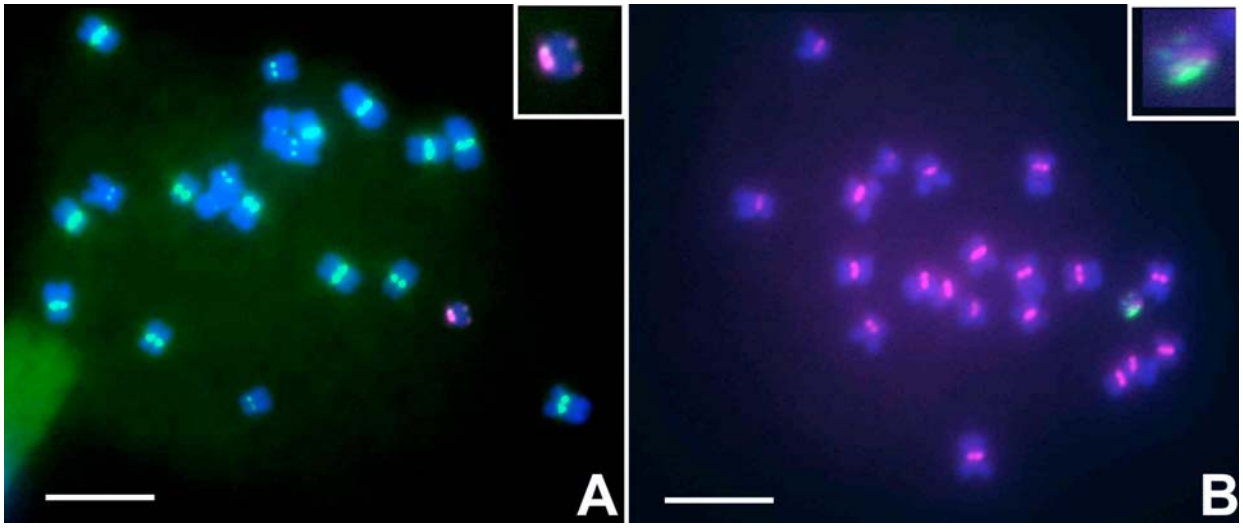
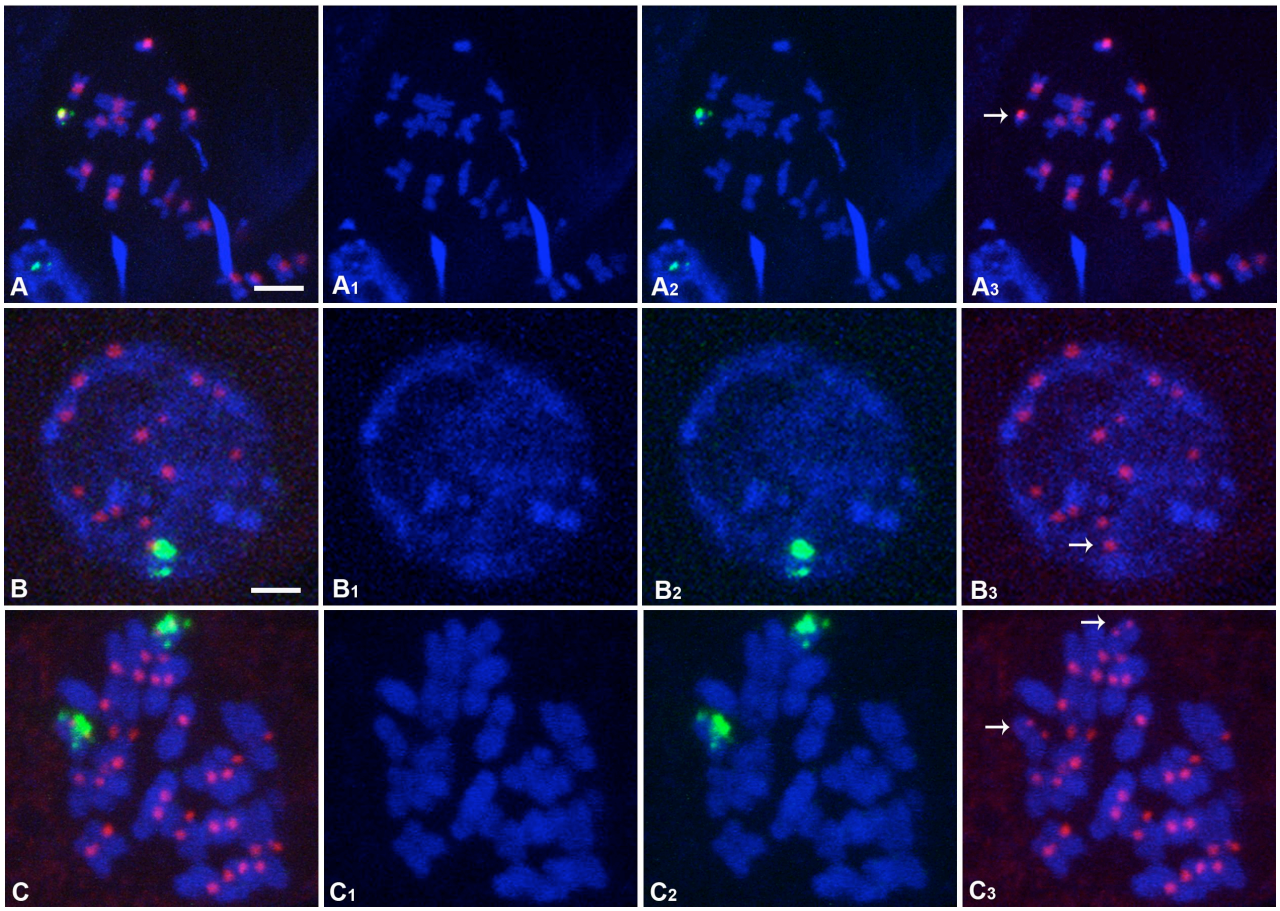
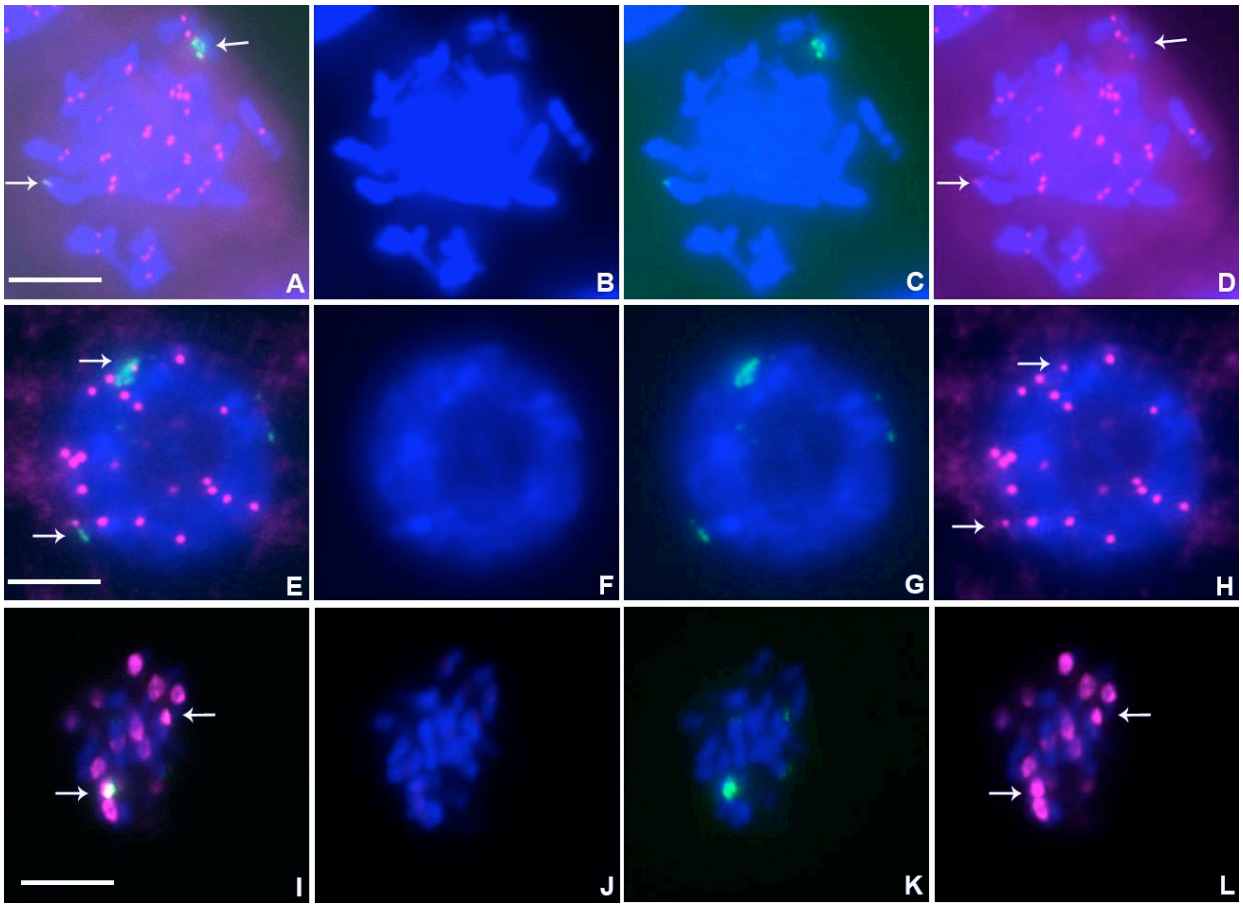
Supplemental Data, Han et al., (2009) Reactivation of an inactive centromere reveals epigenetic and structural components for centromere specification in maize.



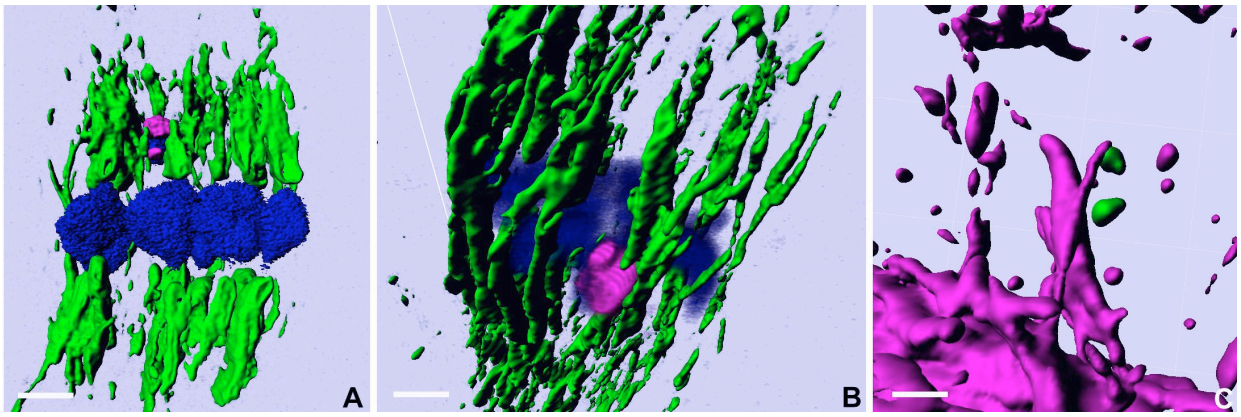
Supplemental Figure 1. Somatic chromosome spread of Dic-15. **(A)** ZmBs is magenta; CentC is green. **(B)** Magenta is CRM and ZmBs is green. Insets at the upper right show the enlarged Dic-15 chromosome. Bar =10 um.



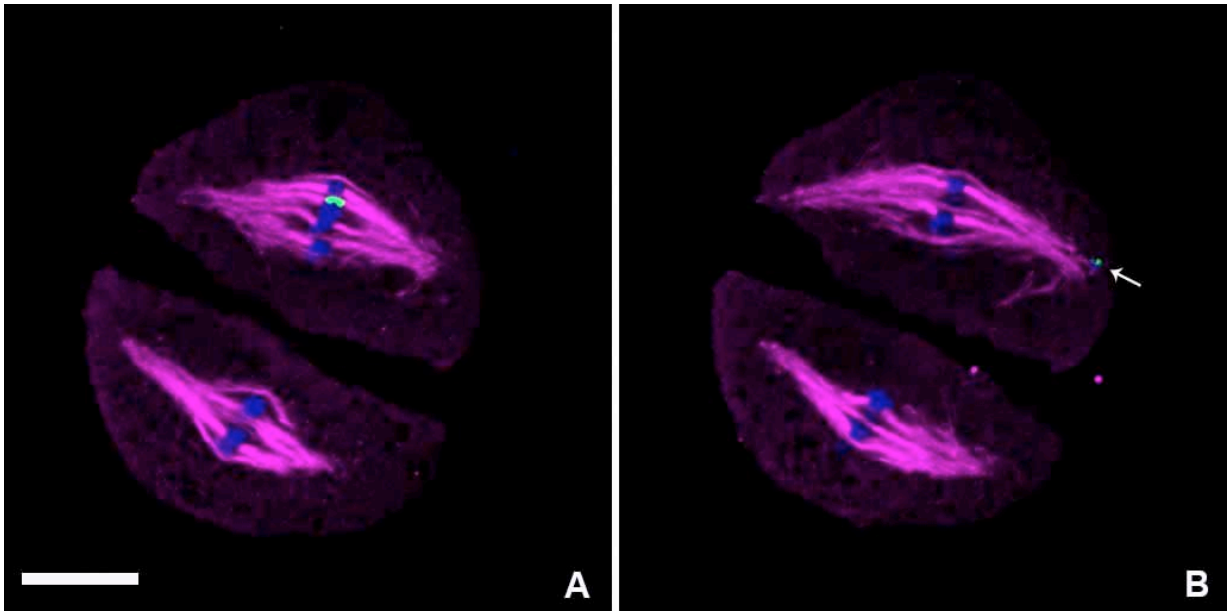
Supplemental Figure 2. Immunolocalization analysis of Dic-15. **(A)** Phosphorylation of histone H3 at Ser-10 (a feature of active centromeres) on a somatic chromosome spread is shown in magenta; ZmBs is green. **(B)** Centromeric histone CENH3 signal is shown in magenta; ZmBs is green. The small B centromere does not label with antibodies to CENH3 (arrow). **(C)** Two copies of Dic-15 chromosomes; CENP-C signals are shown in magenta and ZmBs is green. The small centromeres do not label with CENP-C (arrow). **(A1, B1, C1)** DAPI. **(A2, B2, C2)** ZmBs. (**A3**) H3-Ser10P. (**B3**) CENH3. (**C3**) CENP-C. Bar =10 um.



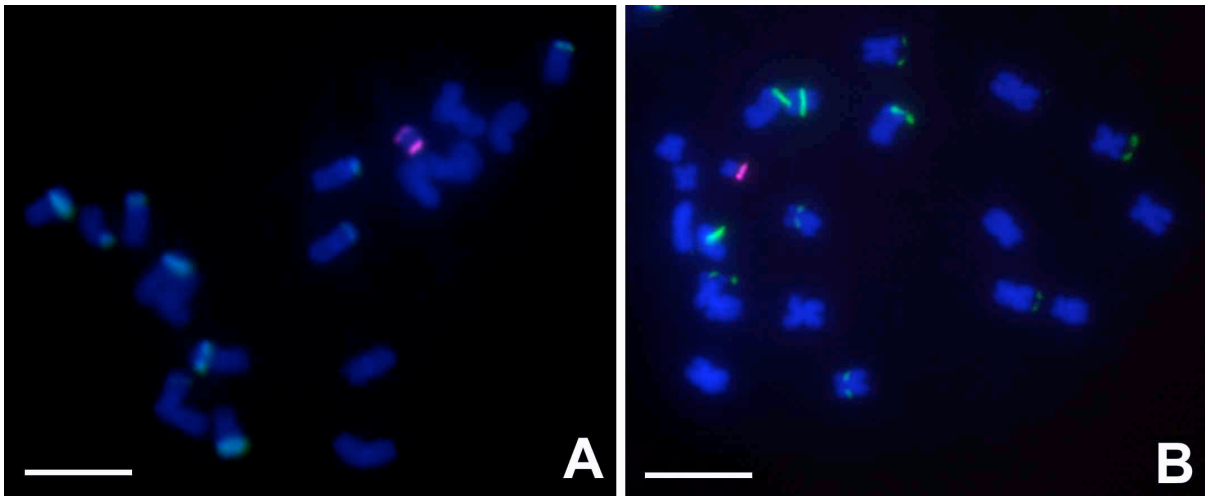
Supplemental Figure 3. Immunocolocalization analysis of a heterozygous plant containing TB-9Sb and Telo 3-5(+). (**A**) CENPC. CENP-C signal is magenta; ZmBs is green; DAPI is blue. Arrows indicate the two chromosomes containing different sized B centromeres. (**B**) DAPI. (**C**) ZmBs. (**D**) CENP-C. Arrows indicate signals on both active centromeres of different size. (**E**) CENH3. CENH3 signal is magenta; ZmBs is green; DAPI is blue. Arrows indicate the two types of B centromeres. (**F**) DAPI. (**G**) ZmBs. (**H**) CENH3. Arrows show signal on both centromeres. (**I**) Phosphorylation of histone H3 at Ser-10. H3Ser10P is magenta; ZmBs is green; DAPI is blue. Arrows indicate the two chromosomes. (**J**) DAPI. (**K**) ZmBs. (**L**) H3Ser10P. Arrows indicate labeling of both centromeres. Bar =10 um.



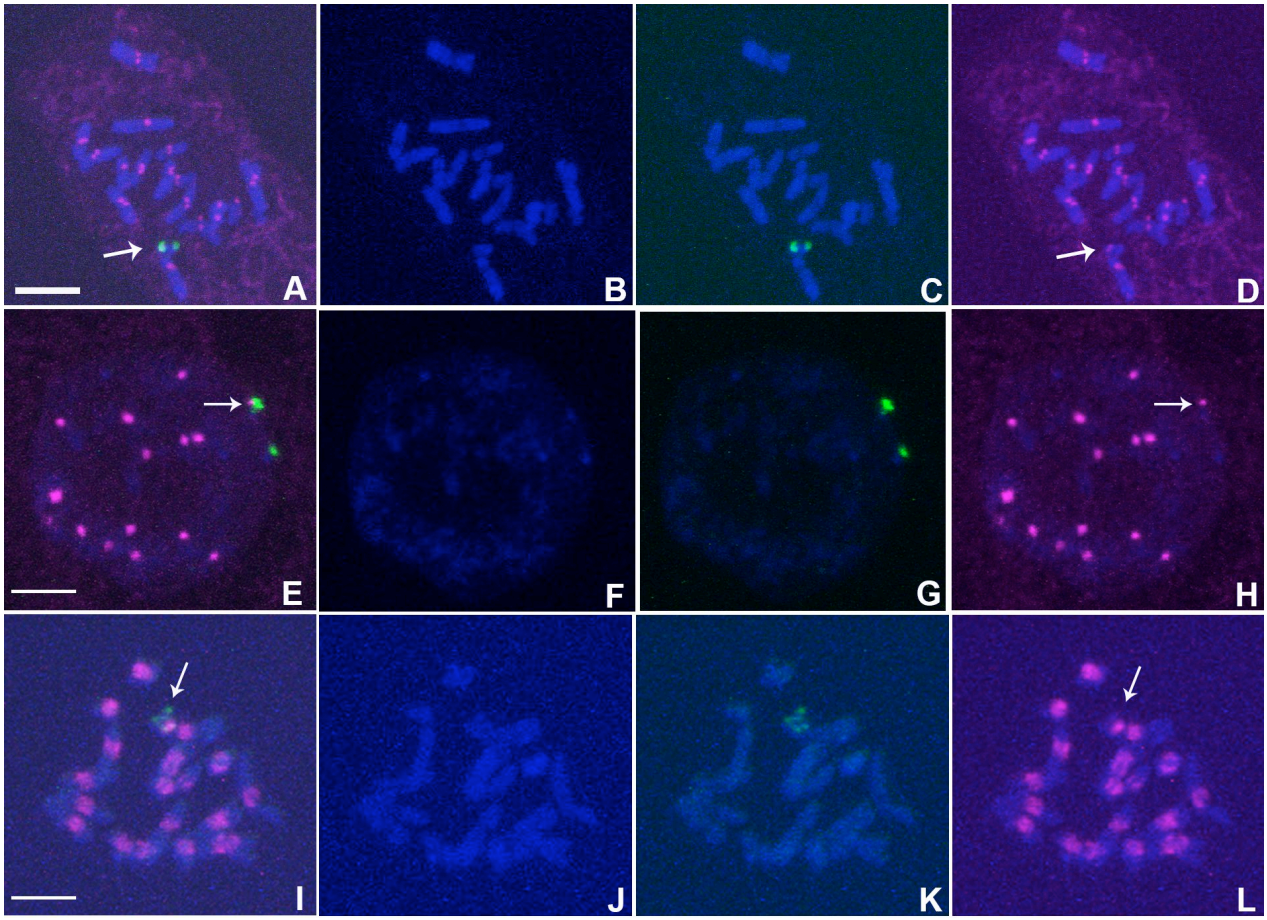
Supplemental Figure 4. Alpha tubulin immunolocalization on Dic-15 at meiosis I and for meiosis II following separation of chromosomes with large or small centromeres. (**A**) Meiosis metaphase I. Tubulin is green; ZmBs is magenta; DAPI is blue. The large centromere of Dic-15 attaches to the spindle but not to the small one. (**B**) Metaphase II. Tubulin is green; ZmBs is magenta; DAPI is blue. The large centromere of Dic-15 attaches to the spindle but not the small one. (**C**) Metaphase II. Tubulin is magenta; ZmBs is green. One centromere of the newly formed chromosome with two small centromeres attaches to the spindle. Bar =10 um.



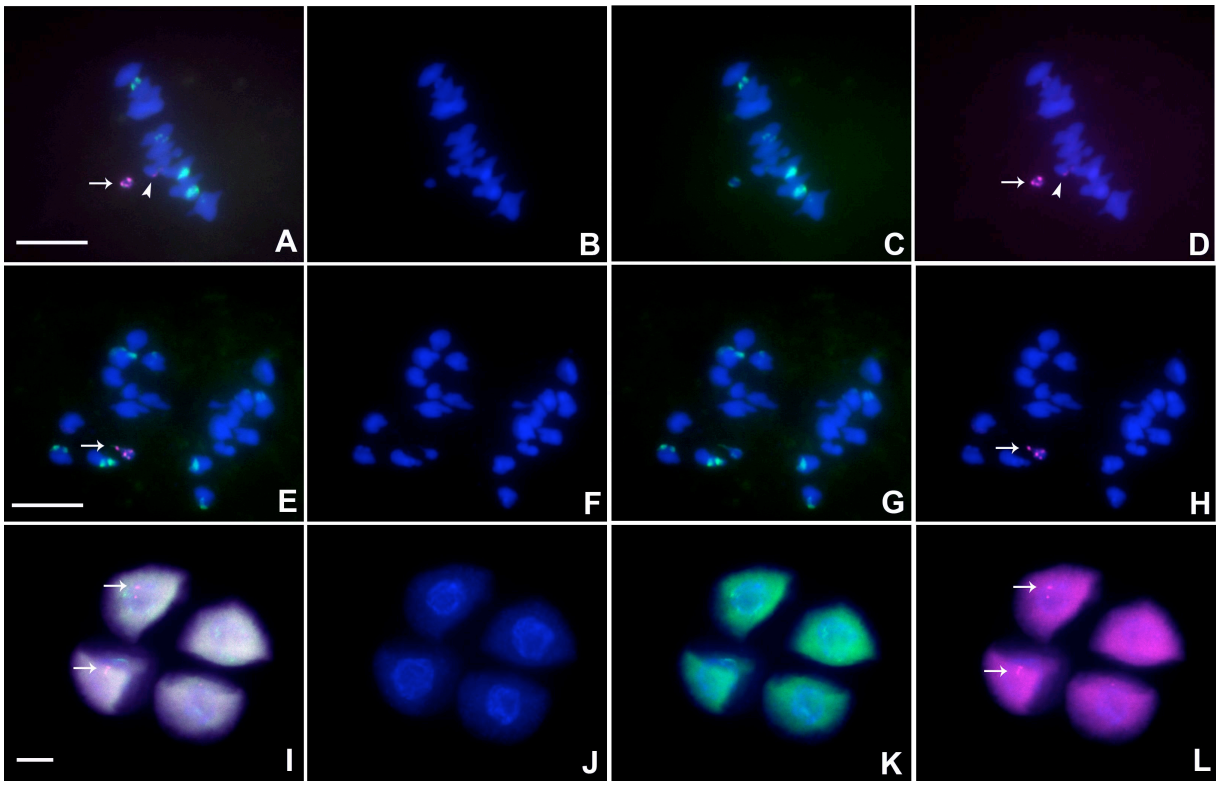
Supplemental Figure 5. Alpha tubulin immunolocalization on Dic-15 at meiosis II following separation of chromosomes with large or small centromeres. **(A)** Tubulin is magenta; ZmBs is green; DAPI is blue. The large centromeres of one chromosome attach to the spindle. **(B)** The same cell as in (A) at a different focal plane shows the chromosome with the smaller centromeres attracting tubulin (ZmBs is green.) (arrow). Bar =10 um.



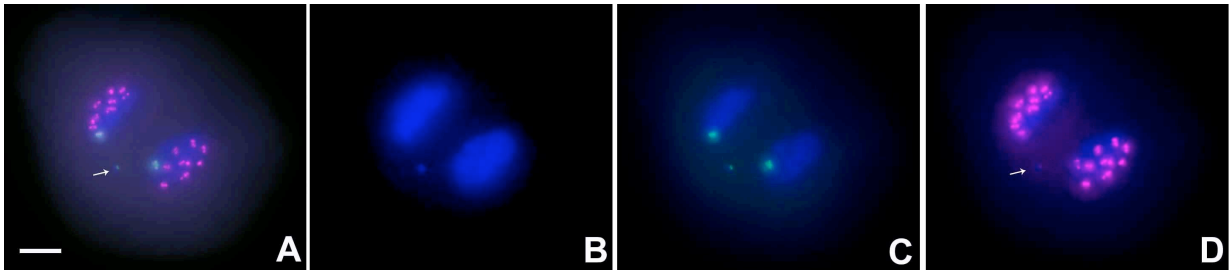
Supplemental Figure 6. Somatic chromosome spreads of progeny derived from Dic-15. **(A)** The dicentric chromosome Dic-15. ZmBs is magenta. In this case, Dic-15 is inherited intact. **(B)** Chromosome has only a large centromere indicating the small centromere has been lost as predicted from recombination in the previous meiosis I and bridge-breakage occurring for the large-large centromere containing chromosome in meiosis II as shown in Figure 4H. ZmBs signal is magenta. Bar =10 um.



Supplemental Figure 7. A chromosome with two smaller centromeres recovered in the progeny of a plant with one copy of Dic-15. Only one centromere has CENP-C, CENH3 and phosphorylation of histone H3 at Ser-10 signals (magenta); ZmBs is green. Arrows indicate the chromosome. **(A, E, I)** Merged image. **(B, F, J)** DAPI. **(C, G, K)** ZmBs. **(D)** CENP-C. Arrow indicates labeling of only one centromere. (**H**) CENH3. Arrow indicates labeling of only one centromere. (Note: this cell is in interphase.) (**L**) Phosphorylated H3 at Ser10. Arrow indicates that only one centromere shows labeling. Bar =10 um.



Supplemental Figure 8. Inheritance and meiotic analysis of a chromosome with a reactivated centromere. **(A)** Merged image of meiotic analysis of a chromosome with two small centromeres. Meiotic metaphase I is shown from a plant inheriting a reactivated small-small centromere structure. ZmBs is labeled in magenta; knob heterochromatin is labeled in green; DAPI is the counterstain in blue. Arrow indicates the dicentric chromosome with two small centromeres. The additional small ZmBs signal (arrowhead) is the long arm tip of the B chromosome on the 9-B chromosome present in the genotype. (**B**) DAPI. (**C**) Knob. (**D**) ZmBs. (**E**) Merged image of anaphase I. Arrow indicates small-small centromere chromosome. (**F**) DAPI. (**G**) Knob. (**H**) ZmBs. (**I**) Merged image of tetrad stage. Arrows indicate the dicentric small-small chromosome. (**J**) DAPI. (**K**) Knob. (**L**) ZmBs. Arrows indicate the dicentric chromosome. Bar =10 um.



Supplemental Figure 9. Immunolocalization analysis of CENP-C in meiosis of the plants containing two Dic-15 chromosomes. CENP-C signals are magenta; ZmBs is green and the DAPI-stained chromosomes are blue. The new dicentric chromosome with small centromeres has a weak CENP-C signal (arrow). **(A)** Merged image. **(B)** DAPI. **(C)** ZmBs. **(D)** CENP-C. Bar =10 um.

Supplementary Table 1. Meiotic analysis of hybrid plants containing TB-9Sb-Dp9 and Telo 3-5(+).

Pairing	No pairing	Total cells
55 (60.43%)	36 (39.56%)	91

Anaphase I (bridge)	Anaphase I (no bridge)	Total cells
40 (54.79%)	33 (45.21%)	73

Five plants containing B-9Sb-Dp-9 and Telo 3-5(+) were used to observe meiosis for chromosome pairing and segregation. Pairing denotes when B-9Sb-Dp-9 and Telo 3-5(+) form a bivalent and will form a bridge at anaphase I if exchange occurs followed by separation of the two centromeres to opposite poles as depicted in Figure 1.

Supplementary Table 2. Meiotic analysis of newly formed dicentric chromosome #15 (Dic-15).

Anaphase I

	Normal segregation	New structures	Bridge	Total
One copy (Dic-15)	67 (73.63%)	24 (26.27%)	0	91
Two copies (Dic-15)	39 (69.6%)	3 (5.35%)	14 (25%)	56

New structures refer to cases in which the large and smaller centromeres were separated; bridges are formed as Figure 5 illustrates.