***Methods***

**Oocyte collection**

Adult female *Xenopus laevis* frogs were anesthetized for 15 minutes in 0.1% MS-222 solution. Oocytes were surgically isolated following an IACUC approved protocol and incubated at 18ºC in OR2 solution, as previously described[23](#_ENREF_23). To remove the follicular layer, oocytes were separated using forceps and incubated in 2 mg/ml collagenase (Sigma) for 1 hour and 20 minutes at 18ºC. Stage V and VI oocytes were used for all experiments and were identified based on their diameter of 1-1.3 mm[29](#_ENREF_29). The oocyte and the subsequently dissected GV were imaged using a Zeiss stereoscope to confirm size.

**DNA and mRNA constructs**

Cloning of DNA constructs was generally completed using either C-terminal EGFP-pCS2+ or RFP-pCS2+ vectors. A Lifeact::GFP construct was cloned using codon-optimized oligos for the small peptide Lifeact (ATGGGAGTGGCTGATCTGATTAAGAAGTTTGAATCTATTTCTAAGGAAGAA ) with a nuclear localization signal (CCAAAGAAGAAGAGAAAGGTG) and later digested with Xho1 and Xmal. Utrophin-230 and Utrophin-261 were cloned from cDNA containing utrophin (gift of Dyche Mullins) into EGFP-pCS2+ vectors with a nuclear localization signal. The full length version of utrophin (Utrophin-261) was used to visualize the filaments, while the shorter version Utrophin-230 did not express correctly[6](#_ENREF_6). Coilin was cloned from a GFP::coilin-pCS2+ vector (gift of Joseph Gall). In the case of visualizing the lamins, a RFP::Lamin B3 construct was made using *X. laevis* cDNA from Lamin B3 (Accession Number: BC078034, Thermo Scientific) and where the RFP was on the N-terminus. NPM1::RFP, NPM1::GFP, and Fibrillarin::GFP were used as previously described[23](#_ENREF_23). mRNA was produced using SP6-based capped RNA transcription kit from linearized DNA and later purified with RNeasy spin columns and stored at -80ºC.

**Fluorescent microspheres for passive microrheology**

Green and red fluorescent microspheres (Invitrogen) of 0.1, 0.25, 0.5 and 1 µm in radius with carboxyl surface chemistry were passivated with PEG-amine (MW 2000 or MW 5000, as purchased) (Rappe Polymere, Germany) to prevent non-specific binding. The PEGylation reaction was completed using EDC chemistry in MES buffer[33](#_ENREF_33). To test the efficacy of the reaction, PEGylated beads were compared to carboxyl beads and beads with BSA adsorbed to the surface with incubation of fluorescent BSA overnight. PEGylated beads had significantly reduced fluorescent BSA adsorption and were deemed passivated[34](#_ENREF_34" \o "Valentine, 2004 #104). Prior to microinjection, the microspheres were sonicated for 1-2 minutes.

**Metallic microspheres for sedimentation experiments**

Metallic Dynabeads Myone Streptavidin C1 (Invitrogen) were used in the sedimentation experiments, with a nominal density of 1.8 g/cc and radius of 0.5 microns. The beads were washed three times with PBS, and incubated with a 25:75 molar ratio of biotin-4-fluorescein and biotin-PEG (MW 5000) in excess for thirty minutes under constant rotation, and were later washed three times with PBS. This ratio of fluorophore to PEG was sufficient to ensure a high signal-to-noise for visualization and high passivation from non-specific binding.

**Microinjection and GV dissection**

Microinjections were performed using a Narishige micromanipulator connected to a PicoPump PV820 on a Zeiss dissecting microscope. Microneedles were pulled from borosilicate glass with O.D. 1 mm and I.D. 0.78 mm using Sutter Instrument Model P-97. After sonication, approximately 5-10 nl of fluorescent microspheres with modified surface chemistry were microinjected directly into the germinal vesicle of the oocytes. Approximately 20-50 nl of NPM1::GFP, NPM1::RFP, Fibrillarin::GFP, Lifeact::GFP, Utrophin-261::GFP, or RFP::Lamin B3 were injected into the cytoplasm of oocytes. All oocytes were incubated overnight for accumulation of translated protein and for the recovery of the GV upon microinjection of microspheres. After overnight incubation, the GVs were manually extracted in mineral oil using forceps and a hair loop[35](#_ENREF_35). The GV was then transferred using a pipette into an imaging chamber, consisting of a glass coverslip and slide separated by a 1 mm thick silicone well (Grace Bio-labs).

**Actin disruption**

Oocytes were incubated in 2 µg/ml of latrunculin A (Sigma) and 0.8% DMSO (Sigma) for approximately one to two hours under constant slow rotation[36](#_ENREF_36). For cytochalasin-D experiments, oocytes were incubated in 20 µg/ml of cytochalasin D (sigma) and 0.4% DMSO for 2-3 hours. For Xpo6 experiments, approximately 10 nl of human Xpo6 (gift of D. Görlich) was microinjected in the GV of oocytes, and after one hour of incubation, nuclei were dissected and prepared for imaging[8](#_ENREF_8). After actin disruption, nuclei became significantly more difficult to dissect and nucleoli were seen to exhibit fusion and sedimentation events.

To stabilize the actin filaments, tropomyosin (Sigma) was dissolved in ultra pure water at a concentration of 5 mg/ml and was microinjected (approximately 5-10 nl) into the nucleus. To bundle the actin filaments, fascin, a gift from Gijsje Koenderink, was microinjected into the GVs at 1.54 mg/ml or 28 µM and incubated for 1-2 hours. To cross-link the actin filaments, alpha-actinin (Sigma) was microinjected into the GVs at 6 mg/ml. To remove ATP, the ATPase apyrase (Sigma) was microinjected into the GV at a concentration of 2 mg/ml and incubated for 30 min – 1 hr. In all of these microinjections, rhodamine dextran was coinjected to confirm that the protein was microinjected prior to completion of the experiment.

**Deformation of GV**

To visualize the location of the microneedle tip, glass microneedles were loaded with 0.02 µm fluorescent blue microspheres (Invitrogen) and imaged with 405 nm light. The needle was mounted on an Eppendorf micromanipulator. The GV was placed in a pool of mineral oil contained by a silicone well and on top of a glass cover slip. The microneedle was placed near the edge of a live GV in immersion oil. During the experiment, the needle was used to deform the edge of the GV under low and high strain repeatedly and brought back to its original location.

**Spinning Disk Confocal Imaging**

Most experiments were performed on a Zeiss inverted microscope equipped with a Yokogawa CSU-X10 confocal spinning disk (Intelligent Imaging Innovations, 3i) using 10X or 20X dry, or 40X, 63X or 100X oil objectives. Time-lapse images were acquired from a QuantEM 512SC camera (Photometrics) using time intervals that were four or five times the exposure time. All experiments were done at room temperature at approximately 20ºC. For passive microrheology using microspheres, the beads were imaged ≥20 µm above the coverslip, to ensure that all beads were at least five bead diameters above the surface.

**Laser Scanning Confocal Microscopy**

Images of the Lifeact::GFP meshwork with nucleoli labeled with NPM1::RFP were acquired on the Leica SP5 laser scanning confocal microscope using a 100X oil immersion objective with 1.46 numerical aperture. Images of the Lifeact::GFP meshwork with red beads of R=1.0 µm were acquired using a 63X oil immersion objective with 1.4 numerical aperture.

**Image analysis**

Slidebook software was used to acquire images and make 3-D renderings of the Z-stacks. ImageJ software was used to format the images, merge channels, and create QuickTime files of image sequences. For some images, a despeckling filter in ImageJ was used for smoothing. In all experiments, the images were analyzed using custom-built Matlab code. The centroid positions and trajectories of the beads in these experiments were detected using Matlab Multiple Particle Tracking Code adapted from[37](#_ENREF_37) (see http://physics.georgetown.edu/matlab/index.html). The particles selected from those identified were based on criteria for particle brightness and radius of gyration. The dynamics of the beads were then analyzed using custom-built Matlab software.

**Image integrity**

Images of the actin meshwork visualized with lifeact or utrophin were highly reproducible, and showed on average a mesh size of ~1 um (Fig. S1). Images of actin meshwork after treatment were variable depending on concentration of agent and length of exposure (Figs. 2G, 3C, 3D, 3E). Distribution of nucleoli and fusion depended on the incubation time of drug and amount of time under the constant force of gravity (Figs. 4E-G, 4H, S4B-D, S4E, S5). All images were repeated at least several times to ensure reproducibility.

**Passive microrheology analysis**

The particle’s position was tracked in two dimensions (XY). The mean squared displacement (MSD) was calculated from time and ensemble average for particles using overlapping intervals for each condition and fit to a power law as a function of lag time, from which the power law exponent was determined as the slope of the curve on a log-log plot and apparent diffusion coefficient as the y-intercept.





For some data on diffusing particles where bulk sample flow was observed, the average velocity of the particle was subtracted from its trajectory. Since experiments were done on different GVs and sometimes with different interval times, the squared displacements were logarithmically binned to obtain the final MSD as a function of binned lag time. The standard error of the mean was calculated for each lag time where the number of independent measurements used was the number of experiments performed at independent z-positions for each condition. Weighted least squares fits were used when fitting log(MSD) vs log(lag time) [38](#_ENREF_38). Error in the slope and y-intercept was used to obtain error in the diffusive exponent and apparent diffusion coefficient, respectively. Error bars were not shown on MSD plots for clarity. For Figure 4B, the MSD from micronucleoli were pooled and binned into groups based on their radius. The number of micronucleoli identified was much smaller than the number of microspheres identified, and the standard error of the mean was obtained using the number of measurements for each time point instead of number of experiments performed.

**Estimation of error in passive microrheology for large microspheres**

To determine the error in the MSD measurements, the largest beads (R=1.0 µm) were immobilized on glass coverslip. Time-lapse movies were acquired using exposure settings that were comparable to those from the passive microrheology settings yielding similar signal-to-noise ratios of intensity. The MSD of these immobilized microspheres was 0.0007 ±0.0003 µm2. Thus, any MSD measurement for these beads above 0.001 µm2 is above the noise floor, and reflects real motion.

**Sedimentation experiments and analysis**

After actin disruption (e.g. Lat-A treatment) under constant, slow rotation, the GVs were quickly dissected, carefully pipetted into an imaging chamber, and mounted onto a spinning disk confocal microscope. This process took approximately 5 minutes. A four-dimensional time-lapse movie was acquired to capture the distribution of objects in Z for a 100-micron thick section as a function of time. The positions (X, Y, and Z) of the objects, either nucleoli, HLBs, or metallic microspheres, were tracked in time; we note that the tracking resolution in the Z-dimension is not sub-pixel and is significantly lower than that for our XY tracking data used to calculate MSDs. The nuclear body size was estimated as the full-width, half-max measured in the Z-plane with maximum brightness. Only objects whose Z-position was at least 5 diameters above the coverslip and showed a good linear fit were analyzed in determining the sedimentation velocity and density differences.

**References**

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