

Figure S1. Predicted TtnuCD32 secondary structure: The secondary structure shown was predicted and the dG value of -22.2 calculated using the mFold web server (http://unafold.rna.albany.edu/?q=mfold/RNA-Fold-ing-Form) [41].



Figure S2. Analysis of putative TtnuCD32 methylation targets: (a) The SnoScan algorithm was applied to predict targets of TtnuCD32 box D and D' associated guide sequences. The SnoScan output from the highest scoring base-pairing interactions with rRNA are given. The D and D' boxes are framed, and predicted target nucleotides are indicated with asterisks above the base-paired nucleotides; (b) Experimental test of predicted nucleotide methylations shown in (a). Primer extension reactions with limiting concentrations of dNTP (1.0, 0.04 and 0.004 mM) were separated next to sequencing reactions of the target RNA. Predicted target of TtnuCD32 is indicated with a black arrow and the nucleotides position in SSU is noted. An experimentally detected methylation unrelated to TtnuCD32 is indicated by a grey arrow.





Figure S3 Analysis of large RNA species in TtnuCD32 wild type and KD cells: (a) Whole cell RNA was isolated from wild type (wt), paromomycin (Paro) resistant cells (resistance cassette inserted at a loci unrelated to rRNA biogenesis) grown in paromomycin (wt+), TtnuCD32 knock-down (KD) grown in Paro, and KD cells allowed to revert towards wt by removing Paro selection pressure for knock-out chromosomes (Rev.). The RNA was seperated on a denaturing formaldehyde agarose gel and stained with ethidium bromide (left panel) and analyzed by northern blotting against ITS-1 and ITS-2 (right two panels). Detected RNA species are noted on the sides and quantifications of each detected band relative to the same signal in wt cells are given below the images. Values of note are highlighted in red; (b) Isolated whole cell RNA from wt and two KD cell lines in exponential growth and under starvation conditions was seperated for an extended time (lane 1-6) and lanes being seperated for standard time (lane 7-12). The gel was stained with SYBR Gold for imaging and known RNA species are noted on the sides.



Figure S4. 5.8S rRNA maturation in TtnuCD32 KD and rescued cells: Small rRNA processing products in wild type cells (wt) compared to paromomycin resistant cells with a paromomycin (Paro) resistance cassette inserted at a loci not important for cell fitness or ribosome biogenesis grown with Paro (wt+), TtnuCD32 knock-down cell line grown with Paro (KD), and TtnuCD32 reverted /'rescue' cells (Rev.). Rescue cells are derived from KD cells but has been propagated without selection for numerous generations allowing for natural selection to reduce the number of genetic copies of the Paro cassette and replace them with wt TtnuCD32 containing copies. Left panel: Image of ethidium bromide (EtBr) stained gel. Molecular sizes derived from known RNA species visible by EtBr staining and the corresponding RNAs are noted on the sides. Right three panels: Northern blot analysis using oligo probes against 5.8S, ITS-1, or ITS-2 as noted above the panels. Signals from the EtBr gel image and the northern blot analysis were quantified and ratios KD/wt for detected rRNA species are noted below the images. Values found to differ between wt and KD cell lines are highlighted in red.

SnoRNA U14 multiple sequence alignment (TCoffee)

Scerevisiae_U14	UC-A-CG-GUGAUG-AAAGACUGGUUC-C-U
Xlaveis U14-1	UC-A-CAGUGAUG-ACUGGUUUC-CA-
Xlaveis_U14-2	UC-A-CAGUGAUG-ACUGGUUUC-CA-
Hsapiens U14A	UC-A-CGUGAUG-AUGGUUUUC-CA-
Hsapiens_U14B	UC-A-CUAUGAUG-AUUGGUUGC-CA-
Xlaveis U14-3	UC-G-CUGUGAUG-AACU-UGUUC-CA-
Xlaveis_U14-4	UC-G-CUGUGAUG-AAUG-UGAUUC-CA-
Xlaveis U14-5	UU-G-CUAAGAUG-AAUA-CGAUUC-CA-
Zmays U14-1	UA-UGG-C-AAUGAUG-AUGAAAGAU-AA-GGCUUGUUUCUCA-
Zmays U14-2	A-UGG-C-AAUGAUG-UUGAA-GUU-AAAGGCUUGUUUCUCA-
Osativa_U14-1	C-AAUGAUG-AUAAAUU-UAAGGCUUGUUUCUCA-
Zmays U14-3	UGCA-UUG-C-AA-GUGAUG-ACAAAAU-CAAGGCUUGUUUCUCG-
Zmays_U14-4	UGCA-UUG-C-AA-GUGAUG-AUGAAGU-CAAGGCUUGUUUCUCU-
Osativa U14-2	UGCA-UGG-C-AA-AUGAUG-CUAAAAG-CAAGGCUUGUUUCUCA-
Osativa_U14-4	UAAUGAUG-CUAAAAUUUCUCA-
Zmays U14-5	CG-GUG-C-CCUAUGAUG-ACAAAAU-CAAGGCUUGUUUCUCU-
Stuberosum_U14	C-UCUGAUG-AUACAAUUGAAGGCUUGUUUCUCU-
Spompe U14	UC-A-AC-AG-GUGAUG-AAAUUUC-CAU
TtnuCD25	A-AU-UC-AA-CUGAUG-AUUAAUUAGUUUCUCU-
TtnuCD32	A-ACAUGAUGUAUAAAACGCAUGGAAA-
TpnuCD32	A-ACAUGAUGUAUAAAACGUAUGGAUA-GA-
Dmelanog. U14	CUUCAAUG-AUUAACUUCAAC
Dyakuba_U14	CUUUAACCUUUAACCUUUAAC
Athaliana_U14A	GGUU-AAUGAUG-AUAA-AU-CCAAAGGCUUGUUUUUCA-
Athaliana_U14B	UC-GAUGAGG-AUAAGAU-GAAGGCUUGUUUCUCA-
Athaliana_U14C	UGAUGAGG-AUUAUAU-UAAGGCUUGUUUCUCA-
Athaliana_U14D	AAAGAUCA-AUGAUG-AUAAACU-UAAGGCUUGUUUCUCA-
Mmusculus_U14i5	UC-G-CUGUGAUG-AUGAUUC-CA-
Mmusculus_U14i6	UC-G-CUGUGAUG-AUGGAUUC-CA-
Mmusculus_U14i8	UC-A-CAA <u>UGAUG-A</u> UGAAUGGUC-CA-
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Box C

Scerevisiae_U14	U-AACAUUCGCAGUUUCCACGGU	-AG-	GAGUACG-CUUACGAA
Xlaveis_U14-1	AUCAUUCGCAGUUUCUACC	-AG-	AAAG-CUU
Xlaveis_U14-2	AUCAUUCGCAGUUUCUACC	-AG-	AAAG-CUU
Hsapiens U14A	ACAUUCGCAGUUUCCACC	-AG-	AAAGG-UUU
Hsapiens U14B	GACAUUCGCAGUUUCCACC	-AG-	AAAUG-UUU-U
Xlaveis U14-3	AAGCCAUUCGUAGUUUCCACC	-AG-	AUGC-CG-AAA
Xlaveis U14-4	AAGCCAUUCGUAGUUUCCACC	-AG-	AUGU-CU-CAC
Xlaveis U14-5	AAGCCAUUCGUAGUUUCCACC	-AG-	AUGU-CG-UAA
Zmays U14-1	ACAUUCGCAGUAGCCGCC-U	A	AGAG-CUUUCGC-
Zmays U14-2	ACAUUCGCAGUAGCCGCC-U	A	AGAG-CUUUCGC-
Osativa U14-2	U-AACAUUCGCAGUUGCCGCC-U	A	AGAG-CUUUCGC-
Osativa U14-4	ACAUUCGCAGUUGCCGCC-U	A	AGAG-CUUUCGC-
Zmays U14-5	ACAUUCGCAGUUGCCGCC-U	A	AGAG-CUUUCGC-
Osativa U14-2	U-AACAUUCGCAGUUGCCGCC-U	A	AGAG-CUUUCGC-
Osativa U14-3	U-AGCAUCUGCAGUUACUGCC-U	A	AUAG-CUUUCGC-
Zmays U14-5	ACAUUCGCAGUUGCCGCC-U	A	AGAG-CUUUCGC-
Stuberosum U14	A-AACAUUCGCAGUGGCCGCC-U	A	AGAU-CUUUCGC-
Spompe U14	UGAACAUUCGCAGUUUCCCC	G	GAGC-GG-CAUACGAA
TtnuCD25	AAA-UUCGCAGUGACCCCCCUAC	UAGU	CCGAAAGGC
TtnuCD32	GAGGUUUGUAGUUAAC-CC-U	A	CUGAA
TpnuCD32	GAGAUGUAGUUAUG-UC-U	A	CUGAA
Dmelanog. U14	ACCUUUUGCGGUUUCCACC	-AG-	AAAG-CUU-CG
Dyakuba U14	ACCUUUUGCGGUUUCCACC	-AG-	AAAG-CUU-CG
Athaliana U14A	AACAUUCGCAGUGGCCGCC-U	A	AGAG-CUUUCGC-
Athaliana U14B	A-AACAUUCGCAGUGGCCGCC-U	A	A-GG-CUUUCGC-
Athaliana U14C	A-AACAUUCGCAGUGGCCGCC-U	A	A-AG-CUUUCGC-
Athaliana U14D	A-AACAUUCGCAGUGGCCGCC-U	A	A-AG-CUUUCGC-
Mmusculus U14i5	AAACCAUUCGUAGUUUCCACC	-AG-	AAGU
Mmusculus U14i6	AAACCAUUCGUAGUUUCCACC	-AG-	AAAU
Mmusculus U14i8	AACAUUCGCGGUUUCCACC	-AG-	AACG-CAA
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Domain A

Scerevisiae_U14	CCCAU-CGUUAGU-ACUCUCGGUGACCGCUCUU-CUU-UAGAGACC
Xlaveis_U14-1	UGCCUGG-UGUUGGCUAGUAACC
Xlaveis_U14-2	UGCUGG-UGUUGGCCUGUAACC
Hsapiens U14A	UCCUUAGUGUUGGGUAAACC
Hsapiens_U14B	UCCUUA-UGUUGGCCAGUUC
Xlaveis U14-3	GGCUGA-UGAUGGCCUAGC-ACC
Xlaveis_U14-4	GACUUA-UGAUGGUUU-AUU-ACC
Xlaveis U14-5	GACUCA-UGAUGGCUCU-ACC
Zmays_U14-1	-CACGCCAG-G-CUCGAGAGCUUGUGCUGUUGA-AUCC
Zmays U14-2	-CCUGCCAG-G-CUUGAGAGCUUGUGCUGUUUA-AUCC
Osativa_U14-1	-CCUGCCAG-G-CUUGAGAGCUAAUGCUGUUAA-UUCC

Zmays U14-3	-CCUGCCAG-G-CUUGAGAGGUAGUGCUGCCAA-AUCC
Zmays U14-4	-CCUGCCAG-G-CUUGAGAGCUAGUGCUGCCAA-AUCC
Osativa U14-2	-CUUGCCAG-G-UUUGAGAGCUAAUGCUGCUAA-UUCC
Osativa U14-4	-CCUGCCAG-G-CUUGAGAGCUAGUGCUGUUAA-AUCC
Zmays U14-5	-CCUGCCAG-G-CUUGAGAGCUAAUGCUGCAGA-AUCC
Stuberosum U14	-CUU-CGCCAG-G-CUUGAGAGAUAAUGCUGCUUU-AUCC
Spompe U14	CCCAAUUUUGCCGCAGA-UGUCG-UGCAUAGUCC
TtnuCD25	G-AGCCUGUAG-GUUAG-AGCC
TtnuCD32	C-CCAGGGUUAAGCCAUCCCUCG-AUGA
TpnuCD32	C-CCAGAUGUAAGCCAUUUCUCC-UUGU
Dmelanog. U14	G-CUUAAU-GAUGGU-CUA-AGGC
Dyakuba U14	G-CUUAAU-GAUGGU-CUA-AGGC
Athaliana U14A	-CUUUCGCCAG-G-CUUGAGAGUUAAUGCUGUUUU-AUCC
Athaliana U14B	-CUUUCGCCGG-G-CUUGAGAGCUUAUGAUGUUUUUAUCC
Athaliana U14C	-CUUUCGCCAG-G-CUUGAGAGCUAAUGCAGCUUU-AUCC
Athaliana U14D	-CUUUCGCCGG-G-CUUGAGAGUUAAUGCAGCUUUUAUCC
Mmusculus U14i5	GCUGUGGCUAGU-UCC
Mmusculus U14i6	GCUGUGGCUAGU-UCC
Mmusculus U14i8	GGCAGUGUUGGCAGUUACC
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Scerevisiae_U14	UUCCUA-GGAU-GUCUGAGUGA
Xlaveis_U14-1	UUCCUU-GGAU-GUCUGAGUGA
Xlaveis_U14-2	UUCCUU-GGAU-GUCUGAGUGA
Hsapiens_U14A	UUCCUU-GGAU-GUCUGAGUGA
Hsapiens_U14B	UUCCUU-GGAU-GUCUGAGUGA
Xlaveis_U14-3	UUCCUU-GGAU-GUCUGAGCGA
Xlaveis_U14-4	UUCCUU-GGAU-GUCUGAGCGA
Xlaveis_U14-5	UUCCUU-GGAU-GUCUGAGCGA
Zmays_U14-1	UUCCUU-GGAU-GUCUGAGCCAUA
Zmays_U14-2	UUCCUU-GGAU-GUCUGAGCCAUA
Osativa_U14-1	UUCCUU-GGAU-GUCUGAG-C
Zmays_U14-3	UUCCUU-GGAU-GUCUGACGCA-AUG-CA
Zmays_U14-4	UUCCUU-GGAU-GUCUGAUGCA-AUG-CA
Osativa_U14-2	UUCCUU-GGAU-GUCUGAUGCA-AU-GCA
Osativa_Ul4-4	UUCGUU-GAAU-GCAUA
Zmays_U14-5	UUCCUU-GGAU-GUCUGAGGGCCGCCG
Stuberosum_U14	UUCCUU-GGAU-GUCUGAGAUC
Spompe_U14	UUCCUUUGGAU-GUCUGA-UGU-UG
TtnuCD25	GUGUCUGA-U
TtnuCD32	AUCCAU-ACCAAUCGUCUGAUU
TpnuCD32	
Dmelanog014	GUCUGACU
Dyakuba_014	GUCUGACU
Athaliana_U14A	UUCCUU-GGAU-GUCUGAAA-CU
Atnaliana_Ul4B	UUCCUU-GGAU-GUCUGA
Atnaliana_U14C	
Athaliana_UI4D	
Mmusculus_UI415	UUCCUU-GGAU-GUCUGAGCGA-A
Mmusculus_UI416	
rmmusculus_01418	*

Domain B Box D

Figure S5. Multiple alignment of U14 snoRNAs and Tetrahymena U14 candidates: Full multiple alignment of the three *Tetrahymena* U14 candidates TtnuCD25, TtnuCD32, and TpnuCD32 with twenty seven U14 snoRNAs from various species as noted for each line. The alignment was created using TCoffee [37] at the phylogeny.fr web server [36]. Box C and D as well as the domain A are underlined in the bottom line and noted under the alignment in red. Alignment of Domain B is highlighted in pink in all sequences. The cluster analysis for the final tree in Figure 5B was based on this alignment including a subsequent curation by Gblocks.



Figure S6. PP7-tagged TtnuCD32 is endogenously trimmed to wild type lenght: (a) Schematic illustration of the targeted replacement of the TtnuCD32 loci with a neo2 and tagged TtnuCD32 containing construct. The position of the PP7 hairpin tag in the 3'end of the mature TtnuCD32 RNA is highlighted (red); (b) Northern blot analysis of TtnuCD32 from wild type (wt) and three independent genetically modified strains having the wt loci fully replaced by the tagged TtnuCD32 construct in the genome (t1-t3) (upper panel). SnRNA U2 stained with SYBR Gold was used as loading control (lower panel).