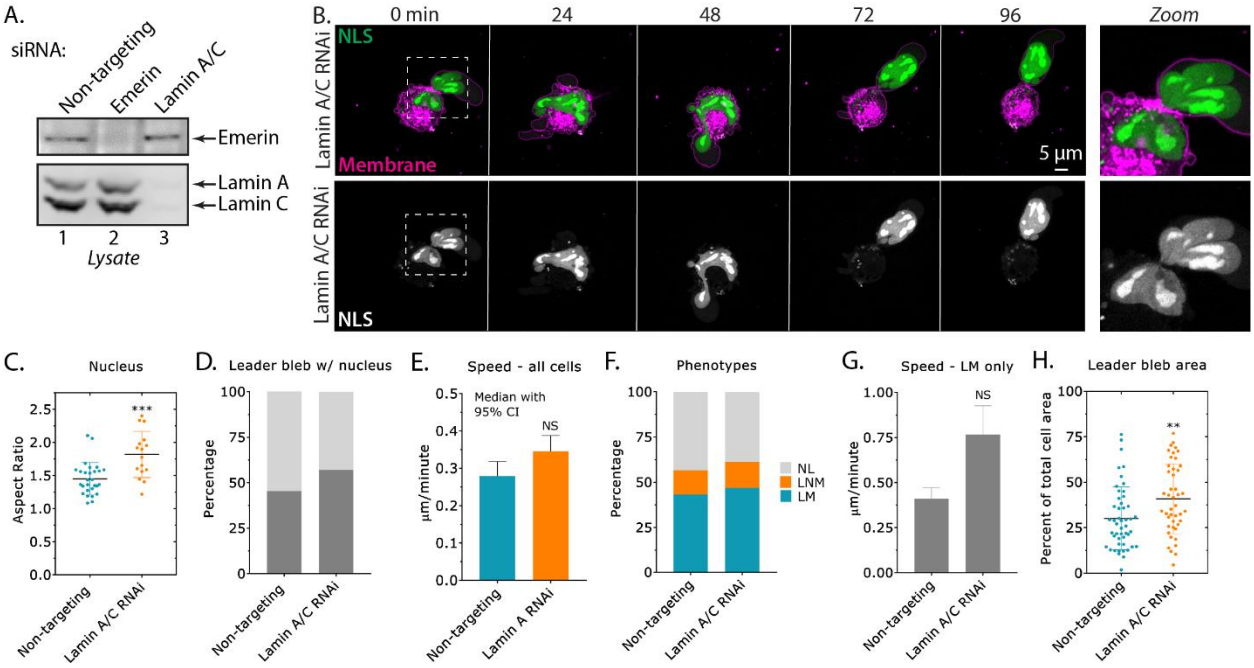


Fig. S1. **A.** Western blot confirmation of emerin and Lamin A/C RNAi in A375-M2 cells. Cells were treated for 5 days with each Locked Nucleic Acid (LNA; 50 nM) to achieve a near complete removal of each protein. **B.** Time-lapse imaging of a A375-M2 cell after Lamin A/C RNAi transiently transfected with Nuclear Localization Sequence (NLS) tagged mEmerald (NLS-mEmerald), which has been confined down to ~3 µm. Cells were stained with membrane dye. Zoom highlights nuclear shape changes in Lamin A/C RNAi cells. **C.** Nuclear aspect ratio for cells after Lamin A/C RNAi (mean +/- SD; two-tailed Student's t-test). **D.** Percent cells with leader blebs containing the nucleus in Lamin A/C RNAi cells. **E.** Instantaneous speeds for all cells after Lamin A/C RNAi (median +/- 95% CI; two-tailed Student's t-test). **F.** Percent NL, LNM, and LM for Lamin A/C RNAi cells. Statistical significance was determined using a Chi-squared test (χ^2=0.748). **G.** Instantaneous speeds for leader mobile (LM) cells after Lamin A/C RNAi (mean +/- SEM; two-tailed Student's t-test). **H.** Leader bleb area (calculated as the percent of total cell area) for cells after Lamin A/C RNAi
(mean +/- SD; two-tailed Student's t-test). All data are representative of at least three independent experiments. * - $p \leq 0.05$, ** - $p \leq 0.01$, *** - $p \leq 0.001$, and **** - $p \leq 0.0001$

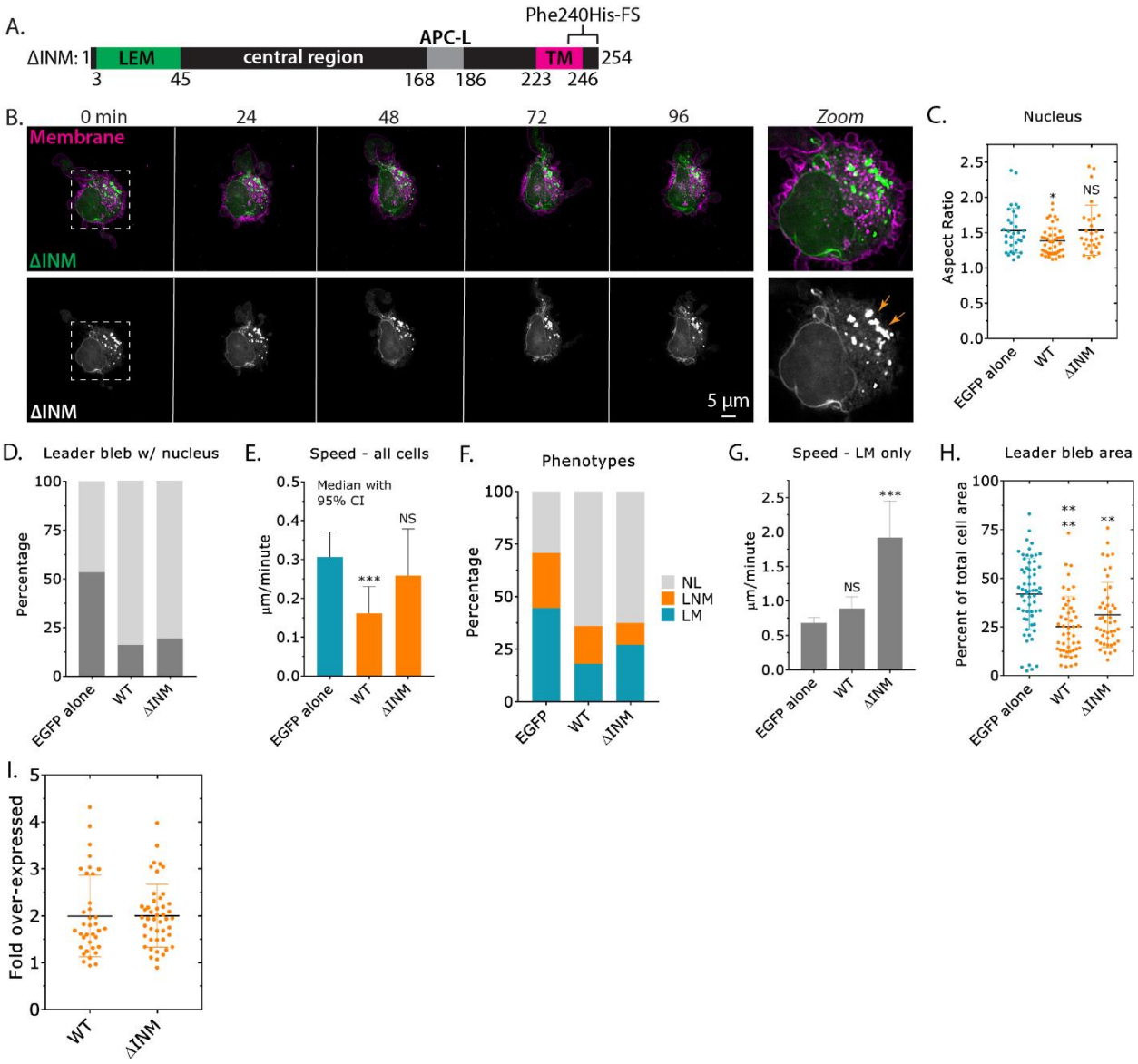


Fig. S2. **A.** Cartoon of the previously described frame shift mutation, Phe240His-FS, near the transmembrane (TM) domain for retaining emerin within the ONM/ER (ΔINM). LEM (Lap2, emerin, MAN1) domain and APC-like (APC-L) domain. **B.** Time-lapse imaging of a A375-M2 cell transiently transfected with emerin-EGFP (ΔINM), which has been confined down to ~3 µm. Cells were stained with membrane dye. Zoom shows emerin (ΔINM) predominantly at the ONM/ER. **C.** Nuclear aspect ratio of cells over-expressing (OE) EGFP alone, emerin WT, and ΔINM (mean +/- SD; multiple-comparison test post-hoc). **D.** Percent cells with leader blebs containing the nucleus for EGFP alone, emerin WT, and ΔINM. **E.** Instantaneous speeds for all cells over-expressing (OE) EGFP alone, emerin WT, and ΔINM. **F.** Percent NL, LNM, and LM for EGFP alone, emerin WT, and ΔINM. Statistical significance was determined using a Chi-squared test (EGFP alone vs. ΔINM; χ^2=2.42 x 10^{-15}). **G.** Instantaneous speeds for leader mobile (LM) cells over-expressing (OE) EGFP alone, emerin WT, and ΔINM (mean +/- SEM;

multiple-comparison test post-hoc). **H.** Leader bleb area (calculated as the percent of total cell area) for cells over-expressing (OE) EGFP alone, emerin WT, and ΔINM (mean +/- SD; multiple-comparison test post-hoc). **I.** Immunofluorescence (IF) was used to conduct a cell-by-cell analysis of emerin WT and ΔINM over-expression (OE; mean +/- SD). All data are representative of at least three independent experiments. * - $p \leq 0.05$, ** - $p \leq 0.01$, *** - $p \leq 0.001$, and **** - $p \leq 0.0001$

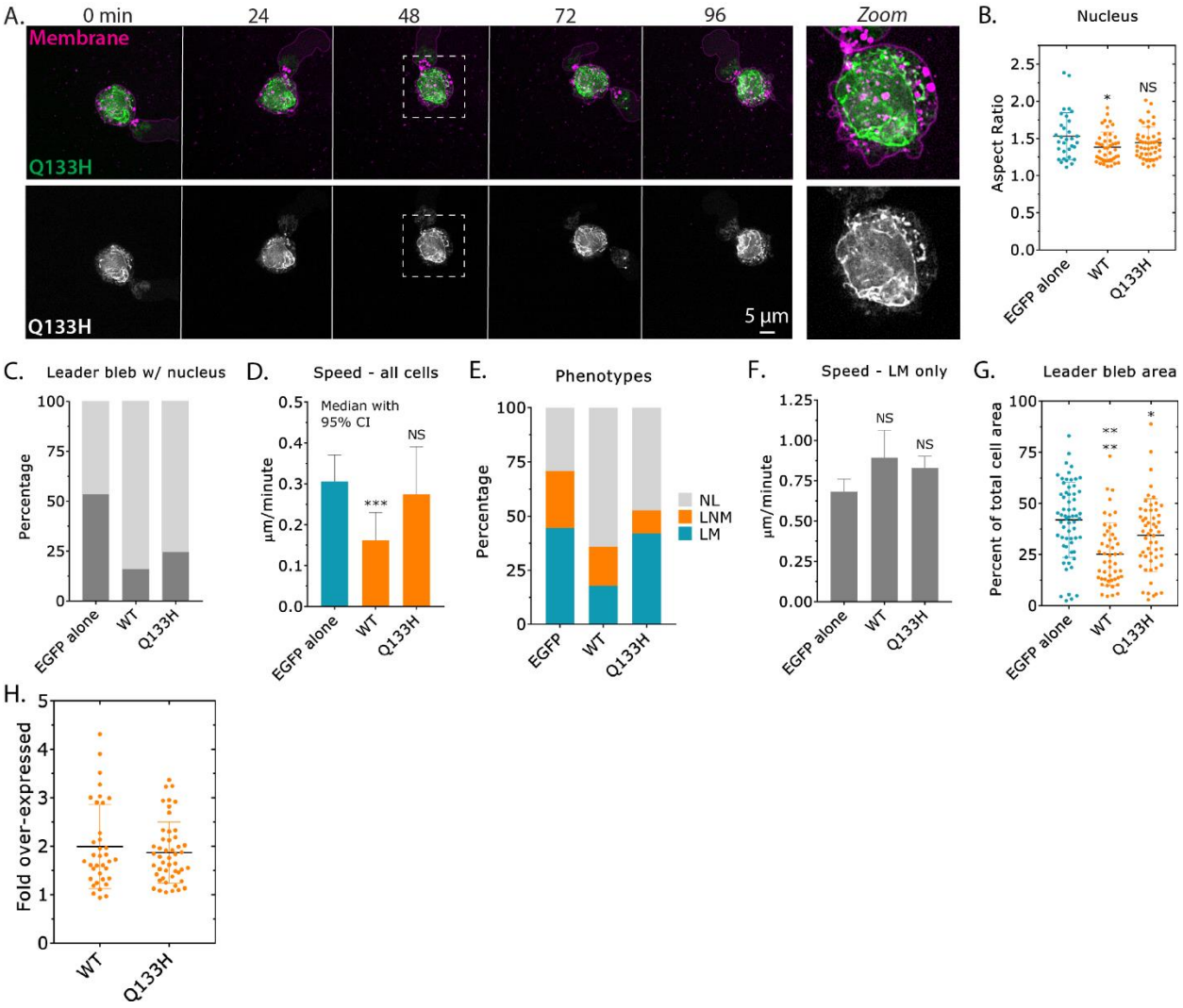


Fig. S3. **A.** Time-lapse imaging of a A375-M2 cell transiently transfected with emerin-EGFP (Q133H), which has been confined down to ~3 µm. Cells were stained with membrane dye. Zoom shows emerin (Q133H) predominantly at the nuclear envelope and ER. **B.** Nuclear aspect ratio for cells over-expressing (OE) EGFP alone, emerin WT, and Q133H (mean +/- SD; multiple-comparison test post-hoc). **C.** Percent cells with leader blebs containing the nucleus for EGFP alone, emerin WT, and Q133H. **D.** Instantaneous speeds for all cells over-expressing (OE) EGFP alone, emerin WT, and Q133H (median +/- 95% CI; multiple-comparison test post-hoc). **E.** Percent NL, LNM, and LM for EGFP alone, emerin WT, and Q133H. Statistical significance was determined using a Chi-squared test (EGFP alone vs. Q133H; $\chi^2=5.07 \times 10^{-6}$). **F.** Instantaneous speeds for leader mobile (LM) cells over-expressing (OE) EGFP alone, emerin WT, and Q133H (mean +/- SEM; multiple-comparison test post-hoc). **G.** Leader bleb area (calculated as the percent of total cell area) for cells over-expressing (OE) EGFP alone, emerin WT, and Q133H (mean +/- SD; multiple-comparison test post-hoc). **H.** IF was used to conduct a cell-by-cell analysis of emerin WT and Q133H over-expression (OE; mean +/- SD). All data are representative of at least three independent experiments. * - $p \leq 0.05$, ** - $p \leq 0.01$, *** - $p \leq 0.001$, and **** - $p \leq 0.0001$

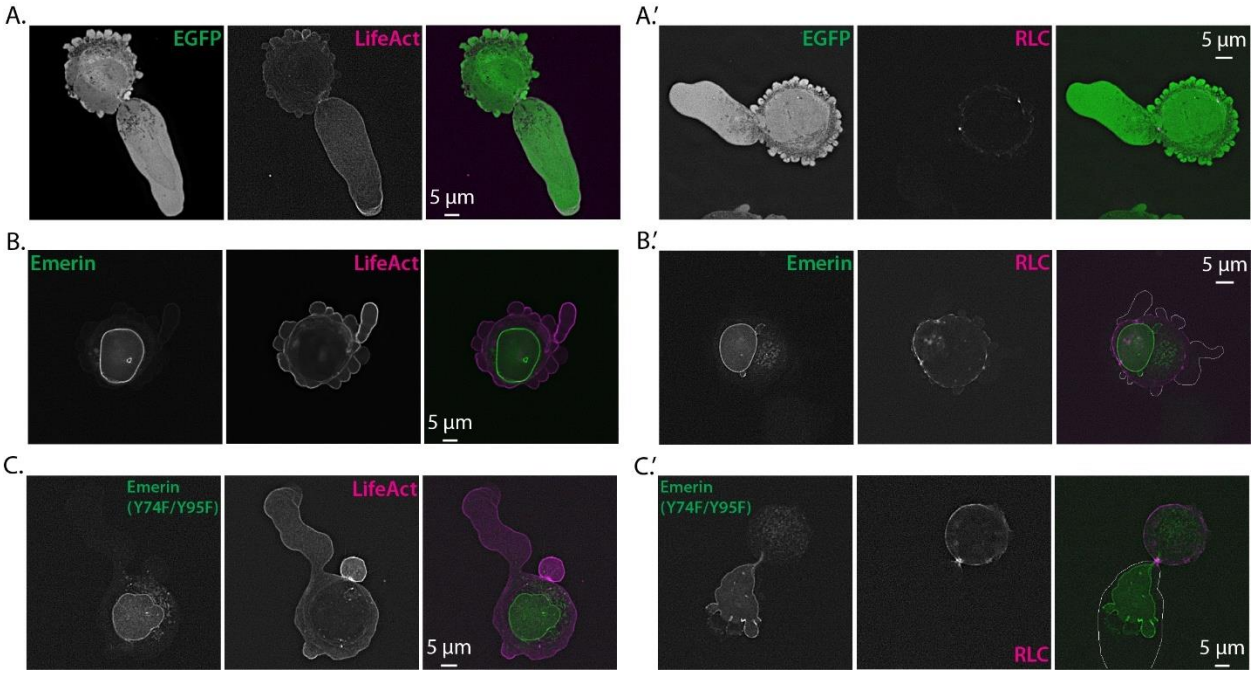


Fig. S4. A. - A.' Representative images of an A375-M2 cell confined down to ~3 µm with (A) EGFP alone and mScarlet-LifeAct and (A') EGFP alone and tdTomato-Regulatory Light Chain (RLC). **B. - B.'** Representative images of an A375-M2 cell confined down to ~3 µm with (B) emerin-EGFP and mScarlet-LifeAct and (B') emerin-EGFP and tdTomato-RLC. The cell boundary was outlined in B'. **C. - C.'** Representative images of an A375-M2 cell confined down to ~3 µm with (C) emerin-EGFP (Y74F/Y95F) and mScarlet-LifeAct and (C') emerin-EGFP (Y74F/Y95F) and tdTomato-RLC. The cell boundary was outlined in C'. All data are representative of at least three independent experiments.

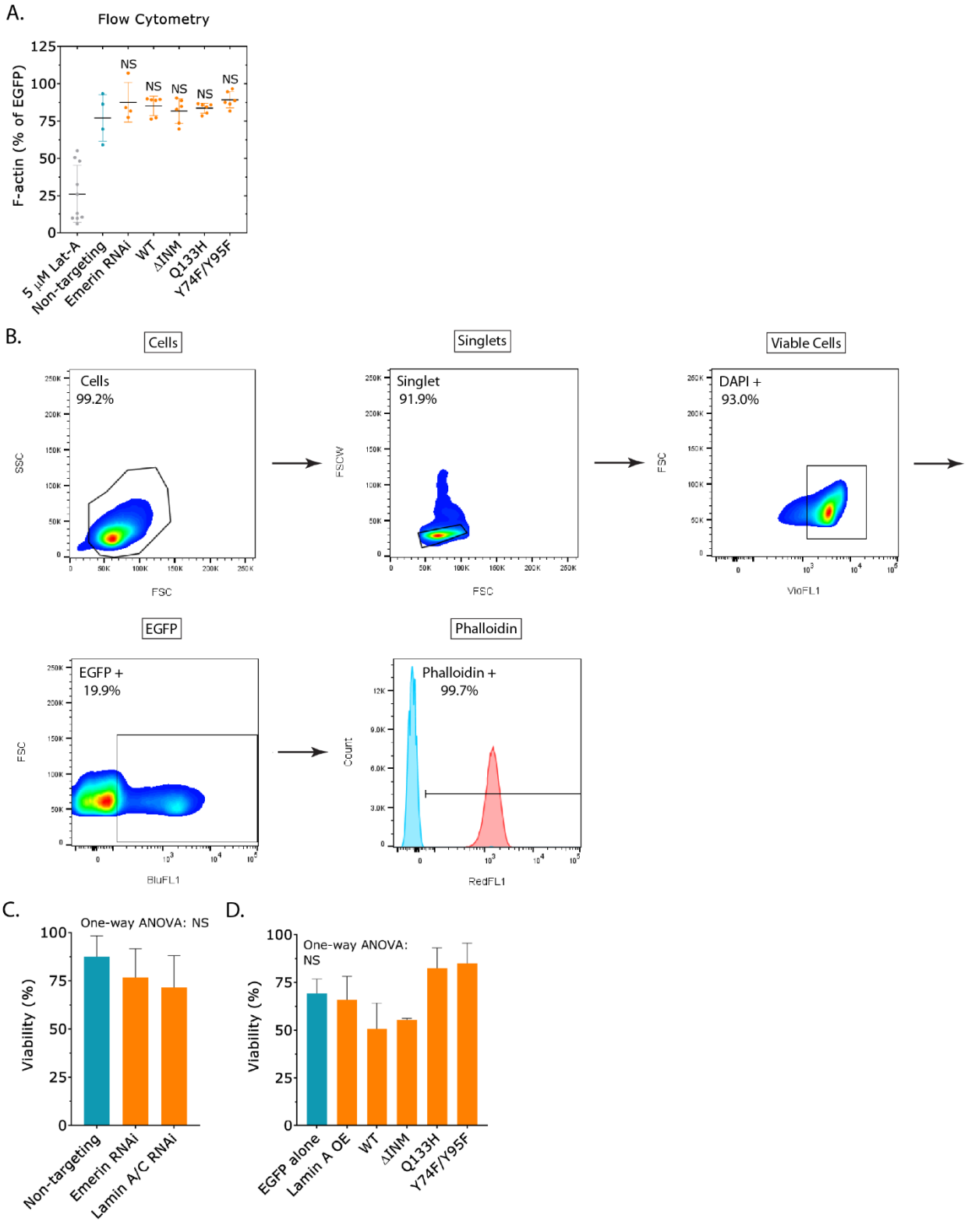
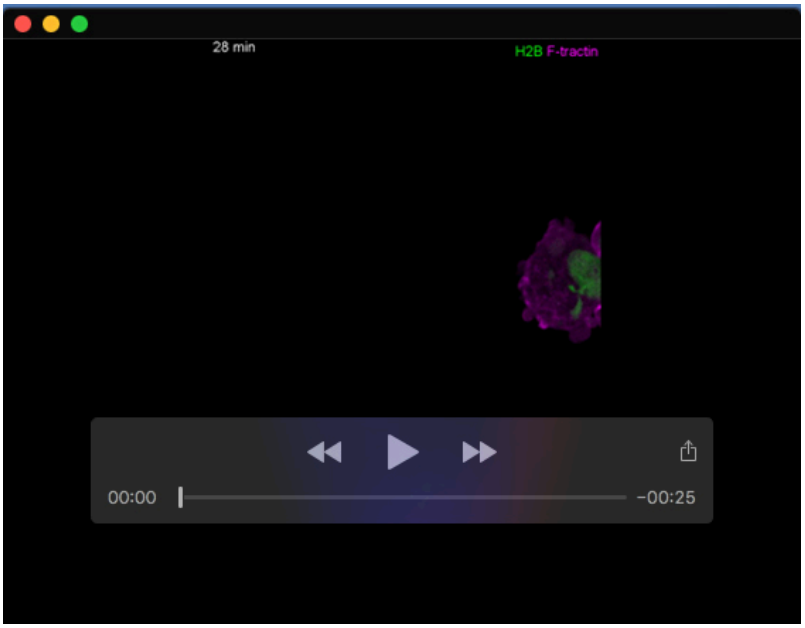
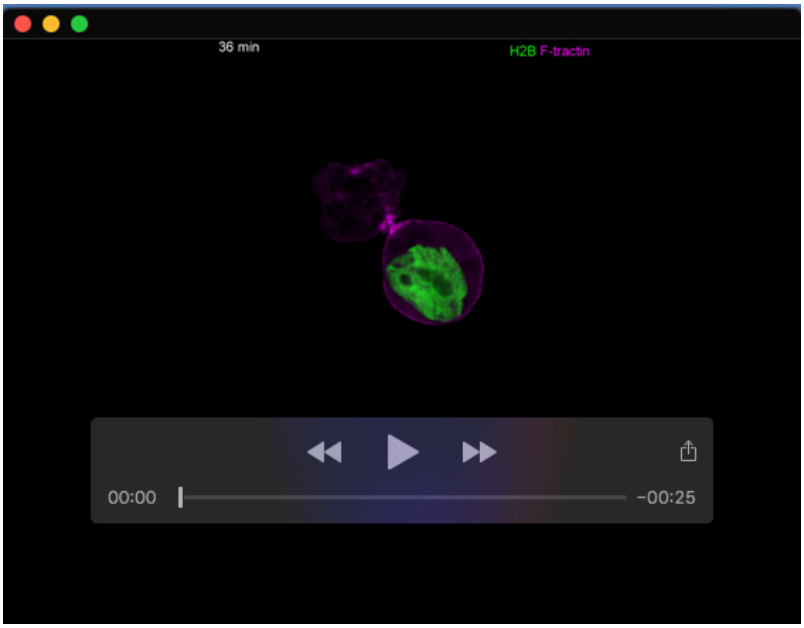


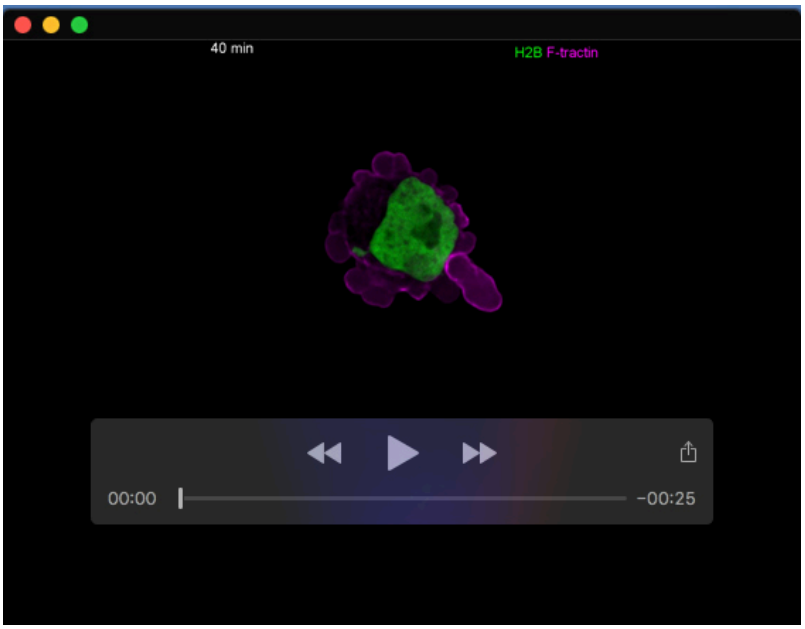
Fig. S5. A. Flow cytometry analysis of total F-actin, as measured by far red fluorescent phalloidin binding in cells treated with Latrunculin-A (Lat-A; 5 µM), non-targeting, emerin RNAi, over-expressing (OE) emerin WT, ΔINM, Q133H, and Y74F/Y95F (relative to EGFP alone) (mean +/- SD; multiple-comparison test post-hoc). Each point corresponds to a Median Fluorescence Intensity (MFI). **B.** Flow gating strategy. Cells are isolated based on instrument calibration. Single cells were isolated by comparing forward scatter width and height. Viable cells were identified using DAPI staining while EGFP fluorescence was used to identify transfected cells. Cells that were positively stained with phalloidin (red curve) were isolated based on a gate created referencing unstained cells (blue curve) for analysis. **C.** Percent A375-M2 cells confined down to ~3 µm alive after 5 hr of fluorescence imaging after non-targeting, emerin, and Lamin A/C RNAi (mean +/- SEM; One-way ANOVA). **D.** Percent A375-M2 cells confined down to ~3 µm alive (viability) after 5 hr of fluorescence imaging over-expressing (OE) EGFP alone, Lamin A, emerin WT, ΔINM, Q133H, and Y74F/Y95F (mean +/- SEM; One-way ANOVA). All data are representative of at least three independent experiments. * - $p \leq 0.05$, ** - $p \leq 0.01$, *** - $p \leq 0.001$, and **** - $p \leq 0.0001$



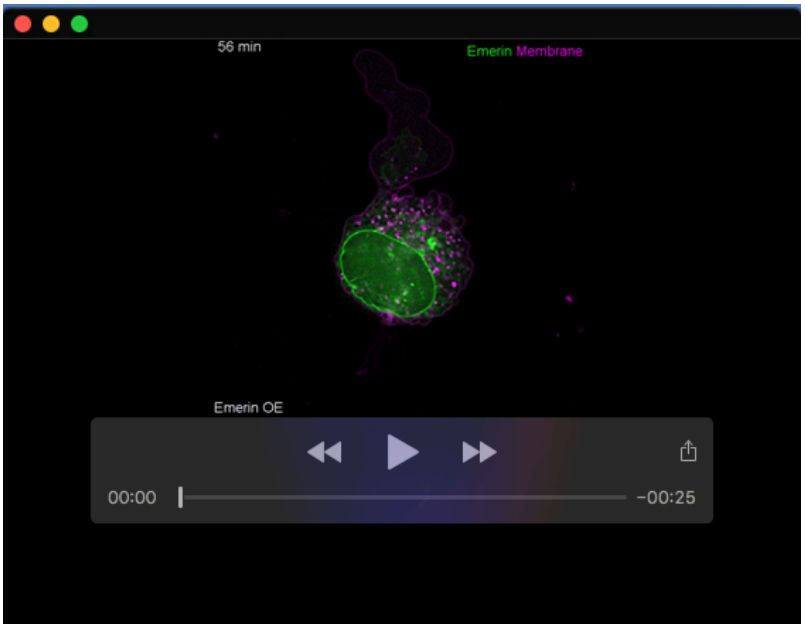
Movie 1. Time-lapse imaging of a leader mobile (LM) cell transiently transfected with H2B-mEmerald and F-tractin-FusionRed, which has been confined down to ~3 µm.



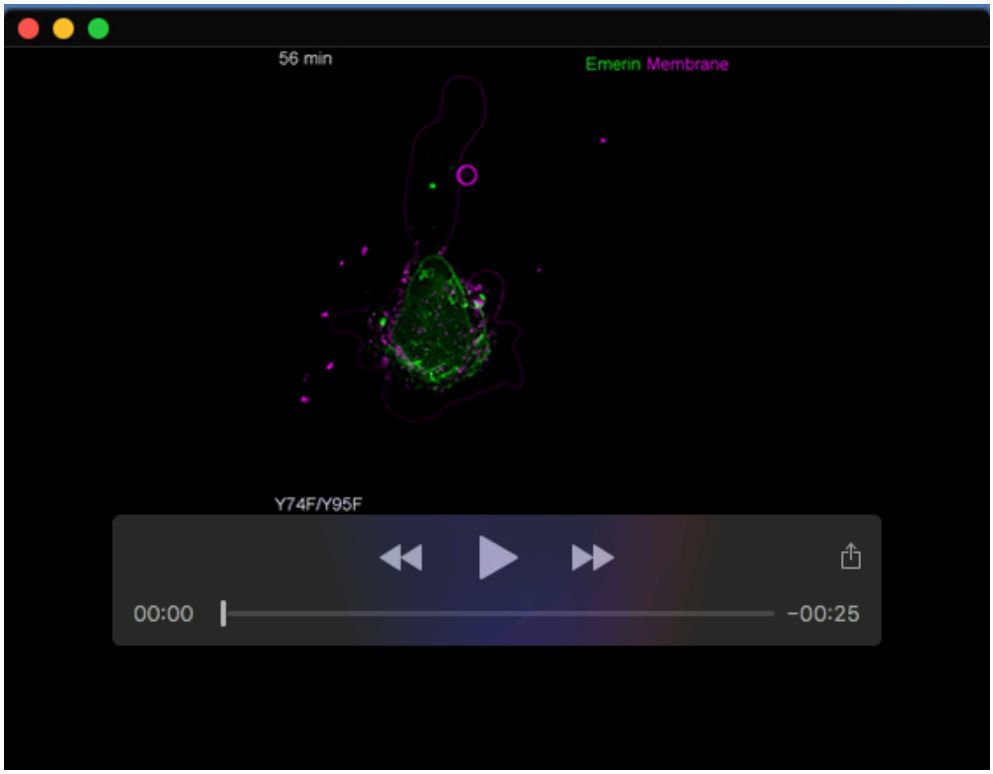
Movie 2. Time-lapse imaging of a leader non-mobile (LNM) cell transiently transfected with H2B-mEmerald and F-tractin-FusionRed, which has been confined down to ~3 µm.



Movie 3. Time-lapse imaging of a no leader (NL) cell transiently transfected with H2B-mEmerald and F-tractin-FusionRed, which has been confined down to ~3 µm.



Movie 4. Time-lapse imaging of an A375-M2 cell over-expressing (OE) emerin-EGFP, which has been confined down to ~3 µm. Cells were stained with membrane dye.



Movie 5. Time-lapse imaging of an A375-M2 cell over-expressing (OE) emerin-EGFP (Y74F/Y95F), which has been confined down to ~3 µm. Cells were stained with membrane dye.