## **Supporting Information**

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**Fig. S1.** Phenotype of Duplication 3a (Dp3a) kernels. A mature kernel containing the Dp3a chromosome with the dominant alleles of *A1* and *Sh2* in a background of anthocyaninless1 (*a1*) and shrunken2 (*sh2*), which are revealed by mosaic loss of Dp3a. The *A1* allele produces purple anthocyanin pigment in the aleurone layer, and the *Sh2* allele produces normal starch in the endosperm. When the Dp3a is lost during development of the kernel, sectors are produced that do not have normal starch production and are lacking pigment in the corresponding aleurone. These indented regions are characteristic of mutant starch production and also have no anthocyanin pigment.

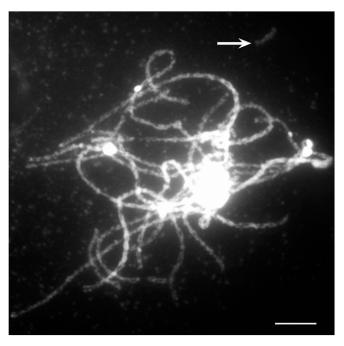


Fig. S2. Duplication 3a (Dp3a) in meiotic pachynema. The DAPI-stained pachytene chromosome image was converted to gray value. Arrow indicates that the Dp3a chromosome did not pair with chromosome 3.

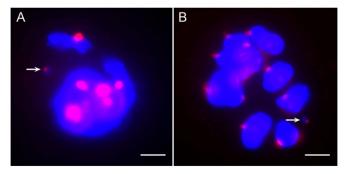


Fig. 53. Immunostaining of Dp3a for CENP-C, a foundational kinetochore protein, at different stages of meiosis. Immunolocalization analysis of CENP-C in meiosis of plants containing one Dp3a chromosome. (A) Diplotene. (B) Metaphase I. Red represents the signal of CENP-C, which is a marker for centromere activity. DAPI-stained chromosomes are blue. The arrow denotes the Dp3a chromosome with one site of CENP-C binding. (Scale bars, 10 μm.)

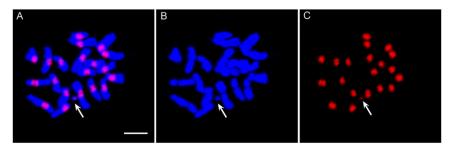


Fig. S4. Phosphorylation of H3-ser-10 on the Dp3a somatic chromosome. Immunofluorescence assay using anti-H3S10ph (red) antibody on mitotic metaphase chromosomes. (A) Merged images. (B) DAPI channel. (C) The red channel is the H3S10 antibody. Arrows indicate the Dp3a chromosome. (Scale bar, 10 μm.)

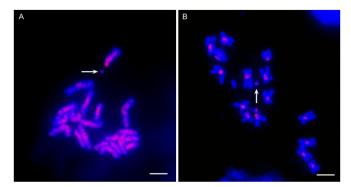


Fig. S5. Sites of H3T3 phosphorylation on the Dp3a somatic chromosome. H3T3 phosphorylation antibody localizes in the inner centromeric region during metaphase. (A) Early metaphase. (B) Metaphase. Arrows indicate the Dp3a chromosome. The red channel is H3T3ph antibody; DAPI-stained chromosomes are blue. (Scale bars, 10 µm.)

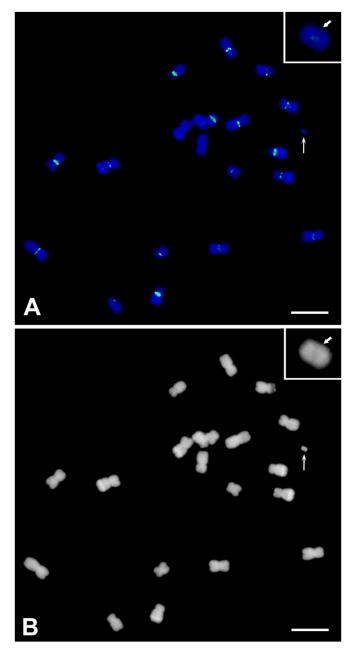


Fig. 56. Confirmation of ChIP-DNA location to centromeric regions. (A) FISH analysis using DNA from ChIP as a probe to detect the location of the ChIP-DNA sequences. The green signals indicate that the ChIP-DNA sequences are located in centromere regions. DAPI-stained chromosomes are blue. Arrow designates Dp3a. *Inset* shows an enlarged image of Dp3a. (B) Gray-value image of A. (Scale bar, 10 μm.)

Table S1. Mapping results from ChIP-Seq

Sequence Reads	Control, pair-end 100 nt		Dp3a, pair-end 100 nt	
	Reads	Percentage	Reads	Percentage
Total reads	51,453,782	100	30,902,058	100
Total mapped	39,326,480	76.43	23,364,933	75.61
Uniquely and nonredundantly mapped	33,992,185	66.06	19,700,469	63.75

Table S2. Genes located in the 350-kb region of Dp3a

Fig. 4B Gene Number CENH3 above designation Name Protein Nucleotide position control GRMZM2G045565 Protein of unknown function DUF538 family protein 218,891,348-218,892,450 2 GRMZM2G045318 218,893,373-218,896,627 Hypothetical protein 3 GRMZM5G815568 Hypothetical protein 218,893,525-218,896,628 4 GRMZM2G045275 Putative early flowering 3-like 218,897,671-218,903,527 5 218,910,518-218,916,860 GRMZM2G045268 Like ubiquinol-cytochrome c reductase complex 6 AC207396.3\_FG004 Hypothetical protein 218,923,790-218,924,035 7 AC207396.3\_FG003 Hypothetical protein 218,926,785-218,927,030 8 AC207396.3\_FG002 Hypothetical protein 218,927,716-218,928,003 9 AC207396.3\_FG001 Hypothetical protein 218,928,547-218,928,792 10 Uncharacterized protein LOC100501661 precursor 219,063,340-219,064,565 GRMZM2G125571 Uncharacterized protein LOC100272281 11 219,075,234-219,076,412 GRMZM2G342653 12 AC209738.3\_FG002 Uncharacterized protein LOC100272281 219,075,562-219,076,050 13 AC209738.3\_FG003 Tetratricopeptide repeat domain 4 219,097,302-219,099,416 14 GRMZM2G342693 Hypothetical protein 219,099,193-219,101,141 15 GRMZM5G882921 Hypothetical protein 219,099,460-219,099,789 16 AC209738.3\_FG005 Zea mays RNA-binding protein (LOC100285689) 219,104,042-219,104,893 17 RNA-binding protein 219,104,793-219,106,389 GRMZM2G342711 18 GRMZM2G342719 Cell division protein ftsZ 219,107,045-219,108,925 19 GRMZM2G508066 Hypothetical protein 219,111,436-219,111,834 Uncharacterized protein LOC100277351 20 GRMZM2G040905 219,117,645-219,126,756

Zea mays B73 monoterpene synthase (tps26) gene

bHLH transcription-like

219,164,362-219,164,942

219,181,998–219,184,295

GRMZM2G702260

GRMZM2G081816

21

22

<sup>+,</sup> binding with CENH3; -, no binding with CENH3.