Supplementary Information

Cuf2 boosts the transcription of APC/C activator Fzr1 to terminate the meiotic division cycle

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Supplementary Methods

Manipulation of Yeast Strains and Plasmids

We constructed strains listed in **Table S1** by conventional genetic crosses and standard PCR-based gene-targeting methods [1-3]. The plasmid pFA6a–5FLAG–kanMX [4] was used to construct the *cuf2–5FLAG* strain. The plasmid to construct GFP–Psy1 strain was a gift from Taro Nakamura [5]. To identify the *sms1* and *sms5* genes, 20 μg of genomic DNA was prepared from mutant cells using zymolyase (Nacalai tesque) and Genomic-tip (QIAGEN). Whole-genome sequencing was performed on the GAIIx system (Illumina) in a 75-base paired-end run. Sequence data was mapped to the reference genome of *S. pombe* (accession numbers : CU329670-2 and X54421) using BWA. For ectopic expression of Fzr1 during meiosis, the plasmid pREP1 was modified as follows. The *nmt1* promoter of pREP1 was replaced with the DNA fragment containing the native promoter (a 1-kb region) and the coding region of the *fzr1* gene fused with the *mei4*–DSR (determinant of selective removal) sequence to suppress mitotic expression. Sporulation agar (SPA) was used for induction of mating, meiosis and sporulation of homothallic h^{90} cells.

Western Blotting

Cultured cells were lysed in HB buffer to prepare whole-cell extracts. The following antibodies

were used for immunoblotting: anti-Fzr1 HY81 (1:2,000; a gift from H. Yamano) [6], anti-Cdc13 6F11/2 (1:2,000; Abcam), anti-GFP (1:1,000; Roche), and anti-Cdk1/Cdc2 PSTAIR (1:2,000; Millipore).

Quantitative RT–PCR

Total RNA was prepared with hot acidic phenol and treated with Turbo DNA-free (Ambion). Reverse transcription (RT) was performed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCR was carried out using Power SYBR Green on the Real-Time PCR 7300 system (Applied Biosystems). Primers used in this study are listed in **Table S2**.

Chromatin Immunoprecipitation

Cells at meiosis I/meiosis II transition were collected using *pat1-114*-driven synchronized meiosis at 3.5 h after temperature shift to 34°C. Extract preparation and immunoprecipitation were carried out as described previously [7]. Anti-Flag M2 (Sigma-Aldrich) and control IgG antibodies were used for immunoprecipitation. DNA in whole-cell extracts or immunoprecipitates was amplified and analyzed with the quantitative PCR method as described above. Primers used for amplification are listed in **Table S2**.

Supplementary Tables

Table S1. Frequency of nuclei that generated ectopic spindles after meiosis II.

Polarity of ectopic spindles assembled after meiosis II	WT	*fzr1*Δ
Bipolar spindles*		
with chromosome segregation	0/44 (0)	10/56 (18)
without chromosome segregation	0/44 (0)	6/56 (11)
Monopolar spindles		
with SPB duplication	0/44 (0)	14/56 (25)
without SPB duplication	0/44 (0)	26/56 (46)

The frequency of nuclei that ectopically generated bipolar or monopolar spindles after meiosis II was calculated in wild-type (WT) and *fzr1*Δ cells based on the behavior of GFP–Atb2 (microtubules), Sid4–2ECFP (SPB), and Mis6–2mRFP (kinetochore) as in **Figs. 2E,F** and **S1B,C**. Some cells also showed abnormal chromosome segregation. Note that V-shape spindles as in the *cut7* mutant were observed in *fzr1*Δ cells (13/56 (23)). The percentages are shown in brackets.

***** All SPBs in nuclei that formed the bipolar spindle were duplicated and separated.

Table S2. Strains used in this study

No.	Genotype	Figures
JY476	*h^{90} leu1 ade6-M210*	1B,C
JZ51	*h^{90} mes1-B44 leu1 ade6-M210*	1B,C
YA304	*h^{90} sms5-1 nat–mes1-B44 leu1 ade6-M210*	1B,C
YA139	*h^{90} sms1-23 nat–mes1-B44 leu1 ade6-M210*	1C
JY450	*h^{90} leu1 ade6-M216*	2A
YA1293	*h^{90} cuf2::ura4$^+$ leu1 ura4 ade6-M216*	2A
KA162	*h^{90} fzr1::ura4$^+$ leu1 ura4 ade6-M210*	2A
YA1310	*h^{90} cuf2::ura4$^+$ fzr1::bsd leu1 ura4 ade6-M216*	2A
YA1984	*h^{90} Z2–GFP–atb2–kan sfi1–mCherry–hph htb1–CFP–nat leu1 ade6-M216*	2B,D
YA1991	*h^{90} cuf2::ura4$^+$ Z2–GFP–atb2–kan sfi1–mCherry–hph htb1–CFP–nat leu1 ura4 ade6-M216*	2C,D
YA1987	*h^{90} fzr1::ura4$^+$ Z2–GFP–atb2–kan sfi1–mCherry–hph htb1–CFP–nat leu1 ura4 ade6-M210 or M216*	2D, S1A
YA2142	*h^{90} Z2–GFP–atb2–kan sid4–2ECFP–nat mis6–2mRFP–hph leu1 ura4 ade6-M210 or M216*	2E
YA2125	*h^{90} fzr1::ura4$^+$ Z2–GFP–atb2–kan sid4–2ECFP–nat mis6–2mRFP–hph leu1 ura4 ade6-M210 or M216*	2F, S1B,C
MO91	*h$^-$/h$^-$ pat1-114/pat1-114 lys1-131::mat-Pc–lys1$^+$/lys1$^+$ leu1/leu1 ade6-M210/ade6-M216*	3A,B
YA1676	*h$^-$/h$^-$ cuf2::kan/cuf2::kan pat1-114/pat1-114 lys1-131::mat-Pc–lys1$^+$/lys1$^+$ leu1/leu1 ade6-M210/ade6-M216*	3A,B
YA2003	*h$^-$/h$^-$ cuf2–5FLAG–kan/cuf2$^+$ pat1-114/pat1-114 lys1-131::mat-Pc–lys1$^+$/lys1$^+$ leu1/leu1 ade6-M210/ade6-M216*	3C
YA2007	*h$^-$/h$^-$ cuf2(R19Q)–5FLAG–kan/cuf2$^+$ pat1-114/pat1-114 lys1-131::mat-Pc–lys1$^+$/lys1$^+$ leu1/leu1 ade6-M210/ade6-M216*	3C
YA126	*h^{90} mes1::ura4$^+$ leu1 ura4 ade6-M216*	3D
YA1303	*h^{90} cuf2::ura4$^+$ mes1::bsd leu1 ura4 ade6-M216*	3D
YA2103	*h$^-$/h$^-$ cuf2–GFP–kan/cuf2$^+$ pat1-114/pat1-114 mes1–3HA–nat/mes1$^+$ lys1-131::mat-Pc–lys1$^+$/lys1$^+$ leu1/leu1 ade6-M210/ade6-M216*	4A
YA2105	*h$^-$/h$^-$ cuf2::kan/cuf2::kan pat1-114/pat1-114 mes1–3HA–nat/mes1$^+$ lys1-131::mat-Pc–lys1$^+$/lys1$^+$ leu1/leu1 ade6-M210/ade6-M216*	4B
YA1705	*h^{90} cdc13–YFP–Tcdc13–kan Z2–CFP–atb2–nat cut11–3mRFP–hph leu1 ura4 ade6-M216*	4C
YA1709	*h^{90} cuf2::ura4$^+$ cdc13–YFP–Tcdc13–kan Z2–CFP–atb2–nat cut11–3mRFP–hph leu1 ura4 ade6-M216*	4C
YA1707	*h^{90} fzr1::ura4$^+$ cdc13–YFP–Tcdc13–kan Z2–CFP–atb2–nat cut11–3mRFP–hph leu1 ura4 ade6-M216*	4C
YA1841	*h^{90} cut2–GFP–kan Z2–CFP–atb2–nat cut11–3mRFP–hph leu1 ura4 ade6-M216*	4D

YA2131	*h^{90} fzr1::ura4^{+} cut2–GFP–kan Z2–CFP–atb2–nat cut11–3mRFP–hph leu1 ura4 ade6-M216*	4E
TM266	*h^{90} GFP–atb2–kan sfi1–CFP–nat leu1-32::GFP–psy1–leu1^{+} ura4 ade6-M210 or M216*	S2A
YA2139	*h^{90} cuf2::ura4^{+} GFP–atb2–kan sfi1–CFP–nat leu1-32::GFP–psy1–leu1^{+} ura4 ade6-M210 or M216*	S2B
YA2137	*h^{90} fzr1::ura4^{+} GFP–atb2–kan sfi1–CFP–nat leu1-32::GFP–psy1–leu1^{+} ura4 ade6-M210 or M216*	S2C
YA1423	*h^{90} cuf2–GFP–kan Z2–CFP–atb2–nat cut11–3mRFP–hph leu1 ura4 ade6-M216*	S3
YA1434	*h^{90} cuf2(R19Q)–GFP–kan Z2–CFP–atb2–nat cut11–3mRFP–hph leu1 ura4 ade6-M216*	S3

Table S3. Oligos used in quantitative PCR analyses in this study

Name	Sequence (5' to 3')	Figures
For RT-PCR assays:		
fzr1_f	GGCACTATTCAAAGGCAATTCAT	3B
fzr1_r	TCCAACATTTTCTTCTCAATTTTCC	3B
cdc13_f	ACAGTGTGCTGCCGCTATGT	3B
cdc13_r	CCACGGTCCACGTCCAA	3B
mes1_f	CGGTCAAAAAGTCTAAGAAGTCGACTA	3B
mes1_r	CGGATGCGGCATGCA	3B
act1_f	TGAGGAGCACCCTTGCTTGT	3B
act1_r	TCTTCTCACGGTTGGATTTGG	3B
For ChIP assays:		
fzr1_f	ATTTTAGGAGTTCTCTTACCATAACATCAC	3C
fzr1_r	TGCACTCATCAAAACAAACTGTTG	3C
cdc13_f	GCCCAGCATAATTTCATAGACAATAA	3C
cdc13_r	CGGAGATATGGAACCACAATTTAA	3C
mes1_f	TGTTGTATATTCGTCGTCCTTATCAATT	3C
mes1_r	CGGAAAATGATTGAAAAGCTTCA	3C

Supplementary Figures

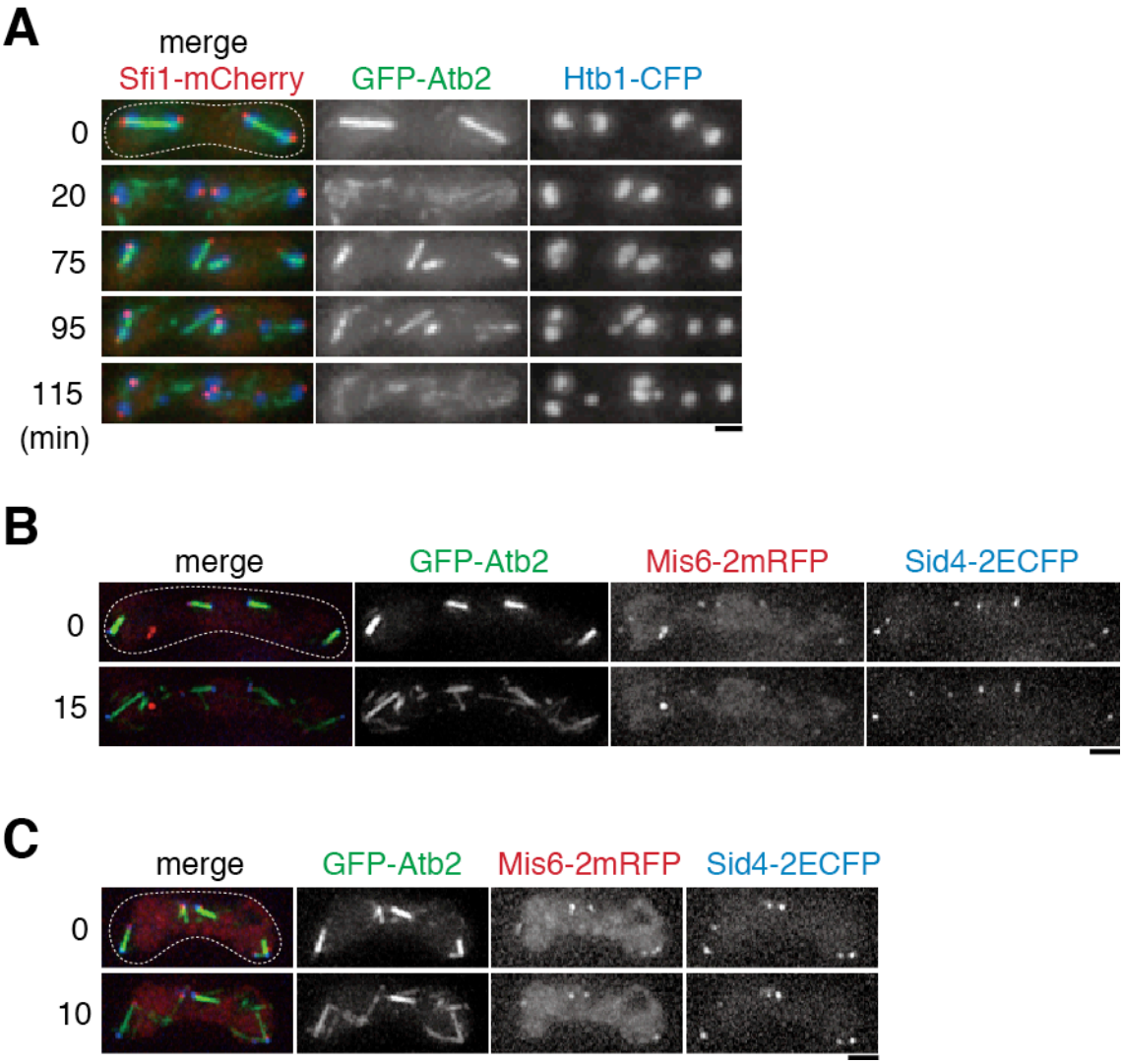

Fig. S1. Abnormal nuclear division, chromosome segregation, and SPB duplication after meiosis II were observed in *fzr1*Δ.

(A) *fzr1*Δ cells expressing GFP–Atb2 (microtubules; green), Htb1–CFP (histone H2B; blue), and Sfi1–mCherry (SPB; red) were filmed from meiosis II. Images for a wild-type cell are shown in **Fig. 2B**. **(B, C)** *fzr1*Δ cells frequently underwent abnormal chromosome segregation (B) and displayed V-shape spindles (C) after meiosis II. Time-lapse imaging of *fzr1*Δ cells expressing GFP–Atb2 (microtubules; green), Mis6–2mRFP (kinetochore; red), and Sid4–2ECFP (SPB; blue) started when ectopic spindles were assembled after meiosis II. Related images are shown in Fig. 2E, F. See also **Table S3** for quantification. Scale bars, 2 μm.

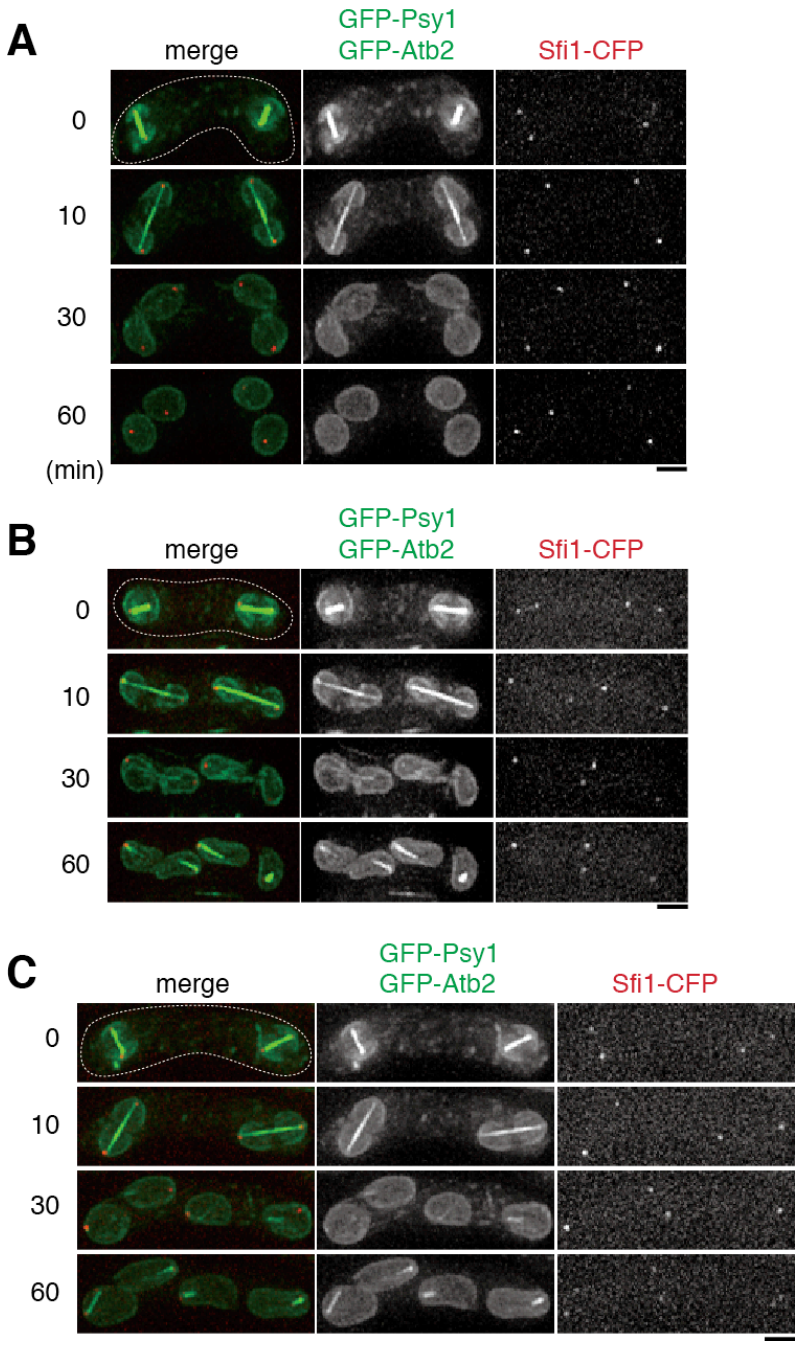

Fig. S2. The ectopic spindle was assembled within a sphere of forespore membrane after meiosis II in *cuf2*Δ and *fzr1*Δ.

(A–C) Time-lapse images of cells expressing GFP–Psy1 (forespore membrane; green), GFP–Atb2 (microtubules; green), and Sfi1–CFP (SPB; red) filmed from meiosis II. **(A)** Wild-type, **(B)** *cuf2*Δ, and **(C)** *fzr1*Δ cells. In *cuf2*Δ and *fzr1*Δ cells, the forespore membrane started to assemble at the early stage of meiosis II as in WT, but the ectopic spindle was reassembled long after meiosis II within a sphere of the forespore membrane. Scale bars, 2 μm.

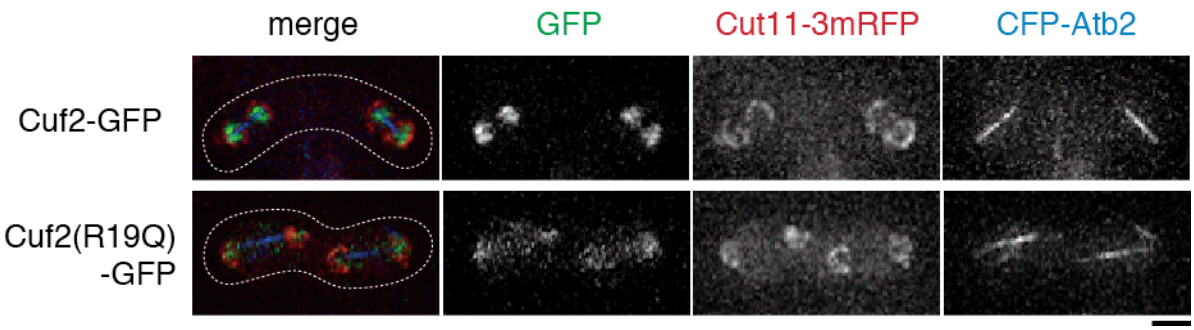

Fig. S3. Cuf2–GFP localizes to the nucleus during meiosis II.

Cells expressing either Cuf2–GFP or Cuf2(R19Q)–GFP and undergoing meiosis II were prepared. GFP (Cuf2–WT or R19Q mutant protein; green), Cut11–3mRFP (nuclear envelope; red) and CFP–Atb2 (microtubules, blue) were detected by fluorescent microscopy. Note that Cuf2–GFP localized to the nucleus in anaphase II (top), whereas Cuf2(R19Q)–GFP dispersed. It was previously shown that the nucleocytoplasmic barrier is invalidated during anaphase II, and it is suggested that proteins bound to the chromatin or spindle tend to remain in the nucleus, whereas those in the nucleoplasm disperse to the cytoplasm (ref. [8, 9]). Scale bar, 2 μm.

Supplementary Movies

Movie S1. Progression of meiosis II in wild-type cells, labeled with GFP–Atb2 (microtubules; green), Htb1–CFP (histone H2B; blue), and Sfi1–mCherry (SPB; red). This movie corresponds to Figure 2B in the main text. Note that the indicted time is different from that shown in the figure.

Movie S2. Progression of meiosis II and meiosis-III like division in *cuf2*Δ cells, labeled with GFP–Atb2 (microtubules; green), Htb1–CFP (histone H2B; blue), and Sfi1–mCherry (SPB; red). This movie corresponds to Figure 2C in the main text.

Movie S3. Behavior of kinetochores during meiosis II in wild-type cells, labeled with GFP-Atb2 (microtubules; green), Mis6-2mRFP (kinetochore; red), and Sid4-2ECFP (SPB; blue). This movie corresponds to Figure 2E in the main text.

Movie S4. Behavior of kinetochores during meiosis II and meiosis-III like division in *fzr1*Δ cells, labeled with GFP-Atb2 (microtubules; green), Mis6-2mRFP (kinetochore; red), and Sid4-2ECFP (SPB; blue). This movie corresponds to Figure 2F in the main text.

Movie S5. Chromosome segregation in meiosis-III like division in *fzr1*Δ cells, labeled with GFP–Atb2 (microtubules; green), Htb1–CFP (histone H2B; blue), and Sfi1–mCherry (SPB; red). Frames were taken every 5 min for 135 min. Note that spindles were assembled ectopically after meiosis II, followed by aberrant chromosome segregation and nuclear division, and were eventually disassembled leaving five nuclei per ascus.

Movie S6. A control movie to Movie S5, showing a wild type cell completing meiosis after meiosis II. Frames were taken every 5 min for 135 min.

References for Supplementary Information

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