

Supplementary Information for

The Cdk1/Cdk2 homolog CDKA;1 controls the recombination landscape in Arabidopsis

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References for SI reference citations

Other supplementary materials for this manuscript include the following:

Dataset S1

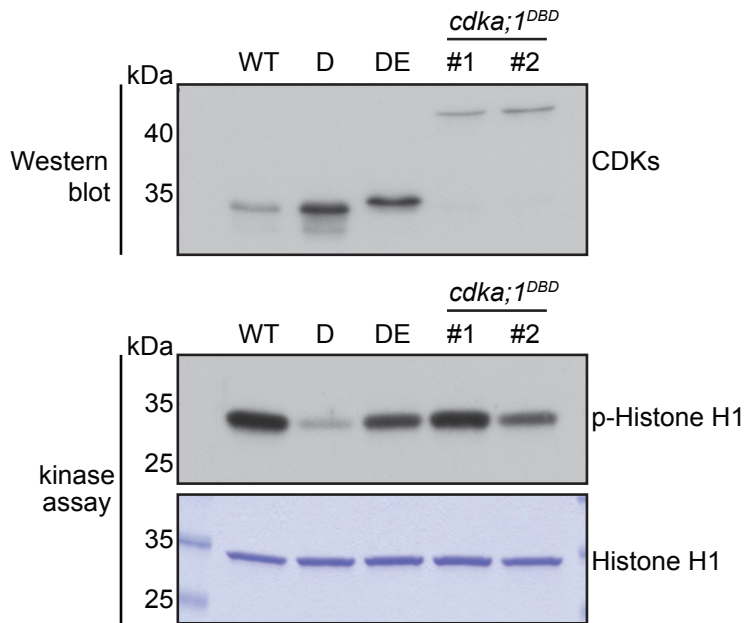


Fig. S1. Histone H1 kinase activity in wildtype and CDKA;1 variants.

p13-associated kinase activities from the wildtype (WT) and the *cdka;1* the previously described hypomorphic alleles D (*cdka;1^{T161D}*), DE (*cdka;1^{T14D;Y15E}*) (1, 2), and the here-presented allele *cdka;1^{DBD}* for which we show two independent transformed lines (#1 and #2). CDKA;1 levels were quantified by Western blotting with a monoclonal antibody against the PSTAIRE cyclin-binding motif (upper panel). The difference in the band shift between D and DE due to the different single and double phosphomimicry substitutions. Hypomorphic mutants in CDKA;1 possess lower kinase activities than wild-type plants against histone H1 as a generic substrate (lower panel, top row). Note that *cdka;1^{DBD}* line #2 shows a lower kinase activity than *cdka;1^{DBD}* line #1. Lower panel, bottom panel shows by Coomassie staining the equal presence of histone H1 in all reactions analyzed.

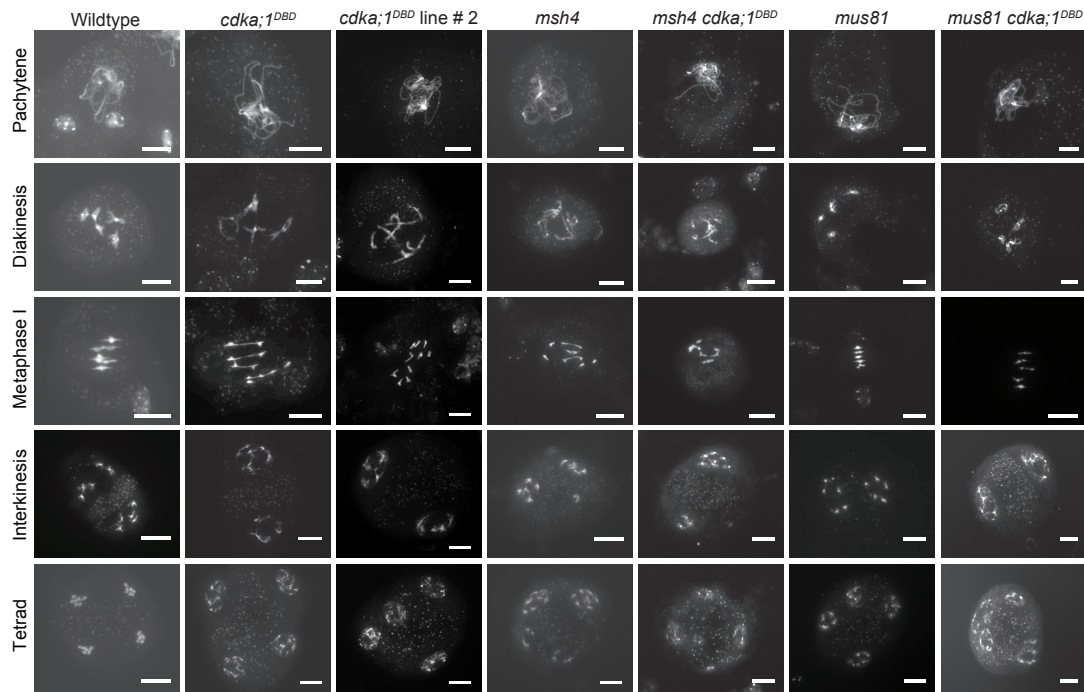


Fig. S2. Meiotic progression in wildtype, *cdk1^{DBD}*, *cdk1^{DBD}* Line #2, *msh4*, *mus81* and double mutants of both *msh4* and *mus81* with *cdk1^{DBD}*.

All images show DAPI labeled DNA. The line *cdk1^{DBD}* (in the text also referred to as “*cdk1^{DBD}* line #1”) is a transgenic line different from *cdk1^{DBD}*, line #2. All single mutant- as well as double mutant combinations show pachytene stages with apparently paired chromosomes. Differences occur at diakinesis and metaphase I in which chiasma numbers differ, as witnessed by the number of ring- and rod bivalents. Some mutants show unbalanced chromosome numbers in nuclei at the second meiotic division as a consequence of the presence of univalents at metaphase I and nondisjunctions at anaphase I. Scale bars 10 μ m.

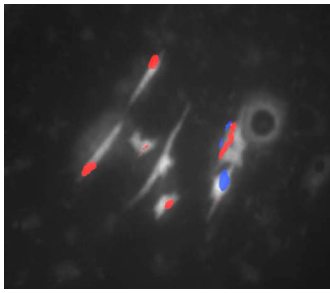


Fig. S3 Fluorescent *in-situ* hybridization (FISH) in *cdka;1^{DBD}* (in the text also referred to as “*cdka;1^{DBD}* line #1”) reveals that bivalents in *cdka;1^{DBD}* are formed between homologous chromosomes.

Image shows a metaphase I cell in which 45S (blue, DEAC) and 5S (red, CY3.5) rDNA are labeled, with a chromatin counterstain of DAPI. One univalent pair is present.

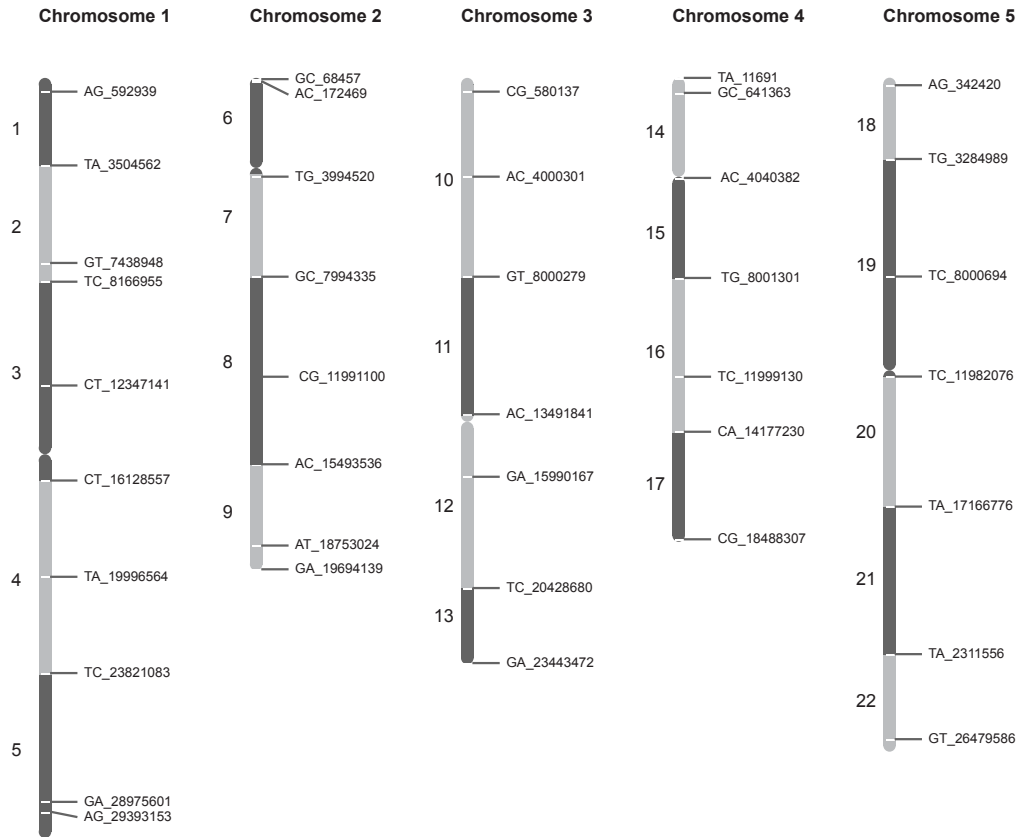


Fig. S4. The positions of markers used to genotype the BC1 populations of wildtype, *cdka;I^{DBD}*, *CDKA;I:YFP* and *FBL17 OE*.

Chromosomes 1 through 5 are shown from left to right. Markers are indicated at their physical positions, with the first two letters indicating the polymorphism followed by the exact genomic position in the (Col-0) reference genome. The chromosomes are shown as dark- and light grey bars. These segments indicate the 22 intervals were used to calculate the genetic interference values as shown in Fig. 3.

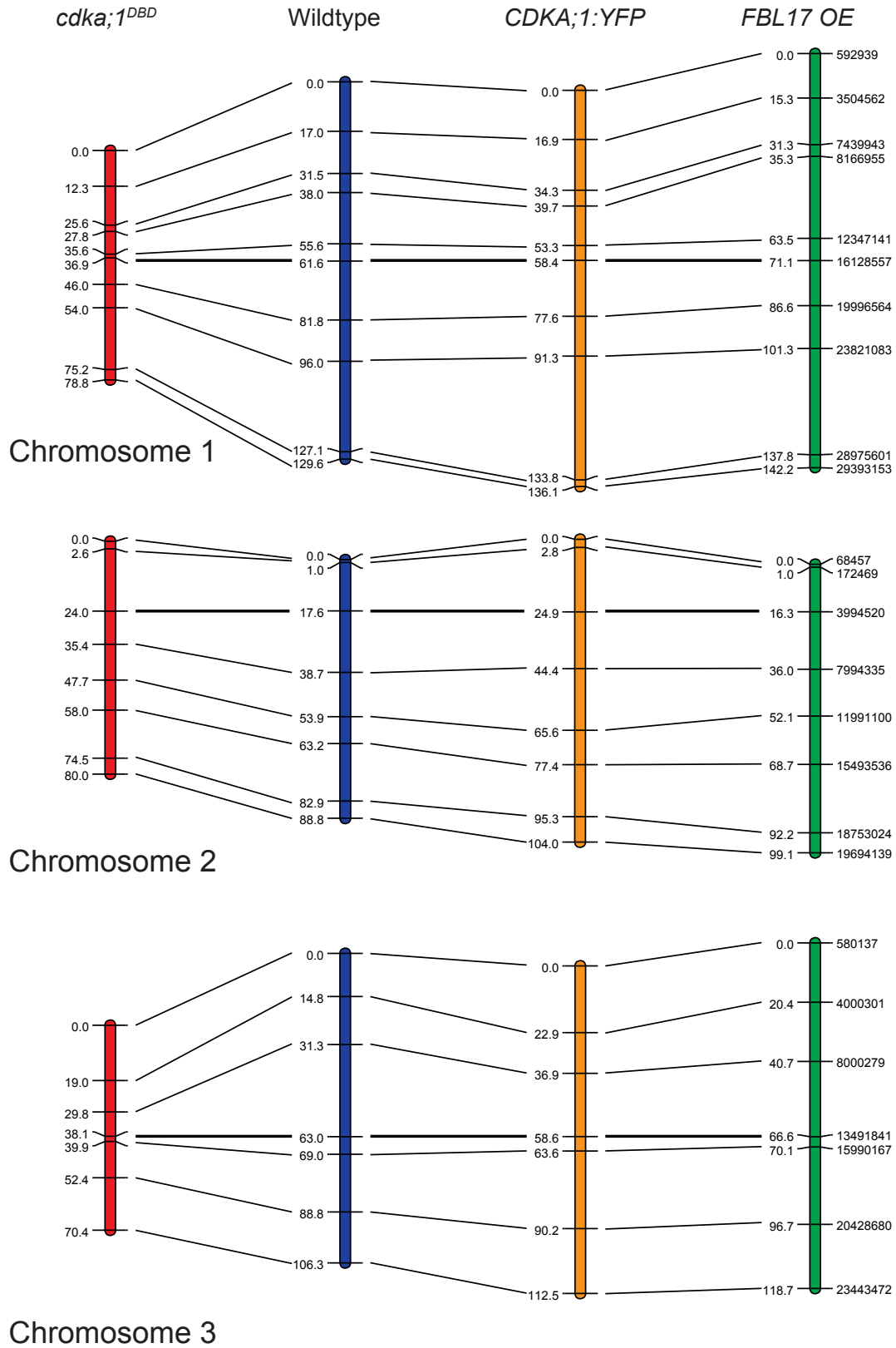


Figure continues on next page.

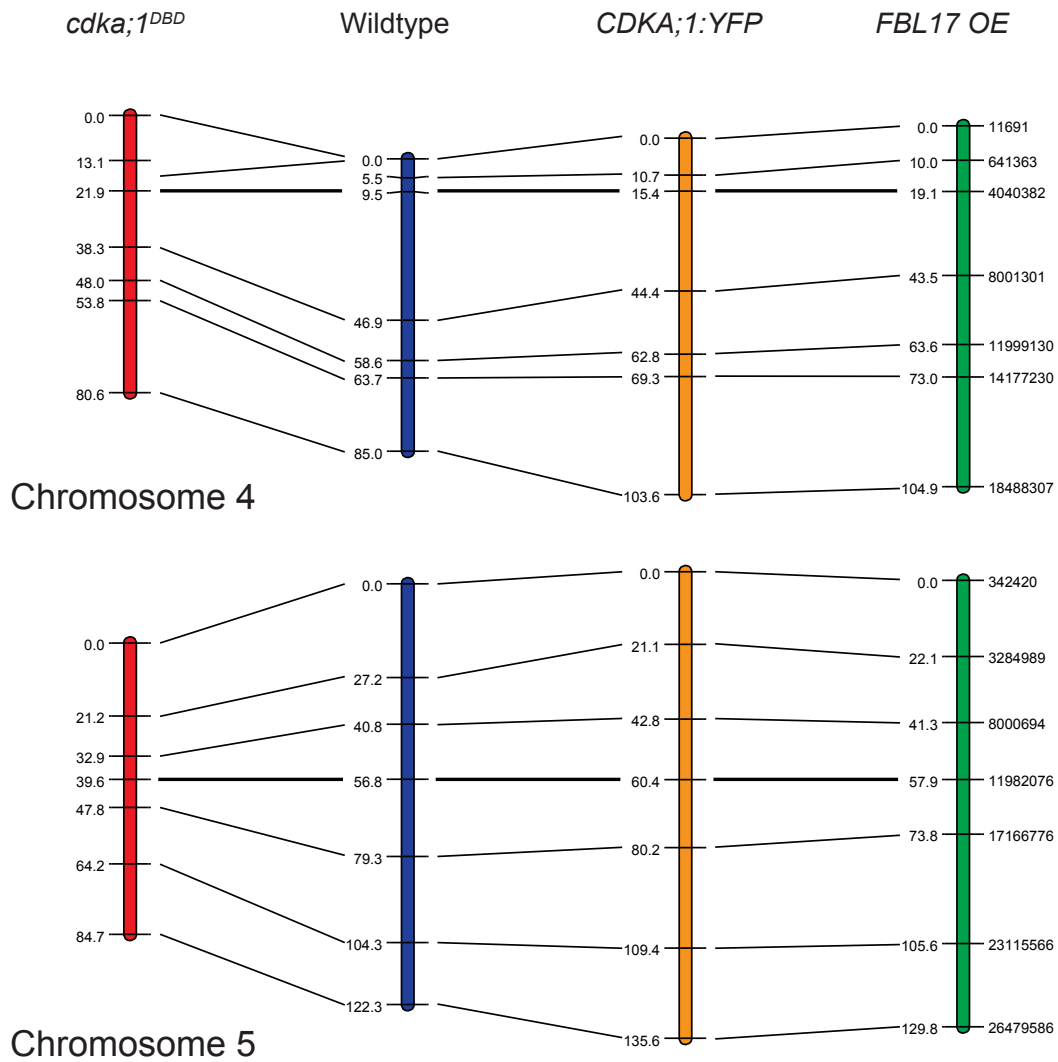


Fig. S5. Genetic maps of BC1 populations generated during this study.

From left to right the genetic maps of *cdka;1^{DBD}* (red), the wildtype (blue), *CDKA;1:YFP* (orange) and *FBL17 OE* (green) are shown, with chromosomes 1, 2, 3, 4 and 5 shown from top to bottom. Maps of each chromosome are aligned using the marker closest to the centromere, also indicated by the thicker bar. All maps were generated with Joinmap using the Kosambi mapping function.

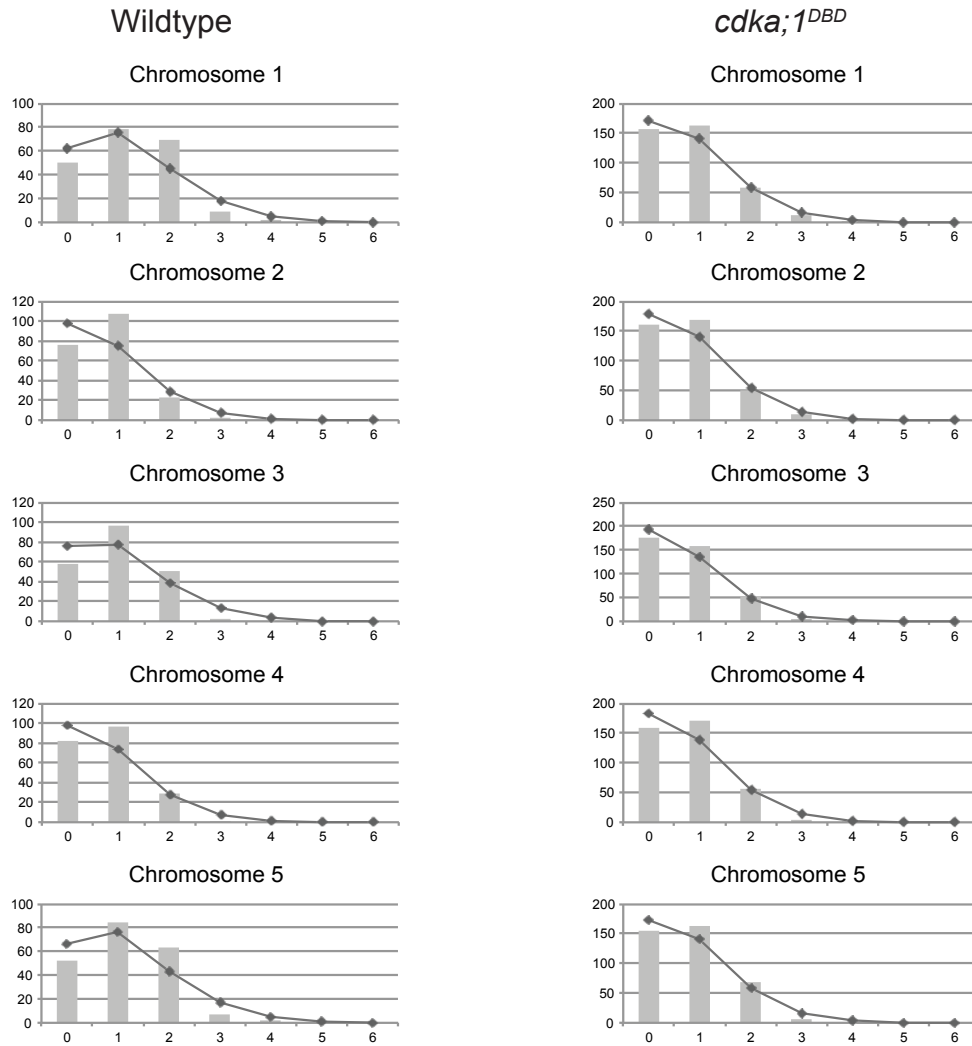


Fig. S6. Comparison of observed CO numbers in wildtype and *cdka;1^{DBD}* backcross populations with the expected crossover numbers under the Poisson distribution.

Observed (grey bars) and expected (black line) numbers deviate more strongly in wildtype than in *cdka;1^{DBD}*, suggesting the latter has a more random distribution of COs. Corresponding p-values are given in Table S1.

Table S1. CO numbers per chromosome in wildtype and *cdka;1^{DBD}*.

The average number of COs per chromosome, and the p-value for the chi-square test, indicating the likeliness that the observed CO number per chromosome fits the Poisson (a random) distribution. In red are p-values smaller than 0,05. The wildtype differs significantly from the Poisson distribution for all chromosomes, likely due to interference. CO numbers per chromosome in *cdka;1^{DBD}* are significantly different for chromosomes 4 and 5, but the CO numbers on *cdka;1^{DBD}* chromosomes 1, 2 and 3 follow a distribution that is not significantly different from the Poisson distribution.

Wildtype

Chromosome	Average nr of COs	p-value	Genetic map length (cM)
1	1,21	7,32E-04	129,6
2	0,77	1,97E-04	88,8
3	1,00	3,65E-04	106,3
4	0,76	1,14E-02	85
5	1,15	1,66E-03	122,3

cdka;1^{DBD}

Chromosome	Average nr of COs	p-value	Genetic map length (cM)	Map length reduction compared to wildtype (%)
1	0,82	2,54E-01	78,8	39,2
2	0,78	8,19E-02	80,0	9,9
3	0,70	5,06E-02	70,4	33,8
4	0,77	4,34E-03	80,6	5,2
5	0,82	1,44E-02	84,7	30,7

Table S2. CO detected in wildtype and *cdka;1^{DBD}* BC1 populations.

Per chromosome, the percentage and absolute number (between brackets) of chromatids in the BC1 offspring is given that show either 0, 1,2 or 3(+) CO. Note that in wildtype meiosis (top) the shorter chromosomes 2 and 4 show the highest percentages of non-recombinant chromatids in BC1 offspring (36 and 39% respectively) as compared to chromosomes 1, 3 and 5. In *cdka;1^{DBD}* (bottom), the number of non-recombinant chromatids slightly increases to 41% in chromosomes 2 and 4, but we recover similar percentages for the other three chromosomes. Chromosomes 1, 3 and 5 show a much stronger increase in the number of non-recombinant chromatids in the BC1.

wildtype	Detected CO per chromatid			
	0	1	2	3 or more
Chr. 1	24,0 (50)	37,5 (78)	33,2 (69)	5,3 (11)
Chr. 2	36,4 (76)	51,7 (108)	11,0 (23)	1,0 (2)
Chr. 3	27,9 (58)	46,1 (96)	24,5 (51)	1,4 (3)
Chr. 4	39,2 (82)	46,4 (97)	13,9 (29)	0,5 (1)
Chr. 5	24,9 (52)	40,7 (85)	30,1 (63)	4,3 (9)
<i>cdka;1^{DBD}</i>	Detected CO per chromatid			
	0	1	2	3 or more
Chr. 1	39,8 (156)	41,8 (164)	14,8 (58)	3,6 (14)
Chr. 2	40,8 (160)	43,4 (170)	12,8 (50)	3,1 (12)
Chr. 3	45,2 (177)	40,6 (159)	13,3 (52)	1,0 (4)
Chr. 4	40,6 (159)	43,9 (172)	14,3 (56)	1,3 (5)
Chr. 5	39,2 (154)	41,3 (162)	17,6 (69)	1,8 (7)

Additional data table S1 (separate file)

BC1 offspring genotypes.

References

1. Dissmeyer N *et al.* (2007) T-Loop Phosphorylation of Arabidopsis CDKA;1 Is Required for Its Function and Can Be Partially Substituted by an Aspartate Residue. *Plant Cell* 19(3):972–985.
2. Dissmeyer N *et al.* (2009) Control of cell proliferation, organ growth, and DNA damage response operate independently of dephosphorylation of the Arabidopsis Cdk1 homolog CDKA;1. *The Plant Cell* 21(11):3641–3654.