## SUPPLEMENTARY DATA

FIG. S1. Diakinesis chromosomes. (A) Sesquidiploid (AAC, 2n = 29): three C chromosomes are incorporated in three trivalents (long arrows pointing at the C chromosomes), one C chromosome is part of a heteromorphic bivalent (short arrow) while the other A chromosome is a univalent (A), and five C chromosomes are univalents (arrowheads). (B) Double MAAL with C6 (short arrow) and C8 (long arrow); (C–F) MAALs. (C, D) Carriers of C7 as a univalent (C, arrow) and as part of a trivalent involving two A chromosomes with median/submedian centromeres (D, arrow). (E) C6 (arrow) as part of a trivalent involving two A chromosomes with submedian/subterminal centromeres. (F) C5 (arrow) as part of a pentavalent involving one pair of A chromosomes with median/submedian centromeres (upper) and one pair with submedian/subterminal centromeres (lower). Scale bars = 10 µm.



FIG. S2. Examples of chromosome structural changes found in progenies of C4 carrier plants (A–E) and of a substitution in a progeny plant of a C5-carrier (F). (A) Diakinesis of a euploid AA plant with 10 II, with a deleted major part of the short arm of one nucleolar chromosome in the heteromorphic bivalent (arrow). (B) Diakinesis of a plant with a C4 larger than usual, apparently with extra duplicated or translocated C-genome chromosomal material, paired with the nucleolar chromosomes of the A-genome forming a trivalent (arrow). (C–E) Meiosis of a monosomic plant containing C4 and an additional minichromosome: (C) metaphase I with C4 as a univalent (arrow) and with the extra mini-chromosome (arrowhead); (D) anaphase I with a lagging C4 (arrow) and a divided mini-chromosome (arrowhead); and (E) a later anaphase I stage with a dividing lagging C4 and separated daughter mini-chromosomes. (F) Diakinesis of a progeny plant with 2n = 20 containing a C5 and lacking the homologue to an A chromosome with a submedian/subterminal centromere. Scale bar = 10 µm.



FIG. S3. Identification of the A and C chromosomes after applying two rounds of multiple target FISH (Xiong and Pires, 2011), using in the first round (A, C, F) probes for 5S rDNA (yellow), 45S rDNA (white), repeated DNA sequences in eight chromosome pairs of *B. rapa* by using BAC KBrB072L17 (green), and repeated DNA sequences specific to two pairs of *B. rapa* by using BAC KBrH092N24 (red), and applying in the second round (B, D, F) probes for the repetitive centromeric DNA sequences CentBr1 (white) and CentBr2 (green) and for repetitive DNA sequences that are C-genome specific by using the BAC BNIH 123L05 (red). (A, B) C4 (LG-C3) in a meiotic metaphase I. (C–F) Mitotic metaphase. (C, D) C3d (LG-C5). (E, F) C6 (LG-C6). Scale bar = 10  $\mu$ m.



FIG. S4. Chromosomes of *Brassica oleracea* designated by linkage group numerals as depicted in an ideogram by Xiong and Pires (2011) and their corresponding chromosomes of *B. oleracea* var. *alboglabra* designated by cytological numerals in a karyotype by Cheng *et al.* (1995). There is a general correspondence regarding chromosome size and position of the centromere between the ideogram and the karyotype, with the exception of LG-C2, which has a more median centromere compared to its corresponding C7.



TABLE S1. Accession numbers of the *Brassica* material deposited in the gene bank NordGen in Alnarp, Sweden (www.nordgen.org) and corresponding linkage groups to the different C-genome chromosomes in the monosomic alien addition lines (MAALs)

Linkage group	Brassica material	Linkage group
NGB23151	MAAL for C1	С9
NGB23152	MAAL for C2	C1
NGB23153	MAAL for C3d	C5
NGB23154	MAAL for C4	C3
NGB23155	MAAL for C4d	C3
NGB23156	MAAL for C5	C4
NGB23157	MAAL for C6	C6
NGB23158	MAAL for C7	C2
NGB23159	MAAL for C8	C7
NGB23160	MAAL for C9	C8
NGB23161	B. rapa var. trilocularis (K-151)	
NGB23162	<i>B. oleracea</i> var. <i>alboglabra</i> (No. 4003)	
NGB23163	B. napus (No. 7406)	