Expanded View Figures

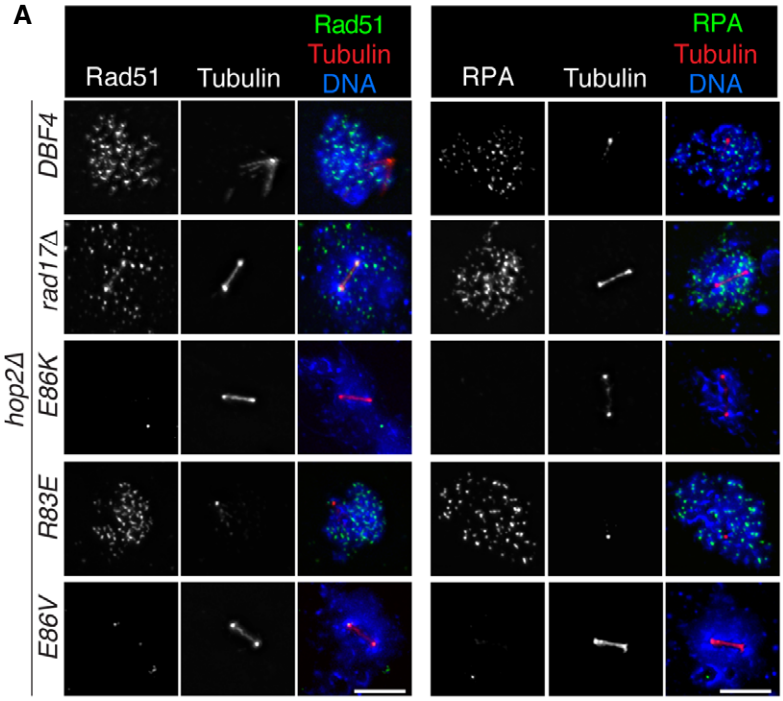

Figure EV1. Rad51-dependent DSB repair occurs before metaphase I.

A *hop2*Δ strains in the BR1919 background were transferred to sporulation media. At 22 h (*hop2*Δ *rad17*Δ) or 30 h (all other strains), cells were harvested and meiotic chromosomes were spread for immunofluorescence microscopy as in Fig 4B. Scale bar, 5 μm.

B Quantification of the results in (A). The percentage of nuclei with metaphase spindle was determined for each strain; this represents cells that have exit pachytene and progressed into metaphase I. From nuclei with metaphase spindles, the percentage that are positive for DNA damage markers was determined; this represents cells that have progressed into metaphase I with unrepaired DSBs (e.g., the *hop2*Δ *rad17*Δ strain, in which the DNA damage checkpoint gene *RAD17* has been deleted). NA, not applicable. At least 150 nuclei were scored for each strain.

B

Genotype	% with metaphase spindle	% with metaphase spindle and damage marker
Rad51 staining		
*hop2*Δ *DBF4*	0.0	NA
*hop2*Δ *rad17*Δ	20.0	100
*hop2*Δ *dbf4-E86K*	14.7	0
*hop2*Δ *dbf4-R83E*	0.0	NA
*hop2*Δ *dbf4-E86V*	15.3	0
RPA staining		
*hop2*Δ *DBF4*	0.0	NA
*hop2*Δ *rad17*Δ	23.3	100
*hop2*Δ *dbf4-E86K*	14.0	0
*hop2*Δ *dbf4-R83E*	0.0	NA
*hop2*Δ *dbf4-E86V*	12.0	0

Figure EV2. The Rad51-dependent DSB repair facilitated by Dbf4-Cdc5 does not efficiently produce interhomolog crossovers.

A Cells were incubated for 48 h on sporulation plates, and tetrads were dissected. After 3 days at 30°C, the number of colonies was counted and expressed as a percentage of spore viability. ND, not determined due to very low levels of sporulation; the spore viability of the *dmc1*Δ mutant was previously shown to be < 1% (Tsubouchi & Roeder, 2006).

B A schematic of the *HIS4-LEU2* recombination hotspot that was utilized to monitor crossover formation. The site where DSBs are formed and the probe for Southern blotting are shown. Note the XhoI polymorphisms between parental chromosomes. rec, recombinant.

C Strains were induced to synchronously enter meiosis. Cells were harvested at the indicated time points. Genomic DNA was extracted and digested with XhoI. Resultant DNA molecules were separated by gel electrophoresis and detected by Southern blotting using the probe shown in (B). rec, recombinant.

D Quantification of the results in (C). The percentage of total DNA corresponding to recombinant DNA molecules was plotted. Data are represented as means from two experiments.

Source data are available online for this figure.

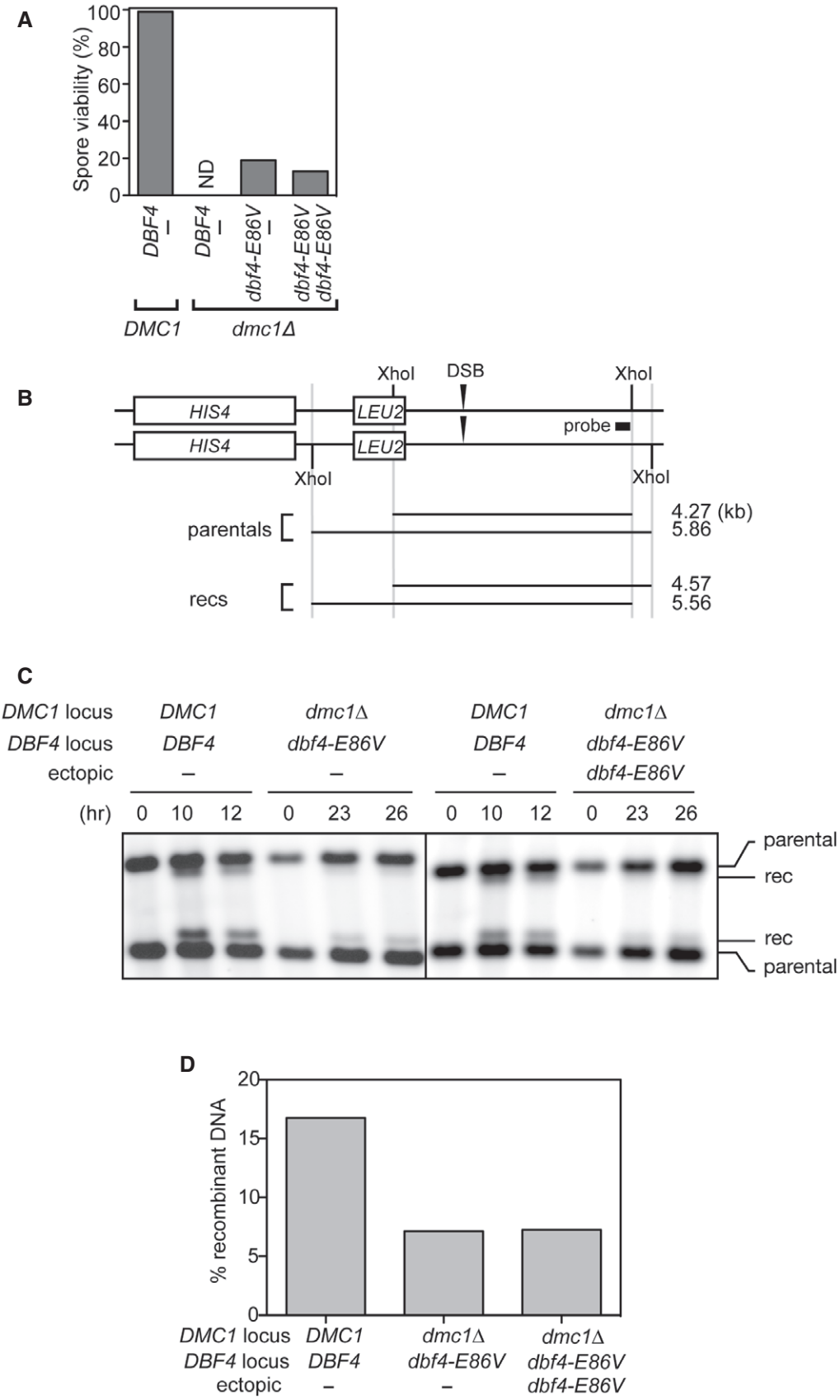

Figure EV2.

Figure EV3. Production of Cdc5 leads to downregulation of DSB formation.

A Strains were induced to synchronously enter meiosis. At 6 h, the *dbf4-E86V* culture was split and either carrier (− Cdc5) or β-estradiol (+ Cdc5) was added. The *dbf4-E86K* culture only received carrier. Cells were harvested at the indicated time points, and proteins were detected as in Fig 1B.

B *ndt80Δ* strains in the BR1919 background were transferred to sporulation media. At 20 h, β-estradiol was added to induce Cdc5 production. Cells were harvested at 2-h intervals after addition of β-estradiol, and proteins were detected as in Fig 1B. Cells are from the same culture as Fig 4B and C.

C Strains were induced to synchronously enter meiosis. Cells were harvested at the indicated time points, and proteins were detected as in Fig 1B. Total, total protein levels (Ponceau S staining).

D Cells were induced to synchronously enter meiosis. At 6 h, the culture was split and either carrier (− Cdc5 induction) or β-estradiol (+ Cdc5 induction) was added. Cells were harvested at the indicated time points, and meiotic chromosomes were monitored as in Fig 3A. Data are represented as the results of an individual experiment (see Fig 5B for the duplicate experiment).

E, F Cells were induced to synchronously enter meiosis. At 3.5 h, the culture was split and either carrier (− Cdc5 induction) or β-estradiol (+ Cdc5 induction) was added. Cells were harvested at the indicated time points to detect proteins as in Fig 1B (E) or monitor meiotic chromosomes as in Fig 3A (F). Cells are from the same culture. Data are represented as mean ± SEM from three experiments (*$P < 0.05$, paired *t*-test).

Source data are available online for this figure.

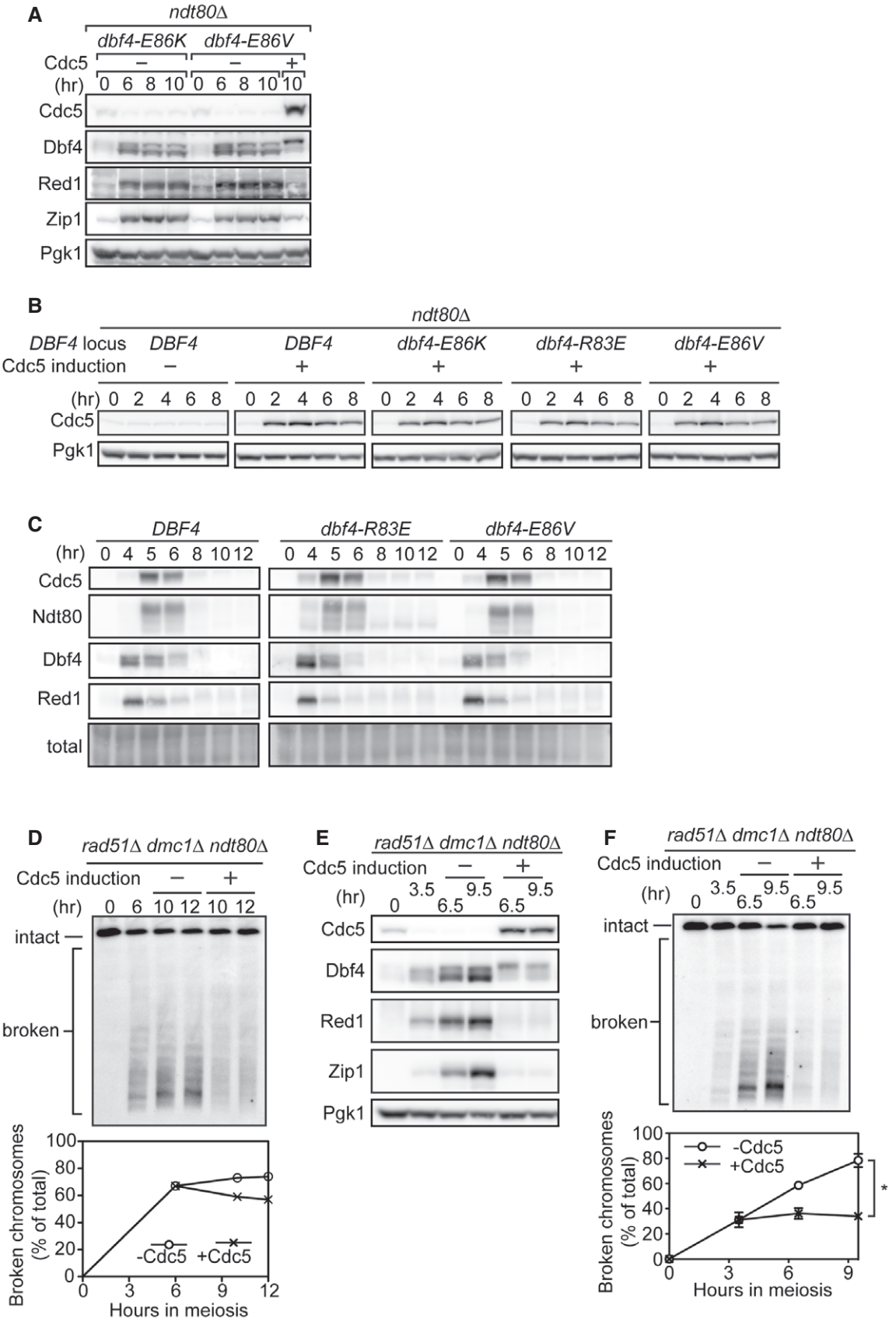

Figure EV3.

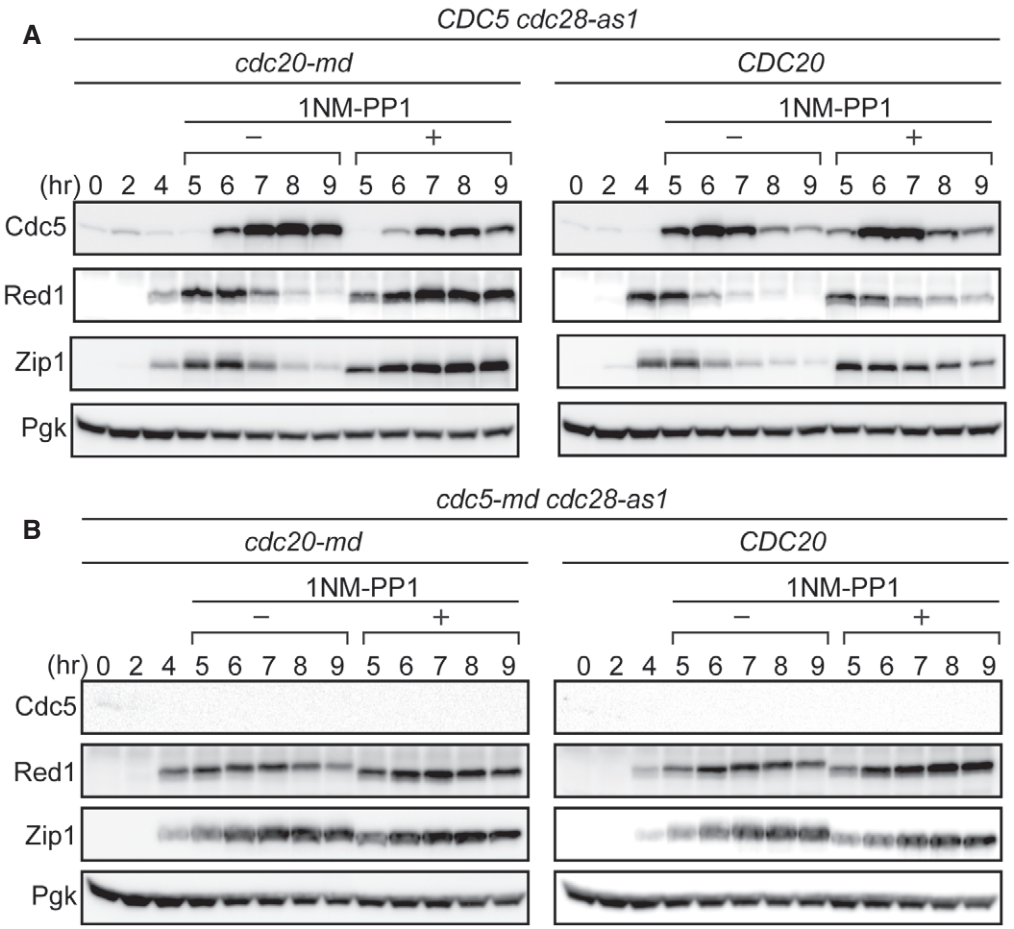

Figure EV4. The requirement for CDK1 in SC destruction is conditional.

A, B Strains were induced to synchronously enter meiosis. At 4 h, cultures were split and either carrier or 1NM-PP1 (an ATP analog that specifically inhibits Cdc28-as1), was added (denoted "–" and "+" under 1NM-PP1, respectively). Cells were harvested at the indicated time points, and proteins were detected as in Fig 1B.

Source data are available online for this figure.

Figure EV5. Conditional depletion of DDK before pachytene exit results in defective SC morphology.

A Single colonies from the indicated strains were streaked on rich media with or without rapamycin. Since *DBF4* and *CDC7* are both essential genes, the observed growth defect in the presence of rapamycin indicates conditional inactivation of DDK.

B The *ndt80Δ DBF4-FRB* strain was induced to synchronously enter meiosis. At 6 h, either carrier (control) or rapamycin (rapamycin) was added. Cells were harvested 2 and 4 h after drug treatment, and chromosomes were spread as in Fig 4B. To quantify these results, nuclei were scored for the presence of fully established SC, as judged by Zip staining, and the presence of Red1 staining (for representative images, see control nuclei). Nuclei in white boxes are expanded to the right. White arrowheads depict polycomplexes, which were larger than usual because of prolonged cell cycle arrest. At least 100 nuclei were scored in the presence and absence of rapamycin at each time point. Data are represented as means from two experiments. Scale bar, 5 μm.

C, D Strains were induced to synchronously enter meiosis. At 6 h, β-estradiol was added to induce production of Cdc5. At the indicated time points, cells were harvested for detection of proteins as in Fig 1B (C). At the 7 and 8 h time points, cells were also harvested for immunoprecipitation with an anti-Dbf4 antibody (D). 5× *4A* denotes that fivefold more *dbf4-4A* sample was loaded than *DBF4*. "–" and "+" indicate the exclusion and inclusion of antibody for IP, respectively, with the no antibody condition serving as a negative control. WCE, whole-cell extract. Sol., soluble fraction.

E, F Strains were induced to synchronously enter meiosis. At 6 h, β-estradiol was added to induce production of Cdc5. Cells were harvested at the indicated time points, and proteins were detected as in Fig 1B.

Source data are available online for this figure.

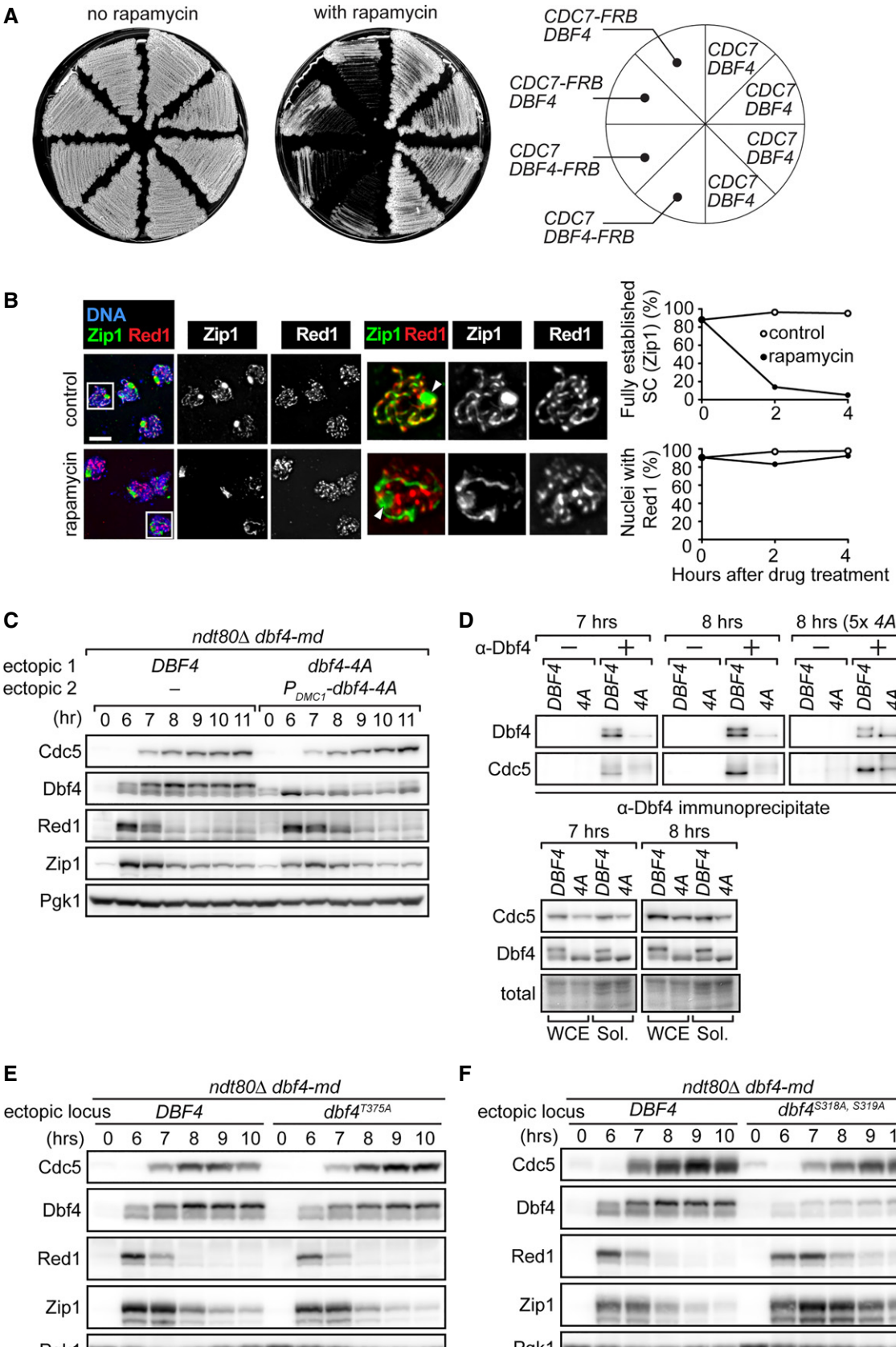

Figure EV5.