

Description of Additional Supplementary Files

Supplementary Movie 1 | Simulated molecular transition of the Smc5/6 hexamer from the I-form to O-form via an external force, related to Fig. 2e.

Supplementary Movie 2 | a-c, HSAFM videos of the wild-type Smc5/6 hexamer in the absence of ATP (a), in the presence of ATP (b), and of the EQ mutant in the presence of ATP (c), observed on the mica imaging stage (related to Fig. 3a). a: Scan area, $100 \times 100 \text{ nm}^2$ at $160 \times 80 \text{ pixels}^2$, cropped to $90 \times 86 \text{ nm}^2$. b: Scan area, $150 \times 150 \text{ nm}^2$ at $160 \times 80 \text{ pixels}^2$, cropped to $110 \times 105 \text{ nm}^2$. c: Scan area, $100 \times 100 \text{ nm}^2$ at $160 \times 80 \text{ pixels}^2$, cropped to $95 \times 90 \text{ nm}^2$.

Supplementary Movie 3 | HS-AFM videos of Smc5/6 pentamer lacking Nse2 displaying I/O (a) and B (b) forms, observed on the mica imaging stage, related to Fig. 3d. Scan area, $120 \times 120 \text{ nm}^2$ at $200 \times 100 \text{ pixels}^2$, cropped to $90 \times 90 \text{ nm}^2$. Imaging rate, 0.63 s per frame.

Supplementary Movie 4 | a,b, Overview HS-AFM videos of DNA-bound wild-type (a) and EQ mutant (b) Smc5/6, observed on the lipid membrane imaging stage (related to Fig. 4e and Supplementary Fig. 10b). a: Scan area, $350 \times 280 \text{ nm}^2$ at $200 \times 80 \text{ pixels}^2$, cropped to $298 \times 238 \text{ nm}^2$. b: Scan area, $300 \times 240 \text{ nm}^2$ at $240 \times 96 \text{ pixels}^2$, cropped to $255 \times 204 \text{ nm}^2$. Imaging rate for both a and b: 1 s per frame.

Supplementary Movie 5 | HS-AFM video of two EQ mutant Smc5/6 complexes bound to a single DNA molecule, observed on the lipid membrane imaging stage (related to Fig. 4f). Scan area, $220 \times 176 \text{ nm}^2$ at $200 \times 80 \text{ pixels}^2$, cropped to $187 \times 134 \text{ nm}^2$. Imaging rate: 0.6 s per frame.

Supplementary Movie 6 | a-c, HSAFM videos of DNA-bound wildtype Smc5/6 in the Hinge-O/I state (a), in the Head-I state (b), and of DNA-bound EQ mutant Smc5/6 in the Head-O state (c), observed on the lipid membrane imaging stage (related to Fig. 5b–d). a: Scan area, $150 \times 150 \text{ nm}^2$ at $160 \times 80 \text{ pixels}^2$, cropped to $150 \times 110 \text{ nm}^2$. b: Scan area, $150 \times 120 \text{ nm}^2$ at $160 \times 64 \text{ pixels}^2$. c: Scan area, $220 \times 176 \text{ nm}^2$ at $200 \times 80 \text{ pixels}^2$, cropped to $172 \times 125 \text{ nm}^2$. Imaging rates: 0.37 s per frame (a), 0.34 s per frame (b), 0.6 s per frame (c).

Supplementary Movie 7 | a-c, HSAFM videos of DNA-bound Smc5/6 oligomers that stabilize DNA twist structures. HS-AFM data and imaging settings are same as those described in Supplementary Figure 17.