

Supplemental Figure legends

Figure S1. Meiotic gene expression in WT, *cdh1* Δ and *mnd2* Δ strains. (A) Wild-type (RSY695), and *cdh1* Δ (RSY1210) strains were induced to enter meiosis and samples taken at the times indicated (in hrs). Total RNA was prepared and the transcription profiles of *IME2*, *CDC20*, *SPS4* and *SPS100* were analyzed by Northern blot analysis. Ethidium bromide-stained rRNA is shown as a loading control. (B) The experiment described in (A) was repeated with the *mnd2* Δ (KCY440) mutant strain.

Figure S2. Ama1p does not target Cdc20p for destruction via Db2 or KEN motifs. (A) Wild-type cells harboring the Cdc20p-18myc expression plasmids indicated on the left were induced to enter meiosis and samples taken for Western analysis at the timepoints indicated. The blots were stripped and reprobbed for Tub1p as loading control. MI and MII indicate the approximate times of meiosis I (MI) and meiosis II (MII) as determined by DAPI analysis. (B) As in (A) but showing the later timepoints only. (C) Quantitation of the degradation kinetics of wild type and the mutated alleles of Cdc20p-18myc from Fig. 5B. Each experiment was repeated 3 times. (D) Quantitation of the degradation kinetics from the experiments described in Fig. 5C.

Figure S3. Ama1p and Cdc20p associate only when co-expressed in the same cell. An *ama1* Δ mutant (RSY562) harboring either Ama1p^{CB Δ /IR Δ} (pKC3048), Cdc20p (pMSC8), vector (pRS426 - lane 1), or both Ama1p^{CB Δ /IR Δ} (pKC3048) and Cdc20p^{CB Δ /IR Δ} (pKC5022) were induced to enter meiosis and cells harvested for analysis at 9 hours when both *AMA1* and *CDC20* are expressed (Cooper *et al.*, 2000). Extracts were prepared as described in Material and methods except equal amounts of extracts containing either vector and Ama1p^{CB Δ /IR Δ} (lane 1) or Cdc20p and Ama1p^{CB Δ /IR Δ} (lane 2) were mixed before immunoprecipitation and Western blot analysis. The top and middle panels control for protein expression. The bottom panel assays co-immunoprecipitation.

Supplemental Table 1

| Strain | Genotype | Source |
|----------|---|---------------------------------|
| RSY10 | <i>MATα ade2 ade6 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</i> | (Cooper <i>et al.</i> , 1997) |
| RSY335 | <i>cyh2^r-z ho::LYS2 leu2::hisG lys2 trp1::hisG ura3</i> | (Cooper <i>et al.</i> , 1997) |
| RSY562* | <i>ama1::KANMX4</i> | (Cooper <i>et al.</i>) |
| RSY695* | <i>CDC20-18MYC::TRP1/CDC20</i> | This study |
| RSY696* | <i>CDC16-6MYC::URA3/CDC16</i> | (Cooper <i>et al.</i>) |
| RSY809 | <i>cdc20-1</i> | (Mallory <i>et al.</i> , 2007) |
| RSY853* | <i>ama1::KANMX4 CDC20-18myc::TRP1/CDC20</i> | This study |
| RSY1055 | <i>Cdc16::TAP/CDC16</i> | (Mallory <i>et al.</i> , 2007) |
| RSY1210* | <i>cdh1::LEU2 CDC20-18myc::TRP1/CDC20</i> | This study |
| KCY224* | <i>TUB4-GFP::URA3/TUB4</i> | This study |
| KCY225* | <i>ama1::KANMX4 TUB4-GFP::URA3/TUB4</i> | This study |
| KCY440* | <i>mnd2::KANMX4 CDC20-18myc::TRP1/CDC20</i> | This study |
| CMY15 | <i>MATα/MATα ho::LYS2 leu2::hisG trp1::hisG lys2/lys2 ura3 ama1::KANMX4 SMK1-HA</i> | (McDonald <i>et al.</i> , 2005) |

Yeast Strains used in this study. Strains marked with an asterisk are isogenic to RSY335. All strains are diploids (except RSY10, RSY1270 and RSY1055) and the alleles in diploid strains are homozygous unless indicated.

Supplemental Table 2

| Name | Gene Target | Mutation Created | Oligonucleotide |
|------|--------------|------------------|--|
| Db1 | <i>CDC20</i> | RXXL-AXXA | GCAATTAGCGGTAACGCGTCTGTAGCTTCTATTGCGTCC |
| Db2 | <i>CDC20</i> | RXXL-AXXA | AACATTAGAAACTCCAAAGCGCCCAGTGCACAGGCC TCT GCCAATTCTATT |
| KEN | <i>CDC20</i> | KEN-AAA | GGTTGTGGAACAAATGATGCGGCCGCTCGTTCCAAAAAT TCG |
| GXEN | <i>CDC20</i> | GXEN-GXAN | GCCACAGTGGGAGGAGATGCAAACCTTAAAATTT |
| CB | <i>CDC20</i> | DRYIPIL-Δ | GCAATTTACAGTAGCTGCCCAAGGAGCTTCGCAAAC |
| IR | <i>CDC20</i> | IR-Δ | AGTACCAGCCAATATTTGTGATAAAATAAAAAAATAAAAG TTCATGTCA |
| CB | <i>AMA1</i> | DRFIPKS-Δ | GCGGAAATTCTACAGAGGTTGTTTCGAGAAATGC |
| IR | <i>AMA1</i> | IR-Δ | GGTATCGAAACAACACATAACAAACGTTAATATAAAAAA GGATATTCATTGC |

Oligonucleotides used in this study with their accompanying mutation identified.

Supplemental Table 3

| Mutation | Gene | Epitope Tag | Plasmid Name | Promotor | 2μ or CEN | Reference |
|-------------|--------------|-------------|--------------|--------------|-----------|--------------------------------|
| WT | <i>CDC20</i> | 18 myc | pMSC7 | <i>CDC20</i> | CEN | This study |
| Db1 | <i>CDC20</i> | 18 myc | pKC5030 | <i>CDC20</i> | CEN | This study |
| Db2 | <i>CDC20</i> | 18 myc | pKC5031 | <i>CDC20</i> | CEN | This study |
| Db1/Db2 | <i>CDC20</i> | 18 myc | pKC5047 | <i>CDC20</i> | CEN | This study |
| KEN | <i>CDC20</i> | 18 myc | pKC5029 | <i>CDC20</i> | CEN | This study |
| Db1/Db2/KEN | <i>CDC20</i> | 18 myc | pKC5034 | <i>CDC20</i> | CEN | This study |
| GXEN | <i>CDC20</i> | 18 myc | pKC5043 | <i>CDC20</i> | CEN | This study |
| Db1/GxEN | <i>CDC20</i> | 18 myc | pKC5045 | <i>CDC20</i> | CEN | This study |
| CB/IR | <i>CDC20</i> | 18 myc | pKC5065 | <i>CDC20</i> | CEN | This study |
| WT | <i>CDC20</i> | 24 myc | pUS995 | <i>GAL1</i> | CEN | (Goh <i>et al.</i> , 2000) |
| Db1 | <i>CDC20</i> | 24 myc | pKC5006 | <i>GAL1</i> | CEN | This study |
| GXEN | <i>CDC20</i> | 24 myc | pKC5009 | <i>GAL1</i> | CEN | This study |
| Db1/GxEN | <i>CDC20</i> | 24 myc | pKC5016 | <i>GAL1</i> | CEN | This study |
| KEN | <i>CDC20</i> | 24 myc | pKC5007 | <i>GAL1</i> | CEN | This study |
| WT | <i>CDC20</i> | 18 myc | pMSC8 | <i>CDC20</i> | 2μ | (Mallory <i>et al.</i> , 2007) |
| CB | <i>CDC20</i> | 18 myc | pKC5020 | <i>CDC20</i> | 2μ | This study |
| IR | <i>CDC20</i> | 18 myc | pKC5021 | <i>CDC20</i> | 2μ | This study |
| CB/IR | <i>CDC20</i> | 18 myc | pKC5022 | <i>CDC20</i> | 2μ | This study |
| Db1 | <i>CDC20</i> | 18 myc | pKC5023 | <i>CDC20</i> | 2μ | This study |
| Db1/GxEN | <i>CDC20</i> | 18 myc | pKC5046 | <i>CDC20</i> | 2μ | This study |
| WT | <i>CDC20</i> | No Tag | pKC5069 | <i>AMA1</i> | CEN | This study |
| Db1/GxEN | <i>CDC20</i> | No Tag | pKC5070 | <i>AMA1</i> | CEN | This study |

| | | | | | | |
|-----------------------|------------------|--------|-----------|---------------|------|---------------------------------|
| WT | <i>CDC20</i> | No Tag | pKC5071 | <i>AMA1</i> | 2μ | This study |
| Db1/GxEN | <i>CDC20</i> | No Tag | pKC5072 | <i>AMA1</i> | 2μ | This study |
| WT (<i>TRP1</i>) | <i>AMA1</i> | 1 T7 | pKC3036 | <i>AMA1</i> | 2μ | This study |
| CB | <i>AMA1</i> | 1 T7 | pKC3045 | <i>AMA1</i> | 2μ | This study |
| IR | <i>AMA1</i> | 1 T7 | pKC3046 | <i>AMA1</i> | 2μ | This study |
| CB/IR (<i>TRP1</i>) | <i>AMA1</i> | 1 T7 | pKC3048 | <i>AMA1</i> | 2μ | This study |
| WT (<i>URA3</i>) | <i>AMA1</i> | 1 T7 | pKC3056 | <i>AMA1</i> | 2μ | This study |
| CB/IR (<i>URA3</i>) | <i>AMA1</i> | 1 T7 | pKC3057 | <i>AMA1</i> | 2μ | This study |
| 201-596 | <i>AMA1</i> | 1 T7 | pKC3084 | <i>AMA1</i> | 2μ | This study |
| - | <i>GST</i> | | pQYAC-GST | <i>ADH1</i> | CEN | This study |
| - | <i>GST</i> | | pEGKT | <i>GAL1</i> * | 2μ | (Burton and Solomon, 2001) |
| 1-200 | <i>GST-AMA1</i> | none | pKC3113 | <i>GAL1</i> * | 2μ | This study |
| WT | <i>CDH1</i> | 3 HA | pKC3078 | <i>AMA1</i> | 2μ | This study |
| 1-200 of Ama1p | <i>AMA1-CDH1</i> | 1 T7 | pKC3077 | <i>AMA1</i> | 2μ | This study |
| 234-566 of Cdh1p | | | | | | |
| WT | <i>TUB4</i> | GFP | pMDE785 | <i>TUB4</i> | int. | V. Guacci |
| WT | <i>SMK1</i> | 3 HA | | | | (McDonald <i>et al.</i> , 2005) |
| WT | <i>CLB1</i> | 3HA | pKC430 | <i>CLB1</i> | CEN | This study |

Plasmids used in this study.

* *CYC1* promoter driven by *GAL1* UAS

Figure S1

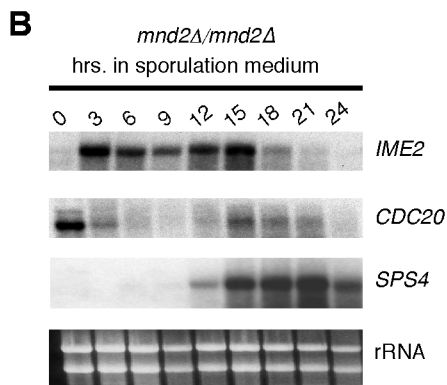
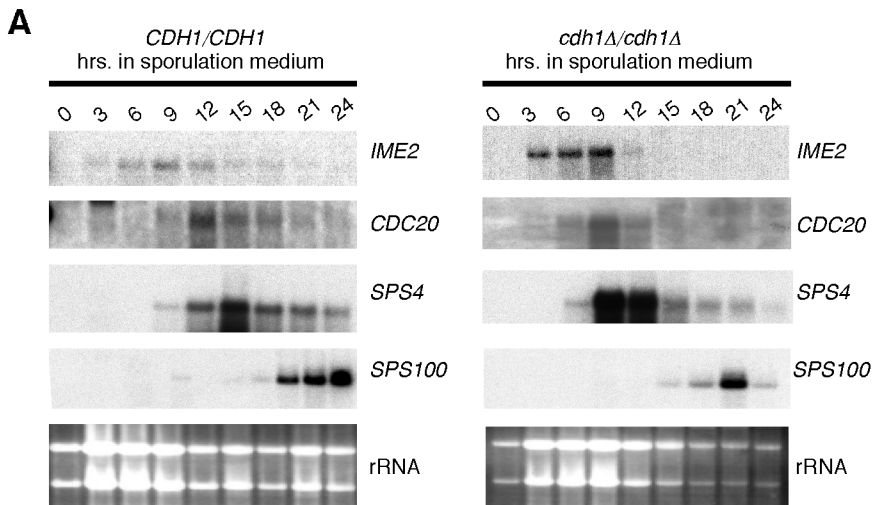
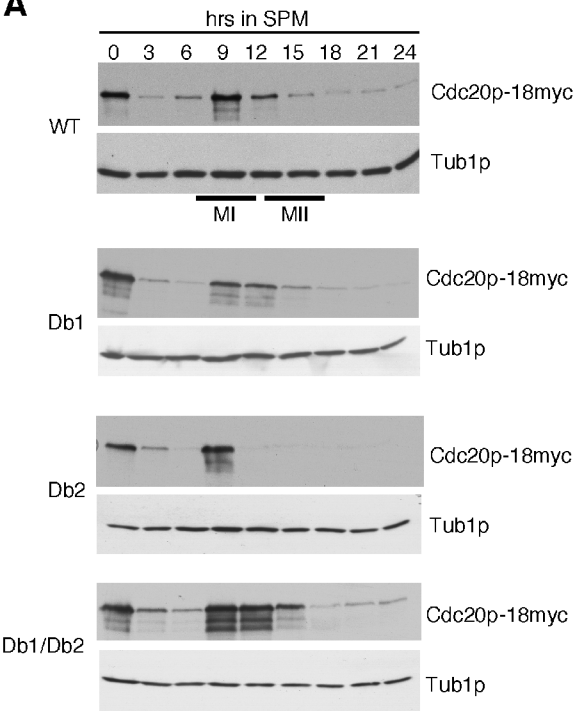
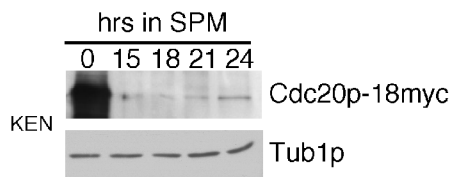


Figure S2

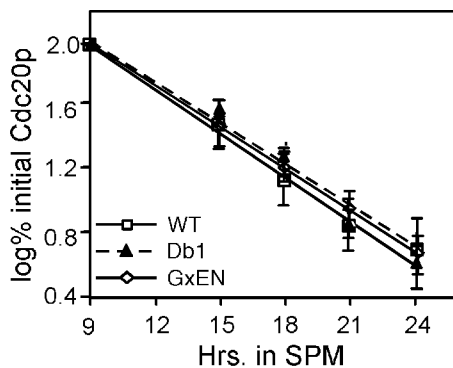
A



B



C



D

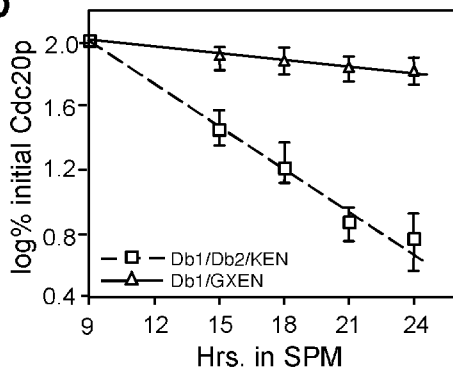


Fig. S3

