

A regulatory module controlling GA-mediated endosperm cell expansion is critical for seed germination in *Arabidopsis*.

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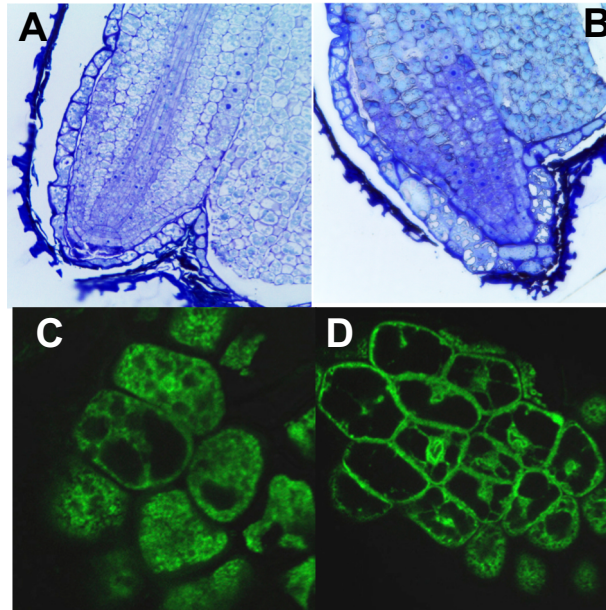


Figure S1. Visualization of changes in endosperm cells during Arabidopsis seed germination.

(A) Semi-thin TBO sections of Arabidopsis endosperm cells at 3 hai.

(B) Semi-thin TBO sections of Arabidopsis endosperm cells at testa rupture.

(C) Confocal image with GFP targeted to vacuole membrane before testa rupture.

(D) Same as **(C)** after testa rupture.

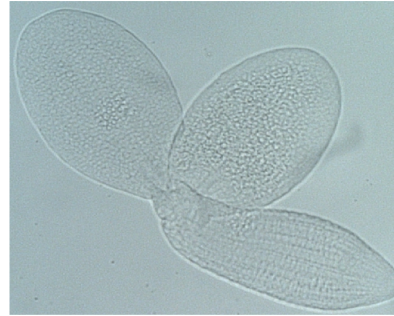


Figure S2. *ProEXPA2:GUS* embryo separated from endosperm at 24 hai and stained overnight to detect GUS activity.

EXPA2 promoters

Arabidopsis thaliana (At)

Arabidopsis lyrata (Al)

Descuriainia sophia (Ds)

Carrichtera annua (Ca)

Hornungia petraene (Hp)

Brassica oleracea (Bo)

Thelungiella halophila (Th)

Capsella rubella (Cr)

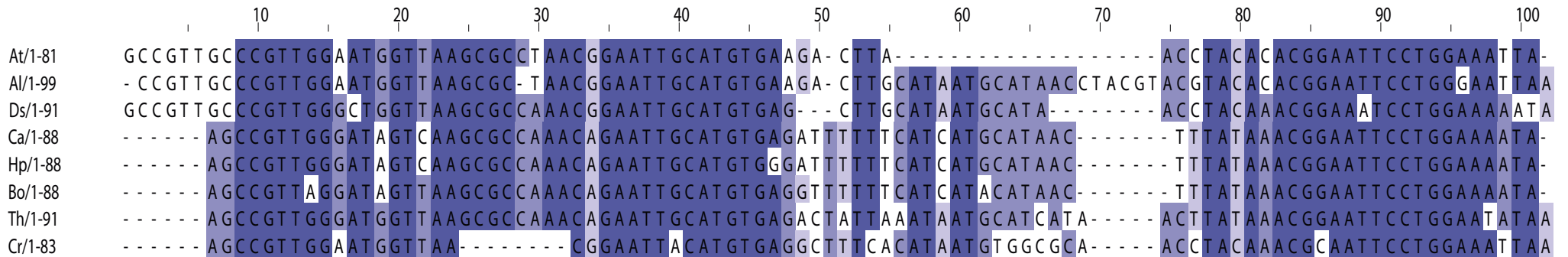


Figure S3. Conserved 80 bp sequenced (*EXPA2-CS*) found after alignment of orthologous *EXPA2* gene promoters from several plant species belonging to the *Brassicaceae* family.

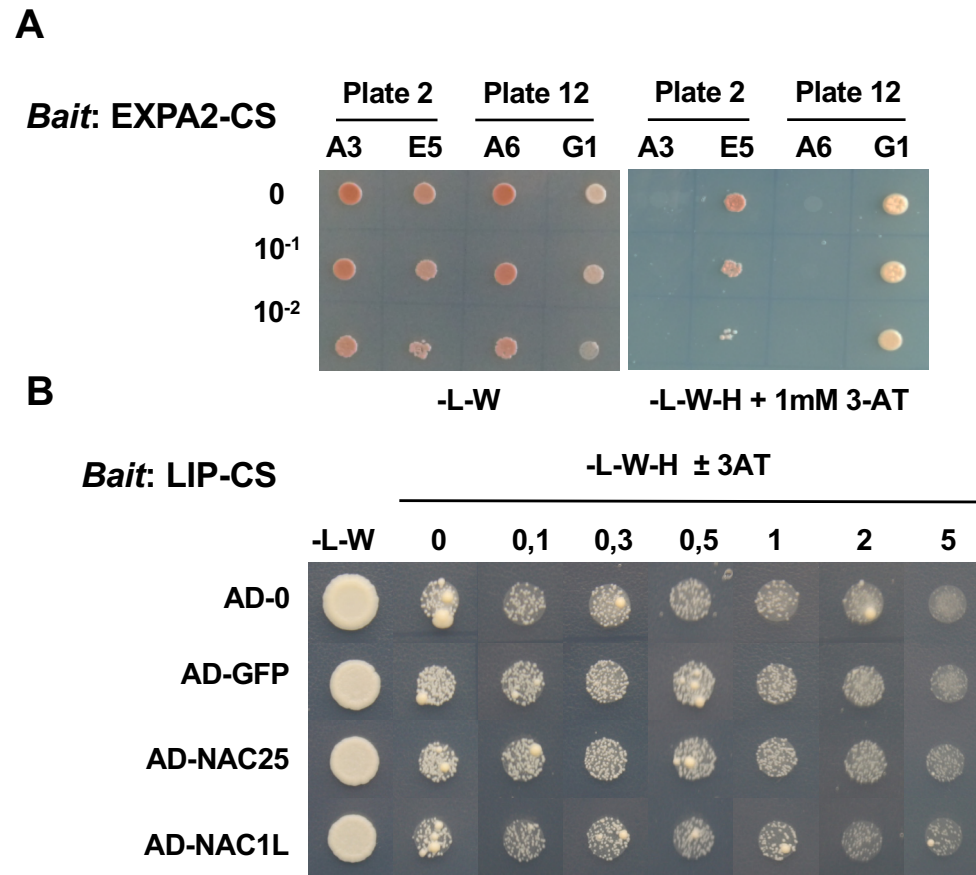


Figure S4. Y1H screening results and control baits.

(A) Y1H assay using the *EXPA2-CS* bait and NAC25 (2-E5), NAC1L (12-G1) and two randomly selected library clones (2-A3 and 12-A6) as preys.

(B) Y1H assay using the *LIP1-CS* bait and NAC25 and NAC1L as preys.

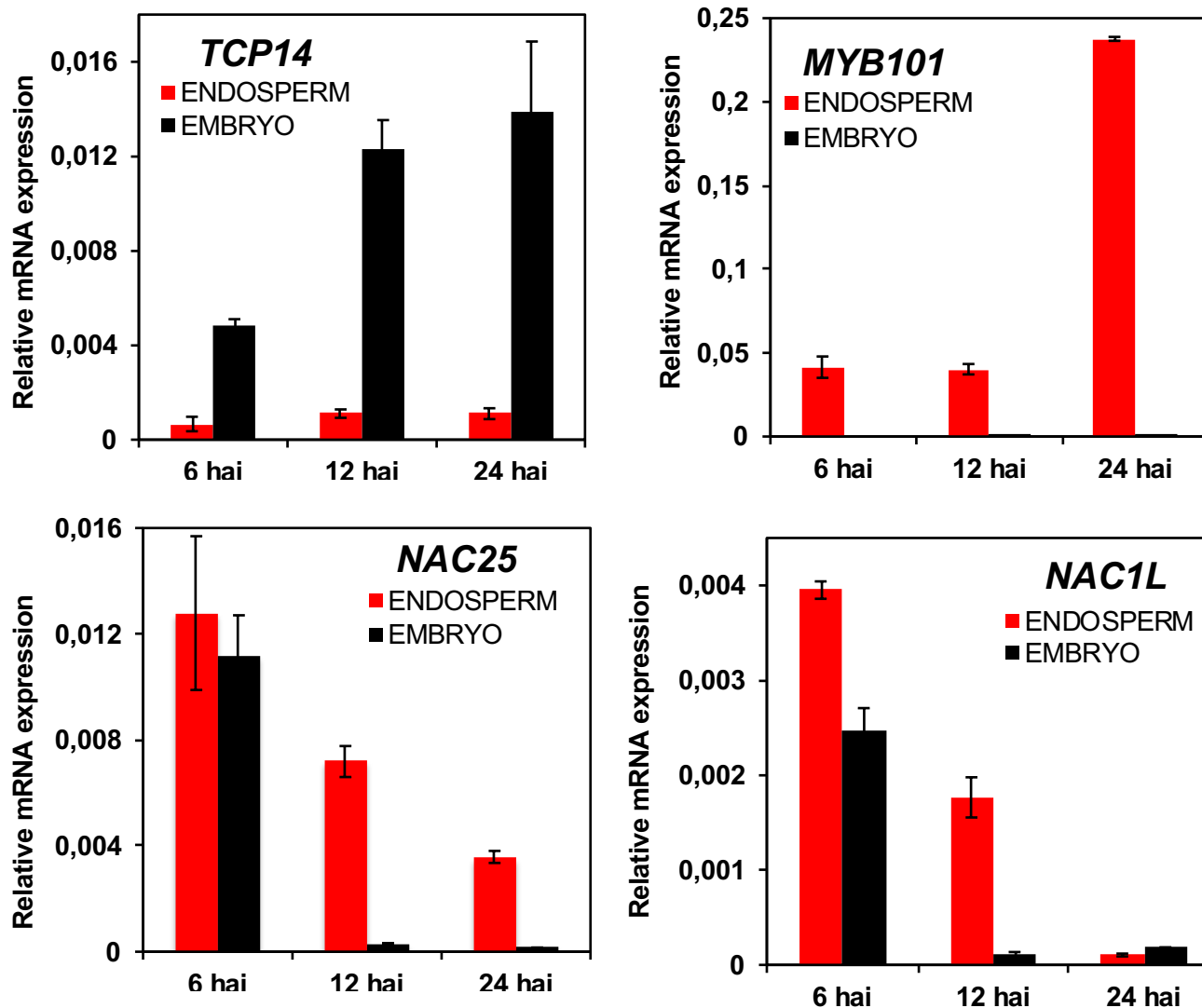


Figure S5. RT-qPCR on RNA isolated from Arabidopsis Col-0 dissected embryos and endosperms at the indicated timepoints. *TCP14* and *MYB101* are expressed throughout imbibition almost exclusively in embryo and endosperm (Dekkers et al., 2013), respectively, and were used to detect significant amounts of tissue-cross contamination in the samples.

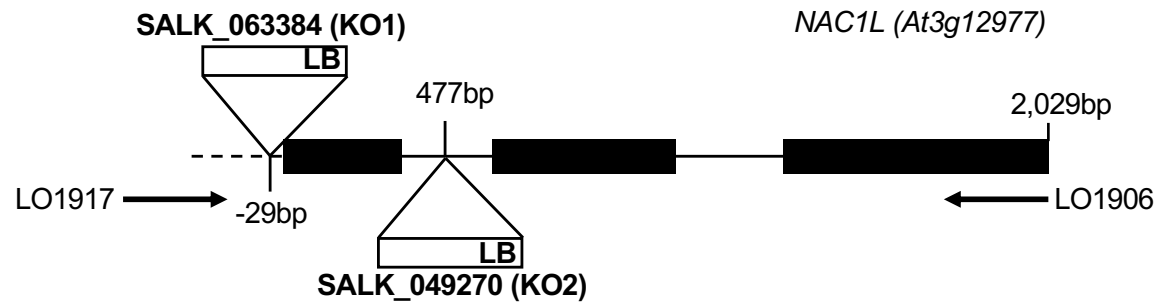
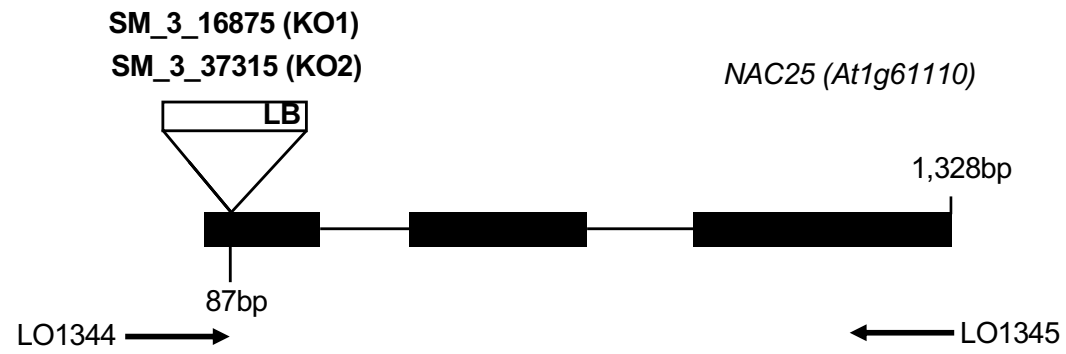
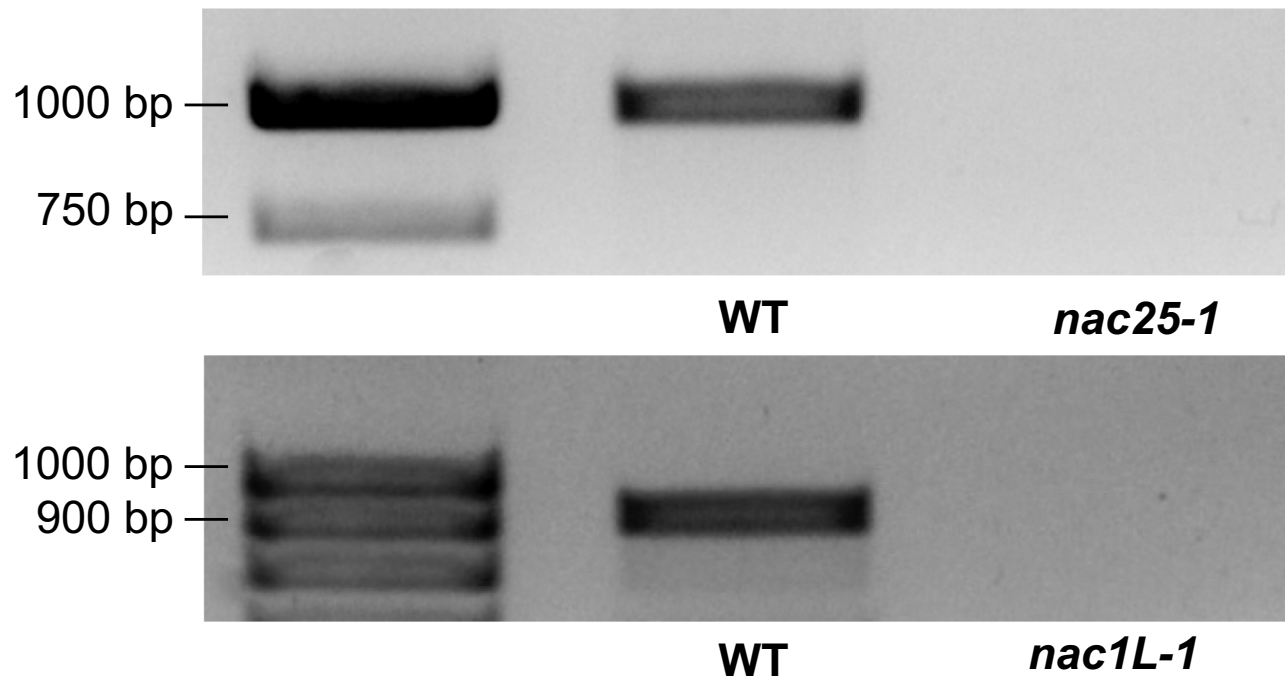
A**B**

Figure S6. Characterization of *NAC25* and *NAC1L* single loss-of-function mutants.

(A) Schematic representation of NAC knock out mutants and oligonucleotides used to amplify full-length cDNAs.

(B) To check that no full-length NAC mRNAs were produced in the homozygous plants, PCR with oligonucleotides spanning the corresponding TDNA insertions was carried out on cDNAs synthesized from RNAs isolated at 12 hai (the same RNAs used for RNA-seq).

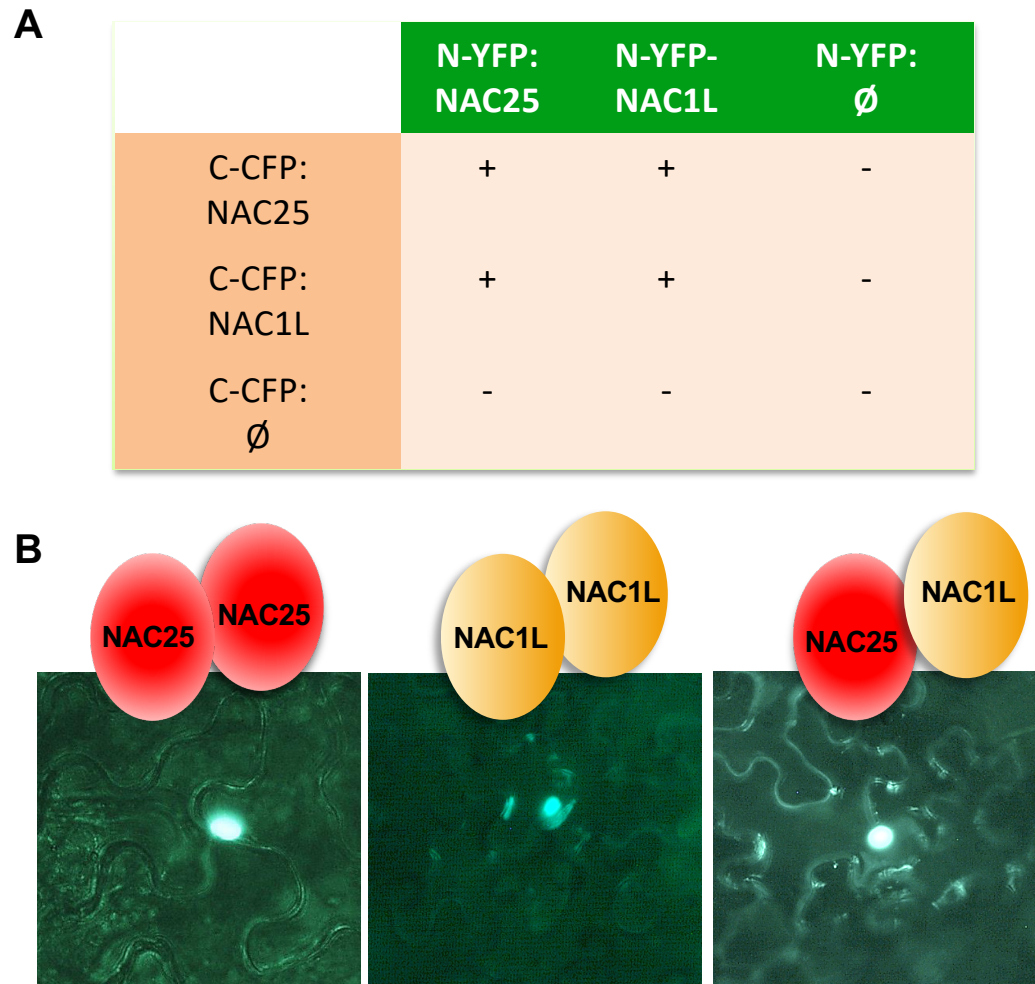


Figure S7. NAC25 and NAC1L form homo and heterodimers *in planta*.

(A) The NAC25 and NAC1L CDSs were fused to the N-YFP (N) or C-CFP (C) CDS and coexpressed in *N. benthamiana* cells in different combinations as indicated in the table. Empty vectors (∅) were included in the combinations as controls. Combinations giving a fluorescent signal in the nuclei of *N. benthamiana* cells or not giving any signal are indicated as (+) and (-), respectively.

(B) Images obtained in a fluorescence microscope for several combinations showing nuclear fluorescence.

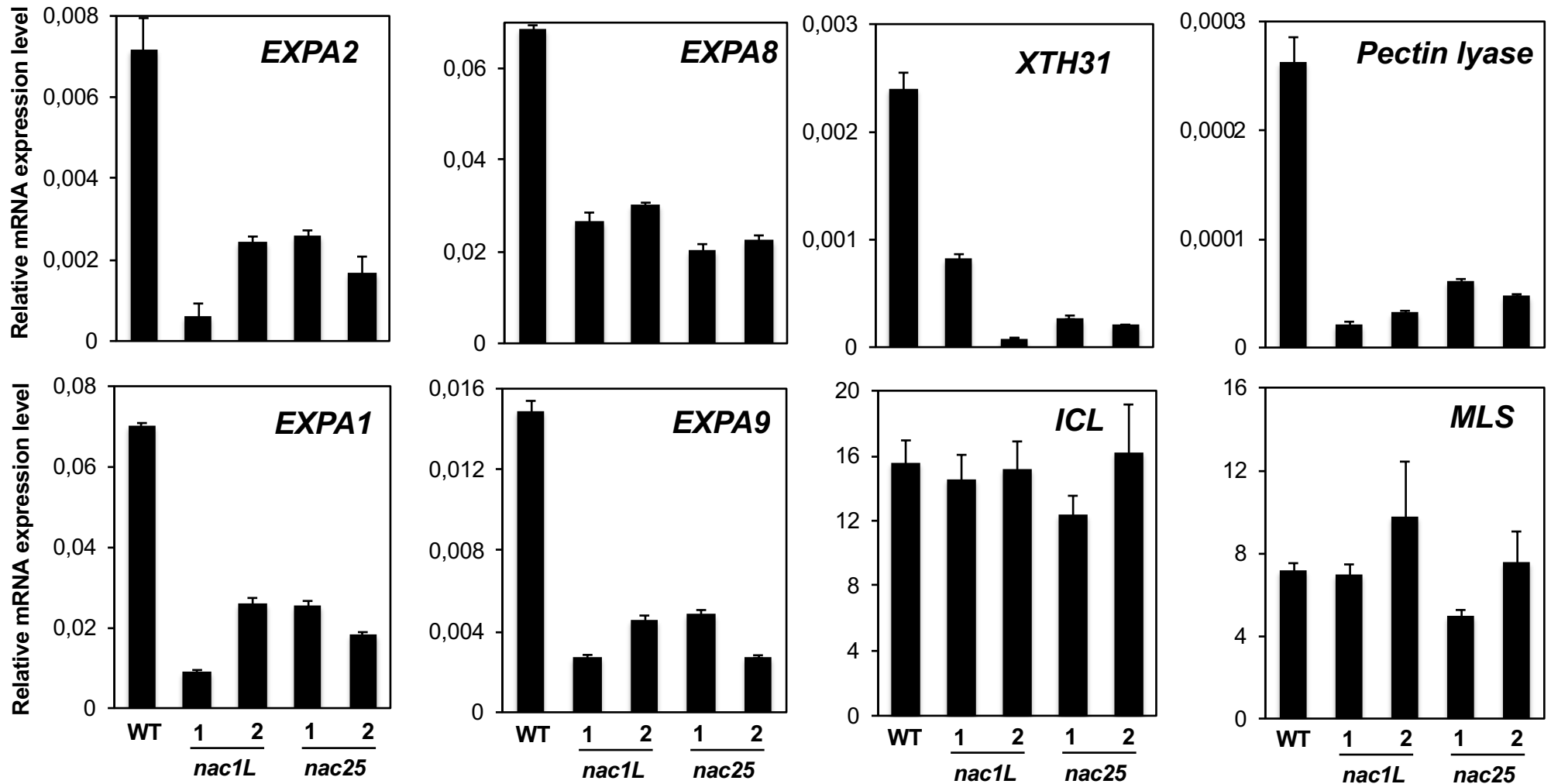


Figure S8. Quantification of CWRE Gene Expression in NAC knock-out mutants. Quantification by qRT-PCR of mRNA levels of CWREs and control genes (ICL and MLS) in wild-type, *nac25-1*, *nac25-2*, *nac1L-1* and *nac1L-2* genotypes. RNAs were isolated from seeds at 12 hai, and their levels are shown relative to those of *ACT8*. Average values and SE for two replicates are shown. Similar results were obtained with a different seed batch.

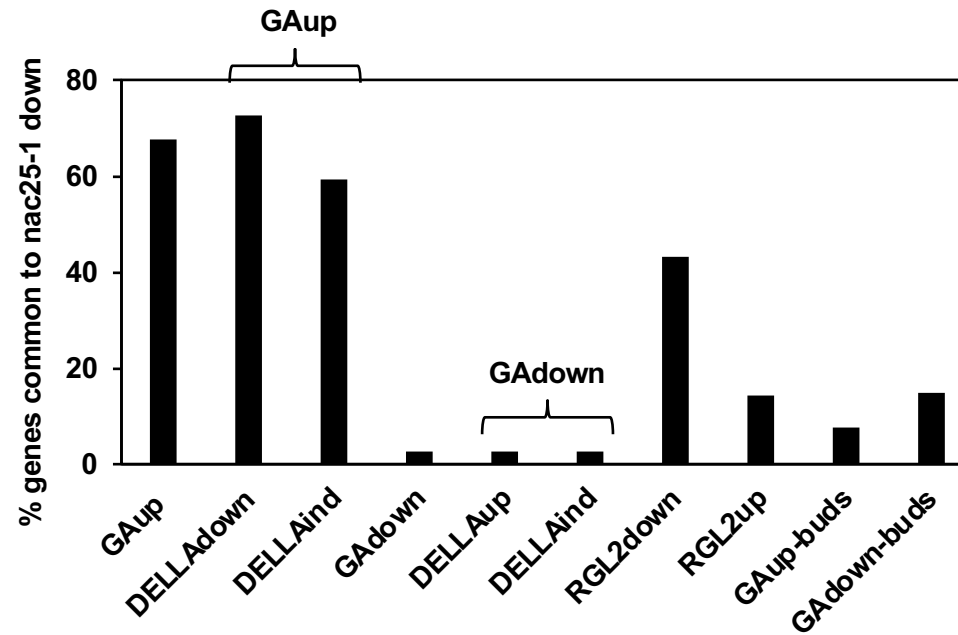


Figure S9. Overlap between genes downregulated >2-fold in the *nac25-1* transcriptome and genes deregulated >2-fold in GA-related transcriptomes.

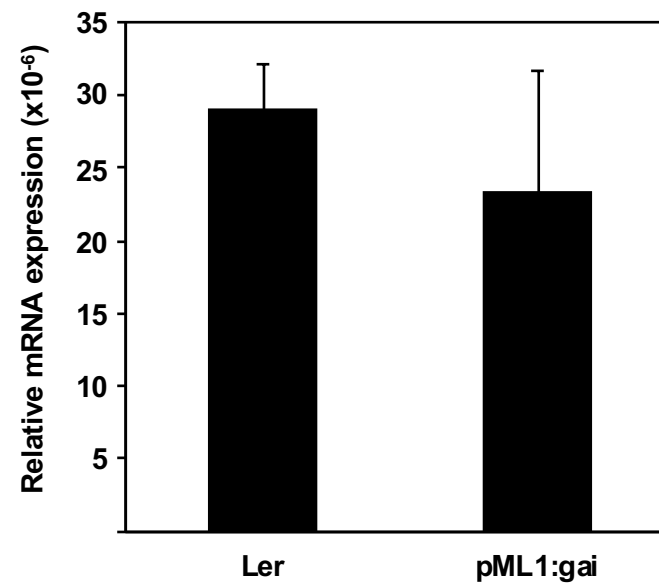


Figure S10. Quantification of *EXPA2* gene expression in WT and *ProML1:GFP-gai-1* seeds, the latter ones having blocked GA signaling in the epidermis (Gallego-Bartolomé et al., 2011; Rombolá-Caldentey et al., 2014).