Supplemental Information

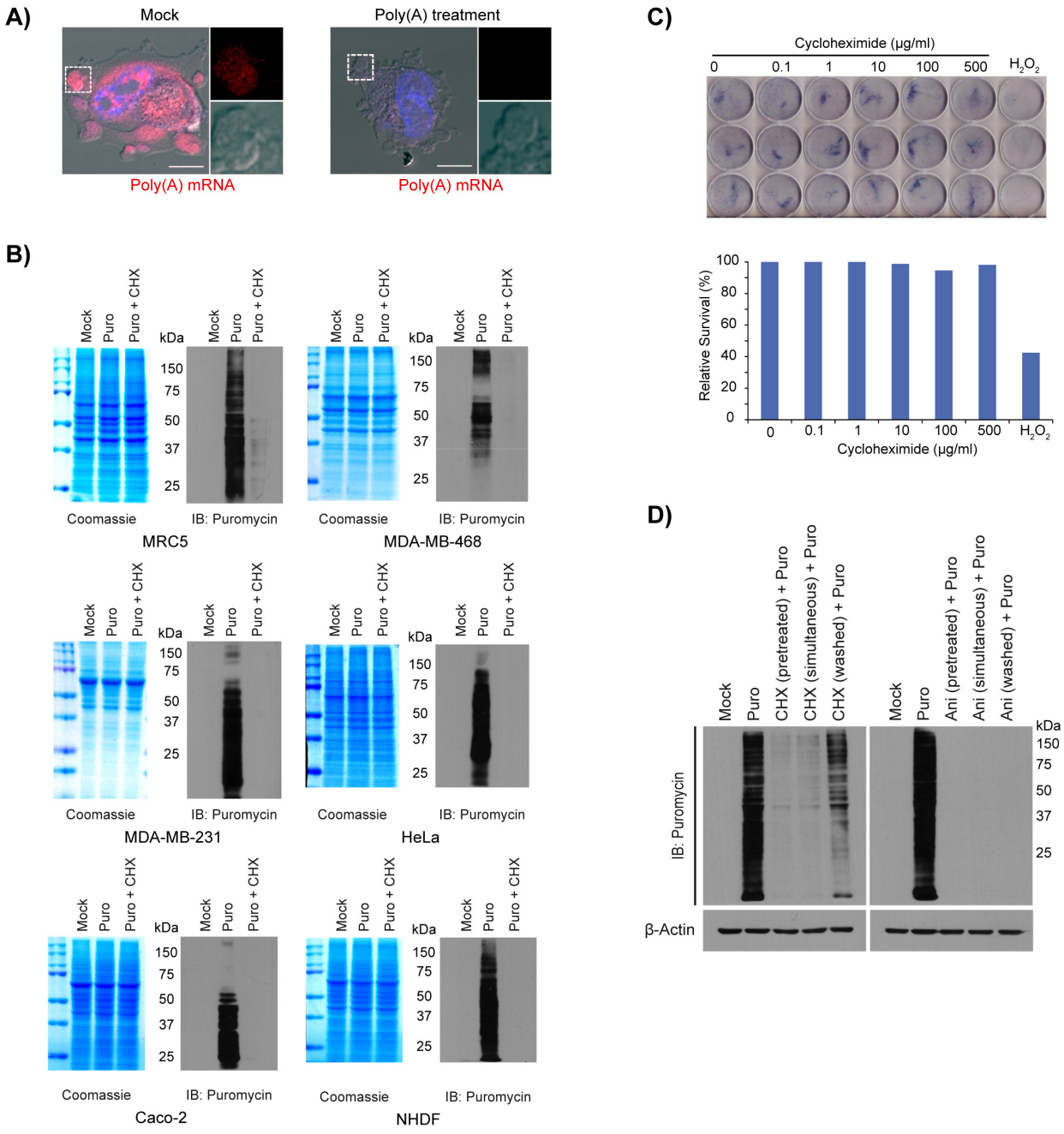

Figure S1: Immunofluorescence negative control.

A) Poly(A) mRNA was detected using 2 μg mL^{-1} of Alexa Fluor® 594 tagged oligo(dt) 25mers (red) left panel. Signal was completely abrogated upon addition of 10 μg mL^{-1} of poly(A) antisens probe. Bars = 10 μm.

B) Assessment of the translation inhibition of cycloheximide in different cell lines. Puromycin incorporation was efficiently blocked using a concentration of 50 µg mL^{-1} of cycloheximide 15 min prior puromycin addition to the media.

C) Cell survival assessment following increasing amount of cycloheximide**.** Cells were treated with increasing amount of cycloheximide (0.10 µg mL^{-1}, 1.00 µg mL^{-1}, 10.00 µg mL^{-1}, 100.00 µg mL^{-1} and 500.00 µg mL^{-1}) for 4 hours. Following this treatment, media was change for standard DMEM media and allowed to grow for 24 hour prior to fixation with 4% paraformaldehyde and crystal violet staining. Visualisation of the cell growth following applied treatment (left panel) and statistical analysis of triplicate survival assays based on the aforementioned treatment compared to the mock treatment (left panel). H_2O_2 (500 µM) was used as positive control.

D) Effect of cycloheximide (left panel) and anisomycin (right panel) on puromycin incorporation. 5 minutes puromycilation efficiency under cycloheximide pretreatment (15 min), simultaneous cycloheximide/puromycin treatment (5 min) or washed cycloheximide pretreatment (15 min). 5 minutes puromycilation efficiency under anisomycin pretreatment (15 min), simultaneous anisomycin/puromycin treatment (5 min) or washed anisomycin pretreatment (15 min).

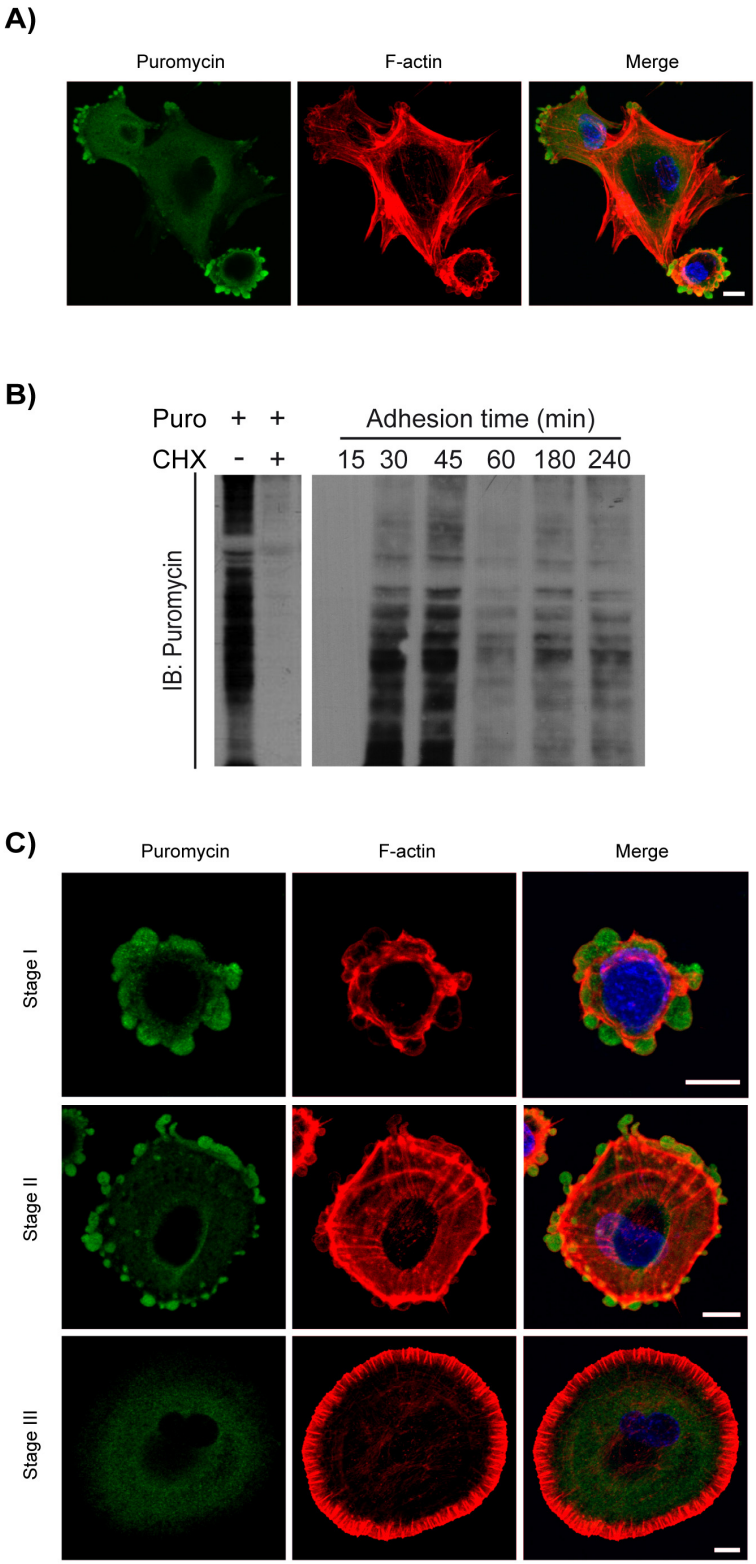

Figure S2: Puromycin incorporation in adhering MRC-5.

A) Representative image of puromycinylated proteins (green) in fully adhered cell compared to a newly adhering cell. F-actin was detected with CF™568 phalloidin (red). Bars = 10 µm.

B) 5 minutes puromycin incorporation during MRC-5 adhesion. 5 µg mL^{-1} of puromycin was added during the last 5 minutes at different time during adhesion.

C) Representative image of puromycinylated proteins (green) in adhering MRC-5 cell at different SIC stages. F-actin was detected with CF™568 phalloidin (red). Bars = 10 µm.

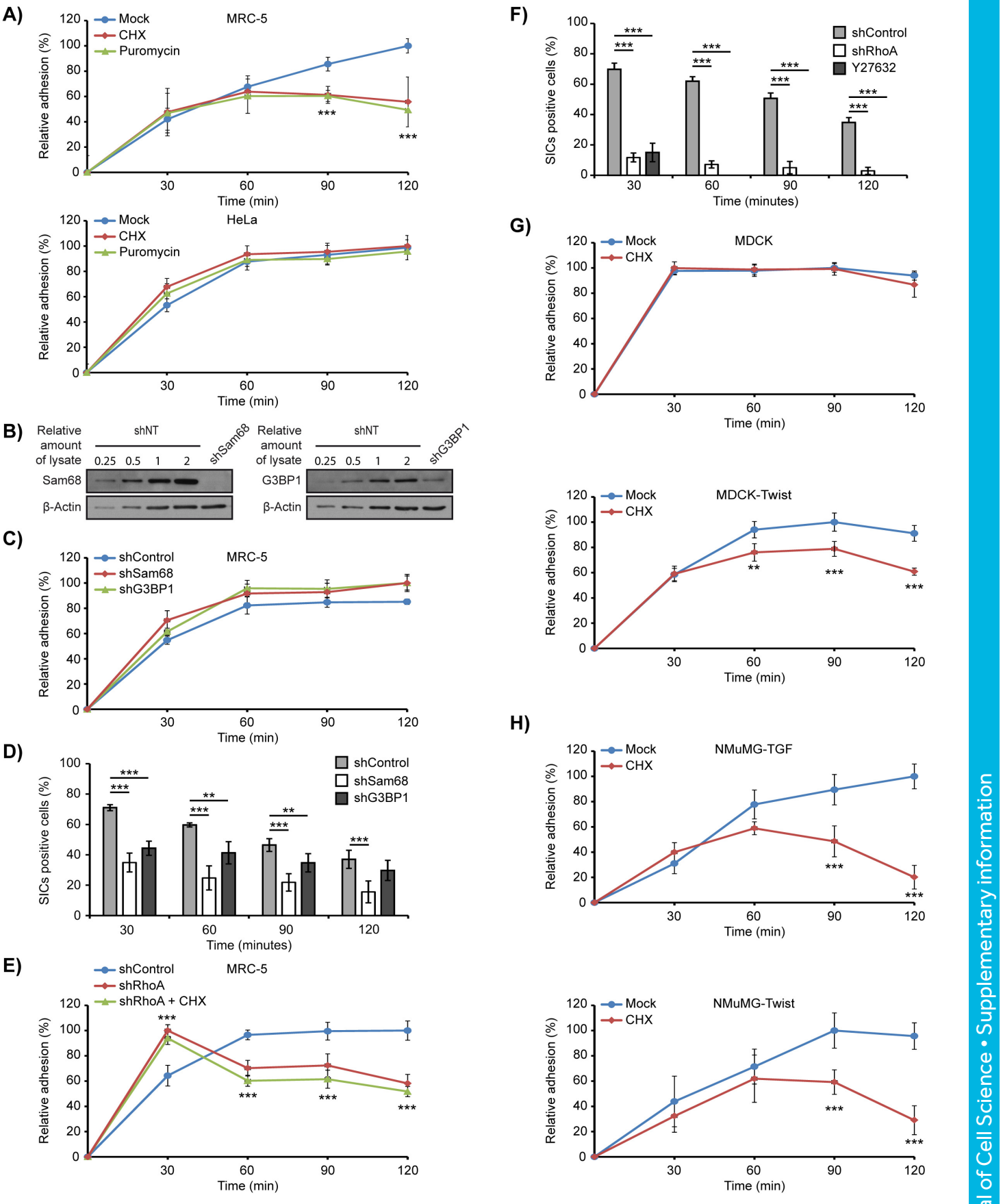

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Figure S3: Relative effect of increasing amount of translational inhibitors on adhesion.

A) Adhesion assay on MRC-5 and HeLa cell lines in presence or absence of cycloheximide (50 µg mL^{-1}) or puromycin (2.5 µg mL^{-1}). Error bar on quantification corresponds to standard deviation. ★★★ = two-tailed t-test P-value ≤ 0.001.

B) Sam68 (left panel) and G3BP1 (right panel) shRNA effect on their respective expression in MRC-5.

C) Adhesion assay on MRC-5 expressing shRNA directed against Sam68 and G3BP1. Error bar on quantification corresponds to standard deviation.

D) Statistical assessment over time of the number of SIC positive cell expressing shRNA directed against Sam68 and G3BP1. Statistical analysis was performed on over 40 cells from triplicate adhesion assays for each condition. Error bar on quantification corresponds to standard deviation. ★★★ = two-tailed t-test P-value ≤ 0.001, ★★ = two-tailed t-test P-value ≤ 0.01.

E) Effect of RhoA inactivation (shRhoA) on adhering MRC-5 cells, in presence or absence of cycloheximide. ★★★ = two-tailed t-test P-value ≤ 0.001.

F) Statistical assessment over time of the number of SIC positive cell, following Y27632 treatment or cell expressing of shRhoA. Statistical analysis was performed on over 40 cells from triplicate adhesion assays for each condition. Error bar on quantification corresponds to standard deviation. ★★★ = two-tailed t-test P-value ≤ 0.001.

G) *Adhesion assay on EMT-induced MDCK cell line using exogenous expression of Twist (lower panel) in presence or absence of cycloheximide (50 ug mL^{-1}). Error bar on quantification corresponds to standard deviation. ★★★ = two-tailed t-test P-value ≤ 0.001, ★★ = two-tailed t-test P-value ≤ 0.01.*

H) Adhesion assay on EMT-induced NMuMG using TGF-β (upper panel) or exogenous expression of Twist (lower panel) in presence or absence of cycloheximide (50 ug mL^{-1}). Error bar on quantification corresponds to standard deviation. ★★★ = two-tailed t-test P-value ≤ 0.001.

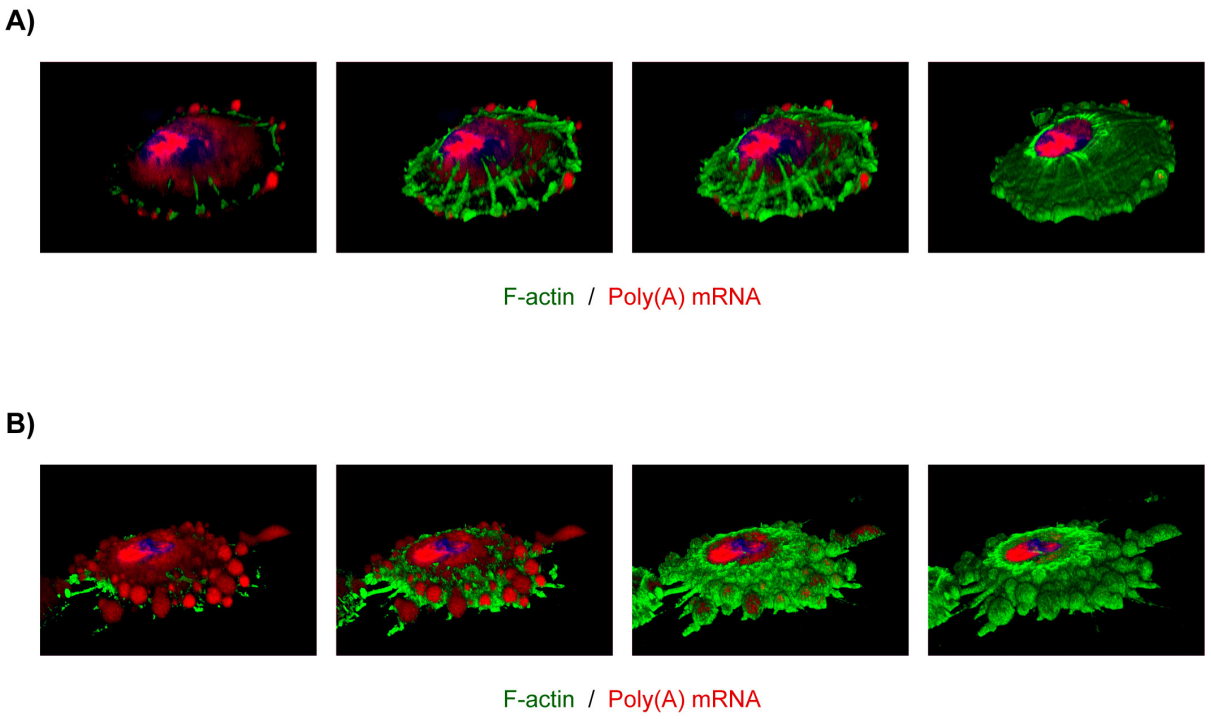
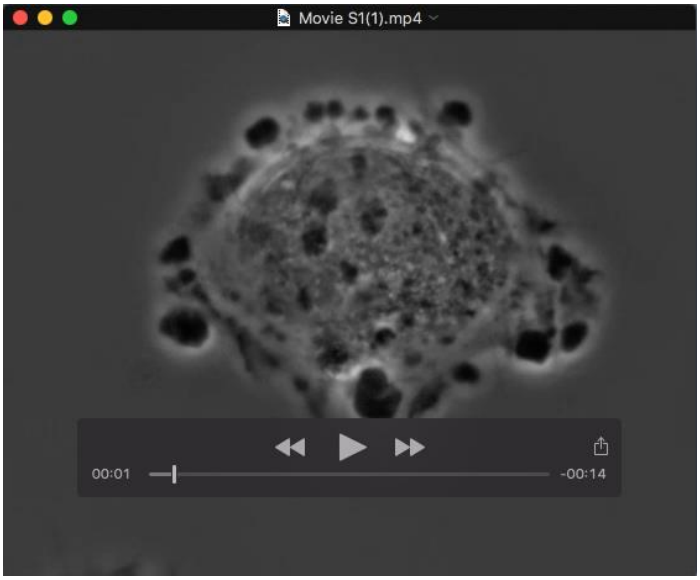
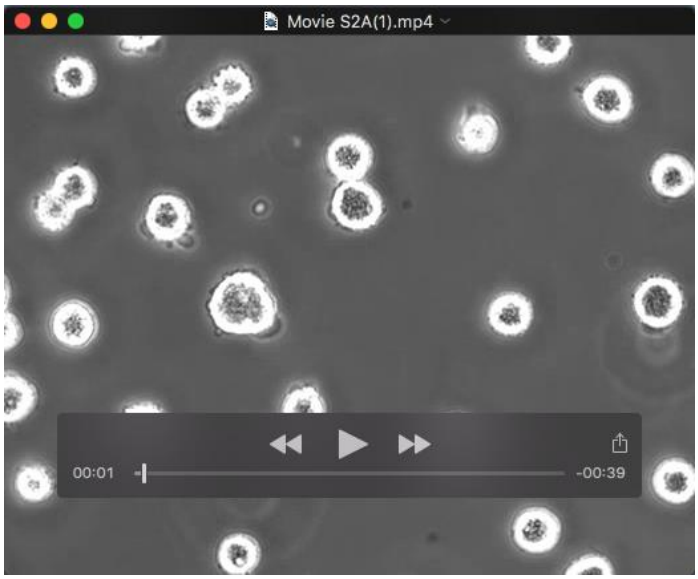


Figure S4: Three-dimensional reconstitution of SIC in adhering MRC-5 cells following A) mock or B) 50 µg mL^{-1} cycloheximide treatment.

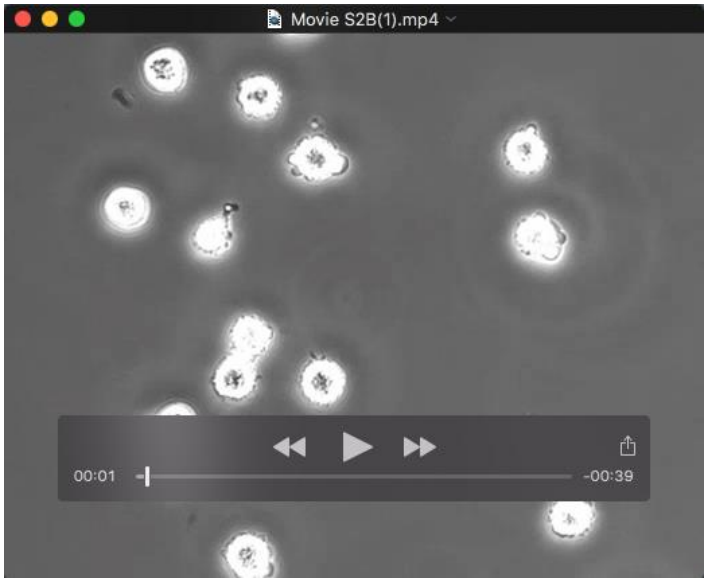


Movie S1: Time-lapse cell imaging of SIC dynamics formed on a typical MRC-5 cell adhering.

A

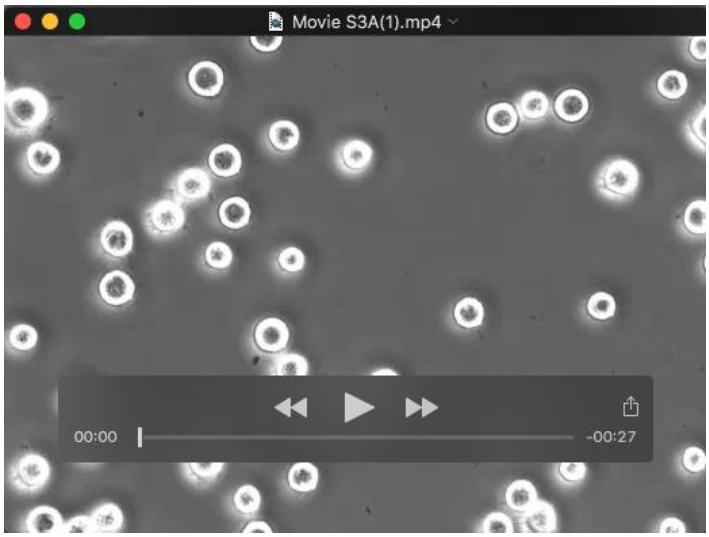


B

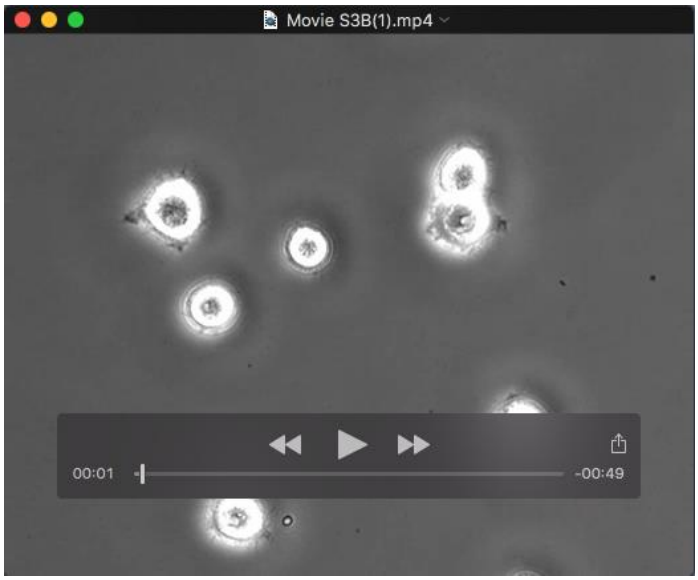


Movie S2: Time-lapse cell imaging of SIC dynamics formed on untreated MRC-5 cell adhering (S2A) and MRC-5 cell adhering in presence of 50 µg mL^{-1} of cycloheximide (S2B).

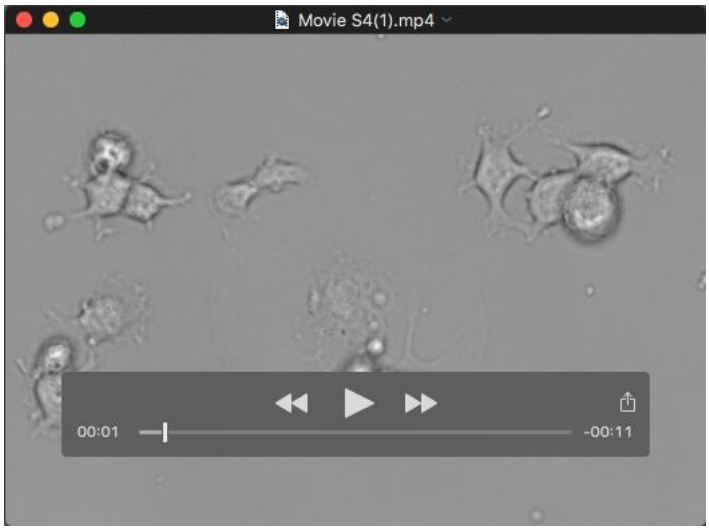
A



B



Movie S3: Time-lapse cell imaging of SIC dynamics formed on untreated HeLa cell adhering (S3A) and HeLa cell adhering in presence of 50 µg mL^{-1} of cycloheximide (S3B).



Movie S4: Time-lapse cell imaging of SIC inhibition and increased spreading of newly adhering MRC-5 cell treated with Y27632 (ROCK inhibitor).