

Figure S1. DNA independent MCM8-9 interaction

Endogenous MCM9 protein was immunoprecipitated either in the presence or absence of EtBr, and endogenous MCM9 or MCM8 protein was detected using indicated antibodies. MCM8 and MCM9 protein interaction did not change by addition of EtBr.

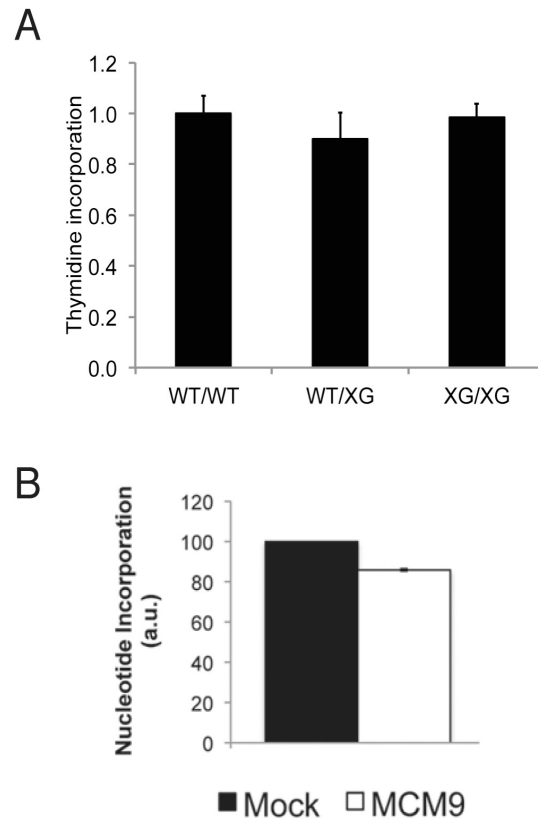


Figure S2. MCM9 is not essential for DNA replication

(A) Thymidine incorporation assay in MEF cells. Cells were labeled with [methyl-³H] thymidine for 10 min and labeled with [2-¹⁴C] thymidine for the preceding 24 hours to normalize for cell recovery. The histogram shows the ([methyl-³H]-thymidine) uptake normalized to total DNA ([2-¹⁴C]-thymidine) in indicated MEF cells. Results are expressed relative to that in wild type MEF cells (WT/WT). Mean ± s.d. of triplicates.

(B) pICL (Fig. 8A) was replicated in Mock- or MCM9-depleted extracts. Uncut DNA samples were analyzed for replication efficiency and quantified.

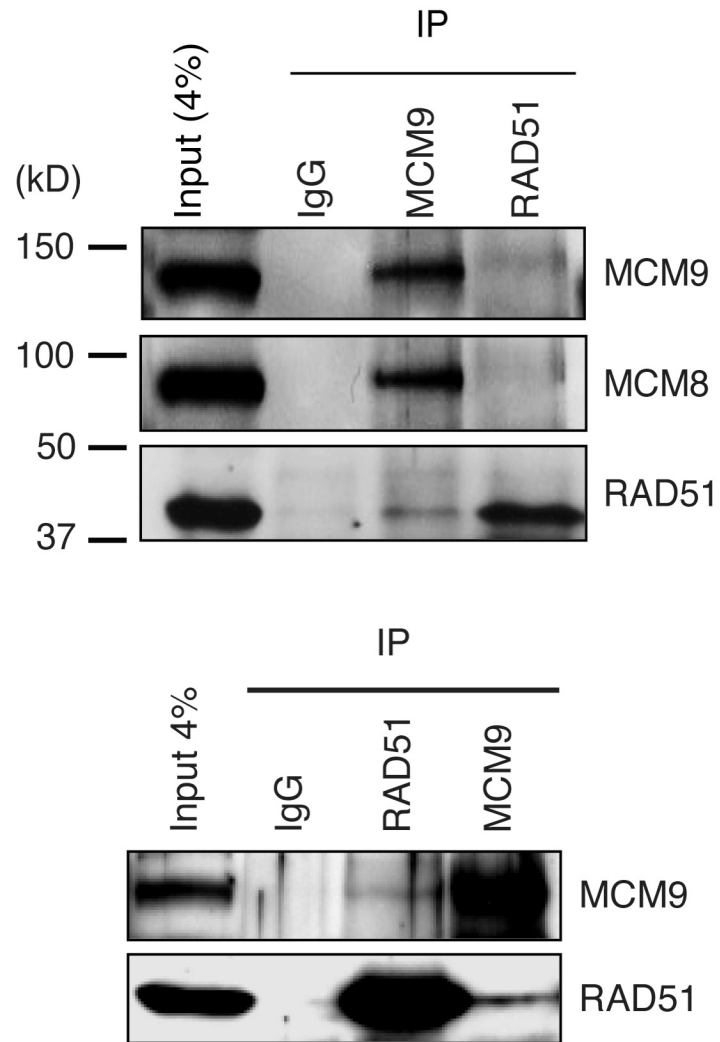


Figure S3. MCM8-9 interacts with RAD51 protein

Endogenous MCM9 or RAD51 protein is immunoprecipitated from U2OS cells and immunoblotted against RAD51, MCM8 or MCM9, respectively. Biological replicates of the experiment performed in Fig. 6A.

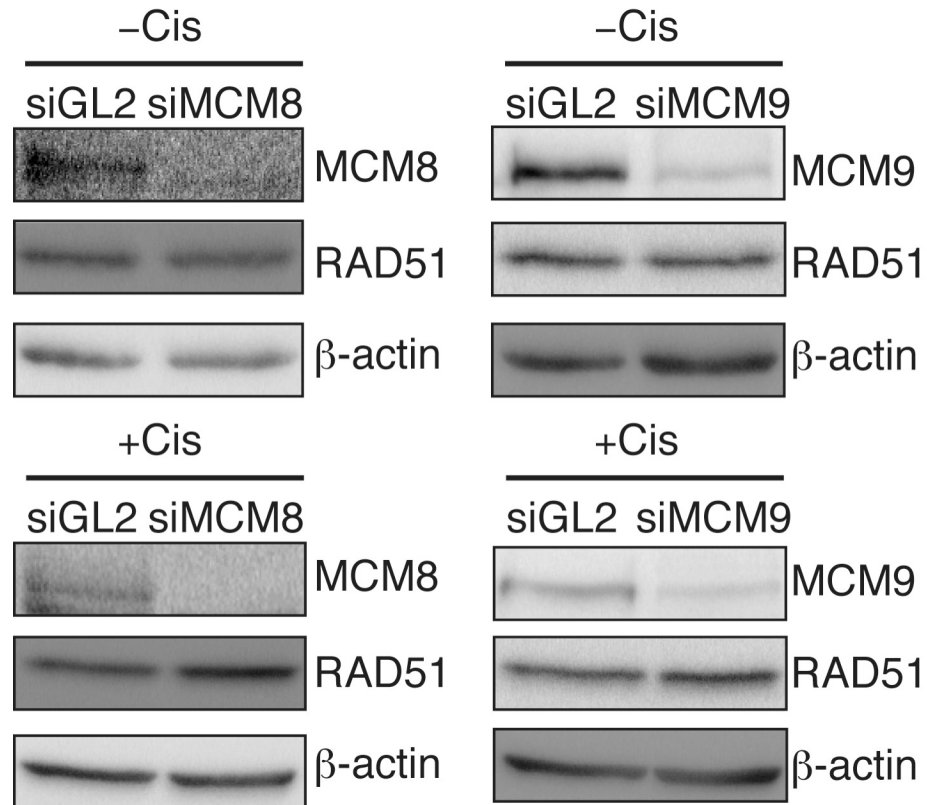


Figure S4. Knockdown of MCM8-9 decreases RAD51 filament formation without affecting RAD51 protein level

Loss of MCM8 or MCM9 does not affect the RAD51 protein steady-state levels. Western blot analysis of the indicated proteins in U2OS cells transfected with control siRNA (siGL2) or with siRNA against MCM8 (siMCM8) or MCM9 (siMCM9) proteins, and treated with 200 μM cisplatin or DMSO for 24 hrs before harvest. β-actin serves as a loading control.

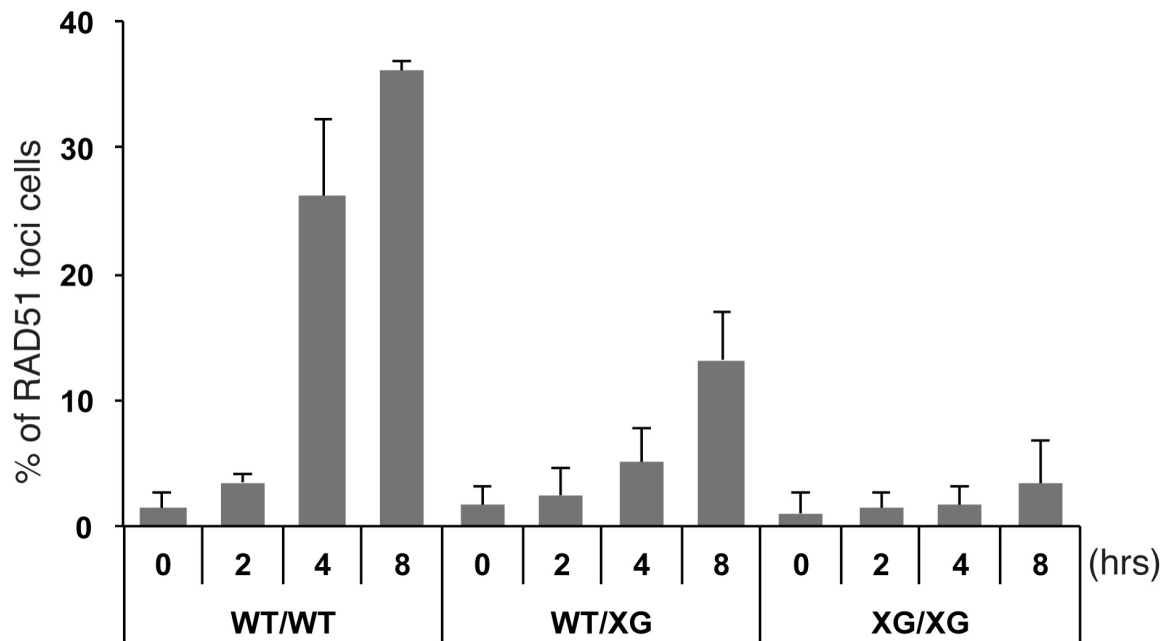


Figure S5. MCM9 protein is important for RAD51 foci formation.

RAD51 foci forming cells after 20 uM cisplatin treatment were counted at the indicated time points in *Mcm9* MEF cells. Y axis is the percentage of RAD51 positive cells (>20 foci/cell) and error bars represent the average of all fields \pm s.d.

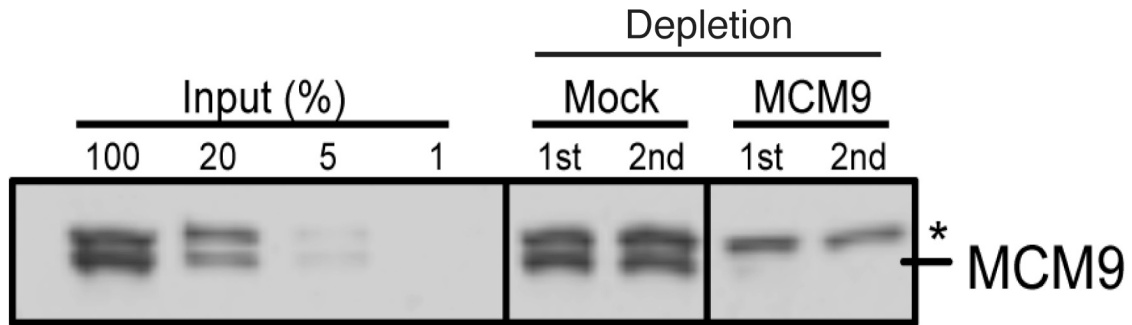


Figure S6. MCM9 protein is efficiently depleted in *Xenopus* egg extracts

Depletion of MCM9 from extract. Pre-immune (*Mock*) or anti-MCM9 (*MCM9*) serum was used to immunodeplete extracts for two rounds (*1st* and *2nd*). MCM9 protein in depleted extracts was compared to a dilution series of untreated (*Input*) extract by Western blot. Asterisk (*) denotes non-specific background band. Irrelevant lanes replaced with a solid line.