Supplemental Information

Aurora B-INCENP Localization at Centromeres/Inner Kinetochores Is Required for Chromosome Bi-orientation in Budding Yeast

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A

| Strain               | TIR1 | TIR1 | TIR1 |
|----------------------|------|------|------|
| bir1-aid / TIR1      | +    | -    | -    |
| mcm21-aid / TIR1     | +    | +    | -    |
| bir1-aid / mcm21-aid | +    | +    | -    |

NAA time after NAA (h) 0 0.5 1 1.5 2

B

| Strain               | NAA  |
|----------------------|------|
| bir1-aid / TIR1      | +    |
| mcm21-aid / TIR1     | +    |
| bir1-aid / mcm21-aid | +    |

C

Pericentromeric cohesion intact
Pericentromeric cohesion weakened
No separation of pericentromeres
Premature separation of pericentromeres

D

One CFP dot (wild type) Two CFP dots (dbf4-myc) Two CFP dots (mcm21Δ)

Scale bar 2μm SPB/NPC-mCherry

% cells with two dots

- wild type
- dbf4-myc
- mcm21Δ

p=0.0008
p<0.0001
p=0.22

(Figure S1, legend on next page)
Figure S1. Evaluation of protein depletion, synthetic growth defects and peri-centromere cohesion. Related to Figure 1.

A. Bir1-aid and Mcm21-aid are depleted to undetectable levels 30 minutes after addition of NAA. Yeast cells carrying TIR with mcm21-aid and bir1-aid individually and in combination were collected every 0.5 h after addition of 2 mM NAA (or 2 h without NAA, as a control) as indicated. Proteins were separated by the SDS-PAGE (NuPAGE 3-8 % Tris-Acetate gel), blotted and probed with an anti-AID tag antibody. The bands corresponding to Bir1-aid and Mcm21-aid are indicated on the right. The non-specific band of ~220 kDa served as a loading control.

B. Bir1 deletion shows synthetic growth defects when combined with Mcm21 depletion. Yeast cells with mcm21-aid TIR and bir1Δ individually and in combination, were serially diluted (10 times dilution each), spotted on plates and incubated for 2 days in the presence (right) and absence (left) of NAA. Cells without AID tag or with stu1-aid were analysed in the same way as controls.

C. Diagram illustrates that sister tetOs at peri-centromeres (e.g. at 15 kb from CEN12, see D) shows frequent separation during metaphase when peri-centromere cohesion is weakened.

D. dbf4-myc shows a defect in peri-centromere cohesion to a similar extent to (or marginally greater than) mcm21 deletion. DBF4+ MCM21+ (wild-type, T10141), dbf4-myc (T10142) and mcm21Δ (T13365) cells with tetOs at 15kb from CEN12, TetR-3×CFP, MET3 promoter-CDC20, SPC42-4×mCherry and NIC96-4×mCherry were cultured in methionine drop-out medium, arrested in G1 with α-factor treatment and released into YPAD plus 2 mM methionine, leading to metaphase arrest (due to Cdc20 depletion). At 2 hours after the release, microscopy images were acquired. Spc42 and Nic96 are components of the spindle pole body (SPB) and the nuclear pore complex (NPC), respectively, and SPB signals were much brighter than NPC signals. Images show representative examples where sister tetOs are, and are not, separated (two and one CFP dots, respectively). Graphs show % of sister tetOs separation during metaphase. n=50–52 for each strain; p-values were obtained using Fisher’s exact test.
A

B

C

| INPUT | PULL-DOWN |
|-------|-----------|
|       | Lane 2 | Lane 3 | Lane 4 | Lane 9 | Lane 10 | Lane 11 |
|       | Score | Coverage | Score | Coverage | Score | Coverage | Score | Coverage | Score | Coverage |
| GST   | 42,125 | 93%     | 22,124 | 92%     | 3,468 | 70%     | 25,120 | 91%     | 84,819 | 95%     | 43,334 | 90%     |
| Sli15 (1-401) | 79,724 | 90%     | 4,174  | 65%     | 3,286 | 67%     | 7,308  | 67%     | 141,988 | 88%     | 8,815  | 70%     |
| Sli15 (393-698) | 404   | 45%     | 29,749 | 89%     | 590   | 59%     | 280    | 24%     | 1,030  | 66%     | 37,360 | 91%     |
| Hisx6-Okp1 | 63     | 7%      | 489    | 29%     | 30,034| 79%     | 1439   | 47%     | 19,069 | 73%     | 17,879 | 73%     |
| Ame1   | 0      | 0%      | 0      | 0%      | 5,977 | 72%     | 200    | 19%     | 3,059  | 61%     | 3,628  | 72%     |
| Mcm21  | 0      | 0%      | 0      | 0%      | 6,889 | 68%     | 306    | 30%     | 7,528  | 72%     | 7,237  | 72%     |
| Ctf19  | 0      | 0%      | 0      | 0%      | 128   | 14%     | 0      | 0%      | 23     | 4%      | 27     | 4%      |

(Figure S2, legend on next page)
Figure S2. Effect of *dbf4-myc* on Ipl1 localization at centromeres, and supplemental data for GST pull-down assay. Related to Figure 2.

**A.** *dbf4-myc* does not affect Ipl1 localization at centromeres in the presence and absence of Bir1. *DBF4*<sup>+</sup> *BIR1*<sup>+</sup> (wild-type, T12858), *bir1-aid* (T12860) *dbf4-myc* (T13385) and *bir1-aid dbf4-myc* (T13386) cells with *IPL1-GFP*, TIR, *GAL1-10* promoter-*CEN3-tetOs*, *TetR-3×CFP*, *mCherry-TUB1* and *MET3* promoter-*CDC20* were treated and analysed as in Figure 2B and C. Ipl1 signals were quantified at *CEN3* in n=25 cells for each strain. Bars show means and SEMs. *p*-values were obtained using t-test.

**B.** The western blot with an anti-His antibody in Figure 2E is shown in whole.

**C.** Mass spectrometry analyses of GST pull-down assay. Gel pieces comprising the area between 33 and 75 kDa were excised from Coomassie stained gel in Figure 2E and subjected to mass spectrometry analysis. The data were analyzed with Mascot 2.4.0. The table shows results from lane 2 [Input: GST-Sli15 (1-401)], lane 3 [Input: GST-Sli15 (393-698)], lane 4 [Input: COMA], lane 9 [Pull-down: GST alone + COMA], lane 10 [Pull-down: GST-Sli15 (1-401) + COMA] and lane 11 [Pull-down: GST-Sli15 (393-698) + COMA]. ‘Score’ represents the probability that the observed match is not a random event [-10×LOG10( *P* ), with *P* = probability that match is random]. ‘Coverage’ represents the percentage of the protein sequence that is covered by the detected and matched peptides. Scores above the following threshold are indicated in bold: Dependent on background signals, the threshold was set to Score = 10,000 for GST alone, GST-Sli15 (1-401), GST-Sli15 (393-698), and Score = 2,000 for His-Okp1, Ame1, Mcm21 and Ctf19. As most samples contained GST either alone or as a fusion protein, GST was detected with high scores throughout all samples. Lanes 10 and 11 showed a higher abundance of COMA proteins than lane 9, indicating their physical interaction with both GST-Sli15 (1-401) and GST-Sli15 (393-698). Whereas Okp1, Ame1 and Mcm21 were detected with high scores, the score for Ctf19 was reproducibly low. Generally, Ctf19 was hard to detect in mass spectrometry even if it was recovered or expressed in high quantity (e.g. after immuno-precipitation of HA-tagged Ctf19) – perhaps its peptide properties did not allow detection in high sensitivity with mass spectrometry.
A  

**Figure S3, legend on next page**

B  

C  

*Figure S3, legend on next page*
Figure S3. Supplemental data for Sli15 tethering to inner kinetochores, and effects of dbf4-myc on growth of bir1Δ sli15ΔN cells. Related to Figure 3 and 4.

A. FKBP12-fused Mif2 recruits FRB-fused Sli15 to the vicinity of CEN2. In the experiments shown in Figure 3B, Sli15-FRB signals were quantified at CEN2 in bir1-aid mcm21-aid cells carrying MIF2 with or without fusion to FKBP12 (T13444 and T13440, respectively), 30 min after addition of rapamycin. After Z sections were projected to 2D images, Sli15-FRB-GFP signals (colocalising at CEN2) were quantified in the area of 2x2 pixels at their maximum intensity. When sister CEN2 showed separation, Sli15-FRB-GFP signals were averaged between sisters in individual cells. Background was subtracted in every measurement. Bars show means and SEMs. p-values were obtained using t-test.

B. Tethering Sli15-FRB to Mif2-FKBP12 makes cells inviable. Yeast cells carrying indicated constructs and mutants (the third to sixth strains from the top were the strains used in Figure 3B) were serially diluted (10 time dilution each), spotted on methionine-dropout media with/without NAA and with/without rapamycin (as indicated), and incubated for 3 days. TOR1-1 cells (T9303) and stu1-aid /TIR (T10133) were used as controls. We speculate the reason for the lethality of tethering Sli15-FRB to Mif2-FKBP12 as follows: It is known that Ipl1–Sli15 normally leaves centromeres and re-locates to the central spindle during anaphase. We suspect that the presence of Ipl1–Sli15 at the centromere/inner kinetochore during anaphase could cause lethality by disrupting kinetochore–microtubule interaction (since tension is weakened) [S1], reengaging cells to the spindle-assembly checkpoint [S2] and/or delaying the spindle disassembly [S3].

C. dbf4-myc does not affect growth of cells with bir1Δ sli15ΔN. Yeast cells with bir1Δ sli15ΔN with DBF4+ or dbf4-myc, were serially diluted (10 times dilution each), spotted on plates and incubated for 2 days.
| Name       | Genotype                                                                 |
|------------|--------------------------------------------------------------------------|
| PJ69-4A    | MAT a trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ |
| T7107      | MAT a ade2-1 trp1-1 leu2-3,112, his311, 15 ura3 can 1-100                |
| T9030      | MAT a::PADH1-OsTIR1-9Myc::URA3                                           |
| T9033      | MAT α TOR1-1 fpr1Δ::natMX4                                                |
| T10133     | MAT α ura3::3CFP::HPH1 MAT a ade1::clonNAT ura3-5-aid::clonNAT MAT α ade1::tetR-3CFP::HPH1 SPC42-4mCherry::natMX6 NIC96-4mCherry::natMX6 tetO224 (LEU2) is integrated at +15-kb from CEN12 |
| T10141     | MAT a::PADH1-OsTIR1-9Myc::URA3 ade1::tetR-3CFP::HPH1 SPC42-4mCherry::natMX6 NIC96-4mCherry::natMX6 tetO224 (LEU2) is integrated at +15-kb from CEN12 |
| T10142     | MAT a::PADH1-OsTIR1-9Myc::URA3 ade1::tetR-3CFP::HPH1 SPC42-4mCherry::natMX6 NIC96-4mCherry::natMX6 tetO224 (LEU2) is integrated at +15-kb from CEN12 |
| T12064     | MAT a::PADH1-OsTIR1-9Myc::URA3 ade1::tetR-3CFP::HPH1 SPC42-4mCherry::natMX6 NIC96-4mCherry::natMX6 tetO224 (LEU2) is integrated at +15-kb from CEN12 |
| T12066     | MAT a::PADH1-OsTIR1-9Myc::URA3 ade1::tetR-3CFP::HPH1 SPC42-4mCherry::natMX6 NIC96-4mCherry::natMX6 tetO224 (LEU2) is integrated at +15-kb from CEN12 |
3CFP::HPH1 trp1::PTEF1-mcherry-TUB1::TRP1 cdc20::PMET-CDC20::TRP1 ura3::PADH1-OsTIR1-9Myc::URA3 mcm21-aid::clonNAT

T12860  MAT a IPL1-yEGFP::SpHis5 cen15::PGAL-CEN3-tetO2x112::URA3 ade1::tetR-3CFP::HPH1 trp1::PTEF1-mcherry-TUB1::TRP1 cdc20::PMET-CDC20::TRP1 ura3::PADH1-OsTIR1-9Myc::URA3 mcm21-aid::clonNAT

T12861  MAT a IPL1-yEGFP::SpHis5 cen15::PGAL-CEN3-tetO2x112::URA3 ade1::tetR-3CFP::HPH1 trp1::PTEF1-mcherry-TUB1::TRP1 cdc20::PMET-CDC20::TRP1 ura3::PADH1-OsTIR1-9Myc::URA3 mcm21-aid::clonNAT

T13199 MAT a TOR1-1 fpr1::K.I. LEU2 SLI15-FRB-GFP::KanMX6 cen15::PGAL-CEN3-tetO2x112::URA3 ade1::tetR-3CFP::HPH1 trp1::PTEF1-mcherry-TUB1::TRP1 cdc20::PMET-CDC20::TRP1 ura3::PADH1-OsTIR1-9Myc::URA3

T13200 MAT a TOR1-1 fpr1::K.I. LEU2 MIF2-2xFKB12::TRP1 SLI15-FRB-GFP::KanMX6 cen15::PGAL-CEN3-tetO2x112::URA3 ade1::tetR-3CFP::HPH1 trp1::PTEF1-mcherry-TUB1::TRP1 cdc20::PMET-CDC20::TRP1 ura3::PADH1-OsTIR1-9Myc::URA3

T13201 MAT a TOR1-1 fpr1::K.I. LEU2 SLI15-FRB-GFP::KanMX6 cen15::PGAL-CEN3-tetO2x112::URA3 ade1::tetR-3CFP::HPH1 trp1::PTEF1-mcherry-TUB1::TRP1 cdc20::PMET-CDC20::TRP1 ura3::PADH1-OsTIR1-9Myc::URA3 bir1-aid::clonNAT

T13202 MAT a TOR1-1 fpr1::K.I. LEU2 MIF2-2xFKB12::TRP1 SLI15-FRB-GFP::KanMX6 cen15::PGAL-CEN3-tetO2x112::URA3 ade1::tetR-3CFP::HPH1 trp1::PTEF1-mcherry-TUB1::TRP1 cdc20::PMET-CDC20::TRP1 ura3::PADH1-OsTIR1-9Myc::URA3 bir1-aid::clonNAT

T13365 MAT a cdc20::MET-CDC20::TRP1 ade1::tetR-3CFP::HPH1 mcm21Δ::KanMX4 SPC42-4mCherry:natMX6 NIC96-4mCherry:natMX6 tetO224 (LEU2) is integrated at +15-κb from CEN12

T13385 MAT a DBF4-9Myc::KanMX4 IPL1-yEGFP::SpHis5 cen15::PGAL-CEN3-tetO2x112::URA3 ade1::tetR-3CFP::HPH1 trp1::PTEF1-mcherry-TUB1::TRP1 cdc20::PMET-CDC20::TRP1 ura3::PADH1-OsTIR1-9Myc::URA3

T13386 MAT a DBF4-9Myc::KanMX4 IPL1-yEGFP::SpHis5 cen15::PGAL-CEN3-tetO2x112::URA3 ade1::tetR-3CFP::HPH1 trp1::PTEF1-mcherry-TUB1::TRP1 cdc20::PMET-CDC20::TRP1 ura3::PADH1-OsTIR1-9Myc::URA3 bir1-aid::clonNAT

T13438 MAT a TOR1-1 fpr1Δ::K.I. LEU2 SLI15-FRB-GFP::KanMX6 ura3::PADH1-OsTIR1-9Myc::URA3 tetO224 (LEU2) is integrated at 0.6-κb from CEN2 ade1::tetR-3CFP::HPH1 SPC42-4mCherry:natMX6 cdc20::MET-CDC20::TRP1

T13440 MAT a TOR1-1 fpr1Δ::K.I. LEU2 bir1-aid::clonNAT mcm21-aid::clonNAT SLI15-FRB-GFP::KanMX6 ura3::PADH1-OsTIR1-9Myc::URA3 tetO224 (LEU2) is integrated at 0.6-κb from CEN2 ade1::tetR-3CFP::HPH1 SPC42-4mCherry:natMX6 cdc20::MET-CDC20::TRP1

T13441 MAT a TOR1-1 fpr1Δ::K.I. LEU2 MIF2-2xFKB12::TRP1 SLI15-FRB-GFP::KanMX6 ura3::PADH1-OsTIR1-9Myc::URA3 tetO224 (LEU2) is integrated at 0.6-κb from CEN2 ade1::tetR-3CFP::HPH1 SPC42-4mCherry:natMX6 cdc20::MET-CDC20::TRP1

T13444 MAT a TOR1-1 fpr1::K.I. LEU2 MIF2-2xFKB12::TRP1 bir1-aid::clonNAT mcm21-aid::clonNAT SLI15-FRB-GFP::KanMX6 ura3::PADH1-OsTIR1-9Myc::URA3 tetO224 (LEU2) is integrated at 0.6-κb from CEN2 ade1::tetR-3CFP::HPH1 SPC42-4mCherry:natMX6 cdc20::MET-CDC20::TRP1

Table S1. The genotypes of budding yeast (Saccharomyces cerevisiae) strains used in this study (Related to STAR Methods). The background of all yeast strains is W303 except for PJ69-4A.
Supplemental References

S1. Tanaka, T.U., Rachidi, N., Janke, C., Pereira, G., Galova, M., Schiebel, E., Stark, M.J., and Nasmyth, K. (2002). Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. Cell 108, 317-329.

S2. Biggins, S., and Murray, A.W. (2001). The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. Genes & development 15, 3118-3129.

S3. Buvelot, S., Tatsutani, S.Y., Vermaak, D., and Biggins, S. (2003). The budding yeast Ipl1/Aurora protein kinase regulates mitotic spindle disassembly. The Journal of cell biology 160, 329-339.