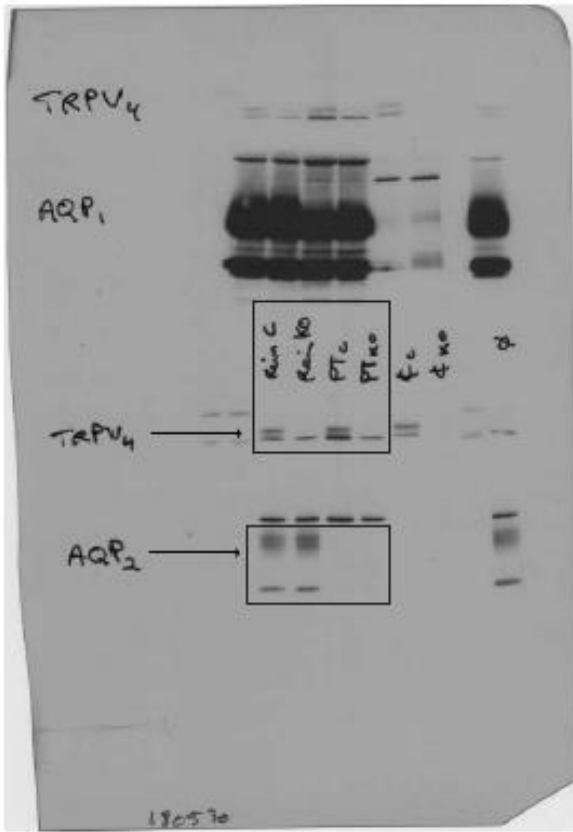


Fig. S1. Complete versions of Western blots presented in Fig. 1.

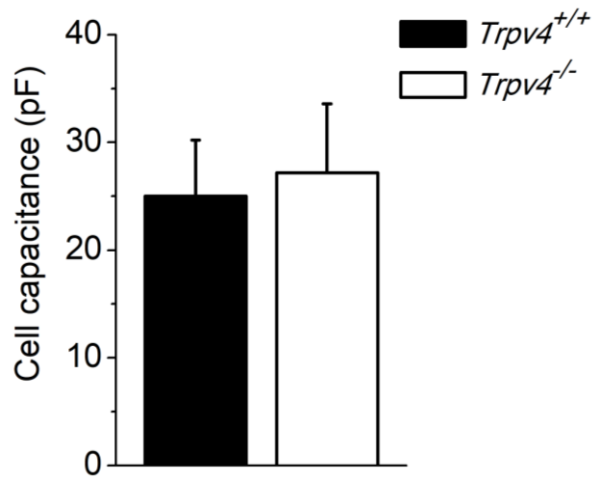


Fig. S2. Capacitance of mPTCs from *Trpv4*^{+/+} and *Trpv4*^{-/-} mice. Mean cell capacitance obtained by patch-clamp recordings (n=30 *Trpv4*^{+/+} cells, n= 27 *Trpv4*^{-/-} cells).

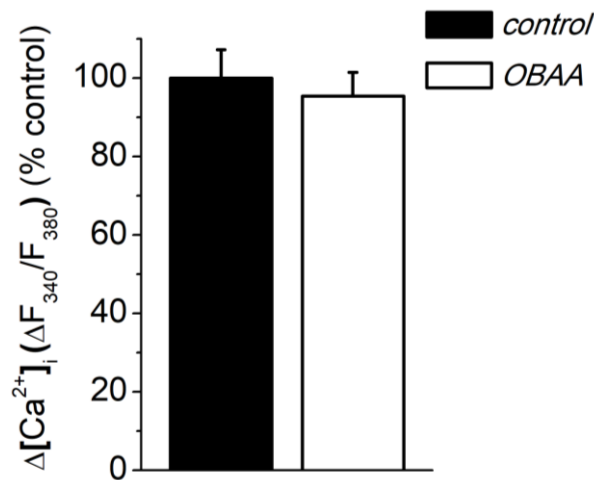


Fig. S3. Effect of OBAA on stretch-induced Ca^{2+} entry in mPTCs. Normalized increase in $[\text{Ca}^{2+}]_i$ in mPTCs from *Trpv4*^{+/+} mice upon cell stretching, in the absence (control) or presence of 100 μM OBAA (n=6 experiments with n>20 cells for each group).

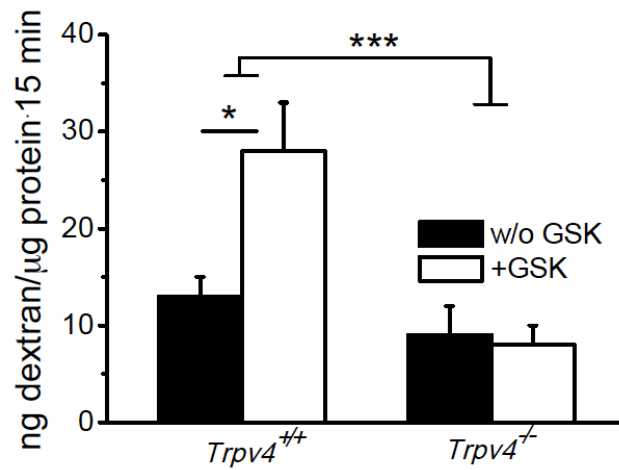


Fig. S4. Dextran uptake induced by TRPV4 activation in mPTCs. Quantification of the total dextran uptake after incubation of *Trpv4*^{+/+} and *Trpv4*^{-/-} mPTCs seeded on collagen-coated membranes, after incubation with 1 mg/ml FITC-dextran for 15 min at 37°C (n= 8 experiments, two-way ANOVA with Bonferroni post hoc test; * p<0.05; *** p<0.001).

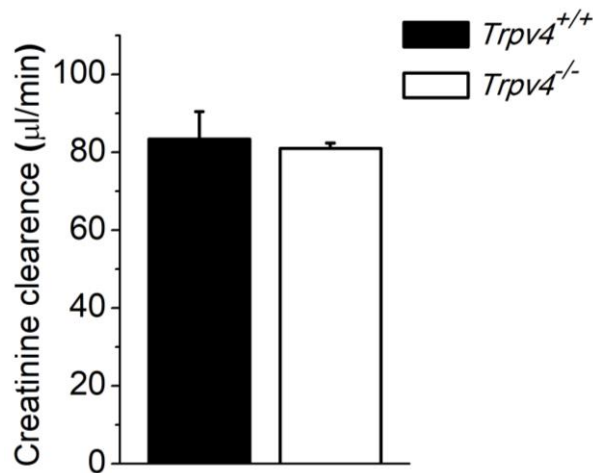


Fig. S5. Creatinine clearance of mice treated with AT2. Creatinine clearance in *Trpv4*^{+/+} and *Trpv4*^{-/-} mice after 2 weeks of AT2 infusion (n = 10 mice for each genotype).

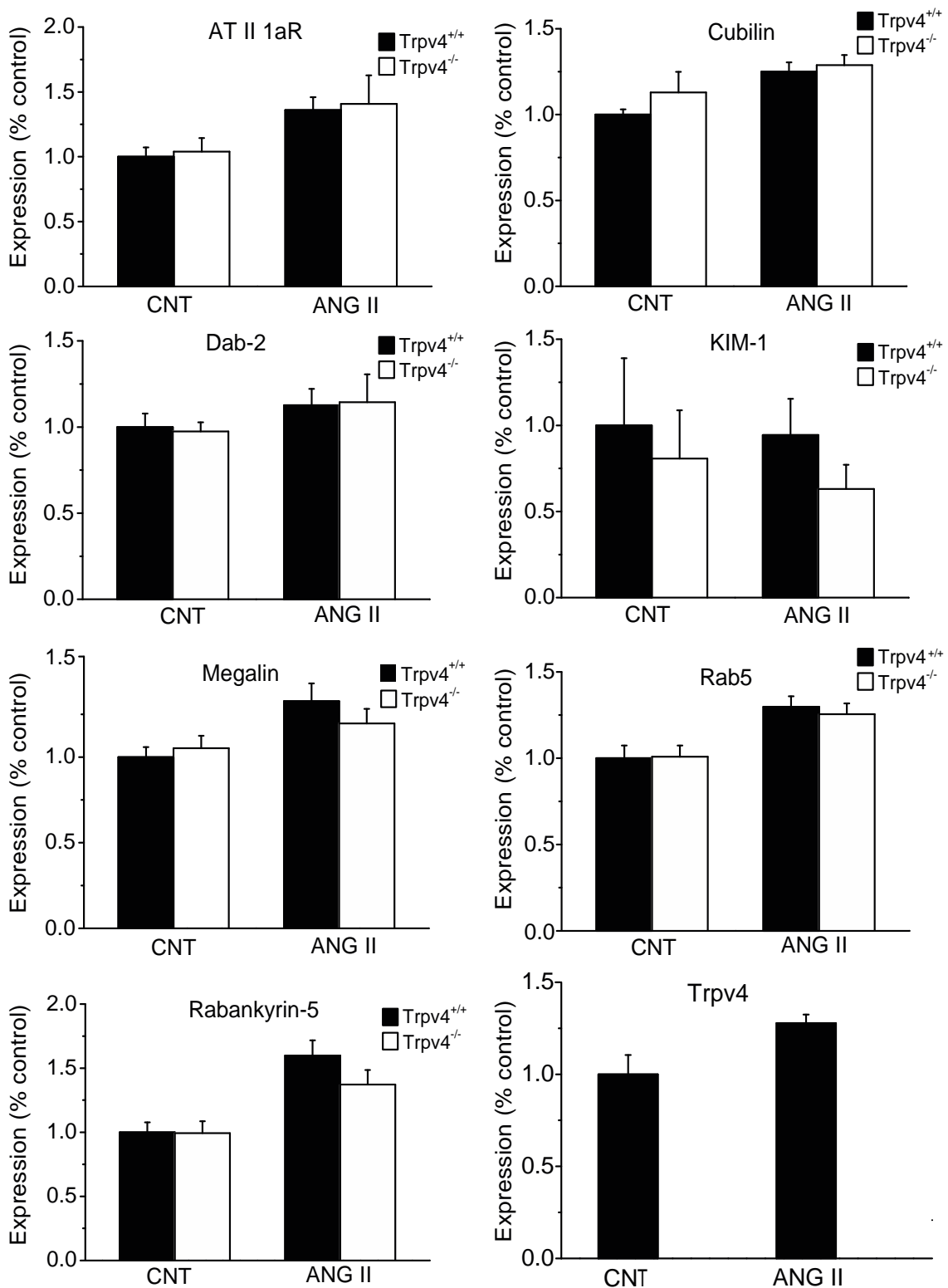


Fig. S6. AT2-induced proteinuria is not caused by down-regulation of genes involved in endocytosis. Quantitative analyses of mRNA expression of the following genes, in PTs isolated from *Trpv4*^{+/+} or *Trpv4*^{-/-} mice before or after injection of AT2 using osmotic minipumps: *Angiotensin II receptor (AT II 1aR)*, *Cubilin*, *Disabled-2 (Dab-2)*, *kidney injury molecule-1 (KIM-1)*, *Megalin*, *Rabankyrin-5*, *Rab5*, *TRPV4* (n=10 mice for each genotype).

Table S1. K_m and V_{max} values extrapolated from Michaelis-Menten kinetics analysis.

Cell Culture Set	K_m	V_{max}
<i>Trpv4^{+/+}</i>	40±6 µg/ml	203±11 ng albumin / µg protein.15 min
<i>Trpv4^{+/+}</i> + GSK	31±4 µg/ml	377±15 ng albumin / µg protein.15 min
<i>Trpv4^{-/-}</i>	57±24 µg/ml	189±30 ng albumin / µg protein.15 min
<i>Trpv4^{-/-}</i> + GSK	36±57 µg/ml	196±108 ng albumin / µg protein.15 min

Table S2. List of DNA primers used in quantitative RT-PCR.

Gene	Forward primer	Reverse primer
<i>Gapdh</i>	TGCACCACCAACTGCTTA	GGATGCAGGGATGATGTT
<i>Trpv4</i>	GAGGCTACTTCTACTTTGGGGAG	GTCCTGTCGCCTCATGTCAG
<i>mATII 1a</i>	GTG GCT GAA GCC AGT ACC AG	CTT GGA GGG TTG CTG TGA GT
<i>Cubilin</i>	AAC CTT TGC CTA CCA GAG CC	GGA CAA TTG CTG GCC GAT TC
<i>Dab-2</i>	ACA GGA GCC CTT TCG GAA ATC	ATA CGT CAA AGC TGG AGC GA
<i>KIM-1</i>	TTG TGA GCA CCG TGG CTA TC	GCA ACC ACG CTT AGA GAT GCT
<i>Megalin</i>	GGG GTG TAC GAT TGC AAG GA	TAC CAT CTC CCT GGC ACT GA
<i>Rabankyrin-5</i>	AGG CAC ATC AGT GCT CAC AA	TTG TCA CCT CAG GAT TCG CA
<i>Rab 5</i>	CGC GGC CCA GGT AAT TTC TC	TCC AGT ATT TGG CCC GTT GG