Sekulic-Jablanovic et al., http://www.jgp.org/cgi/content/full/jgp.201511542/DC1

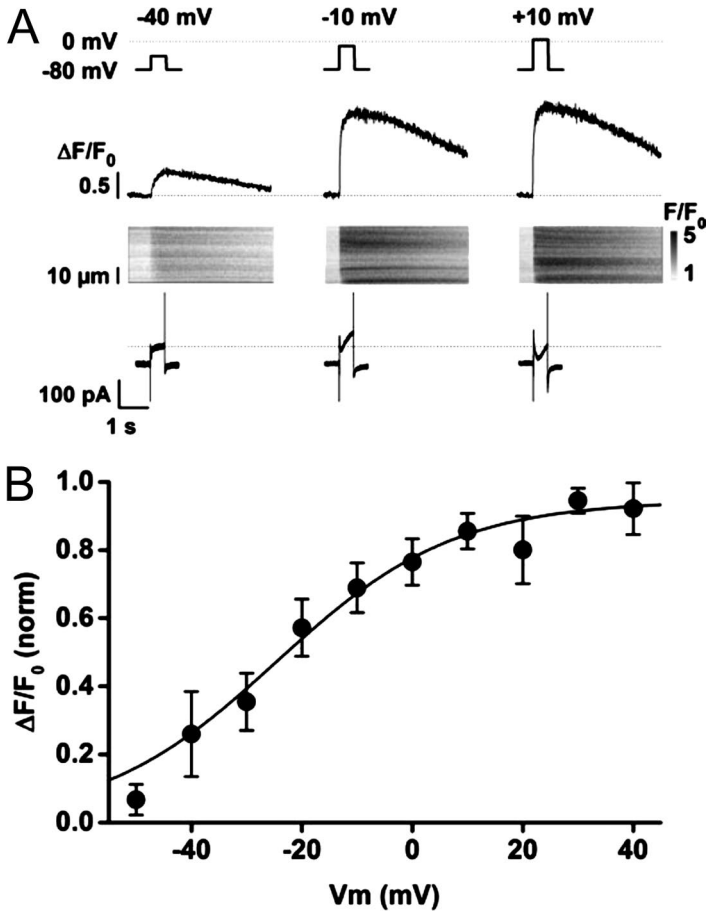

Figure S1. **Voltage dependence of Ca^{2+} release in orbicularis oculi–derived myotubes.** Orbicularis oculi–derived myotubes were patch clamped in the whole cell configuration and stimulated to different depolarizing potentials to elicit SR Ca^{2+} release as described in Materials and methods. (A) Three examples of stimulated Ca^{2+} current (lower traces), line scans imaged in the line scan mode using fluo-3 and corresponding line profiles at −40 mV, −10 mV, and 10 mV, respectively. With increasing membrane depolarization, the amplitude of the triggered Ca^{2+} transient increases. (B) The maximal Ca^{2+} transient amplitudes at each tested membrane potential are summarized. The ECC of orbicularis oculi myotubes demonstrates a V dependence of Ca^{2+} release similar to that observed in quadriceps myotubes (Rokach et al., 2013). At low depolarization, a slow rise in Ca^{2+} initiates and leads to a Ca^{2+} transient of small amplitude. With increasing depolarization, Ca^{2+} release becomes faster and larger, reaching peak amplitudes at about 30 mV. Data are normalized to the respective peak transient amplitude in each cell. The Ca^{2+}-voltage relationship was fitted with a Boltzmann function and derived a half-maximal activation potential of approximately −25 ± 5.3 mV (n = 5–-6 cells). Despite rapid coupling, the Ca^{2+} removal mechanisms after release appear to be very immature. As seen in the line scan images and previously reported in quadriceps-derived myotubes (Rokach et al., 2013), it takes several seconds for $[Ca^{2+}]_i$ to return to basal levels. Error bars indicate SEM.

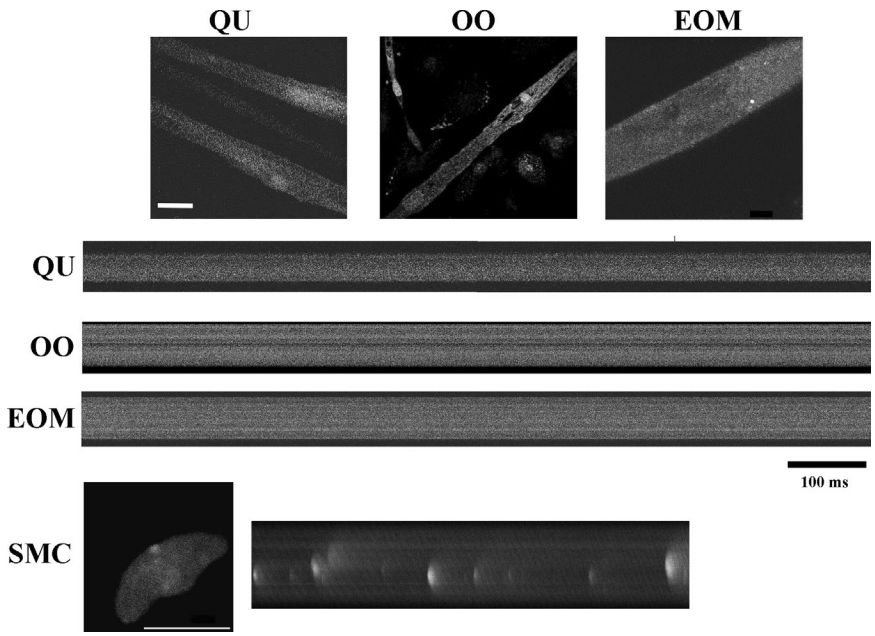

Figure S2. **Human myotubes do not show spontaneous Ca^{2+} release events (SPARKS).** (top) Representative photomicrographs of fluo-4–loaded myotubes. (middle) Representative 2.5-s line scan images. (bottom) Positive control showing that using similar settings sparks can be detected in a vascular smooth muscle cell (SMC). Bars, 20 µm. OO, orbicularis oculi; QU, quadriceps.

Table S1. **Sequence of primers used for qPCR**

Gene name	Primer sequence (5′ to 3′)
RYR1	F: GGACCTCTACGCCCTGTATC R: ATCTCCCGCCTTAGCCATTTT
RYR3	F: CAGTCCCTATCTGTCAGAGCC R: CATGGCCGTATAACAGGGTCC
CACNA1S	F: TTGCCTACGGCTTCTTATTCCA R: GTTCCAGAATCACGGTGAAGAC
CACNA1C	F: TGATTCCAACGCCACCAATTC R: GAGGAGTCCATAGGCGATTACT
SERCA1	F: GGTGCTGGCTGACGACAACT R: AAGAGCCAGCCACTGATGAG
CASQ1	F: CACCCAAGTCAGGGGTACAG R: GTGCCAGCACCTCATACTTCT
JSRP1	F: TGTCGCTCAACAAGTGCCTG R: GCCTGGGCCTCGAACTTAG
UTRN	F: TGAAGGATGTCATGTCGGACC R: GTTGAGGACGTTGACTTGGCT
DMD	F: GAGACACATGCGGCCTTTATG R: CTCTAACTCCTCAAATGGGTGC
ACTN2	F: GACATCGTGAACACCCCTAAAC R: CCGCAAAAGCGTGGTAGAA
MYH13	F: GGAGAGAATCGAGGCTCAAAATC R: GTCTGGATCATGCCTTTCACATA

Conditions were as described in Materials and methods. F, forward; R, reverse.

REFERENCE

Rokach, O., N.D. Ullrich, M. Rausch, V. Mouly, H. Zhou, F. Muntoni, F. Zorzato, and S. Treves. 2013. Establishment of a human skeletal muscle-derived cell line: biochemical, cellular and electrophysiological characterization. *Biochem. J.* 455:169–177. http://dx.doi.org/10.1042/BJ20130698