Expanded View Figures

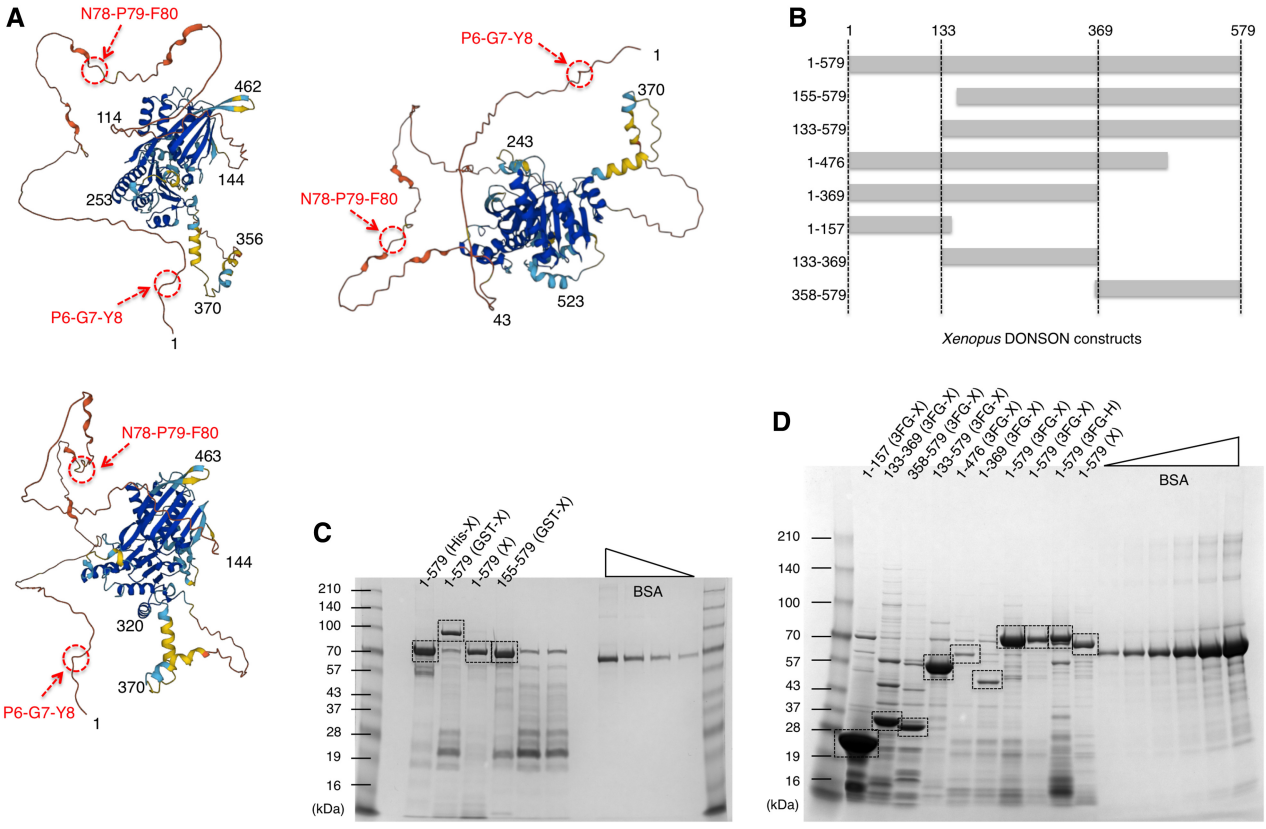


Figure EV1. The structure of DONSON and its deletion construct.

A The three-dimensional structure of human DONSON was predicted by the AlphaFold database. The views from three different directions are shown. The amino acid numbers are indicated in the structure.

B A schematic diagram for the deletion constructs of *Xenopus* DONSON. Each number indicates the residues number of the protein.

C, D Purified recombinant DONSON proteins were subjected to SDS–PAGE and stained with Coomassie Brilliant Blue. His-X, 8 × His-tagged *Xenopus* DONSON. GST-X, GST-tagged *Xenopus* DONSON. X, untagged *Xenopus* DONSON. 3FG-X, 3 × FLAG-tagged *Xenopus* DONSON. 3FG-H, 3 × FLAG-tagged human DONSON. The proper size of each product is indicated with dotted lines.

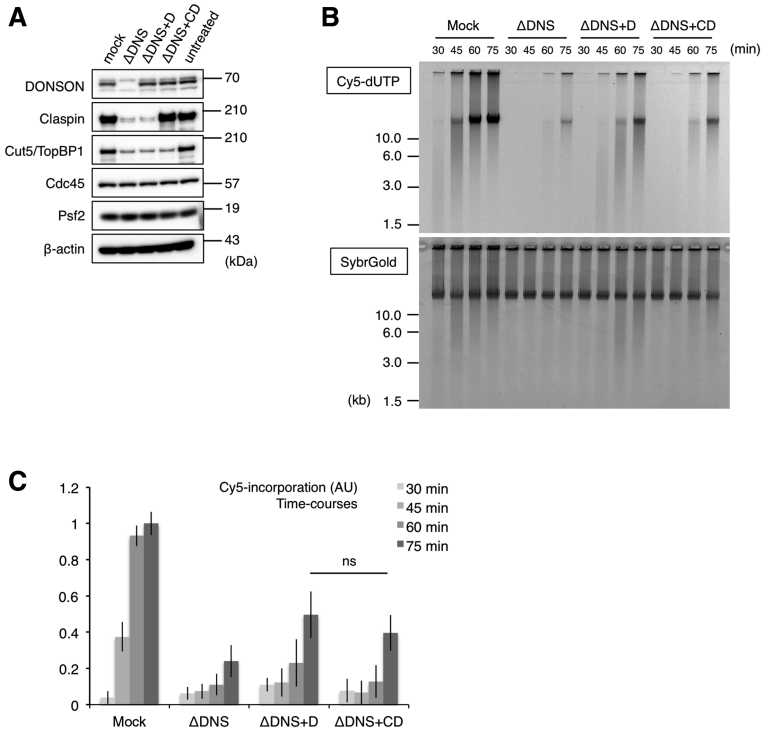


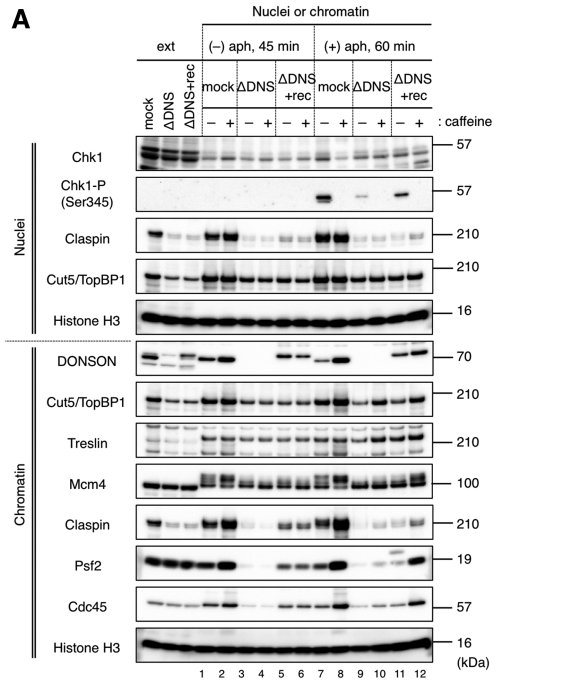
Figure EV2. Requirement of Claspin for DNA replication in the DONSON-depleted extract.

Similar to Fig 3B at different incubation times (30, 45, 60, 75 min) using untagged DONSON with or without His-Claspin (ΔDNS + CD, ΔDNS + C).

A The immunoblotting of the extracts used is shown.

B The isolated genomic DNA was analyzed by agarose gel electrophoresis.

C The relative Cy5 intensities in three independent experiments are shown in the bar graph. Error bar, mean ± SD. *P*-values were calculated using the unpaired *t*-test (two-tailed). ns, not significant.



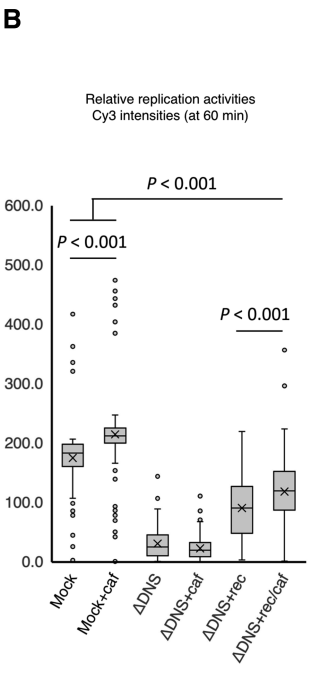


Figure EV3. Involvement of checkpoint signaling in DNA replication in the DONSON-depleted extract.

A Involvement of checkpoint signaling in the replisome assembly. Sperm nuclei (5,000/μl) were incubated in 20 μl of the mock- and DONDON-depleted extracts for 45 or 60 min in the absence (–) or presence (+) of 10 μg/ml aphidicolin (aph). 3 × FLAG-tagged recombinant *Xenopus* DONSON (rec) and caffeine (caf) were added at 0.06 μM and 5 mM, respectively. The nuclear and chromatin fractions were isolated and analyzed by immunoblotting with 0.5 μl of the extracts (ext).

B Involvement of checkpoint signaling in the replication activity. This was the same as the experiment in Fig 3A and was performed using the mock- and DONSON-depleted extracts under aphidicolin-free conditions as in (A). The relative Cy3 intensity of > 100 nuclei was quantified using ImageJ and is shown in the box plot. The boxes define the upper and lower quartiles; the whiskers define max to min values; the X-mark indicates each mean value; the central band indicates each median; the circles indicates outliers. *P*-values were calculated using the Mann–Whitney test. ns, not significant.

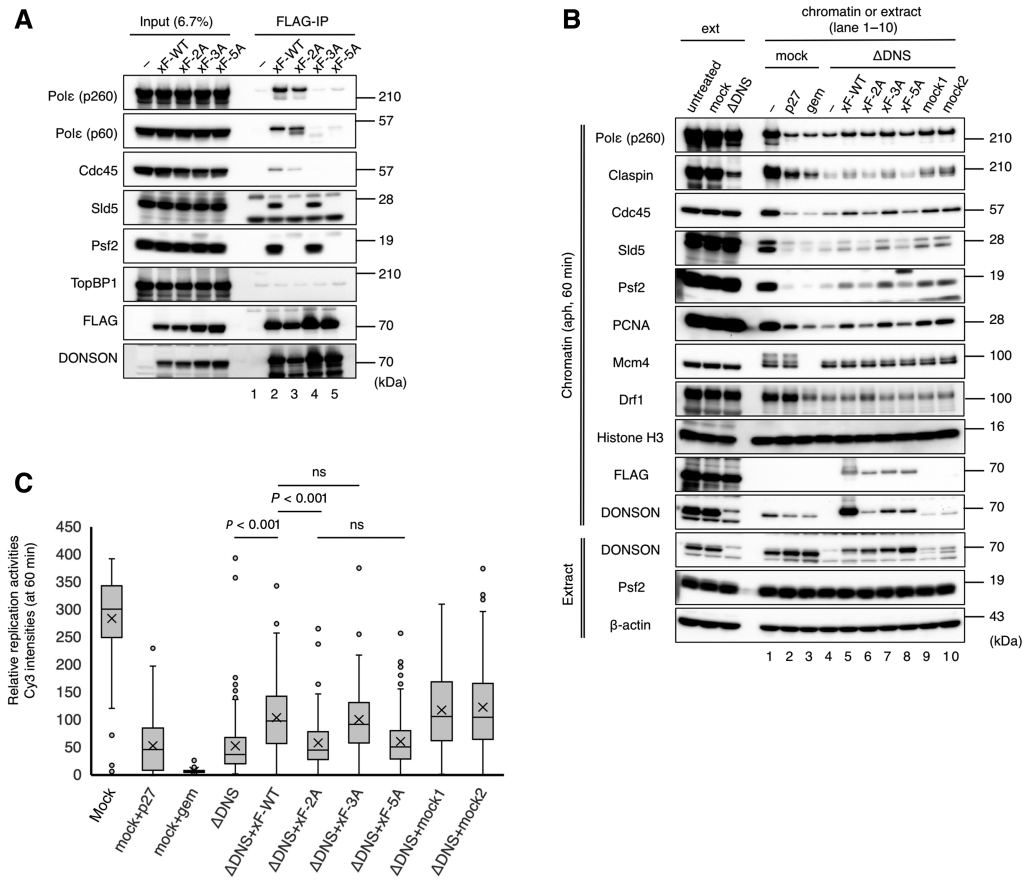


Figure EV4. Interaction of DONSON with GINS is important for DNA replication initiation.

A This FLAG-IP experiment was performed in the same manner as in Fig 5B using 1.2 μM of 3 × FLAG-tagged versions of the wild-type (xF-WT), P6A/Y8A (xF-2A), N67A/P68A/F69A (xF-3A), and P6A/Y8A/N67A/P68A/F69A (xF-5A) of full-length *Xenopus* DONSON. The extract (6.7%) was analyzed for comparison (Input).

B The DONSON depletion experiment, as in Fig 2B, was performed using the same recombinant proteins as in (A) (final concentration of each, 0.06 μM). His-p27 (27) and His-geminin (gem) were used as negative controls. The DONSON-depleted (ΔDNS) and mock-depleted extracts were mixed at 9:1 (mock1, lane 9) and 8:2 (mock2, lane 10), respectively.

C Under the same conditions as in (B) except that it was aphidicolin-free, the replication activity was analyzed in the same manner as Fig 3A. The relative Cy3 intensity of > 100 nuclei was quantified using ImageJ and is shown in the box plot. The boxes define the upper and lower quartiles; the whiskers define max to min values; the X-mark indicates each mean value; the central band indicates each median; the circles indicates outliers. *P*-values were calculated using the Mann–Whitney test. ns, not significant.

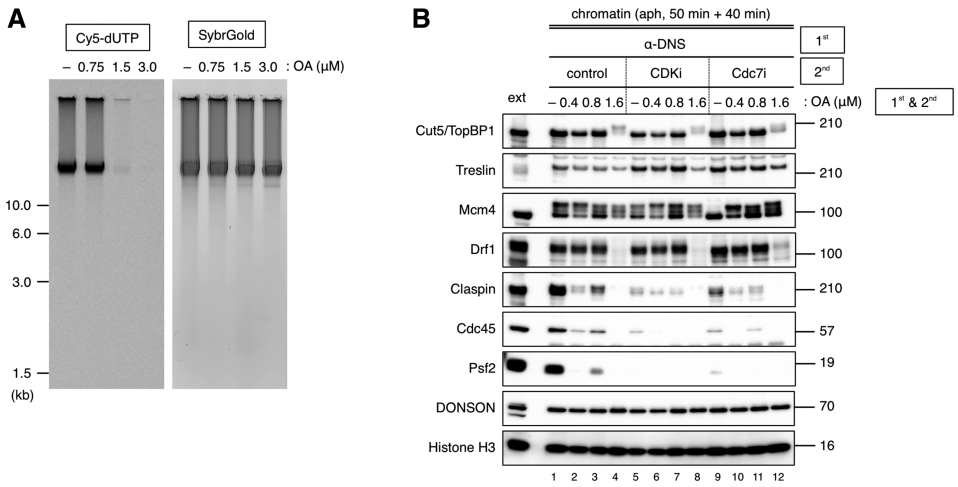


Figure EV5. Effect of phosphatase inhibitors on DNA replication.

A The effect of okadaic acid (OA) on the replication activity. Sperm nuclei (5,000/μl) were incubated for 60 min in 10 μl of extract in the presence of 0 (–), 0.75, 1.5, and 3.0 μM OA. DNA replication activity was detected in the same manner as Fig 3B.

B The effect of phosphatase inhibitors on the replisome assembly after nuclei transfer. The same experiment as in Fig 7C was performed in the presence of phosphatase inhibitors. The indicated concentrations of OA were added to the 1st and 2nd extracts. After the first incubation, nuclei were isolated using a buffer containing a phosphatase inhibitor cocktail.