Supporting Information

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SI Materials and Methods

Cloning of xEco2. Full-length *Xenopus* Eco2 was cloned into pCS2- and pFastBac-derived vectors using primers 5′-ATTTTCAGG-GCCGGCCCGAAATGGCGGCGCGCA and 5′-CGGACCCT-GGCGCGCCCTAACTGACAA.

Cloning of *Xenopus laevis* Eco1 cDNA. Several candidate ESTs encoding proteins with homology to known Eco1 proteins were identified from a publicly available database, a fragment of *Xenopus* Eco1 cDNA was amplified by PCR from a *Xenopus* bacteriophage λ-cDNA library using a gene-specific primer (5′-CCCATGGGGTTTCAGTGC) and vector-specific primer (5′-CTGGAAAGCGGGCAGTGAGCGCAACGC) to identify and sequence the 5′ end of the gene. The 5′ end of the gene was deduced both from sequence analysis and alignment of the ORF with that found in a cDNA in *X. tropicalis* (Fig. S2). The full-length ORF was then amplified from the same bacteriophage library by PCR with the following primers: 5′-GGCCGGCCAGC-CATGGCACAAAAAAGAAAGTTGTCCC and 5′-TCTGGC-GCGCCTTAACTGTGGCCGCT and inserted into vectors for downstream applications.

Development Time Course. Isolated testes were perforated with fine tipped forceps and swirled through freshly laid eggs that were washed and resting in a minimal volume of 1× MMR in a glass dish. After 3 min incubation, the eggs were flooded in 0.1× MMR, causing synchronous fertilization. Thirty minutes after fertilization the eggs were dejellied with 2% cysteine in 1× MMR, pH 7.9. At each time point, five embryos were collected, transferred to 100 μL of Ray's IP buffer (100 mM NaCl, 50 mM β-glycerophosphate, 5 mM EDTA, 0.1% Triton X-100, and protease inhibitors), and immediately homogenized with a 200-μL disposable pipette tip. The homogenates were spun at 12,000 rpm for 5 min at 4 °C and 80 μL of supernatant was collected (avoiding the floating lipid layer) and added to sample buffer. Approximately 0.33 embryo equivalents were run per lane for immunoblot analysis and compared with 1 μL interphase extract.

XTC Cell Lysates. XTC cells were grown in 0.7× Leibowitz' L15 medium (Invitrogen) supplemented with 10% FBS and L-glutamine. Cells were lysed in solubilization buffer (20 mM Hepes pH 7.7, 1.5 mM $MgCl_2$, 5 mM KCl, 0.1% Triton X-100) and dounced 10 times. Nuclei were pelleted (5 min at 1,000 *g*), washed in the same buffer, and resuspended in the sample buffer. Supernatants from the first spin (cytosol) were collected, clarified with an additional spin, and sample buffer was added.

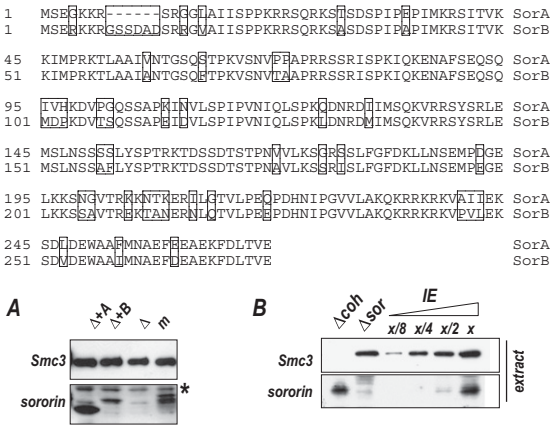

Fig. S1. Characterization of sororin in egg extracts. (*A*) Alignment of sororin A and sororin B from *Xenopus laevis.* A search of *Xenopus laevis* expressed sequence tag (EST) databases revealed the presence of two nearly identical sororin cDNAs (Fig. S1*A*), which likely represent paralogs that have diverged slightly since an ancient genome duplication event in *Xenopus laevis* (1). Differences between the two protein sequences are boxed. (*B*) Immunoblot showing profile of sororin in egg extracts. A rabbit polyclonal antiserum was generated, affinity purified, and tested for recognition of in vitro-translated proteins (Fig. S1*B*). Interphase extract was depleted of sororin and then supplemented with either in vitro-translated sororin A (Δ + A), in vitro-translated sororin B (Δ + B), or nothing (Δ), or mock depleted (*m*). Depleted extracts were probed with anti-Smc3 antibody (*Upper*) or anti-sororin antibody (*Lower*). *Nonspecific band seen in some extracts. (*B*) Effects of sororin and cohesin depletion in extract. Interphase extract was depleted of either sororin or cohesin, probed by immunoblot, and compared against serial twofold dilutions of undepleted extract, where *x* is the amount of extract loaded in each of the first two lanes. In some extracts, particularly in depletion experiments, sororin is difficult to detect in whole extract, although it is readily detectable on chromatin.

1. Evans BJ (2008) Genome evolution and speciation genetics of clawed frogs (Xenopus and Silurana). *Front Biosci* 13:4687–4706.

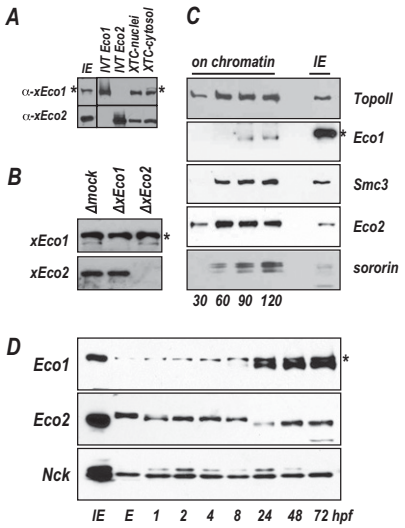

Fig. S2. Analysis of Eco1 and Eco2 in *Xenopus* extracts and embryos. (*A*) Characterization of antibodies against xEco1 and xEco2. Affinity-purified antibodies generated against xEco1 and xEco2 were used to probe interphase extract (IE), in vitro-translated full-length xEco1 (IVT Eco1), xEco2 (IVT Eco2), and nuclear and cytosolic extracts from XTC cells. *A nonspecific band recognized by the anti-xEco1 antibody in both interphase extracts and cytosol. This band is not seen on chromatin or in somatic nuclei. In egg extracts, Eco1 migrates slightly faster than this background band (note weak band below band marked by asterisk). (*B*) Depletion of Eco1 or Eco2 from interphase egg extract The same antibodies in *A* were used to immunodeplete interphase extract, and the depleted extract was then analyzed by immunoblot for both Eco proteins. (*C*) Chromatin association of xEco1 and xEco2 in interphase extract chromatin was isolated from a nuclear assembly reaction at the indicated time points and analyzed by immunoblot for the presence of topoisomerase II (as a loading control), Eco1, Smc3, Eco2, and sororin. IE, interphase extract. (*D*) Developmental time course showing increase in Eco1 levels near MBT. Embryos were collected at the indicated times and lysates were prepared and analyzed by immunoblot for the presence of Eco1, Eco2, and Nck (loading control). IE, interphase extract; E, egg lysates; hpf, hours postfertilization). In some egg extracts we were unable to detect Eco1 due to very low levels of expression.

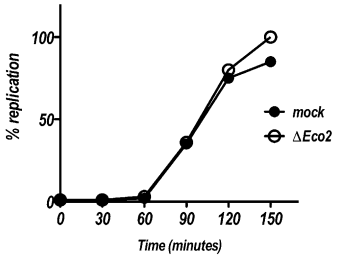

Fig. S3. DNA replication is not grossly affected by Eco2 depletion. Nuclei were added to interphase extract that had been either depleted of Eco2 (*ΔEco2*) or mock depleted (*mock*) and the levels of DNA replication were determined as in Fig. 1.

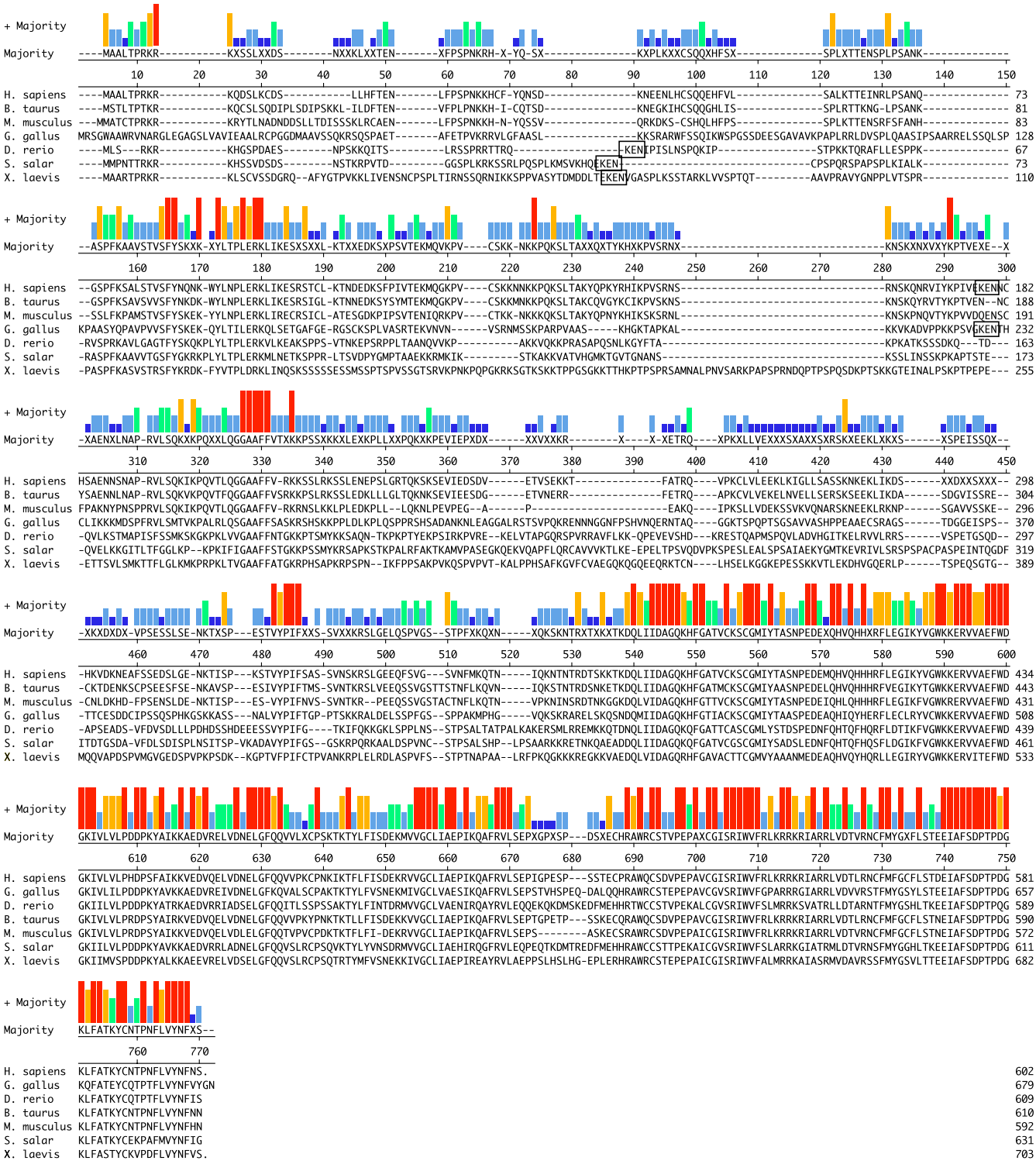

Fig. S4. Alignment of Eco2 proteins from various species. ClustalW alignment of Eco2/Esco2 proteins from various vertebrate species. Included are protein sequences from man (*Homo sapiens*: NP_00101017420), cow (*Bos taurus*: NP_001094652); mouse (*Mus musculus*: NP_082315); chicken (*Gallus gallus*: XP_420012); salmon (*Salmo salar*: NP_001133441); and frog (*Xenopus laevis*: NP_001089603). KEN sequences in the N termini are indicated by black boxes. The strength of homology at each position is indicated by the height of the vertical bars.

Fig. S5. (Continued)

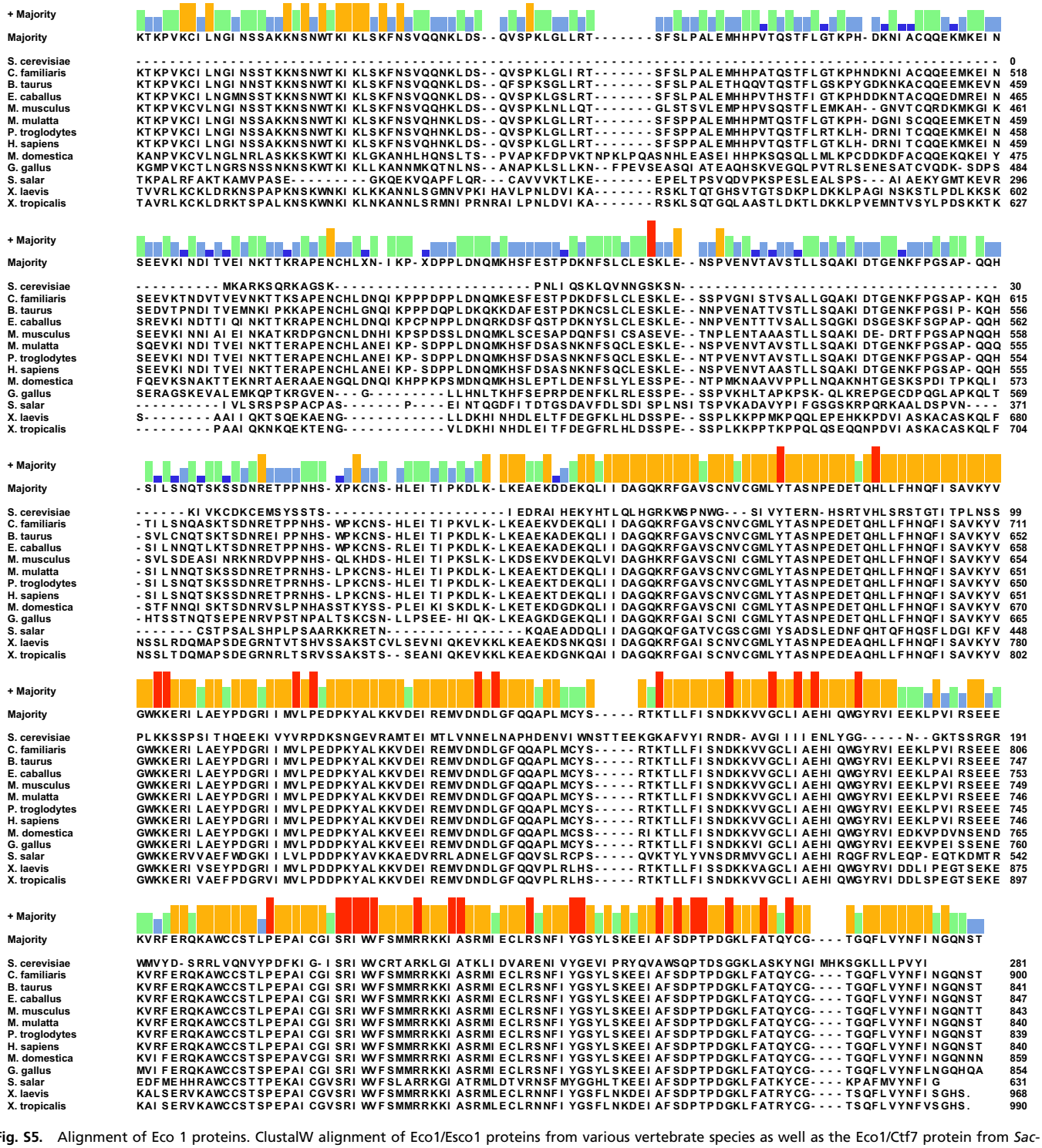

Fig. S5. Alignment of Eco 1 proteins. ClustalW alignment of Eco1/Esco1 proteins from various vertebrate species as well as the Eco1/Ctf7 protein from *Saccharomyces cerevisiae* (NP_116683). Shown are the sequences from dog (*Canis familiaris*: XP_547645); cow (*Bos taurus*: XP_615012); horse (*Eqqus caballus*: XM_001491258); mouse (*Mus musculus*: NP_001074691); rhesus monkey (*Macaca mulatta*: XP_001091733); chimpanzee (*Pan troglodytes*: XP_523883); man (*Homo sapiens*: AAH89426); possum (*Monodelphis domestica*: XP_001367455); chicken (*Gallus gallus*: XP_419155); and salmon (*Salmo salar*: NP_001133441); as well as the protein encoded by the cDNA described in this work from *Xenopus laevis*. Also shown is a hypothetical *Xenopus tropicalis* protein deduced by translation of an assembly of ESTs available through the National Center for Biotechnology Information Web site. KEN sequences in the N termini are indicated by black boxes. The strength of homology at each position is indicated by the height of the vertical bars.

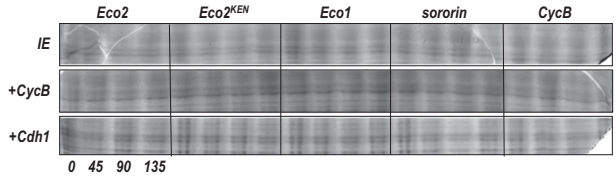

Fig. S6. Coomassie-stained gels of samples shown in Fig. 4*A* to serve as loading controls.