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The Ipl1-Aurora protein kinase activates the spindle checkpoint by creating unattached kinetochores

Benjamin A. Pinsky[1], Charles Kung[2], Kevan M. Shokat[2,3] and Sue Biggins[1,4]

The spindle checkpoint ensures accurate chromosome segregation by delaying cell-cycle progression until all sister kinetochores capture microtubules from opposite poles and come under tension (for reviews, see refs 1, 2). Although the checkpoint is activated by either the lack of kinetochore-microtubule attachments or defects in the tension exerted by microtubule-generated forces, it is not clear whether these signals are linked. We investigated the connection between tension and attachment by studying the conserved budding yeast Ipl1-Aurora protein kinase that is required for checkpoint activation in the absence of tension but not attachment[3]. Here, we show that spindle-checkpoint activation in kinetochore mutants that seem to have unattached kinetochores depends on Ipl1 activity. When Ipl1 function was impaired in these kinetochore mutants, the attachments were restored and the checkpoint was turned off. These data indicate that Ipl1 activates the checkpoint in response to tension defects by creating unattached kinetochores. Moreover, although the Dam1 kinetochore complex has been implicated as a key downstream target, we found the existence of unidentified Ipl1 sites on Dam1 or additional important substrates that regulate both microtuble detachment and the checkpoint.

The spindle checkpoint allows cells time to correct improper microtubule-kinetochore attachments by inhibiting the activity of the anaphase-promoting complex (APC), a ubiquitin ligase that targets the anaphase inhibitor Pds1/Securin for destruction. Although both the lack of kinetochore-microtubule attachments and defects in the tension on kinetochores result in checkpoint activation, kinetochore-microtubule attachments are unstable in the absence of tension[4,5]. Therefore, tension defects may activate the checkpoint through the generation of unattached kinetochores (Fig. 1a). Alternatively, tension and attachment defects may activate the checkpoint through separate signals.

An essential regulator of both kinetochore-microtubule attachments and the spindle checkpoint response to tension defects is the conserved Ipl1-Aurora protein kinase (for a review, see ref. 6). Ipl1-Aurora is thought to facilitate proper attachment by destabilizing kinetochore-microtubule interactions that do not generate tension. The budding yeast Dam1–DASH–DDD kinetochore complex has been proposed to be a key downstream target of Ipl1-Aurora, as a phospho-deficient allele exhibited chromosome segregation defects that were similar to those of *ipl1* mutants[7]. Although the role of Ipl1-Aurora in the spindle checkpoint is unknown, the simplest explanation is that Ipl1-Aurora responds to tension defects by creating unattached kinetochores that activate the checkpoint.

To determine whether the role of Ipl1 in the checkpoint is to generate unattached kinetochores, we analysed a series of temperature-sensitive kinetochore mutants in the presence and absence of Ipl1 function for spindle-checkpoint activity and chromosome attachment. We carried out this study in budding yeast in which there is a single microtubule-binding site on each kinetochore, which enables the attachment state to be precisely determined[8]. Because the yeast kinetochore contains distinct subcomplexes that have been organized into inner, central and outer domains (for a review, see ref. 9), we analysed mutations that impair the inner kinetochore Mif2–Cenp-C protein (*mif2-3*), the central Ndc80–Hec1 and Ctf19 kinetochore complexes (*ndc80-1, nuf2-61* and *okp1-5*), and the outer kinetochore Dam1 complex (*ask1-2*). To determine whether these mutants require Ipl1 to activate the checkpoint, the single kinetochore mutant cells and each *ipl1-321* double mutant were arrested in G1, released to the restrictive temperature (37°C) and monitored by immunoblotting for Pds1 levels (Fig. 1b). As expected, Pds1 levels cycled in wild-type cells and were stabilized in all kinetochore single mutants, indicating a metaphase arrest. By contrast, Pds1 levels cycled in a similar manner to the wild type in the *ipl1* double mutants, demonstrating that these mutants arrest in metaphase due to Ipl1-dependent spindle-checkpoint activation.

We next analysed the chromosome attachment state in the kinetochore mutants that also lack Ipl1 function. Because the only way that chromosomes can segregate to the daughter bud is via attachment to the spindle, we analysed chromosome distribution to the bud as an unambiguous assay for the state of attachment[10]. In *ipl1* mutant cells, sister kinetochores attach to microtubules that arise from the same pole

[1]Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N., PO Box 19024, Seattle, WA 98109, USA. [2]Department of Cellular and Molecular Pharmacology, Box 2280, University of California at San Francisco, San Francisco, CA 94143, USA. [3]Department of Chemistry, University of California at Berkeley, BerkeleyCA 94720, USA. [4]Correspondence should be addressed to S.B. (e-mail: sbiggins@fhcrc.org)

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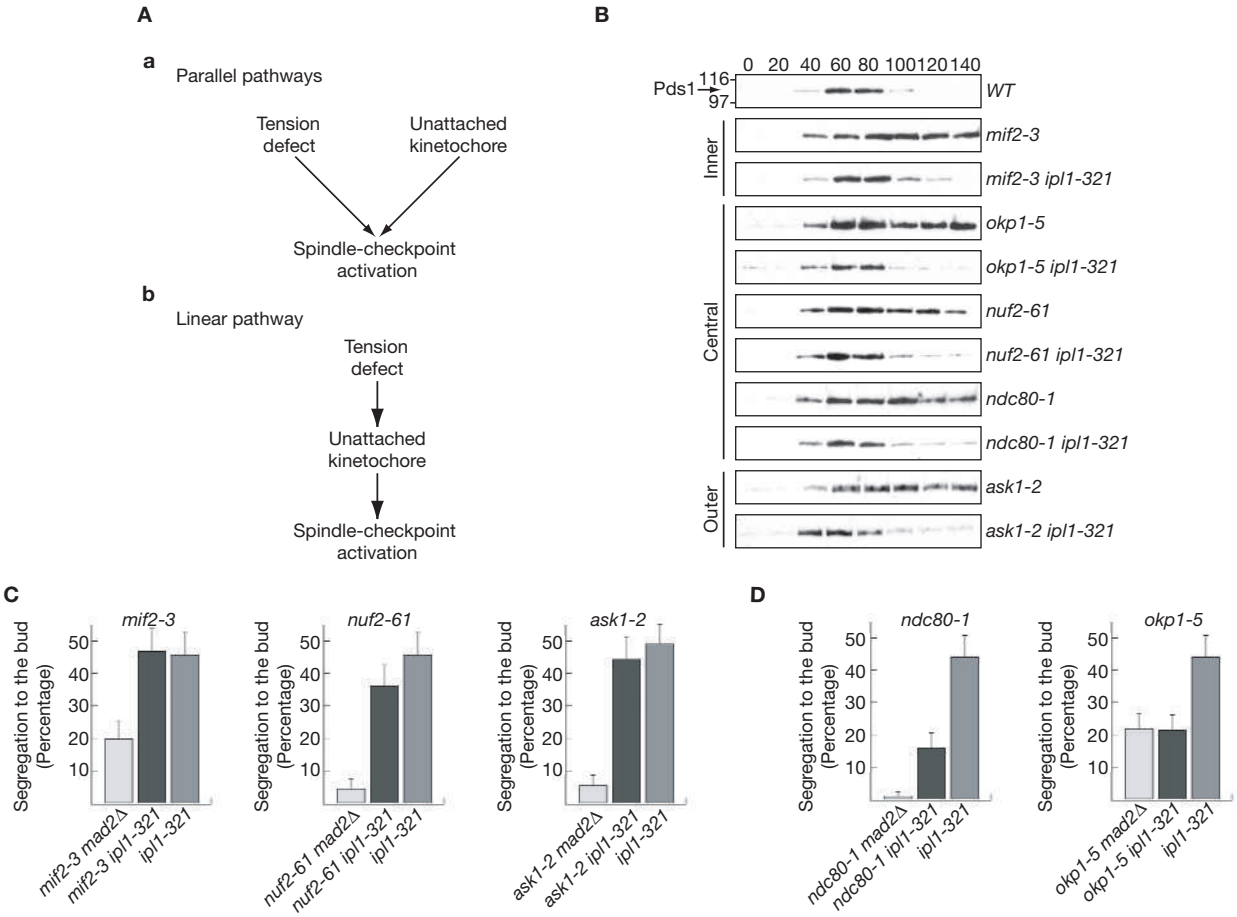

Figure 1 Analysis of kinetochore mutants in the presence and absence of Ipl1 function reveals two classes of mutants. (**A**) Models for the connection between tension and attachment defects in spindle-checkpoint activation. (**a**) Tension defects and unattached kinetochores are sensed separately and lead to spindle-checkpoint activation. (**b**) Tension defects cause microtubule detachment, resulting in an unattached kinetochore that activates the spindle checkpoint. (**B**) Inner-, central- and outer-kinetochore mutants activate the spindle checkpoint in an *IPL1*-dependent manner. Wild-type (WT; SBY818), *mif2-3* (SBY2497), *mif2-3 ipl1-321* (SBY2810), *okp1-5* (SBY2956), *okp1-5 ipl1-321* (SBY2957), *nuf2-61* (SBY2064), *nuf2-61 ipl1*-321 (SBY2063), *ndc80-1* (SBY2250), *ndc80-1 ipl1-321* (SBY2857), *ask1-2* (SBY1444) and *ask1-2 ipl1-321* (SBY1433) cells containing Pds1–Myc18 were arrested in G1 and released to 37ºC. Lysates were prepared at the indicated time points and immunoblotted with anti-Myc antibodies. (**C**) Impairing Ipl1 function restores kinetochore attachment in *mif2-3*, *nuf2-61* and *ask1-2* cells. *ipl1-321* (SBY322), *mif2-3 mad2*Δ (SBY3022), *mif2-3 ipl1-321* (SBY2929), *nuf2-61 mad2*Δ (SBY3148), *nuf2-61 ipl1-321* (SBY3186), *ask1-2 mad2*Δ (SBY3001) and *ask1-2 ipl1-321* (SBY1440) cells containing fluorescently marked Chr IV were arrested in G1 and released to 37ºC for 120 min. Large budded cells were monitored for the segregation of both Chr IV sister chromatids to the bud. (**D**) Impairing Ipl1 function does not fully restore *ndc80-1* and *okp1-5* segregation to the bud. *ipl1-321* (SBY322), *ndc80-1 mad2*Δ (SBY3000), *ndc80-1 ipl1-321* (SBY2857), *okp1-5 mad2*Δ (SBY3023) and *okp1-5 ipl1-321* (SBY3083) cells were processed as in (**C**).

instead of opposite poles[11–13]. This segregation pattern represents the basal state of kinetochore attachment in the absence of the Ipl1-mediated correction mechanism and results in pairs of chromosomes segregating to the bud approximately 50% of the time (Fig. 1c, d).

We analysed the segregation of fluorescently labelled chromosome IV (Chr IV) to the bud in the presence and absence of Ipl1. Because the kinetochore mutants activate the checkpoint, the spindle-checkpoint gene *MAD2* was eliminated to allow cell-cycle progression and comparison with the *ipl1* double mutants. This control also confirmed that the checkpoint itself does not have a role in kinetochore detachment. *Ipl1-321* cells, and each kinetochore mutant combined with either *mad2*Δ or *ipl1-321*, were arrested in G1, released to the restrictive temperature and assayed for Chr IV segregation to the bud at anaphase. Two classes of mutants were identified. In the first class (Fig. 1c), unattached chromosomes were generated by Ipl1 activity. In these mutants (*mif2-3*, *nuf2-61*, *ask1-2* and *ctf13-30*), both copies of Chr IV segregated to the bud less than 20% of the time (Fig. 1c, and see Supplementary Information, Fig. S1a). However, when Ipl1 was also impaired in these mutants, both Chr IV sisters segregated to the bud at the same frequency as *ipl1-321* single mutant cells (approximately 44% of the time). Because impairing Ipl1 both restored the basal state of chromosome segregation and prevented spindle-checkpoint arrest in these mutants, the role of Ipl1 in the checkpoint seems to be linked to the generation of unattached kinetochores.

However, the second class of mutants (*ndc80-1* and *okp1-5*) seemed to have unattached chromosomes in the absence of Ipl1 yet still required Ipl1 for checkpoint activation (Fig. 1d). The difference between the *ndc80-1* and *nuf2-61* mutants probably reflects variation in residual Ndc80 complex function. In *ndc80-1 mad2*Δ cells, both Chr IV sisters segregated to the bud only 1% of the time. Although 44% of *ipl1-321* cells segregated both Chr IV sisters to the bud, this occurred in just 13% of

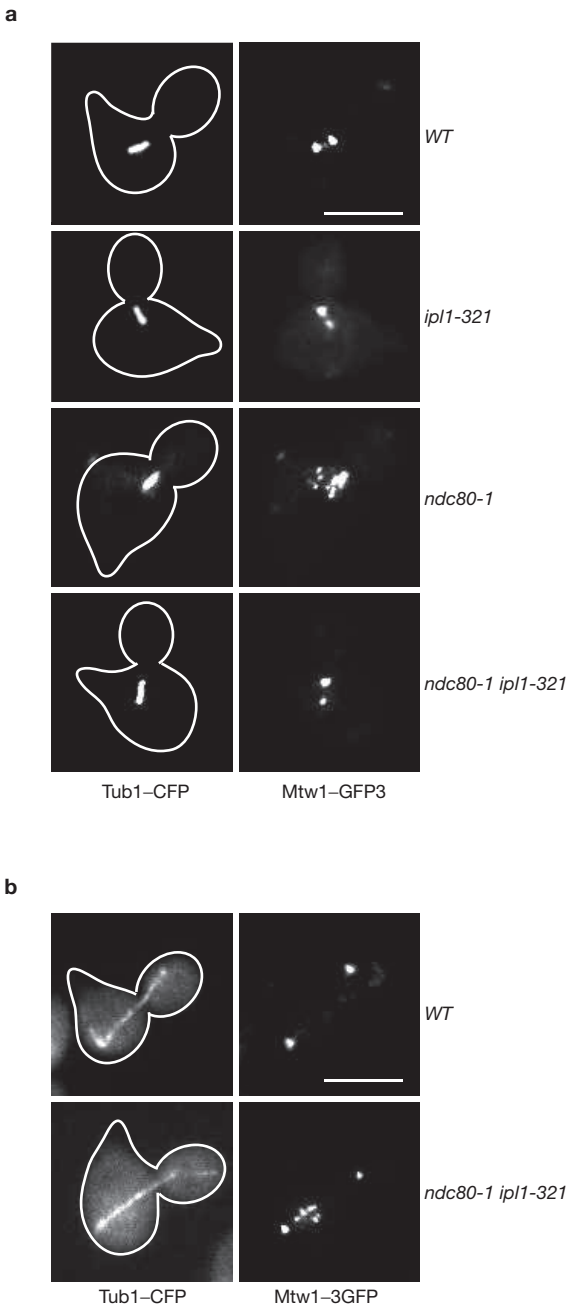

Figure 2 Ipl1 is responsible for generating unattached kinetochores in metaphase *ndc80-1* mutant cells. (**a**) Wild-type (WT; SBY4340), *ipl1-321* (SBY4343), *ndc80-1* (SBY4341) and *ndc80-1 ipl1-321* (SBY4342) cells expressing Tub1–CFP and Mtw1–3GFP were released from G1 to 37ºC. A total of 70 min after release, cells with short spindles were monitored for Mtw1–3GFP kinetochore foci. (**b**) Anaphase *ndc80-1 ipl1-321* cells have Ipl1-independent attachment defects. Wild-type (SBY4340) and *ndc80-1 ipl1-321* (SBY4342) cells were grown as in (**a**) and cells with long spindles were analysed for Mtw1–3GFP localization. Scale bar, 5 μm.

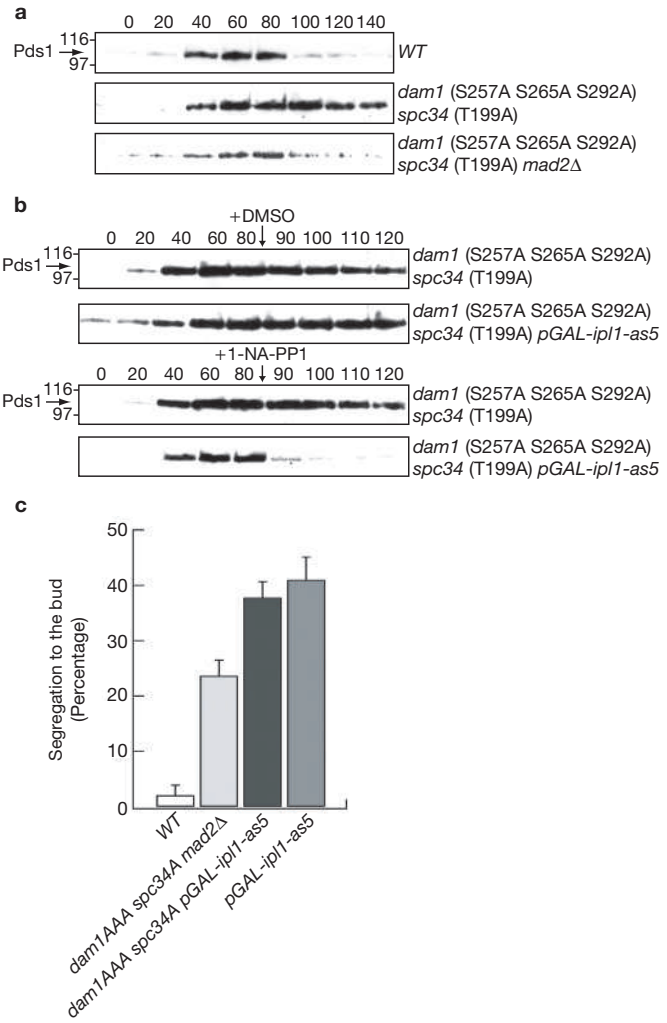

Figure 3 The *dam1* phospho-deficient mutant activates the spindle checkpoint and has unattached chromosomes due to Ipl1 activity. (**a**) Wild-type (WT; SBY818), *dam1* (S257A S265A S292A) *spc34* (T199A) (SBY 3702) and *dam1* (S257A S265A S292A) *spc34* (T199A) *mad2*Δ mutant cells (SBY3801) containing Pds1–Myc18 were arrested in G1 and released to 37ºC. Lysates were prepared at the indicated time points and immunoblotted with anti-Myc antibodies. (**b**) Duplicate cultures of *dam1* (S257A S265A S292A) *spc34* (T199A) (SBY3702) and *dam1* (S257A S265A S292A) *spc34* (T199A) *pGAL-ipl1-as5* (SBY4329) cells containing Pds1–Myc18 were grown in inducing media, arrested in G1 and released to 37ºC in non-inducing media. Control DMSO or 1-naphthyl-pyrazolo[3,4-d]pyrimidine (1-NA-PP1) inhibitor was added at 80 min. Lysates were prepared as in (**a**). (**c**) Wild-type (SBY214), *dam1* (S257A S265A S292A) *spc34* (T199A) *mad2*Δ (SBY4326) (*dam1AAA spc34A mad2*Δ), *dam1* (S257A S265A S292A) *spc34* (T199A) *pGAL-ipl1-as5* (SBY4997) (*dam1AA spc34A pGAL-ipl1-as5*) and *pGAL-ipl1-as5* (SBY4998) cells containing fluorescently marked Chr IV were grown in inducing media, arrested in G1 and released to 37ºC in non-inducing media. 1-NA-PP1 was added following budding and large budded cells were monitored for the segregation of both Chr IV sister chromatids to the bud.

ndc80-1 ipl1-321 cells. This segregation pattern was not due to a defect in spindle elongation, which is necessary to deliver chromosomes to the bud (see Supplementary Information, Fig. S1b).

Although these data indicated that there were unattached chromosomes in *ndc80-1 ipl1-321* mutant cells, the distribution to the bud assay analyses chromosome attachment indirectly by determining the final position of chromosomes after anaphase. This requires that chromosomes undergo the forces of spindle elongation and movement across the bud neck, two processes that could disrupt weak attachments. We therefore monitored attachment prior to segregation in metaphase cells by colocalizing fluorescently labelled spindles using Tub1–CFP and kinetochores using Mtw1–3GFP in wild-type, *ipl1-321*, *ndc80-1* and *ndc80-1 ipl1-321* cells. When Mtw1–3GFP foci localized on the same axis as tubulin, we assumed that the kinetochores were attached. Kinetochore foci that did

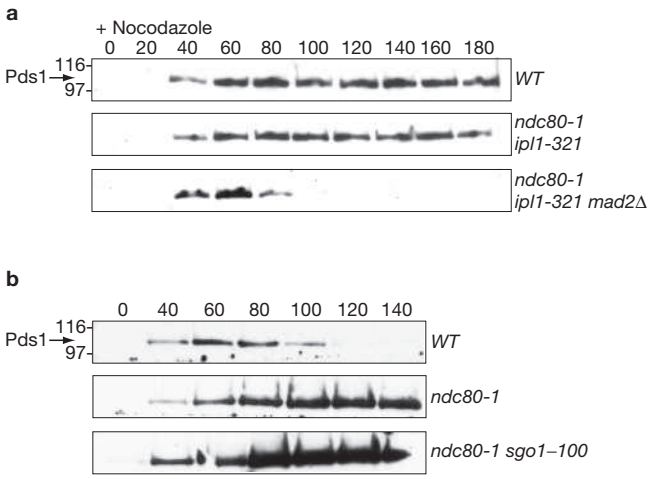

Figure 4 Spindle-checkpoint activity is restored in *ndc80-1 ipl1-321* mutant cells by regenerating unattached kinetochores and Sgo1 is not required to activate the checkpoint in *ndc80-1* cells. (**a**) Wild-type (SBY818), *ndc80-1 ipl1-321* (SBY2857) and *ndc80-1 ipl1-321 mad2*Δ (SBY5005) cells containing Pds1–Myc18 were arrested in G1 and released into nocodazole at 37ºC. Lysates were prepared at the indicated time points and immunoblotted with anti-Myc antibodies. (**b**) Wild-type (SBY818), *ndc80-1* (SBY2250) and *ndc80-1 sgo1-100* (SBY5093) cells containing Pds1–Myc18 were arrested in G1 and released to 37ºC. Lysates were prepared at the indicated time points and immunoblotted with anti-Myc antibodies.

not colocalize with tubulin were considered to be unattached. Wild-type and *ipl1-321* metaphase cells displayed a characteristic bilobular kinetochore localization pattern that was always associated with the spindle and indicates complete attachment (Fig. 2a). By contrast, the kinetochores in *ndc80-1* cells were completely disorganized, with most cells containing more than two foci and at least one focus off the spindle axis, as previously described[14]. Strikingly, the kinetochores in *ndc80-1 ipl1-321* cells appeared as organized as did those in wild-type cells, indicating that impairing Ipl1 function had restored attachments and silenced the checkpoint at metaphase. However, the attachments were not strong enough to withstand the forces of anaphase because *ndc80-1 ipl1-321* cells that had undergone anaphase had multiple kinetochore foci in the mother cell, indicating that chromosomes were unattached (Fig. 2b). Consistent with this, *ndc80 ipl1* cells contained unattached chromosomes if the nucleus was forced through the bud neck in the absence of anaphase (see Supplementary Information, Fig. S2). Taken together, these data indicate that weak attachments are made in the *ndc80-1 ipl1-321* cells until metaphase, and then spindle elongation and movement through the bud neck during anaphase causes them to detach.

If the role of Ipl1 in the checkpoint is to generate unattached kinetochores, Ipl1 phosphorylation of the Dam1–DASH–DDD complex should be required for checkpoint activation. To test this, we used a temperature-sensitive *dam1* phospho-deficient mutant (*dam1* (S257A S265A S292A) *spc34* (T199A)), in which three of the four Ipl1 consensus sites are mutated to alanine in addition to the single site on Spc34, another Dam1 complex component[7]. The *dam1* mutant, with all of the consensus Ipl1 sites changed to alanine, has not been studied because it is inviable. Similar to the S288c background[7], the *dam1* phospho-deficient mutant was temperature-sensitive in the W303 genetic background (see Supplementary Information, Fig. S3a).

To assess spindle-checkpoint activation, Pds1 levels were monitored in wild-type, *dam1* (S257A S265A S292A) *spc34* (T199A) and *dam1* (S257A S265A S292A) *spc34* (T199A) *mad2*Δ cells that were released from G1 to the restrictive temperature (Fig. 3a). Surprisingly, the *dam1* phospho-deficient cells activated the checkpoint because Pds1 was stabilized in a *MAD2*-dependent manner in both strain backgrounds (Fig. 3a, and see Supplementary Information, Fig. S3b). We therefore tested whether the arrest is Ipl1-dependent. Because we found that the *dam1* mutant is synthetically lethal with *ipl1-321*, we used an analogue-sensitive *ipl1* allele (*ipl1-as5*) under the control of the galactose-inducible promoter (*pGAL*) (for a review, see ref. 15). *In vitro* kinase assays verified that this allele was inhibited by the ATP analogue 1-naphthyl-pyrazolo[3,4-d]pyrimidine (1-NA-PP1; see Supplementary Information, Fig. S4). Duplicate cultures of *dam1* (S257A S265A S292A) *spc34* (T199A) and *dam1* (S257A S265A S292A) *spc34* (T199A) *pGAL-ipl1-as5* cells were arrested in G1 under inducing conditions and released into non-inducing media at the restrictive temperature. Some 80 min after release, we added either DMSO as a control or 1-NA-PP1 to inactivate any remaining *ipl1-as5*. Pds1 levels remained high in both strains treated with DMSO, as well as in inhibitor-treated *dam1* phospho-deficient cells (Fig. 3b). However, Pds1 was degraded within 10 min of inhibitor addition in the *dam1* (S257A S265A S292A) *spc34* (T199A) *pGAL-ipl1-as5* cells, indicating that the *dam1* phospho-deficient mutant depends on Ipl1 to activate the spindle checkpoint.

Because the *dam1* phospho-deficient mutant activates the checkpoint, we tested whether it also contained unattached chromosomes due to Ipl1 activity. When Chr IV segregation to the bud was assayed in the same experiment, the *dam1* phospho-deficient mutant segregated Chr IV to the bud 23% of the time (Fig. 3c). By contrast, the *dam1* phospho-deficient *ipl1* mutant cells segregated Chr IV to the bud 39% of the time, similar to that shown in *ipl1-321* cells. Because unattached chromosomes and spindle-checkpoint activation in the *dam1* phospho-deficient mutant require Ipl1, additional Ipl1 sites must exist on Dam1 or other critical targets must be phosphorylated by Ipl1 to mediate its roles in both microtubule destabilization and the spindle checkpoint. Genetic analyses of the *dam1* phospho-deficient cells supported these results (see Supplementary Information, Fig. S3c).

Our analysis of kinetochore mutants is consistent with the hypothesis that Ipl1 activates the spindle checkpoint by creating unattached kinetochores. If this were true, detaching kinetochores in cells lacking Ipl1 should restore checkpoint activation. To examine this, we monitored Pds1 levels in wild-type, *ndc80-1 ipl1-321* and *ndc80-1 ipl1-321 mad2*Δ cells that had been released from G1 to the restrictive temperature in the presence of nocodazole; this enabled depolymerization of microtubules and creation of unattached kinetochores (Fig. 4a). As predicted, Pds1 was stabilized in wild-type and *ndc80-1 ipl1-321* cells, and cycled in *ndc80-1 ipl1-321 mad2*Δ cells, indicating that spindle-checkpoint activity could be restored in the absence of Ipl1 if kinetochore-microtubule interactions were disrupted by another method.

Similar to Ipl1, the Sgo1 protein is required for spindle-checkpoint activation in response to a lack of tension on kinetochores due to a defect in the linkage between sister chromatids[16]. However, in contrast to Ipl1, Sgo1 has not been shown to generate unattached chromosomes. Therefore, if tension defects need to be converted to attachment defects to allow checkpoint activation, kinetochore mutants that depend on Ipl1 for checkpoint activation should not depend on Sgo1. We therefore examined whether Sgo1 was required for checkpoint activation in *ndc80-1* cells. Pds1 levels were examined in *ndc80-1* and *ndc80-1 sgo1-100* cells released from G1 to

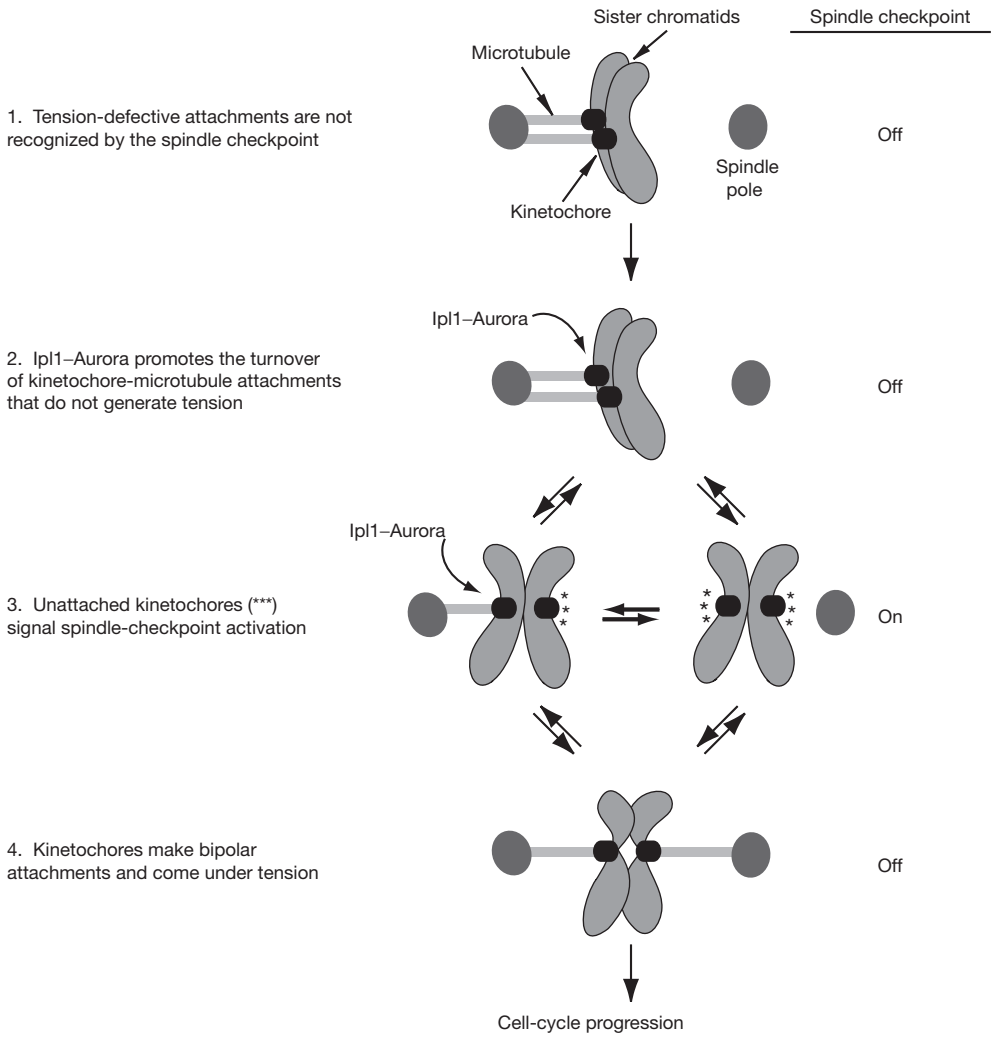

Figure 5 Model for Ipl1-Aurora function: Ipl1-Aurora corrects improper attachments and activates the spindle checkpoint by creating unattached kinetochores. 1. Mono-oriented attachments and other kinetochore-microtubule interactions that do not generate tension are not directly sensed by the spindle checkpoint. 2. Ipl1-Aurora recognizes tension defects and promotes the instability of improper attachments. 3. Ipl1-Aurora-mediated kinetochore detachment creates unoccupied microtubule binding sites that are the primary signal for spindle-checkpoint activation. 4. The checkpoint halts the cell cycle until kinetochores make bipolar attachments and come under tension.

the restrictive temperature. Unlike *ndc80 ipl1* mutant cells (Fig. 1b), Pds1 levels remained elevated in *ndc80 sgo1* mutant cells (Fig. 4b). Therefore, spindle-checkpoint activation in *ndc80* cells depends on Ipl1 but not on Sgo1 activity, which strongly indicates that the checkpoint is active in *ndc80* cells due to the presence of unattached kinetochores.

Our analyses indicate that the attachment defects in many kinetochore mutants are the indirect consequence of Ipl1-mediated detachment and are not due to the direct defect of a particular kinetochore protein. Instead, it seems that most mutant kinetochores actually attach to microtubules, making it crucial that the cell has a mechanism to sense and correct these improper, tension-defective attachments. This work is consistent with the hypothesis that Ipl1 is the tension sensor of the kinetochore and is responsible for the instability of kinetochore attachments that are not under tension. In addition, our data indicate that conclusions about the role of individual kinetochore complexes in microtubule attachment based on the analysis of kinetochore mutants in the presence of Ipl1 function needs to be re-evaluated.

These data strongly indicate that Ipl1 potentiates spindle-checkpoint activation by creating unattached kinetochores. We were unable to identify a situation in which the function of Ipl1 in kinetochore detachment was uncoupled from its role in the spindle checkpoint, and even the severely compromised *ndc80* mutant kinetochores made weak attachments in the absence of Ipl1. In addition, checkpoint activation in *ndc80* mutants did not depend on the activity of the Sgo1 protein, indicating that the checkpoint is activated in *ndc80* cells because Ipl1 created unattached kinetochores. The lack of requirement for Sgo1 may be due to a difference between tension defects that result from defective kinetochores and tension defects that result from a lack of sister-chromatid linkage. Although it is possible that Ipl1 has separate functions in the checkpoint and the generation of unattached chromosomes, we favour the model in which Ipl1 both corrects attachment errors and activates the spindle checkpoint by destabilizing improper kinetochore-microtubule interactions (Fig. 5). In addition, our data provide the strongest demonstration so far that tension defects must be converted to attachment

defects to engage the checkpoint. Therefore, tension and attachment seem to be intimately linked, as previously suggested[5,17].

It is likely that the role of Ipl1 in generating unattached kinetochores to allow spindle-checkpoint activation is conserved because Aurora B has also been shown to be required for tension defects to activate the checkpoint (for a review, see ref. 1). Because both spindle checkpoint and Aurora B dysfunction are associated with many cancers and remain potential targets for cancer therapy (for reviews, see refs 18, 19), our findings may have important implications for our understanding and treatment of human disease. □

METHODS

Microbial techniques. Media and microbial techniques were essentially as described previously[20,21]. All experiments in which cells were released from a G1 arrest were carried out by αF arrest and release, using αF at 1 μg ml^{-1} for *bar1*Δ strains and 10 μg ml^{-1} for *BAR1* strains. 1-NA-PP1 was used at 50 μM and nocodazole was used at 10 μg m^{-1}.

Yeast strains. Yeast strains are listed in the Supplementary Information (Table S1) and were constructed by standard genetic techniques. The *dam1* (S257A S265A S292A) *spc34* (T199A) strains[7] and *mif2-3* strains[22] were backcrossed at least five times into the W303 background. The *nuf2-61* (ref. 23), *ndc80-1* (ref. 24), *ask1-2* (ref. 25), *spc29-3* (ref. 26) and *ipl1-321* (ref. 11) alleles were crossed to make strains for this study. Strains containing *PDS1–Myc18:LEU2* were made by integrating plasmid pSB205 digested with *Hind*III at the *PDS1* locus. Strains containing *SPC42–GFP* were made by integrating plasmid pSB208 digested with *Bsu36*I at the *TRP1* locus. Strains containing *MTW1–3GFP* were generated by integrating plasmid pSB818 digested with *Sna*BI at the *MTW1* locus. Strains containing *TUB1–CFP:URA3* were obtained by integrating plasmid pSB375 digested with *Stu*I at the *URA3* locus. Strains containing *mad2::URA3* were generated by integration of plasmid pSB99 digested with *Eco*RI-*Hind*III. Other deletions in yeast genes were made using a polymerase chain reaction (PCR)-based integration system[27] and were confirmed by PCR. Specific primer sequences are available on request. All fusion proteins are fully functional.

Generation of the *ipl1-as5* strains. The *ipl1-as5* allele has the mutations M181G and T244A, which confer inhibitor sensitivity. The *ipl1-as5:LEU2* integrating plasmid was made by digesting pSB338 with *BamH*I-*Hind*III and ligating the fragment containing *ipl1-as5* into the *BamH*I-*Hind*III sites of plasmid pSB425, to create pSB428. To generate the *pGAL-ipl1-as5* strain, *Mlu*I digested pSB425 was integrated upstream of the *IPL1* locus in an *ipl1* deletion strain covered by the *IPL1, CEN, URA3* plasmid, pSB148. Loss of pSB148 was selected for on 5-FOA and the *pGAL* promoter was introduced using a PCR-based integration system[27].

Plasmid construction. The *MTW1–3GFP* integrating plasmid pSB818 was made by PCR amplification of the carboxy-terminal 600 base pairs of *MTW1* using primers SB844 and SB845, that have *Eco*RI and *BamH*I restriction sites engineered, respectively. The resulting PCR product was digested with *Eco*RI-*BamH*I and ligated into the *Eco*RI-*BamH*I sites of PB1585, to create pSB818.

Microscopy. Analysis of fixed cells was performed as described previously[11]. DAPI was used at a final concentration of 1 μg ml. At least 100 cells were analysed for all reported experiments. The error bars represent the 95% confidence interval.

Protein and immunological techniques. Protein extracts were made and immunoblotted as described previously[28]. 9E10 antibodies that recognize the Myc epitope were obtained from Covance (Princeton, NJ) and used at a 1:10,000 dilution. Equal protein loading was confirmed in all experiments by anti-tubulin immunoblotting (data not shown). Ipl1 kinase assays were performed as described previously[29].

Note: Supplementary Information is available on the Nature Cell Biology website.

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COMPETING FINANCIAL INTERESTS

The authors declare that they have no competing financial interests.

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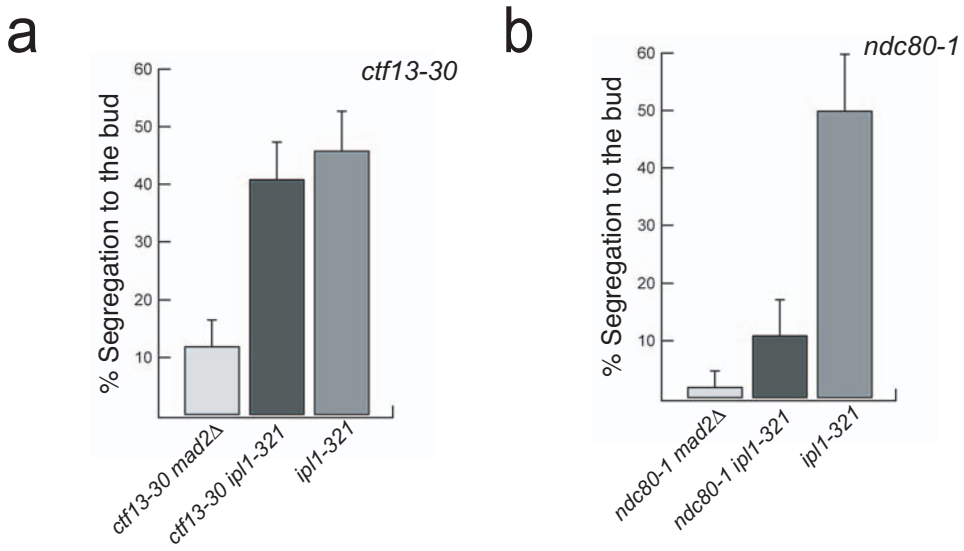

Figure S1. (a) Impairing Ipl1 function restores chromosome segregation to the bud in *ctf13-30* mutant cells. *Ipl1-321* (SBY322), *ctf13-30 mad2*Δ (SBY3015), and *ctf13-30 ipl1-321* (SBY2910) cells containing fluorescently marked Chr IV were arrested in G1 and released to 37 ºC for 120 min. Large budded cells were monitored for the segregation of both Chr IV sister chromatids to the bud. **(b)** The failure to restore segregation to the bud in *ndc80-1 ipl1-321* mutants is not due to defective spindle elongation. Wild type (SBY2877), *ipl1-321* (SBY3025), *ndc80-1 mad2*Δ (SBY4631), and *ndc80-1 ipl1-321* (SBY2987) cells expressing Tub1-CFP and fluorescently marked Chr IV were released from G1 to 37 ºC. 100 minutes after release, large budded cells with only long or broken down spindles were analyzed as in **(a)**.

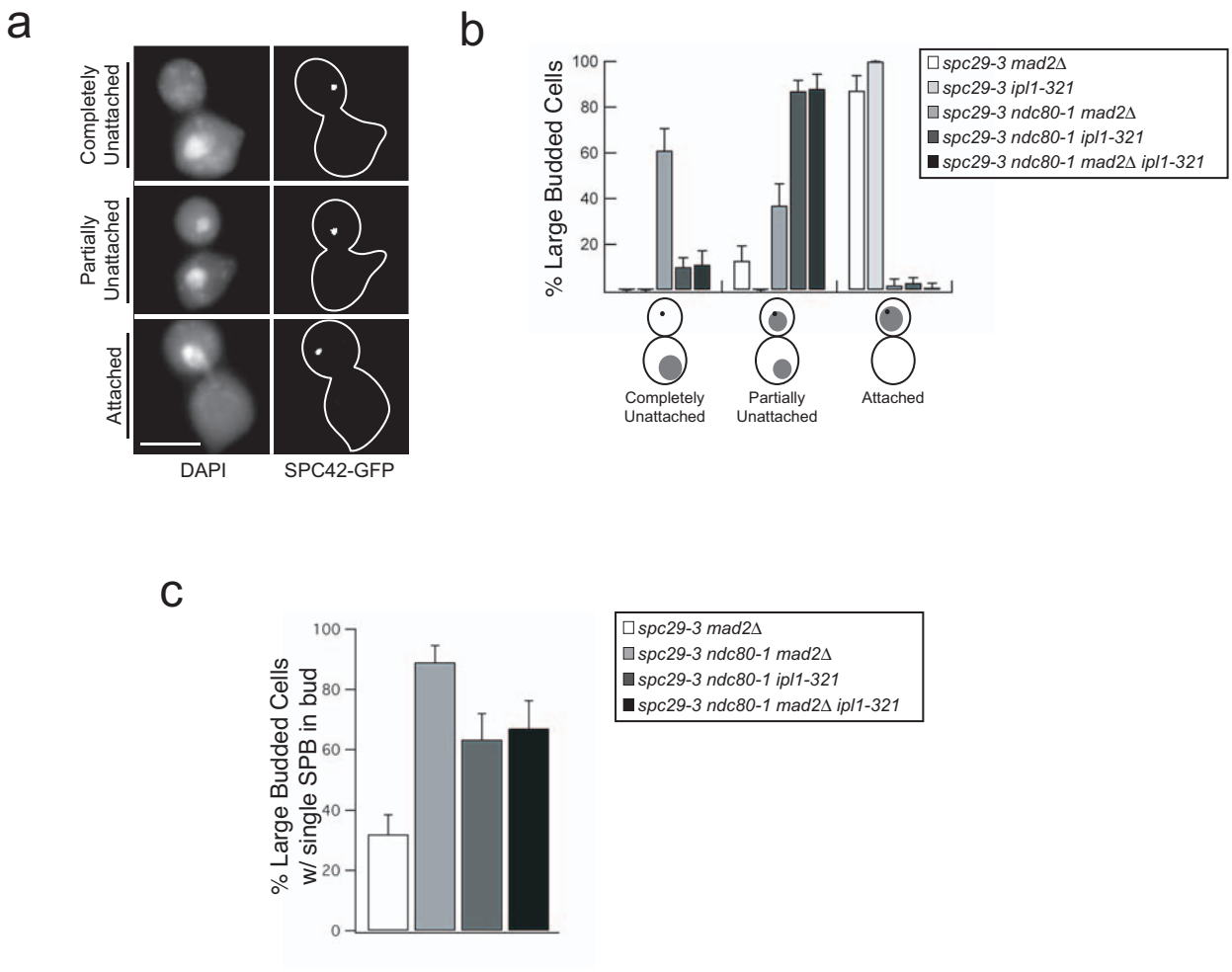

Figure S2. Movement across the bud neck displaces weak attachments. **(a)** *Ndc80-1* mutant cells contain unattached chromosomes when the nucleus moves across the bud neck in the absence of spindle elongation. *SPC29* is an essential component of the spindle pole body (SPB). At the restrictive temperature, *spc29-3* mutant cells fail SPB duplication and therefore cannot undergo spindle elongation. However, the sole functional SPB is pulled into the bud during anaphase [26], allowing visualization of unattached chromosomes by determining whether any non-SPB associated DNA remained in the mother cell. *Spc29-3 mad2*Δ (SBY4393), *spc29-3 ipl1-321* (SBY4006), *spc29-3 ndc80-1 mad2*Δ (SBY4042), *spc29-3 ndc80-1 ipl1-321* (SBY4007), and *spc29-3 ndc80-1 ipl1-321 mad2*Δ (SBY4574) cells expressing Spc42-GFP to mark the SPB were released from G1 to 37 ºC. **(b)** 100 minutes after release, large budded cells with a single SPB in the bud were scored for whether their DNA (DAPI) was completely unattached, partially unattached, or fully attached to the SPB. **(c)** The number of cells with a single SPB in the bud is correlated with the level of unattached DNA. 100 minutes after release, large budded cells with a single SPB were scored for whether the SPB was in the bud.

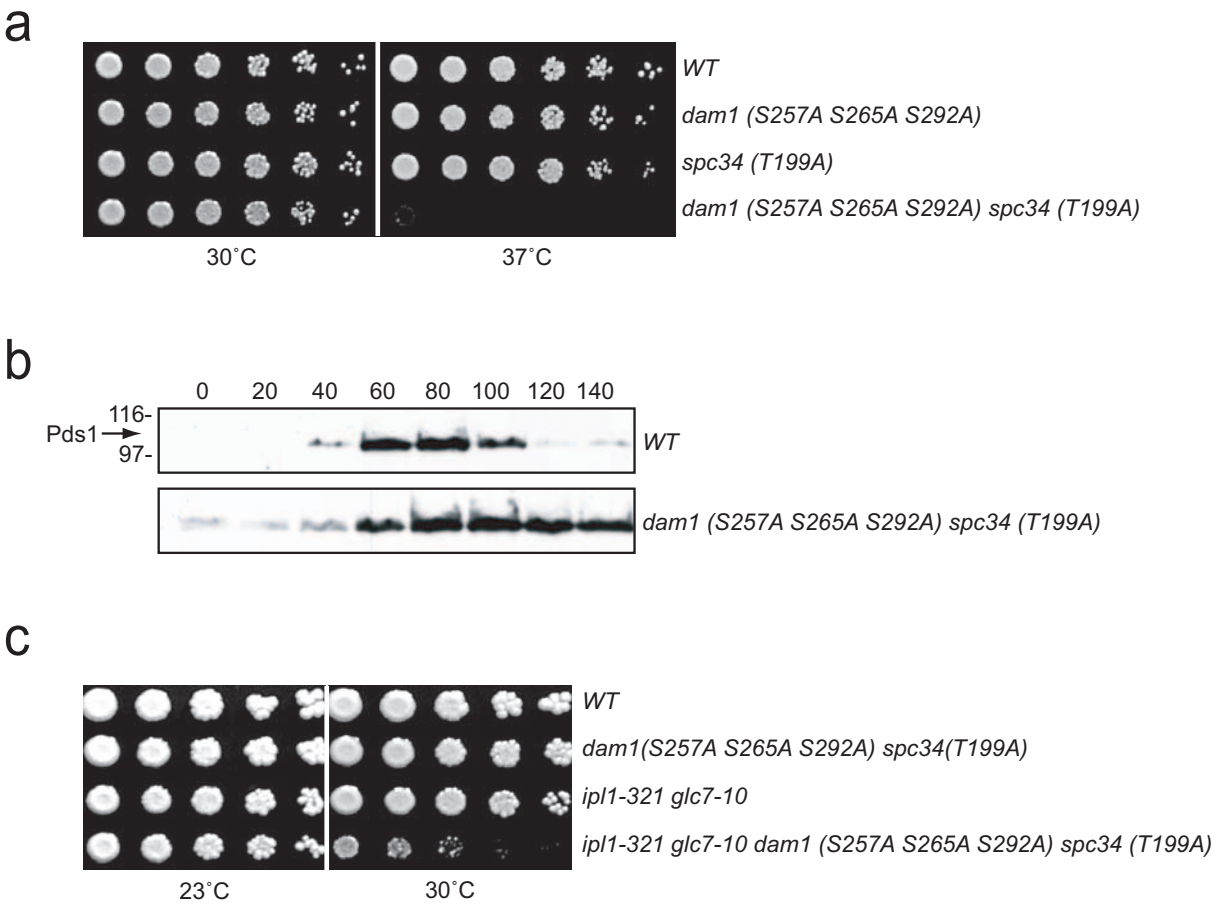

Figure S3. Additional targets of Ipl1 likely exist. **(a)** The *dam1* phospho-deficient complex mutant is temperature sensitive in the W303 strain background. Similar to the S288c background, the Ipl1 consensus sites on Dam1 and Spc34 must be mutated to alanine in combination to create the temperature sensitive *dam1* phospho-deficient mutant. 5-fold serial dilutions of wild type (SBY 214), *dam1 (S257A S265A S292A)* (SBY3783), *spc34 (T199A)* (SBY 4185), *dam1 (S257A S265A S292A) spc34 (T199A)* (SBY 3702), were incubated for 1 day at 37 ºC. (b). The *dam1 (S257A S265A S292A)* (SBY3783), *spc34 (T199A)* mutant activates the spindle checkpoint in the S288c background. Wild type (SBY4172) and *dam1 (S257A S265A S292A) spc34 (T199A)* (SBY4199) cells containing Pds1-myc18 were arrested in G1 and released into nocodazole at 37 ºC. Lysates were prepared at the indicated time points and immunoblotted with anti-myc antibodies. **(c)** Genetic interactions also suggest that the *dam1* phospho-deficient mutant is not the key target of Ipl1. Because Ipl1 is opposed by the Glc7 protein phosphatase 1[30], a reduction in Ipl1 activity is suppressed by a corresponding reduction in Glc7 activity. If the Dam1 phosphorylation sites were the only targets of Ipl1, there should be no genetic interactions between the *glc7-10* allele and the *dam1* phospho-deficient mutant that cannot be phosphorylated by Ipl1. However, *glc7-10* suppressed the synthetic lethality of *ipl1-321 dam1* phospho-deficient double mutant cells. 5-fold serial dilutions of wild type (SBY214), *dam1 (S257A S265A S292A) spc34 (T199A)* (SBY3849), *ipl1-321 glc7-10* (SBY1994), and *ipl1-321 glc7-10 dam1 (S257A S265A S292A) spc34 (T199A)* (SBY4887) mutant cells were incubated for 3 days at 23 ºC and 2 days at 30 ºC. The *ipl1-321 dam1 (S257A S265A S292A) spc34 (T199A)* mutant cells are inviable and could not be shown.

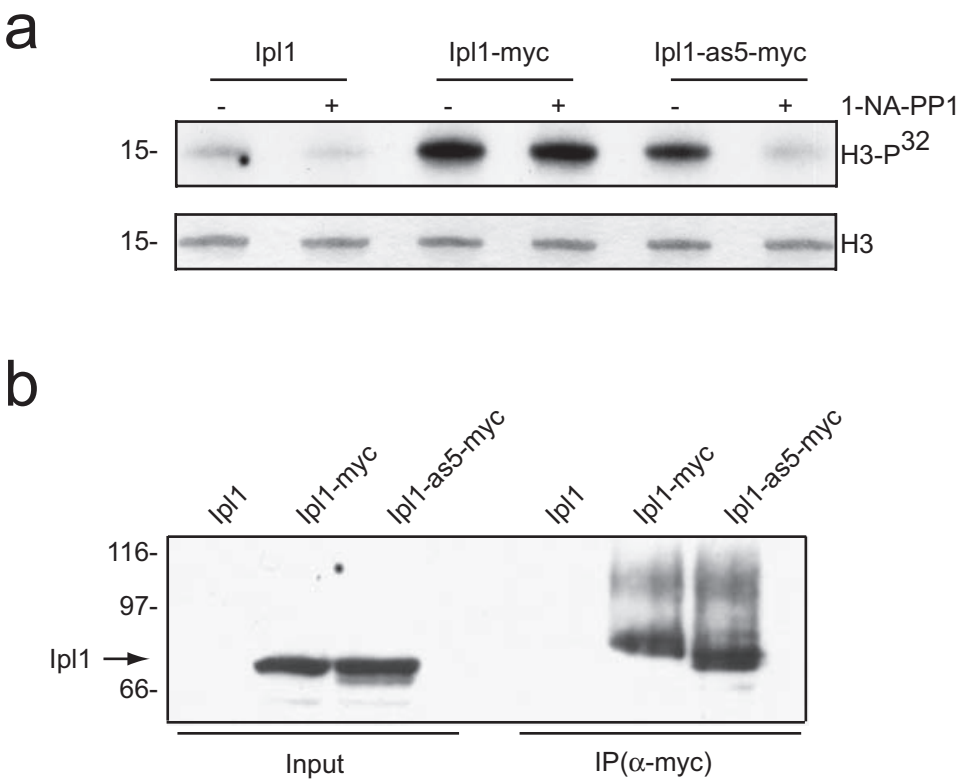

Figure S4. Ipl1-as5 kinase activity is inhibited by 1-NA-PP1. **(a)** Strains containing Ipl1 (SBY3), Ipl1-myc13 (SBY1730) or Ipl1-as5-myc13 (SBY3667) were immunoprecipitated with anti-myc antibodies and used for kinase assays with the substrate histone H3 *in vitro*. DMSO (-) or the inhibitor 1-NA (+) was added to each reaction. Ipl1-as5-myc activity is inhibited to background levels in the presence of 1-NA but Ipl1-myc activity is unaffected. **(b)** Immunoblotting with anti-myc antibodies confirmed that equal amounts of Ipl1-myc and Ipl1-as5-myc were used in the kinase reactions.