

Expanded View Figures

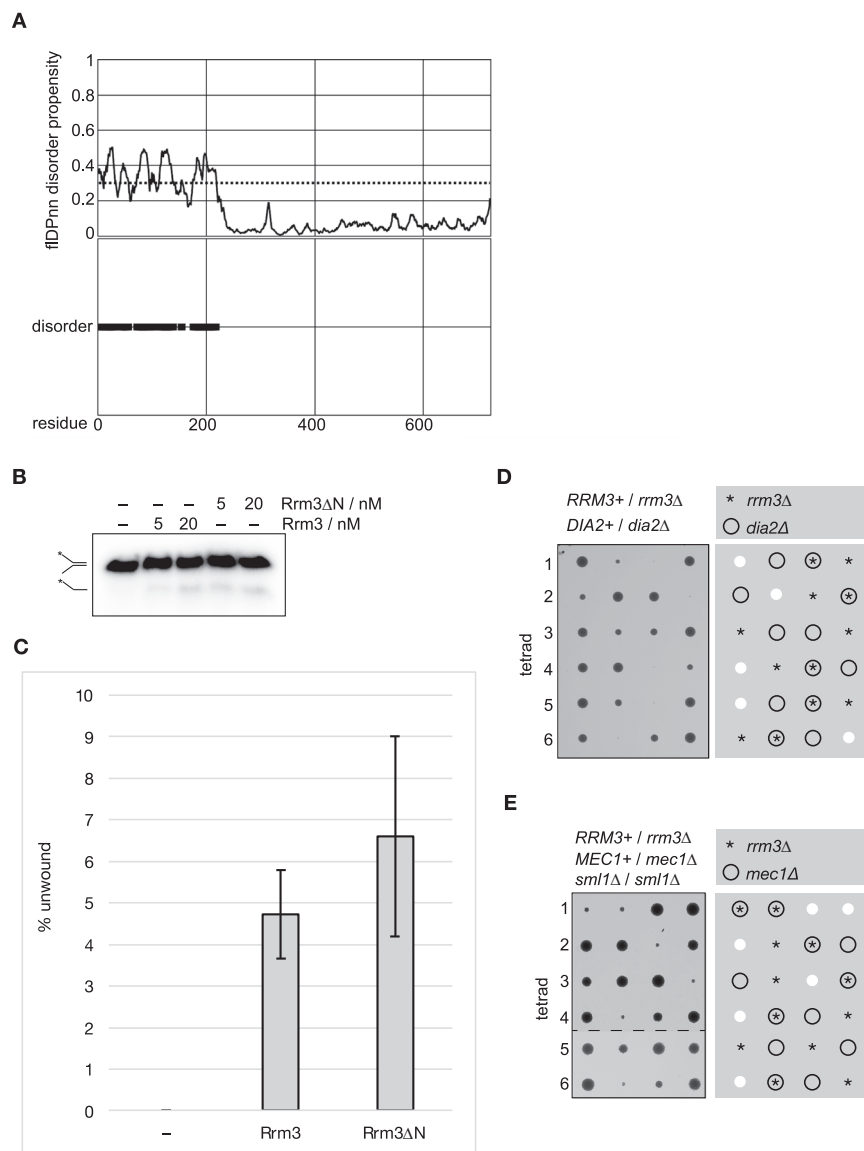


Figure EV1. Characterisation of *rrm3ΔN* mutant in vitro and in vivo.

(A) Disorder prediction for *S. cerevisiae* Rrm3, generated using the fIDPnn webserver. Residue numbers are given on the x-axis. (B) The ability of Rrm3 and Rrm3ΔN to unwind a 25 bp DNA duplex, formed by annealing oligonucleotide TD254 to TD255, was monitored as described in Methods. * indicates 32 P-labelling of TD254. (C) Similar experiments to (B) were performed three times. The percentage of unwound product was quantified in each case for reactions containing 5 nM of Rrm3, and the figure presents the mean values with standard deviations. (D, E) Diploid yeast cells of the indicated genotypes were sporulated and the resulting tetrads were then dissected and grown on YPD medium for 2 days at 30 °C.

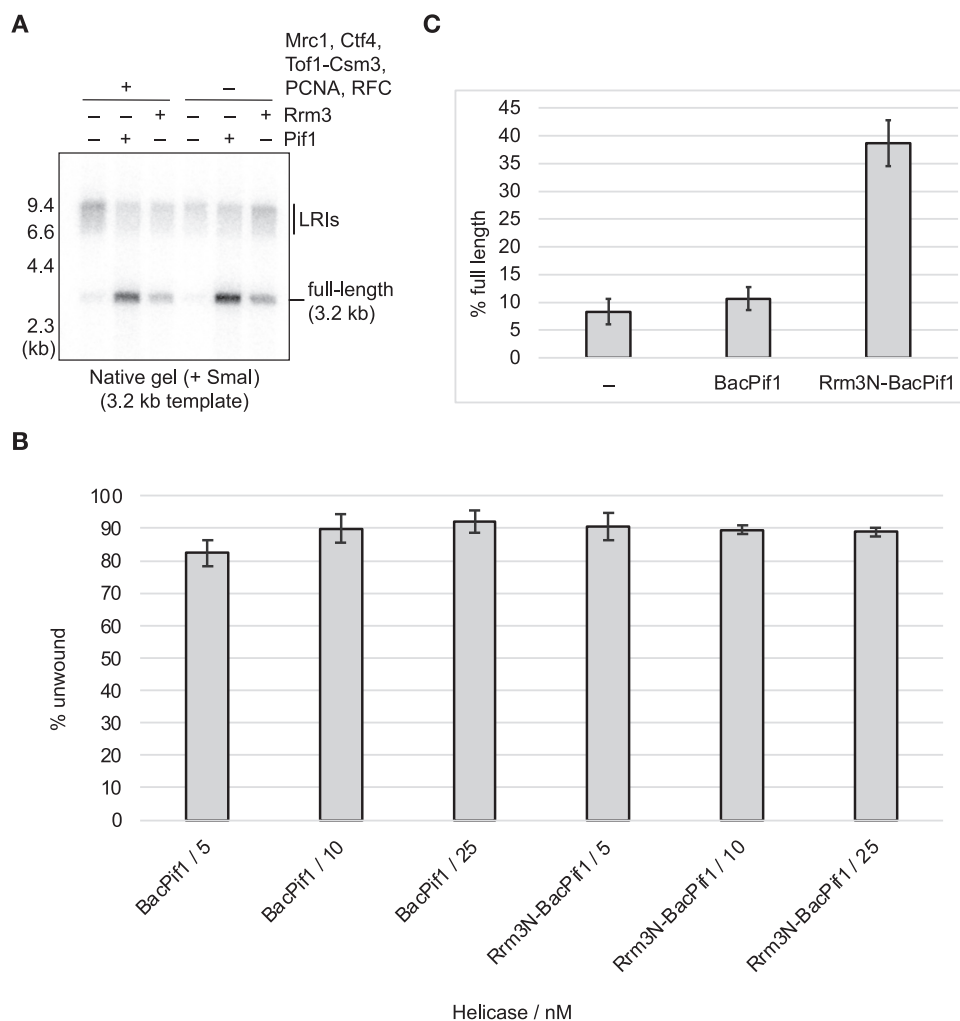


Figure EV2. Supporting data for Fig. 2.

(A) A 3189 bp plasmid template (pBS/ARS1WTA) was replicated in the presence or absence of Rrm3 (12.5 nM) or Pif1 (5 nM) and the indicated replisome components. SmaI-digested radiolabelled replication products were resolved in a native agarose gel and detected by autoradiography. (B) Similar experiments to Fig. 2C were performed three times. The percentage of unwound product was quantified in each case, and the figure presents the mean values with standard deviations. (C) Similar experiments to Fig. 2F were performed three times. The percentage full-length products was quantified in each case, and the figure presents the mean values with standard deviations. Quantification was performed for BacPif1 and Rrm3N-BacPif1 samples that included 5 nM of each helicase.

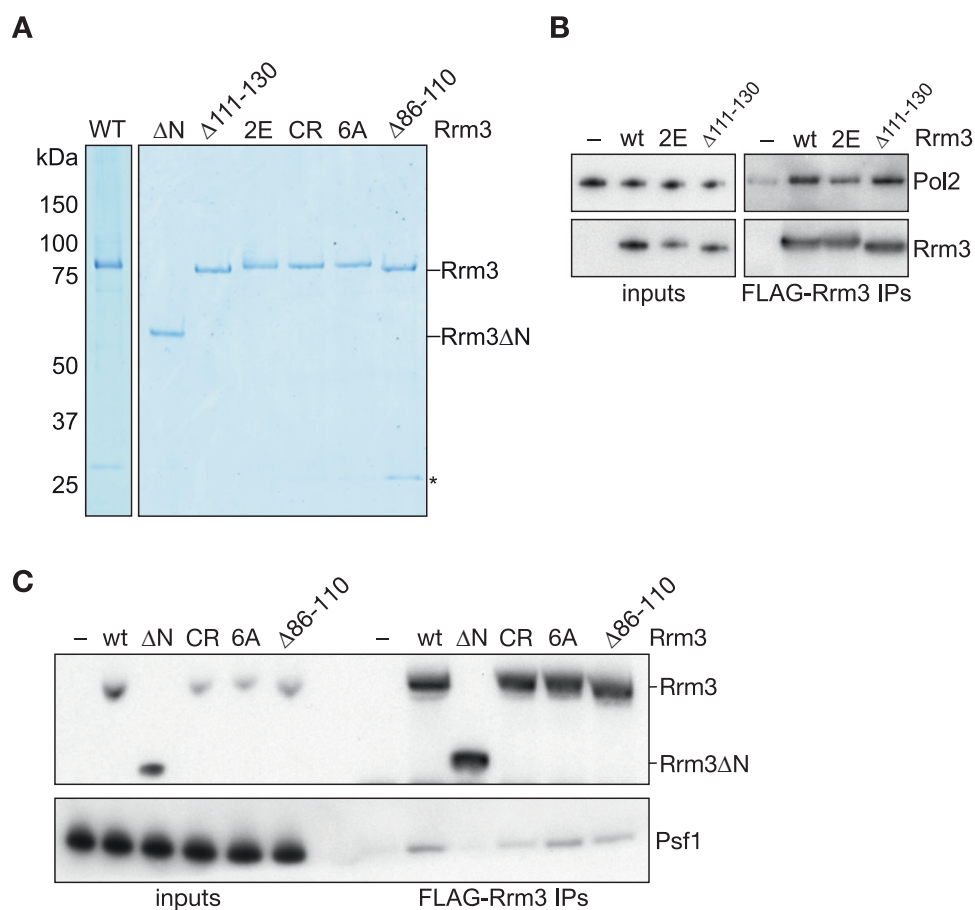


Figure EV3. Generation and characterisation of CMGE-binding mutants of Rrm3.

(A) Purified wild-type or mutant versions of Rrm3 visualised by SDS-PAGE and Coomassie staining. * is a contaminating protein. (B, C) Purified Pole (B) or tetrameric GINS complex (C) were mixed with FLAG-tagged wild-type Rrm3 or the indicated Rrm3 mutants. Resultant complexes were isolated by anti-FLAG immunoprecipitation and detected by SDS-PAGE and immunoblotting. Rrm3 was detected by anti-FLAG immunoblotting.

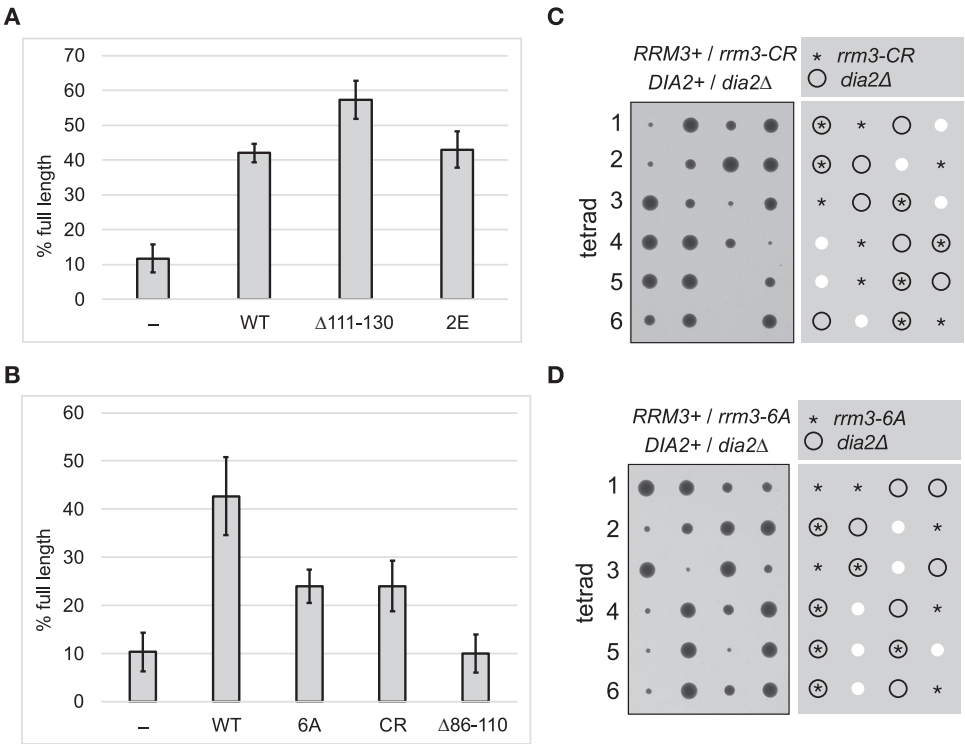


Figure EV4. Supporting data showing that CMGE binding is critical for Rrm3 function.

(A, B) Similar experiments to Fig. 5A (A) and 5B (B) were performed three times. The percentage full-length products was quantified in each case, and the figure presents the mean values with standard deviations. (C, D) Diploid yeast cells of the indicated genotypes were sporulated and the resulting tetrads were then dissected and grown on YPD medium for 2 days at 30 °C.

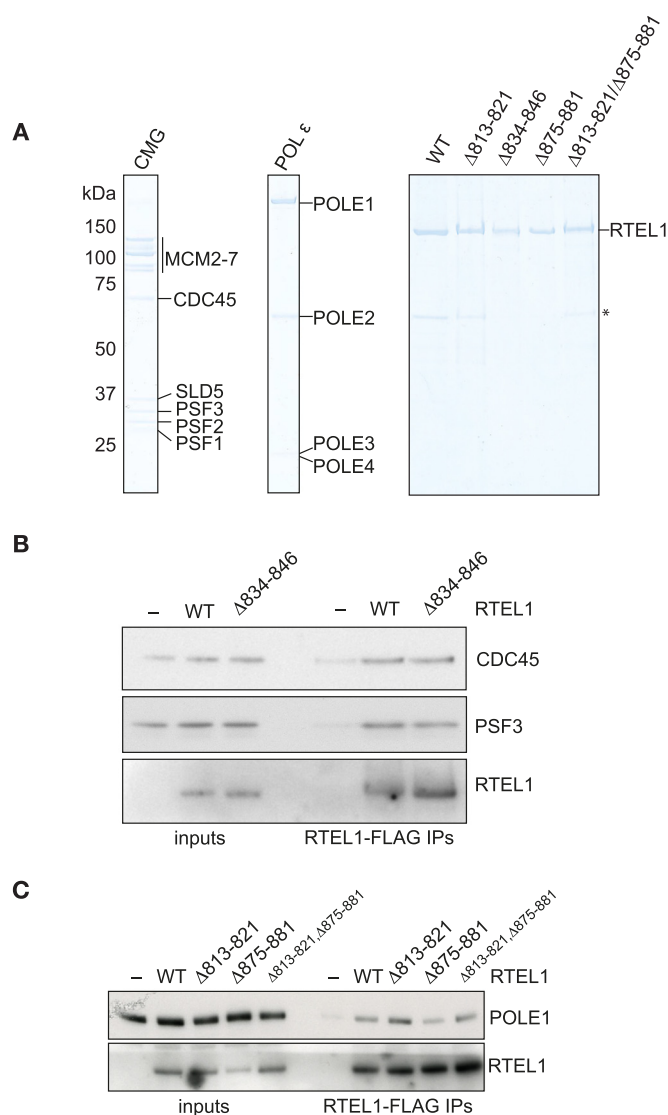


Figure EV5. Generation and characterisation of CMGE-binding mutants of RTEL1.

(A) Wild type or mutant versions of *Homo sapiens* RTEL1, CMG and POL ϵ purified after expression in budding yeast and visualised by SDS-PAGE and Coomassie staining. * indicates a contaminant in purified RTEL1. (B, C) Purified CMG (B) or POL ϵ (C) were mixed with FLAG-tagged wild-type RTEL1 or the indicated RTEL1 mutants. Resultant complexes were isolated by anti-FLAG immunoprecipitation and detected by SDS-PAGE and immunoblotting. RTEL1 was detected by anti-FLAG immunoblotting.