



**Supplemental Figure S7.** Addition of recombinant CTCF to *Xenopus* pronuclei repositions cohesin.

- (A) Immunoblotting of chromatin isolated from demembranated frog sperm in *Xenopus* egg extract after 120' and controls without sperm. CTCF protein was added at different concentrations as indicated
- (B) Semi-quantitative ratios of Smc3 protein to loading control for the immunoblot shown in (A)
- (C) Immunoblotting of supernatant and chromatin pellet from demembranated frog sperm in *Xenopus* egg extract after 120'. CTCF protein was added at different concentrations as indicated. For supernatant samples, only 10% was loaded compared to chromatin pellet samples
- (D) Anti-Smc3 and anti-CTCF ChIP-seq tracks of demembranated frog sperm in *Xenopus* egg extract after 140' (pronuclei 140'), pronuclei 140' with addition of 50 nM CTCF protein and frog XL-177 cells in the region of Chr3L:71.4-71.49Mb. Pronuclei 140' and pronuclei 140' +CTCF datasets were obtained in a calibrated ChIP-seq experiment
- (E) Anti-Smc3 and anti-CTCF ChIP-seq tracks of the same samples as in (D) in the region of Chr3L:107-107.7Mb. Pronuclei 140' and pronuclei 140' +CTCF datasets were obtained in a calibrated ChIP-seq experiment
- (F) Anti-Smc3 and anti-CTCF ChIP-seq tracks of pronuclei 140', pronuclei 140' with addition of 50 nM or 123 nM CTCF protein and XL-177 cells in the region of Chr3L:30.65-30.74Mb. Pronuclei 140' and pronuclei 140' +CTCF datasets were obtained in a calibrated ChIP-seq experiment