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Supplemental Data

The Exosome Subunit Rrp44 Plays a Direct Role in RNA Substrate Recognition

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Table S1. Oligonucleotides Used and Strain Construction

Rrp44-D551N-F	5'-CCTCCAGGAT GTGTTGATAT TAACGATGCC CTACATGC
Rrp44-D551N-R	5'-GCATGTAGGG CATCGTTAAT ATCAACACAT CCTGGAGG
Rrp44-G833D-F	5'-CCTGACTTTA GACACTATGA TTTAGCCGTT GATATCTACA CACATTTC
Rrp44-G833D-R	5'-GAAATGTGTG TAGATATCAA CGGCTAAATC ATAGTGTCTA AAGTCAGG
Rrp44-G916E-F	5'-GGAATAACGA GTCCACAGAA ACTGAATATG TTATTAAGGT ATTTAATAAT GG
Rrp44-G916E-R	5'-CCATTATTAA ATACCTTAAT AACATATTCA GTTTCTGTGG ACTCGTTATT CC
Rrp44-TAP-A	5'-GGATCCAATT ACTAGCAAGC GTAAGGCAGA ATTATTGTTA AAATCCATGG AAAAGAGAAG
Rrp44-TAP-B	5'-CCTTTTCGTT TTTATATCCT GATACTGAAG CATCTTCCAT TACGACTCAC TATAGGG
Rrp44-genF	5'-ACGTACTCGA GGAATATATC CTTTTGAACT GGAGTG
Rrp44-gen(URA)R	5'-ACGTACTCGA GGCTGGATGG GAAGCGTACC
Anti-$tRNA_i^{Met}$	5'-CGATCCGAGG ACATCAGGG
Anti-$tRNA^{Trp\ (CCA)}$	5'-AACCTGCAAC CCTTCGA
Anti-$tRNA^{Tyr\ (G\Psi A)}$	5'-AAGATTTCGT AGTGATAA
Anti-$tRNA^{Pro\ (UGG)}$	5'-ACCCAGGGCC TCTCG
Anti-$tRNA^{Leu\ (CAA)}$	5'-GCATCTTACG ATACCTG
Anti-$tRNA^{Gly\ (GCC)}$	5'-TACCACTAAA CCACTTGC

Oligonucleotides used in these analyses.

Supplemental Experimental Procedures

Strain Construction

S. cerevisiae strains used in this study were derived from the BY4741 wild-type strain (*MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0*). To construct yeast expression plasmids for Rrp44, a C-terminal tag containing a streptavidin-binding peptide (Strep-tag II) (Junttila et al., 2005), TEV cleavage site and two copies of the z-domain of protein A was first added to the wild-type *RRP44* gene in BY4741. This was achieved by genomic integration of a cassette (the template plasmid (*URA3*) was derived from pBS1539 and constructed by Martin Kos, Tollervey lab) using a one-step PCR strategy and the oligonucleotides Rrp44-TAP A and B (Rigaut et al., 1999). The *RRP44*-szz gene including 500 bp of the promoter region was then PCR amplified from the genomic DNA of the tagged strain using the oligos Rrp44-genF and Rrp44-gen(URA)R and cloned into the XhoI restriction site of pRS316. The Rrp44-cat point mutation was created by site-directed mutagenesis as described above. A strain conditionally expressing HA-tagged Rrp44 from the *GAL10* promoter (*GAL::rrp44: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, HisMX6-pGAL-3HA::rrp44* (Houseley and Tollervey, 2006)) was then transformed with an empty cloning vector (pRS316) or with constructs containing either the wild-type or the *rrp44-cat* gene (Gietz et al., 1992). Growth and handling of *S. cerevisiae* were by standard techniques. Strains were grown at 30°C in -His/-Ura dropout medium containing 0.67% nitrogen base (Difco) and either 2% glucose or 2% galactose.

The isolation of the *mtr17-1* allele was previously reported (Kadowaki et al., 1994). Subsequent analysis showed *mtr17-1* to be an allele of *RRP44* (A. Tartakoff, pers. comm.) and it was therefore designated *rrp44-1*. The strain carrying *rrp44-1* was generously provided by Alan Tartakoff (Case Western Reserve University). The *RRP44* ORF was recovered by PCR amplification and the sequence revealed two amino acid exchanges (P4S and G916E), with G916E mapping to the S1 domain of Rrp44 (see Fig. 1A). Rrp44 carrying the G916E mutation (referred to as the "S1" mutation in the text) was expressed in *S.cerevisiae* under control of the endogenous promoter. This conferred a temperature sensitive lethal phenotype following genetic depletion of the endogenous wild-type Rrp44, suggesting that the G916E mutation is responsible for the growth defect observed in *rrp44-1* strains. The GST-fusion protein containing the G916E (S1) point mutation was generated and used for *in vitro* analyses.

Supplemental References

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Kadowaki, T., Chen, S., Hitomi, M., Jacobs, E., Kumagai, C., Liang, S., Schneiter, R., Singleton, D., Wisniewska, J., and Tartakoff, A. M. (1994). Isolation and characterization of Saccharomyces cerevisiae mRNA transport-defective (mtr) mutants. J Cell Biol *126*, 649-659.

Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M., and Seraphin, B. (1999). A generic protein purification method for protein complex characterization and proteome exploration. Nat Biotechnol *17*, 1030-1032.

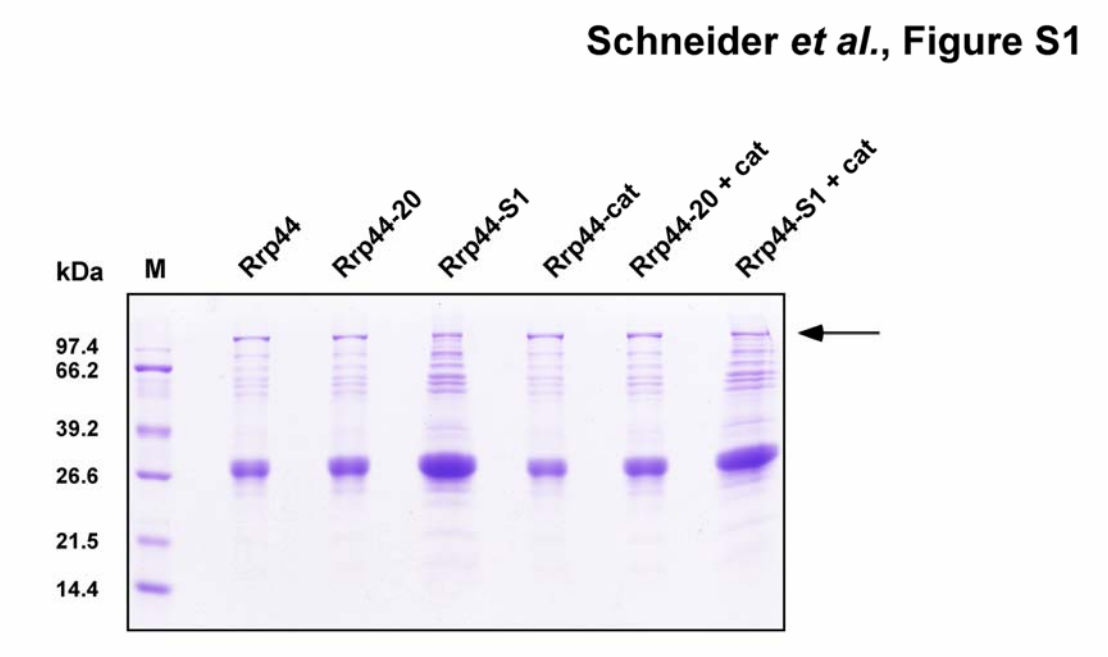

Figure S1. Expression of Recombinant Wild-Type and Mutant GST-Rrp44 Fusion Proteins

GST-fusion proteins were affinity-purified on glutathione-sepharose beads, separated on a denaturing 10% SDS polyacrylamide gel and stained with Coomassie Blue. The amount of wild-type and mutant Rrp44 proteins used in bandshift and exonuclease experiments were normalized to the intensity of the full-length band (marked with an arrow).

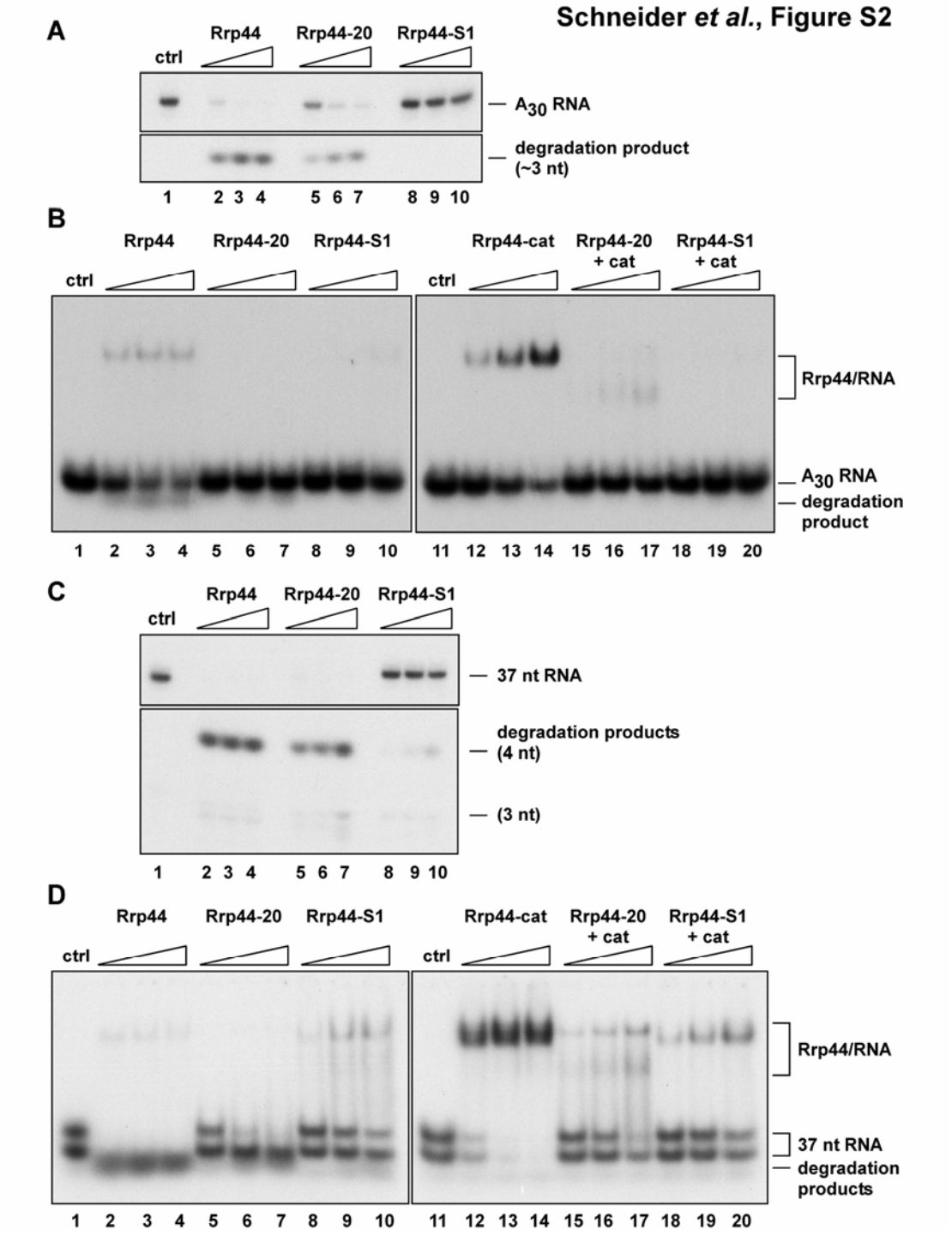

Figure S2. Activities of Mutant Forms of Rrp44 on Other RNA Substrates

(A) Exonuclease activity of wild-type GST-Rrp44, GST-Rrp44-20 or GST-Rrp44-S1 on a 5'-end labeled poly(A_{30}) RNA. Recombinant protein (3, 6 or 12 fmoles) was incubated with 10 fmoles RNA for 90 min at 30°C in the presence of 0.25 mM $MgCl_2$. Reaction products were analyzed as described in Fig. 2A.

(B) RNA binding assay using wild-type or mutant GST-Rrp44 proteins and 5'-end labeled poly(A_{30}) RNA. Recombinant protein (3, 6 or 12 fmoles) was incubated with 10 fmoles RNA for 10 min at 30°C in the presence of 0.25 mM $MgCl_2$. Reactions were analyzed as described in Fig. 2B.

(C) Exonuclease activity of wild-type GST-Rrp44, GST-Rrp44-20 or GST-Rrp44-S1 on a 5'-end labeled 37 nt substrate derived from the pBS polylinker. Recombinant protein (3, 6 or 12 fmoles) was incubated with 10 fmoles RNA for 90 min at 30°C in the presence of 0.25 mM $MgCl_2$. Reaction products were analyzed as described in Fig. 2A.

(D) RNA binding assays using wild-type or mutant GST-Rrp44 proteins and 5'-end labeled 37 nt RNA. Recombinant protein (3, 6 or 12 fmoles) was incubated with 10 fmoles RNA for 10 min at 30°C in the presence of 0.25 mM $MgCl_2$. Reactions were analyzed as described in Fig. 2B.

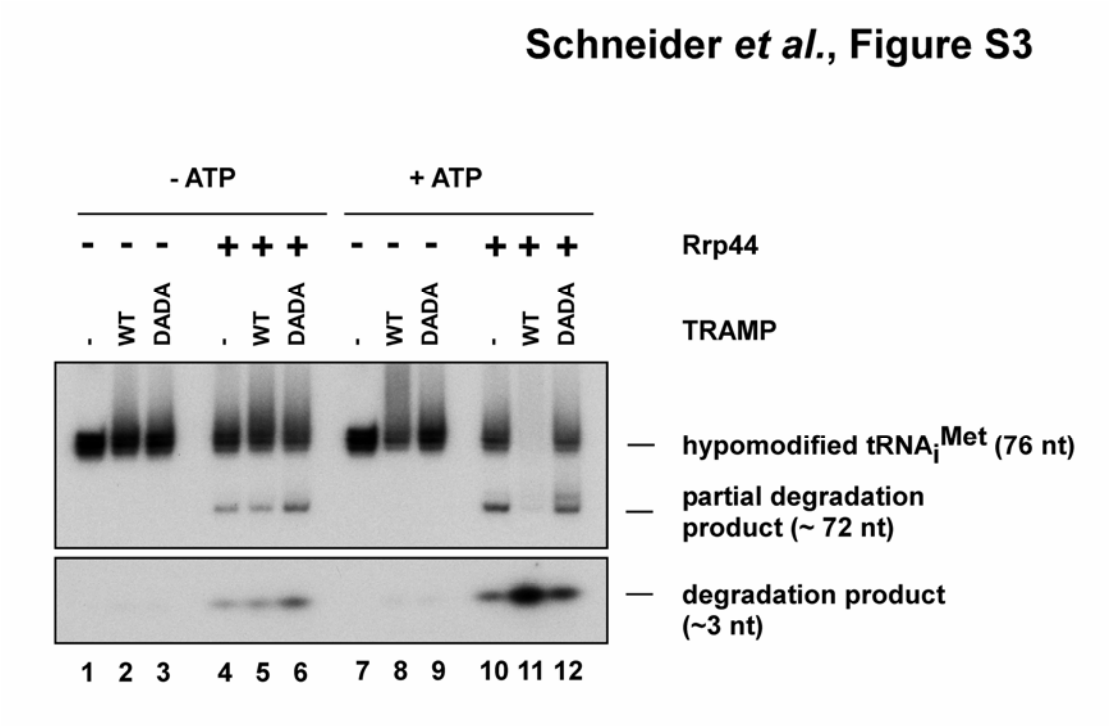

Figure S3. Stimulation of Rrp44-Mediated Degradation of the Hypomodified tRNA$_i^{Met}$ Substrate by the TRAMP Polyadenylation Complex Requires ATP and Catalytically Active Trf4

Combined exonuclease/polyadenylation assay using 0.5 µl TAP-purified TRAMP complex (wildtype (WT) or polyadenylation inactive DADA mutant, see Vanacova *et al.*, 2005), 100 fmoles GST-Rrp44 WT protein and 25 fmoles 5'-end labeled, native hypomodified tRNA$_i^{Met}$ substrate. Reactions were performed for 60 min at 30°C with 0.625 mM $MgCl_2$ and in the absence or presence of 1 mM ATP.

Products were separated on a 12% polyacrylamide/ 8M urea gel and visualized by autoradiography. Full-length tRNA$_i^{Met}$, polyadenylated RNA and degraded RNA species are indicated on the right.