Kerr et al., http://www.jcb.org/cgi/content/full/jcb.201103019/DC1


Figure S1. PP2A ${ }^{\text {Cdc55 }}$ is not required for SC assembly and disassembly but is required for SPB separation during meiosis. (A) Wild-type (Y1843) and cdc55-mn (Y2198) cells were induced to sporulate. Aliquots of the sporulating culture were taken every hour and subjected to chromosome spreading. The spreads were stained using anti-HA (to visualize Rec8) and anti-Zipl (to visualize SC) antibodies. Frequencies of partial SC and full SC observed in chromosome spreads from the two cultures were plotted as a function of time. These are representative data from three experimental repeats. (B) Representative images of full SC, partial SC, dot/minimal SC, and no signal/polycomplexes are shown. (C) CDC55 (Y2111) and cdc55-mn (Y2149) cells containing SPC42-GFP were induced to sporulate. SPB separation was detected by counting the number of Spc42-GFP dots in the two sporulating cultures ( $n=200$ ).



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Figure S2. Nuclear division defect of cdc55-mn cells is not caused by activation of pachytene/spindle assembly checkpoints or lack of Clbl expression or hyperphosphorylation of Cdc28 at Y19 by Swe 1. (A) CDC55 (Y1737), cdc55-mn (Y1738), rad24 (Y2280), cdc55-mn rad24 (Y2282), spoll (Y2270), cdc55-mn spoll (Y2271), mad2 (Y2289), and mad2 cdc55-mn (Y2156) strains were induced to sporulate for 24 h . Cells were harvested, and nuclear division was scored by staining with DAPI ( $n=500$ ). (B) Diploid CDC55 CLB1myc9 (Y2027) and cdc55-mn CLB1myc9 (Y2028) cells were induced to sporulate. Clbl expression was assayed in the two sporulating cultures by immunofluorescence using anti-Myc antibodies. (C) CDC55 (Y1737), cdc55-mn (Y1738), swe $1 \Delta$ (Y2102), cdc55-mn swe $1 \Delta$ (Y2103), cdc28-T18AY19F (Y2241), and cdc55-mn cdc28-T18AY19F (Y2242) strains were induced to sporulate for 24 h . Cells were harvested, and nuclear division was scored by staining with DAPI ( $n=500$ ). Error bars represent SEM.


Figure S3. Premature release of Cdc 14 during meiosis inhibits formation of metaphase I spindles, and PP2A ${ }^{\text {Cdc55 }}$ acts downstream of Slk 19 and Spo 12 in the FEAR pathway. (A) Diploid $P_{G A L}-T A B 6$ GAL4-ER $P_{C L B 2-C D C 20 ~(Y 2285) ~ a n d ~ G A L 4-E R ~} P_{C L B 2-C D C 20 ~(Y 2287) ~ s t r a i n s ~ w e r e ~ a r r e s t e d ~ i n ~ m e t a p h a s e ~ I ~ b y ~ t r a n s-~}^{\text {( }}$ ferring them to SPM for 8 h . Estradiol was added at 3, 4, and 5 h to the cultures, and the effect on spindle formation was determined by immunofluorescence using antitubulin antibodies ( $n=200$ ). These are representative data from three experimental repeats. (B) CDC55 (Y1737), cdc55-mn (Y1738), slk $19 \Delta$ (Y2267), cdc55-mn slk19 $\Delta$ (Y2269), spol24 (Y2272), and cdc55-mn spol24 (Y2274) strains were induced to sporulate for 24 h. Cells were harvested, and nuclear division was scored by staining with DAPI ( $n=500$ ). Error bars represent SEM.

Table S1. List of yeast strains used in the study

| Strain name | Genotype | Used in figure |
| :---: | :---: | :---: |
| Y1737 |  | S2 and S3 |
| Y1738 | MATa/ $\alpha$ | S2 and S3 |
| Y1843 | MATa/ $\alpha$, leu2:tetR-GFP-LEU2/leu2:tetR-GFP-LEU2, REC8-ha3::URA3/REC8-ha3::URA3, PDS1-myc18:: KITRP1/PDS1-myc18::KITRP1, tetO::URA3/ura3 | 1 and S 1 |
| Y2027 | MATa/ , CLB1-myc9::KITRP1/CLB1-myc9::KITRP1 | S2 |
| Y2028 | MATa/ $\alpha$, CLB 1-myc9::KITRP1/CLB1-myc9::KITRP1, cdc55::PClB2-ha3-CDC55::KanMX6/ <br>  | S2 |
| Y2072 | MATa/ $\alpha$, net14 $::$ HIS5/net1 $1::$ HIS5, trp $1::$ NET1-TEV-myc9::TRP1/trp $1::$ NET1-TEV-myc9::TRP1, rec8::REC8-ha3:: URA3/rec8::REC8-ha3::URA3, leu2::tetR-GFP-LEU2/leu2::tetR-GFP-LEU2, ura3::tetO::URA3/ura3 | 4 and 5 |
| Y2075 | MATa/ $\alpha$, net1 $1::$ HIS5/net1 $1::$ HIS5, trp $1::$ NET1-TEV-myc9 $::$ TRP1/trp1 $\because:$ NET1-TEV-myc9::TRP1, rec8::REC8-ha3::URA3/rec8::REC8-ha3-URA3, leu2::tetR-GFP-LEU2/leu2::tetR-GFP-LEU2, cdc55:: P $_{\text {ClB2-ha3-CDC55::KanMX6/cdc55: }}$ : ClB2-ha3-CDC55::KanMX6, ura3::tetO-URA3/ura3 | 4 and 5 |
| Y2078 | ```MATa/\alpha, net1\Delta::HIS5/net1\Delta::HIS5, trp1::net1-6Cdk-TEV-myc9::TRP1/trp1::net1-6Cdk-TEV-myc9:: TRP1, rec8::REC8-ha3::URA3/rec8::REC8-ha3::URA3, leu2::tetR-GFP-LEU2/leu2::tetR-GFP-LEU2, ura3::tetO::URA3/ura3``` | 4 and 5 |
| Y2081 | MATa/ $\alpha$, net1 $1::$ HIS5/net1 $1::$ HIS5, trp $1::$ NET1-TEV-myc9::TRP1/trp $1::$ NET1-TEV-myc9::TRP1, rec8::REC8-ha3::URA3/rec8::REC8-ha3::URA3, leu2::tetR-GFP-LEU2/leu2::tetR-GFP-LEU2, cdc55:: $P_{\text {Clв2 }}$-ha3-CDC55::KanMX6/cdc55:: $P_{\text {ClB2-ha3-CDC55::KanMX6, ura3::tetO::URA3/ura3 }}$ | 4 and 5 |
| Y2102 | MATa/ $\alpha$, swe 1 1 ::HIS3/swe 1 $1:$ :HIS3 | S2 |
| Y2103 | MATa/ , swe 14 ::HIS3/swe 14 ::HIS3, cdc55::PCLB2-ha3-CDC55::KanMX6/cdc55::P ClB2-ha3-CDC55::KanMX6 | S2 |
| Y2111 | MATa/ , SPC42-GFP::HIS3/SPC42-GFP::HIS3 | 2 and S 1 |
| Y2119 | ```MATa/ }\alpha,net1\Delta::HIS5/net1\Delta::HIS5, trp1::NET1-TEV-myc9::TRP1/trp1 ::NET1-TEV-myc9::TRP1, cdc55::P KanMX6/cdc20::P``` | 3 |
| Y2120 | MATa/ $\alpha$, net1 $1::$ HIS5/net1 $1::$ HIS5, trp $1::$ NET1-TEV-myc9::TRP1/trp $1::$ NET1-TEV-myc9::TRP1, cdc20::P ${ }_{\text {ClB2 }}$-CDC20::KanMX6/cdc20::PPCIB2-CDC20::KanMX6 | 3 |
| Y2149 | MATa/ , SPC42-GFP::HIS3/SPC42-GFP::HIS3, cdc55::PClB2-ha3-CDC55::KanMX6/cdc55:: $P_{\text {CLB2-ha3-CDC55::KanMX6 }}$ | 2 |
| Y2156 | MATa/ $\alpha$, cdc55:: $P_{\text {ClB2 } 2-h a 3-C D C 55:: K a n M X 6 / c d c 55:: P ~}^{\text {ClB2 }}$-ha3-CDC55::KanMX6, mad2:: HIS3MX6/mad2::HIS3MX6 | S2 |
| Y2161 | MATa/ $\alpha$, ura3 $:: P_{\text {GPDI }}$-GAL4(848).ER $::$ URA3/ura3::P ${ }_{\text {GPDI }}$-GAL4(848).ER::URA3 | 3 |
| Y2198 | MATa/ $\alpha$, leu2::tetR-GFP-LEU2/leu2::tetR-GFP-LEU2, REC8-HA3::URA3/REC8-ha3::URA3, PDS1-myc18::TRP1/PDS1-myc18::TRP1, cdc55::P ClB2 -ha3-CDC55:: KanMX6/cdc55:: $P_{\text {ClB2-ha3-CDC55::KanMX6, ura3::tetO-URA3/ura3 }}$ | 1 and S 1 |
| Y2212 |  | 3 |
| Y2241 | MATa/ $\alpha$,cdc28:cdc28-T18AY19F::TRP1/cdc28:cdc28-T18AY19F::TRP1 | S2 |
| Y2242 | MATa/ $\alpha$, cdc28:cdc28-T18AY19F::TRP1/cdc28:cdc28-T18AY19F::TRP1, cdc55::PCIB2-ha3-CDC55:: KanMX6/cdc55::P Clв2-ha3-CDC55::KanMX6 | S2 |
| Y2267 | MAT a/ $\alpha$, slk 194::HIS3/slk 194::HIS3 | S3 |
| Y2269 | MAT a/ $\alpha$, slk194 ::HIS3/slk194::HIS3, cdc55::PClB2-ha3-CDC55::KanMX6/cdc55:: $P_{\text {ClB2-ha3-CDC55::KanMX6 }}$ | S3 |
| Y2270 | MAT a/ $\alpha$, spo $114::$ TRP $1 /$ spo $11 \Delta:: T R P 1$ | S2 |
| Y2271 |  | S2 |
| Y2272 | MAT a/ , spo12::KanMX6/spo 12::KanMX6 | S3 |
| Y2274 | MAT a/ , cdc55:: $P_{\text {ClB2 }}$-ha3-CDC55::KanMX6/cdc55::P ClB2-ha3-CDC55::KanMX6, spo12::KanMX6/spo 12::KanMX6 | S3 |
| Y2276 | MATa/ $\alpha$, net14 ::HIS5/net14 ::HIS5, trp1:net1-6Cdk-TEV-myc9::TRP1/trp1:net1-6CDK-TEV-myc9::TRP1, cdc554:: NatMX6/cdc554::NatMX6 | 4 |
| Y2278 | MATa/ $\alpha$, net1 $1::$ HIS5/net1 $1::$ HIS5, trp $1::$ NET1-TEV-myc9::TRP1/trp $1::$ NET1-TEV-myc9::TRP1, cdc554::NatMX6/cdc554::NatMX6 | 4 |
| Y2280 | MATa/ $\alpha$, rad244 : : LEU2/rad24d : LEU2 | S2 |
| Y2282 |  | S2 |
| Y2285 | MATa/ $\alpha$, ura3:: $P_{\text {GPDI }}$-GAL4(848).ER::URA3/ura3 $:: P_{\text {GPDI }}-G A L 4(848) . E R:: U R A 3$, his $3::$ $P_{\text {GAI }}$ CDC14-TAB6::HIS3/his3, cdc20::P $P_{\text {ClB2 } 2}$ CDC20::KanMX6/cdc20::P CIB2 -CDC20::KanMX6 | S3 |
| Y2287 | MATa/ $\alpha$, ura3:: $P_{\text {GPDI }}$ GAL4(848).ER::URA3/Ura3:: $P_{G P D I-G A L 4(848) . E R:: U R A 3, ~ c d c 20:: ~}^{\text {I }}$ $P_{\text {CIB2 }}$ CDC20::KanMX6/cdc20::PC\|B2-CDC20::KanMX6 | S3 |
| Y2289 | MATa/ $\alpha, \operatorname{mad} 24:: H I S 3 M X 6 / \operatorname{mad} 24:: H I S 3 M X 6$ | S2 |
| Y2307 | ```MATa/\alpha, net14::HIS5/net14::HIS5, trp1::net1-6Cdk-TEV-myc9::TRP1/trp1::net1-6Cdk-TEV-myc9::TRP1, cdc55::P PCB2-ha3-CDC55::KanMX6/cdc55::P ClB2-ha3-CDC55::KanMX6, cdc20::P PlB2-CDC20:: KanMX6/cdc20::P(ClB2-CDC20::KanMX6``` | 3 |

All yeast strains are derivatives of SK1 and have the following markers, unless otherwise stated: ho::LYS2/ho::LYS2, ura3/ura3, leu2::hisG/leu2::hisG, trp 1::hisG/ $\operatorname{trp} 1:: h i s G$, his $3:: h i s G / h i s 3:: h i s G$, and lys2/lys2.

