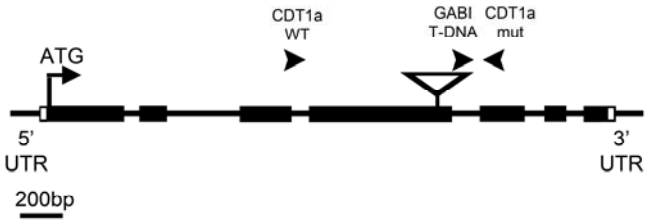
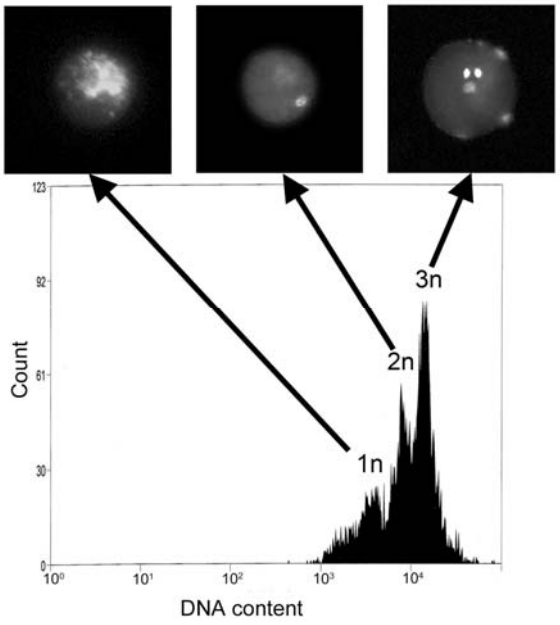


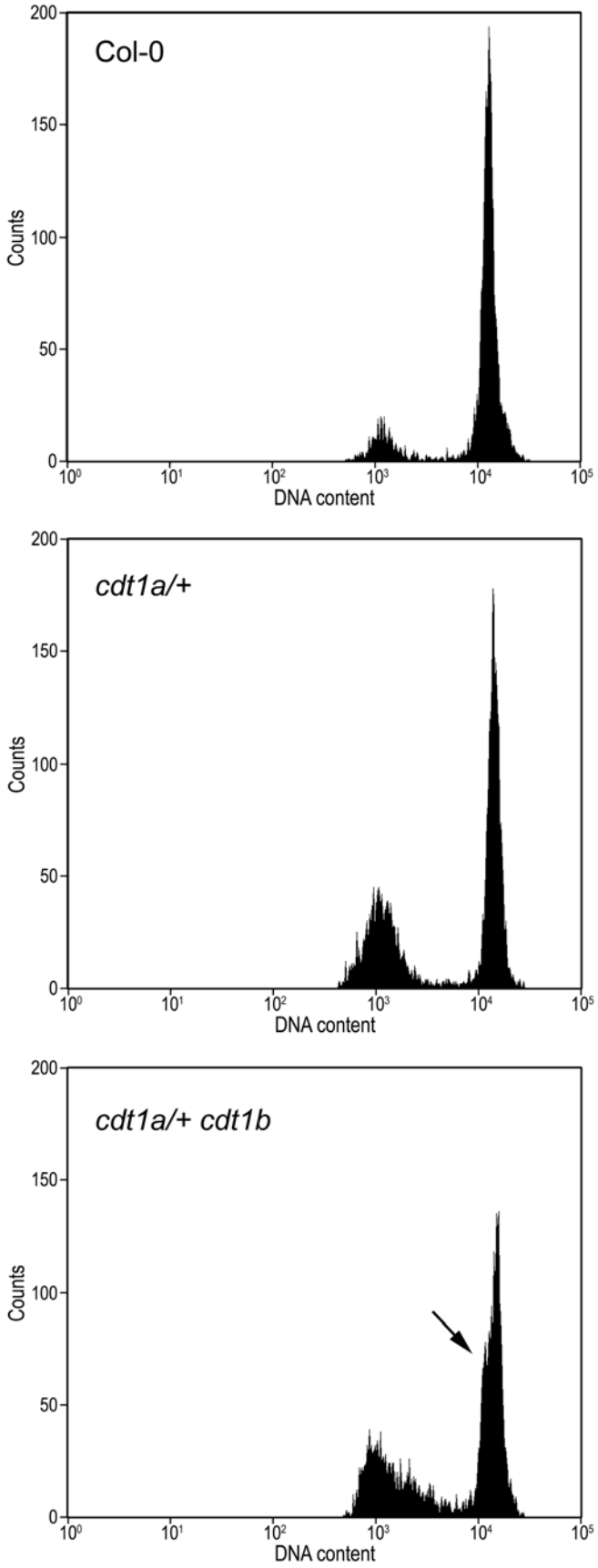
Supplemental Figure 1: Position of the T-DNA insertion in the GABI_025G08 line.

Black boxes represent exons, black lines represent introns. Arrows indicate the position of primers used for genotyping.



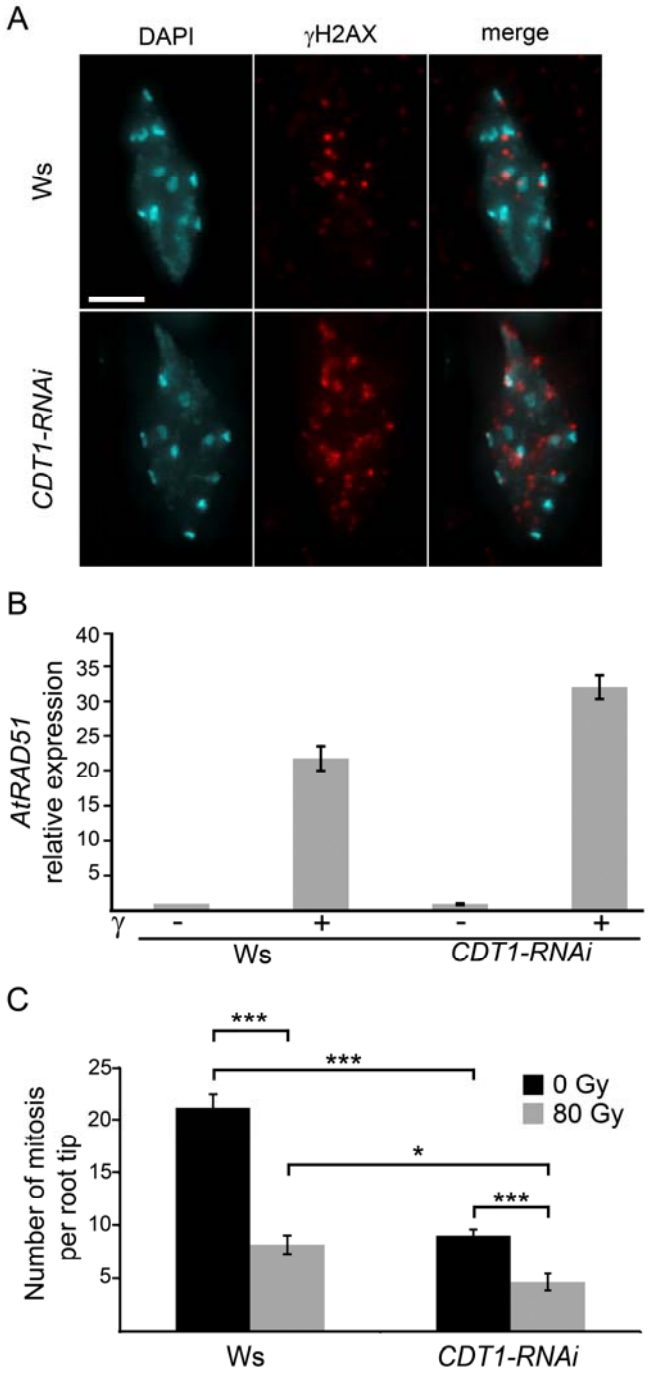
Supplemental Figure 2: DNA content in pollen grains isolated from a whole inflorescence of a Col-0 plant.

Pollen was extracted by dissecting anthers in 45 mM $MgCl_2$, 30 mM sodium citrate, 20 mM MOPS, pH 7, and 0.1% Triton X-100 (Galbraith buffer, Galbraith *Science* 1983) with 5 µg/ml DAPI. DNA content of pollen was analyzed using a MoFlo XDP cytometer (Beckman Coulter) with a 405-nm solid state laser (30 mW) excitation and an emission collected after a 457/50-nm band-pass filter. For microscopy observation, pollen of different DNA levels were sorted using a purify mode with a drop envelop of one. This procedure was applied on pollen extracted from a whole inflorescence. Three major peaks labeled 1n, 2n and 3n could be identified, together with a smaller peak towards the lower DNA content. Microscopic observation of sorted pollen allowed us to confirm that the minor peak corresponded to cell debris while the 1n, 2n, and 3n peaks corresponded to one, two and three celled pollen grains respectively.



Supplemental Figure 3: Altered DNA content in pollen grains isolated from *cdt1a/+ cdt1b* mutants.

Then the same procedure as above was applied to pollen extracted from mature flowers to compare the DNA content of mature pollen grains in wild-type and mutant plants. In Col-0 and *cdt1a/+* plants, pollen grains distributed in a narrow peak corresponding to three cellular pollen grains. In *cdt1a/+ cdt1b* mutants, a shoulder appeared on this peak (black arrow) corresponding to abnormal pollen grains. Comparison with DNA content in two celled and three celled nuclei isolated from Col-0 flowers indicates that the DNA content of abnormal pollen grains is comprised between the DNA content of two celled and three celled pollen grains. The broader peak towards the lower DNA contents corresponds to cell debris and aborted pollen grains. The frequency of aborted pollen grains is higher both in *cdt1a/+* and *cdt1a/+ cdt1b* plants than in the wild type.



Supplemental Figure 4: Cellular response to DNA damage is normal in *CDT1-RNAi* plants

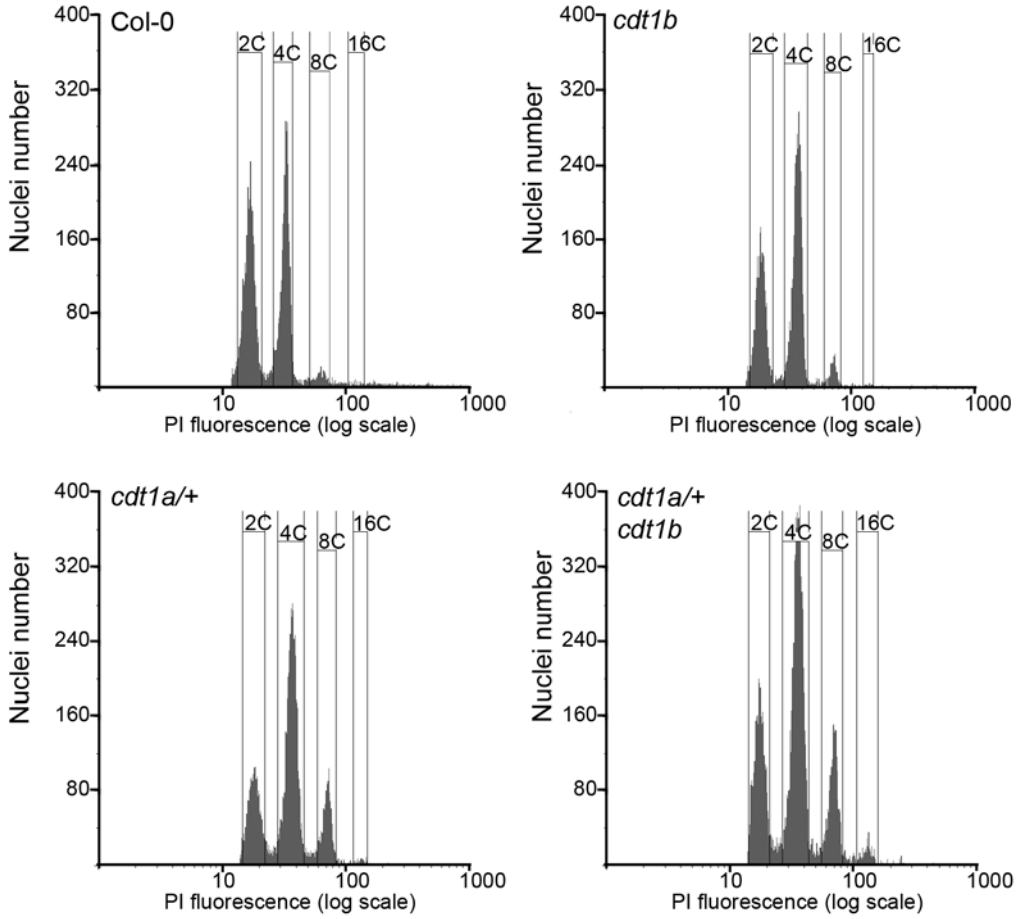
A: Immunofluorescence detection of phosphorylated H2AX in an isolated nucleus of Ws (top) and *CDT1-RNAi* (bottom) plantlets. One of the earliest responses to double-strand breaks is the accumulation of phosphorylated H2AX histones at the site of the break. We therefore measured phosphorylation of the H2AX histone variant after γ-irradiation by immuno-staining on isolated nuclei (see below for detailed procedure). No labelling could be detected in non-irradiated plants. As expected, phosphorylated H2AX is detected as discrete spots in the nuclei. Surprisingly, *CDT1-RNAi* showed a higher proportion of labelled nuclei than the wild-type

(47% vs 13% in the wild-type, n=150) and more intense labelling in the nuclei as illustrated on this Figure, suggesting that either more DSB are formed in these plants, or recognition of DSB occurs faster. Bar=5µm.

B: Q-RTPCR analysis of *RAD51* expression in the wild-type (WS) and *CDT1-RNAi* plants before (-) and after (+) gamma irradiation at 100 Gy. We asked whether later steps of DNA damage response were impaired. After DSB recognition, a signalling pathway induces the expression of genes involved in DNA repair such as *RAD51*. *RAD51* was induced in *CDT1-RNAi* plantlets, and this induction was even stronger than the one observed in the wild-type, suggesting that DSB sensing and response is functional in these plants. Bars are average +/- standard deviation. Data presented here are representative of two biological replicates.

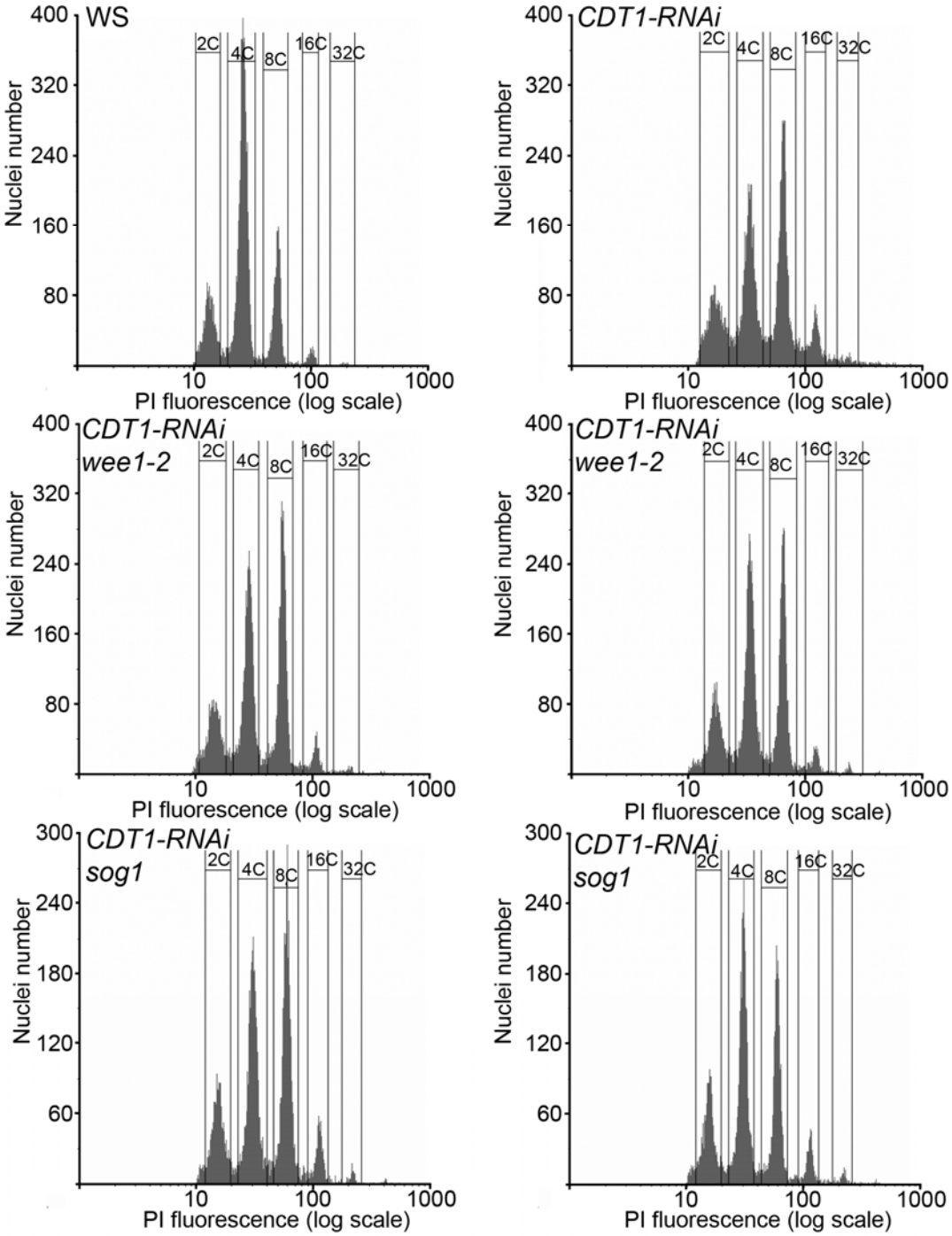
C: Mitotic index in the root tip of wild-type (WS) and *CDT1-RNAi* plants before (blue bars) and after (red bars) gamma irradiation (40 Gy). We assessed the ability of *CDT1-RNAi* plants to induce cell cycle arrest upon DNA damage. To this end we measured the mitotic index in wild-type and *CDT1-RNAi* plants before and 90 minutes after γ-irradiation by counting mitotic events in the root tip of 20 plantlets. To this end, plantlets were fixed in paraformaldehyde (4% w/v), squashed on a glass plate and stained with DAPI. Mitotic events were counted using an epifluorescence microscope. In *CDT1-RNAi* root tips, the mitotic index was 1.5 times lower than in the wild-type, consistent with the previously reported delay in cell cycle progression in these lines (Raynaud et al, 2005). After gamma-irradiation, the mitotic index was reduced both in the wild-type and in *CDT1-RNAi* plants, indicating that their partial resistance to gamma rays is not due to an inability to stop cell cycle progression. Results are representative for two biological replicates.

Bars are average +/- standard error. Stars indicate significantly different values (* Student t test, p value<0.05, *** p value<0.001) Data presented here are representative of two independent experiments.



Supplemental Figure 5: Endoreduplication is increased in *cdt1a/+* and *cdt1a/+cdt1b* mutants.

Nuclei were isolated and their DNA content was analyzed by flow-cytometry as described in the material and methods section. DNA content was measured in at least 6000 nuclei isolated from the first leaf of plantlets harvested 14 days after stratification.



Supplemental Figure 6: Endoreduplication is enhanced in CDT1-RNAi plants in a WEE1 and SOG1 independent manner.

Nuclei were isolated and their DNA content was analyzed by flow-cytometry as described in the material and methods section. DNA content was measured in at least 6000 nuclei isolated from the first cauline leaf of plants. Two representative profiles are shown for CDT1-RNAi *wee1-2* and *CDT1-RNAi sog1* mutants to illustrate that the results are quite reproducible, although there is some variability from one plant to another.

Supplemental Table I: Detailed results for TAP analysis

Protein score: score calculated by the Mascot search engine for each protein. This score is based on the probability that peptide mass matches are non-random events. If the Protein Score is equal to or greater than the Mascot® Significance Level calculated for the database search, the protein match is considered to be statistically non-random at the 95% confidence interval. Protein score = -10*Log(P), where P is the probability that the observed match is a random event

Expect: Protein score expectation value.

RMS error: RMS error of the set of matched mass values, in ppm.

Sequence coverage %: percentage of protein sequence covered by assigned peptide matches.

Unique peptides: number of peptides with unique sequences matching the selected protein.

Total Ion Score A: score calculated by weighting Ion Scores for all individual peptides matched to a given protein.

Peptide Number: Peptide index number within the list of peptides associated with a given protein.

Start: starting position of the peptide in the protein.

End: ending position of the peptide in the protein.

Observed: observed monoisotopic mass of the peptide in the spectrum (m/z).

Mr (Exp): experimental mass of the peptide calculated from the observed m/z value.

Mr (Calc): theoretical mass of the peptide based on its sequence.

Delta (Da): difference between the theoretical (Mr (Calc)) and experimental (Mr (Exp)) masses, in daltons.

Miss: Number of missed Trypsin cleavage sites.

Ions score: the Ions Score is calculated by the Mascot search engine for each peptide matched from MS/MS peak lists. This score is based on the probability that ion fragmentation matches are non-random events. If the Ion Score is equal to or greater than the Mascot® Significance Level calculated for the database search, the peptide match is considered to be statistically non-random at the 95% confidence interval. Ions score = -10*Log(P), where P is the probability that the observed match is a random event.

Best Ions score: highest individual Ion Score for a given protein identification.

Expect: Ions score expectation value.

Peptide: amino acid sequence of the selected peptide.

Variable Modification: Variable modification type on the peptide.

	Identification						PMF data			MSMS data												
Bait	Locus	Protein Name	# Found/ # exp	Database version	Protein Score	Expect	RMS error (ppm)	Sequence coverage %	Unique Peptides	Total Ion Score	Peptide Number	Start	End	Observed	Mr(Exp)	Mr(Calc)	Delta (Da)	Miss	Ions Score	Expect	Peptide	Variable Modification
CDT1a	AT1G08260	EMB2284, POL2A, TIL1, EMB529	2/2	TAIR8	781	2,6E-74	6	27	47	468	1	1984	1990	952.49	951.49	951.49	0.00	0	27	5,10E-02	K.FFDPWK.F	
											2	637	643	1016.57	1015.56	1015.56	-0.00	1	25	9,10E-02	R.KLEWVWR.G	
											3	1821	1830	1160.70	1159.69	1159.70	-0.01	0	34	3,50E-03	K.VFALLLTDLR.R	
											4	197	205	1177.56	1176.56	1176.56	0.00	0	30	2,00E-02	R.EYDVPYHVR.F	
											5	1784	1793	1192.63	1191.62	1191.62	-0.00	0	30	2,70E-02	K.NYADSILQR.L	
											6	1116	1124	1200.63	1199.62	1199.62	-0.00	0	39	2,40E-03	R.LIDWSYYK.Q	
											7	722	731	1239.59	1238.58	1238.59	-0.01	0	42	1,20E-03	R.ENPFYVDTVR.S	
											8	86	95	1263.65	1262.64	1262.64	-0.00	0	24	1,10E-01	K.FRPYFYAATK.D	
											9	33	44	1312.65	1311.64	1311.65	-0.00	0	33	1,10E-02	K.LGFGLFSEGETR.L	
											10	1251	1261	1336.69	1335.68	1335.68	-0.00	0	64	9,70E-06	K.SYNYQGWLELK.K	
											11	1470	1484	1670.87	1669.86	1669.87	-0.00	0	49	2,40E-04	R.SPLVYLYNSTSTGR.A	
											12	1521	1537	1965.91	1964.91	1964.91	-0.01	0	65	4,20E-06	R.DSCLELSLDSLSWDGIR.F	
CDT1a	AT5G22110	DBP2, CYL2	2/2	TAIR8	452	2,10E-41	4	27	10	379	1	408	417	1362.68	1361.68	1361.68	0.00	0	62	1,80E-05	K.FYNQEIVFFR.Q	
											2	474	486	1453.80	1452.79	1452.80	-0.01	0	51	1,10E-04	R.LYPTPHTIVLGDK.S	
											3	392	405	1602.81	1601.80	1601.80	-0.00	0	58	4,00E-05	R.NIPNAIFSSNPCR.V	
											4	252	266	1629.75	1628.74	1628.75	-0.00	0	115	4,60E-11	K.AHSEYDFFGGGTLTK.E	
											5	362	379	1913.02	1912.01	1912.01	-0.00	0	94	5,00E-09	R.FLFIPGPEDAGPSTVLPR.C	
CDT1a	AT2G31270	CDT1a	2/2	TAIR8	88	5,70E-05	9	32	13													
CDT1b	AT1G08260	EMB2284, POL2A, TIL1, EMB529	2/2	TAIR8	911	2,60E-87	6	30	52	527	1	1984	1990	952.50	951.49	951.49	0.00	0	31	1,80E-02	K.FFDPWK.F	
											2	637	643	1016.57	1015.56	1015.56	-0.00	1	32	1,90E-02	R.KLEWVWR.G	
											3	1821	1830	1160.70	1159.69	1159.70	-0.00	0	37	1,40E-03	K.VFALLLTDLR.R	
											4	197	205	1177.57	1176.56	1176.56	0.00	0	48	3,30E-04	R.EYDVPYHVR.F	
											5	1784	1793	1192.63	1191.62	1191.62	-0.00	0	24	9,50E-02	K.NYADSILQR.L	
											6	1116	1124	1200.63	1199.62	1199.62	0.00	0	36	5,90E-03	R.LIDWSYYK.Q	
											7	722	731	1239.60	1238.59	1238.59	-0.01	0	31	1,50E-02	R.ENPFYVDTVR.S	
											8	86	95	1263.65	1262.64	1262.64	-0.00	0	23	1,30E-01	K.FRPYFYAATK.D	
											9	33	44	1312.66	1311.65	1311.65	0.00	0	35	7,00E-03	K.LGFGLFSEGETR.L	
											10	1251	1261	1336.69	1335.68	1335.68	0.00	0	65	8,10E-06	K.SYNYQGWLELK.K	
											11	141	153	1553.75	1552.75	1552.78	-0.04	0	22	1,50E-01	K.ISFDTVQQLMEVK.R	Oxidation (M)
											12	1470	1484	1670.88	1669.87	1669.87	0.00	0	65	5,90E-06	R.SPLVYLYNSTSTGR.A	
											13	1521	1537	1965.92	1964.92	1964.91	0.00	0	78	2,80E-07	R.DSCLELSLDSLSWDGIR.F	
CDT1b	AT5G22110	DBP2, CYL2	2/2	TAIR8	777	6,50E-74	5	37	14	668	1	135	141	894.49	893.48	893.48	-0.00	0	31	2,20E-02	R.FMLLSQR.V	
											2	135	141	910.48	909.48	909.47	0.00	0	(11)	1,90E+00	R.FMLLSQR.V	Oxidation (M)
											3	408	417	1362.68	1361.67	1361.68	-0.00	0	60	2,80E-05	K.FYNQEIVFFR.Q	
											4	474	486	1453.80	1452.79	1452.80	-0.00	0	70	1,40E-06	R.LYPTPHTIVLGDK.S	
											5	392	405	1602.80	1601.80	1601.80	-0.00	0	71	1,80E-06	R.NIPNAIFSSNPCR.V	
											6	252	266	1629.75	1628.74	1628.75	-0.00	0	133	7,10E-13	K.AHSEYDFFGGGTLTK.E	
											7	111	127	1824.87	1823.86	1823.88	-0.02	0	111	1,20E-10	K.FNEHTSSLPIHGEASAK.T	
											8	362	379	1913.01	1912.00	1912.01	-0.00	0	111	1,10E-10	R.FLFIPGPEDAGPSTVLPR.C	
											9	278	299	2624.26	2623.25	2623.25	0.01	0	33	7,00E-03	R.QAVNDTPVILSDIWLDDEEVMR.K	Oxidation (M)
											10	74	101	2862.53	2861.52	2861.52	0.00	0	50	6,80E-05	R.LLGAHNAPEEPTTSASSLAIIDAFLVPK.F	
CDT1b	AT2G27470	CCAAT-box binding transcription fa	2/2	TAIR8						38	1	6	17	1322.80	1321.79	1321.80	-0.01	0	36	5,60E-04	K.VVVDELPLAIVR.R	
CDT1b	AT3G54710	CDT1b	2/2	TAIR8	910	3,30E-87	7	58	25	671	1	83	89	908.48	907.48	907.48	0.00	0	31	2,20E-02	K.IEYLTNR.I	
											2	419	429	1270.71	1269.70	1269.70	-0.00	0	63	8,10E-06	K.IIANQINITDR.R	
											3	172	183	1426.76	1425.75	1425.73	0.03	0	38	2,60E-03	K.ELLPEPFNSPQR.D	
											4	389	400	1453.82	1452.82	1452.83	-0.02	0	69	1,20E-06	K.HNLPQLVNLIHR.V	
											5	184	198	1507.76	1506.75	1506.76	-0.00	0	46	5,50E-04	R.DSYSGIVSVGLGEPK.L	
											6	472	486	1717.87	1716.86	1716.87	-0.01	1	64	6,90E-06	R.ARLEEATSHDISLIY.-	
											7	50	65	1913.96	1912.96	1912.96	-0.00	0	115	6,60E-11	R.FEILEEFFNGLDTAIR.L	
											8	167	183	2009.03	2008.03	2008.03	0.00	1	86	4,90E-08	K.DEIPKELLPEPFNSPQR.D	
											9	384	400	2038.12	2037.11	2037.11	-0.00	1	111	6,60E-11	K.TEPEKHNLPQLVNLIHR.V	
											10	46	65	2409.24	2408.23	2408.24	-0.00	1	49	2,10E-04	K.LPERFEILEEFFNGLDTAIR.L	

Supplemental Table II: Primers sequences

Where applicable, restriction sites introduced in primers are underlined.

Primer name	Sequence 5'->3'
Primers used for genotyping	
GABI T-DNA	CGCCTATAAATACGACGGATCG
CDT1a mut	CAAGCTCTTCTTTTGTGATAACCC
CDT1a WT	CCCTCAGGTATAATGCCCTATCTATTTTAG
HLK3mut	GGGGTCTAATTGTGCACACGTG
Primers used for cloning	
HA-dir	GTTGGTACCTACCCATACGATGTTCCT
HA-rev	TCGAGCTCAGCGTAATCTGGAACGTCATATGG
pCDT1-HindIII	TCAAGCTTGAATAGGGATGTGGTAATATTC
pCDT1-XbaI	AATGGATCTAGAAGAGCCTGGTGTACT
CDT1-ClaI	CAATGTCATCCATTACTTAATTC
CDT1a-KpnI	CTGGTACCAGAAAGCAGTGGAGCCATCTC
CDT1a KpnI dir	GCGGTACCATGAGTACACCAGGCTCT
CDT1a EcoRI rev	CGGAATTCGAAAGCAGTGGAGCCATCTC
CDT1b KpnI dir	GCGGTACCATGAGCTCGTCGTCTAAAATTG
CDT1b EcoRI rev	GCGAATTCCCGTAGATAAGTGAAATGTC
CDT1a rev stop	CGGAATTCTCAAGAAAGCAGTGGAGCCATC
CDT1b rev stop	GCGGAATTCAGTAGATAAGTGAAATGTC
Pol ε EcoRI dir	GGGAATTCATGAGCAGCACCAGTCAGAAGAGG
Pol ε XhoI stop	TCCTCGAGTCACAAAGCAGAGAGTTCGACTTCT
Primers used for qRT-PCR	
CYCB1;1 F	GGAAGCAACAAGAAGAAGGGAG
CYCB1;1 R	AGGGATCAAAGCCACAGCG
RAD51 F	CTTAGGGATGCTGGTCTCTGTAC
RAD51 R	GTCAACCTTGGCATCACTAATTC
PARP2 F	AGCCTGAAGGCCCGGGTAACA
PARP2 R	GCTGTCTCAGTTTTGGCTGCCG
BRCA1 F	AGGTGAACCTGTCTCTGCGGATTT
BRCA1 R	TTCTCCGGCTTCTTGTCAACTCCA
KU70 F	GGTGTAGCTGCTCCTCGCGC
KU70 R	GCATAGTGTCTCTGCAAAGCGGG
XRCC4 F	AGAAGAGCGAGGAAGAAGCCTCAA
XRCC4 R	AACTCTGACCTGAAAATCTCACGGC
PDF2 F	TAACGTGGCCAAAATGATGC
PDF2 R	GTTCTCCACAACCGCTTGGT

Supplemental methods: Immuno-fluorescence

Immuno-detection of the phosphorylated H2AX protein in the wild-type (WS) and CDT1-RNAi plants was performed 15 minutes after γ-irradiation (100 Gy). Seedlings were fixed in PFA 4% in PHEM (PIPES 60mM ; HEPES 25mM ; EGTA 10mM ; $MgCl_2$ 2mM pH 6.9) during 1 hour at room temperature (apply vacuum 20 min to facilitate uptake of the fixation solution). Seedlings were washed 5 min in PHEM and 5 min in PBS pH6.9 and chopped on a petri dish in PBS supplemented with 0.1% triton (w/v). The mixture was filtered (50µm) and centrifugated 10 min at 2000g. Supernatant was carefully removed and pellet was washed once with PBS, gently resuspended in 20µl PBS and a drop was placed on a poly-lysine slide and air dried. Slides were rehydrated with PBS and permealized 2 times by 10min incubation in PBST (PBS, 0.1% Tween20 v/v). Slides were placed in a moist chamber and incubated overnight at 4°C with the primary antibody (Abcam, rabbit gammaH2A.X (phosphor S139)), diluted (1/250 v/v) in PBST supplemented with BSA (3% w/v). Slides were washed 5 x 10 min in PBST (at RT) and incubated 1 h at RT in the dark with the secondary antibody (A11037 Invitrogen, Alexa Fluor 594 goat anti-rabbit) diluted (1/400 v/v) in PBST, 3% BSA. Slides were washed 5x 10 min in PBST. Slides were mounted with a drop of Vectashield with DAPI and observed as described for pollen mitosis analysis with the suitable cube fluorescence filters (BP340-380, DS 400, BP 450-490 for DAPI) (BP570-590, DS595, BP 605-655 for A594). .