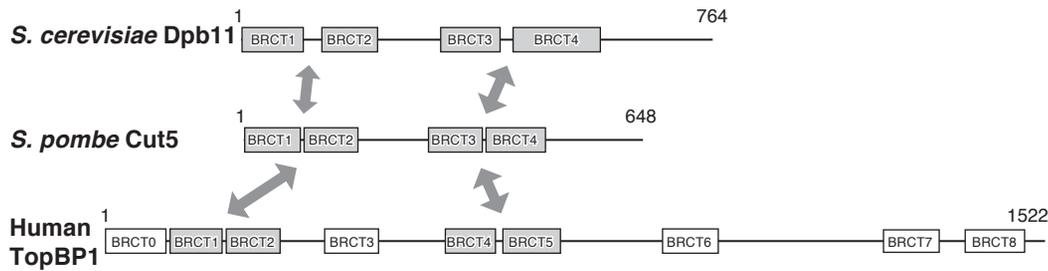


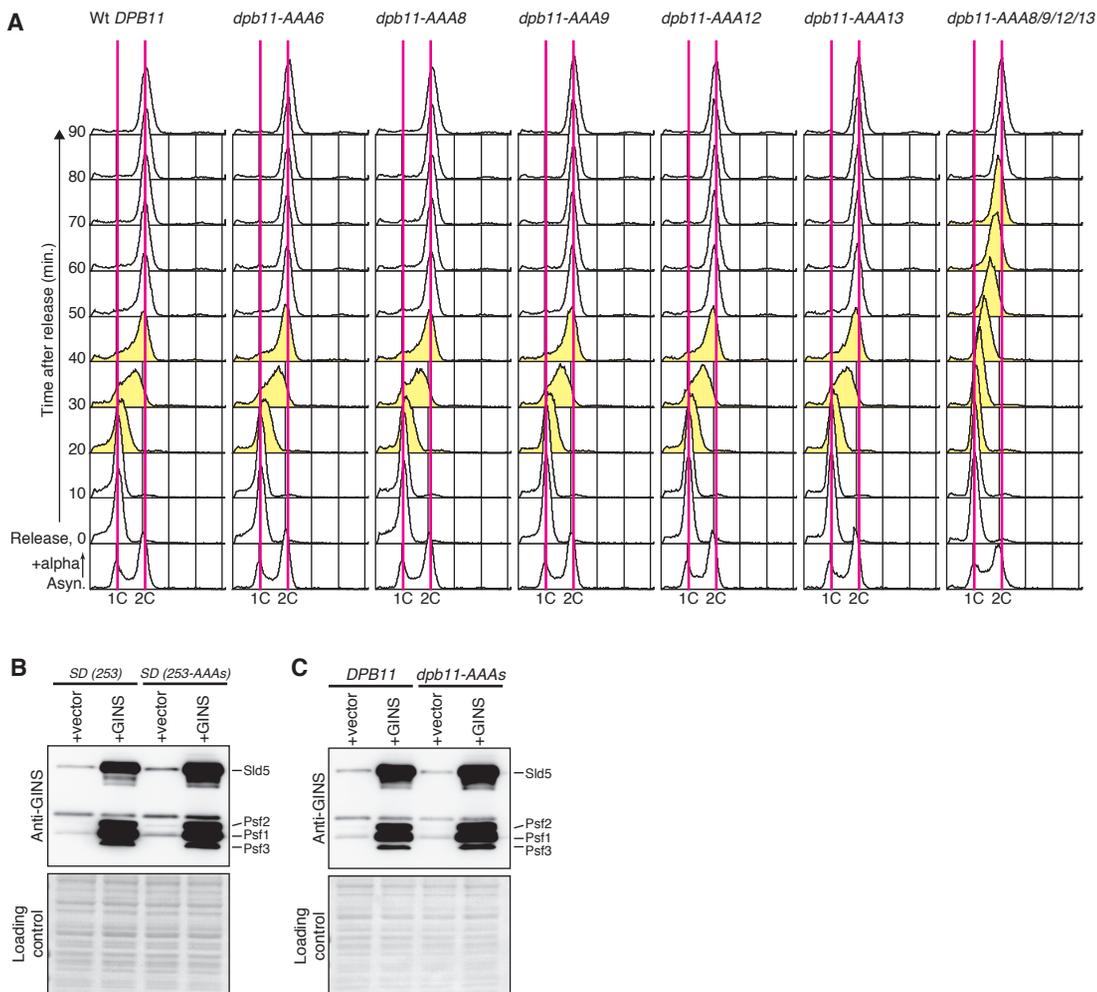
Tanaka et al.\_Supplementary Figure S1



Supplementary Figure S1

Schematic drawings of Dpb11/TopBP1 orthologs. BRCT domains are shown as boxes. BRCTs with high similarity are shown as gray boxes.

Tanaka et al.\_Supplementary Figure S3

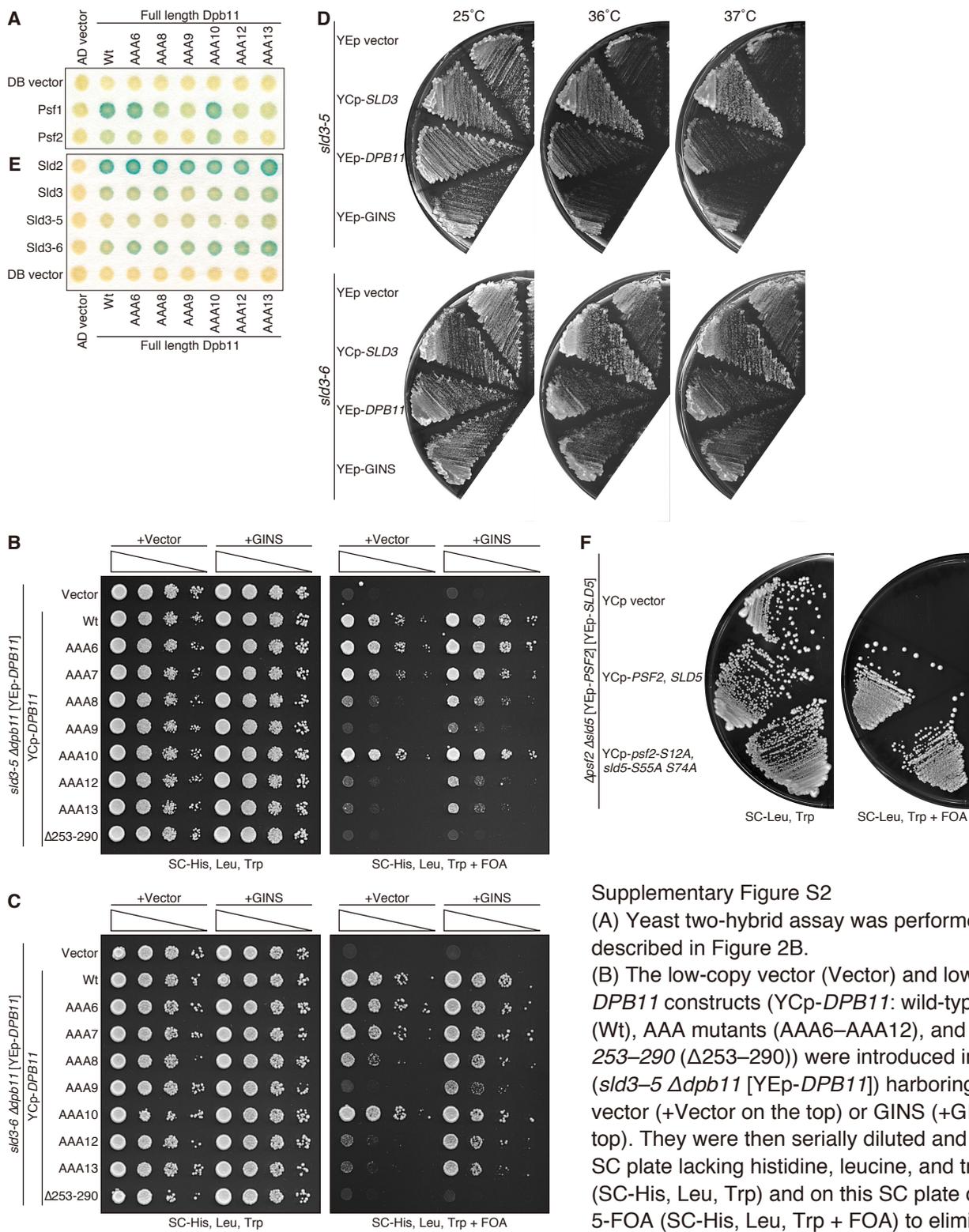


Supplementary Figure S3. Analysis of alanine-substitution mutations in the inter-BRCT region of Dpb11.

(A) W303-1a  $\Delta$ bar1 (Wt *DPB11*), YST1977 (*dpb11-AAA6*), YST1979 (*dpb11-AAA8*), YST1981 (*dpb11-AAA9*), YST1983 (*dpb11-AAA12*), YST1985 (*dpb11-AAA13*), and YST1987 (*dpb11-AAA8/9/12/13*) cells were grown as in Figure 3H.

(B) Whole-cell extracts were prepared from the asynchronous samples described in Figure 4A and the expression level of GINS was examined by western blotting.

(C) Whole-cell extracts were prepared from the asynchronous samples described in Figure 4B and the expression level of GINS was examined by western blotting.



Supplementary Figure S2

(A) Yeast two-hybrid assay was performed as described in Figure 2B.

(B) The low-copy vector (Vector) and low-copy *DPB11* constructs (YEp-*DPB11*: wild-type *DPB11* (Wt), AAA mutants (AAA6–AAA12), and *dppb11*Δ253–290 (Δ253–290)) were introduced into YST1908 (*sld3–5* Δ*dppb11* [YEp-*DPB11*]) harboring a multicopy vector (+Vector on the top) or GINS (+GINS on the top). They were then serially diluted and grown on an SC plate lacking histidine, leucine, and tryptophan (SC-His, Leu, Trp) and on this SC plate containing 5-FOA (SC-His, Leu, Trp + FOA) to eliminate the YEp-*DPB11* plasmid from cells.

(C) The low-copy vector (Vector) and low-copy *DPB11* constructs (YEp-*DPB11*: Wt *DPB11* (Wt), AAA mutants (AAA6–AAA12), and *dppb11*Δ253–290 (Δ253–290)) were introduced into YST1904 (*sld3–6* Δ*dppb11* [YEp-*DPB11*]) harboring the multicopy vector (+Vector at the top) or GINS (+GINS at the top). Cells were grown as in (B).

(D) The high-copy vector (YEp vector), low-copy *SLD3* (YEp-*SLD3*), high-copy *DPB11* (YEp-*DPB11*) and high-copy GINS (YEp-GINS) were introduced into YYK19 (*sld3–5*) and YYK16 (*sld3–6*) cells, respectively, and were grown at the temperatures indicated.

(E) Yeast two-hybrid assay was performed as described in Figure 2B.

(F) The low-copy vector (YEp vector) and low-copy *PSF2* and *SLD5* constructs (wild-type *PSF2* and *SLD5* (YEp-*PSF2*, *SLD5*) and CDK phosphorylation-site mutant of *PSF2* and *SLD5* (YEp-*psf2*-S12A, *sld5*-S55A S74A)) were introduced into YST1296 (Δ*psf2* Δ*sld5* [YEp-*PSF2*] [YEp-*SLD5*]) cells. They were then grown on an SC plate lacking leucine and tryptophan (SC-Leu, Trp) and on this SC plate containing 5-FOA (SC-Leu, Trp + FOA) to eliminate the YEp-*PSF2* and YEp-*SLD5* plasmids from cells.

Tanaka et al.\_Supplementary Figure S4

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Sc Dpb11 -----
Sp Cut5 -----
Hs TopBP1 285 T E F R P E A K T M P N S S T P T S Q I N T I D S R T T S D V S N I S N I N A S C V S E S I C N - S L N S K L E P T L E
Xl TopBP1 283 I E E A S T I K S V E D T S T P T G G N S K P N S R A L V D V S Q I S N I S T S C V N E S A F N S A M A S R L D P P A D

Sc Dpb11 -----
Sp Cut5 -----
Hs TopBP1 343 N L E N L D V S A F Q A P E D L L D G C R I Y L C G F S G R K L D K L R R L I N S G G G V R F N Q L N E D V T H V I V G
Xl TopBP1 343 T L E N L D I S S L Q A P D D L L D G C R I Y L C G F C G R K L D K L R K L I N N G G G V R F N Q L T G D V T H I I V G
                BRCT3

Sc Dpb11 221 -----L P N I K D L P Y D S I G S N S C
Sp Cut5 186 -----D M P A E K I G L G A V R L D P N T T E A K S Y S E
Hs TopBP1 403 D Y D D E L K Q F W N K S A H R P E V V G A K W L L E C F S K G Y M L S E E P Y I H A N Y Q P V E I P V S H Q P E S K A
Xl TopBP1 403 E T D E E L K Q F L N K T O H R P V V L T V K W L L D S F A K G H L O P E E I Y F H S S Y Q T E M P S P F E P ----

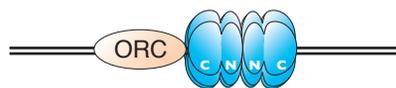
Sc Dpb11 238 D C W D K I N T T F P T N I D A Q S S L Q R Q O S S S T L T P S L P K T S S L L N K F K P K G E K I W D K A M S L Q O H
Sp Cut5 213 N Q K I S K N K E R S G Q S L A A L A E E A D L E P V I M K R G K K R D R S I L W E L N N G K F E F S S R S E E N S V
Hs TopBP1 463 A L L K K K N S S F S K K D F A P S E K H E Q A E D L L S O Y E N G S S T V V E A K T S E A R P F N D S T H A E P L N
Xl TopBP1 459 A I N L T A N K M S S T R G P L N H T R N H Q A E D L L S O Y T E N N S T L I E D E H P K T S N T N S I S Q M S M H E

Sc Dpb11 298 S K T N F S V L G Q S P L S I N N K Q E D L S D -- 321
Sp Cut5 273 L L D D F T P E T V Q P L E E N E L D T E L N I E 297
Hs TopBP1 523 D S T H I S L Q E E N Q S S V S H C V P D V S T I T 547
Xl TopBP1 519 D M T T C T S Q S G L A D T S T I I ----- 536

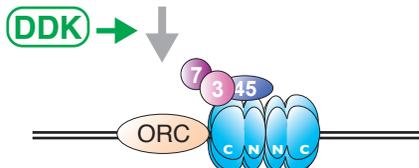
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Supplementary Figure S4. Multiple sequence alignments of inter-BRCT region of Dpb11 orthologs. Multiple sequence alignments of budding yeast Dpb11 (Sc Dpb11, aa 238–321), fission yeast Cut5 (Sp Cut5, aa 213–297), human TopBP1 (Hs TopBP1, aa 284–548), and *Xenopus* TopBP1 (Xl TopBP1, aa 283–536). BRCT3 of Hs TopBP1 and Xl TopBP1 is shown as a blue box. The same and similar amino acid residues are shaded in black and grey, respectively. ClustalW was used for the alignment using a Gap Open value larger than that of the default.

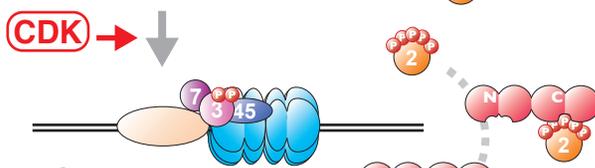
1. Assembly of Pre-RC



2. DDK dependent Sld3-Sld7-Cdc45 association

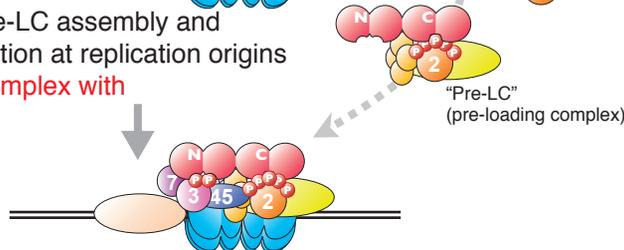


3. CDK dependent Sld2, Sld3 phosphorylation

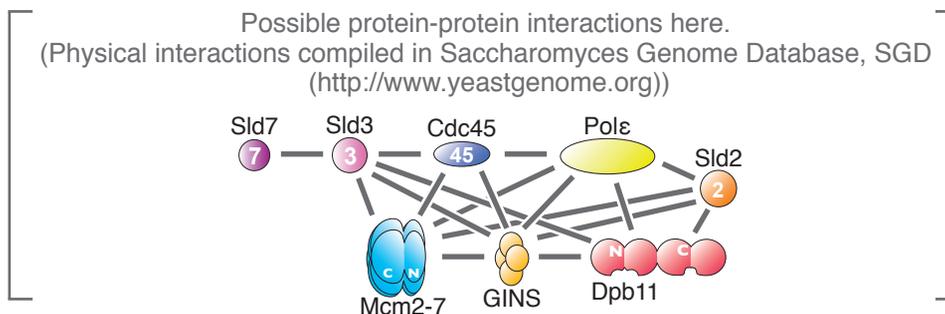
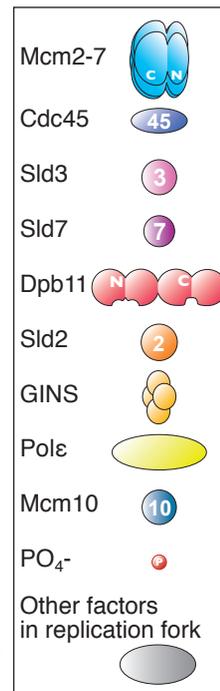
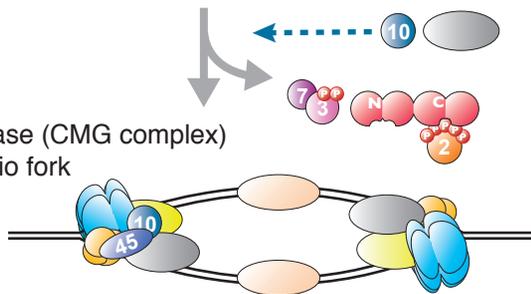


4. Phosphorylation dependent pre-LC assembly and further initiation complex formation at replication origins

Fragile pre-LC and/or initiation complex with Dpb11-AAAs mutant protein



5. Formation of active helicase (CMG complex) and bi-directional replication fork



Supplementary Figure S5. Schematic drawings of the initiation of DNA replication. Dpb11-AAA mutants are likely to have defects at Step 4. See the text for details.