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Supplementary Materials for

An *RYR1* mutation associated with malignant hyperthermia is also associated with bleeding abnormalities

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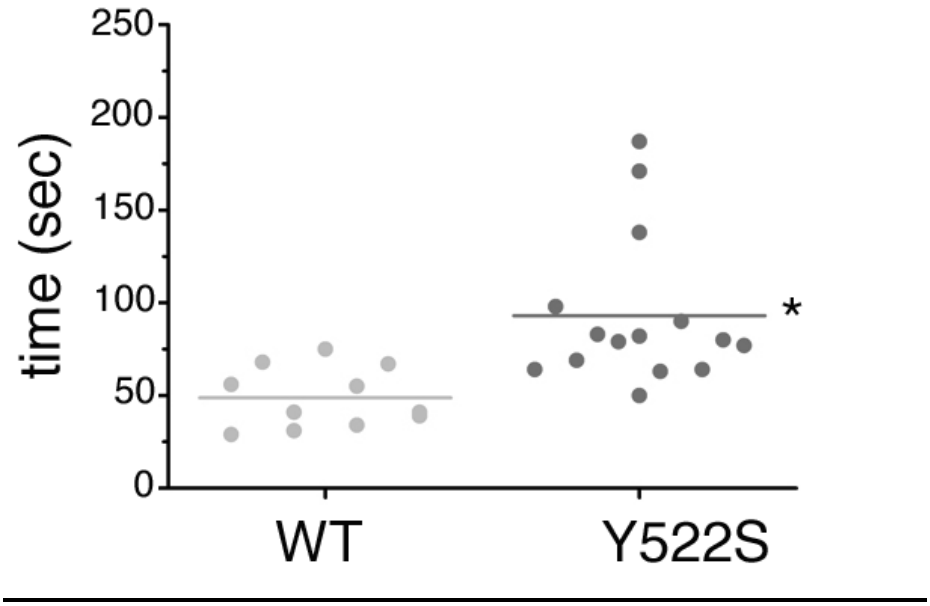
Published 5 July 2016, *Sci. Signal.* **9**, ra68 (2016)
DOI: 10.1126/scisignal.aad9813

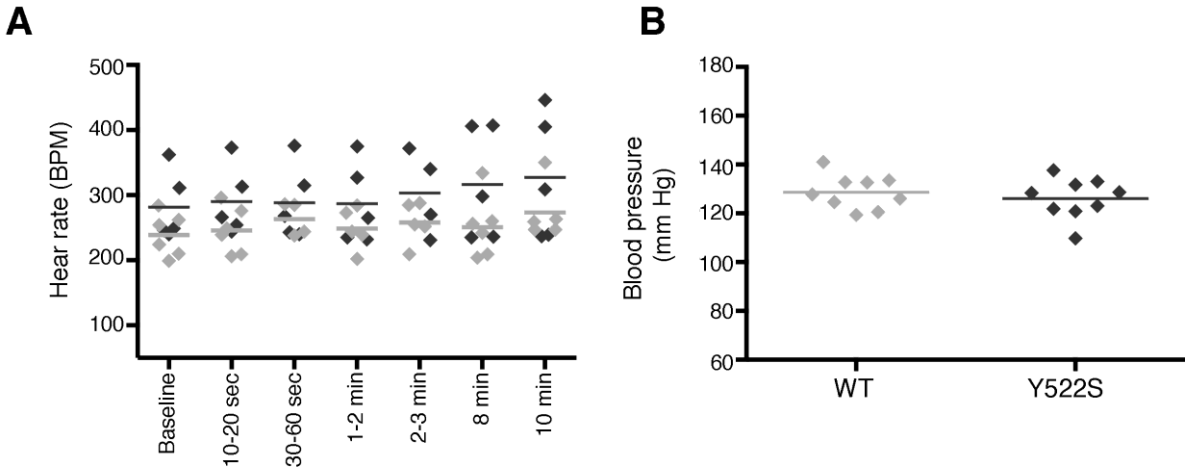
The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/9/435/ra68/DC1)

Video S5 (.avi format). Sparks in smooth muscle cells from $RYR1_{Y522S}$ mice after treatment with 250 nM xestospongin C.

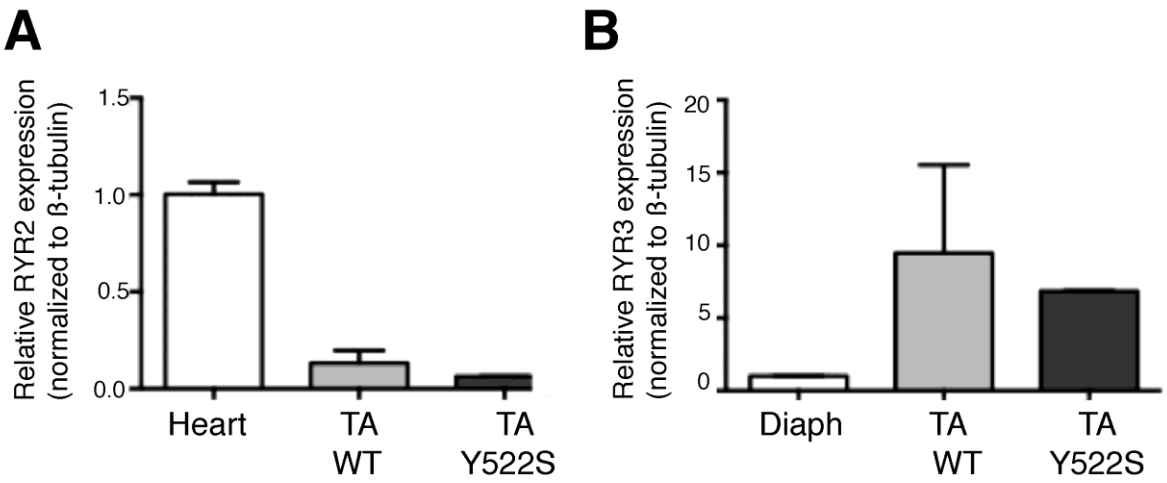


Supplementary Figure 1: RYR1$_{Y522S}$ female mice also exhibit prolonged bleeding times. Each symbol represents the bleeding time (in sec) of a single wild type (light grey) and RYR1$_{Y522S}$ (dark grey) female mouse. *P<0.005 Students *t*-test.

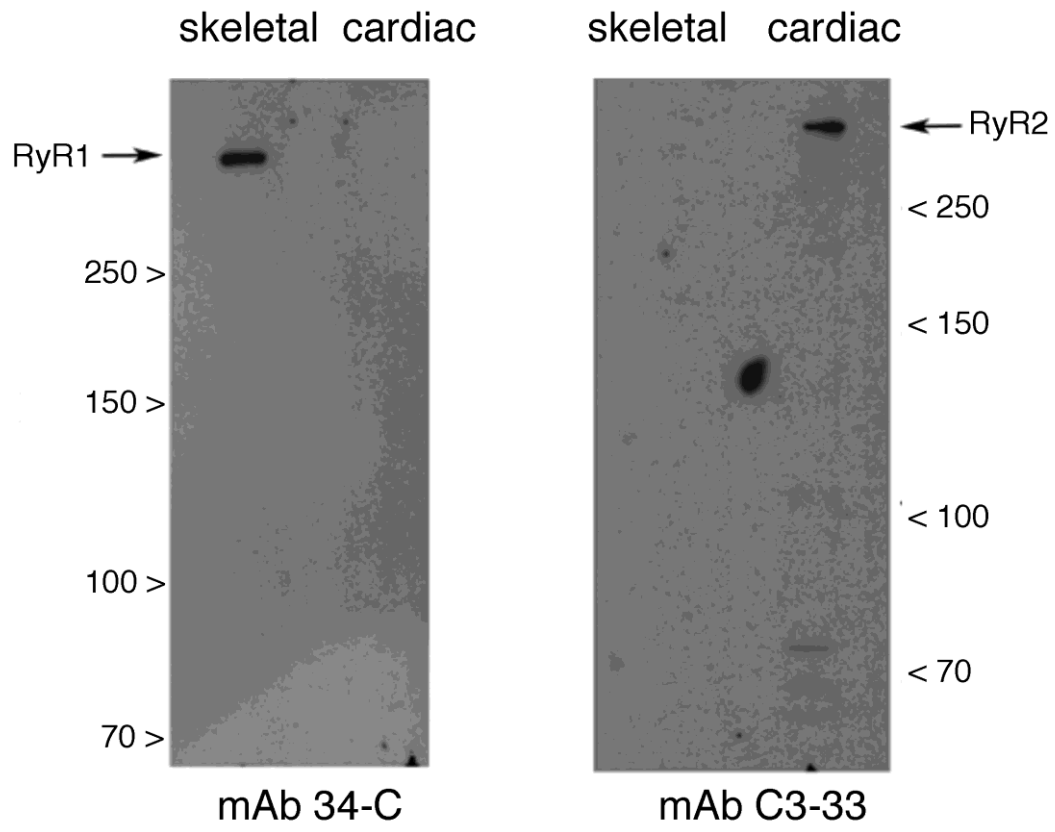


Supplementary Figure 2: Heart rate and systolic blood pressure are similar in wild-type and RYR1$_{Y522S}$ mice. A. Heart rates were obtained while performing Echo Doppler in lightly anesthetized animals at different time points after incision of the caudal artery. Each symbol represents a WT mouse (light grey) and RYR1$_{Y522S}$ mouse (dark grey). There was a trend for

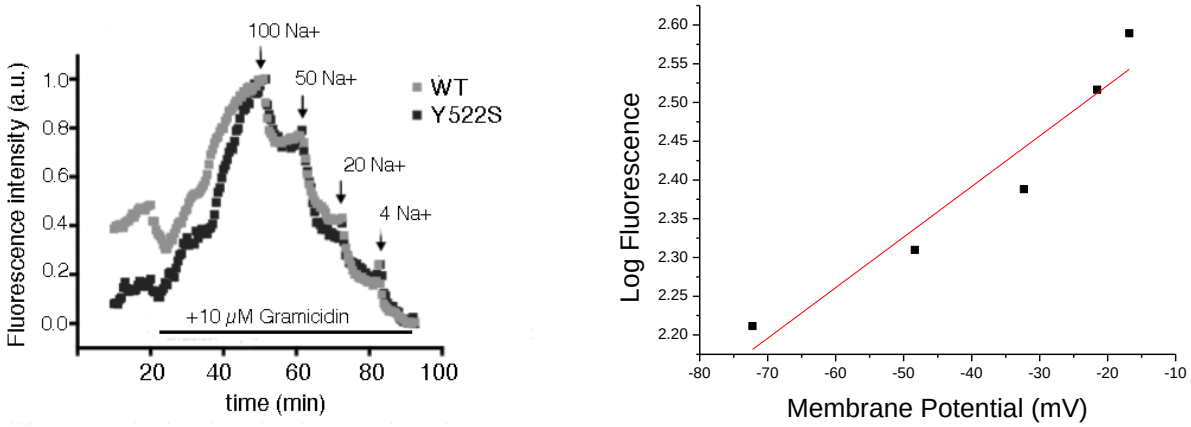
higher heart rates in the $RYR1_{Y522S}$ mice that did not reach statistical significance. **B.** Systolic blood pressure in non-anesthetized animals did not differ between WT (light grey) and $RYR1_{Y522S}$ (dark grey) mice.



Supplementary Figure 3: Expression of *RYR2* and *RYR3* is not significantly different in the tail arteries of wild-type and RYR1$_{Y522S}$ mice. Expression of *RYR2* and *RYR3* were determined by qPCR and was normalized to ß-tubulin. Results are the mean ± SEM of 3 replicates on tail arteries isolated from 4 different mice. Results are expressed as relative expression compared to *RYR2* expression in mouse heart (A) and *RYR3* expression in mouse diaphragm (B).



Supplementary Figure 4: Specificity of the anti-RyR1 antibodies used for immunohistochemistry. Thirty micrograms of sarcoplasmic reticulum proteins from skeletal muscle (left lane) or heart (right lane) were separated on a 6% SDS PAG and blotted onto nitrocellulose. Blots were blocked with 1% blocking buffer (Roche) in TBS for 60 min and probed with 1:5000 dilution monoclonal anti-RyR1 specific mAb34-C (left panel) or anti-RyR2 mAb C3-33 (right panel) for 60 min in TBST, rinsed 3 times with TBST, incubated with peroxidase-conjugate anti-mouse IgG (1:200,000 in TBST), rinsed 3 times and developed using the enhanced chemiluminescence kit from Thermo Scientific. As shown, the anti-RyR1 mAb does not cross react with cardiac RyR2 under the experimental conditions used in our laboratory. The blot on the right shows a positive control for cardiac RyR2. Representative of 2 independent experiments.



Supplementary Figure 5. **Membrane potential measurements using the fluorescence potentiometric probe bis-oxonol**. **Left:** Representative traces of the changes in fluorescence obtained in an arterial smooth muscle cell from WT mice (light symbols and heterozygous $RYR1_{Y522S}$ mice (dark symbols) expressed as Fluorescence Intensity arbitrary units (AU). The arrows indicted addition of Gramicidin or the indicated $[Na^+]$ in mM. **Right:** Values derived from the formula $Em = 60\ Log([Na^+]_o + [K^+]_o)/([Na^+]_i + [K^+]_i)$ with the best linear fitting regression (R^2=0.90; Y = 0.0065X + 2.6525) of the log of the fluorescence obtained at each $[Na^+]$.

Supplementary Table 1. Detailed analysis of full kinetic parameters of sparks in vascular smooth muscle cells from wild-type and RYR1$_{Y522S}$ mice.

	Spark Morphology							
	Amplitude (DF/F_0)	FWHM (μm)	FDHM (ms)	Full Wid (μm)	Full Duration (ms)	TtP (ms)	Tau (ms)	Freq (Sparks/image)
WT (N=877)	0.83±0.01	1.72±0.04	22.09±0.75	4.38±0.10	69.93±2.05	20.72±0.4	128.56±33.6	6.96±0.49
RyR1$_{Y52S}$ (N=1388)	0.79±0.0*	1.56±0.0*	19.52±0.6*	4.04±0.0*	59.32±1.3*	19.76±0.4	72.81±9.13	10.68±0.55*

- Values are expressed as mean ± SEM. * = P< 0.05 Student's *t*-test. Experiments were performed on 28 cells isolated from 6 WT and 6 RYR1$_{Y522S}$ mice respectively.
- †N indicates the number of individual sparks that were analyzed.

Supplementary Video 1: Sparks in smooth muscle cells from WT mice**.**

Supplementary Video 2: Sparks in smooth muscle cells from $RYR1_{Y522S}$ mice.

Supplementary Video 3: Sparks in smooth muscle cells from wild-type mice after treatment with 10 µM ryanodine.

Supplementary Video 4: Sparks in smooth muscle cells from $RYR1_{Y522S}$ mice after treatment with 20 µM dantrolene.

Supplementary Video 5: Sparks in smooth muscle cells from $RYR1_{Y522S}$ mice after treatment with 250 nM xestospongin C.