

Supplementary Information For

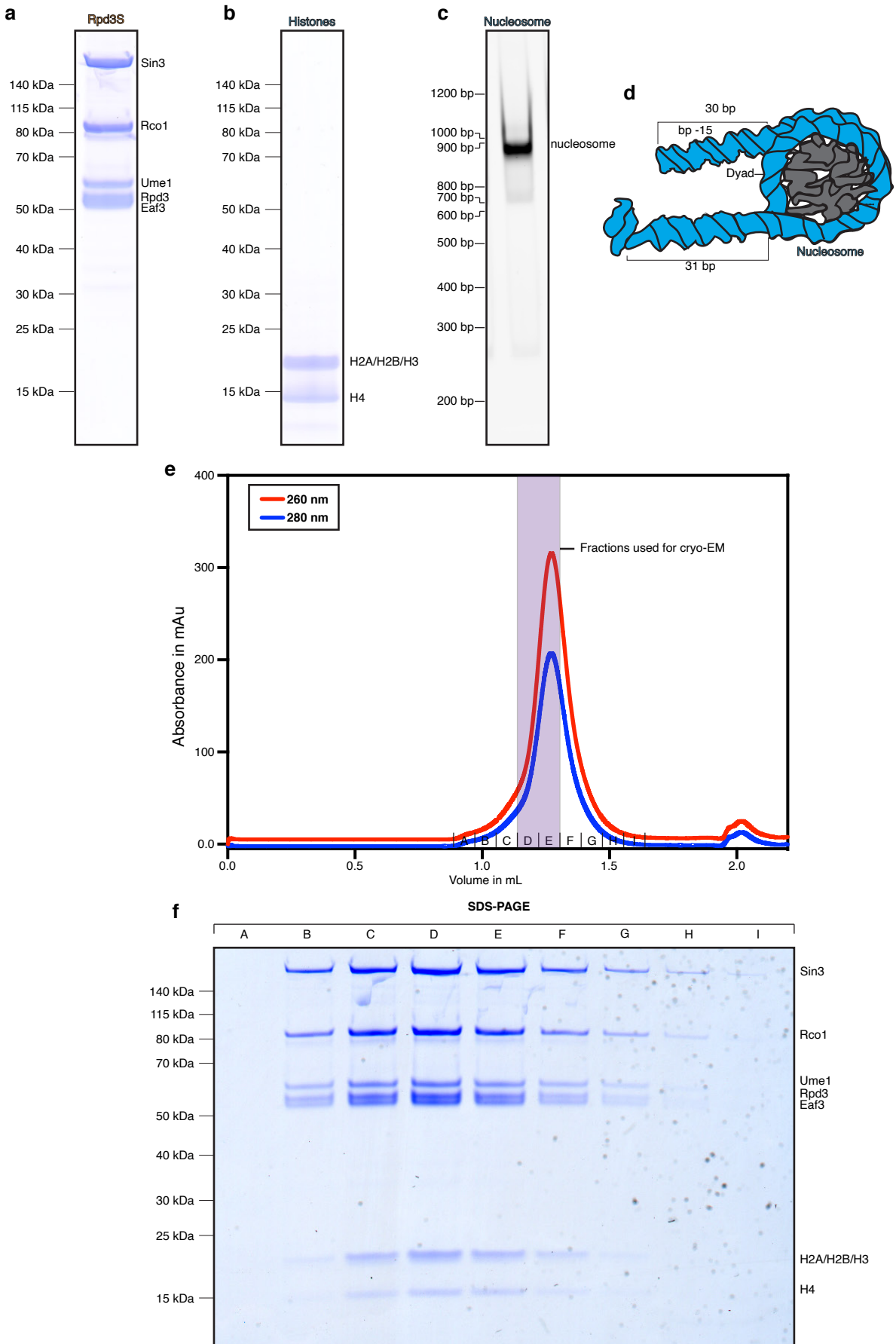
Structure of the complete *Saccharomyces cerevisiae* Rpd3S-nucleosome complex

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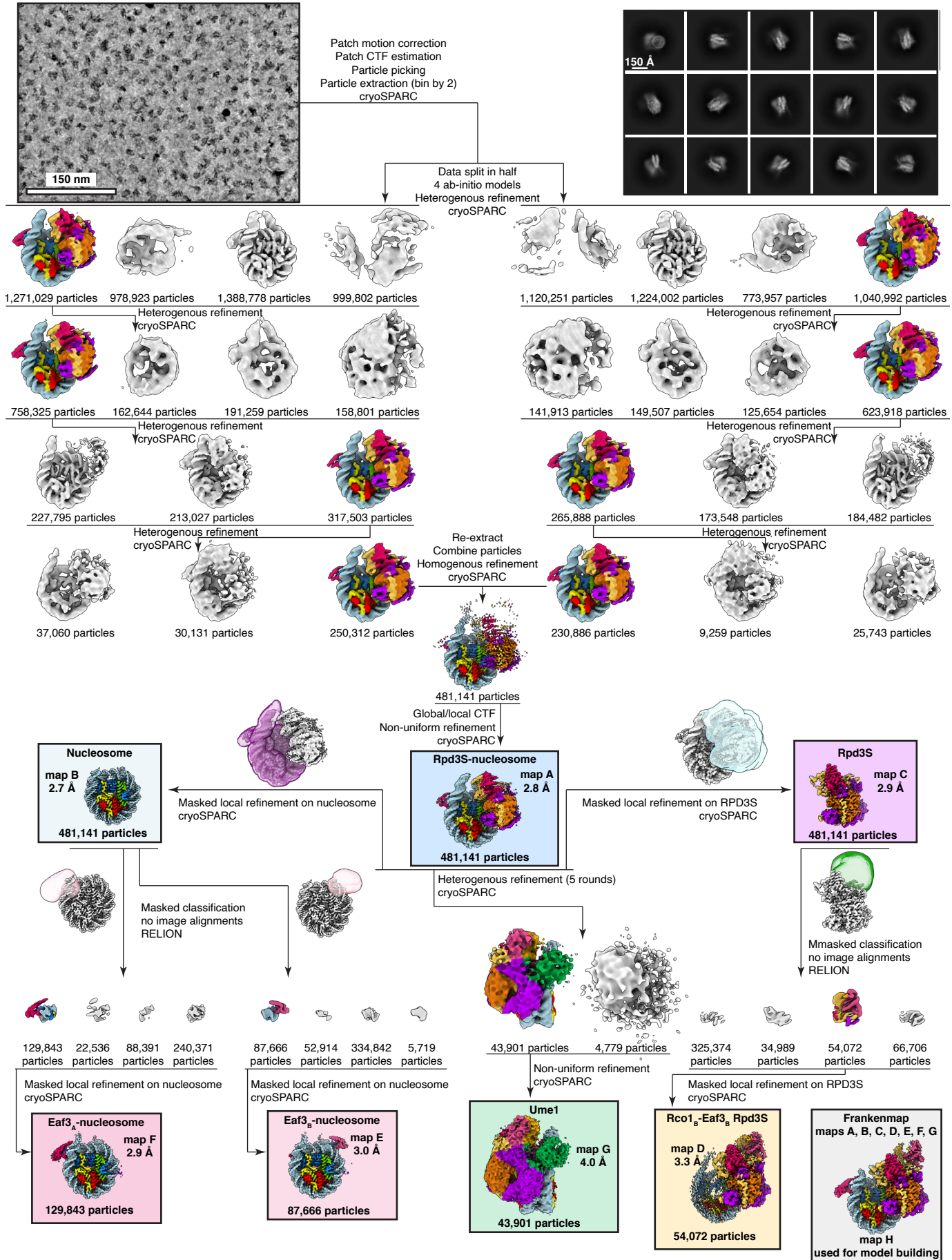
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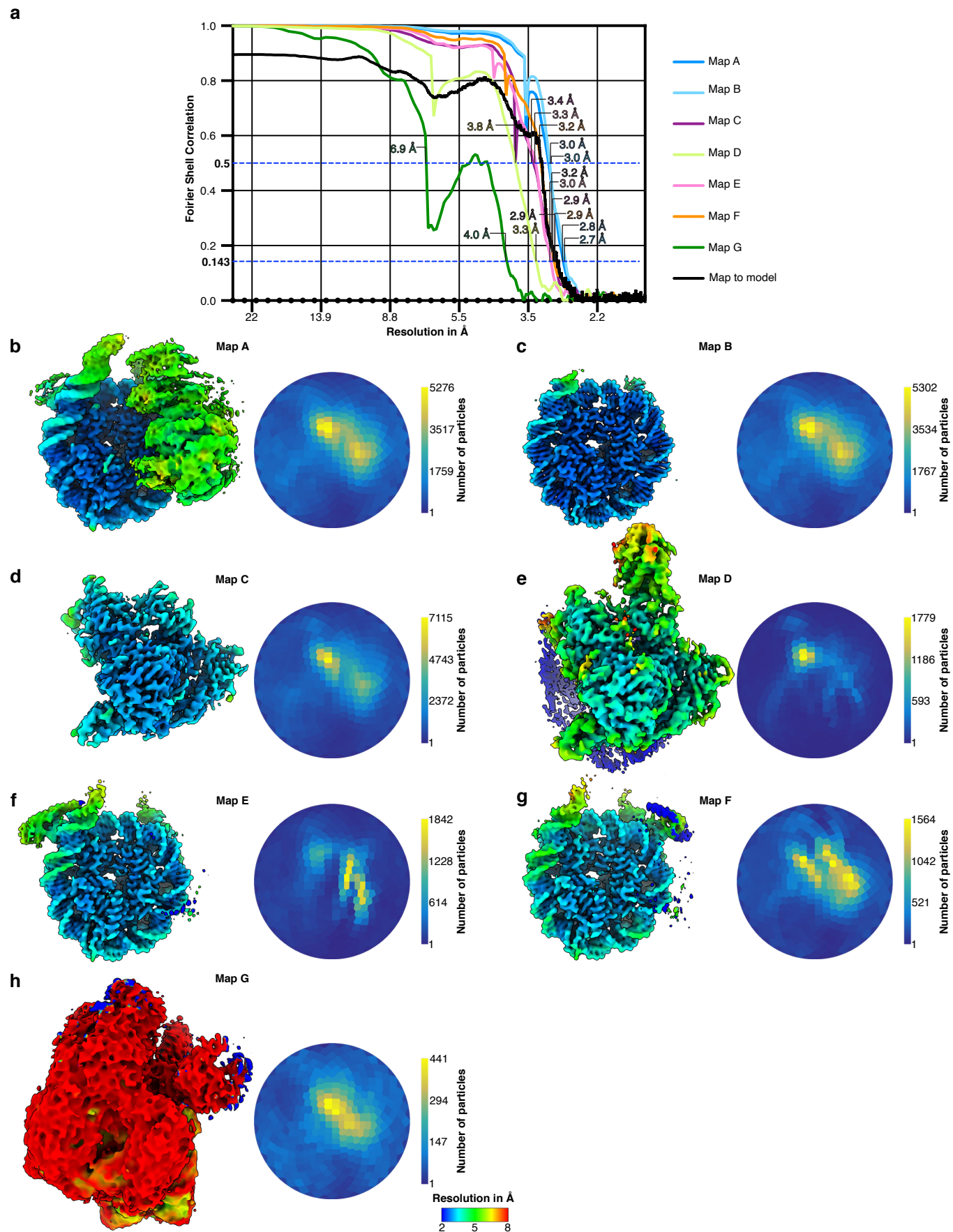
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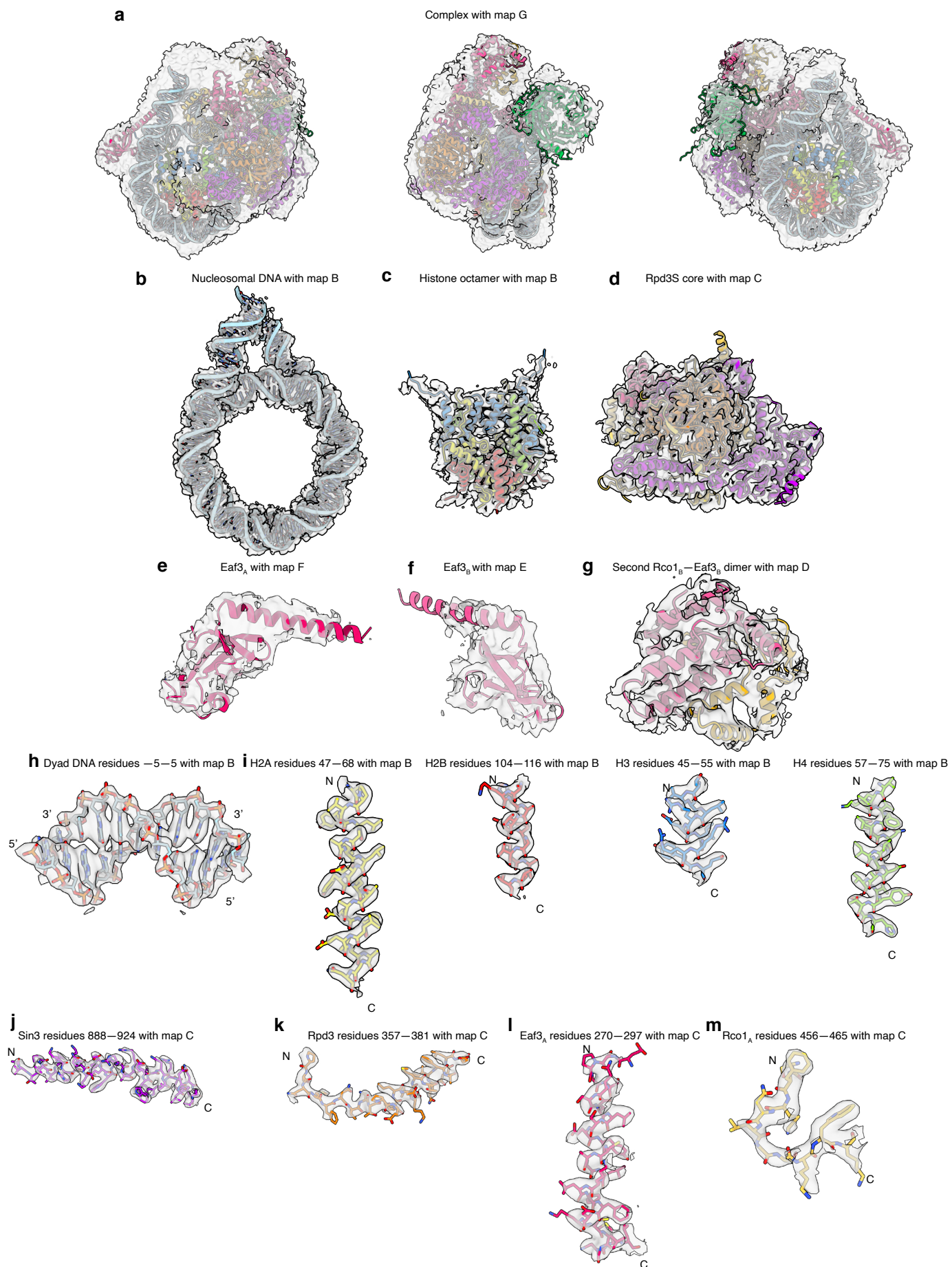


Supplementary Fig. 1 | Complex formation

a, SDS-PAGE of purified Rpd3S complex. **b**, SDS-PAGE of assembled histone octamer. **c**, Native TBE gel of reconstituted nucleosomal substrate. **d**, Schematic of the employed nucleosomal substrate. Extranucleosomal DNA length is indicated. **e**, Chromatogram of Rpd3S-nucleosome complex formation. Absorbance values at 280 and 260 nm are indicated. A Superose 6 Increase 3.2/300 was used for the complex formation. Fractions used for cryo-EM are indicated. **f**, SDS-PAGE of fractions from complex formation as shown in **e**. Fractions on gel correspond to indicated fractions in **e**. Source data are provided as a Source Data file.

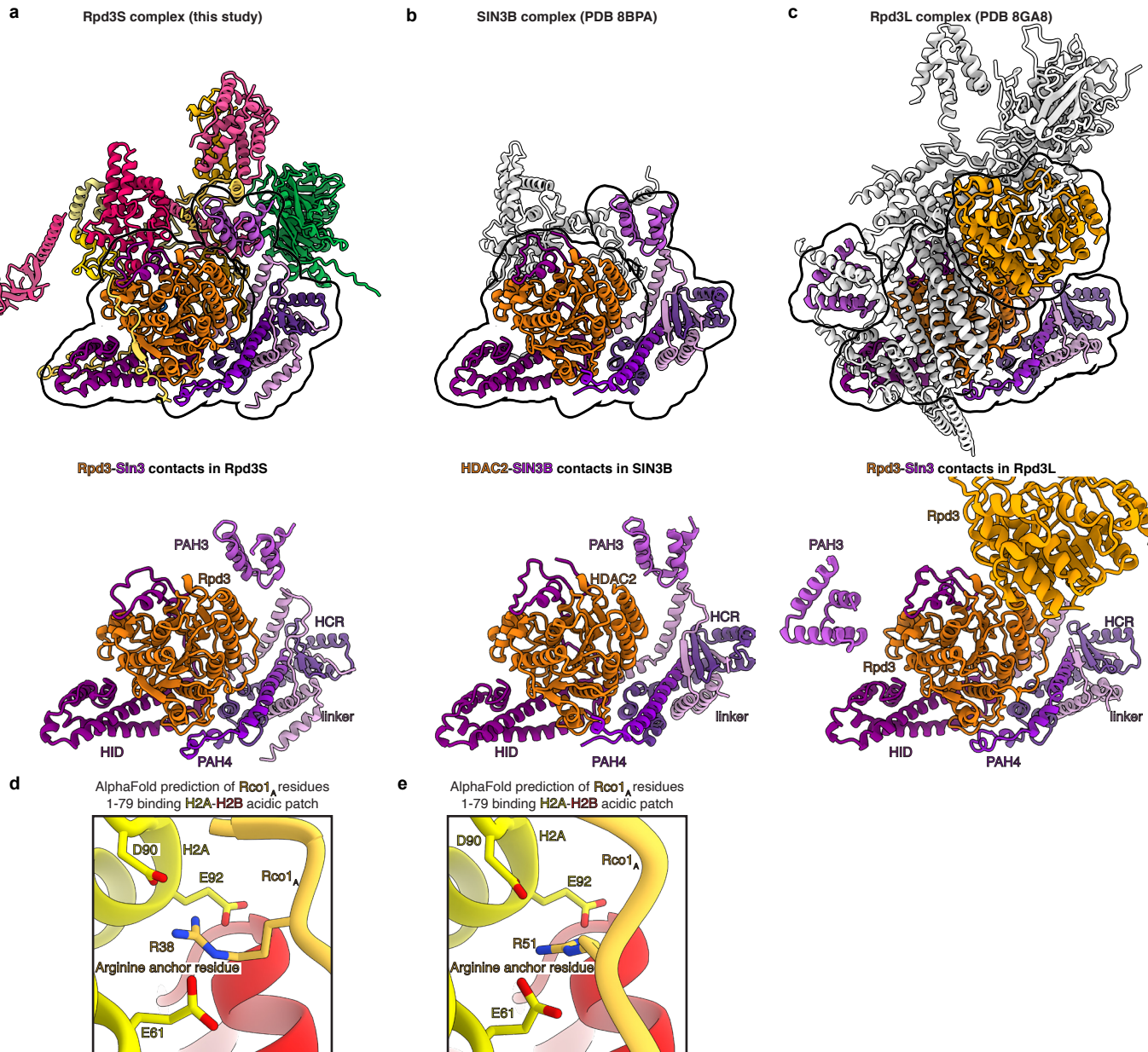






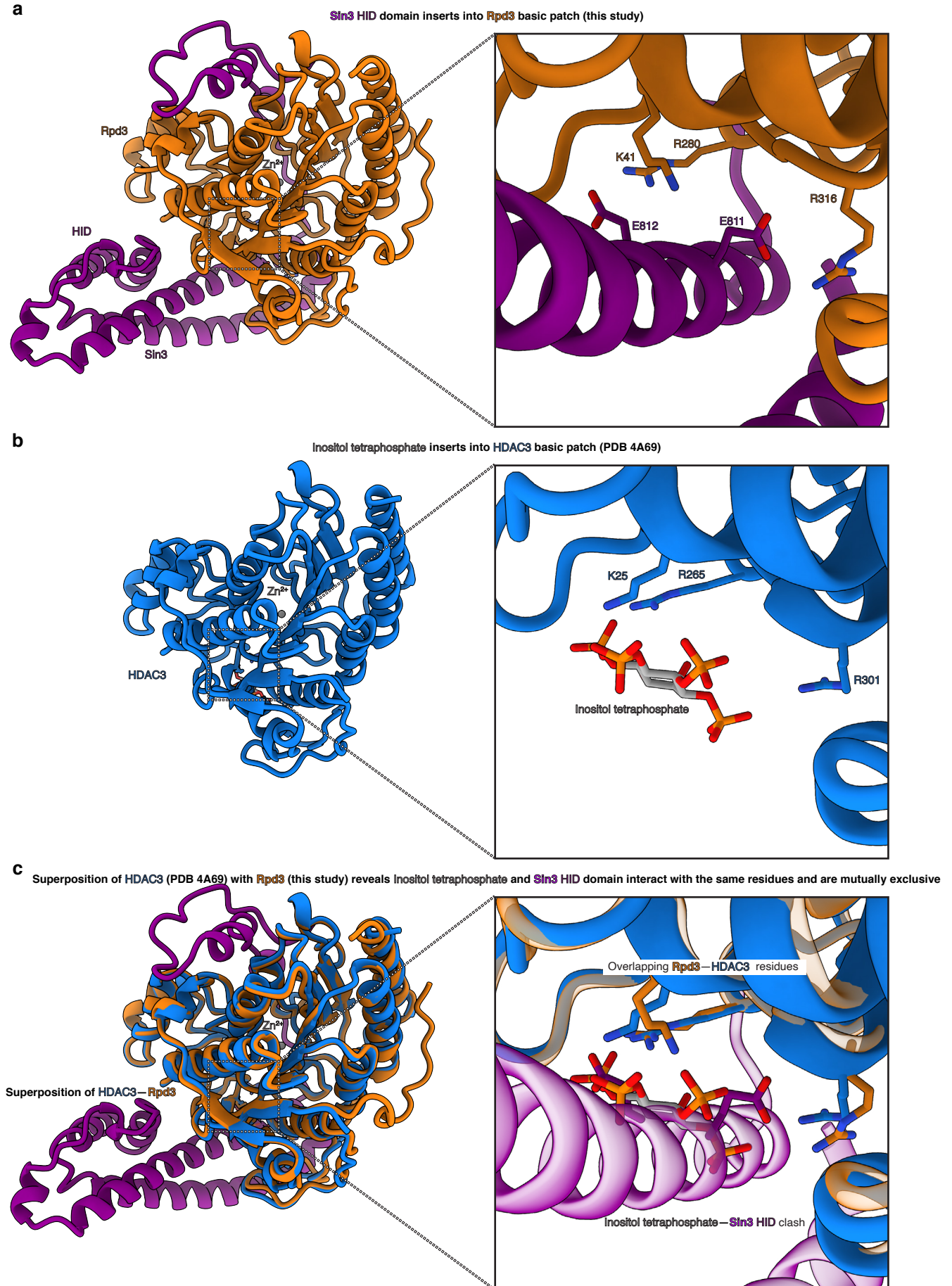
Supplementary Fig. 4 | Representative densities and density fit

a, Coulomb potential map of Rpd3S-nucleosome complex with atomic model in map G. **b**, Coulomb potential map of nucleosomal and extranucleosomal DNA with atomic model in map B. **c**, Coulomb potential map of histone octamer with atomic model in map B. **d**, Coulomb potential map of Rpd3S core module with atomic model in map C. **e**, Coulomb potential map of Eaf3A chromodomain with atomic model in map F. **f**, Coulomb potential map of Eaf3B chromodomain with atomic model in map E. **g**, Coulomb potential map of Rco1B–Eaf3B (auxiliary module) with atomic model in map D. **h**, Nucleosomal DNA dyad residues with atomic model in map B. **i**, Representative coulomb potential maps of histones H2A, H2B, H3, and H4 with atomic model in map B. **j**, Representative coulomb potential map of Sin3 (residues 888–924) with atomic model in map C. **k**, Representative coulomb potential map of Rpd3 (residues 357–381) with atomic model in map C. **l**, Representative coulomb potential map of Eaf3A (residues 270–297) with atomic model in map C. **m**, Representative coulomb potential map of Rco1A (residues 456–465) with atomic model in map C.

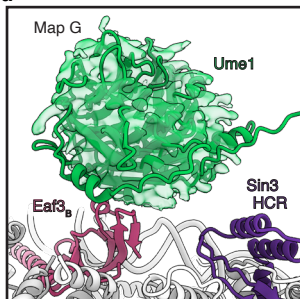
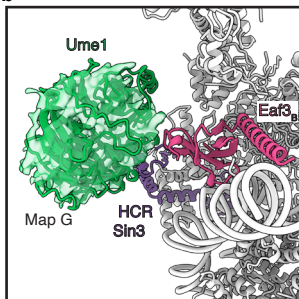


Supplementary Fig. 5 | Comparison of *S. cerevisiae* Rpd3S complex with *H. sapiens* SIN3B and *S. cerevisiae* Rpd3L complex.

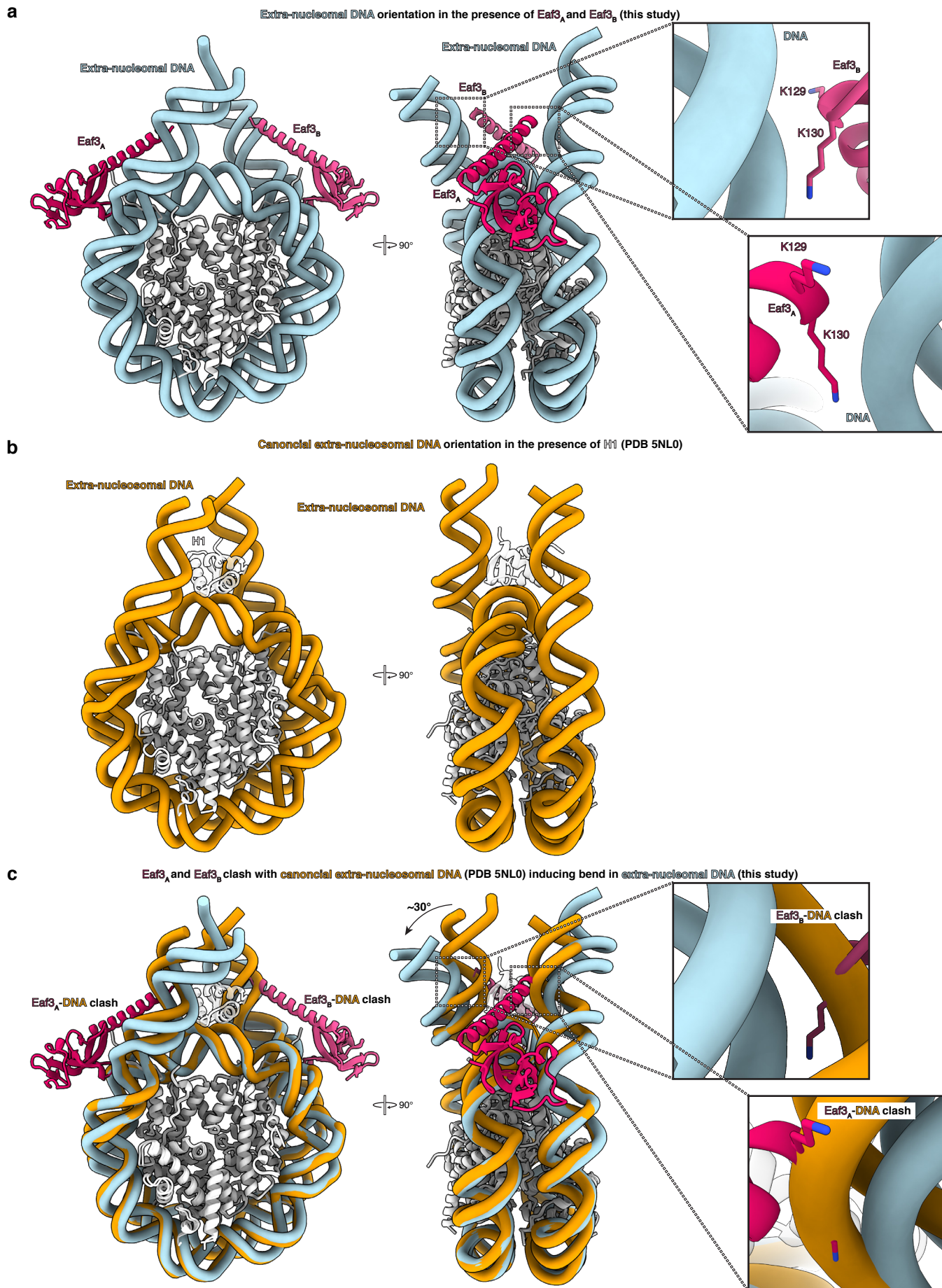
a, *S. cerevisiae* Rpd3S complex with Rpd3-Sin3 contacts. **b**, *H. sapiens* SIN3B with HDAC2-SIN3 contacts (PDB code 8BPA). **c**, *S. cerevisiae* Rpd3L complex with Rpd3-Sin3 contacts (PDB code 8GA8). **d**, **e** AlphaFold2 multimer predictions of H2A-H2B dimer with Rco1 (residues 1-79) reveal binding of potential arginine anchors into the H2A-H2B acidic patch

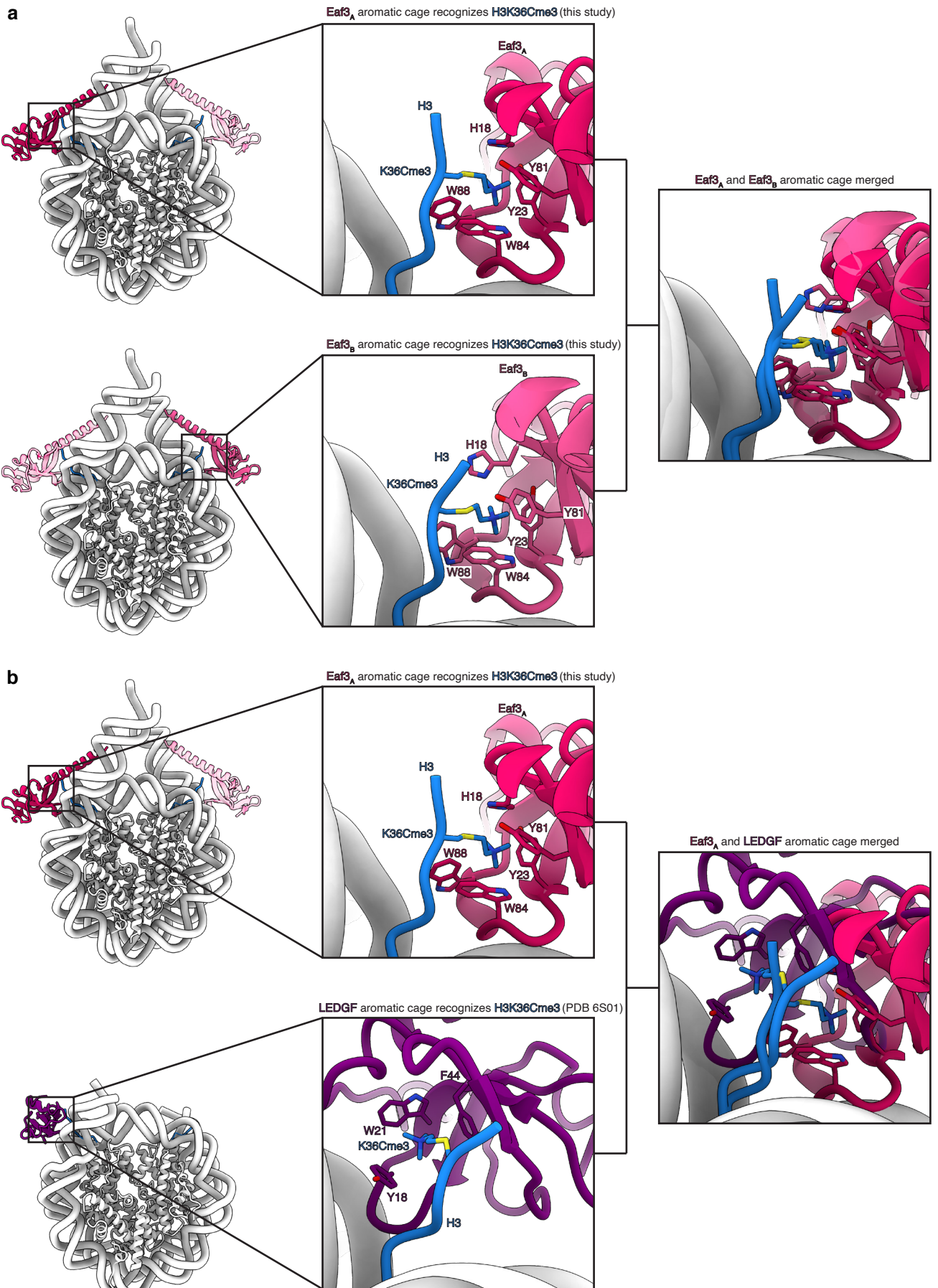


Supplementary Fig. 6 | Comparison of Rpd3S with HDAC3 unveils Sin3's role in bypassing the need for inositol phosphate in the Rpd3S complex
a, Sin3 binds against Rpd3 in the Rpd3S complex and forms an extensive interaction network. **b**, In HDAC3, inositol tetraphosphate inserts into a pocket formed by Rpd3 (PDB code 4A69) **c**, Overlay of the Rpd3S-nucleosome complex structure reveals that Sin3 occupies the inositol phosphate pocket. Sin3 and inositol phosphate binding to Rpd3 is mutually exclusive.

a**b**

Supplementary Fig. 7 | The Ume1 subunit is in close proximity to the Eaf3b CHD and the Sin3 HCR domain. a-b, Density (map G, low-pass filtered) and atomic model for Ume1 are shown in green and are located closely to the Sin3 HCR and the Eaf3B chromodomain.





Supplementary Fig. 9 | Eaf3 binds the H3K36Cme3 analogue.

a, Binding of the EAF3A/B chromodomains to the H3K36Cme3 analogue. **b**, Comparison of the different binding modes of the Eaf3 chromodomains and LEDGF PWWP domain to the H3K36Cme3 analogue. The Eaf3 chromodomains and the LEDGF PWWP domain bind both DNA gyres.

Supplementary Table 1. Cryo-EM data collection statistics

Data collection and Processing				
Microscope	Thermo Fisher			
	Titan Krios			
Voltage (keV)	300			
Camera	Gatan K3			
Magnification	105,000			
Pixel size at detector (Å per pixel)	0.822			
Total electron exposure (e ⁻ Å ⁻²)	51.10			
Exposure rate (e ⁻ Å ⁻² s ⁻¹)	17.983			
Number of frames collected during exposure	50			
Defocus range (µm)	0.5-2.25			
Automation software	EPU			
Energy filter slit width (eV)	20			
Micrographs collected (no.)	32,564			
Micrographs used (no.)	30,088			
Total extracted particles (no.)	8,797,734			
	Rpd3S- nucleosome (Map A) EMD-41449	Nucleosome (Map B) EMD-41449	Rpd3S (Map C) EMD-41449	Rco1 _B -Eaf3 _B - Rpd3S (Map D) EMD-41449
Final particles (no.)	481,141	481,141	481,141	54,072
Resolution (global, Å)				
FSC 0.5 masked	3.0	3.0	3.4	3.8
FSC 0.143 masked	2.8	2.7	2.9	3.3
Map sharpening <i>B</i> factor (Å ²)	82.6	74.5	84.4	62.8
3DFSC sphericity	0.967	0.970	0.875	0.866
Map sharpening methods	cryoSPARC	cryoSPARC	cryoSPARC	cryoSPARC
	Eaf3 _B - nucleosome (Map E) EMD-41449	Eaf3 _A - nucleosome (Map F) EMD-41449	Ume1 (Map G) EMD-41449	Composite map (Map H) EMD-41449
Final particles (no.)	87,666	129,843	43,901	—
Resolution (global, Å)				
FSC 0.5 masked	3.3	3.2	6.9	—
FSC 0.143 masked	3.0	2.9	4.0	—
Map sharpening <i>B</i> factor (Å ²)	56.4	62.7	58.2	25
3DFSC sphericity	0.906	0.955	0.619	0.909
Map sharpening methods	cryoSPARC	cryoSPARC	cryoSPARC	cryoSPARC

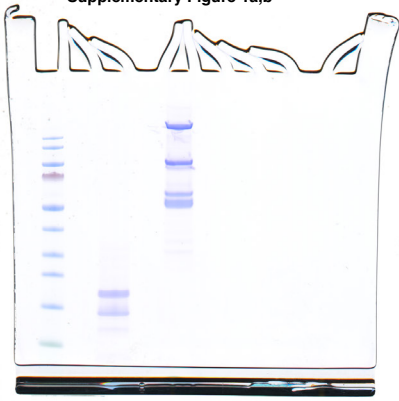
Supplementary Table 2. Model composition, refinement, and validation.

	RPD3S-nucleosome
	PDB-8TOF
	EMD-41449
Model composition	
Non-hydrogen atoms (no.)	29,102
Protein residues (no.)	2,697
Ligands (no.)	7
Nucleotide residues (no.)	352
Model Refinement	
Initial models used (PDB #)	3LZ0, 8I02
Refinement packages	Coot, PHENIX
Model-Map scores	
Cross-correlation coefficient	0.79
Model resolution (Å)	2.9
FSC threshold	0.143
Mean <i>B</i> factors (Å ²)	Composite map values
Protein residues (no.)	113.48
Ligands (no.)	180.45
Nucleotide residues (no.)	81.35
R.m.s. deviations from ideal values	
Bond lengths (Å)	0.010
Bond angles (°)	1.176
Validation	
MolProbity score	2.40
CaBLAM outliers (%)	4.14
Clashscore	11.44
Poor rotamers (%)	3.09
C-beta deviations (%)	0.08
EMRinger score	2.65
Ramachandran plot	
Favored (%)	92.95
Allowed (%)	6.59
Outliers (%)	0.45

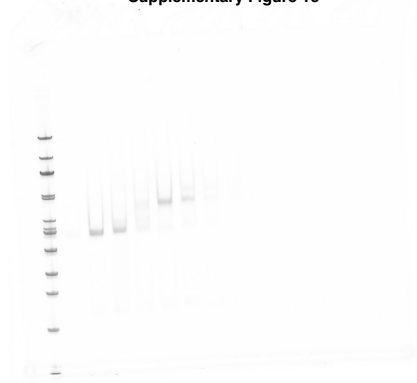
Supplementary Table 3 | Input structural models and model confidence

Complex/domain	Chain id(s)	Input model	Level of confidence	Complex (PDB)
Sin3	A	<i>De novo</i> , Alphafold, 8I02	Atomic	8TOF
Rpd3	B	<i>De novo</i> , Alphafold, 8I02	Atomic	8TOF
Ume1	C	Alphafold, 8I02	Rigid body dock	—
Eaf3A	D	<i>De novo</i> , Alphafold, 8I02	Atomic	8TOF
Eaf3B	E	<i>De novo</i> , Alphafold, 8I02	Secondary	8TOF
Rco1A	G	<i>De novo</i> , Alphafold, 8I02	Atomic	8TOF
Rco1B	F	<i>De novo</i> , Alphafold, 8I02	Secondary	8TOF
Rco1 Acidic patch binding	H	<i>De novo</i>	Atomic/clipped	8TOF
H3	a,e	3LZ0	Atomic	8TOF
H4	b,f	3LZ0	Atomic	8TOF
H2A	c,g	3LZ0	Atomic	8TOF
H2B	d,h	3LZ0	Atomic	8TOF
DNA	N	<i>De novo</i> , 3LZ0	Atomic	8TOF
DNA	T	<i>De novo</i> , 3LZ0	Atomic	8TOF
Active site peptide	x	<i>De novo</i>	Atomic/clipped	8TOF

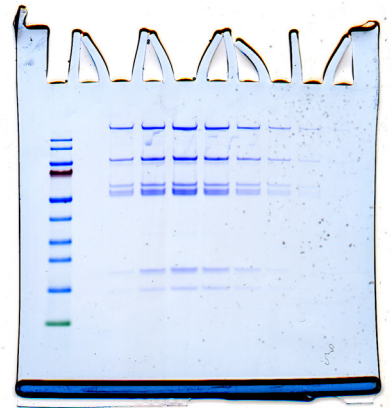
Supplementary Figure 1a,b



Supplementary Figure 1c



Supplementary Figure 1f



Source Data Fig. 1 | SDS-PAGE and TBE gels of the Rpd3S complex, histones, and assembled nucleosome