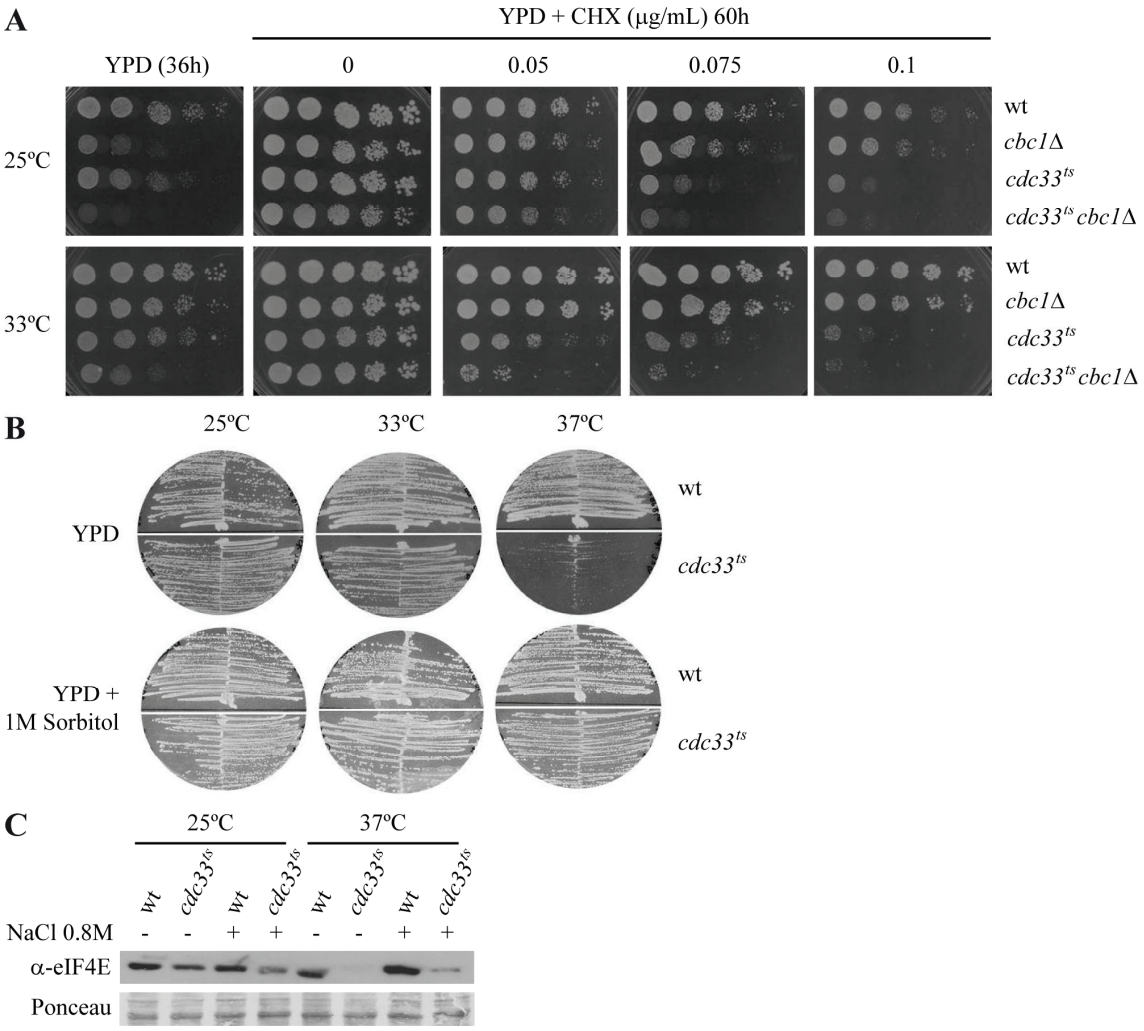

Figure S1. Functional interaction between Cbc1 and eIF4E. (A) Sensitivity to cycloheximide (CHX) of the wt, *cbc1*Δ, *cdc33*^{ts} and *cdc33*^{ts}*cbc1*Δ strains. Cells were grown exponentially in YPD. An aliquot of each culture was diluted and 8μL of the 5-fold serial dilutions were spotted on plates containing YPD or YPD supplemented with 0.05 μg/mL; 0.075 μg/mL and 0.1 μg/mL CHX. Plates were grown for the indicated hours at 30°C. The results in this figure are representative of at least two independent experiments. (B) A mutation in translation initiation factor eIF4E confers resistance to osmotic stress. Growth of the wt and *cdc33*^{ts} strains was tested in YPD and YPD supplemented with 1 M glycerol. Plates were grown for 3 days at 25°C, 33°C and 37°C. The results in this figure are representative of at least two independent experiments. (C) Normal levels of eIF4E protein were not restored at 37ºC by NaCl treatment. The wt and *cdc33*^{ts} mutant were grown exponentially in YPD at 25°C and then were cells incubated at 25ºC or 37ºC for 1 h in YPD or YPD media containing 0.8 M NaCl. The eIF4E protein was analyzed by Western blotting using an anti-eIF4E antibody (kindly provided by M. Ashe). The Ponceau staining of the membrane is shown as a control of the amount of protein loaded.

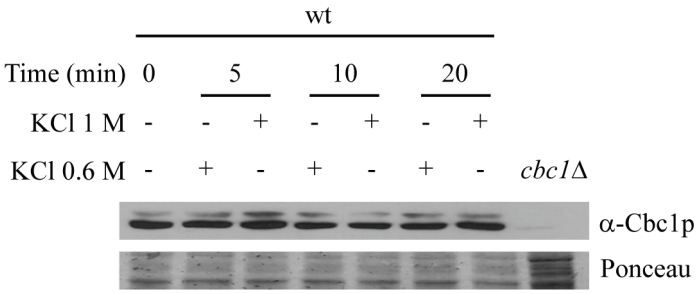

Figure S2. The level and electrophoretic mobility of Cbc1 were not affected by osmotic stress. The wt cells exponentially growing in YPD were treated with 0.6 M or 1 M KCl for 0, 5, 10 and 20 minutes. Total protein extracts were analyzed by Western blotting and Cbc1 was detected using an anti-Cbc1 antibody. A total protein extract from a *cbc1*Δ strain was loaded as the negative control. The Ponceau staining of the membrane is shown as a control of the amount of protein loaded.

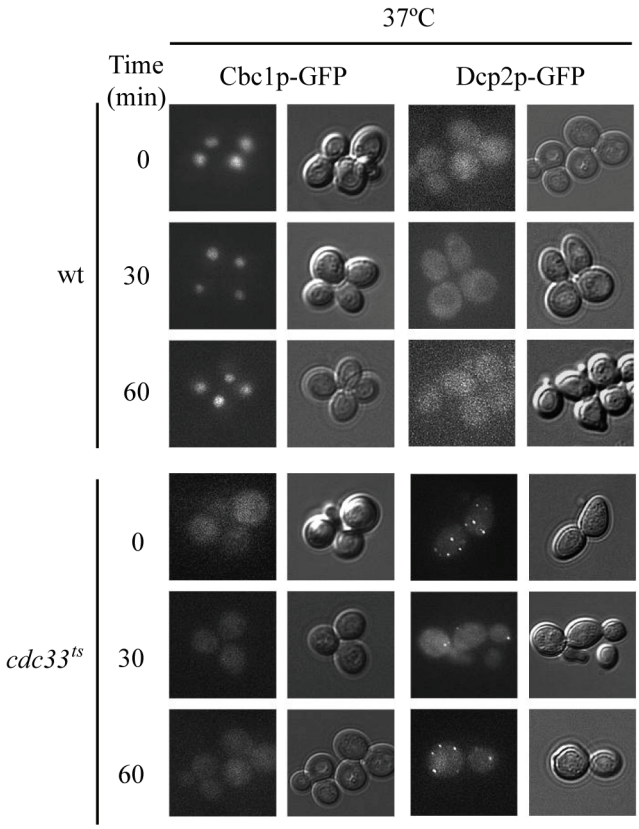

Figure S3. The wt and *cdc33*^{ts} mutant strains expressing Cbc1-GFP or Dcp2-GFP (P-body marker) were grown in YPD at 25ºC until the exponential phase. Cells were incubated at a restrictive temperature (37ºC) for 30 min. After this, Cbc1-GFP or P-bodies (Dcp2-GFP) were visualized at 0, 30 and 60 min by fluorescence microscopy (left panel). Cells were also photographed by Nomarski optics (right panel).

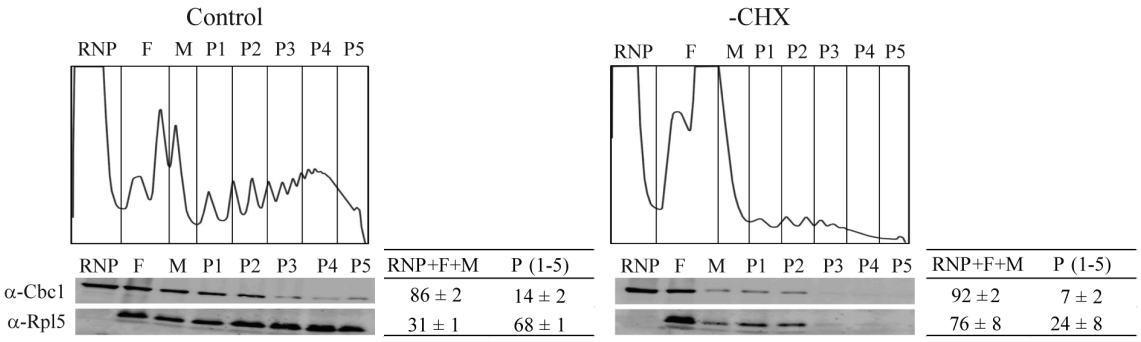

Figure S4. Cbc1p associates with polysomes in a CHX-dependent manner. Wt cells were treated with 0.6 M KCl for 15 min. Polysomal profiles were obtained from one aliquot of the culture (control) as described in Materials and Methods. To disrupt most of the polysomes, another aliquot of the culture was treated with NaN_3 for 30 min at 30°C, and CHX was omitted in the cell extract and sucrose gradient (-CHX). Messenger ribonucleoprotein (RNP), free (F), monosomal (M) and polysomal [P(1-5)] fractions were collected and analyzed by Western blotting with an anti-Cbc1 antibody. Detection of ribosomal protein Rpl5 with a specific antibody was done as a control of polysome disassembly. The polysome profiles and Western blots showed in this figure correspond to a representative experiment. The percentage of Cbc1 and of Rpl5 in the nonpolysomal fractions (RNP+F+M) and the polysomal fractions (P1-P5) is indicated. Data are provided as the mean and standard error of two independent experiments.

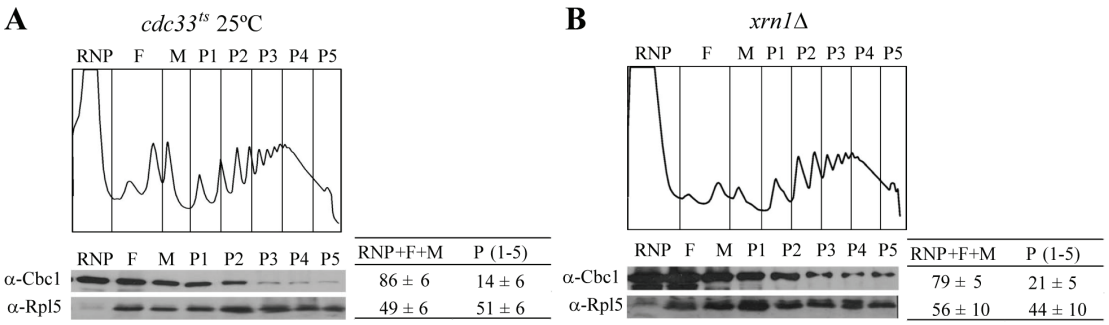

Figure S5. The association of Cbc1 with polysomes remained constant in the *cdc33*^{ts} and *xrn1*Δ mutants. (A) The wt and *cdc33*^{ts} mutant strains were grown in YPD at 25ºC until the exponential phase. Then, one fraction of each culture was kept at 25ºC and another was incubated at 37ºC for 30 minutes. A polysomal analysis was done as described in Materials and Methods, and Cbc1 and Rpl5 were detected in the pooled fractions by Western blots using specific antibodies. (B) The *xrn1*Δ mutant was grown in YPD at 30ºC until the exponential phase, and the association of Cbc1 and Rpl5 with polysomes was analyzed as in (A). The polysome profiles and Western blots shown in this Figure correspond to a representative experiment. The percentage of non associated protein with polysome (RNP+F+M) and the percentage of polysome-associated protein [P(1-5)] are shown in the tables. The data are the mean and standard error of two independent experiments.

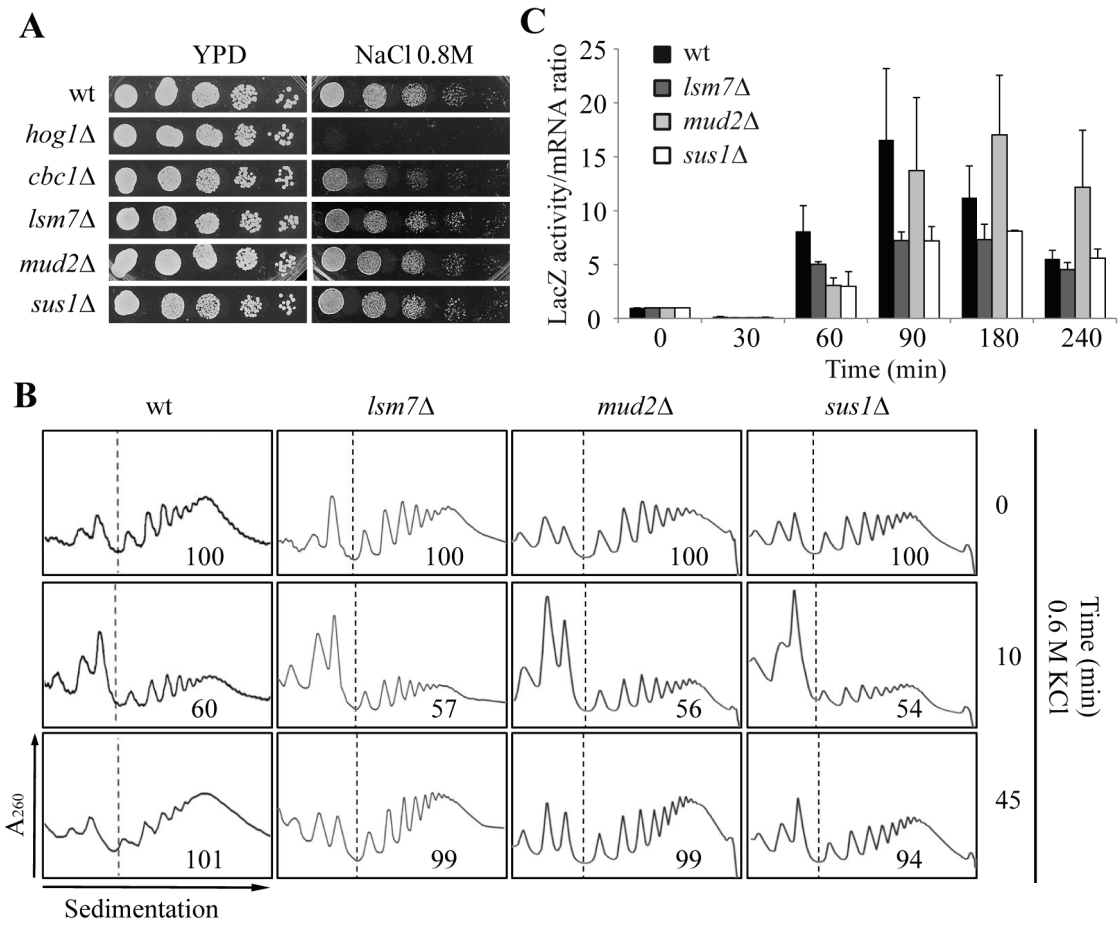

Figure S6. Diverse mutants in genes involved in splicing and mRNA degradation or export had no effect on translation under osmotic stress. (A) Sensitivity to osmotic stress conditions of the strains carrying deletions in the *LSM7* gene, involved in cytoplasmic mRNA decay and nuclear RNA processing; in the *MUD2* gene, involved in pre-mRNA splicing; and in the *SUS1* gene, involved in transcription and mRNA export. Exponentially growing cells were spotted on YPD plates supplemented with 0.8M NaCl. Plates were incubated at 28ºC. (B) Polysome profiles show translation inhibition and recovery during osmotic stress in the *lsm7Δ*, *mud2Δ* and *sus1Δ* strains. The cells grown exponentially in YPD were treated with 0.6 M KCl for the indicated times. Polysomal profiles were obtained as described in Material and Methods. Representative absorbance curves are displayed. The percentage of polysomal area during osmotic stress (0.6 M KCl) is shown in the profile. Data are provided as the mean of two independent experiments. (C) Translation efficiency of *STL1-LacZ* fusion in the *lsm7Δ*, *mud2Δ* and *sus1Δ* mutant cells under osmotic stress. β-galactosidase activity was measured as described in Materials and Methods, and the LacZ mRNA level was determined by Northern blots. Translation efficiency was calculated as β-galactosidase activity/the *LacZ* mRNA ratio, and the results were normalized to the efficiency at time 0. The data shown are the mean and standard error of three independent experiments.