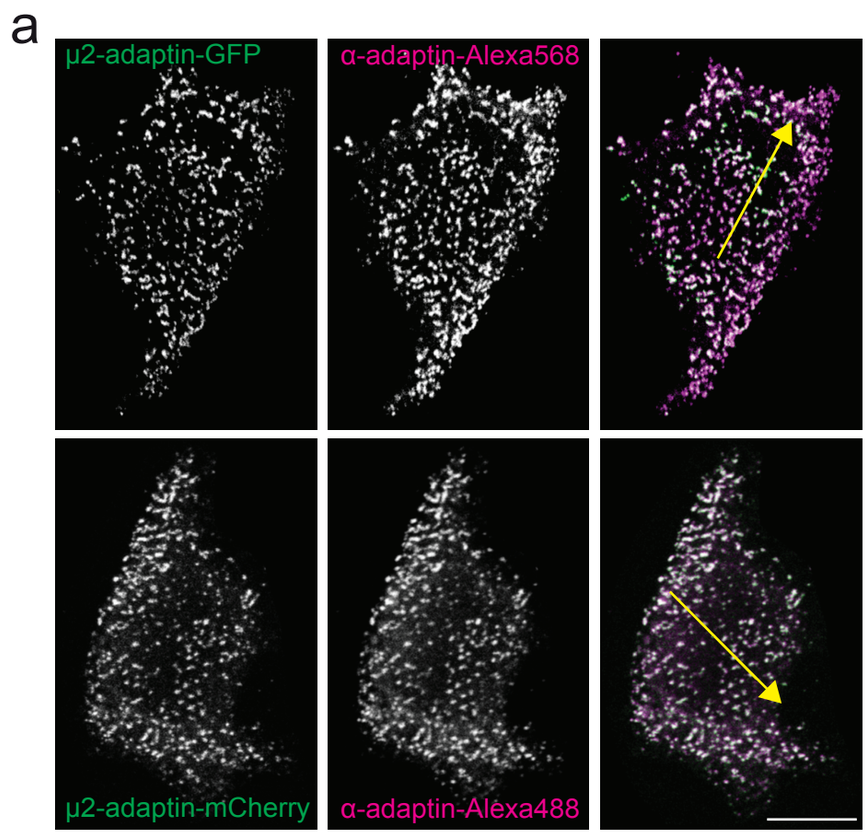
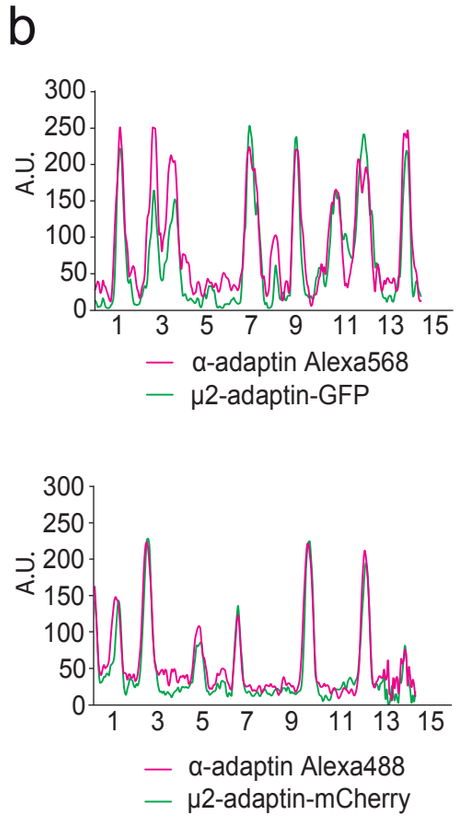
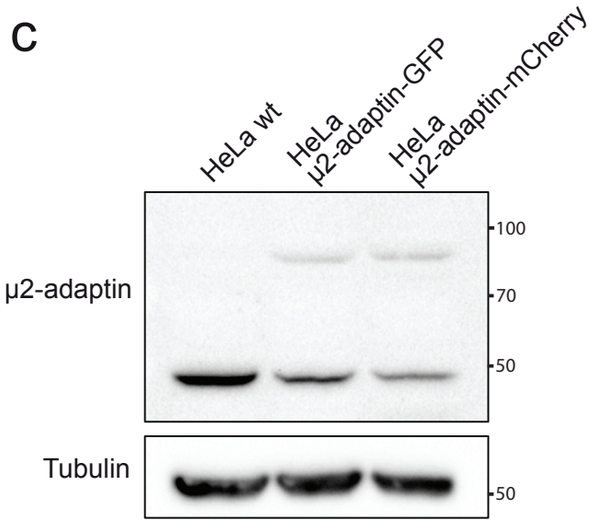
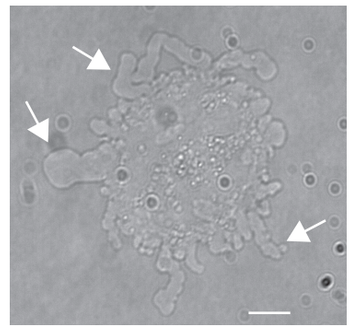
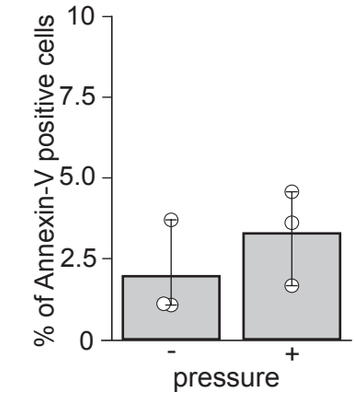
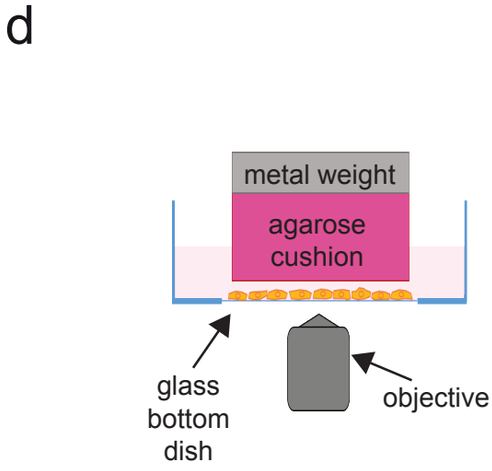


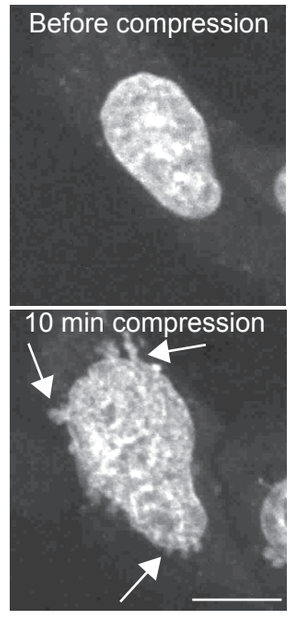












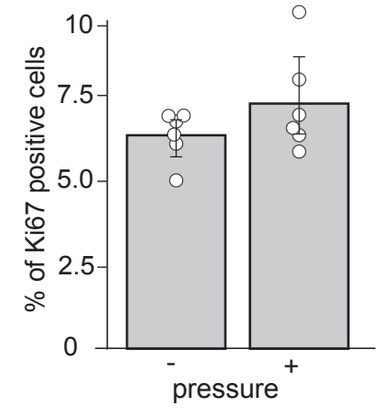
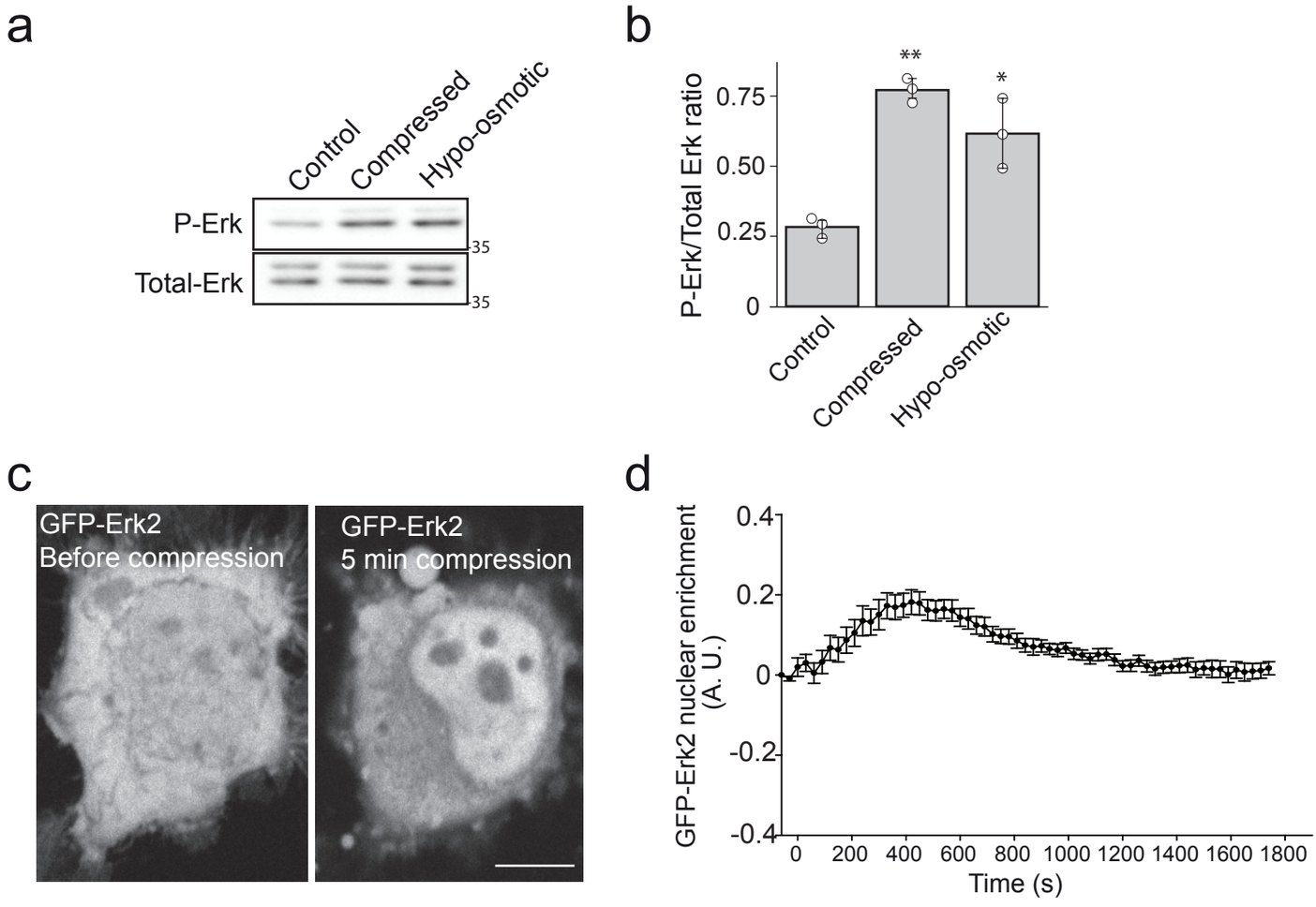


Figure S1

Figure S1. **Validation of the cell compression strategy**. **a**, HeLa cells genome-edited to express a fluorescently-tagged version of µ2-adaptin, a subunit of the clathrin adaptor AP-2, were immunostained using antibodies against α-adaptin, another subunit of the AP-2 complex. Scale bar: 10 µm. **b**, Intensity plot corresponding to the regions marked by yellow arrows in images in a. **c**, Western-blot analysis of µ2-adaptin levels in HeLa cells wt or genome-edited to express a fluorescently-tagged version of µ2-adaptin. Tubulin was used as loading control. **d**,schematic of our cell compression imaging set-up. **e**, Wide-field image of one HeLa cell compressed under and agarose plug. Arrows points to blebs at the plasma membrane. Scale bar: 5µm. **f**, HeLa cells treated with Sir-DNA were imaged by spinning disk microscopy before or after compression under an agarose plug, as indicated. Arrows point to nuclear blebs. Scale bar: 10µm. **g**, HeLa cells were subjected or not to compressive stress for 30 min. Cells were then recovered and stained for Annexin V and DAPI according to the manufacturer's instructions. Cells positive for Annexin V and negative for DAPI were counted in three independent experiments. Results are expressed as mean ± SD (two tailed paired Student's T-test). **h**, HeLa cells plated on glass coverslips at 80% confluency were subjected or not to compressive stress. The stress was then removed and the medium changed to eliminate cell debris. 48 h later, cells were fixed and immunostained for Ki-67 and imaged at 20x. The number of Ki-67 positive cells was counted in each condition. Results are expressed as mean ± SD (Kruskal-Wallis One Way analysis of variance on ranks. Three independent experiments were performed, counting cells in 2 random fields, corresponding to approximately 600 cells per condition per experiment).



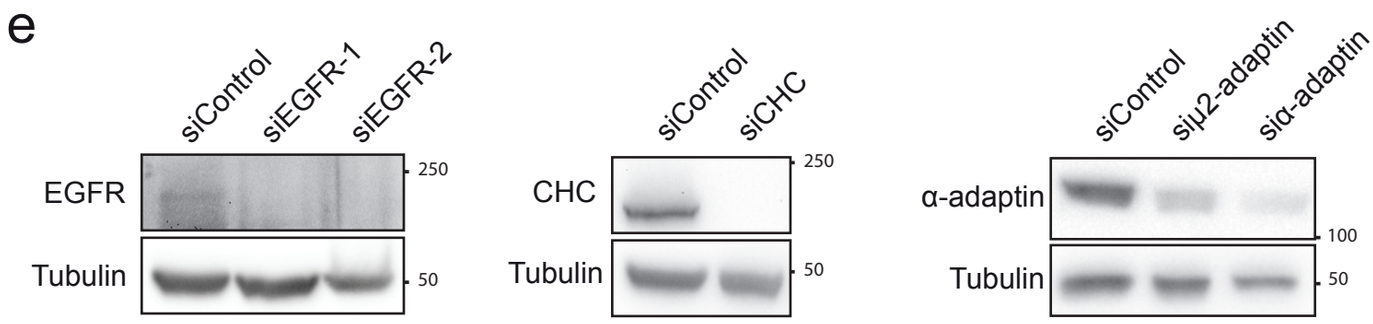


Figure S2

Figure S2. **Analysis on membrane tension and ERK. a**, Western-blot analysis of phospho-ERK (P-ERK) levels in HeLa cells uncompressed (control), compressed, or subjected to hypo-osmotic shock (90% water) for 30 min (representative image of three independent experiments). Total-ERK was used as loading control. **b**, Densitometry analysis of bands obtained in Western-blots as in a. Results are expressed as mean ratio of P-ERK/total ERK ± SD from three independent experiments. (* $P<0.005$, ** $P<0.001$, Kruskal-Wallis One Way analysis of variance on ranks). **c**, HeLa cells transfected with a plasmid encoding eGFP-tagged ERK2 were imaged by spinning disk microscopy before (left panel) or after (right panel) being compressed under an agarose plug. Scale bar: 10µm. **d**, Quantification of eGFP-ERK2 enrichment in the nucleus at the indicated time points after compression. 22 cells from 3 independent experiments were quantified. Results are expressed as mean ± SE.**e,** HeLa cells were transfected with the indicated siRNAs. 72 h after transfection, cells were lysed and subjected to Western-blot analysis using the indicated antibodies. Tubulin was used as a loading control.

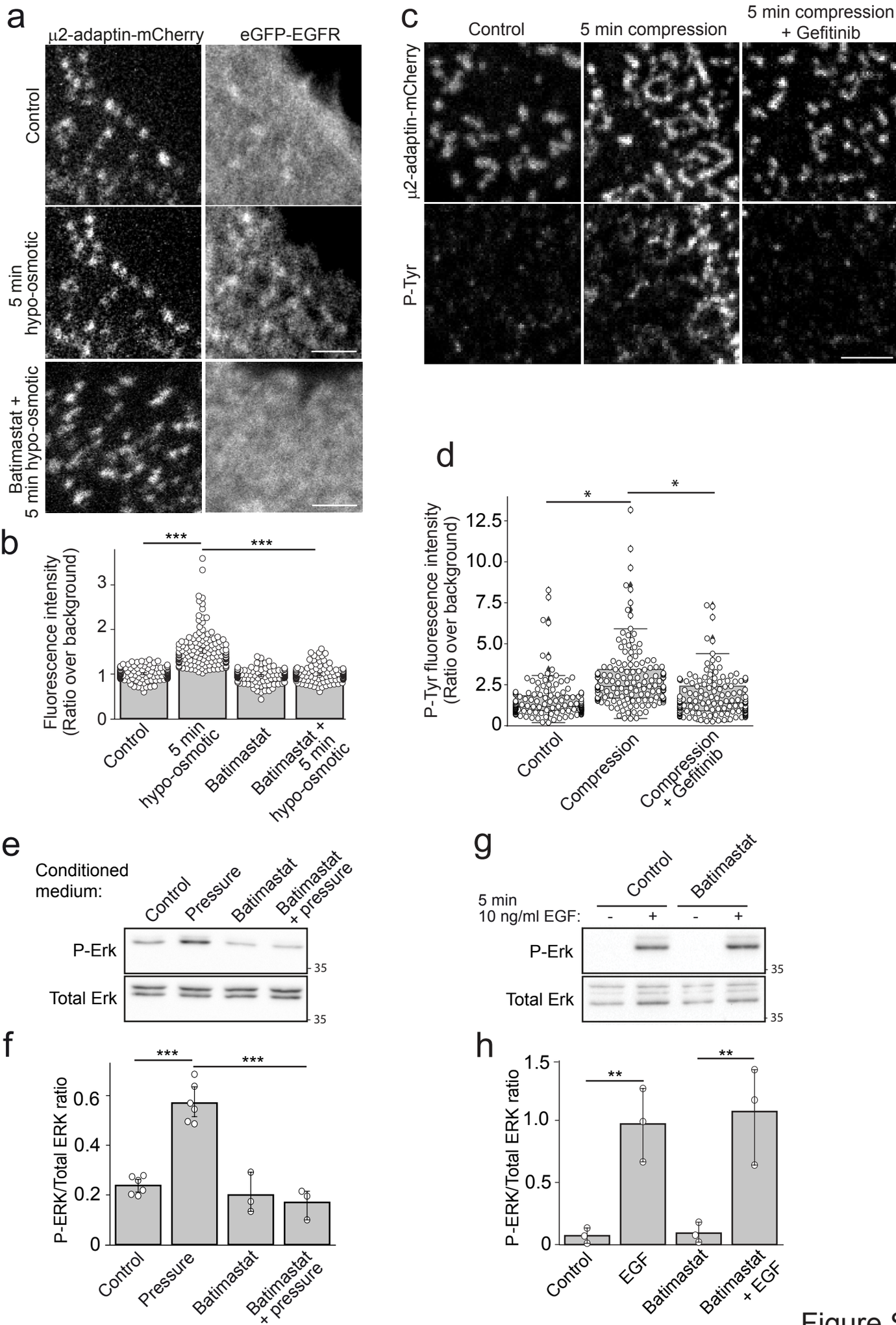


Figure S3

Figure S3. **Characterization of EGFR activation modalities under compression. a**, Genome-edited HeLa cells expressing endogenous mCherry-tagged μ2-adaptin were transfected with a plasmid encoding eGFP-tagged EGFR, seeded on glass, serum-starved for 2 h, subjected to hypo-osmotic shock (90% water) in the presence or not of Batimastat and imaged by TIRF microscopy every 10s for 20 min. Scale bar: 2.5 μm. **b**, Quantification of eGFP-EGFR enrichment at CCSs before or 5 min after hypo-osmotic shock in cells treated as in a and showing some degree of EGFR recruitment at CCSs (*** P<0.001, two tailed paired Student's T-test; 50 structures per experiment in three independent experiments were analysed). **c**, Genome-edited HeLa cells expressing endogenous mCherry-tagged μ2-adaptin were serum starved for 2 h, treated with 10 μM Gefitinib or vehicle for 1 h and subjected or not to compression. Cells were fixed after 5 min of compression, without releasing compression. An immunostaining against phosphorylated tyrosines (P-Tyr) was then performed. Scale bar: 2.5 μm. **d**, Quantification of P-Tyr enrichment at CCSs before (control) or 5 min after compression in cells treated as in e (* P<0.05, Kruskal-Wallis One Way analysis of variance on ranks; 50 structures per experiment in three independent experiments were analysed). **e**, Western-blot analysis of phospho-ERK (P-ERK) levels in HeLa cells serum starved for 2 h, and treated for 5 min with conditioned medium from uncompressed cells (control and Batimastat conditions) or from cells compressed for 5 min in the presence or not of Batimastat (pressure and pressure + Batimastat conditions). **f**, Densitometry analysis of bands obtained in Western-blots as in e. Results are expressed as mean ratio of P-ERK/total ERK ± SD from three independent experiments **(**two tailed paired Student's T-test) **g**, Western-blot analysis of phospho-ERK (P-ERK) levels in HeLa cells serum starved for 2 h, treated or not with Batimastat for 1 h, and stimulated with 10 ng/ml EGF or not as indicated (representative image of three independent experiments). Total-ERK was used as loading control. **h**, Densitometry analysis of bands obtained in Western-blots as in g. Results are expressed as mean ratio of P-ERK/total ERK ± SD from three independent experiments (** *P*<0.01, One Way Analysis of Variance – ANOVA, Student-Newman-Keuls).

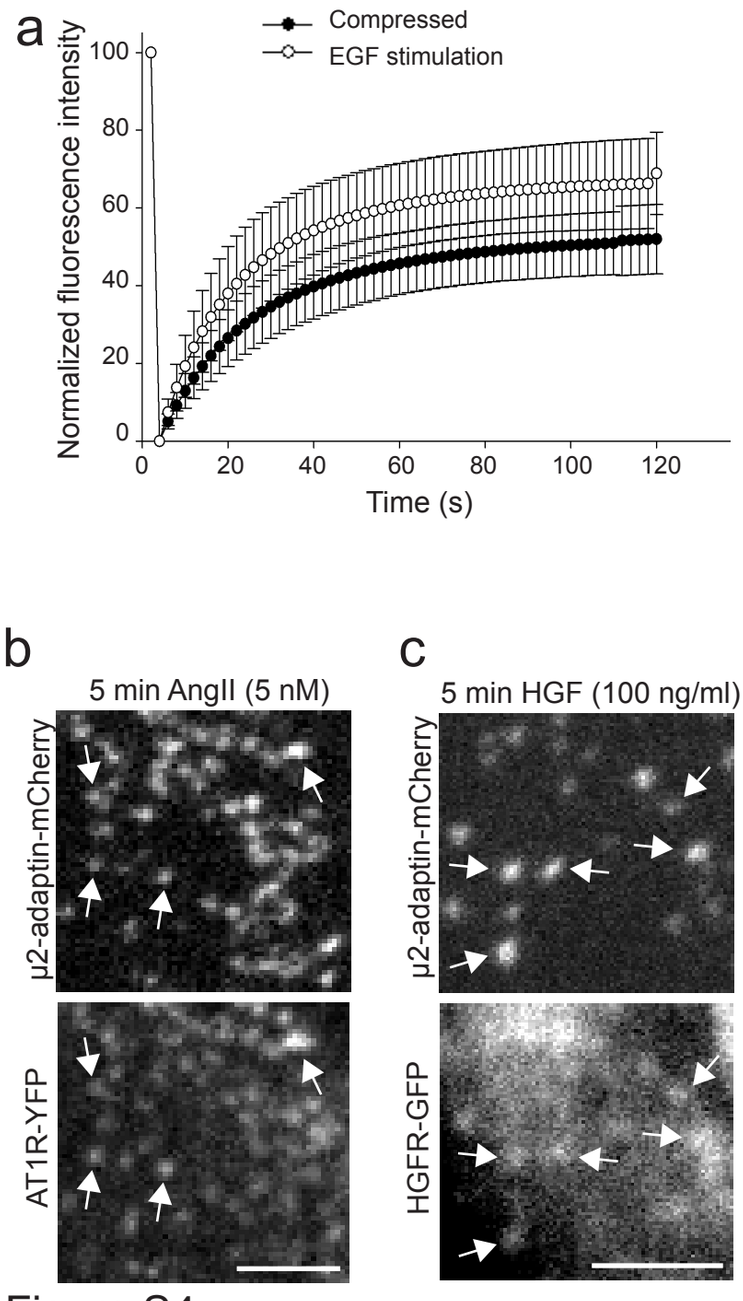
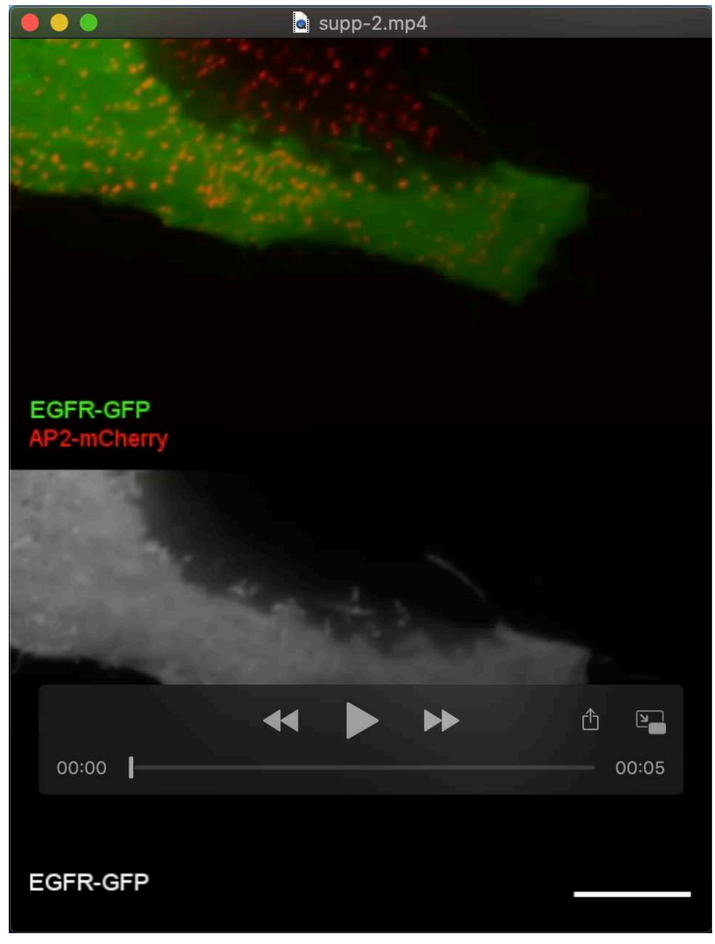


Figure S4

Figure S4. **Alteration of receptor sorting and dynamics under compression. a**, Quantification of fluorescence recovery after photobleaching of eGFP-EGFR fluorescence in individual CCSs in cells stimulated with EGF or compressed under an agarose plug, as indicated. All results are expressed as mean ± SD. **b**, Genome-edited HeLa cells expressing endogenous mCherry-tagged μ2-adaptin were transfected with a plasmid encoding YFP-tagged AT1R. Cells were serum-starved for 2 h, stimulated with 5 nM Angiotensin II for 5 min and imaged by TIRF microscopy every 10s for 20 min. Scale bar: 2.5 μm. **c**, Genome-edited HeLa cells expressing endogenous mCherry-tagged μ2-adaptin were transfected with a plasmid encoding GFP-tagged HGFR. Cells were serum-starved for 2 h, stimulated with HGF (100 ng/ml) for 5 min and imaged by TIRF microscopy every 10 s for 20 min. Scale bar: 2.5 μm.

Supplementary Movies



Movie 1. **EGFR recruitment to CCSs.** Genome-edited HeLa cells expressing endogenous mCherry-tagged μ2-adaptin were transfected with a plasmid encoding eGFP-tagged EGFR, seeded on glass and starved for 2 h. Cells were then compressed under an agarose plug (1 kPa pressure) and imaged by TIRF microscopy every 15s for 30 min. Scale bar: 10 μm.

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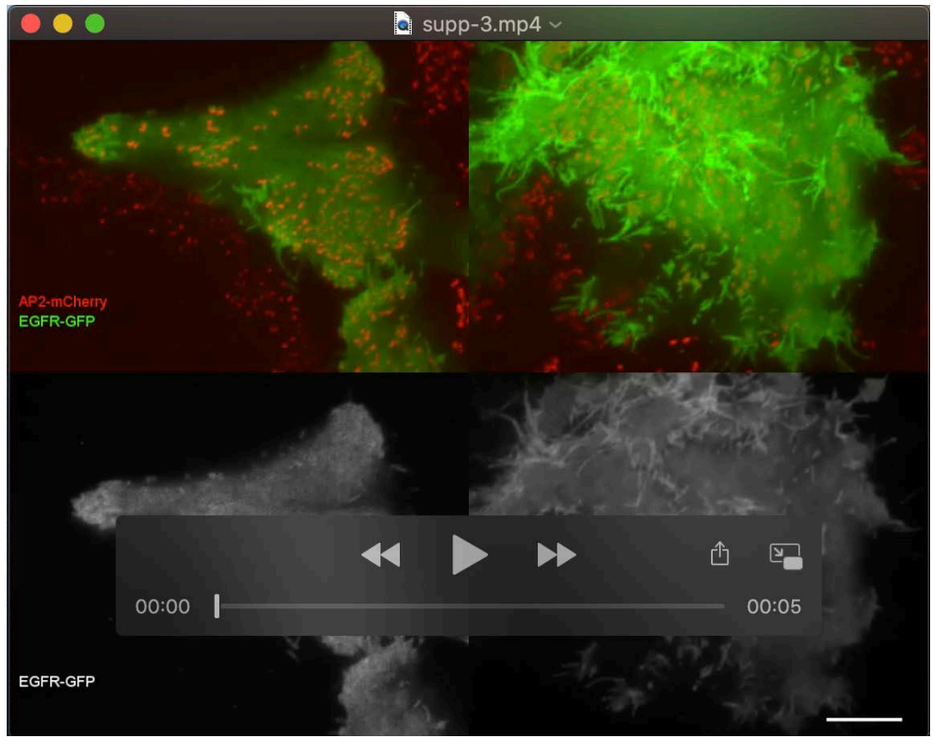
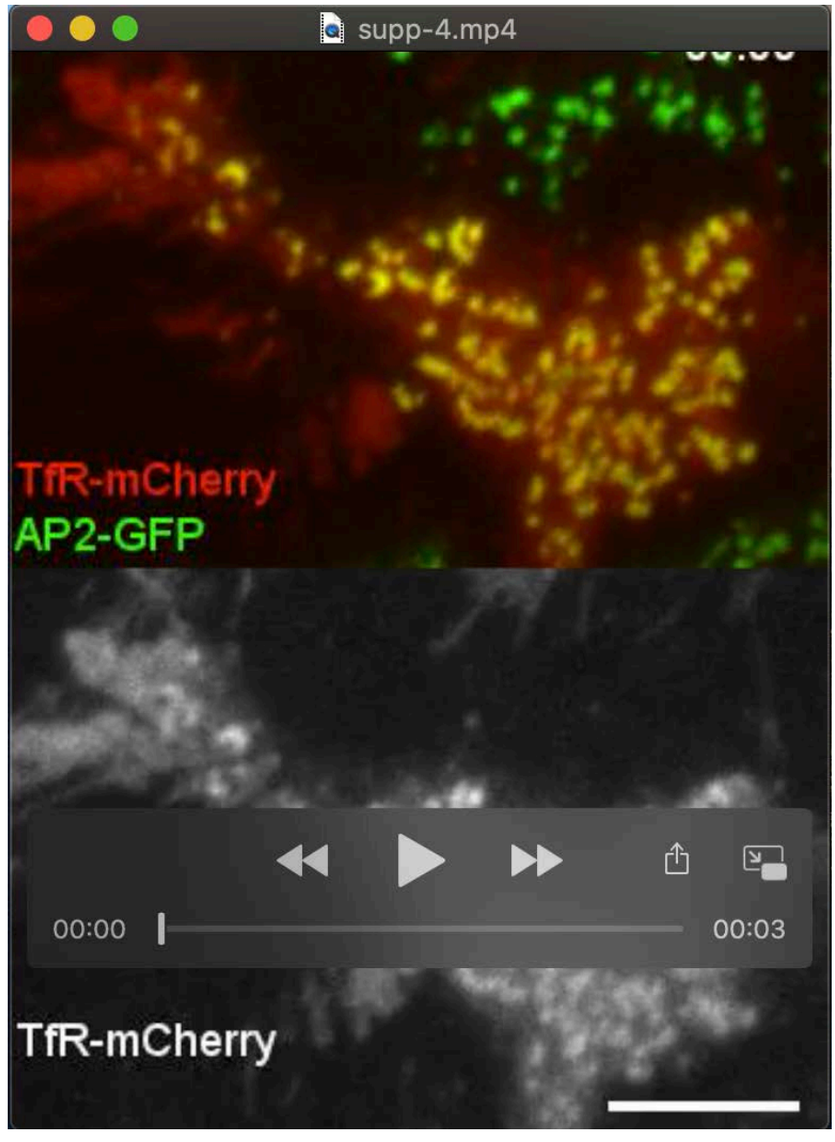


Movie 2. **EGFR recruitment to CCSs in the presence of Gefitinib or Batimastat.** Genome-edited HeLa cells expressing endogenous mCherry-tagged μ2-adaptin were transfected with a plasmid encoding eGFP-tagged EGFR, seeded on glass, starved for 2 h and treated with 10 μM Gefitinib (left) or 10 μM Batimastat (right). Cells were then compressed under an agarose plug (1 kPa pressure) and imaged by TIRF microscopy every 15 s for 30 min. Scale bar: 10 μm.



Movie 3. **TfR is lost from CCSs upon compression.** Genome-edited HeLa cells expressing endogenous GFP-tagged μ2-adaptin were transfected with a plasmid encoding mCherry-tagged TfR, seeded on glass, then compressed under an agarose plug (1 kPa pressure) and imaged by TIRF microscopy every 10 s for 30 min. Scale bar: 5 μm.