Gaik et al., http://www.jcb.org/cgi/content/full/jcb.201411003/DC1

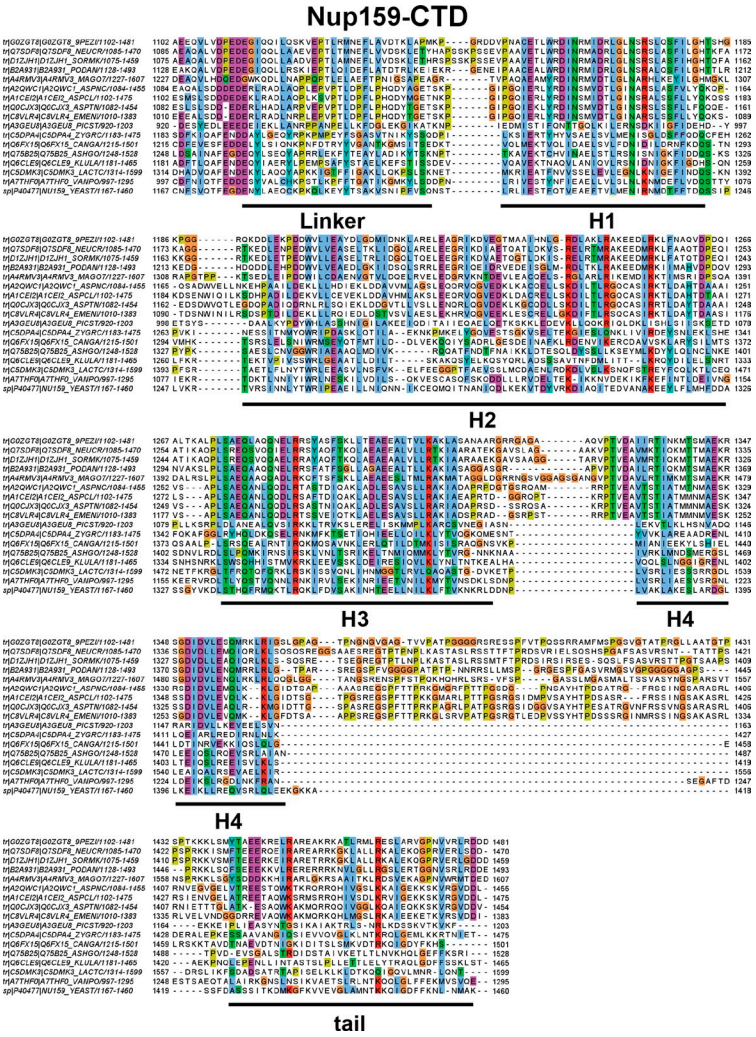

Figure S1. **α-Helically predicted domain of Nup159 are conserved among *fungi*.** Multi-sequence alignment, using T-Coffee (http://www.ebi.ac.uk/Tools/msa/tcoffee/), and Jalview, of the α-helically predicted C domain of Nup159. The default color scheme of ClustalX/Jalview was used with e.g., hydrophobic residues in blue, acidic residues in violet, and basic residues in red.

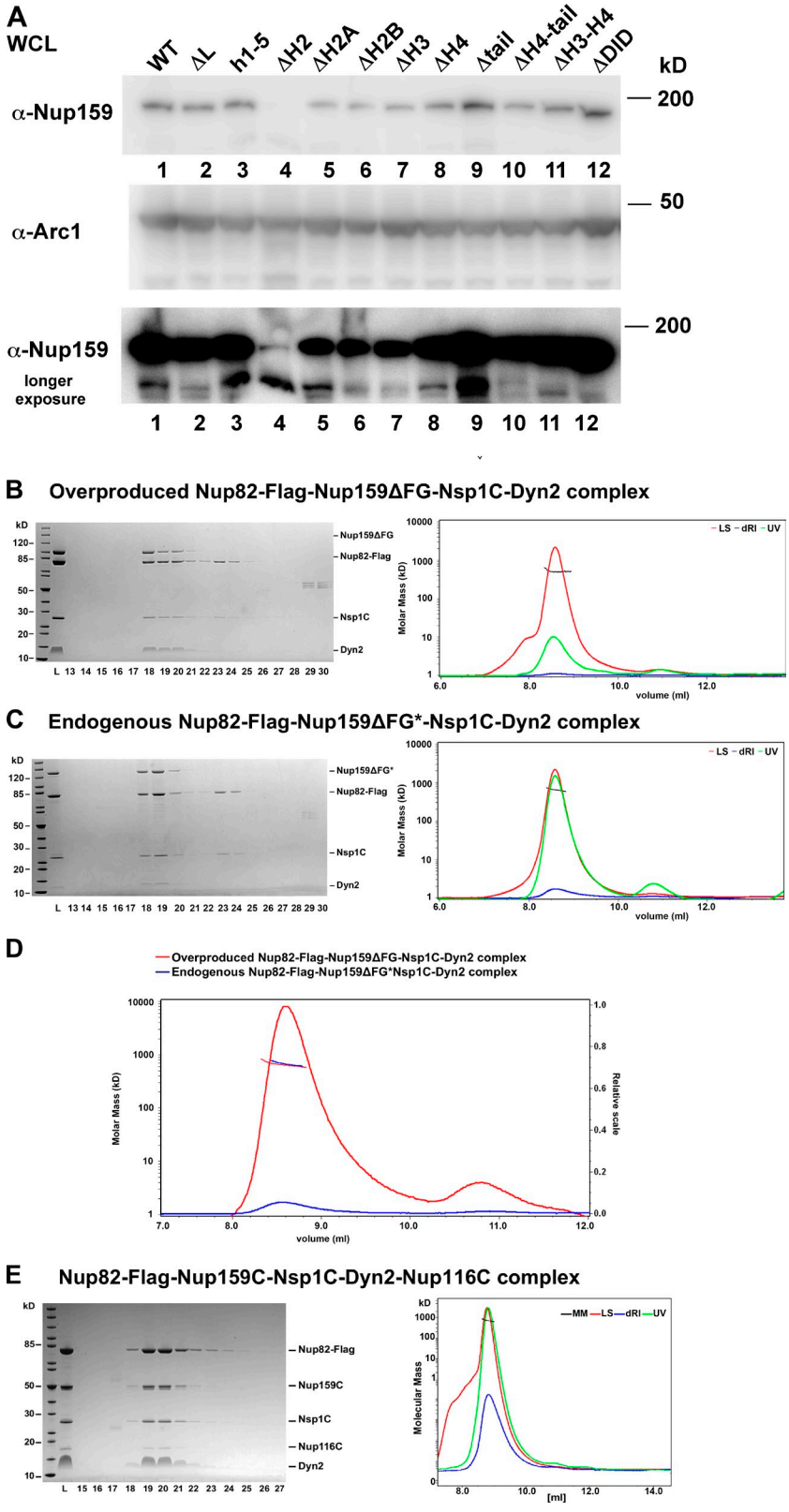

Figure S2. **Overproduced and endogenous Nup82–Nup159ΔFG–Nsp1C–Dyn2 complexes have a similar gel filtration behavior and a related MALS value.** (A) Expression levels of the indicated Nup159 wild-type (WT) and mutant proteins used in this study, which were analyzed by SDS-PAGE of whole-cell lysates (WCL; cells were grown at 30°C) followed by Western blotting using a monoclonal anti-Nup159 antibody (mAB165C10), which allows detection of the Nup159 CTD constructs (Kraemer et al., 1995), and as loading control, we used anti-Arc1 antibodies. (B) SEC-MALS analysis of the overproduced Nup82-Flag–Nup159ΔFG–Nsp1C–Dyn2 complex with a deduced molecular mass of 632 kD. (C) SEC-MALS analysis of the endogenous Nup82-Flag–Nup159ΔFG*–Nsp1C–Dyn2 complex (indicated a molecular mass of 670 kD). The asterisk in Nup159ΔFG* indicates that Nup159 carries additional amino acids between the β-propeller domain and the DID (see Tables S1 and S2). (D) Comparison of yield of overproduced versus endogenous Nup82-Flag–Nup159ΔFG–Nsp1C–Dyn2 complex, as indicated by the differential refractive index (dRI). LS, light scattering; MM, molecular mass. (E) SEC-MALS analysis of the Nup82-Flag–Nup159C–Nsp1C–Dyn2–Nup116C complex (deduced molecular mass 693 kD). In A–D, data shown are from single representative experiments out of two repeats. L, load.

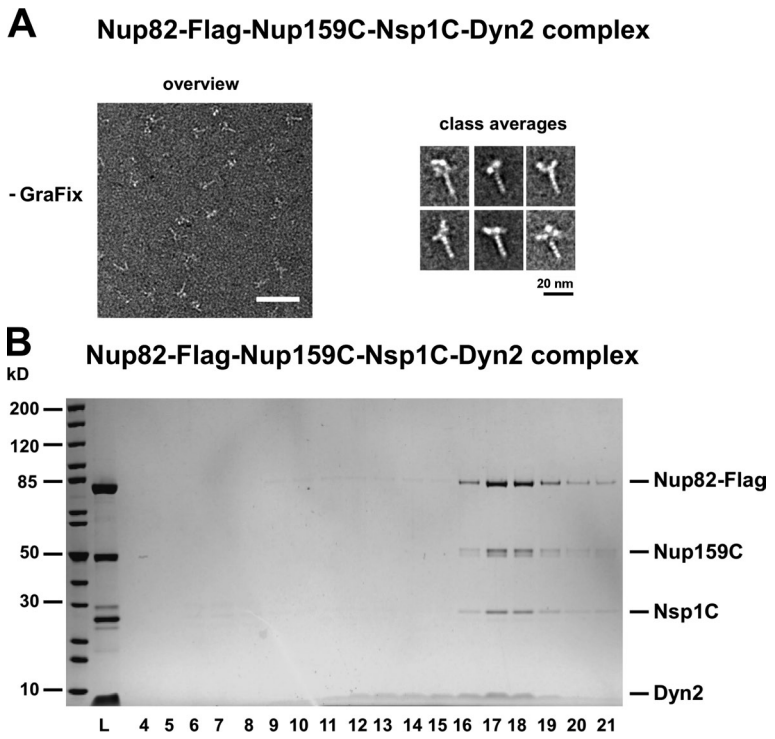

Figure S3. **Negative-staining EM analysis of the unfixed and GraFix-treated Nup82–Nup159C–Nsp1C–Dyn2 complex.** (A) Negative-staining EM of the unfixed Nup82–Nup159C–Nsp1C–Dyn2 complex showing an overview picture (left) and a gallery of class averages (right). Bar, 50 nm. (B) Glycerol gradient centrifugation of affinity-purified mNup82–Nup159C–Nsp1C–Dyn2 complex, analyzed by SDS-PAGE and Coomassie staining, to determine the relevant fractions (17–18) of glycerol–glutaraldehyde (GraFix) gradient used in the EM analysis (Fig. 4 A). L, load.

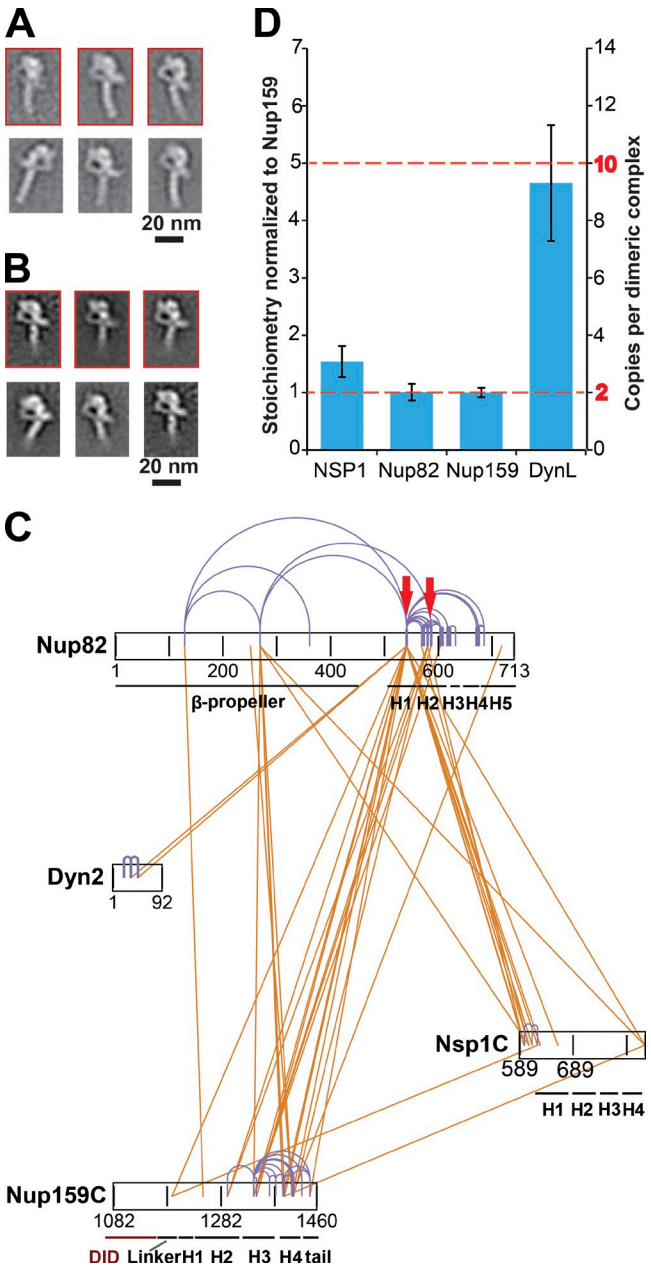

Figure S4. **EM structure, cross-linking, and stoichiometry of the purified Nup82 complex.** (A) Representative 2D class averages of affinity-purified Nup82–Nup159C–Nsp1C–Dyn2 complex based on subtomogram averaging. Parts of the DID_{Nup159}-Dyn2 stalk are averaged out in the first and the third class. Highlighted classes (red) were used for 3D reconstruction (B) Representative class averages of the affinity-purified Nup82–Nup159C–Nsp1C–Dyn2 complex based on subtomogram averaging focused by local masking to the head region such that it is resolved with higher detail. Highlighted classes (red) were used for 3D reconstruction. (C) XL-MS of the affinity-purified Nup82–Nup159C–Nsp1C–Dyn2 complex using DSG. The primary structure of the protein is shown, and specific regions are indicated. Interprotein cross-links are shown in orange; intraprotein cross-links are in purple. For the visualization of cross-links, the xiNET tool from the Rappsilber laboratory was used (http://crosslinkviewer.org/index.php). Because multiple copies of each protein are present, the latter might also occur across multiple instances of the same protein. Homodimeric cross-links that connect the two instances of the same lysine residue are indicated with red arrows. (D) Stoichiometric measurements of purified Nup82–Nup159C–Nsp1C–Dyn2 complex by quantitative, targeted proteomics within an early fraction of the gel filtration peak. Two heavy-labeled reference peptides per protein were used as intrinsic standards. The apparent values were normalized to the abundance of Nup159; error bars correspond to one standard deviation (Fig. 7).

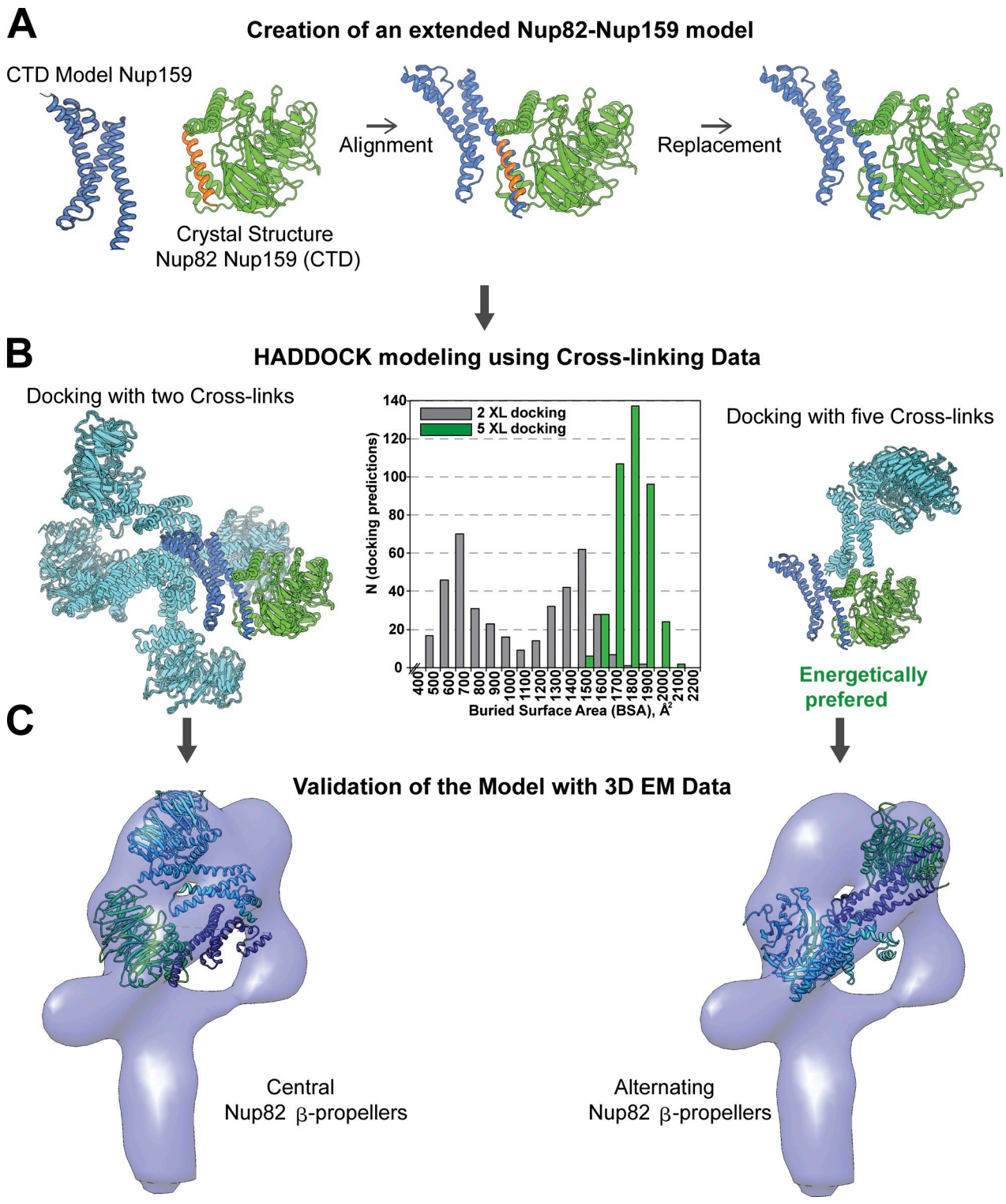

Figure S5. **Structural modeling of the interaction between Nup82 and Nup159.** (A) A structural model of Nup159C (amino acids 1,313–1,434) was generated using I-TASSER. An extended Nup82–Nup159 structural model was built after superposition on top of Nup82, present in the Nup159-Nup116 x-ray structure. The model was further refined using HADDOCK. (B) Interaction of four Nup82 propellers and two Nup159C domains (from amino acids 1,313 to 1,434) were modeled using docking by translating XL-MS information into spatial restraints as implemented in HADDOCK. When only homodimeric spatial restraints (Nup159 K1343 and Nup159 K1414) were considered (left), the approach did not converge into a single solution. When three additional distance restraints were considered (Nup82 K269 with Nup159 K1343, Nup82 K269 with Nup159 K1414, and Nup82 K274 with Nup159 K1343) under the assumption that they occur across two relevant instances of Nup82 and Nup159, an energetically favorable model was generated. (C) Structural models that satisfy the EM volume are shown. The right model is energetically preferred as highlighted in plot in B, showing that calculated buried surface area is much larger compared with the model on the left.

Table S1. **Plasmids used in this study**

Plasmid	Markers and construction details	Reference
pRS414-*NUP159*	CEN, TRP1, AmpR, P_{NUP159} NUP159, T_{ADH1}	Stelter et al., 2007
pRS414-nup159ΔDID	See above, P_{NUP159} Nup159 ΔΔ1,086–1,185 aa	Stelter et al., 2007
pRS414-nup159ΔLinker	See above, P_{NUP159} Nup159 ΔΔ1,179–1,210 aa	This study
pRS414-nup159ΔH1	See above, P_{NUP159} Nup159 ΔΔ1,210–1,244 aa	This study
pRS414-nup159ΔH2	See above, P_{NUP159} Nup159 ΔΔ1,245–1,330 aa	This study
pRS414-nup159ΔH3	See above, P_{NUP159} Nup159 ΔΔ1,330–1,381 aa	This study
pRS414-nup159ΔH4	See above, P_{NUP159} Nup159 ΔΔ1,381–1,425 aa	This study
pRS414-nup159Δtail	See above, P_{NUP159} Nup159 ΔΔ1,425–1,460 aa	This study
pRS414-nup159ΔH3-H4	See above, P_{NUP159} Nup159 ΔΔ1,330–1,425 aa	This study
pRS414-nup159ΔH4-tail	See above, P_{NUP159} Nup159 ΔΔ1,381–1,460 aa	This study
pRS414-nup159 h1-5	See above, P_{NUP159} Nup159 Δ I1232>D,M1235>E	This study
pRS414-GFP-NUP159	*CEN, TRP1, AmpR, P_{NOP1} NUP159, T_{ADH1}*	This study
pRS414-GFP-nup159 h1-5	*CEN, TRP1, AmpR, P_{NOP1} nup159h1-5, T_{ADH1}*	This study
pRS414-NUP159-Flag-TEV-ProtA	*CEN, TRP1, AmpR, P_{NOP1} NUP159-FLAG-TEV-pA, T_{ADH1}*	This study
pRS414-Nup159-h1-5-Flag-TEV-ProtA	*CEN, TRP1, AmpR, P_{NOP1} nup159h1-5-FLAG-TEV-pA, T_{ADH1}*	This study
YEplac181-*NUP159*	*2μ, LEU2, AmpR, $P_{GAL1-10}$ NUP159 T_{ADH1}*	This study
YEplac181-Nup159ΔFG	*2μ, LEU2, AmpR, $P_{GAL1-10}$ NUP159 ΔΔ459–1,083 aa T_{ADH1}*	This study
YEplac181-Nup159C	*2μ, LEU2, AmpR, $P_{GAL1-10}$ NUP159 ΔΔ1–1,082 aa, T_{ADH1}*	This study
Yep351-GAL1-Dyn2	*2μ, LEU2, AmpR, $P_{GAL1-10}$ DYN2, T_{ADH1}*	This study
YEplac195-P1GAL-Dyn2-P2-Nup159C	*YEplac195-P1$_{GAL1-10}$-DYN2-P2-NUP159 ΔΔ1–1,082 aa, URA3*	This study
YEplac181-P1GAL-Nsp1C-P2-Nup82-Flag-TEV-ProtA	*YEplac181-P1$_{GAL1-10}$-NSP1 ΔΔ1–572 aa–P2-NUP82-FLAG-TEV-pA, LEU2*	This study
YEplac195-P1GAL-Dyn2-P2-Nup159Δtail	*YEplac195-P1$_{GAL1-10}$-DYN2-P2-NUP159 ΔΔ1–1,082 aa and 1,425–1,460 aa, URA3*	This study
YEplac195-P1GAL-Dyn2-P2-Nup159ΔFG	*YEplac195-P1$_{GAL1-10}$-DYN2-P2-NUP159 ΔΔ459–1,083 aa, URA3*	This study
YEplac181-P1GAL-HIS-Nup116C	*YEplac181-P1$_{GAL1-10}$-NUP116 ΔΔ1–967 aa, LEU2*	This study
YEplac181-P1GAL-Nsp1C-Flag-TEV-ProtA-P2-Nup82	*YEplac181-P1$_{GAL1-10}$-NSP1 ΔΔ1–572 aa–FLAG-TEV-pA-P2-NUP82, LEU2*	This study
YEplac195-P1GAL-Dyn2-P2-Nup159tail-Flag	*YEplac195-P1$_{GAL1-10}$-DYN2-P2-NUP159 ΔΔ1–1,425 aa–FLAG, URA3*	This study
YEplac112-P1GAL-Nsp1C-P2-Nup82-Flag-TEV-ProtA	*YEplac112-P1$_{GAL1-10}$-NSP1 ΔΔ1–572 aa–P2-NUP82-FLAG-TEV-pA, TRP1*	This study
YEplac112-P1GAL-Nsp1C-P2-Nup82-TEV-ProtA	*YEplac112-P1$_{GAL1-10}$-NSP1 ΔΔ1–572 aa–P2-NUP82-TEV-pA, TRP1*	This study
pET-15b-HIS-TEV-Nup159-QT$_{4-5}$-Linker-H1	*KanR, P_{T7} HIS$_6$-TEV-nup159-1,153–1,241 aa*	This study
pET-15b-HIS-TEV-Nup159-QT$_{4-5}$-Linker-h1-5	*KanR, P_{T7} HIS$_6$-TEV-nup159-1,153–1,241 aa I1232>D,M1235>E*	This study
pRS414-ADHI-*NSP1C*	*CEN, TRP1, AmpR, P_{ADH1} NSP1-ΔΔ 1-605 aa, T_{ADH1}*	Nehrbass et al., 1990
pAM1 (Nup159ΔFG*)	*CEN4, LEU2, AmpR, YCplac111 (Δ[HindIII–SmaI])* containing the *RAT7*-ΔRp*/NUP159* gene (*pLG7*)	Del Priore et al., 1997

Table S2. **Yeast strains used in this study**

Strain	Genotype	Reference
Ds1-2b	*his3-Δ200, leu2-Δ1, trp1-Δ63, ura3-52,* MAT α	Grandi et al., 1995
Nup159Δ shuffle	*nup159*::natNT2, *his3-Δ200, ura3-52, leu2-Δ1, trp1-Δ63,* MAT a, pLG-*URA3-NUP159*	Gorsch et al., 1995
Nup159Δ shuffle, Nup82-Flag-TEV-ProtA	*nup159*::natNT2, *his3-Δ200, ura3-52, leu2-Δ1, trp1-Δ63,* MAT a, Nup82-Flag-TEV-ProtA::HIS3, pLG-*URA3-NUP159*	This study
Nup82-Flag-TEV-ProtA, *Nup159Δ*, *Nsp1Δ* shuffle	*nup159*::HIS3, *nsp1*::HIS3, *ura3-52, leu2-Δ1, trp1-Δ63,* MAT a, Nup82-Flag-TEV-ProtA::natNT2, pLG-*URA3-NUP159,* pRS316-URA3-*NSP1*	This study

Table S3. **Cross-links identified within the Nup82 complex using DSS**

Peptide	Protein 1	Protein 2	AA 1	AA 2	Id score	Comment
KINSWDQVLVK-**K**MLEIDSK	Nsp1	Nup82	661	685	37.2	
IV**K**AQTLGVSIHNR-TEESSTG**K**STADVK	Nup82	Nsp1	541	607	35.5	
IV**K**AQTLGVSIHNR-K^TEESSTG**K**STADVK	Nup82	Nsp1	541	607	36.0	Missed-cleavage derivative to previous peptide
QINSI**K**K-MQ**K**TL	Nsp1	Nup159	822	1,343	31.1	
SLDDNSTSLE**K**QINSIK-MQ**K**TLR	Nsp1	Nup159	816	1,343	31.5	
SLDDNSTSLE**K**QINSIK^K-MQ**K**TLR	Nsp1	Nup159	816	1,343	32.3	Missed-cleavage derivative to previous peptide
FG**K**VDIQK-MQ**K**TLR	Nup82	Nup159	269	1,343	45.3	Restraint used for modeling
FG**K**VDIQK^EYR-MQ**K**TLR	Nup82	Nup159	269	1,343	39.3	Missed-cleavage derivative to previous peptide
FGKVDIQ**K**EYR-MQ**K**TLR	Nup82	Nup159	274	1,343	32.8	Restraint used for modeling
FG**K**VDIQK-LQLEE**K**GK	Nup82	Nup159	269	1,414	37.2	Restraint used for modeling
FG**K**VDIQK-LQLEE**K**GK^K	Nup82	Nup159	269	1,414	37.5	Missed-cleavage derivative to previous peptide
IEAETIKVD**K**K-LFTV**K**NK	Nup82	Nup159	674	1,372	24.7	
IEAETI**K**VDKK-MQ**K**TLR	Nup82	Nup159	671	1,343	36.7	
IIS**K**DDDLRR-MQ**K**TLR	Nup82	Nup159	573	1,343	29.7	
QLQSTCK^IIS**K**DDDLR-MQ**K**TLR	Nup82	Nup159	573	1,343	25.3	Missed-cleavage derivative to previous peptide
IV**K**AQTLGVSIHNR-DALD**K**YQLER	Nup82	Dyn2	541	34	40.4	
IV**K**AQTLGVSIHNR-DGLL**K**EIK	Nup82	Nup159	541	1,397	34.8	
IV**K**AQTLGVSIHNR-DIAGTV**K**K	Nup82	Dyn2	541	46	31.2	
IV**K**AQTLGVSIHNR-DNE**K**KTEESSTGK	Nup82	Nsp1	541	598	36.3	
IV**K**AQTLGVSIHNR-EI**K**LLR	Nup82	Nup159	541	1,400	35.3	
IV**K**AQTLGVSIHNR-E**K**VTDYVR	Nup82	Nup159	541	1,294	32.9	
IV**K**AQTLGVSIHNR-**K**INSWDQVLVK	Nup82	Nsp1	541	661	36.9	
IV**K**AQTLGVSIHNR-**K**TEESSTGK	Nup82	Nsp1	541	599	38.8	
IV**K**AQTLGVSIHNR-LQLEE**K**GK	Nup82	Nup159	541	1,414	37.6	
IV**K**AQTLGVSIHNR-LQLEE**K**GK^K	Nup82	Nup159	541	1,414	26.0	Missed-cleavage derivative to previous peptide
IV**K**AQTLGVSIHNR-MQ**K**TLR	Nup82	Nup159	541	1,343	42.9	
IV**K**AQTLGVSIHNR-QINSI**K**K	Nup82	Nsp1	541	822	32.0	
IV**K**AQTLGVSIHNR-QL**K**EYYTSAK	Nup82	Nup159	541	1,191	34.9	
IV**K**AQTLGVSIHNR-STADV**K**SSDSLK	Nup82	Nsp1	541	613	39.3	
KFEAQNK-MQ**K**TLR	Nup82	Nup159	580	1,343	36.1	
KFEAQNK^K-MQ**K**TLR	Nup82	Nup159	580	1,343	33.1	Missed-cleavage derivative to previous peptide
KFEAQN**K**K-MQ**K**TLR	Nup82	Nup159	586	1,343	34.3	
KMLEIDSK-MQ**K**TLR	Nup82	Nup159	685	1,343	39.5	
KWDAQLSR-LQLEE**K**GK	Nup82	Nup159	587	1,414	27.4	
KWDAQLSR-MQ**K**TLR	Nup82	Nup159	587	1,343	42.5	
MLEIDS**K**IIK-EI**K**LLR	Nup82	Nup159	692	1,400	37.7	
QLQSTC**K**IISK-MQ**K**TLR	Nup82	Nup159	569	1,343	31.4	
QLQSTC**K**IISK^DDDLR-MQ**K**TLR	Nup82	Nup159	569	1,343	26.7	Missed-cleavage derivative to previous peptide
TQGSDY**K**DHDGDYKDDDDK-MQ**K**TLR	Nup82	Nup159	718	1,343	27.1	
E**K**VTDYVRK-KFEAQN**K**K	Nup159	Nup82	1,294	586	29.4	
EYYTSA**K**VSNIPFVSQNSTLR-STADV**K**SSDSLK	Nup159	Nsp1	1,198	613	24.8	
NMDTFFTDQSSIPLV**K**R-IV**K**AQTLGVSIHNR	Nup159	Nup82	1,249	541	34.4	
NMDTFFTDQSSIPLV**K**R-**K**FEAQNK	Nup159	Nup82	1,249	580	25.3	
NMDTFFTDQSSIPLV**K**R-**K**FEAQNK^K	Nup159	Nup82	1,249	580	26.3	Missed-cleavage derivative to previous peptide
DALD**K**YQLER-DIAGTV**K**K	Dyn2		34	46	29.7	
DALD**K**YQLER-DIAGTV**K**K^QLDVK	Dyn2		34	46	33.6	Missed-cleavage derivative to previous peptide
DALD**K**YQLER-**K**QLDVK	Dyn2		34	47	41.2	
DALD**K**YQLER^DIAGTVK-**K**QLDVK	Dyn2		34	47	38.3	Missed-cleavage derivative to previous peptide
DALD**K**YQLER-L**K**EDILTISK	Dyn2		34	21	40.7	

Table S3. **Cross-links identified within the Nup82 complex using DSS** *(Continued)*

Peptide	Protein 1	Protein 2	AA 1	AA 2	Id score	Comment
DNE**K**KTEESSTGK-**K**TEESSTGK	Nsp1		598	599	25.2	
DNE**K**KTEESSTGK-STADV**K**SSDSLK	Nsp1		598	613	25.0	
STADV**K**SSDSLK-**K**TEESSTGK	Nsp1		613	599	38.0	
STADV**K**SSDSLK-DNEK^**K**TEESSTGK	Nsp1		613	599	33.5	Missed-cleavage derivative to previous peptide
KTEESSTG**K**STADVK-**K**TEESSTGK	Nsp1		607	599	27.7	
KTEESSTG**K**STADVK-MGAQ**K**DNEK	Nsp1		607	594	28.5	
LNS**K**PVELKPVSLDNK-STADV**K**SSDSLK	Nsp1		623	613	33.1	
NMDTFFTDQSSIPLV**K**R-MQ**K**TLR	Nup159		1,249	1,343	24.6	
E**K**VTDYVR-MQ**K**TLR	Nup159		1,294	1,343	33.7	
MQ**K**TLR-MQ**K**TLR	Nup159		1,343	1,343	37.5	Homodimeric peptide topology; restraint used for modeling
Q**K**LFDVSAK-MQ**K**TLR	Nup159		1,348	1,343	41.2	
LDDNPLVA**K**LAK-DGLL**K**EIK	Nup159		1,384	1,397	42.2	
LDDNPLVA**K**LAK-EI**K**LLR	Nup159		1,384	1,400	33.8	
LDDNPLVA**K**LAK-LQLEE**K**GK	Nup159		1,384	1,414	31.5	
LDDNPLVA**K**LAK-MQ**K**TLR	Nup159		1,384	1,343	32.7	
DGLL**K**EIK-EI**K**LLR	Nup159		1,397	1,400	35.0	
DGLL**K**EIK-LQLEE**K**GK	Nup159		1,397	1,414	38.8	
DGLL**K**EIK-MQ**K**TLR	Nup159		1,397	1,343	36.8	
EI**K**LLR-MQ**K**TLR	Nup159		1,400	1,343	29.6	
LQLEE**K**GK-DM**K**GFK	Nup159		1,414	1,432	38.5	
LQLEE**K**GK-EI**K**LLR	Nup159		1,414	1,400	36.0	
LQLEE**K**GK^K-EI**K**LLR	Nup159		1,414	1,400	31.5	Missed-cleavage derivative to previous peptide
LQLEE**K**GK-LQLEE**K**GK	Nup159		1,414	1,414	35.8	Homodimeric peptide topology; restraint used for modeling
LQLEE**K**GK^K-LQLEE**K**GK	Nup159		1,414	1,414	38.2	Missed-cleavage derivative to previous peptide
LQLEE**K**GK^K-LQLEE**K**GK^K	Nup159		1,414	1,414	35.7	Missed-cleavage derivative to previous peptide
LQLEE**K**GK-MQ**K**TLR	Nup159		1,414	1,343	44.4	
LQLEE**K**GK^K-MQ**K**TLR	Nup159		1,414	1,343	36.5	Missed-cleavage derivative to previous peptide
LQLEE**K**GKK-Q**K**LFDVSAK	Nup159		1,414	1,348	24.8	
LQLEE**K**GK-LFTV**K**NK	Nup159		1,414	1,372	30.4	
LQLEEKG**K**K-MQ**K**TLR	Nup159		1,416	1,343	31.5	
KASSFDASSSITK-LQLEE**K**GK	Nup159		1,417	1,414	46.4	
KASSFDASSSITK-LQLEEKG**K**K	Nup159		1,417	1,416	29.3	
KASSFDASSSITK-MQ**K**TLR	Nup159		1,417	1343	35.7	
ASSFDASSSIT**K**DMK-LQLEE**K**GKK	Nup159		1,429	1,414	27.7	
DM**K**GFK-MQ**K**TLR	Nup159		1,432	1,343	28.0	
KQIGDFFK-LQLEE**K**GK	Nup159		1,447	1,414	32.9	
FG**K**VDIQK-**K**FEAQNK	Nup82		269	580	26.1	
FG**K**VDIQK-**K**WDAQLSR	Nup82		269	587	28.0	
IV**K**AQTLGVSIHNR-FG**K**VDIQK	Nup82		541	269	35.5	
IV**K**AQTLGVSIHNR-FS**K**LSK	Nup82		541	604	39.7	
IV**K**AQTLGVSIHNR-IEAETIKVD**K**K	Nup82		541	674	35.1	
IV**K**AQTLGVSIHNR-IIS**K**DDDLRR	Nup82		541	573	35.9	
IV**K**AQTLGVSIHNR-IV**K**AQTLGVSIHNR	Nup82		541	541	39.7	Homodimeric peptide topology
IV**K**AQTLGVSIHNR-**K**FEAQNK	Nup82		541	580	43.1	
IV**K**AQTLGVSIHNR-KFEAQN**K**K	Nup82		541	586	35.5	
IV**K**AQTLGVSIHNR-**K**MLEIDSK	Nup82		541	685	43.9	
IV**K**AQTLGVSIHNR-**K**SQNEWDELR	Nup82		541	675	35.2	
IV**K**AQTLGVSIHNR-**K**SQNEWDELR^K	Nup82		541	675	27.4	Missed-cleavage derivative to previous peptide
IV**K**AQTLGVSIHNR-**K**WDAQLSR	Nup82		541	587	48.4	
IV**K**AQTLGVSIHNR-QLQSTC**K**IISK	Nup82		541	569	36.7	
IV**K**AQTLGVSIHNR-QLQSTC**K**IISK^DDDLR	Nup82		541	569	38.6	Missed-cleavage derivative to previous peptide
QLQSTC**K**IISK-**K**FEAQNK	Nup82		569	580	38.7	

Table S3. **Cross-links identified within the Nup82 complex using DSS** *(Continued)*

Peptide	Protein 1	Protein 2	AA 1	AA 2	Id score	Comment
QLQSTC**K**IISK-KFEAQN**K**K	Nup82		569	586	26.2	
IIS**K**DDDLR-R**K**FEAQNK	Nup82		573	580	25.2	
IIS**K**DDDLR^R-**K**FEAQNK	Nup82		573	580	41.9	Missed-cleavage derivative to previous peptide
IIS**K**DDDLR^R-**K**FEAQNK^K	Nup82		573	580	28.9	Missed-cleavage derivative to previous peptide
QLQSTCK^IIS**K**DDDLR-R**K**FEAQNK	Nup82		573	580	31.6	Missed-cleavage derivative to previous peptide
IIS**K**DDDLRR-KFEAQN**K**K	Nup82		573	586	29.4	
IIS**K**DDDLRR-**K**WDAQLSR	Nup82		573	587	38.3	
KFEAQN**K**K-**K**MLEIDSK	Nup82		586	685	25.2	
KWDAQLSR-FEAQN**K**K	Nup82		587	586	36.6	
FEAQNK^**K**WDAQLSR-KFEAQN**K^**K	Nup82		587	586	26.5	Missed-cleavage derivative to previous peptide
KWDAQLSR-FS**K**LSK	Nup82		587	604	40.0	
KWDAQLSR-**K**FEAQNK	Nup82		587	580	42.4	
KWDAQLSR-R^**K**FEAQNK	Nup82		587	580	35.1	Missed-cleavage derivative to previous peptide
KLSQIAESNK-FS**K**LSK	Nup82		608	604	40.6	
KLSQIAESNK^FK-FS**K**LSK	Nup82		608	604	41.9	Missed-cleavage derivative to previous peptide
KISHGEMK-WF**K**EIR	Nup82		622	632	30.3	
IEAETI**K**VDKK-FG**K**VDIQK	Nup82		671	269	37.3	
IEAETI**K**VDKK-FS**K**LSK	Nup82		671	604	28.7	
IEAETI**K**VDKK-**K**FEAQNK	Nup82		671	580	28.5	
IEAETI**K**VDKK-**K**MLEIDSK	Nup82		671	685	35.4	
IEAETI**K**VDKK-**K**WDAQLSR	Nup82		671	587	37.2	
IEAETI**K**VDK-**K**SQNEWDELR	Nup82		671	675	38.3	
SELTR^IEAETI**K**VDK-**K**SQNEWDELRK	Nup82		671	675	35.0	Missed-cleavage derivative to previous peptide
IEAETI**K**VDK-**K**SQNEWDELR^K	Nup82		671	675	42.0	Missed-cleavage derivative to previous peptide
IEAETIKVD**K**K-**K**FEAQNK	Nup82		674	580	34.4	
IEAETIKVD**K**K-**K**MLEIDSK	Nup82		674	685	31.7	
IEAETIKVD**K**K-**K**WDAQLSR	Nup82		674	587	28.3	
KSQNEWDELR-**K**MLEIDSK	Nup82		675	685	38.2	
KSQNEWDELRK-MLEIDS**K**IIK	Nup82		675	692	29.5	
TQGSDY**K**DHDGDYKDDDDK-IV**K**AQTLGVSIHNR	Nup82		718	541	34.2	
TQGSDYKDHDGDY**K**DDDDK-FEAQN**K**K	Nup82		725	586	26.7	

Cross-linked peptides are separated by hyphens; cross-linked lysine residues of the peptides are in bold. ^ indicates a missed-cleavage derivative of previous peptide, which comprises an independent experimental evidence. Id, linear discriminant.

Table S4. **Cross-links identified within the Nup82 complex using DSG**

Peptide	Protein 1	Protein 2	AA 1	AA 2	Id score	Comment
KTEESSTG**K**STADVK-**K**QLDVK	Nsp1	Dyn2	607	47	24.9	
KTEESSTG**K**STADVK-FG**K**VDIQK	Nsp1	Nup82	607	269	27.6	
KTEESSTGK -IV**K**AQTLGVSIHNR	Nsp1	Nup82	541	599	36.6	
KTEESSTGK^STADVK-IV**K**AQTLGVSIHNR	Nsp1	Nup82	599	541	28.5	Missed-cleavage derivative to previous peptide
LNS**K**PVELKPVSLDNK-IV**K**AQTLGVSIHNR	Nsp1	Nup82	623	541	29.7	
LNSKPVEL**K**PVSLDNK-IV**K**AQTLGVSIHNR	Nsp1	Nup82	628	541	28.2	
KTEESSTG**K**STADVK-QLQSTC**K**IISK	Nsp1	Nup82	607	569	25.3	
DNE**K**KTEESSTGK-**K**FEAQNK	Nsp1	Nup82	598	580	28.2	
LNS**K**PVELKPVSLDNK-QL**K**EYYTSAK	Nsp1	Nup159	623	1,191	27.6	
QINSI**K**K-EI**K**LLR	Nsp1	Nup159	822	1,400	28.0	
NMDTFFTDQSSIPLV**K**R-**K**VLFHPK	Nup159	Nup82	1,249	129	25.3	
DGLL**K**EIK-FG**K**VDIQK	Nup159	Nup82	1,397	269	25.4	
LQLEE**K**GK-FG**K**VDIQK	Nup159	Nup82	1,414	269	25.8	
LQLEE**K**GK^K-FG**K**VDIQK	Nup159	Nup82	1,414	269	28.5	Missed-cleavage derivative to previous peptide
E**K**VTDYVRK-**K**FEAQNK	Nup159	Nup82	1,294	580	29.4	
KASSFDASSSITK-**K**FEAQNKK	Nup159	Nup82	1,417	580	28.5	
QL**K**EYYTSAK-**K**TEESSTGK	Nup159	Nsp1	1,191	599	24.5	
IV**K**AQTLGVSIHNR-DALD**K**YQLER	Nup82	Dyn2	541	34	26.5	
IV**K**AQTLGVSIHNR-**K**QLDVK	Nup82	Dyn2	541	47	29.8	
IV**K**AQTLGVSIHNR-DNE**K**KTEESSTGK	Nup82	Nsp1	541	598	31.0	
IV**K**AQTLGVSIHNR-DNEK^**K**TEESSTGK	Nup82	Nsp1	541	599	32.2	Missed-cleavage derivative to previous peptide
IV**K**AQTLGVSIHNR-TEESSTG**K**STADVK	Nup82	Nsp1	541	607	25.6	
IV**K**AQTLGVSIHNR-K^TEESSTG**K**STADVK	Nup82	Nsp1	541	607	37.5	Missed-cleavage derivative to previous peptide
IV**K**AQTLGVSIHNR-STADV**K**SSDSLK	Nup82	Nsp1	541	613	33.9	
IV**K**AQTLGVSIHNR-**K**INSWDQVLVK	Nup82	Nsp1	541	661	27.9	
FG**K**VDIQK-QINSI**K**K	Nup82	Nsp1	269	822	26.3	
IV**K**AQTLGVSIHNR-QINSI**K**K	Nup82	Nsp1	541	822	28.3	
IV**K**AQTLGVSIHNR-QL**K**EYYTSAK	Nup82	Nup159	541	1,191	31.1	
IV**K**AQTLGVSIHNR-E**K**VTDYVR	Nup82	Nup159	541	1,294	32.1	
IV**K**AQTLGVSIHNR-E**K**VTDYVR^K	Nup82	Nup159	541	1,294	36.9	Missed-cleavage derivative to previous peptide
FG**K**VDIQK-MQ**K**TLR	Nup82	Nup159	269	1,343	37.1	
FG**K**VDIQK^EYR-MQ**K**TLR	Nup82	Nup159	269	1,343	31.5	Missed-cleavage derivative to previous peptide
IV**K**AQTLGVSIHNR-MQ**K**TLR	Nup82	Nup159	541	1,343	37.2	
IIS**K**DDDLRR-MQ**K**TLR	Nup82	Nup159	573	1,343	24.8	
KFEAQNK-MQ**K**TLR	Nup82	Nup159	580	1,343	27.2	
KFEAQNK^K-MQ**K**TLR	Nup82	Nup159	580	1,343	26.3	Missed-cleavage derivative to previous peptide
KWDAQLSR-MQ**K**TLR	Nup82	Nup159	587	1,343	26.7	
FS**K**LSK-MQ**K**TLR	Nup82	Nup159	604	1,343	25.9	
IV**K**AQTLGVSIHNR-Q**K**LFDVSAK	Nup82	Nup159	541	1,348	32.7	
IV**K**AQTLGVSIHNR-DGLL**K**EIK	Nup82	Nup159	541	1,397	30.2	
FG**K**VDIQK-EI**K**LLR	Nup82	Nup159	269	1,400	29.2	
IV**K**AQTLGVSIHNR-EI**K**LLR	Nup82	Nup159	541	1,400	31.	
NVI**K**QLQFVSK-LQLEE**K**GK	Nup82	Nup159	251	1,414	26.4	
IV**K**AQTLGVSIHNR-LQLEE**K**GK	Nup82	Nup159	541	1,414	27.8	
TQGSDY**K**DHDGDYKDDDDK-LQLEE**K**GKK	Nup82	Nup159	718	1,414	27.3	
IV**K**AQTLGVSIHNR-**K**QIGDFFK	Nup82	Nup159	541	1,447	32.5	
DALD**K**YQLER-**K**QLDVK	Dyn2		34	47	31.9	
DALD**K**YQLER-L**K**EDILTISK	Dyn2		34	21	27.5	
STADV**K**SSDSLK-**K**TEESSTGK	Nsp1		613	599	26.5	
LNS**K**PVELKPVSLDNK-STADV**K**SSDSLK	Nsp1		623	613	26.4	
E**K**VTDYVR-MQ**K**TLR	Nup159		1,294	1,343	26.7	

Table S4. **Cross-links identified within the Nup82 complex using DSG** *(Continued)*

Peptide	Protein 1	Protein 2	AA 1	AA 2	Id score	Comment
E**K**VTDYVR^K-MQ**K**TLR	Nup159		1,294	1,343	28.4	Missed-cleavage derivative to previous peptide
Q**K**LFDVSAK-MQ**K**TLR	Nup159		1,348	1,343	31.7	
LFTV**K**NK-MQ**K**TLR	Nup159		1,372	1,343	25.5	
RLDDNPLVA**K**LAK-LFTV**K**NK	Nup159		1,384	1,372	29.2	
LDDNPLVA**K**LAK-EI**K**LLR	Nup159		1,384	1,400	27.7	
LDDNPLVA**K**LAK-MQ**K**TLR	Nup159		1,384	1,343	25.8	
LDDNPLVA**K**LAK-DGLL**K**EIK	Nup159		1,384	1,397	24.8	
DGLL**K**EIK-MQ**K**TLR	Nup159		1,397	1,343	25.5	
DGLL**K**EIK-EI**K**LLR	Nup159		1,397	1,400	25.1	
LQLEE**K**GK-EI**K**LLR	Nup159		1,414	1,400	32.0	
EI**K**LLR^EQVSR-LQLEE**K**GK	Nup159		1,400	1,414	26.9	Missed-cleavage derivative to previous peptide
EI**K**LLREQVSR-MQ**K**TLR	Nup159		1,400	1,343	24.6	
LQLEE**K**GK-MQ**K**TLR	Nup159		1,414	1,343	28.5	
LQLEEKG**K**K-MQ**K**TLR	Nup159		1,416	1,343	25.9	
KASSFDASSSITK-LQLEE**K**GK	Nup159		1,417	1,414	32.9	
KASSFDASSSITK-MQ**K**TLR	Nup159		1,417	1,343	26.1	
DM**K**GFK-MQ**K**TLR	Nup159		1,432	1,343	27.0	
KQIGDFFK-LQLEE**K**GK	Nup159		1,447	1,414	28.6	
KQIGDFFK-EI**K**LLR	Nup159		1,447	1,400	27.3	
KQIGDFFK-MQ**K**TLR	Nup159		1,447	1,343	25.5	
FG**K**VDIQK-**K**VLFHPK	Nup82		269	129	34.4	
FG**K**VDIQK-**K**WDAQLSR	Nup82		269	587	25.9	
EI**K**SLITLPEQLGK-**K**VLFHPK	Nup82		361	129	26.1	
IV**K**AQTLGVSIHNR-**K**WDAQLSR	Nup82		541	587	39.5	
IV**K**AQTLGVSIHNR-FS**K**LSK	Nup82		541	604	38.5	
IV**K**AQTLGVSIHNR-FG**K**VDIQK	Nup82		541	269	37.8	
IV**K**AQTLGVSIHNR-IEAETIKVD**K**K	Nup82		541	674	33.1	
IV**K**AQTLGVSIHNR-**K**FEAQNK	Nup82		541	580	32.2	
IV**K**AQTLGVSIHNR-R^**K**FEAQNK	Nup82		541	580	29.2	Missed-cleavage derivative to previous peptide
IV**K**AQTLGVSIHNR-**K**VLFHPK	Nup82		541	129	31.8	
IV**K**AQTLGVSIHNR-**K**MLEIDSK	Nup82		541	685	31.3	
IV**K**AQTLGVSIHNR-IIS**K**DDDLR	Nup82		541	573	28.0	
IV**K**AQTLGVSIHNR-IIS**K**DDDLR^R-	Nup82		541	573	30.0	Missed-cleavage derivative to previous peptide
IV**K**AQTLGVSIHNR-IV**K**AQTLGVSIHNR	Nup82		541	541	28.0	Homodimeric peptide topology
IV**K**AQTLGVSIHNR-QLQSTC**K**IISK	Nup82		541	569	28.0	
IV**K**AQTLGVSIHNR-LSQIAESN**K**FK	Nup82		541	617	27.7	
IV**K**AQTLGVSIHNR-IEAETI**K**VDKK	Nup82		541	671	26.6	
IV**K**AQTLGVSIHNR-KFEAQN**K**K	Nup82		541	586	26.1	
QLQSTC**K**IISK-**K**WDAQLSR	Nup82		569	587	29.1	
QLQSTC**K**IISKDDDLR-IV**K**AQTLGVSIHNR	Nup82		569	541	26.3	
QLQSTC**K**IISK-**K**FEAQNK	Nup82		569	580	25.8	
IIS**K**DDDLRR-**K**FEAQNK	Nup82		573	580	29.6	
IIS**K**DDDLRR-FS**K**LSK	Nup82		573	604	27.0	
IIS**K**DDDLR-**K**WDAQLSR	Nup82		573	587	25.7	
IIS**K**DDDLR^R-**K**WDAQLSR	Nup82		573	587	25.5	Missed-cleavage derivative to previous peptide
KFEAQNK-FS**K**LSK	Nup82		580	604	32.4	
KFEAQNK-**K**FEAQNK	Nup82		580	580	24.9	Homodimeric peptide topology
KFEAQN**K**K-FS**K**LSK	Nup82		586	604	25.9	
KWDAQLSR-R^**K**FEAQNK	Nup82		587	580	26.3	Missed-cleavage derivative to previous peptide
KWDAQLSR-**K**FEAQNK	Nup82		587	580	33.0	
KWDAQLSR-FS**K**LSK	Nup82		587	604	29.4	
FS**K**LSKK-**K**FEAQNK	Nup82		604	580	28.8	
KLSQIAESNK-FS**K**LSK	Nup82		608	604	35.2	

Table S4. **Cross-links identified within the Nup82 complex using DSG** *(Continued)*

Peptide	Protein 1	Protein 2	AA 1	AA 2	ld score	Comment
KLSQIAESNK^FK-FS**K**LSK	Nup82		608	604	31.3	Missed-cleavage derivative to previous peptide
LSQIAESN**K**FK-E**K**KISHGEMK	Nup82		617	621	25.7	
LSQIAESN**K**FK-**K**ISHGEMK	Nup82		617	622	25.4	
KISHGEMK-WF**K**EIR	Nup82		622	632	28.2	
IEAETI**K**VDK-**K**SQNEWDELR	Nup82		671	675	27.5	
SELTRIEAETI**K**VDK-**K**SQNEWDELR^K	Nup82		671	675	29.5	Missed-cleavage derivative to previous peptide
IEAETI**K**VDKK-**K**MLEIDSK	Nup82		671	685	26.5	
KSQNEWDELR-**K**MLEIDSK	Nup82		675	685	35.8	

Cross-linked peptides are separated by hyphens; cross-linked lysine residues of the peptides are in bold. ^ indicates missed-cleavage derivative of previous peptide, which comprises an independent experimental evidence. ld, linear discriminant.

Table S5. **Protein stoichiometry of the Nup82 complex using SRM-MS**

Protein	Peptide	Early gel filtration fraction		Apex gel filtration fraction	
		Intensity per peptide[a]	Abundance per protein[b]	Intensity per peptide[a]	Abundance per protein[b]
Nsp1	IDQSLQYIER	0.24	3.08	7.66	1.78
Nsp1	ILNSHFDALR	0.35	–	8.51	–
Nup82	AQTLGVSIHNR	0.22	2.02	13.19	2.39
Nup82	SLQQDLSYLK	0.17	–	8.54	–
Nup159	DLSTHQFR	0.21	2.00	9.16	2.00
Nup159	SINNLYTWR	0.18	–	8.98	–
Dyn2	NFGSYVTHEK	0.70	9.31	35.27	10.56
Dyn2	YGNTWHVIVGK	1.09	–	60.49	–

[a]Summed intensity of the five most intense transitions per peptide.
[b]Peptide intensities corresponding to the same protein were averaged and normalized to the Nup159 signal, resulting in the final stoichiometric read out per protein.

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