

## **Supplementary Materials**

### **Supplementary Methods**

#### **Phylogenetic analysis**

43 amino acid sequences of Dis3, Dis3L and Dis3L2 proteins, including the SPAC2C4.07c gene product, used in the phylogenetic analyses, were obtained from NCBI Entrez and the Genome Portal of the Department of Energy Joint Genome Institute (Grigoriev et al, 2012) and are described in Supplementary Table 3.

CLANS (Frickey & Lupas, 2004) was used to visualize the relationships between different Dis3 proteins according to their pairwise sequence similarities with P-value cutoff at  $10^{-10}$ .

Sequences were aligned using SeaView (Gouy et al, 2010). To estimate phylogenetic relationships the protein sequences were analysed using the Neighbour Joining (NJ), Maximum Parsimony (MP) and Bayesian inference (BI) methods. NJ analysis was performed using BioNJ (Gascuel, 1997) with the Kimura or Poisson distance and 1000 bootstrap replicates. For MP analysis PAUP\* (Swofford, 2003) version 4.0b10 was used, with 1000 bootstrap replicates to assess the reliability of nodes. Consensus tree (50%) with bootstrap support values was calculated using the SumTrees program of the DendroPy package version 3.12.0 (Sukumaran & Holder, 2010). Bayesian inference analysis was performed using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003) with 500 000 generations of each MCMC search sampled every 10 generations, and mixed fixed-rate models. Burnin values corresponded to 25% of the samples. Trees were visualized using Dendroscope (Huson et al, 2007).

#### **Microarray analysis**

Yeasts were grown up to early exponential phase at YES media and total RNA was extracted as described. The quality was assessed with a BioAnalyzer (Agilent Technology). RNA was processed for use on GeneChip Yeast Genome 2.0 Array from Affymetrix, according to the manufacturer's protocol. Three independent biological samples were analysed for each strain. Hybridization, scanning and detection procedures were done at the Genomics Unit of the Instituto Gulbenkian de Ciência.

Subsequent exploration, normalization, summarization and analyses of the generated Affymetrix CEL files were performed using R free statistical software (<http://cran.r-project.org/>) and its associated tool for high-throughput genomic data, Bioconductor (<http://www.bioconductor.org/>).

The reliability of the data set, before and after normalization, was estimated through its statistical exploration. For each strain, the summarized probe set intensities were calculated using the Robust MultiArray Averaging (RMA) method (Bolstad et al, 2003; Irizarry et al, 2003). The empirical Bayes statistics was used to analyze the data because it provides a robust estimate of variance for each gene. It indeed assumes that genes expressed at similar levels exhibit similar variance which leads to the smoothing of standard errors associated to the fold-change logs (Hatfield et al, 2003). The multiple testing issue was furthermore taken into account through the calculation of the False Discovery Rate (FDR) according to Benjamini-Hochberg method (Benjamini et al, 2001).

#### **pFA6a TAP Kan MX6 plasmid construction**

GFP sequence was removed from the pFA6a GFP kan MX6 vector using restriction enzymes PacI AscI (Fermentas). The TAP tag sequence was then cloned into the plasmid using SLIC method according to the protocol (Li & Elledge, 2007). TAP tag sequence was amplified using pJL72 vector template. Primers used are listed in Supplementary Table 2.

#### **Dis3L2 protein purification**

Proteins were expressed in BL(21) pRIL cells grown in full media at 37°C until OD<sub>600</sub> 0.6 and then expression was induced for overnight at 20°C, subsequently cells were harvested, resuspended in lysis buffer (300mM NaCl, 1mMDTT, 50mM Phosphate buffer pH 7) and lysed using French press. Lysates were clarified by ultracentrifugation and protein purification was performed using GST-trap column according to manufacturer's instructions (GE Healthcare). The proteins were additionally purified using cation exchange column (HiTrap SP HP) according to manufacturer's instructions (GE Healthcare). After purification the proteins were concentrated to about 100 µg/µl using viva spin 500 columns (Sartorius stedim). The concentration of proteins was monitored using Bradford method and SDS-PAGE gels. Protein solutions were finally diluted until 50% in glycerol and kept at -80°C.

### **Substrate labeling**

For all assays described in the text HPLC, purified RNA or DNA oligonucleotides were used (<http://www.stabvida.net/>). Oligonucleotides were 5' labeled with radioactive phosphate using PNK (Fermentas) and  $\gamma\text{P}^{32}\text{ATP}$  and the substrate purified using G-50 columns (GE Healthcare). Substrates were then added to the reactions directly or supplemented with a known concentration of the non-labeled oligonucleotide. To create double-stranded substrates a complementary non-labeled DNA oligonucleotide was mixed with the radioactive oligo in a 2:1 molar ratio, samples were then denatured and cooled down slowly for annealing. Integrity of the double-stranded substrates was checked using native acrylamide gels (Malecki et al, 2010).

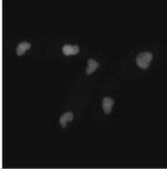

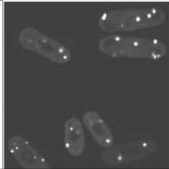
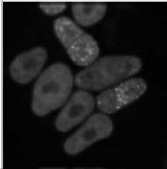
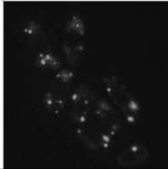
For the synthesis of the internally labeled substrate, in vitro transcription was carried out using the purified PCR product as template in the presence of an excess of  $[\text{32P}]\text{-}\alpha\text{-UTP}$  over unlabeled UTP with 'Riboprobe in vitro Transcription System' (Promega) and T7 RNA polymerase. Radioactive transcripts were purified by electrophoresis on an denaturing polyacrylamide gel. The gel slice was crushed and the RNA eluted with elution buffer (3 M ammonium acetate pH 5.2, 1mM EDTA, 2.5% (v/v) phenol pH 4.3), overnight at room temperature. The RNA was ethanol precipitated and resuspended in RNase free water.

### **RNA isolation**

Cells were thawed on ice, resuspended in the cold AES buffer (0.5 % SDS, 50mM NaAc pH 5.2, 10mM EDTA) and then transferred to the tubes containing phenol solution and glass beads (Sigma 425-600  $\mu\text{m}$ ). Cells were lysed in phenol using FastPrep-24 equipment (MP Biomedicals) – 3 times maximum speed for 25 s. Subsequently the solution was incubated in  $65^{\circ}\text{C}$  for 30 min with vortex every 10 min, than centrifuged and aqueous fraction was transferred to the fresh tube with phenol. Solution was vortex for 5 min, centrifuged and the aqueous fraction was transferred to the fresh tube with chlorophorm: isoamyl alcohol solution (24:1). Solution was vortex for 5 min, centrifuged and aqueous fraction was transferred to the fresh tube. Subsequently NaAc (pH 5.3) was added to 300 mM and RNA was precipitated with ethanol.

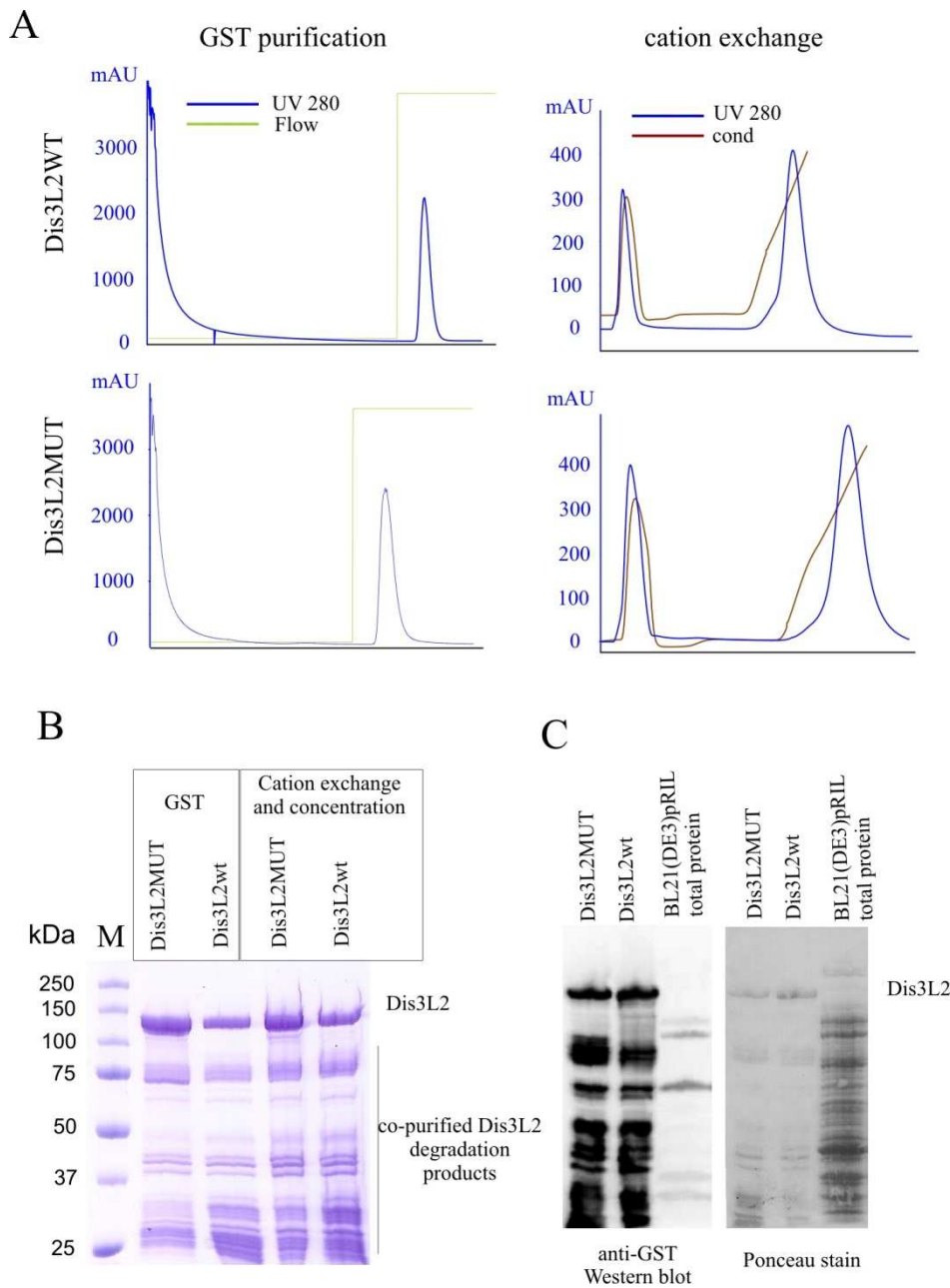
## Supplementary Figures

### Supplementary Figure 1

SPBC26H8.10	SPAC2C4.07c	SPBC609.01	SPCC16C4.09	SPCC23B6.06	systematic name
					localisation of overexpressed GFP fusion (data from RIKEN www.riken.jp/SPD/)
inviable	viable	inviable	viable	viable	deletion phenotype
Dis3	-	-	Ssd1	Dss1	<i>S. cerevisiae</i> orthologs
yes	yes	no	no	yes	conservation of exoribonucleolytic active site

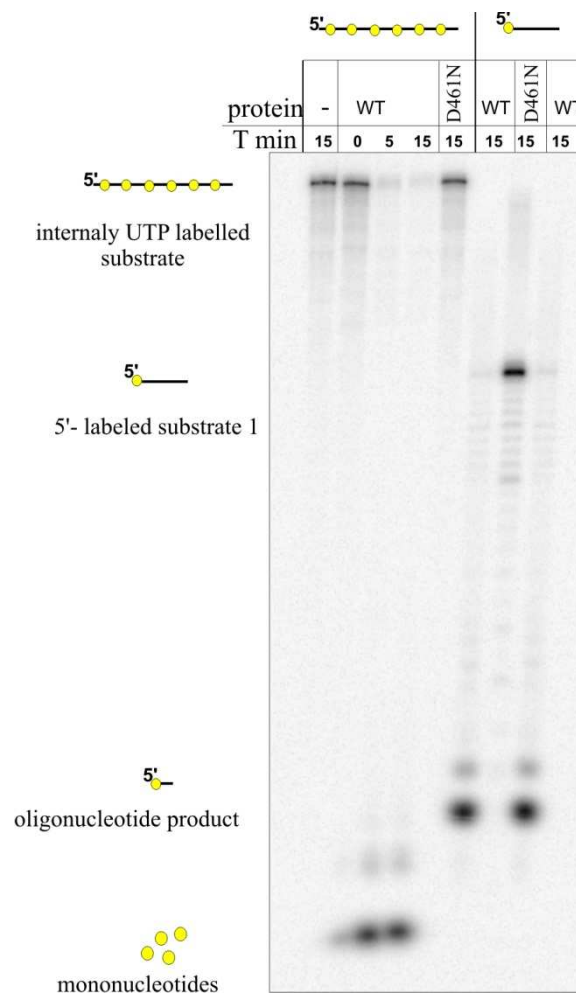
**Figure S1** Listed *S. pombe* genes that encode proteins with RNase II/R domain, with localization data from (Matsuyama et al, 2006). Phenotype and budding yeast homologue information from Pombase (<http://www.pombase.org/>) (Wood et al, 2012).

## Supplementary Figure 2



**Figure S2** Dis3L2 purification. **(A)** GST fusions of Dis3L2 wild type (Dis3L2WT) and mutated version (Dis3L2MUT) were purified using GST affinity chromatography followed by cation exchange chromatography. **(B)** SDS-PAGE analysis of purification products. The size of the full length Dis3L2 protein is indicated on the right. Preparations obtained after cation exchange and protein concentration were used in the assays. **(C)** Purified protein preparations were subjected to Western blot analysis against GST to investigate the nature of lower molecular weight contaminants. Contaminations consisted on Dis3L2-GST protein degradation by-products as indicated in the picture. The total protein extract from the bacteria used for protein expression was used (BL21(DE3)pRIL) as control. The weaker signal of target protein in Western blot is due to signal saturation.

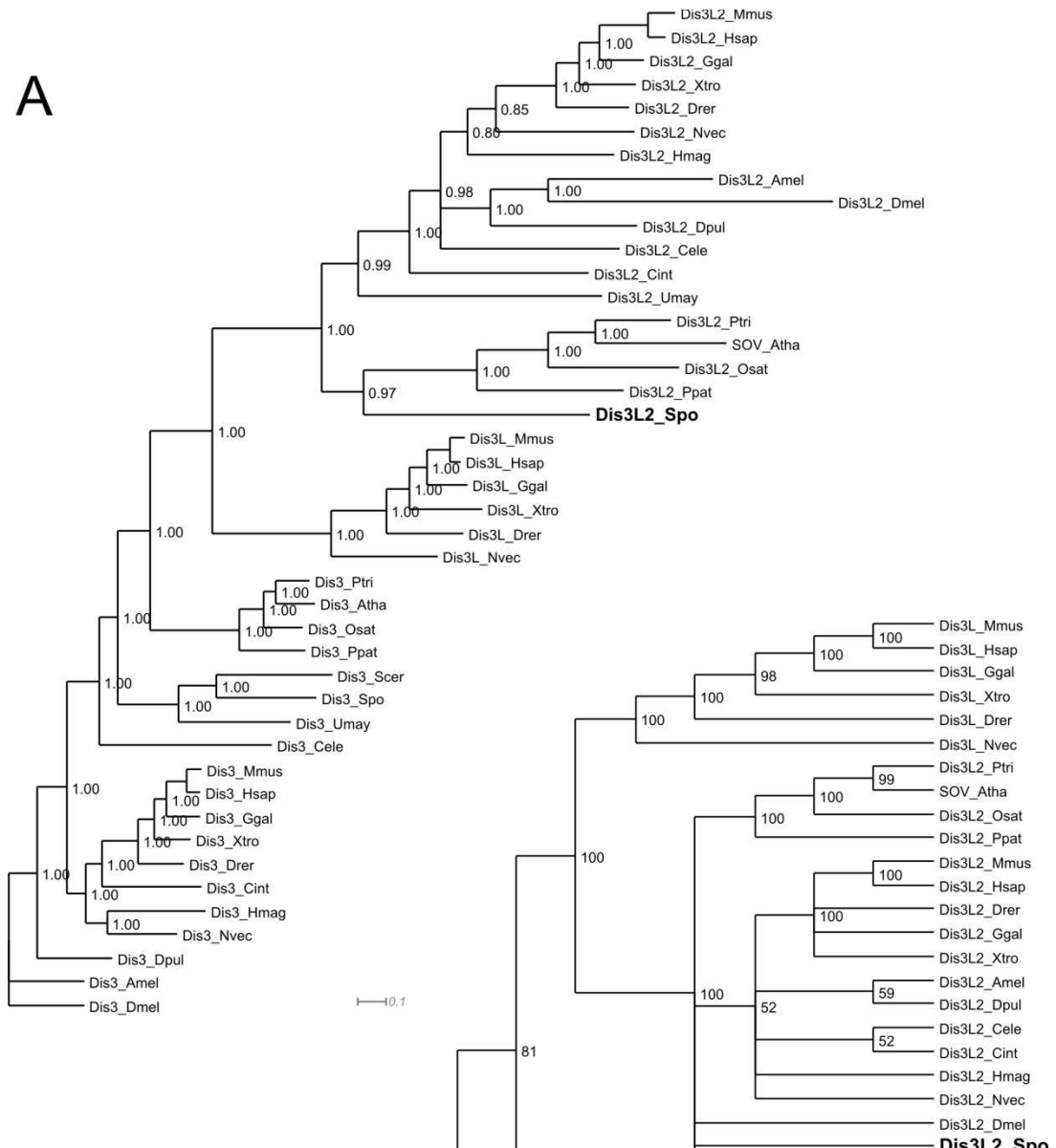
### Supplementary Figure 3



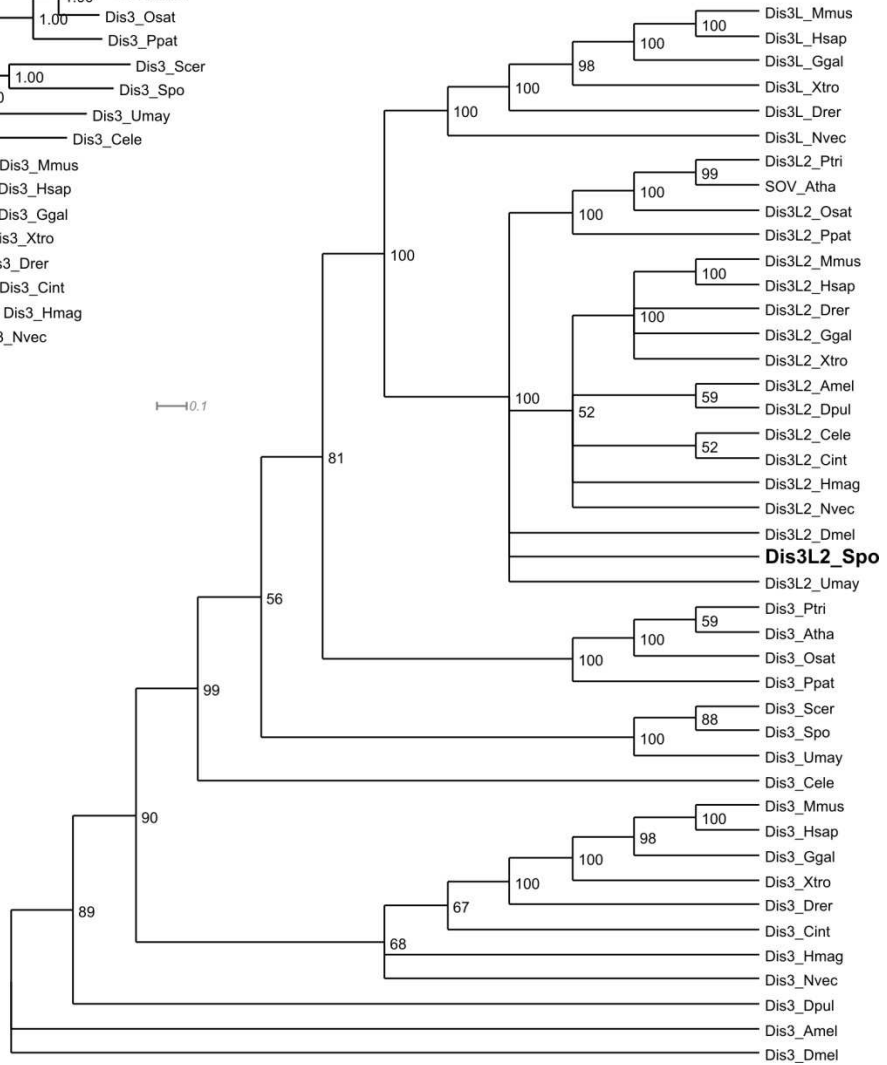
**Figure S3** Dis3L2 is a processive 3-5' exonuclease. Around 0.5 pmols of purified wild type protein Dis3L2 (WT) and its mutated version (D461N) were incubated with 0.2 pmols of either an internally labeled RNA substrate (see Supplementary Table 2) or a 5'-end labeled substrate (substrate 1). Reactions were stopped at the indicated times and products were separated on denaturing polyacrylamide gel. The size of the different substrates and reaction products are indicated.

Supplementary Figure 4

A



B



C

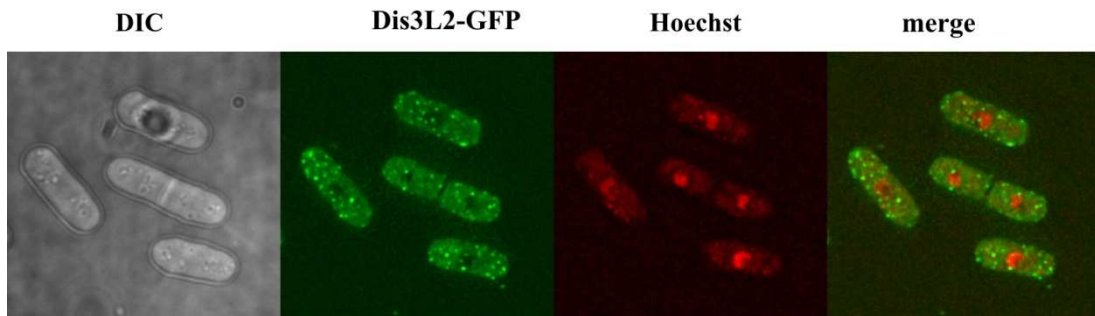
						10							
<i>Dis3_gi 328786997_Apis_mallifera/455-467</i>	P	P	G	C	T	D	I	D	D	A	L	H	C
<i>Dis3_gi 24649634_Drosophila_melanogaster/487-499</i>	P	P	G	C	T	D	I	D	D	A	L	H	C
<i>Dis3_gi 321478602_Daphnia_pulex/477-489</i>	P	P	G	C	T	D	I	D	D	A	L	H	F
<i>Dis3_gi 198429347_Ciona_intestinalis/452-464</i>	P	P	G	C	T	D	I	D	D	C	L	H	H
<i>Dis3_gi 190014623_Homo_sapiens/480-492</i>	P	P	G	C	T	D	I	D	D	A	L	H	C
<i>Dis3_gi 145207992_Mus_musculus/480-492</i>	P	P	G	C	T	D	I	D	D	A	L	H	C
<i>Dis3_gi 118084745Gallus_gallus/483-495</i>	P	P	G	C	T	D	I	D	D	A	L	H	C
<i>Dis3_gi 171847251_Xenopus_tropicalis/475-487</i>	P	P	G	C	T	D	I	D	D	A	L	H	C
<i>Dis3_gi 326664018_Danio_reiro/477-489</i>	P	P	G	C	T	D	I	D	D	A	L	H	C
<i>Dis3_ 10544 gw.129.26.1_Nematostella_vactensis/478-490</i>	P	P	G	C	T	D	I	D	D	A	L	H	W
<i>Dis3_gi 221101666_Hydra_magnipapillata/470-482</i>	P	P	G	C	T	D	I	D	D	A	L	H	W
<i>Dis3_gi 212645896_Caenorhabditis_elegans/492-504</i>	P	L	G	C	T	D	I	D	D	A	L	H	C
<i>Dis3_gi 224071355_Populus_trichocarpa/484-496</i>	P	P	G	C	K	D	I	D	D	A	L	H	C
<i>Dis3_gi 18398450_Arabidopsis_thaliana/482-494</i>	P	P	G	C	K	D	I	D	D	A	L	H	C
<i>Dis3_gi 222624135_Oryza_sativa/469-481</i>	P	P	G	C	R	D	I	D	D	A	L	H	C
<i>Dis3_gi 168027129_Physcomitrella_patens/501-513</i>	P	L	G	C	R	D	I	D	D	A	L	H	C
<i>Dis3_gi 6324552_Saccharomyces_cerevisiae/544-556</i>	P	P	G	C	V	D	I	D	D	A	L	H	A
<i>Dis3_gi 19113445_Schizosaccharomyces_pombe/509-521</i>	P	P	G	C	Q	D	I	D	D	A	L	H	A
<i>Dis3_gi 154340223_Leishmania_braziliensis/410-422</i>	P	L	G	C	R	D	I	D	D	A	L	H	C
<i>Dis3L_gi 219521928_Homo_sapiens/479-491</i>	P	K	G	C	E	D	V	D	D	T	L	S	V
<i>Dis3L_gi 295293138_Mus_musculus/479-491</i>	P	K	G	C	E	D	V	D	D	T	L	S	V
<i>Dis3L_gi 363737633_Gallus_gallus/476-488</i>	P	K	G	C	E	D	V	D	D	A	L	S	V
<i>Dis3L_gi 1118403694_Xenopus_tropicalis/394-406</i>	P	K	G	C	E	D	V	D	D	A	L	S	I
<i>Dis3L_gi 160333118_Danio_reiro/473-485</i>	P	K	G	C	E	D	V	D	D	T	L	S	V
<i>Dis3L_gi 156382055_Nematostella_vactensis/479-491</i>	P	K	G	C	E	D	V	D	D	T	L	S	I
<i>Dis3L2_gi 17553506_Caenorhabditis_elegans/315-326</i>	P	K	T	A	R	D	L	D	D	A	L	H	-
<i>Dis3L2_gi 198421184Ciona_intestinalis/403-414</i>	P	P	S	A	R	D	L	D	D	A	L	H	-
<i>Dis3L2_gi 134288890_Homo_sapiens/384-395</i>	P	S	T	A	R	D	L	D	D	A	L	S	-
<i>Dis3L2_gi 24233556_Mus_musculus/382-393</i>	P	S	T	A	R	D	L	D	D	A	L	A	-
<i>Dis3L2_gi 363737173_Gallus_gallus/384-395</i>	P	S	T	A	K	D	L	D	D	A	L	S	-
<i>Dis3L2_gi 118404918_Xenopus_tropicalis/355-366</i>	P	A	T	A	R	D	L	D	D	A	L	S	-
<i>Dis3L2_gi 292610486_Danio_reiro/348-359</i>	P	A	T	A	R	D	L	D	D	A	L	S	-
<i>Dis3L2_gi 156388005_Nematostella_vactensis/301-312</i>	P	L	T	A	R	D	L	D	D	A	L	H	-
<i>Dis3L2_gi 321453433_Daphnia_pulex/426-438</i>	P	A	D	A	R	D	L	D	D	A	V	S	G
<i>Dis3L2_gi 221123214_Hydra_magnipapillata/273-284</i>	P	A	T	A	R	D	L	D	D	A	V	S	-
<i>Dis3L2_gi 328786136_Apis_mallifera/301-312</i>	P	D	A	A	V	D	L	D	D	S	V	S	-
<i>Dis3L2_gi 24654592_Drosophila_melanogaster/573-584</i>	P	M	T	A	R	D	L	D	D	A	V	S	-
<i>Dis3L2_gi 19115422_Schizosaccharomyces_pombe/454-465</i>	P	E	T	A	R	D	L	D	D	A	V	S	-
<i>Dis3L2_gi 224066863_Populus_trichocarpa/405-416</i>	P	S	S	A	T	D	L	D	D	A	L	S	-
<i>Dis3L2_gi 15220899_Arabidopsis_thaliana/489-500</i>	P	S	T	A	T	D	L	D	D	A	L	S	-
<i>Dis3L2_gi 115448745_Oryza_sativa/476-487</i>	P	P	T	A	T	D	L	D	D	A	I	S	-
<i>Dis3L2_gi 168042407_Physcomitrella_patens/481-492</i>	P	P	T	A	R	D	L	D	D	A	L	S	-
<i>Dis3L2_gi 154340243_Leishmania_braziliensis/320-332</i>	P	A	T	A	R	D	L	D	D	A	L	S	I

**Figure S4** Phylogenetic analyses of the Dis3 homologues in eukaryotes. Three distinct groups, corresponding to Dis3, Dis3L and Dis3L2 are apparent in all trees, and the SPAC2C4.07c gene product of *S. pombe* (Dis3L2\_Spo) is consistently classified with the Dis3L2 group. (A) 50% majority-rule consensus tree from 1000 bootstrap replicates of a maximum parsimony (MP) analysis, bootstrap support values shown for nodes. (B) Bayesian inference (BI) tree, with posterior probabilities of each node. (C) Conservation of active site consensus between different Dis3 like proteins.

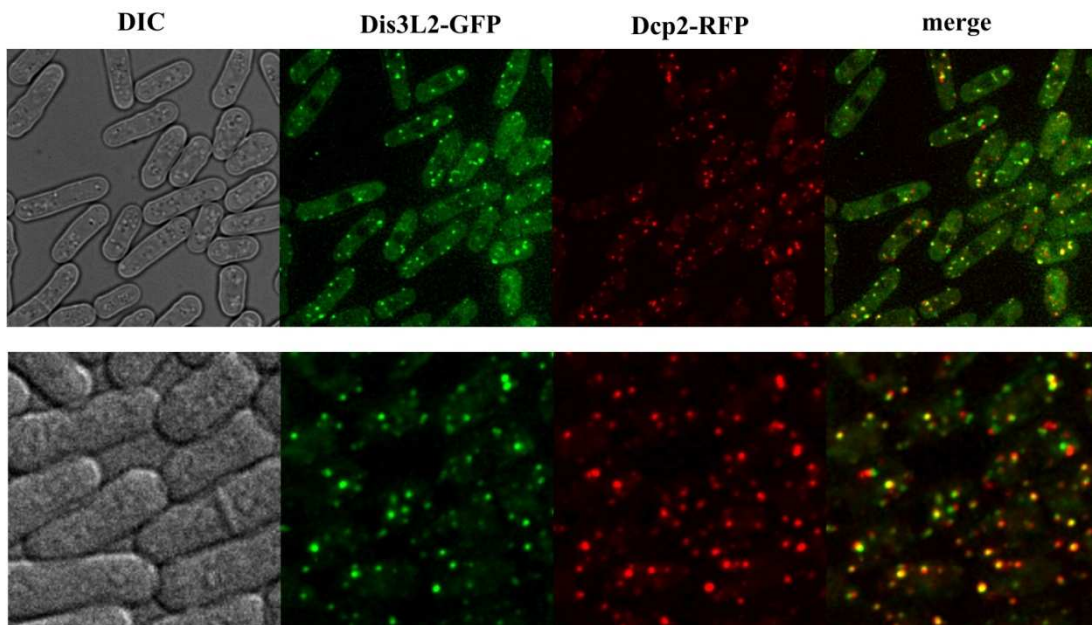


## Supplementary Figure 5

A

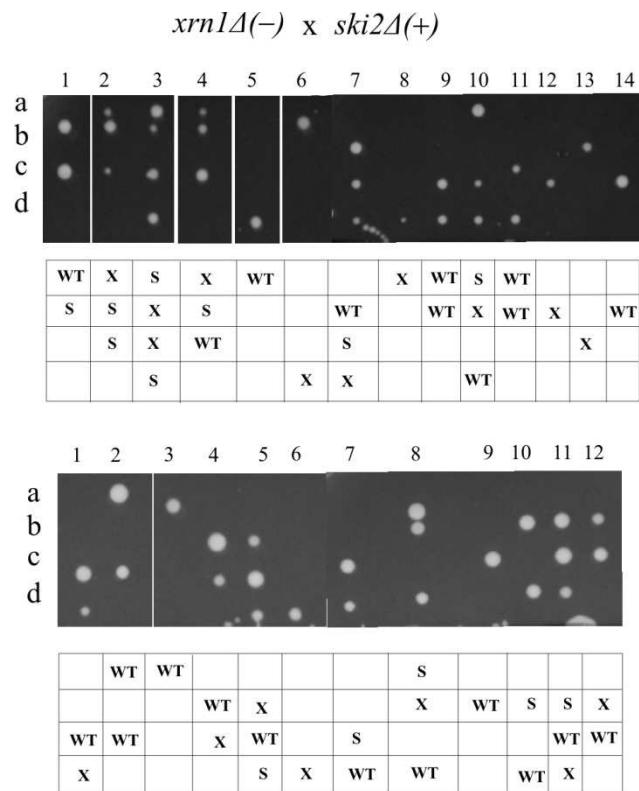


B



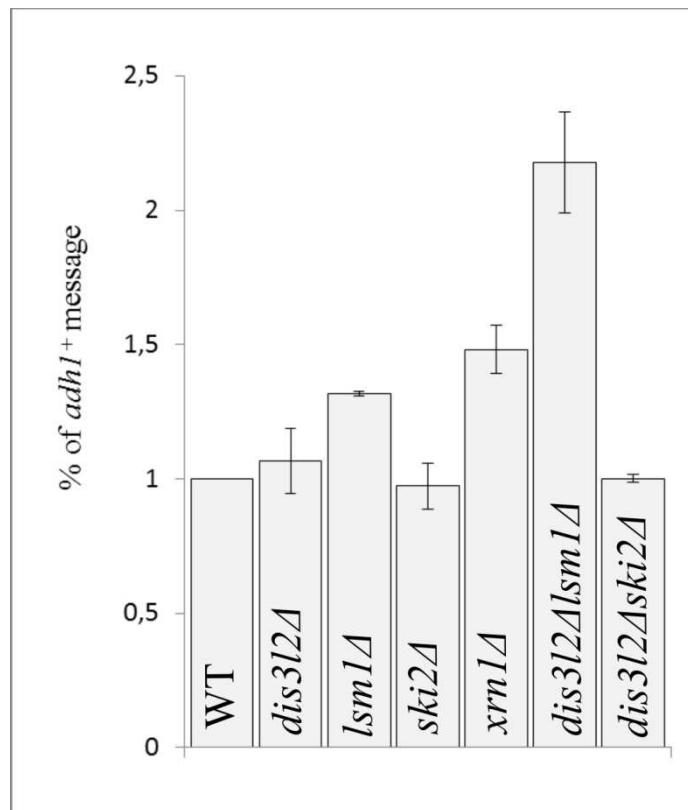
**Figure S5** Dis3L2 localizes in the cytoplasm and cytoplasmic foci. **(A)** Cells expressing Dis3L2-GFP were grown to mid-log phase in minimal medium (EMM) and the localization of epitope tagged protein was determined by fluorescence microscopy. Nucleus were stained with Hoechst and its co-localisation with Dis3L2-GFP signal was examined. **(B)** Dis3L2-GFP was examined for co-localization with Dcp2-RFP. Cells expressing Dis3L2-GFP and Dcp2-RFP were grown to mid-log phase in minimal medium (EMM) and then immediately subjected to microscopy.

**Supplementary Figure 6**



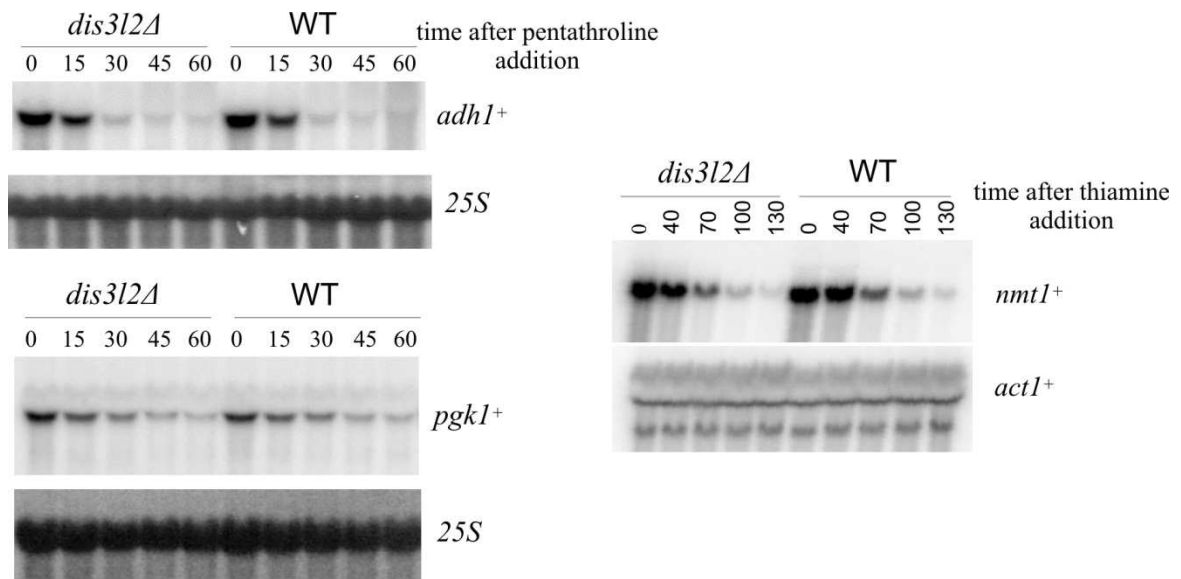
**Figure S6** *xrn1<sup>+</sup>* deletion is synthetically lethal with deletion of *ski2<sup>+</sup>*. Haploid *ski2<sup>+</sup>::hph* cells were crossed with *xrn1<sup>+</sup>::hph* strain. Resulting diploids were sporulated and tetrads were dissected on YES plates. Genotypes of the spores were analyzed by colony PCR and are described in the bottom tables: WT- wild type strain, X- *xrn1Δ*, S- *ski2Δ*.

### Supplementary Figure 7



**Figure S7** *adh1*<sup>+</sup> transcript levels accumulates in a *dis3l2*Δ*lsm1*Δ background. Graph represents Northern blot results of *adh1*<sup>+</sup> mRNA analysis over total RNA from the wild type and different deletion mutant strains, from three independent experiments. Error bars represent standard deviation.

## Supplementary Figure 8



**Figure S8** No detectable difference was observed in mRNAs degradation rates between wild type yeast strain (WT) and *dis3l2Δ* mutant. Wild type and *dis3l2Δ* strains were grown in the full media (for *adh1*<sup>+</sup> and *pgk1*<sup>+</sup>) or minimal media (for *nmt1*<sup>+</sup>) until mid-log phase, subsequently transcription was stopped by either 1,10-pentanthroline (for *adh1*<sup>+</sup> and *pgk1*<sup>+</sup>) or thiamine (for *nmt1*<sup>+</sup>) addition. Cells were harvested at the indicated time points after transcriptional arrest, total RNA was isolated and mRNA decay analyzed by Northern blot.

## Supplementary Figure 9

### WT

CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (15)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (28)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA 21  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (31)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (19)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (21)  
CCCCAAAAAAAAAAAAAAAAAAAAAAT (30)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (27)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (21)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (31)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (19)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (31)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (14)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAAT (17)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAAT (14)

### *dis3l2Δ*

CCCATCCTTTAAAAAAAAAAAAAAAAAAAAAT (23)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (34)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (49)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAAT (29)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (23)  
CCCATCCTTTAAAAAAAAAAAAAT (12)  
CCCCAAAAAAAAAAAAAAAAAAAAA (15)  
CCCATCCTTTAAAAAAAAAAAAAT (13)  
CCCCAAAAAAAAAAAAAAAAAAAAA (25)  
CCCCAAAAAAAAAAAAAT (9)  
CCCATCCTTTAAAAAAAAAAAAAT (14)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAA (18)

### *lsm1Δ*

CTTTTCTTTTCTCCTCCTCCTCGTTCAT (-171)  
CAAACCATCTCGGGGTTAGAGT (-246)  
CCCATCCTTTAAAAAAAT (7)  
CCCATCCTTTAAAAAAAAAAAAATTT (14)  
CCCATCCTTTAAAAAAAAAAAAATTT (12)  
CCCATCCTTTAAAAAAAAAAAAAT (10)  
CCCATCCTTTAAAAAAAT (6)  
CCCATCCTTTAAAAAAAAAAAA (10)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAA (26)  
CCCATCCTTTAAAAAAAAAAAAAT (10)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAA (26)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAA (18)  
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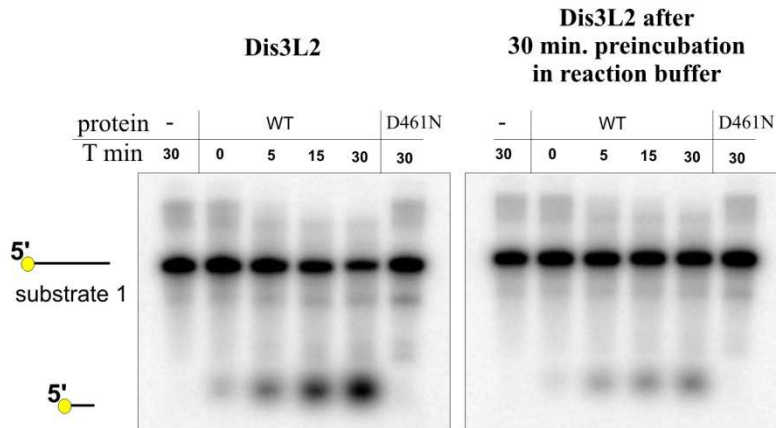
### *dis312Δ lsm1Δ*

TGTTTTGTATAGAAATCAATGTTTT (-20)  
GGACGATTGTACCTTTGAAAATTTT (-55)  
GATTGTACCTTTGAAAACCAAATTT (-52)  
GGACGATTGTACCTTTGAAAACCAACT (-50)  
TGTTTTGTATAGAAATCAATGT (-20)  
TGTTTTGTATAGAAATTTTT (-25)  
CCTTTGAAAATTTT (-55)  
TTTTGCATGTTTTTTTATT (-35)  
CCCATCCTTTAAAAAAAAAAAAAAAAAAAAATTT (18)  
CAACTACTTTTGCATGTTTT (-34)  
CCCATCCTTTAAAAAAAAGAAAAAAATTT (17)  
TTAGAATCCCATTT (-4)  
CAACTACTTTTGCATGTTTT (-34)  
TTTTGTATAGAAATC (-23)  
TTAGAATCCCATTT (-4)  
TTTTGCATGTTCTTTTTT (-36)  
TTAGAATCCCATTTTTTTTTT (-4)  
CCCATCCTTTAAAAAAATTT (8)

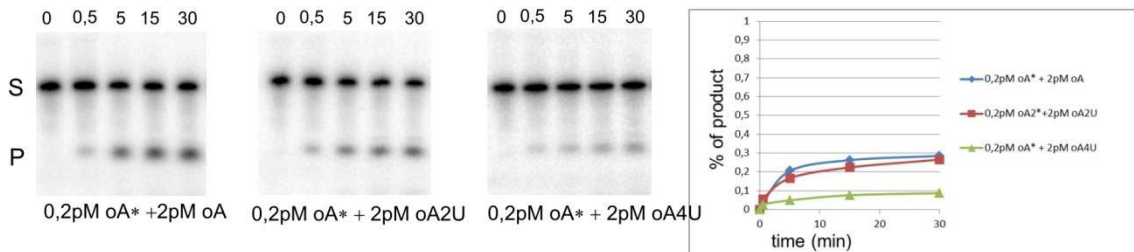
**Figure S9** Sequences of 3'-ends of *adh1*<sup>+</sup> transcripts obtained in 3' RACE experiment from different yeast strains. The non-genome encoded thymines are colored in red. Length of poly(A) tail is represented by the positive values in the brackets, and the extension of the 3'-end trimming is represented by the negative value. Nucleotides were counted from polyadenylation site (zero point).

## Supplementary Figure 10

A



B



**Figure S10 (A)** Dis3L2 loses its activity over time in the reaction conditions. 0.5 pmols of Dis3L2 or mutated protein version (D461N) were incubated with 2pmols of 5' labeled substrate 1 for 30 minutes or first pre-incubated 30 minutes in reaction buffer in 30°C and subsequently supplemented with the substrate and incubated another 30 minutes. Reactions were stopped at indicated times and products were separated on denaturing polyacrylamide gels **(B)** Uracil residues added to the 3' end can effectively target RNA substrates for degradation by Dis3L2 *in vitro*. Addition of non-labeled uridylated substrates inhibits degradation of labeled adenylated RNAs. Equal amounts of Dis3L2 protein were incubated with the indicated amounts of different RNA substrates (RNA oligonucleotides sequence in Fig.7 and Supplementary Table 1). Reactions with radioactive substrates (labeled with \*) were supplemented with non-labeled oligonucleotides. Reactions were stopped at the indicated time points (top of the gels) and separated on denaturing polyacrylamide gels. Graphs on the right side depict the accumulation of the reaction product at different time points as calculated using Image Quant.

## Supplementary Tables

**Supplementary Table 1** Strains used in this study

Strain	Genotype	Creator
MGF10	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18</i>	J Cooper
MGF11	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18</i>	J Cooper
MGF1	<i>h-</i>	
MGF2	<i>h+</i>	
MGF3	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18 dis312::KanMX4</i>	Bioneer
MM1	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18 dis312::dis312-GFP-KanMS6</i>	this study
MM2	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18 dis312::dis312-GFP-KanMS6 dcp2::dcp2-mRFP-hph</i>	this study
MM3	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18 dis312::dis312-GFP-KanMS6 pabp::pabp-mRFP-hph</i>	this study
MM4	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18 dis312::dis312-GFP-KanMS6 dis3::dis3-mRFP-hph</i>	this study
MM5	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18 dis3::dis3-GFP-KanMS6</i>	this study
MM6	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18 rrp43::rrp43-GFP-KanMS6</i>	this study
MM7	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18 dis3::dis3-TAPtag-KanMS6</i>	this study
MM8	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18 rrp43::rrp43-TAPtag-KanMS6</i>	this study
MM9	<i>h- ade6-M210 his3-D1 leu1-32 ura4-D18 dis312::dis312-TAPtag-KanMS6</i>	this study
MM10	<i>h- dis312::KanMS6</i>	this study
MM11	<i>h- dis312::hph</i>	this study
MM12	<i>h+ dis312::hph</i>	this study
MM13	<i>h- xrn1::hph</i>	this study
MM14	<i>h+ xrn1::hph</i>	this study
MM15	<i>h- dis312::KanMS6 ski2::hph</i>	this study
MM16	<i>h- ski2::hph</i>	this study
MM17	<i>h- dis312::KanMS6 lsm1::hph</i>	this study
MM18	<i>h- lsm1::hph</i>	this study
MM19	<i>h- dcp2::dcp2-GFP-KanMS6</i>	this study
MM20	<i>h- dis312::hph dcp2::dcp2-GFP-KanMS6</i>	this study



**Supplementary Table 2** Oligonucleotides and substrates used in this study.

RNA substrates	
Substrate 1	GUUUUGUAUAGAAAUCA AUG
Substrate 2	CCCGACACCAACCACUAAAAAAAAAAAAAAAA
oA	CACCAACCACUAAAAAAAAAAAAAAAA
oA2U	CACCAACCACUAAAAAAAAAAAAAAAAU
oA4U	CACCAACCACUAAAAAAAAAAAAUUUU
Poli(U)	UUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUU
Poli(A)	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Internally labeled substrate	GAAAUAAUUUGUAAUUGCAACUAAUUUUCUGUAACAUAGUGUGAUUAAUUUU CAGUAGGGGUUUAUAAAAUUGAAGGAUAAGAUUAUUGAUUAACGCAA UAACCUAAUUUUCAGAG
DNA oligonucleotides	
Substrate3 (DNA)	AGTGGTTGGTGTCTCGG
18SrRNAprobe	ACCAGACTTGCCCTCCAATTGTTCC
25SrRNAprobe	ACCTTGGAGACCTGCTGCGGTTATG
Primers	
L_fba1_probe	CATCCCTGCCATTAACGTCAC
R_fba1_probe	GACGTAGGCGAATTGGGTATC
L_pabp_probe	TACTCGCCGTTCTTTGGG
R_pabp_probe	ACCTGCTTCTGCTTTCC
L_hsc1_probe	AGGGCAATGCTCGTCCTAC
R_hsc1_probe	GAGGCAGCCAAACCAGAAC
L_pyk1_probe	CTCCGTGATGCTGGTATG
R_pyk1_probe	GCTGGTGGACAAGACAAC
L_pgk1_probe	CACCAACAATGCCCGTATCG
R_pgk1_probe	TCCACAGTCCAAACCCATCC
L_act1_probe	CTATGTATCCCGGTATTGCC
R_act1_probe	GGAGCTTAGAAGCACTTACG
L_rrg1_probe	CACGAACAGCCTTCTTACTC
R_rrg1_probe	GAGCCAAAGTCTTCTTACC
L_adh1_probe	CACCGATTTACACGCTCTTC
R_adh1_probe	GAAAGGTCCAAGACGATACG
L_pik1_probe	GCTGGTAAAAATGTTGTTAC
R_pik1_probe	TAGTAAATTCCGTTCCG
L_nmt1_probe	TATGAGCGTGAAGGGATTGAG
R_nmt1_probe	CGACATAACGAAGTGAGTTGC
1 <sup>st</sup> _adh_PASE	CAAGATTGCCGCGCGTATCG
2 <sup>nd</sup> _adh_PASE	TTTCACCACACGTTTATACC
L_TAPtagSLIC	CGCTGCAGGTCGACGGATCCCCGGGTTAATTAACATGG AAAAGAGAAGATGGA
R_TAPtagSLIC	TAAGAAATTCGCTTATTTAGAAGTGCGCGCCTCAGGT TGACTTCCCCGCGG
L_mut_Dis3l2	CAGCTCGAGACTTGAATGATGCTGTTTC
P_mut_Dis3l2	GAAACAGCATCATTCAAGTCTCGAGCTG
L_clon_Dis3l2	ATGAATTCATGGATTTAAAACCAAATATTAG
P_clon_Dis3l2	TACTCGAGTTAATTCAAAGAACTAGAC

\*all the primers used for *S. pombe* gene deletions and integrations were designed using PPP: Pombe PCR Primer Programs tool (Penkett et al, 2006) and are not included in this list, as well as primers used for checking integration in the obtained strains.

Standard primers for 3' linker used in PASE reaction (Alexander et al, 2010) are not included in the table.

**Supplementary Table 3** Sequences used in protein comparison and phylogenetic analyzes. Accession numbers from NCBI Entrez with the exception of \*.

Name	Family	Species	Accession#
Dis3_Amel	Dis3	<i>Apis mellifera</i>	XP_397381
Dis3L2_Amel	Dis3_L2	<i>Apis mellifera</i>	XP_624734
Dis3_Cele	Dis3	<i>Caenorhabditis elegans</i>	NP_501835
Dis3L2_Cele	Dis3_L2	<i>Caenorhabditis elegans</i>	NP_498160
Dis3_Cint	Dis3	<i>Ciona intestinalis</i>	XP_002132066
Dis3L2_Cint	Dis3_L2	<i>Ciona intestinalis</i>	XP_002119190
Dis3_Drer	Dis3	<i>Danio rerio</i>	XP_001336850
Dis3L_Drer	Dis3L	<i>Danio rerio</i>	NP_001103928
Dis3L2_Drer	Dis3L2	<i>Danio rerio</i>	XR_084214
Dis3_Dpul	Dis3	<i>Daphnia pulex</i>	EFX89559
Dis3L2_Dpul	Dis3L2	<i>Daphnia pulex</i>	EFX64669
Dis3_Dmel	Dis3	<i>Drosophila melanogaster</i>	NP_651246
Dis3L2_Dmel	Dis3L2	<i>Drosophila melanogaster</i>	NP_728490
Dis3_Ggal	Dis3	<i>Gallus gallus</i>	XP_417016
Dis3L_Ggal	Dis3L	<i>Gallus gallus</i>	XP_003641875
Dis3L2_Ggal	Dis3L2	<i>Gallus gallus</i>	XP_422741
Dis3_Hmag	Dis3	<i>Hydra magnipapillata</i>	XR_053815
Dis3L2_Hmag	Dis3L2	<i>Hydra magnipapillata</i>	XP_002154262
Dis3_Mmus	Dis3	<i>Mus musculus</i>	NP_082591
Dis3L_Mmus	Dis3L	<i>Mus musculus</i>	NP_001001295
Dis3L2_Mmus	Dis3L2	<i>Mus musculus</i>	NP_705758
Dis3L_Nvec	Dis3L	<i>Nematostella vectensis</i>	XP_001632370
Dis3L2_Nvec	Dis3L2	<i>Nematostella vectensis</i>	XP_001634492
Dis3_Nvec	Dis3	<i>Nematostella vectensis</i>	10544 gw.129.26.1*
Dis3_Osat	Dis3	<i>Oryza sativa</i>	EEE58267
Dis3L2_Osat	Dis3L2	<i>Oryza sativa</i>	NP_001048152
Dis3_Ppat	Dis3	<i>Physcomitrella patens</i>	XP_001766083
Dis3L2_Ppat	Dis3L2	<i>Physcomitrella patens</i>	XP_001773680
Dis3_Scer	Dis3	<i>Saccharomyces cerevisiae</i>	NP_014621
Dis3_Spo	Dis3	<i>Schizosaccharomyces pombe</i>	NP_596653
Dis3L2_Spo	Dis3_L2	<i>Schizosaccharomyces pombe</i>	NP_594510
Dis3_Umay	Dis3	<i>Ustilago maydis</i>	XP_759849
Dis3L2_Umay	Dis3L2	<i>Ustilago maydis</i>	XP_757367
Dis3_Xtro	Dis3	<i>Xenopus (Silurana) tropicalis</i>	NP_001120564
Dis3L_Xtro	Dis3L	<i>Xenopus (Silurana) tropicalis</i>	NP_001072835
Dis3L2_Xtro	Dis3L2	<i>Xenopus (Silurana) tropicalis</i>	NP_001072804
Dis3L_Hsap	Dis3L	<i>Homo sapiens</i>	NP_001137160
Dis3_Hsap	Dis3	<i>Homo sapiens</i>	NP_055768
Dis3L2_Hsap	Dis3L2	<i>Homo sapiens</i>	NP_689596
Dis3_Ptri	Dis3	<i>Populus trichocarpa</i>	XP_002303419
Dis3L2_Ptri	Dis3L2	<i>Populus trichocarpa</i>	XP_002302251
Dis3_Atha	Dis3	<i>Arabidopsis thaliana</i>	NP_565418
SOV_Atha	Dis3_L2	<i>Arabidopsis thaliana</i>	NP_177891

\* Sequence not available in NCBI Entrez at the time of writing. Protein ID and Name from the Genome Portal of the Department of Energy Joint Genome Institute (Grigoriev et al, 2012) provided instead.

**Supplementary Table 4** Proteins co-purified with Dis3TAP or Rrp43TAP and identified by mass-spectrometry.

Protein or gene names are listed by score order. Known exosome complex subunits and interacting proteins are underlined

Dis3TAP	Rrp43TAP
<u>dis3</u>	<u>dis3</u>
<u>tdh1</u>	<u>rrp45</u>
<u>rrp46</u>	<u>rrp4</u>
<u>rrp45</u>	<u>rrp42</u>
<u>rrp42</u>	<u>rrp46</u>
<u>rrp4</u>	<u>fas2</u>
<u>rrp40</u>	<u>rrp40</u>
<u>fas2</u>	<u>rrp43</u>
<u>rrp43</u>	<u>fas1</u>
<u>rrp41</u>	<u>rrp41</u>
<u>fas1</u>	<u>rrp6</u>
<u>gpm1</u>	<u>mtr3</u>
<u>rp14a</u>	<u>csl4</u>
<u>csl4</u>	<u>sks2</u>
<u>tef1a</u>	<u>act1</u>
<u>mtr3</u>	<u>ssa2</u>
<u>pyk1</u>	<u>rpp201</u>
<u>tef3</u>	<u>tef1b</u>
<u>eno101</u>	<u>gpd3</u>
<u>egd2</u>	<u>rpa5</u>
<u>ssa2</u>	<u>SPAC24C9.12c</u>
<u>pgk1</u>	<u>gua1</u>
<u>pda1</u>	<u>ura1</u>
<u>swo1</u>	<u>pda1</u>
<u>SPBC16A3.08c</u>	<u>rp14b</u>
<u>gua1</u>	<u>arg5</u>
<u>act1</u>	<u>SPAP8A3.05</u>
<u>rpp201</u>	<u>SPACUNK4.11c</u>
<u>rrp6</u>	<u>pdb1</u>
<u>SPCC794.07</u>	<u>SPCC794.07</u>
<u>his1</u>	<u>rps18a</u>
<u>pdb1</u>	<u>hsp60</u>
<u>rp17c</u>	<u>rpp203</u>
<u>ura1</u>	<u>rp114</u>
<u>SPAC1F8.07c</u>	<u>SPCC1235.01</u>
<u>rpp203</u>	<u>rp125a</u>
<u>adh1</u>	<u>rps7</u>
<u>rpa5</u>	<u>rpp202</u>
<u>rps3</u>	<u>rp130a</u>
<u>hsp60</u>	<u>SPCC1827.03c</u>
<u>fba1</u>	<u>SPCC1739.07</u>
<u>sce3</u>	<u>rp118b</u>
<u>rps2</u>	<u>cct4</u>
<u>wis2</u>	<u>fta5</u>
<u>cct2</u>	<u>rps23a</u>
<u>rps7</u>	<u>cct5</u>
<u>SPBC8D2.18c</u>	<u>rp128e</u>
<u>rps18a</u>	
<u>vas2</u>	
<u>sgt2</u>	
<u>arg5</u>	
<u>rpa1</u>	
<u>rps6a</u>	
<u>cdc48</u>	
<u>rps5b</u>	
<u>sla1</u>	
<u>rp16</u>	
<u>rps0b</u>	
<u>SPCC1739.07</u>	
<u>rp11201</u>	
<u>rp125a</u>	
<u>rp118a</u>	
<u>lys4</u>	
<u>rps22a</u>	
<u>cdc48</u>	

fta5	sla1
tef-1	tif471
ssp1	ssp1
rps19a	htb1
SPCC1259.09c	puf6
SPAC9.09	eft1
gpd1	SPCC364.06
eft1	rpl27a
SPAC24C9.12c	rpl7c
SPAC22A12.16	SPAC222.08c
ilv5	rpp0
btf3	rpt3
rpl11a	hsp10
SPBC24C6.04	rpl35
eca39	rpl7b
rpl30a	swol
pab1	pfk1
mbf1	SPAC12G12.07c
sua1	rpl37b
cof1	rpl37b
SPCC1827.03c	cct6
rps16a	mlo3
rpl23a	tea3
SPAC1782.11	rpn7
rpl7b	rps4c
rpl14	btf3
cct6	rps14a
<u>SPAP8A3.05</u>	rpl22
SPCC1235.01	dld1
rpl27a	rpl6
rpl28e	rps15b
p23fy	tfs1
vma10	rps102
rps6b	tif213
krs1	ubi3
SPCC1223.14	rpl8
rps0a	rps2
rpp202	rpl17b
rps14a	rpc19
SPCC191.02c	rps5b
gly1	tif211
hsp10	rpl13
rps102	rpl36a
rpl35	rpl16a
rpl18b	SPAC1F8.07c
cut6	rpl1201
rpl24b	SPBC1734.11
sti1	tcg1
SPAC12G12.07c	cut6
SPBC354.10	ppt1
rps20	
rps4c	
rps23a	
SPCC584.01c	
SPAPB1E7.07	
srp2	
aro4	
SPBC660.16	
tif211	
rpp0	
rpl13	
rps21	
rps12a	
but2	
mge1	
SPCC364.06	
sir1	
rpl2a	
SPAC222.08c	
rki1	
inh1	
rpl31	
cdc37	
ura5	
rpl36a	

gpx1  
cdc8  
rpl10b  
htb1  
SPBP8B7.05c  
SPAC26F1.13c  
nup61  
arg4  
mlo3  
rps4b  
rpn2  
rpl17b  
SPAC1805.02c  
fim1  
rps17a  
rpt3  
tea3  
SPAC4F10.19c  
arg1  
dbp2  
rps9a  
hub1  
rps0b  
SPAC17A5.15c  
atp2  
pmt3  
tif1  
SPAC24H6.10c  
SPBC1711  
lys3  
rpl37b  
rps101  
SPBC1703.07  
SPBC19C7.06  
SPBC18E5.07  
rpl28a  
rps10b  
pmp20  
SPAC8C9.04  
mmf1  
rpl22  
rad25  
tim13  
rpb4  
rpl15b  
erg10  
SPBC2F12.05c  
bip1  
hsp9  
SPBC21B10.03c  
cct8  
cct4  
SPAPB24D3.07c  
rpl5a  
tif471  
ypt1  
rkp1  
hts1  
tal1  
lys1  
SPBC1539.06  
grx4  
rpl20a  
rad24  
SPBC776.15c  
SPCC1183.02  
rpl3b  
cpy1  
dld1  
shm2  
plb1  
shg1  
tpx1  
SPBC713.03  
rps8b

rps27  
cyc1  
rpl8  
ubi3  
SPBC1734.11  
rps30a  
tpi1  
sui1  
clc1  
mpd2  
tef3  
mri1  
eif3f  
SPBC1271.04c  
SPAC10F6.16  
rps13  
tif213  
pan1  
SPBC359.05  
SPCC965.06  
SPAC25G10.08  
SPAC29E6.06c  
SPAC13G7.06  
arc4  
vip1  
SPBC1198.05  
tif11  
rps15a  
SPBC776.03  
teg1  
SPAC1F5.11c  
ppt1  
cct5  
rps24b  
rpl16a  
SPAC9E9.09c  
utp22  
SPAC1F5.06  
mug125  
mts2  
eif3m  
hmt2  
rrf1  
nhp6  
SPAC22E12.19  
SPAC23H3.15c  
uba2  
dps1  
SPBP4H10.15  
srp54  
hvk1  
hrq1  
aru1  
SPAC5H10.03  
SPBC30D10.05c  
SPBC14C8.04  
ino80  
SPBC29A3.16  
rhh1  
mdn1  
srp72  
ded1  
SPAC926.08c  
prs3  
SPAC458.04c  
let1  
SPBC337.07c  
smd1  
utp20  
SPMIT.03

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