Sec66-Dependent Regulation of Yeast Spindle-Pole Body Duplication Through Pom152
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ABSTRACT In closed mitotic systems such as Saccharomyces cerevisiae, the nuclear envelope (NE) does not break down during mitosis, so microtubule-organizing centers such as the spindle-pole body (SPB) must be inserted into the NE to facilitate bipolar spindle formation and chromosome segregation. The mechanism of SPB insertion has been linked to NE insertion of nuclear pore complexes (NPCs) through a series of genetic and physical interactions between NPCs and SPB components. To identify new genes involved in SPB duplication and NE insertion, we carried out genome-wide screens for suppressors of deletion alleles of SPB components, including Mps3 and Mps2. In addition to the nucleoporins POM152 and POM34, we found that elimination of SEC66/SEC71/KAR7 suppressed lethality of cells lacking MPS2 or MPS3. Sec66 is a nonessential subunit of the Sec63 complex that functions together with the Sec61 complex in import of proteins into the endoplasmic reticulum (ER). Cells lacking Sec66 have reduced levels of Pom152 protein but not Pom34 or Ndc1, a shared component of the NPC and SPB. The fact that Sec66 but not other subunits of the ER translocon bypass deletion mutants in SPB genes suggests a specific role for Sec66 in the control of Pom152 levels. Based on the observation that sec66Δ does not affect the distribution of Ndc1 on the NE or Ndc1 binding to the SPB, we propose that Sec66-mediated regulation of Pom152 plays an NPC-independent role in the control of SPB duplication.

KEYWORDS Mps3; Sec66/Sec71/Kar7; spindle-pole body; Pom152; Nbp1

Accurate transmission of genetic material to daughter cells during cell division requires two precise duplication events: DNA replication and centrosome duplication. In addition, the cell must increase the number of organelles and protein complexes such as ribosomes and nuclear pore complexes (NPCs) so that the daughter cells have material to continue cell growth, metabolism, transcription, translation, and other vital cellular processes. While much is known about the mechanism and regulation of DNA replication, less is known about how cells duplicate protein-based structures such as the centrosome once per cell cycle.

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The spindle-pole body (SPB) is the Saccharomyces cerevisiae centrosome-equivalent organelle and is perhaps one of the best-characterized microtubule organizing centers (Jaspersen and Winiey 2004; Kilmartin 2014). Cytologic studies of the SPB showed that it is embedded in the nuclear envelope (NE) throughout the yeast life cycle, where it nucleates cytoplasmic microtubules that are important for nuclear positioning and mating and spindle microtubules that are essential for chromosome segregation during mitosis and meiosis (Winey and Bloom 2012). Conventional electron microscopy (EM) and electron tomography revealed that the SPB is a multilayered structure that is attached to the NE via hooklike appendages (Byers and Goetsch 1974, 1975; O’Toole et al. 1999). One side of the SPB is associated with an electron-dense region of the NE known as the half-bridge, which plays a key role in the control of SPB duplication and is likely involved in insertion of the SPB into the NE.

SPB duplication begins as cells exit mitosis by elongation of the half-bridge and deposition of the satellite (the SPB precursor) at its distal cytoplasmic tip (Byers and Goetsch 1975; Adams and Kilmartin 1999). During G1, the satellite matures
into a structure known as the *duplication plaque*, and the extended bridge fuses at its tip, retracts, and bends underneath the duplication plaque. Finally, the duplication plaque is inserted into the NE, at which time nuclear SPB components can assemble to result in duplicated side-by-side SPBs.

A combination of molecular, biochemical, and genetic approaches led to the identification of 18 core components of the SPB, and all have been assigned a position within the SPB structure based on immuno-EM localization and physical interaction studies (Jaspersen and Winey 2004; Kilmartin 2014). Of the 18 core components, all but one or two (Cnm67 and, in some strains, Spc72) are essential for growth. Analysis of conditional hypomorphic mutations or degron alleles has suggested an order and a function for each gene product in SPB duplication, microtubule nucleation, or other functions such as exit from mitosis. Most mutations in components of the half-bridge, such as the membrane proteins Mps3 and Kar1 and the soluble filamentous Sfl1 protein and its binding partner Cdc31 (centrin), have defects in the early steps of SPB duplication (Baum et al. 1986; Vallen et al. 1992, 1994; Spang et al. 1993, 1995; Higgins and Rose 1994; Jaspersen et al. 2002; Kilmartin 2003), while most mutations in membrane pore components (Mps2 and Ndc1) and their binding partners (Bbp1 and Nbp1, respectively) arrest at the late step of SPB duplication, with a mature duplication plaque that is unable to insert into the NE (Winey et al. 1993; Chial et al. 1998; Munoz-Centeno et al. 1999; Schramm et al. 2000; Araki et al. 2006). Recent super-resolution imaging of duplicating SPBs suggests that membrane insertion may be coupled with SPB assembly in wild-type cells (Burns et al. 2015). The localization of Ndc1 and/or Nbp1 to the new SPB appears to be involved in the final step of SPB duplication, consistent with genetic analyses (Winey et al. 1993; Chial et al. 1998; Munoz-Centeno et al. 1999; Schramm et al. 2000; Araki et al. 2006).

In yeast as in other eukaryotes, NPCs are present at multiple locations in the NE to facilitate movement of macromolecules into and out of the nucleus. Like SPBs, NPCs are composed of a relatively small number of molecules that are present in multiple copies (Strambio-De-Castillia et al. 2010; Aitchison and Rout 2012). NPC assembly in postmitotic cells or in or-ganisms such as yeast that undergo a closed mitosis, in which the nuclear membrane does not break down, occurs by a *de novo* pathway in which NPCs are inserted into an intact NE (Hetzer and Wente 2009). Cytologic, genetic, and molecular studies have linked the mechanism of *de novo* NPC assembly with that of SPB membrane insertion. Membrane-associated proteins that bind, bend, or stabilization curved membranes, including reticulons and ALPS (for ArfGAP1 lipid packing sensor) domain-containing proteins, are involved in SPB duplication and NPC assembly (Dawson et al. 2009; Doucet et al. 2010; Drin and Antony 2010; Kupke et al. 2011; Casey et al. 2012; Kim et al. 2014). In addition, Ndc1 encodes a conserved integral membrane protein that localizes to both SPBs and NPCs, and analysis of yeast cells lacking Ndc1 function shows defects in NE insertion of both complexes (Chial et al. 1998; West et al. 1998; Lau et al. 2006; Madrid et al. 2006; Onischenko et al. 2009). The observation that mutation or deletion of genes encoding membrane and membrane-associated components of the SPB can be suppressed by elimination of Ndc1 binding partners at the NPC such as Pom152 or Pom34 has led to the idea that the SPB and NPC may compete for Ndc1 or other NE insertion factors (Chial et al. 1998; Sezen et al. 2009; Witkin et al. 2010; Casey et al. 2012). Consistent with this idea, we recently demonstrated that the distribution of Ndc1 between the NPC and SPB was altered by deletion of Pom152 (Chen et al. 2014). However, other suppression mechanisms may exist, including a translational control pathway that is activated following Pom34 deletion and/or alteration in the properties of the NE that may facilitate insertion of large complexes such as the SPB (Sezen et al. 2009; Witkin et al. 2010; Friederichs et al. 2011).

Here we characterized cells lacking Mps3 and Pom152 to determine whether elimination of the nucleoporin Pom152 is able to suppress all known functions of Mps3, including its essential role in SPB duplication in mitosis and meiosis and its nonessential functions in chromosome organization within the nucleus. We then used genome-wide screening to identify other bypass suppressors of mps3Δ as well as other deletions in membrane (mps2Δ and nbp1Δ) and half-bridge (cdc31Δ, kar1Δ, and sfl1Δ) components of the SPB to better understand interactions between the SPB and NPC and to discover new factors that regulate SPB duplication and NE insertion. Further characterization of one bypass suppressor, sec66Δ, suggests that post-transcriptional control of Pom152 affects SPB duplication in a manner that is distinct from its role in the NPC.

**Materials and Methods**

**Yeast methods**

Standard techniques were used for DNA and yeast manipulations. All strains are listed in Supporting Information, Table S4. With the exception of strains used for chromosome loss and for the suppressor screen, strains are derivatives of W303. The yeast deletion collection was purchased from Open Biosystems in 2004. Deletion and tagging of genes were done using PCR-based methods and verified by PCR (Longtine et al. 1998; Sheff and Thorn 2004).

pRS425-derived plasmids (2μ-LEU2) containing Pom152 (pSJ998) or Pom34 (pSJ1652) were created by PCR amplification of the ORF plus ~500 bp of upstream sequence and ~200 bp of downstream sequence from genomic DNA derived from W303. The ORF of Pom152 also was amplified and cloned together with the GAL1/10 promoter into pRS305 to create pRS305-GAL1-POM152 (pSJ1655).

**SPB suppressor screen**

The SPB suppressor screen was done using a modified version of the protocol for synthetic genetic analysis described by Tong and Boone (2006). A centromeric *URA3*-marked plasmid containing a SPB gene and its flanking sequence was
transformed into the SGA query strain, and then the genomic copy of the SPB gene was deleted with a NATMX cassette using PCR, creating MATα spbΔ::NATMX can1Δ::STE2pr-HIS3MX lyr1Δ ura3 his3 YFG1 pURA3-SPB. Each was mated to the deletion collection (MATα yfgΔ::KANMX LYP1 CAN1 his3 ura3 SPB) using the Singer RoToR, and diploids were selected on YPD + G418 + cloNAT. Cells were pinned to 5-fluoro-otic-ac (5-FOA) twice to select for loss of the covering plasmid, pURA3-SPB. Diploids were sporulated for 3–4 weeks, and then haploids were selected twice on SD-His-Lys-Arg + thialysine + canavanine before pinning to SD/MSG-His-Lys-Arg + thialysine + canavanine + G418 + cloNAT. All plates were incubated at 23°C. Each screen was done in triplicate, and genes that rescued growth in at least two of the three screens are shown in Figure 2C, Table S1, and Table S2.

Suppressors of mps3Δ were further verified by complementation of the suppression phenotype using clones from the 2μ tiling library, if available (Jones et al. 2008). In addition, deletions were recreated in the W303 strain background using PCR-based methods. Only pom152Δ, pom34Δ, and sec66Δ suppressed mps3Δ in both BY and W303 strains.

Chromosome 13

Deletions in a small region of chromosome 13 were identified in all but one of our screens (Table S2). Many have been previously isolated in suppressor screens for other essential genes using synthetic genetic array (SGA) methodology (Copic et al. 2012). We performed array-based comparative genomic hybridization using eight deletion strains from this region in our copy of the MATα deletion collection and the isogenic wild-type strain BY4741 to determine whether the deletion strains carried a linked mutation that was responsible for the suppression phenotype. Six of eight strains (UFO1, AIM33, ALO1, YML083C, YML082W, and DUS1) contained the correct deletion; in the remaining two, one had the adjacent gene removed (GAL80 instead had a deletion of YML082W), and the other (RPS1B) contained no detectable deletion. All eight strains also have a deficiency in YML050W/AIM32, a gene of unknown function. Although present in the deletion collection, this ORF was never recovered as a hit. Direct knockout of genes in this region individually or with AIM32 did not bypass any SPB deletions. We suspect that deletion of one of these genes allows cells to escape a growth condition used in high-throughput screening, and thus diploids or one of the individual knockouts lives.

Chromosome assays

The position of GFP telomere spots was determined as described previously (Hediger et al. 2002). Briefly, a Zeiss Axio Imager with a 100× Zeiss alpha Plan-Fluar objective (NA = 1.45) and a Hamamatsu ORCA-ER digital camera were used to capture 19-image stacks of 170-nm step size through nuclei of log-phase cells at room temperature. The spot-top eriphery distance and the nuclear diameter were determined in a single-Z-stack image where the spot was most concentrated using Axiovision 4.6.3 (Zeiss), except in cases where the spot fell into one of the top or bottom three focal planes. By dividing the spot-to-periphery distance by the diameter, each spot fell into one of three zones of equal surface (Figure 1A). Zone 1 has a width of 0.184 × the nuclear radius r, zone 2 has a width of 0.184 × r to 0.422 × r, and zone 3 has a width of 0.422 × r. Confidence values (P-values) for the χ² test were calculated for each data set between random and test distributions.

Sister-chromatid cohesion was assayed in logarithmically growing cells that were arrested with 10 μg/ml nocodazole for 3 hr. Cells were briefly fixed with 4% paraformaldehyde and stained with DAPI before cell counting. In addition, aliquots of cells were removed for flow cytometric analysis of DNA content to verify the mitotic arrest. Chromosome loss assays and flow cytometric analysis of DNA content were performed as described previously (Spencer et al. 1990; Jaspersen et al. 2002). Fractional statistics were analyzed via the binomial distribution (Bevington and Robinson 2003). In Figure 1E, variation between colony replicates was somewhat larger than allowed by the binomial distribution. In such cases, P-values also were generated via t-tests between three colony replicates of each sample: mps3Δ 75–150 and pom152Δ mps3Δ had P-values below 0.001, while pom152Δ had a P-value below 0.02, all indicating statistical significance.

Quantitative imaging

Live-cell imaging data were acquired on a PerkinElmer Ultra-view Confocal Microscope with a Yokagawa CSU-X1 Spinning Disk on an inverted Zeiss 200 base. Emission was collected onto a C9100-13 Hamamatsu EM-CCD using Velocity software (PerkinElmer). An α Plan-Apochromat 100× 1.46-NA oil-immersion objective was used. GFP and mCherry images were acquired with 488- and 561-nm excitation, respectively, using a 405/488/561/640 dichroic. Emission filters were a 415- to 475-nm/580- to 650-nm dual-band filter for mCherry and a 500- to 550-nm filter for GFP. mTurquoise2 and yellow fluorescent protein (YFP) were excited with 440 and 514 nm, respectively, via a 405/440/514/640 dichroic. Emission filters were a 456- to 484-nm filter for mTurquoise2 and a 525- to 575-nm filter for YFP. All spinning-disk data were acquired in alternating excitation mode. For Z-stacks, a slice size of 400 nm was used.

Acceptor-photobleaching fluorescence resonance energy transfer (FRET)

Using the PhotoKinesis accessory on the PerkinElmer Ultra-view Spinning Disk Confocal Microscope, cells expressing mTurquoise2 (donor) and YFP (acceptor) were imaged at maximum speed for a total of 10 frames. YFP at the SPB was bleached following frame 5 using 15 iterations with 100% of the 514-nm line. YFP fluorescence was checked following acquisition to ensure complete bleaching. Controls indicated no significant crosstalk of YFP fluorescence into the mTurquoise2 channel. Analysis was performed using ImageJ software: the center of the SPB was determined via a two-dimensional (2D) Gaussian fit, and the total intensity over
Deletion of POM152 only bypasses the requirement for Mps3 at the SPB. (A) Schematic of chromosome 6 with ~256 copies of the LacO<sub>6</sub> and four lexA<sup>OP</sup> binding sites integrated at ARS609, near a truncated version of telomere VI-R that contains the ADE2 reporter linked to copies of the TG1<sup>-3</sup> telomeric repeats (Hediger et al. 2002). Expression of GFP-Lac and Nup49-GFP (a nucleoporin) in these cells allows the subnuclear position of the telomere to be scored with respect to the distance from the NE in a single-plane image and assigned a position in one of three zones of equal volume. (B) Single-plane images showing the localization of truncated telomere VI-R in wild-type (SLJ2602), mps3<sup>D</sup>75–150 (SLJ3081), pom152<sup>D</sup> (SLJ4333), and mps3<sup>D</sup> pom152<sup>D</sup> (SLJ4330) cells. Note that in some cases the telomeric focus may be above or below the focal plane shown. The position of telomeres was scored in three dimensions. Bar, 5 μm. (C) Using bud morphology as a marker for cell cycle position, the location of telomeres in S-phase cells was determined. Zone 1, black bars; zone 2, white bars; and zone 3, gray bars. The red horizontal bar at 33% corresponds to a random distribution. Confidence intervals (P-values) for the χ² test were calculated for each data set between random and test distributions. The number of cells examined in each data set is indicated (n). (D) Expression of the telomeric ADE2 gene in strains from B was assayed by streaking cells to SD plates containing 10 μg/ml adenine. Following growth for 3 days at 30°C, plates were incubated for 1 week at 4°C to allow the red pigment to develop. Expression of ADE2 results in white-colored cells and blocks the accumulation of the red pigment in this strain background; this occurs in cells that have lost telomeric silencing. (E) Sister-chromatid cohesion was tested by arresting cells containing GFP-LacI and an ARS609-bp LacO<sub>6</sub> array on the arm of chromosome 4 in mitosis using nocodazole. Under these conditions, a single GFP focus is indicative of cohesion, while the appearance of two foci indicates that cohesion has been lost. The cell outline is based on the DIC image. Bar, 5 μm. (F) Maximum-intensity projections of wild-type (SLJ1982), mps3<sup>Δ</sup>75–150 (SLJ3131), pom152<sup>Δ</sup> (SLJ4328), and mps3<sup>Δ</sup> pom152<sup>Δ</sup> (SLJ4325) cells. Bar, 5 μm. (G) The percentage of large-budded cells from F that contained two distinct GFP foci was determined in two independent experiments using three biological replicates. Average values from the six samples are shown; error bars show the SD from the mean. **P < 0.0001. Statistical analysis of individual isolates using the t-test also showed significance between samples at P = 0.02 or greater. (H) Loss of an artificial chromosome 3 fragment containing SUP17 was assayed in wild-type (SLJ2156), mps3<sup>Δ</sup>75–150 (SLJ4547), and mps3<sup>Δ</sup> pom152<sup>Δ</sup> (SLJ4548) cells. The average loss rate from three independent experiments is shown along with the SEM. **P < 0.0001. (I) Diploid strains of the indicated genotypes were grown overnight at 23°C in YPA before being transferred to sporulation medium for 5 days at 23°C. Meiotic progression was analyzed based on DAPI staining and DIC images. The percentage of unsporulated cells and sporulated cells that formed dyads (two DNA masses in two spores per ascus) or tetrads (four DNA masses in four spores per ascus) was determined (n = 200) in three independent experiments (error bars, SEM; *P < 0.01; **P < 0.0001).
time was calculated for a 9 × 9 square centered at the initial position of the SPB. The background-subtracted values for the pre- and postbleach time series then were each fit to a linear regression, and the value of each at time point 5.5 was interpolated. FRET was then calculated from the donor intensity as 100 × (postbleach − prebleach)/postbleach. To eliminate artifacts owing to SPB movement, the 2D Gaussian fit was calculated over time, and SPBs were removed from analysis if, in either pre- or postbleach time series, SPB centers moved more than 1 pixel in x or y or the SD of the Gaussian fit changed more than 0.5 pixel.

**RNA-Seq analysis**

Starting with total RNA from three replicates of wild-type and sec66Δ cells, polyA RNA was isolated and prepared for sequencing using a TruSeq RNA Sample Prep Kit (Illumina). Indexed samples were pooled and sequenced in a single-flow cell on an Illumina HiSeq. Five of six samples generated 10–11 million reads each, and one wild-type sample generated 5.6 million reads. Using TopHat 2.0.10, reads were aligned to the saCer3 genome from UCSC with gene annotations from Ensembl 72., and counts were normalized using edgeR. Differentially expressed genes listed in Table S3 showed a twofold increase (27) or decrease (91) in the mutant vs. wild-type cells [P < 0.05 and average log2(normalized counts) > 0]. SPB, NPC, and membrane encoding genes depicted in Figure 3E were identified based on annotations in the Saccharomyces Genome Database (SGD).

**Western blotting**

Whole-cell extracts were prepared by bead beating into SDS sample buffer. The following primary antibody dilutions were used: 1:1000 anti-GFP (Cell Signaling Technology) and 1:5000 anti-Pgk1 (Life Technologies). Alkaline phosphatase–conjugated secondary antibodies were used at 1:10000 (Promega), and fluorescently labeled secondaries were used at 1:5000 (LiCor).

**Data availability**

Original data underlying this manuscript can be downloaded from the Stowers Original Data Repository at [http://www.stowers.org/pubs/LIBPB-1004](http://www.stowers.org/pubs/LIBPB-1004).

**Results**

**Characterization of cells lacking MPS3**

The Mps3 C-terminus, which includes the conserved SUN domain, is essential for SPB duplication (Nishikawa et al. 2003; Jaspersen et al. 2002, 2006). Previously, we showed that the growth defect of C-terminal MPS3 mutants could be rescued if POM152 was eliminated from the genome. In addition, the lethality associated with deletion of MPS3 is suppressed by removal of POM152, suggesting that the essential SPB function of Mps3 is bypassed (Withkin et al. 2010; Friederichs et al. 2011). At least two models could explain this suppression: first, POM152 might act as a “dosage suppressor” by freeing factors from the NPC that are involved in both NPC and SPB assembly, and second, reduced NPC assembly in POM152 may indirectly result in NE changes that allow SPB duplication in the absence of MPS3 (Jaspersen and Ghosh 2012).

To better understand the mechanism of POM152-based suppression, we analyzed the nonessential functions of Mps3 in POM152 mps3Δ cells. Although the Mps3 N-terminus is not required for mitotic growth, cells lacking amino acids 75–150 (mps3Δ75–150) display defects in chromosome organization, including loss of telomere tethering and silencing and defects in sister-chromatid cohesion following DNA replication (Antoniacci et al. 2004; Bupp et al. 2007; Schober et al. 2009; Ghosh et al. 2012). A different region of the Mps3 N-terminus (amino acids 2–64) is required for meiotic chromosome reorganization and movement (Conrad et al. 2007). We hypothesized that if POM152 suppresses the requirement for Mps3 by liberating a SPB insertion factor, it would likely suppress only the SPB function of Mps3, and POM152 mps3Δ cells would be defective in other Mps3-dependent processes, similar to mps3Δ75–150 or mps3Δ2–64 mutants. However, if suppression arises as a consequence of altered NE properties, then deletion of POM152 may reduce or eliminate the need for Mps3 in its other functions, such as chromosome positioning during S phase, sister-chromatid cohesion, or meiotic progression.

In yeast, the location of a particular chromosome within the nucleus and its association with its sister chromatid following DNA replication are commonly assayed using arrays of the lactose-operator DNA binding site (LacOα) and a GFP fusion to the DNA binding region of the lac repressor (GFP-LacI) (Figure 1, A and E). If the array is inserted into a telomeric region of the genome in a strain containing a NE marker, the distance between the chromosomal focus of GFP-LacI and the NE, as well as the nuclear diameter, can be measured, and each spot is assigned into one of three concentric zones of equal volume that approximates the position of the telomere within the nucleus (Figure 1A) (Hediger et al. 2002). If the array is integrated into a chromosomal region near the centromere, it can be used to assay cohesion between sister chromatids—the linkage between sisters resists chromosome separation (and that of the arrays) until the onset of anaphase, so a single focus is observed in metaphase-arrested cells if cohesion is maintained (Figure 1E) (Straight et al. 1996). Both assays have been used previously to show that the Mps3 N-terminus is required for telomere tethering and sister-chromatid cohesion (Bupp et al. 2007; Schober et al. 2009; Ghosh et al. 2012).
telomere tethering (43% foci in zone 1), consistent with previous findings that NPC components affect the peripheral recruitment of chromosome ends (Galy et al. 2000; Therizols et al. 2006; Van de Vosse et al. 2013). However, if MPS3 also was deleted, chromosomes assumed a random distribution (Figure 1, B and C). This loss of tethering in cells lacking MPS3 correlated with a loss of telomeric silencing, which was assayed using an ADE2 marker located at a truncated version of telomere VI-R. In wild-type cells, where telomeres were tethered, stochastic expression of ADE2 led to the formation of red and white colonies after growth on plates containing limiting amounts of adenine (Figure 1D). A similar expression pattern was observed in pom152Δ cells, but pom152Δ mps3Δ cells appeared light pink to white in color, similar to mps3Δ75–150 mutants, as a result of increased expression of ADE2 at the truncated telomere (Figure 1D) (Bupp et al. 2007). In the cohesion assay, 79% of wild-type and 74% of pom152Δ cells maintained cohesion in a metaphase arrest (Figure 1, F and G). Only 63% mps3Δ pom152Δ cells established cohesion (Figure 1, F and G), which is similar to the amount of cohesion observed in mps3Δ75–150 mutants (64%) (Ghosh et al. 2012). These data demonstrate that deletion of POM152 does not suppress the requirement for MPS3 in sister-chromatid cohesion, telomere tethering, or silencing during mitotic growth.

To determine whether pom152Δ suppresses the need for Mps3 in meiosis, we constructed a series of heterozygous and homozygous diploids containing POM152 or pom152Δ and MPS3 or mps3Δ. In the construction of these strains, we noticed that it was difficult to obtain diploids if both parental cells were of the pom152Δ mps3Δ genotype. This defect was suppressed by the addition of a covering plasmid containing MPS3 but not POM152 in one or both of the haploid parents. The covering plasmid could be lost in the diploid, indicating that pom152Δ/pom152Δ mps3Δ/mps3Δ diploids are viable, although they do show an increased rate of chromosome loss and a slight growth defect (Figure 1H; data not shown). These data support the recent finding from Rogers and Rose (2014) that Mps3 is required for karyogamy (nuclear fusion following mating) and that elimination of POM152 cannot bypass this requirement. Sporulation of diploids showed that POM152 deletion does not rescue Mps3 function during meiosis. Cells lacking both copies of MPS3 were unable to form tetrads after 5 days in sporulation medium (Figure 1I). The sporulation frequency between wild-type (35 ± 2%) and pom152Δ homo- and heterozygotes (ranging from 23–30%) (Figure 1I) was only moderately decreased, indicating that deleting POM152 had a minor effect on meiosis, sporulation, and/or germination. However, if MPS3 is deleted, virtually no meiotic products are formed, including dyads or tetrads, pointing to an arrest early in the meiotic program (Figure 1I). Thus, although pom152Δ suppresses the essential function of Mps3 during mitosis, it is unable to bypass the need for Mps3 during meiosis and sporulation. Collectively, these data lend support to the idea that deletion of POM152 specifically affects the SPB function of MPS3 during mitotic growth.

**A genome-wide screen for suppressors of mps3Δ**

To better understand how the requirement for Mps3 at the SPB can be bypassed, we conducted a genome-wide screen for other deletions that can suppress the lethality of mps3Δ. Because the deletion collection of nonessential yeast genes was made in the BY strain background, we constructed a query strain containing mps3Δ covered by MPS3 on a URA3-marked centromeric plasmid in this background for use in high-throughput studies (Figure 2, A and B). Following mating and diploid selection, the pCEN-URA3-MPS3 covering plasmid was removed by two rounds of growth on 5-FOA, and then diploids were sporulated and haploids selected using markers engineered into the strain (see Materials and Methods). Because MPS3 is essential for growth, only deletions that bypass the requirement for Mps3 function should be viable on plates that select for both mps3Δ and the gene knockout. Of the ~4900 deletion mutants in the collection, only 35 genes reproducibly (in three iterations of the screen) rescued mps3Δ cells (Table S1, Table S2, and Figure 2C). Thirteen deletions were along a region of chromosome 13 that contains an unknown suppressor of other essential genes (Copic et al. 2012), so these hits were not considered further (see Table S2 and Materials and Methods for a discussion of these genes).

To ensure that suppression in the remaining cases did not occur through a secondary mutation that cosegregated with mps3Δ or the deletion mutant, knockouts were made de novo in W303, a strain background commonly used in studies of SPB duplication. Of the remaining 22 deletions, only three hits, pom152Δ, pom34Δ, and sec66Δ, suppressed growth of mps3Δ mutants in W303 (Figure 2D). A single gene was not responsible for the strain-specific differences between W303 and BY that we observed for the other deletions because multiple outcrosses were required to convert a BY-like phenotype to a W303-like phenotype or vice versa (data not shown).

The robust suppression by pom152Δ in the genome-wide screen was consistent with our previous work showing that its elimination resulted in loss of Ndc1 from the NPC and increased Ndc1 binding at the SPB (Chen et al. 2014). NDC1 overexpression and/or pom152Δ can suppress a number of conditional alleles in genes encoding SPB components (Chial et al. 1998; Araki et al. 2006; Jaspersen et al. 2006; Anderson et al. 2007; Sezen et al. 2009; Witkin et al. 2010), but elimination of POM152 was only able to bypass deletions of MPS3 or MPS2 and not other membrane or half-bridge components of the SPB (Figure 2C; data not shown). Our observation that pom152Δ is unable to suppress the growth of mps2Δ mps3Δ double mutants suggests that Mps2 is required for pom152Δ-dependent bypass of mps3Δ and that Mps3 is required for pom152Δ-dependent bypass of mps2Δ (Figure 2E).

**sec66Δ suppresses mps3Δ and mps2Δ**

SEC66/SEC71/KAR7 encodes an integral membrane component of the Sec63 complex (Feldheim et al. 1993; Kurihara and Silver 1993; Brizzio et al. 1999). Together with the Sec61 translocon, the Sec63 complex is important for post-translational
Figure 2 Suppressors of SPB deletions. (A) Schematic of the mps3Δ strain (SLJ1888) used for our suppressor screen. Genes encoding the lysine permease LYP1 and the arginine permease CAN1 were deleted in the query strain. This strain also contained S. pombe his5+ (HIS3MX) expressed from the MATa-specific STE2 promoter. After addition of a URA3-based covering plasmid containing a wild-type copy of MPS3, the genomic copy of MPS3 was deleted using the NATMX marker. (B) Outline of screening strategy used for identification of suppressors. Query strains were mated to the MATa version of the yeast deletion collection, and diploids were selected on YPD + G418 + clonNAT. The URA3-marked plasmid was removed from the diploids by two rounds of growth on 5-FOA; then the diploids were sporulated. Haploids were selected by growth on SD-His-Lys-Arg + thiawine + canavanine; then cells containing both deletions were selected by addition of G418 + clonNAT. Because SPB genes are essential, most double-mutant
translocation of proteins into the ER (Figure 3A) (Park and Rapoport 2012; Ast and Schuldiner 2013; Mandon et al. 2013). Previous studies showed that SEC66 is nonessential for growth at 25 and 30°C, but sec66Δ cells show reduced growth at 37°C (Feldheim et al. 1993; Kurihara and Silver 1993; Fang and Green 1994). We found this temperature sensitivity to be largely dependent on strain background (Figure 2D, Figure 3, B and C, and Figure S1). Genetic or physical interactions between Sec66 and SPB components have not been reported, and Sec66 does not localize to the SPB (Figure 3E). sec66Δ was not identified as a suppressor of other SPB deletion mutants in our genome-wide screens (Figure 2C); however, direct testing in the W303 strain background showed that sec66Δ bypassed the requirement for mps2Δ at 23°C in addition to mps3Δ at all temperatures, but it had no effect on growth of other SPB deletions (Figure 3, B and C, and Figure S1). Unlike mps3Δ pom152Δ or mps3Δ pom34Δ cells that remained haploid, mps3Δ sec66Δ mutants showed a partial increase in ploidy, and mps2Δ sec66Δ mutants completely diploidized (Figure 2D; data not shown). This suggests that Mps3 and Mps2 have functions that are not or are incompletely bypassed by deletion of SEC66. Alternatively, given that sec66Δ alone shows a small 4N peak (Figure 2D), this change in chromosome content may be associated with a mitotic defect in the deletion in SEC66. Our observation that deletion of SEC66 and POM152 did not have additive effects on the growth of mps3Δ is most consistent with the idea that Sec66 and Pom152 are in the same pathway, although we cannot completely rule out the possibility of a threshold effect because mps2Δ sec66Δ pom152Δ cells grew better than mps2Δ sec66Δ cells (Figure 3, B and C). Elimination of other nonessential components of the Sec61 (SBH1) or Sec63 (SEC72) complex did not suppress mps3Δ, nor did deletion of the Sec61 homolog SSH1 (Figure 3D). Conditional mutants in SEC61 and SEC63 also did not bypass mps3Δ (data not shown), lending evidence to the idea that Sec66 is specifically involved in the control of SPB duplication via a pathway related to Mps3.

Characterization of SEC66

Previous work showed that cells lacking SEC66 have defects in translocation/insertion of a subset of proteins at the ER membrane, including the ER resident protein Kar2/Bip and the secreted proteins invertase/Suc2, α-factor, and vacuolar carboxypeptidase Y (Feldheim et al. 1993). A recent study using ribosome profiling implicated Sec66 in processing and ER docking of proteins with a looped signal sequence (Jan et al. 2014). Although nucleoporins and SPB components were not among the list of genes controlled by Sec66 and lack these sequence motifs, one could envision that loss of Sec66 function partially blocks ER import or folding of proteins needed directly or indirectly for SPB or NPC function, perhaps fully or partially mimicking pom152Δ and/or pom34Δ. Therefore, we examined NPC distribution, nucleocytoplasmic transport, SPB duplication, and spindle assembly in wild-type and sec66Δ cells.

Fusions between GFP and the nucleoporins Nic96, Nup192, Nup188, and Nup49 were created by PCR at endogenous loci in a strain containing the ER reporter HDEL-dsRed (a Kar2 signal sequence followed by dsRed and the yeast ER retrieval sequence HDEL) (Rossanese et al. 2001). The cytoplasmic signal of HDEL-dsRed was slightly increased in sec66Δ mutants compared to wild-type cells, which could be due to partial defects in its ER translocation because previous studies showed impaired import of full-length Kar2 in cells with mutant versions of SEC66 (Figure 4A) (Green et al. 1992; Feldheim et al. 1993; Kurihara and Silver 1993). NPC assembly defects often manifest as foci of one or more nucleoporins in the nucleus or cytoplasm. The lack of cytoplasmic or nuclear Nic96-GFP, Nup192-GFP, Nup188-GFP, and GFP-Nup49 foci in sec66Δ mutants suggests that removal of SEC66 does not result in major defects in NPC assembly, even if cells were shifted to 37°C for 4 hr (Figure 4A and Figure S2A; data not shown). Wild-type and sec66Δ cells showed similar nuclear sizes, and NPCs were evenly distributed over the surface of the NE. To confirm that NPCs were intact and functional, we analyzed nucleocytoplasmic transport with a series of reporters containing nuclear localization sequences (NLSs) and/or nuclear export sequences (NESs). Shown in Figure 4 (B and C) is the steady-state distribution of the cNLS-GFP2 and rgNLS-NESmut-GFP2 reporters that use the Kap60/Kap95 and Kap104 pathways, respectively (Stade et al. 1997; Lee and Aitchison 1999; Chook and Blobel 2001). A minor transport defect for cNLS-GFP2, but not rgNLS- NESmut-GFP2, was observed in mps3Δ sec66Δ cells, but in cells lacking SEC66 alone, the distribution of reporters was unaffected, similar to wild-type and pom152Δ pom34Δ cells (Miao et al. 2006). These data suggest that sec66Δ alone does not result in major defects in NPC structure and transport.
Comparison of the transcriptome of wild-type and sec66Δ cells grown at 23°C showed no difference in the expression levels of SPB or NPC genes, and machinery involved in protein folding or quality control was not induced, including components of the unfolded protein and stress-response pathways (Figure 4D and Table S3). Among the transcripts downregulated in sec66Δ compared to wild-type cells were a number of dubious ORFs (26 of 91), many of which appear to overlap with genes encoding ribosome subunits (11 of 26, 34.6% compared to a genome frequency of 5.5%). Most, but not all, of the dubious ORF transcripts are located on the noncoding strand and may represent antisense transcripts. However, decreased levels of the overlapping protein-coding gene were not observed, so the biological function and origin of the dubious ORF RNA were not investigated further (Figure S2B).

Examination of SPBs and microtubules using Spc42-mCherry and GFP-Tub1 in asynchronously grown cells revealed that a significant fraction of sec66Δ mutants assembled bipolar metaphase and anaphase spindles that were indistinguishable from wild-type spindles (Figure 4, E and F). However, broken spindles with microtubules from each SPB that do not overlap were observed in 12% (n = 102) of sec66Δ mutants vs. 4% (n = 105) of wild-type cells. Monopolar spindles in which a single SPB nucleates nuclear microtubules also were present in 7% (n = 102) of sec66Δ cells compared to <1% (n = 105) of wild-type cells. The same or more severe spindle defects were observed in cells lacking SEC72 and in temperature-sensitive mutants of SEC63, an essential subunit of the Sec63 complex (Figure 4, E and F, and Figure S3). Spindles within the bud and multipolar spindles were seen, particularly in sec63–104 at both 23 and 37°C, but no accumulation of cells in mitosis was detected either by flow cytometric analysis of DNA content or by budding index (Figure S3). Thus, perturbation of Sec63 complex function leads to spindle defects similar to other components of the secretory pathway (Winey et al. 1991; Duden et al. 1994; Yu et al. 2006). However, given that suppression is specific to sec66Δ (Figure 3D; data not shown), it is unlikely that the role of the Sec63 complex in spindle assembly or maintenance is related to the bypass pathway of MPS2 and MPS3.

**Elimination of SEC66 decreases Pom152 levels**

Pom34 and Pom152 are integral membrane proteins that are presumably inserted into the ER via the Sec61 translocon together with the Sec63 complex (Wozniak et al. 1994;
Figure 4 Characterization of NPCs and SPBs in cells lacking SEC66. (A) Wild-type and sec66Δ cells containing the ER marker HDEL-dsRed (red) and the NPC subunits Nic96-GFP (green, top panels) or Nup192-GFP (green, bottom panels) were grown at 30°C and imaged. Bar, 2 μm. (B and C) Wild-type, sec66Δ, and mps3Δ sec66Δ cells containing Ndc1-mCherry (red) and the nuclear transport reporters cNLS-GFP (green) or rgNLS-GFP (not shown) were imaged, and the intensity of the reporter in the nucleus and cytoplasm was quantitated in each cell (n = 100). The long bar shows the average ratio of nuclear to cytoplasmic signal, and the shorter bars show SEM. P-values were calculated using the Student’s t-test; only localization of cNLS-GFP in mps3Δ sec66Δ cells was statistically different from that of wild-type cells, as indicated. Bar, 2 μm. (D) Differentially expressed genes in wild-type (SLJ173)
Tcheperegin et al. 1999; Rout et al. 2000; Miao et al. 2006). Therefore, Sec66 may inhibit SPB duplication by altering the levels of Pom152 or Pom34, which would partially or completely mimic pom152Δ or pom34Δ. To test this idea, we fused the endogenous copy of POM152 or POM34 to YFP in wild-type, sec66Δ, and mps3Δ sec66Δ cells. Western blotting showed an increase in levels of Pom34-YFP in both sec66Δ and mps3Δ sec66Δ cells compared to wild-type cells, but analysis of its localization showed little change in the amount of Pom34-YFP at the NE (Figure 5, A–C). In contrast, Pom152-YFP levels decreased by over 50% in both assays in sec66Δ and mps3Δ sec66Δ cells compared to wild-type cells (Figure 5, A–C).

To test whether sec66Δ suppresses the growth of mps3Δ mutants via downregulation of Pom152, we added extra POM152 to cells using a 2-µm plasmid. Overexpression of POM152, but not POM34, exacerbated growth of mps3Δ sec66Δ mutants, particularly at 37°, but did not affect the growth of wild-type cells (Figure 5D). A similar phenotype was observed if POM152 was overproduced using the strong constitutive GAL1/10 promoter (Figure 5E). Taken together, these observations suggest that Sec66 is required to maintain wild-type levels of Pom152 in the cell and that a decrease in Pom152 levels is at least partially responsible for the ability of sec66Δ to bypass the requirement for Mps3.

**Sec66 effects at the SPB are independent of Ndc1**

Previously, we proposed that a shift in Ndc1 binding from the NPC to the SPB underlay the ability of pom152Δ to bypass the requirement for Mps3. This conclusion was based in part on our finding that deletion of POM152 resulted in decreased amounts of NPC-associated Ndc1 and increased Ndc1 levels at the SPB (Chen et al. 2014). Thus, we anticipated finding more Ndc1 at the SPB in sec66Δ mutants as a result of decreased Pom152 levels (Figure 5, A–C).

Quantitative Western blotting of Ndc1-GFP in wild-type and sec66Δ cells showed little change in total intracellular levels of Ndc1 (Figure 5C), and imaging showed that the distribution of protein on the NE and SPB also was similar (Figure 6, A–C). To study the spatial relationship between Ndc1 and Nbp1 at the SPB, we used acceptor-photobleaching FRET. In this method of FRET, the fluorescence intensity of the FRET donor is measured before and after bleaching the FRET acceptor. If energy transfer occurs between the FRET pairs, fluorescence of the FRET donor should increase after bleaching of the acceptor, which is expressed in a FRET efficiency score (see Materials and Methods). Because molecules must be in close proximity for the energy transfer to occur, FRET efficiency is related to the distance between donor and acceptor. Two SPB components (Spc42-mTurquoise2 and Cnm67-YFP) previously shown to exhibit high FRET gave 11.0 ± 1.0% (n = 159) FRET efficiency in our system (Muller et al. 2005). In contrast, if molecules are unable to transfer energy, no change in fluorescence of the FRET donor should be observed after bleaching. YFP-Spc110-mTurquoise2 exhibits 0.4 ± 1.1% (n = 79) FRET owing to the fact that the N- and C-termini of Spc110 are separated by 600–800 Å (Muller et al. 2005). Using Nbp1-mTurquoise2 as the donor and Ndc1-YFP as the acceptor, we observed 4.8 ± 1.1% (n = 113) FRET at the SPB in wild-type cells and 6.5 ± 1.8% (n = 79) FRET in sec66Δ cells, a change that was not statistically significant. This result suggests that Ndc1 and Nbp1 are in close proximity at the SPB and that their location is unchanged on loss of Sec66.

In mps3Δ sec66Δ mutants, several notable differences between wild-type and sec66Δ cells were observed. First, although all strains were haploid at the beginning of our experiment, the heterogeneity in nuclear (Figure 6, A and B) and cell size (not shown) in mps3Δ sec66Δ suggested that at least some fraction of cells diploidized during the course of our experiment. Because SPB and nuclear size scale with ploidy (Byers and Goetsch 1974; Bullitt et al. 1997; Jorgensen et al. 2007), levels of Ndc1-YFP at the SPB and NE were higher in a fraction of the double-mutant cells compared to wild-type and sec66Δ cells (Figure 6, A and B). However, the ratio of Ndc1-YFP at the SPB and NE, using values derived from each cell, showed that the distribution of Ndc1 is largely unaffected (Figure 6B). Second, levels of Nbp1-mTurquoise2 at the SPB were reduced in mps3Δ sec66Δ cells compared to wild-type or sec66Δ cells (Figure 6, A and C). Despite this reduction, FRET between Ndc1-YFP and Nbp1-mTurquoise2 in mps3Δ sec66Δ cells (6.5 ± 2.2%, n = 114) is virtually indistinguishable from that in sec66Δ cells and is not statistically different from wild-type cells. The fact that FRET does not decrease despite a reduction in levels of the Nbp1 donor in mps3Δ sec66Δ cells suggests that Nbp1 is the limiting factor at the SPB (Figure 6E), although it is formally possible that conformational differences between

vs. sec66Δ (SLJ5281) cells are shown in red in the plot, with average counts on the x-axis and the change in expression on the y-axis. Dashed lines at −1 and 1 indicate a twofold change in expression. The position of membrane and NPC- and SPB-encoding genes within the expression data are also shown. (E and F) Wild-type (SLJ6834), sec66Δ (SLJ10759), sec72Δ (SLJ10775), sec63–101 (SLJ10773), sec63–104 (SLJ10774), and sec63–105 (SLJ10807) cells containing Spc42-mCherry (red) and GFP-Tub1 (green) to visualize the SPB and microtubules, respectively, were grown to log phase at 23° and imaged. Examples images of large budded cells are shown in E, with dashed lines drawn based on bright-field images. Bar, 2 µm. (F) Spindle morphology was analyzed in at least 100 large budded cells of each genotype. Monopolar spindles (large budded cells containing a single SPB or containing two SPBs, only one of which nucleates nuclear microtubules) and broken spindles (large budded cells with two separated SPBs that contain nonoverlapping arrays of nuclear microtubules) were observed. The percentage of metaphase and anaphase spindles was determined using the natural gap in spindle length that occurred at 2 µm in all strains. Average length is shown by the long bar, and the shorter bars show SEM. *P*-values calculated using the Student’s t-test are listed. While none are statistically different from wild-type cells, the fraction of anaphase cells with intact spindles decreased particularly in sec63–104 and sec63–105 cells.
Pom152 protein levels are reduced in cells lacking SEC66. Mid-log phase cultures of wild-type (SLJ8167, SLJ7824), sec66Δ (SLJ8168, SLJ7854), and mps3Δ sec66Δ (SLJ8339, SLJ7895) cells containing Pom152-YFP or Pom34-YFP grown in SC-complete medium at 30°C were examined. (A) Projection images of three Z-slices in the center of the nucleus showing NE localization. Bar, 2 μm. (B) Levels of Pom152-YFP or Pom34-YFP were quantitated as described in Materials and Methods. For the 60 cells examined, the total fluorescence intensity is shown in arbitrary fluorescence units. The average fluorescence intensity is indicated by the long bar, and the shorter bars show SEM. P-values calculated by Student’s t-test are listed. (C) Extracts were prepared from the previous strains, Ndc1-GFP (SLJ7936, SLJ7937, and SLJ7938) and wild type (SLJ1070). Western blotting using anti-GFP antibodies was used to determine the total level of Pom152-YFP, Pom34-YFP, or Ndc1-GFP in the cell. Pgk1 is a loading control. Samples were normalized so that wild type cells had a value of 1. Molecular weight markers are shown on the left. (D) Wild-type (SLJ8666) and mps3Δ sec66Δ (SLJ8669) cells containing GAL-POM152 were serially diluted 10-fold, spotted onto plates containing 2% raf and 0.5% galactose, and incubated for 2 days at 37°C or 3 days at 23°C. (E) Wild-type (SLJ9253), sec66Δ (SLJ9259), and mps3Δ sec66Δ (SLJ9262) cells containing GAL-POM152 were serially diluted 10-fold, spotted onto plates containing 2% raffinose and 0.5% galactose, and incubated for 2 days at 37°C or 3 days at 23°C.
Ndc1-YFP and Nbp1-mTurquoise2, rather than changes in the number of bound molecules, account for FRET observed. Third, the observation that Ndc1-YFP/Ndc1-GFP levels at the SPB are not decreased in mps3Δ sec66Δ cells suggests that Ndc1 binds to additional SPB components besides Nbp1. While Sec66 may act by lowering Pom152 levels, the decreased dosage of Pom152 does not affect Ndc1 distribution and thus does not phenocopy pom152Δ (Chen et al. 2014). This suggests that the mechanism of suppression in sec66Δ cells is Ndc1 independent and that Pom152 may affect the SPB in more than one way.

Reduction of Pom152 is able to bypass Mps3 function

To test the idea that Pom152 dosage is important for SPB duplication, we compared the effects of removing one or both copies of POM152 in diploid cells lacking MPS3. At 23 or 30°C,
elimination of one copy of POM152, which decreases the dosage of the gene by 50%, is able to suppress the growth arrest of mps3Δ/mps3Δ cells (Figure 7A). The effect is weaker at 37°C in both hetero- and homozygous pom152Δ cells, but the dosage seems to be Pom152 specific. Elimination of one copy of POM34 produced an identical phenotype as deletion of both copies—suppression of growth at 30°C but not at 23 or 37°C. Deletion of one copy of POM152 is unable to suppress the haploinsufficiency of ndc1-L562S (Figure 7B), a mutant allele of NDC1 that displays Pom152-dependent binding to Mps3 (Chen et al. 2014). These findings are consistent with the idea that Pom152 levels play a key role in the control of SPB duplication independent of Ndc1.

Discussion

Because the SPB is the sole site of microtubule nucleation in budding yeast, virtually all components of the SPB are essential for viability. Therefore, it is somewhat surprising that bypass suppressors of SPB deletion mutants were recovered. Although previous work revealed genetic connections between the NPC and the SPB (Chial et al. 1998; Sezen et al. 2009; Greenland et al. 2010; Witkin et al. 2010; Friederichs et al. 2011; Casey et al. 2012; Chen et al. 2014), our work is the first to show that Sec66 plays a role in SPB duplication. Based on the fact that deletion of SEC66 bypasses the requirement for MPS3 and, to a lesser extent, MPS2, it seems that in wild-type cells, Sec66 functions to inhibit SPB duplication.

Our observation that deletion of POM152 and SEC66 did not have additive effects on growth of mps3Δ or mps3Δ cells suggests that Sec66 is likely acting in the same pathway as Pom152. However, unlike cells lacking POM152 (Chen et al. 2014), the distribution of Ndc1 between the NPC and the SPB was largely unaffected. Perhaps this is because sec66Δ mutants have some Pom152 protein (~50% reduction from wild-type cells compared with 100% in pom152Δ cells based on gene dosage). The fact that Ndc1 distribution is unchanged and that overproduction of Pom152 alone is detrimental to mps3Δ sec66Δ mutants under conditions where it does not affect the growth of wild-type or sec66Δ cells leads us to hypothesize that Pom152 has a direct role in blocking SPB duplication and that simply lowering levels is adequate to overcome the requirement for certain SPB components. This mechanism of suppression is not as efficient as pom152Δ, which not only removes Pom152 from the cell but also causes a redistribution of Ndc1 (Chen et al. 2014). Unlike mps3Δ pom152Δ cells that are stable haploids with no obvious SPB abnormalities (Witkin et al. 2012), mps3Δ sec66Δ cells exhibit a partial increase in ploidy, and mps2Δ sec66Δ mutants spontaneously diploidyze, which is commonly observed in cells with SPB duplication defects.

Multiple models have been proposed to explain the genetic relationship between NPCs and the SPB, including the idea that NPC components such as Pom152 may directly inhibit SPB duplication. Pom152 has been recovered in purified SPB preparations, and NPCs are often observed by EM in the vicinity of the duplicating SPB (Wigge et al. 1998; Adams and Kilmartin 1999). The model of Pom152-dependent inhibition predicts that more Nbp1 should localize to the SPB in mps3Δ sec66Δ mutants than in wild-type cells owing to reduced levels of Pom152. However, reduced levels of Nbp1 may have been observed because Mps3 is required to stabilize Nbp1 at the SPB. Mps3 and Nbp1 copurify, and overexpression of NBP1 is able to restore growth to certain MPS3 mutants (Jaspersen et al. 2006; Kupke et al. 2011). It is unknown whether the stability of Nbp1 is controlled or whether Mps3 and Nbp1 form a SPB-associated complex. Reduced levels of Pom152 also may result in decreased transport of Nbp1 into the nucleus or affect NE lipids (Friederichs et al. 2011; Kupke et al. 2011; Jaspersen and Ghosh 2012). Future studies will be

Figure 7 Pom152 dosage-dependent suppression of SPB mutants. (A) The ability of a hetero- or homozygous deletion of POM152 or POM34 was tested for its ability to rescue the growth defect of diploid strains lacking MPS3 by plating 10-fold serial dilutions of cells on SC-Ura (which selects for the pURA3-MPS3 plasmid) or 5-FOA (which selects for loss of the plasmid). Growth at 23°C (4 days), 30°C (4 days), or 37°C (3 days) is compared to a wild-type diploid. (B) Similarly, the growth of diploid cells containing the indicated combinations of NDC1 and POM152 alleles was compared.
required to elucidate the bypass mechanism, but our FRET, together with recent super-resolution imaging data (Burns et al. 2015), lends evidence to the idea that Nbp1 is a key SPB insertion factor.

Sec66 is a nonessential subunit of the budding yeast Sec63 complex, which is involved in targeting and translocation of a subset of proteins into the ER (Feldheim et al. 1993; Kurihara and Silver 1993; Brizzio et al. 1999). Sec66 might be involved in the membrane insertion of Pom152, but it is unclear why other components of the Sec63 complex, including temperature-sensitive sec63 alleles and sec72Δ, do not share the same ability to rescue mps3Δ or mps2Δ. We favor the idea that Sec66 plays a specialized role in the control of SPB duplication via Pom152 that may be independent of its role as a component of the Sec63 complex. This might explain why SEC66 is present only in lower eukaryotes that typically undergo a closed mitosis in which the SPB must assemble into the NE. Sec66 together with Pom152 may control events needed for SPB insertion into the membrane. This function would be similar, but not identical, to post-translational assembly of integral membrane proteins at the ER (Shao and Hegde 2011) and suggests that the primary role of Mps2 and Mps3 during SPB duplication is to facilitate membrane insertion of the newly duplicated pole.

While it is not surprising that bypass suppressors of MPS3 such as pom152Δ do not rescue the function of Mps3 in telomere tethering or sister-chromatid cohesion, it is unclear why deletion of POM152 is unable to rescue mps3Δ during meiosis because the SPB is key to the formation of the meiosis I and II spindles (Moens and Rapport 1971). Therefore, it seems likely that Mps3 has an essential meiotic function not suppressed by pom152Δ, such as its role in meiotic chromosome movement or linking SPBs prior to meiosis I (Conrad et al. 2007, 2008; Koszul et al. 2008; Lee et al. 2012; Li et al. 2015).

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SPB Control by Sec66 and Pom152

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Sec66-Dependent Regulation of Yeast Spindle-Pole Body Duplication Through Pom152

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Figure S1. Deletion of SEC66 partially bypasses the requirement for MPS2 but not other SPB components.

In the W303 background, sec66Δ (SLJ6997) was crossed to mps2Δ pURA3-MPS2 (SLJ5099), kar1Δ pURA3-KAR1 (SLJ5102), nbp1Δ pURA3-NBP1 (SLJ4282), sfi1Δ pURA3-SFI1 (SLJ9449) and bbp1Δ pURA3-BBP1 (SLJ9338) and sec66Δ (SLJ6968) to ndc1Δ pURA3-NDC1 (SLJ6059). Cells were sporulated and tetrads dissected and analyzed. 10-fold serial dilutions of cells from a tetratype tetrad were spotted onto SC-Ura or 5-FOA and incubated at 23°C for 3 d or 37°C for 2 d.
Figure S2. Expression of genes adjacent to dubious ORFs.
A. Wild-type and sec66Δ cells containing the ER marker HDEL-dsRed (red) and the NPC subunits Nic96-GFP (green) (SLJ10647 and SLJ10648) were grown at 23°C then half the culture was shifted to 37°C for 4 h prior to imaging. Bar, 2 μm. B. Genes in wild-type (SLJ173) versus sec66Δ (SLJ5281) cells are shown in the plot, with average counts on the x-axis and the change in expression on the y-axis. Dashed lines at -1 and 1 indicate a 2-fold change in expression. The position of genes upstream and downstream of the dubious ORFs is highlighted.
Figure S3. Spindle defects in sec63 mutants.

sec63-101 (SLJ10773), sec63-104 (SLJ10774) and sec63-105 (SLJ10807) cells containing Spc42-mCherry (red) and GFP-Tub1 (green) to visualize the SPB and microtubules, respectively, were grown to log phase at 23°C then half the culture was shifted to 37°C for 4 h. A. Flow cytometric analysis of DNA content. B. Examples images of large budded cells are shown, with dashed lines drawn based on brightfield image. Bar, 2 µm. C. In at least 100 large budded cells, spindle morphology was analyzed. Monopolar spindles (large budded cells containing a single SPB or containing two SPBs, only one of which nucleates nuclear microtubules) and broken spindles (large budded cells with two separated SPBs that contain non-overlapping arrays of nuclear microtubules) were observed. The percentage of metaphase and anaphase spindles was determined using the natural gap in length that occurred at 2 µm in all strains. D. Average spindle length is shown by the long bar; shorter bars show SEM. p values calculated using Student’s t-test are listed. In addition to broken and monopolar spindles, we observed cells that contained bipolar spindles within the daughter cell (see sec63-104 at both 23°C and 37°C).
Table S1.Suppressors of *mps3Δ*.

| ORF name   | gene | alias       | *mps3Δ* | function                                                                                                                                 |
|------------|------|-------------|---------|------------------------------------------------------------------------------------------------------------------------------------------|
| YBR171W    | SEC66| HSS1, SEC71 | yes     | Non-essential subunit of Sec63 complex; with Sec61 complex, Kar2p/BiP and Lhs1p forms a channel competent for SRP-dependent and post-translational SRP-independent protein targeting and import into the ER |
| YBR196C-A  |      |             | no      | Putative protein of unknown function Putative aryl-alcohol dehydrogenase; mutational analysis has not yet revealed a physiological role       |
| YCR107W    | AAD3 |             | no      | Subunit of a complex that inhibits sister chromatid cohesion                                                                                |
| YDR014W    | RAD61| WPL1        | nd      | Protein component of the small (40S) ribosomal subunit                                                                                 |
| YDR025W    | RPS11A|            | nd      | Inositol phosphotransferase; involved in synthesis of mannose-(inositol-P)2-ceramide (M(IP)2C), the most abundant sphingolipid              |
| YDR072C    | IPT1 | MIC,2, KTI6, SYR4 | no      | Subunit of THO/TREX complex to couple transcription elongation with mitotic recombination and with mRNA metabolism and export; regulates lifespan; involved in telomere maintenance |
| YDR138W    | HPR1 | TRF1        | no      |                                                                                                                                          |
| Gene       | Protein Name | Expression |
|------------|--------------|------------|
| YDR273W    | DON1         | nd         |
| YGL136C    | MRM2         | no         |
| YGL151W    | NUT1         | no         |
| YGL206C    | CHC1         | no         |
| YGL261C    | PAU11        | no         |
| YGR143W    | SKN1         | no         |
| YLR018C    | POM34        | yes        |
| YLR338W    | OPI9 (VRP1)  | nd         |

**YDR273W (DON1)**: Meiosis-specific component of the spindle pole body; part of the leading edge protein (LEP) coat, forms a ring-like structure at the leading edge of the prospore membrane during meiosis II.

**YGL136C (MRM2)**: Mitochondrial 2'-O-ribose methyltransferase; required for methylation of U(2791) in 21S rRNA.

**YGL151W (NUT1)**: Component of the RNA polymerase II mediator complex.

**YGL206C (CHC1)**: Clathrin heavy chain; subunit of the major coat protein involved in intracellular protein transport and endocytosis.

**YGL261C (PAU11)**: Putative protein of unknown function; member of the seripauperin multigene family encoded mainly in subtelomeric regions.

**YGR143W (SKN1)**: Protein involved in sphingolipid biosynthesis; subunit of the transmembrane ring of the nuclear pore complex (NPC); contributes to nucleocytoplasmic transport, NPC biogenesis and spindle pole body duplication.

**YLR018C (POM34)**: Dubious open reading frame; paritally overlaps with VRP1, a proline-rich actin-associated protein; involved in actin nucleation, endocytosis and cytokinesis.
| Gene Name | Description                                                                                                                                   |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------|
| YLR368W  | **MDM30** DSG1  
F-box component of an SCF ubiquitin protein ligase complex; associates with and is required for Fzo1p ubiquitination and for mitochondria fusion; stimulates nuclear export of specific mRNAs |
| YMR129W  | **POM152**  
Glycoprotein subunit of transmembrane ring of nuclear pore complex; contributes to nucleocytoplasmic transport, nuclear pore complex (NPC) biogenesis and spindle pole body duplication |
| YOR239W  | **ABP140** YOR240 W, **TRM140**  
AdoMet-dependent tRNA methyltransferase and actin binding protein |
| YPL090C  | **RPS6A**  
Protein component of the small (40S) ribosomal subunit |
| YPR043W  | **RPL43A**  
Ribosomal 60S subunit protein L43A  
Protein that may have a role in transcription elongation; forms a complex with Csn12p that is recruited to transcribed genes |
| YPR045C  | **THP3** **MNI2**  
Dubious open reading frame that overlaps with NVJ2, a lipid-binding ER protein, enriched at nucleus-vacuolar junctions (NVJ); may be involved in sterol metabolism or signaling at the NVJ |
| YPR092W  | (NVJ2)  
|
Table S2. Chromosome XIII hits.

|   | MPS3 | MPS2 | NBP1 | KAR1 | CDC31 | SFI1 | Combined | Gene  |
|---|------|------|------|------|-------|------|----------|-------|
|   |      |      |      |      |       |      | YML048W  | GSF2  |
|   |      |      |      |      |       |      | YML051W  | GAL80 |
|   |      |      |      |      |       |      | YML061C  | PIF1  |
|   |      |      |      |      |       |      | YML062C  | MFT1  |
|   |      |      |      |      |       |      | YML063W  | RPS1B |
|   |      |      |      |      |       |      | YML078W  | CPR3  |
|   |      |      |      |      |       |      | YML079W  |       |
|   |      |      |      |      |       |      | YML080W  | DUS1  |
|   |      |      |      |      |       |      | YML081W  | TDA9  |
|   |      |      |      |      |       |      | YML082W  |       |
|   |      |      |      |      |       |      | YML083C  |       |
|   |      |      |      |      |       |      | YML084W  |       |
|   |      |      |      |      |       |      | YML086C  |       |
|   |      |      |      |      |       |      | YML087C  |       |
|   |      |      |      |      |       |      | YML088W  | ALO1  |
|   |      |      |      |      |       |      | YML089C  | AIM33 |
|   |      |      |      |      |       |      |          | UFO1  |

Black boxes show hits from the indicated region along chromosome XIII that came up as hits in the SPB bypass suppressor screen.
Table S3. Sec66-dependent transcripts.

| ORF         | Gene Name | sec66Δ vs WT | pval |
|-------------|-----------|-------------|------|
| YBR171W     | SEC66     | -10.2880004 | 0    |
| YLR062C     | BUD28     | -5.48643455 | 0.000156377 |
| YDR209C     | YDR209C   | -4.295857687 | 0.017130425 |
| tF(GAA)M    | tF(GAA)M  | -4.06645471 | 0.034235269 |
| YHR079C-A   | SAE3      | -4.05487497 | 0.033219158 |
| YLR076C     | YLR076C   | -3.64276427 | 0.000713944 |
| YGR190C     | YGR190C   | -2.781392901 | 0.042814708 |
| YHR056W-A   | YHR056W-A | -2.757566482 | 0.002351011 |
| YNL194C     | YNL194C   | -2.600037779 | 1.45E-08 |
| YLL044W     | YLL044W   | -2.394228307 | 0.001732815 |
| YJL188C     | BUD19     | -2.38132712 | 1.08E-05 |
| YNR034W-A   | YNR034W-A | -2.353961449 | 6.29E-19 |
| YLR076C     | YLR076C   | -2.325364627 | 0.000713944 |
| YHR056W-A   | YHR056W-A | -2.302547703 | 0.002351011 |
| YPL250W-A   | YPL250W-A | -2.27526846 | 0.005605854 |
| YOR178C     | GAC1      | -2.147570558 | 2.87E-09 |
| YER078W-A   | YER078W-A | -2.132870744 | 0.016353188 |
| YCR021C     | HSP30     | -2.107740157 | 0.002656172 |
| YBR054W     | YRO2      | -2.05641111 | 2.57E-09 |
| YER067W     | RGI1      | -2.000876054 | 7.76E-10 |
| YGL255W     | ZRT1      | -1.99782713 | 1.05E-29 |
| YNL174W     | YNL174W   | -1.87993942 | 0.025228476 |
| YGR102C     | YGR102C   | -1.847132559 | 0.000174266 |
| YBR093C     | PHO5      | -1.842011975 | 1.54E-25 |
| YMR245W     | YMR245W   | -1.840916334 | 0.00912187 |
| YAL059C-A   | YAL059C-A | -1.832830554 | 0.015773239 |
| YPR123C     | YPR123C   | -1.82179286 | 0.017209026 |
| YER150W     | SPI1      | -1.73567611 | 0.00148842 |
| YFR036W-A   | YFR036W-A | -1.707286044 | 0.03669005 |
| YMR122C     | YMR122C   | -1.621641101 | 0.04078201 |
| YPR142C     | YPR142C   | -1.615796291 | 0.003216244 |
| YLR255C     | YLR255C   | -1.611382332 | 0.039099586 |
| YAL037C-B   | YAL037C-B | -1.6092561 | 0.00401517 |
| YGR052W     | FMP48     | -1.588796836 | 1.28E-13 |
| YEL053W-A   | YEL053W-A | -1.581634522 | 0.033508252 |
| YBO49W      | MOH1      | -1.579161933 | 6.56E-07 |
| YGL123C-A   | YGL123C-A | -1.56179132 | 0.001303258 |
| YGR088W     | CT1       | -1.55400821 | 8.30E-11 |
| YOR393W     | ERR1      | -1.540342252 | 0.012763129 |
| YDR171W     | HSP42     | -1.513674929 | 0.000187228 |
| YMR085W     | YMR085W   | -1.503429012 | 7.47E-05 |
| YMR084W     | YMR084W   | -1.503326472 | 0.001949641 |
| tK(UUU)P    | tK(UUU)P  | -1.47341834 | 0.01418737 |
| YPR160W-A   | YPR160W-A | -1.4229929 | 0.005901096 |
| YHR087W     | RTC3      | -1.418151914 | 1.10E-06 |
| YOR053W     | YOR053W   | -1.373040332 | 0.019455409 |
| YEL034C-A   | YEL034C-A | -1.362287935 | 0.017523617 |
| YAR071W     | PHO11     | -1.360624145 | 9.05E-19 |
| YHR215W     | PHO12     | -1.357642361 | 8.35E-20 |
| YPL187W     | MF(ALPHA) | -1.34276221 | 0.032556093 |
| Gene      | Symbol | Value   | P-value  |
|-----------|--------|---------|----------|
| YGR138C   | TPO2   | -1.337626195 | 0.000922046 |
| YGR160W   | YGR160W | -1.322036041 | 0.023910501 |
| YNR014W   | YNR014W | -1.306601308 | 6.94E-05 |
| YPL250C   | ICY2   | -1.303993147 | 2.65E-10 |
| YOR273C   | TPO4   | -1.303292941 | 0.000736433 |
| YGL096W   | TOS8   | -1.257521507 | 0.00187126 |
| YPR157W   | TDA6   | -1.255323373 | 0.000194205 |
| YML100W   | TSL1   | -1.25403863  | 1.64E-13 |
| YPL014W   | YPL014W | -1.24223574  | 0.001187976 |
| YHR139C   | SPS100 | -1.238293895 | 0.000750221 |
| YDR119W-A | YDR119W-A | -1.237340604 | 0.000338856 |
| YFR015C   | GSY1   | -1.225193838 | 2.22E-14 |
| YAL061W   | BDH2   | -1.219536416 | 0.000585374 |
| YGR008C   | STF2   | -1.210841353 | 1.97E-05 |
| YGR142W   | BTN2   | -1.201197112 | 5.23E-07 |
| YFR032C   | RRT5   | -1.181384605 | 4.94E-05 |
| YDL204W   | RTN2   | -1.161468728 | 4.72E-08 |
| YIL113W   | SDP1   | -1.160566552 | 0.000685696 |
| YMR081C   | ISF1   | -1.159914343 | 0.002268085 |
| YOL084W   | PHM7   | -1.148366818 | 1.57E-05 |
| YGR248W   | SOL4   | -1.142884304 | 6.74E-05 |
| YJR018W   | YJR018W | -1.14010918  | 0.019030745 |
| YER188W   | YER188W | -1.136101848 | 0.00137426 |
| YJL142C   | IRC9   | -1.128668222 | 0.000663983 |
| YOL153C   | YOL153C | -1.117237892 | 0.003449447 |
| YMR250W   | GAD1   | -1.114849554 | 7.22E-06 |
| YGL179C   | TOS3   | -1.114362892 | 1.73E-05 |
| YGR249W   | MGA1   | -1.109988486 | 0.010968082 |
| YNL077W   | APJ1   | -1.091393604 | 2.18E-06 |
| YBL048W   | RRT1   | -1.075328329 | 0.005400329 |
| YDR074W   | TPS2   | -1.062314516 | 5.90E-07 |
| snR190    | SNR190 | -1.060490288 | 0.024823597 |
| YDL037C   | BSC1   | -1.05149752  | 1.29E-12 |
| YPL165C   | SET6   | -1.050013994 | 6.23E-06 |
| YEL011W   | GLC3   | -1.046633537 | 3.49E-09 |
| YLL052C   | AQY2   | -1.032058858 | 4.03E-06 |
| YDL039C   | PRM7   | -1.028681093 | 1.26E-12 |
| YER053C   | PIC2   | -1.028188955 | 0.000502348 |
| YHR216W   | IMD2   | -1.023757369 | 2.27E-10 |
| YLR161W   | YLR161W | -1.011504658 | 0.046144344 |
| YJL141C   | YAK1   | -1.009501865 | 6.57E-05 |
| YMR244W   | YMR244W | -1.001061547 | 0.020577729 |
| YEL065W   | SIT1   | -1.039044351 | 4.60E-11 |
| YJR079W   | YJR079W | -1.054224459 | 0.023831712 |
| YKL065W-A | YKL065W-A | -1.083089191 | 0.006233041 |
| YLR109W   | AHP1   | 1.085865436  | 2.77E-15 |
| YOR338W   | YOR338W | 1.096599904  | 7.80E-13 |
| YDR216W   | ADR1   | 1.152002753  | 1.86E-05 |
| YFR052C-A | YFR052C-A | 1.154190348 | 0.000856472 |
| YDR379C-A | YDR379C-A | 1.160552069 | 5.97E-08 |
| YNL276C   | YNL276C | 1.163607333  | 0.007991201 |
| Gene   | Gene  | Value1  | Value2  |
|--------|-------|---------|---------|
| YBL043W | ECM13 | 1.195707174 | 6.57E-08 |
| YLL062C | MHT1  | 1.213716162  | 4.35E-11 |
| YDR214W | AHA1  | 1.224462224  | 4.45E-17 |
| YNL198C | YNL198C | 1.230980296 | 0.001493988 |
| YIL141W | YIL141W | 1.234164358 | 5.90E-06 |
| YAL067C | SEO1  | 1.239996885  | 3.85E-05 |
| YGR109C | CLB6  | 1.265496689  | 0.001307086 |
| YBR294W | SUL1  | 1.418032243  | 2.13E-06 |
| YFR053C | HXK1  | 1.431347425  | 7.49E-07 |
| YHR069C-A | YHR069C-A | 1.448600162 | 0.008446839 |
| YOL038C-A | YOL038C-A | 1.530245849 | 0.000108017 |
| YDL186W | YDL186W | 1.533401345 | 0.02267933 |
| snR62 | SNR62 | 1.555935314  | 0.002834202 |
| YLR438W | CAR2  | 1.636506697  | 3.81E-08 |
| YEL045C | YEL045C | 1.644600039 | 0.001665849 |
| tY(GUA)F1 | SUP11 | 4.113705585  | 0.033577853 |
| YBR072C-A | YBR072C-A | 4.1164138  | 0.033303304 |
| Q0275 | COX3  | 4.117769535  | 0.033552379 |
## Table S4. Yeast Strains.

| Strain      | Relevant Genotype                                                                 | Experiment |
|-------------|----------------------------------------------------------------------------------|------------|
| SLJ2602     | Mata his3::GFP-LACI-HIS3 NUP49-GFP-URA3 TELVIR-LACOR-lexAo-TRP1 ADE2-TG1-3        | Figure 1B-D |
|             | Mata mps3Δ::mps3Δ75-150-NATMX his3::GFP-LACI-                                  |            |
|             | H33 NUP49-GFP-URA3 TELVIR-LACOR-lexAo-TRP1 ADE2-TG1-3                          |            |
| SLJ3081     | SLJ4333                                                                          | Figure 1B-D |
|             | NUP49-GFP-URA3 TELVIR-LACOR-lexAo-TRP1 ADE2-TG1-3                              |            |
| SLJ4333     | SLJ3081                                                                          | Figure 1B-D |
|             | Mata mps3Δ::NATMX his3::GFP-LACI-                                               |            |
|             | HIS3 NUP49-GFP-URA3 TELVIR-LACOR-lexAo-TRP1 ADE2-TG1-3                          |            |
| SLJ4330     | SLJ1982                                                                          | Figure 1E-G |
|             | Mata leu2::PDS1-18xmyc-LEU2 his3::GFP-LACI-HIS3 trp1::256x LACOR-TRP1           |            |
| SLJ1982     | SLJ3131                                                                          | Figure 1F-G |
|             | Mata mps3Δ::mps3Δ75-150-NATMX leu2::PDS1-18xmyc-LEU2 his3::GFP-LACI-HIS3 trp1:: |            |
|             | 256x LACOR-TRP1                                                                 |            |
| SLJ4328     | SLJ4325                                                                          | Figure 1F-G |
|             | Mata pom152Δ::HYGMX leu2::PDS1-18xmyc-LEU2 his3::GFP-LACI-HIS3 trp1::256x       |            |
|             | LACOR-TRP1                                                                       |            |
| SLJ4325     | SLJ2156                                                                          | Figure 1H   |
|             | Mata ade2-1 ura3 leu2 trp1-1 lys-801 his3 CAN1 (CEN 3.L. YPH278) URA3-SUPII     |            |
| SLJ2156     | SLJ4547                                                                          | Figure 1H   |
|             | Mata pom152Δ::HYGMX ade2-1 ura3 leu2 trp1-1 lys-801 his3 CAN1 (CEN 3.L. YPH278) |            |
|             | URA3-SUPII                                                                       |            |
| SLJ4547     | SLJ4548                                                                          | Figure 1H   |
|             | Mata pom152Δ::HYGMX mps3Δ::NATMX ade2-1 ura3 leu2 trp1-1 lys-801 his3 CAN1      |            |
|             | CFIII (CEN 3.L. YPH278) URA3-SUPII                                              |            |
| SLJ4548     | SLJ1888                                                                          | Figure 2A,  |
|             | Mata mps3Δ::NATMX can1Δ::STE2pr-SpHIS5 his3ΔO ura3ΔO leu2ΔO met15ΔO pURA3-MPS3 | C, Table S1,|
| SLJ1888     | SLJ4779                                                                          | S2         |
|             | Mata kar1Δ::NATMX can1Δ::STE2pr-SpHIS5 his3ΔO ura3ΔO leu2ΔO met15ΔO pURA3-KAR1  | Figure 2C,  |
|             | SLJ4781                                                                          | S2         |
|             | Mata mps2Δ::NATMX can1Δ::STE2pr-SpHIS5 his3ΔO ura3ΔO leu2ΔO met15ΔO pURA3-MPS2  |            |
| SLJ4781     | SLJ5872                                                                          | Figure 2C,  |
|             | Mata nbp1Δ::NATMX can1Δ::STE2pr-SpHIS5 his3ΔO ura3ΔO leu2ΔO met15ΔO pURA3-NBP1  | S2         |
| SLJ5872     | SLJ6357                                                                          | Figure 2C,  |
|             | Mata cdc31Δ::NATMX can1Δ::STE2pr-SpHIS5 his3ΔO ura3ΔO leu2ΔO met15ΔO pURA3-CDC31| S2         |
| SLJ6357     | SLJ6358                                                                          | Figure 2C,  |
|             | Mata sfi1Δ::NATMX can1Δ::STE2pr-SpHIS5 his3ΔO ura3ΔO leu2ΔO met15ΔO pURA3-SFI1  | S2         |
| SLJ6358     | SLJ8666                                                                          | Figure 2D,  |
|             | Mata pURA3-MPS3                                                                  | 3B, 3D, 5D |
| SLJ8666     | SLJ8667                                                                          | Figure 2D   |
|             | Mata mps3Δ::NATMX pURA3-MPS3                                                     |            |
| SLJ8667     | SLJ9550                                                                          | Figure 2D   |
|             | Mata pom152Δ::HYGMX pURA3-MPS3                                                  |            |
| SLJ9550     | SLJ4738                                                                          | Figure 2D   |
|             | Mata mps3Δ::NATMX pom152Δ::HYGMX pURA3-MPS3                                     |            |
SLJ9309  Mata pom3Δ::KANMX pURA3-MPS3
SLJ9310  Mata mps3Δ::NATMX pom3Δ::KANMX pURA3-MPS3
SLJ8668  Mata sec66Δ::KANMX pURA3-MPS3
SLJ8669  Mata mps3Δ::NATMX sec66Δ::KANMX pURA3-MPS3
SLJ4807  Mata pURA3-MPS2
SLJ4808  Mata mps2Δ::KANMX pURA3-MPS2
SLJ4809  Mata pom152Δ::HYGMX pURA3-MPS2
SLJ4810  Mata mps2Δ::KANMX pom152Δ::HYGMX pURA3-MPS2
SLJ4811  Mata mps3Δ::NATMX pom152Δ::HYGMX pURA3-MPS2
SLJ4812  Mata mps2Δ::KANMX mps3Δ::NATMX pom152Δ::HYGMX pURA3-MPS2
SLJ8670  Mata sec66Δ::KANMX pURA3-MPS2
SLJ7687  Mata mps2Δ::NATMX mps2Δ::KANMX pom152Δ::HYGMX pURA3-MPS2
SLJ9469  Mata sec72Δ::KANMX pURA3-MPS3
SLJ9489  Mata mps3Δ::NATMX sec72Δ::KANMX pURA3-MPS3
SLJ9792  Mata sbh1Δ::KANMX pURA3-MPS3
SLJ9791  Mata mps3Δ::NATMX sbh1Δ::KANMX pURA3-MPS3
SLJ7637  Mata mps3Δ::NATMX ssh1Δ::KANMX pURA3-MPS3
SLJ10508  Mata SEC66-GFP-NATMX pLEU2-SPC42-mCherry-HIS3MX
SLJ10647  Mata NIC96-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ10648  Mata sec66Δ::KANMX NIC96-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ10649  Mata NUP192-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ10650  Mata sec66Δ::KANMX NUP192-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ7996  Mata NDC1-mCherry-HYGMX 2µ-LEU2-rgNLS-mutNES-GFP
SLJ7997  Mata NDC1-mCherry-HYGMX pLEU2-cNLS-GFP
SLJ7998  Mata sec66Δ::KANMX NDC1-mCherry-HYGMX 2µ-LE
SLJ7999  Mata sec66Δ::KANMX NDC1-mCherry-HYGMX pLEU2-cNLS-GFP
SLJ8000  Mata mps3Δ::NATMX sec66Δ::KANMX NDC1-mCherr
SLJ8001  Mata mps3Δ::NATMX sec66Δ::KANMX NDC1-mCherr
SLJ6834  Mata SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10759  Mata sec66Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10775  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10773  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10774  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10807  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ7992  Mata sbh1Δ::KANMX pURA3-MPS3
SLJ9791  Mata mps3Δ::NATMX sbh1Δ::KANMX pURA3-MPS3
SLJ7637  Mata mps3Δ::NATMX ssh1Δ::KANMX pURA3-MPS3
SLJ10508  Mata SEC66-GFP-NATMX pLEU2-SPC42-mCherry-HIS3MX
SLJ10647  Mata NIC96-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ10648  Mata sec66Δ::KANMX NIC96-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ10649  Mata NUP192-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ10650  Mata sec66Δ::KANMX NUP192-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ7996  Mata NDC1-mCherry-HYGMX 2µ-LEU2-rgNLS-mutNES-GFP
SLJ7997  Mata NDC1-mCherry-HYGMX pLEU2-cNLS-GFP
SLJ7998  Mata sec66Δ::KANMX NDC1-mCherry-HYGMX 2µ-LE
SLJ7999  Mata sec66Δ::KANMX NDC1-mCherry-HYGMX pLEU2-cNLS-GFP
SLJ8000  Mata mps3Δ::NATMX sec66Δ::KANMX NDC1-mCherr
SLJ8001  Mata mps3Δ::NATMX sec66Δ::KANMX NDC1-mCherr
SLJ6834  Mata SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10759  Mata sec66Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10775  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10773  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10774  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10807  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ7992  Mata sbh1Δ::KANMX pURA3-MPS3
SLJ9791  Mata mps3Δ::NATMX sbh1Δ::KANMX pURA3-MPS3
SLJ7637  Mata mps3Δ::NATMX ssh1Δ::KANMX pURA3-MPS3
SLJ10508  Mata SEC66-GFP-NATMX pLEU2-SPC42-mCherry-HIS3MX
SLJ10647  Mata NIC96-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ10648  Mata sec66Δ::KANMX NIC96-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ10649  Mata NUP192-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ10650  Mata sec66Δ::KANMX NUP192-GFP-HIS3MX trp1::HDEL-DsRed-TRP1 ADE2
SLJ7996  Mata NDC1-mCherry-HYGMX 2µ-LEU2-rgNLS-mutNES-GFP
SLJ7997  Mata NDC1-mCherry-HYGMX pLEU2-cNLS-GFP
SLJ7998  Mata sec66Δ::KANMX NDC1-mCherry-HYGMX 2µ-LE
SLJ7999  Mata sec66Δ::KANMX NDC1-mCherry-HYGMX pLEU2-cNLS-GFP
SLJ8000  Mata mps3Δ::NATMX sec66Δ::KANMX NDC1-mCherr
SLJ8001  Mata mps3Δ::NATMX sec66Δ::KANMX NDC1-mCherr
SLJ6834  Mata SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10759  Mata sec66Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10775  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10773  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10774  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ10807  Mata sec72Δ::KANMX SPC42-mCherry-HYGMX GFP-TUB1-NATMX
SLJ6968  Mata sec66Δ::KANMX  Figure S1
SLJ6997  Mata sec66Δ::KANMX  Figure S1
SLJ5102  Mata kar1Δ::NATMX pURA3-KAR1  Figure S1
SLJ5099  Mata mps2Δ::NATMX pURA3-MPS2  Figure S1
SLJ9338  Mata bbp1Δ::NATMX pURA3-BBP1  Figure S1
SLJ6059  Mata ndc1Δ::NATMX pURA3-NDC1  Figure S1
SLJ4282  Mata nbp1Δ::HIS3MX pURA3-ADE3-NBP1  Figure S1
SLJ9449  Mata sfi1Δ::NATMX pURA3-SFI1  Figure S1
SLJ7686  Mata sec66Δ::KANMX pURA3-MPS2  Figure S1
SLJ7687  Mata mps2Δ::NATMX pURA3-MPS2  Figure S1
SLJ7688  Mata sec66Δ::KANMX mps2Δ::NATMX pURA3-MPS2  Figure S1
SLJ7690  Mata sec66Δ::KANMX pURA3-KAR1  Figure S1
SLJ7691  Mata kar1Δ::NATMX pURA-KAR1  Figure S1
SLJ7692  Mata sec66Δ::KANMX kar1Δ::NATMX pURA-KAR1  Figure S1
SLJ7706  Mata nbp1Δ::HIS3MX pURA3-ADE3-NBP1  Figure S1
SLJ7707  Mata sec66Δ::KANMX pURA3-ADE3-NBP1  Figure S1
SLJ7708  Mata nbp1Δ::HIS3MX sec66Δ::KANMX pURA3-ADE3-NBP1  Figure S1
SLJ7709  Mata ndc1Δ::NATMX pURA3-NDC1  Figure S1
SLJ7710  Mata sec66Δ::KANMX pURA3-NDC1  Figure S1
SLJ7711  Mata sec66Δ::KANMX ndc1Δ::NATMX pURA3-NDC1  Figure S1
SLJ9530  Mata sfi1Δ::NATMX pURA3-SFI1  Figure S1
SLJ9531  Mata sec66Δ::KANMX pURA3-SFI1  Figure S1
SLJ9532  Mata sfi1Δ::NATMX sec66Δ::KANMX pURA3-SFI1  Figure S1
SLJ9539  Mata bbp1Δ::NATMX pURA3-BBP1  Figure S1
SLJ9540  Mata sec66Δ::HYGMX pURA3-BBP1  Figure S1
SLJ9541  Mata bbp1Δ::NATMX sec66Δ::HYGMX pURA3-BBP1  Figure S1