Supplemental Materials

Molecular Biology of the Cell

Chrupcala *et al*.

Figure S1.

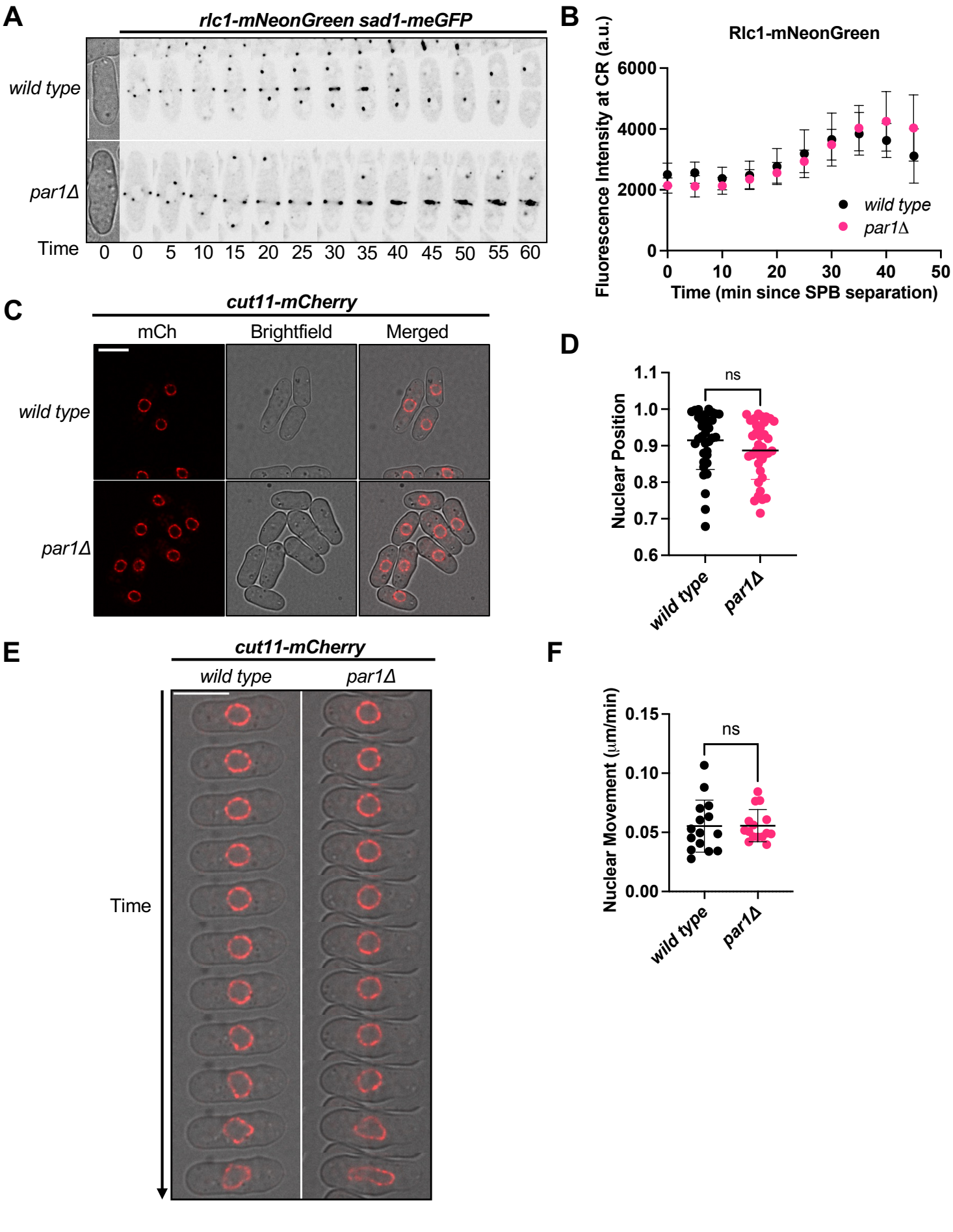
A
rlc1-mNeonGreen sad1-meGFP
wild type
par1Δ
Time
0 0 5 10 15 20 25 30 35 40 45 50 55 60
B
Rlc1-mNeonGreen
Fluorescence Intensity at CR (a.u.)
6000
4000
2000
0
0 10 20 30 40 50
Time (min since SPB separation)
wild type
par1Δ
C
cut11-mCherry
mCh
Brightfield
Merged
wild type
par1Δ
D
Nuclear Position
1.1
1.0
0.9
0.8
0.7
0.6
ns
wild type
par1Δ
E
cut11-mCherry
wild type
par1Δ
Time
F
Nuclear Movement (μm/min)
0.15
0.10
0.05
0.00
ns
wild type
par1Δ

Figure S2.

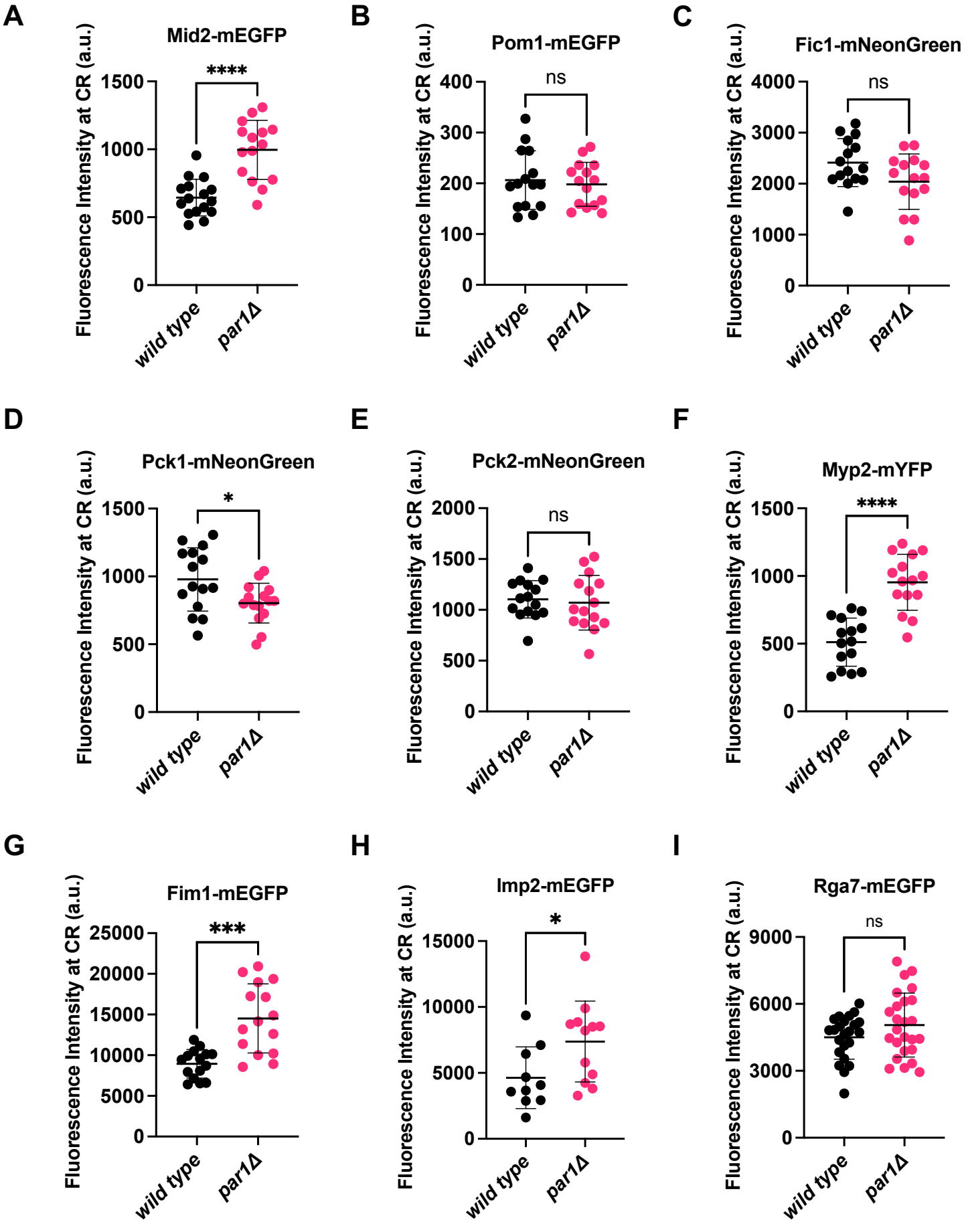
A
Mid2-mEGFP
****
Fluorescence Intensity at CR (a.u.)
1500
1000
500
0
wild type
par1Δ
B
Pom1-mEGFP
ns
400
300
200
100
0
C
Fic1-mNeonGreen
ns
4000
3000
2000
1000
0
D
Pck1-mNeonGreen
*
E
Pck2-mNeonGreen
ns
2000
1500
1000
500
F
Myp2-mYFP
****
G
Fim1-mEGFP
***
25000
20000
15000
10000
5000
H
Imp2-mEGFP
*
I
Rga7-mEGFP
ns
9000
6000
3000

Figure S3.

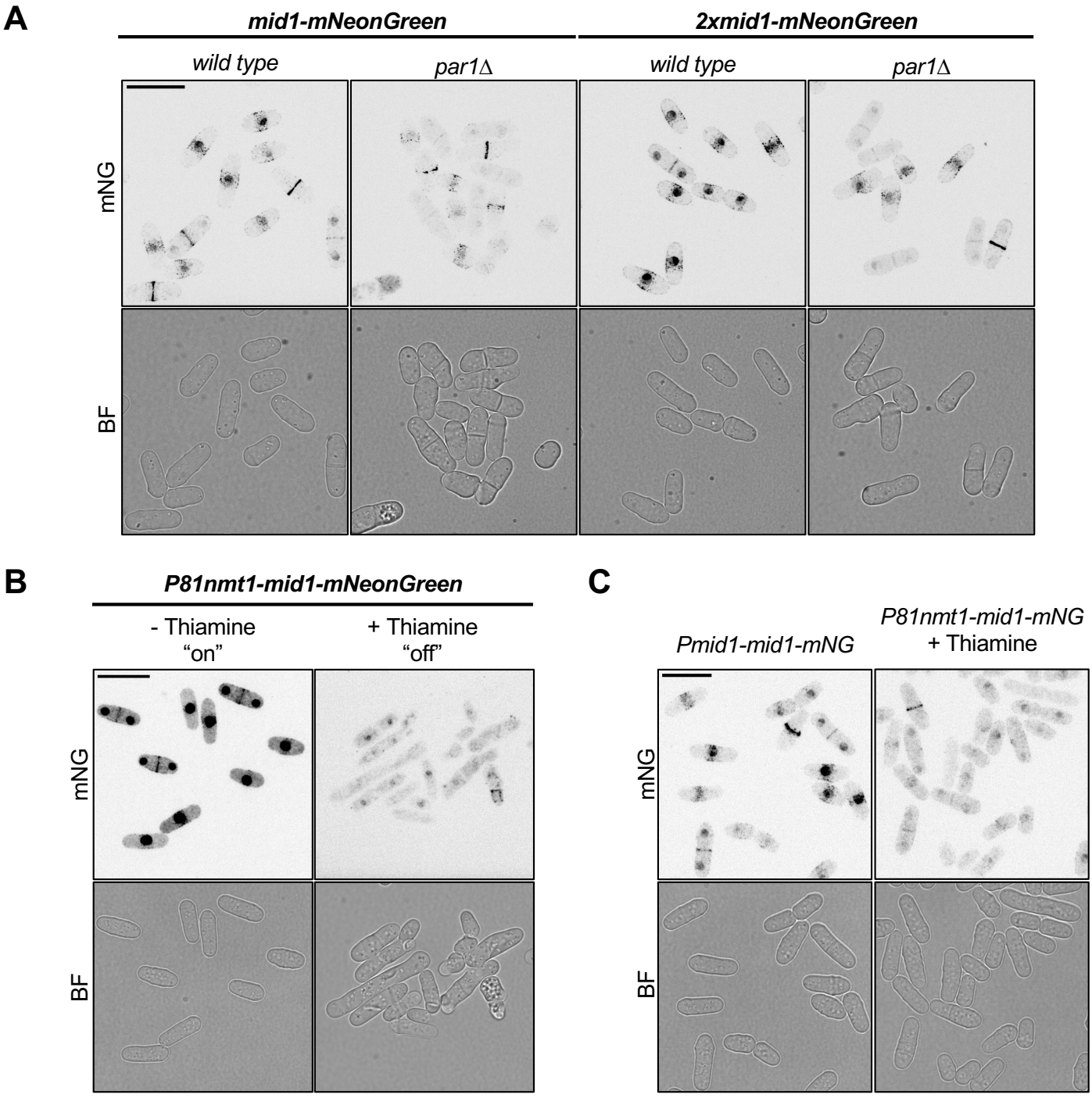
A
mid1-mNeonGreen
2xmid1-mNeonGreen
wild type
par1Δ
wild type
par1Δ
mNG
BF
B
P81nmt1-mid1-mNeonGreen
- Thiamine
"on"
+ Thiamine
"off"
mNG
BF
C
Pmid1-mid1-mNG
P81nmt1-mid1-mNG
+ Thiamine
mNG
BF

Figure S4.

A

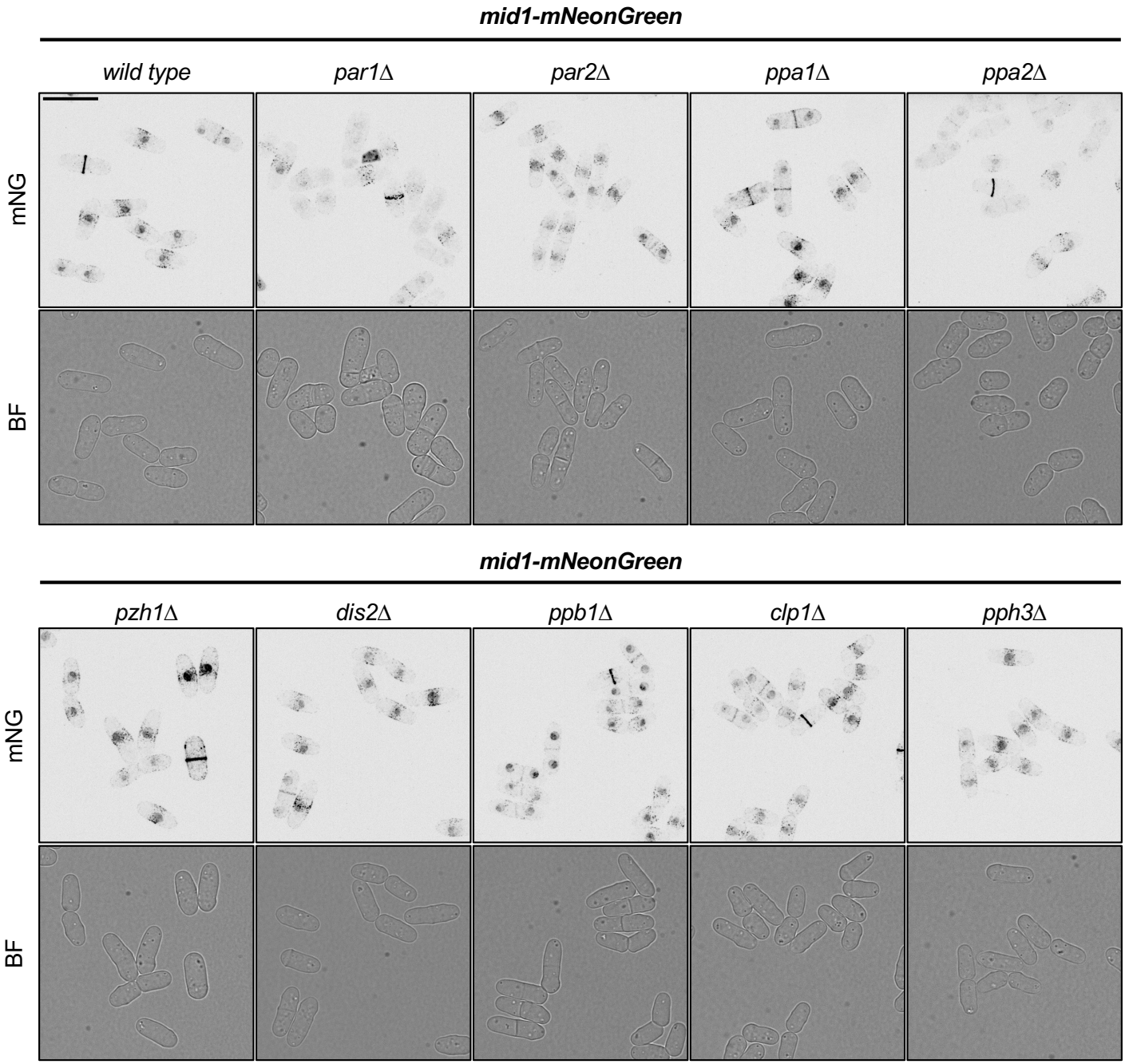
mid1-mNeonGreen
wild type
par1Δ
par2Δ
ppa1Δ
ppa2Δ
mNG
BF
mid1-mNeonGreen
pzh1Δ
dis2Δ
ppb1Δ
clp1Δ
pph3Δ
mNG
BF

Figure S5.

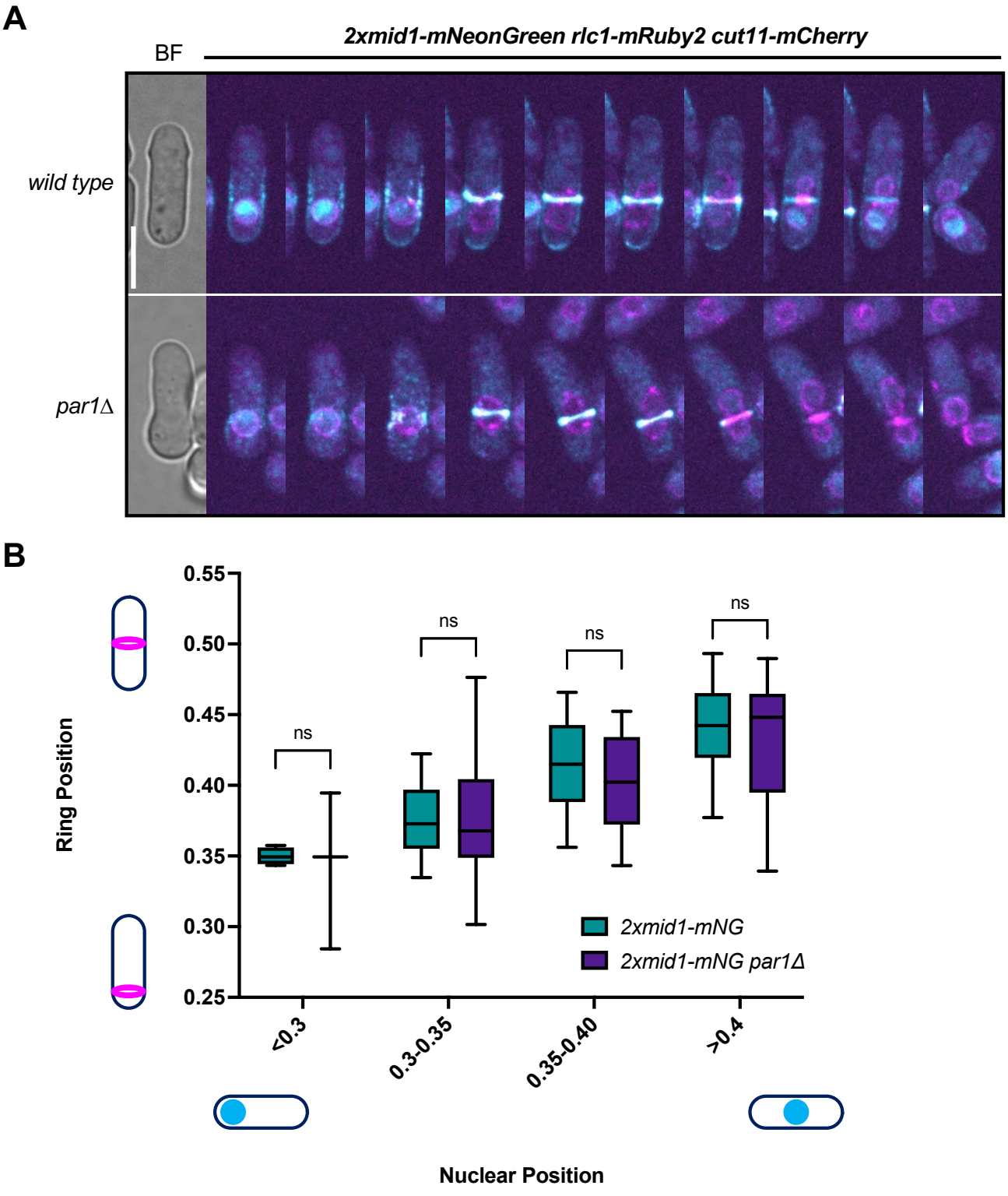
A
2xmid1-mNeonGreen rlc1-mRuby2 cut11-mCherry
BF
wild type
par1Δ
B
Ring Position
0.55
0.50
0.45
0.40
0.35
0.30
0.25
ns
ns
ns
ns
2xmid1-mNG
2xmid1-mNG par1Δ
<0.3
0.3-0.35
0.35-0.40
>0.4
Nuclear Position

Figure S6.

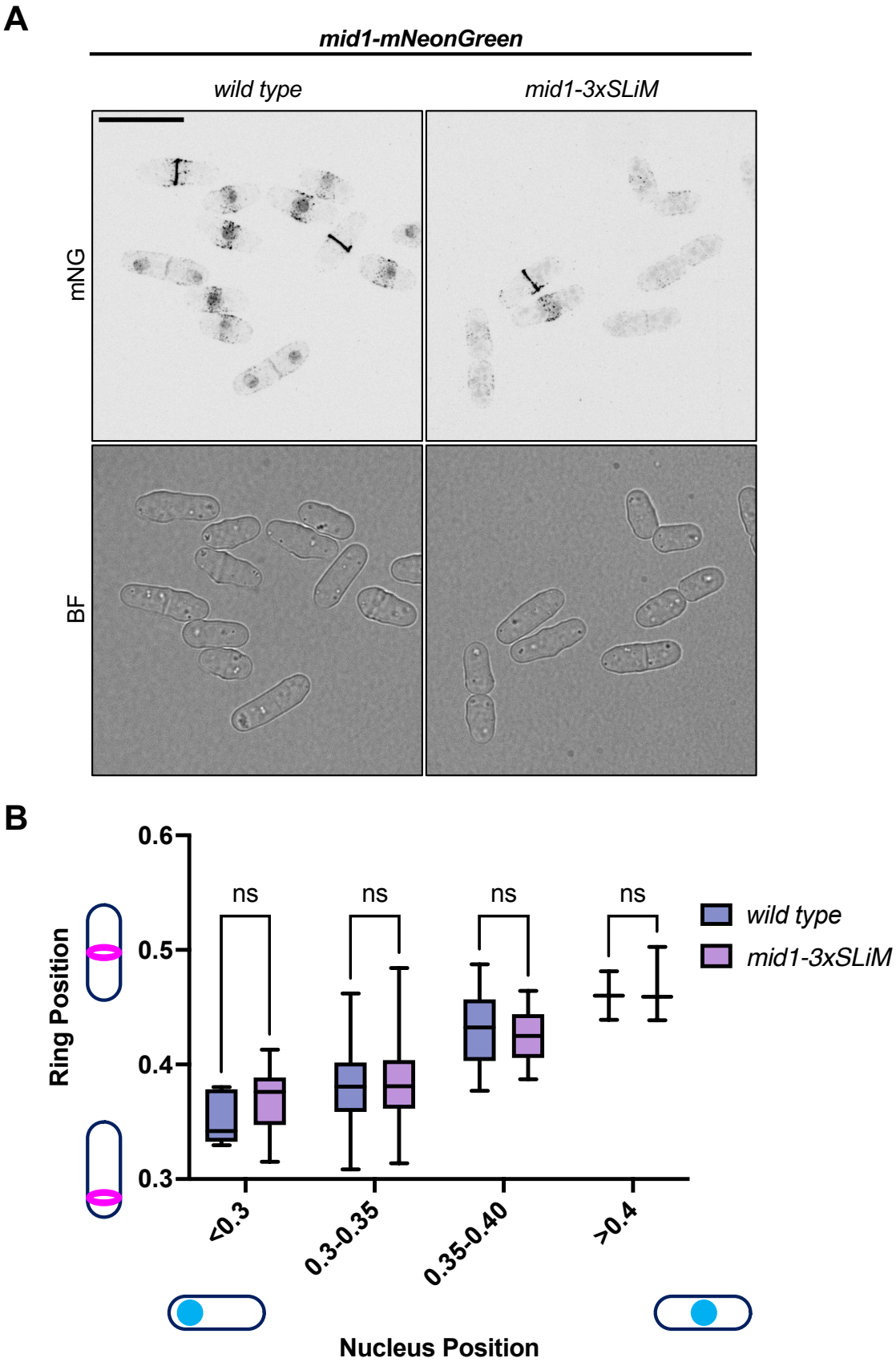
A
mid1-mNeonGreen
wild type
mid1-3xSLiM
mNG
BF
B
Ring Position
0.6
0.5
0.4
0.3
ns
ns
ns
ns
wild type
mid1-3xSLiM
<0.3
0.3-0.35
0.35-0.40
>0.4
Nucleus Position

Figure S1. Additional characterization of *par1*Δ cells. (A) Representative time-lapse montages of wild type and *par1*Δ cells expressing Rlc1-mNeonGreen and Sad1-mEGFP. Images are a single middle focal plane with inverted fluorescence. Cells were captured in 5-minute intervals. Spindle pole body separation was used as time point 0, which was determined by Sad1-meGFP signal. Scale bar is 7μm. (B) Quantification of Rlc1-mNG recruitment to the cytokinetic ring in wild type and *par1*Δ. Time corresponds to minutes since spindle pole body separation. N ≥ 15 cells each. Errors bars indicate mean and error ± standard deviation. (C) Single middle focal plane images of representative wild type and *par1*Δ cells expressing Cut11-mCherry. Scale bar is 7μm. (D) Quantification of nuclear position. The distance from each cell tip to the center of the nucleus was measured, and then the nuclear position was determined by dividing the short length by the long length. N ≥ 34 cells each. ns indicates *p* value > 0.05 determined by Welch's unpaired t-test. (E) Time-lapse montage of representative wild type and *par1*Δ cells expressing Cut11-mCherry. Images are a single middle focal plane with mCherry overlaid onto brightfield to visualize the cell tips. Images are captured in 3-minute intervals. Scale bar is 7μm. (F) Quantification of nuclear movement. The distance from the cell tip to the center of the nucleus was measured at every time point. Nuclear movement was determined by averaging the difference between this distance at every time point and dividing it by 3 minutes. N ≥ 15 cells each. ns indicates *p* value > 0.05 determined by Welch's unpaired t-test.

Figure S2. Concentration of cytokinesis proteins at the CAR in *par1*Δ cells. Quantification of the fluorescence intensity for the indicated proteins at the contractile ring (CR) in wild type and par1Δ cells. N ≥ 10 cells each. ns indicates *p* value > 0.05; * indicates *p* value <0.05; ** indicates *p* value < 0.005; *** indicates *p* value <0.0005; **** indicates *p* value <0.0001. All statistical tests performed in this figure are Welch's unpaired t-tests.

Figure S3. Mid1 protein levels affect division plane positioning. (A) Representative images of the indicated strains, mNG images are maximum intensity projections with inverted fluorescence. Scale bar is 14μm. (B) Representative images of cells expressing Mid1-mNG under the control of the thiamine repressible *P81nmt1* promoter. "On" cells were grown with no thiamine for 40 hours (left). "Off" cells were grown in 2.5 μg/ml thiamine (right) for 24 hours. Brightfield images are single middle focal planes. mNG images are sum projections with inverted fluorescence. Scale bar is 12μm. (C) Representative images of the indicated strain. *P81nmt1-mid1-mNG* cells were grown in 0.005μg/ml thiamine for 23 hours. Brightfield images are single middle focal plane. mNG images are sum projections with inverted fluorescence. Scale bar is 12μm.

Figure S4. Reduced Mid1 levels are specific to PP2A mutants. (A) Representative images of the indicated strains. Brightfield images are a single middle focal plane. mNG images are maximum intensity projections with inverted fluorescence. Scale bar is 14μm.

Figure S5. Restoring Mid1 levels in *par1*Δ mutant restores division plane repositioning following nuclear displacement. (A) Time-lapse montage of wild type and *par1*Δ cells expressing 2xMid1-mNeonGreen, Rlc1-mRuby2, and Cut11-mCherry following MBC treatment and centrifugation. Images are captured in 15-minute intervals.

Representative maximum intensity projections are shown with Cut11-mCherry and Rlc1-mRuby2 in magenta and Mid1-mNG in cyan. Brightfield image is a single middle focal plane from the first time point shown. Scale bar is 7μm. (B) Quantification of nuclear displacement experiments. Positions are expressed as a fraction of the cell length. Nuclear positions were separated into <0.3, 0.3-0.35, 0.35-0.40, and >0.4 bins. Error bars correspond to minimum and maximum ring position for each bin. N ≥ 56 cells for each strain.

Figure S6. Additional examination of Mid1-3xSLiM cells. (A) Representative images of wild type and *mid1-3xSLiM* cells expressing Mid1-mNeonGreen. Brightfield images are a single middle focal plane. mNG images are maximum intensity projections with inverted fluorescence. Scale bar is 14μm. (B) The relationship between the cytokinetic ring and the displaced nucleus in wild type and *mid1-3xSLIM* cells (as in Figures 7C and S5B). Nuclear positions were separated into <0.3, 0.3-0.35, 0.35-0.40, and >0.4 bins. Error bars correspond to minimum and maximum ring position for each bin. N ≥ 56 cells each. All statistical tests performed in this figure are Welch's unpaired t-tests.

Table S1. Strains used in this study.

Table S2. PP2A-B56 SLiM motifs in cytokinetic node and cytokinetic ring proteins.