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Supporting Material

Moving Cell Boundaries Drive Nuclear Shaping during Cell Spreading

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Supplemental Figures

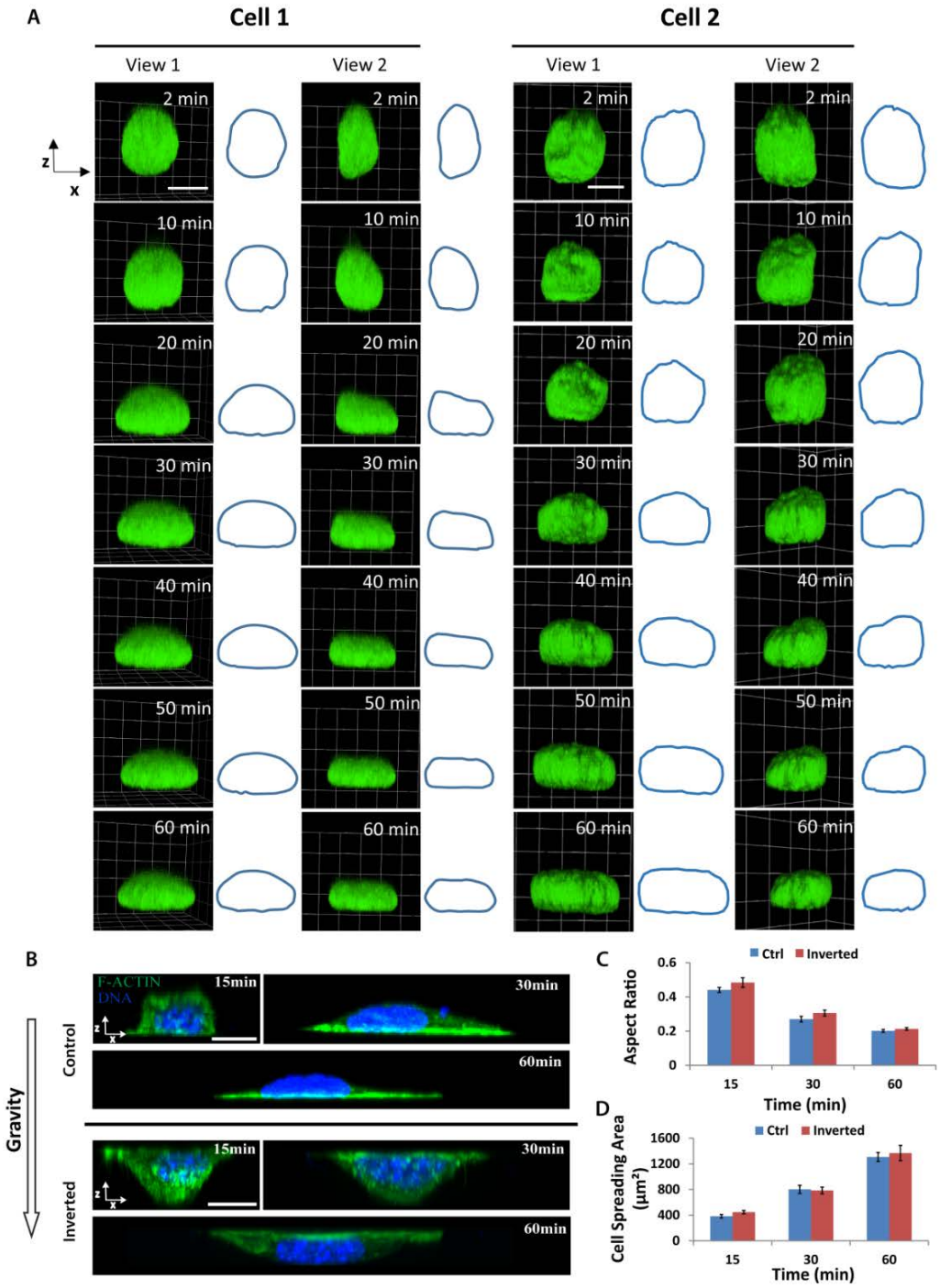

Figure S1. The dynamics of nuclear flattening against the substratum is not influenced by gravity. (A) Images show *x*-*z* view of the nuclear shape from two different view angles in two cells (corresponding to cells in Movie S1 and S2, respectively) at different time. Scale bar is 5 μm. (B) Nuclear shape changes during cell spreading are shown for normal (top) and inverted samples (bottom) at different time points in *x*-*z* view. For the inverted case, cells were allowed to first settle and attach for 5min before inverting the sample. Neither nuclear aspect ratio (C) nor cell spreading area (D) has a significant difference between control and inverted ($n \geq 32$). Scale bar is 10 μm. All data are shown as Mean ± SEM.

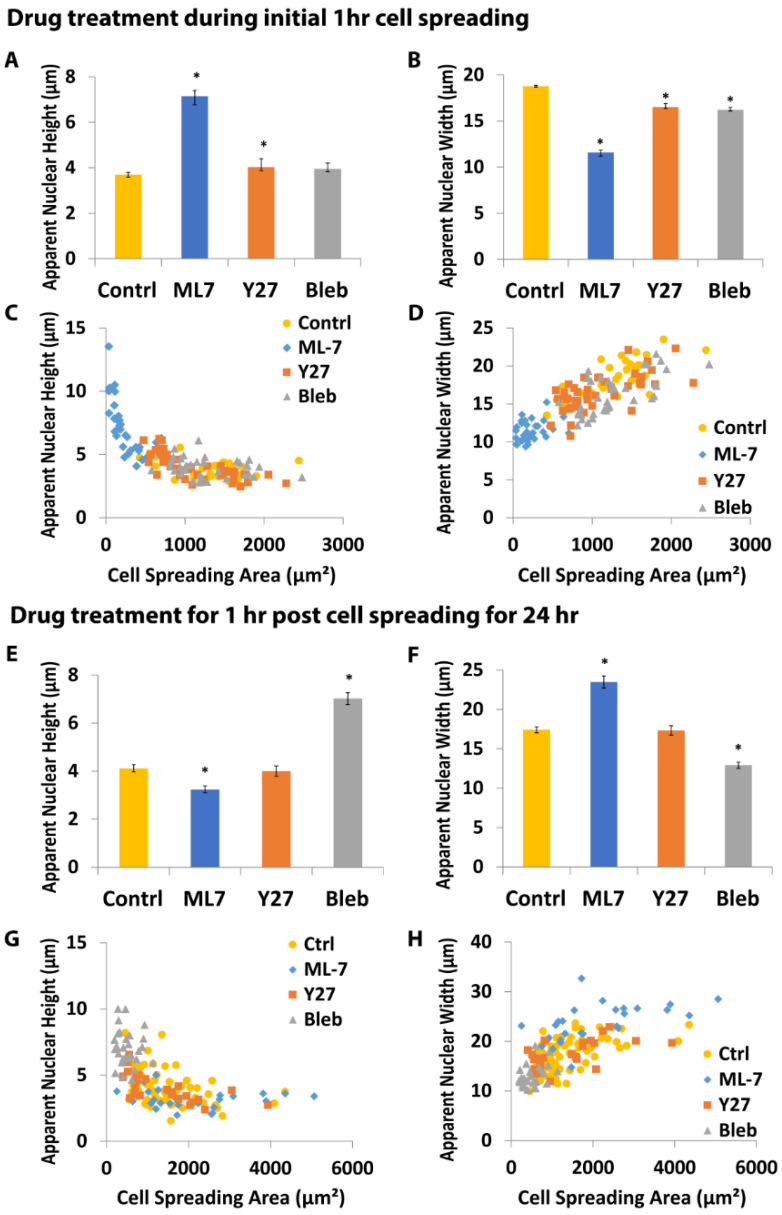

Figure S2. Nuclear flattening is independent of actomyosin contraction. (A). Shown are nuclear height and width (B) during initial cell spreading under myosin inhibition. Cells were pretreated with myosin inhibitors for 1 h, suspended and seeded onto glass bottomed dishes where they were allowed to spread in the presence of the drug for 1 h. The average nuclear height measurements show that ML-7 treated cells have a rounded nucleus and correspondingly small average nuclear width in (B). C. Nuclear height correlates with the degree of cell spreading. In ML-7 treated cells, the large nuclear heights correlated with the small area of cell spreading. D. Nuclear widths were smaller reflecting unflatten nuclei only in ML-7 treated cells, consistent with the fact that the cells were themselves not spread. $n \geq 31$, * indicates $p<0.05$; all comparisons are with untreated controls. The experiments in E-H show the corresponding results of treating well-spread cells (cultured overnight on fibronectin-coated glass-bottomed dishes) with myosin inhibitors. $n \geq 24$, * indicates $p<0.05$; all comparisons are with untreated control. All data are shown as Mean $\pm$ SEM.

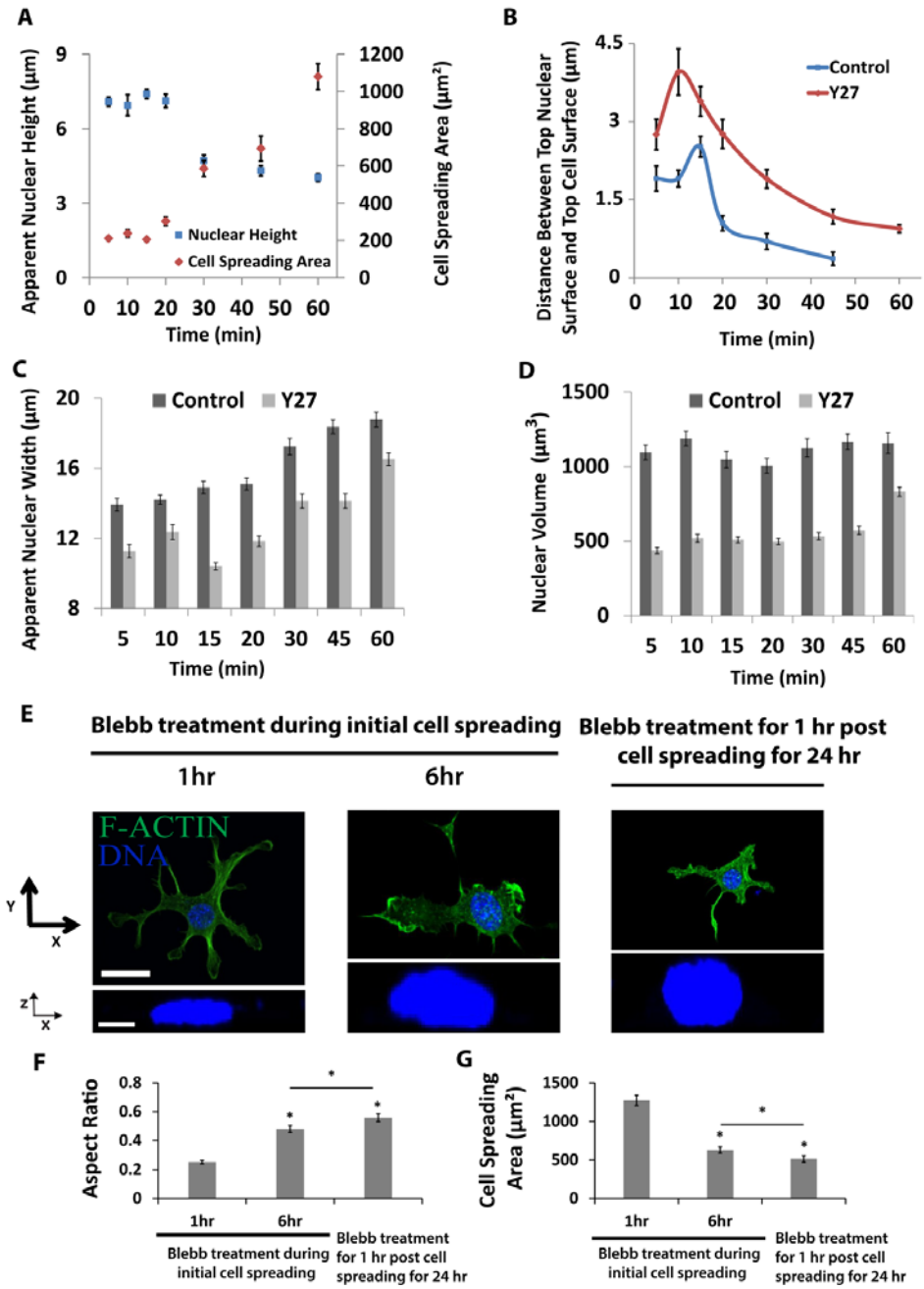

Figure S3. Inhibition of myosin activity with Y-27632 does not alter the qualitative features of dynamic nuclear flattening during cell spreading, and blebbistatin prevents cell spreading at later times resulting in final nuclear rounding. As seen in (A), the time-dependent changes in nuclear height ($n \geq 33$) still occur on Y-27632 pre-treatment while the cells spread (an initial lag time in the flattening of the nucleus is attributable to an initial lag time in the spreading). Y-27632 treatment increased the separation between the apical cell and nuclear surfaces (B) ($n \geq 29$). The decrease in nuclear width (C) and volume (D) in Y-27632 treated cells suggests that myosin contraction plays a role in the nuclear widening process ($n \geq 29$). E. At 1 hr images show the spreading of blebbistatin-treated cells ($n \geq 30$) in *x*-*y* view and the associated nuclear height in *x*-*z* view. Given more time (6 hours), the area of cell spreading decreased significantly and the nucleus was rounded. Consistent with this, nuclear aspect ratio (F) at 6 hours was higher than control (G). * indicates $p<0.05$; all comparisons are with untreated control. Scale bar is 10 µm in both *x*-*y* view and 5 µm for the *x*-*z* view. All data are shown as Mean ± SEM.

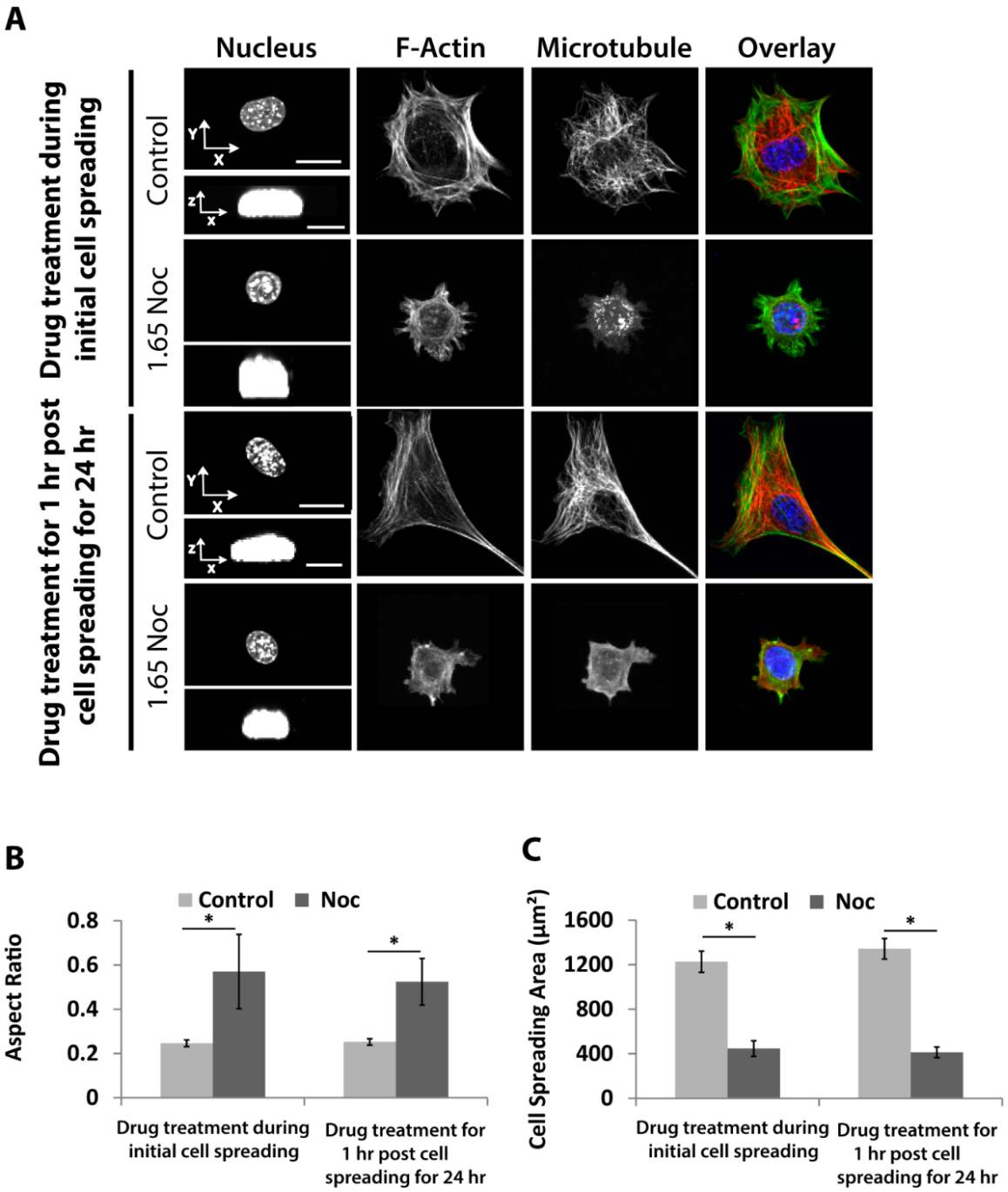

Figure S4. Disruption of microtubules by nocodazole rounds up the nucleus but also prevents cell spreading. (A) Immunofluorescence images are shown of 1.65 μM nocodazole treated cells in two kinds of experiments, one in which the drug was treated during initial cell spreading, and the other in which cells that were well-spread were treated with nocodazole. For both experiments, no distinct microtubules were visible, but nuclei were not flat as the cells had rounded morphologies. The quantifications of nuclear aspect ratio (B) and cell spreading area (C) again show that the nuclear rounding is correlated with the extent of cell spreading. $n \geq 30$, * indicates $p<0.05$; all comparisons are with untreated control. Scale bar is 10 μm in both *x*-*y* and *x*-*z* view. All data are shown as Mean ± SEM.

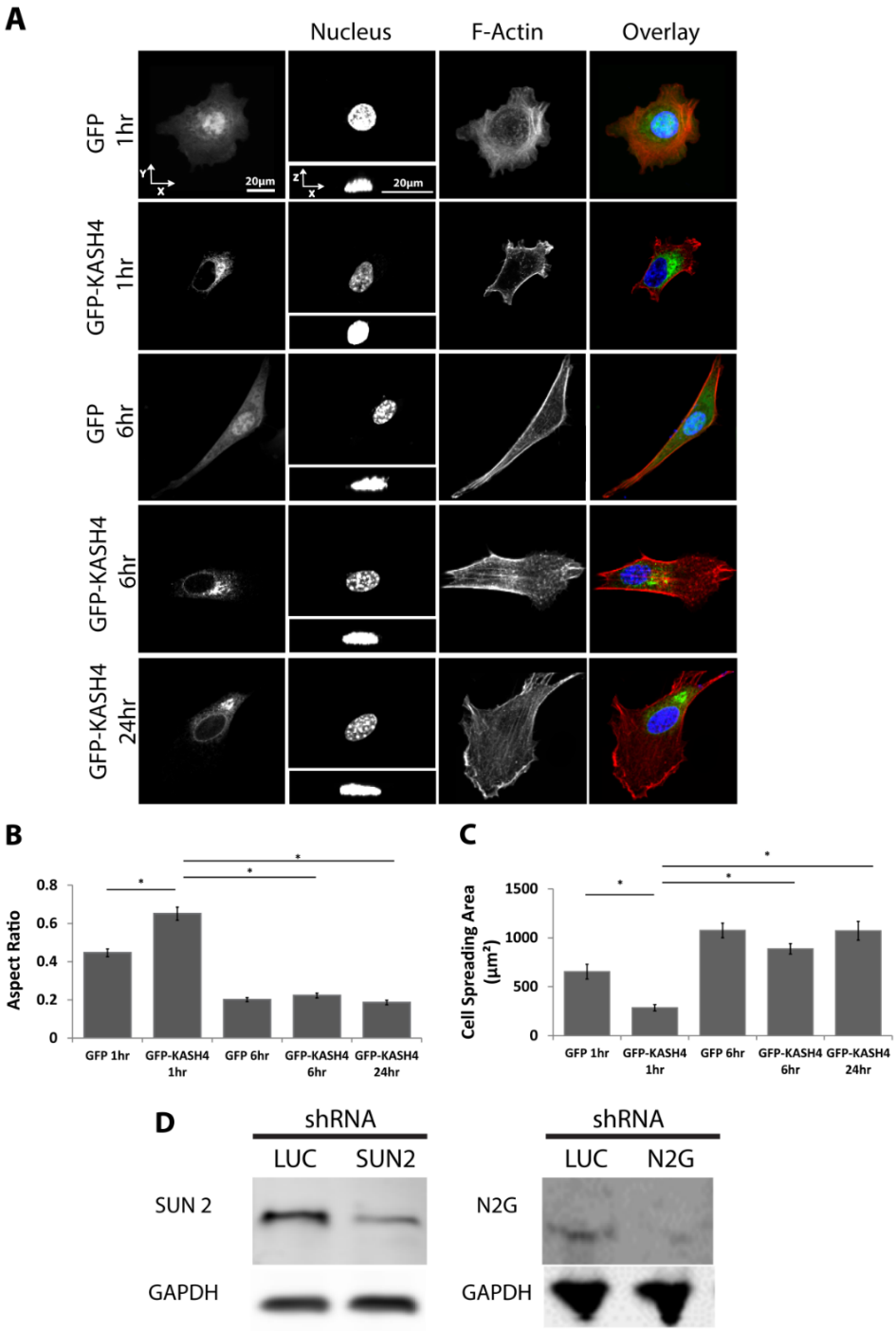

Figure S5. GFP-KASH4 overexpression slows down but does not prevent cell spreading and nuclear flattening. A. Images show GFP-KASH4 cells after one hour of spreading have unflattened nuclei and not so-well spread cells; but nuclei become flat at long times (6 and 24 hours) with fully spread cells. The changes of nuclear aspect ratio and cell spreading area in (B) and (C) respectively, indicate that the rounded nucleus in cells expressing GFP-KASH4 at 1 hour is correlated with a slower cell spreading rate. D. Western blots of indicated proteins immunoprecipitated by SUN2 or Nesprin 2G antibodies from NIH 3T3 cell lysates. $n \geq 37$, * indicates $p<0.05$. Scale bar is 20 μm in both *x-y* and *x-z* view. All data are shown as Mean ± SEM.

Supplemental Movies

Movie 1: Movie of *x*-*z* view from two different angles of a GFP-histone labeled nucleus during the flattening process.

Movie 2: Movie of *x*-*z* view from two different angles of another GFP-histone labeled nucleus during the flattening process.

Movie 3. Simulation of cell spreading and nuclear flattening, using parameters shown in Table S1. The nucleus distends and translates to the substratum then widens as the cell spreads.

Movie 4. Simulation of cell spreading and nuclear flattening with assembly of network at the apical cortex turned off ($v_a = 0$). All other parameters are shown in Table S1. The nucleus shape mimics changes in boundary shapes.

Movie 5. Simulation of cell spreading and nuclear flattening with lateral friction turned off ($\eta = 0$). The cell stops spreading and the nucleus stops flattening at a steady state.

Movie 6. Simulation of cell spreading and nuclear flattening with network contractile tension turned off ($\sigma_c = 0$), showing no effect of network contractility on cell and nuclear shape *changes*.

Movie 7. Simulation of cell spreading and nuclear flattening with nuclear surface area tension turned off ($T_{nuc} = 0$) showing the nucleus continues to flatten with cell spreading when there is no area constraint on the nucleus.