### 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;  $\beta$ -strands are represented with green arrows and  $\alpha$ -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 EGFPhDis3L1 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<sup>2+</sup>. (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<sup>2+</sup>. 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- $\gamma$ -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  $\beta$ -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

|                                                  |                          |                                                                                                                                                                                                                                                                                                                                                                                                      | PIN domain                                                                                                                                         |
|--------------------------------------------------|--------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 1<br>1<br>1              | MSVPAIAPRRKRLADGLSVTQKVFVRSRNGGATKIVREHYLRSDIPCLSRSCTKCPQIVVPDAQNELPKFILSDSPLELSAP<br>- MLKSKTFLKKTRAGGVMKIVREHYLRDDIGCGAPGCAACGGAHEGPALEPQPQDPASSVCF<br>- MLQKREKVLLLRTFQGRTLRIVREHYLRPCVPCHSPLC                                                                                                                                                                                                    | GKHYVVLDTNVVLQAI99<br>QPHYLLPDTNVLLHQI77<br>VTHYVIPDWKVVQDYL70<br>GPH26                                                                            |
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 100<br>78<br>71<br>27    | DLLENPNCFFDVIVPQIVLDEVRN-KSYPVYTRLRTLCRDSDDHKRFIVFHNEFSEHTFVERLENETINDRNDRAIRKTCOW<br>DVLEDPA-IRNVIVLQTVLQEVRN-RSAPVYKRIRDVTNNQEKHFYTFTNEHHRETYVEQEQGENANDRNDRAIRKTCOW<br>EILEFPE-LKGIIFMQTAGQAVQHORGRQYNKLRNLLKDAR-HDCILFANEFQQCCYLPRERGESMEKWQTSJVNAAVW<br>DIGASPG                                                                                                                                 | SEHLK PYD I NVV 193<br>NEHLKKMSADNQLQV I 172<br>YHHCQ D - RMP I V 161<br>48                                                                        |
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 194<br>173<br>162<br>49  | LVTNDRLNREAATKEVESNIITKSLVQYIELLPNADDIRDSIPQMDSFDKDLERDTFSDFTFPEYYSTARVMGGLKNGV<br>FITNDRRNKEKAIEEG-IPAFTCEEYVKSLTANPELIDRLACLSEEGNEIESGKIIFSEHLPLSKLQQGIKSGT<br>MVTEDEEAIQQYGSETEG-VFVITFKNYLDNFWPDLKAAHELCDSILQSRRERENESQESHGK-EYPEHLPLEVLEAGIKSGT<br>- IFETYMSKEDVSEGLKRGT                                                                                                                        | Y L Y Q G N L Q I SEYNF - L E 287<br>Y L Q G T F A S R E NY - L E 260<br>Y I Q G I L N V N K H R A Q I E 258<br>L I Q G V L R I N P K K F - H E 82 |
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 288<br>261<br>259<br>83  | GSVSLPRFSKPVLIVGOKNLNRAFNGDQVIVELLPQSEWK                                                                                                                                                                                                                                                                                                                                                             | SEHFDVNDNPD I EAGD 351<br>IDE GQ 315<br>IND CD 317<br>IND SPDV I VEAQFDGSD 174                                                                     |
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 352<br>316<br>318<br>175 | Cold shock domai<br>DDD                                                                                                                                                                                                                                                                                                                                                                              | PQSSSTQNVFVILMDK 429<br>SDIKESRRHLFTPADK 378<br>ISQCKNAQKILVTPWDY 372<br>INSELFRKYALFSPSDH 273                                                     |
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 430<br>379<br>373<br>274 | RNB domain<br>CLPKVR IRTRRAAELLDKR IVIS IDSWPTTHKYPLGHFVRDLGT IES AGAETEALLLEHDVEYRPFSKKVLEOLF<br>RIPRIR IETRQASTLEGRR I IVAIDGWPRNSRYPNGHFVRNLGDVGEKETETEVLLLEHDVPHQPFSQAVLSFLF<br>RIPKIR ISTQQAETLQDFRVVVRIDSWESTSVYPNGHFVRVLGRIGDLEGEIATILVENSISVIPFSEAQMCEME<br>RVPR I YVPLKDCPQDFVARPKDYANTLFICR IVDWKEDCNFALGQLAKSLGQAGE IEPETEGILTEYGVDFSDFSSEVLECLF                                          | AE GHDWKAPTKLDD 518<br>KMPWSITEK 462<br>VNTPESPWKVSPE 460<br>QGLPWTIPPE 366                                                                        |
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 519<br>463<br>461<br>367 | D486N  PEAVSKDPLLTKRKDLRD-KLICSIDPPGCVDIDDALHAKKLPNGNWEVGVHIADVTHFVKPGTALDAEGAARGTSVYLVDKE DMKNREDLRH-LCICSVDPPGCTDIDDALHCRELENGNLEVGVHIADVSHFIRPGNALDQESARRGTTVYLCEKF COKRKDLRKSHLVFSIDPKGCEDVDDTLSVRTLNNGNLELGVHIADVTHFVAPNSYIDIEARTRATTYYLADRE COKRKDLRKSHLVFSIDPKGCEDVDDTLSVRTLNNGNLELGVHIADVSHFIPGSDLDKVAAERATSVYLVDK COKRKDLRKSHLVFSIDPKGCEDVDDTLSVRTLNNGNLELGVHIADVSHFVPEGSDLDKVAAERATSVYLVDK | IDMLPMLLGTDLCSLK 616<br>IDMVPELLSSNLCSLK 552<br>YDMLPSVLSADLCSLL 551<br>VPMLPRLLCEELCSLN 456                                                       |
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 617<br>553<br>552<br>457 | PYVDR FAFSV IWELDDSA-NIVNVNFMKSVIRSREAFSYEQAOLRIDD                                                                                                                                                                                                                                                                                                                                                   | KLSVKLKOKRLEAGALN 695<br>KLAKILKKRRIEKGALT 631<br>FARHVRAKRDGCGALE 650<br>STAKQLROORFVDGALR 550                                                    |
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 696<br>632<br>651<br>551 | LASPEVKVHMDSETSDPNEVEIKKLLATNSLVEEFMLLANISVARKIYDAFPQTAMLRRHAAPPSTNFEILNEMLNTRKNMS<br>LSSPEVRFHMDSETHDPIDLQTKELRETNSMVEEFMLLANISVAKKIHEEFSEHALLRKHPAPPPSNYEILVKAARSS-NLE<br>LEGVEVCVQLD-DKKNIHDLIPKQPLEVHETVAECMILANHWVAKKIWESFPHQALLRQHPPPHQEFFSELRECAKAK-GFF<br>LDQLKLAFTLDHETGLPQGCHIYEYRESNKLVEEFMLLANMAVAHKIHRAFPEQALLRRHPPPQTRMLSDLVEFCDQM-GLP                                                 | SLESSKALADSLDRC - 793<br>KTDTAKSLAESLDQA - 728<br>DTRSNKTLADSLDNA - 746<br>DFSSAGALNKSLTQTF 648                                                    |
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 794<br>729<br>747<br>649 | - VDPEDPYFNTLVR IMSTRCMMAAQYFYSGAYSYP - DFRHYGLAVD IYTHFTSP IRRYCDVVAHRQLAGA IGYEP LSLT<br>- ESPTFPYLNTLLR ILATRCMMQAYYFCSGMDN DFHHYGLASP IYTHFTSP IRRYADV IVHRLLAVA IGADC TYPEL<br>- NDPHDP IVNRLLRSMATGAMSNALYFSTGSCAEE - EFHHYGLALDKYTHFTSP IRRYSD IVVHRLLAVA IGADC TYPEL<br>GDDKYSLARKEVLTNMCSRPMQMALYFCSGLLQDPAQFRHYALNVPLYTHFTSP IRRFADVLVHSLLAAALGYRERLDMAPD                                  | RDKNKMDMICRNINRK 887<br>TDKHKLADICKNLNFR 820<br>FSNKDLEELCRHINNR 843<br>LQKQADHCNDR 742                                                            |
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 888<br>821<br>844<br>743 | HRNAQFAGRAS I EYYVGQVMRNNEST ET GYVI KVFNNG I VVL VPKFGVEGL I RLDNLT EDPNSAAFDEVEY<br>HKMAQYAQRASVAFHTQLFFKSKG I VSEE AY I LFVRKNA I VVL I PKYGLEGTVFFEEKO KPNP<br>NQAAQHSQKQSTELFQCMYFKDKDPATEERC I SDGVI YS I RTNGVLLF I PRFG I KGAAYL KNKDGLV I SCOPDSCSEWKPGSL<br>RMASKRVQELSTSLFFAVLVKESGPLESE AMVMG I LKQAFDVLVLRYGVQKR I YCNALA LRSHHFQKVQKKPELT                                              | LTFVPTNSDKP<br>01 YDDEIPSLKIED 898<br>0RFQNKITSTTDGES 942<br>1 WEPEDMEQEPAQ 831                                                                    |
| yDis3<br>hDis3<br>hDis3L1<br>hDis3L2<br>jnetpred | 971<br>899<br>943<br>832 | RDVYVFDKVEVQV                                                                                                                                                                                                                                                                                                                                                                                        | 1001<br>958<br>/RQTKGRSLYTLLEEIR 1041<br>885                                                                                                       |

P D62N

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



(B)

(A)

EGFP-hDis3l1



## Staals et al., Supplementary Figure S3



# Staals et al., Supplementary Figure S4A-B



## Staals et al., Supplementary Figure S5

