Supplementary Materials for

Architecture of the symmetric core of the nuclear pore

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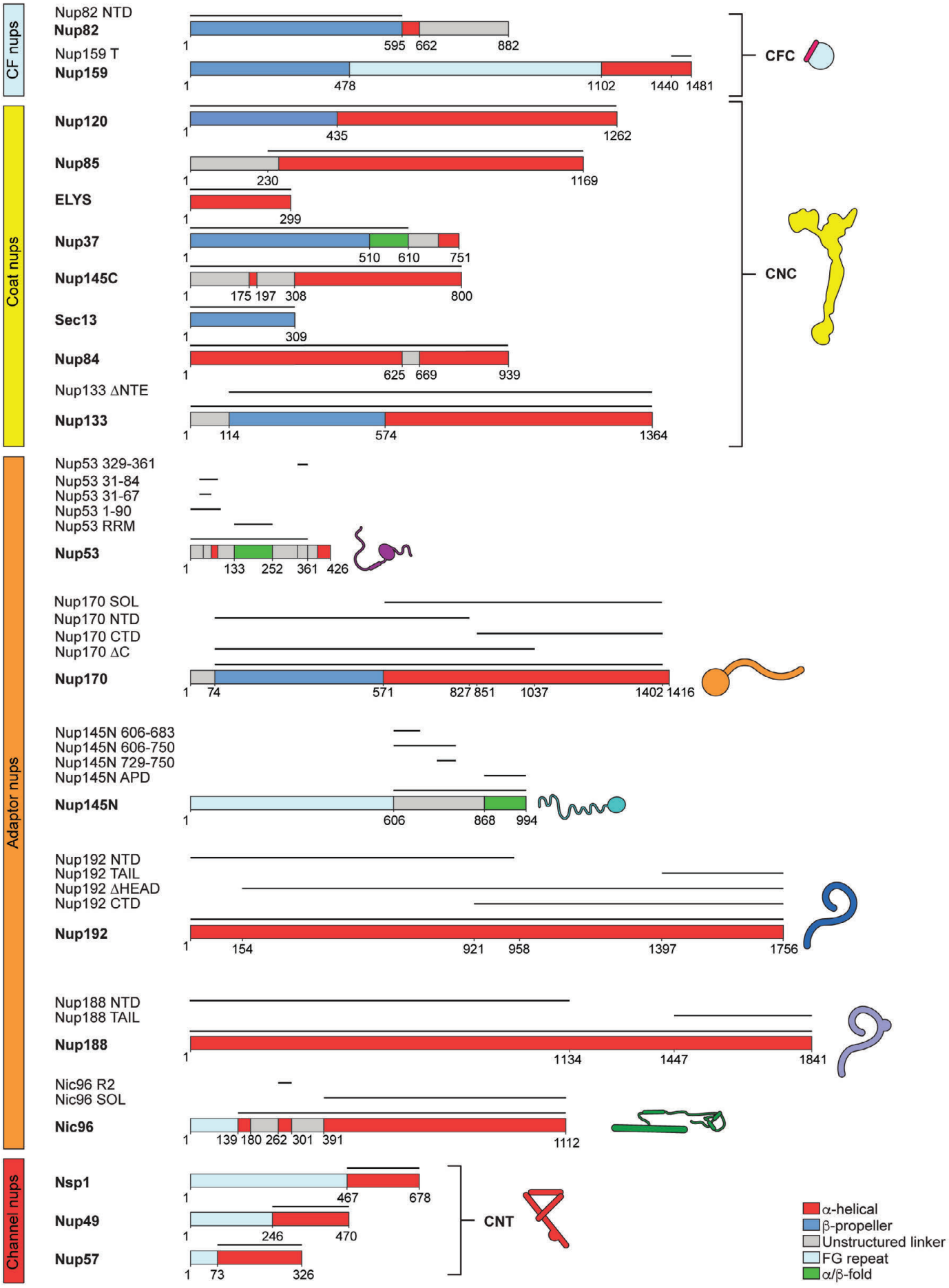

CF nups
Coat nups
Adaptor nups
Channel nups
Nup82 NTD
Nup82
Nup159 T
Nup159
1
595
662
882
1
478
1102
1440
1481
CFC
Nup120
Nup85
ELYS
Nup37
Nup145C
Sec13
Nup84
Nup133 ΔNTE
Nup133
1
435
1262
1
230
1169
1
299
1
510
610
751
1
175
197
308
800
1
309
1
625
669
939
1
114
574
1364
CNC
Nup53 329-361
Nup53 31-84
Nup53 31-67
Nup53 1-90
Nup53 RRM
Nup53
1
133
252
361
426
Nup170 SOL
Nup170 NTD
Nup170 CTD
Nup170 ΔC
Nup170
1
74
571
827
851
1037
1402
1416
Nup145N 606-683
Nup145N 606-750
Nup145N 729-750
Nup145N APD
Nup145N
1
606
868
994
Nup192 NTD
Nup192 TAIL
Nup192 ΔHEAD
Nup192 CTD
Nup192
1
154
921
958
1397
1756
Nup188 NTD
Nup188 TAIL
Nup188
1
1134
1447
1841
Nic96 R2
Nic96 SOL
Nic96
1
139
180
262
301
391
1112
Nsp1
Nup49
Nup57
1
467
678
1
246
470
1
73
326
CNT
α-helical
β-propeller
Unstructured linker
FG repeat
α/β-fold

Fig.S1.
Nucleoporin fragments. Domain boundaries of all nucleoporin fragments used throughout the text are indicated by black lines.

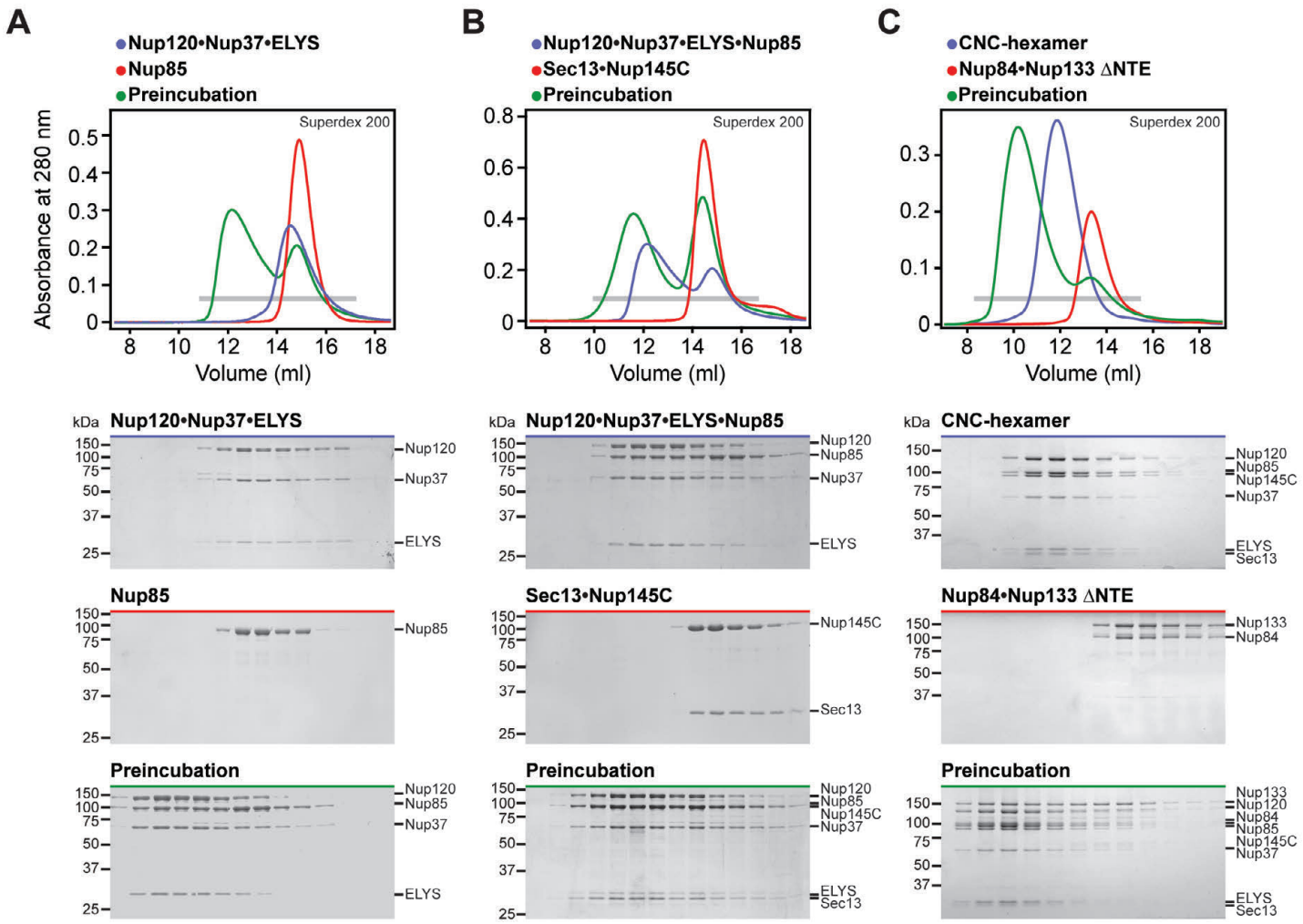

A
Nup120•Nup37•ELYS
Nup85
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
B
Nup120•Nup37•ELYS•Nup85
Sec13•Nup145C
Preincubation
Superdex 200
Volume (ml)
C
CNC-hexamer
Nup84•Nup133 ΔNTE
Preincubation
Superdex 200
Volume (ml)
kDa
Nup120•Nup37•ELYS
Nup120
Nup37
ELYS
Nup85
Nup85
Preincubation
Nup120
Nup85
Nup37
ELYS
kDa
Nup120•Nup37•ELYS•Nup85
Nup120
Nup85
Nup37
ELYS
Sec13•Nup145C
Nup145C
Sec13
Preincubation
Nup120
Nup85
Nup145C
Nup37
ELYS
Sec13
kDa
CNC-hexamer
Nup120
Nup85
Nup145C
Nup37
ELYS
Sec13
Nup84•Nup133 ΔNTE
Nup133
Nup84
Preincubation
Nup133
Nup120
Nup84
Nup85
Nup145C
Nup37
ELYS
Sec13

Fig. S2.
Reconstitution of the CNC. SEC and SDS-PAGE analysis for the reconstitution of (**A**) the Nup120•Nup37•ELYS•Nup85 hetero-tetramer, (**B**) the CNC-hexamer, and (**C**) the CNC-octamer. SEC profiles of nucleoporins or nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 10/300 GL column. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. The CNC-octamer could only be reconstituted with a Nup133 construct lacking the NTE.

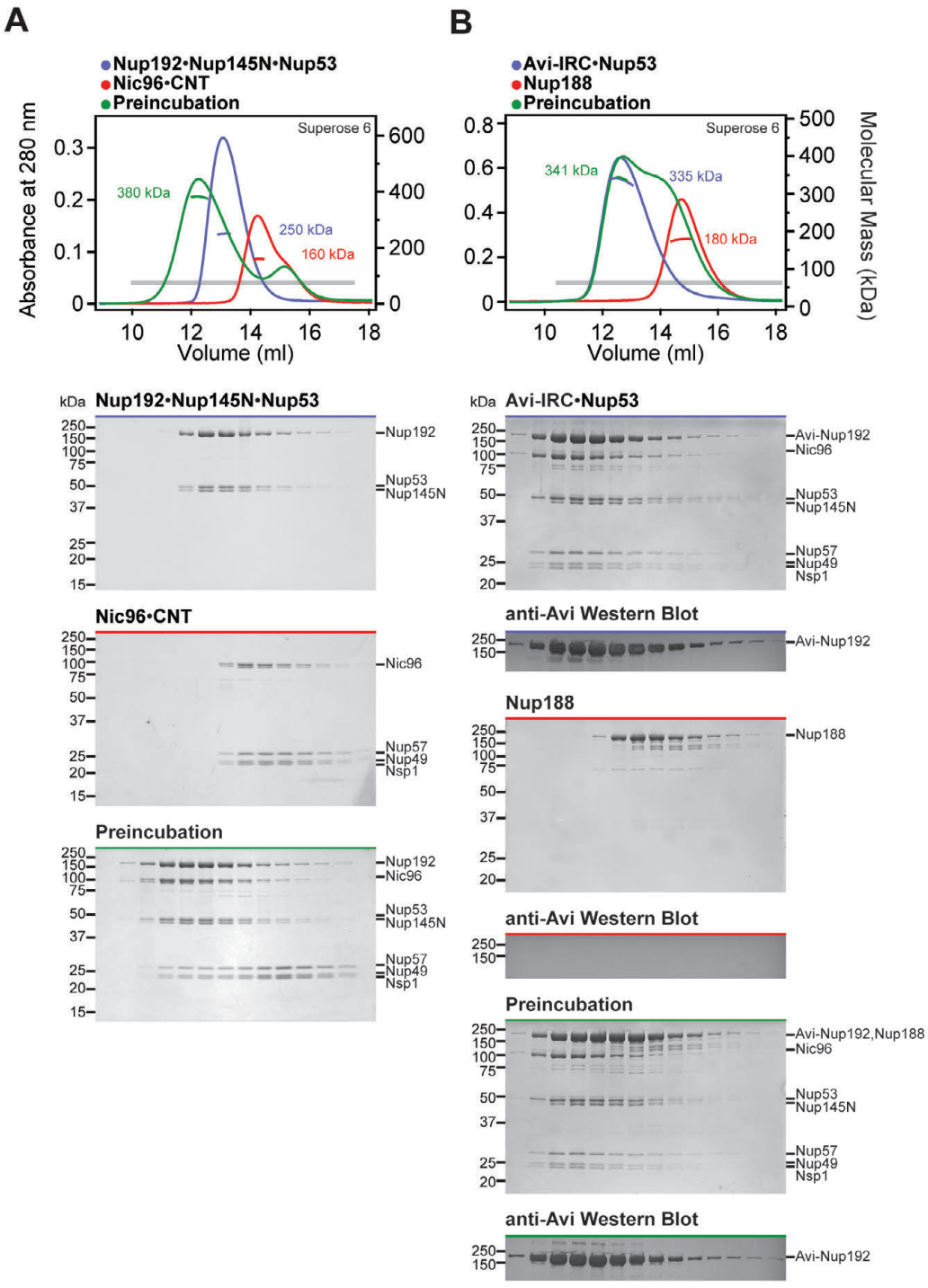
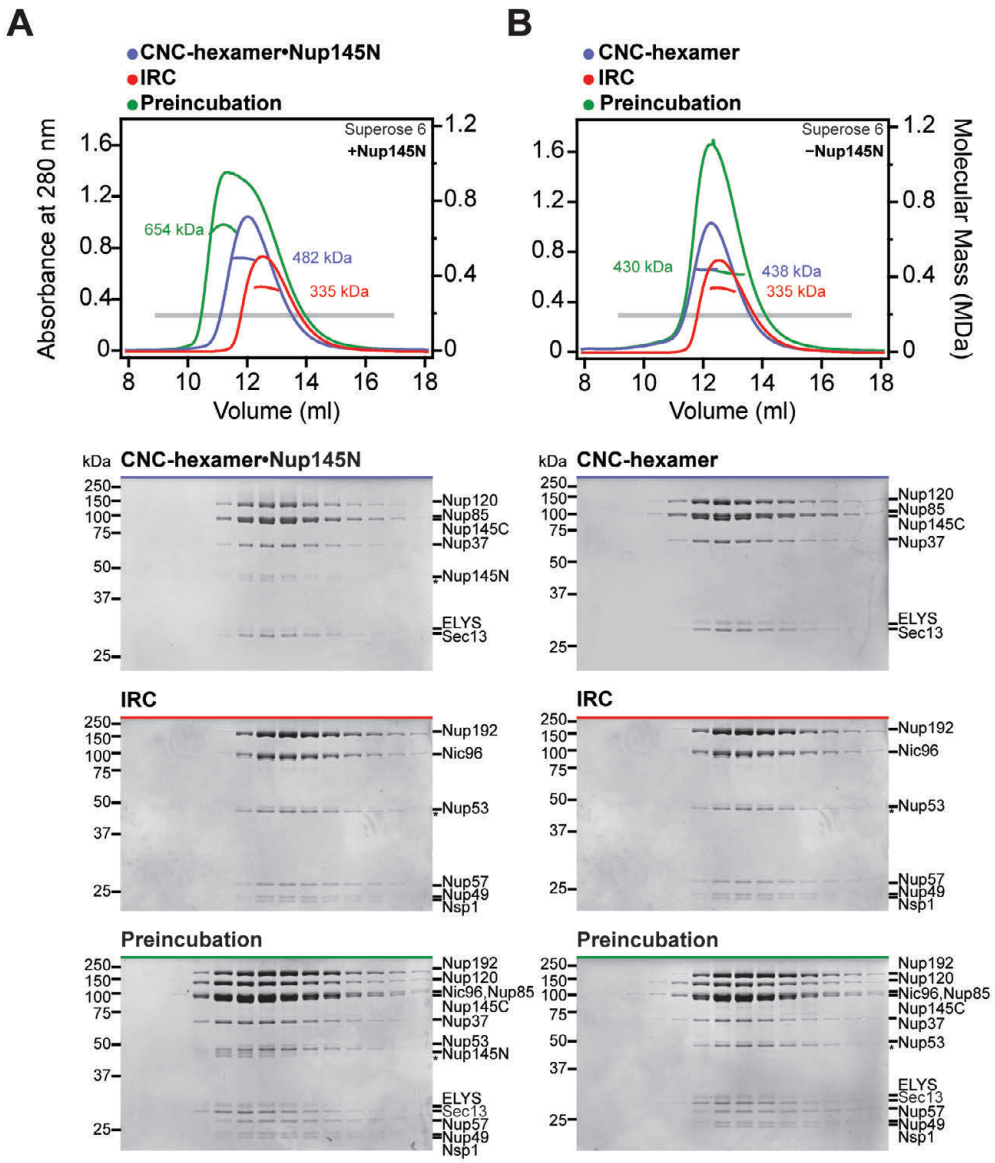

A
B
Nup192•Nup145N•Nup53
Nic96•CNT
Preincubation
Superose 6
Absorbance at 280 nm
380 kDa
250 kDa
160 kDa
Volume (ml)
Avi-IRC•Nup53
Nup188
341 kDa
335 kDa
180 kDa
Molecular Mass (kDa)
kDa
Nup192•Nup145N•Nup53
Nup192
Nup53
Nup145N
Nic96•CNT
Nic96
Nup57
Nup49
Nsp1
Preincubation
Avi-IRC•Nup53
Avi-Nup192
Nic96
anti-Avi Western Blot
Nup188
Avi-Nup192,Nup188

Fig. S3.
Reconstitution of the IRC•Nup53 complex. SEC-MALS and SDS-PAGE analysis of (**A**) the reconstitution of the IRC•Nup53 hetero-heptamer and (**B**) the interaction of IRC•Nup53 with Nup188. SEC-MALS profiles of nucleoporins or nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superose 6 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining or by immunoblotting with a mouse anti-AviTag antibody. As Nup188 and Nup192 cannot be distinguished by SDS-PAGE analysis, lack of Nup192 displacement from the IRC by Nup188 was confirmed by western blotting against Avi-tagged Nup192.


A
CNC-hexamer•Nup145N
IRC
Preincubation
Superose 6
+Nup145N
654 kDa
482 kDa
335 kDa
Absorbance at 280 nm
Volume (ml)
B
CNC-hexamer
IRC
Preincubation
Superose 6
−Nup145N
430 kDa
438 kDa
335 kDa
Molecular Mass (MDa)
Volume (ml)
kDa
CNC-hexamer•Nup145N
Nup120
Nup85
Nup145C
Nup37
Nup145N
ELYS
Sec13
IRC
Nup192
Nic96
Nup53
Nup57
Nup49
Nsp1
Preincubation
Nup192
Nup120
Nic96,Nup85
Nup145C
Nup37
Nup53
Nup145N
ELYS
Sec13
Nup57
Nup49
Nsp1
kDa
CNC-hexamer
Nup120
Nup85
Nup145C
Nup37
ELYS
Sec13
IRC
Nup192
Nic96
Nup53
Nup57
Nup49
Nsp1
Preincubation
Nup192
Nup120
Nic96,Nup85
Nup145C
Nup37
Nup53
ELYS
Sec13
Nup57
Nup49
Nsp1

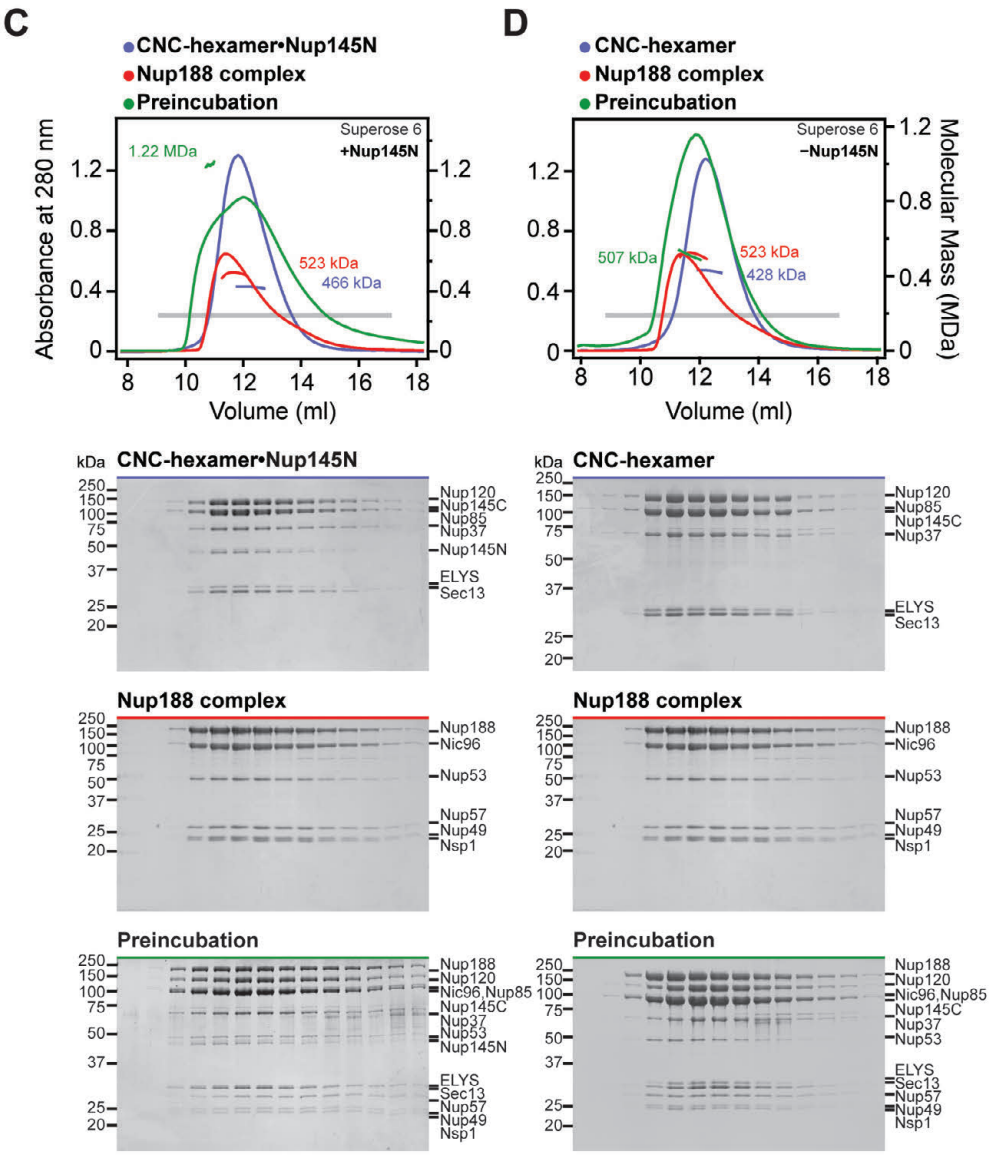

C
CNC-hexamer•Nup145N
Nup188 complex
Preincubation
Superose 6
+Nup145N
1.22 MDa
523 kDa
466 kDa
Absorbance at 280 nm
Volume (ml)
D
CNC-hexamer
Nup188 complex
Preincubation
Superose 6
−Nup145N
507 kDa
523 kDa
428 kDa
Molecular Mass (MDa)
Volume (ml)
kDa
CNC-hexamer•Nup145N
Nup120
Nup145C
Nup85
Nup37
Nup145N
ELYS
Sec13
CNC-hexamer
Nup120
Nup85
Nup145C
Nup37
ELYS
Sec13
Nup188 complex
Nup188
Nic96
Nup53
Nup57
Nup49
Nsp1
Preincubation
Nup188
Nup120
Nic96,Nup85
Nup145C
Nup37
Nup53
Nup145N
ELYS
Sec13
Nup57
Nup49
Nsp1

Fig. S4.
Reconstitution of NPC core protomers. (**A**-**D**) SEC-MALS and SDS-PAGE analysis corresponding to Fig. 1, D to G. SEC-MALS profiles of nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superose 6 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining.

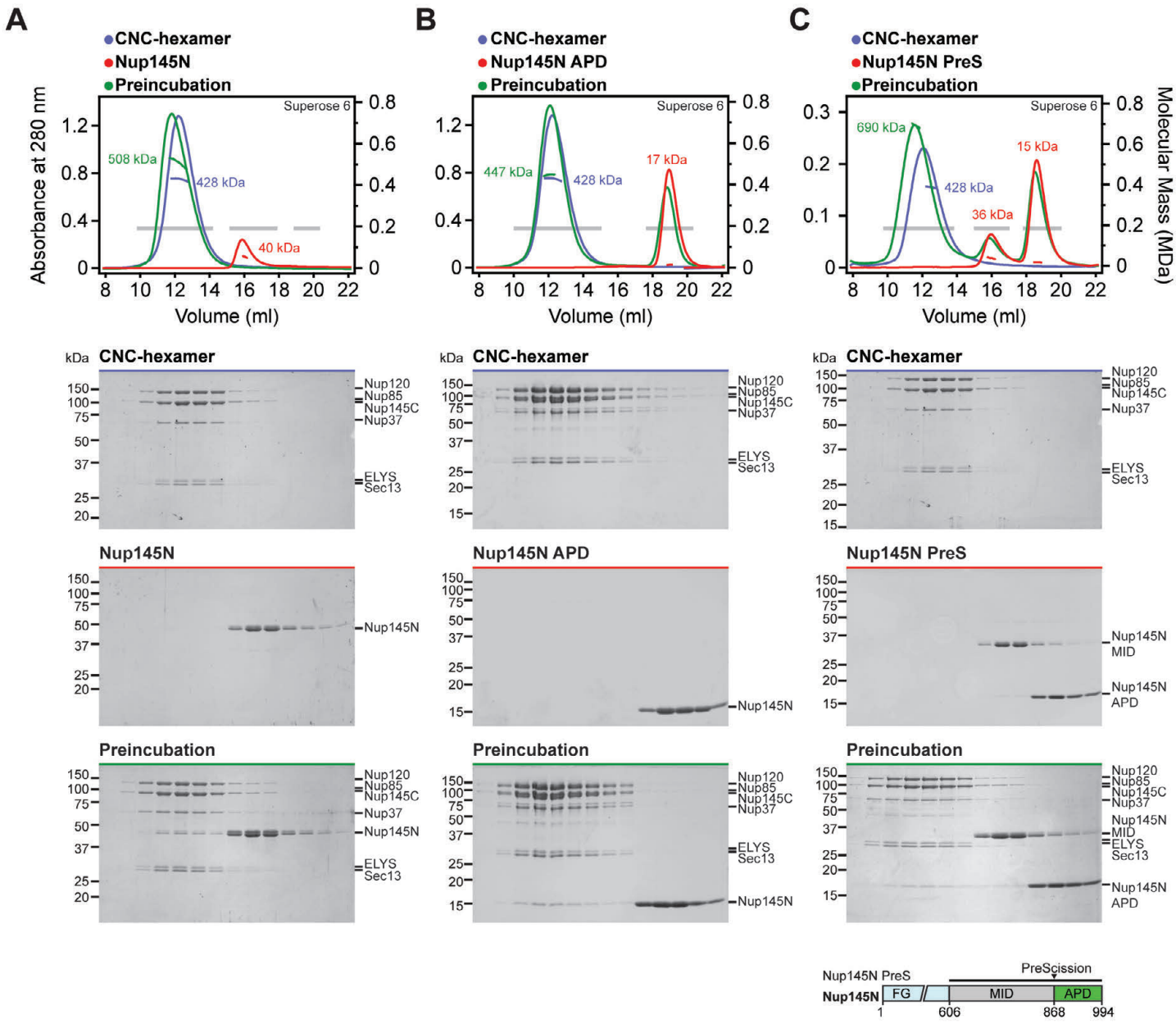

A
CNC-hexamer
Nup145N
Preincubation
Superose 6
508 kDa
428 kDa
40 kDa
Absorbance at 280 nm
Volume (ml)
B
CNC-hexamer
Nup145N APD
Preincubation
Superose 6
447 kDa
428 kDa
17 kDa
Volume (ml)
C
CNC-hexamer
Nup145N PreS
Preincubation
Superose 6
690 kDa
428 kDa
36 kDa
15 kDa
Molecular Mass (MDa)
Volume (ml)
kDa
CNC-hexamer
Nup120
Nup85
Nup145C
Nup37
ELYS
Sec13
Nup145N
Nup145N APD
Nup145N PreS
Nup145N MID
Nup145N APD
Preincubation
Nup145N PreS
PreScission
Nup145N
FG
MID
APD
1
606
868
994

Fig. S5.
The CNC-hexamer interacts with Nup145N^{APD}. SEC-MALS and SDS-PAGE analysis of the CNC-hexamer interaction with (**A**) Nup145N, (**B**) Nup145N^{APD}, and (**C**) Nup145N PreS preincubated with PreScission protease. SEC-MALS profiles of nucleoporins or nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superose 6 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. As reference, the domain structure of Nup145N PreS is shown, indicating the construct boundaries and PreScission cleavage site with a black bar and a black triangle, respectively. After cleavage with PreScission protease, Nup145N^{APD} co-elutes with the CNC-hexamer, whereas Nup145N^{MID} does not incorporate into the complex.

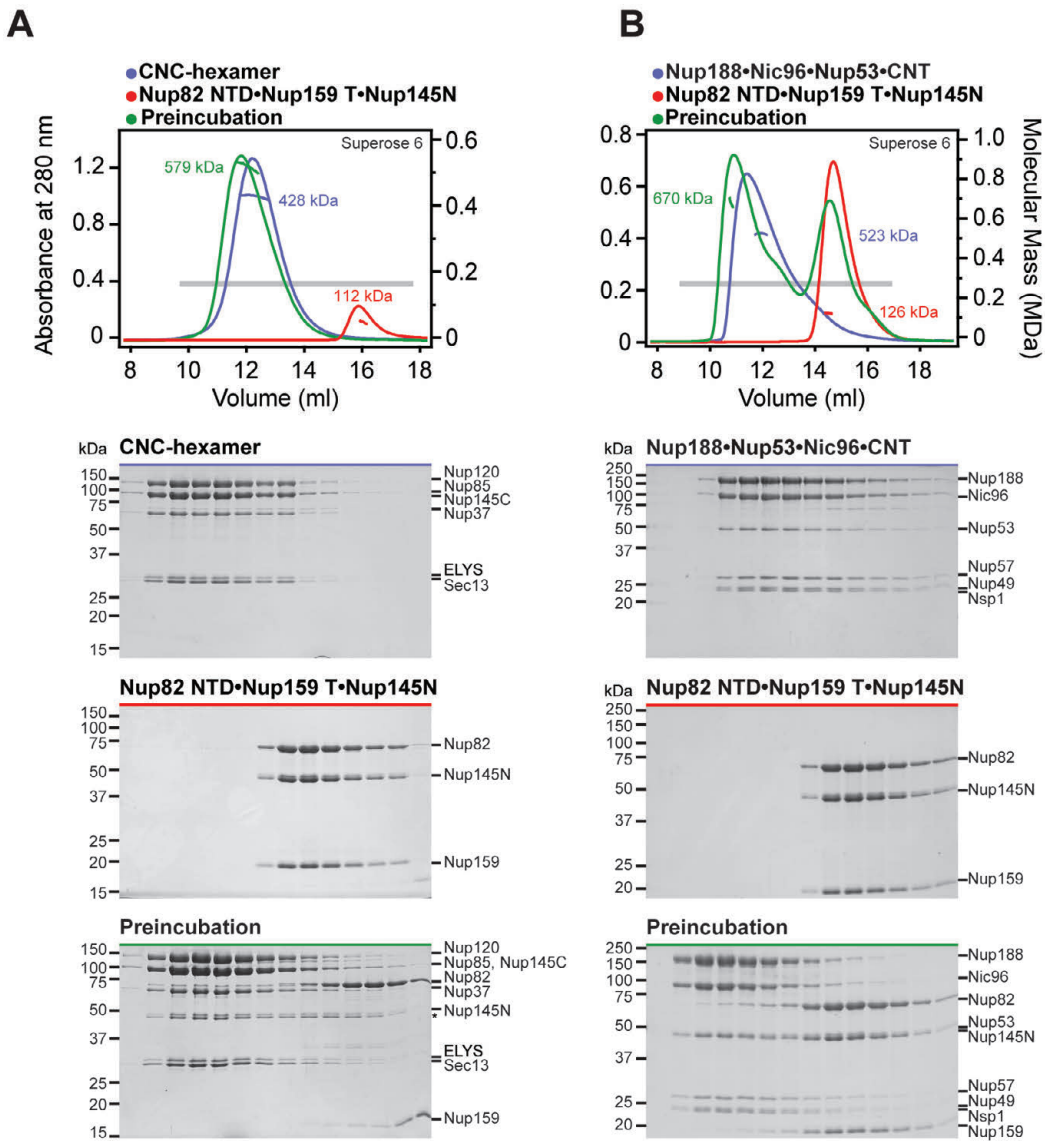

A
CNC-hexamer
Nup82 NTD•Nup159 T•Nup145N
Preincubation
Superose 6
579 kDa
428 kDa
112 kDa
Absorbance at 280 nm
Volume (ml)
kDa
CNC-hexamer
Nup120
Nup85
Nup145C
Nup37
ELYS
Sec13
Nup82 NTD•Nup159 T•Nup145N
Nup82
Nup145N
Nup159
Preincubation
Nup120
Nup85, Nup145C
Nup82
Nup37
Nup145N
ELYS
Sec13
Nup159
B
Nup188•Nic96•Nup53•CNT
Nup82 NTD•Nup159 T•Nup145N
Preincubation
Superose 6
670 kDa
523 kDa
126 kDa
Molecular Mass (MDa)
Volume (ml)
kDa
Nup188•Nup53•Nic96•CNT
Nup188
Nic96
Nup53
Nup57
Nup49
Nsp1
Nup82 NTD•Nup159 T•Nup145N
Nup82
Nup145N
Nup159
Preincubation
Nup188
Nic96
Nup82
Nup53
Nup145N
Nup57
Nup49
Nsp1
Nup159

Fig. S6.

Incorporation of Nup145N into complexes with CNC-hexamer or Nup188•Nic96•Nup53•CNT is exclusive of Nup145N binding to Nup82^{NTD}•Nup159^{T}. SEC-MALS and SDS-PAGE analysis of the CFC interaction with (**A**) the CNC-hexamer or (**B**) the Nup188•Nic96•Nup53•CNT complex. SEC-MALS profiles of nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). SEC profiles were obtained using a Superose 6 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. In both preincubations, Nup145N incorporates stoichiometrically into the complexes, but the Nup82^{NTD}•Nup159^{T} hetero-dimer does not. However, Nup82^{NTD}•Nup159^{T} interacts weakly with the Nup188-containing complex.

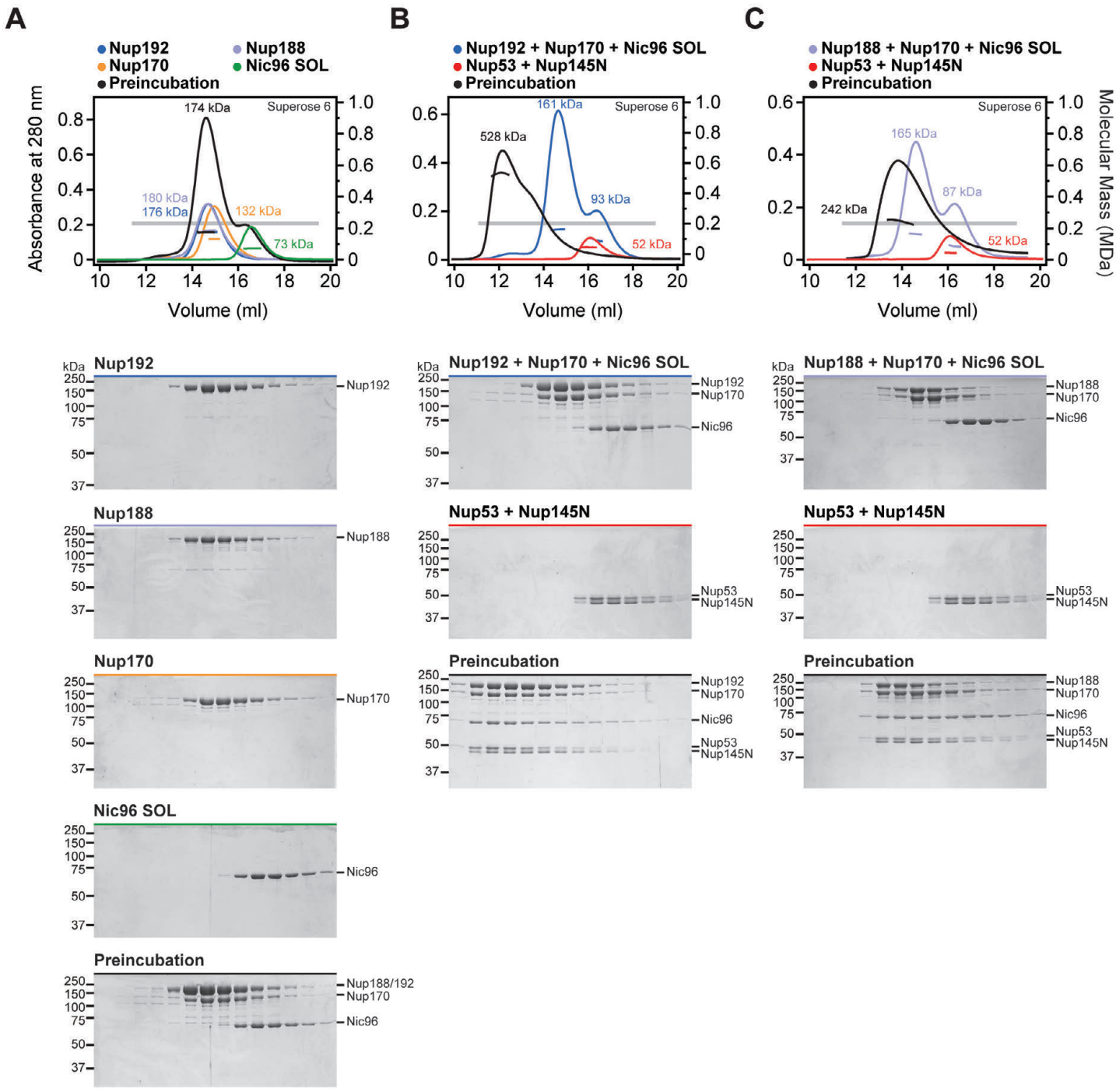

A
Nup192
Nup188
Nup170
Nic96 SOL
Preincubation
Absorbance at 280 nm
174 kDa
Superose 6
180 kDa
176 kDa
132 kDa
73 kDa
Volume (ml)
B
Nup192 + Nup170 + Nic96 SOL
Nup53 + Nup145N
Preincubation
161 kDa
528 kDa
93 kDa
52 kDa
C
Nup188 + Nup170 + Nic96 SOL
Nup53 + Nup145N
Preincubation
165 kDa
87 kDa
242 kDa
52 kDa
Molecular Mass (MDa)
kDa
Nup192
Nup188
Nup170
Nic96 SOL
Preincubation
Nup188/192
Nup192 + Nup170 + Nic96 SOL
Nup53 + Nup145N
Nic96

Fig. S7.
Scaffold nucleoporins are assembled by the linker nucleoporins. SEC-MALS and SDS-PAGE analysis corresponding to Fig. 2, A to C. (**A**) For interaction analysis between scaffold nucleoporins, SEC-MALS profiles of nucleoporins are shown individually (blue, purple, orange, or green) and after their preincubation (black). (**B**) To analyze the assembly of Nup192, Nup170, and Nic96^{SOL} with the linker nucleoporins Nup53 and Nup145N, SEC-MALS profiles of the scaffold mixture (blue), the linker mixture (red), or after their preincubation (black) are shown. (**C**) To analyze the assembly of Nup188, Nup170 and Nic96^{SOL} with the linker nucleoporins Nup53 and Nup145N, SEC-MALS profiles of the scaffold mixture (purple), the linker mixture (red), or after their preincubation (black) are shown. All SEC profiles were obtained using a Superose 6 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Because Nup188 could not be incorporated into the IRC, Nup188 and Nup192 were not simultaneously included in preincubations.

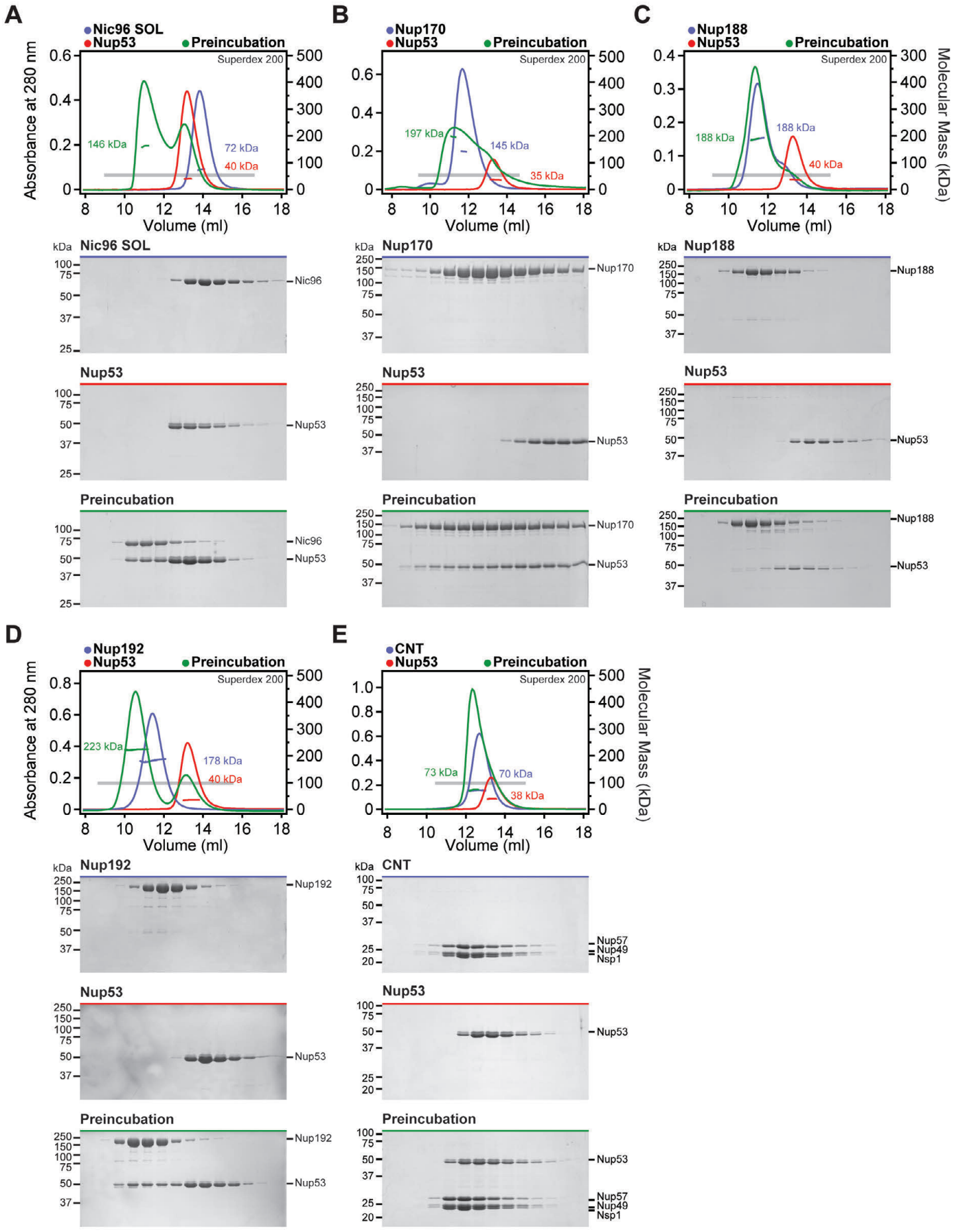

A
Nic96 SOL
Nup53
Preincubation
Superdex 200
Absorbance at 280 nm
Molecular Mass (kDa)
Volume (ml)
146 kDa
72 kDa
40 kDa
kDa
Nic96 SOL
Nic96
Nup53
Preincubation
B
Nup170
Nup53
Preincubation
Superdex 200
197 kDa
145 kDa
35 kDa
Volume (ml)
Nup170
Nup53
Preincubation
C
Nup188
Nup53
Preincubation
Superdex 200
188 kDa
188 kDa
40 kDa
Volume (ml)
Nup188
Nup53
Preincubation
D
Nup192
Nup53
Preincubation
Superdex 200
223 kDa
178 kDa
40 kDa
Volume (ml)
Nup192
Nup53
Preincubation
E
CNT
Nup53
Preincubation
Superdex 200
73 kDa
70 kDa
38 kDa
Volume (ml)
CNT
Nup57
Nup49
Nsp1
Nup53
Preincubation

Fig. S8.
Identification of interactions between Nup53 and the scaffold nucleoporins. (**A**-**E**) SEC-MALS and SDS-PAGE analysis for Fig. 2D. SEC-MALS profiles of nucleoporins are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Robust complex formation with Nup53 was observed for Nic96^{SOL}, Nup170, and Nup192. Interactions of Nup53 with Nup188 and the CNT were only barely detectable.

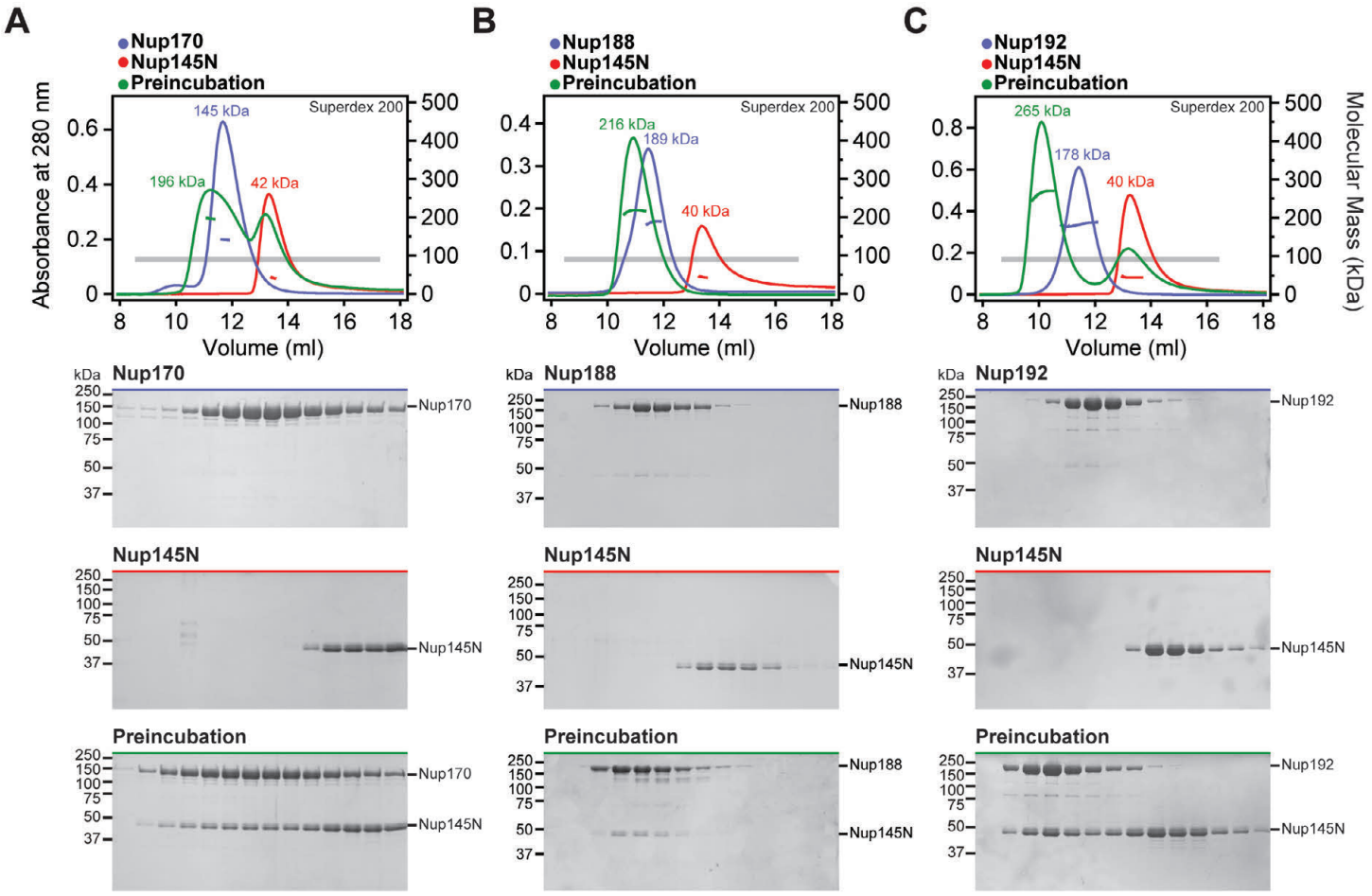

A
Nup170
Nup145N
Preincubation
Superdex 200
145 kDa
196 kDa
42 kDa
Absorbance at 280 nm
Volume (ml)
Molecular Mass (kDa)
Nup170
Nup145N
Preincubation
B
Nup188
Nup145N
Preincubation
Superdex 200
216 kDa
189 kDa
40 kDa
Volume (ml)
Nup188
Nup145N
Preincubation
C
Nup192
Nup145N
Preincubation
Superdex 200
265 kDa
178 kDa
40 kDa
Volume (ml)
Nup192
Nup145N
Preincubation
kDa

Fig. S9.
Identification of interactions between Nup145N and scaffold nucleoporins. (**A**-**C**) SEC-MALS and SDS-PAGE analysis for Fig. 2E. SEC-MALS profiles of nucleoporins are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Robust complex formation with Nup145N was observed for Nup170, Nup188, and Nup192. We previously demonstrated interactions between Nup145N and Nic96 and the CNT (*15*).

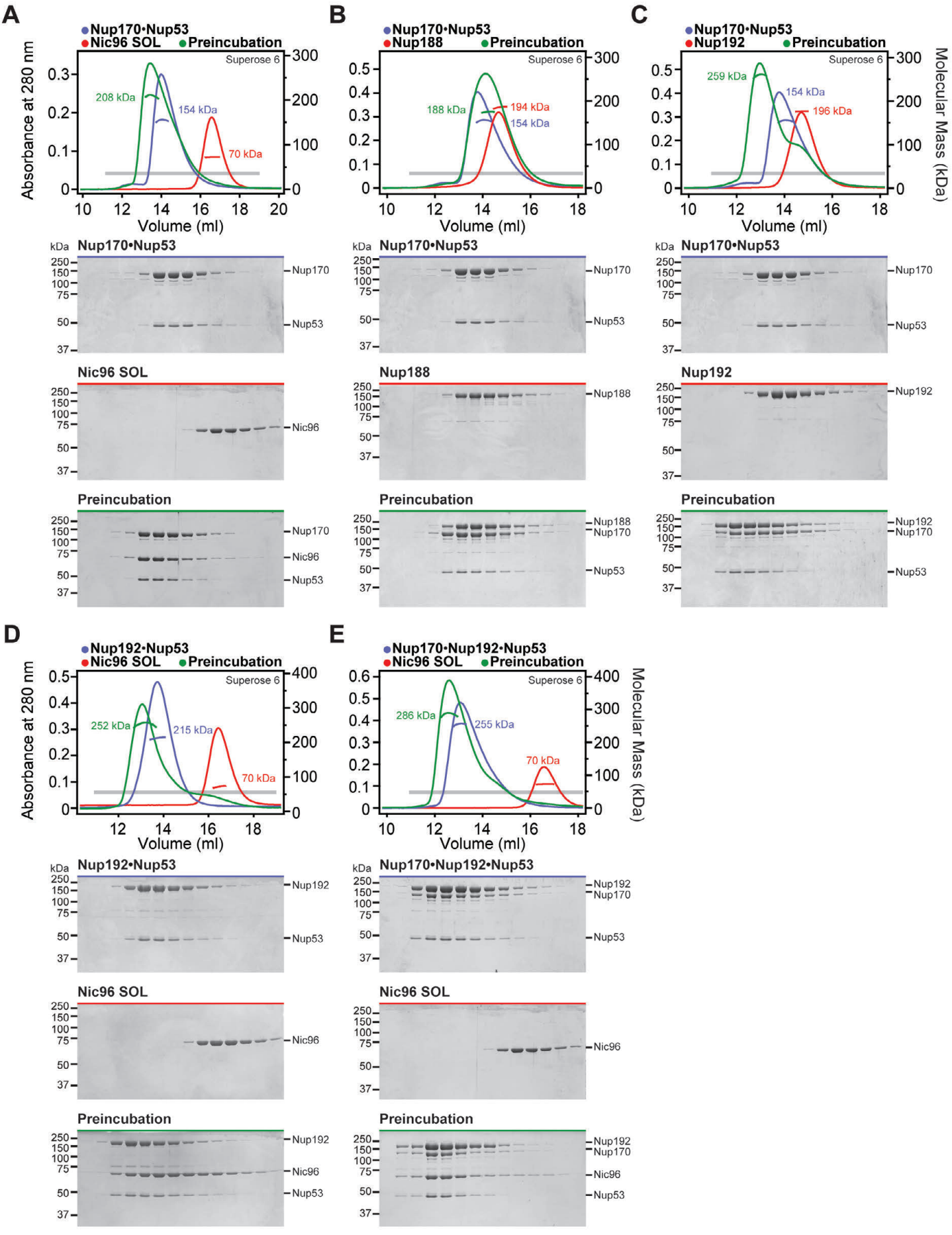
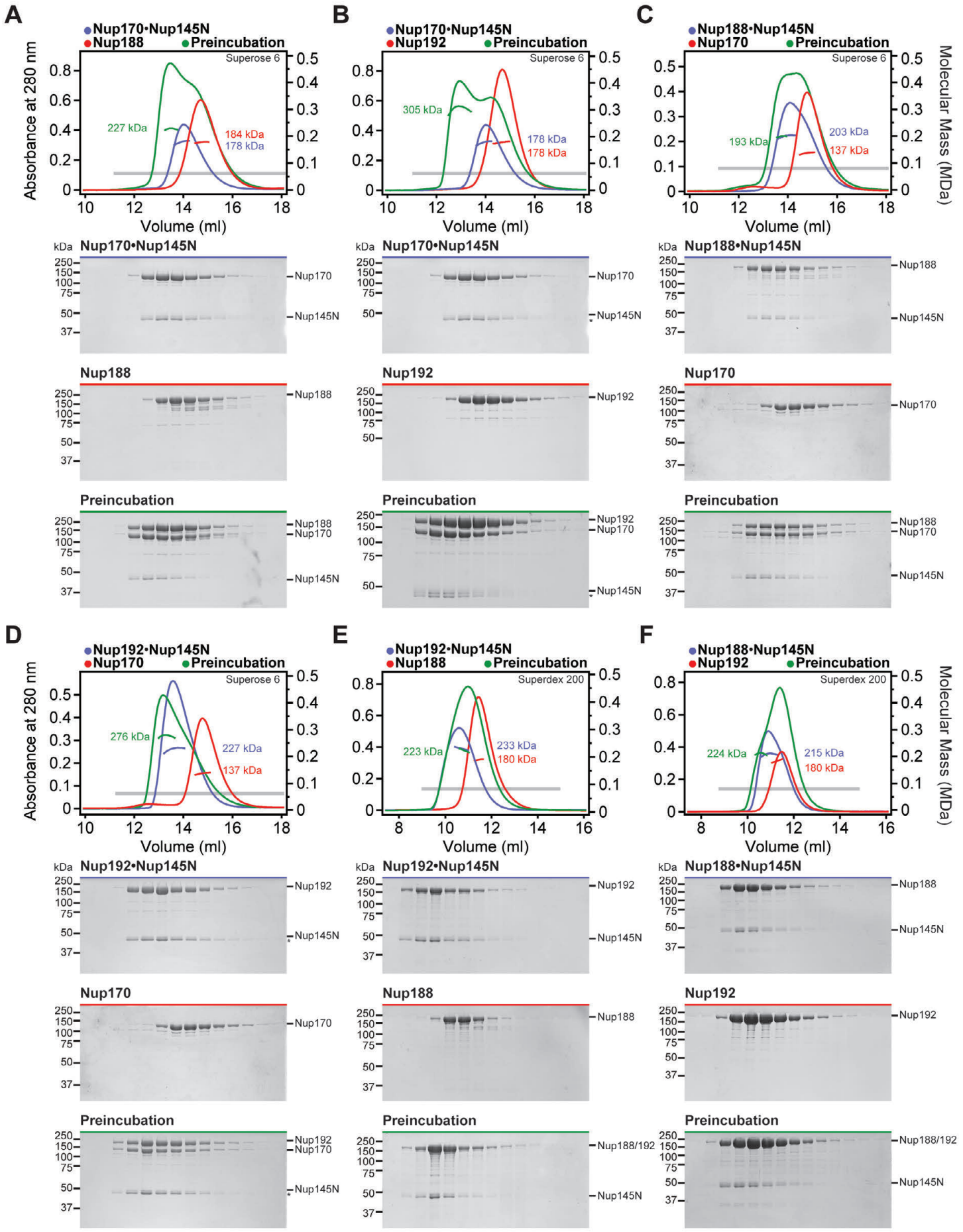

A
Nup170•Nup53
Nic96 SOL
Preincubation
Superose 6
Absorbance at 280 nm
Molecular Mass (kDa)
Volume (ml)
208 kDa
154 kDa
70 kDa
kDa
Nup170•Nup53
Nic96 SOL
Preincubation
Nup170
Nup53
Nic96
B
Nup170•Nup53
Nup188
Preincubation
188 kDa
194 kDa
154 kDa
Nup188
C
Nup170•Nup53
Nup192
Preincubation
259 kDa
154 kDa
196 kDa
Nup192
D
Nup192•Nup53
Nic96 SOL
Preincubation
252 kDa
215 kDa
70 kDa
E
Nup170•Nup192•Nup53
Nic96 SOL
Preincubation
286 kDa
255 kDa
70 kDa

Fig. S10.
Identification of hetero-trimeric complexes assembled by Nup53. (**A**-**E**) SEC-MALS and SDS-PAGE analysis corresponding to Fig. 2D. Purified hetero-dimeric Nup53 complexes were tested for their ability to form hetero-trimers with an additional scaffold nucleoporin. SEC-MALS profiles of nucleoporins or nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superose 6 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Nup53 is able to bind simultaneously to Nup170, Nic96^{SOL}, and Nup192. The interaction of the Nup170•Nup53 hetero-dimer with Nup188 was only barely detectable.


A
Nup170•Nup145N
Nup188
Preincubation
Superose 6
227 kDa
184 kDa
178 kDa
Absorbance at 280 nm
Molecular Mass (MDa)
Volume (ml)
kDa
Nup170•Nup145N
Nup170
Nup145N
Nup188
Nup188
Preincubation
Nup188
Nup170
Nup145N
B
Nup170•Nup145N
Nup192
Preincubation
Superose 6
305 kDa
178 kDa
178 kDa
Volume (ml)
Nup170•Nup145N
Nup170
Nup145N
Nup192
Nup192
Preincubation
Nup192
Nup170
Nup145N
C
Nup188•Nup145N
Nup170
Preincubation
Superose 6
193 kDa
203 kDa
137 kDa
Volume (ml)
Nup188•Nup145N
Nup188
Nup145N
Nup170
Nup170
Preincubation
Nup188
Nup170
Nup145N
D
Nup192•Nup145N
Nup170
Preincubation
Superose 6
276 kDa
227 kDa
137 kDa
Volume (ml)
Nup192•Nup145N
Nup192
Nup145N
Nup170
Nup170
Preincubation
Nup192
Nup170
Nup145N
E
Nup192•Nup145N
Nup188
Preincubation
Superdex 200
223 kDa
233 kDa
180 kDa
Volume (ml)
Nup192•Nup145N
Nup192
Nup145N
Nup188
Nup188
Preincubation
Nup188/192
Nup145N
F
Nup188•Nup145N
Nup192
Preincubation
Superdex 200
224 kDa
215 kDa
180 kDa
Volume (ml)
Nup188•Nup145N
Nup188
Nup145N
Nup192
Nup192
Preincubation
Nup188/192
Nup145N

G

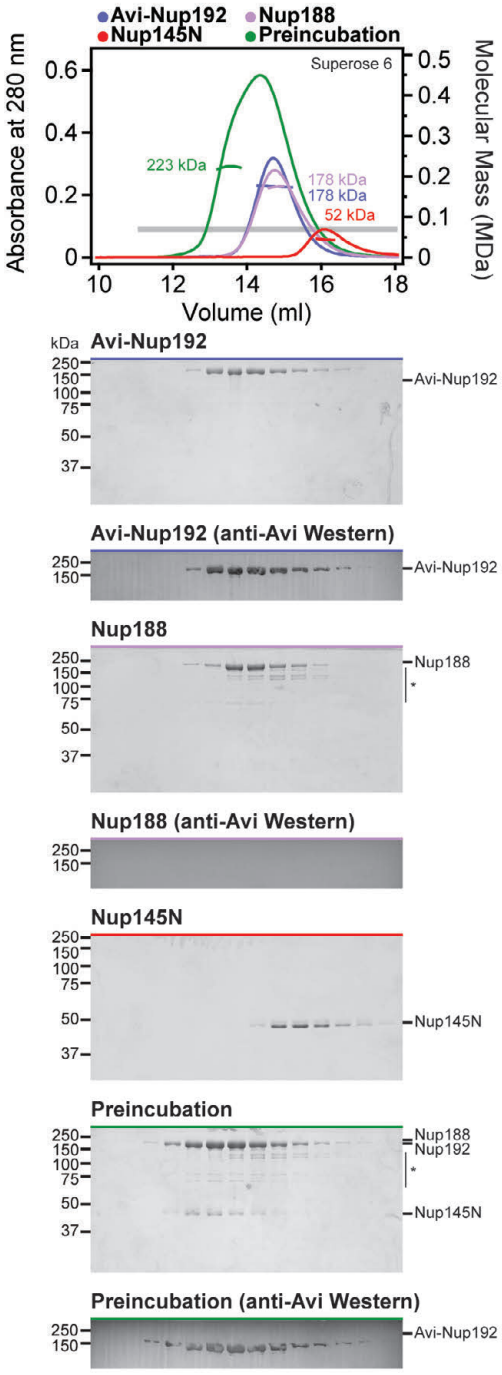

Avi-Nup192
Nup188
Nup145N
Preincubation
Superose 6
Absorbance at 280 nm
Molecular Mass (MDa)
223 kDa
178 kDa
178 kDa
52 kDa
Volume (ml)
kDa
Avi-Nup192
Avi-Nup192 (anti-Avi Western)
Nup188
Nup188 (anti-Avi Western)
Nup145N
Preincubation
Nup188
Nup192
Nup145N
Preincubation (anti-Avi Western)

Fig. S11.
Identification of hetero-trimeric complexes assembled by Nup145N. (**A**-**G**) SEC-MALS and SDS-PAGE analysis corresponding to Fig. 2E. Purified hetero-dimeric Nup145N complexes were tested for their ability to form hetero-trimers with an additional scaffold nucleoporin. SEC-MALS profiles of nucleoporins or nucleoporin complexes are shown individually (blue, red, and purple) and after their preincubation (green). SEC profiles were obtained using either a Superose 6 10/300 GL column or a Superdex 200 10/300 GL column as indicated. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining or by western blot analysis with a mouse anti-AviTag antibody. Hetero-trimeric complex formation was determined on the basis of a shift in elution volume, an increase in measured molecular mass, and the presence of all three proteins in the higher molecular mass fractions. For preincubations with Nup188 or Nup188•Nup145N, only minor shifts in elution volume and no increases in the measured molecular masses were observed, indicating the formation of a mixture of species rather than stable hetero-trimeric complexes as seen between Nup170, Nup192, and Nup145N. As Nup188 and Nup192 cannot be distinguished by SDS-PAGE, western blot analysis was employed to confirm that Nup188 and Nup192 bind to Nup145N in a mutually exclusive fashion.

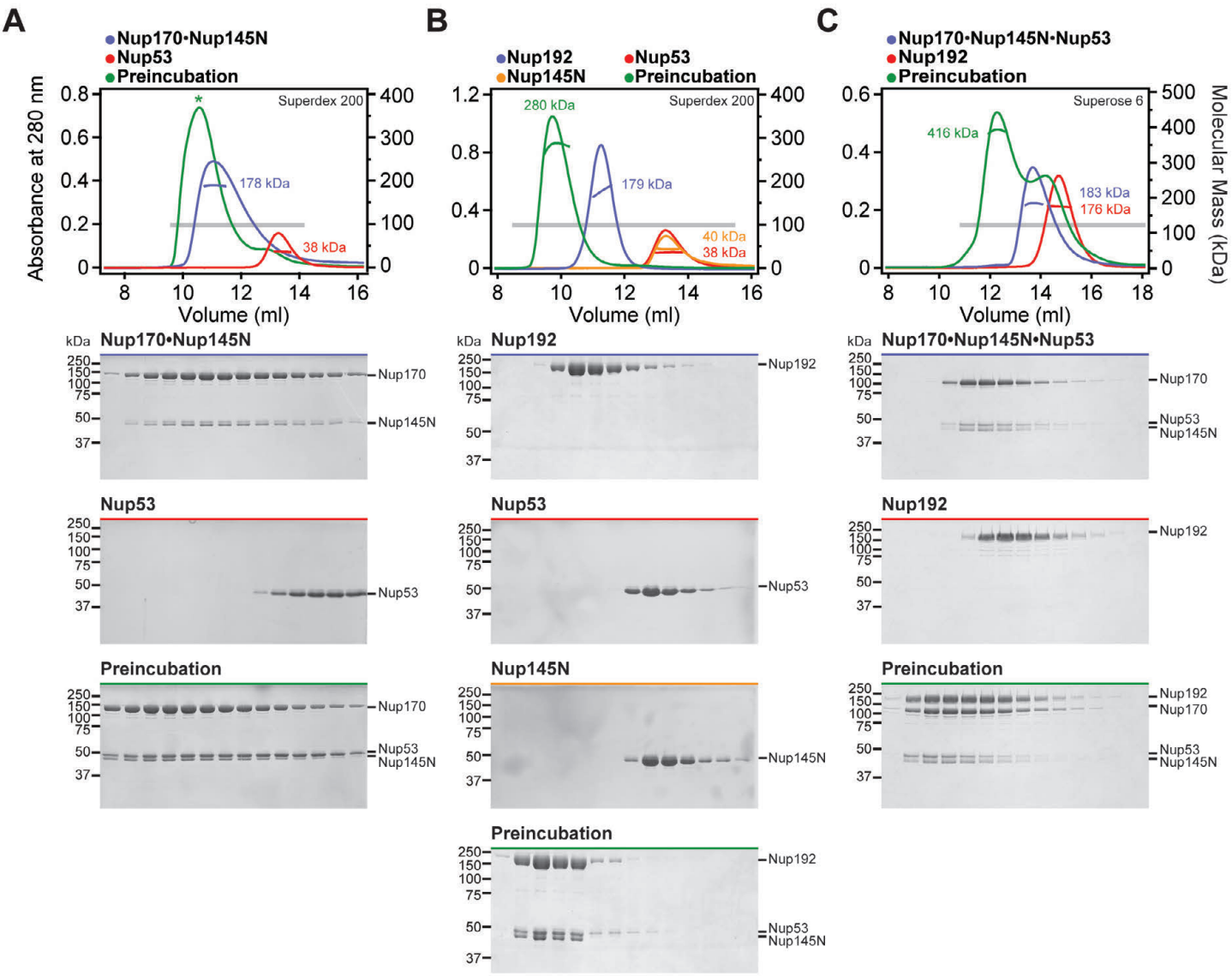

A
Nup170•Nup145N
Nup53
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
178 kDa
38 kDa
kDa
Nup170•Nup145N
Nup170
Nup145N
Nup53
Nup53
Preincubation
Nup170
Nup53
Nup145N
B
Nup192
Nup145N
Nup53
Preincubation
Superdex 200
280 kDa
179 kDa
40 kDa
38 kDa
Nup192
Nup53
Nup145N
Preincubation
Nup192
Nup53
Nup145N
C
Nup170•Nup145N•Nup53
Nup192
Preincubation
Superose 6
416 kDa
183 kDa
176 kDa
Molecular Mass (kDa)
Nup170•Nup145N•Nup53
Nup170
Nup53
Nup145N
Nup192
Nup192
Preincubation
Nup192
Nup170
Nup53
Nup145N

Fig. S12.
Binding sites for linker nucleoporins on Nup170 and Nup192 are distinct and compatible. (**A**-**C**) SEC-MALS profiles of nucleoporins or nucleoporin complexes are shown individually (blue, red, or yellow) and after their preincubation (green). SEC profiles were obtained using a Superdex 200 10/300 GL column for panels (A, B) and a Superose 6 10/300 GL column for panel (C). Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. We were unable to obtain an accurate molecular mass measurement for the preincubation of Nup53 with Nup170•Nup145N, as indicated by an asterisk above the chromatogram.

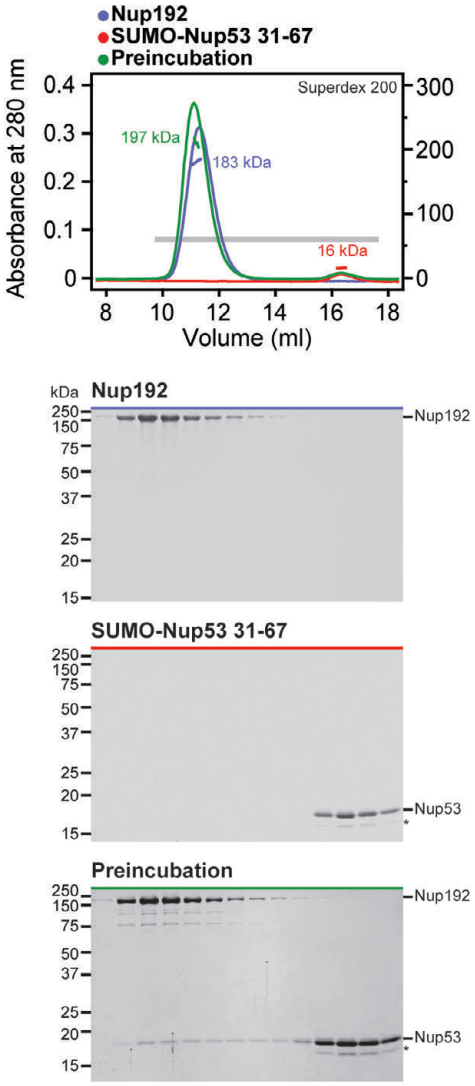

Nup192
SUMO-Nup53 31-67
Preincubation
Absorbance at 280 nm
Superdex 200
197 kDa
183 kDa
16 kDa
Volume (ml)
kDa
Nup192
SUMO-Nup53 31-67
Preincubation
Nup53

Fig. S13.
Nup192 recognizes Nup53$^{31-67}$. SEC-MALS and SDS-PAGE analysis corresponding to Fig. 2F. SEC-MALS profiles of nucleoporins are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining.

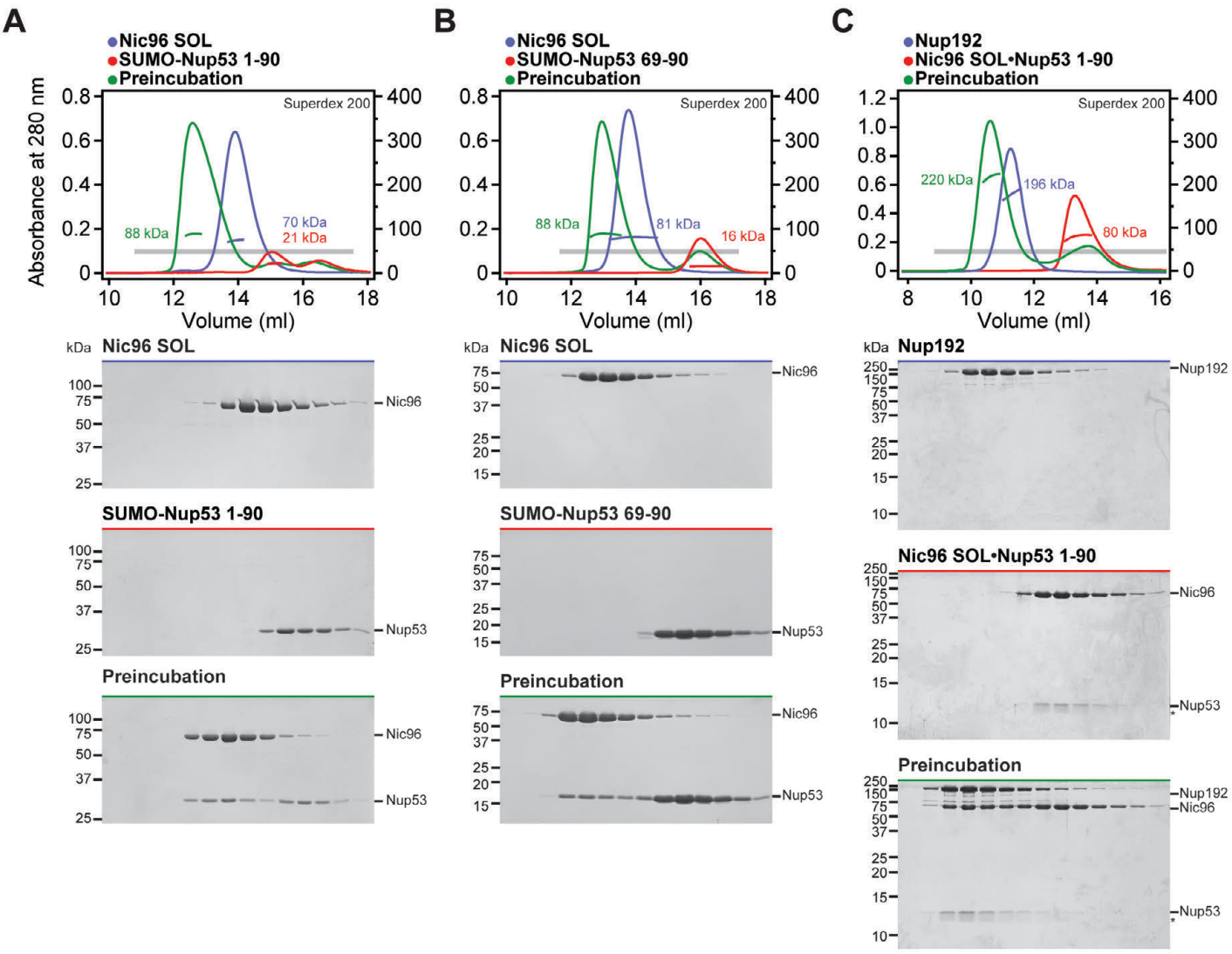

A
Nic96 SOL
SUMO-Nup53 1-90
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
88 kDa
70 kDa
21 kDa
kDa
Nic96 SOL
Nic96
SUMO-Nup53 1-90
Nup53
Preincubation
B
Nic96 SOL
SUMO-Nup53 69-90
Preincubation
Superdex 200
Volume (ml)
88 kDa
81 kDa
16 kDa
Nic96 SOL
Nic96
SUMO-Nup53 69-90
Nup53
Preincubation
C
Nup192
Nic96 SOL•Nup53 1-90
Preincubation
Superdex 200
Volume (ml)
220 kDa
196 kDa
80 kDa
Nup192
Nic96 SOL•Nup53 1-90
Nic96
Nup53
Preincubation
Nup192
Nic96
Nup53

Fig. S14.
Nic96^{SOL} recognizes Nup53$^{69-90}$ and Nup53$^{1-90}$ binds simultaneously to Nup192 and Nic96^{SOL}. (**A-C**) SEC-MALS and SDS-PAGE analysis corresponding to Fig. 2F. SEC-MALS profiles of nucleoporins or nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. The Nup53 binding sites for Nic96^{SOL} and Nup192 map to directly adjacent sequence fragments and are not mutually exclusive.

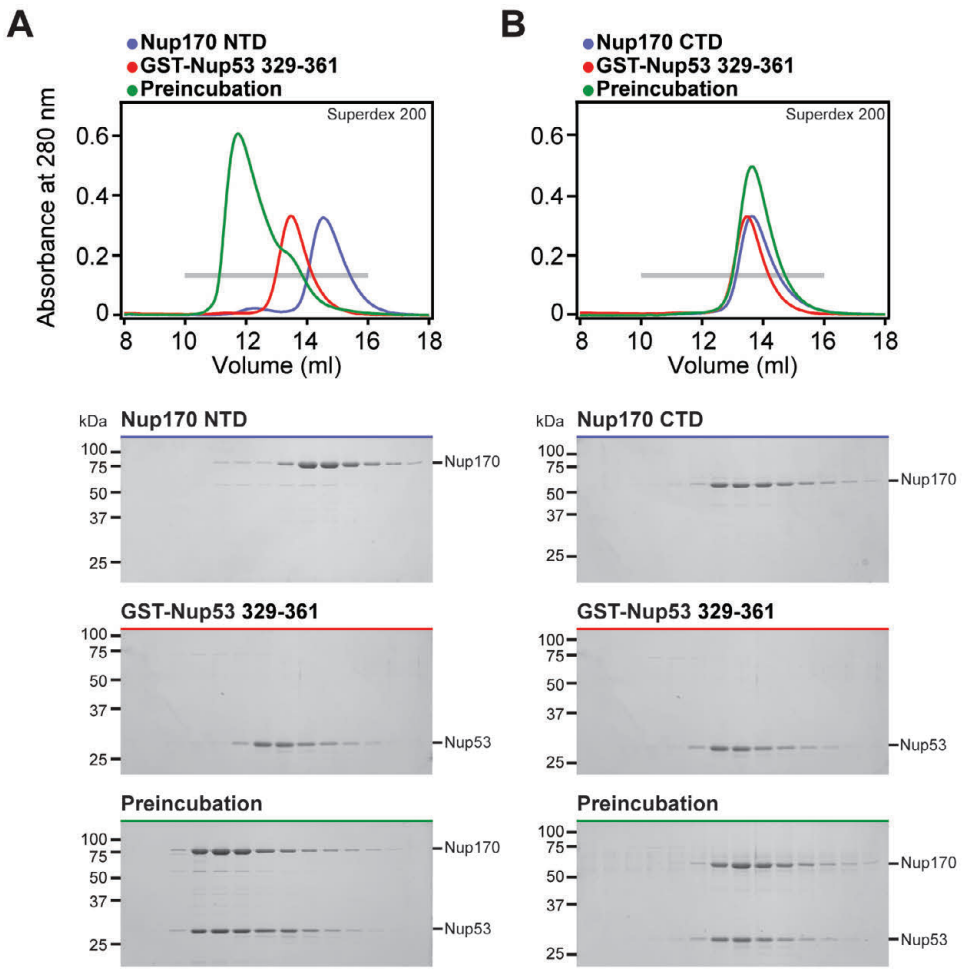

A
Nup170 NTD
GST-Nup53 329-361
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
B
Nup170 CTD
GST-Nup53 329-361
Preincubation
Superdex 200
Volume (ml)
kDa
Nup170 NTD
Nup170
GST-Nup53 329-361
Nup53
Preincubation
Nup170
Nup53
Nup170 CTD
Nup170
GST-Nup53 329-361
Nup53
Preincubation
Nup170
Nup53

Fig. S15.
Nup170^{NTD} recognizes Nup53$^{329-361}$. (**A**, **B**) SEC and SDS-PAGE analysis corresponding to Fig. 2F. SEC profiles of nucleoporins are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining.

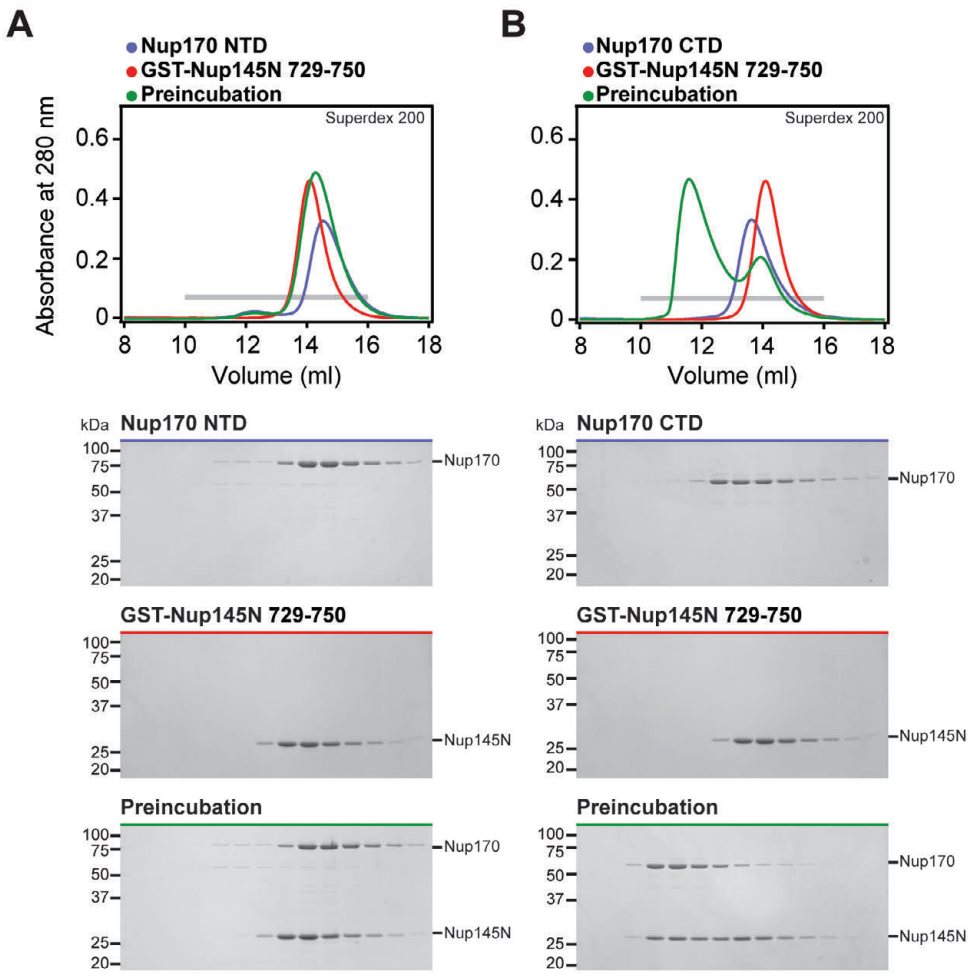
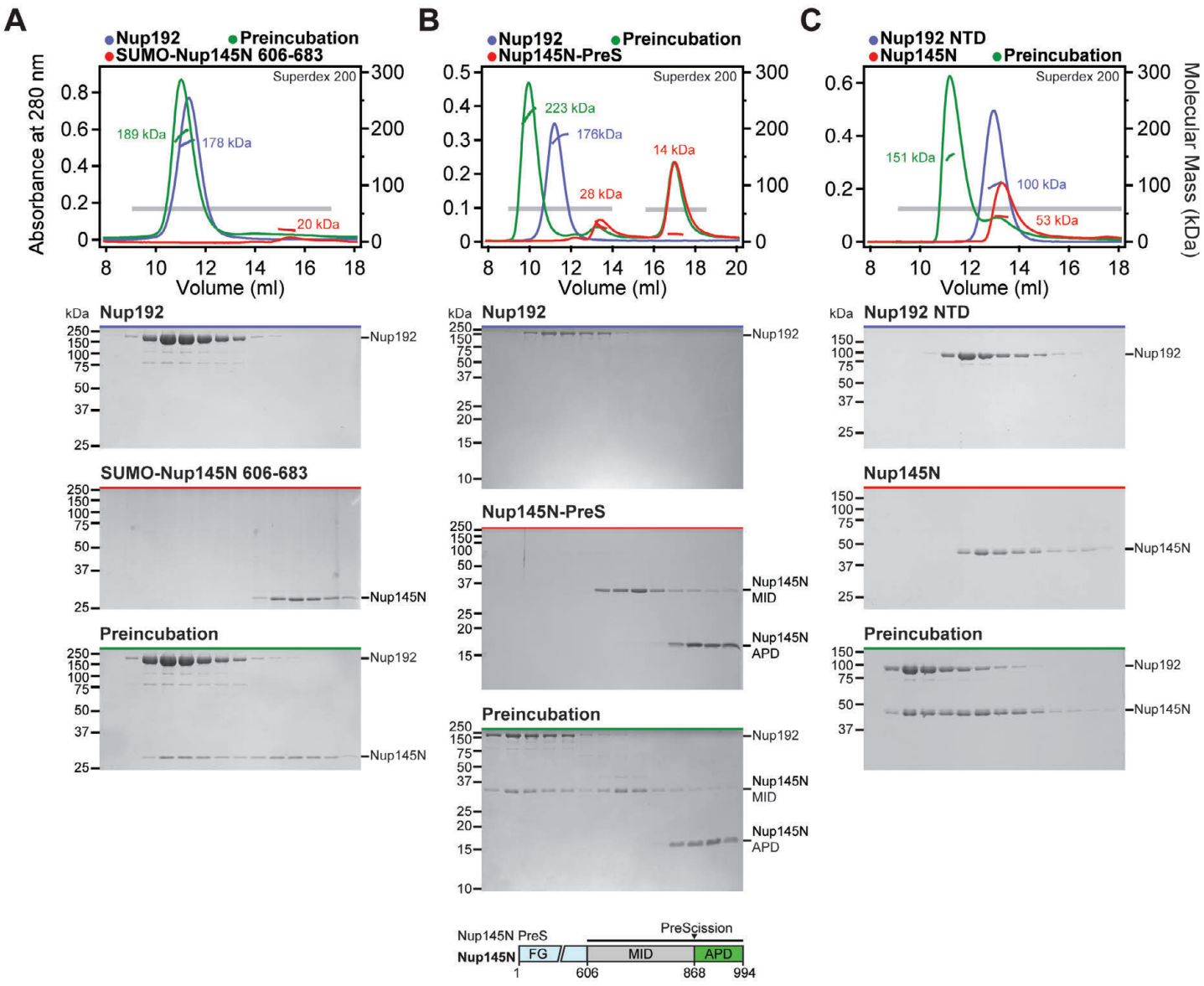

A
Nup170 NTD
GST-Nup145N 729-750
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
B
Nup170 CTD
GST-Nup145N 729-750
Preincubation
Superdex 200
Volume (ml)
kDa
Nup170 NTD
Nup170
GST-Nup145N 729-750
Nup145N
Preincubation
Nup170
Nup145N
kDa
Nup170 CTD
Nup170
GST-Nup145N 729-750
Nup145N
Preincubation
Nup170
Nup145N

Fig. S16.
Nup170^{CTD} recognizes Nup145N$^{729-750}$. (**A**, **B**) SEC and SDS-PAGE analysis corresponding to Fig. 2F. SEC profiles of nucleoporins are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining.


A
Nup192
Preincubation
SUMO-Nup145N 606-683
Superdex 200
Absorbance at 280 nm
Molecular Mass (kDa)
189 kDa
178 kDa
20 kDa
Volume (ml)
kDa
Nup192
SUMO-Nup145N 606-683
Nup145N
Preincubation
B
Nup192
Preincubation
Nup145N-PreS
223 kDa
176kDa
14 kDa
28 kDa
Nup145N MID
Nup145N APD
Nup145N PreS
PreScission
FG
MID
APD
1
606
868
994
C
Nup192 NTD
Nup145N
151 kDa
100 kDa
53 kDa

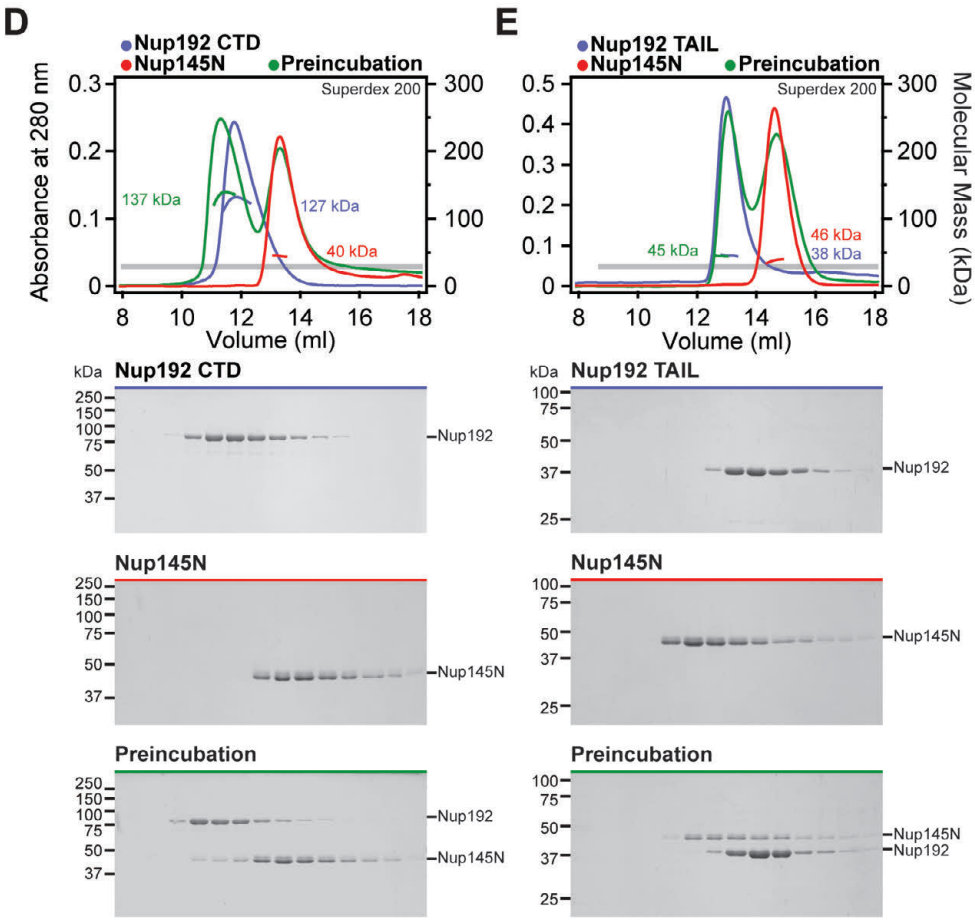
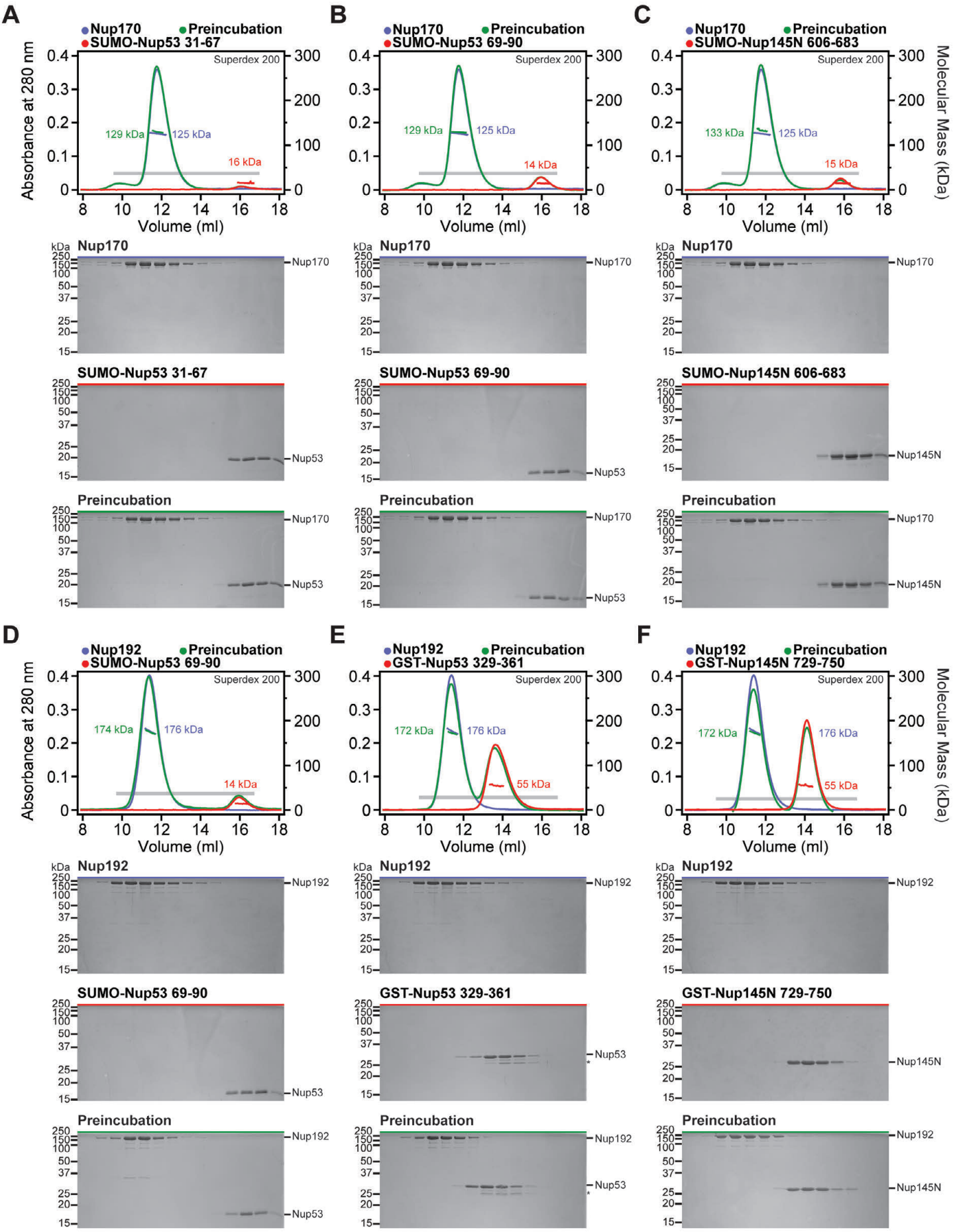

D
Nup192 CTD
Nup145N
Preincubation
Superdex 200
Absorbance at 280 nm
137 kDa
127 kDa
40 kDa
Volume (ml)
kDa
Nup192 CTD
Nup192
Nup145N
Nup145N
Preincubation
Nup192
Nup145N
E
Nup192 TAIL
Nup145N
Preincubation
Superdex 200
Molecular Mass (kDa)
45 kDa
46 kDa
38 kDa
Volume (ml)
kDa
Nup192 TAIL
Nup192
Nup145N
Nup145N
Preincubation
Nup145N
Nup192

Fig. S17.
Nup192 recognizes Nup145N$^{606-683}$ primarily with its NTD. (**A**-**E**) SEC-MALS and SDS-PAGE analysis corresponding to Fig. 2F. SEC-MALS profiles of nucleoporins are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Nup192 binds to a minimal sequence on Nup145N between residues 606-683. After cleavage of Nup145N^{PreS} with PreScission protease, Nup145N^{MID} co-elutes with Nup192, whereas Nup145N^{APD} does not interact with Nup192. As a reference, the domain structure of Nup145N^{PreS} is shown, indicating the construct boundaries and PreScission cleavage site with a black bar and a black triangle, respectively. Nup145N forms a stoichiometric complex with Nup192^{NTD}, but Nup145N binding to Nup192^{CTD} and Nup192^{TAIL} were barely detectable and undetectable, respectively.


A
Nup170
Preincubation
SUMO-Nup53 31-67
Superdex 200
Absorbance at 280 nm
Molecular Mass (kDa)
Volume (ml)
129 kDa
125 kDa
16 kDa
kDa
Nup170
SUMO-Nup53 31-67
Nup53
Preincubation
B
Nup170
Preincubation
SUMO-Nup53 69-90
Superdex 200
129 kDa
125 kDa
14 kDa
Nup170
SUMO-Nup53 69-90
Nup53
Preincubation
C
Nup170
Preincubation
SUMO-Nup145N 606-683
Superdex 200
133 kDa
125 kDa
15 kDa
Nup170
SUMO-Nup145N 606-683
Nup145N
Preincubation
D
Nup192
Preincubation
SUMO-Nup53 69-90
Superdex 200
174 kDa
176 kDa
14 kDa
Nup192
SUMO-Nup53 69-90
Nup53
Preincubation
E
Nup192
Preincubation
GST-Nup53 329-361
Superdex 200
172 kDa
176 kDa
55 kDa
Nup192
GST-Nup53 329-361
Nup53
Preincubation
F
Nup192
Preincubation
GST-Nup145N 729-750
Superdex 200
172 kDa
176 kDa
55 kDa
Nup192
GST-Nup145N 729-750
Nup145N
Preincubation

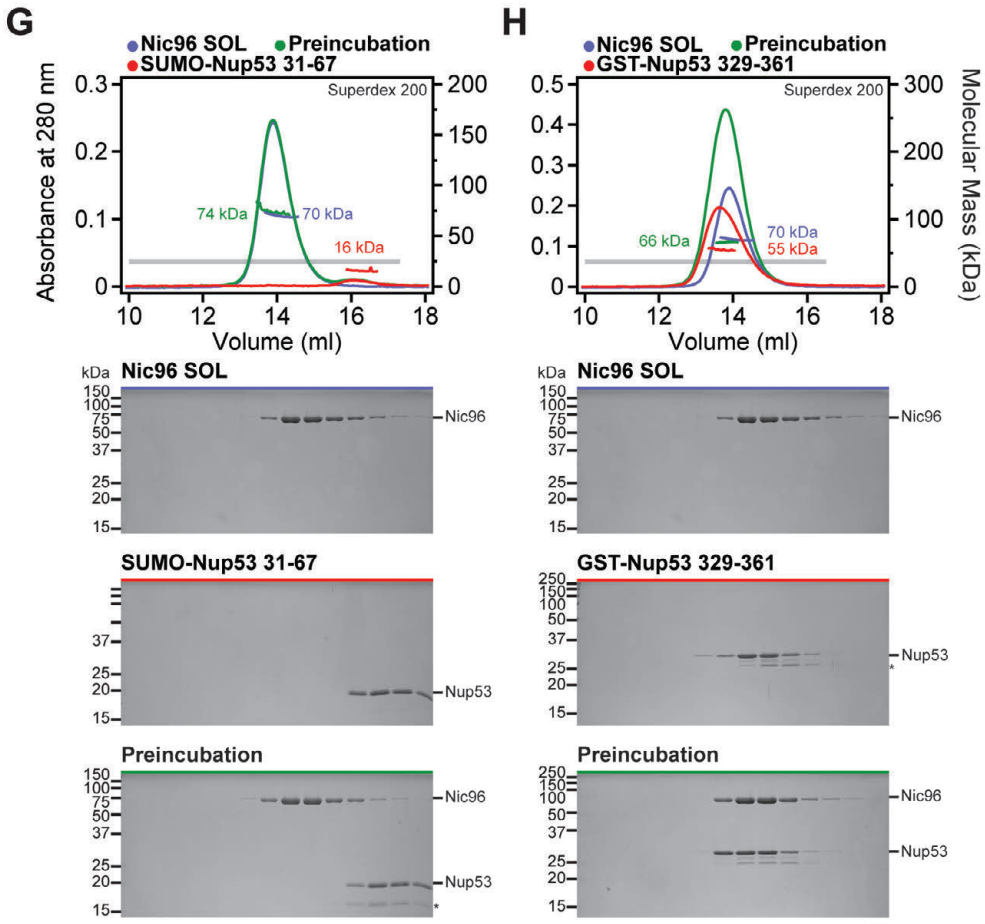
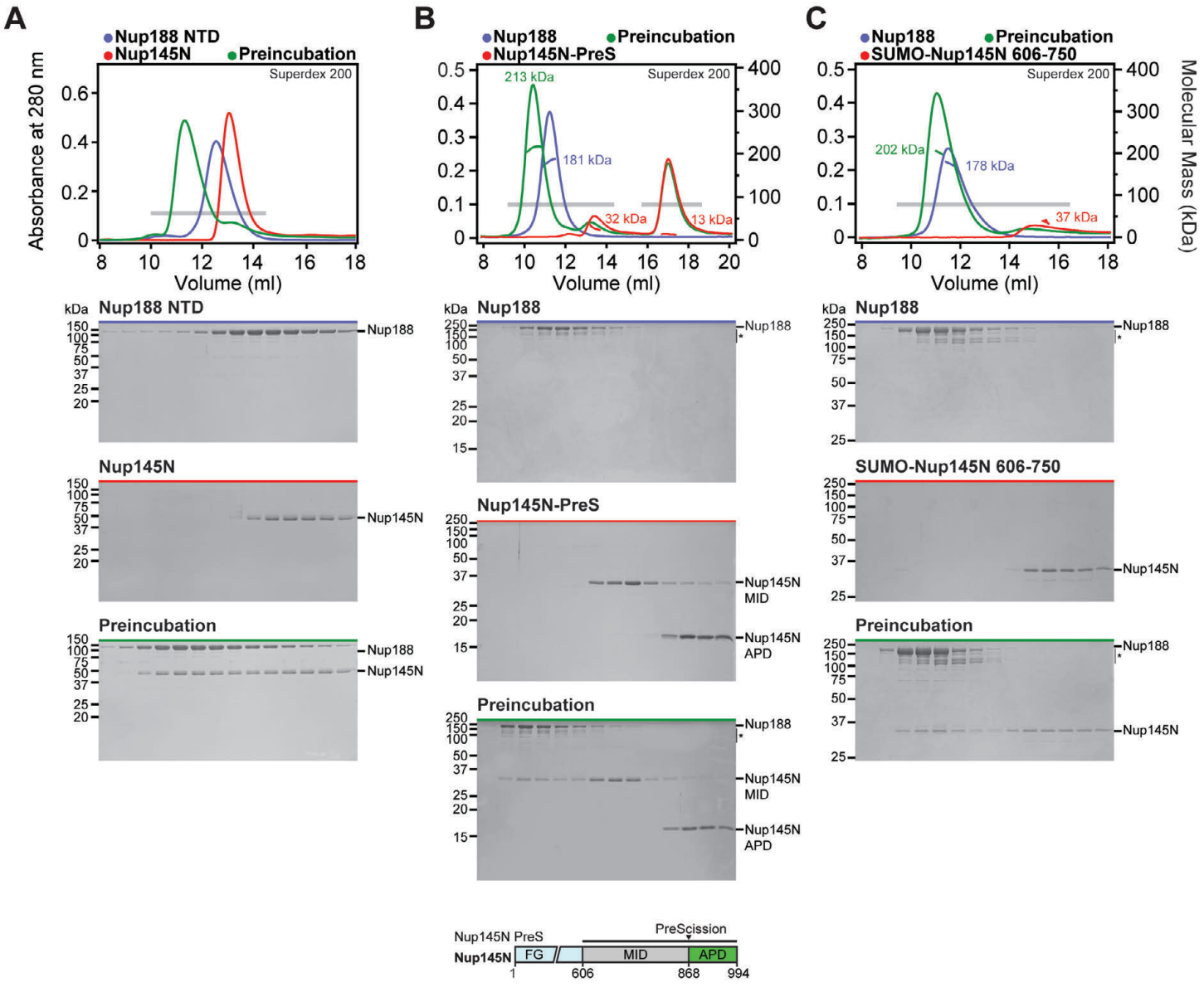

G
Nic96 SOL
Preincubation
SUMO-Nup53 31-67
Superdex 200
Absorbance at 280 nm
74 kDa
70 kDa
16 kDa
Volume (ml)
kDa
Nic96 SOL
Nic96
SUMO-Nup53 31-67
Nup53
Preincubation
Nic96
Nup53
H
Nic96 SOL
Preincubation
GST-Nup53 329-361
Superdex 200
Molecular Mass (kDa)
66 kDa
70 kDa
55 kDa
Volume (ml)
kDa
Nic96 SOL
Nic96
GST-Nup53 329-361
Nup53
Preincubation
Nic96
Nup53

Fig. S18.
Mapped minimal linker nucleoporin fragments are specific for the identified binding partners. (**A**-**H**) SEC-MALS profiles of nucleoporins are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. The minimal sequence fragments of the linker nucleoporins Nup53 and Nup145N did not interact with other scaffold nucleoporins, demonstrating their specificity for a single scaffold nucleoporin.


A
Nup188 NTD
Nup145N
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
kDa
Nup188 NTD
Nup188
Nup145N
Preincubation
B
Nup188
Preincubation
Nup145N-PreS
213 kDa
181 kDa
32 kDa
13 kDa
Nup145N MID
Nup145N APD
C
Nup188
Preincubation
SUMO-Nup145N 606-750
202 kDa
178 kDa
37 kDa
Molecular Mass (kDa)
Nup145N PreS
PreScission
FG
MID
APD
1
606
868
994

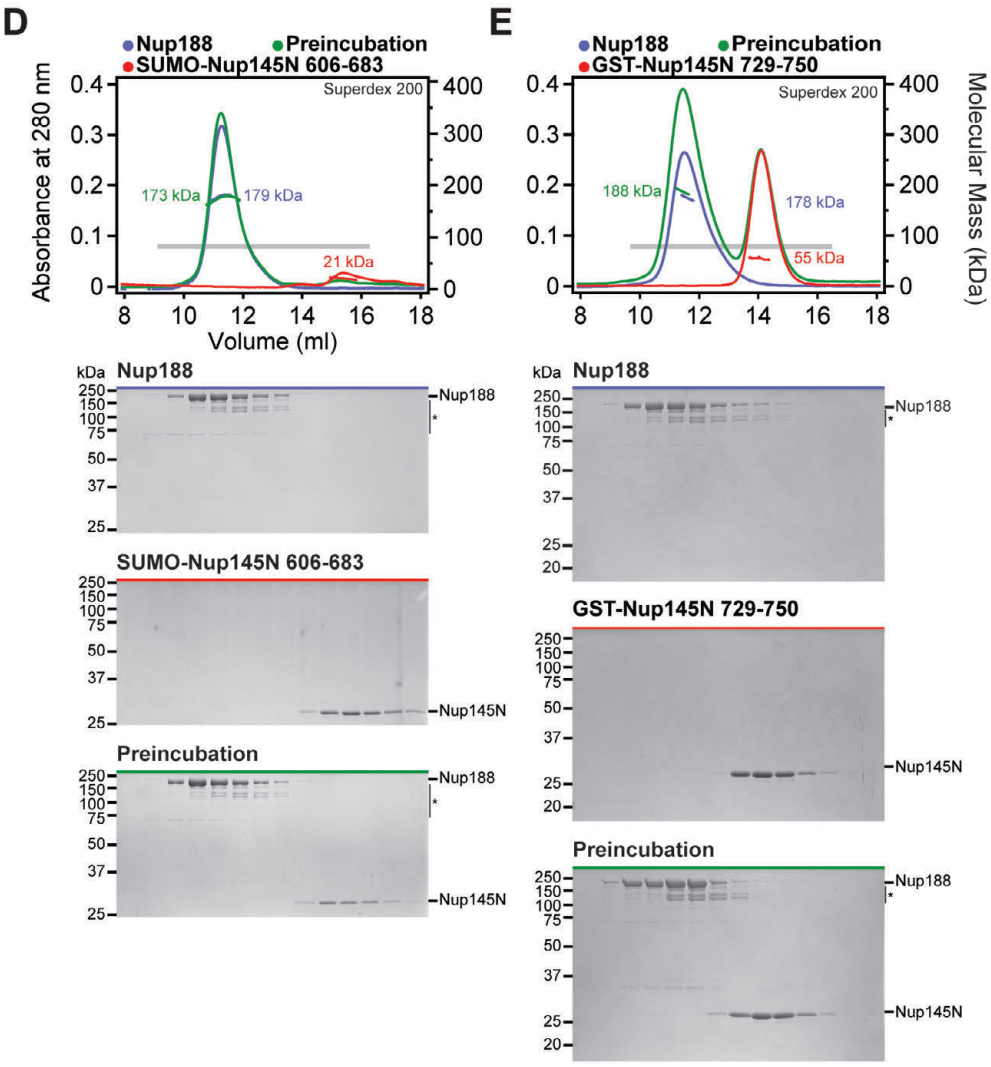

D
Nup188
Preincubation
SUMO-Nup145N 606-683
Superdex 200
Absorbance at 280 nm
Volume (ml)
173 kDa
179 kDa
21 kDa
kDa
Nup188
SUMO-Nup145N 606-683
Preincubation
Nup145N
E
Nup188
Preincubation
GST-Nup145N 729-750
Superdex 200
Molecular Mass (kDa)
188 kDa
178 kDa
55 kDa
Nup188
GST-Nup145N 729-750
Preincubation
Nup145N

Fig. S19.
Nup188 recognizes Nup145N$^{606-750}$ primarily via its NTD. (**A**-**E**) SEC-MALS and SDS-PAGE analysis corresponding to Fig. 2F. SEC-MALS profiles of nucleoporins are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Nup188^{NTD} is responsible for binding Nup145N. After Nup145N^{PreS} cleavage with PreScission protease, Nup145N^{MID} co-elutes with Nup188, whereas Nup145N^{APD} does not interact with Nup188. As reference, the domain structure of Nup145N^{PreS} is shown, indicating the construct boundaries and PreScission cleavage site with a black bar and a black triangle, respectively. Nup188 binds to a minimal sequence on Nup145N between residues 606-750, but does not interact with the minimal Nup145N binding sequences for Nup192 or Nup170.

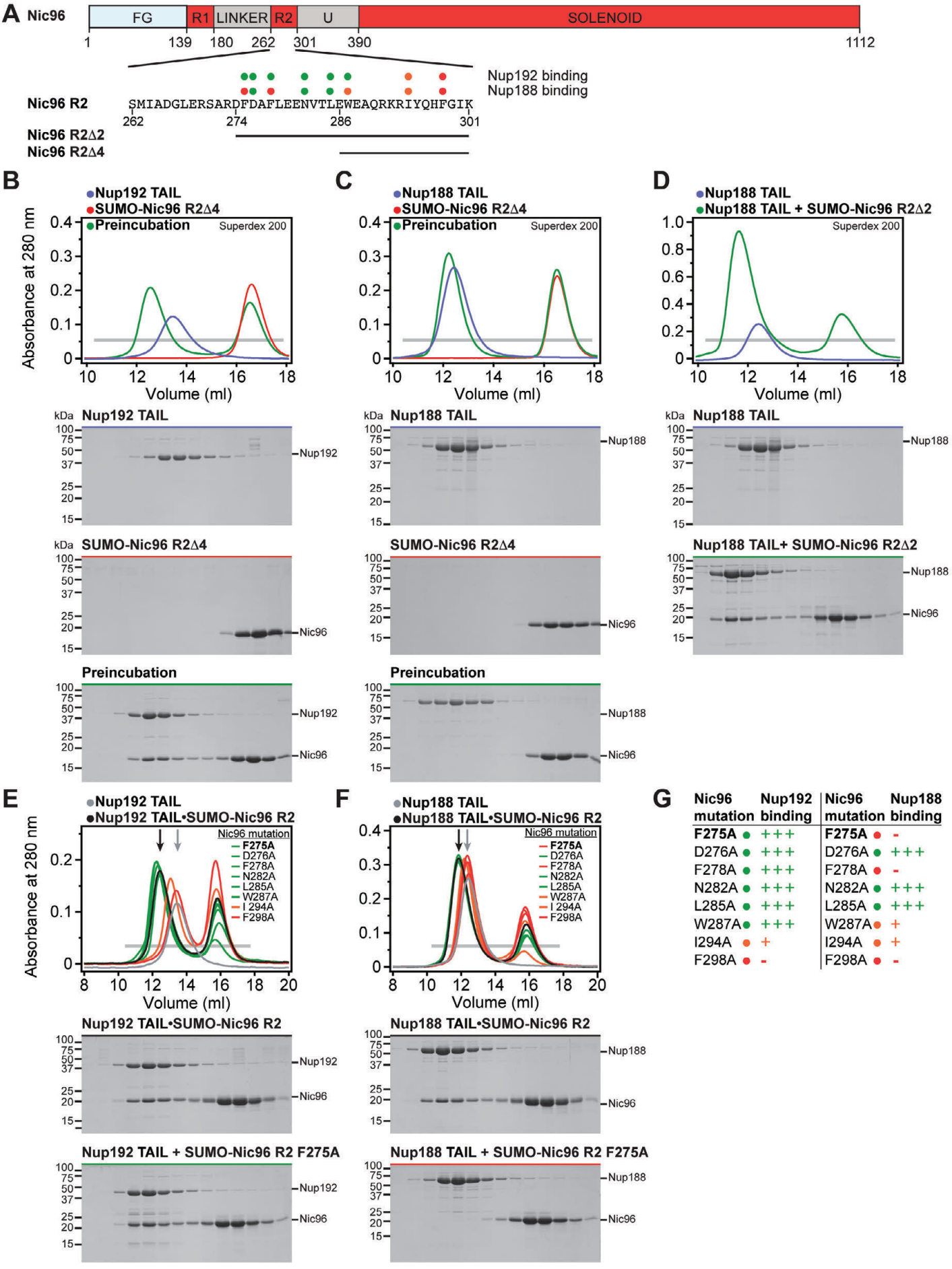
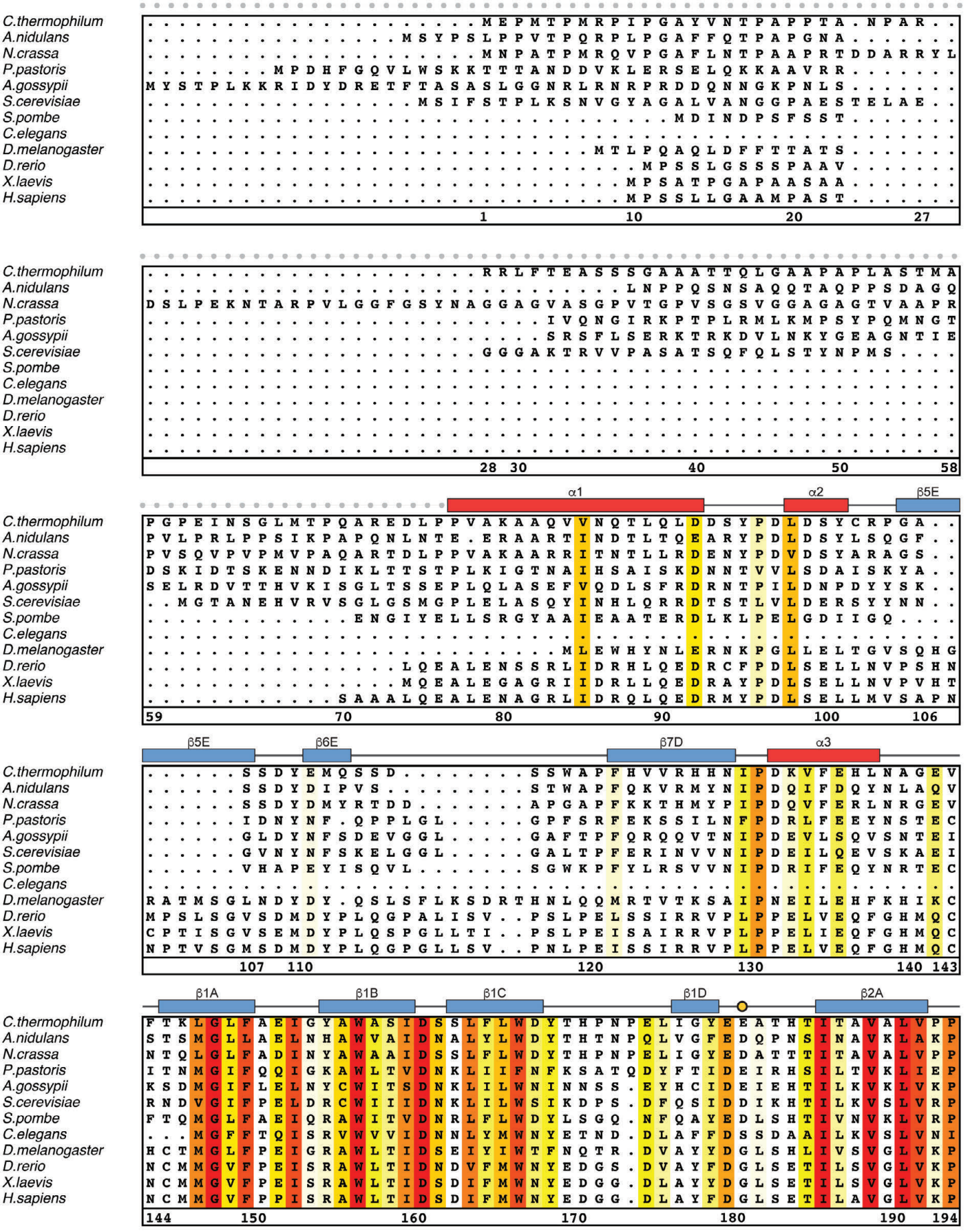
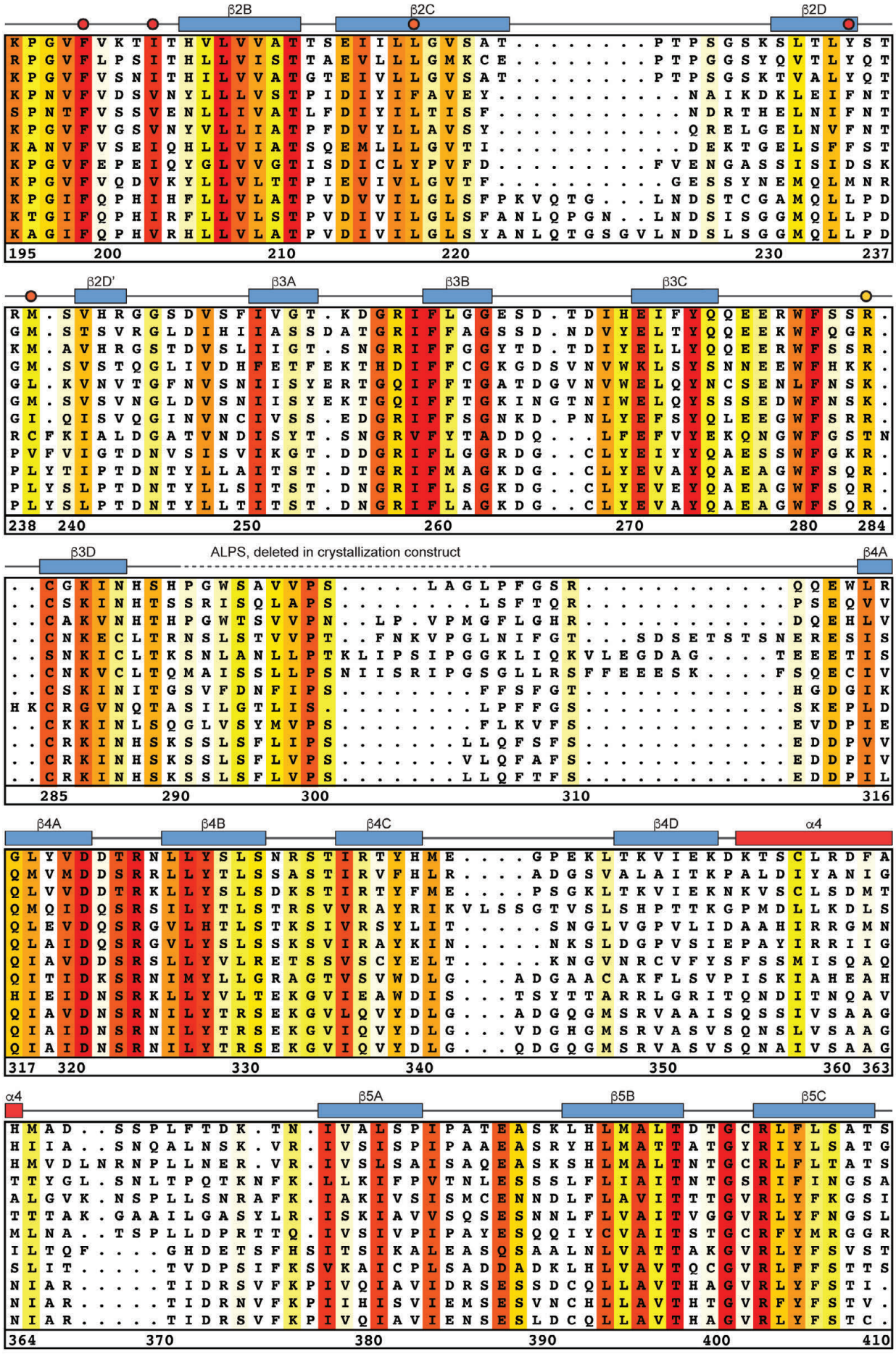

A
Nic96
FG
R1
LINKER
R2
U
SOLENOID
1
139
180
262
301
390
1112
Nup192 binding
Nup188 binding
Nic96 R2
SMIADGLERSARDFDAFLEENVTLEWEAQRKRIYQHFGIK
262
274
286
301
Nic96 R2Δ2
Nic96 R2Δ4
B
Nup192 TAIL
SUMO-Nic96 R2Δ4
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
kDa
Nup192 TAIL
Nup192
SUMO-Nic96 R2Δ4
Nic96
Preincubation
Nup192
Nic96
C
Nup188 TAIL
SUMO-Nic96 R2Δ4
Preincubation
Superdex 200
Volume (ml)
Nup188 TAIL
Nup188
SUMO-Nic96 R2Δ4
Nic96
Preincubation
Nup188
Nic96
D
Nup188 TAIL
Nup188 TAIL + SUMO-Nic96 R2Δ2
Superdex 200
Volume (ml)
Nup188 TAIL
Nup188
Nup188 TAIL+ SUMO-Nic96 R2Δ2
Nup188
Nic96
E
Nup192 TAIL
Nup192 TAIL•SUMO-Nic96 R2
Nic96 mutation
F275A
D276A
F278A
N282A
L285A
W287A
I 294A
F298A
Absorbance at 280 nm
Volume (ml)
Nup192 TAIL•SUMO-Nic96 R2
Nup192
Nic96
Nup192 TAIL + SUMO-Nic96 R2 F275A
Nup192
Nic96
F
Nup188 TAIL
Nup188 TAIL•SUMO-Nic96 R2
Nic96 mutation
F275A
D276A
F278A
N282A
L285A
W287A
I 294A
F298A
Volume (ml)
Nup188 TAIL•SUMO-Nic96 R2
Nup188
Nic96
Nup188 TAIL + SUMO-Nic96 R2 F275A
Nup188
Nic96
G
Nic96 mutation
Nup192 binding
F275A ● +++
D276A ● +++
F278A ● +++
N282A ● +++
L285A ● +++
W287A ● +++
I294A ● +
F298A ● -
Nic96 mutation
Nup188 binding
F275A ● -
D276A ● +++
F278A ● -
N282A ● +++
L285A ● +++
W287A ● +
I294A ● +
F298A ● -

Fig. S20.
Nup192^{TAIL} and Nup188^{TAIL} bind to partially overlapping sites in Nic96^{R2}. (**A**) Domain boundaries of Nic96 with the protein sequence corresponding to the R2 region shown below. Black lines indicate construct boundaries for Nic96^{R2}, Nic96$^{R2\Delta2}$, and Nic96$^{R2\Delta4}$. The effects of Nic96^{R2} alanine mutations on Nup192^{TAIL} and Nup188^{TAIL} binding are indicated by colored dots; no effect (green), reduced binding (orange), and complete disruption (red). (**B**-**D**) SEC and SDS-PAGE analysis corresponding to Fig. 2F. SEC profiles of nucleoporins are shown individually (blue and red) and after their preincubation (green). Nic96$^{R2\Delta2}$ is insoluble when expressed alone and therefore was expressed and purified in the presence of Nup188^{TAIL}. (**E**, **F**) Mutational analysis of the Nic96^{R2} interaction with Nup192^{TAIL} and Nup188^{TAIL}. SEC profiles of Nup192^{TAIL} or Nup188^{TAIL} are shown individually (gray) and after preincubation with wild type Nic96^{R2} (black) and are annotated with arrows of the same color. SEC profiles of mutant proteins preincubated with Nic96^{R2} are colored according to measured effect as in panel (A). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. (**G**) Table summarizing the measured effects of Nic96^{R2} mutation on Nup192^{TAIL} and Nup188^{TAIL} binding colored according to panel (A): no effect (+++), moderate effect (+), abolished binding (-). The Nic96^{R2} F275A mutant abolished binding to Nup188^{TAIL} but not to Nup192^{TAIL}.

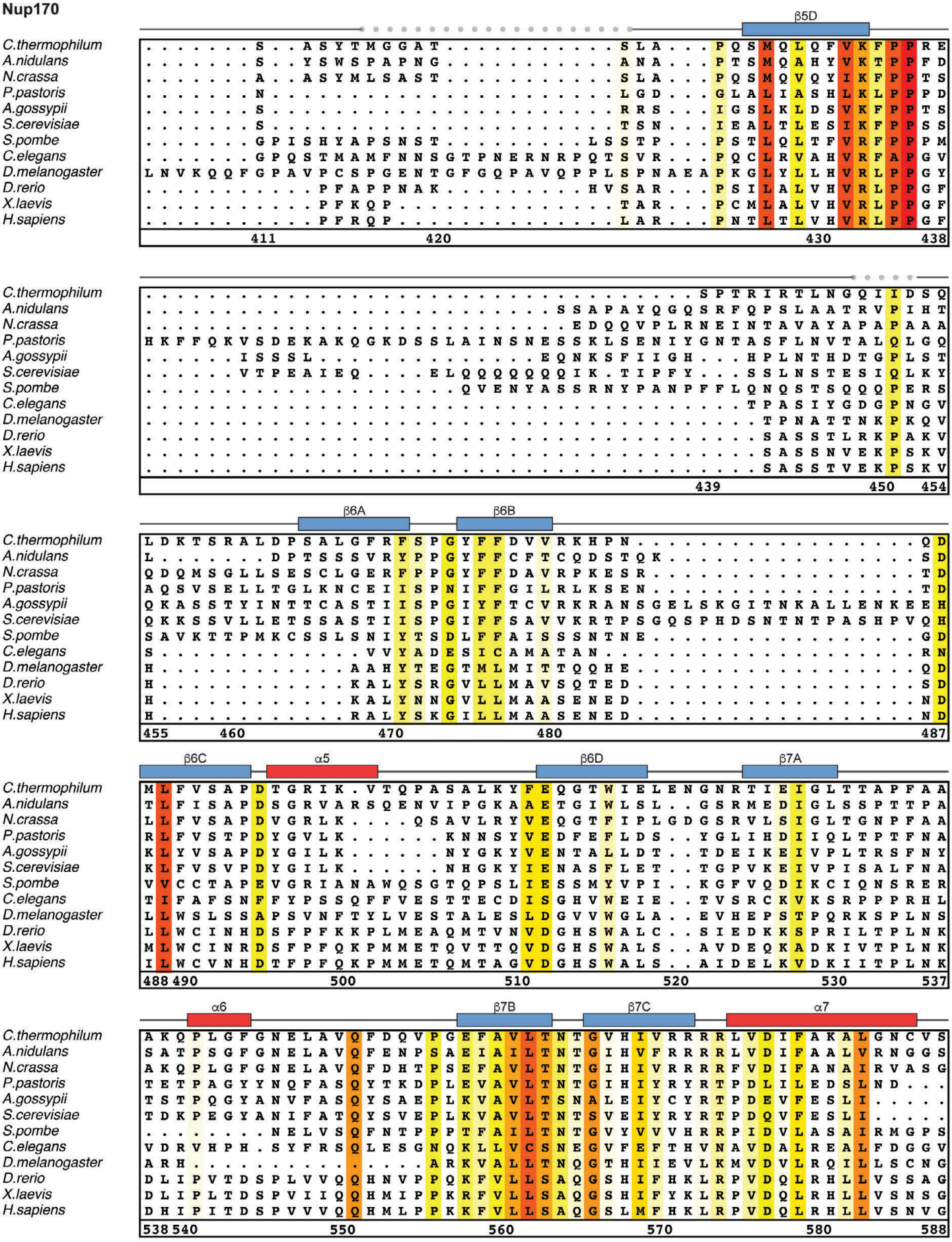
Nup170


C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
α1
α2
β5E
β5E
β6E
β7D
α3
β1A
β1B
β1C
β1D
β2A

Nup170


β2B
β2C
β2D
C.thermophilum K P G V F V K T I T H V L V V A T T S E I I L L G V S A T . . . . . P T P S G S K S L T L Y S T
A.nidulans R P G V F L P S I T H L L V I S T T A E V L L L G M K C E . . . . . P T P G G S Y Q V T L Y Q T
N.crassa K P G V F V S N I T H I L V V A T G T E I V L L G V S A T . . . . . P T P S G S K T V A L Y Q T
P.pastoris K P N V F V D S V N Y L L L V S T P I D I Y I F A V E Y . . . . . N A I K D K L E I F N T
A.gossypii S P N T F V S S V E N L L I V A T L F D I Y I L T I S F . . . . . N D R T H E L N I F N T
S.cerevisiae K P G V F V G S V N Y V L L I A T P F D V Y L L A V S Y . . . . . Q R E L G E L N V F N T
S.pombe K A N V F V S E I Q H L L V I A T S Q E M L L L G V T I . . . . . D E K T G E L S F F S T
C.elegans K P G V F E P E I Q Y G L V V G T I S D I C L Y P V F D . . . . . F V E N G A S S I S I D S K
D.melanogaster K P G V F V Q D V K Y L L V L T T P I E V I V L G V T F . . . . . G E S S Y N E M Q L M N R
D.rerio K P G I F Q P H I H F L L V L A T P V D V V I L G L S F P K V Q T G . . . L N D S T C G A M Q L L P D
X.laevis K T G I F Q P H I R F L L V L S T P V D I V I L G L S F A N L Q P G N . . L N D S I S G G M Q L L P D
H.sapiens K A G I F Q P H V R H L L V L A T P V D I V I L G L S Y A N L Q T G S G V L N D S L S G G M Q L L P D
195 200 210 220 230 237
β2D'
β3A
β3B
β3C
C.thermophilum R M . S V H R G G S D V S F I V G T . K D G R I F L G G E S D . T D I H E I F Y Q Q E E R W F S S R .
A.nidulans G M . S T S V R G L D I H I I A S S D A T G R I F F A G S S D . N D V Y E L T Y Q Q E E K W F Q G R .
N.crassa K M . A V H R G S T D V S L I I G T . S N G R I F F G G Y T D . T D I Y E L L Y Q Q E E R W F S S R .
P.pastoris G M . S V S T Q G L I V D H F E T F E K T H D I F F C G K G D S V N V W K L S Y S N N E E W F H K K .
A.gossypii G L . K V N V T G F N V S N I I S Y E R T G Q I F F T G A T D G V N V W E L Q Y N C S E N L F N S K .
S.cerevisiae G M . S V S V N G L D V S N I I S Y E K T G Q I F F T G K I N G T N I W E L Q Y S S S E D W F N S K .
S.pombe G I . Q I S V Q G I N V N C I V S S . E D G R I F F S G N K D . P N L Y E F S Y Q L E E G W F S R R .
C.elegans R C F K I A L D G A T V N D I S Y T . S N G R V F Y T A D D Q . . . L F E F V Y E K Q N G W F G S T N
D.melanogaster P V F V I G T D N V S I S V I K G T . D D G R I F L G G R D G . . C L Y E I Y Y Q A E S S W F G K R .
D.rerio P L Y T I P T D N T Y L L A I T S T . D T G R I F M A G K D G . . C L Y E V A Y Q A E A G W F S Q R .
X.laevis P L Y S L P T D N T Y L L S I T S T . D N G R I F L S G K D G . . C L Y E V E Y Q A E A G W F S Q R .
H.sapiens P L Y S L P T D N T Y L L T I T S T . D N G R I F L A G K D G . . C L Y E V A Y Q A E A G W F S Q R .
238 240 250 260 270 280 284
β3D
ALPS, deleted in crystallization construct
β4A
C.thermophilum . . C G K I N H S H P G W S A V V P S . . . . . L A G L P F G S R . . . . . Q Q E W L R
A.nidulans . . C S K I N H T S S R I S Q L A P S . . . . . L S F T Q R . . . . . P S E Q V V
N.crassa . . C A K V N H T H P G W T S V V P N . . L P . V P M G F L G H R . . . . . D Q E H L V
P.pastoris . . C N K E C L T R N S L S T V V P T . . F N K V P G L N I F G T . . . S D S E T S T S N E R E S I S
A.gossypii . . S N K I C L T K S N L A N L L P T K L I P S I P G G K L I Q K V L E G D A G . . . . T E E E T I S
S.cerevisiae . . C N K V C L T Q M A I S S L L P S N I I S R I P G S G L L R S F F E E E S K . . . . F S Q E C I V
S.pombe . . C S K I N I T G S V F D N F I P S . . . . . F F S F G T . . . . . H G D G I K
C.elegans H K C R G V N Q T A S I L G T L I S . . . . . L P F F G S . . . . . S K E P L D
D.melanogaster . . C K K I N L S Q G L V S Y M V P S . . . . . F L K V F S . . . . . E V D P I E
D.rerio . . C R K I N H S K S S L S F L I P S . . . . . L L Q F S F S . . . . . E D D P V V
X.laevis . . C R K I N H S K S S L S F L V P S . . . . . V L Q F A F S . . . . . E D D P I V
H.sapiens . . C R K I N H S K S S L S F L V P S . . . . . L L Q F T F S . . . . . E D D P I L
285 290 300 310 316
β4A
β4B
β4C
β4D
α4
C.thermophilum G L Y V D D T R N L L Y S L S N R S T I R T Y H M E . . . . G P E K L T K V I E K D K T S C L R D F A
A.nidulans Q M V M D D S R R L L Y T L S S A S T I R V F H L R . . . . A D G S V A L A I T K P A L D I Y A N I G
N.crassa Q L V V D D T R K L L Y S L S D K S T I R T Y F M E . . . . P S G K L T K V I E K N K V S C L S D M T
P.pastoris Q M Q I D Q S R S I L Y T L S T R S V V R A Y R I K V L S S G T V S L S H P T T K G P M D L L K D L S
A.gossypii Q L E V D Q S R G V L H T L S T K S I V R S Y L I T . . . . . S N G L V G P V L I D A A H I R R G M N
S.cerevisiae Q L A I D Q S R G V L Y S L S S K S V I R A Y K I N . . . . . N K S L D G P V S I E P A Y I R R I I G
S.pombe Q I A V D D S R S L L Y V L R E T S S V S C Y E L T . . . . . K N G V N R C V F Y S F S S M I S Q A Q
C.elegans Q I T I D K S R N I M Y L L G R A G T V S V W D L G . . . A D G A A C A K F L S V P I S K I A H E A H
D.melanogaster H I E I D N S R K L L Y V L T E K G V I E A W D I S . . . T S Y T T A R R L G R I T Q N D I T N Q A V
D.rerio Q I A V D N S R N I L Y T R S E K G V L Q V Y D L G . . . A D G Q G M S R V A A I S Q S S I V S A A G
X.laevis Q I A I D N S R N I L Y T R S E K G V I Q V Y D L G . . . V D G H G M S R V A S V S Q N S L V S A A G
H.sapiens Q I A I D N S R N I L Y T R S E K G V I Q V Y D L G . . . Q D G Q G M S R V A S V S Q N A I V S A A G
317 320 330 340 350 360 363
α4
β5A
β5B
β5C
C.thermophilum H M A D . . S S P L F T D K . T N . I V A L S P I P A T E A S K L H L M A L T D T G C R L F L S A T S
A.nidulans H I I A . . S N Q A L N S K . V R . I V S I S P I P A A E A S R Y H L M A T T A T G Y R I Y L S A T G
N.crassa H M V D L N R N P L L N E R . V R . I V S L S A I S A Q E A S K S H L M A L T N T G C R L F L T A T S
P.pastoris T T Y G L . S N L T P Q T K N F K . L L K I F P V T N L E S S S L F L I A I T N T G S R I F I N G S A
A.gossypii A L G V K . N S P L L S N R A F K . I A K I V S I S M C E N N D L F L A V I T T T G V R L Y F K G S I
S.cerevisiae T T T A K . G A A I L G A S Y L R . I S K I A V V S Q S E S N N L F L V A I T V G G V R L Y F N G S L
S.pombe M L N A . . T S P L L L D P R T T Q . I V S I V P I P A Y E S Q Q I Y C V A I T S T G C R F Y M R G G R
C.elegans I L T Q F . . . . G H D E T S F H S I T S I K A L E A S Q S A A L N L V A T T A K G V R L Y F S V S T
D.melanogaster S L I T . . . . . T V D P S I F K S V K A I C P L S A D D A D K L H L V A V T Q C G V R L F F S T T S
D.rerio N I A R . . . . . T I D R S V F K P I V Q I A V I D R S E S S D C Q L L A V T H A G V R L Y F S T I .
X.laevis N I A R . . . . . T I D R N V F K P I I H I S V I E M S E S V N C H L L A V T H T G V R F Y F S T V .
H.sapiens N I A R . . . . . T I D R S V F K P I V Q I A V I E N S E S L D C Q L L A V T H A G V R L Y F S T C .
364 370 380 390 400 410

Nup170


β5D
C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
411
420
430
438
439
450
454
β6A
β6B
455
460
470
480
487
β6C
α5
β6D
β7A
488 490
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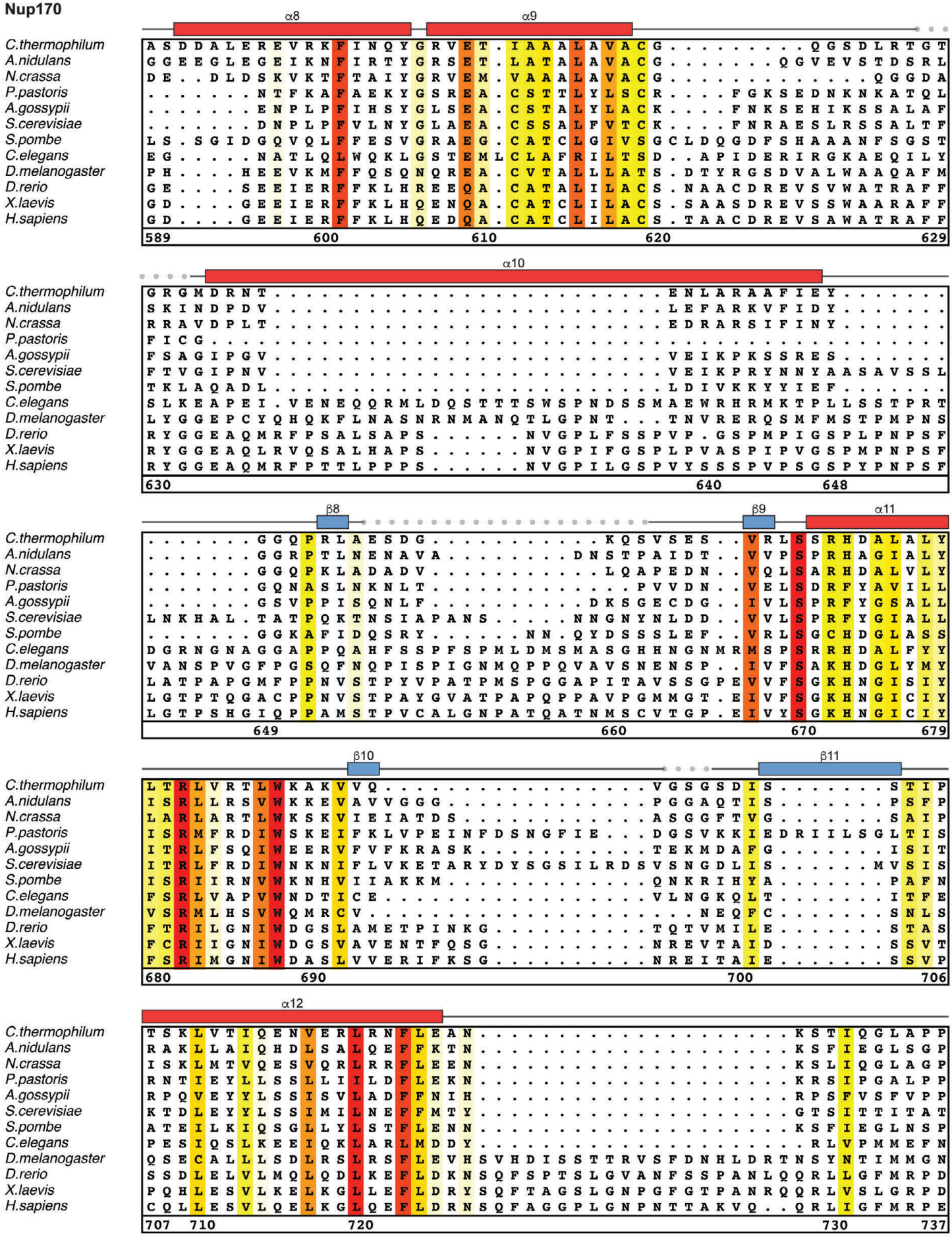
537
α6
β7B
β7C
α7
538 540
550
560
570
580
588

Nup170


α8
α9
C.thermophilum A S D D A L E R E V R K F I N Q Y G R V E T . I A A A L A V A C G . . . . . Q G S D L R T G T
A.nidulans G G E E G L E G E I K N F I R T Y G R S E T . L A T A L A V A C G . . . . . Q G V E V S T D S R L
N.crassa D E . . D L D S K V K T F T A I Y G R V E M . V A A A L A V A C G . . . . . Q G G D A
P.pastoris . . . . . N T F K A F A E K Y G S R E A . C S T T L Y L S C R . . . . F G K S E D N K N K A T Q L
A.gossypii . . . . . E N P L P F I H S Y G L S E A . C S T A L Y L A C K . . . . F N K S E H I K S S A L A F
S.cerevisiae . . . . . D N P L P F V L N Y G L A E A . C S S A L F V T C K . . . . F N R A E S L R S S A L T F
S.pombe L S . S G I D G Q V Q L F F E S V G R A E G . C A T C L G I V S G C L D Q G D F S H A A A N F S G S T
C.elegans E G . . . . . N A T L Q L W Q K L G S T E M L C L A F R I L T S D . . A P I D E R I R G K A E Q I L Y
D.melanogaster P H . . . . H E E V K M F F Q S Q N Q R E A . C V T A L L L A T S . D T Y R G S D V A L W A A Q A F M
D.rerio G E . . . . S E E I E R F F K L H R E E Q A . C A T A L I L A C S . N A A C D R E V S V W A T R A F F
X.laevis G D . . . . G E E I E R F F K L H Q E N Q A . C A T C L I L A C S . S A A S D R E V S S W A A R A F F
H.sapiens G D . . . . G E E I E R F F K L H Q E D Q A . C A T C L I L A C S . T A A C D R E V S A W A T R A F F
589
600
610
620
629
α10
C.thermophilum G R G M D R N T . . . . . E N L A R A A F I E Y . . . . . .
A.nidulans S K I N D P D V . . . . . L E F A R K V F I D Y . . . . . .
N.crassa R R A V D P L T . . . . . E D R A R S I F I N Y . . . . . .
P.pastoris F I C G . . . . . .
A.gossypii F S A G I P G V . . . . . V E I K P K S S R E S . . . . . .
S.cerevisiae F T V G I P N V . . . . . V E I K P R Y N N Y A A S A V S S L
S.pombe T K L A Q A D L . . . . . L D I V K K Y Y I E F . . . . . .
C.elegans S L K E A P E I . V E N E Q Q R M L D Q S T T T S W S P N D S S M A E W R H R M K T P L L S S T P R T
D.melanogaster L Y G G E P C Y Q H Q K F L N A S N R N M A N Q T L G P N T . . . T N V R E R Q S M F M S T P M P N S
D.rerio R Y G G E A Q M R F P S A L S A P S . . . . . N V G P L F S S P V P . G S P M P I G S P L P N P S F
X.laevis R Y G G E A Q L R V Q S A L H A P S . . . . . N V G P I F G S P L P V A S P I P V G S P M P N P S F
H.sapiens R Y G G E A Q M R F P T T L P P P S . . . . . N V G P I L G S P V Y S S S P V P S G S P Y P N P S F
630
640
648
β8
β9
α11
C.thermophilum . . . . . G G Q P R L A E S D G . . . . . K Q S V S E S . . V R L S S R H D A L A L Y
A.nidulans . . . . . G G R P T L N E N A V A . . . . . D N S T P A I D T . . V V P S P R H A G I A L Y
N.crassa . . . . . G G Q P K L A D A D V . . . . . L Q A P E D N . . V Q L S A R H D A L V L Y
P.pastoris . . . . . G Q N A S L N K N L T . . . . . P V V D N . . V E L S D R F Y A V I L L
A.gossypii . . . . . G S V P P I S Q N L F . . . . . D K S G E C D G . . I V L S P R F Y G S A L L
S.cerevisiae L N K H A L . T A T P Q K T N S I A P A N S . . . . . N N G N Y N L D D . . V V L S P R F Y G I A L L
S.pombe . . . . . G G K A F I D Q S R Y . . . . . N N . Q Y D S S S L E F . . V R L S G C H D G L A S S
C.elegans D G R N G N A G G A P P Q A H F S S P F S P M L D M S M A S G H H N G N M R M S P S R R H D A L F Y Y
D.melanogaster V A N S P V G F P G S Q F N Q P I S P I G N M Q P P Q V A V S N E N S P . . I V F S A K H D G L Y M Y
D.rerio L A T P A P G M F P P N V S T P Y V P A T P M S P G G A P I T A V S S G P E V V F S G K H N G I S I Y
X.laevis L G T P T P T Q G A C P P N V S T P A Y G V A T P A P Q P P A V P G M M G T . E I V F S G K H N G I C I Y
H.sapiens L G T P S H G I Q P P A M S T P V C A L G N P A T Q A T N M S C V T G P . E I V Y S G K H N G I C I Y
649
660
670
679
β10
β11
C.thermophilum L T R L V R T L W K A K V V Q . . . . . V G S G S D I S . . . . . S T I P
A.nidulans I S R L L R S V W K K E V A V V G G G . . . . . P G G A Q T I S . . . . . P S F P
N.crassa L A R L A R T L W K S K V I E I A T D S . . . . . A S G G F T V G . . . . . S A I P
P.pastoris I S R M F R D I W S K E I F K L V P E I N F D S N G F I E . . . D G S V K K I E D R I I L S G L T I S
A.gossypii I T R L F S Q I W E E R V F V F K R A S K . . . . . T E K M D A F G . . . . . I S I T
S.cerevisiae I T R L F R D I W N K N I F L V K E T A R Y D Y S G S I L R D S V S N G D L I S . . . . . M V S I S
S.pombe I S R I I R N V W K N H V I I A K K M . . . . . Q N K R I H Y A . . . . . P A F N
C.elegans F S R L V A P V W N D T I C E . . . . . V L N G K Q L T . . . . . I T F E
D.melanogaster V S R M L H S V W Q M R C V . . . . . N E Q F C . . . . . S N L S
D.rerio F T R I L G N I W D G S L A M E T P I N K G . . . . . T Q T V M I L E . . . . . S T A S
X.laevis F C R I I G N I W D G S V A V E N T F Q S G . . . . . N R E V T A I D . . . . . S S V T
H.sapiens F S R I M G N I W D A S L V V E R I F K S G . . . . . N R E I T A I E . . . . . S S V P
680
690
700
706
α12
C.thermophilum T S K L V T I Q E N V E R L R N F L E A N . . . . . K S T I Q G L A P P
A.nidulans R A K L L A I Q H D L S A L Q E F F K T N . . . . . K S F I E G L S G P
N.crassa I S K L M T V Q E S V Q R L R R F L E E N . . . . . K S L I Q G L A G P
P.pastoris R N T I E Y L L S S L L I I L D F L E K N . . . . . K R S I P G A L P P
A.gossypii R P Q V E Y Y L S S I S V L A D F F N I H . . . . . R P S F V S F V P P
S.cerevisiae K T D L E Y Y L S S I M I L N E F F M T Y . . . . . G T S I T T I T A T
S.pombe A T E I L K I Q S G L L Y L S T F L E N N . . . . . K S F I E G L N S P
C.elegans P E S I Q S L K E E I Q K L A R L M D D Y . . . . . R L V P M M E F N
D.melanogaster Q S E C A L L L S D L R S L R S F L E V H S V H D I S S T T R V S F D N H L D R T N S Y N T I M M G N
D.rerio S S D L E L V L M Q L Q D L K E F L D K N S Q F S P T S L G V A N F S S P A N L Q Q R L L G F M R P D
X.laevis P Q H L E S V L K E L K G L L E F L D R Y S Q F T A G S L G N P G F G T P A N R Q Q R L V S L G R P D
H.sapiens C Q L L E S V L Q E L K G L Q E F L D R N S Q F A G G P L G N P N T T A K V Q . . Q R L I G F M R P E
707
710
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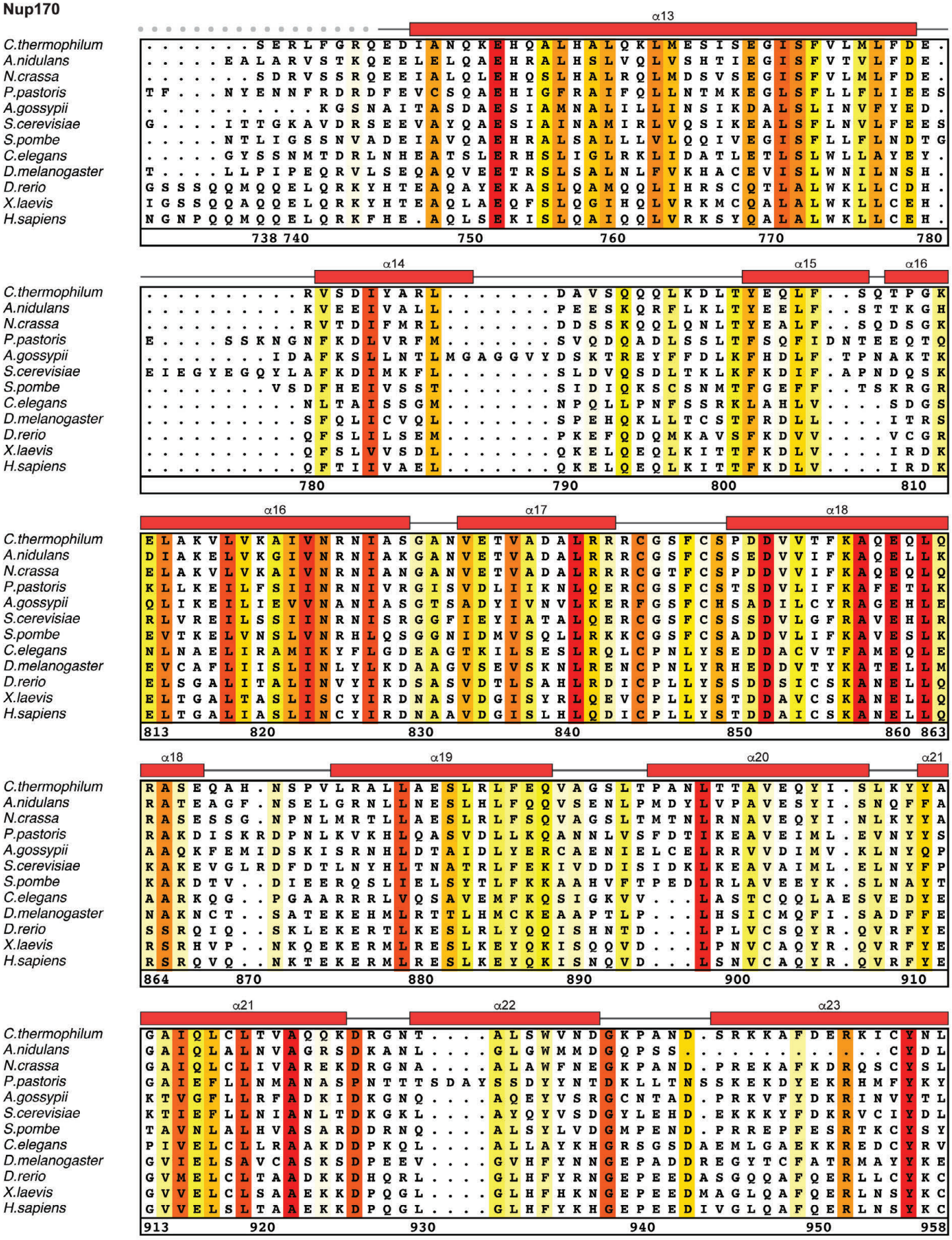
730
737

Nup170


α13
C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
738 740
750
760
770
780
α14
α15
α16
780
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α18
α19
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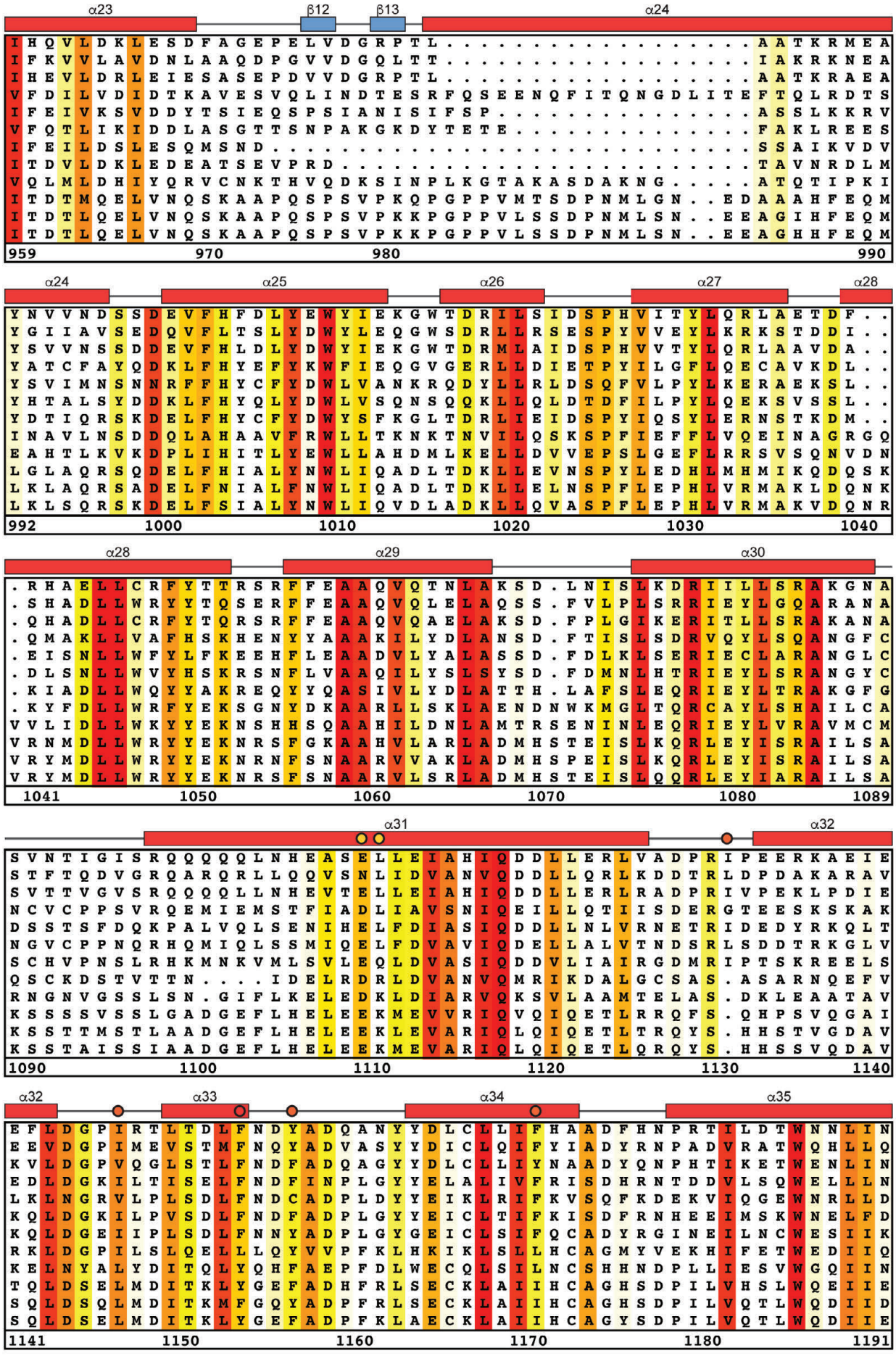
958

Nup170


α23 β12 β13 α24
C.thermophilum I H Q V L D K L E S D F A G E P E L V D G R P T L . . . . . A A T K R M E A
A.nidulans I F K V V L A V D N L A A Q D P G V V D G Q L T T . . . . . I A K R K N E A
N.crassa I H E V L D R L E I E S A S E P D V V D G R P T L . . . . . A A T K R A E A
P.pastoris V F D I L V D I D T K A V E S V Q L I N D T E S R F Q S E E N Q F I T Q N G D L I T E F T Q L R D T S
A.gossypii I F E I V K S V D D Y T S I E Q S P S I A N I S I F S P . . . . . A S S L K K R V
S.cerevisiae V F Q T L I K I D D L A S G T T S N P A K G K D Y T E T E . . . . . F A K L R E E S
S.pombe I F E I L D S L E S Q M S N D . . . . . S S A I K V D V
C.elegans I T D V L D K L E D E A T S E V P R D . . . . . T A V N R D L M
D.melanogaster V Q L M L D H I Y Q R V C N K T H V Q D K S I N P L K G T A K A S D A K N G . . . . . A T Q T I P K I
D.rerio I T D T M Q E L V N Q S K A A P Q S P S V P K Q P G P P V M T S D P N M L G N . . E D A A A H F E Q M
X.laevis I T D T L Q E L V N Q S K A A P Q S P S V P K K P G P P V L S S D P N M L S N . . E E A G I H F E Q M
H.sapiens I T D T L Q E L V N Q S K A A P Q S P S V P K K P G P P V L S S D P N M L S N . . E E A G H H F E Q M
959 970 980 990
α24 α25 α26 α27 α28
C.thermophilum Y N V V N D S S D E V F H F D L Y E W Y I E K G W T D R I L S I D S P H V I T Y L Q R L A E T D F . .
A.nidulans Y G I I A V S E D Q V F L T S L Y D W Y L E Q G W S D R L L R S E S P Y V V E Y L K R K S T D D I . .
N.crassa Y S V V N S S D D E V F H L D L Y D W Y I E K G W T D R M L A I D S P H V V T Y L Q R L A A V D A . .
P.pastoris Y A T C F A Y Q D K L F H Y E F Y K W F I E Q G V G E R L L D I E T P Y I L G F L Q E C A V K D L . .
A.gossypii Y S V I M N S N N R F F H Y C F Y D W L V A N K R Q D Y L L R L D S Q F V L P Y L K E R A E K S L . .
S.cerevisiae Y H T A L S Y D D K L F H Y Q L Y D W L V S Q N S Q Q K L L Q L D T D F I L P Y L Q E K S V S S L . .
S.pombe Y D T I Q R S K D E L F H Y C F Y D W Y S F K G L T D R L I E I D S P Y I Q S Y L E R N S T K D M . .
C.elegans I N A V L N S D D Q L A H A A V F R W L L T K N K T N V I L Q S K S P F I E F F L V Q E I N A G R G Q
D.melanogaster E A H T L K V K D P L I H I T L Y E W L L A H D M L K E L L D V V E P S L G E F L R R S V S Q N V D N
D.rerio L G L A Q R S Q D E L F H I A L Y N W L I Q A D L T D K L L E V N S P Y L E D H L M H M I K Q D Q S K
X.laevis L K L A Q R S A D E L F N I A L F N W L I Q A D L T D K L L E L N S P F L E P H L V R M A K L D Q N K
H.sapiens L K L S Q R S K D E L F S I A L Y N W L I Q V D L A D K L L Q V A S P F L E P H L V R M A K V D Q N R
992 1000 1010 1020 1030 1040
α28 α29 α30
C.thermophilum . R H A E L L C R F Y T T R S R F F E A A Q V Q T N L A K S D . L N I S L K D R I I L L S R A K G N A
A.nidulans . S H A D L L W R Y Y T Q S E R F F E A A Q V Q L E L A Q S S . F V L P L S R R I E Y L G Q A R A N A
N.crassa . Q H A D L L C R F Y T Q R S R F F E A A Q V Q A E L A K S D . F P L G I K E R I T L L S R A K A N A
P.pastoris . Q M A K L L V A F H S K H E N Y Y A A A K I L Y D L A N S D . F T I S L S D R V Q Y L S Q A N G F C
A.gossypii . E I S N L L W F Y L F K E E H F L E A A D V L Y A L A S S D . F D L K L S E R I E C L A R A N G L C
S.cerevisiae . D L S N L L W V Y H S K R S N F L V A A Q I L Y S L S Y S D . F D M N L H T R I E Y L S R A N G Y C
S.pombe . K I A D L L W Q Y Y A K R E Q Y Y Q A S I V L Y D L A T T H . L A F S L E Q R I E Y L T R A K G F G
C.elegans . K Y F D L L W R F Y E K S G N Y D K A A R L L S K L A E N D N W K M G L T Q R C A Y L S H A I L C A
D.melanogaster V V L I D L L W K Y Y E K N S H H S Q A A H I L D N L A M T R S E N I N L E Q R I E Y L V R A V M C M
D.rerio V R N M D L L W R Y Y E K N R S F G K A A H V L A R L A D M H S T E I S L K Q R L E Y I S R A I L S A
X.laevis V R Y M D L L W R Y Y E K N R N F S N A A R V V A K L A D M H S P E I S L K Q R L E Y I S R A I L S A
H.sapiens V R Y M D L L W R Y Y E K N R S F S N A A R V L S R L A D M H S T E I S L Q Q R L E Y I A R A I L S A
1041 1050 1060 1070 1080 1089
α31 α32
C.thermophilum S V N T I G I S R Q Q Q Q Q L N H E A S E L L E I A H I Q D D L L E R L V A D P R I P E E R K A E I E
A.nidulans S T F T Q D V G R Q A R Q R L L Q Q V S N L I D V A N V Q D D L L Q R L K D D T R L D P D A K A R A V
N.crassa S V T T V G V S R Q Q Q Q L L N H E V T E L L E I A H I Q D D L L E R L R A D P R I V P E K L P D I E
P.pastoris N C V C P P S V R Q E M I E M S T F I A D L I A V S N I Q E I L L Q T I I S D E R G T E E S K S K A K
A.gossypii D S S T S F D Q K P A L V Q L S E N I H E L F D I A S I Q D D L L N L V R N E T R I D E D Y R K Q L T
S.cerevisiae N G V C P P N Q R H Q M I Q L S S M I Q E L F D V A V I Q D E L L A L V T N D S R L S D D T R K G L V
S.pombe S C H V P N S L R H K M N K V M L S V L E Q L D V A S I Q D D V L I A I R G D M R I P T S K R E E L S
C.elegans Q S C K D S T V T T N . . . . I D E L R D R L D V A N V Q M R I K D A L G C S A S . A S A R N Q E F V
D.melanogaster R N G N V G S S L S N . G I F L K E L E D K L D I A R V Q K S V L A A M T E L A S . D K L E A A T A V
D.rerio K S S S S V S S L G A D G E F L H E L E E K M E V V R I Q V Q I Q E T L R R Q F S . Q H P S V Q G A I
X.laevis K S S T T M S T L A A D G E F L H E L E E K L E V A R I Q L Q I Q E T L T R Q Y S . H H S T V G D A V
H.sapiens K S S T A I S S I A A D G E F L H E L E E K M E V A R I Q L Q I Q E T L Q R Q Y S . H H S S V Q D A V
1090 1100 1110 1120 1130 1140
α32 α33 α34 α35
C.thermophilum E F L D G P I R T L T D L F N D Y A D Q A N Y Y D L C L L I F H A A D F H N P R T I L D T W N N L I N
A.nidulans E E V D G P I M E V S T M F N Q Y A D V A S Y Y D I C L Q I F Y I A D Y R N P A D V R A T W Q H L L Q
N.crassa K V L D G P V Q G L S T L F N D F A D Q A G Y Y D L C L L I Y N A A D Y Q N P H T I K E T W E N L I N
P.pastoris E D L D G K I L T I S E L F N D F I N P L G Y Y E L A L I V F R I S D H R N T D D V L S Q W E L L L N
A.gossypii L K L N G R V L P L S D L F N D C A D P L D Y Y E I K L R I F K V S Q F K D E K V I Q G E W N R L L D
S.cerevisiae K Q L D G K I L P V S D L F N D F A D P L G Y Y E I C L T I F K I S D F R N H E E I M S K W N E L F D
S.pombe K Q L D G E I I P L S D L F N N Y A D P L G Y G E I C L S I F Q C A D Y R G I N E I L N C W E S I I K
C.elegans R K L D G P I L S L Q E L L L Q Y Y V P F K L H K I K L S L L H C A G M Y V E K H I F E T W E D I I Q
D.melanogaster K E L N Y A L Y D I T Q L Y Q H F A E P F D L W E C Q L S I L N C S H H N D P L L I E S V W G Q I I N
D.rerio T Q L D S E L M D I T K L Y G E F A D H F R L S E C K L A I I H C A G H S D P I L V H S L W Q E I I E
X.laevis S Q L D S Q L M D I T K M F G Q Y A D P F R L S E C K L A I I H C A G H S D P I L V Q T L W Q D I I D
H.sapiens S Q L D S E L M D I T K L Y G E F A D P F K L A E C K L A I I H C A G Y S D P I L V Q T L W Q D I I E
1141 1150 1160 1170 1180 1191

Nup170

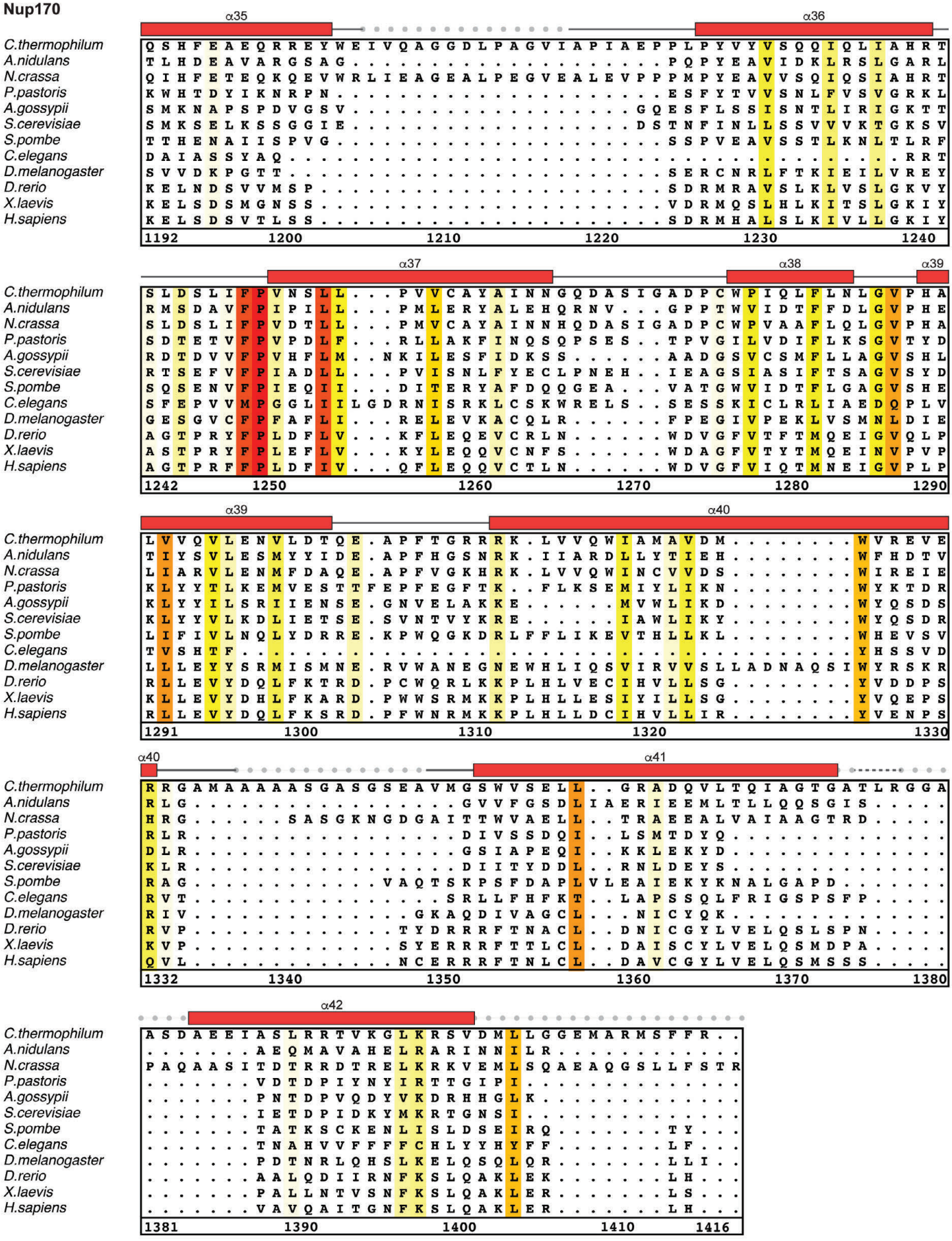

α35
α36
C.thermophilum Q S H F E A E Q R R E Y W E I V Q A G G D L P A G V I A P I A E P P L P Y V Y V S Q Q I Q L I A H R T
A.nidulans T L H D E A V A R G S A G . . . . . P Q P Y E A V I D K L R S L G A R L
N.crassa Q I H F E T E Q K Q E V W R L I E A G E A L P E G V E A L E V P P P M P Y E A V V S Q I Q S I A H R T
P.pastoris K W H T D Y I K N R P N . . . . . E S F Y T V V S N L F V S V G R K L
A.gossypii S M K N A P S P D V G S V . . . . . G Q E S F L S S I S N T L I R I G K T T
S.cerevisiae S M K S E L K S S G G I E . . . . . D S T N F I N L L S S V V V K T G K S V
S.pombe T T H E N A I I S P V G . . . . . S S P V E A V S S T L K N L T L R F
C.elegans D A I A S S Y A Q . . . . . R R T
D.melanogaster S V V D K P G T T . . . . . S E R C N R L F T K I E I L V R E Y
D.rerio K E L N D S V V M S P . . . . . S D R M R A V S L K L V S L G K V Y
X.laevis K E L S D S M G N S S . . . . . V D R M Q S L H L K I T S L G K I Y
H.sapiens K E L S D S V T L S S . . . . . S D R M H A L S L K I V L L G K I Y
1192 1200 1210 1220 1230 1240
α37
α38
α39
C.thermophilum S L D S L I F P V N S L L . . . P V V C A Y A I N N G Q D A S I G A D P C W P I Q L F L N L G V P H A
A.nidulans R M S D A V F P I P I L L . . . P M L E R Y A L E H Q R N V . . . G P P T W V I D T F F D L G V P H E
N.crassa S L D S L I F P V D T L L . . . P M V C A Y A I N N H Q D A S I G A D P C W P V A A F L Q L G V P H A
P.pastoris S D T E T V F P V P D L F . . . R L L A K F I N Q S Q P S E S . . T P V G I L V D I F L K S G V T Y D
A.gossypii R D T D V V F P V H F L M . . N K I L E S F I D K S S . . . . . A A D G S V C S M F L L A G V S H L
S.cerevisiae R T S E F V F P I A D L L . . . P V I S N L F Y E C L P N E H . . I E A G S I A S I F T S A G V S Y D
S.pombe S Q S E N V F P I E Q I I . . . D I T E R Y A F D Q Q G E A . . . V A T G W V I D T F L G A G V S H E
C.elegans S F E P V V M P G G L I I L G D R N I S R K L C S K W R E L S . . S E S S K I C L R L I A E D Q P L V
D.melanogaster G E S G V C F P F A F L I . . . R E L E V K A C Q L R . . . . . F P E G I V P E K L V S M N L D I E
D.rerio A G T P R Y F P L D F L V . . . K F L E Q E V C R L N . . . . . W D V G F V T F T M Q E I G V Q L P
X.laevis A S T P R Y F P L E F L V . . . K Y L E Q Q V C N F S . . . . . W D A G F V T Y T M Q E I N V P V P
H.sapiens A G T P R F F P L D F I V . . . Q F L E Q Q V C T L N . . . . . W D V G F V I Q T M N E I G V P L P
1242 1250 1260 1270 1280 1290
α39
α40
C.thermophilum L V V Q V L E N V L D T Q E . A P F T G R R R K . L V V Q W I A M A V D M . . . . . W V R E V E
A.nidulans T I Y S V L E S M Y Y I D E . A P F H G S N R K . I I A R D L L Y T I E H . . . . . W F H D T V
N.crassa L I A R V L E N M F D A Q E . A P F V G K H R K . L V V Q W I N C V V D S . . . . . W I R E I E
P.pastoris K L Y Y T L K E M V E S T T F E P F E G F T K . . F L K S E M I Y L I K N . . . . . W Y K T D R
A.gossypii K L Y Y I L S R I I E N S E . G N V E L A K K E . . . . . M V W L I K D . . . . . W Y Q S D S
S.cerevisiae K L Y Y V L K D L I E T S E . S V N T V Y K R E . . . . . I A W L I K Y . . . . . W Y Q S D R
S.pombe L I F I V L N Q L Y D R R E . K P W Q G K D R L F F L I K E V T H L L K L . . . . . W H E V S V
C.elegans T V S H T F . . . . . Y H S S V D
D.melanogaster L L L E Y Y S R M I S M N E . R V W A N E G N E W H L I Q S V I R V V S L L A D N A Q S I W Y R S K R
D.rerio R L L E V Y D Q L F K T R D . P C W Q R L K K P L H L V E C I H V L L S G . . . . . Y V D D P S
X.laevis K L L E V Y D H L F K A R D . P W W S R M K K P L H L L E S I Y I L L S G . . . . . Y V Q E P S
H.sapiens R L L E V Y D Q L F K S R D . P F W N R M K K P L H L L D C I H V L L I R . . . . . Y V E N P S
1291 1300 1310 1320 1330
α40
α41
C.thermophilum R R G A M A A A A A S G A S G S E A V M G S W V S E L L . . G R A D Q V L T Q I A G T G A T L R G G A
A.nidulans R L G . . . . . G V V F G S D L I A E R I E E M L T L L Q Q S G I S . . . . .
N.crassa H R G . . . . . S A S G K N G D G A I T T W V A E L L . . T R A E E A L V A I A A G T R D . . . . .
P.pastoris R L R . . . . . D I V S S D Q I . . L S M T D Y Q . . . . . .
A.gossypii D L R . . . . . G S I A P E Q I . . K K L E K Y D . . . . . .
S.cerevisiae K L R . . . . . D I I T Y D D L . . R N L D E Y S . . . . . .
S.pombe R A G . . . . . V A Q T S K P S F D A P L V L E A I E K Y K N A L G A P D . . . . . .
C.elegans R V T . . . . . S R L L F H F K T . . L A P S S Q L F R I G S P S F P . . . . . .
D.melanogaster R I V . . . . . G K A Q D I V A G C L . . . N I C Y Q K . . . . . .
D.rerio R V P . . . . . T Y D R R R F T N A C L . . D N I C G Y L V E L Q S L S P N . . . . .
X.laevis K V P . . . . . S Y E R R R F T T L C L . . D A I S C Y L V E L Q S M D P A . . . . .
H.sapiens Q V L . . . . . N C E R R R F T N L C L . . D A V C G Y L V E L Q S M S S S . . . . .
1332 1340 1350 1360 1370 1380
α42
C.thermophilum A S D A E E I A S L R R T V K G L K R S V D M L L G G E M A R M S F F R . .
A.nidulans . . . . . A E Q M A V A H E L R A R I N N I L R . . . . . .
N.crassa P A Q A A S I T D T R R D T R E L K R K V E M L S Q A E A Q G S L L F S T R
P.pastoris . . . . . V D T D P I Y N Y I R T T G I P I . . . . . .
A.gossypii . . . . . P N T D P V Q D Y V K D R H H G L K . . . . . .
S.cerevisiae . . . . . I E T D P I D K Y M K R T G N S I . . . . . .
S.pombe . . . . . T A T K S C K E N L I S L D S E I R Q . . . . . T Y . . .
C.elegans . . . . . T N A H V V F F F F C H L Y Y H Y F F . . . . . L F . . .
D.melanogaster . . . . . P D T N R L Q H S L K E L Q S Q L Q R . . . . . L L I . .
D.rerio . . . . . A A L Q D I I R N F K S L Q A K L E K . . . . . L H . . .
X.laevis . . . . . P A L L N T V S N F K S L Q A K L E R . . . . . L S . . .
H.sapiens . . . . . V A V Q A I T G N F K S L Q A K L E R . . . . . L H . . .
1381 1390 1400 1410 1416

Fig. S21.
Multispecies sequence alignment of Nup170. Sequences from twelve diverse species were aligned and colored by sequence similarity according to the BLOSUM62 matrix from white (less than 55 % similarity), to yellow (55 % similarity), to red (100 % identity). Numbering below alignment is relative to the *C. thermophilum* sequence. Secondary structure observed in the Nup170 structures is shown above the alignment: α-helices (red bars), β-sheets (blue bars), and unstructured regions (black lines). Mutations that affect Nup53 or Nup145N binding identified by a mutational analysis (Fig. 3; figs. S22; fig. S23) are indicated by circles above the alignment and colored according to the measured effect; weak effect (yellow), moderate effect (orange), abolished binding (red). Dashed lines indicate loops that were deleted in crystallization constructs. Disordered regions are indicated by gray dots.

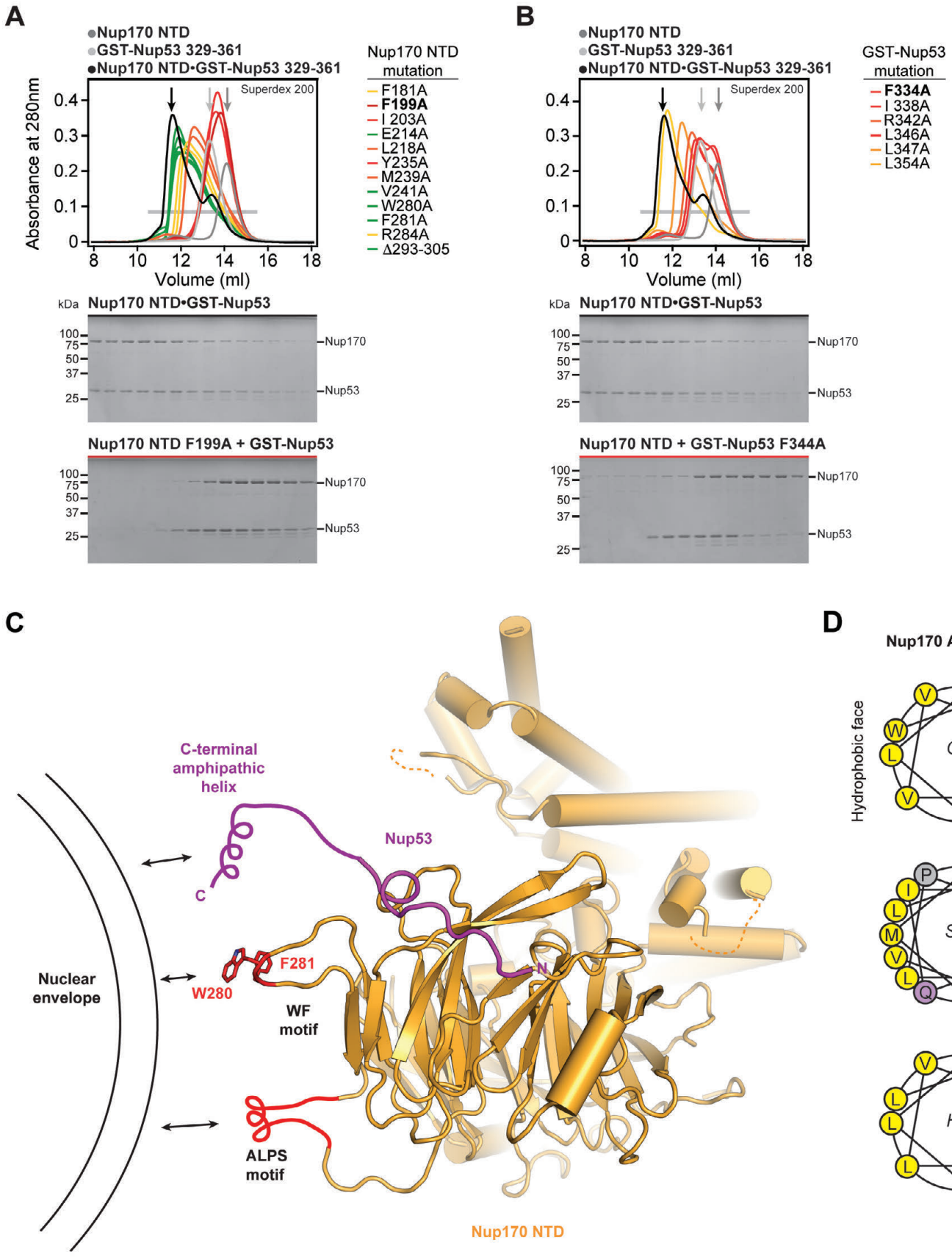

A
Nup170 NTD
GST-Nup53 329-361
Nup170 NTD•GST-Nup53 329-361
Superdex 200
Absorbance at 280nm
Volume (ml)
Nup170 NTD mutation
F181A
F199A
I 203A
E214A
L218A
Y235A
M239A
V241A
W280A
F281A
R284A
Δ293-305
kDa
Nup170 NTD•GST-Nup53
Nup170
Nup53
Nup170 NTD F199A + GST-Nup53
B
GST-Nup53 mutation
F334A
I 338A
R342A
L346A
L347A
L354A
Nup170 NTD + GST-Nup53 F344A
C
C-terminal amphipathic helix
Nup53
C
N
F281
W280
WF motif
Nuclear envelope
ALPS motif
Nup170 NTD
D
Nup170 ALPS motif
Hydrophobic face
C.t.
S.c.
H.s.

Fig. S22.
Mutational analysis of Nup170^{NTD}•Nup53 and proposed interactions with the nuclear envelope. SEC interaction experiments performed with mutants of (**A**) Nup170^{NTD} and (**B**) GST-Nup53$^{329-361}$. SEC profiles of wild type Nup170^{NTD} (dark gray) and GST-Nup53$^{329-361}$ (light gray) are shown individually and after preincubation (black) and are annotated with arrows of the same color. Mutant SEC profiles are colored according to the measured effect: no effect (green), weak effect (yellow), moderate effect (orange), and abolished binding (red). All SEC profiles were obtained using a Superdex 200 10/300 GL column. (**C**) Nup170^{NTD} is shown in cartoon representation and possible membrane interaction motifs are highlighted. The Nup170 β-propeller domain contains two sequence motifs, WF and ALPS, which are located on the same face directly adjacent to a C-terminal amphipathic helix of Nup53 that has previously been shown to anchor Nup53 to the nuclear envelope (*18, 19*). (**D**) Helical wheel diagrams of the Nup170 ALPS motif in *C. thermophilum*, *S. cerevisiae*, and *H. sapiens* are shown with hydrophobic and polar residues colored in yellow and purple, respectively. The universally conserved proline residue on the polar face of the helix, a feature reminiscent of antimicrobial membrane-destabilizing peptides, is colored in red (*34*).

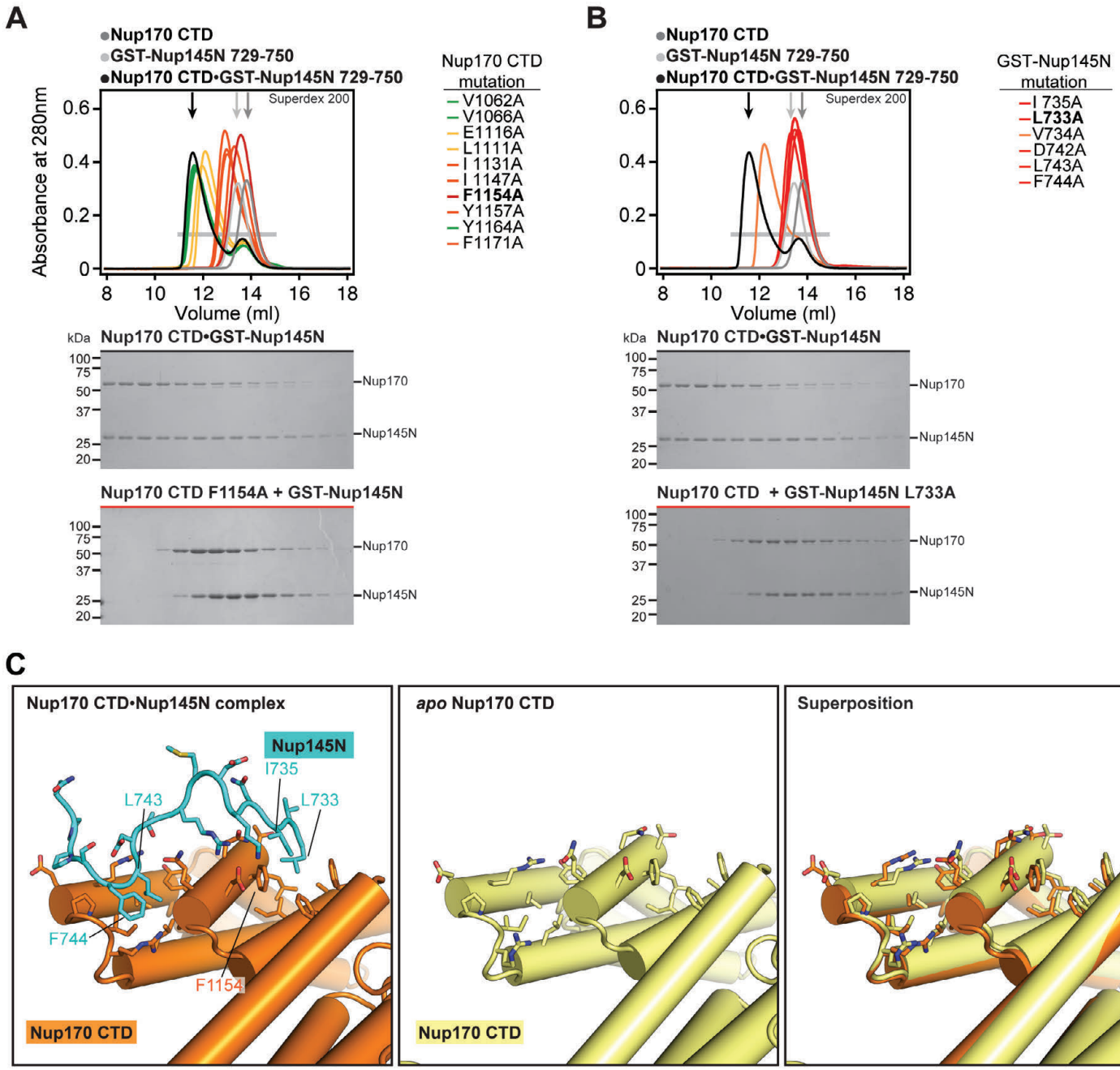

A
Nup170 CTD
GST-Nup145N 729-750
Nup170 CTD•GST-Nup145N 729-750
Superdex 200
Absorbance at 280nm
Volume (ml)
Nup170 CTD mutation
V1062A
V1066A
E1116A
L1111A
I 1131A
I 1147A
F1154A
Y1157A
Y1164A
F1171A
kDa
Nup170 CTD•GST-Nup145N
Nup170
Nup145N
Nup170 CTD F1154A + GST-Nup145N
B
Nup170 CTD
GST-Nup145N 729-750
Nup170 CTD•GST-Nup145N 729-750
Superdex 200
Volume (ml)
GST-Nup145N mutation
I 735A
L733A
V734A
D742A
L743A
F744A
Nup170 CTD•GST-Nup145N
Nup170 CTD + GST-Nup145N L733A
C
Nup170 CTD•Nup145N complex
Nup145N
I735
L743
L733
F744
F1154
Nup170 CTD
apo Nup170 CTD
Nup170 CTD
Superposition

Fig. S23.
Mutational and structural analyses of the Nup170^{CTD}-Nup145N interaction. SEC interaction experiments performed with mutants of (**A**) Nup170^{CTD} and (**B**) GST-Nup145N$^{729-750}$. SEC profiles of wild type Nup170^{CTD} (dark gray) and GST-Nup145N$^{729-750}$ (light gray) are shown individually and after preincubation (black) and are annotated with arrows of the same color. Mutant SEC profiles colored according to measured effect: no effect (green), weak effect (yellow), moderate effect (orange), and abolished binding (red). All SEC profiles were obtained using a Superdex 200 10/300 GL column. (**C**) Comparison of the Nup145N binding pocket in Nup170 in the bound and *apo* states reveals minimal conformational changes upon binding.

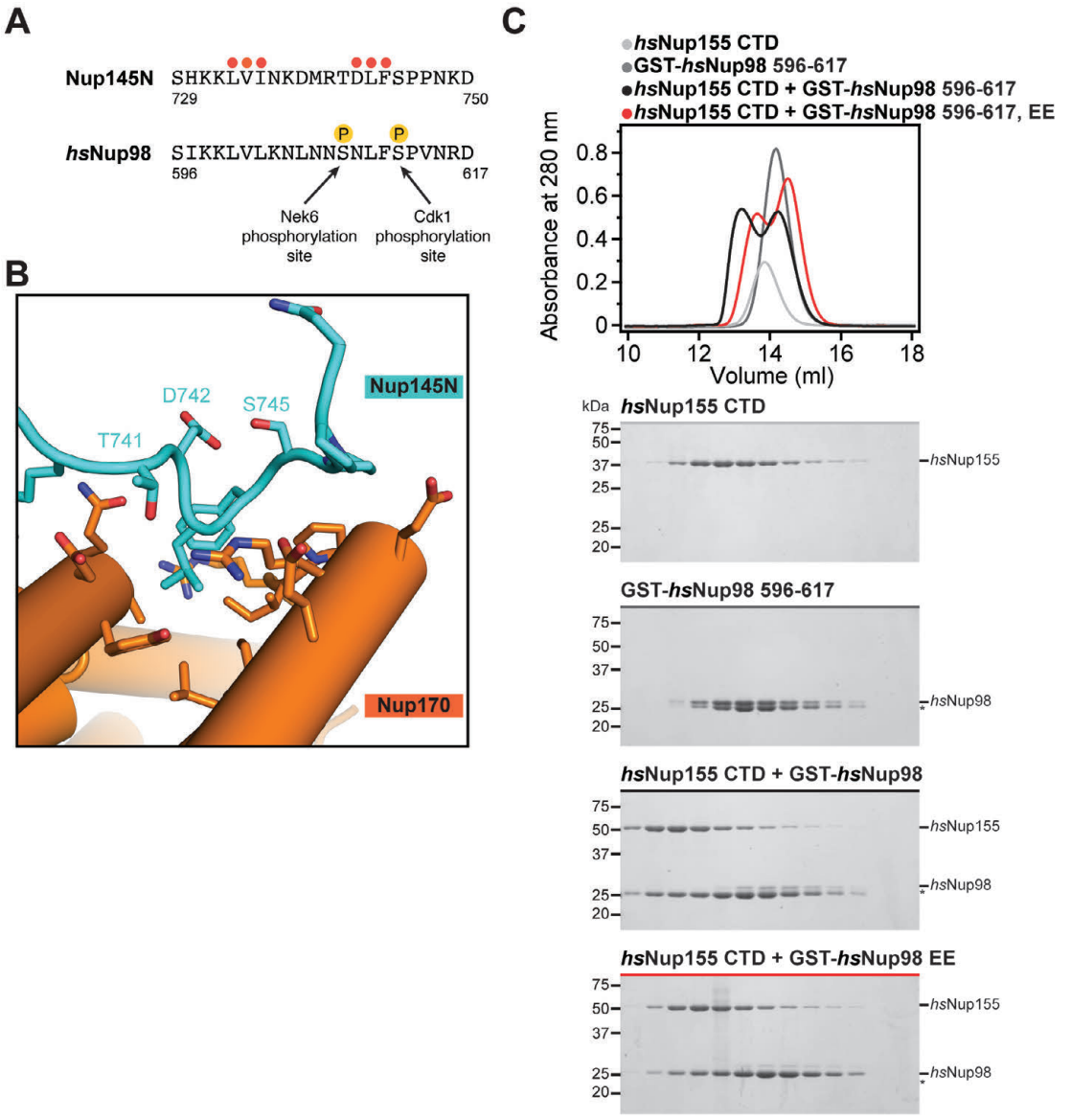
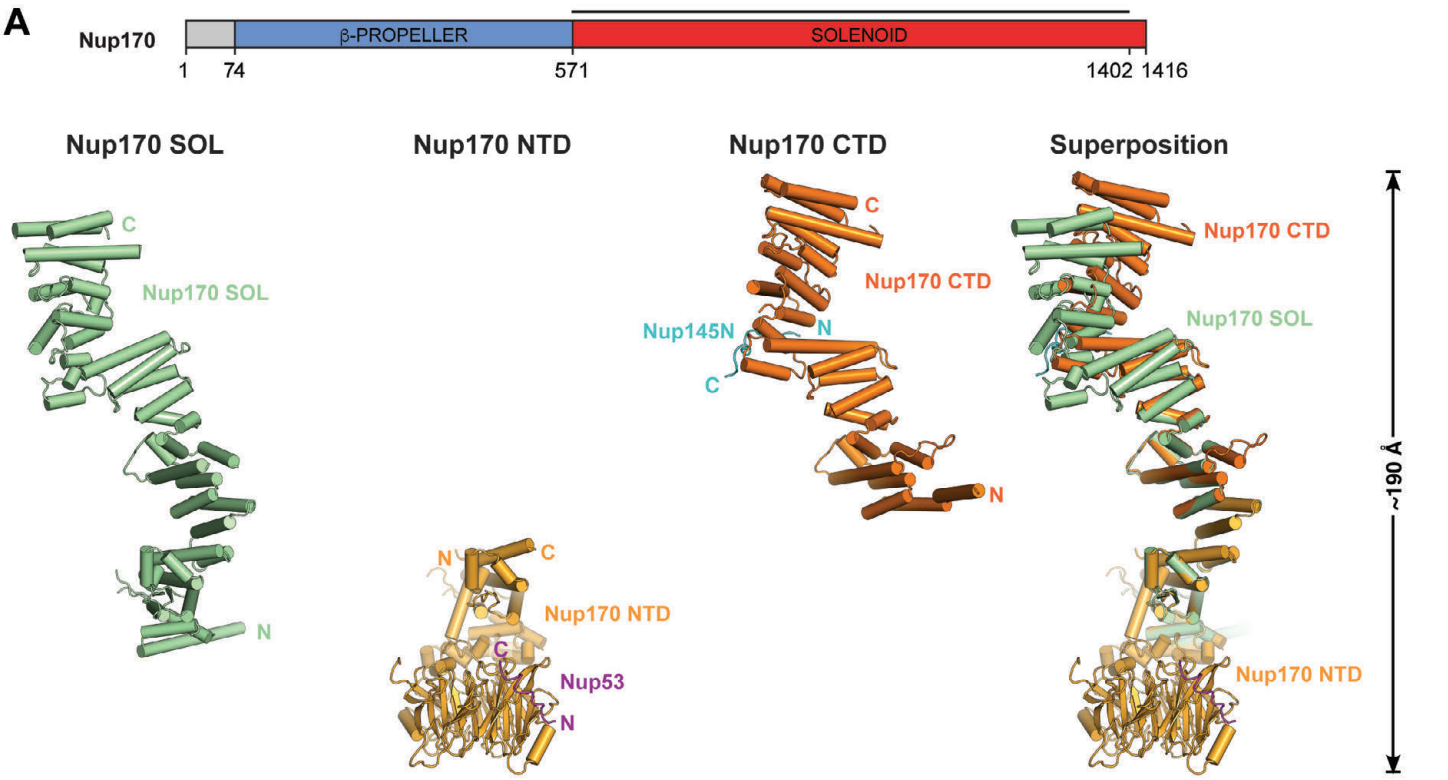

A
Nup145N SHKKLVINKDMRTDLFSPPNKD
729
750
hsNup98 SIKKLVLKNLNNSNLFSPVNRD
596
617
P
P
Nek6 phosphorylation site
Cdk1 phosphorylation site
B
D742
S745
T741
Nup145N
Nup170
C
hsNup155 CTD
GST-hsNup98 596-617
hsNup155 CTD + GST-hsNup98 596-617
hsNup155 CTD + GST-hsNup98 596-617, EE
Absorbance at 280 nm
0.8
0.6
0.4
0.2
0
10
12
14
16
18
Volume (ml)
kDa
hsNup155 CTD
75
50
37
25
25
20
hsNup155
GST-hsNup98 596-617
75
50
37
25
20
hsNup98
hsNup155 CTD + GST-hsNup98
75
50
37
25
20
hsNup155
hsNup98
hsNup155 CTD + GST-hsNup98 EE
75
50
37
25
20
hsNup155
hsNup98

Fig. S24.
The Nup170^{CTD}-Nup145N interaction is conserved in humans and partially disrupted by phosphomimetic mutations. (**A**) Sequence alignment of Nup145N$^{729-750}$ and *hs*Nup98$^{596-617}$. Nup145N$^{729-750}$ mutants are indicated by circles and colored as in Fig. 3J. *hs*Nup98 residues S608 and S612 that are phosphorylated during mitosis are indicated (*35*). (**B**) Close-up view of the Nup170^{CTD}-Nup145N interaction indicating the positioning of T741 and S745 in Nup145N, which correspond to the mitotically phosphorylated *hs*Nup98 residues S608 and S612. (**C**) SEC interaction analysis of the *hs*Nup155^{CTD}-*hs*Nup98$^{596-617}$ interaction. SEC profiles of *hs*Nup155^{CTD} (light gray) and *hs*Nup98$^{596-617}$ (dark grey), the preincubation of the wild type proteins (black), and the preincubation of *hs*Nup155^{CTD} with the phosphomimetic *hs*Nup98$^{596-617}$ S608E/S612E double mutant (red) are shown. All SEC profiles were obtained using a Superdex 200 10/300 GL column. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining.


A
Nup170
β-PROPELLER
SOLENOID
1
74
571
1402
1416
Nup170 SOL
Nup170 NTD
Nup170 CTD
Superposition
C
Nup170 SOL
N
N
C
Nup170 NTD
C
Nup53
N
C
Nup170 CTD
Nup145N
N
C
N
Nup170 CTD
Nup170 SOL
Nup170 NTD
~190 Å

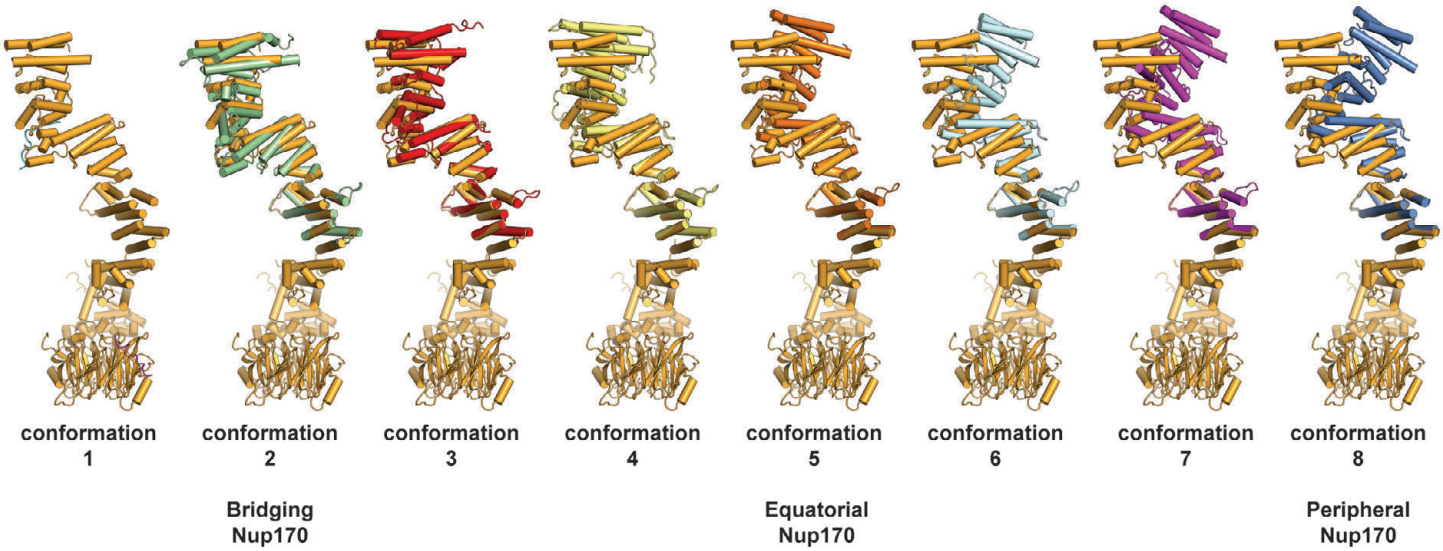

B
conformation 1
conformation 2
conformation 3
conformation 4
conformation 5
conformation 6
conformation 7
conformation 8
Bridging Nup170
Equatorial Nup170
Peripheral Nup170

Fig. S25.
Superposition-generated structures of full-length Nup170. (**A**) The construct boundaries of Nup170^{SOL} are indicated by a black line above the domain structure. The crystal structures of Nup170^{SOL} (green), Nup170^{NTD} (light orange), and Nup170^{CTD} (dark orange), and their superposition are shown in cartoon representation. See also Movie 1. (**B**) Complete conformational range of Nup170 generated by superposing different conformations of Nup170^{CTD} with the structure of Nup170^{SOL}. Conformations were obtained from the Nup170^{SOL} structure (conformation 1), different molecules in the asymmetric unit of the *apo* Nup170^{CTD} structures (conformations 2, 3, and 4), and different molecules in the asymmetric unit of the Nup170^{CTD}•Nup145N complex structure (conformations 5, 6, 7, and 8). The conformations that were docked into cryoET reconstruction of the NPC are indicated below. See also Movie 2.

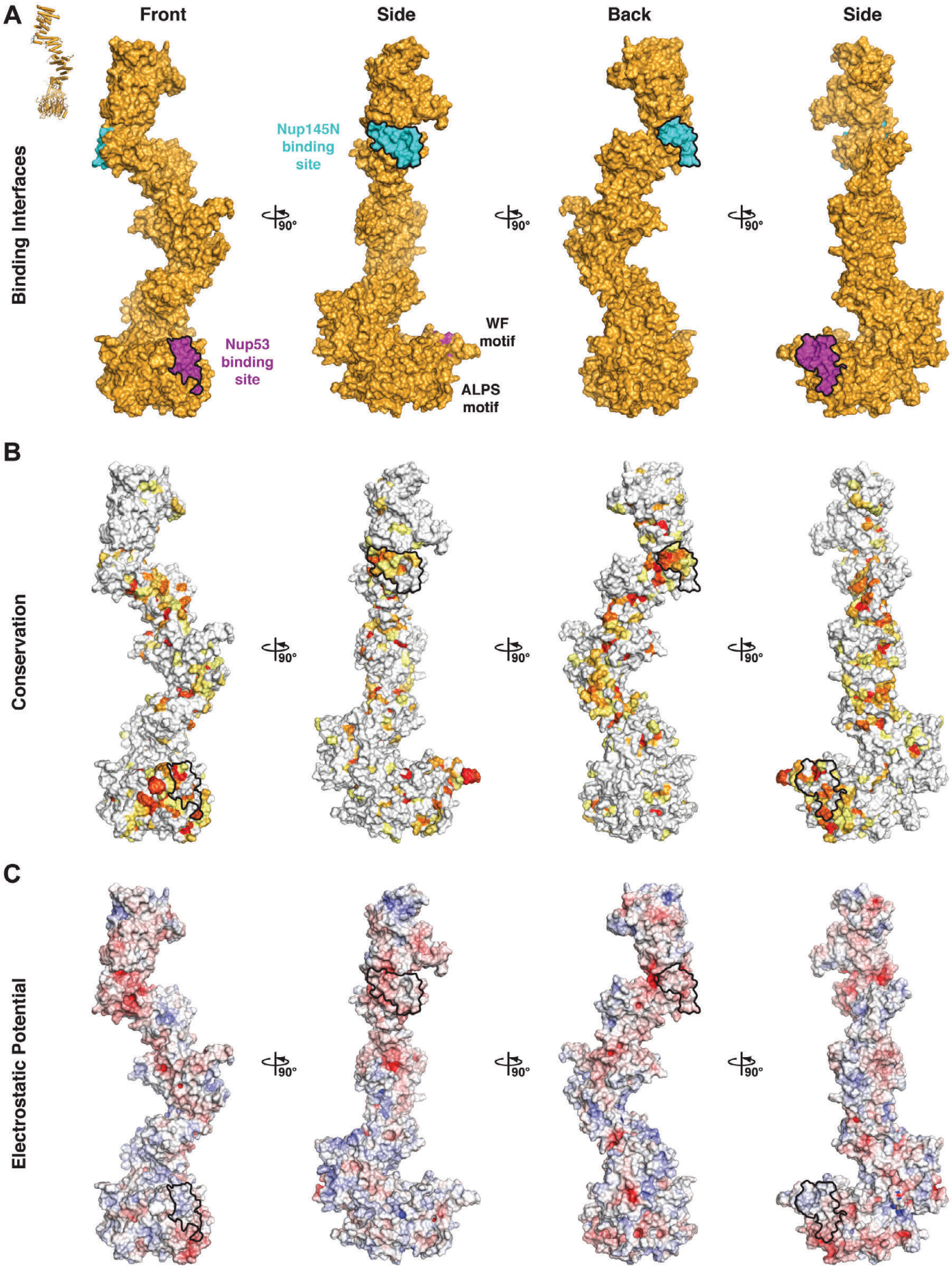
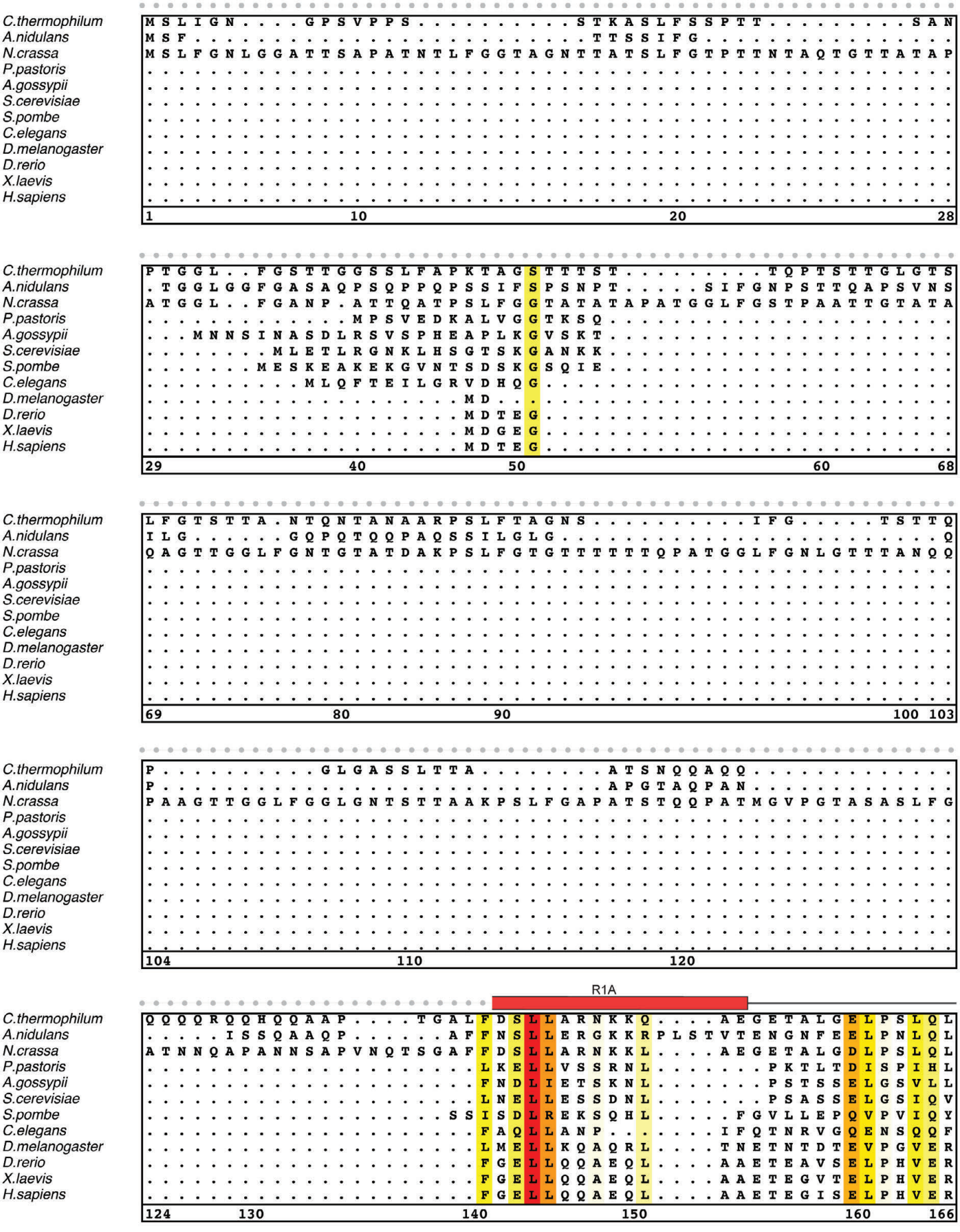

A
Front
Side
Back
Side
Binding Interfaces
Nup145N binding site
Nup53 binding site
WF motif
ALPS motif
90°
B
Conservation
C
Electrostatic Potential

Fig. S26.
Surface properties of Nup170. Surface representations of Nup170 is shown in four different orientations related by 90° rotations. Nup53 and Nup145N binding interfaces are outlined in black. (**A**) Surface representation with the Nup53 and Nup145N binding sites colored in purple and cyan, respectively. (**B**) Surface representation colored according to sequence identity based on the alignment in fig. S21. (**C**) Surface representation colored according to electrostatic potential from -10 k_BT/e (red) to +10 k_BT/e (blue).

Nic96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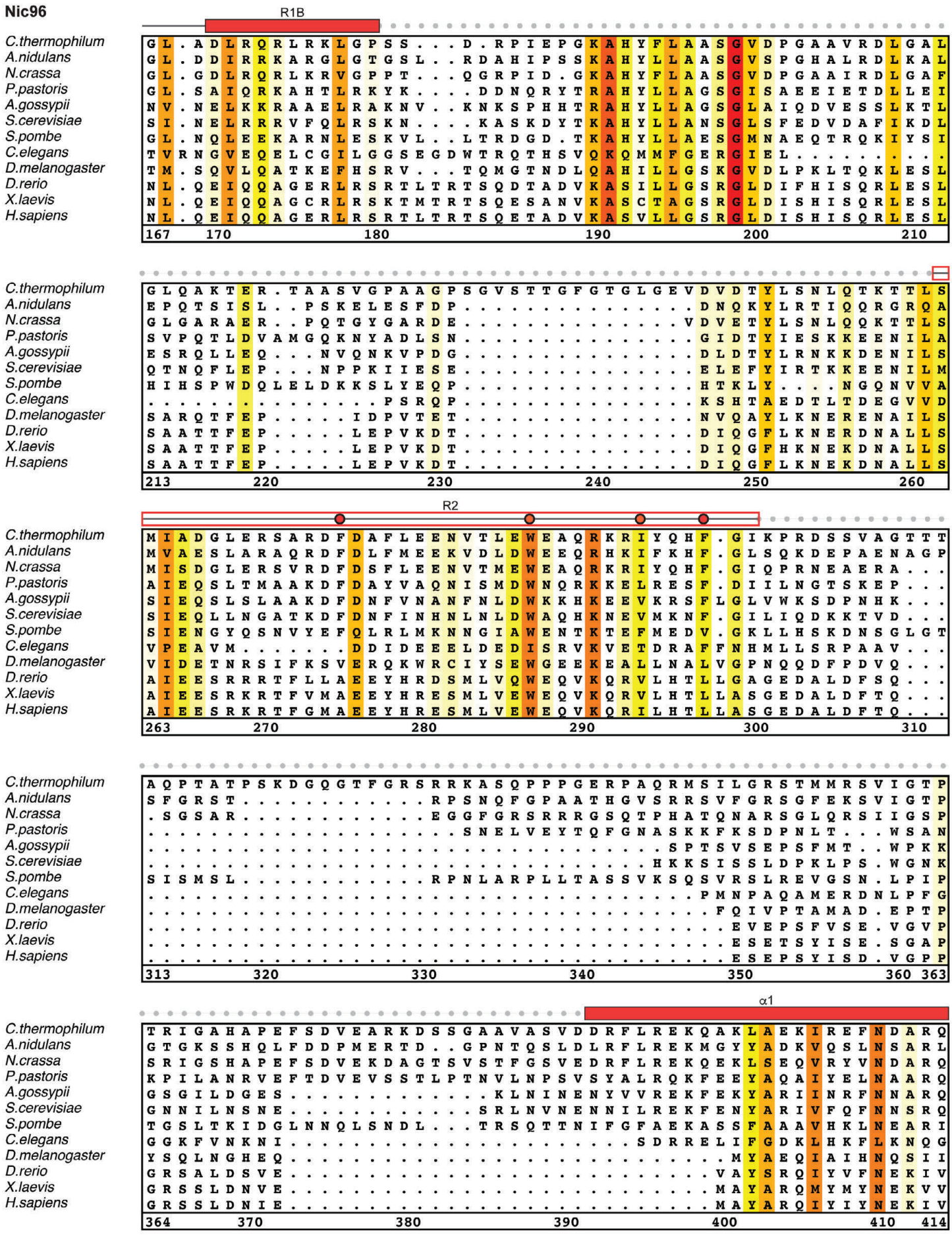
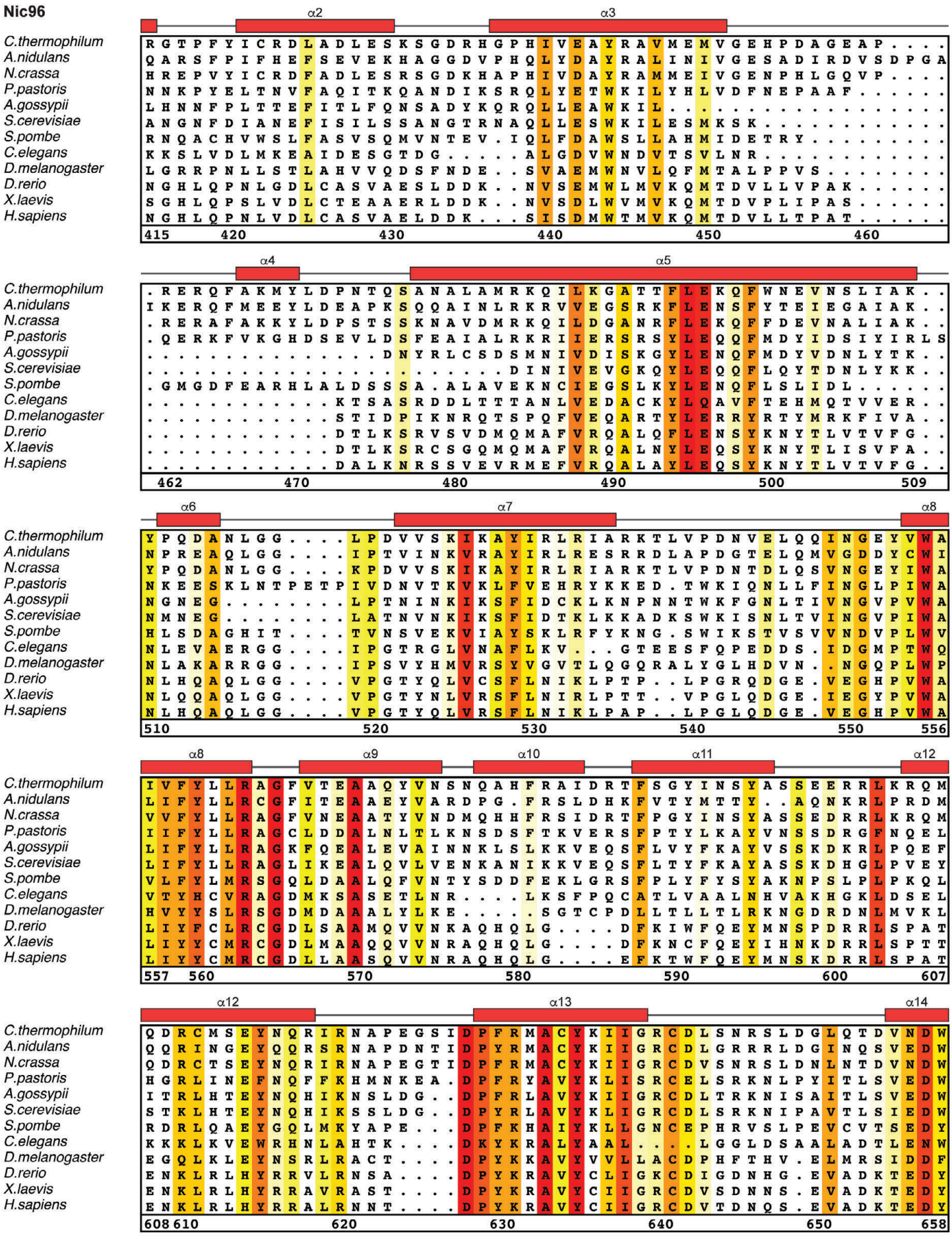
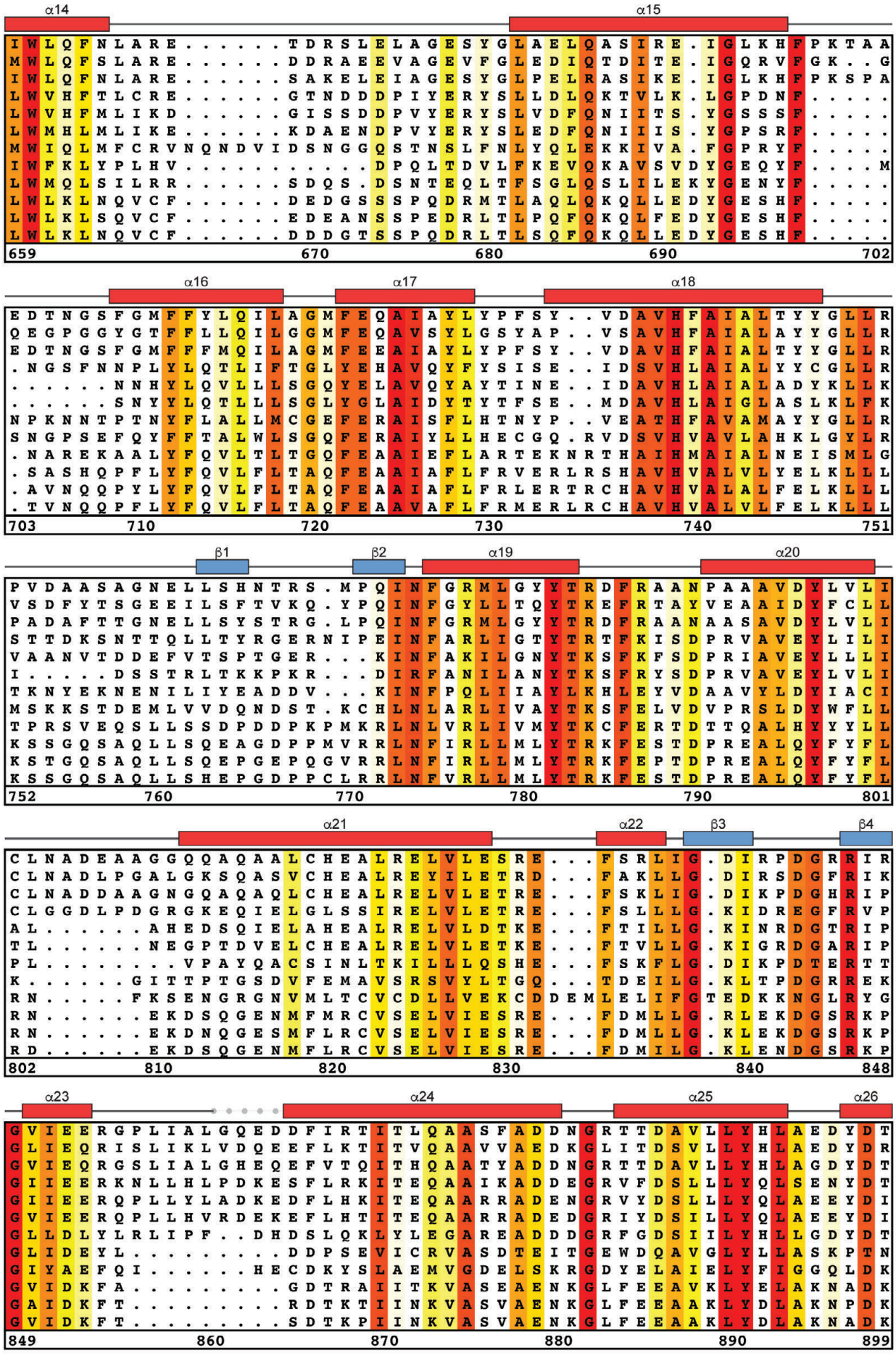
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
1
10
20
28
29
40
50
60
68
69
80
90
100
103
104
110
120
R1A
124
130
140
150
160
166


Nic96
R1B
C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
167 170 180 190 200 210
213 220 230 240 250 260
R2
263 270 280 290 300 310
313 320 330 340 350 360 363
α1
364 370 380 390 400 410 414


Nic96
α2
α3
C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
415 420 430 440 450 460
α4
α5
462 470 480 490 500 509
α6
α7
α8
510 520 530 540 550 556
α8
α9
α10
α11
α12
557 560 570 580 590 600 607
α12
α13
α14
608 610 620 630 640 650 658

Nic96


α14
α15
C.thermophilum I W L Q F N L A R E . . . . . T D R S L E L A G E S Y G L A E L Q A S I R E . I G L K H F P K T A A
A.nidulans M W L Q F S L A R E . . . . . D D R A E E V A G E V F G L E D I Q T D I T E . I G Q R V F G K . . G
N.crassa I W L Q F N L A R E . . . . . S A K E L E I A G E S Y G L P E L R A S I K E . I G L K H F P K S P A
P.pastoris L W V H F T L C R E . . . . . G T N D D D P I Y E R Y S L L D L Q K T V L K . L G P D N F . . . . .
A.gossypii L W V H F M L I K D . . . . . G I S S D D P V Y E R Y S L V D F Q N I I T S . Y G S S S F . . . . .
S.cerevisiae L W M H L M L I K E . . . . . K D A E N D P V Y E R Y S L E D F Q N I I I S . Y G P S R F . . . . .
S.pombe M W I Q L M F C R V N Q N D V I D S N G G Q S T N S L F N L Y Q L E K K I V A . F G P R Y F . . . . .
C.elegans I W F K L Y P L H V . . . . . D P Q L T D V L F K E V Q K A V S V D Y G E Q Y F . . . . M
D.melanogaster L W M Q L S I L R R . . . . . S D Q S . D S N T E Q L T F S G L Q S L I L E K Y G E N Y F . . . . .
D.rerio L W L K L N Q V C F . . . . . D E D G S S S P Q D R M T L A Q L Q K Q L L E D Y G E S H F . . . . .
X.laevis L W L K L S Q V C F . . . . . E D E A N S S P E D R L T L P Q F Q K Q L F E D Y G E S H F . . . . .
H.sapiens L W L K L N Q V C F . . . . . D D D G T S S P Q D R L T L S Q F Q K Q L L E D Y G E S H F . . . . .
659 670 680 690 702
α16
α17
α18
C.thermophilum E D T N G S F G M F F Y L Q I L A G M F E Q A I A Y L Y P F S Y . . V D A V H F A I A L T Y Y G L L R
A.nidulans Q E G P G G Y G T F F L L Q I L G G M F E Q A V S Y L G S Y A P . . V S A V H F A I A L A Y Y G L L R
N.crassa E D T N G S F G M F F F M Q I L A G M F E E A I A Y L Y P F S Y . . V D A V H F A I A L T Y Y G L L R
P.pastoris . N G S F N N P L Y L Q T L I F T G L Y E H A V Q Y F Y S I S E . . I D S V H L A I A L Y Y C G L L R
A.gossypii . . . . . N N H Y L Q V L L L S G Q Y E L A V Q Y A Y T I N E . . I D A V H L A I A L A D Y K L L K
S.cerevisiae . . . . . S N Y Y L Q T L L L S G L Y G L A I D Y T Y T F S E . . M D A V H L A I G L A S L K L F K
S.pombe N P K N N T P T N Y F F L A L L M C G E F E R A I S F L H T N Y P . . V E A T H F A V A M A Y Y G L L R
C.elegans S N G P S E F Q Y F F T A L W L S G Q F E R A I Y L L H E C G Q . R V D S V H V A V L A H K L G Y L R
D.melanogaster . N A R E K A A L Y F F Q V L T L T G Q F E A A I E F L A R T E K N R T H A I H M A I A L N E I S M L G
D.rerio . S A S H Q P F L Y F F Q V L F L T A Q F E A A I A F L F R V E R L R S H A V H V A L V L Y E L K L L L
X.laevis . A V N Q Q P Y L Y F F Q V L F L T A Q F E A A I A F L F R L E R T R C H A V H V A L A L F E L K L L L
H.sapiens . T V N Q Q P F L Y F F Q V L F L T A Q F E A A V A F L F R M E R L R C H A V H V A L V L F E L K L L L
703 710 720 730 740 751
β1
β2
α19
α20
C.thermophilum P V D A A S A G N E L L S H N T R S . M P Q I N F G R M L G Y Y T R D F R A A N P A A A V D Y L V L I
A.nidulans V S D F Y T S G E E I L S F T V K Q . Y P Q I N F G Y L L T Q Y T K E F R T A Y V E A A I D Y F C L L
N.crassa P A D A F T T G N E L L S Y S T R G . L P Q I N F G R M L G Y Y T R D F R A A N A A S A V D Y L V L I
P.pastoris S T T D K S N T T Q L L T Y R G E R N I P E I N F A R L I G T Y T R T F K I S D P R V A V E Y L I L I
A.gossypii V A A N V T D D E F V T S P T G E R . . . K I N F A K I L G N Y T K S F K F S D P R I A V E Y L L L I
S.cerevisiae I . . . . . D S S T R L T K K P K R . . . D I R F A N I L A N Y T K S F R Y S D P R V A V E Y L V L I
S.pombe T K N Y E K N E N I L I Y E A D D V . . . K I N F P Q L I I A Y L K H L E Y V D A A V Y L D Y I A C I
C.elegans M S K K S T D E M L V V D Q N D S T . K C H L N L A R L I V A Y T K S F E L V D V P R S L D Y W F L L
D.melanogaster T P R S V E Q S L L S S D P D D P K P M K R L N L V R L I V M Y T K C F E R T D T T Q A L H Y Y Y L L
D.rerio K S S G Q S A Q L L S Q E A G D P P M V R R L N F I R L L M L Y T R K F E S T D P R E A L Q Y F Y F L
X.laevis K S T G Q S A Q L L S Q E P G E P Q G V R R L N F I R L L M L Y T R K F E P T D P R E A L Q Y F Y F L
H.sapiens K S S G Q S A Q L L S H E P G D P P C L R R L N F V R L L M L Y T R K F E S T D P R E A L Q Y F Y F L
752 760 770 780 790 801
α21
α22
β3
β4
C.thermophilum C L N A D E A A G G Q Q A Q A A L C H E A L R E L V L E S R E . . . F S R L I G . D I R P D G R R I R
A.nidulans C L N A D L P G A L G K S Q A S V C H E A L R E Y I L E T R D . . . F A K L L G . D I R S D G F R I K
N.crassa C L N A D D A A G N G Q A Q A Q L C H E A L R E L V L E T R E . . . F S K L I G . D I K P D G H R I P
P.pastoris C L G G D L P D G R G K E Q I E L G L S S I R E L V L E T R E . . . F S L L L G . K I D R E G F R V P
A.gossypii A L . . . . . A H E D S Q I E L A H E A L R E L V L D T K E . . . F T I L L G . K I N R D G T R I P
S.cerevisiae T L . . . . . N E G P T D V E L C H E A L R E L V L E T K E . . . F T V L L G . K I G R D G A R I P
S.pombe P L . . . . . V P A Y Q A C S I N L T K I L L L Q S H E . . . F S K F L G . D I K P D T E R T T
C.elegans K . . . . . G I T T P T G S D V F E M A V S R S V Y L T G Q . . . T D E I L G . K L T P D G R R E K
D.melanogaster R N . . . . . F K S E N G R G N V M L T C V C D L L V E K C D D E M L E L I F G T E D K K N G L R Y G
D.rerio R N . . . . . E K D S Q G E N M F M R C V S E L V I E S R E . . . F D M L L G . R L E K D G S R K P
X.laevis R N . . . . . E K D N Q G E S M F L R C V S E L V I E S R E . . . F D M L L G . K L E K D G S R K P
H.sapiens R D . . . . . E K D S Q G E N M F L R C V S E L V I E S R E . . . F D M I L G . K L E N D G S R K P
802 810 820 830 840 848
α23
α24
α25
α26
C.thermophilum G V I E E R G P L I A L G Q E D D F I R T I T L Q A A S F A D D N G R T T D A V L L Y H L A E D Y D T
A.nidulans G L I E Q R I S L I K L V D Q E E F L K T I T V Q A A V V A E D K G L I T D S V L L Y H L A E D Y D R
N.crassa G V I E Q R G S L I A L G H E Q E F V T Q I T H Q A A T Y A D D N G R T T D A V L L Y H L A G D Y D T
P.pastoris G I I E E R K N L L H L P D K E S F L R K I T E Q A A I K A D D E G R V F D S L L L Y Q L S E N Y D T
A.gossypii G I I E E R Q P L L Y L A D K E D F L H K I T E Q A A R R A D E N G R V Y D S L L L Y Q L A E E Y D I
S.cerevisiae G V I E E R Q P L L H V R D E K E F L H T I T E Q A A R R A D E D G R I Y D S I L L Y Q L A E E Y D I
S.pombe G L L D L Y L R L I P F . . D H D S L Q K L Y L E G A R E A D D D G R F G D S I I L Y H L L G D Y D T
C.elegans G L I D E Y L . . . . . D D P S E V I C R V A S D T E I T G E W D Q A V G L Y L L A S K P T N
D.melanogaster G I Y A E F Q I . . . . . H E C D K Y S L A E M V G D E L S K R G D Y E L A I E L Y F I G G Q L D K
D.rerio G V I D K F A . . . . . G D T R A I I T K V A S E A E N K G L F E E A V K L Y E L A K N A D K
X.laevis G A I D K F T . . . . . R D T K T I I N K V A S V A E N K G L F E E A A K L Y D L A K N P D K
H.sapiens G V I D K F T . . . . . S D T K P I I N K V A S V A E N K G L F E E A A K L Y D L A K N A D K
849 860 870 880 890 899

Nic96

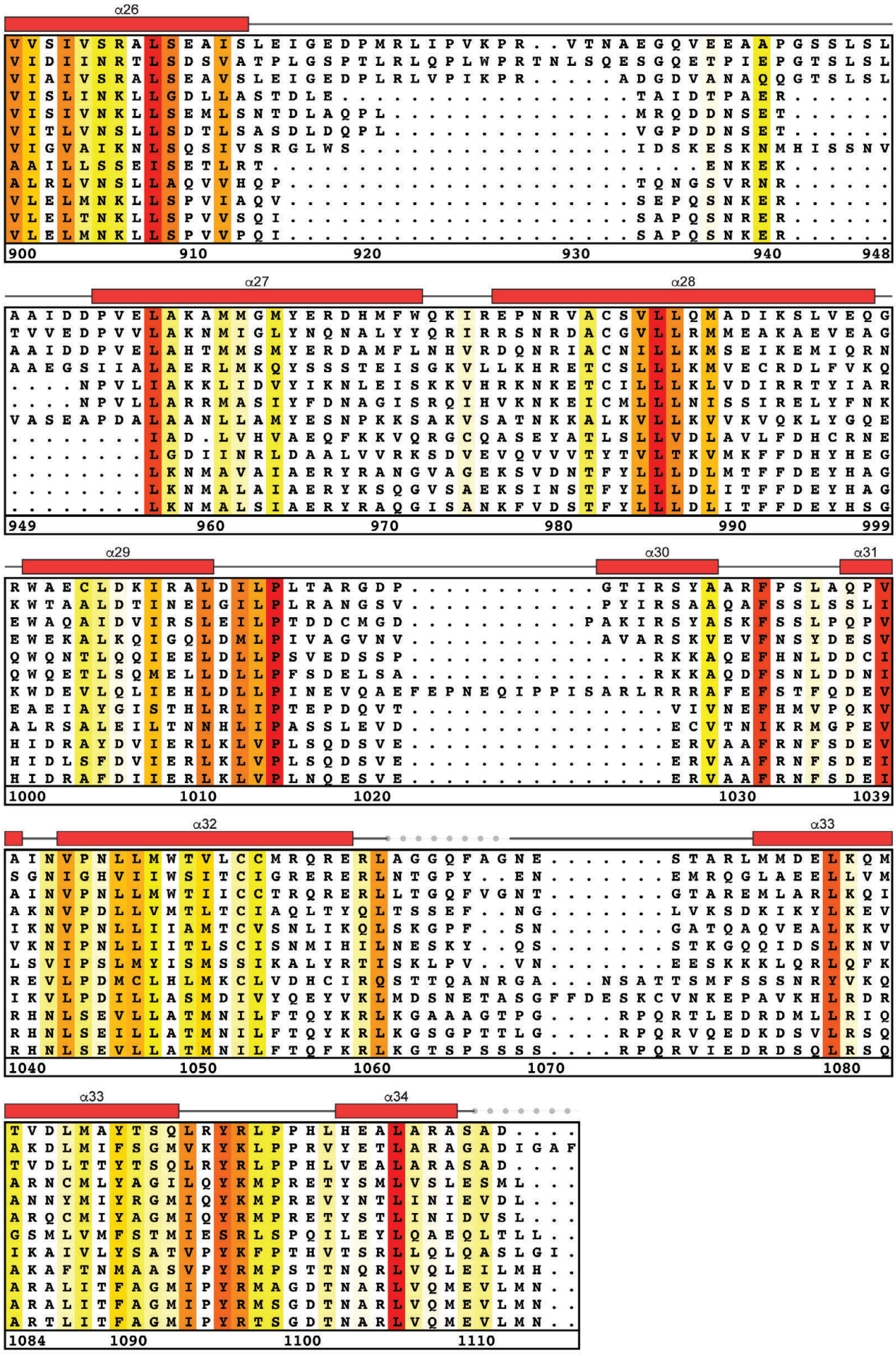

α26
C.thermophilum VVSIVSRALSEAISLEIGEDPMRLIPVKPR..VTNAEGQVEEAAPGSSLSL
A.nidulans VIDIINRTLSDSVATPLGSPTLRLQPLWPRTNLSQESGQETPIEPGTSLSL
N.crassa VIAIVSRALSEAVSLEIGEDPLRLVPIKPR.....ADGDVANAQQGTSLSL
P.pastoris VISLINKLLGDLLASTDLE..................TAIDTPAER.....
A.gossypii VISIVNKLLSEMLSNTDLAQPL..............MRQDDNSET.....
S.cerevisiae VITLVNSLLSDTLSASDLDQPL..............VGPDDNSET.....
S.pombe VIGVAIKNLSQSIVSRGLWS..............IDSKESKNMHISSNV
C.elegans AAILLSSEISETLRT..................ENKEK.....
D.melanogaster ALRLVNSLLAQVVHQP..............TQNGSVRNR.....
D.rerio VLELMNKLLSPVIAQV..............SEPQSNKER.....
X.laevis VLELTNKLLSPVVSQI..............SAPQSNRER.....
H.sapiens VLELMNKLLSPVVPQI..............SAPQSNKER.....
900 910 920 930 940 948
α27 α28
C.thermophilum AAIDDPVELAKAMMGMYERDHMFWQKIREPNRVACSVLLQMADIKSLVEQG
A.nidulans TVVEDPVVLAKNMIGLYNQNALYYQRIRRSNRDACGVLLRMMEAKAEVEAG
N.crassa AAIDDPVELAHTMMSMYERDAMFLNHVRDQNRIACNILLKMSEIKEMIQRN
P.pastoris AAEGSIIALAERLMKQYSSSTEISGKVLLKHRETCSLLLKMVECRDLFVKQ
A.gossypii ....NPVLIAKKLIDVYIKNLEISKKVHRKNKETCILLLKLVDIRRTYIAR
S.cerevisiae ....NPVLLARRMASIYFDNAGISRQIHVKNKEICMLLLNISSIRELYFNK
S.pombe VASEAPDALAANLLAMYESNPKKSAKVSATNKKALKVLLKVVKVQKLYGQE
C.elegans ....IAD.LVHVAEQFKKVQRGCQASEYATLSLLVDLAVLFDHCRNE
D.melanogaster .........LGDIINRLDAALVVRKSDVEVQVVVTYTVLTKVMKFFDHYHEG
D.rerio .........LKNMAVAIAERYRANGVAGEKSVDNTFYLLLDLMTFFDEYHAG
X.laevis .........LKNMALAIAERYKSQGVSAEKSINSTFYLLLDLITFFDEYHAG
H.sapiens .........LKNMALSIAERYRAQGISANKFVDSTFYLLLDLITFFDEYHSG
949 960 970 980 990 999
α29 α30 α31
C.thermophilum RWAECLDKIRALDILPLTARGDP..........GTIRSYAARFPSLAQPV
A.nidulans KWTAALDTINELGILPLRANGSV..........PYIRSAAQAFSSLSSLI
N.crassa EWAQAIDVIRSLEILPTDDCMGD..........PAKIRSYASKFSSLPQPV
P.pastoris EWEKALKQIGQLDMLPIVAGVNV..........AVARSKVEVFNSYDESV
A.gossypii QWQNTLQQIEELDLLPSVEDSSP..........RKKAQEFHNLDDCI
S.cerevisiae QWQETLSQMELLDLLPFSDELSA..........RKKAQDFSNLDDNI
S.pombe KWDEVLQLIEHLDLLPINEVQAEFEPNEQIPPISARLRRRAFEFSTFQDEV
C.elegans EAEIAYGISTHLRLIPTEPDQVT..........VIVNEFHMVPQKV
D.melanogaster ALRSALEILTNNHLIPASSLEVD..........ECVTNIKRMGPEV
D.rerio HIDRAYDVIERLKLVPLSQDSVE..........ERVAAFRNFSDEV
X.laevis HIDLSFDVIERLKLVPLSQDSVE..........ERVAAFRNFSDEI
H.sapiens HIDRAFDIIERLKLVPLNQESVE..........ERVAAFRNFSDEI
1000 1010 1020 1030 1039
α32 α33
C.thermophilum AINVPNLLMWTVLCCMRQRERLAGGQFAGNE.......STARLMMDELKQM
A.nidulans SGNIGHVIIWSITCIGRERERLNTGPY..EN.......EMRQGLAEELLVM
N.crassa AINVPNLLMWTIICCTRQRERLLTGQFVGNT.......GTAREMLARLKQI
P.pastoris AKNVPDLLVMTLTCIAQLTYQLTSSEF..NG.......LVKSDKIKYLKEV
A.gossypii IKNVPNLLIIAMTCVSNLIKQLSKGPF..SN.......GATQAQVEALKKV
S.cerevisiae VKNIPNLLIITLSCISNMIHILNESKY..QS.......STKGQQQIDSLKNV
S.pombe LSVIPSLMYISMSSIKALYRTISKLPV..VN.......EESKKKLQRLQFK
C.elegans REVLPDMCLHLMKCLVDHCIRQSTTQANRGA...NSATTSMFSSSNRYVKQ
D.melanogaster IKVLPDILLASMDIVYQEYVKLMDSNETASGFFDESKCVNKEPAVKHLRDR
D.rerio RHNLSEVLLATMNILFTQYKRLKGAAAGTPG....RPQRTLEDRDMLLRIQ
X.laevis RHNLSEILLATMNILFTQYKRLKGSGPTTLG....RPQRVQEDKDSVLRSQ
H.sapiens RHNLSEVLLATMNILFTQFKRLKGTSPSSSS....RPQRVIEDRDSQLRSQ
1040 1050 1060 1070 1080
α33 α34
C.thermophilum TVDLMAYTSQLRYRLPPHLHEALARASAD....
A.nidulans AKDLMIFSGMVKYKLPPRVYETLARAGADIGAF
N.crassa TVDLTTYTSQLRYRLPPHLVEALARASAD....
P.pastoris ARNCMLYAGILQYKMPRETYSMLVSLESML...
A.gossypii ANNYMIYRGMIQYKMPREVYNTLINIEVDL...
S.cerevisiae ARQCMIYAGMIQYRMPRETYSTLINIDVSL...
S.pombe GSMLVMFSTMIESRLSPQILEYLQAEQLTLL..
C.elegans IKAIVLYSATVPYKFPTHVTSRLLQLQASLGI.
D.melanogaster AKAFTNMAASVPYRMPSTTNQRLVQLEILMH..
D.rerio ARALITFAGMIPYRMAGDTNARLVQMEVLMN..
X.laevis ARALITFAGMIPYRMSGDTNARLVQMEVLMN..
H.sapiens ARTLITFAGMIPYRTSGDTNARLVQMEVLMN..
1084 1090 1100 1110

Fig. S27.
Multispecies sequence alignment of Nic96^{SOL}. Sequences from twelve diverse species were aligned and colored by sequence similarity according to the BLOSUM62 matrix from white (less than 55 % similarity), to yellow (55 % similarity), to red (100 % identity). Numbering below alignment is relative to the *C. thermophilum* sequence. Secondary structure observed in the Nic96 structure is shown above the alignment: α-helices (red bars), β-sheets (blue bars), and unstructured regions (black lines). The secondary structure of R2 is unknown, but predicted to be helical, which is indicated by a red outline. Mutations that affect binding to Nup53, Nup188, or Nup192 found by mutational analysis (figs. S20 and S28) are indicated by circles above the alignment and colored according to the measured effect; weak effect (yellow), moderate effect (orange), and abolished binding (red). Disordered regions are indicated by gray dots.

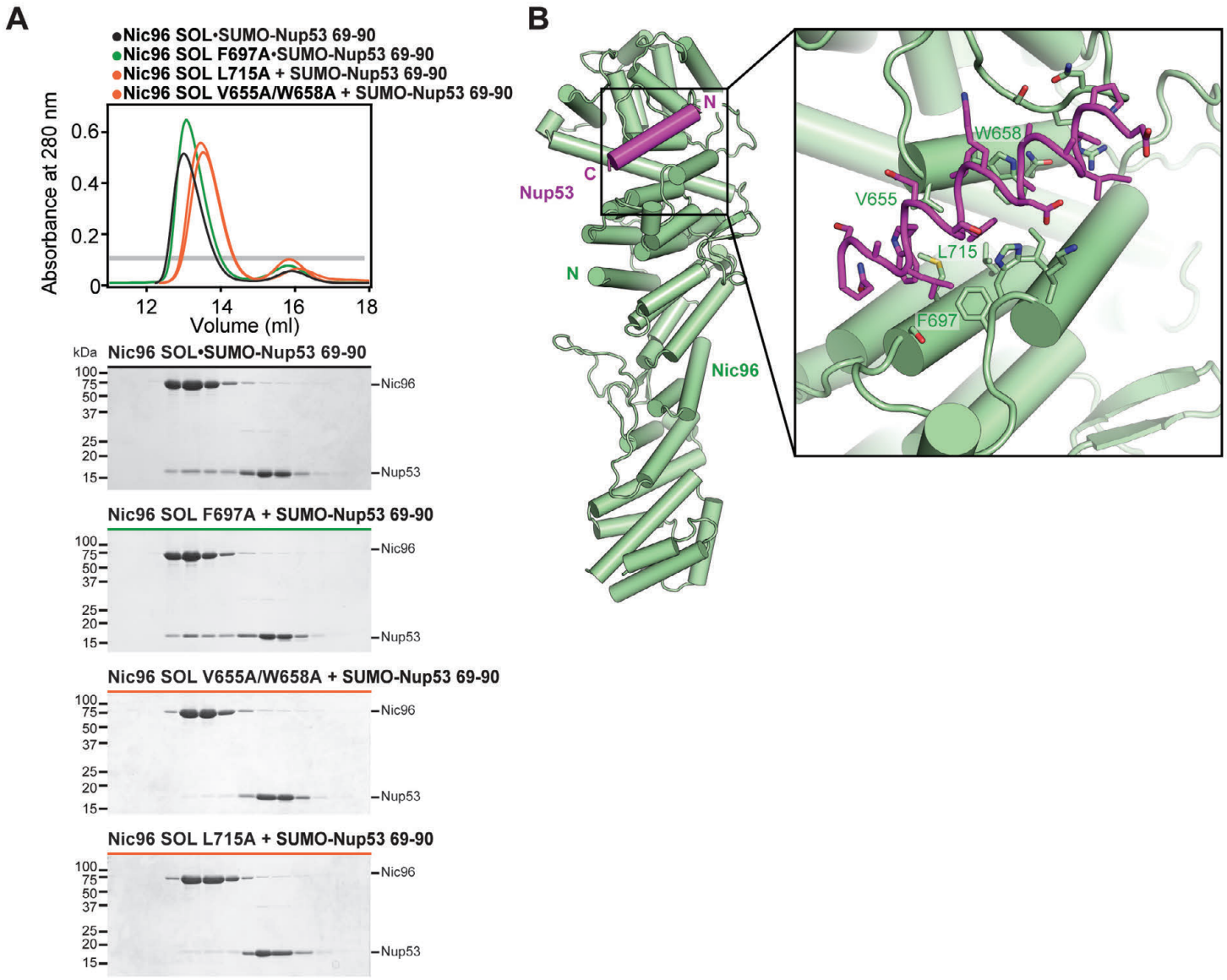

A
Nic96 SOL•SUMO-Nup53 69-90
Nic96 SOL F697A•SUMO-Nup53 69-90
Nic96 SOL L715A + SUMO-Nup53 69-90
Nic96 SOL V655A/W658A + SUMO-Nup53 69-90
Absorbance at 280 nm
0.6
0.4
0.2
0
12
14
16
18
Volume (ml)
kDa
Nic96 SOL•SUMO-Nup53 69-90
100
75
50
37
25
20
15
Nic96
Nup53
Nic96 SOL F697A + SUMO-Nup53 69-90
Nic96 SOL V655A/W658A + SUMO-Nup53 69-90
Nic96 SOL L715A + SUMO-Nup53 69-90
B
N
C
Nup53
N
Nic96
W658
V655
L715
F697

Fig. S28.
Identification of Nic96^{SOL} mutants that disrupt Nup53 binding. (**A**) SEC interaction analysis between Nic96^{SOL} mutants and SUMO-Nup53^{R2}. SEC profiles are colored according to the effect on SUMO-Nup53^{R2} binding; no effect (green), moderate effect (orange), or abolished binding (red). For reference, the SEC profile of the wild type Nic96^{SOL}•SUMO-Nup53^{R2} hetero-dimer (black) is shown. All SEC profiles were obtained using a Superdex 200 10/300 GL column. A gray bar indicates fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. (**B**) The structure of Nic96^{SOL}•Nup53^{R2} is shown in cartoon representation. The inset illustrates the region that is expanded on the right with mutated residues labeled in green.

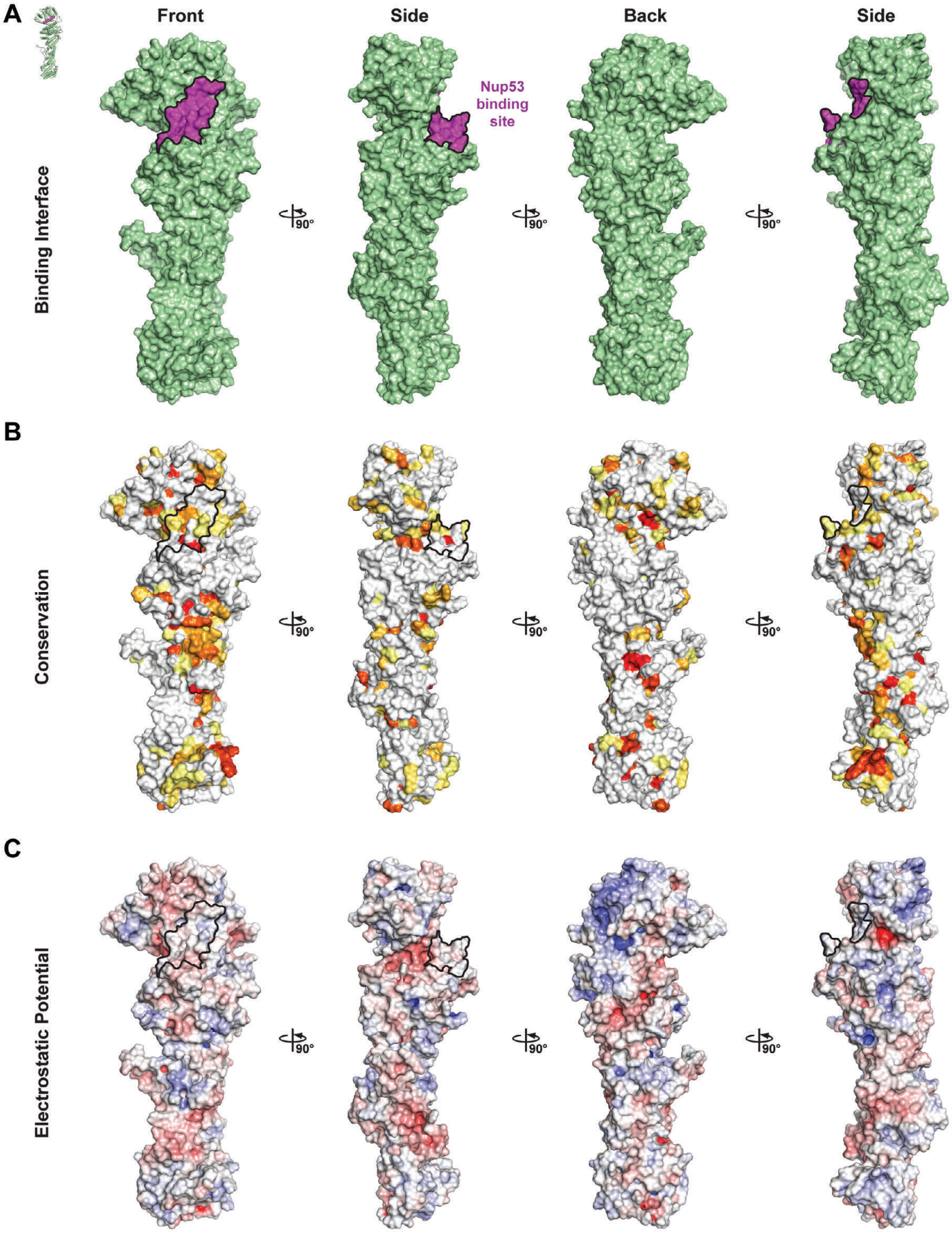
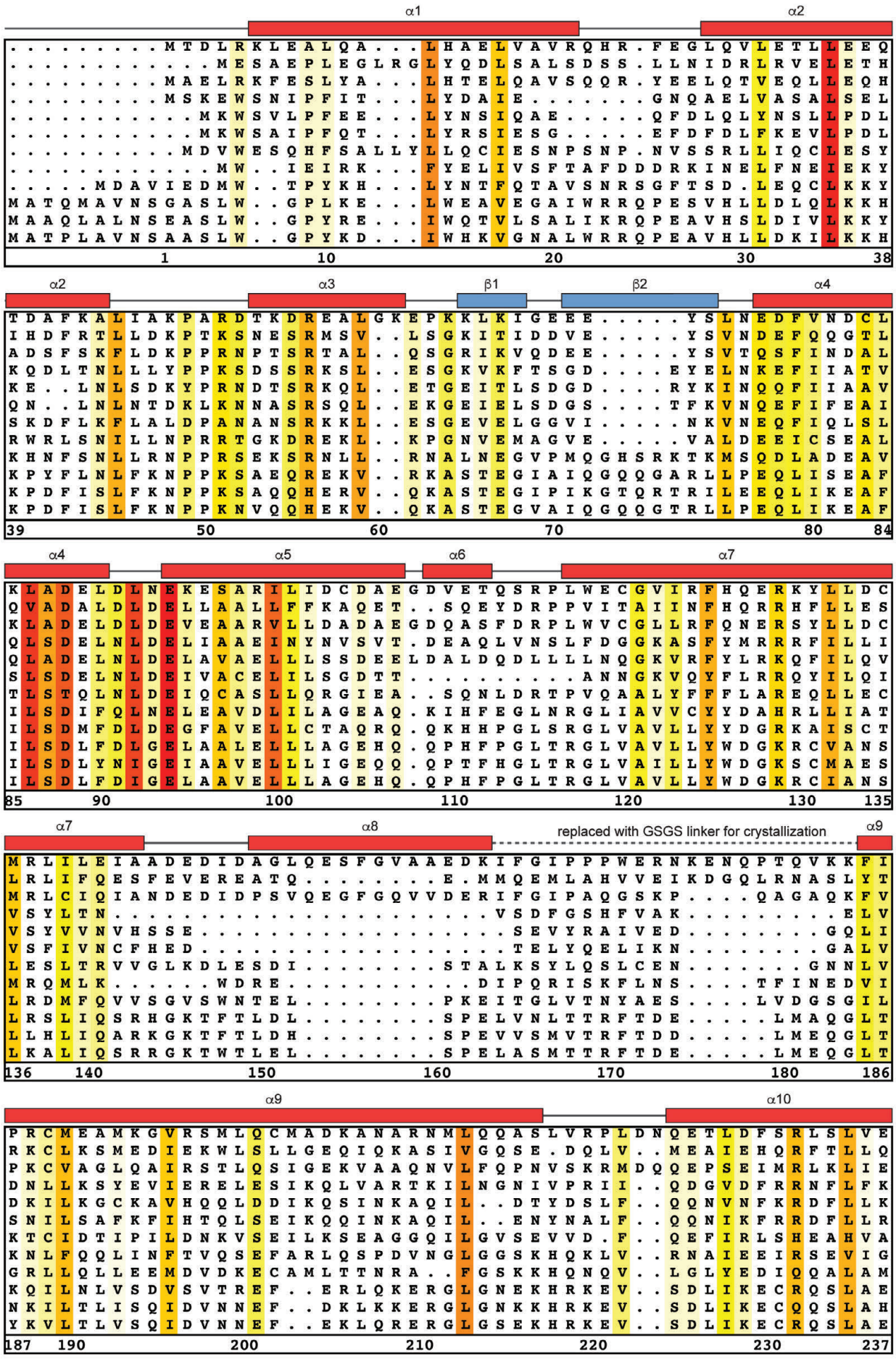
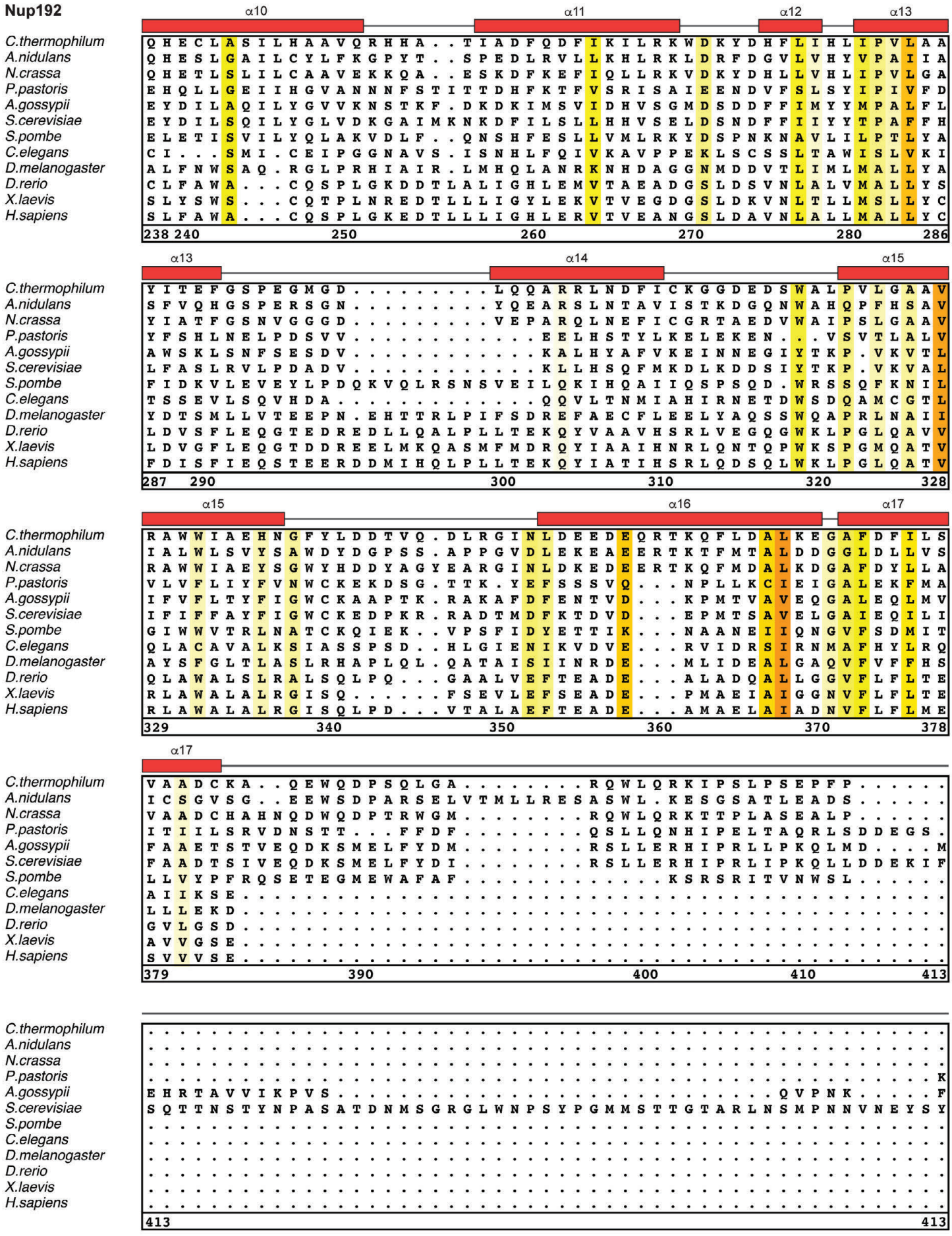
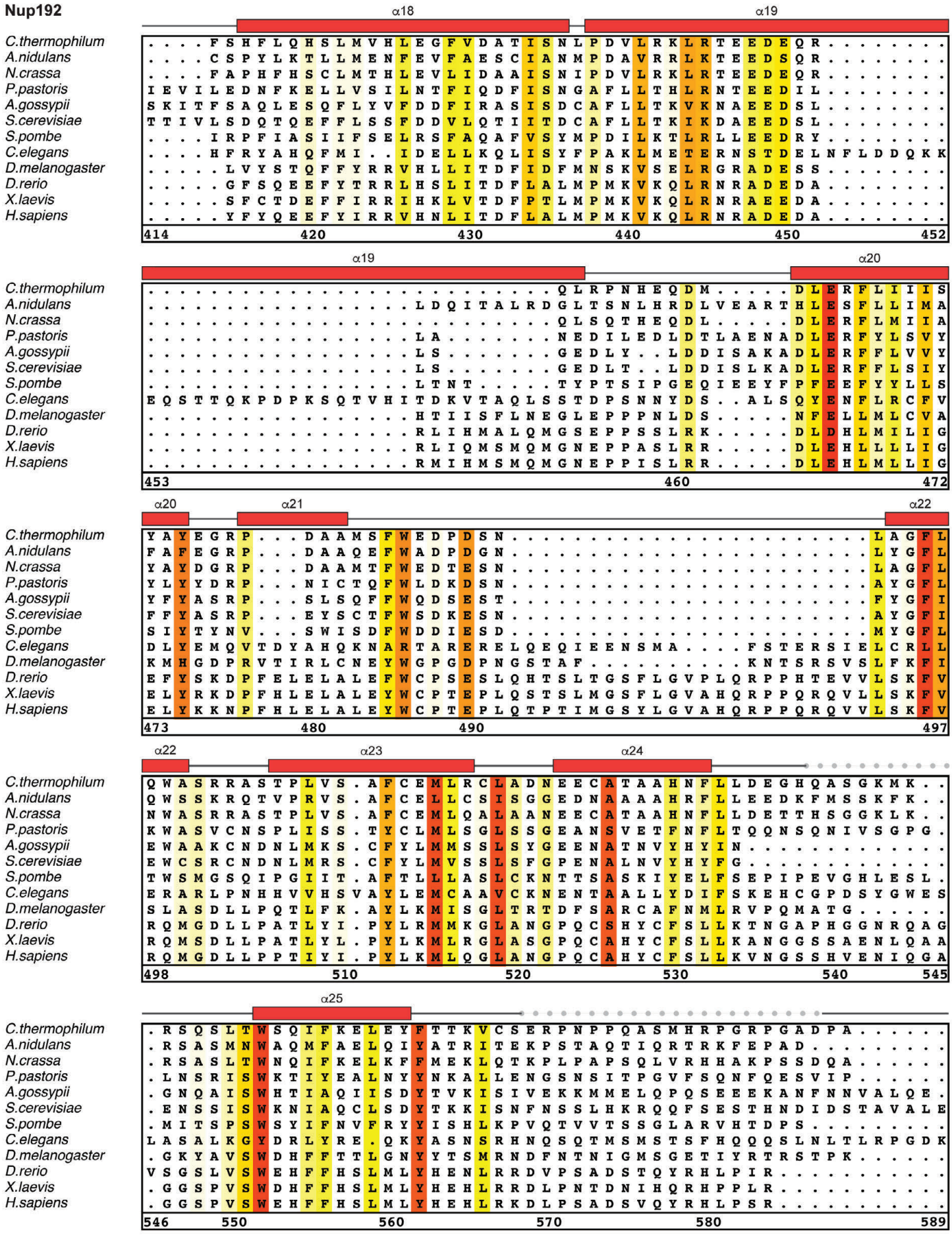

A
Front
Side
Back
Side
Binding Interface
Nup53 binding site
90°
90°
90°
B
Conservation
90°
90°
90°
C
Electrostatic Potential
90°
90°
90°

Fig. S29.
Surface properties of Nic96^{SOL}. Surface representations of Nic96^{SOL} are shown in four different orientations related by 90° rotations. The Nup53 binding interface is outlined in black. (**A**) Surface representation with the Nup53 binding site colored in purple. (**B**) Surface representation colored according to sequence identity based on the alignment in fig. S27. (**C**) Surface representation colored according to electrostatic potential from -10 k_BT/e (red) to +10 k_BT/e (blue).

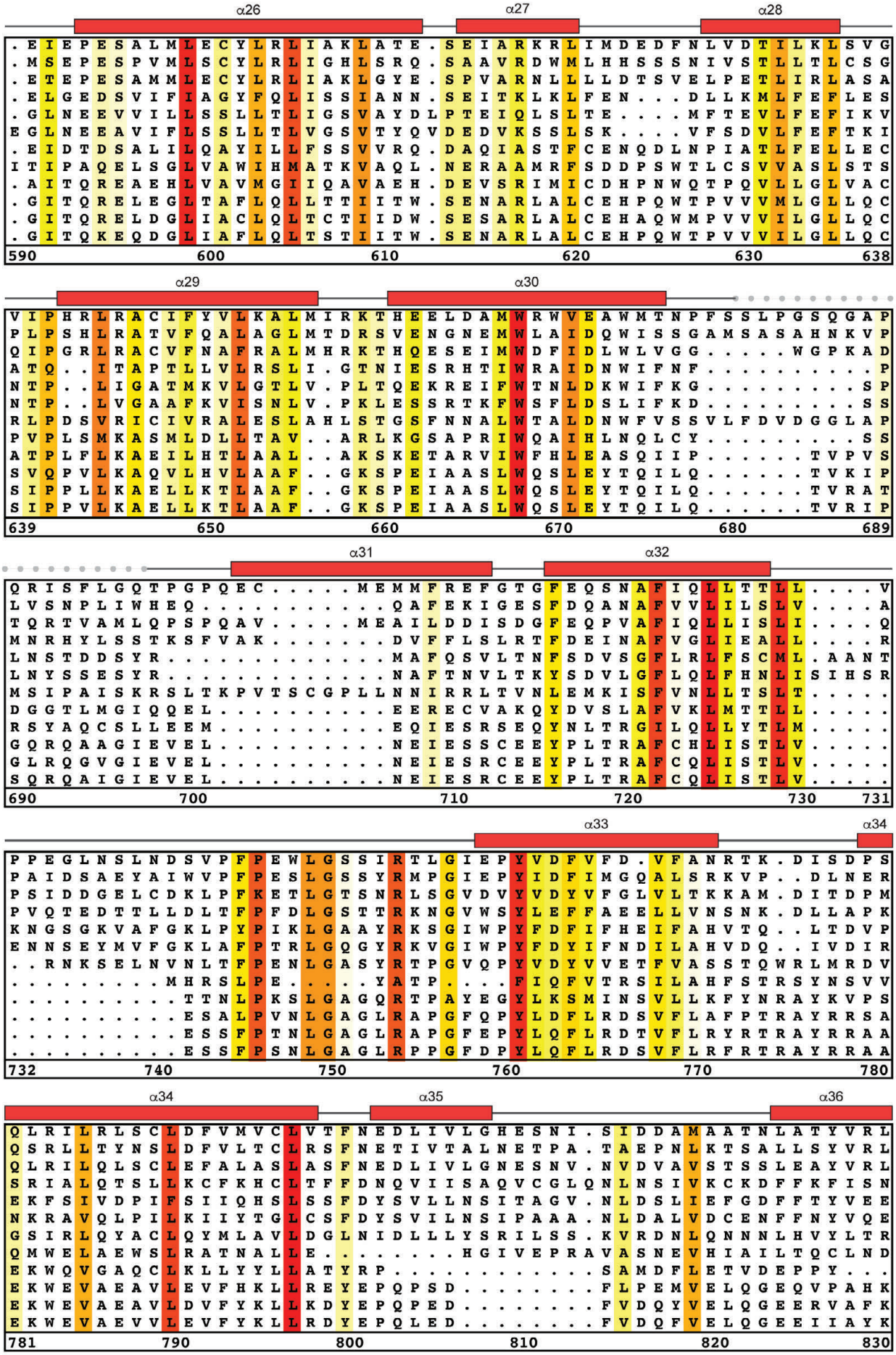
Nup192

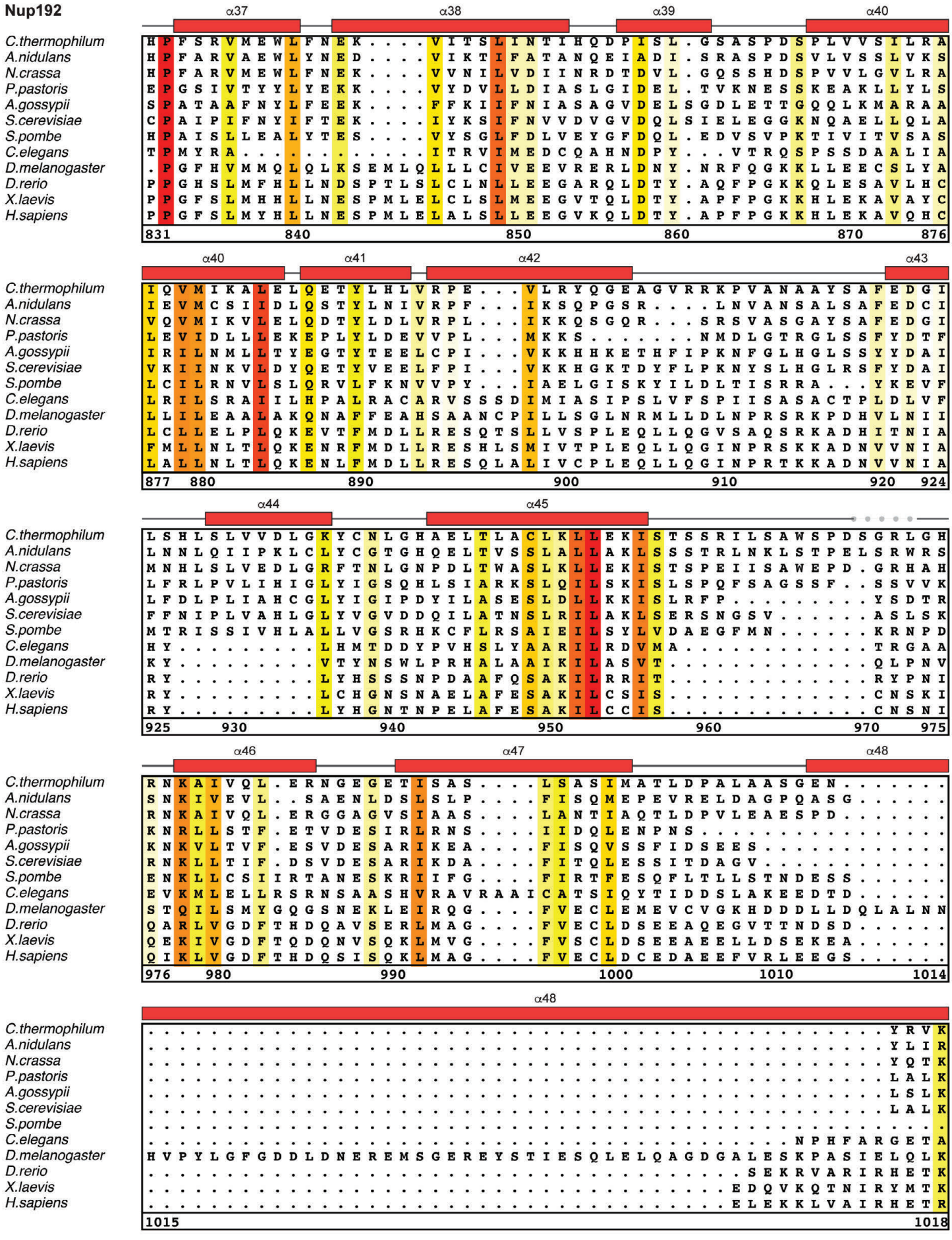

α1
α2
C.thermophilum . . . . . MTDLRKLEALQA . . . LHAELVAVRQHR . FEGLQVLETLLEEQ
A.nidulans . . . . . MESAEPLEGLRG LYQDLSALSDSS . LLNIDRLRVELETH
N.crassa . . . . . MAELRKFESLYA . . . LHTELQAVSQQR . YEELQTVEQLLEQH
P.pastoris . . . . . MSKEWSNIPFIT . . . LYDAIE . . . . . GNQAELVASALSEL
A.gossypii . . . . . MKWSVLPFEE . . . LYNSIQAE . . . . . QFDLQLYNSLLPDL
S.cerevisiae . . . . . MKWSAIPFQT . . . LYRSIESG . . . . . EFDFDLFKEVLPDL
S.pombe . . . . . MDVWESQHFSALLY LLQCIESNPSNP . NVSSRLLIQCLESY
C.elegans . . . . . MW . . IEIRK . . . FYELIVSFTAFDDDRKINELFNEIEKY
D.melanogaster . . . . . MDAVIEDMW . . TPYKH . . . LYNTFQTAVSNRSGFTSD . LEEQCLKKY
D.rerio MATQMAVNSGASLW . . GPLKE . . . LWEAVEGAIWRRQPESVHLLDLQLKKH
X.laevis MAAQLALNSEASLW . . GPYRE . . . IWQTVLSALIKRQPEAVHSLDIVLKKY
H.sapiens MATPLAVNSAASLW . . GPYKD . . . IWHKVGNALWRRQPEAVHLLDKILKKH
1 10 20 30 38
α2
α3
β1
β2
α4
C.thermophilum TDAFKALIAKPARDTKDREALGKEPKKLKIGEEE . . . . . YSLNEDFVNDCL
A.nidulans IHDFRTLLDKPTKSNESRMSV . . LSGKITIDDVE . . . . . YSVNDEFQQGTL
N.crassa ADSFSKFLDKPPRNPTSRTAL . . QSGRIKVQDEE . . . . . YSVTQSFINDAL
P.pastoris KQDLTNLLLYPPKSDSSRKSL . . ESGKVKFTSGD . . . . EYELNKEFIIATV
A.gossypii KE . . LNLSDKYPRNDTSRKQL . . ETGEITLSDGD . . . . RYKINQQFIIAAV
S.cerevisiae QN . . LNLNTDKLKNNASRSQL . . EKGEIELSDGS . . . . TFKVNQEFIFEAI
S.pombe SKDFLKFLALDPANANSRKKL . . ESGEVELGGVI . . . . . NKVNEQFIQLSSL
C.elegans RWRLSNILLNPRRTGKDREKL . . KPGNVEMAGVE . . . . . VALDEEICSEAL
D.melanogaster KHNFSNLLRNPPRSEKSRNLL . . RNALNEGVPMQGHSRKTKMSQDLADEAV
D.rerio KPYFLNLFKNPPKSAEQREKV . . RKASTEGIAIQGQQGARLLPEQLISEAF
X.laevis KPDFISLFKNPPKSAQQHERV . . QKASTEGIPIKGTQRTRILEEQLIKEAF
H.sapiens KPDFISLFKNPPKNVQQHEKV . . QKASTEGVAIQGQQGTRLLPEQLIKEAF
39 50 60 70 80 84
α4
α5
α6
α7
C.thermophilum KLADELDLNEKESARILIDCDAEGDVETQSRPLWECGVIRFHQERKYLLDC
A.nidulans QVADALDLDELLAALLFFKAQET . . SQEYDRPPVITAIINFHQRRHFLLES
N.crassa KLADELDLDEVEAARVLLDADAEGDQASFDRPLWVCGLLRFQNERSYLLDC
P.pastoris QLSDELNLDELIAAEINYYNVSVT . DEAQLVNSLFDGGKASFYMRRFILLI
A.gossypii QLADELNLDELAVAELLLSSDEELDALDQDLLLLNQGKVRFYLRKQFILQV
S.cerevisiae SLSDELNLDEIVACELILSGDTT . . . . . ANNGKVQYFLRRQYILQI
S.pombe TLSTQLNLDEIQCASLLQRGIEA . . SQNLDRTPVQAALYFFFLAREQLLEC
C.elegans ILSDIFQLNELEAVDLILAGEAQ . KIHFEGLNRGLIAVVCYYDAHRLLIAT
D.melanogaster ILSDMFDLDEGFAVELLCTAQRQ . QKHHPGLSRGLVAVLLYYDGRKAISCT
D.rerio ILSDLFDLGELAALELLLAGEQQ . QPHFPGLTRGLVAVLLYWDGKRCVANS
X.laevis ILSDLYNIGEIAAVELLLIGEQQ . QPTFHGLTRGLVAILLYWDGKSCMAES
H.sapiens ILSDLFDIGELAAVELLLAGEHQ . QPHFPGLTRGLVAVLLYWDGKRCIANS
85 90 100 110 120 130 135
α7
α8
replaced with GSGS linker for crystallization
α9
C.thermophilum MRLILEIAADEDIDAGLQESFGVAAEDKIFGIPPPWERNKENQPTQVKKFI
A.nidulans LRLIFQESFEVEREATQ . . . . . E . MMQEMLAHVVEIKDGQLRNASLYT
N.crassa MRLCIQIANDEDIDPSVQEGFGQVVDERIFGIPAQGSKP . . . . QAGAQKFV
P.pastoris VSYLTN . . . . . VSDFGSHFVAK . . . . . ELV
A.gossypii VSYVVNVHSSE . . . . . SEVYRAIVED . . . . . GQLI
S.cerevisiae VSFIVNCFHED . . . . . TELYQELIKN . . . . . GALV
S.pombe LESLTRVVGLKDLESDI . . . . . STALKSYLQSLCEN . . . . . GNNLV
C.elegans MRQMLK . . . . . WDRE . . . . . DIPQRISKFLNS . . . . TFINEDVI
D.melanogaster LRDMFQVVSGVSWNTEL . . . . . PKEITGLVTNYAES . . . . LVDGSGIL
D.rerio LRSLIQSRHGKTFTLDL . . . . . SPELVNLTTRFTDE . . . . . LMAQGLT
X.laevis LLHLIQARKGKTFTLDH . . . . . SPEVVSMVTRFTDD . . . . . LMEQGLT
H.sapiens LKALIQSRRGKTWTLEL . . . . . SPELASMTTRFTDE . . . . . LMEQGLT
136 140 150 160 170 180 186
α9
α10
C.thermophilum PRCMEAMKGVRSMLQCMADKANARNMLQQASLVRPLDNQETLDFSRLSLVE
A.nidulans RKCLKSMEDIEKWLSLLGEQIQKASIVGQSE . DQLV . . MEAIEHQRFTLLQ
N.crassa PKCVAGLQAIRSTLQSIGEKVAAQNVLFQPNVSKRMDQQEPSEIMRLKLIE
P.pastoris DNLLKSYEVIERELESIKQLVARTKILNGNIVPRII . . QDGVDFRRNFLFK
A.gossypii DKILKGCKAVHQQLDDIKQSINKAQIL . . DTYDSLF . . QQNVNFKRDFLLK
S.cerevisiae SNILSAFKFIHTQLSEIKQQINKAQIL . . ENYNALF . . QQNIKFRRDFLLR
S.pombe KTCIDTIPILDNKVSEILKSEAGGQIL GVSEVVD . F . . QEFIRLSHEAHVA
C.elegans KNLFQQLINFTVQSEFARLQSPDVNGL GGSKHQKLV . . RNAIEEIRSEVIG
D.melanogaster GRLLQLLEEMDVDKECAMLTTNRA . . FGSKKHQNQV . . LGLYEDIQQALAM
D.rerio KQILNLVSDVSVTREF . . ERLQKERGL GNEKHRKEV . . SDLIKECRQSLAE
X.laevis NKILTLISQIDVNNEF . . DKLKKERGL GNKKHRKEV . . SDLIKECQQSLAH
H.sapiens YKVLTLVSQIDVNNEF . . EKLQRERGL GSEKHRKEV . . SDLIKECRQSLAE
187 190 200 210 220 230 237


Nup192
α10
α11
α12
α13
C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
238 240 250 260 270 280 286
α13
α14
α15
287 290 300 310 320 328
α15
α16
α17
329 340 350 360 370 378
α17
379 390 400 410 413
413 413

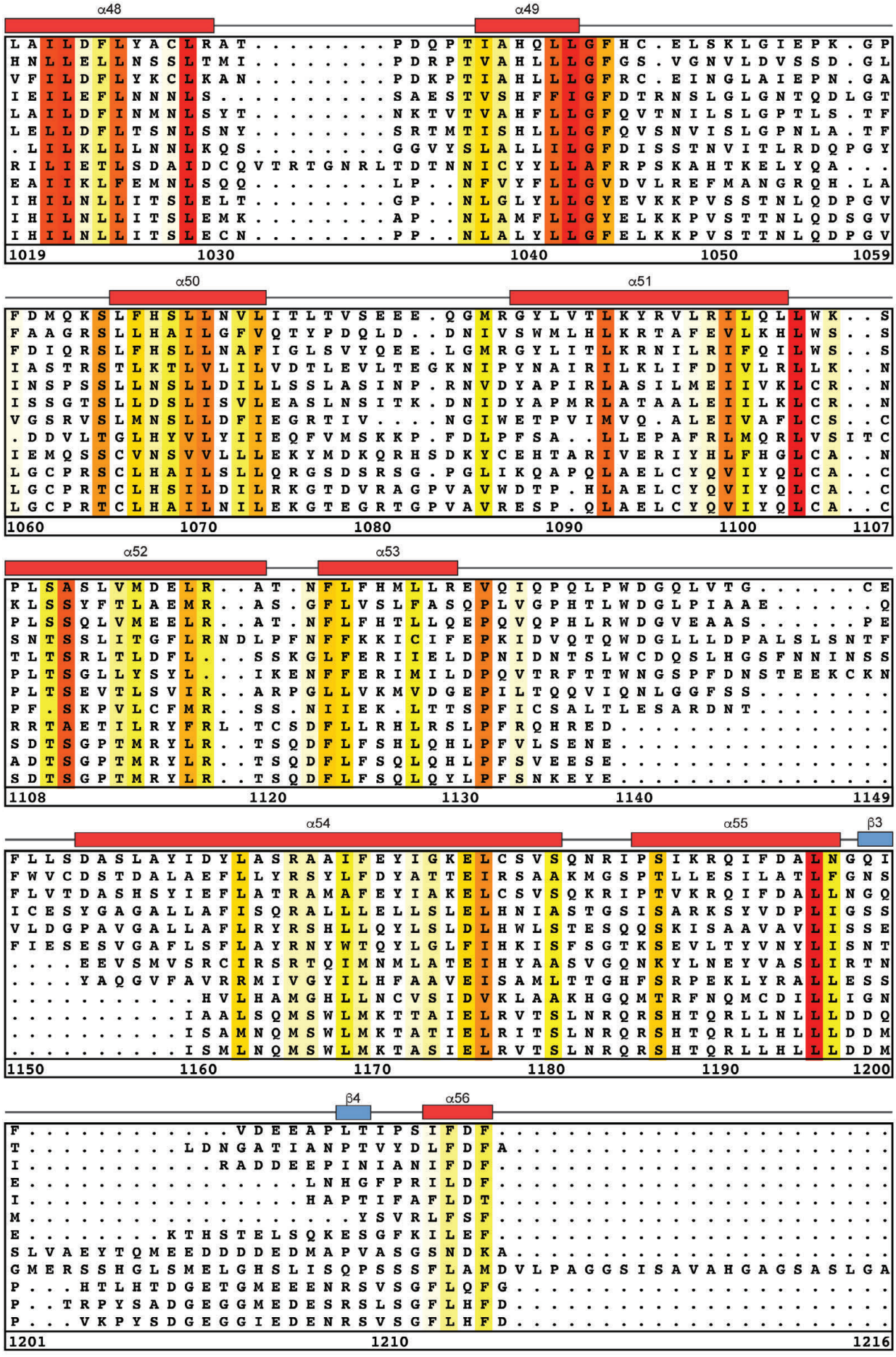

Nup192
α18
α19
C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
414
420
430
440
450
452
α19
α20
453
460
472
α20
α21
α22
473
480
490
497
α22
α23
α24
498
510
520
530
540
545
α25
546
550
560
570
580
589

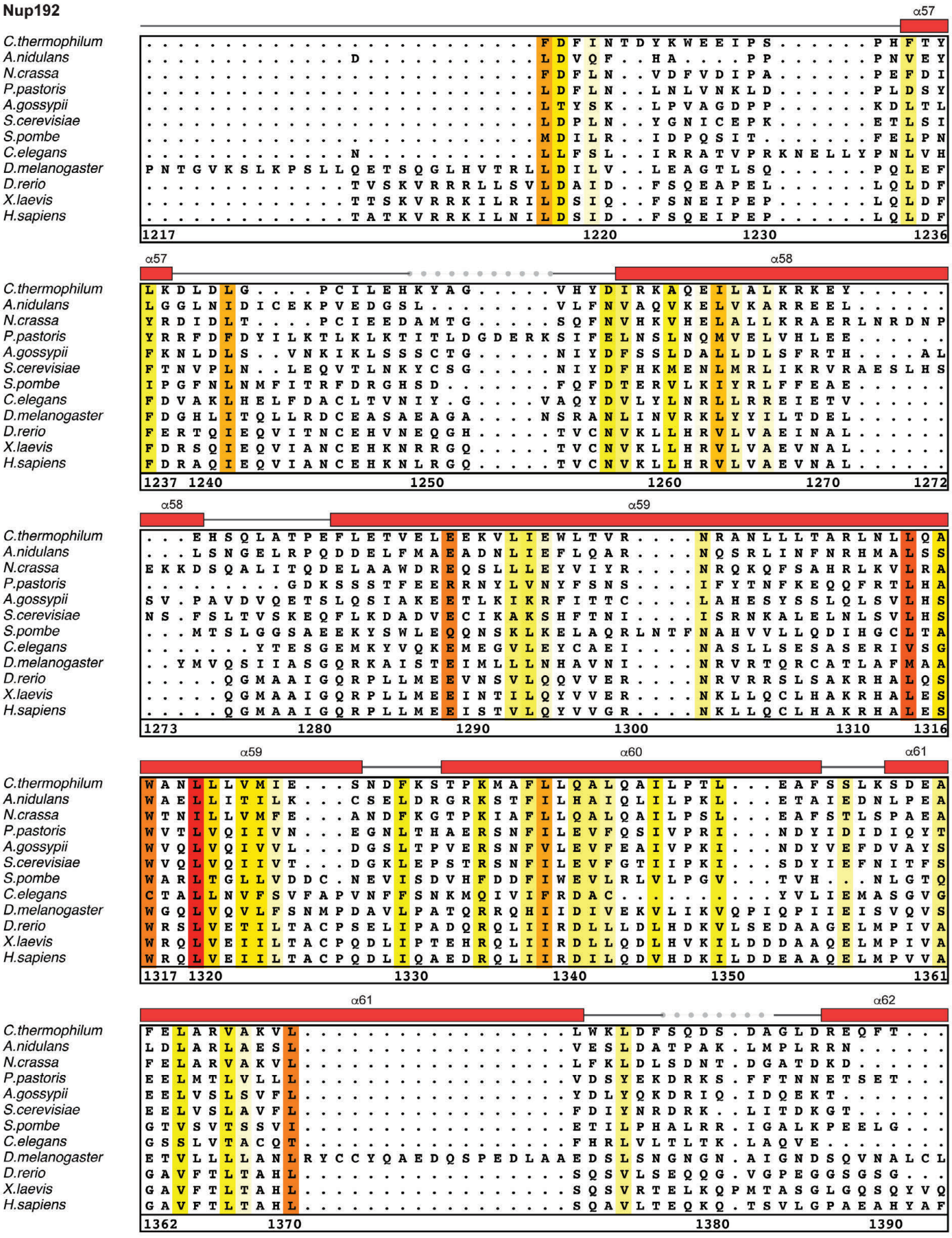
Nup192


α26
α27
α28
C.thermophilum . E I E P E S A L M L E C Y L R L I A K L A T E . S E I A R K R L I M D E D F N L V D T I L K L S V G
A.nidulans . M S E P E S P V M L S C Y L R L I G H L S R Q . S A A V R D W M L H H S S S N I V S T L L T L C S G
N.crassa . E T E P E S A M M L E C Y L R L I A K L G Y E . S P V A R N L L L L D T S V E L P E T L I R L A S A
P.pastoris . E L G E D S V I F I A G Y F Q L I S S I A N N . S E I T K L K L F E N . . . D L L K M L F E F L E S
A.gossypii . G L N E E V V I L L S S L L T L I G S V A Y D L P T E I Q L S L T E . . . . M F T E V L F E F I K V
S.cerevisiae E G L N E E A V I F L S S L L T L V G S V T Y Q V D E D V K S S L S K . . . . V F S D V L F E F T K I
S.pombe . E I D T D S A L I L Q A Y I L L F S S V V R Q . D A Q I A S T F C E N Q D L N P I A T L F E L L E C
C.elegans I T I P A Q E L S G L V A W I H M A T K V A Q L . N E R A A M R F S D D P S W T L C S V V A S L S T S
D.melanogaster . A I T Q R E A E H L V A V M G I I Q A V A E H . D E V S R I M I C D H P N W Q T P Q V L L G L V A C
D.rerio . G I T Q R E L E G L T A F L Q L L T T I I T W . S E N A R L A L C E H P Q W T P V V V M L G L L Q C
X.laevis . G I T Q R E L D G L I A C L Q L T C T I I D W . S E S A R L A L C E H A Q W M P V V V I L G L L Q C
H.sapiens . G I T Q K E Q D G L I A F L Q L T S T I I T W . S E N A R L A L C E H P Q W T P V V V I L G L L Q C
590 600 610 620 630 638
α29
α30
C.thermophilum V I P H R L R A C I F Y V L K A L M I R K T H E E L D A M W R W V E A W M T N P F S S L P G S Q G A P
A.nidulans P L P S H L R A T V F Q A L A G L M T D R S V E N G N E M W L A I D Q W I S S G A M S A S A H N K V P
N.crassa Q I P G R L R A C V F N A F R A L M H R K T H Q E S E I M W D F I D L W L V G G . . . . . W G P K A D
P.pastoris A T Q . . I T A P T L L V L R S L I . G T N I E S R H T I W R A I D N W I F N F . . . . . P
A.gossypii N T P . . L I G A T M K V L G T L V . P L T Q E K R E I F W T N L D K W I F K G . . . . . S P
S.cerevisiae N T P . . L V G A A F K V I S N L V . P K L E S S R T K F W S F L D S L I F K D . . . . . S S
S.pombe R L P D S V R I C I V R A L E S L A H L S T G S F N N A L W T A L D N W F V S S V L F D V D G G L A P
C.elegans P V P L S M K A S M L D L L T A V . . A R L K G S A P R I W Q A I H L N Q L C Y . . . . . S S
D.melanogaster A T P L F L K A E I L H T L A A L . . A K S K E T A R V I W F H L E A S Q I I P . . . . . T V P V S
D.rerio S V Q P V L K A Q V L H V L A A F . . G K S P E I A A S L W Q S L E Y T Q I L Q . . . . . T V K I P
X.laevis S I P P L L K A E L L K T L A A F . . G K S P E I A A S L W Q S L E Y T Q I L Q . . . . . T V R A T
H.sapiens S I P P V L K A E L L K T L A A F . . G K S P E I A A S L W Q S L E Y T Q I L Q . . . . . T V R I P
639 650 660 670 680 689
α31
α32
C.thermophilum Q R I S F L G Q T P G P Q E C . . . . . M E M M F R E F G T G F E Q S N A F I Q L L T T L L V
A.nidulans L V S N P L I W H E Q . . . . . Q A F E K I G E S F D Q A N A F V V L I L S L V . . . . A
N.crassa T Q R T V A M L Q P S P Q A V . . . . . M E A I L D D I S D G F E Q P V A F I Q L L I S L I . . . . Q
P.pastoris M N R H Y L S S T K S F V A K . . . . . D V F F L S L R T F D E I N A F V G L I E A L L . . . . . R
A.gossypii L N S T D D S Y R . . . . . M A F Q S V L T N F S D V S G F L R L F S C M L . A A N T
S.cerevisiae L N Y S S E S Y R . . . . . N A F T N V L T K Y S D V L G F L Q L F H N L I S I H S R
S.pombe M S I P A I S K R S L T K P V T S C G P L L N N I R R L T V N L E M K I S F V N L L T S L T . . . . .
C.elegans D G G T L M G I Q Q E L . . . . . E E R E C V A K Q Y D V S L A F V K L M T T L L . . . . .
D.melanogaster R S Y A Q C S L L E E M . . . . . E Q I E S R S E Q Y N L T R G I L Q L L Y T L M . . . . .
D.rerio G Q R Q A A G I E V E L . . . . . N E I E S S C E E Y P L T R A F C H L I S T L V . . . . .
X.laevis G L R Q G V G I E V E L . . . . . N E I E S R C E E Y P L T R A F C Q L I S T L V . . . . .
H.sapiens S Q R Q A I G I E V E L . . . . . N E I E S R C E E Y P L T R A F C Q L I S T L V . . . . .
690 700 710 720 730 731
α33
α34
C.thermophilum P P E G L N S L N D S V P F P E W L G S S I R T L G I E P Y V D F V F D . V F A N R T K . D I S D P S
A.nidulans P A I D S A E Y A I W V P F P E S L G S S Y R M P G I E P Y I D F I M G Q A L S R K V P . . D L N E R
N.crassa P S I D D G E L C D K L P F K E T L G T S N R L S G V D V Y V D Y V F G L V L T K K A M . D I T D P M
P.pastoris P V Q T E D T T L L D L T F P F D L G S T T R K N G V W S Y L E F F A E E L L V N S N K . D L L A P K
A.gossypii K N G S G K V A F G K L P Y P I K L G A A Y R K S G I W P Y F D F I F H E I F A H V T Q . . L T D V P
S.cerevisiae E N N S E Y M V F G K L A F P T R L G Q G Y R K V G I W P Y F D Y I F N D I L A H V D Q . . I V D I R
S.pombe . . R N K S E L N V N L T F P E N L G A S Y R T P G V Q P Y V D Y V E T F V A S S T Q W R L M R D V
C.elegans . . . . . M H R S L P E . . . . . Y A T P . . . . F I Q F V T R S I L A H F S T R S Y N S V V
D.melanogaster . . . . . T T N L P K S L G A G Q R T P A Y E G Y L K S M I N S V L L K F Y N R A Y K V P S
D.rerio . . . . . E S A L P V N L G A G L R A P G F Q P Y L D F L R D S V F L A F P T R A Y R R S A
X.laevis . . . . . E S S F P T N L G A G L R A P G F E P Y L Q F L R D T V F L R Y R T R A Y R R A A
H.sapiens . . . . . E S S F P S N L G A G L R P P G F D P Y L Q F L R D S V F L R F R T R A Y R R A A
732 740 750 760 770 780
α34
α35
α36
C.thermophilum Q L R I L R L S C L D F V M V C L V T F N E D L I V L G H E S N I . S I D D A M A A T N L A T Y V R L
A.nidulans Q S R L L T Y N S L D F V L T C L R S F N E T I V T A L N E T P A . T A E P N L K T S A L L S Y V R L
N.crassa Q L R I L Q L S C L E F A L A S L A S F N E D L I V L G N E S N V . N V D V A V S T S S L E A Y V R L
P.pastoris S R I A L Q T S L L K C F K H C L T F F D N Q V I I S A Q V C G L Q N L N S I V K C K D F F K F I S N
A.gossypii E K F S I V D P I F S I I Q H S L S S F D Y S V L L N S I T A G V . N L D S L I E F G D F F T Y V E E
S.cerevisiae N K R A V Q L P I L K I I Y T G L C S F D Y S V I L N S I P A A A . N L D A L V D C E N F F N Y V Q E
S.pombe G S I R L Q Y A C L Q Y M L A V L D G L N I D L L L Y S R I L S S . K V R D N L Q N N N L H V Y L T R
C.elegans Q M W E L A E W S L R A T N A L L E . . . . . H G I V E P R A V A S N E V H I A I L T Q C L N D
D.melanogaster E K W Q V G A Q C L K L L Y Y L L A T Y R P . . . . . S A M D F L E T V D E P P Y . . .
D.rerio E K W E V A E A V L E V F H K L L R E Y E P Q P S D . . . . . F L P E M V E L Q G E Q V P A H K
X.laevis E K W E V A E A V L D V F Y K L L K D Y E P Q P E D . . . . . F V D Q Y V E L Q G E E R V A F K
H.sapiens E K W E V A E V V L E V F Y K L L R D Y E P Q L E D . . . . . F V D Q F V E L Q G E E I I A Y K
781 790 800 810 820 830

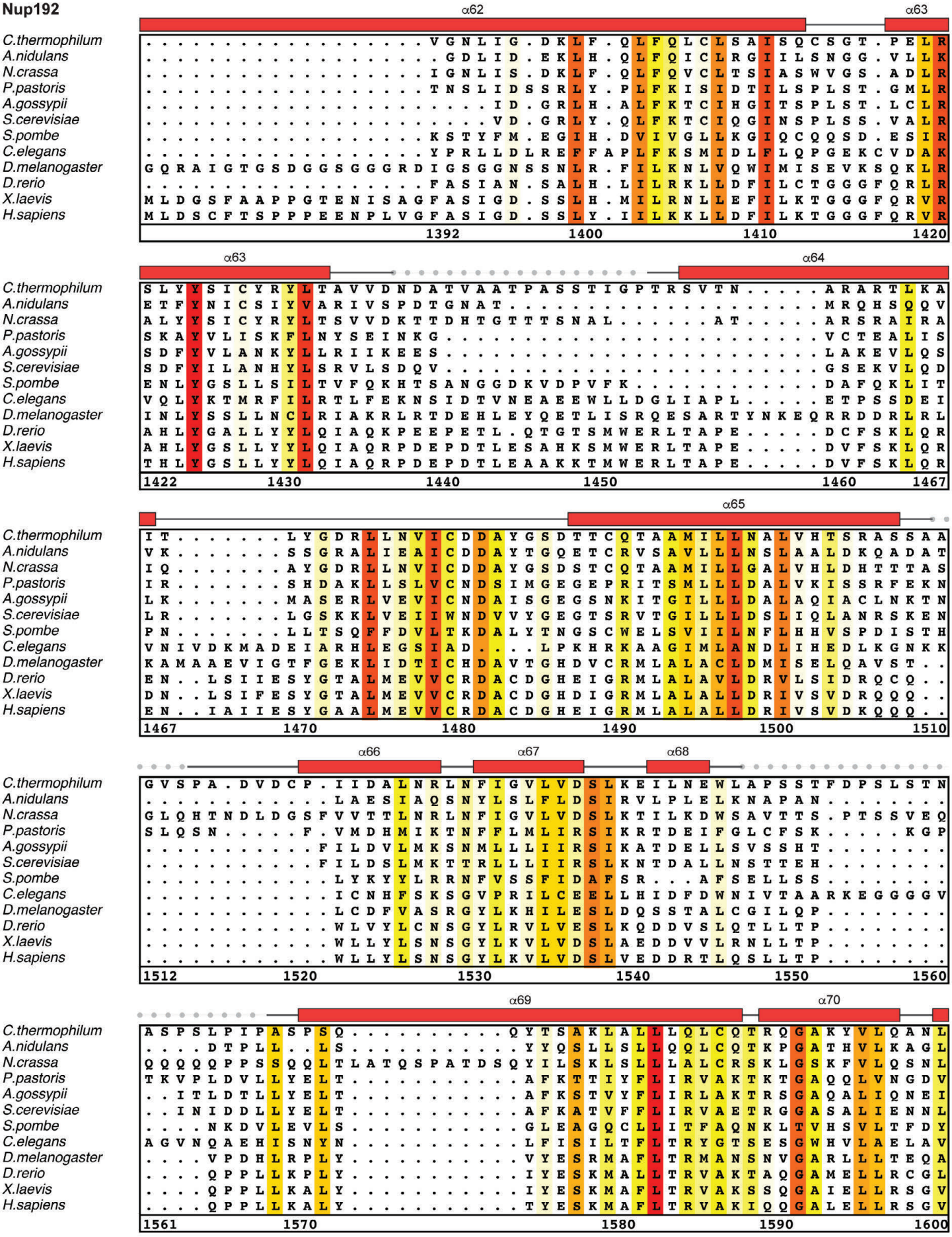

Nup192
α37
α38
α39
α40
C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
831
840
850
860
870
876
α40
α41
α42
α43
877
880
890
900
910
920
924
α44
α45
925
930
940
950
960
970
975
α46
α47
α48
976
980
990
1000
1010
1014
α48
1015
1018

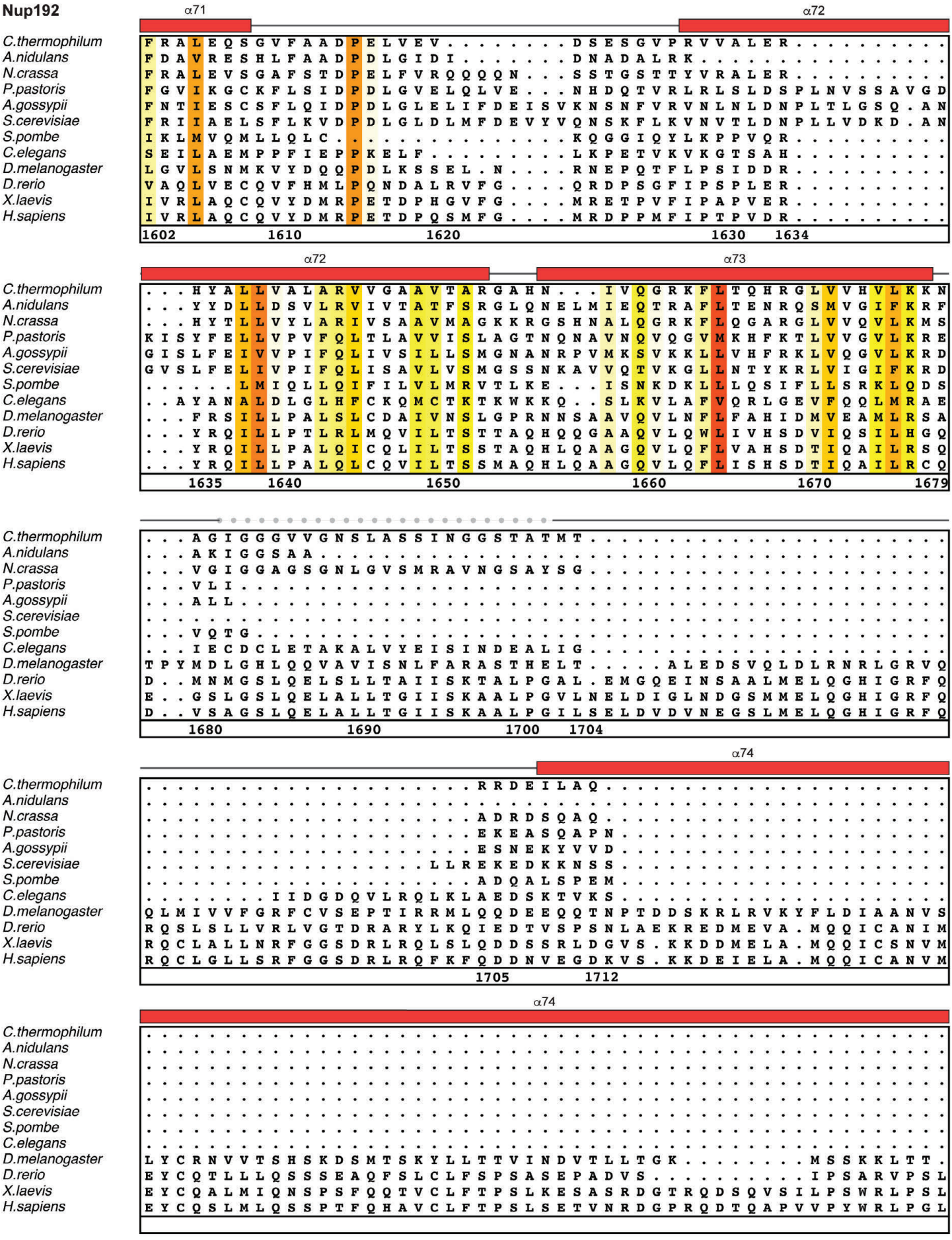
Nup192


α48
α49
C.thermophilum L A I L D F L Y A C L R A T . . . . . P D Q P T I A H Q L L G F H C . E L S K L G I E P K . G P
A.nidulans H N L L E L L N S S L T M I . . . . . P D R P T V A H L L L G F G S . V G N V L D V S S D . G L
N.crassa V F I L D F L Y K C L K A N . . . . . P D K P T I A H L L L G F R C . E I N G L A I E P N . G A
P.pastoris I E I L E F L N N N L S . . . . . S A E S T V S H F F I G F D T R N S L G L G N T Q D L G T
A.gossypii L A I L D F I N M N L S Y T . . . . . N K T V T V A H F L L G F Q V T N I L S L G P T L S . T F
S.cerevisiae L E L L D F L T S N L S N Y . . . . . S R T M T I S H L L L G F Q V S N V I S L G P N L A . T F
S.pombe . L I L K L L L N N L K Q S . . . . . G G V Y S L A L L I L G F D I S S T N V I T L R D Q P G Y
C.elegans R I L I E T L S D A I D C Q V T R T G N R L T D T N N I C Y Y L L A F R P S K A H T K E L Y Q A . . .
D.melanogaster E A I I K L F E M N L S Q Q . . . . . L P . . N F V Y F L L G V D V L R E F M A N G R Q H . L A
D.rerio I H I L N L L I T S L E L T . . . . . G P . . N L G L Y L L G Y E V K K P V S S T N L Q D P G V
X.laevis I H I L N L L I T S L E M K . . . . . A P . . N L A M F L L G Y E L K K P V S T T N L Q D S G V
H.sapiens I H I L N L L I T S L E C N . . . . . P P . . N L A L Y L L G F E L K K P V S T T N L Q D P G V
1019 1030 1040 1050 1059
α50
α51
C.thermophilum F D M Q K S L F H S L L N V L I T L T V S E E E . Q G M R G Y L V T L K Y R V L R I L Q L L W K . . S
A.nidulans F A A G R S L L H A I L G F V Q T Y P D Q L D . . D N I V S W M L H L K R T A F E V L K H L W S . . S
N.crassa F D I Q R S L F H S L L N A F I G L S V Y Q E E . L G M R G Y L I T L K R N I L R I F Q I L W S . . S
P.pastoris I A S T R S T L K T L V L I L V D T L E V L T E G K N I P Y N A I R I L K L I F D I V L R L L K . . N
A.gossypii I N S P S S L L N S L L D I L L S S L A S I N P . R N V D Y A P I R L A S I L M E I I V K L C R . . N
S.cerevisiae I S S G T S L L D S L I S V L E A S L N S I T K . D N I D Y A P M R L A T A A L E I I L K L C R . . N
S.pombe V G S R V S L M N S L L D F I E G R T I V . . . . N G I W E T P V I M V Q . A L E I V A F L C S . . C
C.elegans . D D V L T G L H Y V L Y I I E Q F V M S K K P . F D L P F S A . . L L E P A F R L M Q R L V S I T C
D.melanogaster I E M Q S S C V N S V V L L L E K Y M D K Q R H S D K Y C E H T A R I V E R I Y H L F H G L C A . . N
D.rerio L G C P R S C L H A I L S L L Q R G S D S R S G . P G L I K Q A P Q L A E L C Y Q V I Y Q L C A . . C
X.laevis L G C P R T C L H S I L D I L R K G T D V R A G P V A V W D T P . H L A E L C Y Q V I Y Q L C A . . C
H.sapiens L G C P R T C L H A I L N I L E K G T E G R T G P V A V R E S P . Q L A E L C Y Q V I Y Q L C A . . C
1060 1070 1080 1090 1100 1107
α52
α53
C.thermophilum P L S A S L V M D E L R . . A T . N F L F H M L L R E V Q I Q P Q L P W D G Q L V T G . . . . . C E
A.nidulans K L S S Y F T L A E M R . . A S . G F L V S L F A S Q P L V G P H T L W D G L P I A A E . . . . . Q
N.crassa P L S S Q L V M E E L R . . A T . N F L F H T L L Q E P Q V Q P H L R W D G V E A A S . . . . . P E
P.pastoris S N T S S L I T G F L R N D L P F N F F K K I C I F E P K I D V Q T Q W D G L L L D P A L S L S N T F
A.gossypii T L T S R L T L D F L . . . S S K G L F E R I I E L D P N I D N T S L W C D Q S L H G S F N N I N S S
S.cerevisiae P L T S G L L Y S Y L . . . I K E N F F E R I M I L D P Q V T R F T T W N G S P F D N S T E E K C K N
S.pombe P L T S E V T L S V I R . . A R P G L L V K M V D G E P I L T Q Q V I Q N L G G F S S . . . . . .
C.elegans P F . S K P V L C F M R . . S S . N I I E K . L T T S P F I C S A L T L E S A R D N T . . . . . .
D.melanogaster R R T A E T I L R Y F R L . T C S D F L L R H L R S L P F R Q H R E D . . . . . .
D.rerio S D T S G P T M R Y L R . . T S Q D F L F S H L Q H L P F V L S E N E . . . . . .
X.laevis A D T S G P T M R Y L R . . T S Q D F L F S Q L Q H L P F S V E E S E . . . . . .
H.sapiens S D T S G P T M R Y L R . . T S Q D F L F S Q L Q Y L P F S N K E Y E . . . . . .
1108 1120 1130 1140 1149
α54
α55
β3
C.thermophilum F L L S D A S L A Y I D Y L A S R A A I F E Y I G K E L C S V S Q N R I P S I K R Q I F D A L N G Q I
A.nidulans F W V C D S T D A L A E F L L Y R S Y L F D Y A T T E I R S A A K M G S P T L L E S I L A T L F G N S
N.crassa F L V T D A S H S Y I E F L A T R A M A F E Y I A K E L C S V S Q K R I P T V K R Q I F D A L L N G Q
P.pastoris I C E S Y G A G A L L A F I S Q R A L L L E L L S L E L H N I A S T G S I S A R K S Y V D P L I G S S
A.gossypii V L D G P A V G A L L A F L R Y R S H L L Q Y L S L D L H W L S T E S Q Q S K I S A A V A V L I S S E
S.cerevisiae F I E S E S V G A F L S F L A Y R N Y W T Q Y L G L F I H K I S F S G T K S E V L T Y V N Y L I S N T
S.pombe . . . . E E V S M V S R C I R S R T Q I M N M L A T E I H Y A A S V G Q N K Y L N E Y V A S L I R T N
C.elegans . . . . Y A Q G V F A V R R M I V G Y I L H F A A V E I S A M L T T G H F S R P E K L Y R A L L E S S
D.melanogaster . . . . . H V L H A M G H L L N C V S I D V K L A A K H G Q M T R F N Q M C D I L L I G N
D.rerio . . . . . I A A L S Q M S W L M K T T A I E L R V T S L N R Q R S H T Q R L L N L L L D D Q
X.laevis . . . . . I S A M N Q M S W L M K T A T I E L R I T S L N R Q R S H T Q R L L H L L L D D M
H.sapiens . . . . . I S M L N Q M S W L M K T A S I E L R V T S L N R Q R S H T Q R L L H L L L D D M
1150 1160 1170 1180 1190 1200
β4
α56
C.thermophilum F . . . . . V D E E A P L T I P S I F D F . . . . . .
A.nidulans T . . . . . L D N G A T I A N P T V Y D L F D F A . . . . . .
N.crassa I . . . . . R A D D E E P I N I A N I F D F . . . . . .
P.pastoris E . . . . . L N H G F P R I L D F . . . . . .
A.gossypii I . . . . . H A P T I F A F L D T . . . . . .
S.cerevisiae M . . . . . Y S V R L F S F . . . . . .
S.pombe E . . . . . K T H S T E L S Q K E S G F K I L E F . . . . . .
C.elegans S L V A E Y T Q M E E D D D D E D M A P V A S G S N D K A . . . . . .
D.melanogaster G M E R S S H G L S M E L G H S L I S Q P S S S F L A M D V L P A G G S I S A V A H G A G S A S L G A
D.rerio P . . . H T L H T D G E T G M E E E N R S V S G F L Q F G . . . . . .
X.laevis P . . T R P Y S A D G E G G M E D E S R S L S G F L H F D . . . . . .
H.sapiens P . . . V K P Y S D G E G G I E D E N R S V S G F L H F D . . . . . .
1201 1210 1216


Nup192
α57
C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
1217
1220
1230
1236
α57
α58
1237
1240
1250
1260
1270
1272
α58
α59
1273
1280
1290
1300
1310
1316
α59
α60
α61
1317
1320
1330
1340
1350
1361
α61
α62
1362
1370
1380
1390

Nup192


α62
α63
C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
1392
1400
1410
1420
α63
α64
1422
1430
1440
1450
1460
1467
α65
1467
1470
1480
1490
1500
1510
α66
α67
α68
1512
1520
1530
1540
1550
1560
α69
α70
1561
1570
1580
1590
1600


Nup192
α71
α72
C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
1602
1610
1620
1630
1634
α72
α73
1635
1640
1650
1660
1670
1679
1680
1690
1700
1704
α74
1705
1712
α74

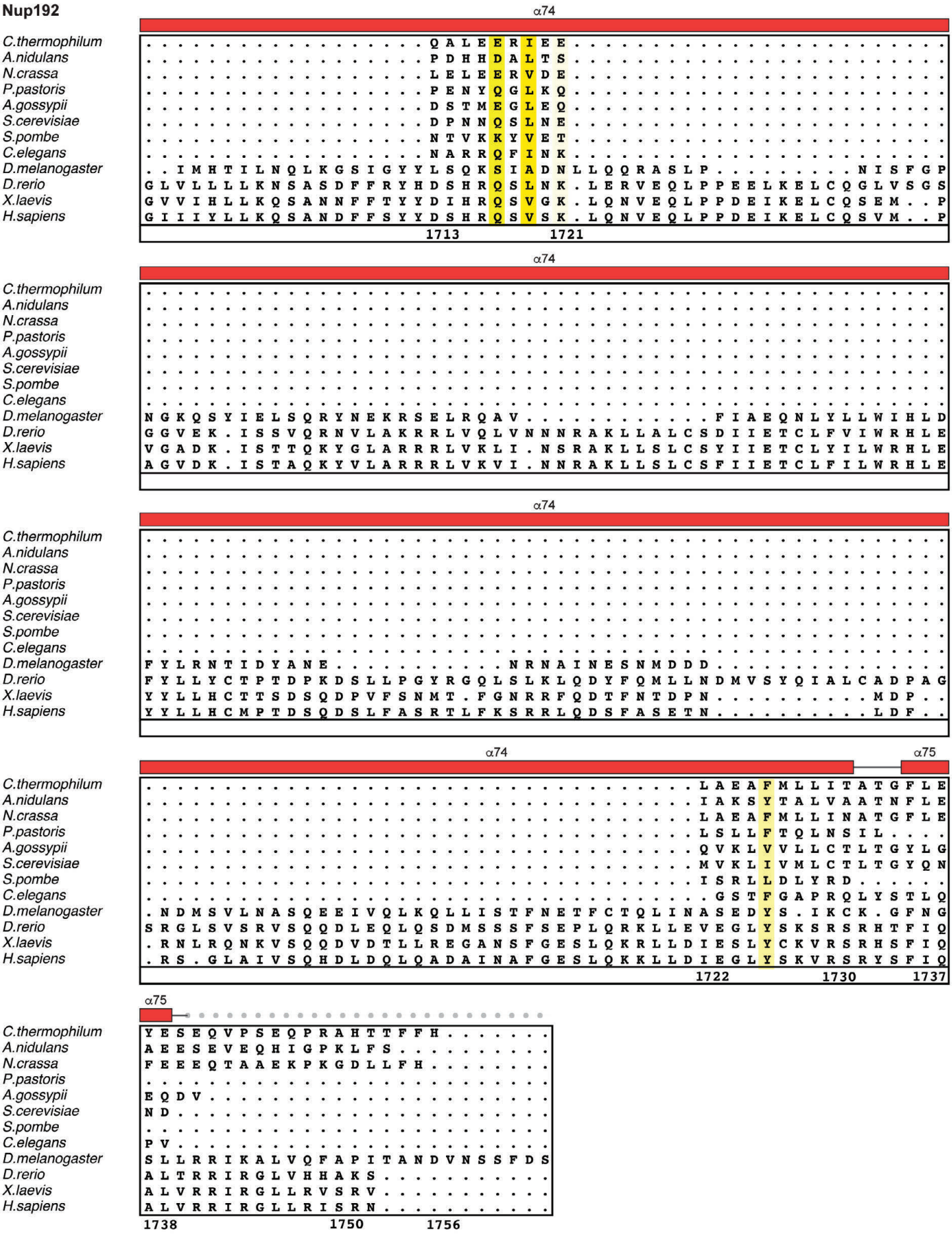

Nup192
α74
C.thermophilum
A.nidulans
N.crassa
P.pastoris
A.gossypii
S.cerevisiae
S.pombe
C.elegans
D.melanogaster
D.rerio
X.laevis
H.sapiens
1713
1721
α74
α74
α74
α75
1722
1730
1737
α75
1738
1750
1756

Fig. S30.
Multispecies sequence alignment of Nup192. Sequences from twelve diverse species were aligned and colored by sequence similarity according to the BLOSUM62 matrix from white (less than 55 % similarity), to yellow (55 % similarity), to red (100 % identity). Numbering below alignment is relative to the *C. thermophilum* sequence. Secondary structure observed in the Nup192 structure is shown above the alignment: α-helices (red bars), β-sheets (blue bars), and unstructured regions (black lines). A dashed line indicates the loop that was deleted for crystallization. Disordered regions are indicated by gray dots.

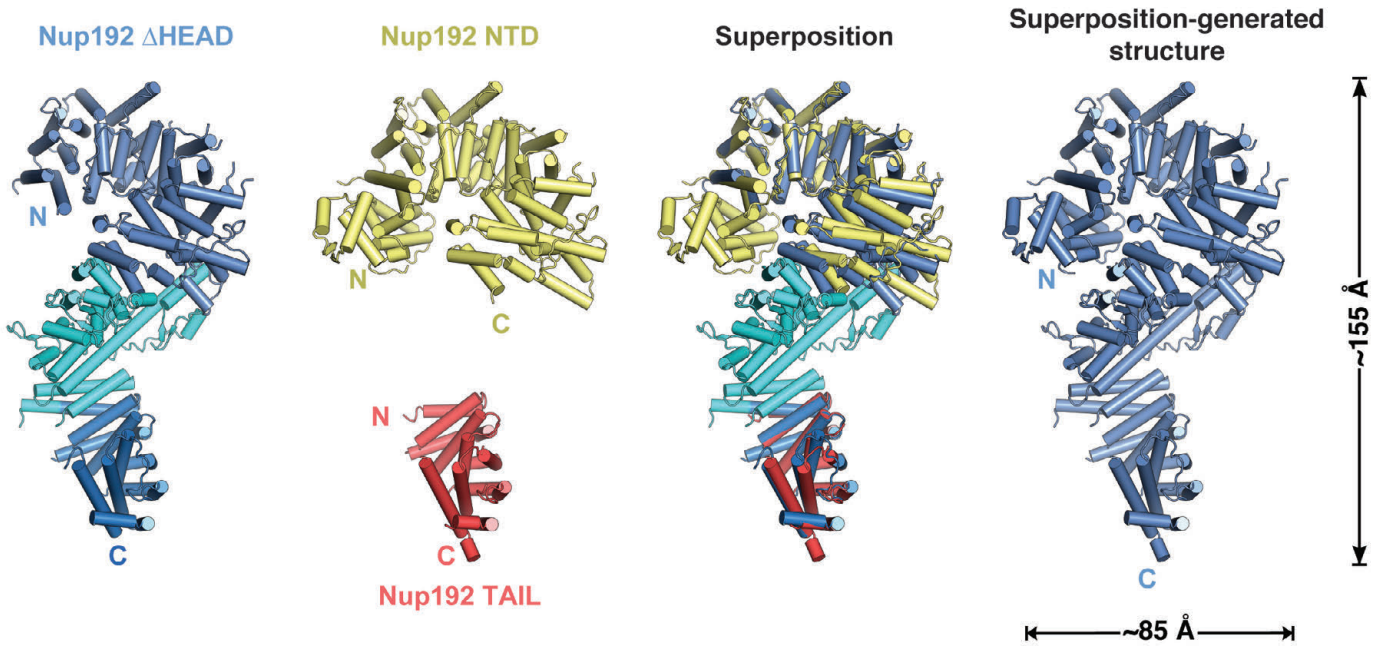

Nup192 ΔHEAD
N
C
Nup192 NTD
N
C
N
C
Nup192 TAIL
Superposition
Superposition-generated structure
N
C
~155 Å
~85 Å

Fig. S31.
Superposition-generated structure of full-length Nup192. Cartoon representations of the crystal structures of Nup192$^{\Delta HEAD}$ (colored as in Fig. 4F), Nup192^{NTD} (yellow), and Nup192^{TAIL} (red) and their superposition are shown. A cartoon representation of the superposition-generated structure of full-length Nup192 (blue) generated from the superposition is shown on the right. See also Movie 4.

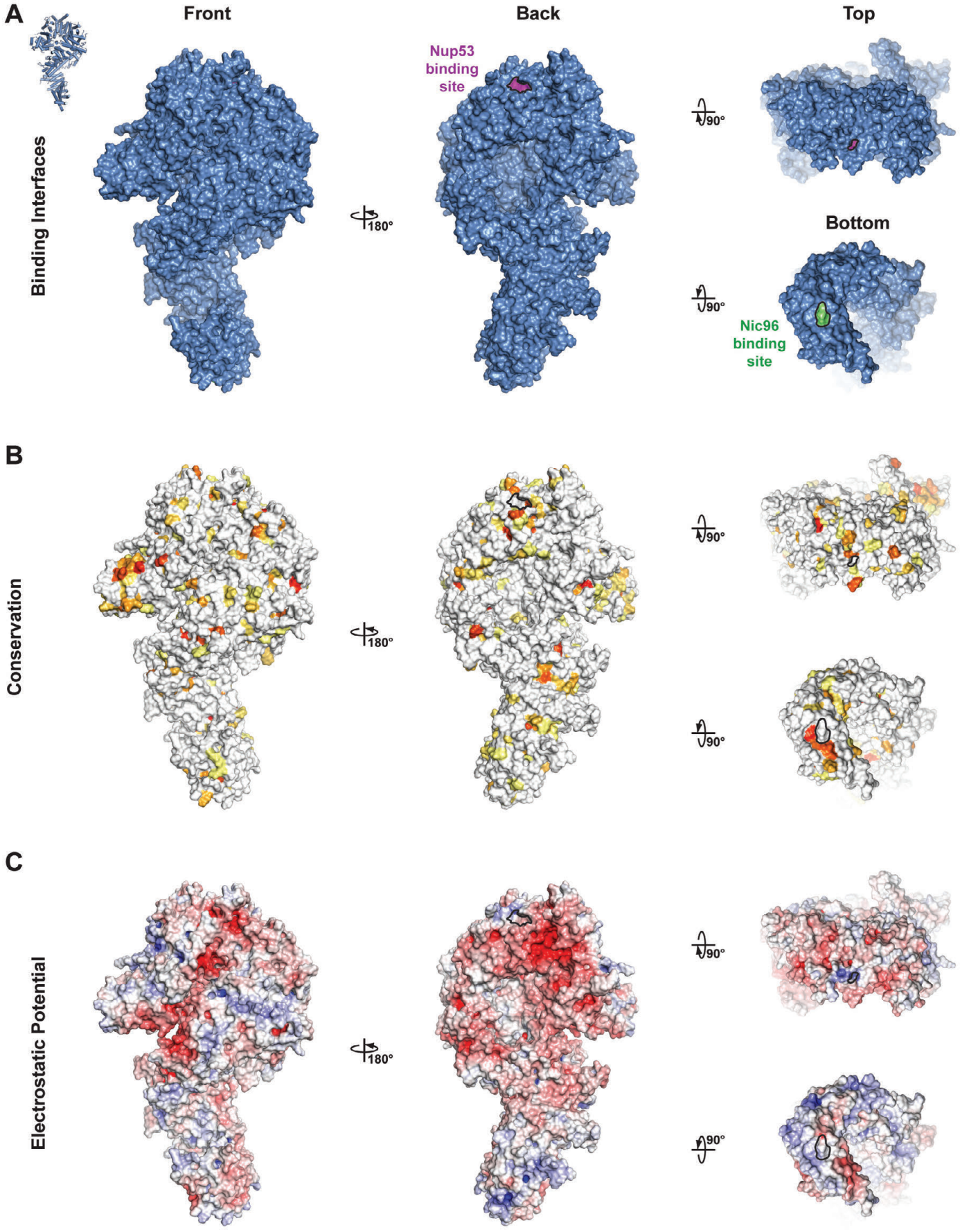

A
Front
Back
Top
Binding Interfaces
Nup53 binding site
180°
90°
Bottom
Nic96 binding site
B
Conservation
C
Electrostatic Potential

Fig. S32.
Surface properties of Nup192. Surface representations of Nup192 are shown in four different orientations. The surfaces corresponding to previously identified point mutations that disrupt interactions with Nup53 or Nic96 are outlined in black. (**A**) A surface representation of Nup192 with previously identified point mutations that disrupt interactions with Nup53 or Nic96 colored purple or green, respectively (*15, 20*). (**B**) Surface representation colored according to sequence identity based on the alignment in fig. S30. (**C**) Surface representation colored according to electrostatic potential from -10 k_BT/e (red) to +10 k_BT/e (blue).


Coat nups
Adaptor nups
Channel nups
hsNup160 (15%)
1 34 453 1436
hsNup85 (9%)
1 66 656
hsNup37 (8%)
1 326
hsNup96 (5%)
1 230 936
hsSec13 (46%)
1 332
hsNup107 (15%)
1 150 647 665 995
hsNup133 (20%)
1 71 508 1156
hsNup53 (7%)
1 161 251 326
hsNup155 (21%)
1 20 512 1391
hsNup98 (22%)
1 520 738 880
hsNup205 (15%)
1 2012
hsNup188 (11%)
1 1749
hsNup93 (15%)
1 44 98 147 179 819
hsNup62 (20%)
1 467 522
hsNup58 (15%)
1 250 467 599
hsNup54 (21%)
1 212 242 331 507

Fig. S33.
Domain structures of symmetric core nucleoporins are conserved between *H. sapiens* and *C. thermophilum*. Predicted domain boundaries are shown for the symmetric core nucleoporins from *H. sapiens* using *H. sapiens* nomenclature. Percent sequence identity to *C. thermophilum* is shown in parenthesis.

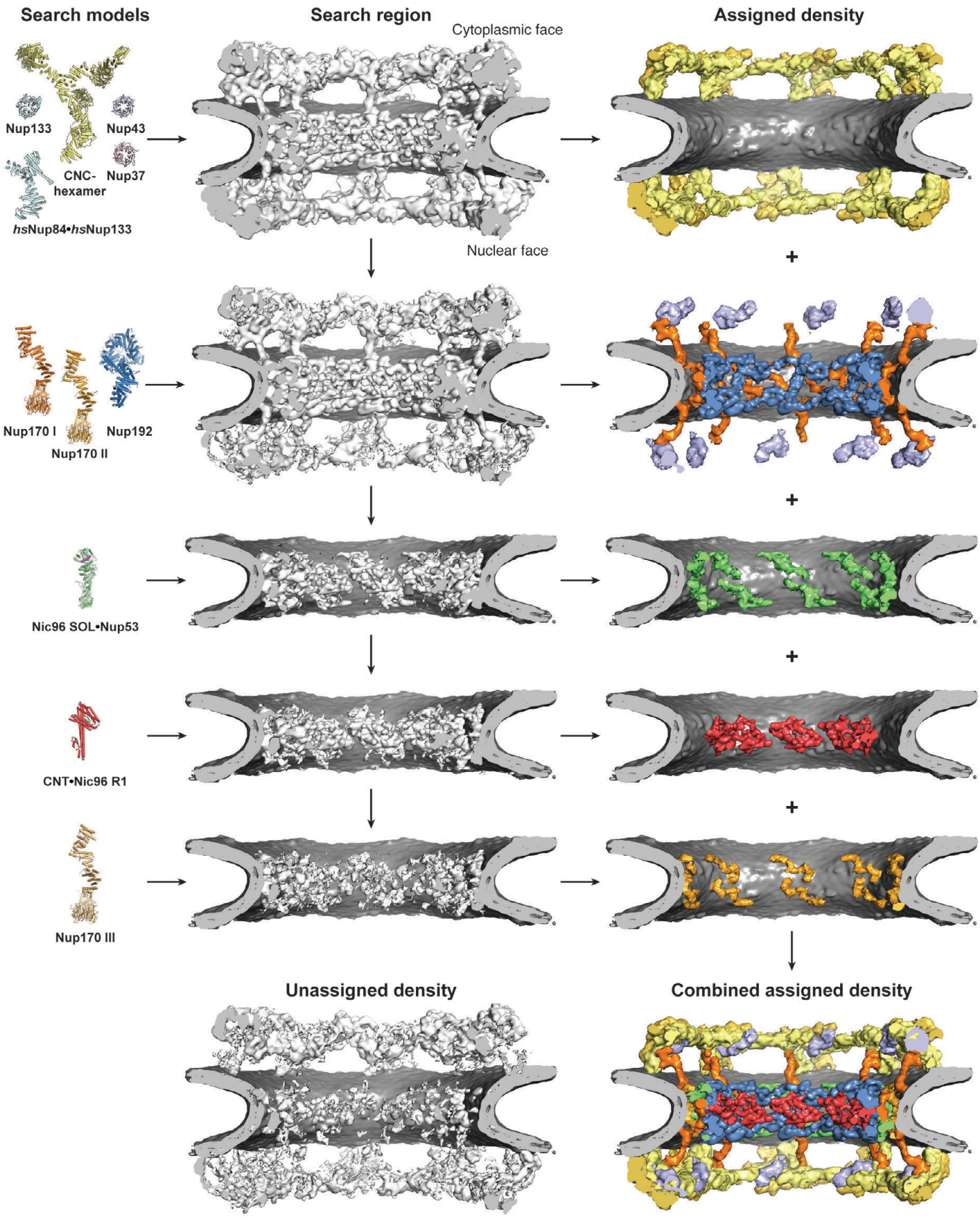
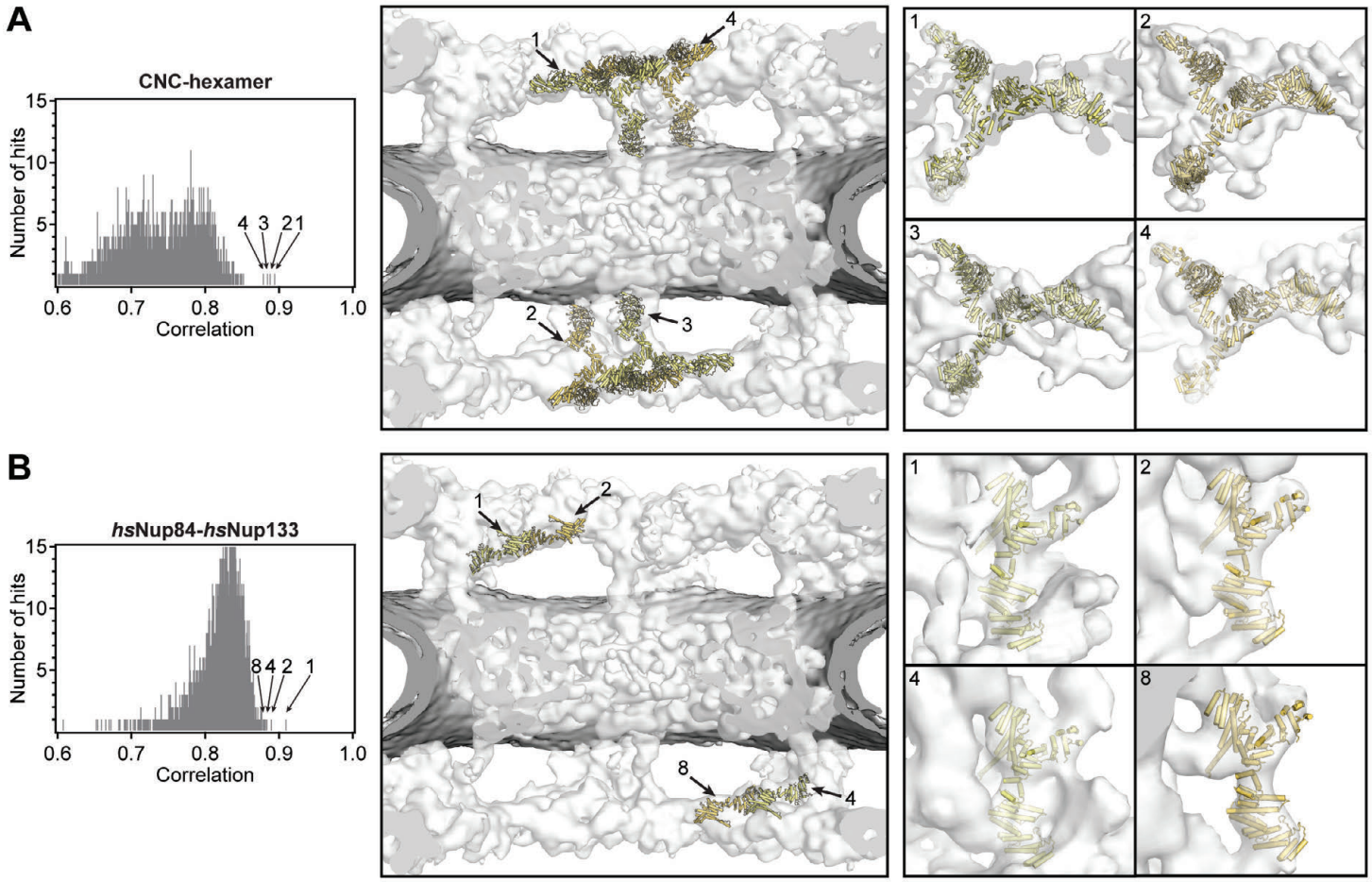

Search models
Search region
Assigned density
Cytoplasmic face
Nup133
Nup43
CNC-
hexamer
Nup37
hsNup84•hsNup133
Nuclear face
Nup170 I
Nup192
Nup170 II
Nic96 SOL•Nup53
CNT•Nic96 R1
Nup170 III
Unassigned density
Combined assigned density

Fig. S34.
Flowchart of the incremental approach used to dock crystal structures into the cryoET reconstruction of the intact human NPC. For each docking step, the crystal structures used for global searches are shown on the left (search model) and the cryoET reconstruction the searches were performed with is shown in the middle (search region). Newly assigned density that was removed from the cryoET reconstruction in subsequent searches is shown on the right and colored as the crystal structure that was placed (assigned density). The remaining unassigned density and combined assigned density are shown at the bottom middle and right, respectively.


A
CNC-hexamer
Number of hits
15
10
5
4 3 21
0.6
0.7
0.8
0.9
1.0
Correlation
1
4
2
3
1
2
3
4
B
hsNup84-hsNup133
Number of hits
15
10
5
8 4 2 1
0.6
0.7
0.8
0.9
1.0
Correlation
1
2
8
4
1
2
4
8

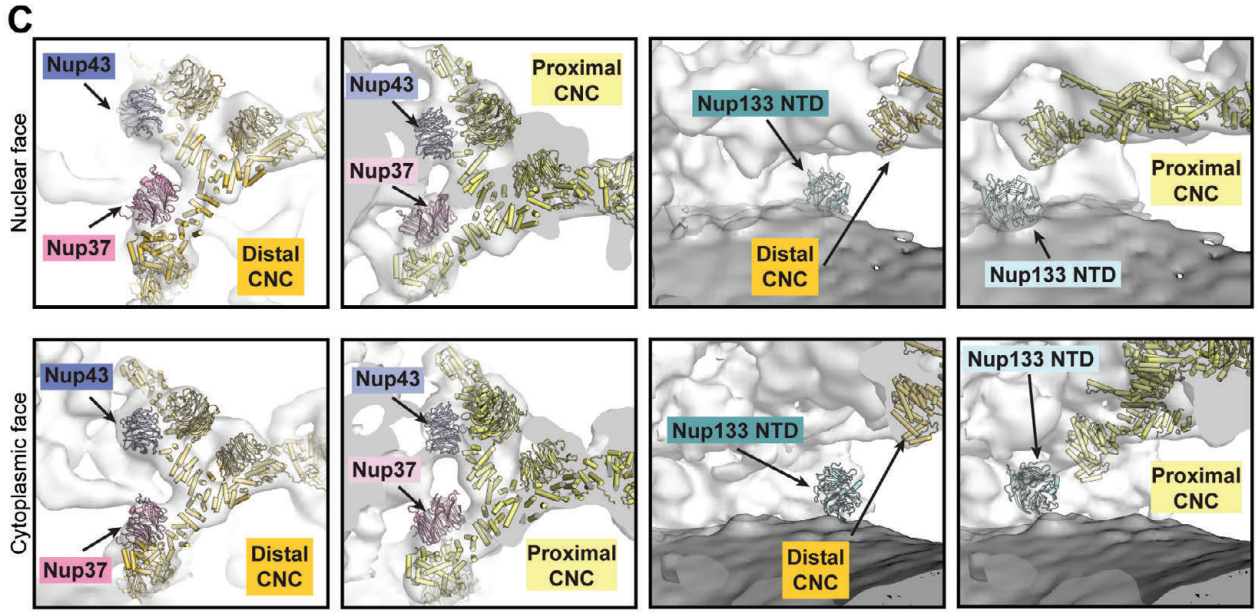

C
Nuclear face
Cytoplasmic face
Nup43
Nup37
Distal CNC
Proximal CNC
Nup133 NTD

Fig. S35.
Docking of the CNC-hexamer and Nup84•Nup133 crystal structures into the cryoET reconstruction of the intact human NPC. On the left, histograms of the cross-correlation scores from a global search with 50,000 random initial placements are shown for (**A**) the yeast CNC-hexamer (PDB ID 4XMM) and (**B**) *hs*Nup84•*hs*Nup133 hetero-dimer (PDB ID 3I4R) (*14, 23*). Arrows and corresponding numbers indicate the unique solutions that were accepted and the rank of the score, respectively. The arrangement of the unique solutions in one spoke is shown in the middle. The densities corresponding to the nuclear envelope and the NPC are colored in dark gray and white, respectively. Representative views illustrating the quality of the fits are shown on the right. The numbers on the top left of the box indicate which solution is depicted. (**C**) Manual docking of Nup43 (PDB ID 4I79; periwinkle), Nup37 (PDB ID 4FHM; pink), and $Nup133^{NTD}$ (PDB ID 1XKS; turquoise) β-propellers into cryoET density guided by previously published biochemical and structural data (*39-41, 63*).

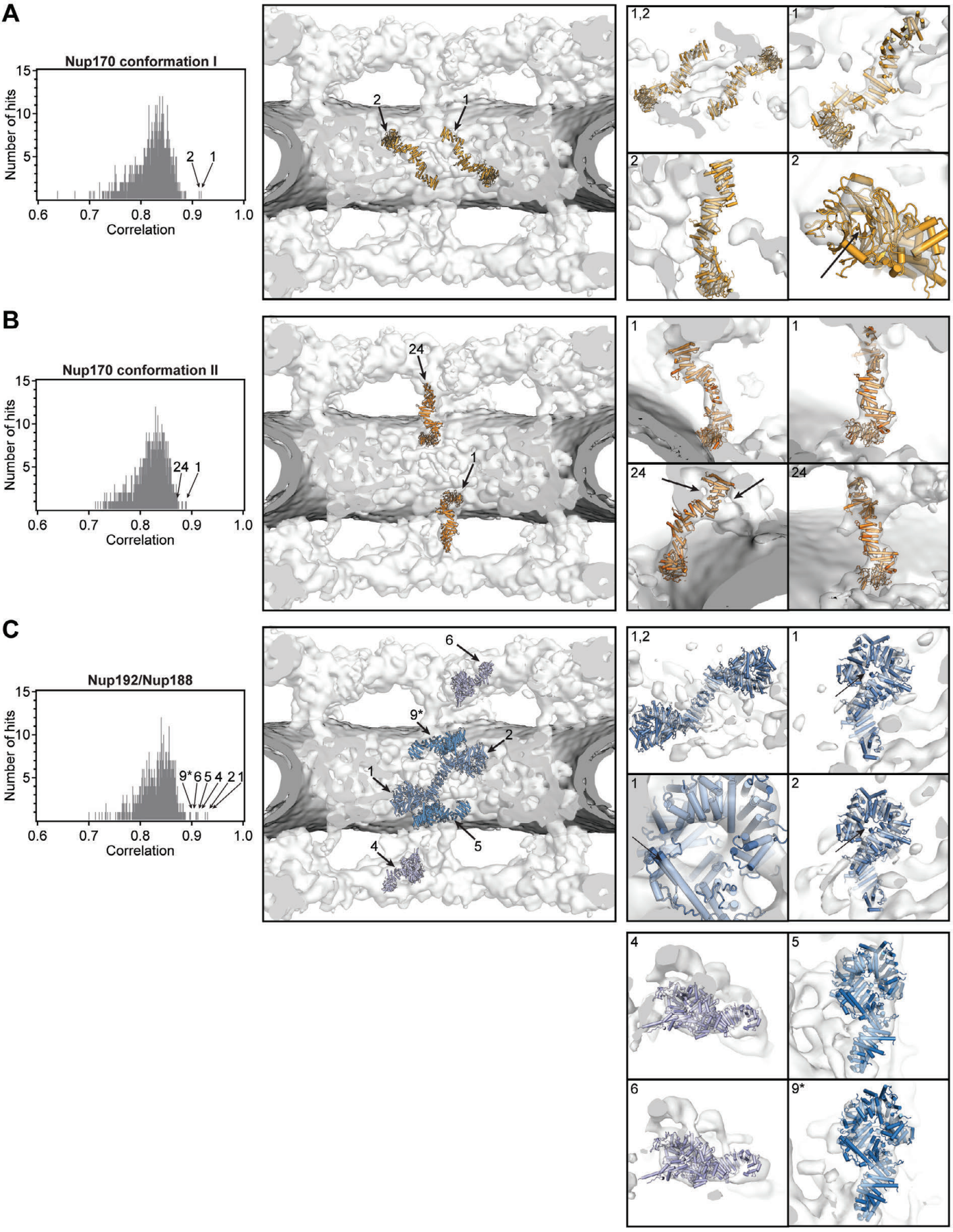

A
Nup170 conformation I
Number of hits
Correlation
2 1
B
Nup170 conformation II
24 1
C
Nup192/Nup188
9* 6 5 4 2 1
1,2

Fig. S36.
Docking of the superposition-generated full-length Nup170 and Nup192 structures into the cryoET reconstruction of the intact human NPC. On the left, histograms of the cross-correlation scores from a global search with 50,000 random initial placements are shown for (**A**) Nup170 conformation I (light orange), (**B**) Nup170 conformation II (dark orange), and (**C**) Nup192 (blue), Nup188 (purple). The Nup170 conformations I and II correspond to conformations 5 and 2, respectively, in fig. S25. Arrows and corresponding numbers indicate the unique solutions that were accepted and the rank of the score, respectively. For Nup192, we accepted 5 placements from the global search results, two placements in the inner ring, two placements in the outer rings, and one placement on the nuclear peripheral side of the inner ring. A matching cytoplasmic peripheral placement was not found in global searches, but the manual placement on the cytoplasmic peripheral side of the inner ring generated a score which would rank as the 9^{th} highest score (asterisk). The arrangement of the unique solutions in one spoke is shown in the middle. The densities corresponding to the nuclear envelope and the NPC are colored in dark gray and white, respectively. Representative views illustrating the quality of the fits are shown on the right. The numbers on the top left of the box indicate which solution is depicted.

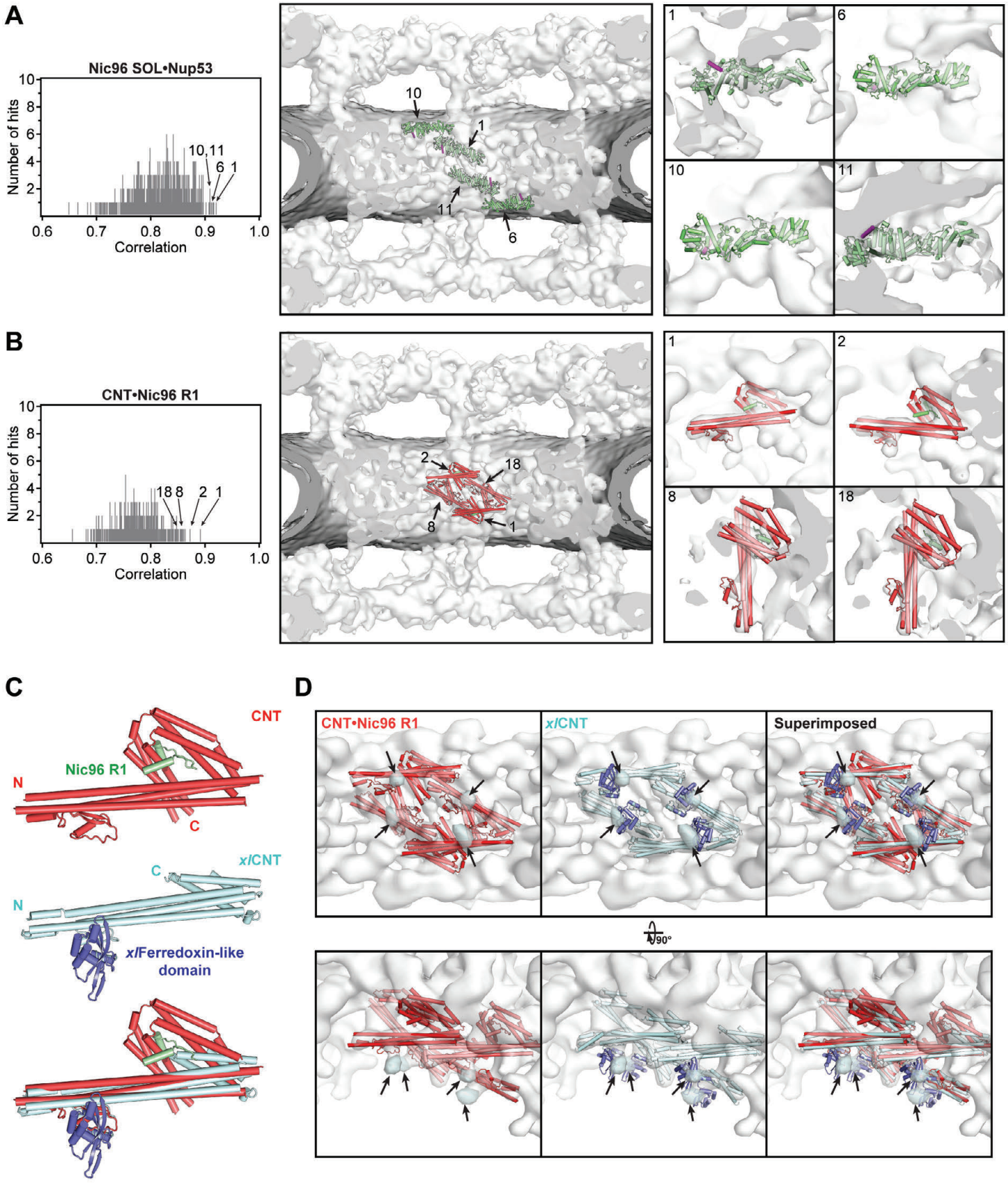

A
Nic96 SOL•Nup53
Number of hits
Correlation
10,11
6
1
10
1
11
6
B
CNT•Nic96 R1
18
8
2
1
C
CNT
Nic96 R1
N
C
x/CNT
x/Ferredoxin-like domain
D
CNT•Nic96 R1
x/CNT
Superimposed
90°

Fig. S37.
Docking of Nic96^{SOL} and CNT crystal structures into the cryoET reconstruction of the intact human NPC. On the left, histograms of the cross-correlation scores from a global search with 50,000 random initial placements are shown for (**A**) Nic96^{SOL}•Nup53^{R2} (green) and (**B**) CNT•Nic96^{R1} (PDB 5CWS; red). Arrows and corresponding numbers indicate the unique solutions that were accepted and the rank of the score, respectively. The arrangement of the unique solutions in one spoke is shown in the middle. The densities corresponding to the nuclear envelope and the NPC are colored in dark gray and white, respectively. Representative views illustrating the quality of the fits are shown on the right. The numbers on the top left of the box indicate which solution is depicted. (**C**) Crystal structures of the *C. thermophilum* CNT•Nic96^{R1} hetero-tetramer (PDB ID 5CWS; red and green; top), the *X. laevis* CNT hetero-trimer (PDB ID 5C3L; light cyan; middle) and ferredoxin-like domain (PDB ID 5C2U; purple; middle), and their superposition (bottom) are shown in cartoon representation (*15, 16*). (**D**) The four knobs of unexplained density directly adjacent to the docked CNT•Nic96^{R1} crystal structures (colored in cyan and marked with arrows) are readily explained by the presence of the ferredoxin-like domain, a metazoan-specific insertion in Nup57. Side views from within the central transport channel of the NPC (top) and top views from the cytoplasm (bottom) are shown of the cryoET reconstruction with the docked CNT•Nic96^{R1} crystal structures (left), the model of *X. laevis* CNT with ferredoxin-like domain (middle), and their superposition (right).

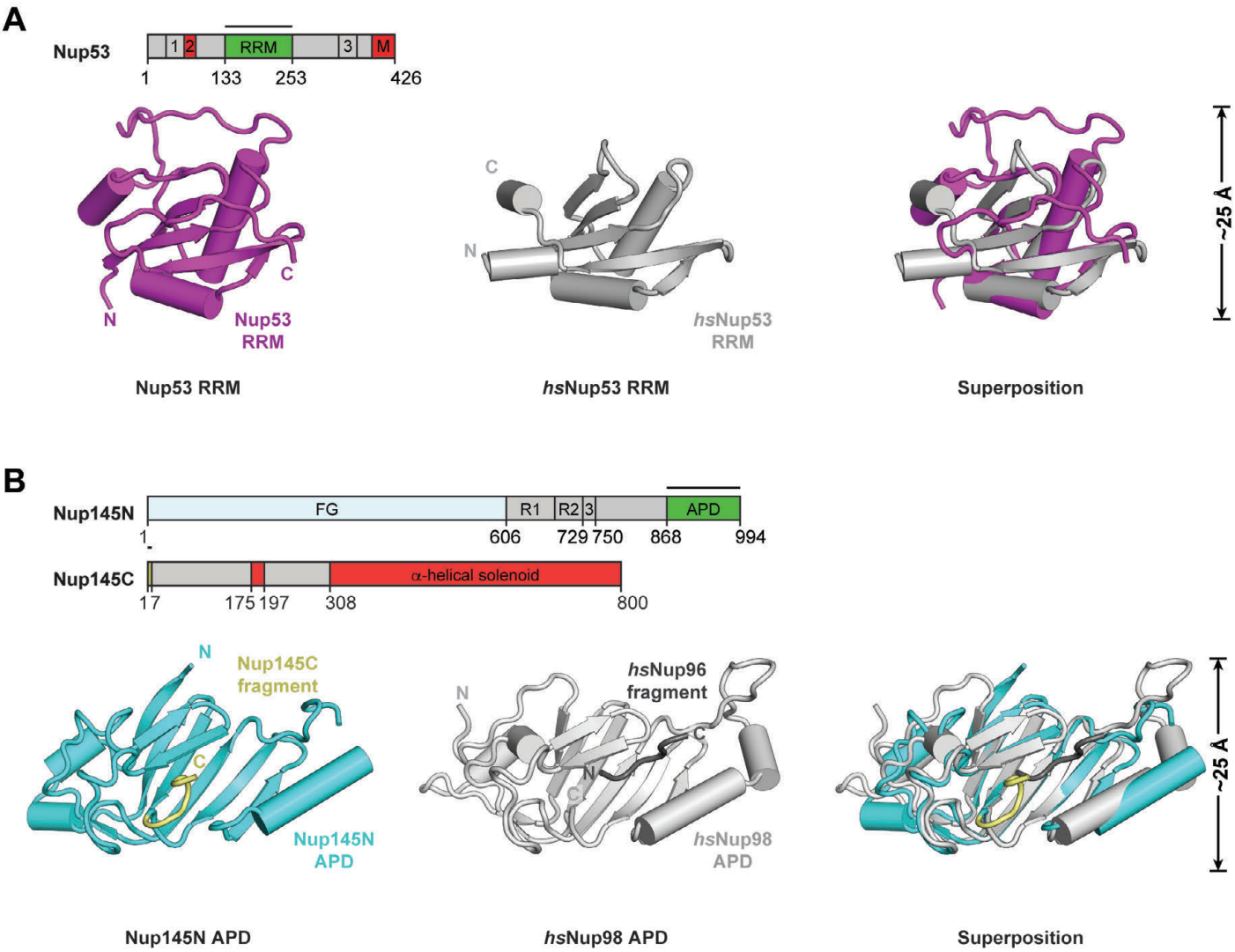
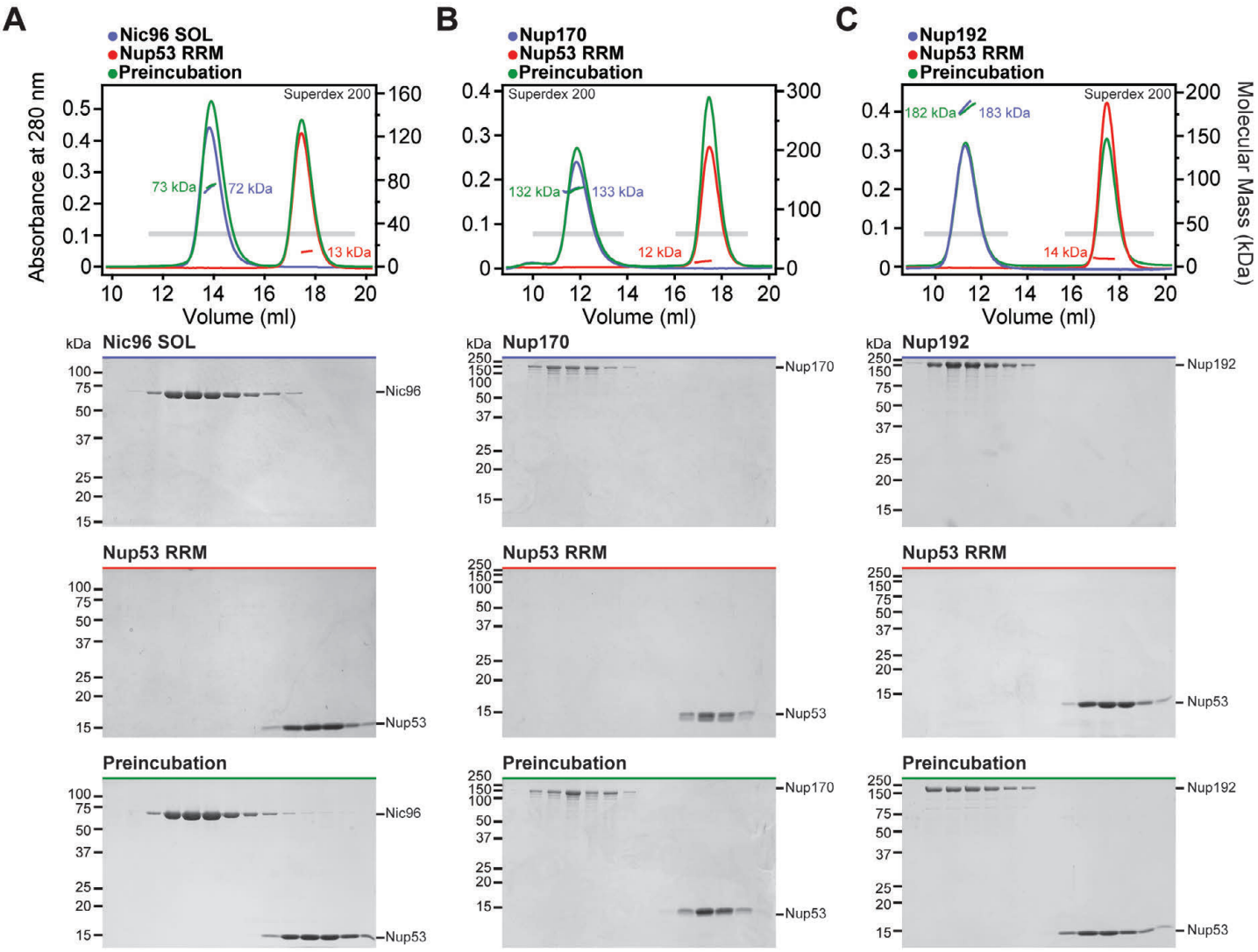

A
Nup53
1
2
RRM
3
M
1
133
253
426
N
C
Nup53
RRM
C
N
hsNup53
RRM
~25 Å
Nup53 RRM
hsNup53 RRM
Superposition
B
Nup145N
FG
R1
R2
3
APD
1
606
729
750
868
994
Nup145C
α-helical solenoid
17
175
197
308
800
N
Nup145C
fragment
C
Nup145N
APD
N
hsNup96
fragment
C
N
C
hsNup98
APD
~25 Å
Nup145N APD
hsNup98 APD
Superposition

Fig. S38.
Crystal structures of Nup53^{RRM} and Nup145N^{APD}•Nup145C^{N}. (**A**) Crystal structures of *C. thermophilum* Nup53^{RRM} (purple), *H. Sapiens* Nup53^{RRM} (PDB ID 4LIR; gray), and their superposition are shown in cartoon representation. The core fold is conserved, but there are minor alterations in the size of helices and loops. (**B**) Crystal structures of the *C. thermophilum* Nup145N^{APD}•Nup145C^{N} complex (cyan), the *H. sapiens* Nup98^{APD}•Nup96^{N} complex (PDB ID 1KO6; light gray), and their superposition are shown in cartoon representation. As previously observed for the *hs*Nup98^{APD}•*hs*Nup96^{N} hetero-dimer, fusion of the Nup145C^{N} peptide to Nup145N^{APD} and introduction of the catalytically inactive T994A mutation allowed for structure determination of the Nup145N^{APD}•Nup145C^{N} complex. Nup145C^{N} occupied the same binding grove as previously observed in the *hs*Nup98•*hs*Nup96 complex (*30*). As references, the domain structures of Nup53, Nup145N, and Nup145C are shown above the crystal structures with black bars indicating the crystallized fragments.


A
Nic96 SOL
Nup53 RRM
Preincubation
Superdex 200
Absorbance at 280 nm
Molecular Mass (kDa)
Volume (ml)
73 kDa
72 kDa
13 kDa
kDa
Nic96 SOL
Nic96
Nup53 RRM
Nup53
Preincubation
Nic96
Nup53
B
Nup170
Nup53 RRM
Preincubation
Superdex 200
132 kDa
133 kDa
12 kDa
Volume (ml)
kDa
Nup170
Nup170
Nup53 RRM
Nup53
Preincubation
Nup170
Nup53
C
Nup192
Nup53 RRM
Preincubation
Superdex 200
182 kDa
183 kDa
14 kDa
Volume (ml)
kDa
Nup192
Nup192
Nup53 RRM
Nup53
Preincubation
Nup192
Nup53

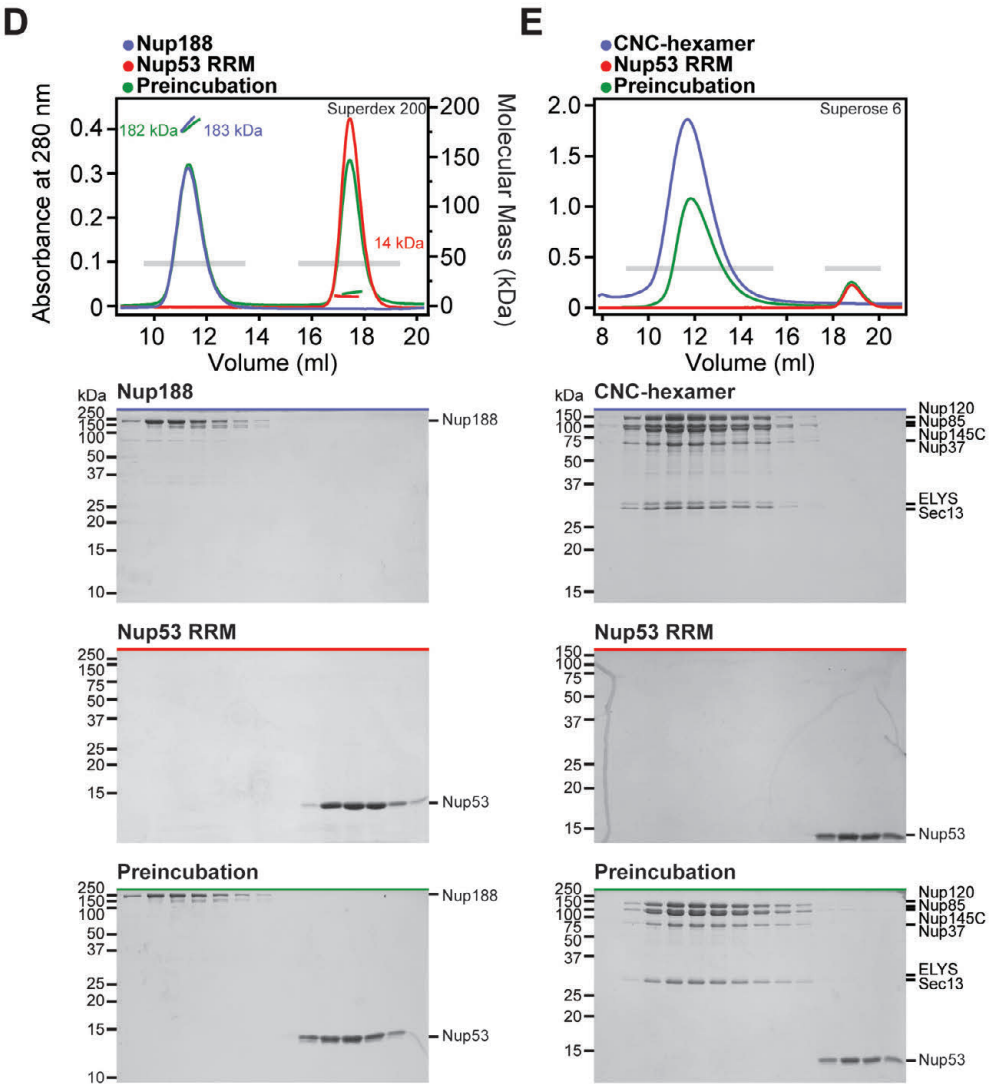
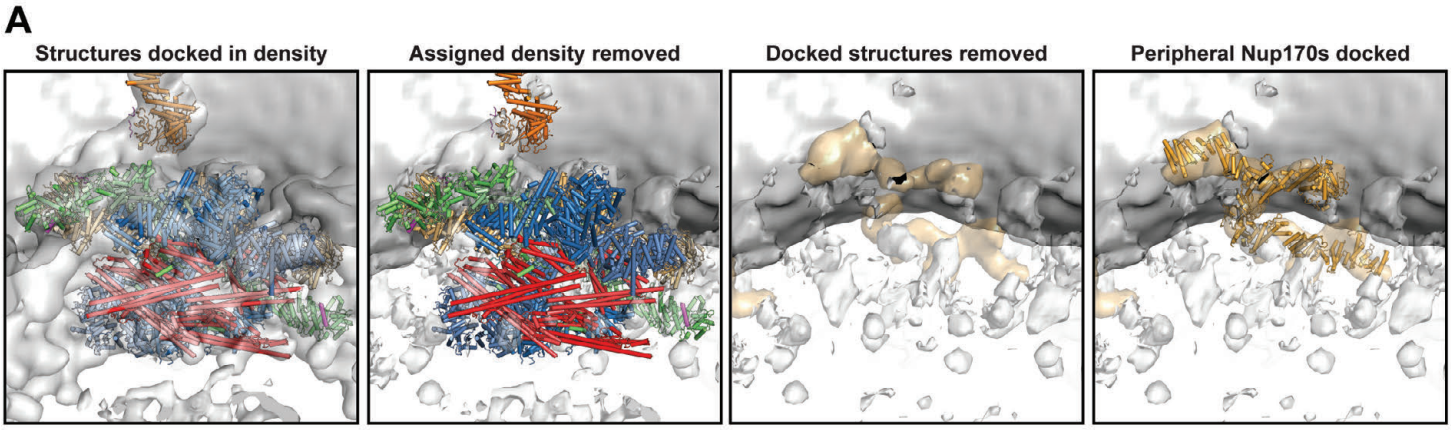
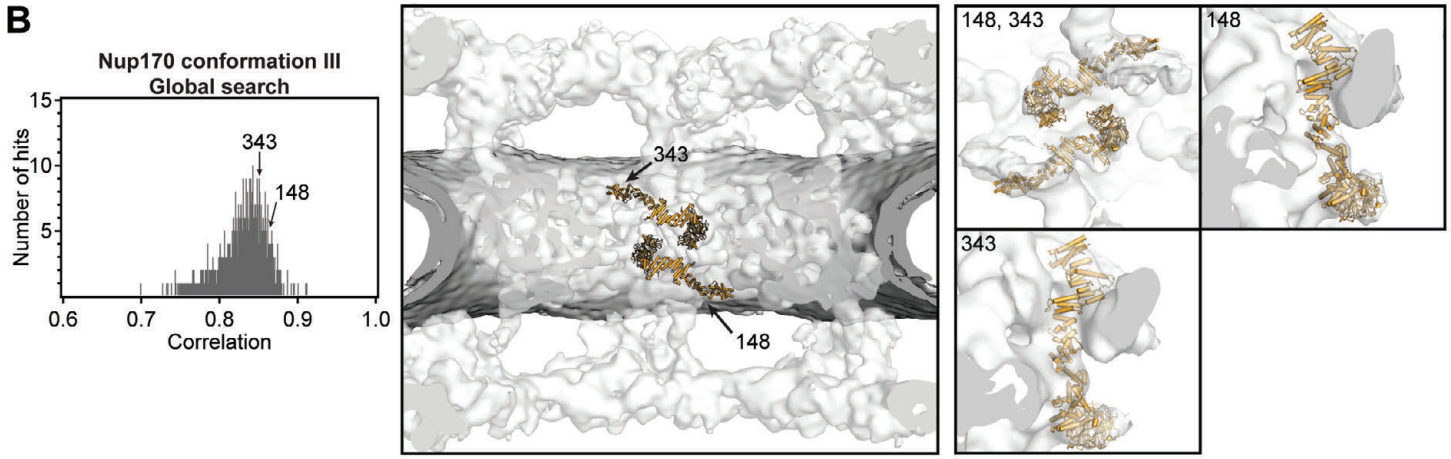

D
Nup188
Nup53 RRM
Preincubation
Absorbance at 280 nm
Molecular Mass (kDa)
Superdex 200
182 kDa
183 kDa
14 kDa
Volume (ml)
E
CNC-hexamer
Nup53 RRM
Preincubation
Superose 6
Volume (ml)
kDa
Nup188
Nup53 RRM
Preincubation
CNC-hexamer
Nup120
Nup85
Nup145C
Nup37
ELYS
Sec13
Nup53

Fig. S39.
Nup53^{RRM} does not interact with inner ring scaffold nucleoporins or the CNC-hexamer. (**A**-**E**) SEC-MALS profiles of nucleoporins or nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). SEC profiles were obtained using a Superdex 200 10/300 column or Superose 6 10/300 column as indicated. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining.


A
Structures docked in density
Assigned density removed
Docked structures removed
Peripheral Nup170s docked


B
Nup170 conformation III
Global search
Number of hits
15
10
5
343
148
0.6
0.7
0.8
0.9
1.0
Correlation
343
148
148, 343
148
343

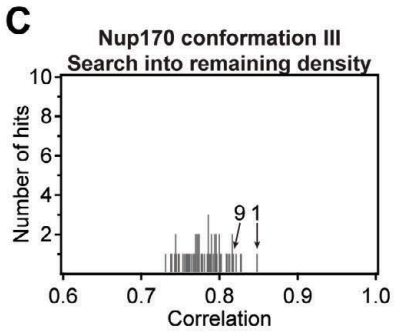

C
Nup170 conformation III
Search into remaining density
Number of hits
10
8
6
4
2
9
1
0.6
0.7
0.8
0.9
1.0
Correlation

Fig. S40.
Identification of a third pair of Nup170 molecules in the inner ring of the cryoET reconstruction of the intact human NPC. (**A**) Views of the cryoET density in the inner ring illustrating all density and the docked proteins from the unbiased search (far left), the cryoET density remaining after removal of the assigned density and the docked proteins (middle left), the remaining cryoET density without the docked proteins from the unbiased search revealing density that is shaped like a Nup170 molecule colored in orange (middle right), and the same density with a third conformation of Nup170 docked (far right). (**B**) Search results for two additional Nup170 molecules fitted into a cryoET map from which the density for the CNCs had been removed. The histogram on the left shows the cross-correlation scores for a pair of Nup170 conformation III (light orange) from a global search with 50,000 random initial placements. Nup170 conformation III corresponds to conformation 8 in fig. S25. Arrows and corresponding numbers indicate the unique solutions that were accepted and the rank of the score, respectively. The arrangement of the unique solutions in one spoke is shown in the middle. The densities corresponding to the nuclear envelope and the NPC are colored in dark gray and white, respectively. Representative views illustrating the quality of the fits are shown on the right. The numbers on the top left of the box indicate which solution is depicted. (**C**) A histogram illustrating the search results for fitting Nup170 conformation III into a cryoET map of the inner ring from which the density corresponding to all other components had been removed.

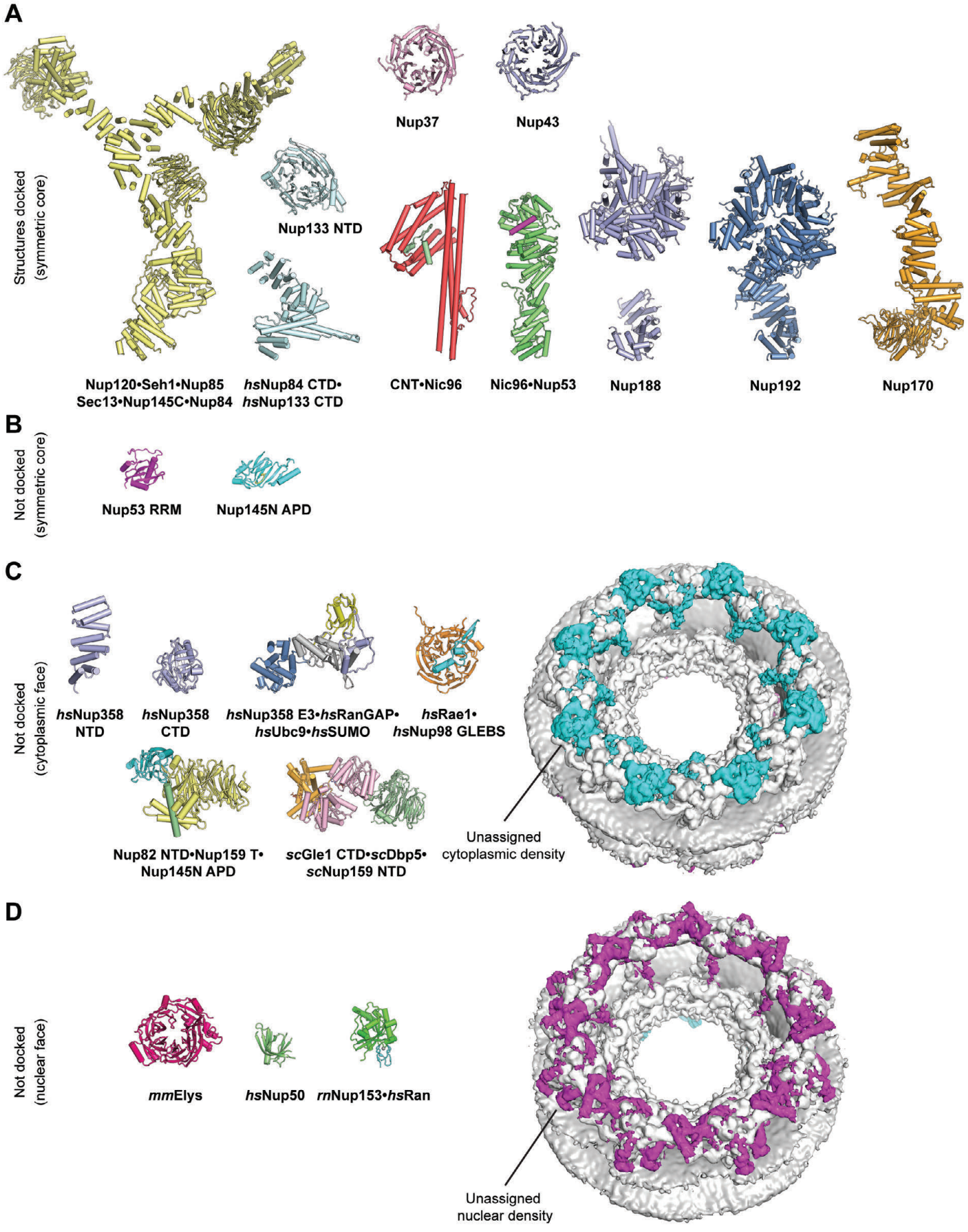
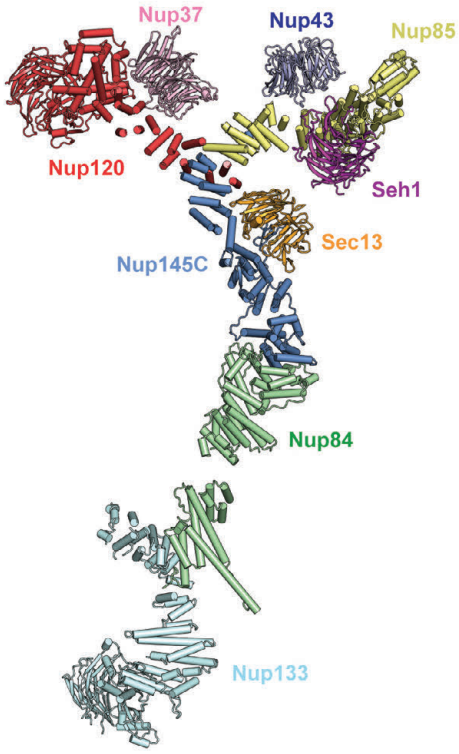
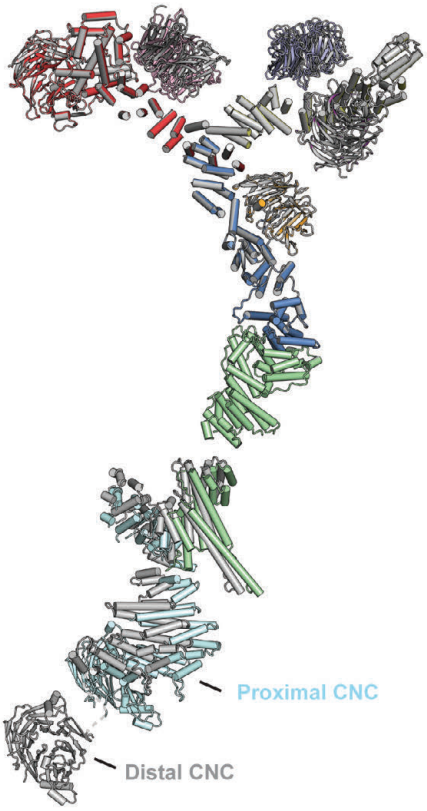

A
Structures docked (symmetric core)
Nup37
Nup43
Nup133 NTD
Nup120•Seh1•Nup85 Sec13•Nup145C•Nup84
hsNup84 CTD• hsNup133 CTD
CNT•Nic96
Nic96•Nup53
Nup188
Nup192
Nup170
B
Not docked (symmetric core)
Nup53 RRM
Nup145N APD
C
Not docked (cytoplasmic face)
hsNup358 NTD
hsNup358 CTD
hsNup358 E3•hsRanGAP• hsUbc9•hsSUMO
hsRae1• hsNup98 GLEBS
Nup82 NTD•Nup159 T• Nup145N APD
scGle1 CTD•scDbp5• scNup159 NTD
Unassigned cytoplasmic density
D
Not docked (nuclear face)
mmElys
hsNup50
rnNup153•hsRan
Unassigned nuclear density

Fig. S41.
Summary of crystal structure docking into the cryoET reconstruction of the intact human NPC. (**A**) Crystal structures of the symmetric core nucleoporins that could be successfully docked into a cryoET reconstruction of the intact human NPC. (**B**-**D**) Crystal structures of symmetric core nucleoporin domains, the cytoplasmic filament nucleoporins, and nuclear basket nucleoporins, which were not docked due to their small size, indistinctive shape, or lack of biochemical restraints. Cytoplasmic and nuclear views of the cryoET reconstruction of the intact human NPC are shown on the right. The assigned density of the symmetric NPC core is colored in gray and the unassigned densities on the cytoplasmic and nuclear faces are colored in cyan and purple, respectively. The volume of the unassigned density appears to be sufficient to accommodate the majority of the remaining structured protein mass of the asymmetric cytoplasmic filament and nuclear basket nucleoporins. All crystal structures are shown to scale in cartoon representation.



Proximal CNC

Distal CNC



Superposition

Fig. S42.
Comparison of the distal and proximal CNC docking solutions. Individual distal (multicolored; left) and proximal (gray; middle) CNCs are shown in cartoon representation. Their superposition reveals a dramatically different orientation for $Nup133^{NTD}$ and slightly different orientations of the Nup133 and Nup84 solenoids (right).

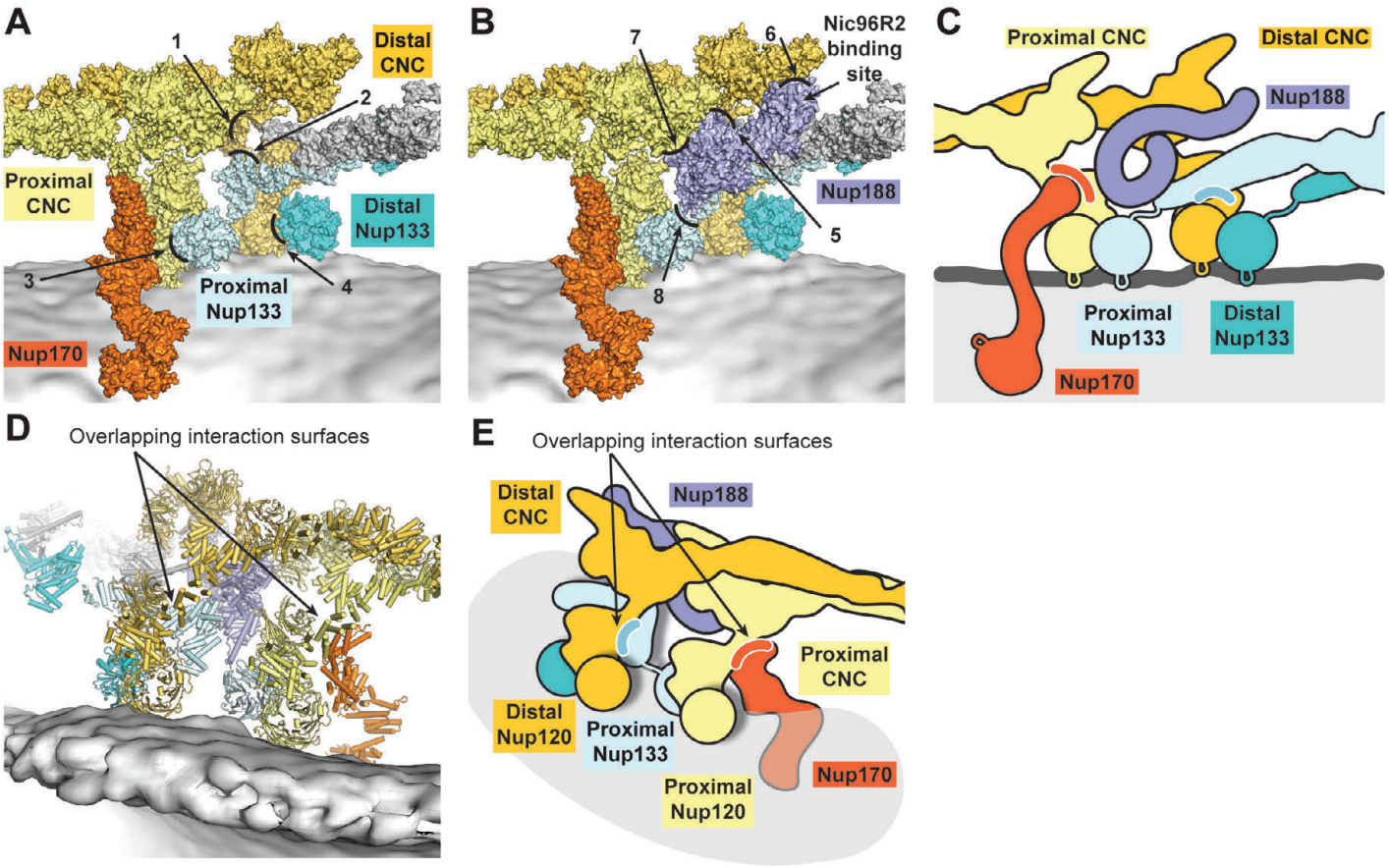

A
1
2
3
4
Distal CNC
Proximal CNC
Distal Nup133
Proximal Nup133
Nup170
B
7
6
5
8
Nic96R2 binding site
Nup188
C
Proximal CNC
Distal CNC
Nup188
Proximal Nup133
Distal Nup133
Nup170
D
Overlapping interaction surfaces
E
Overlapping interaction surfaces
Distal CNC
Nup188
Proximal CNC
Distal Nup120
Proximal Nup133
Nup170
Proximal Nup120

Fig. S43.
Interactions mediating inter-spoke assembly. (**A**) The outer ring inter-spoke interface is shown in surface representation, but with Nup188 removed for clarity. Contacts observed between CNCs from different spokes are indicated by arrows and numbered from 1 to 4, corresponding to the contacts described in the text. (**B**) The same view as in panel (A), but with Nup188 also shown in a surface representation. Contacts observed between Nup188 and the CNCs at the inter-spoke interface are indicated by arrows and numbered from 5 to 8, corresponding to the contacts described in the text. An arrow highlights the $Nic96^{R2}$ binding site on Nup188. (**C**) Schematic of the inter-spoke interface, from the same view as in panel (A). (**D**) The outer ring inter-spoke interface, shown in cartoon representation, ~180° rotated from the view in panel (A). Nup170 and Nup133 contact overlapping interfaces on proximal and distal Nup120 molecules. (**E**) Schematic of the outer ring inter-spoke interface drawn in the same view as in panel (D).

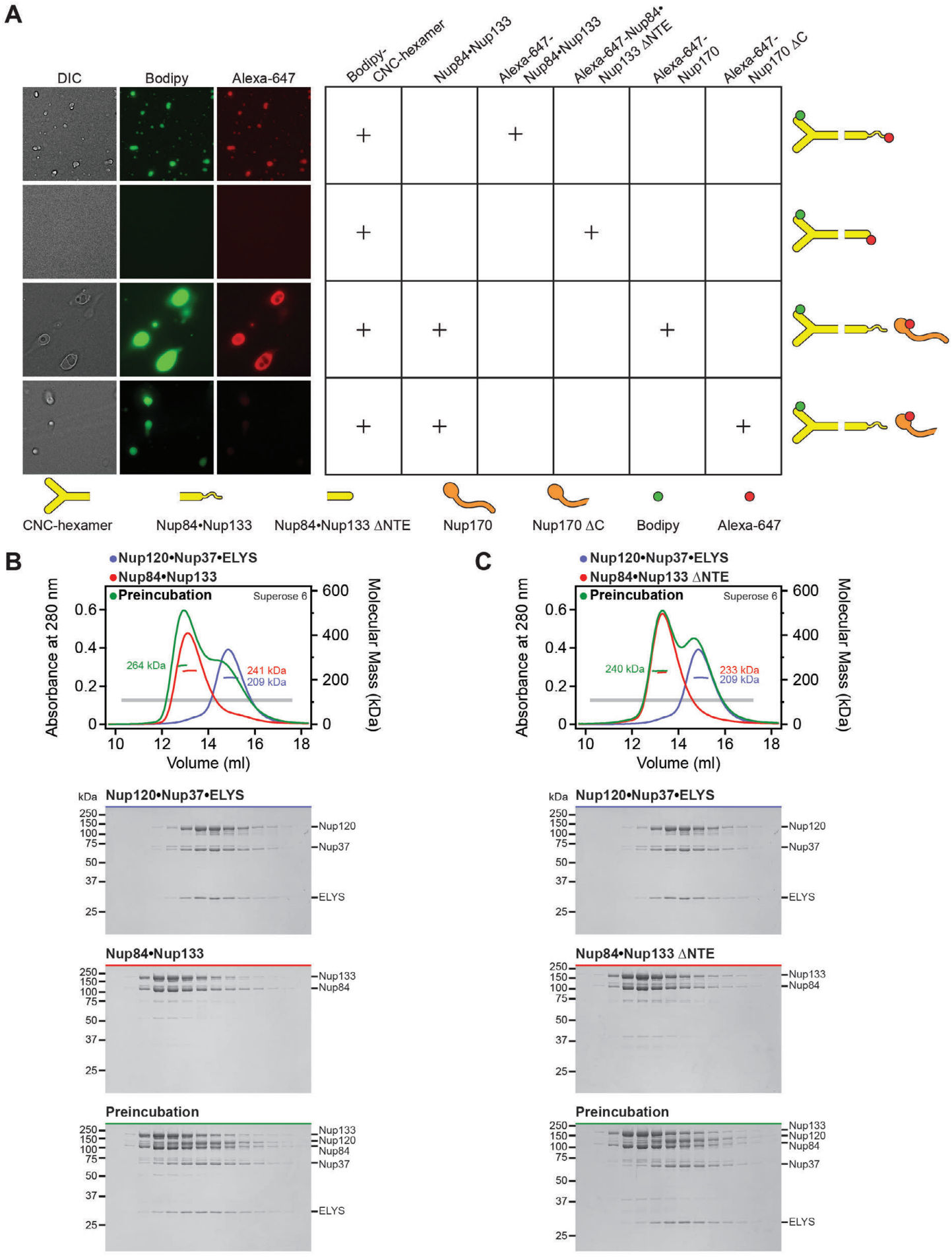

A
DIC
Bodipy
Alexa-647
Bodipy-CNC-hexamer
Nup84•Nup133
Alexa-647-Nup84•Nup133
Alexa-647-Nup84•Nup133 ΔNTE
Alexa-647-Nup170
Alexa-647-Nup170 ΔC
CNC-hexamer
Nup84•Nup133
Nup84•Nup133 ΔNTE
Nup170
Nup170 ΔC
Bodipy
Alexa-647
B
Nup120•Nup37•ELYS
Nup84•Nup133
Preincubation
Superose 6
264 kDa
241 kDa
209 kDa
Absorbance at 280 nm
Molecular Mass (kDa)
Volume (ml)
kDa
Nup120•Nup37•ELYS
Nup120
Nup37
ELYS
Nup84•Nup133
Nup133
Nup84
Preincubation
Nup120
Nup133
Nup84
Nup37
ELYS
C
Nup120•Nup37•ELYS
Nup84•Nup133 ΔNTE
Preincubation
Superose 6
240 kDa
233 kDa
209 kDa
Absorbance at 280 nm
Molecular Mass (kDa)
Volume (ml)
kDa
Nup120•Nup37•ELYS
Nup120
Nup37
ELYS
Nup84•Nup133 ΔNTE
Nup133
Nup84
Preincubation
Nup120
Nup133
Nup84
Nup37
ELYS

Fig. S44.
Oligomerization of the CNC. (**A**) The CNC forms oil droplets in the presence of the unstructured N-terminal extension (NTE) of Nup133. The CNC-hexamer was labeled with Bodipy and various other nucleoporins were labeled with Alexa-647 and tested for their ability to form oil droplets. Nup170 incorporates CNC oil droplets, but this incorporation is ablated by deletion of C-terminal helices. Tested protein combinations are indicated in the table and shown schematically on the right. All experiments were repeated at least three times. (**B**, **C**) SEC-MALS profiles of nucleoporin complexes are shown individually (blue or red) and after their preincubation (green). There is a weak interaction between Nup120•Nup37•ELYS and Nup84•Nup133 only in the presence of the NTE of Nup133. SEC profiles were obtained using a Superose 6 10/300 GL column. Measured molecular masses are indicated for the peak fractions. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining.

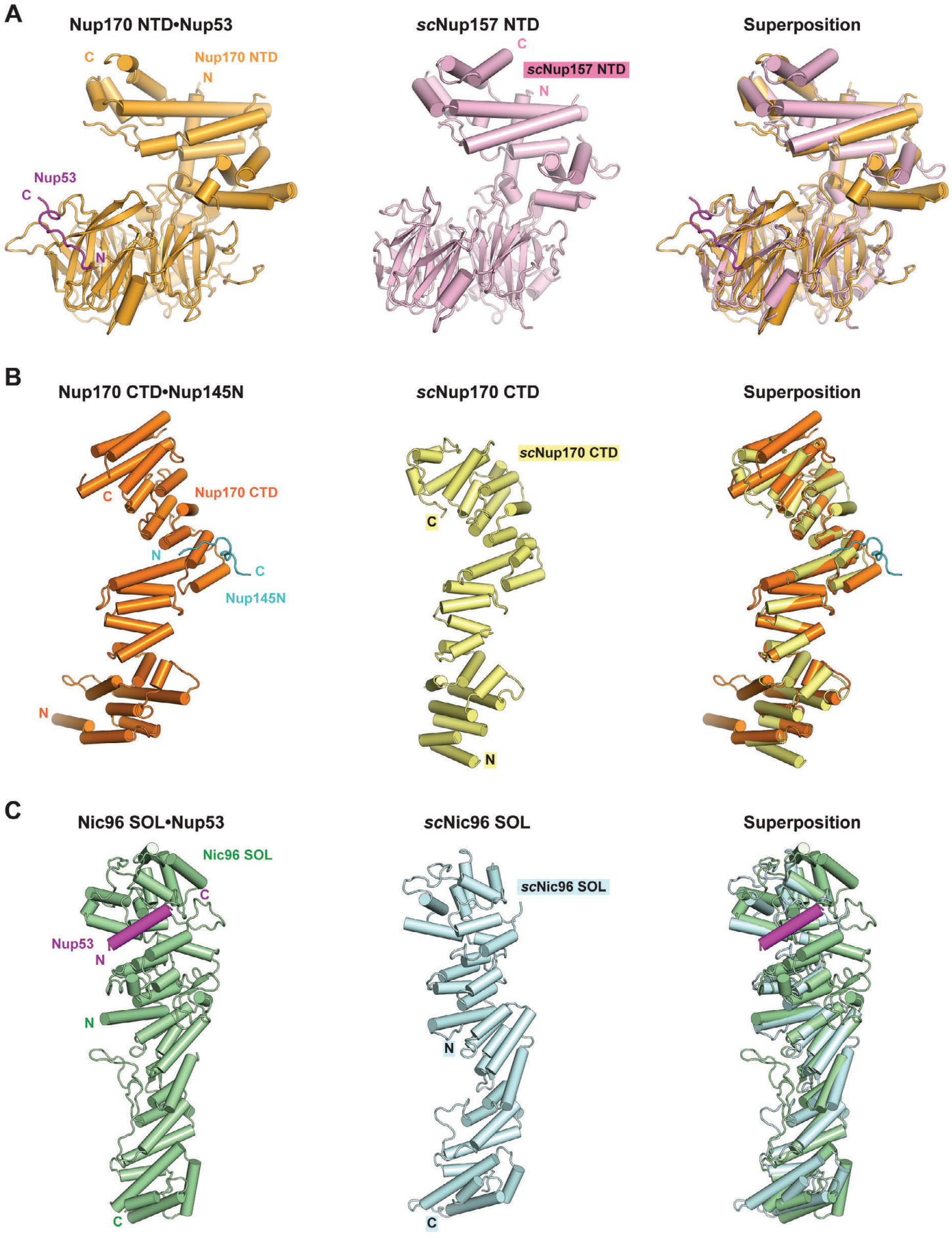

A
Nup170 NTD•Nup53
C
Nup170 NTD
N
Nup53
C
N
scNup157 NTD
C
scNup157 NTD
N
Superposition
B
Nup170 CTD•Nup145N
C
Nup170 CTD
N
C
Nup145N
N
scNup170 CTD
scNup170 CTD
C
N
Superposition
C
Nic96 SOL•Nup53
Nic96 SOL
C
Nup53
N
N
C
scNic96 SOL
scNic96 SOL
N
C
Superposition

Fig. S45.
Overall nucleoporin folds are evolutionarily conserved. Structural comparison of *C. thermophilum* Nup170 and Nic96 complexes reported here and previously determined *apo S. cerevisiae* crystal structures, revealing extensive evolutionary conservation of the overall folds. (**A**) $Nup170^{NTD}$•$Nup53^{R3}$ (left), *sc*$Nup157^{NTD}$ (PDB ID 4MHC; middle), and their superposition (right) are shown in cartoon representation (*21*). (**B**) $Nup170^{CTD}Nup145N^{R3}$ (left), *sc*$Nup170^{CTD}$ (PDB ID 3I5P; middle), and their superposition (right) are shown in cartoon representation (*23*). (**C**) $Nic96^{SOL}$•$Nup53^{R2}$ (left), *sc*$Nic96^{SOL}$ (PDB ID 2RFO; middle), and their superposition (right) are shown in cartoon representation (*24*).

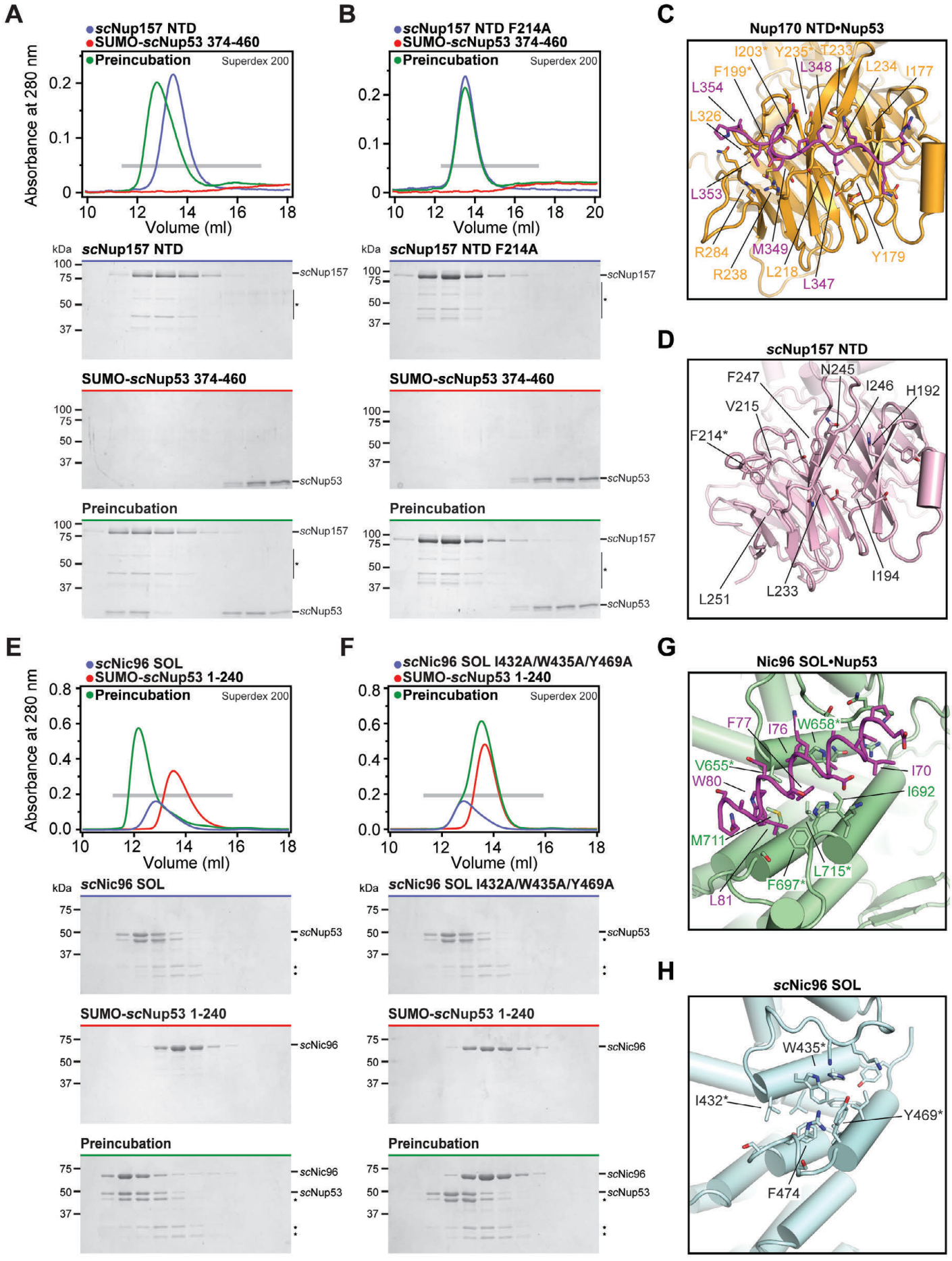

A
scNup157 NTD
SUMO-scNup53 374-460
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
kDa
scNup157 NTD
SUMO-scNup53 374-460
Preincubation
scNup157
scNup53
B
scNup157 NTD F214A
SUMO-scNup53 374-460
Preincubation
Superdex 200
Volume (ml)
kDa
scNup157 NTD F214A
SUMO-scNup53 374-460
Preincubation
scNup157
scNup53
C
Nup170 NTD•Nup53
I203*
Y235*
T233
F199*
L348
L234
I177
L354
L326
L353
R284
M349
R238
L218
L347
Y179
D
scNup157 NTD
F247
N245
I246
H192
V215
F214*
I194
L233
L251
E
scNic96 SOL
SUMO-scNup53 1-240
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
kDa
scNic96 SOL
SUMO-scNup53 1-240
Preincubation
scNup53
scNic96
F
scNic96 SOL I432A/W435A/Y469A
SUMO-scNup53 1-240
Preincubation
Superdex 200
Volume (ml)
kDa
scNic96 SOL I432A/W435A/Y469A
SUMO-scNup53 1-240
Preincubation
scNup53
scNic96
G
Nic96 SOL•Nup53
F77
I76
W658*
V655*
W80
I70
I692
M711
L715*
F697*
L81
H
scNic96 SOL
W435*
I432*
Y469*
F474

Fig. S46.
Binding pockets on *sc*Nup157 and *sc*Nic96 for *sc*Nup53 are evolutionarily conserved. (**A**, **B**) SEC interaction experiments were performed with constructs of *S. cerevisiae* $Nup157^{NTD}$ and Nup53 homologous to the crystallized $Nup170^{NTD}$•$Nup53^{R3}$ complex and with a mutant $Nup157^{NTD}$ construct in which a conserved F214 residue in the Nup53 binding pocket was mutated to alanine. SEC profiles of nucleoporins are shown individually (blue and red) and after their preincubation (green). (**C**) A close-up view of the $Nup170^{NTD}$•$Nup53^{R3}$ complex, same as Fig. 3C. (**D**) A close-up view of the corresponding surface of *sc*Nup157 (PDB ID 4MHC) reveals that the hydrophobic binding pocket for $Nup53^{R3}$ is intact (*21*). An asterisk highlights the *sc*$Nup157^{NTD}$ mutant that abolished the interaction with *sc*Nup53. (**E**, **F**) Interaction experiments were performed with fragments of *S. cerevisiae* $Nic96^{SOL}$ and Nup53 homologous to the crystallized $Nic96^{SOL}$•$Nup53^{R2}$ complex and with mutant *sc*$Nic96^{SOL}$ in which conserved hydrophobic residues within the Nup53 binding pocket were mutated to alanine. SEC profiles of nucleoporins are shown individually (blue and red) and after their preincubation (green). (**G**) A close-up view of the $Nic96^{SOL}$•$Nup53^{R2}$ complex, same as Fig. 4C. (**H**) A close-up view of the corresponding surface of *sc*$Nic96^{SOL}$ (PDB ID 2RFO) reveals that the hydrophobic groove that recognizes $Nup53^{R2}$ is intact (*24*). Asterisks highlight the *sc*$Nic96^{SOL}$ mutants that abolished the interaction with *sc*Nup53. All SEC profiles were obtained using a Superdex 200 10/300 GL column. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining.

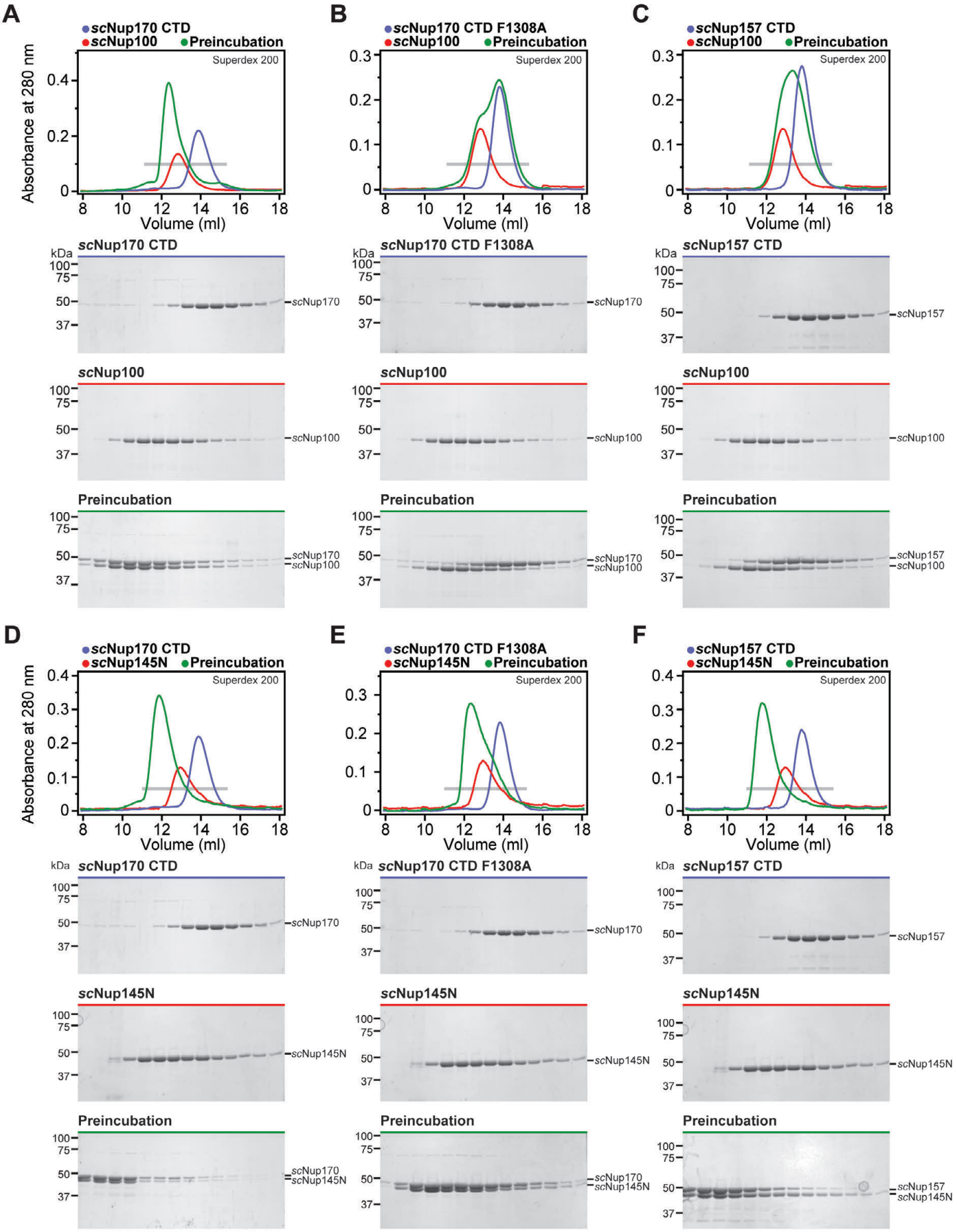

A
B
C
D
E
F
scNup170 CTD
scNup100
Preincubation
scNup170 CTD F1308A
scNup157 CTD
scNup145N
Superdex 200
Absorbance at 280 nm
Volume (ml)
kDa
scNup170
scNup157
scNup145N

Fig. S47.
Binding pockets on *sc*Nup157 and *sc*Nup170 for the Nup145N paralogs *sc*Nup100 and *sc*Nup145N are conserved. (**A**-**F**) SEC profiles of nucleoporins or nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining.

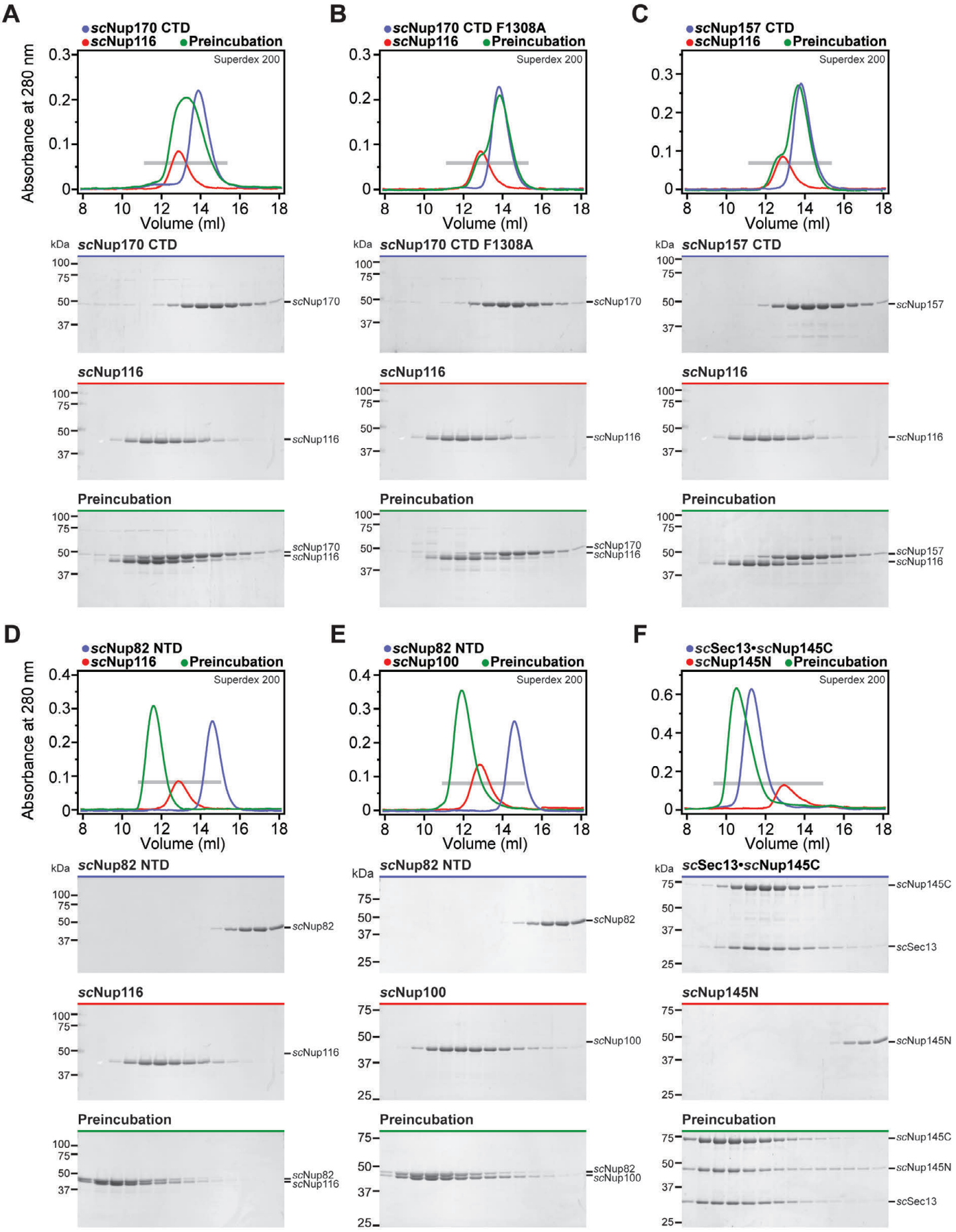

A
scNup170 CTD
scNup116
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
kDa
scNup170 CTD
scNup170
scNup116
Preincubation
scNup170
scNup116
B
scNup170 CTD F1308A
scNup116
Preincubation
Superdex 200
Volume (ml)
scNup170 CTD F1308A
scNup170
scNup116
Preincubation
scNup170
scNup116
C
scNup157 CTD
scNup116
Preincubation
Superdex 200
Volume (ml)
scNup157 CTD
scNup157
scNup116
Preincubation
scNup157
scNup116
D
scNup82 NTD
scNup116
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
scNup82 NTD
scNup82
scNup116
scNup116
Preincubation
scNup82
scNup116
E
scNup82 NTD
scNup100
Preincubation
Superdex 200
Volume (ml)
scNup82 NTD
scNup82
scNup100
scNup100
Preincubation
scNup82
scNup100
F
scSec13•scNup145C
scNup145N
Preincubation
Superdex 200
Volume (ml)
scSec13•scNup145C
scNup145C
scSec13
scNup145N
scNup145N
Preincubation
scNup145C
scNup145N
scSec13

Fig. S48.
Other Nup145N interactions are conserved in the three *S. cerevisiae* paralogs *sc*Nup100, *sc*Nup145N, and *sc*Nup116, but *sc*Nup116 only binds very weakly to *sc*Nup170^{CTD} and *sc*Nup157^{CTD}. (**A**-**F**) SEC profiles of nucleoporins or nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining.

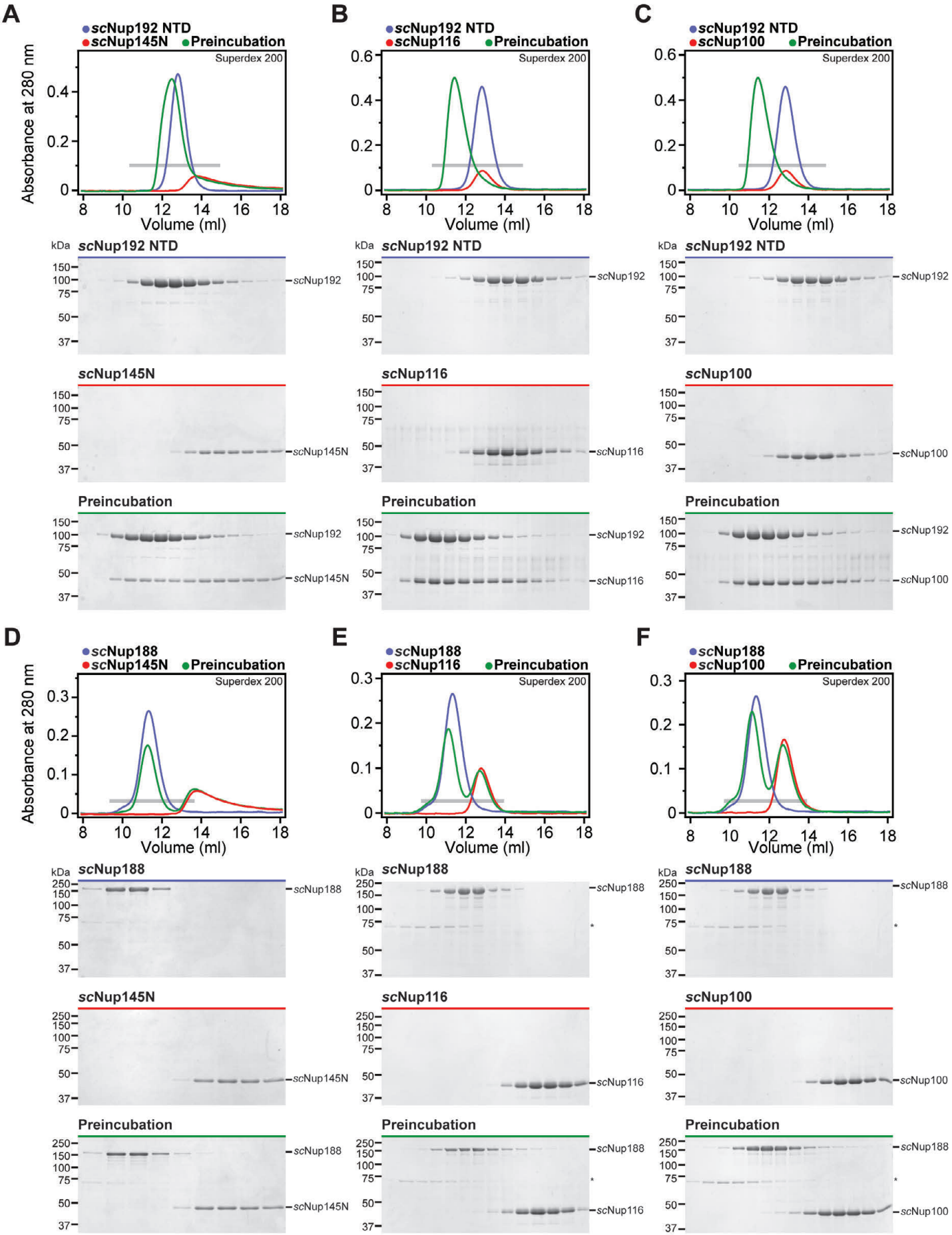
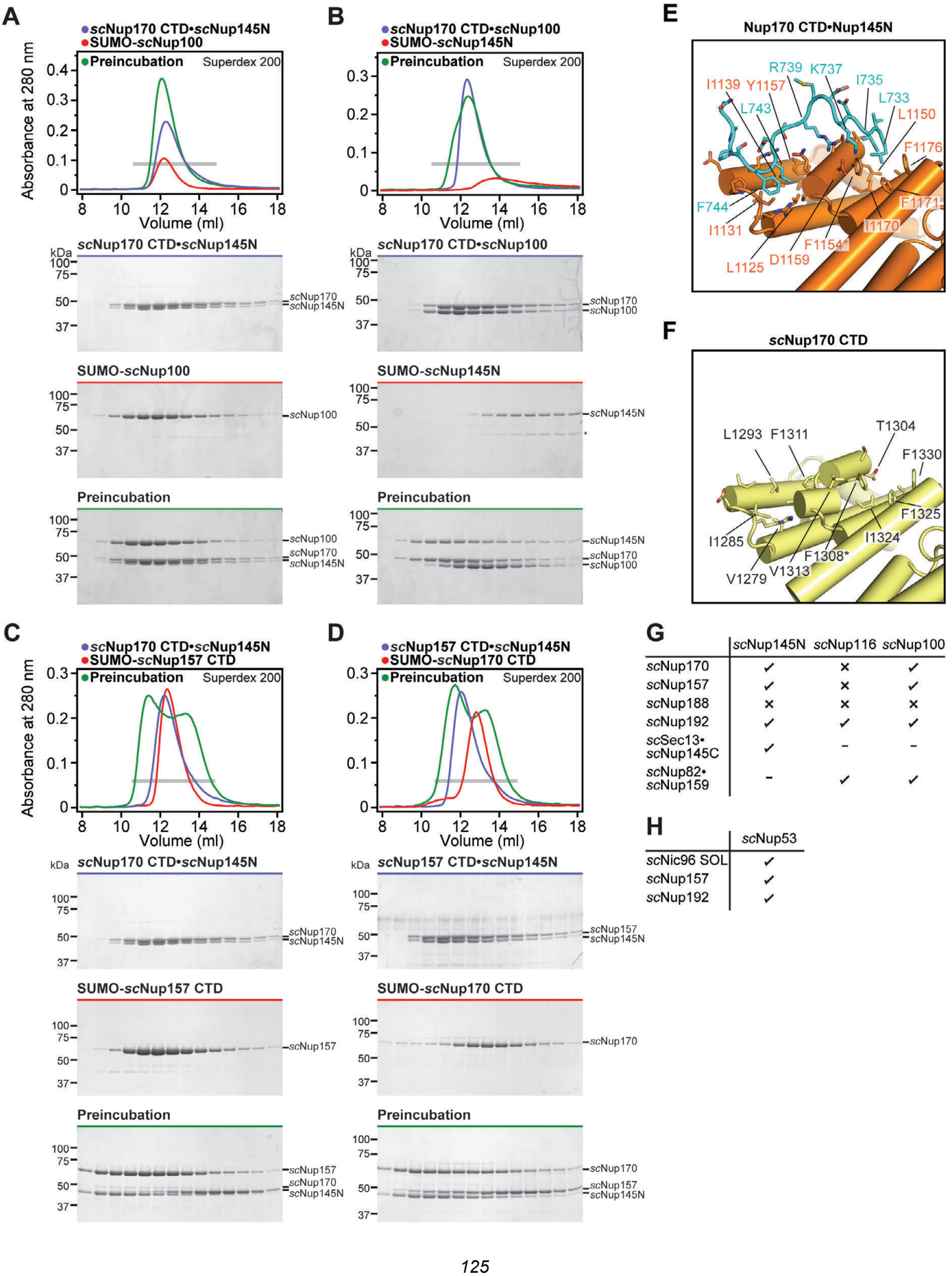

A
B
C
D
E
F
scNup192 NTD
scNup145N
scNup116
scNup100
scNup188
Preincubation
Superdex 200
Absorbance at 280 nm
Volume (ml)
kDa
scNup192
scNup145N
scNup116
scNup100
scNup188
Preincubation
*

Fig. S49.
All three *S. cerevisiae* Nup145N paralogs bind to *sc*Nup192^{NTD}, but none bind to *sc*Nup188. (**A**-**F**) SEC profiles of nucleoporins or nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining.



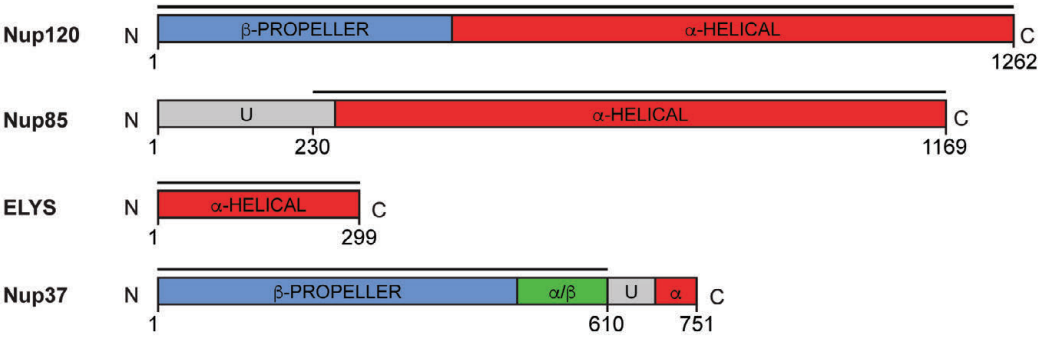
G

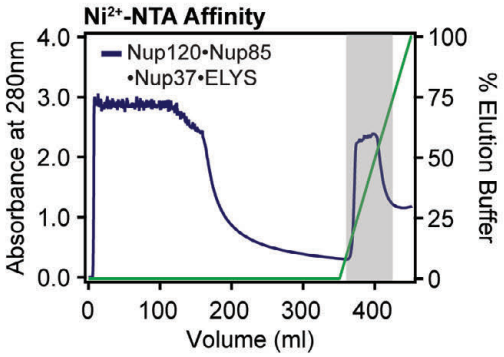
	scNup145N	scNup116	scNup100
scNup170	✓	×	✓
scNup157	✓	×	✓
scNup188	×	×	×
scNup192	✓	✓	✓
scSec13•scNup145C	✓	-	-
scNup82•scNup159	-	✓	✓

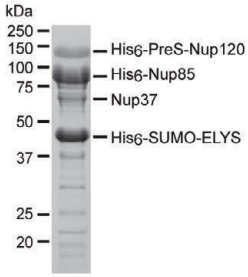
H

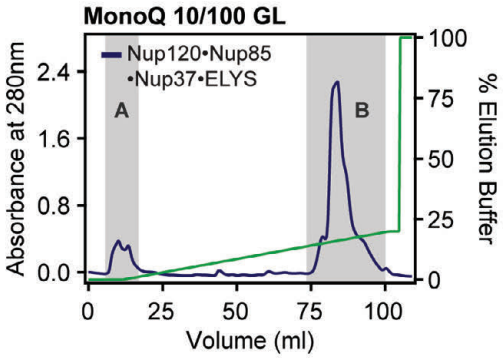
	scNup53
scNic96 SOL	✓
scNup157	✓
scNup192	✓

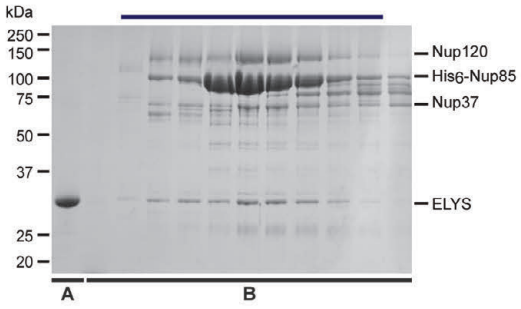
Fig. S50.
***sc*Nup145N and *sc*Nup100 bind to *sc*Nup170^{CTD} and *sc*Nup157^{CTD} in a mutually exclusive fashion.** (**A**, **B**) *sc*Nup145N and *sc*Nup100 bind to *sc*Nup170^{CTD} in a mutually exclusive fashion and *sc*Nup145N outcompetes *sc*Nup100. (**C**, **D**) *sc*Nup157^{CTD} and *sc*Nup170^{CTD} bind to *sc*Nup145N in a mutually exclusive fashion. SEC profiles of nucleoporins or nucleoporin complexes are shown individually (blue and red) and after their preincubation (green). All SEC profiles were obtained using a Superdex 200 10/300 GL column. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. (**E**) Close-up view of the Nup170^{CTD}•Nup145N^{R3} complex. (**F**) Close-up view of the corresponding surface in *sc*Nup170^{CTD} (PDB ID 3I5P) revealed that both hydrophobic binding pockets for Nup145N^{R3} are conserved (*23*). The conserved residue, F1308, which was mutated in the interaction experiments in figs. S47 and S48 is indicated by an asterisk. (**G**) Summary of the results of interaction experiments performed with *S. cerevisiae* Nup145N homologs. Check marks indicate complexes that can form in SEC experiments, crosses indicate complexes that do not form, and dashes indicate complexes that were not tested. (**H**) Summary of the results of interaction experiments performed with *S. cerevisiae* Nup53. Check marks indicate complexes that can form in SEC experiments, crosses indicate complexes that do not form, and dashes indicate complexes that were not tested.

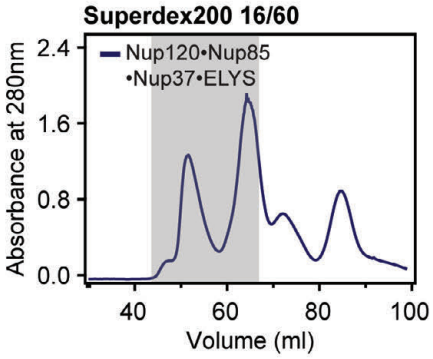

Nup120
N
β-PROPELLER
α-HELICAL
C
1
1262
Nup85
N
U
α-HELICAL
C
1
230
1169
ELYS
N
α-HELICAL
C
1
299
Nup37
N
β-PROPELLER
α/β
U
α
C
1
610
751


Ni²⁺-NTA Affinity
Nup120•Nup85
•Nup37•ELYS
Absorbance at 280nm
% Elution Buffer
Volume (ml)


kDa
His6-PreS-Nup120
His6-Nup85
Nup37
His6-SUMO-ELYS


MonoQ 10/100 GL
Nup120•Nup85
•Nup37•ELYS
A
B
Absorbance at 280nm
% Elution Buffer
Volume (ml)


kDa
Nup120
His6-Nup85
Nup37
ELYS
A
B


Superdex200 16/60
Nup120•Nup85
•Nup37•ELYS
Absorbance at 280nm
Volume (ml)

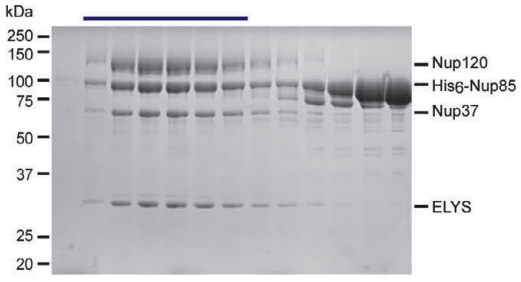
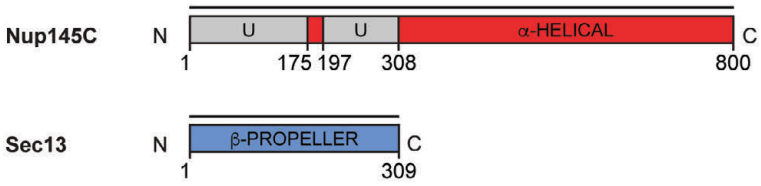
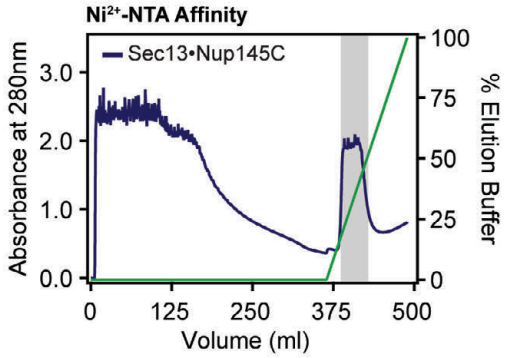
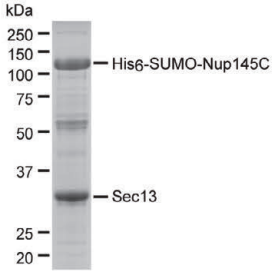
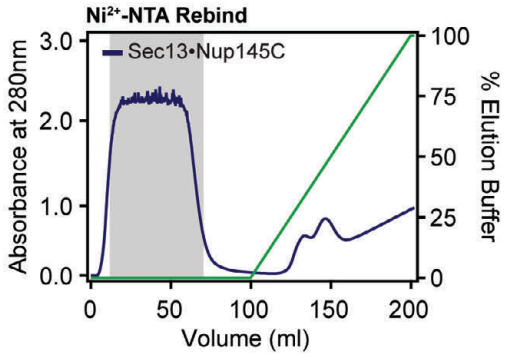

kDa
Nup120
His6-Nup85
Nup37
ELYS

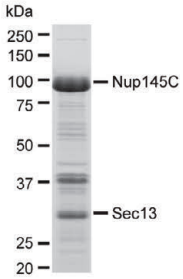
Fig. S51.
Purification protocol for Nup120•Nup37•ELYS•Nup85 hetero-tetramer. Domain boundaries of the purified nucleoporins are shown with black lines indicating the construct boundaries. Sequential chromatography purification steps are shown from top to bottom with the employed columns indicated. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Pooled fractions are indicated with a black bar above the SDS-PAGE gels. For details of the buffer conditions see Table S2.

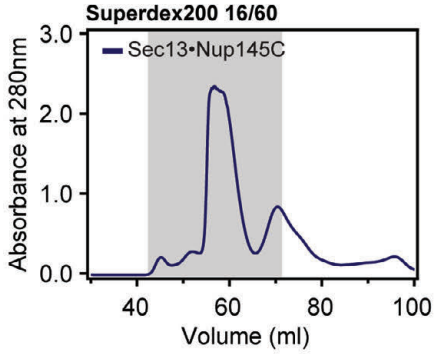

Nup145C
N
U
U
α-HELICAL
C
1
175
197
308
800
Sec13
N
β-PROPELLER
C
1
309


Ni²⁺-NTA Affinity
Sec13•Nup145C
Absorbance at 280nm
% Elution Buffer
Volume (ml)
0.0
1.0
2.0
3.0
0
25
50
75
100
0
125
250
375
500


kDa
250
150
100
75
50
37
25
20
His6-SUMO-Nup145C
Sec13


Ni²⁺-NTA Rebind
Sec13•Nup145C
Absorbance at 280nm
% Elution Buffer
Volume (ml)
0.0
1.0
2.0
3.0
0
25
50
75
100
0
50
100
150
200


kDa
250
150
100
75
50
37
25
20
Nup145C
Sec13


Superdex200 16/60
Sec13•Nup145C
Absorbance at 280nm
Volume (ml)
0.0
1.0
2.0
3.0
40
60
80
100

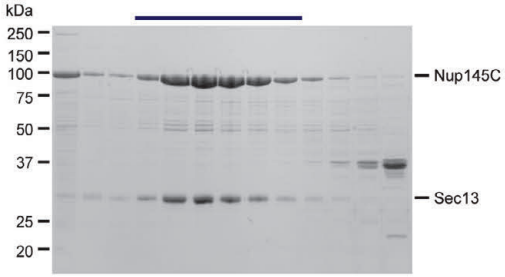
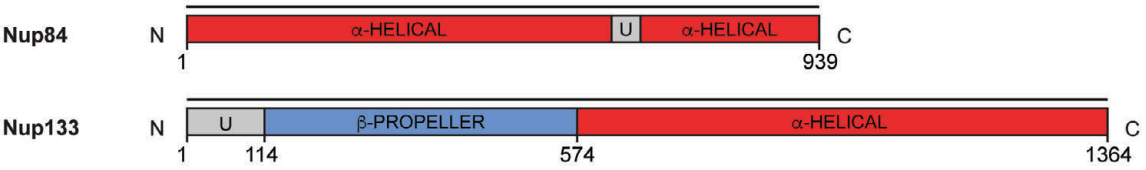
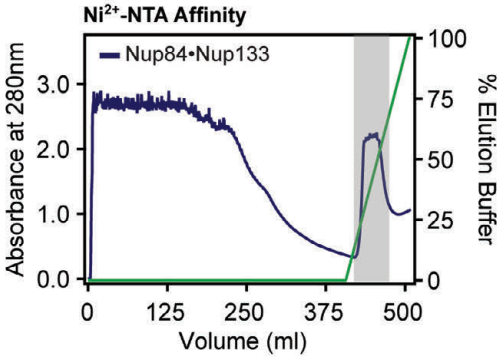
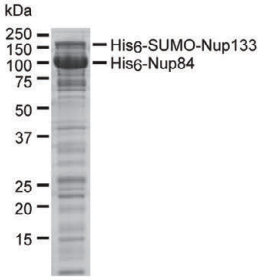
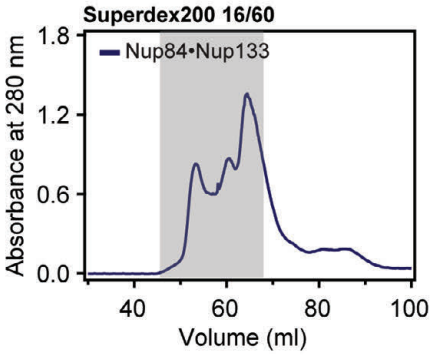

kDa
250
150
100
75
50
37
25
20
Nup145C
Sec13

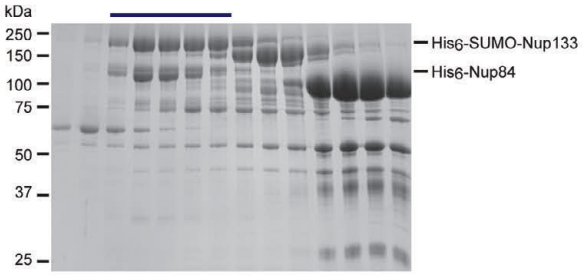
Fig. S52.
Purification protocol for the Sec13•Nup145C hetero-dimer. Domain boundaries of the purified nucleoporins are shown with black lines indicating the construct boundaries. Sequential chromatography purification steps are shown from top to bottom with the employed columns indicated. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Pooled fractions are indicated with a black bar above the SDS-PAGE gels. For details of the buffer conditions see Table S2.

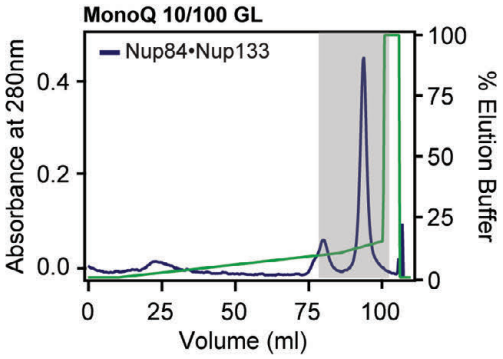

Nup84
N
α-HELICAL
U
α-HELICAL
C
1
939
Nup133
N
U
β-PROPELLER
α-HELICAL
C
1
114
574
1364


Ni2+-NTA Affinity
Nup84•Nup133
Absorbance at 280nm
% Elution Buffer
Volume (ml)


kDa
His6-SUMO-Nup133
His6-Nup84


Superdex200 16/60
Nup84•Nup133
Absorbance at 280 nm
Volume (ml)


kDa
His6-SUMO-Nup133
His6-Nup84


MonoQ 10/100 GL
Nup84•Nup133
Absorbance at 280nm
% Elution Buffer
Volume (ml)

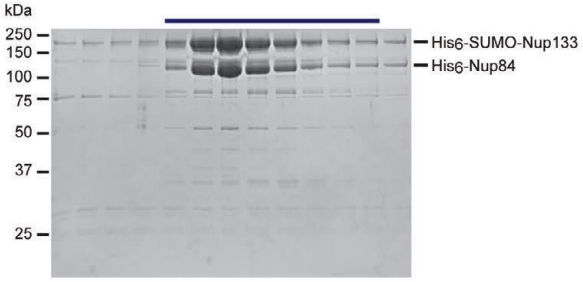
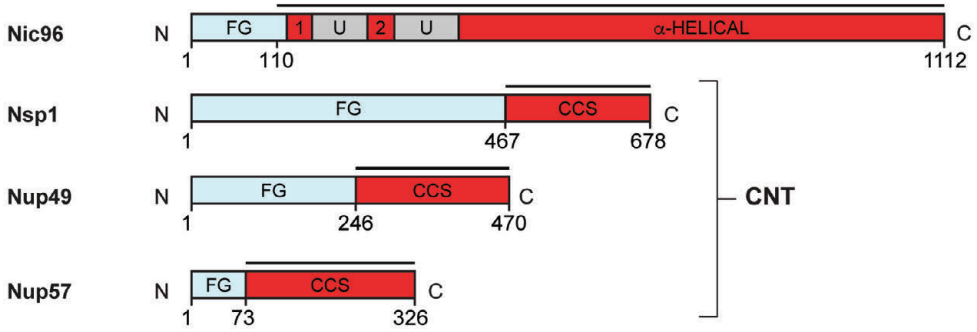
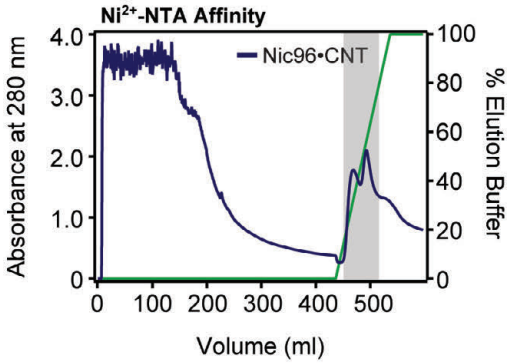
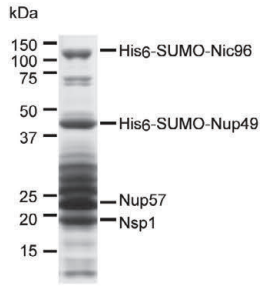
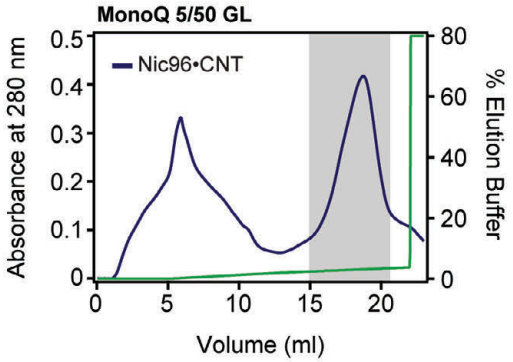

kDa
His6-SUMO-Nup133
His6-Nup84

Fig. S53.
Purification protocol for the Nup84•Nup133 hetero-dimer. Domain boundaries for the purified proteins are shown above with black lines indicating the construct boundaries. Sequential steps of purification via chromatography are shown from top to bottom, with the fractions pooled for the subsequent step highlighted in grey. SDS-PAGE gels for each step of the purification are shown next to each chromatogram. Buffer conditions can be found in Table S2.


Nic96
N
FG
1
U
2
U
α-HELICAL
C
1
110
1112
Nsp1
FG
CCS
467
678
Nup49
246
470
Nup57
73
326
CNT


Ni2+-NTA Affinity
Nic96•CNT
Absorbance at 280 nm
% Elution Buffer
Volume (ml)


kDa
His6-SUMO-Nic96
His6-SUMO-Nup49
Nup57
Nsp1


MonoQ 5/50 GL
Nic96•CNT
Absorbance at 280 nm
% Elution Buffer
Volume (ml)

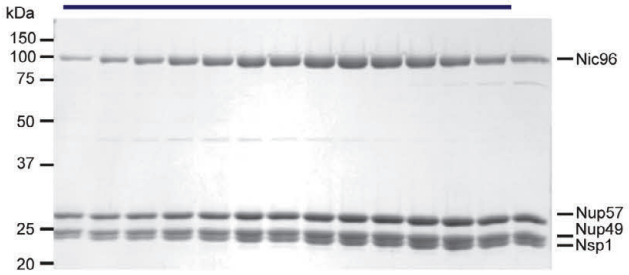
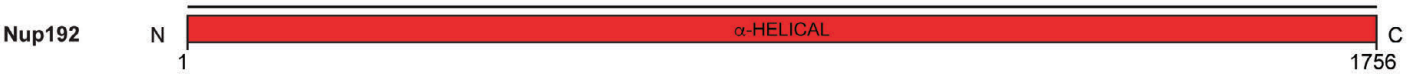
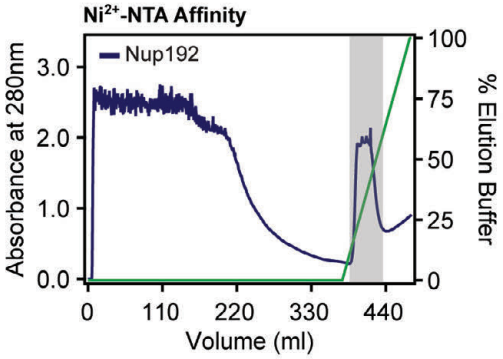
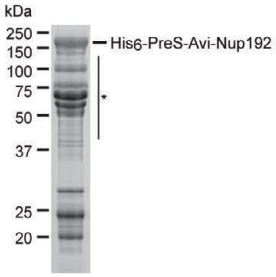
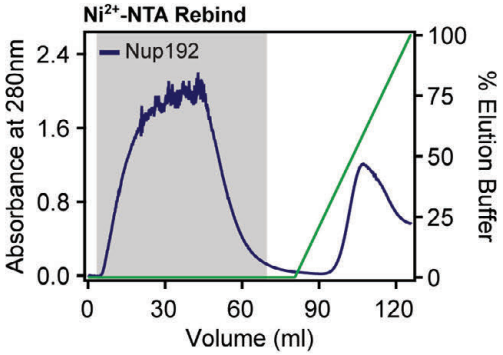

kDa
Nic96
Nup57
Nup49
Nsp1

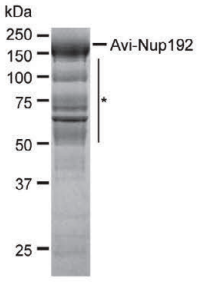
Fig. S54.
Purification protocol for the CNT•Nic96 hetero-tetramer. Domain boundaries of the purified nucleoporins are shown with black lines indicating the construct boundaries. Sequential chromatography purification steps are shown from top to bottom with the employed columns indicated. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Pooled fractions are indicated with a black bar above the SDS-PAGE gels. For details of the buffer conditions see Table S2.

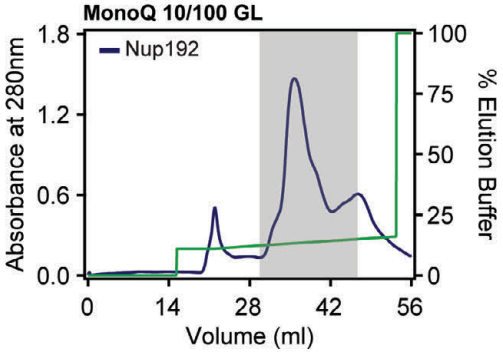

Nup192
N
α-HELICAL
C
1
1756

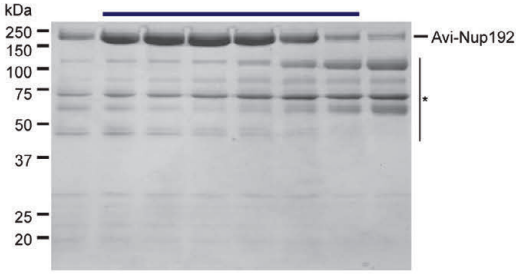

Ni2+-NTA Affinity
Nup192
Absorbance at 280nm
% Elution Buffer
Volume (ml)

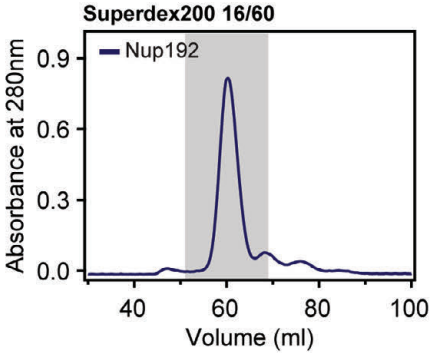

kDa
His6-PreS-Avi-Nup192
*


Ni2+-NTA Rebind
Nup192
Absorbance at 280nm
% Elution Buffer
Volume (ml)


kDa
Avi-Nup192
*


MonoQ 10/100 GL
Nup192
Absorbance at 280nm
% Elution Buffer
Volume (ml)


kDa
Avi-Nup192
*


Superdex200 16/60
Nup192
Absorbance at 280nm
Volume (ml)

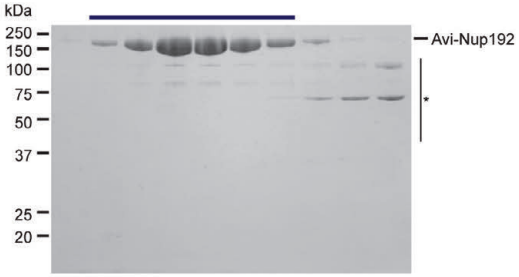
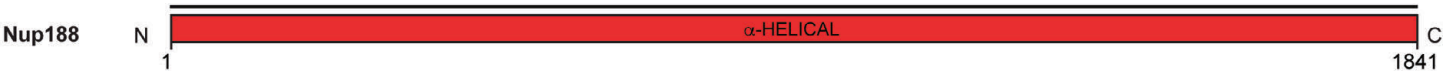
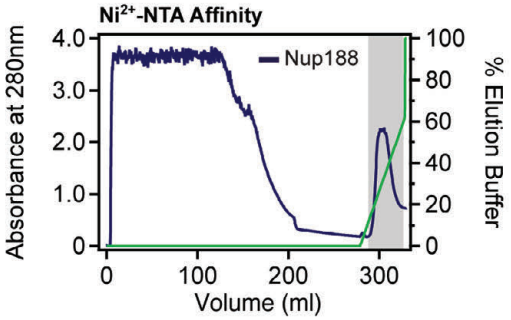
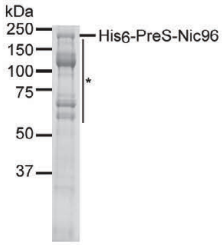
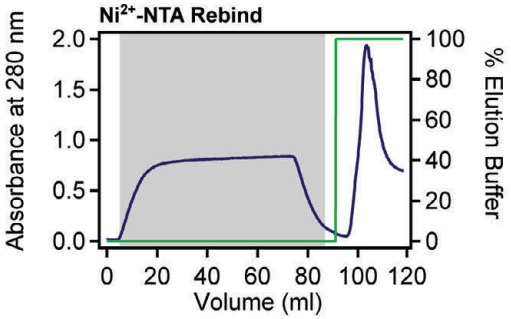

kDa
Avi-Nup192
*

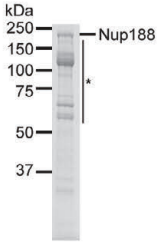
Fig. S55.
Purification protocol for Nup192. Domain boundaries of the purified nucleoporins are shown with black lines indicating the construct boundaries. Sequential chromatography purification steps are shown from top to bottom with the employed columns indicated. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Pooled fractions are indicated with a black bar above the SDS-PAGE gels. For details of the buffer conditions see Table S2.

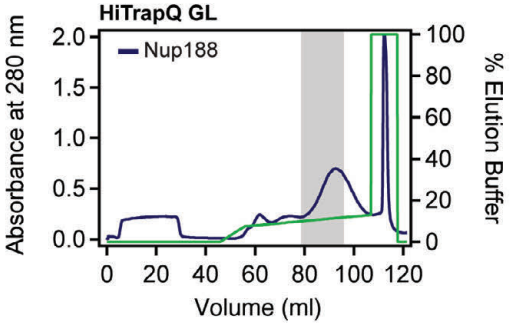

Nup188
N
α-HELICAL
C
1
1841

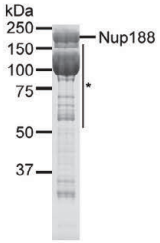

Ni2+-NTA Affinity
Nup188
Absorbance at 280nm
% Elution Buffer
Volume (ml)

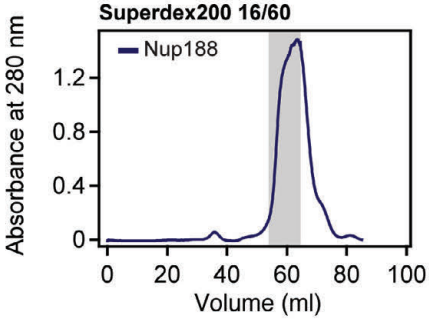

kDa
His6-PreS-Nic96
*

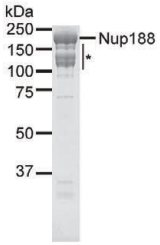

Ni2+-NTA Rebind
Absorbance at 280 nm
% Elution Buffer
Volume (ml)

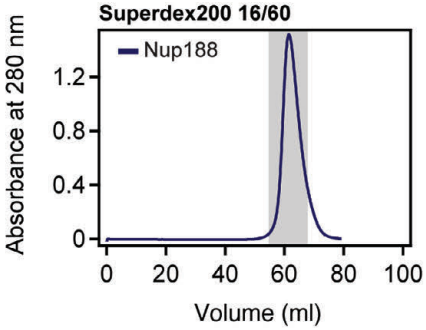

kDa
Nup188
*


HiTrapQ GL
Nup188
Absorbance at 280 nm
% Elution Buffer
Volume (ml)


kDa
Nup188
*


Superdex200 16/60
Nup188
Absorbance at 280 nm
Volume (ml)


kDa
Nup188
*


Superdex200 16/60
Nup188
Absorbance at 280 nm
Volume (ml)

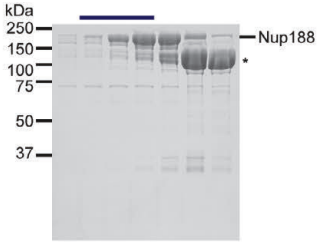
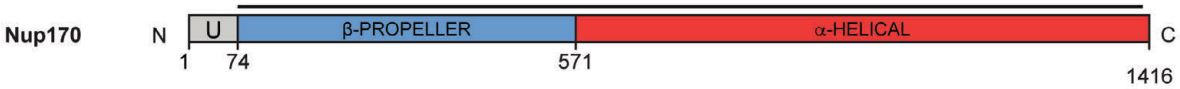
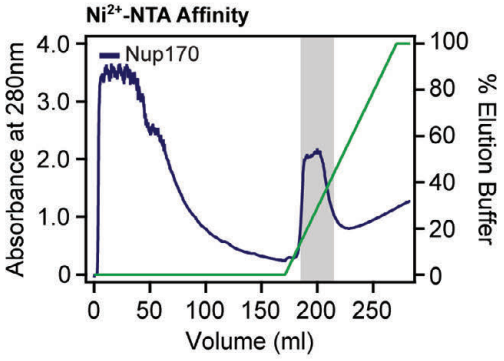
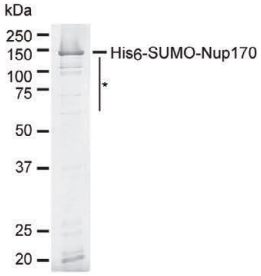
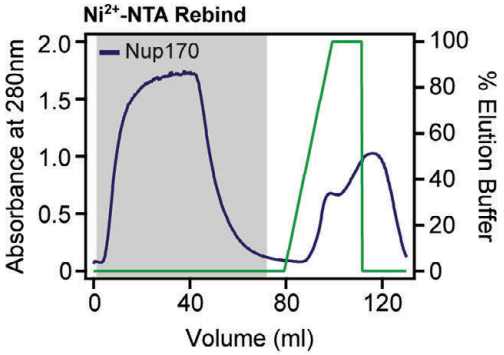

kDa
Nup188
*

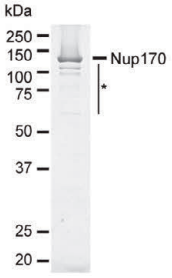
Fig. S56.
Purification protocol for Nup188. Domain boundaries of the purified nucleoporins are shown with black lines indicating the construct boundaries. Sequential chromatography purification steps are shown from top to bottom with the employed columns indicated. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Pooled fractions are indicated with a black bar above the SDS-PAGE gels. For details of the buffer conditions see Table S2.

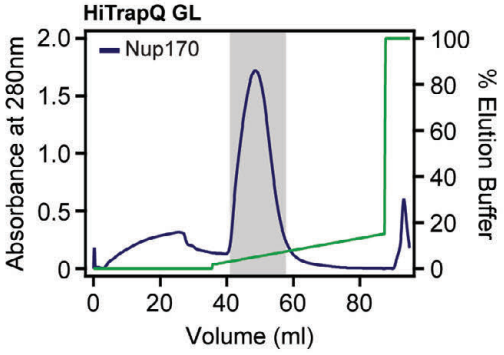

Nup170
N
U
β-PROPELLER
α-HELICAL
C
1
74
571
1416

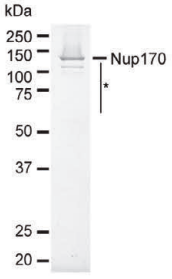

Ni2+-NTA Affinity
Nup170
Absorbance at 280nm
% Elution Buffer
Volume (ml)

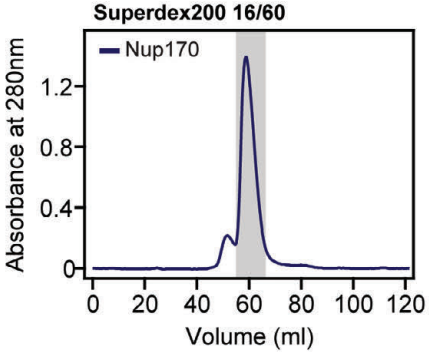

kDa
His6-SUMO-Nup170
*


Ni2+-NTA Rebind
Nup170
Absorbance at 280nm
% Elution Buffer
Volume (ml)


kDa
Nup170
*


HiTrapQ GL
Nup170
Absorbance at 280nm
% Elution Buffer
Volume (ml)


kDa
Nup170
*


Superdex200 16/60
Nup170
Absorbance at 280nm
Volume (ml)

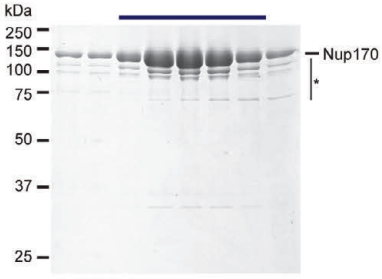
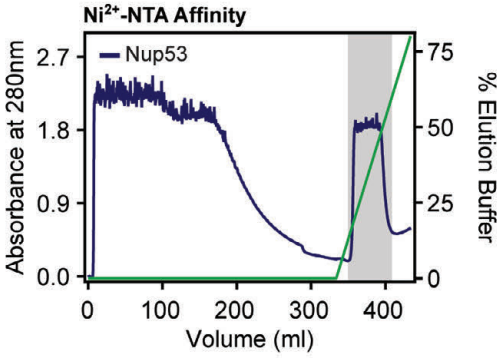
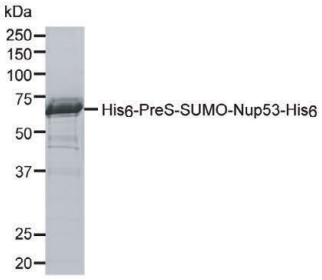
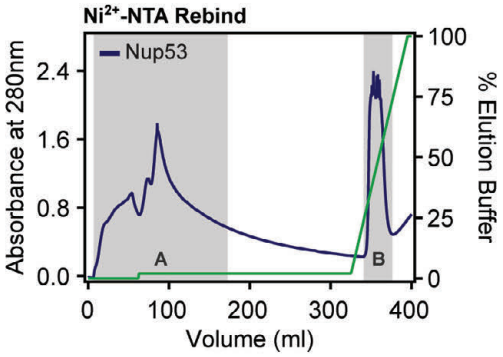

kDa
Nup170
*

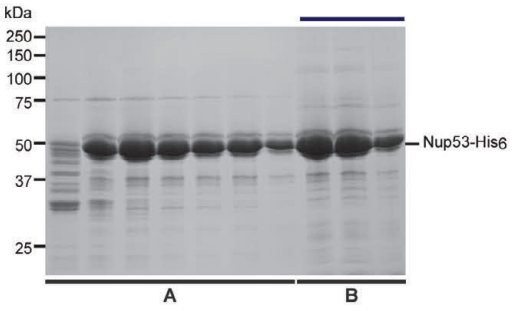
Fig. S57.
Purification protocol for Nup170. Domain boundaries of the purified nucleoporins are shown with black lines indicating the construct boundaries. Sequential chromatography purification steps are shown from top to bottom with the employed columns indicated. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Pooled fractions are indicated with a black bar above the SDS-PAGE gels. For details of the buffer conditions see Table S2.

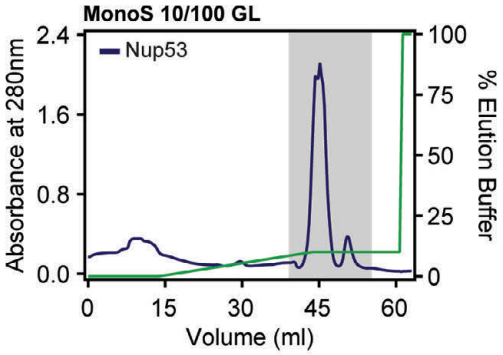

Nup53
N
1
2
RRM
3
M
C
1
133
252
361
426

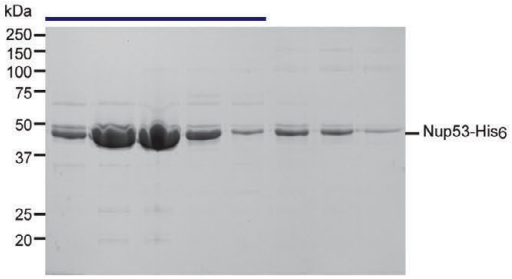

Ni2+-NTA Affinity
Nup53
Absorbance at 280nm
% Elution Buffer
Volume (ml)

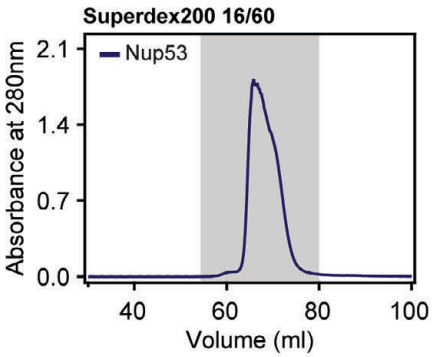

kDa
His6-PreS-SUMO-Nup53-His6


Ni2+-NTA Rebind
Nup53
Absorbance at 280nm
% Elution Buffer
A
B
Volume (ml)


kDa
Nup53-His6
A
B


MonoS 10/100 GL
Nup53
Absorbance at 280nm
% Elution Buffer
Volume (ml)


kDa
Nup53-His6


Superdex200 16/60
Nup53
Absorbance at 280nm
Volume (ml)

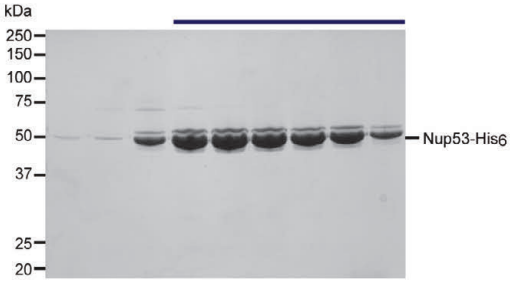
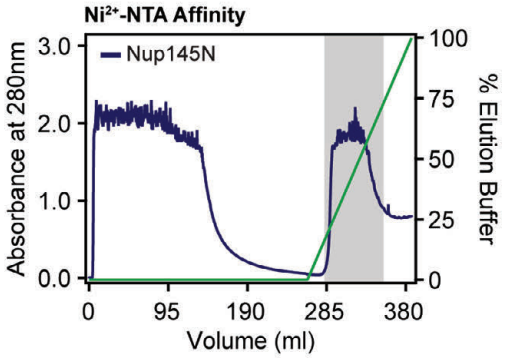
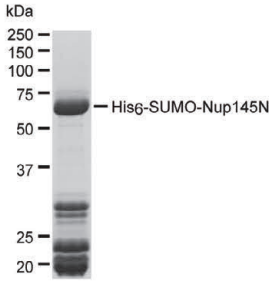
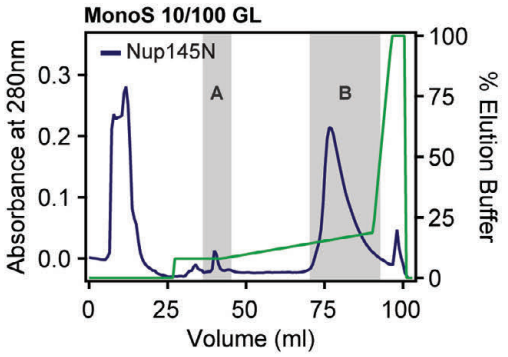

kDa
Nup53-His6

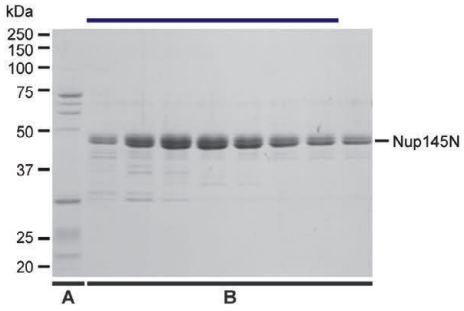
Fig. S58.
Purification protocol for Nup53. Domain boundaries of the purified nucleoporins are shown with black lines indicating the construct boundaries. Sequential chromatography purification steps are shown from top to bottom with the employed columns indicated. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Pooled fractions are indicated with a black bar above the SDS-PAGE gels. For details of the buffer conditions see Table S2.

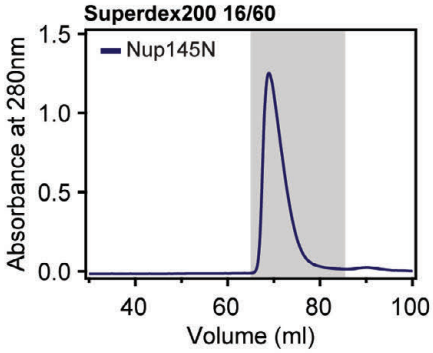

Nup145N
FG
R1
R2
3
APD
1
606
868
994


Ni²⁺-NTA Affinity
Nup145N
Absorbance at 280nm
% Elution Buffer
Volume (ml)


kDa
His6-SUMO-Nup145N


MonoS 10/100 GL
Nup145N
A
B
Absorbance at 280nm
% Elution Buffer
Volume (ml)


kDa
Nup145N
A
B


Superdex200 16/60
Nup145N
Absorbance at 280nm
Volume (ml)

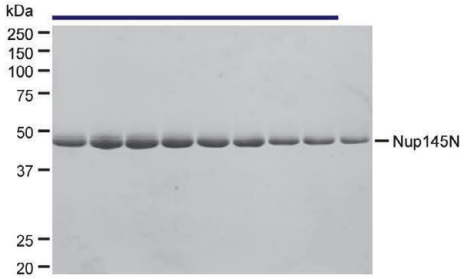

kDa
Nup145N

Fig. S59.
Purification protocol for Nup145N. Domain boundaries of the purified nucleoporins are shown with black lines indicating the construct boundaries. Sequential chromatography purification steps are shown from top to bottom with the employed columns indicated. Gray bars indicate fractions that were resolved on SDS-PAGE gels and visualized by Coomassie staining. Pooled fractions are indicated with a black bar above the SDS-PAGE gels. For details of the buffer conditions see Table S2.

Table S1.

Bacterial expression constructs and expression conditions

#	Protein	Residues	Expression vector	Restriction sites 5', 3'	N-terminal overhang	C-terminal overhang	Expression conditions
1	Nup82 NTD	1-595	pET28a-PreS	NdeI, NotI	GPH	none	18 °C / 18 hours
2	Nup159 T	1440-1481	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 18 hours
3	Nup120 Nup37 ELYS	1-1262 1-610 1-299	pET-MCN-SUMO	NdeI, BamHI NdeI, BamHI BamHI, NotI	GPHMHHHHHH Q S	none none none	37 °C / 2 hours
4	Nup85	230-1169	pET24a	NdeI, BamHI	MGHHHHHH	none	37 °C / 2 hours
5	Sec13	1-309	pETMCN	NdeI, BamHI	Q	none	23 °C / 18 hours; coexpressed with Nup145C
6	Nup145C	1-800	pET-MCN-SUMO	NcoI, NotI	S	none	23 °C / 18 hours; coexpressed with Sec13
7	Nup84	1-939	pET24a	NdeI, BamHI	MGHHHHHH	none	23 °C / 18 hours; coexpressed with Nup133
8	Nup133	1-1364	pET-MCN-SUMO	EcoRI, HindIII	SEF	none	23 °C / 18 hours; coexpressed with Nup84
9	Nup133 ΔNTE	105-1364	pET-MCN-SUMO	EcoRI, HindIII	SEF	none	23 °C / 18 hours; coexpressed with Nup84
10	Nup53	1-361	pET28a-SUMO	BamHI, XhoI	S	LEHHHHHH	23 °C / 18 hours
11♦	Nup53 RRM	133-253	pET28a-PreS	NdeI, XhoI	GPHM	none	23 °C / 18 hours
12	Nup53 R2	69-90	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
13♦	Nup53	31-84	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
14	Nup53 R1	31-67	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
15	Nup53	1-90	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
16	Nup53 R3	329-361	pGEX-6P-1	BamHI, NotI	not cleaved	none	37 °C / 2 hours
17	Nup53 R3 (F334A)	329-361	pGEX-6P-1	BamHI, NotI	not cleaved	none	37 °C / 2 hours
18	Nup53 R3 (I338A)	329-361	pGEX-6P-1	BamHI, NotI	not cleaved	none	37 °C / 2 hours
19	Nup53 R3 (R342A)	329-361	pGEX-6P-1	BamHI, NotI	not cleaved	none	37 °C / 2 hours
20	Nup53 R3 (K343A)	329-361	pGEX-6P-1	BamHI, NotI	not cleaved	none	37 °C / 2 hours
21	Nup53 R3 (L346A)	329-361	pGEX-6P-1	BamHI, NotI	not cleaved	none	37 °C / 2 hours
22	Nup53 R3 (L347A)	329-361	pGEX-6P-1	BamHI, NotI	not cleaved	none	37 °C / 2 hours
23	Nup53 R3 (E350A)	329-361	pGEX-6P-1	BamHI, NotI	not cleaved	none	37 °C / 2 hours
24	Nup53 R3 (E351A)	329-361	pGEX-6P-1	BamHI, NotI	not cleaved	none	37 °C / 2 hours
25	Nup53 R3 (L353A)	329-361	pGEX-6P-1	BamHI, NotI	not cleaved	none	37 °C / 2 hours
26	Nup53 R3 (L354A)	329-361	pGEX-6P-1	BamHI, NotI	not cleaved	none	37 °C / 2 hours
27	Nup170	74-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
28	Nup170 ΔC	74-1037	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
29♦	Nup170 SOL	575-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
30	Nup170 CTD	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
31♦	Nup170 CTD (ΔTLR)	851-1402 (Δ1375-1377)	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
32♦	Nup170 NTD	74-827 (Δ293-305)	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
33♦	Nup170 NTD	74-843 (Δ293-305)	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
34	Nup170 NTD (F199A)	74-843	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
35	Nup170 NTD (Y235A)	74-843	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
36	Nup170 NTD (V241A)	74-843	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
37	Nup170 NTD (W280A)	74-843	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
38♦	Nup170 CTD	851-1416	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
39	Nup170 NTD (F281A)	74-843	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
40	Nup170 NTD (R284A)	74-843	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
41	Nup170 NTD (I203A)	74-843	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
42	Nup170 NTD (M239A)	74-843	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
43	Nup170 NTD (L218A)	74-843	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
44	Nup170 NTD (E214A)	74-843	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
45	Nup170 NTD (E181A)	74-843	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
46	Nup170 CTD (Y1050A)	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
47	Nup170 CTD (V1062A)	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
48	Nup170 CTD (L1066A)	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
49	Nup170 CTD (E1110A)	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
50	Nup170 CTD (L1111A)	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
51	Nup170 CTD (I1131A)	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
52	Nup170 CTD (I1147A)	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
53	Nup170 CTD (Y1164A)	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
54	Nup170 CTD (F1171A)	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
55	Nup170 CTD (F1154A)	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
56	Nup170 CTD (Y1157A)	851-1402	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
57♦	Nup170 CTD (Y905M/ L1007M/L1183M/V1292M)	832-1416	pET28a-SUMO	BamHI, NotI	S	none	37 °C / 2 hours

#	Protein	Residues	Expression vector	Restriction sites 5', 3'	N-terminal overhang	C-terminal overhang	Expression conditions
58	Nup145N	606-993	pET28a-SUMO	BamHI, NotI	S	none	23 °C / 18 hours
59♦	Nup145N APD	858-993	pET28a-PreS	NdeI, XhoI	GPHM	none	23 °C / 18 hours
60♦	Nup145N APD (T994A) •Nup145C N	858-1000	pET28a-PreS	NdeI, XhoI	GPHM	none	23 °C / 18 hours
61	Nup145N R1	606-683	pET28a-SUMO	BamHI, NotI	S	AAALEHHHHHH	37 °C / 2 hours
62	Nup145N	606-750	pET28a-SUMO	BamHI, NotI	S	AAALEHHHHHH	37 °C / 2 hours
63	Nup145N	649-750	pET28a-SUMO	BamHI, NotI	S	AAALEHHHHHH	37 °C / 2 hours
64	Nup145N R3	729-750	pGEX-6P-1	BamHI, NotI	not cleaved	none	18 °C / 18 hours
65	Nup145N R3 (L733A)	729-750	pGEX-6P-1	BamHI, NotI	not cleaved	none	18 °C / 18 hours
66	Nup145N R3 (V734A)	729-750	pGEX-6P-1	BamHI, NotI	not cleaved	none	18 °C / 18 hours
67	Nup145N R3 (I735A)	729-750	pGEX-6P-1	BamHI, NotI	not cleaved	none	18 °C / 18 hours
68	Nup145N R3 (M739A)	729-750	pGEX-6P-1	BamHI, NotI	not cleaved	none	18 °C / 18 hours
69	Nup145N R3 (D742A)	729-750	pGEX-6P-1	BamHI, NotI	not cleaved	none	18 °C / 18 hours
70	Nup145N R3 (L743A)	729-750	pGEX-6P-1	BamHI, NotI	not cleaved	none	18 °C / 18 hours
71	Nup145N R3 (F744A)	729-750	pGEX-6P-1	BamHI, NotI	not cleaved	none	18 °C / 18 hours
72	Avi-Nup192	1-1756	pET28a-PreS	NdeI, NotI	GPLMSGLNDIFEAQKI EWHEGSAGGSGH	none	37 °C / 2 hours
73♦	Nup192 ΔHEAD	153-1756 (Δ167-184, replaced with GSGS)	pET28a-PreS	NdeI, NotI	GPHM	none	37 °C / 2 hours
74	Nup192 NTD	1-958	pET28a-PreS	NdeI, NotI	GPH	none	37 °C / 2 hours
75	Nup192 CTD	921-1756	pET28a-PreS	NdeI, NotI	GPHM	none	23 °C / 18 hours
76	Nup192 TAIL	1397-1756	pET28a-PreS	NdeI, NotI	GPHM	none	37 °C / 2 hours
77	Nic96	110-1112	pET28a-SUMO	BamHI, NotI	S	AAALEHHHHHH	18 °C / 18 hours
78♦	Nic96 SOL	391-1112	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 18 hours
79	Nic96 R2Δ2	274-301	pET-MCN-SUMO	BamHI, NotI	S	none	37 °C / 2 hours coexpressed with Nup188 TAIL
80	Nic96 R2Δ4	286-301	pET-MCN-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
81	Nic96 R2	262-301	pET-MCN-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
82	Nic96 R2 (F275A)	262-301	pET-MCN-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
83	Nic96 R2 (D276A)	262-301	pET-MCN-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
84	Nic96 R2 (F278A)	262-301	pET-MCN-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
85	Nic96 R2 (N282A)	262-301	pET-MCN-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
86	Nic96 R2 (L285A)	262-301	pET-MCN-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
87	Nic96 R2 (W287A)	262-301	pET-MCN-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
88	Nic96 R2 (I294A)	262-301	pET-MCN-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
89	Nic96 R2 (F298A)	262-301	pET-MCN-SUMO	BamHI, NotI	S	none	37 °C / 2 hours
90	Nic96 SOL (L715A)	391-1112	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 18 hours
91	Nic96 SOL (V655A/W658A)	391-1112	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 18 hours
92	Nic96 SOL (F697A)	391-1112	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 18 hours
93	Nup188	1-1858	pET28a-PreS	AseI, BamHI	GPHN	none	23 °C / 18 hours
94	Nup188 NTD	1-1134	pET28a-PreS	AseI, BamHI	GPHN	none	23 °C / 18 hours
95	Nup188 TAIL	1447-1858	pET28a-SUMO	BamHI, NotI	SM	none	18 °C / 18 hours
96	Nsp1	467-674	pETDuet1	NcoI, NotI	None	none	18 °C / 18 hours
	Nup57	74-319		NdeI, XhoI	M	none	coexpressed with Nup49
97	Nup49	246-470	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 18 hours coexpressed with Nsp1 and Nup57
98	*hs*Nup155 CTD	870-1391	pET28a-PreS	NdeI, NotI	GPHM	none	37 °C / 2 hours
99	*hs*Nup98	596-617	pGEX-6P-1	BamHI, NotI	not cleaved	none	23 °C / 18 hours
100	*hs*Nup98 (S608E/S612E)	596-617	pGEX-6P-1	BamHI, NotI	not cleaved	none	23 °C / 18 hours
101	*sc*Nup159 T	1425-1460	pETDuet1	NcoI, NotI	GPHM	none	18 °C / 16 hours
	*sc*Nup82 NTD	1-452		NdeI, XhoI	none	none	
102	*sc*Nup145C	1-712	pETDuet1	BamHI, NotI	MGSSHHHHHHSQDP	none	18 °C / 16 hours
	*sc*Sec13	1-297		NdeI, XhoI	N/A	none	
103	*sc*Nup53	374-460	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 16 hours
104	*sc*Nup170 CTD	999-1502	pET28a-SUMO	SacI, NotI	SEFEL	none	18 °C / 8 hours
105	*sc*Nup170 CTD (F1308A)	999-1502	pET28a-SUMO	SacI, NotI	SEFEL	none	18 °C / 8 hours
106	*sc*Nup157 CTD	894-1391	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 16 hours
107	*sc*Nup157 NTD	70-893	pGEX-6P-1	BamHI, NotI	GPLGS	none	18 °C / 8 hours
108	*sc*Nup157 NTD (F214A)	70-893	pGEX-6P-1	BamHI, NotI	GPLGS	none	18 °C / 8 hours
109	*sc*Nup100	571-959	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 16 hours
110	*sc*Nup116	752-1113	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 16 hours
111	*sc*Nup145N	210-605	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 16 hours
112	*sc*Nup192 NTD	1-960	pET28a-PreS	NdeI, NotI	GPH	none	21 °C / 8 hours
113	*sc*Nic96 SOL (I432A/W435A/Y469A)	204-839	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 16 hours
114	*sc*Nup53	1-240	pET28a-SUMO	BamHI, NotI	S	none	18 °C / 16 hours
115	*sc*Nup188	1-1655	pET28a-SUMO	BamHI, NotI	S	none	21 °C / 16 hours
116	Nup145N PreS	606-993	pET28a-SUMO	BamHI, NotI	S	none	23 °C / 18 hours

Proteins are from *C. thermophilum* unless otherwise noted

♦ Constructs that were used for crystallization

Table S2.

Protein purification protocols

Protein(s)	Expression constructs	Purification step	Buffer A	Buffer B
Avi-Nup192*	Individual (#72)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. MonoQ 10/100 GL 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. IEX-A2, pH 8.0, 5 mM β-ME / PreS 3. Ni-A2, 5 mM β-ME 4. IEX-A2, pH 8.0, 5 mM DTT 5. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. IEX-B, pH 8.0, 5 mM DTT 5. N/A
Nup192 ΔHEAD♦	Individual (#73)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. MonoQ 10/100 GL 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. IEX-A2, pH 8.0, 5 mM β-ME / PreS 3. Ni-A2, 5 mM β-ME 4. IEX-A2, pH 8.0, 5 mM DTT 5. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. IEX-B, pH 8.0, 5 mM DTT 5. N/A
Nup192 NTD	Individual (#74)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A2, 5 mM β-ME / PreS 3. Ni-A2, 5 mM β-ME 4. IEX-A2, pH 8.0, 5 mM DTT 5. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. IEX-B, pH 8.0, 5 mM DTT 5. N/A
Nup192 CTD	Individual (#75)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. MonoQ 10/100 GL 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. IEX-A2, pH 8.0, 5 mM β-ME / PreS 3. Ni-A2, 5 mM β-ME 4. IEX-A2, pH 8.0, 5 mM DTT 5. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. IEX-B, pH 8.0, 5 mM DTT 5. N/A
Nup192 TAIL	Individual (#76)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A3, 5 mM β-ME / PreS 3. Ni-A3, 5 mM β-ME 4. SEC-C, 1 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. N/A
Nup188*	Individual (#93)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG 6. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. IEX-A2, pH 8.0, 5 mM β-ME / PreS 3. Ni-A2, 5 mM β-ME 4. IEX-A2, pH 8.0, 5 mM DTT 5. SEC-A, 5 mM DTT 6. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. IEX-B, pH 8.0, 5 mM DTT 5. N/A 6. N/A
Nup188 NTD	Individual (#94)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A2, 5 mM β-ME / PreS 3. Ni-A2, 5 mM β-ME 4. IEX-A2, pH 8.0, 5 mM DTT 5. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. IEX-B, pH 8.0, 5 mM DTT 5. N/A
Nup188 TAIL	Individual (#95)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A2, 5 mM β-ME / ULP1 3. Ni-A2, 5 mM β-ME 4. IEX-A2, pH 8.0, 5 mM DTT 5. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. IEX-B, pH 8.0, 5 mM DTT 5. N/A
Nic96 SOL♦ wild-type and mutants	Individual (#78, 82-92)	1. Ni-NTA 2. HiPrep 26/20 Desalting/Cleavage 3. Ni-NTA 4. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. IEX-A2, pH 8.0, 5 mM β-ME / ULP1 3. Ni-A2, 5 mM β-ME 4. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. N/A
Nup170*	Individual (#27)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME, 5 % Glycerol 2. Ni-A3, 5 mM β-ME, 5 % Glycerol / ULP1 3. Ni-A3, 5 mM β-ME, 5 % Glycerol 4. IEX-A4, pH 8.0, 5 mM DTT, 5 % Glycerol 5. SEC-C, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 5 mM β-ME, 5 % Glycerol 2. N/A 3. Ni-B1, 5 mM β-ME, 5 % Glycerol 4. IEX-B, pH 8.0, 5 mM DTT, 5 % Glycerol 5. N/A
Nup170 SOL♦	Individual (#29)	1. Ni-NTA 2. Dialysis/Cleavage (48 hr) 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME, 2. Ni-A3, 5 mM β-ME / ULP1 3. Ni-A3, 5 mM β-ME 4. IEX-A4, pH 8.0, 5 mM DTT 5. SEC-C, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. IEX-B, pH 8.0, 5 mM DTT 5. N/A
Nup170 NTD♦ wild-type and mutants	Individual (#32-47, 39-45)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME, 5 % Glycerol 2. Ni-A3, 5 mM β-ME, 5 % Glycerol / ULP1 3. Ni-A3, 5 mM β-ME, 5 % Glycerol 4. IEX-A2, pH 8.0, 5 mM DTT, 5 % Glycerol 5. SEC-C, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 5 mM β-ME, 5 % Glycerol 2. N/A 3. Ni-B1, 5 mM β-ME, 5 % Glycerol 4. IEX-B, pH 8.0, 5 mM DTT, 5 % Glycerol 5. N/A
Nup170 CTD♦ wild-type and mutants	Individual (#30-31, 38, 46-57)	1. Ni-NTA 2. HiPrep 26/20 Desalting/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A2, 5 mM β-ME / ULP1 3. Ni-A2, 5 mM β-ME 4. IEX-A2, pH 8.0, 5 mM DTT 5. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. IEX-B, pH 8.0, 5 mM DTT 5. N/A
***hs*Nup155 CTD**	Individual (#98)	1. Ni-NTA 2. HiPrep 26/20 Desalting/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME, 5 % Glycerol 2. Ni-A3, 5 mM β-ME, 5 % Glycerol / PreS 3. Ni-A3, 5 mM β-ME, 5 % Glycerol 4. IEX-A3, pH 8.0, 5 mM DTT, 5 % Glycerol 5. SEC-B, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 5 mM β-ME, 5 % Glycerol 2. N/A 3. Ni-B1, 5 mM β-ME, 5 % Glycerol 4. IEX-B, pH 8.0, 5 mM DTT, 5 % Glycerol 5. N/A
Nup120•Nup37•ELYS*	Co-expression (#3)	1. Ni-NTA 2. Dialysis/Cleavage 3. HiTrap Q HP 4. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME, 5 % Glycerol 2. Ni-A3, 5 mM DTT, 5 % Glycerol / ULP1-PreS 3. IEX-A4, pH 8.0, 5 mM DTT, 5 % Glycerol 4. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME, 5 % Glycerol 2. N/A 3. IEX-B, pH 8.0, 5 mM DTT, 5 % Glycerol 4. N/A
Nup85*	Individual (#4)	1. Ni-NTA 2. Dialysis/Cleavage 3. HiTrap Q HP 4. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A2, 5 mM DTT / PreS 3. IEX-A2, pH 8.0, 5 mM DTT 4. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME, 5 % Glycerol 2. N/A 3. IEX-B, pH 8.0, 5 mM DTT 4. N/A
Sec13•Nup145C*	Co-expression (#5, 6)	1. Ni-NTA 2. HiPrep 26/20 Desalting /Cleavage 3. Ni-NTA 4. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A2, 5 mM β-ME / ULP1 3. Ni-A2, 5 mM β-ME 4. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B2, 5 mM β-ME 4. N/A

Protein(s)	Expression constructs	Purification step	Buffer A	Buffer B
Nup84•Nup133*	Co-expression (#7, 8)	1. Ni-NTA 2. Dialysis 3. HiLoad Superdex 200 16/60 PG 4. MonoQ 10/100 GL	1. Ni-A1, 5 mM β-ME 2. SEC-A, 5 mM DTT 3. SEC-A, 5 mM DTT 4. IEX-A2, pH 8.0, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. N/A 4. IEX-B, pH 8.0, 5 mM DTT
Nup84•Nup133 ΔNTE	Co-expression (#7, 9)	1. Ni-NTA 2. Dialysis 3. HiLoad Superdex 200 16/60 PG 4. MonoQ 10/100 GL	1. Ni-A1, 5 mM β-ME 2. SEC-A, 5 mM DTT 3. SEC-A, 5 mM DTT 4. IEX-A2, pH 8.0, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. N/A 4. IEX-B, pH 8.0, 5 mM DTT
Nup145N* wild-type and PreS	Individual (#58, 116)	1. Ni-NTA 2. HiPrep 26/20 Desalting/Cleavage 3. MonoS 5/50 GL 4. HiLoad Superdex 75 16/60 PG	1. Ni-A1, 5 mM β-ME 2. IEX-A2, pH 7.0, 5 mM DTT / ULP1 3. IEX-A2, pH 7.0, 5 mM DTT 4. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. IEX-B, pH 7.0, 5 mM DTT 4. N/A
Nup145N APD♦	Individual (#59)	1. Ni-NTA 2. Dialysis/cleavage 3. Ni-NTA 4. HiLoad Superdex 75 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A2, pH 8.0, 5 mM β-ME / PreS 3. Ni-A2, 5 mM β-ME 4. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. N/A
GST-Nup145N wild-type and mutants	Individual (#64, 65-71)	1. GST Affinity 2. HiTrap Q HP 3. HiLoad Superdex 200 16/60 PG	1. GST-A, 5 mM DTT 2. IEX-A2, pH 8.0, 5 mM DTT 3. SEC-A, 5 mM DTT	1. GST-B, 5mM DTT 2. IEX-B, pH 8.0, 5 mM DTT 3. N/A
GST-*hs*Nup98 wild-type and mutant	Individual (#99, 100)	1. GST Affinity 2. HiTrap Q HP 3. HiLoad Superdex 200 16/60 PG	1. GST-A, 5 mM DTT 2. IEX-A2, pH 8.0, 5 mM DTT 3. SEC-A, 5 mM DTT	1. GST-B, 5mM DTT 2. IEX-B, pH 8.0, 5 mM DTT 3. N/A
SUMO-Nup145N various fragments	Individual (#61-63)	1. Ni-NTA 2. HiPrep 26/20 Desalting /Cleavage 3. Ni-NTA 4. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A2, 5 mM β-ME / ULP1 3. Ni-A2, 5 mM β-ME 4. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B2, 5 mM β-ME 4. N/A
Nup53*	Individual (#10)	1. Ni-NTA 2. HiPrep 26/20 Desalting/Cleavage 3. Ni-NTA 4. HiPrep 26/20 Desalting 5. MonoS 5/50 GL 6. HiLoad Superdex 75 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A1, 5 mM β-ME / ULP1 3. Ni-A1, 5 mM β-ME 4. IEX-A2, pH 7.0, 5 mM DTT 5. IEX-A2, pH 7.0, 5 mM DTT 6. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. N/A 5. EX-B, pH 7.0, 5 mM DTT 6. N/A
Nup53 RRM♦	Individual (#11)	1. Ni-NTA 2. Desalting/Cleavage 3. Ni-NTA 4. HiLoad Superdex 75 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A2, pH 8.0, 5 mM β-ME / PreS 3. Ni-A2, 5 mM β-ME 4. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. Ni-B1, 5 mM β-ME 4. N/A
SUMO-Nup53 various fragments	Individual (#12-15)	1. Ni-NTA 2. HiPrep 26/20 Desalting 3. HiLoad Superdex 75 16/60 PG	1. Ni-A1, 5 mM β-ME 2. SEC-A, 5 mM DTT 3. SEC-A, 5 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. N/A
GST-Nup53 R3 wild-type and mutants	Individual (#16, 17-26)	1. GST Affinity 2. HiTrap Q HP 3. HiLoad Superdex 200 16/60 PG	1. GST-A, 5 mM DTT 2. IEX-A2, pH 8.0, 5 mM DTT 3. SEC-A, 5 mM DTT	1. GST-B, 5mM DTT 2. IEX-B, pH 8.0, 5 mM DTT 3. N/A
CNT	Co-expression (#96, 97)	1. Ni-NTA 2. HiPrep 26/20 Desalting/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 4 mM β-ME 2. Ni-A2, 4 mM β-ME / ULP1 3. Ni-A2, 4 mM β-ME 4. IEX-A2, pH 8.0, 5 mM DTT 5. SEC-A, 5 mM DTT	1. Ni-B1, 4 mM β-ME 2. N/A 3. Ni-B1, 4 mM β-ME 4. IEX-B, pH 8.0, 5 mM DTT 5. N/A
Nup82 NTD• SUMO-Nup159 T	Individual (#1, 2) co-lysis ratio 1:1	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. Ni-A1, 5 mM β-ME/ PreS / ULP1 3. Ni-A1, 5 mM β-ME 4. SEC-A, 5 mM DTT	1. Ni-B1, 4 mM β-ME 2. N/A 3. Ni-B1, 4 mM β-ME 4. N/A
His_6-Nup192 TAIL	Individual (#76)	1. Ni-NTA 2. Dialysis 3. HiTrap Q HP 4. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. IEX-A1, pH 9.0, 1 mM DTT 3. IEX-A1, pH 9.0, 1 mM DTT 4. SEC-C, 1 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. IEX-B, pH 9.0, 1 mM DTT 4. N/A
SUMO-Nup188 TAIL	Individual (#95)	1. Ni-NTA 2. Dialysis 3. MonoQ 10/100 GL 4. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. IEX-A2, pH 8.0, 1 mM DTT 3. IEX-A2, pH 8.0, 1 mM DTT 4. SEC-C, 1 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. IEX-B, pH 8.0, 1 mM DTT 4. N/A
SUMO-Nup188 TAIL• SUMO-Nic96-R2Δ2	Co-expression (#95, 79)	1. Ni-NTA 2. Dialysis 3. HiTrap Q HP 4. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 5 mM β-ME 2. IEX-A2, pH 8.0, 1 mM DTT 3. IEX-A2, pH 8.0, 1 mM DTT 4. SEC-C, 1 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. IEX-B, pH 8.0, 1 mM DTT 4. N/A
SUMO-Nic96 R2	Individual (#81)	1. Ni-NTA 2. Dialysis 3. HiTrap Q HP 4. HiLoad Superdex 75 16/60 PG	1. Ni-A1, 5 mM β-ME 2. IEX-A2, pH 8.0, 1 mM DTT 3. IEX-A2, pH 8.0, 1 mM DTT 4. SEC-C, 1 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. IEX-B, pH 8.0, 1 mM DTT 4. N/A
SUMO-Nic96 R2Δ4	Individual (#80)	1. Ni-NTA 2. Dialysis 3. HiTrap Q HP 4. HiLoad Superdex 75 16/60 PG	1. Ni-A1, 5 mM β-ME 2. IEX-A2, pH 8.0, 5 mM β-ME 3. IEX-A2, pH 8.0, 1 mM DTT 4. SEC-C, 1 mM DTT	1. Ni-B1, 5 mM β-ME 2. N/A 3. IEX-B, pH 8.0, 1 mM DTT 4. N/A
SUMO-*sc*Nup53	Individual (#103)	1. Ni-NTA 2. Dialysis 3. HiTrap Q HP 4. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A3, pH 8.0, 4 mM β-ME, 5 % Glycerol 3. IEX-A3, pH 8.0, 4 mM β-ME, 5 % Glycerol 4. SEC-B, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. IEX-B, pH 8.0, 4 mM β-ME 4. N/A
***sc*Nup100**	Individual (#109)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A3, pH 8.0, 4 mM β-ME, 5 % Glycerol / ULP1 3. IEX-A3, pH 8.0, 4 mM β-ME 5 % Glycerol 4. IEX-A3, pH 8.0, 4 mM β-ME 5 % Glycerol 5. SEC-B, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. Ni-B1, 4 mM β-ME 4. IEX-B, pH 8.0, 4 mM β-ME 5. N/A
SUMO-*sc*Nup100	Individual (#109)	1. Ni-NTA 2. Dialysis 3. HiTrap Q HP 4. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A3, pH 8.0, 4 mM β-ME, 5 % Glycerol 3. IEX-A3, pH 8.0, 4 mM β-ME, 5 % Glycerol 4. SEC-B, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. IEX-B, pH 8.0, 4 mM β-ME 4. N/A

Protein(s)	Expression constructs	Purification step	Buffer A	Buffer B
***sc*Nup116**	Individual (#110)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A3, pH 8.0, 4 mM β-ME, 5 % Glycerol / ULP1 3. IEX-A3, pH 8.0, 4 mM β-ME 5 % Glycerol 4. IEX-A3, pH 8.0, 4 mM β-ME 5 % Glycerol 5. SEC-B, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. Ni-B1, 4 mM β-ME 4. IEX-B, pH 8.0, 4 mM β-ME 5. N/A
***sc*Nup145N**	Individual (#111)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A3, pH 8.0, 4 mM β-ME, 5 % Glycerol / ULP1 3. IEX-A3, pH 8.0, 4 mM β-ME 5 % Glycerol 4. IEX-A3, pH 8.0, 4 mM β-ME 5 % Glycerol 5. SEC-B, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. Ni-B1, 4 mM β-ME 4. IEX-B, pH 8.0, 4 mM β-ME 5. N/A
SUMO-*sc*Nup145N	Individual (#111)	1. Ni-NTA 2. Dialysis 3. HiTrap Q HP 4. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A3, pH 8.0, 4 mM β-ME, 5 % Glycerol 3. IEX-A3, pH 8.0, 4 mM β-ME, 5 % Glycerol 4. SEC-B, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. IEX-B, pH 8.0, 4 mM β-ME 4. N/A
***sc*Nup188**	Individual (#115)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A2, pH 8.0, 4 mM β-ME, 5 % Glycerol / ULP1 3. IEX-A2, pH 8.0, 4 mM β-ME 5 % Glycerol 4. IEX-A2, pH 8.0, 4 mM β-ME 5 % Glycerol 5. SEC-A, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. Ni-B1, 4 mM β-ME 4. IEX-B, pH 8.0, 4 mM β-ME 5. N/A
***sc*Nup192 NTD**	Individual (#112)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A2, pH 8.0, 4 mM β-ME, 5 % Glycerol / PreS 3. IEX-A2, pH 8.0, 4 mM β-ME 5 % Glycerol 4. IEX-A2, pH 8.0, 4 mM β-ME 5 % Glycerol 5. SEC-A, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. Ni-B1, 4 mM β-ME 4. IEX-B, pH 8.0, 4 mM β-ME 5. N/A
***sc*Nup170 CTD** wild-type and mutant	Individual (#104, 105)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A4, pH 8.0, 4 mM β-ME, 5 % Glycerol / ULP1 3. IEX-A4, pH 8.0, 4 mM β-ME 5 % Glycerol 4. IEX-A4, pH 8.0, 4 mM β-ME 5 % Glycerol 5. SEC-C, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. Ni-B1, 4 mM β-ME 4. IEX-B, pH 8.0, 4 mM β-ME 5. N/A
SUMO-*sc*Nup170 CTD	Individual (#104)	1. Ni-NTA 2. Dialysis 3. HiTrap Q HP 4. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A4, pH 8.0, 4 mM β-ME, 5 % Glycerol 3. IEX-A4, pH 8.0, 4 mM β-ME 5 % Glycerol 4. SEC-C, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. IEX-B, pH 8.0, 4 mM β-ME 4. N/A
***sc*Nup157 CTD**	Individual (#106)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A4, pH 8.0, 4 mM β-ME, 5 % Glycerol / ULP1 3. IEX-A4, pH 8.0, 4 mM β-ME 5 % Glycerol 4. IEX-A4, pH 8.0, 4 mM β-ME 5 % Glycerol 5. SEC-C, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. Ni-B1, 4 mM β-ME 4. IEX-B, pH 8.0, 4 mM β-ME 5. N/A
SUMO-*sc*Nup157 CTD	Individual (#106)	1. Ni-NTA 2. Dialysis 3. HiTrap Q HP 4. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A4, pH 8.0, 4 mM β-ME, 5 % Glycerol 3. IEX-A4, pH 8.0, 4 mM β-ME 5 % Glycerol 4. SEC-C, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. IEX-B, pH 8.0, 4 mM β-ME 4. N/A
***sc*Nup82 NTD•*sc*Nup159 T**	Co-expression (#101)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A2, pH 8.0, 4 mM β-ME, 5 % Glycerol / ULP1 3. IEX-A2, pH 8.0, 4 mM β-ME 5 % Glycerol 4. IEX-A2, pH 8.0, 4 mM β-ME 5 % Glycerol 5. SEC-A, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. Ni-B1, 4 mM β-ME 4. IEX-B, pH 8.0, 4 mM β-ME 5. N/A
***sc*Sec13•*sc*Nup145C**	Co-expression (#103)	1. Ni-NTA 2. Dialysis/Cleavage 3. Ni-NTA 4. HiTrap Q HP 5. HiLoad Superdex 200 16/60 PG	1. Ni-A4, 4 mM β-ME, 5 % Glycerol 2. IEX-A2, pH 8.0, 4 mM β-ME, 5 % Glycerol / ULP1 3. IEX-A2, pH 8.0, 4 mM β-ME 5 % Glycerol 4. IEX-A2, pH 8.0, 4 mM β-ME 5 % Glycerol 5. SEC-A, 5 mM DTT, 5 % Glycerol	1. Ni-B1, 4 mM β-ME 2. N/A 3. Ni-B1, 4 mM β-ME 4. IEX-B, pH 8.0, 4 mM β-ME 5. N/A
***sc*Nic96 SOL** triple mutant	Individual (#113)	1. Ni-NTA 2. HiPrep 26/20 Desalting/Cleavage 3. HiLoad Superdex 200 16/60 PG	1. Ni-A1, 4 mM β-ME 2. SEC-A, 5 mM DTT / ULP1 3. SEC-A, 5 mM DTT	1. Ni-B1, 4 mM β-ME 2. N/A 3. N/A
SUMO-*sc*Nup53	Individual (#114)	1. Ni-NTA 2. Dialysis 3. HiTrap Q HP 4. HiLoad Superdex 75 16/60 PG	1. Ni-A1, 4 mM β-ME 2. IEX-A1, pH 8.0, 5 mM DTT 3. IEX-A1, pH 8.0, 5 mM DTT 4. SEC-A, 5 mM DTT	1. Ni-B1, 4 mM β-ME 2. N/A 3. IEX-B, pH 8.0, 5 mM DTT 4. N/A
***sc*Nup157 NTD** wild-type and mutant	Individual (#107-108)	Detailed description in SI methods		
Nic96•CNT	Individual (#77, 96, 97) co-lysis	Detailed description in SI methods		

♦ Constructs that were used for crystallization

* Detailed purification provided in supplementary figures

Ni-A1: 20 mM TRIS (pH 8.0), 500 mM NaCl, 20 mM imidazole
Ni-A2: 20 mM TRIS (pH 8.0), 100 mM NaCl, 20 mM imidazole
Ni-A3: 20 mM TRIS (pH 8.0), 200 mM NaCl, 20 mM imidazole
Ni-A4: 75 mM TRIS (pH 8.0), 500 mM NaCl, 20 mM imidazole
Ni-B1: 20 mM TRIS (pH 8.0), 500 mM NaCl, 500 mM imidazole
Ni-B2: 20 mM TRIS (pH 8.0), 100 mM NaCl, 500 mM imidazole

GST-A: 20 mM TRIS (pH 8.0), 200 mM NaCl
GST-B: 20 mM TRIS (pH 8.0), 200 mM NaCl, 20 mM glutathione

IEX-A1: 20 mM TRIS, 50 mM NaCl
IEX-A2: 20 mM TRIS, 100 mM NaCl
IEX-A3: 20 mM TRIS, 150 mM NaCl
IEX-A4: 20 mM TRIS, 200 mM NaCl
IEX-B: 20 mM TRIS, 2.0 M NaCl

SEC-A: 20 mM TRIS (pH 8.0), 100 mM NaCl
SEC-B: 20 mM TRIS (pH 8.0), 150 mM NaCl
SEC-C: 20 mM TRIS (pH 8.0), 200 mM NaCl

Table S3.

SEC-MALS analysis

Figure	Nucleoporin or nucleoporin complex	Experimental mass (kDa)	Theoretical mass (kDa)	Stoichiometry
fig. S3A	Nup192•Nup145N•Nup53	250	278	
	Nic96•CNT (Nic96•Nup57•Nup49•Nsp1)	160	187	
	IRC•Nup53 (Nup192•Nic96•Nup145N•Nup53•CNT)	380	465	conc. dependent
fig. S3B	IRC•Nup53 (Nup192•Nic96•Nup145N•Nup53•CNT)	335	423	
	Nup188	180	204	
	IRC•Nup53 + Nup188	341 / 220	627	no interaction
Fig. 1D fig. S4A	CNC-hexamer•Nup145N (Nup120•Nup37•ELYS•Nup85•Sec13•Nup145C•Nup145N)	482	507	
	Nup192•Nic96•Nup53•CNT	335	423	
	CNC-hexamer•IRC•Nup53	654	930	conc. dependent
Fig. 1E fig. S4B	CNC-hexamer (Nup120•Nup37•ELYS•Nup85•Sec13•Nup145C)	438	465	
	Nup192•Nic96•Nup53•CNT	335	423	
	CNC-hexamer + IRC•Nup53	430	888	no interaction
Fig. 1F fig. S4C	CNC-hexamer•Nup145N	466	507	
	Nup188•Nic96•Nup53•CNT	523	431	
	CNC-hexamer•Nup188•Nic96•Nup145N•Nup53•CNT	1220	938	superstoichiometric
Fig. 1G fig. S4D	CNC-hexamer	428	465	
	Nup188•Nic96•Nup53•CNT	523	431	
	CNC-hexamer + Nup188•Nic96•Nup53•CNT	507	896	no interaction
fig. S5A	CNC-hexamer	428	465	
	Nup145N	40	42	
	CNC-hexamer•Nup145N	508	507	stoichiometric
fig. S5B	CNC-hexamer	428	465	
	Nup145N APD	17	15	
	CNC-hexamer•Nup145N APD	447	480	stoichiometric
fig. S5C	CNC-hexamer	428	465	
	Nup145N PreS	36 / 15	27 / 15	
	CNC-hexamer•Nup145N APD + Nup145N MID	690	480	superstoichiometric
fig. S6A	CNC-hexamer	428	465	
	Nup82 NTD•Nup159 T•Nup145N	112	122	
	CNC-hexamer•Nup145N + Nup82 NTD•Nup159 T	579	587	stoichiometric
fig. S6B	Nup188•Nic96•Nup53•CNT	523	431	
	Nup82 NTD•Nup159 T•Nup145N	126	122	
	Nup188•Nic96•Nup145N•Nup53•CNT + Nup82 NTD•Nup159 T	670	553	superstoichiometric
Fig. 2A fig. S7A	Nup192	176	196	
	Nup188	180	204	
	Nup170	132	147	
	Nic96 SOL	73	81	
	Nup192 + Nup188 + Nup170 + Nic96 SOL	174	628	no interaction
Fig. 2B fig. S7B	Nup192 + Nup170 + Nic96 SOL	161 / 93	172[a] / 81	
	Nup53 + Nup145N	52	41[a]	
	Nup192•Nup170•Nic96 SOL•Nup53•Nup145N	528	506	stoichiometric
Fig. 2C fig. S7C	Nup188 + Nup170 + Nic96 SOL	165 / 87	176[a] / 81	
	Nup53 + Nup145N	52	41[a]	
	Nup188 + Nup170 + Nic96 SOL + Nup53 + Nup145N	242	512	weak interaction
Fig. 2D fig. S8A	Nic96 SOL	72	81	
	Nup53	40	40	
	Nic96 SOL•Nup53	146	121	superstoichiometric
Fig. 2D fig. S8B	Nup170	145	147	
	Nup53	35	40	
	Nup170•Nup53	197	187	stoichiometric
Fig. 2D fig. S8C	Nup188	188	204	
	Nup53	40	40	
	Nup188 + Nup53	188	244	weak interaction
Fig. 2D fig. S8D	Nup192	178	196	
	Nup53	40	40	
	Nup192•Nup53	223	236	stoichiometric
Fig. 2D fig. S8E	CNT (Nup57, Nup49, Nsp1)	70	77	
	Nup53	38	40	
	CNT + Nup53	73	117	weak interaction
Fig. 2E fig. S9A	Nup170	145	147	
	Nup145N	42	42	
	Nup170•Nup145N	196	189	stoichiometric
Fig. 2E fig. S9B	Nup188	189	204	
	Nup145N	40	42	
	Nup188•Nup145N	216	246	stoichiometric
Fig. 2E fig. S9C	Nup192	178	196	
	Nup145N	40	42	
	Nup192•Nup145N	265	238	superstoichiometric

Figure	Nucleoporin or nucleoporin complex	Experimental mass (kDa)	Theoretical mass (kDa)	Stoichiometry
Fig. 2D fig. S10A	Nup170•Nup53	154	187	
	Nic96 SOL	70	81	
	Nup170•Nup53•Nic96 SOL	208	268	stoichiometric
Fig. 2D fig. S10B	Nup170•Nup53	154	187	
	Nup188	194	204	
	Nup170•Nup53 + Nup188	188	391	no interaction
Fig. 2D fig. S10C	Nup170•Nup53	154	187	
	Nup192	196	196	
	Nup170•Nup53•Nup192	259	383	conc. dependent
Fig. 2D fig. S10D	Nup192•Nup53	215	236	
	Nic96 SOL	70	81	
	Nup192•Nup53•Nic96 SOL	252	317	conc. dependent
fig. S10E	Nup170•Nup53•Nup192	255	383	
	Nic96 SOL	70	81	
	Nup170•Nup53•Nup192•Nic96 SOL	286	464	conc. dependent
Fig. 2E fig. S11A	Nup170•Nup145N	178	189	
	Nup188	184	204	
	Nup170•Nup145N + Nup188•Nup145N	227	393	partial exchange
Fig. 2E fig. S11B	Nup170•Nup145N	178	189	
	Nup192	178	196	
	Nup170•Nup145N•Nup192	305	385	conc. dependent
Fig. 2E fig. S11C	Nup188•Nup145N	203	246	
	Nup170	137	147	
	Nup188•Nup145N + Nup170•Nup145N	193	393	partial exchange
Fig. 2E fig. S11D	Nup192•Nup145N	227	238	
	Nup170	137	147	
	Nup192•Nup145N•Nup170	276	385	conc. dependent
Fig. 2E fig. S11E	Nup192•Nup145N	233	238	
	Nup188	190	204	
	Nup192•Nup145N + Nup188•Nup145N	223	442	partial exchange
Fig. 2E fig. S11F	Nup188•Nup145N	215	246	
	Nup192	180	196	
	Nup188•Nup145N + Nup192•Nup145N	224	442	partial exchange
Fig. 2E fig. S11G	Avi-Nup192	178	194	
	Nup188	178	204	
	Nup145N	52	42	
	Avi-Nup192•Nup145N + Nup188•Nup145N	223	440	partial exchange
fig. S12A	Nup170•Nup145N	178	189	
	Nup53	38	40	
	Nup170•Nup145N•Nup53	*inconclusive	229	stoichiometric
fig. S12B	Nup192	179	196	
	Nup53	38	40	
	Nup145N	40	42	
	Nup192•Nup53•Nup145N	280	278	stoichiometric
fig. S12C	Nup170•Nup53•Nup145N	183	229	
	Nup192	176	196	
	Nup170•Nup53•Nup145N•Nup192	416	425	stoichiometric
Fig. 2F fig. S13A	Nup192	183	196	
	SUMO-Nup53 31-67	16	14	
	Nup192•SUMO-Nup53 31-67	197	210	stoichiometric
Fig. 2F fig. S14A	Nic96 SOL	70	81	
	SUMO-Nup53 1-90	21	21	
	Nic96 SOL•SUMO-Nup53 1-90	88	102	stoichiometric
Fig. 2F fig. S14B	Nic96 SOL	81	81	
	SUMO-Nup53 69-90	16	14	
	Nic96 SOL•SUMO-Nup53 69-90	88	95	stoichiometric
Fig. 2F fig. S14C	Nup192	176	196	
	Nic96 SOL•SUMO-Nup53 1-90	81	102	
	Nup192•Nic96 SOL•SUMO-Nup53 1-90	220	298	conc. dependent
Fig. 2F fig. S17A	Nup192	178	196	
	SUMO-Nup145N 606-683	20	19	
	Nup192•SUMO-Nup145N 606-683	189	215	stoichiometric
Fig. 2F fig. S17B	Nup192	176	196	
	Nup145N PreS	28 / 14	27 / 15	
	Nup192•Nup145N MID + Nup145N APD	223	223	stoichiometric
Fig. 2F fig. S17C	Nup192 NTD	100	109	
	Nup145N	53	42	
	Nup192 NTD•Nup145N	151	151	stoichiometric
Fig. 2F fig. S17D	Nup192 CTD	127	91	
	Nup145N	40	42	
	Nup192 CTD + Nup145N	137	133	stoichiometric
Fig. 2F fig. S17E	Nup192 TAIL	38	41	
	Nup145N	46	42	
	Nup192 TAIL + Nup145N	45 / 40	83	stoichiometric

Figure	Nucleoporin or nucleoporin complex	Experimental mass (kDa)	Theoretical mass (kDa)	Stoichiometry
fig. S18A	Nup170	125	147	
	SUMO-Nup53 31-67	16	14	
	Nup170 + SUMO-Nup53 31-67	129	161	no interaction
fig. S18B	Nup170	125	147	
	SUMO-Nup53 69-90	14	14	
	Nup170 + SUMO-Nup53 69-90	129	161	no interaction
fig. S18C	Nup170	125	147	
	SUMO-Nup145N 606-683	15	19	
	Nup170+ SUMO-Nup145N 606-683	133	166	no interaction
fig. S18D	Nup192	176	196	
	SUMO-Nup53 69-90	14	14	
	Nup192 + SUMO-Nup53 69-90	174	210	no interaction
fig. S18E	Nup192	176	196	
	GST-Nup53 329-361	55	61	
	Nup192 + GST-Nup53 329-361	172	257	no interaction
fig. S18F	Nup192	176	196	
	GST-Nup145N 729-750	55	59	
	Nup192 + GST-Nup145N 729-750	172	255	no interaction
fig. S18G	Nic96 SOL	70	81	
	SUMO-Nup53 31-67	16	14	
	Nic96 + SUMO-Nup53 31-67	74	95	no interaction
fig. S18H	Nic96 SOL	70	81	
	GST-Nup53 329-361	55	61	
	Nic96 + GST-Nup53 329-361	66	142	no interaction
Fig. 2F fig. S19B	Nup188	181	204	
	Nup145N PreS	28 / 14	27 / 15	
	Nup188•Nup145N MID + Nup145N APD	213	231	stoichiometric
Fig. 2F fig. S19C	Nup188	178	204	
	SUMO-Nup145N 606-750	37	27	
	Nup188•SUMO-Nup145N 606-750	202	231	stoichiometric
Fig. 2F fig. S19D	Nup188	179	204	
	SUMO-Nup145N 606-683	21	19	
	Nup188 + SUMO-Nup145N 606-683	173	223	no interaction
Fig. 2F fig. S19E	Nup188	178	204	
	GST-Nup145N 729-750	55	59	
	Nup188 + GST-Nup145N 729-750	188	263	weak interaction
fig. S39A	Nic96 SOL	72	81	
	Nup53 RRM	13	13	
	Nic96 SOL + Nup53 RRM	73	94	no interaction
fig. S39B	Nup170	133	147	
	Nup53 RRM	12	13	
	Nup170 + Nup53 RRM	132	160	no interaction
fig. S39C	Nup192	183	196	
	Nup53 RRM	14	13	
	Nup192 + Nup53 RRM	182	209	no interaction
fig. S39D	Nup188	183	204	
	Nup53 RRM	14	13	
	Nup188 + Nup53 RRM	182	209	no interaction
fig. S44B	Nup120•Nup37•ELYS	209	239	
	Nup84•Nup133	241	258	
	Nup120•Nup37•ELYS•Nup84•Nup133	264	497	weak interaction
fig. S44C	Nup120•Nup37•ELYS	209	239	
	Nup84•Nup133 ΔNTE	233	246	
	Nup120•Nup37•ELYS +Nup84•Nup133 ΔNTE	240	485	no interaction

Table S4.

Crystallization and cryoprotection conditions

Protein(s)	Concentration	Crystallization condition	Cryo protection condition
$Nup170^{NTD}$•$Nup53^{R3}$	20 mg/ml $Nup170^{NTD}$ 3-fold molar excess $Nup53^{R3}$	1.0 M Na/K phosphate (pH 6.9)	Mother liquor gradually supplemented to 25 % (v/v) ethylene glycol in 5 % steps
$Nup170^{CTD}$•$Nup145N^{R3}$	20 mg/ml $Nup170^{CTD}$ 3-fold molar excess $Nup145N^{R3}$	12 % (w/v) PEG 3,350 0.2 M lithium acetate	Mother liquor gradually supplemented to 25 % (v/v) ethylene glycol in 5 % steps
$Nup170^{CTD}$ SeMet	15 mg/ml	0.1 M MES (pH 6.3) 10 % PEG 20,000 10 % (v/v) ethylene glycol 0.2 M potassium thiocyanate	Mother liquor gradually supplemented to 25 % (v/v) ethylene glycol in 5 % steps
$Nup170^{CTD}$	30 mg/ml	0.1 M HEPES (pH 7.0) 1.0 M sodium acetate	Mother liquor gradually supplemented to 25 % (v/v) ethylene glycol in 5 % steps
$Nup170^{SOL}$	7 mg/ml	0.1 M HEPES (pH 7.1) 0.5 M ammonium sulfate	Mother liquor gradually supplemented to 25 % (v/v) ethylene glycol in 5 % steps
$Nic96^{SOL}$•$Nup53^{R2}$	12 mg/ml	0.1 M TRIS (pH 7.0) 12 % (w/v) PEG 6,000 1.2 M sodium chloride	Paratone N
$Nic96^{SOL}$	12 mg/ml	4 % Tacsimate (pH 7.4) 14 % (w/v) PEG 3,350	Paratone N
$Nup192^{\Delta HEAD}$	7 mg/ml	0.1 M TRIS (pH 7.9) 6 % (w/v) PEG 4,000 5 % (v/v) polypropylene glycol	Stabilized in 0.1 M TRIS (pH 7.9) 7 % (w/v) PEG 4,000 6 % (v/v) polypropylene glycol Solution gradually supplemented to 25 % (v/v) polypropylene glycol in 5 % steps
$Nup53^{RRM}$	15 mg/ml	0.1 M HEPES (pH 7.0) 24 % (w/v) PEG 3,350 0.2 M potassium iodide	Mother liquor supplemented to 25 % ethylene glycol
$Nup53^{RRM}$	15 mg/ml	4 % Tacsimate (pH 4.1) 15 % (w/v) PEG 3,350	32 % Tacsimate (pH 4.1) 19 % (w/v) PEG 3,350
$Nup145N^{APD}$	10 mg/ml	0.1 M citric acid (pH 3.0) 25 % (w/v) PEG 3,350	Mother liquor supplemented to 25 % ethylene glycol
$Nup145N^{APD}$•$Nup145C^{N}$	10 mg/ml	0.1 M TRIS (pH 8.8) 32 % (w/v) PEG 4,000 0.2 M lithium sulfate	Mother liquor supplemented to 10 % glycerol

Table S5.

X-ray crystallography analysis of Nup170^{NTD}•Nup53^{R3} and Nup170^{SOL}

Data collection					
Protein	Nup170^{NTD}•Nup53^{R3}	Nup170^{NTD}•Nup53^{R3}	Nup170^{SOL}	Nup170$^{SOL\ g}$	Nup170$^{SOL\ g}$
PDB ID		5HAX		5HB1	
Synchrotron	APS[a]	APS	SSRL[b]	SSRL	SSRL
Beamline	23-ID-D	23-ID-D	BL12-2	BL12-2	BL12-2
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12$	$P2_12_12$	$P2_12_12$
Cell dimensions					
a, *b*, *c* (Å)	68.7, 104.0, 119.0	69.2, 106.2, 120.1	93.2, 136.9, 89.0	96.0, 138.5, 89.2	96.0, 138.5, 89.2
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
	Se Peak		*Se Peak*		
Wavelength	0.9792	1.0332	0.9791	1.0330	
Resolution (Å)	50.0 – 2.5	50.0 – 2.1	50.0 – 4.5	40.0 – 4.0	40.0 – 4.3
R_{meas} (%)[c]	8.5 (127.7)	8.0 (188.5)	13.1 (290.0)	5.7 (353.7)	5.1 (121.5)
R_{pim} (%)[c]	1.7 (24.9)	2.2 (51.1)	3.7 (85.0)	1.7 (98.5)	1.5 (33.0)
$CC_{1/2}$[c]	100.0 (86.6)	100.0 (68.1)	99.9 (56.0)	100.0 (55.5)	100.0 (92.2)
$< I / \sigma I >$[c]	30.4 (2.4)	20.3 (1.8)	11.6 (1.0)	18.5 (0.8)	22.8 (2.5)
Completeness (%)[c]	98.9 (90.7)	100.0 (100.0)	99.3 (94.1)	99.7 (98.5)	99.8 (99.9)
No. of observations	776,896	676,933	91,230	135,558	108,324
No. of unique reflections[c,d]	30,229 (2,703)	52,358 (5,120)	7,200 (649)	10,614 (1,027)	8,524 (845)
Redundancy[c]	25.7 (25.2)	12.9 (13.5)	12.7 (11.1)	12.8 (12.4)	12.7 (13.4)
Refinement					
Resolution (Å)		50.0 – 2.1		40.0 – 4.0	
No. of reflections		52,342		7,867[e]	
No. of reflections test set		2,618 (5.0%)		789 (10.0%)	
R_{work} / R_{free}		19.9 / 23.0		30.4 / 35.2	
No. atoms		5,998		5,895	
Protein		5,581		5,895	
Water		387		0	
Ligand/Ions		30		0	
B-factors		62		222	
Protein		63		222	
Water		57		-	
Ligand/Ions		79		-	
RMSD					
Bond lengths (Å)		0.002		0.003	
Bond angles (°)		0.5		0.5	
Ramachandran plot[f]					
Favored (%)		97.6		96.8	
Additionally allowed (%)		2.4		3.2	
Outliers (%)		0.0		0.0	
MolProbity					
Clashscore[f]		0.18 (100th percentile)		1.95 (100th percentile)	
Molprobity score[f]		0.79 (100th percentile)		1.46 (100th percentile)	

[a]APS, Advanced Photon Source
[b]SSRL, Stanford Synchotron Radiation Lightsource
[c]Highest-resolution shell is shown in parentheses
[d]Friedel pairs were merged
[e]Refinement was performed with ellipsoidally truncated data
[f]As determined by MolProbity
[g]As a reference, two different high-resolution cutoffs are shown

Table S6.

X-ray crystallography analysis of *apo* Nup170^{CTD} and Nup170^{CTD}•Nup145N^{R3}

Data collection			
Protein	Nup170^{CTD} Y905M/L1007M/L1183M/V1292M	Nup170^{CTD}	Nup170^{CTD}•Nup145N^{R3}
PDB ID	5HAY	5HAZ	5HB0
Synchrotron	SSRL[a]	APS[b]	APS
Beamline	BL12-2	23-ID-D	23-ID-D
Space group	$P2_12_12_1$	$P2_12_12_1$	P1
Cell dimensions			
a, *b*, *c* (Å)	61.8, 101.7, 193.8	69.2, 106.2, 120.1	74.6, 111.47, 111.75
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0	91.8, 92.5, 91.4
	Se Peak		
Wavelength	0.9795	1.0332	1.0332
Resolution (Å)	50.0 – 2.8	50.0 – 2.1	50.0 – 3.5
R_{meas} (%)[c]	11.6 (99.9)	5.4 (89.5)	41.4 (179.9)
R_{pim} (%)[c]	2.8 (25.2)	2.1 (33.8)	15.6 (67.6)
$CC_{1/2}$[c]	99.9 (81.8)	99.9 (85.0)	98.4 (51.7)
$< I / \sigma I >$[c]	22.4 (2.9)	21.0 (2.0)	6.2 (1.3)
Completeness (%)[c]	98.2 (82.2)	97.8 (93.9)	98.8 (94.9)
No. of observations	500,568	325,202	317,882
No. of unique reflections[c]	30,966 (2,527)	47,908 (4,589)	45,493 (4,380)
Redundancy[c]	16.2 (14.2)	6.8 (6.8)	7.0 (6.9)
Refinement			
Resolution (Å)	50.0 – 2.8	50.0 – 2.1	50.0 – 3.5
No. of reflections	30,817	47,882	45,466
No. of reflections test set	1,544 (5.0%)	2,395 (5.0%)	2,011 (4.4%)
R_{work} / R_{free}	20.9 / 25.1	20.6 / 23.1	21.0 / 26.0
No. atoms (non-hydrogen)	8,613	4,919	16,998
Protein	8,564	4,517	16,998
Water	48	378	0
Ligand/Ions	1	24	0
B-factors	65	69	89
Protein	65	69	89
Water	57	64	-
Ligand/Ions	62	117	-
RMSD			
Bond lengths (Å)	0.003	0.004	0.005
Bond angles (°)	0.6	0.6	0.6
Ramachandran plot[e]			
Favored (%)	97.2	97.5	95.1
Additionally allowed (%)	2.8	2.5	4.9
Outliers (%)	0.0	0.0	0.0
MolProbity			
Clashscore[e]	1.35 (100th percentile)	1.44 (100th percentile)	1.98 (100th percentile)
Molprobity score[e]	1.29 (100th percentile)	1.27 (100th percentile)	1.55 (100th percentile)

[a]SSRL, Stanford Synchotron Radiation Lightsource
[b]APS, Advanced Photon Source
[c]Highest-resolution shell is shown in parentheses
[d]Friedel pairs were merged
[e]As determined by MolProbity

Table S7.

X-ray crystallography analysis of *apo* Nic96^{SOL}, Nup96^{SOL}•Nup53^{R2} and Nup192$^{\Delta HEAD}$

Data collection				
Protein	Nic96^{SOL}	Nup96^{SOL}•Nup53^{R2}	Nup192$^{\Delta HEAD\ f}$	Nup192$^{\Delta HEAD\ f}$
PDB ID	5HB2	5HB3	5HB4	
Synchrotron	APS[a]	SSRL[b]	SSRL	SSRL
Beamline	23-ID-B	BL12-2	BL12-2	BL12-2
Space group	$P2_1$	P1	C2	C2
Cell dimensions				
a, *b*, *c* (Å)	53.0, 72.9, 122.7	59.5, 87.0, 98.1	190.7, 53.5, 171.8	190.7, 53.5, 171.8
α, β, γ (°)	90.0, 93.6, 90.0	100.7, 99.6, 95.7	90.0, 108,3, 90.0	90.0, 108,3, 90.0
	Se Peak		*Os Peak*	*Os Peak*
Wavelength	0.9792	1.0000	1.1250	1.1250
Resolution (Å)	50.0 – 3.3	50.0 – 2.65	50.0 – 3.2	50.0 – 3.5
R_{meas} (%)[c]	13.7 (104.2)	7.7 (83.7)	20.8 (325.0)	16.5 (151.7)
R_{pim} (%)[c]	5.0 (38.7)	3.9 (49.4)	5.7 (92.5)	4.5 (40.9)
$CC_{1/2}$[c]	99.8 (85.1)	99.9 (81.5)	99.9 (37.5)	99.9 (74.7)
$< I / \sigma I >$[c]	12.0 (2.5)	15.1 (2.1)	12.1 (0.8)	15.5 (2.1)
Completeness (%)[c]	100.0 (99.8)	88.6 (82.4)	98.6 (92.0)	99.4 (99.1)
No. of observations	107,569	189,415	367,182	280,883
No. of unique reflections[c,d]	14,334 (1,400)	48,566 (4,513)	27,800 (2,557)	21,186 (2,077)
Redundancy[c]	7.5 (7.0)	3.9 (3.8)	13.2 (11.8)	13.3 (13.7)
Refinement				
Resolution (Å)	50.0 – 3.3	50.0 – 2.65	50.0 – 3.2	
No. of reflections	14,284	48,536	27,512	
No. of reflections test set	1,410 (9.9%)	2,368 (4.9%)	1,377 (5.0%)	
R_{work} / R_{free}	23.1 / 27.8	21.1 / 24.9	23.3 / 26.5	
No. atoms (non-hydrogen)	5,674	11,569	11,035	
Protein	5,674	11,495	11,030	
Water	-	71	0	
Ligand/Ions	-	3	5	
B-factors	127	64	136	
Protein	127	65	136	
Water	-	45	-	
Ligand/Ions	-	57	167	
RMSD				
Bond lengths (Å)	0.003	0.004	0.002	
Bond angles (°)	0.6	0.9	0.5	
Ramachandran plot[e]				
Favored (%)	94.2	96.0	95.2	
Additionally allowed (%)	5.8	4.0	4.8	
Outliers (%)	0.0	0.0	0.0	
MolProbity				
Clashscore[e]	3.10 (100th percentile)	3.71 (99th percentile)	1.27 (100th percentile)	
Molprobity score[e]	1.84 (100th percentile)	1.43 (100th percentile)	1.24 (100th percentile)	

[a]APS, Advanced Photon Source
[b]SSRL, Stanford Synchotron Radiation Lightsource
[c]Highest-resolution shell is shown in parentheses
[d]Friedel pairs were merged
[e]As determined by MolProbity
[f]As a reference, two different high-resolution cutoffs are shown

Table S8.

X-ray crystallography analysis of Nup145N^{APD} and Nup145N^{APD}•Nup145C^{N}

Data collection		
Protein	Nup145N^{APD}	Nup145N^{APD}•Nup145C^{N}
PDB ID	5HB5	5HB6
Synchrotron	SSRL[a]	ALS[b]
Beamline	BL12-2	8.2.2
Space group	$P2_1$	P1
Cell dimensions		
a, *b*, *c* (Å)	46.1, 34.9, 78.2	43.0, 44.1, 45.3
α, β, γ (°)	90.0, 99.4, 90.0	99.8, 111.0, 105.9
Wavelength	0.9795	1.0000
Resolution (Å)	30.0 – 1.5	30.0 – 1.3
R_{meas} (%)[c]	9.4 (100.4)	5.9 (110.5)
R_{pim} (%)[c]	3.6 (39.1)	2.2 (56.0)
$CC_{1/2}$[c]	99.9 (87.6)	99.9 (61.7)
$< I / \sigma I >$[c]	12.4 (2.3)	17.2 (1.4)
Completeness (%)[c]	97.2 (92.8)	94.7 (88.9)
No. of observations	257,064	461,854
No. of unique reflections[c,d]	38,743 (3,651)	66,722 (6,234)
Redundancy[c]	6.6 (6.3)	6.9 (3.8)
Refinement		
Resolution (Å)	30.0 – 1.5	30.0 – 1.3
No. of reflections	38,683	66,707
No. of reflections test set	2,012 (5.2%)	1,792 (2.7%)
R_{work} / R_{free}	16.1 / 18.9	15.1 / 17.4
No. atoms (non-hydrogen)	2,753	2,848
Protein	2,331	2,351
Water	331	472
Ligand/Ions	91	25
B-factors	21	25
Protein	18	23
Water	32	36
Ligand/Ions	61	37
RMSD		
Bond lengths (Å)	0.005	0.008
Bond angles (°)	0.8	1.0
Ramachandran plot[e]		
Favored (%)	95.9	94.6
Additionally allowed (%)	4.1	5.1
Outliers (%)	0.0	0.3
MolProbity		
Clashscore[e]	0.42 (100th percentile)	1.47 (99th percentile)
Molprobity score[e]	0.99 (99th percentile)	1.31 (91st percentile)

[a]SSRL, Stanford Synchotron Radiation Lightsource
[b]ALS, Advanced Light Source
[c]Highest-resolution shell is shown in parentheses
[d]Friedel pairs were merged
[e]As determined by MolProbity

Table S9.

X-ray crystallographic analysis of $Nup53^{RRM}$

Data collection					
Protein	$Nup53^{RRM\ e}$	$Nup53^{RRM\ e}$	$Nup53^{RRM}$	$Nup53^{RRM}$	$Nup53^{RRM}$
PDB ID	5HB7			5HB8	
Synchrotron	SSRL[a]	SSRL[a]	SSRL	SSRL	SSRL
Beamline	BL12-2	BL12-2	BL12-2	BL12-2	BL12-2
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P3_121$	$P3_121$
Cell dimensions					
a, *b*, *c* (Å)	35.0, 50.4, 60.0	35.0, 50.4, 60.0	34.9, 50.4, 60.0	94.7, 94.7, 115.5	93.8, 97.8, 114.1
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 120.0	90.0, 90.0, 120.0
			Se Peak		*Os Peak*
Wavelength	0.7293	0.7293	0.9794	0.9794	1.1399
Resolution (Å)	40.0 – 0.80	40.0 – 1.0	40.0 – 1.1	50.0 – 1.7	20.0 – 3.1
R_{meas} (%)[b]	4.1 (94.5)	3.8 (12.1)	10.4 (111.0)	7.9 (155.6)	13.5 (154.4)
R_{pim} (%)[b]	1.3 (33.2)	1.2 (3.7)	2.1 (28.1)	1.3 (29.6)	3.7 (41.8)
$CC_{1/2}$[b]	99.9 (66.4)	99.9 (99.6)	99.9 (66.6)	100.0 (74.5)	99.8 (76.1)
$< I / \sigma I >$[b]	29.3 (2.3)	43.5 (18.8)	19.8 (2.1)	37.3 (2.2)	20.2 (1.8)
Completeness (%)[b]	88.4 (50.3)	97.1 (94.8)	95.4 (66.8)	91.1 (93.1)	99.9 (99.3)
No. of observations	890,036	550,262	984,014	2,169,719	147,214
No. of unique reflections[b,c]	92,712 (5,211)	56,349 (5,425)	42,372 (2,914)	60,735 (6,155)	10,957 (1,073)
Redundancy[b]	9.6 (7.6)	9.8 (10.3)	23.2 (14.6)	35.7 (25.9)	13.4 (13.2)
Refinement					
Resolution (Å)	40.0 – 0.8			50.0 – 1.7	
No. of reflections	92,709			59,806	
No. of reflections test set	4,650 (5.0%)			1,812 (3.0%)	
R_{work} / R_{free}	11.8 / 13.2			16.3 / 21.0	
No. atoms (non-hydrogen)	1,448			4,176	
Protein	1,192			3,632	
Water	253			466	
Ligand/Ions	3			78	
B-factors	15			38	
Protein	11			37	
Water	31			43	
Ligand/Ions	16			73	
RMSD					
Bond lengths (Å)	0.008			0.010	
Bond angles (°)	1.1			1.0	
Ramachandran plot[d]					
Favored (%)	98.6			99.3	
Additionally allowed (%)	1.4			0.7	
Outliers (%)	0.0			0.0	
MolProbity					
Clashscore[d]	0.43 (95^{th} percentile)			0.96 (99^{th} percentile)	
Molprobity score[d]	0.65 (98^{th} percentile)			0.88 (100^{th} percentile)	

[a]SSRL, Stanford Synchotron Radiation Lightsource
[b]Highest-resolution shell is shown in parentheses
[c]Friedel pairs were merged
[d]As determined by MolProbity
[e]As a reference, two different high-resolution cutoffs are shown

Table S10.

Molecular masses and nucleoporin stoichiometry in composite structure

C. thermophilum Protein	Stoichiometry	Molecular Mass (kDa)	Total Mass (kDa)
Symmetric Core (observed in composite structure)			
Nup120	32	141.8	4537.6
Nup37	32	80.3	2569.6
-	-	-	-
Nup85	32	128.1	4099.2
-	-	-	-
Sec13	32	33.8	1081.6
Nup145C	32	88.8	2841.6
Nup84	32	108.1	3459.2
Nup133	32	150.7	4822.4
Nup170	48	156.2	7497.6
Nup192	32	196.9	6300.8
Nic96	32	121.5	3888.0
Nup57	32	36.9	1180.8
Nup49	32	48.6	1555.2
Nsp1	32	67.1	2147.2
Nup53	48	47.0	2256.0
Nup145N	48	99.8	4790.4
Nup188	16	203.8	3260.8
TOTAL			**56288.0**
Cytoplasmic Filaments			
-	-	-	
Nup159	-	156.2	
Nup82	-	98.1	
Gle1	-	58.0	
Gle2	-	39.4	
Nup42	-	58.0	
Nuclear Basket			
Mlp1	-	231.1	
Nup152	-	152.2	
Nup56	-	56.2	
ELYS	-	33.1	
POMs/Other			
Pom152	-	141.5	
-	-	-	
Ndc1	-	71.8	
Pom34	-	34.2	
-	-	-	

H. Sapiens Protein	Stoichiometry	Molecular Mass (kDa)	Total Mass (kDa)
Symmetric Core (observed in composite structure)			
Nup160	32	162.1	5187.2
Nup37	32	36.7	1174.4
Nup43	32	42.2	1350.4
Nup85	32	75.0	2400.0
Seh1	32	39.7	1270.4
Sec13	32	35.5	1136.0
Nup96	32	105.9	3388.8
Nup107	32	106.4	3404.8
Nup133	32	129.0	4128.0
Nup155	48	155.2	7449.6
Nup205	32	227.9	7292.8
Nup93	32	93.5	2992.0
Nup54	32	55.4	1772.8
Nup58	32	53.2	1702.4
Nup62	32	53.3	1705.6
Nup53	48	34.8	1670.4
Nup98	48	91.7	4401.6
Nup188	16	196.0	3136.0
TOTAL			**55563.2**
Cytoplasmic Filaments			
Nup358	-	358.2	
Nup214	-	213.6	
Nup88	-	83.5	
Gle1	-	79.8	
Rae1	-	41.0	
Nupl2	-	44.9	
Nuclear Basket			
TPR	-	267.3	
Nup153	-	153.9	
Nup50	-	50.1	
ELYS	-	252.5	
POMs/Other			
Gp210	-	205.1	
POM121	-	127.7	
NDC1	-	76.3	
ALADIN	-	59.6	