Supplemental materials

Comparison of catalytic site point mutations and domain deletions suggests that the exoribonuclease domain of Rrp44 has non-catalytic functions.

To investigate whether the exonuclease domain of Rrp44 had any non-catalytic functions we compared the effect of a mutation in the exonuclease active site (*rrp44-exo*$^-$) to the effect of completely deleting the exonuclease domain (*rrp44-*Δ*exo*). While both the *rrp44-exo*$^-$ and *rrp44-*Δ*exo* mutations cause a slow growth as previously reported (Dziembowski et al., 2007; Lebreton et al., 2008; Schaeffer et al., 2009; Schneider et al., 2009), the phenotype caused by deletion of exonuclease domain is much more severe (Supplemental figure S1A). These results indicate that the exonuclease domain has a noncatalytic function, although they do not indicate what the noncatalytic function might be.

Overexpressing Rrp44 alleles suggest that the exonuclease domain contributes to interaction with the RNA exosome core.

To better understand which interactions are critical for RNA exosome function *in vivo*, we choose to overexpress catalytically inactive Rrp44. We reasoned (supplemental figure S1B) that the catalytically inactive subunit of a complex should displace the endogenous subunit and result in a dominant negative phenotype. To validate this reasoning we used a galactose-inducible promoter to conditionally overexpress either wild-type Rrp44, a catalytically inactive Rrp44 (Rrp44-endo$^-$-exo$^-$) that lacks both endo and exonuclease activities. As expected, the catalytically inactive Rrp44 caused slow growth (Supplemental figure 1C). We also overexpressed an additional Rrp44 (Rrp44-CR3-endo$^-$-exo$^-$) that combines the catalytic site mutations with a mutation previously shown to inhibit RNA exosome association (Schaeffer et al., 2012). This catalytically inactive Rrp44 that was unable to interact with exo9 had no effect on growth. Western blot analysis indicated that each mutant was overexpressed to a similar extent (Supplemental figure 1D). These results suggest that overexpression of the catalytically inactive mutant is indeed detrimental because it displaces the endogenous Rrp44 from the RNA exosome.

The endonuclease domain of Rrp44 has previously been shown to be a major exosome interacting domain. To test whether the exonuclease domain of Rrp44 is also important for the interaction with the RNA exosome core, we overexpressed just the catalytically inactive endonuclease domain (Rrp44-endo$^-$-Δexo). Both Rrp44-endo$^-$-Δexo and Rrp44-endo$^-$-exo$^-$ lack exonuclease activity, but in the latter the exonuclease domain is available to serve non-catalytic functions, while this domain is entirely deleted in the former. If the endonuclease domain were the only critical interaction site between Rrp44 and the core, overexpression of Rrp44-endo$^-$-Δexo and Rrp44-endo$^-$-exo$^-$ would similarly displace the endogenous Rrp44 and cause growth inhibition. However, we did not observe any dominant negative growth defect when Rrp44-endo$^-$-Δexo was overexpressed (Supplemental figure 1E). The expression level of *rrp44-*Δ*exo* can not be directly compared to full length Rrp44 alleles because the antibody was raised against full length Rrp44. Therefore, we used C-terminal TAP-tagged variants of *RRP44* alleles to assess the expression levels by Protein A antibody. Overexpression of Rrp44-endo$^-$-Δexo-TAP gives a similar result to untagged variants (Supplemental figure 1E). Western

blot analysis with anti TAP antibodies indicated that the full length Rrp44-endo$^-$-exo$^-$ and Rrp44-endo$^-$-Δexo are expressed at similar levels (Supplemental Figure 1E). Overexpression of any of the full length Rrp44 constructs also caused the accumulation of degradation intermediates, but these degradation products are similar for wild-type Rrp44 and any of the mutants tested (Supplemental Figure 1C and E). These degradation products detected by the antibody to the C-terminal TAP tag do not possess the N-terminal region and are unlikely to efficiently associate with the exo-9 core. The results from these overexpression experiments are consistent with the exonuclease domain of Rrp44 contributing to exo-9 interaction, and led us to further pursue this hypothesis as described in the main text.

Table S1: Strains used.

Name	Genotype	reference
BY4741	*MATa/leu2-Δ0/ura3-Δ0/his3Δ1/met15Δ0*	Giaever et al., 2002
yAV1115	*MATa/leu2-Δ0/ura3-Δ0/his3Δ-Δ1/rrp44Δ::NEO [RRP44, URA3]*	Schaeffer et al., 2009
yAV1143	*MATα/trp1/ura3/leu2/dcp1-2ts::TRP1/rrp44::NEO [RRP44, URA3]*	Schaeffer and van Hoof, 2011
yAV1117	*MATa/leu2-Δ0/ura3-Δ0/his3Δ1/met15Δ0 /rrp43::myc::HIS3*	Schaeffer et al., 2012
yAV1137	*MATa/leu2-Δ0/ura3-Δ0/his3-Δ1/rrp44Δ::HYG/rrp6Δ::NEO [RRP44, URA3]*	Schaeffer et al., 2012
yAV1234	*MATα/ura3-Δ0/leu2-Δ0/his3-Δ1/rrp41Δ::NEO [RRP41, URA3] [RRP41, LEU2]*	Wasmuth and Lima, 2012
yAV1244	*MATα/ura3-Δ0/leu2-Δ0/his3-Δ1/rrp45Δ::NEO [RRP45, URA3] [RRP45, LEU2]*	Wasmuth and Lima, 2012
yAV1420	*MATα/rrp44-20/trm6-504/gcn2-101/his1-29/ura3-52::HIS4-lacZ/ino1*	Kadaba et al., 2004
yAV1634	*MATa/ura3-Δ0/leu2-Δ0/his3-Δ1/rrp44Δ::NEO/trp1Δ::hisG [RRP44, LEU2]*	This study
yAV1642	*MATα/ura3-Δ0/leu2-Δ0/his3-Δ1/rrp44Δ::NEO/rrp41Δ::NEO/trp1Δ::hisG [RRP44, URA3] [RRP41, TRP1]*	This study
yAV1751	*MATa/ura3-Δ0/leu2-Δ0/his3-Δ1/rrp44Δ::NEO/rrp45Δ::NEO/trp1Δ::hisG [RRP44, LEU2] [RRP45, URA3]*	This study
yAV1713	*MATα/ura3-Δ0/leu2-Δ0/his3-Δ1/rrp44Δ::NEO/rrp41Δ::NEO/trp1Δ::hisG [RRP44, LEU2] [RRP41, URA3]*	This study
yAV1714	*MATα/ura3-Δ0/leu2-Δ0/his3-Δ1/rrp44Δ::NEO/rrp41Δ::NEO/trp1Δ::hisG [rrp44-da, LEU2] [RRP41, URA3]*	This study
yAV1715	*MATα/ura3-Δ0/leu2-Δ0/his3-Δ1/rrp44Δ::NEO/rrp41Δ::NEO/trp1Δ::hisG [rrp44-CR3, LEU2] [RRP41, URA3]*	This study
yAV1716	*MATα/ura3-Δ0/leu2-Δ0/his3-Δ1/rrp44Δ::NEO/rrp41Δ::NEO/trp1Δ::hisG [rrp44-exo-, LEU2] [RRP41, URA3]*	This study
yAV1717	*MATα/ura3-Δ0/leu2-Δ0/his3-Δ1/rrp44Δ::NEO/rrp41Δ::NEO/trp1Δ::hisG [rrp44-endo-, LEU2] [RRP41, URA3]*	This study
yAV1718	*MATα/ura3-Δ0/leu2-Δ0/his3-Δ1/rrp44Δ::NEO/rrp41Δ::NEO/trp1Δ::hisG [rrp44-endo-da, LEU2] [RRP41, URA3]*	This study
yAV1966	*MATα/rrp44Δ::NEO/trm6-504/gcn2-101/his1-29/ura3-52::HIS4-lacZ/ino1/leu2-Δ0 [RRP44, URA3]*	This study

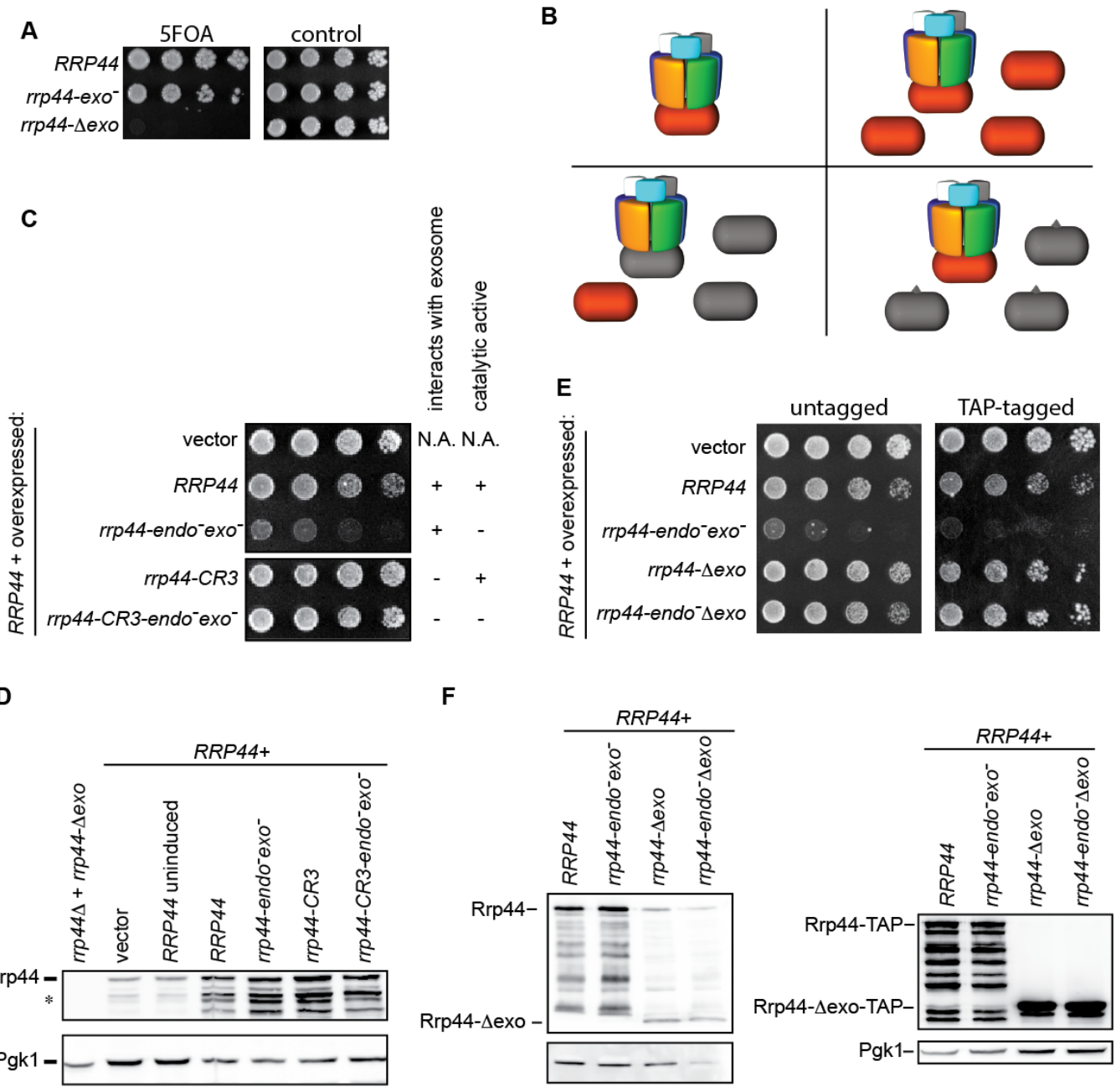
Table S2: plasmids used.

Name	Short description	Description	Marker	reference
pAV952	pNKY1009	for generation of trp1Δ::hisG strain	NA	Alani et al., 1987
pAV948	pAD1	for generation of leu2Δ0 strains	NA	Brachmann et al., 1998
pAV241	HIS3-Rz-stop	HIS3-Hammerhead ribozyme-stop reporter	URA3	Meaux and van Hoof, 2006
pAV958	rrp44-CR3	RRP44 promoter, RRP44 ORF (C47S, C52S, C55S)	LEU2	Schaeffer et al., 2009
pAV503	rrp44-endo-	RRP44 promoter, RRP44 ORF (D171A)	LEU2	Schaeffer et al., 2009
pAV501	rrp44-exo-	RRP44 promoter, RRP44 ORF (D551N)	LEU2	Schaeffer et al., 2009
pAV344	RRP44	RRP44 promoter, RRP44 ORF 1-1001	LEU2	Schaeffer et al., 2009
pAV777	rrp44-yrd	RRP44 promoter, RRP44 ORF (Y40A, R42A, D44A)	LEU2	Schaeffer and van Hoof, 2011
pAV189	HIS3-nonstop	HIS3 nonstop reporter	URA3	van Hoof et al., 2002
pAV361	RRP44	RRP44 promoter, RRP44 ORF	URA3	Wasmuth and Lima, 2012
pAV1085	rrp41-L	RRP41 promoter, rrp41-L (GESEGESEGEL inserted after K62)	LEU2	Wasmuth and Lima, 2012
pAV1043	RRP41	RRP41 promoter, RRP41 ORF	URA3	Wasmuth and Lima, 2012
pAV1039	RRP41	RRP41 promoter, RRP41 ORF	LEU2	Wasmuth and Lima, 2012
pAV1044	RRP45	RRP45 promoter, RRP45 ORF	URA3	Wasmuth and Lima, 2012
pAV883	da fragment	da fragment (R439A, R440A, H466A, L500A, D602A) synthesized by GENEWIZ	NA	This study
pAV959	rrp44-CR3-TAP	RRP44 promoter, RRP44 ORF (C47S, C52S, C55S), TAP	LEU2	This study
pAV921	rrp44-da-TAP	RRP44 promoter, RRP44 ORF (R439A, R440A, H466A, L500A, D602A), TAP	LEU2	This study
pAV920	rrp44-yrd-TAP	RRP44 promoter, RRP44 ORF (Y40A, R42A, D44A), TAP	LEU2	This study
pAV917	RRP44-TAP	RRP44 promoter, RRP44 ORF, TAP	LEU2	This study
pAV912	rrp44-CR3-da	RRP44 promoter, RRP44 ORF (C47S, C52S, C55S, R439A, R440A, H466A, L500A, D602A)	LEU2	This study
pAV911	rrp44-endo-da	RRP44 promoter, RRP44 ORF (D171A, R439A, R440A, H466A, L500A, D602A)	LEU2	This study
pAV910	rrp44-da	RRP44 promoter, RRP44 ORF (R439A, R440A, H466A, L500A, D602A)	LEU2	This study
pAV1109	rrp45-L	RRP45 promoter, rrp45-L (GESEGESEGEL inserted after G94)	TRP1	This study
pAV1089	rrp41-L	RRP41 promoter, rrp41-L (GESEGESEGEL inserted after K62)	TRP1	This study
pAV1079	rrp44-da-endo-exo-	GAL promoter, RRP44 ORF (D171A, R439A, R440A, H466A, L500A, D551N, D602A)	MET15	This study
pAV1077	rrp44-CR3-endo-exo-	GAL promoter, RRP44 ORF (C47S, C52S, C55S, D171A, D551N)	MET15	This study
pAV1076	rrp44-CR3	GAL promoter, RRP44 ORF (C47S, C52S, C55S)	MET15	This study
pAV1074	rrp44-da	GAL promoter, RRP44 ORF (R439A, R440A, H466A, L500A, D602A)	MET15	This study
pAV1058	rrp44-endo-exo-	GAL promoter, RRP44 ORF (D171A, D551N)	MET15	This study
pAV1053	RRP44	GAL promoter, RRP44 ORF	MET15	This study
pAV1049	RRP41	RRP41 promoter, RRP41 ORF	TRP1	This study
pAV1033	rrp44-endo-Δexo	GAL promoter, residues 1-235 of RRP44 ORF (D171A)	MET15	This study
pAV1032	rrp44-Δexo	GAL promoter, residues 1-235 of RRP44 ORF	MET15	This study
pAV1029	rrp44-exo-da	RRP44 promoter, RRP44 ORF (R439A, R440A, H466A, L500A, D551N, D602A)	LEU2	This study
pAV1065	RRP45	RRP45 promoter, RRP45 ORF	TRP1	This study

Table S3: oligonucleotides used.

Name	Short description	Sequence	Description
Norterhn blot probes			
oAV224	SRP	gtctagccgcgaggaagg	
oAV910	U14	tcctaccgtggaaactgcg	
oAV911	pre U14	gatactacagtatacgatcactc	
oAV1151	5'ETS	cgaacgacaagcctactcg	
oAV1233	7S pre-rRNA	tgagaaggaaatgacgct	
oAV1234	tRNAiMET	tcggtttcgatccgaggacatcagggttatga	
oAV1235	tRNAcaaLEU	tggttgctaagagattcgaac	
oAV1278	5S	tcgcgtatggtcacccactaca	
Site-directed mutagenesis			
oAV482	CR3 sense	gatcggacatcccaagtctttctagaagtagtaccaagagtccgcaaattgtcg	RRP44 to rrp44-CR3
oAV569	CR3 antisense	cgacaatttgcggactcttggtactacttctagaaagacttgggatgtccgatc	RRP44 to rrp44-CR3
oAV565	exo- sense	ctccaggatgtgttcatatcaacgatgccctacatgcg	RRP44 to rrp44-D551N
oAV566	exo- antisense II	cgcatgtagggcatcgttgatatcaacacatcctggag	RRP44 to rrp44-D551N
oAV572	endo- sense	cgattaatgacagaaacgcgcgcgctataaggaaaacctgtcaatgg	RRP44 to rrp44-D171A
oAV573	endo- antisense	ccatgacaggttttccttatagcgcgcgcgtttctgtcattaatcg	RRP44 to rrp44-D171A
oAV847	YRD sense	cgtaagagaacacgcgttagcttcggctatcccatgtctttcg	RRP44 to rrp44-yrd
oAV848	YRD antisense	cgaaagacatgggatagccgaagctaacgcgtgttctcttacg	RRP44 to rrp44-yrd
Cloning			
oAV1163	3Rrp44-F	tagaggcaggtgccttgaacttagcttctcctgaggttaaggtccatatg	cloning TAP-tagged RRP44 by homologous recombination
oAV1164	TAP3UTR-R	cgtaatacgactcactatagggcgaattgggtaccgggccccccctcgagtgccggtagaggtgtggtcaataa	cloning TAP-tagged RRP44 by homologous recombination
oAV1339	41F	atgcactagtaagtggagaattgtttgtttattt	Cloning of RRP41 (SpeI/XhoI)
oAV1340	41R	atgcctcgagttcatagctgaggagtataagc	Cloning of RRP41 (SpeI/XhoI)
oAV1341	45F	atgcactagttgctgaaagagaattactgatg	Cloning of RRP45 (SpeI/XhoI)
oAV1342	45R	atgcctcgagatgacgatgacgaagtttttgt	Cloning of RRP45 (SpeI/XhoI)

Supplemental movie (related to figure 1). The movie shows the large movement of the exonuclease domain of Rrp44 going from the channeling conformation to the direct access conformation. Grey: Rrrp44. Orange: exo-9 core. Black: RNA. Yellow: endonuclease active site (D171). Red: exonuclease active site (D551). Green: amino acids mutated in *rrp44-da* allele. The movie is linear interpolation of two exosome structures (PDB ID: 5C0W and 4IFD; (Makino et al., 2013a; Makino et al., 2015) by using UCSF chimera (Pettersen et al., 2004)



Supplemental figure S1 (related to figure 1). **A.** The effect on growth of deleting the exonuclease domain of Rrp44 (*rrp44-Δexo*) is much more severe than the effect of a point mutation inactivating the exonuclease domain (*rrp44-exo-*) **B.** Rationale behind overexpression experiments in C and E. Top left, normal RNA exosome. Top right: Overexpressing wild-type Rrp44 should not affect RNA exosome function. Bottom left: Overexpressing inactive Rrp44 should displace wild-type Rrp44 and inhibit RNA exosome function dominant negatively. Bottom right: Overexpressing inactive Rrp44 unable to interact with the RNA exosome should not displace wild-type Rrp44 and should not affect RNA exosome activity. Red oval: Active Rrp44. Grey oval: Catalytically inactive Rrp44. Grey triangle: Mutation that interferes with RNA exosome binding. **C.** Overexpression of catalytically inactive Rrp44 causes a dominant negative phenotype. Wild-type yeast strains

carrying various alleles of *RRP44* under a galactose inducible promoter were serially diluted and spotted on solid media containing galactose. No growth defect was observed on media containing glucose (data not shown). The white line between the third and fourth row indicates that lanes containing other irrelevant mutants were cut from the image. The five rows shown are from the same plate. **D, F, G.** Western blot analysis indicates mutant *RRP44* or *RRP44-TAP* alleles are overexpressed to similar extents. Cell lysates were subjected to western blot with α-Rrp44 (untagged Rrp44), α-Protein A (TAP-tagged Rrp44) and α-Pgk1 (loading control) antibodies. The position of full length Rrp44 is indicated, as is the position of several degradation products (*). The first lane of panel D contains a lysate from a strain that expresses only a truncated Rrp44. The absence of the signals for both full length Rrp44 and degradation products in this lane indicate that these signals are specific. The third lane of panel D is from the same strain as lane four but grown in glucose containing (noninducing) medium. Note that in the strain that contains endogenous Rrp44 and overexpressed rrp44-Δexo (panel F lane 3) the band intensities for the two proteins are approximately equal even though the truncated Rrp44 likely lacks many of the epitopes recognized by the polyclonal αRrp44 antisera. **(E)** Overexpression of catalytically inactive endonuclease domain (*rrp44-endo-*Δ*exo*) does not cause a dominant negative phenotype.

A

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ScRrp44     GQLAPSSVD-PQSSSTQNVFVILMDKCLPKVRIRTRRAAELLDKRI 450
SpDis3      GHVDNATIAQSKGGSQQTVLLTPMDRRVPKIRFRTRQAPRLVGRRI 421
hDis3       GMLSKSDIK-----ESRRHLFTPADKRIPRIRIETRQASTLEGRRI 398
hDis3L      GMLSKSDIK-----ESRRHLFTPADKRIPRIRIETRQASTLEGRRI 368
DmDis3      GILQPSLIE-----DTNRHIFVPADRKIPRIRIETRQAAMLQNQRI 405
DmDis3L     GILQPSLIE-----DTNRHIFVPADRKIPRIRIETRQAAMLQNQRI 405

ScRrp44     LGHFVRDLGTIESAQAETEALLLEHDVEYRPFSKKVLECLPAEGHD 509
SpDis3      EGHFVRDLGEMETKEAETEALLLEYDVQHRPFPKAVLDCLPEEGHN 481
hDis3       NGHFVRNLGDVGEKETETEVLLLEHDVPHQPFSQAVLSFLP----- 453
hDis3L      NGHFVRNLGDVGEKETETEVLLLEHDVPHQPFSQAVLSFLP----- 423
DmDis3      HGHFVRSLGPLGDMATENEVILLEHDVPHCKFSDEVLSFLP----- 460
DmDis3L     HGHFVRSLGPLGDMATENEVILLEHDVPHCKFSDEVLSFLP----- 460

ScRrp44     DAEGAARGTSVYLVDKRIDMLPMLLGTDLCSLKPYVDRFAFSVIWE 629
SpDis3      DSEAASRGTTVYLVDKRIDMLPMLLGTDLCSLRPYVERFAFSCIWE 594
hDis3       DQESARRGTTVYLCEKRIDMVPELLSSNLCSLKCDVDRLAFSCIWE 565
hDis3L      DQESARRGTTVYLCEKRIDMVPELLSSNLCSLKCDVDRLAFSCIWE 535
DmDis3      DMEAAARGTTVYLVGKRIDMVPELLSSNLCSLVGGVERFAFSCVWE 572
DmDis3L     DMEAAARGTTVYLVGKRIDMVPELLSSNLCSLVGGVERFAFSCVWE 572
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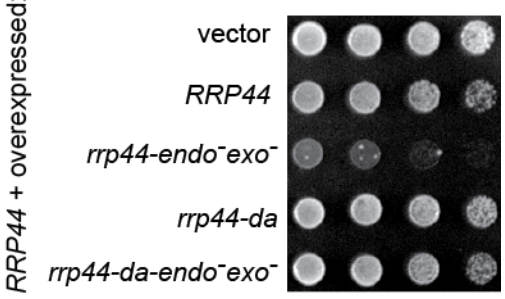
B



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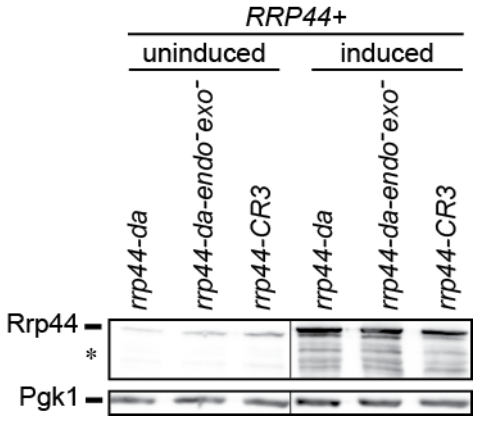

Supplemental figure S2 (related to figure 1). **A.** Sequence alignment of part of Rrp44 with homologs from *Schizosaccharomyces pombe* (SpDis3), human (hDis3 and hDis3L) and *Drosophila melanogaster* (DmDis3 and DmDis3L). Blue and purple boxes highlight residues in the CSD2 domain and RNB domain, respectively, that are mutated in the *rrp44-da* allele. **B.** *rrp44-da-endo⁻exo⁻* is not dominant negative when overexpressed. This is consistent with the results from co-immunoprecipitation that show that the *rrp44-da* mutation affects interaction with the RNA exosome core. Wild-type yeast strains carrying *RRP44* variants under a galactose inducible promoter were serially diluted and spotted on solid media containing galactose. No growth defect was observed on media containing glucose (data not shown). **C.** The *rrp44-da*, and *rrp44-da-endo⁻exo⁻* mutants were successfully overexpressed. The black line between the third and fourth lane indicates that a lane containing an irrelevant mutant was cut from the image. The lanes shown are from the same blot.

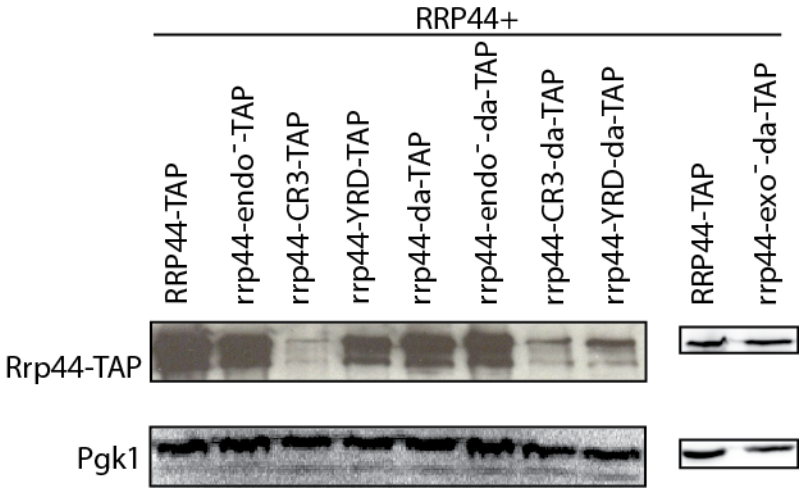

Figure S3: related to figures 2 and 3. The lethality of *rrp44-CR3-da*, *rrp44-yrd-da*, and *rrp44-exo⁻-da* is not correlated with their expression level. Specifically, the lethal *rrp44-CR3-da-TAP*, and *rrp44-yrd-da-TAP* expressed at higher levels than *rrp44-CR3-TAP*, the slow growing *rrp44-endo⁻-da-TAP* is expressed similar to either single mutant (*rrp44-endo⁻-TAP* and *rrp44-da-TAP*) and the lethal *rrp44-exo⁻-da-TAP* is expressed similar to wild-type *RRP44-TAP*.

SUPPLEMENTAL REFERENCES CITED

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