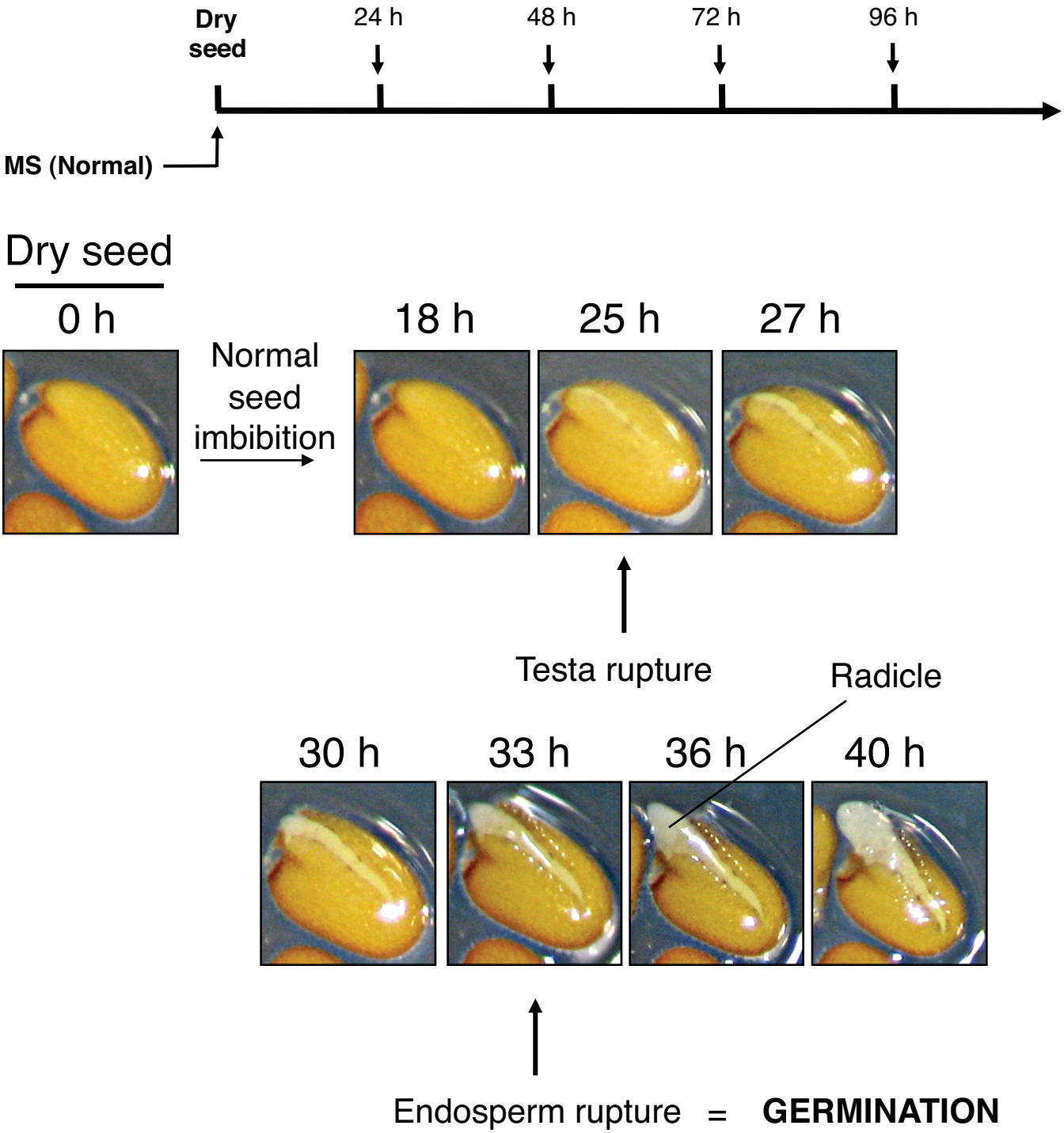
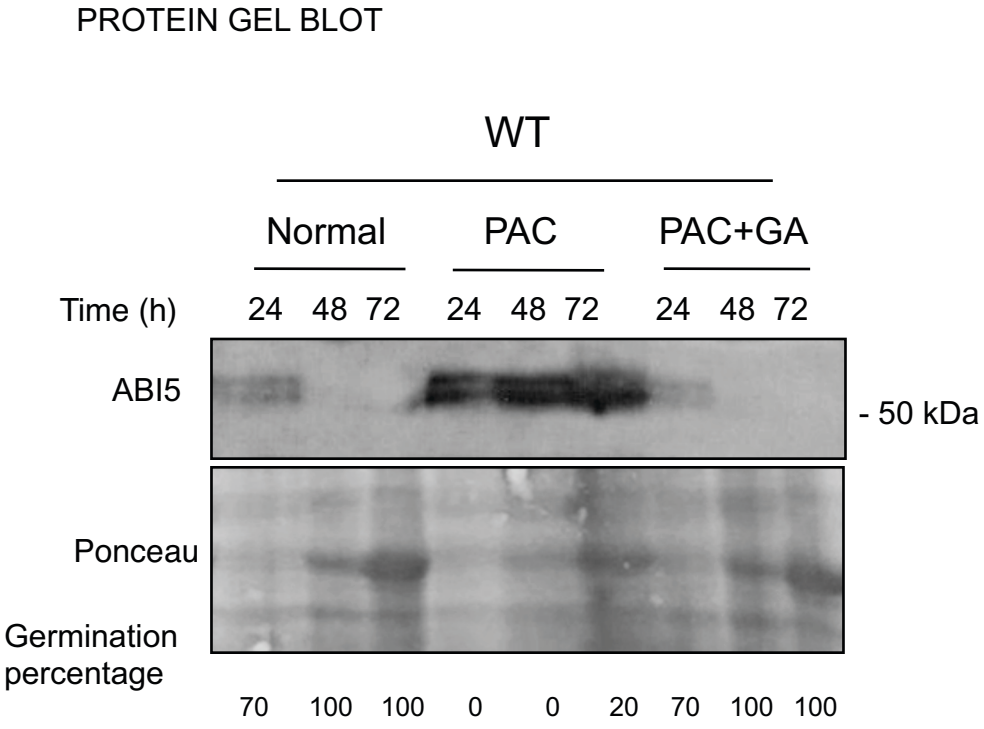
Supplemental Data Piskurewicz et al. (2008) The GA-signaling repressor RGA-Like 2 inhibits Arabidopsis seed germination by stimulating ABA synthesis and ABA-Insensitive 5 activity



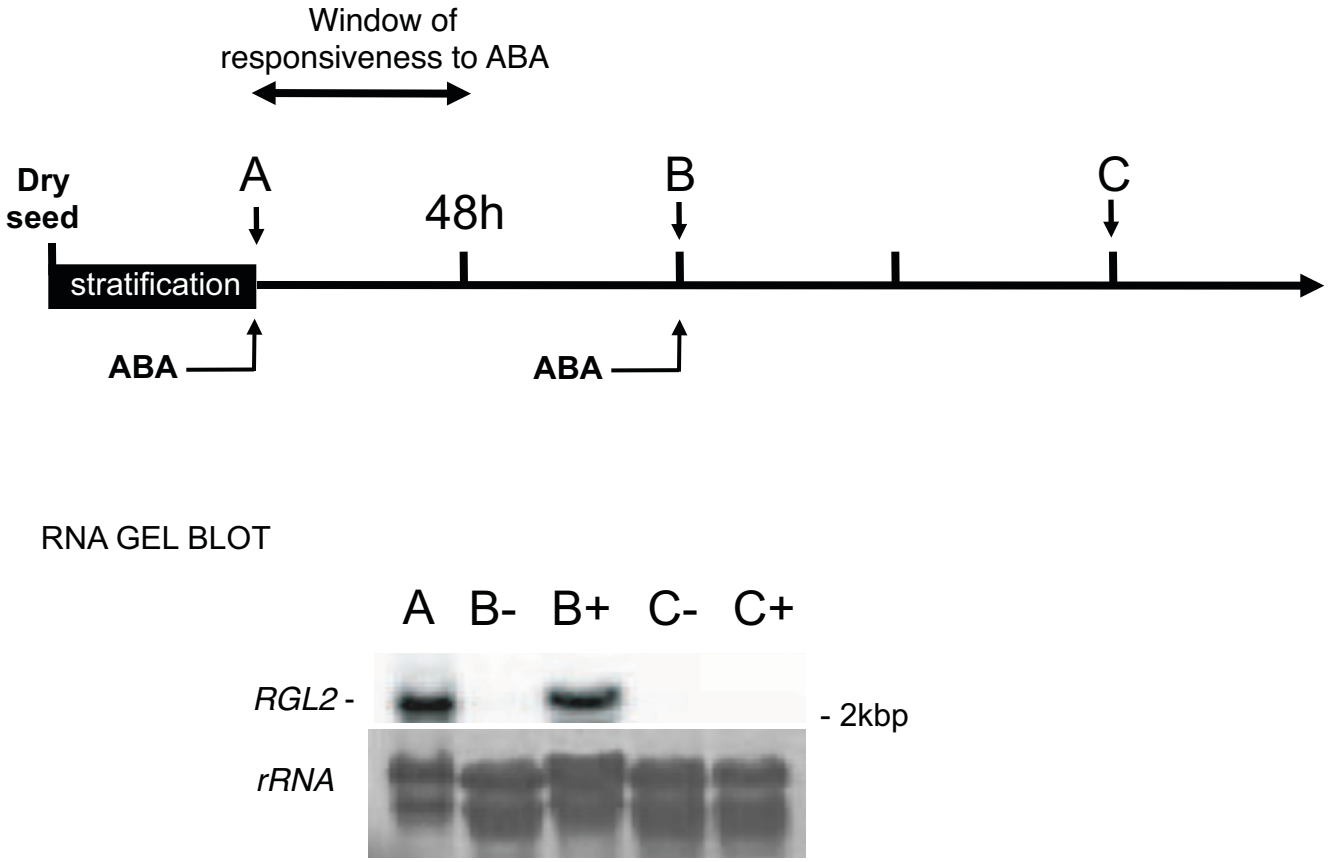
Supplemental Figure 1: Early developmental steps upon seed imbibition

WT seeds (Col) at different times upon imbibition under normal conditions. Testa and endosperm rupture events are indicated by arrows.



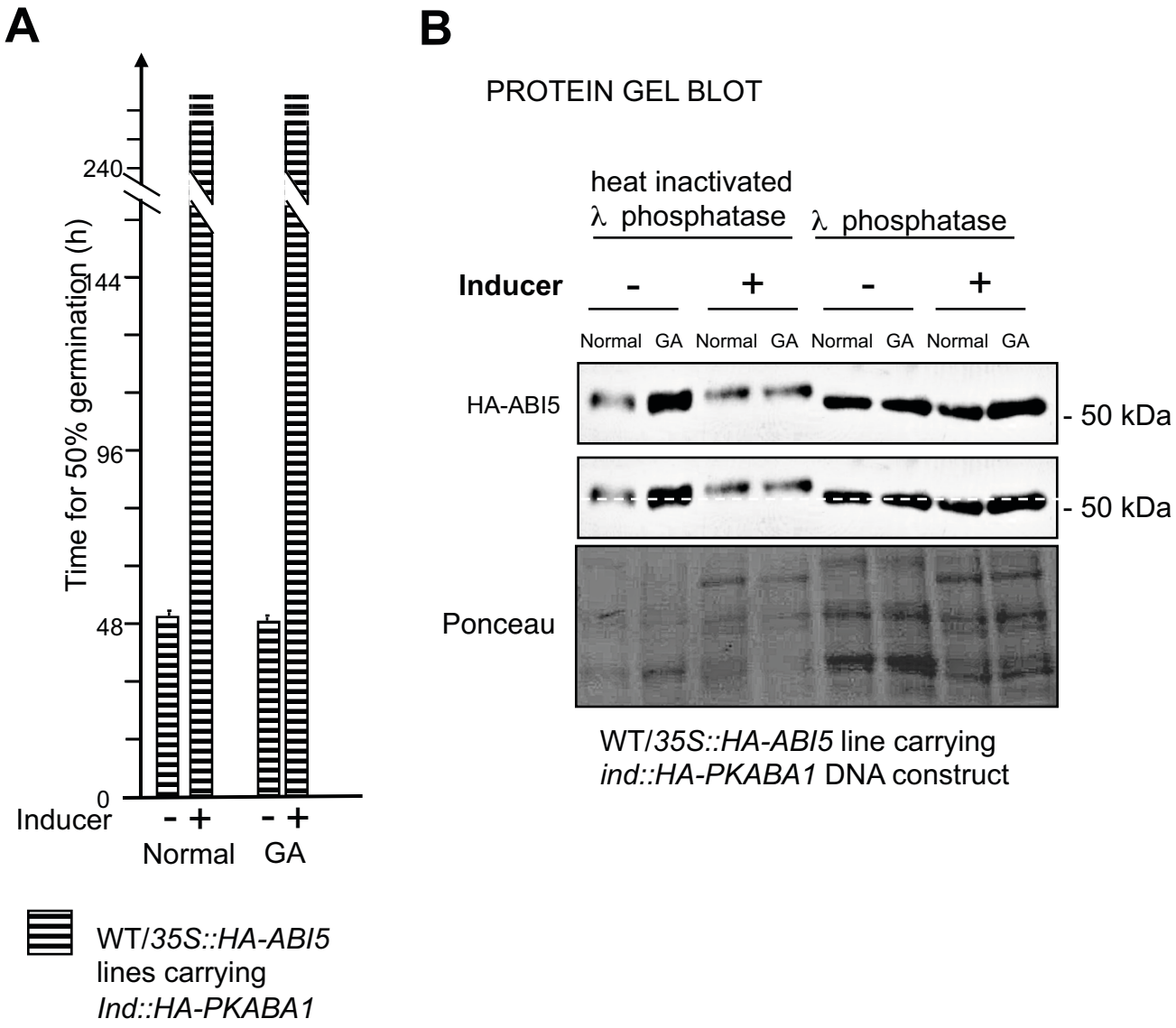
Supplemental Figure 2: GA treatment prevents high ABI5 protein accumulation in PAC-treated seeds.

Protein gel blot analysis of ABI5 protein levels upon imbibition of WT (Col) seeds in absence (normal) or presence of PAC (5μM) or PAC and GA (50μM). Protein extracts from 100 WT seeds were loaded in each lane. Germination percentage at each time point is indicated.



Supplemental Figure 3: ABA regulates *RGL2* expression within the same developmental window previously characterized for *ABI5.*

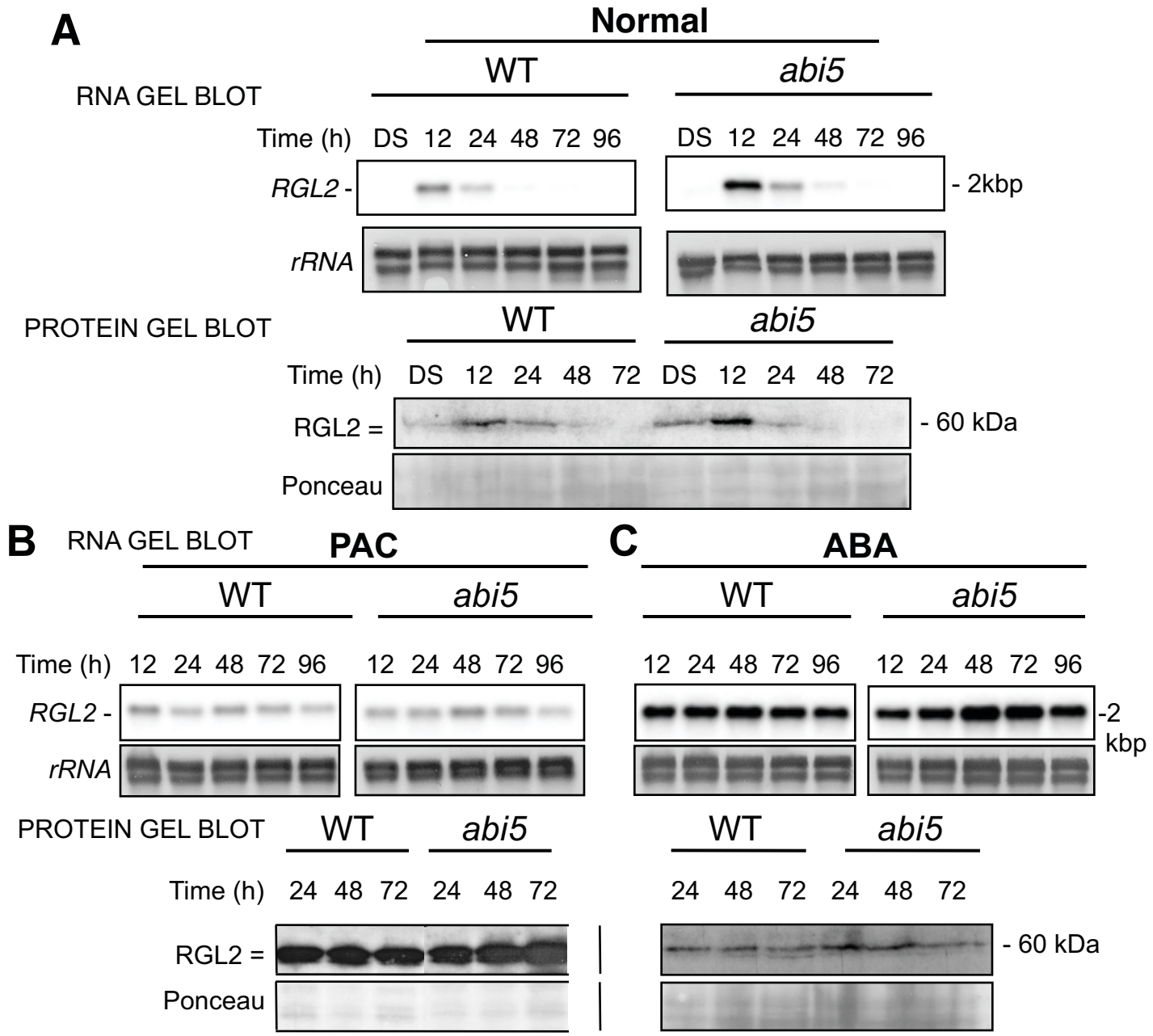
This experiment is performed as in (Lopez-Molina et al., 2001). WT (Col) seeds were kept in darkness at 4°C for 3 days in absence of ABA and then either left 8 days in absence of ABA or transferred to ABA (2.5μM) plates as follows: just after stratification (A) or 4 days after stratification (B). Samples for *RGL2* mRNA analysis were collected at the end of stratification (A), 4 days after stratification in absence (B-) or presence of ABA (B+) or 8 days after stratification in absence (C-) or presence of ABA (C+). *RGL2* mRNA levels were monitored by RNA gel blot as described in Fig. 1.



Supplemental Figure 4: Co-expression of ABI5 and PKABA1 is sufficient to block seed germination.

A: Seeds of WT/*35S:HA-ABI5* lines carrying a *ind:HA-PKABA* transgene were sown in absence or presence of inducer (50µM 17ß-estradiol). Percentage of seed germination was scored over time and the resulting germination percentage curves were used to determine the time when half of the seed population had germinated. Histograms show the times obtained for three independent seed batches ($t < 0.05$).

B: Protein gel blot analysis showing differences in HA-ABI5 protein mobility as shown in Fig. 2B. Ponceau S staining of the membrane prior to antibody incubation is shown.

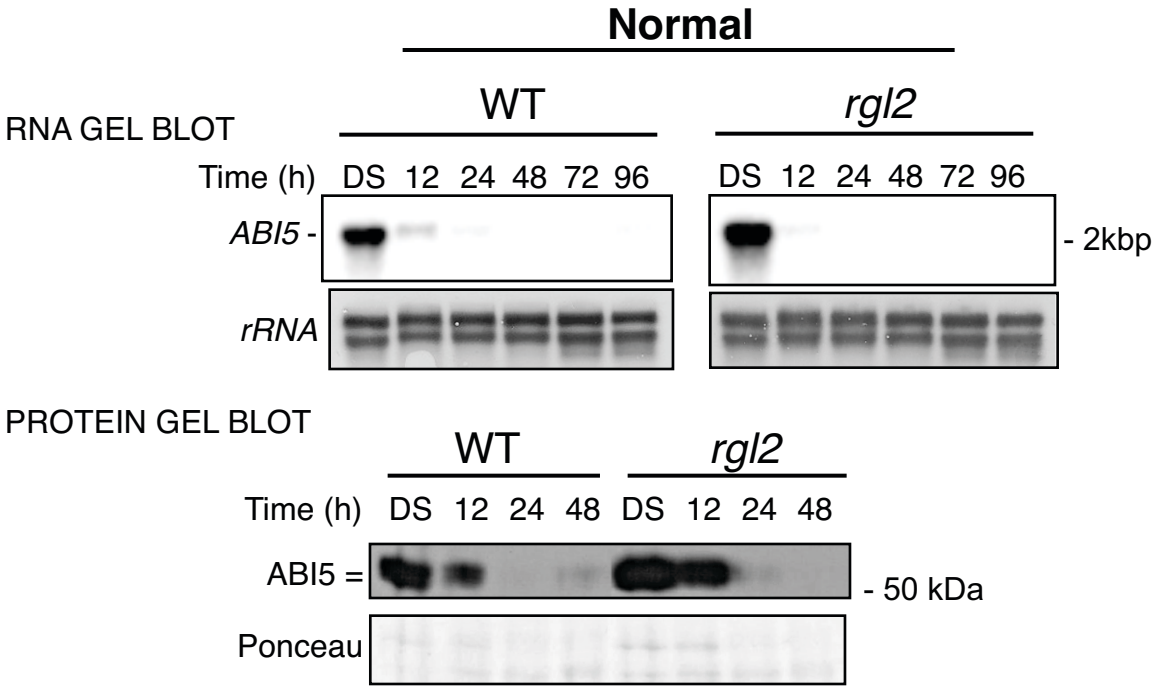


Supplemental Figure 5: *RGL2* expression in *abi5* mutant is similar to WT under all germination conditions examined.

A: RNA and protein gel blot analysis of a time course of *RGL2* mRNA and protein levels upon WT (Col) and *abi5-3* seed imbibition under normal conditions. 2µg of total RNA per lane. rRNA, ribosomal RNA loading control; DS, dry seeds. 10µg of total protein per lane. Protein extracts were stained with Ponceau S as a loading control (Ponceau) prior to detection with antibodies against RGL2.

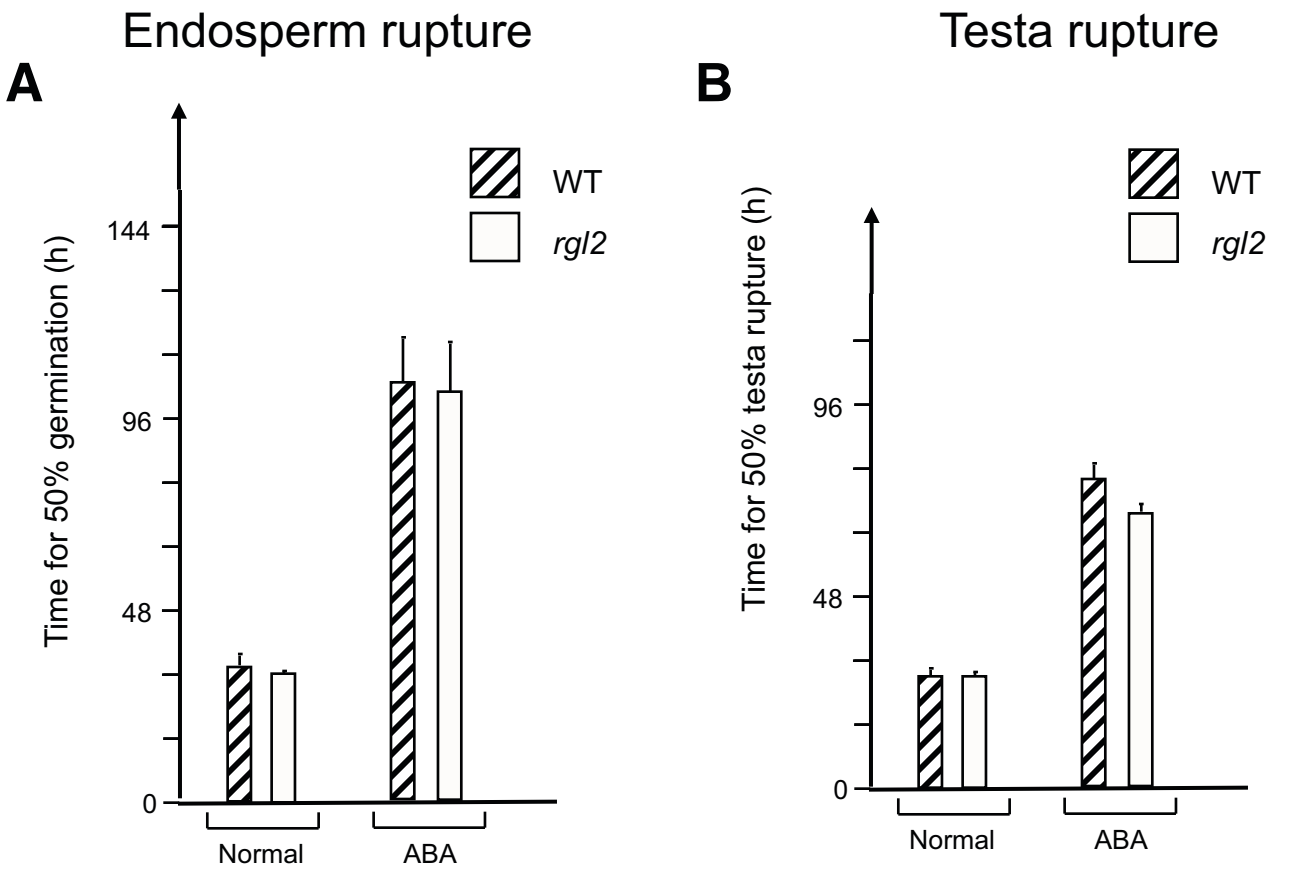
B: RNA and protein gel blot analysis of a time course of *RGL2* mRNA and protein levels upon WT (Col) and *abi5-3* seed imbibition in presence of 5µM paclobutrazol (PAC), an inhibitor of GA synthesis. 2µg of total RNA per lane. rRNA, ribosomal RNA loading control; DS, dry seeds. 10µg of total protein per lane. Protein extracts were stained with Ponceau S as a loading control (Ponceau) prior to detection with antibodies against RGL2.

C: RNA and protein gel blot analysis of a time course of *RGL2* mRNA and protein levels upon WT (Col) and *abi5-3* seed imbibition in presence of 5µM ABA (ABA). 2µg of total RNA per lane. rRNA, ribosomal RNA loading control; DS, dry seeds. 10µg of total protein per lane. Protein extracts were stained with Ponceau S as a loading control prior to detection with antibodies against RGL2.



Supplemental Figure 6: *ABI5* expression in *rgl2* mutant is similar to WT under normal germination conditions.

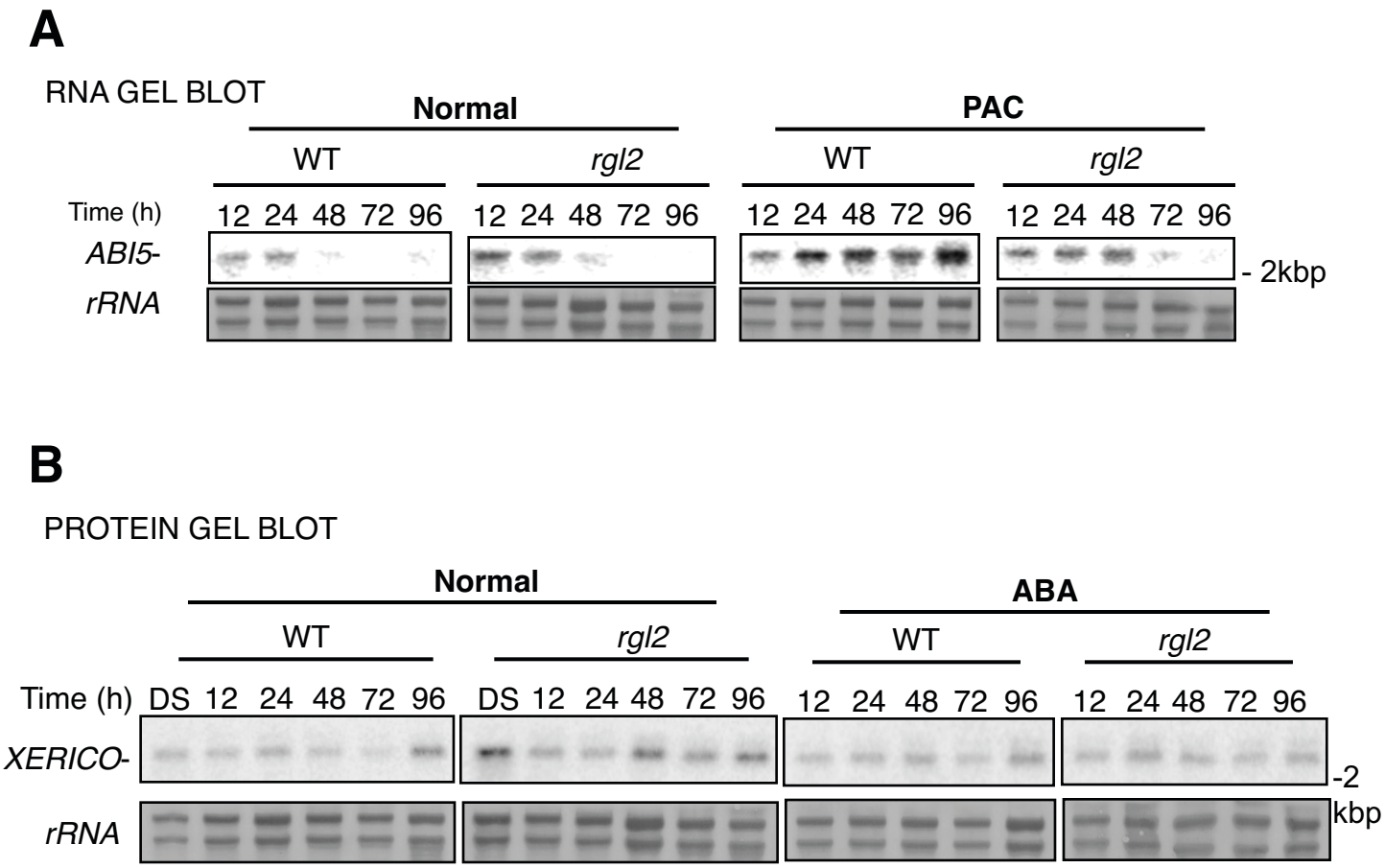
RNA and protein gel blot analysis of a time course of *ABI5* mRNA and protein levels upon WT (Col) and *rgl2-13* seed imbibition under normal conditions. 2µg of total RNA per lane. rRNA, ribosomal RNA loading control; DS, dry seeds. 10µg of total protein per lane. Protein extracts were stained with Ponceau S as a loading control (Ponceau) prior to detection with antibodies against ABI5.



Supplemental Figure 7: Inhibition of endosperm rupture by ABA is similar between *rgl2* and WT. Testa rupture of *rgl2* seed is moderately resistant to high ABA concentrations.

A: Seeds of WT (Col) and *rgl2-13* were sown in absence (Normal) or presence of ABA (1µM). Percentage of seed germination (endosperm rupture) was scored over time and the resulting germination percentage curves were used to determine the time when half of the seed population had ruptured the endosperm. Histograms show the times obtained for three independent seed batches ($t < 0.05$).

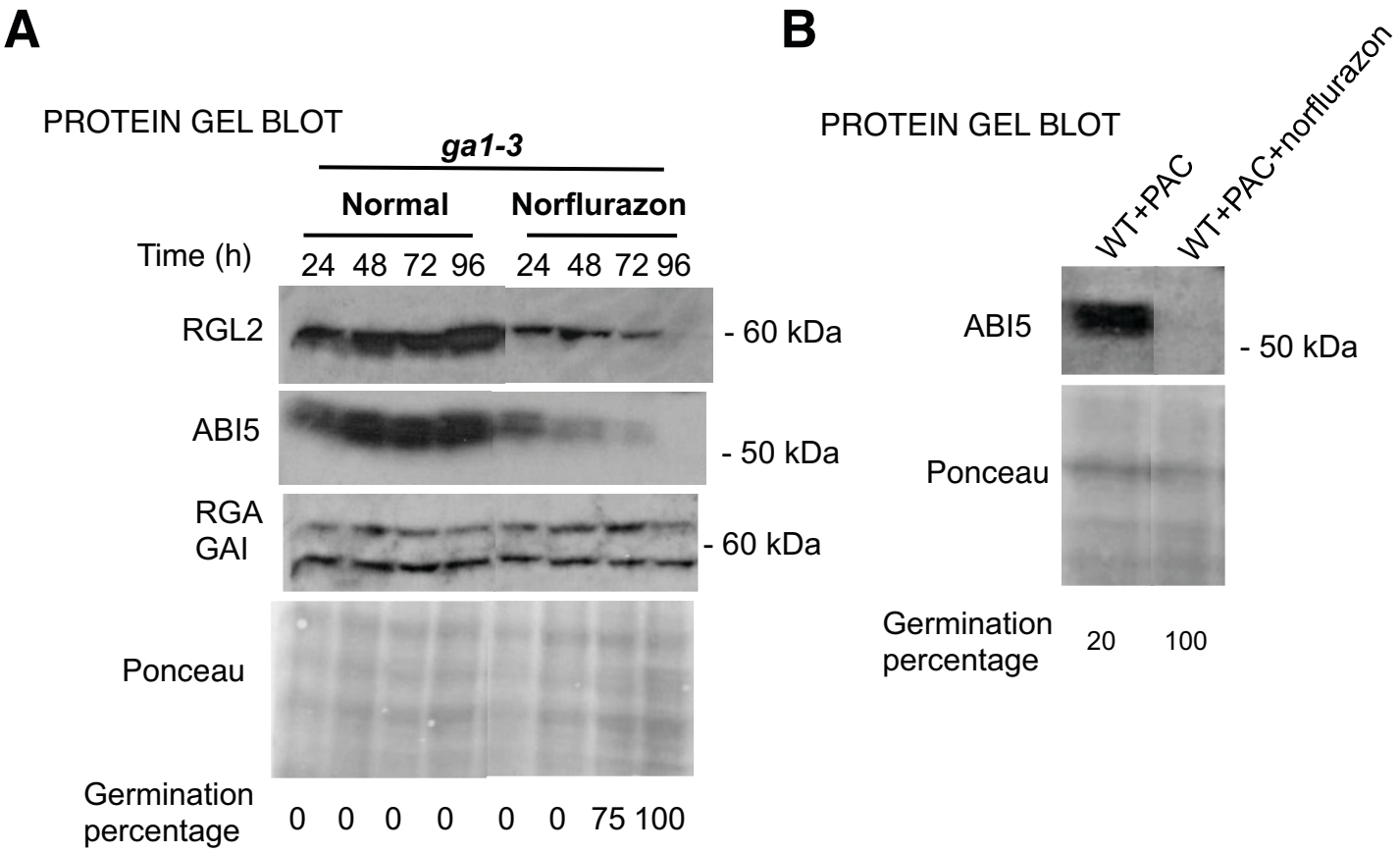
B: Seeds of WT (Col) and *rgl2-13* were sown in absence (Normal) or presence of ABA (25µM). Percentage of testa rupture events was analyzed as in A. Histograms show the times obtained for three independent seed batches ($t < 0.05$).



Supplemental Figure 8: *ABI5* and *XERICO* mRNA expression levels in samples from Figure 7

A: Time course of *ABI5* mRNA levels in WT (Col) and *rgl2-13* mutant seeds under normal conditions and in presence of PAC (5µM). The same plant material used to measure endogenous ABA levels and ABI5 protein levels was used (Figure 7).

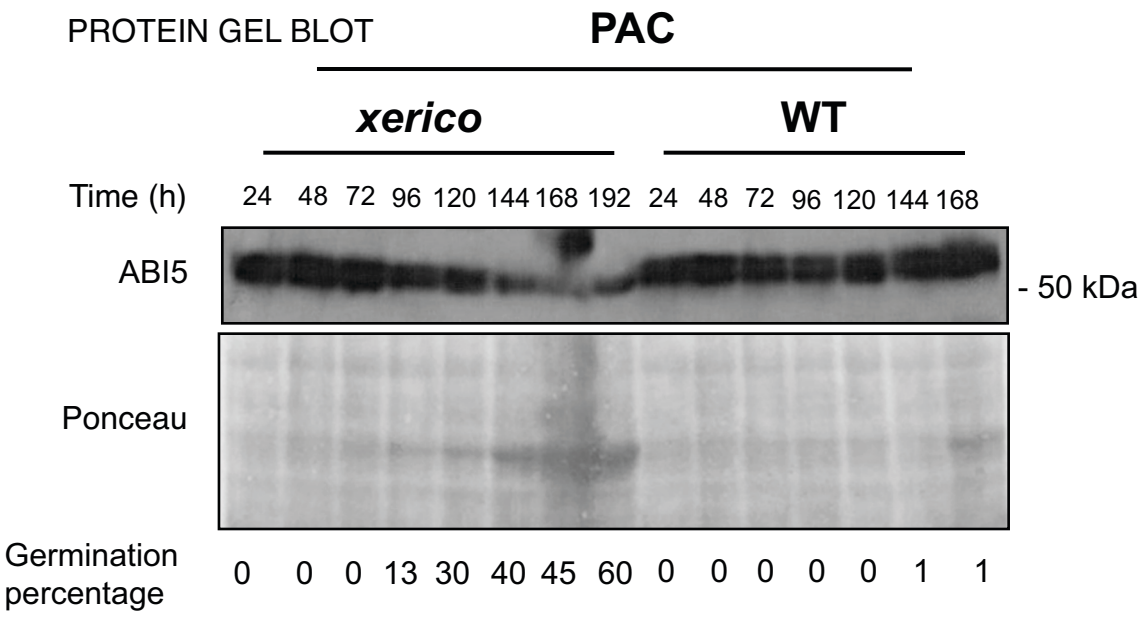
B: Time course of *XERICO* mRNA accumulation in WT(Col) and *rgl2-13* mutant seeds under normal conditions and in presence of PAC (5µM).). The same plant material used to measure endogenous ABA levels and ABI5 protein levels was used (Figure 7).



Supplemental Figure 9: **Inhibition of ABA synthesis in *ga1* or in PAC-treated WT seeds triggers seed germination and correlates with decreased ABI5 protein levels.**

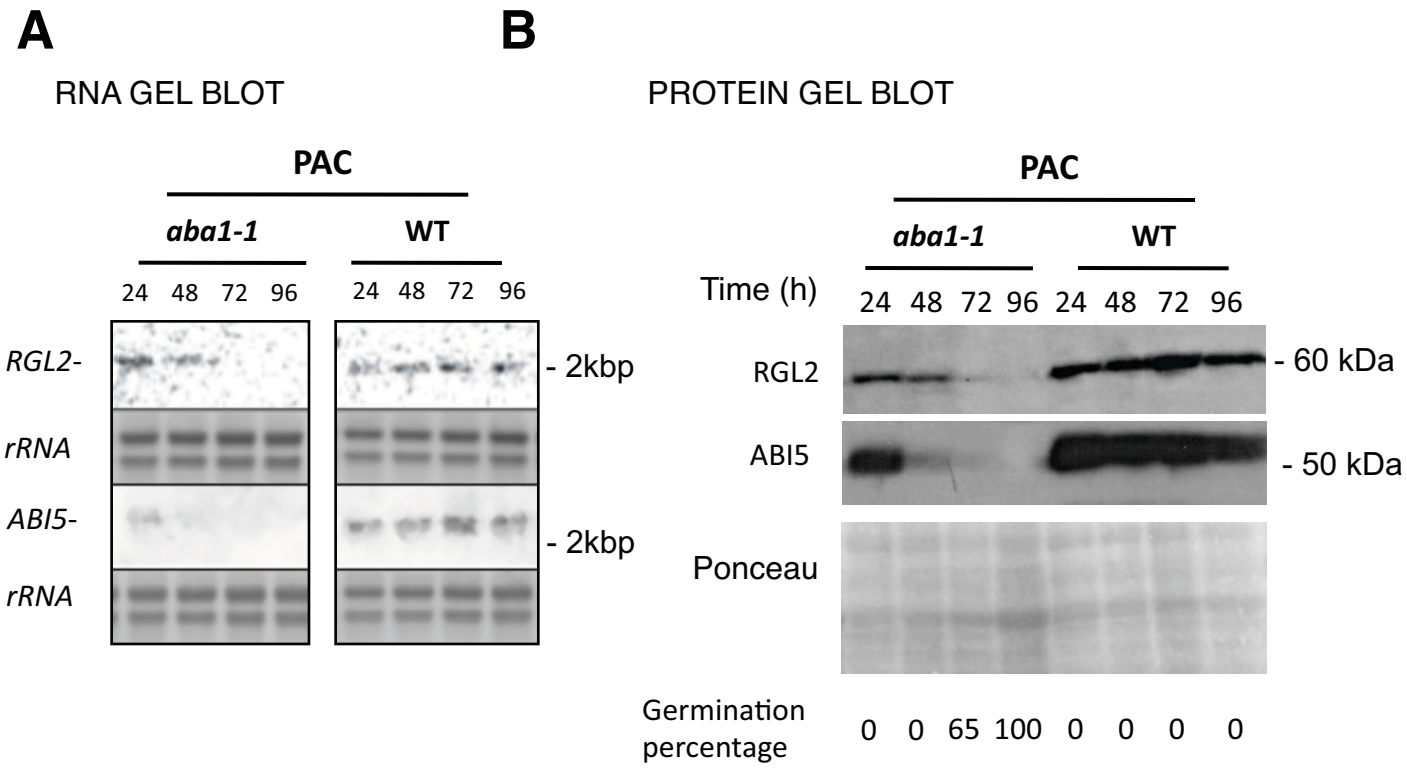
A: Protein gel blot analysis of RGL2, ABI5, GAI and RGA protein levels in *ga1-3* mutant seeds in absence (Normal) and presence of norflurazon (50µM). Protein extracts from 100 seeds of *ga1-3* mutant were loaded in each lane. Germination percentage at each time point is indicated.

B: Protein gel blot analysis of ABI5 protein levels in WT (Col) seeds in presence of PAC (5µM) with or without norflurazon (50µM) present in the medium. Protein extracts from 100 seeds were loaded in each lane. Germination percentage at each time point is indicated.



Supplemental Figure 10: Germination of *xerico* mutant seeds under low GA conditions coincides with ABI5 protein disappearance.

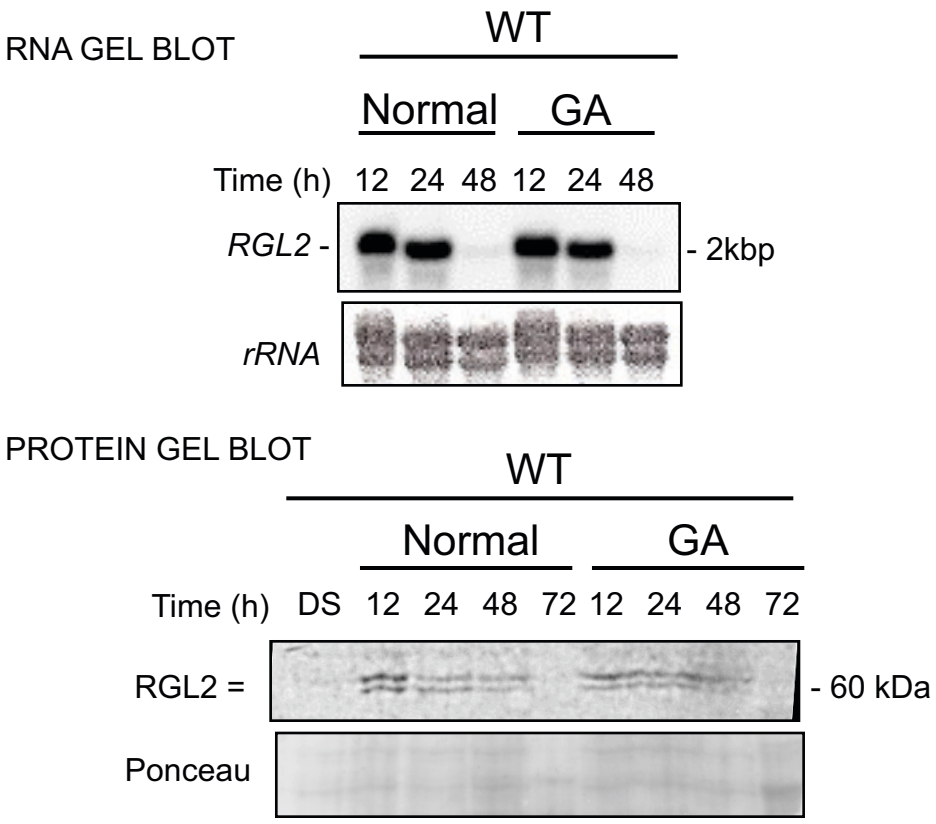
Protein gel blot analysis of ABI5 protein levels in *xerico* mutant and WT (Col) seeds germinating on PAC (2.5μM). Protein extracts from 100 seeds were loaded in each lane. Germination percentage at each time point is indicated.



Supplemental Figure 11: Seeds of *aba1-1* mutant germinate under low GA conditions and this coincides with RGL2 and ABI5 protein disappearance.

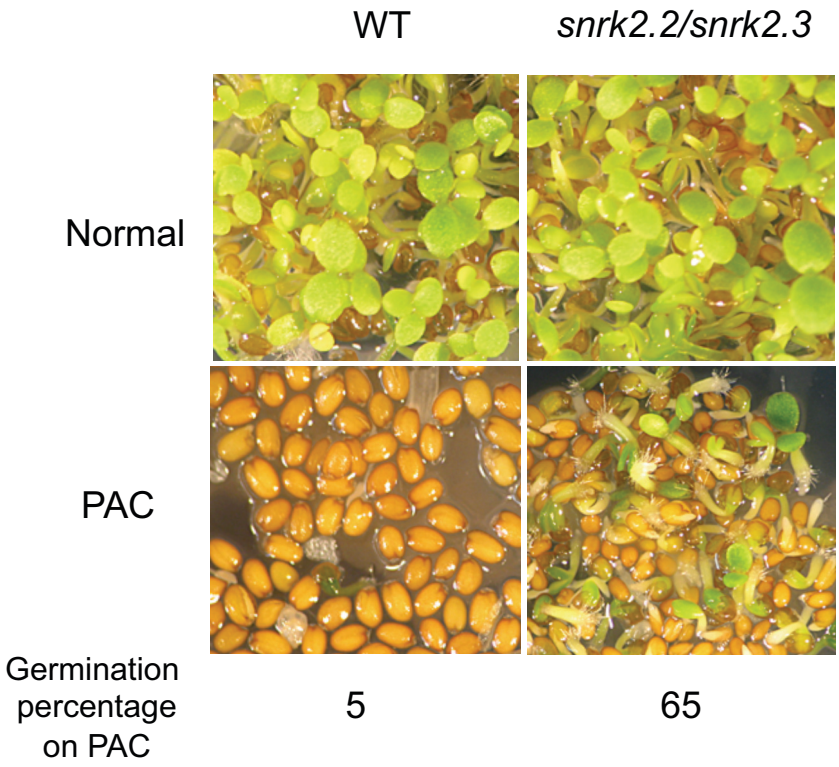
A: RNA gel blot analysis of time course of *RGL2* and *ABI5* mRNA levels in *aba1-1* and WT (Ler) seeds germinating under low GA conditions (PAC 5µM).

B: Protein gel blot analysis of RGL2 and ABI5 protein levels in *aba1-1* and WT (Ler) seeds germinating in presence of PAC (5µM). Protein extracts from 100 seeds were loaded in each lane. Germination percentage at each time point is indicated.



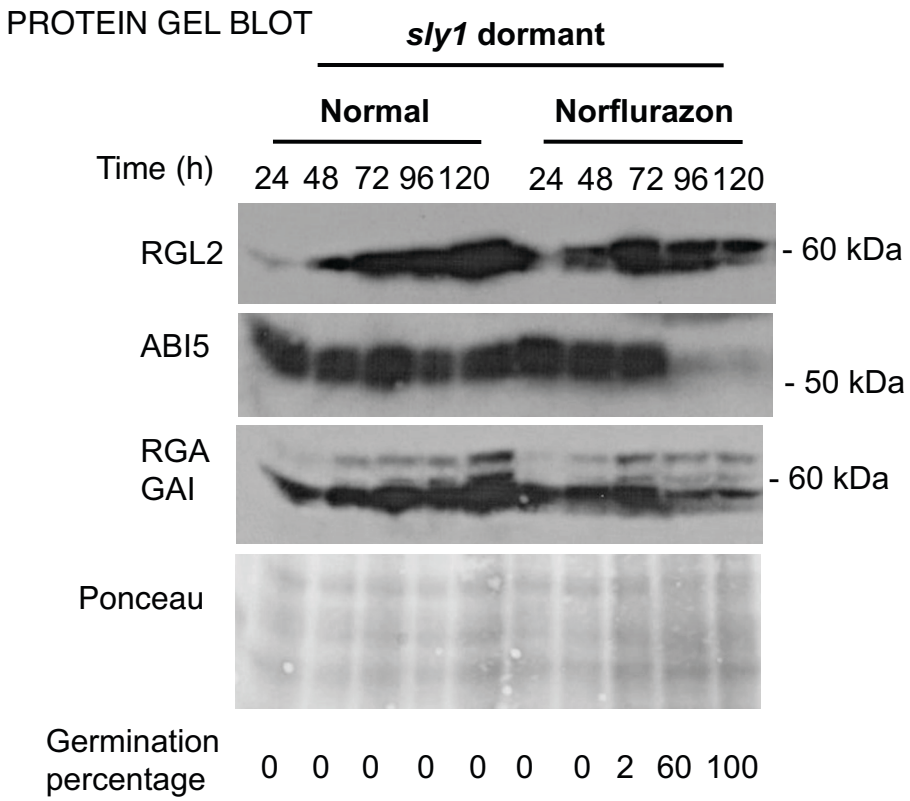
Supplemental Figure 12: Exogenous GA does not affect *RGL2* mRNA and protein levels relative to normal conditions.

RNA gel blot analysis of time course of *RGL2* mRNA and protein expression upon imbibition of WT (Col) seeds in the absence (Normal) or presence of GA (50 µM). rRNA and Ponceau S staining were used as loading controls. Plant material was harvested at the indicated times after seed imbibition. DS, dry seeds.



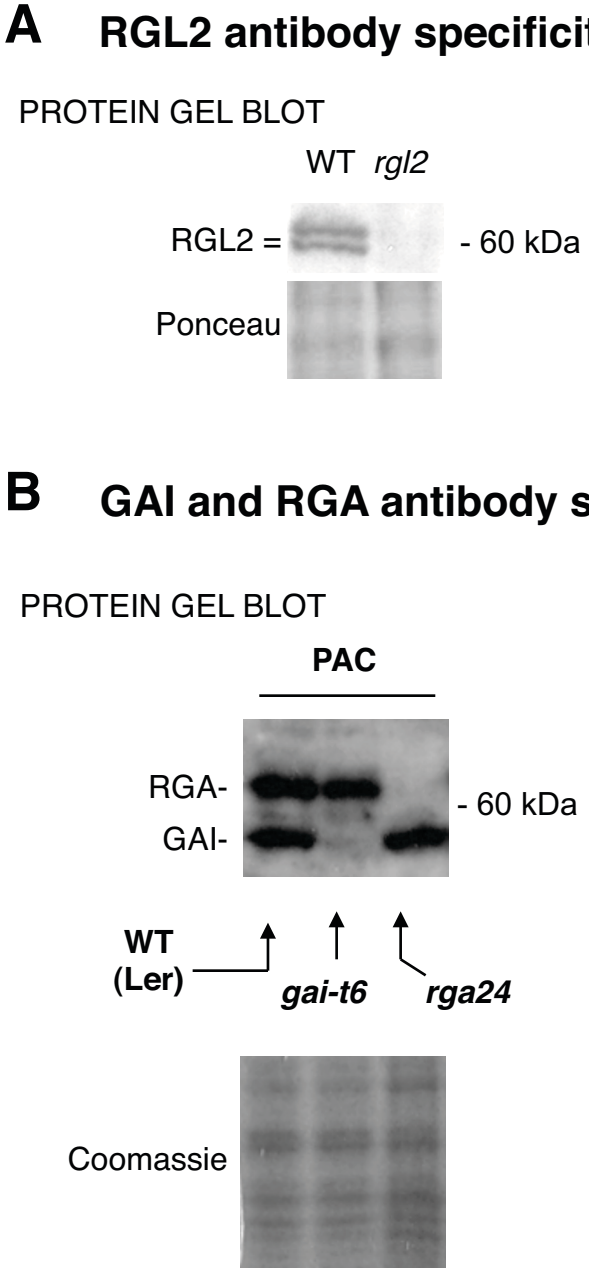
Supplemental Figure 13: *snrk2.2/snrk2.3* double mutant seed germination is resistant to low GA conditions.

Pictures of WT (Col) and *snrk2.2/snrk2.3* seeds germinating in absence (Normal) and presence of PAC (5µM). Pictures taken 96 h after imbibition. Germination percentage at this time point is indicated.



Supplemental Figure 14: Dormant *sly1-10* mutants germination in presence of norflurazon, an inhibitor of ABA synthesis, coincides with ABI5 protein disappearance but not that of RGL2, GAI nor RGA.

Protein gel blot analysis of RGL2, ABI5, GAI and RGA protein levels in dormant *sly1-10* mutant seeds in absence (Normal) and presence of norflurazon (50µM). Protein extracts from 100 seeds of *sly1-10* were loaded in each lane. Germination percentage at each time point is indicated.



Supplemental Figure 15: Antibody specificity.

A: Protein gel blot analysis using antiRGL2 antibody. Protein extracts from WT (Col) and *rgl2-13* seeds harvested 48 h after seed imbibition in presence of 5µM PAC.

A: Protein gel blot analysis using antiGAI antibody that also recognizes RGA. Protein extracts from WT (Ler), *gai-t6* and *rga24* seeds harvested 24 h after seed imbibition in presence of 5µM PAC.