Expanded View Figures

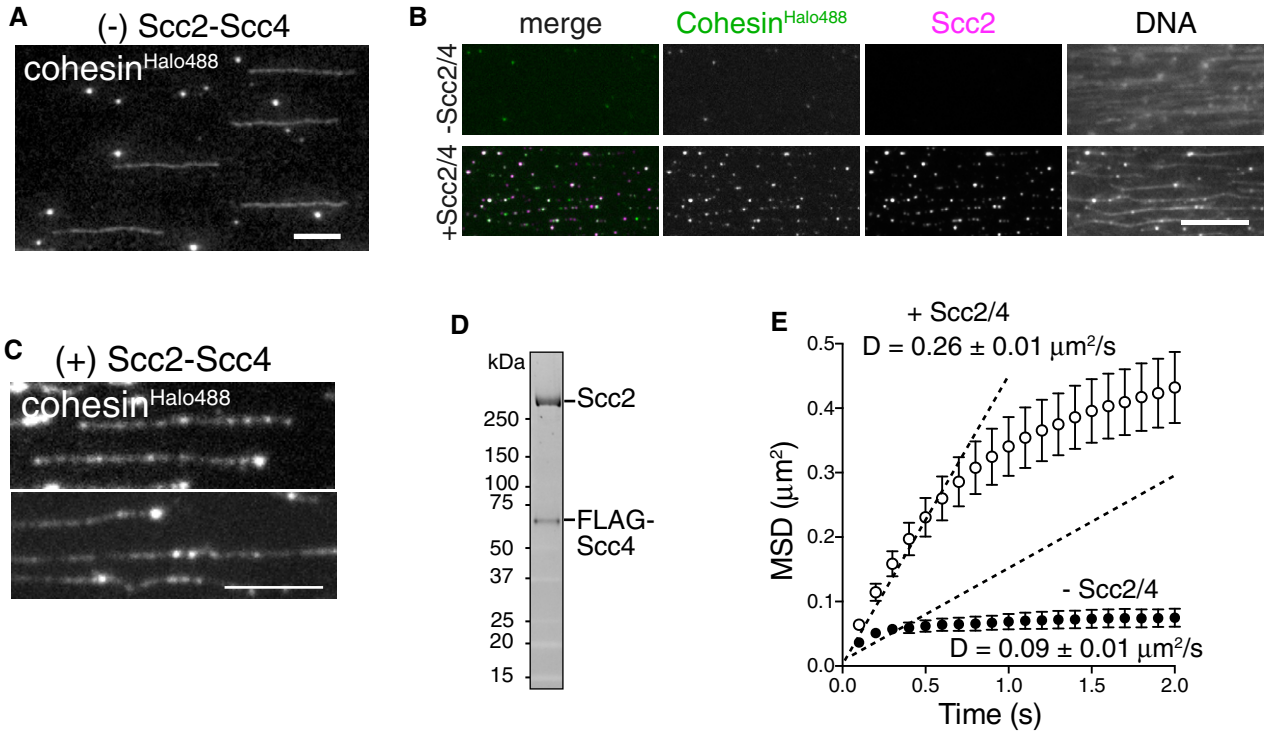

Figure EV1. Proper loading of cohesin by Scc2-Scc4 is required for translocation ability (related to Fig 1).

A Cohesin^{Halo488} is specifically bound to DNA in the absence of Scc2-Scc4. Cohesin^{Halo488} (3.25 nM) was directly loaded onto DNA and DNA-bound cohesin^{Halo488} was observed. Cohesin^{Halo488} signals were detected all along DNA. Scale bar, 10 μm.

B Cohesin^{Halo488} was loaded onto DNA in the presence or absence of Scc2-Scc4 and washed in high-salt buffer. Scc2 was immunostained by anti-Scc2 antibody. DNA was counterstained with SYTOX. Scale bar, 10 μm.

C Cohesin^{Halo488} specifically binds to DNA in the presence of Scc2-Scc4. Cohesin^{Halo488} (3.25 nM) was loaded onto DNA after Scc2-Scc4 bound to DNA, and cohesin^{Halo488} was observed. Note that cohesin^{Halo488} signals were detected all along the DNA. Scale bar, 5 μm.

D SYPRO Ruby staining of the purified Scc2-Scc4 complex after separation by SDS–PAGE.

E Cohesin^{Halo488} was loaded onto DNA as in (B). After high-salt wash, residual cohesin particles on DNA were observed in the presence of ATP and 100 mM KCl. MSD vs. time is shown. D indicates the diffusion coefficient (n = 45, mean ± s.e.m.).

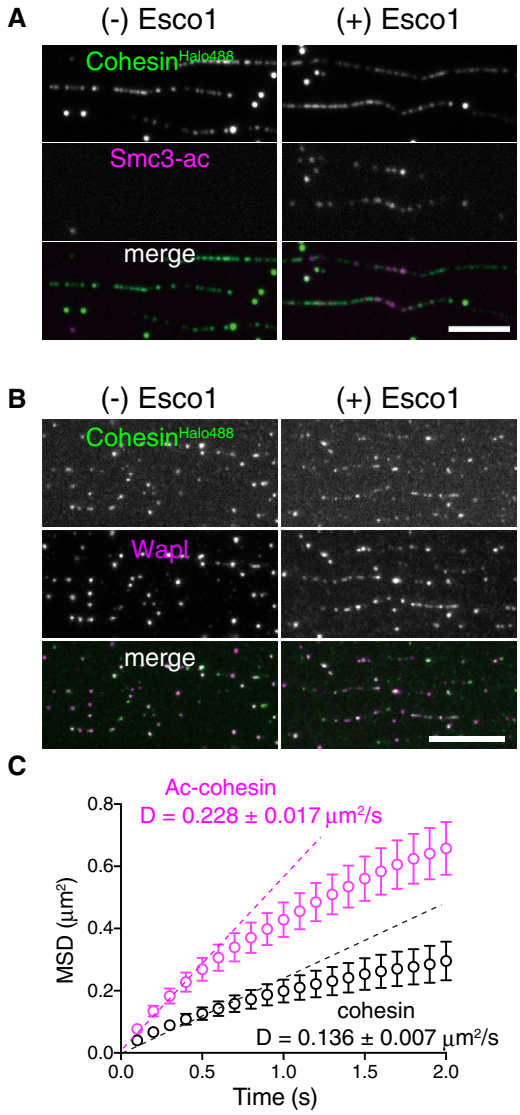

Figure EV2. Acetylation of Smc3 facilitates cohesin translocation (related to Fig 2).

A Topologically loaded cohesin^{Halo488} (−Esco1) or ac-cohesin^{Halo488} (+Esco1) was washed in high-salt buffer. Acetylated Smc3 (Smc3-ac) was detected by immunostaining. Scale bar, 5 μm.

B Topologically loaded cohesin^{Halo488} (−Esco1) or ac-cohesin^{Halo488} (+Esco1) was washed in high-salt buffer and treated with Wapl-Pds5. Wapl was detected by immunostaining. Scale bar, 10 μm.

C Cohesin^{Halo488} was treated as in (A) and cohesin^{Halo488} (cohesin) or acetylated cohesin^{Halo488} (Ac-cohesin) particles were observed. MSD vs. time is shown. D indicates the diffusion coefficient (n = 45, mean ± s.e.m.).