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Supplemental Data

Functional Specificity

among Ribosomal Proteins

Regulates Gene Expression

Suzanne Komili, Natalie G. Farny, Frederick P. Roth, and Pamela A. Silver

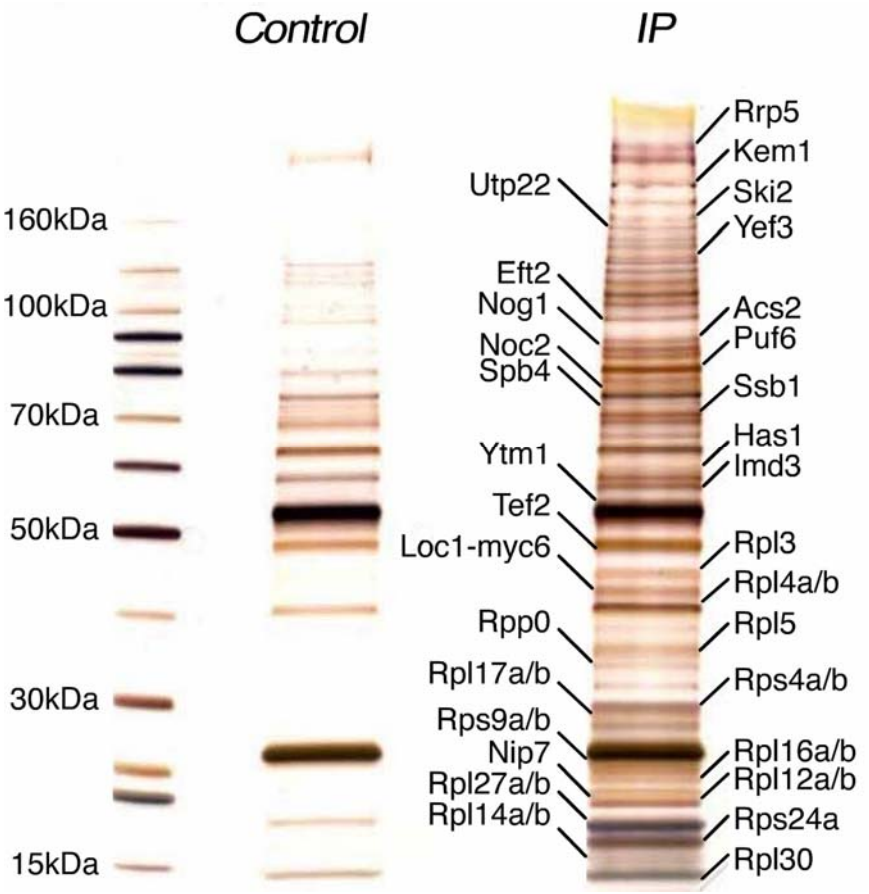

Figure S1: Loc1 associates with ribosomal proteins and ribosomal assembly factors. Loc1 was immunoprecipitated using the myc tag and associated proteins were analyzed by SDS-PAGE followed by silver staining. Associated proteins not present in the untagged control were excised and identified using MALDI-TOF mass spectrometry.

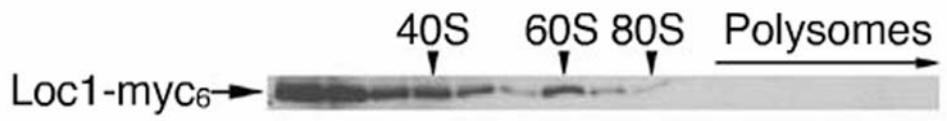

Figure S2: Loc1 does not sediment with polysomal fractions. A strain expressing Loc1-myc$_6$ was subjected to sucrose gradient sedimentation and the resulting fractions were analyzed by immunoblotting. Fractions were identified by sedimentation of the rRNA (data not shown).

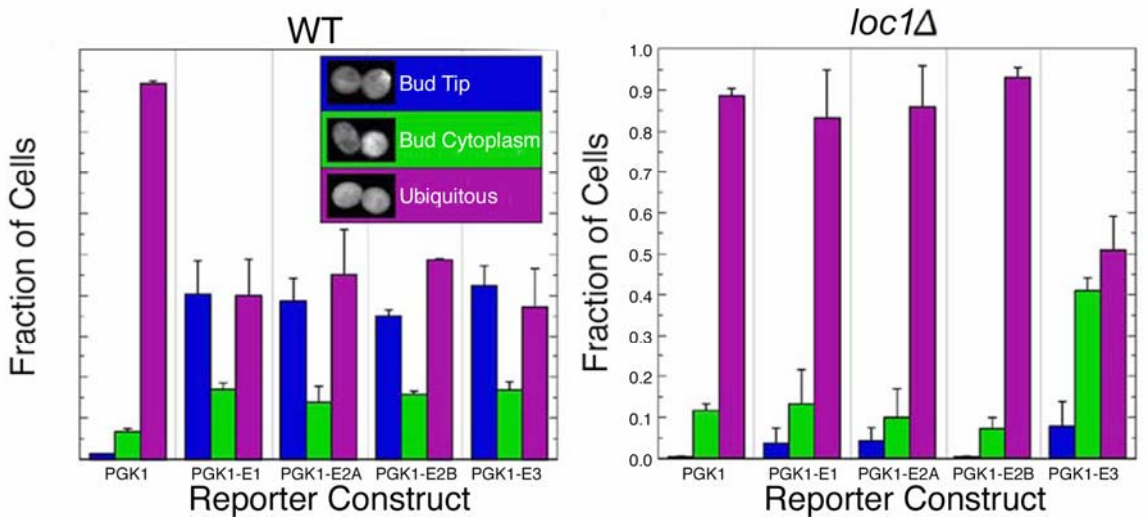

Figure S3: Localizations of reporters bearing all four ASH1 regulatory elements. Left: Assays in wild-type cells demonstrate functionality of reporter constructs. As expected, all four reporters bearing ASH1 sequences localize primarily to the bud-tip, while the negative control exhibits ubiquitous localization. Right: Reporter constructs are mis-localized in *loc1Δ* cells. Reporter constructs bearing E1, E2A, and E2B sequences exhibit ubiquitous localizations, while the reporter construct containing the E3 sequence is primarily bud-cytoplasmic.

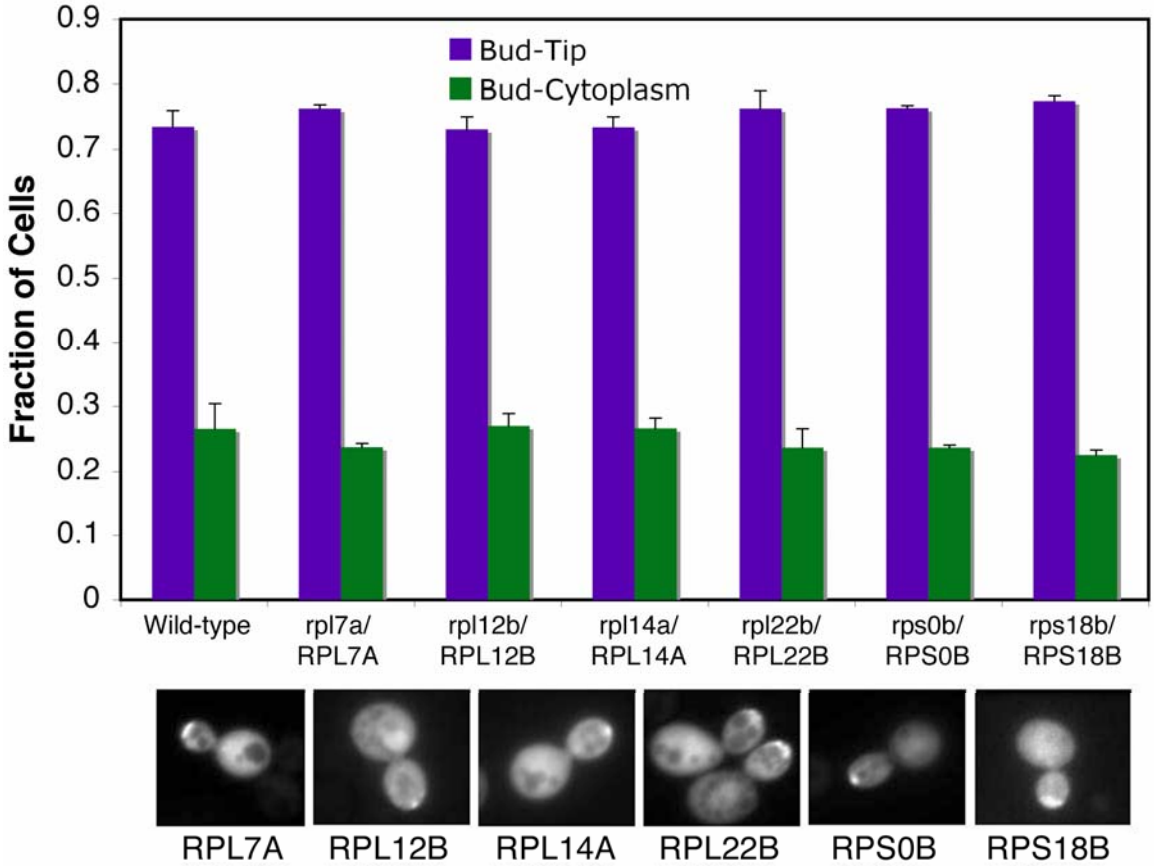

Figure S4: Re-introduction of deleted ribosomal protein gene rescues ASH1 localization defects. The indicated ribosomal protein was expressed off of a CEN plasmid in a strain lacking the corresponding gene. Plasmid expression restores bud-tip localization, confirming that the defect observed in the strains lacking the corresponding ribosomal proteins was not due to secondary mutations.

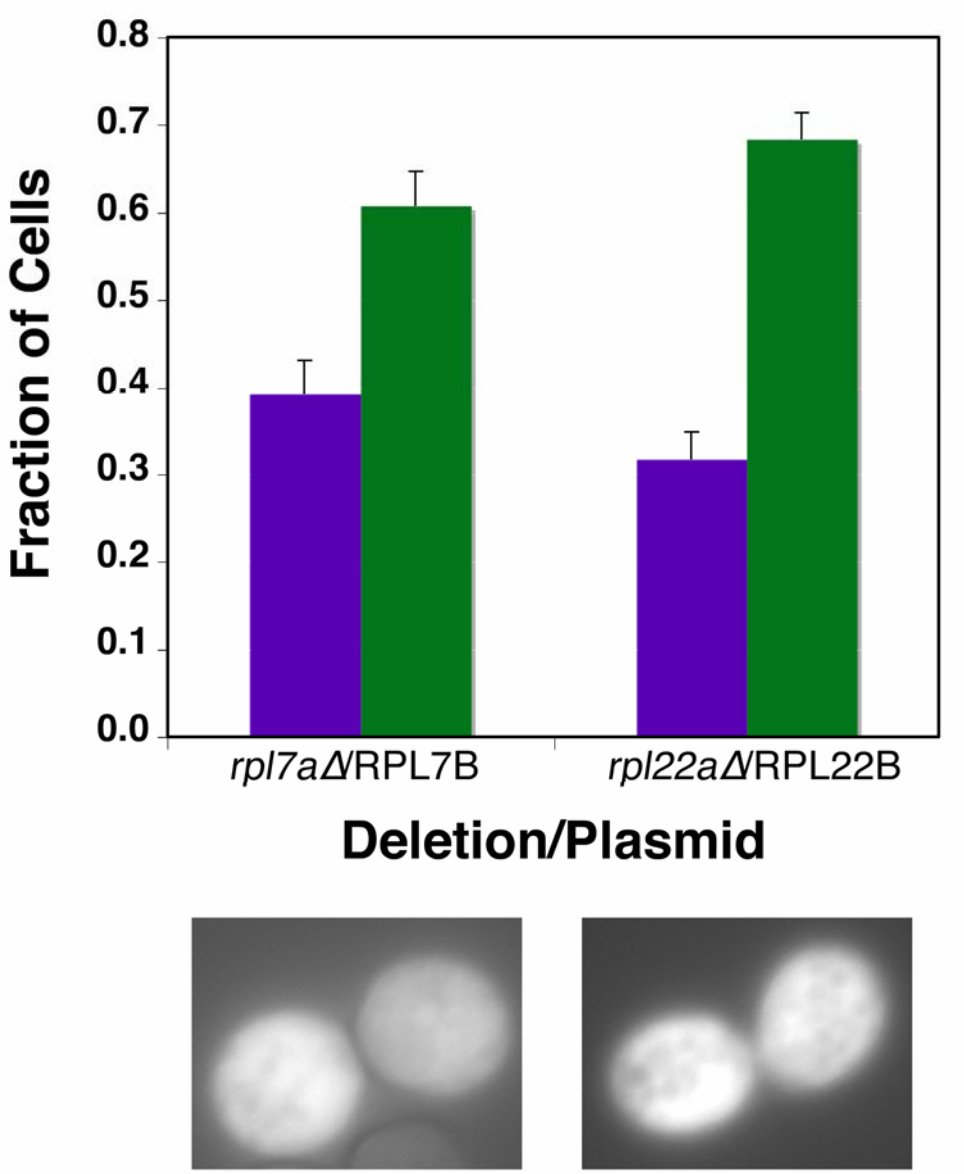

Figure S5: Overexpression of the paralogous ribosomal protein gene does not rescue the ASH1 localization defect. The indicated ribosomal protein was expressed off of a CEN plasmid in a strain lacking the paralogous gene.

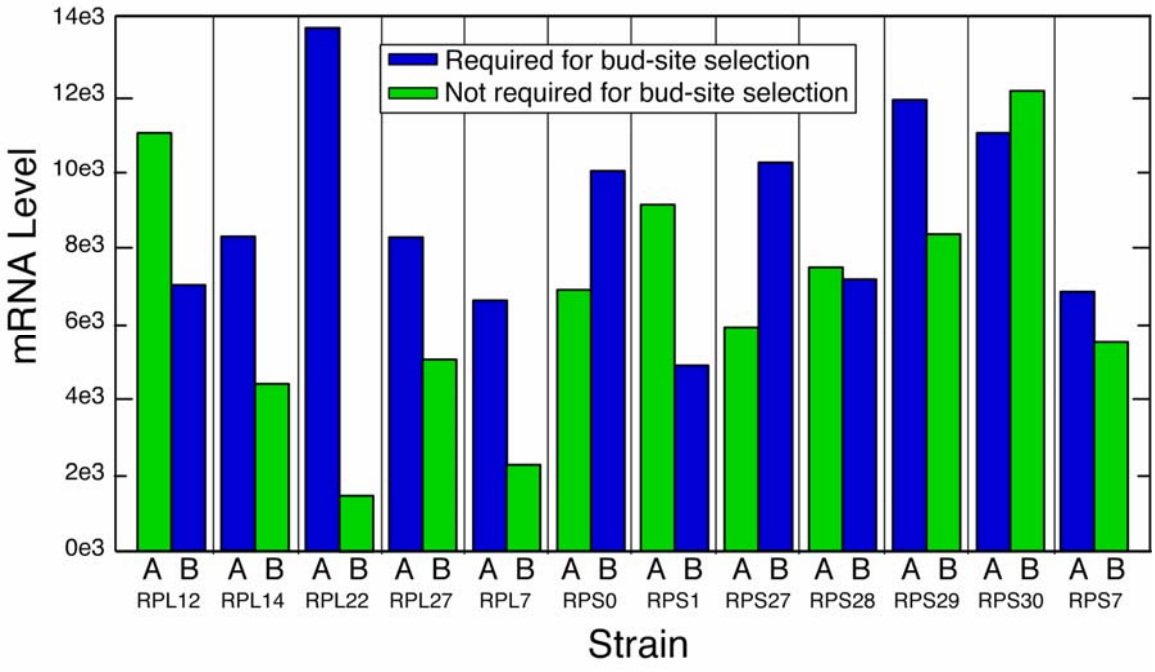

Figure S6: mRNA expression levels of ribosomal proteins required for bud-site selection and their paralogs do not account for ASH1 localization defects. Expression levels were obtained from transcriptional profiling of the wild-type strain used in this study (see Transcriptional Profiling in Experimental Procedures).

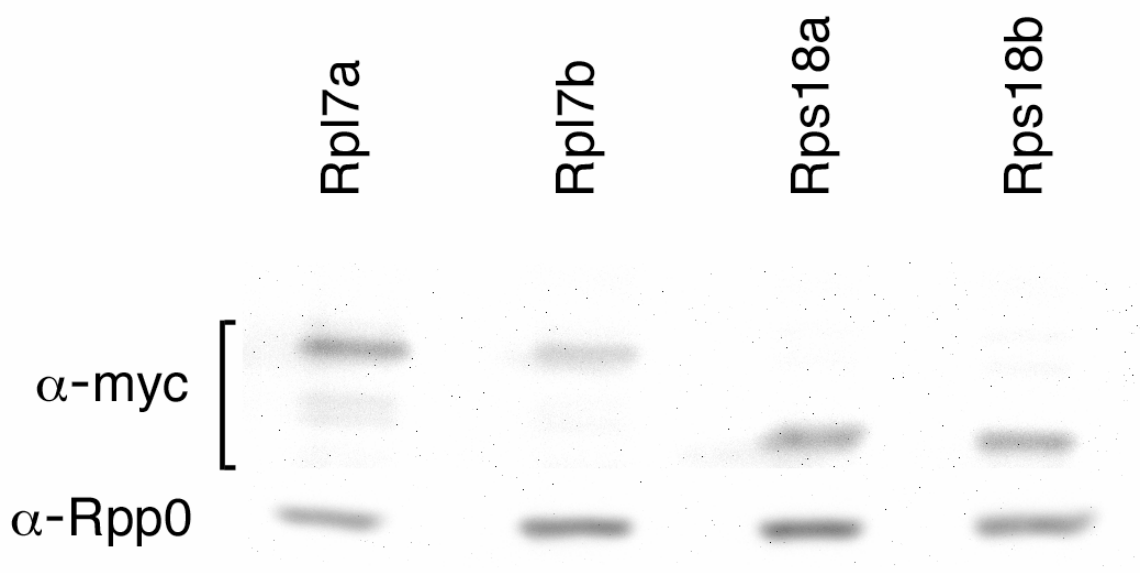

Figure S7: Protein expression levels of two pairs of duplicated ribosomal proteins required for bud-site selection demonstrate that gene dosage is not responsible for defects in *ASH1* localization. Rpl7a, Rpl7b, Rps18a, and Rps18b were N-terminally tagged with myc at the endogenous locus with the same method employed for Ash1 tagging (see Experimental Procedures). Lysates of exponentially-growing cells were normalized to 1mg/mL and analyzed using SDS-PAGE and immunoblotted for myc and Rpp0 (a loading control). Equal amounts of protein were loaded into each lane.

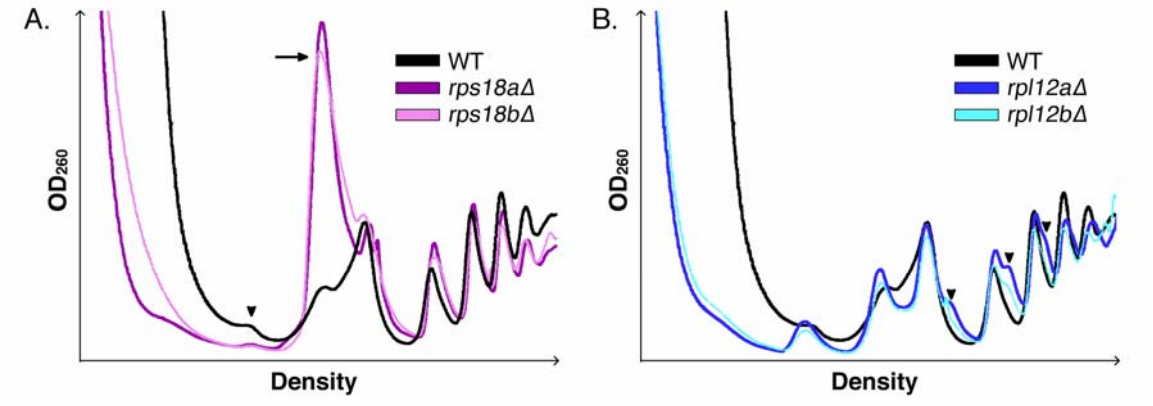

Figure S8: Polysome profiles of cells lacking duplicated ribosomal proteins indicate that paralog specificity observed for *ASH1* localization is not due to differential requirements for ribosomal assembly. (A) Polysome profiles of cells lacking Rps18a and Rps18b overlaid with wild-type. Both deletions have similarly severe accumulation of the 60S fraction (arrow) and decreased abundance of the 40S fraction (arrowhead). (B) Polysome profiles of cells lacking Rpl12a and Rpl12b overlaid with wild-type. Both deletions exhibit mild 60S assembly defects, indicated by the presence of halfmer polysomes (arrowheads).

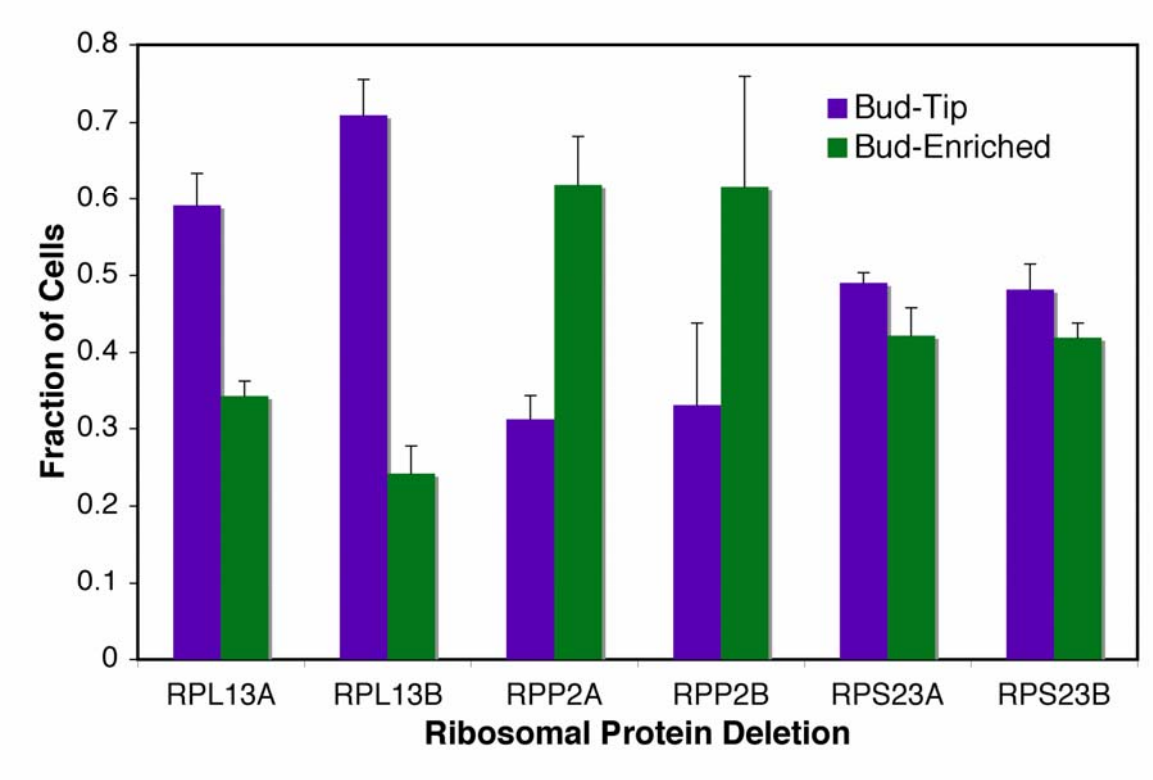

Figure S9: Duplicated ribosomal proteins not implicated in bud-site selection do not exhibit differences in E3 localization between paralogs. A representative subset of E3 localization assays in cells lacking ribosomal proteins in which neither copy is needed for bud-site selection is shown.

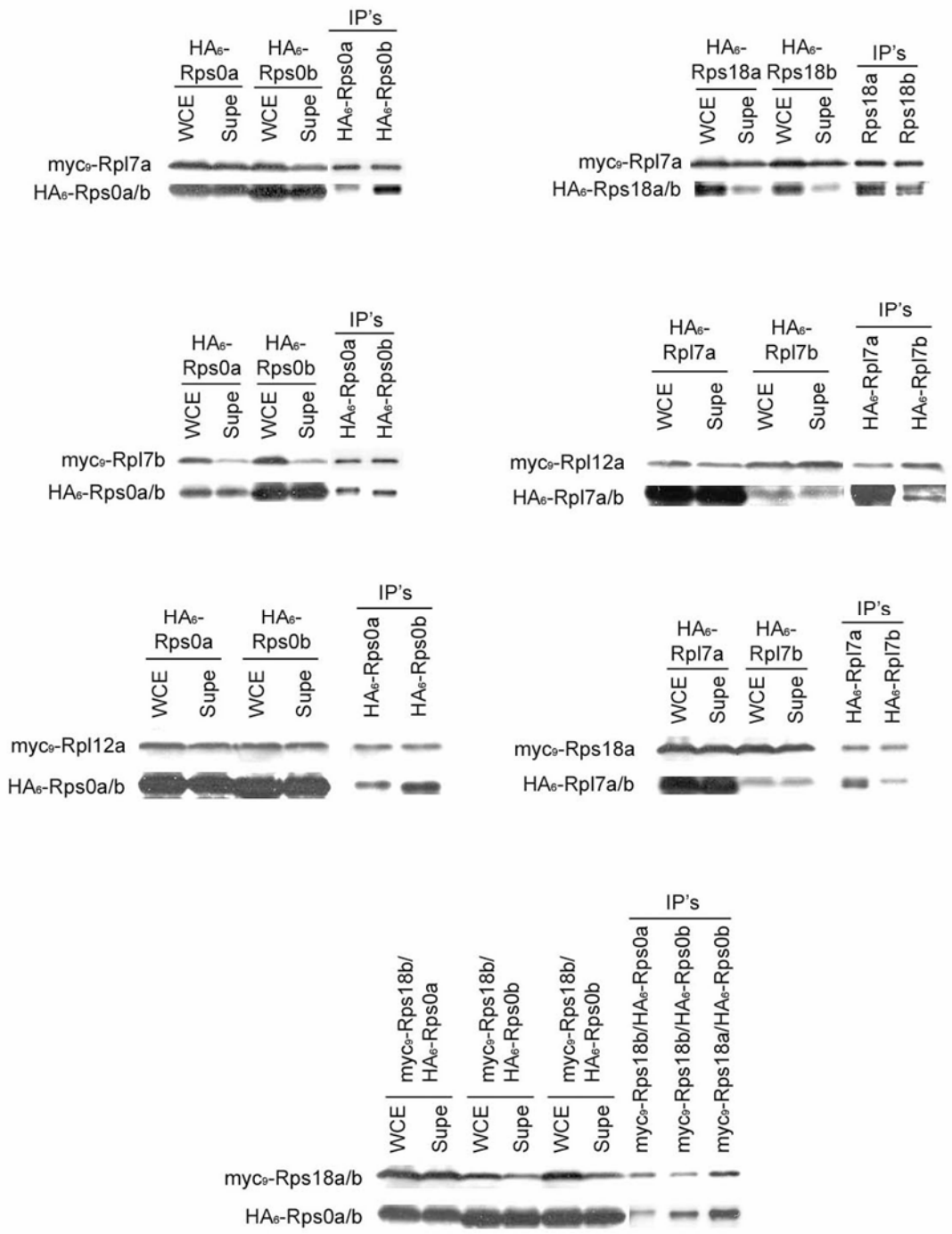

Figure S10: Epitope-tagged ribosomal proteins associate with each other in all combinations tested. Ribosomal proteins were N-terminally tagged at the endogenous locus with the same method employed for Ash1 tagging (see Experimental Procedures). Lysates were prepared from cells flash-frozen in exponential phase, normalized to 1 mg/mL, immunoprecipitated overnight using α-myc, and RNAse-treated. Whole-cell extracts, supernatants, and immunoprecipitated samples were analyzed by SDS-PAGE followed by immunoblotting.