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Supplementary Materials for

RSK2 contributes to myogenic vasoconstriction of resistance arteries by activating smooth muscle myosin and the Na^+/H^+ exchanger

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at www.sciencesignaling.org/cgi/content/full/11/554/eaar3924/DC1)

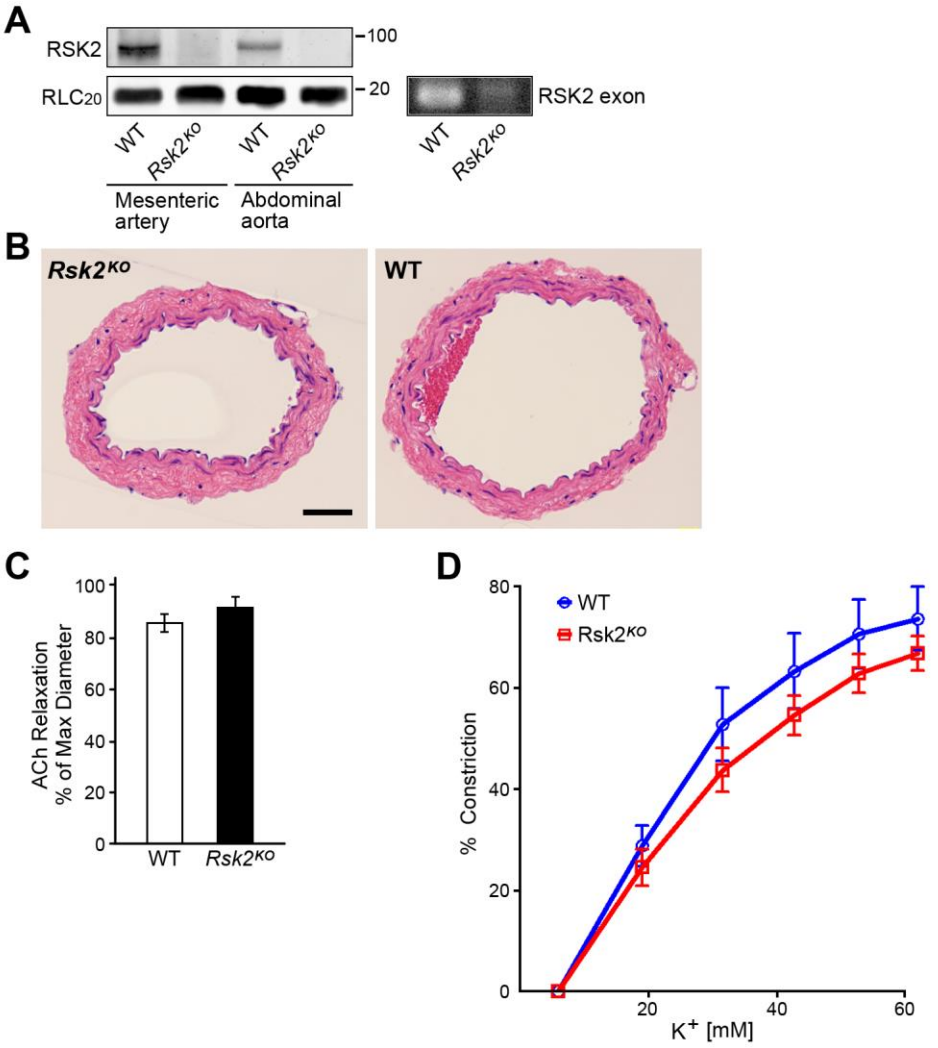

Fig. S1. Characterization of *Rsk2*^{KO} mice. A) Because the RSK2 gene *Rps6ka3* is located on the X chromosome, analysis was performed on *Rps6ka3*$^{-/-}$ knockout (KO*)* male mice and *Rps6ka3*$^{-/-}$ KO female mice. Controls were littermate *Rps6ka3*$^{-/+}$ males and *Rps6ka3* $^{+/+}$ female mice. *Rsk2*^{KO} mice were identified by Western blots of mesenteric arteries and PCR amplification of aortic homogenates. B) Representative hematoxylin and eosin stained transverse sections through 4th order mesenteric arteries from WT and *Rsk2*^{KO} mice showing similar vessel wall structures and smooth muscle composition. 6 *Rsk2*^{KO} and 4 WT 4th order vessel from 1 pair of mice were examined. The number of smooth muscle layers and number of smooth muscle nuclei are presented in Table S1. C) Acetylcholine (ACh) induced relaxation of pressurized mesenteric arteries was used as an indication of vessel viability. Maximum diameter = diameter in Ca^{2+} free solution and represents the maximum dilation. Two tailed homoscedastic Student's t-test. D) Constriction of WT and *Rsk2*^{KO} 4th order mesenteric arteries at 60 mm Hg pressure in response to increasing concentrations of high [K^+], n = 3 mice/group, 6-7 vessels/group.

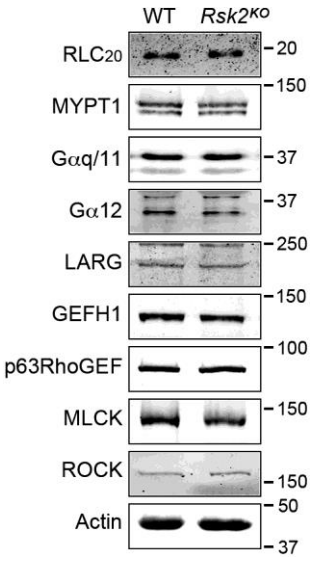

Fig. S2. Protein abundance of myosin, RhoGEFs, G$\alpha_{q/11/12}$, MYPT1, MLCK, and ROCK in WT and *Rsk2*^{KO} smooth muscle. MA arcades equilibrated in the resting state were immunoblotted for the indicated proteins. Protein abundance was normalized to actin. N=4 WT and *Rsk2* KO mice.

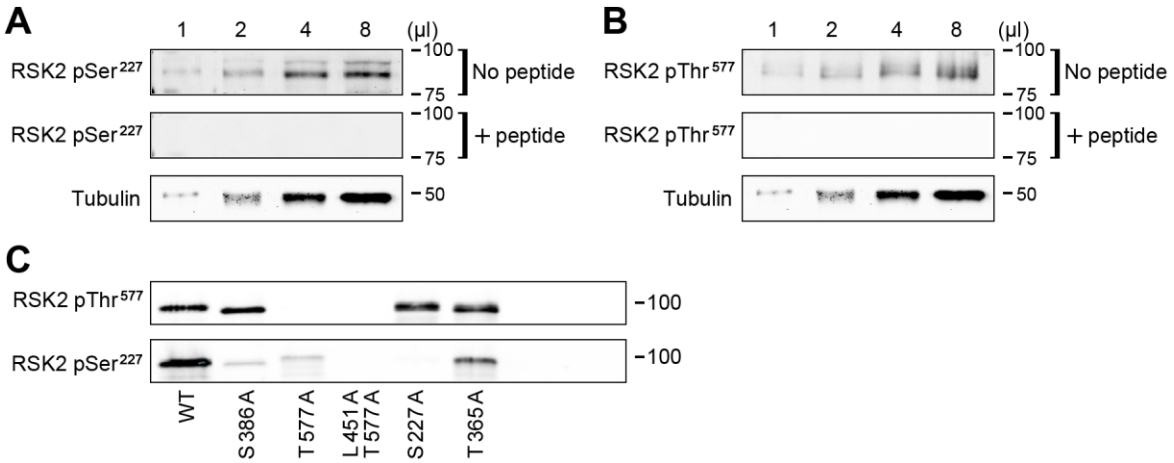

Fig. S3. Demonstration of specificity of Ser^{227} and Thr^{577} phospho-specific RSK2 antibodies. A) and B) Representative Western blots showing blocking peptides specific to phospho-Ser^{227} and phospho-Thr^{577} blocked the immuno-reactivity to phospho-Ser^{227} and phospho-Thr^{577} of aortae homogenates. n = 3 mice. C) S227A and T577A but not S386A or T365A mutations blocked the immunoreactivity to phospho-Ser^{227} and phospho-Thr^{577} in recombinant RSK2.

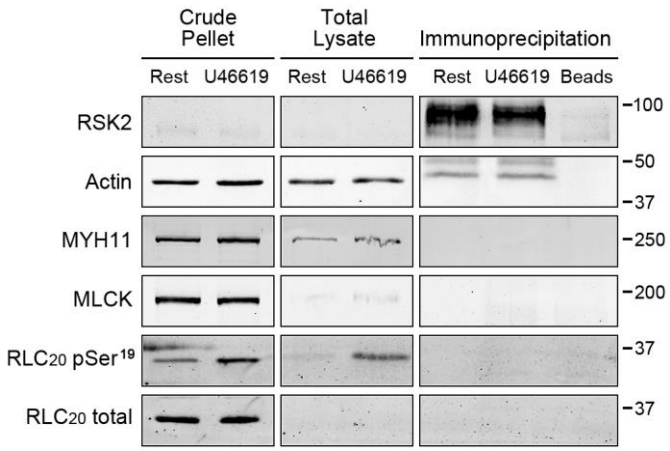

Fig. S4. RSK2 immunoprecipitated with actin, but not myosin, from mouse abdominal aorta. Western blots of isolated aortae stimulated with thromboxaneA2 agonist U46619 (1μM). Actin but not myosin (MYH11), MLCK, or RLC_{20} immunoprecipitated with RSK2 antibody in both stimulated and unstimulated vessels, n = 5 aortae from 5 mice.

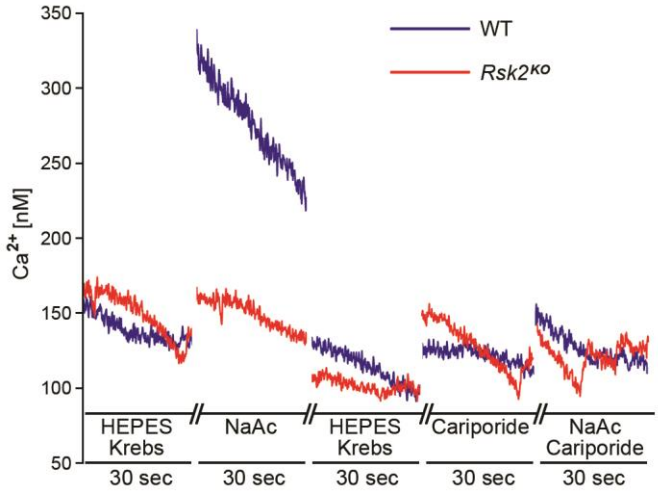

Fig. S5. Typical Ca^{2+} measurements in Fluo-4–loaded cultured normal smooth muscle cells before and after treatment with Na acetate. Imaging was briefly interrupted with each solution change in the cell culture chamber, as indicated //.

Table S1. Histological analysis of WT and *Rsk2*^{KO} mesenteric arteries.

	Number of SM layers		Number of SM nuclei ± SEM*				
	WT	*Rsk2*$^{-/-}$	WT	N	*Rsk2*$^{-/-}$	N	*p-values*
4th order	1-2	1-2	12.1±2.1	4	15.6±2.1	5	0.11
3th order	2-3	2-3	15.6±1.1	5	17.8±0.5	4	0.11
2d order	2-3	2-3	14.3±1.3	3	15.4±0.8	3	0.42

*Number of SM cell nuclei measured per vessel cross section, expressed as number of SM nuclei per 500 μm length of vessel circumference. N = number of mesenteric arteries from one WT and one *Rsk*$^{-/-}$ mouse.

Table S2. Cardiac functions measured by MRI in WT and *Rsk2*^{KO} littermates.

	Original measurements			Values scaled to body weight		
	WT	*Rsk2*^{KO}	p-values	WT	*Rsk2*^{KO}	p-values
Heart Rate, bpm	542.2±17.3	509.4±47.0	0.47	542.2±17.3	509.4±47.0	0.5
R-R, ms	110.8±3.5	110.8±10.7	0.42	110.8±3.5	110.8±10.7	0.4
LVM, ml [μl/g]	0.118±0.01	0.076±0.004	0.02	3.7±0.4	3.9±0.6	0.8
LVM, g [mg/g]	0.124±0.01	0.080±0.004	0.02	3.9±0.4	4.0±0.6	0.8
EDV, ml [μl/g]	0.045±0.002	0.036±0.001	0.01	1.4±0.2	1.8±0.2	0.2
ESV, ml [μl/g]	0.018±0.001	0.014±0.001	0.05	0.6±0.1	0.7±0.1	0.2
SV, ml [μl/g]	0.028±0.001	0.022±0.0004	0.001	0.9±0.1	1.1±0.1	0.2
EF, %	61.2±1.0	61.6±2.6	0.85	61.2±1.0	61.6±2.6	0.8
CO, l/min [ml/min/g]	0.015±0.0001	0.011±0.001	0.005	0.5±0.0	0.6±0.0	0.1
LVM measured at ED, g [mg/g]	0.102±0.01	0.077±0.004	0.08	3.2±0.3	3.9±0.6	0.3
LVM measured at ES g [mg/g]	0.133±0.01	0.076±0.004	0.01	4.2±0.4	3.8±0.5	0.5

LVM – Left Ventricular Mass, CO – Cardiac Output, EF – Ejection Fraction, ESV – End Systolic Volume, EDV - End Diastolic Volume, SV Stroke Volume, R-R – R-R wave ECG interval, ED – End Diastole, ES - End Systole.

Movie S1. Ca^{2+} transients in mesenteric arterial smooth muscle.