Supplementary data

Supplementary data

Supplementary Figures S1-S7 and Legends

Supplementary Tables 1S-S4

Supplementary materials and methods

Supplementary references

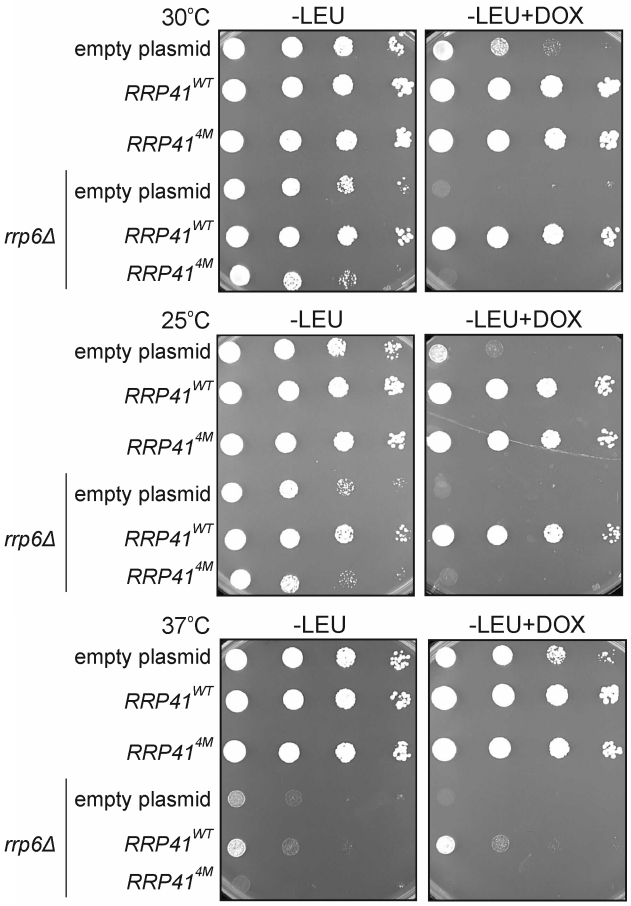

Figure S1. *Rrp41*^{4M} exosome central channel mutation is synthetically lethal with deletion of *RRP6*. LEU-marked plasmids containing *RRP41*^{WT}, *RRP41*^{4M} or no insert (empty plasmid control) were transformed into two strains harboring endogenous *RRP41* under control of a doxycycline-repressible promoter, the second was also an *rrp6* deletion strain. Growth phenotypes of the resulting strains were analyzed after 60 h at 25, 30 and 37℃ in the absence (-LEU) or presence (-LEU+DOX) of doxycycline.

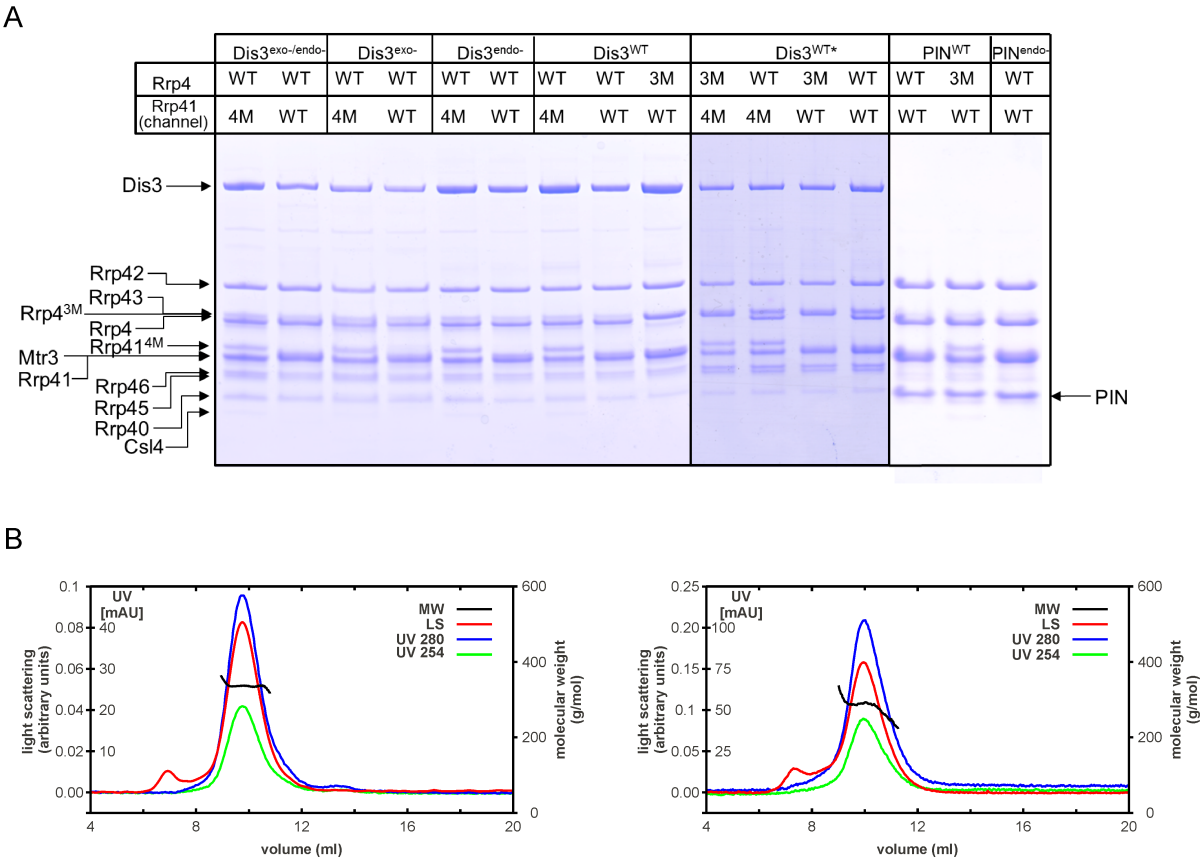

Figure S2. Reconstruction of WT and various mutant forms of the exosome complex from *C. thermophilum.* (**A**) SDS-PAGE analysis of reconstructed complexes. (**B**) Size exclusion chromatography combined with Multi Angle Light Scattering (SEC-MALS) analysis of reconstructed WT exosome complex. Black, red, blue and green lines represent calculated molecular weight, scattering signal, 280 nm and 254 nm absorbance respectively.

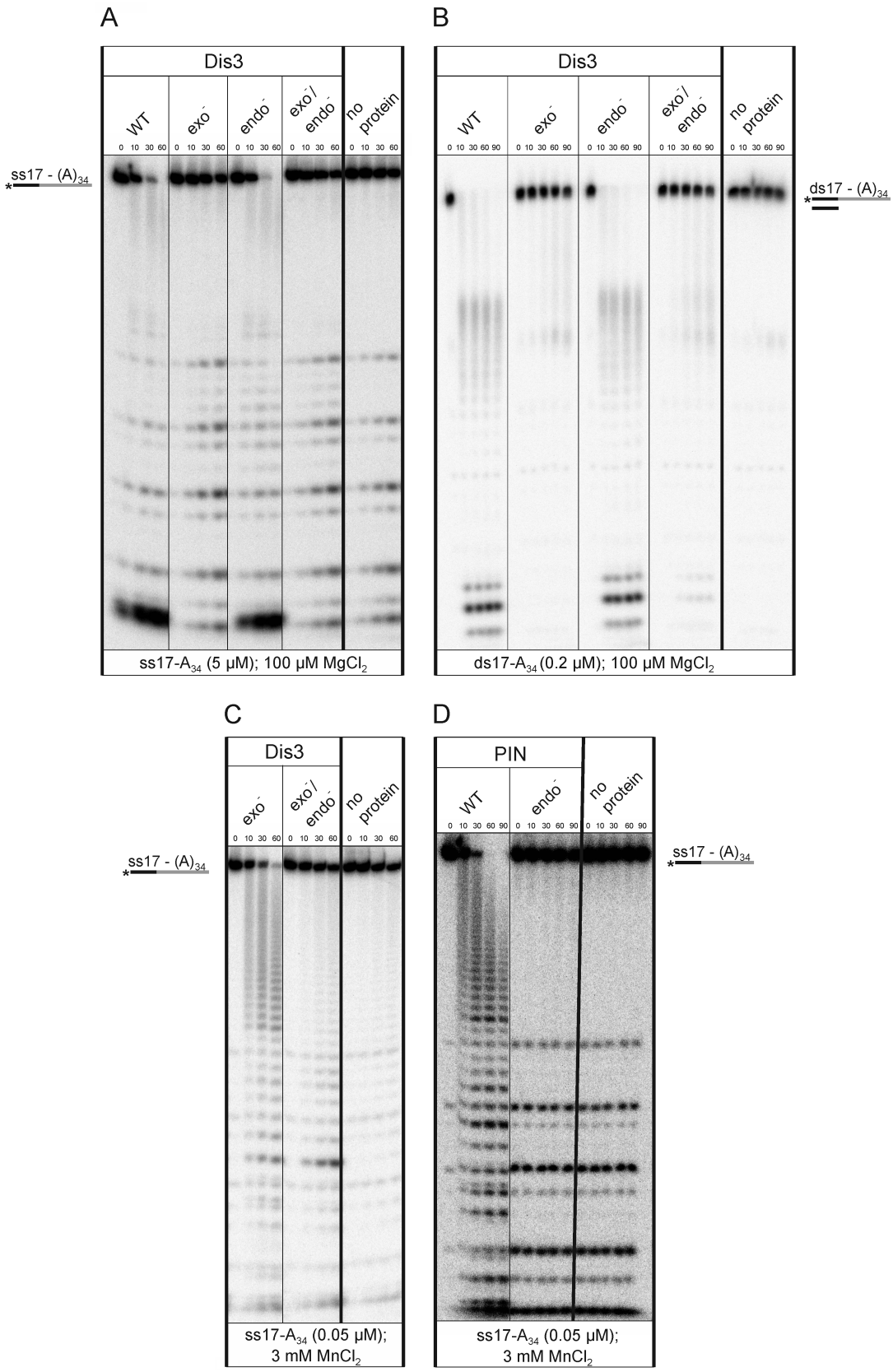

Figure S3. Biochemical characterization of *C. thermophilum* Dis3 ribonucleolytic activities. (**A**) and (**B**) Recombinant full-length *C. thermophilum* Dis3 protein displays exoribonucleolytic activity able to degrade both single- and double-stranded RNA substrates, which is strictly dependent on the intact aspartic acid residue in the active site of the RNB domain. 5´-labeled

ss17-$(A)_{34}$ (**A**) or ds17-$(A)_{34}$ (**B**) substrate was incubated in buffer containing 100 µM magnesium with different versions of Dis3 protein from *C. thermophilum* ($Dis3^{WT}$, $Dis3^{exo-}$, $Dis3^{endo-}$, $Dis3^{exo-/endo-}$) or in the absence of added protein. Samples were collected at the indicated time points (minutes) and analyzed by denaturing PAGE and phosphorimaging. (**C**) and (**D**) Recombinant full-length *C. thermophilum* Dis3 protein and its PIN domain alone both possess endoribonucleolytic activity, which is abolished by mutation of the conserved aspartate within the catalytic center of the PIN domain. 5´-labeled ss17-$(A)_{34}$ substrate was incubated in buffer containing 3 mM manganese with different versions of full-length *C. thermophilum* Dis3 ($Dis3^{exo-}$, $Dis3^{exo-/endo-}$) (**C**), isolated PIN domain (PIN^{WT}, PIN^{endo-}) (**D**) or in the absence of added protein. Samples were collected at the indicated time points (minutes) and analyzed by denaturing PAGE and phosphorimaging.

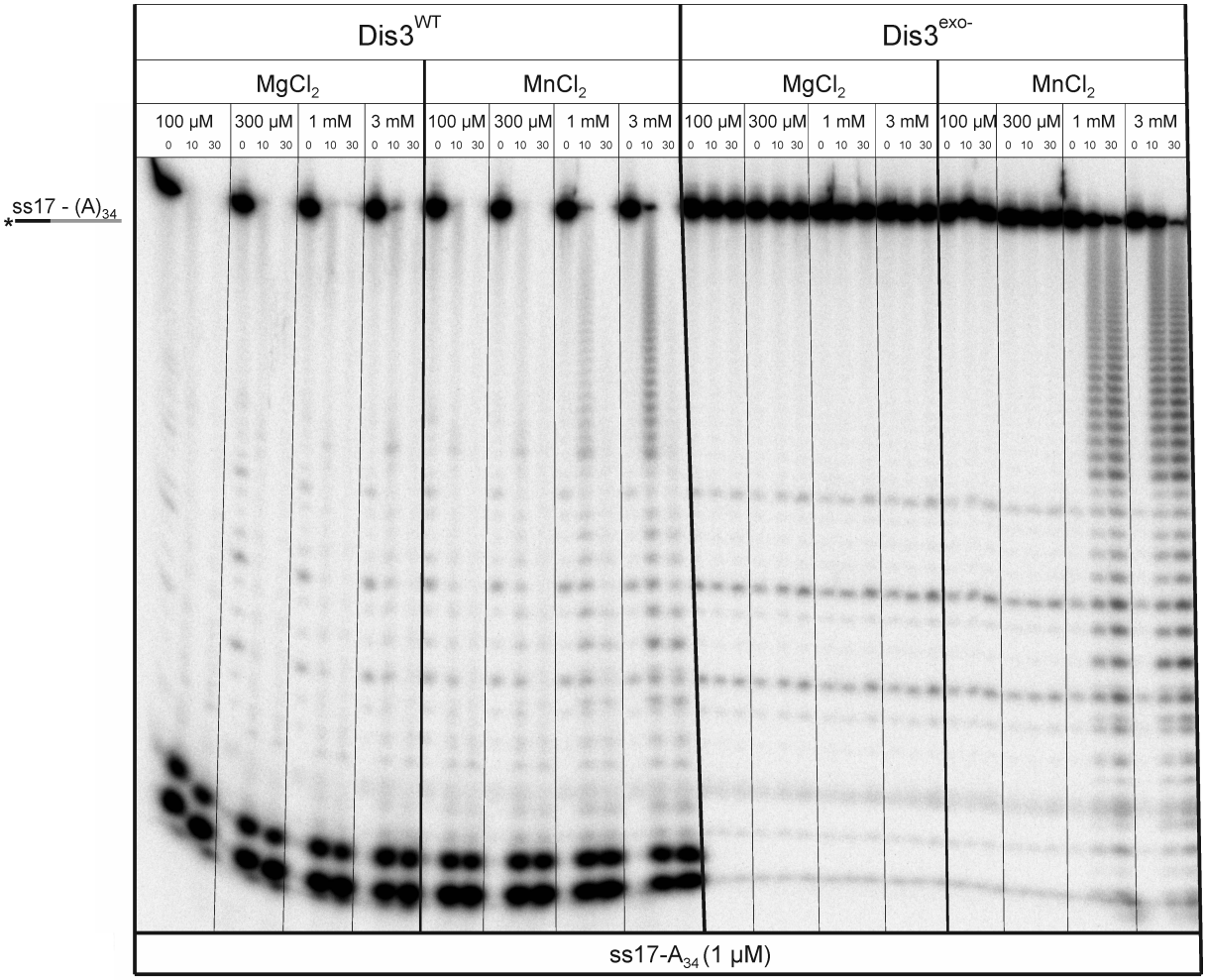

Figure S4. *C. thermophilum* Dis3 exoribonucleolytic activity can be observed within a broad range of both magnesium and manganese concentrations while PIN domain-dependent endoribonuclease activity is detectable exclusively in buffers containing manganese at a high concentration. 5´-labeled ss17-$(A)_{34}$ RNA substrate was incubated with *C. thermophilum* $Dis3^{WT}$ or $Dis3^{exo-}$ in buffers containing magnesium or manganese cations at different concentrations as indicated. Reactions were terminated at the indicated time points (minutes) followed by denaturing PAGE and phosphorimaging. $Dis3^{WT}$ displays mainly exoribonuclease activity, which is readily visible in all cases while $Dis3^{exo-}$ endoribonuclease activity is strictly dependent on the presence of manganese as a cofactor and can be revealed only when the concentration of this cation is at least 1 mM.

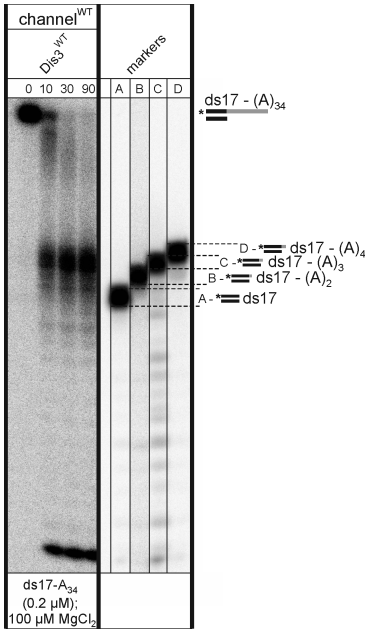

Figure S5. Degradation of double-stranded ds17-$(A)_{34}$ RNA by *in vitro* reconstructed *C. thermophilum* exosome results in accumulation of decay intermediates possessing 2-3 nt-long single-stranded extensions. ds17-$(A)_{34}$ substrate was incubated in buffer containing 100 µM magnesium with reconstituted exosome containing non-mutated channel and $Dis3^{WT}$. Samples were collected at the indicated time points (minutes) and analyzed by denaturing PAGE and phosphorimaging. Blunt-ended dsRNA (ds17) or its counterparts with short single-stranded extensions (ds17-$(A)_2$, ds17-$(A)_3$ and ds17-$(A)_4$) were run in parallel, as indicated in the right, to enable estimation of the size of decay intermediates.

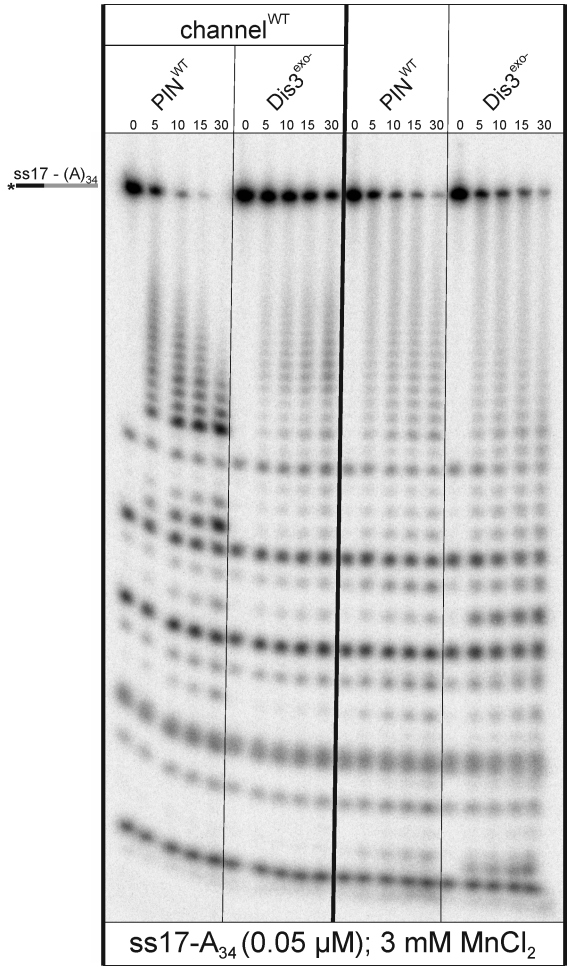

Figure S6. Direct comparison of degradation rates and patterns performed for reconstituted exosomes and individual proteins that display endoribonucleolytic activity. 5´-labeled ss17-$(A)_{34}$ substrate was incubated in a buffer containing 3 mM manganese with *C. thermophilum* exosome complexes with PIN domain (PIN^{WT}) or full-length Dis3 lacking exonuclease activity ($Dis3^{exo-}$) as well as with PIN^{WT} and $Dis3^{exo-}$ proteins alone. Samples were collected at the indicated time points (minutes) and analyzed by denaturing PAGE and phosphorimaging.

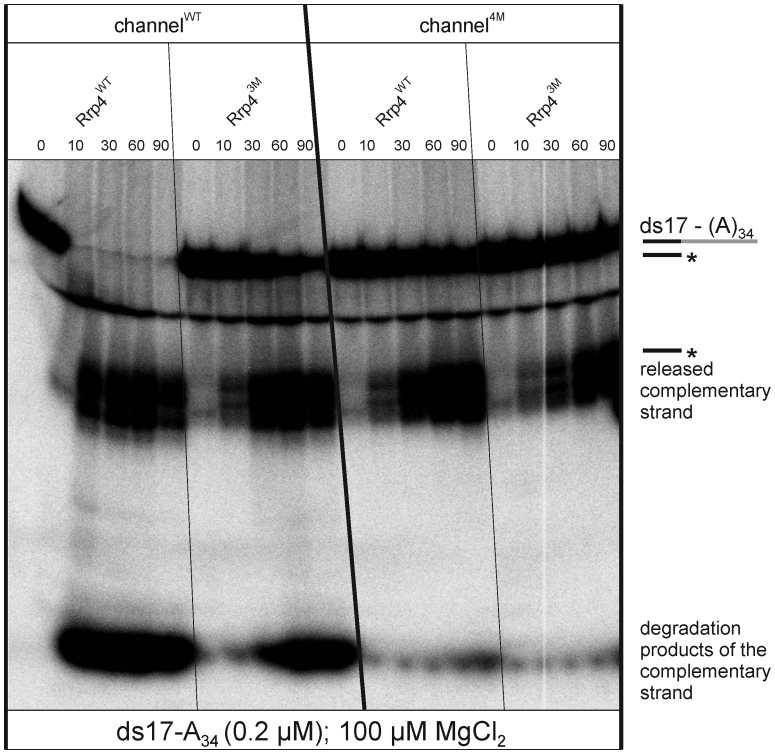

Figure S7. Unwinding of dsRNA substrate by reconstructed *C. thermophilum* exosome complexes. Reactions were carried out using ds17-$(A)_{34}$ duplex RNA substrate, which had been prepared in the presence of 5'-labeled complementary ss17 oligonucleotide and incubated with *in vitro* reconstituted *C. thermophilum* exosomes containing different versions of the channel (either with $Rrp41^{WT}$ or $Rrp41^{4M}$) and Rrp4 cap subunit ($Rrp4^{WT}$ or $Rrp4^{3M}$), as indicated. Samples were collected at the indicated time points (minutes) and analyzed by electrophoresis in 15% native polyacrylamide gel, followed by phosphorimaging. Positions of the initial duplex RNA substrate, the radioactive complementary strand released by unwinding of dsRNA and the final degradation products of the complementary strand are shown in the right.

Table S1. Oligonucleotides used for strain construction, northern blot analysis and biochemical assays. "*r*" before sequence in brackets indicates that the oligo is composed of ribonucleotides.

Oligonucleotide	Sequence
ADZKD100	TTAATAGTAGTCTATGCTGG
ADZKD103	CTGTTTTTTATGTACTTTTATATAAACAGTGGCAATTAATGGCG TTTTTTATTtttaaactggatggcggcgt
ADZKD106	TCGGTCATATGAGAGTGTGTTGCG
ADZKD107	AGTGGTTTAGTGGTAAAATCCAACGTTGCCATCGTTGGGCCC CCGGTTCG
ADZKD113	TAGACGAAATAGGAACAACAAACAGCTTATAAGCACCCAATAA GTGCGTTagatctgtttagcttgcc
ADZKD114	ATGAAAATTACCATAATTTATAAATAAAAAAATACGCTTGTTTT ACATAAtcgatgaattcgagctcg
ADZKD117	TTTTGATCCCGTAGAACACG
ADZKD118	TGGCTTTGGTGGTGTTTTGC
5S rRNA probe	CTACTCGGTCAGGCTC
7S rRNA probe	GGCCAGCAATTTCAAGTTA
5.8S rRNA probe	GCGTTGTTCATCGATGC
SCR1 probe	ATCCCGGCCGCCTCCATCAC
ss17	r(CCCCACCACCAUCACUU)
ss17-$(A)_2$	r(CCCCACCACCAUCACUUAA)
ss17-$(A)_3$	r(CCCCACCACCAUCACUUAAA)
ss17-$(A)_4$	r(CCCCACCACCAUCACUUAAAA)
ss17-$(A)_{14}$	r(CCCCACCACCAUCACUUAAAAAAAAAAAAAA)
ss17-$(A)_{34}$	r(CCCCACCACCAUCACUUAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAA)
compl	r(AAGUGAUGGUGGUGGGG)

Table S2. Yeast strains employed in this study.

Strain	Mutations	References
ADZY522	*rrp41*^{4M} (K62E S63D R95E R96E)	This study
ADZY531	*dis3*$^{endo-}$ (D171N)	This study
ADZY537	*rrp41*^{4M} (K62E S63D R95E R96E) *dis3*$^{endo-}$ (D171N)	This study
BSY1735	*dis3*$^{exo-}$ (D551N)	(1)
ADZY539	*rrp41*^{4M} (K62E S63D R95E R96E) *dis3*$^{exo-}$ (D551N)	This study
BSY1665	*dcp*$^{1-2}$	(1)
ADZY649	*dcp*$^{1-2}$ *rrp41*^{4M} (K62E S63D R95E R96E)	This study
ADZY712	*RRP4 tet-off mtr4*gfp	This study
TH_6151	*RRP4 tet-off*	(2)
TH_3687	*RRP41 tet-off*	(2)
ADZY524	*RRP41 tet-off rrp6Δ*	This study

Table S3. Constructs used for *C. thermophilum* exosome reconstruction.

Construct	Plasmid backbone
HisTAG-SUMO-CtRrp46 (WT) + CtRrp43 (WT)	pET28
HisTAG-SUMO-CtRrp41 (WT) + CtRrp45 (WT)	pET28
HisTAG-SUMO-CtRrp41 (R62E R63D K114E R115E) + CtRrp45 (WT)	pET28
HisTAG-SUMO-CtMtr3 (WT)	pET28
HisTAG-SUMO-CtRrp42 (WT)	pET28
HisTAG-SUMO-CtRrp4 (WT)	pET28
HisTAG-SUMO-CtRrp4 (R158E K159D R160D)	pET28
HisTAG-SUMO-CtCsl4 (WT)	pET28
HisTAG-SUMO-CtRrp40 (WT)	pET28
HisTAG-SUMO-CtDis3 (WT)	pET28
HisTAG-SUMO-CtDis3 (D168N)	pET28
HisTAG-SUMO-CtDis3 (D536N)	pET28
HisTAG-SUMO-CtDis3 (D168N D536N)	pET28
HisTAG-SUMO-CtPIN (WT)	pET28
HisTAG-SUMO-CtPIN (D168N)	pET28

Table S4. Alignment of amino acid sequences of the exosome subunits from *S. cerevisiae*, *C. thermophilum and H. sapiens.* Sites of mutations analyzed in this study are indicated.

Rrp41

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Conservation:                          9           9 9999  999           9999 9 9 9  9    9 99 9
RRP41_Homo_sapiens                   1 MA-GLELLSDQGYRVDGRRAGELRKIQARMGVFAQ-ADGSAYIEQGNTKALAVVYGPHEIRG--------   60
Rrp41p_S._cerevisiae                 1 MS-RLEIYSPEGLRLDGRRWNELRRFESSINTHPHAADGSSYMEQGNNKIITLVKGPKEPR---------   60
Rrp41p_Ch._thermophilum              1 MPLDTSTYKLALLRVDGRRWNELRRVHAQIRTQAA-ADGSSYLEMGHTKVMCVVTGPSEPGPRRGTGAGT   69
Consensus_ss:                             hhhhhh          hhheeeeeeee        eeeeeee   eeeeeeee

Conservation:                                          9          99   9           9                  9
RRP41_Homo_sapiens                  61 ----------SRARALPDRALVNCQYSSATFSTGERKRR-PHGDRKSCEMGLQLRQTFEAAILTQLHPR  118
Rrp41p_S._cerevisiae                61 ----------LKSQMDTSKALLNVSVNITKFSKFERSKSSHKNERRVLEIQTSLVRMFEKNVMLNIYPR  119
Rrp41p_Ch._thermophilum             70 TGGGAGGAGGGGSGGQGKEAEVVVSIVIAGFSSVDRKRH-GRNDKRIIEMQSTVANALSASLHTHLFPH  138
Consensus_ss:                                        eeeeeeeee                hhhhhhhhhhhhhhhhh  hhh

Conservation:                            9 9   99  99       9  999  9999 9 9       9              9 9    99
RRP41_Homo_sapiens                 119 SQIDIYVQVLQADGGTYAACVNAATLAVLDAGIPMRDFVCACSAGFVD--------GTALADLSHVEEAA  180
Rrp41p_S._cerevisiae               120 TVIDIEIHVLEQDGGIMGSLINGITLALIDAGISMFDYISGISVGLYD--------TTPLLDTNSLEENA  181
Rrp41p_Ch._thermophilum            139 SQITISLHVLSQDGSLLAALINAATLACVDAGIPMTDYVVACTAGSTSTYAANDENADPLLDLNHQEEQE  208
Consensus_ss:                          eeeeeeeeeee    hhhhhhhhhhhhhhh        eeeeeeeee          eeee   hhhhhh

Conservation:                                    9     9          99  9              9   9
RRP41_Homo_sapiens                 181 GGPQLALALLPASGQIALLEMDARLHEDHLERVLEAAAQAARDVHTLLDRVVRQHVREASILLGD-----  245
Rrp41p_S._cerevisiae               182 MS-TVTLGVVGKSEKLSLLLVEDKIPLDRLENVLAIGIAGAHRVRDLMDEELRKHAQKRVSNASAR----  246
Rrp41p_Ch._thermophilum            209 LP-WLTVATLGESDKVAVLVCESRVQVSRLEGMLAVGVDGCKQIRAILDHVVRQKGRRMIREGAVEKGVS  277
Consensus_ss:                            eeeee     eeeee      hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh

Conservation:
RRP41_Homo_sapiens                      -------
Rrp41p_S._cerevisiae                    -------
Rrp41p_Ch._thermophilum            278 LDDMDED  284
Consensus_ss:
```

Rrp45

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Conservation:                          9      9  9  9 9 9     99999     9     99   9   9  9 9 9    9     9
RRP45_Homo_sapiens                   1 MK-ETPLSNCERRFLLRAIEEKKRLDGRQTYDYRNIRISFGTDYGCCIVELGKTRVLGQVSCELVSPKLN   69
Rrp45p_S._cerevisiae                 1 MAKDIEISASESKFILEALRQNYRLDGRSFDQFRDVEITFGKEFGDVSVKMGNTKVHCRISCQIAQPYED   70
Rrp45p_Ch._thermophilum              1 MPREVEPSLSERQFVLQALQEGLRLDGRQLDQYRPLSLTFGDQYGVADVTFGKTRVLAKASAEVTVPYAD   70
Consensus_ss:                              hhhhhhhhhh         hhheeeeeeee     eeeeee   eeeeeee

Conservation:                          9   9      9 9 9    99         9   9  9   9 9   9 9 99 999 9 9  9 9 9
RRP45_Homo_sapiens                  70 RATEGILFFNLELSQMAAPAFEPG-RQSDLLVKLNRLMERCLRNSKCIDTESLCVVAGEKVWQIRVDLHL  138
Rrp45p_S._cerevisiae                71 RPFEGLFVISTEISPMAGSQFENGNITGEDEVLCSRIIEKSVRRSGALDVEGLCIVAGSKCWAVRADVHF  140
Rrp45p_Ch._thermophilum             71 RPLDGIFTIATELSPMTSPTFEVN-RPTETEVLLSRLLEKTIRRSGALDTESLCLVAGQKCWSIRVDVHV  139
Consensus_ss:                             eeeeeeeee               hhhhhhhhhhhhhhhh     hh          eeeeeeeee

Conservation:                            99   99  99    9 99  99             99 9999   9 9 99   99
RRP45_Homo_sapiens                 139 LNHDGNIIDAASIAAIVALCHFRRPDVSVQGDEVTLYTPEERDPVPLSIHHMPICVSFAFFQQG------  202
Rrp45p_S._cerevisiae               141 LDCDGGFIDASCIAVMAGLMHFKKPDITVHGEQIIVHPVNEREPVPLGILHIPICVTFSFFNPQDTEENI  210
Rrp45p_Ch._thermophilum            140 MSHDGNLVDAACIAVVAALRHFRKPDTSIESGVLTIYTPAEREPVPLSWLHTPFCVTWSFFGDE------  203
Consensus_ss:                          ee    hhhhhhhhhhhhhhh    eeee     eeee              eeeeeeeee

Conservation:                                    9    99    9      99  9       99          9   9
RRP45_Homo_sapiens                 203 ------TYLLVDPNEREERVMDGLLVIAMNKHREICTIQSSGGIMLLKDQVLRCSKIAGVKVAEITELIL  266
Rrp45p_S._cerevisiae               211 KGETNSEISIIDATLKEELLRDGVLTVTLNKNREVVQVSKAGGLPMDALTLMKCCHEAYSIIEKITDQIL  280
Rrp45p_Ch._thermophilum            204 -----GEIAVLDATWLEEQVRVGSCTISMNKHGEICQIAKLGGTPVEAVSLLQCTSIALTKVKEFSDLVD  268
Consensus_ss:                             eeee   hhhhhh   eeeeee     eeeeee       hhhhhhhhhhhhhhhhhhhhhhhh

Conservation:                            9  9   9           9
RRP45_Homo_sapiens                 267 KALENDQKVRKEGG--KFGFAESIANQRITAFKMEKAPIDTSDVEEKAEEIIAEAEPPSEVVSTPVLWTP  334
Rrp45p_S._cerevisiae               281 QLLKEDSEKRNKYA--AMLTSENARE------------------------------------------  304
Rrp45p_Ch._thermophilum            269 KKLAEDFKRRNPGGLLKELKAENDR-------------------------------------------  293
Consensus_ss:                          hhhhhhhhh        eee

Conservation:
RRP45_Homo_sapiens                 335 GTAQIGEGVENSWGDLEDSEKEDDEGGGDQAIILDGIKMDTGVEVSDIGSQDAPIILSDSEEEEMIILEP  404
Rrp45p_S._cerevisiae               305 ----------------------------------------------------I---------------  305
Rrp45p_Ch._thermophilum                ------------------------------------------------------------------------
Consensus_ss:

Conservation:
RRP45_Homo_sapiens                 405 DKNPKKIRTQTTSAKQEKAPSKKPVKRRKKKRAAN  439
Rrp45p_S._cerevisiae                   ---------------------------------
Rrp45p_Ch._thermophilum                ---------------------------------
Consensus_ss:
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Rrp42

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Conservation:                                99 99  9           9 999     9     9       99 9          9
RRP42_Homo_sapiens              1  MAS-----VTLSEAEKVYIVHGVQE--DLRVDGRGCEDYRCVEVETDVVSNTSGSARVKLGH-TDILVGV   62
Rrp42p_S._cerevisiae            1  --------MSLSVAEKSYLYDSLASTPSIRPDGRLPHQFRPIEIFTDFLPSSNGSSRIIASDGSECIVSI   62
Rrp42p_Ch._thermophilum         1  MSAASSQHVLLSPAELAYLHASLSLTPPIRPDGRSPTQFRPLIAETGILPGANGSARVCFADGTEAIVGV   70
Consensus_ss:                             hhhhhhhhhhhh           hhheeeeeeee        eeeeee    eeeeee

Conservation:                            9                9
RRP42_Homo_sapiens             63  KAEMGTPKLEKPNEGYLEFFVDCSASATPEFEGRG-------------------------GDDLGTEIA  106
Rrp42p_S._cerevisiae           63  KSKVVDHH---VENELLQVDVDIAGQRDD---------------------------------ALVVETIT   96
Rrp42p_Ch._thermophilum        71  KAEVEKTVSRSKEDEEVGLLVASAGDMDVDDEEGYAKVGADNRTGEASWVEITVEIPGVRDDDSGMVFLA  140
Consensus_ss:                            e             eeeeeeee                                  hhhhhhh

Conservation:                              9                          9 9             9     99  9  9      9
RRP42_Homo_sapiens             107 NTLYRIFNNKSSVDLKTLCISPREHCWVLYVDVLLLECGGN-LFDAISIAVKAALFNTRIPRVRVLEDEE  175
Rrp42p_S._cerevisiae            97 SLLNKVLKSGSGVDS-SKLQLTKKYSFKIFVDVLVISSHSH-PVSLISFAIYSALNSTYLPKLISAFDDL  164
Rrp42p_Ch._thermophilum        141 QLLGEALLADGEFV--KKLWINRRYHWKLYIDILLISPPLSYPLPLLSLTTHLALLSTRLPRLKSEGDED  208
Consensus_ss:                      hhhhhhhhh     h hh        eeeeeeeeeee     hhhhhhhhhhhhhhh

Conservation:                                                 9        9     9    9       9  9
RRP42_Homo_sapiens             176 GSKDIELSDDPYDC------------IRLSVENVPCIVTLCKIGYRHVVDATLQEEACSLASLLVSVTS  232
Rrp42p_S._cerevisiae           165 EVEELPTFHDYDMVK--------------LDINPPLVFILAVVGNNMLLDPAANESEVANNGLIISWSN  219
Rrp42p_Ch._thermophilum        209 PYFDDWAVAPYLFPRSSSASKSSKSSPTQPTTRPPITLLVMAVGNNILFDPSKEELAVADVALAVSVTA  278
Consensus_ss:                        ee e                           eeeeeeeee  eeee   hhhhh   eeeeee

Conservation:                                    9                      9
RRP42_Homo_sapiens             233 KGV---------VTCMRKVGKGSLDPESIFEMMETGKRVGKVLHASLQSV-----------------VHK  276
Rrp42p_S._cerevisiae           220 GKITSPIR----SVALNDSNVKSFKPHLLKQGLAMVEKYAPDVVRSLENL--------------------  265
Rrp42p_Ch._thermophilum        279 TDVDPDESDAQKETATATAGPDSADAAKRGRKLRLLSIRTIDPPSRLTPPGVPNSTNPAAIYGTTSSSGT  348
Consensus_ss:                        eeeee    eeeee        hhhhhhhhhhhhhhhhhhhhhhh                hhh

Conservation:
RRP42_Homo_sapiens             277 EESLGPKR------------QKV---------------------------------GFLG----  291
Rrp42p_S._cerevisiae                ------------------------------------------------------------------
Rrp42p_Ch._thermophilum        349 NGNGQPQQKVESGKISEPIEPIEGVWRAPRGGAKRLVLGALVQKVLEKGGVVDEVLDALEGVELT  413
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Mtr3

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Conservation:                            9   9 99  99                                        9      999  9
MTR3_Homo_sapiens               1  MP-GDHRRIRGPEESQPPQ------LYAADEEEAPGTRDPTRLRPV---YARAGLLSQAKGSAYLEAGGT   60
Mtr3p_Ch._thermophilum          1  M--TDRRRINGPAGATIPPVYEDS-GISEVKALKIRSRPSNIIRKICTHHLKTGVTPSASGSAYLELETS   67
Mtr3p_S._cerevisiae             1  MNVQDRRRLLGPAAAKPMAFSNTTTHVPEKKSTDLTPKGNESEQEL---SLHTGFIENCNGSALVEARSL   67
Consensus_ss:                                                      hhheeee   eeee       eeeeeee

Conservation:                                 9 999
MTR3_Homo_sapiens               61  --------KVLCAVSGPRQAEGGERGGGPAGAGGEAPAALRGRLLCDFRRAPFAGRRRRAPPGGCEEREL  122
Mtr3p_Ch._thermophilum          68  ANSGVSGLKLSCTVHGPRSLPRSSP------------FSPHMVVSTHVKYAPFATKQRRGYLRDPTERDL  125
Mtr3p_S._cerevisiae             68  G----HQTSLITAVYGPRSIRGSFTS-----------QGTISIQLKNGLLEKYNTNE---------LKEV  113
Consensus_ss:                            eeeeeee                          eeeeeeeeee              hhhhhh

Conservation:                              9           9 9                                        9    99999
MTR3_Homo_sapiens               123 ALALQEALEPAVRLGRYPRAQLEVSALLLEDGG--------------------SALAAALTAAALALADA  172
Mtr3p_Ch._thermophilum          126 GIHLEAALRGAIIADRWPKSGVDIIISIIEGDQDR----EASKTQGDEVWDMMNTLSGCITVASAALADA  191
Mtr3p_S._cerevisiae             114 SSFLMGIFNSVVNLSRYPKSGIDIFVYLTYDKDLTNNPQDDDSQSKMMSSQISSLIPHCITSITLALADA  183
Consensus_ss:                       hhhhhhhhhhhh  hhh   eeeeeeeeeee           hhhhhhhhhh   hhhhhhhhhhhhhhh

Conservation:                            9    9                        9
MTR3_Homo_sapiens               173 GVEMYDLVVGCGLSLAPGP--APTWLLDPTRLEEERAAAGLTVALMPVLNQVAGLLGSGEGGLTESWAEA  240
Mtr3p_Ch._thermophilum          192 GIDCVDTVAGGVAALVQDSDGSPEIVVDPIPSEHRKILAACCVAYLPMRDEVTNLWFRGDL-PASDMDLY  260
Mtr3p_S._cerevisiae             184 GIELVDMAGAGEA--------NGTVVSFIKNGEEIV-----------------GFWKDDGD--DEDLLEC  226
Consensus_ss:                             eeeeeeeee       eeee   hhhhhh    eeeeee     eeeeee      hhhhhhh

Conservation:                                  9
MTR3_Homo_sapiens               241 VRLGLEGCQRL---YPVLQQSLVRAARRRGAAAQP  272
Mtr3p_Ch._thermophilum          261 TELVEKGIQASRSANRVLVDCLTETV---G-----  287
Mtr3p_S._cerevisiae             227 LDRCKEQYNRY---RDLMISCLMNQE---T-----  250
Consensus_ss:                       hhhhhhhhhhh   hhhhhhhhhhh
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Rrp43

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Conservation:                            9                      9     9             9   9   9 9         9
RRP43_Homo_sapiens              1  M-----------------AAGFKTVEPLEYYRRF--------LKENCRPDGRELGEFRTTTVNIGSISTA   45
Rrp43p_Ch._thermophilum         1  MATDA---ASAPHSLTFPRGIFAKLSPHPYLLRTLCPDPSNSSSTPQRTNGRRPNEARPFRVNLGSLSHA   67
Rrp43p_S._cerevisiae            1  MAESTTLETIEIHPITFPPEVLARISPELSLQRH--------LSLGIRPCLRKYEEFRDVAIENNTLSRY   62
Consensus_ss:                                    hhhhhhh hhhhhhhh        hh         hhheeeeeeee ee

Conservation:                              99     9 9 9
RRP43_Homo_sapiens              46  ------------DGSALVKLGNTTVICGVKAEFAAPSTDA--------------------PDKGYVVPN   82
Rrp43p_Ch._thermophilum         68  ------------HGSALVRAGDTTVLCGVRGEVLPVERIPLFRQPDVNGSGIGATVGRGELKEYDLLVPN  125
Rrp43p_S._cerevisiae            63  ADAGNIDTKNNILGSNVLKSGKTIVITSITGGIIEETSAA--------------------IKDLDDFGE  111
Consensus_ss:                              eeeeee   eeeeeeee                                      eeeee

Conservation:                            9                                  9                9
RRP43_Homo_sapiens              83  VDLPPLCSSRFR-----------------SGPPGEEAQVASQFIADVIENSQIIQKEDLCISP-------  128
Rrp43p_Ch._thermophilum         126 IELATGSAPQFL---------------PGVPPTALAQTLSTRVYSLLHSTRLVSAEELRIWYRPVATNR  179
Rrp43p_S._cerevisiae            112 EELFEVTKEEDIIANYASVYPVVEVERGRVGACTDEEMTISQKLHDSILHSRILPKKALKVKAGVRSANE  181
Consensus_ss:                      ee                                  hhhhhhhhhhhhhhh      hhhhee

Conservation:                                      999     9   9   9        99    99
RRP43_Homo_sapiens              129 ------------------------GKLVWVLYCDLICLDYDGNILDACTFALLAALKNVQLPEVTINEE  173
Rrp43p_Ch._thermophilum         180 SKEGEDVEMEEECQEESGE--EQDRVVAYWVLYIDLVFLSFDGNPFDVAWAAVVAALRDTKLPVARWDPD  247
Rrp43p_S._cerevisiae            182 DGTFSVLYPDELEDDTLNETNLKMKRKWSYVLYAKIVVLSRTGPVFDLCWNSLMYALQSVKLPRAFIDER  251
Consensus_ss:                            e                      eeeeeeeeeee    hhhhhhhhhhhhhhh  hhhhh
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Conservation:                                                 9
RRP43_Homo_sapiens           174  TALAEVN--------------------LKKKSYLNIRTHPVATSFAVFD--------------------  202
Rrp43p_Ch._thermophilum      248  REMVVCS--------------------KTETMKLTIKGLPIACSAAVFLEKE------------HGEVA  284
Rrp43p_S._cerevisiae         252  ASDLRMTIRTRGRSATIRETYEIICDQTKSVPLMINAKNIAFASNYGIVELDPECQLQNSDNSEEEEVDI  321
Consensus_ss:                       eeee                                eeeeeeeee

Conservation:                              9     99                          99                 9
RRP43_Homo_sapiens           203  -----DTLLIVDPT-GEEEHLATGTLTIVMDEEGK---LCCLHKP-GGSGLTGAKLQDCMSRAVTRHKEV  262
Rrp43p_Ch._thermophilum      285  VGEKNRHWILLDPD-RLEESLCKEVITMVVDFSDGETRIRAIEKQ-GGTVFGRELIRSFALVAEDRWKVV  352
Rrp43p_S._cerevisiae         322  DMDKLNTVLIADLDTEAEETSIHSTISILAAPSGN---YKQLTLVGGGAKITPEMIKRSLLLSRVRADDL  388
Consensus_ss:                           eeee    hhhhhh    eeeee        eeeeee        hhhhhhhhhhhhhhhhhh

Conservation:
RRP43_Homo_sapiens           263  KKLMDEVIKSMKPK  276
Rrp43p_Ch._thermophilum      353  KEVMK---------  357
Rrp43p_S._cerevisiae         389  STRFNI--------  394
Consensus_ss:                     hhhh
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Rrp46

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Conservation:                     9                                  9   999         9   9 99 9     9
RRP46_Homo_sapiens             1  MEEETHTDAKIRAENGTGSSPRGPGCSLRHFACEQNLLSRPDGSASFLQGDTSVLAGVYGPAEVKVSKEI   70
Rrp46p_S._cerevisiae           1  M----------------------------SVQAEIGILDHVDGSSEFVSQDTKVICSVTGPIEPKARQEL   42
Rrp46p_Ch._thermophilum        1  M---------------------TTTTATTAPEAALGVLPRADGSARYSHAGYTVTASVNGPIEAQRRDEH   49
Consensus_ss:                                          eeeeeeee       eeeeeee   eeeeeee

Conservation:                        9 99  9     9   9                99       9
RRP46_Homo_sapiens            71  FNKATLEVILRPKIGLPGVAEKSRERLIRNTCEAVVLGTLHPRTSITVVLQVVSDAGS------------  128
Rrp46p_S._cerevisiae          43  PTQLALEIIVRPAKGVATTREKVLEDKLRAVLTPLITRHCYPRQLCQITCQILESGEDEAEFS-------  105
Rrp46p_Ch._thermophilum       50  PYEAHVDVIVRPAAGVGGTRERHLESILQSSFAQIILVKSFPRSLIQIVLQVEESPENEYVNTKLVQASL  119
Consensus_ss:                        eeeeeeee       hhhhhhhhhhhhhhhhh  hhh   eeeeeeeeeee       hh

Conservation:                              9  99  99           9           99   9      9   9
RRP46_Homo_sapiens           129  ---LLACCLNAACMALVDAGVPMRALFCGVACALDSD---GTLVLDPTSKQEKEARAVLTFALDSV----  188
Rrp46p_S._cerevisiae         106  -LRELSCCINAAFLALVDAGIALNSMCASIPIAIIKDT--SDIIVDPTAEQLKISLSVHTLALEFVNGGK  172
Rrp46p_Ch._thermophilum      120  NFAVMPALFQTAMLALLSAGVPMRATATATAIALASENGATKTLIDPSPRQVELAQSVHVFAFTSQ----  185
Consensus_ss:                        hhhhhhhhhhhhhhh         eeeeeeeee     eeee   hhhhhh    eeeee

Conservation:                           9     9
RRP46_Homo_sapiens           189  -ERKLLMSSTKGLYSDTELQQCLAAAQAASQHVFRFYRESLQRRYSKS----------------------  235
Rrp46p_S._cerevisiae         173  VVKNVLLLDSNGDFNEDQLFSLLELGEQKCQELVTNIRRIIQDNISPRLVV------------------  223
Rrp46p_Ch._thermophilum      186  --DELLLAESEGDFTIKEWDAAYETAKNICCRPSPTMDGVQMMAIDDDRLVGPDLRHFIRSTMEAKVATD  253
Consensus_ss:                        eeeeee      hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh

Conservation:
RRP46_Homo_sapiens                -----
Rrp46p_S._cerevisiae              -----
Rrp46p_Ch._thermophilum      254  LHWKS  258
Consensus_ss:
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Rrp40

```
Conservation:                                    99                                          999
RRP40_Homo_sapiens             1  MAEPASVAAESLAGSRARAARTVLGQVVLPGEELLLPEQEDAEGPGGAVERPLSLNARACSRVRVVCGPG   70
Rrp40p_Ch._thermophilum        1  MSTT-------------------TRPFVLPGETIDPSLV------------------PTHPKHPLRLGPG   33
Rrp40p_S._cerevisiae           1  ----------------------MSTFIFPGDSFPVDP----------------------TTPVKLGPG   24
Consensus_ss:                                      eee  eeee                                  eee

Conservation:                          9 9                     99 9   9 9         9
RRP40_Homo_sapiens            71  LRRCG--DRLLVTKCGRLRHKEPGSGSGGGVYWVDSQQKRYVPVKGDHVIGIVTAKSGDIFKVDVGGSE-  137
Rrp40p_Ch._thermophilum       34  LRHVPP-SDIIPTVAGQLITNLN-----KNSMWVEYNSQRYVPTQNDLVLAQVLRSTQDSYLCLITPHTP   97
Rrp40p_S._cerevisiae          25  IYCDPNTQEIRPVNTGVLHVSAKGK-SGVQTAYIDYSSKRYIPSVNDFVIGVIIGTFSDSYKVSLQNFSS   93
Consensus_ss:                     eee     eeeeeeeeeeeee        eeeeeee           eeeeeeeee  eeeeee

Conservation:                        9    9  9 9  99  9 9 9 9     9      9  9      9   9 9     9    9 9
RRP40_Homo_sapiens            138  PASLSYLSFEGATKRNRPNVQVGDLIYGQFVVANKDMEPEMVCIDS-CGRANGMGVIGQDGLLFKVTLGL  206
Rrp40p_Ch._thermophilum        98  PATLPHLAFESATKKTRPQLQPGQLVYARVSLANRHMDPELECVNPSTGKADGLGPITGPGCVFEVSLGF  167
Rrp40p_S._cerevisiae           94  SVSLSYMAFPNASKKNRPTLQVGDLVYARVCTAEKELEAEIECFDSTTGRDAGFGILE-DGMIIDVNLNF  162
Consensus_ss:                        eeeehhh               eeeeeeeee     eeeeee hh   hh   ee    eeeee hhh

Conservation:                         9 99                              9   9 99  99
RRP40_Homo_sapiens            207  IRKLLAPD----------------CEIIQEVGKLYPLEIVFGMNGRIWVKAKTIQQTLILANILEACEH-  259
Rrp40p_Ch._thermophilum       168  ARRLLMAKSREEGKVGVLEMLAGEDPSIGEAGAGLAFETAVGRNGRVWVGSEDVKTVIIVGRALQETDRG  237
Rrp40p_S._cerevisiae          163  ARQLLFNND---------------FPLLKVLAAHTKFEVAIGLNGKIWVKCEELSNTLACYRTIMECCQK  217
Consensus_ss:                     h hhh                   hhhhhhhh    eeeeee  eeee    hhhhhhhhhhhhhhhh

Conservation:                            9
RRP40_Homo_sapiens            260  -MTSDQRKQIFSRLAES-------  275
Rrp40p_Ch._thermophilum       238  NLTIEGQRKLVRRLLREMR-----  256
Rrp40p_S._cerevisiae          218  -NDTAAFKDIAKRQFKEILTVKEE  240
Consensus_ss:                        hhhhhhhhhhhhhh
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Rrp4

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Conservation:                                                                                                  9 99
RRP4_Homo_sapiens                1  ----MAMEMRLPVARKP---------------------------------LSERLGRDTKKHLVVPGD   31
Rrp4p_S._cerevisiae              1  MSEVITITKRNGAFQNSSNLSYNN------TGISDDENDEEDIYMHD-----VNSASKSESDSQIVTPGE   59
Rrp4p_Ch._thermophilum           1  ----MPITIHAPLPPPRLHDEDSDVDMSSDSDSSSEGGVPLTSNLPSRAKPKSSLFTTTKSSSDIVTPGE   66
Consensus_ss:                         eeee                                          h         eee  e

Conservation:                          9     9999999          9999   9 9 9  9  9  99  9 99 99999 99   99 9
RRP4_Homo_sapiens               32  TITTDTGFMRGHGTYMGE--EKLIASVAGSVERVNKLICVKALKTRYIGEVGDIVVGRITEVQQKRWKVE   99
Rrp4p_S._cerevisiae             60  LVTDDPIWMRGHGTYFLD--NMTYSSVAGTVSRVNRLLSVIPLKGRYAPETGDHVVGRIAEVGNKRWKVD  127
Rrp4p_Ch._thermophilum          67  LITTSPQFMRGHGTYIPPGTTSIISSVAGTILRTNKLLSVRPLRARYTPEVGDLVVGRIIEVQARRWRVD  136
Consensus_ss:                       e      eee    eee    eeeeeeeeeeeee  eeeeee           eeeeeeeee  eeeee

Conservation:                             9 9   99999 99     999 99 9  9999  9999    99   99999999999  9
RRP4_Homo_sapiens               100 TNSRLDSVLLLSSMNLPGGELRRRSAEDELAMRGFLQEGDLISAEVQAVFSDGAVSLHTRSLKYGKLGQG  169
Rrp4p_S._cerevisiae             128 IGGKQHAVLMLGSVNLPGGILRRKSESDELQMRSFLKEGDLLNAEVQSLFQDGSASLHTRSLKYGKLRNG  197
Rrp4p_Ch._thermophilum          137 VGSTQFASLPLSAINLPGGILRKRTETDELQMRSFFSEGDLLVAEVQGVYGDGGAVLHTRSLKYGKLRNG  206
Consensus_ss:                       ee      ee                 hhhhhhhh     eeeeeeeee     eeeeee    eee

Conservation:                           9                         9 99 99  9
RRP4_Homo_sapiens               170 VLVQVSPSLVKRQKTHFH---------DLPCGASVILGNNGFIWIYPTPEHKEEEAGGFIANLEP-----  225
Rrp4p_S._cerevisiae             198 MFCQVPSSLIVRAKNHTH---------NLPGNITVVLGVNGYIWLRKTSQMDLARDTPSANNSSSIKSTG  258
Rrp4p_Ch._thermophilum          207 VFVAVSGMGGGGGVVRSRRQVWTLEGANGAGLIDVVLGVNGYVWIAKHTEDGPGEDPNASTKQVGITNLE  276
Consensus_ss:                       eeeee hhh    h                   eeeeee  eeee    hhhhhhhhhhhhhhhh  hhh

Conservation:                                                    9 9       9               9
RRP4_Homo_sapiens               226 -----------------------------VSLADREVISRLRNCIISLVTQRMMLYDTSILYCYEASLP  265
Rrp4p_S._cerevisiae             259 PTGAVSLNPSITRLEESSWQIYSDENDPSISNNIRQAICRYANVIKALAFCEIGITQQRIVSAYEASMV  328
Rrp4p_Ch._thermophilum          277 EGMSANMYSS---------------QNDRIEAETMREIARLRGVVMALVENGLRVDEDMVMRGYREAVE  330
Consensus_ss:                       hhhhhhh                   hhhhhhhhhhhhhhhhhhh      hhhhhhhhhhhhh

Conservation:
RRP4_Homo_sapiens               266 HQ-IKDILKPEIMEEIVMET--RQRLLEQEG---  293
Rrp4p_S._cerevisiae             329 YSNVGELIEKNVMESIGSDILTAEKM---RGNGN  359
Rrp4p_Ch._thermophilum          331 MALVSPEGPEDVYLGGERGRQLAAAL---TA---  358
Consensus_ss:                       hhhhhhh  hhhhhhhhhhhhhhhhh
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Csl4

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Conservation:                          9          99                    99 99                 9 9
CSL4_Homo_sapiens                1  MAP---PVRYCIPGERLCNL------------EEGSPGSGTYTRH--------GYIFSSLAGCLMKSSEN   47
Csl4p_S._cerevisiae              1  MACNFQFPEIAYPGKLICPQYGTENKDGEDIIFNYVPGPGTKLIQYEHNGRTLEAITATLVGTVRCEEEK   70
Csl4p_Chaetomium_thermoph        1  MTTT--QPTLALPGQLLGPI------------SKYQPGPGTHVHE--------SNLYSSLLGTVHVTQPA   48
Consensus_ss:                             eee  ee                  ee    eeee          eeeeeeeeeeee

Conservation:                                                                                    99  9 99
CSL4_Homo_sapiens                48  G---------------------------------------------ALPVVSVVRETESQLLPDVGAIVT   72
Csl4p_S._cerevisiae              71  KTDQEEEREGTDQSTEEEKSVDASPNDVTRRTVKNILVSVLPGTEKGRKTNKYANNDFANNLPKEGDIVL  140
Csl4p_Chaetomium_thermoph        49  RAPGPVKRLNRITPAP-------------TPAELPTISVSAARPAGSAASGLVTGRKREILPEVGNIVL  104
Consensus_ss:                                                  e  eeeee          eeee     ee      eee

Conservation:                          9       9 9 99                                    9     9 99  9
CSL4_Homo_sapiens                73  CKVSSINSRFAKVHILYVG---------------------------------SMPLKNSFRGTIRKEDV  108
Csl4p_S._cerevisiae             141  TRVTRLSLQRANVEILAVEDKPSPIDSGIGSNGSGIVAGGGSGAATFSVSQASSDLGETFRGIIRSQDV  210
Csl4p_Chaetomium_thermoph       105  CRVIRITPRQAVVTILVCG---------------------------------DTVLDAEWQGLIRVQDI  140
Consensus_ss:                       eeeeee   eeeeeeeee                                    ee        eehhh

Conservation:                       9 9  9 9     9 99999 9 9 9999   99 9 99 9 999  9      9  9    9     9
CSL4_Homo_sapiens                109  RATEKDKVEIYKSFRPGDIVLAKVISLGDAQSNYLLTTAENELGVVVAHSES--GIQMVPISWCEMQCPK  176
Csl4p_S._cerevisiae              211  RSTDRDRVKVIECFKPGDIVRAQVLSLGDG-TNYYLTTARNDLGVVFARAANGAGGLMYATDWQMMTSPV  279
Csl4p_Chaetomium_thermoph        141  RATEKDRVKVYESFRPGDIVRAEVISLGDQ-ANYYLSTARNELGVILATSEA--GNTMYPVSWREYRDPI  207
Consensus_ss:                             hhhh     eeeeeeeee     eeeeee      eeeee       eeeee   eee

Conservation:                       9   9 99 9
CSL4_Homo_sapiens                177  THTKEFRKVARVQPEFLQT  195
Csl4p_S._cerevisiae              280  TGATEKRKCAKPF------  292
Csl4p_Chaetomium_thermoph        208  TGLTELRKVAKPY------  220
Consensus_ss:                          eeeee
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Dis3

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Conservation:                                    9         9   99999 999 99 9    9  9       9
DIS3_Homo_sapiens                1  M-------------------LKSKTFLKKT-RAGGVMKIVREHYLRDDIGCGAPGCAACGGAHEGPALEP   50
Dis3p_S._cerevisiae              1  MSVPAIAPRRKRLADGLS--VTQKVFVR-S-RNGGATKIVREHYLRSDIPCLSRSCTKCPQIVVPDAQNE   66
Dis3p_Ch._thermophilum           1  ----MASLKRSLESDPLAANISSKVYVR-STKSGKVQKIVREVYLRTDIPCSSKLCKSCITGAPRDAQGR   65
Consensus_ss:                        hhhhh          e  eeeeeeeeee     eeeeeeeeee       hhhhhh

Conservation:                              99   999  9   9  9       999 9 99 9 99 9 9 9 9
DIS3_Homo_sapiens                51  QP------QDPASSVCPQPHYLLPDTNVLLHQIDVLEDP-AIRNVIVLQTVLQEVRNRSAPVYKRIRDVT  113
Dis3p_S._cerevisiae              67  LPKFILSDSPLELSAPIGKHYVVLDTNVVLQAIDLLENPNCFFDVIVPQIVLDEVRNKSYPVYTRLRTLC  136
Dis3p_Ch._thermophilum           66  IAPFVLSDQPAGTKAFPQGHYLVPDTNAFLTAMDLFESSAAFYDVIVLQVVLEELRNRSLPLYNRLINLT  135
Consensus_ss:                            eeeeeehhhhhhhhhhh        eeeehhhhhhhhh  hhhhhhhhhh

Conservation:                         9 9  9 99    9 9     9  9999999 9    99 9999            9    99
DIS3_Homo_sapiens                114  NNQ--EKHFYTFTNEHHRETYVEQEQGENANDRNDRAIRVAAKWYNEHLKKMSADNQ----LQVIFITND  177
Dis3p_S._cerevisiae              137  RDSDDHKRFIVFHNEFSEHTFVERLPNETINDRNDRAIRKTCQWYSEHLKPY----D----INVVLVTND  198
Dis3p_Ch._thermophilum           136  KSED--KRFYVFFNEFRFETHVARLEGESVNDRNDRAVRKAVAWYAEHLKKT----GKKNVPAVVMLSND  199
Consensus_ss:                           eeee    hhhh         hhh  hhhhhhhhhhhhhhhhh           eeeee h
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Conservation:                      9 9   9  9    9       9   9       9                        9         9
DIS3_Homo_sapiens           178    RRNKEKAIEE---GIPAFTCEEYVKSLTANPELIDRLACLSEEGNEIE---SGKIIFSEHLPLSKLQQGI  241
Dis3p_S._cerevisiae         199    RLNREAATKEVESNIITKSLVQYIELLPNADDIRDSIPQMDSFDKDLERDTFSDFTFPEYYSTARVMGGL  268
Dis3p_Ch._thermophilum      200    RANLRKAKEE---GIHACSLAEYVSQLPDGDALLDMVPATEE-EETIKEKRPNEMLYPEYYSMSKMMTGI  265
Consensus_ss:                      hhhhhhhhh      eeeehhhhhhh    hhhhhhhhhhhhhhh            hhhhhhhhhhhhh

Conservation:                      9 9   99    9  9 99           9     9    999   9 9 99 99   9  99  9
DIS3_Homo_sapiens           242    KSGTYLQGTFRASRENYLEATVWIHGDNEENKEIILQGLKHLNRAVHEDIVAVELLPKSQWVAPSSVVLH  311
Dis3p_S._cerevisiae         269    KNGVLYQGNIQISEYNFLEGSVSLPR---FSKPVLIVGQKNLNRAFNGDQVIVELLPQSEWKAPSSIVLD  335
Dis3p_Ch._thermophilum      266    KNGLLHQGIFNVSPYNYLEGSIRVPA---FQKPLLILGRENINRAIDGDLVVVEVLPKDQWKEPSTQVIE  332
Consensus_ss:                      h   eeeeeeee      eeeeee         eeehhhhhh       eeeeeeeehhh     eeeee

Conservation:                       9                                                  99  99    9
DIS3_Homo_sapiens           312    DEGQ-----------------------NEEDVEKEEETERMLKTAVSEKML----KPTGRVVGIIKRN  352
Dis3p_S._cerevisiae         336    SEHFDVNDNPDIEAGDDDDNNESSSNTTVISDKQRRLLAKDAMIAQRSK--KI----QPTAKVVYIQRRS  399
Dis3p_Ch._thermophilum      333    EEAITKNENPDAE----------ESTDLVSEKERKALQEEVK-RMHSK--TTEGHPQPTARVVGVIKRN  388
Consensus_ss:                      e                       ee hhhhhhhh   eeeeeee          eeeeeeeeee

Conservation:                      99 9 9    9                    9   9  9  99    9   99   99 9      9 99
DIS3_Homo_sapiens           353    WRPYCGMLSKSDIK-------ESRRHLFTPADKRIPRIRIETRQASTLEGRRIIVAIDGWPRNSRYPNGH  415
Dis3p_S._cerevisiae         400    WRQYVGQLAPSSVD---PQSSSTQNVFVILMDKCLPKVRIRTRRAAELLDKRIVISIDSWPTTHKYPLGH  466
Dis3p_Ch._thermophilum      389    WRQYVGHIDQSSISQSAQQGRKQDSVFVIPMDRRIPKIRLRTRQVAELVGKRILVAIDAWDRSSRHPTGH  458
Consensus_ss:                       eeeeeeeee              eeeeee      eeeee    hhh   eeeeeeee         ee

Conservation:                        9 99       999 9999 99   99   99  99                        9 999
DIS3_Homo_sapiens           416    FVRNLGDVGEKETETEVLLLEHDVPHQPFSQAVLSFLPKMP------------WSITEKDMKNREDLRH  472
Dis3p_S._cerevisiae         467    FVRDLGTIESAQAETEALLLEHDVEYRPFSKKVLECLPAEGHDWKAPTKLDDPEAVSKDPLLTKRKDLRD  536
Dis3p_Ch._thermophilum      459    LIRSLGEMETNAAETEALLLEYDVQYRPFPKTVLDCLPKEGHDWRVPEDKNDPG-------WRDREDLRH  521
Consensus_ss:                      eeeee       hhhhhhhhh          hhhhhhhhh        hhhhhhh    hhhh     hh

Conservation:                        999 99 99 9999999   9 999 999999999 99  9  9 9 9   999 999  99999 9
DIS3_Homo_sapiens           473    LCICSVDPPGCTDIDDALHCRELENGNLEVGVHIADVSHFIRPGNALDQESARRGTTVYLCEKRIDMVPE  542
Dis3p_S._cerevisiae         537    KLICSIDPPGCVDIDDALHAKKLPNGNWEVGVHIADVTHFVKPGTALDAEGAARGTSVYLVDKRIDMLPM  606
Dis3p_Ch._thermophilum      522    LLICSIDPIGCQDIDDALHSRPLPNGNFEVGVHIADVSHFVRPNNAMDLEASIRGTTVYLVDKRIDMLPT  591
Consensus_ss:                       eeeee          eeeeee     eeeeeee  hhhh     hhhhhhhh   eee    ee    h

Conservation:                      99   99999  9 9 999  99    9 9    9 9999 9     9  99 9 9     9  9   9
DIS3_Homo_sapiens           543    LLSSNLCSLKCDVDRLAFSCIWEMNHNAEILKTKFTKSVINSKASLTYAEAQLRIDSANMNDDITTSLRG  612
Dis3p_S._cerevisiae         607    LLGTDLCSLKPYVDRFAFSVIWELDDSANIVNVNFMKSVIRSREAFSYEQAQLRIDDKTQNDELTMGMRA  676
Dis3p_Ch._thermophilum      592    LLGTDLCSLKPYVERYAFSVLWELTPDADIVSVRFTKSVIKSREAFSYEQAQMRVDDGSYQDDLTKSIRT  661
Consensus_ss:                      hhhhhhh        eeeeeeeee     eeeeeeeeeeee      hhhhhhhhh     hhhhhhhhh

Conservation:                      9  9   99  9   999 9 9999  99 999 99     9    9 9 9999999999999 99   9
DIS3_Homo_sapiens           613    LNKLAKILKKRRIEKGALTLSSPEVRFHMDSETHDPIDLQTKELRETNSMVEEFMLLANISVAKKIHEEF  682
Dis3p_S._cerevisiae         677    LLKLSVKLKQKRLEAGALNLASPEVKVHMDSETSDPNEVEIKKLLATNSLVEEFMLLANISVARKIYDAF  746
Dis3p_Ch._thermophilum      662    LLALSRKLKQKRMDAGALSLSSPEVKIHMESETSDPIDIKTKKHLPTMSLVEEFMLLANISVARKIYEAF  731
Consensus_ss:                      hhhhhhhhhhhhhh          eeeeee     eeeeeee   hhhhhhhhhhhhhhhhhhhhhhhh

Conservation:                         9 99 9 999  9   9                9 99 999    9  9  999 99  99999 9
DIS3_Homo_sapiens           683    SEHALLRKHPAPPPSNYEILVKAARSRN-LEIKTDTAKSLAESLDQAESPTFPYLNTLLRILATRCMMQA  751
Dis3p_S._cerevisiae         747    PQTAMLRRHAAPPSTNFEILNEMLNTRKNMSISLESSKALADSLDRCVDPEDPYFNTLVRIMSTRCMMAA  816
Dis3p_Ch._thermophilum      732    PQTAILRRHAAPPKTNFDELANQLRVKRGLELSVESSKALADSLDRCVDPNEPFFNTLVRIMATRCMMAA  801
Consensus_ss:                         eeeee     hhhhhhhhhhhhhh   ee       hhhhhhhhh      hhhhhhhhhhhhh

Conservation:                       99 99      9 99999  999999999999 9   99 99 999                9 9 9
DIS3_Homo_sapiens           752    VYFCSGM--DNDFHHYGLASPIYTHFTSPIRRYADVIVHRLLAVAIGADCTYPELTDKHKLADICKNLNF  819
Dis3p_S._cerevisiae         817    QYFYSGAYSYPDFRHYGLAVDIYTHFTSPIRRYCDVVAHRQLAGAIGYEPLSLTHRDKNKMDMICRNINR  886
Dis3p_Ch._thermophilum      802    EYFCSGTQAYPEFRHYGLASEIYTHFTSPIRRYADLVAHRQLAAAIGYEAIDPSVRSRGRLEAVCKNINV  871
Consensus_ss:                      eeee           hh  hhhh     hhhhhhhhhhhhhhhhhhh          hhhhhhhhhhhhh

Conservation:                       9  99 9 999               9         9  999 9  9 99               9
DIS3_Homo_sapiens           820    RHKMAQYAQRASVAFHTQLFFKSKGIVSEEAYILFVRKNAIVVLIPKYGLEGTVFFEEKDKPNPQLIYDD  889
Dis3p_S._cerevisiae         887    KHRNAQFAGRASIEYYVGQVMRNNES-TETGYVIKVFNNGIVVLVPKFGVEGLIRLDNLTEDPNSAAFDE  955
Dis3p_Ch._thermophilum      872    RHRNAQLAGRASIAYYVGQALKGKAE-EEDGFVMKIFSNGFVVLVPRFGIEGLIRLRDLAEPEPQAEYDA  940
Consensus_ss:                      hhhhhhhhhhhhhhhhhhhhhhhh    eeeeeeeeee  eeeeeee   eeeeeehhh      eeeee

Conservation:                         9              9 99 9            9      9
DIS3_Homo_sapiens           890    EIPSLKIE------DTVFHVFDKVKVKIMLDSSNLQHQKI--RMSLVEPQIPGISIPTDTSNMDLNGPKK  951
Dis3p_S._cerevisiae         956    VEYKLTFVPTNSDKPRDVYVFDKVEVQVRSVMDPIT-SKR--KAELLLK---------------------- 1001
Dis3p_Ch._thermophilum      941    ENYVLK---TKGSREVTVELFQKVRVRVSDQKDEAT-GKRGVKMELVKTY---------------------  986
Consensus_ss:                       eeeeeeeee     eeeee  eeeeeeeeee      ee  eeeeee

Conservation:
DIS3_Homo_sapiens           952    KKMKLGK  958
Dis3p_S._cerevisiae                -------
Dis3p_Ch._thermophilum             -------
Consensus_ss:
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Supplementary materials and methods

Strain construction

rrp41^{4M} mutant

Mutations K62E S63D R95E R96E were introduced into the *RRP41* gene by *in vivo* recombination into a BMA64 wild type diploid strain. A DNA fragment containing the *RRP41* gene with K62E S63D R95E R96E mutations and Sp.*HIS5* selection marker was amplified using ADZKD100 and ADZKD101 primers in overlap PCR with two templates: first – *RRP41* gene with K62E S63D R95E R96E mutations amplified on the pRS415 plasmid with the cloned *RRP41* gene containing K62E S63D R95E R96E mutations (pADZ433), second – Sp.*HIS5* gene amplified on pFA6A-link-yEGFP-Sp.*HIS5* vector. Spores were dissected and those harboring *RRP41* mutations yielded strain ADZY522.

dis3^{endo-} mutant

The D171N mutation was introduced into the *DIS3* gene in the BMA64 wild type haploid strain by *in vivo* recombination. The *DIS3* gene with the D171N mutation, protein A sequence and Kl*TRP1* selection marker was amplified using ADZKD102 and ADZKD103 primers and pBS3278 vector as a template. This gave strain ADZY531.

rrp41^{4M} *dis3*$^{endo-}$ mutant

The ADZY531 strain was modified by introducing *RRP41* K62E S63D R95E R96E mutations and Sp.*HIS5* selection marker by *in vivo* recombination using a DNA fragment amplified from ADZY522 total DNA as a template using ADZKD100 and ADZKD101 primers. This gave the ADZY537 strain.

rrp41^{4M} *dis3*$^{exo-}$ mutant

A DNA fragment containing the *RRP41* gene with K62E S63D R95E R96E mutations and Sp.*HIS5* selection marker was amplified using primers ADZKD100 and ADZKD101 with ADZY522 total DNA as a template and introduced into the BSY1735 (1) strain by *in vivo* recombination. This gave strain ADZY539.

dcp$^{1-2}$ *rrp41*^{4M} mutant

A DNA fragment containing the *RRP41* gene with K62E S63D R95E R96E mutations and Sp.*HIS5* selection marker was amplified using primers ADZKD100 and ADZKD101 with ADZY522 total DNA as a template and introduced into the BSY1665 (1) strain by *in vivo* recombination. This gave strain ADZY649.

The strain was transformed with pRP485 (containing *MFA2*pG), pRP603 (containing wild type *PGK1*pG), pRP611 (containing *PGK1*pG with PTC), pRP1079 (containing nonstop *PGK1*pG) and pRP1252 (containing *PGK1*pG with stem loops).

RRP41 tet-off *rrp6Δ*

A DNA fragment containing Sp.*HIS5* selection marker and sequences flanking the *RRP6* gene was amplified using primers ADZKD113 and ADZKD114 with pFA6A-link-yEGFP-Sp.*HIS5* vector as a template and introduced into the endogenous *RRP6* locus in the TH_3687 strain (2). This gave strain ADZY524.

RRP4 tet-off Mtr4gfp

A DNA fragment containing the *MTR4* gene with gfp sequence and Sp*HIS3*MX6 selection marker was amplified using ADZKD117 and ADZKD118 primers with total DNA from a yeast strain with Mtr4 gfp-tagged (3) as a template and introduced into the TH_6151 strain (2) with the *RRP4* gene under the control of the tet-off promoter by *in vivo* recombination. This gave strain ADZY712.

Supplementary references

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3. Huh,W.K., Falvo,J.V., Gerke,L.C., Carroll,A.S., Howson,R.W., Weissman,J.S. and O'Shea,E.K. (2003) Global analysis of protein localization in budding yeast. *Nature*, **425**, 686-691.