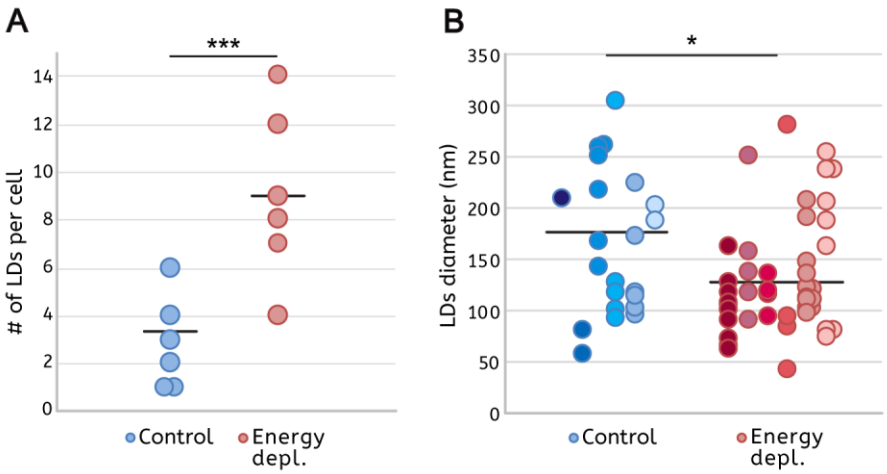
Supplemental Materials

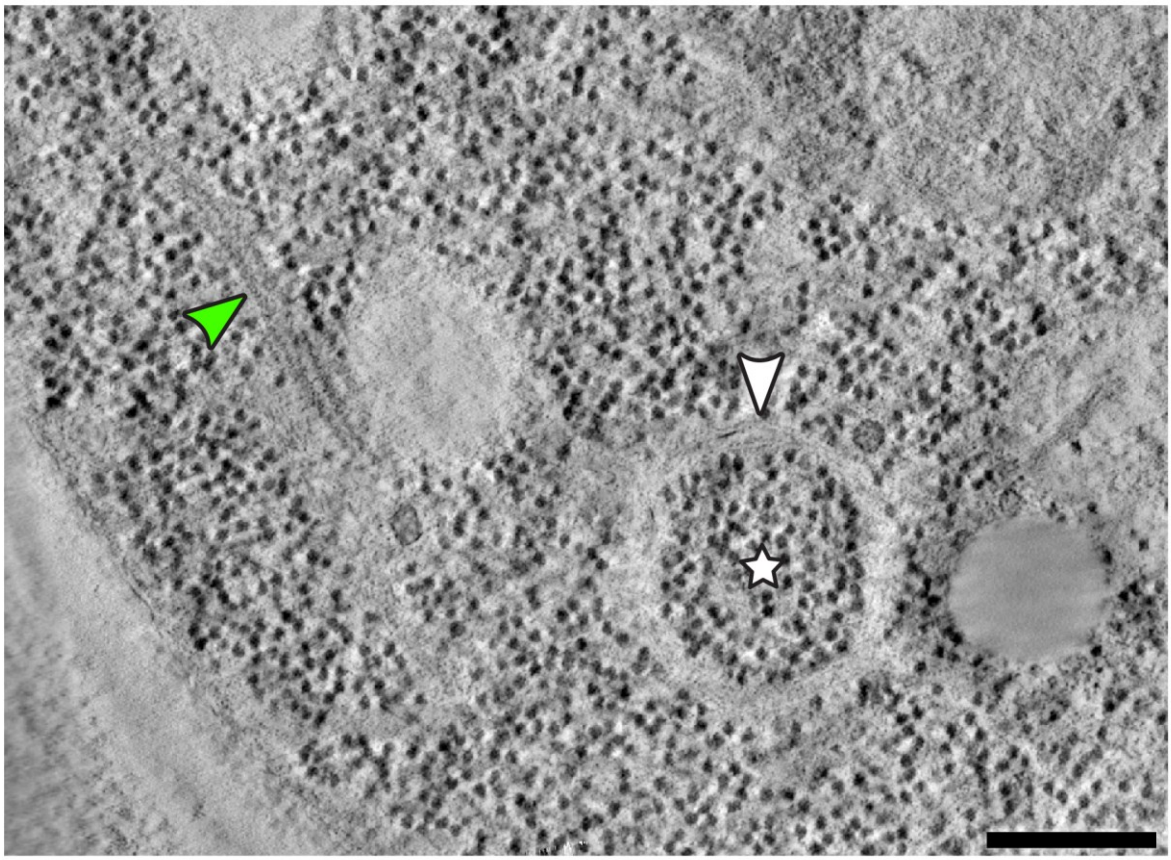
Molecular Biology of the Cell

Marini et al.

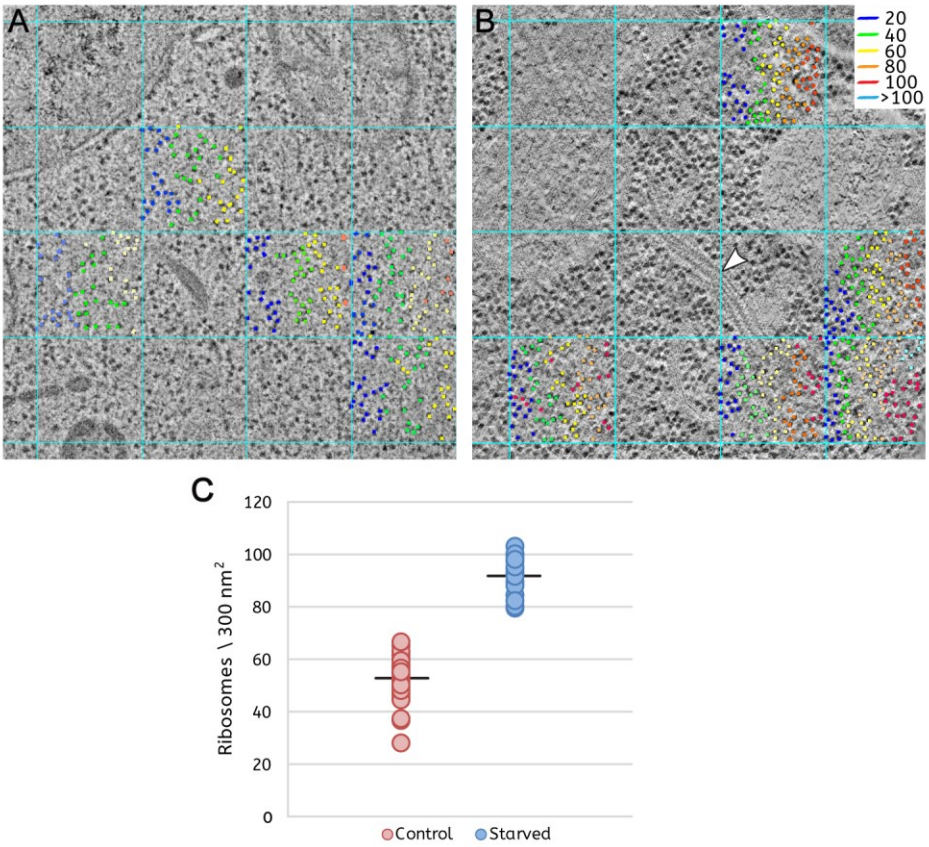
Supplemental figures and videos legends



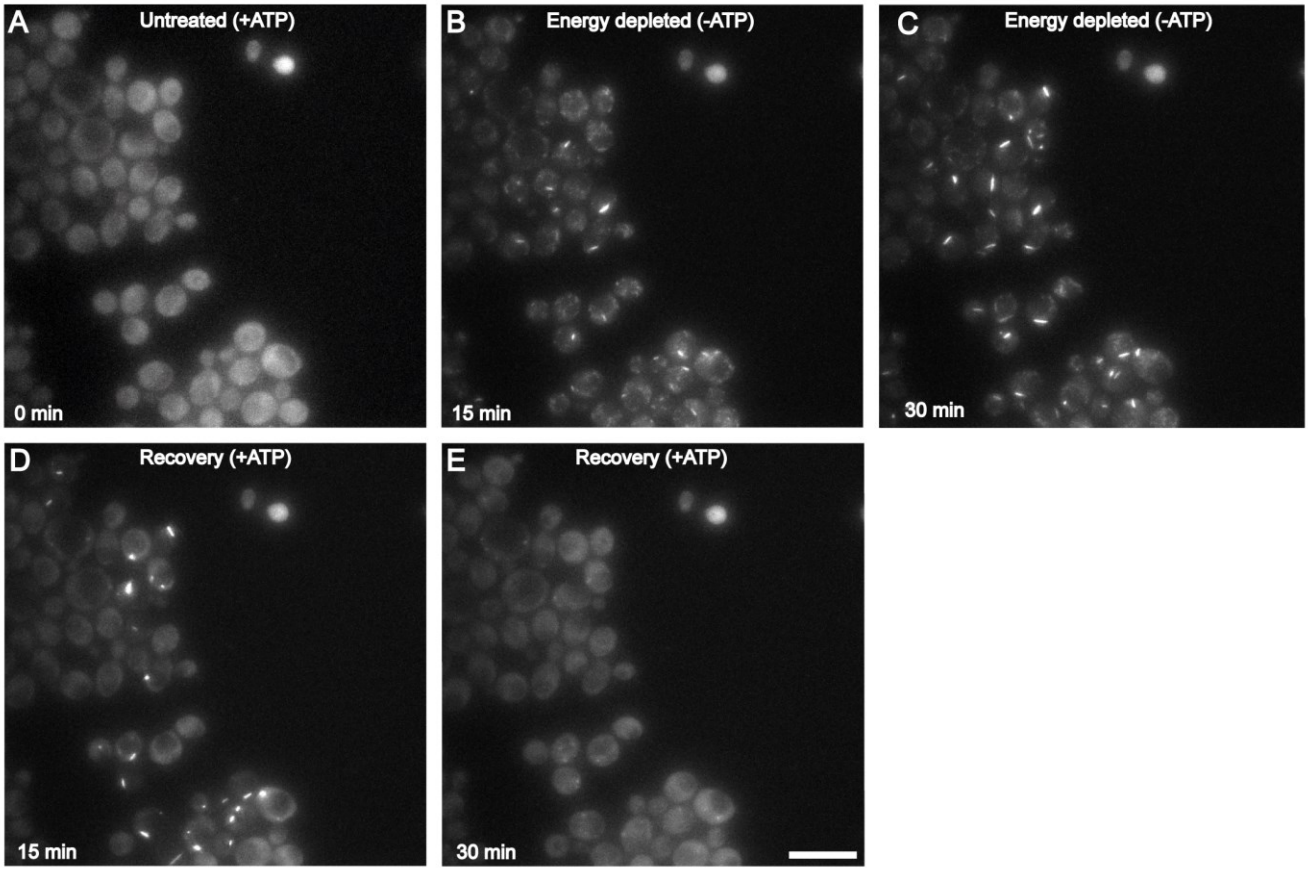
Supplemental Figure S1. LDs measurement and quantification. (A) Energy depleted cells contain a significantly higher number of LDs compared to control cells. Each point in the plot represents the number of LDs per cell. ***P-value = .0001 (B) The average LDs diameter is larger in control yeast cells than in energy depleted cells. Each column of points in the graph, shown with different shades of blue or red, corresponds to LDs measured in a single tomogram. *P-value = .026. In both plots 6 tomograms where analyzed per condition.



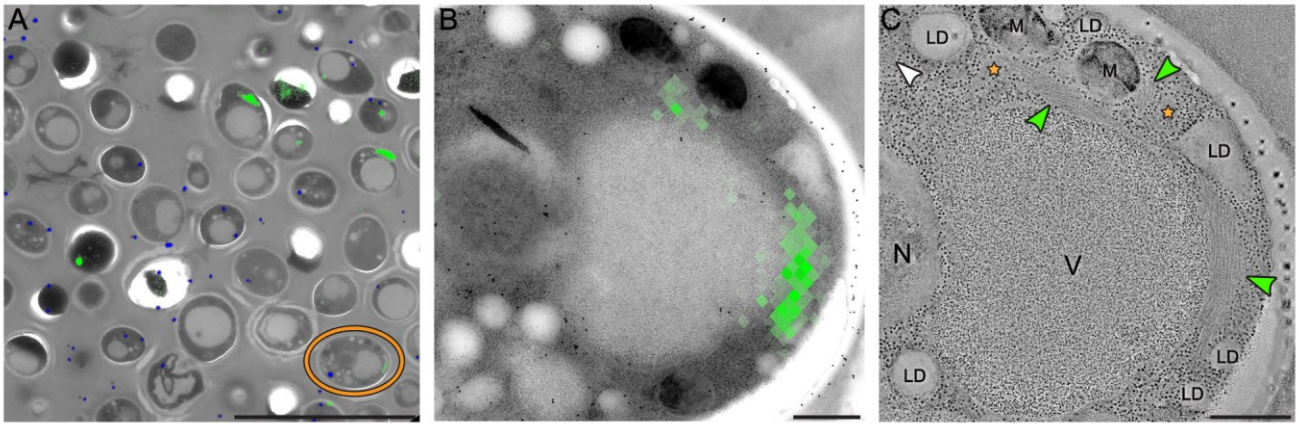
Supplemental Figure S2. Vesicle containing ribosomes in a stressed yeast cell. A double membrane vesicle, highlighted with a white arrow, contains tightly packed ribosomes visible in the lumen (white star). It might be identified as an autophagosomal vesicle in the process of bringing its content to the vacuole for degradation. The green arrow points to a filament in the cytoplasm of the energy depleted yeast. Scale bar = 200 nm.



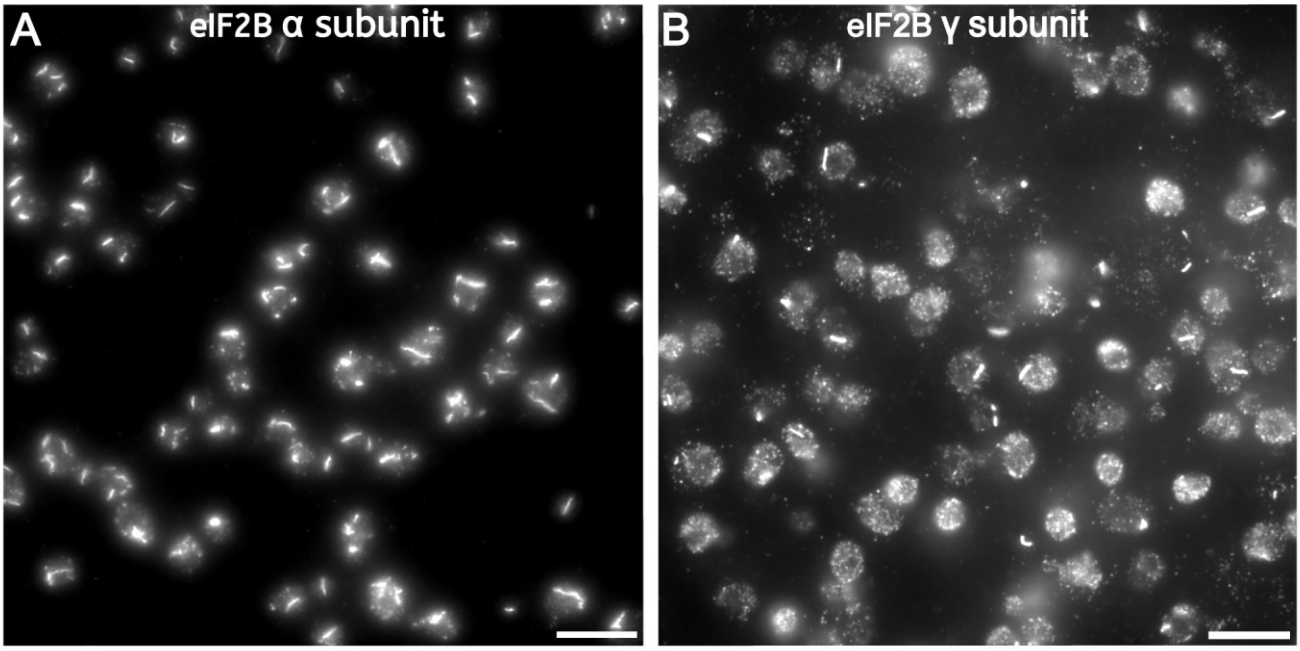
Supplemental Figure S3. Manual method for ribosome counting. A 300 × 300 nm grid is superimposed on the central slice of a tomogram. Ribosomes in 5 cytoplasmic squares are counted, avoiding those comprising mainly large organelles, vacuole or nucleus. Ribosomes in the upper and left edge of each chosen square are included in the count. (A) Yeast control cells have usually 50-70 ribosomes per 300 nm^2 area. (B) Energy depleted yeast cells have usually 100 or more ribosomes per 300 nm^2 area. White arrow highlights a filament in the stressed cell. (C) The result of the manual ribosome counting is shown in the plot. Five tomograms are analyzed for each condition. The almost-two-fold increase (42%) in ribosome density in the stressed yeast cells is confirmed.



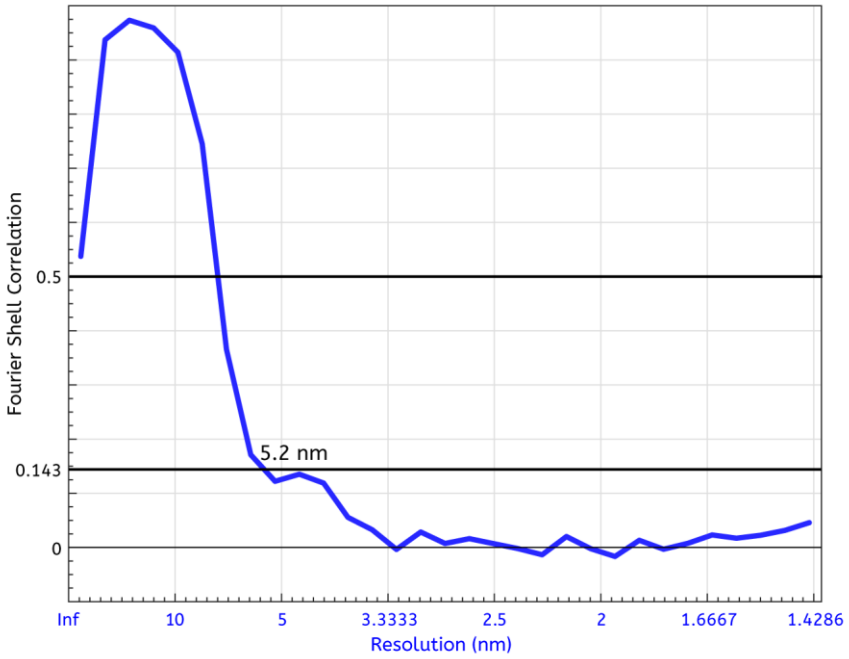
Supplemental Figure S4. eIF2B enzymes change their cytoplasmic distribution between log-phase growing and energy-depleted yeast cells. (A) According to the fluorescent signal, the sfGFP-labeled eIF2B complexes have a diffuse distribution throughout the cytoplasm in log-phase growing and dividing cells. (B) The fluorescence signal of sfGFP-tagged eIF2B condenses into foci-like or elongated structures in stressed cells that have been energy-depleted for 15 minutes. (C) After 30 minutes of energy depletion the number of foci is reduced in favor of larger condensates. (D, E) The fluorescence signal rapidly recovers a diffuse distribution as cells reenter the cell cycle upon energy replenishment. Scale bar = 10 μm.



Supplemental Figure S5. Correlative fluorescence and electron microscopy analysis reveals organization of sfGFP-tagged eIF2B into bundles of parallel filaments. (A) The fluorescent signal of sfGFP-eIF2B is overlaid on low-magnification TEM image of energy-depleted yeast cells previously embedded in Lowicryl HM-20 and then sectioned. (B) Close up of the cell highlighted in A. (C) Magnified views of the tomographic slices shown in C. Two big bundles of filamentous structures and one small one (green arrows) corresponds to the fluorescence signal. A smaller non-fluorescently labelled filamentous structure (white arrow) is visible. Small non-membrane-bound compartment with amorphous appearance are labelled with magenta stars. LD = lipid droplet; M = mitochondrion; V = vacuole; N = nucleus. Scalebars: A=10 µm, B and C=500 nm.



Supplemental Figure S6. Immunofluorescence labeling of two HA-tagged eIF2B subunits show filament bundles in energy-depleted yeast cells. (A) Immunolabeling of the HA-tagged GCN3 (α) and (B) GCD1 (γ) subunits of eIF2B show that the formation of filament bundles in energy-depleted cells occurs in the absence of the sfGFP tag. This demonstrates that separation of eIF2B in non-membrane bound compartments is not induced or enhanced by the sfGFP tag. Scale bars = 10 μm.



Supplemental Figure S7. The estimated resolution of the 3D model shown in Figure 7 is 5.2 nm at Henderson's gold standard cutoff of 0.143 [Rosenthal and Henderson, 2003].