

Germann et al., <http://www.jcb.org/cgi/content/full/jcb.201305157/DC1>

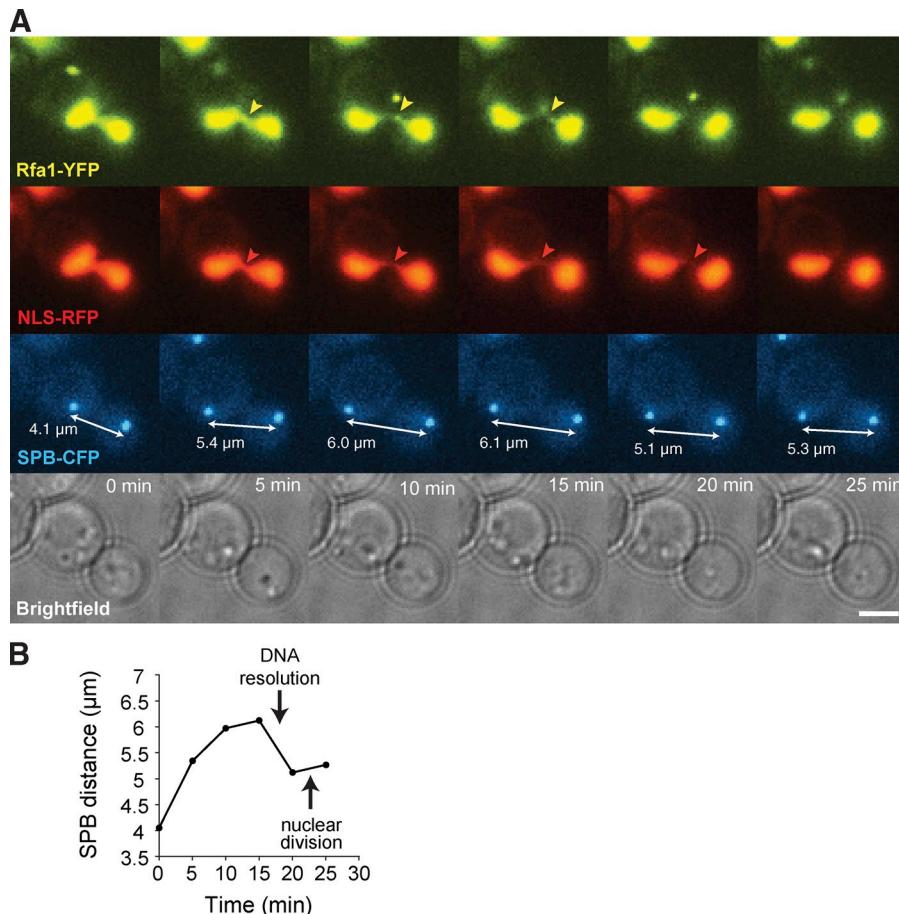


Figure S1. Rfa1 binds yeast anaphase DNA bridges. (A) Time-lapse microscopy of Rfa1. Cells (ML658-4C) expressing Rfa1-YFP, Spc110-CFP, and NLS-RFP were grown to exponential phase and subjected to time-lapse microscopy ($n = 7$). Bar, 3 μm . (B) Resolution of Rfa1 bridges is coincidental with relaxation of the mitotic spindle. The distance between the SPBs (Spc110-CFP) in A was measured in 3D and plotted as a function of time.

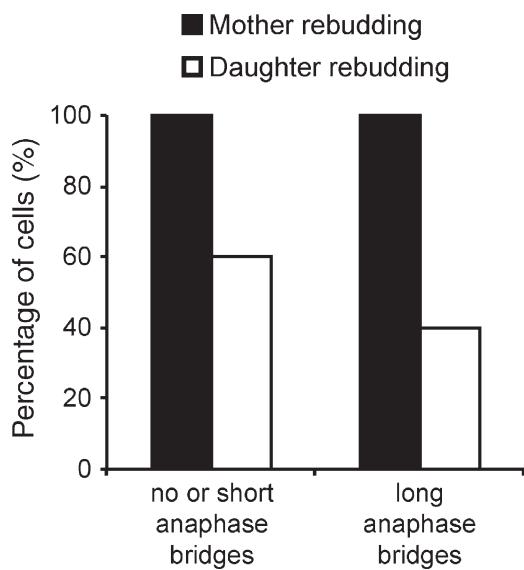


Figure S2. Rebudding after resolution of anaphase bridges. Cells (ML628) expressing (*Tet-off* promoter) Dpb11-YFP, Spc110-CFP, and NLS-RFP were followed by time-lapse microscopy in SC+Ade medium for 12.4 h to determine the fate of cells containing Dpb11 bridges. The cells were divided into two populations exhibiting either no bridges or bridges shorter than the median length or exhibiting bridges longer than the median. No statistically significant difference was found between the two populations ($n = 37$).

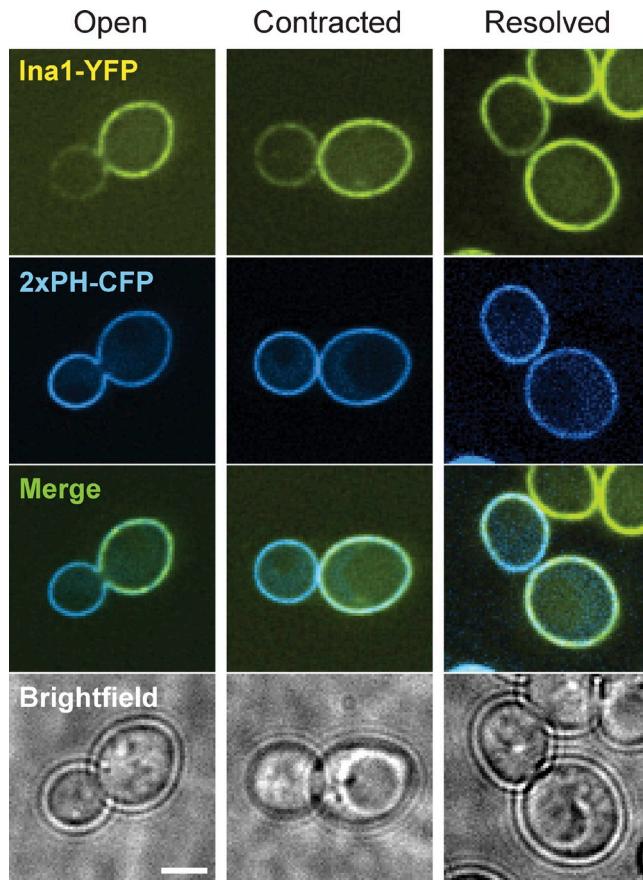


Figure S3. Validation of Ina1 as a marker for abscission. Cells (ML719) expressing Ina1-YFP from its endogenous promoter and 2xPH-CFP from a 2- μ m-based plasmid (pML105) were grown in SC-Ura. (A) Ina1-YFP and 2xPH-CFP colocalize. Images of representative cells at different stages (open, contracted, and resolved) of abscission are shown. The abscission index (contracted/resolved) was calculated to 0.37 and 0.48 based on inspection of the Ina1-YFP and 2xPH-CFP markers, respectively, in 200–300 cells. The obtained abscission indices are not significantly different for the two markers ($P = 0.50$, χ^2 test).

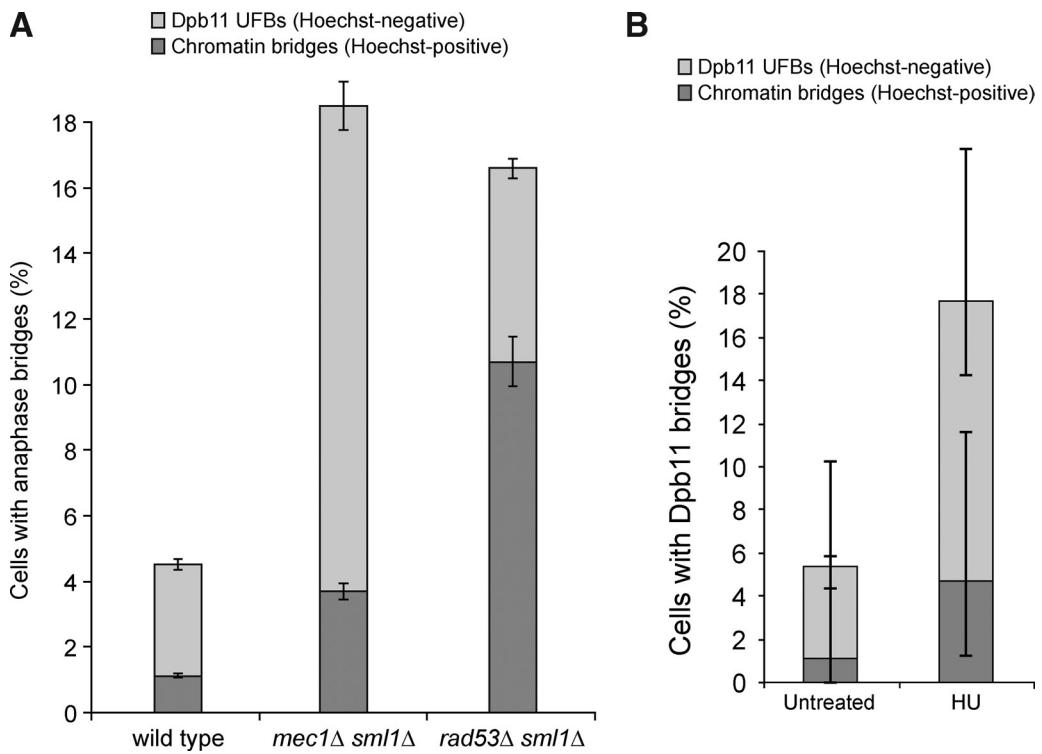


Figure S4. Dpb11 bridges accumulate in checkpoint mutants and during replication stress. (A) Dpb11 bridges accumulate in *mec1Δ* and *rad53Δ* mutants. Wild-type (ML628), *mec1Δ sml1Δ* (ML689-2B), and *rad53Δ sml1Δ* (ML692-6A) mutant strains were grown to exponential phase at 25°C, stained with Hoechst, and examined for Dpb11-YFP and Hoechst bridges. (B) Hydroxyurea induces Dpb11 UFBs. Wild-type (ML628) cells were treated with 20 mM hydroxyurea (HU) at 25°C for 1 h, stained with Hoechst, and examined for Dpb11-YFP and Hoechst bridges.

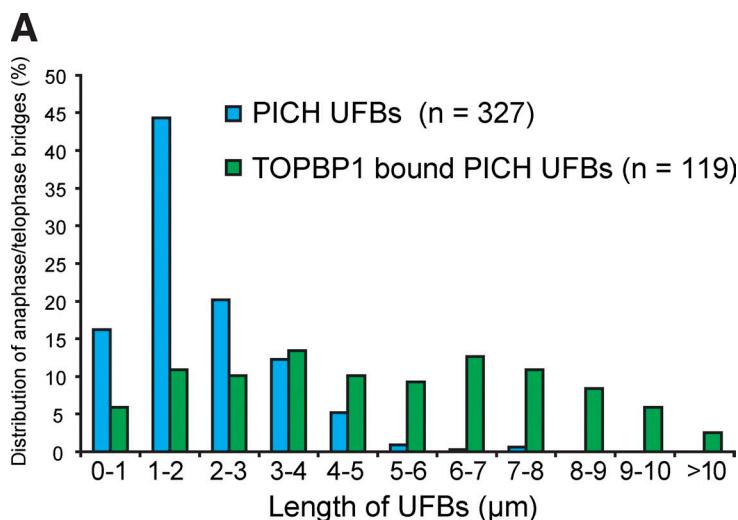
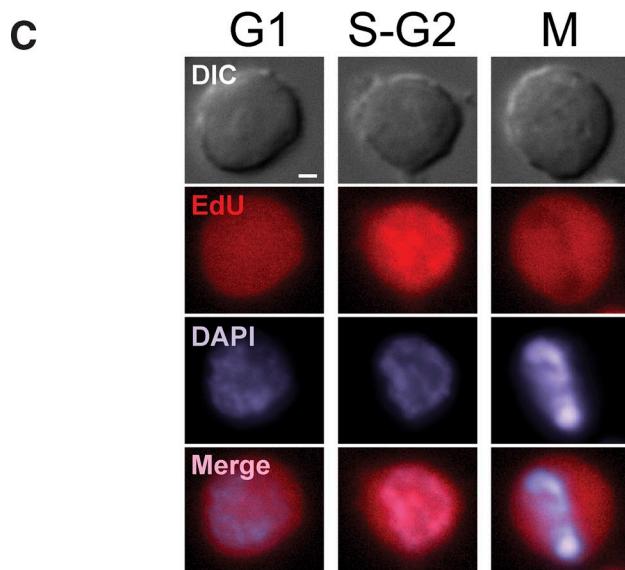
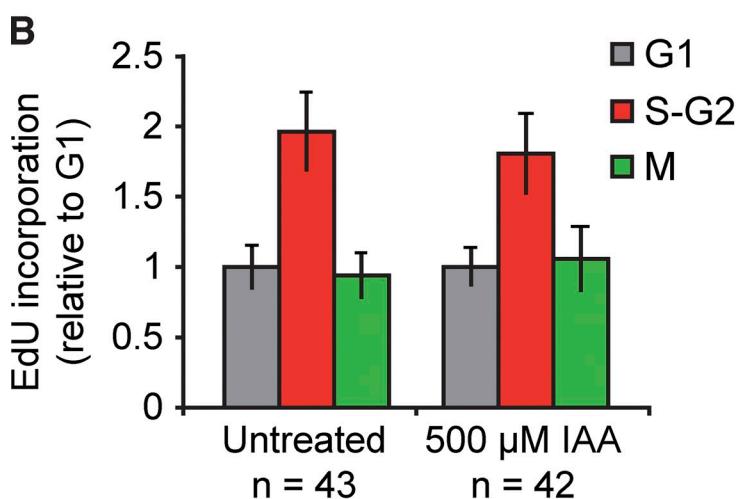
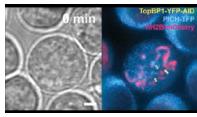
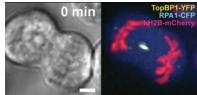


Figure S5. Replication in TopBP1-depleted cells and localization of TopBP1 in DT40 cells. (A) PICH UFBs that extend beyond 5 μm are exclusively bound by TopBP1. Length distribution of TopBP1-positive and -negative PICH UFBs was quantified based on the measured length of UFBs in Fig. 7 F (IAA), Fig. 7 G (APH) and Fig. 7 H (ATR). (B) DNA synthesis is not detectable in cells 30 min before mitotic onset regardless of TopBP1 depletion. Cells expressing TopBP1-YFP-AID, PICH-TFP, and randomly integrated OsTIR and hH2B-mCherry (RTP143) were treated with 10 μM EdU and either 500 μM IAA or 0.2% ethanol (vol/vol, untreated) for 30 min before imaging. G1, S-G2, and M phase cells were identified based on DAPI staining and Alexa Fluor 594 staining of incorporated EdU. EdU incorporation was quantified based on the intensity of the Alexa Fluor 594 signal. Error bars represent SDs. The number of cells analyzed is indicated in the graphs (n). (C) EdU incorporation is only detectable in S-G2 phase cells. Representative images of cells from B.





Video 1. TopBP1 marks sites to become TopBP1-bound UFBs from prometaphase. Representative time-lapse microscopy of TopBP1-YFP-AID (yellow), PICH-TFP (blue), and hH2B-mCherry (red) in cell line RTP151 used for quantification in Fig. 7 E (ATRi). Images were acquired every 2 min for 18 min. Bar, 3 μ m.



Video 2. RPA foci colocalize with TopBP1 bridges and persist into the next G1 phase. Representative time-lapse microscopy of TopBP1-YFP (yellow), RPA1-CFP (blue), and hH2B-mCherry (red) in cell line RTP156 used for quantification in Fig. 7 B (untreated). Images were acquired every 2 min for 18 min. Bar, 3 μ m.

Table S1. Yeast strains used in this study

| Strain | Genotype | Source |
|------------|--|-------------------------|
| ML253 | MAT α DPB11-4ala-YFP | Germann et al., 2011 |
| ML412 | MAT α/α LYS2/lys2 trp1-1/TRP1 leu2-ΔBs β Ell/leu2-ΔEcoRI | Altmannova et al., 2010 |
| ML533 | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX | Germann et al., 2011 |
| ML533-5D | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-1-YFP::KanMX hmrΔ::URA3 | This study |
| ML628 | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX NLS-yEmRFP _Y Prv::URA3 TKp404::TRP1 SPC110-CFP::KAN | This study |
| ML658-4C | MAT α RFA1-8ala-YFP NLS-yEmRFP _Y Prv::URA3 SPC110-CFP::KAN | This study |
| ML689-2B | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX NLS-yEmRFP _Y Prv::URA3 SPC110-CFP::KAN mec1::TRP1 sml1::HIS3 | This study |
| ML692-6A | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX NLS-yEmRFP _Y Prv::URA3 SPC110-CFP::KAN rad53::HIS3 sml1::URA3 | This study |
| ML719 | MAT α INA1-4ala-YFP | This study |
| ML734-9B | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX NLS-yEmRFP _Y Prv::URA3 SPC110-CFP::KAN INA1-4ala-CFP | This study |
| ML734-11D | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX NLS-yEmRFP _Y Prv::URA3 SPC110-CFP::KAN INA1-4ala-CFP top2-1ts | This study |
| ML737-3C | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX NLS-yEmRFP _Y Prv::URA3 SPC110-CFP::KAN INA1-4ala-CFP ahc1::NatMX | This study |
| ML735-1C | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX NLS-yEmRFP _Y Prv::URA3 SPC110-CFP::KAN INA1-4ala-CFP slk19::NatMX | |
| ML735-13A | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX NLS-yEmRFP _Y Prv::URA3 SPC110-CFP::KAN INA1-4ala-CFP slk19::NatMX top2-1ts | |
| ML737-11A | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX NLS-yEmRFP _Y Prv::URA3 SPC110-CFP::KAN INA1-4ala-CFP ahc1::NatMX top2-1ts | This study |
| ML762 | MAT α/α LYS2/lys2 trp1-1/TRP1 leu2-ΔBs β Ell/leu2-ΔEcoRI ahc1::NatMX/ahc1::NatMX | This study |
| ML767 | MAT α/α LYS2/lys2 trp1-1/TRP1 leu2-ΔBs β Ell/leu2-ΔEcoRI tTA(tetR-VP16)-tetO ₂ - DPB11::KanMX/ tTA(tetR-VP16)-tetO ₂ -DPB11::KanMX | This study |
| ML768 | MAT α/α LYS2/lys2 trp1-1/TRP1 leu2-ΔBs β Ell/leu2-ΔEcoRI ahc1::NatMX/ahc1::NatMX tTA(tetR-VP16)-tetO ₂ -DPB11::KanMX/ tTA(tetR-VP16)-tetO ₂ -DPB11::KanMX | This study |
| SMG216-10A | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-YFP::KanMX RFA1-CFP | This study |
| SMG247-4A | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-CFP::KanMX TOP3-YFP | This study |
| SMG219-5D | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-YFP::KanMX HTZ1-CFP | This study |
| SMG220-15A | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-YFP::KanMX RSC1-CFP | This study |
| SMG221-15D | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-YFP::KanMX NHP10-CFP | This study |
| SMG260-7C | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-CFP::KanMX RAD52-RFP YFP-SML7 | This study |
| SMG223-2C | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-YFP::KanMX sgs1Δ::HIS3 | This study |
| SMG258-10A | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-YFP::KanMX YFP-SGS1 | This study |
| SMG266-6D | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-YFP::KanMX smc6-9::NatMX | This study |
| VS3-7A | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-YFP::KanMX SPC110-CFP::KanMX | This study |
| VS11-13D | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX top2-1ts | This study |
| VS16-3C | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX rad51Δ | This study |
| VS17-1C | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX rad54::LEU2 | This study |
| VS19-5A | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX rad52::HIS5 | This study |
| VS21 | MAT α TOP2-CFP | This study |
| VS22-7B | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX TOP2-CFP | This study |
| VS23-1B | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-4ala-YFP::KanMX NLS-yEmRFP _Y Prv::URA3 HTA1-CFP | This study |
| VS26-15A | MAT α lys2Δ tTA(tetR-VP16)-tetO ₂ -DPB11-YFP::KanMX sgs1Δ::HIS3 rad51Δ | This study |
| VS28 | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-YFP::KanMX SPC110-CFP::KanMX TRP1::BrdU-Inc | This study |
| VS34-1A | MAT α tTA(tetR-VP16)-tetO ₂ -DPB11-CFP::KanMX DDC2-4ala-YFP | This study |

Yeast strains in this study are derivatives of ML8-9A, a RAD5 ADE2 derivative of W303-1A (MAT α BAR1 LYS2 ade2-1 can1-100 ura3-1 his3-11,15 leu2-3,112 trp1-1 rad5-535; Thomas and Rothestein, 1989).

Table S2. DT40 cell lines used in this study

| Cell line | Genotype | Source |
|-----------|---|-----------------------------|
| DT40 | Wild type | Buerstedde and Takeda, 1991 |
| RTP82 | PICH ^{YFP/YFP} (loxed) hH2B-mCherry (CMV promoter, Neo ^R) | This study |
| RTP143 | TopBP1 ^{YFP-AID/YFP-AID/YFP-AID} (loxed) PICH ^{TFP/WT} (loxed) OsTIR (CMV promoter, Neo ^R) | This study |
| RTP149 | PICH ^{TFP/WT} (loxed) TopBP1 ^{YFP/WT/WT} (loxed) hH2B-mCherry (CMV promoter, Neo ^R) | This study |
| RTP151 | TopBP1 ^{YFP-AID/YFP-AID/YFP-AID} (loxed) PICH ^{TFP/WT} (loxed) OsTIR (CMV promoter, Neo ^R) hH2B-mCherry (β -actin promoter, Puro ^R) | This study |
| RTP156 | TopBP1 ^{YFP/WT/WT} (loxed) RPA ^{CFP/WT} (loxed) hH2B-mCherry (β -actin promoter, Neo ^R) | This study |
| RTP177 | TopBP1 ^{YFP-AID/YFP-AID/YFP-AID} (loxed) PICH ^{TFP/WT} (loxed) hH2B-mCherry (β -actin promoter, Puro ^R) | This study |

DT40 cell lines in this study are derivatives of DT40 wild type (Buerstedde and Takeda, 1991).

Table S3. Plasmids used in this study

| Plasmid | Relevant markers | Source |
|------------------------|--|------------------------|
| pBlueScript SK+ | AMP ^r | Fermentas GmbH |
| pWJ1299 | AMP ^r HIS3 NOP1-CFP | Shor et al., 2005 |
| pWJ1323 | AMP ^r HIS3 CFP-NUP49 | Shor et al., 2005 |
| pKW1219 | AMP ^r LEU2 NLS-mRFP1 | Madrid et al., 2006 |
| pML96 | AMP ^r URA3 NLS-yEmRFP | This study |
| pML104 | AMP ^r HIS3 NLS-yEmRFP | This study |
| pML105 | AMP ^r URA3 CFP-2xPH(PLC δ) | This study |
| pNEB30 | AMP ^r <i>K. lactis</i> URA3 yEmRFP | Silva et al., 2012 |
| pYES2 | AMP ^r URA3 PGAL1 | Shah et al., 2010 |
| pYES-GAL-RAD51 | AMP ^r URA3 PGAL1-RAD51 | Shah et al., 2010 |
| pYES-GAL-rad51K191A | AMP ^r URA3 PGAL1-rad51K191A | Shah et al., 2010 |
| pVHO3 | AMP ^r TopBP1-YFP PAC | Germann et al., 2011 |
| pLOX-BSR | AMP ^r BSR | Arakawa et al., 2001 |
| pLOX-PURO | AMP ^r PAC | Arakawa et al., 2001 |
| pLOX-NEO | AMP ^r NEO | Arakawa et al., 2001 |
| pExpress | AMP ^r chicken β -actin promoter | Arakawa et al., 2001 |
| pEYFP-C1 | KAN ^r EYFP | Takara Bio Inc. |
| pH2B_mCherry_IRES_neo3 | AMP ^r human H2B-mCherry-NEO CMV promoter | Nam and Benezra, 2009 |
| pmTurquoise2-N1 | KAN ^r mTurquoise2 | Goedhart et al., 2012 |
| pNHK65 | KAN ^r OsTIR1 NEO | Nishimura et al., 2009 |
| pMK43 | AMP ^r IAA17 NEO | Nishimura et al., 2009 |
| pRTP6 | AMP ^r PICH-YFP BSR | This study |
| pRTP9 | AMP ^r PICH-YFP PAC | This study |
| pRTP14 | AMP ^r TopBP1-YFP-AID NEO | This study |
| pRTP15 | AMP ^r TopBP1-YFP-AID BSR | This study |
| pRTP16 | AMP ^r TopBP1-YFP-AID PAC | This study |
| pRTP17 | AMP ^r PICH-TFP BSR | This study |
| pRTP23 | AMP ^r Human H2B-mCherry (chicken β -actin promoter) PAC | This study |

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