Sun et al., http://www.jcb.org/cgi/content/full/jcb.201407082/DC1

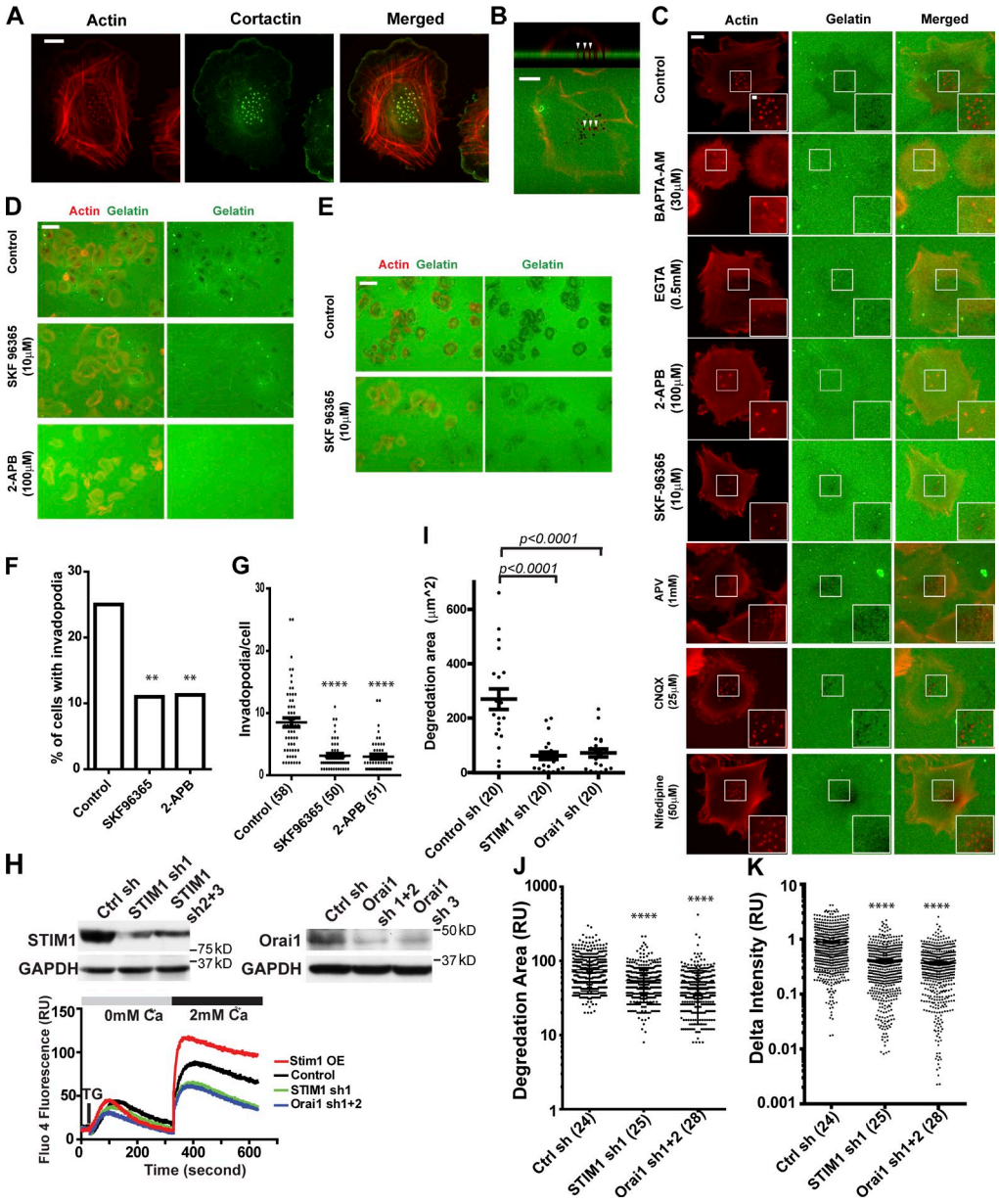


Figure S1. **Effects of Ca^{2+} influx blockade on invadopodium formation and ECM degradation.** Corresponds to Fig. 1. (A) Actin (red) and cortactin (green) staining revealed invadopodia (actin- and cortactin-positive dots) on the ventral side of WM793 cells. Bar, 10 µm. (B) Confocal microscopy revealed that invadopodia (arrowheads, Alexa Fluor 594–phalloidin staining) degraded Alexa Fluor 488 gelatin and protruded into the gelatin film (green). Orthogonal view of the confocal z stack is shown above the main image. Bar, 10 µm. (C) Representative images showing that 30 µM BAPTA-AM, 0.5 mM EGTA, 100 µM 2-APB, and 10 µM SKF96365 inhibited the formation of invadopodia and degradation of Alexa Fluor 488–labeled gelatin by WM793 melanoma cells. Treatment of WM793 cells with APV (NMDA inhibitor), CNQX (AMPA receptor inhibitor), and nifedipine (L-type voltage-gated channel inhibitor) had no significant effects on invadopodium formation and ECM degradation. Insets are magnified views of the boxed areas in the main images. Bars: (main images) 10 µm; (insets) 2 µm. (D) SOCE blockers SKF96365 and 2-APB robustly inhibited ECM degradation by WM245 cells. Gelatin degradation was initiated by removing GM6001 and stopped 4 h later with 4% paraformaldehyde fixation. Bar, 50 µm. (E) SKF96365 blocked ECM degradation by CHL-1 cells. Cells were fixed 2 h after removing GM6001. (F and G) Effects of SOCE blockers on invadopodium formation in WM 245 cells. (F) Treatment of SKF96365 and 2-APB decreased the proportion of WM245 cells with invadopodia from 25% in control group (95% confidence interval [CI] = 19.3–31.7%, n = 184) to 11.6% (95% CI = 7.7–17.8%, n = 172) and 11.9% (95% CI = 8.1–17.3, n = 192), respectively. **, $P < 0.01$, as determined by two-tailed Fisher's exact test. (G) SKF96365 and 2-APB treatments decreased the mean numbers of invadopodia in invadopodia-positive WM245 cells. Data presented are means ± SEM. ****, $P < 0.0001$, as determined by two-tailed Mann–Whitney test. (H, top) Western blotting showing knockdown of STIM1 and Orai1 by shRNA. (bottom) Effects of STIM1 knockdown, Orai1 knockdown, and STIM1 overexpression (STIM1 OE) on thapsigargin (TG)-induced SOCE. The data shown are representative of three repeats. (I) Effects of STIM1 and Orai1 knockdown on gelatin degradation by WM 793 cells (means ± SEM). (J and K) Degradation area per invadopodium (J) and ΔIntensity per invadopodium (K) used to calculate IDX in Fig. 1 F. The numbers of cells used for quantitation were indicated in the parenthesis of respective figure labeling, and representative results from at least three similar independent experiments were presented. Data presented are means ± SEM. RU, relative unit; Ctrl sh, control shRNA.

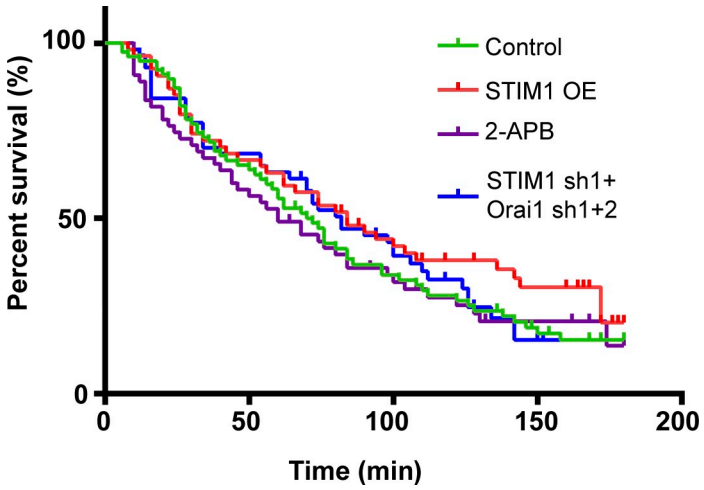


Figure S2. **The effect of STIM1 overexpression, 2-APB treatment, and STIM1 and Orai1 knockdown on invadopodium lifetime.** FBS-induced invadopodium formation in WM793 cells was recorded through live cell imaging. The effects of STIM1 overexpression, 2-APB treatment, and STIM1 and Orai1 knockdown on invadopodium lifetime were not statistically significant (Kaplan–Meier survival analysis). OE, overexpression.

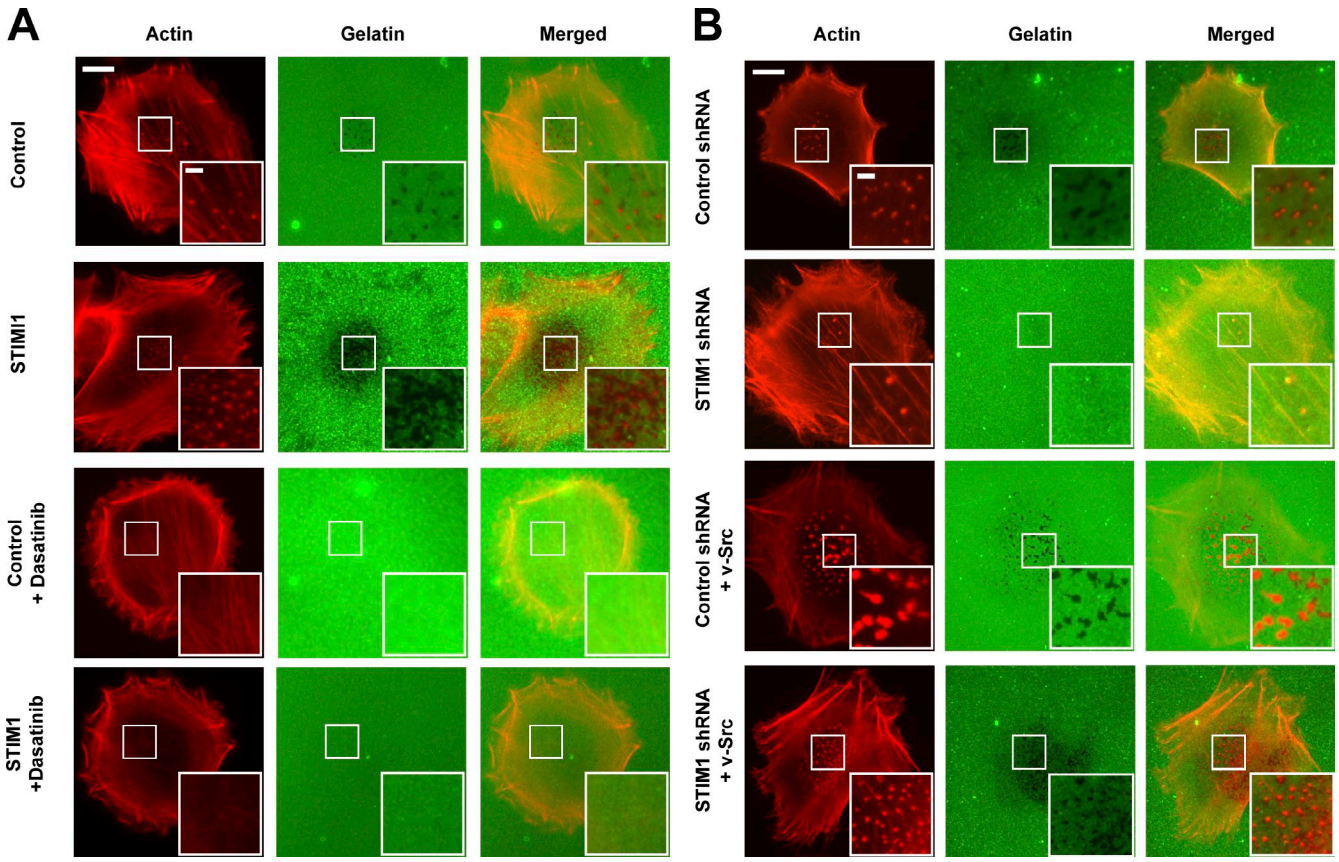
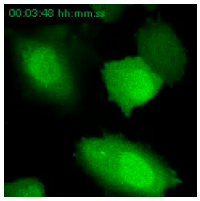
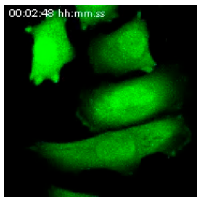


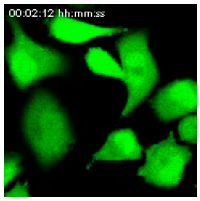
Figure S3. **The role of Src in SOCE-mediated invadopodium formation.** (A) Representative images showing inhibition of Src activity with 2.5 nM dasatinib abolished STIM1-mediated invadopodia formation. (B) Representative images showing that ectopic expression of constitutively active v-Src rescued the inhibition of invadopodia formation by STIM1 shRNA. Insets are magnified regions of the boxed areas. Bars: (main images) 10 µm; (insets) 2 µm.



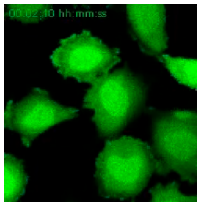
Video 1. **Ca^{2+} oscillation in WM793 cells stimulated by addition of 10% FBS.** WM793 cells loaded with Fluo4-AM were analyzed for FBS-stimulated calcium signaling using time-lapse confocal microscopy (LSM 710; Carl Zeiss). Frames were taken every 2 s for 4,000 s.



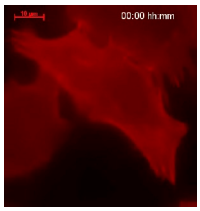
Video 2. **Chelating of extracellular Ca^{2+} with EGTA abrogated Ca^{2+} oscillations in WM793 cells.** WM793 cells loaded with Fluo4-AM were analyzed for FBS-stimulated calcium signaling in the absence of extracellular Ca^{2+} (buffered with 2 mM EGTA) using time-lapse confocal microscopy (LSM 710; Carl Zeiss). Frames were taken every 2 s for 4,000 s.



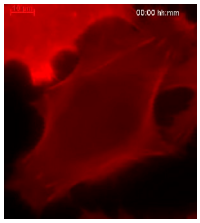
Video 3. **Adding back extracellular Ca^{2+} (at 30 min after FBS stimulation) restored oscillation in WM793 cells.** WM793 cells loaded with Fluo4-AM were analyzed for FBS-stimulated calcium signaling in the absence of extracellular Ca^{2+} (buffered with 2 mM EGTA) using time-lapse confocal microscopy (LSM 710; Carl Zeiss). The extracellular Ca^{2+} was restored to 2 mM at 30 min after FBS stimulation. Frames were taken every 2 s for 4,000 s.



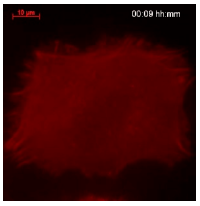
Video 4. **Inhibition of Ca^{2+} oscillation in WM793 cells by SOCE blocker SKF96365.** WM793 cells loaded with Fluo4-AM were analyzed for FBS-stimulated calcium signaling in the in the presence of SOCE blocker SKF96365 using time-lapse confocal microscopy (LSM 710; Carl Zeiss). Frames were taken every 2 s for 4,000 s.



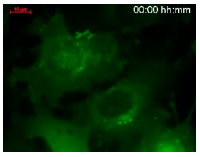
Video 5. **Invadopodium precursor assembly in WM793 cells stimulated by 10% FBS.** WM793 cells stably expressing Lifeact-mAPPLE were used for the imaging experiment. Cells were starved overnight in RPMI 1640 medium containing 1% FBS. Invadopodium precursor assembly was stimulated by directly adding 10% FBS to the medium. Frames were taken every 0.5 min for 60 min.



Video 6. **Abrogation of Ca^{2+} oscillation with EGTA inhibited invadopodium precursor assembly.** WM793 cells stably expressing Lifeact-mAPPLE were used for the imaging experiment. Cells were starved overnight in RPMI 1640 medium containing 1% FBS. The extracellular Ca^{2+} was chelated with EGTA. Invadopodium precursor assembly was stimulated by directly adding 10% FBS to the medium. Frames were taken every 0.5 min for 60 min.



Video 7. **Abrogation of Ca^{2+} oscillation with SKF96365 inhibited invadopodium precursor assembly.** WM793 cells stably expressing Lifeact-mAPPLE were used for the imaging experiment. Cells were starved overnight in RPMI 1640 medium containing 1% FBS. Invadopodium precursor assembly was stimulated by directly adding 10% FBS to the medium in the presence of SOCE blocker SKF96365. Frames were taken every 0.5 min for 60 min.



Video 8. **Treatment with SOCE blocker 2-APB induced translocation of MT1-MMP from plasma membrane to intracellular vesicles.** WM793 cells stably expressing MT1-MMP–EGFP were used for the imaging experiment. Cells were treated with 100 µM 2-APB, and the effect of MT1-MMP–EGFP subcellular localization was recorded for 3 h (1.5-min interval between frames).