Rossio et al., http://www.jcb.org/cgi/content/full/jcb.201007025/DC1

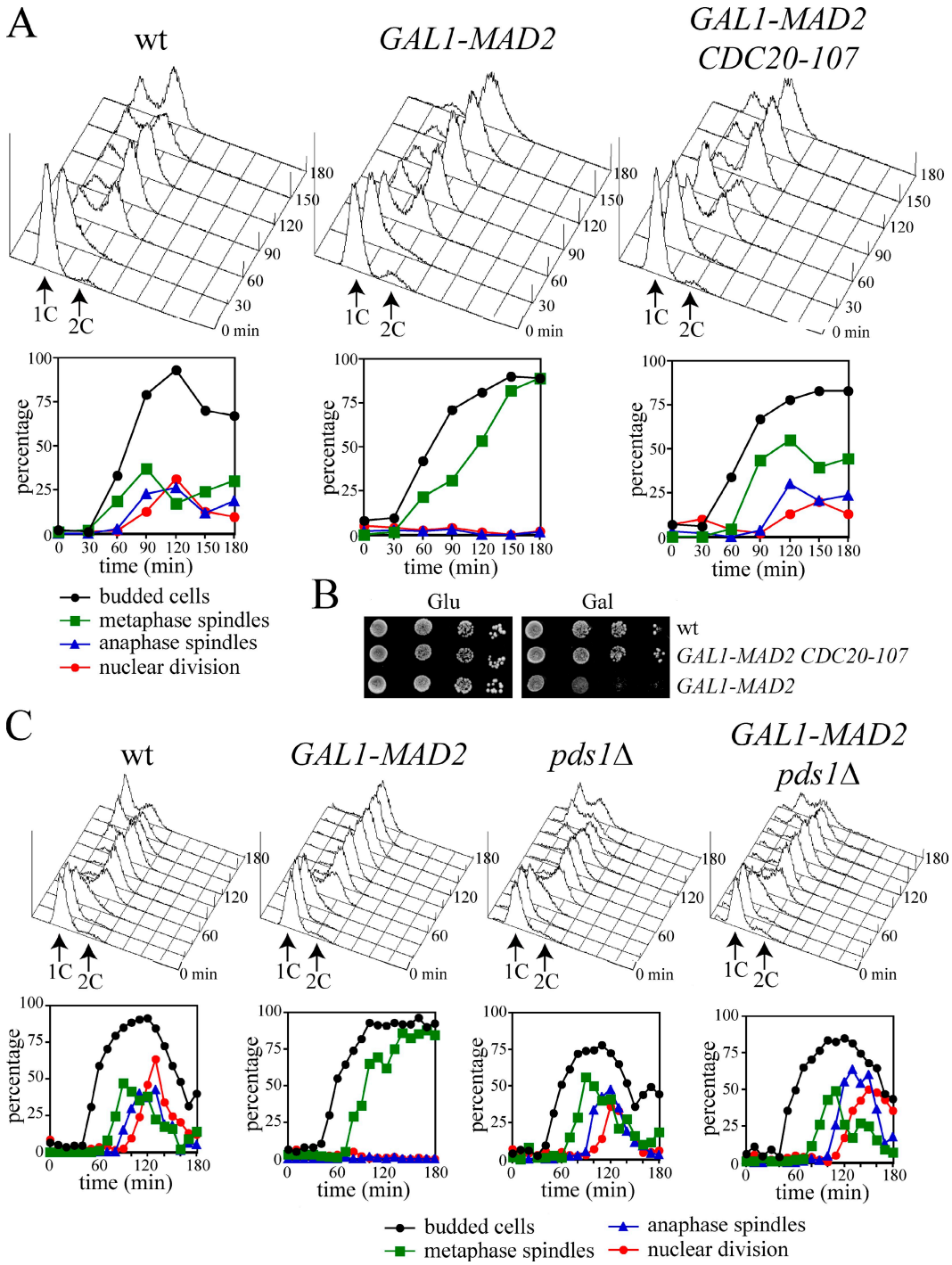

Figure S1. **The metaphase arrest caused by *MAD2* overexpression is only caused by SAC hyperactivation.** (A) Wild-type (wt; ySP5697), *GAL1-MAD2* (ySP6220), and *GAL1-MAD2 CDC20-107* (ySP6217) cells were grown in synthetic selective medium with raffinose, arrested in G1 with α-factor, and then released in YEPRG medium (t = 0). (B) Serial dilutions of the same strains used in A were spotted on appropriate selective glucose- and galactose-containing medium (Glu, *GAL1* promoter off; Gal, *GAL1* promoter on) and incubated for 2 d at 30°C. (C) Wild-type (W303), *GAL1-MAD2* (ySP6170), *pds1Δ* (ySP586), and *GAL1-MAD2 pds1Δ* (ySP6516) cells were grown in YEPR at 23°C, arrested in G1 with α-factor, and then released in YEPRG medium (t = 0) at 23°C. Samples were collected at the indicated times for FACS analysis of DNA contents and to follow kinetics of budding, nuclear division, and mitotic spindle formation/elongation.

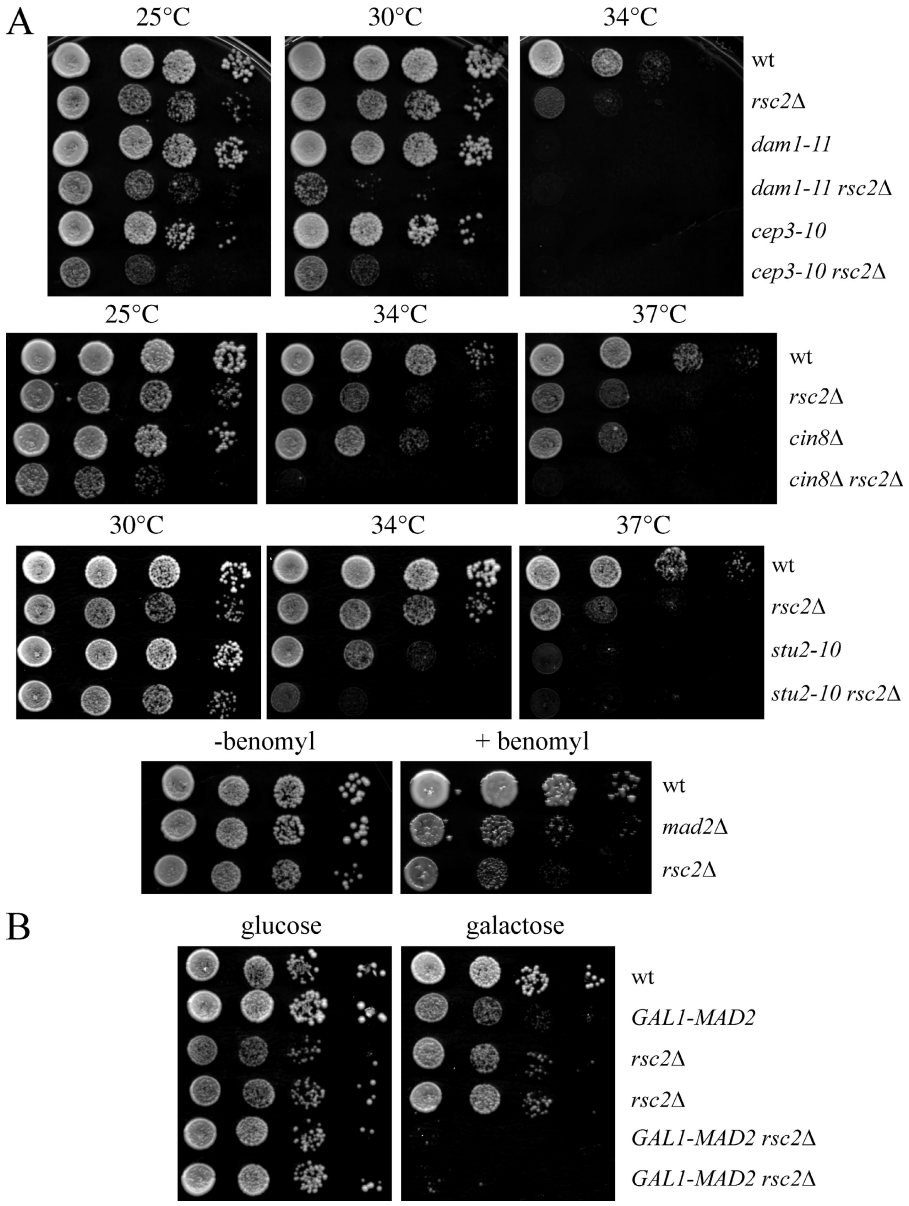

Figure S2. ***RSC2* deletion impairs growth efficiency of mutants defective in kinetochore components and microtubule-binding proteins.** (A) Serial dilutions of strains with the indicated genotypes were spotted on YEPD plates either lacking or containing benomyl and incubated for 2 d at the indicated temperatures. (B) Serial dilutions of strains with the indicated genotypes were spotted on YEPD (-Gal, *GAL1* promoter off) and YEPRG (+Gal, *GAL1* promoter on) plates and incubated for 2 d at 30°C. wt, wild type.

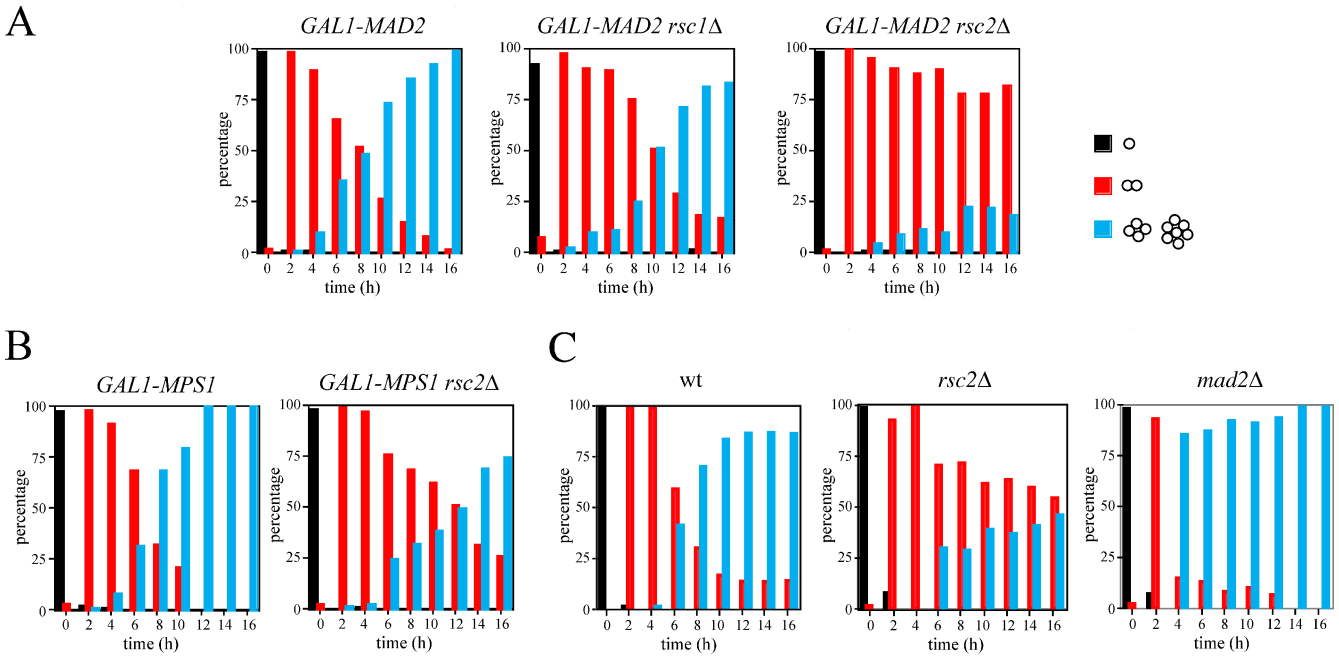

Figure S3. **Adaptation to SAC activation requires Rsc2 but not its paralogue Rsc1.** (A) *GAL1-MAD2* (ySP6170), *GAL1-MAD2 rsc1Δ* (ySP7279), and *GAL1-MAD2 rsc2Δ* (ySP6850) cells were grown in uninduced conditions, arrested in G1 with α-factor (unbudded cells), and spotted on YEPRG plates (t = 0). (B) *GAL1-MPS1* (ySP1941) and *GAL1-MPS1 rsc2Δ* (ySP7143) cells were grown in uninduced conditions, arrested in G1 with α-factor (unbudded cells), and spotted on YEPRG plates (t = 0). (C) Wild-type (wt; W303), *rsc2Δ* (ySP6858), and *mad2Δ* (ySP1070) cells were arrested in G1 with α-factor (unbudded cells) and spotted on YEPD plates containing benomyl (t = 0). 200 cells were scored at each time point for microcolony formation.

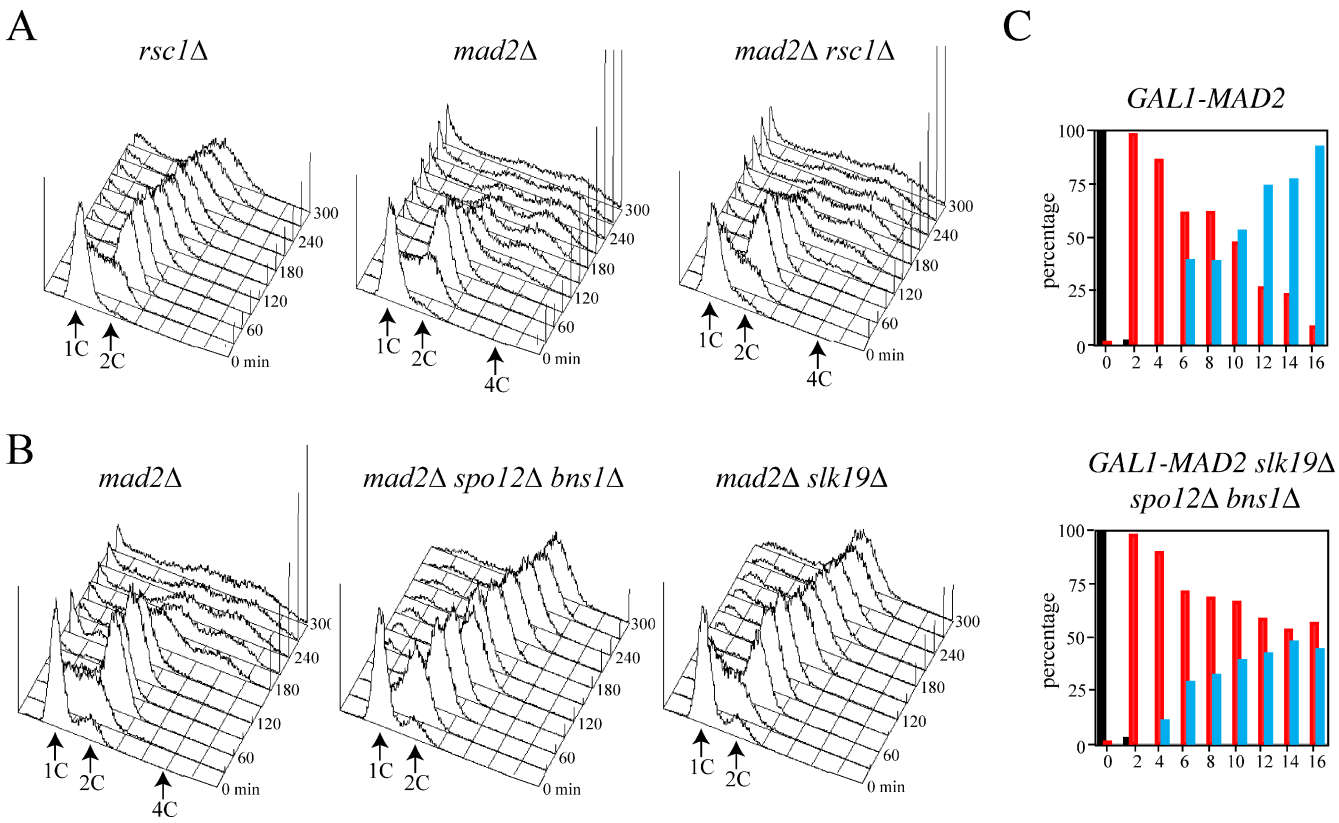

Figure S4. **The FEAR pathway, but not Rsc1, is required for SAC mutants to exit from mitosis upon spindle disruption and for adaptation to the SAC.** (A and B) Cultures of *rsc1Δ* (ySP7296), *mad2Δ* (ySP1070), and *mad2Δ rsc1Δ* (ySP8198; A) and cultures of *mad2Δ* (ySP1070), *mad2Δ spo12Δ bns1Δ* (ySP7810), and *mad2Δ slk19Δ* (ySP7637; B) cells were grown in YEPD, arrested in G1 by α-factor, and then released into medium containing nocodazole (t = 0). At the indicated times, cells were collected for FACS analysis of DNA contents. (C) *GAL1-MAD2* (ySP6170) and *GAL1-MAD2 slk19Δ spo12Δ bns1Δ* (ySP8505) were grown in uninduced conditions, arrested in G1 with α-factor (unbudded cells), and spotted on YEPRG plates (t = 0) to follow microcolony formation over time. Data are representative of three independent repeats.

Table S1. **Strains used in this study**

Name	Relevant genotype
ySP51	*MATa cdc15-2*
ySP284	*MATa cdc14-3*
ySP311	*MATα dbf2-2*
ySP324	*MATa cdc5-2::URA3*
ySP586	*MATa pds1::URA3*
ySP591	*MATa mad1::LEU2*
ySP993	*MATa cin8::TRP1*
ySP1070	*MATa mad2::TRP1*
ySP1084	*MATa mad2::TRP1 PDS1-myc18::LEU2*
ySP1131	*MATa cep3-10*
ySP1283	*MATa GAL1-BFA1-myc::URA3*
ySP1455	*MATa stu2-10*
ySP1941	*MATa GAL1-MPS1::URA3*
ySP2589	*MATa dam1-11::KanMX*
ySP3417	*MATa tem1::URA3 YCplac111-tem1-3*
ySP3418	*MATa lte1::URA3*
ySP3344	*MATa leu2::LEU2::4X GAL1-MAD2*
ySP4806	*MATa PDS1-myc18::LEU2*
ySP5697	*MATa YCplac22 TRP1*
ySP6170	*MATa ura3::4X URA3::GAL1-MAD2*
ySP6217	*MATa ura3::4X URA3::GAL1-MAD2 YCplac22-CDC20-107*
ySP6220	*MATa ura3::4X URA3::GAL1-MAD2 YCplac22*
ySP6273	*MATα ura3::4X URA3::GAL1-MAD2*
ySP6367	*MATa cdc14-1*
ySP6516	*MATa ura3::4X URA3::GAL1-MAD2 pds1::URA3*
ySP6769	*MATa leu2::LEU2::4X GAL1-MAD2 15::HIS3tetR-GFP ura3::3XURA3tetO$_{112}$ PDS1-myc18::LEU2*
ySP6850	*MATa ura3::URA3::4X GAL1-MAD2 rsc2::KanMX*
ySP6857	*MATα ura3::4X URA3::GAL1-MAD2 rsc2::KanMX*
ySP6858	*MATa rsc2::KanMX*
ySP6859	*MATα rsc2::KanMX*
ySP6970	*MATa rsc2::KanMX mad1::LEU2*
ySP6976	*MATa rsc2::KanMX dam1-11::KanMX*
ySP6997	*MATa rsc2::KanMX PDS1-myc18::LEU2*
ySP6699	*MATa bar1::kanMX4 leu2::LEU2 tetR-GFP ura3::URA3 224XtetO ura3::4X URA3::GAL1-MAD2*
ySP7022	*MATα rsc2::KanMX cep3-10*
ySP7088	*MATa mad2::TRP1 rsc2::KanMX*
ySP7092	*MATa RSC2-HA3::KlURA3*
ySP7143	*MATa GAL1-MPS1::URA3 rsc2::KanMX,*
ySP7279	*MATa rsc1::KanMX ura3::4X URA3::GAL1-MAD2*
ySP7296	*MATa rsc1::KanMX*
ySP7378	*MATa rsc2::KanMX cin8::TRP1*
ySP7471	*MATa rsc2::KanMX stu2-10*
ySP7543	*MATa mad2::TRP1 rsc2::KanMX PDS1-myc18::LEU2*
ySP7637	*MATa slk19::HIS3 mad2::TRP1*
ySP7644	*MATa ura3::4X URA3::GAL1-MAD2 trp1::TRP1::CDC14 $^{TAB6\text{-}1}$*
ySP7645	*MATa mad2::TRP1 rsc2::KanMX trp1::TRP1::CDC14 $^{TAB6\text{-}1}$*
ySP7677	*MATa ura3::4X URA3::GAL1-MAD2 bub2::HIS3*
ySP7764	*MATa GAL1-BFA1-myc::URA3 rsc2::kanMX*
ySP7797	*MATa CDC5-Flag3::KanMX*
ySP7803	*MATa bns1::KanMX spo12::HIS3 GAL1-BFA1-myc::URA3*
ySP7808	*MATa GAL1-UBR1::HIS3 sth1:: CUP1-HA-STH1^{td}::URA3 ura3::4X URA3::GAL1 MAD2*
ySP7810	*MATa bns1::KanMX spo12::HIS3 mad2::TRP1*
YSP7814	*MATa RSC2-HA3::KlURA3 CDC5-Flag3::KanMX*
ySP7838	*MATa cdc15-2 rsc2::KanMX*
ySP7841	*MATa dbf2-2 rsc2::KanMX*
ySP7842	*MATα tem1::URA3 YCplac111-tem1-3 rsc2::KanMX*
ySP7869	*MATa GAL1-UBR1::HIS3 sth1:: CUP1-HA-STH1^{td}::URA3 mad2::TRP1*
ySP7947	*MATa leu2::LEU2::GAL1-RSC2*

Table S1. **Strains used in this study** *(Continued)*

Name	Relevant genotype
ySP7958	*MATa net1::his5 NET1-6Cdk-TEV-myc9::TRP1 ura3::4X URA3::GAL1-MAD2*
ySP8018	*MATα leu2::LEU2::GAL1-RSC2, cdc15-2*
ySP8080	*MATa cdc14-1 rsc2::KanMX*
ySP8138	*MATa ura3::4X URA3::GAL1-MAD2 cdc20::MET3-HA3-CDC20::TRP1*
ySP8198	*MATa mad2::TRP1 rsc1::TRP1*
ySP8200	*MATa CDC5-Flag3::KanMX rsc2::KanMX*
ySP8226	*MATa ura3::4X URA3::GAL1-MAD2 cdc5::ura4 (Schizosaccharomyces pombe), leu2::LEU2::MET3-CDC5*
ySP8505	*MATa bns1::KanMX spo12::HIS3 slk19::HIS3 ura3::4X URA3::GAL1-MAD2*
ySP8526	*MATa PDS1-myc18::LEU2 ura3::4X URA3::GAL1-MAD2*
ySP8534	*MATa PDS1myc18::LEU2 tetR-GFP::LEU2 tetOs::URA3 bar1::kanMX*
ySP8573	*MATa CDC14-3HA NET1-3Myc*
ySP8596	*MATa rsc2::KanMX NET1-3Myc CDC14-3HA*
ySP8599	*MATa PDS1myc18::LEU2 ura3::4X URA3::GAL1-MAD2, bar1::kanMX*
ySP8704	*MATa cdc28::hisG::CDC28-F19::TRP1 ura3::4X URA3::GAL1-MAD2*
ySP8706	*MATa ura3::4X URA3::GAL1-MAD2 sic1::HIS3*
ySP8709	*MATa BUB3-HA3-klURA3 bar1::hisG*
ySP8710	*MATa ura3::URA3::GAL-CLB2-ΔDB leu2::LEU2::4X GAL1-MAD2*