Structure of the cohesin loader Scc2

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The functions of cohesin are central to genome integrity, chromosome organization and transcription regulation through its prevention of premature sister-chromatid separation and the formation of DNA loops. The loading of cohesin onto chromatin depends on the Scc2–Scc4 complex; however, little is known about how it stimulates the cohesion-loading activity. Here we determine the large ‘hook’ structure of Scc2 responsible for catalysing cohesin loading. We identify key Scc2 surfaces that are crucial for cohesin loading in vivo. With the aid of previously determined structures and homology modelling, we derive a pseudo-atomic structure of the full-length Scc2–Scc4 complex. Finally, using recombinantly purified Scc2–Scc4 and cohesin, we performed crosslinking mass spectrometry and interaction assays that suggest Scc2–Scc4 uses its modular structure to make multiple contacts with cohesin.
he cohesin complex (Smc1, Smc3, Scc1 and Scc3) safeguards genome integrity by ensuring correct sister-chromatid segregation during mitosis and meiosis1–3. The topological entrapment of chromosomes by cohesin is catalysed by the cohesin–loader complex Scc2–Scc4 (refs 4,5). In yeast, Scc2–Scc4 coordinates with the remodels structure of chromatin (RSC) chromatin-remodelling complex to load cohesin at nucleosome-free regions, while mutations of the Scc4-conserved surface disrupt centromeric loading of cohesin6,7. Cohesin alone can bind DNA in vitro; however, the presence of the Scc2–Scc4 complex substantially accelerates its topological loading onto circular DNA in an ATP-dependent manner8. Functional studies have revealed that the in-vitro-loading activity of Scc2–Scc4 resides in the C terminus of Scc2, in contrast, Scc4 is likely a chromatin adaptor for targeting cohesin in vivo9–11. A number of Scc2–Scc4 contacts have been mapped on different domains of cohesin subunits that are spatially separated9. This has led to the suggestion that cohesin undergoes a large conformational change during loading8,9. Both rotary shadowed electron microscopy (EM) and atomic force microscopy studies have shown considerable flexibility in the Smc-coiled coils that could allow such a change to occur10–13. Apart from sister-chromatid cohesion, cohesin also plays important roles in organizing chromosome structure and regulating transcription1–3. Cohesin interacts with NipblScc2 and the mediator complex to connect enhancers and promoters of actively transcribing genes14. Cohesin conformation capture assays show that cohesin helps organize key cell identity genes into insulated neighbourhoods by the formation of chromosomal loop structures14,15. While in zebrafish and mice, NipblScc2 and the mediator cooperate to recruit cohesin to gene promoters16, while in zebrafish and mice, NipblScc2 and the mediator cooperatively regulate gene expression during limb development17. The intimate relation between cohesin and NipblScc2 manifests its importance in Cornelia de Lange syndrome (CdLS), one of a family of genetic disorders known as cohesinopathies18–20. Symptoms of CdLS include facial dysmorphism, intellectual disability and abnormal limb development, with 60% of CdLS patients carrying heterozygous mutations in the NipblScc2 protein. Cells with one functional copy of NipblScc2 have normal chromosome number and are not defective in sister-chromatid cohesion21. The disease is thought to be a result of abnormal gene expression, predominantly driven by loss-of-function mutations in NipblScc2. To gain further insight into the molecular function of the cohesin–loader Scc2–Scc4, which has such broad implications for both chromosome biology and genetic diseases, we determined the large modular structure of C-terminal Scc2 and derived a pseudo-atomic structure of the full-length Scc2–Scc4 complex. Our functional assays reveal key Scc2 surfaces that are crucial for cohesin loading in vivo. By using crosslinking mass spectrometry (XL-MS), we suggest that Scc2–Scc4 utilizes its modular structure to make multivalent contact with cohesin.

**Results**

**Modular structure of C-terminal Scc2.** The 130 kDa structure of *Ashyba gossypii* Scc2178–1479 was determined experimentally to 2.9 Å resolution by multiple isomorphous replacement with anomalous scattering (Supplementary Table 1). The Scc2 structure consists of an N-terminal globular domain (GD1) connected to 14 contiguous HEAT-repeat neck regions (Fig. 1a,b). The 15th and 16th repeats form part of an oval-shape globular domain (GD2) with an extended loop folded back to the domain body connecting the capping helix at the extreme C terminus. The crystal structure of Scc2 hook bears good resemblance to the corresponding 2D class averages from negative-stain EM6 (Fig. 1c). A small domain (residues 169–377, GD0), visible in the EM classes was not present in our crystallized construct (Fig. 1a,c). Sequence analysis and homology fold prediction suggest this missing domain is predominantly α-helical, and has a tertiary fold related to that of human symplekin22 (Supplementary Fig. 1a). When the Scc2 hook structure is combined with the previously determined tetratricopeptide repeat (TPR) structure of Scc21–168–Scc434–620 (Scc2N–Scc4) and the homology fold of GD0, a model of the full-length Scc2–Scc4 complex, which resembles the EM class averages, can be derived (Fig. 1a,d,e).

Our previous EM studies showed that the hook structure of Scc2 can adopt either open or closed conformations8. Analysis of the atomic structure shows there are a number of loops between adjacent HEAT repeats with high crystallographic temperature factors. In addition, normal mode analyses of the structure suggest a pincer-like opening and closing of the HEAT repeats around these loops (Supplementary Fig. 1b). These loops may permit considerable motion of the hook structure as suggested by the conformational variability observed in the Scc2 hook EM images8. In addition, the structure of the Scc2 hook contains a number of conserved buried residues that are mutated in CdLS18,20,23–27. These mutations result in significant changes in their side-chain chemical properties (Supplementary Fig. 1c; Supplementary Fig. 2). Patients carrying these mutations show severe phenotypes, suggesting that the disruption of Scc2 structural integrity can be a causal factor.

**Surface analysis of Scc2.** Sequence alignment and conservation analysis show that the Scc2 surface is relatively poorly conserved protein with only two highly conserved patches at the neck and base regions (Fig. 2a). To investigate the importance of these conserved surfaces, we designed three sets of conserved neck surface mutations D749A/S751A (Group I), K788A/R792A (Group II) and E821G/E822S/D823A (Group III), and one set of conserved base mutations Y1279A/E1280S/T1281G (Group IV) in *Saccharomyces cerevisiae* (Supplementary Table 2). Mutant yeast strains were subjected to viability, as well as in vivo chromatin-binding assays (Fig. 2b,c). Our results show that both Group I and Group IV have wild-type (WT) phenotype. However, the neck mutants Groups II and III reduce cell viability, and result in cohesin-binding defects at three known cohesin chromosome-binding sites (POA1, MET10 and CEN3). Scc2 shares strong structural similarity with the human SA2Scc3 and Pds5 (refs 28–30; Fig. 3), with all three proteins exhibiting a hook-like curve built from HEAT repeats. Interestingly, the respective HEAT-repeat neck region of each protein is conserved between species, with SA2Scc3 utilizing its neck region to interact with Scc1 (ref. 31). Scc2 may also adopt a similar mode of interaction with its binding partners by utilizing its equivalent conserved neck surface. Due to the low affinity between Scc2–Scc4 and cohesin5,6, we employed an interaction assay using glycerol gradient centrifugation and were able to form a stable complex between recombinant cohesin and WT Scc2–Scc4. We further performed interaction assays between cohesin and Scc2–Scc4 Groups II and III mutants to investigate the significance of the Scc2 neck region in cohesin interaction (Supplementary Fig. 3a). To our surprise, neither neck mutants, which result in reduced viability and cohesin-binding in vivo (Fig. 2b,c), had any impact on Scc2–Scc4 interaction with cohesin as assessed by co-sedimentation in vitro (Supplementary Fig. 3b–g) or communoprecipitation in vivo (Supplementary Fig. 3h). This suggests that the reduction in viability and impairment of cohesin binding to chromatin in vivo could be due to non-productive interaction between Scc2–Scc4 mutants and cohesin or due to the disruption of Scc2 interacting with a crucial yet unidentified binding partner through its neck region.
Interaction studies between Scc2–Scc4 and cohesin. To map interactions between cohesin and Scc2–Scc4, we performed amine XL-MS using recombinantly purified *S. cerevisiae* cohesin and WT Scc2–Scc4 (Fig. 4a; Supplementary Fig. 4; Supplementary Data 1). The inter- and intra-protein crosslinks observed are generally consistent with a similar study performed with full-length human cohesin12, as well as known crystal structures of Smc3–Scc1N and Scc2N–Scc4 7,32, with the corresponding crosslinks highlighted in the interaction diagram (Supplementary Fig. 4a; Supplementary Data 1). The cohesin–loader crosslinks show that Scc2–Scc4 utilizes its modular structure (Fig. 1a,d) to create multiple contacts with cohesin core subunits, notably between GD0/GD2 domains and the base of the Smc1/Smc3-coiled coils. To better validate these interactions, we purified a GD0 domain in isolation (isolated GD2 could not be expressed) and tested its interaction with cohesin by glycerol gradient centrifugation (Fig. 4b-f). We were able to observe co-migration of the GD0 domain with cohesin, supporting the multivalent interactions suggested by our crosslink data. Given the high flexibility of both cohesin and the loader8,12,33, some caution is required in the interpretation of the crosslinks as different transient conformations of the complex might be trapped. This would explain the adjacent sites on Smc1 (residues 377 and 379) crosslinked to GD0 (residue 269) and GD2 (residue 1,491) respectively. In addition, there are also intra-molecular crosslinks within Scc2 that are consistent with the flexible conformations exhibited by the motion of the Scc2 hook and the Scc2N–Scc4 region observed in Scc2–Scc4 complex under EM30 (Supplementary Fig. 4a; Supplementary Data 1).

We also attempted to visualize the cohesin–loader interaction by negative-stain EM for *Saccharomyces pombe* cohesin and the cohesin–loader complex (Supplementary Fig. 5). Cohesin and cohesin–loader complexes were purified by glycerol gradient centrifugation (Supplementary Fig. 5a). Complexes could be purified in the absence of crosslinker, but more homogenous particles were observed when using mild crosslinking (Grafix) treatment34. When visualized, both apo-cohesin and the cohesin–loader complex adopt a rod-like conformation with a head and an extended tail that is consistent with the human cohesin recently observed under EM33, as well as similar structures reported in atomic force microscopy and EM studies of cohesin and condensin10,11,13,35,36. The heterogeneity and limited resolution of the particles does not allow detailed analysis of the interaction, but particles with a bulkier head can be readily distinguished when the loader is bound (compare Supplementary Fig. 5b,c). We propose that this represents the loader binding at or near ATPase domains of cohesin, which is consistent with our crosslinking data (Fig. 4a), and known binding sites for both Pds5 and Scs3 (refs 12,28,29,31,33).

**Figure 1** | Structure of Scc2 hook and the full-length Scc2–Scc4 model. (a) Schematics showing the linear domain organizations of Scc2 and Scc4. The same colouring scheme is used in the corresponding crystal structures of Scc2 and Scc4 as shown in (b,d). (b) The modular structure of Scc2 resembles a ‘hook’. The overall fold comprises an N-terminal globular domain (GD1; violet), 14 contiguous HEAT repeats (yellow) and an oval-shaped C-terminal globular domain (GD2; wheat) with an extended loop connecting the C-terminal capping helix. (c) An EM 2D class average of Scc2 hook with GD0 indicated. (d) Reconstruction of a pseudo-full-length structure of Scc2–Scc4 with the previously determined crystal structure of Scc2-168–Scc434–620 (Scc2N–Scc4, PDB I.D. 5C6G; purple and salmon), a homology fold of Scc2169–377 (GD0; mauve) from human symplekin (PDB I.D. 3O2T) and Scc2 hook (violet, yellow and wheat). (e) An EM 2D class average of full-length Scc2–Scc4.
Discussion

We present here the large HEAT-repeat structure of the cohesion–loader Scc2 and reconstruct a pseudo-atomic structure of the modular Scc2–Scc4 complex. Our viability assays and in vivo chromatin-binding results indicate that the conserved surfaces at the Scc2 neck region are important for cohesin binding to chromatin (Fig. 2). However, the in vitro interaction assay and in vivo coimmunoprecipitation assay suggest that the mutations at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region in SA2 and Pds5 are highly conserved and is important in cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3). Given the equivalent region at the neck region do not impair Scc2–Scc4 interaction with cohesin (Supplementary Fig. 3).

Figure 2 | Scc2 surface analysis (a) Scc2 surface conservation with the positions of mutations indicated. (b) SCC2 mutants were tested for the ability to restore viability to a strain carrying an SCC2 degron allele. Cells of each strain were serially diluted and spotted on synthetic minimal medium without methionine (CSM–Met) plates or in plates containing indoleacetic acid (IAA). (c) Cohesin levels detected by chromatin immunoprecipitation against three cohesin-binding loci (POA1, MET10 and CEN3) and one negative control locus (TUB2) in either WT or SCC2 mutant strains arrested in mitosis. Error bars represent s.e.’s of assays each with three repeats.

regions (Fig. 4). In the Kikuchi et al.37 study, no interaction is detected between Scc2 and Smc1–Smc3 dimer or between Scc2 and Scc3 using in vitro translation-generated proteins. This could reflect that the binding of Scc2 to Smc1/3 is favoured by certain cohesin conformations, which may only exist in the context of the full tetrameric cohesin complex. The cohesin conformation that is required for Scc2 binding may involve an interaction between the Smc1/3 ATPase domains and between the adjacent Smc1/3 coiled coils (Fig. 4; Supplementary Fig. 5), which may only exist in the presence of Scc1 and Scc3. This mode of interaction between Scc2–Scc4 is similar to the interaction between full-length human cohesin and Pds5B (ref. 12). Interestingly, interactions are also seen between different Scc2–Scc4 modules and the DNA-entry/exit gate formed by Scc1 and Smc3 (refs 12,32), suggesting that the loader might play a role in DNA gate opening. Since Pds5 also binds to Scc1 and Smc3 close to DNA-entry/exit gate28,29,38, this could explain how Pds5 inhibits in vitro loading of cohesin by Scc2–Scc4 through competitive binding to the same site on cohesin9,37.

Our Scc2 structure contains a number of residues that are mutated in CdLS and are conserved between human and yeasts18,20,23–27 (Supplementary Fig. 1c; Supplementary Fig. 2). Patients carrying these mutations show classic phenotype of CdLS. In GD1 and the HEAT-repeat neck region lie a few buried conserved CdLS mutations, one of which (human R1895T) is required for the recruitment of histone deacetylase 3 in CdLS patients23 (Supplementary Fig. 1c; Supplementary Fig. 2). Further conserved CdLS mutations are located along the base region, all of which are buried within the HEAT-repeat structure. Adjacent to CdLS mutation L1584R is a buried, conserved temperature-sensitive (ts) mutation (A. gossypii D524K, S. cerevisiae E534K), which compromises ncRNA biogenesis, translational fidelity, and changes gene expression pattern in yeast1,39. Although different mechanisms have been proposed, the buried nature of the ts and CdLS mutations and their distribution along the Scc2 HEAT-repeat neck and base suggest that destabilizing these regions by CdLS mutations would compromise NipblScc2 functions in human in ways similar to that in yeast.

Our comparative structural analysis shows that cohesin subunits Scc2, Scc3 and Pds5 are likely descendants of a common
HEAT-repeat ancestor (Fig. 3), with all three proteins having been shown to bind to DNA. In condensin, there are two core HEAT-repeat subunits, Ycg1 and Ycs4, both of which are required for efficient DNA binding. It is possible that cohesin has evolved with two detached regulatory HEAT subunit, Scc2 and Pds5, and retained a core HEAT subunit, Scc3, whereas condensin retained both HEAT subunits as core. While yeast condensin is constitutively present on chromatin, a separate loader may offer more fine-tuned regulation of cohesin loading.

### Methods

#### Cloning and purification of Scc2 and Scc2-Scc4

*An. gossypii* Scc2<sup>378–1,479</sup> and *S. cerevisiae* Scc2–Scc4 were amplified by PCR from genomic DNA (LGC Standards) and cloned into a modified version of the MultiBac vector pFBDM<sup>42,43</sup>. A double Strep-tag II (ds) together with a tobacco etch virus cleavage site were introduced into the C terminus of Scc2. The resultant protein expression cassettes were recombined with the DH10MultiBac cells to create a bacmid. Both constructs were expressed using the baculovirus and insect cell (High 5 cells) systems, and purified by a combination of Strep-Tactin (Qiagen), anion exchange chromatography on Poros HQ50 and Superdex 200 size-exclusion chromatography (GE Healthcare) in a final buffer of 10 mM imidazole pH 7, 300 mM NaCl and 0.5 mM tris (2-carboxyethyl) phosphine (TCEP). Mutants of *An. gossypii* Scc2 and *S. cerevisiae* Scc2–Scc4 were generated using a USER cloning method.<sup>43</sup>

#### Crystallization and heavy-atom derivatization

*An. gossypii* Scc2<sup>378–1,479</sup> in size-exclusion buffer 10 mM imidazole pH 7, 300 mM NaCl and 0.5 mM TCEP was concentrated to 6 mg ml<sup>−1</sup>. Crystals were grown at 4 °C in a hanging-drop manner by seeding protein solution. Seeded protein solution was mixed with an equal volume of crystallization solution containing 100 mM imidazole (pH 6.8), 200 mM lithium

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**Figure 3** | Surface conservation comparison of cohesin HEAT repeat subunits. The Scc2, SA<sub>2</sub>Scc<sub>3</sub> (PDB I.D. 4PK7) and Pds5 (PDB I.D. 5F0O) structures all exhibit a hook-like curve built with strong conservation at their respective neck regions. All three structures can be presented as three regions: neck, cleft and base. (a) Surface conservation of Scc2 hook showing invariant (ruby) to less conserved (cyan) surfaces. (b) Surface conservation of SA<sub>2</sub>Scc<sub>3</sub> in the SA<sub>2</sub>Scc<sub>3</sub>–Scc1 complex showing invariant (ruby) to less conserved (cyan) SA<sub>2</sub>Scc<sub>3</sub> surfaces. (c) Surface conservation of Pds5 in the Pds5–Scc1 complex showing invariant (ruby) to less conserved (cyan) Pds5 surfaces.
sulfate and 4.5% polyethylene glycol 5,000 monomethyl ether (MME) at 4\degree C. Crystals started to appear after 3 days and continue to grow for 2 months. Crystals were collected either as native crystals in a cryo buffer containing 18% of polyethylene glycol 500 MME or as gold derivatives with an addition of 5 mM potassium tetrachloroaurate(III) hydrate.

Structure solution. The structure of Sc22 hook was solved by multiple isomorphous replacement with anomalous scattering using gold and selenomethionine derivatives. Native, selenomethionine and gold data were collected on beamlines IO2, IO3 and IO4 at the Diamond Light Source. The structure was solved using the AutoSHARP package\(^44\) and phases (overall figure of merit = 0.245) were improved using iterative solvent flattening (solvent content of 0.58) by SOLOMON. An initial model was traced using phenix.autobuild\(^45\). Iterative rounds of rebuilding and refinement were carried out using Coot\(^46\) and REFMAC5 (Ref. 47). The sequence was validated by referencing the 47 selenomethionine sites located by molecular replacement of the final model against single-wavelength anomalous diffraction data. Final refinement was carried out against a native data set at 2.9 Å. Full data collection and refinement statistics are

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**Figure 4 | Cohesin-loader interaction analysis.** (a) Diagram indicating intermolecular crosslinks ($n = 30$) between cohesin subunits and Sc22-Sc4. Grey boxes are structured domains of cohesin and Sc2-Sc4 (Smc-coiled coils are not indicated). Red crosslinks indicate interaction between GD0 and cohesin. Crosslinks that are consistent with the published human cohesin crosslinks\(^12\) are in blue. Crosslinks that are consistent with published Smc3-Sc1N and Sc2N-Sc4 crystal structures\(^7,32\) are in yellow. Crosslinks that are consistent with both the published human cohesin crosslinks and crystal structures are in green. A crosslink that would require a conformation change in the loader to occur for distance restraints to be satisfied is in orange. Positions of Sc2 mutations I-IV, DNA-entry/exit gate\(^9\) and the documented Pds5-binding sites\(^12,28,29,38\) are marked. (b) Silver-stain gel showing glycerol gradient fractions of GD0 domain. M indicates protein marker. (c) Silver-stain gel showing glycerol gradient fractions of cohesin. (d) Over-stained silver-stain gel showing glycerol gradient fractions of cohesin. (e) Silver-stain gel showing glycerol gradient fractions of $S.\text{cerevisiae}$ cohesin plus GD0 domain. (f) Over-stained silver-stain gel showing glycerol gradient fractions of cohesin plus GD0 with cohesin-bound GD0 being more visible.
given in Supplementary Table 1. A total of 95.99% of residues were in the Ramachandran favoured region, with 0.72% outliers. A stereo image of a portion of the electron density map can be seen in Supplementary Fig. 6.

**Sequence alignments and homology modelling.** Multiple sequence alignments were generated using ClustalOmega. Scc2169–377 was used to as a search sequence for homology structures using pGENTHREADER on the Phyre2 website. The human symplekin structure (3O2T) was used as a homology structure in reconstituting the pseudo-full-length structure of Scc2–Scc4 (ref. 22).

**EM image processing.** Class averages of the Scc2 hook domain and full-length Scc2–Scc4 complex were re-calculated using the data presented in ref. 8. The Scc2 hook data set consists of 5,221 particles and the Scc2–Scc4 complex 2,936. Reference-free class-averages were calculated using Relion50.

**Normal mode analysis.** Normal mode analyses of the Scc2 structure were carried out using PyANm plugin in PyMOL. A 12 Å cutoff was employed for modelling of the inter-residue interactions.

**Surface conservation analysis.** Surface conservation analyses were performed using the ConSurf server. Alignment sequences used for Scc2 are same as in Supplementary Fig. 52.

**In vivo survival assay.** A strain carrying an auxin-inducible degron allel of Scc2 was transformed with integrative plasmids carrying either WT Scc2, D749A, S751A (I), K788A/R792A (II), E821G/E822S/D823A (III) or Y1279A/E1280S/T1281G (IV) SCC2. Protein levels were checked by western blot. A total of 8 × 10^5 cells of each strain were serially diluted and spotted on synthetic minimal medium for homology structures using pGENTHREADER on the Phyre2 website. The human symplekin structure (3O2T) was used as a homology structure in reconstituting the pseudo-full-length structure of Scc2–Scc4 (ref. 22).

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**Sample preparation for EM.** S. pombe Scc2–Scc4 (200 nM) and cohesin (80 nM) were mixed and incubated at 4 °C for 30 min in 120 μg/ml Hepes-KOH (pH 7.5), 0.5 mM TCEP, 25 mM NaCl. A measure of 100 μl of the mixture was mounted on 5 μl of 20–50% glycerol gradient in buffer GG. The protein complexes were separated by ultracentrifugation at 4 °C at 48,000 r.p.m. for 15 h (beckman, MLA-55 rotor). The samples were fractionated by 200 μl each and analysed by SDS–PAGE and silver staining. Grafix were performed as the same protocol above but containing a linear gradient of 0–0.1% glutaraldehyde. Equivalent fractions from native and Grafix conditions were used for EM analyses.

**Electron microscopy.** A measure of 4 μl of cohesin and cohesin–loader complex purified from glycerol gradient centrifugation were directly applied to glow-discharged carbon-coated Quantifoil 2/2 grids and left to blot for 12–24 h in a humidified chamber. All samples were subsequently stained with 2% uranyl acetate and grids were visualized on a Tecnai G2 electron microscope operating at 120 kV. Images were recorded on a Gatan Ultrascan 2 K at × 30,000 giving a nominal sampling of 3.45 Å per pixel.

**Data availability.** Coordinates and structure factors of the Scc2 protein have been deposited in the Protein Data Bank under accession code 5ME3. The data support the findings of this study are available from the corresponding author upon request.

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