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Supplemental Information

Fuzzy Interactions Form and Shape the Histone Transport Complex

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Fuzzy interactions form and shape the histone transport complex

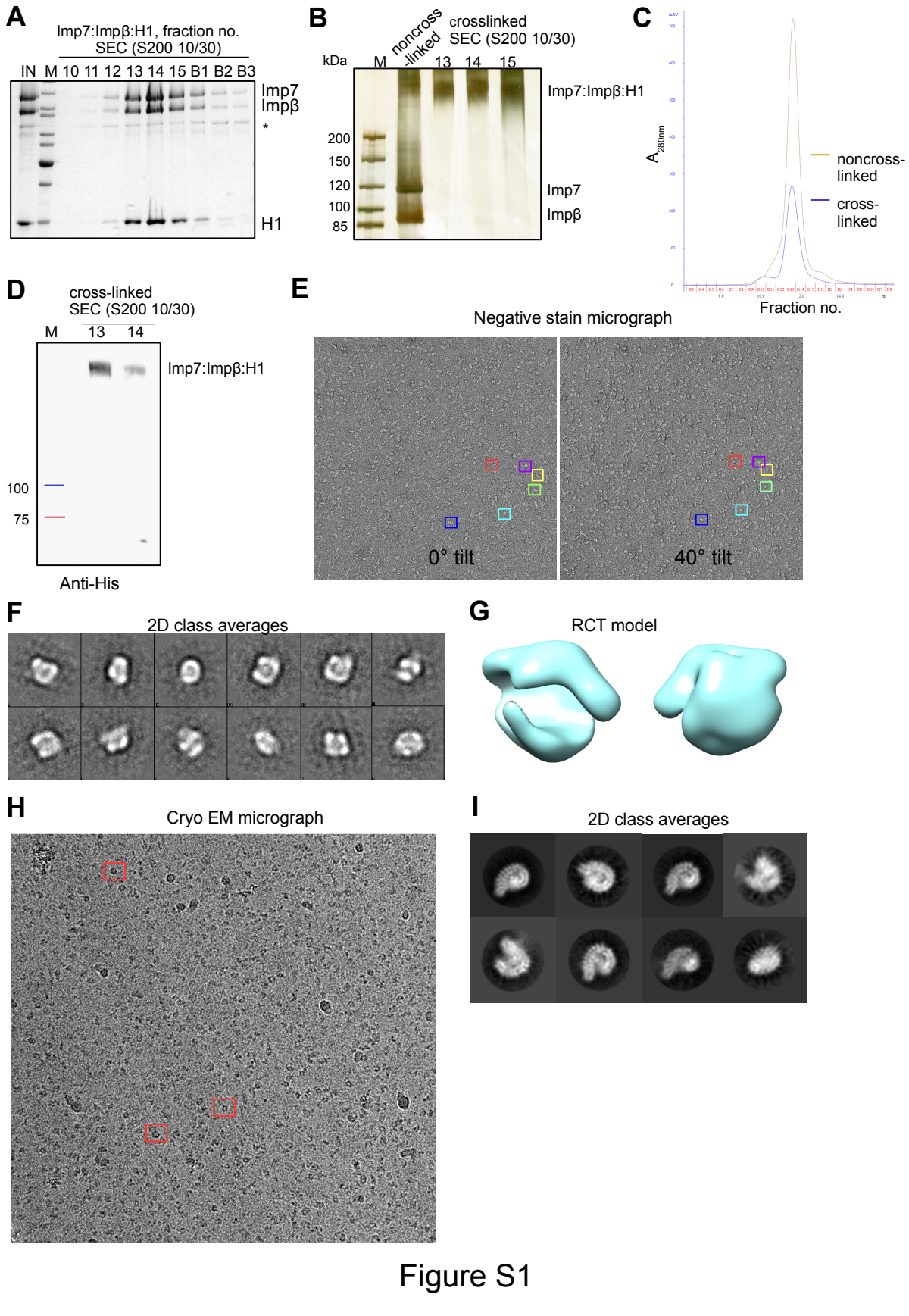
A
Imp7:Impβ:H1, fraction no.
SEC (S200 10/30)
IN M 10 11 12 13 14 15 B1 B2 B3
Imp7
Impβ
*
H1
B
kDa
M
noncross-linked
crosslinked
SEC (S200 10/30)
13 14 15
Imp7:Impβ:H1
200
150
120
100
85
Imp7
Impβ
C
A280nm
noncross-linked
cross-linked
Fraction no.
D
cross-linked
SEC (S200 10/30)
M 13 14
Imp7:Impβ:H1
100
75
Anti-His
E
Negative stain micrograph
0° tilt
40° tilt
F
2D class averages
G
RCT model
H
Cryo EM micrograph
I
2D class averages

Figure S1

Figure S1. Related to Figure 1. Assembly and Random Conical Tilt of the Imp7:Impβ:H1.0 Complex.

(**A**) SDS-PAGE analysis of the *in vitro* reconstituted complex after size-exclusion chromatography.

(**B**) SDS-PAGE analysis of the cross-linked Imp7:Impβ:H1.0 after size-exclusion chromatography.

(**C**) Size-exclusion chromatogram: comparison of native and cross-linked Imp7:Impβ:H1.0 indicating that both complexes have the same molecular weight.

(**D**) Anti-His Western blot analysis of cross-linked Imp7:Impβ:H1.0 complex showing the presence of H1.0-His protein in the Imp7:Impβ:H1.0 complex.

(**E**) A pair of untilted (left) and tilted (right) negative stain micrographs. Six tilt-pair particles are indicated with colored boxes, with each color indicating one molecule.

(**F**) 2D class averages of the untilted particle images.

(**G**) Representative initial 3D model obtained by the random conical tilt method.

(**H**) Representative cryo-EM micrograph of the Imp7:Impβ:H1.0 complex.

(**I**) 2D class averages of particle images.

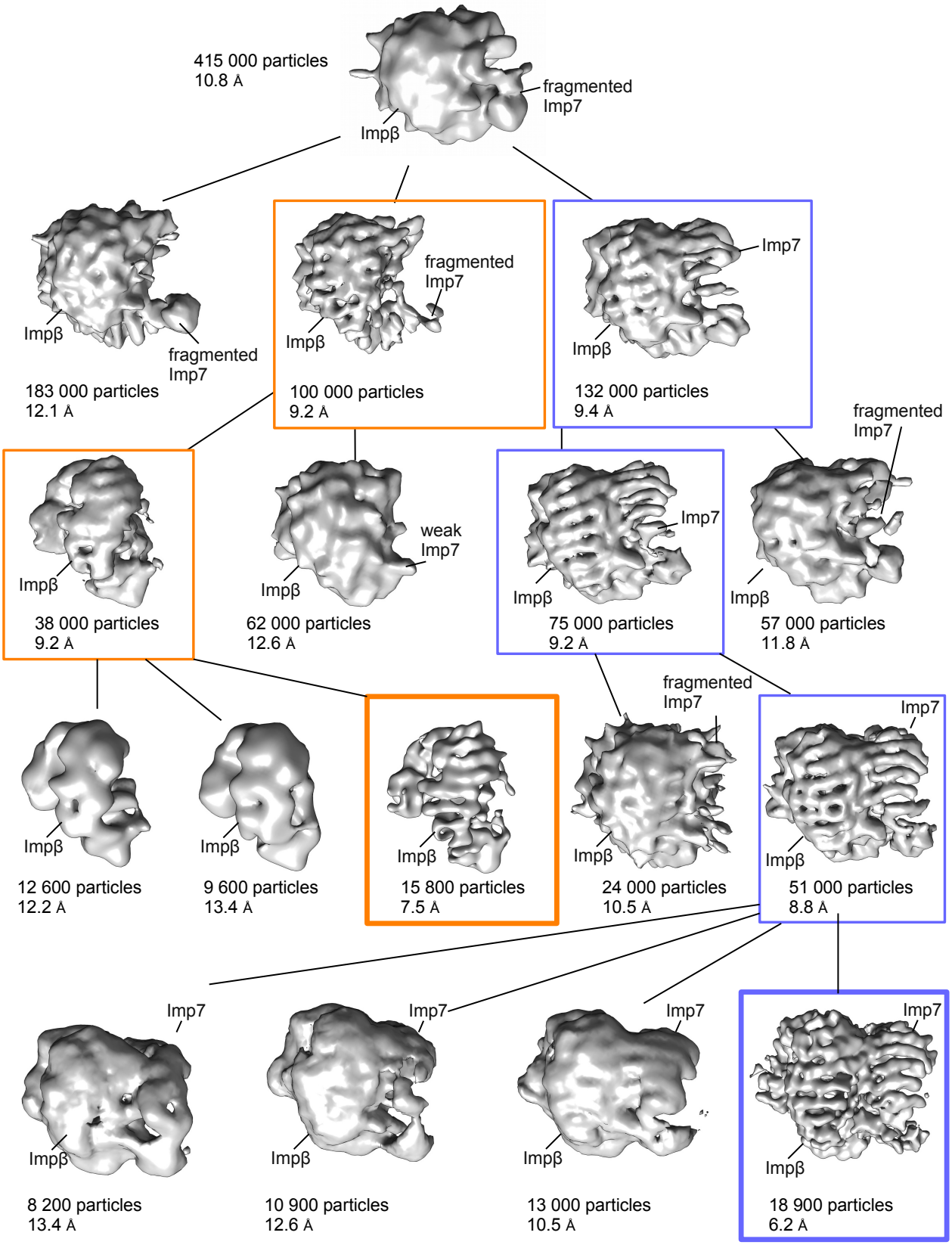
415 000 particles
10.8 Å
fragmented Imp7
Impβ
Impβ
fragmented Imp7
183 000 particles
12.1 Å
fragmented Imp7
Impβ
100 000 particles
9.2 Å
Imp7
Impβ
132 000 particles
9.4 Å
Impβ
38 000 particles
9.2 Å
weak Imp7
Impβ
62 000 particles
12.6 Å
Imp7
Impβ
75 000 particles
9.2 Å
fragmented Imp7
Impβ
57 000 particles
11.8 Å
Impβ
12 600 particles
12.2 Å
Impβ
9 600 particles
13.4 Å
Impβ
15 800 particles
7.5 Å
fragmented Imp7
Impβ
24 000 particles
10.5 Å
Imp7
Impβ
51 000 particles
8.8 Å
Imp7
Impβ
8 200 particles
13.4 Å
Imp7
Impβ
10 900 particles
12.6 Å
Imp7
Impβ
13 000 particles
10.5 Å
Imp7
Impβ
18 900 particles
6.2 Å

Figure S2

Figure S2. Related to Figure 1. Classification of Cryo-EM Images.

The overall dataset comprising 415000 particles was extensively 3D classified. It was first subdivided into three classes of different sizes, two of which were further classified (orange and blue box). The second (orange) class yielded after further sub-classification rounds the final Impβ:H1.0 reconstruction and the third (blue) class the Imp7:Impβ:H1.0.

Other interesting sub-classes contained Impβ and fragmented Imp7 or the Imp7:Impβ:H1.0 with the N-terminal HEAT-repeats of Imp7 missing.

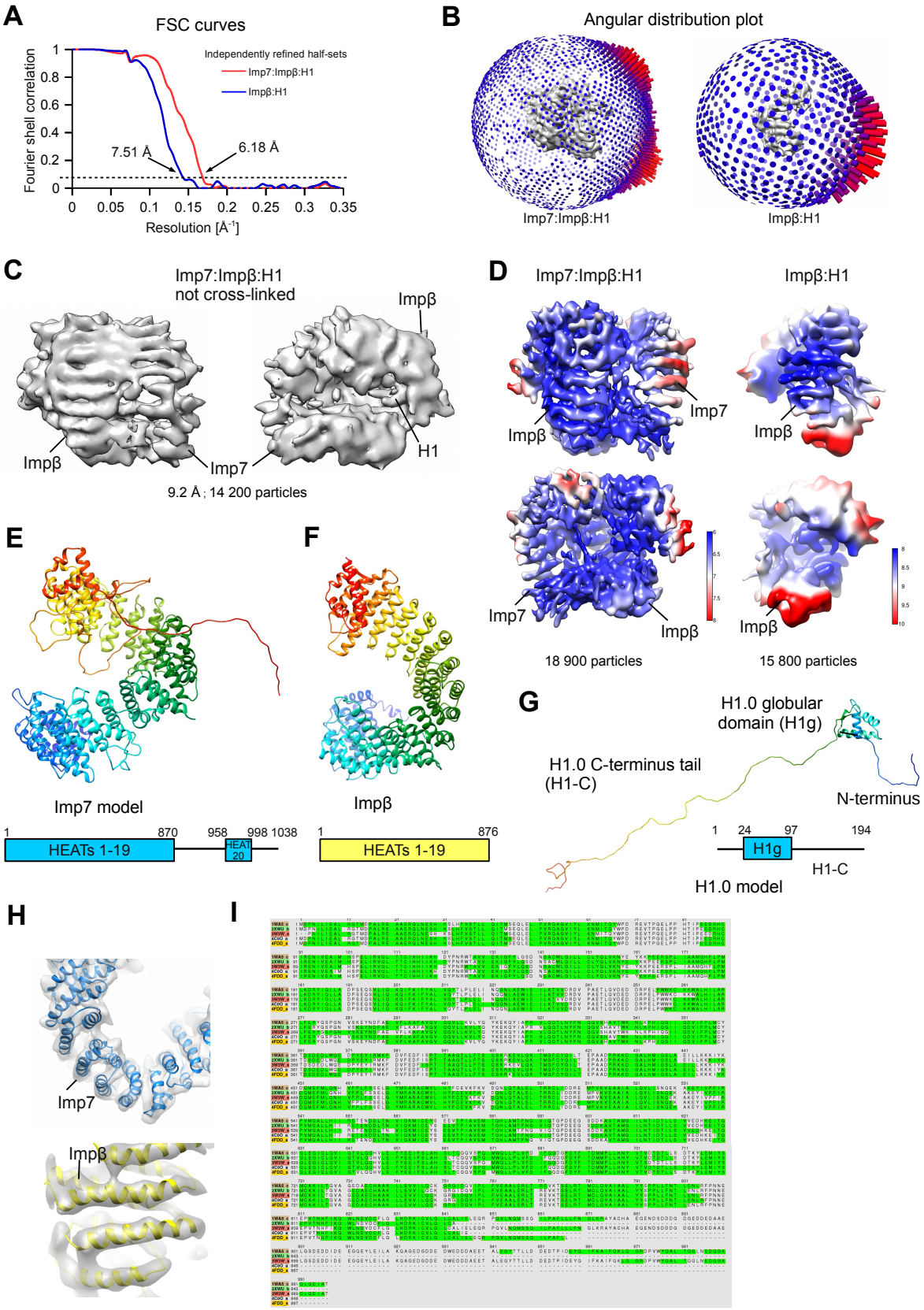
A
FSC curves
Independently refined half-sets
Imp7:Impβ:H1
Impβ:H1
Fourier shell correlation
7.51 Å
6.18 Å
Resolution [Å⁻¹]
B
Angular distribution plot
Imp7:Impβ:H1
Impβ:H1
C
Imp7:Impβ:H1
not cross-linked
Impβ
H1
Imp7
9.2 Å ; 14 200 particles
D
Imp7:Impβ:H1
Impβ:H1
Imp7
Impβ
18 900 particles
15 800 particles
E
Imp7 model
1
870
958
998 1038
HEATs 1-19
HEAT 20
F
Impβ
1
876
HEATs 1-19
G
H1.0 globular domain (H1g)
H1.0 C-terminus tail (H1-C)
N-terminus
1
24
97
194
H1g
H1-C
H1.0 model
H
Imp7
Impβ
I

Figure S3

Figure S3. Related to Figure 1. Cryo-EM Reconstruction of the Imp7:Impβ:H1.0 Complex and Fitting of Models into the Cryo-EM Map.

(**A**) FSC curves for Imp7:Impβ:H1.0 and Impβ:H1.0 reconstructions. The resolutions according to the Fourier shell cutoff at 0.143 are 6.2 Å and 7.5 Å, respectively.

(**B**) Angular distribution of the Imp7:Impβ:H1.0 and Impβ:H1.0 reconstructions.

(**C**) Cryo-EM map of the not–cross-linked Imp7:Impβ:H1.0 complex resolved to 9.2 Å.

(**D**) Front and back views of not-sharpened Imp7:Impβ:H1.0 and Impβ:H1.0 reconstructions colored by local resolution. All the densities are shown at the same contour level and are filtered to local resolution. Resolution bars are shown on the right.

(**E**) Model prediction for Imp7 obtained by the MODELLER software, using PDB 1WA5 as the template.

(**F**) Crystal structure of Impβ (PDB code: 3W5K).

(**G**) Model of the histone H1.0 obtained by MODELLER, using PDB 5NL0 as the template.

(**E–G**) Each model is colored using a spectrum from blue to red, which correspond to the N-terminus and C-terminus, respectively. A scheme showing the prediction for structured (filled box) and unstructured regions is shown below the model.

(**H**) Representative EM density (gray) with fitted HEAT repeats from Imp7 (blue) and Impβ (yellow).

(**I**) Secondary structure predictions for Imp7, using various templates.

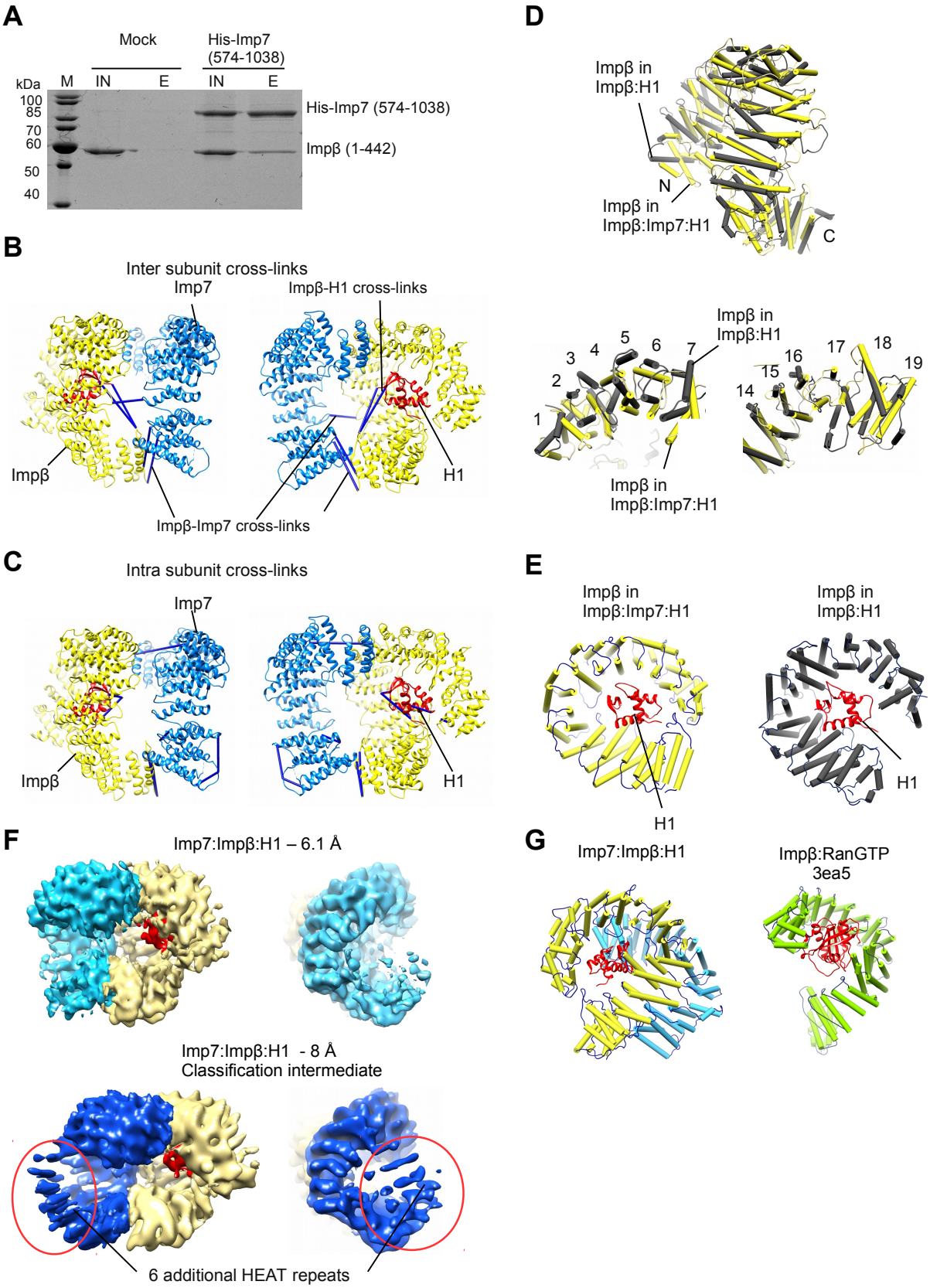
A
Mock
His-Imp7 (574-1038)
kDa
M
IN
E
IN
E
100
85
70
60
50
40
His-Imp7 (574-1038)
Impβ (1-442)
B
Inter subunit cross-links
Imp7
Impβ-H1 cross-links
Impβ
H1
Impβ-Imp7 cross-links
C
Intra subunit cross-links
Imp7
Impβ
H1
D
Impβ in Impβ:H1
N
Impβ in Impβ:Imp7:H1
C
Impβ in Impβ:H1
1
2
3
4
5
6
7
14
15
16
17
18
19
Impβ in Impβ:Imp7:H1
E
Impβ in Impβ:Imp7:H1
Impβ in Impβ:H1
H1
H1
F
Imp7:Impβ:H1 – 6.1 Å
Imp7:Impβ:H1 - 8 Å
Classification intermediate
6 additional HEAT repeats
G
Imp7:Impβ:H1
Impβ:RanGTP
3ea5

Figure S4

Figure S4. Related to Figure 1. Cross-Linking Mass Spectrometry Supports the Imp7:Impβ:H1.0 Model.

(**A**) Results of a pull-down experiment showing that the N-terminal part of Impβ (1-442) binds the C-terminal part of Imp7 (574-1038). SDS-PAGE analysis of input (IN) and elution (E) fractions of the pull-down experiment with His-tagged Imp7 (574-1038) bound to the resin.

(**B**) A model depicting inter-subunit lysine-lysine cross-links in the structured part of the Imp7:Impβ:H1.0 complex. The spacer arm of the BS3 cross-linker reagent is 11.4 Å. The cross-links between lysine residues are within 25 Å of the backbone in this structure. The cross-links are consistent with the model.

(**C**) A model depicting intra-subunit lysine-lysine cross-links in the structured part of the Imp7:Impβ:H1.0 complex. The cross-links between lysine residues are within 25 Å of the backbone in this structure. The cross-links are consistent with the model.

(**D**) Superimposition of Impβ from the Imp7:Impβ:H1.0 (yellow) and the Impβ:H1.0 (gray) complex. Although the middle HEAT repeats of Impβ are well aligned, the N- and C-terminal HEAT repeats are more open in the Impβ:H1.0 complex. HEAT repeats of Impβ are numbered.

(**E**) Comparison of Impβ:H1.0 interaction in Imp7:Impβ:H1.0 and Impβ:H1.0. H1.0 is shown as a red ribbon. Impβ (yellow) from the Imp7:Impβ:H1.0 complex and Impβ (gray) from Impβ:H1.0 are displayed as cylinders.

(**F**) Comparison of the final map and a 3D classification intermediate of the Imp7:Impβ:H1.0 complex. The final refined map of the Imp7:Impβ:H1.0 complex is shown in the upper panel. In the final map, the Imp7 N-terminus is absent due to flexibility. The 3D classification intermediate, shown in the lower panel, has a density for the Imp7 N-terminus (HEAT repeats 1–6) at lower contour levels (red circle).

(G) Structures of Impβ in complex with Imp7 and H1.0 and RanGTP. H1 and RanGTP are shown as red ribbons, Imp7 as blue cylinders, Impβ in Imp7:Impβ:H1.0 complex as yellow cylinders, and Impβ in complex with RanGTP as green cylinders.

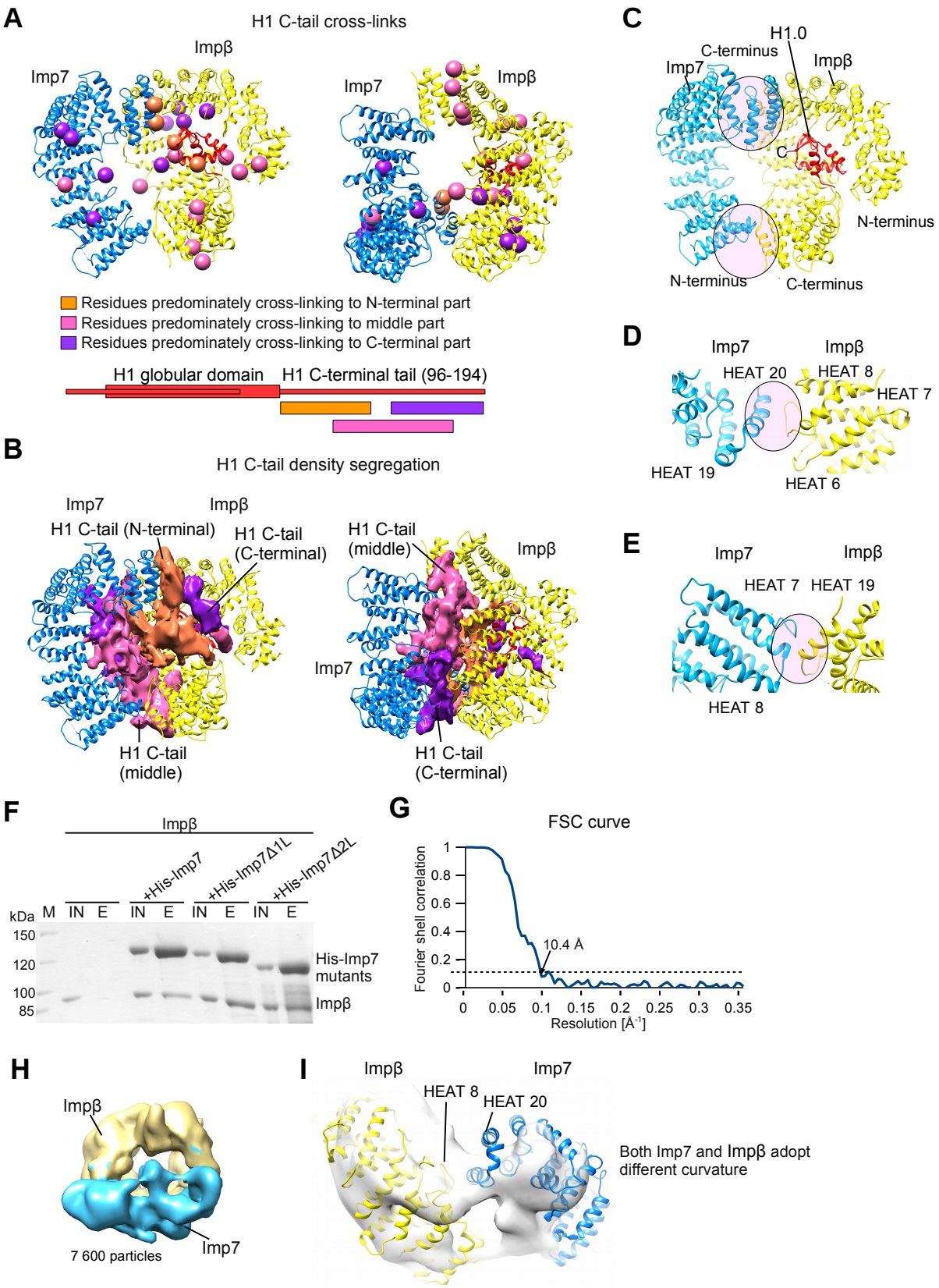
A
H1 C-tail cross-links
Impβ
Imp7
Residues predominately cross-linking to N-terminal part
Residues predominately cross-linking to middle part
Residues predominately cross-linking to C-terminal part
H1 globular domain
H1 C-terminal tail (96-194)
B
H1 C-tail density segregation
H1 C-tail (N-terminal)
H1 C-tail (C-terminal)
H1 C-tail (middle)
C
H1.0
C-terminus
N-terminus
D
HEAT 20
HEAT 8
HEAT 7
HEAT 19
HEAT 6
E
HEAT 7
HEAT 19
HEAT 8
F
+His-Imp7
+His-Imp7Δ1L
+His-Imp7Δ2L
kDa
M
IN
E
150
120
100
85
His-Imp7 mutants
G
FSC curve
Fourier shell correlation
10.4 Å
Resolution [Å^{-1}]
H
7 600 particles
I
HEAT 8
HEAT 20
Both Imp7 and Impβ adopt different curvature

Figure S5

Figure S5. Related to Figure 2 and 3. The H1 C-Terminal Tail Stabilizes the Imp7:Impβ:H1.0 Complex.

(**A**) Residues that cross-link with the C-terminal tail of H1 are depicted as spheres. Residues that cross-link predominantly to the N-terminal part of the H1 C-terminal tail are shown in orange; those that cross-link predominantly to the middle part in pink; and those that cross-link predominantly to the C-terminal part in violet. DMTMM, a zero-length cross-linker, was used in the reaction.

(**B**) Disordered density in the cradle and in between two importins was segregated based on the cross-linking mass-spectrometry data. The density in proximity to the importin residues that cross-link to the N-terminal part of the H1 C-terminal tail is shown in orange; that in proximity to the importin residues that cross-link to the middle part in pink; and that in proximity to the importin residues that cross-link to the C-terminal part in violet.

(**C**) Structural model of the Imp7:Impβ:H1.0 complex. The N- and C- termini of each protein, as well as the contact areas between the importins (pink transparent circles), are indicated. Imp7 and Impβ are shown in blue and yellow, respectively.

(**D**) Close-up view at the contact area between Imp7 HEAT repeat 20 and the loop connecting Impβ HEAT repeats 7 and 8.

(**E**) Close-up view of the contact area between Imp7 HEAT repeats 7–8 and the inner helix of Impβ HEAT repeat 19.

(**F**) Results of a pull-down experiment showing that Impβ binds wild-type Imp7, Imp7Δ1L (884–912), and Imp7Δ2L (884–912 and 923–954) mutants with glycine-serine residues replacing acidic loops. SDS-PAGE analysis of input (IN) and elution (E) fractions of the pull-down experiment with His-tagged wild-type and mutant Imp7 bound to the resin.

(**G**) FSC curves for the Imp7:Impβ reconstruction. The resolution according to the Fourier shell cutoff at 0.143 is 10.4 Å.

(**H**) Cryo-EM map of Imp7:Impβ without H1.0 resolved to 10.4 Å. Impβ is yellow, Imp7 is blue.

(**I**) Model of the Imp7:Impβ:H1.0 complex superimposed on the Imp7:Impβ map. Imp7 and Impβ are shown in blue and yellow, respectively. Imp7 and Impβ adopt different curvatures.

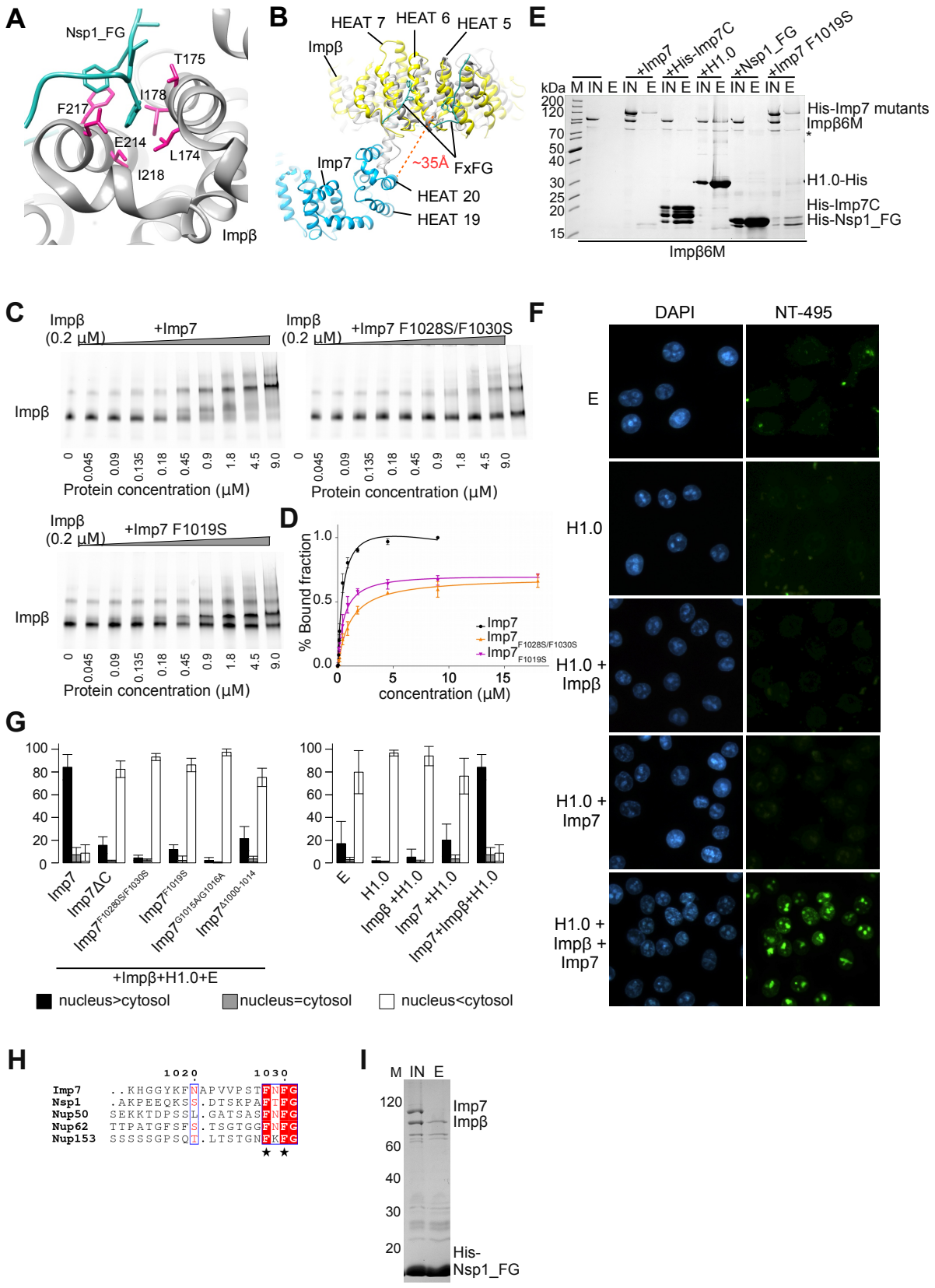
A
Nsp1_FG
T175
I178
F217
E214
L174
I218
Impβ
B
HEAT 7
HEAT 6
HEAT 5
Impβ
Imp7
~35Å
FxFG
HEAT 20
HEAT 19
E
+Imp7
+His-Imp7C
+H1.0
+Nsp1_FG
+Imp7 F1019S
kDa M IN E IN E IN E IN E IN E IN E
200
120
70
50
40
30
25
20
15
His-Imp7 mutants
Impβ6M
*
H1.0-His
His-Imp7C
His-Nsp1_FG
Impβ6M
C
Impβ (0.2 μM)
+Imp7
Impβ
0 0.045 0.09 0.135 0.18 0.45 0.9 1.8 4.5 9.0
Protein concentration (μM)
Impβ (0.2 μM)
+Imp7 F1028S/F1030S
0 0.045 0.09 0.135 0.18 0.45 0.9 1.8 4.5 9.0
Protein concentration (μM)
Impβ (0.2 μM)
+Imp7 F1019S
Impβ
0 0.045 0.09 0.135 0.18 0.45 0.9 1.8 4.5 9.0
Protein concentration (μM)
D
% Bound fraction
1.0
0.5
0.0
0
5
10
15
concentration (μM)
Imp7
Imp7 F1028S/F1030S
Imp7 F1019S
F
DAPI
NT-495
E
H1.0
H1.0 + Impβ
H1.0 + Imp7
H1.0 + Impβ + Imp7
G
100
80
60
40
20
0
Imp7
Imp7ΔC
Imp7 F1028S/F1030S
Imp7 F1019S
Imp7 G1015A/G1016A
Imp7 Δ1000-1014
+Impβ+H1.0+E
E
H1.0
Impβ +H1.0
Imp7 +H1.0
Imp7+Impβ+H1.0
nucleus>cytosol
nucleus=cytosol
nucleus<cytosol
H
1020
1030
Imp7 ..KHGGYKFNAPVVPSTFNFG
Nsp1 .AKPEEQKSS.DTSKPAFTFG
Nup50 SEKKTDPSSL.GATSASFNFG
Nup62 TTPATGFSFS.TSGTGGFNFG
Nup153 SSSSSGPSQT.LTSTGNFKFG
I
M IN E
120
60
40
30
20
Imp7
Impβ
His-Nsp1_FG

Figure S6

Figure S6. Related to Figure 4 and 5. Interaction of the Imp7 C-Terminal Tail with the Impβ:FG-Nucleoporin Binding Site.

(**A**) Close-up view of Nsp1p FxFG repeats (green) binding to Impβ (gray) between HEAT repeats 5–6 and 6–7 (PDB ID: 1F59). The primary FG binding site on Impβ (gray) is shown with key residues in sticks. Residues mutated in the Impβ6M mutant are shown in pink.

(**B**) The Imp7:Impβ:H1.0 cryo-EM model superimposed on the Impβ:Nsp1p crystal structure (PDB ID: 1F59). HEAT repeats involved in binding are numbered, and FxFG repeats from the crystal structure are highlighted. Imp7 is shown in blue, Nsp1p in green, Impβ from Imp7:Impβ:H1.0 in yellow, and Impβ from Impβ:Nsp1p in gray.

(**C**) Results of a gel-shift assay showing the Imp7:Impβ complex formation. Impβ (0.2μM) was mixed with increasing amounts of Imp7 and Imp7 mutants.

(**D**) Results of a quantification of three independent gel-shift experiments showing Imp7 and Imp7-mutant binding to Impβ. The Kd for Imp7/Impβ interaction was estimated from the binding curve. The Kd for wild-type Imp7 is 0.38 μM, for Imp7 F1028S/F1030S 2.9 μM and for Imp7 F1019S 1.2 μM.

(**E**) Results of a pull-down experiment showing that the canonical FG-nucleoporin binding site in Impβ is essential for interaction with Imp7. SDS-PAGE analysis of input (IN) and elution (E) fractions of the pull-down experiment with Impβ6M (Impβ L174S, T175A, I178D, E214A, F217A, and I218D) and resin-bound His-tagged Imp7, the Imp7 C-terminal tail, H1.0, Nsp1_FG, and the Imp7 F1019S mutant. Degradation products of Impβ and Imp7 are indicated with an asterisk.

(**F**) Results of a nuclear import assay in permeabilized HeLa S3 cells, using NT-495–labeled H1.0 in the presence of an energy-regenerating system (E), Impβ, or wild type Imp7 was performed as described in the Methods section. H1 import into the nucleus is possible only when Imp7 and Impβ transport receptors are present. In the panels in the left column, cell nuclei are stained with DAPI.

(**G**) Quantitative analysis of H1.0 nuclear import using an energy-regenerating system (E), Impβ, and the wild-type Imp7 or its mutants. The fluorescence intensity of NT-495–labeled H1.0 was measured in 50 to 150 cells by using the ImageJ software (National Institutes of Health) and was scored into three categories: nucleus > cytosol; nucleus = cytosol; and nucleus < cytosol.

(**H**) Sequence alignment of Imp7 C-terminal tail and representatives of FG-nucleoporins. Phe residues in the FxFG motif are indicated with an asterisk.

(**I**) Results of a pull-down experiment showing that the FG-repeats from Nsp1 compete with Imp7 for binding site in Impβ. SDS-PAGE analysis of input (IN) and elution (E) fractions of the pull-down experiment with the His-tagged Nsp1.

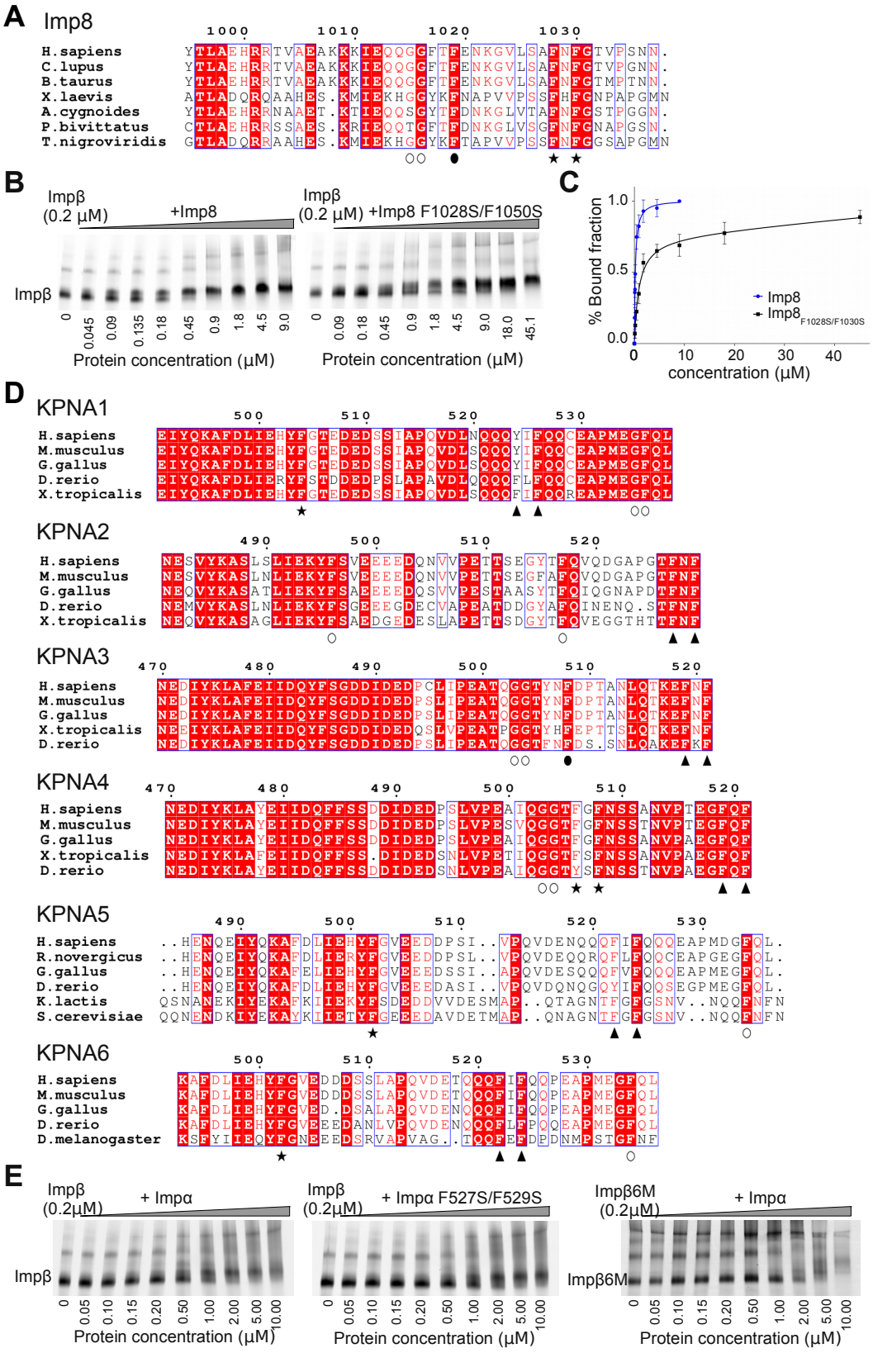
A
Imp8
H.sapiens
C.lupus
B.taurus
X.laevis
A.cygnoides
P.bivittatus
T.nigroviridis
B
Impβ (0.2 μM)
+Imp8
Impβ (0.2 μM) +Imp8 F1028S/F1050S
Impβ
Protein concentration (μM)
C
% Bound fraction
Imp8
Imp8F1028S/F1030S
concentration (μM)
D
KPNA1
KPNA2
KPNA3
KPNA4
KPNA5
KPNA6
E
Impβ (0.2μM)
+ Impα
Impβ (0.2μM)
+ Impα F527S/F529S
Impβ6M (0.2μM)
Impβ6M
Protein concentration (μM)

Figure S7

Figure S7. Related to Figure 6. Sequence Alignment Showing That Other Importins Contain Conserved FxFG or Similar Motifs.

(**A**) Sequence alignment of Imp8 C-terminal tail (aas 995–1038). Phe residues in the FxFG motif are indicated with an asterisk. Gly and Phe residues in the GGxxF motif are indicated by white and black circles, respectively.

(**B**) Results of a gel-shift assay showing the Imp8:Impβ complex formation. Impβ (0.2 μM) was mixed with increasing amounts of Imp8 and Imp8 F1028S/F1030S mutant. Imp8 F1028S/F1030S mutant shows a ~10-fold reduction in binding to Impβ.

(**C**) Results of a quantification of three independent gel-shift experiments showing Imp8 and Imp8-mutant binding to Impβ. The Kd was estimated from the binding curve. The Kd for wild type Imp8 is 0.19 μM and for Imp8 F1028S/F1030S 1.7 μM.

(**D**) Sequence alignments of Impα (Kapα) C-terminal tails. Conserved Phe residues in FxF, GGxxF, FG, and GF motifs are highlighted.

(**E**) Results of a gel-shift assay showing the Impα:Impβ complex formation. Impβ (0.2 μM) was mixed with increasing amounts of Impα and Impα F527S F529S mutant. Impβ6M mutant (0.2 μM) was mixed with increasing amounts of Impα. Both, Impα and Impβ mutants show reduced binding to wild type proteins.