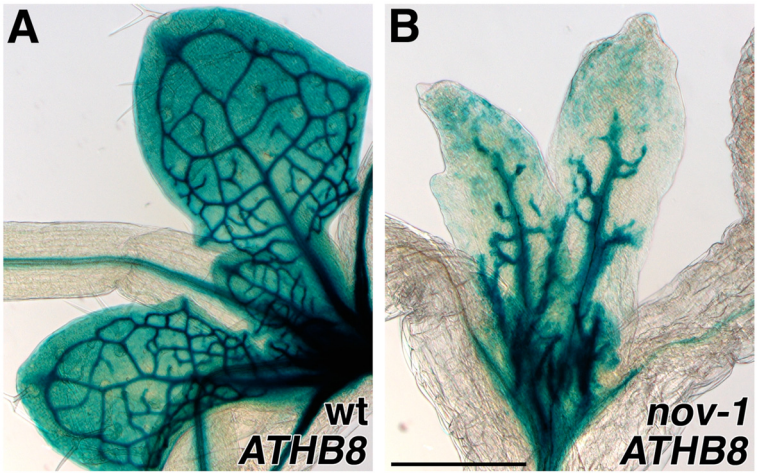
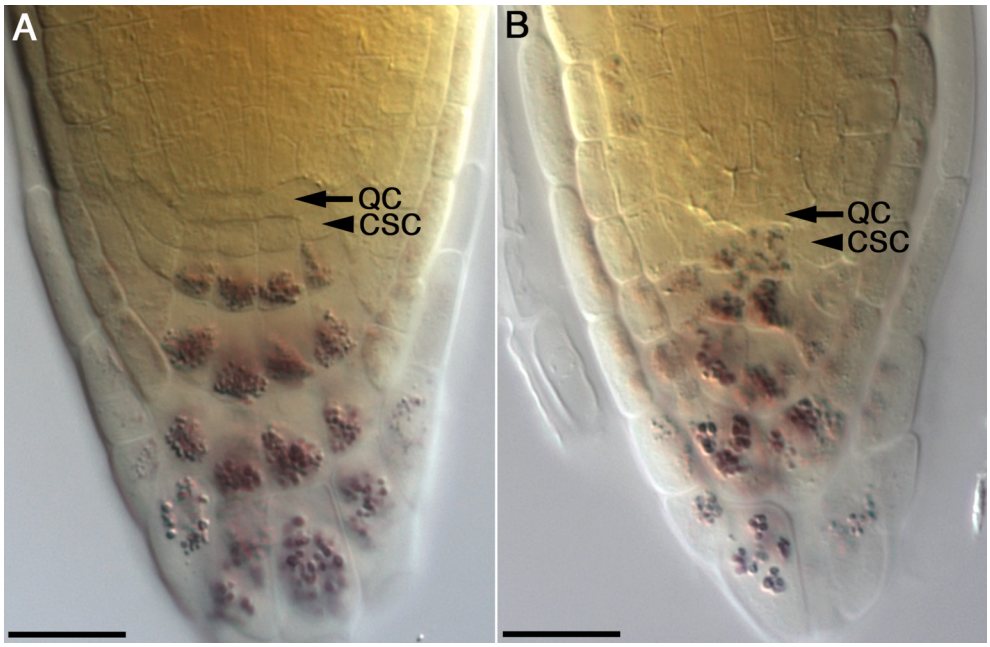
Supplemental data. Tsugeki et al. (2009) NO VEIN Mediates Auxin-Dependent Specification and Patterning in the *Arabidopsis* Embryo, Shoot and Root



Supplemental Figure 1. *ATHB8*$_{pro}$*:GUS* Expression in *nov-1*.

(A) and **(B)** *ATHB8*$_{pro}$*:GUS* expression in leaf primordia of wild type **(A)** and *nov-1* **(B)**. Seedlings containing *ATHB8*$_{pro}$*:GUS* were grown at 21˚C and subjected to GUS staining.

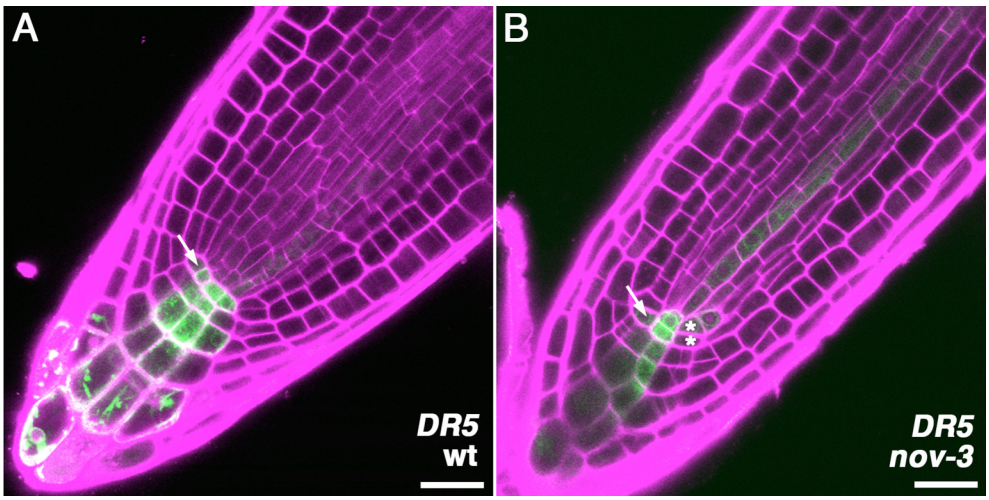
Scale bar: 0.5 mm (**[B]**; equal scale in **[A]** and **[B]**).



Supplemental Figure 2. Maintenance of Columella Root-Cap Stem Cells Is Defective in *nov-3*.

(A) and **(B)** Starch staining of wild-type **(A)** and *nov-3* **(B)** root tips. Arrows and arrowheads indicate positions of the quiescent center (QC) and columella stem cells (CSC), respectively.

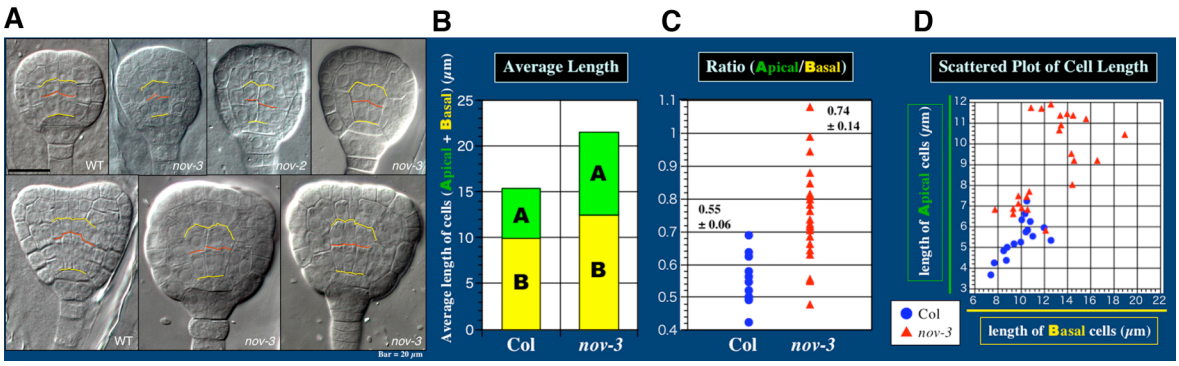
Scale bars: 20 μm (**[A]** and **[B]**).



Supplemental Figure 3. Maintenance of Cortex/Endodermis Stem Cells Is Defective in *nov-3* Root Tips.

(A) and **(B)** Propidium iodide staining of wild type **(A)** and *nov-3* **(B)** containing $DR5_{pro}$*:GFP*. The cell boundary, stained with propidium iodide, and GFP expression are indicated in magenta and green, respectively. Arrows indicate the position of the quiescent center. Cells resulting from an ectopic periclinal division in the cortex/endodermis stem cell are marked with white asterisks.

Scale bars: 20 μm (**[A]** and **[B]**).

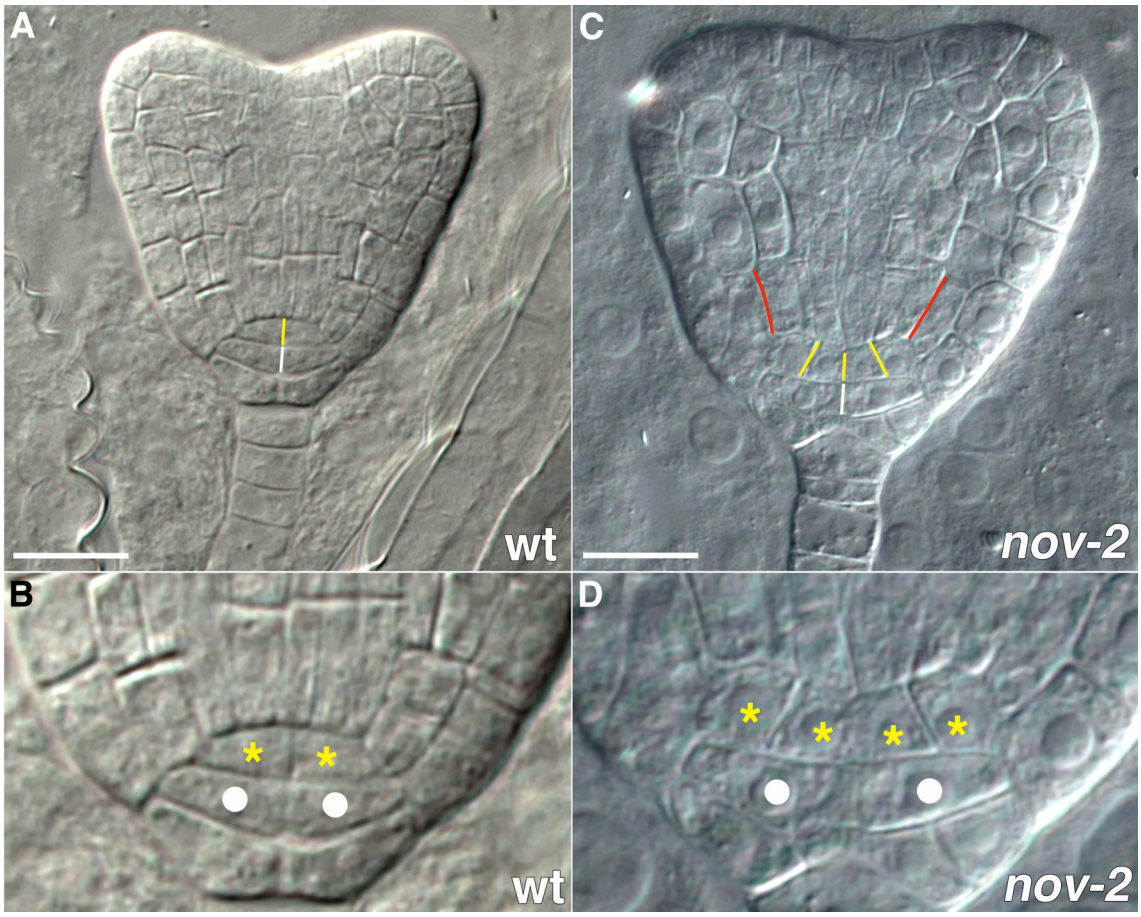


Supplemental Figure 4. Abnormality in Formation of Vascular Stem Cells in *nov* Mutants during Embryogenesis.

(A) In wild-type embryos during the globular stage, centrally located vascular primordial cells undergo asymmetric horizontal division (WT in the upper side). The resulting basal cells, which are longer than the apical cells, divide periclinally, giving rise to vascular (inner) and pericycle (outer) stem cells (WT in the lower side). In *nov-2~5*, the length of apical and basal daughter cells is less asymmetric than in wild-type embryos. Examples of *nov-2* and *nov-3* embryos are shown.

(B) to **(D)** The length of the apical and basal vascular-stem cells in wild-type and *nov-3* embryos was measured. **(B)** Average length of the apical and basal cells was schematically viewed, with the apical cell stacked onto the basal one. **(C)** The ratio of the apical to basal length of each sample was plotted. The averages of these ratios were 0.55 ± 0.06 for wild type and 0.74 ± 0.14 for *nov-3*. **(D)** Scatter plot of length of apical (Y axis) and basal cells (X axis) in wild type and *nov-3*.

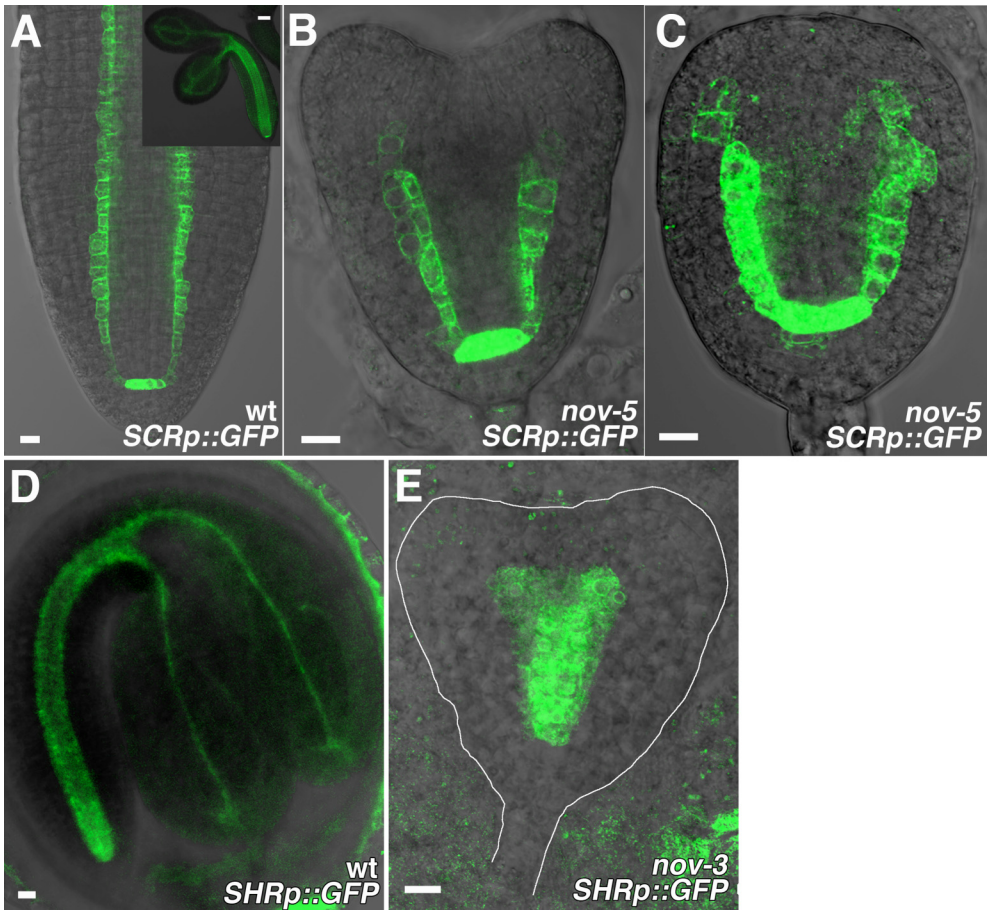
Scale bar: 20 μm (**[A]**; all photos to equal scale).



Supplemental Figure 5. Ectopic Cell Division in the Quiescent Center of *nov-2* during Embryogenesis.

(A) to **(D)** Embryos of wild type **(A)** and *nov-2* **(C)**. Photographs in **(B)** and **(D)** are enlarged regions around the quiescent centers in **(A)** and **(C)**, respectively. In *nov-2* embryos, precocious periclinal division in cortex/endodermis stem cells (marked by red lines in **[C]**) occurred concomitantly with ectopic periclinal division in quiescent cells (**[C]** and **[D]**), presumabably replacing those cortex/endodermis stem cells lost during precocious differentiation. Yellow and white lines indicate boundaries between cells of the quiescent center and between adjacent columella stem cells, respectively (**[A]** and **[C]**). In **(B)** and **(D)**, yellow asterisks and white dots indicate cells at the position of quiescent center and columella stem cells, respectively.

Scale bars: 20 μm (**[A]** and **[C]**).

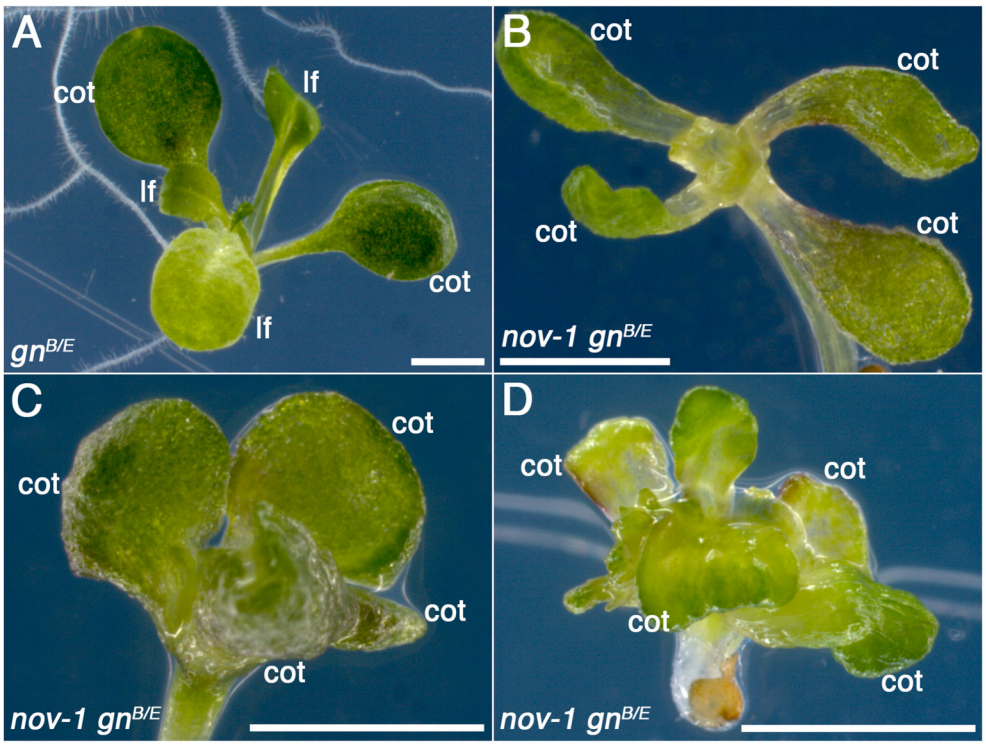


Supplemental Figure 6. Expression of *SCR$_{pro}$:GFP* and *SHR$_{pro}$:GFP* in Embryos with Strong *nov* Alleles.

(A) to **(C)** *SCR$_{pro}$:GFP* expression in embryos of wild type **(A)** and *nov-5* (**[B]** and **[C]**). In the basal side of *nov-5* embryos (**[B]** and **[C]**), *SCR$_{pro}$:GFP* expression was as seen in the wild-type embryonic root and hypocotyl **(A)**. An inset shows a whole embryo in **(A)**.

(D) and **(E)** *SHR$_{pro}$:GFP* expression in wild type **(D)** and *nov-3* **(E)**. In the basal side of *nov-3* embryos **(E)**, *SHR$_{pro}$:GFP* expression was as seen in the wild-type embryonic root and hypocotyl **(D)**.

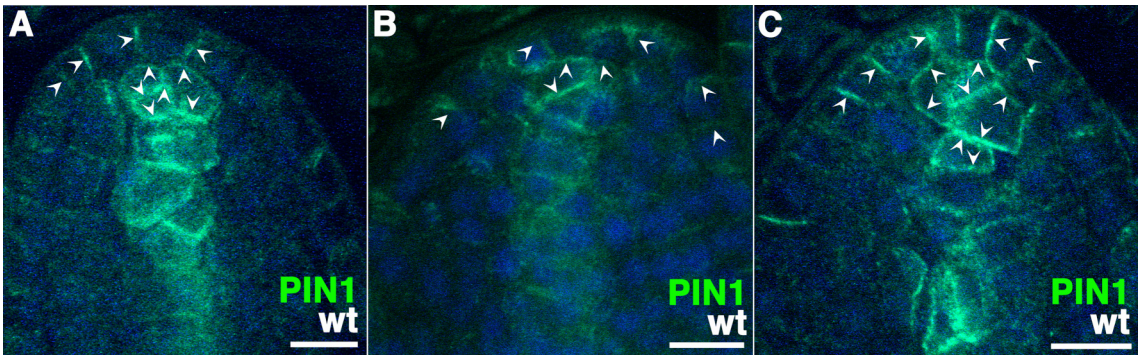
Scale bars: 10 μm (**[A]** to **[C]** and **[E]**), 50 μm (inset in **[A]**) and 20 μm **(D)**.



Supplemental Figure 7. Seedling Phenotype in *nov-1 gnom*$^{B/E}$ Double Mutant.

(A) to **(D)** Twelve-day-old seedlings of *gnom*$^{B/E}$ **(A)** and of *nov-1 gnom*$^{B/E}$ double mutant plants with multiple cotyledons (**[B]** to **[D]**). cot, cotyledon; lf, rosette leaf; *gn*$^{B/E}$, *gnom*$^{B/E}$.

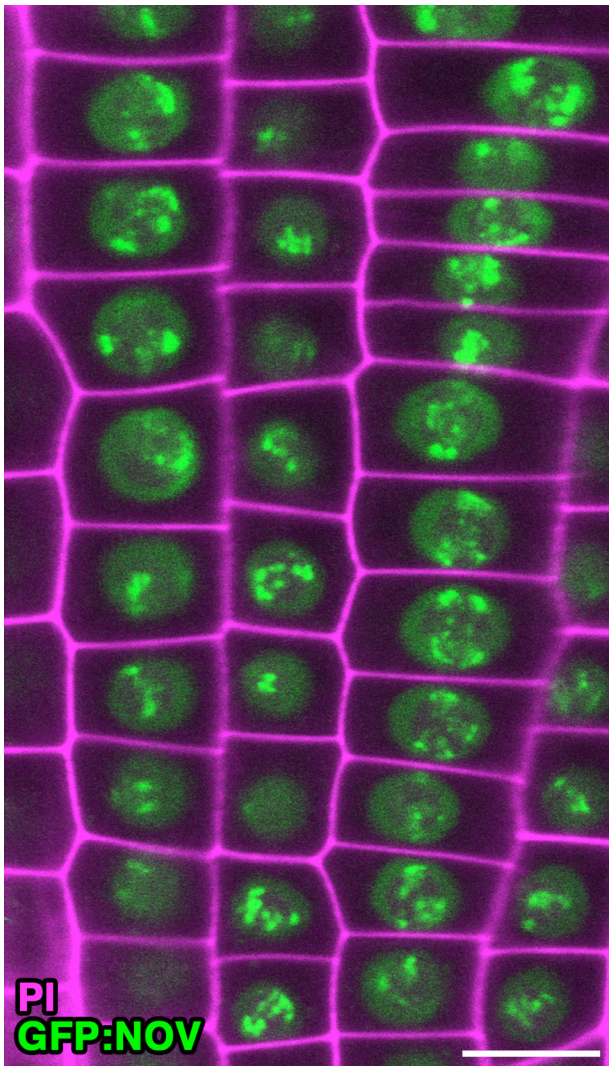
Scale bars: 2 mm (**[A]** to **[D]**).



Supplemental Figure 8. Subepidermal Cells at the Apical End of the Midvein Provascular Strand Exhibit Non-Polar PIN1 Localization.

(A) to **(C)** Immunolocalization of PIN1 in wild-type leaf primordia. Images are focused on the subepidermal cells in the apical domain of midvein provascular cells. Leaf primordia in **(A)** and **(B)** are identical to those in Figures 6A and 6C. Arrowheads indicate the polarity of PIN1 in some cells for clarity.

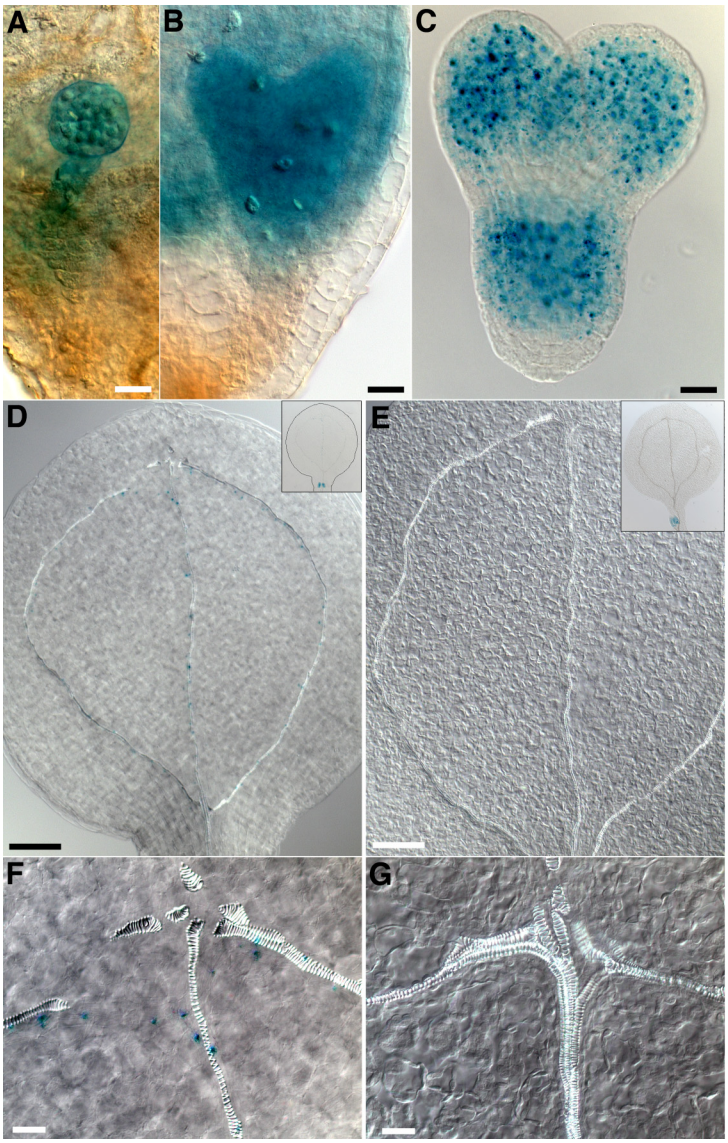
Scale bars: 10 μm (**[A]** to **[C]**).



Supplemental Figure 9. Nuclear Localization of GFP:NOV.

Subcellular localization of GFP:NOV was examined in root epidermal cells of seedlings containing *NOV*$_{pro}$*:GFP:NOV* (GFP:NOV). GFP:NOV and the cell boundary stained with propidium iodide are indicated in green and magenta, respectively. GFP:NOV is specifically localized in the nucleus.

Scale bar: 10 μm.

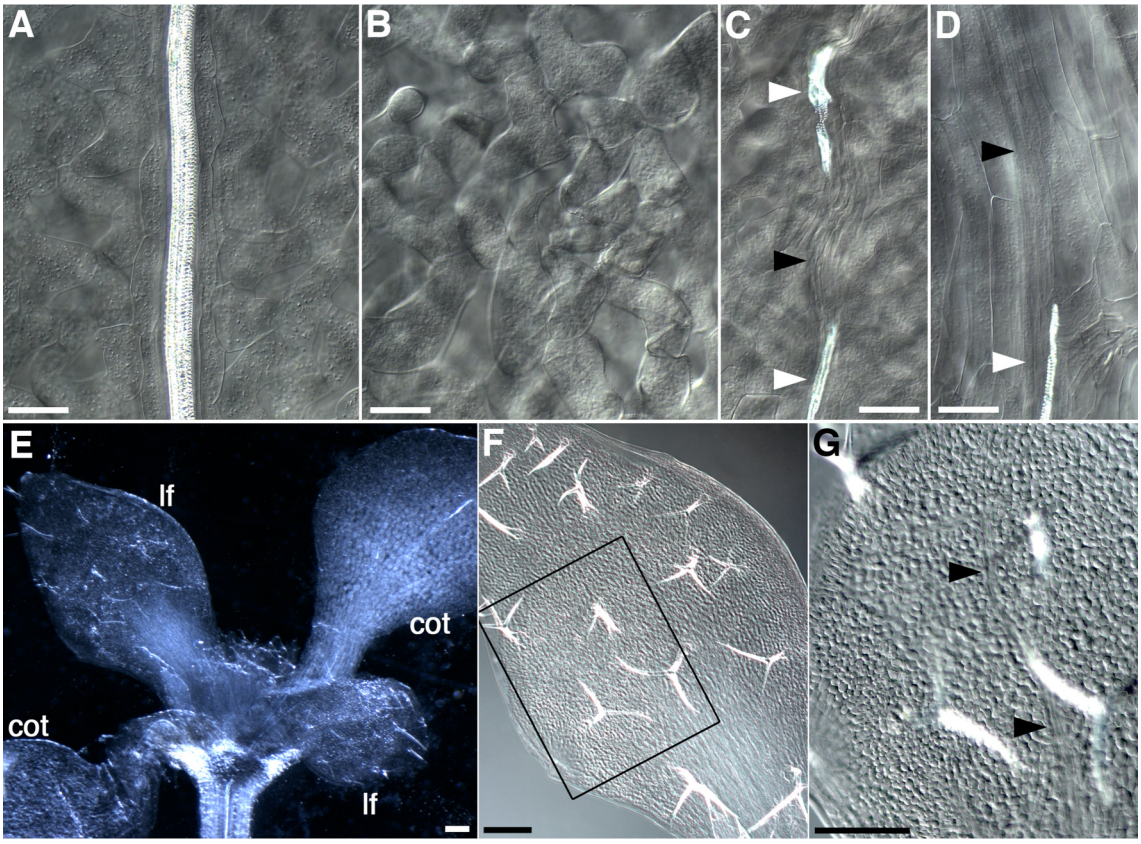


Supplemental Figure 10. *NOV_{pro}:NOV:GUS* Expression in Embryos and in Cotyledons of Developing Seedlings.

(A) to **(C)** *NOV_{pro}:NOV:GUS* expression in embryos at the globular **(A)**, heart **(B)** and torpedo **(C)** stages.

(D) to **(G)** *NOV_{pro}:NOV:GUS* expression in cotyledons of seedlings 3 days **(D)** and 4 days **(E)** after germination. Insets in **(D)** and **(E)** are zoom-out views, where developing leaf primordia expressing *NOV_{pro}:NOV:GUS* can be seen. Photographs in **(F)** and **(G)** are magnified regions of apical junctions of the midvein and secondary vein in **(D)** and **(E)**, respectively. *NOV_{pro}:NOV:GUS* expression was seen in cotyledon provascular cells of 3-day-old seedlings (**[D]** and **[F]**), but disappeared 1 or 2 days later (**[E]** and **[G]**).

Scale bars: 20 μm (**[A]** to **[C]**, **[F]** and **[G]**) and 100 μm (**[D]** and **[E]**).



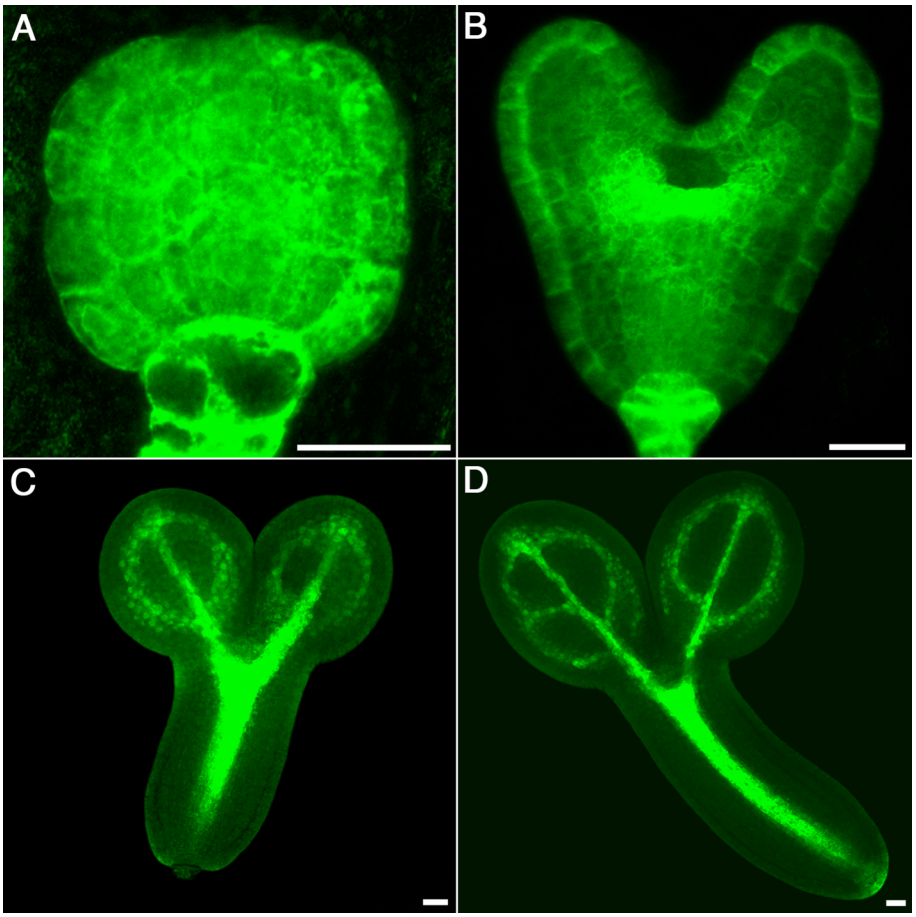
Supplemental Figure 11. Vascular and Ground-Tissue Phenotypes in *nov-1* and *nov-3* Leaves.

(A) to **(D)** Vascular and ground-tissue cells in the first or second rosette leaves, where midvein vascular path might be predicted. Wild type **(A)** and *nov-1* (**[B]** to **[D]**). The middle regions of the leaf blade (**[A]** to **[C]**) and the basal region of the petiole **(D)**.

(E) to **(G)** An example of a *nov-3* rosette leaf without lignified xylem strands. The aerial part of a *nov-3* seedling has first and second rosette leaves without xylem strands **(E)**; detail of first rosette leaf (**[F]**, the left leaf in **[E]**). The boxed area in **(F)** is enlarged in **(G)**.

Ground tissue cells in *nov-1* and *nov-3* rosette leaves, where the xylem strand might be predicted in wild type (like in **[A]**), either do not include elongated vascular cells (as in **[B]**) or include elongated cells with (white arrowheads in **[C]** and **[D]**) or without differentiated xylem cells (black arrowheads in **[C]**, **[D]** and **[G]**). Samples were taken from seedlings grown for 21 days (**[A]** to **[D]**) and 27 days (**[E]** to **[G]**). cot, cotyledon; lf, rosette leaf.

Scale bars: 50 μm (**[A]** to **[C]**) and 200 μm (**[D]** to **[F]**).



Supplemental Figure 12. *J1511-GFP* Expression during embryogenesis.

(A) to **(C)** *J1511-GFP* expression in embryos at the late globular **(A)**, heart **(B)** late torpedo **(C)** and walking stick stages **(D)**. See also Figure 3D, 3I and 3J.

Scale bars: 20 μm (**[A]** to **[D]**).

Supplemental Table 1. Leaf and Petiole Lengths of the First Two Rosette Leaves of Seedlings Grown in the Absence or Presence of NPA.

Wild type (C24) and *nov-1* were grown for 21 days in the absence or presence of NPA. The lengths of the leaf (blade and petiole) and petiole were measured.

Data represent mean (± SE) of the 24 leaves (the first or second rosette leaves; n = 24). Lengths are in mm.

	Untreated[a]		— NPA[b]		+ NPA[c]	
Strain	Leaf	Petiole	Leaf	Petiole	Leaf	Petiole
C24	13.7 ± 0.4	7.5 ± 0.2	14.2 ± 0.4	7.9 ± 0.3	9.2 ± 0.3	2.9 ± 0.2
nov-1	6.6 ± 0.3	3.4 ± 0.2	7.4 ± 0.2	3.6 ± 0.2	4.6 ± 0.1	0.1 ± 0.04[d]

[a] The "Untreated" growth medium includes no additional reagent.

[b] The "— NPA" growth medium includes 0.01% (v/v) dimethyl sulfoxide (DMSO).

[c] The "+ NPA" growth medium includes 10 μM NPA and 0.01% (v/v) DMSO.

[d] For 20 of 24 *nov-1* leaves treated with NPA, petiole length was 0 mm, as petioles were not recognized under the dissection microscope.