Figure S1

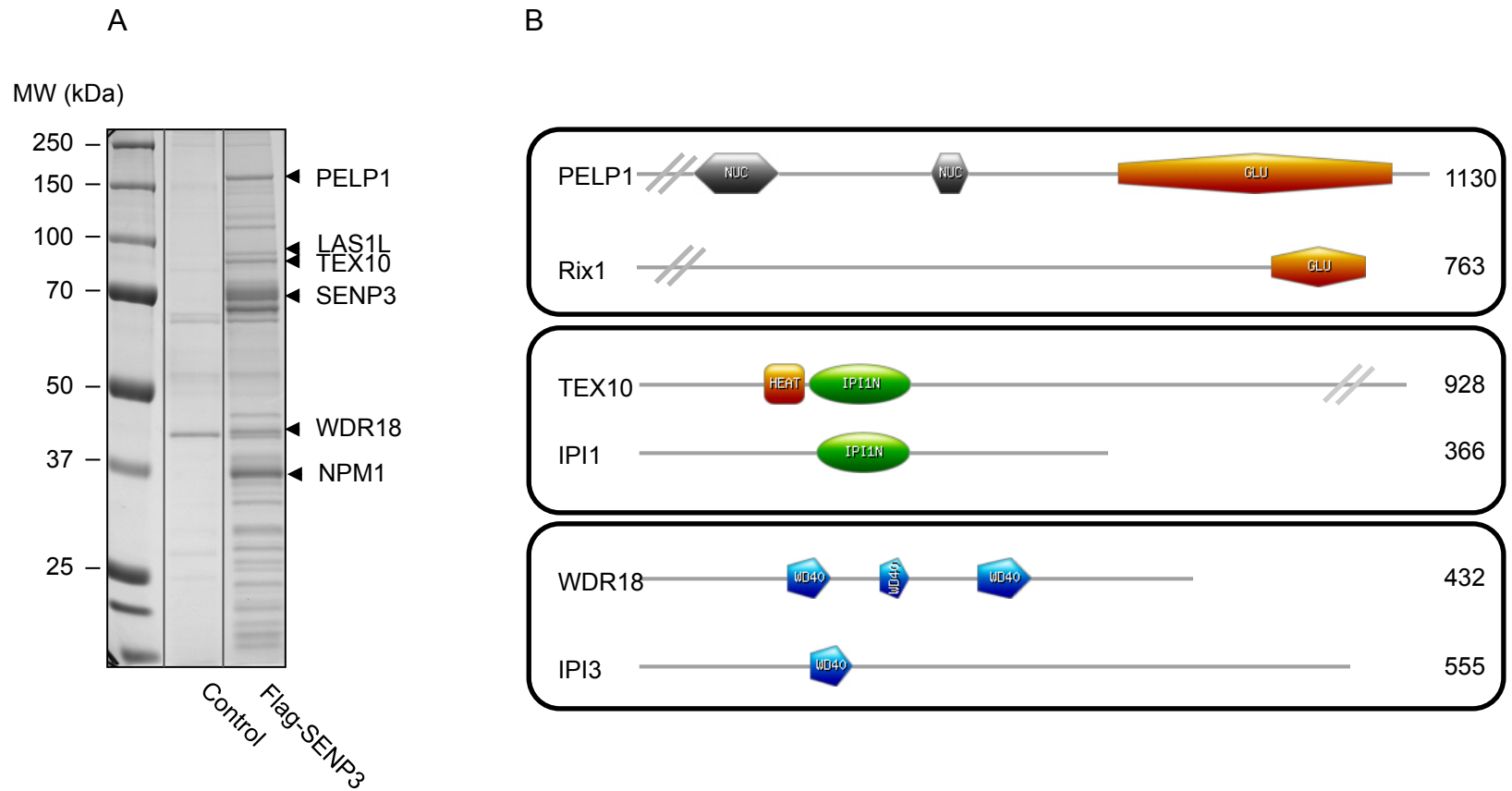

A
MW (kDa)
250
150
100
70
50
37
25
PELP1
LAS1L
TEX10
SENP3
WDR18
NPM1
Control
Flag-SENP3
B
PELP1
NUC
NUC
GLU
1130
Rix1
GLU
763
TEX10
HEAT
IPI1N
928
IPI1
IPI1N
366
WDR18
WD40
WD40
WD40
432
IPI3
WD40
555

Figure S2 A

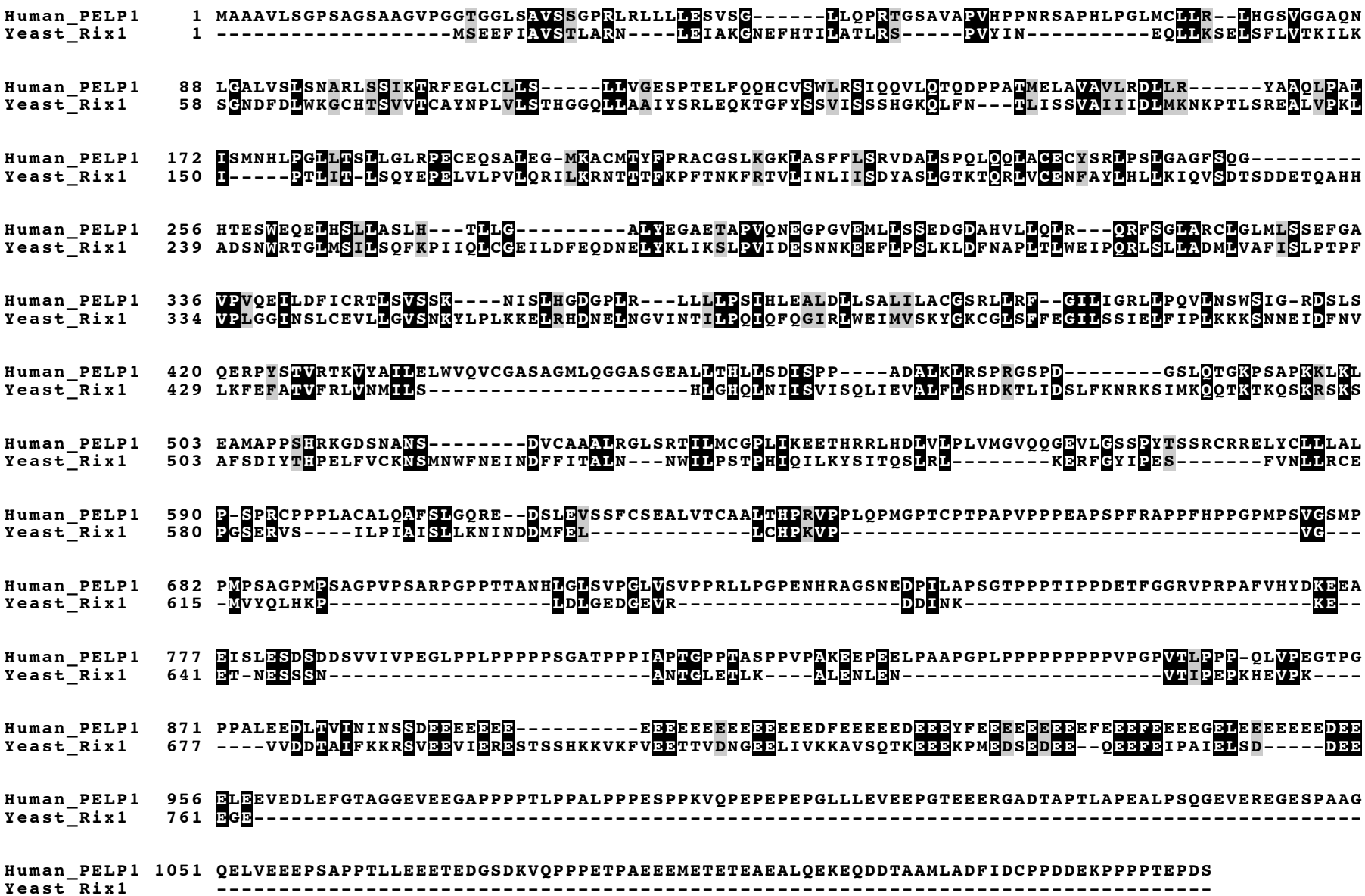

Human_PELP1 1
Yeast_Rix1 1
Human_PELP1 88
Yeast_Rix1 58
Human_PELP1 172
Yeast_Rix1 150
Human_PELP1 256
Yeast_Rix1 239
Human_PELP1 336
Yeast_Rix1 334
Human_PELP1 420
Yeast_Rix1 429
Human_PELP1 503
Yeast_Rix1 503
Human_PELP1 590
Yeast_Rix1 580
Human_PELP1 682
Yeast_Rix1 615
Human_PELP1 777
Yeast_Rix1 641
Human_PELP1 871
Yeast_Rix1 677
Human_PELP1 956
Yeast_Rix1 761
Human_PELP1 1051
Yeast_Rix1

Figure S2 B

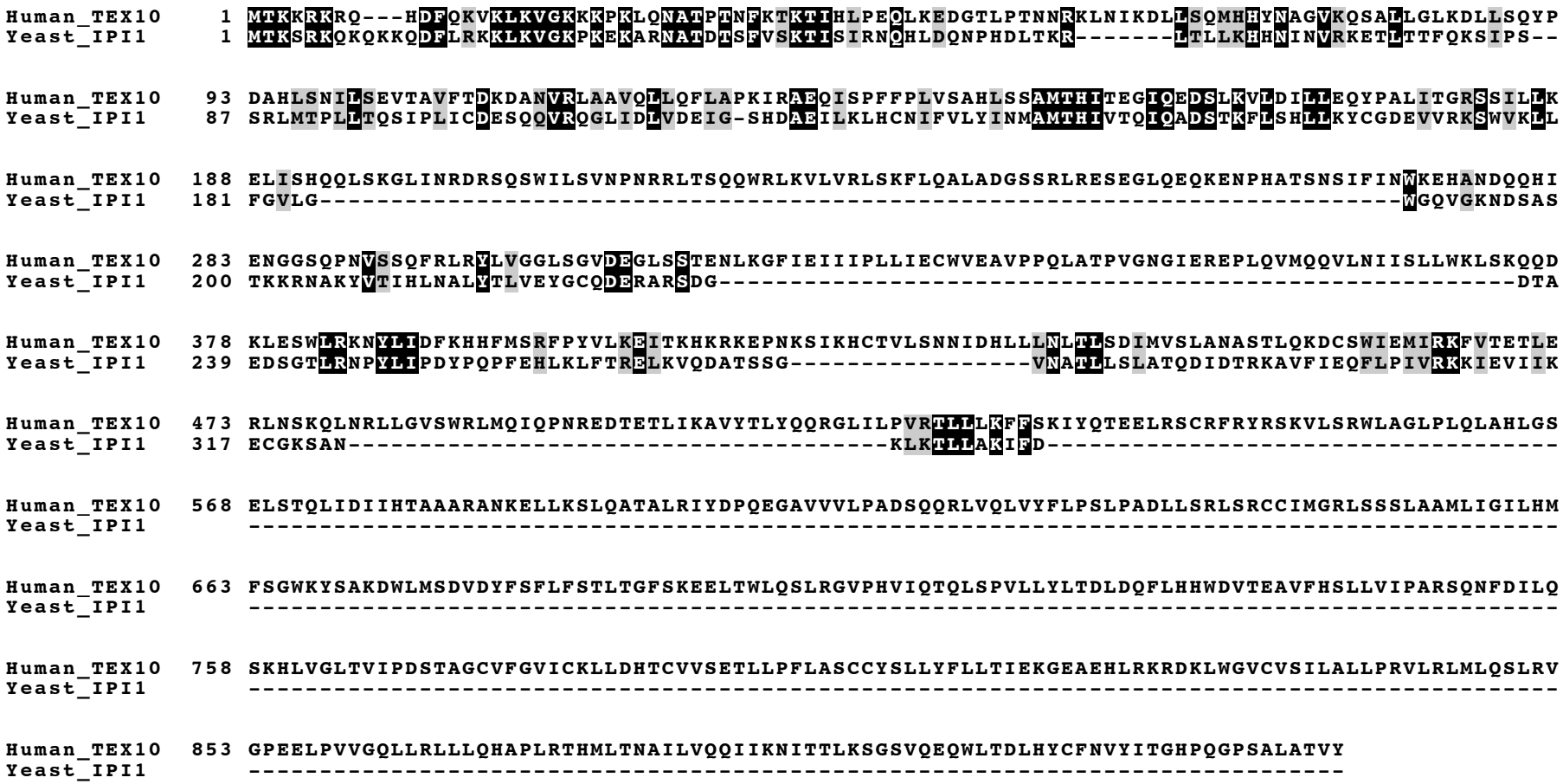
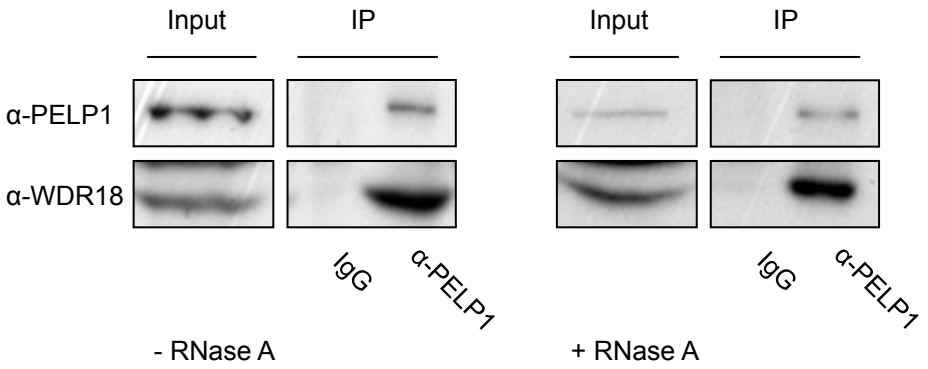

Human_TEX10 1 MTKKRKRQ---HDFQKVKLKVGKKKPKLQNATPTNFKTKTIHLPEQLKEDGTLPTNNRKLNIKDLLSQMHHYNAGVKQSALLGLKDLLSQYP
Yeast_IPI1 1 MTKSRKQKQKKQDFLRKKLKVGKPKEKARNATDTSFVSKTISIRNQHLDQNPHDLTKR-------LTLLKHHNINVRKETLTTFQKSIPS--
Human_TEX10 93 DAHLSNILSEVTAVFTDKDANVRLAAVQLLQFLAPKIRAEQISPFFPLVSAHLSSAMTHITEGIQEDSLKVLDILLEQYPALITGRSSILLK
Yeast_IPI1 87 SRLMTPLLTQSIPLICDESQQVRQGLIDLVDEIG-SHDAEILKLHCNIFVLYINMAMTHIVTQIQADSTKFLSHLLKYCGDEVVRKSWVKLL
Human_TEX10 188 ELISHQQLSKGLINRDRSQSWILSVNPNRRLTSQQWRLKVLVRLSKFLQALADGSSRLRESEGLQEQKENPHATSNSIFINWKEHANDQQHI
Yeast_IPI1 181 FGVLG----------WGQVGKNDSAS
Human_TEX10 283 ENGGSQPNVSSQFRLRYLVGGLSGVDEGLSSTENLKGFIEIIIPLLIECWVEAVPPQLATPVGNGIEREPLQVMQQVLNIISLLWKLSKQQD
Yeast_IPI1 200 TKKRNAKYVTIHLNALYTLVEYGCQDERARSDG----------DTA
Human_TEX10 378 KLESWLRKNYLIDFKHHFMSRFPYVLKEITKHKRKEPNKSIKHCTVLSNNIDHLLLNLTLSDIMVSLANASTLQKDCSWIEMIRKFVTETLE
Yeast_IPI1 239 EDSGTLRNPYLIPDYPQPFEHLKLFTRELKVQDATSSG----------VNATLLSLATQDIDTRKAVFIEQFLPIVRKKIEVIIK
Human_TEX10 473 RLNSKQLNRLLGVSWRLMQIQPNREDTETLIKAVYTLYQQRGLILPVRTLLLKFFSKIYQTEELRSCRFRYRSKVLSRWLAGLPLQLAHLGS
Yeast_IPI1 317 ECGKSAN----------KLKTLLAKIFD----------
Human_TEX10 568 ELSTQLIDIIHTAAARANKELLKSLQATALRIYDPQEGAVVVLPADSQQRLVQLVYFLPSLPADLLSRLSRCCIMGRLSSSLAAMLIGILHM
Yeast_IPI1 ----------
Human_TEX10 663 FSGWKYSAKDWLMSDVDYFSFLFSTLTGFSKEELTWLQSLRGVPHVIQTQLSPVLLYLTDLDQFLHHWDVTEAVFHSLLVIPARSQNFDILQ
Yeast_IPI1 ----------
Human_TEX10 758 SKHLVGLTVIPDSTAGCVFGVICKLLDHTCVVSETLLPFLASCCYSLLYFLLTIEKGEAEHLRKRDKLWGVCVSILALLPRVLRLMLQSLRV
Yeast_IPI1 ----------
Human_TEX10 853 GPEELPVVGQLLRLLLQHAPLRTHMLTNAILVQQIIKNITTLKSGSVQEQWLTDLHYCFNVYITGHPQGPSALATVY
Yeast_IPI1 ----------

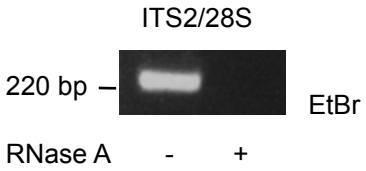
Figure S2 C


Human_WDR18 1 MAAPMEVAVCTDSAAPMWSCIVWELHSGANLLTYRGGQAGPRGLALLNGEYLLAAQLGKNYISAWELQ---RKDQLQQKIMCPGPVTCLTAS
Yeast_IPI3 1 --MDEQVIFTTNTSGTIASVHSFEQIN----LRQCSTQSRNSCVQVGN-KYLFIAQAQKALINVYNLSGSFKRESVEQRLPLPEILKCLEVV
Human_WDR18 93 -------------------LYVLAGVAESIHL--WEVSTGNLL-VILSRHYQDVSCLQFTGDSSHFISGGKDCLVLVWS---LCSVLQADPS
Yeast_IPI3 89 GVQYDRIQGVNHNLPDFNLPYLLLGSTESGKLYIWELNSGILLNVKPMAHYQSITKIKSILNGKYIITSGNDSRVIIWQTVDLVSASNDDP-
Human_WDR18 163 APRHVWSHHALPITDLHCG--FGGPLARVAT--------------------SSLDQTVKLWEVSSGE---LLLSVLFDVSIMAVTMDLAEHH
Yeast_IPI3 180 KPLCILHDHTLPVTDFQVSSSQGKFLSCTDTKLFTVSQDATIRCYDLSLIGSKKKQKANENDVSIGKTPVLLATFTTPYSIKSIVLDPADRA
Human_WDR18 233 GGSEGSIFQVDLFTWPGQRERSFHPEQDAGK--VFKG------HRN----------------------------QVTCLSVSTDGSVLLSGSH
Yeast_IPI3 275 GTAEG-CFSLNLFYKLKG-NAIVNLLQSAGVNTVQKGRVFSLVQRNSLTGGENEDLDALYAMGQLVCENVLNSNVSCLEISMDGTLLLIGDT
Human_WDR18 293 VRLWDVQSKQCIRTVALK-------GPVTNAAILLAPVSML----------SSDFRPS------------LPLPHFNKHLLGAEHGDEPRHG
Yeast_IPI3 368 VSIAEIYSKQIIRTIQTLTTSQDSVGEVTN---LLTNPYRLERGNLLFEGESKGKQPSNNNGHNFMKIPNLQRVIFDGKNKGHLHDIWYQIG
Human_WDR18 357 ------LTLRLG-----LHQ---QGSEPS--------------YLDRTEQLQAVLCSTMEKSVLGG-QDQLRVRVTELEDEVRNLRKINRDL
Yeast_IPI3 460 AETDPNLALPLNDFNAYLEQVKTQESIFSHIGKVSSNVKVIDNKIDATSSLDSNAAKDEEITELKTNIEALTHAYKELRDMHEKLYEEHQQM
Human_WDR18 423 STRFITRPAK
Yeast_IPI3 555 Q---------

Figure S3

A


Input
IP
Input
IP
α-PELP1
α-WDR18
IgG
α-PELP1
IgG
α-PELP1
- RNase A
+ RNase A


ITS2/28S
220 bp
EtBr
RNase A
-
+

B

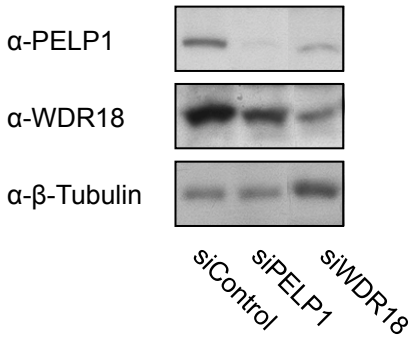
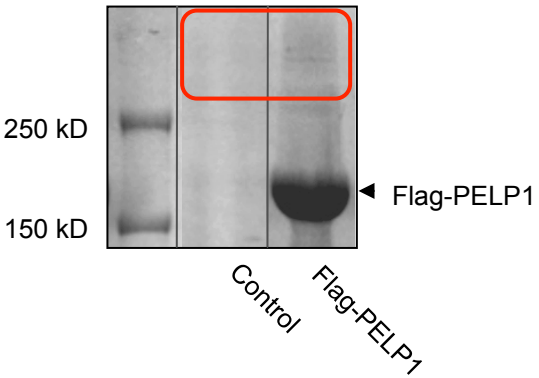

α-PELP1
α-WDR18
α-β-Tubulin
siControl
siPELP1
siWDR18

Figure S4

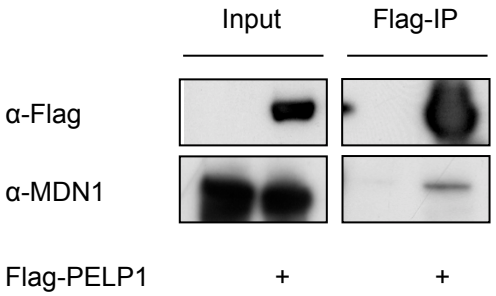
A



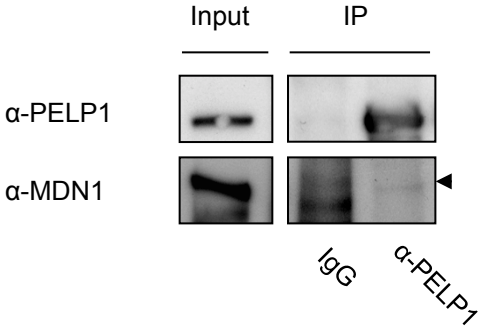
Unique peptides of MDN1 identified by MS/MS :

155 FLQQEQSVFR 164
222 LLEEAQLQDLEK 233
2489 IMQSPSPENLK 2499
3269 NLSSQLQTGR 3278
3340 APAVQDLLTR 3349
3361 SAQVAQSLLK 3370
4206 LNAALATPAK 4215
5509 VLAAVQAAR 5517
5547 GPGEMPEIR 555

B



C



D

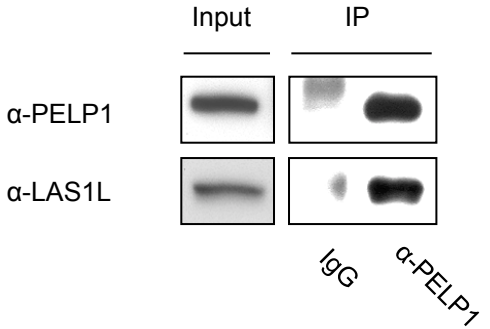


Figure S5

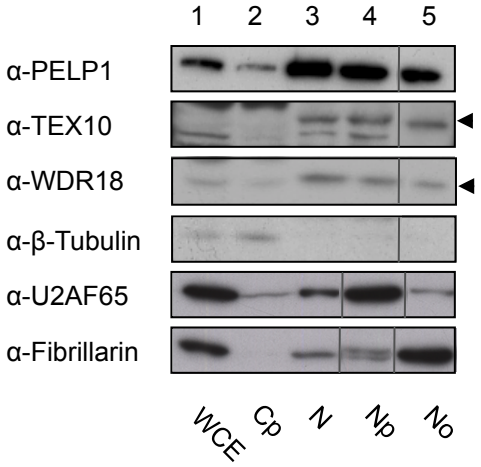

1
2
3
4
5
α-PELP1
α-TEX10
α-WDR18
α-β-Tubulin
α-U2AF65
α-Fibrillarin
WCE
Cp
N
Np
No

Figure S6

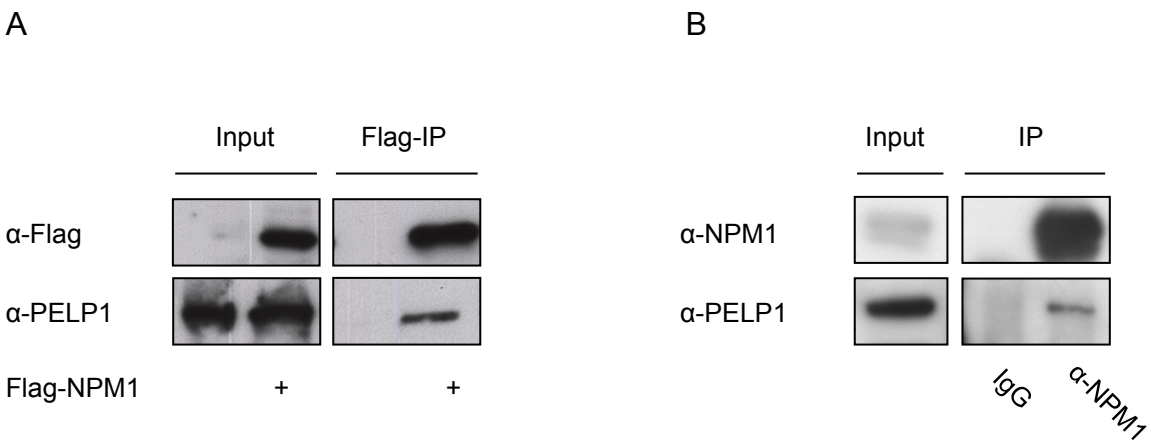

A
Input
Flag-IP
α-Flag
α-PELP1
Flag-NPM1
+
+
B
Input
IP
α-NPM1
α-PELP1
IgG
α-NPM1

Figure S7

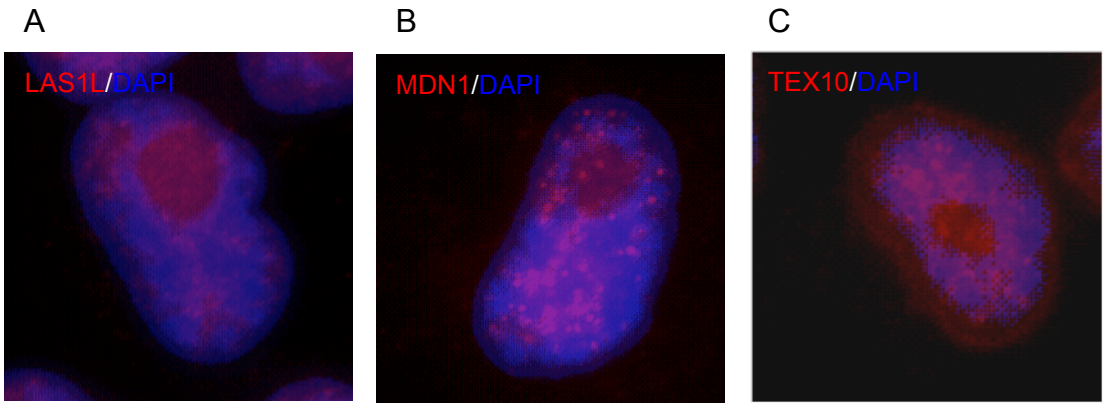


Figure S8

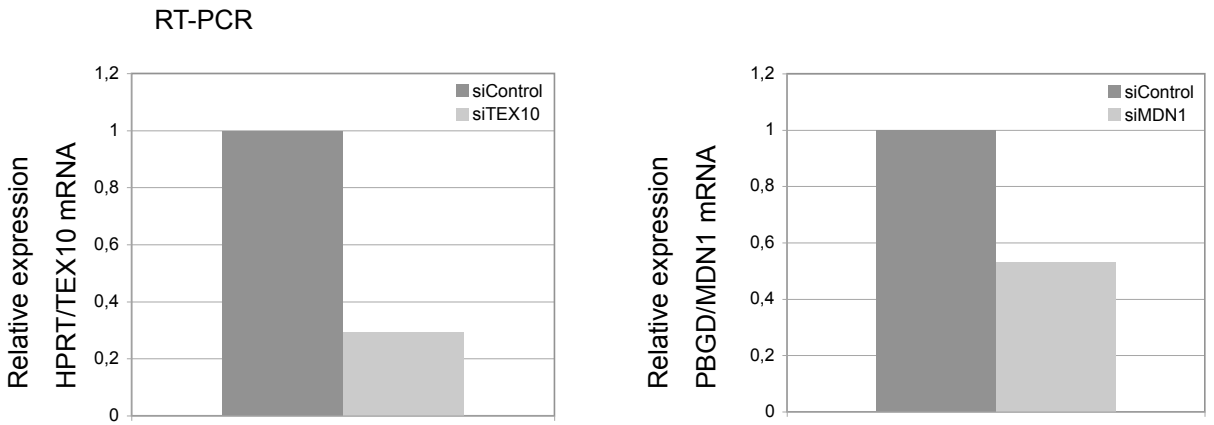

RT-PCR
Relative expression
HPRT/TEX10 mRNA
1,2
1
0,8
0,6
0,4
0,2
0
siControl
siTEX10
Relative expression
PBGD/MDN1 mRNA
1,2
1
0,8
0,6
0,4
0,2
0
siControl
siMDN1

Figure S9

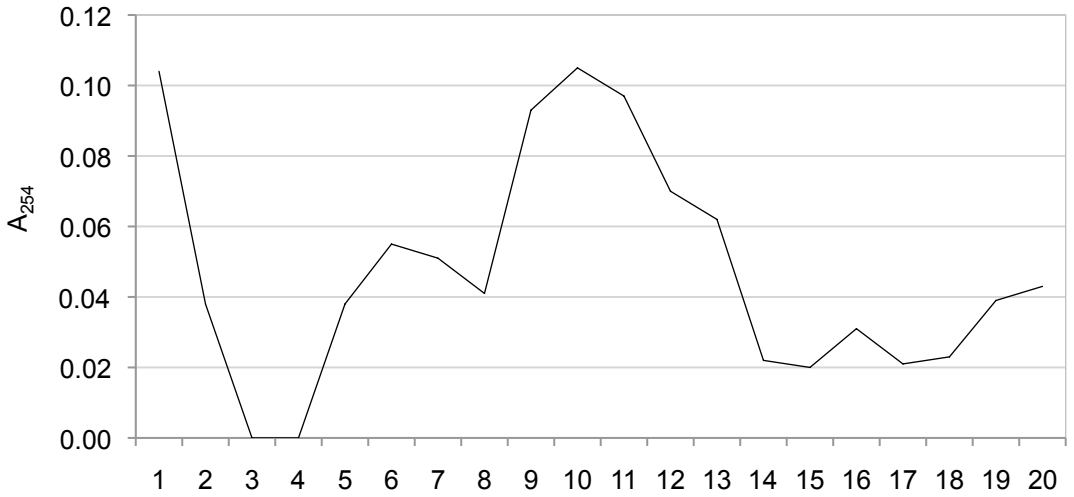
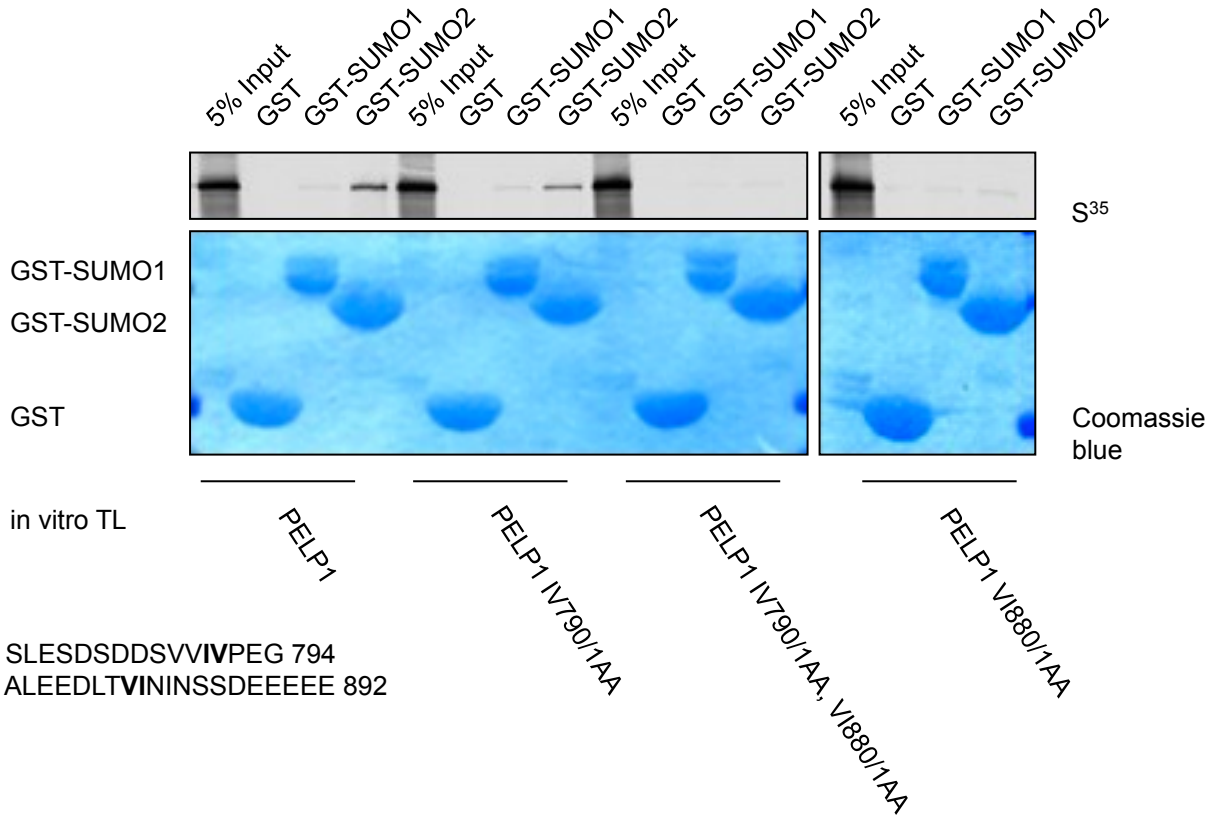

0.12
0.10
0.08
0.06
0.04
0.02
0.00
A_{254}
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

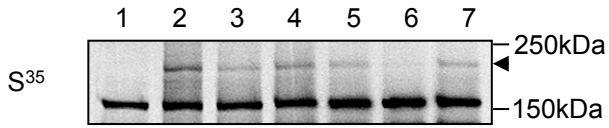
Figure S10

A



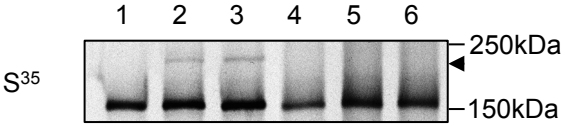
SIM1: 779 SLESDSDDSVV**IV**PEG 794
SIM2: 873 ALEEDLT**VI**NINSSDEEEEE 892

B



PELP1	+	+	+	+	+	+	+
SUMO1		+	+	+			
SUMO2					+	+	+
SENP3			+			+	
SENP3^{C532S}				+			+

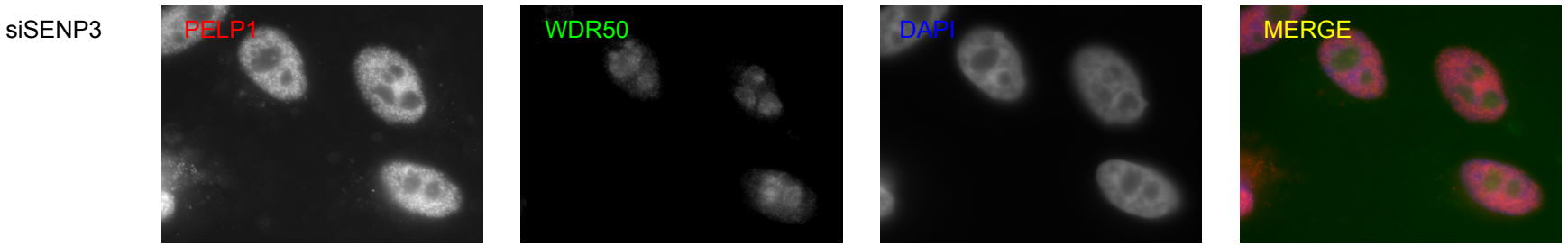
C



PELP1	+	+	+			
PELP1^{K826R}				+	+	+
SUMO1		+			+	
SUMO2			+			+

Figure S11

A



B

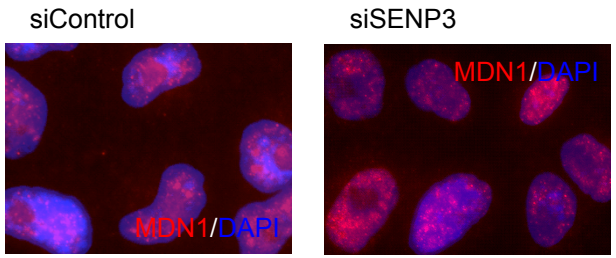


Figure S12

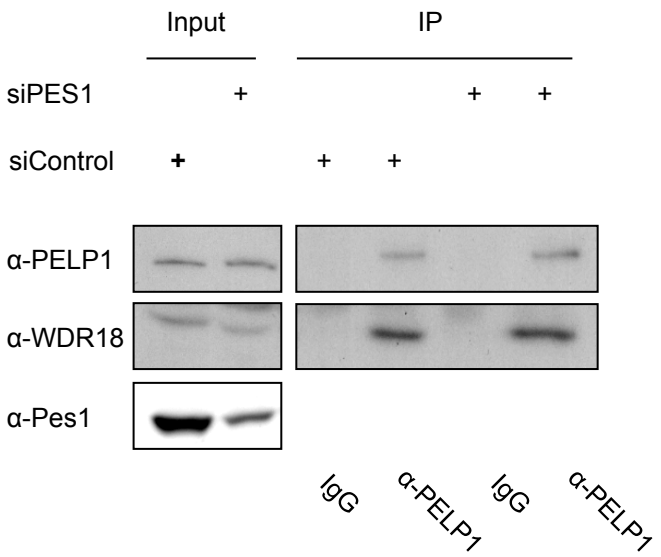
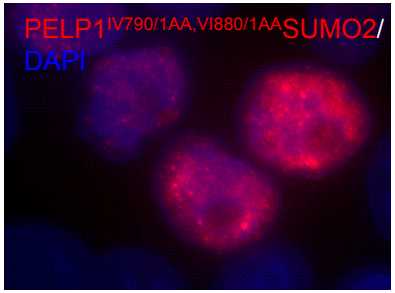

Input
IP
siPES1
+
+
+
siControl
+
+
+
α-PELP1
α-WDR18
α-Pes1
IgG
α-PELP1
IgG
α-PELP1

Figure S13

A



B

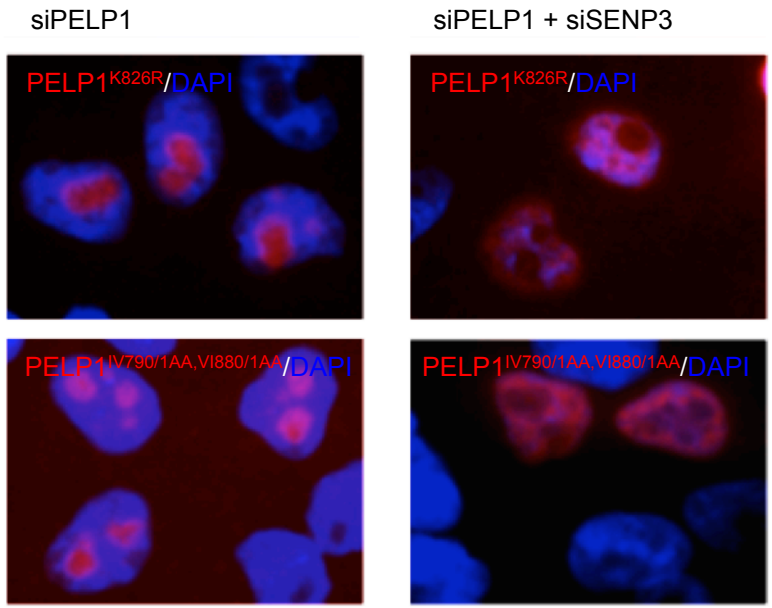


Figure S14

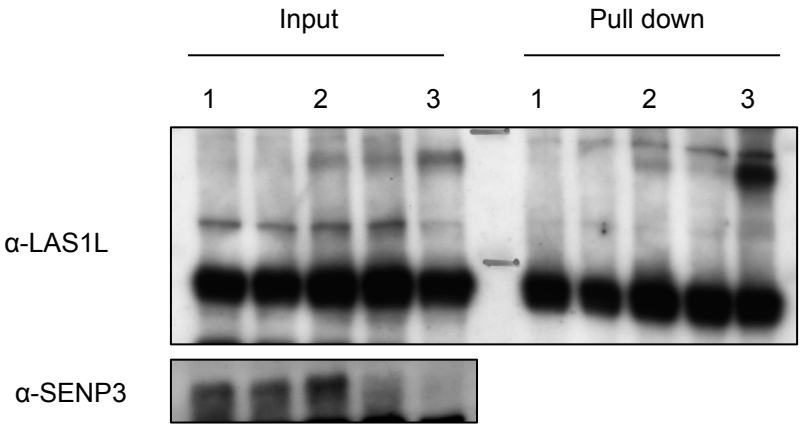


Figure S15

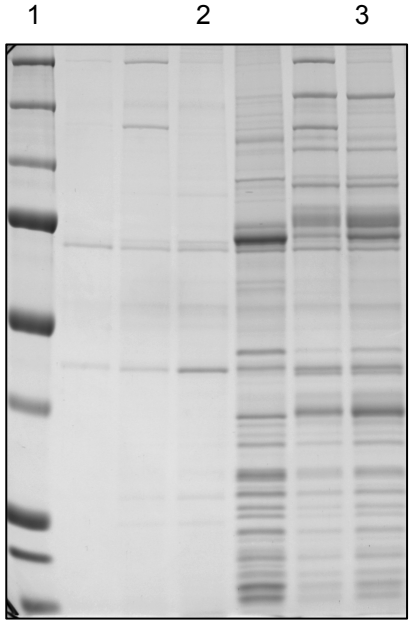


Figure S16

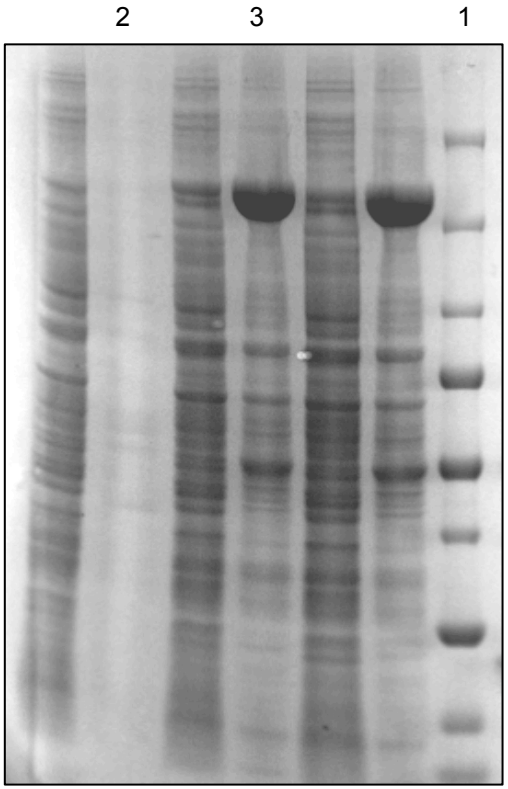
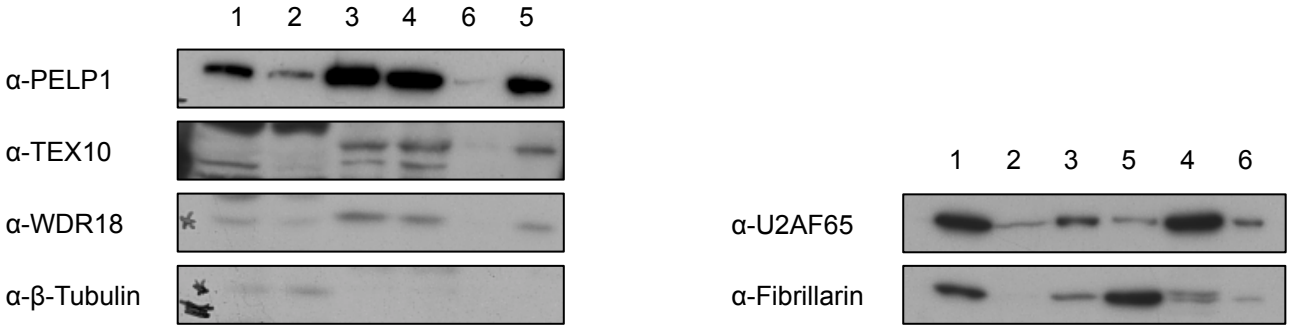


Figure S17


1 2 3 4 6 5
α-PELP1
α-TEX10
α-WDR18
α-β-Tubulin
1 2 3 5 4 6
α-U2AF65
α-Fibrillarin

Legends to Supplementary Figures

Fig S1: PELP1, TEX10 and WDR18 co-purify with Flag-SENP3.

(A) Flag-tagged SENP3 was expressed in HEK293T cells and purified on a Flag-affinity column. Bound proteins were eluted with Flag-peptide, separated by SDS-PAGE and analyzed by mass-spectrometry. PELP1, LAS1L, TEX10, WDR18 and NPM1 were identified by MASCOT algorithms in individual bands as indicated. Vertical lines indicate removal of irrelevant neighbouring lanes from the initial gel. The corresponding original scan is provided in Supplementary Figure 15. (B) Diagram showing conserved domains of PELP1, TEX10 and WDR18 and their corresponding yeast counterparts. The PELP1 family of higher eukaryotes harbours a NUC201/202 domain (Pfam accession: PF08167), which is found in hypothetical nucleolar proteins. GLU represents the glutamic acid rich C-terminal region of PELP1 and Rix1. Tex10 and Ipi1 share the Ipi1N domain (Pfam accession: PF12333), which defines the eukaryotic Tex10/Ipi1 family. In TEX10 a heat repeat (Pfam accession: PF00514) can be found. WDR18 and IPI3 have WD40 repeats (Pfam accession: PF00400) in common.

Fig S2: Sequence comparison of PELP1-TEX10-WDR18 with Rix1-Ipi1-Ipi3.

(A) Human PELP1 (accession Q8IZL8) was aligned to *S. cerevisiae* Rix-1 (accession P38883). (B) Human TEX10 (accession Q9NXF1) was aligned to *S. cerevisiae* IPI1 (accession P38803). (C) Human WDR18 (accession Q9BV38) was aligned to S. *cerevisiae* IPI3 (accession P53877).

Fig S3: Interdependency of PELP1 and WDR18.

(A) Endogenous PELP1 was immunoprecipitated from HeLa cells with a rabbit polyclonal antibody from control extracts or extracts treated with RNase A respectively. Immunocomplexes were probed for the presence of endogenous WDR18 by western blotting with an anti-WDR18 antibody. Right panel: Efficient digestion of RNA by RNase A treatment was verified by RT-PCR using primers for the indicated rRNA region. (B) HeLa cells were transfected with the indicated siRNAs and protein levels were monitored by western blotting as indicated. Detection of β-tubulin served as loading control.

Fig S4: MDN1 and LAS1L are associated with PELP1.

(A) Flag-tagged PELP1 was expressed in HeLa cells and purified on a Flag-affinity column. Bound proteins were separated by SDS-PAGE and the higher molecular weight region above 250 kDa was cut out of the gel and analyzed by mass-spectrometry. MS/MS identified nine unique peptides corresponding to human MDN1 as indicated. In the respective control sample only one

peptide of MDN1 was identified. Vertical lines indicate removal of irrelevant neighbouring lanes from the initial gel. The corresponding original scan is provided in Supplementary Figure 16. (B) Flag-PELP1 was expressed in HeLa cells, captured on Flag-beads, and the presence of endogenous MDN1 was tested by immunoblotting as indicated. (C, D) Endogenous PELP1 was immunoprecipitated from HeLa cells with a rabbit polyclonal antibody and probed using western blotting with the indicated antibodies. The inputs represent 2.5 % of the total cell lysate.

Fig S5: A subfraction of PELP1, TEX10 and WDR18 localizes to the nucleolus.

HeLa cells were fractionated into cytoplasmic (Cp), nuclear (N), nucleoplasmic (Np) and nucleolar (No) fractions and the respective fractions were investigated for the presence of PELP1, TEX10 and WDR18 by western blotting as indicated. The following proteins served as markers for the respective fractions. Cp: beta-tubulin, Np: U2AF65 (a splicing factor), No: anti-Fibrillarin. WCE: whole cell extract. Vertical lines indicate removal (upper four panels) or swapping (lower two panels) of neighbouring lanes. The original scan is provided in Supplementary Figure 17.

Fig S6: PELP1 coimmunoprecipitates with NPM1.

(A) Flag-tagged NPM1 was expressed in U2OS cells, captured on Flag-beads and immunoblotted for the presence of endogenous PELP1 as indicated. (B) Endogenous NPM1 was immunoprecipitated from OCI-AML3 cells using a mouse monoclonal antibody (Invitrogen) and immunocomplexes were probed for the presence of endogenous PELP1. The inputs represent 2.5 % of the total cell lysate.

Fig S7: LAS1L, MDN1 and TEX10 can be found in the nucleolus.

Localization of LAS1L, MDN1 or TEX10 was examined by indirect fluorescence using anti-LAS1L, anti-MDN1 or anti-TEX10 antibodies as indicated. Nuclei were counterstained with DAPI.

Fig S8: siRNA-mediated downregulation of TEX10 and MDN1.

Real time quantitative PCR was performed to verify the decreased mRNA levels of TEX10 and MDN1 following siRNA treatment. Relative expression levels are calculated by dividing the corresponding values for TEX10 and MDN1 by values for the indicated control genes (HPRT= Hypoxanthine-guanine phosphoribosyltransferase/ PBGD=porphobilinogen deaminase). Relative expression levels in control siRNA treated cells were set at 1.

Fig S9: Profile of UV absorbance obtained upon fractionation of nuclear extracts on a sucrose gradient.

After ultracentrifugation of the gradients 20 fractions were collected and A_{254} was recorded. As a result two major peaks appeared containing small and large ribosomal subunits, respectively, and their precursors.

Fig S10: PELP1 binds to SUMO in a covalent and non-covalent fashion.

(A) GST-tagged versions of SUMO1 and SUMO2 as well as GST alone were purified in *E. coli* and incubated with ^{35}S-labeled in vitro translated versions of full length PELP1 and PELP1 mutants as indicated. (B) PELP1 was generated by in vitro transcription/translation and incubated with recombinant E1 and E2 enzymes and SUMO1 or SUMO2 respectively in the presence of ATP. In the control reaction (lane 1) SUMO was omitted. Where indicated wild-type SENP3 or the catalytically inactive SENP3^{C532S} version - generated by in vitro transcription/translation - was added to the reactions. (C) PELP1^{WT} and PELP1^{K826R} were generated by in vitro transcription/translation and sumoylated in the presence of recombinant E1, E2, SUMO1 or SUMO2, respectively, and ATP. In the control lanes (lane 1 and 4) SUMO was not added.

Fig S11: SENP3 depletion leads to delocalization of MDN1, but not of WDR50/Utp18.

(A, B) HeLa cells were transfected with SENP3 siRNA. After fixation and permeabilization the localization of PELP1, WDR50 or MDN1 respectively was monitored by indirect fluorescence using anti-PELP1, anti-WDR50 or anti-MDN1 antibodies as indicated. Nuclei were counterstained with DAPI.

Fig S12: Depletion of PES1 does not affect binding of PELP1 to WDR18.

Endogenous PELP1 was immunoprecipitated from cells treated with a control siRNA and cells treated with an siRNA directed against PES1, respectively. The presence of endogenous WDR18 was tested by western blotting. Inputs represent 2.5 % of the total cell lysate.

Fig S13: Localization of Flag-PELP1$^{IV790/1AA,VI880/1AA}$-SUMO2, HA-PELP1^{K826R} and HA-PELP1$^{IV790/1AA,VI880/1AA}$.

(A, B) HeLa cells were transfected with the indicated siRNAs and PELP1 versions. After fixation and permeabilization their localization was analyzed using anti-Flag or anti-HA antibodies, respectively. Nuclei were counterstained with DAPI.

Fig S14: Original scan of Figure 7C.

Lanes 1, 2 and 3 of both input and pull down were taken to assemble Figure 7C. The other lanes were removed as they were not relevant for the actual figure.

Fig S15: Original scan of Figure S1A.

Lanes 1, 2 and 3 were composed to arrange Figure S1A. The remaining lanes were not included as they were irrelevant in this context.

Fig S16: Original scan of Figure S4A.

Lanes 1, 2 and 3 were merged to form Figure S4A. The other lanes were not included as they were negligible for the actual figure.

Fig S17: Original scans of Figure S5.

Lanes 6 in the original scans were depleted as they were not relevant for Figure S5. In addition, for anti-U2AF65 as well as for anti-Fibrillarin (right panel), lanes 4 and 5 of the original scan were swapped in order to be in line with the order of the other immunoblots of the actual figure.