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.

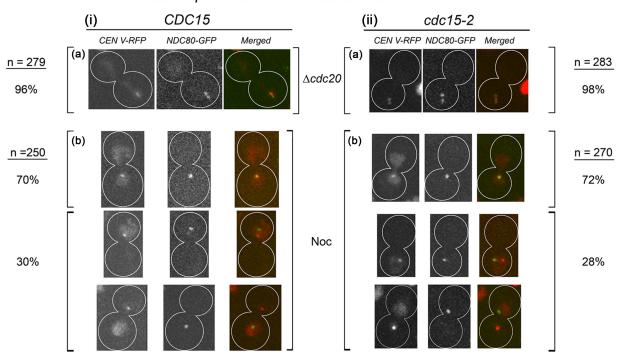
Fig S6 Ipl1p and Sli15p localized to *cdc15-2* cells similarly as in wild-type cells. Wild-type (i) and mutant (ii) cells carrying *IPL1-GFP MYO1-REDSTAR2* (A) and *SLI15-GFP MYO1-REDSTAR2* (B) were treated as in Fig 4.

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



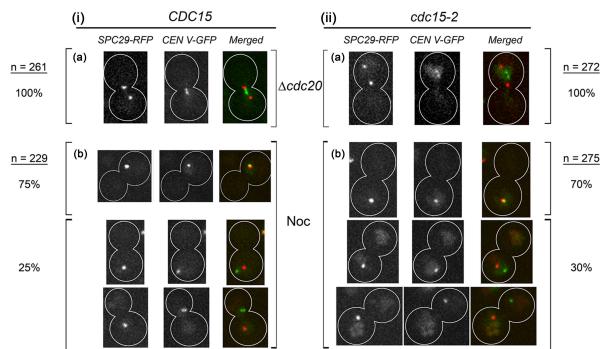
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С

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



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
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Tracking lines for CEN V-GFP

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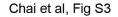
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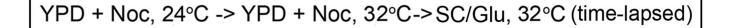
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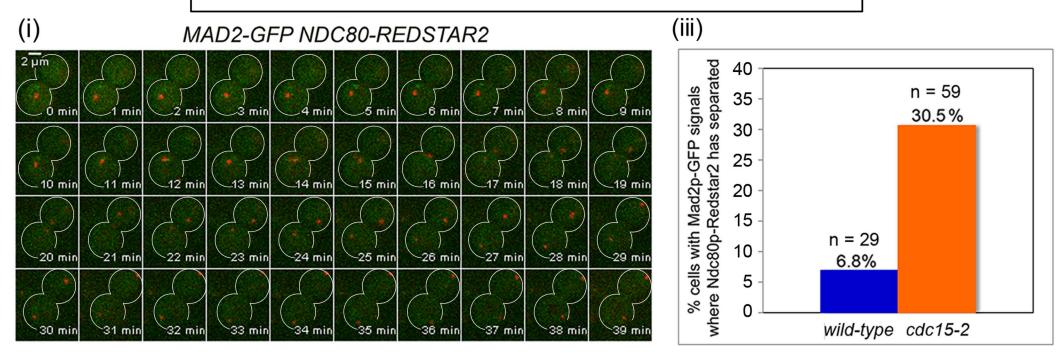
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(ii)

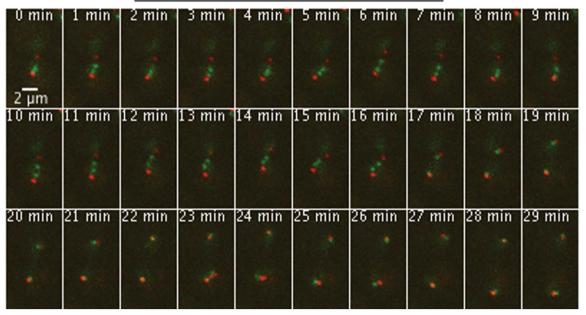
cdc15-2 MAD2-GFP NDC80-REDSTAR2

2 µm 0 min		2 min	3 min	4 min	5 min	6 min	7 min	8 min	9 min
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YPD arrest ____ release into YP/Raff/Gal (cdc20-depletion)

Chai et al, Fig S4

cdc20∆ GAL-CDC20 CEN V-GFP SPC29-RFP

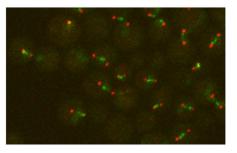


YPD, 24°C ->YPD, 32°C->SC/Glu, 32°C

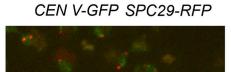
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27 min	28 min	29 min	30 min	31 min	32 min	33 min	34 min	35 min	27 min	28 min	29 min	30 min	31 min	32 min	33 min	34 min	35 min
36 min	37 min	38 min	39 min	40 min	41 min	42 min	43 min	44 min	36 min	37 min	38 min	39 min	40 min	41 min	42 min	43 min	44 min
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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

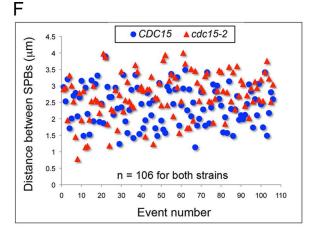
D cdc20∆ GAL-CDC20 CEN V-GFP SPC29-RFP



G



E cdc15-2 cdc20∆ GAL-CDC20



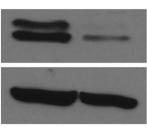
YPD, 24°C -> YPD, 32°C

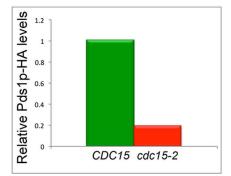
cdc20∆ GAL-CDC20 CEN V-GFP SPC29-RFP PDS1-HA

CDC15 cdc15-2

Pds1p-HA

Pgk1p







Α

IPL1-GFP MYO1-REDSTAR2

0 min	16 min	22 min	34 min	40 min	42 min
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(i)

cdc15-2 IPL1-GFP MYO1-REDSTAR2

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В

(i)

SLI15-GFP MYO1-REDSTAR2

0 min 2 μm	6 min	32 min	34 min	36 min	38 min	40 min
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(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
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Chai et al, Fig S7

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

