Supporting Information

Roszak and Köhler 10.1073/pnas.1117111108

SI Materials and Methods

Plant Materials. The *VRN2pro:VRN2-GUS* line has been previously published (1) and was kindly provided by Caroline Dean (John Innes Centre, Norwich, UK). The *PHE1pro:PHE1-EGFP* line has been described previously (2). The *35Spro:NLS-3xGFP* line contains three copies of the GFP sequence fused to a nuclear localization signal (NLS) under control of the 35S promoter (kindly provided by Rita Gross-Hardt and Ronny Völz, University of Tübingen, Tübingen, Germany).

Microscopy. Staining of seeds to detect GUS activity was done as described previously (3). To generate sections of GUS-stained seeds, seeds were dehydrated in four 1-h steps, followed by an overnight incubation in 100% ethanol. After two wash steps with 100% ethanol, the seeds were stepwise infiltrated with Technovit 7100 (Heraeus Kulzer) preparation solution and incubated for 16 h in 100% preparation solution. For embedding, seeds were mixed with polymerization solution and allowed to polymerize for at least 2 h. The embedded samples were sectioned into 4-μm sections using a Leica RM2255 Microtome (Leica). The sections were collected on water-covered SuperFrost Plus microscope slides (Menzel) and dried on a heating plate at 72 °C.

Transcript Level Analysis. Total RNA from 1- to 5-d-old seeds was extracted using the RNeasy kit (Qiagen). RNA was treated with DNaseI (Applied Biosystems) and reverse transcribed with the first-strand cDNA synthesis kit (Fermentas). Fast SYBR Green Master Mix (Applied Biosystems) and gene specific primers were used for RT-PRC performed on a 7500 Fast Real-Time PCR device (Applied Biosystems).

1. Choi J, et al. (2009) Resetting and regulation of Flowering Locus C expression during Arabidopsis reproductive development. *Plant J* 57:918–931.
2. Weinhofer I, Hehenberger E, Roszak P, Hennig L, Köhler C (2010) H3K27me3 profiling of the endosperm implies exclusion of polycomb group protein targeting by DNA methylation. *PLoS Genet* 6:e1001152.
3. Köhler C, et al. (2003) The Polycomb-group protein MEDEA regulates seed development by controlling expression of the MADS-box gene *PHERES1*. *Genes Dev* 17: 1540–1553.

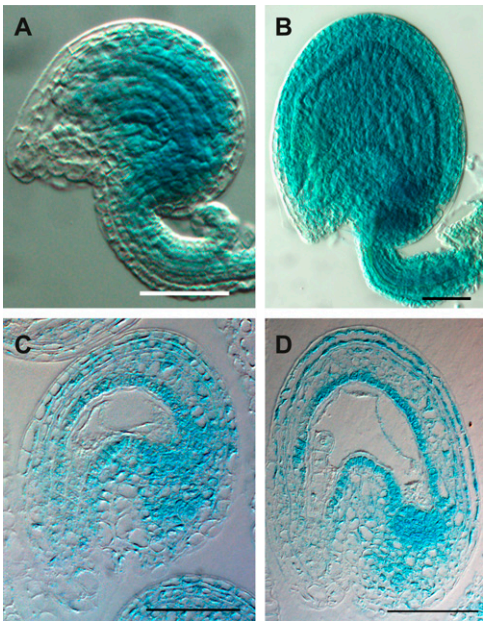

Fig. S1. Expression of *VRN2pro:VRN2-GUS* (1) in integuments and seed coat. GUS expression in integuments of unfertilized ovules (*A* and *C*) and seed coat of seeds at 3 DAP (*B* and *D*) analyzed by clearings (*A* and *B*) and sections (*C* and *D*).

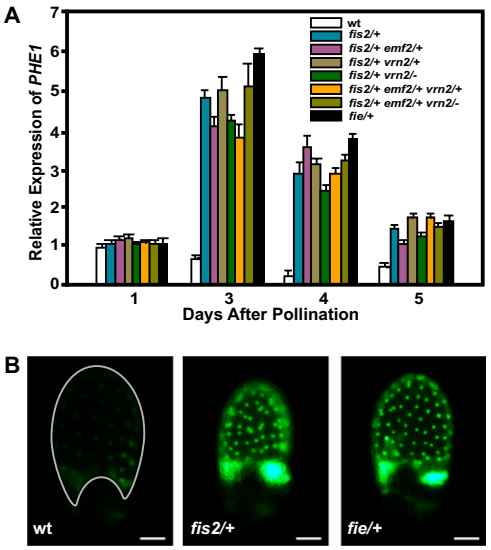

Fig. S2. Expression analysis of the FIS target gene *PHE1* in seeds of *fis* mutants and mutant combinations with *vrn2* and *emf2*. (*A*) Quantitative RT-PCR analysis of *PHE1* expression in wild type, *fis2-1*, and *fie-2* single mutants and *fis2-1* in combination *vrn2-1* and *emf2-5* mutants. Error bars indicate SEM. (*B*) Expression of the *PHE1pro:PHE1-EGFP* reporter gene in wild-type (WT), *fie-2*, and *fis2-1* seeds at 3 DAP. Gray line depicts border between seed coat and endosperm in wild type. (Scale bar, 50 μm.)

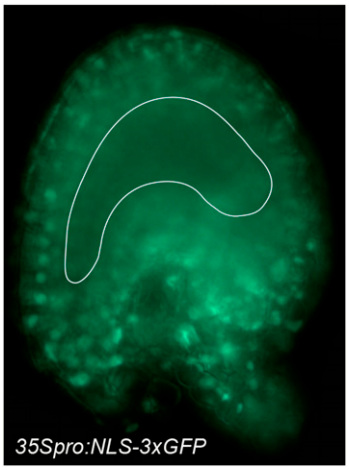

Fig. S3. Expression of *35Spro:NLS-3xGFP* in ovules. No GFP expression can be detected in the mature female gametophyte. The position of the female gametophyte is encircled.

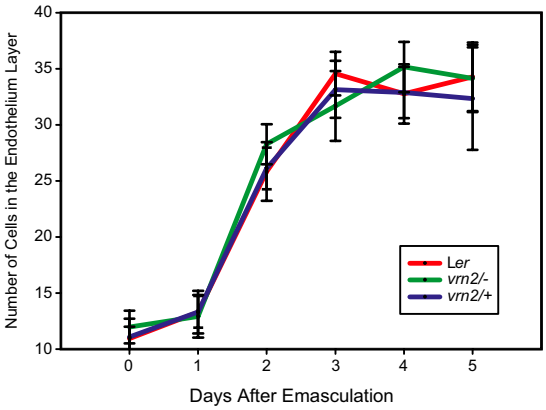

Fig. S4. Number of cells in the endothelium layer of wild type, *vrn2-1*/+, and *vrn2*/– ovules from 0 to 5 DAE. Cells were counted in triplicates from sectioned ovules.

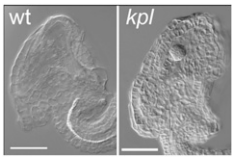

Fig. S5. Seeds without endosperm degenerate at the same time as wild-type ovules without fertilization. Degenerating ovule of wild type (WT) at 6 DAE (*Left*) and seed developing after fertilization with *kpl* pollen at 3 DAP (*Right*). Seed contains only an embryo and no endosperm. (Scale bars, 50 μm.)

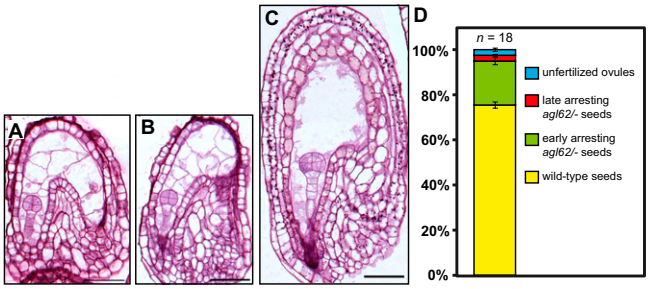

Fig. S6. Phenotype of *agl62-2*/– seeds. (*A* and *B*) Most seeds arrest development containing few endosperm cells and no developed seed coat. (*C*) Few *agl62*/– seeds arrest at a later stage containing about 50 endosperm cells and a developed seed coat (*Right*). (Scale bars, 50 μm.) (*D*) Percentage of different seed classes in siliques of self-fertilized *agl62*/+ plants. *N*, number of siliques analyzed.