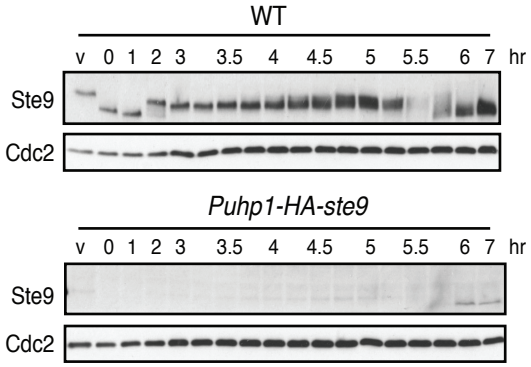


Figure S1. Regulation of Ste9 in wild type and ste9-shut off diploid cells during meiosis

Meiosis was induced from G1 phase using *pat1-114* mutation in wild type and *Puhp1-HA-ste9* diploid strains. Ste9 and Cdc2 were analyzed by immunoblotting using anti-Ste9 and Cdc2 antibodies. Ste9 is mostly phosphorylated in the vegetatively growing phase (indicated as v). Ste9 was dephosphorylated in G1 caused by nitrogen starvation (0 hr). Following this Ste9 became highly phosphorylated at 2 hrs after meiosis induction and remained phosphorylated until the end of meiosis. In *Puhp1-HA-ste9* strain, Ste9 protein levels are almost undetectable until 6 hrs after meiotic induction.

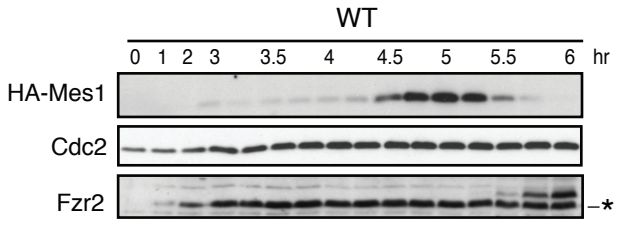


Figure S2. Expression of Fzr2 in meiosis.

Samples from *pat1*-induced synchronized meiosis were analyzed by immunoblotting using anti-HA, anti-Cdc2 and anti-Fzr2 antibodies. Asterisk indicates a non-specific crossreacting band.

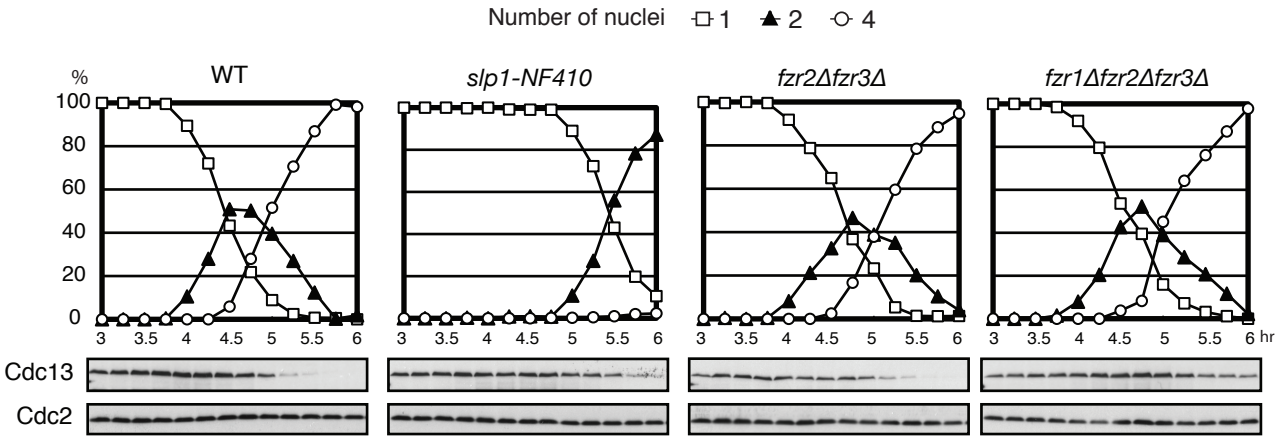


Figure S3. Synchronized meiosis in WT, *slp1-NF410*, *fzr2*Δ*fzr3*Δ and *fzr1*Δ*fzr2*Δ*fzr3*Δ diploid cells.

pat1-114-induced synchronization of meiosis was performed in WT, *slp1-NF410, fzr2*Δ*fzr3*Δ and *fzr1*Δ*fzr2*Δ*fzr3*Δ mutant diploid strains. Meiotic progression was examined microscopically for the number of nuclei in a cell. Protein levels were analyzed by immunoblotting using specific antibodies at indicated time points.

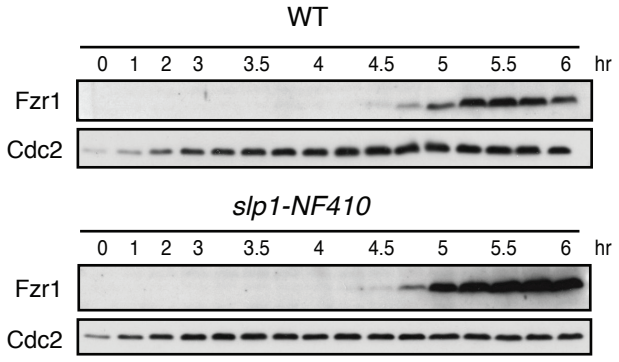


Figure S4. Fzr1/Mfr1 protein levels in synchronized meiosis in wild type and *slp1-NF410* cells.

Samples from *pat1-114*-induced synchronized meiosis in wild type (WT) and *slp1-NF410* were analyzed by immunoblotting using anti-Fzr1 and anti-Cdc2 antibodies. Fzr1/Mfr1 expression started around 4.5 hrs and continued till the end of meiosis. Fzr1/Mfr1 was more accumulated in *slp1-NF410* mutant cells than WT.

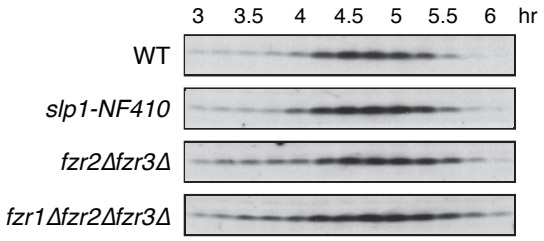


Figure S5. Mes1 levels in *fzr2*Δ*fzr3*Δ and *fzr1*Δ*fzr2*Δ*fzr3*Δ mutant cells.

pat1-114-induced synchronization of meiosis was performed in WT, *slp1-NF410, fzr2*Δ*fzr3*Δ and *fzr1*Δ*fzr2*Δ*fzr3*Δ mutant diploid strains expressing HA-tagged Mes1 under the native promoter. HA-Mes1 levels were analyzed by immunoblotting using anti-HA antibody.

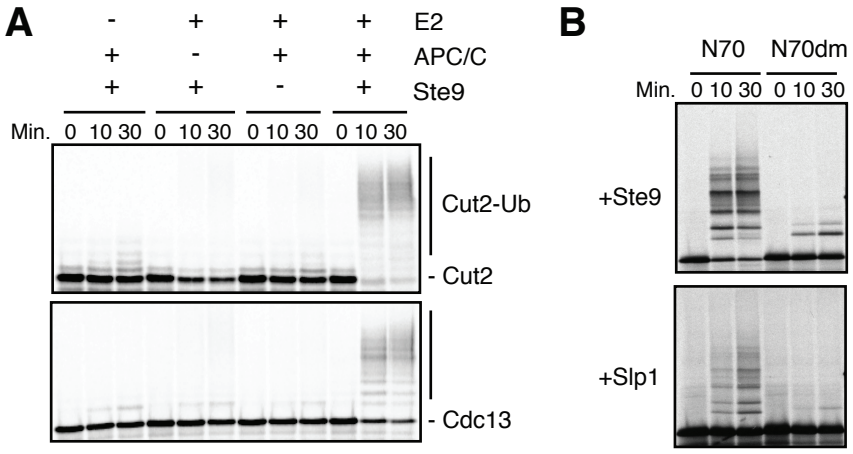
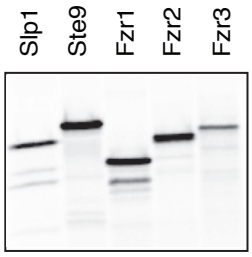


Figure S6. ***In vitro*** **ubiquitylation assay with** ***S. pombe*** **APC/C**

(A) *S. pombe* APC/C purified from *lid1*$^{+}$*-TAP* strain was used for in vitro ubiquitylation assay with Ste9 and *S. pombe* E2s. Efficient ubiquitylation of Cut2 and Cdc13 was observed only when APC/C, E2s and Ste9 were all present. (B) Destruction box-dependency of the reconstituted ubiquitylation reaction. In vitro ubiquitylation was performed using ^{35}S-labeled wild type or D-box mutant of N-terminal 70 residues of Cdc13 (N70 or N70dm, respectively) as subtrates. Both Slp1 and Ste9 induced ubiquitylation of N70 but not N70dm.



Band intensities	1	3.57	2.70	2.45	0.46
No. of Met	4	12	9	7	10
Relative levels	1	1.19	1.20	1.40	0.18

Figure S7. ***In vitro translation of S. pombe APC/C co-activators***

Slp1, Ste9, Fzr1/Mfr1, Fzr2 and Fzr3 were prepared in coupled transcription/translation in reticulocyte lysates in the presence of ^{35}S-methionine (TNT; Promega). Band intensities quantified are indicated at the bottom. Knowing the number of methionine residues in the co-activators allowed estimation of the relative levels of each. To prepare co-activators for cell-free destruction and ubiquitylation assay in this paper, cold methionine (0.02mM) was used instead of ^{35}S-methionine.

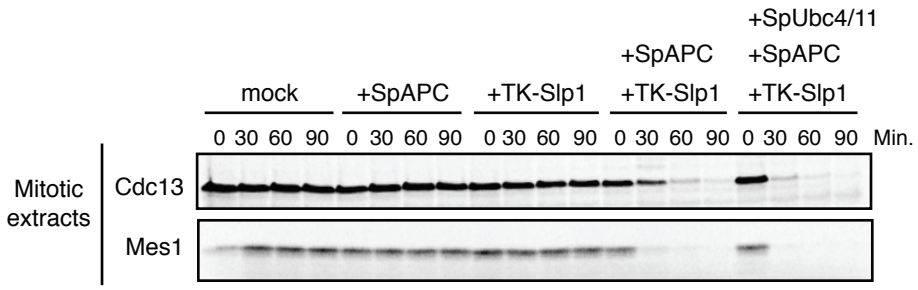


Figure S8. *S. pombe* APC/C-dependent destruction assay in *Xenopus* egg extracts.

Endogenous APC/C and Fizzy/Cdc20 were depleted from *Xenopus* CSF extracts using anti-Apc3 and anti-Fizzy antibodies. Purified *S. pombe* APC/C and in vitro translated Slp1 as well as recombinant *S. pombe* E1 (Uba1) and E2s (Ubc4 and Ubc11) were supplemented into the extracts and destruction assays were performed with ^{35}S-labelled Cdc13 or Mes1 as a substrate. Cdc13 and Mes1 were destroyed in a *S. pombe* APC/C and Slp1-dependent manner. Addition of Ubc4 and Ubc11 enhanced the destruction.

Table S1. Strain List

Strains	Genotype	Source
HYY718	*h-/h- pat1-114/pat1-114 mes1::kanMX/mes1::kanMX slp1-NF410::kanMX/slp1-NF410::kanMX ade6-M210/ade6-M216 ura4-d18/ura4-d18*	This study
HYY719	*h-/h- pat1-114/pat1-114 mes1::kanMX/mes1::kanMX fzr1::hphMX/fzr1::hphMX ade6-M210/ade6-M216 ura4-d18/ura4-d18*	This study
HYY729	*h-/h- pat1-114/pat1-114 mes1WT::ura4+::kanMX/mes1WT::ura4+::kanMX ade6-M210/ade6-M216 ura4-d18/ura4-d18*	Our stock
HYY733	*h-/h- pat1-114/pat1-114 mes1::Pint-3HA-ura4+-kanMX/mes1::Pint-3HA-ura4+-kanMX ade6-M210/ade6-M216 ura4-d18/ura4-d18*	Our stock
HYY734	*h-/h- pat1-114/pat1-114 HA-mes1WT::ura4+::kanMX/HA-mes1WT::ura4+::kanMX ade6-M210/ade6-M216 ura4-d18/ura4-d18*	Our stock
HYY738	*h-/h- pat1-114/pat1-114 HA-K0mes1::ura4+::kanMX/HA-K0mes1::ura4+::kanMX ade6-M210/ade6-M216 ura4-d18/ura4-d18*	Our stock
HYY742	*h-/h- pat1-114/pat1-114 HA-mes1WT::ura4+::kanMX/HA-mes1WT::ura4+::kanMX ade6-M210/ade6-M216 ura4-d18/ura4-d18 slp1-NF410::kanMX/slp1-NF410::kanMX*	This study
HYY743	*h-/h- pat1-114/pat1-114 HA-mes1WT::ura4+::kanMX/HA-mes1WT::ura4+::kanMX ade6-M210/ade6-M216 ura4-d18/ura4-d18 fzr1::hphMX/fzr1::hphMX*	This study
HYY744	*h-/h- pat1-114/pat1-114 HA-mes1WT::ura4+::kanMX/HA-mes1WT::ura4+::kanMX ade6-M210/ade6-M216 ura4-d18/ura4-d18 slp1-NF410/slp1-NF410 fzr1::hphMX/fzr1::hphMX*	This study
HYY745	*h-/h- pat1-114/pat1-114 HA-mes1WT::ura4+::kanMX/HA-mes1WT::ura4+::kanMX ade6-M210/ade6-M216 ura4-d18/ura4-d18 fzr2::ura4+/fzr2::ura4+*	This study
HYY793	*h-/h- pat1-114/pat1-114 HA-mes1WT::ura4+::kanMX/HA-mes1WT::ura4+::kanMX ade6-M210/ade6-M216 ura4-d18/ura4-d18 Puhp1-HA-ste9::hphMX/Puhp1-HA-ste9::hphMX*	This study
HYY790	*h-/h- pat1-114/pat1-114 HA-mes1WT::ura4+::kanMX/HA-mes1WT::ura4+::kanMX ade6-M210/ade6-M216 ura4-d18/ura4-d18 fzr3::kanMX/fzr3::kanMX*	This study
HYY1026	*h-/h- pat1-114/pat1-114 HA-mes1WT::ura4+::natMX/HA-mes1WT::ura4+::natMX ade6-M210/ade6-M216 ura4-d18/ura4-d18 fzr2Δ::ura4+/ fzr2Δ::ura4+ fzr3::kanMX/fzr3::kanMX*	This study
HYY1027	*h-/h- pat1-114/pat1-114 HA-mes1WT::ura4+::natMX/HA-mes1WT::ura4+::natMX ade6-M210/ade6-M216 ura4-d18/ura4-d18 fzr1Δ::hphMX/ fzr1Δ::hphMX fzr2Δ::ura4+/ fzr2Δ::ura4+ fzr3::kanMX/fzr3::kanMX*	This study
KGY2050	*h- lid1-TAP::KanMX mts3-1 mad2::ura4+ mad3::ura4+*	K. Gould