Supplemental Information

Supplemental Data

Movie 1. Whi3 forms droplet-like assemblies in *Ashbya* cells. Related to Fig. 1A. A *Ashbya* hypha with GFP tagged Whi3 visualized with oblique angle total internal reflection fluorescence microscope (TIRF).Time format mm:ss.

Movie 2. Whi3 assemblies at a branch site in *Ashbya* cells. Related to Fig. 1B. A *Ashbya* hypha with a new branch site showing Whi3-GFP assemblies visualized with oblique angle TIRF.Time format mm:ss.

Movie 3. Whi3 forms liquid droplets in vitro. Related to Fig. 1D. Recombinantly-expressed Whi3 (28 µM) at relatively low salt (75 mM) phase separates to condensed liquid-like droplets, visualized with 10% of Whi3 protein labeled with GFP under a spinning disc confocal microscope. Time format mm:ss.

Movie 4A. Add *CLN3* mRNA to Whi3 droplets-part I. Related to Fig. 2A. 53 nM *CLN3* mRNA (cy3-labled, red) was added to phase-separated Whi3 (10%GFP labeled, green) at 60 mM salt at time 0. New droplets (yellow, consist of Whi3 and *CLN3* mRNA) start to appear about 1 min 30 seconds. Time format mm:ss.

Movie 4B. Add *CLN3* mRNA to Whi3 droplets-part II. Related to Fig. 2A. Continue of Movie 4A. Whi3-only droplets (green) continue to shrink, new droplets consist of Whi3 and *CLN3* mRNA (yellow) continue to grow. Time format mm:ss.

Movie 5. Whi3ΔRRM forms tubular structures in *Ashbya* cells. Related to Fig. 6A. A *Ashbya* hypha with GFP tagged Whi3ΔRRM visualized with oblique angle TIRF.Time format mm:ss.
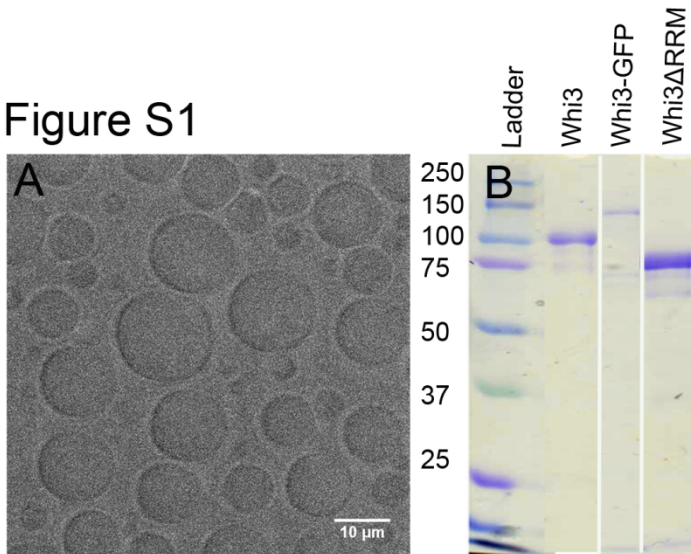
Figure S1. Whi3 droplet formation is not a consequence of the GFP tag. Related to Figure 1. (A) 34 µM unlabeled Whi3 forms droplets with 75mM salt. (B) SDS-PAGE gel for unlabeled Whi3, Whi3-GFP and Whi3ΔRRM expressed in and purified from *E. coli. Lanes are digitally cut from three gels, with white lines indicating the cut.*
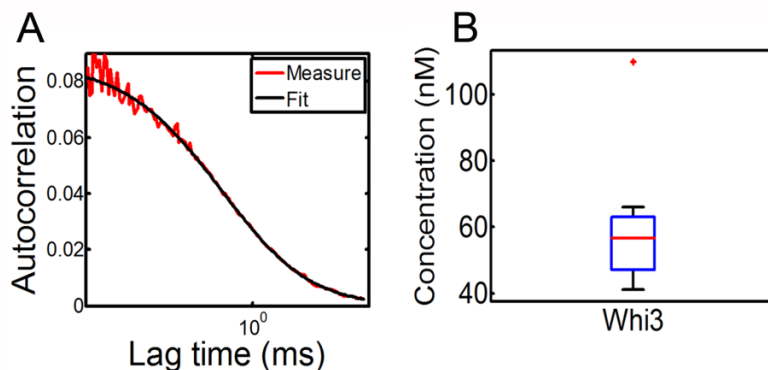

Figure S2. Whi3 concentration in *Ashbya* measured from FCS. Related to Figure 2. (A) Fitting of autocorrelation curve to an anomalous diffusion model to obtain Whi3 concentration. (B) Box plot of Whi3 concentrations measured in *Ashbya* cells.
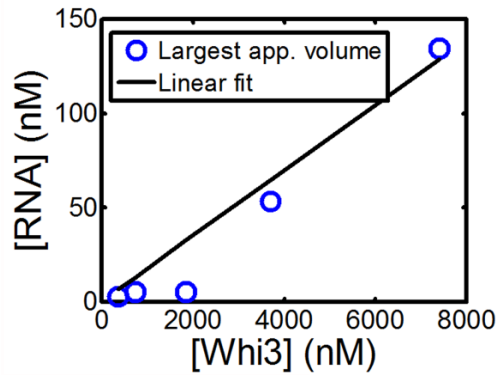
Figure S3

Figure S3. Molar ratio of *CLN3* mRNA to Whi3 that gives the largest apparent droplet volume. Related to Figure 2.   Circles indicates the positions of *CLN3* RNA concentration at which the largest apparent droplet volume is found for each Whi3 concentration in Fig. 2D, as judged by eye from the images. A rough linear fit gives a *CLN3* RNA to Whi3 molar ratio of 0.02, below and above which droplet formation is less favored.
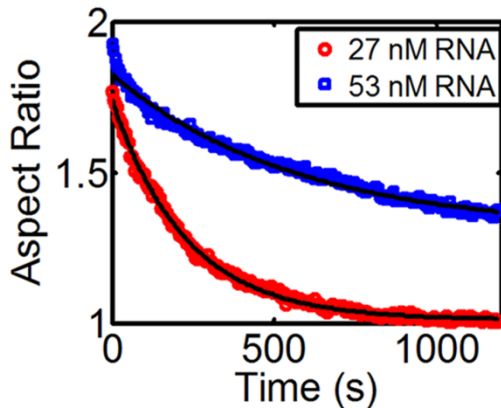
Figure S4

Figure S4   Decay of aspect ratio over time when droplets fuse. Related to Figure 4. Exponential fits (black lines) give a larger decay time 570 s for 53nM *CLN3* and 230 s for 27nM *CLN3* RNA.
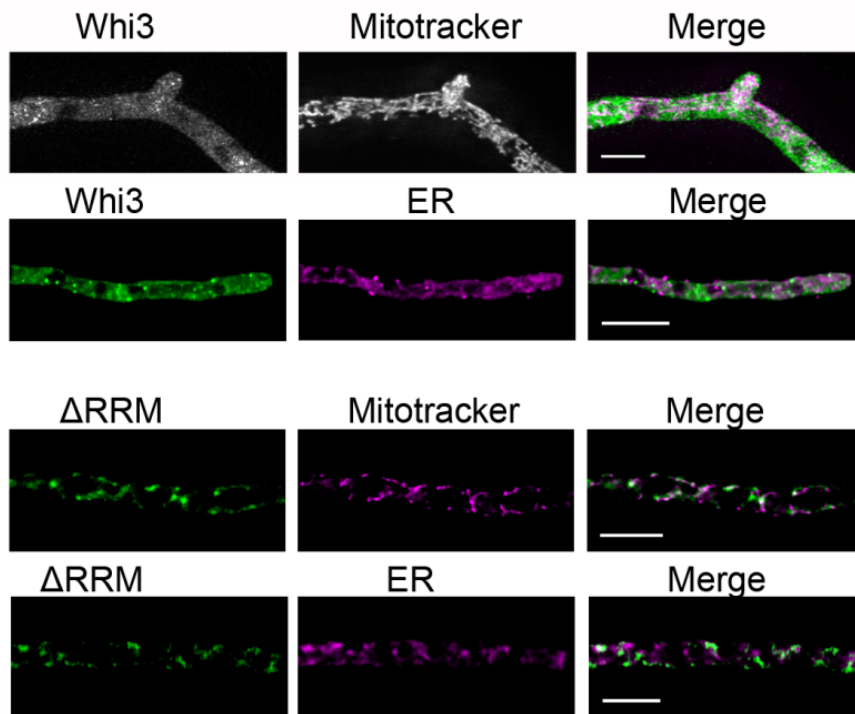
Figure S5. Neither Whi3 nor ΔRRM colocalize with ER or mitochondria.
Related to Figure 6. Scale bars are 5 μm.

Supplemental Experimental Procedures

Protein purification

6-His tag was cloned to the 5' of the gene of interest and inserted into pET30b
backbone for *E. coli* expression. Protein expression was induced with 1 mM IPTG and
proteins were purified with Ni_NTA beads with The plasmids were transformed into 5-
Alpha competent *E coli* cells (NEB) for storage and for producing more plasmids. When
ready for expression, plasmids were transformed into BL21 competent *E coli* cells
(NEB). One newly transformed colony was transferred to 30 mL LB and incubated at 37
°C overnight and then diluted 1:60 into 1L terrific broth to grow till OD reaches 0.6-0.8.

Cells were then cooled down in a cold room. 1 mM IPTG was then added to induce protein expression and the cells were transferred to an 18 $^{\circ}$C incubator. After 16 hours growth, cells were spun down and the pellet was resuspended into 25ml lysis buffer (1.5 M NaCl, 20 mM Tris pH 8, 20 mM Imidazole pH8, 1 mM DTT, 1 tablet of Roche protease inhibitor cocktail, 5 µl of Benzonase nuclease). Cells were incubated on ice for 30 mins and then sonicated for 10 s for 5 times with 30 s ice-incubation in between. The lysed sample was then spun down at 10,000 g for 30 min at 4 $^{\circ}$C. The supernatant was then filtered with 0.2 µm filter (Millipore), and incubated with 2 ml Ni-NTA (Qiagen) rotating at 4 $^{\circ}$C for 30min. The sample was then transferred into a gravity column and washed with at least 10CV wash buffer (the same as lysis buffer except there is no protease inhibitor and Benzonase nuclease). Finally, protein was eluted the 6CV elution buffer(150 mM NaCl, 20 mM Tris pH8.0, 200 mM Imidazole pH8 1 mM DTT).

After checking with SDS-PAGE, the fraction containing pure protein of interest was then dialyzed into droplet formation buffer (150 mM NaCl, 50 mM Tris pH8, 1 mM DTT), and concentration was measured with Bradford. Finally aliquots were flash frozen and stored at -80 $^{\circ}$C.

To avoid RNA contamination, we used high salt (1.5M) and Benzonase nuclease (Sigma) in our lysis buffer. This appears to be effective as 260/280 is ~0.7 measured with nanodrop and no RNA was detected with agarose gel.

Fluorescence correlation spectroscopy (FCS)

Fluorescence lifetime correlation spectroscopy (FLCS) was used to measure GFP-tagged Whi3 concentration in live *Ashbya* cells after growing overnight. FLCS was done with commercial PicoQuant set up on a Nikon A1 LSM, using a Plan Apo IR 60×WI 1.27NA objective. Time trace was collected with a 485 nm pulsed laser at 20 MHz repetition frequency. The effective volume V was calibrated with 1.2 nM fluorescence dye ATTO488 (ATTO-TEC GmbH, Germany) by fitting the autocorrelation function to a triplet triplet-state model

$$G(\tau) = (1 - T + Te^{-\frac{\tau}{\tau_t}})\frac{\gamma}{N}(1 + \frac{\tau}{\tau_D})^{-1}(1 + \frac{\tau}{\kappa^2\tau_D})^{-0.5}$$

where T is the (triplet) fraction of molecules, τ_t is the life time of the triplet state. The length to diameter ratio of the focal volume $\kappa = 5.15 \pm 0.35$ was obtained from the fitting. With the known diffusion coefficient $D = 400 \mu m^2/s$ of ATTO 488, the fitted τ_D from the autocorrelation curve, a confocal volume $V = 0.25 \pm 0.05$ fl was obtained.

Whi3 autocorrelation function (ACF) was fitted to an anomalous diffusion model in SymPhoTime (PicoQuant GmbH, Germany):

$$G(\tau) = \frac{\gamma}{N}[1 + (\frac{\tau}{\tau_D})^\alpha]^{-1}[1 + \frac{1}{\kappa^2}(\frac{\tau}{\tau_D})^\alpha]^{-0.5}$$

Were N is number of Whi3 molecule and the Whi3 concentration was obtained from $C = N/V$ (Fig. S2).

Wide field fluorescence microscopy setup and image processing

An AxioImager-M1 upright light microscope (Carl Zeiss, Jena, Germany) equipped with the following Zeiss oil immersion objectives was used: EC Plan_Neofluar 40X/1.3 numerical aperture (NA), Plan-Apochromat 63X/1.4 NA, Plan-Neofluar 100X/1.3 NA, and a Plan-Fluar 100X/1.45 NA. Chroma filter set 41025 and Zeiss filter set 38HE were used for GFP. An Exfo X-Cite 120 lamp was employed as the fluorescence light source. A Hammamatsu Orca-ER (C4742-80-12AG) CCD camera driven by Micromanager was used for acquisition of images. Z-stacks of still images were acquired at different slice sizes and resulting images were processed by iterative deconvolution (45 iterations) using calculated point spread functions in Volocity 4. All still images were linearly contrast enhanced in imageJ.