Supplementary Figure Legends

Figure S1. Purification and characterization of budding yeast cohesin and its loader.

(A) Purification scheme for the cohesin tetramer complex. Size exclusion chromatography was the final purification step. Fractions from the column were analyzed by SDS-PAGE followed by Coomassie Blue staining. Peak fractions corresponding to the cohesin tetramer complex were pooled and used for biochemical experiments described in this manuscript. The cohesin tetramer complex contained a substoichiometric contamination from chaperones of the Hsp70 family (Ssa1-4, Ssb1-2, identified by mass spectrometry), indicated by '*'. The UV absorbance elution profile from the size exclusion column is also shown, together with the profiles of size markers used to calibrate the column. **(B)** As (A), but the purification scheme and final size exclusion chromatography step of the Scc2-Scc4 cohesin loader complex purification are shown.

Figure S2. Characterization of budding yeast cohesin and its loader.

(A) DNA binding of cohesin to covalently closed circular DNA (CCC) and to linear DNA (L) was measured using an electrophoretic mobility shift assay. **(B)** DNA binding of the Scc2-Scc4 cohesin loader complex, next to the Scc2C fragment, was analyzed in an electrophoretic mobility shift assay. **(C)** Dose-dependent stimulation of cohesin loading by the cohesin loader. Gel image and quantification of the recovered DNA from cohesin loading assays performed with the indicated concentrations of the Scc2-Scc4 complex. The means and

standard deviations from three independent experiments are shown. **(D)** Stimulation of ATP hydrolysis by the Scc2C fragment. Rates of ATP hydrolysis were determined in reactions containing cohesin and DNA, without or with the cohesin loader or with the Scc2C fragment. The means and standard deviations from three independent experiments are shown.

Figure S3. Cohesin retains its oligomeric state during incubation at low ionic strength. 15 pmol of tetrameric cohesin was diluted to 150 nM in cohesin loading buffer (35 mM Tris-HCl pH 7.0, 20 mM NaCl, 0.5 mM $MgCl_2$, 13.3% Glycerol, 0.5 mM ATP, 0.003% Tween, 1 mM TCEP) to mimic the conditions of a cohesin loading reaction. The final cohesin concentration was somewhat higher than in a typical loading reaction, which was required to detect the protein in the following analysis. The sample was separated on a Superose 6 Increase 10/300 GL column equilibrated in R buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 10% glycerol, 0.5mM TCEP), or was first incubated at 29 ℃ for 120 minutes and then separated. In both samples, cohesin eluted at the expected size of the tetrameric complex (compare Fig S1A). At later elution volumes, the absorptions of detergent and ATP become apparent. The positions of the separation peaks of blue dextran (>2 MDa), thyroglobulin (660 kDa), aldolase (158 kDa), carbonic anhydrase (29 kDa) and aprotinin (6.5kDa), under the same conditions, are shown as a reference.

Figure S4. Topological loading of the budding yeast cohesin ring onto DNA.

(A) Gel image of input DNA of various topologies and of recovered DNA following cohesin loading. CCC, covalently closed circular DNA; RC, relaxed circular DNA; NC, nicked circular DNA; L, linear DNA. **(B)** Topological DNA loading promoted by Scc2C was assessed by DNA release following linearization with PstI. Supernatant (S) as well as beads-bound fractions (B) were retrieved following PstI or mock treatment. DNA in both fractions was analyzed by agarose gel electrophoresis.

Figure S5. Topological loading onto DNA without added ATP.

(A) A DNA release experiments by restriction enzyme cleavage was carried out following a cohesin loading reaction in the presence or absence of added ATP. The immobilized cohesin-DNA complex was incubated with or without PstI. Supernatant (S) as well as beads-bound (B) fractions were collected and DNA in each fraction was analyzed by agarose gel electrophoresis. **(B)** Gel image and quantification of recovered DNA from cohesin loading reactions performed with or without added ATP using Scc2C as the cohesin loader. **(C)** Topological DNA loading by Walker B motif mutant EQ-cohesin, in the absence or presence of the cohesin loader, was analyzed by DNA release following linearization with PstI. Supernatant (S) as well as beads-bound fractions (B) were retrieved following PstI or mock treatment. DNA in both fractions was analyzed by agarose gel electrophoresis.