Supplementary Information

Table S1. Statistics of Cryo-EM data collection, processing and refinement for IMP4•H3-H4•ASF1 and IMP4•RanGTP.

	IMP4•H3-H4•ASF1	IMP4•RanGTP
Data collection and processing		
Magnification	105,000	81,000
Voltage (kV)	300	300
Electron exposure (e^{-}/Å^2)	52	50
Defocus range (μm)	-2.5 to -1.0	-1.2 to -2.7
Pixel size (Å)	0.83	1.09
Symmetry imposed	C1	C1
Initial particle images (no.)	2,636,349	1,226,438
Final particle images (no.)	146,050	16,089
Map resolution (Å)	3.5	7.1
FSC threshold	0.143	0.143
Refinement		
Initial model used (PDBID)	2HUE, 3W3T	3W3Z
Model resolution (Å)	2.9/3.2/3.6	6.4/6.9/8.0
FSC threshold	0/0.143/0.5	0/0.143/0.5
Map sharpening *B* factor (Å^2)	-140	-657
Model composition		
Nonhydrogen atoms	11,014	9,296
Protein residues	1401	1196
R.m.s. deviations		
Bond lengths (Å)	0.003	0.003
Bond angles (°)	0.644	0.872
Validation		
MolProbity score	1.59	2.31
Clashscore	7.59	19.52
Poor rotamers (%)	0.00	0.00
Ramachandran plot		
Favored (%)	97.03	90.82
Allowed (%)	2.97	8.75
Disallowed (%)	0.00	0.42

Supplementary 1

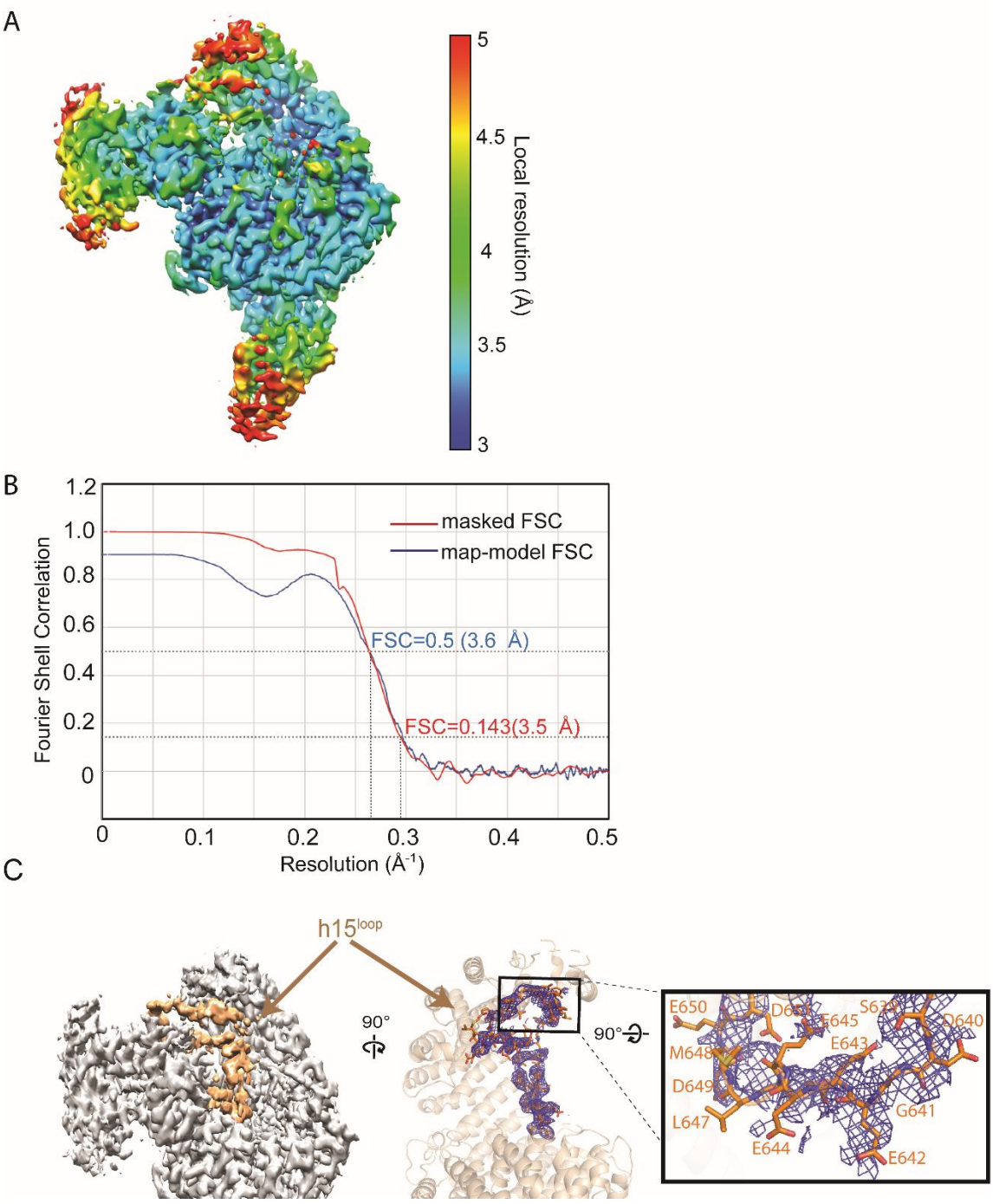

Figure S1. **Cryo-EM map of IMP4•H3-H4•ASF1** and density of an IMP4 loop. A) Cryo-EM map colored by local resolution as indicated by the color key. B) The Fourier Shell Correlation (FSC) for the EM density map calculated from cryoSPARC (red line; masked FSC) and phenix.refine (blue line; map-model FSC). C) Left, Cryo-EM map showing the $h15^{loop}$ in orange. Middle, the density of the $h15^{loop}$ shown as blue mesh' contour level = 4 σ. Right, details of the density at $h15^{loop}$ residues 639-651.

Supplementary 2

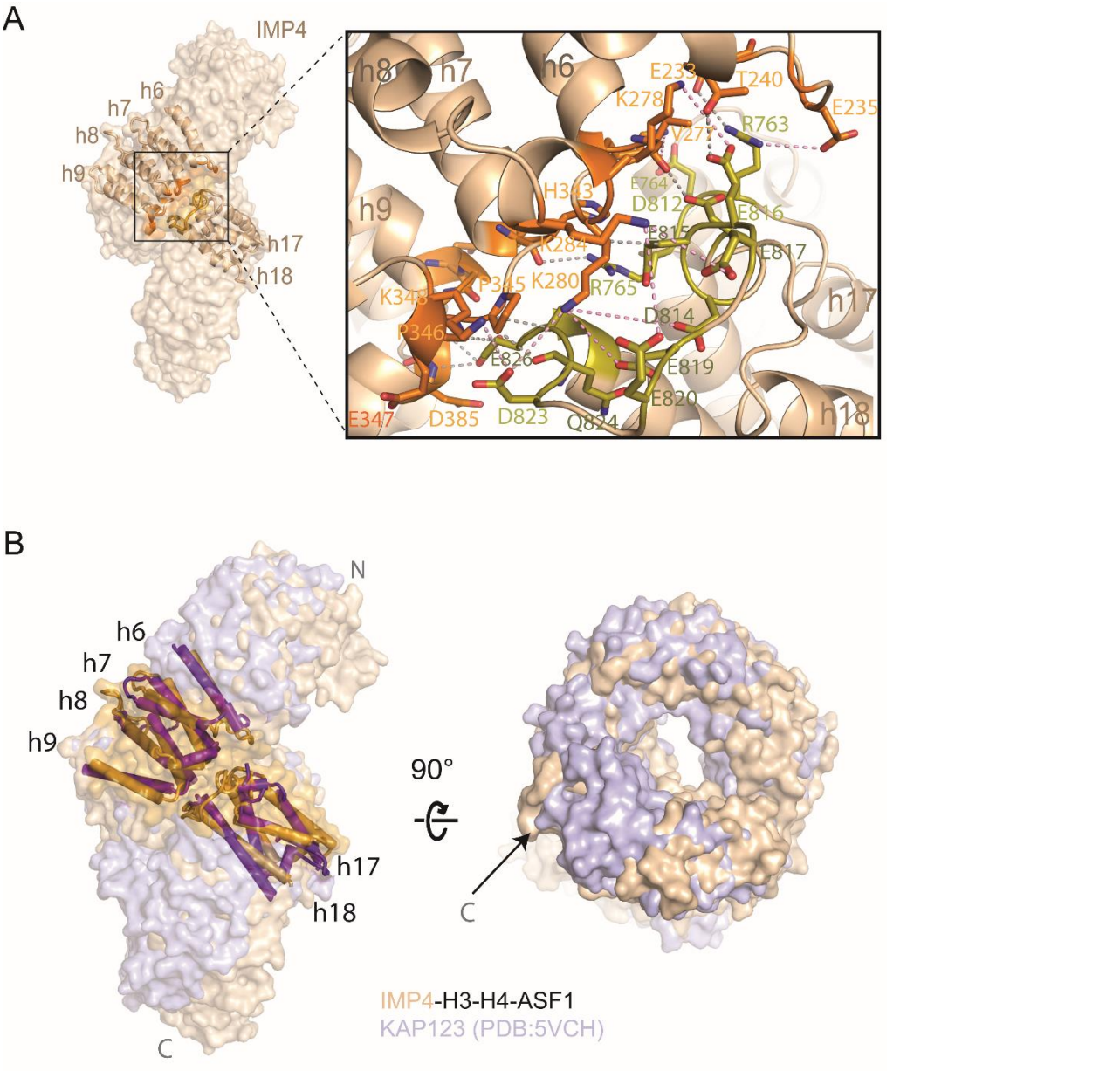

Figure S2. **Details of interactions between h6-h9 and h17-h18.** A) Details of the interactions between repeats h6-h9 (orange) and h17-18 (yellow) of IMP4 (beige). Hydrophobic and hydrogen-bonds interactions are shown with gray dashed lines and electrostatic interactions with pink dashes. B) Surface representations of unliganded KAP123 (lilac; PDBID 5VCH) superimposed on the ASF1•H3-H4-bound IMP4 (beige). The helices of repeats h6-h9 and h17-18 are shown as cylinders to highlight the similarities of both importins in this region. The right panel is a 90 ° rotation about the horizontal axis of the left panel to show the central rings of unliganded KAP123 and ASF1•H3-H4-bound IMP4.

Supplementary 3

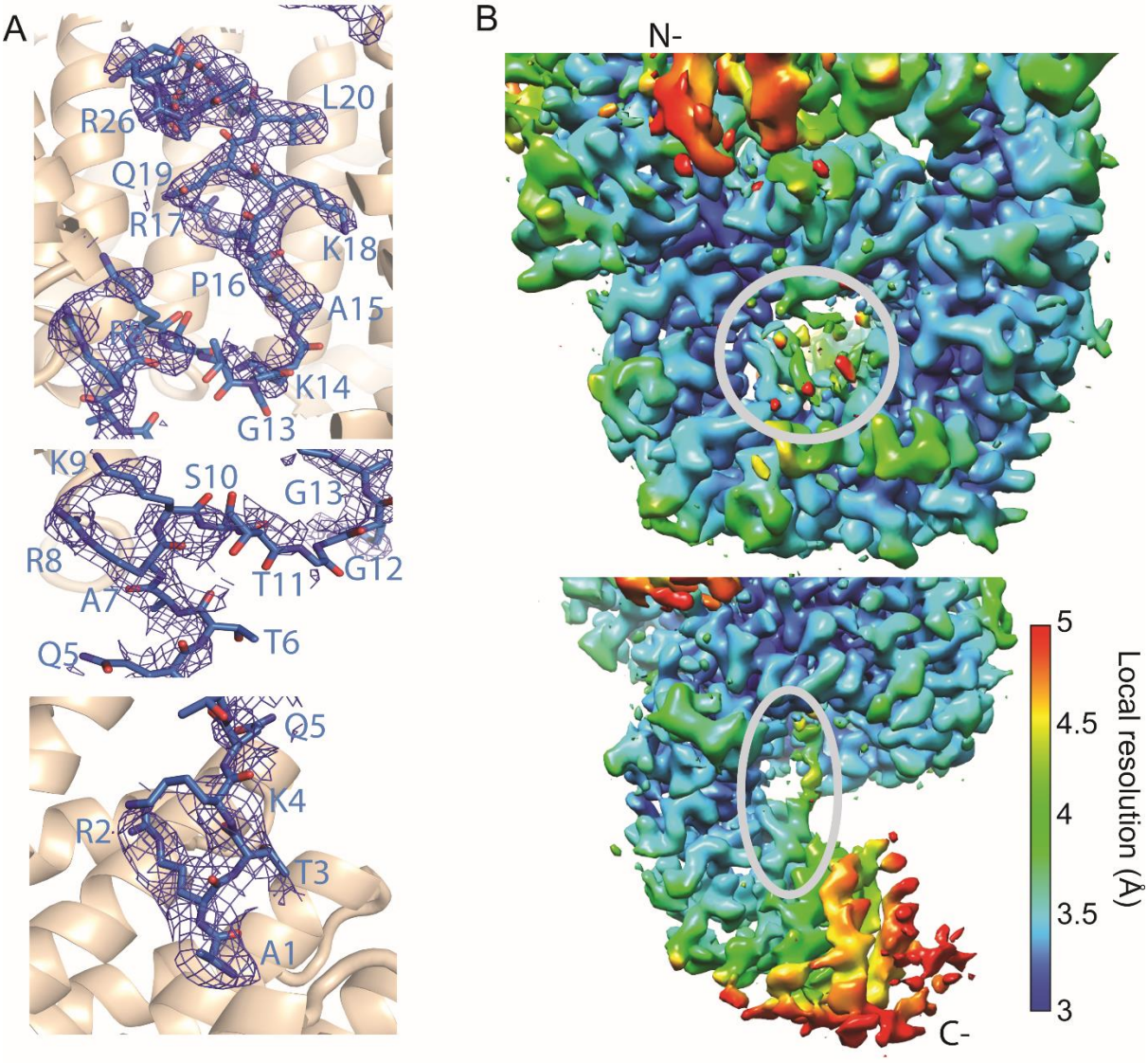

Figure S3. EM density of the H3^{tail}. A) Cryo-EM density for the H3^{tail} of IMP4•H3-H4•ASF1 (blue mesh; contour level = 4 σ). The H3^{tail} is shown in the same views as in Figure 3B-D. B) EM density of IMP4•H3-H4•ASF1 colored by the local resolution. Top panel, a view of the IMP4 central ring with the H3^{tail} indicated by a light grey circle. Bottom panel, the C-terminal half of IMP4 with the light grey oval highlighting the N-terminal most residues of the H3^{tail}.

Supplementary 4

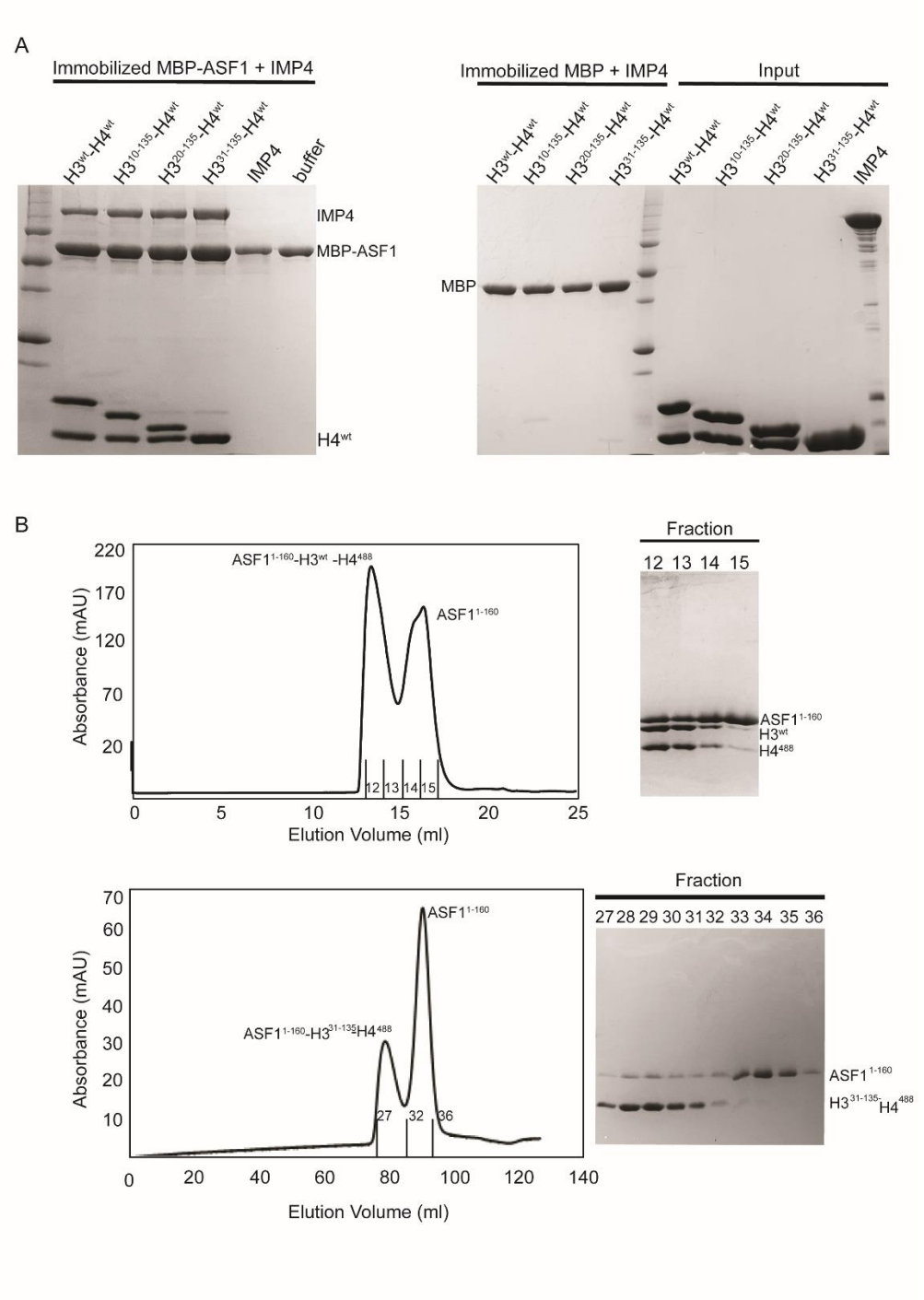

Figure S4. Binding assays and size-exclusion chromatography of ASF1•H3-H4 with truncated H3^{tail}. A) Pulldown binding assays of immobilized MBP-ASF1 with IMP4 and H3-H4 constructs (SDS-PAGE/Coomassie Blue). B) Size-exclusion chromatography of the ASF1•H3^{WT}-H4^{488} and ASF1•H3$^{30-135}$-H4^{488} complexes used in the fluorescence polarization assays in Figure 3F, and SDS-PAGE of the fractions containing the complexes.

Supplementary 5

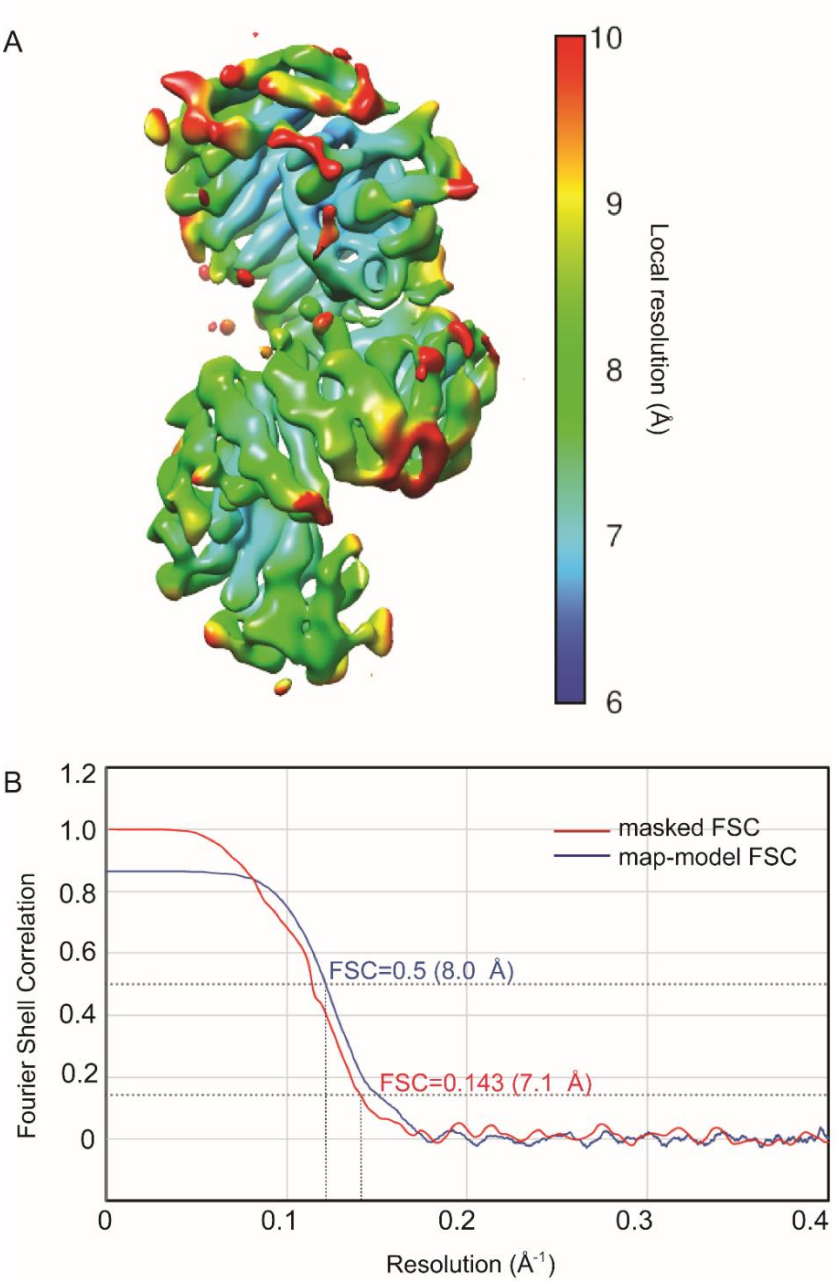

Figure S5. **Cryo-EM map of IMP4·RanGTP**. A) Cryo-EM map colored by local resolution as indicated by the color key. B) The Fourier Shell Correlation (FSC) for the EM density map calculated from cryoSPARC (red; masked FSC) and phenix.refine (blue; map-model FSC).

Supplementary 6

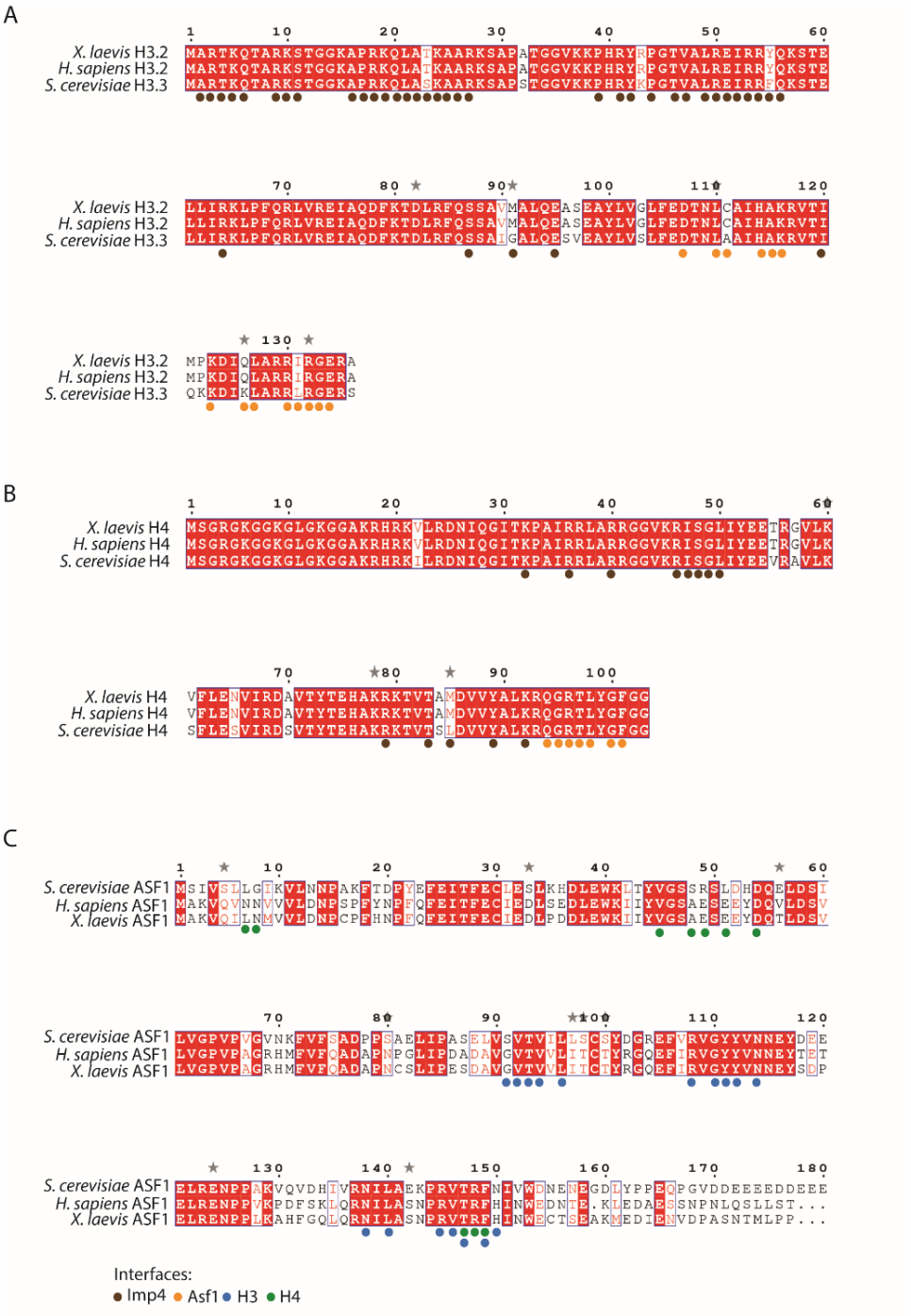

Figure S6. Sequence alignments of Asf1 and histones H3 and H4 from different organisms. Primary sequences from (A) Asf1 (residues 1-180), (B) histone H3 and (C) histone H4 from *X. laevis*, *H. sapiens* and *S. cerevisiae* were aligned using Clustal Omega. Residues highly conserved between species were highlighted in red. Residues that bind IMP4 are marked as brown circles, residues that bind Asf1 are marked as yellow circles and residues that bind histones H3 or H4 are marked as blue or green circles, respectively.

Video S1. **Conformational changes of Imp4.** Morphing of IMP4 bound to H3-H4•ASF1 and to RanGTP. The video shows the large conformational changes of the N-terminal half of the protein to accommodate RanGTP leading to cargo release in the nucleus. This video was generated in PyMOL.