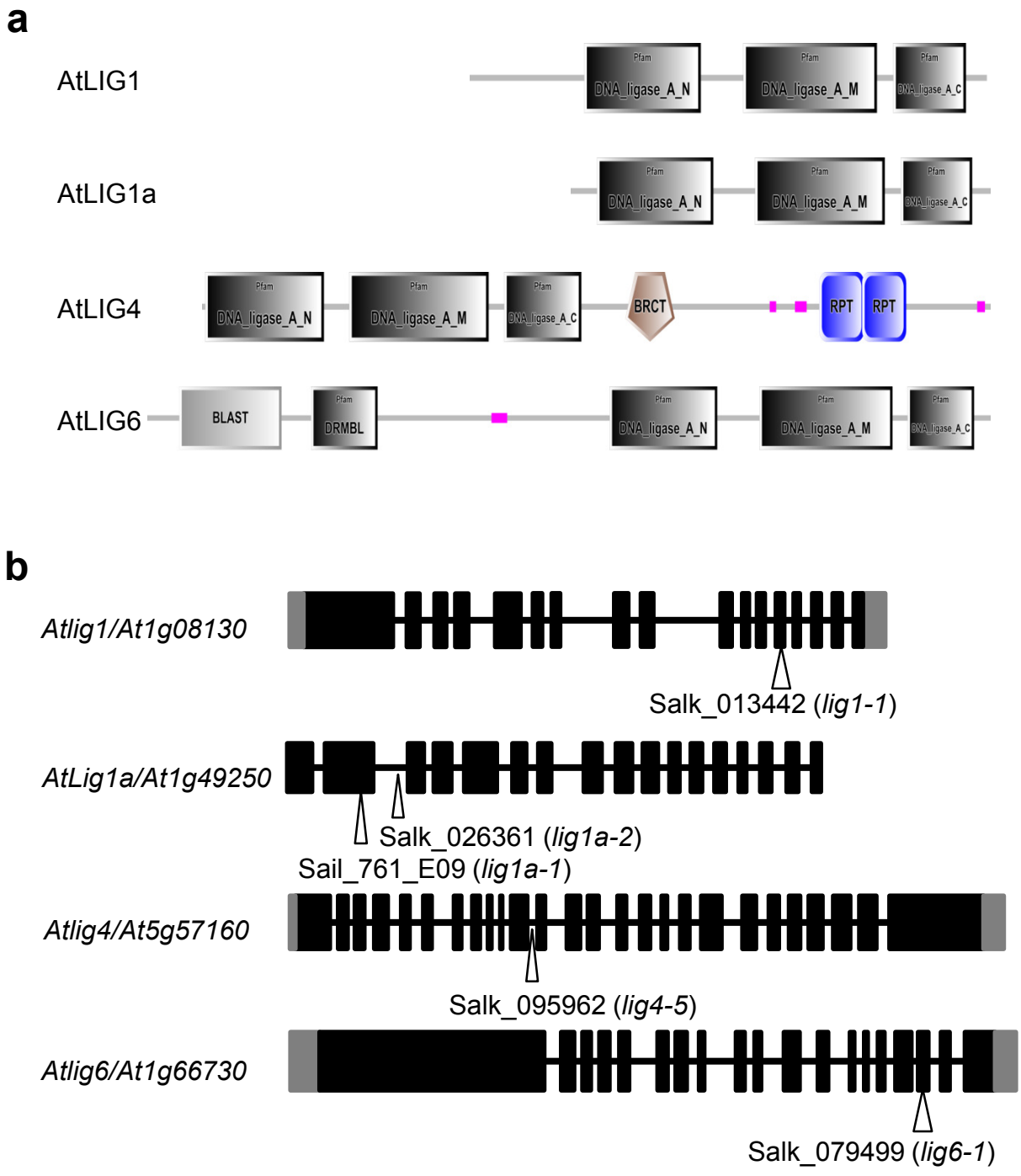
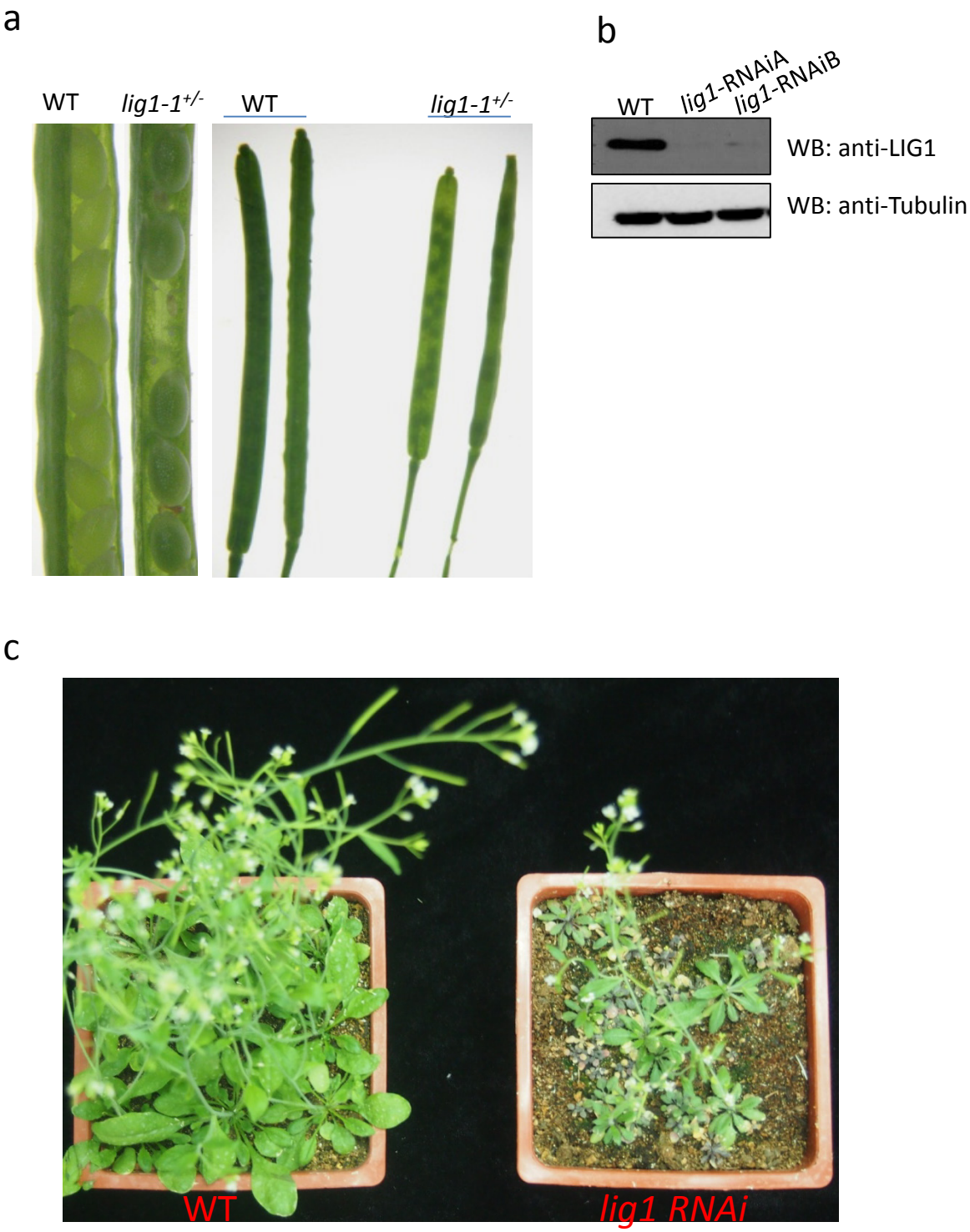
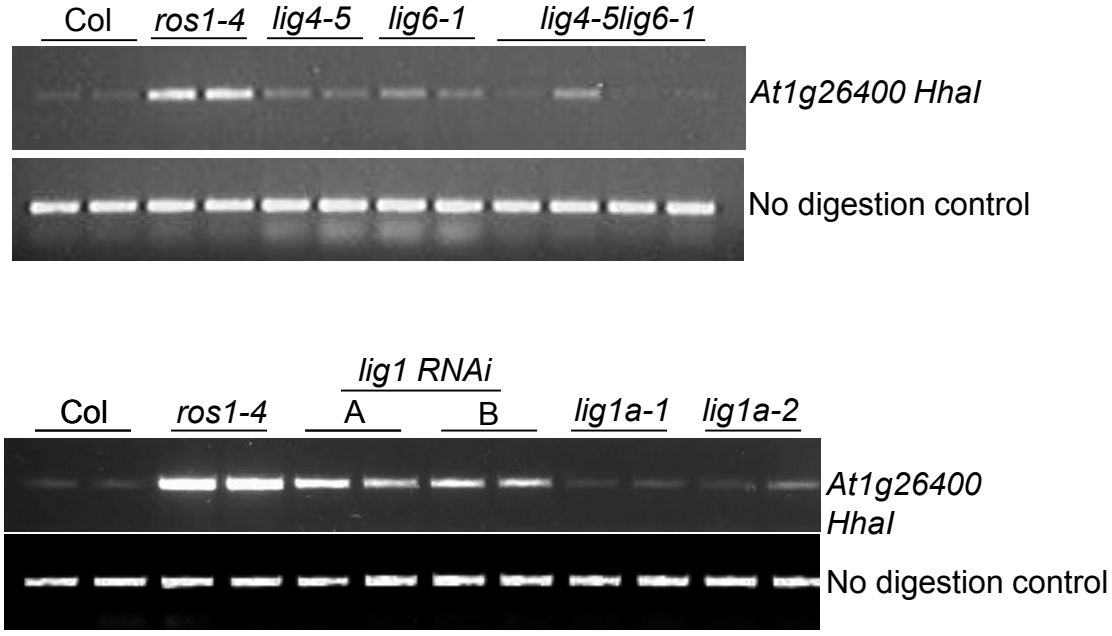


Supplementary Figure 1. Characterization of DNA Ligases from *Arabidopsis*.
(a) Domain structure for Ligase proteins predicted in SMART (http://smart.embl-heidelberg.de/).
(b) Gene structure and T-DNA position for *ligases* used in this study.

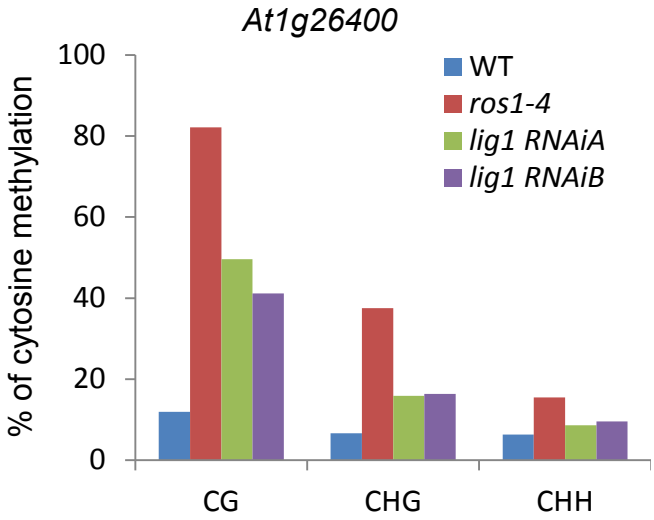


Supplementary Figure 2. Characterization of *lig1* RNAi lines.
(a) Developmental phenotype in *lig1-1*$^{+/-}$ lines.
(b) Determine the LIG1 protein level in different RNAi lines by Western blotting. Tubulin as a loading control.
(c) Developmental phenotype in *lig1* RNAi plant.

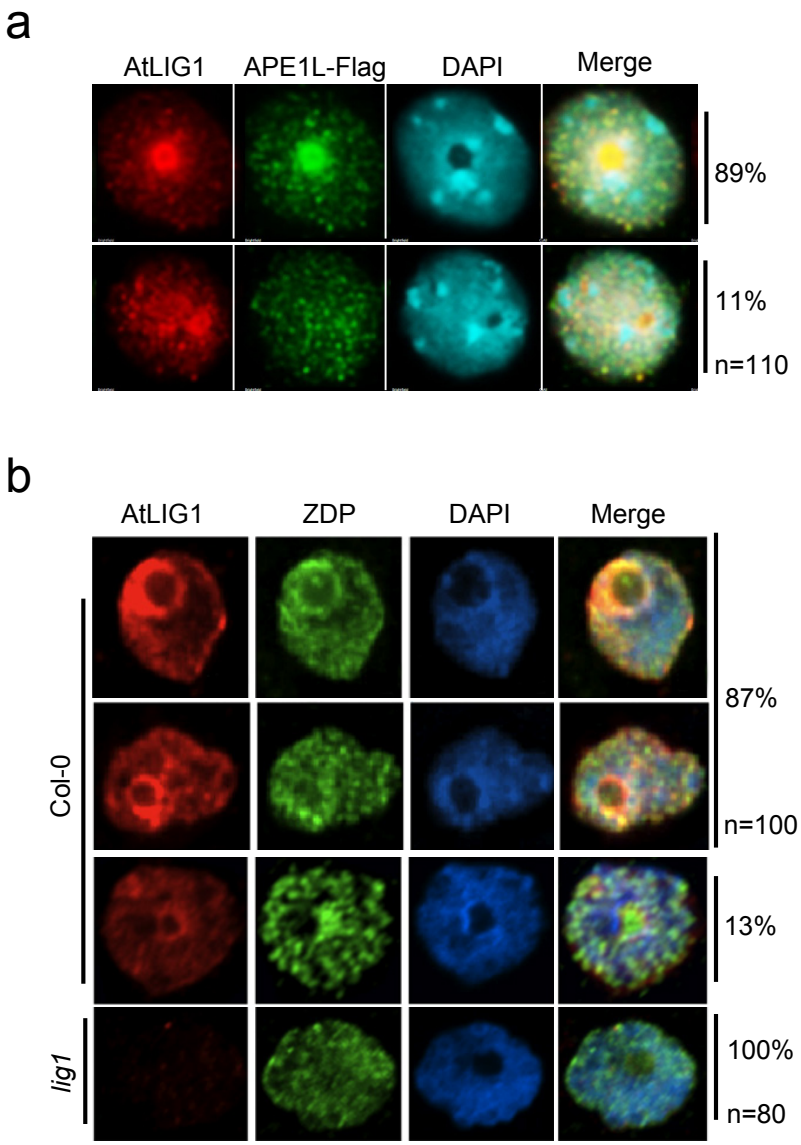
a



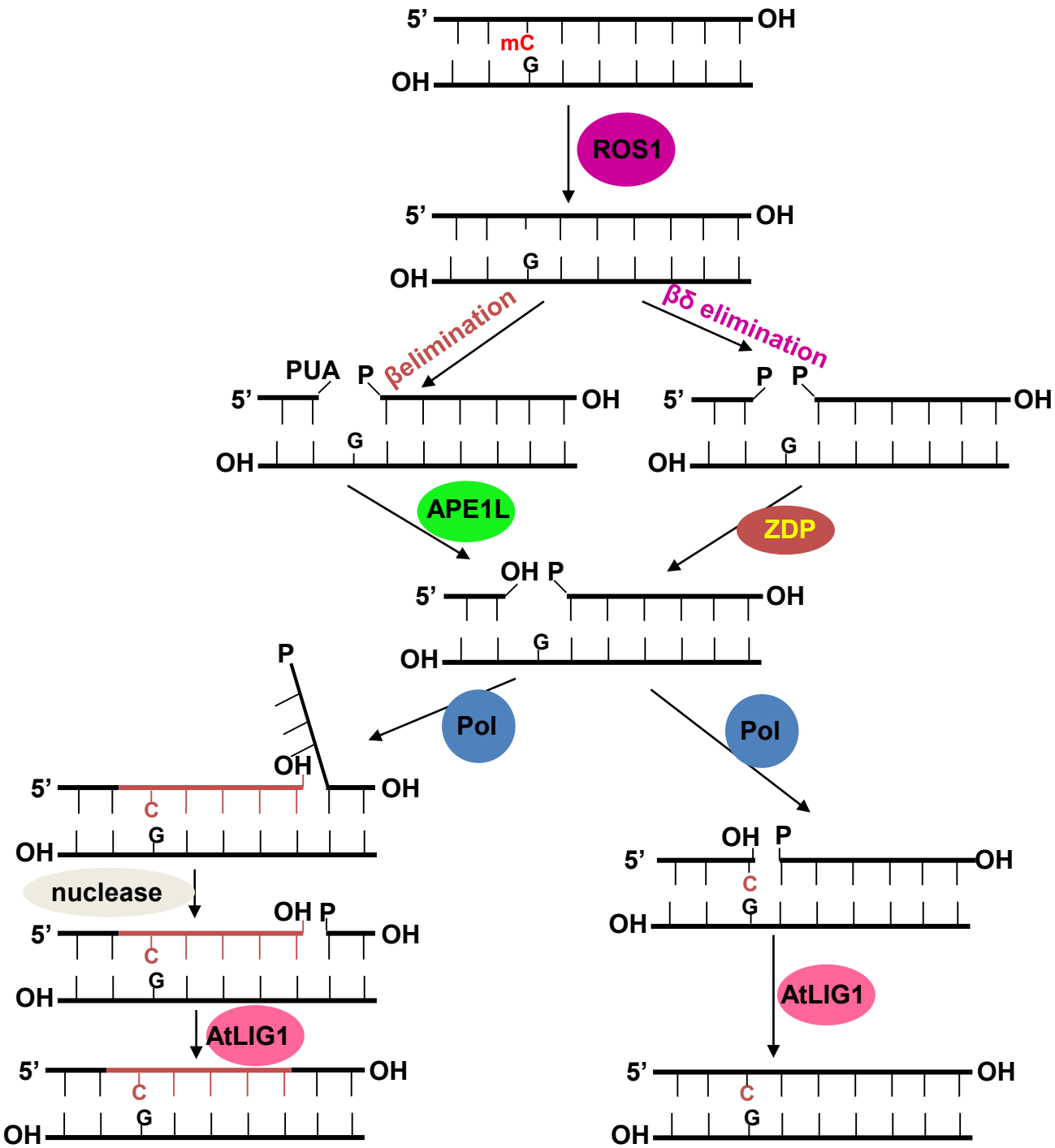
b



Supplementary Figure 3. *Lig1* mutation show DNA hypermethylation at 3' end of *At1g26400* region.
(a) Analysis of DNA methylation level at the *At1g26400* locus by CHOP PCR. HhaI digestion is sensitive to CG DNA methylation, so DNA hypermethylation results in increased levels of the PCR product. Undigested DNA is shown as a control.
(b) Percent methylation of 3' end *At1g26400* locus in different genetic background. Methylation level for each context were determined by bisulfite sequencing.



Supplementary Figure 4. Co-localization analysis of AtLIG1, APE1L and ZDP. **(a)** Dual immunofluorescence using anti-LIG1 (red) in transgenic lines expressing APE1L-Flag (green). **(b)** Dual immunofluorescence using anti-LIG1 (red) and anti-ZDP (green) in wild type plants. In all panels the DNA was stained with DAPI (blue). The frequency of nuclei displaying each interphase pattern is shown on the right.



Supplementary Figure 5. Diagram of ROS1-initiated active DNA demethylation by the base excision repair (BER) pathway. ROS1 is a bifunctional DNA glycosylase/lyase that removes the 5-methylcytosine base and then cleaves the DNA backbone at the abasic site via β or β, δ elimination reaction, resulting in a gap with 3' dRP or only phosphate at the 3' terminus which are removed by APE1L or ZDP. Then the gap is filled with an unmethylated cytosine nucleotide by an as yet unknown DNA polymerase and AtLIG1 through single nucleotide repair (SN-BER) or long-patch BER (LP-BER) pathway. The diagram is based on the model in Li et al (Li et al., 2015).

Supplementary information, Materials and Methods

Plant materials and growth condition

T-DNA insertion mutant *atlig1-1* (salk_013442), *atlig1a-1* (sail_761_E09) and *atlig1a-2* (salk_026361) were obtained from the Arabidopsis Biological Resource Center (http://www.arabidopsis.org). *Arabidopsis* seedlings were grown on Murashige-Skoog (MS) nutrient agar plates or soil at 23℃ with 16 h of light and 8 h of darkness.

Immunolocalization

Immunofluorescence localization was performed in 2-3 week-old leaves as described by Pontes et al [12]. Nuclei preparations were incubated overnight at room temperature with sheep anti-AtLIG1 and mouse anti-Flag (F3165, Sigma) or rabbit anti-ZDP [3]. Primary antibodies were visualized using mouse Alexa 488-conjugated and rabbit Alexa-594 secondary antibody at a 1:200 dilution (Molecular Probes) for 2 h at 37℃. DNA was counterstained using DAPI in Prolong Gold (Invitrogen). Nuclei were examined using a Nikon Eclipse E800i epifluorescence microscope equipped with a Photometrics Coolsnap ES.

Bisulfite sequencing

Female C24 wild type or *atlig1-3*$^{+/-}$ plants (C24 background) were crossed with male wild type plants (Col-0) to enable identification of the maternal and paternal alleles. F1 seeds were dissected under a microscope and the endosperm tissues were collected in a tube floating on liquid nitrogen [13]. DNA was extracted using the CTAB method and treated with RNase. DNA was then subjected to bisulfite conversion using BisulFlash DNA Modification Kit (EPIGENTEK) according to the manufacturer's instructions. The bottom strand of 5 ´SINE related repeats in *FWA* gene promoter was amplified by two rounds of PCR (for primer sequence see Table S2). Primers *FWA*-bottom F and *FWA*-bottom R were used for the first round PCR. Primers *FWA*-BF1 and *FWA*-BF2 were used for the second round PCR. The bottom strand of *MEA ISR* was amplified using the primers JP1026 and JP1027. The PCR conditions were 94 ℃ for 3 min, 45 cycles of 94 ℃ for 30 sec, 50 ℃ for 30 sec and 72 ℃ for 2 min, then 72 ℃ for 10 min. The PCR

products were cloned into T-easy vector (Promega) and more than 30 colonies were selected for sequencing. The maternal and paternal alleles were separated based on the DNA sequence polymorphism.

RNA purification and Real-Time PCR analysis

To obtain immature seeds at 3 DAP, *atlig1-1*$^{+/-}$ (contain *pFWA::ΔFWA-GFP*) mutant plants were pollinated with wild-type pollen, and then 200 GFP-positive and 200 GFP-negative seeds were harvested under a fluorescence microscope. Total RNA was isolated using the Trizol method. DNAase treatment and LiCl precipitation were applied to remove DNA and polysaccharide contaminations, respectively. RNA was reverse transcribed into cDNA by the SuperScript® *III* First-Strand Synthesis System (Invitrogen) with an oligo dT primer. Real time-PCR analysis was performed using SYBR Premix Ex Taq (TaKaRa) and CFX96 real-time system (Bio-Rad). *ACT11* was used as the internal control.

Microscopy

Images of seed phenotypes were captured using an Olympus SZX7 microscope equipped with a Canon Powershot A640 camera. Fluorescence was detected with an Olympus BX53 fluorescence microscope equipped with an Olympus DP80 digital camera.

Whole genome bisulfite sequencing and DMR analysis

Bisulfite conversion, Illumiana library construction, sequencing and the bioinformatics analysis were performed exactly as described by Qian et al [14].

CHOP-PCR

Genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN). 100 ng of genomic DNA was digested with the CG methylation sensitive enzyme *HhaI* in a 20 μl reaction mixture (no enzyme for control). After digestion, PCR was performed using 1 μl of the digested DNA as template in a 10 μl PCR system using *At1g26400* locus primers (Table S2).

Supplementary Table 1. DNA methylome analysis by whole genome bisulfite sequencing in the different mutants.

Samples	Hyper-DMRs	Hypo-DMRs
lig4lig6	78	60
ros1-4	4991	106
rdd	9290	1052

Supplementary Table 2. Primers used in this study.

Primer name	Sequence 5´to 3´	References
FWA bottom F	TAAAGTATTTTATATATAAGYGAAAAA TAGATAAATTGG	first round amplification of *FWA* for bisulfite sequencing
FWA bottom R	AATTCTATACTAATATCAAAAAATTATA AACCRAAACCC	
FWA-BF1	TTCTATACTAATATCAAARARTTATRRR CCRAARCCC	Second round amplification of *FWA* for bisulfite sequencing
FWA-BF2	TAAAYAAAATGTAAAAAAATYTGATTT TTGGYTGA	
MEA JP1026	AAAGTGGTTGTAGTTTATGAAAGGTTTT AT	amplification of *MEA ISR*
MEA JP1027	CTTAAAAAATTTTCAACTCATTTTTTTT AAAAAA	
*FWA*TkF2	GTGACTCTGGTCAAGACT	q-PCR using endosperm RNA
*FWA*6135r	TTGGTTCCACCAGAACCGGTA	
GFP26r	TCCTCGCCCTTGCTCACCAT	
FWA4910f	GCGCACTGCTTATCAAACTCAAGAACT	
MEA-F	ATTGTGGTCTCAGATCCAAAC	
MEA-R	TCTAGGCACGTCTTAAGCC	
DME-RTf	CAGAAGTGTGGAGGGAAAGCGTCTGGC	
DME-RTr4	AAATATGTCCCGTTGAGCGGAA	
FIS2RTf	GCAAAGAGGAAAAGCGATACATGT	
FIS2RTr2	TGAGACCGTTGTTCCACAGT	
ACT11-F	GGAACAGTGTGACTCACACCATC	

ACT11-R	AAGCTGTTCTTTCCCTCTACGC	
At1g26400-F1	TGACCTGCATAGGCTATAACACA	chop-PCR
At1g26400-R1	ATTGGAATCAATCCGAGTGG	
At1g26400-F2	GTAGTTTGAGATGATTAATGATAGAGTT	Bisulfite sequencing
At1g26400-R2	AAACTTATTCAATCTTCAATACTCTAC	