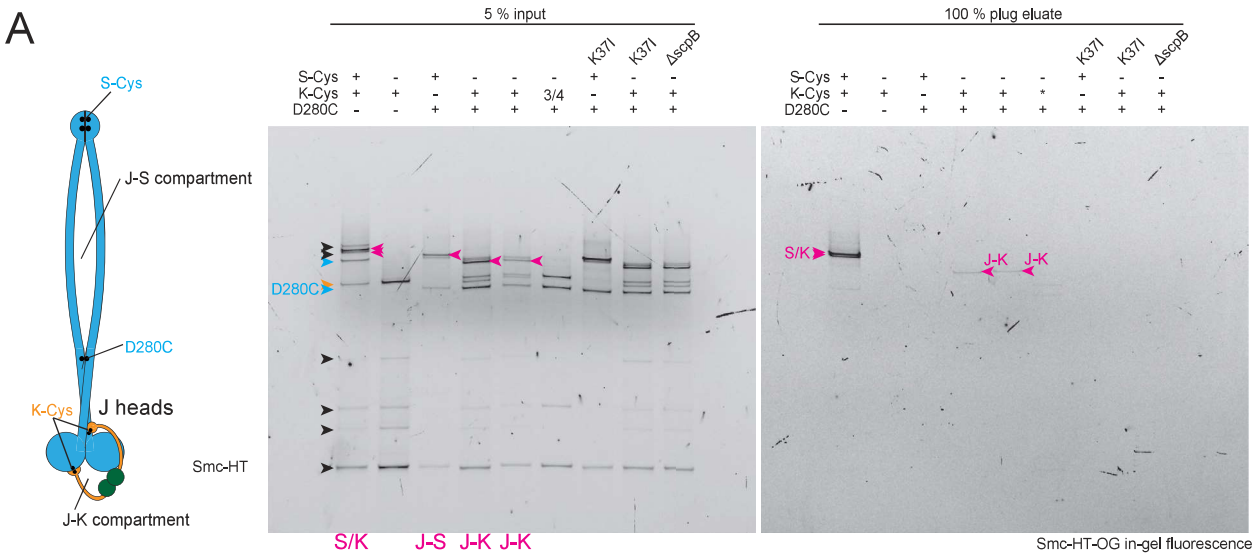
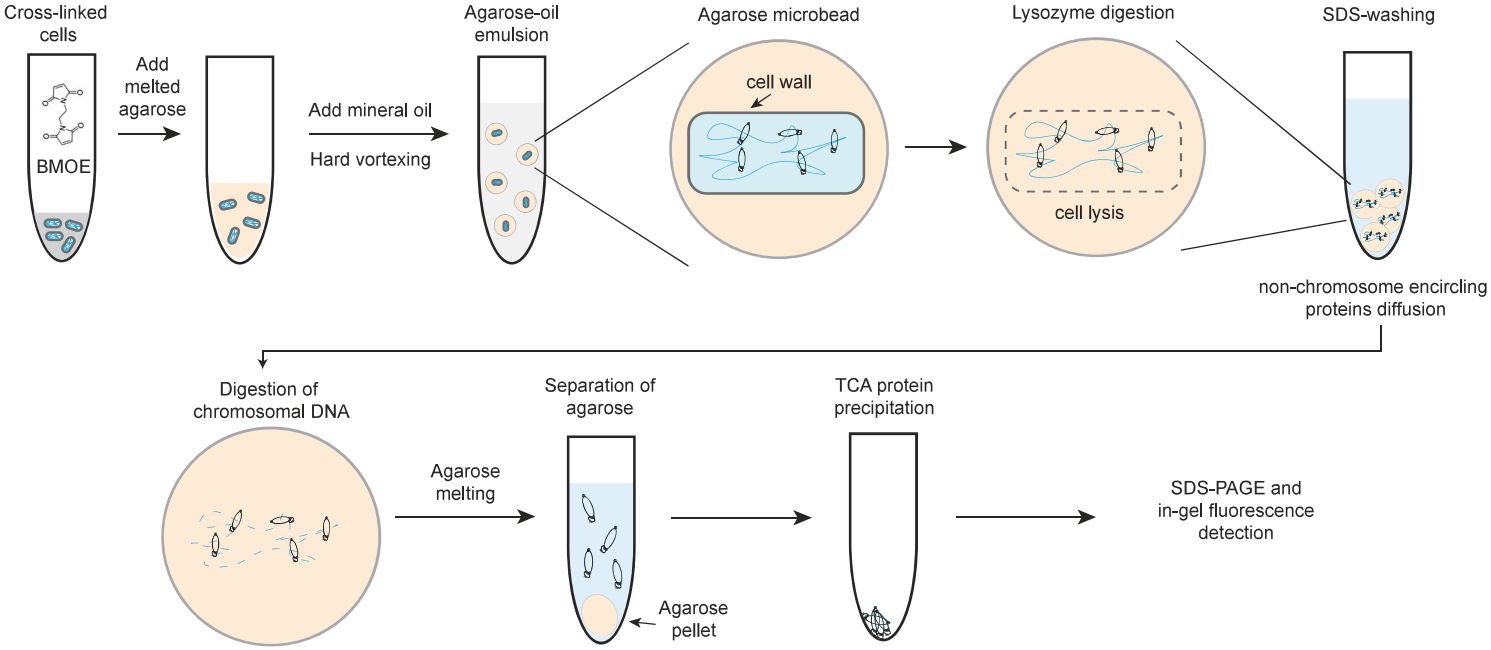

A
5 % input
100 % plug eluate
S-Cys
K-Cys
D280C
K37I
ΔscpB
S-Cys
J-S compartment
D280C
K-Cys
J heads
Smc-HT
J-K compartment
S/K
J-S
J-K
Smc-HT-OG in-gel fluorescence


B
Cross-linked cells
Add melted agarose
BMOE
Add mineral oil
Hard vortexing
Agarose-oil emulsion
Agarose microbead
cell wall
Lysozyme digestion
cell lysis
SDS-washing
non-chromosome encircling proteins diffusion
Digestion of chromosomal DNA
Agarose melting
Separation of agarose
Agarose pellet
TCA protein precipitation
SDS-PAGE and in-gel fluorescence detection

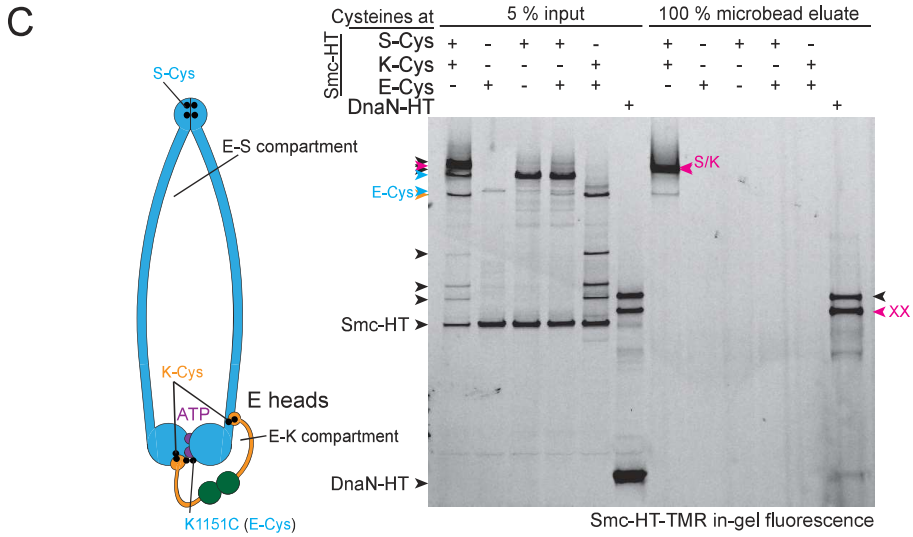

C
Cysteines at
5 % input
100 % microbead eluate
Smc-HT
S-Cys
K-Cys
E-Cys
DnaN-HT
S-Cys
E-S compartment
K-Cys
ATP
E heads
E-K compartment
K1151C (E-Cys)
E-Cys
S/K
Smc-HT
XX
DnaN-HT
Smc-HT-TMR in-gel fluorescence

Figure S1

Figure S1, related to Figure 1.

(A) Chromosome entrapment assay using agarose plugs (as in Figure 1C) for strains harboring the Smc arm cross-linking residue D280C. Strains in lane 4 and 5 are identical except for mutations in the HaloTag and cysteine-free Smc (lane 5). Strain in lane 6 harbors only three out of four cysteines for K-Cys cross-linking and thus cannot produce circular species. Labelling of bands as in Figure 1B-D.

(B) Scheme of the optimized chromosome entrapment assay using agarose microbeads instead of agarose plugs. Cells are incubated with BMOE and rapidly mixed with molten agarose solution and with mineral oil. An emulsion is formed by continuous vortexing until the agarose forms gel microbeads. The oil is removed and the cells in the agarose microbeads are labelled with HaloTag ligands and lysed. The microbeads are washed in presence of SDS. Proteins retained in the agarose microbeads are released by DNA digestion and precipitated to finally resolve them by SDS-PAGE and in-gel fluorescence detection.

(C) Chromosome entrapment assay with agarose microbeads. As in Figure 1D for E heads Smc/ScpAB instead of J heads Smc/ScpAB. Labelling as in Figure 1D. Species cross-linked by E-Cys (K1151C) ('E') are indicated by an arrowhead in blue colors. Please note that E-Cys cross-linking is inefficient in wt Smc. Species for E-S and E-K compartments are difficult to discern in the input samples and absent from agarose eluates. A mix of cells carrying wt DnaN (80 %), DnaN-HT (10 %) and DnaN(Cys)-HT (10 %) is included as positive and negative control for DNA entrapment. Arrowheads in magenta colors denote circular protein species. 'XX' indicates the double cross-linked, circular DnaN-HT species. The single cross-linked, X-shaped DnaN-HT species exhibits slightly slower mobility (labeled by arrowhead in black colors).

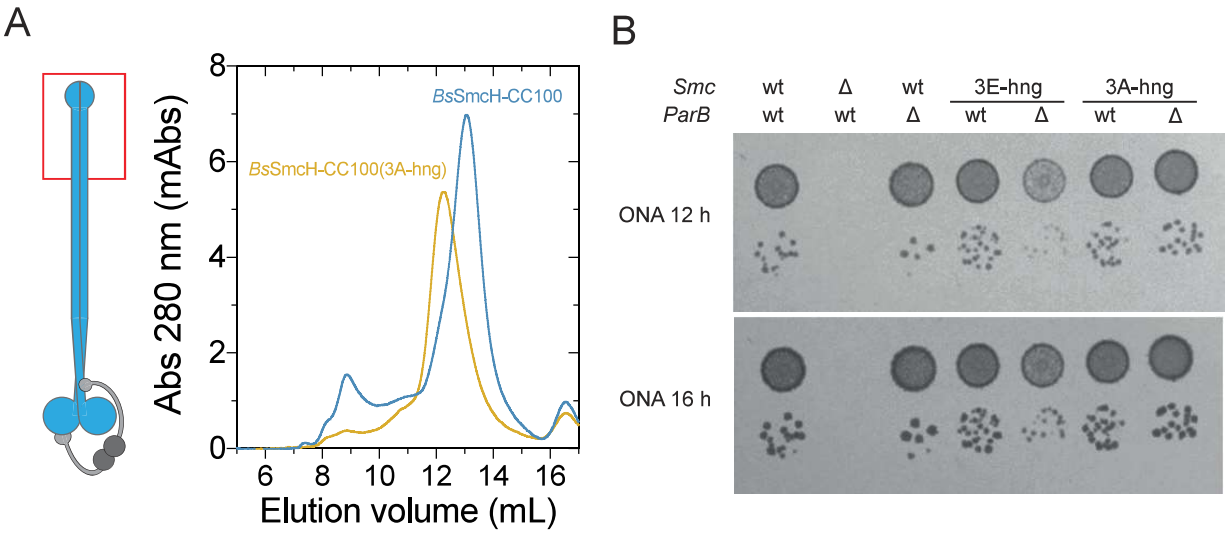

A
B
Abs 280 nm (mAbs)
Elution volume (mL)
BsSmcH-CC100
BsSmcH-CC100(3A-hng)
Smc
ParB
wt
wt
Δ
wt
Δ
wt
3E-hng
wt
Δ
3A-hng
wt
Δ
ONA 12 h
ONA 16 h

Figure S2

Figure S2, related to Figure 2.

(A) Size exclusion chromatography of SmcH-CC100 proteins. Gel filtration profile on a Superdex 200 10/300 column. The 3A-hng mutation does not affect dimer formation but appears to increase the fraction of an extended ('open-arm') conformation.

(B) Dilution spotting of *smc(3E-hng)* and *smc(3A-hng)* cells on nutrient-rich medium (ONA) grown for 12 hours (upper panel) or 16 hours (lower panel). The lower panel is identical to the one shown in Figure 2D.

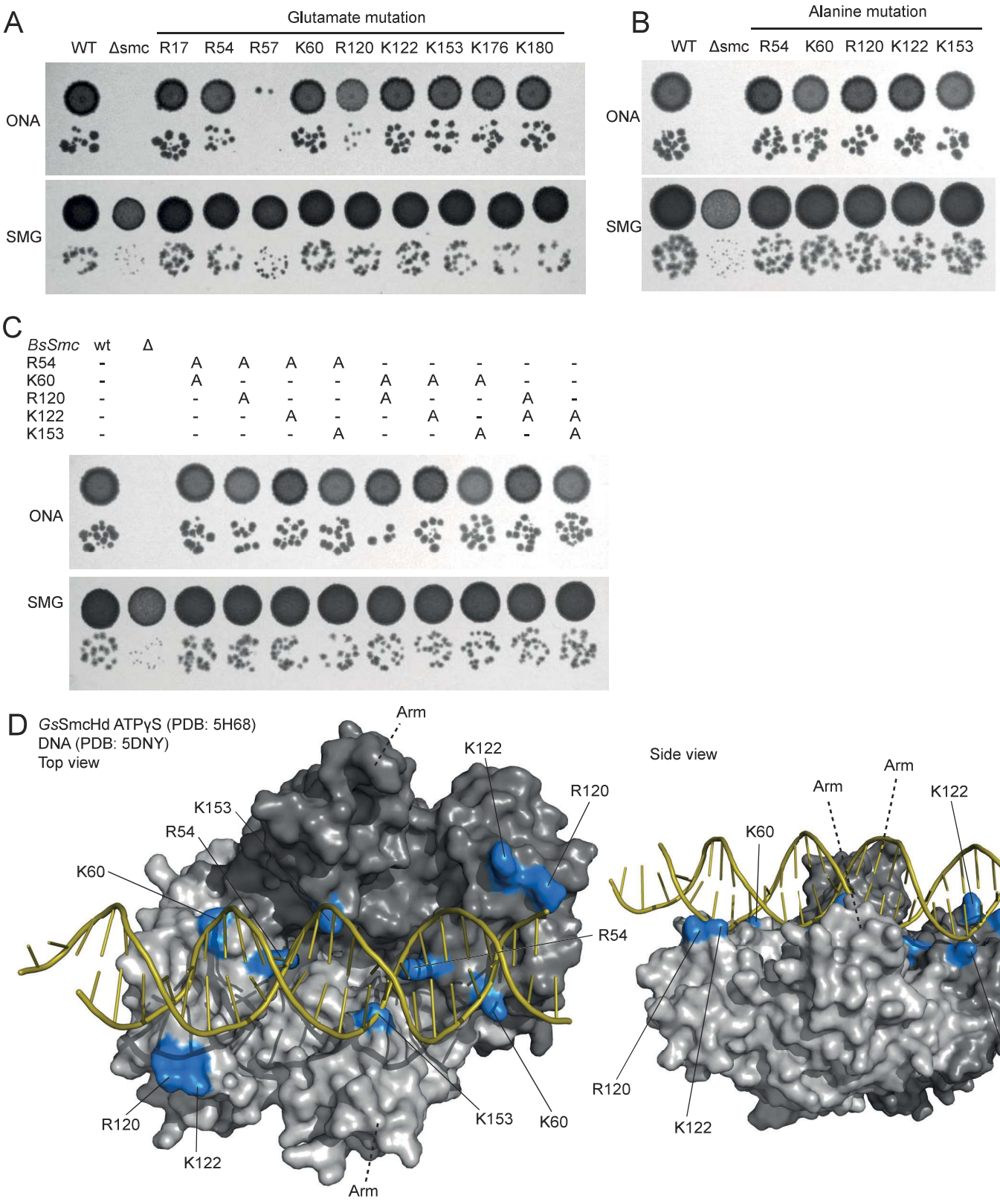

A
Glutamate mutation
WT Δsmc R17 R54 R57 K60 R120 K122 K153 K176 K180
ONA
SMG
B
Alanine mutation
WT Δsmc R54 K60 R120 K122 K153
ONA
SMG
C
BsSmc wt Δ
R54 - A A A A - - - - -
K60 - A - - - A A A - -
R120 - - A - - A - - A -
K122 - - - A - - A - A A
K153 - - - - A - - A - A
ONA
SMG
D
GsSmcHd ATPγS (PDB: 5H68)
DNA (PDB: 5DNY)
Top view
Arm
K122
R120
K153
R54
K60
R54
R120
K122
K153
K60
Arm
Side view
Arm
Arm
K122
K60
R120
R120
K122
K60

Figure S3

Figure S3, related to Figure 3.

(A) Dilution spotting of single glutamate mutants. R57 is buried in E heads Smc and contacts ATP, thus it was not considered to be a DNA binding residue. As in Figure 2B.

(B) Dilution spotting of five single alanine mutants. As in (A).

(C) Dilution spotting of ten double alanine mutant combinations. As in (A).

(D) Top view of *G. stearothermophilus* SmcHd-ATPγS structure (PDB: 5H68) in gray, superimposed with the Rad50Hd-ATPγS-DNA structure (PDB: 5DNY). Only DNA is shown in yellow colors. In blue colours, the five positively-charged residues identified in Figure 3B and 3C are shown. ATPγS is not shown. As in Figure 3D using a different Rad50-ATPγS-DNA co-structure for superimposition. Top view (left panel) and side view (right panel).

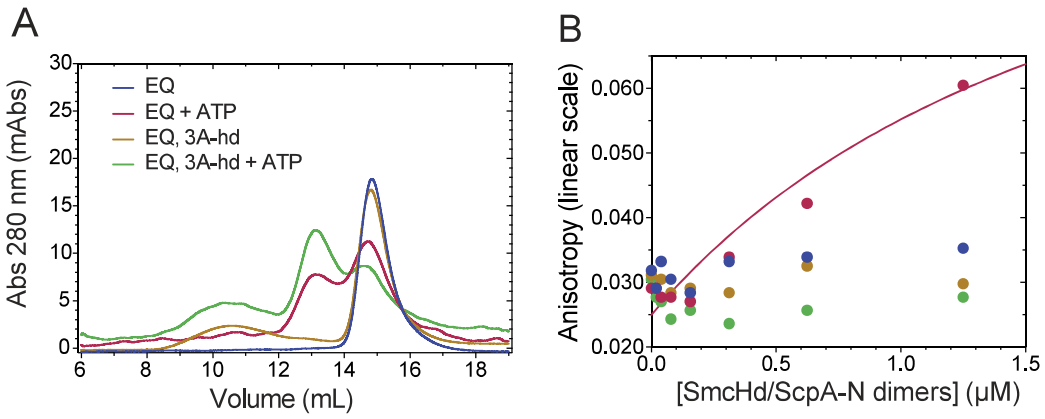

A
Abs 280 nm (mAbs)
Volume (mL)
EQ
EQ + ATP
EQ, 3A-hd
EQ, 3A-hd + ATP
B
Anisotropy (linear scale)
[SmcHd/ScpA-N dimers] (μM)

Figure S4

Figure S4, related to Figure 4.

(A) Chromatogram of SmcHd/ScpA-N elution in a Superdex 200 10/300 size exclusion column. Same experiments as in Figure 4C showing a wider volume range.

(B) DNA binding curves represented by the fluorescence anisotropy (linear scale) for low protein concentrations. Same data as in Figure 4D.

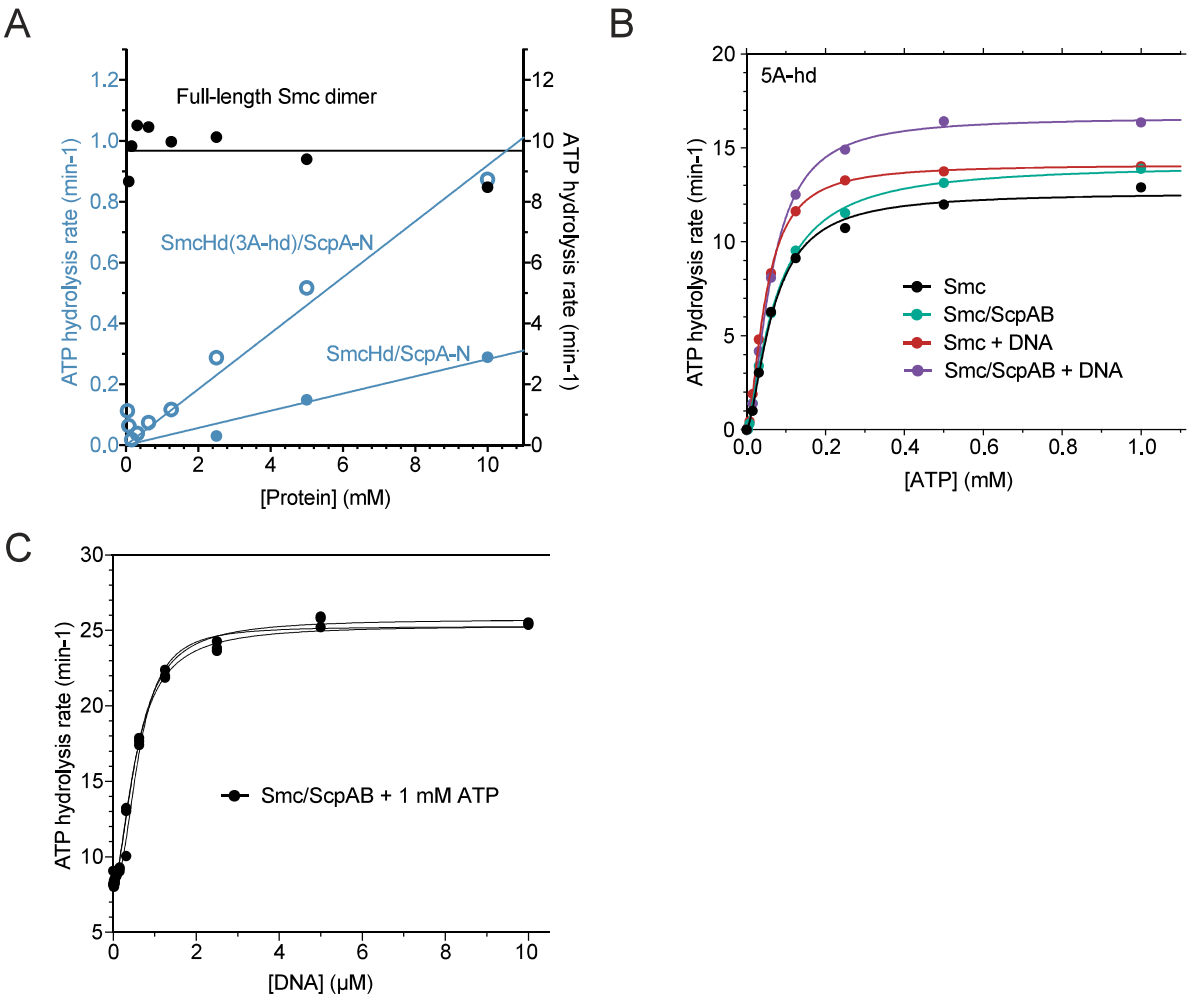

A
Full-length Smc dimer
SmcHd(3A-hd)/ScpA-N
SmcHd/ScpA-N
ATP hydrolysis rate (min-1)
ATP hydrolysis rate (min-1)
[Protein] (mM)
B
5A-hd
Smc
Smc/ScpAB
Smc + DNA
Smc/ScpAB + DNA
ATP hydrolysis rate (min-1)
[ATP] (mM)
C
Smc/ScpAB + 1 mM ATP
ATP hydrolysis rate (min-1)
[DNA] (μM)

Figure S5

Figure S5, related to Figure 5.

(A) ATP hydrolysis activity of SmcHd/ScpA-N mutants (axis and data in blue colors) and full-length Smc (axis and data in black colors) at increasing protein concentrations. Data points for the 3A-hd mutant of SmcHd/ScpA-N are shown in open circles. Each rate was divided by the corresponding protein concentration and fit to the first order k_{cat} equation for full-length Smc dimer, or to the second order k_{cat} equation for the SmcHd/ScpA-N variants (see STAR*Methods for details). The k_{cat} values for full-length Smc, SmcHd/ScpA-N and SmcHd(3A-hd)/ScpA-N are 9.6 + 0.2 min^{-1}, 28.5 ± 2.5 mM^{-1} min^{-1}, and 91.9 ± 4.75 mM^{-1} min^{-1}, respectively.

(B) ATP hydrolysis rate for full-length Smc(5A-hd) protein. As in Figure 5C.

(C) DNA titration of wt Smc/ScpAB in the presence of 1 mM ATP. Data points and fits for three experiments are shown.

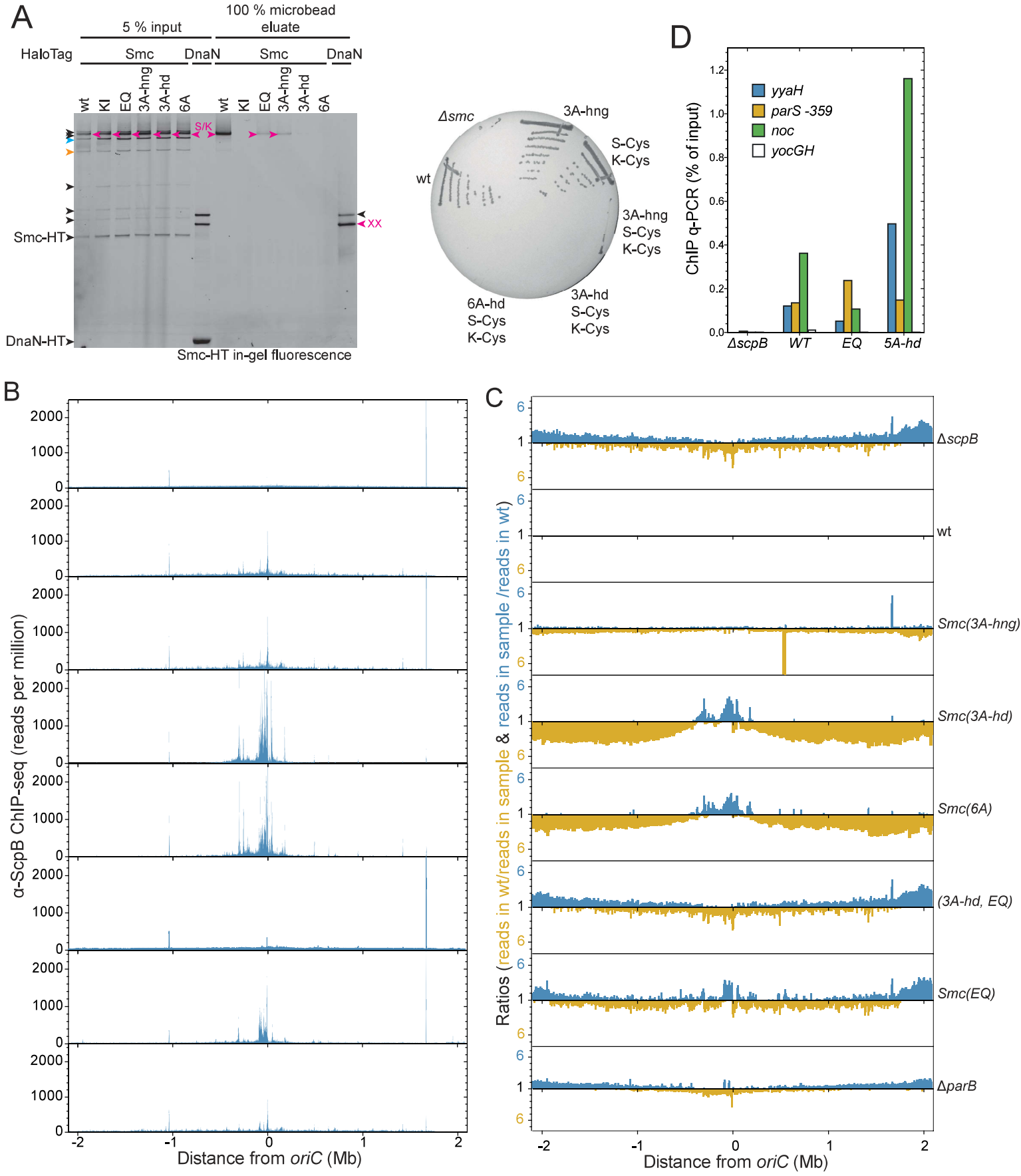

A
5 % input
100 % microbead eluate
HaloTag
Smc
DnaN
wt
KI
EQ
3A-hng
3A-hd
6A
S/K
Smc-HT
DnaN-HT
XX
Smc-HT in-gel fluorescence
Δsmc
wt
3A-hng
S-Cys
K-Cys
3A-hng
S-Cys
K-Cys
3A-hd
S-Cys
K-Cys
6A-hd
S-Cys
K-Cys
D
yyaH
parS -359
noc
yocGH
ChIP q-PCR (% of input)
ΔscpB
WT
EQ
5A-hd
B
α-ScpB ChIP-seq (reads per million)
Distance from oriC (Mb)
C
Ratios (reads in wt/reads in sample & reads in sample /reads in wt)
ΔscpB
wt
Smc(3A-hng)
Smc(3A-hd)
Smc(6A)
(3A-hd, EQ)
Smc(EQ)
ΔparB
Distance from oriC (Mb)

Figure S6

Figure S6, related to Figure 6.

(A) Chromosome entrapment assay for S/K rings in mutant Smc/ScpAB using agarose microbeads (left panel). As in Figure 6A but including the 3A-hng and 6A mutants. Growth of strains with DNA binding mutations and cross-linking cysteines on nutrient rich medium by single-colony streaking (right panel). Incubation at 37° C for 16 hr.

(A) Chromosome-wide ChIP-seq profiles (in reads per million) for data shown in Figure 6B.

(B) Ratiometric analysis of chromosome-wide profiles shown in (B). Ratios were calculated as described in Figure 6C.

(D) α-ScpB-ChIP-qPCR. As in Figure 6D for the 5A-hd mutant.

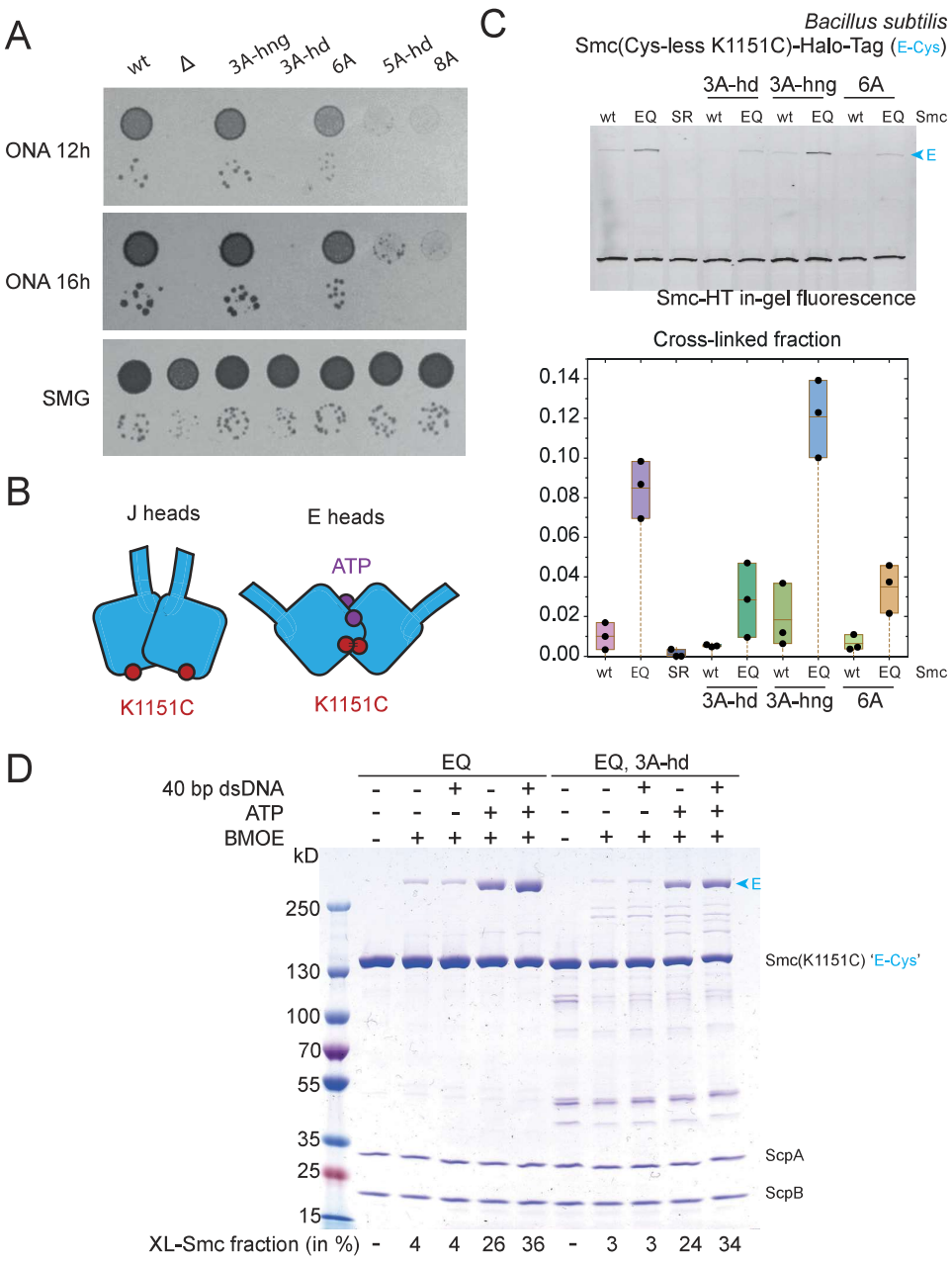

A
wt
Δ
3A-hng
3A-hd
6A
5A-hd
8A
ONA 12h
ONA 16h
SMG
B
J heads
E heads
ATP
K1151C
K1151C
C
Bacillus subtilis
Smc(Cys-less K1151C)-Halo-Tag (E-Cys)
3A-hd
3A-hng
6A
wt EQ SR wt EQ wt EQ wt EQ Smc
E
Smc-HT in-gel fluorescence
Cross-linked fraction
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
wt EQ SR wt EQ wt EQ wt EQ Smc
3A-hd
3A-hng
6A
D
EQ
EQ, 3A-hd
40 bp dsDNA
ATP
BMOE
kD
250
130
100
70
55
35
25
15
E
Smc(K1151C) 'E-Cys'
ScpA
ScpB
XL-Smc fraction (in %) - 4 4 26 36 - 3 3 24 34

Figure S7

Figure S7, related to Figure 7.

(A) Dilution spotting of *smc(6A)* and *smc(5A-hd, 3A-hng)* ('8A') strains. Growth on ONA was monitored after 12 and 16 hr (upper and middle panels). As in Figure 2D.

(B) Schematic view of K1151C cross-linking in E heads but not J heads Smc/ScpAB.

(C) Head engagement levels in 3A-hd, 3A-hng and 6A mutants as measured by *in vivo* cross-linking of E-Cys (K1151C). In gel-fluorescence of HaloTag-TMR ligand conjugate is shown in the top panel and the intensity quantification ratio in the bottom panel. Each dot in the graph represents an experimental data point. The boxes span from the lowest to the highest value and the horizontal line denotes the mean from three biological replicates. SR denotes the Signature motif mutation (S1090R), EQ (E1118Q).

(D) Head engagement levels in EQ and (3A-hd, EQ) mutant Smc/ScpAB as measured by in vitro cross-linking of E-Cys. Quantification of cross-linked fractions is given below the gel image. Please note that the purified preparation of Smc(3A-hd, EQ) contains a number of impurities.

Supplemental tables

Table S1, related to Figures 1-7. Strain usage. Genotypes of all strains are given in Table S6.

Figure	Strains
1B	BSG1682, BSG1681, BSG1719, BSG3109, BSG2264, BSG1782, BSG2360, BSG2370
1C	BSG1782, BSG2360, BSG2370, BSG2709, BSG2695
1D	BSG1782, BSG2360, BSG2370, BSG2709, BSG2695, BSG1002, BSG1449, BSG1459
2D	BSG1002, BSG1007, BSG1050, BSG1993, BSG2011, BSG1992, BSG2046
3B	BSG1002, BSG1007, BSG2879, BSG2870, BSG2907, BSG2871, BSG2881, BSG2971, BSG2873, BSG2899, BSG2882, BSG2910
3C	BSG1002, BSG1007, BSG2873, BSG2903, BSG2909, BSG2911
6A	BSG1782, BSG1784, BSG1786, BSG3447, BSG1002, BSG1449, BSG1459
6B, C	BSG1489, BSG1002, BSG1993, BSG2873, BSG3213, BSG1008
6D	BSG1489, BSG1002, BSG1008, BSG2873
7A	BSG1002, BSG1007, BSG1008, BSG1993, BSG2873, BSG3214, BSG3213
7C	BSG1682, BSG1681, BSG1719, BSG3109, BSG1457, BSG1488, BSG1782, BSG3107
7D	BSG1782, BSG1786, BSG1726, BSG3107, BSG3799, BSG3800, BSG1002, BSG1449, BSG1459
S1A	BSG1782, BSG1810, BSG2361, BSG2371, BSG2363, BSG2712, BSG2710, BSG2725
S1C	BSG1782, BSG1457, BSG3109, BSG3097, BSG3098, BSG1002, BSG1449, BSG1459
S2B	BSG1002, BSG1007, BSG1050, BSG1993, BSG2046, BSG1992, BSG2011
S3A	BSG1002, BSG1007, BSG2812, BSG2813, BSG2833, BSG2834, BSG2835, BSG2836, BSG2972, BSG2837, BSG2838
S3B	BSG1002, BSG1007, BSG2868, BSG2880, BSG2874, BSG2876, BSG2904
S3C	BSG1002, BSG1007, BSG2902, BSG2878, BSG2869, BSG2905, BSG2877, BSG2872, BSG2896, BSG2875, BSG2906
S6A	BSG1782, BSG1784, BSG1786, BSG3780, BSG3447, BSG3775, BSG1002, BSG1449, BSG1459, BSG1002, BSG1007, BSG1992, BSG1782, BSG3780, BSG3775, BSG3447
S6B, C	BSG1489, BSG1002, BSG1993, BSG2873, BSG3213, BSG3214, BSG1008, BSG1050
S6D	BSG1489, BSG1002, BSG1007, BSG2911
S7A	BSG1002, BSG1007, BSG1993, BSG2873, BSG3213, BSG2911, BSG3068
S7C	BSG1457, BSG1488, BSG1600, BSG3599, BSG3600, BSG3618, BSG3619, BSG3616, BSG3617

Table S2, related to Figures 1 and 7. Fraction of cross-linked species.

Cross-linked species	K-Cys (N only)	K-Cys (C only)	K-Cys	S-Cys	J-Cys	E-Cys (wt)	E-Cys (EQ)	K-Cys/ S-Cys	S-Cys/ J-Cys	K-Cys/ J-Cys
Smc-HT	0.65 ± 0.014	0.67 ± 0.002	0.46 ± 0.007	0.13 ± 0.011	0.33 ± 0.035	0.99 ± 0.003	0.84 ± 0.003	0.09 ±0.002	0.14 ± 0.008	0.20 ± 0.011
C		0.32 ± 0.001	0.05 ± 0.004					0.02 ± 0.002		0.02 ± 0.002
N	0.34 ± 0.009		0.06 ± 0.001					0.017 ±0.002		0.02 ± 0.003
X			0.02 ± 0.001					0.009 ± 0.003		0.02 ± 0.003
J					0.66 ± 0.031				0.11 ± 0.007	0.19 ± 0.005
K			0.38 ± 0.001					0.074 ±0.007		0.17± 0.003
S				0.86 ± 0.01				0.17 ± 0.008	0.30 ± 0.035	
E						0.003 ± 0.001	0.13 ± 0.021			
JC										0.36 ± 0.002
JN										0.055 ± 0.003
Y										0.05 ± 0.010
S/K								n.d		
Calc.								*0.33*		
J-S									0.43 ± 0.038	
Calc.									*0.569*	
J-K										0.19 ± 0.018
Calc.										*0.257*

Table S3, related to Figures 2 and 4. Fluorescence anisotropy DNA-binding fit values.

Figure	Construct	K_d (μM)	A_{min}	A_{max}	replicates
1D	SmcH-CC100	0.16 ± 0.01	0.034 ± 0.001	0.21 ± 0.002	3
1D	SmcH-CC100 (3A-hng)	5.8 ± 2.3	0.034 ± 0.0009	0.10 ± 0.02	3
3D	SmcHd(EQ)/ ScpA-N	n.d	n.d	n.d	3
3D	SmcHd(EQ)/ ScpA-N + 1mM ATP	1.90 ± 0.31	0.024 ± 0.001	0.088 ± 0.003	3
3D	SmcHd(EQ, 3A-hd)/ ScpA-N	n.d	n.d	n.d	3
3D	SmcHd(EQ, 3A-hd)/ ScpA-N + 1 mM ATP	n.d	n.d	n.d	3

Table S4, related to Figure 3 and S3.
parB deletion in single glutamate *smc* mutants

Mutant	Number of clones checked for Δ*parB*	Number of positive clones for Δ*parB*
wt	8	8
Δsmc	2	0
R17E	8	7
R54E	16	0
R57E	5	0
K60E	24	0
R120E	24	1
K122E	24	0
K153E	16	0
K176E	8	8
K180E	8	6

Table S5, related to Figure 5. Kinetic parameters of the Smc ATPase.

Construct	k_{cat} (min^{-1})	$K_{0.5}$ (mM)	h	Repli-cates
SmcHd/ScpA-N (10μM)	0.0002 ± 0.000009	0.20 ± 0.01	1.4 ± 0.1	2
SmcHd(3A-hd)/ScpA-N (10 μM)	0.0009 ± 0.00003	0.48 ± 0.03	1.12 ± 0.05	2
Smc wt	10.9 ± 0.8	0.35 ± 0.06	1.07 ± 0.086	3
Smc wt/ScpAB	6.66 ± 0.45	0.20 ± 0.03	0.88 ± 0.07	3
Smc wt + 3 μM dsDNA	14.15 ± 0.55	0.063 ± 0.006	1.8 ± 0.2	3
Smc wt/ScpAB + 3 μM dsDNA	24.12 ± 0.84	0.112 ± 0.008	1.92 ± 0.22	3
Smc(R57A)	4.76 ± 0.86	0.51 ± 0.22	0.89 ± 0.11	2
Smc(R57A)/ScpAB	6.34 ± 0.3	0.32 ±0.03	0.94 ± 0.04	2
Smc(R57A) + 3 μM DNA	4.85 ± 0.55	0.43 ± 0.1	1.08 ± 0.1	2
Smc(R57A)/ScpAB + 3 μM DNA	10.6 ± 0.72	0.45 ±0.07	0.93 ± 0.04	2
Smc(3A-hd)	10.1 ± 0.2	0.051 ± 0.003	1.3 ± 0.1	3
Smc(3A-hd)/ScpAB	9.9 ± 0.1	0.065 ± 0.001	1.41 ± 0.04	3
Smc(3A-hd) + 3 μM dsDNA	10.8 ± 0.1	0.034 ± 0.001	1.68 ± 0.09	3
Smc(3A-hd)/ScpAB + 3 μM dsDNA	17.2 ± 0.3	0.037 ± 0.002	1.82 ± 0.16	3
Smc(3A-hng)	18.5 ± 3.6	0.87 ± 0.38	0.86 ± 0.08	3
Smc(3A-hng)/ScpAB	121.7 ± 13.0	1.06 ± 0.19	1.1 ± 0.05	3
Smc(3A-hng) + 3 μM dsDNA	14.6 ± 0.4	0.17 ± 0.01	1.28 ± 0.07	3
Smc(3A-hng)/ScpAB + 3 μM dsDNA	82.2 ± 2.2	0.47 ± 0.02	1.33 ± 0.03	3
Smc(6A)	10.7 ± 0.6	0.18 ± 0.02	1.14 ± 0.09	2
Smc(6A)/ScpAB	43.04 ± 0.46	0.151 ± 0.003	1.68 ± 0.04	2
Smc(6A) + 3 μM dsDNA	10.07 ± 0.43	0.17 ± 0.01	1.17 ± 0.08	2
Smc(6A)/ScpAB + 3 μM dsDNA	43.3 ± 0.4	0.146 ± 0.003	1.72 ± 0.05	2
Smc(5A-hd)	12.6 ± 0.3	0.066 ± 0.005	1.5 ± 0.1	2
Smc(5A-hd)/ScpAB	14.4 ± 0.2	0.075 ± 0.003	1.36 ± 0.05	2
Smc(5A-hd) + 3 μM dsDNA	14.1 ± 0.1	0.048 ± 0.0009	1.63 ± 0.04	2
Smc(5A-hd)/ScpAB + 3 μM dsDNA	16.6 ± 0.2	0.063 ± 0.002	1.67 ± 0.09	2