***Supplementary Figure Legends***

**Figure S1 Actin disruption with latrunculin A or Xpo6. a,** Row shows how actin network, visualized with Lifeact::GFP, is disrupted at 15 minute intervals after incubation with latrunculin A. Scale bar = 10 μm. Each image is from a different GV. **b**, Row shows how nuclear actin structure is disrupted due to actin export at 15-minute intervals after Xpo6 microinjection. Each image is from a different GV. Scale bar = 10 μm. **c**, **d**, The probability distribution of F-actin mesh size for GVs under latrunculin A, **c,** and Xpo-6, **d,** conditions, for each time point shown above (blue: 15 minutes, yellow: 30 minutes, and red: 45 minutes). Black data points are for the intact Lifeact::GFP structure with no actin disruption (13 z-stacks from 9 GVs). The exponential behavior of the distributions is consistent with a Poisson interval distribution, where the mesh size is ~1 µm for untreated GVs and ~10 µm for actin-disrupted GVs after 45 minutes of treatment.

**Figure S2 Visualization of the nuclear actin network.** **a**, Image of Lifeact::GFP labeled network within the GV. **b**, Image of Utrophin-261::GFP labeled network within the GV, showing similar structure as Lifeact::GFP. Scale bar = 10 µm.

**Figure S3 Expression ofLifeact::GFP does not alter microrheology of the GV.** **a**, MSD versus lag time of R=0.1 µm (green) (n=24 z-positions from 9 GVs, 10,648 particles identified), R=0.25 µm (blue) (n=16 z-positions from 8 GVs, 2,053 particles identified), R=0.5 µm (black) n=19 z-positions from 6 GVs, 1,867 particles identified), and R=1 µm (red) (n=35 z-positions from 14 GVs, 3,011 particles identified) microspheres in native GV (circles) compared with MSD versus lag time of R=0.1 µm (green) (n=4 z-positions from 2 GVs, 7,639 particles identified), R=0.25 µm (blue) (n=18 z-positions from 6 GVs, 7,250 particles identified), R=0.5 µm (black) n=10 z-positions from 4 GVs, 702 particles identified), and R=1 µm (red) (n=5 z-positions from 4 GVs, 237 particles identified) microspheres in Lifeact::GFP expressing GVs (triangles). **b**, Diffusive exponent as a function of microsphere radius, with untreated case in blue and Lifeact::GFP in green. **c,** MSD at 5 s for each bead size, with untreated case in blue and Lifeact::GFP in green. Error bars = s.e.m.

**Figure S4 Actin disruption leads to nucleolar sedimentation and fusion.** Top images show a maximum intensity projection of a 100-micron thick section of nucleoli (labeled with NPM1::GFP & Fibrillarin::GFP) and bottom images show a 3-D rendering in the X-Z plane. **a**, Nucleoli are suspended in an untreated GV. For **b-d**, time-lapse images are from the same GV after Lat-A disruption of actin. **e,** Large nuclear bodies that form overnight after Lat-A treatment. Scale bar = 50 μm and grid size = 50 μm.

**Figure S5 Actin disruption after Xpo6 microinjection also leads to formation of a few massive nucleoli at the bottom of the GV.** Top row shows XY maximum intensity projection of an untreated GV (left) and one after overnight incubation after Xpo6 microinjection (right). Bottom row shows the XZ projection of a 100-μm thick section for the corresponding GVs. Scale bar = 50 μm and grid size = 50 μm.

***Supplementary Video Legends***

**Supplementary Video S1**

Diffusion of R=0.1 μm red microspheres within the Lifeact::GFP actin meshwork. These beads were the smallest bead size probed and showed diffuse-like behavior. Time reported as minutes : seconds.

**Supplementary Video S2**

Diffusion of R=0.25 μm red microspheres within the Lifeact::GFP actin meshwork. These intermediate beads showed cage-hopping behavior, during which the beads diffuse inside a pore and after some time, jump to a new pore. Time reported as minutes : seconds.

**Supplementary Video S3**

Diffusion of R=1 μm red microspheres within Lifeact::GFP actin meshwork. These beads were much larger than the average mesh size and exhibited highly-subdiffusive behavior, leading to their trapped dynamics.Time reported as minutes : seconds.

**Supplementary Video S4**

Diffusion of NPM1::RFP micronucleoli within Lifeact::GFP actin meshwork. The diameter of these micronucleoli was approximately equal to or smaller than the pore size, leading to intermittent dynamics and cage-hopping behavior. Time reported as minutes : seconds.

**Supplementary Video S5**

Increased mobility of GFP::coilin labeled HLBs upon actin disruption by latrunculin A. Top panel shows the XY projection of a 100-μm thick section. HLBs are more motile and show more diffusive like behavior than in unperturbed GVs. Bottom panel shows XZ projection of a 100-micron thick section. HLBs sediment to the bottom of the GV on the scale of ~1 hour. Time reported as minutes : seconds.

**Supplementary Video S6**

Sedimentation and fusion of NPM1::GFP & Fibrillarin::GFP labeled nucleoli upon actin disruption by latrunculin A. Top panel shows the XY projection of a 100-μm thick section. Nucleoli are more motile and show more diffusive like behavior than in unperturbed GVs. Bottom panel shows XZ projection of a 100-μm thick section. Nucleoli rapidly sediment to the bottom of the GV on the scale of ~15 minutes. Time reported as minutes : seconds.

**Supplementary Video S7**

Sedimentation of NPM1::RFP labeled nucleoli and GFP::coilin labeled HLBs upon actin disruption by latrunculin A. Top panel shows the XY projection of a 100-μm thick section. Nucleoli and HLBs are more motile and show more diffusive like behavior than in unperturbed GVs. Bottom panel shows XZ projection of a 100-μm thick section. Nucleoli rapidly sediment to the bottom of the GV on the scale of ~5-10 minutes, while HLBs sediment on a longer time scale of ~30 min. Time reported as minutes : seconds.

**Supplementary Video S8**

Actin disruption after 75-minute treatment with cytochalasin D. Actin is labeled in green with Lifeact::GFP and nucleoli are labeled in red with NPM1::RFP. Cyo-D causes the network to become disrupted and results in puncta formation of the actin. The nucleoli become more mobile and can be seen moving in and out of plane as they sediment. Time reported as minutes : seconds.

**Supplementary Video S9**

Actin disruption after 30-minute treatment with Latrunculin-A. Actin is labeled in green with Lifeact::GFP. Lat-A disrupts the actin meshwork and results in small, unconnected filaments that are diffusive. Dark bodies are unlabeled nucleoli that can be seen diffusing and moving in and out of plane as they sediment. Time reported as minutes : seconds.