Figures and figure supplements

Distinct mechanisms regulating mechanical force-induced Ca\textsuperscript{2+} signals at the plasma membrane and the ER in human MSCs

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Figure 1. Intracellular Ca\(^{2+}\) oscillations in response to mechanical force in HMSCs with Ca\(^{2+}\)-free medium. (A) A schematic drawing of the activation mechanism of the Ca\(^{2+}\) FRET biosensor. (B) Beads coated with Fn or BSA were seeded onto the cell and mechanical force was applied by pulling a Fn-coated bead using optical laser tweezers. Both Ca\(^{2+}\) influx and ER Ca\(^{2+}\) release can contribute to force-induced Ca\(^{2+}\) signals. (C) Color images represent the YPet/ECFP emission ratio of the cytoplasmic Ca\(^{2+}\) biosensor. The color scale bars represent the range of emission ratio, with cold and hot colors indicating low and high levels of Ca\(^{2+}\) concentration, respectively. (D) The time courses represent the YPet/ECFP emission ratio averaged over the cell body outside of nucleus upon seeding of Fn or BSA-coated beads and force application. (E–F) Bar graphs represent the frequency or ratio of the intracellular Ca\(^{2+}\) oscillations evoked by mechanical force. Error bars indicate standard error of mean; *p < 0.05, n = 14. (Scale bar: 10 µm). DOI: 10.7554/eLife.04876.003
Figure 1—figure supplement 1. Laser-tweezer pulling of a Fn-coated bead on a BAEC in Ca^{2+}-free medium. Color images (upper panels) represent the YPet/ECFP emission ratio of the cytoplasmic Ca^{2+} biosensor. The color scale bars represent the range of emission ratio, with cold and hot colors indicating low and high levels of Ca^{2+} concentration, respectively. The time courses of the YPet/ECFP emission ratio averaged over the cell body outside of nucleus is shown in the lower graph.

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Figure 1—figure supplement 2. Mechanical force doesn't induce any increase in IP₃ level. The time courses represent the YPet/ECFP emission ratio of cytoplasmic Ca²⁺ in HMSCs pretreated with (A) Thapsigargin (TG, 10 µM), a SERCA pump blocker, or (B) 2-APB (100 µM), an IP₃R blocker. (C) IP₃ production is monitored by a FRET-based IP₃ biosensor, LIBRAvIIIs. ATP treatment induces IP₃ increase, which can be clearly detected by an IP₃ biosensor LIBRAvIIIs (upper panels). However, laser-tweezer pulling of a Fn-coated bead to produce the mechanical force did not cause any increase in IP₃ (lower panels).

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Figure 2. Cytoskeletal support, actomyosin contractility, and TRPM7 channels mediate the force-induced intracellular Ca$$^{2+}$$ oscillations. The time courses represent the YPet/ECFP emission ratio of cytoplasmic Ca$$^{2+}$$ in HMSCs in the absence of extracellular Ca$$^{2+}$$ when these cells were pretreated with (A) 2 μM Cyto D (n = 8), (B) 1 μM Noc (n = 8), (C) 5 μM ML-7 (n = 8), and (D) 5 μM Bleb (n = 8). (E) Color images represent the YPet/ECFP emission ratio of the cytoplasmic Ca$$^{2+}$$ biosensor in HMSCs transfected with NT or TRPM7 siRNA. The color scale bars represent the range of emission ratio, with cold and hot colors indicating low and high levels of Ca$$^{2+}$$ concentration, respectively. (F) The time courses represent the YPet/ECFP emission ratio averaged over the cell bodies outside of nucleus treated with siRNA as indicated.

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Figure 2—figure supplement 1. Stretch-activated or store-operated channels at the plasma membrane mediate force-induced Ca\(^{2+}\) release from ER. The time courses represent the YPet/ECFP emission ratio of cytoplasmic Ca\(^{2+}\) in HMSCs pretreated with (A) GdCl\(_3\) (5 µM), a broad spectrum Ca\(^{2+}\) channel inhibitor, (B) LaCl\(_3\) (100 µM), a broad spectrum Ca\(^{2+}\) channel blocker, (C) streptomycin (200 µM), a mechanosensitive channel inhibitor, or (D) Nifedipine (10 µM), an L-type Ca\(^{2+}\) channel inhibitor, all in the absence of extracellular Ca\(^{2+}\).

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Figure 2—figure supplement 2. TRPM7 channels as well as cytoskeletal support and actomyosin contractility mediate the force-induced intracellular Ca\(^{2+}\) oscillations. (A) HMSCs expressing Ca\(^{2+}\) biosensor and transfected with non-targeting (NT) or TRPM7 siRNA were immunostained (upper images) or immunoblotted (lower panels) with polyclonal TRPM7 antibody to assess the amount of TRPM7. TRPM7 specific siRNA induced knockdown of TRPM7. (B) Bar graphs represent the percentile of HMSCs showing intracellular Ca\(^{2+}\) oscillations. Three groups of cells (1: Ca\(^{2+}\) biosensor only, 2: biosensor and non-targeting (NT) siRNA, and 3: biosensor and TRPM7 siRNA) were measured and compared. The number of cells displaying Ca\(^{2+}\) oscillations in both control group (55%, 11 of 20 cells) and NT-siRNA group (45%, 9 of 20 cells) was approximately 9–11 fold higher than that of TRPM7 siRNA group (4.76%, 1 of 21 cells). (C–D) Bar graphs represent the frequency or ratio of the intracellular Ca\(^{2+}\) oscillations induced by mechanical force in the presence of different inhibitors. Error bars indicate standard error of mean; *p < 0.05. DOI: 10.7554/eLife.04876.009
Figure 3. The visualization of force-induced Ca\textsuperscript{2+} release from ER using a FRET-based ER Ca\textsuperscript{2+} biosensor. (A) The time course and (B) the color images of YPet/ECFP emission ratio in HMSCs expressing the D3ER biosensor before and after force application. The red arrows indicated episodes of Ca\textsuperscript{2+} release from ER. (C) The bar graphs represent the normalized changes of YPet/ECFP emission ratio of the D3ER in HMSCs upon force application without extracellular Ca\textsuperscript{2+} in the untreated cells as the control group (n = 3) or cells pretreated with CytoD (n = 5), Noc (n = 5), ML-7 (n = 6), Bleb (n = 5), or TRPM7 siRNA (n = 9) as indicated. * represents p < 0.05. DOI: 10.7554/eLife.04876.010
Figure 4. Ca\textsuperscript{2+} influx in response to mechanical force in Ca\textsuperscript{2+}-containing medium. Ca\textsuperscript{2+} release from ER in all the HMSCs was blocked by pretreatment with 2-APB. (A, C) Color images represent the YPet/ECFP emission ratio of the cytoplasmic Ca\textsuperscript{2+} biosensor in control cells treated by 2-APB only (n = 5) or those co-treated by Nifedipine (n = 5), Gd\textsuperscript{3+} (n = 3), La\textsuperscript{3+} (n = 6), STM (n = 8), or TRPM7 siRNA (n = 9), CytoD (n = 8), Noc (n = 4), ML-7 (n = 9) or blebbistatin (n = 6). Arrows in DIC images point to the direction of applied force. (B, D) Bar graphs represent the normalized change of YPet/ECFP emission ratio of the cytoplasmic Ca\textsuperscript{2+} biosensor under different conditions as indicated in (A, C). Error bars indicate standard errors of mean; * represents p < 0.05. (E) The models depicting the mediators of mechanical force-induced Ca\textsuperscript{2+} influx or ER Ca\textsuperscript{2+} release.

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Figure 4—figure supplement 1. Src, FAK or PI3K has no effect on the mechanical force-induced Ca\(^{2+}\) signals. (A) The inhibition of neither Src by PP1 (n = 4), FAK by PF228 (n = 4), nor PI3K by LY294002 (n = 3) abolished the force-induced cytosolic Ca\(^{2+}\) oscillations in HMSCs without extracellular Ca\(^{2+}\). (B) The Ca\(^{2+}\) release from ER in all the HMSCs was blocked by pretreatment with 2-APB. Color images represent the YPet/ECFP emission ratio of the cytoplasmic Ca\(^{2+}\) biosensor in cells pretreated by 2-APB together with PP1 (n = 5), PF228 (n = 4), or LY294002 (n = 6). Arrows in DIC images point to the direction of applied force. Bar graphs represent the normalized change of YPet/ECFP emission ratio of the cytoplasmic Ca\(^{2+}\) biosensor under different conditions as indicated in (A). (C) The average delay time between force application and the first Ca\(^{2+}\) signals in three groups. First two groups were ER Ca\(^{2+}\) release monitored by either cytoplasmic or ER Ca\(^{2+}\) biosensor. The last group was Ca\(^{2+}\) influx in the presence of 2-APB monitored by cytoplasmic Ca\(^{2+}\) biosensor. (***, p < 0.001) Error bars indicate standard errors of mean.

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