

Expanded View Figures

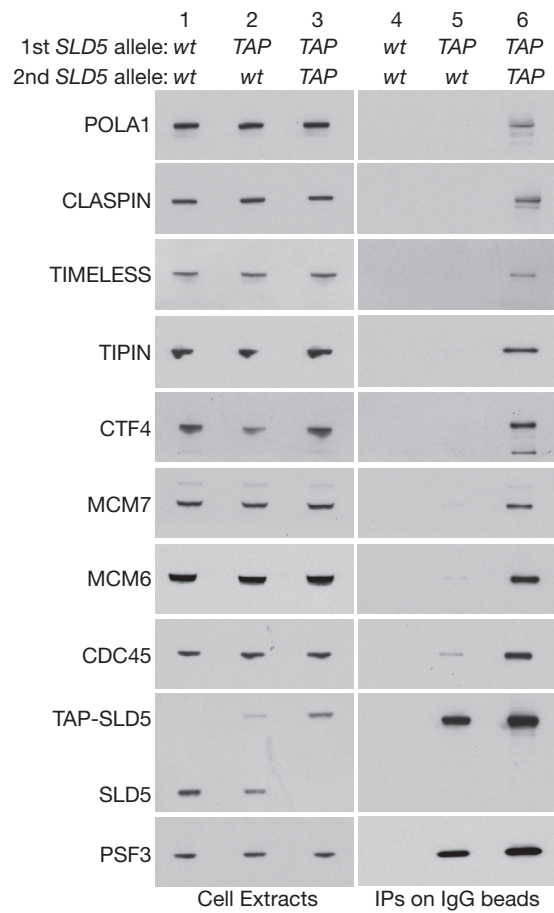
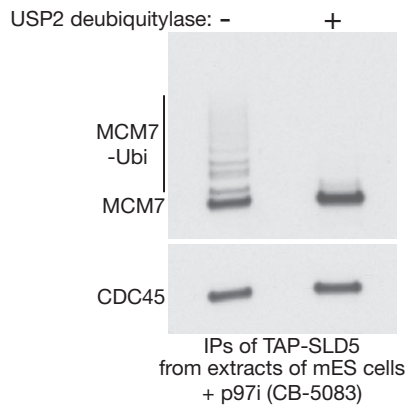


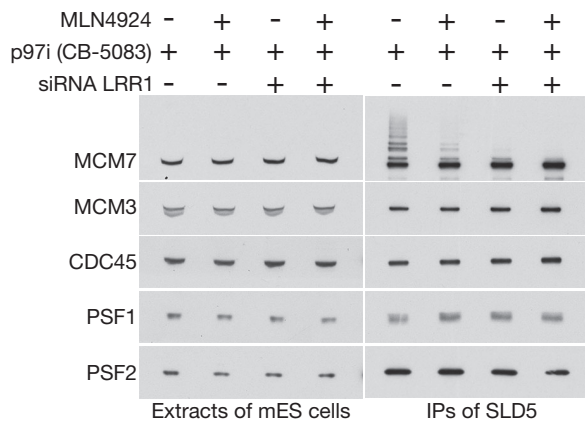
Figure EV1. GINS co-purifies with a range of replisome components from homozygous *TAP-*SLD5 mouse ES cells.**

Cell extracts from mouse ES lines with the indicated genotypes were incubated with IgG beads. The isolated proteins were analysed by immunoblotting in order to monitor the factors that co-purified with *TAP-*SLD5**. Note that isolation of the CMG was more effective from homozygous *TAP-*SLD5** / *TAP-*SLD5** cells than from the equivalent heterozygote

A



B



C

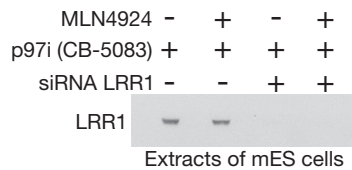


Figure EV2. Ubiquitylation of CMG-MCM7 by CUL2^{LRR1}.

- A Ubiquitylated CMG was isolated from extracts of mouse ES cells treated with 5 μM CB-5083, by immunoprecipitation of TAP-SLD5. Subsequently, the immunoprecipitates were treated with the USP2 deubiquitylase as indicated, confirming that the observed modifications of CMG-MCM7 corresponded to ubiquitylation.
- B TAP-SLD5 cells were treated as indicated and TAP-SLD5 was then isolated from cell extracts by immunoprecipitation. The indicated proteins were monitored by immunoblotting.
- C Depletion of LRR1 was monitored by immunoblotting for the experiment in (B).

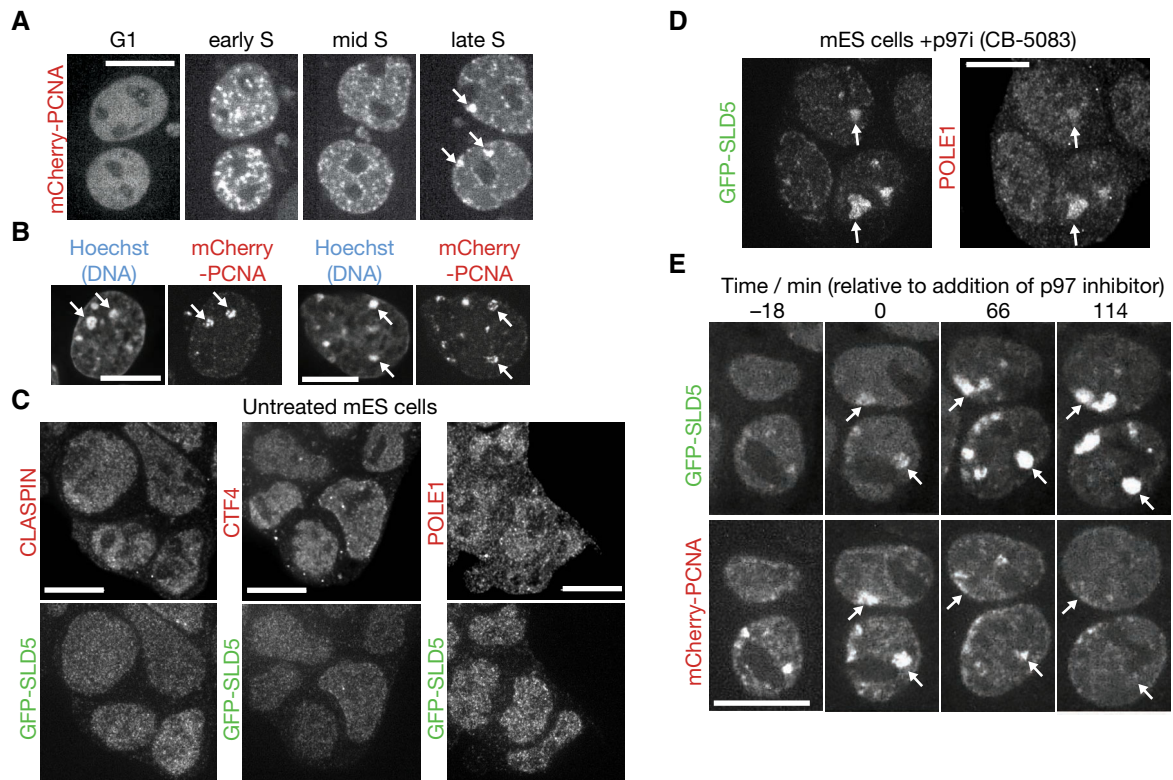


Figure EV3. Accumulation of replisome components on chromatin in response to p97 inhibition.

- A Time-lapse analysis of mCherry-PCNA as mouse ES cells enter and progress through S-phase (arrows denote large patches that are seen in late S-phase).
- B Cells expressing mCherry-PCNA were fixed and stained with Hoechst, revealing that large mCherry-PCNA patches in late S-phase correspond to replication of constitutive heterochromatin (examples marked by arrows). In 94% of cells with large PCNA patches, these co-localised with condensed DNA, $n = 53$.
- C The indicated replisome factors were analysed by immunofluorescence in untreated mouse ES cells expressing GFP-SLD5.
- D Similar experiment to that in (C), using cells treated with $5 \mu\text{M}$ CB-5083. The arrows indicate the co-localisation of POLE1 and GFP-SLD5 on heterochromatic patches (88% of cells, $n = 49$).
- E Time-lapse analysis of mouse ES cells expressing GFP-SLD5 and mCherry-PCNA, following treatment with the p97 inhibitor CB-5083. The arrows illustrate the persistence of GFP-SLD5 on heterochromatic patches after the disappearance of mCherry-PCNA (100% of cells, $n = 77$).

Data information: Scale bars in all panels correspond to $10 \mu\text{m}$.

Figure EV4. *TRAIP*^{-/-} mouse ES cells are viable but are sensitive to DNA damaging agents that induce inter-strand DNA crosslinks.

- A Procedure for creating small deletion at 5' end of first exon of the *TRAIP* gene in mouse ES cells.
- B Location of gRNAs that were used to target the Cas9-D10A "nickase" to the *TRAIP* locus. The panel also indicates the location of two PCR oligos that were used subsequently to check the integrity of the targeted region.
- C PCR analysis of genomic DNA from cells transfected with DNA expressing Cas9-D10A and the indicated gRNA(s) from (B).
- D DNA sequence analysis of the targeted region from control cells and two clones exposed to Cas9-D10A in the presence of gRNAs 1 + 2 (PAM = Protospacer Adjacent Motif). See Materials and Methods for further details.
- E Immunoblots of cell extracts from control cells and *TRAIP* Δ clones, using the indicated anti-*TRAIP* antibodies. Asterisks indicate non-specific bands.
- F (i) Flow cytometry analysis of DNA content for asynchronously growing wild-type and *TRAIP*^{-/-} mouse ES cells. (ii) Doubling times were calculated as described in Materials and Methods.
- G Procedure for expressing wild-type or mutated *TRAIP* at the *ROSA26* locus in *TRAIP*^{-/-} cells.
- H Cells with the indicated genotypes were grown for 24 h in the presence of varying concentrations of the DNA damaging agent Mitomycin C as shown, before continued growth in the absence of drug.

Data Information: In (F), the doubling time data are presented as the mean values of three experiments \pm standard deviation.

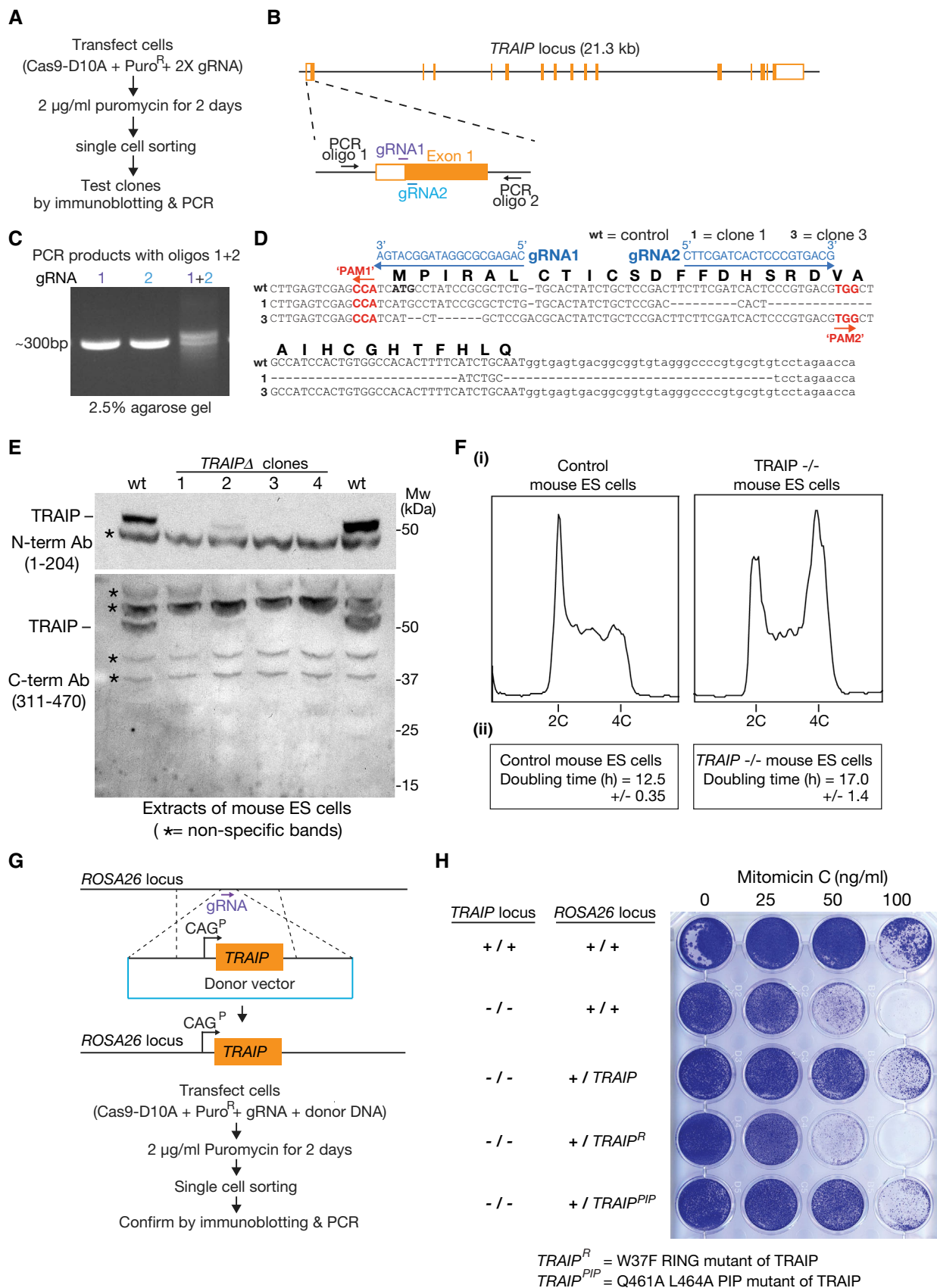


Figure EV4.

Figure EV5. Models for disassembly of the metazoan replisome. The figure summarises data from this manuscript together with previous studies with early embryos of *Caenorhabditis elegans* and extracts of *Xenopus laevis* eggs.

- A During DNA replication termination, CUL2^{LRR1} promotes ubiquitylation of CMG-MCM7, leading to disassembly by p97-UFD1-NPL4.
- B In cells that lack CUL2^{LRR1} activity, the CMG replisome remains on chromatin after DNA replication termination. Upon entry into mitosis, the TRAIPI ubiquitin ligase activates a second pathway of CMG disassembly, dependent upon p97.
- C Previous work showed that TRAIPI is required in human cells to process sites of incomplete DNA replication during mitosis, by a process of Mitotic DNA Synthesis or "MiDAS" (Sonneville et al, 2019). It is likely that the first step in this pathway is TRAIPI-dependent CMG disassembly upon entry into mitosis.

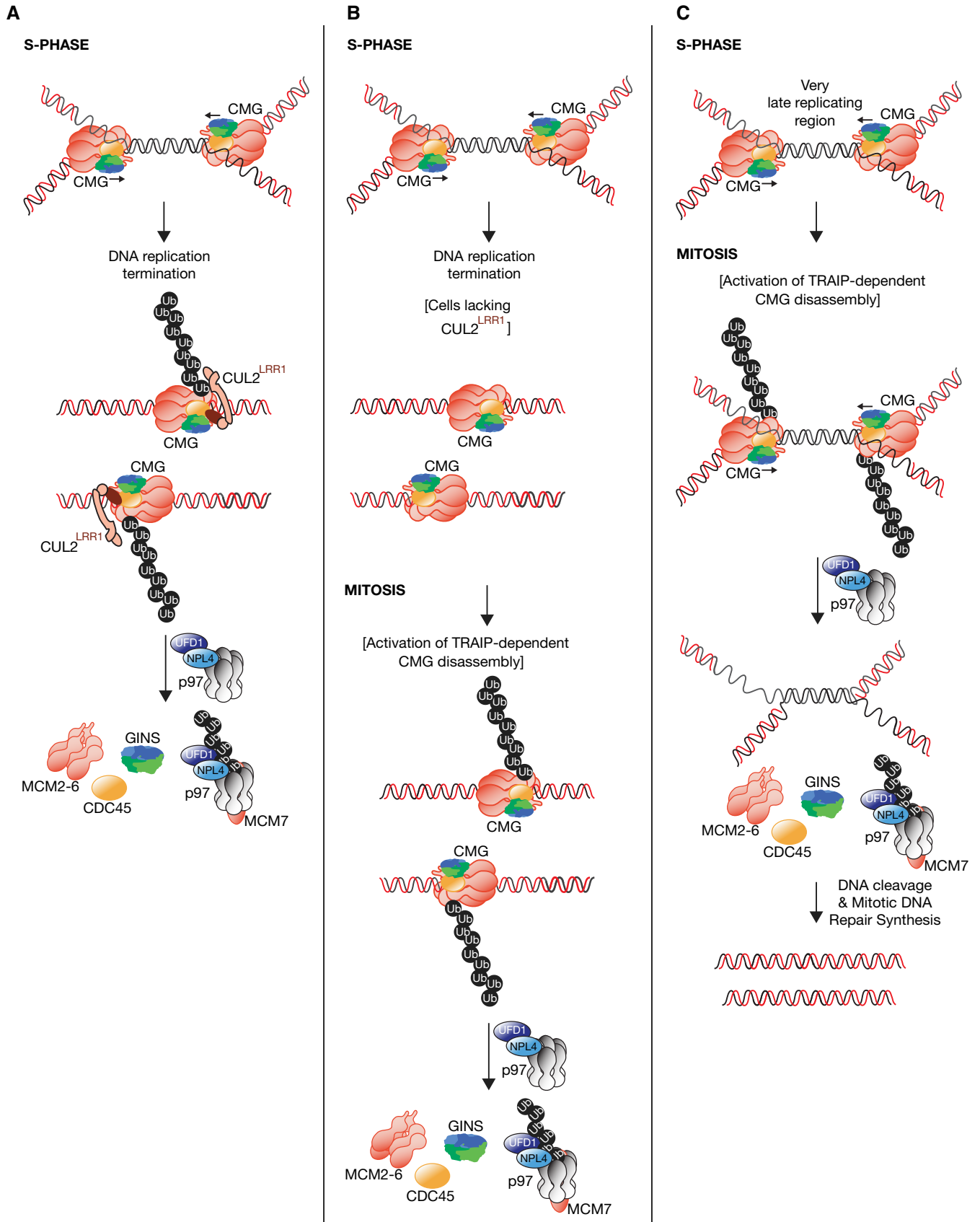


Figure EV5.