**Supplemental Figure Legends**

**Figure S1. Stoichiometry of the endogenous Nup82 holo-complex.** Related to Figure 1.

(A) Affinity-purified Nup82 holo-complex was loaded into 5-20% sucrose density gradients. The resulting fractions were analyzed by Sypro Ruby stained SDS-PAGE. A representative example is shown. The resulting sedimentation coefficient (S20,w) value corresponding to the main fractions was estimated from n=4 gradients.

(B) Size-exclusion chromatography was used to estimate the Stokes radius (Rs) value for the affinity-purified Nup82 holo-complex. Sypro Ruby stained SDS-PAGE gel of a representative experiment is shown. The resulting mass (M) of the holo-complex was calculated using the Siegel-Monte equation (Erickson, 2009).

(C) Sypro-Ruby stained SDS-PAGE gel showing the affinity purified Nup82 holo-complex from diploid strains containing one PrA tagged copy of the indicated nucleoporin. Colored dots indicate the bands identified by mass spectrometry, with the protein ID indicated below. Blue indicates the tagged protein, and green indicates components of the Nic96 complex co-purifying with Nsp1-PrA. Molecular weight standards are shown on the left.

(D) Sucrose density gradient purified Nup82 holocomplex was analyzed by quantitative proteomics using an internal standard (QconCat) as described in the STAR Methods. The relative stoichiometry was normalized to Nup82. Error

bars represent the standard error of the mean for 2 in 2 biological and technical replicas.

(E) Affinity purified native Nup82 holo-complex from a strain carrying an empty plasmid (wild type) or a Dyn2 overexpression plasmid (Dyn2 overex.) were analyzed by SDS-PAGE and stained with Sypro-Ruby. The intensity of the resulting bands was quantified and normalized to the abundance of Nup159. The relative amount of each protein between the wt and Dyn2 overex. was obtained and plotted. Error bars represent the standard error of the mean for n=6.

**Figure S2. Cross-linking-MS and negative stain electron microscopy analyses of the Nup82 holo-complex.** Related to Figure 1.

(A) Circos-XL plots showing the distribution of all DSS (left plot) and EDC (middle plot) cross-links identified within the native Nup82 holo-complex and to the substoichiometric component Nup116. On the right side, a similar plot showing the DSS cross-links identified on the exogenous-skNup82-containing complex (see STAR Methods) is shown. Each protein is represented as a colored segment, with the amino acid residue indicated on the outside of the plot and relevant domains indicated inside each segment; regions without clear fold assignment are identified by clear tone colors. Inter-molecular cross-links are depicted as purple lines and intra-molecular cross-links as gray lines. The internal circles include histograms representing the density of cross-links per 10 residues in DSS and EDC (blue and light blue color for inter-molecular cross-

links and intra-molecular cross-links, respectively) and the density of lysines in DSS (orange and light orange bars for cross-linked and uncross-linked residues, respectively) or the density of lysine/carboxylic acid in EDC (pink and light pink bars for cross-linked and uncross-linked residues, respectively).

(B) An example of a cross-link MS/MS spectrum (mass = 9,264 Da, z = 6) is shown. The corresponding b and y ion series and their charge states are assigned.

(C) Negative stain EM 2D class averages of the endogenous Nup82 holo-complex. 4,266 single particles were classified in 23 class averages using EMAN (Ludtke et al., 1999). The number of particles per class is indicated in the upper-left corner of each panel. The two class averages where a double Dyn2 dimer was observed are indicated with an arrow. Scale bar, 10nm.

(D) The unstructured FG repeats are not revealed in the negative-stain EM holo-complex shape. Negative-stain EM 2D analysis of the difference between a wild type (WT, left panels) Nup82 holo-complex and a version where the FG and FxFG regions of Nsp1 have been genomically deleted (Nsp1ΔFG, middle panels) (Strawn et al., 2004). The difference map on the right panels was calculated by subtracting the Nsp1ΔFG class averages from the WT class averages. Scale bar, 10nm.

**Figure S3. Structural and evolutionary relationship between the Nup82 and Nic96 complexes and four-stage scheme for integrative structure determination of the Nup82 holo-complex.** Related to Figure 1.

Closest homologs of the *Saccharomyces cerevisiae* Nsp1 (A), Nup159 (B), and Nup82 (C) coiled-coil regions were detected by HHPred (Soding, 2005) (Table S1). The multiple sequence alignment was visualized using SeaView 4.6 (Gouy et al., 2010), and numbering above alignment is relative to *S. cerevisiae*. Remarkably, the top and highly significant hit is another complex from the NPC, also containing a heterotrimer of coiled-coils: the *Xenopus laevis* Nup93:Nup62:Nup58:Nup54 complex (PDB 5C3L) (Chug et al., 2015) and its *Chaetomium thermophilum* Nic96:Nsp1:Nup57:Nup49 complex homolog (PDB 5CWS) (Stuwe et al., 2015a). The C-termini of both complexes share a common domain arrangement, formed by three consecutive helical coiled-coil regions of different lengths, connected by flexible linkers (Figure 1), and both complexes even share a common component, Nsp1.

D) Our integrative structure determination proceeds through four stages: (1) gathering of data, (2) representation of subunits and translation of the data into spatial restraints, (3) configurational sampling to produce an ensemble of structures that satisfies the restraints, and (4) analysis and validation of the ensemble structures. Further details are provided in Table 1, as well as the STAR Methods. Files containing the input data, scripts, and output structures are available online (http://salilab.org/nup82; https://github.com/salilab/nup82).

**Figure S4. Validation of the Nup82 holo-complex structure (I).** Related to Figure 2.

(A) Clustering based on the RMSD distance matrix identified a single dominant cluster containing 370 of the 463 refined top-scoring models. The RMSD values are colored from dark blue (0 Å) to dark red (30 Å).

(B) Representative em2d score distributions of initial structures show the cross-correlation coefficient ranging from 0.76 to 0.91 for the EM 2D class averages 4 (blue filled circle) and 19 (red filled square). We filtered structures above a cross-correlation threshold of 0.89 (black dotted line) for refinement. The final set of 650 filtered structures satisfies at least 10 class averages above the threshold.

(C) The positional precisions for each component of the Nup82 holo-complex were calculated as average RMSF across all pairs of structures in the cluster, after least-squares superposition onto the centroid structure (Shi et al., 2014). The 9.0 Å precision of the core structured region in the dominant cluster was sufficiently high to pinpoint the locations and orientations of the constituent proteins and domains, demonstrating the quality of the data.

(D) We assessed the DSS (left plot) and EDC (right plot) chemical cross-links in the dominant cluster; a cross-link restraint is satisfied by the cluster ensemble if the median Cα-Cα distance of the corresponding residue pairs (considering restraint ambiguity) is < 35 Å and 30 Å for the DSS and the EDC cross-links, respectively. Satisfied cross-links (93.3% DSS and 74.1% EDC) were represented by blue filled circles and the violated cross-links as blue empty circles. Same-residue cross-links between two copies of the same protein are represented by red triangles.

**Figure S5. Validation of the Nup82 holo-complex structure (II).** Related to Figure 2.

(A) Comparison of the localization probability density computed from our structure of the Nup82 holo-complex (left, light blue), with the previously published negative-

stain EM tomography map of a truncated version of the Nup82 holo-complex (right, darker blue) (Gaik et al., 2015). The common and specific structural features are indicated. Scale bar, 20 Å.

(B) Comparison between the major (370 structures) and minor (93 structures) cluster ensembles of the Nup82 holo-complex solutions. The average RMSD between the major and minor clusters is relatively low at approximately 20Å, considering the resolution of the data, the resolution of the coarse-grained molecular representation, and the variation within each cluster (Schneidman-Duhovny et al., 2014) (Figure S4A). As a result, localization of all components is effectively identical between the major and minor clusters, differing only in the orientation of the Nup82 β-propeller. Most importantly, our functional interpretation of the structure is completely robust with regard to the differences between the means of the two clusters.

(C) Trimeric coiled-coil-like structure predicted between the helical regions Nup82 (562-612) (dark blue), Nsp1 (667-722) (cyan) and Nup159 (1283-1327) (navy blue). The model is computed using the chemical cross-linking data, crystallographic structures of domains, secondary structure predictions, and assuming a 1:1:1 stoichiometry of the complex. The shown ribbon is the backbone structure of a representative model chosen from the best scoring cluster of solutions. The localization densities are calculated for the three helical regions on the best scoring cluster.

(D) , (E), and (F) SAXS analyses of the recombinantly expressed Nup82 (4-220) (D), Nup82 (4-452) (E), and Nup82 (572-690) (F) constructs.

(LEFT) the experimental (black dots) and calculated SAXS profiles (red lines) using FoXS (Schneidman-Duhovny et al., 2010) are shown. The lower left plot presents the residuals (calculated intensity/experimental intensity) of the corresponding SAXS sample.

(MIDDLE) Upper-middle inset shows the SAXS profiles in the Guinier plot with the calculated $R_g$ fit value in Å. The linear behavior of the Guinier plots confirms a high degree of homogeneity for all Nup82 SAXS samples in solution. Lower-middle inset shows the correspondent Kratky plot. The extrapolation curves (red lines) are added to the Kratky plots. The Kratky plots are used to visually depict the level of macromolecular flexibility. A sample with a high degree of flexibility has a monotonous increase in the Kratky curve, such as Nup82 (572-690) (F). In contrast, Nup82 (4-220) (D) and Nup82 (4-452) (E) show well-defined "bell-shaped" curves, indicating folded structures with less flexibility.

(RIGHT) Shown is a view of the *ab initio* shape (represented as a transparent envelope) computed from the experimental SAXS profile, with the best fit of a ribbon representations of each construct. In (F), two ribbon representations of the equivalent Nup82 fragments are shown in the conformation they adopt within the Nup82 holo-complex structure subunits 1 (red) and 2 (blue).

**Figure S6. Fluorescence in situ hybridization analysis of mRNA export defects on Nup84 complex truncation mutants.** Related to Figure 4.

The upper image of each row shows representative images of the localization of polyA mRNA by FISH (red) for each of the analyzed Nup84 truncation mutants (Fernandez-

Martinez et al., 2012). The lower image on each row shows the merged localization of polyA mRNA (red) and DNA stained with DAPI (blue). Bar, 5 μm.

**Figure S7. Validation of the Nup82-Nup84 complex assembly.** Related to Figures 3, 5, and 6.

(A) Clustering based on the RMSD distance matrix identified three clusters containing 86, 70, and 44 structures of the 200 top-scoring structures, respectively. The RMSD values are colored from dark blue (0 Å) to dark red (135 Å).

(B) Comparison among the three cluster ensembles of the Nup82-Nup84 complex assembly. The localization probability density map for each of the three clusters was shown as a transparent envelope. All our solutions were similar, differing only in the degree of the Nup82 complex rotation along its long axis, relative to the Nup84 complex. Precisions of the Nup82 holo-complex in the 3 clusters were 30.2, 11.0, and 39.0 Å, respectively.

(C) Fitting of the Nup82-Nup84 complex assemblies to the *S. cerevisiae* NPC localization probability density map. Two views of the optimized alignment of two *S. cerevisiae* Nup82-Nup84 complex assemblies into the *S. cerevisiae* NPC map (transparent gray), together with a side view of the detailed alignment (Alber et al., 2007b); Nup85 (green), Nup133 (red), and two Nup82 units (blue and orange) are indicated. Among the three clusters, only cluster C satisfied both the cross-links used to compute them (Table S3) and the S. cerevisiae NPC localization probability density map (fit score by overlapping volume = 0.46).

(D) Comparison of the Nup82-Nup84 complex assemblies with the human NPC tomographic cryo-EM map (EMDB 2444) (Bui et al., 2013). Two views of the optimized alignment of two S. cerevisiae Nup82-Nup84 complex assemblies into the human NPC map. Cluster C is the only one that aligns to the wild-type human NPC tomographic cryo-EM map (CCC = 0.72).

**Supplemental Tables**

**Table S1. Representation of the Nup82 holo-complex components.** Related to Figure 1.

**Table S2. CX-MS analysis of the Nup82 holo-complex.** Related to Figure 1, 2 and S2.

**Table S3. Mapping of the cytoplasmic mRNA export platform using CX-MS**. Related to Figures 3 and 6.

**Table S4. Summary of SAXS experiments.** Related to Figure 2.

**Supplemental Movie Legends**

**Movie S1. Structure of the core Nup82 holo-complex.** Related to Figure 1.

First, each of the core Nup82 holo--complex components is shown in a sequential order (Nup82, Nup159, Nsp1, and Dyn2), along with its localization probability density. Then, the two subunits of the Nup82 holo-complex are highlighted in red (subunit 1) and blue (subunit 2). Finally, the whole Nup82 complex is presented by a 360° rotation along y and x axes.

**Movie S2. Structure of the Nup82-Nup84 complex assembly.** Related to Figures 3, 5, and 6.

First, the whole Y--shaped Nup84 complex is shown along with its localization probability density. Then we present the optimal arrangement of the whole Nup82 holo-complex, as well as its localization probability density. Notably, two copies of the Nup116 C-terminal domain were docked into the Nup82 β-propellers in the movie. Finally, the whole Nup82-Nup84 complex assembly is presented by a 360° rotation along y and x axes.