Supplementary data

Figure S1: Induction of I-PpoI by dexamethosone results in cell death in Arabidopsis roots of 7 d seedlings.

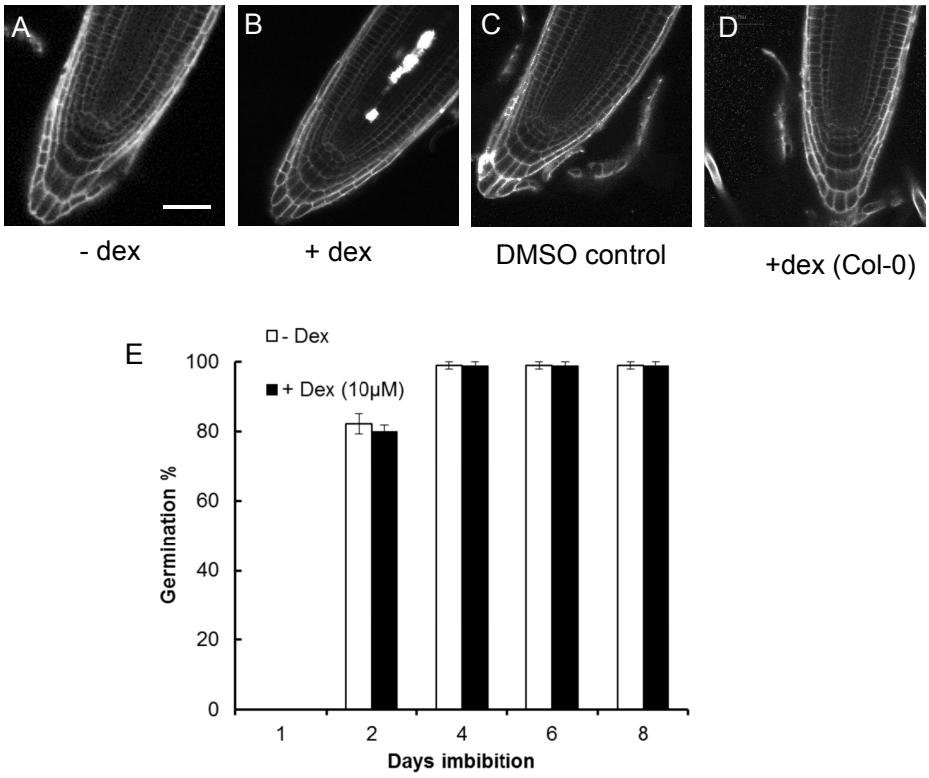

Figure S1 Induction of I-PpoI by dexamethosone results in cell death in Arabidopsis roots of 7 d seedlings.
Cell death in lines expressing inducible I-PpoI was visualized by propidium iodide staining and fluorescence microscopy **(A-D).** I-PpoI lines - dex **(A)**, + dex **(B),** DMSO control **(C)**, wild type col0 +Dex **(D)**. PI stains cell walls and in the presence of dexamethasone, dead root meristem cells can also be observed. Scale bar is 50µm. Dexamethasone does not affect germination performance of wild type col0 seeds **(E).** Seeds were plated onto germination paper pre-wetted with 10 µM dexamethasone or 0.5% DMSO (control) and stratified at 4°C for 48h before transfer to 20°C. Error bars show the standard error of the mean of 3 replicates of 50 seeds.

Figure S2: *RAD51* transcript levels in imbibing Col-0 and *atm-3* seeds.

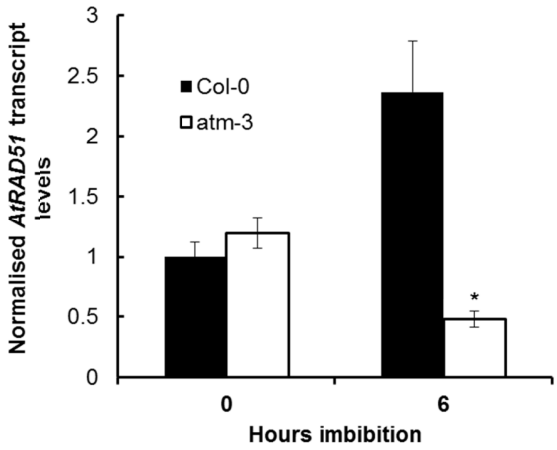

Figure S2. *RAD51* transcript levels during imbibition.
Q-PCR analysis of *AtRAD51* expression using cDNA synthesized from RNA isolated from imbibing Col-0 or *atm3* mutant Arabidopsis seed. Control Q-PCR was performed using primers specific to *AtACTIN2*. Significant differences in mean values are indicated *: P<0.05 (T-Test).

Table S1: Heat map of DNA repair gene transcript levels in dormant seeds.

Magnitude of change	0	40	80	120	160	200	240	280	320	360	>400

* For *SMR5* maximum colour intensity set at 4000

		PDD	DDL	PD24h	PD48h	PD30d	SD1	SD2	PDC	PDL	ND	PDN	PDLN	LIG	Imbibed seeds Max	Min	Fold change
AT3G48190	*ATM*	295	185	304	445	728	515	323	165	171	115	217	128	159	728	115	6
AT5G40820	*ATR*	92	88	76	79	132	172	166	48	53	57	57	46	91	172	46	4
AT1G66730	*LIG6*	56	44	79	142	397	252	149	72	59	50	61	44	47	397	44	9
AT1G07500	*SMR5*	3958	5101	2610	3657	4675	2637	1815	1394	1677	103	1235	205	208	4675	103	45
AT2G31970	*RAD50*	152	171	142	137	395	274	343	90	142	83	207	153	139	395	83	5

Data are mean absolute transcript values obtained from Arabidopsis eFP browser (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi) based on published microarray experiments (1-4).

Abbreviation	Treatment
PDD	Primary dormant: seeds dry (will not complete germinate when imbibed in Light or dark).
DDL	Primary dormant seeds, dry after-ripened: seeds dry (will germinate when imbibed in the light).
PD24h	Primary dormant seeds imbibed for 24 h (will not complete germination).
PD48h	Primary dormant: seeds imbibed for 48 h in the dark (will not complete germination).
PD30d	Primary dormant: seeds imbibed for 30 days in the dark (will not complete germination).
SD1	Secondary dormant: DL seeds imbibed in the dark for a further 24 days (sensitive to nitrate).
SD2	Secondary dormant: SD1 seeds imbibed at 3°C in the dark for 20 days (insensitive to nitrate).
PDC	Primary dormant seeds after-ripened for 117 days and then imbibed for 4 days at 3°C (will not complete germination unless exposed to light).
PDL	Primary dormant seeds after-ripened for 91 days and then imbibed for 24 h in the light (will not complete germination).
ND	Dry after-ripened seeds imbibed for 24 h (will germinate if placed in the light).
PDN	Primary dormant seeds after-ripened for 91 days and then imbibed for 24 h on a 10 mM KNO_3 solution (will not complete germination unless exposed to light).
PDLN	Primary dormant seeds after-ripened for 91 days and then imbibed in white light for 24 h on a solution 10 mM KNO_3 (will complete germination).
LIG	Dry after-ripened seeds imbibed for 20 h in the dark and then 4 h in red light (will complete germination).

Figure S3: Mean germination time of *atm* and *atr* mutant seed and wild type lines after accelerated ageing.

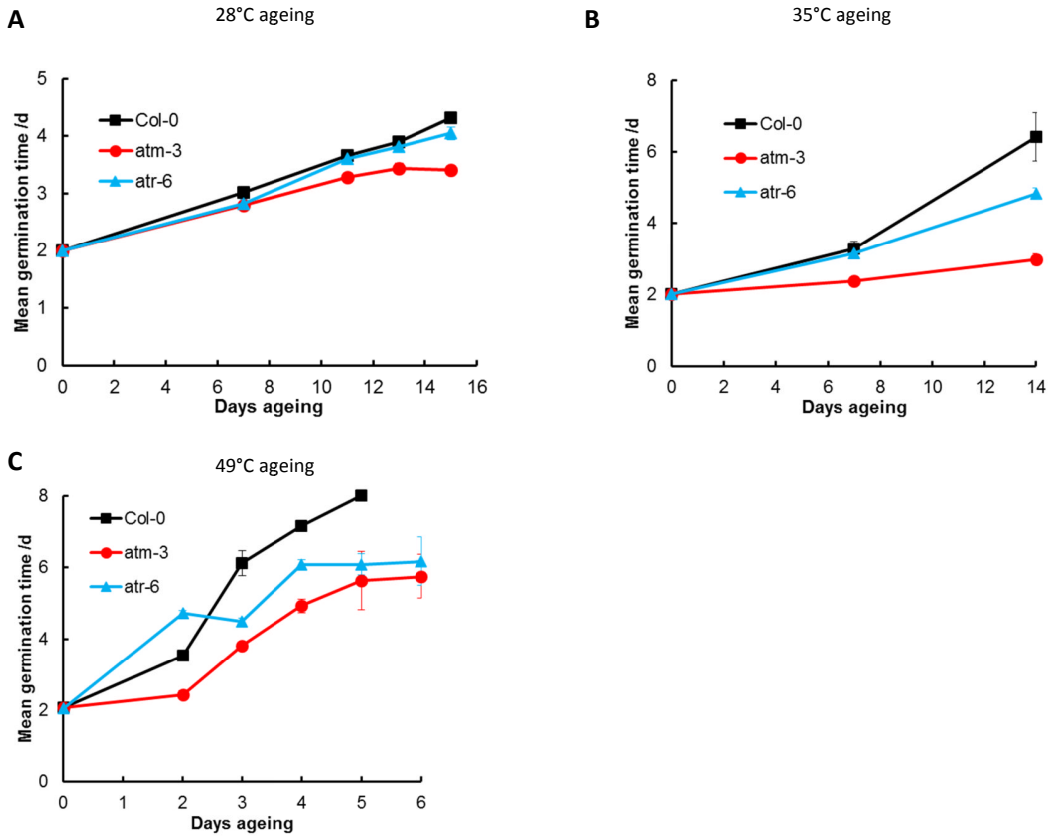

Figure S3. Mean germination time (MGT) of *atm* and *atr* mutant seed and wild type lines after accelerated ageing

Analysis of *atm-3, atr-6* and WT mean germination time after accelerated ageing at **(A)** 28°C, **(B)** 35°C and **(C)** 49°C. Accelerated ageing was performed for up to 15 days over a saturated solution of KCl. Seeds were plated and stratified at 4°C for 48h before transfer to an environmental growth chamber at 22°C and seeds scored for radicle emergence each day post-imbibition. Error bars show the standard error of the mean of 3 replicates of 50 seeds.

Figure S4: Germination of *atm* and *atr* mutant seed is resistant to accelerated ageing at 28°C, 84.6% RH.

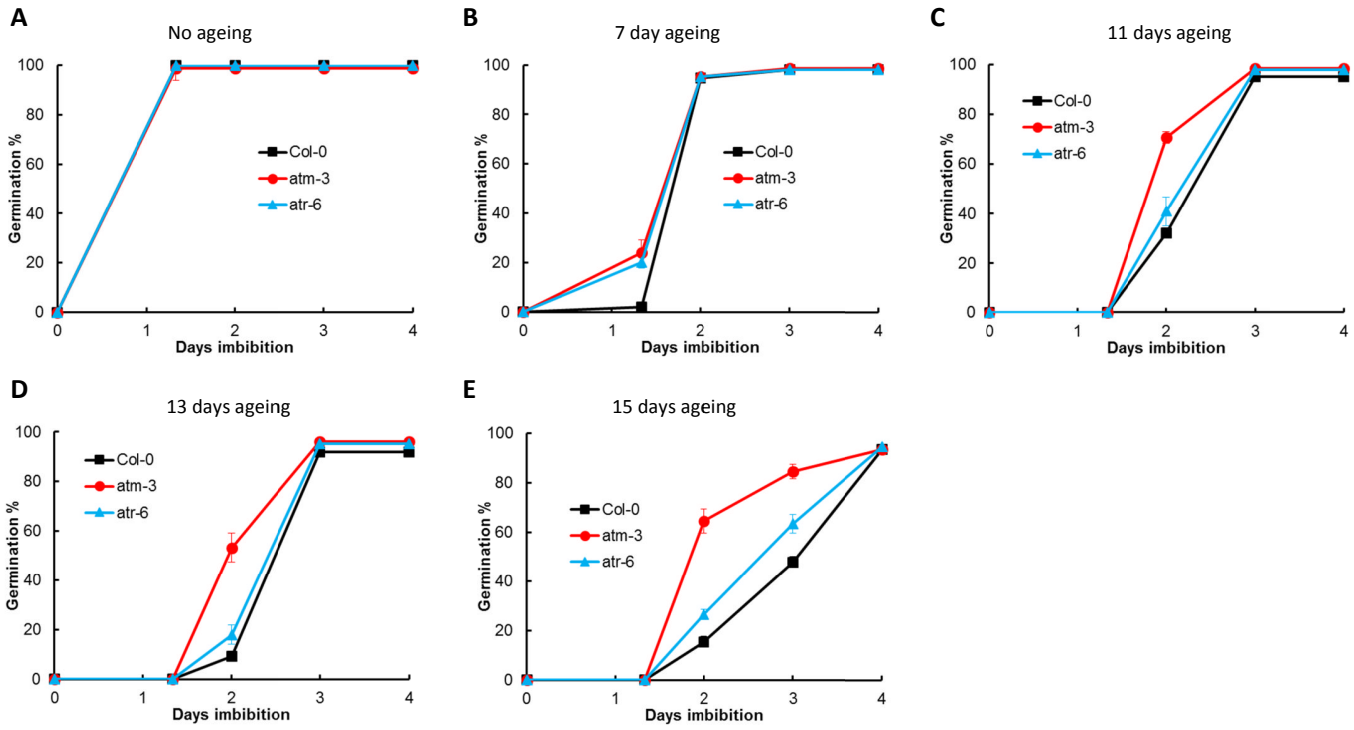

Figure S4. Germination of atm and atr mutant seed is resistant to accelerated ageing at 28°C, 84.6%. Analysis of *atm-3*, *atr-6* and WT seed germination performance after accelerated ageing. Seed viability and vigor after **(A)** 0 days accelerated ageing **(B)** 7 days accelerated ageing **(C)** 11 days accelerated ageing **(D)** 13 days ageing **(E)** 15 days ageing. Accelerated ageing was performed at 28°C for between 0 to 7 days over a saturated solution of KCl. Seeds were plated and stratified at 4°C for 48h before transfer to an environmental growth chamber at 22°C and seeds scored for radicle emergence each day post-imbibition. Error bars show the standard error of the mean of 3 replicates of 50 seeds.

Figure S5: Germination of *atm* and *atr* mutant seed is resistant to accelerated ageing at 49°C, 80.6%RH.

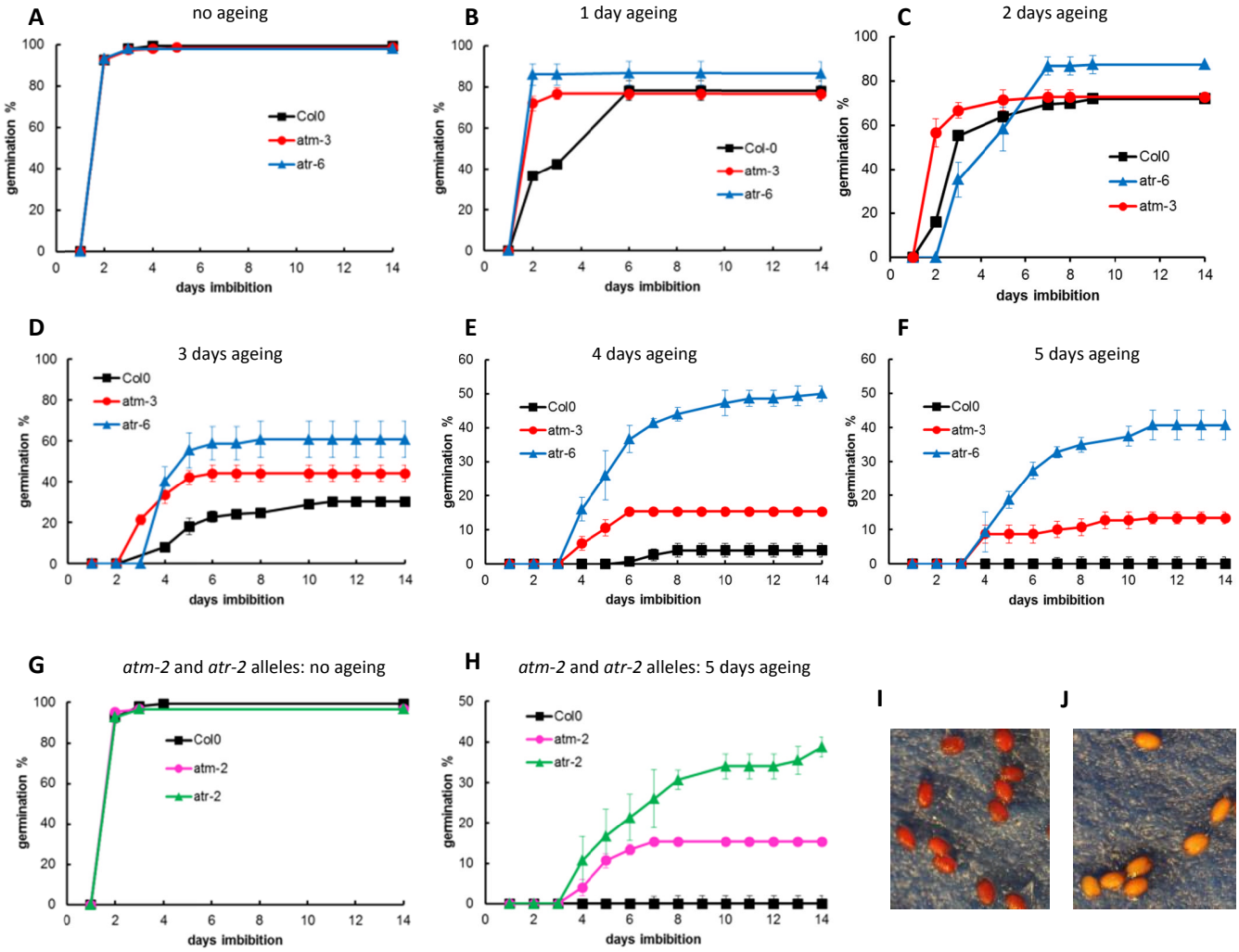

Figure S5 Germination of *atm* and *atr* mutant seed is resistant to accelerated ageing at 49°C, 80.6%.
Analysis of *atm-3*, *atr*-6 and WT seed germination performance after accelerated ageing. Seed viability and vigor after **(A)** 0 days accelerated ageing **(B)** 1 day accelerated ageing **(C)** 2 days accelerated ageing **(D)** 3 days ageing **(E)** 4 days accelerated ageing **(F)** 5 days accelerated ageing **(G)** Germination of wild type, *atm-2* and *atr-2* mutant seed after 0d ageing **(H)** Germination of wild type, atm-2 and atr-2 mutant seed after no ageing. Analysis of *atm-2*, *atr-2* and WT seed germination performance after 5 days accelerated ageing. Accelerated ageing was performed at 49°C for between 0 to 7 days over a saturated solution of KCl. Seeds were plated and stratified at 4°C for 48h before transfer to an environmental growth chamber at 22°C and seeds scored for radicle emergence each day post-imbibition. Error bars show the standard error of the mean of 3 replicates of 50 seeds. Example of viability staining with 2,3,5-triphenyltetrazolium chloride **(I)** Unaged seeds imbibed for 20h **(J)** Aged seeds that failed to germinate.

Figure S6: Initiation of S-phase in germination

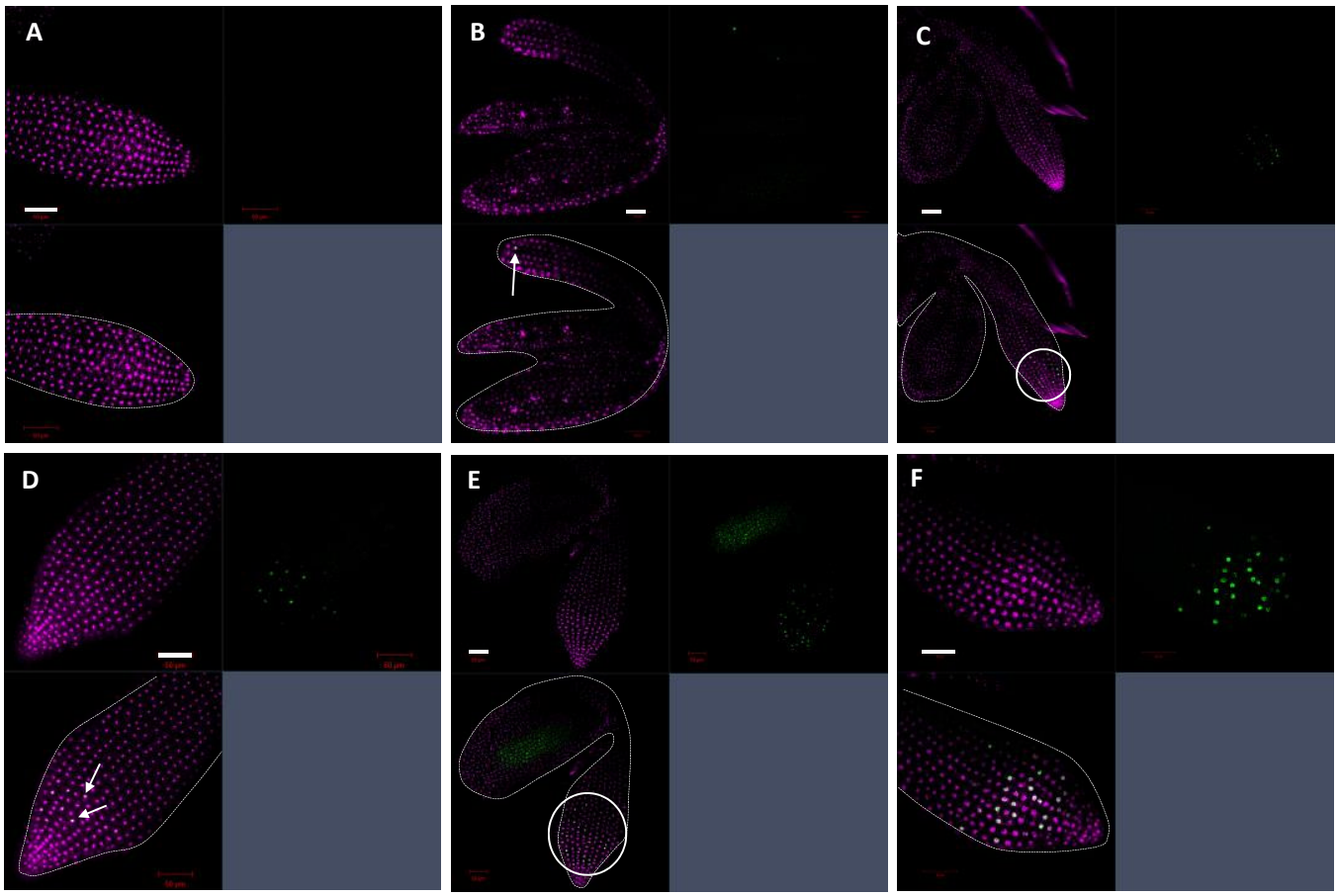

Figure S6: Initiation of S-phase in germination
EdU labelling of unaged wild type seeds reveals S-phase cells in the tip of the embryonic root and spreading up the root axis as germination progresses. Autofluorescence is observed in cotyledons. DAPI staining of nuclear DNA is shown as magenta and newly synthesized EdU labeled DNA is colored green and indicated by arrows in panel B and D and circled in panels C and E. The outline of the embryo is inducted in the merged images by a dotted white line. Panels A to F are seeds at progressively further stages though germination. **(A)** ungerminated **(B-D)** seed coat split **(E-F)** germinated with the radicle fully emerged from the seed coat. Bar is 50µm.

Figure S7: Initiation of S-phase in seeds aged for 14d .

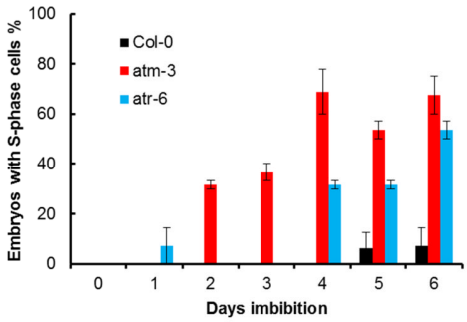

Figure S7. DNA replication in seeds aged for 14 days at 35°C, 83% RH. Embryos were scored for the presence of cells in S-phase as described in Fig.4. Both *atr* and *atm* mutants display greater number of embryos with S-phase cells relative to wild type controls, correlating with increased viability observed in the mutant lines (Fig. 3).

Figure S8: Analysis of WEE1 in seed germination.

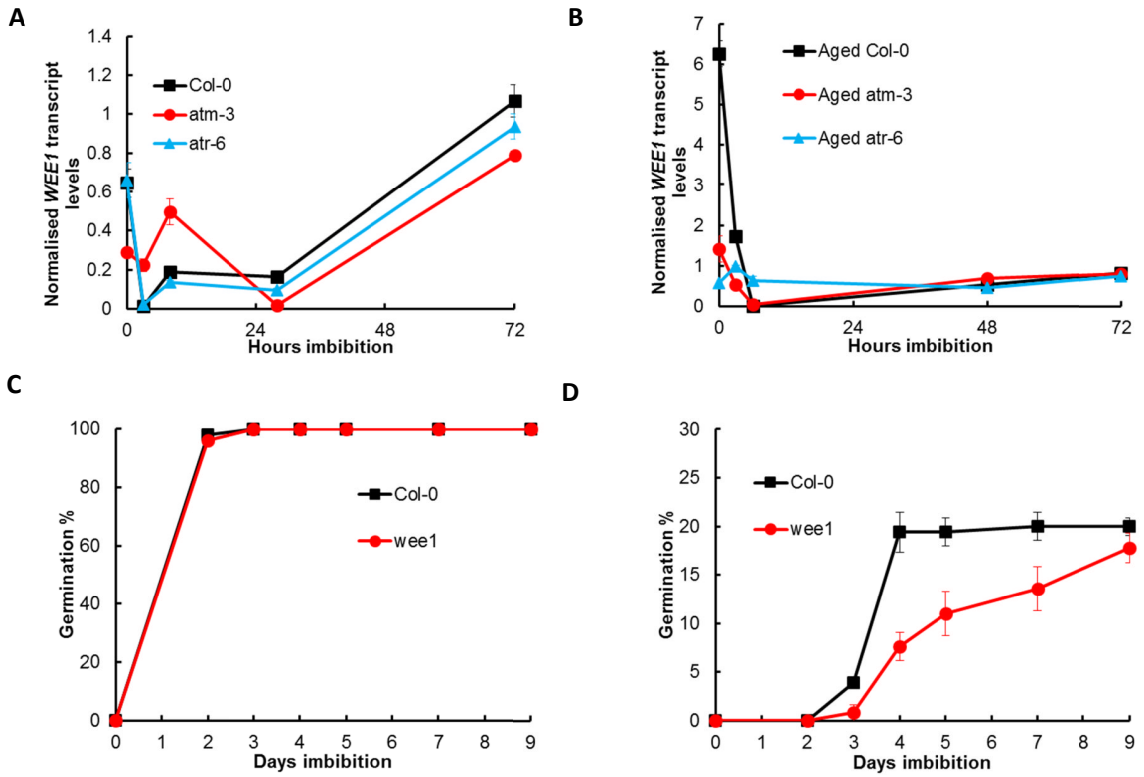

Figure S8 Analysis of WEE1 in seed germination

Transcript levels of *WEE1* in germinating seeds is independent of ATM and ATR **(A-B).** *WEE1* transcript levels in seeds at 0-48h imbibition in **(A)** unaged Col-0 and **(B)** *wee1* seed aged at 35°C, 83% RH for 7 days. Seeds deficient in the cell cycle regulator WEE1 show slowed germination relative to Col-0 **(C-D)**. Analysis of *wee1* and wild type seed germination performance after accelerated ageing. Seed viability and vigor of unaged seeds **(C)** and after 14 days **(D)** accelerated ageing at 35°C, 83% RH. Seeds were plated and stratified at 4°C for 48h before transfer to an environmental growth chamber at 22°C and seeds scored for radicle emergence each day post-imbibition. Error bars show the standard error of the mean of 3 replicates of 50 seeds.

Figure S9: Transcript levels of *SMR5*, *SMR7* and *RAD51* in Arabidopsis seeds.

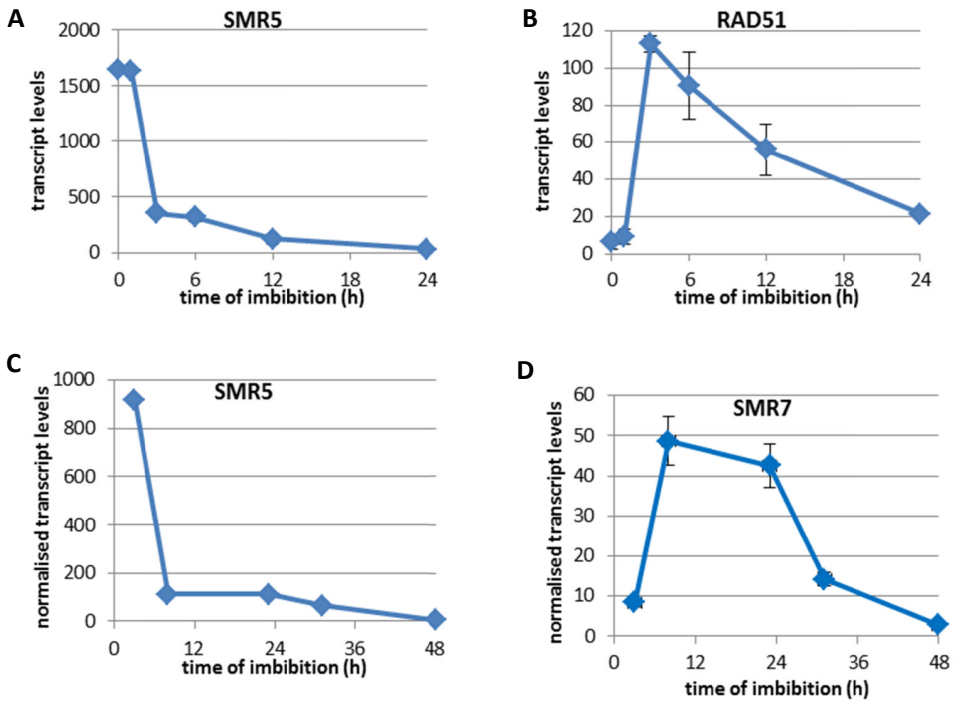

Figure S9: Transcript levels of SMR5, SMR7 and RAD51 in wild type Arabidopsis seeds. Transcript levels of *SMR5* **(A)** and *RAD51* **(B)** in imbibing seeds were analyzed using publically available microarray data (1). *SMR7* is not represented on microarrays used in the study by Nakabayashi et al (2005) (1). Real-time PCR data normalized to ACTIN7 expression (AT5G09810) shown for *SMR5* **(C)** and *SMR7* **(D)**.

Figure S10: Transcript levels of *SMR5* Arabidopsis seeds during dormancy cycling in the seed soil bank.

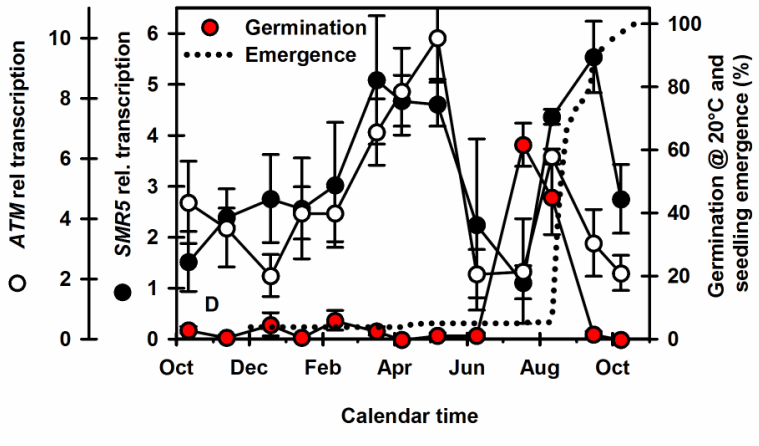

Figure S10: Transcription of *ATM* and *SMR5* and germination potential of seeds in the soil seed bank. Transcription profiles of *ATM* and *SMR5* in seeds recovered from the soil over 12 months from October 2007.Germination at 20°C in the light of seeds recovered from the soil at monthly intervals. Seedling emergence (% of total emerged) in the field following monthly soil disturbance (n=4). Error bars indicate the standard error of the mean, n=3. Germination and emergence data are from Footitt et al., 2011 (19) and with *ATM* transcript levels are also displayed in Figure 2 of the main text.

Figure S11: Mean germination time of *smr5, smr7, smr5 smr7* mutant seed after accelerated ageing.

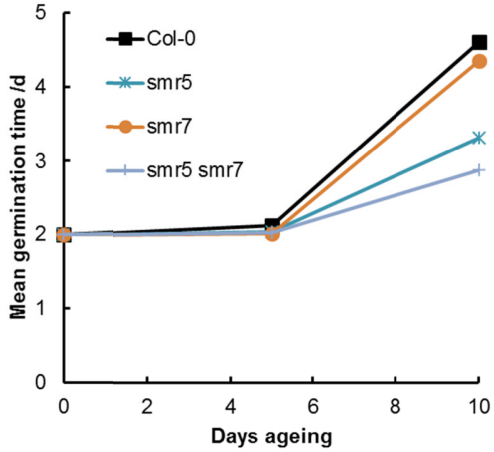

Figure S11: Mean germination time of *smr5, smr7, smr5 smr7* mutant seed after accelerated ageing
Analysis of *smr5*, *smr7*, *smr5 smr7* and WT mean germination time after accelerated ageing. MGT after accelerated ageing of *smr5*, *smr7*, *smr5 smr7* and WT at 35°C. Accelerated ageing was performed for up to 14d days over a saturated solution of KCl. Seeds were plated and stratified at 4°C for 48h before transfer to an environmental growth chamber at 22°C and seeds scored for radicle emergence each day post-imbibition. Error bars show the standard error of the mean of 3 replicates of 50 seeds.

Figure S12: Development of true leaves is delayed in seedlings germinated from viable *atm-3* and *atr-6* mutant seeds relative to Col-0 after accelerated ageing.

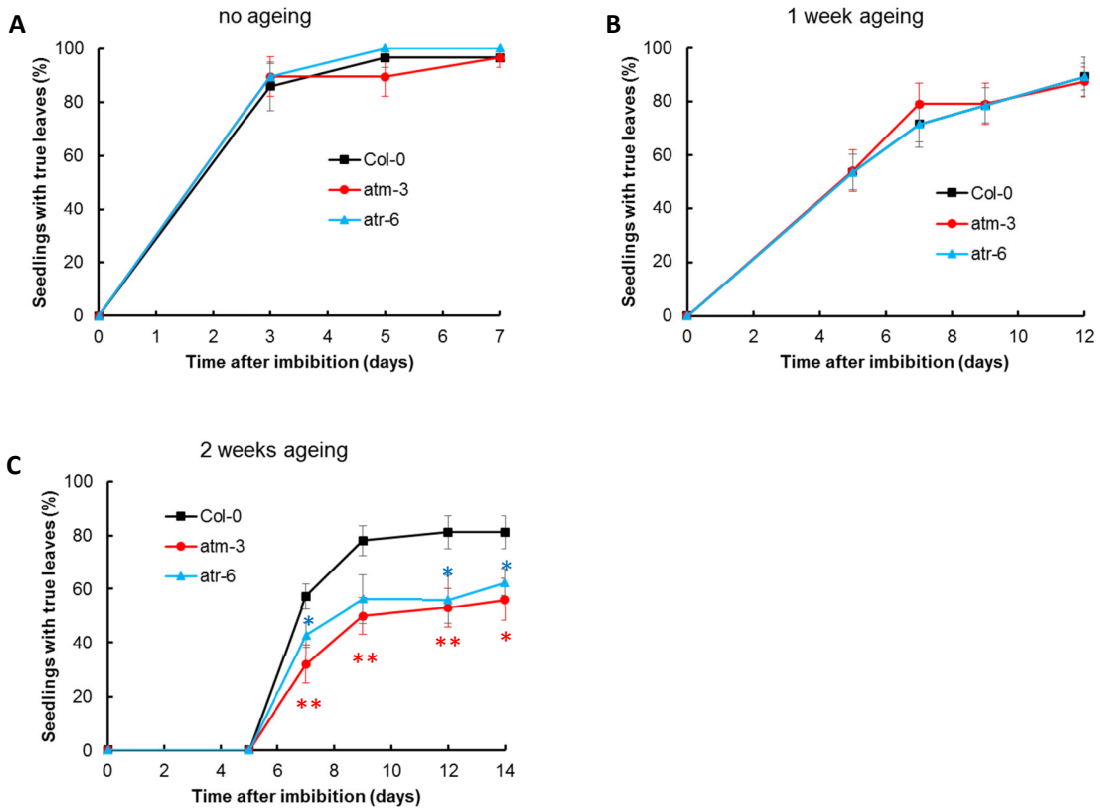

Figure S12 Development of true leaves is delayed in seedlings germinated from viable aged *atm* and *atr* mutant seeds relative to Col-0. Analysis of development in those plants which germinated from *atm-3*, *atr-6* and Col-0 seed after accelerated ageing at 35°C over a saturated solution of KCl for 0 day **(A)**, 7 day **(B)** and 14 day **(C)**. Seeds were stratified at 4°C for 48h before transfer to 22°C/Light. After 7 days seedlings germinated from viable seed were transferred to soil and plants subsequently scored for development of true leaves. Error bars show the standard error of the mean of 10 replicates of 4 plants. Significant differences in mean values are indicated *: P<0.05, **: P<0.01 (T-Test).

Figure S13: Isolation of the *atr-6* mutant line (SALK_054383).

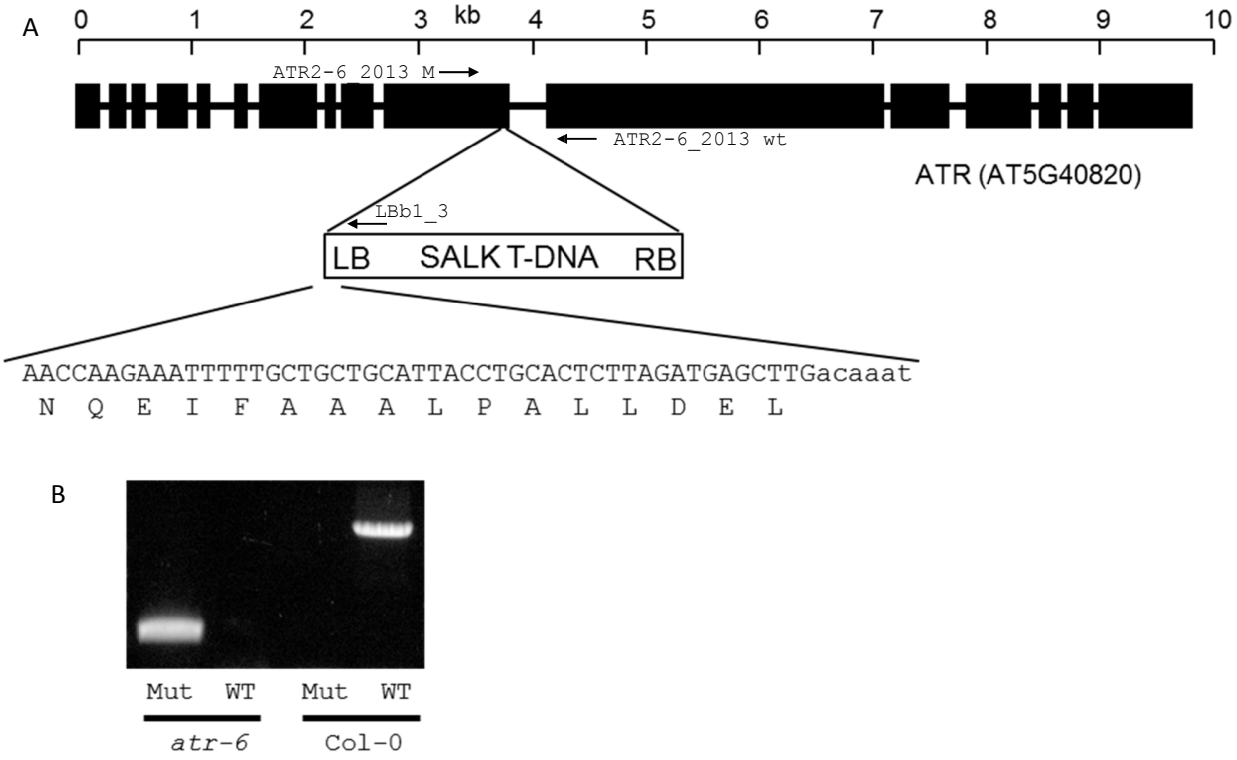

Figure S13. Isolation of the *atr-6* mutant line. A) The T-DNA insertion (SALK_054383) is located in the 10th exon. T-DNA sequence is in lower case. B) PCR confirmed the *atr-6* homozygous mutant had no wild type *ATR* allele. Mutant (Mut) primers: ATR-6_2013 M CAGAGTTCTTGCATTTGCTCTGA LBb1_3_SALK ATTTTGCCGATTTCGGAAC. Wild type (WT) primers: ATR-6_2013 M CAGAGTTCTTGCATTTGCTCTGA ATR-6_2013 wt AGTTCGAGATTCTTCTGCACACC

Figure S14: Construction of the pPPOΔGR vector: Codon optimized I-PpoI.

```
atggctaagtctaatcaagctagagttaacggaggatctaattatgattctcttactcct
 M  A  K  S  N  Q  A  R  V  N  G  G  S  N  Y  D  S  L  T  P
cttaatatggctcttactaatgctcaaattcttgctgttattgattcttgggaagaaact
 L  N  M  A  L  T  N  A  Q  I  L  A  V  I  D  S  W  E  E  T
gttggacaatttcctgttattactcatcatgttcctcttggaggaggacttcaaggaact
 V  G  Q  F  P  V  I  T  H  H  V  P  L  G  G  G  L  Q  G  T
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 L  H  C  Y  E  I  P  L  A  A  P  Y  G  V  G  F  A  K  N  G
cctactagatggcaatataagagaactattaatcaagttgttcatagatggggatctcat
 P  T  R  W  Q  Y  K  R  T  I  N  Q  V  V  H  R  W  G  S  H
actgttccttttcttcttgaacctgataatatcaacggaaagacttgtactgcttctcat
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ctttgtcataatactagatgtcataatcctcttcatctttgttgggaatctcttgatgat
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aataagggaagaaattggtgtcctggacctaacggaggatgtgttcatgctgttgtttgt
 N  K  G  R  N  W  C  P  G  P  N  G  G  C  V  H  A  V  V  C
cttagacaaggacctctttatggacctggagctactgttgctggacctcaacaaagagga
 L  R  Q  G  P  L  Y  G  P  G  A  T  V  A  G  P  Q  Q  R  G
tctcattttgttgtt
 S  H  F  V  V
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Figure S15: Map of pPPOΔGR.

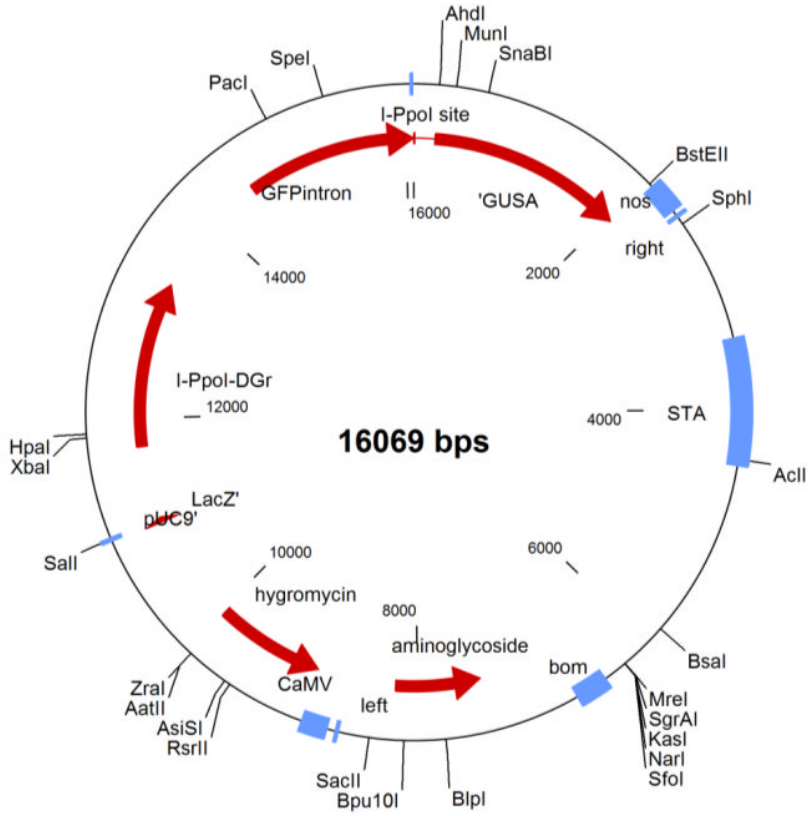

Table S2: QPCR primers

		Forward	Reverse
At4g34270	*Tip 41-like*	gtgaaaactgttggagagaagcaa	tcaactggatacccttcgca
At4g12590		gagatgaaaatgccattgatgac	gcacccagactctttgatg
AT3g48190	*ATM*	gatggccatgaggcattatt	tctcctttgaggaggttaccaa
AT5g40820	*ATR*	ctggagaagcctgagttggt	atgcccaaaccatcaatcat
AT1g66730	*LIG6*	cagaaagctgtttcagggaga	aggaacaaccacgtccagag
AT3G18780	Actin	tccctcagcacattccagcagat	aacgaattctggacctgcctcatc
AT1G49250	AtLIG4	ctttagtttcgaaaagcgaaatg	cttgtagtggatcctcatgg
AT5G20850	RAD51	gttcttgagaagtcttcagaagttag	gctgaaccatctacttgcgcaactac
At5g09810	ACT7	ctttaggatgcttgtgatgatgct	gcgccaatataacaatcgacaata
At1g07500	SMR5	tacggtgacggttgatgatg	gctgctaccaccgagaagaa
At3g27630	SMR7	ccggtgaagacgaaactcat	caccaactcgaaatctgaagg

Supplementary methods

Nucleic acid purification and cloning

DNA procedures and bacterial manipulations were by established protocols (36). RNA was isolated from above ground tissues of flowering Arabidopsis using the SV total RNA isolation kit (Promega) according to the manufacturer's instructions. Plasmid DNA was prepared using QIAGEN columns according to the manufacturer's instructions (Qiagen). cDNA synthesis was performed with Superscript II reverse transcriptase (Invitrogen) and followed by amplification with iProof DNA polymerase (Bio-Rad). Analytical PCR was performed using PCR Reddymix (ABGene) and analyzed by agarose gel electrophoresis. Real-time RT-PCR analysis was performed on a CFX96 thermocycler (Bio-Rad), as described previously (5), using SYBR Green Supermix (Bio-Rad). A plant codon-optimized I-PpoI gene was synthesized (Genscript, SI Appendix, Fig S14) and cloned into pBI-ΔGR and the expression cassette subcloned into pCB1300 carrying a I-PpoI recognition site to create pPPOΔGR (SI Appendix, Fig S15). Propidium iodide (PI) staining was performed using 10µg.ml^{-1} PI in water and EdU labeling was performed as described previously (6) and analyzed on a Zeiss LSM700 Inverted confocal microscope. Quantification of anaphase figures was performed on radicle tissue isolated 6h into the 16h light phase of a 16/8h light dark cycle and analyzed using a Zeiss LSM880 Upright confocal laser scanning Airyscan deconvolution microscope.

Dormancy analysis

Seeds production, harvest, storage and details of seed burial in, and recovery from field soils and post-recovery seed handling are as described previously (7). RNA was extracted from seeds as described elsewhere (8). cDNA synthesis and Quantitative PCR was performed in triplicate on each of three independent biological samples as in ref 13 using a 1/25 dilution of cDNA and the following touchdown PCR thermal cycle: one cycle at 95°C for 10 min followed by 50 cycles at 95 °C for 30s, 70°C (decreasing by 0.2°C/cycle to a target temperature of 67°C) for 30s, and 72 °C for 30s. Data was analyzed using LightCycler® 480 software (version 1.5; Roche Diagnostics). Gene expression levels were determined using a cDNA dilution series of the primer pairs of each gene (SI Appendix, Table S2) of interest with normalization against the combined mean at each time point of the reference genes *At4g34270* (Tip 41-like) and *At4g12590*. These reference genes have highly stable transcripts in Arabidopsis seeds in both microarray and QPCR studies (2, 3, 9).

References

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