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Supplementary Materials for

Microtubule structures underlying the sarcoplasmic reticulum support peripheral coupling sites to regulate smooth muscle contractility

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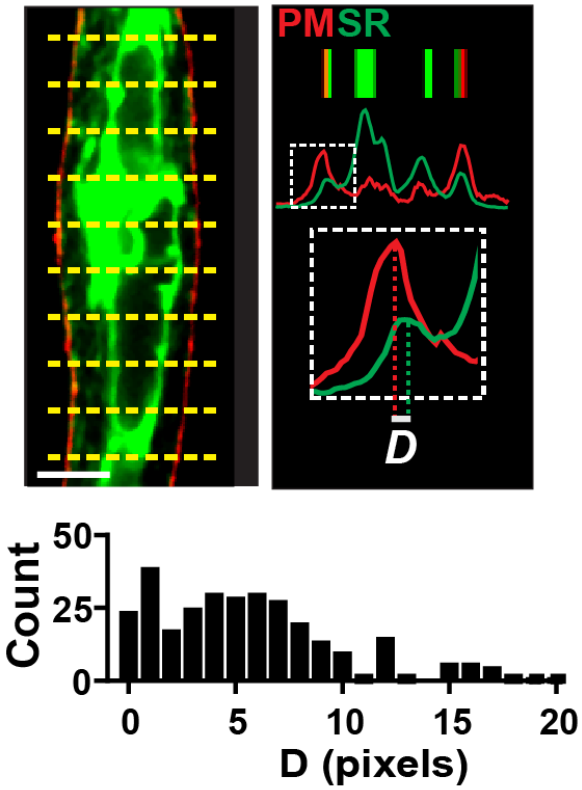
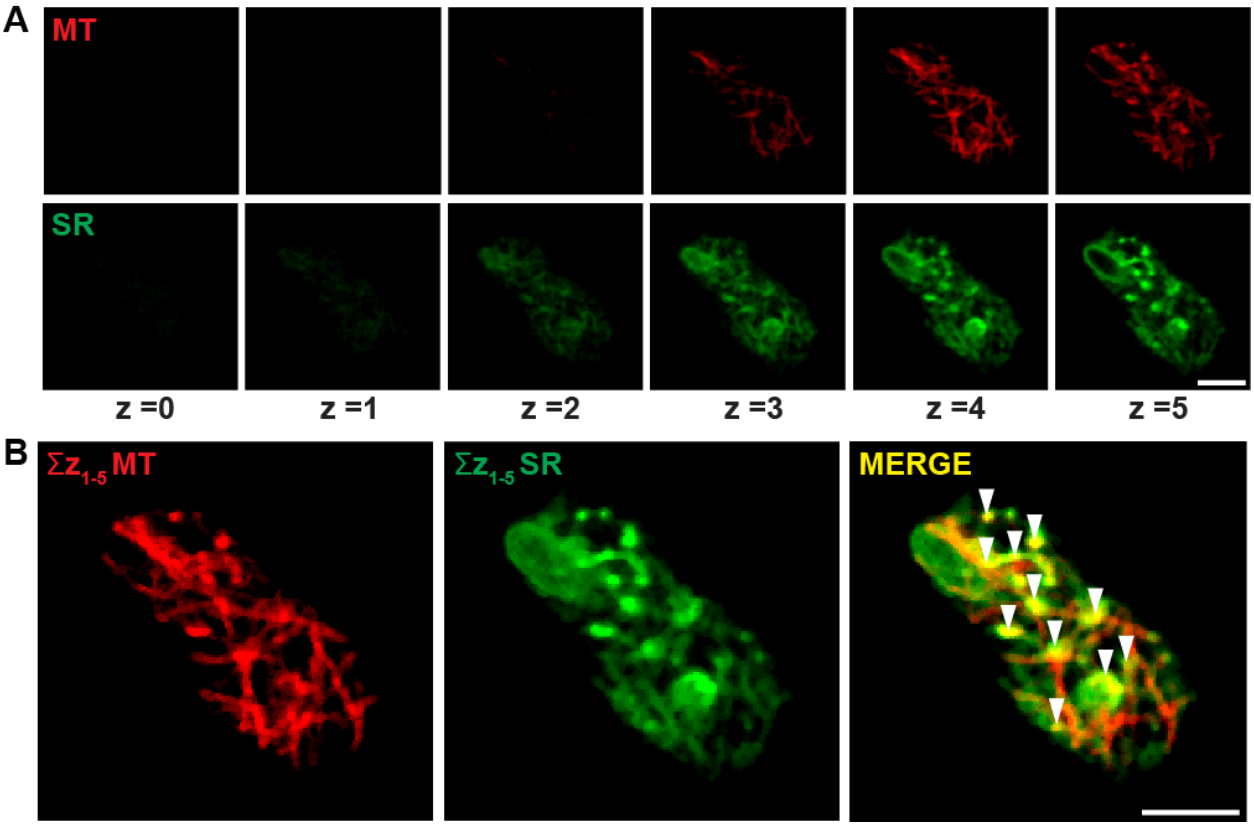
The PDF file includes:

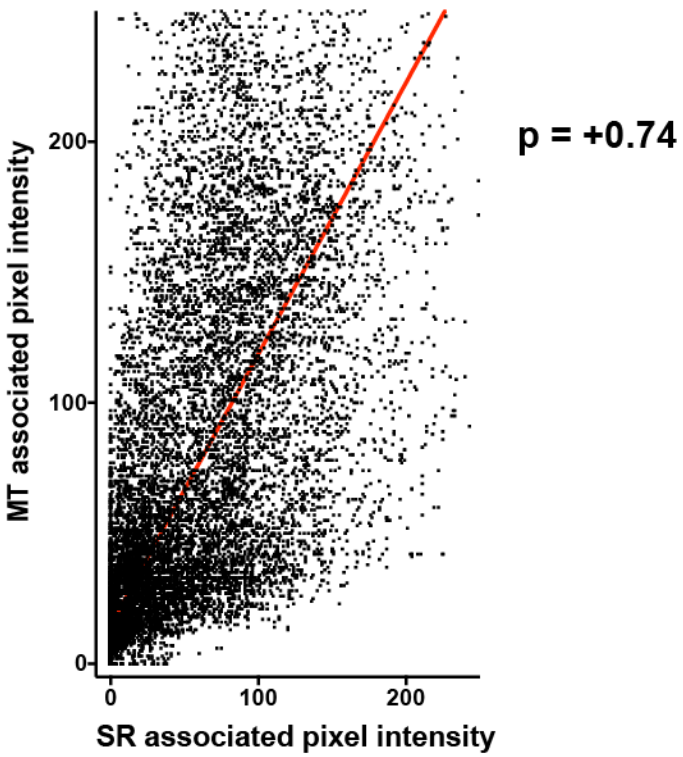
Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/10/497/eaan2694/DC1)



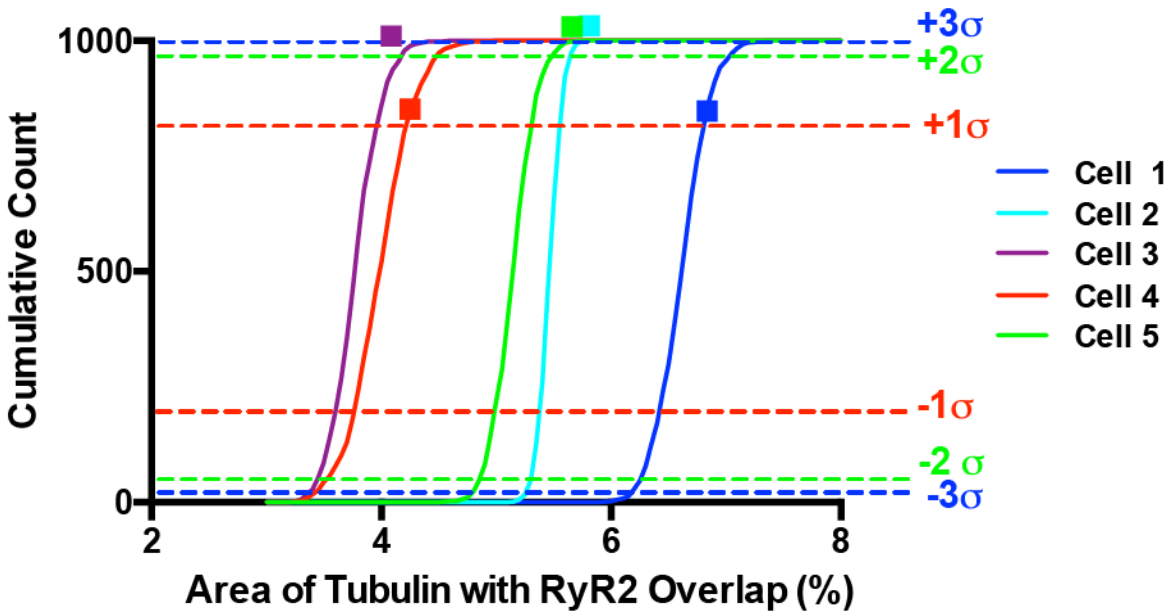
Supplemental Figure 1: Method used to estimate SR-PM separation distance. Left: Representative image of an isolated smooth muscle cell stained with ER-Tracker (green) to label the sarcoplasmic reticulum (SR) and Cell Mask Red (red) to label the plasma membrane (PM). Right: The SR-PM separation distance was estimated by plotting relative fluorescence intensity (F/F_0) for line segments drawn perpendicular to the long axis of the cell. Note that the peak fluorescence for the PM (red) is juxtaposed to the peripheral SR membrane (green). The distance between the peaks (D), determined by counting pixels, is representative of the SR-PM separation distance. The histogram shows the distribution of D values obtained for n = 10 cells from N= 5 animals. The mean separation distance under these conditions was 4.4 ± 0.2 pixels.



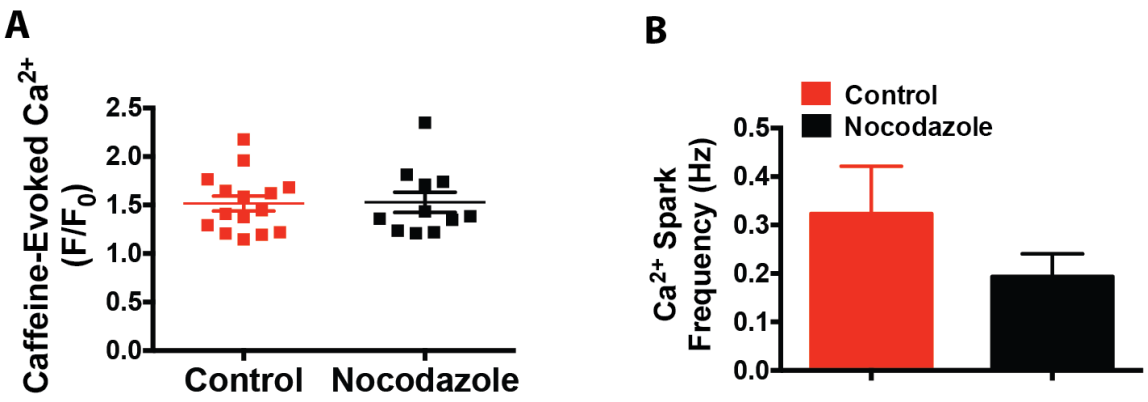
Supplemental Figure 2: *Z*-stack analysis of interactions between the SR and microtubule arches. A: Representative z-stack images of an isolated smooth muscle cell loaded with Tubulin-Tracker (red) and ER-Tracker (green) to stain microtubules (MT) and the SR, respectively (n = 8 cells, N = 3 animals). Confocal images were obtained at increments of 0.25 µm, starting at the bottom of the cell in contact with the glass coverslip (z = 0). B: Representative compressed (Σz1-5, 1.25 µm) z-stack images of the bottom of the isolated smooth muscle cell. White arrowheads indicate areas where microtubules are in contact with the SR proximal to the plasma membrane. Scale bars = 5 µm.



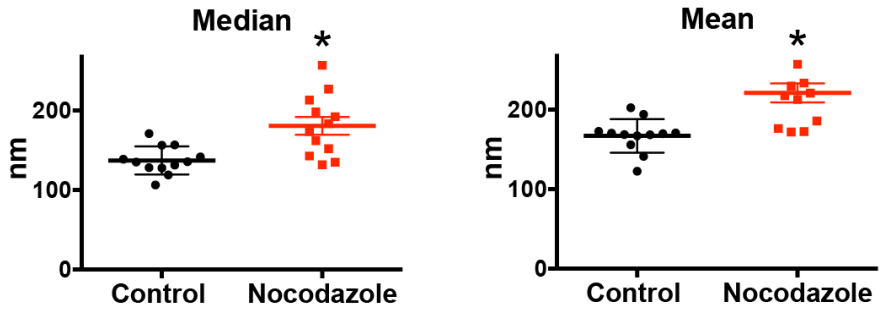
Supplemental Figure 3: Microtubules underlie the peripheral SR. Relationship between microtubule (MT)- and SR-associated fluorescence for each pixel for the images shown in Figure 2B. The red line is a plot of the linear regression of these data. The Pearson's coefficient (p) was +0.74.



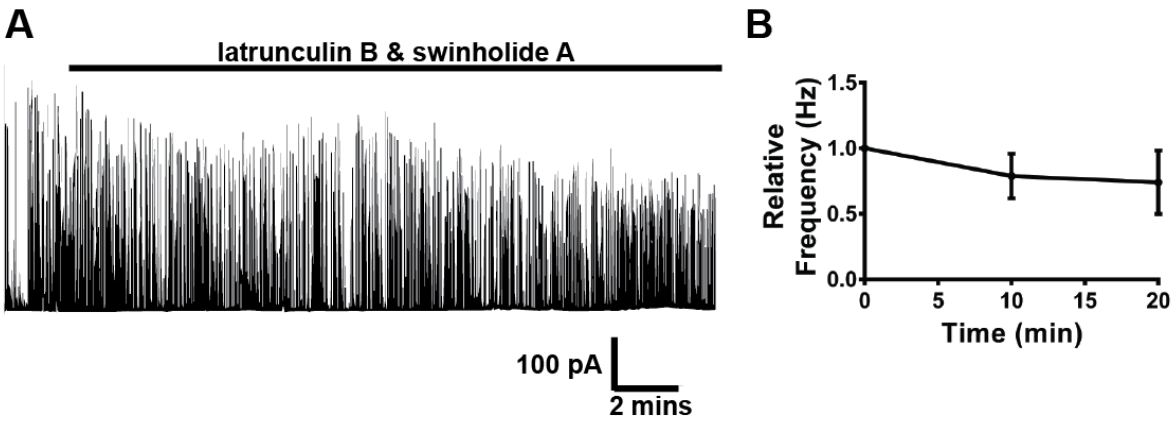
Supplemental Figure 4: Overlap of RyR2 channel clusters with tubulin. RyR2 channel cluster overlap with tubulin filaments was determined by measuring the percentage of the area of tubulin that contained overlapping RyR2 immunoreactivity, and vice versa. Whether the degree of overlap between RyR2 and tubulin was greater than random was determined by randomizing the position of each RyR2 cluster within a radius of 285 nm of its real position (creating 1000 random images) and measuring the degree of overlap. The cumulative histograms show the percent overlap of randomized RyR2 positions compared with tubulin for each cell (n = 5 cells, N = 3 animals).



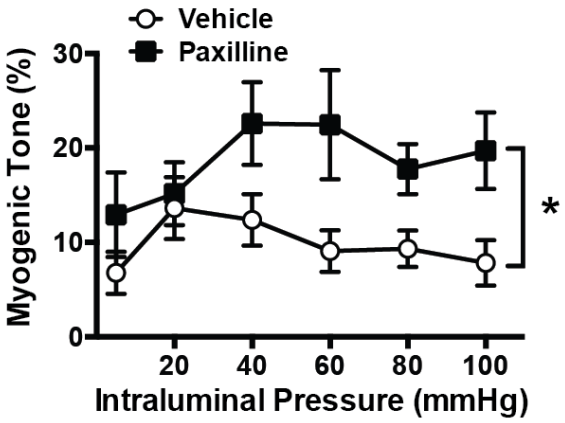
Supplemental Figure 5: Nocodazole treatment has no effect on total SR Ca^{2+} store load or Ca^{2+} spark frequency. A: Summary data indicating that fractional changes in global intracellular [Ca^{2+}] (F/F_0) following caffeine (5 mM) administration do not differ between control and nocodazole (10 μM)-treated cerebral arterial myocytes (n = 11–15 cells/group, N = 3 animals). B: Summary data indicating that Ca^{2+} spark frequency was not significantly altered following treatment with nocodazole (10 μM) (n= 5/cells/group, N= 3 animals).



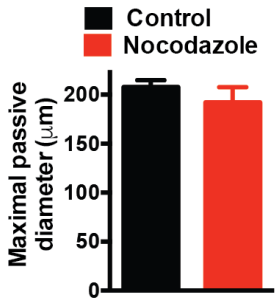
Supplemental Figure 6: BKα and RyR2 nearest neighbor analysis. The absolute positions of all BKα and RyR2 protein clusters in cerebral arterial myocytes imaged using GSDIM (Figure 5) were determined using the open-source software CellProfiler (version 2.0) (*50, 52*). Nearest-neighbor analysis was used to determine the distance between all BKα puncta centroids and their closest RyR2 centroid. Both the mean and median distance between nearest neighbors were greater for cells treated with nocodazole (10 μM) compared with controls (n = 12 cells, N = 3 animals, $*P \leq 0.05$).



Supplemental Figure 7. Actin disruption does not alter STOC frequency. (A) Representative data showing the effects of depolymerization of actin filaments with latrunculin B (1 μM) and swinholide A (0.1 μM) on STOCs. (B) Summary data showing relative STOC frequency (Hz) as a function of time when STOCs were recorded in the presence of latrunculin B and swinholide A for 20 min; V_H = -30 mV (n = 5 cells, N = 3 animals) There were no significant differences.



Supplemental Figure 8. Inhibition of BK channels increases myogenic tone. Summary data showing the effects of blockade of BK channels with paxilline (1 μM) on myogenic tone of cerebral arteries as a function of intraluminal pressure (n = 6 arteries, N = 3 animals; *$P \leq 0.05$).



Supplemental Figure 9. Effects of nocodazole on passive luminal diameter. Nocodazole has no effect on the maximal passive diameter of cerebral arteries bathed in Ca^{2+}-free PSS (intraluminal pressure = 100 mmHg; n = 5 arteries, N = 3 animals).

Supplemental Movie S1: Microtubule depolymerization increases the separation distance between the SR and the plasma membrane. Movie showing the SR (green) moving away from the plasma membrane (red) in response to depolymerization of microtubules by nocodazole (10 μM).

Supplemental Movie S2: Microtubule structure in freshly isolated smooth muscle cells. 3D reconstruction of a cerebral arterial myocyte stained with Tubulin Tracker (red) to label the microtubule network.

Supplemental Movie S3: SR structure in freshly isolated smooth muscle cells. 3D reconstruction of a cerebral arterial myocyte stained with a dye (green) that specifically labels the SR.