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Supplementary Information for

An intermediate step of cohesin's ATPase cycle allows cohesin to entrap DNA

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Supplementary Information Text

Elution of cohesin from DNA-beads with DNase I

For DNase treatment, DNA-cohesin complexes were assembled as described above (DNA-bead assay), resuspended in CL1 buffer in the presence of 2U DNase I (Sigma) and incubated at 30°C for 30 minutes. Input proteins (10%), starting beads, and supernatant and pellet after the DNase I treatment were run on SDS-PAGE. Proteins were visualized by silver staining.

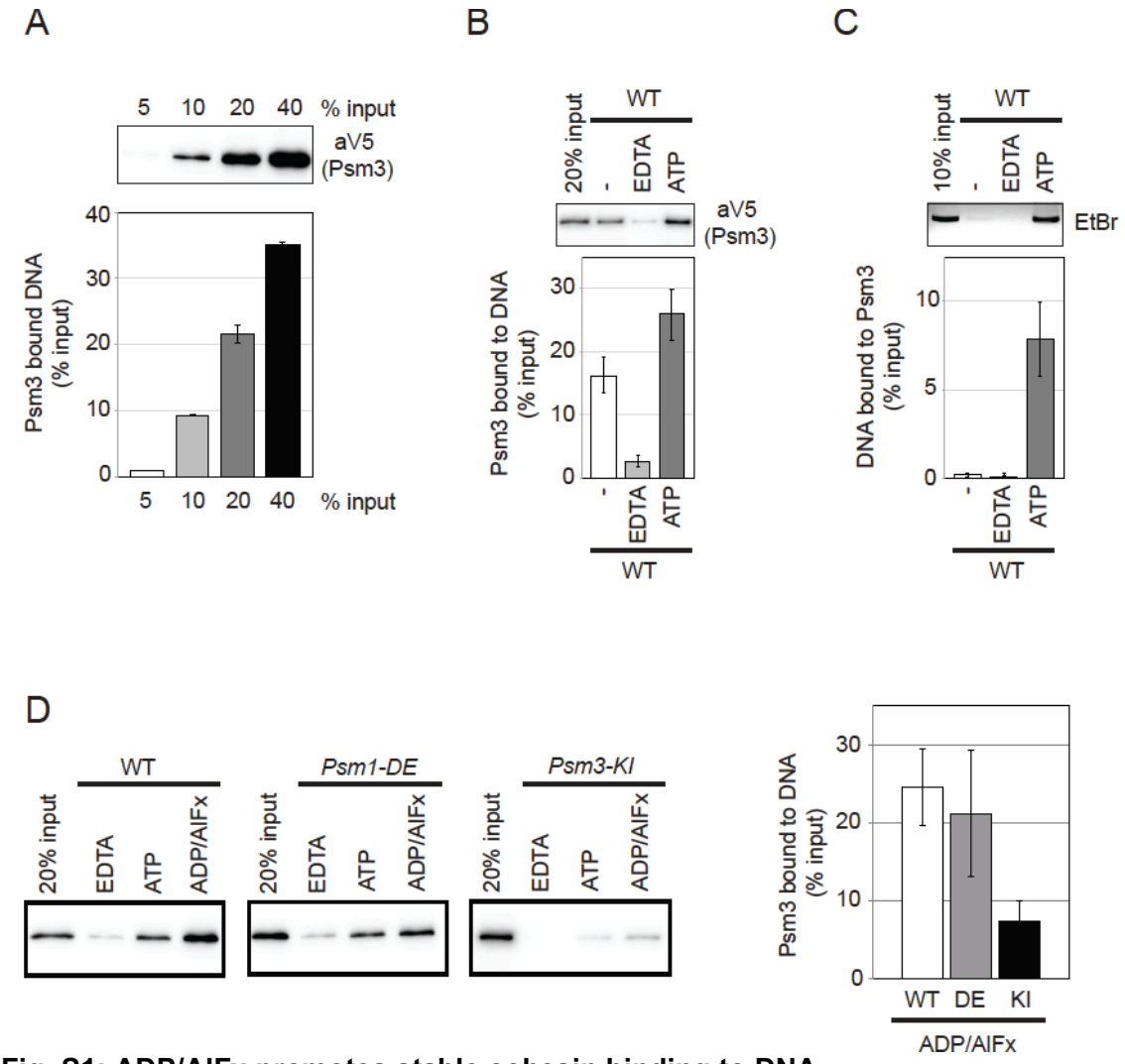

Fig. S1: ADP/AlFx promotes stable cohesin binding to DNA

(A) Titration of cohesin. Top panel: 5%, 10%, 20% or 40% input of a 80 nM WT cohesin mix were loaded and analyzed on SDS-PAGE followed by detection by Western blotting using anti-V5 antibodies (Psm3-3V5). Bottom panel: Quantitation of cohesin loaded from Western blots in top panel. (B) Effect of omitting EDTA in the reaction on DNA binding of WT cohesin using DNA-bead assay. Top panel: DNA-cohesin complexes were assembled in absence of EDTA (-), in presence of EDTA, ATP or ADP/AlFx and detected by Western Blot using anti-V5 antibodies (Psm3-3V5). Bottom panel: Quantitation of Western blots. (C) Effect of omitting EDTA in the reaction on DNA binding of WT cohesin using protein-bead assay. Top panel: DNA bound to immunoprecipitated cohesin complexes assembled in absence of EDTA (-), presence of EDTA, ATP or ADP/AlFx and detected using ethidium bromide stained agarose gels. Bottom panel: Quantitation of DNA. (D) Effect of ADP/AlFx on DNA binding of WT, *Psm1-DE* and *Psm3-KI* cohesin. Left panel: Same blots as in Fig.1C with the addition of the ADP/AlFx lane. DNA-cohesin complexes were assembled with EDTA, ATP or ADP/AlFx and detected by Western Blot using anti-V5 antibodies (Psm3-3V5). Right panel: Quantitation of ADP/AlFx lanes. All error bars represent standard deviation of data from at least two independent experiments.

A

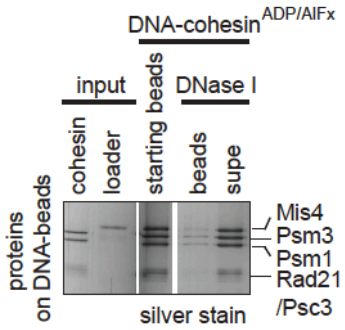

Fig. S2: Assembly of cohesin$^{ADP/AlFx}$-DNA complexes using DNA-bead assay is dependent on DNA.

(A) Effect of DNase on DNA binding of WT cohesin with ADP/AlFx using DNA bead assay. Silver stained gel showing cohesin and loader after assembly in the presence of ADP/AlFx and treatment with DNase I. Beads and supernatant fractions were loaded.

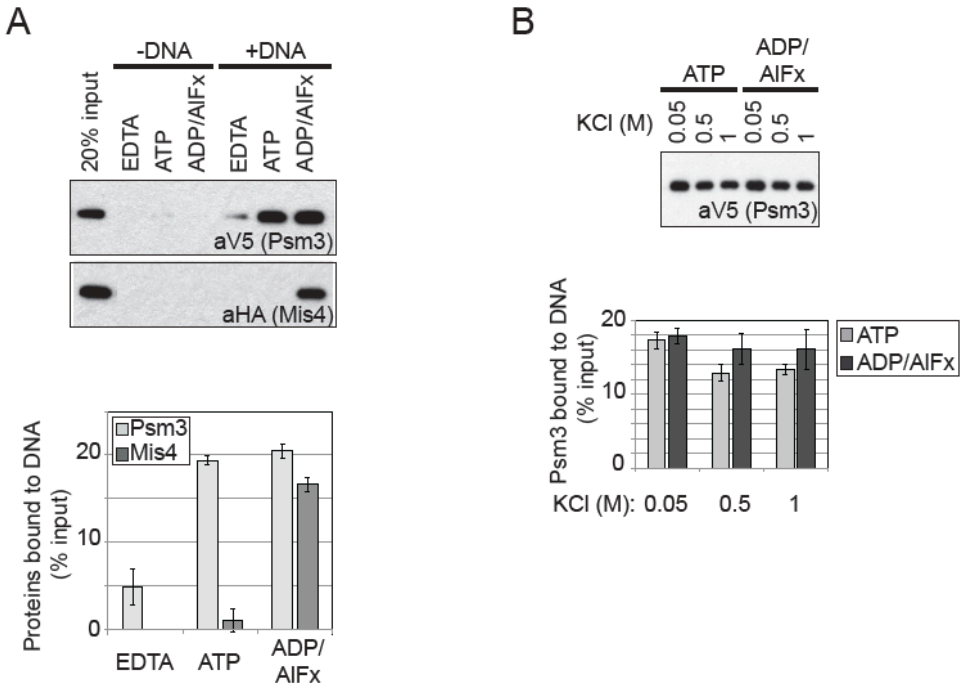

Fig. S3: Loader remains bound stably and stoichiometrically to cohesin$^{ADP/AlFx}$-DNA complexes.

(A) Effect of ADP/AlFx on loader binding to cohesin using DNA-bead assay. WT cohesin and an equimolar amount of loader was assayed for binding to DNA-beads in the presence or ATP or ADP/AlFx. Top panel: Cohesin and loader bound to DNA beads detected by Western Blot using anti-V5 (Psm3-3V5) and anti-HA (Mis4-HA) antibodies. Bottom panel: Quantitation of cohesin and loader bound to DNA-beads detected by Western blots. (B) Effect of increasing salt concentration on cohesin binding to DNA beads in the presence of ATP or ADP/AlFx. Top panel: Cohesin bound to DNA at increased salt washes was analyzed by SDS-PAGE and Western blot using anti-V5 antibodies (Psm3-3V5). Bottom panel: Quantitation of cohesin binding derived from Western Blots. Error bars represent standard deviation from at least two independent experiments.