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## Supplemental Information

# Transcriptome-wide Analysis of Exosome Targets

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**Table S1: Oligonucleotides used in these studies.**
Oligonucleotides are indicated (5'-3'), rN indicates RNA nucleotide

| | |
|---|---|
| Construction of Rrp44-HTP plasmids, derived from Rrp44-szz (Schneider et al., 2009) | |
| Rrp44-genF | ACGTACTCGAGGAATATATCCTTTTGAACTGGAGTG |
| Rrp44-gen(URA)R | ACGTACTCGAGGCTGGATGGGAAGCGTACC |
| Rrp44-$His_6$-overlap-F | GAGAAGCCACCATCACCATCACCATACTGCTAGCGAGAATTTG |
| Rrp44-$His_6$-overlap-R | GTTAAAATCCATGGAAAAGAGAAGCCACCATCACCATCACCAT |
| Insertion of a PreScission Protease (PP) cleavage site into Rrp44-HTP or Rrp44-szz plasmids | |
| Rrp44-PP-F | CTGGAAGTTCTGTTCCAGGGGCCCGATAAAGATCTTGAAAGGG ACACATTTTCAG |
| Rrp44-PP-R | GGGCCCCTGGAACAGAACTTCCAGAAAAGAATCCATTTGAGGAATA GAGTCTCTG |
| Rrp44-$His_6$-PP-F | CACCATCACCATCACCATCTGGAAGTTCTGTTCCAGGGGCCCGA TAAAGATCTTGAAAGGGACACATTTTCAG |
| Rrp44-$His_6$-PP-R | GGGCCCCTGGAACAGAACTTCCAGATGGTGATGGTGATGGTG AAAAGAATCCATTTGAGGAATAGAGTCTCTG |
| Construction of HTP-tagged yeast strains | |
| Rrp41-HTP-F | GGAAACATGCTCAGAAAAGAGTCAGTAACGCCTCTGCTAGGG AGCACCATCACCATCACC |
| Rrp41-TAP-R | ACTTTTATATAAACAGTGGCAATTAATGGCGTTTTTTATTTACG ACTCACTATAGGG |
| Csl4-HTP-F | TTACAGGCGCTACAGAAAAGCGCAAATGTGCCAAACCTTTTG AGCACCATCACCATCACC |
| Csl4-TAP-R | TATATACGCGTCTATATGCACTGTAGATAAGCTGTTACATATA CGACTCACTATAGGG |
| Rrp6-HTP-F | AAAGAGGAGGCCTGCCGCCAAAGGTAAGAATCTGTCATTTAA AAGGTCCATGGAGCACCATC |
| Rrp6-TAP-R | TAACTCCATGACACAGATATTCGATTAGATGAATTTAGAGGTC TTAATACGACTCACTATAGGG |
| pBS1539-HTP-URA | plasmid for HTP tagging, Granneman *et al.*, 2009 |
| CRAC 5' linkers (IDT) | |
| 5'L | 5InvddT/GTTCArGrArGrUrUrCrUrArCrArGrUrCrCrGrArCrGrArUrC |
| L5c | 5InvddT/ACACrGrArCrGrCrUrCrUrUrCrCrGrArUrCrUrGrA |
| L5d | 5InvddT/ACACrGrArCrGrCrUrCrUrUrCrCrGrArUrCrUrArCrArArGrC |
| L5e | 5InvddT/ACACrGrArCrGrCrUrCrUrUrCrCrGrArUrCrUrCrArCrArGrC |
| L5f | 5InvddT/ACACrGrArCrGrCrUrCrUrUrCrCrGrArUrCrUrGrCrGrArGrC |
| L5Aa | 5InvddT/ACACrGrArCrGrCrUrCrUrUrCrCrGrArUrCrUrNrNrNrUrArArGrC |
| L5Ad | 5InvddT/ACACrGrArCrGrCrUrCrUrUrCrCrGrArUrCrUrNrNrNrCrGrCrUrUrArGrC |
| CRAC 3′ pre-activated, adenylated cloning linker (IDT) | |
| miRCat-33™ 3'-L | rAppTGGAATTCTCGGGTGCCAAGG/ddC/ |

CRAC RT and PCR

| | |
|---|---|
| miRCat-33™ RT | CCTTGGCACCCGAGAATT |
| PCR-F1 | AATGATACTGCGACCACCGACAGGTTCAGAGTTCTACAGTCCGA |
| PCR-R1 | CAAGCAGAAGACGGCATACGA |
| PCR-F2 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGA CGCTCTTCCGATCT |
| PCR-R2 | CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGGC CTTGGCACCCGAGAATTCC |

Northern probes

| | |
|---|---|
| #304 anti-tRNA$^{Pro}$(UGG) mature | ACCCAGGGCCTCTCG |
| #305 anti-tRNA$^{Trp}$(CCA) mature | AACCTGCAACCCTTCGA |
| W331 anti-tRNA$^{Arg}$(UCU) mature | GGGGTCGAACCCATAATCTT |
| #250 anti-scR1 | ATCCCGGCCGCCTCCATCAC |
| #261 anti-U6 snRNA | AAAACGAAATAAATTCTTTGTAAAAC |

**Table S2: Yeast strains used in these studies.**

| | | |
|---|---|---|
| BY4741 | *MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0* | Euroscarf |
| Δ*rrp44* shuffle strain | *MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; rrp44Δ::kanMX6; [pRS316/RRP44-szz]* | (Schneider et al., 2009) |
| *Gal::rrp44* | *MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, His3MX6-pGAL1-3HA::rrp44* | (Houseley and Tollervey, 2006) |
| Trf4-HTP | *MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; TRF4-HTP-URA3* | (Wlotzka et al., 2011) |
| Rrp6-HTP | *MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; RRP6-HTP-URA3* | This study |
| Csl4-HTP | *MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; CSL4-HTP-URA3* | This study |
| Rrp41-HTP | *MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; RRP41-HTP-URA3* | This study |

**Supplementary References**


Gietz, D., St. Jean, A., Woods, R.A., and Schiestl, R.H. (1992). Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. *20*, 1425.

Granneman, S., Kudla, G., Petfalski, E., and Tollervey, D. (2009). Identification of protein binding sites on U3 snoRNA and pre-rRNA by UV cross-linking and high throughput analysis of cDNAs. Proc. Natl. Acad. Sci. U.S.A. *106*, 9613-9818.

Granneman, S., Petfalski, E., and Tollervey, D. (2011). A cluster of ribosome synthesis factors regulate 5.8S rRNA maturation. EMBO J. *30*, 4006-4019.

Houseley, J., and Tollervey, D. (2006). Yeast Trf5p is a nuclear poly(A) polymerase. EMBO Rep. *7*, 205-211.

Schneider, C., Anderson, J.T., and Tollervey, D. (2007). The exosome subunit Rrp44 plays a direct role in RNA substrate recognition. Mol. Cell *27*, 324-331.

Schneider, C., Leung, E., Brown, J., and Tollervey, D. (2009). The N-terminal PIN domain of the exosome subunit Rrp44 harbors endonuclease activity and tethers Rrp44 to the yeast core exosome. Nucleic Acids Res *37*, 1127-1140.

Wlotzka, W., Kudla, G., Granneman, S., and Tollervey, D. (2011). The nuclear RNA polymerase II surveillance system targets polymerase III transcripts. EMBO J. *30*, 1790-1803.

**Table S3 (Excel file):**
**Hit densities on functionally grouped RNAs recovered with all individual biological replicate experiments**
Dataset used for making the heatmaps presented in Figures 1, 5, 6 and S1. Numbers of hits per million mapped reads are shown.

**Supplementary Figures:**

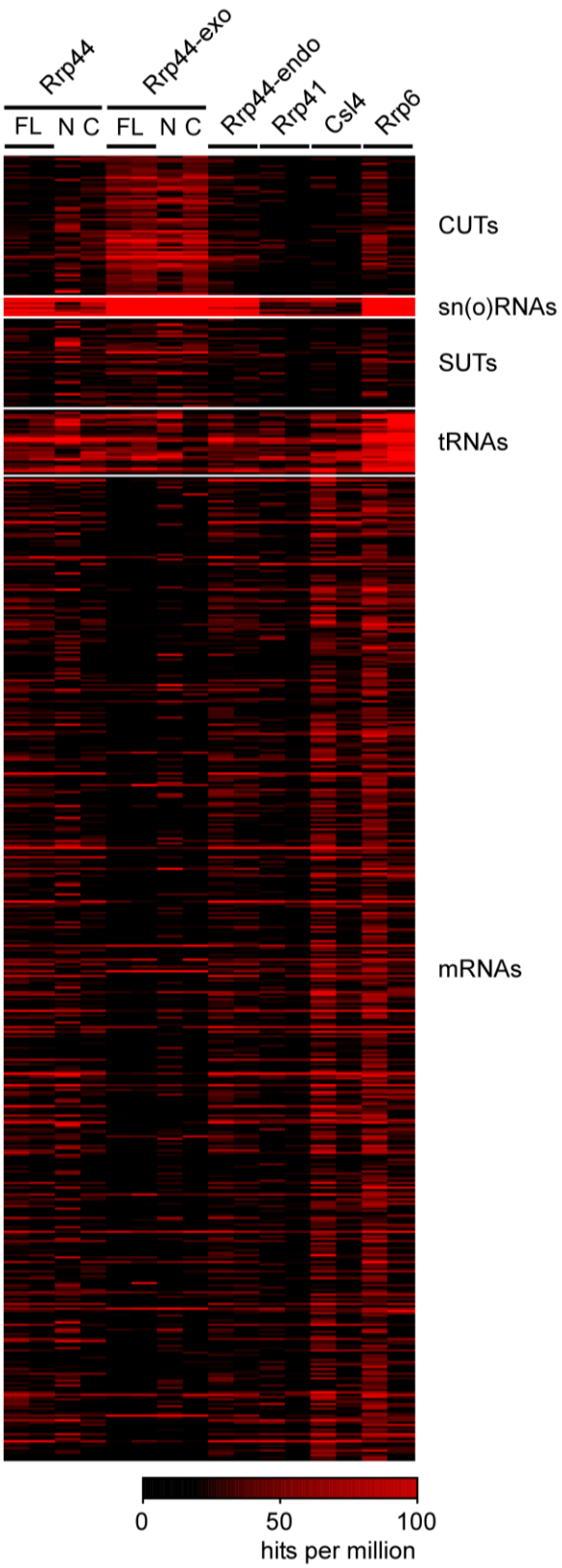


**Figure S1: Heat maps for main substrate groups and all individual biological replicate experiments.** Hits per million mapped reads of recovered RNAs are shown in shades of red.

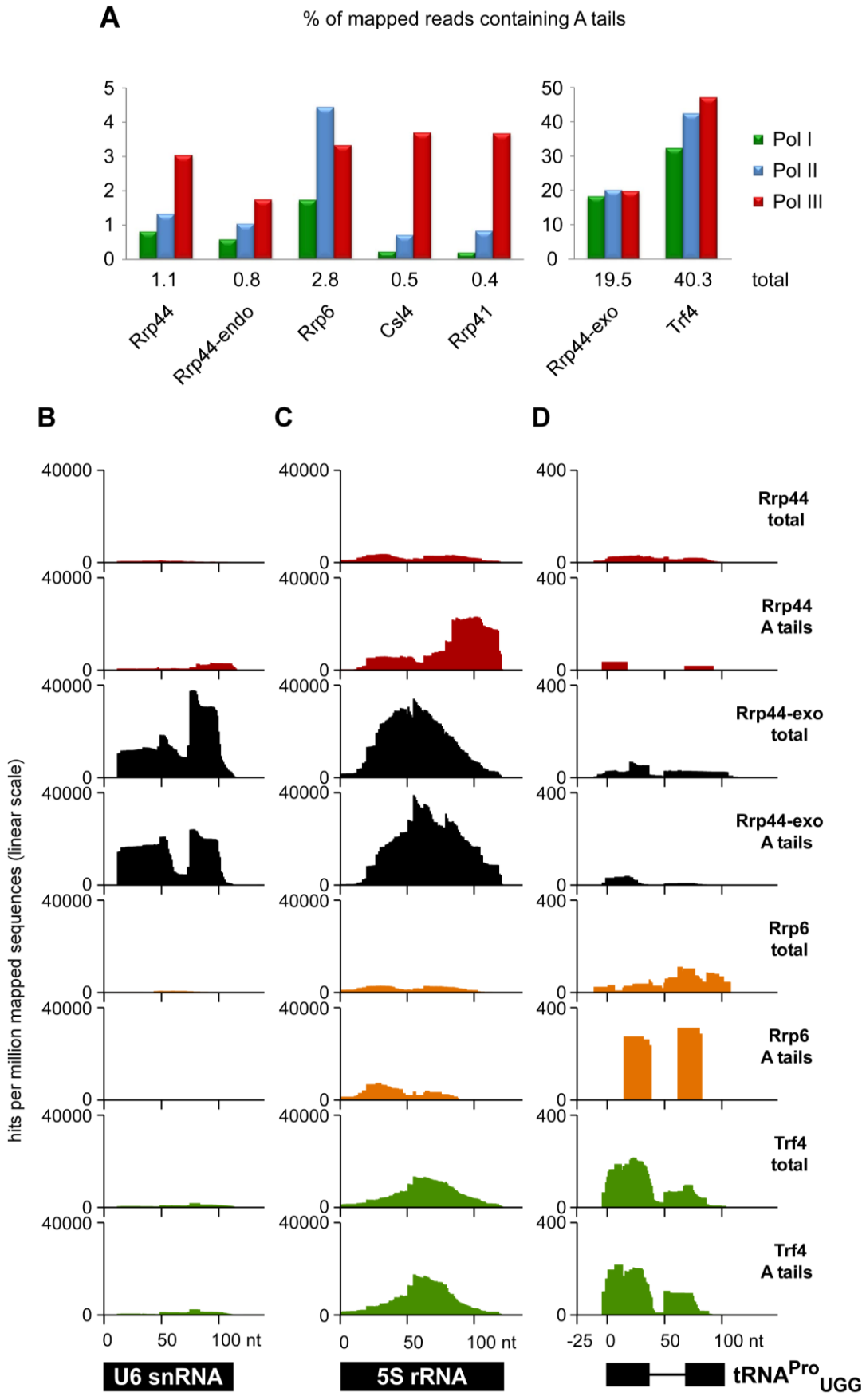
A
% of mapped reads containing A tails
5
4
3
2
1
0
1.1
0.8
2.8
0.5
0.4
Rrp44
Rrp44-endo
Rrp6
Csl4
Rrp41
50
40
30
20
10
0
19.5
40.3
total
Rrp44-exo
Trf4
Pol I
Pol II
Pol III
B
C
D
hits per million mapped sequences (linear scale)
40000
0
400
Rrp44 total
Rrp44 A tails
Rrp44-exo total
Rrp44-exo A tails
Rrp6 total
Rrp6 A tails
Trf4 total
Trf4 A tails
0
50
100 nt
-25
U6 snRNA
5S rRNA
tRNAPro UGG

**Figure S2**

**Figure S2:**

**A: Frequencies of non-templated terminal oligo(A) sequence reads in core exosome, Rrp6 and Trf4 datasets.** Datasets are either filtered for total reads, or for Pol I, Pol II and Pol III transcripts, that contain 2 or more non-templated As.

**B, C, D: Oligoadenylated sequence reads derived from representative Pol III RNAs**

Densities of high-throughput sequencing reads, either unfiltered (total) or filtered for reads containing 2 or more non-templated As (A-tails), from Rrp44, Rrp44-exo, Rrp6 and Trf4 datasets were mapped to the U6 snRNA (B), 5S rRNA (C) and $tRNA^{Pro}_{UGG}$ (D).

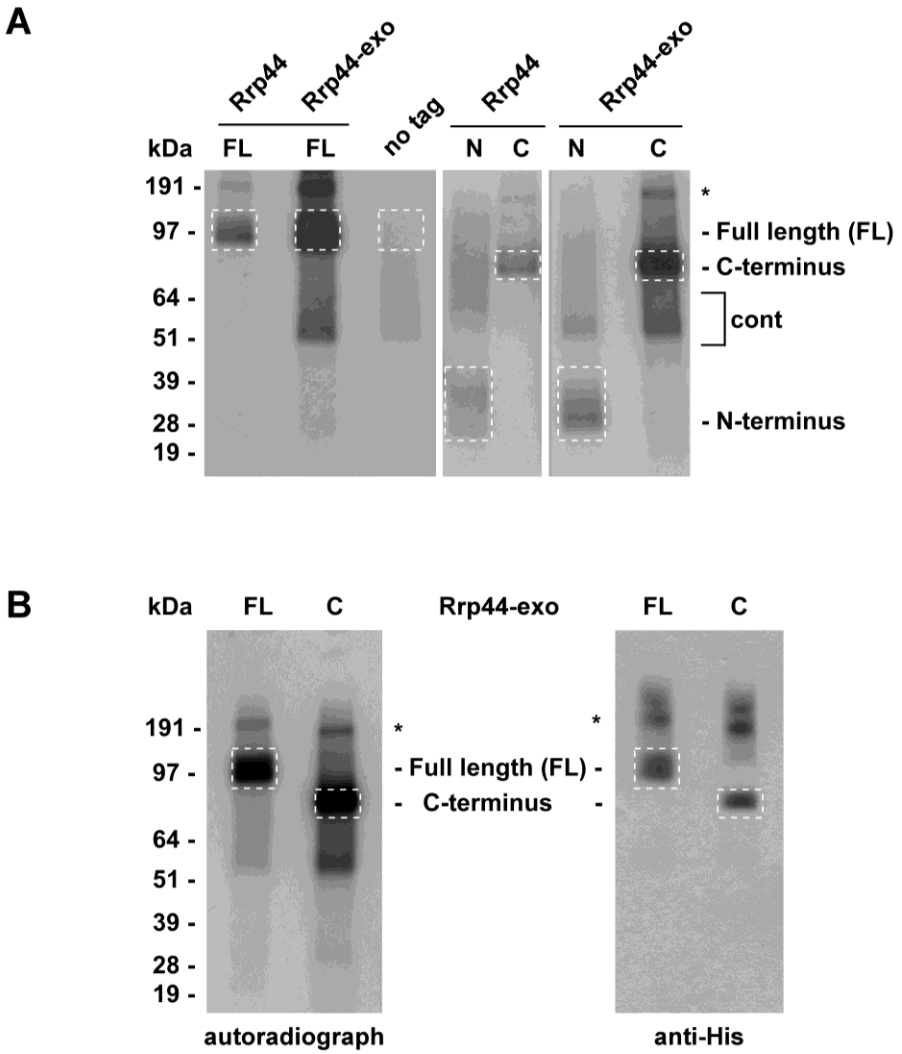


**Figure S3: Separation of full length Rrp44 and cleaved fragments**

**A**: Autoradiography of a denaturing SDS PAGE separating full length Rrp44 and N- and C-terminal fragments. Proteins purified from yeast strains expressing either wild type Rrp44 or the Rrp44-exo mutant are visualized by labeling of crosslinked RNA fragments. No tag: untagged strain; cont: common contaminant ~55 kDa band (Granneman *et al.*, 2009).

**B**: Full length Rrp44-exo and the cleaved C-terminal fragment are visualized by autoradiography (left, see panel A) or immunoblotting using an anti-His antibody (right).

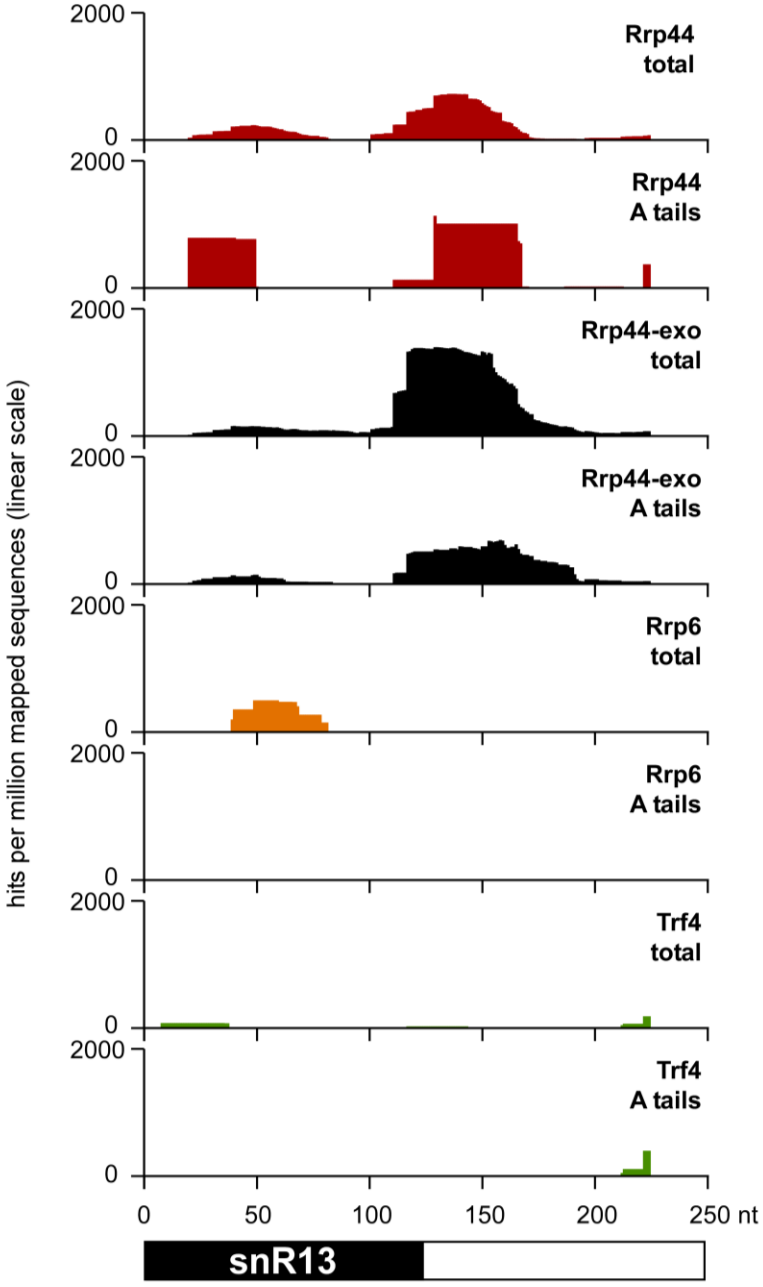


**Figure S4: Functional overlap between Rrp44 and Rrp6 on snR13**
Densities of high-throughput sequencing reads, either unfiltered (total) or filtered for reads containing 2 or more non-templated As (A-tails), from Rrp44, Rrp44-exo, Rrp6 and Trf4 datasets were mapped to the box C/D snoRNA snR13 (1-124 nt, black box) and downstream regions. The snoRNA terminator elements I and II are located at nt 125-190 (I) and nt 190-249 (II).

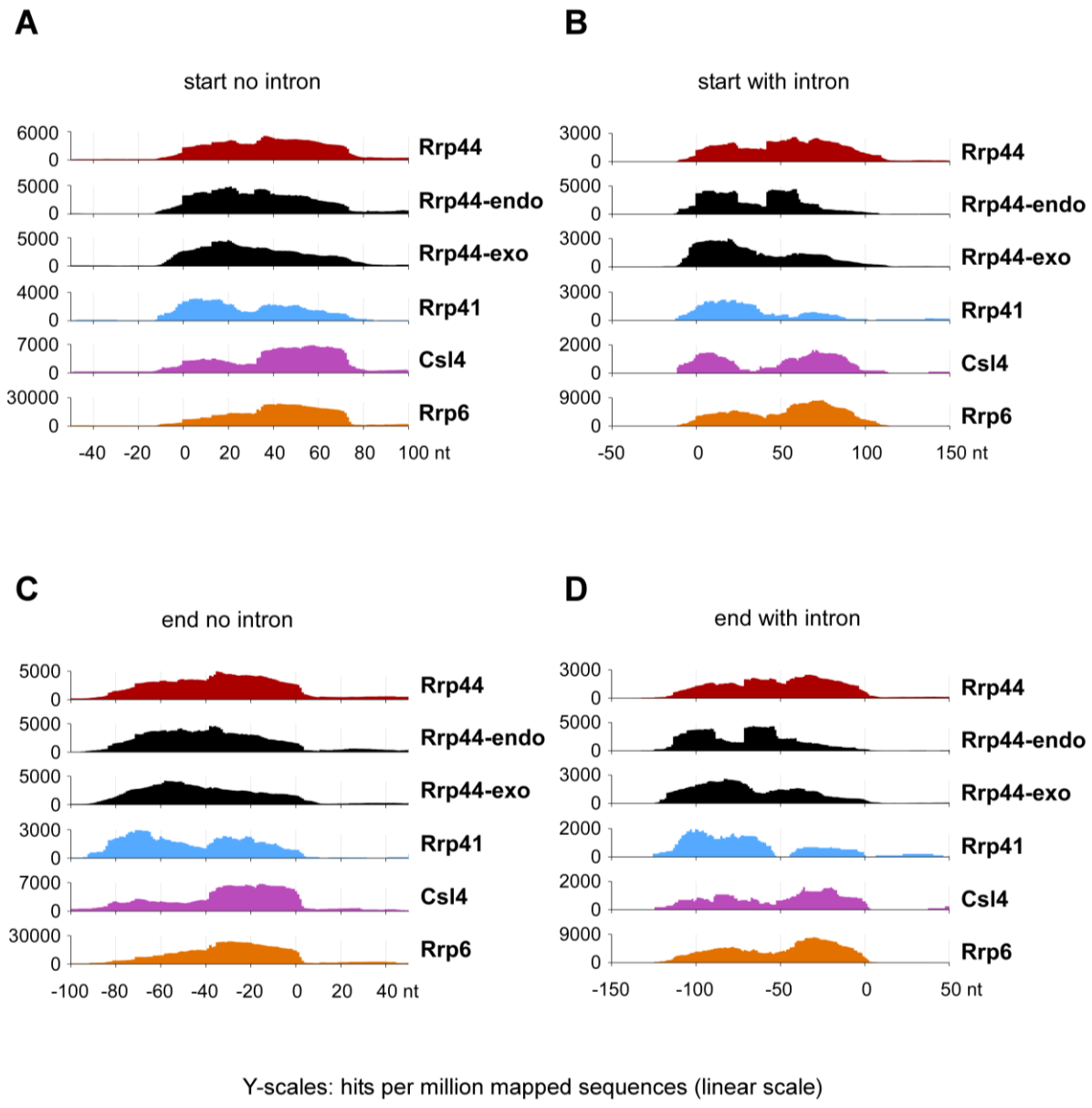


**Figure S5: Distribution of high-throughput sequencing reads from core exosome and Rrp6 datasets over all yeast tRNA genes.**

All yeast tRNA genes were filtered by the presence or absence of an intron and aligned at either 5' or 3' end of the mature tRNAs (indicated as position 0 on the plots). Total hit densities per million mapped reads are indicated for the proteins tested. Note that different scales are used in the Y-axis, reflecting differences in overall tRNA association for the different proteins.