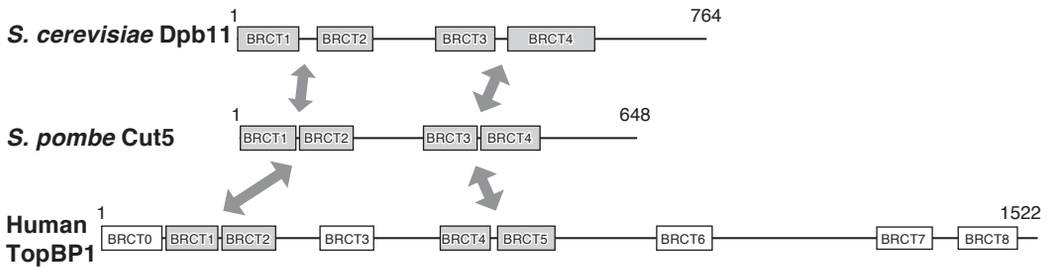
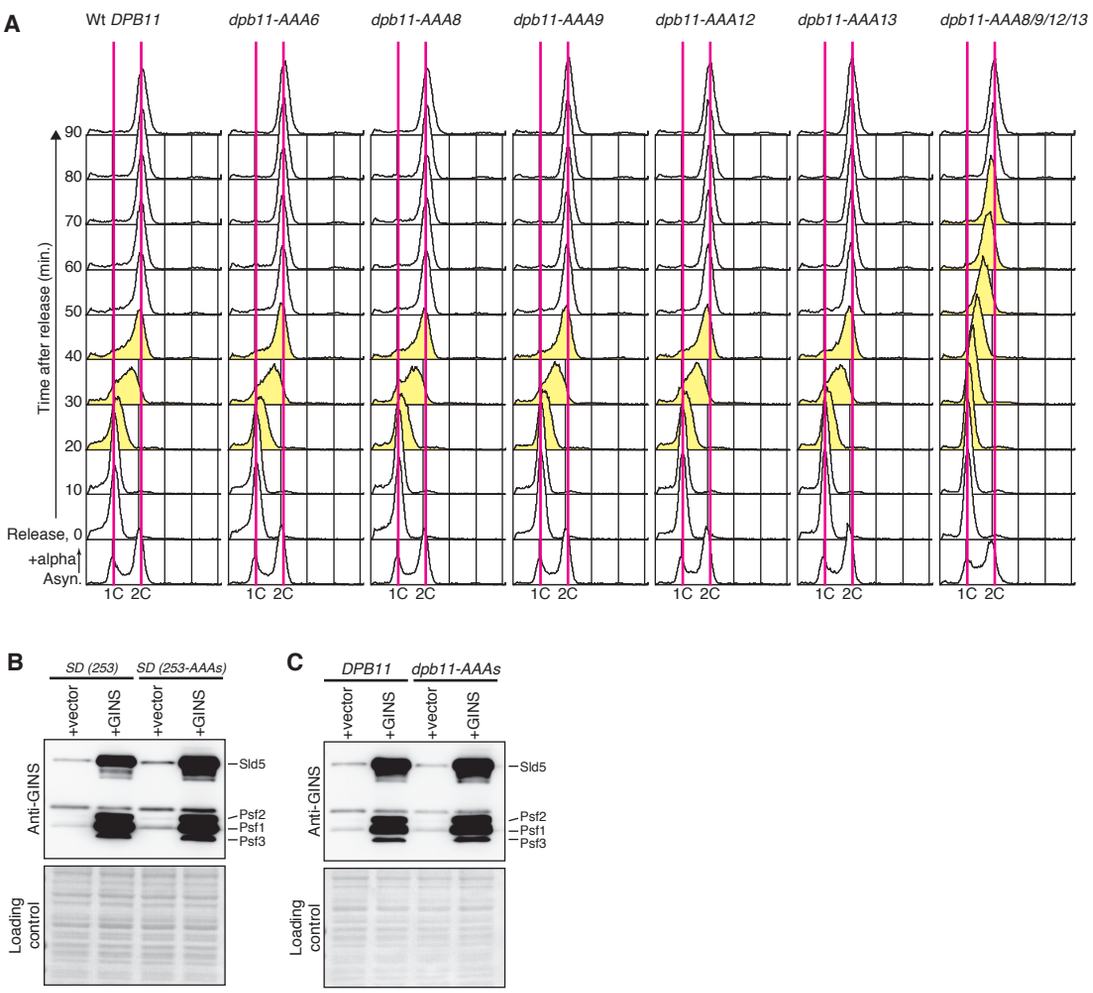
Tanaka et al._Supplementary Figure S1

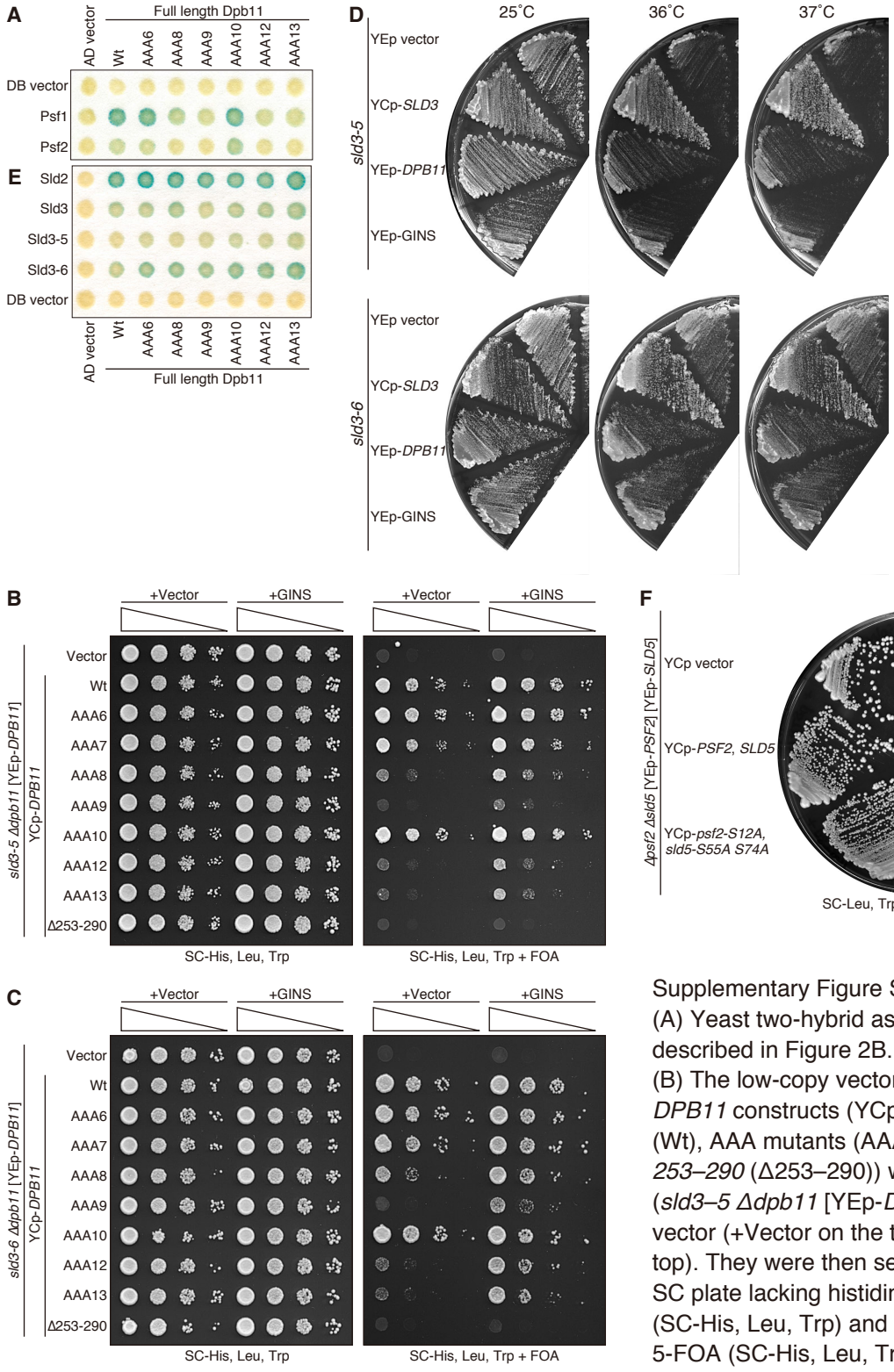


Supplementary Figure S1
Schematic drawings of Dpb11/TopBP1 orthologs. BRCT domains are shown as boxes. BRCTs with high similarity are shown as gray boxes.

Tanaka et al._Supplementary Figure S3



Supplementary Figure S3. Analysis of alanine-substitution mutations in the inter-BRCT region of Dpb11.
(A) W303-1a Δbar1 (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.

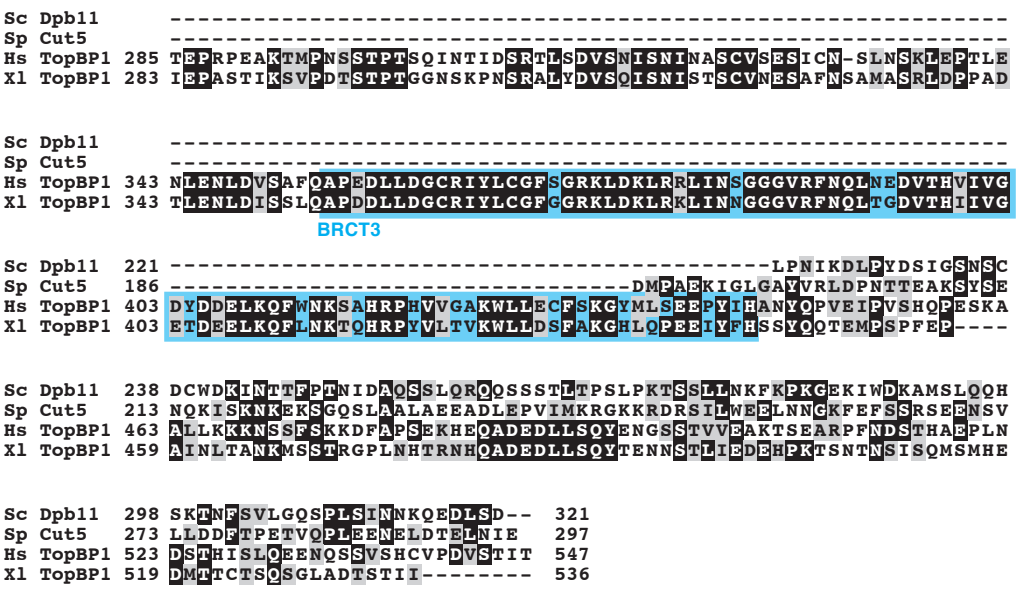
(B) The low-copy vector (Vector) and low-copy *DPB11* constructs (YCp-*DPB11*: wild-type *DPB11* (Wt), AAA mutants (AAA6–AAA12), and *dpb11Δ 253–290* (Δ253–290)) were introduced into YST1908 (*sld3–5 Δdpb11* [YEp-*DPB11*]) harboring a multicopy vector (+Vector on the top) or GINS (+GINS on the top). 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 (YCp-*DPB11*: Wt *DPB11* (Wt), AAA mutants (AAA6–AAA12), and *dpb11Δ253–290* (Δ253–290)) were introduced into YST1904 (*sld3–6 Δdpb11* [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* (YCp-*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.

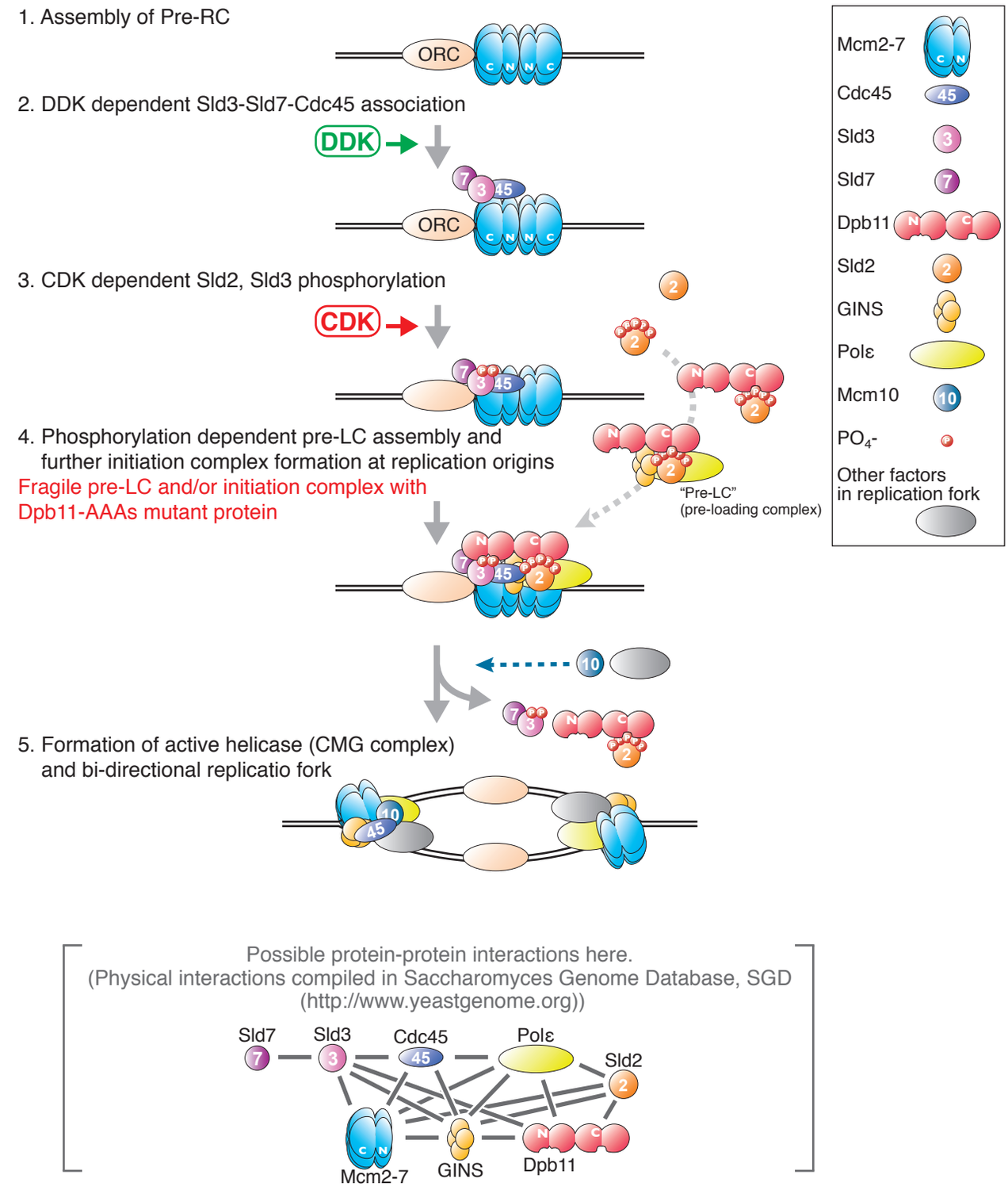
(E) Yeast two-hybrid assay was performed as described in Figure 2B.

(F) The low-copy vector (YCp vector) and low-copy *PSF2* and *SLD5* constructs (wild-type *PSF2* and *SLD5* (YCp-*PSF2*, *SLD5*) and CDK phosphorylation-site mutant of *PSF2* and *SLD5* (YCp-*psf2-S12A*, *sld5-S55A S74A*)) were introduced into YST1296 (*Δpsf2 Δsld5* [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.



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.

Tanaka et al._Supplementary Figure S5



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.