Fig S1 A population of kinetochores can be found at a distance from SPBs in Noc-treated CDC15 and cdc15-2 cells. (A) CEN V-RFP can be found at a distance from Ndc80p-GFP in Noc-arrested cells. Cells carrying cdc20::pMET-CDC20 NDC80-GFP CEN V-RFP in CDC15 (i) and *cdc15-2* (ii) backgrounds were arrested in metaphase by cdc20-depletion at 32°C (a). The CEN5::tetO2X112 located 1.4kb left of CEN V (Tanaka et al., 2000) together with the tetR-RFP served as a marker for the localization of kinetochore on chromosome V and allowed us to examine the relative position of a specific kinetochore in relation to the rest of the kinetochores marked by Ndc80p-GFP. In parallel experiments, the strains were arrested in Noc 32°C (b). There appeared to be no significant difference in the percentages of cells with such kinetochores losing association in CDC15 and cdc15-2 cells. (B) Ndc80p-GFP can be found at a distance from Spc29p-RFP in Noc-arrested cells. Cells carrying cdc201 GAL-CDC20 NDC80-GFP SPC29-RFP in CDC15 (i) and cdc15-2 (ii) backgrounds were arrested in metaphase by cdc20-depletion at 32°C (a). In parallel experiments, the strains were arrested in Noc 32°C (b). Both the CDC15 and cdc15-2 strains showed that a small population of the cells exhibited weak Ncd80p-GFP signals at a distance from the Spc29p-RFP (Noc, bottom panels, and magnified regions with white arrows). This was consistent with data from a previous report (Gillett et al., 2004). (C) CEN V-GFP can be found at a distance from Spc29p-RFP in Noc-arrested cells. cdc20A::GAL-CDC20 CEN V-GFP SPC29-RFP in CDC15 (i) and cdc15-2 (ii) backgrounds arrested in metaphase by cdc20-depletion at 32°C (a). In parallel experiments, the strains were arrested in Noc 32°C (b). Similar percentages of CDC15 and cdc15-2 cells were found to display CEN V-GFP signals at a distance away from the Spc29p-RFP. All images were taken at the arrested stage using spinning disk confocal microscopy.

Fig S2 CEN V-GFP drifts away from SPBs in some Noc-treated cells. Wild-type *tetR-GFP CEN5::tetO2X112 SPC29-RFP MYO1-Redstar2* cells were treated in alpha-factor and then released into Noc-containing media on slides for time-lapsed imaging. (A) In most cells, the CEN V-GFP spots were closely-associated with Spc29p-RFP (i). The tracking of Spc29p-RFP and CEN V-GFP as indicated by the pathways taken by them (ii) suggested that the SPBs and CEN V undergo dynamic movements. (B) In some cells maintained in Noc-containing media, the CEN V-GFP spots were found to move away from Spc29p-RFP (i). The tracking of lines Spc29p-RPF and CEN V-GFP are shown in (ii).

Fig S3 Time-lapsed imaging of Mad2p-GFP in wild-type (i) and *cdc15-2* cells (ii) released from Noc arrest to YPD at 32°C as described in Fig 4. (iii) Graph shows the percentages of wild-type and *cdc15-2* cells with persistent Mad2p-GPF signals after Ndc80p-Redstar2 has separated.

Fig S4 Time-lapsed images showing *cdc20 GAL-CDC20 CEN V-GFP SPC29-RFP* released from Cdc20p-depletion into YPD at 32°C. CEN V-GFP and Spc29p-RFP do not show oscillation through the neck as the cell separated Spc29p-RFP and CEN V-GFP.

Fig S5 Biorientation of CEN V-GFP spots is normal in *cdc20*-arrest in *cdc15-2* cells. *cdc20* Δ *GAL-CDC20 CEN V-GFP SPC29-RFP* (A) and *cdc15-2 cdc20* Δ *GAL-CDC20 CEN V-GFP SPC29-RFP* (B) cells were arrested in YPD for 2.5 hrs at 24°C and YPD for 2.5 hrs at 32°C before being transferred into SC/Glu and imaged on the heated-stage. (C) Cell counts showing percentage of cells with biorientated CEN V-GFP. (D) and (E) close-up images of CEN V-GFP in *CDC15* and *cdc15-2* cells reveal longer distances between Spc29p-RFP in *cdc15-2* cells. (F)

Plots show the distance between Spc29p-RFP in 106 cells for each strain in the experiment as described in (A) and (B). In parallel experiments, $cdc20\Delta$ GAL-CDC20 CEN V-GFP SPC29-RFP PDS1-HA and cdc15-2 $cdc20\Delta$ GAL-CDC20 CEN V-GFP SPC29-RFP PDS1-HA cells were arrested as described in (A) and (B) and Western blot analysis performed on lysates obtained at the Cdc20p-depleted stage to determine Pds1p and Pgk1p levels.

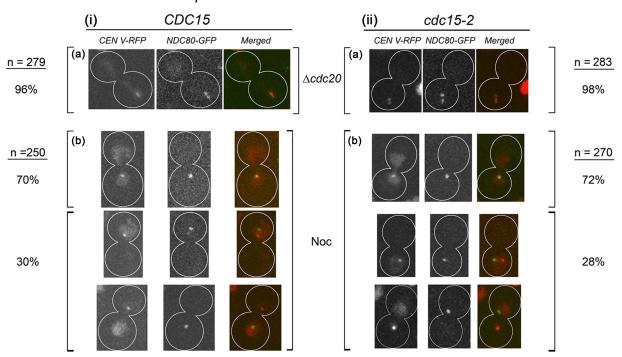
Fig S6 Ipl1p and Sli15p localized to *cdc15-2* cells similarly as in wild-type cells. Wild-type (i) and mutant (ii) cells carrying *IPL1-GFP MYO1-REDSTAR2* (A) and *SLI15-GFP MYO1-REDSTAR2* (B) were treated as in Fig 4.

Fig S7 Flow-chart showing the regime for the experiment performed as described in Fig 7.

Movie 1 Dynamic movement of Spc29p-RFP across the neck in relation to CEN V-GFP and Myo1p-Redstar2 in wild-type cell. The movie shown is of the cell described in Fig 4Ai.

Movie 2 Dynamic movement of Spc29p-RFP across the neck in relation to CEN V-GFP and Myo1p-Redstar2 in *cdc15-2* cell. The movie shown is of the cell described in Fig 4Aii.

cdc20::pMET-CDC20 NDC80-GFP CEN V-RFP



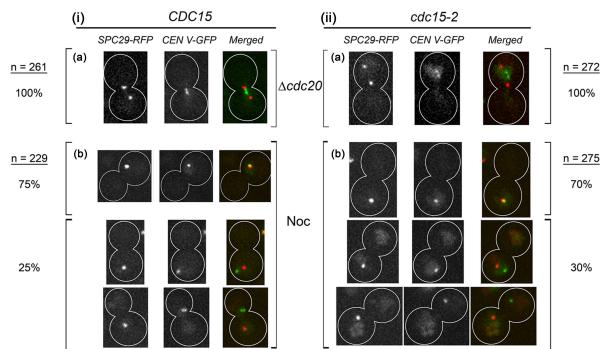
В

Α

 $dcc20\Delta GAL-CDC20 NDC80-GFP SPC29-RFP$ (i) cdc15-2 SPC29-RFP NDC80-GFP Merged SPC29-RFP NDC80-GFP Merged $\int SPC29-RFP NDC80-GFP Merged$ $\int SPC29-RFP$

С

 $cdc20 \Delta \text{ GAL-CDC20 CEN V-GFP SPC29-RFP}$



CEN V-GFP SPC29-RFP MYO1-REDSTAR2

αF arrest → release into YPD + Noc for 1 min → SC/Glu + Noc on slide for time-lapsed imaging

| A(i) | 2 µm 0 min | 1 min | ► 2 min | ► 3 min | 4 min | 5 min | ► 6 min | 7 min | 8 min | 9 min | (ii) | Tr | ackir | ng lir | nes f | for S | pc29 | 9p-R | RFP | | | Trac | cking | g |
|------|---------------|---------------|-------------|-------------|---------------|----------------|-------------|----------------|-------------|---------------|---------|----|----------|--------|-------|-------|------|----------|-----|--------|---------------|------|-------|---|
| | 10 min | | 12 min | 13 min | - 1 14 min | 1 5 min | ► 16 min | - 17 min | ► 18 min | - 19 min | 2 µm | | à | | | ٣ | ۲ | P | 4 | 4 | | 9 | e. | |
| | | | | | | | | | | | ł. | A | P | þ | 4 | ¢ | ¢ | A | A | þ | | | | |
| | 20 min | 21 min | | | 24 min | 25 min | 28 min | 27 min | 28 min | | Ą | A | Ą | ¢. | ø | Å | þ | ą. | ě. |) A | • | * | • | |
| | 30 min | 31 min | 32 min | 33 min | 34 min | 35 min | 36 min | 37 min | 38 min | 39 min |). F | 3 | \$ | * | ¥ | ¥ | × | * | \$ | \$ | × | ٨ | ۶ | |
| | ► 40 min | ► 41 min | ► 42 min | 43 min | 44 min | 45 min | 46 min | 47 min | ► 48 min | ► . 49 min | 1 | * | 1 | ¥. | 2 | 2 | ¥ | ¥ | 8 | 3 | * | * | * | |
| | | | | | | | > | | | | * | ¥ | ¥., | * | * | 8 | ¥ | 8 | * | 7 | * | * | * | |
| | 50 min | 51 min | 52 min | 53 min | 54 min | 55 min | 58 min | 57 min | 58 min | 59 min | 4 | * | * | 4 | 4 | * | 4 | 4 | 4 | 4 | * | * | * | |
| | 60 min | 61 min | 62 min | 63 min | 64 min | 65 min | 66 min | 67 min | 68 min | | 44 | ** | 4 | - | ** | ** | ** | ** | ** | * | 5 \$\$ | * | * | |
| | ► 70 min | ► - 71 min | ► 72 min | ► 73 min | ►. 74 min | ► • 75 min | ▶ 76 min | 7 7 min | 78 min | 79 min | | | | | | | | | | | | | | |

Tracking lines for CEN V-GFP

4

4

1

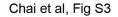
Ŷ

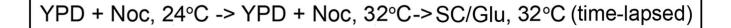
8

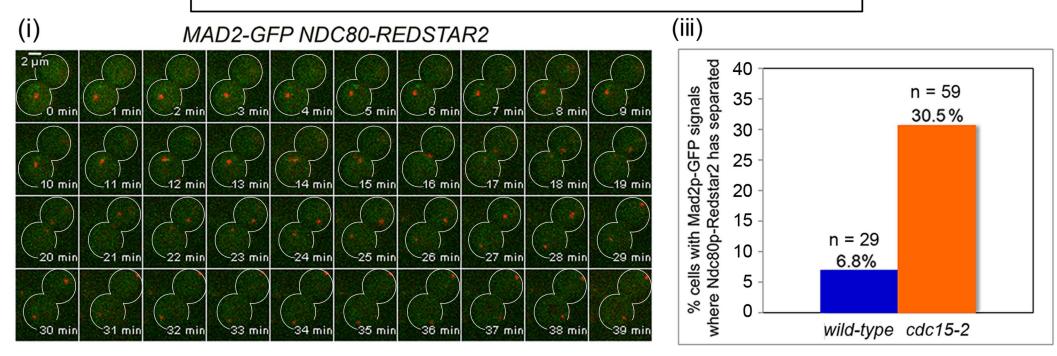
| * | * | 4 | * | * | 4 | 4 | s. | 4 | | * | * | * | * | * | * | * | * | * | 5 | |
|-----|--------------|------|----------|------|------------|----------|----|------|----|------------|------|----|------|-------|------|-------|----------|-----------|----|----------|
| 5 | * | ** | ** | ** | * | ** | ** | 1 | | 5 ¥ | * | - | * | * | * | * | * | * | * | |
| | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| (ii | | Trac | king | line | s for | Spo | 29 | p-RF | -P | | | Т | rack | ing l | ines | for (| CEN | V-G | FP | |
| 2 µ | im · | N | ۲. | 7 | * . | K | ~ | ٠. | | * | - 28 | 1 | X | ų | | \$ | \$ | 8 | × | * |
| | K . 1 | 5 | • | • | • | s - 1 | ę | ۴ | ¥ | ٤ | × | * | 8 | \$ | * | *4 | 8.Q | SS. | * | *4 |
| 1 | ŧ. | ۹ - | < | ج ا | ¢ (| ŧ . | ¢ | ۲ | Ķ | ¥ | ** | *4 | * | N. | \$¥ | 8 | Å. | 8 | 8 | 42 |
| | ę – | ٤ - | ¢ 🛛 | ę i | | | ę | 4 | Ł | 4 | * | 43 | * | 49 | * | 47 | 48 | 49 | 4 | A |
| | • | \$ | 2 | (| | | < | 4 | K | 4 | 4 | 4 | \$ | ¥3 | ×. | * | No. | ¥₹ | * | No. |
| | ٠ | ¢ j | ¢ | ¢ | ¢ (| • | 4 | * | ¢ | * | * | * | * | 2 | * | * | R. | \$ | \$ | atta. |
| | * | ¢ | * | * | ¢ (| * | 4 | 4 | ¢ | * | * | 4 | 4 | ** | * | 4 | * | * | * | * |
| | ¢ | ¢ | * | ¢ | • | • | 4 | 4 | * | 4 | * | 4 | 4 | * | - | 4 | * | 4 | 4 | * |
| | | | | | | | | | | | | | | | | | | | | |

B(i)

| 2 µm | | | 12223 | State P | | 1000 | 1 Acres | and the second | a starter |
|--------|--------|--------|--------|--------------|--------|--------|---------|----------------|-----------|
| 0 min | 1 min | | 3 min | 4 min | 5 min | 6 min | 7 min | 8 min | 9 min |
| | | + | | | - | | | - | - |
| 10 min | 11 min | 12 min | 13 min | 14 min | 15 min | 16 min | 17 min | 18 min | 19 min |
| - | - | - | - | - | - | - | - | - | - |
| 20 min | 21 min | 22 min | 23 min | 24 min | 25 min | 26 min | 27 min | 28 min | 29 min |
| | | | - | - | - | - | | | - |
| 30 min | 31 min | 32 min | 33 min | 34 min | 35 min | 36 min | 37 min | 38 min | 39 min |
| - | - | | - | - | - | - | - | | |
| 40 min | 41 min | 42 min | 43 min | 44 min | 45 min | 48 min | 47 min | 48 min | 49 min |
| - | - | | | - | | | | - | - |
| 50 min | 51 min | 52 min | 53 min | 54 min | 55 min | 56 min | 57 min | 58 min | 59 min |
| | ►. | - | - | | - | - | | | |
| 60 min | 61 min | 62 min | 63 min | 64 min | 65 min | 66 min | 67 min | 68 min | 69 min |
| | - | | ►, | - | - | ►. | | - | |
| 70 min | 71 min | 72 min | 73 min | 74 min | 75 min | 76 min | 77 min | 78 min | 79 min |







(ii)

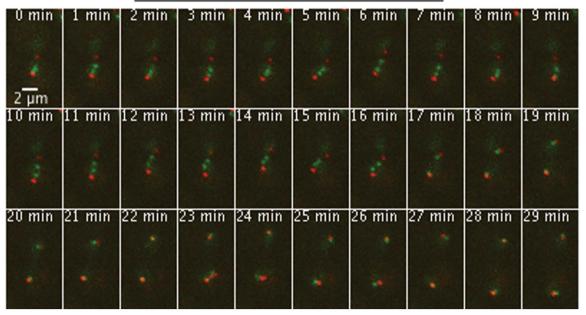
cdc15-2 MAD2-GFP NDC80-REDSTAR2

| 2 µm 0 min | | 2 min | 3 min | 4 min | 5 min | 6 min | 7 min | 8 min | 9 min |
|---------------|--------|--------|---|--|---------------------------------|--|--|--|--|
| 10 min | 11 min | 12 min | 13 min | 14 min | 15 min | 16 min | 17 min | 18 min | 19 min |
| | | | The second se | CONTRACTOR OF THE OWNER AND A DESCRIPTION OF THE OWNE | THE R. LEWIS CO., LANSING MICH. | The second s | No. of Concession, Name of Street, or other Designation of Str | NAMES OF TAXABLE PARTY OF TAXABLE PARTY. | the state of the second se |
| 20 min | 21 min | 22 min | 23 min | 24 min | 25 min | 26 min | 27 min | 28 min | 29 min |

YPD arrest ____ release into YP/Raff/Gal (cdc20-depletion)

Chai et al, Fig S4

cdc20∆ GAL-CDC20 CEN V-GFP SPC29-RFP

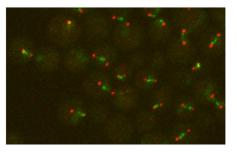


YPD, 24°C ->YPD, 32°C->SC/Glu, 32°C

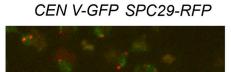
| А | | | | | | | | | В | | cdc15 | 5-2 cd | c20∆ (| GAL-C | CDC20 |) | |
|---------------|--------|--------|----------|----------|-------------|--------|---------------------|--------|---------------|--------|--------|--------|--------|--------|---------|--------|--------|
| | cdc20/ | GAL- | CDC2 | 0 CEN | V-GFI | SPC | 29-RF | Р | | | CE | N V-G | FP SI | PC29- | RFP | | |
| 2 µm 0 min | 1 min | 2 min | 3 min | 4 min | 5 min | 6 min | 7 min | 8 min | 0 min 2 µm | 1 min | 2 min | 3 min | 4 min | 5 min | 6 min | 7 min | 8 min |
| 9 min | 10 min | 11 min | 12 min * | 13 min * | 14 min | 15 min | 16 min | 17 min | 9 min | 10 min | 11 min | 12 min | 13 min | 14 min | 15 min | 18 min | 17 min |
| 18 min | 19 min | 20 min | 21 min | 22 min | 3 23 min | 24 min | 25 min | 28 min | 18 min | 19 min | 20 min | 21 min | 22 min | 23 min | 24 min | 25 min | 28 min |
| 27 min | 28 min | 29 min | 30 min | 31 min | 32 min | 33 min | 34 min | 35 min | 27 min | 28 min | 29 min | 30 min | 31 min | 32 min | 33 min | 34 min | 35 min |
| 36 min | 37 min | 38 min | 39 min | 40 min | 41 min | 42 min | 43 min | 44 min | 36 min | 37 min | 38 min | 39 min | 40 min | 41 min | 42 min | 43 min | 44 min |
| 45 min | 48 min | 47 min | 48 min | 49 min | 50 min | 51 min | 52 min | 53 min | 45 min | 48 min | 47 min | 48 min | 49 min | 50 min | 51 min | 52 min | 53 min |
| 54 min | 55 min | 56 min | 57 min | 58 min | 59 min | 60 min | %. 61 min | 62 min | 54 min | 55 min | 56 min | 57 min | 58 min | 59 min | 60 min | 61 min | 62 min |
| 63 min | 64 min | 65 min | 66 min | 67 min | 68 min | 69 min | 70 min | 71 min | 83 min | 64 min | 65 min | 86 min | 87 min | 68 min | 69 min | 70 min | 71 min |
| 5 | s. | 1 | e., | 1 | 1 | 2 | 2 | * | • • • | • • | | 00 min | o/ min | os min | 109 min | 70 min | |
| 72 min | 73 min | 74 min | 75 min | 76 min | 77 min | 78 min | 79 min | 80 min | 72 min | 73 min | 74 min | 75 min | 76 min | 77 min | 78 min | 79 min | 80 min |

| С | Strain background | % cells showing biorientation of CEN V-GFP spots |
|---|-------------------------|--|
| | <i>CDC15</i> (n= 49) | 100 |
| | <i>cdc15-2</i> (n = 44) | 97.7 |

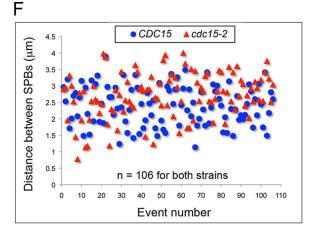
D cdc20∆ GAL-CDC20 CEN V-GFP SPC29-RFP



G



E cdc15-2 cdc20∆ GAL-CDC20



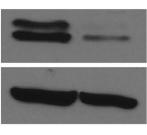
YPD, 24°C -> YPD, 32°C

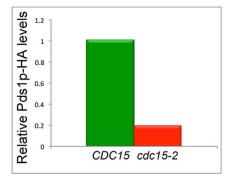
cdc20∆ GAL-CDC20 CEN V-GFP SPC29-RFP PDS1-HA

CDC15 cdc15-2

Pds1p-HA

Pgk1p







Α

IPL1-GFP MYO1-REDSTAR2

| 0 min | 16 min | 22 min | 34 min | 40 min | 42 min |
|----------------|---------|--------|--------|--------|--------|
| 2 μm 44 min | 48 min | 48 min | 50 min | 52 min | |
| S. St. N | No. No. | | | | |



(i)

cdc15-2 IPL1-GFP MYO1-REDSTAR2

| 0 min 2 µm | 16 min | 24 min | 38 min | 48 min | 52 min | 54 min | 58 min | 60 min |
|---------------|--------|----------------|--------|--------|--------|--------|--------|--------|
| 64 min | 66 min | 68 min | 70 min | 72 min | 74 min | 76 min | 78 min | 80 min |
| | 1 | 4 2 4 * | •* | • • • | | | | |

В

(i)

SLI15-GFP MYO1-REDSTAR2

| 0 min 2 μm | 6 min | 32 min | 34 min | 36 min | 38 min | 40 min |
|---------------|--------|--------|--------|--------|--------|--------|
| 42 min | 44 min | 46 min | 48 min | 50 min | 52 min | |
| 1 | * | | | | | |
| A SA | | | | | | |

(ii)

cdc15-2SLI15-GFP MYO1-REDSTAR2

| 0 min | 4 min | 20 min | 36 min | 38 min | 40 min |
|----------------|--------|--------|--------|--------|--------|
| 2 µm 42 min | 44 min | 46 min | 48 min | 50 min | 52 min |
| | | Stor. | | | |

Chai et al, Fig S7

cdc15-2 pMET-PDS1-myc CEN V-GFP SP29-RFP MYO1-Redstar2

