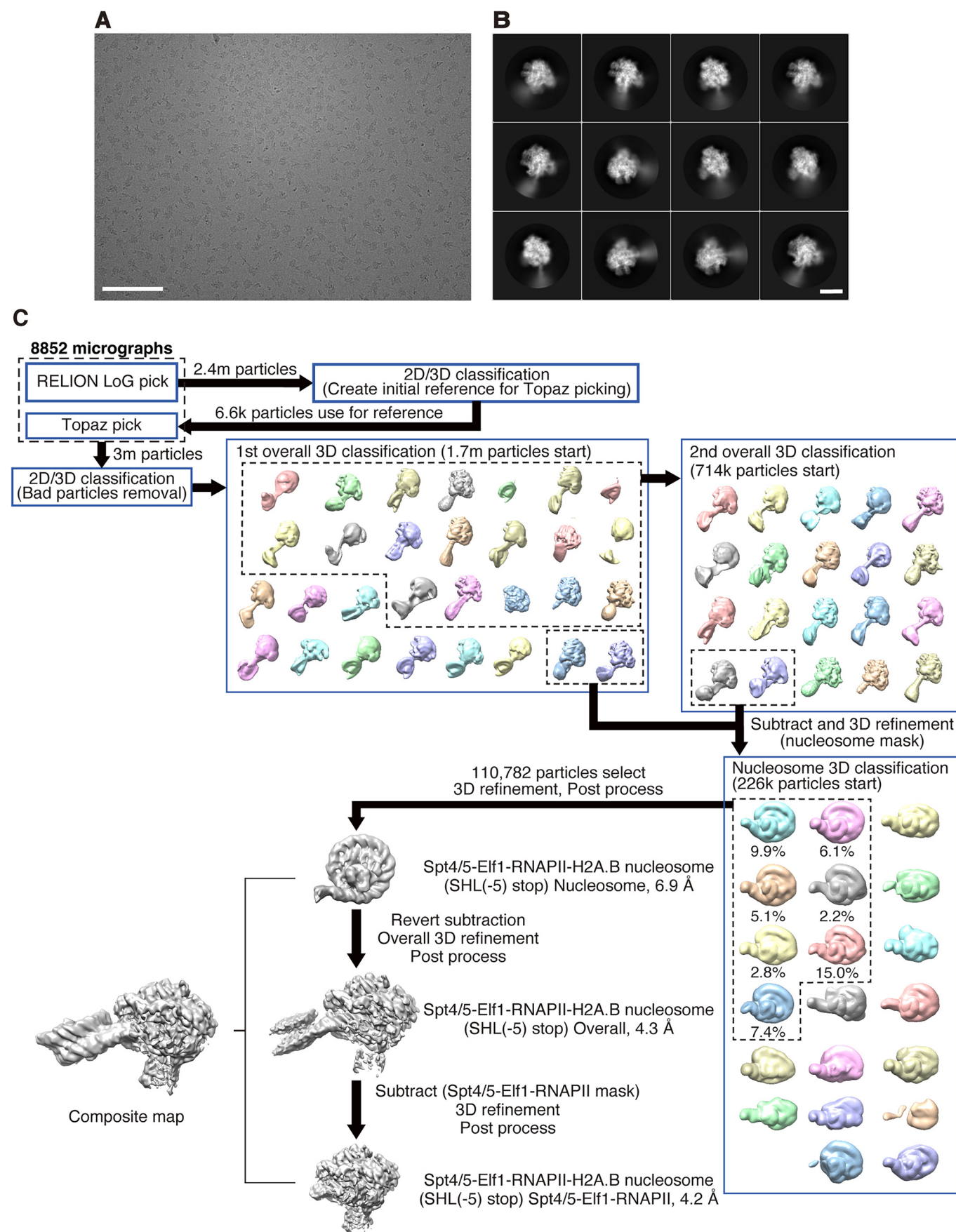


Expanded View Figures

Figure EV1. Cryo-EM analysis of the EC-H2A.B nucleosome complex paused at the SHL(−5) position.

(A) Representative micrograph of the EC-H2A.B nucleosome complex paused at the SHL(−5) position. Scale bar: 100 nm. (B) Representative 2D class averages for the final map of the EC-H2A.B nucleosome complex paused at the SHL(−5) position. Scale bar: 10 nm. (C) Workflow of the image processing of the EC-H2A.B nucleosome complex paused at the SHL(−5) position.



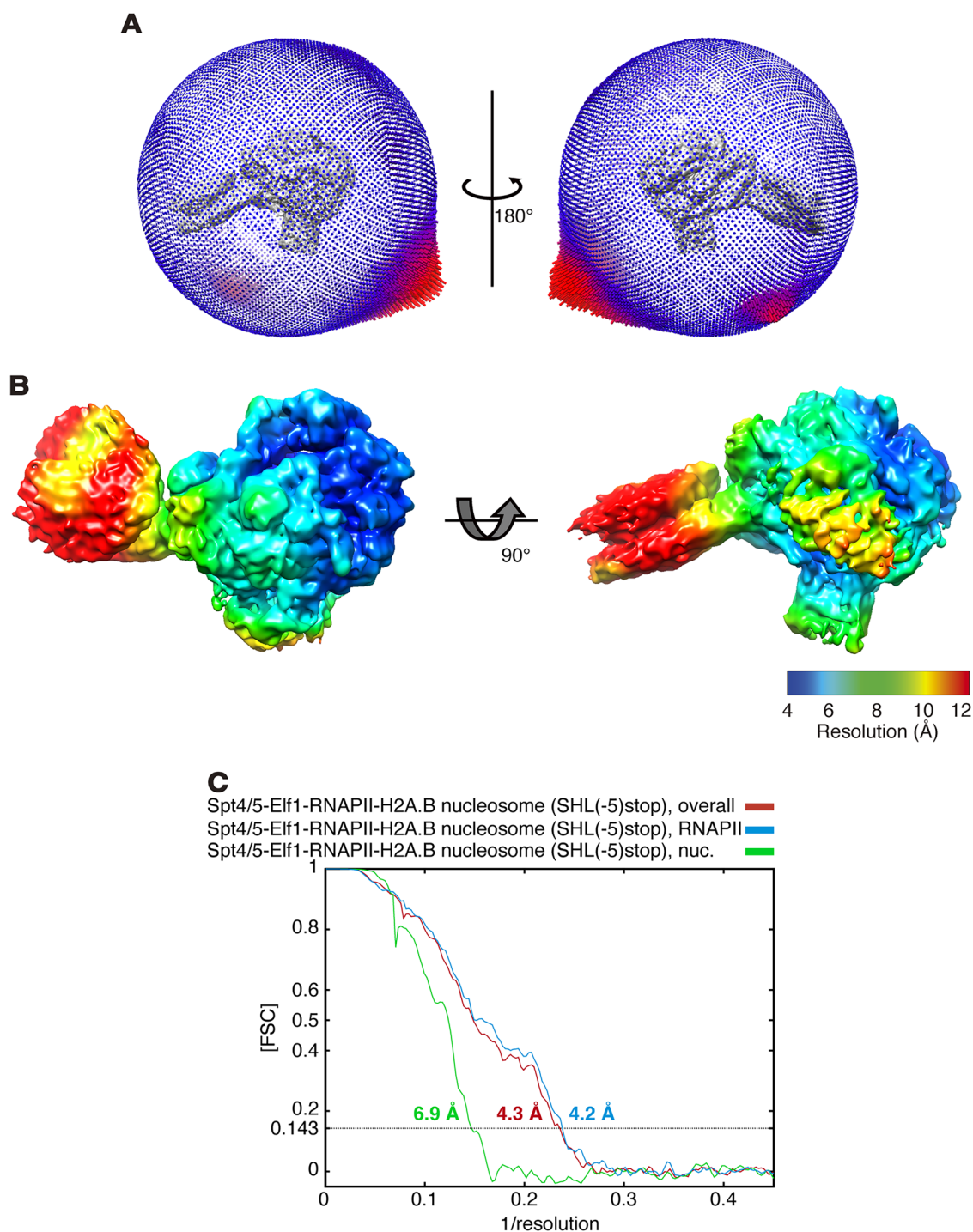
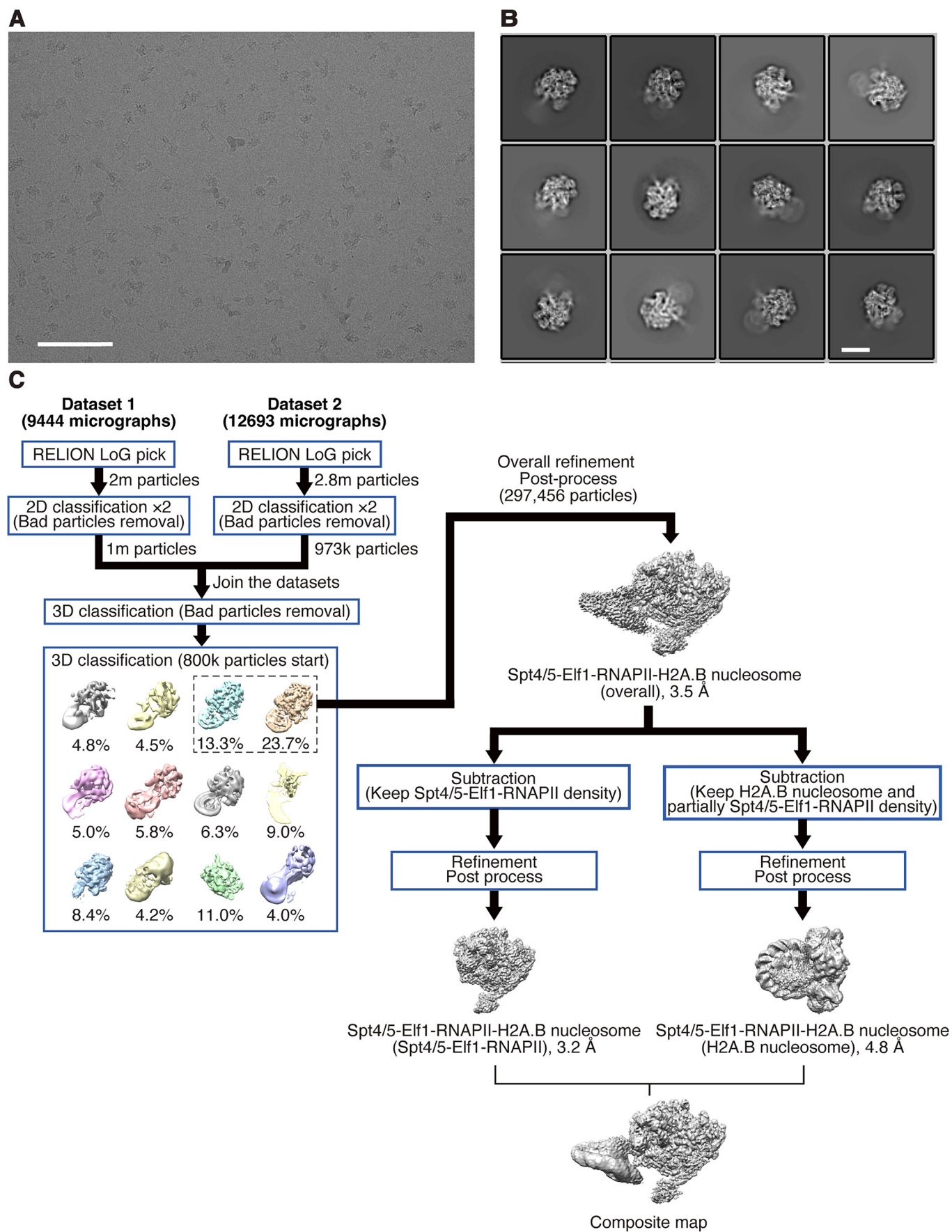


Figure EV2. Cryo-EM map and model qualities of the EC-H2A.B nucleosome complex paused at the SHL(-5) position.

(A) Angular distribution of the EC-H2A.B nucleosome complex paused at the SHL(-5) position. (B) Local resolution map of the EC-H2A.B nucleosome complex paused at the SHL(-5) position. (C) Fourier Shell Correlation (FSC) curve of the EC-H2A.B nucleosome complex paused at the SHL(-5) position. The final resolutions of the structures were estimated at 4.3 Å (overall), 4.2 Å (Spt4/5-Elf1-RNAPII), and 6.9 Å (nucleosome) (FSC = 0.143).



◀ Figure EV3. Cryo-EM analysis of the EC-H2A.B nucleosome complex paused at the SHL(−1) position.

(A) Representative micrograph of the EC-H2A.B nucleosome complex paused at the SHL(−1) position. Scale bar: 100 nm. (B) Representative 2D class averages for the final map of the EC-H2A.B nucleosome complex paused at the SHL(−1) position. Scale bar: 10 nm. (C) Workflow of the image processing of the EC-H2A.B nucleosome complex paused at the SHL(−1) position.

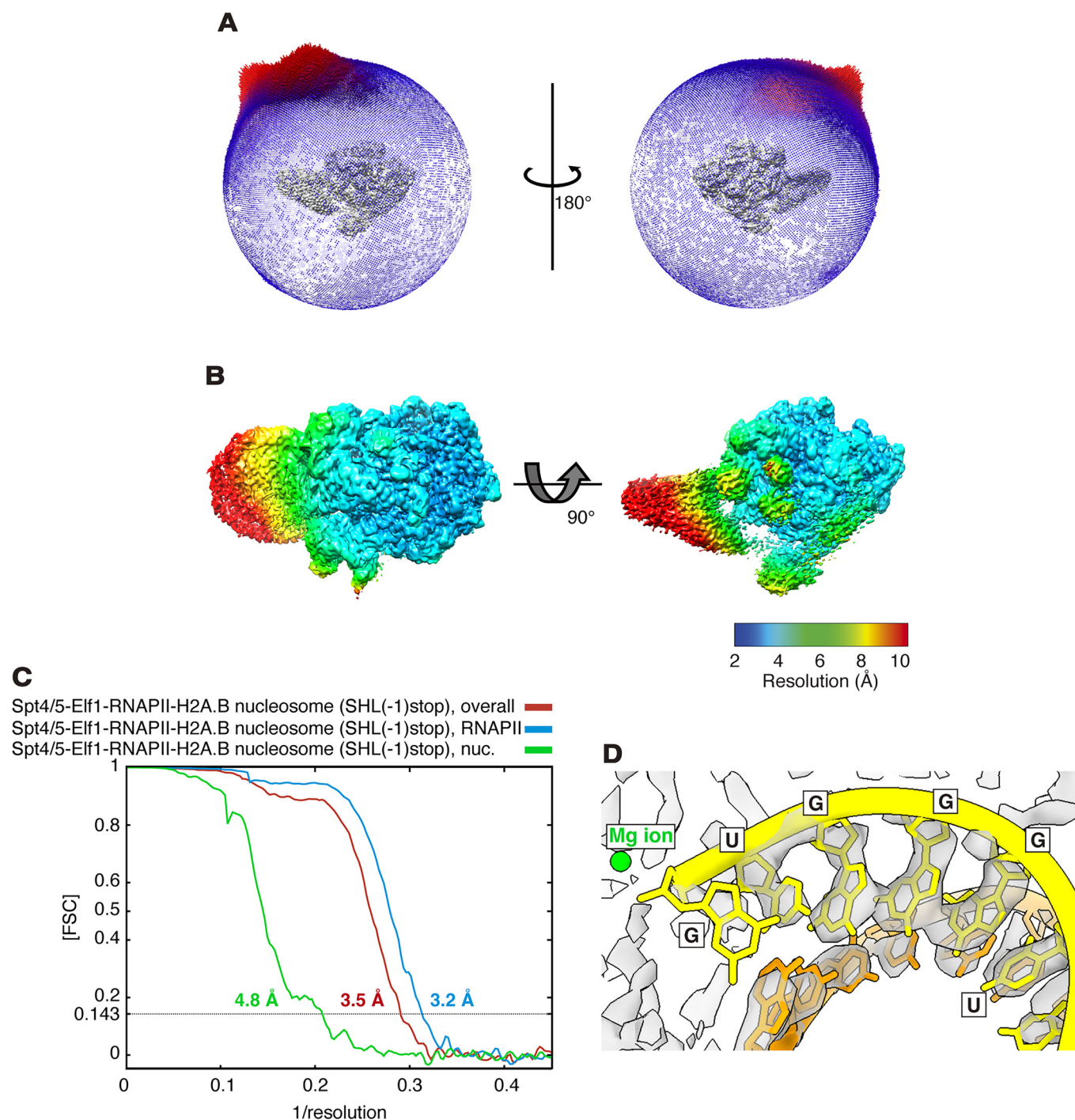
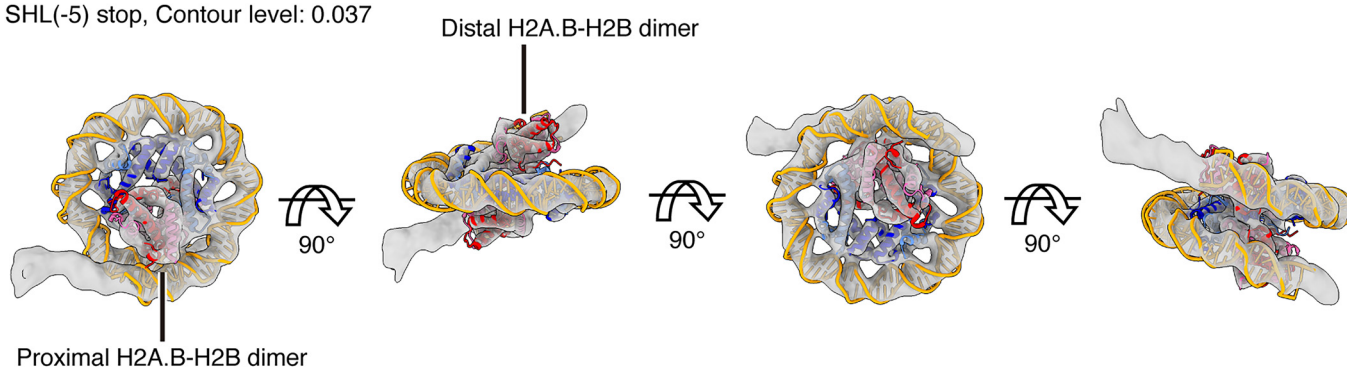


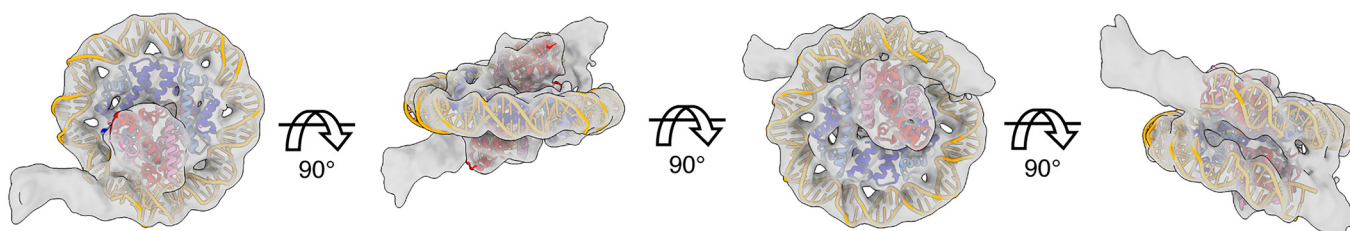
Figure EV4. Cryo-EM map and model qualities of the EC-H2A.B nucleosome complex paused at the SHL(-1) position.

(A) Angular distribution plots of the EC-H2A.B nucleosome complex paused at the SHL(-1) position. (B) Local resolution map of the EC-H2A.B nucleosome complex paused at the SHL(-1) position. (C) Fourier Shell Correlation (FSC) curve of the EC-H2A.B nucleosome complex paused at the SHL(-1) position. The final resolutions of the structures were estimated at 3.5 Å (overall), 3.2 Å (Spt4/5-Elf1-RNAPII), and 4.8 Å (nucleosome) (FSC = 0.143). (D) Close-up view of the RNA in the RNAPII catalytic center. Nascent RNA, template DNA, and magnesium atom are colored yellow, orange, and green, respectively. The RNAPII pausing site is determined based on the RNA sequence (5'-UGGGUG-3') shown in the panel.

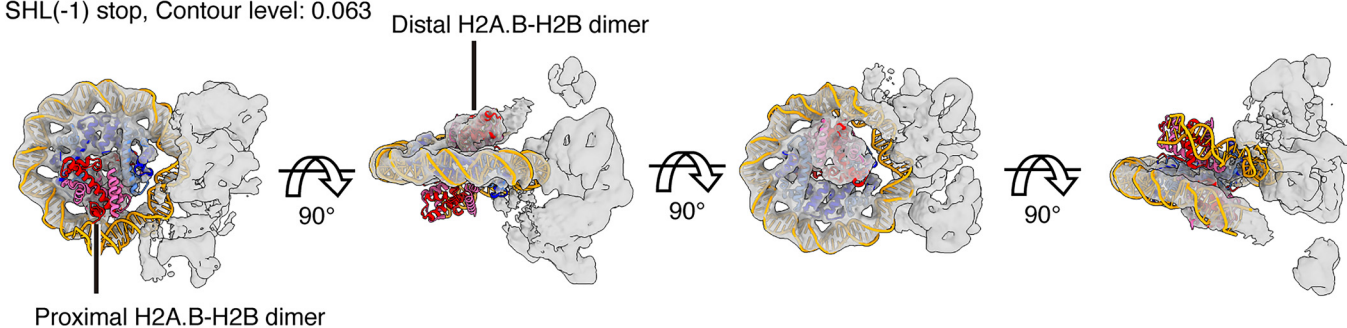
SHL(-5) stop, Contour level: 0.037



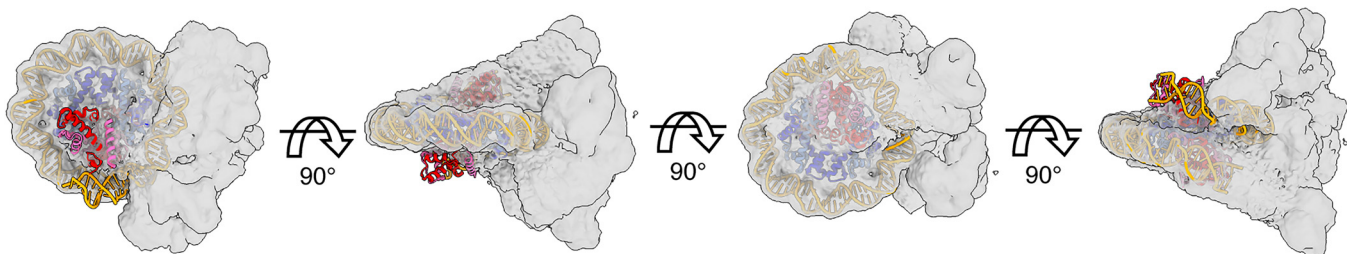
SHL(-5) stop, Contour level: 0.031



SHL(-1) stop, Contour level: 0.063



SHL(-1) stop, Contour level: 0.057

**Figure EV5. Cryo-EM maps of the nucleosome regions in the EC-H2A.B nucleosome complexes paused at the SHL(-5) and SHL(-1) positions.**

Focused refinement maps of the nucleosome regions with different contour levels. The atomic model of the H2A.B nucleosome (PDB: [6M4G](#)) is superimposed.