Expanded View Figures

Figure EV1. Biochemical characterization and data processing of the H2A.B-NCP.

A Native-PAGE analysis of the purified human H2A-NCP and H2A.B-NCP.
B SDS–PAGE analysis of the purified human H2A.B-NCP. The linked H2B-H2A.B dimer (lnkH2B-H2A.B) was used for nucleosome reconstitution.
C, D SDS–PAGE (C) and Native-PAGE (D) analyses of pre-crosslinked H2A.B-NCP subject to GraFix treatment. Peak fractions (18–19) containing intact H2A.B-NCP were processed for further electron microscopy analysis. Experiments were repeated multiple times ($n > 5$).
E Representative cryo-EM image (left) and 2D class averages (right) of H2A.B-NCP particles.
F Flowchart of data processing of the H2A.B-NCP.
G FSC curve of the final density maps of the H2A.B-NCP. Reported resolutions were based on the FSC = 0.143 criteria.
H The final 3D density map of H2A.B-NCP shown in the indicated views is colored according to the local resolution estimated by the software ResMap.
I Examples of cryo-EM density maps of the histones and DNA generated in Pymol.

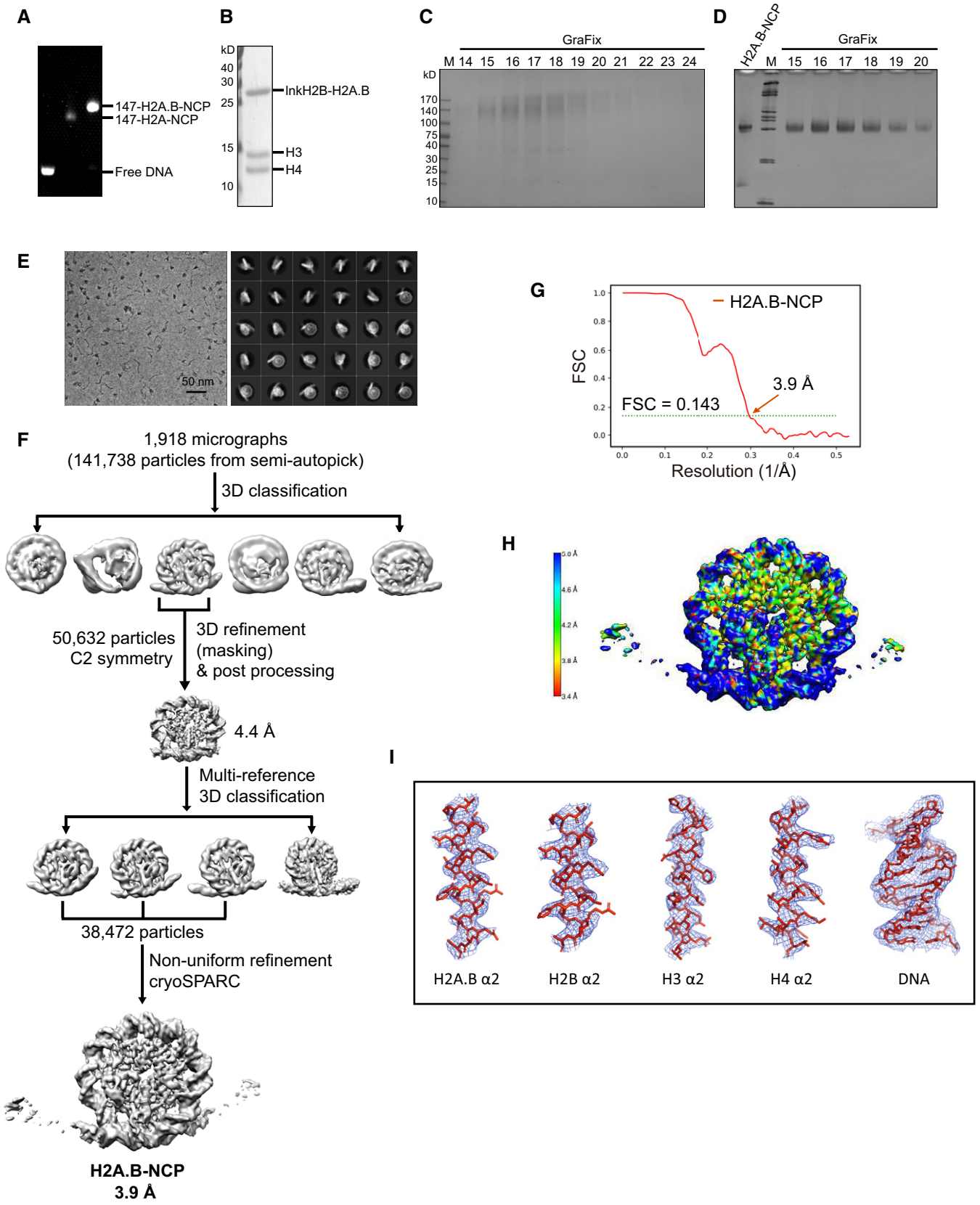

Figure EV1.

Figure EV2. Biochemical characterization and data processing of the PARP1-DBD bound H2A.B-NCP (Pb-H2A.B-NCP).

A SDS–PAGE analysis of the purified human PARP1 DNA binding domain (hPARP1-DBD).
B EMSA analyses of H2A.B-NCP interacting with linker histone H5 and PARP1 DNA binding domain. Chicken H5 residues 22–102 (GH5-GD) and human PARP1 DNA binding domain (hPARP1-DBD) were added in two- or fourfold molar excess.
C, D SDS–PAGE (C) and Native-PAGE (D) analyses of Pb-H2A.B-NCP subject to GraFix treatment. H2A.B-NCP bound to PARP1 DNA binding domain (Pb-H2A.B-NCP) were subjected to 10–30% glycerol gradient centrifugation with 0–0.15% glutaraldehyde. Peak fractions (19–20) containing intact Pb-H2A.B-NCP were processed for further electron microscopy analysis. Experiments were repeated multiple times ($n > 10$).
E Representative cryo-EM image (left) and 2D class averages (right) of Pb-H2A.B-NCP particles.
F Flow chart of data processing of the Pb-H2A.B-NCP particles. Highlighted with green color refers to the extra density corresponding to hPARP1-DBD.
G The final 3D density map of Pb-H2A.B-NCP shown in the indicated views is colored according to the local resolution estimated by the software ResMap.
H FSC curves of the final density maps of the Pb-H2A.B-NCP C1 (blue) and the Pb-H2A.B-NCP C2 (yellow). Reported resolutions were based on the FSC = 0.143 criteria.
I Examples of cryo-EM density maps of the histones and DNA in Pb-H2A.B-NCP generated in Pymol.

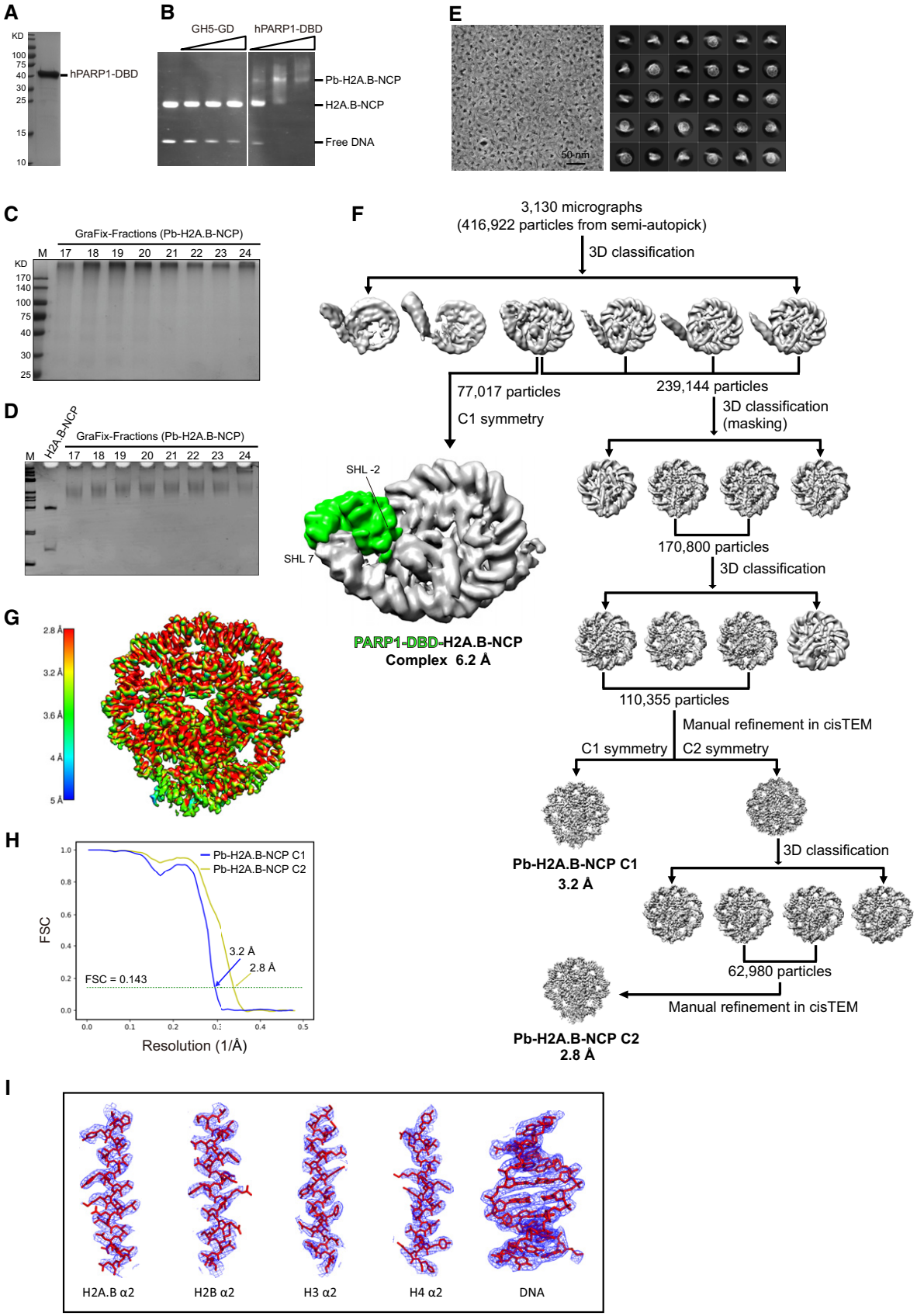

Figure EV2.

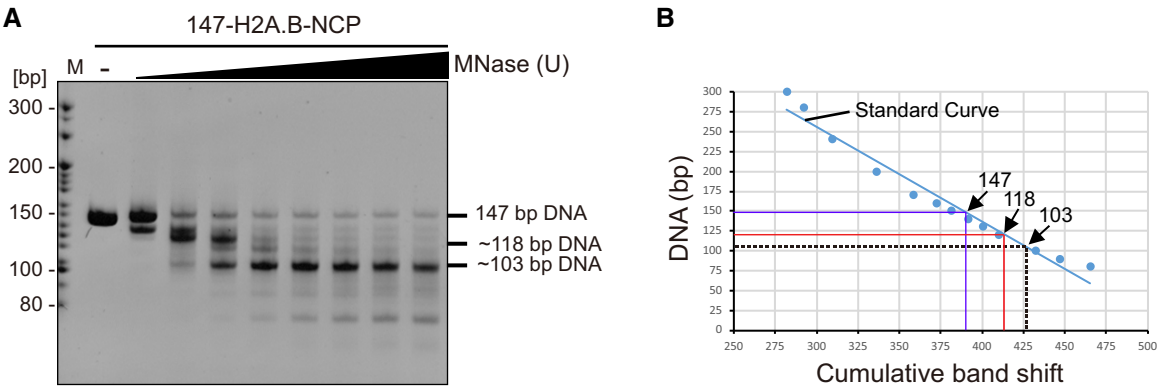

Figure EV3. Characterization of DNA in H2A.B-NCP.

A, B Protection of H2A.B-NCP DNA against MNase digestion. The H2A.B nucleosomes containing a 147-bp Widom 601 sequence DNA were treated with increasing amounts of MNase at 30°C. After 30 min incubation, the reactions were quenched by adding EGTA and proteinase K. The protected DNA was extracted and analyzed by 10% Native-PAGE with ethidium bromide staining (A). Length of DNA protected against MNase digestion. A standard curve is prepared by plotting the length of each DNA standard versus the mobility measured from the EMSA. The length of the 147-bp DNA, 118-bp DNA, and 103-bp DNA are then calculated from the standard curve (B).

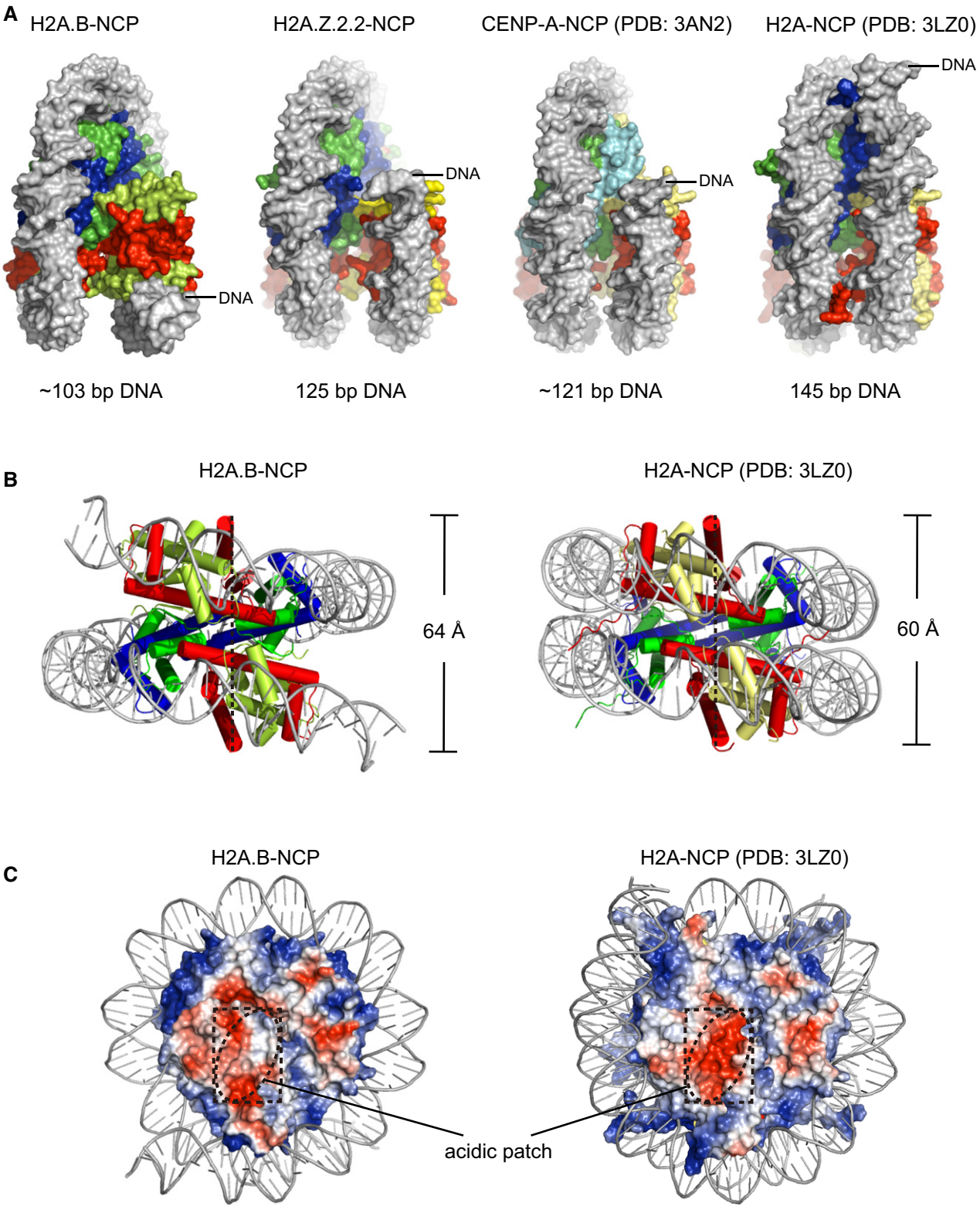

Figure EV4.

Figure EV4. Structural comparison of nucleosomes containing H2A.B, H2A.Z.2.2, and other histone variants.

A Structural comparison of nucleosome containing H2A.B, H2A.Z.2.2, CENP-A (PDB: 3AN2), and H2A (PDB: 3LZ0) in gyre view. The DNA visible in these structures are colored in gray for comparison. The histones H2A or variants, H2B, H3, or CENP-A are colored in yellow, red, blue, and green, respectively. The nucleosomal DNA is colored in gray.

B Structural comparison of nucleosome containing H2A.B (left) and H2A (right, PDB: 3LZ0) in bottom view. The dashed lines indicate the distance between the two H2B S113.

C Structural comparison of nucleosome containing H2A.B (left) and H2A (right, PDB: 3LZ0) in disc view. Structures of core histones are shown in the surface mode and colored according to the electrostatic potential. The acidic patches of H2A.B-NCP and canonical nucleosome are highlighted by dashed line for comparison.

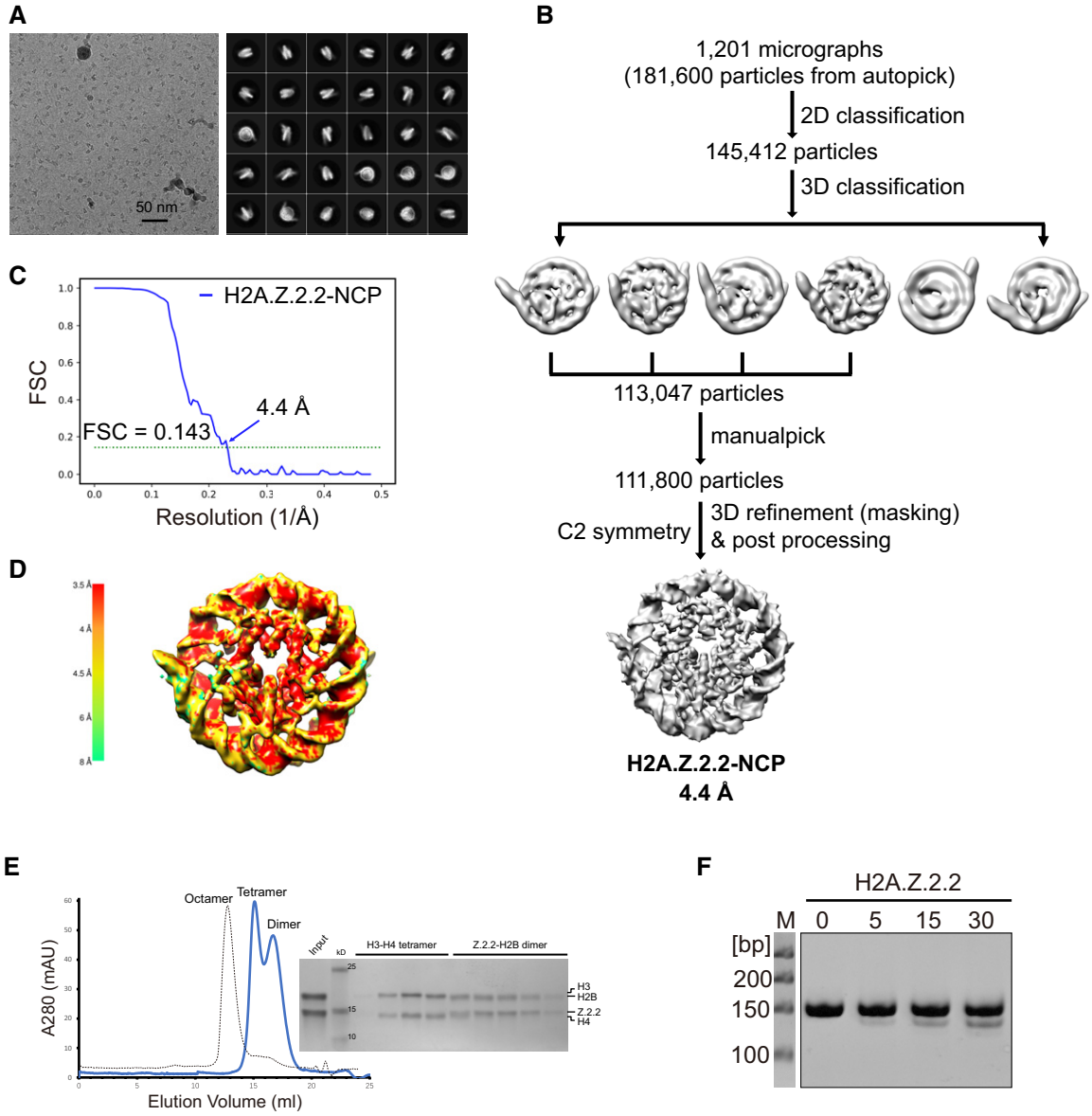

Figure EV5. Data processing of the H2A.Z.2.2-NCP.

A Representative cryo-EM image (left) and 2D class averages (right) of H2A.Z.2.2-NCP particles.

B Flow chart of data processing of the Z.2.2-NCP particles.

C FSC curves of the final density map of the Z.2.2-NCP. Reported resolutions were based on the FSC = 0.143 criteria.

D The final 3D density map of Z.2.2-NCP shown in the indicated views is colored according to the local resolution estimated by the software ResMap.

E Z.2.2 does not constitute stable histone octamers with H2B, H3, and H4 *in vitro*. Left: Size-exclusion chromatography of refolding reactions using recombinant human H3, H4, and H2B proteins together with either H2A (dashed line) or Z.2.2 (blue line). Peaks corresponding to histone octamers, tetramers, or dimers are labeled, respectively. Right: Fractions corresponding to blue line were analyzed by 4–16% gradient SDS–PAGE and stained with Coomassie brilliant blue.

F Effects of the Z.2.2 on nucleosome resistance to MNase digestion. MNase digested nucleosomal DNA is analyzed by 10% Native-PAGE.