Table S3. Yeast strains.

Strain ID	Background	Genotype	Source/Reference
YAM1	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf
YAM6	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xrn1Δ::kanMX4*	Euroscarf
YAM202	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 upf1Δ::kanMX4*	Euroscarf
YAM2831	YAM1	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 adh2Δ::URA3*	This work
YAM2852	YAM2831	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-a*	This work
YAM2853	YAM2831	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-b*	This work
YAM2854	YAM2831	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-d*	This work
YAM2855	YAM2831	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-f*	This work
YAM2862	YAM2852	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-a upf1Δ::kanMX4*	This work
YAM2863	YAM2853	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-b upf1Δ::kanMX4*	This work
YAM2864	YAM2854	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-d upf1Δ::kanMX4*	This work
YAM2865	YAM2855	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-f upf1Δ::kanMX4*	This work
YAM2893	YAM2831	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-e*	This work
YAM2896	YAM2893	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-e upf1Δ::kanMX4*	This work
YAM2898	YAM2831	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-c*	This work
YAM2901	YAM2898	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-c upf1Δ::kanMX4*	This work
YAM2903	YAM2831	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-chimera*	This work
YAM2904	YAM2903	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-chimera upf1Δ::kanMX4*	This work
YAM2908	YAM2831	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-b-3FLAG*	This work
YAM2911	YAM2908	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-b-3FLAG upf1Δ::kanMX4*	This work
YAM2934	YAM2831	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SL-xut0741-b-3FLAG*	This work
YAM2937	YAM2934	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SL-xut0741-b-3FLAG upf1Δ::kanMX4*	This work
YAM2951	YAM2908	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xut0741-b-3FLAG xrn1Δ::kanMX4*	This work
YAM2952	YAM2934	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SL-xut0741-b-3FLAG xrn1Δ::kanMX4*	This work

Table S4. **Oligonucleotides.**

ID	Sequence 5’-3’	Target	Use
AMO34	CTTCTTCCGTACTTATAATCGGG	*XRN1*	PCR
AMO35	GGTGTCCACAGATCAAATGGC	*XRN1*	PCR
AMO190	GGCGAACTCCGTAATTCGCC	*UPF1*	PCR
AMO193	GGCTGTAATGGCTTTCTGG	*scR1*	qPCR
AMO415	GTGCGGAATAGAGAACTATCC	*scR1*	RT + qPCR
AMO496	TCTTGCCCAGTAAAAGCTCTCATG	ITS1 & 20S pre-rRNA	Northern blot
AMO1482	ATCCCGGCCGCCTCCATCAC	*scR1*	Northern blot
AMO1595	GGGAAAAGTTTGTTTGGCTTATTCTGGTGGTTTAG	*XUT1678/SUT768*	Northern blot
AMO1669	GGAAATCAGGGCTCAAGAGCC	*XRN1*	PCR
AMO1762	GACAGTGTTCGAAGTTTCACGA	*XUT0741*	RT & Northern blot
AMO2710	TGGGAGGGACACCTTTATACGC	*UPF1*	PCR
AMO2711	CTAGGATATCAAGTCCATGCCC	*UPF1*	PCR
AMO2712	CTTTATTACGCATTGCAGTGCG	*UPF1*	PCR
AMO3350	GAAGTCGTTCGTACTAGCAACATGG	*XUT0741/ADH2*	PCR
AMO3247	CGTCAAACTAAGTACTTACGACTTCC	*XRN1*	PCR
AMO3351	CAGGCGGGAAACCATCCACTTCAC	*XUT0741/ADH2*	PCR
AMO3354	GGCTGGAAGATCGGTGACTA	*XUT0741*	RT
AMO3359	CAACTTGAGAGCAGGCCACT	*XUT0741*	Cloning
AMO3379	GGGGTACCGCCGGCCCGCTATATTTTGG	*XUT0741*	Cloning
AMO3382	GCTCTAGAGCCGGCATCTCCAACTTATAAGTTGG	*XUT0741*	Cloning
AMO3471	GGGGTACCAGATCTGAATTCAGGAATGGGTACAACTCACAGG	*XUT0741*	Cloning
AMO3497	TACCCATTCCTGAATTCTTAGTTGGTGGTCACGAAGGTGCCGGT	*XUT0741*	Cloning
AMO3530	CCTTGTAATCGATGTCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTCGGTTCTTTGGCTGTTCAATATG	*XUT0741 + 3FLAG*	Cloning
AMO3531	TATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGCTAGGATCCTAGACCACCAGCAGCACCAGAAA	*XUT0741 + 3FLAG*	Cloning
AMO3549	ACTGATCCCGCGGTTCGCCGCGGTTTGTTGGAGGATGCCGTA	*XUT0741*	Cloning
AMO3550	AAACCGCGGCGAACCGCGGGATCAGTCGGTTATAGTTTGTCC	*XUT0741*	Cloning
AMO3558	CGAACACTGCTGAAGCTACC	*XUT0741*	qPCR
AMO3559	CTACTTTTTGCTCCCACCGC	*XUT0741*	qPCR
AMO3581	TAAGGATATCCCAGTTCCAAAGCC	*XUT0741*	Northern blot
5S_44	/5BiotinTEG/ACT+ACTCGGTCAGGCTCT+TACCAGCTTAACT+ACAGTT	5S rRNA	Ribo-Seq (ribo-depletion)
5.8S_125	/5BiotinTEG/AA+ATGACGCTCA+AACAGGCATGCCCCCTGGA+AT	5.8S rRNA	Ribo-Seq (ribo-depletion)
18S_1712	/5BiotinTEG/AA+ATGACCAAGT+TTGTCCAA+ATTCTCCGCTC	18S rRNA	Ribo-Seq (ribo-depletion)

18S_rDNA1	/5BiotinTEG/TGATGCCCCCGACCGTCCCTAT+TAATCATTACGACCA+AGTTTGTCCAA+ATTCTCCGCTCTGAGA	18S rRNA	Ribo-Seq (ribo-depletion)
25S_557	/5BiotinTEG/GACTT+ACGTCGCAGTCCTC+AGTCCCAGCTGGCAGT+ATTCCCACAGGCTA	25S rRNA	Ribo-Seq (ribo-depletion)
25S_698	/5BiotinTEG/CGAGGCCCCA+ACCTACGTTCACT+TTCATT+ACGCGT	25S rRNA	Ribo-Seq (ribo-depletion)
25S_rDNA2	/5BiotinTEG/GCTAGCCTGCT+ATGGTTCAGCGACGCCACAACTGATCA+AATGCCCTTCCCTTTCAACAA+TTTCACG	25S rRNA	Ribo-Seq (ribo-depletion)
25S_rDNA3	/5BiotinTEG/TTCCAGCTCCGCTTCATTGAATAAGTA+AAGAACTAT+TTTGCCGACTTCCCTTATCTACATT+ATTCTA	25S rRNA	Ribo-Seq (ribo-depletion)
25S_rDNA5	/5BiotinTEG/ATTCTATT+ATTCCATGCTAAT+ATATTCGAGCAAGCGGTTATCAGTACGACCTGGCATGAA+AAC	25S rRNA	Ribo-Seq (ribo-depletion)

Note: + corresponds to a Locked nucleic acid base.

Table S5. Mapping statistics.

Sample	Data	Figure	Read pairs in fastq	Read pairs mapped	Read pairs filtered
WT-1-DMSO	RNA-Seq	1	14 503 671	13 972 248	10 726 366
WT-2-DMSO	RNA-Seq	1	30 921 123	29 567 896	21 617 872
WT-1-CHX	RNA-Seq	1	15 161 829	14 660 499	12 496 933
WT-2-CHX	RNA-Seq	1	17 931 225	17 208 697	14 344 426
upf1-1-DMSO	RNA-Seq	1	30 166 640	29 037 586	22 634 261
upf1-2-DMSO	RNA-Seq	1	31 948 976	30 715 056	16 772 544
upf1-1-CHX	RNA-Seq	1	35 856 855	34 740 967	26 361 859
upf1-2-CHX	RNA-Seq	1	15 904 538	15 590 456	9 328 222
WT-1-DMSO	RNA-Seq	1 & S1	13 775 833	13 509 645	7 169 855
WT-2-DMSO	RNA-Seq	1 & S1	13 441 382	13 140 538	9 480 390
WT-1-ANS	RNA-Seq	1 & S1	13 421 285	13 182 950	7 337 498
WT-2-ANS	RNA-Seq	1 & S1	13 438 583	13 146 920	8 715 834
upf1-1-DMSO	RNA-Seq	1 & S1	13 909 130	13 700 767	9 731 949
upf1-2-DMSO	RNA-Seq	1 & S1	14 591 424	14 325 138	10 950 987
upf1-1-ANS	RNA-Seq	1 & S1	13 087 188	12 897 985	9 889 436
upf1-2-ANS	RNA-Seq	1 & S1	14 556 757	14 317 925	12 329 660
WT-1	RNA-Seq	2 & S2	35 688 357	31 863 303	16 712 627
WT-2	RNA-Seq	2 & S2	32 174 963	28 880 548	15 505 733
WT-1-GD	RNA-Seq	2 & S2	29 818 665	27 350 132	13 730 561
WT-2-GD	RNA-Seq	2 & S2	27 553 301	25 480 615	12 754 661
WT-1-CHX	RNA-Seq	2 & S2	33 551 448	32 677 828	12 458 944
WT-2-CHX	RNA-Seq	2 & S2	33 064 352	32 234 831	12 073 738
WT-2-GD-CHX	RNA-Seq	2 & S2	30 786 212	29 499 680	5 495 599
WT-1-GD-CHX	RNA-Seq	2 & S2	28 524 919	27 584 517	6 720 423
xrn1-1	RNA-Seq	2 & S2	39 462 002	35 373 186	25 848 284
xrn1-2	RNA-Seq	2 & S2	28 933 960	26 044 163	19 226 873
xrn1-1-GD	RNA-Seq	2 & S2	39 798 609	36 233 080	26 753 494
xrn1-2-GD	RNA-Seq	2 & S2	33 148 328	29 802 594	21 719 642
upf1-1	RNA-Seq	2 & S2	34 841 355	30 582 692	17 908 638
upf1-2	RNA-Seq	2 & S2	32 640 573	29 703 654	16 921 445
upf1-1-GD	RNA-Seq	2 & S2	31 998 193	28 912 284	15 861 157
upf1-2-GD	RNA-Seq	2 & S2	35 046 407	31 617 414	15 877 798
WT-1	Ribo-Seq	3 & S3	77 424 622	72 489 228	10 396 011
WT-2	Ribo-Seq	3 & S3	82 084 394	75 850 401	9 045 008
upf1-1	Ribo-Seq	3 & S3	88 048 157	81 706 396	13 198 391
upf1-2	Ribo-Seq	3 & S3	78 102 685	72 561 775	11 883 099
WT-1-CHX	Ribo-Seq	3 & S3	78 440 785	72 655 432	14 157 109
WT-2-CHX	Ribo-Seq	3 & S3	78 718 754	73 187 204	15 092 093
upf1-1-CHX	Ribo-Seq	3 & S3	70 990 693	66 382 238	12 523 870
upf1-2-CHX	Ribo-Seq	3 & S3	75 224 757	69 802 815	13 429 514