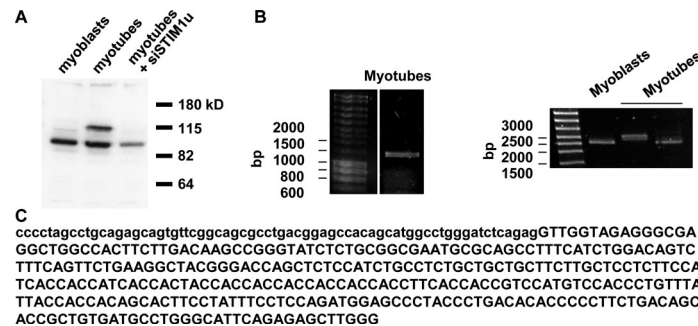
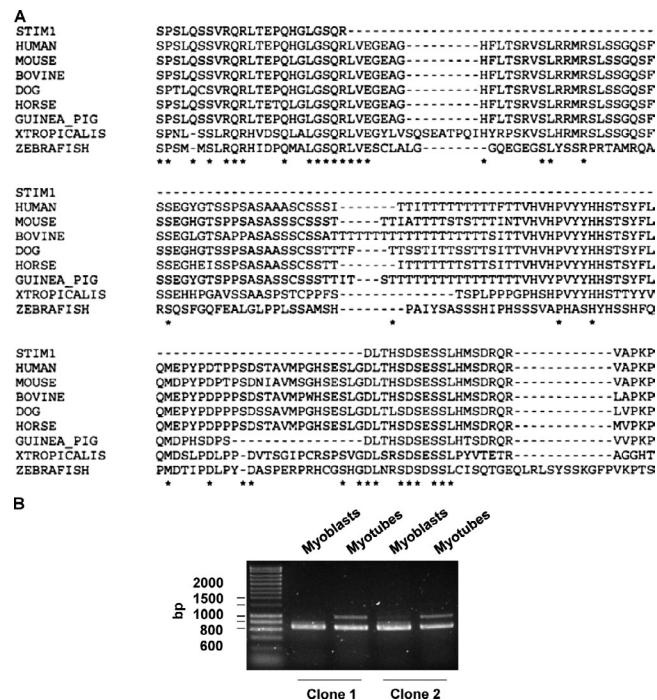


Darbellay et al., <http://www.jcb.org/cgi/content/full/jcb.201012157/DC1>

**Figure S1. STIM1 identification by Western blotting and RACE and the nucleotide sequence of the spliced exon.** (A) STIM1 expression assessed by Western blotting in proliferating myoblasts and in differentiated myotubes. C-terminal STIM1 antibody detected both STIM1 proteins. Transfection with si-STIM1u (see Materials and Methods) decreased the amounts of both proteins. (B) Identification of an alternative splicing of STIM1 mRNA by RACE. Products of the 3' and 5' RACE were removed from the gels and reamplified by nested PCR. (left) Only one band of the expected size (~1.5 kbp) was amplified from the 5' end of STIM1 mRNA obtained from myotubes. (right) 3' RACE of STIM1 mRNA was performed in proliferating myoblasts and in myotubes (120 h of differentiation). One band of the expected size of ~2.6 kbp was detected in myoblasts, whereas two bands appeared in myotubes (~2.6 and ~2.9 kbp). (C) Nucleotide sequence of human exon 11 of STIM1. The sequence added by alternative splicing is in capital letters. The STIM1 exon 11 is in lowercase letters.



**Figure S2. Sequence alignment of the putative alternative splicing domain of STIM1 in several species and confirmation of the presence of two STIM1 mRNAs in myotubes by RT-PCR.** (A) Multiple sequence alignment of the putative alternative splicing domain of STIM1 in several species on the basis of the genome sequence in silico analysis. The stars indicate conserved amino acids. Note the high degree of amino acid sequence conservation in mammals. Human, STIM1; STIM1, STIM1S. (B) Amplification of STIM1 cDNAs by RT-PCR using exon 10 forward and exon 12 reverse STIM1 primers. Total RNAs were extracted from proliferating myoblasts and myotubes after 120 h of differentiation. Only one band of ~800 bp was found in myoblasts, whereas two bands appeared in myotubes (~800 and ~1,100 bp). Two clones are shown.

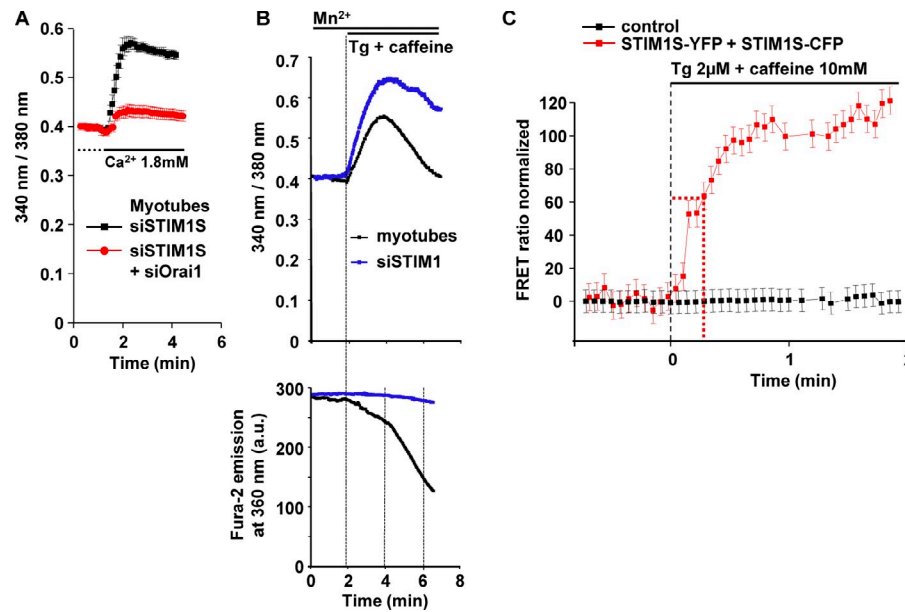


Figure S3. **STIM1L-related SOCE relies on Orai1.** (A) Cytoplasmic  $\text{Ca}^{2+}$  assessed with Fura-2 in myotubes 3 d after transfections with either siSTIM1S or siSTIM1S + siOrai1.  $\text{Ca}^{2+}$  stores were depleted with 2  $\mu\text{M}$  Tg and 10  $\mu\text{M}$  caffeine in 250 nM of external  $\text{Ca}^{2+}$  and 1.8 mM of external  $\text{Ca}^{2+}$  added to reveal SOCE (mean  $\pm$  SEM;  $n = 3$  experiments). (B, top) Cytoplasmic  $\text{Ca}^{2+}$  assessed with Fura-2 in control myotubes and in myotubes 3 d after transfections with siSTIM1.  $\text{Ca}^{2+}$  stores were depleted with 2  $\mu\text{M}$  Tg and 10  $\mu\text{M}$  caffeine in 1.8 mM of external  $\text{Ca}^{2+}$  and 0.5 mM of external  $\text{Mn}^{2+}$ . (bottom) Fura-2 fluorescence at 360 nm in 0.5 mM  $\text{Mn}^{2+}$  (to quench fluorescence). Cytoplasmic  $\text{Ca}^{2+}$  and Fura-2 fluorescence quench were measured simultaneously.  $n = 3$  experiments. a.u., arbitrary unit. (C) FRET increase between STIM1S-CFP and -YFP (red) after the application of 2  $\mu\text{M}$  Tg and 10 mM caffeine (mean  $\pm$  SD; mean of three independent experiments). FRET before Tg and caffeine application was set to 0 and maximum FRET to 100%. The black squares represent FRET signals in myoblasts expressing STIM1S-CFP and -YFP after application of DMSO (control). The dotted red line shows the time required to reach 50% of maximal FRET increase ( $20 \pm 3$  s). The vertical dashed line represents the application of Tg and caffeine at time 0.

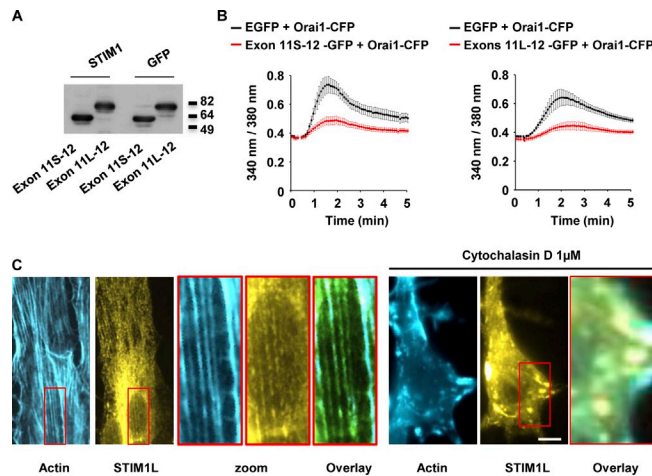


Figure S4. **STIM1L-GFP distribution relies on actin fibers.** (A) Expression of the GFP-tagged cytosolic last two exons (11 and 12) of STIM1S and -L assessed by Western blots 24 h after transfection by electroporation. STIM1: anti-STIM1 C-terminal antibody, 1:2,000; YFP: anti-YFP antibody, 1:2,000. Molecular mass is indicated in kilodaltons. (B) Cytoplasmic  $\text{Ca}^{2+}$  assessed with Fura-2 in myoblasts 2 d after transfection with the indicated plasmids.  $\text{Ca}^{2+}$  stores were depleted with 2  $\mu\text{M}$  Tg in 250 nM of external  $\text{Ca}^{2+}$  and 1.8 mM of external  $\text{Ca}^{2+}$  subsequently added to reveal SOCE (mean  $\pm$  SEM; one representative experiment out of three). (C) Distribution and colocalization of STIM1L-GFP and actin (detected by phalloidin-TRITC) in myoblasts. Myoblasts were electroporated with STIM1L-GFP and then cultured in proliferation medium for 24 h. Actin and GFP distribution were observed in fixed cells. 1  $\mu\text{M}$  cytochalasin D was applied to disrupt the actin fibers. Red rectangles show enlarged images. Bar, 20  $\mu\text{m}$ .

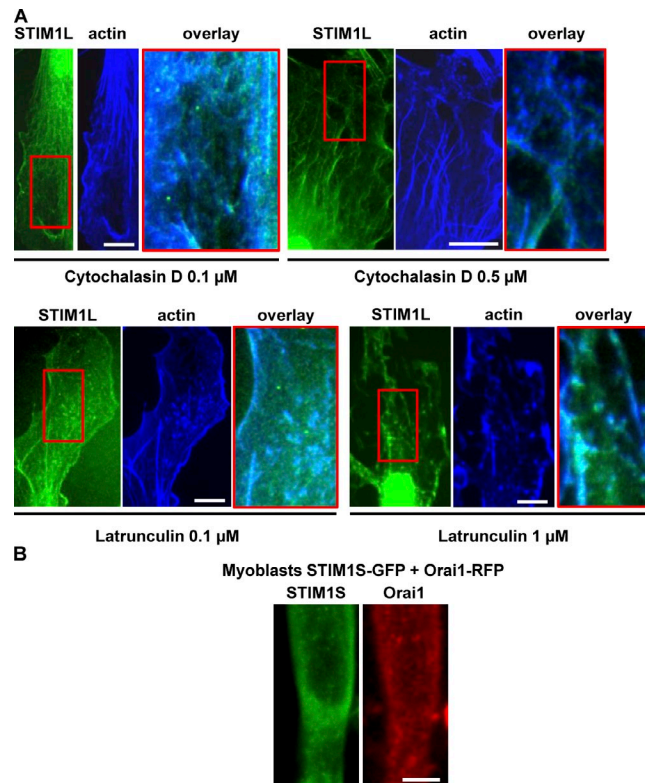


Figure S5. **Effects of cytochalasin D and latrunculin on STIM1L distribution and the distribution of STIM1S-GFP and Orai1-RFP in store-filled myoblasts.** (A) Distribution and colocalization of STIM1L-GFP and actin (detected by phalloidin-Atto 390) in myoblasts. Cells were electroporated with STIM1L-GFP and then kept in proliferation medium for 24 h. Actin and STIM1L distribution were observed in fixed cells. 0.1 and 0.5  $\mu$ M cytochalasin D and 0.1 and 1  $\mu$ M latrunculin B were applied to disrupt the actin fibers. Red rectangles show enlarged images. Bars, 20  $\mu$ m. (B) Predepleted myoblasts 24 h after electroporation with STIM1S-GFP and Orai1-RFP. Bar, 15  $\mu$ m.