Supporting Information

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SI Materials and Methods

Cytogenetic Characterization. Tissues were fixed in ice-cold Carnoys fixative (1:3 acetic acid:ethanol), except those for volume measurements, which were fixed in 1% formaldehyde under vacuum infiltration for 30 min and washed thereafter in PBS buffer, as described previously (1, 2). Spread preparations of nuclei were prepared as described previously (1–5), with a modified enzymatic cell wall degrading mixture comprising 0.6% Cellulase R10 (Yakult) and 0.25% Macerozyme R10 (Duchefa) in 10 mM citrate buffer (pH 4.5). Slides were mounted in Vectashield (Vector Laboratories) with DAPI (2 μg mL^{-1}) before observation with an Axioplan 2 fluorescence microscope (Zeiss). Hoechst 33258 staining was performed in essence similar as DAPI staining described above, with the exception that the slides were washed in 2× SSC for at least 3 h to remove excess stain.

Nuclear size (area of the spread nucleus) and relative heterochromatic fraction [i.e., fluorescence intensity of intensely DAPI-stained patches (chromocenters) relative to the fluorescence of the entire nucleus (1, 3–6)] measurements were performed using a custom-made macro (1) in ImagePro-Plus (Media Cybernetics) designed and kindly provided by Penka Pavlova (Wageningen University, Wageningen, The Netherlands). For each data point (biological replicate), >50 nuclei of at least 100 pooled embryonic cotyledons were measured. Nuclear volumes were measured and calculated from Z-stacked images (10 slices, total stack depth 3–5 μm) on nuclei stained with propidium-iodide (5 μg mL^{-1}), taken with a confocal microscope as described previously (2), using Imaris 6.2.0 software (Bitplane).

FISH Analysis. FISH experiments were carried out as described previously (1, 3–5). Nuclei were counterstained with DAPI (2 μg mL^{-1} in Vectashield) before observation. Slides were examined with an Olympus BX6000 epifluorescence microscope coupled to a CCD camera (Coolsnap FX; Photometrics). After acquisition, the images were processed, pseudocolored and merged using Adobe Photoshop software. Plasmid pAL1 (7) was used to detect the 180-bp centromeric tandem repeat. BAC F28D6 (GenBank accession no. AF147262; Nottingham Arabidopsis Stock Centre) in pBeloBAC-Kan vector was used for the detection of pericentromeric repeats. The 45S rDNA probe was described previously (8).

5-Methylcytosine Detection by Immunolabeling. Slides were dried at 60 °C for 30 m, treated with 10 μg mL^{-1} of RNase A (Roche) for 60 min at 37 °C, rinsed twice for 5 min in PBS, fixed in 1% formaldehyde, dehydrated in successive ethanol baths, and air-dried. Denaturation was carried out by adding 50 μL of HB50 (50% formamide in 1× SSC) and heating at 80 °C for 2 min. The slides where then washed in 70% ice-cold ethanol and dehydrated by successive ethanol baths. Slides were incubated for 1 h in 1% BSA to prevent aspecific binding and washed three times for 5 min in TNT [1 M Tris/HCl (pH 8.0), 1 M NaCl, and 0.5% Tween 20]. Incubation with the antibody against 5-methylcytosine raised in mouse, 1:50 in 1% BSA in PBS (Eurogentec), was carried out at 37 °C for a minimum of 1 h. Antibody detection was performed with the same antibodies used for FISH digoxigenin-labeled probes as described above. The nuclei were counterstained with DAPI (2 μg mL^{-1}) in Vectashield before observation.

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4. Tessadori F, et al. (2009) *Phytochrome B* and *histone deacetylase 6* control light-induced chromatin compaction in *Arabidopsis thaliana*. *PLoS Genet* 5:e1000638.
5. van Zanten M, et al. (2010) Photoreceptors CRYTOCHROME2 and phytochrome B control chromatin compaction in *Arabidopsis*. *Plant Physiol* 154:1686–1696.
6. Soppe WJ, et al. (2002) DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in *Arabidopsis*. *EMBO J* 21:6549–6559.
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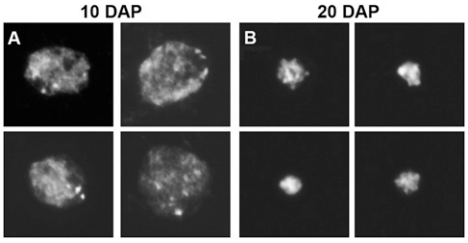

Fig. S1. Nuclear size in Landsberg *erecta* embryonic cotyledons during seed maturation. Representative DAPI-stained spread nuclei at 10 d after pollination (DAP) (*A*) and 20 DAP (*B*) are shown.

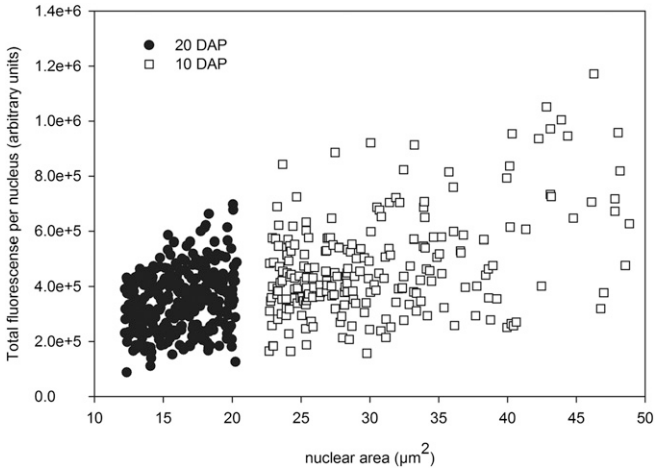

Fig. S2. DAPI intensity measurements of small and large nuclei revealed no major differences, indicating equal ploidy levels. Cotyledons were isolated from embryos from 10 DAP and 20 DAP tissues in equal amounts and pooled. Four independent biological replicas were used. Spread preparations were made from each pooled sample and stained with equal amounts of DAPI, as described in the text. Approximately 200 nuclei were photographed from each replica (total, ~800) in a single session without changing the settings of the fluorescence microscope. Thereafter, the nuclear size in combination with intensity per pixel and overall intensity was measured from each group of ~800 nuclei. All nuclei of apparent size within 2 times the SE from the mean were taken into account. As expected, this resulted in two size classes of nuclei, small/20 DAP (black circles in graph) and large/10 DAP (white squares in graph). Plotting the intensity of the whole nucleus revealed a correlation between area and intensity per nucleus, but importantly, the intensities did not fall into two distinguishable intensity classes. Given that a doubling of ploidy levels should result in a doubling of the DAPI staining, we conclude that nuclei at 10 DAP have a similar ploidy as nuclei from 20 DAP, and thus that changes in ploidy levels cannot account for the changes in nuclear size observed during seed maturation.

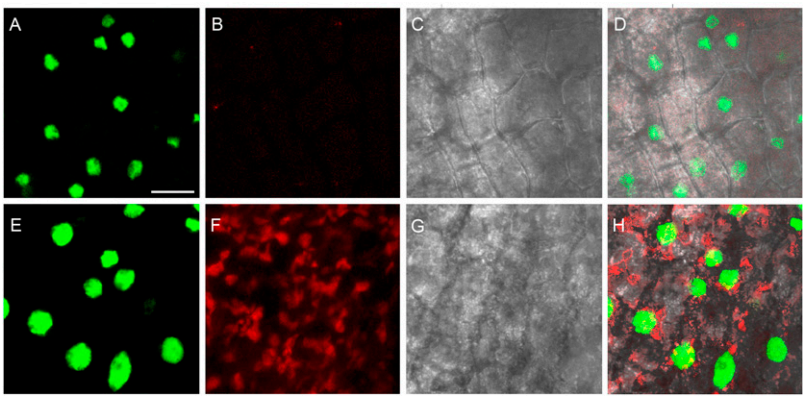

Fig. S3. In vivo analysis of nuclear size during imbibition and germination. Z-minimum projection–derived confocal microscopy images of 2-h (*A*–*D*) and 72-h (*E*–*F*) imbibed transgenic seeds expressing the nuclear marker Histone 2B fused to fluorescent GFP (pH2B:H2B::GFP). (*A* and *E*) GFP signal. (*B* and *F*) Chlorophyll autofluorescence signal. (*C* and *D*) Bright-field image. (*D* and *H*) All signals merged. Note that germinating seeds accumulate chlorophyll (*F*), which is largely absent in the 2-h imbibed seeds (*B*). (Scale bar: 10 μm.)

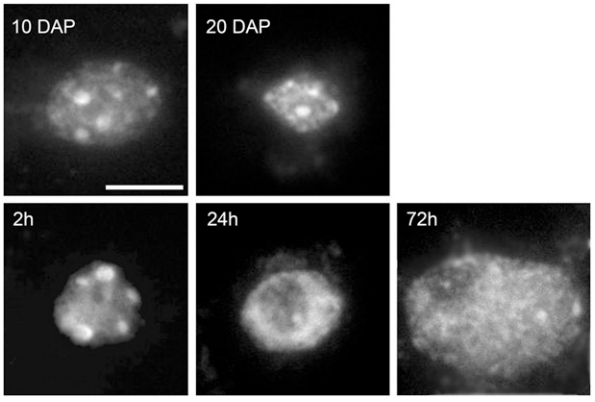

Fig. S4. Cytogenetic characterization of embryonic cotyledon nuclei using Hoechst 33258 staining. Representative Columbia-0 WT nuclei during seed maturation (*Upper*; 10 and 20 DAP) and imbibition/germination (*Lower*; 2 h, 24 h, and 72 h) are shown. (Scale bar: 5 μm.)

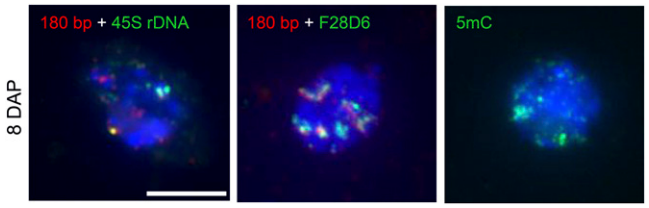

Fig. S5. Cytogenetic characterization of embryonic cotyledon nuclei at the end of embryo development. Representative FISH signals for the centromeric 180-bp repeat (red, *Left* and *Middle*) and subtelomeric 45S rDNA repeats (green; *Left*) or pericentromeric F28D6 (green, *Middle*) and immunolabeling of 5mC (green, *Right*), during late embryo development (8 DAP). Each nucleus is counterstained with DAPI (blue). (Scale bar: 5 μm.)

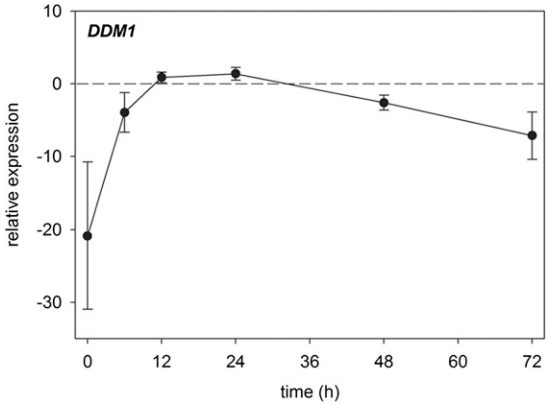

Fig. S6. Relative expression of *DDM1* and *ACTIN8* during imbibition and germination. *DDM1* (black circles)-specific primers were used as described previously (9). Error bars represent SE; $n = 4$.

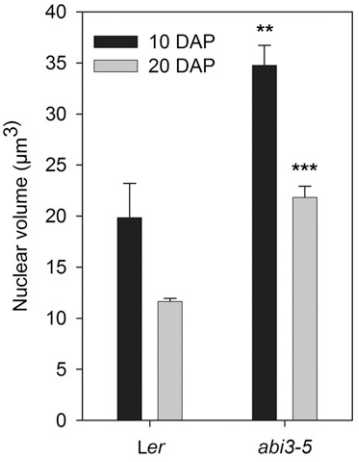

Fig. S7. Nuclear volume of the *abi3-5* mutant at 10 DAP (black bars) and 20 DAP (gray bars). Significance levels: $^{**}0.001 > P < 0.01$, $^{***}0.0001 > P < 0.001$, two-tailed Student *t*-test compared with control. Error bars represent SE; $n \geq 25$.

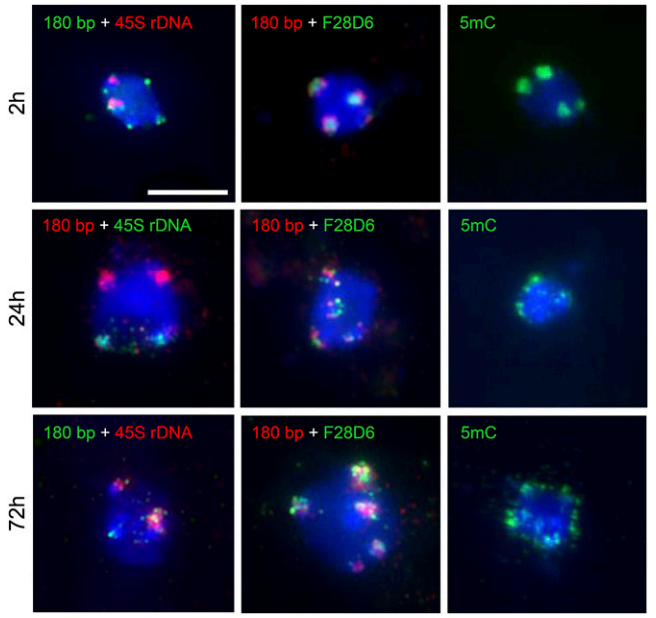

Fig. S8. Cytogenetic characterization of *linc1-1 linc2-1* embryonic cotyledon nuclei during seed imbibition and germination. Representative FISH signals for the centromeric 180-bp repeat and subtelomeric 45S rDNA repeat (*Left*), 180-bp repeat and pericentromeric F28D6 (*Middle*), and immunolabeling of 5mC (*Right*) during imbibition/germination (2 h, 24 h, and 72 h) are shown. Each nucleus was counterstained with DAPI (blue). (Scale bar: 5 μm.)

Table S1. Average size (μm^2) and number of nuclei analyzed for all nuclear size measurements in the study

Figure	Developmental phase	Genotype	Time point/stage	Average size, μm^2 ± SE	Nuclei measured
Fig. 1C	Seed maturation	Col-0	8 DAP	63.04 ± 8.89	149
			10 DAP	35.92 ± 6.63	150
			12 DAP	21.49 ± 2.95	99
			14 DAP	21.06 ± 2.11	102
			16 DAP	24.68 ± 3.07	209
			18 DAP	18.61 ± 2.05	102
			20 DAP	16.31 ± 2.06	157
		Ler	8 DAP	56.69 ± 9.82	155
			10 DAP	32.22 ± 3.93	155
			12 DAP	30.25 ± 4.13	154
			14 DAP	27.37 ± 2.54	105
			16 DAP	26.35 ± 2.46	102
			18 DAP	16.32 ± 2.14	105
			20 DAP	12.77 ± 1.54	154
Fig. 1*E*	Imbibition/germination	Col-0	2 h	16.85 ± 1.90	98
			24 h	21.38 ± 1.83	92
			48 h	31.54 ± 4.28	86
			72 h	99.91 ± 9.52	100
		Ler	2 h	12.97 ± 1.80	104
			24 h	15.87 ± 1.67	108
			48 h	23.97 ± 3.94	142
			72 h	86.38 ± 10.36	92
Fig. 1*H*		Col-0	Dormant	9.21 ± 0.66	101
			Germinating	56.91 ± 5.01	103
		Ler	Dormant	12.29 ± 1.09	107
			Germinating	68.75 ± 6.84	105
Fig. 4*A*	Seed maturation	*abi3-5*	10 DAP	49.18 ± 7.11	155
			20 DAP	38.10 ± 5.25	99
		Ler	10 DAP	32.22 ± 3.93	155
			20 DAP	12.77 ± 1.54	154
Fig. 4*B*	Seed maturation	*linc1-1 linc2-1*	10 DAP	17.91 ± 1.83	105
			20 DAP	10.59 ± 0.94	103
		Col-0	10 DAP	35.92 ± 6.63	150
			20 DAP	16.31 ± 2.06	157

Table S1. Cont.

Figure	Developmental phase	Genotype	Time point/stage	Average size, $\mu m^2 \pm$ SE	Nuclei measured
Fig. 4*C*	Seed maturation	Col-0	2 h	13.90 ± 1.50	103
			24 h	25.19 ± 2.44	110
			48 h	63.45 ± 7.52	103
			72 h	100.52 ± 11.45	89
		linc1-1 linc2-1	2 h	7.63 ± 0.61	101
			24 h	15.50 ± 1.64	100
			48 h	14.60 ± 4.75	105
			72 h	31.31 ± 4.20	104
Fig. 5*A*	Seed maturation	Col-0	10 DAP	35.92 ± 6.63	150
			20 DAP	16.31 ± 2.06	157
		L*er*	10 DAP	32.22 ± 3.93	155
			20 DAP	68.75 ± 6.84	105
		Cvi-0	10 DAP	35.92 ± 3.10	107
			20 DAP	17.56 ± 1.89	108
		NIL-*DOG1*	10 DAP	35.41 ± 4.25	103
			20 DAP	16.08 ± 1.49	104
		dog1-1	10 DAP	25.45 ± 1.85	103
			20 DAP	12.30 ± 1.14	106
		dog1-2	10 DAP	30.61 ± 2.19	104
			20 DAP	12.48 ± 1.58	104
		rdo2-1	10 DAP	31.01 ± 3.17	100
			20 DAP	12.25 ± 1.20	105
		hub1-2	10 DAP	26.08 ± 2.67	105
Fig. 5*C*		*C. plantagineum*	Fresh	136.30 ± 16.61	102
			Dried	74.80 ± 8.46	102

Table S2. Quantitative RT-PCR primers used in this study

ACT8	5′-CTCAGGTATTGCAGACCGTATGAG-3′
	5′-CTGGACCTGCTTCATCATACTCTG-3′
RDO2	5′-CCACTGGAAGTTCTGTTGAGG-3′
	5′-CTGCTAGCAAATGGACACGA-3′
HUB1	5′-TGGGGCATTAGAACTGGAAC-3′
	5′-GGCCGATGATCCTTCTATGA-3′