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1 **Title: In cell architecture of the nuclear pore complex and snapshots of its**
2 **turnover**

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31 **Summary:**

32 Nuclear pore complexes (NPCs) mediate exchange across the nuclear envelope. They consist of
33 hundreds of proteins called nucleoporins (Nups) that assemble in multiple copies to fuse the inner
34 and outer nuclear membranes. Elucidating the molecular function and architecture of NPCs
35 imposes a formidable challenge and requires the convergence of *in vitro* and *in situ* approaches.
36 How exactly NPC architecture accommodates processes such as mRNA export or NPC assembly
37 and turnover inside of cells remains poorly understood. Here we combine integrated *in situ*
38 structural biology, correlative light and electron microscopy with yeast genetics to structurally
39 analyze NPCs within the native context of *Saccharomyces cerevisiae* cells under conditions of
40 starvation and exponential growth. We find an unanticipated *in situ* layout of nucleoporins with
41 respect to overall dimensions and conformation of the NPC scaffold that could not have been
42 predicted from previous *in vitro* analysis. Particularly striking is the configuration of the Nup159
43 complex, which appears critical to spatially accommodate not only mRNA export but also NPC
44 turnover by selective autophagy. We capture structural snapshots of NPC turnover, revealing that
45 it occurs through nuclear envelope herniae and NPC-containing nuclear vesicles. Our study
46 provides the basis for understanding the various membrane remodeling events that happen at the
47 interface of the nuclear envelope with the autophagy apparatus and emphasizes the need of
48 investigating macromolecular complexes in their cellular context.

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49 **Text**

50 Nuclear pore complexes (NPCs) are giant macromolecular assemblies with a very intricate
51 architecture. About 30 different genes termed nucleoporins (Nups) encode for components of
52 NPCs. Scaffold Nups contain folded domains and form a cylindrical central channel. This channel
53 is lined with FG-Nups, which harbor intrinsically disordered FG-rich repeats that interact with
54 nuclear transport receptors. Nups assemble in multiple copies to form an eight-fold rotationally
55 symmetric complex, totaling in ~550 protein building blocks in yeast and ~1000 in mammals¹.
56 The NPC consists of two outer rings, also called nuclear (NR) and cytoplasmic rings (CR), which
57 are placed distally to the inner ring (IR) that resides at the fusion plane between the inner and outer
58 nuclear membranes. Within the three-ringed architecture, Nups are organized into subcomplexes
59 that form specific substructures. The Y-complex is the key scaffolding component of the outer
60 rings, whereas the IR scaffold is built by the inner ring complex². Further, the yeast Nup159
61 complex (Nup214 complex in mammals) associates asymmetrically with the Y-complex at the CR
62 and facilitates the terminal steps of mRNA export. Its core consists of two Nup159-Nup82-Nsp1
63 heterotrimers that dimerize into a characteristic P-shaped structure^{3,4}.

64 Several studies have highlighted that the accurate spatial positioning of the Nup159 complex with
65 respect to the central channel at the cytoplasmic face of the NPC is critical for the spatial
66 organization and directionality of mRNA export^{3,5,6}. While mRNAs are chaperoned through the
67 FG repeats within the central channel in a Mex67-dependent (but Ran independent) manner,
68 exported mRNPs encounter the ATPase activity of the DEAD-box RNA helicase Dpb5 at the
69 cytoplasmic face that removes Mex67 and ratchets the RNA into the cytoplasm^{1,6,7}. Dpb5
70 recruitment and positioning is ensured by the N-terminal beta propeller of Nup159, which is
71 separated by a flexible linker from the C-terminal coiled coils that anchor Nup159 to the NPC
72 scaffold⁷.

73 However, understanding how Nup subcomplexes are positioned to each other within the context
74 of the nuclear membranes and the overall architecture of NPCs imposes a considerable challenge
75 to structural biologists and requires the convergence of *in vitro* and *in situ* approaches⁸. While
76 several high-resolution structures of Nups have been solved by X-ray crystallography, biochemical
77 analysis and cross-linking mass spectrometry revealed the interaction topology. Cryo-electron
78 microscopy (cryo-EM) maps provided an overall framework for the positioning of subcomplexes

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79 using systematic fitting approaches^{9,10,11,12,13}. Cryo-electron tomography (cryo-ET) with
80 subsequent subtomogram averaging is the method of choice to generate such cryo-EM maps,
81 because it allows structural analysis of NPCs within the native context of the nuclear membranes
82 that have to be considered as an integral part of their architecture. Such *in situ* structural analysis
83 is available for both mammals and algae (reviewed in ¹) and revealed that key features of the NPC
84 architecture, such as the stoichiometry of Y-complexes within the outer rings, are not conserved.
85 Intriguingly, the P-shaped outline of the yeast Nup159 complex⁴ was not apparent in any of the
86 cryo-EM maps available to date, either suggesting structural diversity, or alternatively, questioning
87 its physiological relevance.

88 To date, *in situ* structural analysis is still missing for the *S. cerevisiae* NPC (*ScNPC*), which has
89 been extensively studied as a model organism not only for Nup structure, NPC architecture and
90 the mechanism of mRNA export, but also NPC surveillance and turnover^{1,14}. Thus far, only
91 detergent extracted, purified NPC species have been analyzed¹³ that bear neither the nuclear
92 membranes nor any distinct features of Y-complexes (reviewed in ¹). An integrative model of the
93 entire *ScNPC* architecture based on extensive experimental analysis *in vitro* has been recently put
94 forward¹³ and in principle could enable structure-to-function analyses based on the very powerful
95 yeast genetics approaches. However, it remains unknown to what extent the native architecture of
96 the *ScNPC* has been preserved *in vitro*. Therefore, there has been a pressing need to elucidate the
97 spatial organization of subcomplexes of the *ScNPC in situ* within the cellular context.

98 *Structural analysis of the ScNPC in the cellular context*

99 To characterize *ScNPC* architecture *in situ*, we prepared thin cryo-FIB lamellae of exponentially
100 growing, plunge frozen cells¹⁵. We acquired 230 cryo-electron tomograms (Fig. 1a) containing
101 ~500 *ScNPCs* and determined the structures by subtomogram averaging (see Methods). The
102 resulting cryo-EM map at ~25 Å resolution (Extended Data Fig. 1a-b) provides the most detailed
103 overview of the native configuration and conformation of subcomplexes of actively transporting
104 NPCs in cells of any species to date (Fig. 1b-c, Extended Data Fig. 1b-c). A visual inspection of
105 the structure reveals striking disparities, not only compared to the previously analyzed structures
106 of the algal NPC¹⁰ and human NPC⁹ that likely account for species-specific differences, but also
107 in comparison to previous cryo-EM maps of the biochemically isolated and detergent-extracted
108 *ScNPC* (Extended Data Fig. 1c-d, Source Data 1 attached for review process). The previously

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109 published integrative model of one asymmetric unit of the IR¹³ fits unambiguously into the
110 observed density (p-value 1.6×10^{-12} Extended Data Fig. 2), however the model of the entire IR has
111 to be dilated by ~20 nm in diameter in order to fit the *in situ* conformation (Extended Data Fig.
112 3a), thereby spatially separating the eight individual spokes (Extended Data Fig. 3a). This analysis
113 underlines the plasticity of the NPC within cells¹⁵, which might be physiologically relevant for the
114 transport of large cargos and inner nuclear membrane proteins.

115 As expected^{13,16}, 16 nuclear and cytoplasmic rings of Y-complexes are apparent in our cryo-EM
116 map (Fig. 1c-d, Extended Data Fig. 1c), although some additional, yet unexplained density is
117 observed in the nuclear ring. While the crystal structures and homology models of the yeast Y-
118 complex vertex¹⁷ (Sec13–Nup145C–Nup84–ScNup120–ScSeh1–ScNup85) or its fragments fit
119 into the observed Y-shape density with significant p-values (Extended Data Fig. 2), the previous
120 integrative model of the Y-complex¹³ does not fit due to the curvature of its tail (Extended Data
121 Fig. 3c). We therefore applied an integrative modeling procedure (Methods) to take the observed
122 *in situ* conformation into account and obtained complete structures of the Y-complexes in both CR
123 and NR. Due to the larger circumference of the NPC, the Y-complexes occupy a more extended
124 conformation (Extended Data Fig. 3b). These findings further emphasize that the combination of
125 *in vitro* and *in situ* structural analysis is essential to understand NPC architecture in physiological
126 conditions.

127 A prominent feature of the CR, which remains yet unassigned after localization of the Y-
128 complexes, is the P-shaped density of the Nup159 complex that is strikingly reminiscent of the
129 previous *in vitro* analysis of the isolated, negatively stained Nup159 complex^{3,4} (Fig. 2).
130 Systematic fitting of the Nup159 complex negative stain map⁴ into our *in situ* structure confirmed
131 this fit (Fig. 2b, p-value 0.0027, Extended Data Fig. 4a, see Methods). Based on the top resulting
132 fit, we superimposed a previously published representative integrative model of the Nup159
133 complex^{3,13} and we locally fit it into our cryo-EM map (Extended Data Fig. 4b). In comparison to
134 previous architectural models of the entire ScNPC^{3,13}, the P-shape is flipped around the axis that
135 points towards the central channel (Extended Data Fig. 4b). Thereby, the Nup82 β -propellers are
136 positioned towards the inner ring (Extended Data Fig. 4b). The previous architectural model was
137 in part guided by distance restraints from crosslinking mass spectrometry. In our updated Nup159
138 complex configuration, all four of the previously published crosslinks¹³ between the Nup159 and

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139 Y-complexes that could be mapped to the structure are satisfied (Extended Data Fig. 4e). The arm
140 of the P-shape that consists of Nup159 DID tandem repeats binding multiple Dyn2 dimers^{4,18} is
141 clearly apparent *in situ* (Fig. 2). It projects towards the cytoplasm at an angle of $\sim 45^\circ$ with respect
142 to the nucleocytoplasmic axis (Fig. 2), while it had been previously thought to rather face the IR³
143 (Extended Data Fig. 4b). Instead, the protein termini preceding FG-repeats of Nsp1 face the central
144 channel.

145 *Yeast genetics confirms orientation of the RNA export platform*

146 To independently confirm this arrangement, we combined yeast genetics with *in situ* structural
147 analysis. An important interactor of Nup82 is Nup116, one out of three yeast homologues of the
148 essential vertebrate Nup98. This is a key Nup for establishing the NPC permeability barrier^{19,20},
149 mRNA export^{6,19} and pre-ribosome translocation²¹. It is also an architecturally critical linker Nup
150 that connects the inner ring, Y and Nup159 complexes through short linear motifs in its
151 intrinsically disordered domains^{12,22,23}. We superimposed the crystal structure of Nup116(966-
152 1111)-Nup82(1-452)-Nup159(1425-1458)²⁴ with the respective parts of two copies of Nup159 and
153 Nup82 contained in the P-complex (Extended Data Fig. 4c-d). This analysis predicts that the
154 autoproteolytic domain of Nup116 is placed into two yet unassigned densities that are proximate
155 to each other and project towards Nup188 of the IR (Fig. 2c, Extended Data Figs. 4c-d and 5a).
156 Crosslinking analysis¹³ and previous biochemical data suggesting that Nup116 links to Nup188
157 and Nup192 of the IR^{22,23} agree well with this configuration (Extended Data Figs. 4e and 5a). To
158 validate this assignment, we structurally analyzed the ScNPC in *nup116Δ* cells (knock-out, k. o.)
159 cells at permissive temperature, at which NPCs have an ordinary morphological appearance in
160 electron micrographs²⁵. We acquired ~ 120 tomograms and obtained a cryo-EM map as described
161 above (see Methods). The *nup116Δ* structure lacks density exactly at positions proximate to
162 Nup188 as predicted by superposition of the Nup116-Nup82-Nup159 X-ray structure (Fig. 2c and
163 Extended Data Figs. 4c and 5c). This finding corroborates our spatial positioning of Nup116 at the
164 interface between the IR and Nup159-complexes and previous biochemical work that has
165 established Nup116 as a linker nucleoporin associated with the inner ring components^{22,23}. The
166 positioning of the two copies of Nup116 agrees with only one of the two that were previously
167 assigned by integrative modeling¹³ (Extended Data Fig. 5b). Our new model of NPC architecture
168 spatiotemporally accommodates extensive biochemical analysis of mRNP export^{5,7,19}. In fact, it

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169 places the FG-repeats that interact with Mex67 and are relevant earlier during the process, more
170 towards the equatorial plane. Dbp5 and the N-terminus of Nup159 that facilitate the terminal
171 release of Mex67 are positioned more towards the cytoplasm²⁶ (Fig. 2d).

172 *The Nup159 complex mediates selective autophagy through nuclear envelope herniae*

173 At nonpermissive temperature, the *nup116Δ* strain forms nuclear envelope (NE) herniae (Extended
174 Data Fig. 6a), a phenotype in which NPCs are engulfed by the NE membranes and not accessible
175 to the cytosol²⁵. Interestingly, this phenotype was also observed when other Nup159 complex
176 associated Nups, such as Gle2 were genetically perturbed²⁷, and has been linked to the surveillance
177 of NPC assembly by ESCRT proteins^{28,29}. Since herniae were also observed under seemingly
178 unrelated conditions, such as genetic perturbation of inner nuclear membrane proteins^{30,31} in both
179 yeast and human cells³², their exact functional relevance remained to be determined^{14,33}. We
180 wondered if NPC architecture under the herniae is altered and addressed this using cryo-FIB
181 milling, cryo-ET (~40 tomograms) and subtomogram averaging of NPCs at the herniae basis in
182 the *nup116Δ* strain at non-permissive temperature. We found that in these trapped NPCs not only
183 Nup116, but the entire cytoplasmic ring, including the Nup159 and Y-complexes are missing
184 (Extended Data Fig. 6c). Concurrently, the nuclear envelope is less curved at the fusion plane,
185 which is typical for the neck of herniae (Extended Data Fig. 6a-c). This result is in line with models
186 that conceptualize herniae as failed inside-out assembly intermediates of NPCs, in which the lack
187 of fusion of the NE membranes prevented the assembly of the cytoplasmic components (reviewed
188 in^{14,33}). Another finding further corroborates this model. We noticed that as compared to WT cells,
189 the NE of *nup116Δ* cells at permissive temperature contains a high number of mushroom-shape
190 evagination of the inner nuclear membrane (Extended Data Fig. 6d-e). The structures are
191 morphologically reminiscent of interphase assembly intermediates previously characterized in
192 human cells³⁴. These data point to a direct or indirect contribution of Nup116 to membrane fusion
193 during NPC assembly.

194 A recent study identified Nup159 as an intrinsic receptor for selective autophagy of nuclear pores
195 (Lee et al in revision; attached for review process). The AIM (Atg8-interacting motif), located
196 proximate to the DID-Dyn2 arm of Nup159, mediates NPC turnover under conditions of low
197 nitrogen supply. It recruits the ubiquitin-like autophagosomal protein Atg8 that is essential for

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198 anchoring autophagic protein cargo to autophagosomal membranes. Above findings suggest that
199 the AIM is exposed into the cytoplasm together with the DID-Dyn2 arm (Fig. 3a), thus favoring
200 the recruitment of cytoplasmic Atg8. We hypothesized that the herniae might be cleared by
201 selective autophagy. This would make sense if they would not only occur upon genetic
202 perturbation as previously observed, but also upon stress conditions that trigger autophagy, which
203 was indeed the case. Under conditions of nitrogen starvation, NE herniae were observed, although
204 they were less abundant as compared to the *nup116Δ* strain at nonpermissive temperature (Fig.
205 3b). How exactly herniae clearance could be mediated by the AIM of Nup159 in the *nup116Δ*
206 background is however not obvious because Nup159 complex should not be present at the herniae
207 according to the NPC structure determined in *nup116Δ* cells at not permissive temperature
208 (Extended Data Fig. 6c).

209 To visualize how the autophagy machinery is recruited to the NPC, we employed a split-Venus
210 approach specifically targeting the Nup159-Atg8 interaction. To enhance autophagic clearance of
211 spatially clustered NPCs, we used a *nup120Δ* background³⁵ as described in (Lee et al) but at shorter
212 nitrogen starvation exposure (Fig. 3c-d and Extended Data Fig. 7a-b). We identified the regions of
213 interest using correlative light and electron microscopy (CLEM) on plastic sections³⁶ and 3D cryo-
214 CLEM³⁷. We acquired tomograms at positions of NPCs engaged in Atg8 interaction. These data
215 revealed that agglomerates of herniae were engulfed by additional double membranes (Fig. 3c-d
216 and Supplementary Video 1). Concurrently, the membrane topology of the herniae was more
217 complex and displayed NPCs budding out of the NE, indicating additional membrane remodeling
218 (Fig. 3c-d, Extended Data Fig. 7a and Supplementary Video 1). Thereby, many NPCs and thus
219 AIMs are exposed to the cytoplasm (Fig. 3c-d, Supplementary Video 1). This finding would
220 predict that herniae initially form independently from Atg8, possibly in the context of NPC
221 surveillance²⁸. Indeed, herniae formed in *atg8Δ* cells exposed to nitrogen starvation (Fig. 3b and
222 Extended Data Fig. 8). We further noted, that under conditions where hernia formation was
223 triggered but not selective autophagy, as in the *nup116Δ* strain at non-permissive temperature
224 without starvation, herniae accumulate (Fig. 3b, Extended Fig. 6a) and are also observed as NPC-
225 containing nuclear vesicles in the cytoplasm (Fig. 4c, Extended Data Fig. 9c and Supplementary
226 Video 2), which agrees with the biochemical analysis of Lee et al. Instead, under conditions in
227 which selective autophagy is triggered by starvation, NPC-containing nuclear vesicles (~340 nm

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228 diameter) are surrounded by ribosomes and double membranes typical of autophagosomes (Fig.
229 4e-f and Extended Data Figs 7c and 9e-f), that are *en route* towards the vacuole as demonstrated
230 by Lee et al (Extended Data Fig. 9g). Taken together, we observed several snapshots of NPC
231 turnover, although the transitions between them, such as the budding of herniae into nuclear
232 vesicles or fusion of autophagosome-engulfed NPC-containing-nuclear-vesicles to the vacuole
233 need to be further investigated in the future (Extended Data Fig. 9). It will be also interesting to
234 elucidate if herniae triggered under alternative genetic conditions, also in human cells³², ultimately
235 feed into the autophagy pathway.

236 In conclusion, our *in situ* structural analysis of ScNPC clarifies the layout of the major Nup
237 subcomplexes under native conditions. It confirms the physiological relevance of the P-shaped
238 configuration of the Nup159 complex but identifies an unexpected orientation with respect to the
239 scaffold that accommodates both the terminal steps of mRNA export (Fig 2d) and exposure of the
240 intrinsic AIM (Fig. 4d). Our findings highlight the power of in cell structural biology to provide
241 novel insights into fundamental processes of eukaryotic life.

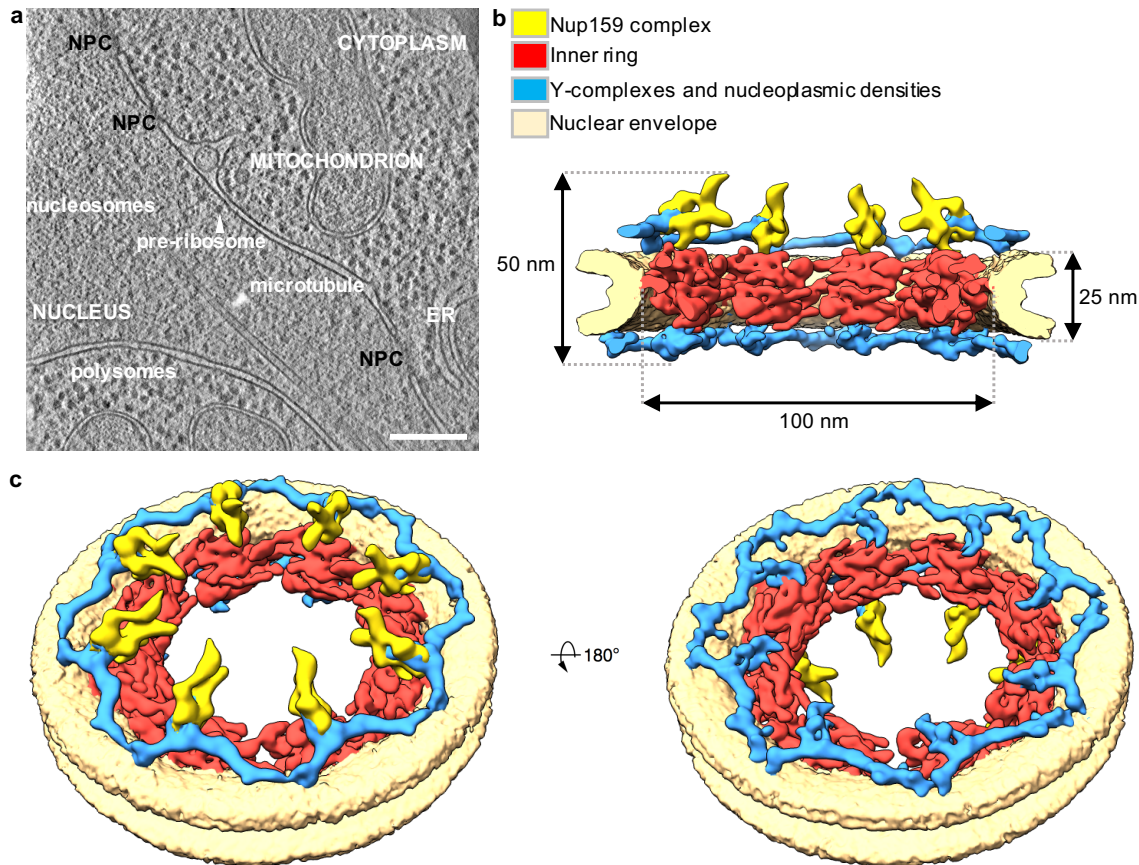
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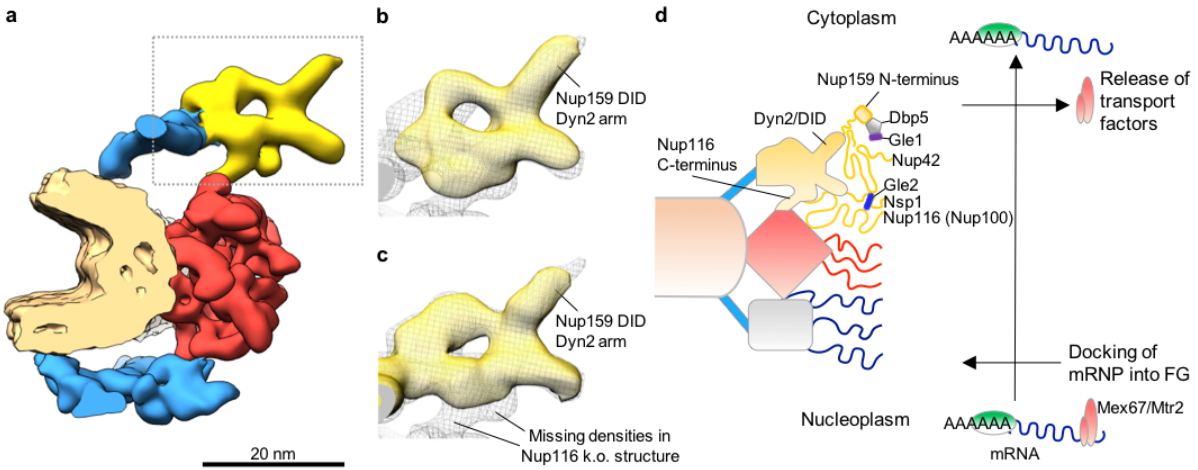
354 **Figures**



355
356 **Fig. 1: In cell structure of *S. cerevisiae* NPC.** **a**, Tomographic slice of a *S. cerevisiae* cell during
357 division. Scale bar 200 nm. **b**, Segmentation of the cryo-EM map of the *Sc*NPC cut in half. **c**,
358 Tilted view of the entire NPC showing both the cytoplasmic (left) and the nucleoplasmic (right)
359 face.

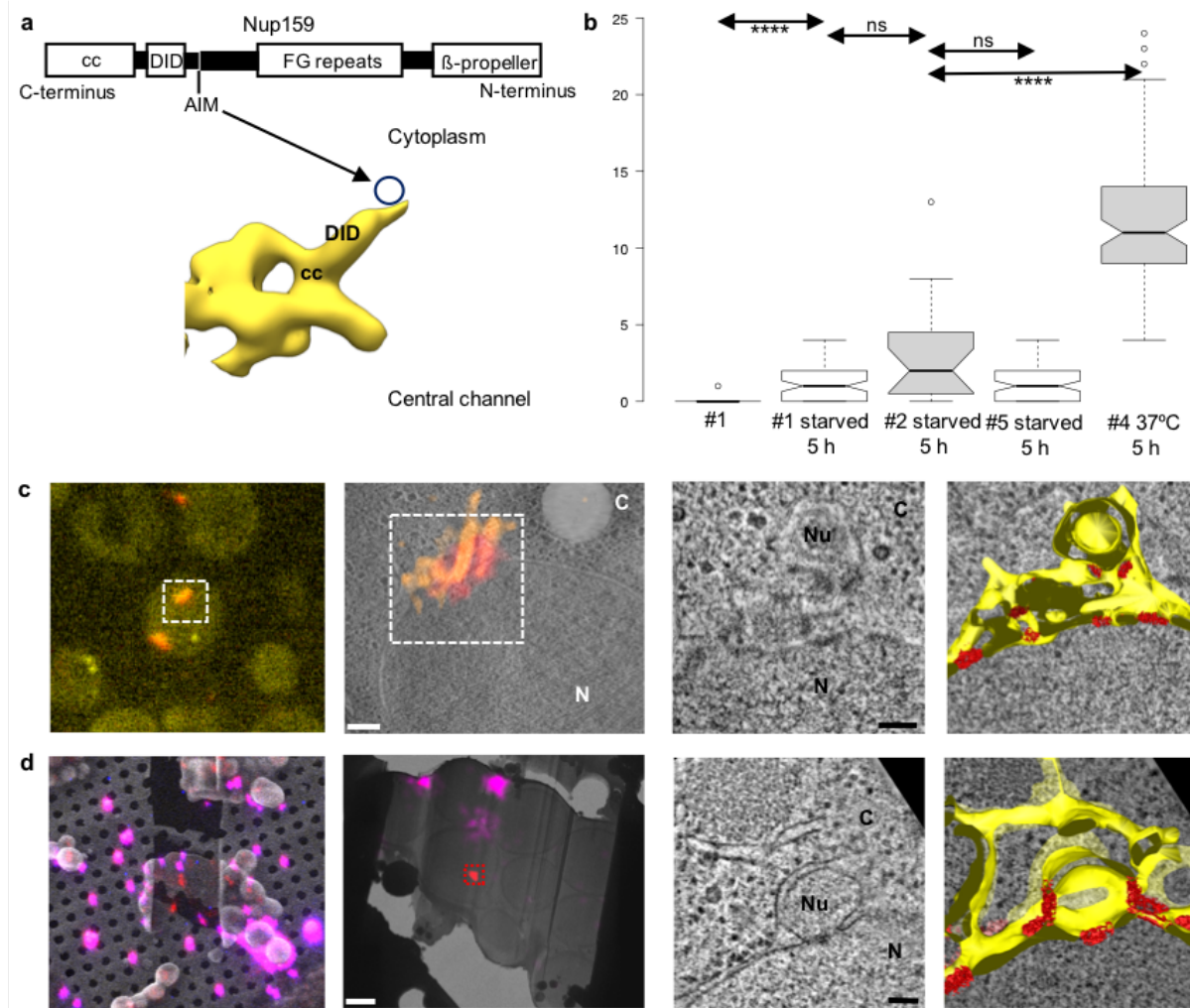
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368 **Fig. 2: Nup159 complex architecture.** **a**, Individual spoke of the *ScNPC* (color-code as in Fig.
369 1). Nup159 complex is highlighted with a dotted frame. **b**, Nup159 complex region of the in cell
370 *ScNPC* map (gray mesh) superimposed with the top scoring systematic fit (see Methods) of the
371 negative stain map of the Nup159 complex⁴ (yellow surface). **c**, Same as **b** but superimposed with
372 the in cell *ScNPC* map of a *nup116Δ* strain grown at permissive temperature (yellow surface). **d**,
373 Spatial model of how mRNA export is accommodated by the orientation of the Nup159 complex.
374 A poly-A mRNA with transport factors Mex67/Mtr2 (red) and proteins that protect the poly-A tail
375 from degradation (light green) docks to the unstructured region of the basket Nups⁵ (grey box with
376 blue filaments). Nup159 N-terminal domain that mediates the release of the transport factors from
377 the mRNA⁷ is anchored at the cytoplasmic side.
378

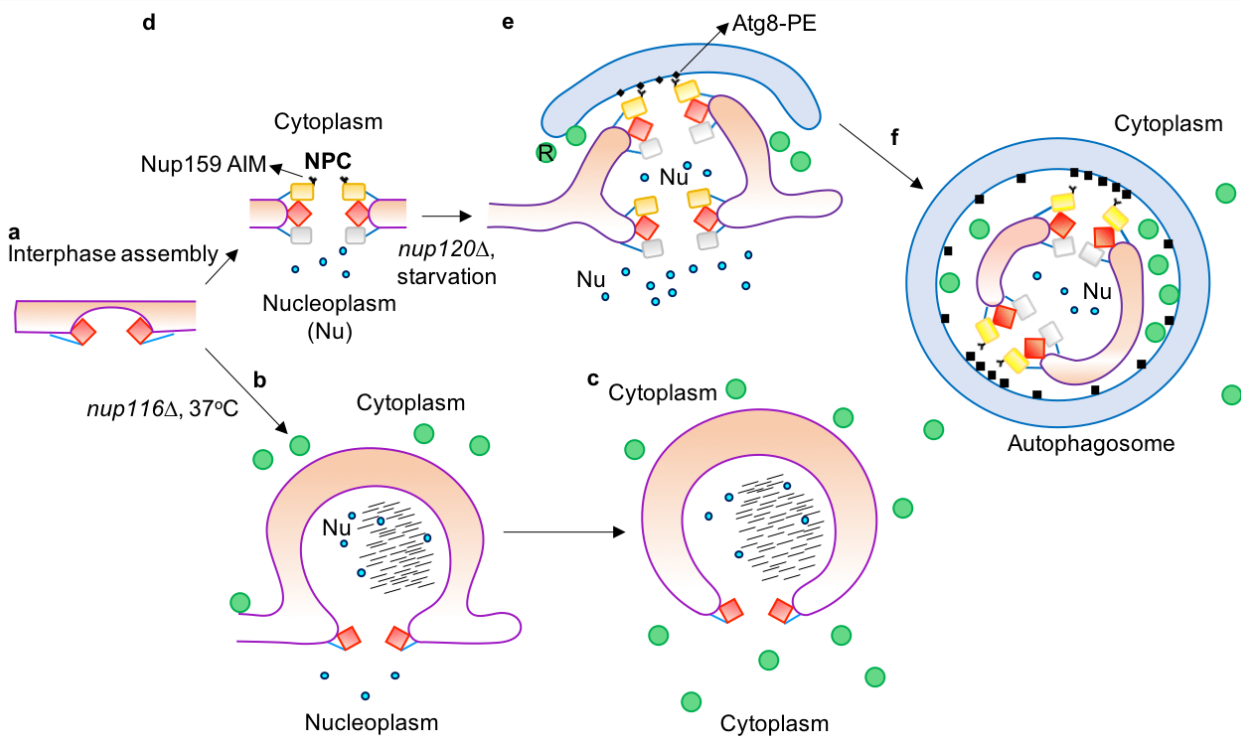
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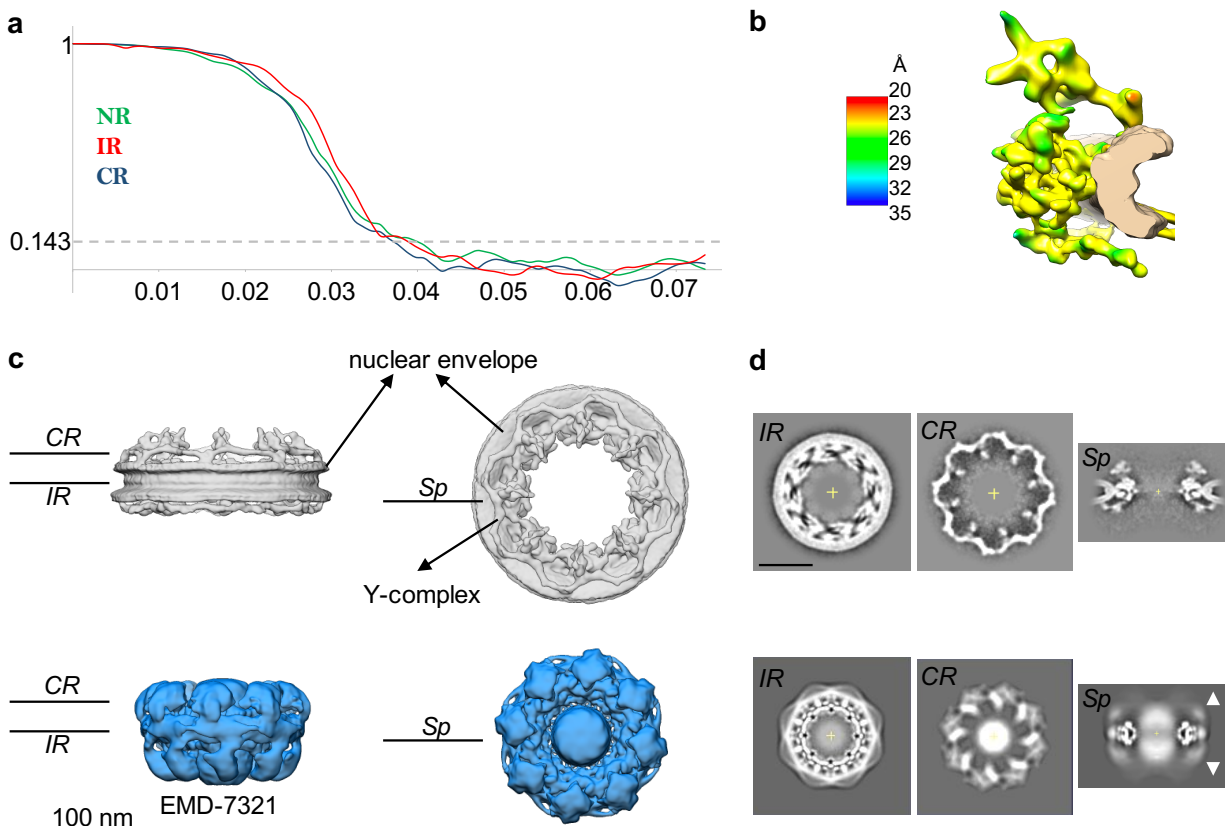
379
 380 **Fig. 3: Cellular visualization of Nup159-Atg8 interaction by CLEM.** **a**, Domain structure of
 381 Nup159 with corresponding map showing the position of the Atg8 interacting motif (AIM) (Lee
 382 et al). **b**, Box plot (median, 1st quartile) showing the number of herniae (see Methods) in *S.*
 383 *cerevisiae* strains before and after starvation according to Extended Data Table 1 (#1 control cells;
 384 #2 Nup159-Atg8-splitVenus, Nup170-Mars, *nup120Δ* cells; #4 *nup116Δ* cells; #5 *atg8Δ* cells).
 385 *nup116Δ* cells (#4) were shifted to the non-permissive Temperature of 37°C; herniae are induced
 386 (**** $p < 0.0001$; Mann Whitney, two-sided). **c**, On section CLEM of strain #2 after 5.5h of
 387 starvation. The left panel shows the light microscopy signal on plastic section. SplitVenus signal
 388 arising from the interaction of Nup159 with Atg8 is shown in yellow. Nup170-Mars is shown in
 389 red. The second panel zooms in the area of interest (white dashed square in the previous panel)
 390 showing the overlay of the fluorescent signal on the tomographic EM slice. Scale bar: 200 nm.
 391 The framed area is shown as tomographic slice and segmented on the right (N nucleus; C
 392 cytoplasm; Nu nucleoplasm; scale bar: 100 nm; NPCs in red; membranes in yellow). **d**, 3D cryo-
 393 CLEM of strain #2 after 5.5 h of starvation. SEM top view of a lamella with overlaid fluorescent
 394 signal. Red is NPC signal; magenta is fiducial-bead signal. Second panel: cryo-TEM overview (~2

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395 nm/pix) of the same lamella. Scale bar: 2 μ m. The framed area is shown as tomographic slice and
396 segmented on the right. Scale bar: 100 nm.
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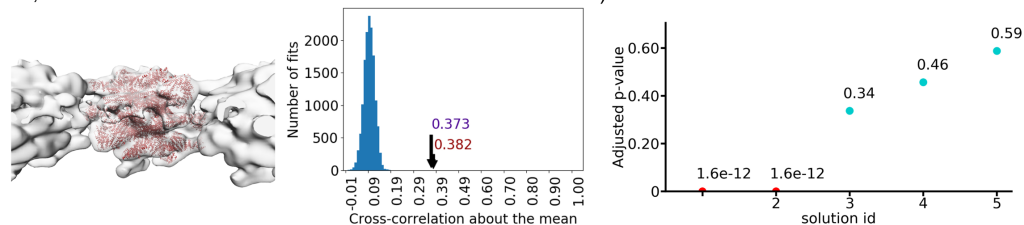


398
399 **Fig. 4: Cartoon model of membrane remodeling during NPC turnover by selective**
400 **autophagy.** **a**, Inside-out assembly intermediates³⁴ or inner nuclear membrane evaginations
401 (Extended Data Fig. 6d) progress into **b**, NE herniae as in the *nup116Δ* strain at non-permissive
402 temperature (Extended Data Fig. 6a); or mature into **d**, fully assembled NPCs. **e**, When autophagy
403 is triggered by Nitrogen starvation (or NPC clustering), NPCs are cytoplasmically exposed at the
404 herniations by membrane remodeling (Fig. 3 c-d, Supplementary Video 1). Nup159 AIM interacts
405 with Atg8 and double membranes are recruited to the nuclear envelope. **c**, If hernia accumulation
406 is enforced in *nup116Δ* cells at non permissive temperature, nuclear vesicles are observed in the
407 cytosol. **e**, **f**, If autophagy is triggered, nuclear vesicles are engulfed by double membranes
408 (Extended Data Fig. 7c) and, as shown by Lee et al, ultimately end up in the lysosome (Extended
409 Data Fig. 9g). Color-code as in Fig. 2d. R is ribosome depicted as a green circle; black lines
410 represent dark densities visible in the cryo-tomograms.

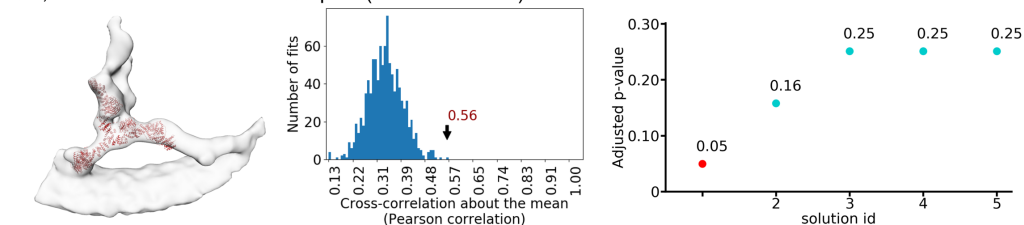


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 412 **Extended Data Fig. 1: In cell structure of the *Sc*NPC vs detergent-extracted *Sc*NPC (EMD-**
 413 **7321).** **a**, Gold standard FSCs of in cell cryo-EM map of the *Sc*NPC. All the curves (nuclear ring
 414 NR, cytoplasmic ring CR, inner ring IR) intersect the 0.143 criterium at around 25 Å resolution.
 415 **b**, Local resolution analysis⁵¹ with color-coded bar. **c**, In cell cryo-EM map (gray) in comparison
 416 to cryo-EM map of detergent-extracted *Sc*NPCs (blue, EMD-7321 at the suggested contour level)
 417 show significant differences in diameter and in interpretable features. The nuclear membranes and
 418 the Y-complexes are clearly discerned in the in cell cryo-EM map, in contrast to EMD-7321. **d**,
 419 Slices through the maps at the level of the cytoplasmic (CR) and inner rings (IR) and an individual
 420 spoke (Sp). Lines in **c** indicate slicing position shown in **d**. Arrowheads indicate blurred features
 421 in the outer rings. Scale bar: 50 nm. See also: Source Data 1 attached for review process.

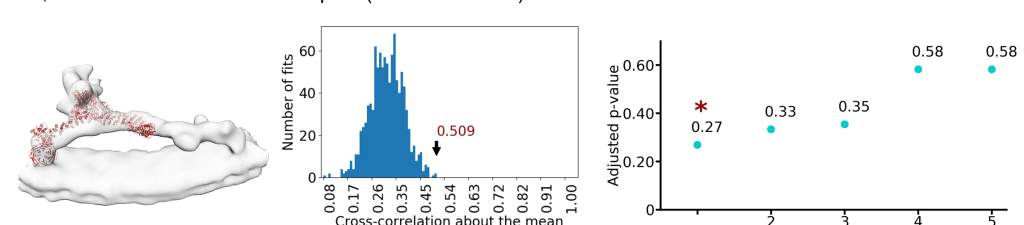
IR, fitted structure: Yeast IR complex (PDB DEV ID: 00000010)



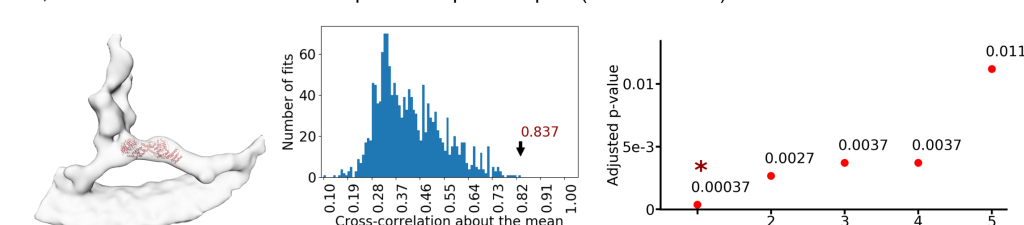
CR, fitted structure: Yeast Y complex (PDB ID: 4XMM)



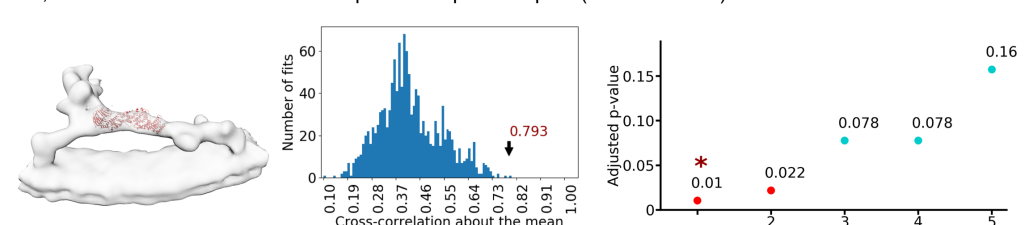
NR, fitted structure: Yeast Y complex (PDB ID: 4XMM)



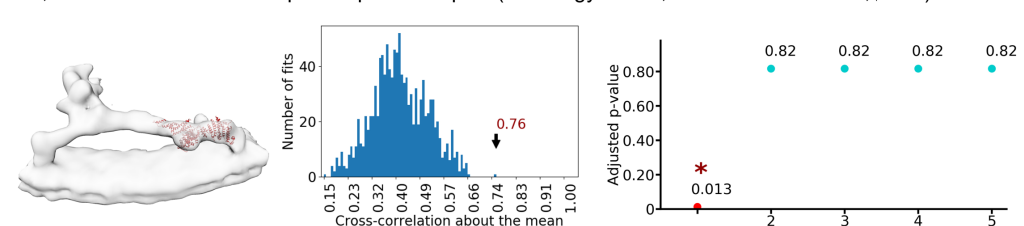
CR, fitted structure: Yeast Sec13-Nup145C-Nup84 complex (PDB ID: 3IKO)



NR, fitted structure: Yeast Sec13-Nup145C-Nup84 complex (PDB ID: 3IKO)



NR, fitted structure: Yeast Nup84-Nup133 complex (homology model, Fernandez-Martinez, 2016)

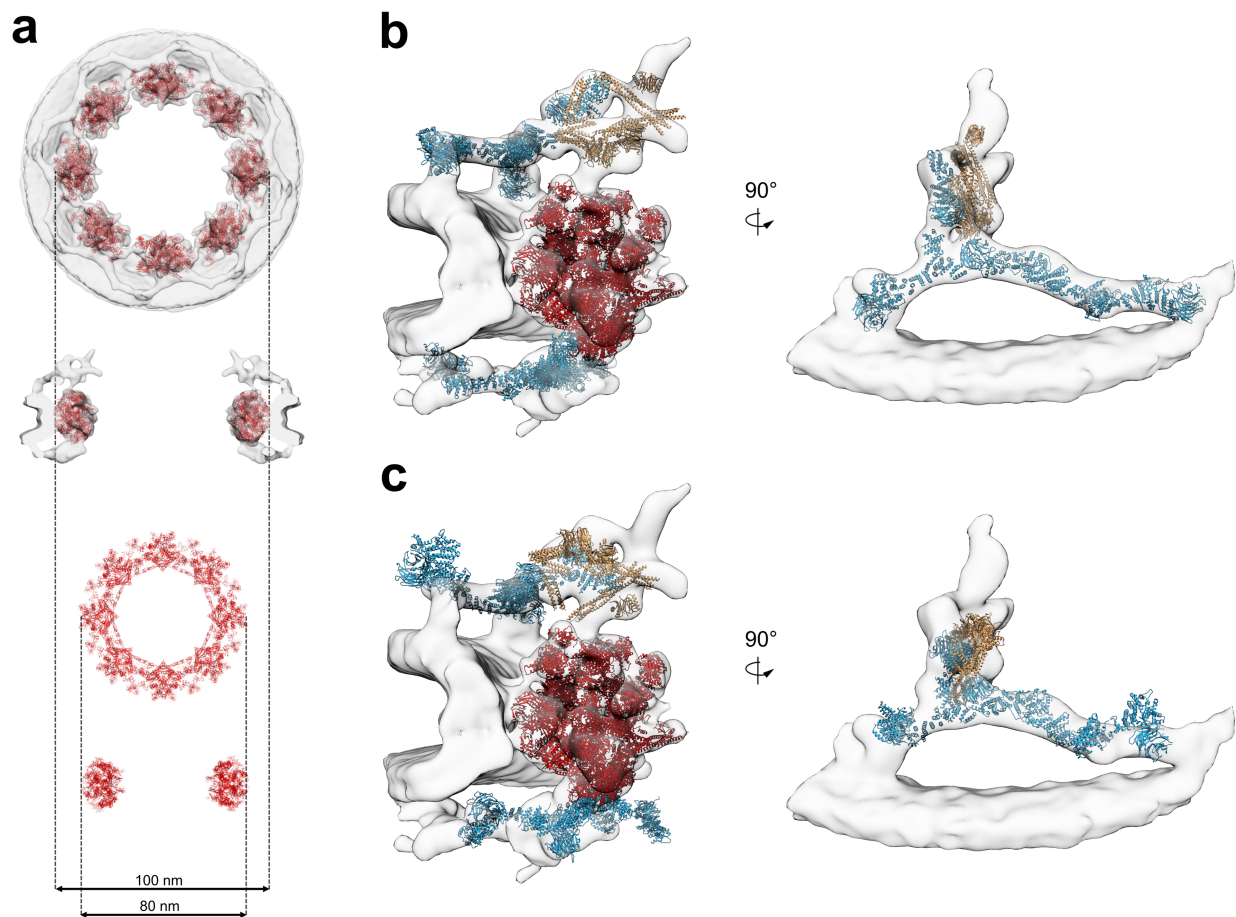


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Extended Data Fig. 2: Systematic fitting of inner and outer ring components into the ScNPC map. Each row shows the visualization of the most significant fits (left), the histogram of raw

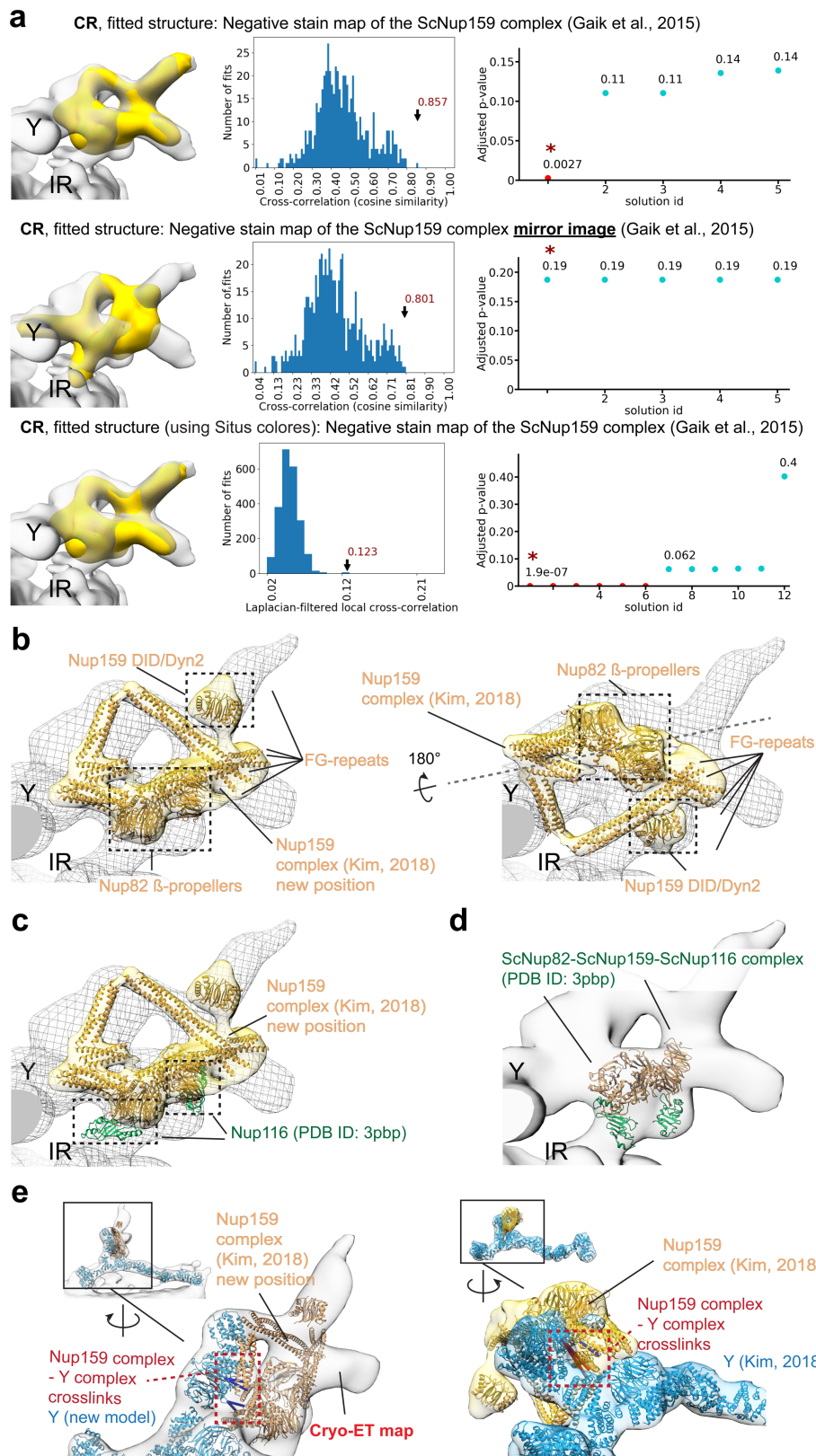
425 scores (middle), and a plot of the top five p-values (right). The statistically significant fits (p-value
426 < 0.05 , assessed as described in Methods) or the first top fit are marked with asterisk. The number
427 of sampled fits used to calculate p-values after clustering of similar solutions was 14348, 1015,
428 1039, 1479, 1354, and 1183 for the rows from top to bottom. For the IR, the integrative model of
429 the single spoke of the IR¹³ was used as the fitted structure. For the outer rings (CR and NR), the
430 crystal structure of the yeast Y-complex was fitted or its parts corresponding to subcomplexes. The
431 structures were fitted by an unbiased global search using UCSF Chimera⁵² and scored using the
432 cross-correlation score about the mean as explained in the Methods. The IR complex was fitted to
433 the entire spoke map, while the other structures were fitted to individual CR and NR segments.

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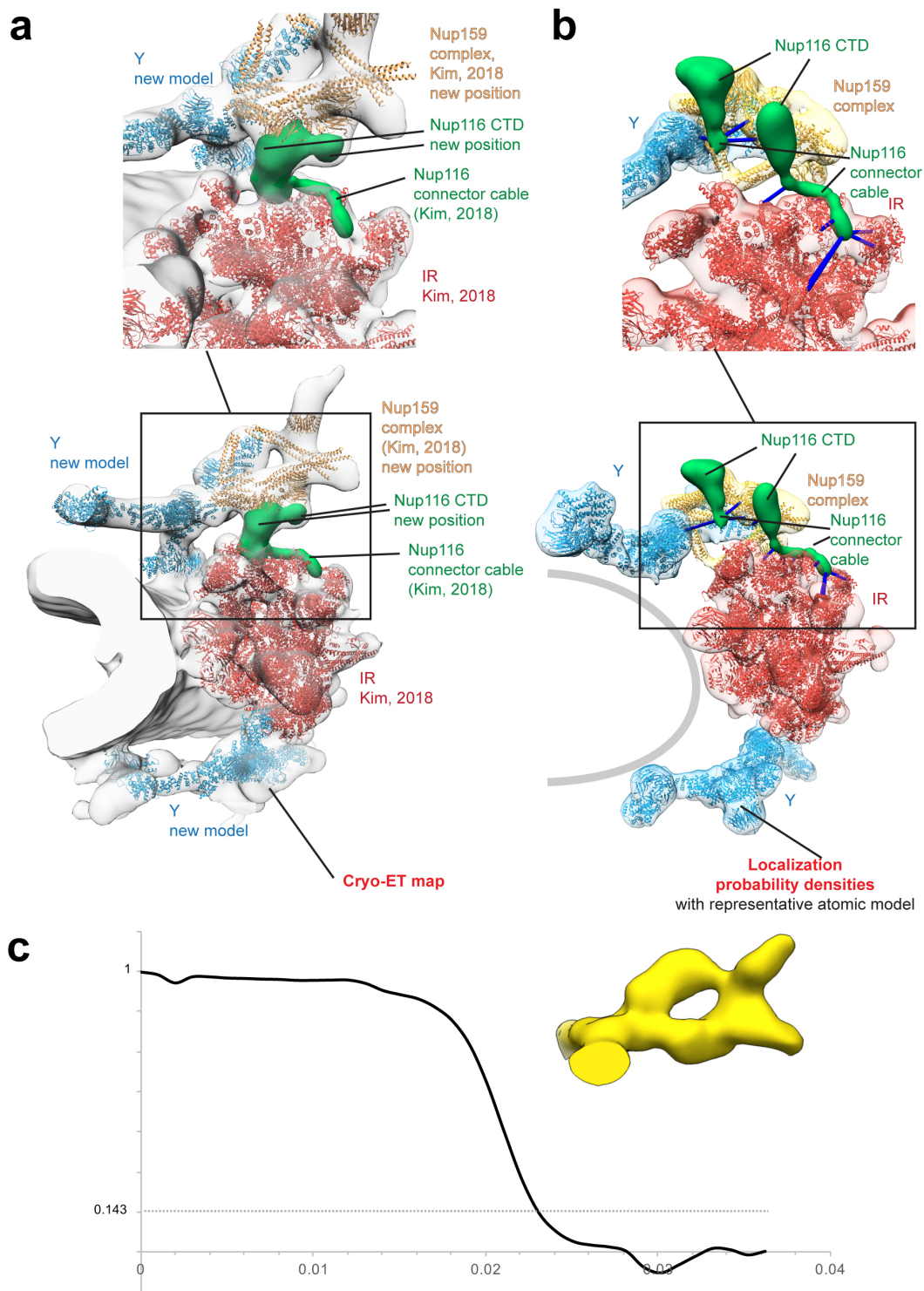
435

436 **Extended Data Fig. 3: Architectural model of ScNPC.** **a**, Comparison of fitted (to the cryo-ET
 437 map from this study, depicted in gray) integrative Inner Ring complex (IRC) models (red ribbons)
 438 from¹³ with 20 nm diameter difference. **b**, Representative structural models of CR and NR Y-
 439 complex (blue ribbons), P-complex (yellow ribbons) and IR (red ribbons) built in this work (see
 440 Methods). The Y-complexes are more extended as compared to reference ¹³ version by ~40 Å. **c**,
 441 Representative integrative structural models of CR and NR Y-complex (blue ribbons), P-complex
 442 (yellow ribbons) and inner ring complex (red ribbons) from ¹³ fitted to cryo-ET map (grey density)
 443 from this study, with respect to spatial reference frame from ¹³.



444
 445 **Extended Data Fig. 4: Validation of the orientation of the Nup159 complex.** a, Systematic
 446 fitting of the negative stain map of the Nup159 complex into the ScNPC map using UCSF

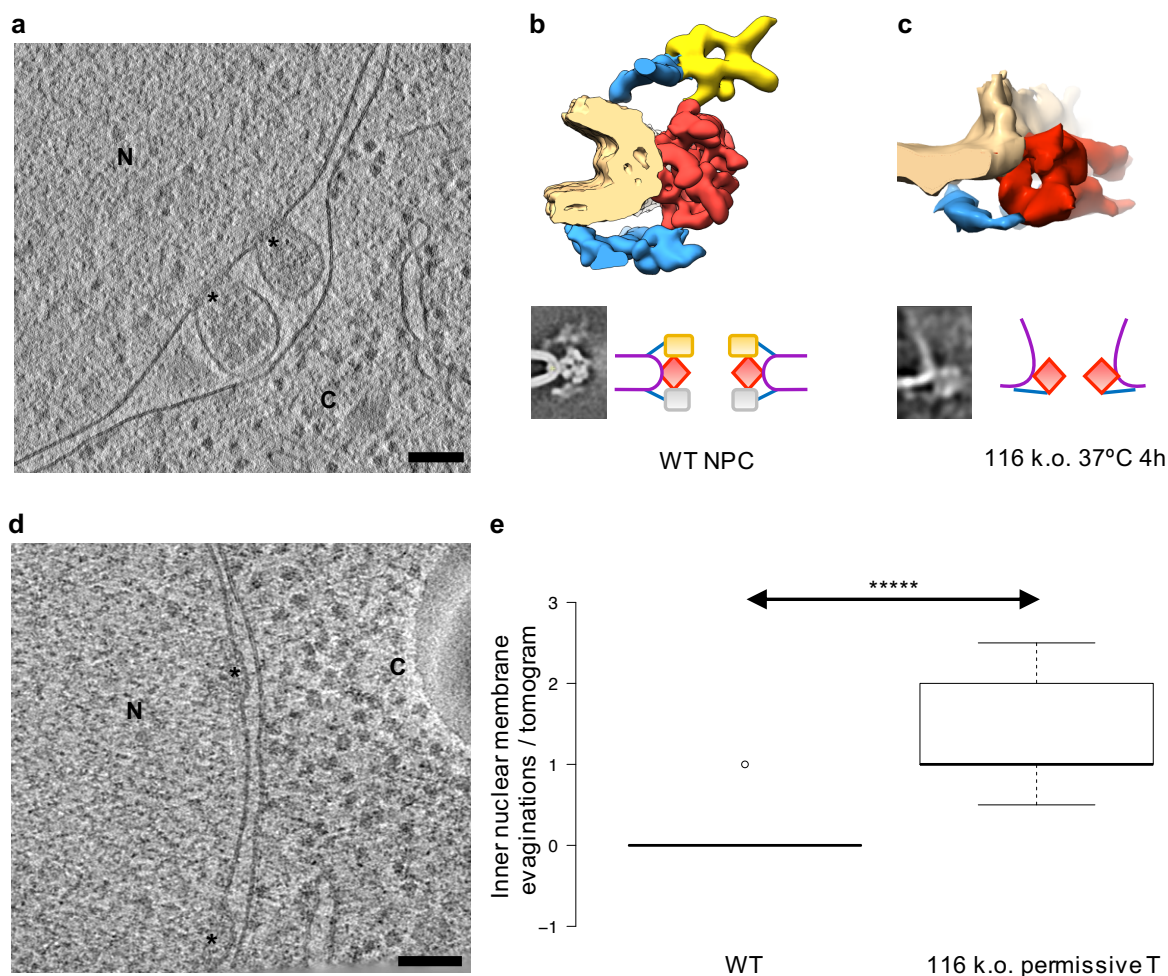
447 Chimera⁵² and Colores program from the Situs package⁵⁴. For comparison, both mirror images of
448 the negative stain map (yellow) were fitted and only single one led to significant scores,
449 underlining the unambiguous nature of the fit. Each row shows the visualization of the most
450 significant fits (left), the histogram of raw scores (middle), and a plot of the top five p-values
451 (right). The statistically significant fits (p-value < 0.05, assessed as described in Methods) or the
452 first top fit are marked with asterisk. The number of sampled fits used to calculate p-values after
453 clustering of similar solutions was 585, 599, and 2243, for top, middle, and bottom rows
454 respectively. **b**, Representative integrative Nup159 complex model from¹³ inside the in cell ScNPC
455 map (gray mesh) in the orientation determined in this work (*left*) versus the previously published
456 orientation (*right*). The Nup159 complex model is shown in orange ribbons within yellow
457 localization probability density locally fitted (with UCSF Chimera⁵²). The previous orientation
458 was reproduced by first fitting the entire model from¹³ to the in cell cryo-EM map and then locally
459 fitting the Nup159 complex to the density (which was needed to bring the Nup159 complex into
460 the density and preserved the orientation). The dashed gray line indicates the flipping axis between
461 the two fits. **c**, Superimposition of the crystal structure 3PBP²⁴ onto representative integrative
462 Nup159 complex model from reference¹³ in the newly determined orientation (left) predicts the
463 position of Nup116, as confirmed by our knock-out study (Fig. 2c). **d**, Visualization of two of the
464 top resulting systematic fits of the 3PBP crystal structure into the cryo-ET map presented in this
465 study confirms our *nup116Δ* structure (Fig. 2c). **e**, Crosslinks between the Nup159 complex and
466 the Y complex from¹³ support the new orientation (left) compared to the published orientation
467 (right). Satisfied and violated crosslinks are depicted as blue and red bars, respectively.



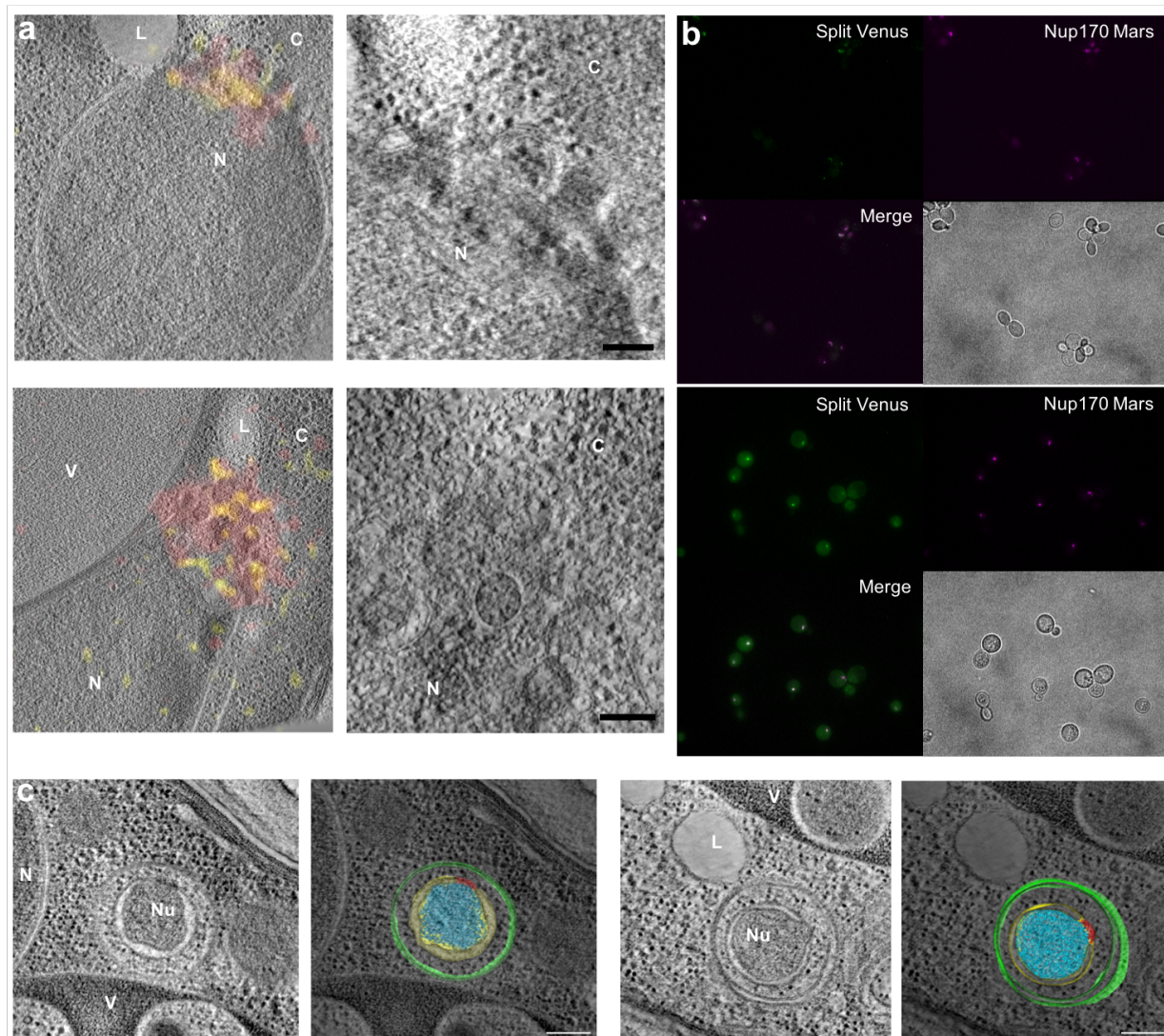
468
 469 **Extended Data Fig. 5: Nup116 positioning.** a, New positioning of the Nup116 (left) versus
 470 previously published integrative model (PDB DEV ID: 00000010 from¹³, right). The two *Sc*NPC
 471 models were superimposed such that the IRs are aligned to the same reference frame. The Nup116
 472 position is shown either as the density assigned to Nup116 based on the Nup116 knock-out
 473 structure (left) or as localization probability densities retrieved from reference¹³ model (right). The

474 major structural elements of the NPC are indicated. The Nup116 connector cable in the in cell
475 model (left) has been taken from reference ¹³ based on its position relatively to the IR. Blue bars
476 represent crosslinks from ¹³ from Nup116 to other Nups. For the in cell model, the cryo-ET map
477 is displayed; for the model from ¹³, the localization probability densities (not an EM map) are
478 shown instead. **b**, FSC of *nup116Δ* cytoplasmic filaments structure intersects the 0.143 line at ~40
479 Å, the cryo map is depicted in yellow as in Fig. 2c.
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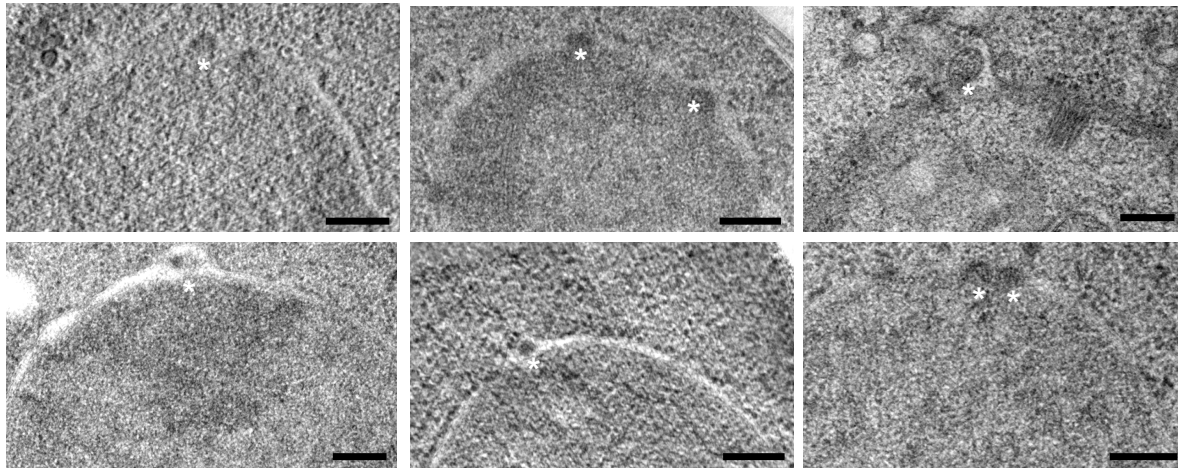
482
 483 **Extended Data Fig. 6: NPC and nuclear envelope morphology under *nup116Δ* conditions. a,**
 484 tomographic slice of nuclear envelope with herniations (*nup116Δ* 4h at 37°C); N is nucleus, C is
 485 cytoplasm, stars depict density at the basis of the herniations that was subjected to subtomogram
 486 averaging; scale bar: 100 nm. **b,** Map and cartoon model of an individual spoke of the WT ScNPC
 487 as in Fig. 2a and Fig. 4, shown isosurface rendered and as slice. **c,** same as **b** but as result of the
 488 subtomogram averaging at the basis of the herniations (see Methods). The average shows that the
 489 cytoplasmic ring, including the Nup159 complex is missing. **d,** cryo tomographic slice of nuclear
 490 envelope with inner nuclear membrane evagination depicted as stars (*nup116Δ* 25°C). Labels as
 491 in **a.** **e,** Box plot (median, 1st quartile) showing that the number of inner nuclear membrane
 492 evaginations are significantly higher in the NEs of *nup116Δ* cells at 25°C (28 in 130 cryo
 493 tomograms) in comparison to WT envelopes (1 in 230 cryo tomograms); $p < 0.00001$ (Mann
 494 Whitney, two-sided).



495
 496 **Extended Data Fig. 7: Snapshots of selective autophagy at the nuclear envelope. a**, Gallery of
 497 tomographic slices overlaid with fluorescence images as obtained by on section-CLEM of
 498 Nup170-Mars, Nup159-Atg8-SplitVenus cells. The Nup159-Atg8 interaction is observed as the
 499 yellow SplitVenus signal, Nup170-Mars is shown in red. In the right panel, zoomed views of the
 500 same tomographic slices are shown, emphasizing the clustering of herniae and nuclear vesicles
 501 arising through further membrane remodeling (See also Fig. 3 c-d and Supplementary Video 1). N
 502 is nucleus, V is vacuole, L is lipid droplet, C is cytoplasm. Scale bar: 100 nm. **b**, Fluorescence and
 503 phase contrast microscopy images of Nup170-Mars (magenta), Nup159-Atg8-SplitVenus (green)
 504 cells before (top) and after 5,5h of nitrogen starvation (bottom). Upon starvation, Nup159-Atg8-
 505 SplitVenus signal increases and co-localizes with Nup170-Mars (white dots). **c**, tomographic slice
 506 (left) as well as the corresponding segmentation and isosurface rendering (right) of NPC-
 507 containing nuclear vesicles (~340 nm in diameter) observed in the cytoplasm. They are surrounded
 508 by cytosol content (ribosomes) and another two membranes (~540 nm in diameter). *Sce atg15Δ*

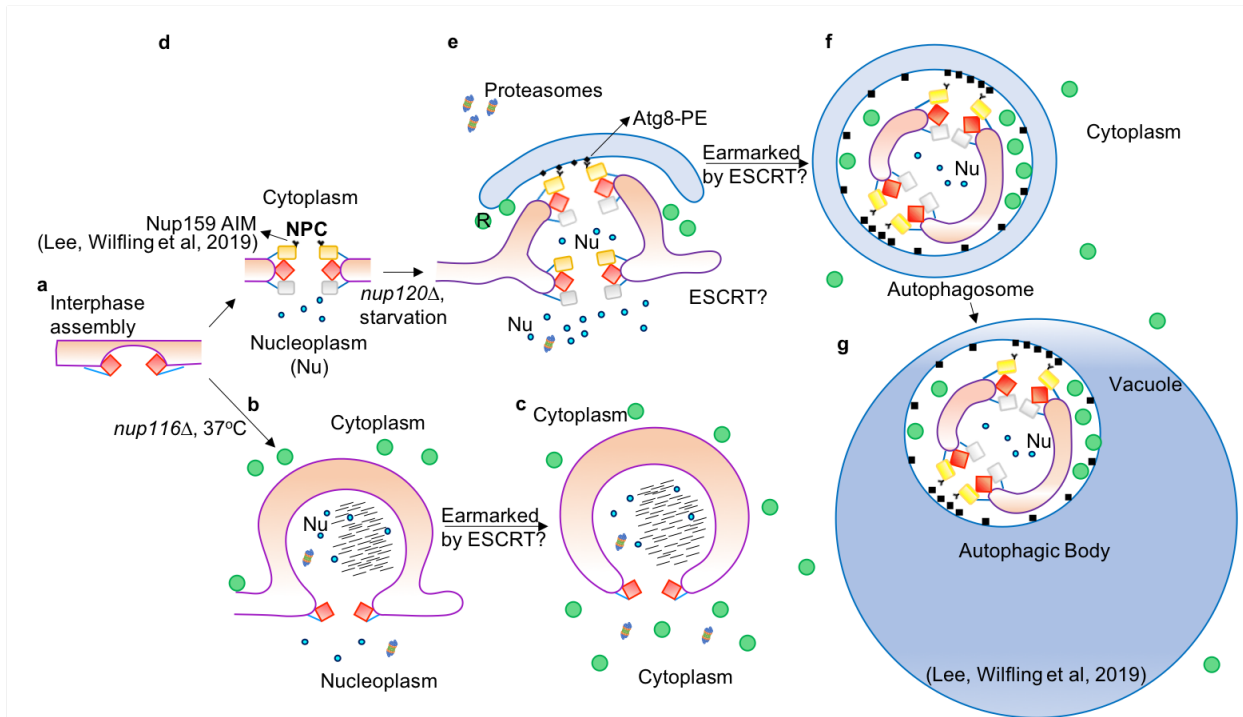
509 cells were starved for ~24 h to enrich NPC autophagy events. NPCs in red, Nuclear content (Nu)
510 in cyan, autophagosomal double membranes in green. Scale bar: 200 nm.

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517 **Extended Data Fig. 8: Herniations under *atg8Δ* conditions after 5h of nitrogen starvation.**
518 Small herniations appears in the nuclear envelope and are marked with *. Statistics in Figure 3b.
519 Scale bar: 200 nm.

520



521

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523

Extended Data Fig. 9: Cartoon summarizing the results from this paper and the accompanying paper Lee et al. Color code as in Fig. 4.

524 **Legends for Supplementary items**

525

526 **Video Supplementary 1: 3D cryo-CLEM tomogram of Nup159-Atg8 interaction.** The video
527 shows how clustered NPCs depicted in red are surrounded by double membranes depicted in
528 yellow after 5,5h starvation. Fig. 3b for statistics and 3d for scale bar.

529

530 **Video Supplementary 2: Cryo electron tomogram of *nup116Δ* strain #4 after shifting 5h to**
531 **37°C shows budded herniae.** NPCs at the herniae are red, membranes are pink. Scale bar: 100
532 nm.

533

534 **Extended Data Table 1: *S. cerevisiae* strains used in this study.**

535

536 **Source Data 1: Comparison of Cryo-EM maps of the *Sc*NPC.** Zip file for reviewers containing
537 the in cell cryo-EM map of the *Sc*NPC (this study), the cryo-EM map (EMD-7321) of detergent
538 extracted NPCs previously obtained by Kim et al. and a Chimera session that displays both drawn
539 to scale at suggested contour level (in cell cryo-EM map in grey, EMD-7321 in cyan).

540 **Methods**

541 Yeast strains, growth conditions, fluorescence imaging

542 Strains used in this study are listed in Extended Data Table 1. Fresh cells were grown to mid-log
543 phase in YPAD (1% Yeast Extract + 2% Peptone (YP), adenine (A), 2% Dextrose (D)). Nitrogen
544 starvation was carried out by switching cells grown at OD₆₀₀ ~0.5 with YPAD into SD-N medium
545 (synthetic minimal medium lacking nitrogen; 0.17% yeast nitrogen base, without aminoacids and
546 ammonium sulfate, supplemented with 2% glucose) for indicated times. Fluorescent images of
547 unfixed yeast cells upon starvation (strains #2, 3) were acquired using a Zeiss Axio Observer. Co-
548 localization figure was made in FIJI³⁸.

549

550 Correlative fluorescence and electron tomography in plastic sections

551 CLEM analysis was conducted as previously described^{39,40}. *S cerevisiae* (*Sc*) cells strain #2 after
552 ~5h of starvation were high pressure frozen (HPM010, AbraFluid) and freeze substituted (EM-
553 AFS2, Leica) with 0.1% uranyl acetate in acetone for 55h at -90°C. The temperature was then
554 raised to -45°C at 3.5°C/h and samples were further incubated for 5h. After rinsing in acetone, the
555 samples were infiltrated in Lowicryl HM20 and the resin was polymerized under UV light. 300
556 nm sections were cut with a microtome (EM UC7, Leica) and placed on carbon coated 200 mesh
557 copper grids (S160, Plano).

558 The fluorescence microscopy (FM) imaging of the sections was carried out as previously
559 described^{36,40} using a widefield fluorescence microscope (Olympus IX81) equipped with an
560 Olympus PlanApo 100X 1.40 NA oil immersion objective and a CCD camera (Orca-ER;
561 Hamamatsu Photonics).

562 After fluorescence imaging, grids were poststained with uranyl acetate and lead citrate. 15 nm
563 protein A-coupled gold beads were also added as fiducial markers used for overlaying low-mag
564 with high-mag tomograms. 60° to 60° tilt series of the cells of interest were acquired semi-
565 automatically on a Tecnai F30 (Thermofisher, FEI) at 300 kV with Serial-EM⁴¹ at 20000x and at
566 4700x to facilitate ease of correlation.

567 Tomograms were reconstructed automatically with IMOD batchruntomo⁴². Tilts were aligned by
568 patch tracking. 3DMOD software was used for manual segmentation of the tomograms and for
569 making the videos. Overlays of fluorescence spots and tomograms was performed with ec-CLEM
570 Plugin⁴³ in ICY⁴⁴ by clicking manually on corresponding pairs of notable features in the two
571 imaging modalities.

572

573 2D electron microscopy

574 Strains #1, 2, 3, 5 upon starvation were high pressure frozen as described above and freeze
575 substituted with 0.2% uranyl acetate 0.1% glutaraldehyde in acetone for 60h at -90°C. The
576 temperature was then raised to -45°C at 3°C/h and samples were further incubated for 9h. After
577 rinsing in acetone, the samples were infiltrated in Lowicryl HM20 resin and the resin was
578 polymerized under UV light. Acquisition was performed with a Tecnai F30 (Thermofisher, FEI)
579 at 300 kV. Herniation counting was performed using 2D electron microscopy of 70-100 nm

580 sections and 3D electron microscopy using 300 nm sections. Boxplots were done with BoxPlotR
581 (<http://shiny.chemgrid.org/boxplotr/>) using around 150 nuclei counted per each strain or condition.

582

583 Cryo-FIB milling and cryo-CLEM

584 *Sc* wild type strain #1 and knock-out #4 were grown in YPAD liquid medium to an OD₆₀₀ of 0.2
585 at 30°C, OD 0.5 for correlative studies using strain #2. Per grid, 3.5µl of cell suspension were
586 applied on 200-mesh copper grids coated with R 2/1 holey carbon or SiO₂ films (Quantifoil Micro
587 Tools GmbH) and plunge frozen in liquid ethane at ~-186°C using a Leica EM GP grid plunger.
588 The blotting chamber conditions were adjusted to 30°C, 95% humidity and 1,5-2 seconds blotting
589 time. For cryo-CLEM, Crimson FluoSpheres™ Carboxylate-Modified Microspheres, 1.0 µm,
590 crimson fluorescent (625/645), were washed in 1×PBS and added to the cell suspension at 1:20
591 dilution, and 1 second blotting time was used.

592 The frozen grids were fixed in modified autogrids to allow milling at a shallow angle⁴⁵ and
593 transferred into an Aquilos (cryo-FIB-SEM dual beam, Thermofisher). For correlative studies, the
594 clipped grids were imaged on a prototype Leica cryo-confocal microscope based on Leica TCS
595 SP8 CFS equipped with a Cryo Stage (similar to the commercially available EM Cryo CLEM
596 Widefield system). Imaging was performed using a 50× objective, NA 0.90, 552 nm laser
597 excitation, and detecting simultaneously at 560–620 nm and 735–740 nm.

598 In the Aquilos, samples were sputter-coated with inorganic platinum and coated with an
599 organometallic protective platinum layer using the Aquilos gas injection system (GIS)⁴⁶. Lamellae
600 were produced using the Gallium ion beam at 30 kV and stage tilt angles of 17°-19° by milling in
601 two parallel rectangular patterns. The lamella preparation was conducted in a step-wise fashion,
602 gradually reducing the current of the ion beam until the final polishing of a thin slab of biological
603 material of around 150-250 nm.

604 For correlative studies, beads (5 to 10) were picked in the squares of interested and overlaid with
605 fluorescence signals coming from the confocal stacks using 3DCT in both the electron and ion
606 beam images³⁷. After choosing the signal of interest in the confocal stacks, 3DCT provides the
607 position to place the milling patterns in x, y and zed. Milling is then performed as described above.
608 After polishing, the signal of interest is retained in the lamellae provided that the milling did not
609 result in deformation of the carbon film.

610 Autogrids with lamellae were unloaded and placed in storage boxes. In some cases, a further
611 inorganic Pt layer was added for reducing charging during TEM imaging.

612

613 Cryo-electron tomography data collection and processing

614 Grids with lamellae were loaded into the Krios cassette. Cryo-electron tomographic tilt series (TS)
615 were acquired on a Titan Krios (Thermofisher FEI) operating at 300 kV equipped with a Gatan K2
616 summit direct electron detector and energy filter. The autogrids were carefully loaded with the
617 lamella orientation perpendicular to the tilt-axis of the microscope prior to TS acquisition.

618 Lamellae were mapped at low mag (2 nm/pix, 30 kV slit) and for correlative studies the maps were
619 overlaid with the electron beam images from the Aquilos using serialEM registration points. Spots
620 of interested were chosen accordingly.

621 All data was collected using the K2 operating in dose-fractionation mode at 4k x 4k resolution
622 with a nominal pixel size of 0.34 nm. TS collection was automated at using a modified version of
623 the dose-symmetric scheme⁴⁷ taking the lamella pre-tilt into account. Defocus TS were acquired
624 over a tilt range of +65 to -45 for a positive pre-tilt with a tilt increment of 2-3°, a total dose of
625 ~140 e-/Å² and a targeted defocus of around 2 to 4.5 μm.

626 All images were pre-processed and dose-filtered as described in¹¹. Tilt-series alignment was
627 performed using IMOD software package 4.9.2.⁴², by patch tracking function on bin4 image
628 stacks. Initial tilt series alignment was manually inspected and improved by removing contours
629 showing large deviations (i.e. a large mean residual error) from the alignment model function. The
630 software package gCTF⁴⁸ was used for CTF estimation. 3dCTF correction and tomogram
631 reconstruction was performed with NovaCTF⁴⁹.

632

633 Subtomogram alignment and averaging

634 Subtomogram alignment and averaging was performed with slight modifications from a previously
635 described workflow⁵⁰ using the Matlab TOM package re-implemented in C++. In brief: Particle
636 coordinates and initial orientations were manually picked and assigned. Initial NPC alignment was
637 performed on 8 and 4 times binned subtomograms followed by a manual inspection and curation
638 of the initial alignment for each particle. From the whole aligned NPCs all 8 spokes were assigned
639 according to an 8-fold rotational symmetry and the subunits were extracted from the tomograms
640 removing subunits which are located outside of the lamellae. Subunits were further aligned. The
641 alignment was once more manually inspected, and all misaligned subunits were removed. Final
642 alignments were done binning two times the subtomograms using focused masks on the
643 cytoplasmic, inner and nucleoplasmic ring of the NPC.

644 In the *S. cerevisiae* wild type data set ~500 NPC (4000 asymmetric units) were initially picked
645 from ~230 tomograms, ~200 NPCs (1600 asymmetric units) from 120 tomos for the *nup116Δ*
646 strain at permissive temperature and ~40 NPCs from 40 tomos for the *nup116Δ* strain at not-
647 permissive temperature (37°C). The particles were split in two half datasets for gold-standard
648 processing. After alignment and manual curation 2000 subunits were used in the final average for
649 the WT NPC and ~800 for the *nup116Δ* at permissive temperature and 320 for the *nup116Δ* strain
650 at 37°C. For the latter NPCs filtered volumes were used for alignment and weighted back
651 projection volumes to get the final structure. Gold standard Fourier shell correlation (FSC) was
652 calculated with Fourier Shell Correlation Server
653 (www.ebi.ac.uk/pdbe/emdb/validation/fsc/results/) with half-maps as inputs. Local resolution was
654 calculated with Resmap⁵¹, B-factor sharpening was estimated empirically as in⁹ being between
655 2000 Å² for the inner ring and 3000 Å² for the cytoplasmic ring. For counting the number of inner
656 nuclear membrane evaginations (Extended Data Fig. 6d-e), all the WT tomograms and ~120
657 tomograms from *nup116Δ* cells at permissive temperature were used.

658

659 Systematic fitting of ScNPC components to the cryo-ET map

660 An unbiased systematic (global) fitting approach was performed using structural models of various
 661 ScNPC subcomplexes derived from previously published structures^{3,13}. All structural models were
 662 low-pass filtered to 40 Å prior the fitting. The resulting model maps were then independently fitted
 663 into the ScNPC cryo-EM map using global fitting as implemented in UCSF Chimera⁵². The fitting
 664 of the Y complex structures and Nup159 complex was performed for the isolated maps of the
 665 cytoplasmic and nuclear rings. The inner ring model was fitted to the IR map. The nuclear envelope
 666 density was erased prior to the fitting. The regions of the nuclear envelope distant from apparent
 667 contact points between membrane and protein densities were erased prior to the fitting to eliminate
 668 fits significantly overlapping with the membrane. All fitting runs were performed using 100,000
 669 random initial placements and the requirement of at least 30% of the model map to be covered by
 670 the ScNPC density envelope defined at low threshold. For each fitted model, this procedure yielded
 671 between 500-17,000 fits after clustering.

672 For fitting the filtered atomic models, the cross-correlation about the mean (cam score, equivalent
 673 to Pearson correlation) score from UCSF Chimera was used as a fitting metric as in our previous
 674 work^{9,10,11,12}.

675

$$676 \quad cam = \frac{\langle \mathbf{u} - \mathbf{u}_{ave} | \mathbf{v} - \mathbf{v}_{ave} \rangle}{|\mathbf{u} - \mathbf{u}_{ave}| |\mathbf{v} - \mathbf{v}_{ave}|}$$

677 where \mathbf{u}_{ave} is a vector with all components equal to the average of the components of \mathbf{u} and \mathbf{v}_{ave} is
 678 defined analogously.

679 The negative stain map of the Nup159 complex was fitted using Chimera's cross-correlation about
 680 zero (equivalent to cosine similarity).

$$681 \quad cc = \frac{\langle \mathbf{u} | \mathbf{v} \rangle}{|\mathbf{u}| |\mathbf{v}|}$$

682

683 This score was used instead of the cam score because the cam score tests the linear dependence of
 684 the two fitted maps, which is not the case when fitting negative stain maps, which contain the
 685 signal of the electron density at the surface of the complex.

686 To confirm this fit, the Nup159 complex was also fitted using the colors program from the Situs
 687 package⁵⁴ with settings appropriate for low resolution negative stain maps (i.e. using Laplacian
 688 filtering that emphasizes on contour matching over interior volume matching).

689 For each fitting run, the statistical significance of the fits was assessed as a p-value calculated from
 690 the normalized cross-correlation scores. To calculate the p-values, the cross-correlation scores
 691 were first transformed to z-scores (Fisher's z-transform) and centered, from which two-sided p-
 692 values were computed using standard deviation derived from an empirical null distribution
 693 (derived from all obtained fits and fitted using fdrtool⁵⁵ R-package). All p-values were corrected
 694 for multiple testing using Benjamini-Hochberg procedure. Figures were made using UCSF
 695 Chimera⁵² and Xlink Analyzer⁵⁶.

696

697 Integrative modeling

698 To build the integrative model of ScNPC, the IR model from¹³ was fitted as a rigid body using the
 699 procedure described above. The models of Y-complex fitted into CR and NR could not be obtained
 700 by rigid body fitting of the published models¹³. Therefore, we have divided the available crystal
 701 structures and homology models¹³ into six smaller rigid bodies (with cut points corresponding to
 702 boundaries of published crystal structures of Y-subcomplexes) and fitted them simultaneously into
 703 the cryo-EM maps of the CR and NR using the integrative modeling procedure implemented with
 704 using Integrative Modeling Platform⁵⁷ version 2.9.0 as described previously by^{11,53}. Firstly, each
 705 of the rigid bodies has been independently fitted to the EM map using UCSF Chimera⁵² as
 706 described above (structural models were low-pass filtered to 40 Å) to generate libraries of
 707 alternative fits for each rigid body. Then, we generated configurations of all the rigid bodies by
 708 recombining the above fits using simulated annealing Monte Carlo optimization. Each
 709 configuration was generated by an independent Monte Carlo optimization comprising 30,000 steps
 710 resulting in total 20,000 models and scored. The scoring function for the optimization was a linear
 711 combination of the normalized EM cross-correlation scores of the precalculated domain fits,
 712 domain connectivity restraint, a term preventing overlap of the Y components with the nuclear
 713 envelope and the assigned Nup159 complex density) and clash score (see ref.¹¹ for the
 714 implementation details). The structures were simultaneously represented at two resolutions: in C α -
 715 only representation and a coarse-grained representation, in which each 10-residue stretch was
 716 converted to a bead. The 10-residue bead representation was used for the clash score to increase
 717 computational efficiency, the C α -only representation was used for crosslinking and domain
 718 connectivity restraints. Since the EM restraint was derived from the original EM fits generated
 719 with UCSF Chimera, it was derived from the full atom representation. The final models for
 720 visualization were selected as the top scoring model.

721

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- 779

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793 **Author Contributions**

794 MA, CEZ conceived the project, designed experiments, collected data, analyzed data, wrote the
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796 designed experiments, collected data, analyzed data. HKHF, CWL, XZ collected data, analyzed
797 data. WH collected data. BT analyzed data. KK performed computational analysis. CWM, JM, YS
798 supervised the project. BP, JK, MB conceived the project, designed experiments, supervised the
799 project, wrote the manuscript.

800

801 **Depositions**

802 The EM maps associated with this manuscript will be deposited in Electron Microscopy Data Bank
803 upon publication. The integrative model of ScNPC will be deposited in PDB-dev ([https://pdb-
804 dev.wwpdb.org/](https://pdb-dev.wwpdb.org/)). An in cell cryo-EM map of the ScNPC is attached for the review process (Source
805 Data 1).