Force-Induced Changes in Subnuclear Movement and Rheology

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Force-Induced Subnuclear response

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Supplemental Figures

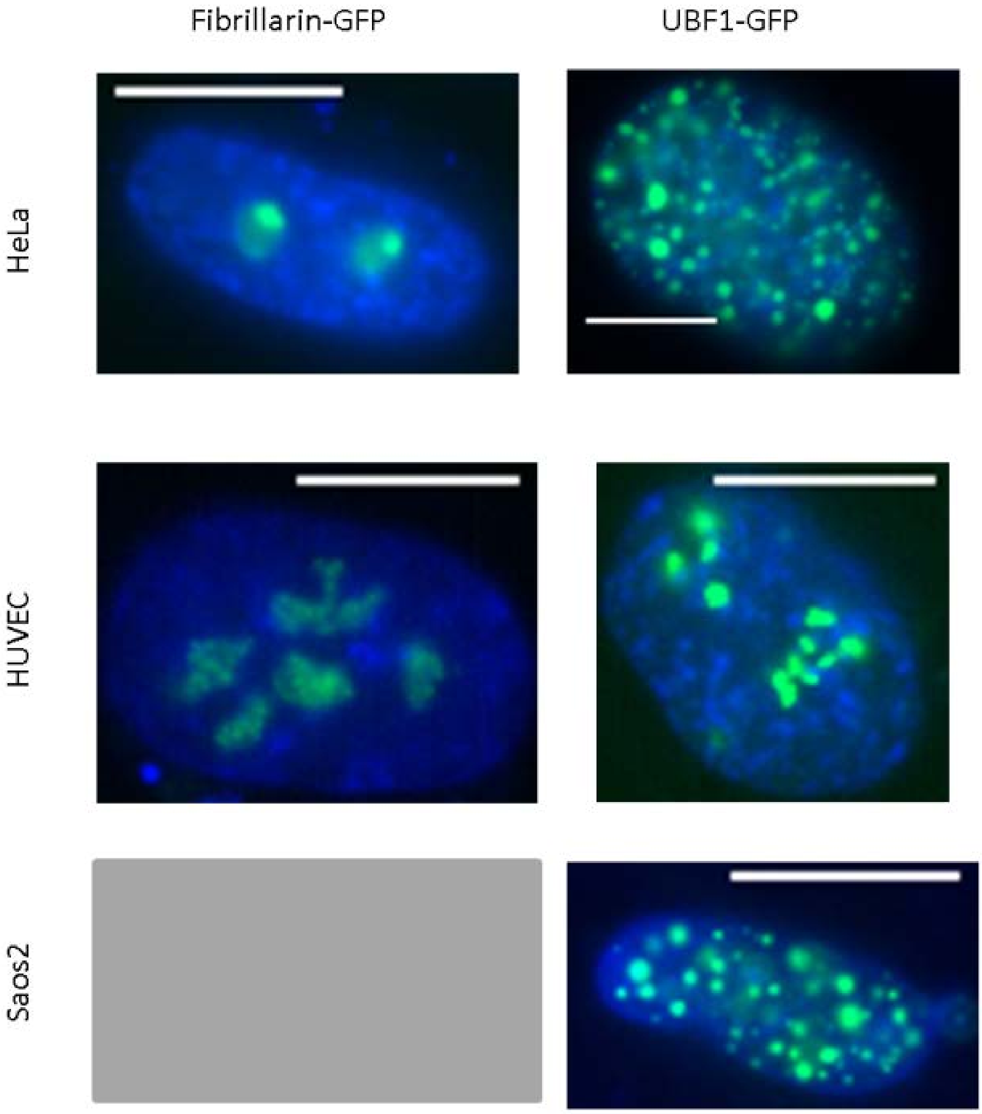

Figure S1: Representative images of cells transfected with Fibrillarin-GFP and UBF1-GFP. HeLa cells and HUVECs were transfected with Fibrillarin-GFP a nucleolar marker and UBF1-GFP. Saos-2 cells were transfected with UBF1-GFP. Blue color is from Hoechst 33342 to show nuclear compartmentalization.

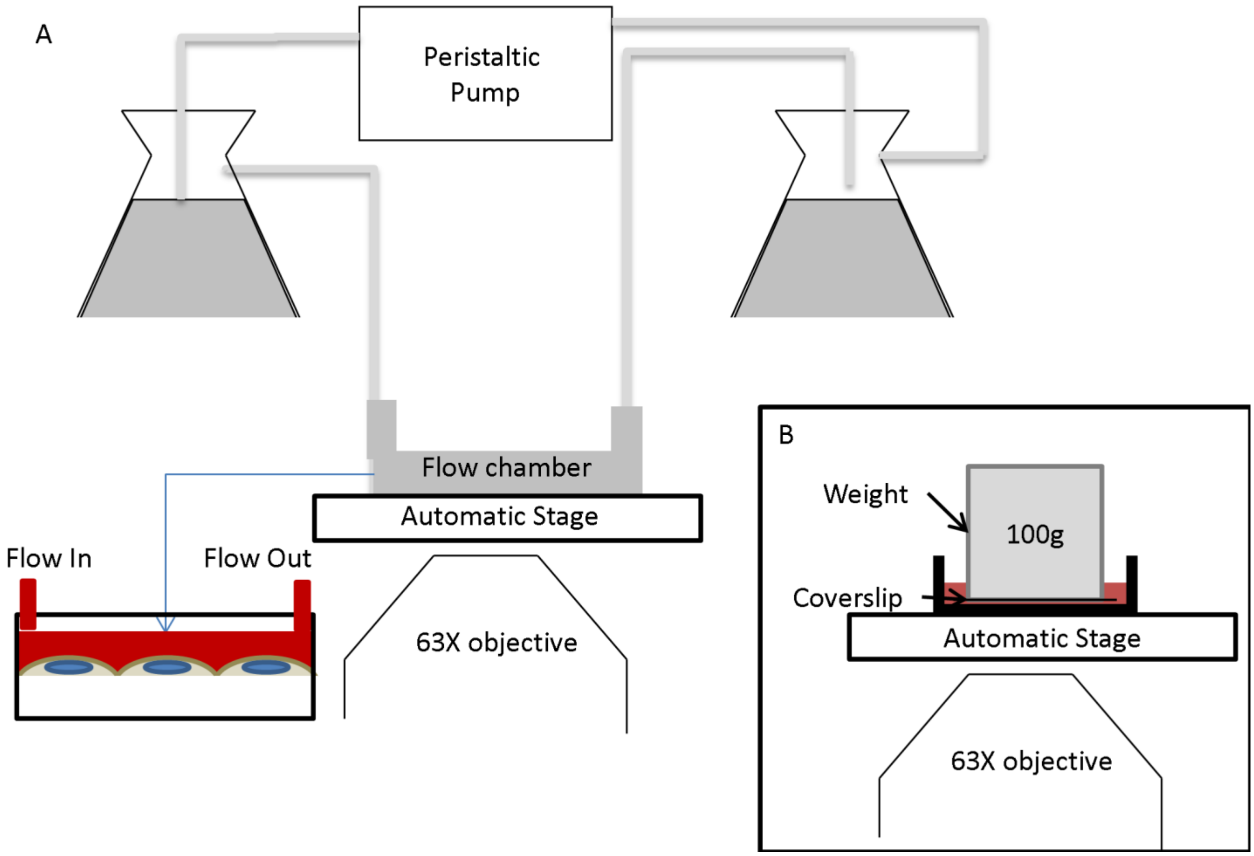

Figure S2: Shear and compression apparatuses used for mechanical stimulation of cells. A) The shear stress apparatus consisted of two media reservoirs, a peristalitic pump, and ibidi parallel plate flow chambers with coverslip bottoms for imaging. Transfected cells were seeded in the chambers, given 24 h to adhere, and then subjected to either 5, 10, 20, or 40 dyn/cm^2 shear stress for 2 h. B) Cells were seeded in Matek dishes with coverslip bottoms and imaged for 2 h as they experienced either no force, or compressive force of 0.1 MPa from a 100 g weight.

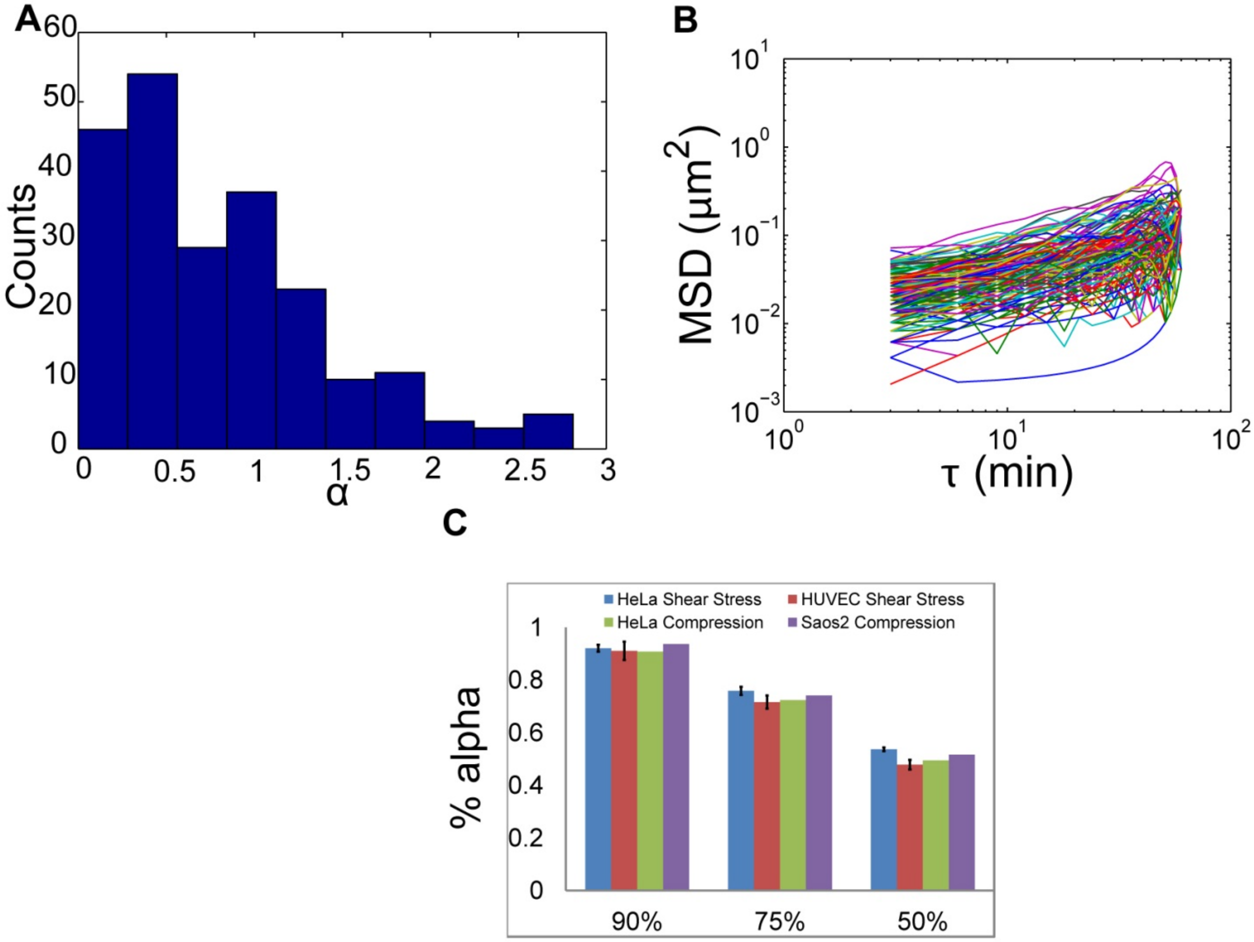

Figure S3: Intranuclear tracking data shows a range of subcellular response. A)The distribution of the values of α of HUVECs exposed to 20 dyn/cm^2 shear stress for 1 h with 3 min time steps. Individual tracks were fit to $\langle \Delta r^2 \rangle \propto \tau^{\alpha}$ and the values for α were binned. There is a wide distribution of values of α, showing the presence of sub-diffusive, diffusive, and enhanced diffusive characterized motions between different fiducial points. B) Raw data of individual track of HUVECs exposed to 20 dyn/cm^2 shear stress. Consistent with the binned values of alpha, there is a range of response sub-diffusive, diffusive, and enhanced diffusion. C) Contributions of 90%, 75%, and 50% lowest MSD values to the mean value of α measured for different data sets. The percentage values of α are approximately equal to the percentage of the data they represent, suggesting that the data is weighing into the summary statistic α evenly and outlying data is not driving the outcome.

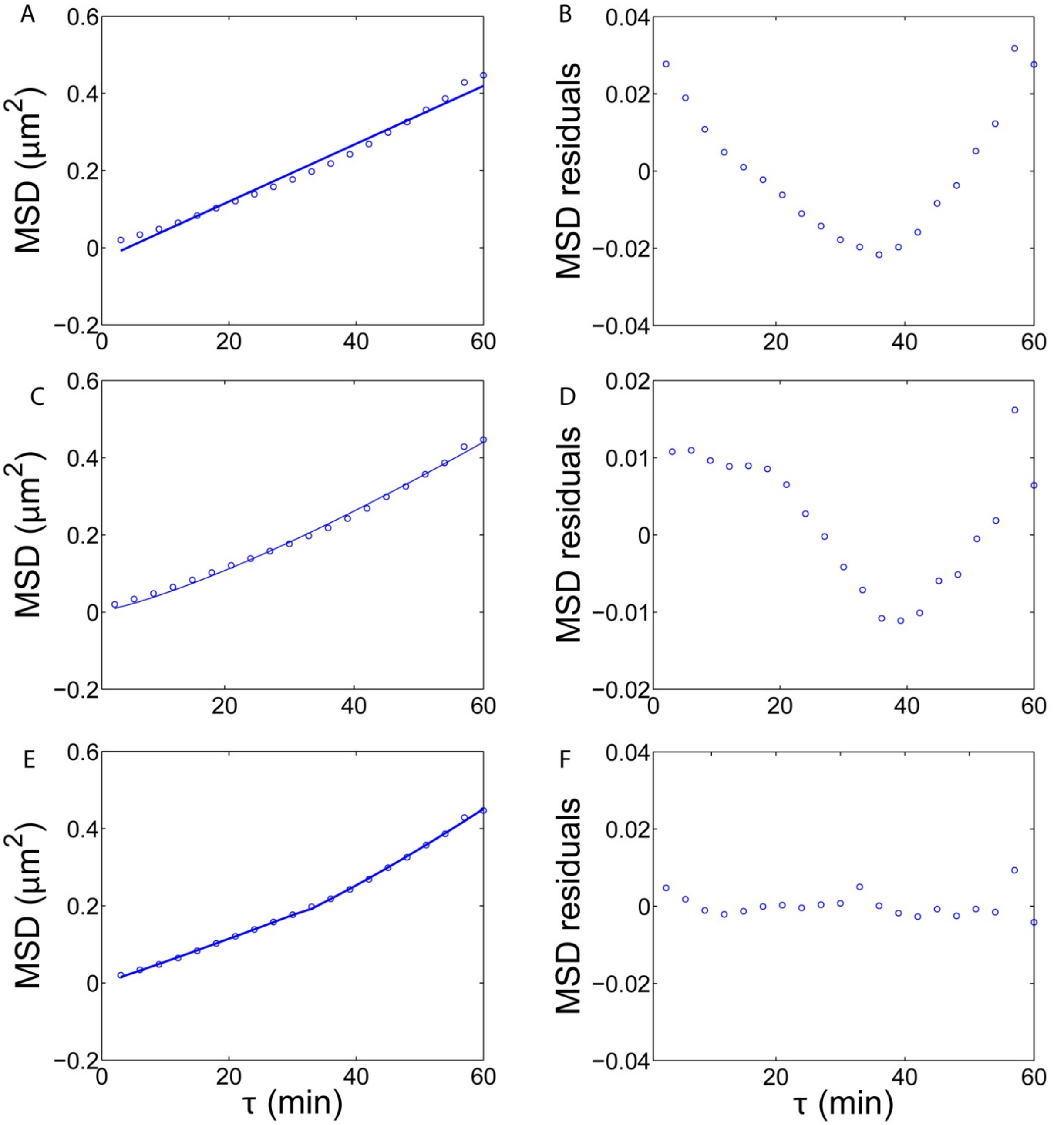

Figure S4: **Plots of MSD values, fitted curves, and associated residuals.** To further demonstrate the improved fit by using the anomalous diffusion model and the anomalous diffusion model with crossover time a series of MSD plots for the HeLa cell data sheared at 10 dyn/cm^2 for 1 hour have been prepared. A) A linear fit of MSD data and the B) corresponding residuals which show a clear parabolic order suggesting that an improved fit can be found. C) An anomalous diffusion model fit and the D) corresponding residuals. The residuals suggest that the fit is better than the linear fit but deviates both at early times and late times and shows some order similar to the linear fit. E) the crossover time model with two anomalous diffusion curves for early and late time and the F) corresponding residuals. The residuals are greatly reduced and do not show ordering as seen in the prior examples. Additionally, the data is not overfit as indicated by AIC (Table 1).

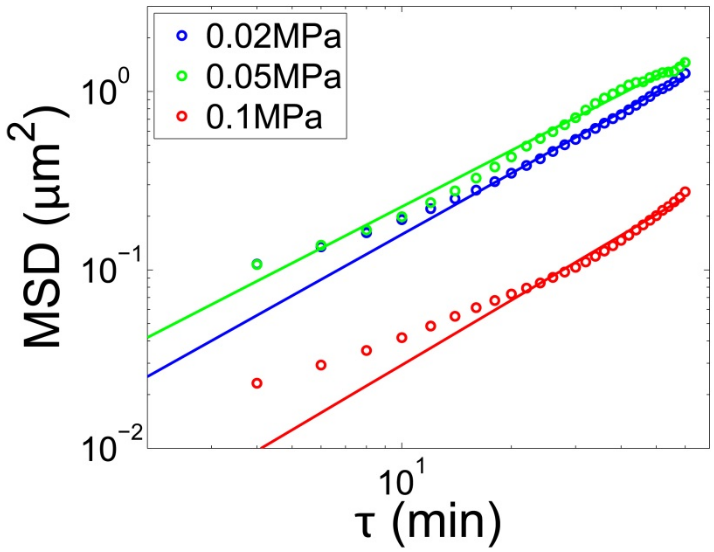

Figure S5: Plots of 0.02, 0.05, and 0.1MPa compressive stress. The 0.02 and 0.05 MPa compressive stresses are very similar and were experimentally problematic. In the presence of a second apical substrate and the lower compressive stresses the cells would move out of the pre-designated focal plane often causing a loss of focus and quantitatively showing a large increase in MSD. Due to the experimental difficulties and the corresponding results the lower compressive stresses were not continued and additional focus was placed on the 0.1 MPa compressive stress which showed results more consistent with shear stress.

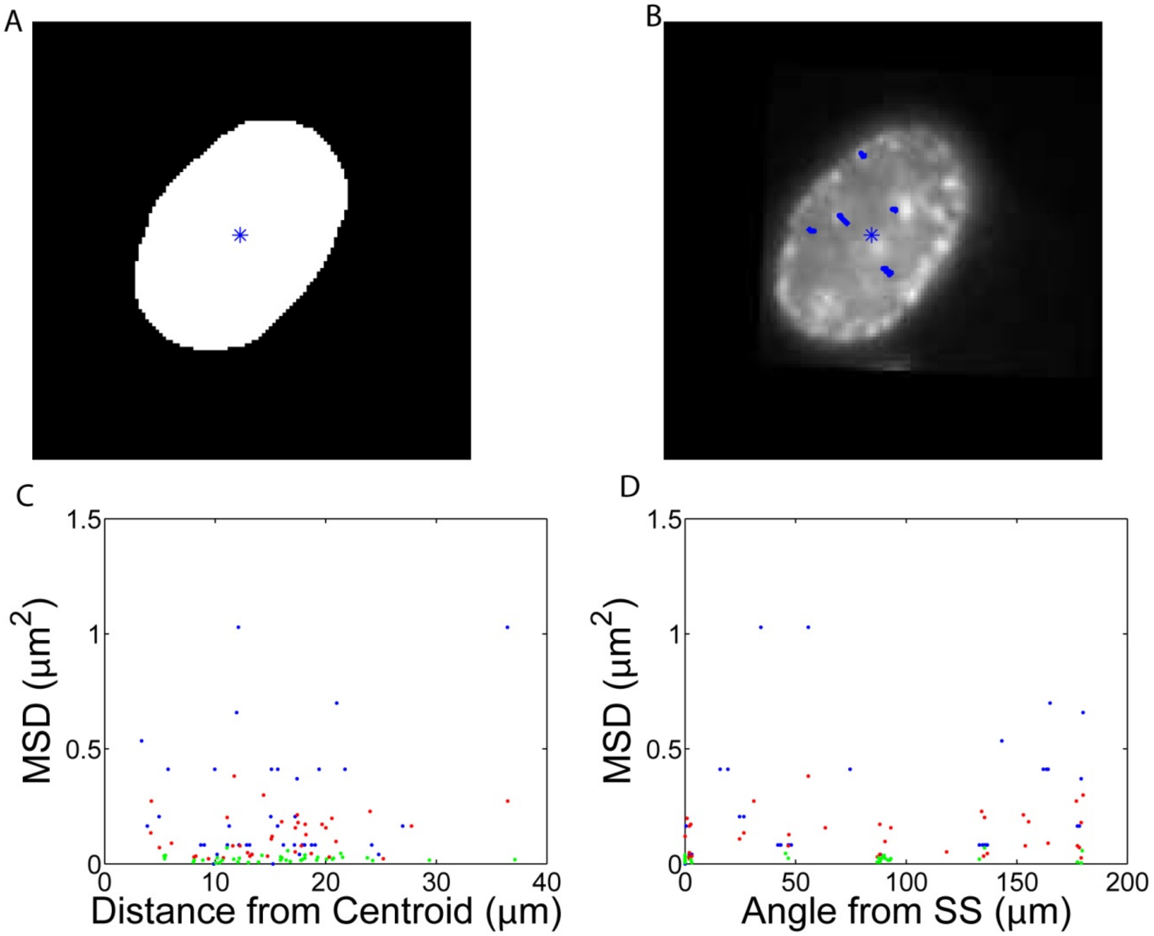

Figure S6: Mapping MSD response to radial nuclear location and orientation with respect to shear stress. A) Mapping of the subnuclear movements was done by first utilizing the Hoechst 33342 single to create a nuclear mask and locating the centroid. B) The radial distance of the traced points from the centroid for every tracked point was calculated over the course of the experiment. C) Data from HUVECs sheared for 1 h at 40 dyn/cm^2 were plotted as MSD versus distance (green = 3 min, red = 30 min, and blue = 60 min). There appears to be a consistent spread of high MSD values at all radial locations. D) The angle of the tracked point with respect the shear stress was also plotted such that MSD values as green = 3 min, red = 30 min, and blue = 60 min. Again, there was no detectable correlation suggesting that the shear stress did not consistently bias directed subnuclear motion.

Table S1: Subnuclear localization and angle with respect to shear stress.

Time Lag (min)	Shear Stress Applied	Slope (μm) (MSD vs distance from centroid)	p value (MSD vs distance from centroid)	p value (MSD vs angle from shear stress)
3	5 dyn/cm^2	2.7563e-04	0.5687	0.6050
30	5 dyn/cm^2	0.0460	0.4309	0.8592
60	5 dyn/cm^2	0.0120	0.3223	0.6676
3	40 dyn/cm^2	-3.7122e-05	0.9511	0.3082
30	40 dyn/cm^2	9.4712e-04	0.7812	0.5478
60	40 dyn/cm^2	0.0016	0.8570	0.8850

To determine if there was any correlation of fiducial marker localization or angle of shear stress with MSD, we performed a t-test for existence of a significant slope, Figure S6. In all cases, the null hypothesis that there is no linear relationship between radial location and MSD values held or angle to shear stress and MSD. The movement of the fiducial markers is affected by their immediate microenvironment and does not show associations with changes in the location throughout the nucleus.