**Highlights**

- Smc4 and Smc2 ATPase head structures reorganize upon ATP binding and dimerization.

- A Q-loop-mediated switch releases the Ycs4 HEAT-repeat subunit from the Smc4 head.

- The Smc2 head engages with the ATP-bound Smc4 head into an asymmetric heterodimer.

- Head dimerization releases the Brn1 kleisin from Smc2 via coiled-coil rotation.

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**In Brief**

Hassler et al. report structural and functional insights into the enzymatic core of the condensin protein complex that reveal large-scale conformational changes upon ATP binding by and subsequent dimerization of its catalytic SMC head domains. These movements presumably power the condensin-mediated extrusion of DNA loops during mitotic chromosome formation.
Structural Basis of an Asymmetric Condensin ATPase Cycle

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SUMMARY

The condensin protein complex plays a key role in the structural organization of genomes. How the ATPase activity of its SMC subunits drives large-scale changes in chromosome topology has remained unknown. Here we reconstruct, at near-atomic resolution, the sequence of events that take place during the condensin ATPase cycle. We show that ATP binding induces a conformational switch in the Smc4 head domain that releases its hitherto undescribed interaction with the Ycs4 HEAT-repeat subunit and promotes its engagement with the Smc2 head into an asymmetric heterodimer. SMC head dimerization subsequently enables nucleotide binding at the second active site and disengages the Brm1 kleisin subunit from the Smc2 coiled coil to open the condensin ring. These large-scale transitions in the condensin architecture lay out a mechanical path for its ability to extrude DNA helices into large loop structures.

INTRODUCTION

Multi-subunit protein complexes of the structural maintenance of chromosomes (SMC) family direct large-scale organizational changes in genome architecture that are essential for all aspects of chromosome biology. In addition to their central functions in the segregation of replicated genomes during prokaryotic and eukaryotic cell divisions (Hirano, 2016; Uhlmann, 2016), SMC complexes of cohesin (Onn et al., 2007) and cohesin, might be their ability to extrude DNA into loop structures (Goloborodko et al., 2016; Nasmyth, 2001). This hypothesis is consistent with the recent discoveries that condensin complexes purified from budding yeast are able to translocate along DNA double helices and processively expand loops of DNA of several kilobase pairs in length in a fashion that depends on their ability to hydrolyze ATP (Ganji et al., 2018; Terakawa et al., 2017). How the energy of nucleotide binding, hydrolysis, and release is converted into DNA translocation and looping movements has remained unknown, but currently available models rely on mechanochemical coupling of the ATPase cycle to large conformational transitions that affect chromosome interactions by creating topological compartments or contact sites that entrap or directly bind DNA, respectively (Gruber, 2017; Hassler et al., 2018).

Based on their homology to ATP binding cassette (ABC) transmembrane transporters and the Rad50 DNA damage repair protein (Hopfner, 2016), the two globular ATPase “head” domains situated at the ends of ~50-nm-long intra-molecular coiled coils of a heterodimer of condensin’s Smc2 and Smc4 subunits are thought to sandwich a pair of ATP molecules between catalytic sites, each composed of Walker A (P loop) and Walker B motifs of one head and a so-called ABC signature motif of the opposite head. Each head domain (hd) can be subdivided into a “RecA”-like lobe that contains the ATP-binding pocket and a “helical” lobe that merges into the coiled coils; they connect the heads to a half-doughnut-shaped “hinge” dimerization domain. Smc2_Hd and Smc4_Hd are furthermore connected by their binding to opposite ends of the Bm1_Cnd2/NCPA31 kleisin subunit (Onn et al., 2007). Several crystal structures have revealed the formation of a helical bundle between the kleisin N-terminal domain and the coiled-coil “neck” region immediately adjacent to one SMC head (the •SMC_Hd) and the interaction of the kleisin C-terminal winged helix domain (WHD) with the “cap” region located at the distal surface of the other SMC head (the •SMC_Hd) of cohesin (Gilgioris et al., 2014; Haering et al., 2004) or bacterial SMC complexes (Bürmann et al., 2013; Diebold-Durand et al., 2017; Kamada et al., 2017; Woo et al., 2009). No structural information has so far been available for the...
homologous ATPase domains or interfaces of the condensin complex. The condensin SMC-kleisin ring structure has been proposed to topologically encircle chromosomal DNA (Guylen et al., 2011), which is consistent with the finding that other tripartite SMC-kleisin rings that have been covalently circularized retain their association with chromosomal DNA even after protein denaturation (Gilgonis et al., 2014; Haering et al., 2008; Wilhelm et al., 2015). Data from cohesin suggest that DNA release from these rings relies on the opening of the interface between the kleisin N terminus and the SMC coiled coil (cc), which serves as a DNA exit (Buheitel and Stemmann, 2013; Chan et al., 2012; Huis in ’t Veld et al., 2014) and, potentially, also as an entry gate (Murayama and Uhlmann, 2015).

The central role of the ATPase cycle to SMC complex function is underscored by the fact that the majority of mutations that affect nucleotide binding, head dimerization, or ATP hydrolysis render cohesin or condensin non-functional (Arumugam et al., 2003; Hudson et al., 2008; Kinoshita et al., 2015; Palou et al., 2018; Thadani et al., 2018; Weitzer et al., 2003). Although these mutations largely abolish the association of cohesin with chromosomes, some of the homologous mutations in condensin have less dramatic effects on the chromosomal levels of condensin. This difference might be due to the presence of an ATPase-independent DNA binding site formed at the interface between the Brn1 kleisin subunit and the Ycg1Cnd3/NCAPG subunit, which is composed of multiple repeats of α-helical HEAT (huntingtin, elongation factor 3, protein phosphatase 2A, Tor1 kinase) motifs (Kschonsak et al., 2017). The function of the second HEAT-repeat subunit, named Ycs4Cnd1/NCAPDS2, has so far remained unclear, despite its presence being similarly essential for the association of condensin with chromosomes (Kinoshita et al., 2015; Lavoie et al., 2002).

Here we report high-resolution structures of the Smc2 and Smc4 heads, including their interfaces with the N- and C-terminal domains of the Brn1 kleisin subunit, and of the Ycs4-Brn1 complex. We provide evidence for rearrangements of key residues that take place during sequential ATP binding to the two catalytic sites and describe how these structural transitions trigger large-scale conformational changes that result in the dissociation of the Ycs4 subunit from a highly conserved binding site within the Smc4 head, Smc2-Smc4 head dimerization, and, ultimately, release of the Brn1 kleisin subunit from the Smc2 coiled coil.

RESULTS

Structural Basis for Asymmetric ATP Binding by the Condensin SMC Head Domains

To gain functional insights into the condensin ATPase cycle, we solved the crystal structures of Smc2\textsubscript{hd} and the Smc4\textsubscript{hd}-Brn1\textsubscript{C} complex of the thermophilic yeast Chaetomium thermophilum (Ct; Figure S1A; Table S1) to 2.6- or 3.0-Å resolution, respectively (Figure 1A; Table 1). As expected, both structures revealed canonical two-lobed SMC ATPase folds that display a high degree of evolutionary surface conservation at their ATP-binding and head dimerization interfaces (Figure S1B). Although included in the crystallization construct, crystals of the Ct Smc2\textsubscript{hd} domain showed no electron density for the N-terminal Brn1 region, which presumably dissociated during crystallization (see below). In contrast, the Ct Smc4\textsubscript{hd} crystal structure displayed distinct density for the C-terminal Brn1 region, which folds into a wHD and binds to the cap’ face of the SMC ATPase.

A comparison of the nucleotide-free Ct Smc2\textsubscript{hd} and Smc4\textsubscript{hd}-Brn1\textsubscript{C} structures of condensin to the adenosine 5’-[γ-thio]triphosphate (ATP\textsubscript{γS})-bound Saccharomyces cerevisiae (Sc) Smc1\textsubscript{NAD}-Scc1\textsubscript{C} and Smc3\textsubscript{NAD}-Scc1\textsubscript{C} structures of cohesin or to bacterial SMC ATPase head structures revealed differences in the orientations of the helical lobes and attached coiled coils relative to the RecA lobes, which can be explained by flexion movements of ~25° or ~15°, respectively (Figure 1B). These motions displace strictly conserved glutamine residues in the Smc2 and Smc4 Q loops, which are thought to coordinate the catalytic Mg\textsuperscript{2+} ion and contribute to nucleotide binding. Mutation of this residue in Smc4 to leucine indeed drastically reduced the low micromolar affinity of Ct Smc4\textsubscript{hd}-Brn1\textsubscript{C} for ATP (Figure 1C). In contrast, even the wild-type (WT) version of Ct Smc2\textsubscript{hd}-Brn1\textsubscript{N} was unable to bind ATP. This finding is readily explained by the Ct Smc2\textsubscript{hd} structure, where the more pronounced flexion of the Smc2 helical lobe not only repositions the Q loop but also induces a cascade of structural displacement events that alter the P loop of the ATP-binding pocket into a conformation that is incompatible with nucleotide binding (Figure 1B). This incompatibility with ATP binding is even more obvious in a second crystal form of Ct Smc2\textsubscript{hd} (Figure S1C).

Distinct Contributions of the Two ATPase Sites to SMC Head Dimerization

Despite these structural differences, we were able to trap a stable heterodimer of Ct Smc2\textsubscript{hd}-Brn1\textsubscript{N} and Ct Smc4\textsubscript{hd}-Brn1\textsubscript{C} when we prevented ATP hydrolysis by mutation of the catalytic Walker B glutamate residues in both heads (Ct Smc2\textsubscript{hd} E1116G, Ct Smc4\textsubscript{hd} E1475Q; Figures 1D and S2A). This is consistent with the absence of discernible steric clashes in a structural model of an ATP-dimerized Ct Smc2\textsubscript{hd}-Smc4\textsubscript{hd}-Brn1\textsubscript{C} complex (Figure S2B). Consistent with the inability of Ct Smc2\textsubscript{hd} to bind ATP (Figure 1C), preventing ATP hydrolysis only at the Smc2 active site was insufficient for dimer formation, whereas mutation of only the Smc4 active site was sufficient (Figures 1D and S2A). Mutation of the Smc2 signature motif serine residue that contacts the nucleotide bound at the Smc4 active site (Ct Smc2\textsubscript{hd} S1088R) prevented dimerization with Walker B mutant Ct Smc4\textsubscript{hd} E1475Q, whereas simultaneous mutation of the Smc4 signature motif (Ct Smc4\textsubscript{hd} S1447R, E1475Q) still allowed formation of a dimer that, however, eluted at a different retention volume during size-exclusion chromatography (Figure S2A). This suggests that a dimer with a distinct conformation can be mediated solely by ATP sandwiched between the Smc4 Walker A, Walker B, and Smc2 signature motifs. However, the second site formed by the Smc2 Walker A, Walker B, and Smc4 signature motifs must nevertheless be capable of binding and hydrolysing ATP in the context of the heterodimer because mutation of the Smc2 Walker B motif had an even more severe effect on the basal ATPase activity of Ct Smc2\textsubscript{hd}-Brn1\textsubscript{N} and Ct Smc4\textsubscript{hd}-Brn1\textsubscript{C} complexes than mutation of the Smc4 Walker B motif (Figures S2C and S2D). ATP binding to the Smc4 active site is therefore sufficient to induce Smc2-Smc4 head dimerization, which then renders the Smc2 active site capable of binding and hydrolyzing ATP.
Figure 1. Structures and Dimerization of Smc2 and Smc4 ATPase Head Domains
(A) Cartoon models of the Ct Smc2hd (crystal form I) and the Ct Smc4hd-Brn1C complex.
(B) Structural alignment based on the RecA-like lobe of Ct Smc2hd (I) and Ct Smc4hd structures to ATPγS-bound structures of the Sc cohesin Smc1hd (PDB: 1W1W; Cα RMSD = 0.892 and 0.839) and Smc3hd (PDB: 4UX3; Cα RMSD = 2.582 and 1.068) or the nucleotide-free structures of B. subtilis (Bs) SMChd (PDB: 3ZGX; Cα RMSD = 1.828 and 1.914) and P. yayanosii (Py) SMChd (PDB: 5XEI; Cα RMSD = 0.977 and 0.818). Close-up views highlight the positions of the conserved Q-loop glutamine and Walker B glutamate residues aligned to Sc cohesin Smc1hd with ATPγS (gray).
(C) Isothermal titration calorimetry (ITC) of ATP binding by WT Ct Smc2hd-Brn1N and WT or Q-loop mutant Ct Smc4hd-Brn1C (fit ± error of the fit).
(D) Size exclusion chromatography profiles of double or single Walker B mutant combinations of Ct Smc2hd-Brn1N and Ct Smc4hd-Brn1C in the absence (-ATP) or presence (+ATP) of nucleotide.
See also Figures S1 and S2.
Table 1. Crystallography Data Collection and Refinement Statistics

|                     | Ct Smc2hd (I) (SeMet- SAD) | Ct Smc2hd (I) (Native) | Ct Smc4hd-Brn1C | Ct Ycs4-Bm1Y4 (SeMet-SIRAS)* | Ct Ycs4-Bm1Y4 (Native-SIRAS) | Ct Ycs4-Bm1Y4-Smc4hd-Brn1C |
|---------------------|-----------------------------|------------------------|-----------------|------------------------------|-----------------------------|----------------------------|
| **Data collection** |                             |                        |                 |                              |                             |                            |
| Space group         | P 2₁, 2₁, 2₁                | P 6₅                   | P 6₄            | P 2₁                         | P 2₁                        | P 2₁                       |
| Molecules per       | 1                           | 1                      | 1               | 1                            | 1                           | 1                          |
| asymmetric unit     |                             |                        |                 |                              |                             |                            |
| **Cell dimensions (Å)** |                           |                        |                 |                              |                             |                            |
| a                   | 47.32                       | 93.75                  | 132.72          | 86.61                        | 86.07                       | 84.40                      |
| b                   | 107.98                      | 93.75                  | 132.72          | 81.76                        | 80.79                       | 82.38                      |
| c                   | 174.25                      | 117.97                 | 75.55           | 132.88                       | 130.84                      | 177.83                     |
| α                   | 90.00                       | 90.00                  | 90.00           | 90.00                        | 90.00                       | 90.00                      |
| β                   | 90.00                       | 90.00                  | 90.00           | 93.15                        | 90.00                       | 90.00                      |
| γ                   | 90.00                       | 120.00                 | 120.00          | 93.40                        | 90.00                       | 90.00                      |
| **Resolution (Å)**  | 45.94–2.50 (2.57–2.50)      | 81.19–2.00 (2.11–2.00) | 45.74–2.90 (3.06–2.90) | 45.11–3.30 (3.50–3.30) | 44.63–3.38 (3.56–3.38) | 47.74–5.50 (5.80–5.50) |
| Rmerge              | 0.082 (1.854)               | 0.103 (1.733)          | 0.086 (1.497)   | 0.015 (1.108)                | 0.096 (0.763)               | 0.121 (1.359)              |
| lσl                 | 20.09 (1.50)                | 12.3 (1.40)            | 12.4 (1.10)     | 11.5 (2.1)                   | 8.8 (1.7)                   | 8.1 (0.9)                  |
| CC (%)              | 1.0 (5.515)                 | 1.0 (5.558)            | 1.0 (0.497)     | 1.0 (0.704)                  | 1.0 (0.456)                 | 0.99 (0.358)               |
| Completeness (%)    | 99.8 (97.6)                 | 99.9 (99.2)            | 99.9 (99.7)     | 99.3 (96.1)                  | 99.4 (99.5)                 | 97.6 (99.6)                |
| Redundancy          | 16.09 (15.64)               | 10.3 (10.5)            | 6.9 (7.1)       | 12.6 (7.2)                   | 3.4 (3.4)                   | 3.3 (3.5)                  |
| **Refinement**      |                             |                        |                 |                              |                             |                            |
| Resolution (Å)      | 45.90–2.56                  | 66.88–2.00             | 43.45–3.00      | 45.11–3.30                   | 44.63–3.38                   | 47.74–5.80                 |
| No. reflections (total) | 29,681                     | 39,658                 | 15,305          | 27,912                       | 6,655                       | 6,655                      |
| Rwork/Rfree         | 0.23/0.26                   | 0.19/0.22              | 0.22/0.24       | 0.23/0.28                    | 0.29/0.30                   | 0.29/0.30                  |
| No. atoms           |                             |                        |                 |                              |                             |                            |
| Protein             | 3,205                       | 3,178                  | 3,474           | 7,704                        | 10,778                      | 10,778                     |
| Ligand or ion       | 0                           | 0                      | 30              | 0                            | 0                           | 0                          |
| Water               | 41                          | 163                    | 0               | 0                            | 0                           | 0                          |
| B-factors           |                             |                        |                 |                              |                             |                            |
| Protein             | 88.71                       | 59.86                  | 124.61          | 121.21                       | 210.41                      |
| Ligand or ion       | NA                          | NA                     | 172.37          | NA                           | NA                          |
| Water               | 74.31                       | 56.64                  | NA              | NA                           | NA                          |
| RMSDs               |                             |                        |                 |                              |                             |                            |
| Bond lengths (Å)    | 0.003                       | 0.007                  | 0.004           | 0.064                        | 0.004                       |
| Bond angles (°)     | 0.629                       | 0.855                  | 0.771           | 1.220                        | 0.692                       |

Values in parentheses are for the highest-resolution shell; *from two merged datasets (used for refinement). NA, not available; SAD, single-wavelength anomalous diffraction; SeMet, selenomethionine; SIRAS, single isomorphous replacement with anomalous scattering.
A Conserved Patch on the Smc4 Head Binds to the Ycs4 HEAT-Repeat Subunit

A striking feature of the Smc4hd helical lobe is a highly conserved surface patch formed by residues within a loop that surround a strictly conserved tryptophan residue (W-loop; Figure 2A). The corresponding region in the homologous Smc1hd of cohesin also displays some degree of conservation, whereas the regions in the Smc2hd or Smc3hd structures show no obvious sequence conservation (Figure S3A; Table S2). Mutation to alanine of the strictly conserved tryptophan residue of Smc4 rendered budding yeast cells non-viable (Sc Smc4W1317A), as did mutation to aspartate of the neighboring serine residue (Sc Smc4S1298bpa; Figure 2B; Table S3). Mutation of the latter to alanine (Sc Smc4S1316A), of the arginine residue following the tryptophan or lysine residues in Sc Smc2 (Sc Smc2W1077A, Sc Smc2K1078E) had a less dramatic effect on cell proliferation (Figure 2B; Table S3). Mutation of the latter to alanine (Sc Smc4S1316A), of the arginine residue following the tryptophan or lysine residues in Sc Smc2 (Sc Smc2W1077A, Sc Smc2K1078E) had a less dramatic effect on cell proliferation (Figure 2B; Table S3). Mutation of the latter to alanine (Sc Smc4S1316A), of the arginine residue following the tryptophan or lysine residues in Sc Smc2 (Sc Smc2W1077A, Sc Smc2K1078E) had a less dramatic effect on cell proliferation (Figure 2B; Table S3). Mutation of the latter to alanine (Sc Smc4S1316A), of the arginine residue following the tryptophan or lysine residues in Sc Smc2 (Sc Smc2W1077A, Sc Smc2K1078E) had a less dramatic effect on cell proliferation (Figure 2B; Table S3).

To identify proteins that potentially interact with the W-loop surface patch of Smc4, we introduced the non-natural amino acid p-benzoyl-L-phenylalanine (bpa) into one of several positions surrounding the Sc Smc4 W-loop for in vivo photo-crosslinking (Chen et al., 2007). Three of seven different Sc Smc42bo constructs produced upshifted bands in immunoblots for the PKα epitope tag fused to the C terminus of Sc Smc4 after UV crosslinking in live yeast cells (Figure S3C). Mass spectrometry of the upshifted band identified, in addition to Sc Smc4 itself, peptides of the Sce Ycs4 HEAT-repeat subunit and, to a lesser extent, the Sc Brm1 kleisin subunit (Figure 2E; Table S4). Western blotting against HAe epitope tags fused to the C termini of Sc Ycs4 or Sc Brm1 confirmed the presence of these subunits in the same retention volume as the dimer formed between Ct Smc2nad-Brn1N and signature mutant Smc4nad-Brn1C (Figure S2A), it is likely that the Smc4 W-loop mutation prevents the neighboring signature motif from sandwiching ATP bound to the Smc2 active site, which explains the reduction in ATPase rates. As an expected consequence of their inability to complete a full ATPase cycle, chromosome binding of yeast (Figure 2C) and human (Figure 2D) condensin complexes with Smc4 W-loop mutations was dramatically reduced when measured by chromatin immunoprecipitation followed by qPCR (ChIP-qPCR) or live-cell microscopy, respectively. We conclude that the conserved W-loop of Smc4 is essential for condensin’s ATPase activity and for the stable association of condensin complexes with chromosomest.

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the upshifted bands, whereas the Ycg1 HEAT-repeat subunit did not shift under these conditions (Figure S3D).

**Ycs4 Forms a Complex with Brn1 via Two Independent Binding Interfaces**

Because Ycs4 has so far been thought to assemble into the condensin complex exclusively through its constitutive interaction with the Brn1 kleisin subunit (Onn et al., 2007; Piazza et al., 2014), we mapped the part of Ct Brn1 required for Ct Ycs4 binding to a high-affinity (Kd = 0.70 nM) core region of 175 residues (Ct Brn1336–512) and to an extended region that includes the preceding 690 residues (Ct Brn1225–512; Figures S4A and S4B). The additional segment of the extended region displays a higher degree of sequence conservation (Figures 3A and S4C) but lower affinity for Ct Ycs4 binding (Figure S4). Further truncation experiments showed that the N-terminal ~690 residues of Ct Ycs4 are sufficient for binding to the extended Ct Brn1 segment (Figure S4D) but with strongly reduced affinity (Kd = 1.22 μM) when compared to full-length Ct Ycs4 (Figure S4B). These results point to the presence of two distinct sites of contact between Ct Ycs4 and Ct Brn1: a high-affinity interface between the conserved C-terminal region of Ycs4 and the less conserved kleisin core binding region (Ct Brn1336–512), and a low-affinity interface between the less conserved N-terminal part of Ycs4 and the more highly conserved extension of the kleisin core region (Ct Brn1225–335).

**Structure of the Ct Ycs4-Brn1 Complex**

To reveal the interaction between the Brn1 kleisin and Ycs4 HEAT-repeat subunits at near-atomic resolution, we solved the co-crystal structure of Ct Ycs4 bound to Ct Brn1225–418 to 3.3-Å resolution (Ct Ycs4-Brn1Y4; Figure 3B; Table 1). The structure revealed a hook-shaped conformation of the 21 HEAT-repeat motifs of Ycs4 (Figure S5A), with the low-affinity binding region of the kleisin subunit (Ct Brn1241–299) winding along the concave surface of the HEAT-repeat solenoid (Figure 3B) and entirely filling the space between the two lobes of the sharp U-turn in the C-terminal part of the Ycs4 subunit (Figure 3C). A marked drop in the quality of the electron density map in the second lobe because of crystallographic disorder only allowed modeling of a polyalanine chain into unaccounted electron density alongside the tip of the Ycs4 U-turn, which presumably matches residues of the Brn1Y4 high-affinity interaction region included in the crystallization construct. Localization of selenomethionine residues based on anomalous difference maps showed that the N-terminal ~690 residues of Ct Ycs4 are sufficient for binding to the extended Ct Brn1 segment (Figure S4D) but with strongly reduced affinity (Kd = 1.22 μM) when compared to full-length Ct Ycs4 (Figure S4B). These results point to the presence of two distinct sites of contact between Ct Ycs4 and Ct Brn1: a high-affinity interface between the conserved C-terminal region of Ycs4 and the less conserved kleisin core binding region (Ct Brn1336–512), and a low-affinity interface between the less conserved N-terminal part of Ycs4 and the more highly conserved extension of the kleisin core region (Ct Brn1225–335).

**Figure 3. Structure of the Ycs4-Brn1Y4 Complex**

(A) Partial alignment of the extended Brn1Y4 region that binds Ycs4. Colors indicate conservation scores calculated from an alignment of sequences from 40 species. Residues and secondary elements highlighted in the Ct Ycs4–Brn1Y4 structure are indicated.

(B) Cartoon model of the 21 HEAT-repeat motifs of Ycs4 (yellow) bound to residues 241 to 299 of Ct Brn1Y4 (green). A helical insertion between Ycs4 HEAT-repeat motifs 8 and 9 creates an extended “trunk” (proboscis).

(C) Close-up view of Brn1Y4 electron density in the Ycs4 U-turn. Anomalous difference density marking selenomethionine positions is shown in blue.

(D) Close-up view of additional electron density at the distal tip of the Ycs4 U-turn that presumably matches residues of the Brn1Y4 high-affinity interaction region included in the crystallization construct.

See also Figures S4 and S5.
and secondary structure prediction nevertheless allowed the assignment of residue numbers for almost all of Ycs4.

A search for similar structures in the PDB using the DALI server (Holm and Sander, 1996) returned several HEAT-repeat proteins that fold into similarly curved shapes, including the cohesin subunits Pds5 and Sec35KSS2 (Hara et al., 2014; Ouyang et al., 2016) and the condensin subunit Ycg1 (Kachonsk et al., 2017; Figure S5B). Comparison with the structure of the Scc2 subunit of the cohesin loader complex (Kikuchi et al., 2016) also revealed a strikingly similar overall shape despite the fact that this protein was not included in the list from the DALI server. A feature apparently unique to Ycs4 is an N-terminal extension of HEAT-repeat motif 10 (‘‘proboscis’’; Figure 3B), which showed, however, no apparent primary sequence conservation among Ycs4 homologs (Figure S5A).

**Structure of the Ct Ycs4-Brn1Y4-Smc4hd-Brm1C Complex**

Consistent with the finding that the Sc Smc4 W-loop cross-links to Sc Ycs4 and Sc Brm1 in vivo (Figure 2E), Ct Ycs4-Brm1v4 and Ct Smc4hd-Brm1C formed a stable stoichiometric complex in vitro (Kd = 0.63 μM; Figures 4A and S6A). We solved the crystal structure of this complex to 5.8-Å resolution (Table 1) and used the high-resolution structures of the individual subcomplexes (Figures 1A and 3B) and deformable elastic network refinement to build a coarse model of the Ct Ycs4-Brm1v4-Smc4hd-Brm1C complex (Figure 4B). As expected, the Smc4 W-loop mediates the majority of interactions with the HEAT-repeat subunit and does so by contacting the second lobe of the Ycs4 U-turn. In addition, a strictly conserved phenylalanine-arginine residue pair adjacent to the so-called D-lobe of the Ct Smc4 ATPase head contacts a helical extension of Ycs4 HEAT repeat 15. Despite the low resolution in the C-terminal half of Ycs4, we identified the highly conserved peptide loop that connects HEAT repeats 18 and 19 and contains a conserved lysine-glycine pair (KG-loop; Figure S5A) as the most likely candidate to mediate the interaction with the Smc4 W-loop by correlating sequence and structure based on the conserved steric clash between Smc2 and Ycs4 (Figure S6E). This implies that ATP-dependent Smc2-Smc4 head dimerization is incompatible with simultaneous binding of Ycs4-Brm1v4 to the Smc4 head. Consistent with this prediction, we found that addition of Ct Smc2-N-Brm1N and ATP disrupted the interaction between Ct Ycs4-Brm1v4 and Ct Smc4hd-Brm1C in pull-down assays (Figure S6F). Surprisingly, addition of ATP alone was similarly sufficient to compete with complex formation in this assay (Figure S6F) and during size-exclusion chromatography (Figures 4G and S6G). This effect was not caused by Smc4hd homodimerization in the presence of ATP, which has been observed for the homologous Smc1hd of cohesin (Haering et al., 2004), because ATP addition similarly disrupted Ycs4-Brm1v4 binding to a version of Smc4hd-Brm1C that was unable to dimerize because of a mutation in the signature motif (Ct Smc4hdQ1447R; Figure S6G).

Addition of AMP, but not of GTP or guanosine triphosphate (GTP), prevented Ct Ycs4-Brm1v4 binding to Ct Smc4hd-Brm1C with a similar efficiency as addition of ATP (Figure S6H). If ATP or AMP binding to the Smc4 RecA lobe induced dissociation of Ycs4-Brm1v4 from the helical lobe via a flexion movement of the two lobes (Figure 1B), then mutation of the central Q-loop should render the Ycs4-Brm1v4-Smc4hd-Brm1C complex insensitive to nucleotide addition. This was indeed the case (Ct Smc4hdQ421L; Figures 4G and S6G). To rule out that the continued association of the Smc4 Q-loop mutant complex in the presence of ATP was not merely due to the reduced affinity for nucleotide binding that results from a failure to coordinate Mg2+ at the active site (Figure 1C), we repeated the experiment under conditions (10 mM MgCl2) that still allowed ATP binding by the Smc4hd Q-loop mutant with micromolar affinity (Kd = 18.35 μM; Figure S6I). Even under these conditions, ATP addition (1 mM) failed to release Ct Ycs4-Brm1v4 from Ct Smc4hd-Brm1C (Figure S6G). These results strongly support a central role of the Q-loop motif in a conformational switch that transmits an allosteric change from the nucleotide-binding pocket of Smc4hd to its Ycs4-Brm1v4 W-loop interface.

**The Smc2 Neck Region Binds Brm1**

Because the Smc2Ost-Brm1N complex was refractory to crystallization, we determined an NMR structure of this dynamic interface by fusing the Ct Brm1N domain to the coiled-coil neck region of Ct Smc2 (Figures 5A and S7A; Table S5). Similar to the Smc3hd-Scc1N interface in cohesin (Gilgoris et al., 2014), the third “contact” helix (α3) of the kleisin subunit forms a ~50-Å-long helical bundle with the SMC neck coiled coil (Figure S7B). This contact is stabilized by a salt bridge between highly conserved arginine and aspartate residues in the Brm1 contact...
helix and the C-terminal Smc2 neck helix, respectively (Ct Brn1R183, Ct Smc2D1013; Figures 5A and S7C). Mutations of these and neighboring residues rendered condensin nonfunctional in budding yeast (Sc Brn1R89D and Ct Smc2DK-AE; Figures 5B and S7D) and disrupted the Ct Smc2cc-Brn1N interaction (Figure 5C). Mutation of conserved residues in the N-terminal Smc2 neck helix that make no direct contact with Brn1 had, in contrast, no effect on the Ct Smc2cc-Brn1N interaction (Ct
Smc2TKK-AEE; Figure 5C) while nevertheless rendering condensin non-functional in yeast (Figure 5B). This suggests that the role of the Smc2 neck region might go beyond providing a binding platform for the kleisin subunit.

Comparison of the coiled-coil conformations in the Ct Smc2nd and Ct Smc2cc-Brn1N structures implies that the formation of the three-helix bundle requires rotation of one helix by 60°−90° relative to the other helix and spreading of the two helices by 2.4 Å (Figure 5D). The deformation of the coiled coil is caused by the insertion of a preserved tyrosine residue located within the Brn1 contact helix (Ct Brn11-141; Figure 5A). A similar insertion of a conserved kleisin tyrosine residue between the coiled-coil helices can also be observed in the Sc Smc3nd-Scc1N crystal structure of cohesin (Sc Scc1183; Figure S7B), which suggests that this binding mode might be generally conserved among kleisin-SMC protein complexes. Mutation of this tyrosine residue had a more gradual effect on the Smc2cc-Brn1N condensin ring. We therefore purified Smc2TKK-AEE; Figure 5C) while nevertheless rendering condensin function (Figure 5B) and condensin function (Figure 5C) and cell proliferation (Ct Smc2cc-Brn1N; Figures 5B and S7D). ATP-dependent release of the Brn1N subunit from the Smc2cc-Brn1N interface following nucleotide binding induces condensin ring opening at the Smc2-Brn1 interface independent of the presence of either HEAT-repeat subunit.

**DISCUSSION**

Based on the current work, we propose a multi-step model of the condensin ATPase cycle (Figure 7). In the nucleotide-free state, only the Smc4 head is able to bind an ATP molecule (Figure 1C). Correlation of the nucleotide-free Smc4hd-Brn1N structure with the ATPγS-bound structure of the homologous Smc1hd-Scc1G complex (Haering et al., 2004) suggests that, upon ATP binding to the P loop of the Smc4 RecA lobe, the O-loop glutamine residue repositions to form hydrogen bonds with the Mg2+ ion and the γ-phosphate (Figure 1B). As a consequence, the helical lobe tilts, relative to the RecA lobe, by 15° degrees (Video S1), which is similar to the flexion recently described for bacterial
SMC proteins (Kamada et al., 2017). The comparison of the Smc4$_{hd}$-Bm1$_{C}$ and Smc1$_{C}$-Scc1$_{C}$ structures furthermore reveals an inversion of the side-chain conformations of a conserved tyrosine-lysine (tyr or arg) pair in a region of the helical-lobe that we define as the W-loop (Figure S7F). It is likely that tilting and repositioning of W-loop residues dissociate Ycs4 (Figure 4G), which binds to this part of Smc4$_{hd}$ (Figure 4B) and sterically blocks access of Smc2$_{hd}$ (Figure S6E).

Ycs4, whose role for condensin function has so far remained incompletely understood, binds to two distinct sequence stretches of the Brn1 kleisin subunit (Figure S4): the N-terminal, low-affinity binding stretch winds along the inner surface of the hook-shaped HEAT-repeat solenoid (Figure 3C), whereas the high-affinity core binding region stretch is not clearly resolved in the crystal structure, although it is essential for the interaction of the Ycs4-Brn1$_{1-141}$ subcomplex with Smc4$_{hd}$ (Figure S6D). It is conceivable that contacts with this part of the kleisin subunit support the essential contacts between the Ycs4 KG-loop and the Smc4 W-loop and between the helical extension of Ycs4 HEAT-repeat motif 15 and the conserved polyalanine-arginine residue pair at the tip of the Smc4 D-loop. Notably, the conservation of the KG-loop sequence extends to the NCAPD3 HEAT-repeat subunits of metazoan condensin II complexes (Figure S5A) and the identity of several residues in the loop segment of Smc1$_{hd}$ that corresponds to the Smc4$_{hd}$ W-loop has been maintained throughout evolution (Figure S3A). These findings raise the possibility that the interaction of a HEAT-repeat subunit with the $\gamma$-SMC$_{hd}$ is a central feature of all condensin and cohesin complexes. In cohesin, the most likely HEAT-repeat subunit to bind Smc1$_{hd}$ might be Pds5, which is most similar in structure to Ycs4 (Figure S5B).

Smc2-Smc4 head dimerization commences in an asymmetric state in which only the Smc4 ATP-binding pocket is occupied (Figure 7). The Smc2 head fails to bind ATP on its own (Figure 1C), presumably because of the deformation of the Smc2 P loop observed in two different crystal forms (Figures 1B and S1C), but readily binds and hydrolyses ATP when in complex with the Smc4 head (Figure S2C). This transition is presumably the consequence of a pronounced reorientation of the Smc2 helical-lobe and coiled-coil by $\sim$25° upon head dimerization, which is transmitted via D-loop and adjacent helices to create a P loop conformation that is compatible with nucleotide binding (Figure 1B). The subsequent sandwiching of the second ATP by the Smc4 signature motif presumably also depends on the prior reorientation of the neighboring W-loop because mutation of either of the two motifs results in head dimers with distinct elution profiles during size-exclusion chromatography (Figure S2A).

Our data furthermore suggest that, during formation of a pseudo-symmetric Smc2-Smc4 head dimer with both active sites occupied by ATP, changes in the Smc2 coiled-coil conformation markedly reduce the binding affinity to the Brn1 N terminus (Figure 6A). Because we observe Brn1 dissociation in the context of the full-length Smc2-Smc4 dimers but not for the Smc2$_{cd}$-Bm1$_{N}$ subcomplex (Figure S1A), the conformational transition of the coiled coil might require a pivot point, which could be situated in the “joint” region approximately one-third up the length of the coiled coil (Diebold-Durand et al., 2017) or generated by folding back of the SMC hinge domain onto the coiled coils (Bürmann et al., 2019). In either case, helix rotation provides a straightforward mechanism to control the propensity of the coiled coils to spread apart and accommodate insertion of the kleisin tyrosine side chain (Figure 5A). The fact that the
It is important to note that the asymmetry of condensin differs from that described for certain heterodimeric ABC transporters, which operate in an asymmetric fashion because only one of the two ATPase sites seems to be capable of hydrolyzing ATP (Procko et al., 2009). Furthermore, it has been suggested that many homodimeric ABC transporters use their two catalytic sites in an alternating fashion (Jones and George, 2013). Because even the two ATPase heads of bacterial SMC homodimers are embedded asymmetrically into the holocomplex by their binding to different ends of the kleisin subunit (Bürmann et al., 2013; Zawadzka et al., 2018), the asymmetric model put forward by our analysis of condensin might not only explain functional differences between the two ATPase sites of condensin’s Smc2-Smc4 subunits or cohesin’s Smc1-Smc3 subunits (Elbatsh et al., 2016) but, conceivably, apply to all SMC protein complexes. Future studies will need to complete the structural landscape of SMC holocomplexes to uncover how the asymmetric ATPase motor drives the diverse functions of this class of chromosome organizers.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **CONTACT FOR REAGENT AND RESOURCE SHARING**
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Cell Lines
  - Yeast Strains
  - Bacterial Strains
- **METHOD DETAILS**
  - Protein Expression and Purification
  - Crystallization and Data Collection
  - Structure Determination and Refinement
  - NMR spectroscopy and structure calculation
  - Multiple-sequence alignments
  - ATP Hydrolysis Assays
  - Isothermal Titration Calorimetry
  - Analytical Size-Exclusion Chromatography
  - GST Pulldown
  - Release of the amino-terminal Brn1 fragment
  - Condensin Immunoprecipitation and Western Blotting
  - Bpa Crosslinking
  - Mass Spectrometry
  - ChiP-qPCR
  - Microscopy of Human Condensin Complexes
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
- **DATA AND SOFTWARE AVAILABILITY**

**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.molcei.2019.03.037.

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AUTHOR CONTRIBUTIONS
M.H., M.K., S.B., L.T., and H.J.B. purified proteins and conducted biochemical assays. M.H., M.K., and L.T. crystallized the proteins. M.H. and M.K. collected data and solved the X-ray structures. B.S.S., J. Maco, and J.H. solved the NMR structure. F.M. carried out imaging experiments with human cells. J. Metz performed and analyzed ChiP-qPCR experiments. I.A.S. purified condensin holocomplexes and performed biochemical assays on the Smc2-Bm16 interface. M.H. and C.H.H. designed the study. J.H. and C.H.H. supervised the work. M.H. and C.H.H. wrote the manuscript with input from all authors.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT OR RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Mouse monoclonal anti V5-tag (anti PK6-tag) | AbD Serotec | Cat# MCA1360, RRID: AB_322378 |
| Rabbit polyclonal anti HA-tag | Abcam | Cat# ab9110, RRID: AB_307019 |
| Mouse monoclonal anti tubulin (TAT1) | Woods et al., 1989 | N/A |
| Mouse monoclonal anti HA-tag (12CA5) | EMBL Protein Expression and Purification Core Facility | N/A |
| Rabbit polyclonal anti Sc Ycg1 | Piazza et al., 2014 | N/A |
| Bacterial and Virus Strains | | |
| *Escherichia coli* Rosetta (DE3) pLysS | Merck | Cat# 70954 |
| Chemicals, Peptides, and Recombinant Proteins | | |
| *C. thermophilum* 6 × HIS–Brn1112–204 in complex with Smc22–224/981–1179 | This work | N/A |
| *C. thermophilum* 6 × HIS–Brn1112–204 in complex with Smc22–224/981–1179, E1116Q | This work | N/A |
| *C. thermophilum* 6 × HIS–Brn1112–204 in complex with Smc22–224/981–1179, Q147L | This work | N/A |
| *C. thermophilum* 6 × HIS–Brn1112–204 in complex with Smc22–224/981–1179, S1088R | This work | N/A |
| *C. thermophilum* 6 × HIS–Brn1112–204 in complex with Smc22–224/981–1179, W1080A | This work | N/A |
| *C. thermophilum* 6 × HIS–Brn1112-204 in complex with Smc22–215/990–1179 | This work | N/A |
| *C. thermophilum* Brn1112–204 in complex with Smc22–224/981–1179–6 × HIS | This work | N/A |
| *C. thermophilum* Brn1112–204 in complex with Smc22–224/981–1179, T184A, K187E, K188E–6 × HIS | This work | N/A |
| *C. thermophilum* Brn1112–204, D1013A, K1016E–6 × HIS | This work | N/A |
| *C. thermophilum* Brn1112–204, R183D in complex with Smc22–224/981–1179–6 × HIS | This work | N/A |
| *C. thermophilum* Brn1112–204, Y180A in complex with Smc22–224/981–1179–6 × HIS | This work | N/A |
| *C. thermophilum* Brn1112–204, Y180I in complex with Smc22–224/981–1179–6 × HIS | This work | N/A |
| *C. thermophilum* Brn1112–204, Y180F in complex with Smc22–224/981–1179–6 × HIS | This work | N/A |
| *C. thermophilum* 6 × HIS–Smc2981–1031/170–224 in complex with Smc22–224/981–1179–6 × HIS | This work | N/A |
| *C. thermophilum* Brn1765–898 in complex with Smc4264–466/1367–1542–8 × HIS | This work | N/A |
| *C. thermophilum* Brn1765–898 in complex with Smc4264–466/1367–1542, E1475Q–8 × HIS | This work | N/A |
| *C. thermophilum* Brn1765–898 in complex with Smc4264–466/1367–1542, Q421L–8 × HIS | This work | N/A |
| *C. thermophilum* Brn1765–898 in complex with Smc4264–466/1367–1542, E1475Q, S1447R–8 × HIS | This work | N/A |

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Continued

| REAGENT OR RESOURCE | SOURCE | IDENTIFIER |
|----------------------|--------|------------|
| C. thermophilum Brn1765–898 in complex with Smc4264–466/1367–1542, E1475Q, W1440A–8 | This work | N/A |
| C. thermophilum Brn1765–898 in complex with Smc4264–466/1367–1542, S1447R–8 | This work | N/A |
| C. thermophilum Brn1765–898 in complex with Smc4264–466/1367–1542, S1439D, W1440A–8 | This work | N/A |
| C. thermophilum Brn1765–898 in complex with Smc4264–466/1367–1542, F1482A, R1483D–8 | This work | N/A |
| C. thermophilum Brn1765–898 in complex with Smc4264–466/1367–1542, W1440A–8 | This work | N/A |
| C. thermophilum Brn1225–512 in complex with Ycs43–1222 modified from Piazza et al., 2014 | This work | N/A |
| C. thermophilum Brn1225–1222 in complex with Ycs43–1222 | This work | N/A |
| C. thermophilum Brn1225–512 in complex with Ycs43–512 modified from Piazza et al., 2014 | This work | N/A |
| C. thermophilum Brn1225–418 in complex with Ycs43–828, 869–915, 939–1222 | This work | N/A |
| C. thermophilum Brn1225–418 in complex with Ycs43–828, 869–915, 939–1222, K1094D, V1095S, K1096D, Q1098D, L1099S | This work | N/A |
| C. thermophilum GST–Brn1225–340 modified from Kschonsak et al., 2017 | This work | N/A |
| C. thermophilum GST Brn1225–512 modified from Kschonsak et al., 2017 | This work | N/A |
| C. thermophilum GST Brn1336–512 modified from Kschonsak et al., 2017 | This work | N/A |
| C. thermophilum GST Brn1336–714 modified from Kschonsak et al., 2017 | This work | N/A |
| C. thermophilum GST Brn1513–714 modified from Kschonsak et al., 2017 | This work | N/A |
| C. thermophilum GST Brn1636–714 modified from Kschonsak et al., 2017 | This work | N/A |
| S. cerevisiae Smc2 in complex with Smc4–3 StrepII, Brn1–12 × HIS–3 × HA, Ycg1, Ycs4 | modified from Kschonsak et al., 2017 | N/A |
| S. cerevisiae Smc2 in complex with Smc4–3 StrepII, Brn1(ybbR tag replacing residues 13–23, TEV site inserted at residue 141)–12 × HIS–3 × HA, Ycg1, Ycs4 | This work | N/A |
| S. cerevisiae Smc2 in complex with Smc4–3 StrepII, Brn1(ybbR tag replacing residues 13–23, TEV sites inserted at residues 141 and 373)–12 × HIS–3 × HA, Ycg1, Ycs4 | This work | N/A |
| S. cerevisiae Smc2 in complex with Smc4–3 StrepII, Brn1(ybbR tag replacing residues 13–23, 1 × TEV sites inserted at residues 141 and 373)–12 × HIS–3 × HA, Ycg1, Ycs4 | This work | N/A |
| S. cerevisiae Smc2 in complex with Smc4–3 StrepII, Brn1(ybbR tag replacing residues 13–23, 3 × TEV site inserted at residue 141)–12 × HIS–3 × HA, Ycg1, Ycs4 | This work | N/A |

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CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Christian H. Haering (christian.haering@embl.de).
EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell Lines
HeLa Kyoto H2B-mCherry cells (Neumann et al., 2010) were cultivated in DMEM (Life Technologies) containing 10% FBS (Life Technologies), 1% PenStrep (Invitrogen), and 1% glutamine (Invitrogen) at 37°C, 5% CO₂.

Yeast Strains
Saccharomyces cerevisiae strains are derived of W303. Genotypes are listed in the Table S3.

Bacterial Strains
Proteins for crystallography and biochemistry were expressed in Escherichia coli Rosetta (DE3) pLysS cells (Merck, 70954) pre-grown at 37°C and grown at 18°C for induction in 2× TY or Terrific Broth (TB) medium.

METHOD DETAILS

Protein Expression and Purification
Expression of Ct Ycs4, Ct Ycs4–Brn1, or Ct Smc2hd–Brn1N constructs (see Table S1) was induced for 18 h from pET-MCN vectors (Romier et al., 2006) in Escherichia coli Rosetta (DE3) pLysS (Merck) grown at 18°C in 2× TY medium or M9 minimal medium (for NMR). The Ct Smc4hd–Brn1C constructs were expressed in St21 cells using the Multibac expression system (Fitzgerald et al., 2006). Cells were lysed by sonication at 4°C in lysis buffer (50 mM TRIS-HCl pH 7.5, 200–500 mM NaCl, 20 mM imidazole, 5 mM β-mercaptoethanol) containing complete protease inhibitor cocktail tablets without EDTA (cOm–EDTA, Roche). The lysate was cleared by centrifugation at 45,000 × gmax and loaded onto Ni-Sepharose 6FF (GE Healthcare). After washing with 30–40 column volumes (cv) lysis buffer, proteins were eluted in 7–10 cv elution buffer (lysis buffer plus 300 mM imidazole). The eluate was dialyzed overnight in dialysis buffer (25 mM TRIS-HCl pH 7.5, 200–300 mM NaCl, 1 mM DTT) at 4°C and amino-terminal His6-tags were cleaved by addition of TEV protease, where applicable.

Ct Smc2hd–Brn1N was loaded onto a Superdex 200 26/60 column (GE Healthcare) equilibrated in SEC-buffer (25 mM TRIS-HCl pH 7.5, 300 mM NaCl, 1 mM DTT). Peak fractions were pooled and diluted with low-salt buffer (25 mM TRIS-HCl pH 7.5, 100 mM NaCl, 1 mM DTT) to a final salt concentration of 100 mM NaCl and loaded onto an 8-ml Superose 26/60 column pre-equilibrated with low-salt buffer. After washing with 3–5 cv low-salt buffer, proteins were eluted by increasing NaCl concentrations to 1 M in a linear gradient of 60 mL. Peak fractions were pooled and concentrated by ultrafiltration (Vivaspin 10,000 MWCO, Sartorius).

Ct Smc4hd–Brn1C was loaded onto a Superdex 200 26/60 column (GE Healthcare) equilibrated in SEC-buffer (25 mM TRIS-HCl pH 7.5, 500 mM NaCl, 1 mM DTT) to a final salt concentration of 100 mM NaCl and loaded onto an 8-ml Resource Q (GE Healthcare) cation exchange column pre-equilibrated with low-salt buffer. Flow through fractions containing Ct Smc4hd–Brn1C were pooled and concentrated by ultrafiltration (Vivaspin 10,000 MWCO, Sartorius).

Ct Ycs4 and Ct Ycs4–Brn1 were diluted with low-salt buffer (25 mM TRIS-HCl pH 7.5, 100 mM NaCl, 1 mM DTT) to a final salt concentration of 150 mM NaCl and loaded onto a 6-ml Resource Q (GE Healthcare) anion exchange column pre-equilibrated with low-salt buffer. After washing with 3–5 cv low-salt buffer, proteins were eluted by increasing NaCl concentrations to 1 M in a linear gradient of 60 mL. Peak fractions were pooled and loaded onto a Superdex 200 26/60 column (GE Healthcare) equilibrated in SEC-buffer (25 mM TRIS-HCl pH 7.5, 500 mM NaCl, 1 mM DTT). Peak fractions were pooled and concentrated by ultrafiltration (Vivaspin 30,000 MWCO, Sartorius).

Histidine-tagged Ct Brn1 fragments spanning residues 225–512 or 336–512 where co-expressed and co-purified with untagged Ct Ycs4 using the above protocol. Excess Ct Brn1 was separated from the Ct Ycs4–Brn1V4 complex during the size-exclusion chromatography step.

Ct Smc2hd–Brn1N fusion protein (NMR) was dialyzed overnight in dialysis buffer (10 mM Na/K phosphate pH 6.5, 50 mM NaCl, 1 mM DTT) at 4°C and the amino-terminal His6-tag was cleaved by addition of TEV protease before loading onto a Superdex 75 26/60 column (GE Healthcare) equilibrated in SEC-buffer (10 mM Na/K phosphate pH 6.5, 50 mM NaCl, 1 mM DTT). Peak fractions were pooled and concentrated by ultrafiltration (Vivaspin 10,000 MWCO, Sartorius).

Various Ct Brn1 fragments (see Table S1) were expressed as amino-terminal GST fusion constructs from a pGEX6P-1 vector (Smith and Johnson, 1988) as described above. Cells were lysed at 4°C by sonication in lysis buffer (50 mM TRIS-HCl pH 7.5, 500 mM NaCl, 2 mM DTT containing cOm–EDTA). The lysate was cleared by centrifugation at 45,000 × gmax and loaded onto Glutathione Sepharose 4B beads (GE Healthcare). The GST-fusion protein was eluted from the beads with lysis buffer containing 10 mM L-glutathione. The eluate was dialyzed and purified over RESOURCE Q as described above. Peak fractions were pooled and concentrated by ultrafiltration (Vivaspin 10,000 MWCO).

Condensin holocomplexes were expressed (St-Pierre et al., 2009) and purified as described previously (Kschonsak et al., 2017). YbbR-tagged holocomplexes were covalently coupled to Coenzyme A-ATTO488 (New England Biolabs) with Sfp synthase as
described previously (Ganjii et al., 2018). Selenomethionine-labeled Ct Ycs4–Brn1$_Y$ and Ct Smc2$_{hd}$–Brn1$_N$ were expressed applying methionine pathway inhibition (Doublié, 1997) and purified as described above.

**Crystallization and Data Collection**

Crystals of selenomethionine-labeled Ct Smc2$_{hd}$ (form I) (Table 1) were grown at 20°C by hanging-drop vapor diffusion. A volume of 1 µL protein Ct Smc2hd$_{222/248-983/1179}$–Brn1$_{12-204}$ (8 mg/mL in 25 mM TRIS–HCl pH 7.5, 100 mM NaCl, 1 mM DTT) was mixed with 1 µL crystallization solution (18% (w/v) PEG3350, 0.2 M Succinate pH 7.0, 2 mM MnCl$_2$). Crystals were cryo-protected by addition of crystallization solution containing 20% (v/v) glycerol before flash freezing in liquid nitrogen. Single-wavelength anomalous dispersion data were collected at a wavelength of 0.976 Å (peak) at beamline ID29, European Synchrotron Radiation Facility (ESRF, Grenoble, France) (de Sanctis et al., 2012). Data were processed with XDS and XSCEAL (Kabsch, 2010).

Ct Smc2$_{hd}$ crystals (form II) (Table 1) were grown by hanging-drop vapor diffusion after mixing 1 µL protein Smc2$_{215/990-1179}$–Brn1$_{12-204}$ (12.7 mg/mL in 25 mM TRIS–HCl pH 7.5, 100 mM NaCl, 1 mM DTT) and 1 µL crystallization solution (10%–17% (w/v) PEG 8,000, 0.1 M Na cacodylate pH 6.0) at 20°C. Crystals were cryo-protected by addition of 20% (v/v) glycerol before flash freezing in liquid nitrogen. The dataset was collected at a wavelength of 0.976 Å at beamline ID29, ESRF (de Sanctis et al., 2012). Data were processed with XDS (Kabsch, 2010) and POINTLESS (Evans, 2011) and scaled with SCALA of the CCP4 suite (Evans and Murshudov, 2013; Winn et al., 2011).

Ct Smc4$_{cr}$–Brn1$_C$ crystals (Table 1) were grown by sitting drop vapor diffusion after mixing 1 µL protein Smc4$_{264-466/1367-1542}$–Brn1$_{65-98}$ (6.5 mg/mL in 25 mM TRIS–HCl pH 7.5, 150 mM NaCl, 1 mM DTT, 1 mM MgCl$_2$, 1 mM ATP$_2$S) and 1 µL crystallization solution (3% (v/v) ETOH, 0.1 M Na citrate pH 6.0, 1.5 M LiSO$_4$) at 20°C. Crystals were cryo-protected in 2 mM LiSO$_4$ before flash freezing in liquid nitrogen. The dataset was collected at a wavelength of 1.000 Å at beamline ID29, ESRF (de Sanctis et al., 2012). Data were processed as described above.

Native and selenomethionine-labeled Ct Ycs4–Brn1$_Y$ crystals (Table 1) were grown by sitting drop vapor diffusion after mixing 100 nL Ycs4$_{loops}$–Brn1$_{1255-418}$ and 100 nL crystallization solution in an MRC 2-well plate (Hampton Research). Native crystals were harvested after 30 days (5 mg/mL in 10 mM TRIS–HCl pH 7.5, 200 mM NaCl, 1 mM DTT) with crystallization buffer (12% (w/v) PEG 8,000, 0.1 M ADA pH 6.8, 0.1 M NaCl) at 7°C. Selenomethionine-labeled crystals were harvested after 30 days (6 mg/mL in 10 mM TRIS–HCl pH 7.5, 200 mM NaCl, 1 mM DTT) with crystallization buffer (13% (w/v) PEG 8,000, 0.1 M ADA pH 7.1, 0.13 M NaCl) at 7°C. All crystals were flash frozen in liquid nitrogen after addition of 2 µL crystallization buffer containing 37% (v/v) PEG 400. Datasets of selenomethionine-labeled Ct Ycs4–Brn1$_Y$ were collected at a wavelength of 0.966 Å at beamline MASSIF1 (ID30A–1), ESRF (Bowler et al., 2015; Svensson et al., 2015). The dataset of native Ct Ycs4–Brn1$_Y$ was collected at a wavelength of 1.0 Å at beamline ID29, ESRF (de Sanctis et al., 2012). All datasets were processed as described above using AIMLESS (Evans and Murshudov, 2013).

Ct Ycs4–Brn1$_{Y\rightarrow Y4}$–Smc4$_{cr}$–Brn1$_C$ crystals (Table 1) were grown by sitting drop vapor diffusion after mixing 100 nL sample (10 mg/mL complex in 25 mM TRIS–HCl pH 7.5, 150 mM NaCl, 1 mM DTT) and 100 nL crystallization solution (0.1 M TRIS–HCl pH 8.5, 8% PEG 8,000, 1 mM TCEP) in an MRC 2-well plate (Hampton Research) at 7°C. Crystals were cryo-protected by addition of 2 µL crystallization buffer containing 37% (v/v) PEG 400 before flash freezing in liquid nitrogen. The dataset was collected at a wavelength of 0.976 Å at beamline ID30B, ESRF (Mueller-Dieckmann et al., 2015). Data were processed as described above using SCALA (Evans, 2006).

**Structure Determination and Refinement**

Single anomalous dispersion data for Ct Smc2$_{hd}$ (crystal form I), was used to locate 16 selenium sites with autoSHARP (Vonrhein et al., 2007) followed by site refinement, phasing, and density modification. An initial model was built using Phenix AutoBuild and manual adjustment in Coot (Emsley et al., 2010; Terwilliger et al., 2008). The model was further improved by iterative rounds of restrained refinement with phenix.refine and manual adjustment with Coot (Afonine et al., 2012; Emsley et al., 2010).

The Ct Smc2$_{hd}$ (crystal form II) structure was solved by molecular replacement with an adapted Ct Smc2$_{hd}$ (crystal form I) as search model using Phenix Phaser-MR (McCoy, 2007). The structure was finalized in iterative rounds of manual correction with Coot (Emsley et al., 2010) and restrained refinement with phenix.refine (Afonine et al., 2012).

The Ct Smc4$_{cr}$–Brn1$_C$ structure was solved by molecular replacement with an adapted Sc Smc1$_{hd}$–Scc1$_C$ structure (pdb 1W1W) as search model using Phenix Phaser-MR (McCoy, 2007). An initial model was built using Phenix AutoBuild and manual adjustments with Coot (Emsley et al., 2010; Terwilliger et al., 2008). The structure was further improved in iterative rounds of manual correction with Coot (Emsley et al., 2010) and restrained refinement with phenix.refine (Afonine et al., 2012).

Single anomalous dispersion data, merged from two independent datasets, and native data for Ct Ycs4–Brn1$_Y$ were used to locate 34 selenium sites with Phenix AutoSol (Adams et al., 2010), followed by site refinement, phasing, and density modification. An initial model was built using Phenix AutoBuild and manual adjustment in Coot (Emsley et al., 2010; Terwilliger et al., 2008). The model was further improved by iterative rounds of manual adjustments with Coot (Emsley et al., 2010) and restrained refinements with phenix.refine (Afonine et al., 2012) against the anomalous dataset.

The Ct Ycs4–Brn1$_Y$–Smc4$_{cr}$–Brn1$_C$ low-resolution co-structure was solved by a molecular replacement search with adapted Ct Ycs4–Brn1$_Y$ and Ct Smc4$_{cr}$–Brn1$_C$ as individual search components using Phenix Phaser-MR (McCoy, 2007). The initial model was refined using the deformable elastic network (DEN) protocol with CNS over a grid-enabled web server hosted by SBGrid (O’Donovan et al., 2012; Schröder et al., 2010) using standard settings and the input structure as both starting and reference models (Bürger...
ments were used to map surface sequence conservation with Consurf (relaxed conservation scores) (Ashkenazy et al., 2016). To align with MAFFT (Katoh et al., 2002) using the Smith-Waterman local algorithm (L-INS-i) with default settings. The Ycs4 alignments were initialized by addition of ATP and incubated at 30°C. A volume of 1.0 µL of the reaction mix was spotted onto PEI cellulose F TLC plates (Merck) every 3 min for a total of 15 min. The reaction products were resolved on TLC plates using 0.5 M LiCl and 1 M formic acid staining.

**ATP Hydrolysis Assays**

Reactions (10 µL) were set up with 5 µM SMC head proteins, as indicated, in ATPase buffer (50 mM TRIS-HCl pH 7.5, 215 mM NaCl, 2% (v/v) glycerol, 10 mM MgCl₂, 5 mM ATP, 1.3 mM DTT and 33 mM [32P]-ATP; Hartmann Analytic). ATP hydrolysis reactions were initiated by addition of ATP and incubated at 30°C. A volume of 1.0 µL of the reaction mix was spotted onto PEI cellulose F TLC plates (Merck) every 3 min for a total of 15 min. The reaction products were resolved on TLC plates using 0.5 M LiCl and 1 M formic acid solution and detected by exposing the TLC plates to a phosphorimager screen and analysis on a Typhoon FLA 9,500 scanner (GE Healthcare). ATP hydrolysis rates were calculated from the ADP/ATP ratios from time points in the linear range of the reaction.

**Analytical Size-Exclusion Chromatography**

For Ct constructs, aliquots of 80 µL of protein samples at a concentration of 15 µM where incubated (with 1 mM ATP where indicated) at a flow rate of 0.05 mL/min using the ÄKTA Ettan System (GE Healthcare). Fractions of 100 µL were collected and analyzed by SDS-PAGE and Coomassie staining.

NMR spectroscopy and structure calculation

NMR experiments were recorded on Bruker AVIII NMR spectrometers operating at field strengths corresponding to proton Larmor frequencies of 600 and 800 MHz equipped with a cryogenic TXI probe. All spectra were acquired at 298 K, processed with NMRPipe (Delaglio et al., 1995), and analyzed using NMRview (Johnson and Blevins, 1994). Initial backbone assignments were achieved from TROSY-HNCA, -HN(CO)CA, -HNCACB and -HN(CO)CACB recorded on a 2H,13C,15N labeled sample (Pervushin et al., 1997; Salzmann et al., 1998). Backbone and side-chain assignments were completed mainly on a set of 3D NOESY spectra – 1H-NOESY-1H,13N-HSQC, (1H),13C-HMQC-NOESY-1H,13N-HSQC, 1H,13C-HMQC-NOESY-1H,15N-HMQC. The same experiments were used for deriving NOE-based distance restraints to feed structure calculation using CNS1.2 (Brunger, 2007) and ARIA1.2 (Linge et al., 2003). Due to the size and for NMR disadvantageous tumbling behavior of coiled-coil proteins, conventional side chain assignment experiments yielded too low signal-to-noise. Therefore, side chain assignments have been achieved using the above listed NOE-type experiments. Consequently, NOEs were thereby manually assigned by the iterative ARIA approach to quantify, merge, and decrease assignment ambiguities, with the (1H),13C-HMQC-NOESY-1H,15N-HMQC data included as a 4D peak list. Backbone torsion angles were determined from chemical shifts using TALOS+ (Shen et al., 2009). Structural quality after refinement of the ten lowest energy structures out of 100 calculated structures in iteration 8 was validated using PROCHECK (Laskowski et al., 1996) and WHATIF (Vriend, 1990) (Table S5).

Multiple-sequence alignments

Smc2, Smc4, Brn1 and Ycs4 sequences from 40 divergent species (10 animals, 10 plants, 10 yeasts, 10 protists; Table S2) were aligned with MAFFT (Katoh et al., 2002) using the Smith-Waterman local algorithm (L-INS-i) with default settings. The Ycs4 alignments were used to map surface sequence conservation with Consurf (relaxed conservation scores) (Ashkenazy et al., 2016). Out of the resulting models, the one with the lowest R_free value was used for a final round of manual adjustments with Coot (Emsley et al., 2010) and real-space refinement with phenix.refine (Afonine et al., 2012).

All structures were refined with hydrogens (‘riding’ model) and validated using MolProbity (Chen et al., 2010). Structures (Table 1) have the following Ramachandran statistics: Ct Smc2hd (crystal form I) favored 96.0%, outliers 0.2%; Ct Smc2id (crystal form II) favored 97.0%, outliers 0%; Ct Smc4id-Bm1C favored 94.0%, outliers 0.5%; Ct Ycs4–Bm1Y4 favored 92.0%, outliers 0.8%; Ct Ycs4–Bm1–Smc4id favored 90.0%, outliers 0.1%.

Structures were visualized with PyMOL (Schrödinger, LLC). The electrostatic surface potential graph was created with APBS (Baker et al., 2001).

**ATPase assays with condensin holocomplexes**

Isothermal Titration Calorimetry

Ct Smc2id-Bm1N or Ct Smc4id-Bm1C proteins were dialyzed to ITC buffer 1 (25 mM TRIS-HCl pH 7.5, 200 mM NaCl, 1 mM MgCl₂) or buffer 2 (25 mM TRIS-HCl pH 7.5, 200 mM NaCl, 10 mM MgCl₂, 2% glycerol, 0.5 mM DTT). ATP was dissolved in ITC buffer 1 or buffer 2. ATP was injected at a concentration of 340 µM into 37.5–42.0 µM protein at 25°C (buffer 1) or 190–400 µM into 23.6–40.0 µM protein at 20°C (buffer 2). For the interaction studies of Ct Brn1 and Ct Ycs4 or of Ct Smc4id-Bm1C and Ct Ycs4–Bm1Y4, proteins were dialyzed against ITC buffer 3 (25 mM TRIS-HCl pH 7.5, 300 mM NaCl, 0.5 mM DTT) and injected at 25°C or 10°C.

ITC measurements were performed on a MicroCal ITC200 or a PEAQ-ITC microcalorimeter (Malvern Panalytical). ITC data were corrected for the dilution heat and fitted with the MicroCal Origin software package applying one set of binding sites model. Standard deviation values of the fit were calculated from the original data points.

Analytical Size-Exclusion Chromatography

For Ct constructs, aliquots of 80 µL of protein samples at a concentration of 15 µM where incubated (with 1 mM ATP where indicated) on ice for 15 min and injected onto a Superdex 200 Increase 3.2/300 column (GE Healthcare) and separated in a buffer containing 175 mM NaCl, 25 mM TRIS-HCl pH 7.5, 1 mM MgCl₂ and 1 mM DTT (and 100 µM ATP where indicated) at a flow rate of 0.05 mL/min using the ÄKTA Ettan System (GE Healthcare). Fractions of 100 µL were collected and analyzed by SDS-PAGE and Coomassie staining.
For Sc condensin holocomplexes, 20 μL aliquots at a concentration of ~3 μM were incubated 16 h on ice with 1.5 μg TEV protease in the presence of 1 mM EDTA, 0.2 mM PMSF and 0.01% (v/v) Tween-20. After adjustment to 1.0 μM condensin concentration and 125 mM NaCl, 50 mM KOCl, 50 mM TRIS-HCl pH 7.5, 5 mM MgCl₂ and 1 mM DTT (with 1 mM ATP where indicated), 50 μL was injected onto a Superose 6 Increase 3.2/300 column (GE Healthcare) and separated in same buffer (with 100 μM ATP where indicated) at a flow rate of 0.05 mL/min using the AKTA Ettan System (GE Healthcare). Fractions of 100 μL were collected and protein precipitated with 10% (w/v) trichloroacetic acid before SDS-PAGE and silver staining.

GST Pulldown
40 μg of glutathione S-transferase (GST) fusion protein was incubated with 60 μg of each untagged protein and 30 μL Glutathione Sepharose 4B (GE Healthcare) in 200 mM NaCl, 50 mM TRIS-HCl pH 7.5, 1 mM DTT, 1 mM MgCl₂ in a total volume of 0.5 mL for 1 h at 4°C. The beads were gently centrifuged at 1,200 rpm for 3 min and washed 6 times with the same volume of buffer before boiling and analysis by SDS-PAGE.

Release of the amino-terminal Brn1fragment
Aliquots of 10–20 μL of ~3 μM Sc condensin holocomplexes with CoA-ATTO488-labeled Brn1 were treated 16 h on ice with 1.5 μg TEV protease in the presence of 1 mM EDTA, 0.2 mM PMSF and 0.01% (v/v) Tween-20. Next, 5.5 pmol of condensin was immobilized on 20 μL Protein A-coupled Dynabeads (ThermoFisher Scientific) that had been pre-bound to 3 μg anti-HA antibody (12CA5). Beads were washed four times with 50 mM TRIS–HCl pH 7.5, 125 mM NaCl, 5 mM MgCl₂, 5% (v/v) glycerol, 1 mM DTT, 0.2 mM PMSF and 0.01% (v/v) Tween-20. Release of the Brn1 amino-terminal cleavage fragment was subsequently assayed by washing three times with same buffer (including 1 mM ATP where indicated) with 5 min incubation at 25°C each. The three washes were collected and protein precipitated with 10% (w/v) trichloroacetic acid. Proteins bound to beads were eluted in 2 × SDS loading buffer (100 mM TRIS–HCl pH 6.8, 4% (w/v) SDS, 20% glycerol (v/v) 0.2% (w/v) bromophenol blue, 0.2 M DTT) by heating to 65°C for 5 min. Individual proteins were resolved by SDS-PAGE and fluorescence was analyzed in-gel on a Typhoon FLA9500 imager (GE Healthcare) with a 473-nm laser and a 510-nm long pass filter.

Condensin Immunoprecipitation and Western Blotting
Immunoprecipitation of endogenous condensin complexes from yeast was performed as described previously (Kschonsak et al., 2017). Yeast strains were grown at 30°C in 2 L YPAD media to an OD₆₀₀ of 1.0, harvested by centrifugation and lysed by cryogenic grinding (SPEX Freezer/Mill 6970) in lysis buffer (50 mM TRIS–HCl pH 8.0, 100 mM NaCl, 2.5 mM MgCl₂, 0.25% (v/v) Triton X-100, 1 mM DTT, 0.25% (v/v) EDTA) containing 2 × cOm–EDTA before lysis by cryogenic grinding (SPEX Freezer/Mill 6970). Condensin complexes were immunoprecipitated as described above, using 100 μL Protein A-coupled Dynabeads that had been pre-bound to 10 μg anti-PK antibody. After elution and SDS-PAGE, gels were silver stained using a formaldehyde-free protocol. The cross-linked band and a band at the same height in the –UV control were excised for analysis by mass spectrometry.
Mass Spectrometry

Silver-stained bands were excised, chopped into small pieces and transferred to 0.5-mL tubes. For all following steps, buffers were exchanged by two consecutive 15 min incubation steps in 200 μL of acetonitrile, which was removed after each step. Proteins were reduced by the addition of 200 μL of 10 mM DTT, 100 mM (NH₄)HCO₃ at 56 °C for 30 min and alkylated by the addition of 200 μL of 55 mM iodoacetamide, 100 mM (NH₄)HCO₃ for 20 min in the dark. A volume of 50 μL of 1 ng/μL trypsin in 50 mM (NH₄)HCO₃ was added and samples were incubated for 30 min on ice and then over night at 37 °C. Gel pieces were sonicated for 15 min, spun down and the supernatant was transferred into a glass vial (VDS OptiLab, 93908556). The gel pieces were washed once with 50 μL of an aqueous solution of 50% acetonitrile and 1% formic acid and sonicated for 15 min. The combined supernatants were dried and reconstituted in 10 μL of 0.1% (v/v) formic acid.

Peptides were separated using the nanoAcquity UPLC system (Waters) with nanoAcquity trapping (nanoAcquity Symmetry C18, 5 μm, 180 μm × 20 mm) and analytical (nanoAcquity BEH C18, 1.7μm, 75 μm × 200 mm) columns, which were coupled to an LTQ Orbitrap Velos (Thermo Fisher Scientific) using the Proxeon nanospray source. Peptides were loaded for 6 min using a constant flow of solvent A (0.1% formic acid) at 5 μL min⁻¹. Peptides were then separated via the analytical column using a constant flow of 0.3 μL min⁻¹. The percentage of solvent B (acetonitrile, 0.1% formic acid) was increased from 3 to 10% within 5 min, followed by an increase to 40% within 10 min. Eluting peptides were ionized with a Pico-Tip Emitter 360 μm OD × 20 μm ID (10 μm tip, New Objective), applying a spray voltage of 2.2 kV at 300 °C. Peptides were analyzed with an Orbitrap Velos Pro system (Thermo). Full scan MS spectra with a mass range of 300–1,700 m/z were acquired in profile mode with a resolution of 30,000 and a filling time of 500 ms, applying a limit of 106 ions. The 15 most intense ions were fragmented in the LTQ using a normalized collision energy of 40%. Three times 104 ions were selected within 100 ms and fragmented upon accumulation of selected precursor ions. MS/MS data were acquired in centroid mode of multiple charged (2+, 3+, 4+) precursor ions. The dynamic exclusion list was restricted to 500 entries with a maximum retention period of 30 s and relative mass window of 10 ppm. In order to improve the mass accuracy, a lock mass correction using a background ion (m/z 445.12003) was applied.

Data were processed using IsobarQuant (Franken et al., 2015) and Mascot (v2.2.07), including carbamidomethyl (C), acetyl (N-term) and oxidation (M) modifications. The mass error tolerance for full scan MS spectra was set to 10 ppm and for MS/MS spectra to 0.02 Da. A maximum of 2 missed cleavages were allowed. A minimum of two unique peptides with a peptide length of at least seven amino acids and a false discovery rate below 0.01 were required on the peptide and protein level (Table S4).

ChIP-qPCR experiments were performed as described previously (Cuylen et al., 2011; Kschonsak et al., 2017). Yeast strains were grown in 42 mL YPAD at 30 °C, pH 8.0, 10 mM EDTA, 1% (w/v) SDS) at 65 °C. Fixation was stopped by addition of glycerol to 125 mM (final concentration), followed by washing steps in PBS and PIPES buffer (100 mM PIPES-KOH pH 8.3). Cells were lysed by spheroplasting with 0.5 mg/mL zymolase T-100 (AMS Biotechnology) in HEMS buffer (100 mM HEPES-KOH pH 7.5, 1 mM EDTA, 1 mM MgSO₄, 1.2 M Sorbitol, 1 mM PMSF) containing cOm–EDTA, followed by resuspension of cells in 1.5 mL lysis buffer (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% (v/v) Triton X-100, 0.1% (w/v) sodium deoxycholate, 1 mM PMSF) containing cOm–EDTA. Chromatin was sheared by sonication to a length of ~500 bp using a Bioruptor UCD-200 (Diagenode) for 9 min, ‘high level’ setting (30 s on, 60 s off).

Lysates were cleared by centrifugation at 16,800 × gmax and pre-cleared with 50 μL Protein A Dynabeads (ThermoFisher Scientific) for 90 min at 4 °C. 10% of the cleared lysate was used to check sonication, 12% was kept on ice as input sample. 2 μg anti-PK (V5) tag antibody (Abd Serotec MCA1360) was added to the remaining lysate and samples were incubated at 4 °C for 16 h before addition of 100 μL Protein A Dynabeads (ThermoFisher Scientific) for another 4 h at 4 °C. Beads were washed with lysis buffer, wash buffer (10 mM TRIS-HCl pH 8.0, 0.25 M LiCl, 0.5% (w/v) sodium deoxycholate, 1 mM EDTA, 1 mM PMSF) containing cOm–EDTA and TE buffer (10 mM TRIS-HCl pH 8.0, 1 mM EDTA) containing cOm–EDTA. TE buffer was added to 300 μL TES buffer (50 mM TRIS–HCl pH 8.0, 10 mM EDTA, 1% (w/v) SDS) at 65 °C for 8 h. Addition of 30 μg RNaseA (Roche) for 90 min at 37 °C and 200 μg Proteinase K (Roche) for 90 min at 65 °C, DNA was purified via a spin column (QIAGEN) and eluted in 50 μL EB buffer.

qPCR reactions were set up for 5 μL of 1:5 and 1:25 dilutions for immunoprecipitated samples and 1:5, 1:50, 1:500 and 1:5,000 dilutions for input samples with SYBR green PCR Master mix (Applied Biosystems) and 5 μM qPCR primers (see Key Resources Table) on an Applied Biosystems 7,500 Fast Real-Time PCR System. Data were calculated from two independent experiments with two qPCR runs each.

Microscopy of Human Condensin Complexes

HeLa Kyoto H2B-mCherry cells (Neumann et al., 2010) were transiently transfected with pC1 FLAG-EGFP-NCAPH or SMCM-FLAG-EGFP as described previously (Kschonsak et al., 2017).

Images were analyzed with Fiji (Schindelin et al., 2012). First, background was subtracted using the rolling ball algorithm. Chromatin regions were segmented based on the mCherry fluorescence signal and the whole cell was segmented based on the bright field image. Cytoplasmic regions were selected after subtracting the areas of chromatin from the whole cell regions. Mean fluorescence intensities of EGFP images were measured for chromatin and cytoplasmic regions. Data were calculated from two independent experiments. Cells were tested for mycoplasma contamination.
QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical details of experiments can be found in the figure legends or Method Details section.

DATA AND SOFTWARE AVAILABILITY

The accession numbers for the coordinate files reported in this paper are PDB: 6Q6E, 6QJ0, 6QJ1, 6QJ2, 6QJ3, 6QJ4. The accession number for the NMR chemical shifts and restraints reported in this paper is BMRB: 34336. Original image files are available at Mendeleev Data: https://doi.org/10.17632/rk9hdmj8tk.1.
Supplemental Information

Structural Basis of an Asymmetric Condensin ATPase Cycle

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Figure S1. Structures of Smc2 and Smc4 ATPase Head Domains (related to Figure 1)

A Coomassie-stained SDS-PAGE lanes of wild-type Ct Smc2_{hd}–Brn1_{N} and wild-type or Q-loop mutant Ct Smc4_{hd}–Brn1_{C} complexes used for crystallization and ITC.

B Cartoon, electrostatic surface potential and surface conservation models of Ct Smc2_{hd} and Ct Smc4_{hd}–Brn1_{C}.

C Structural alignment based on the RecA lobe of Ct Smc2_{hd} (crystal form II) to ATPγS-bound structures of the Sc cohesin Smc1 (pdb 1W1W, C_{α} RMSD = 0.866) and Smc3 (pdb 4UX3, C_{α} RMSD = 2.880) or the nucleotide-free structures of B. subtilis (Bs) SMC (pdb 3ZGX, C_{α} RMSD = 2.866) and P. yayanosii (Py) SMC (pdb SXEI, C_{α} RMSD = 0.988). Close-up views highlight the position of the conserved Q-loop glutamine and Walker B glutamate residues.
Figure S2. Smc2–Smc4 ATPase Head Dimerization (related to Figure 1)

A Size exclusion chromatography and Coomassie-stained SDS-PAGE analysis of elution fractions of wild-type, Walker B, signature motif or W-loop mutant combinations of Ct Smc2hd–Bm1N and Ct Smc4hd–Bm1C. Dotted lines indicate elution profiles of Ct Smc2hd E1116Q–Bm1N and Ct Smc4hd E1475Q–Bm1C in the presence (black) or absence (light blue) of ATP, Ct Smc2hd E1116Q–Bm1N and Ct Smc4hd–Bm1C (blue) or Ct Smc2hd–Bm1N and Ct Smc4hd E1475Q–Bm1C (red) combinations.

B Model of a Ct Smc2hd–Ct Smc4hd–Bm1C complex built on the ATPγS-dimerized Sc Smc1hd–Scc1C homodimer structure (pdb 1W1W).

C ATPase assays with wild-type or Walker B mutant combinations of Ct Smc2hd–Bm1N and Ct Smc4hd–Bm1C (mean ± SD of 3 independent experiments).

D Coomassie-stained SDS-PAGE of wild-type, Walker B or W-loop mutants of Ct Smc2hd–Bm1N and Ct Smc4hd–Bm1C.

E ATPase assays with wild-type and W-loop mutant combinations of purified Ct Smc2hd–Bm1N and Ct Smc4hd–Bm1C complexes (mean ± SD of 3 independent experiments).
or Smc2K1078E were dissected and incubated for 3 days at 30°C. Protein expression levels were tested by western blotting of whole cell extracts against HA or PK epitope tags.

Whole cells extracts of strains (C4656, C4672, C4669, C4657, C4670, C4671, C4673, C4681) expressing Smc4-PK6 with bpa substitutions at the indicated position were tested by western blot analysis of Smc4 cross-linking products by probing against the PK epitope of Smc4-HA6.

Smc4 x bpa-PK6 strains co-expressing bpa-substituted versions of Smc4 with endogenously HA6-tagged versions of Ycs4 (C4715, C4697, C4700, C4754), Brn1 (C4713, C4695, C4698, C4752) or Ycg1 (C4714, C4696, C4699, C4753).
Figure S4. Mapping of Ct Ycs4–Brn1 Interaction Domains (related to Figure 3)

A Binding of purified Ct Ycs4 protein (input) to glutathione beads pre-bound with various GST-Ct Brn1 fragments tested by SDS-PAGE and Coomassie staining of bound fractions. B ITC of the binding of full-length Ct Ycs43-1222 or truncated Ycs43-689 to Ct Brn1225-512 or Ct Brn1336-512 (fit ± error of the fit). C Graphical representation of the Brn1 sequence conservation. Colors indicate conservation scores calculated from an alignment of sequences from 40 species (Table S1). Brn1 stretches used for detailed Ycs4 mapping and regions interacting with other condensin subunits are highlighted. D Size exclusion chromatography of complexes formed between full-length or truncationed versions of Ct Ycs4 and Ct Brn1225-512 or Ct Brn1336-512. Coomassie-stained SDS-PAGE of elution fractions.

Figure S5. Structure of the Ct Ycs4–Brn14 Complex (related to Figure 3)

A Cartoon, electrostatic surface potential and surface conservation models of the Ct Ycs4–Brn14 complex. Surfaces were not built for the carboxy-terminal portion of Ycs4 due to the crystallographic disorder of this part of the complex. The colored box indicates conservation scores in Ycs4 calculated from an alignment of sequences from 40 species and the positions of the Proboscis and conserved KG-loop are highlighted (Table S1). The cartoon model indicates the arrangement of the 21 HEAT-repeat motifs in Ct Ycs4 (H1–H21). B Side-by-side comparison of the Ct Ycs4–Brn1 structure to structures of the Hs Pds5 HEAT-repeat subunit and the Hs SA2–Scc1 HEAT-repeat-kleisin complex of cohesin (pdb 5HD1, 4PJJ), the Ct Ycg1–Brn1 HEAT-repeat-kleisin complex of condensin (pdb 5OQQ) and the Ct Scc2 subunit of the cohesin loading complex (pdb 5T8V).
Figure S6. ATP-dependent Ycs4–Brn1Y4 release from Smc2hd–Brn1N (related to Figure 4)
A Size exclusion chromatography of complexes formed between Ct wild-type, Smc4 W-loop mutant (Smc4S1439D, W1440A), Smc4 D-loop mutant (Smc4F1482A, R1483D) or Ycs4 KG-loop mutant (Ycs4KG-loop) versions of Smc4hd–Brn1C with Ycs4–Brn1225-418. B Sc Ycs4–PK6 protein expression levels tested by western blotting of whole cell extracts against the PK epitope tag (strains C5003, C5005, C5007). C Coomassie-stained SDS-PAGE of wild-type, Ycs4 KG-loop and Smc2–Smc4 Q-loop mutant Sc condensin holocomplexes used for ATPase assays. D Binding of combinations of purified Ct Ycs4–Brn11396-512, Ct Ycs4 and Ct Smc4hd–Brn1C proteins (input) to glutathione beads pre-bound with GST-Ct Brn11838-714 or GST-Ct Brn11225-340 tested by SDS-PAGE and Coomassie staining of bound fractions. E Model of the Ct Ycs4–Brn114–Smc4hd–Brn1C complex bound to Ct Smc2hd based on the ATPɣS-dimerized Sc Smc1hd–Scc1C homodimer structure (pdb 1W1W). Steric clashes are indicated by blue surfaces. F Binding of purified Ct Ycs4–Brn11396-512, Ct Ycs4, Ct Smc2hd–Brn1N and Ct Smc4hd–Brn1C protein combinations (input) to glutathione beads pre-bound with GST-Ct Brn11225-340, in the absence (–ATP) or presence of 600 μM ATP (+ATP) tested by SDS-PAGE and Coomassie staining of bound fractions. G Size exclusion chromatography of complexes formed between Ct Ycs4–Brn114 and wild-type, signature motif mutant (Smc4hd S1447R) or Q-loop mutant (Smc4hd Q421L) versions of Ct Smc4hd–Brn1C in the absence (–ATP) or presence of nucleotide (+ATP), or in the presence of ATP and 10 mM MgCl₂. Coomassie-stained SDS-PAGE of elution fraction in the presence of ATP. H Effect of different nucleotides on the interaction between Ct Smc4–Brn1C and Ct Ycs4–Brn11396-512 immobilized on glutathione beads via its binding to GST-Ct Brn11225-340, ITC in buffer containing 10 mM MgCl₂ of ATP binding by wild-type Ct Smc2hd–Brn1N and wild-type or Q-loop mutant Ct Smc4hd–Brn1C (fit ± error of the fit).
Figure S7. Structure and ATP-dependent release of the Smc2cc–Brn1N from condensin (related to Figures 5 and 6)

A Ensemble of the 10 lowest energy NMR conformers of Ct Smc2cc–Brn1N. B Superimposition of the lowest energy Ct Smc2cc–Brn1N NMR structure onto the Sc Smc3hd–Scc1N crystal structure (pdb 4UX3), using Smc2 coiled coil and Brn1 α3 helices as guide. Close-up views highlight the intercalation of the conserved kleisin tyrosine residues between the SMC coiled coils.

C Partial alignment of Smc2 amino-terminal (left) and carboxy-terminal (middle) neck regions as well as Brn1 Nα3 regions from 12 divergent species. Colors indicate conservation scores calculated from an alignment of sequences from 40 species (Table S1).

D Expression levels of wild-type (C5277) and mutant (C5278, C5279) versions of Smc2–PK6 or of wild-type (C5239) and mutant (C5261–C5263) versions of Brn1–PK6 in budding yeast probed by western blotting of whole cell extracts against the PK epitope tag.

E Size exclusion chromatography of purified Sc condensin complexes cleaved by TEV protease at position 141 in Brn1 in the absence of nucleotide or presence of ATP. Silver-stained SDS-PAGE of TCA-precipitated elution fractions.

F Comparison of W-loop residue conformations in nucleotide-free condensin Ct Smc2 and Ct Smc4 and ATPγS-bound Sc Smc1 (pdb 1W1W) head structures.
### Table S1. Recombinant DNA (related to Figures 1 to 6)

| Entry | Description |
|-------|-------------|
| 1771  | pETMCN 6×HIS-TEV-Bm1112-204-Smc2hd (res. 112–204 of Ct Bm1 and res. 2–224, 981–1179 of Ct Smc2) |
| 2858  | pETMCN 6×HIS-TEV-Bm1112-204-Smc2hd (res. 112–204 of Ct Bm1 and res. 2–224, 981–1179 of Ct Smc2 with E1116Q) |
| 3138  | pETMCN 6×HIS-TEV-Bm1112-204-Smc2hd (res. 112–204 of Ct Bm1 and res. 2–224, 981–1179 of Ct Smc2 with Q147L) |
| 3388  | pETMCN 6×HIS-TEV-Bm1112-204-Smc2hd (res. 112–204 of Ct Bm1 and res. 2–224, 981–1179 of Ct Smc2 with S1088R) |
| 2936  | pETMCN 6×HIS-TEV-Bm1112-204-Smc2hd (res. 112–204 of Ct Bm1 and res. 2–224, 981–1179 of Ct Smc2 with W1080A) |
| 1911  | pETMCN 6×HIS-TEV-Bm1112-204-Smc2hd (res. 112–204 of Ct Bm1 and res. 2–215, 990–1179 of Ct Smc2) |
| 3427  | pETMCN Brn1112–204-Smc2hd TKK-AEE 6×HIS |
| 3428  | pETMCN Brn1112–204-Smc2hd TKK-AEE 6×HIS |
| 3426  | pETMCN Brn1112–204-Smc2hd TKK-AEE 6×HIS |
| 3577  | pETMCN Brn1112–204, Y180A-Smc2hd |
| 3578  | pETMCN Brn1112–204, Y180A-Smc2hd |
| 3627  | pETMCN Brn1112–204, Y180A-Smc2hd |
| 2676  | pETMCN 6×HIS-Brn1112–204-Smc2hd (res. 112–204 of Ct Bm1 and res. 981–1031, 170–224 of Ct Smc2) |
| 2566  | Multibac cre pIDC Brn1765–898–pFL Smc4m-m8-HIS (res. 765–898 of Ct Bm1 and res. 264–466, 1367–1542 of Ct Smc4) |
| 2775  | Multibac cre pIDC Brn1765–898–pFL Smc4m-m8-HIS (res. 765–898 of Ct Bm1 and res. 264–466, 1367–1542 of Ct Smc4 with E1475Q) |
| 3224  | Multibac cre pIDC Brn1765–898–pFL Smc4m-m8-HIS (res. 765–898 of Ct Bm1 and res. 264–466, 1367–1542 of Ct Smc4 with Q421L) |
| 3580  | Multibac cre pIDC Brn1765–898–pFL Smc4m-m8-HIS (res. 765–898 of Ct Bm1 and res. 264–466, 1367–1542 of Ct Smc4 with S1439D, W1440A) |
| 2944  | Multibac cre pIDC Brn1765–898–pFL Smc4m-m8-HIS (res. 765–898 of Ct Bm1 and res. 264–466, 1367–1542 of Ct Smc4 with S1447R, W1440A) |
| 3405  | Multibac cre pIDC Brn1765–898–pFL Smc4m-m8-HIS (res. 765–898 of Ct Bm1 and res. 264–466, 1367–1542 of Ct Smc4 with T184A, K187E, K188E) |
| 3406  | Multibac cre pIDC Brn1765–898–pFL Smc4m-m8-HIS (res. 765–898 of Ct Bm1 and res. 264–466, 1367–1542 of Ct Smc4 with W1080A) |
| 3404  | Multibac cre pIDC Brn1765–898–pFL Smc4m-m8-HIS (res. 765–898 of Ct Bm1 and res. 264–466, 1367–1542 of Ct Smc4 with F1482A, R1483D) |
| 2943  | Multibac cre pIDC Brn1765–898–pFL Smc4m-m8-HIS (res. 765–898 of Ct Bm1 and res. 264–466, 1367–1542 of Ct Smc4 with W1440A) |
| 1896  | pETMCN 6×HIS-TEV-Ycs4m3–1222 (res. 3–1222 of Ct Ycs4) |
| 2319  | pETMCN 6×HIS-TEV-Ycs4m3–1222 (res. 3–827 of Ct Ycs4) |
| 2322  | pETMCN 6×HIS-TEV-Ycs4m3–1222 (res. 3–689 of Ct Ycs4) |
| 1857  | pETMCN 6×HIS-TEV-Ycs4m3–1222 (res. 3–518 of Ct Ycs4) |
| 2221  | pETMCN 6×HIS-TEV-Bm1125–512=1225–512 (res. 225–512 of Ct Bm1 and res. 3–1222 of Ct Ycs4) |
| 2220  | pETMCN 6×HIS-TEV-Bm1125–512=1225–512 (res. 225–512 of Ct Bm1 and res. 3–1222 of Ct Ycs4) |
| 2777  | pETMCN 6×HIS-TEV-Bm1125–512=1225–512 (res. 225–512 of Ct Bm1 and res. 3–1222 of Ct Ycs4) |
| 3176  | pETMCN 6×HIS-TEV-Bm1125–512=1225–512 (res. 225–512 of Ct Bm1 and res. 3–1222 of Ct Ycs4) |

Continued on next page
Table S1. Recombinant DNA (related to Figures 1 to 6) continued from previous page

| ID   | Constructs                                                                 | Notes                                                                 |
|------|-----------------------------------------------------------------------------|----------------------------------------------------------------------|
| 3314 | 2μ pGAL7 Smc4-3×StrepII, pGAL10 Smc2, pGAL1 Brn1(ybbR13–23, 3×TEV141)-12×HIS-3×HA, TRP1 (Sc Smc4, Smc2, Brn1 with ybbR tag replacing res. 13–23, 3×TEV site inserted at res. 141) |                                                                                   |
| 3349 | 2μ pGAL7 Smc4E1352Q-3×StrepII, pGAL10 Smc2E1113Q, pGAL1 Brn1(ybbR13–23, 3×TEV141)-12×HIS-3×HA, TRP1 (Sc Smc4 with E1352Q, Smc2 with E1113Q, Brn1 with ybbR tag replacing res. 13–23, 3×TEV site inserted at res. 141) |                                                                                   |
| 3371 | 2μ pGAL7 Smc4Q302L-3×StrepII, pGAL10 Smc2Q147L, pGAL1 Brn1(ybbR13–23, 3×TEV141)-12×HIS-3×HA, TRP1 (Sc Smc4 with Q302L, Smc2 with Q147L, Brn1 with ybbR tag replacing res. 13–23, 3×TEV site inserted at res. 141) |                                                                                   |
| 3378 | 2μ pGAL7 Smc4S1324R-3×StrepII, pGAL10 Smc2S1085R, pGAL1 Brn1(ybbR13–23, 3×TEV141)-12×HIS-3×HA, TRP1 (Sc Smc4 with S1324R, Smc2 with S1085R, Brn1 with ybbR tag replacing res. 13–23, 3×TEV site inserted at res. 141) |                                                                                   |
| 3373 | 2μ pGAL7 Smc4-3×StrepII, pGAL10 Smc2, pGAL1 Brn1(ybbR13–23, 1×TEV141, 1×TEV373)-12×HIS-3×HA, TRP1 (Sc Smc4, Smc2, Brn1 with ybbR tag replacing residues 13–23, 1×TEV sites inserted at res. 141 and 373) |                                                                                   |
| 1999 | pSMC4-FLAG-EGFP (Hs SMC4)                                                   |                                                                                   |
| 1725 | pSMC4-FLAG-EGFPWA (Hs SMC4 with W1185A)                                     |                                                                                   |
| 1732 | pSMC4-FLAG-EGFPSD, WA (Hs SMC4 with S1184D, W1185A)                         |                                                                                   |
| 1455 | pFLAG-EGFP-NCAPD2 (Hs NCAPD2)                                               |                                                                                   |
| 3649 | pFLAG-EGFP-NCAPD2 (Hs NCAPD2 with K1384D, V1385S, K1386D, Q1388D, V1389S) |                                                                                   |
| 1288 | 6.4kb (plasmid backbone with E. coli ori and ampR)                          |                                                                                   |
| Animals | Protists | Plants | Fungi | Prokaryotes |
|---------|----------|--------|-------|-------------|
| Birds   | Galleria sulphuraria | Homo sapiens | Saccharomyces cerevisiae | Cryptomonads |
| Reptiles| Chelydra serpentina | Mus musculus | Candida albicans | Guillardia theta |
| Amphibians| Xenopus laevis | Gallus gallus | Chaetomium thermophilum | Leishmania major |
| Fish | Danio rerio | Chelonia mydas | Neurospora crassa | Trypanosoma brucei |
| Arthropods | Drosophila melanogaster | Thalassiosira pseudonana | Dictyostelium | Saprolegnia parasitica |
| Nematodes | Emericella nidulans | Phytophthora infestans | Neurospora crassa | Guillardia theta |
| Flatworms | Schistosoma mansoni | Synechococcus sp. | Neurospora crassa | Leishmania major |

| Vertebrates | Eucaryotes | Eudicots | Monocots | Nematodes |
|-------------|------------|----------|----------|----------|
| Mammals | Arabidopsis thaliana | Theobroma cacao | Oryza sativa | Amborella family |
| Birds | Thelecitons latifrons | Eucalyptus grandis | W1P9A4 | Amborella trichopoda |
| Reptiles | Eucalyptus grandis | Myrtus communis | W1PTT0 | Physcomitrella patens |
| Amphibians | Solanum lycopersicum | Glycine max | W1N1C1 | Physcomitrella patens |
| Fish | Arabidopsis thaliana | Vitis vinifera | Somaticidae | Amborella trichopoda |
| Insects | Arabidopsis thaliana | Solanum lycopersicum | Physcomitrella patens | Amborella trichopoda |

| Table S2. Uniprot Identifiers for Sequence Alignments (related to Figures 2, S1, S3, S5 and S7) |
|-----------------------------------------------|
| Homo sapiens | Q9K3J5 | Q9A617 | Q10037 | Q1021 |
| Mus musculus | Q60482 | Q84CG4 | Q10166 | Q10166 |
| Gallus gallus | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Chelydra mydas | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Xenopus laevis | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Drosophila melanogaster | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Eucalyptus grandis | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Solanum lycopersicum | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Arabidopsis thaliana | Q9Q073 | Q84CG4 | Q10166 | Q10166 |

| Homo sapiens | Q9K3J5 | Q9A617 | Q10037 | Q1021 |
| Mus musculus | Q60482 | Q84CG4 | Q10166 | Q10166 |
| Gallus gallus | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Chelydra mydas | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Xenopus laevis | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Drosophila melanogaster | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Eucalyptus grandis | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Solanum lycopersicum | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Arabidopsis thaliana | Q9Q073 | Q84CG4 | Q10166 | Q10166 |
| Yeast Genotypes (related to Figures 2, 4, 5, 6, S3, S6 and S7) |
|---------------------------------------------------------------|
| C4568  MATα, smo4::HIS3/SMC4, ura3::SMC4-HA::URA3/ura3       |
| C4592  MATα, smo4::HIS3/SMC4, ura3::SMC4_A1276amb::PK6/LEU2 |
| C4595  MATα, smo4::HIS3/SMC4, ura3::SMC4S1298amb::PK6/LEU2  |
| C4570  MATα, smo4::HIS3/SMC4, ura3::SMC4Q1283amb::PK6/LEU2  |
| C4590  MATα, smo4::HIS3/SMC4, ura3::SMC4S1298amb::PK6/LEU2  |
| C4589  MATα, smo4::HIS3/SMC4, ura3::SMC4S1298amb::PK6/LEU2  |
| C4564  MATα, smo2::hphMX4/SMC2, trp1::SMC2-PK6::TRP1/tra1    |
| C4567  MATα, smo2::hphMX4/SMC2, trp1::SMC2W1077A::PK6::TRP1  |
| C4608  MATα, smo2::hphMX4/SMC2, trp1::SMC2K1078E::PK6::TRP1  |
| C4582  MATα, smo2::hphMX4/SMC2, trp1::SMC2S1085R::PK6::TRP1  |
| C4656  MATα, smo4::natMX, [YCplac111 SMC4-PK6 LEU2], [pLH157::TRP1] |
| C4672  MATα, smo4::natMX, [YCplac111 SMC4A1276amb::PK6 LEU2], [pLH157 TRP1] |
| C4669  MATα, smo4::natMX, [YCplac111 SMC4S1298amb::PK6 LEU2], [pLH157 TRP1] |
| C4657  MATα, smo4::natMX, [YCplac111 SMC4Q1283amb::PK6 LEU2], [pLH157 TRP1] |
| C4670  MATα, smo4::natMX, [YCplac111 SMC4S1298amb::PK6 LEU2], [pLH157 TRP1] |
| C4671  MATα, smo4::natMX, [YCplac111 SMC4Q1283amb::PK6 LEU2], [pLH157 TRP1] |
| C4673  MATα, smo4::natMX, [YCplac111 SMC4S1298amb::PK6 LEU2], [pLH157 TRP1] |
| C4681  MATα, smo4::natMX, [YCplac111 SMC4S1298amb::PK6 LEU2], [pLH157 TRP1] |
| C4715  MATα, smo4::natMX, YCS4-HA::HIS3, [YCplac111 SMC4-PK6 LEU2], [pLH157 TRP1] |
| C4697  MATα, smo4::natMX, YCS4-HA::HIS3, [YCplac111 SMC4A1276amb::PK6 LEU2], [pLH157 TRP1] |
| C4700  MATα, smo4::natMX, YCS4-HA::HIS3, [YCplac111 SMC4S1298amb::PK6 LEU2], [pLH157 TRP1] |
| C4754  MATα, smo4::natMX, YCS4-HA::HIS3, [YCplac111 SMC4Q1283amb::PK6 LEU2], [pLH157 TRP1] |
| C4713  MATα, smo4::natMX, BRN1-HA::HIS3, [YCplac111 SMC4-PK6 LEU2], [pLH157 TRP1] |
| C4695  MATα, smo4::natMX, BRN1-HA::HIS3, [YCplac111 SMC4A1276amb::PK6 LEU2], [pLH157 TRP1] |
| C4698  MATα, smo4::natMX, BRN1-HA::HIS3, [YCplac111 SMC4S1298amb::PK6 LEU2], [pLH157 TRP1] |
| C4752  MATα, smo4::natMX, BRN1-HA::HIS3, [YCplac111 SMC4Q1283amb::PK6 LEU2], [pLH157 TRP1] |
| C4714  MATα, smo4::natMX, YCG1-HA::HIS3, [YCplac111 SMC4-PK6 LEU2], [pLH157 TRP1] |
| C4696  MATα, smo4::natMX, YCG1-HA::HIS3, [YCplac111 SMC4A1276amb::PK6 LEU2], [pLH157 TRP1] |
| C4699  MATα, smo4::natMX, YCG1-HA::HIS3, [YCplac111 SMC4S1298amb::PK6 LEU2], [pLH157 TRP1] |
| C4753  MATα, smo4::natMX, YCG1-HA::HIS3, [YCplac111 SMC4Q1283amb::PK6 LEU2], [pLH157 TRP1] |
| C5003  MATα, ycs4::kanMX6/YCS4, URA3/ura3                   |
| C5005  MATα, ycs4::kanMX6/YCS4, ura3::YCS4::PK6::URA3/ura3  |
| C5007  MATα, ycs4::kanMX6/YCS4, ura3::YCS4K1048D, V1049S, K1050D, C1052D, L1053R::PK6::URA3/ura3 |
| C5277  MATα, smo2::HIS3/SMC2, ura3::SMC2-PK6::URA3/ura3     |
| C5278  MATα, smo2::HIS3/SMC2, ura3::SMC2T184A, K187E, K188E::PK6::URA3/ura3 |
| C5279  MATα, smo2::HIS3/SMC2, ura3::SMC2D1010A, K1013E::PK6::URA3/ura3 |
| C4239  MATα, brn1::HIS3/BRN1, ura3::BRN1-PK6::URA3/ura3    |
| C5261  MATα, brn1::HIS3/BRN1, ura3::BRN1Y166::PK6::URA3/ura3 |
| C5262  MATα, brn1::HIS3/BRN1, ura3::BRN1Y166::PK6::URA3/ura3 |
| C5263  MATα, brn1::HIS3/BRN1, ura3::BRN1Y166::PK6::URA3/ura3 |

Continued on next page
### Table S3. Yeast Genotypes (related to Figures 2, 4, 5, 6, S3, S6 and S7) continued from previous page

| Yeast Strain | Detailed Genotype Description |
|--------------|--------------------------------|
| C4491        | MATα, lys2::pGAL1 GAL4::LYS2, pep4::HIS3, bar1::hisG, [2μ, pGAL7 SMC4-(StrepII)3, pGAL10 SMC2, pGAL1 BRN1-HA3-His12 TRP1]. [2μ, pGAL1 YCG1, pGAL10 YCS4 URA3] |
| C4724        | MATα, lys2::pGAL1 GAL4::LYS2, pep4::HIS3, bar1::hisG, [2μ, pGAL7 SMC4-(StrepII)3, pGAL10 SMC2 Q147L, pGAL1 BRN1-HA3-His12 TRP1]. [2μ, pGAL1 YCG1, pGAL10 YCS4 URA3] |
| C5050        | MATα, lys2::pGAL1 GAL4::LYS2, pep4::HIS3, bar1::hisG, [2μ, pGAL7 SMC4-(StrepII)3, pGAL10 SMC2, pGAL1 BRN1-HA3-His12 TRP1]. [2μ, pGAL1 YCG1, pGAL10 YCS4 URA3] |
| C4896        | MATα, lys2::pGAL1 GAL4::LYS2, pep4::HIS3, bar1::hisG, [2μ, pGAL7 SMC4-(StrepII)3, pGAL10 SMC2, pGAL1 BRN1 (TEV141)3-HA3-His12 TRP1]. [2μ, pGAL1 YCG1, pGAL10 YCS4 URA3] |
| C5066        | MATα, lys2::pGAL1 GAL4::LYS2, pep4::HIS3, bar1::hisG, [2μ, pGAL7 SMC4-(StrepII)3, pGAL10 SMC2, pGAL1 ybbR(12-24)-BRN1 (TEV141)3-HA3-His12 TRP1]. [2μ, pGAL1 YCG1, pGAL10 YCS4 URA3] |
| C5125        | MATα, lys2::pGAL1 GAL4::LYS2, pep4::HIS3, bar1::hisG, [2μ, pGAL7 SMC4-(StrepII)3, pGAL10 SMC2 Q147L, pGAL1 ybbR12-24BRN1 (TEV141)3-HA3-His12 TRP1]. [2μ, pGAL10 YCS4, pGAL1 YCG1 URA3] |
| C5139        | MATα, lys2::pGAL1 GAL4::LYS2, pep4::HIS3, bar1::hisG, [2μ, pGAL7 SMC4-(StrepII)3, pGAL10 SMC2 Q147L, pGAL1 ybbR12-24BRN1 (TEV141)3-HA3-His12 TRP1]. [2μ, pGAL10 YCS4, pGAL1 YCG1 URA3] |
| C5142        | MATα, lys2::pGAL1 GAL4::LYS2, pep4::HIS3, bar1::hisG, [2μ, pGAL7 SMC4-(StrepII)3, pGAL10 SMC2 E113Q, pGAL1 ybbR12-24BRN1 (TEV141)3-HA3-His12 TRP1]. [2μ, pGAL10 YCS4, pGAL1 YCG1 URA3] |
| C5110        | MATα, lys2::pGAL1 GAL4::LYS2, pep4::HIS3, bar1::hisG, [2μ, pGAL7 SMC4-(StrepII)3, pGAL10 SMC2, pGAL1 ybbR12-24BRN1 (TEV141)3-HA3-His12 TRP1]. [2μ, pGAL10 YCS4, pGAL1 YCG1 URA3] |
| C5122        | MATα, lys2::pGAL1 GAL4::LYS2, pep4::HIS3, bar1::hisG, [2μ, pGAL7 SMC4-(StrepII)3, pGAL10 SMC2, pGAL1 ybbR12-24BRN1 (TEV141, TEV373)3-HA3-His12 TRP1]. [2μ, pGAL10 YCS4, pGAL1 YCG1 URA3] |
## Table S4. Mass Spectrometry Data (related to Figure 2E)

| Protein Name | Uniprot Identifier | Mass (kDa) | Score  | Number Peptides | Sequence Coverage (%) |
|--------------|--------------------|------------|--------|-----------------|----------------------|
| Smc4S1298bpΔ–UV | None | | | | |
| Sc Smo4 | Q12267 | 162.1 | 2,236 | 59 | 35.4 |
| Sc Ycs4 | Q06156 | 132.9 | 1,638 | 38 | 26.1 |
| Sc Bm1 | P38170 | 86.2 | 392 | 10 | 13.9 |
| Sc Smc2 | P38989 | 133.8 | 272 | 5 | 4.6 |
### Table S5. NMR Statistics (related to Figure 5)

#### Experimental restraints

| Distance restraints                  |         |
|-------------------------------------|---------|
| Total NOEs (unambiguous/ambiguous)  | 2527/317|
| Short range (|i–j| ≤ 1)              | 1884/202|
| Medium range (|i–j| < 5)             | 265/66  |
| Long range (|i–j| > 5)              | 378/49  |
| Hydrogen bonds                      | 61      |
| Dihedral restraints (φ/ψ)           | 170/170 |

#### Structural quality

| Coordinate precision (Å, residues 58–80,130–160,167–193)          |         |
|------------------------------------------------------------------|---------|
| Backbone (N, Cα, C')                                            | 0.65 ± 0.16 |
| Heavy atoms                                                      | 1.16 ± 0.15 |

| Coordinate precision (Å, residues 16-46,51–56,58–80,130–160,167–193) |         |
|------------------------------------------------------------------|---------|
| Backbone (N, Cα, C')                                            | 0.79 ± 0.15 |
| Heavy atoms                                                      | 1.25 ± 0.15 |

| Restraint RMSD                  |         |
|---------------------------------|---------|
| Distance restraints (Å)         | 0.024 ± 0.005 |
| Dihedral restraints (°)         | 0.91 ± 0.47  |

| Deviation from idealized geometry |         |
|-----------------------------------|---------|
| Bond lengths (Å)                  | 0.0032 ± 0.0001 |
| Bond angles (°)                   | 0.46 ± 0.02  |

| Ramachandran analysis (%)        |         |
|----------------------------------|---------|
| Favoured regions                 | 91.1 ± 1.4  |
| Allowed regions                   | 8.0 ± 1.5  |
| Generously allowed               | 0.4 ± 0.3   |
| Disallowed                        | 0.6 ± 0.5 |

| Whatcheck analysis              |         |
|----------------------------------|---------|
| 1st generation packing           | −0.717 ± 0.311  |
| 2nd generation packing           | −2.019 ± 0.342  |
| Ramachandran plot appearance    | −1.889 ± 0.427  |
| Chi-1/Chi-2 rotamer normality    | −2.958 ± 0.385  |
| Backbone conformation            | −0.666 ± 0.435  |