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^{o}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^{o}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 *cdc20Δ GAL-CDC20 NDC80-GFP SPC29-RFP* in *CDC15* (i) and *cdc15-2* (ii) backgrounds were arrested in metaphase by cdc20-depletion at 32^{o}C (a). In parallel experiments, the strains were arrested in Noc 32^{o}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. *cdc20Δ::GAL-CDC20 CEN V-GFP SPC29-RFP* in *CDC15* (i) and *cdc15-2* (ii) backgrounds arrested in metaphase by cdc20-depletion at 32^{o}C (a). In parallel experiments, the strains were arrested in Noc 32^{o}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^{o}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 *cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP* released from Cdc20p-depletion into YPD at 32^{o}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. *cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP* (A) and *cdc15-2 cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP* (B) cells were arrested in YPD for 2.5 hrs at 24^{o}C and YPD for 2.5 hrs at 32^{o}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)

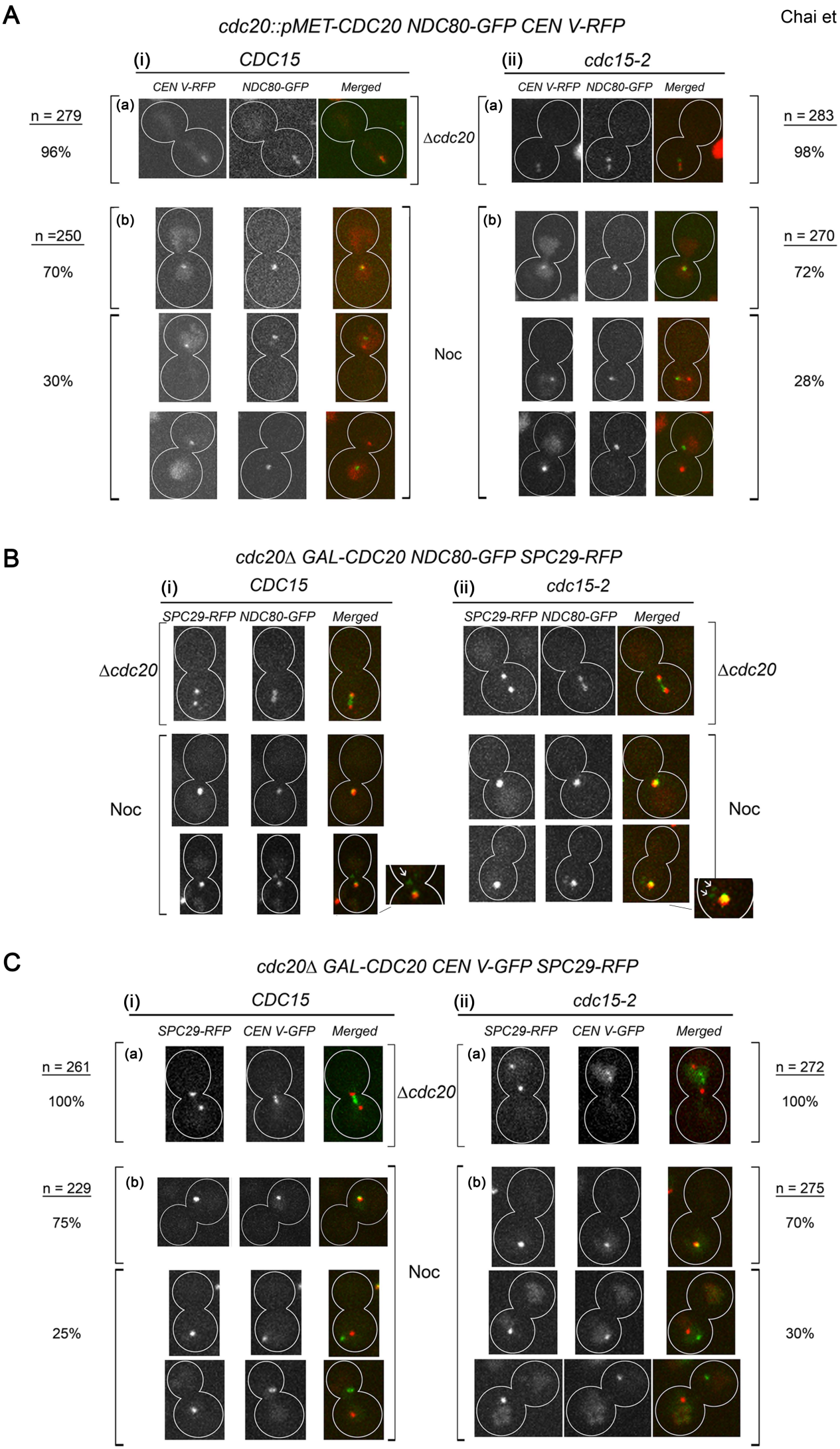
Plots show the distance between Spc29p-RFP in 106 cells for each strain in the experiment as described in (A) and (B). In parallel experiments, *cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP PDS1-HA* and *cdc15-2 cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP PDS1-HA* cells were arrested as described in (A) and (B) and 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.

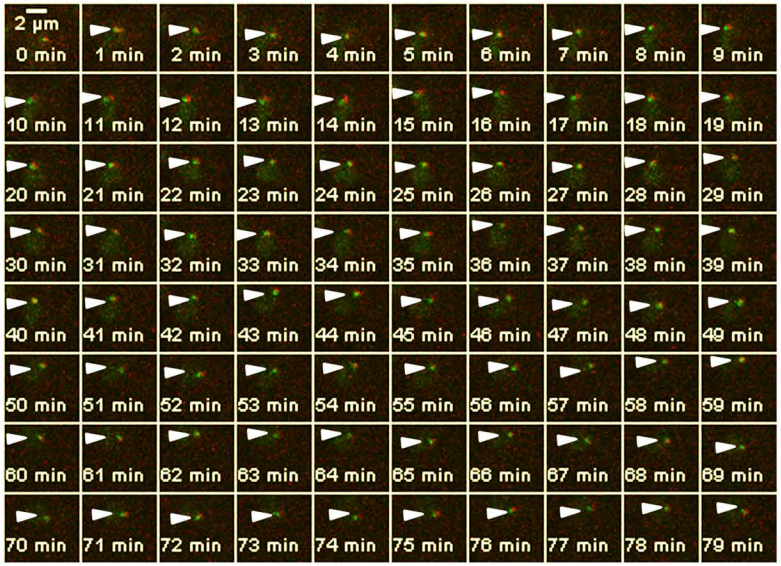
Movie 2 Dynamic movement of Spc29p-RFP across the neck in relation to CEN V-GFP and Myo1p-Redstar2 in *cdc15-2* cell. The movie shown is of the cell described in Fig 4Aii.


A
cdc20::pMET-CDC20 NDC80-GFP CEN V-RFP
(i) CDC15
(ii) cdc15-2
CEN V-RFP
NDC80-GFP
Merged
(a)
(b)
n = 279
96%
n = 283
98%
Δcdc20
n =250
70%
n = 270
72%
30%
28%
Noc
B
cdc20Δ GAL-CDC20 NDC80-GFP SPC29-RFP
(i) CDC15
(ii) cdc15-2
SPC29-RFP
NDC80-GFP
Merged
Δcdc20
Noc
C
cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP
(i) CDC15
(ii) cdc15-2
SPC29-RFP
CEN V-GFP
Merged
(a)
(b)
n = 261
100%
n = 272
100%
Δcdc20
n = 229
75%
n = 275
70%
25%
30%
Noc

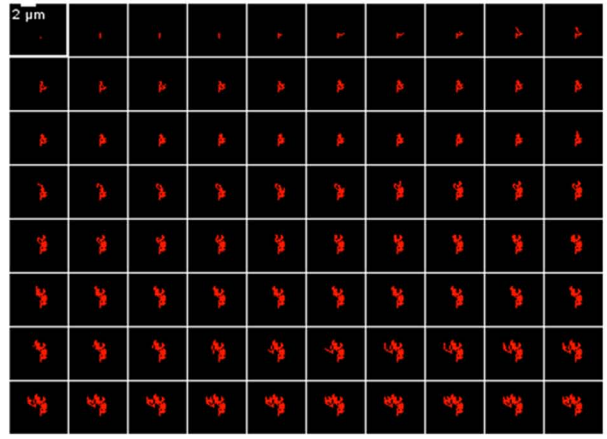
CEN V-GFP SPC29-RFP MYO1-REDSTAR2

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

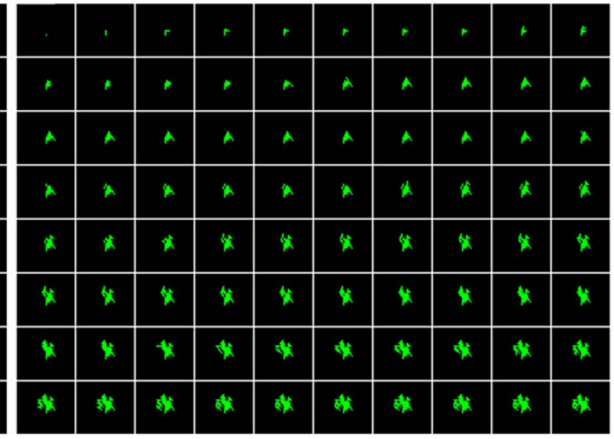
A(i)



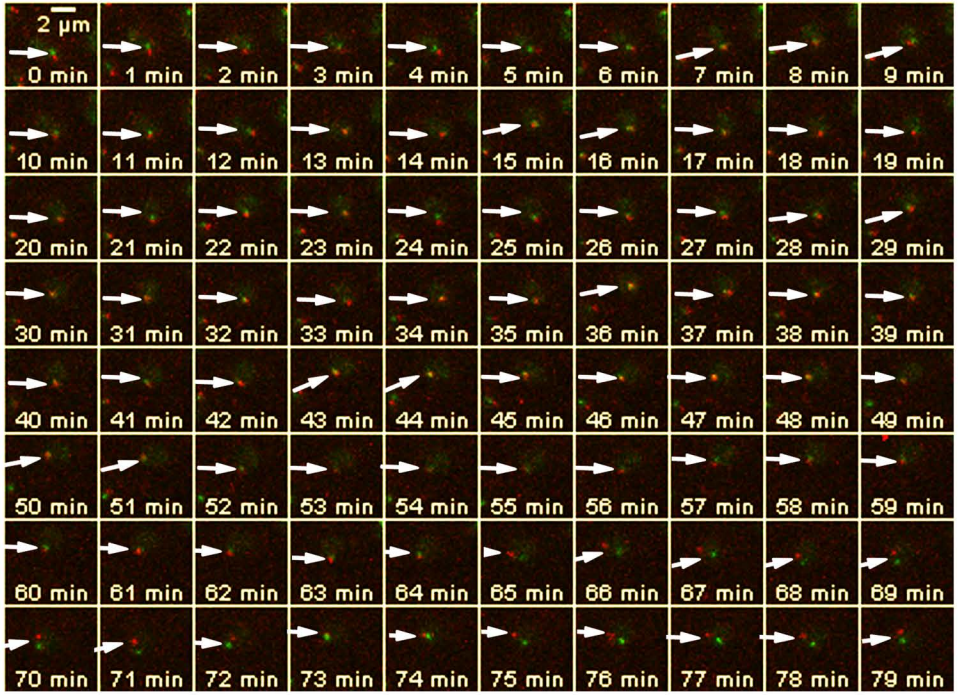
(ii) Tracking lines for Spc29p-RFP



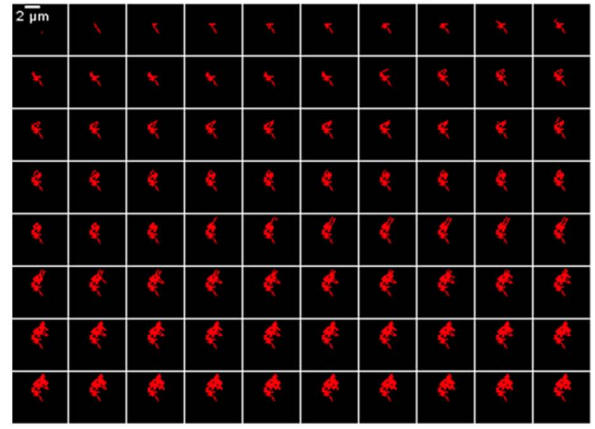
Tracking lines for CEN V-GFP



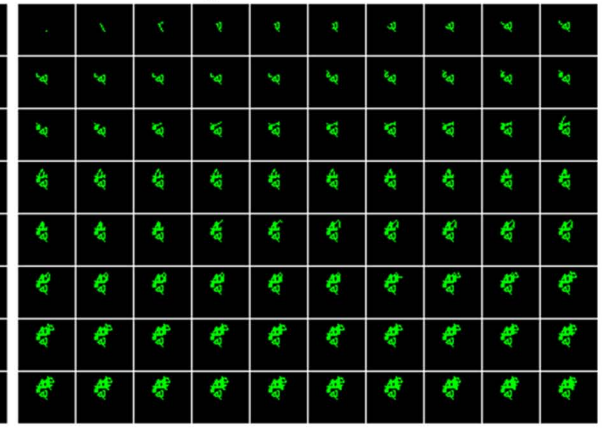
B (i)



(ii) Tracking lines for Spc29p-RFP



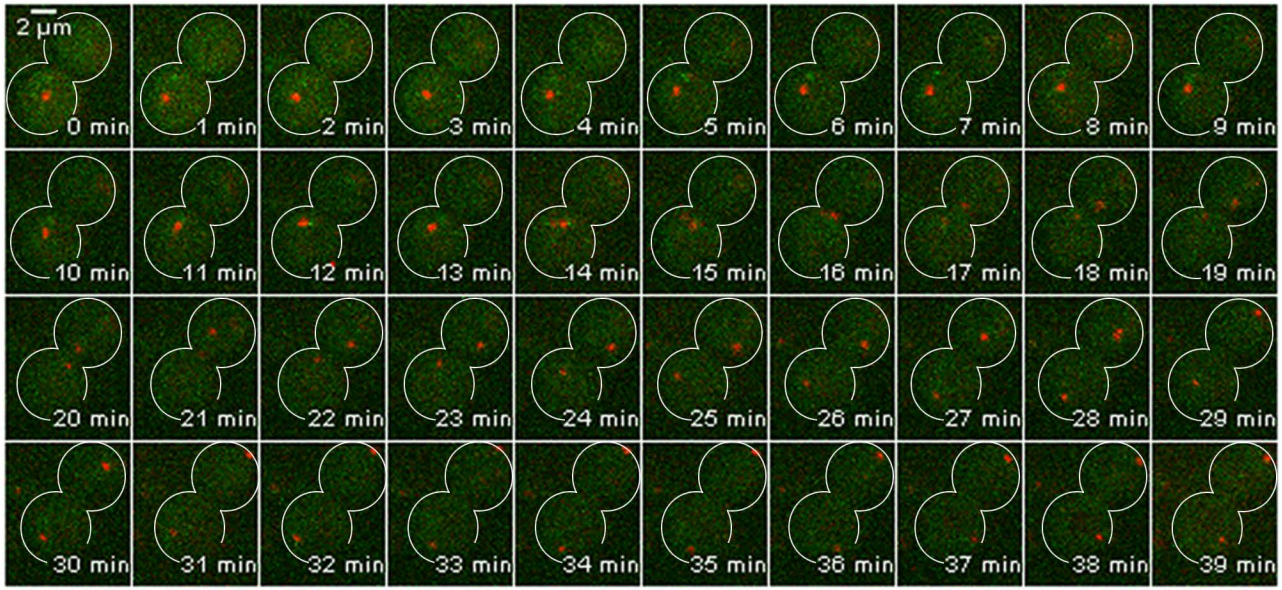
Tracking lines for CEN V-GFP



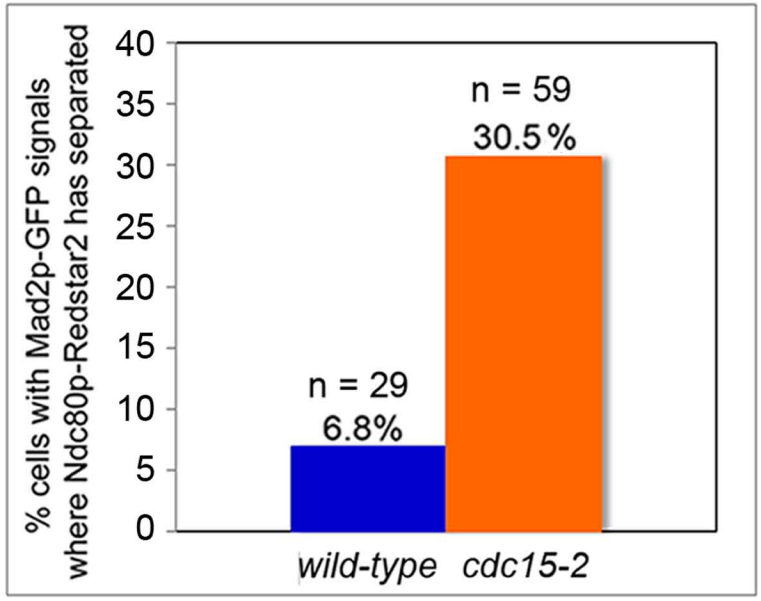
YPD + Noc, 24°C -> YPD + Noc, 32°C-> SC/Glu, 32°C (time-lapsed)

(i)

MAD2-GFP NDC80-REDSTAR2

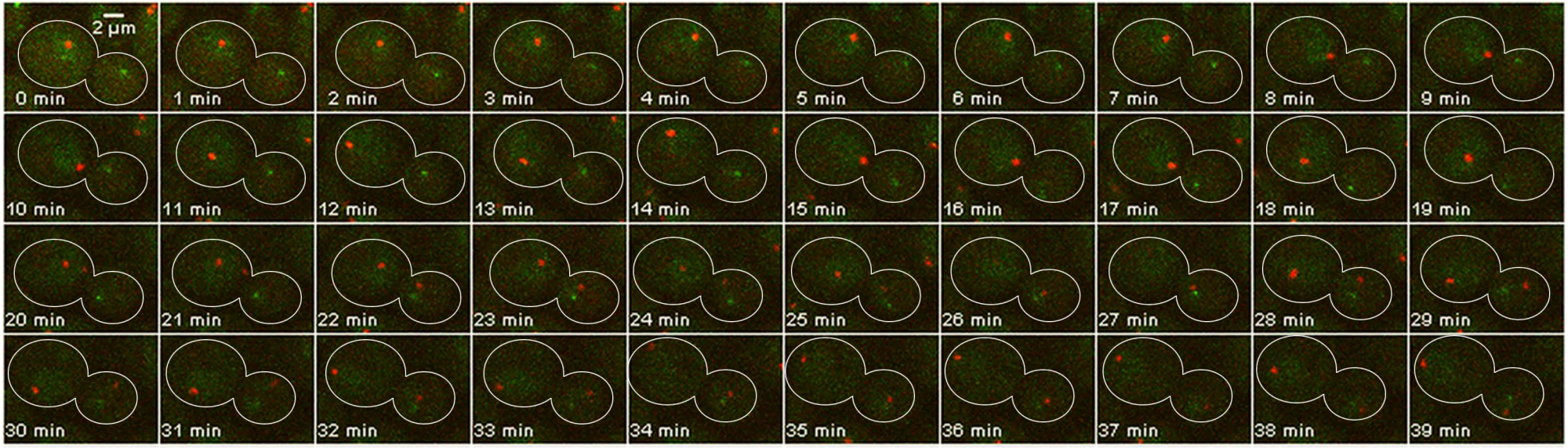


(iii)



(ii)

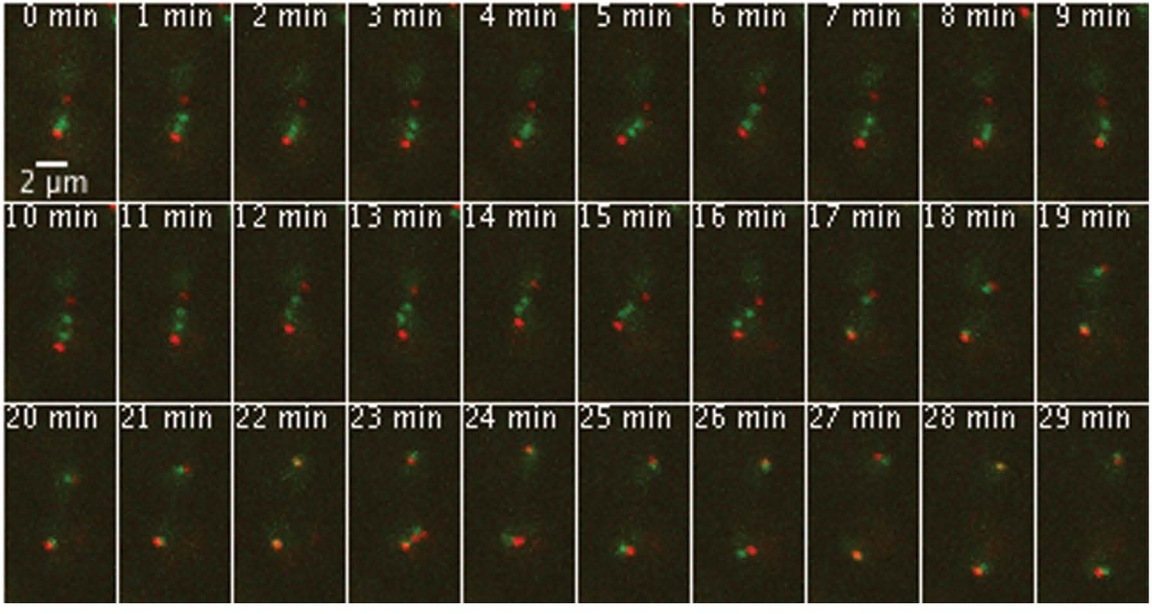
cdc15-2 MAD2-GFP NDC80-REDSTAR2



Chai et al, Fig S4

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

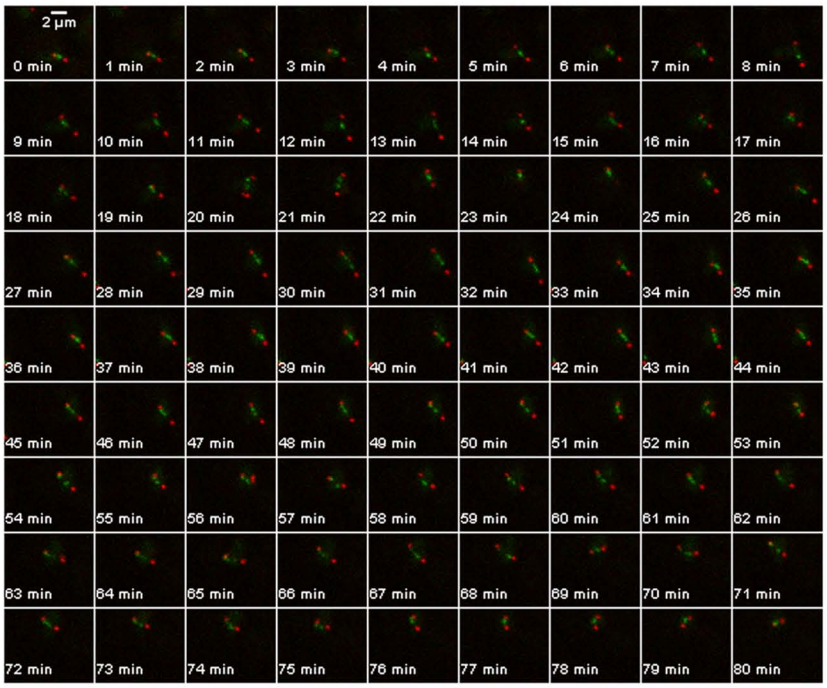
cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP



YPD, 24°C ->YPD, 32°C->SC/Glu, 32°C

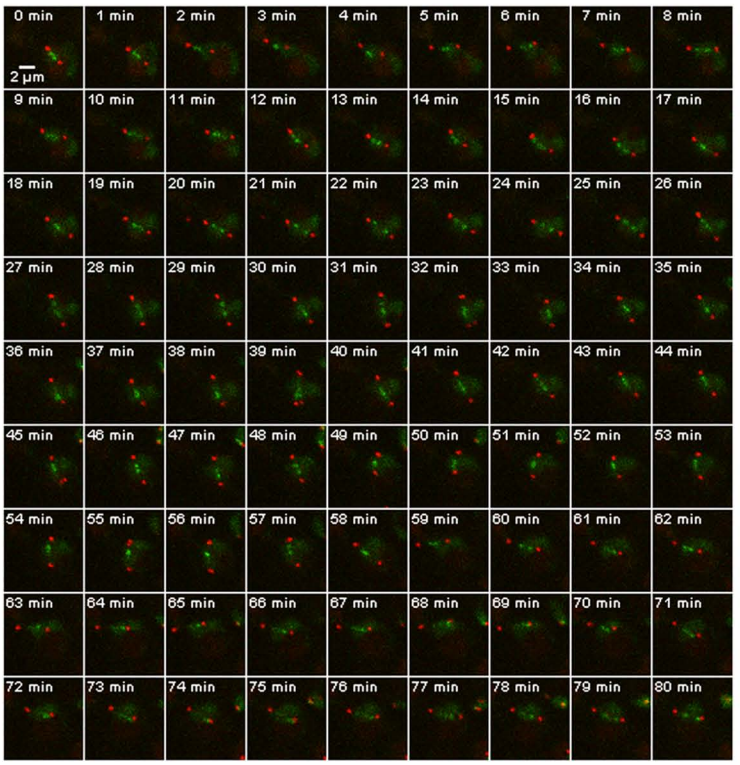
A

cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP



B

cdc15-2 cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP

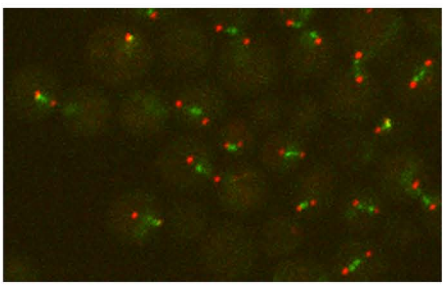


C

Strain background	% cells showing biorientation of CEN V-GFP spots
CDC15 (n= 49)	100
cdc15-2 (n = 44)	97.7

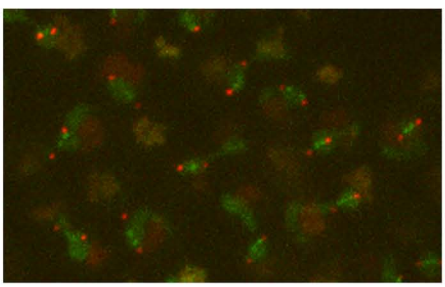
D

cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP

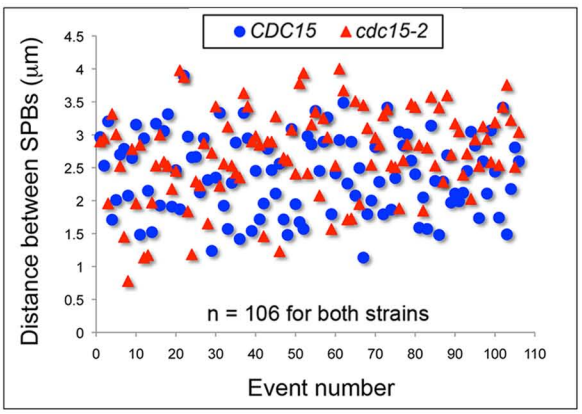


E

cdc15-2 cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP



F

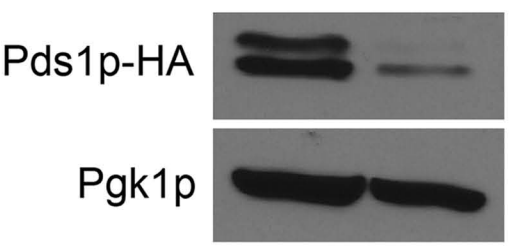


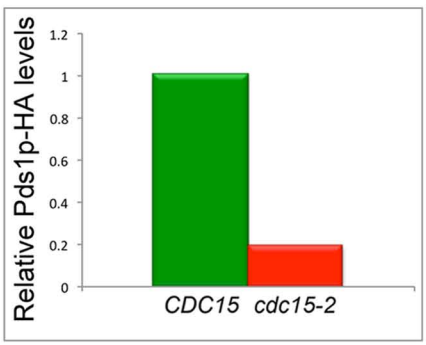
G

YPD, 24°C -> YPD, 32°C

cdc20Δ GAL-CDC20 CEN V-GFP SPC29-RFP PDS1-HA

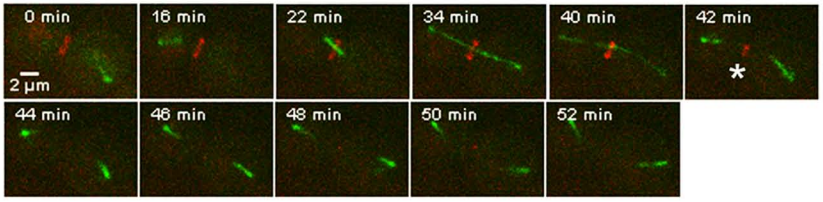
CDC15 cdc15-2



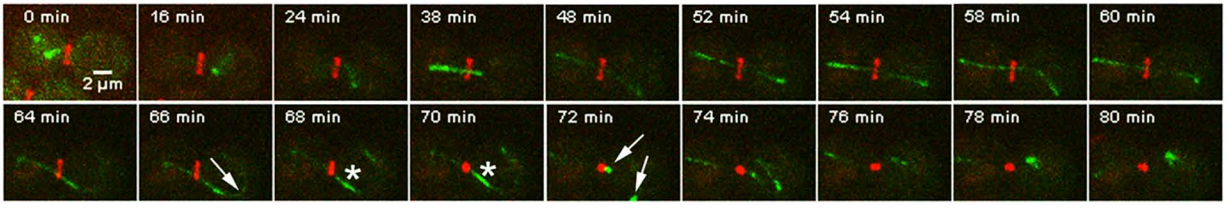


A

(i) *IPL1-GFP MYO1-REDSTAR2*

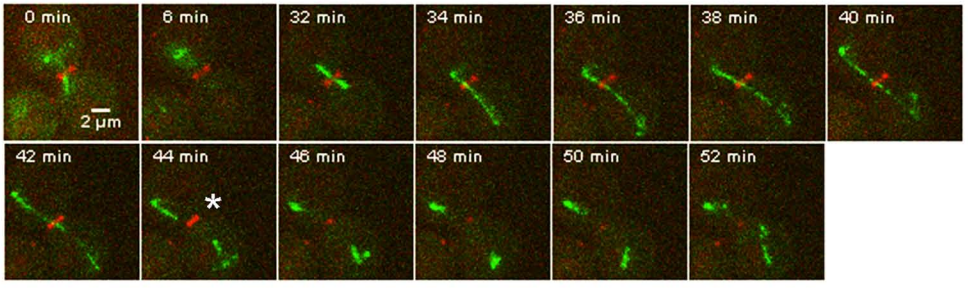


(ii) *cdc15-2 IPL1-GFP MYO1-REDSTAR2*

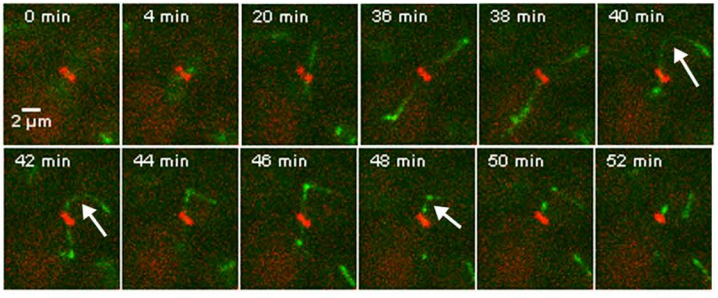


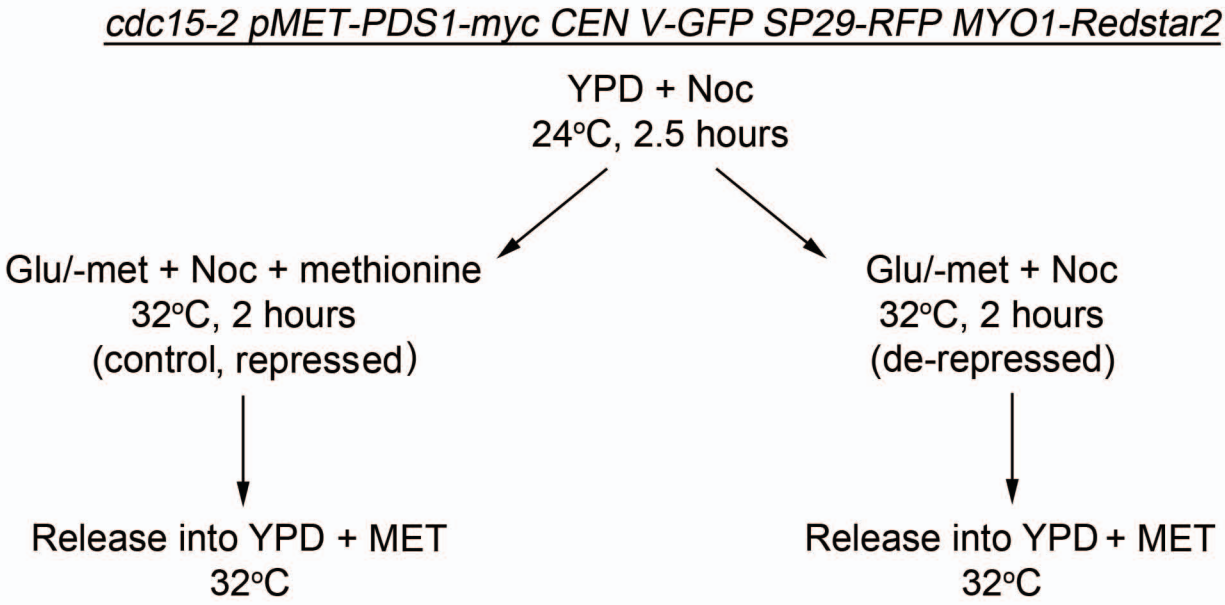
B

(i) *SLI15-GFP MYO1-REDSTAR2*



(ii) *cdc15-2 SLI15-GFP MYO1-REDSTAR2*




cdc15-2 pMET-PDS1-myc CEN V-GFP SP29-RFP MYO1-Redstar2
YPD + Noc
24°C, 2.5 hours
Glu/-met + Noc + methionine
32°C, 2 hours
(control, repressed)
Glu/-met + Noc
32°C, 2 hours
(de-repressed)
Release into YPD + MET
32°C
Release into YPD + MET
32°C