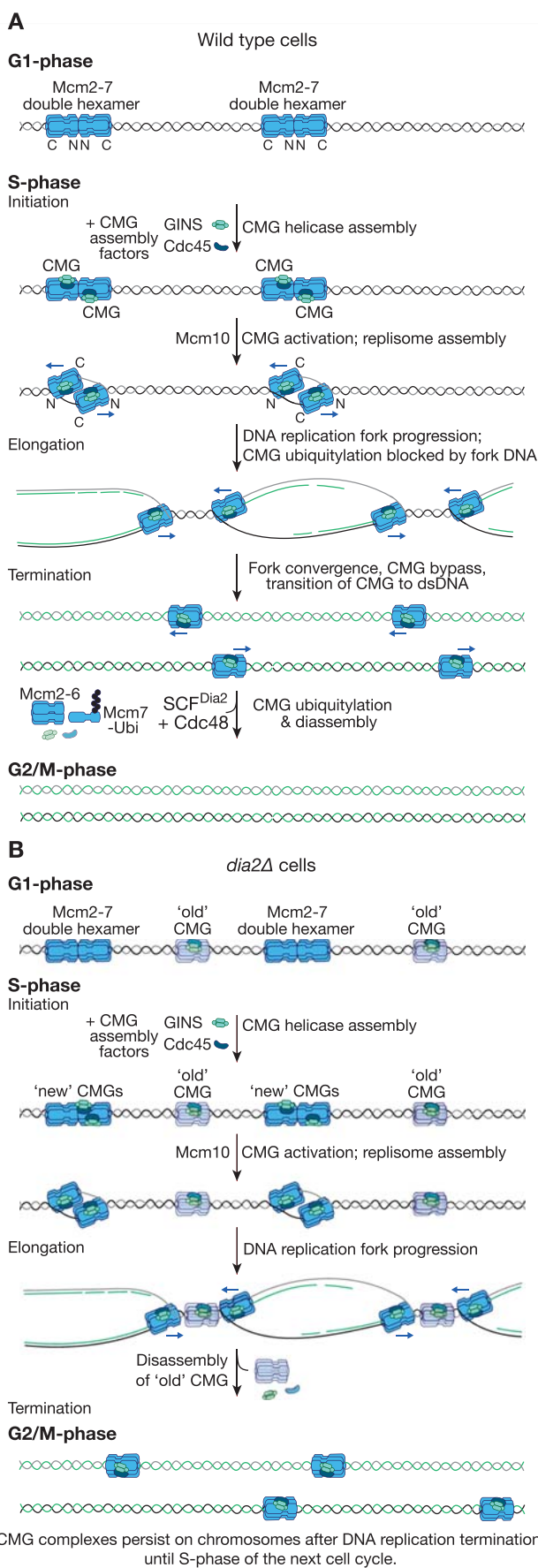


Expanded View Figures

Figure EV1. Model for disassembly of CMG helicase in wild-type cells and *dia2Δ*.

(A) In wild-type budding yeast cells, ubiquitylation of CMG by SCF^{Dia2} is sterically impeded at replication forks, by the parental DNA strand that is excluded from the Mcm2-7 ring of the helicase. This inhibition is released during DNA replication termination, when a pair of forks converge and the two CMG helicases bypass each other, thereby breaking the association between CMG and the excluded parental DNA strand. (B) In *dia2Δ* cells, CMG cannot be ubiquitylated during DNA replication termination and persists on chromatin until the next cell cycle. However, once *dia2Δ* enter S-phase, the old CMG complexes are disassembled by a previously unknown pathway, likely coupled to the encounter between new replication forks and old CMG.





◀ **Figure EV2. Location of TEV cleavage sites and SCF^{Dia2} ubiquitylation sites in Mcm7.**

(A) Mcm7 from multiple yeast species (*S. cerevisiae* = *Saccharomyces cerevisiae*; *E. gossypii* = *Eremothecium gossypii*; *S. mikatae* = *Saccharomyces mikatae*; *K. lactis* = *Kluyveromyces lactis*; *S. kudriavzevii* = *Saccharomyces kudriavzevii*) were aligned using Clustal Omega software. Mcm7-K29 is marked in black, whereas the three sites used for insertion of TEV cleavage sites (M167, A219 and T394) are shown in red with an asterisk. (B) The structure of *S. cerevisiae* Mcm7 (from PDB file 7PMK), illustrating the location of K29 and the three sites within disordered loops that were used to insert three consecutive TEV cleavage sites (after M167, A219 or T394 of Mcm7). Also see Movie EV1. (C) Structure of yeast Mcm7 (from PDB file 7PMK) and location in red of the 10 lysines mutated in Mcm7-10R. Note that K217 and K218 are in a disordered loop not visible in the cryoEM structure of the yeast replisome. (D) The structure of SCF^{Dia2} was predicted with AlphaFold2-Multimer and then docked onto the cryoEM structure of the yeast replisome (adapted from PDB file 7PMK) with UCSF Chimera software. The RING domain of Dia2, and the 10 lysines mutated in Mcm7-10R, are shown in red. Also see Movie EV2.

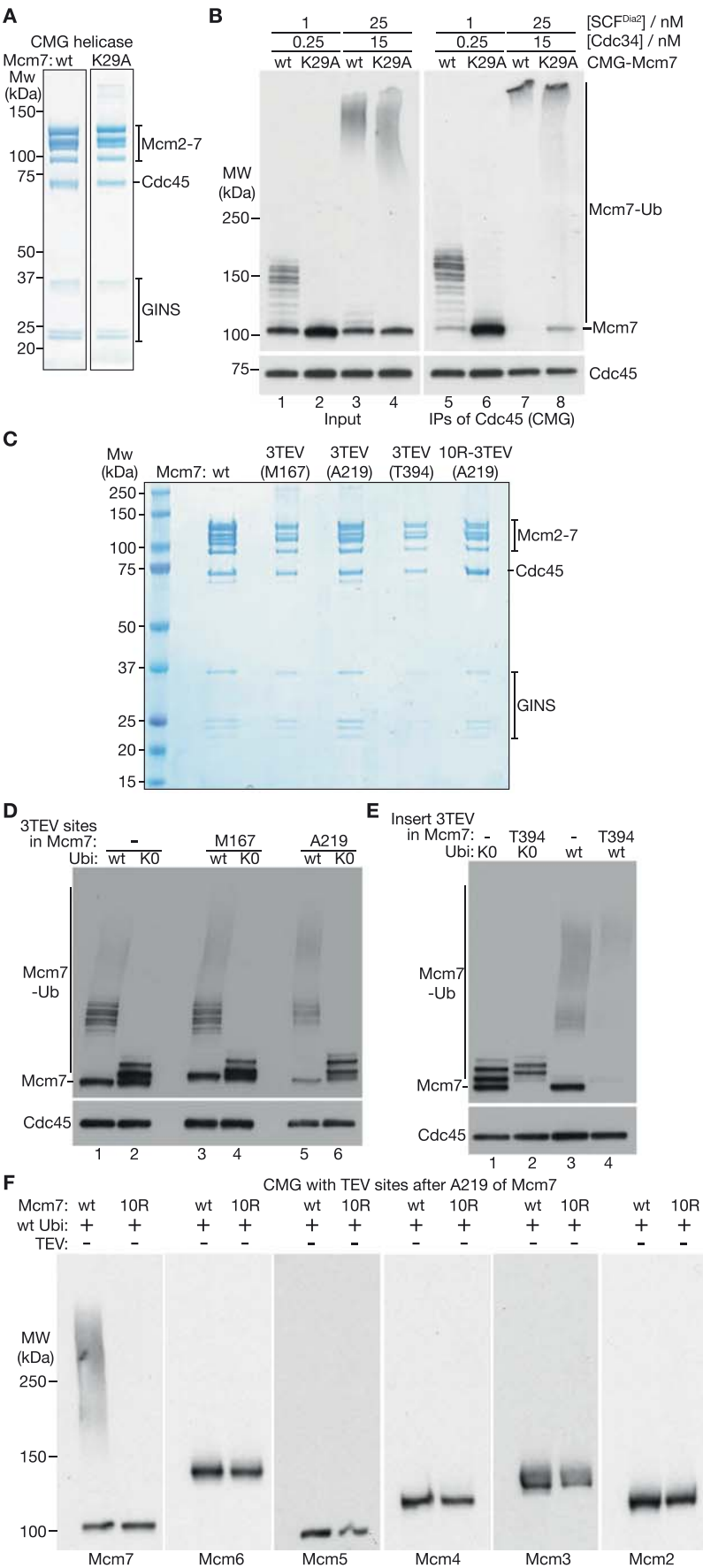


Figure EV3. Generation and characterisation of recombinant CMG complexes with mutated alleles of Mcm7.

(A) Purified recombinant budding yeast CMG helicase, with wild-type Mcm7 or Mcm7-K29A, were resolved by SDS-PAGE, before staining of the gel with Coomassie blue. (B) CMG containing wild-type (wt) Mcm7 or Mcm7-K29A was ubiquitylated in the presence of the indicated concentrations of SCF^{Dia2} and Cdc34. The reactions were analysed by immunoblotting. (C) Coomassie-stained gel with purified recombinant CMG containing wild-type Mcm7 or the indicated variants. (D, E) Recombinant CMG with the indicated versions of Mcm7 were ubiquitylated in vitro by SCFDia2 and Cdc34, using either wild-type ubiquitin (wt Ubi) or lysine-free ubiquitin (KO Ubi). Reactions were then analysed by immunoblotting. Note that the ubiquitylated forms in lanes 3–6 of (D), and lanes 2 and 4 of (E), are shifted by comparison with the control, due to the insertion into Mcm7 of peptide sequences containing TEV cleavage sites (the sites were not cleaved in these reactions). (F) Analogous reactions to those in D–E were performed with wild-type ubiquitin. The Mcm2–7 subunits of CMG were monitored by immunoblotting. Source data are available online for this figure.

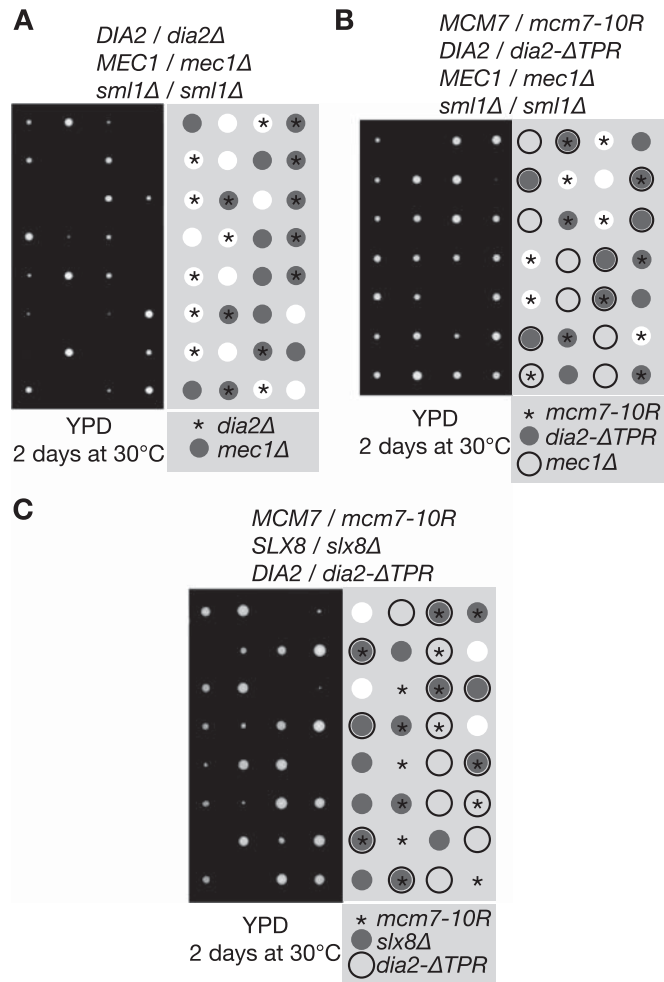


Figure EV4. The S-phase checkpoint and Slx8 become essential when the ubiquitylation of CMG-Mcm7 is blocked.

(A–C) Tetrad analysis of diploid budding yeast cells of the indicated genotypes (YCPR486, YCPR500, YCPR222). YPD = medium comprising Yeast Extract, Peptone, Dextrose. Source data are available online for this figure.

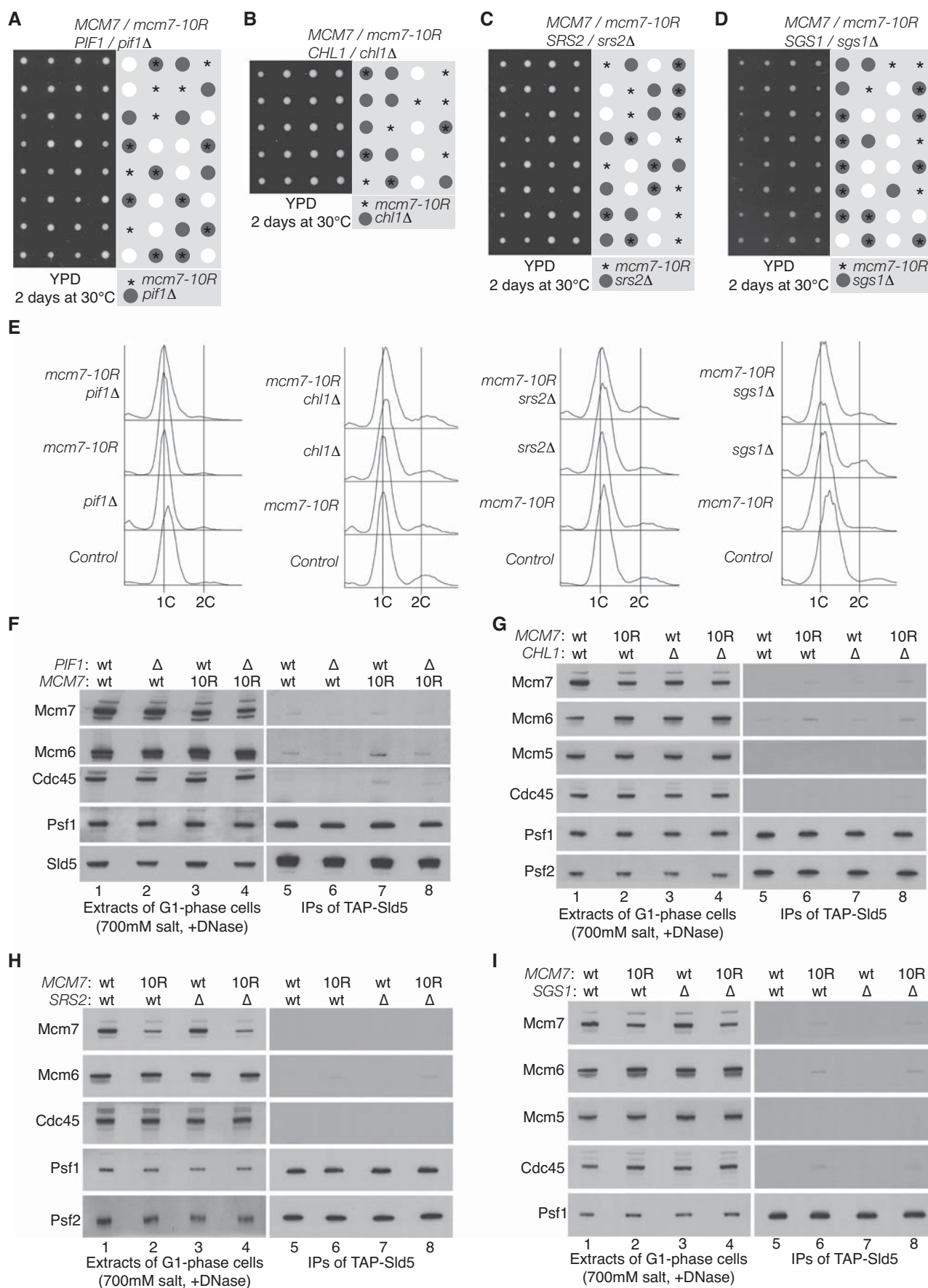


Figure EV5. Combination of *mcm7-10R* with *pif1Δ*, *chl1Δ*, *srs2Δ* or *sgs1Δ* does not cause synthetic lethality or accumulation of CMG during G1 phase.

(A–D) Tetrad analysis of diploid budding yeast cells of the indicated genotypes (YCPR75, YCPR435, YCPR434, YCPR171). YPD = Yeast Extract, Peptone, Dextrose. (E) The indicated strains (YCPR141, YCPR406, YCPR412, YCPR428) were arrested in G1 phase at 30 °C by addition of mating pheromone. DNA content was monitored by flow cytometry. (F–I) Cell extracts were prepared from the samples in (E) and used to isolate GINS and the CMG helicase by immunoprecipitation of TAP-Sld5. The indicated factors were monitored by immunoblotting. Source data are available online for this figure.