

Supplemental Materials

Molecular Biology of the Cell

Estrem and Moore

Table S1. Strains used in this study

Strain	Genotype	Source
yJM0596/ YEF473A	<i>MATA ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	(Bi, E., Pringle, J. R.,1996)
yJM0301	<i>MATA tub2-430Δ +331::TRP1 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-Tub1 his3-Δ200 trp1-Δ63</i>	(Aiken et al. 2014)
yJM0562	<i>MATA ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-Tub1 his3-Δ200 trp1-Δ63</i>	(Aiken et al. 2014)
yJM1345	<i>MATA kip3Δ::HIS3 ura3-52 leu2-Δ1::LEU2-GFP-Tub1 lys2-801 leu2-Δ1 his3- Δ200 trp1-Δ63</i>	This study
yJM2094	<i>MATA nip100ΔCAP-Gly leu2-Δ1::LEU2-GFP-Tub1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM2306	<i>MATA PDR1::pdr1-DBD-CYC8::LEU2 ura3-52::URA3-GFP-Tub1 lys2-801 leu2- Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM1008	<i>MATA promURA3::tetR::GFP::LEU2 CenIV::tetOx448::URA3 Spc110- tdTomato::HIS3 ura3-1 his3-11. 15 leu2-3. 112 trp1-1 can1-100</i>	(Fees et al. 2016)
yJM1018	<i>MATA promURA3::tetR::GFP::LEU2 CenIV::tetOx448::URA3 Spc110- tdTomato::HIS3 tub2-430Δ +331::TRP1 ura3-1 his3-11. 15 leu2-3. 112 trp1-1 can1-100</i>	(Fees et al. 2016)

yJM1019	<i>MATα promURA3::tetR::GFP::LEU2 CenIV::tetOx448::URA3 Spc110-tdTomato::HIS3 tub2-430Δ +331::TRP1 ura3-1 his3-11. 15 leu2-3. 112 trp1-1 can1-100</i>	(Fees et al. 2016)
yJM1449	<i>MATA kar9Δ::HIS3MX6 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-Tub1 his3-Δ200 trp1-Δ63</i>	This study
yJM1680	<i>MATα mcm21Δ::KanMX promURA3::tetR::GFP::LEU2 CenIV::tetOx448::URA3 Spc110-tdTomato::HIS3 tub2-430Δ +331::TRP1 ura3-1 his3-11. 15 leu2-3. 112 trp1-1 can1-100</i>	(Fees et al. 2016)
yJM1771	<i>MATA ase1Δ::HIS leu2-Δ1::LEU2-GFP-Tub1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM2093	<i>MATA nip100ΔCAP-Gly tub2-430Δ +331::TRP1 leu2-Δ1::LEU2-GFP-Tub1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM2094	<i>MATA nip100ΔCAP-Gly leu2-Δ1::LEU2-GFP-Tub1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM2096	<i>MATα nip100ΔCAP-Gly leu2-Δ1::LEU2-GFP-Tub1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM2495	<i>MATA RAD52-tdTomato::HIS3MX ura3-52 lys2-801 leu2::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study

yJM2496	<i>MATA RAD52-tdTomato::HIS3MX tub2-430Δ+331::TRP1 ura3-52 lys2-801 leu2::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJM2662	<i>MATα RAD52-tdTomato::HIS3MX tub2-430Δ+331::TRP1 leu2::LEU2-GFP-TUB1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM2671	<i>MATα kar9Δ::hphMX4 RAD52-tdTomato::HIS3MX6 leu2::LEU2-GFP-TUB1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM2672	<i>MATA kar9Δ::hphMX4 RAD52-tdTomato::HIS3MX6 leu2::LEU2-GFP-TUB1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM2674	<i>MATA RAD52-tdTomato::HIS3MX tub2-430Δ+331::TRP1 kip3Δ::HIS ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJM2675	<i>MATα RAD52-tdTomato::HIS3MX tub2-430Δ+331::TRP1 kip3Δ::HIS3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJM2788	<i>MATA kip3Δ::HIS3 RAD52-tdTomato::HIS3MX ura3-52 lys2-801 leu2::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJM2803	<i>MATα tub2-430Δ +331::TRP1 nip100ΔCAP-Gly RAD52-tdTomato::HIS3MX leu2-Δ1::LEU2-GFP-TUB1 ura3-52 lys2-801 his3-Δ200 trp1-Δ63</i>	This study
yJM2804	<i>MATA nip100ΔCAP-Gly RAD52-tdTomato::HIS3MX leu2-Δ1::LEU2-GFP-TUB1 ura3-52 lys2-801 his3-Δ200 trp1-Δ63</i>	This study

yJM2951	<i>MATα mcm21Δ::KanMX tub2-430Δ +331::TRP1 RAD52-tdTomato::HIS3MX</i> <i>ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM2953	<i>MATα ase1Δ::HIS3 RAD52-tdTomato::HIS3MX ura3-52 lys2-801 leu2-</i> <i>Δ1::LEU2-GFP-Tub1 his3-Δ200 trp1-Δ63</i>	This study
yJM2960	<i>MATα mcm21Δ::KanMX RAD52-tdTomato::HIS3MX ura3-52 lys2-801 leu2-</i> <i>Δ1::LEU2-GFP-Tub1 his3-Δ200 trp1-Δ63</i>	This study
yJM2961	<i>MATα mcm21Δ::KanMX RAD52-tdTomato::HIS3MX kar9Δ::hphMX4 ura3-52</i> <i>lys2-801 leu2-Δ1::LEU2-GFP-Tub1 his3-Δ200 trp1-Δ63</i>	This study
yJM3047	<i>MATα MRE11-Neon::HIS3 mRuby2-Tub1::LEU lys2-801 leu2-Δ1 his3-Δ200</i> <i>trp1-Δ63</i>	This study
yJM3051	<i>MATα RAD52-tdTomato::HIS3MX leu2-Δ1::LEU2-GFP-TUB1 Myo1-</i> <i>EGFP::HPH ura3-52 lys2-801 his3-Δ200 trp1-Δ63</i>	This study
yJM3056	<i>MATα RAD52-tdTomato::HIS3MX leu2::LEU2-GFP-TUB1 bni1Δ::TRP1</i> <i>Myo1-EGFP::HPH ura3-52 lys2-801 his3-Δ200 trp1-Δ63</i>	This study
yJM3098	<i>MATα dam1-S221F(765)::His3Mx6 RAD52-tdTomato::HIS3MX leu2-</i> <i>Δ1::LEU2-GFP-TUB1 ura3-52 lys2-801 his3-Δ200 trp1-Δ63</i>	This study

yJM3126	<i>MATα bni1Δ::TRP1 kar9Δ::hphMX4 RAD52-tdTomato::HIS3MX leu2- Δ1::LEU2-GFP-TUB1 ura3-52 lys2-801 his3-Δ200 trp1-Δ63</i>	This study
yJM3128	<i>MATA bni1Δ::TRP1 kar9Δ::hphMX4 RAD52-tdTomato::HIS3MX leu2- Δ1::LEU2-GFP-TUB1 Myo1-EGFP::hphMX4 ura3-52 lys2-801 his3-Δ200 trp1- Δ63</i>	This study
yJM3166	<i>MATα MRE11-Neon::HIS3 kar9Δ::HIS3MX6 mRuby2-Tub1::LEU lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM3167	<i>MATA mec1::Trp sml1::HIS3 tub2-430Δ +331::TRP1 bar1::LEU2 RAD52-YFP ADE2 LYS2</i>	This study
yJM3169	<i>MATA pU-GAL-HO::URA RAD52-tdTomato::HIS3MX CSE4- mNeonGreen::spHIS5 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJM3194	<i>MATA MRE11-Neon::HIS3 mRuby2-Tub1::LEU tub2-430Δ +331::TRP1 ura3- 52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM3230	<i>MATA mec1::Trp sml1::HIS3 tub2-430Δ +331::TRP1 bar1::LEU2 RAD52-YFP mRuby2-Tub1+3'UTR::URA3 ADE2 LYS2</i>	This study
yJM3232	<i>MATA mec1::Trp sml1::HIS3 bar1::LEU2 RAD52-YFP mRuby2- Tub1+3'UTR::URA3 ADE2 LYS2</i>	This study

yJM3264	<i>MATA HTB2-mNeonGreen::HIS3 mRuby2-Tub1+3'UTR::LEU ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM3265	<i>MATA HTB2-mNeonGreen::HIS3 tub2-430Δ +331::TRP1 mRuby2-Tub1+3'UTR::LEU ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM3284	<i>MATA Spc110-tdTomato::HIS3 ase1Δ::HIS3 promURA3::tetR::GFP::LEU2 CenIV::tetOx448::URA3 ura3-1 his3-11. 15 leu2-3. 112 trp1-1 can1-100</i>	This study
yJM3308	<i>MATA HTB2-mNeonGreen::HIS3 mRuby2-Tub1+3'UTR::LEU bni1Δ::TRP1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ</i>	This study
yJM3352	<i>MATA pU-GAL-HO::URA RAD52-tdTomato::HIS3MX tub2-430Δ+331::TRP1 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJM3354	<i>MATα pU-GAL-HO::URA kar9Δ::hphMX4 RAD52-tdTomato::HIS3MX6 leu2-Δ1::LEU2-GFP-TUB1 ura3-52 lys2-801 his3-Δ200 trp1-Δ63</i>	This study
yJM3405	<i>MATA kar9Δ::HIS3MX6 HTB2-mNeonGreen::HIS3 mRuby2-Tub1+3'UTR::LEU ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ</i>	This study
yJM3411	<i>MATα HTB2-mNeonGreen::HIS3 bni1Δ::TRP1 mRuby2-Tub1+3'UTR::LEU kar9Δ::HIS3 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM0970	<i>MATA NLS-GFP::LEU SPC110-DsRED::KanMX ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study

yJM0971	<i>MATα tub2-430Δ +331::TRP1 NLS-GFP::LEU SPC110-DsRED::KanMX ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJM3598	<i>MATA RAD52-tdTomato::HIS3 nba1Δ::HIS3MX ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJM3599	<i>MATα RAD52-tdTomato::HIS3 nba1Δ::HIS3MX ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJM3590	<i>MATA RAD52-tdTomato::HIS3 rga1Δ::HIS3MX ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study

Table S2. Plasmids used in this study

Plasmid	Description	Source
yJM261	<i>pFA6a-mNeonGreen-SpHIS5</i>	This study
yJM309	<i>pLEU2:HIS3p:mRuby2-TUB1+3'UTR</i> , integrating plasmid	(Markus et al. 2015)
yJM464	<i>URA3::pU-GAL-HO, YCp50</i>	R.A Sclafani

SUPPLEMENTAL FIGURE 1. Confirming genetic interactions *tub2-430Δ* and DNA repair mutants.

A) Genes involved in response to DNA damage stimulus that exhibit genetic interactions with *tub2-430Δ*. Molecular functions are gene ontologies from the Saccharomyces Genome Database (www.yeastgenome.org). SGA scores are based on a previously published genetic interaction screen (REF Aiken 2014). Negative values indicate negative genetic interactions between the indicated null mutants and *tub2-430Δ* (REF PMID: 21076421). Deletion mutants *ctf18Δ*, *mms22Δ*, *rad52Δ* and *mus81Δ* were constructed in the YEF473 strain background, confirmed by PCR, and crossed to *tub2-430Δ* mutants. Double mutant progeny within tetrads were predicted based on expected 2:2 segregation. Double mutant phenotypes were defined as follows: '-', no detectable difference compared to single mutants; 'slow growth', double mutants grew slower under optimal conditions than either single mutant; "lethal", double mutants were inviable. B) 10-fold dilution series of indicated haploid progeny from tetrad dissections strains were spotted to rich media (YPD) and incubated at indicated temperatures.

SUPPLEMENTAL FIGURE 2 DNA damage and increased spindle migration mutants

(A) Percent of cells in G1, M, and anaphase containing Rad52-tdTomato foci for WT and *tub2-430Δ* mutant. Percentages are calculated by the number of cells with Rad52 foci separated by the cell cycle stage then divided by the total number of cells observed. WT, n=17322; *tub2-430Δ*, n=6246 cells. (B) Percent of cells containing Rad52 foci in pre-anaphase of the cell cycle. Percentages are calculated by number of cells containing Rad52 foci, divided by number of cells in pre-anaphase of the cell cycle. WT, n=2581; *mec1Δ sml1Δ*, n=604; *tub2-430Δ mec1Δ sml1Δ*, n=880; *tub2-430Δ*, n=2278 cells. Asterisks denote a significant difference. **, p-value < 0.001; determined by Fishers exact test. (C) Mean astral microtubule length in μm . WT, n=76; *tub2-430Δ*, n=77; *kip3Δ*, n=133; *tub2-430Δ kip3Δ*, n=142; *kar9Δ*, n=95 microtubules. Asterisks denote a significant different from WT. *, p-value < 0.0001; determined by t test. (D) Mean sliding events per minute per cell. WT, n=91; *tub2-430Δ*, n=73; *kip3Δ*, n=53; *tub2-430Δ kip3Δ*, n=33; *kar9Δ*, n=28 cells. Asterisks denote a significant different from WT. *, p-value < 0.0001; determined by t test. (E) Mean sliding events per minute per cell. *tub2-430Δ*, n=23; *tub2-430Δ nip100CGΔ*, n=31 cells. Asterisks denote P < 0.0001; determined by t test.

SUPPLEMENTAL FIGURE 3 Duration of Rad52 and Mre11 foci

Box and whisker plot of duration of Rad52 and Mre11 foci in minutes. Center bar denotes the median, box denotes the first and third quartiles, and whiskers are maxima and minima. The maximum duration is 60 minutes. Black bars represent the 95% CI. Rad52: WT, n =61; *kar9Δ*, n=28. Mre11: WT, n=28; *kar9Δ*, n=36 cells. p-values determined by Mann-Whitney test.

SUPPLEMENTAL FIGURE 4 Widening the bud neck

(A) Scatter plot depicting WT and *bni1Δ* mutant distributions comparing bud neck size in μm on the y-axis to total cell length in μm on the x-axis. Black dots represent WT cells. Green dots represent *bni1Δ* mutant cells. WT, n =180; *bni1Δ*, n= 108 cells. (B) Dot plot of maximum eccentricity reached in pre-anaphase. Red bars represent the standard error of the mean. WT, n=19; *kar9Δ*, n=17; *bni1Δ*, n=11; *kar9Δ bni1Δ*, n=23 cells. Asterisks denote a significant difference from WT. **, p-value <0. 0.001; determined by t test.

SUPPLEMENTAL Video 1

GFP-NLS and SPC110-DsRed in a WT cell. Time-lapse images of a WT cell expressing GFP-NLS and SPC110-DsRed were captured on a spinning-disk confocal microscope at 10-s intervals for 10 min. Each image represents a composite of 13 planes separated by 450 nm. The video plays at four frames per second. Strain: WT, yJM970.

SUPPLEMENTAL Video 2

GFP-NLS and SPC110-DsRed in a *tub2-430Δ* cell. Time-lapse images of a *tub2-430Δ* cell expressing GFP-NLS and SPC110-DsRed were captured on a spinning-disk confocal microscope at 10-s intervals for 10 min. Each image represents a composite of 13 planes separated by 450 nm. The video plays at four frames per second. Strain: *tub2-430Δ*, yJM971.

SUPPLEMENTAL Video 3

HTB2-mNeonGreen and mRuby2-Tub1 in a WT cell. Time-lapse images of a WT cell expressing HTB2-mNeonGreen and mRuby2-Tub1 were captured on a spinning-disk confocal microscope at 3-minute intervals for 1 hour. Each image represents a composite of 13 planes separated by 450 nm. The video plays at four frames per second. Strain: WT, yJM3264.

SUPPLEMENTAL Video 4

HTB2-mNeonGreen and mRuby2-Tub1 in a *kar9Δ* cell. Time-lapse images of a *kar9Δ* cell expressing HTB2-mNeonGreen and mRuby2-Tub1 were captured on a spinning-disk confocal microscope at 3-minute intervals for 1 hour. Each image represents a composite of 13 planes separated by 450 nm. The video plays at four frames per second. Strain: *kar9Δ*, yJM3405.

SUPPLEMENTAL Video 5

HTB2-mNeonGreen and mRuby2-Tub1 in a *bni1Δ* cell. Time-lapse images of a *bni1Δ* cell expressing HTB2-mNeonGreen and mRuby2-Tub1 were captured on a spinning-disk confocal microscope at 3-minute intervals for 1 hour. Each image represents a composite of 13 planes separated by 450 nm. The video plays at four frames per second. Strain: *bni1Δ*, yJM3308.

SUPPLEMENTAL Video 6

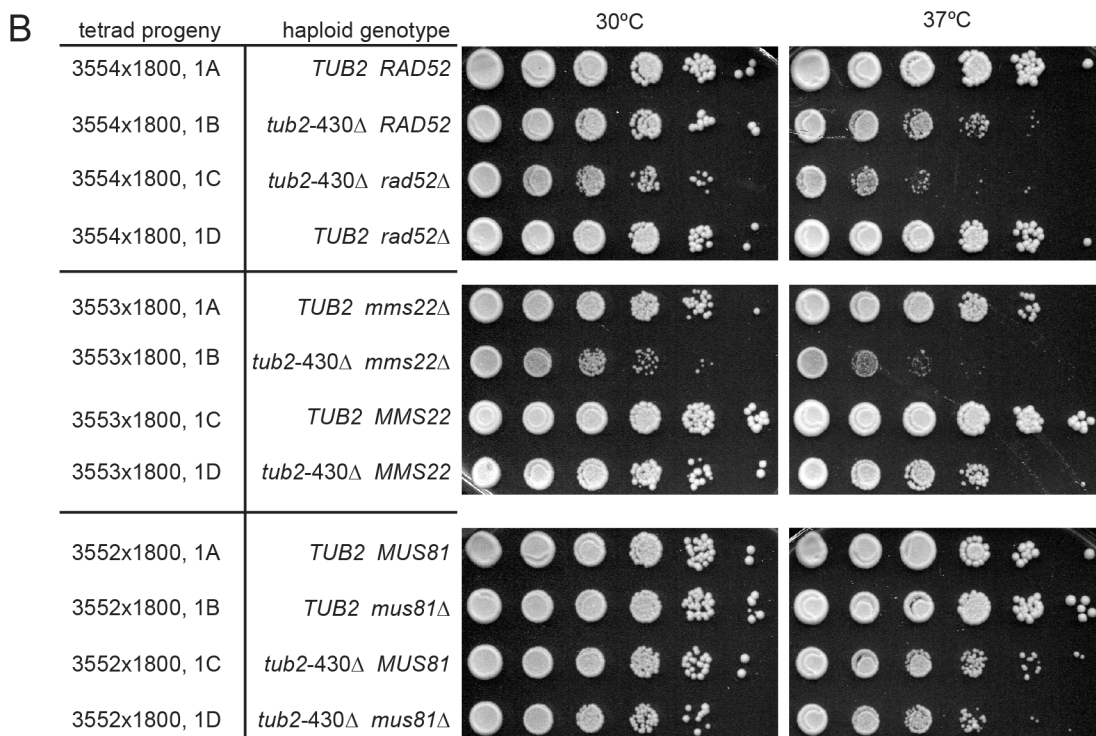
HTB2-mNeonGreen and mRuby2-Tub1 in a *kar9Δ bni1Δ* cell. Time-lapse images of a *kar9Δ bni1Δ* cell expressing HTB2-mNeonGreen and mRuby2-Tub1 were captured on a spinning-disk

confocal microscope at 3-minute intervals for 1 hour. Each image represents a composite of 13 planes separated by 450 nm. The video plays at four frames per second. Strain: *kar9Δ bni1Δ*, yJM3411.

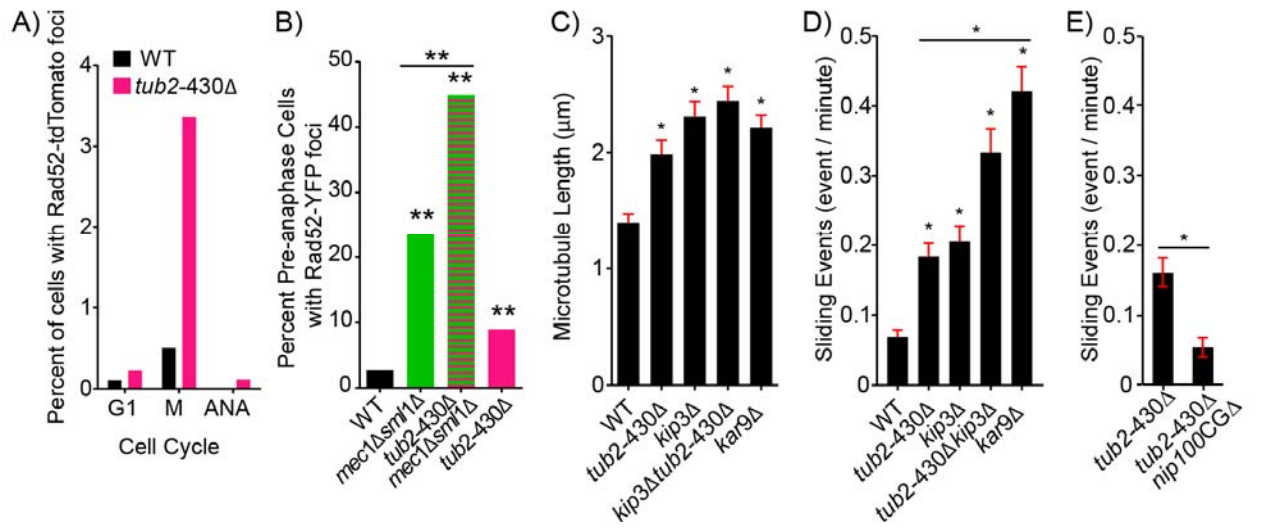
Supplemental Figure 1

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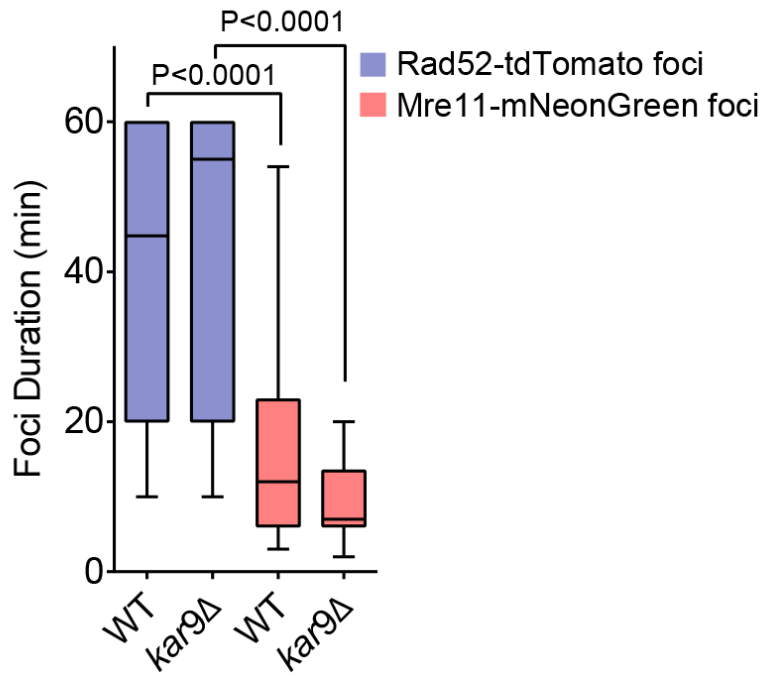
deletion mutant	Molecular Function	SGA score	double mutants combined with <i>tub2-430Δ</i> (recovered/predicted)	double mutant phenotype
<i>asf1Δ</i>	Nucleosome assembly factor	-0.248	nd	nd
<i>bmh1Δ</i>	DNA replication origin binding	-0.164	nd	nd
<i>cka2Δ</i>	Alpha' catalytic subunit of casein kinase 2	-0.178	nd	nd
<i>ckb1Δ</i>	Beta regulatory subunit of casein kinase 2	-0.131	nd	nd
<i>ctf18Δ</i>	Ctf18 RFC-like complex	-0.269	0/8	lethal
<i>ctf8Δ</i>	Ctf18 RFC-like complex	-0.286	nd	nd
<i>ctk3Δ</i>	Gamma subunit of C-terminal domain kinase I	-0.177	nd	nd
<i>dcc1Δ</i>	Ctf18 RFC-like complex	-0.204	nd	nd
<i>lrp1Δ</i>	Nuclear exosome-associated nucleic acid binding	-0.144	nd	nd
<i>mms1Δ</i>	Cul8 RING ubiquitin ligase complex	-0.206	nd	nd
<i>mms22Δ</i>	Cul8 RING ubiquitin ligase complex	-0.194	5/12	slow growth
<i>mrc1Δ</i>	Claspin homolog; DNA replication checkpoint	-0.124	nd	nd
<i>ptc2Δ</i>	Type 2C protein phosphatase	-0.231	nd	nd
<i>rad27Δ</i>	5' to 3' exonuclease, 5' flap endonuclease	-0.182	nd	nd
<i>rad52Δ</i>	Single stranded DNA annealing	-0.254	15/15	slow growth
<i>rad57Δ</i>	Stimulates strand exchange	-0.207	nd	nd
<i>rif2Δ</i>	p90 subunit of the CAF-1 Complex	-0.239	nd	nd
<i>snf6Δ</i>	SWI/SNF chromatin remodeling complex	-0.191	nd	nd
<i>wss1Δ</i>	SUMO-ligase and SUMO-targeted metalloprotease	-0.191	nd	nd
<i>mus81Δ</i>	Mms4-Mus81 endonuclease complex	na	10/11	-



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4

