The Title and Legends for Extended Data Tables.

Extended Data Table 1. Yeast strains used in this study.

Extended Data Table 2. Plasmids used in this study.

Extended Data Table 3. Adapters used for the CRAC experiments.

5'-invddT indicates an inverted dideoxy Thymidine. "N" indicates random nucleotide

sequences.

The Legends for Extended Data Figures

Extended Data Figure 1. Multiple sequence alignment of various Nug2 orthologs. Multiple Sequence alignment of YlqF (Bacterial homolog of Nug2; *Bacillus cereus*), *Ct*Nug2 (*Chaetomium thermophilum*), *Dm*Nug2 (*Drosophila melanogaster*), *Dr*Nug2 (*Danio rerio*), *Hs*Nug2 (Homo sapiens), *Kl*Nug2 (*Kluyveromyces lactis*), *Mm*Nug2 (*Mus musculus*), *Sc*Nug2 (*Saccharomyces cerevisiae*), *Sp*Nug2 (*Schizosaccharomyces pombe*), *Xl*Nug2 (*Xenopus laevis*), *Yl*Nug2 (*Yarrowia lipolytica*), using T-Coffee multiple sequence alignment (http://www.ebi.ac.uk/Tools/msa/tcoffee) and Jalview. Indicated above the alignment are the different Nug2 domains including N-, G-, C-domain and C-terminal Extension. Moreover, DAR, G1-, G3-, G4-motifs, point mutation sites (in red) and truncated site of *ct*Nug2 510aa (red line) are indicated.

Extended Data Figure 2. Nug2 and Nmd3 are not found on the same pre-60S particles. Indicated different TAP-tagged bait proteins were affinity purified from yeast wild type cells. The final eluates were analyzed by SDS-PAGE and Coomassie staining (upper panel), and by Western blotting using indicated antibody (lower panel). The asterisks mark the position of each bait protein. Rea1 has been identified by mass spectrometry. All affinity-purifications and Western analyses were performed at least twice, yielding highly reproducible datasets.

Extended Data Figure 3. *Chaetomium thermophilum* Nug2 (*ct*Nug2) can complement the lethal phenotype of a *nug2Δ* null mutant. Serial dilutions of the yeast Nug2 shuffle strain (*Mat*a, *ade2*, *ade3*, *his3*, *ura3*, *leu2*, *trp1*, *nug2*::*kan*MX4, pHT4467-NUG2) transformed with either empty plasmid, yeast *scNUG2*, *ctNUG2* and *ctNUG2-510* (trunction of the non-conserved C-terminal extension; see Extended data Fig. 1) under the control of the constitutive *ADH1* promoter in a single-copy (YCplac111) or multi-copy (pRS425) number plasmid (see Supplementary Table 2) were spotted on SDC-Leu (loading control) and SDC plates containing 5-FOA at indicated temperatures for 6 days. Note that *ct*Nug2 only partially complements the *nug2* null mutant.

Extended Data Figure 4. Mutations in ATP-binding or MIDAS domain of Rea1 inhibit release of Rsa4 and Nug2 from the pre-60S particle. (**a**, **b**) Wild-type *REA1* and the *rea1* mutants mapping in the ATP-binding site of the AAA2 domain (K659A) or in the MIDAS domain (DAA)[21] were N-terminally tagged with GFP and expressed in a *REA1* shuffle strain (**a**) or overexpressed under the control of the inducible *GAL1-10* promoter in *REA1* wild-type strain DS1-2b (**b**). Transformants were spotted in 10-fold serial dilution steps on the indicated plates and incubated at 30ºC for 3 days. Both the *rea1* mutant alleles do not complement the *rea1* null strain (**a**, SDC + 5-FOA) and cause a dominant-negative phenotype upon overexpression by replacing endogenous Rea1 (**b**, Galactose). (**c**) Overnight pre-cultures were grown in SRC -Leu to prevent plasmid loss,

followed by shifting cells (OD 0.75) to galactose medium (YPG) for 7 hours. Rix1-particles, which were affinity-purified from a Rix1-TAP/RpL3-Flag strain containing either endogenous wild-type or overexpressed wild-type GFP-Rea1, GFP-Rea1DAA and GFP-Rea1K659A, respectively, were incubated with or without 4 mM ATP in KCl buffer, before the different *in vitro* matured pre-60S particles were re-isolated by affinity-purification via the RpL3-Flag on Flag beads. Subsequently, the *in vitro* matured pre-60S particles (eluates) were analyzed by SDS-PAGE and Coomassie staining. Relevant bands are indicated on the right of the gel. Note that in the case of the *rea1* mutants, the release of Nug2, Rsa4, but also of Rea1 and the Rix1-complex is significantly inhibited. All *in vitro* assays were performed at least twice, yielding highly reproducible datasets.

Extended Data Figure 5. Nug2 depletion assay using Auxin inducible degron system. (**a**) Growth of Nug2 auxin degron strains (*sAid-NUG2-sAid*) in the P_{ADH}-*OsTIR1* background on YPD plates with or without 500 μM Auxin (IAA). The cell growth of *sAid-NUG2-sAid* strain was inhibited by the addition of Auxin. (**b**) Western blotting of sAid-Nug2-sAid after auxin treatment. The depletion of sAid-Nug2-sAid occurred within about 30 minutes after addition of Auxin.