Supplemental Figures:

Supplemental Figure 1: Variability in the pairing analysis illustrated for the *LYS2* and *LEU2* loci.

(A) Five independent experiments are shown analyzing pairing as described in Figure 1 in wild-type cells with homologous *LYS2* dots (A9828, closed squares) and wild-type cells with non-homologous *LYS2/URA3* dots (A9829, open squares), both deleted for *NDT80*.

(B) Five independent experiments are shown analyzing pairing as described in Figure 1 in wild-type cells with homologous *LEU2* dots (A5111, closed circles) and wild-type cells with non-homologous *LEU2/LYS2* dots (A11474, open circles), both deleted for *NDT80*.

Supplemental Figure 2: DSBs are essential for homolog pairing.

(A) Wild-type (A5111, black squares), *SPO11-HA/SPO11-HA* (A16376, dark blue squares), *spo11-D290A/spo11-D290A* (A16399, dark green squares), *SPO11-HA/spo11-Y135F* (A16391, light green squares) and *spo11-Y135F-HA/spo11-Y135F-HA* (A13346, yellow squares) cells all carrying homologous *LEU2* dots, and wild-type cells with non-homologous *LEU2/LYS2* dots (A11474, open squares), all deleted for *NDT80,* were assayed for pairing as described in Figure 1. The percentage of DSBs created by the various *spo11* alleles was determined by (Henderson and Keeney, 2004).

(B) Wild-type (A9828, black squares), *SPO11-HA/SPO11-HA* (A16292, dark blue squares), *spo11-D290A/spo11-D290A* (A16126, dark green squares), *SPO11-HA/spo11-Y135F* (A16147, light green squares) and *spo11-Y135F-HA/spo11-Y135F-HA* (A16133, yellow squares) cells all carrying homologous *LYS2* dots, and wild-type cells with non-homologous *LEU2/LYS2* dots (A11474, open squares), all deleted for *NDT80,* were assayed for pairing as described in Figure 1.

(C - E) Wild-type cells with homologous *LYS2* dots (A9828, black squares), *clb5Δ clb6Δ* cells with homologous *LYS2* dots (A11268, closed triangles) and wild-type cells with non-homologous *LEU2/LYS2* dots (A11474, open squares), all deleted for *NDT80,* were induced to sporulate. At the indicated time points, samples were assayed for pairing as described in Figure 1 (C) and DNA content analysis by flow cytometry (D, E).

(F - H) Wild-type cells with homologous *LEU2* dots (A5111, black squares), *clb5Δ clb6Δ* cells with homologous *LEU2* dots (A11326, closed triangles) and wild-type cells with non-homologous *LEU2/LYS2* dots (A11474, open squares), all deleted for *NDT80,* were were induced to sporulate. At the indicated times, samples were taken and assayed for pairing as described in Figure 1 (F) and DNA content analysis by flow cytometry (G, H).

(I) Wild-type cells with homologous *LYS2* dots (A9828, closed squares), *mer2-S30A* cells with homologous *LYS2* dots (A16149, closed triangles), wild-type cells with non-homologous *URA3/LYS2* dots (A9829, open squares), all deleted for *NDT80,* were assayed for pairing as described in Figure 1.

Supplemental Figure 3: Meiotic progression of strains in Figure 3.
(A) Wild-type (A7097, closed squares), *spo11*Δ (A8477, closed triangles), *rec8*Δ A16664, closed circles), *cdc6-mn* (A15880, open squares), *cdc6-mn rec8*Δ (A17021, open triangles), and *clb5*Δ *clb6*Δ (A16113, open circles) were induced to sporulate. At the indicated times, samples were taken to determine the percentage of cells with unassembled spindles.

(B) Wild-type (A1972, closed squares) and *scc3-mn* (A20163, closed triangles) were induced to sporulate. At the indicated times, samples were to determine the percentage of cells with unassembled spindles.

(C) *REC8-3HA* (A13946, closed squares), *pREC8-SCC1-3HA* (A16132, closed triangles), and *REC8-NC* (A13539, closed circles) were induced to sporulate. At the indicated times, samples were taken to determine the percentage of cells with unassembled spindles.

Supplemental Figure 4: Zip1 categorization for strains analyzed in Figure 3B.

Wild-type (A7097; A), *spo11*Δ (A8477; B), *rec8*Δ (A16664; C), *clb5*Δ *clb6*Δ (A16113; D), *cdc6-mn* (A15880; E), and *cdc6-mn rec8*Δ (A17021; F) were induced to sporulate. At the indicated times, cells were harvested and chromosome spreads were assayed for Zip1 staining according to the categories shown in Figure 3A. 100 mononucleate cells were counted per time point

Supplemental Figure 5: Zip1 categorization for strains analyzed in Figure 3C.

Wild-type (A1972, A) and *scc3-mn* (A20163, B) were induced to sporulate. At the indicated times, cells were harvested and chromosome spreads were assayed for Zip1 staining according to the categories shown in Figure 3A. 100 mononucleate cells were counted per time point.

Supplemental Figure 6: Zip1 categorization for strains analyzed in Figure 3D.

REC8-3HA (A13946; A), *pREC8-SCC1-3HA* (A16132; B), and *REC8-NC* (A13539; C) were induced to sporulate. At the indicated times, cells were harvested and chromosome spreads were assayed for Zip1 staining according to the categories shown in Figure 3A. 100 mononucleate cells were counted per time point.

Supplemental Figure 7: Association of Hop1 with chromatin is not affected in *rec8-6A* and *rec8-29A* mutants.

Wild-type (A20066), *rec8-29A* (A14385), and *rec8-6A* (A15042) cells were induced to sporulate. At 4 hours, cells were harvested and chromosome spreads were assayed for Hop1 staining. Representative “Full Hop1” cells are pictured for each strain. Hop1 is shown in green and DAPI is shown in blue.

Supplemental Figure 8: Zip1 categorization for strains analyzed in Figure 7D, 7E

Wild-type (A20066, A), *rec8*Δ (A3528, B), *rec8-29A* (A14385, C), and *rec8-6A* (A15042, D) cells were induced to sporulate. At the indicated times, cells were harvested and chromosome spreads were assayed for Zip1 staining according to the categories shown in Figure 3A. 100 mononucleate cells were counted per time point. counted per timepoint in A and C.

Supplemental Figure 9: The Zip1 assembly defect is variable in *rec8-6A* mutants.

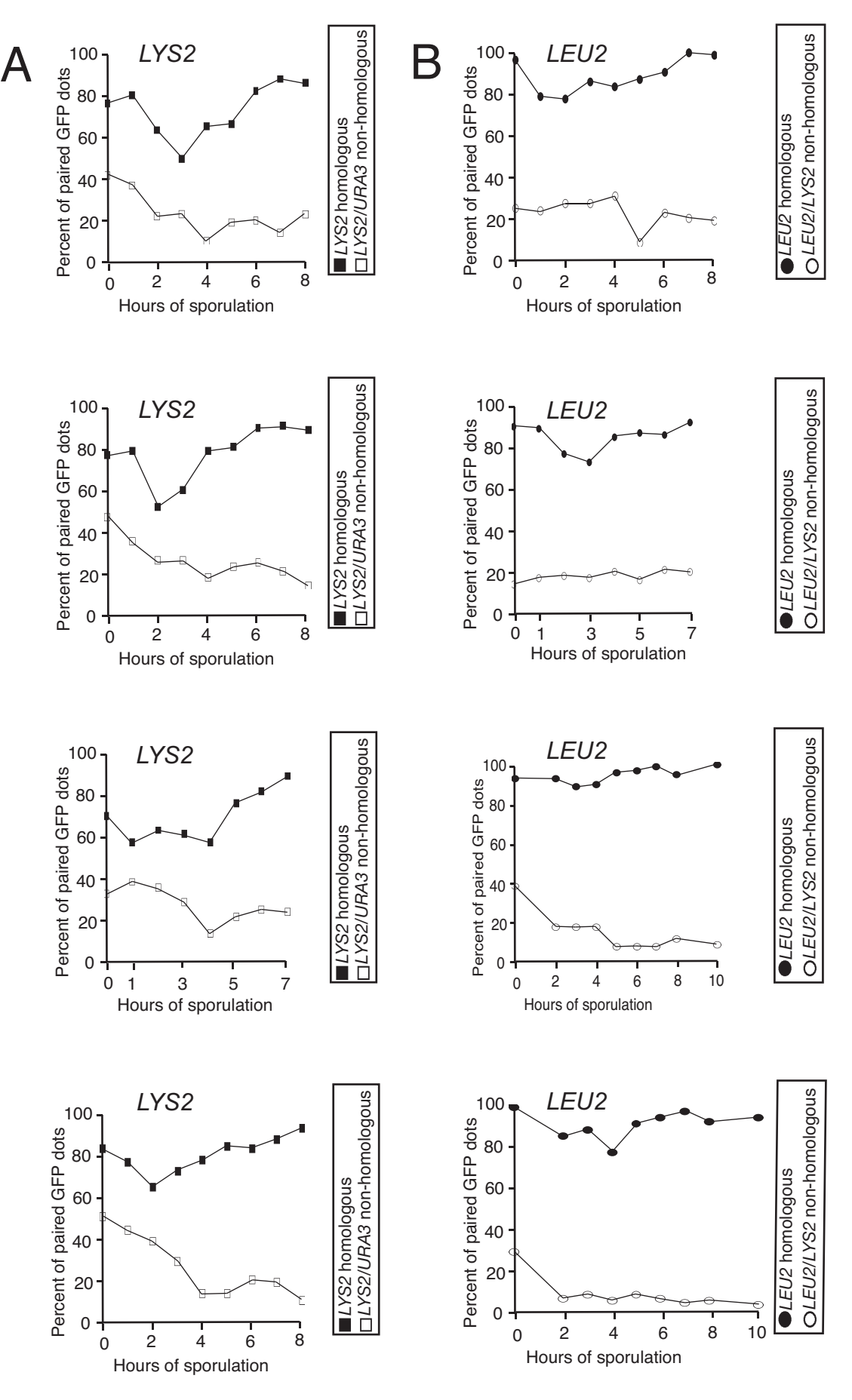
A comparison of Zip1 assembly in independent experiments using wild-type (A20066), *rec8*Δ (A3528), and *rec8-6A* (A15042) cells. Zip1 staining is quantified in (B, D), the percentage of cells with unassembled meiotic spindles in (A, C). 100 mononucleate cells were counted per time point in B and D, 200 cells were counted per timepoint in A and C.

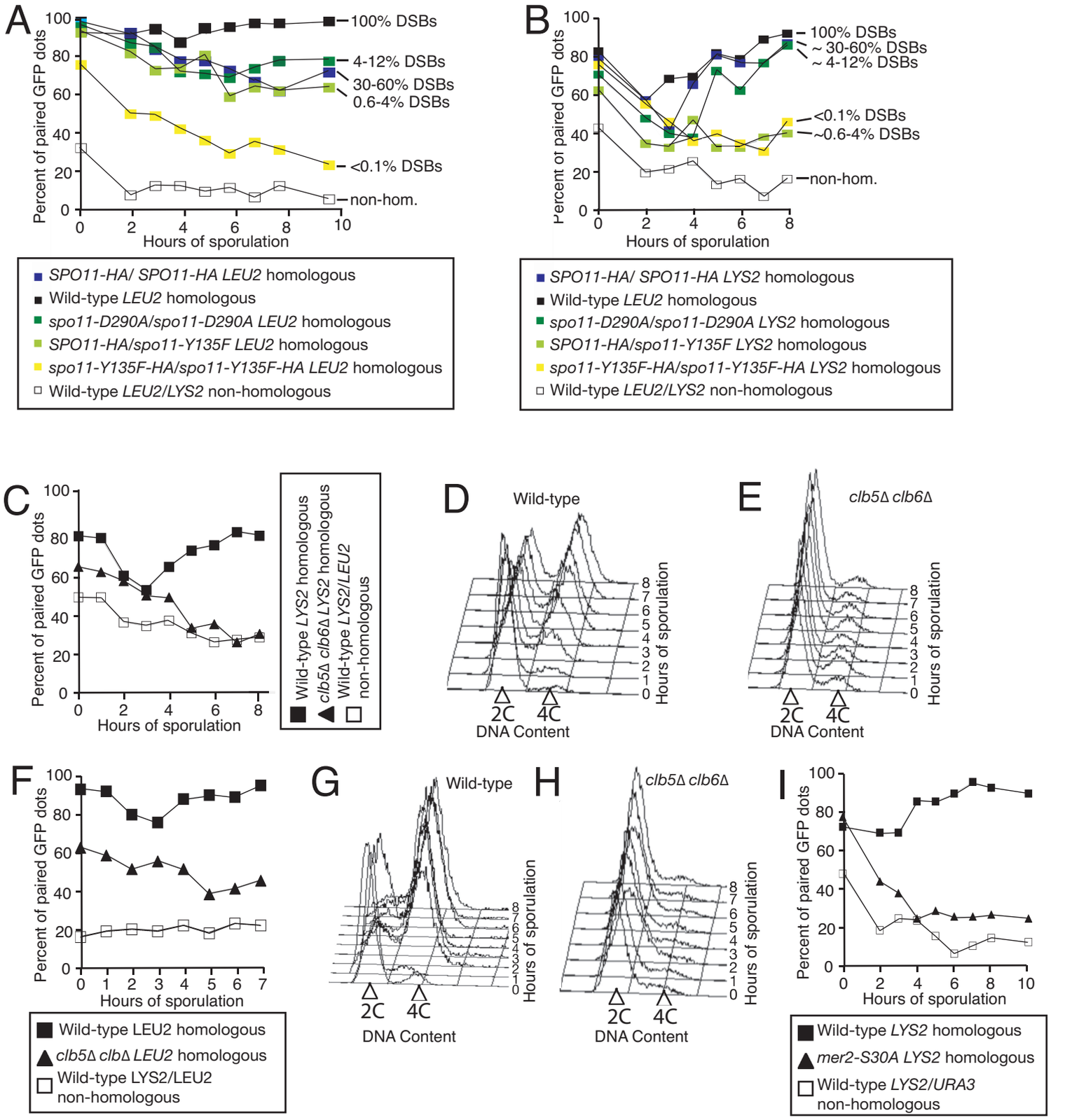
Supplemental Figure 10: Zip1 categorization for strains analyzed in Figure 8A - F.

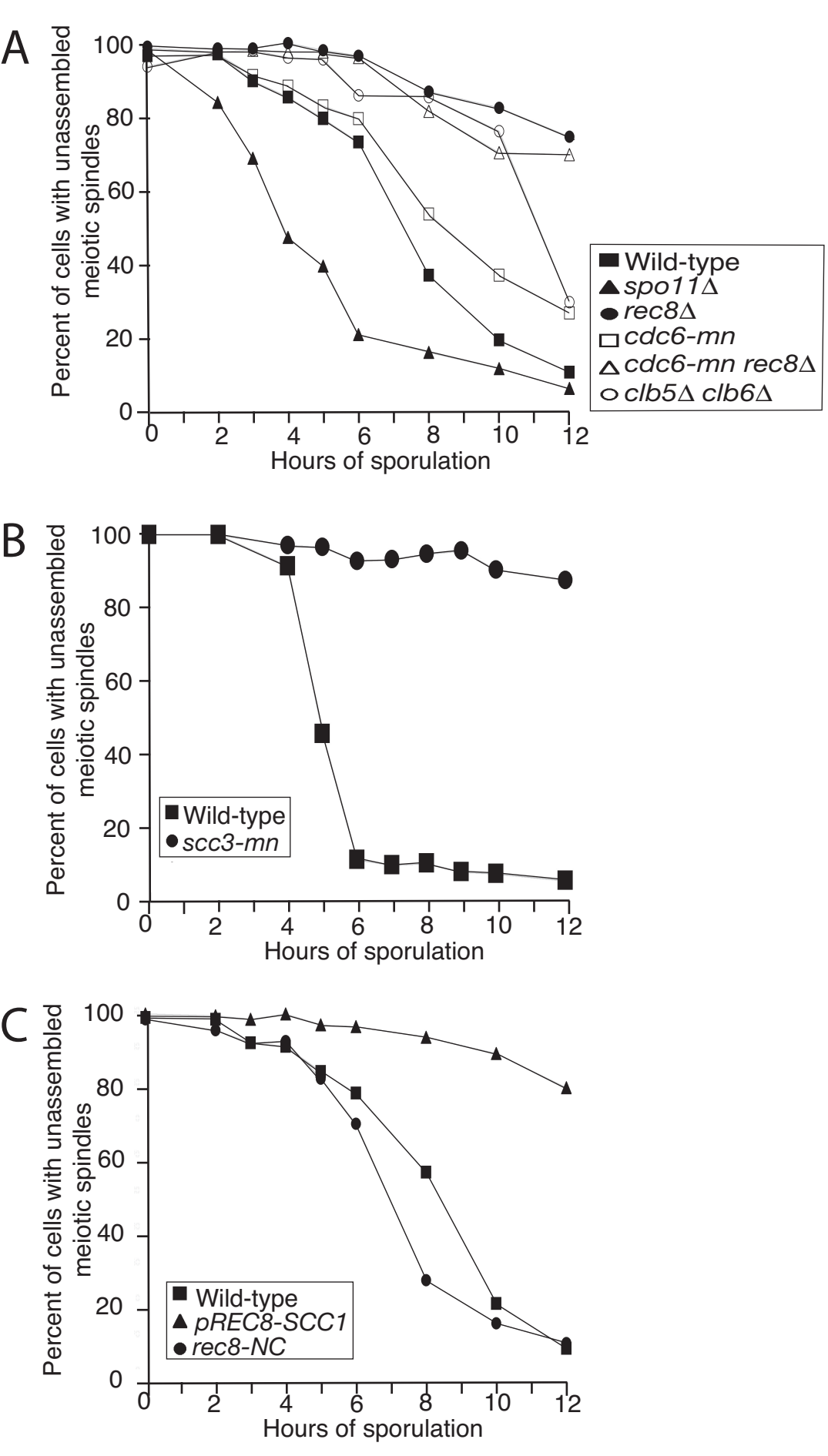
Wild-type (A20066; A), *cdc5-mn* (A5844; B) cells and *rec8-psa* (A15364; D) cells and a corresponding wild-type (A14655; C) were induced to sporulate. At the indicated times, cells were harvested and chromosome spreads were assayed for Zip1 staining according to the categories shown in Figure 3A. 100 mononucleate cells were counted per time point.

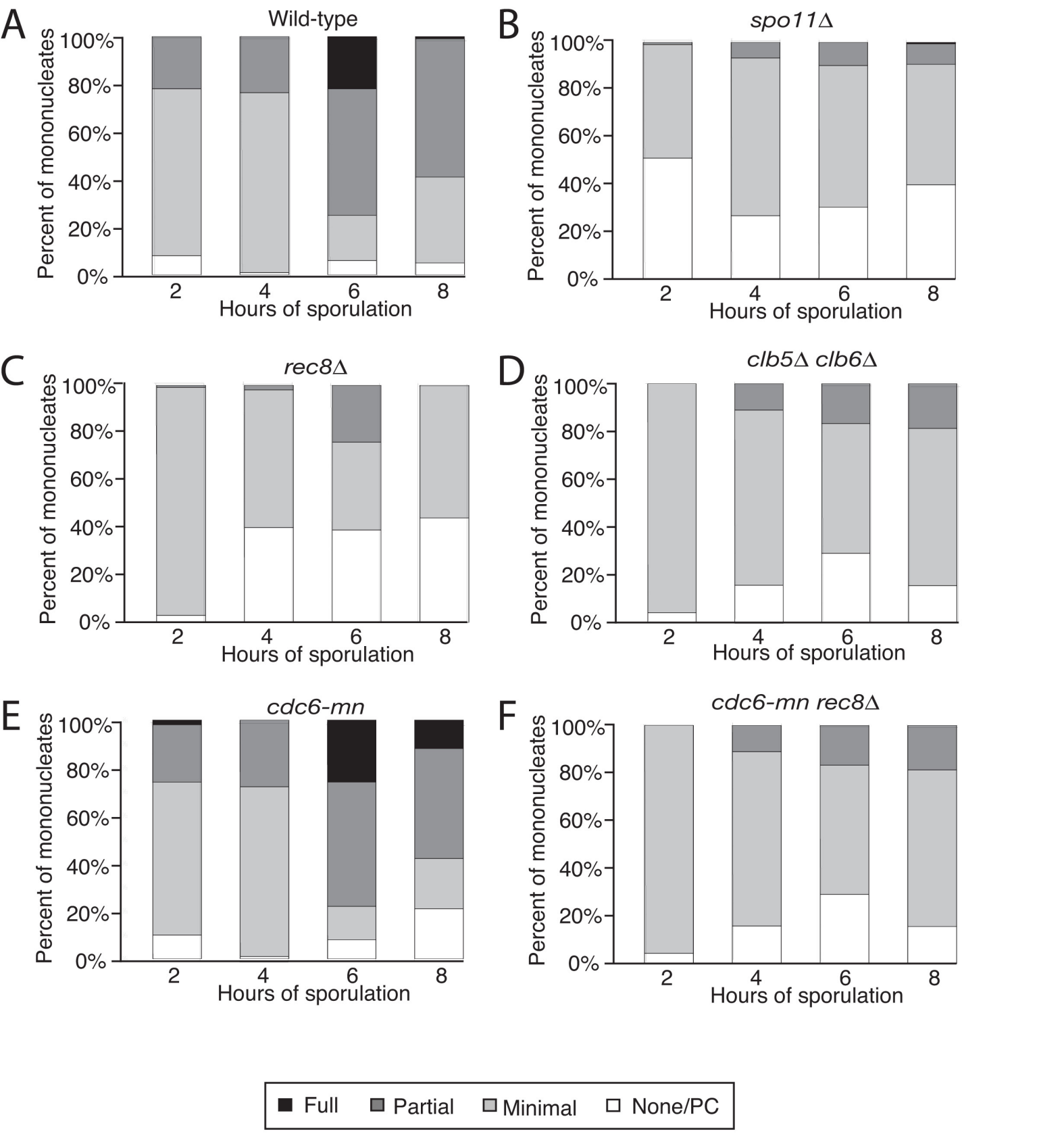
Supplemental Figure 11: The rec8-6D and rec8-6E proteins are not stable.

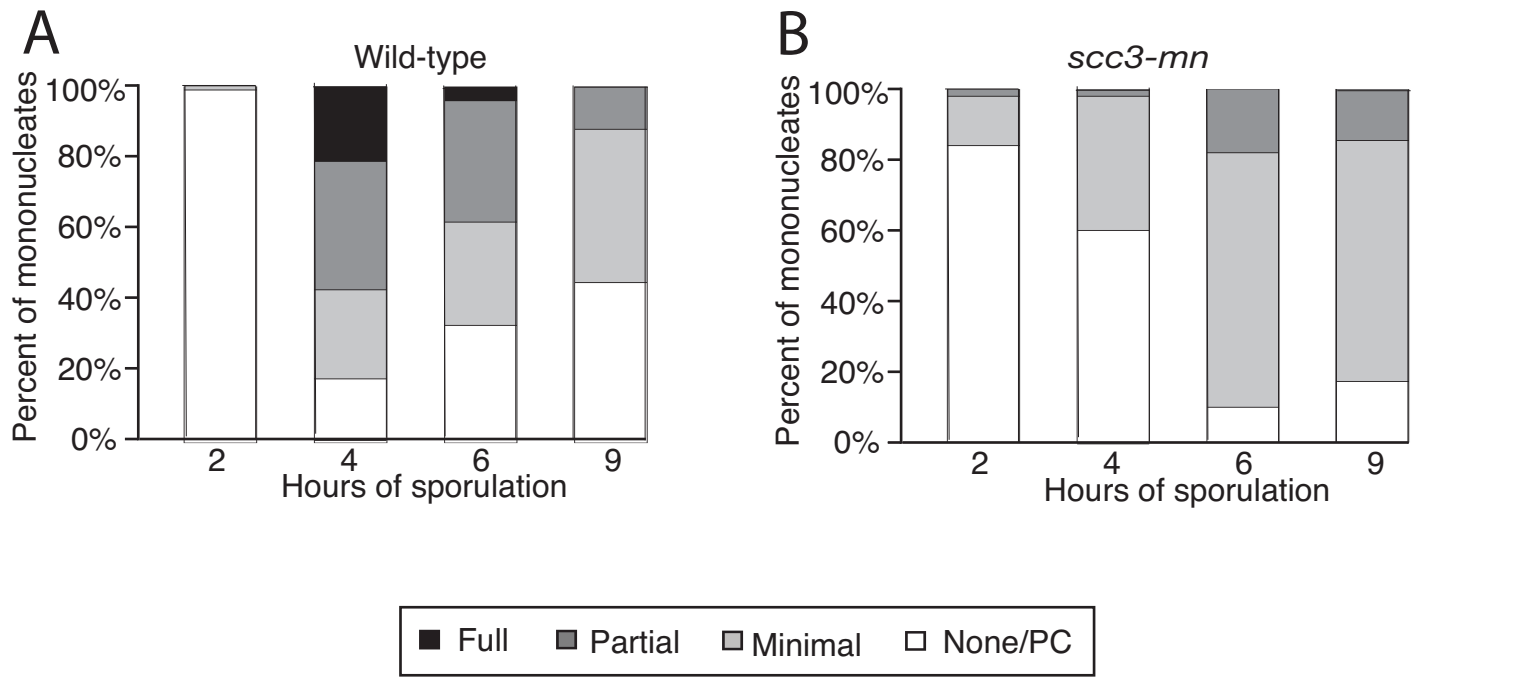
Wild-type (A20066), *rec8-6D* (A20072) and *rec8-6E* (A20075) cells, all carrying HA-tagged versions of Rec8, were induced to sporulate. At 0 hours and 5 hours, cells were collected and Rec8-3HA levels were analyzed by Western blot analysis. α-PGK blotting is included as a loading control.

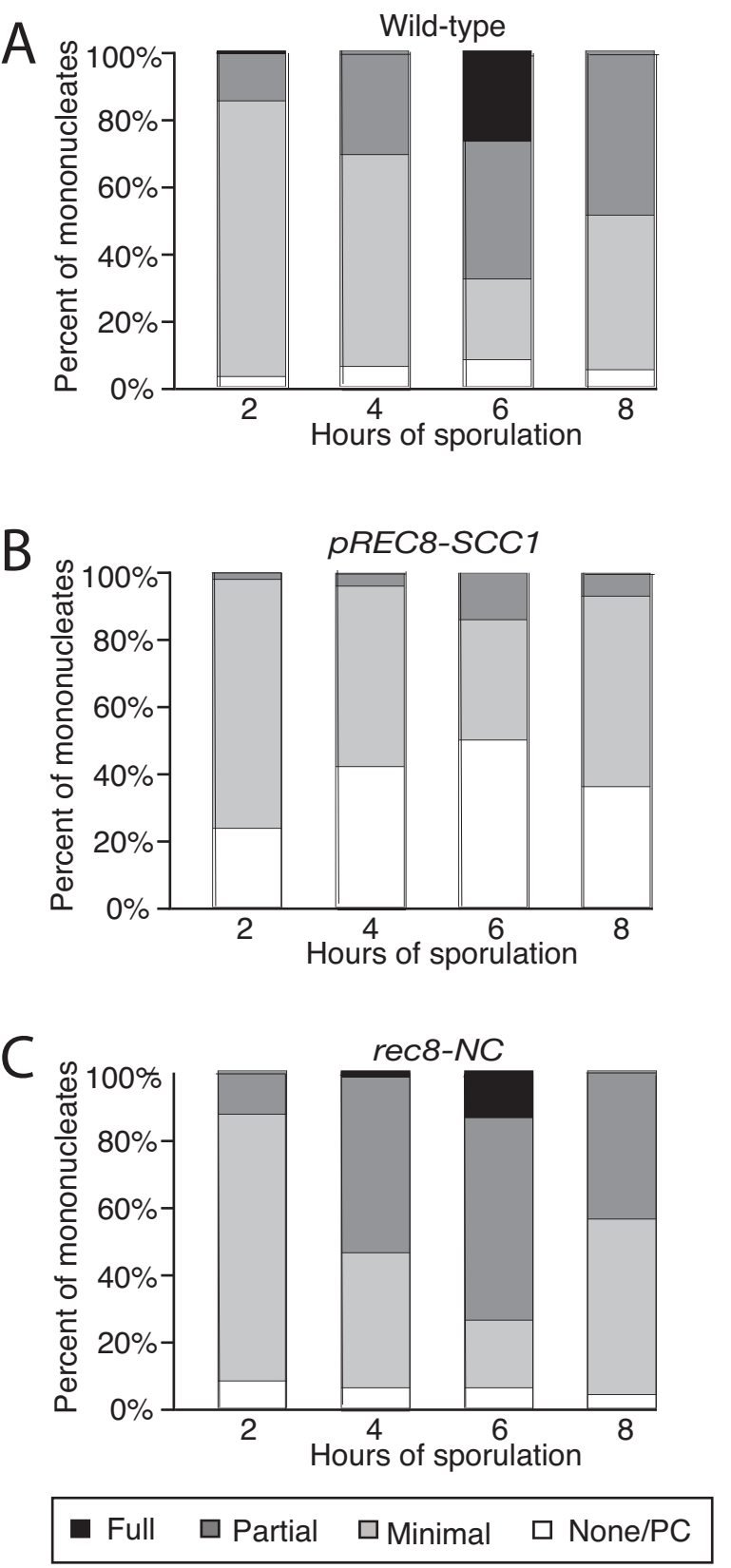

A
LYS2
Percent of paired GFP dots
Hours of sporulation
LYS2 homologous
LYS2/URA3 non-homologous
B
LEU2
LEU2 homologous
LEU2/LYS2 non-homologous

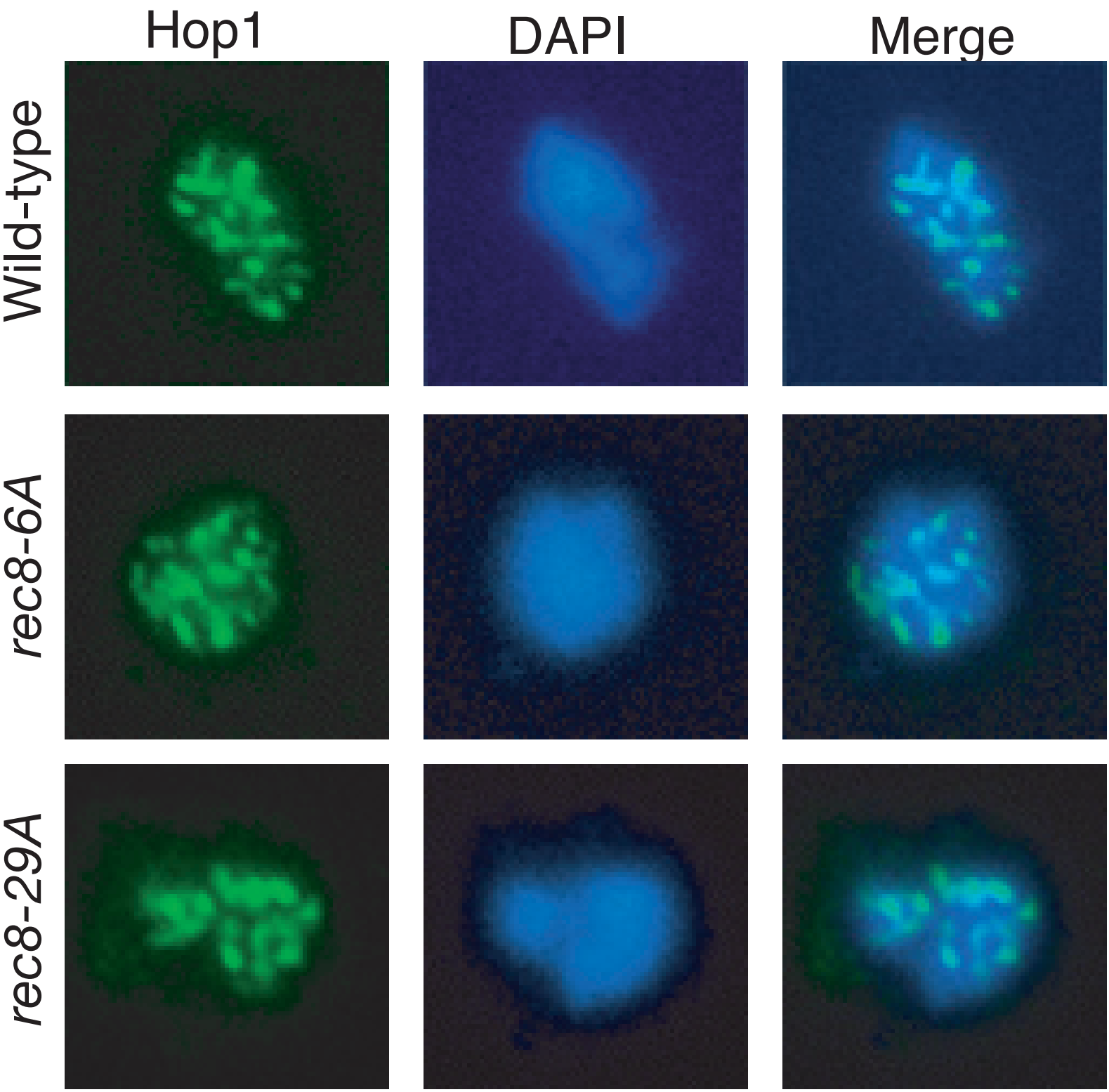

A
Percent of paired GFP dots
Hours of sporulation
100% DSBs
4-12% DSBs
30-60% DSBs
0.6-4% DSBs
<0.1% DSBs
non-hom.
SPO11-HA/ SPO11-HA LEU2 homologous
Wild-type LEU2 homologous
spo11-D290A/spo11-D290A LEU2 homologous
SPO11-HA/spo11-Y135F LEU2 homologous
spo11-Y135F-HA/spo11-Y135F-HA LEU2 homologous
Wild-type LEU2/LYS2 non-homologous
B
Percent of paired GFP dots
Hours of sporulation
100% DSBs
~ 30-60% DSBs
~ 4-12% DSBs
<0.1% DSBs
~0.6-4% DSBs
non-hom.
SPO11-HA/ SPO11-HA LYS2 homologous
Wild-type LEU2 homologous
spo11-D290A/spo11-D290A LYS2 homologous
SPO11-HA/spo11-Y135F LYS2 homologous
spo11-Y135F-HA/spo11-Y135F-HA LYS2 homologous
Wild-type LEU2/LYS2 non-homologous
C
Percent of paired GFP dots
Hours of sporulation
Wild-type LYS2 homologous
clb5Δ clb6Δ LYS2 homologous
Wild-type LYS2/LEU2 non-homologous
D
Wild-type
2C
4C
DNA Content
Hours of sporulation
E
clb5Δ clb6Δ
2C
4C
DNA Content
Hours of sporulation
F
Percent of paired GFP dots
Hours of sporulation
Wild-type LEU2 homologous
clb5Δ clbΔ LEU2 homologous
Wild-type LYS2/LEU2 non-homologous
G
Wild-type
2C
4C
DNA Content
Hours of sporulation
H
clb5Δ clb6Δ
2C
4C
DNA Content
Hours of sporulation
I
Percent of paired GFP dots
Hours of sporulation
Wild-type LYS2 homologous
mer2-S30A LYS2 homologous
Wild-type LYS2/URA3 non-homologous

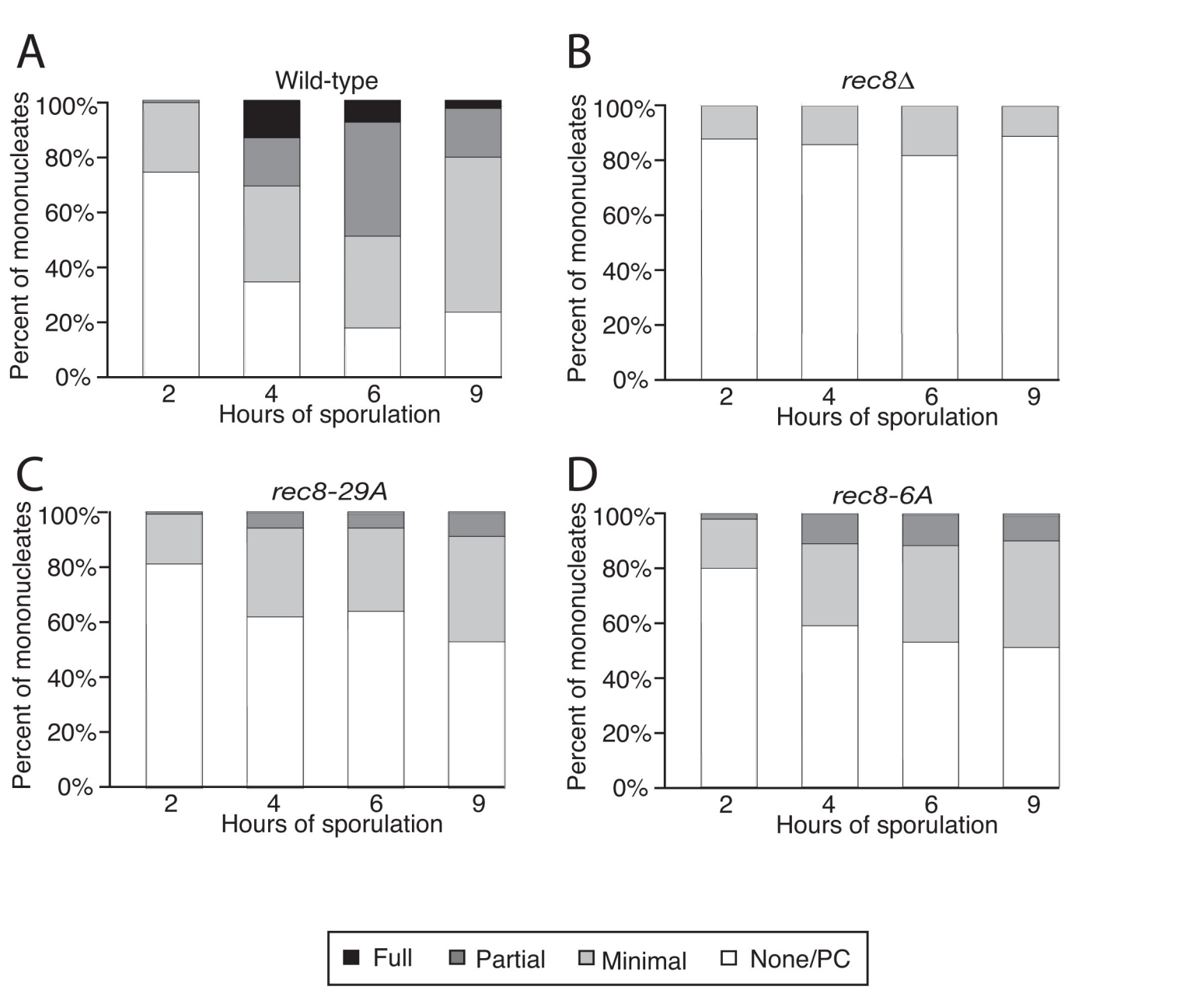

A
Percent of cells with unassembled meiotic spindles
100
80
60
40
20
0
0
2
4
6
8
10
12
Hours of sporulation
Wild-type
spo11Δ
rec8Δ
cdc6-mn
cdc6-mn rec8Δ
clb5Δ clb6Δ
B
Percent of cells with unassembled meiotic spindles
Wild-type
scc3-mn
Hours of sporulation
C
Percent of cells with unassembled meiotic spindles
Wild-type
pREC8-SCC1
rec8-NC
Hours of sporulation

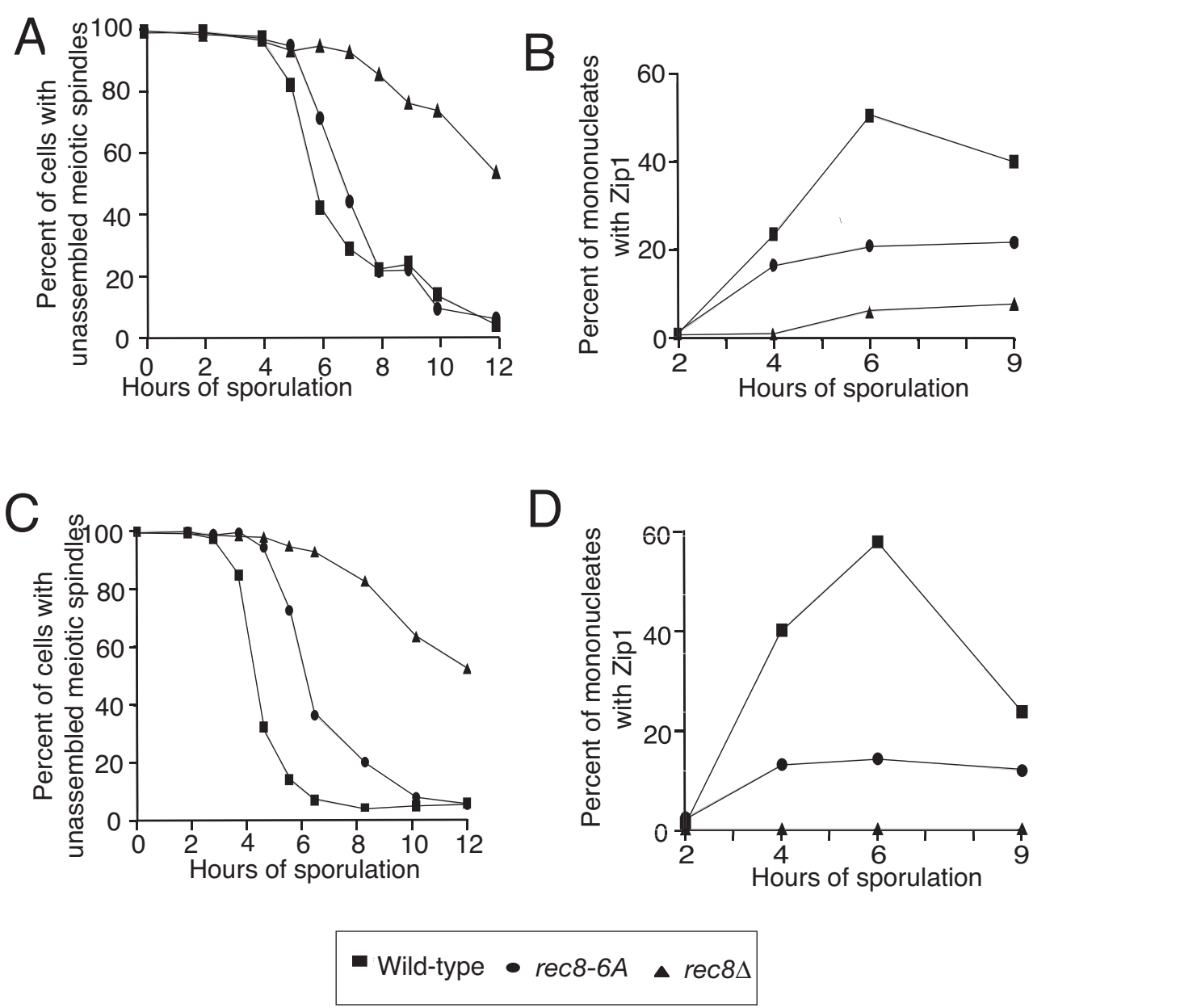

A
Wild-type
B
spo11Δ
C
rec8Δ
D
clb5Δ clb6Δ
E
cdc6-mn
F
cdc6-mn rec8Δ
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
8
Hours of sporulation
Full
Partial
Minimal
None/PC

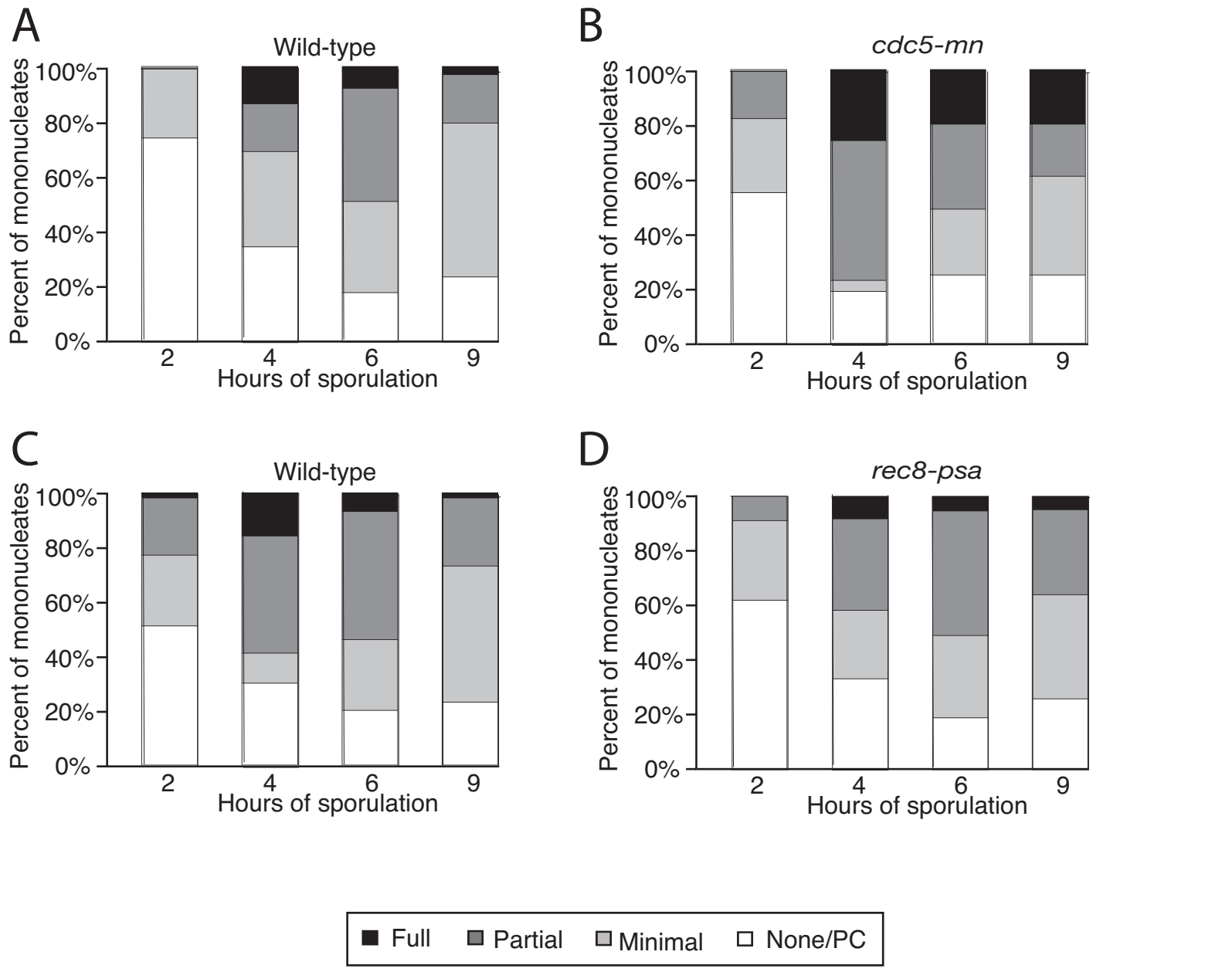

A
Wild-type
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
9
Hours of sporulation
B
scc3-mn
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
9
Hours of sporulation
Full
Partial
Minimal
None/PC

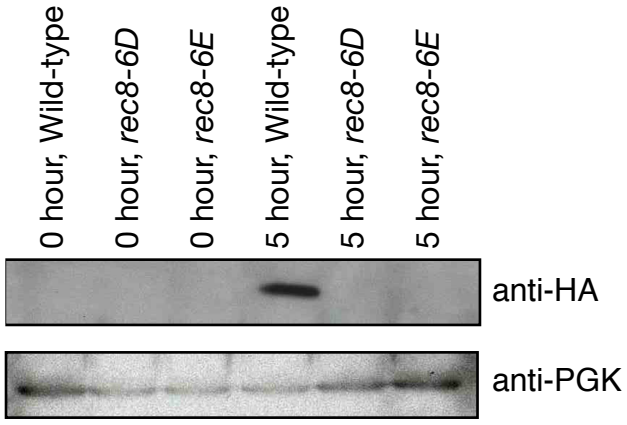

A
Wild-type
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
8
Hours of sporulation
B
pREC8-SCC1
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
8
Hours of sporulation
C
rec8-NC
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
8
Hours of sporulation
Full
Partial
Minimal
None/PC


Hop1
DAPI
Merge
Wild-type
rec8-6A
rec8-29A


A
Wild-type
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
9
Hours of sporulation
B
rec8Δ
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
9
Hours of sporulation
C
rec8-29A
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
9
Hours of sporulation
D
rec8-6A
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
9
Hours of sporulation
Full
Partial
Minimal
None/PC


A
Percent of cells with unassembled meiotic spindles
100
80
60
40
20
0
0 2 4 6 8 10 12
Hours of sporulation
B
Percent of mononucleates with Zip1
60
40
20
0
2 4 6 9
Hours of sporulation
C
Percent of cells with unassembled meiotic spindles
100
80
60
40
20
0
0 2 4 6 8 10 12
Hours of sporulation
D
Percent of mononucleates with Zip1
60
40
20
0
2 4 6 9
Hours of sporulation
Wild-type
rec8-6A
rec8Δ


A
Wild-type
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
9
Hours of sporulation
B
cdc5-mn
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
9
Hours of sporulation
C
Wild-type
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
9
Hours of sporulation
D
rec8-psa
Percent of mononucleates
100%
80%
60%
40%
20%
0%
2
4
6
9
Hours of sporulation
Full
Partial
Minimal
None/PC


0 hour, Wild-type
0 hour, rec8-6D
0 hour, rec8-6E
5 hour, Wild-type
5 hour, rec8-6D
5 hour, rec8-6E
anti-HA
anti-PGK