Expanded View Figures

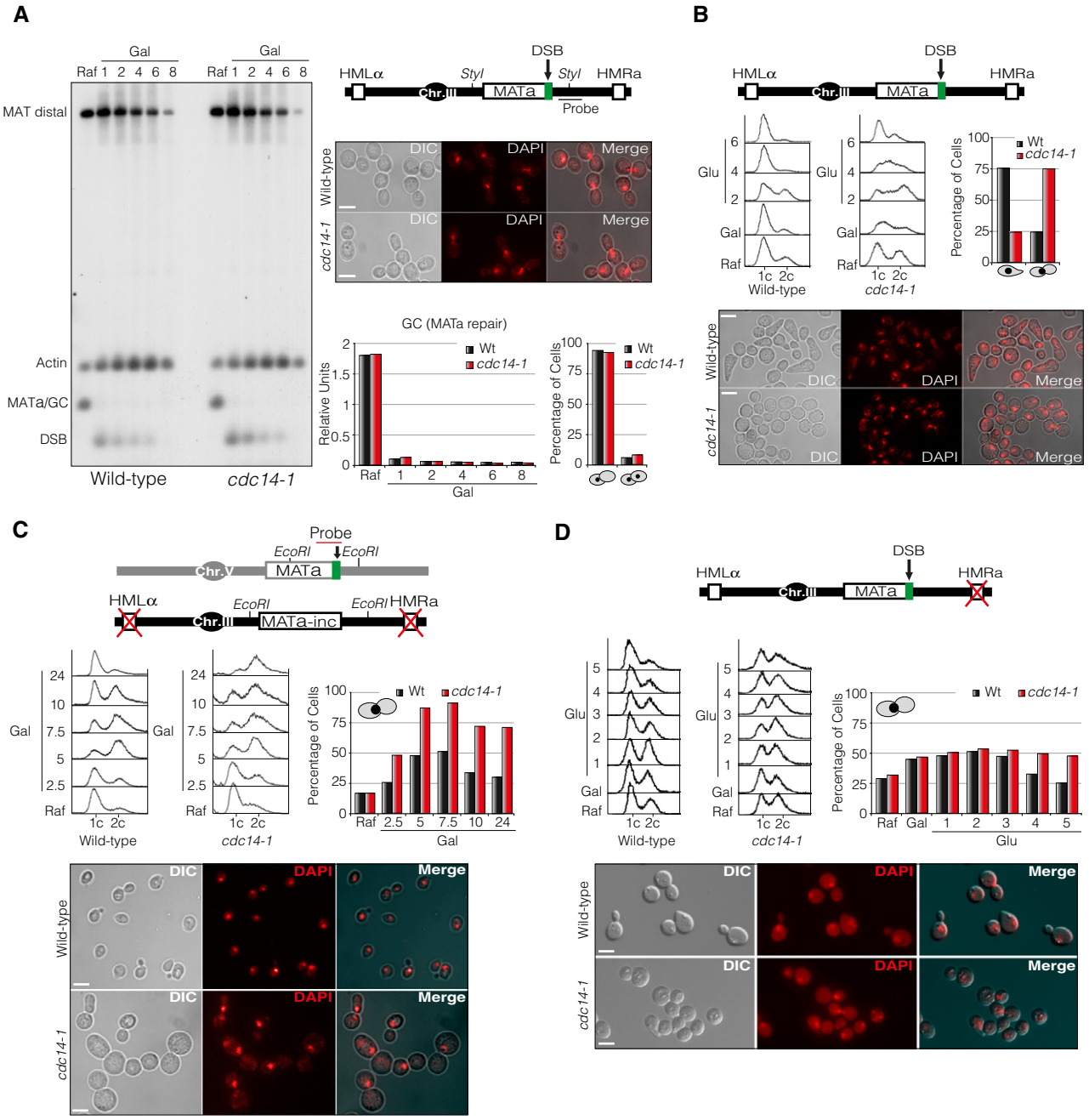

Figure EV1.

Figure EV1. Cdc14 is required for recombinational DNA repair.

A Constitutive expression of HO produces a constant DSB at the *MAT* locus in wild-type and *cdc14-1* cultures at semipermissive temperature. Southern blot of wild-type and *cdc14-1* cells showing the efficiency and stability of the DSB. A diagram with the genomic information, the restriction sites used and the location of the *MAT*-distal probe is shown. Micrographs depict cultures blocked in G2/M 8 h after DSB induction. Scale bar: 5 μm. Graphs show quantification of gene conversion (GC) leading to the re-establishment of *MAT*a and the percentage of cells blocked in G2/M after 8 h from the galactose addition.

B Analysis of mating-type switching through formation of G1 shmoo cells in wild-type and *cdc14-1* cultures at semipermissive temperature. Glucose was added to the cultures after DSB formation to repress HO, thus allowing repair with *HM* donor sequences (top diagram). FACS analysis was performed on cells collected at each time point indicated. Graph shows percentage of cells blocked at G1 (shmoos) or mono-nucleated arrested G2/M (by DAPI staining) 4 h after glucose addition. Micrographs show cultures 4 h after incubation with glucose. Scale bar: 5 μm.

C Wild-type and *cdc14-1* mutant strains carrying the ectopic gene conversion assay depicted were grown overnight and exposed to expression of HO through addition of galactose, thus producing a DSB on chromosome V (top diagram). Samples were taken at 0, 2.5, 5, 7.5, 10 and 24 h. FACS analysis of each time point is shown. Graphs show percentage of mono-nucleated G2/M cells of each sample. Micrographs show cultures 24 h after DSB induction. Scale bar: 5 μm.

D Wild-type and *cdc14-1* mutant strains harbouring the repair pathway choice assay described (top diagram) were grown overnight and exposed to expression of HO through addition of galactose for 1.5 h. Samples were taken before and at 1, 2, 3, 4 and 5 h after supplementing with glucose. FACS analysis of each time point is shown. Quantification of mono-nucleated G2/M arrested cells of wild-type and *cdc14-1* cells during the time course is shown. Micrographs show cultures 5 h after glucose addition. Scale bar: 5 μm.

Data information: DAPI, 4′,6-diamidino-2′-phenylindole dihydrochloride; DSB, double-strand break; Glu, glucose; Raf, raffinose; Gal, galactose; GC, gene conversion.

Figure EV2. Single or multiple DSBs generated outside the *MAT* locus are recruited to the SPBs.

A Cdc14 is released from the nucleolus into the nucleoplasm in response to a single DSB induced by the HO endonuclease. A strain carrying Cdc14-YFP was grown in raffinose before inducing a non-reparable DSB by expressing the HO endonuclease. Cnm67-RFP and Ddc2-CFP were used as SPB and DSB reporters, respectively. Arrow depicts Cdc14 signal at the nucleoplasm. Scale bar: 3 μm.

B Schematic representation of the approach used to analyse DSB recruitment to the nuclear rim. The Ddc2-GFP foci proximity to a circumference connecting both SPBs was calculated by determining the position of the DSB into three concentric zones of equal area. Graphs represent the percentage of Ddc2 foci falling into each zone. Values represent the mean ± SD of three independent experiments. At least 100 cells per experiment were scored. *P*-values were calculated using a two-tailed unpaired Student's *t*-test.

C Diagram depicting the genomic background used to induce a single DSB outside the *MAT* locus. Spc110-RFP and Ddc2-GFP were used as SPB and DSB markers, respectively. D1: inter-SPB distance; D2: DSB distance to proximal SPB; D3: DSB distance to distal SPB. Graph represents the mean ± SD of D1, D2 and D3 distances of at least 100 cells scored using the maximum projection of three *z*-planes. A representative picture is shown. Scale bar: 3 μm.

D Diagram showing the genomic background used to induce multiple DSBs. Spc110-RFP and Ddc2-GFP were used as SPB and DSB markers, respectively. D1: inter-SPB distance; D2: DSB distance to proximal SPB; D3: DSB distance to distal SPB. Graph represents the mean ± SD of D1, D2 and D3 distances of at least 100 cells scored using the maximum projection of three *z*-planes. A representative picture is shown. Scale bar: 3 μm.

E Diagram illustrating the workflow used to analyse DSB-SPB recruitment of DNA lesions induced by phleomycin treatment. Phleomycin was added to a final concentration of 1 μM. Spc110-RFP and Ddc2-GFP were used as SPB and DSB reporters, respectively. D1: inter-SPB distance; D2: DSB distance to proximal SPB; D3: DSB distance to distal SPB. Graph represents the mean ± SD of D1, D2 and D3 distances of at least 100 cells scored using the maximum projection of seven *z*-planes. A representative picture is shown. Scale bar: 3 μm.

F Rad52 foci are recruited to the SPBs in response to a non-reparable DSB generated by expressing the HO endonuclease. Rad52-GFP and Spc110-RFP were used as DSB and SPB reporters, respectively. Graph represents the mean ± SD of D1, D2 and D3 distances of at least 100 cells scored using the maximum projection of nine *z*-planes. Scale bar: 3 μm.

Data information: DSB, double-strand break; SPB, spindle pole body.

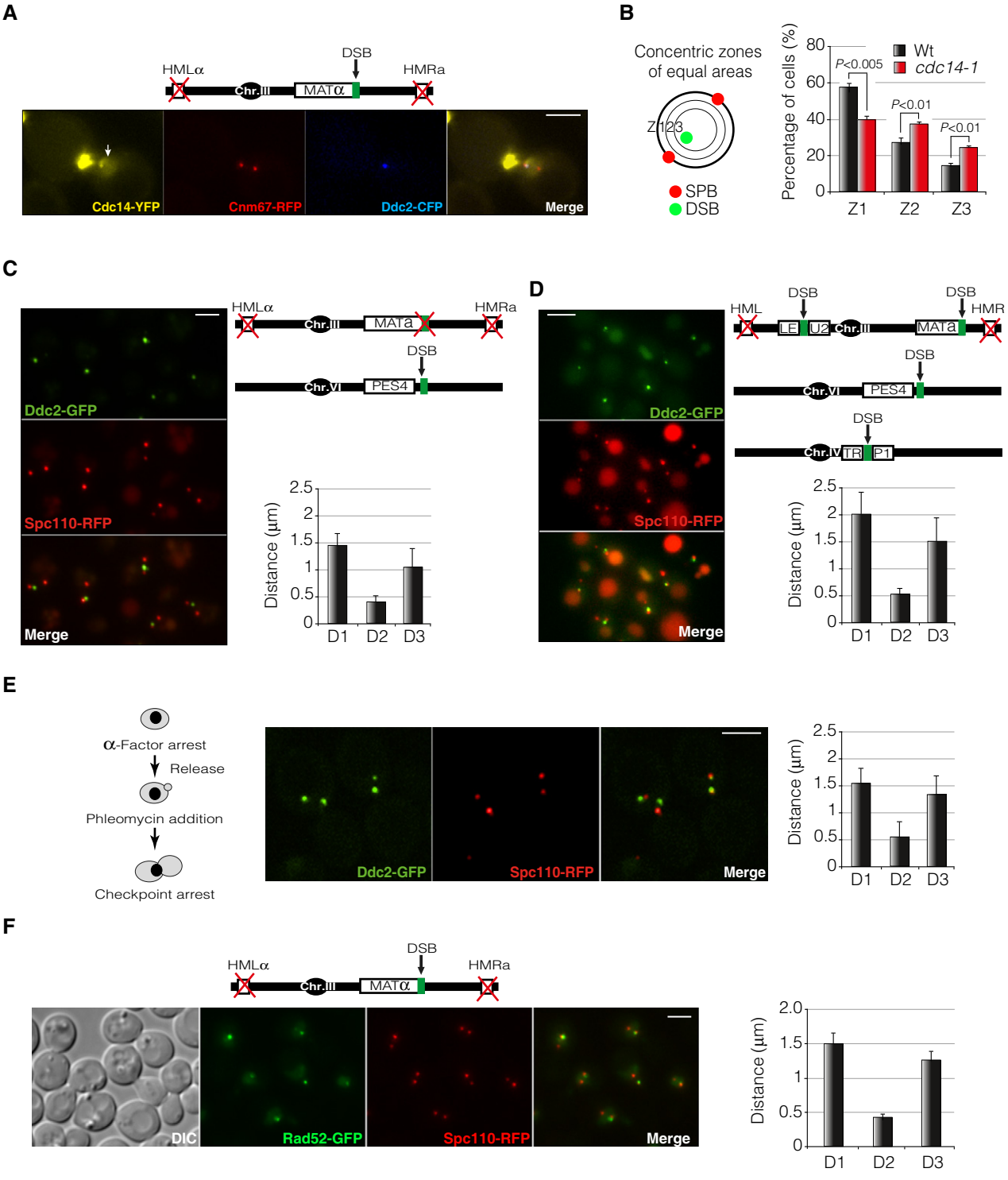

Figure EV2.

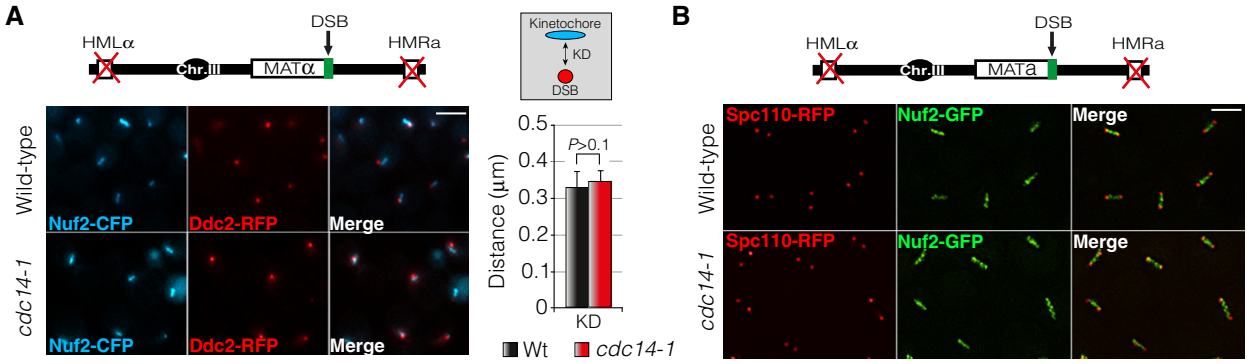

Figure EV3. Chromatin compaction or kinetochore closeness to SPBs is not affected in cells lacking Cdc14 activity in response to a DSB.

A Chromatin compaction during the DDR is not affected in the absence of Cdc14. A single DSB was generated by expressing the HO endonuclease in both wild-type and *cdc14-1* mutant cells. Nuf2-CFP and Ddc2-RFP were used as kinetochore and DSB markers, respectively. Micrographs were generated by using the maximum projection of nine *z*-planes images. Scale bar: 3 μm. The kinetochore–DSB distance in both wild-type and *cdc14-1* backgrounds was measured and plotted. Graph depicts the mean ± SD of three replicates of at least 100 cells each. *P*-value was calculated using a two-tailed unpaired Student's *t*-test.

B Cdc14 is not required to promote DSB-SPB interaction by generating pulling forces that move kinetochores regions close to the SPBs during the DNA damage response. A single DSB was produced by inducing the HO endonuclease expression in both wild-type and *cdc14-1* mutant cells. Spc110-RFP and Nuf2-GFP were used to visualize the SPBs and the kinetochore, respectively. Micrographs were created by using the maximum projection of nine *z*-planes images. Scale bar: 3 μm.

Data information: KD, kinetochore distance; DSB, double-strand break.

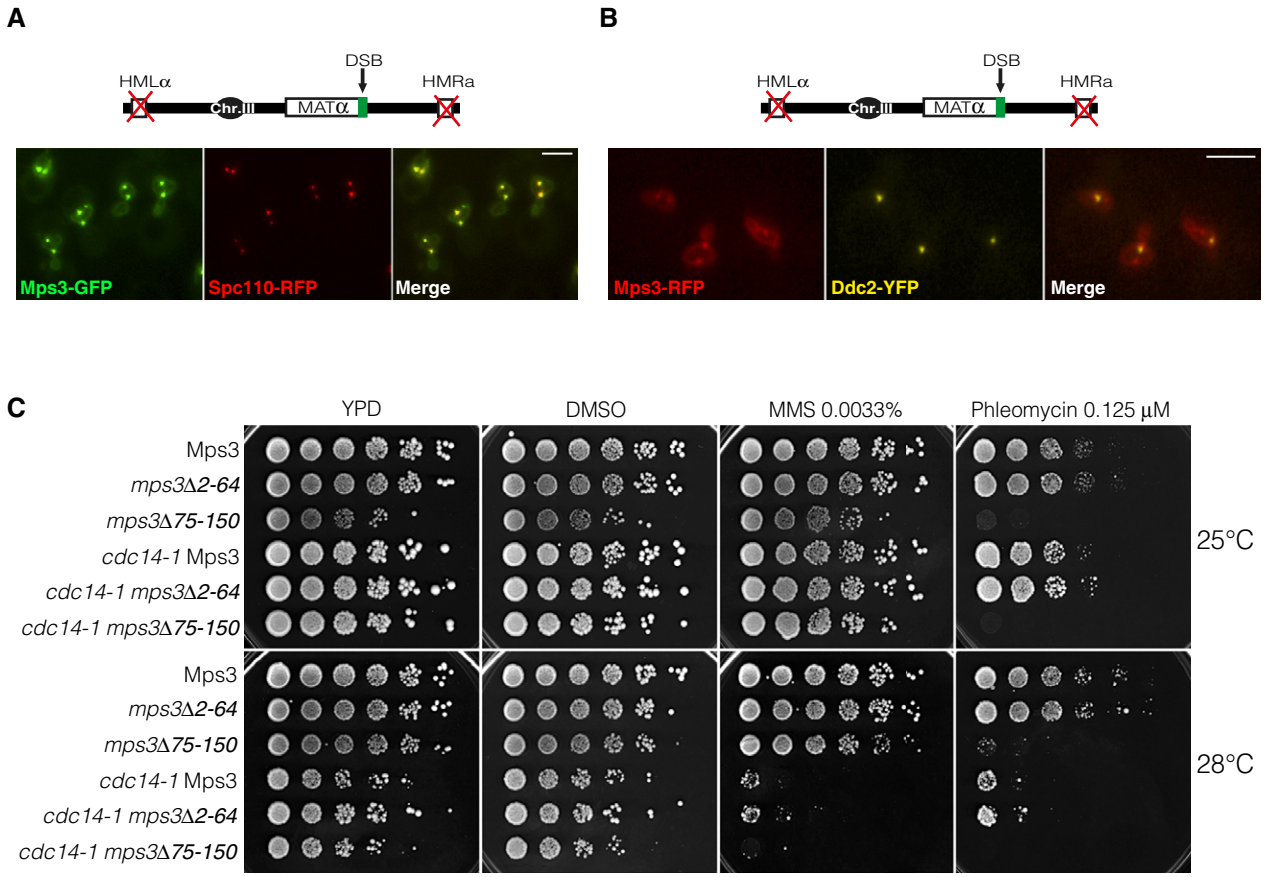

Figure EV4. DSBs are preferentially recruited to Mps3 loaded onto the SPBs.

A Mps3 localizes to the nuclear membrane and SPBs in response to a single DSB. A non-reparable DSB was induced in cells expressing Mps3-GFP. Spc110-RFP was used as a SPB reporter. Scale bar: 3 μm.

B DSBs are preferentially recruited to Mps3 bound to the SPBs. A non-reparable DSB was induced in cells harbouring Mps3-RFP. Ddc2-YFP was used as a DSB reporter. Scale bar: 3 μm.

C Elimination of the amino acids 75–150 of the N-terminus domain of Mps3 sensitizes cells to phleomycin. Tenfold serial dilutions from overnight cultures of cells expressing truncated versions of the N-terminus domain of Mps3 in wild-type and *cdc14-1* mutants. Cells were dropped and grown on solid rich media or media containing DMSO (mock), MMS or Phleomycin at 25 or 28°C.

Data information: DSB, double-strand break; DMSO, dimethyl sulphoxide; MMS, methyl methanesulphonate.

Figure EV5. Low Spc110 activity results in high SPB dynamics, loss of DSB-SPB interaction and inefficient DSB repair by homologous recombination.

A D1, D2 and D3 distances measured in wild-type and *spc110-220* cells after inducing a non-reparable DSB at the *MAT* locus. Cnm67-CFP and Ddc2-RFP were used as SPB and DSB markers, respectively. Graph represents the mean ± SD of D1, D2 and D3 distances of three independent experiments. At least 100 cells per experiment were scored using the maximum projection of three *z*-planes. *P*-values were calculated using a two-tailed unpaired Student's *t*-test. A diagram with the genome information and a representative picture are shown. Scale bar: 3 μm.

B Time-lapse experiments in living cells were carried out after expressing the HO endonuclease in wild-type and *spc110-220* cells. Three *z*-planes every 10-s intervals over a period of 5 min were captured and used to quantify the average SPB track length and velocity using Spc110-RFP as SPB marker. At least 100 cells were scored.

C Physical analysis of wild-type and *spc110-220* mutant strains carrying the inter-chromosomal gene conversion assay depicted (top diagram). Cells were grown overnight and exposed to HO expression by adding galactose at 32°C, thus producing a DSB on chromosome V. Samples were taken at different time points, genomic DNA was extracted, digested with *EcoRI* and analysed by Southern blot. Blots were hybridized with a *MAT*a-only and *ACT1* (loading control) DNA sequences. FACS profiles for DNA content are included. Graphs represent quantification of gene conversion, DSB induction and crossover versus non-crossover ratio. All data were normalized with the actin control. Graphs show the mean ± SD from three independent experiments. *P*-values were calculated using a two-tailed unpaired Student's *t*-test. Asterisk denotes an overexposed film to visualize crossover formation. Data information: DSB, double-strand break; SPB, spindle pole body; Raf, raffinose; Gal, galactose; GC, gene conversion; CO, crossover.

Source data are available online for this figure.

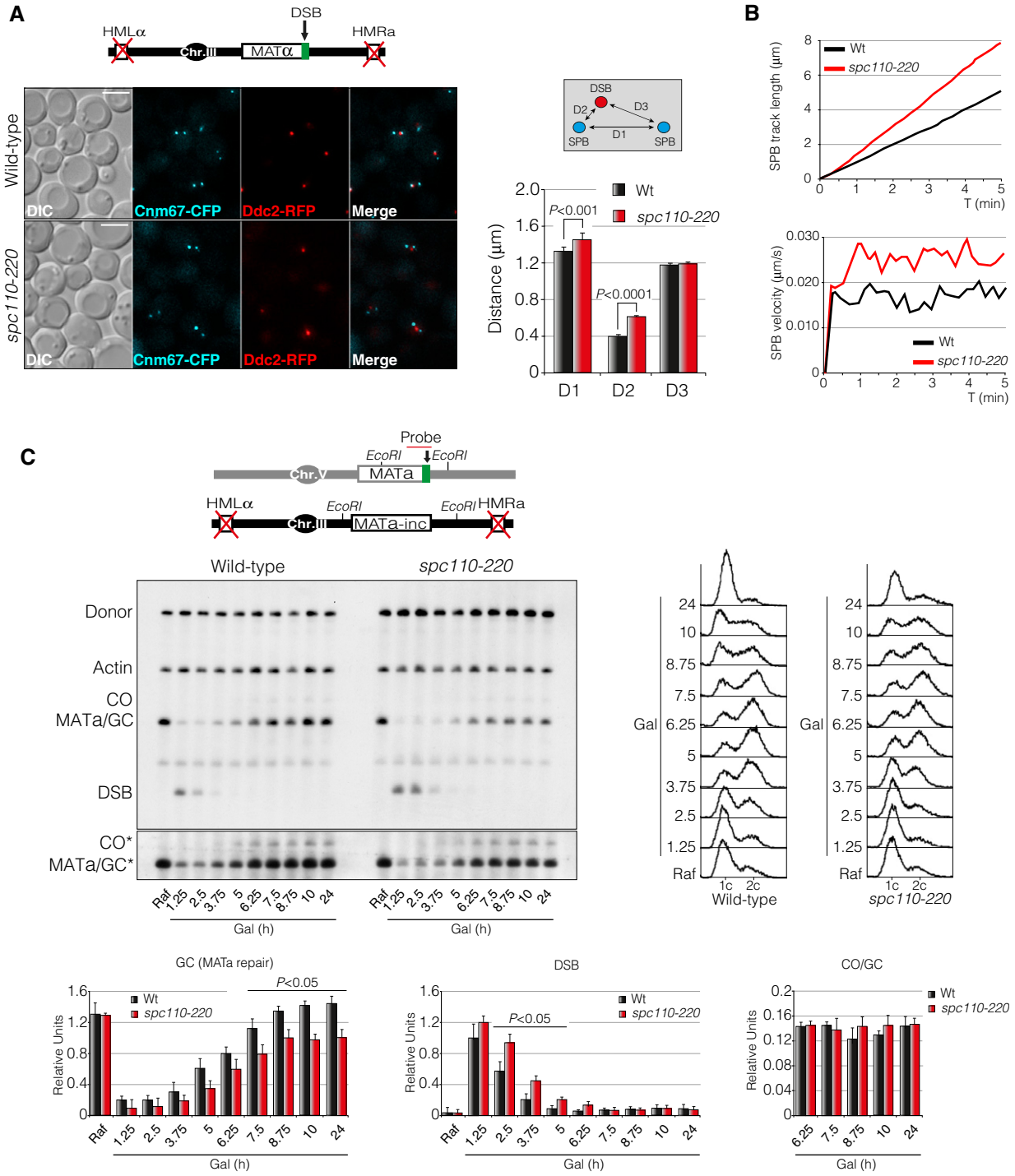
A
HMLα
DSB
HMRa
Chr.III
MATα
Wild-type
spc110-220
DIC
Cnm67-CFP
Ddc2-RFP
Merge
DSB
D2
D3
D1
SPB
SPB
Distance (μm)
Wt
spc110-220
P<0.001
P<0.0001
D1
D2
D3
B
SPB track length (μm)
T (min)
SPB velocity (μm/s)
C
Probe
EcoRI
Chr.V
MATa
HMLα
MATa-inc
HMRa
Donor
Actin
CO
MATa/GC
DSB
CO*
MATa/GC*
Gal (h)
Gal
Raf
1c
2c
GC (MATa repair)
DSB
CO/GC
Relative Units
P<0.05

Figure EV5.