**Figure S3:** Overexpressing eIF5 (hc TIF5) increases eIF5 ribosome-binding, but not 43S assembly, in the 66-70 mutant. A. 43S assembly was measured in WT (H2999 harboring empty vector YEplac195) and 66-70 (harboring either YEplac195 or YEpTIF5-U) strains after cross-linking cells with 1% v/v HCHO. WCEs were resolved by sedimentation through 7.5-30% sucrose gradients and gradient fractions were subjected to Western analysis using the indicated antibodies. 1% and 0.2% aliquots of each input WCE (IN) were analyzed in parallel. The fractions containing free 40S subunits are boxed. B. Initiation factor binding to 40S subunits in the experiment shown in (A), and in two replicate experiments, was quantified by calculating the ratio of the 40S signal in the mutant relative to the WT. The results are means ± SE (n=2). The line at y=1.0 indicates the WT level of binding.