

ALE1 relative level

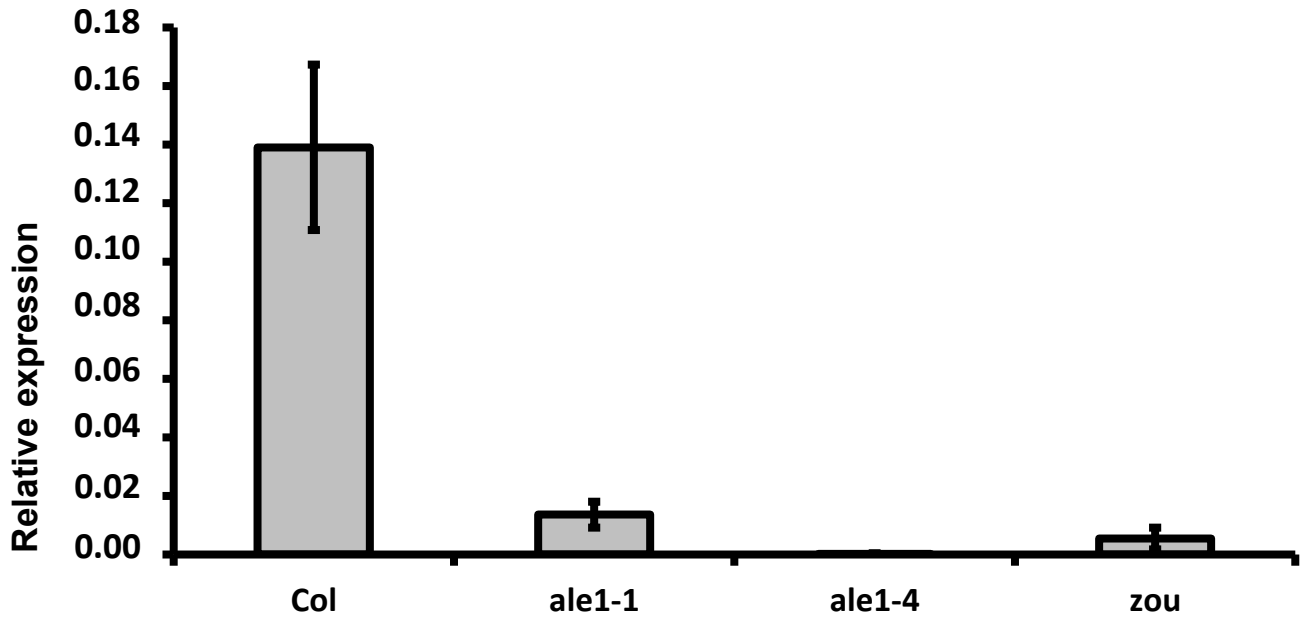


Fig. S1. Transcription *ALE1* relative to that of *EIF4* in Col0, *ale1-1*, *ale1-4* and *zou-4*. It should be noted that the transcripts detected in *ale1-1* may be non-functional owing to the presence of a transposon in the 3' coding region of *ALE1* in this allele. Seed samples were taken at the late heart stage. Results are from biological triplicates, each of which was analysed in technical triplicate. Error bars represent s.d. between biological triplicates.

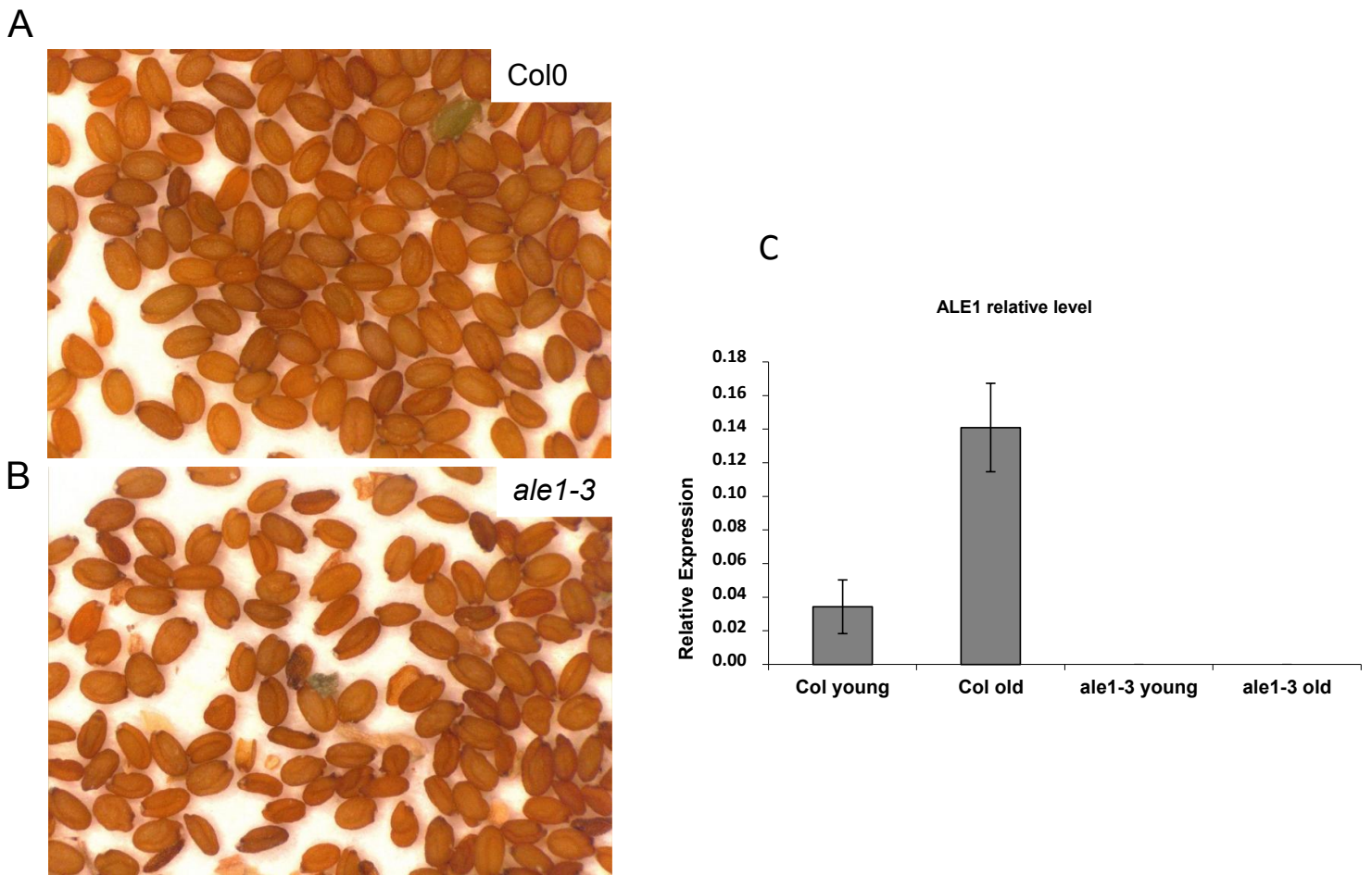


Fig. S2. Characterization of seed defects and *ALE1* expression in the *ale1-3* allele. (A,B) Seeds of wild-type (A), and concomitantly harvested *ale1-3* (B) homozygous plants. Seed-shape defects are observed in the *ale1-3* mutant. No *ALE1* transcript is detectable by Q-RT-PCR in *ale1-3* siliques either at the globular and heart stages (young), or at the torpedo stage (old). Results are from biological triplicates, each of which was analysed in technical triplicate. Error bars represent s.d. between biological triplicates.

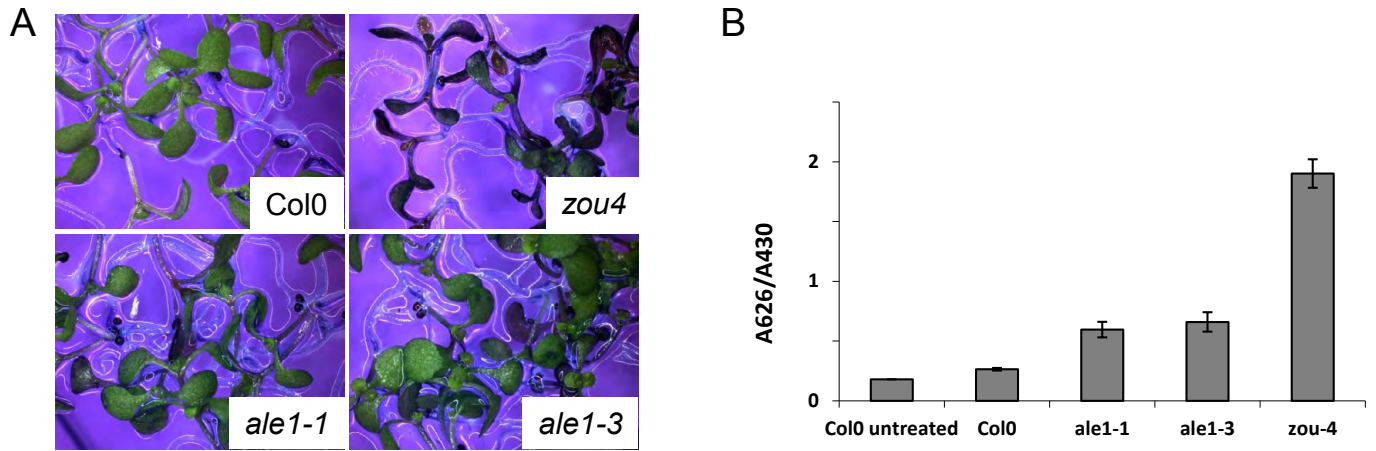


Fig. S3. Characterization of seedling cuticle defects in the *ale1-3* allele. (A) Toluidine Blue staining of wild-type, *ale1-1*, *ale1-3* and *zou-4* seedlings. (B) Toluidine Blue uptake in these lines was quantified spectrophotometrically, showing a significant increase in staining of *ale1-1* and *ale1-3* seedlings compared with wild type. *zou-4* seedlings stain very heavily. Error bars represent s.d. between three biological replicates each containing 20 seedlings.

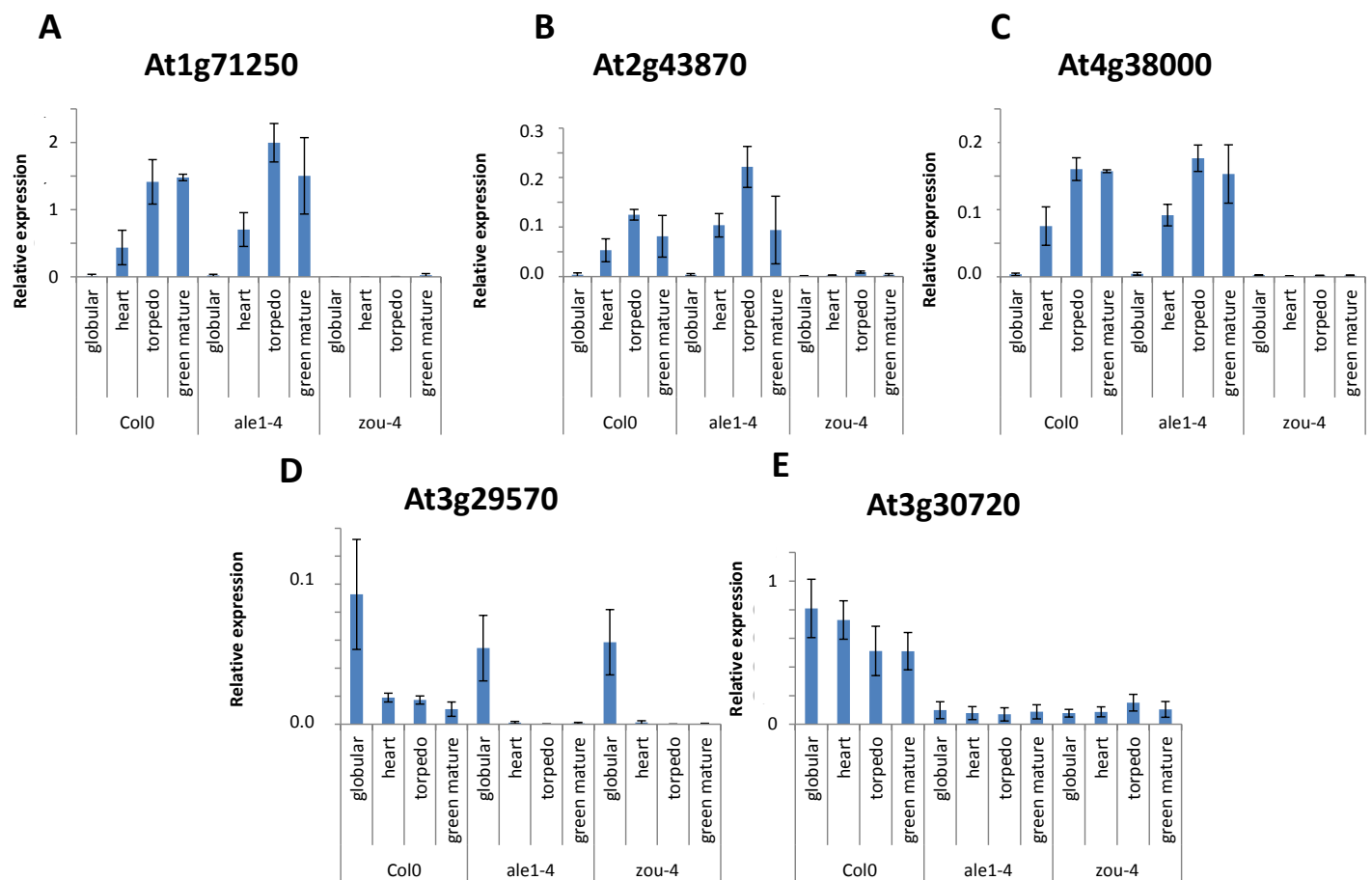


Fig. S4. ZOU and ALE1 have overlapping functions. (A-C) Transcription of three genes relative to that of *EIF4*, the expression of which is lost in *zou-4* mutants, but is not significantly affected in *ale1-4* mutant seeds. (D,E) Transcription of two genes relative to that of *EIF4*, the expression of which is downregulated both in *zou-4* mutants, and in *ale1-4* mutant seeds. In most cases, significant differences are seen only from the heart stage onwards. Seed samples were taken at the globular, heart, torpedo and mature green developmental stages. Results are from biological triplicates, each of which was analysed in technical triplicate. Error bars represent s.d. between biological triplicates.

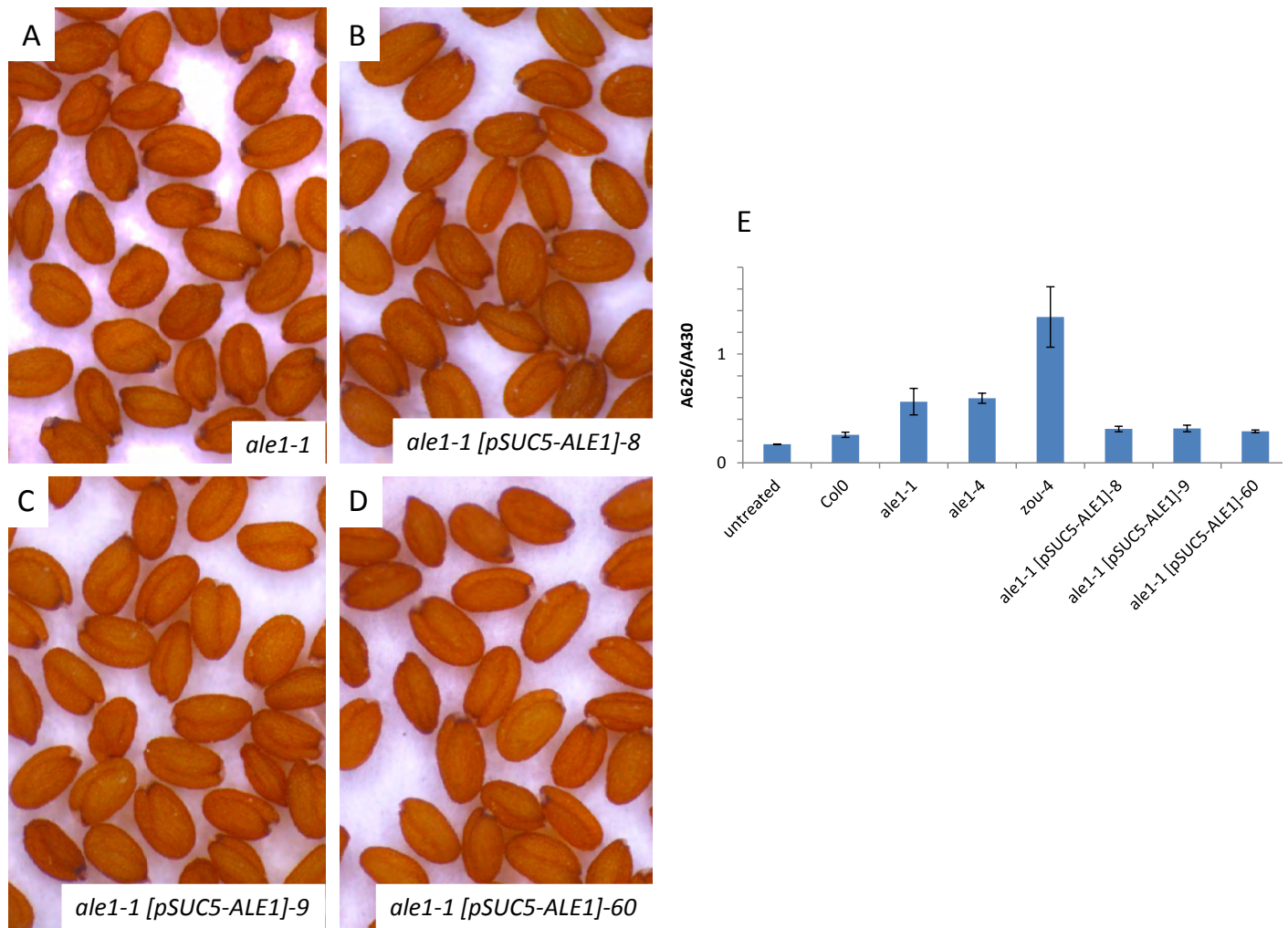


Fig. S5. Seed phenotypes. (A-D) *ale1-1* (A) and three independent lines of *ale1-1[pSUC5::ALE1]* (B-D), showing total rescue of the *ale1* seed phenotype. (E) These same lines were subjected to Toluidine Blue staining which was quantified spectrophotometrically. The presence of the *pSUC5::ALE1* transgene rescues the cuticle phenotype of *ale1-1*. Error bars represent s.d. between three biological replicates each containing 20 seedlings.

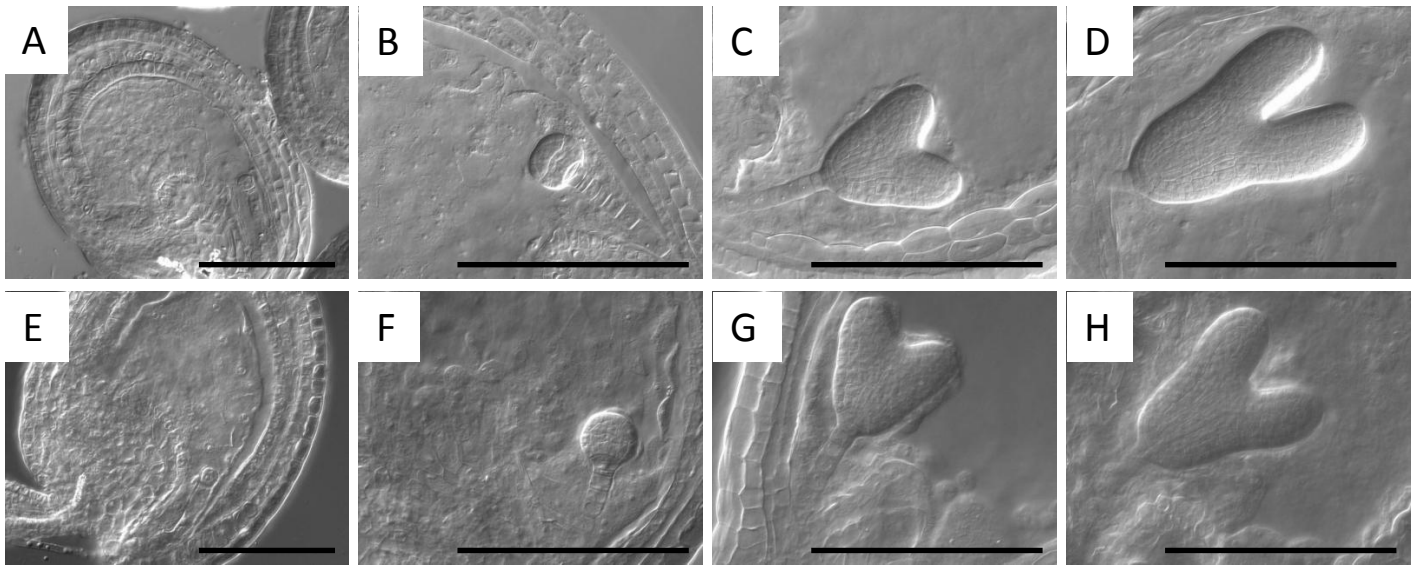


Fig. S6. Developmental staging for samples used in Fig. 5. Staging for Col0 (A-D) and *zou-4* (E-H) is shown. Developmental stages are pre-globular (A,E), globular (B,F), heart (C,G) and early torpedo (D,H). Scale bar: 50 μ m.

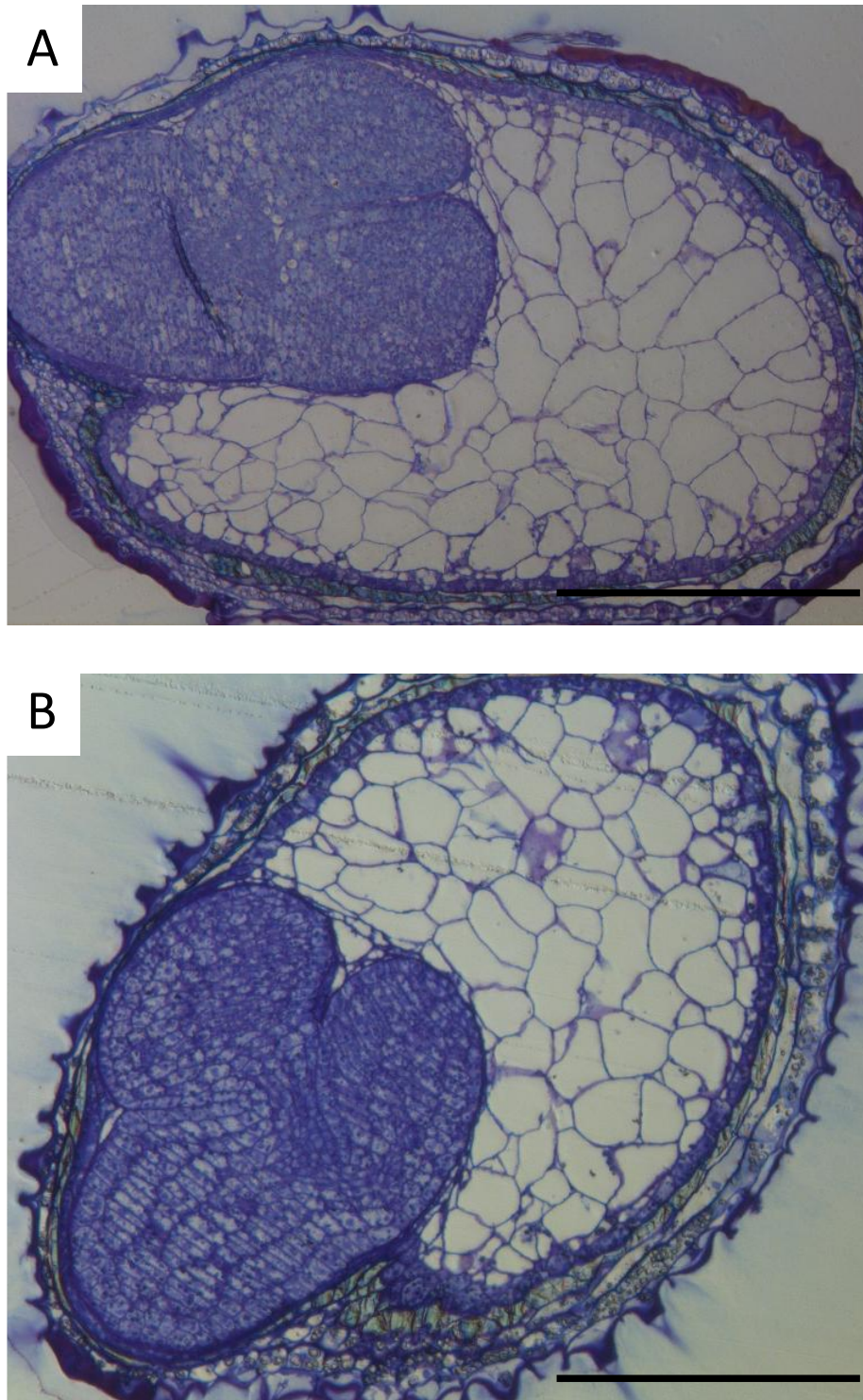


Fig. S7. Comparison of persistent endosperm phenotype. (A,B) *zou-4* (A) and *zou-4[pSUC5::ALE1]-line 8* (B). No significant loss of endosperm persistence is observed due to the introduction of the *pSUC5::ALE1* transgene. Scale bar: 100 μ m

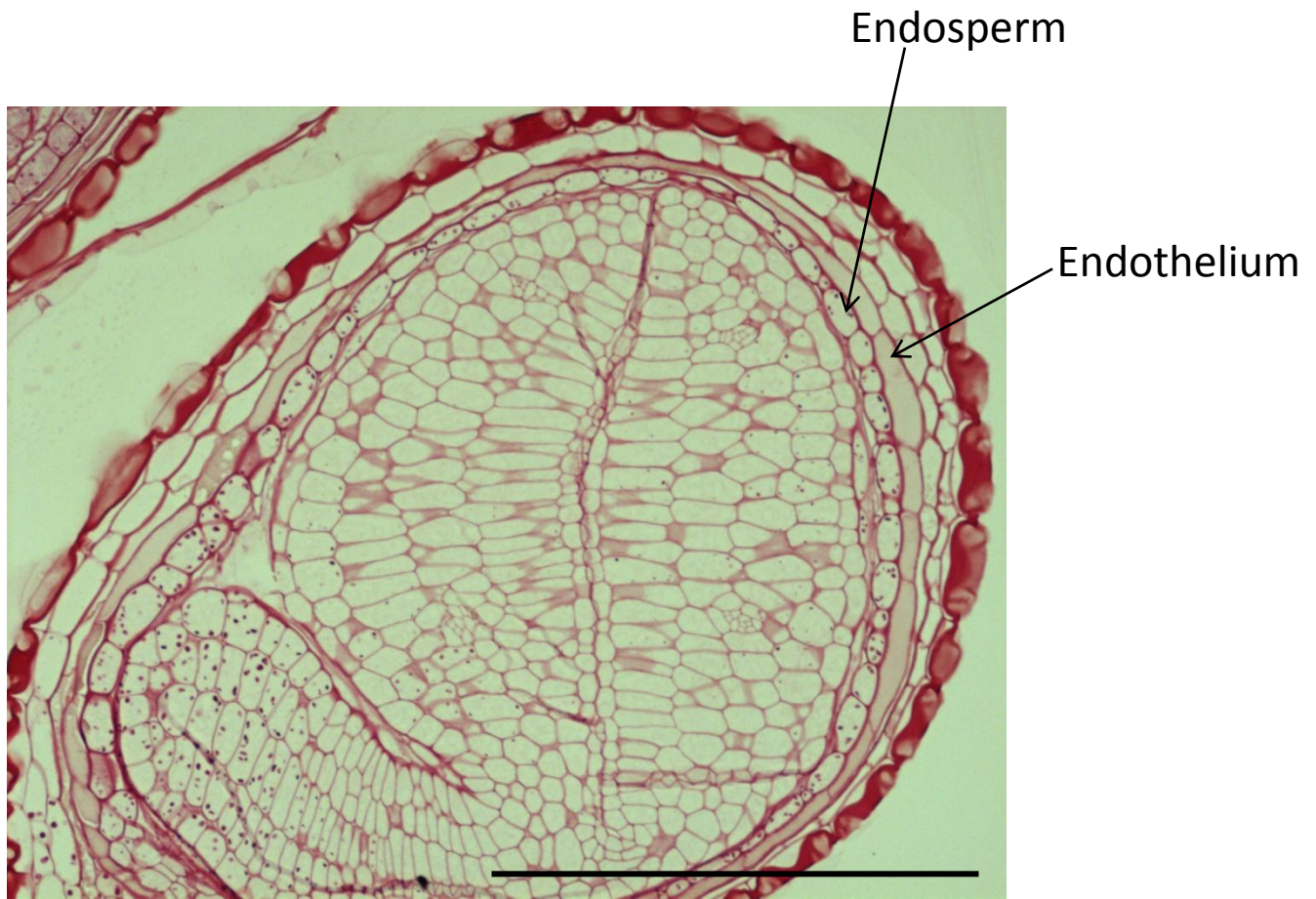


Fig. S8. *gso1-1/gso2-1* double mutant seeds do not contain a persistent endosperm. Mature, green seeds from *gso1-1/gso2-1* homozygous plants were fixed, embedded, sectioned and stained using periodic acid and Schiff's reagent (Sigma Aldrich). Only a single layer of endosperm is visible by this stage in development. Scale bar: 100 μ m

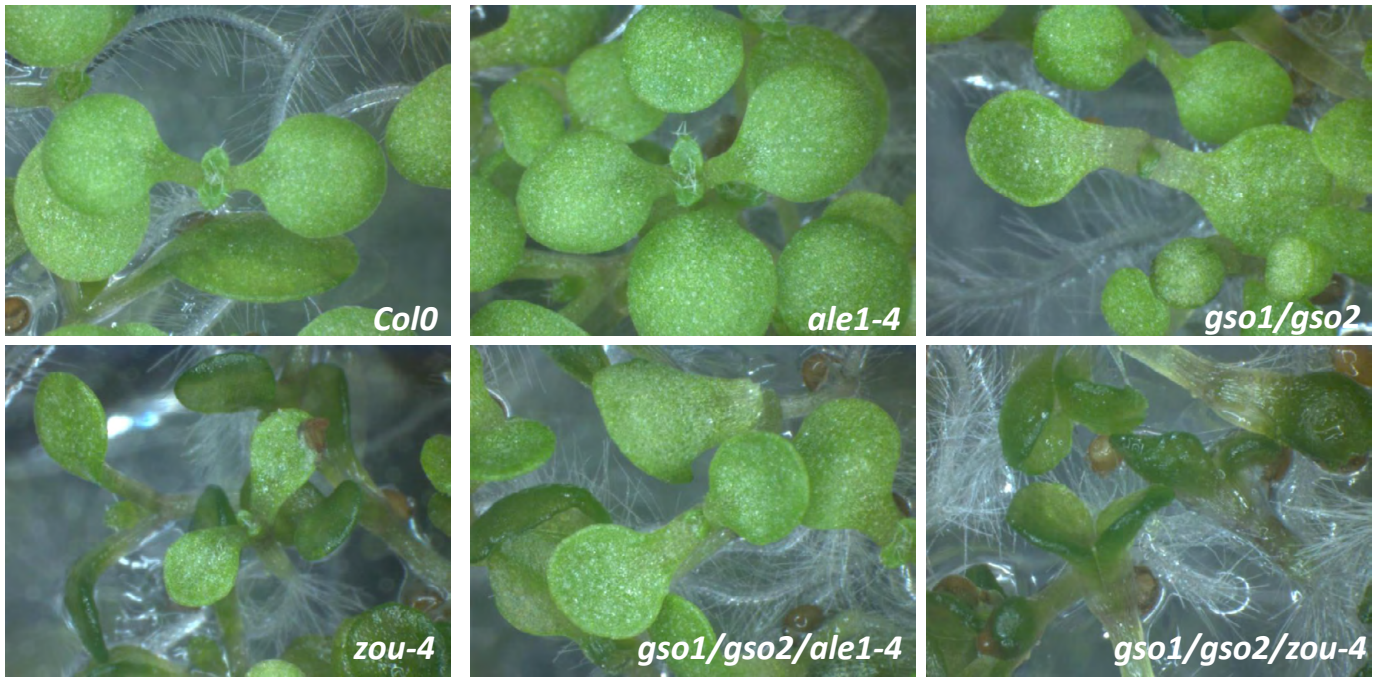


Fig. S9. Seedling phenotypes of single and multiple mutant combinations between *gso1*, *gso2*, *ale1-4* and *zou-4*. Toluidine Blue uptake in these seedlings is quantified in Fig. 7.

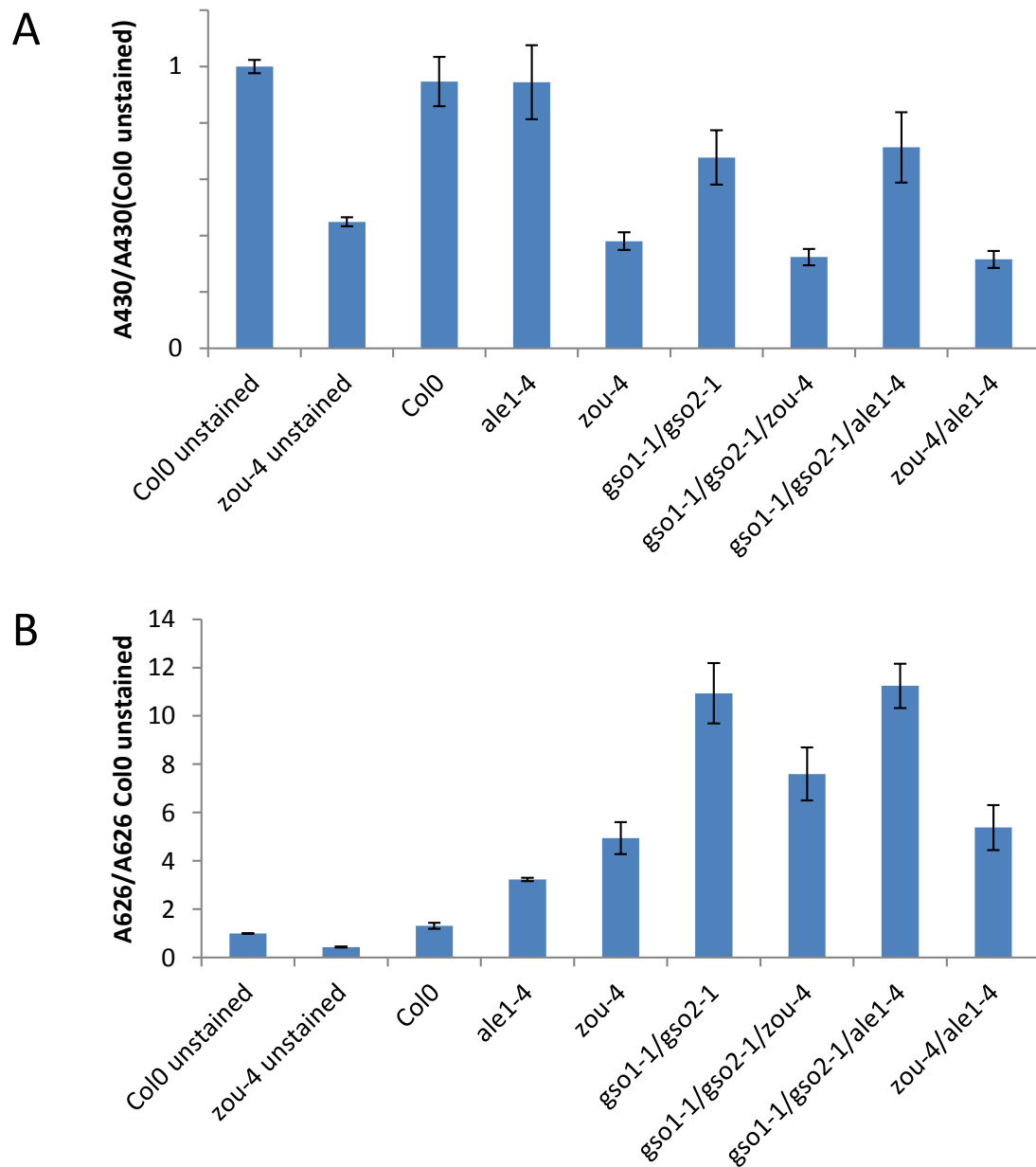


Fig. S10. Absorbance values for samples shown in Fig. 7G. Each sample contained exactly 20 seedlings and was extracted in 1 ml 80% ethanol. **(A)** A430 values (absorbance maximum for Chlorophyll A) relative to A430 for unstained Col0. The smaller size of seedlings from mutant combinations containing *zou-4* and/or *gso1-1/gso2-1* is reflected in their lower chlorophyll content. **(B)** A626 values (absorbance peak for Toluidine Blue) relative to A626 for unstained Col0.

Table S1. Primers used for Q-PCR analysis

| Primer Name | Sequence |
|-------------------------|-----------------------------|
| EIF4A-F (RT) | TTCGCTCTTCTCTTTGCTCTC |
| EIF4A-R (RT) | GAACTCATCTTGTCCCTCAAGTA |
| ALE1-F (RT) | AGGGCGTTGGACTATCAGG |
| ALE1-R (RT) | TGGCTAAGACAAGTCTGTGTTGA |
| At1g71250-F (RT) | TCTTAGACCTAGGCTGTAAAGAGTCA |
| At1g71250-R (RT) | TCAACAACCTACCTTATGCCTAATCTC |
| At2g43870-L (RT) | aGCTTCTTTATGGGATTGTAAAAAGT |
| At2g43870-R (RT) | TGAGCTCTGAAATCCTATTGTCTG |
| At4g38000-L (RT) | AAACAAGAACAAGCCTTGCG |
| At4g38000-R (RT) | GATGACGTCCCGTTCGAG |
| At3g29570-F (RT) | TTTGCAATTTTGCATTCAGTTT |
| At3g29570-R (RT) | TCTGCTTCTTATTGCATCTGACTT |
| At3g30720-F (RT) | GGTTTGAAGCTTCTTTCAACG |
| At3g30720-R (RT) | TTTCTCCACAGCGACCAGTT |