Supporting Information

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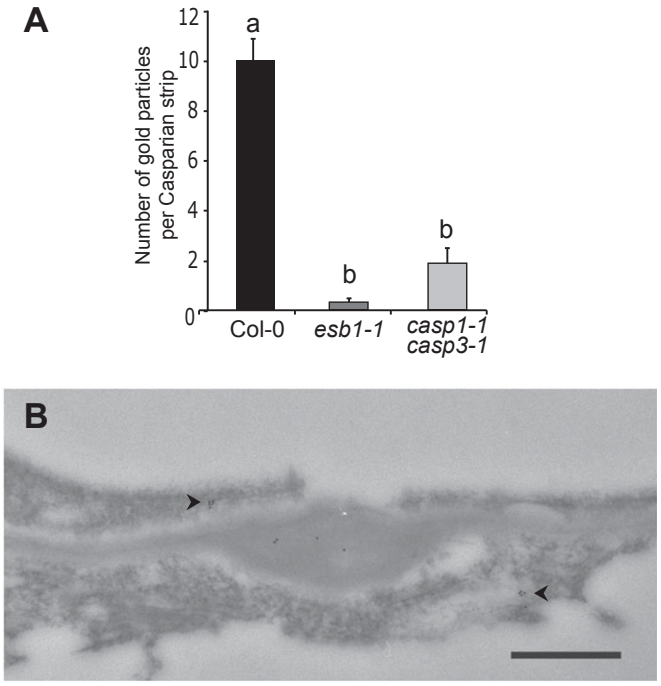


Fig. S1. Casparian strip domain proteins (CASPs) are necessary for correct localization of dirigent-domain protein ESB1. (*A*) Plot of number of gold particles per Casparian strip from an immunogold localization experiment by using anti-ESB1 antibodies. Different letters (a and b) indicate statistically significant differences between means by one-way analysis of variance (ANOVA) with Tukey–Kramer separation of means ($P < 0.05$), $n = 8–16$. (*B*) ESB1 mislocalized in *casp1-1casp3-1*. Immunogold-electron micrograph of the Casparian strip in *casp1-1casp3-1* using anti-ESB1 antibodies. Arrowheads show gold particles corresponding to ESB1 protein in the cytosol of endodermal cells. (Scale bar: 500 nm.)

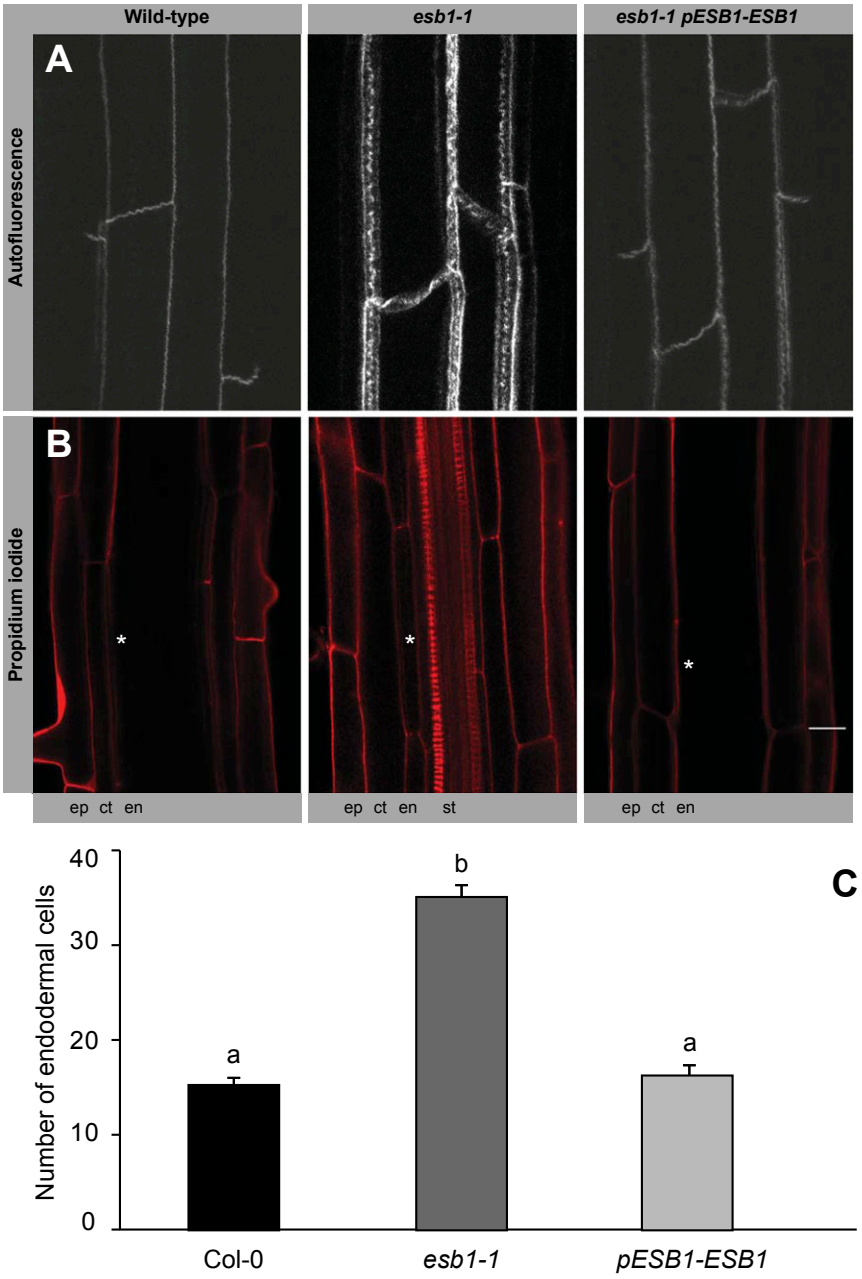


Fig. S2. Wild-type *ESB1* complements *esb1-1* Casparian strip defects including morphology and diffusion barrier properties. Casparian strip morphology defect of *esb1-1* is complemented by wild-type *ESB1* expressed from its native promoter. (*A*) Casparian strips visualized by autofluorescence of lignin. Delayed establishment of a functional barrier to propidium iodide diffusion observed in *esb1-1* is complemented by wild-type *ESB1*. (*B*) Propidium iodide fluorescence. Asterisks mark the 30th endodermal cell after onset of elongation. (*C*) Propidium iodide penetration into the stele of roots quantified as number of endodermal cells after onset of elongation. Different letters (a and b) indicate statistically significant differences between means by one-way ANOVA with Tukey–Kramer separation of means ($P < 0.05$), $n = 5$ roots. (Scale bar: 10 μm.) ct, cortex; en, endodermis; ep, epidermis; st, stele.

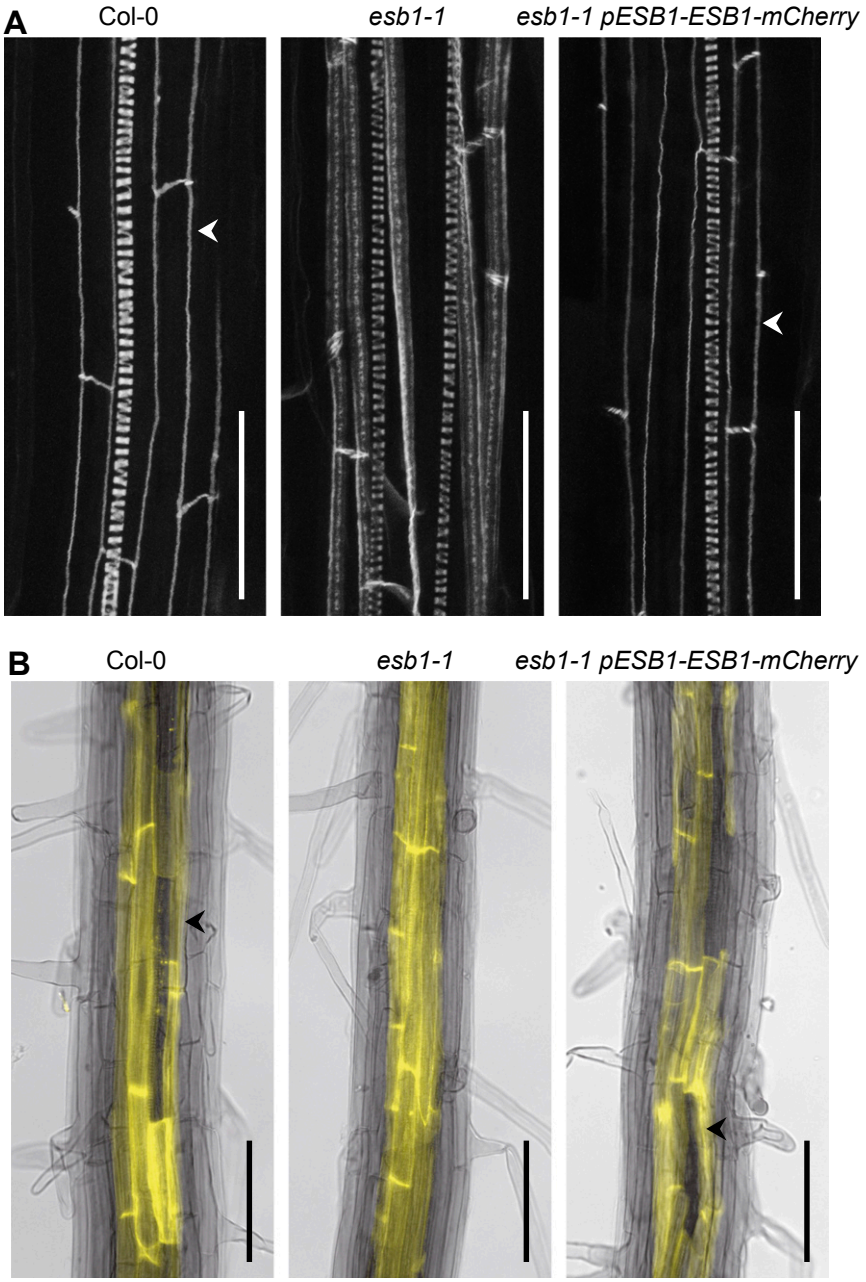


Fig. S3. *ESB1-mCherry* fusion construct complements *esb1-1* Casparian strip morphology defect and the loss of unsuberised endodermal passage cells. (*A*) Casparian strips imaged by confocal microscopy of lignin autofluorescence. Arrows indicate wild-type Casparian strip morphology. (*B*) Endodermal suberin lamella formation detected by Fluorol Yellow 088 staining. Arrowheads marks transfer cells. (Scale bars: *A*, 50 μm; *B*, 100 μm.)

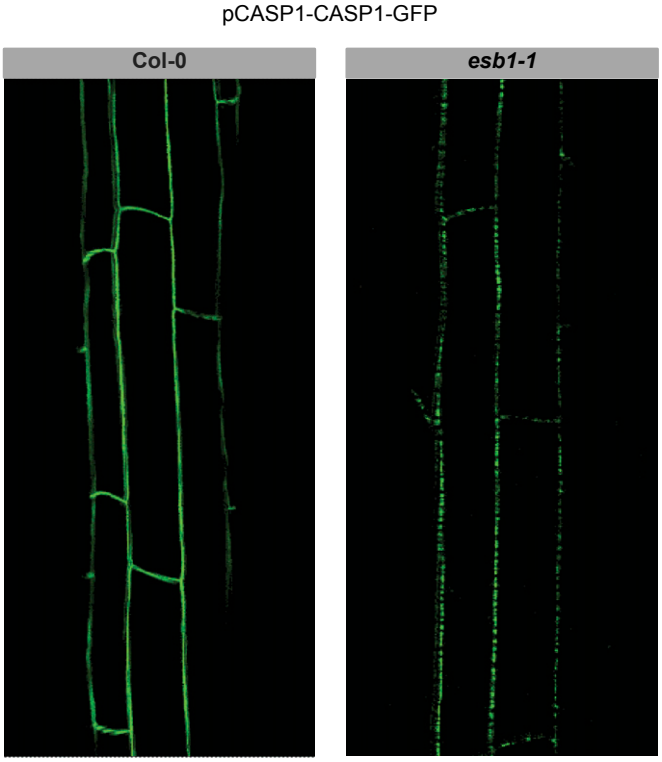


Fig. S4. CASP1 localization is disrupted in *esb1-1*. *Z*-stack confocal image (green) of plants expressing *CASP1-GFP* from the *CASP1* native promoter in *esb1-1* shows a patchy distribution of CASP1-GFP along the Casparian strip domain.

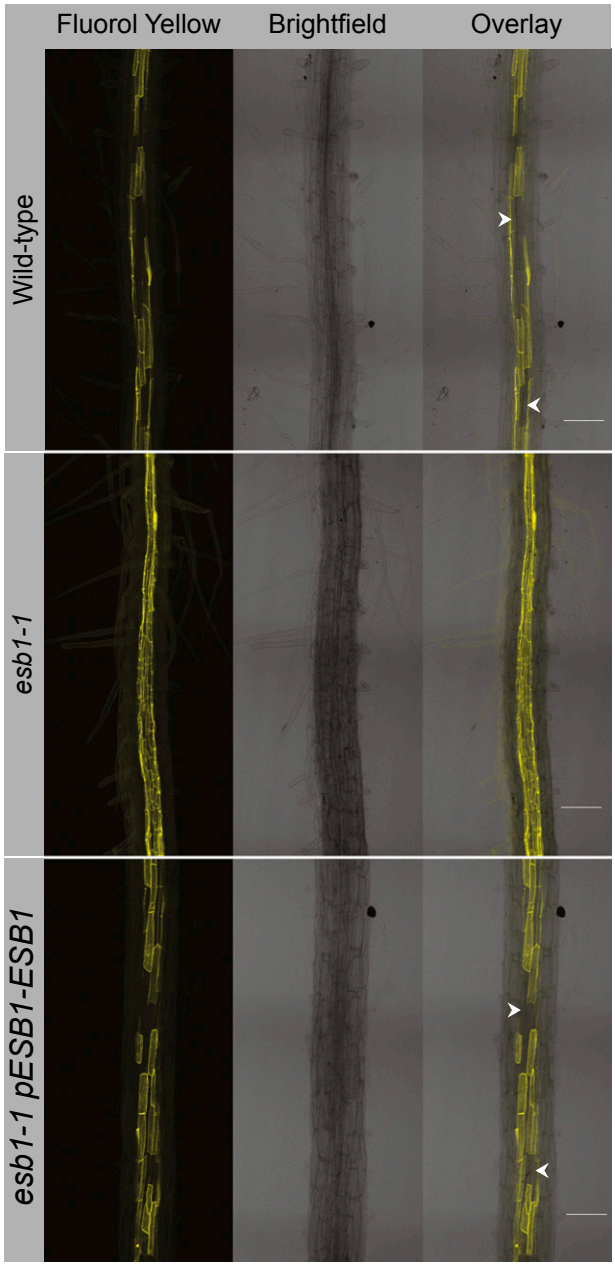
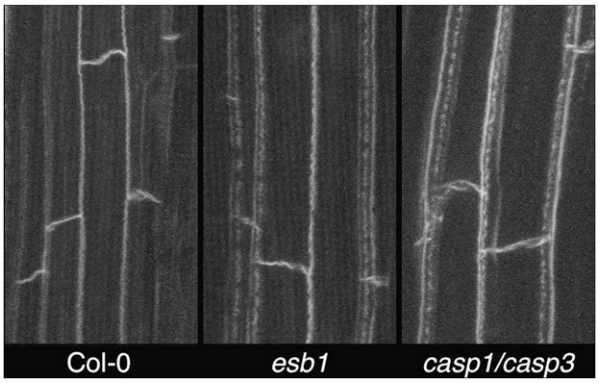


Fig. S5. Wild-type *ESB1* complements *esb1-1* loss of unsuberised passage cells. Fluorol Yellow staining of suberin in wild-type, *esb1-1*, and *esb1-1* expressing *pESB1-ESB1*. Arrowheads indicate passage cells. (Scale bars: 100 μm.)



Movie S1. Autofluorescence of wild type Col-0, *esb1-1*, and *casp1-1casp3-1*. Three-dimensional view of the Casparian strip in wild-type (*Left*), *esb1-1* (*Center*), and *casp1-1casp3-1* (*Right*) visualized as autofluorescence after root clearing. For constructing 3D animation images were acquired every 0.95 μm with a depth of at least one endodermal cell.

Movie S1

Dataset S1. Elemental profile of *esb1-1* is similar to that of *casp1-1/casp3-1*

Dataset S1

Plants were grown for 2 wk on MGRL medium (1) with 1% sucrose solidified with 1.2% type E agar (Sigma). Shoot element concentrations were determined by inductively coupled plasma mass spectrometry. *P* values were calculated by Dunnett's multiple comparison with wild-type Col-0. Element concentrations significantly different from wild-type are colored with red (decrease in mutant) and blue (increase in mutant). $n = 10$ biological replicates.

1. Fujiwara T, Hirai MY, Chino M, Komeda Y, Naito S (1992) Effects of sulfur nutrition on expression of the soybean seed storage protein genes in transgenic petunia. *Plant Physiol* 99(1): 263–268.