Legends of Supplemental Figures

Supplemental Figure S1 Extracts prepared from yeast cells treated with EDTA were fractionated in 10-50% linear sucrose gradients. An OD_{254} plot corresponding to the non-polysomal and polysomal fractions is shown. ASH1 mRNA was shifted into non-polysomal fractions after EDTA treatment.

Supplemental Figure S2 (A) MBP-MCP fusion protein was expressed in E. Coli and was affinity-purified using amylose beads (NEB). Purified recombinant proteins were separated in SDS-PAGE following by Coomassie blue staining. (B) Recombinant His-tagged Dhh1p was expressed in E. Coli and purified. The purified proteins were separated in SDS-PAGE following by Coomassie blue staining. Purified Dhh1p was stored in -80C and was used for RNA gel shift assays. Figures of (A) and (B) were modified after removing some unnecessary lanes.

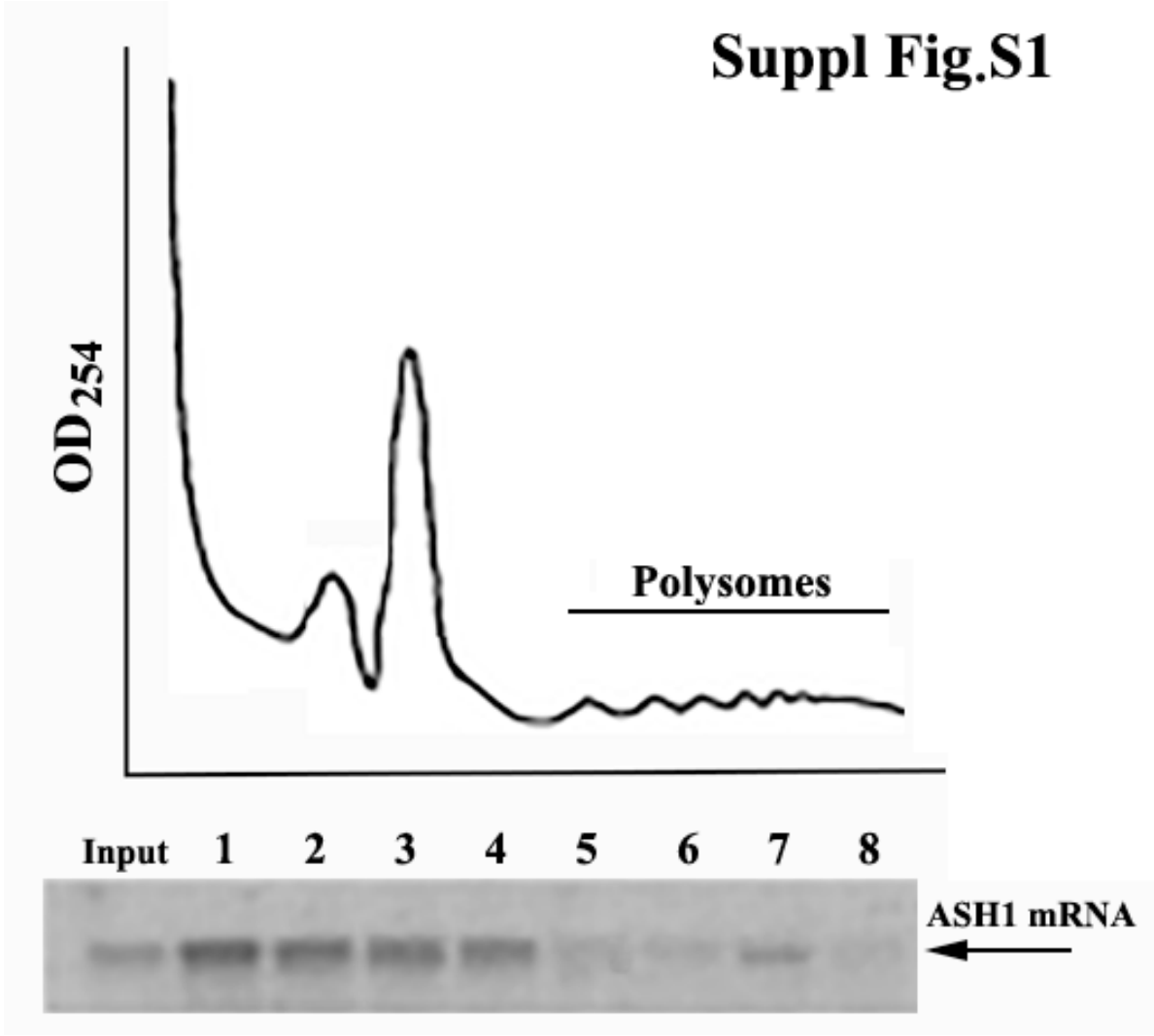
Supplemental Figure S3 (A) Equal amounts of protein from cells treated with or without CHX were subjected to Western blot for detecting the expression of Dhh1p and Puf6p. GAPDH was used as an internal control. (B) Quantitative analysis of Dhh1p and Puf6p expression in the cell extracts treated with or without CHX. Mean values (+SD) were from three independent western experiments. (C) Relative levels of Dhh1p and Puf6p associated with ASH1 mRNA in the cells treated with or without CHX were shown. Mean values (+SD) were from two independent western experiments.

Supplemental Figure S4 (A) Yeast cells expressing Puf6p-TAP were treated with cycloheximide for 20 min. Co-IP experiments were performed to analyze the association of Puf6p with Dhh1p and MID2 or SRL1 mRNAs. Both MID2 mRNA and SRL1 mRNA co-precipitated with Puf6p in CHX non-treated cells, but not co-precipitated with Puf6p in CHX treated cells (middle panels). Dhh1p was also greatly decreased in the precipitates of CHX treated cells (lower panel). (B) MS2(6) repeats were fused into the 3' ends of *5'UTR-ASH1ORF-3'UTR* (WT) and *ADH2-5'UTR-ASH1ORF-3'UTR* (5'mut) constructs. Recombinant chimeric MBP-MCP was used to precipitate ASH1-MS2(6) mRNA in the cell extracts. Upper, RT-PCR was performed to detect MS2(6) tagged ASH1 mRNAs. Lower, Western blots were used to detect Dhh1 and Puf6 that co-precipitated with WT and 5'mut of *ASH1* mRNA.

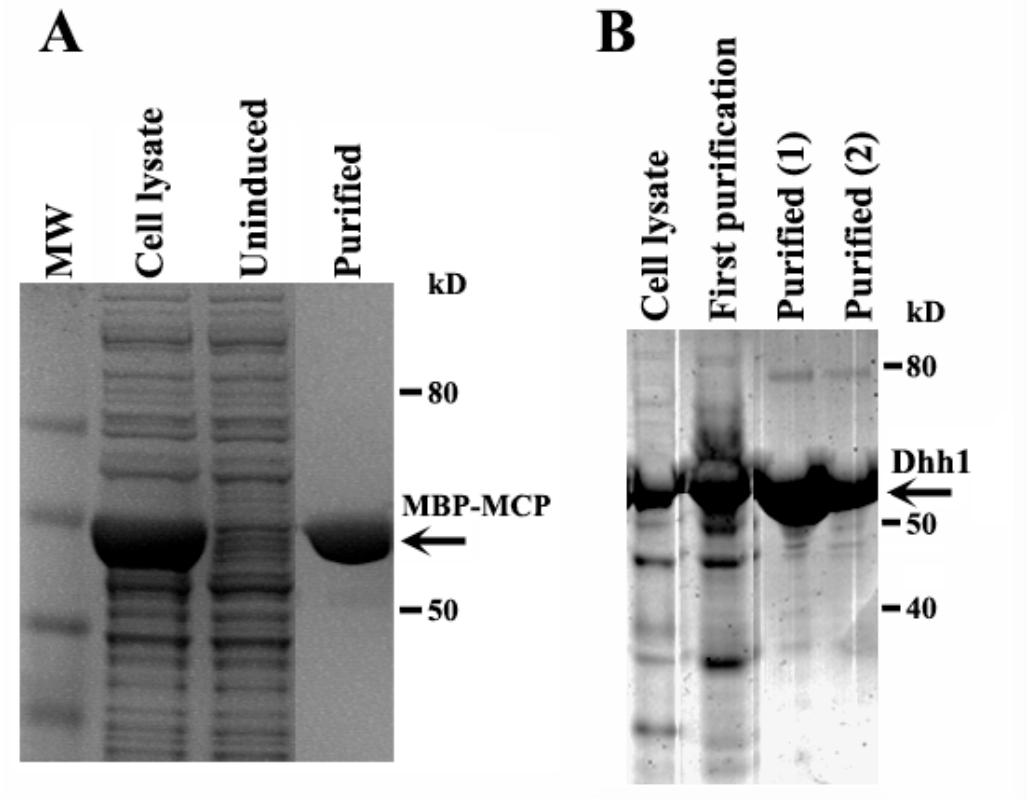
Supplemental Figure S5 Expression of Tap-tagged Dhh1, Puf6 and She2 in yeast strains Dhh1-TAP, Puf6-TAP and She2-TAP cells were cultured to OD_{600} around 1.0. Cells were harvested and extracts were prepared. Western blots were performed to examine the expression of Tap-tagged Dhh1, Puf6 and She2 proteins. The arrows indicated the detected target proteins. Tubulin was used as a loading control. (B) Co-IP and RT-PCR experiments were performed to detect the association of Dhh1p-Tap with ASH1 mRNA in Tap-tagged dhh1p cells. Upper panels: western blots indicate the precipitation of Dhh1p-Tap. Lower panels: RT-PCR indicate that cytoplasmic ASH1 mRNA co-precipitated with Dhh1p-Tap.

Supplemental Figure S6 (A) In vitro translation of luciferase reporters in yeast extracts prepared from a *dhh1* strain. Autoradiography of in vitro translated [35S]methionine-labeled luciferase protein using the RNA templates containing the 5'UTR of ASH1 mRNA or the 5'UTR of ADH2 mRNA in the presence of 1x, 4x, and 16x excess molar ratio of recombinant Dhh1. (B) Relative translational efficiency of RNA template containing the 5'UTRs of ASH1 mRNA was shown after normalizing to the RNA templates containing the 5' UTR of ADH2 mRNA. Mean values (+ SD) were from three independent experiments. *$P<0.05$.

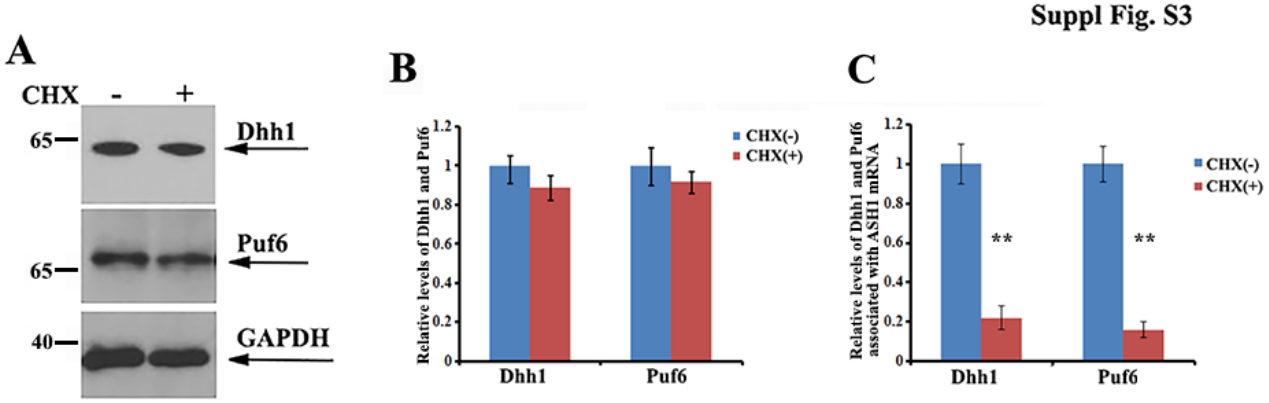
Suppl Fig.S1



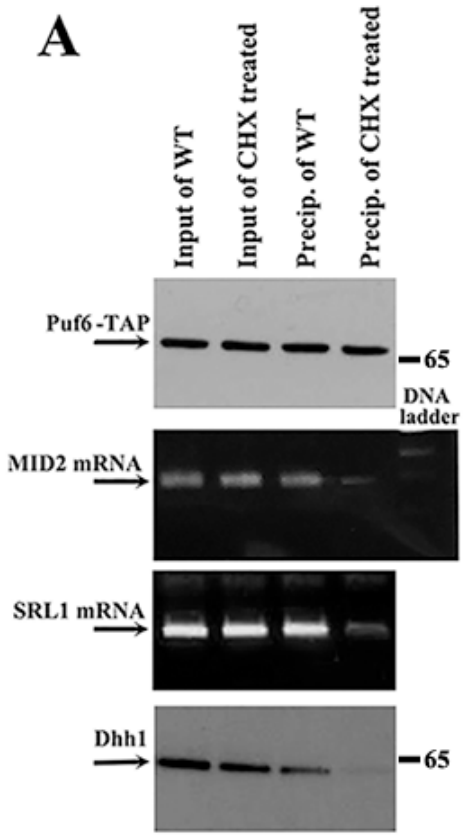
Suppl Fig. S2

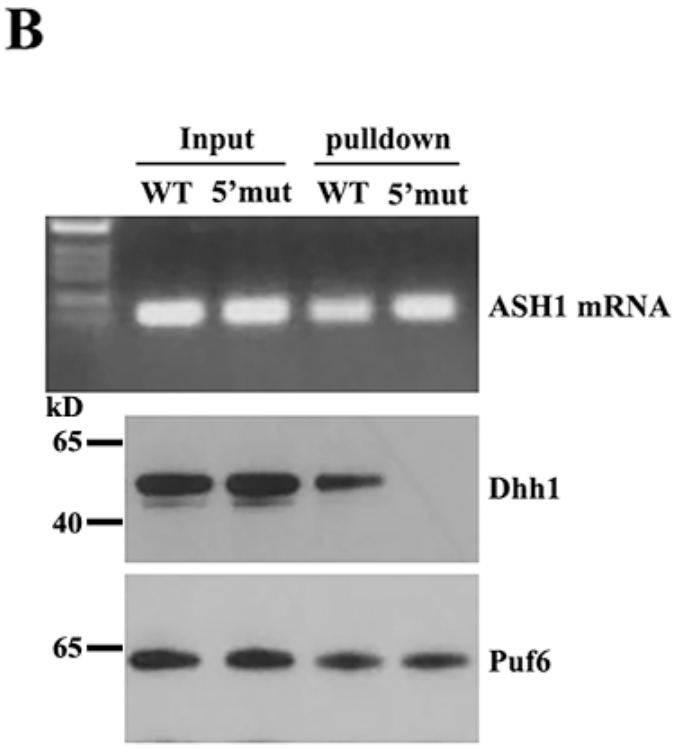

A
MW
Cell lysate
Uninduced
Purified
kD
80
MBP-MCP
50
B
Cell lysate
First purification
Purified (1)
Purified (2)
kD
80
Dhh1
50
40

Suppl Fig. S3

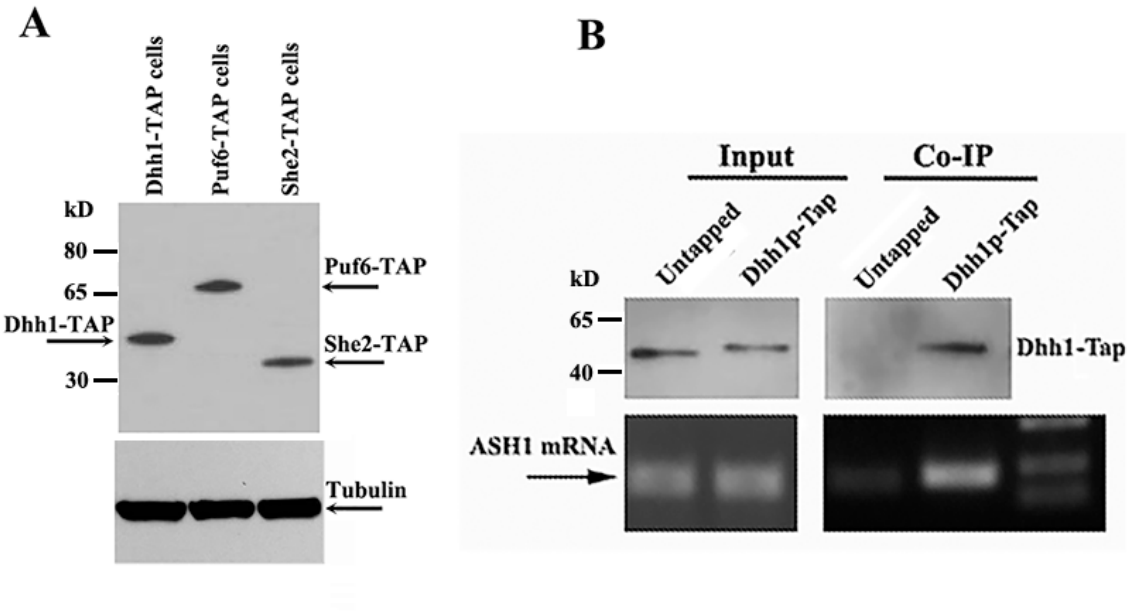

A
CHX - +
65
Dhh1
65
Puf6
40
GAPDH
B
Relative levels of Dhh1 and Puf6
1.2
1
0.8
0.6
0.4
0.2
0
CHX(-)
CHX(+)
Dhh1
Puf6
C
Relative levels of Dhh1 and Puf6 associated with ASH1 mRNA
1.2
1
0.8
0.6
0.4
0.2
0
**
**
CHX(-)
CHX(+)
Dhh1
Puf6

Suppl Fig. S4

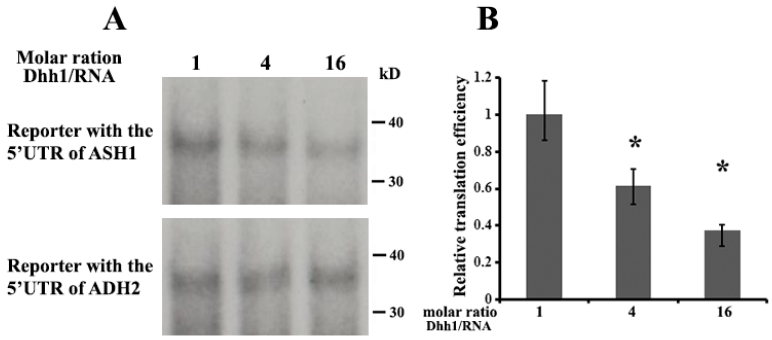

A
Input of WT
Input of CHX treated
Precip. of WT
Precip. of CHX treated
Puf6 -TAP
65
DNA ladder
MID2 mRNA
SRL1 mRNA
Dhh1
65


B
Input
pulldown
WT 5'mut WT 5'mut
ASH1 mRNA
kD
65
40
Dhh1
65
Puf6

Suppl. Fig.5


A
Dhh1-TAP cells
Puf6-TAP cells
She2-TAP cells
kD
80
65
30
Dhh1-TAP
Puf6-TAP
She2-TAP
Tubulin
B
Input
Co-IP
Untapped
Dhh1p-Tap
Untapped
Dhh1p-Tap
kD
65
40
Dhh1-Tap
ASH1 mRNA

Suppl. Fig. 6


A
Molar ration Dhh1/RNA
1
4
16
kD
Reporter with the 5'UTR of ASH1
40
30
Reporter with the 5'UTR of ADH2
40
30
B
Relative translation efficiency
1.2
1
0.8
0.6
0.4
0.2
0
*
*
molar ratio Dhh1/RNA
1
4
16