

Table S1 Yeast strains used in this study. All strains are isogenic with the W303 background and were generated by standard procedures. Plasmids are indicated in brackets.

Strain	Genotype
SBY2318	<i>MATa ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3]</i>
SBY5948	<i>MATa ura3-1 leu2,3-112 his3-11::DSN1::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3][pSB1097; DSN1, HIS3]</i>
SBY5949	<i>MATa ura3-1 leu2,3-112 his3-11::dsn1-S240A,S250A::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3][pSB1099; dsn1-S240A,S250A, HIS3]</i>
SBY5950	<i>MATa ura3-1 leu2,3-112 his3-11::dsn1-S240D,S250D::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3][pSB1104; dsn1-S240D,S250D, HIS3]</i>
SBY5952	<i>MATa ura3-1 leu2,3-112 his3-11::dsn1-S240A,S250A,S264A::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3][pSB1108; dsn1-S240A,S250A,S264A, HIS3]</i>
SBY5954	<i>MATa ura3-1 leu2,3-112 his3-11::dsn1-S240D,S250D::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB1104; dsn1-S240D,S250D, HIS3]</i>
SBY5956	<i>MATa ura3-1 leu2,3-112 his3-11::dsn1-S240A,S250A,S264A::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB1108; dsn1-S240A,S250A,S264A, HIS3]</i>
SBY6072	<i>MATa ura3-1 leu2,3-112 his3-11::dsn1-S264A::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3][pSB944; dsn1-S264A, HIS3]</i>
SBY7441	<i>MATa ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 lys2Δ can1-100 bar1Δ DSN1-3FLAG::KanMX</i>
SBY7774	<i>MATa ura3-1 leu2,3-112 his3-11::DSN1-3GFP::HIS3 trp1-1 ade2-1 LYS2</i>

can1-100 dsn1::KanMX bar1Δ

SBY7775 *MATa ura3-1 leu2,3-112 his3-11::DSN1-S240D,S250D-3GFP::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ*

SBY7776 *MATa ura3-1 leu2,3-112 his3-11::DSN1-S240A,S250A,S264A-3GFP::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ*

SBY7823 *MATa ura3-1::pCMV-LacI-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LYS2 BARI NDC80-13myc::KAN ipl1-321 DSN1-3HA::HIS3 [WT CEN3 Minichromosome, TRP1]*

SBY7824 *MATa ura3-1::pCMV-LacI-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LYS2 BARI NDC80-13myc::KAN DSN1-3HA::HIS3 [WT CEN3 Minichromosome, TRP1]*

SBY7902 *MATa ura3-1::DSN1-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 can1-100 bar1Δ dsn1::KanMX*

SBY7904 *MATa ura3-1::DSN1-S240A,S250A,S264A-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 can1-100 bar1Δ dsn1::KanMX*

SBY8037 *MATa ura3-1::DSN1-S240D,S250D-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 can1-100 bar1Δ dsn1::KanMX*

SBY8120 *MATa ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 lys2Δ can1-100 bar1Δ ipl1-321 DSN1-3FLAG::KanMX*

SBY8123 *MATa ura3-1::DSN1-S264A-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 can1-100 bar1Δ dsn1::KanMX*

SBY8338 *MATa ura3-1::DSN1-12MYC::URA3 leu2,3-112::LacI-FLAG::LEU2 his3-11 trp1-1 ade2-1 can1-100 LYS2 bar1 dsn1::KanMX [pSB963, CEN3, 8lacO, TRP1]*

SBY8339 *MATa ura3-1::DSN1-S240D,S250D-12MYC::URA3 leu2,3-112::LacI-FLAG::LEU2 his3-11 trp1-1 ade2-1 can1-100 LYS2 bar1 dsn1::KanMX [pSB963, CEN3, 8lacO, TRP1]*

SBY8340 *MATa ura3-1::DSN1-S240A,S250A,S264A-12MYC::URA3 leu2,3-*

*112::LacI-FLAG::LEU2 his3-11 trp1-1 ade2-1 can1-100 LYS2 bar1
 dsn1::KanMX [pSB963, CEN3, 8lacO, TRP1]*

SBY8341 *MATa ura3-1::DSN1-S264A-12MYC::URA3 leu2,3-112::LacI-
 FLAG::LEU2 his3-11 trp1-1 ade2-1 can1-100 LYS2 bar1 dsn1::KanMX
 [pSB963, CEN3, 8lacO, TRP1]*

SBY11599 *MATa ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 lys2Δ can1-100
 mad2::URA3 bar1Δ*

SBY11600 *MATa ura3-1 leu2,3-112 his3-11::dsn1-S240D,S250D::HIS3 trp1-1 ade2-1
 lys2Δ can1-100 mad2::URA3 bar1Δ dsn1::KanMX bar1Δ [pSB1104; dsn1-
 S240D,S250D, HIS3]*

SBY11601 *MATa ura3-1 leu2,3-112 his3-11:: dsn1-S240A,S250A,S264A::HIS3 trp1-1
 ade2-1 lys2Δ can1-100 mad2::URA3 dsn1::KanMX bar1Δ [pSB1108; dsn1-
 S240A,S250A,S264A, HIS3]*

Media and genetic and microbial techniques were essentially as described (Rose et al., 1990). Phospho-mutants were made by Quickchange site-directed mutagenesis (Stratagene) and are described in (AKIYOSHI *et al.* 2013).

Supplemental Literature Cited

AKIYOSHI, B., C. R. NELSON, N. DUGGAN, S. CETO, J. A. RANISH *et al.*, 2013 The Mub1/Ubr2 ubiquitin ligase complex regulates the conserved Dsn1 kinetochore protein. *PLoS Genet* **9**: e1003216.