Supporting Information

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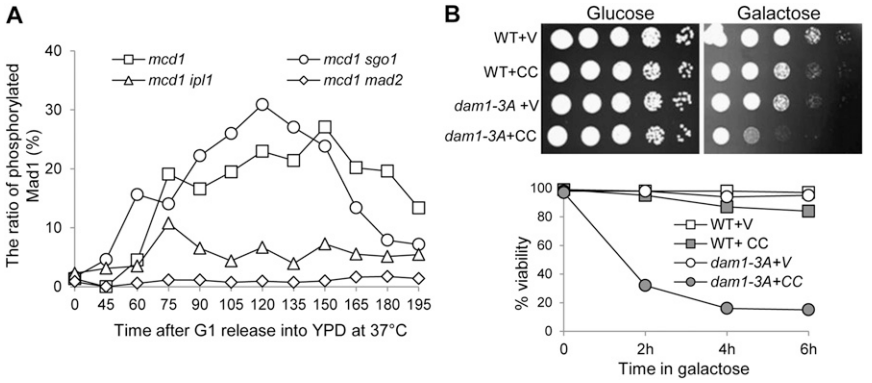

Fig. S1. (*A*) The relative level of phosphorylated mitotic arrest-deficient 1 (Mad1) in Fig. 1*B*. ImageJ program was used to quantify the percentage of phosphorylated Mad1 based on the Western blotting result in Fig. 1*B*. (*B*) *dam1–3A* (Duo1 and Mps1 interacting) mutant cells lose viability after *CIK1-CC* (the coiled-coil domain of CIK1 gene) overexpression. Serial 10-fold dilutions of WT and *dam1–3A* cells with a vector (V) or a P_{GAL}*CIK1-CC* (CC) plasmid were plated onto glucose and galactose plates and incubated at 25 °C for 3 d. G_1-arrested cells with the indicated genotypes were released into galactose medium at 25 °C. Cells were collected at 0, 2, 4, and 6 h and spread onto yeast peptone dextrose (YPD) plates to count the plating efficiency after overnight growth ($n > 300$).

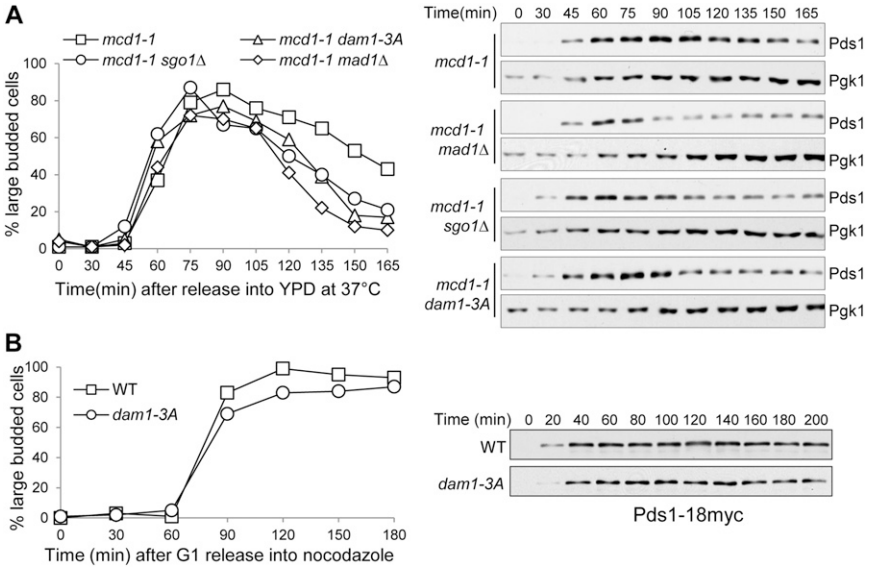

Fig. S2. *dam1–3A* mutant cells enter anaphase prematurely in the absence of tension. (*A*) *dam1–3A* mutation alleviates the delay of precocious dissociation of sisters 1 (Pds1) degradation in *mitotic chromosome determinant 1-1* (*mcd1-1*) mutants. *PDS1–18myc* (c-Myc) cells with the indicated genotypes were synchronized in G_1 phase at 25 °C, and then released into 37 °C YPD medium. Cell lysates were prepared every 15 min to determine the protein level of Pds1. Here we also show phosphoglycerate kinase 1 (Pgk1) protein levels as a loading control. (*B*) *dam1–3A* mutant cells show efficient cell cycle arrest in response to nocodazole treatment. G_1-arrested *PDS1–18myc* and *dam1–3A PDS1–18myc* cells were released into medium containing 20 μg/mL of nocodazole and incubated at 30 °C. Pds1 protein levels were determined after Western blotting. The budding index is shown on the *Left*, and Pds1 levels are shown on the *Right*.

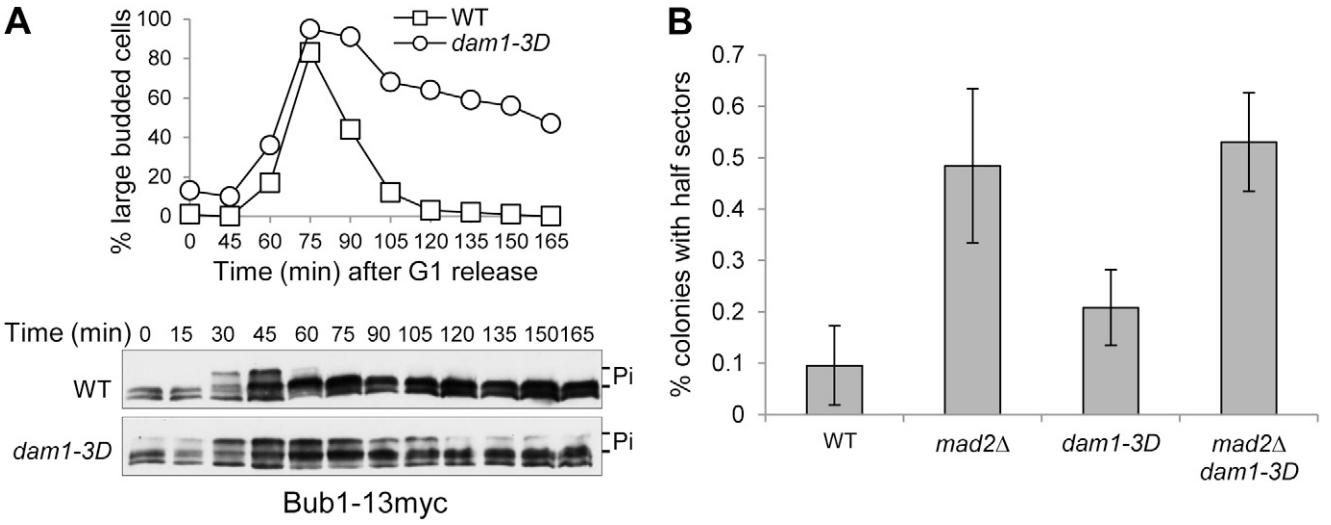

Fig. S3. (*A*) *dam1–3D* cells show impaired budding uninhibited by benzimidazole 1 (Bub1) dephosphorylation. *BUB1–13myc* and *dam1–3D BUB1–13myc* cells were first arrested in G_1 phase and then released into YPD medium at 30 °C. The budding index and cell-cycle–regulated Bub1 modification are shown. (*B*) Determination of chromosome loss rates. Strains harboring chromosome III fragment (CFIII) were grown in uracil drop-out medium at 30 °C to log-phase and then shifted to YPD medium for 3 h. The cells were plated out on YPD plates at 30 °C and incubated for 3 d. The percentage of colonies with half sectors is calculated. The experiment was performed four times, and more than 2,000 colonies were counted for each sample.

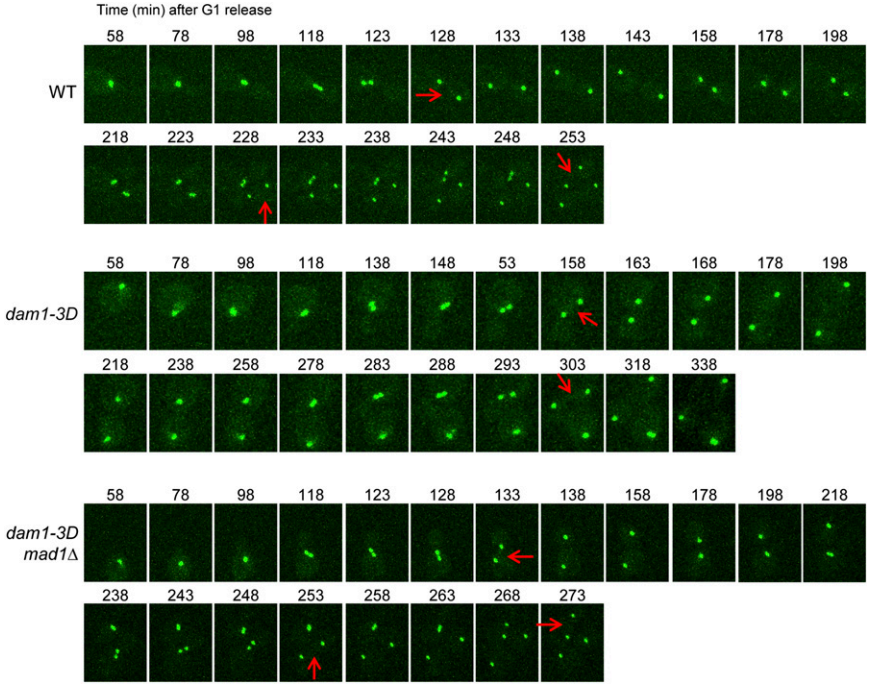

Fig. S4. Live-cell images showing kinetochore cluster segregation in WT, *dam1–3D*, and *dam1–3D mad1*Δ cells. Shown is the segregation of mis twelve-like 1 (Mtw1)-GFP clusters in a representative cell for each genotype. The experimental procedure was described in Fig. 4*C*. One daughter cell of the *dam1–3D* mutant did not segregate kinetochore clusters until 368 min, which is not shown in this figure.

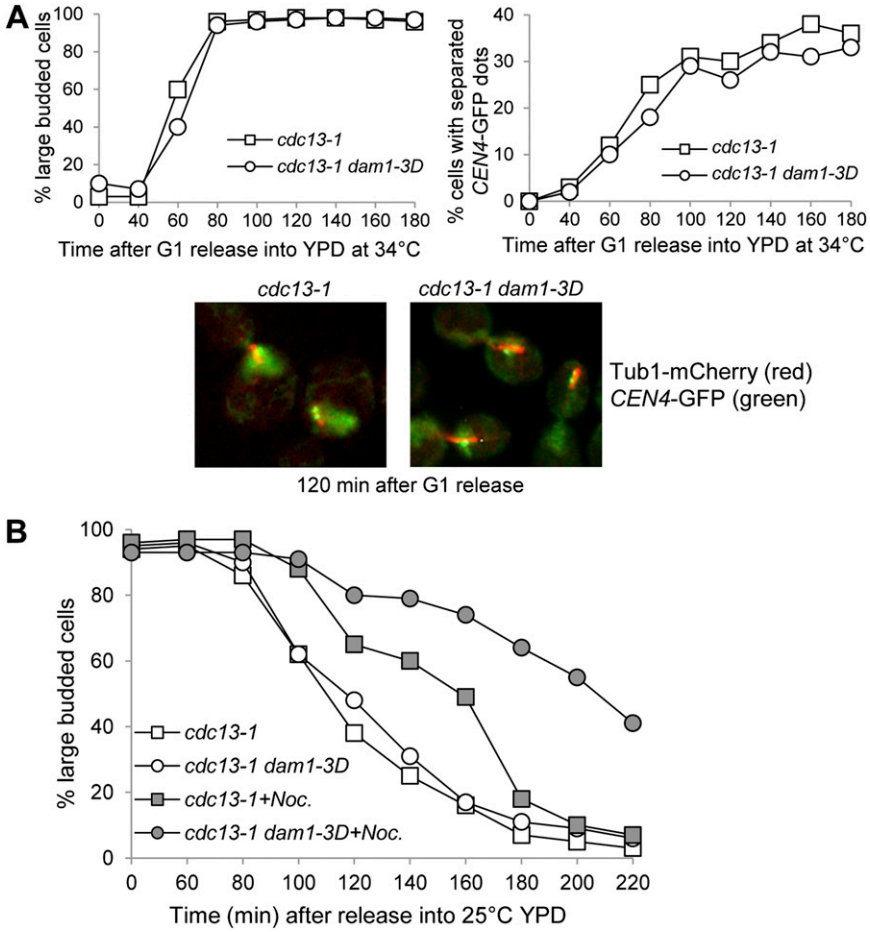

Fig. S5. *dam1–3D* mutants show spindle assembly checkpoint silencing defect. (*A*) *dam1–3D* mutant cells show normal kinetics of sister centromere separation. *Cell division cycle 13-1* (*cdc13-1*) and *cdc13-1 dam1–3D* cells with *TUB1–mCherry CEN4–GFP* were synchronized in G_1 phase and then released into the cell cycle at 34 °C, the restrictive temperature for *cdc13-1*. Cells were collected every 20 min to examine the spindle morphology (Tub1–mCherry, red) and separation of centromere on chromosome IV (*CEN4*–GFP, green). The appearance of two GFP dots indicates bipolar attachment that applies tension and separates sister centromeres. The budding index and the percentage of cells with two GFP dots are shown in the *Upper* panels. Some representative images for cells at 120 min are shown in the *Lower* panel. (*B*) The kinetics of anaphase onset in *dam1–3D* cells after nocodazole treatment. G_1-arrested *cdc13-1* and *cdc13-1 dam1–3D* cells were released into 32 °C YPD medium with or without 20 μg/mL nocodazole (Noc) for 2 h. After nocodazole washout, the cells were released into 25 °C YPD medium. α-factor was added back to block the second round of cell cycle. The percentage of large-budded cells was counted over time ($n > 100$).

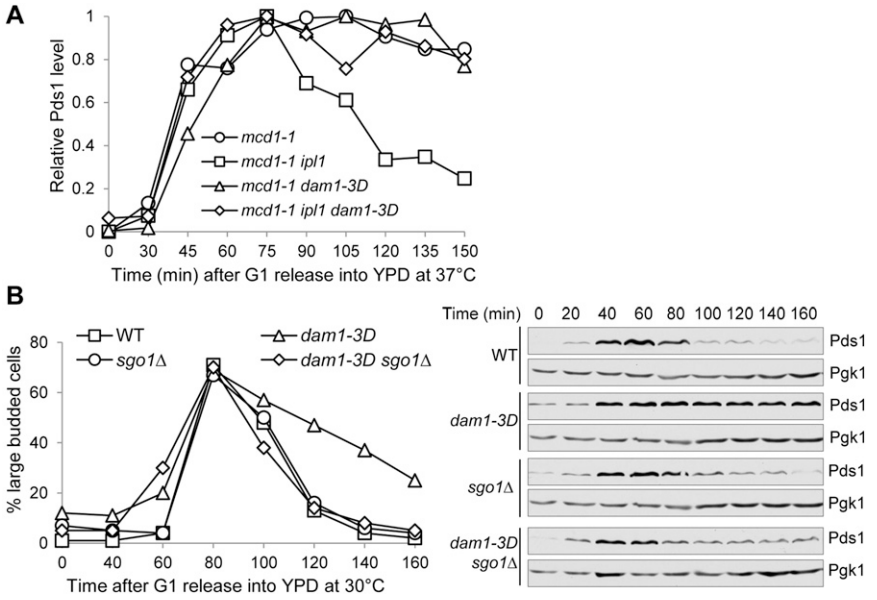

Fig. S6. Genetic analysis of the SAC silencing network network. (*A*) The relative Pds1 protein levels for Fig. 5*A*. ImageJ program was used to quantify the protein level of Pds1 in Fig. 5*A*. For each strain, we set the protein level at 75 min after G_1 release as 1. The relative Pds1 protein levels during the cell cycle were calculated. (*B*) *sgo1Δ* (ShuGOshin) suppresses the anaphase entry delay in *dam1–3D* mutants. G_1-synchronized cells with the indicated genotypes were released into cell cycle at 30 °C. α-factor was added back to block the subsequent cell cycle. Cells were collected over time to determine Pds1 protein levels. Pgk1 protein levels are used as a loading control.

Table S1. Strains used in this study

Strains	Relevant genotypes	Reference
Y300	*MAT***a** *ura3-1 his3–11,15 leu2-3,112 trp1-1 ade2-1 can1–100*	Lab stock
2781–9-1	*MAT***a** *mcd1-1 trp1::Sphis5*$^{+}$ *MAD1–HA3–URA3*	This study
2730–1-3	*MAT***a** *mcd1-1 trp1::Sphis5*$^{+}$ *ipl1–321 MAD1–HA3–URA3*	This study
2731–3-1	*MAT***a** *mcd1-1 trp1::Sphis5*$^{+}$ *sgo1Δ::KanMX MAD1–HA3–URA3*	This study
2781–5-1	*MAT***a** *mcd1-1 trp1::Sphis5*$^{+}$ *mad2Δ::LEU2 MAD1–HA3–URA3*	This study
2719–3-4	*MAT***a** *MAD1–HA3–URA3*	This study
2723–4-4	*MAT***a** *sgo1Δ::KanMX MAD1–HA3–URA3*	This study
2722–2-2	*MAT***a** *ipl1–321 MAD1–HA3–URA3*	This study
YYW141	*MAT***a** *promURA3::tetR::GFP–LEU2 CENIV::tetOX448::URA3 TUB1–mCherry::URA3*	Lab stock
2320–2-4	*MAT***a** *dam1(S257A S265A S292A)::KanMX promURA3::tetR::GFP-LEU2 CENIV::tetOX448::URA3 TUB1–mCherry::URA3*	This study
2425–11-3	*MAT***a** *mcd1-1 PDS1–18myc::LEU2*	This study
2450–1-1	*MAT***a** *mcd1-1 mad1Δ::HIS3 PDS1–18myc::LEU2*	This study
2436–3-3	*MAT***a** *mcd1-1 sgo1Δ::KanMX PDS1–18myc::LEU2*	This study
2425–6-4	*MAT***a** *mcd1-1 dam1(S257A S265A S292A)::KanMX PDS1–18myc::LEU2*	This study
300–1-1	*MAT***a** *PDS1–18myc::LEU2*	Lab stock
2376–9-3	*MAT***a** *dam1(S257A S265A S292A) –KanMX PDS1–18myc::LEU2*	This study
2718–7-2	*MAT***a** *dam1(S257A S265A S292A)::KanMX MAD1–HA3–URA3*	This study
2782–2-2	*MAT***a** *mcd1-1 trp1::Sphis5*$^{+}$ *dam1(S257A S265A S292A)::KanMX MAD1–HA3–URA3*	This study
2862–9-4	*MAT***a** *mcd1-1 trp1::Sphis5*$^{+}$ *BUB1–13myc::Sphis5*$^{+}$	This study
2862–4-4	*MAT***a** *mcd1-1 trp1::Sphis5*$^{+}$ *dam1(S257A S265A S292A)::KanMX BUB1–13myc::Sphis5*$^{+}$	This study
DDY2496	*MAT***a** *his3Δ200 leu2–3,112 ura3–52 dam1(S257D S265D S292D)::KanMX*	Georjana Barnes (University of California, Berkeley, CA)
2769–1-2	*MAT***a** *dam1(S257D S265D S292D)::KanMX MAD1–HA3–URA3*	This study
2859–1-4	*MAT***a** *dam1(S257D S265D S292D)::KanMX BUB1–13myc::Sphis5*$^{+}$	This study
771–4-1	*MAT***a** *mad1Δ::HIS3–PDS1–18myc::LEU2*	This study
2848–8-3	*MAT***a** *dam1(S257D S265D S292D)::KanMX PDS1–18myc::LEU2*	This study
2848–2-2	*MAT***a** *mad1Δ::HIS3 dam1(S257D S265D S292D)::KanMX PDS1–18myc::LEU2*	This study
2686–1-2	*MAT***a** *mad2Δ::URA3 PDS1–18myc::LEU2*	This study
2686–4-1	*MAT***a** *mad2Δ::URA3 dam1(S257D S265D S292D)::KanMX PDS1–18myc::LEU2*	This study
2849–2-2	*MAT***a** *ipl1–321 dam1(S257A S265A S292A)::KanMX MAD1–HA3–URA3*	This study
2880–14-1	*MAT***a** *mcd1-1 trp1::Sphis5*$^{+}$ *PDS1–18myc::LEU2*	This study
2880–3-1	*MAT***a** *mcd1-1 trp1::Sphis5*$^{+}$ *dam1(S257D S265D S292D)::KanMX–PDS1–18myc::LEU2*	This study
2880–2-3	*MAT***a** *mcd1-1 trp1::Sphis5*$^{+}$ *ipl1–321 PDS1–18myc::LEU2*	This study
2880–9-2	*MAT***a** *mcd1-1 trp1::Sphis5*$^{+}$ *ipl1–321 dam1(S257D S265D S292D)::KanMX PDS1–18myc::LEU2*	This study
1091–5-3	*MAT***a** *cdc13-1 promURA3::tetR::GFP–LEU2 CENIV::tetOX448::URA3 TUB1–mCherry::URA3*	Lab stock
2419–1-1	*MAT***a** *cdc13-1 dam1(S257D S265D S292D)::KanMX promURA3::tetR::GFP–LEU2 CENIV::tetOX448::URA3 TUB1–mCherry::URA3*	This study
2902–3-2	*MAT***a** *DAM1–3HA:: Sphis5*$^{+}$	This study
2902–1-1	*MAT***a** *sgo1Δ::KanMX DAM1–3HA:: Sphis5*$^{+}$	This study
683–15-3	*MAT***a** *MTW1–3GFP::HIS3*	Lab stock
2975–2-1	*MAT***a** *dam1(S257D S265D S292D)::KanMX MTW1–3GFP::HIS3*	This study
2971–3-3	*MAT***a** *dam1(S257D S265D S292D)::KanMX mad1Δ::HIS3 MTW1–3GFP::HIS3*	This study
2973–3-2	*MATα CFIII(URA3,sup11)*	This study
2973–12-3	*MAT***a** *dam1(S257D S265D S292D)::KanMX CFIII(URA3,sup11)*	This study
2973–2-3	*MAT***a** *mad2Δ::LEU2 CFIII(URA3,sup11)*	This study
2973–4-1	*MAT***a** *dam1(S257D S265D S292D)::KanMX mad2Δ::LEU2CFIII(URA3,sup11)*	This study
2851–4-2	*MAT***a** *sgo1Δ::TRP1 PDS1–18myc::LEU2*	This study
2851–3-3	*MAT***a** *sgo1Δ::TRP1 dam1(S257D S265D S292D)::KanMX PDS1–18myc::LEU2*	This study

Ade, adenine requiring; *can*, canavanine resistance; *CENIV*, centromere on chromosome IV; *ipl*, increase in ploidy; *KanMX*, kanamycin resistance; *MATa*, mating type a; *prom*, promoter; *Sphis*, histidine gene from S. pombe; *sup*, suppressor; *tetO*, tetracyclin operator; *trp*, tryptophan requiring; *ura*, uracil requiring.