Table S1 Sporulation of *syb1-S1* at various temperatures.

	sporulation rate (%)					
	23°C	25°C	28°C	30°C	32°C	34°C
Wild type	100	99.1	99.2	100	98.6	N.T.
syb1-S1	92.3	94.5	90.6	93.0	91.9	N.T.

Wild-type (TY235) and *syb1-S1* (TY234) strains were incubated on MEA at various temperatures. For each sample, >100 cells were counted.

N.T., not tested because the mating was completely inhibited at 34°C.

Table S2 Spore size of *syb1-S1* at various temperatures

	Long axis of spores (μm) at					
	23°C	25°C	28°C	30°C	32°C	34°C
Wild type	3.56 ± 0.36	3.52 ± 0.44	3.40 ± 0.29	3.57 ± 0.50	3.42 ± 0.46	N.T.
syb1-S1	2.66 ± 0.34	2.61 ± 0.31	2.71 ± 0.30	2.75 ± 0.44	2.76 ± 0.43	N.T.

Wild-type (TY235) and *syb1-S1* (TY234) strains were incubated on MEA at various temperatures.

The mean values with standard deviations are presented ($n > 50$).

N.T., note tested because the mating was completely inhibited at 34°C.

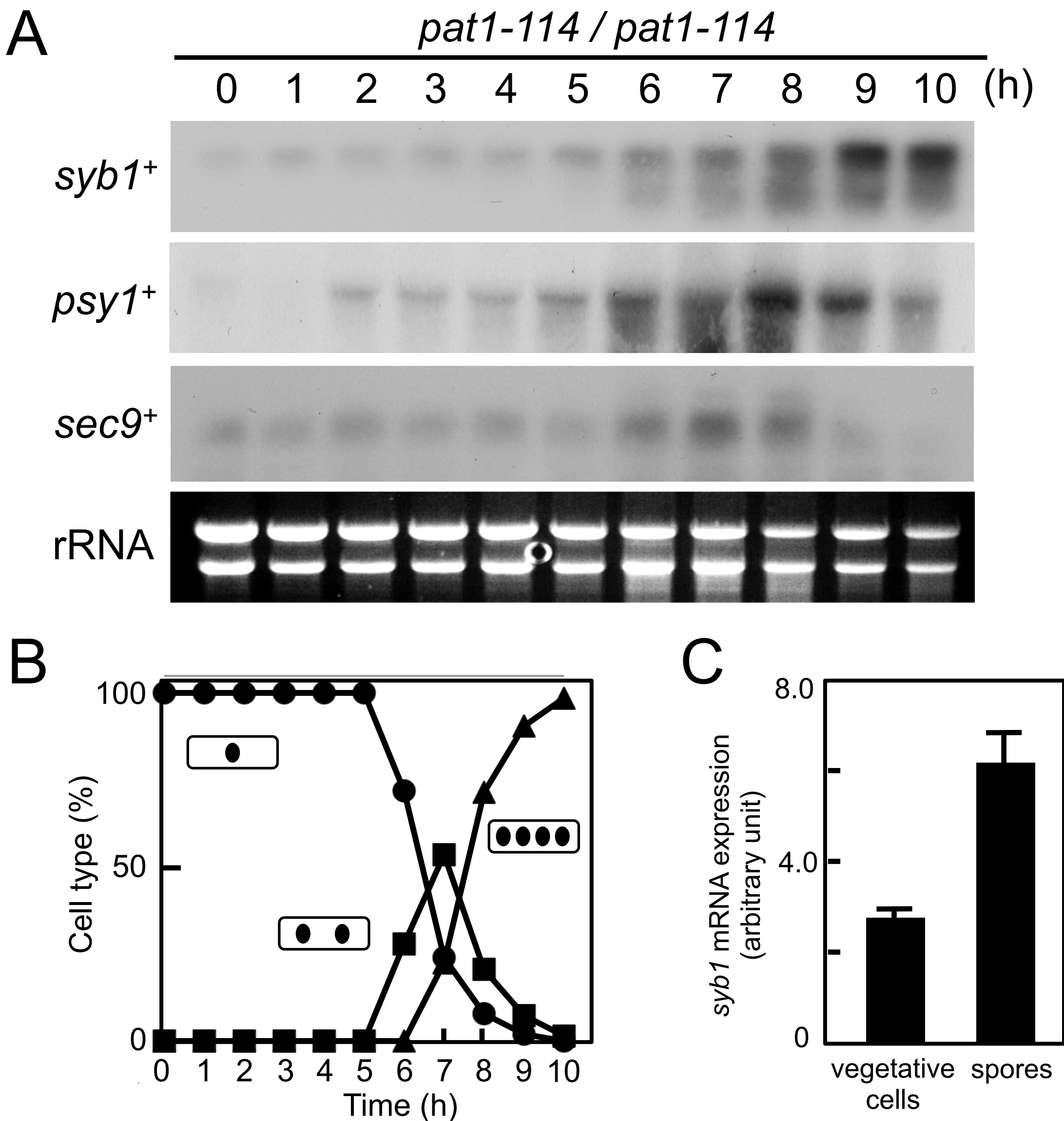

Fig. S1. Transcription of *syb1*$^{+}$ in *pat1*-driven synchronous meiosis. (A) Meiosis of diploid strains harboring homozygous *pat1-114* (JZ670) was synchronous. At hourly intervals, total RNA was prepared and blotted with a radiolabeled *syb1*$^{+}$ or *sec9*$^{+}$ DNA fragment. (B) Meiotic nuclear division of (A) was monitored by counting the number of nuclei per cell. Circles, mononucleate; squares, binucleate; triangles, tetranucleate cells. (C) RT-qPCR analysis of *syb1* in vegetative cells and spores. Total RNA was prepared from vegetative growing cells and gradient-purified spores of the wild-type strain (MKW5). 0.5 μg of total RNA was used for RT-qPCR analysis. A reverse transcriptional reaction was performed using a High Capacity cDNA Reverse Transcription Kit (Applied biosystems, Foster city, CA). The qPCR reaction was set up with a GoTaq(R) qPCR Master Mix (Promega, Madison, WI). The reactions and analyses were carried out with a 7500 Real Time PCR system (Applied biosystems). To amplify the *syb1* cDNA, the primers syb1-F (5' CCTTCTGCAGCTGTTCGTAGTG) and syb1-R (5' CTGAATTGCAGCTGTCTTCATATTC) were used. To amplify the *act1* cDNA, the primers act1-F (5' TGTTGACTGAGGCTCCTTTGAA) and act1-R (5' GGTGCATTAAAGGTTTCGAAAATAA) were used. The *syb1* expression level was normalized against *act1* expression level.

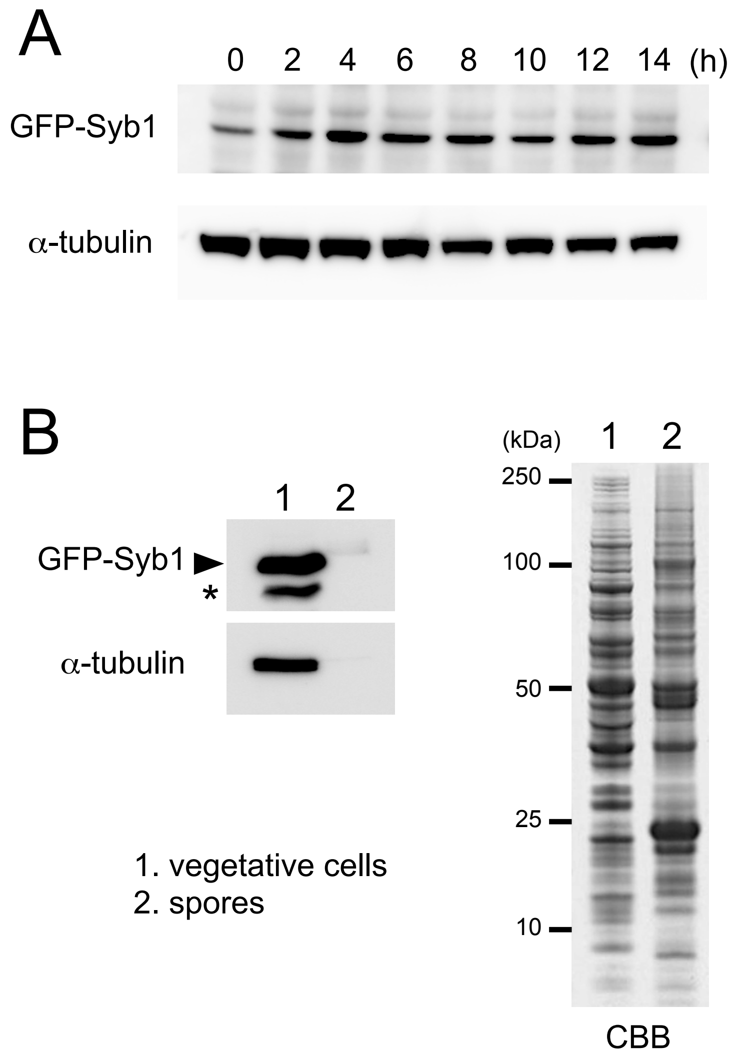

Fig. S2. Changes in the abundance of Syb1 during sporulation. (A) Cells expressing GFP-Syb1 (TY149) were allowed to proceed through synchronous meiosis. Aliquots were removed every 2 hr, and the protein extracts were subjected to Western blot analysis with mouse anti-GFP antibody. α-tubulin was used as a loading control. (B) Abundance of GFP-Syb1 in spores. Protein extracts from vegetative growing cells and gradient-purified spores of the wild-type (TY1) strain were subjected to Western blot analysis with mouse anti-GFP and anti-α-tubulin antibodies. The band indicated by an asterisk is presumed to be a degradation product of Syb1. The extracts were also analyzed by SDS-PAGE, and the gel was stained with Coomassie brilliant blue (CBB).

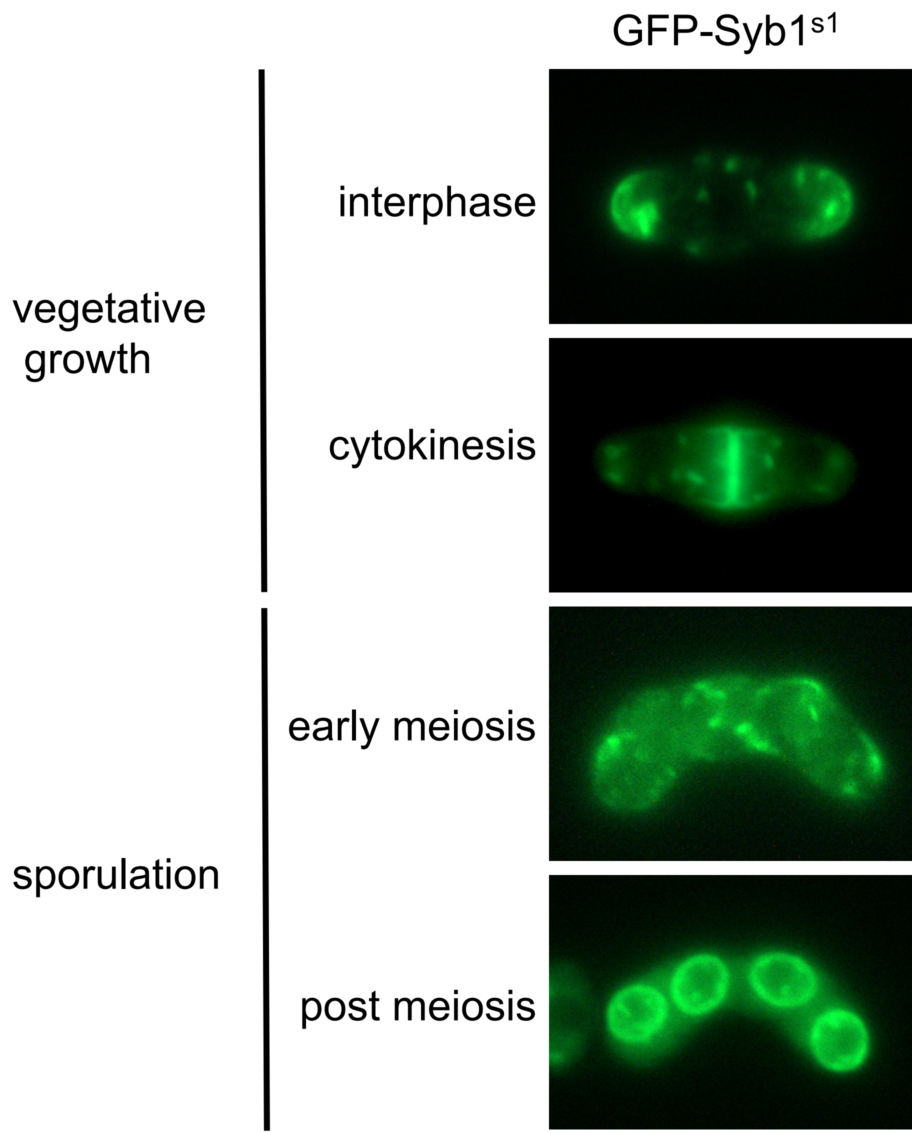

Fig. S3. Localization of Syb1[s1] protein. TY227 expressing Syb1[s1]-GFP was incubated in MM+N (vegetative growth) and sporulated in MM-N medium (sporulation). Bar, 10 μm

Supplementary Movie Legends

Movie S1. Behavior of GFP-Syb1 and mCherry-Psy1 during mitotic interphase. The movie essentially corresponds to the frames shown in Fig. 3A. The movie plays at 10 frames per second.

Movie S2. Behavior of GFP-Syb1 and mCherry-Psy1 during mitotic M-phase. The movie essentially corresponds to the frames shown in Fig. 3B. The movie plays at 10 frames per second.

Movie S3. Behavior of GFP-Syb1 and mCherry-Psy1 during meiotic interphase. The movie essentially corresponds to the frames shown in Fig. 3C. The movie plays at 10 frames per second.

Movie S4. Behavior of GFP-Syb1 and mCherry-Psy1 during FSM formation. The movie plays at 10 frames per second.