**SUPPLEMENTARY INFORMATION**

Supplemental Figure Legends

**Supplementary Figure S1:** Sequence alignment of *Xenopus* and human MCM-BP, using MultAlin (http://multalin.toulouse.inra.fr/multalin/). Identical amino acid residues are shaded.

**Supplementary Figure S2:** Specificity of the rabbit polyclonal antibody against *Xenopus* MCM-BP.

Rabbit polyclonal antibodies were raised against full length recombinant MCM-BP and were affinity purified against the antigen as described in Methods.

(A) Detection of *in vitro* translated *Xenopus* MCM-BP with the purified rabbit polyclonal anti-MCM-BP antibody. 1 μl of reticulocyte-lysate translated *Xenopus* MCM-BP was analyzed by immunoblotting.

(B) MCM-BP is localized in the germinal vesicles (GV) of *Xenopus* oocytes.

Total oocyte and germinal vesicle (GV) fractions were prepared and analyzed by SDS-PAGE. Blots were incubated with the rabbit polyclonal antibody against MCM-BP and antibodies against other replication proteins as well as Tubulin. The asterisk denotes a cross-reacting protein, present in total oocytes.

(C) Co-depletion of MCM-BP by MCM7-depletion from egg extracts.

1 μl of mock- and MCM7-depleted (using the rabbit polyclonal anti-MCM7 antibody) interphase egg extracts were analyzed by western blotting with the indicated antibodies to confirm MCMs and MCM-BP depletion.
Supplementary Figure S3: Analysis of the MCM-BP/MCM7 complex.

(A) The MCM sub-complex is accumulated after DNA replication. Sperm nuclei (6,000 per µl) were added to *Xenopus* interphase egg extracts and incubated in the presence or absence of Geminin for 3 hr. Reaction mixtures were diluted with 4 volumes of XB buffer containing 0.2% TritonX-100 and insoluble material was removed by centrifugation at 12,000 g for 10 min. Clarified extracts were subjected to sucrose density gradient centrifugation. The indicated proteins were detected by western blot analysis.

(B) Dissociation of MCM proteins and MCM-BP from MCM7 immuno-precipitates after high salt and alkaline treatment.

*Xenopus* interphase egg extracts were incubated with anti-MCM7 antibodies coupled to protein A beads. MCM7 immuno-precipitates were then eluted with high salt buffer containing 0.8 M NaCl (lane 1), followed by alkaline buffer (pH 11.0) (lane 2). High salt and alkaline eluates were analyzed with beads-bound proteins (lane 3) by western blotting using the indicated antibodies.

(C) MCM-BP binding to MCMs in *Xenopus* mitotic egg extracts.

Mitotic (M) or interphase (I) egg extracts were immuno-precipitated with pre-immune serum (lanes 3 and 6), anti-MCM-BP (lanes 4 and 7) or -MCM7 antibodies (lanes 5 and 8) coupled to protein A beads. Immuno-precipitated proteins were analyzed by western blotting using anti-MCM4 (upper panel), anti-MCM7 (middle panel) and -MCM-BP antibodies (lower panel). 0.5 µl of M and I extracts (LSS, low speed supernatants) before immuno-precipitations were also analyzed (lanes 1 and 2). A mitosis-specific mobility shift of MCM4 was used to identify the cell cycle state of extracts.
**Supplementary Figure S4: GST pull-down of wild type and mutant MCM7**

(A) Western blotting of Myc-tagged wild type MCM7 and deletion mutants described in Figure 2D.

(B) GST or GST-MCM-BP were bound to GSH-Sepharose beads and incubated either with *in vitro* translated Myc-tagged, recombinant, full length MCM7 (WT) or mutant MCM7 (KA), which has a lysine to alanine point mutation in its Walker A motif. Co-precipitated proteins were analyzed by western blotting. Wild type and mutant MCM7 bound equally well to MCM-BP.

**Supplementary Figure S5: Immuno-depletion of MCM-BP from *Xenopus* interphase egg extracts.**

(A) The indicated amounts of mock-depleted (lanes 2, 4-7) or MCM-BP-depleted *Xenopus* interphase egg extracts (lane 3) were separated on SDS-PAGE and probed with anti-MCM-BP and -RPA34 antibodies; 100%=1 μl.

**Supplementary Figure S6: pre-RC formation in MCM-BP-depleted high-speed extracts**

*Xenopus* interphase high-speed egg extracts were immuno-depleted with IgG fractions purified from pre-immune serum (lane 1) or with the rabbit polyclonal anti-MCM-BP antibody (lane 2). Sperm nuclei were added to *Xenopus* egg extracts in the absence (lanes 3 and 5) or presence (lanes 4 and 6) of recombinant geminin and chromatin fractions were isolated after 30 min incubation at room temperature. Chromatin-bound proteins (lanes 3-6) were analyzed by western blotting using the indicated antibodies.
Supplementary Figure S7: Dissociation of MCM2-7 is inhibited in MCM-BP-depleted egg extracts, without changes in DNA chain elongation.

(A) *Xenopus* interphase egg extracts were immuno-depleted with IgG fractions purified from pre-immune serum (upper panels; ΔMock) or with the rabbit polyclonal anti-MCM-BP antibody (lower panels; ΔBP). Sperm nuclei were added to *Xenopus* egg extracts and chromatin fractions were isolated at the indicated times during DNA replication. Chromatin-bound proteins were analyzed by western blotting using the indicated antibodies.

(B) Replication efficiency of the reaction described in (A).

(C) Autoradiography of α-[\(^{32}\)P]dCTP-labeled DNA synthesized in mock-depleted (left panel), or MCM-BP-depleted (right panel). Total DNA was extracted at the indicated time during S phase and analyzed by 0.8% alkaline agarose gel electrophoresis. Standard DNA molecular weight markers (kb) were run in parallel.

Supplementary Figure S8: Recombinant MCM-BP rescues MCM2-7 dissociation from chromatin in MCM-BP-depleted extracts.

(A) Purified recombinant MCM-BP rescues MCM7 unloading from chromatin in MCM-BP-depleted *Xenopus* egg extracts. Egg extracts were mock-depleted with IgG fractions purified from pre-immune serum (lanes 1 and 2; ΔMock) or immuno-depleted with the rabbit polyclonal anti-MCM-BP antibody (lanes 3 and 4; ΔBP). Subsequently, buffer alone (lanes 1 and 3) or recombinant MCM-BP (rMCM-BP, 15 ng/μl) (lanes 2 and 4) was added to the depleted extracts before sperm addition. Chromatin fractions were isolated 120 min after sperm addition and analyzed by western blotting for MCM7 and ORC2 expression.
(B and C) Replication kinetics in mock- and MCM-BP-depleted extracts described in Figure 4A and 4C, respectively.

**Supplementary Figure S9: Excess MCM-BP destabilizes the interaction between MCM3 and other MCM proteins**

*Xenopus* interphase egg extracts were incubated or not with 2 μM recombinant MCM-BP for 30 min as described in Fig. 5A. They were then fractionated on a linear 7% to 19% sucrose gradient. Pooled high (H) and low (L) molecular weight fractions (left panels) were immuno-precipitated with anti-MCM3 antibodies (right panels) and analyzed by western blotting with the indicated antibodies.

**Supplementary Figure S10: ATP hydrolysis is not necessary for the disassembly of immuno-purified MCM2-7 by recombinant MCM-BP**

The MCM2-7 complex was immuno-precipitated from *Xenopus* interphase egg extracts with anti-MCM7 antibodies. MCM7 precipitates were washed and incubated with purified recombinant MCM-BP for 30 min in the absence or presence of 5 mM ATP or ATPγS. The proteins released in the eluate were separated from antibody beads by centrifugation and analyzed by western blotting with antibodies against different MCM proteins.