Supplemental Data

Supplemental Figure S1. Using different housekeeping genes (*ACTIN*, *TIP41* and *PP2C1A*) gave similar results in gene expression analyses in tomato seeds.

Supplemental Figure S2. Sequence analyses of *ait1.1* #2 and *ait1.2* CRISPR mutant alleles.

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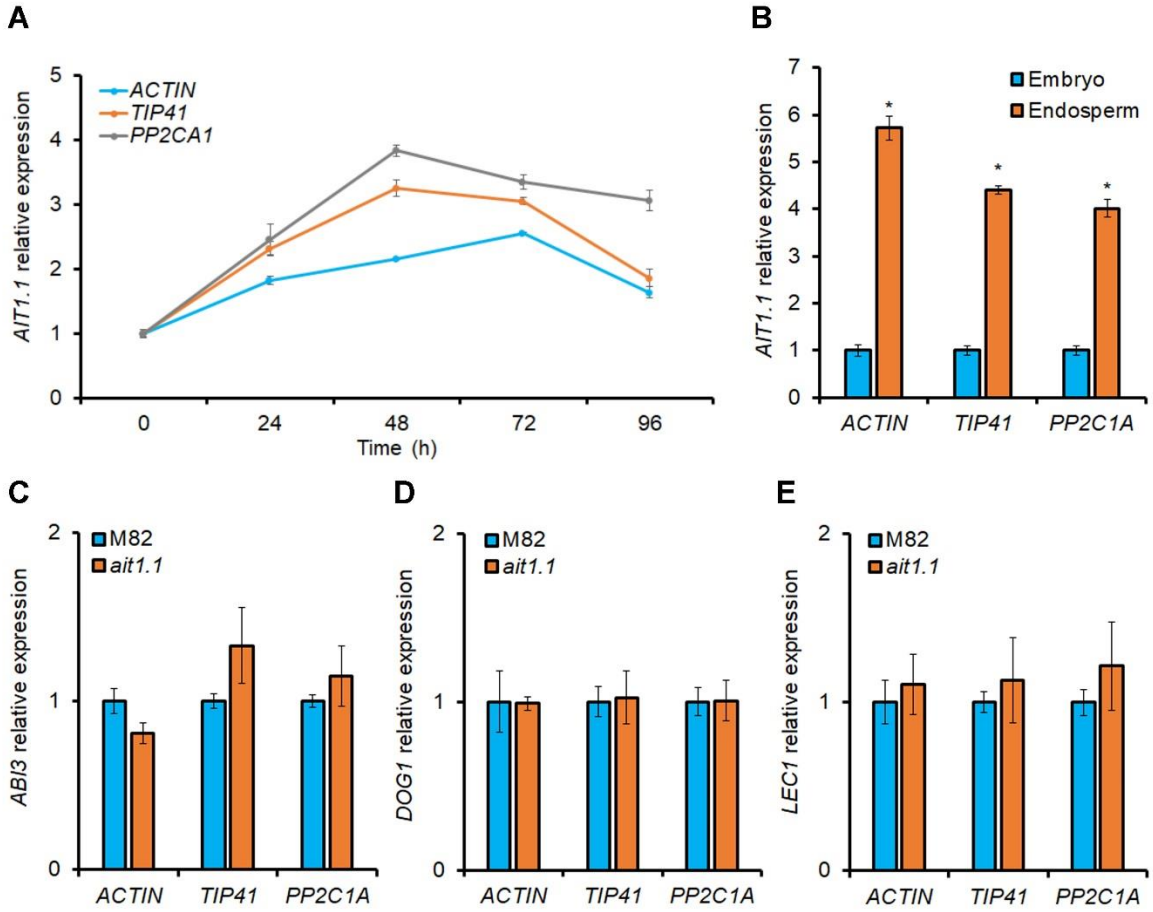
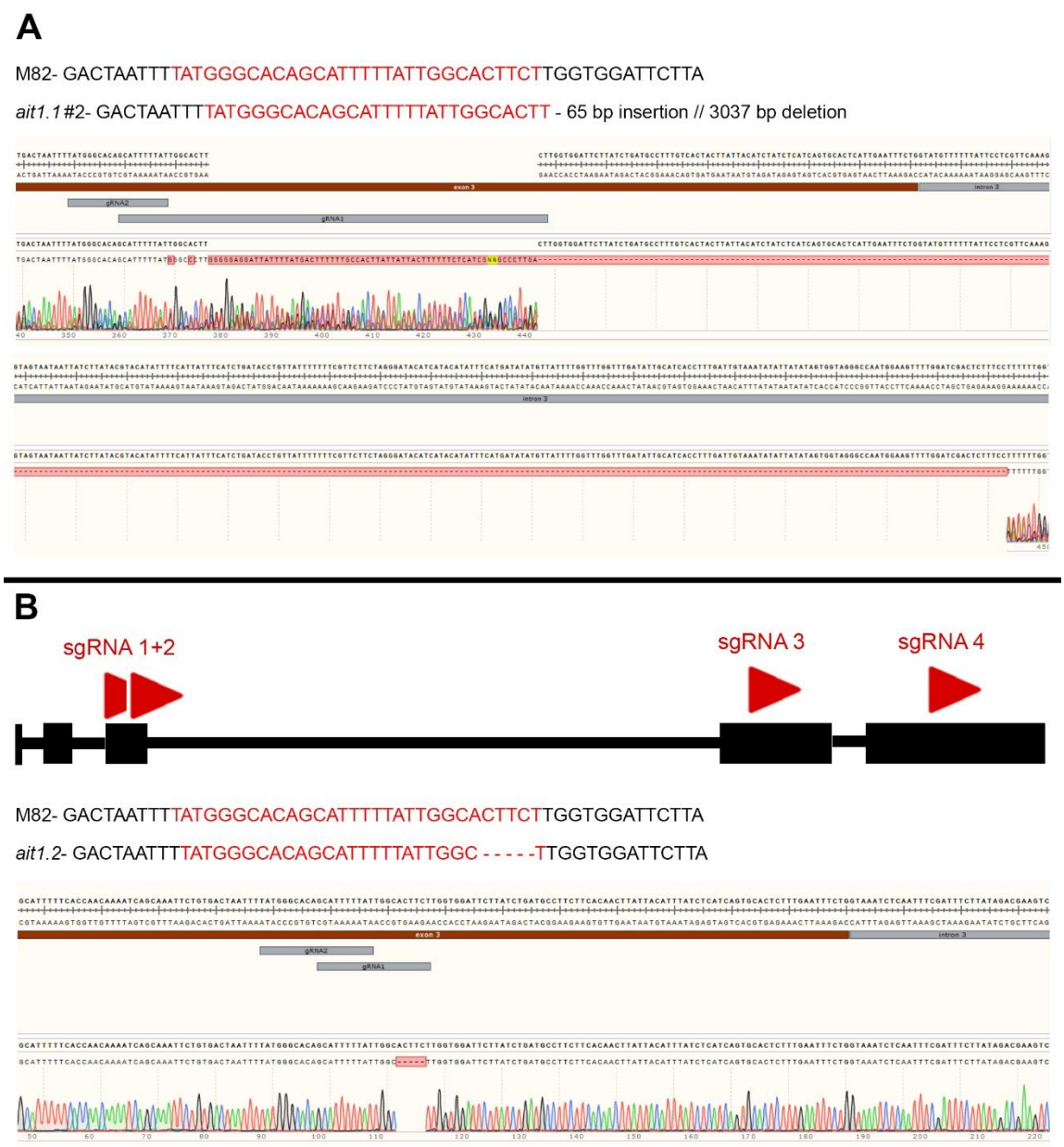
Supplemental Table S1. gRNAs used in this study.

Supplemental Table S2. Primers used in this study.

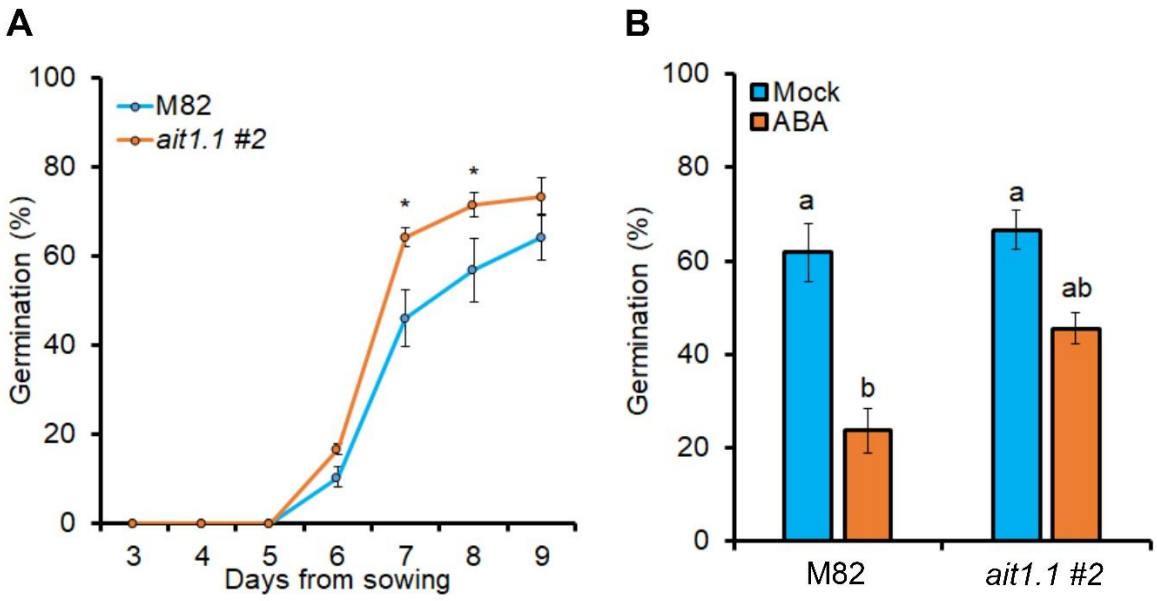
Supplemental Figures



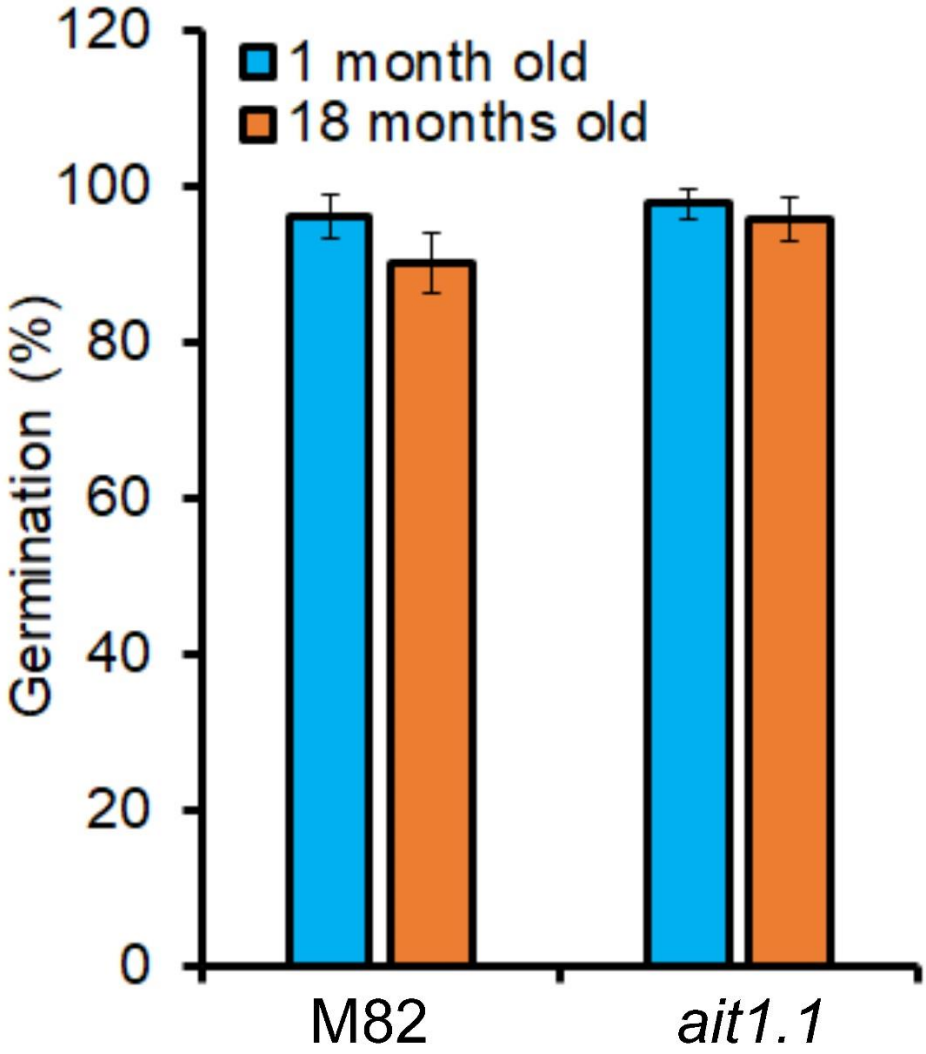
Supplemental Figure S1. Using different housekeeping genes (*ACTIN*, *TIP41* and *PP2C1A*) gave similar results in gene expression analyses in tomato seeds. (A) Relative expression of *AIT1.1* in dry (0h) seeds and following imbibition (24-96h). (B) Relative expression of *AIT1.1* in isolated embryos or embryo-less endosperms. (C-E) Relative expression of the central dormancy-related genes ABI3 (C), DOG1 (D) and LEC1 (E) in M82 and *ait1.1* dry seeds stored for 1 month. Values in A-E are means of 4 replicates, each contains 10 seeds ±SE. Stars in B represent significant differences between respective treatments by Student's *t* test ($P < 0.05$).



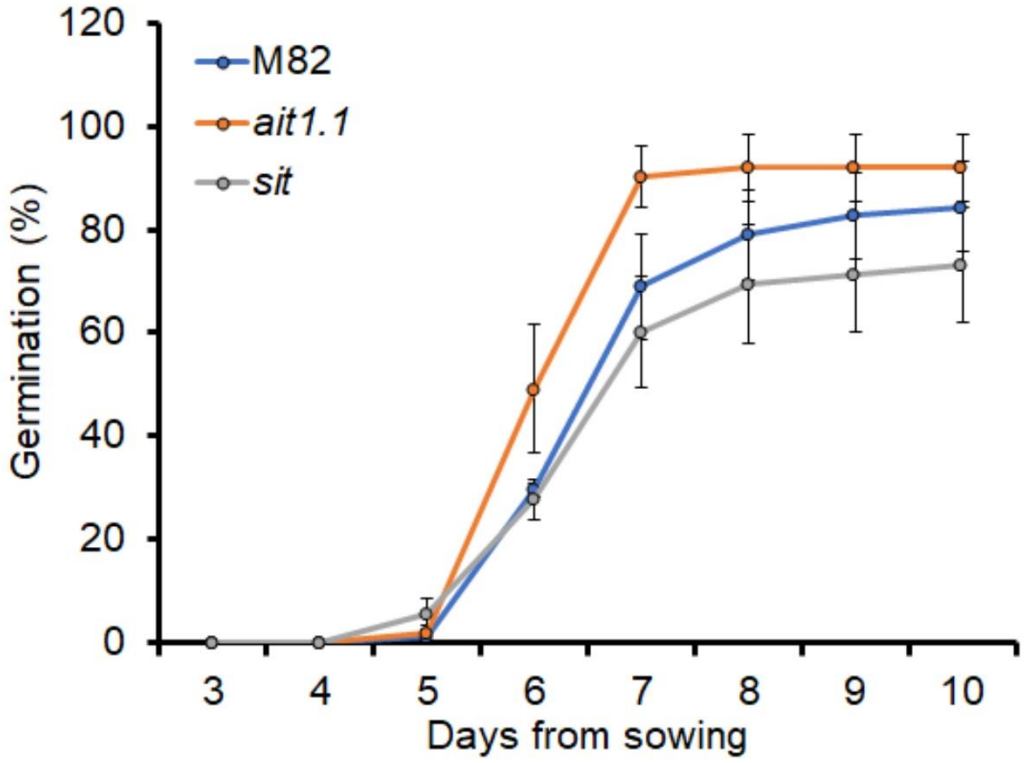
Supplemental Figure S2. Sequence analyses of *ait1.1 #2* and *ait1.2* CRISPR mutant alleles. (A) The sequence of *ait1.1#2*. RNA guide1 and 2 (red letters) and the chromatograms *ait1.1#2*. (B) Schematic structure of *AIT1.2* with the positions of four RNA guides (red arrows) in the third, fourth and fifth exons. Also shown RNA guide1 and 2 (red letters) and the chromatograms of *ait1.2*.



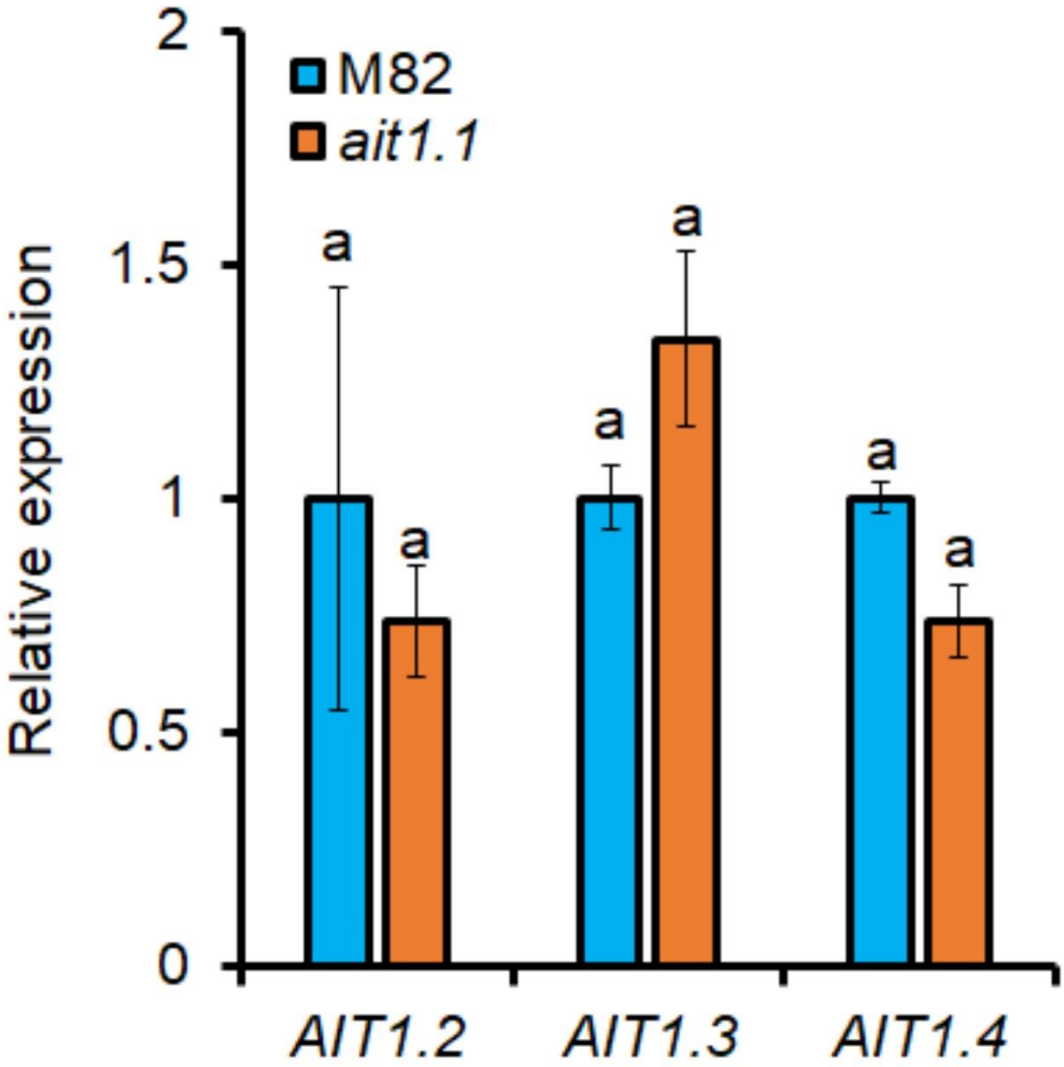
Supplemental Figure S3. Loss of *AIT1.1* promoted germination in additional independent allele. (A) Germination of M82 and *ait1.1 #2* (3037 bp deletion in exon and intron 3 (see also Fig. S2). (B) Percentages of germinated seeds after 10 days in petri dishes with or without 5μM abscisic acid (ABA). Values are means of 3 replicates each contains 50 seeds ±SE. Stars in A represent significant differences between respective treatments by Student's *t* test ($P < 0.05$). Small letters in B represent significant differences between respective treatments by Tukey-Kramer HSD test ($P < 0.05$).



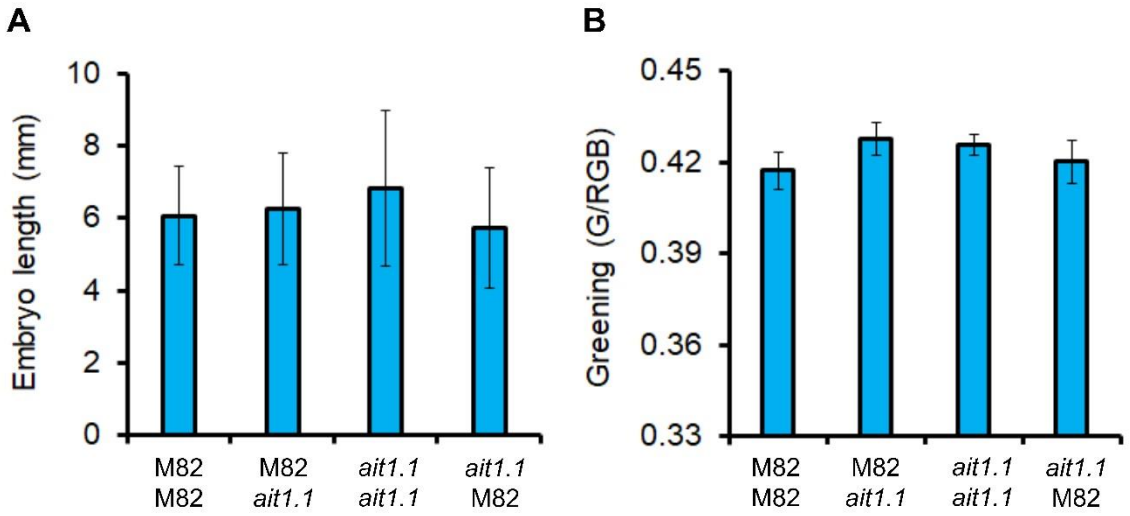
Supplemental Figure S4. Loss of *AIT1.1* did not affect seed longevity. Germination of M82 and *ait1.1* seeds stored for 1 or 18 months. Values are means of 3 replicates each contains 50 seeds ±SE.



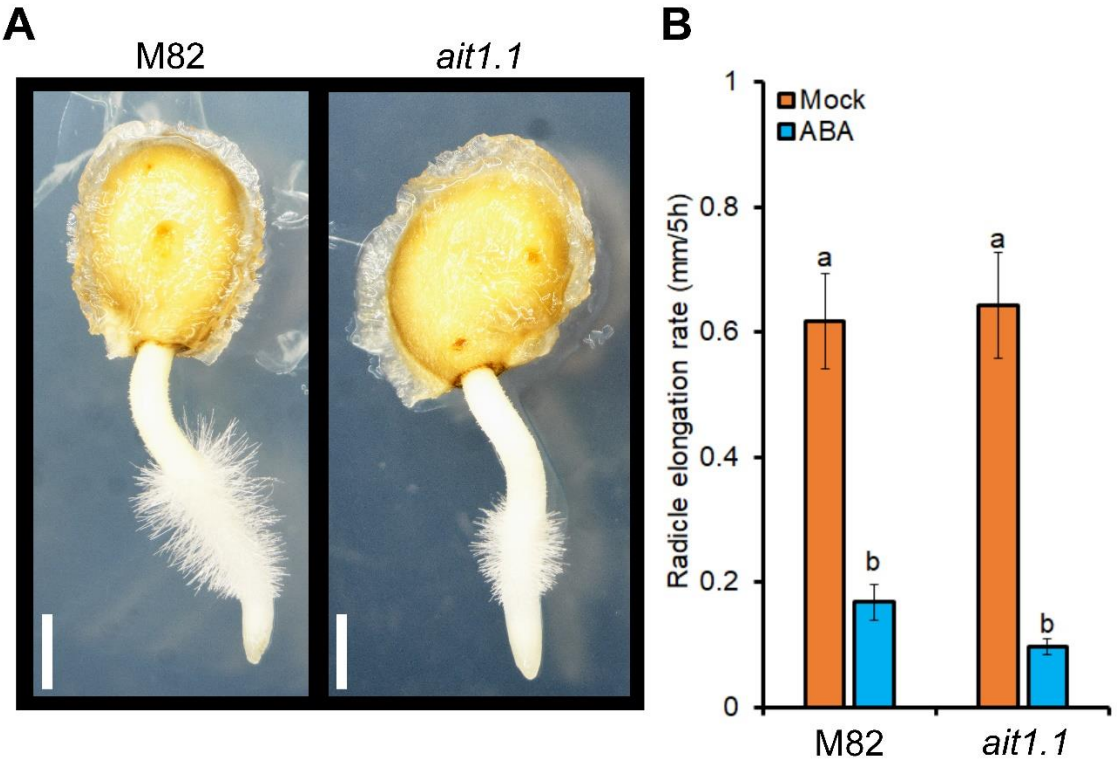
Supplemental Figure S5. Abscisic acid (ABA) deficiency reduced seed longevity. Germination of M82, ABA-deficient mutant *sit* and *ait1.1* seeds stored for 18 months. Values are means of 3 replicates each contains 50 seeds ±SE.



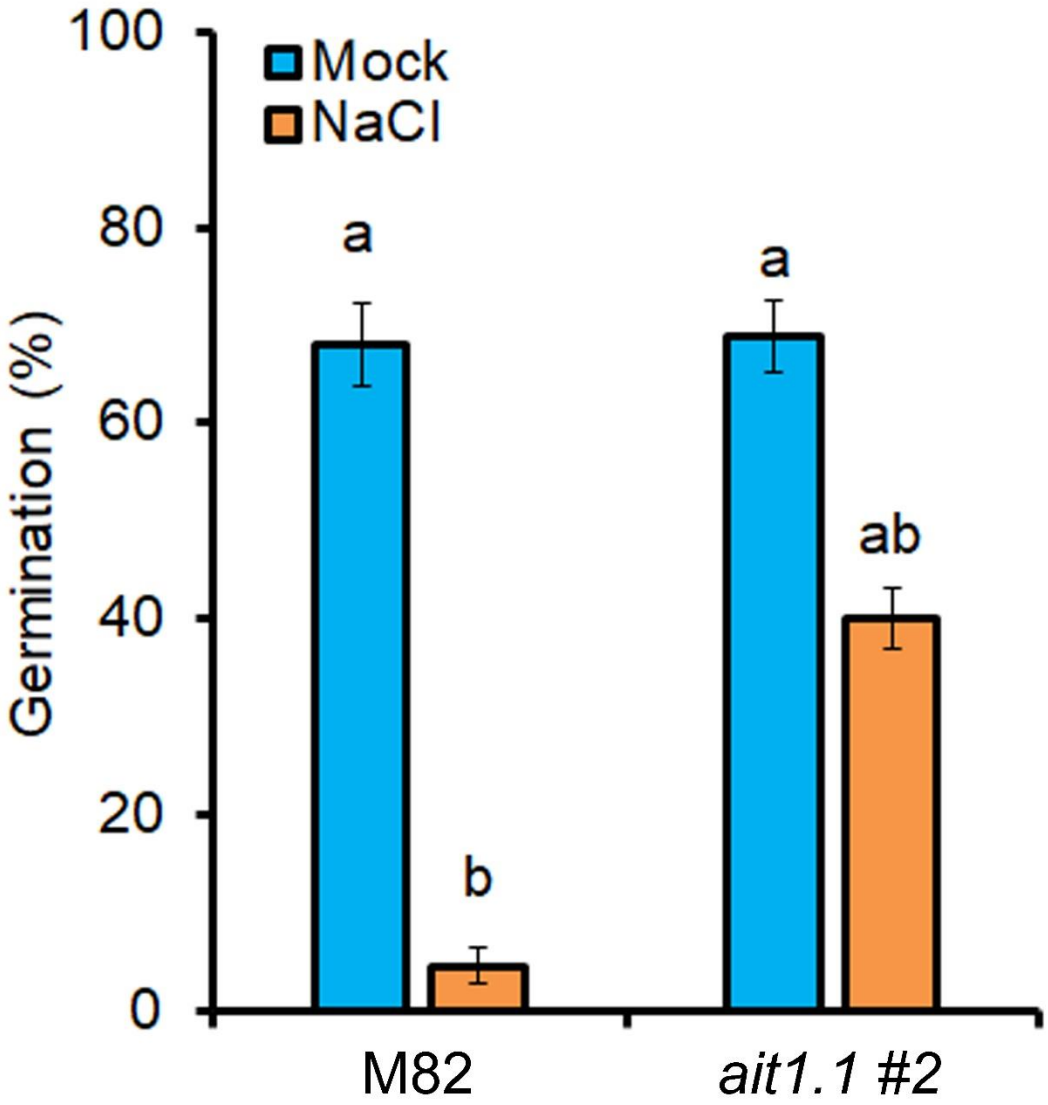
Supplemental Figure S6. Loss of *AIT1.1* did not affect the expression of the other *AIT1* genes. Relative expression of *AIT1.2*, *AIT1.3* and *AIT1.4* in M82 and *ait1.1* seeds 48h following imbibition. Values are means of 4 replicates each contains 10 seeds ±SE. Small letters represent significant differences between respective lines by Tukey-Kramer HSD test (*P < 0.05)*.



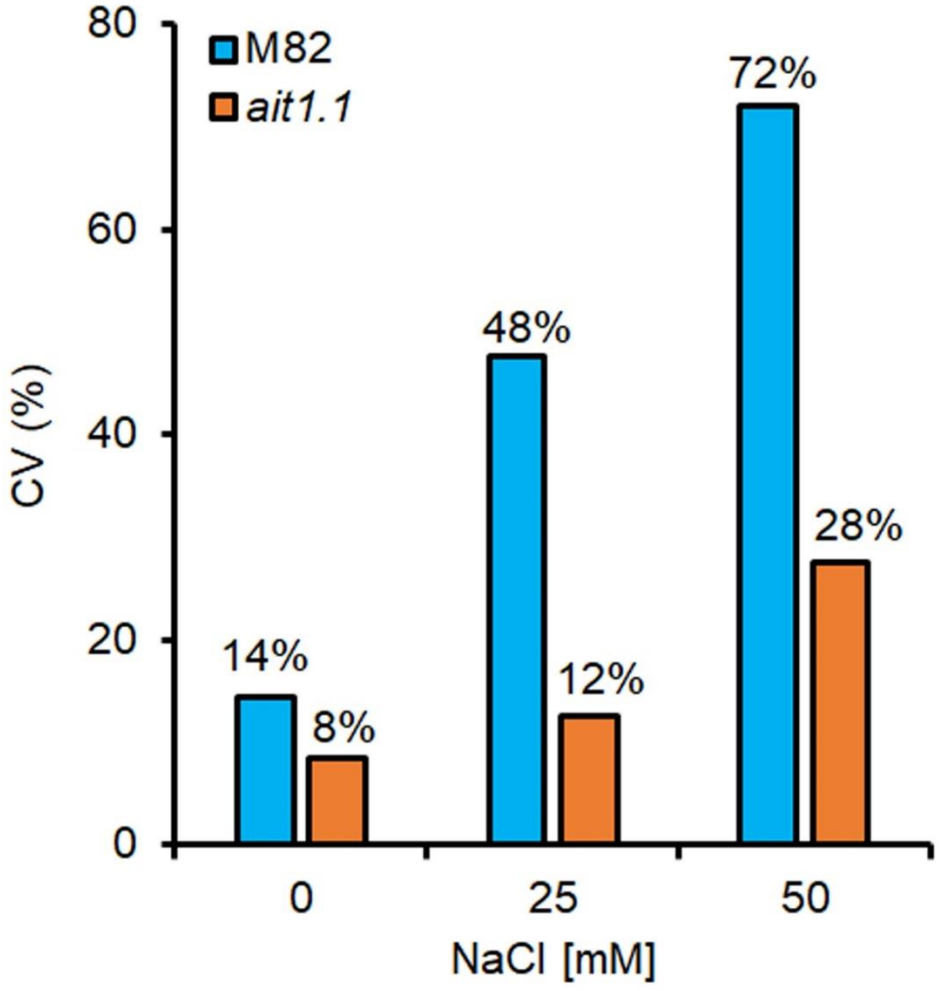
Supplemental Figure S7. *AIT1.1* did not affect endosperm inhibition of embryo growth. Length (A) and greening (G/RGB, see methods)(B) of M82 and *ait1.1* isolated embryos (upper), placed for 72h on a layer of endosperms (lower). Values are means of 10 biological replicates ±SE.



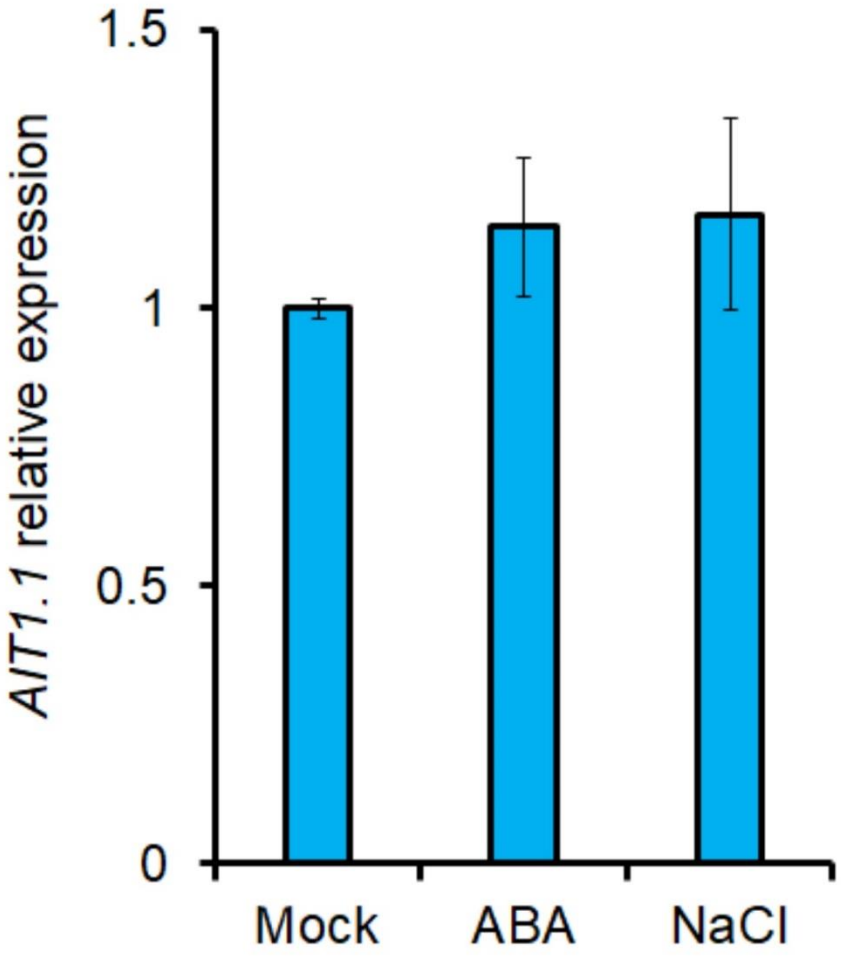
Supplemental Figure S8. Loss of *AIT1.1* did not affect post-emergence radicle elongation. (A) Representative M82 and *ait1.1* germinating seeds following scarification. (B) Radicle elongation-rate of M82 and *ait1.1* following scarification in petri dishes containing Murashige & Skoog (MS) medium with (or without) 5µM abscisic acid (ABA). Values are means of 8 biological replicates ±SE. Small letters (B) represent significant differences between respective treatments by Tukey-Kramer HSD test (*P < 0.05)*. Scale bar = 1 mm.



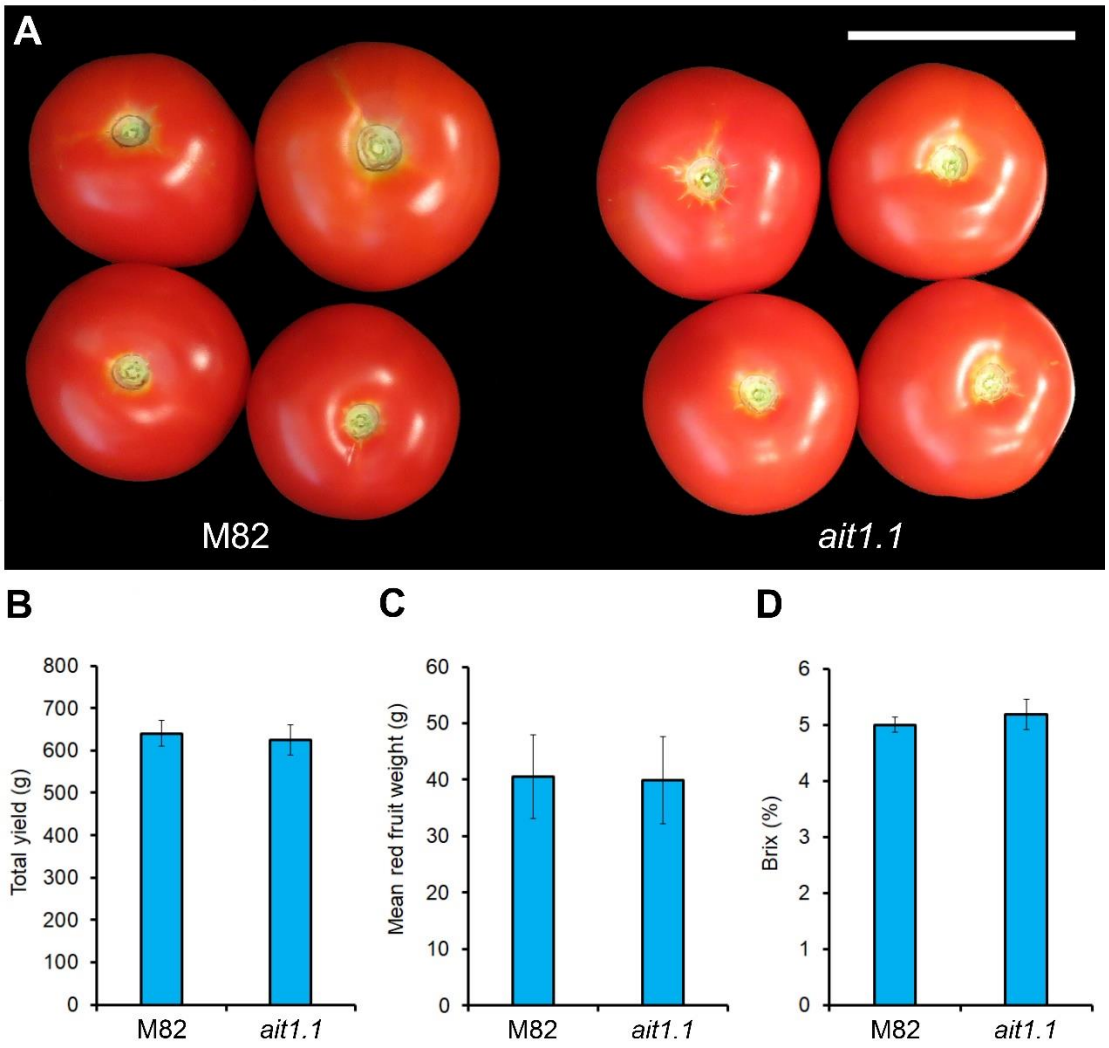
Supplemental Figure S9. Increased germination under salinity conditions in additional independent *ait1.1* allele. Percentages of germinated seeds after 7 days on Petri dishes with or without 50mM NaCl. Values are means of 3 independent replicates each contained 50 seeds ±SE. Small letters represent significant differences between respective treatments by Tukey-Kramer HSD test (*P < 0.05)*.



Supplemental Figure S10. Loss of *AIT1.1* increased germination uniformity under normal and stress conditions. Co-efficient variance (CV- standard deviation/mean) of M82 and *ait1.1* germination under different concentrations of NaCl. Sample size for each line and treatment was approximately 100 seeds.



Supplemental Figure S11. Salt or abscisic acid (ABA) treatments did not affect *AIT1.1* expression in seeds. Relative expression of *AIT1.1* in seeds treated for 48h with (or without) 10µM ABA or 50mM NaCl. Values are means of 4 replicates each contains 10 seeds ±SE.



Supplemental Figure S12. Loss of *AIT1.1* did not affect fruit yield in the field. (A) Representative M82 and *ait1.1* red-ripen fruits. The images were digitally extracted for comparison. (B) Total fruit yield per plant (green and red fruits). (C) Mean weight of red-ripen fruit. (D) Brix of red-ripen fruits. Values are means of 20 plants ±SE. Scale bar in A = 5 cm.

Supplemental Tables

Supplemental Table S1. gRNAs used in this study.

Gene	Used for	Sequence (5'-3')
AIT1.1/2 gRNA-1	CRISPR	GCATTTTTATTGGCACTTCT
AIT1.1/2 gRNA-2	CRISPR	TATGGGCACAGCATTTTTAT
AIT1.1/2 gRNA-3	CRISPR	TTAGGCGTTGGAGGTATAAA
AIT1.1/2 gRNA-4	CRISPR	CGTAGTATTCATCATGATCT

Supplemental Table S2. Primers used in this study.

Gene	Used for	Sequence (5'-3')
ACTIN	RT-qPCR	Forward- GTCCTCTTCCAGCCATCCAT Reverse- ACCACTGAGCACAATGTTACCG
TIP41	RT-qPCR	Forward- ATCGAGTGTCGCAAGCTTTT Reverse- CGGCAAGTGAGTTGTCTGAA
PP2AC1	RT-qPCR	Forward- ATGGAGTGCAAGCCGTTATC Reverse- GCACATTCCATTCCTCCACT
AIT1.1	RT-qPCR	Forward- GTGGAAGCCCTCTCACAACA Reverse- CTTCGCCATTTTCCTTGCCC

AIT1.2	RT-qPCR	Forward- ATCGAGAAGCCTTAGCTTCCTT Reverse- GAAAATCGGGAACAGTTTCATC
AIT1.3	RT-qPCR	Forward- GGCATTGGGTGTTGGAGGTA Reverse- TCGCCGTCAAATTGTTCAGC
AIT1.4	RT-qPCR	Forward- CTTGTGGCGGCATTTTCCAA Reverse- CCATGTCCTTCTTCCGCACT
ABI3	RT-qPCR	Forward- TTGGGTATGTTGGCCTTCTC Reverse- TCTGCTGGTTCTGTGATTGC
FUS3	RT-qPCR	Forward- GCGGTATTCCGAGGTTATGA Reverse- GTTGTCCATTGCAGGGAAGT
DOG1	RT-qPCR	Forward- CGAGCTAAACGACGACGAAT Reverse- CACCAAGTAGGGGCAAAGAA
LEC1	RT-qPCR	Forward- GCTACCGTAGGTTCCCACAA Reverse- TCGCGTCGTCTGATATCTTG
β1-3-glucanase	RT-qPCR	Forward- AATGCAGCAACATGCTTGAG Reverse- GGGGGAAAACAGACCAAAAT
EXPA2	RT-qPCR	Forward- GTGCAATGTCAATGCTGACC Reverse- TTAGCCAGGGCATAGTTTGG
MAN2	RT-qPCR	Forward- AGGAACGATGGGAGGAAGTT Reverse- CCAGCAGTGGATGGATTTTT
RAB18	RT-qPCR	Forward- CTCAAGGCATGGGTACTGGT Reverse- CCTTCTTTCTCCTCCCACCT
RD29	RT-qPCR	Forward- CGGAAGCAACACGAATGAG

		Reverse- TTCCATGATCAAATTGAAAATCC