

SUPPLEMENTAL FIGURES

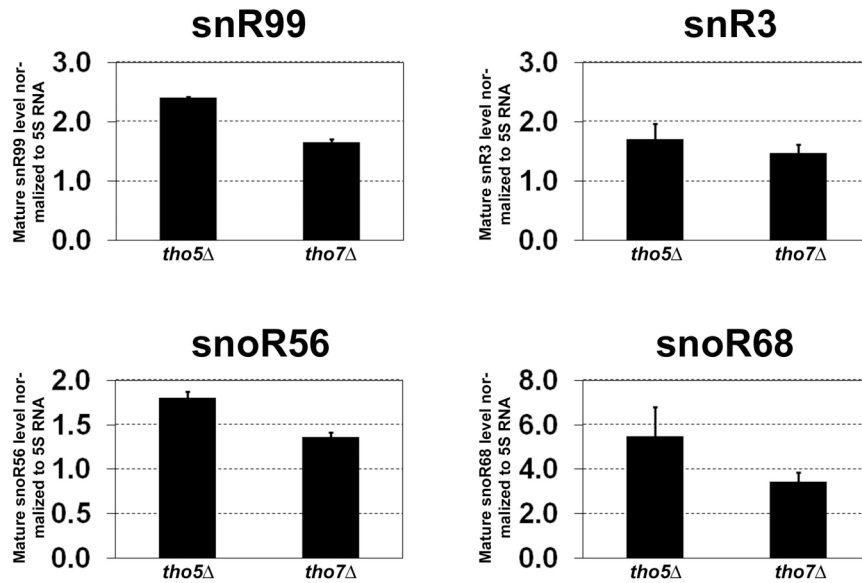


Figure S1. Increased levels of mature snoRNAs in *tho5Δ* and *tho7Δ* strains. Quantification of Northern blot data for the indicated snoRNAs. Values were normalized to the 5S rRNA and are relative to wild-type cells. The data and error bars represent the average and standard deviation from at least three independent experiments.

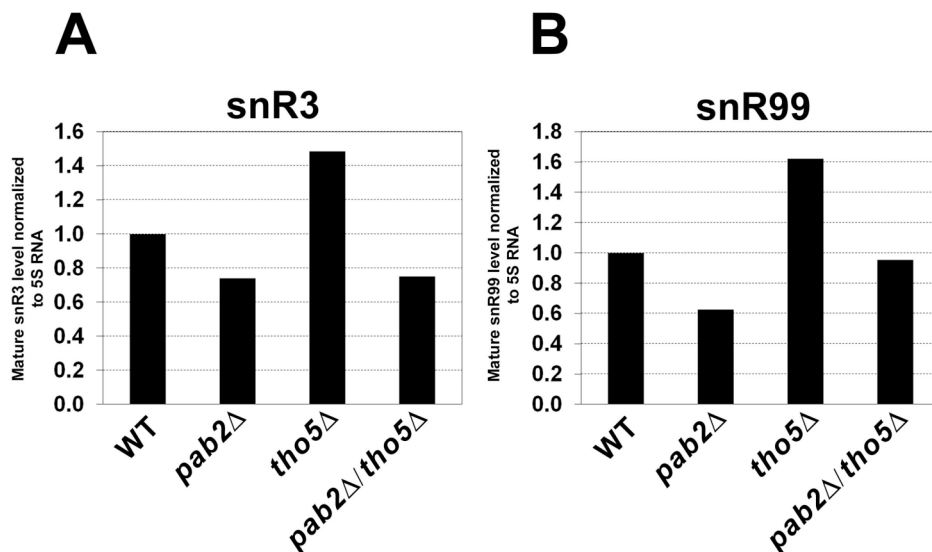
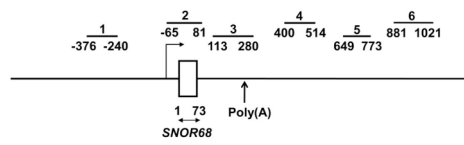
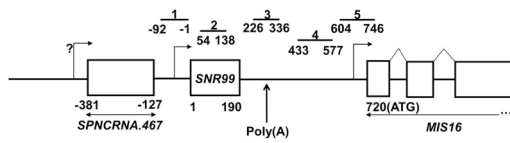
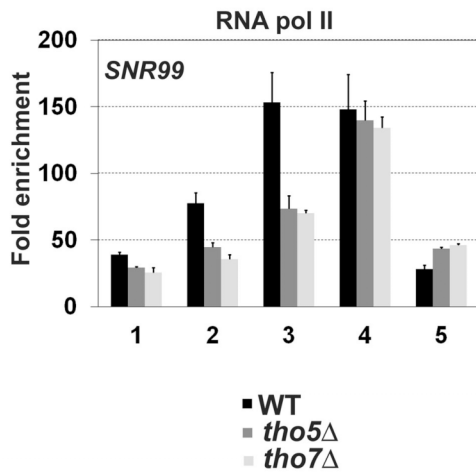


Figure S2. Pab2-dependent maturation is required for the accumulation of mature snoRNA in a THO mutant. Quantification of Northern blot data for snR3 (A) and snR99 (B). Values were normalized to the 5S rRNA and are relative to wild-type cells. The data were calculated based on the Northern blots shown in Fig. 1C-1D.



A



B

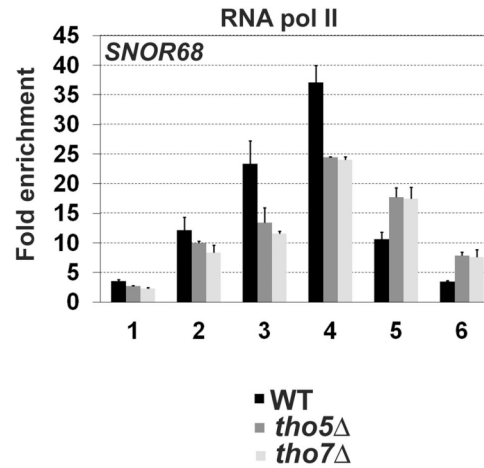


Figure S3. The accumulation of snoRNAs in THO deletion strains is not associated with increased transcription. ChIP assays were performed using an RNA Pol II-specific antibody at *SNR99* (A) and *SNOR68* (B) genes using primers shown above, as described in Fig. 2A. The data are presented as the fold enrichment compared to a nontranscribed intergenic region. Data and error bars represent the average and standard deviation from three biological replicates.

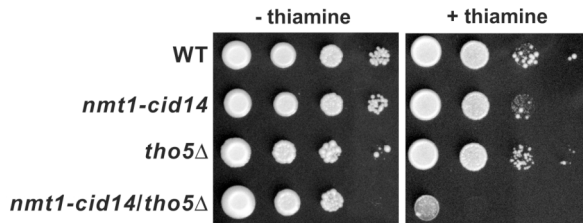


Figure S4. Genetic interaction between *tho5* and *cid14*. 10-fold serial dilutions of WT, *nmt1-cid14*, *tho5*Δ, and *nmt1-cid14/tho5*Δ strains were cultured in the presence or in the absence of thiamine.

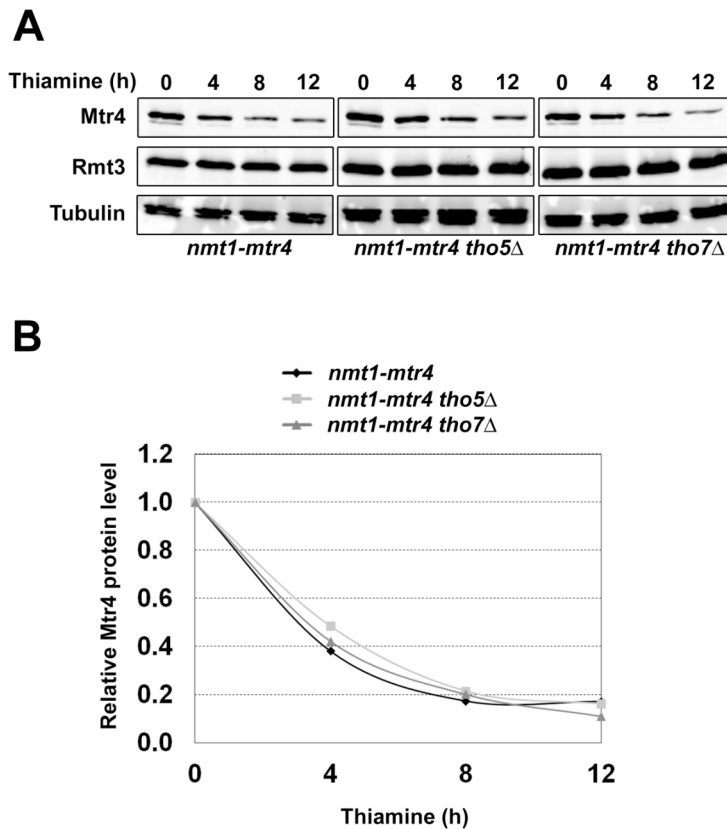


Figure S5. Analysis of thiamine-dependent Mtr4 depletion in wild-type and THO-depleted strains. (A) Wild-type, *tho5*Δ, and *tho7*Δ cells that express Mtr4 under the control of the thiamine-sensitive *nmt1* promoter were grown to early log-phase, and shifted to medium containing thiamine for 0-12 h. Total cell extracts were prepared and analyzed by immunoblotting using antibodies specific to Mtr4 (upper panel) and control proteins: Rmt3 (middle panel) and tubulin (lower panel). (B) Quantification of Mtr4 depletion kinetics were determined by calculating Mtr4/Rmt3 ratios for each time point and expressed relative to the initial zero time point (t=0h). Similar results were obtained using data normalized to tubulin (not shown).

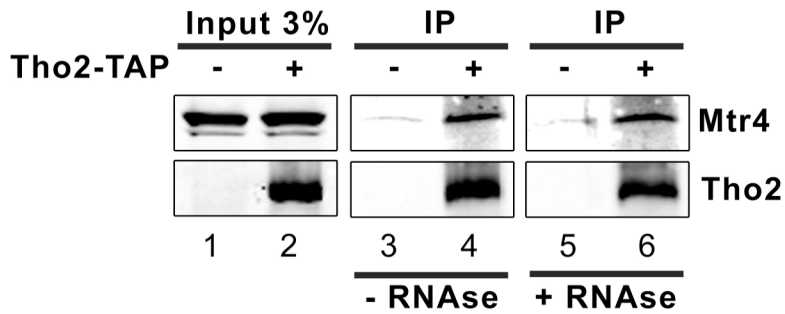


Figure S6. The association between Tho2 and Mtr4 is not sensitive to RNase. Western blot analysis of total cell extract (lanes 1-2) and IgG-sepharose purifications (lanes 3-6) prepared from cells that expressed a TAP-tagged version of Tho2 (lanes 2, 4, and 6) and untagged control cells (lanes 1, 3, and 5). Following IgG purification, the samples were subsequently divided and treated (lanes 5-6) or not treated (lanes 3-4) with RNase A and T1 for 10 min at 25°C. Tho2-TAP and Mtr4 were detected by probing the blot with Mtr4 (*top*) and TAP (*bottom*) antibodies.

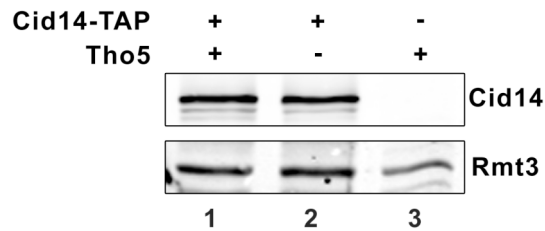


Figure S7. The absence of Tho5 does not affect Cid14 expression. Western blot analysis of total extracts prepared from wild-type (lane 1) and *tho5Δ* cells (lane 2) that express a TAP-tagged version of Cid14. A total extract prepared from wild-type cells that do not express Cid14-TAP (lane 3) were used to control the specific detection of Cid14-TAP. An antibody specific to the Rmt3 arginine methyltransferase (*bottom*) was used as a loading control.

SUPPLEMENTARY TABLE

Table S1. Yeast strains used in this study

Strain	Genotype	Reference
FBY106	<i>h+ ade6-M216 leu1-32 ura4-D18 his3-D1</i>	(20)
FBY176	<i>h+ leu1- ura4- his2- dis3-54</i>	(20)
FBY245	<i>h- ade6-M210 leu1-32 ura4-D18 his3-D1 cid14::URA4</i>	(20)
FBY542	<i>h+ ade6-M21? leu1-32 ura4-D18 his3-D1 pab2::KanMX6</i>	This study
FBY250	<i>h- ade6-M216 leu1-32 ura4-D18 his3-D1 cid14::URA4 pab2::KanMX6</i>	(20)
FBY543	<i>h- ade6-M21? leu1-32 ura4-D18 his3-D1 tho5::URA4</i>	This study
FBY544	<i>h+ ade6-M21? leu1-32 ura4-D18 his3-D1 tho5::URA4 pab2::KanMX6</i>	This study
FBY564	<i>h+ ade6-M216 leu1-32 ura4-D18 his3-D1 tex1::URA4</i>	This study
FBY611	<i>h+ ade6-M216 leu1-32 ura4-D18 his3-D1 tho7::URA4</i>	This study
FBY621	<i>h+ ade6-M216 leu1-32 ura4-D18 his3-D1 air1::KanMX6</i>	This study
FBY648	<i>h+ ade6-M216 leu1-32 ura4-D18 his3-D1 air1::KanMX6 pab2::his3</i>	This study
FBY644	<i>h- ade6-M210 leu1-32 ura4-D18 his3-D1 Tho2-TAP::KanMX6</i>	This study
FBY645	<i>h- ade6-M210 leu1-32 ura4-D18 his3-D1 Tho5-TAP::KanMX6</i>	This study
FBY737	<i>h? ade6-M21? leu1-32 ura4-D18 his3-D1 Cid14-TAP::KanMX6</i>	This study
FBY739	<i>h? ade6-M21? leu1-32 ura4-D18 his3-D1 Cid14-TAP::KanMX6 tho5::URA4</i>	This study
FBY963	<i>h? ade6-M21? leu1-32 ura4-D18 his3-D1 KanMX6::p81nmt1-mtr4</i>	(35)
FBY964	<i>h? ade6-M21? leu1-32 ura4-D18 his3-D1 p81nmt1::kanMX6-mtr4 pab2::ura4</i>	(35)
FBY984	<i>h+ ade6-M216 leu1-32 ura4-D18 his3-D1 pFB366::ade6</i>	This study
FBY987	<i>h- ade6-M210 leu1-32 ura4-D18 his3-D1 cid14::URA4 pFB366::ade6</i>	This study
FBY994	<i>h- ade6-M210 leu1-32 ura4-D18 his3-D1 cid14::URA4 pFB494::ade6</i>	This study
FBY1063	<i>h- ade6-M210 leu1-32 ura4-D18 his3-D1 cid14::URA4 pFB495::ade6</i>	This study
FBY1116	<i>h? ade6-M21? leu1-32 ura4-D18 his3-D1 Cid14-TAP::KanMX6 tho7::URA4</i>	This study
FBY1190	<i>h? ade6-M21? leu1-32 ura4-D18 his3-D1 KanMX6::p81nmt1-cid14 tho5::URA4</i>	This study
FBY1192	<i>h? ade6-M21? leu1-32 ura4-D18 his3-D1 KanMX6::p81nmt1-cid14</i>	This study
FBY1196	<i>h? ade6-M21? leu1-32 ura4-D18 his3-D1 KanMX6::p81nmt1-mtr4 tho5::URA4</i>	This study
FBY1197	<i>h? ade6-M21? leu1-32 ura4-D18 his3-D1 KanMX6::p81nmt1-mtr4 tho7::URA4</i>	This study