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SUPPLEMENTARY INFORMATION

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A folded conformation of MukBEF and cohesin

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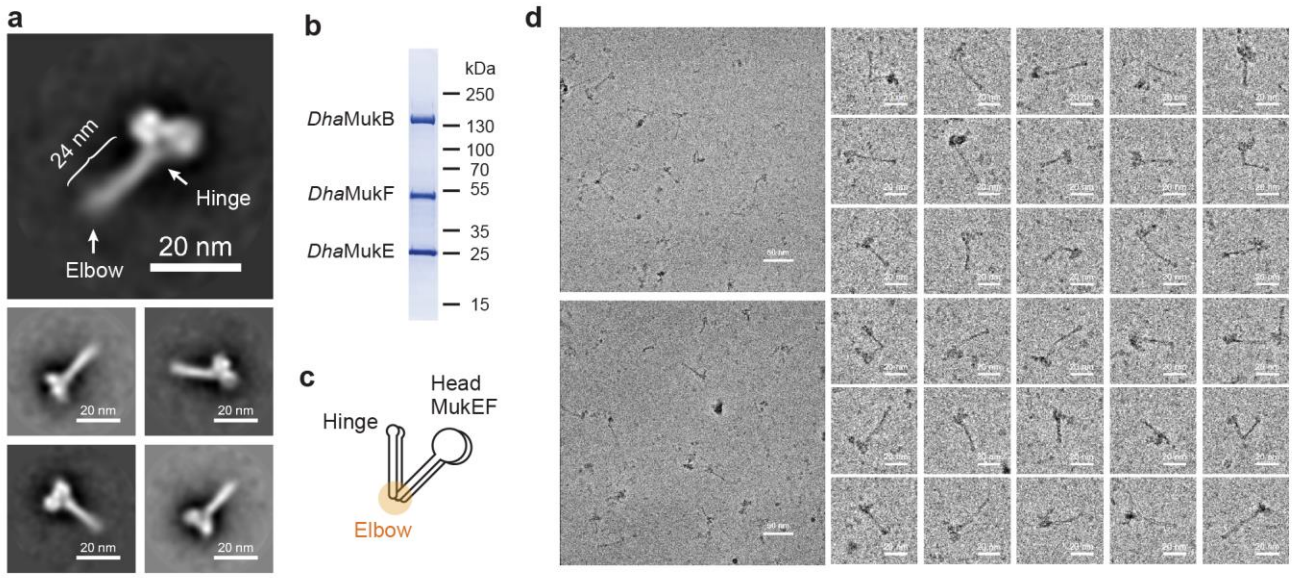
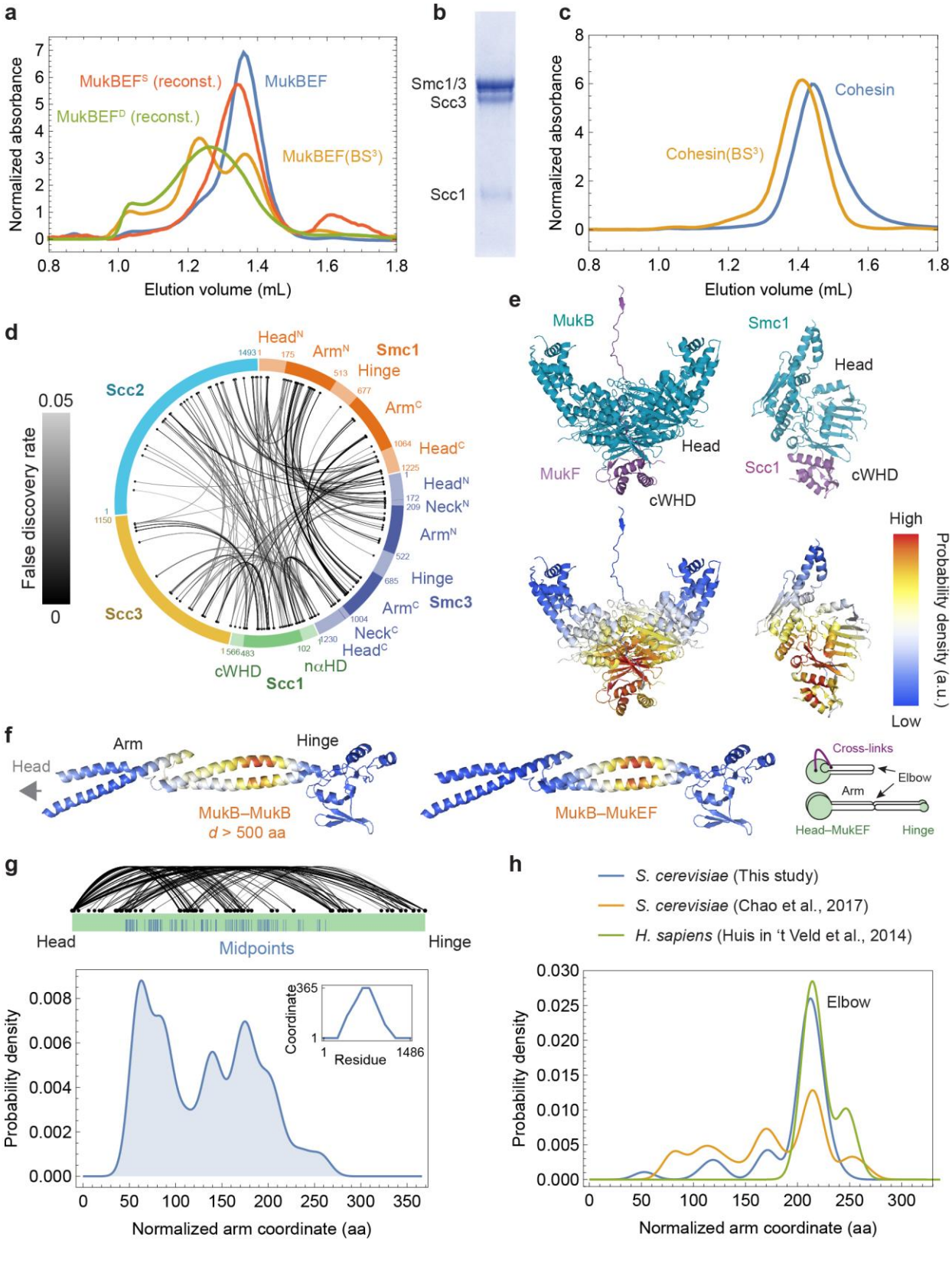


Supplementary Figure 1

EM analysis of MukBEF.

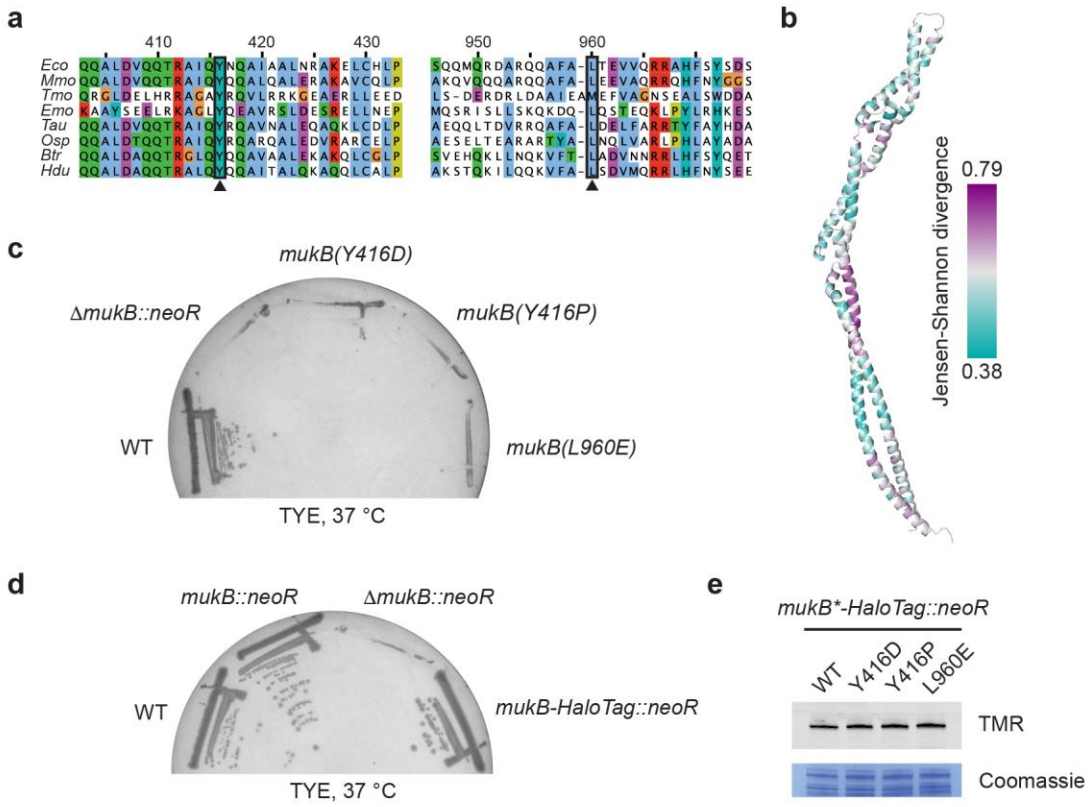
a, Negative stain 2D class averages for the folded conformation of native *E. coli* MukBEF using a circular mask of 640 Å. **b**, SDS-PAGE analysis of purified *Desulfovermiculus halophilus* MukBEF. The gel was stained with Coomassie. **c**, Cartoon of intermediate particle shapes of *D. halophilus* MukBEF indicating the presence of a coiled-coil elbow in different conformations. **d**, Cryo-EM imaging of *D. halophilus* MukBEF in unsupported vitreous ice. Contrast was enhanced by use of a Volta phase plate and high total electron dose. Typical fields of view are shown on the left, examples of single particle images are shown on the right. We estimate that approximately 35 % of particles may adopt a fully folded conformation under the conditions used. Low particle abundance and sample heterogeneity prevented further structural analysis.


a
Normalized absorbance
Elution volume (mL)
MukBEF^S (reconst.)
MukBEF
MukBEF^D (reconst.)
MukBEF(BS^3)
b
Smc1/3
Scc3
Scc1
c
Normalized absorbance
Elution volume (mL)
Cohesin
Cohesin(BS^3)
d
False discovery rate
0.05
0
Head^N
Arm^N
Smc1
Hinge
Arm^C
Head^C
Head^N
Neck^N
Arm^N
Hinge
Arm^C
Smc3
Neck^C
Head^C
nαHD
Scc1
cWHD
Scc3
Scc2
e
MukB
Smc1
Head
Head
MukF
Scc1
cWHD
cWHD
High
Low
Probability density (a.u.)
f
Arm
Hinge
Head
MukB–MukB
d > 500 aa
MukB–MukEF
Cross-links
Elbow
Arm
Head–MukEF
Hinge
g
Head
Midpoints
Hinge
Probability density
Coordinate
Residue
Normalized arm coordinate (aa)
h
S. cerevisiae (This study)
S. cerevisiae (Chao et al., 2017)
H. sapiens (Huis in 't Veld et al., 2014)
Probability density
Elbow
Normalized arm coordinate (aa)

Supplementary Figure 2

Cross-linking and mass spectrometry of MukBEF and cohesin.

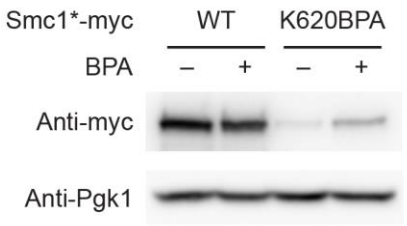
a, SEC profiles of native co-expressed MukBEF (blue), BS^3 treated co-expressed MukBEF (orange), singlet MukBEF ($MukBEF^S$) reconstituted in buffer containing 40 mM NaCl, 2 mM $MgCl_2$ (red) and doublet MukBEF ($MukBEF^D$) reconstituted in buffer containing 200 mM NaCl (green). Reconstitution was similar to protocols established previously (*J. Biol. Chem*. **281**, 34208–34217, 2006). **b**, SDS-PAGE analysis of a purified cohesin complex containing Smc1, Smc3, Scc1 and Scc3. The gel was stained with Coomassie. **c**, SEC profiles of the cohesin complex containing Smc1, Smc3, Scc1 and Scc3 before and after treatment with BS^3 (see Fig. 1h). **d**, Inter-subunit cross-links of a cohesin complex containing Smc1, Smc3, Scc1, Scc3 and Scc2. As in Fig. 2a. **e**, Kernel density estimates for the position of cross-link sites mapped onto the partial structure of the *H. ducreyi* MukBEF head module (PDB ID 3EUH) and the cohesin Smc1–Scc1 cWHD interface (PDB ID 1W1W). **f**, Kernel density estimates for long-distance cross-links at the MukB hinge. Probability density for MukB cross-links to MukB sites located at least 500 aa away (left) or to MukEF (middle). The cartoon (right) illustrates an explanation for the observed cross-linking pattern. **g**, Cross-link midpoint analysis for MukB performed as in Fig. 2c but using random resampling without replacement before data processing. **h**, Cross-link midpoint analysis for various cohesin datasets (as in Fig. 2). Peak density for human cohesin corresponds to residues 375 and 813 (Smc1) and 379 and 811 (Smc3).



Supplementary Figure 3

Conservation analysis and mutagenesis of the MukB elbow.

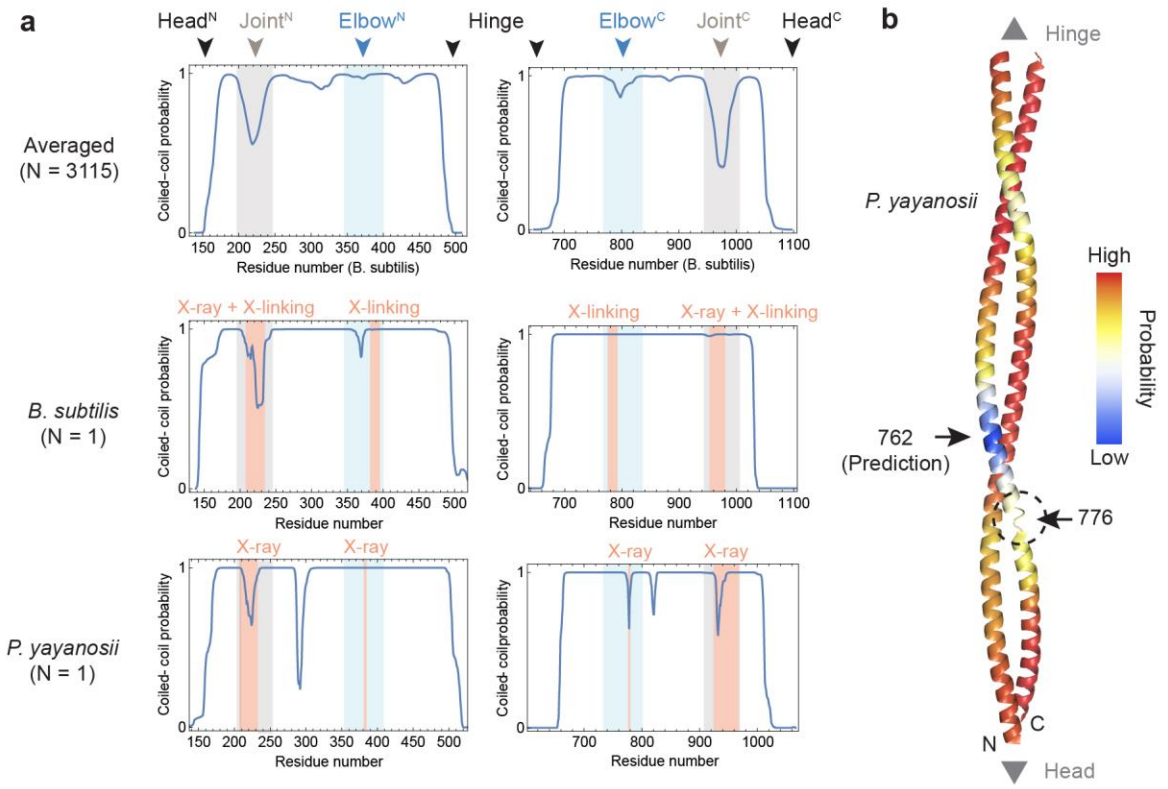
a, Sequence alignment of the N-terminal (left) and C-terminal (right) parts of the MukB elbow. Residues chosen for mutagenesis are highlighted by triangles. *Eco*, *Escherichia coli*; *Mmo, Morganella morganii*; *Tmo*, *Thioflavicoccus mobilis*; *Emo*, *Endozoicomonas montiporae*; *Tau*, *Tolumonas auensis*; *Osp*, *Oceanimonas sp.* GK1; *Btr*, *Bibersteinia trehalosi*; *Hdu*, *Haemophilus ducreyi*. **b**, Sequence conservation (Jensen-Shannon divergence) was mapped onto the structure (high conservation is purple, low conservation is cyan). **c**, Growth of strains containing point mutations at the elbow in the endogenous *mukB* gene. **d**, Construction of a functional *mukB-HaloTag* allele. **e**, Protein levels of elbow mutants fused to a HaloTag. Extracts were labelled with a HaloTag-TMR substrate and were analyzed by in-gel fluorescence (top) and Coomassie staining (bottom) after SDS-PAGE. WT, wild-type.



Supplementary Figure 4

BPA-dependent expression of Smc1(K620BPA).

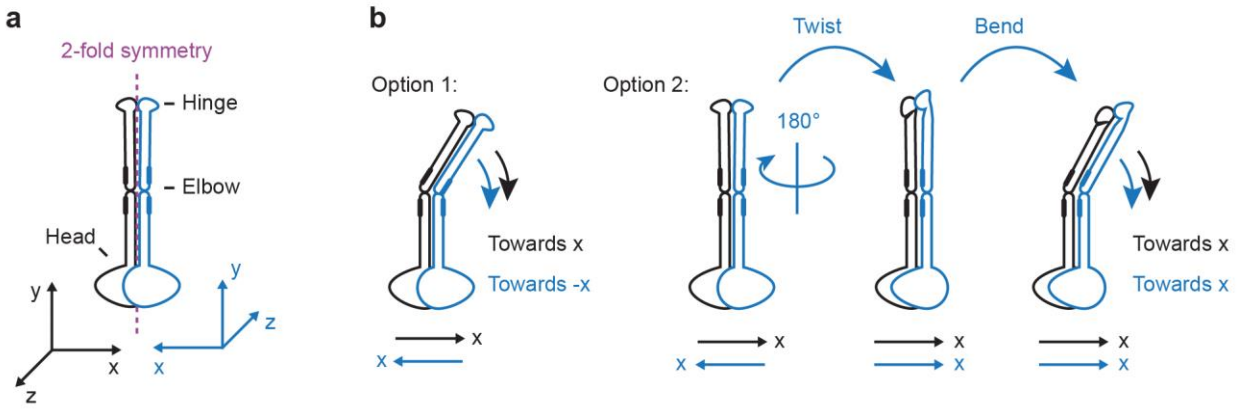
Strains were grown either in the absence or presence of 1 mM BPA, and extracts were analyzed by Western blotting.



Supplementary Figure 5

Locations of coiled-coil discontinuities in bacterial and archaeal Smc proteins.

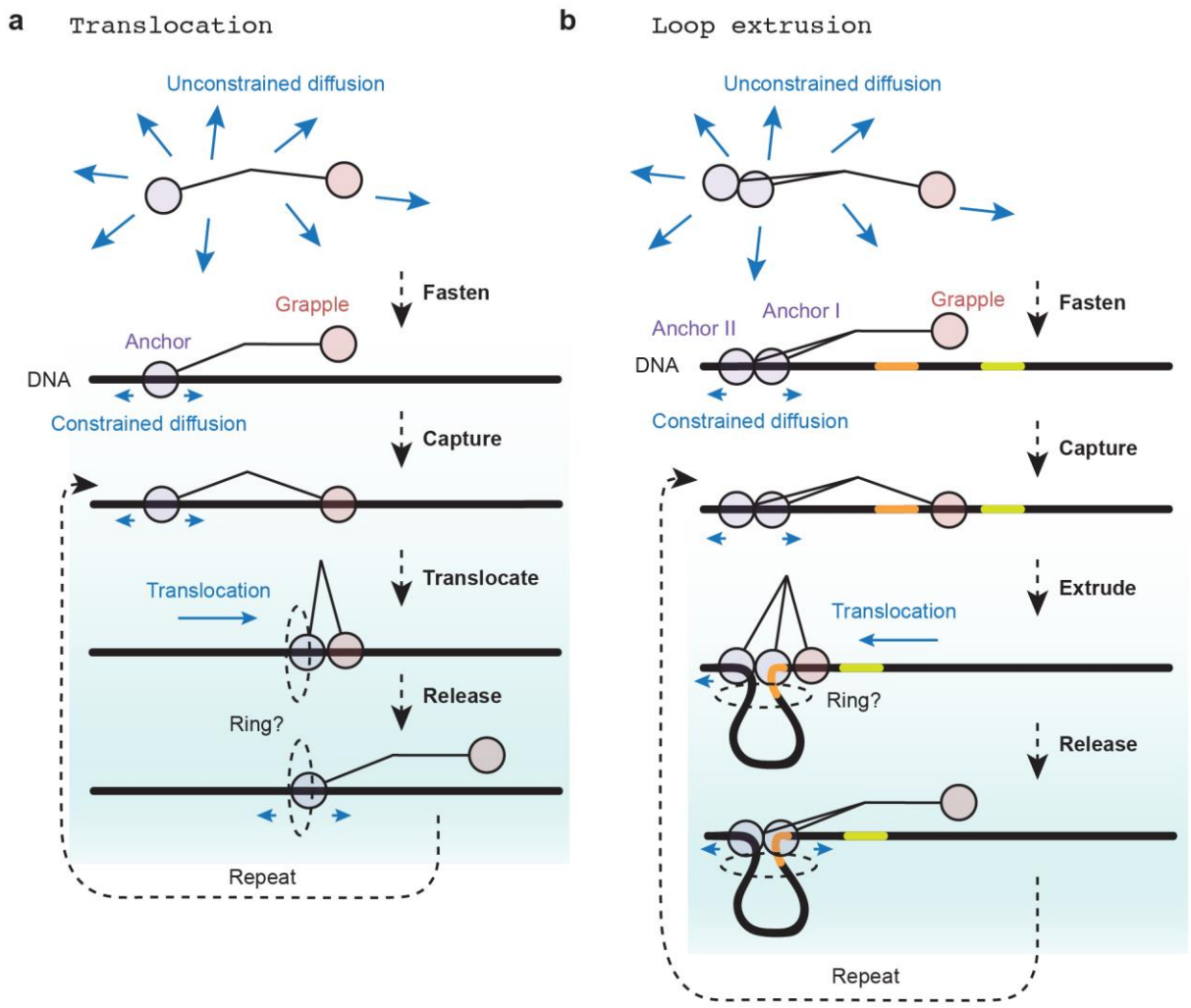
a, Aggregate coiled-coil probability profile (same as in Fig. 5) and single-sequence profiles for *B. subtilis* Smc (bacterial) and *Pyrococcus yayanosii* Smc (archaeal). Positions of coiled-coil discontinuities experimentally determined by X-ray crystallography (*Mol. Cell* **67**, 334-347.e5, 2017) or disulfide cross-linking (*Proteins* **83**, 1027–1045, 2015) are highlighted in red. **b**, The elbow region of *P. yayanosii* Smc. The predicted coiled-coil probability from aggregate analysis (see **a** and Fig. 5) is mapped onto the crystal structure of a central arm fragment (PDB ID 5XG2). Positions of the predicted and crystallographically determined discontinuities are shown.



Supplementary Figure 6

Bending of SMC dimers.

a, An SMC dimer with C2 symmetry. Monomers and their body-frame coordinate systems are shown in black or blue. The symmetry axis of the dimer is shown in purple. **b**, Symmetry breaking upon elbow bending. Option 1: monomers bend into opposite directions; Option 2: monomers twist and bend into the same direction. Orientations of the relevant body-frame coordinate axes are shown at the bottom.



Supplementary Figure 7

Inchworm models for DNA and translocation and loop extrusion.

a, DNA translocation model requiring a regulated grapple DNA binding site and a sliding anchor DNA binding site. DNA binding may or may not involve a DNA entrapping ring that could be used to enhance processivity. **b**, Loop extrusion using a second anchor site. DNA binding may or may not involve a DNA entrapping ring that could be used to enhance processivity.

Fig. 1a

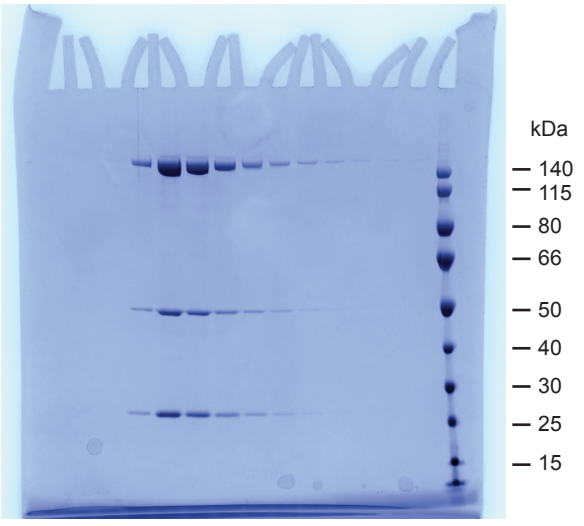
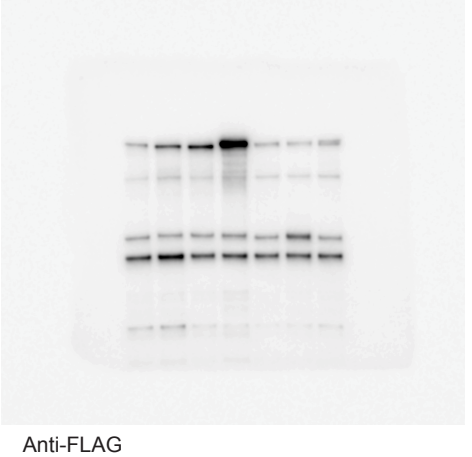
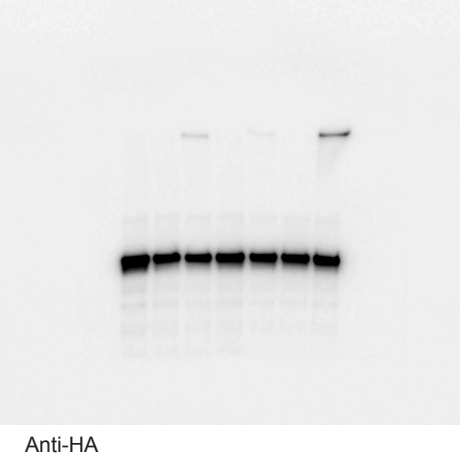


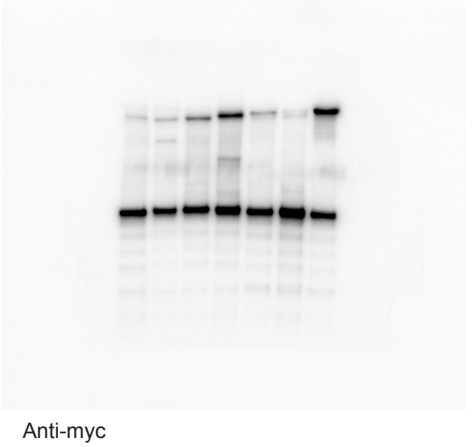
Fig. 4b



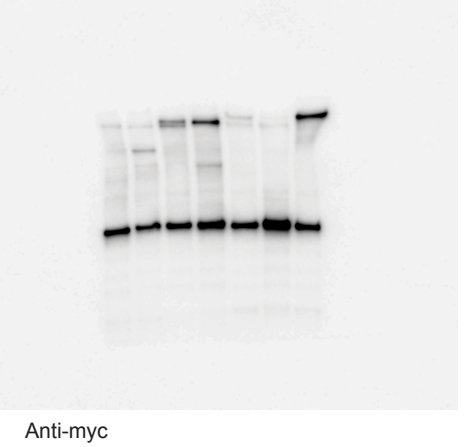
Anti-FLAG



Anti-HA



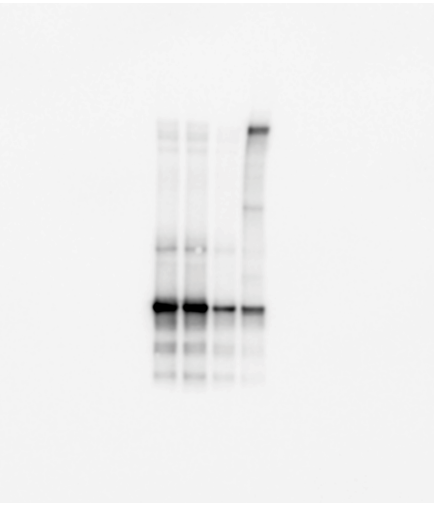
Anti-myc



Anti-myc

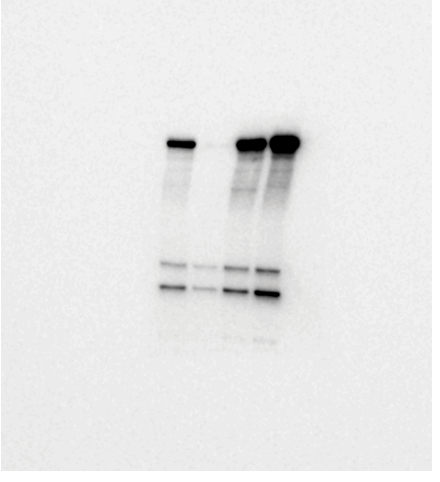
Fig. 4c

Anti-FLAG

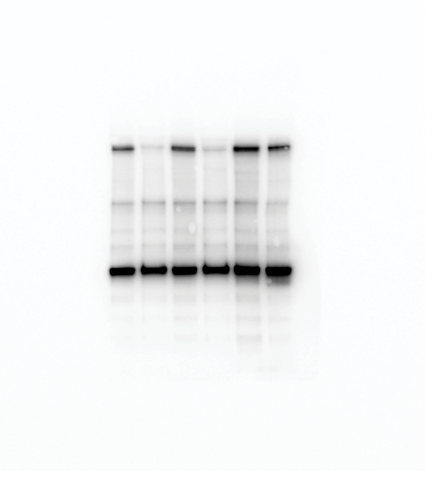


Anti-myc

Fig. 4d



Anti-FLAG



Anti-myc

Supplementary Table 1 | Microbial strains.

Strain ID	Genotype	Figures
***E. coli* strains**		
MG1655	F-, λ-, *rph*-1, *fnr+*	**S3c, S3d**
SFB012	MG1655, *mukB::neoR*	**S3d**
SFB017	MG1655, *mukB-HaloTag(C61V, C262A)::neoR*	**S3d, S3e**
SFB018	MG1655, *ΔmukB::neoR*	**S3c, S3d**
SFB022	MG1655, *mukB(Y416D)*	**S3c**
SFB025	MG1655, *mukB(Y416P)*	**S3c**
SFB026	MG1655, *mukB(L960E)*	**S3c**
SFB030	MG1655, *mukB(Y416D)-HaloTag(C61V, C262A)::neoR*	**S3e**
SFB031	MG1655, *mukB(Y416P)-HaloTag(C61V, C262A)::neoR*	**S3e**
SFB032	MG1655, *mukB(L960E)-HaloTag(C61V, C262A)::neoR*	**S3e**
***S. cerevisiae* strains**		
W303	*Mat a, ade2-1, trp1-1, can1-100, leu2-3, 112, his3-11, 15, ura3, GAL, psi*	**-**
2017	W303, Smc3-HA6::HIS3, Scc1-PK9::NatMX, pBH826 (Smc1(D588TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2018	W303, Smc3-HA6::HIS3, Scc1-PK9::NatMX, pBH827 (Smc1(E562TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2019	W303, Smc3-HA6::HIS3, Scc1-PK9::NatMX, pBH828 (Smc1(T565TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2020	W303, Smc3-HA6::HIS3, Scc1-PK9::NatMX, pBH829 (Smc1(K620TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2021	W303, Smc3-HA6::HIS3, Scc1-PK9::NatMX, pBH830 (Smc1(E591TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2022	W303, Smc3-HA6::HIS3, Scc1-PK9::NatMX, pBH831 (Smc1(D592TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2023	W303, Smc3-HA6::HIS3, Scc1-PK9::NatMX, pBH832 (Smc1(D593TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2069	W303, Pds5-6xHis-6xFLAG::KanMX, Scc1-PK9::NatMX, pBH826 (Smc1(D588TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2070	W303, Pds5-6xHis-6xFLAG::KanMX, Scc1-PK9::NatMX, pBH827 (Smc1(E562TAG)-myc9 in YEplac181), pBH61 (BPA crosslink, Trp1)	**4b**
2071	W303, Pds5-6xHis-6xFLAG::KanMX, Scc1-PK9::NatMX, pBH828 (Smc1(T565TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2072	W303, Pds5-6xHis-6xFLAG::KanMX, Scc1-PK9::NatMX, pBH829 (Smc1(K620TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b, c, S4**
2073	W303, Pds5-6xHis-6xFLAG::KanMX, Scc1-PK9::NatMX, pBH830 (Smc1(E591TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2074	W303, Pds5-6xHis-6xFLAG::KanMX, Scc1-PK9::NatMX, pBH831 (Smc1(D592TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2075	W303, Pds5-6xHis-6xFLAG::KanMX, Scc1-PK9::NatMX, pBH832 (Smc1(D593TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4b**
2221	W303, Pds5-6xHis-6xFLAG::KanMX, ura3::Scc1-PK9::URA3, pBH829 (Smc1(K620TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4d**
2223	W303, Pds5-6xHis-6xFLAG::KanMX, ura3::Scc1(V137K)-PK9::URA3, pBH829 (Smc1(K620TAG)-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4d**
2357	W303, Pds5-6xHis-6xFLAG::KanMX, Scc1-PK9::NatMX, pBH768 (Smc1-myc9 in YEplac181), pBH61 (BPA cross-link, Trp1)	**4c, S4**