SUPPLEMENTARY DATA

Dis3-like 1: a novel exoribonuclease associated with the human exosome

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Yeast and human Dis3 and Dis3-like sequence alignment.

A multiple alignment of the amino acid sequences of yeast Dis3 (yDis3), human Dis3 (hDis3), human Dis3-like 1 (hDis3L1) and human Dis3-like 2 (hDis3L2) was generated by the MUSCLE algorithm (Edgar, 2004). The secondary structure of hDis3L1, as predicted by PsiPred, is depicted below the sequence alignment; β-strands are represented with green arrows and α-helices with red bars. Protein domains predicted by SMART are indicated by colored boxes surrounding the sequences. The graphical presentation of the

alignments was generated with Jalview (Waterhouse *et al.*, 2009) using the default colour scheme used for alignments in Clustal X.

Figure S2: hDis3L1 lacks endonuclease activity.

(**A**) HEp-2 cells were transfected with expression constructs encoding EGFP or EGFP-hDis3L1 and after 48 hrs cell lysates were subjected to immunoprecipitation with anti-GFP antibodies. Precipitated material was incubated with a radiolabeled substrate RNA (Input) and the reaction products were subsequently analyzed by denaturing polyacrylamide gel electrophoresis followed by autoradiography. The incubations were performed in the presence the indicated concentrations of Mn^{2+}. (**B**) Ribonuclease assay as described above (A), but with immunoprecipitated hDis3L1 mutants D62N, D166N, D486N (in addition to the wild type protein) in the presence of 1 mM Mn^{2+}. Mononucleotide degradation products, most likely resulting from low levels of exoribonuclease activity, are indicated.

Figure S3: SiRNA-mediated hDis3L1 knock-down.

The lysates from HEp-2 cells transfected with siRNAs for EGFP or hDis3L1 that were used for the activity assay, as depicted in Figure 4B, were analyzed by incubating western blots with anti-hDis3L1 and anti-hRrp4 antibodies. Anti-γ-tubulin antibodies were used as a loading control.

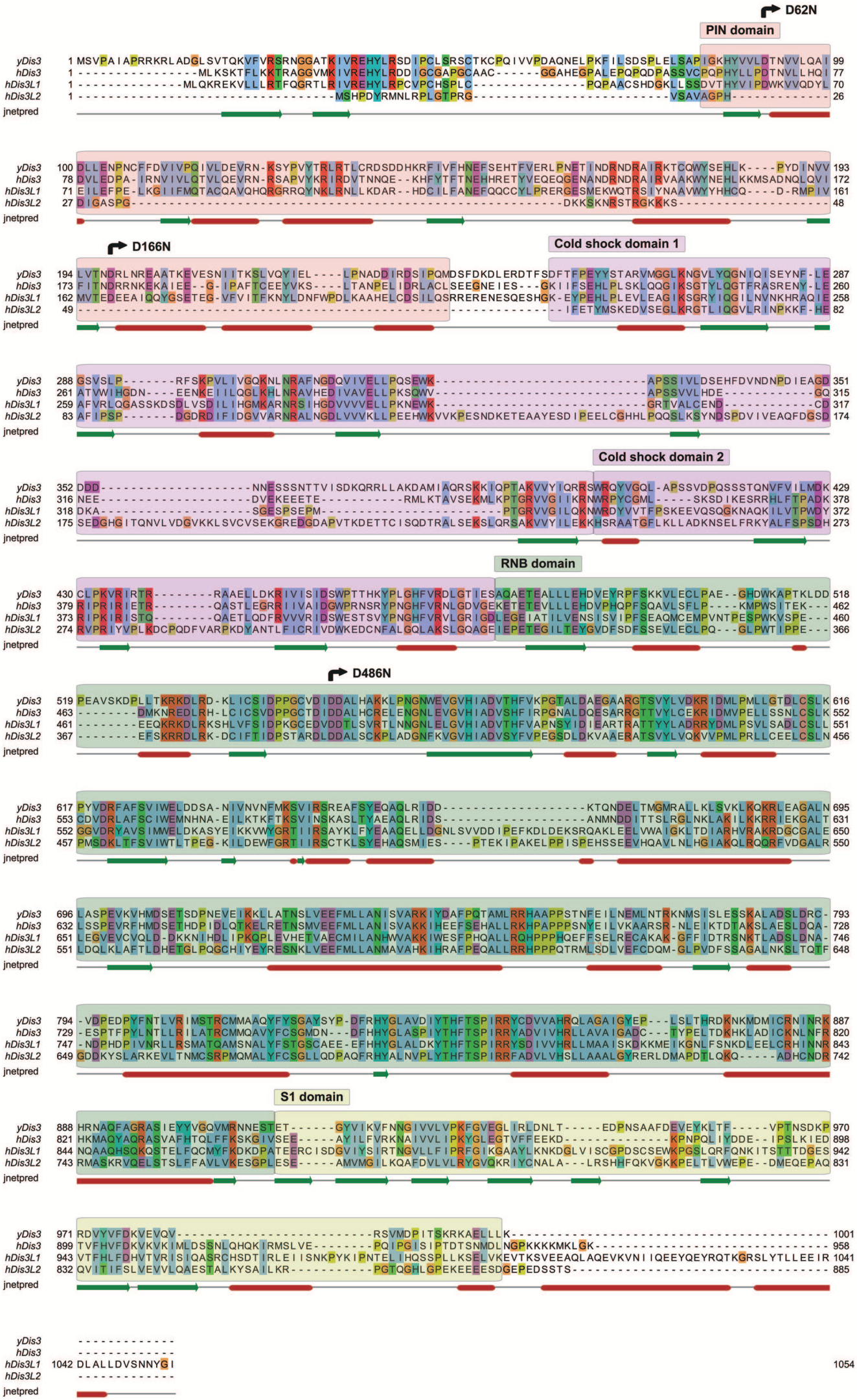
Figure S4: Analysis of cell fractionation and efficiency of knock-down for cells used to study cytoplasmic rRNA degradation.

(**A**) Following cell fractionation, RNA purified from the cytoplasmic and nuclear fractions was stained with EtBr and the rRNA and tRNA distribution was compared with that of total cellular RNA. Tot., total cell RNA; Cyt., cytoplasmic RNA; Nuc., nuclear RNA. In addition, the RNA samples were subjected to northern blot hybridization using a probe for the nuclear U2 snRNA. The positions of the various RNAs are indicated. (**B**) The efficiency of siRNA-mediated silencing of exosome subunits hDis3L1, hRrp40 and PM/Scl-100 was monitored by RT-PCR using RNA isolated from the respective cells. RT-PCR analysis of β-actin mRNA was performed in parallel to control for equal amounts of starting material with the RNA from mock and siRNA-transfected cells.

Figure S5: hDis3 does not stably interact with the exosome core.

Anti-hRrp40 antibodies were used to precipitate exosome complexes from a HEp-2 cell lysate in the presence of NaCl concentrations ranging from 25 mM to 150 mM, as indicated. The coprecipitation of hDis3 and hRrp4 (exosome core control) was monitored by western blotting using polyclonal and monoclonal antibodies, respectively, to these proteins. In the 'Input' lane the total cell lysate was loaded.

Staals *et al.*, Supplementary Figure S1



Staals *et al*., Supplementary Figure S2A-B

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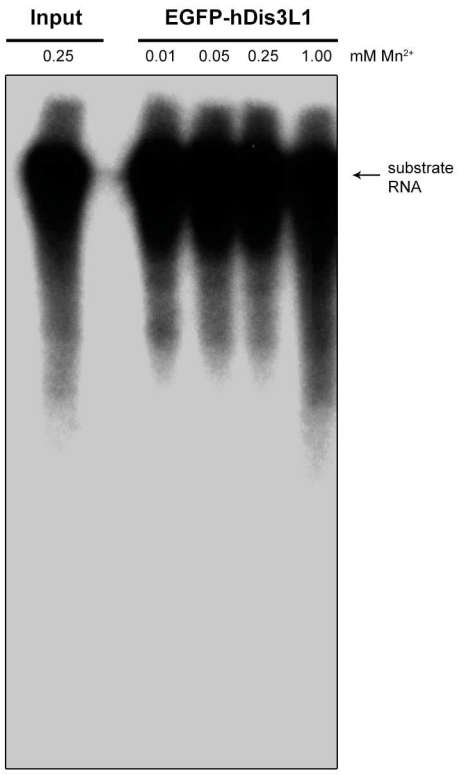
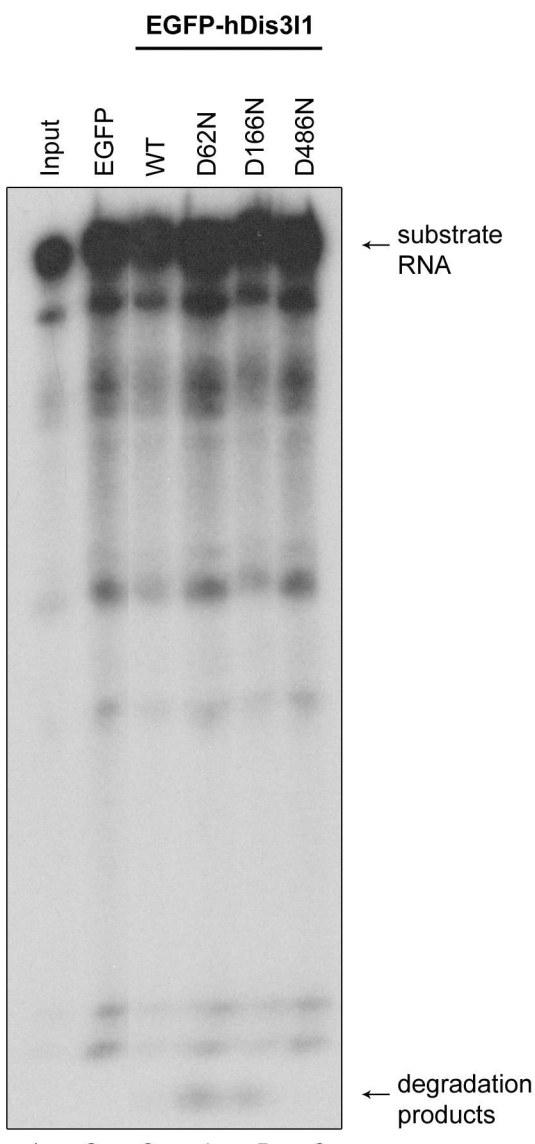
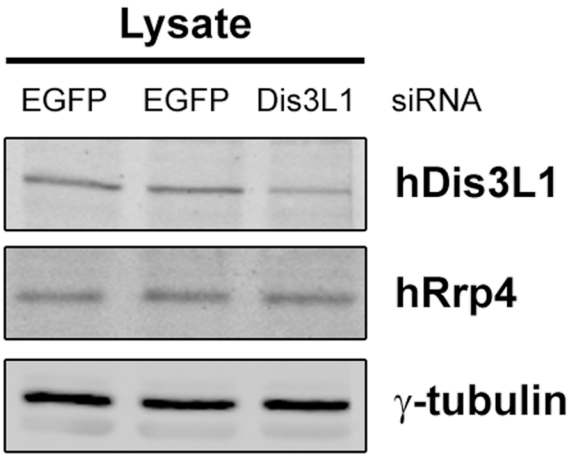


(B)

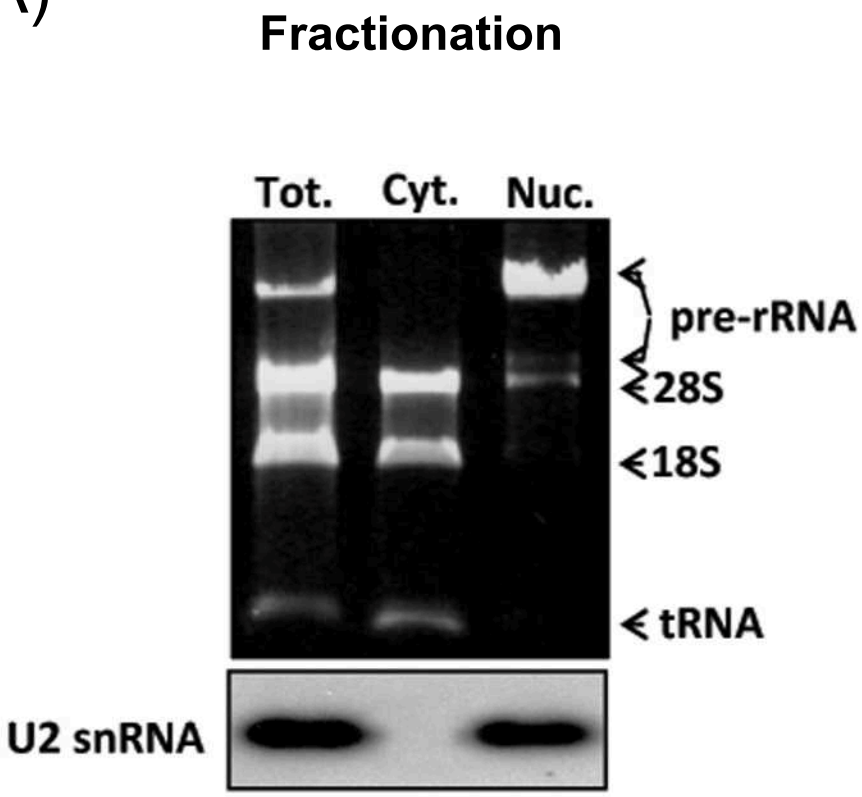


Staals *et al*., Supplementary Figure S3

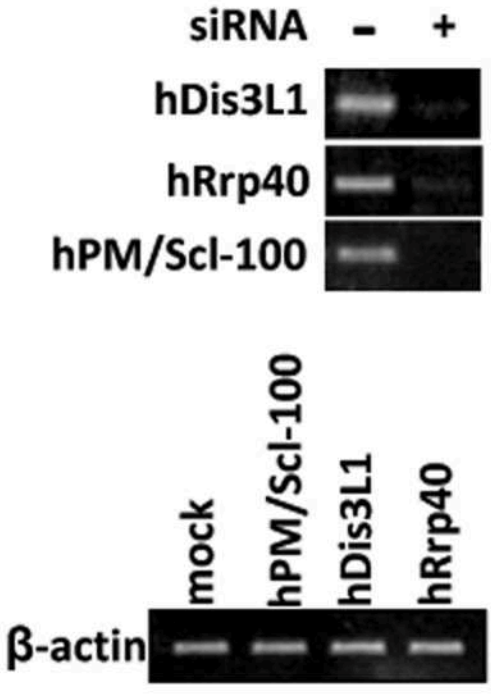


Staals *et al*., Supplementary Figure S4A-B

(A)



(B)



Staals *et al*., Supplementary Figure S5