Expanded View Figures

Figure EV1. CENP-A nucleosome structural features.

A Virtual gels from Bioanalyzer showing DNA digestion for CENP-A and H3 nucleosomes on three different DNA templates.

B (Top) Sequence overlay of the N-terminus of H3 and CENP-A, indicating swapped sequences used in CENP-A$^{H3(N\text{-}tail)}$ and H3$^{CENP\text{-}A(N\text{-}tail)}$ constructs. (Bottom) Virtual gels of MNase digestion for CENP-A$^{H3(N\text{-}tail)}$ and H3$^{CENP\text{-}A(N\text{-}tail)}$ nucleosomes assembled on 601 DNA.

C Representative cryo-EM map of the CENP-A nucleosome, illustrating quality of map and model fitting.

D EM density of CENP-A-specific features on the nucleosome: αN helix (2 different sides), C-terminal tail and RG-loop.

© 2019 The Authors

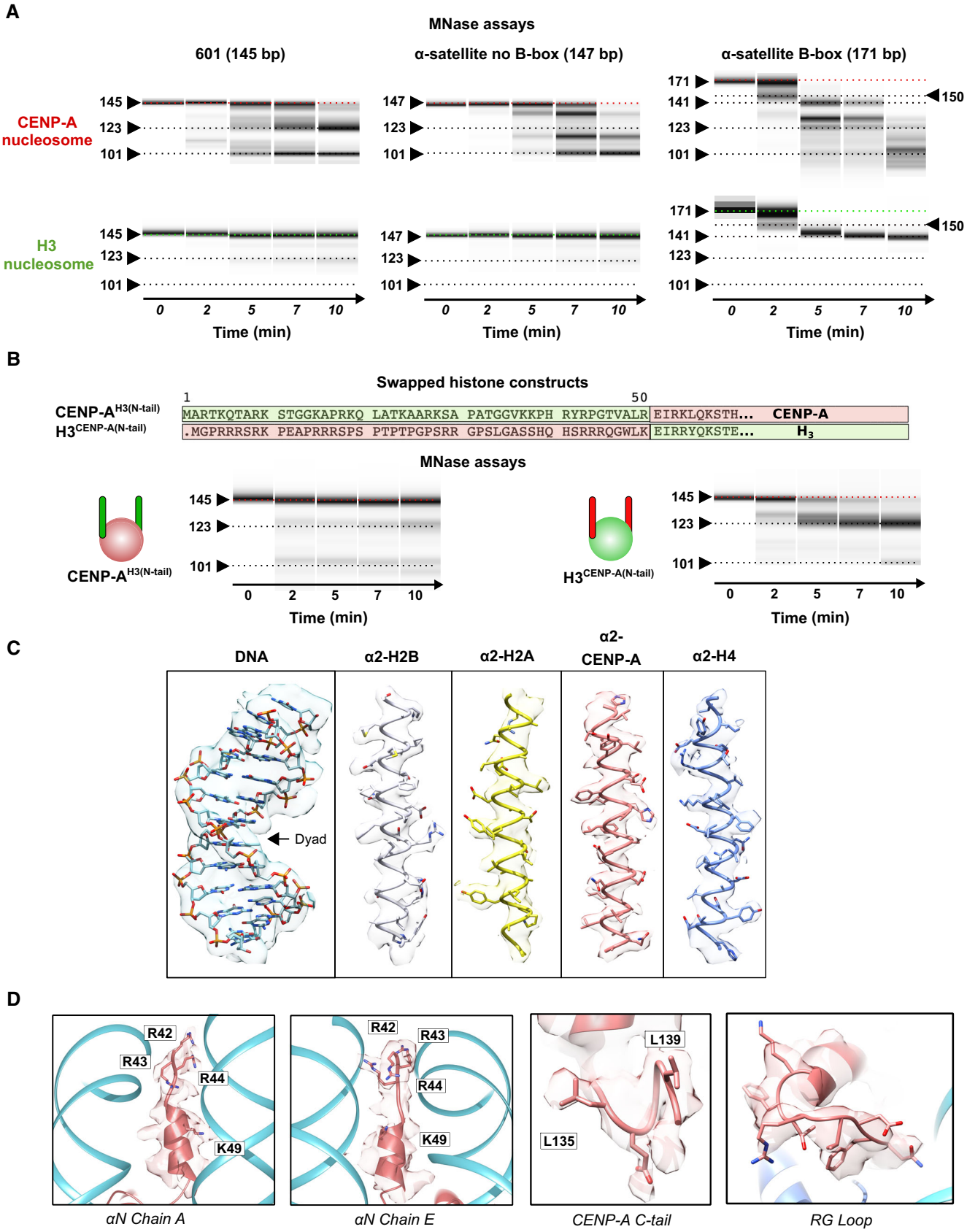
A
MNase assays
601 (145 bp)
α-satellite no B-box (147 bp)
α-satellite B-box (171 bp)
CENP-A nucleosome
H3 nucleosome
145
123
101
147
171
141
150
0 2 5 7 10
Time (min)
B
Swapped histone constructs
1
50
CENP-A^H3(N-tail)
H3^CENP-A(N-tail)
MARTKQTARK STGGKAPRKQ LATKAARKSA PATGGVKKPH RYRPGTVALR EIRKLQKSTH... CENP-A
.MGPRRRSRK PEAPRRRSPS PTPTPGPSRR GPSLGASSHQ HSRRRQGWLK EIRRYQKSTE... H3
MNase assays
C
DNA
α2-H2B
α2-H2A
α2-CENP-A
α2-H4
Dyad
D
R42
R43
R44
K49
L139
L135
αN Chain A
αN Chain E
CENP-A C-tail
RG Loop

Figure EV1.

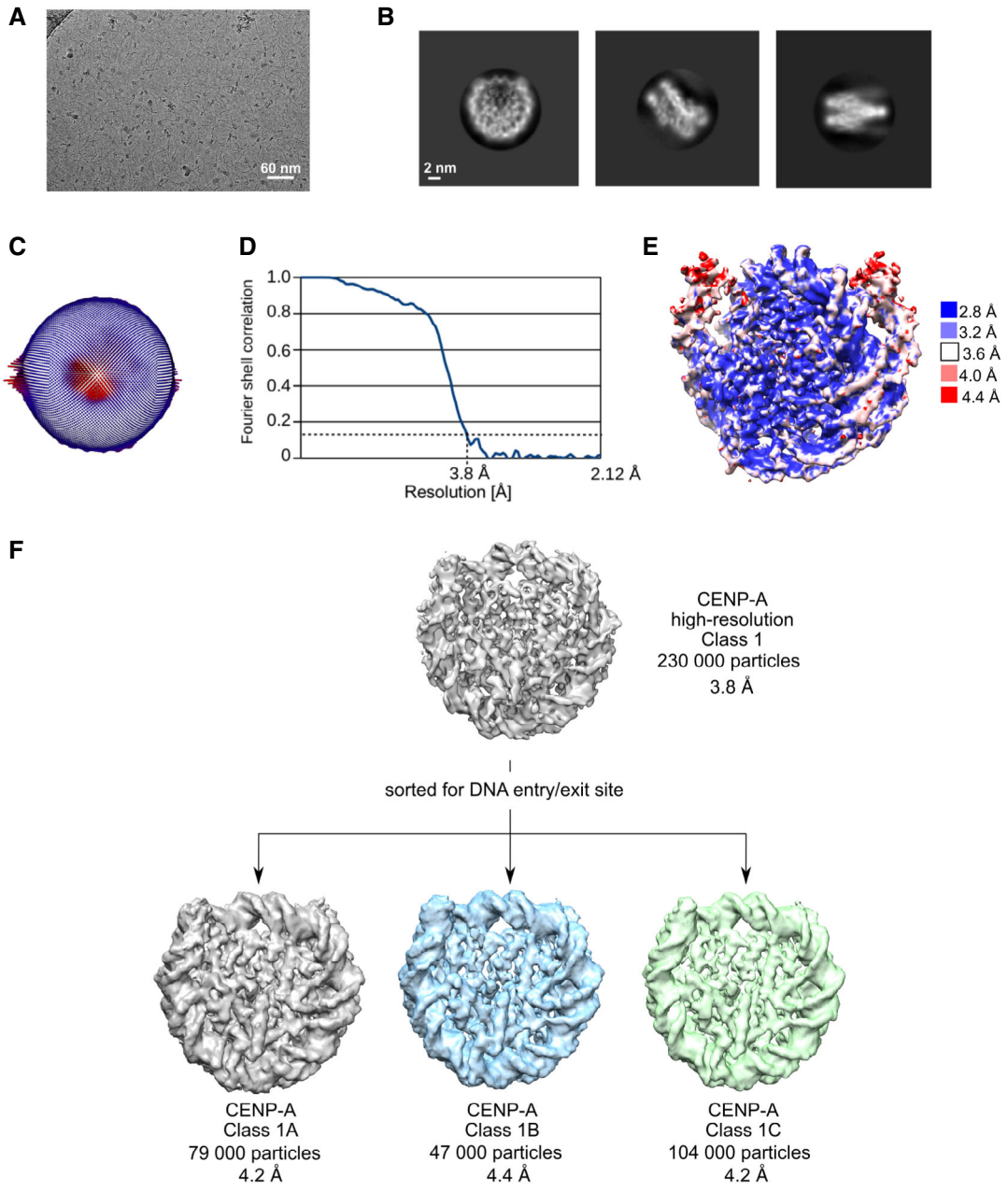

Figure EV2. Cryo-EM analysis of CENP-A nucleosome.

A Representative cryo-EM raw micrograph.
B Subset of selected 2D class averages.
C Euler angle distribution of particles used in the final 3D reconstruction.
D Fourier shell correlation (FSC) curves of the final density map (CENP-A high resolution).
E Local resolution of the final 3D density map.
F Particles used for the high-resolution CENP-A map were further classified for DNA entry/exit site in order to highlight differences at this part of the nucleosome. Gray map (Class 1A) has loosest DNA wrap, and green map (Class 1C) has tightest DNA wrap. The blue map represents particles that were in-between two extreme conformations.

© 2019 The Authors

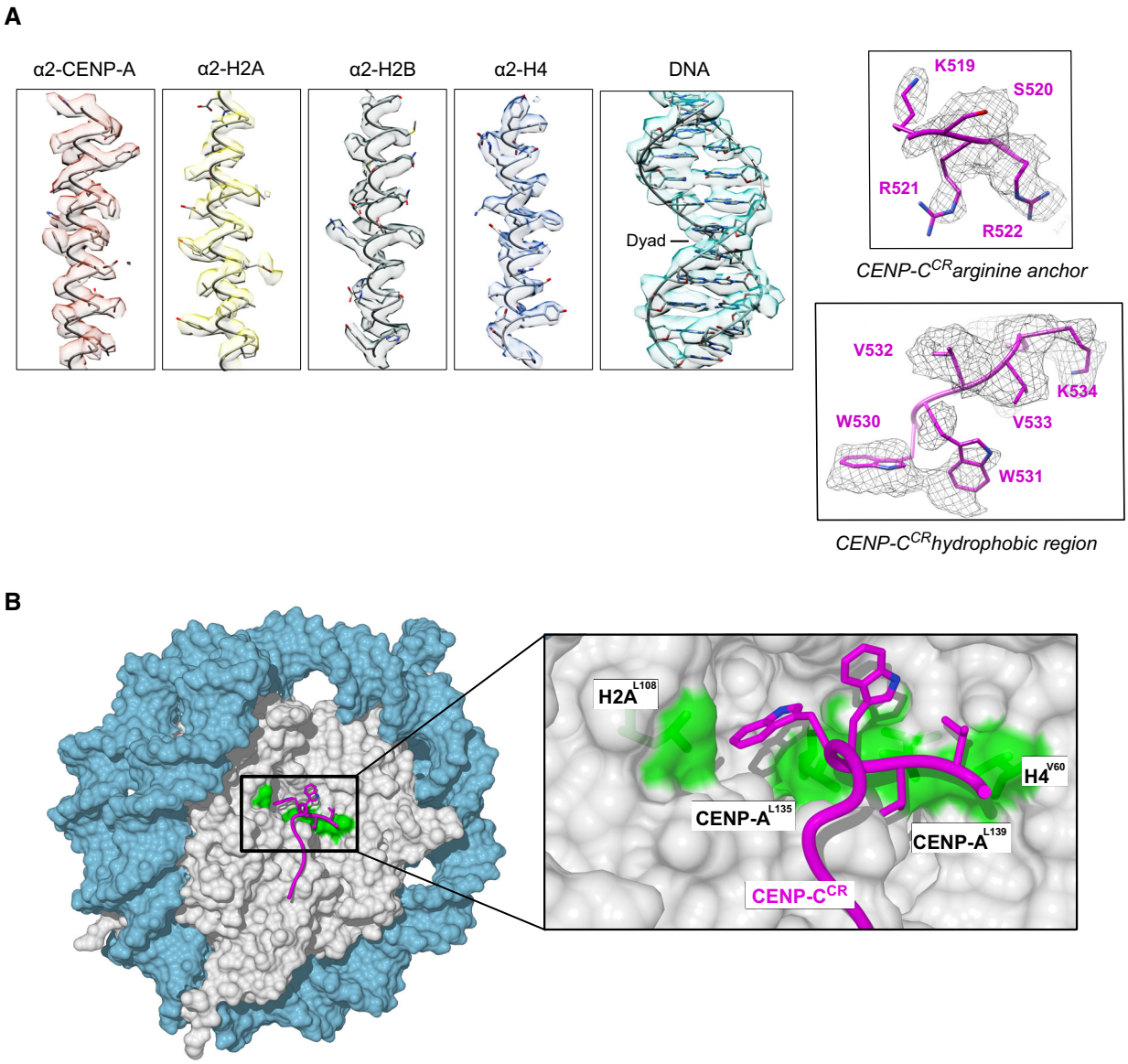

Figure EV3. CENP-A nucleosome/CENP-C^{CR} complex structure.

A Representative cryo-EM densities showing fitted model for DNA and each of the histones (left), arginine anchor, and hydrophobic regions of CENP-C^{CR} (right).

B Surface representation of nucleosome (histone core—gray, DNA—blue), showing a hydrophobic groove (green) on the nucleosome formed by H2A^{L108}, CENP-A^{L135}, CENP-A^{L139}, and H4^{V60}. CENP-C^{CR} is shown as a purple coil with hydrophobic sidechains in stick representation.

Figure EV4. Cryo-EM analysis of CENP-A/CENP-C^{CR} complex.

A Representative cryo-EM raw micrograph.

B Subset of selected 2D class averages.

C Euler angle distribution of particles used in the final 3D reconstruction for CENP-A/CENP-C^{CR} high resolution and CENP-A/CENP-C^{CR} complex enriched for CENP-C^{CR}.

D Fourier shell correlation (FSC) curves of the final density map for CENP-A/CENP-C^{CR} high resolution and CENP-A/CENP-C^{CR} enriched for CENP-C^{CR}.

E Local resolution of the final 3D density maps.

F First, particles were sorted for high resolution, and this map was used for initial model building. A map enriched in CENP-C^{CR} was generated to increase map quality around CENP-C^{CR}. Particles used for the later map were further classified for DNA entry/exit site in order to highlight extend of DNA unwrapping. The gray map (Class 2A) has the loosest DNA wrap, and the green map (Class 2C) has tightest DNA wrap. The blue map presents particles that were in-between two extreme conformations.

© 2019 The Authors

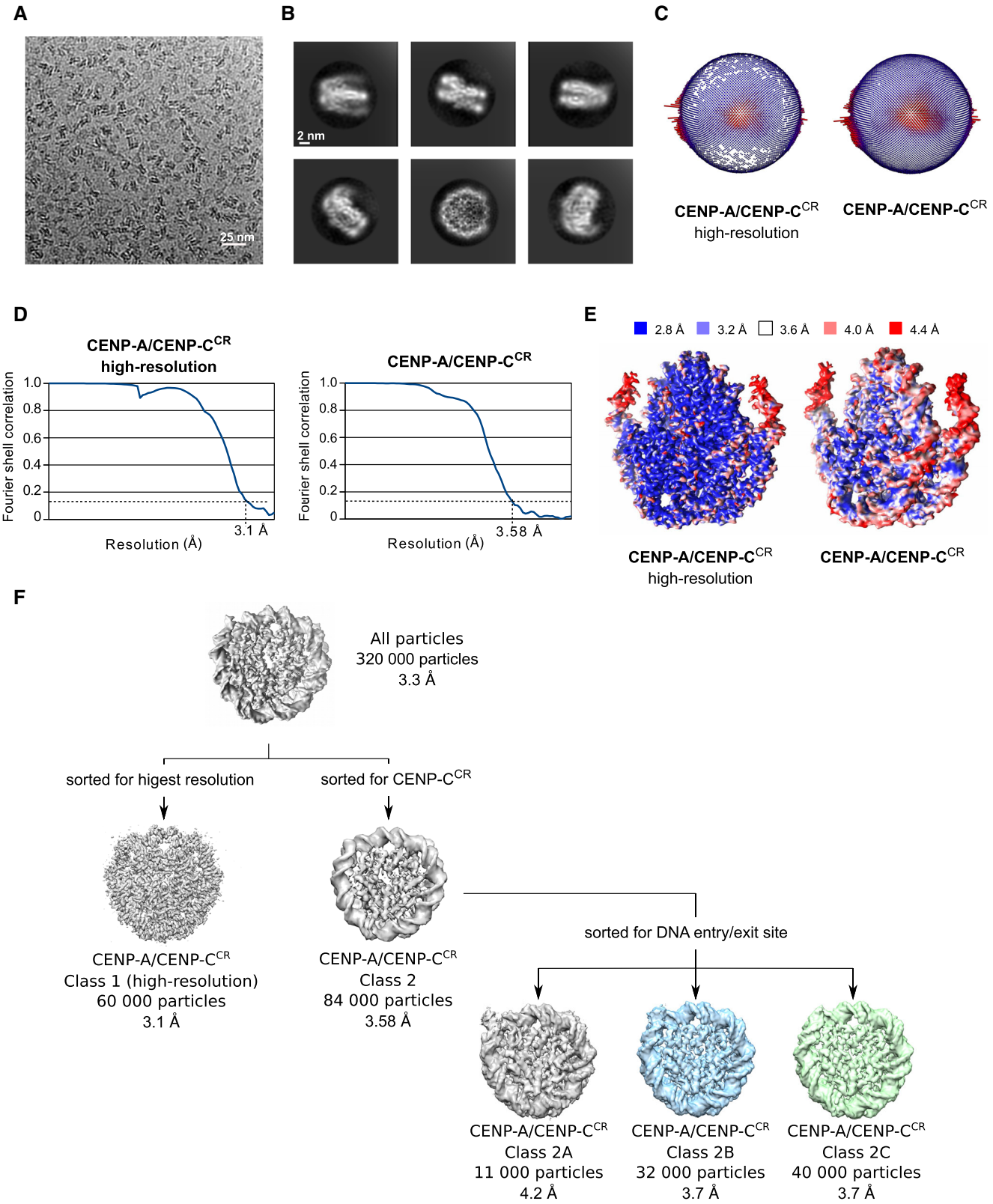

Figure EV4.

© 2019 The Authors

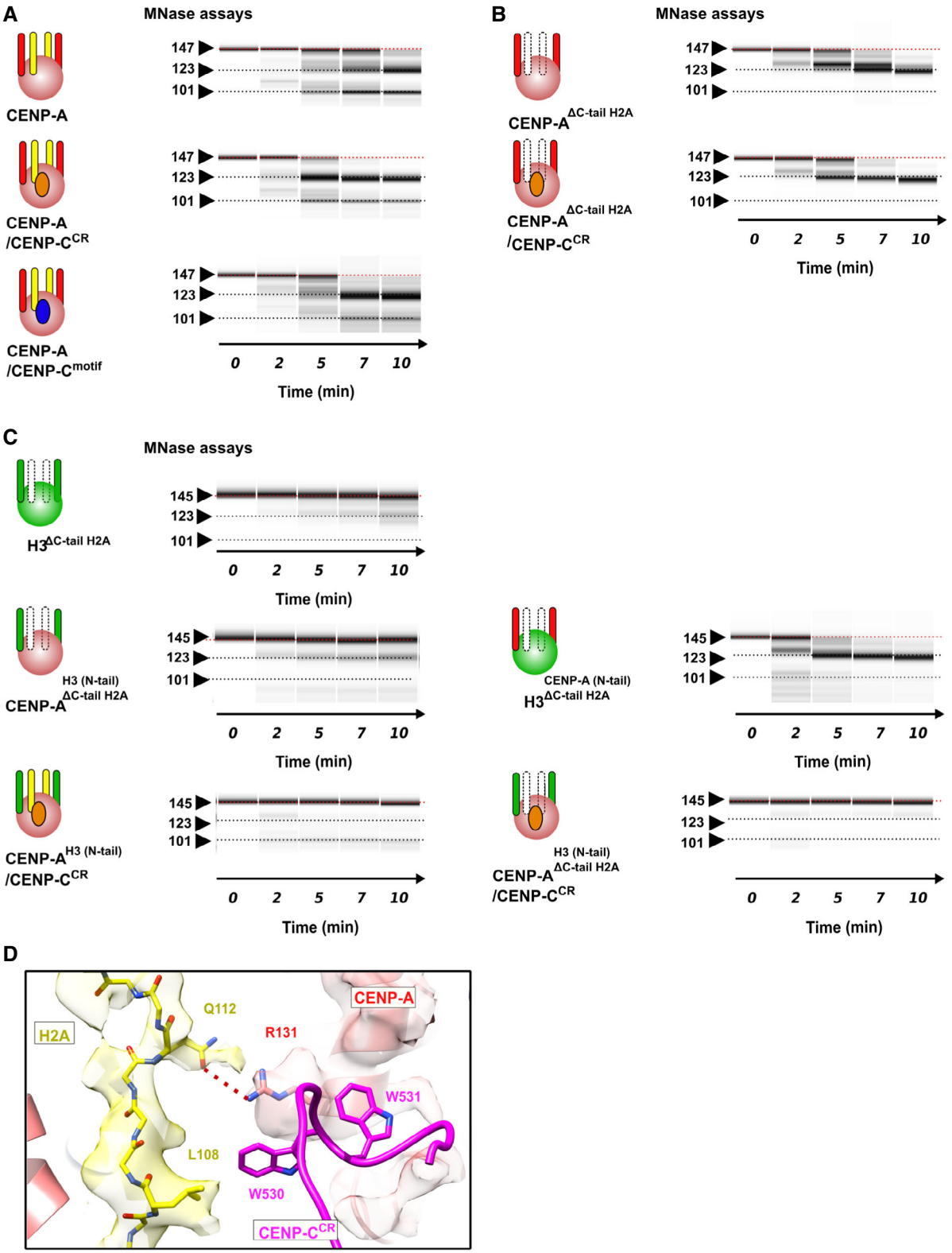
A
MNase assays
CENP-A
CENP-A /CENP-C^CR
CENP-A /CENP-C^motif
147
123
101
0 2 5 7 10
Time (min)
B
MNase assays
CENP-A^ΔC-tail H2A
CENP-A^ΔC-tail H2A /CENP-C^CR
C
MNase assays
H3^ΔC-tail H2A
145
123
101
CENP-A^H3 (N-tail) ΔC-tail H2A
CENP-A^H3 (N-tail) ΔC-tail H2A /CENP-C^CR
CENP-A (N-tail) H3^ΔC-tail H2A
D
H2A
CENP-A
Q112
R131
W531
L108
W530
CENP-C^CR

Figure EV5.

Figure EV5. Conformational changes on the CENP-A nucleosome upon CENP-C^{CR} binding.

A–C Virtual gels for MNase digestion. CENP-A nucleosomes are pink, and H3 nucleosomes are green balls. CENP-A N-terminal tail (aa 1–49) is red stick, H3 N-terminal tail (aa 1–50) is green stick, H2A C-terminal tail (aa 110–130) is yellow stick, CENP-C^{CR} is orange circle, and CENP-C^{motif} is blue circle. Deleted H2A C-terminal tail is indicated as dotted white stick. (A) MNase digestion of the CENP-A nucleosome alone and in complex with CENP-C^{CR} or CENP-C^{motif} (also in Fig 4A). (B) MNase digestion of the CENP-A nucleosome assembled with H2A lacking 110–130 residues, alone (top) or in complex with CENP-C^{CR} (bottom), showing similar magnitude of digestion. (C) (Top) MNase digestion of the H3$^{\Delta C\text{-tail H2A}}$, indicating that removal of H2A$^{110–130}$ does not have an effect on the DNA digestion speed in the context of H3 nucleosome. (Middle) MNase digestion of CENP-A$^{H3(N\text{-tail}),\ \Delta C\text{-tail H2A}}$ and H3$^{CENP\text{-}A(N\text{-tail}),\ \Delta C\text{-tail H2A}}$, indicating that removal of H2A$^{110–130}$ does not have an effect on the DNA digestion speed in the context of CENP-A$^{H3(N\text{-tail})}$ nucleosome, but digestion is slightly increased in the context of H3$^{CENP\text{-}A(N\text{-tail})}$. (Bottom) MNase digestion of the CENP-A$^{H3(N\text{-tail})}$ is unaffected with CENP-C^{CR} binding independently of the presence of H2A$^{110–130}$.

D Interactions between H2A C-terminal tail and CENP-C^{CR}.

© 2019 The Authors