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Supplementary Materials for

Cdc48 and a ubiquitin ligase drive disassembly of the CMG helicase at the end of DNA replication

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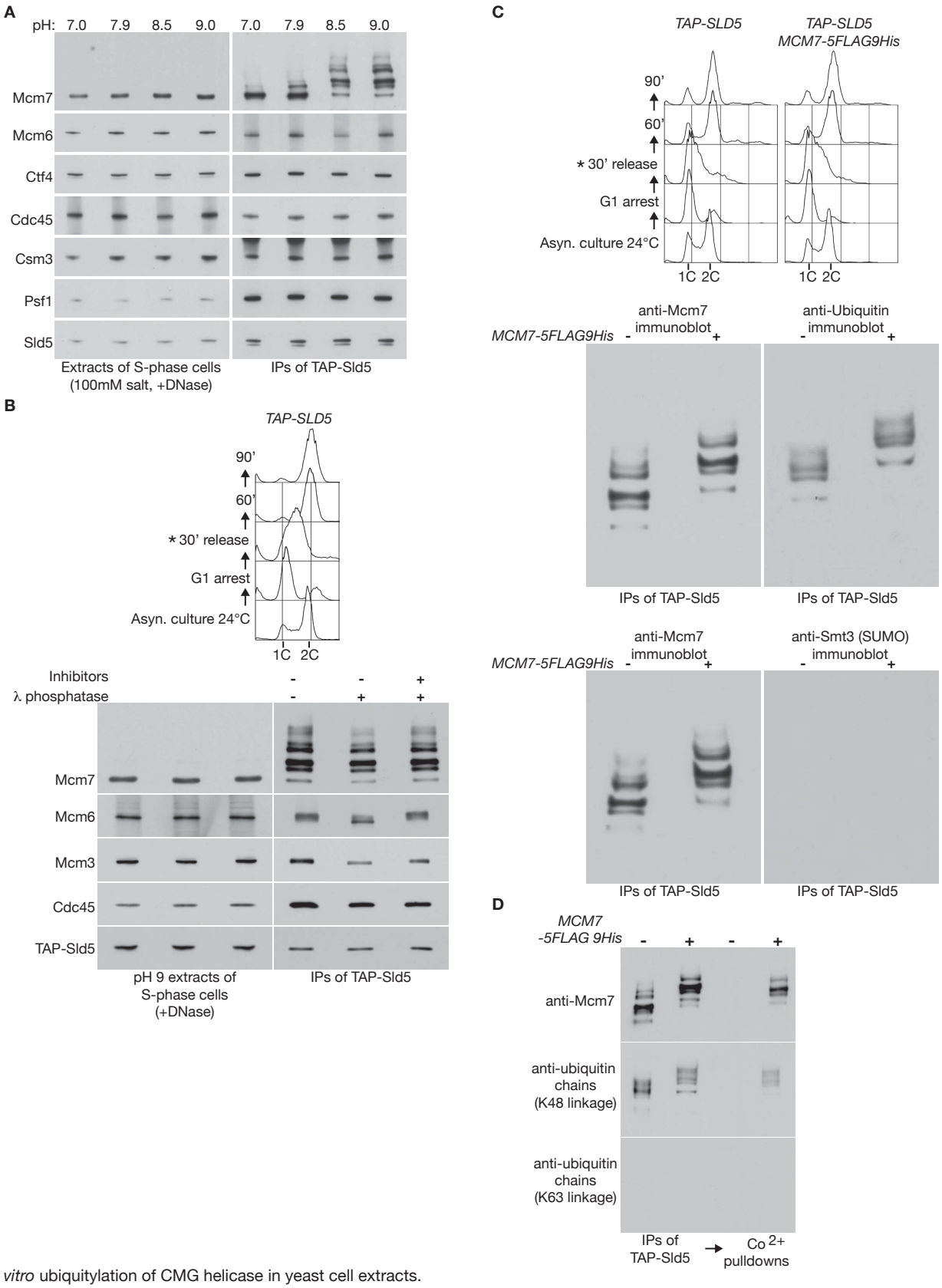
Supplementary Text: Author Contributions

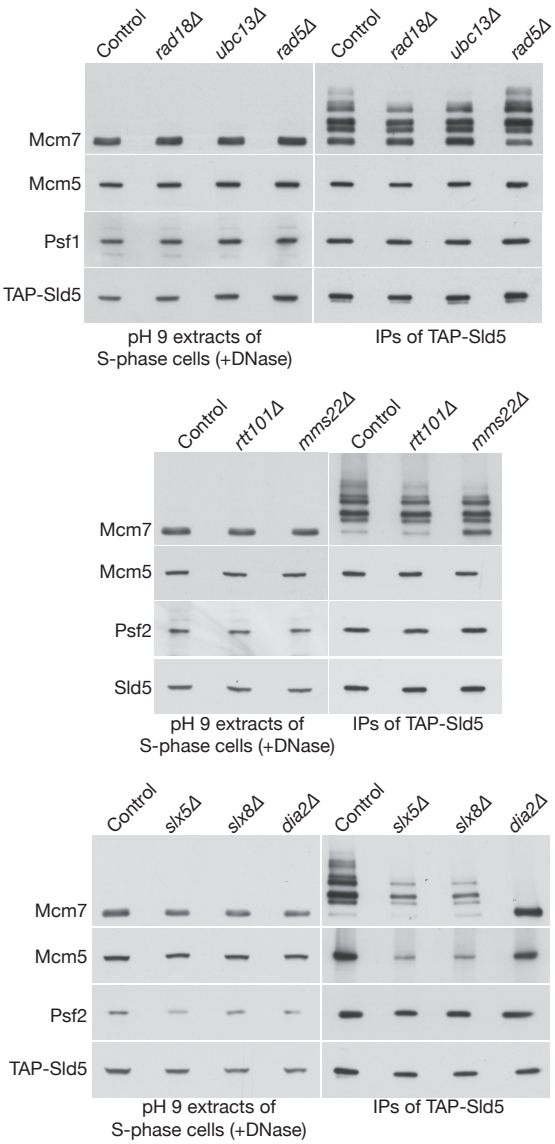
GDP made the initial discovery of ubiquitylated Mcm7. MM performed the experiments shown in Fig. 1, Fig. 2A, Fig. 3, Figs. 7-8, Fig. S1, Fig. S3, Fig. S4, and Figs. S10-S14. TM performed the experiments in Fig. 2B-D, Figs. 4-6, Fig. S2, and Figs. S5-S9. KL designed and interpreted all the experiments, in collaboration with MM, TM and GDP. KL wrote the manuscript together with MM and TM.

Maric-Maculins Supplementary Figure 1



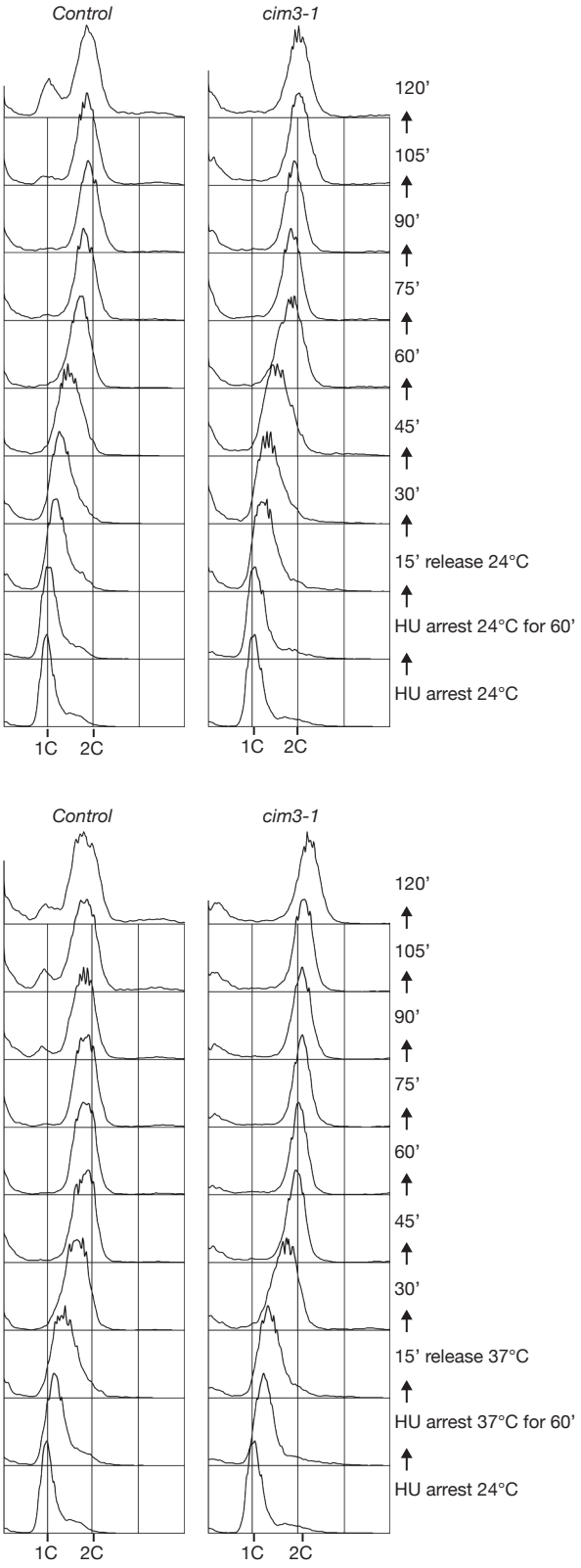
In vitro ubiquitylation of CMG helicase in yeast cell extracts.
(**A**) Extracts of S-phase *TAP-SLD5* cells (YASD375) were generated at the indicated pH, before digestion of chromosomal DNA and isolation of the GINS component of the CMG helicase, via a TAP tag on the Sld5 subunit. The indicated CMG components and the other replisome factors were detected by immunoblotting.
(**B**) Extracts of S-phase *TAP-SLD5* cells (YASD375) were prepared at pH 9 as above (DNA content was measured by flow cytometry; the asterisk denotes the sample that was used to prepare the cell extracts), before immunoprecipitation of the TAP-Sld5 subunit of GINS. As indicated, the immunoprecipitates were treated with λ phosphatase (with or without phosphatase inhibitors), as described in Methods.
(**C**) TAP-Sld5 was isolated from S-phase extracts of *TAP-SLD5* (YASD375) and *TAP-SLD5 MCM7-5FLAG9HIS* (YGDP483), before immunoblotting with antibodies specific to Mcm7, ubiquitin (P4D1) or Smt3 (SUMO). DNA content was measured by flow cytometry; the asterisk again denotes the samples that were used to prepare the cell extracts.
(**D**) A similar experiment was performed with control cells (YASD375) or cells with tagged Mcm7 (YGDP483). The isolated proteins were analysed by immunoblotting with the indicated antibodies.
Additional flow cytometry data for the experiments in this Fig. can be found in Fig. S14.

Maric-Maculins Supplementary Figure 2

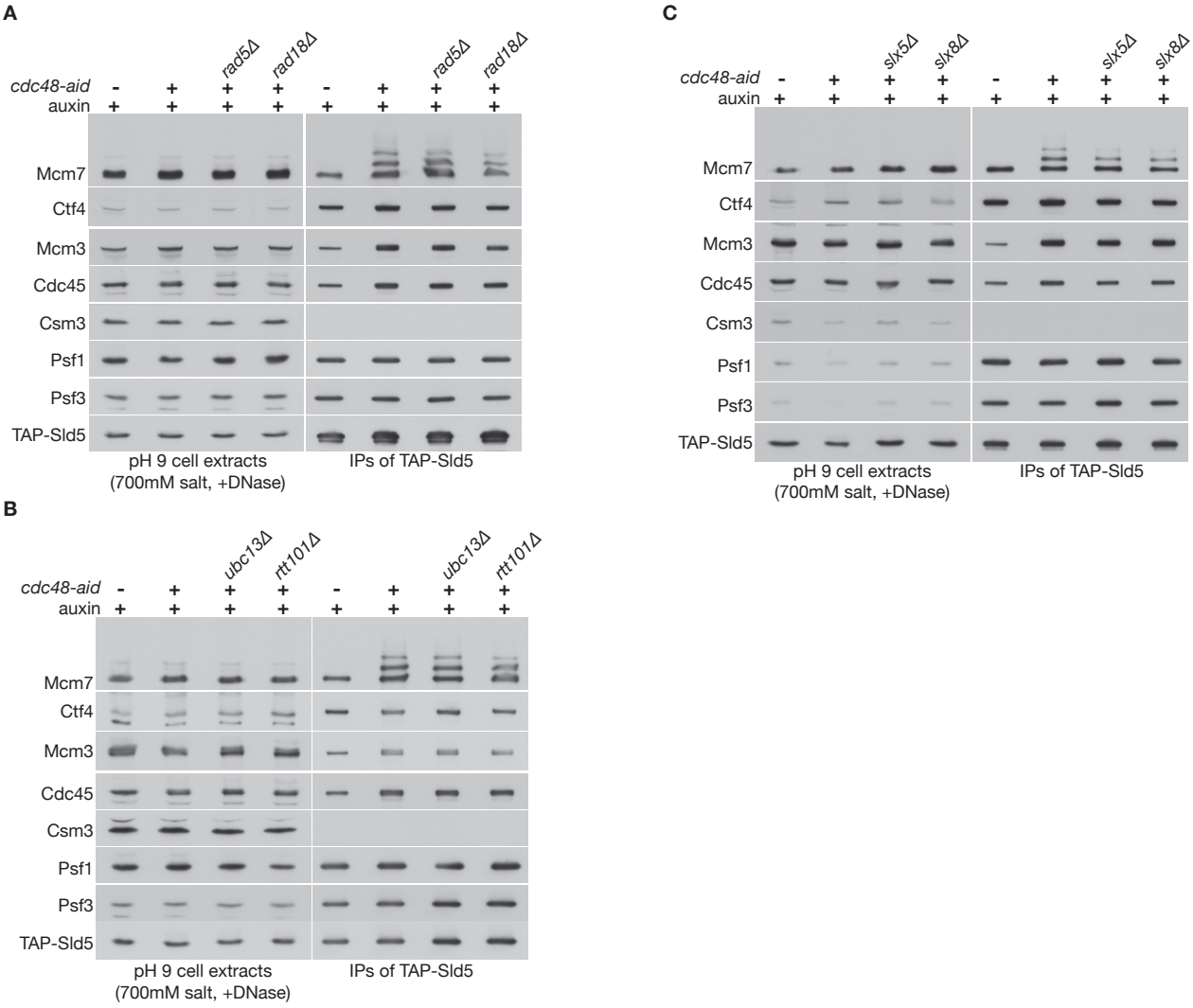


The CMG helicase is ubiquitylated in vitro in yeast cell extracts by the SCFDia2 ubiquitin ligase.
The indicated strains were synchronised in S-phase at 30°C as above, and then used to prepare cell extracts at pH 9, in order to promote the specific ubiquitylation of the CMG helicase on its Mcm7 subunit. GINS was isolated as above, by immunoprecipitation of TAP-tagged Sld5.

Maric-Maculins Supplementary Figure 3

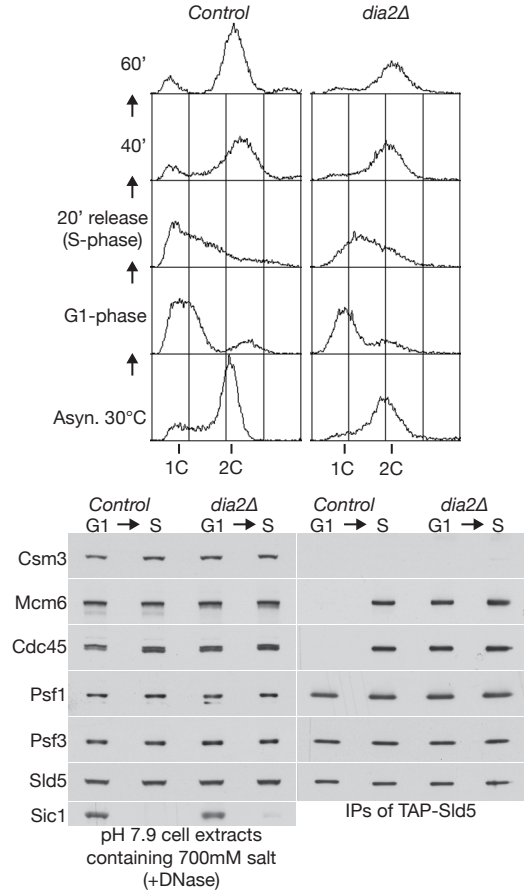


cim3-1 cells are able to complete DNA replication at 37°C.
The indicated cells were grown as shown and DNA content was monitored by flow cytometry.



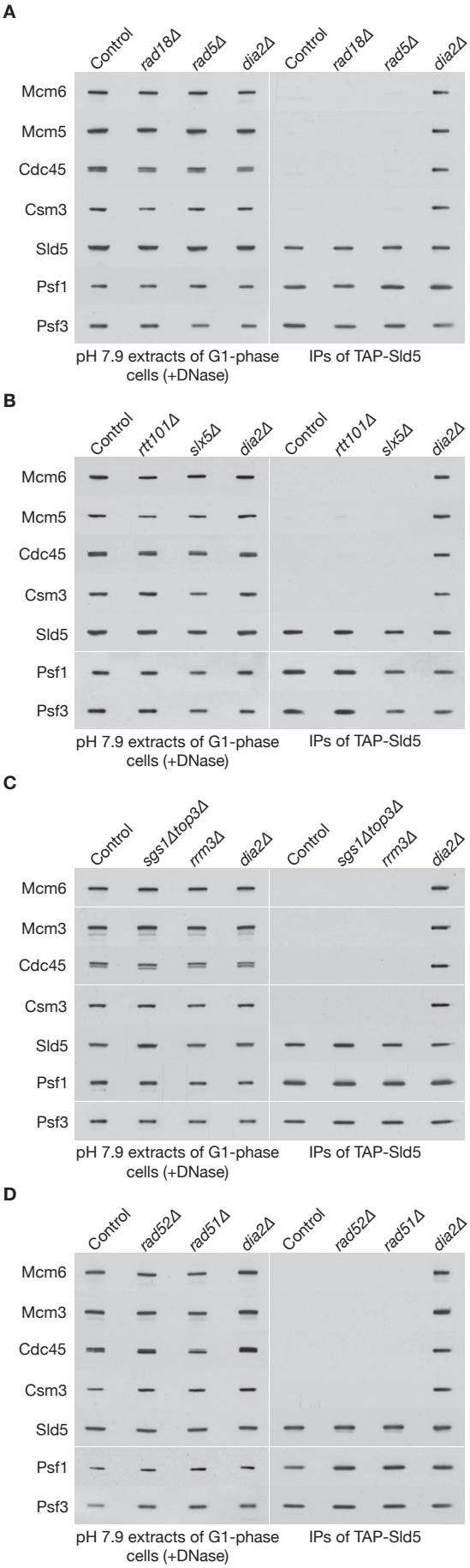
In vivo ubiquitylation of the CMG helicase requires the E3 ubiquitin ligase SCFDia2.
(**A**) Control (YMM256), *cdc48-aid* (YMM228), *cdc48-aid rad5Δ* (YMM340) and *cdc48-aid rad18Δ* (YMM341) cells were grown at 30°C, then incubated for 2 hours in the presence of auxin, before processing as in Fig. 3F.
(**B**) *cdc48-aid ubc13Δ* (YMM346) and *cdc48-aid rtt101Δ* (YMM351) were compared with controls in a similar experiment.
(**C**) An analogous experiment including *cdc48-aid slx5Δ* (YMM347) and *cdc48-aid slx8Δ* (YMM350).

Maric-Maculins Supplementary Figure 5



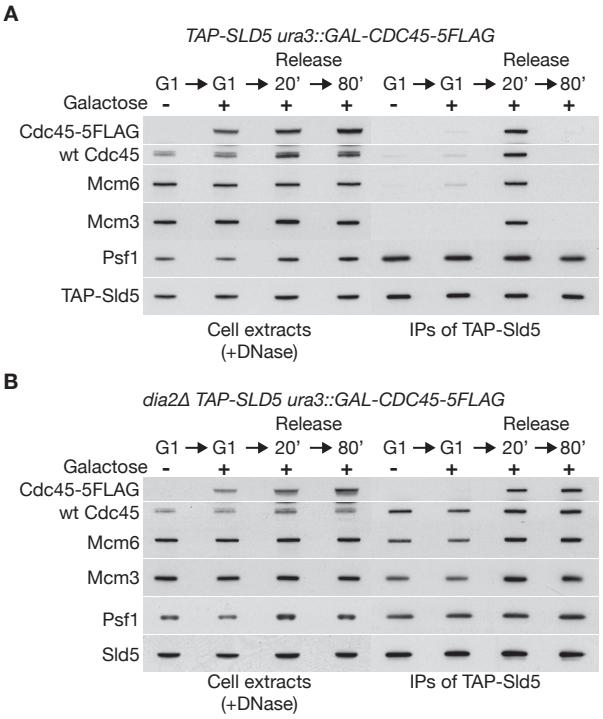
Stable CMG complexes are present during the G1-phase of the cell cycle in *dia2Δ* cells.
The experiment in Fig. 4A was repeated, but the cell extracts contained 700mM potassium acetate, instead of 100mM.
The CMG helicase is stable in these 'high salt extracts', although its interaction with other factors such as Csm3 is impaired (Ref. 1).

Maric-Maculins Supplementary Figure 6



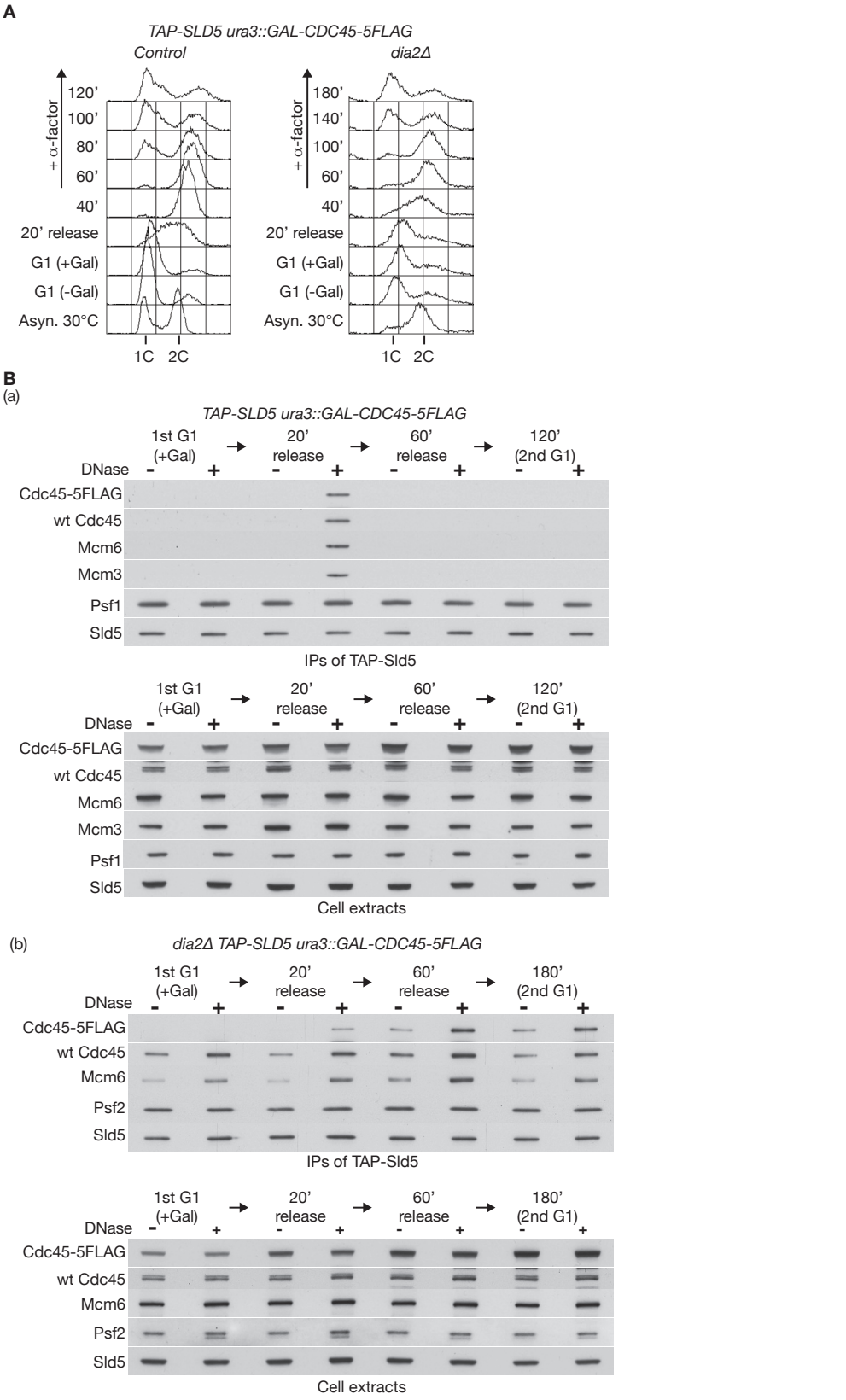
Persistence of the CMG helicase during G1-phase is unique to *dia2Δ* cells. (**A**-**D**) The indicated strains were arrested in G1-phase with mating pheromone, before isolation of GINS from cell extracts as above.

Maric-Maculins Supplementary Figure 7



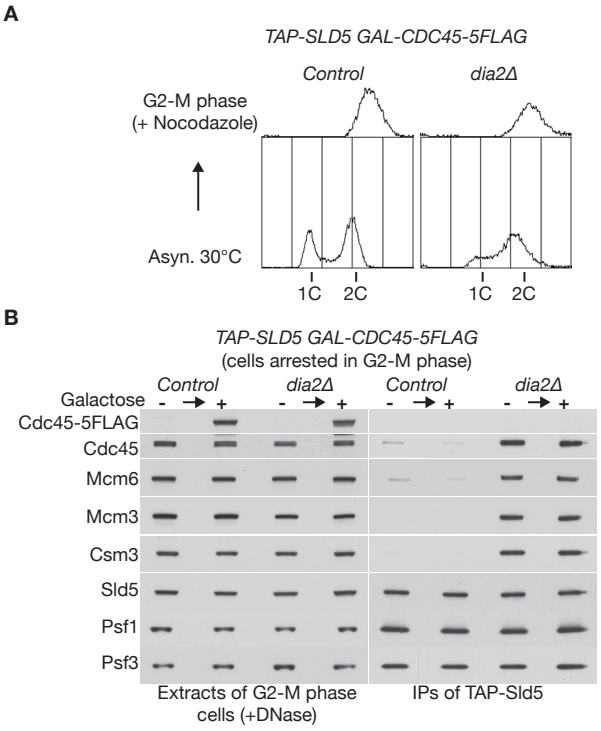
Dia2 is required for disassembly of the CMG helicase at the end of S-phase.
A similar experiment to that in Fig. 5 was performed with control ((**A**), YTM577) and *dia2Δ* ((**B**), YTM576) cells, in which a *GAL-CDC45-5FLAG* construct had been integrated at the *ura3* locus. In this case, the cells expressed TAP-tagged Sld5, which was used to monitor the assembly and disassembly of the CMG helicase as above.

Maric-Maculins Supplementary Figure 8



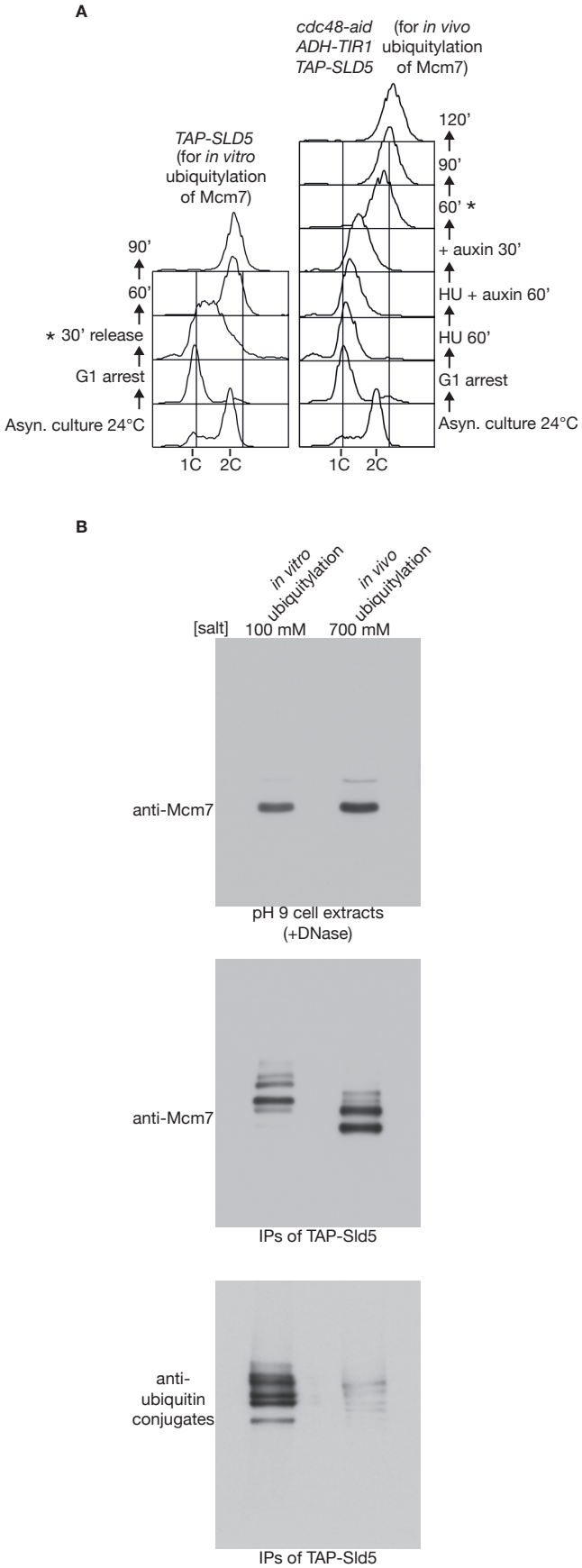
In the absence of Dia2, the CMG helicase persists into the next cell cycle, mostly associated with chromatin. A similar experiment to that in Supplementary Fig. 7 was performed, but the timecourse was extended to include G1-phase of the second cell cycle. Moreover, each cell extract was split into two aliquots, only one of which was treated with DNase to release protein complexes from chromatin.

Maric-Maculins Supplementary Figure 9



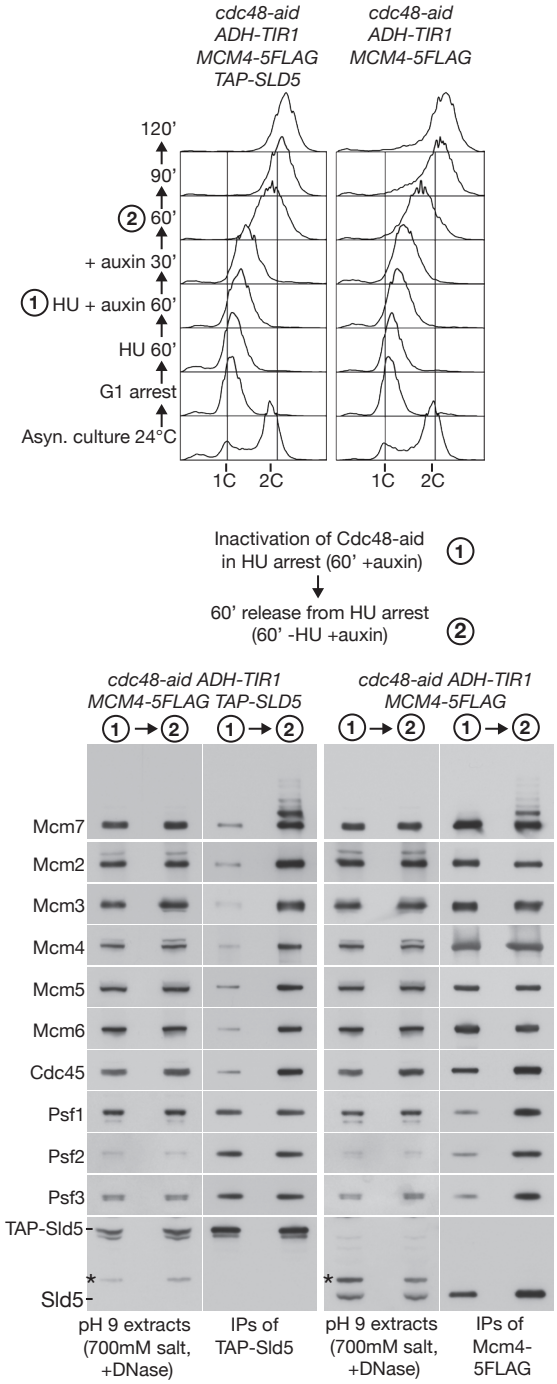
Persistence of the CMG helicase after S-phase in *dia2Δ* cells is not due to ongoing helicase assembly.
The same strains as in Fig. S8 were arrested in G2-M phase by addition of nocodazole to the culture medium, before addition of galactose to induce expression of Cdc45-5FLAG from the *GAL* promoter.
Extracts were then generated and used to isolate CMG complexes, by immunoprecipitation of TAP-Sld5.
Tagged Cdc45-5FLAG was not incorporated into the CMG complexes that persisted in *dia2Δ* cells.

Maric-Maculins Supplementary Figure 10



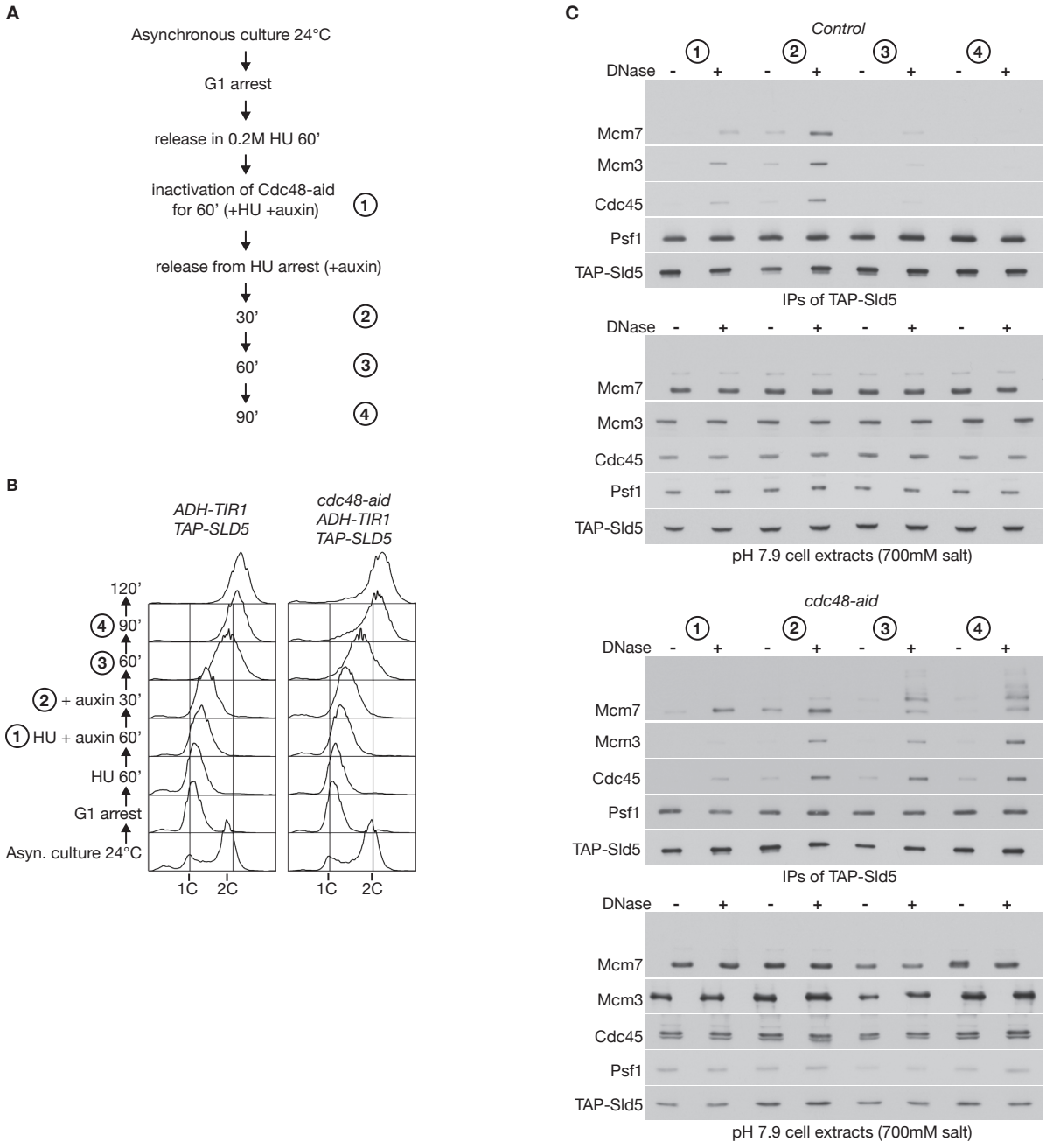
Comparison of *in vitro* and *in vivo* ubiquitylated forms of the Mcm7 subunit of the CMG helicase.
(**A**) Analogous experiments to those shown in Fig. S1A (YASD375, for in vitro ubiquitylation in 'pH9 cell extracts') and Fig. 8B (YMM228, for in vivo ubiquitylation) were performed, and the samples indicated by asterisks were were used to prepare 'pH 9 cell extracts' in the presence of 100mM salt (for in vitro ubiquitylation) or 700mM salt (for analysis of in vivo ubiquitylation).
(**B**) Immunoprecipitates of TAP-Sld5 were probed with an anti-Mcm7 antibody, or with an antibody specific for conjugates of ubiquitin (FK2 antibody; see Methods for details). Although ubiquitylated Mcm7 is detected in both the *in vitro* and *in vivo* samples, the number of attached ubiquitin moieties is reproducibly different in the two cases. At present we cannot say whether this reflects *in vitro* and *in vivo* differences in the activity of deubiquitylase enzymes, or in the efficiency of ubiquitylation in the two cases, or both of the above.

Maric-Maculins Supplementary Figure 11



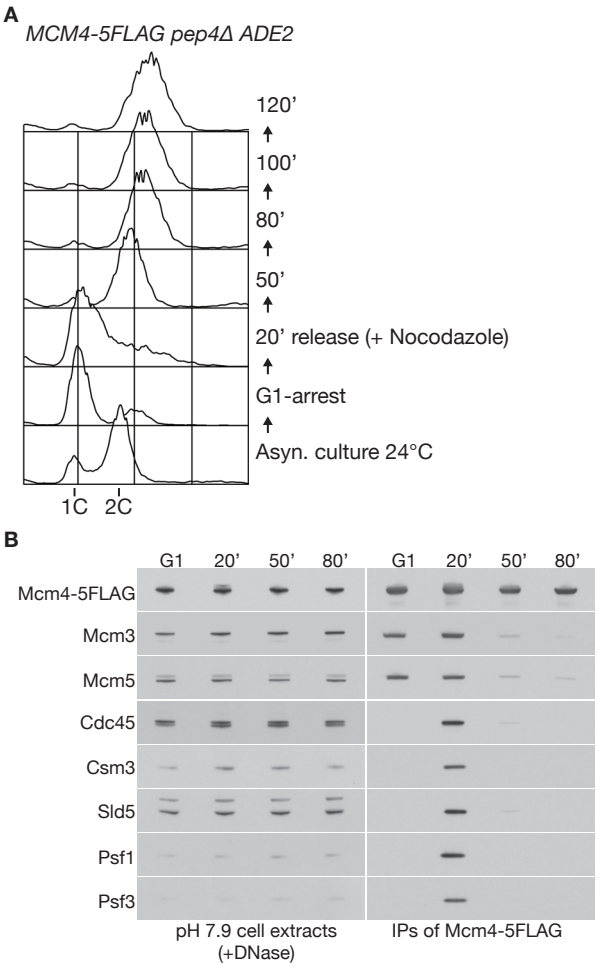
Ubiquitylation of CMG *in vivo* occurs on the Mcm7 subunit.
As in Fig. 7, Cdc48-aid was inactivated in HU-arrested cells (YMM320 and YMM339), before release for 60' into fresh medium lacking HU.
To examine modification of all 11 subunits of CMG, we compared immunoprecipitates of the Sld5 subunit of GINS (from YMM320, to examine associated Cdc45 and Mcm2-7), and the Mcm4 subunit of Mcm2-7 (from YMM339, to examine associated Cdc45 and GINS).
The asterisk in the Sld5 immunoblots denotes a non-specific signal in the cell extracts.

Maric-Maculins Supplementary Figure 12



Ubiquitylated CMG helicase persists on chromatin in the absence of Cdc48 activity.
(**A**) The experiment in Fig. 8B was repeated.
(**B**) DNA content was measured by flow cytometry.
(**C**) Cell extracts were prepared at pH 7.9 in the presence of 700mM salt, with or without DNase treatment to digest chromosomal DNA, before immunoprecipitation of the TAP-tagged Sld5 subunit of CMG.

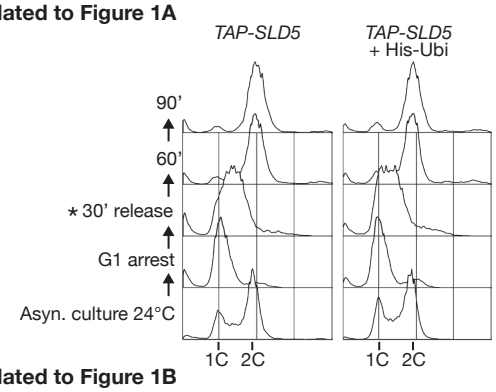
Maric-Maculins Supplementary Figure 13



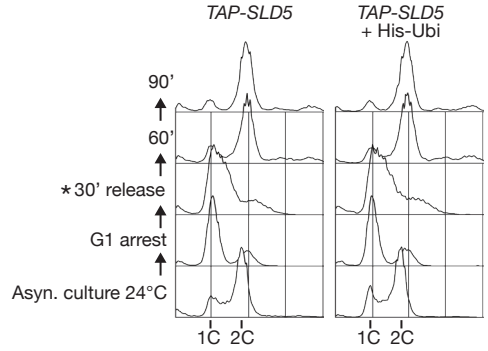
Stable Mcm2-7 complexes are lost during completion of DNA replication.
MCM4-5FLAG (YHM42) cells were arrested in G1-phase and then released for the indicated times. Nocodazole was added after 20', to prevent progression through mitosis. Cell extracts were treated with DNase to release complexes from chromatin, and then Mcm4-5FLAG was isolated by immunoprecipitation. Stable Mcm2-7 complexes were detected during G1-phase and S-phase (in the latter case these were a mixture of inactive Mcm2-7 complexes and the CMG helicase), but then were lost when cells completed S-phase. These data indicate that the Mcm2-7 component of CMG is no longer stable when CMG disassembles at the end of S-phase. It thus seems likely that all three components of CMG (Cdc45, Mcm2-7 and GINS) are released from chromatin in a co-ordinated fashion during completion of replication.

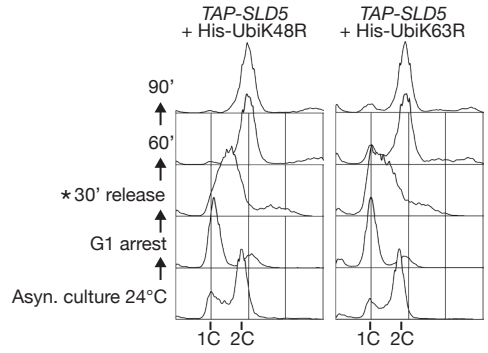
Maric-Maculins Supplementary Figure 14

Related to Figure 1A

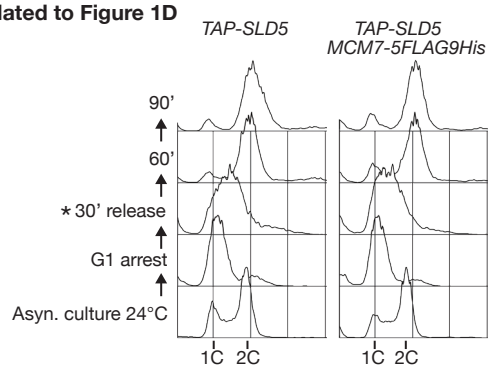


Related to Figure 1B

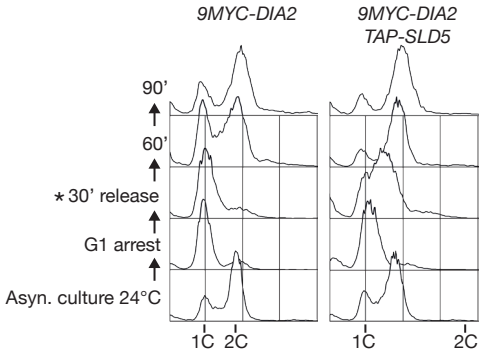




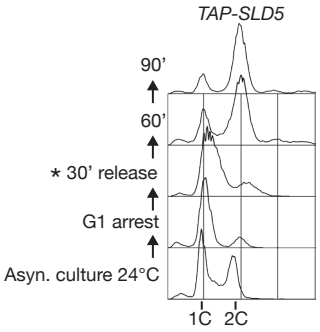
Related to Figure 1D



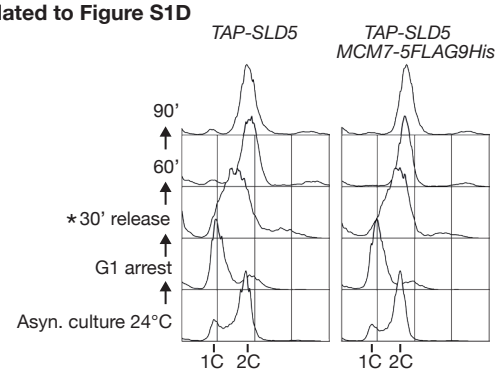
Related to Figure 2A



Related to Figure S1A



Related to Figure S1D



Additional flow cytometry data for the indicated experiments
(where included, the asterisks denote the samples that were used to prepare cell extracts).

Strain	Genotype
W303-1	*MAT**a** ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 / MAT*α *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*
W303-1a	*MAT**a** ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*
YAG230-3	*MAT**a** TAP-SLD5 (kanMX) MCM4-5FLAG (hphNT) pep4Δ::URA3 ADE2*
YASD375	*MAT**a** TAP-SLD5 (kanMX) pep4Δ::URA3 ADE2*
YGDP483	*MAT**a** TAP-SLD5 (kanMX) MCM7-5FLAG 9His (hphNT) pep4Δ::URA3 ADE2*
YGDP671	*MAT**a** TAP-SLD5 (kanMX) rtt101Δ::kanMX pep4Δ::ADE2*
YGDP673	*MAT**a** TAP-SLD5 (kanMX) mms22Δ::kanMX pep4Δ::ADE2*
YHM42	*MAT**a** MCM4-5FLAG (hphNT) pep4Δ::URA3 ADE2*
YHM117	*MAT**a** 9MYC-DIA2 (kanMX) HRT1-6HA (K.l.TRP1) pep4Δ::URA3*
YHM130	*MAT**a** TAP-SLD5 (kanMX) dia2Δ::HIS3 pep4Δ::URA3 ADE2*
YHM132	*MAT**a** TAP-SLD5 (kanMX) 9MYC-DIA2 (kanMX) HRT1-6HA (K.l.TRP1) pep4Δ::URA3 ADE2*
YJW15	*MAT**a** ura3-1::ADH1-OsTIR1-9MYC (URA3)*
YMM13	*MAT**a** TAP-SLD5 (kanMX) rad5Δ::hphNT pep4Δ::URA3 ADE2*
YMM15	*MAT**a** TAP-SLD5 (kanMX) ubc13Δ::hphNT pep4Δ::URA3 ADE2*
YMM22	*MAT**a** TAP-SLD5 (kanMX) pep4Δ::URA3 ADE2 pRS423 (HIS3)*
YMM23	*MAT**a** TAP-SLD5 (kanMX) pep4Δ::URA3 ADE2 pRS423-CUP-His7-Ubi (HIS3)*
YMM24	*MAT**a** TAP-SLD5 (kanMX) rad18Δ::hphNT pep4Δ::URA3 ADE2*
YMM70	*MAT**a** dia2Δ::HIS3 pep4Δ::URA3 ADE2*
YMM74	*MAT**a** MCM4-5FLAG (hphNT) cdc45-td (kanMX) GAL-UBR1 (HIS3) pep4Δ::URA3 ADE2*
YMM89	*MAT**a** TAP-SLD5 (kanMX) pep4Δ::URA3 ADE2 pRS423-CUP-His7-UbiK48R (HIS3)*
YMM90	*MAT**a** TAP-SLD5 (kanMX) pep4Δ::URA3 ADE2 pRS423-CUP-His7-UbiK63R (HIS3)*
YMM203	*MAT**a** cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1)*
YMM206	*MAT**a** TAP-SLD5 (kanMX) cim3-1 pep4Δ::URA3 ADE2*
YMM214	*MAT**a** TAP-SLD5 (kanMX) cdc48-3 pep4Δ::URA3 ADE2*
YMM228	*MAT**a** TAP-SLD5 (kanMX) cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3 ADE2*
YMM256	*MAT**a** TAP-SLD5 (kanMX) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3 ADE2*
YMM283	*MAT**a** TAP-SLD5 (kanMX) GALL-DIA2 (kanMX) cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::ADE2*
YMM309	*MAT**a** TAP-SLD5 (kanMX) dbf4-4A (HIS3) sld3-37A-10HIS-13MYC (kanMX) cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3 ADE2*
YMM320	*MAT**a** TAP-SLD5 (kanMX) MCM4-5FLAG (hphNT) cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1)*

	pep4Δ::URA3 ADE2
YMM339	*MAT**a** MCM4-5FLAG (hphNT) cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3 ADE2*
YMM340	*MAT**a** TAP-SLD5 (kanMX) rad5Δ::hphNT cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3 ADE2*
YMM341	*MAT**a** TAP-SLD5 (kanMX) rad18Δ::hphNT cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3 ADE2*
YMM346	*MAT**a** TAP-SLD5 (kanMX) ubc13Δ::hphNT cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3 ADE2*
YMM347	*MAT**a** TAP-SLD5 (kanMX) slx5Δ::kanMX cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3 ADE2*
YMM350	*MAT**a** TAP-SLD5 (kanMX) slx8Δ::kanMX cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3 ADE2*
YMM351	*MAT**a** TAP-SLD5 (kanMX) rtt101Δ::kanMX cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3 ADE2*
YMM366	*MAT**a** TAP-SLD5 (kanMX) sml1Δ::HIS3 cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3 ADE2*
YMM368	*MAT**a** TAP-SLD5 (kanMX) sml1Δ::HIS3 mec1Δ::ADE2 cdc48-aid (hphNT) ura3-1::ADH1-OsTIR1-9MYC (URA3 & K.l.TRP1) pep4Δ::URA3*
YSS184	*MAT**a** MCM4-5FLAG (hphNT) GAL-UBR1 (HIS3) pep4Δ::URA3 ADE2*
YTM214	*MAT**a** TAP-SLD5 (kanMX) slx5Δ::kanMX pep4Δ::URA3 ADE2*
YTM216	*MAT**a** TAP-SLD5 (kanMX) slx8Δ::kanMX pep4Δ::URA3 ADE2*
YTM305	*MAT**a** MCM7-5FLAG 9His (hphNT) pep4Δ::URA3 ADE2*
YTM306	*MAT**a** MCM7-5FLAG 9His (hphNT) dia2Δ:::HIS3 pep4Δ::URA3 ADE2*
YTM312	*MAT**a** MCM7-5FLAG 9His (hphNT) ura3-1::GAL-5FLAG-DIA2 (URA3) pep4Δ::URA3*
YTM330	*MAT**a** GAL-UBR1 (HIS3) pep4Δ::URA3 ADE2*
YTM371	*MAT**a** TAP-SLD5 (kanMX) rrm3Δ::hphNT pep4Δ::ADE2*
YTM376	*MAT**a** cdc34-2 pep4Δ::ADE2*
YTM396	*MAT**a** CDC34-TAP (kanMX) pep4Δ::ADE2*
YTM415	*MAT**a** CDC45-ProteinA (kanMX) dia2Δ::HIS3 pep4Δ::ADE2*
YTM444	*MAT**a** td-cdc53-1 (kanMX) GAL-UBR1 (HIS3) pep4Δ::URA3 ADE2*
YTM445	*MAT**a** td-cdc4-1 (kanMX) GAL-UBR1 (HIS3) pep4Δ::URA3 ADE2*
YTM532	*MAT**a** MCM7-5FLAG 9His (hphNT) ura3-1::GAL-ProteinA-DIA2 (URA3) dia2Δ::HIS3 pep4Δ::URA3 ADE2*
YTM568	*MAT**a** TAP-SLD5 (kanMX) GALL-DIA2 (kanMX) pep4Δ::ADE2*
YTM576	*MAT**a** TAP-SLD5 (kanMX) ura3-1::GAL-CDC45-5FLAG 9His (URA3) dia2Δ::HIS3 pep4Δ::ADE2*
YTM577	*MAT**a** TAP-SLD5 (kanMX) ura3-1::GAL-CDC45-5FLAG 9His (URA3) pep4Δ::ADE2*
YTM592	*MAT**a** TAP-MCM3 (kanMX) ura3-1::GAL-PSF2-5FLAG (URA3) dia2Δ::HIS3 pep4Δ::ADE2*
YTM593	*MAT**a** TAP-MCM3 (kanMX) ura3-1::GAL-PSF2-5FLAG (URA3) pep4Δ::ADE2*

YTM687	*MAT**a** TAP-SLD5 (kanMX) MCM4-5FLAG (hphNT) dia2Δ::HIS3 pep4Δ::URA3 ADE2*
YTM688	*MAT**a** TAP-SLD5 (kanMX) rad52Δ::kanMX pep4Δ::ADE2*
YTM689	*MAT**a** TAP-SLD5 (kanMX) rad51Δ::kanMX pep4Δ::ADE2*
YTM709	*MAT**a** TAP-SLD5 (kanMX) sgs1Δ::URA3CP top3Δ::kanMX pep4Δ::ADE2*

Table S1

Yeast strains used in this study. All strains are based on the W303 background.

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