Supporting Information

Buzas et al. 10.1073/pnas.1404058111

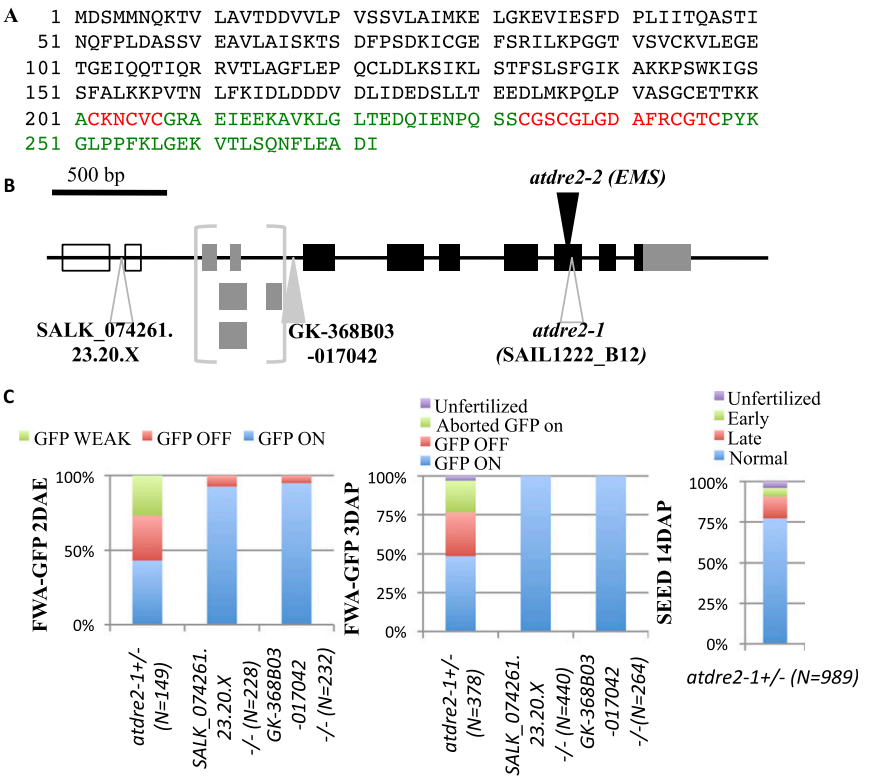

Fig. S1. *ALAC4* mapping and allele complementation. (*A*) To identify the *ALAC4* gene, we identified recombinant F2 plants between *alac4*$^{+/-}$;*pFWA-GFP* in the Col background and L*er*, using dCAPs PCR markers (http://helix.wustl.edu/dcaps) designed to detect single nucleotide polymorphisms (http://polymorph-clark20.weigelworld.org). The coding regions of all of the 14 genes defined by the two closest recombination breakpoints were sequenced. The mutation creates a putative frameshift at amino acid 201 and a stop codon after a further 16 chimeric residues. Amino acids indicated in green and red are probably not translated. The conserved motifs for binding Fe-S clusters are indicated in red. (*B*) Sites of T-DNA insertions. (*C*) We introgressed T-DNA insertions in the genomic region of At5g18400 into the *pFWA-GFP* line. The line with the T-DNA insertion in the 5′ upstream region (SALK_074261; open triangle in the upstream gene) and the GABI-KAT line with the insertion in the first intron (GK-368B03; gray triangle) could be made homozygous and had no *FWA-GFP* phenotype before or after fertilization. Another T-DNA insertion site [SAIL_1222, named *atdre2-1*(6); open triangle in the coding region] was near the EMS mutation and shared with *atdre2-2* gametophytic lethality, *pFWA-GFP* inactivation (monitored at 2 DAE and 3 DAmP), and seed phenotypes (monitored at 14 DAmP).

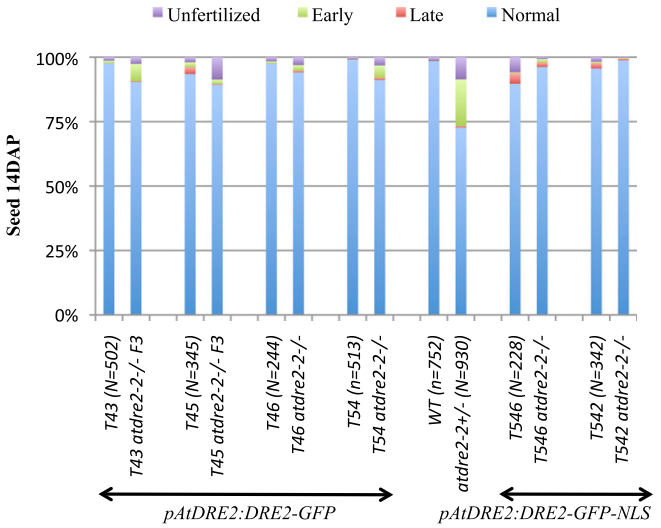

Fig. S2. Seed complementation in *atdre2-2*$^{-/-}$ background. The *pAtDRE2:AtDRE2-EGFP* and *pAtDRE2:AtDRE2-EGFP-NLS* constructs were transformed into Col-0 WT, and T2 transgenics were crossed into *atdre2-2*$^{+/-}$. F2 seeds selected for the transgene and genotyped for *atdre2-2* were selfed, and the seed phenotype of F3 plants homozygously segregating for both the transgene and the *alac4* allele was recorded. For each of the six independent transgenes (T43, T45, T46, T54, T546, and T542) data from the control transgene are presented as well. N indicates the number of seeds analyzed.

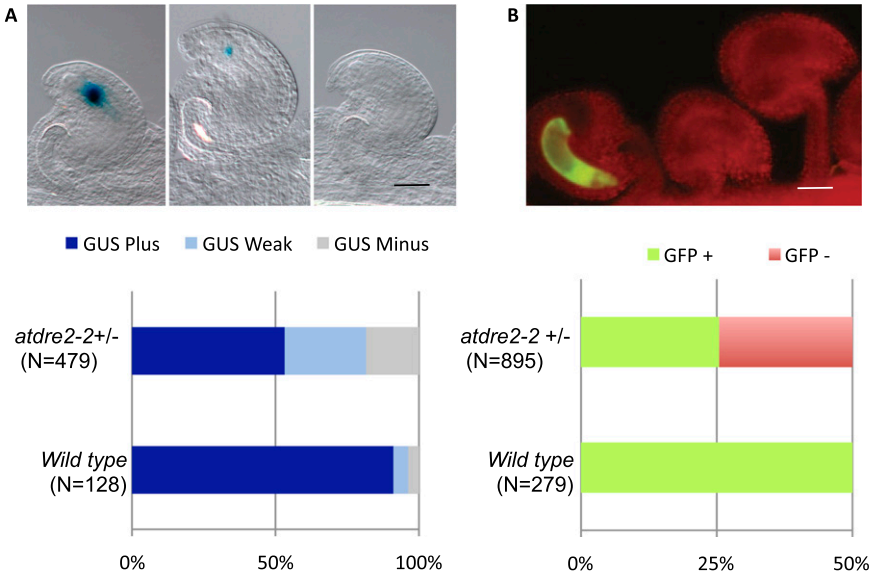

Fig. S3. Gametophytic phenotype of *atdre2-2*$^{+/-}$ monitored with *pFIS2:GUS* (*A*) and *pMEDEA:GFP* (*B*) at 2 DAE. N indicates the number of ovules examined. *pFIS2:GUS* was homozygous, and *pMEDEA:GFP* was heterozygous. (Scale bar: 50 μm.)

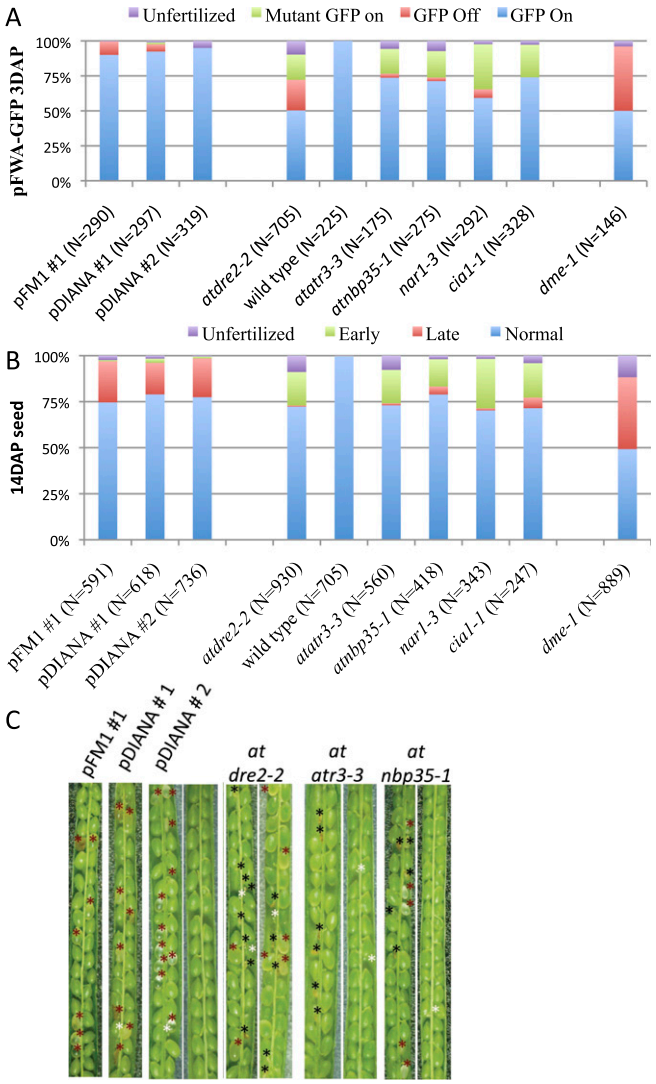

Fig. S4. *pFWA-GFP* and seed phenotype of transgenics and CIA mutants (corresponding to Fig. 3). (*A*) *FWA-GFP* in *atdre2-2*$^{+/-}$;*pFM1:AtDRE2*#1, *atdre2-2*$^{+/-}$;*pDIANA:AtDRE2*#1, *atdre2-2*$^{+/-}$;*pDIANA:AtDRE2*#2, and CIA and DME mutants (*atdre2-2, atatr3-3*, *atnbp35-1*, *nar1-3*, *cia1-1*, and *dme-1*) at 3 DAmP. (*B*) Seed phenotype at 14 DAmP for the same genotypes. The numbers of ovules/seeds analyzed are indicated below each graph. (*C*) Seed phenotype in dissected siliques at 14 DAP (*Left*) or 14 DAmP with WT pollen (*Right*) in the indicated genotypes. White asterisks, unfertilized seeds; black asterisks, early-aborted seeds; red asterisks, late-aborted seeds.

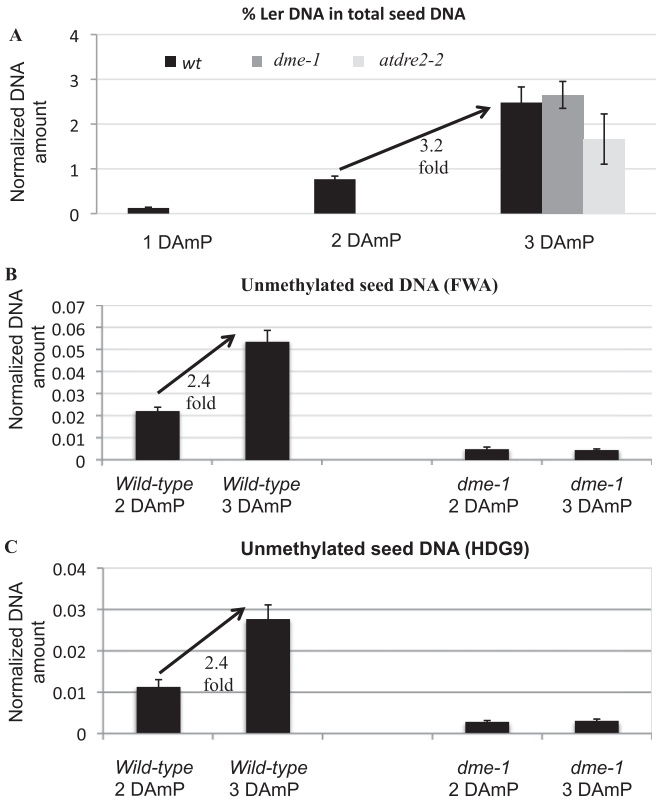

Fig. S5. Quantitative DNA methylation assay in 3 DAmP seeds. To estimate the DNA methylation level of DME target genes at the 3 DAmP stage in *atdre2-2* and WT, we first estimated the amount of endosperm DNA in total DNA extracted from early seed development stages by pollinating with a different genotype (L*er*), followed by PCR amplification using ecotype-specific loci. Extracted DNA was quantified using a NanoDrop spectrophotometer. After double fertilization, at 1 DAmP, 2 DAmP and 3 DAmP, only the paternal allele of the endosperm and of the embryo will inherit the L*er* genome, with the amount of embryo DNA negligible compared with that of the endosperm. (The divisions in the embryo are delayed and reduced in number relative to those in the endosperm.) (*A*) We found that we could recover increasing amounts of L*er*-specific loci at 1 DAmP, 2 DamP, and 3 DAmP, suggesting that most of this DNA is endosperm DNA. At 3 DAmP, 2.5% of the total seed DNA was L*er* DNA. Therefore, 5% of the total DNA should be maternal endosperm DNA, and is predicted to be mostly hypomethylated in specific regions of the DME targets in the WT and hypermethylated in control *dme* mutant seeds. (*B* and *C*) We found that the amount of DNA recovered after digestion with the DNA methylation-specific enzyme *McrBC* at *FWA* (*B*) and *HDG9* (*C*) increased from 2 DAmP to 3 DAmP proportionally with the increase in endosperm DNA (2.4- and 2.4-fold, respectively, compared with 3.2-fold). We also found that at 3 DAmP, *FWA, FIS2, HDG8*, and *HDG9* had drastically reduced amounts of DNA across the methylated region in *dme-1* GFP off seed pools compared with WT GFP on seeds (*C*). These controls indicated that a difference in DNA methylation levels at these genes is quantifiable in our assay. When we assayed 3 DAmP *atdre2-2* pools of GFP off seeds, we also found that the amount of DNA recovered after *McrBC* digestion was reduced compared with WT, although increased compared with *dme-1* (Fig. 4*C*). We note that the amount of endosperm DNA in the total seed DNA, estimated using a L*er*-specific locus, was comparable between wild-type, *dme-1* and *atdre2-2* seed pools at 3 DAmP (*A*). WT, *dme-1*, and *atdre2-2* seed pools were dissected from crosses between *pFWA-GFP*, *dme-1*;*pFWA-GFP*, *atdre2-2*;*pFWA-GFP*, and *Ler*. GFP on seeds from WT and GFP off seeds from *dme1* and *atdre2-2* were used. A minimum of 250 seeds was used for each pool at 3 DAmP, and 1 μg of DNA (quantified using a NanoDrop spectrophotometer) was digested for each sample with *McrBC* (*B* and *C*).

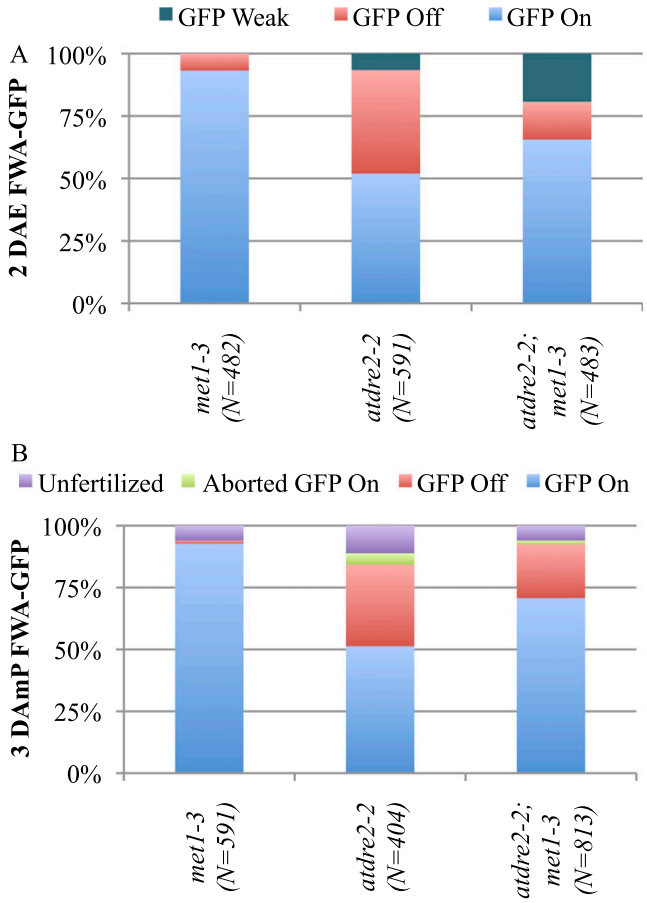

Fig. S6. Genetic interaction between *atdre2-2*$^{+/-}$ and *met1-3*$^{+/-}$ monitored in double mutants by FWA-GFP at 2 DAE (*A*) and 3 DAmP (*B*). N represents numbers of ovules/seeds analyzed.

Table S1. Seed phenotype in self and reciprocal crosses between *atdre2-2;pFWA-GFP* and *pFWA-GFP*

		%			
Parental genotype	*n*	WT	Late aborted	Early aborted	Unfertilized
pFWA-GFP	895	96.7	0.3	0.4	2.5
atdre2-2 self set 1	491	68.3	16.5	5.6	9.6
atdre2-2 self set 2	930	72.36	0.62	18.15	8.86
pFWA-GFP × pFWA-GFP	668	89.9	1.3	3.7	5.2
atdre2-2 × *atdre2-2*	710	70.0*	5.0*	12.9*	12.1*
atdre2-2 × pFWA-GFP	775	75.1*	9.8*	3.0	12.1*
pFWA-GFP × *atdre2-2*	689	83.6	3.7*	1.9	10.9

While investigating the distinct *atdre2-2*$^{+/-}$ seed phenotypes and the gametophytic vs. zygotic nature of the defects in detail, we found that the percentage of each mutant seed class varied among experiments (see, e.g., set 1 and set 2), whereas the total mutant seed percentage was less variable. Seed phenotype was analyzed at 14 d after self or manual pollination. *P* values were calculated relative to the WT control, and the values that reject the null hypothesis are indicated with asterisks. *n* indicates the number of seeds analyzed for each genetic background.

Table S2. Transmission of single and double mutants

Parental genotype							
♀	♂	*n*	WT	*atdre2-2*	*met1-3*	*atdre2-2;met1-3*	λ^2
atdre2-2	WT	107	78	22	—	—	32.53
WT	*atdre2-2*	94	83	17	—	—	40.89
atdre2-2; met1-3	WT	152	34	28	22	16	9.73
WT	*atdre2-2; met1-3*	103	47	13	28	12	35.05

n indicates the number of seedlings genotyped. λ^2 was calculated based on expected ratios of 1:1 for single mutants and 1:1:1:1 for double mutants.

Table S3. Oligonucleotides used in this study

Oligo name	Used for
Vector construction	
CACCGGCCTTCTACCACCTTA	*pAtDRE:2DRE2-EGFP*
TATGTCAGCTTCAAGGAAGTTTT	
CACCGGCCTTCTACCACCTTA	*pAtDRE2:DRE2-NLS-EGFP*
ACCTTTCTCTTCTTCTTAGGTATGTCAGCTTCAAGGAAG	
GAGCTCGGTACCATACTAGCATGTATCCAC	*pFM1:AtDRE2*
GGGAGCTCGGTACCTGGAACTTTATCGGTTTT	
GGTACCCACCTGACAAATGCCCGAAACC	*pDIANA:AtDRE2*
TACGGGTACCTTGTAATTACTCGGCTTGCC	
Gene expression	
GTGACTCTGGTCAAGACT	*FWA*
TTGGTTCCACCAGAACCGGTA	
TCCTCGCCCTTGCTCACCAT	*FWA-GFP*
GCGCACTGCTTATCAAACTCAAGAACT	
ACATGCAGAGCGGTGCGAGG	*FIS2*
ACATGCAGAGCGGTGCGAGG	
TGATCTGGTCGTGCGGCGTG	*MEA*
TTTGCACCAAAAATCACAGG	
CCATTGCCTCTTTGACCATT	*mPAC*
CTTCGCTGGATCTAGACTCGGGCTC	
GCCCGATGCTCACTCCAAGGTAATG	*HDG8*
GGGTGGTTGACCGGCCTCAAT	
TGGTTTGCCCTCCATGTGGTGG	*HDG9*
GGTGTGGCCTCGACGTTATGCA	
TTCTCTCAATTCTCTCTACCGT	*UBIQUITIN10*
TGGCCTTAACGTTGTCGA	
TCTCCTGGCGAGGGAGCTTCA	*FIE*
GGAGGGCAACTTTTCAAATCCCAGACA	
TGCCAACTCCTCTCCCCGCA	*FUS3*
AGCCTCCGCGGCTTTCTTCG	
McrBC assay	
AAACAACAAAAATCTGATTGTCAGTATC	*FWA*
ATTCATACGAGCACCGCTTTACG	
GTTGATTACCAAACCCGAAGAAGA	*FIS2*
GATTTACTTTATGATTTCGCAGCGG	
TCGCGATGACAACTCAAGTG	*HDG9*
AGACGTCGCTGAAATTCGTC	
CACCGTAATCGTAGTCTAGCCA	*HDG8*
AGGTGTGTAGCATGAGGAAAGAA	
AAGCCAGCGCTATCACTAAACTTT	*FLC* (internal control)
TCGGCAGATTGAAAATGACATT	
GGCTCTAAGAGGGTGCTGAA	Ler-specific locus
TACCACCACCTCACACCAAA	