

**Figure S1.** Kinetics of meiosis in wild type and the *spo7* $\Delta$  mutant.

Synchronous meiosis was induced in the diploid strains JZ670 (pat1-114/pat1-114) and MN49 ( $pat1-114 spo7\Delta/pat1-114 spo7\Delta$ ). A portion of the culture was stained with DAPI. Meiotic cells were classified by the number of nuclei per cell. For each sample, about 500 cells were counted. This number is based on a representative result of three independent experiments that gave similar results. Diamonds, mononucleate; triangles, binucleate; squares, tri- or tetranucleate cells.



Figure S2. Relationship of Spo7 with other meiotic SPB components.

(A) Localization of Spo7 in *spo15* $\Delta$ , *spo2* $\Delta$ , and *spo13* $\Delta$ . *spo15* $\Delta$  (MN28), *spo2* $\Delta$  (MN24), and *spo13* $\Delta$  (MN26) strains expressing Spo7-GFP were sporulated on SSA for 2 days and analyzed by fluorescence microscopy. (B) Localization of Spo15, Spo2, and Spo13 in *spo7* $\Delta$ . *spo7* $\Delta$  strains expressing Spo15-GFP (MN101), Spo2-GFP (MN99), or Spo13-GFP (MN100) were sporulated on SSA for 2 days and analyzed by fluorescence microscopy. (C) Yeast two-hybrid analysis between Spo7 and other meiotic SPB components. *spo7*<sup>+</sup> was cloned into pGBT9, which contains the DNA-binding domain of the *GAL4* gene (BD). *spo7*<sup>+</sup>, *spo15*<sup>+</sup>, *spo13*<sup>+</sup> and *spo2*<sup>+</sup> ORFs were cloned into pGAD424, which contains the activation domain of the *GAL4* gene (AD). Plasmids carrying these fusion constructs were introduced into the *S. cerevisiae* tester strain (AH109). The assay was done by monitoring growth of the host cells on adenine-depleted medium. "AD (vector)" indicates control pGAD424.



Figure S3. Spo7 is expressed slightly before Spo13.

(A) Dual observation of Spo7 and Spo13 in early meiosis. Homothallic haploid wild-type cells expressing Spo7-CFP and Spo13-GFP (MN69) were sporulated on SSA medium and analyzed by fluorescence microscopy. (B) Western analysis of Spo7 with Spo13. Cells expressing Spo7-HA and Spo13-GFP (MN78) were allowed to proceed through synchronous meiosis. Aliquots were removed at hourly intervals and the protein extract was subjected to Western blot analysis with the rat anti-HA antibody 3F10, mouse anti-GFP antibody and the anti- $\alpha$ -tubulin antibody TAT-1 as a loading control. Meiotic nuclear division was monitored by counting the number of nuclei per cell. Circles, mononucleate; squares, binucleate; triangles, tetranucleate cells.



Figure S4. Yeast two-hybrid analysis between Spo7 and Spo7 deletion mutants.

 $spo7^+$  was cloned into pGAD424, which contains the activation domain of the *GAL4* gene (AD).  $spo7^+$ ,  $spo7-\Delta C2$ ,  $spo7-\Delta C3$  and  $spo7-\Delta PH$  were cloned into pGBT9, which contains the DNA-binding domain of the *GAL4* gene (BD). Plasmids carrying these fusion constructs were introduced into the *S. cerevisiae* tester strain (AH109). The assay was done by monitoring growth of the host cells on histidine-depleted medium. "BD (vector)" indicates control pGBT9.