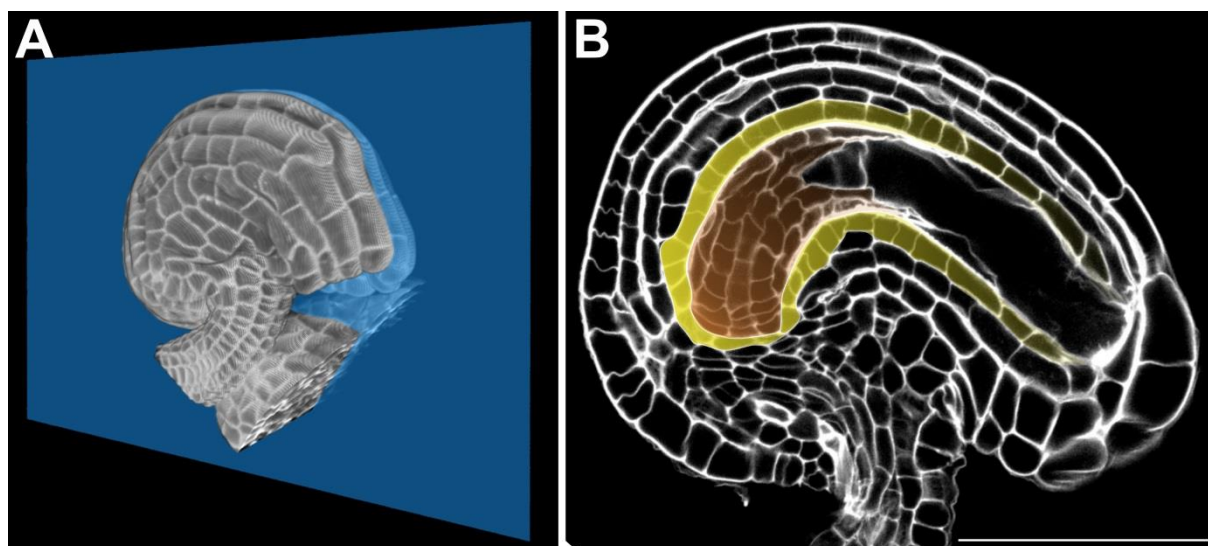


SUPPLEMENTAL MATERIALS

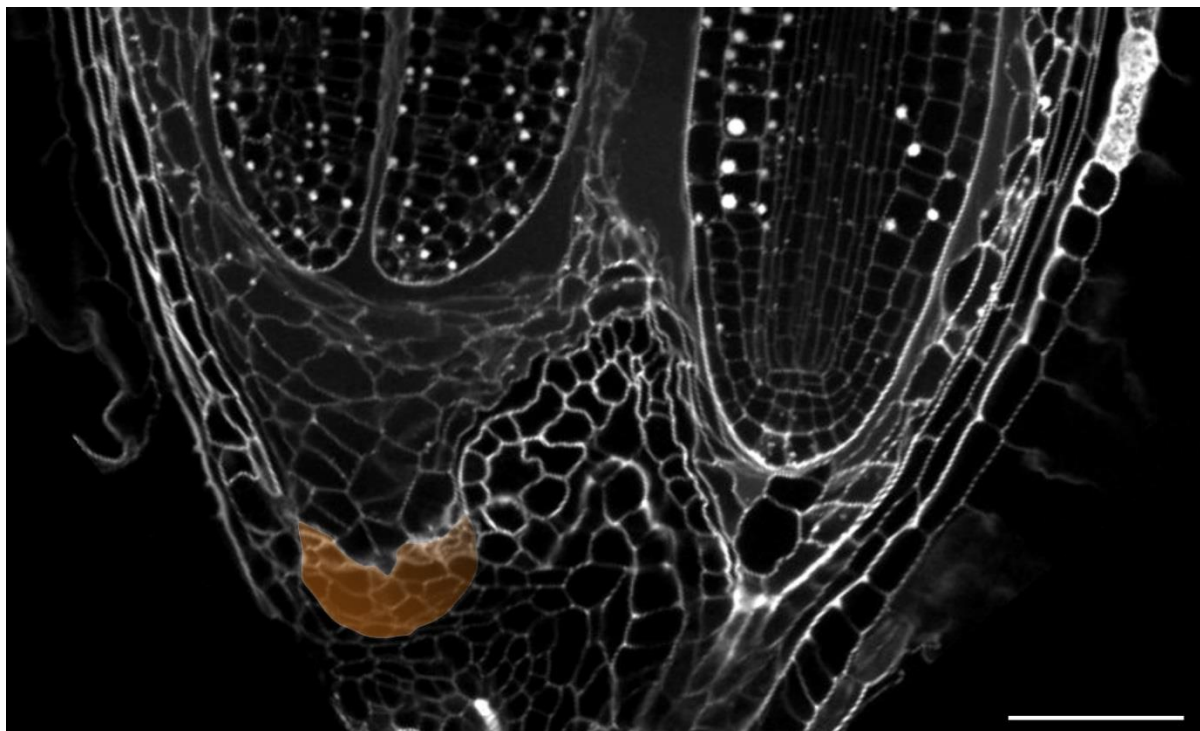


Supplemental Figure 1. Imaging the Nucellus Using the mPS-PI Technique.

(A) Three-dimensional reconstruction of a wild-type ovule 0 DAF imaged using the mPS-PI technique. The median longitudinal plane is highlighted in blue. Ecotype Col-0.

(B) Central longitudinal section of a wild-type ovule 0 DAF imaged using the mPS-PI technique. The inner integument 1 and pigment strand are highlighted in yellow whereas the nucellus is highlighted in orange. Ecotype Col-0.

Scale bar, 50 μ m.

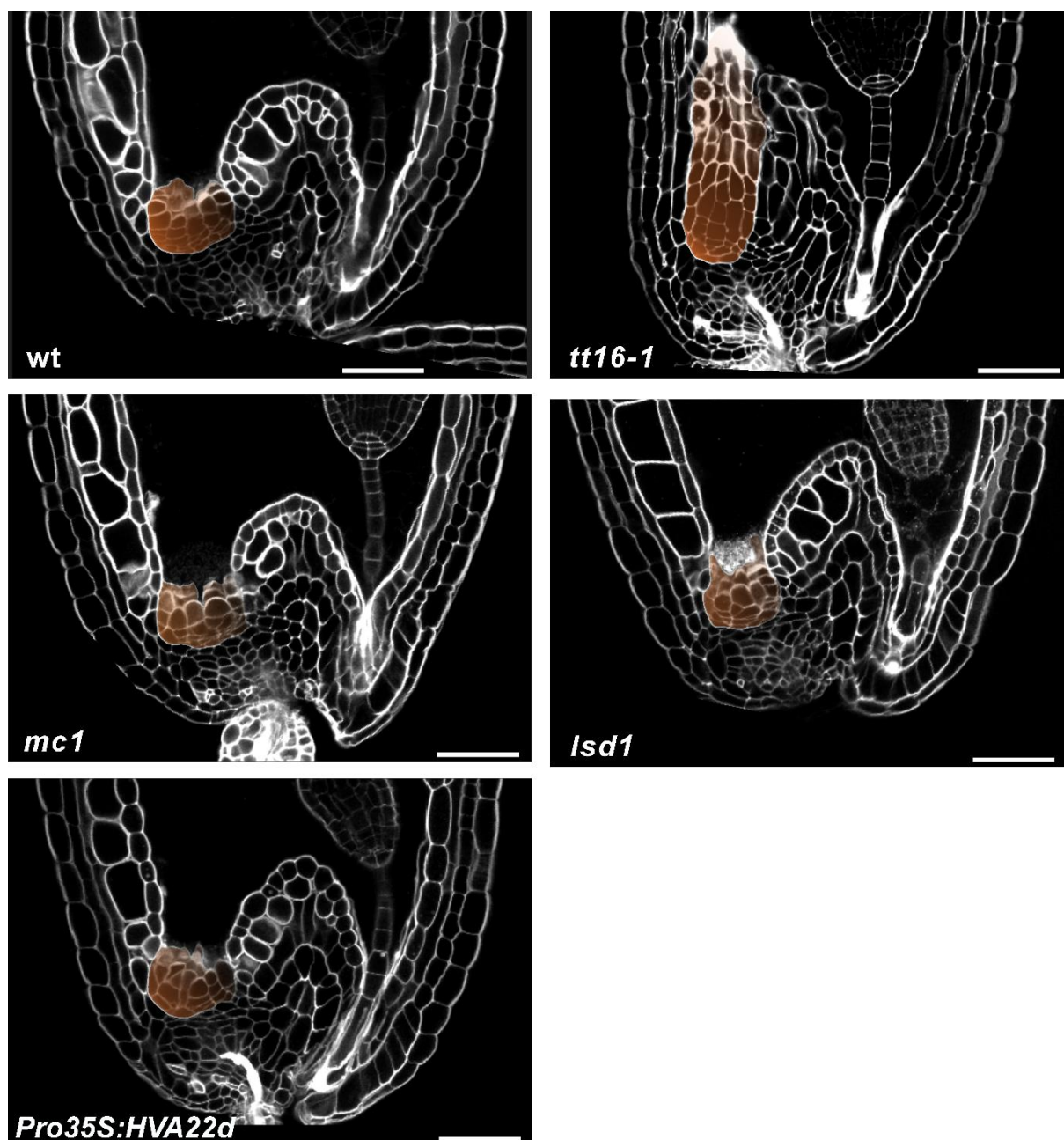


Supplemental Figure 2. The Nucellus Does Not Entirely Degenerate.

Central longitudinal section of a wild-type mature seed imaged using the mPS-PI technique.

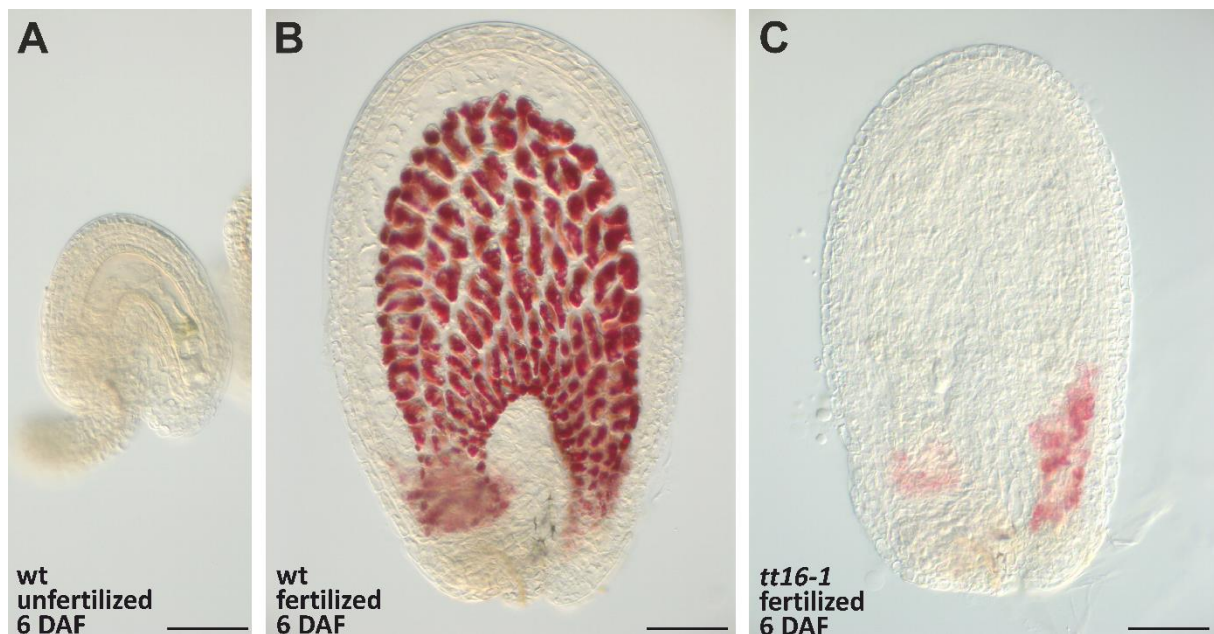
The nucellus is highlighted in orange. Ecotype Ws-2.

Scale bar, 50 μ m.



Supplemental Figure 3. Nucellus Development in PCD Mutants and Overexpression Lines.

Central longitudinal sections of wild-type, *tt16-1*, *mc1*, *lsd1*, and *Pro35S:HVA22d* seeds 6 DAF imaged using the mPS-PI technique. The nucellus is highlighted in orange. Ecotype Col-0. DAF, days after flowering; wt, wild-type. Scale bars, 50 μ m.



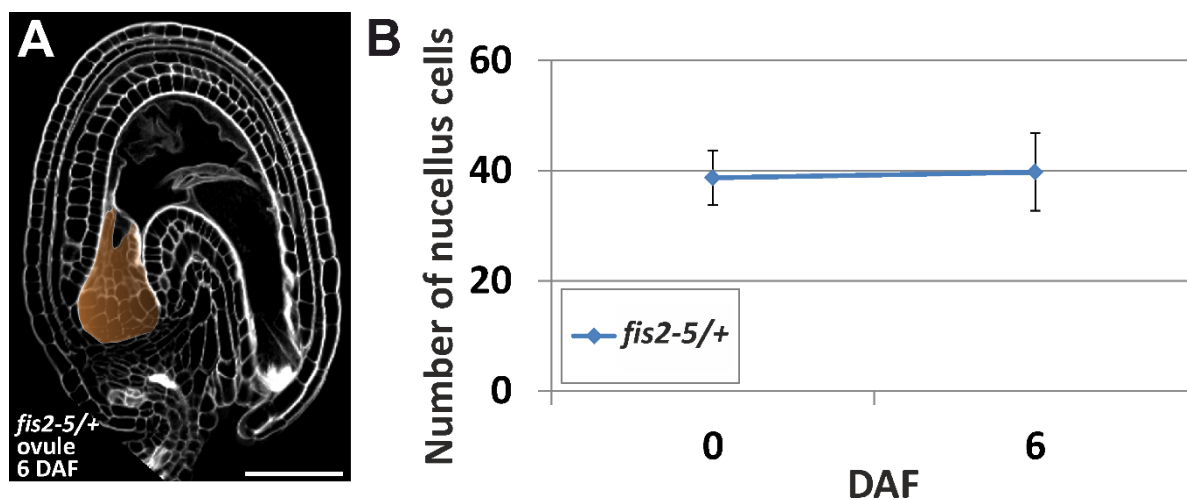
Supplemental Figure 4. PA Deposition.

(A) Vanillin staining of an unfertilized wild-type ovule (6 DAF). Ecotype Col-0.

(B) Vanillin staining of a fertilized wild-type seed (6 DAF). Ecotype Col-0.

(C) Vanillin staining of a fertilized *tt16-1* seed (6 DAF). Col-0.

DAF, days after flowering; wt, wild-type. Scale bars, 50 μ m.

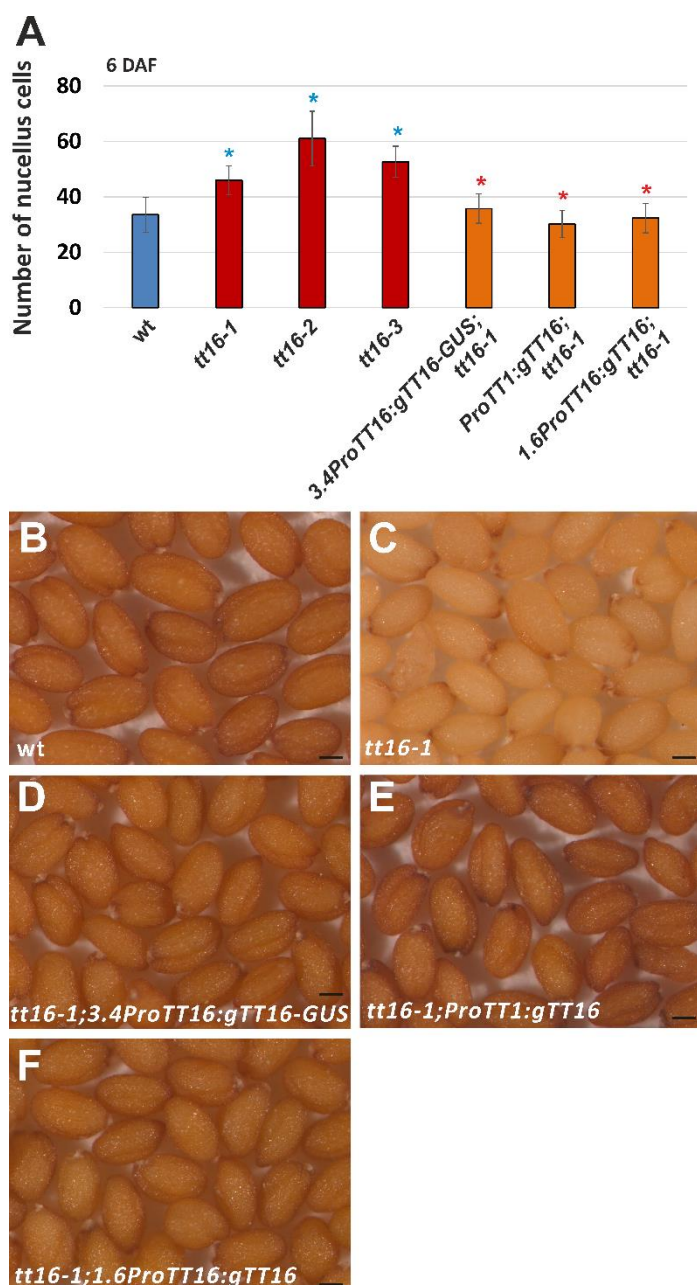


Supplemental Figure 5. FIS2 Does Not Repress the Degeneration of the Nucellus before Fertilization.

(A) Central longitudinal section of an unfertilized *fis2-5/+* ovule (6 DAF) imaged through the mPS-PI technique. The nucellus is highlighted in orange. Ecotype Col-0.

(B) Average number of nucellus cells in the central longitudinal sections of *fis2-5/+* ovules (0 and 6 DAF). Ecotype Col-0.

DAF, days after flowering. Standard deviations (error bars) were calculated from more than 30 individuals. Scale bar, 50 μ m.

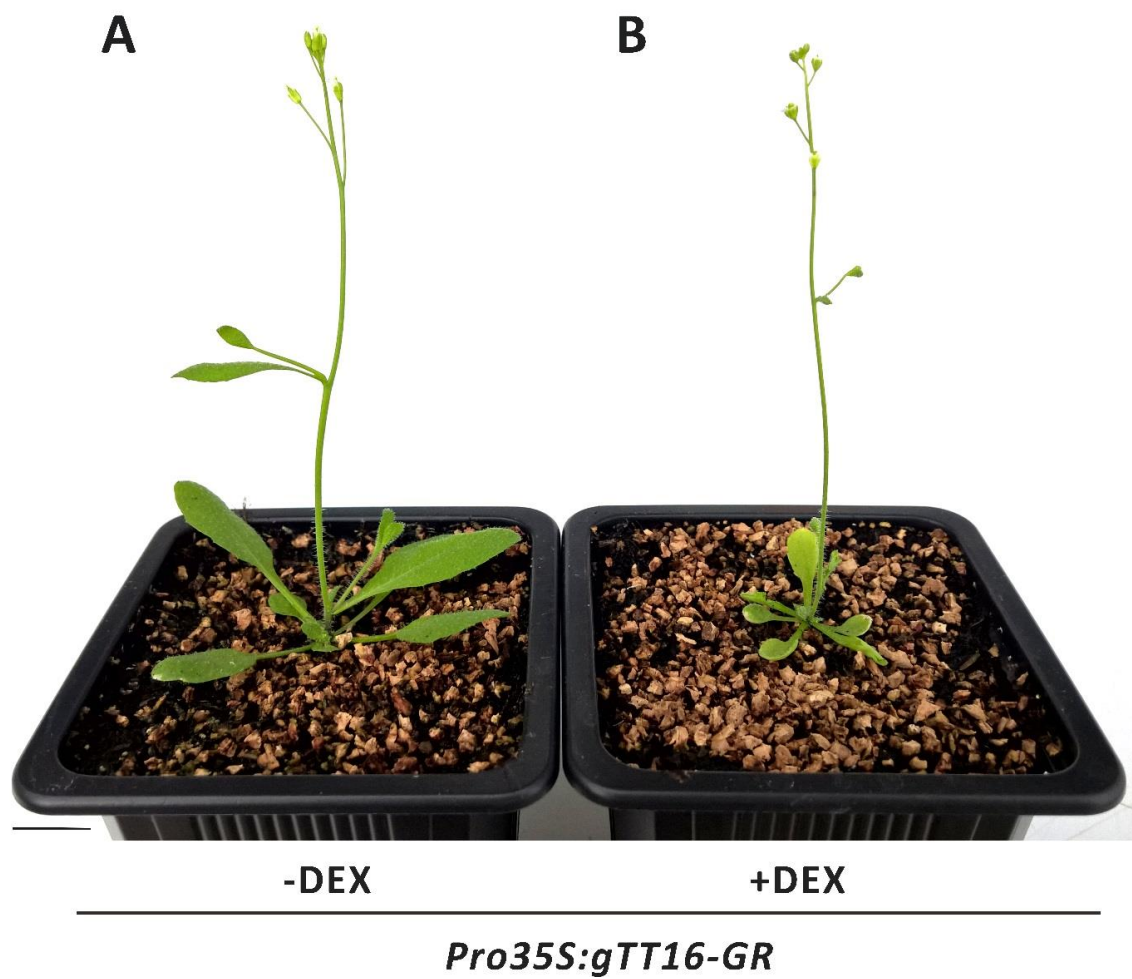


Supplemental Figure 6. TT16 Promotes Nucellus Degeneration.

(A) Average number of nucellus cells in the central longitudinal sections of wild-type, *tt16-1*, *tt16-2*, *tt16-3*, *tt16-1*;3.4PTT16:gTT16-GUS, *tt16-1*;PTT1:gTT16, and *tt16-1*;1.6PTT16:gTT16 seeds (6 DAF). Ecotype Ws-2.

(B-F) Dry seeds of wild-type, *tt16-1*, *tt16-1*;3.4PTT16:gTT16-GUS, *tt16-1*;PTT1:gTT16, and *tt16-1*;1.6PTT16:gTT16 plants.

DAF, days after flowering; wt, wild-type. Standard deviations (error bars) were calculated from more than 30 individuals. Colored asterisks indicate a statistical difference compared to wild-type (blue) or *tt16-1* (red) (Student's *t* test, $P < 0.01$). Scale bars, 200 μ m.

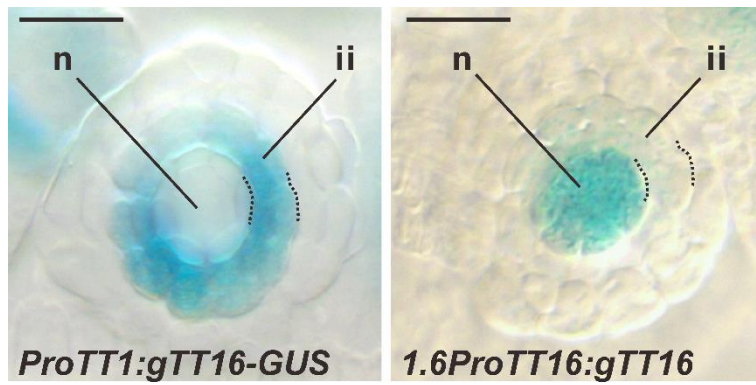


Supplemental Figure 7. A TT16-Inducible Line.

(A) A *Pro35S:TT16-GR Arabidopsis* plant.

(B) A *Pro35S:TT16-GR Arabidopsis* plant treated with DEX.

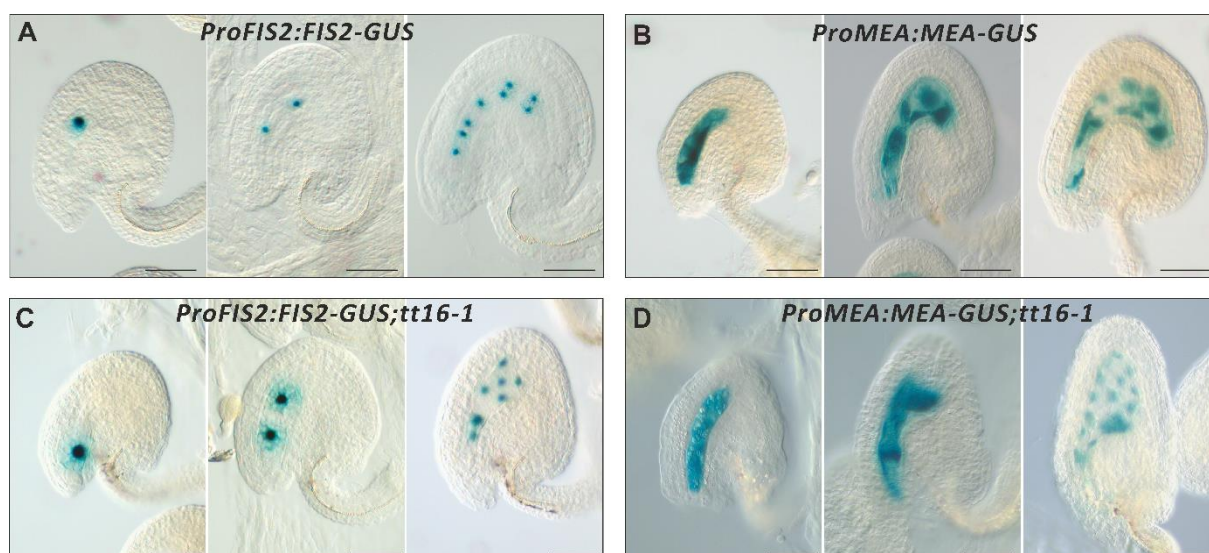
Scale bar, 1 cm.



Supplemental Figure 8. *TT16* Expression in the Inner Integuments or in the Nucellus.

β -glucuronidase activity in cleared whole mounts of *ProTT1:gTT16-GUS* and *1.6ProTT16:gTT16-GUS* ovules (stage 3-V). Ecotype Col-0.

n, nucellus; ii, inner integument. Scale bars, 20 μ m.



Supplemental Figure 9. Early Endosperm Development is Unaffected in the *tt16* Mutant.

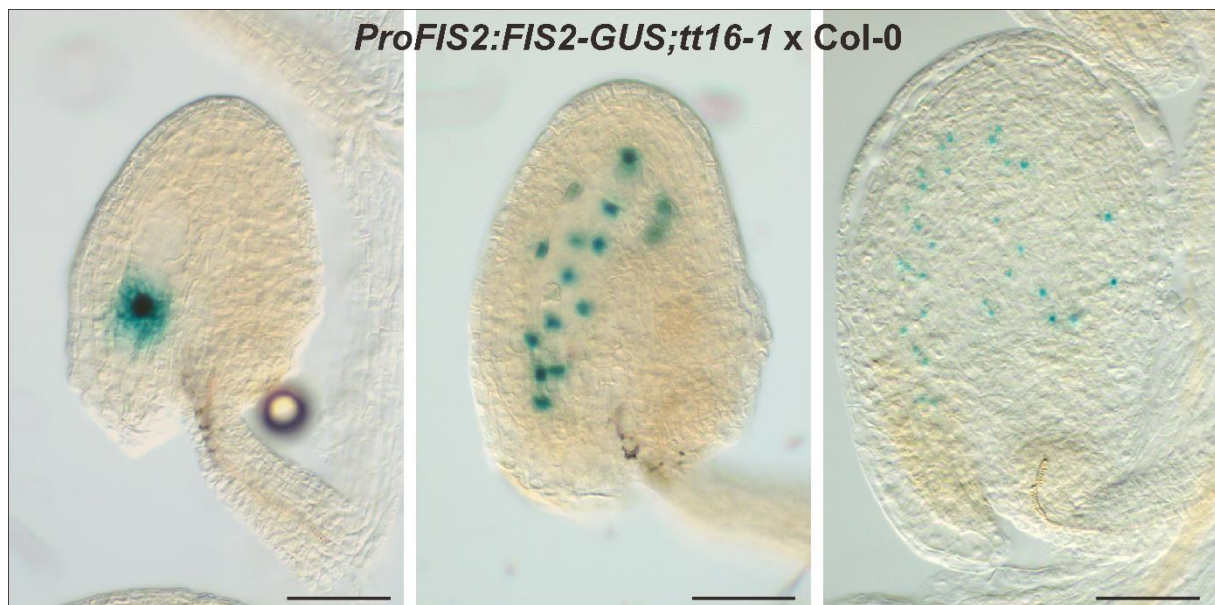
(A) β -glucuronidase activity in cleared whole mounts of *ProFIS2:FIS2-GUS* wild-type seeds (stage 4-I, 4-II, and 1 DAF from left to right). Ecotype Col-0.

(B) β -glucuronidase activity in cleared whole mounts of *ProMEA:MEA-GUS* wild-type seeds (stage 4-II, 1 DAF, and 2 DAF from left to right). Ecotype Col-0.

(C) β -glucuronidase activity in cleared whole mounts of *ProFIS2:FIS2-GUS;tt16-1* seeds (stage 4-I, 4-II, and 1 DAF from left to right). Ecotype Col-0.

(D) β -glucuronidase activity in cleared whole mounts of *ProMEA:MEA-GUS;tt16-1* seeds (stage 4-II, 1 DAF, and 2 DAF from left to right). Ecotype Col-0.

Scale bars, 50 μ m.



Supplemental Figure 10. TT16 Maternal Effect on Endosperm Development.

β -glucuronidase activity in cleared whole mounts seeds (stage 4-I, 2 DAF, and 4 DAF from left to right) obtained from the cross between *ProFIS2:FIS2-GUS;tt16* (female) and wild-type (male) plants. Ecotype Col-0.

Scale bars, 50 μ m.

Gene code	Gene symbol
<i>At4g24960</i>	<i>AtHVA22d</i>
<i>At3g57240</i>	<i>BETA-1,3-GLUCANASE 3</i>
<i>At5g20885</i>	/
<i>At2g41100</i>	<i>TOUCH 3</i>
<i>At4g14365</i>	<i>XB3 ORTHOLOG 4</i>
<i>At1g77380</i>	<i>AMINO ACID PERMEASE 3</i>
<i>At1g19180</i>	<i>JASMONATE-ZIM-DOMAIN PROTEIN 1</i>
<i>At3g55470</i>	/
<i>At3g56240</i>	<i>COPPER CHAPERONE</i>
<i>At1g09970</i>	<i>RECEPTOR-LIKE KINASE 7</i>
<i>At4g03550</i>	<i>GLUCAN SYNTHASE-LIKE 5</i>
<i>At4g18950</i>	/
<i>At1g51680</i>	<i>4-COUMARATE:COA LIGASE 1</i>
<i>At2g31260</i>	<i>AUTOPHAGY 9</i>
<i>At3g07525</i>	<i>AUTOPHAGY 10</i>

Supplemental Table 1. TT16 Target Genes.

Genes up- (red) or down- (green) regulated more than two fold in *tt16* versus wild-type seeds at seven days post anthesis according to (Dean et al. 2011).