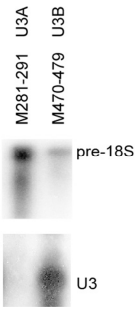

Figure S1. Northern blot analysis of 5' ETS mutant 90S.

RNA (1µg) was extracted from 90S particles purified via MS2-tagged pre-18S and Utp9-TAP and separated in an 8% urea denaturing polyacrylamide gel. RNA was hybridized with ^{32}P-labeled DNA probes binding the MS2-tag and U3 snoRNA.

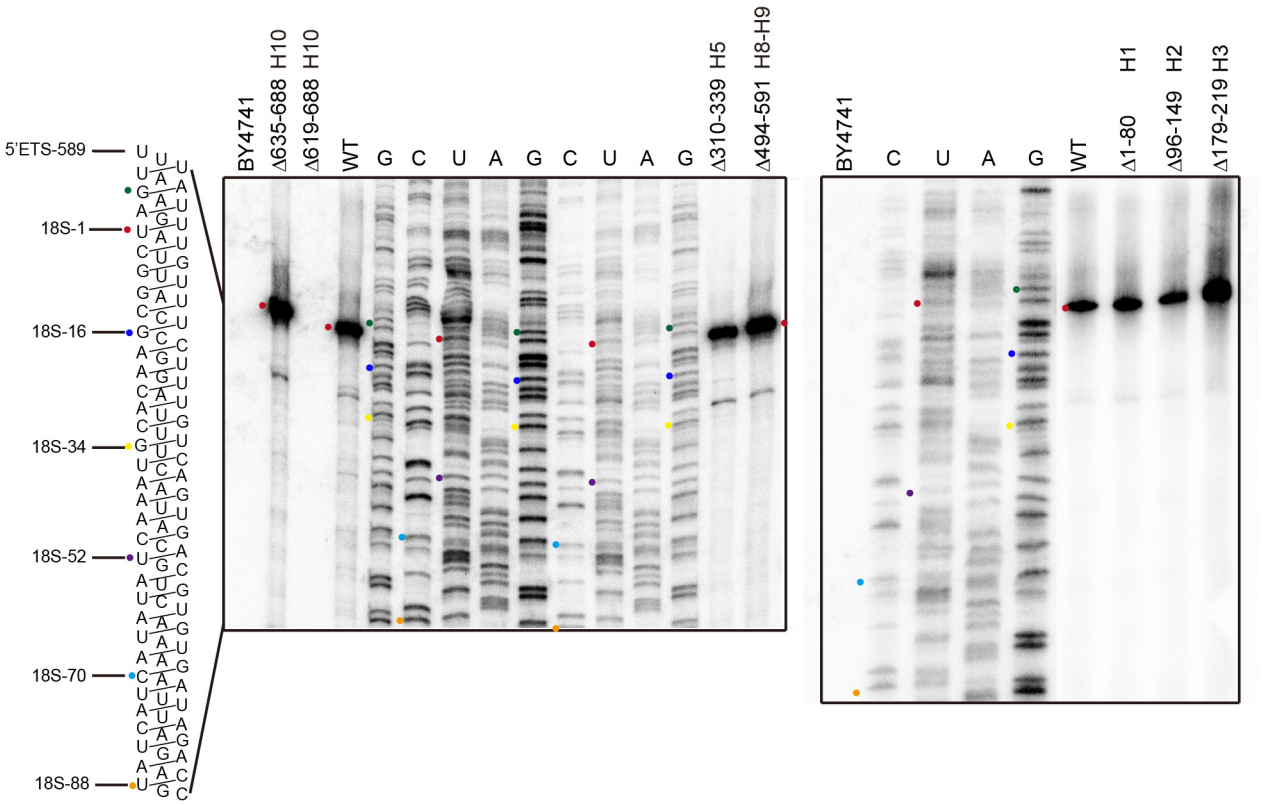

Figure S2. Mapping of 5' end of plasmid-derived 18S rRNA. RNA was extracted from BY4741 (control) or Utp9-TAP strains expressing the indicated pre-18S fragments and subjected to primer extension using the 18S-tag probe that is complementary to a plasmid-specific sequence inserted after position 232 of 18S rRNA. Sequencing ladders (A, T, G, C) are generated from plasmid pLM104. The reference sequence is shown in a zigzag manner at the left. A few positions are labeled with colored dots at both the sequence ladders and the sequence.

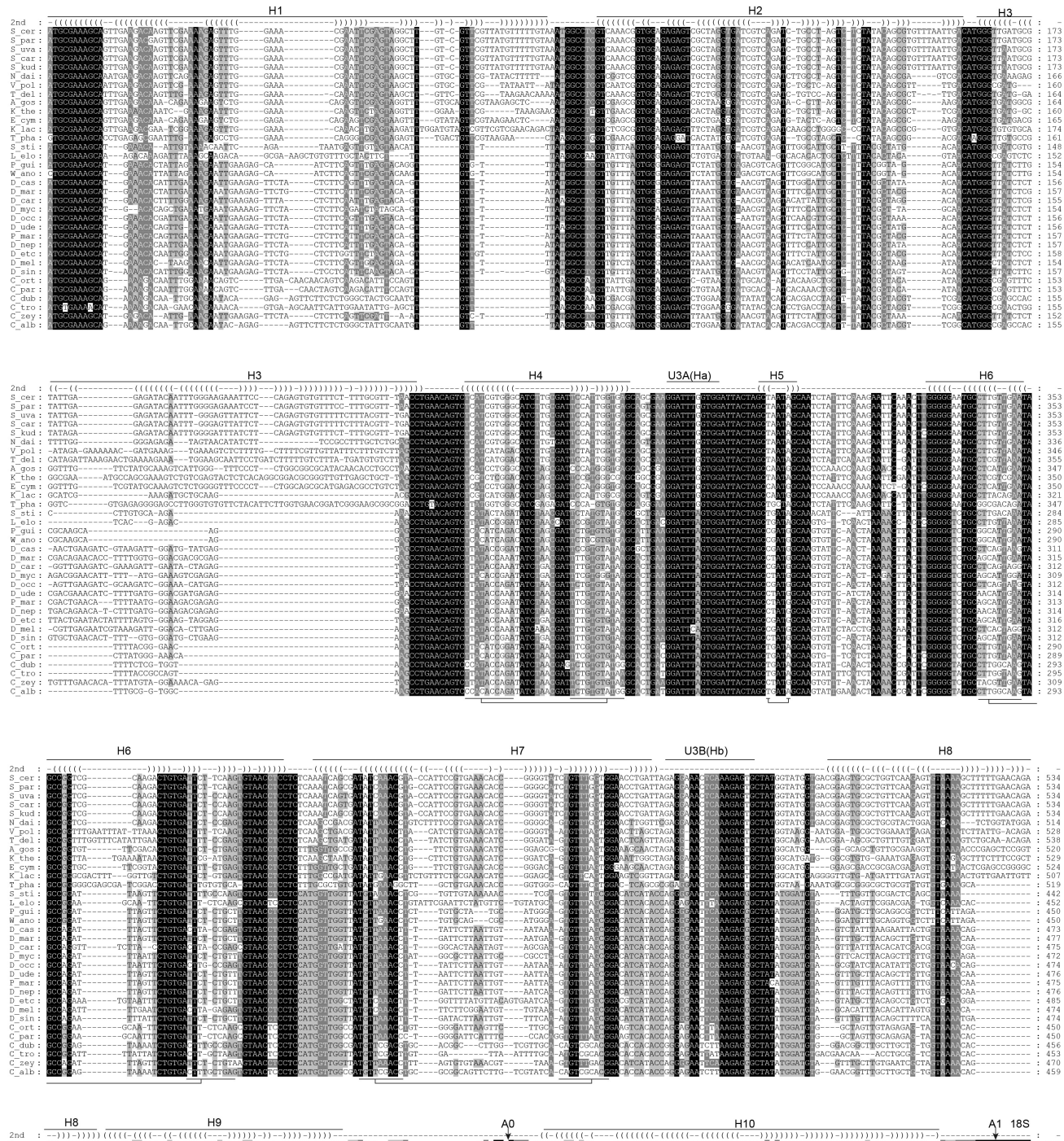

Figure S3. Multiple sequence alignment of yeast 5' ETS.

5’ ETS sequences from 34 yeasts were aligned by MUSCLE and manual adjustment and presented in Genedoc. Residues with >95%, 95-80% and 80-60% identity are shaded with black, grey and light grey, respectively. Based paired residues are annotated with parentheses on the top. Positions with strong co-variation are connected at the bottom. Species and abbreviations are listed below:

Saccharomycotina/Saccharomycetaceae: Saccharomyces cerevisiae (S_cer), Saccharomyces paradoxus (S_par), Saccharomyces uvarum (S_uva), Saccharomyces carlsbergensis (S_car), Saccharomyces kudriavzevii (S_kud), Naumovozyma dairenensis (N_Dai), Vanderwaltozyma polyspora (V_pol), Torulaspora delbrueckii (T_del), Ashbya gossypii (A_gos), Kluyveromyces thermotolerans (K_the), Eremothecium cymbalariae (E_cym), Kluyveromyces lactis (K_lac), Tetrapisispora phaffii (T_pha)

Saccharomycotina/Debaryomycetaceae: Scheffersomyces stipitis (S_sti), Lodderomyces elongisporus (L_elo), Pichia guilliermondii (P_gui), Debaryomyces castellii (D_cas), Debaryomyces maramus (D_mar), Debaryomyces carsonii (D_car), Debaryomyces mycophilus (D_myc), Debaryomyces occidentalis (D_occ), Debaryomyces udenii (D_ude), Debaryomyces nepalensis (D_nep), Debaryomyces etchellsii (D_etc), Debaryomyces melissophilus (D_mel), Debaryomyces singareniensis (D_sin), Candida orthopsilosis (C_oth), Candida parapsilosis (C_par), Candida dubliniensis (C_dub), Candida tropicalis (C_tro), Candida zeylanoides (C_zey), Candida albicans (C_alb)

Saccharomycotina/Phaffomycetaceae: Wickerhamomyces anomalus (W_ano)

Pezizomycotina/Trichocomaceae: Penicillium marneffei (P_mar)

Supplementary Dataset 1. Mass spectrometric data for pre-ribosomal particles. The SCPHR and RSAF values of identified proteins are displayed in two separate sheets. The RSAF is normalized against six UTPB proteins, Efg1 or Rrp5 (highlighted). The top rows include the summed SCPHR or RSAF values for 90S proteins, pre-40S proteins, pre-60S proteins, small subunit ribosomal proteins (RPS), large subunit ribosomal proteins (RPL) and total identified proteins and the molar percentage of 90S and pre-40S AFs over all detected proteins. (Excel table)