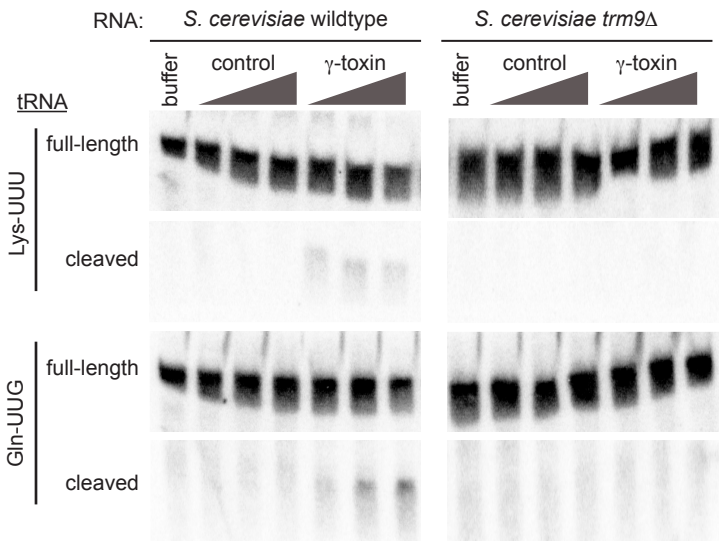
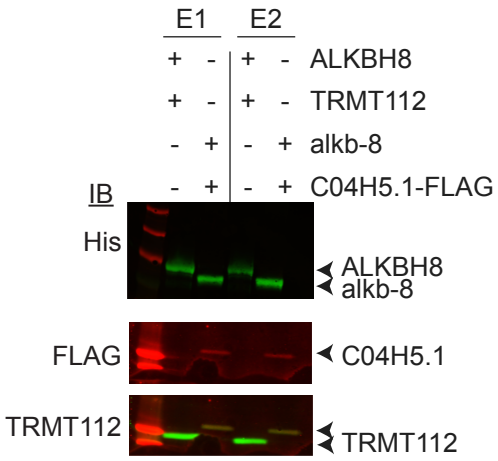
Supplemental Table 1. Oligonucleotides

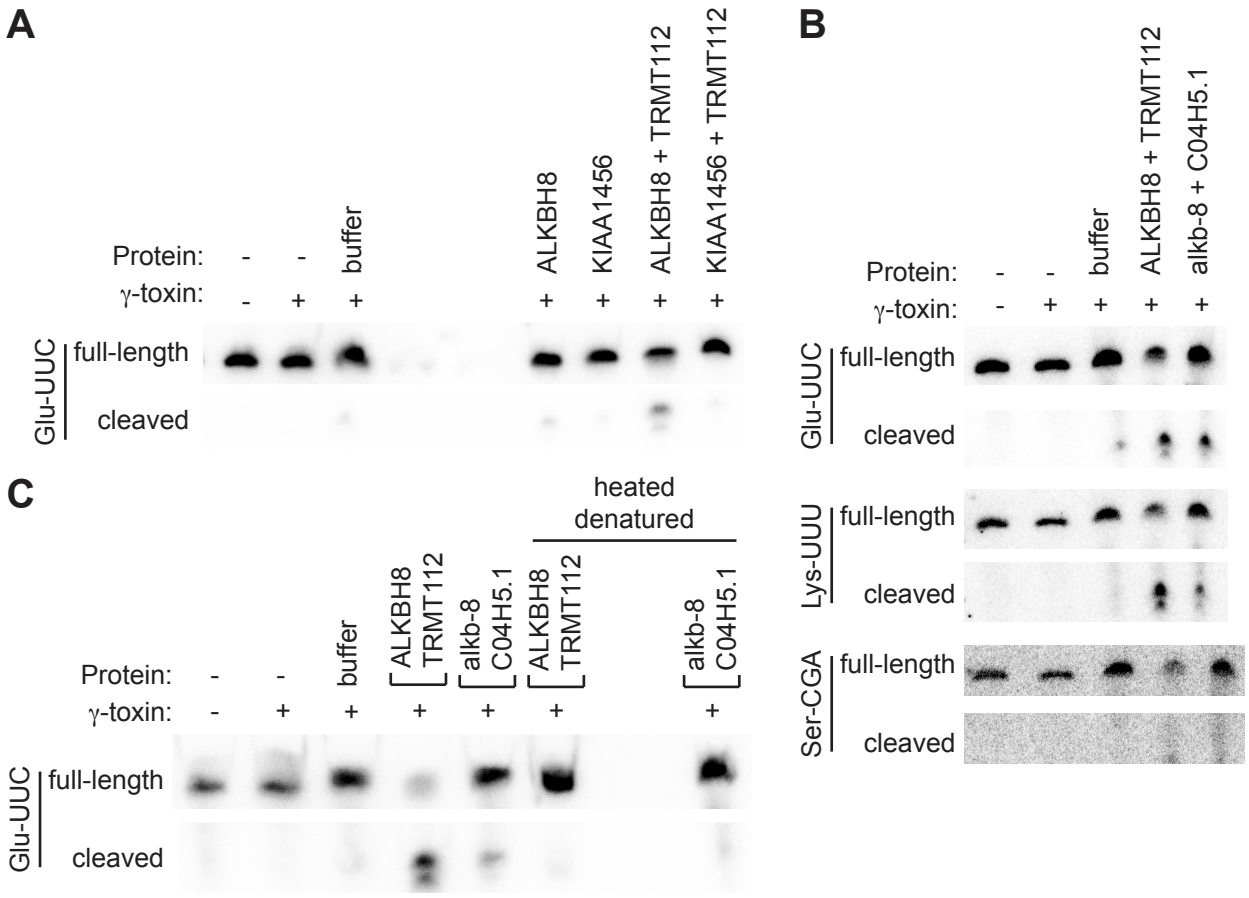
Northern blot probes	**Sequence (5′-3′)**
Yeast Glu TTC 5'	AGCCGTTACACTATATCGGA
Yeast Ser CGA 5'	CTCTCGCCTTAACCACTCGGCCATAGTGCC
Yeast Gln TTG 5'	GAAAGTGATAACCACTACACTATAGGACC
Yeast Lys TTT 3'	CTCCTCATAGGGGGCTC
Human Glu TTC 5'	CCAGGAATCCTAACCGCTAGACCATRTGGGA
Human Ser CGA 5'	GCCTTAACCACTCGGCCATCACAGC
Human Lys TTT 5'	TGATGCTCTACCRACTGAGCTATCCRGGC
Human Gln TTG 3'	AGGTCCCACCGAGATTTGAACTCG
Human Arg TCT	CCAATGCGCTATCCATTGCGCCACAGA
Human Arg TCG 3'	GGCAGGACTCGAACCTGCAaTCTTC
Human Gly TCC 5'	CAGCTATGCTAACCACTATACCACCAACGC
Human Sec TGA 5'	CTGCACCCCAGACCACTGAGGATCAT
Human U6 snRNA	CGTTCCAATTTTAGTATATGTGCTGCCGAAGCGA
E. coli Glu	GCGGTGTCCTGGGCCTCTAGACG
Tetrahymena Glu TTC 5'	CCTAACCTGCTAGACTATAGGGGA
Tetrahymena Ser CGA 5'	AACCTCCTTAACCACTCGGACAAACTGAC
C. elegans Glu TTC 5'	GAATCCTAACCACTAGACCACATGGGA
C. elegans Ser CGA 5'	ACTCCTTAACCACTCGGACATGACTGC
D. melanogaster Glu TTC 5'	CCAGATATCCTaGCCACTAGACCATATGGGA
D. melanogaster Ser AGA/CGA 5'	ACGCCTTAACCACTCGGCCACGACTGC
X. laevis Glu TTC 5'	GGAATCCTAACCGCTAGACCATAT
X. laevis Ser CGA *5'*	GCCTTAACCACTCGGCCATCACA
Mouse Glu TTC 5'	GGAATCCTAACCGCTAGACCATGTGGGA
Mouse Ser CGA 5'	GCCTTAACCACTCGGCCATCACAGC
QRT-PCR primers	
sc-tRNA-Glu-TTC-F1	TCCGATATAGTGTAACGGCtAT
sc-tRNA-Glu-TTC-R1	CTCCGATACGGGGAGTCG
Yeast 25s rRNA Forward	GAAATCTGGTACCTTCGGTG
Yeast 25s rRNA Reverse	GATTCTCACCCTCTATGACG
hs-tRNA-Glu-TTC-F1	TGGTCTAGYGGYtAGGATTC
hs-tRNA-Glu-TTC-R1	ACCGGGARTCGAACCCGGGC
Human 5.8s Forward	GGTGGATCACTCGGCTCGT
Human 5.8s Reverse	GCAAGTGCGTTCGAAGTGTC



Supplemental Figure 1. Gamma-toxin cleavage of *S. cerevisiae* tRNA-Gln-UUG and Lys-UUU. Northern blots of *S. cerevisiae* Wild-type and trm9delta RNA incubated with buffer alone or increasing amounts of control purification or purified gamma-toxin.
The blot was hybridized with probes against tRNA-Lys-UUU and tRNA-Gln-UUG.



Supplemental Figure 2. Immunoblot verification of Trm9-Trm112 copurification. Elutions 1 and 2 of purified protein expressed in *E. coli* bacterial cells were visualized by western blotting and antibodies against hexa-histidine tag, 3X-FLAG tag, and TRMT112 were used.



Supplemental Figure 3. Gamma-toxin cleavage of *Trm9*Δ RNA requires active Trm9-Trm112 enzyme complexes and is specific for mcm5s2U-containing tRNAs. Northern blots of *S. cerevisiae Trm9*Δ RNA pre-incubated with the indicated buffers or proteins followed by treatment with purified gamma-toxin. (A) Purified eukaryotic Trm9 proteins with or without Trm112 subunit were pre-incubated with *Trm9*Δ RNA followed by the gamma-toxin assay. KIAA1456 represents another human protein with homology to yeast Trm9 but displays no methyltransferase activity. (B) Purified *H. sapiens* or *C. elegans* Trm9-Trm112 complexes were pre-incubated with *Trm9*Δ RNA followed by treatment with purified gamma-toxin. (C) *Trm9*Δ RNA was pre-incubated with *H. sapiens* or *C. elegans* Trm9-Trm112 complexes that were either mock-treated or heat denatured followed by the gamma-toxin assay.