Figure S1

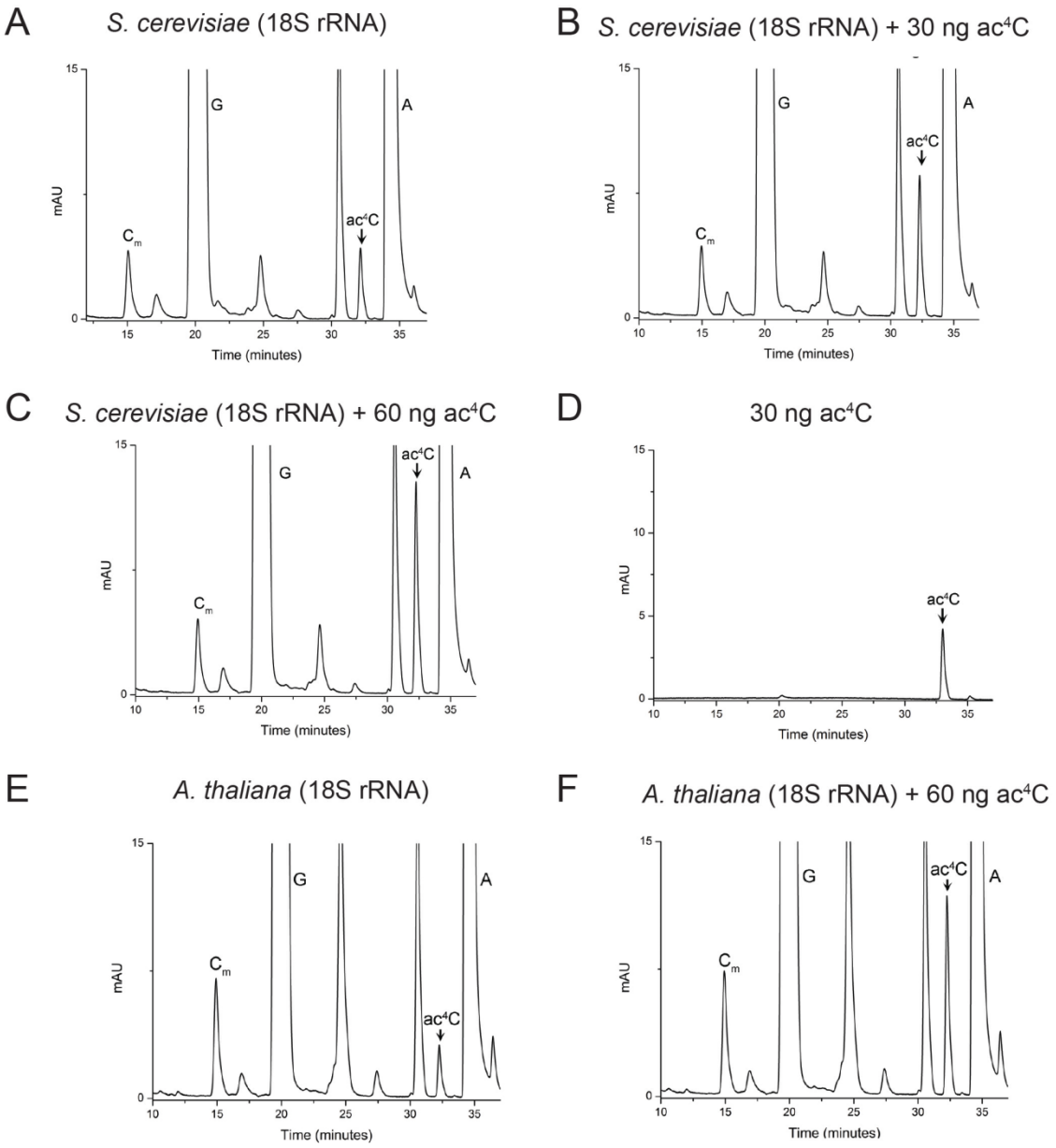

Fig S1: Detecting ac4C by quantitative HPLC in budding yeast and plants
Purified yeast and plant 18S rRNA digested and analyzed by HPLC. A commercial ac4C marker (Carbosynth Ltd., U.K.) used alone or in combination with yeast or plant RNA established the peak at 32.5 min as N4-acetylcytidine.
A, budding yeast 18S rRNA
B, comigration of budding yeast 18S rRNA and 30 ng synthetic ac4C
C, same as in B with 60 ng marker
D, 30 ng ac4C marker
E, plant 18S rRNA
F, plant 18S rRNA and 60 ng ac4C

Figure S2

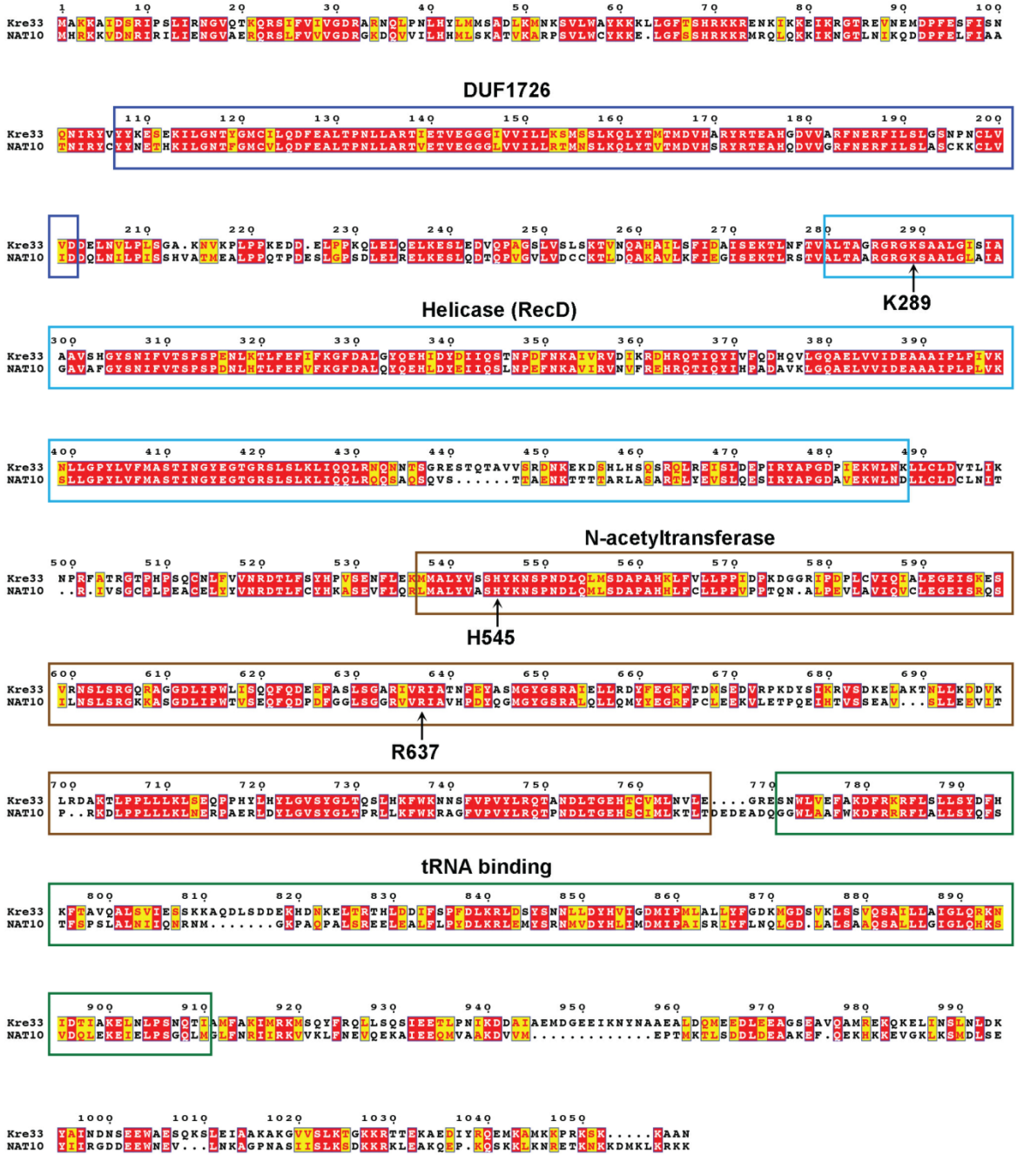

Fig S2 Yeast Kre33 is homologous to human NAT10

Multiple protein alignment between S. cerevisiae Kre33 and H. sapiens NAT10 depicting conserved boxes and residues mutated in this work.

Figure S3

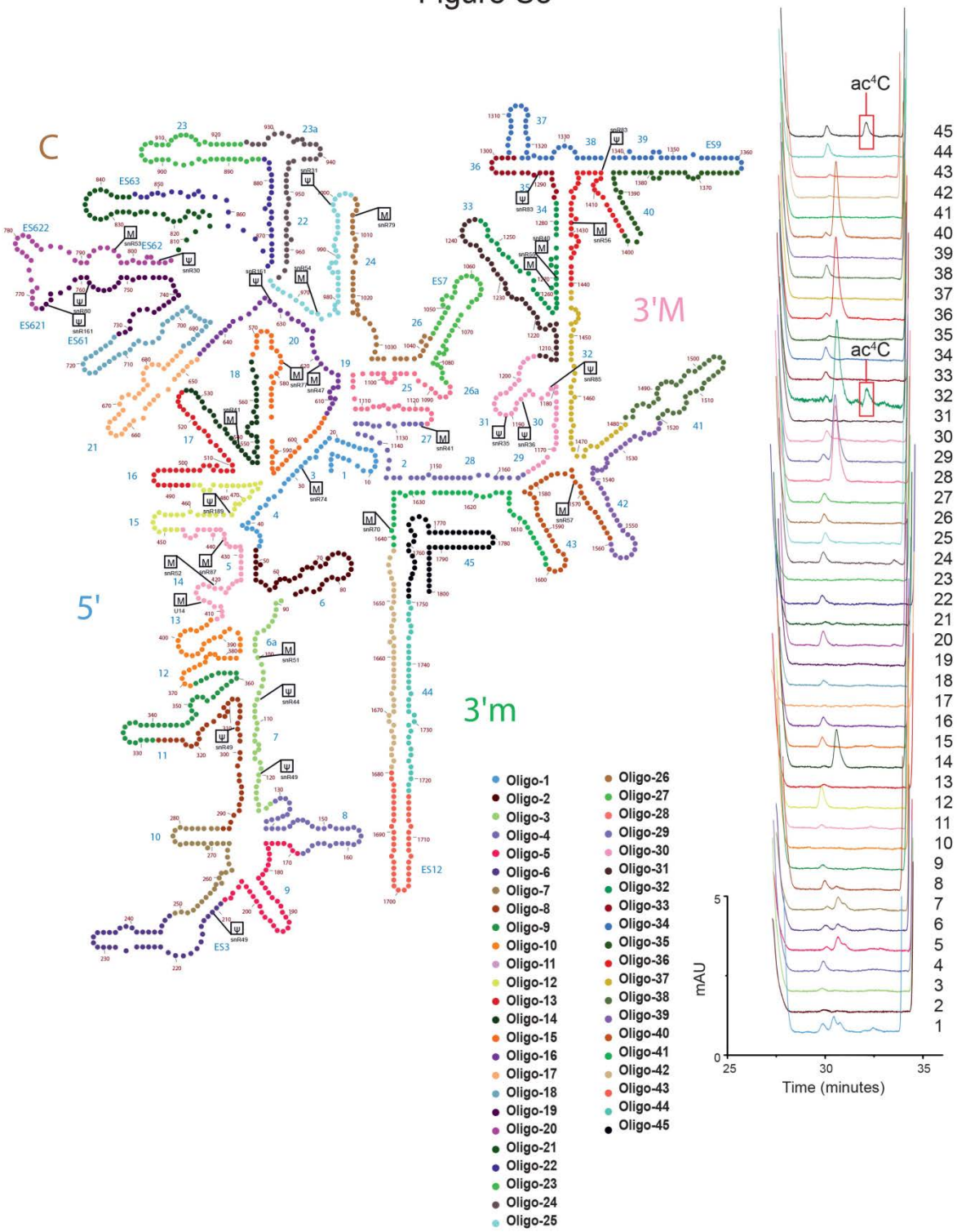

Fig S3: Scanning the entire budding yeast 18S rRNA for acetylated cytosines by a mung bean nuclease protection assay reveals two ac4C

Forty-five probes were used to scan the entire small subunit rRNA sequence by mung bean nuclease protection assay for the presence of acetylated cytosines.

Left, secondary structure of yeast 18S rRNA with color-coded probes. The four 18S rRNA domains are indicated as 5', C (central), 3'M (3' major) and 3'm (3' minor). Boxed M, 2'-O methylated residues

(with snoRNA underneath). Boxed Ψ, pseudouridines (with snoRNA underneath). Helices and expansion segments (ES) numbered in blue.
Right, HPLC profile obtained with each probe. Probe n°32, complementary to helix 34 (residues 1245-1283) in the 3' major domain, and probe n°45, complementary to helix 45 (residues 1751 to 1800) at the 3' end of the 18S rRNA, both protect an ac4C modification. Similar results were obtained with probe n°1280, protecting 1267-1317, and probe n°1770, protecting 1747-1796, respectively.

Figure S4

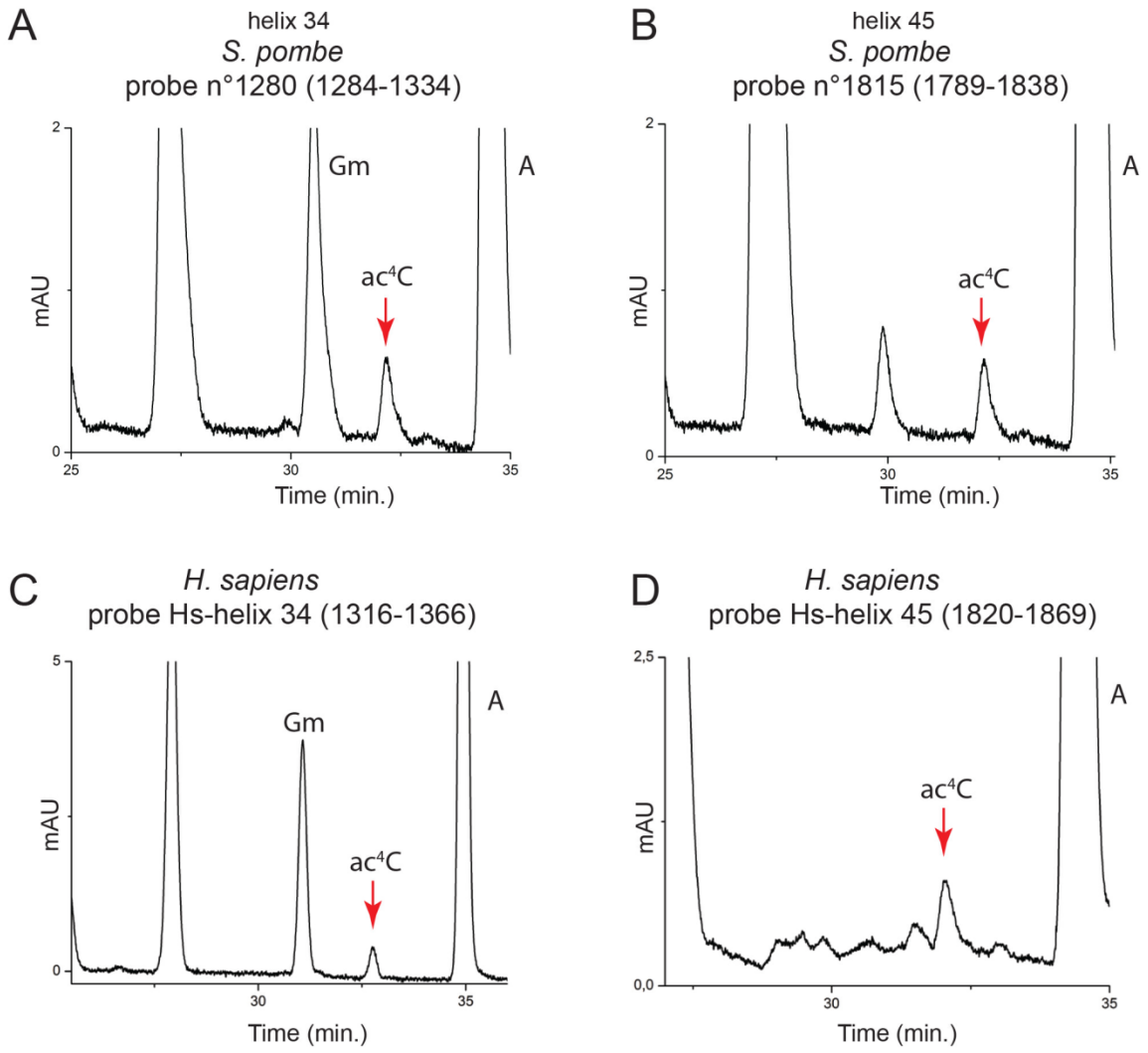

Fig S4: S. pombe and H. sapiens cells carry one ac4C in helix 34 and one in helix 45

A, total RNA from S. pombe annealed to probe n°1280, protecting nt 1284-1334 (helix 34 in 3' major domain)

B, total RNA isolated from S. pombe annealed to probe n°1815, protecting nt 1789-1838 (helix 45 in 3' minor domain)

C, total RNA from H. sapiens annealed to probe Hs-helix 34, protecting nt 1316-1336 (3' major domain)

D, total RNA isolated from H. sapiens annealed to Hs-helix 45, protecting nt 1820-1869 (3' minor domain)

Figure S5

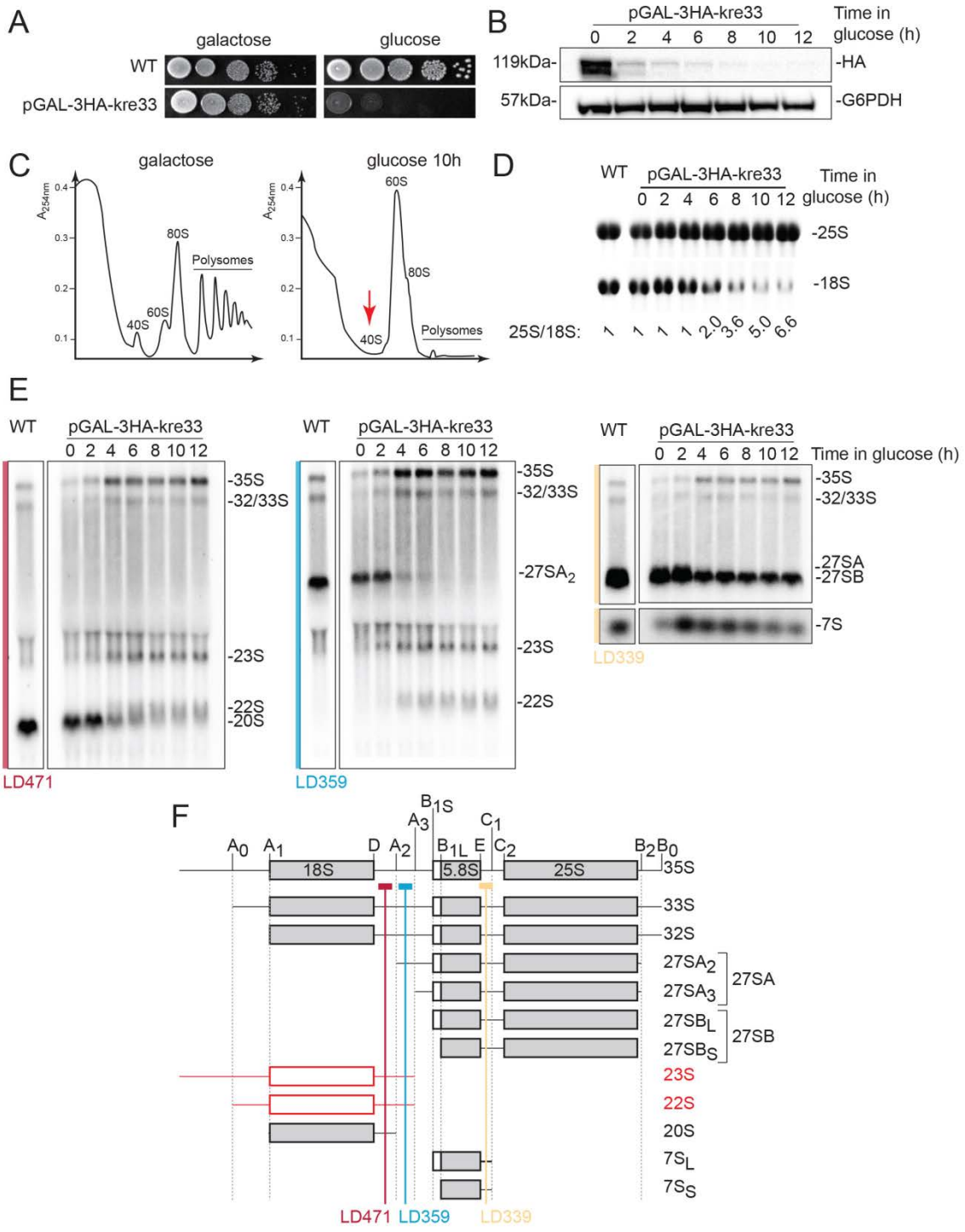

Fig S5: Budding yeast Kre33 is required for 40S subunit synthesis

A, The conditional pGAL-3HA-kre33 strain is galactose-dependent. Growth assay on plates: serial dilutions (1x to 104x from left to right) of pGAL-3HA-kre33 cells spotted on galactose or glucose-based rich medium and incubated for 3 d at 30°C.

B, In pGAL-3HA-kre33 cells, depletion of Kre33 is efficient upon transfer to glucose. Western blot analysis of Kre33 depletion. Cells were grown to mid-log phase in galactose, washed, and transferred to glucose for the indicated times. Total protein was extracted and analyzed by anti-HA western blotting. Detection of G6PDH provides a loading control.

C, Kre33 is required for small subunit accumulation. Polysomes profile analysis upon Kre33 depletion. Polysomes formed in cells grown in galactose or transferred to glucose for 10 h.
D, Kre33 is required for 18S rRNA synthesis. Total RNA extracted at the indicated time points of Kre33 depletion was analyzed on denaturing agarose gels stained with ethidium bromide. 28S/18S ratio determined from Agilent Bioanalyzer electropherograms.
E, Pre-rRNA processing analysis upon Kre33 depletion. The RNAs in panel D were transferred to a nylon membrane and hybridized with three probes (LD471, LD359, LD339).
F, Schematic representation of the major pre-rRNA intermediates detected with the probes used. Cleavage sites indicated on top of the 35S primary transcript (A0 to D).

Figure S6

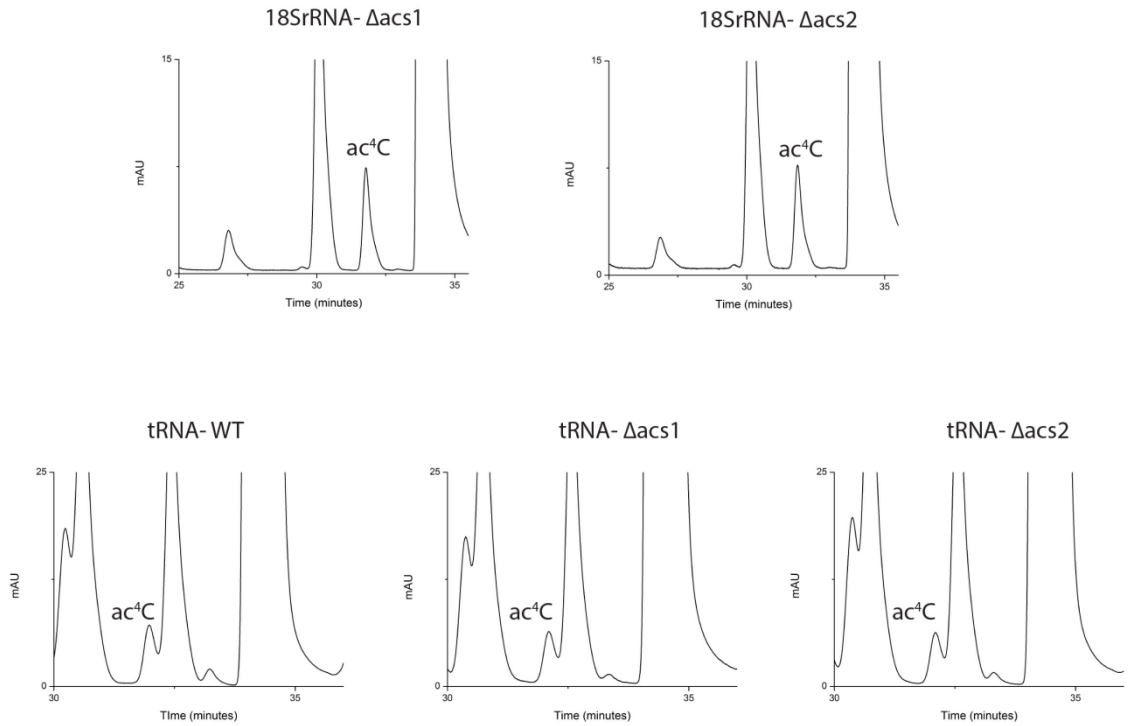

Fig S6: Yeast acetyl-CoA synthetases 1 and 2 (Acs1 and Acs2) are each dispensable for 18S rRNA and tRNA acetylation.
18S rRNA and tRNAs purified from the indicated yeast strains were analyzed by HPLC. Acs2 is essential to growth on glucose-based medium but is dispensable on galactose or sucrose (83). Therefore, the acs1Δ, acs2Δ and isogenic wild-type cells were grown on galactose. Identical results were obtained on sucrose.

83.Van den Berg, M.A. and Steensma, H.Y. (1995) ACS2, a Saccharomyces cerevisiae gene encoding acetyl-coenzyme A synthetase, essential for growth on glucose. European journal of biochemistry / FEBS, 231, 704-713.