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Supplemental information

**Structure of a backtracked hexasomal
intermediate of nucleosome transcription**

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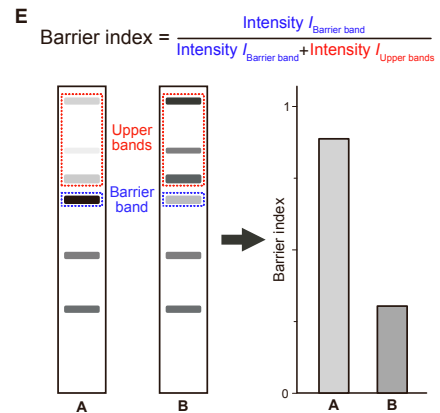
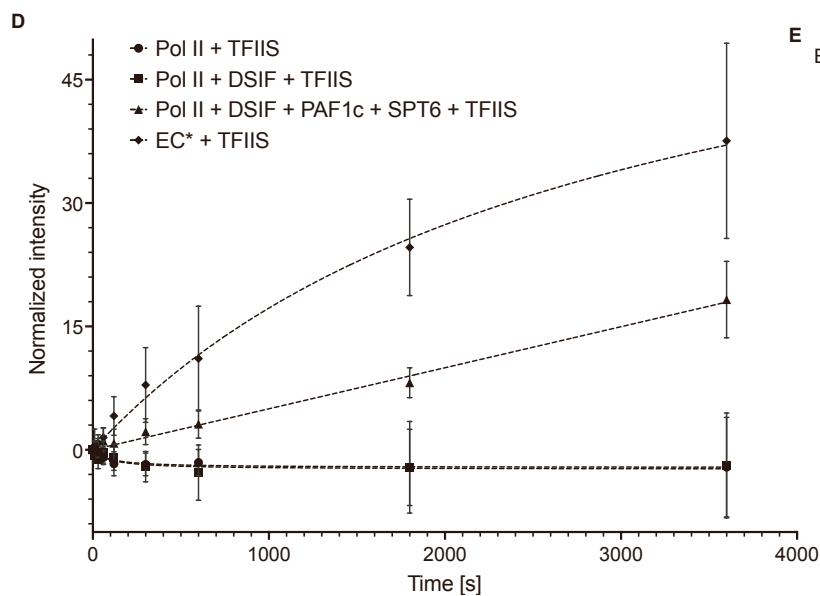
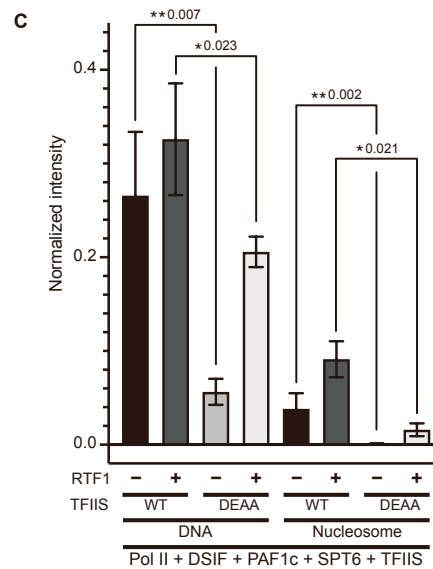
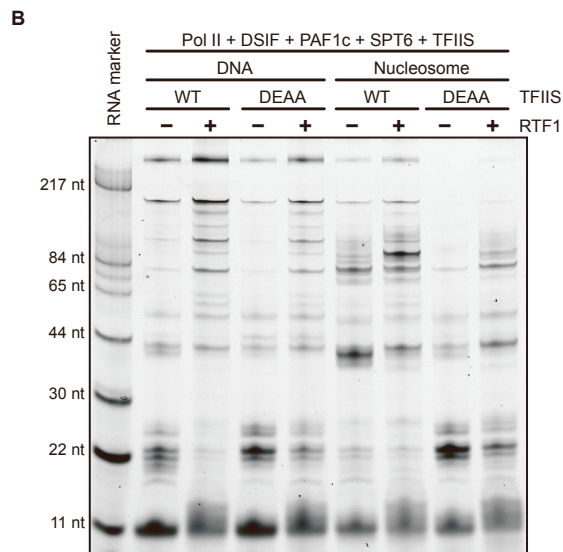
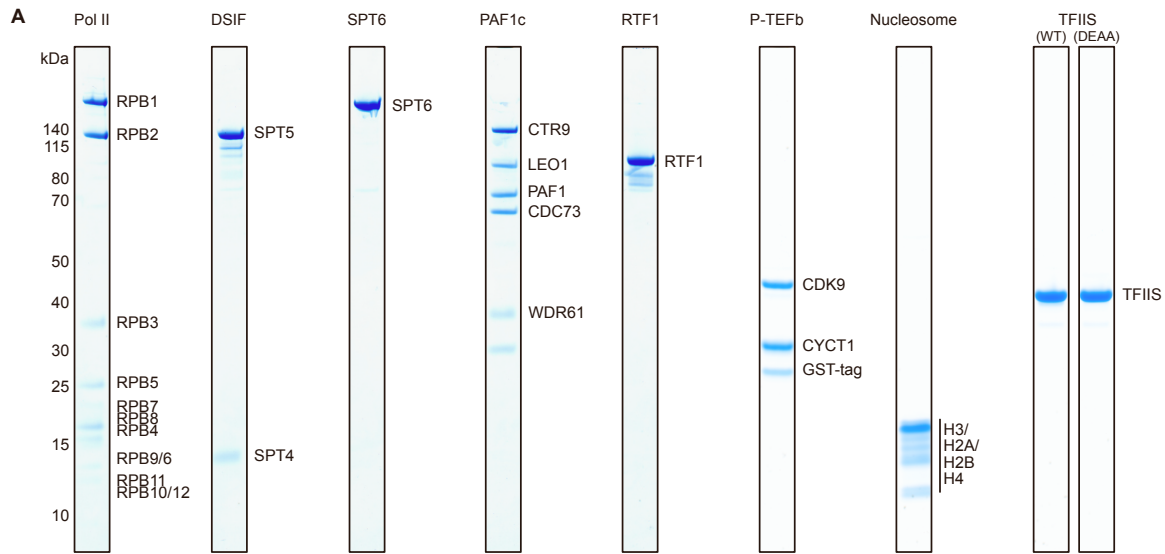


Figure S1. Protein components and transcription assays. Related to Figure 1 and 4.

(A) SDS-PAGE of all purified protein components (0.75 µg proteins per lane).

(B) Transcription assay of the EC* with and without RTF1 on either linear or a nucleosomal template DNA in the presence of wild-type TFIIS or a catalytically dead TFIIS variant (DEAA).

(C) Quantification of the full-length RNA product of the transcription assay depicted in (B) (n = 4). The full-length RNA signal was determined by measuring the integrated signal density, subtracting the background and normalizing the value through the signal of the lane and the gel (Methods). Mean ± SD values are shown. P-values were determined using unpaired two-tailed t tests (*P < 0.05, **P < 0.01).

(D) Quantification of the full-length RNA product from transcription assay time courses of Pol II elongation complexes on a nucleosomal template in the presence of different combinations of elongation factors DSIF, TFIIS, SPT6, PAF1, RTF1 (n = 3). Mean ± SD values are shown. P-values were determined using unpaired two-tailed t tests (*P < 0.05, **P < 0.01).

(E) Schematic of the principle and calculation of the barrier index.

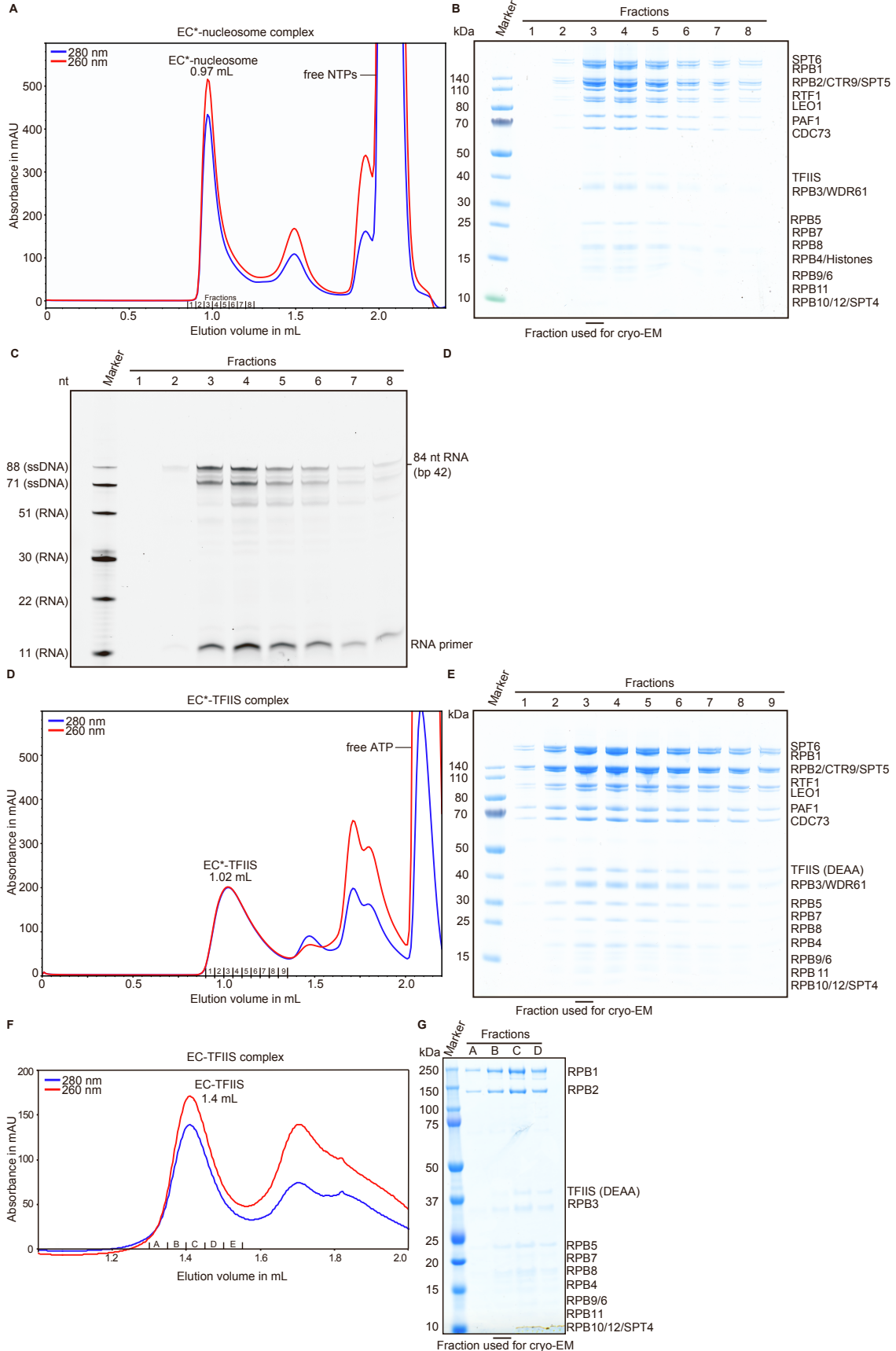


Figure S2. Formation of complexes. Related to Figure 2, 5 and 6.

(A) Chromatogram of EC*-nucleosome complex formation (Superose 6 Increase 3.2/300).

(B) SDS-PAGE of EC*-nucleosome complex formation.

(C) Denaturing gel of EC*-nucleosome complex formation.

(D) Chromatogram of EC*-TFIIS complex formation (Superose 6 Increase 3.2/300).

(E) SDS-PAGE of EC*-TFIIS complex formation.

(F) Chromatogram of Pol II-TFIIS elongation complex formation (Superose 6 Increase 3.2/300).

(G) SDS-PAGE of Pol II-TFIIS elongation complex formation.

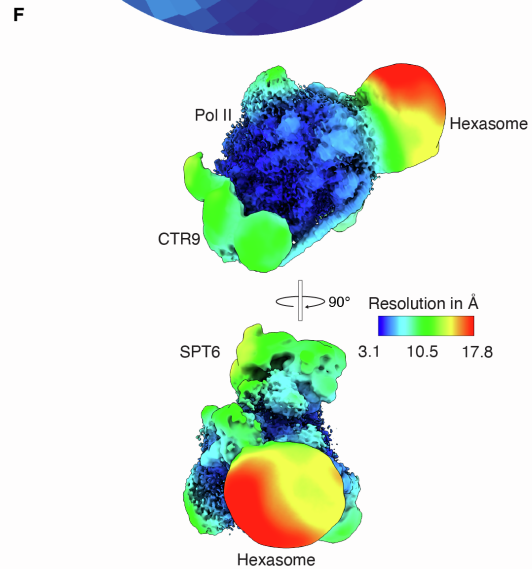
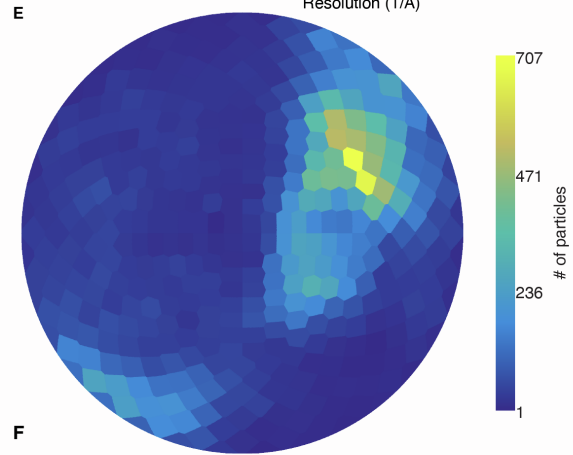
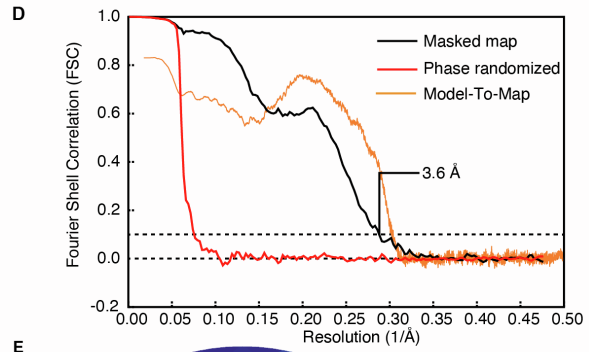
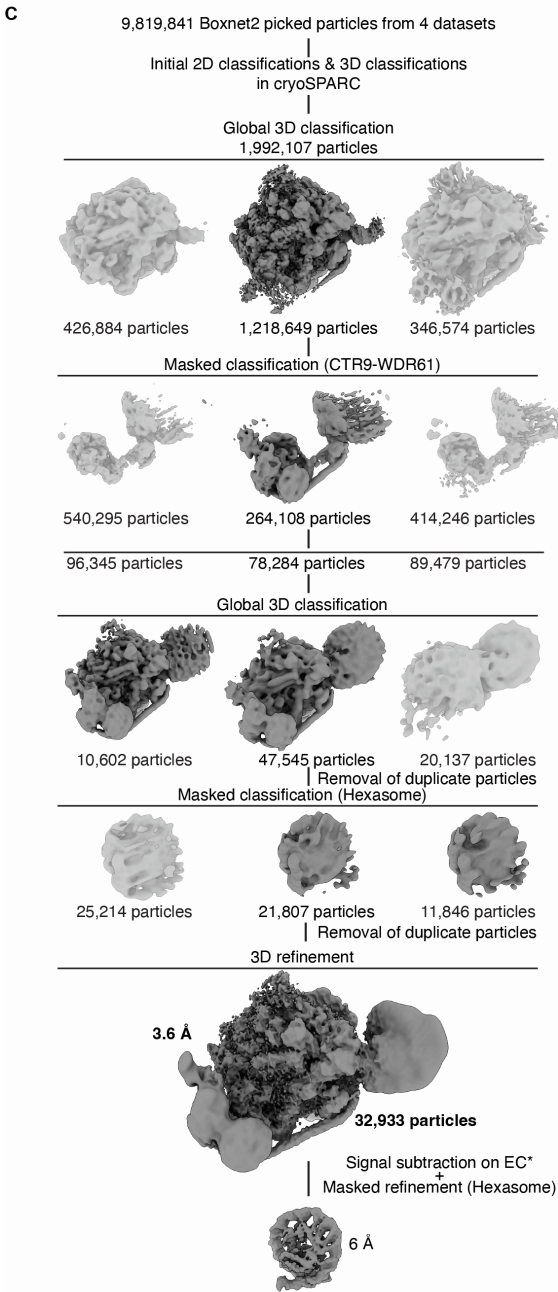
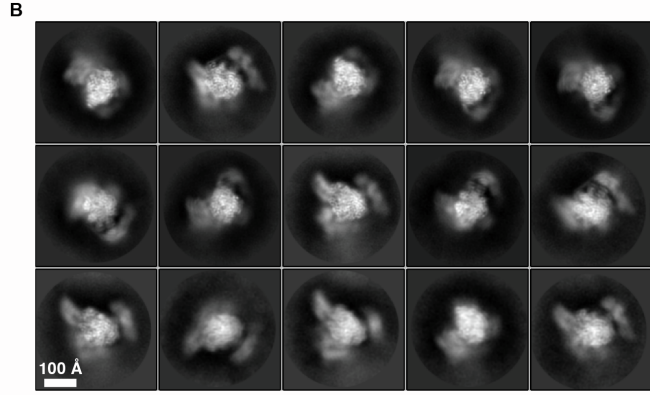
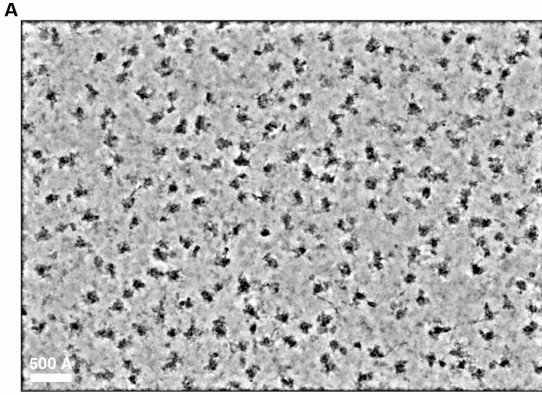


Figure S3. Data acquisition, processing, and data quality metrics for the EC*-hexasome complex. Related to Figure 2.

- (A)** Representative denoised micrograph of data collection with scale bar (50 nm).
- (B)** 2D classes of final refinement show RNA polymerase II, transcription elongation factors, and nucleosome-like shape with scale bar of 10 nm.
- (C)** Sorting and classification tree of EC*-hexasome complex dataset.
- (D)** FSC curve of overall map. Resolution at FSC threshold criterion 0.143 is indicated.
- (E)** Angular distribution of particles employed to reconstruct EC*-hexasome complex.
- (F)** Local resolution of EC*-hexasome complex.

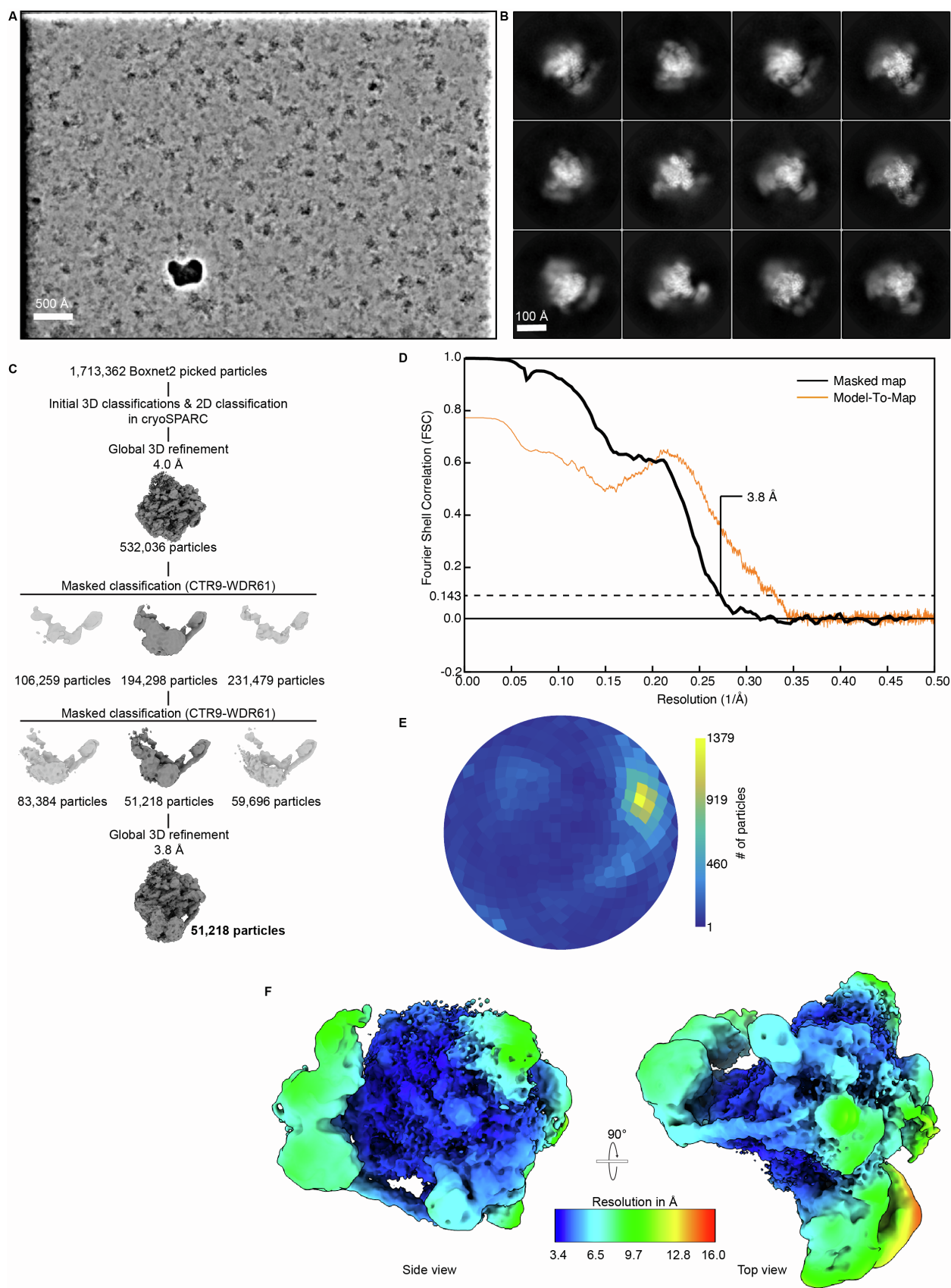


Figure S4. Data acquisition, processing, and data quality metrics for the EC*-TFIIS complex. Related to Figure 5.

- (A)** Representative denoised micrograph of data collection with scale bar (50 nm).
- (B)** 2D classes of final refinement show RNA polymerase II, transcription elongation factors, and TFIIS with scale bar of 10 nm.
- (C)** Sorting and classification tree of EC*-TFIIS complex dataset.
- (D)** FSC curves of EC*-TFIIS complex. Resolution at FSC threshold criterion 0.143 is indicated.
- (E)** Angular distribution of particles employed to reconstruct EC*-TFIIS complex.
- (F)** Local resolution of map EC*-TFIIS complex.

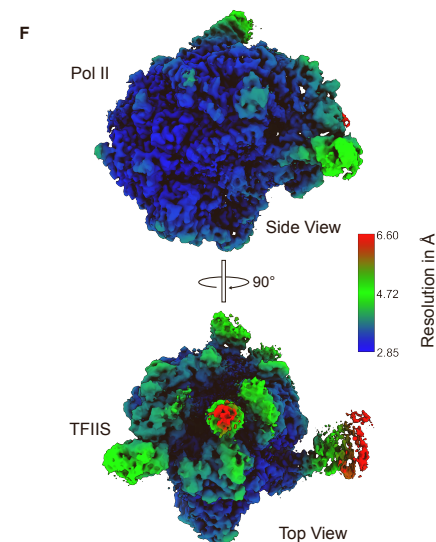
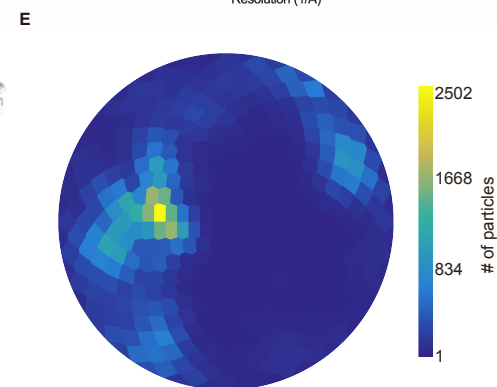
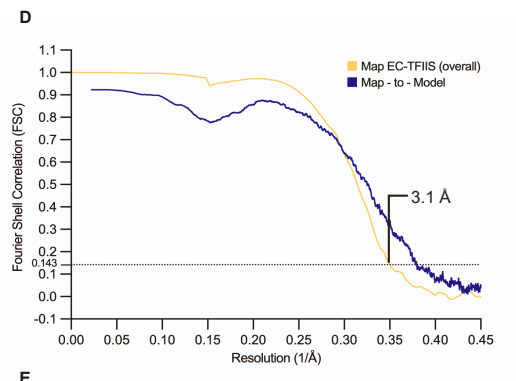
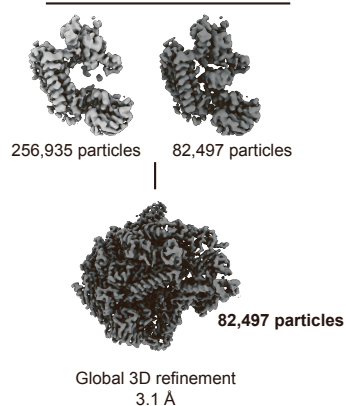
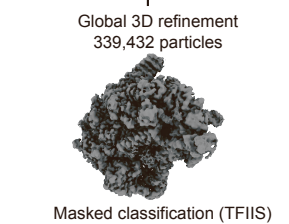
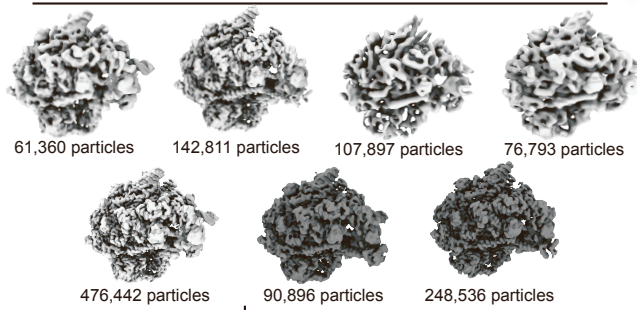
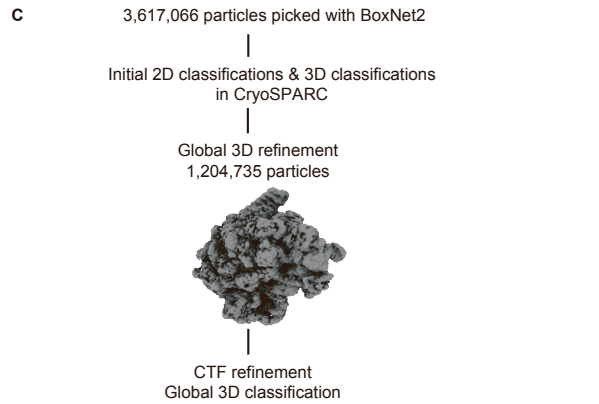
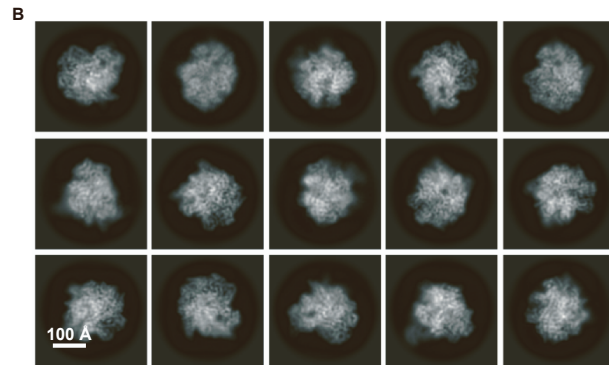
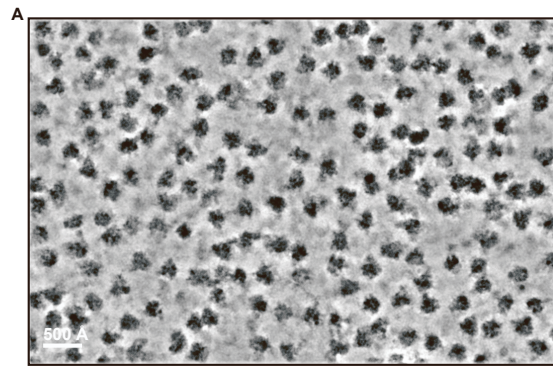


Figure S5. Data acquisition, processing, and data quality metrics for the Pol II-TFIIS EC complex. Related to Figure 6.

(A) Representative denoised micrograph of data collection with scale bar (50 nm).

(B) 2D classes of final refinement show RNA polymerase II and TFIIS with scale bar of 10 nm.

(C) Sorting and classification tree of Pol II-TFIIS EC complex dataset.

(D) FSC curve of Pol II-TFIIS elongation complex and map-to-model. Resolution at FSC threshold criterion 0.143 is indicated.

(E) Angular distribution of particles employed to reconstruct Pol II-TFIIS elongation complex.

(F) Local resolution of Pol II-TFIIS elongation complex.

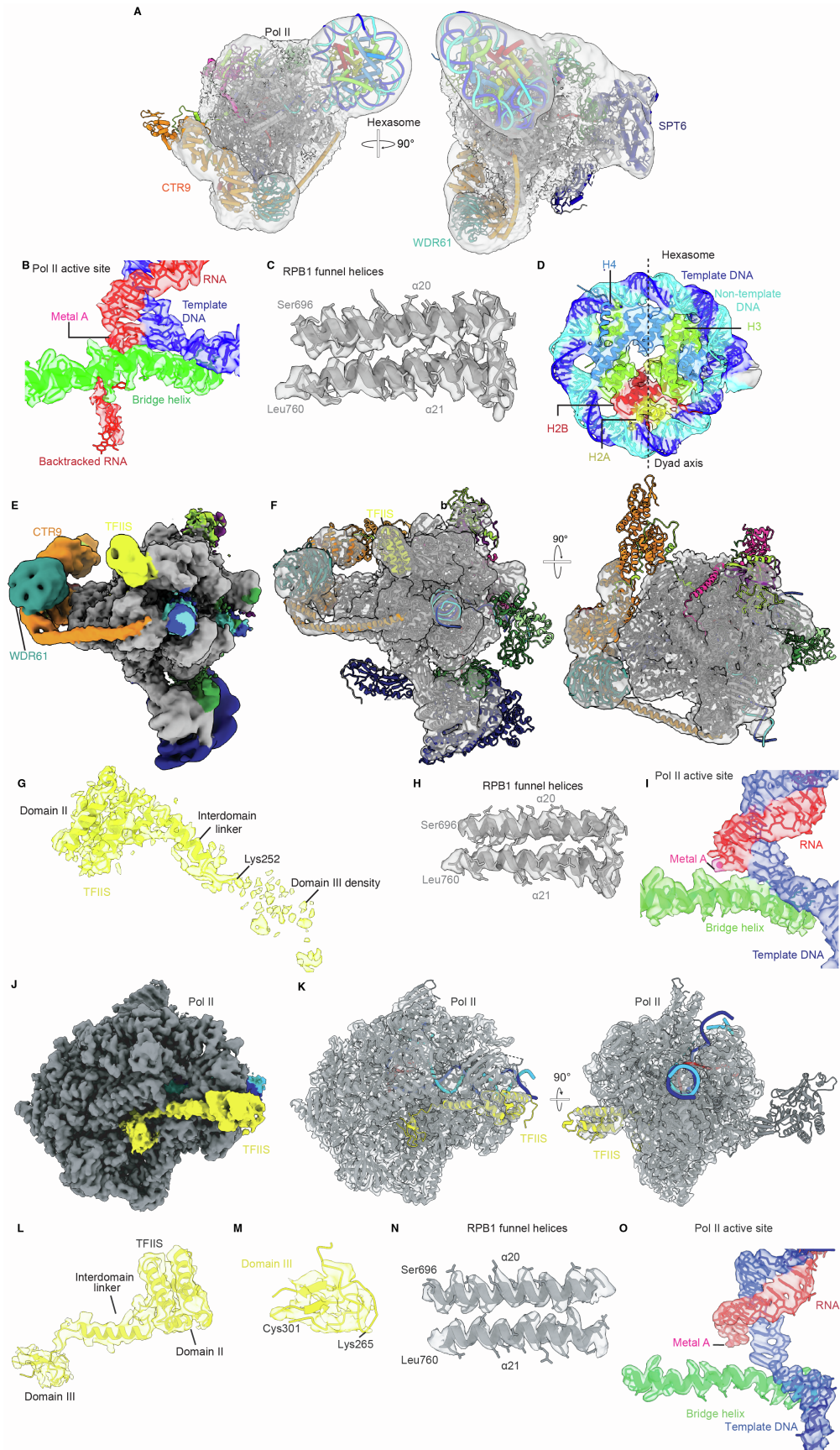


Figure S6. Cryo-EM densities of complexes. Related to Figure 2, 5, and 6.

- (A)** EC*-hexasome structure with corresponding cryo-EM map. Cryo-EM map is shown in grey.
- (B)** Pol II active site of EC*-hexasome structure with corresponding density. Metal A is shown as a pink sphere.
- (C)** RPB1 funnel helices with corresponding density from EC*-hexasome complex.
- (D)** Hexasome with corresponding density (map from masked refinement) from EC*-hexasome complex.
- (E)** Cryo-EM map of EC*-TFIIS complex.
- (F)** EC*-TFIIS structure with corresponding cryo-EM map. Cryo-EM map is shown in grey.
- (G)** TFIIS with corresponding density from EC*-TFIIS complex. Domain III is indicated.
- (H)** RPB1 funnel helices with corresponding density from EC*-TFIIS complex.
- (I)** Pol II active site of EC*-TFIIS structure with corresponding density. Metal A is shown as a pink sphere.
- (J)** Cryo-EM map of Pol II-TFIIS EC complex.
- (K)** Pol II-TFIIS elongation complex structure with corresponding cryo-EM map. Cryo-EM map is shown in grey.
- (L)** TFIIS with corresponding density from Pol II-TFIIS elongation complex.
- (M)** TFIIS domain density from Pol II-TFIIS elongation complex.
- (N)** RPB1 funnel helices with corresponding density from Pol II-TFIIS elongation complex.
- (O)** Active site of Pol II-TFIIS EC structure with corresponding density. Metal A is shown as a pink sphere.

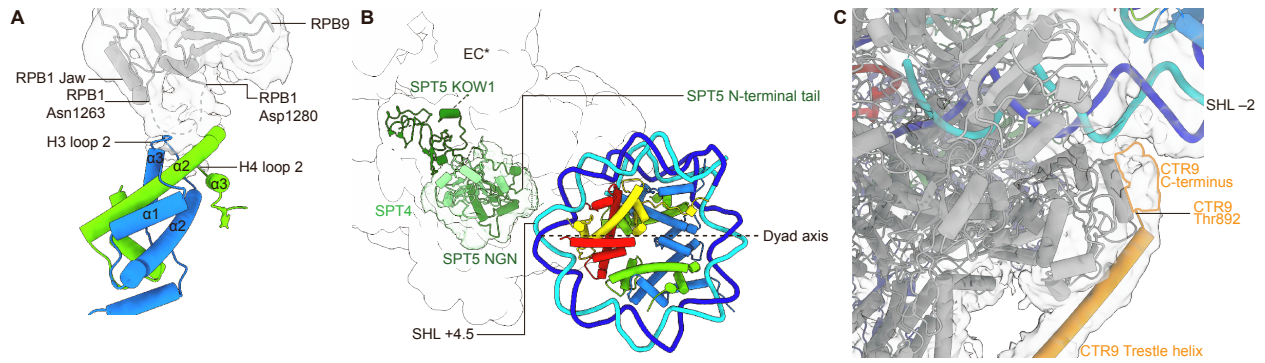


Figure S7. Interactions of Pol II and transcription elongation factors with the hexasome. Related to Figure 2.

(A) Interaction of the RPB1 jaw domain with H3-H4. Density in grey.

(B) Interaction of DSIF with nucleosomal DNA at SHL +4.5. Density in green. EC* indicated as outline. SPT5 N-terminal tail indicated as dashed line.

(C) Interaction of the CTR9 C-terminus with nucleosomal DNA at SHL -2. Density shown in grey and putative CTR9 density highlighted in orange.

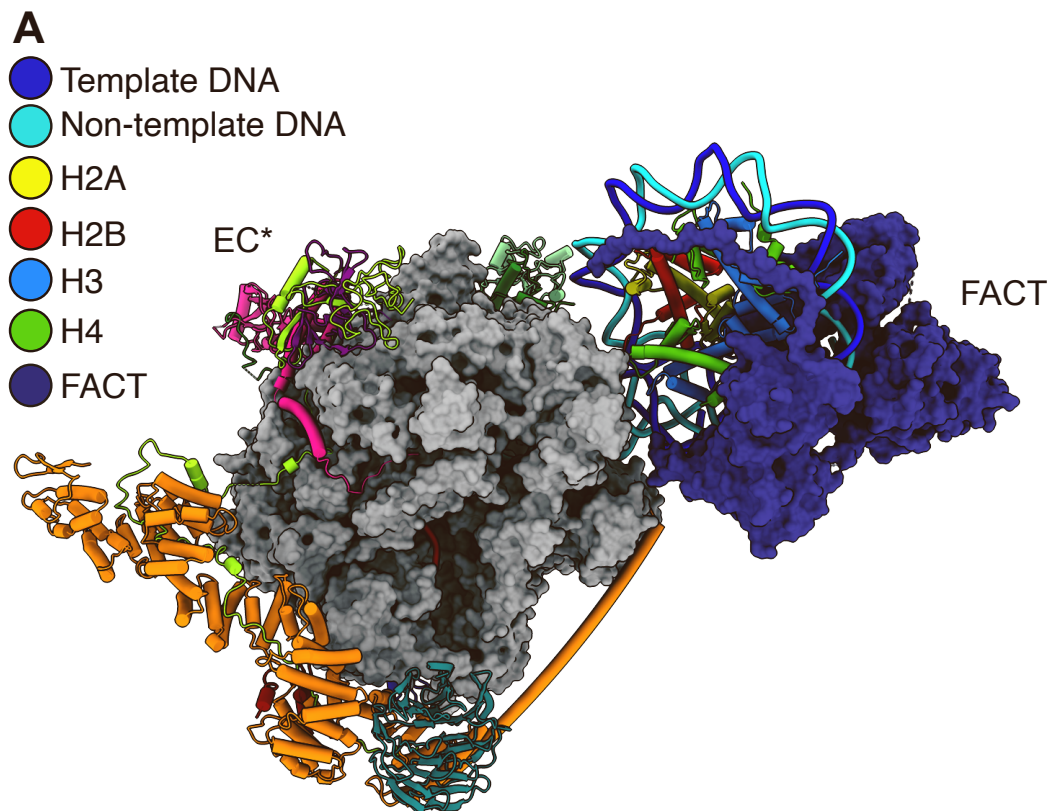


Figure S8. Binding of FACT is compatible with the observed nucleosome position in front of the transcribing EC* complex. Related to Figure 2.

The nucleosome–FACT structure (PDB ID 6UPK) was superimposed onto the EC*-hexasome structure. No clashes are observed between FACT and the EC*. It is possible that FACT can redeposit a H2A–H2B dimer in the observed conformation. FACT with subunits SPT16 and SSRP1 is shown in purple.

Table S1. Cryo-EM data collection, refinement, and validation statistics. Related to Figures 2, 5, and 6

	EC*-hexasome (EMD-15127) (PDB 8A3Y)	EC*-TFIIS (EMDB-15128) (PDB 8A3Z)	EC-TFIIS (EMDB-15129) (PDB 8A40)
Data collection and processing			
Magnification	81,000	81,000	105,000
Voltage (kV)	300	300	300
Electron exposure (e ⁻ /Å ²)	43-45	43-45	39.87
Defocus range (μm)	0.25-4	0.25-4	0.25-4
Pixel size (Å)	1.05	1.05	0.834
Symmetry imposed	C1	C1	C1
Initial particle images (no.)	9,819,841	1,713,362	3,617,066
Final particle images (no.)	32,933	51,218	82,497
Map resolution (Å)	3.6	3.8	3.1
FSC threshold	0.143	0.143	
Map resolution range (Å)	3.1-17.8	3.4-16	2.8-4.7
Refinement			
Initial models used (PDB code)	6TED, 3LZ0	6TED, 5IYB	5IYB, 5OIK, 6GMH
Map sharpening <i>B</i> factor (Å ²)	-36	-86	-10
Model composition			
Non-hydrogen atoms	65,421	61,283	34,003
Protein residues	7894	7437	4054
Nucleotides	293	106	81
Ligands	10	10	7
<i>B</i> factors (Å ²)			
Protein	201	49.80	47.41
Nucleotide	486	111.84	70.06
Ligand	170	68.20	67.48
R.m.s. deviations			
Bond lengths (Å)	0.005	0.003	0.004
Bond angles (°)	0.867	0.552	0.698
Validation			
MolProbity score	1.91	2.05	1.45
Clashscore	7.91	10.34	4.43
Poor rotamers (%)	0.76	1.67	0.00
Ramachandran plot			
Favored (%)	92.16	95.02	96.53
Allowed (%)	7.28	4.94	3.44
Disallowed (%)	0.55	0.04	0.02