Soto Hinojosa et al., https://doi.org/10.1083/jcb.201702010

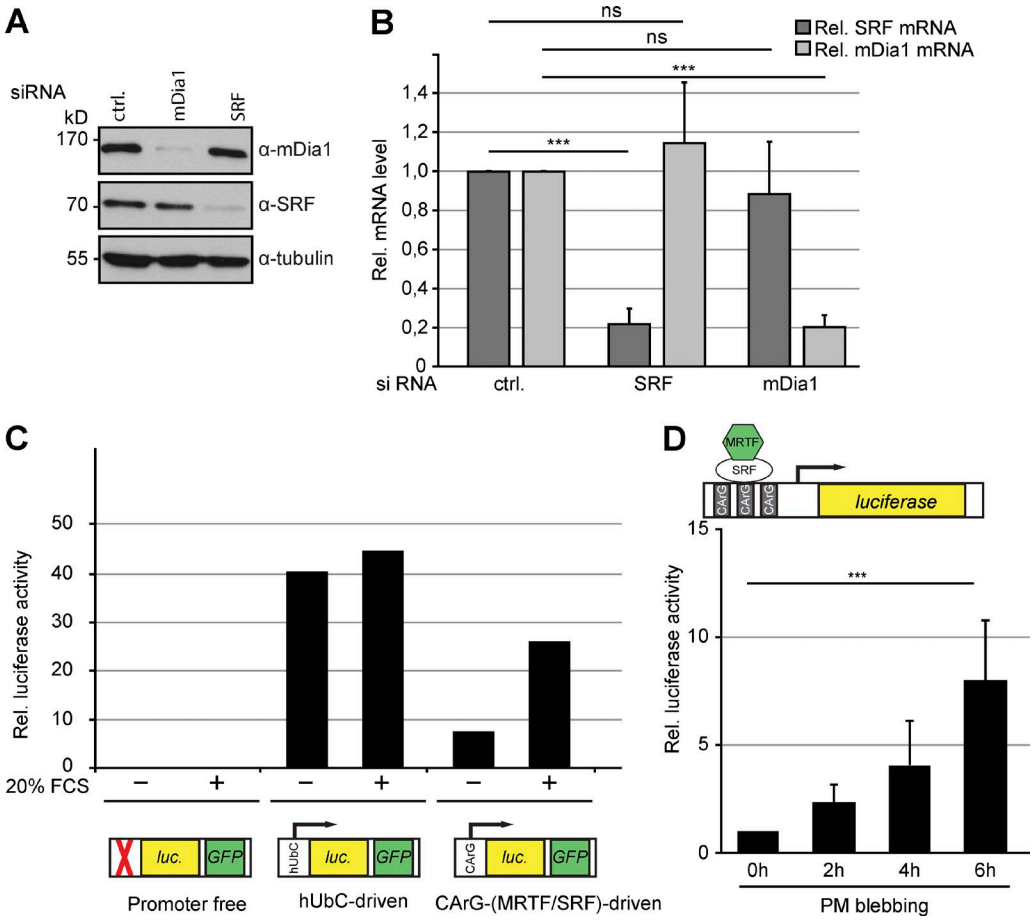


Figure S1. **The SRF pathway does not affect expression of mDia1 but is induced by PM blebbing.** (A and B) Western blot (A) and quantitative RT-PCR (B) of MCF10A cells treated with the indicated siRNAs illustrates no impact of SRF silencing on the expression of mDia1. (A) Tubulin served as a loading control. (B) Results are shown as means from three independent experiments. Asterisks indicate statistical significance (***, $P \leq 0.001$). ns indicates no significance ($P > 0.05$). (C) MCF10A cells stably expressing the indicated luciferase reporter constructs were serum-deprived for 24 h and stimulated either with or without 20% FCS for 7 h. The amount of firefly luciferase was measured luminometrically for each condition. Note the serum-responsive luciferase expression of the MRTF–SRF-specific, CArG box–driven reporter in contrast to the constitutive hUbC promoter. Results are shown from one experiment. (D) MCF10A cells stably expressing an MRTF–SRF-specific luciferase reporter were cultured on poly-HEMA to induce PM blebbing. The amount of firefly luciferase was measured luminometrically at the indicated times and expressed relative to time 0. Results are shown as means from four independent experiments. Error bars indicate SD. Asterisks indicate statistical significance (***, $P \leq 0.001$).

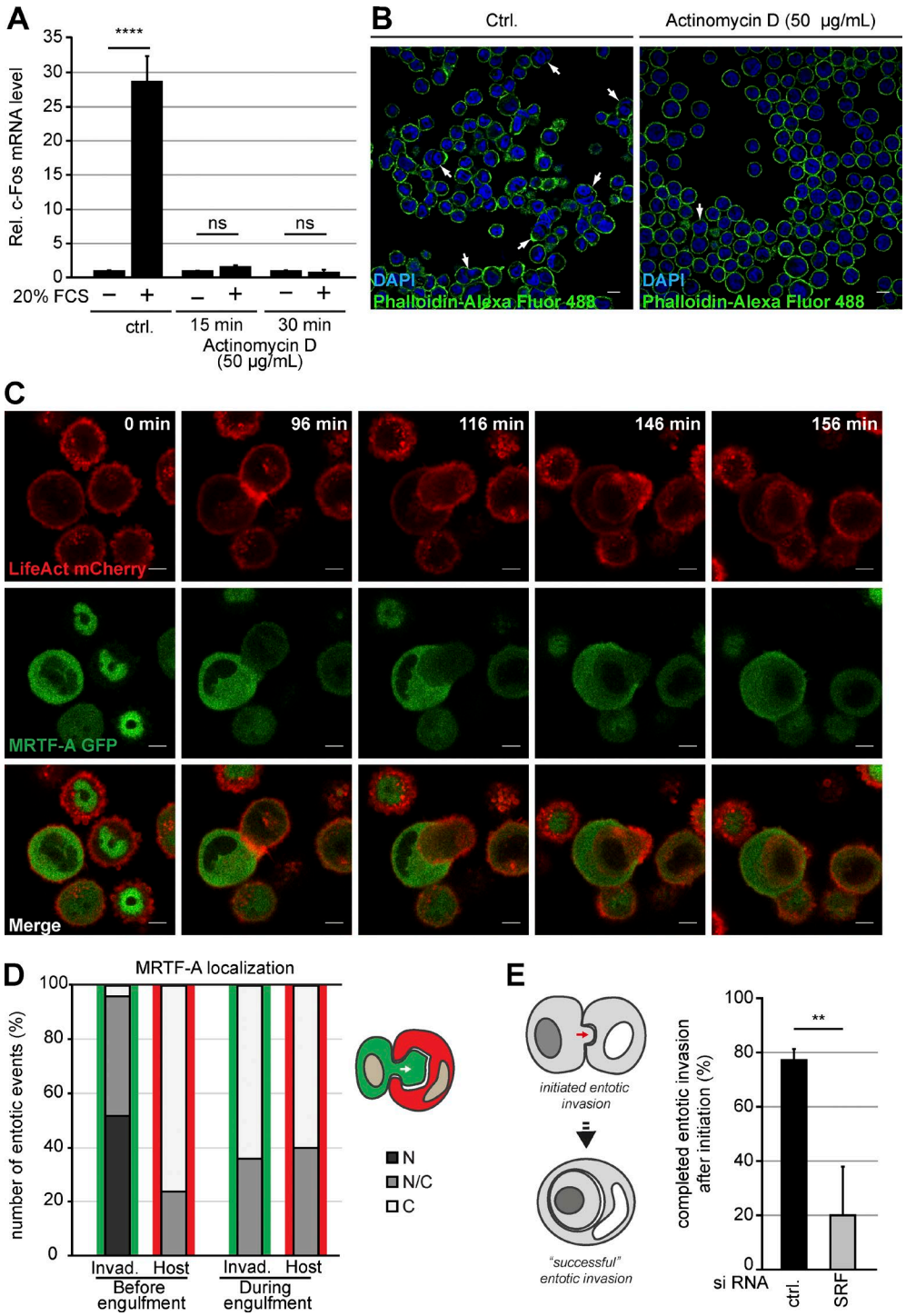
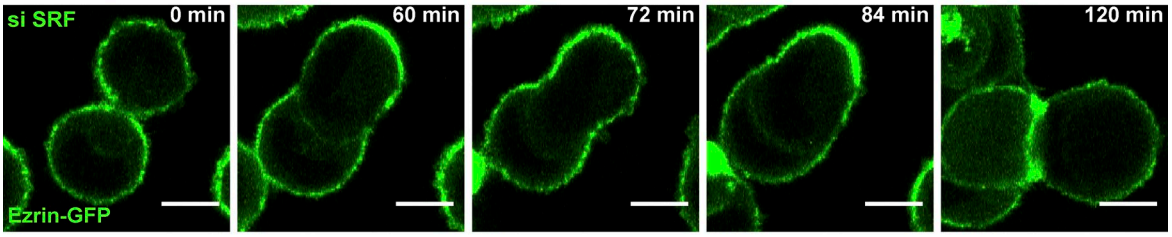


Figure S2. **Entotic invasion relies on active transcription and the MRTF–SRF pathway.** (A) Quantitative RT-PCR of serum-inducible c-Fos expression confirms transcriptional inhibition by actinomycin D. MCF10A cells were serum deprived for 24 h and treated with 50 µg/ml actinomycin D for the indicated times before stimulation either with or without 20% FCS for 30 min. Note the absence of serum-mediated up-regulation of c-Fos in the presence of actinomycin D. Results are shown as means from two independent experiments. Asterisks indicate statistical significance (****, $P \leq 0.0001$). ns indicates no significance ($P > 0.05$). (B) Image examples of entosis quantification corresponding to Fig. 4 D. F-actin was labeled with phalloidin–Alexa Fluor 488, and nuclei were stained with DAPI. Arrows indicate entotic events. Bars, 10 µm. (C) MCF10A cells stably expressing MRTF-A–GFP (green) together with LifeAct-mCherry (red) were imaged over time to follow the subcellular localization of MRTF-A before and during the process of entotic invasion. Bars, 5 µm. Also see Video 6. (D) Quantification of MRTF-A–GFP subcellular localization before and during cell engulfment as in C. Localization was scored as predominantly cytoplasmic (C), pancellular (N/C), or predominantly nuclear (N), considering a total of 25 entotic events. Note that predominantly nuclear MRTF-A imparts invader status. (E) MCF10A cells silenced for SRF fail to complete entotic invasion. MCF10A cells were either treated with control or siRNA directed against SRF and imaged over time by confocal microscopy. Events of entotic invasion initiation were identified by an initial protrusion of one cell into another and followed over time to assess the proportion of subsequent successful invasions. Numbers represent the percentages of successful cell-in-cell invasions. ≥40 initiated entotic events were analyzed from 10 independent experiments. Error bars indicate SD. Asterisks indicate statistical significance (**, $P \leq 0.01$).

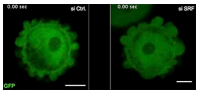
A



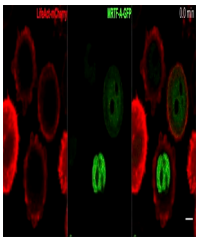
B

MCF10A Ezrin-GFP	si ctrl.	si SRF
	time (min)	time (min)
1	84	60
2	100	132
3	124	84
4	88	72
5	96	96
6	164	48
7	64	84
8	128	60
9	80	64
10	52	168
Mean	98 ± 33.05	86.8 ± 37.14

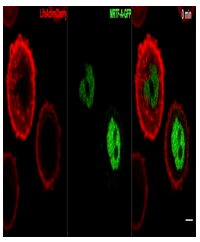
Figure S3. **Overexpression of Ezrin-GFP restores entotic invasion in SRF-depleted cells.** (A) MCF10A cells stably expressing Ezrin-GFP were treated with siRNA directed against SRF, plated on poly-HEMA and imaged during the process of entotic cell-in-cell invasion. Bars, 10 µm. (B) Table comparing the periods of time necessary for the completion of individual entotic events in MCF10A cells expressing Ezrin-GFP, which were treated either with control or siRNA directed against SRF.



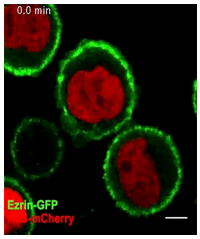
Video 1. **Silencing of SRF affects PM blebbing.** Video corresponding to Fig. 1 C shows dynamic PM blebbing of MCF10A cells stably expressing GFP (green). Before imaging, cells were treated with either control or siRNA directed against SRF and plated on poly-HEMA–coated dishes. Bars, 5 µm.



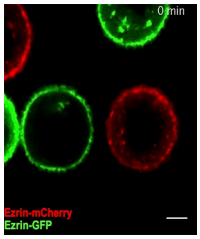
Video 2. **PM blebbing induces nuclear accumulation of MRTF-A.** Video corresponding to Fig. 2 A shows MCF10A cells stably expressing MRTF-A–GFP (green) together with the actin marker LifeAct-mCherry (red). Cells were plated on poly-HEMA–coated dishes to induce oscillations of PM blebbing, and the subcellular distribution of MRTF-A–GFP was followed over time. Arrows indicate periods of increased PM bleb activity. Bars, 5 µm.



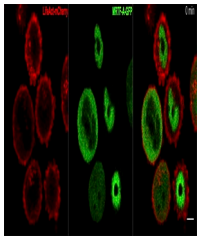
Video 3. **MRTF-A nuclear localization depends on cortical contractility.** Video corresponding to Fig. 2 C shows MCF10A cells stably expressing MRTF-A–GFP (green) together with the actin marker LifeAct-mCherry (red). Cells were plated on poly-HEMA–coated dishes, and the subcellular distribution of MRTF-A–GFP was followed over time and upon treatment with 100 µM blebbistatin (0 min) to interfere with cortical contractility and PM blebbing. Bar, 5 µm.



Video 4. **Ezrin shows polar and dynamic redistribution during entotic cell-in-cell invasion.** Video corresponding to Fig. 4 A shows MCF10A cells stably expressing Ezrin-GFP (green) together with H2B-mCherry (red). Cells were plated on poly-HEMA–coated dishes to follow the distribution of Ezrin-GFP during the process of entotic cell-in-cell invasion. Bar, 5 µm.



Video 5. **The redistribution of Ezrin during entotic invasion specifically occurs at the rear of the invading cell.** Video corresponding to Fig. 4 B shows two MCF10A cells stably expressing either Ezrin-mCherry (invading cell, red) or Ezrin-GFP (invaded cell, green), which undergo entotic invasion. Note that the observed redistribution and enrichment of Ezrin is restricted to the invading cell. Bar, 5 µm.



Video 6. **Nuclear MRTF imparts invader status.** Video corresponding to Fig. S2 C shows MCF10A cells stably expressing MRTF-A–GFP (green) together with LifeAct-mCherry (red). Cells were plated on poly-HEMA–coated dishes to follow the distribution of MRTF-A during the process of cell-in-cell invasion. Bar, 5 µm.