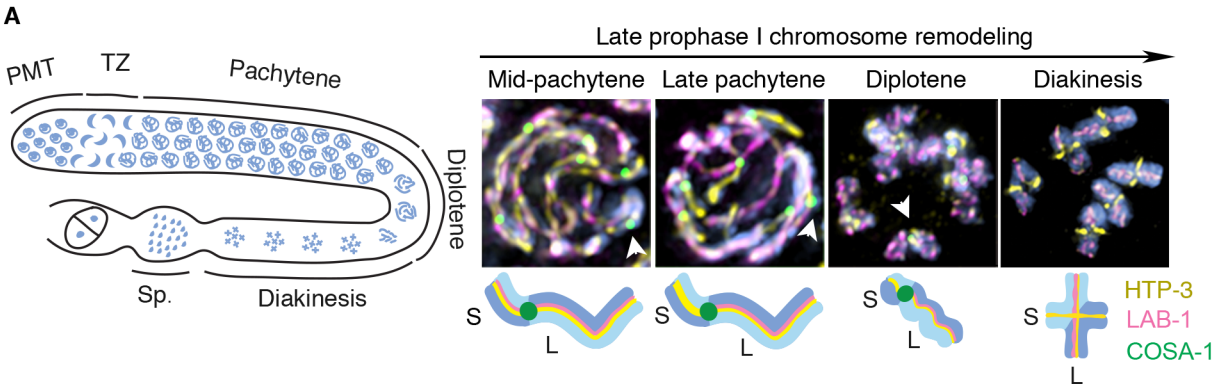

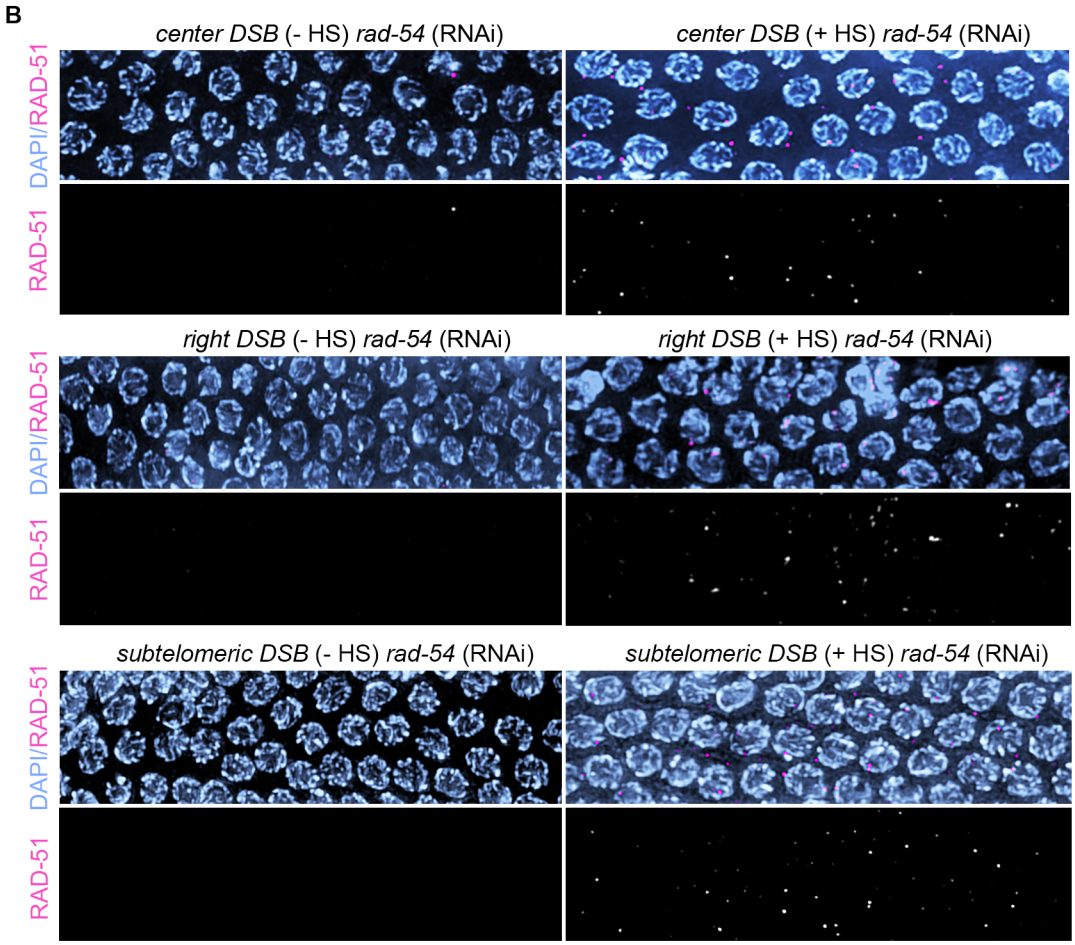

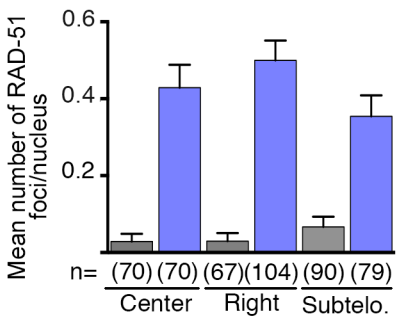

Comparison	P value
Center III DSB (-HS) vs Center III DSB (+HS)	<0.0001 (****)
Right III DSB (-HS) vs Right III DSB (+HS)	<0.0001 (****)
Subtelo. III DSB (-HS) vs Subtelo. III DSB (+HS)	<0.0001 (****)
Center III DSB (+HS) vs Right III DSB (+HS)	0.3556 (n.s.)
Center III DSB (+HS) vs Subtelo. III DSB (+HS)	0.4250 (n.s.)
Right III DSB (+HS) vs Subtelo. III DSB (+HS)	0.0644 (n.s.)

Figure S1. Genomic *Mos1* transposon insertion sites and assessment of DSB and bivalent formation post heat shock-induced *Mos1* excision. Related to Figure 1.

(A) Illustration of a *C. elegans* gonad and immunofluorescence images of individual germline nuclei undergoing chromosome remodeling. Nuclei at the premeiotic tip (PMT) are undergoing mitotic proliferation before entering meiosis at transition zone (TZ; leptotene/zygotene stages), followed by pachytene, diplotene and diakinesis. Sp indicates the spermatheca where oocytes are fertilized. Immunofluorescence images showing the progression of chromosome remodeling in wild-type nuclei from mid-pachytene through diakinesis. Illustrations in the bottom depict a single bivalent for simplicity. At mid-pachytene, CO precursor sites are marked by COSA-1 (green; 1 focus per chromosome; 6 foci per nucleus), and LAB-1 (magenta) fully co-localizes with the chromosome axes marker HTP-3 (yellow) along the length of the chromosomes. In late pachytene, LAB-1 localization is restricted to the long arm of the chromosomes (L), which is separated by the CO site (marked by the COSA-1 focus, white arrow) from the short arm (S). During diplotene, chromosomes undergo condensation, and at diakinesis, 6 cruciform-shaped bivalents with LAB-1 only on the long arm (L) are visible. **(B)** DSBs are generated by heat shock-induced *Mos1* excision and marked by the DNA repair protein RAD-51 (magenta). Images show germline nuclei at pachytene in animals subjected to *rad54*(RNAi) and carrying a *Mos1* insertion at the center, off-center (right arm) and subtelomeric position of chromosome *III* either without (left; -HS) or with heat shock (right, +HS). Histogram at the bottom shows the mean number of RAD-51 foci observed in mid-pachytene nuclei, control= -HS, n= number of nuclei scored. Only RAD-51 foci overlapping with DAPI were quantified. Error bars represent SEM. Table indicates statistical analysis results for the indicated pairwise comparisons by the two-tailed Mann-Whitney test, 95% C.I.

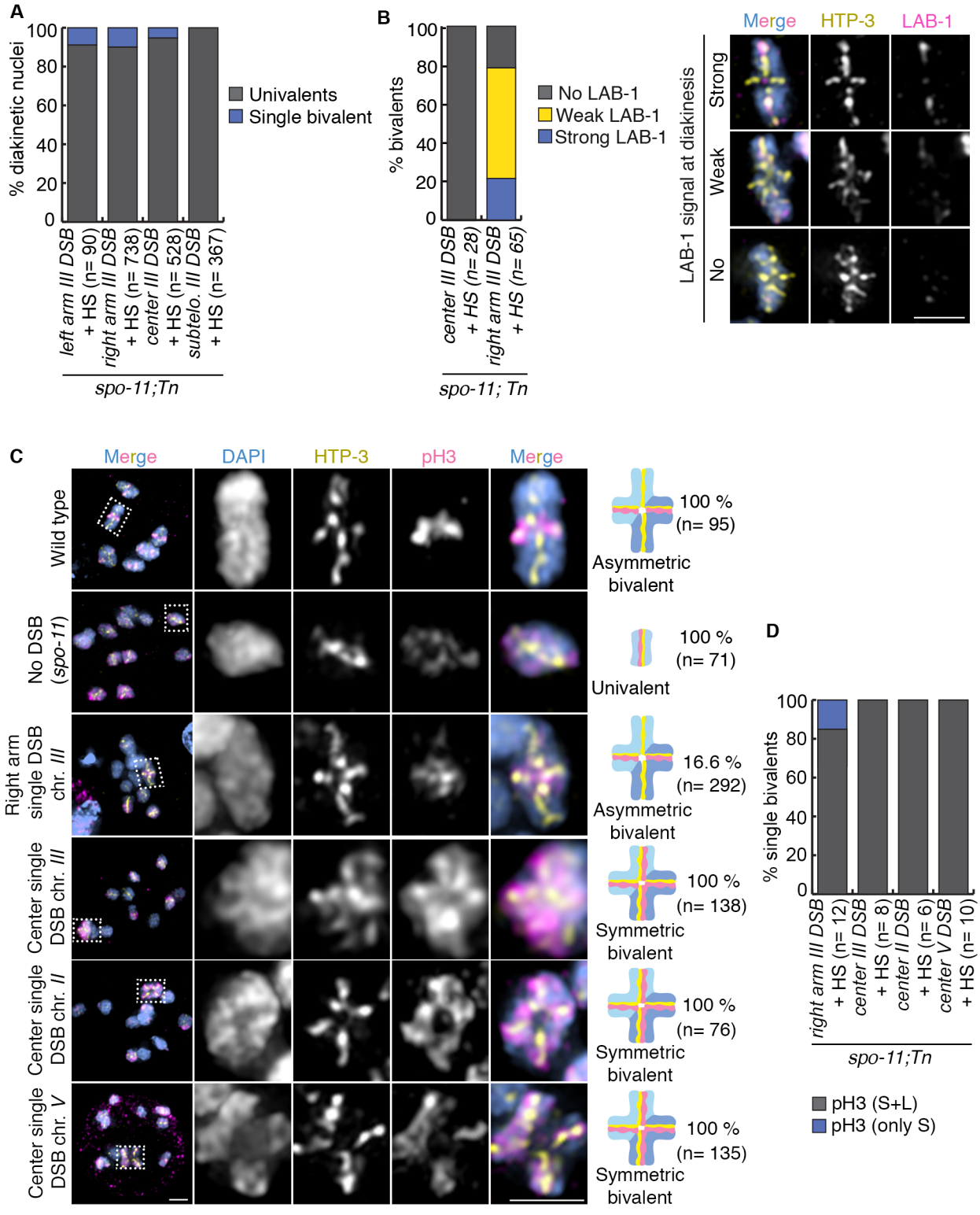
A
% diakinetic nuclei
100
80
60
40
20
0
Univalents
Single bivalent
left arm III DSB + HS (n= 90)
right arm III DSB + HS (n= 738)
center III DSB + HS (n= 528)
subtelo. III DSB + HS (n= 367)
spo-11;Tn
B
% bivalents
No LAB-1
Weak LAB-1
Strong LAB-1
center III DSB + HS (n= 28)
right arm III DSB + HS (n= 65)
spo-11; Tn
Merge
HTP-3
LAB-1
LAB-1 signal at diakinesis
Strong
Weak
No
C
Merge
DAPI
HTP-3
pH3
Merge
Wild type
No DSB (spo-11)
Right arm single DSB chr. III
Center single DSB chr. III
Center single DSB chr. II
Center single DSB chr. V
100 % (n= 95)
Asymmetric bivalent
100 % (n= 71)
Univalent
16.6 % (n= 292)
Asymmetric bivalent
100 % (n= 138)
Symmetric bivalent
100 % (n= 76)
Symmetric bivalent
100 % (n= 135)
Symmetric bivalent
D
% single bivalents
right arm III DSB + HS (n= 12)
center III DSB + HS (n= 8)
center II DSB + HS (n= 6)
center V DSB + HS (n= 10)
spo-11;Tn
pH3 (S+L)
pH3 (only S)

Figure S2: Chromosome remodeling and phosphorylation of Histone H3 on the short arms of the chromosomes are affected by the position of the crossover. Related to Figure 1.
(A) Quantification of bivalent formation after heat shock induced *Mos1* excision at the indicated positions on chromosome *III*. n= number of diakinesis nuclei scored. **(B)** Quantification and immunofluorescence images of the LAB-1 localization detected on bivalents following *Mos1* excision from the indicated positions on chromosome *III*. Both weak and strong signal was observed for LAB-1 as indicated. n = number of bivalents scored. **(C)** Immunofluorescence images of phosphorylated histone H3 (pH3; magenta) and HTP-3 (yellow) localization on DAPI-stained chromosomes (blue) in oocytes at diakinesis. Dashed boxes indicate the bivalents shown at higher magnification. n= number of diakinesis nuclei scored. Bar, 2 μm. **(D)** Quantification of the localization pattern of pH3 on the observed bivalents. n = number of bivalents scored.

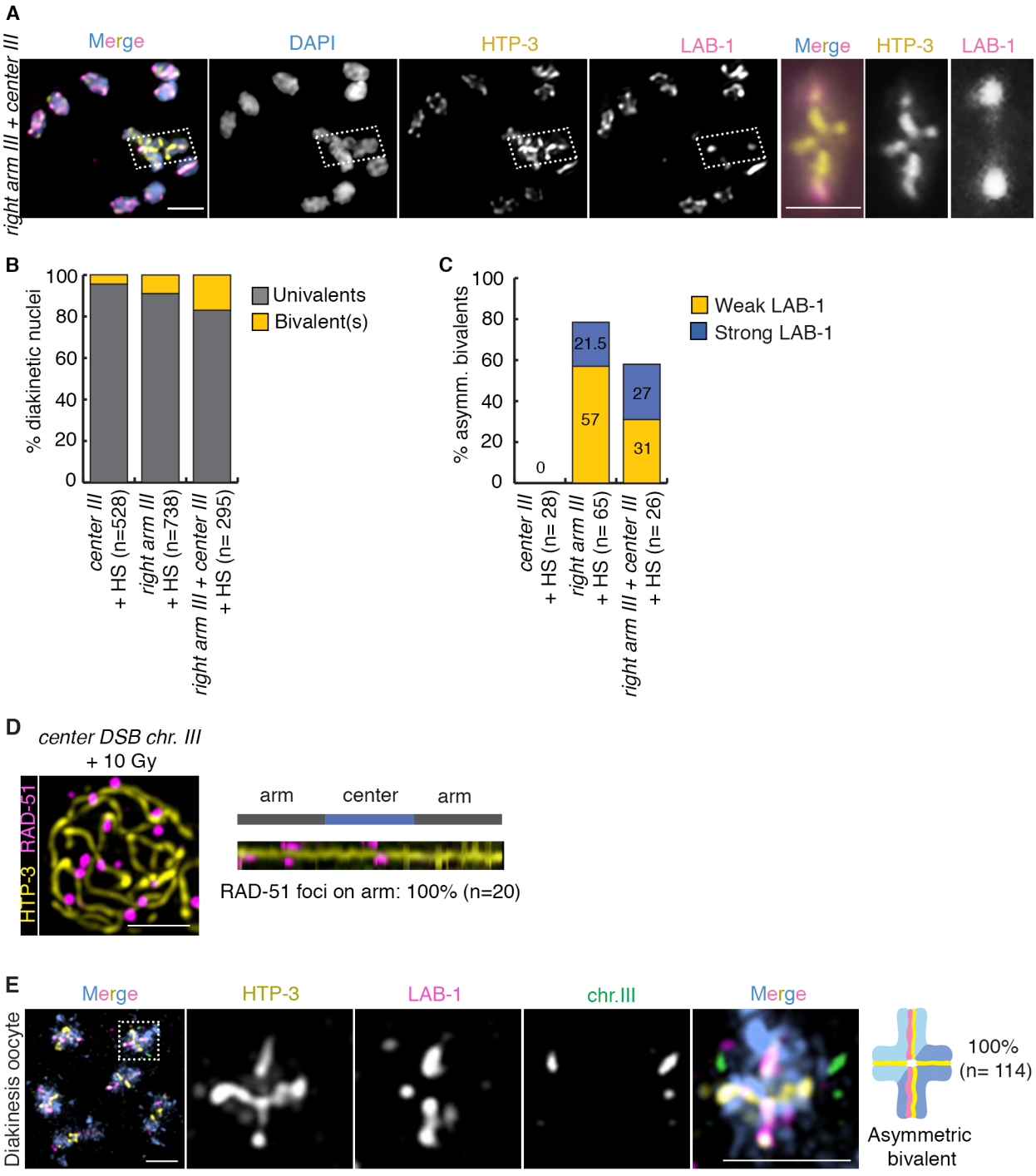
A
right arm III + center III
Merge
DAPI
HTP-3
LAB-1
Merge
HTP-3
LAB-1
B
% diakinetic nuclei
100
80
60
40
20
0
Univalents
Bivalent(s)
center III + HS (n=528)
right arm III + HS (n=738)
right arm III + center III + HS (n= 295)
C
% asymm. bivalents
100
80
60
40
20
0
Weak LAB-1
Strong LAB-1
0
21.5
57
27
31
center III + HS (n= 28)
right arm III + HS (n= 65)
right arm III + center III + HS (n= 26)
D
center DSB chr. III
+ 10 Gy
HTP-3 RAD-51
arm
center
arm
RAD-51 foci on arm: 100% (n=20)
E
Diakinesis oocyte
Merge
HTP-3
LAB-1
chr.III
Merge
100%
(n= 114)
Asymmetric bivalent

Figure S3: Bivalent formation and LAB-1 localization in worms carrying two *Mos1* sites and after exposure to exogenous DSBs. **Related to Figure 3**.

(A) Immunostaining of chromosomes in diakinetic oocytes of worms harboring two *Mos1* transposon insertion sites, one at the center and another at the right arm of chromosome *III*. Dashed box shows the asymmetric bivalent depicted at higher magnification on the right with LAB-1 (magenta) on the long arm and HTP-3 (yellow) in both axes. High-magnification image has not been deconvolved (processed) to show the LAB-1 signal extends along the long arm but is lost during image processing due to the stronger signal at the ends. Bar, 2 μm. **(B)** Quantification of bivalent frequency upon heat shock. n= number of diakinesis nuclei scored. **(C)** Quantification of both weak and strong signal observed for LAB-1 on asymmetric bivalents following *Mos1* excision from the indicated positions on chromosome *III*. Numbers in columns indicate observed percentages. n = number of bivalents scored. **(D)** Representative image of a pachytene nucleus exposed to IR (10 Gy) after heat shock-induced DSB formation at the center of chromosome *III*. Chromosome axes are marked with HTP-3 (yellow) and sites undergoing DSB repair following DSB formation are marked with RAD-51 (magenta). A computationally straightened chromosome is shown horizontally on the right. Chromosome axis length was divided into thirds to assess RAD-51 foci formation at chromosome arms (see schematic drawing). n value reflects the number of nuclei from which chromosomes have been computationally straightened. Bar, 2 μm. **(E)** Diakinesis nuclei exposed to 10 Gy after heat shock induced DSB formation at chromosome *III* center. HTP-3 (yellow), LAB-1 (magenta) and chromosome *III* FISH probe (green). Bar, 2 μm.

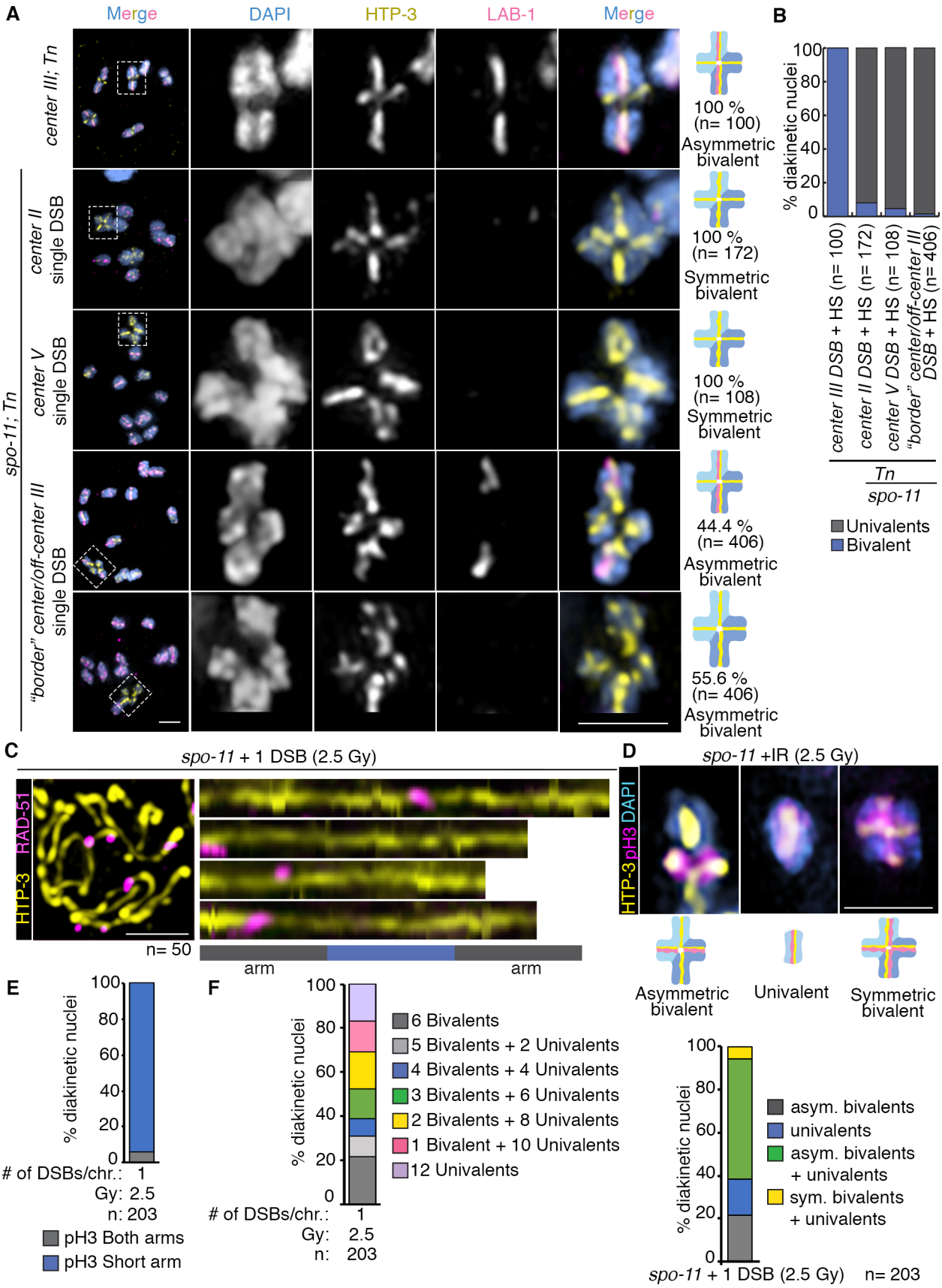
A
Merge
DAPI
HTP-3
LAB-1
Merge
spo-11; Tn
center III; Tn
center II single DSB
center V single DSB
"border" center/off-center III single DSB
100 % (n= 100) Asymmetric bivalent
100 % (n= 172) Symmetric bivalent
100 % (n= 108) Symmetric bivalent
44.4 % (n= 406) Asymmetric bivalent
55.6 % (n= 406) Asymmetric bivalent
B
% diakinetic nuclei
100
80
60
40
20
0
center III DSB + HS (n= 100)
center II DSB + HS (n= 172)
center V DSB + HS (n= 108)
"border" center/off-center III DSB + HS (n= 406)
Tn
spo-11
Univalents
Bivalent
C
spo-11 + 1 DSB (2.5 Gy)
HTP-3 RAD-51
n= 50
arm
arm
D
spo-11 +IR (2.5 Gy)
HTP-3 pH3 DAPI
Asymmetric bivalent
Univalent
Symmetric bivalent
E
% diakinetic nuclei
100
80
60
40
20
0
# of DSBs/chr.: 1
Gy: 2.5
n: 203
pH3 Both arms
pH3 Short arm
F
% diakinetic nuclei
100
80
60
40
20
0
# of DSBs/chr.: 1
Gy: 2.5
n: 203
6 Bivalents
5 Bivalents + 2 Univalents
4 Bivalents + 4 Univalents
3 Bivalents + 6 Univalents
2 Bivalents + 8 Univalents
1 Bivalent + 10 Univalents
12 Univalents
% diakinetic nuclei
100
80
60
40
20
0
asym. bivalents
univalents
asym. bivalents + univalents
sym. bivalents + univalents
spo-11 + 1 DSB (2.5 Gy) n= 203

Figure S4: Chromosome remodeling defects following a centered DSB/CO are a global feature of autosomes and pH3 localization after induction of DSB by γ-IR. Related to Figure 4.
(A) Immunofluorescence images of diakinesis nuclei from the indicated genotypes. Dashed boxes show the DAPI-stained bodies depicted at higher magnification on the right accompanied by illustrations of the merge images. Top row shows the control line harboring the *Mos1* transposon at the center of chromosome *III* and the transposase (Tn) in a *spo-11*+ background, with LAB-1 restricted only to the long arm of the bivalent. The following two rows depict the lack of LAB-1 localization observed for single bivalents obtained by heat shock-induced *Mos1* excision at the center of chromosomes *II* and *V*. The two bottom rows show that when a DSB is induced at a position close to the border between the center region and the arms (defined based on the genetic map) on chromosome *III*, two types of bivalents were observed: either carrying the restricted localization of LAB-1 along the long arms, as in wild type, or lacking LAB-1, as observed when the DSB is induced in the physical middle of the chromosome. HTP-3 (yellow), LAB-1 (magenta) and DAPI-stained chromosomes (blue). n= number of diakinesis oocytes scored. Bar, 2 μm. **(B)** Histogram showing the percentage of oocytes at diakinesis observed carrying a bivalent after heat shock-induced *Mos1* excision at the center of chromosomes *III*, *II* and *V* and at the "border" between center/off-center regions of chromosome *III*. **(C)** Representative pachytene nucleus of *spo-11* mutant worm exposed to IR (2.5 Gy) inducing ~1 DSB per homologous chromosome pair. Computationally straightened chromosomes are displayed on the right. Chromosome axis is shown in yellow (HTP-3) and RAD-51 in magenta. n value indicates the number of nuclei from which chromosomes were straightened. Bar, 2 μm. **(D)** Representative DAPI-stained bodies (blue) showing the localization of HTP-3 (yellow) and pH3 (magenta) observed in diakinesis nuclei of *spo-11* mutants subjected to a γ-IR dose (2.5 Gy) producing 1 DSB per chromosome pair. Illustrations are shown below the immunofluorescence images. Bar, 2 μm. Histogram depicting the categories of bivalent/univalent configurations observed at diakinesis after exposure to indicated γ-IR dose. n= number of diakinesis nuclei examined. **(E)** Quantification of pH3 localization in diakinesis nuclei from *spo-11* mutants following exposure to 2.5 Gy. **(F)** Histogram showing quantification of the observed number of bivalents and/or univalents at 2.5 Gy.