# Supporting Information

## Lee et al. 10.1073/pnas.1012896107

### SI Materials and Methods

**Germination Assays.** Seeds were plated on Murashige and Skoog media (Sigma-Aldrich) with 0.8% (wt/vol) agar. Medium was supplemented with 100 μM $GA_3$ (Sigma), 5 μM (±)-ABA (Sigma), or 50 μM Norflurazon (Supelco). Dormant Cvi seeds were stratified for 9 d at 4 °C in darkness to break dormancy. Seeds were grown under long day condition (16 h light/8 h dark, 80 μmol $m^{-2}$ $s^{-1}$) at 20–21 °C. Germination rates were scored on the basis of radical emergence. Between 150 and 300 seeds were used to check radical emergence and endosperm rupture.

**Bedding Assay with Seed Coat Extract.** A total of 150 seed coats from dormant and nondormant Cvi seeds were collected 24 h and 48 h after seed imbibition on MS agar medium and frozen under liquid nitrogen and homogenized. The ground materials were resuspended in 15 μL MS liquid medium. The resulting extract was mixed with 15 μL of MS/agar medium and was let to solidify in a Petri dish. Col embryos dissected 4 h after imbibition where then laid on the solidified medium separated by a nylon filter.

**RNA Extraction and Analysis.** Total RNA was extracted as described in ref. 1. RNA gel blot analysis was performed as described in ref. 2. The RGL2, ABi3, and ABi5 probes were described in ref. 2. A *SLY1* and a *GID1A* DNA probe were generated using the following primers: SLY1, 5′-ATGAAGCGCAGTACTACCGACTCTG-3′ and 5′-TTATTTGGATTCTGGAAGAGGTCTC-3′; and GID1A, 5′-ATGGCTGCGAGCGATGAAGTTAATCTTATTGAGAG-3′ and 5′-ATTCCGCGTTTACAA ACGCCG-3′.

**GUS Assay.** GUS assays were performed as described in ref. 3. The dissected coatless embryos with GUS assay buffer [50 mM sodium phosphate buffer (pH 7.0), 10 mM EDTA (pH 8.0), 0.1% (vol/vol) Triton X-100, 0.5 mg/mL X-GlcA] were infiltrated by vacuum for 5 min and incubated at 37 °C for 1.5 h.


1. Vicient CM, Delseny M (1999) Isolation of total RNA from Arabidopsis thaliana seeds. *Anal Biochem* 268:412–413.
2. Piskurewicz U, et al. (2008) The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. *Plant Cell* 20:2729–2745.
3. Jefferson RA (1987) Assaying chimeric genes in plants: GUS gene fusion system. *Plant Mol Biol Rep* 5:387–405.


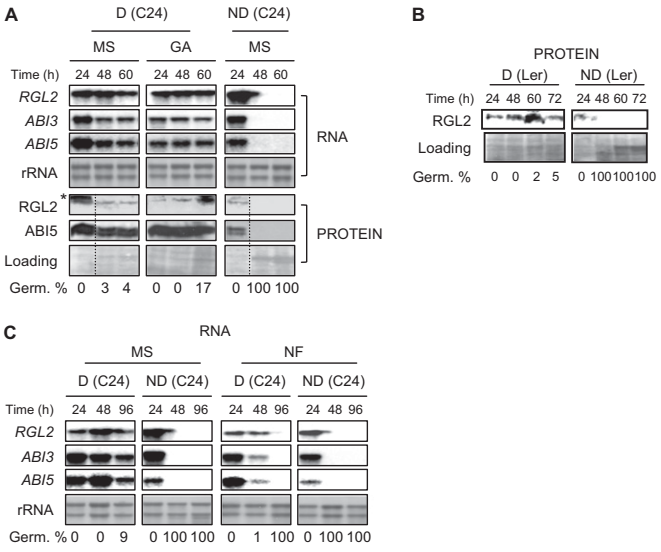


**Fig. S1.** (*A*) Time course of *RGL2, ABI3*, and *ABI5* expression in dormant (D) and nondormant (ND) C24 seeds in absence (MS) or presence of 100 μM GA (GA). Germination rate (Germ. %) at each time point is indicated. (*) aspecific signal. (*B*) Time course of RGL2 protein levels in freshly harvested, i.e., dormant (D) and after-ripened, i.e., nondormant (ND), Ler seeds upon imbibition in MS medium. (*C*) Same as *A* with seeds treated with Norflurazon (NF). Germination rate (Germ. %) at each time point is indicated. Vertical dashed lines within the pictures indicate that the separated lanes were from the same gel but were not directly next to each other.

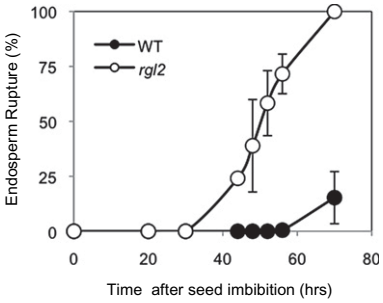


**Fig. S2.** Time course of percentage of endosperm rupture events (i.e., germination events) in freshly harvested Ler (WT) and *rgl2-1* (*rgl2*) seeds after imbibition on normal germination medium (MS). Data represent the average of two repetitions and error bars represent SD.


**Fig. S3.** RGL2 protein levels in seed coat dissected from Ler and *rgl2-1* (*rgl2*). One hundred seed coats from freshly harvested Ler and *rgl2* seeds were dissected 72 h after seed imbibition on MS medium (*A*) and dissected 4 h after seed imbibition and incubated for 68 h on MS medium (*B*).

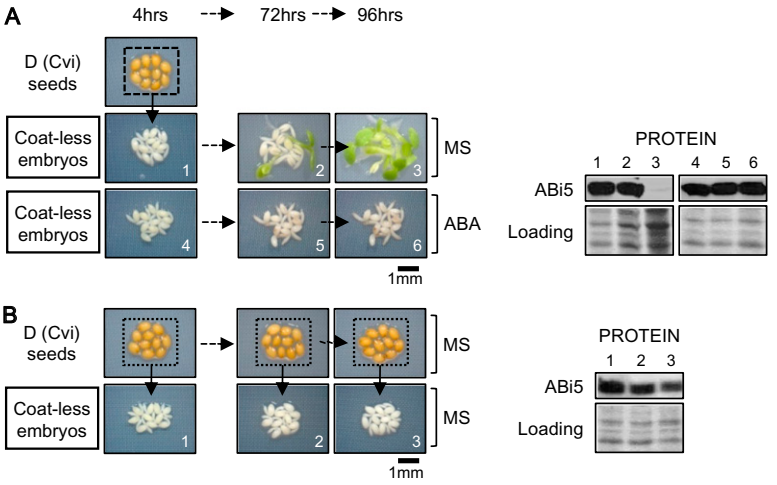


**Fig. S4.** (*A*) Pictures (*Left*) show embryos dissected from dormant (D) Cvi seeds 4 h after imbibition in MS medium. Pictures (*Right*) show embryos incubated in absence (MS) or presence of 0.5 μM ABA (ABA) at the indicated times. Proteins were extracted from the embryo material shown. (*B*) Dormant (D) Cvi seeds at different times upon imbibition in a normal medium (MS). Arrows indicate Cvi embryos upon their dissection from dormant seeds at each time point. Proteins were extracted from the embryo material shown.

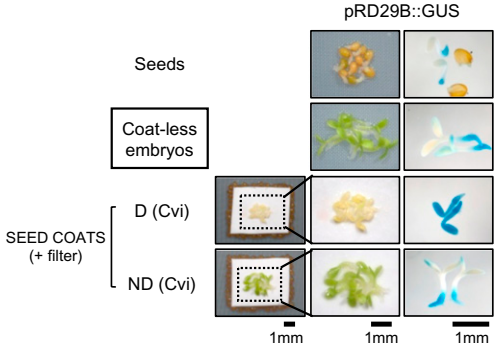


**Fig. S5.** Seed coat bedding assays using embryos dissected from nondormant p*RD29B-GUS* seeds and seed coats dissected from dormant (D) or nondormant (ND) Cvi seeds (coats are hidden by the filter). Pictures were taken 54 h after imbibition in MS medium. GUS staining was performed from photographed material.

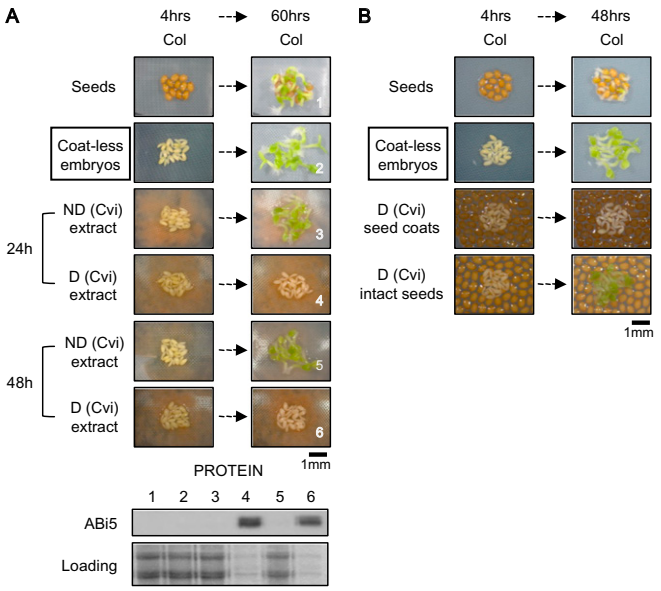


**Fig. S6.** (*A*) Embryos dissected from nondormant Col seeds were laid on a layer of extracts of seed coats dissected from dormant (D) or nondormant (ND) Cvi seeds 24 h and 48 h after seed imbibition. Proteins were extracted from the embryo material shown. (*B*) Embryos dissected from nondormant Col seeds were laid on a layer of dormant Cvi seed coats or intact seeds.

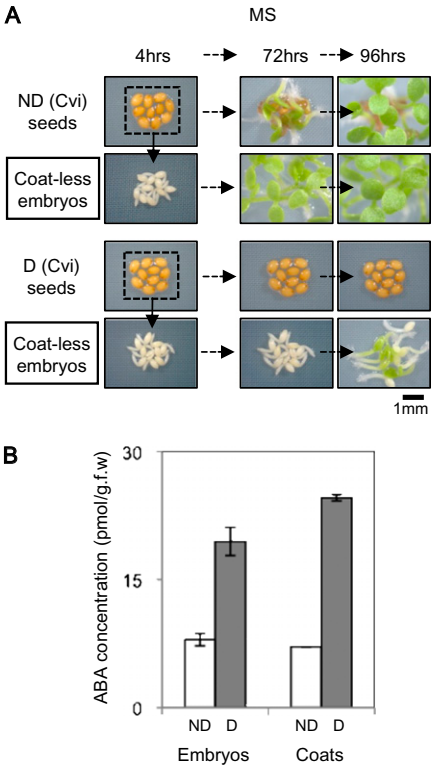


**Fig. S7.** (*A*) Pictures (*Left*) show seeds and embryos dissected from dormant (D) and nondormant (ND) Cvi seeds 4 h after imbibition in MS medium. Pictures (*Right*) show seeds and embryos incubated in MS medium at the indicated times. (*B*) Endogenous ABA levels in coatless embryos and seed coats dissected from dormant (D) and nondormant (ND) Cvi seeds 24 h after seed imbibition in MS medium. Error bars indicate SD ($n$ = 3).

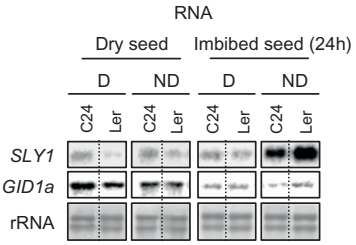


**Fig. S8.** *SLY1* and *GID1a* mRNA expression in dormant (D) and after-ripened, i.e., nondormant (ND) WT seeds (C24 and Ler). Vertical dashed lines within the pictures indicate that the separated lanes were from the same gel but were not directly next to each other.