Supplemental Materials

Translation suppression promotes stress granule formation and cell survival in response to cold shock

Sarah Hofmann, Valeria Cherkasova, Peter Bankhead, Bernd Bukau, Georg Stoecklin

The Supplemental Materials include one movie, nine figures and one table.

MOVIE S1: Cold shock-induced SGs remain assembled during 10ºC hypothermia. Human Huh7 hepatocarcinoma cells stably expressing a GFP-tagged G3BP as a SG marker were seeded onto glass bottom plates. A Pelltier element was employed to cool the microscope stage to 10°C prior to imaging. Cells were exposed to cold shock and images were recorded during the course of the cold shock using an Olympus xcellence TIRF microscope and a Hamamatsu Orca-05G camera. The movie was assembled in ImageJ.

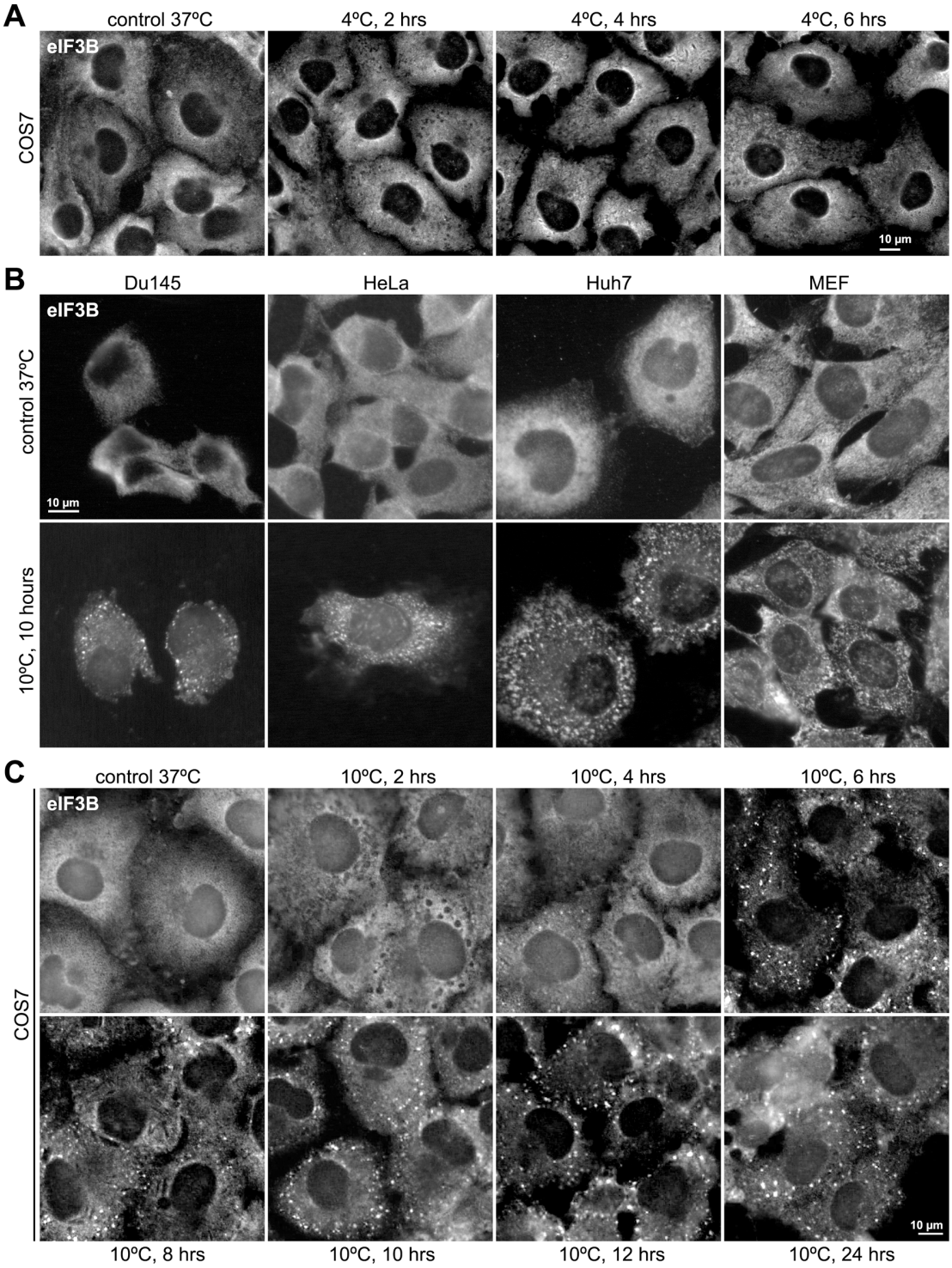

FIGURE S1: Cold shock-induced SG assembly in mammalian cell lines. (**A**) COS7 cells were grown under control conditions at 37ºC or exposed to 4ºC for 2, 4 or 6 hours. Cells were fixed in the cold and the subcellular localization of eIF3B was determined by IF staining followed by wide-field fluorescence microscopy. (**B**) Human Du145 prostate carcinoma cells, human HeLa cervix carcinoma cells, human Huh7 hepatocellular carcinoma cells and mouse embryonic fibroblasts (MEFs) were grown under control conditions at 37ºC or exposed to 10ºC for 10 hours, and analysed as above. (**C**) To assess kinetics of cold shock-induced SG formation, African green monkey COS7 kidney cells were exposed to 10ºC for increasing periods of time, as indicated, and analysed as above.

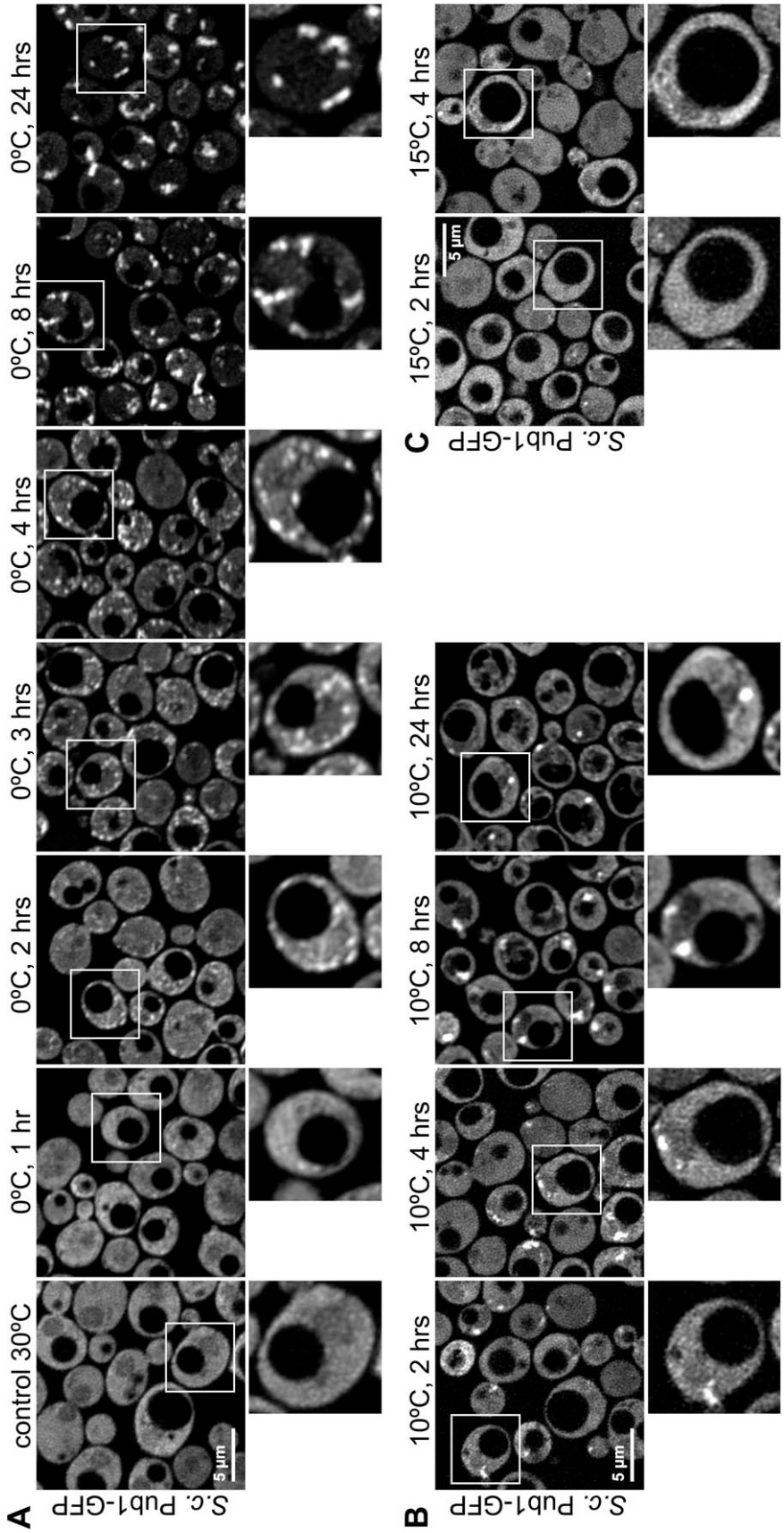

FIGURE S2: Kinetics and temperature dependence of cold shock-induced SG formation in *S. cerevisiae*. (**A**) A genomically tagged yeast strain expressing Pub1-GFP was either grown under control conditions or exposed to 0°C for increasing periods of time, as indicated. Cells were fixed in the cold and subcellular localization of Pub1-GFP was analyzed by confocal microscopy. Four optical sections were used for deconvolution and the resulting maximum projection is depicted. (**B**) The same strain was exposed to milder cold shock at 10ºC for 2, 4, 8 and 24 hours and analyzed as above. (**C**) Exposure to 15ºC for 2 and 4 hours was examined as above.

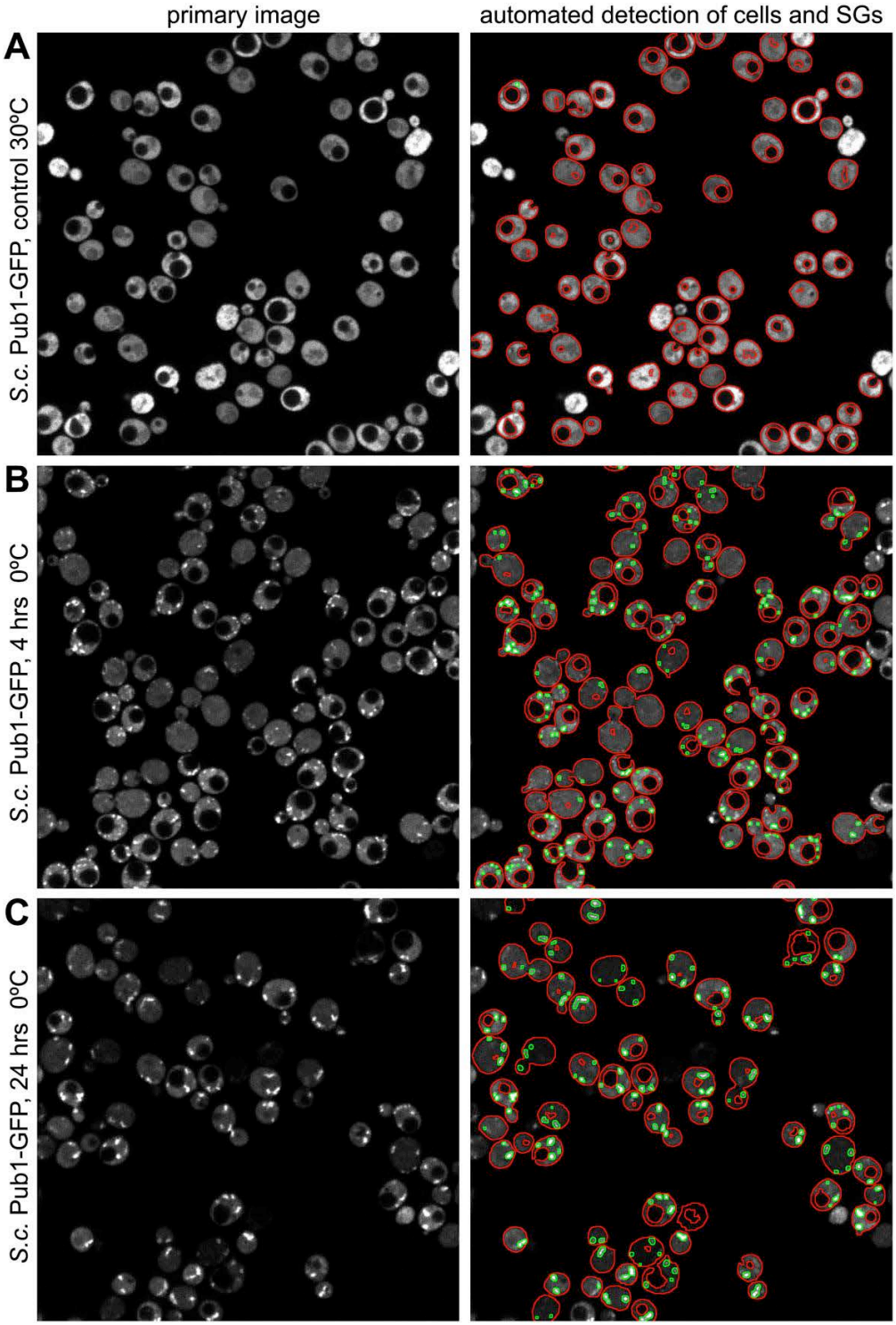

FIGURE S3: Automated quantification of SGs in *S. cerevisiae*. (**A–C**) A yeast strain expressing genomically tagged Pub1-GFP was either (**A**) grown under control conditions or (**B**) exposed to 0°C for 4 or (**C**) 24 hours. Cells were fixed in the cold and the Pub1-GFP signal was visualized by confocal microscopy. A single plane was chosen from the non-deconvoluted image stack and normalized to exposure time (images on the left side). Automated cell detection was used to delineate cell boundaries and exclude nuclei (red borders in the images on the right side). Aggregates were defined as pixels inside the cytoplasm at which the fluorescence increase is greater than 0.75 times the local baseline (green borders). The information was used to calculate cell number, cell area, signal intensity within each cell, number of aggregates, area of aggregates and signal intensity within aggregates.

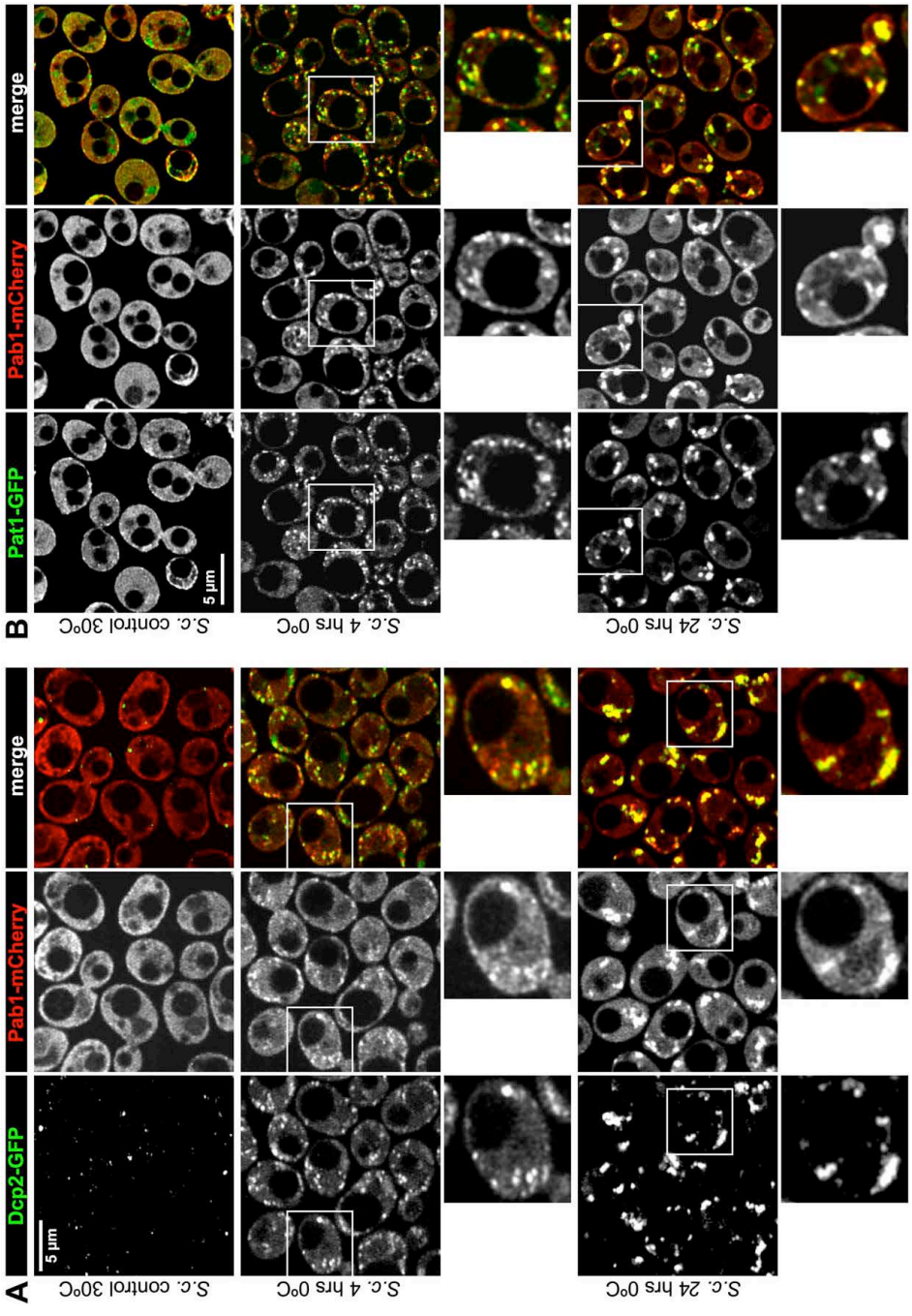

FIGURE S4: Cold shock induces SGs and P-bodies in *S. cerevisiae*. (**A**) A yeast strain expressing genomically tagged Dcp2-GFP (green, P-body marker) and Pab1-mCherry (red, SG marker) was either grown under control conditions or exposed to cold shock at 0°C for 4 and 24 hours. Cells were fixed and subcellular localization of the tagged proteins was analyzed by confocal microscopy. Three optical sections were used for deconvolution and the resulting maximum projection is depicted. (**B**) A yeast strain expressing genomically tagged Pat1-GFP (green, P-body marker) and Pub1-mCherry (red, SG marker) was analyzed as in panel A.

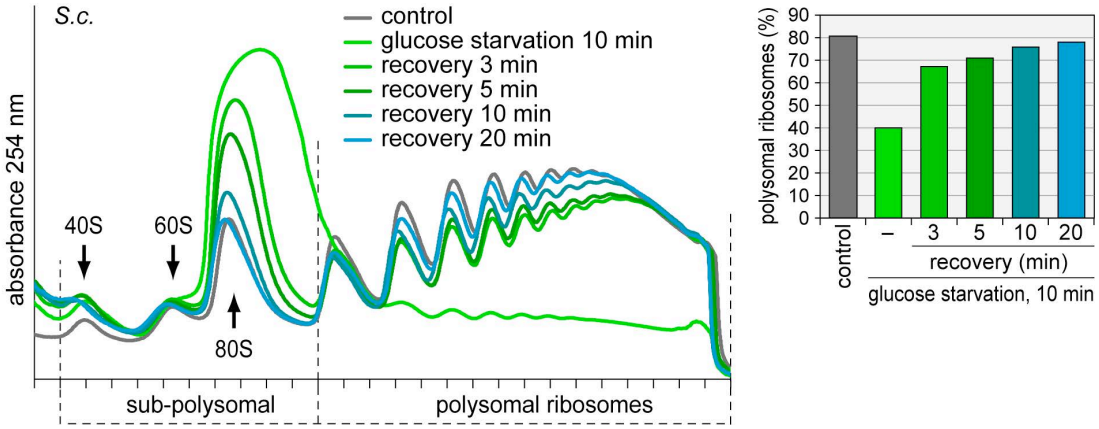

FIGURE S5: Re-assembly of polysomes after glucose starvation in *S. cerevisiae*. Polysome profiles were recorded from yeast cells grown under control conditions, grown in glucose-free medium for 10 minutes, and after return to full medium for the indicated time periods. In the bar graph, the percentage of polysomal ribosomes was quantified as described for Figure 1K.

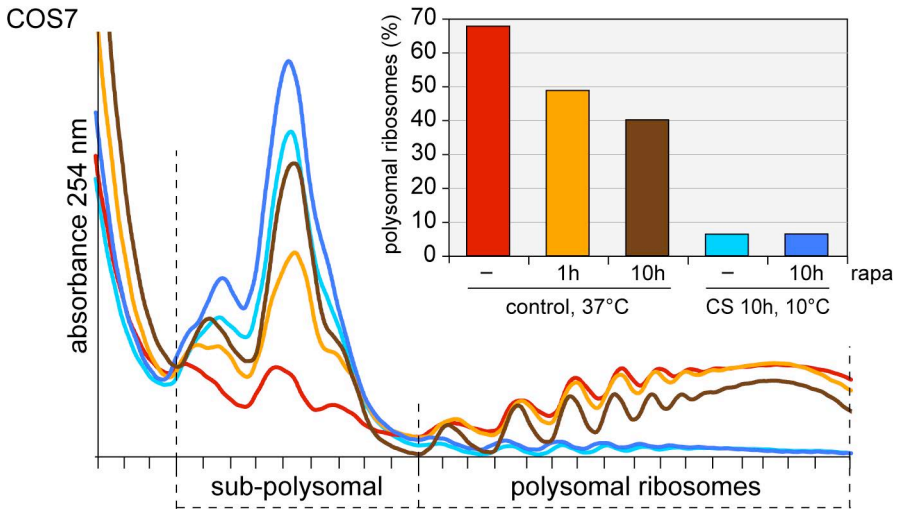

FIGURE S6: Effect of mTOR inhibition on cold shock-induced translation suppression. Polysome profiles were recorded from COS7 cells grown under control conditions, treated with 0.2 μM rapamycin for one or 10 hours, or subjected to 10 hours cold shock at 10°C in the absence or presence of 0.2 μM rapamycin. In the bar graph, the percentage of polysomal ribosomes was quantified, as described for Figure 1K.

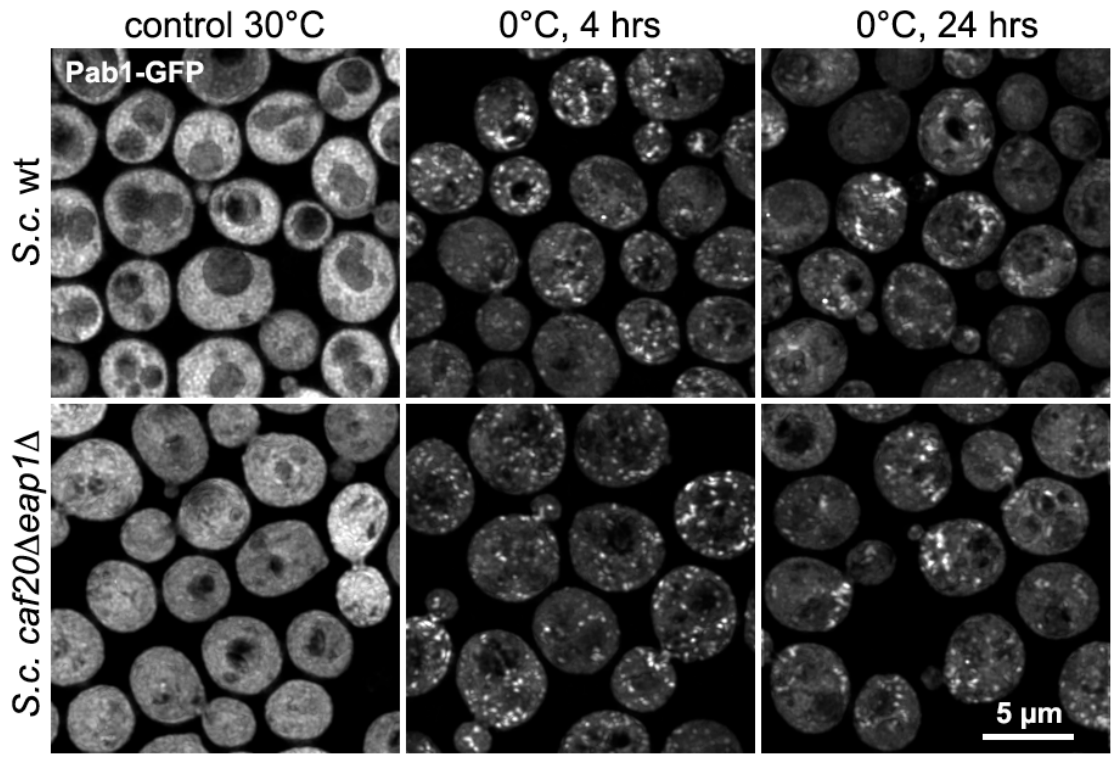

FIGURE S7: Deletion of *EAP1* and *CAF20* in *S. cerevisiae* does not affect cold shock-induced SG assembly. Pab1-GFP was genomically tagged in wt *S. cerevisiae* and in a *caf20*Δ*eap1*Δ double deletion strain. Cells were either grown under control conditions or exposed to 0˚C for 4 and 24 hours. Cells were then fixed in the cold and subcellular localization of Pab1-GFP was analysed by confocal microscopy. Optical sections through the entire cell volume were used for deconvolution and the resulting maximum projection is depicted.

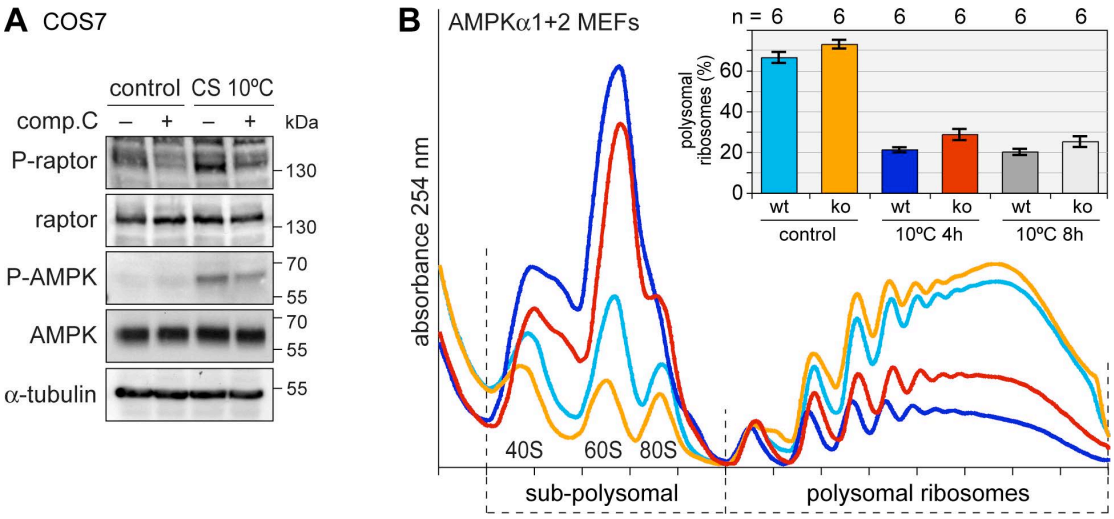

FIGURE S8: Effect of AMPK inhibition on cold shock-induced translation suppression. (**A**) COS7 cells were grown under control conditions at 37°C or subjected to cold shock at 10°C for 10 hours. In addition, cells were treated for 10 hours with the AMPK inhibitor compound C (20 μM) under both conditions. Total protein lysates were analyzed by Western blotting for phospho(S792)-raptor, total raptor, phospho(T172)-AMPK, total AMPK and α-tubulin as loading control. (**B**) AMPKα1+2 double ko MEFs as well as wt counterparts were grown under control conditions or subjected to 4 hours of cold shock at 10°C. In the bar graph, the percentage of polysomal ribosomes was quantified, as described for Figure 1K. Average values ± SE from 6 repeat experiments are shown, including values from an 8 hour cold shock.

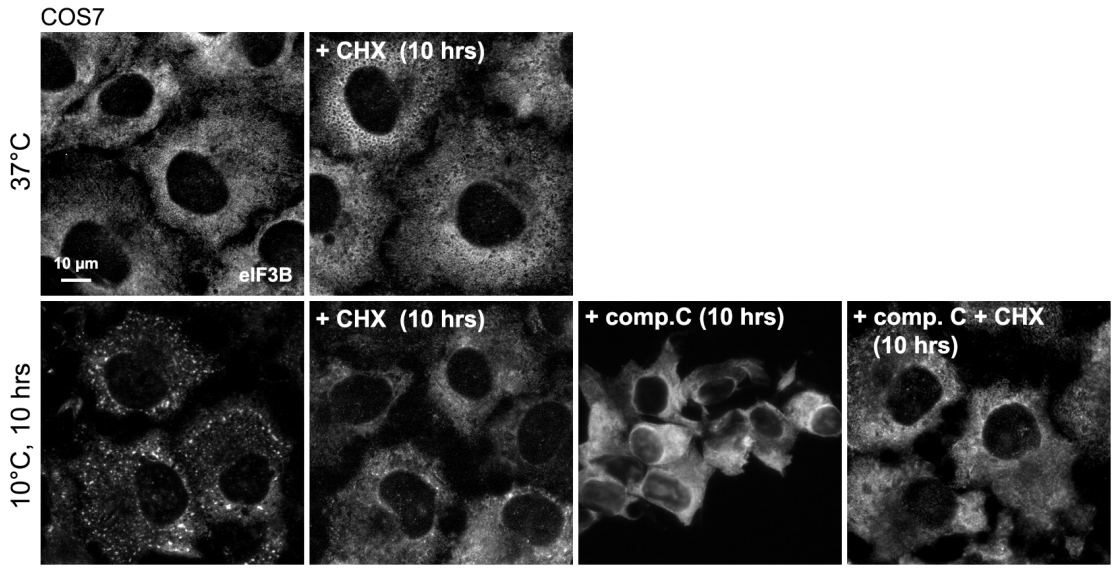

FIGURE S9: Effect of cycloheximide (CHX) on cold shock-induced SG formation. COS7 cells were grown under control conditions at 37ºC or exposed to 10ºC for 10 hours. During the 10 hour period, cells were treated either with cycloheximide (10 μg/ml), compound C (20 μM) or both. Cells were then fixed in the cold and the subcellular localization of eIF3B was determined by IF staining followed by wide-field fluorescence microscopy. CHX and compound C prevent the formation of SGs.

TABLE S1: *S. cerevisiae* strains used in this study

Strain	Genotype
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*
VCY064	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Pab1GFP::kan*
VCY128	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pub1Δ::kan (EUROSCARF)*
VCY196	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 gcn2Δ::kan (EUROSCARF)*
VCY204	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 gcn2Δ::kan Pab1GFP::hph*
VCY210	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Pab1-myc-TAP::kan*
VCY238	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Pub1GFP::kan Pab1mCherry::hph*
VCY251	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pat1Δ::kan (EUROSCARF)*
VCY262	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pub1Δ::kan Pab1GFP::hph*
VCY263	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pat1Δ::kan Pab1GFP::hph*
VCY272	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Pat1GFP::kan Pab1mCherry::hph*
VCY274	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Dcp2GFP::kan Pub1mCherry::hph*
VCY275	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Ngr1GFP::kan Pab1mCherry::hph*
VCY335	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Pab1GFP::URA3*
VCY459	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 caf20Δ::nat eap1Δ::hph Pab1GFP::kan*