

APPENDIX

Deprotection of Centromere Cohesin at Meiosis II Requires APC/C Activity but not Kinetochore Tension

Valentina Mengoli, Katarzyna Jonak, Oleksii Lyzak, Mahdi Lamb, Lisa M. Lister, Chris Lodge, Julie Rojas, Ievgeniia Zagoriy, Mary Herbert, and Wolfgang Zachariae

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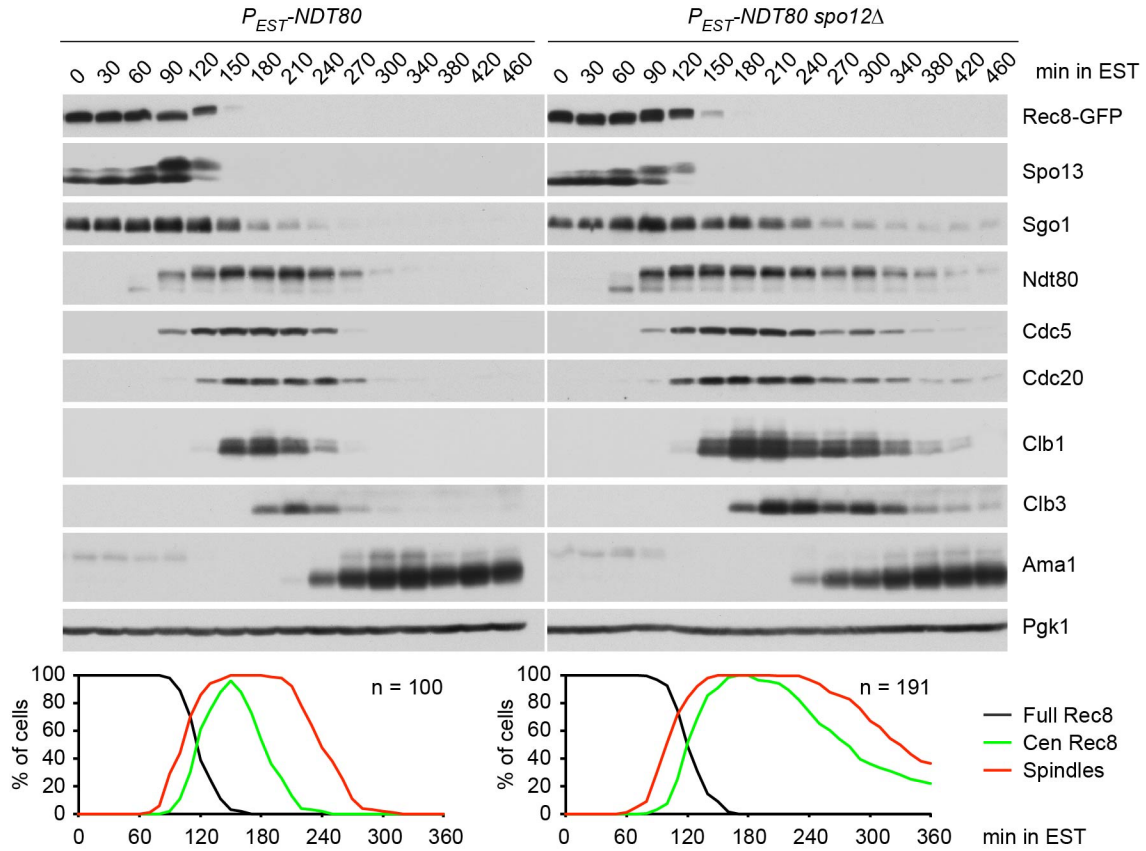
Appendix Figure S2. *mam1* Δ mutants prolong metaphase I in a Mad2-dependent manner

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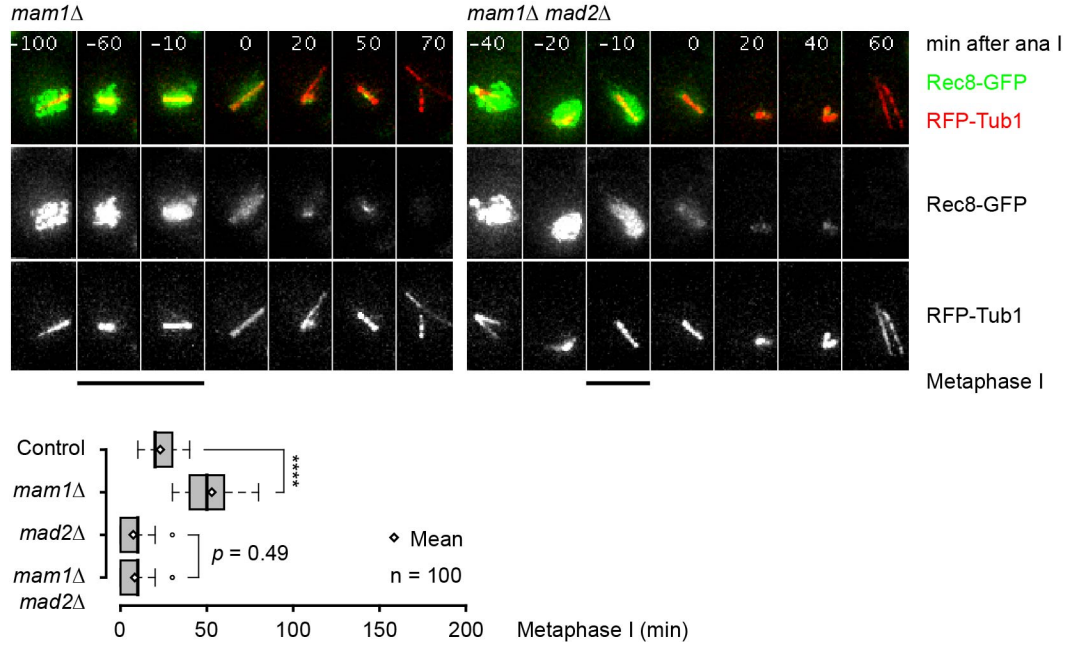
Appendix Figure S5. Deletion of *SPO11* does not prolong metaphase I in the *mad2* Δ mutant

Appendix Table S1. *Saccharomyces cerevisiae* SK1 Strains Used in this Study



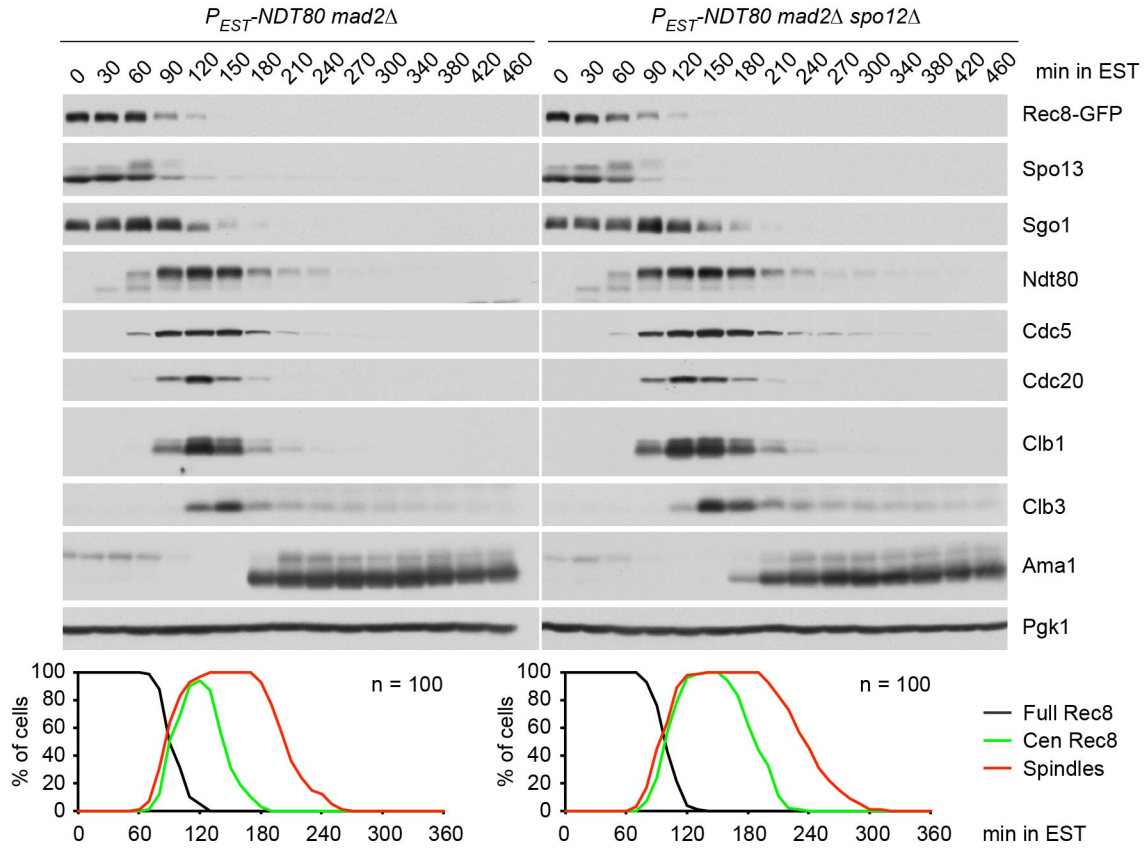
Appendix Figure S1. The *SPO12* deletion delays APC/C-dependent proteolysis at meiosis II

Control and *spo12Δ* strains synchronized with an estradiol-inducible *NDT80* gene (*P_{EST}-NDT80*) were analysed by immunoblot detection of proteins in whole cell extracts (panels). In parallel, Rec8-GFP and RFP-tubulin were followed by live-imaging (graphs). Deletion of *SPO12* delays degradation of APC/C substrates, removal of centromeric Rec8, and spindle disassembly at meiosis II by ~2 hr. Data are representative of 2 independent experiments.



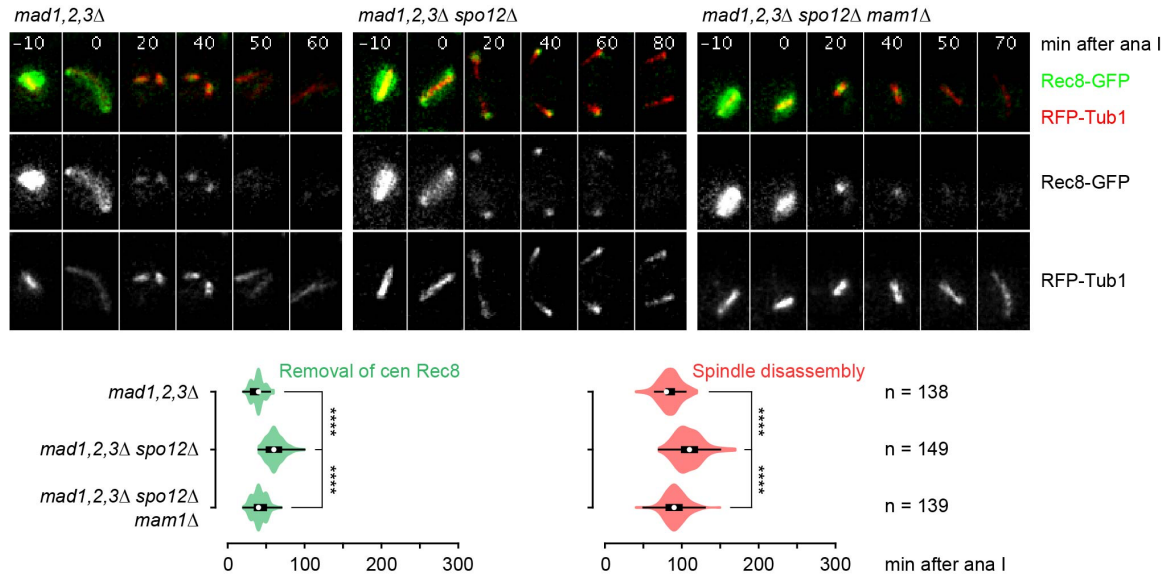
Appendix Figure S2. *mam1Δ* mutants prolong metaphase I in a Mad2-dependent manner

Imaging of Rec8-GFP and RFP-tubulin to determine the duration of metaphase I in control and *mam1Δ* cells containing or lacking Mad2. Top, representative time-lapse series. Bottom, duration of metaphase I (spindle formation to Rec8 cleavage at meiosis I) in the indicated strains. **** $p < 0.0001$, Mann-Whitney test. Data are representative of 2 independent experiments.



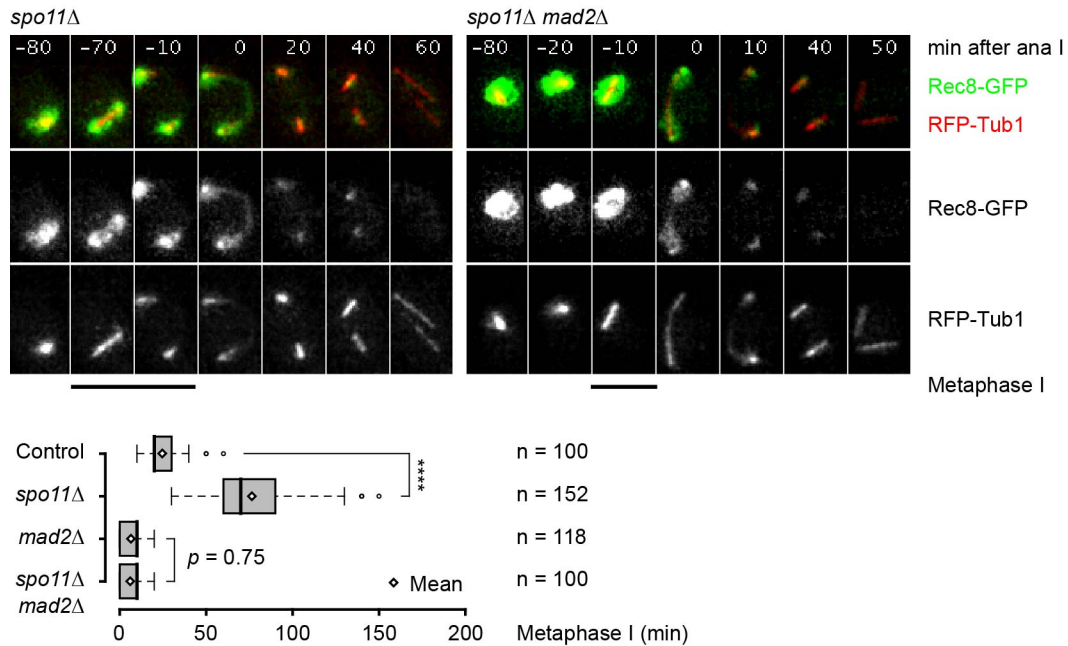
Appendix Figure S3. Effect of the *SPO12* deletion on APC/C-dependent proteolysis in the *mad2Δ* mutant

mad2Δ and *mad2Δ spo12Δ* strains synchronized with an estradiol-inducible *NDT80* gene (*P_{EST}-NDT80*) were analysed by immunoblot detection of proteins in whole cell extracts (panels). In parallel, Rec8-GFP and RFP-tubulin were followed by live-imaging (graphs). Deletion of *SPO12* in *mad2Δ* cells delays degradation of APC/C substrates, removal of centromeric Rec8, and spindle disassembly at meiosis II by only ~0.5 hr. Data are representative of 2 independent experiments.



Appendix Figure S4. Effects of deleting *SPO12* or *SPO12* and *MAM1* in *mad1Δ mad2Δ mad3Δ* triple mutants

Top, time-lapse series from the imaging of Rec8-GFP and RFP-tubulin. Bottom, times from cohesin cleavage at anaphase I to the removal of centromeric Rec8 and to spindle disassembly. **** $p < 0.0001$, Mann-Whitney test. Data are representative of 2 independent experiments.



Appendix Figure S5. Deletion of *SPO11* does not prolong metaphase I in the *mad2Δ* mutant

Imaging of Rec8-GFP and RFP-tubulin to determine the duration of metaphase I in control and *spo11Δ* cells containing or lacking Mad2. Top, representative time-lapse series. Bottom, duration of metaphase I (spindle formation to Rec8 cleavage at meiosis I) in the indicated strains. **** $p < 0.0001$, Mann-Whitney test.

Appendix Table S1. *Saccharomyces cerevisiae* SK1 Strains Used in this Study

Figure	Strain ¹	Genotype ²
1B	Z32945	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3</i>
1B	Z32944	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 spo12Δ::NatMX4</i>
1C	Z33612	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 CEN5/CEN5::tetO₂₂₄::HIS3 leu2/leu2::P_{URA3}-tetR-tdTomato::LEU2</i>
1C	Z33613	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 CEN5/CEN5::tetO₂₂₄::HIS3 leu2/leu2::P_{URA3}-tetR-tdTomato::LEU2 spo12Δ::NatMX4</i>
1D	Z33261	<i>MAD2-mNeonGreen::KITR1 CNM67-tdTomato::NatMX4</i>
1D	Z33262	<i>MAD2-mNeonGreen::KITR1 CNM67-tdTomato::NatMX4 spo12Δ::NatMX4</i>
2B	Z35330	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mam1Δ::HIS3</i>
2B	Z35329	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mam1Δ::HIS3 spo12Δ::NatMX4</i>
2C	Z33614	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 CEN5/CEN5::tetO₂₂₄::HIS3 leu2/leu2::P_{URA3}-tetR-tdTomato::LEU2 mam1Δ::HIS3</i>
2C	Z33615	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 CEN5/CEN5::tetO₂₂₄::HIS3 leu2/leu2::P_{URA3}-tetR-tdTomato::LEU2 mam1Δ::HIS3 spo12Δ::NatMX4</i>
2D	Z33264	<i>MAD2-mNeonGreen::KITR1 CNM67-tdTomato::NatMX4 mam1Δ::HIS3</i>
2D	Z33262	<i>MAD2-mNeonGreen::KITR1 CNM67-tdTomato::NatMX4 mam1Δ::HIS3 spo12Δ::NatMX4</i>
3A	Z33827	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 CEN5/CEN5::tetO₂₂₄::HIS3 leu2/leu2::P_{URA3}-tetR-tdTomato::LEU2 mad2Δ::KIURA3</i>
3A	Z33828	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 CEN5/CEN5::tetO₂₂₄::HIS3 leu2/leu2::P_{URA3}-tetR-tdTomato::LEU2 mad2Δ::KIURA3 spo12Δ::NatMX4</i>
3A	Z33948	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 CEN5/CEN5::tetO₂₂₄::HIS3 leu2/leu2::P_{URA3}-tetR-tdTomato::LEU2 mad2Δ::KIURA3 spo12Δ::NatMX4 mam1Δ::HIS3</i>
3B	Z30453	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KIURA3</i>
3B	Z30454	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KIURA3 spo12Δ::NatMX4</i>
3B	Z30618	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KIURA3 spo12Δ::NatMX4 mam1Δ::HIS3</i>
3C	Z34176	<i>RTS1-eGFP::KanMX4 MTW1-mCherry::HphMX4 mad2Δ::KIURA3 spo12Δ::NatMX4</i>
3C	Z33507	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 MTW1-mCherry::HphMX4 mad2Δ::KIURA3 spo12Δ::NatMX4</i>
3D	Z35525	<i>RTS1-mNeonGreen::KITR1 rec8Δ::KanMX4::REC8-mScarlet::LEU2 mad2Δ::KIURA3</i>
3D	Z35526	<i>RTS1-mNeonGreen::KITR1 rec8Δ::KanMX4::REC8-mScarlet::LEU2 mad2Δ::KIURA3 spo12Δ::NatMX4</i>
3D	Z35527	<i>RTS1-mNeonGreen::KITR1 rec8Δ::KanMX4::REC8-mScarlet::LEU2 mad2Δ::KIURA3 spo12Δ::NatMX4 mam1Δ::HIS3</i>
4A, B	Z27968	<i>cdc20::P_{CLB2}-CDC20::KanMX6 trp1::P_{CUP1}-CDC20::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 PDS1-myc18::HIS3MX6</i>

4A, B	Z31712	<i>cdc20::P_{CLB2}-CDC20::KanMX6 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 PDS1-myc18::HIS3MX6</i>
4C	Z37459	<i>cdc20::P_{HSL1}-CDC20::HphMX4 trp1/trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 RTS1-eGFP::BleMX4 MTW1-mCherry::HphMX4</i>
4C	Z37470	<i>cdc20::P_{HSL1}-CDC20::HphMX4 trp1/trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 MTW1-mCherry::HphMX4</i>
5A, B	Z36435	<i>cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 PDS1-AID*::KanMX4</i>
5A, B	Z36436	<i>cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 PDS1-AID*::KanMX4 mps1-as</i>
5A, B	Z35713	<i>cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 PDS1-AID*::KanMX4 ura3::P_{CUP1}-OsTIR-myc3::URA3</i>
5A, B	Z35714	<i>cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 PDS1-AID*::KanMX4 mps1-as ura3::P_{CUP1}-OsTIR-myc3::URA3</i>
6A, B	Z37076	<i>cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 SGO1-AID*::KanMX4</i>
6A, B	Z37077	<i>cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 SGO1-AID*::KanMX4 ura3::P_{CUP1}-OsTIR-myc3::URA3</i>
6C, D	Z37078	<i>cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 SGO1-AID*::KanMX4 PDS1-AID*::KanMX4</i>
6C, D	Z37079	<i>cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 SGO1-AID*::KanMX4 PDS1-AID*::KanMX4 ura3::P_{CUP1}-OsTIR-myc3::URA3</i>
EV1A	Z33310	<i>SPC42/SPC42-eGFP::HIS3MX6 ura3::P_{HIS3}-mCherry-TUB1::URA3</i>
EV1A	Z33309	<i>SPC42/SPC42-eGFP::HIS3MX6 ura3::P_{HIS3}-mCherry-TUB1::URA3 spo12Δ::NatMX4</i>
EV1B	Z33305	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 MTW1-mCherry::HphMX4</i>
EV1B	Z33506	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 MTW1-mCherry::HphMX4 spo12Δ::NatMX4</i>
EV1C	Z15736	<i>RTS1-eGFP::KanMX4 MTW1-mCherry::HphMX4</i>
EV1C	Z34178	<i>RTS1-eGFP::KanMX4 MTW1-mCherry::HphMX4 spo12Δ::NatMX4</i>
EV1D	Z34052	<i>RTS1-mNeonGreen::KITRP1 rec8Δ::KanMX4::REC8-mScarlet::LEU2</i>
EV1D	Z34053	<i>RTS1-mNeonGreen::KITRP1 rec8Δ::KanMX4::REC8-mScarlet::LEU2 spo12Δ::NatMX4</i>
EV2A	Z34890	<i>REC8-ha3::URA3 CEN5/CEN5::tetO₂₂₄::HIS3 leu2/leu2::P_{URA3}-tetR-eGFP::LEU2 mad2Δ::KIURA3</i>
EV2A	Z34891	<i>REC8-ha3::URA3 CEN5/CEN5::tetO₂₂₄::HIS3 leu2/leu2::P_{URA3}-tetR-eGFP::LEU2 mad2Δ::KIURA3 spo12Δ::NatMX4</i>
EV2B	Z37473	<i>REC8-ha3::URA3 BMH1/BMH1::tetO₂₂₄::URA3 leu2/leu2::P_{URA3}-tetR-eGFP::LEU2 mad2Δ::KIURA3</i>
EV2B	Z37472	<i>REC8-ha3::URA3 BMH1/BMH1::tetO₂₂₄::URA3 leu2/leu2::P_{URA3}-tetR-eGFP::LEU2 mad2Δ::KIURA3 spo12Δ::NatMX4</i>

EV2C	Z34907	<i>ndt80Δ::NatMX4 leu2::P_{GALI}-NDT80::LEU2 his3::P_{GPD}-GAL4⁴⁸⁴-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KIURA3</i>
EV2C	Z34909	<i>ndt80Δ::NatMX4 leu2::P_{GALI}-NDT80::LEU2 his3::P_{GPD}-GAL4⁴⁸⁴-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KIURA3 trp1::P_{CUP1}-CDC20::TRP1</i>
EV2C	Z34908	<i>ndt80Δ::NatMX4 leu2::P_{GALI}-NDT80::LEU2 his3::P_{GPD}-GAL4⁴⁸⁴-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KIURA3 spo12Δ::NatMX4</i>
EV2C	Z34910	<i>ndt80Δ::NatMX4 leu2::P_{GALI}-NDT80::LEU2 his3::P_{GPD}-GAL4⁴⁸⁴-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KIURA3 spo12Δ::NatMX4 trp1::P_{CUP1}-CDC20::TRP1</i>
EV3 A, B	Z35712	<i>cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 PDS1-AID*::KanMX4</i>
EV4	Z23511	<i>cdc20::P_{CLB2}-CDC20::HphMX4 trp1::P_{CUP1}-CDC20::TRP1 his3::P_{GPD}-GAL4⁴⁸⁴- ER::HIS3 ura3::P_{GALI}-Ha3-REC8::URA3 PDS1-myc18::KITRPI gal4Δ::NatMX4</i>
EV4	Z23496	<i>cdc20::P_{CLB2}-CDC20::HphMX4 trp1::P_{CUP1}-CDC20::TRP1 his3::P_{GPD}-GAL4⁴⁸⁴- ER::HIS3 ura3::P_{GALI}-Ha3-rec8-24A::URA3 PDS1-myc18::KITRPI gal4Δ::NatMX4</i>
S1	Z33877	<i>ndt80Δ::NatMX4 leu2::P_{GALI}-NDT80::LEU2 his3::P_{GPD}-GAL4⁴⁸⁴-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3</i>
S1	Z33878	<i>ndt80Δ::NatMX4 leu2::P_{GALI}-NDT80::LEU2 his3::P_{GPD}-GAL4⁴⁸⁴-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 spo12Δ::NatMX4</i>
S2	Z36167	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3</i>
S2	Z36169	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mam1Δ::HIS3</i>
S2	Z36168	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KIURA3</i>
S2	Z36170	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mam1Δ::HIS3 mad2Δ::KIURA3</i>
S3	Z34026	<i>ndt80Δ::NatMX4 leu2::P_{GALI}-NDT80::LEU2 his3::P_{GPD}-GAL4⁴⁸⁴-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KIURA3</i>
S3	Z34025	<i>ndt80Δ::NatMX4 leu2::P_{GALI}-NDT80::LEU2 his3::P_{GPD}-GAL4⁴⁸⁴-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KIURA3 spo12Δ::NatMX4</i>
S4	Z35132	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad1Δ::KanMX4 mad2Δ::KIURA3 mad3Δ::KITRPI</i>
S4	Z35133	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad1Δ::KanMX4 mad2Δ::KIURA3 mad3Δ::KITRPI spo12Δ::NatMX4</i>
S4	Z35134	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad1Δ::KanMX4 mad2Δ::KIURA3 mad3Δ::KITRPI spo12Δ::NatMX4 mam1Δ::HIS3</i>
S5	Z32945	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3</i>

S5	Z33724	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3</i> <i>spo11Δ::NatMX4</i>
S5	Z30453	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3</i> <i>mad2Δ::KIURA3</i>
S5	Z33723	<i>rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3</i> <i>spo11Δ::NatMX4 mad2Δ::KIURA3</i>

¹Strains are listed for each figure from left to right and/or top to bottom. ²All SK1 strains are diploid with the background *MATa/MATα ho::LYS2 lys2 ade2Δ::hisG trp1Δ::hisG leu2Δ::hisG his3Δ::hisG ura3*. Mutations are homozygous unless stated otherwise.