Journal Club

A Mechanism for Localized Lignin Deposition in the Endodermis

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Why and How I like this Paper?

Plant lignification is a cellular process generating lignin polymer in the cell wall of many different cell types and in response to various environmental stresses. As lignin remains a major obstacle for the utilization of cellulosic biofuels , understanding and manipulation lignin formation are therefore of fundamental importance.

In general, lignification occurs in three stages (Liu, 2012):
Biosynthesis of monolignols in the cytosol
Transportation of these monolignols to the cell wall
Oxidative dehydrogenation and polymerization to form heterogeneous macromolecules

While the biosynthesis of monolignols are well studied, the transportation and polymerization mechanisms of monolignols are largely unknown. This paper provide substantial studies to understand the third stage, showing how lignin deposition can be localized to specific cell domain called Casparian strip domain (CSD) in root endodermis cell, therefore established a good model that may be applied to other cell types undergoing lignification.

Background

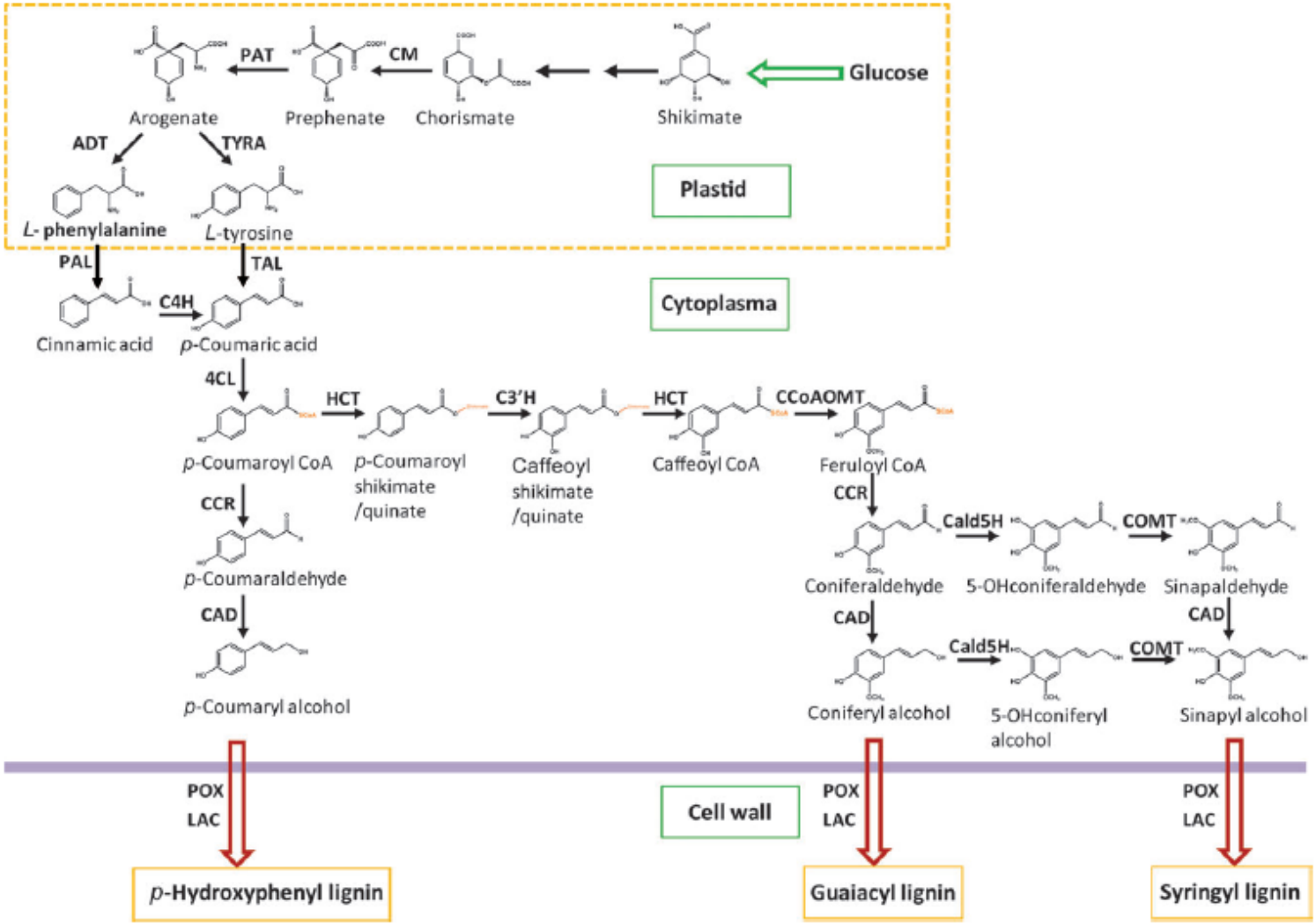


Figure 1. The Scheme of the Simplified Shikimate–Phenylpropanoid–Lignin Biosynthetic Pathway, Illustrating Different Compartmentalization of the Biosynthesis.

Chang-Jun Liu Molecular Plant • Volume 5 • Number 2 • Pages 304–317

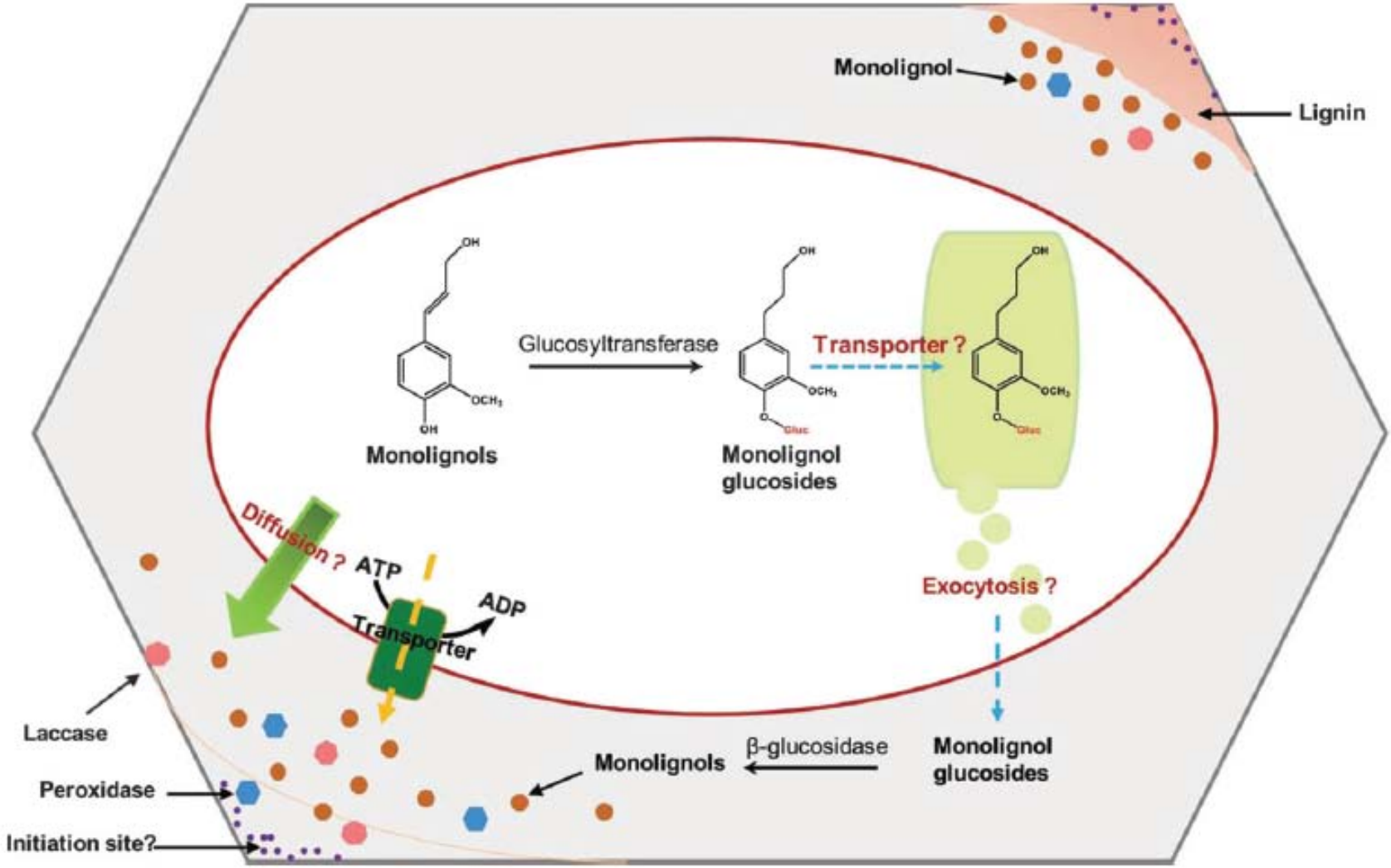
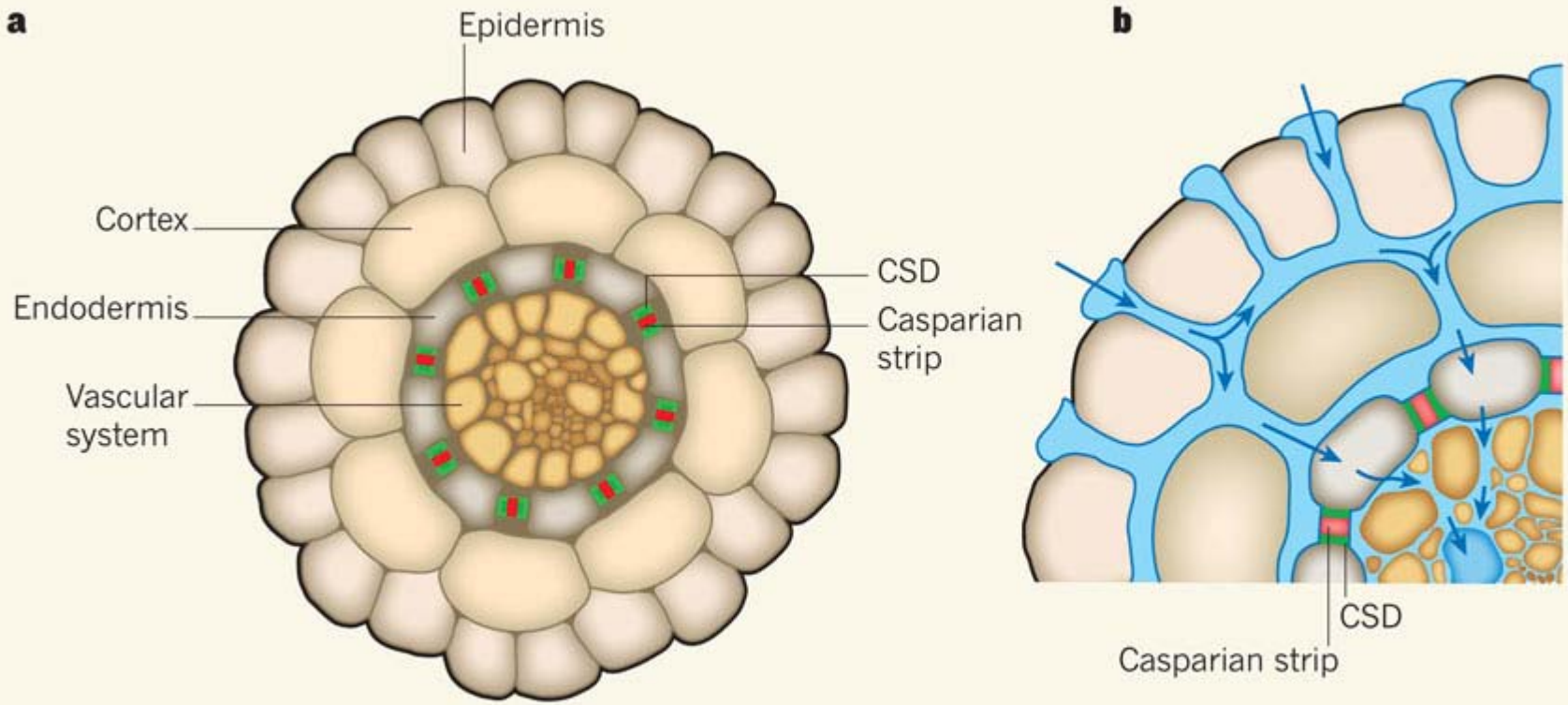


Figure 2. The Scheme for Monolignol Deposition and the Subsequent Initiation of Lignin Polymerization within the Cell Wall.

The Casparian strip in plant roots is a diffusion barrier that directs water and solutes from the soil to the water-conducting tissues.

First described by Robert Caspary, 1865



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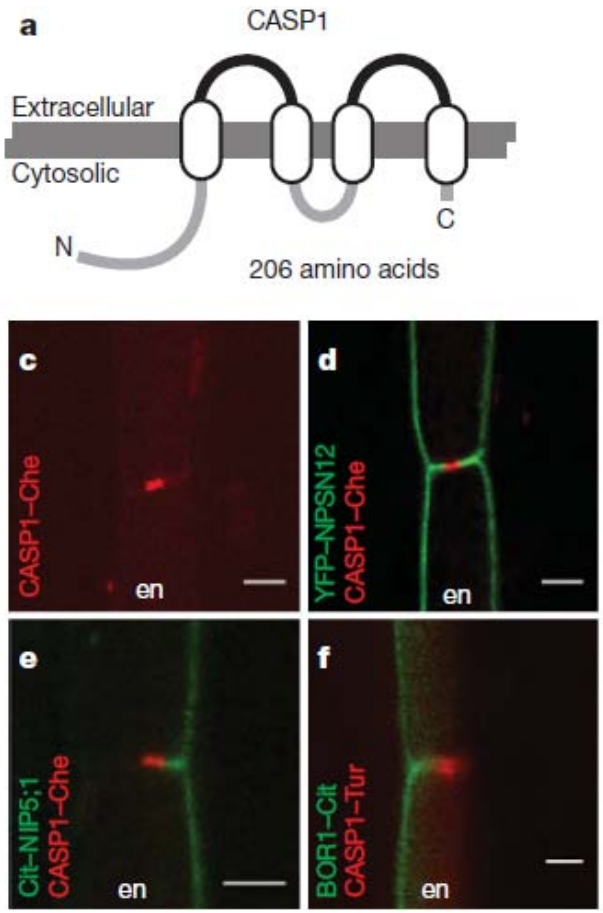


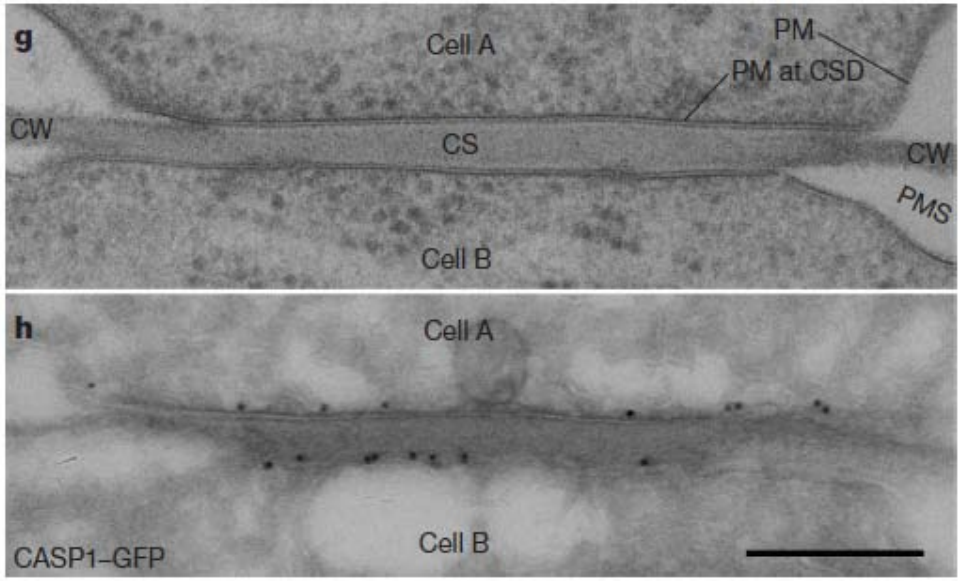
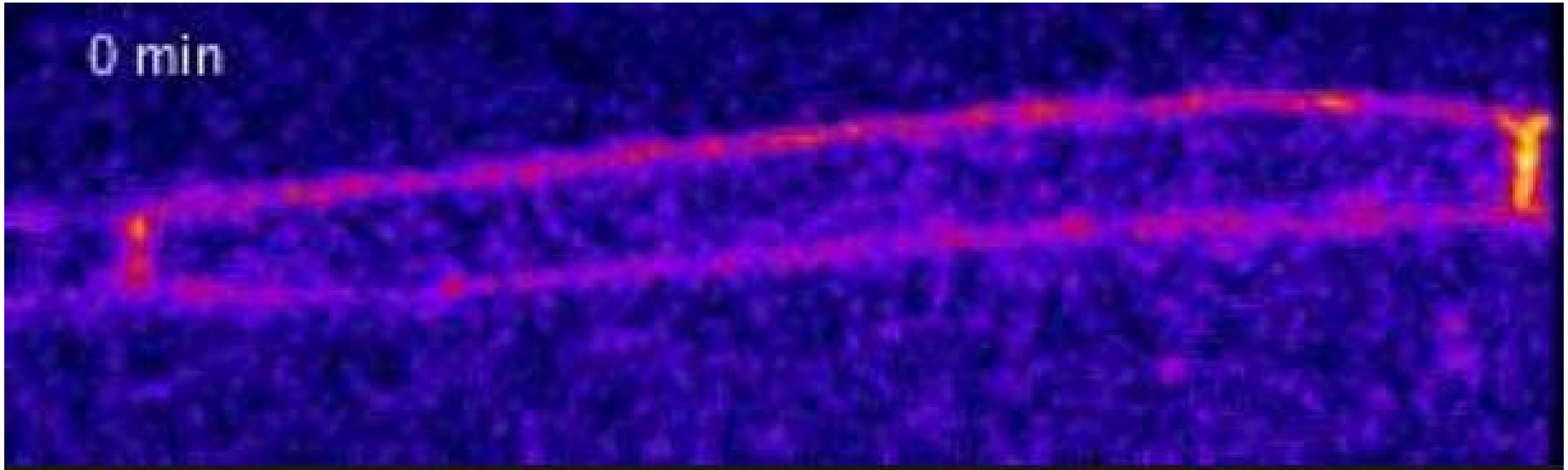


Figure 1 | A new protein family localizes to the Casparian strip membrane domain. **a**, Predicted topology of CASP1. **b**, Cartoon of the observed dots in an optical section that represent a longitudinal band in three dimensions. **c–f**, CASP1 (red) is confined to the CSD (**c**), is complementary to NPSN12 (**d**), and outer (NIP5;1, **e**) and inner (BOR1(Y373A/Y398A/Y405A), **f**) markers. Che, mCherry; Cit, citrine; en, endodermis; Tur, mTurquoise. **g**, Casparian strips (CS) appear homogeneous in electron micrographs. CSD adheres to Casparian strips. CW, cell wall; PM, plasma membrane; PMS, space generated by plasmolysis. **h**, Immunogold-electron micrograph of CASP1–GFP. Gold particles reside at either side of the Casparian strip. Scale bars: **c–f**, 5 μm; **h**, **i**, 250 nm.

A novel protein family mediates Casparian strip formation in the endodermis

Daniele Roppolo[1], Bert De Rybel[2]†, Valérie Dénervaud Tendon[1], Alexandre Pfister[1], Julien Alassimone[1], Joop E. M. Vermeer[1], Misako Yamazaki[1], York-Dieter Stierhof[3], Tom Beeckman[2] & Niko Geldner[1]

Nature, 2011, 473(19) , 380-384



Supplementary Movie 2: Initially non-localised CASP1-GFP rapidly accumulates in a central ring.
Time-lapse movies of CASP1-GFP localisation dynamics in a central optical cut. Total duration 108 min, 3 min frame rate. Movie played at 10 fps. Note the initially equal distribution of PM signals in the first frames that quickly accumulate at the dots at the two cell ends (the equatorial ring in 3D).

Roppolo et al., 2011 Nature

Casparian strip autofluorescence

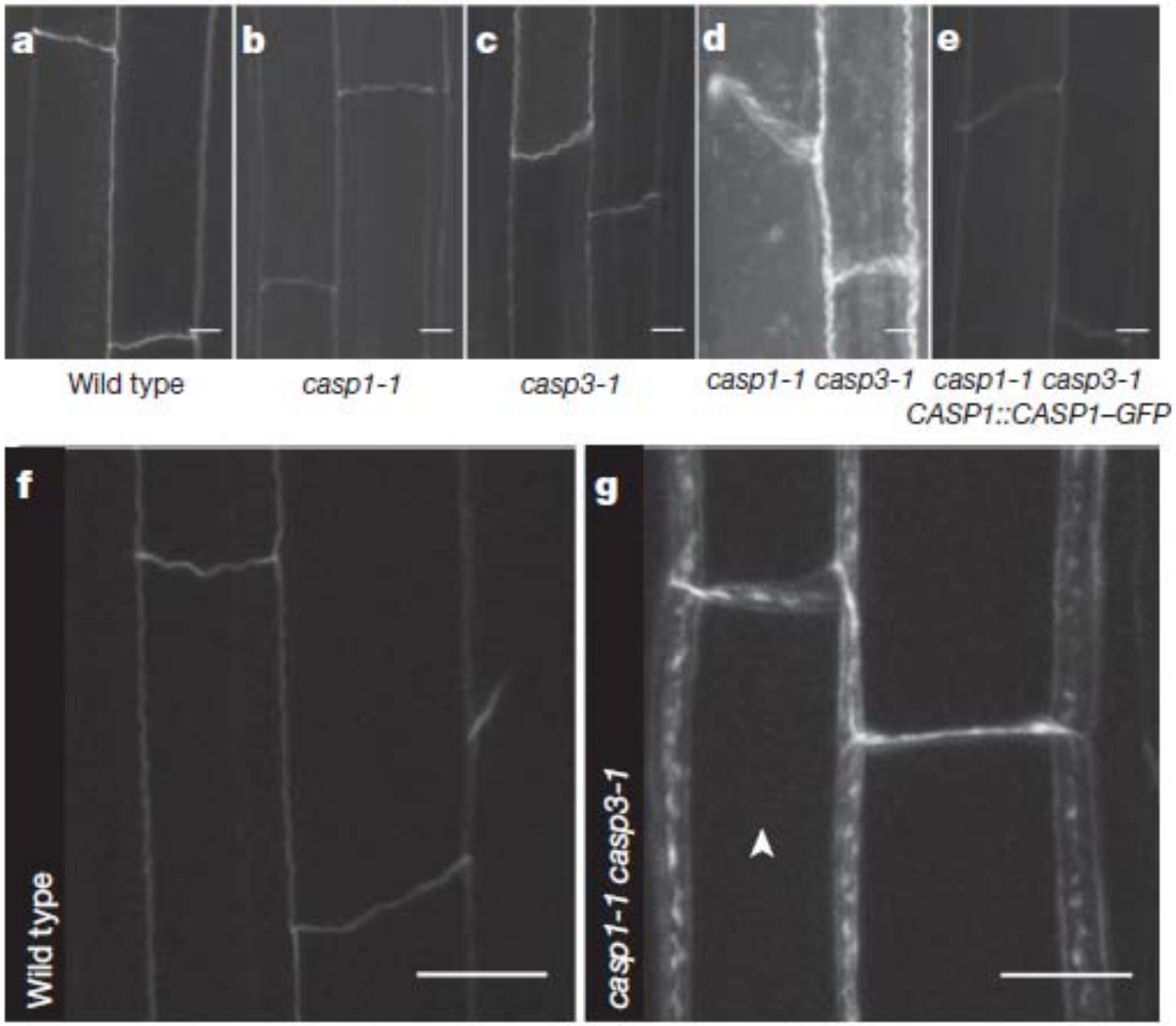
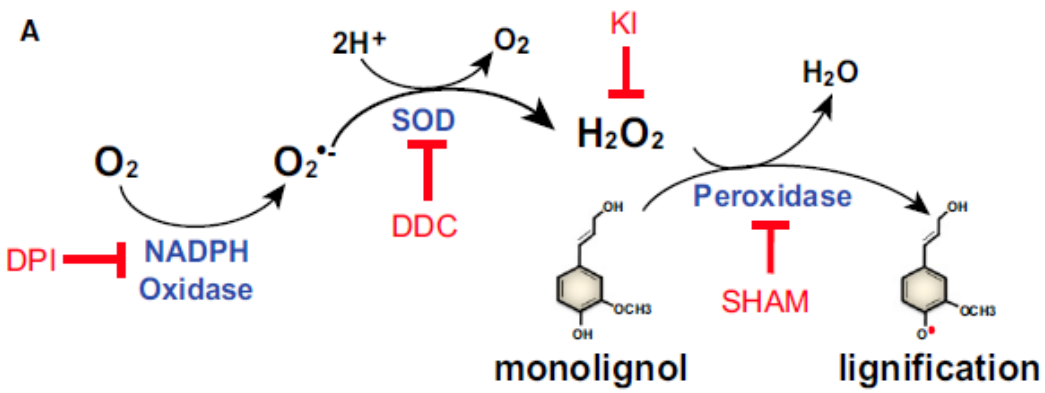


Figure 4 | CASP proteins are necessary for the correct structure and localization of the Casparian strips. a–e, Surface view of Casparian strip

Roppolo et al., 2011 Nature

In order to identify factors involved in Casparian strip formation, Niko Geldner group undertook a forward genetic screen for mutants with an impaired endodermal barrier. Among which, *sgn4/rbohf* mutants show a very strong delay in the formation of an apoplastic diffusion barrier, as visualized by the penetration of externally applied propidium iodide (PI) into the stele. The causal gene is one of the NADPH oxidase genes, *Respiratory burst oxidase homolog F (RBOHF).*



Lignin formation requires oxidation of monolignols, which is thought to be catalyzed by peroxidases or laccase. Peroxidases require hydrogen peroxide (H202) for this oxidation and NADPH oxidases have been implicated as major sources of regulated superoxide production, which can be enzymatically or nonenzymatically dismutated into H202. Therefore, they considered it most straightforward that SGN4/RBOHF would be needed for the production of H202 as a peroxidase cosubstrate.

***RBOHF* is required for Casparian Strip formation but not for CASP1 localization**

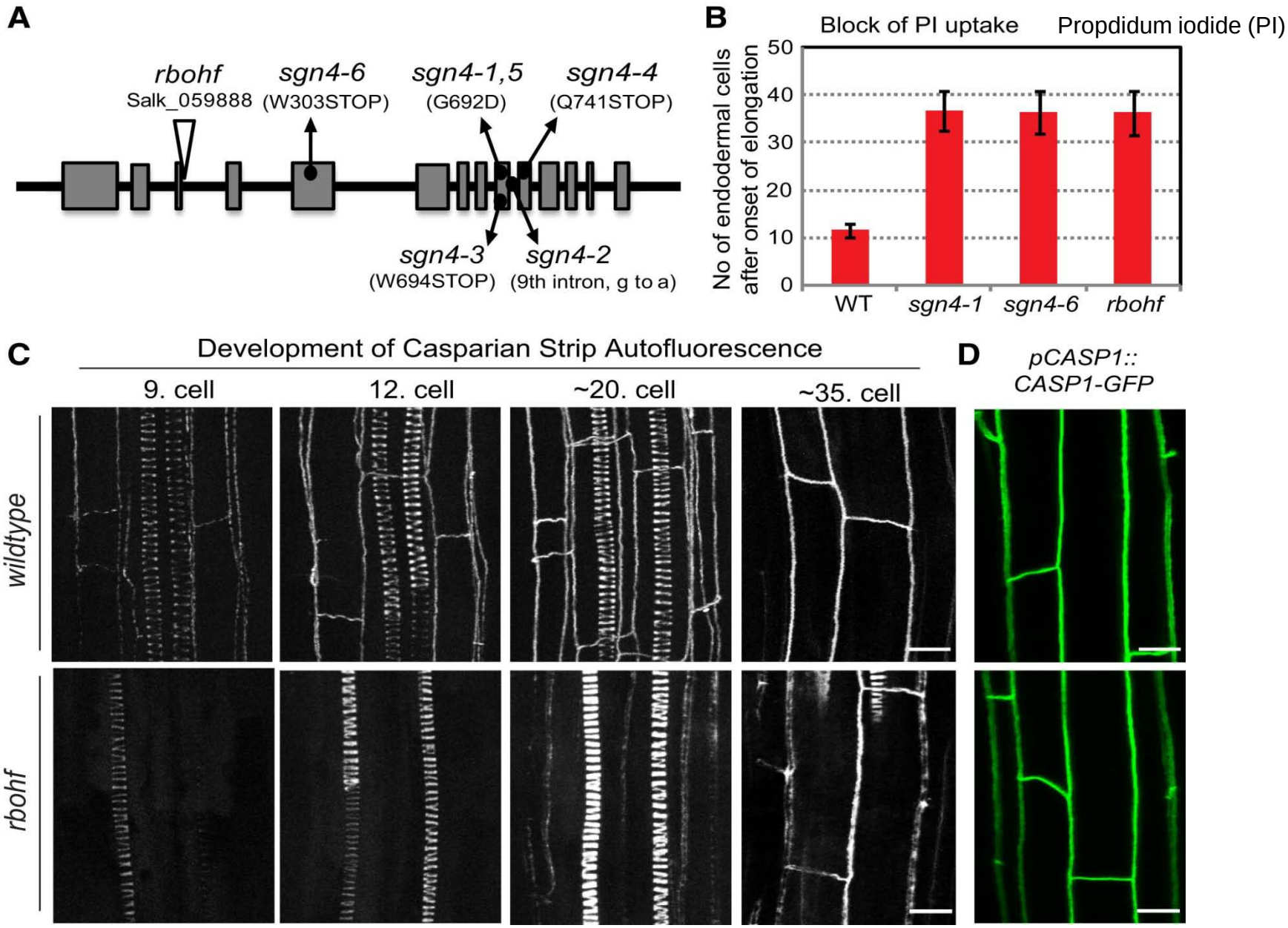


Figure 1 Casparian Strip Formation Is Delayed in sgn4/rbohf Mutants

***RBOHF* is not required for CASP1 localization**

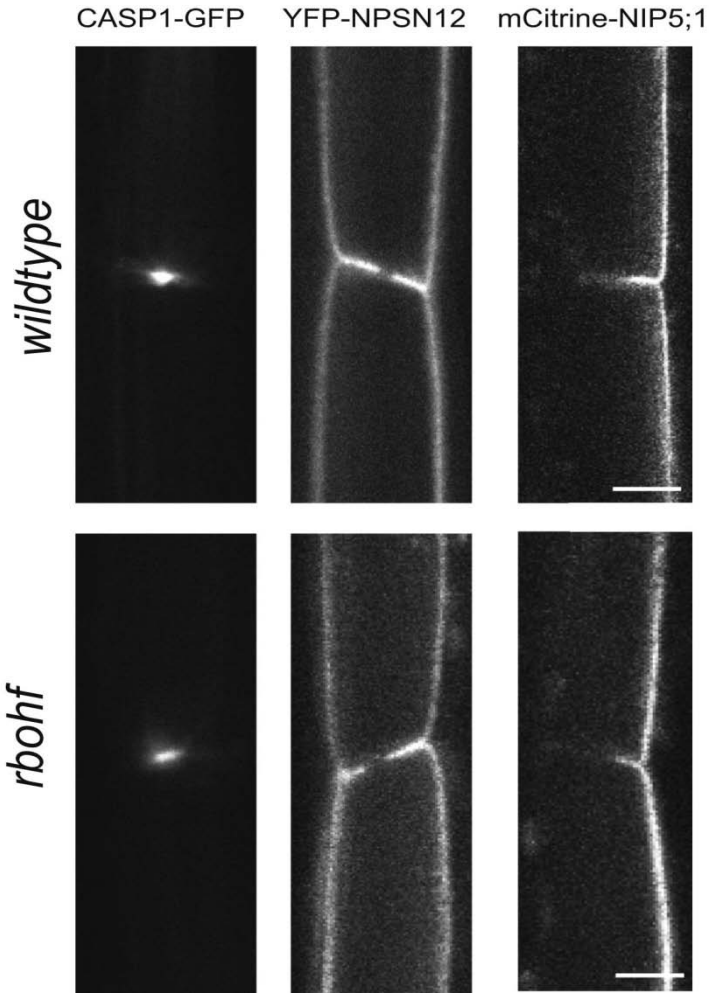
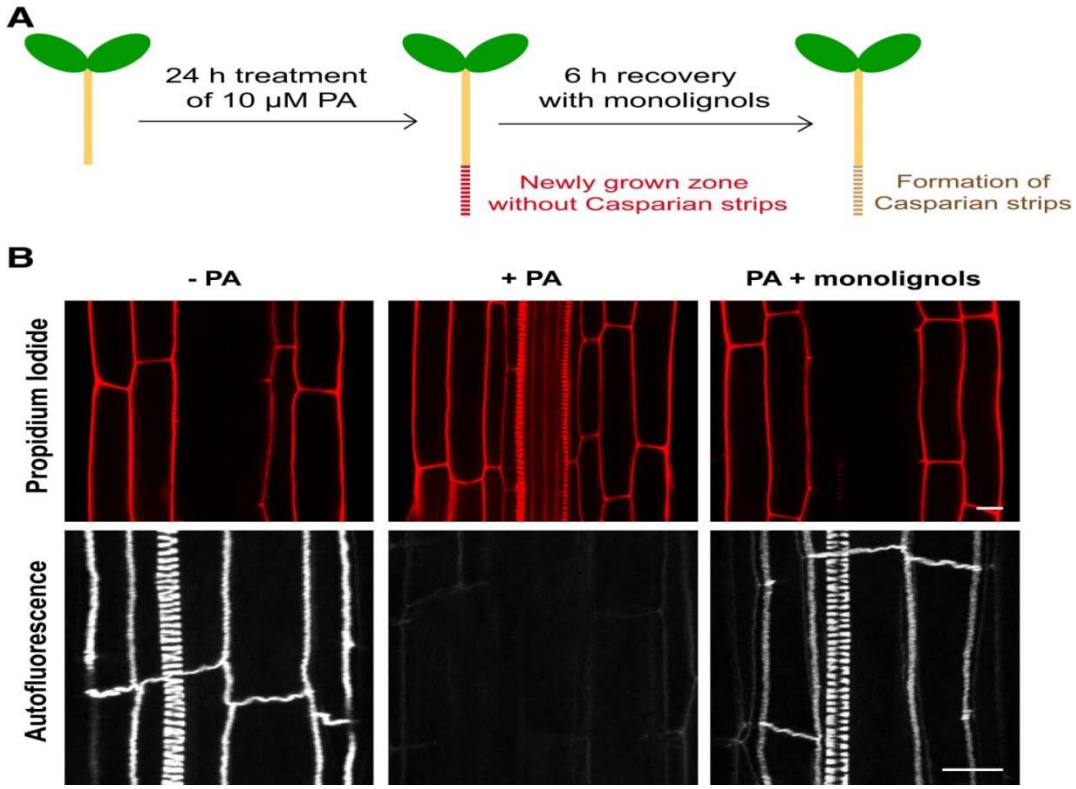


Figure S1. Casparian Strip Membrane Domain and Plasma Membrane Polarity Are Retained in *rbohf* Mutant, Related to Figure 1

A system allowing investigation of the immediate effect of NADPH oxidases on Casparian strip formation

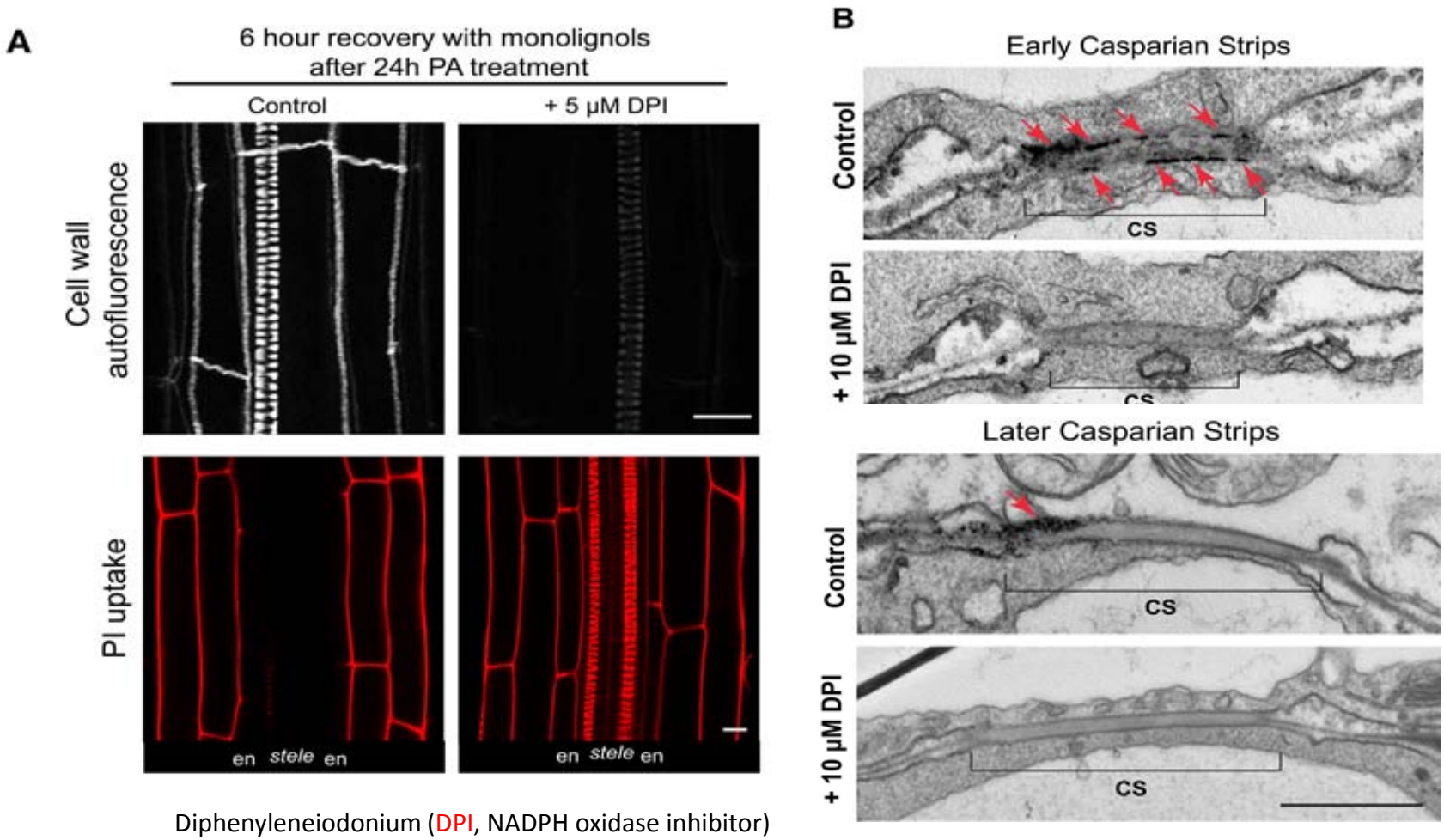


lignin inhibitor:
piperonylic acid (PA, 10 µM)

Figure S3. Casparian Strip Formation Blocked by Monolignol Biosynthesis Inhibitor Is Complemented by Exogenous Application of Monolignols, Related to Figure 2

(A) Schematic representation of a seedling explaining how block in Casparian strip formation is complemented by exogenously applied monolignols.
(B) PA blocks establishment of the PI diffusion barriers and Casparian strip formation in newly forming cells. This effect is complemented by monolignol addition.

NADPH Oxidase Is Essential for Casparian Strip Formation and Localized ROS Production



Diphenyleneiodonium (DPI, NADPH oxidase inhibitor)

Figure 2. NADPH Oxidase Is Essential for Casparian Strip Formation and Localized ROS Production

(A) The NADPH oxidase inhibitor DPI abolishes complementation observed by monolignol addition in the newly grown root zone of 6-day-old seedlings

(B) Localized H_2O_2 is detected in electron micrographs by its reaction with 10 mM cerium chloride (CeCl3) to produce electron-dense deposits.

Only RbohF and RbohB are specifically localized in endodermis

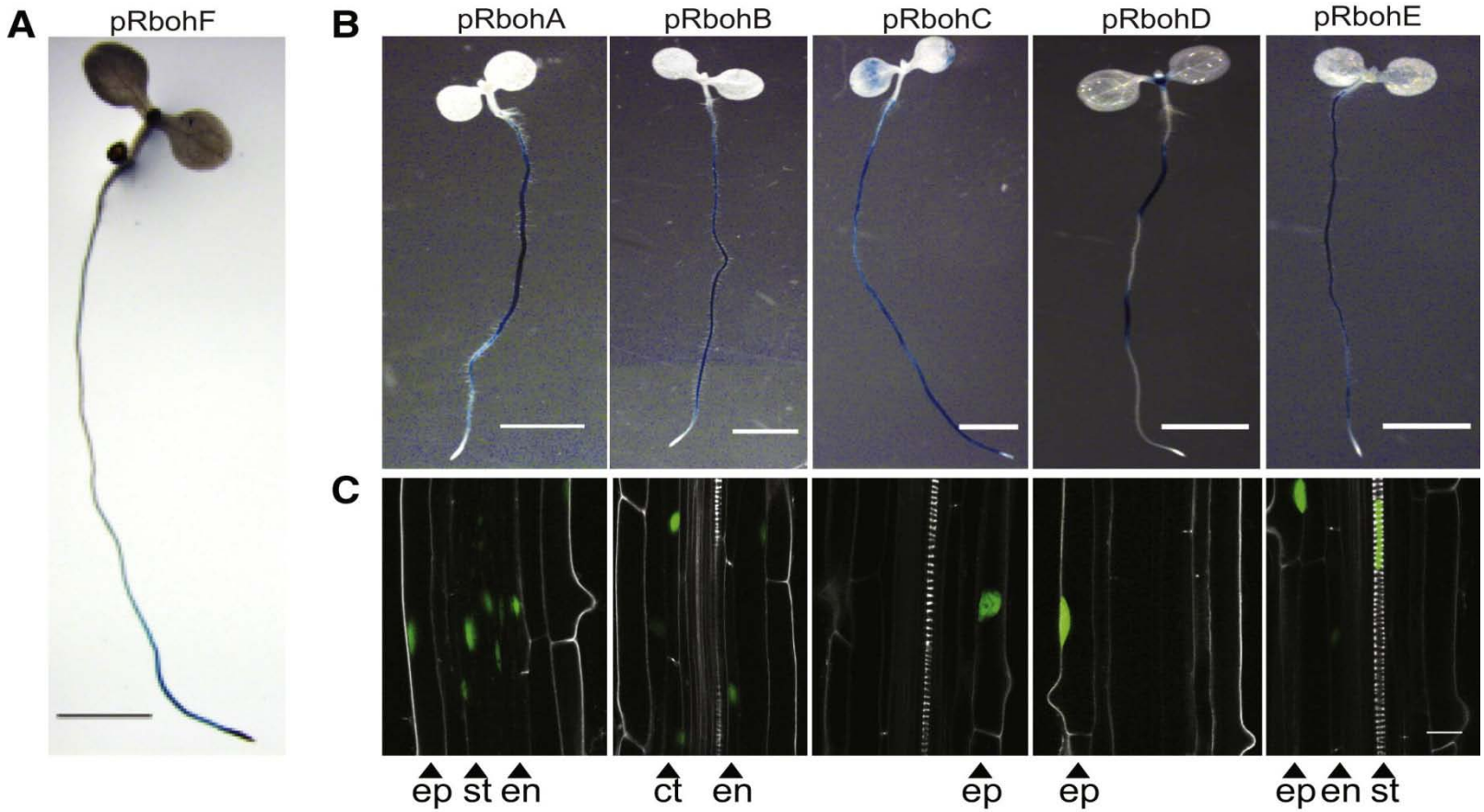


Figure S4. Promoter Analysis of RBOH Genes

(A) Promoter analysis of RBOHF by GUS activity.

(B) (B and C) Promoter analysis of RBOH genes by GUS activity (B) or nuclear localized GFP fluorescence (C) driven by own promoter. Analysis reveals that RBOHB is strongly expressed in the endodermis. Reporter gene is GFP-GUS with nuclear targeting

ep: epidermis, ct: cortex, en: endodermis, st: stele.

CSD Localization and Regulatory Input Determine Specificity of RBOHF Action in Casparian Strip Formation

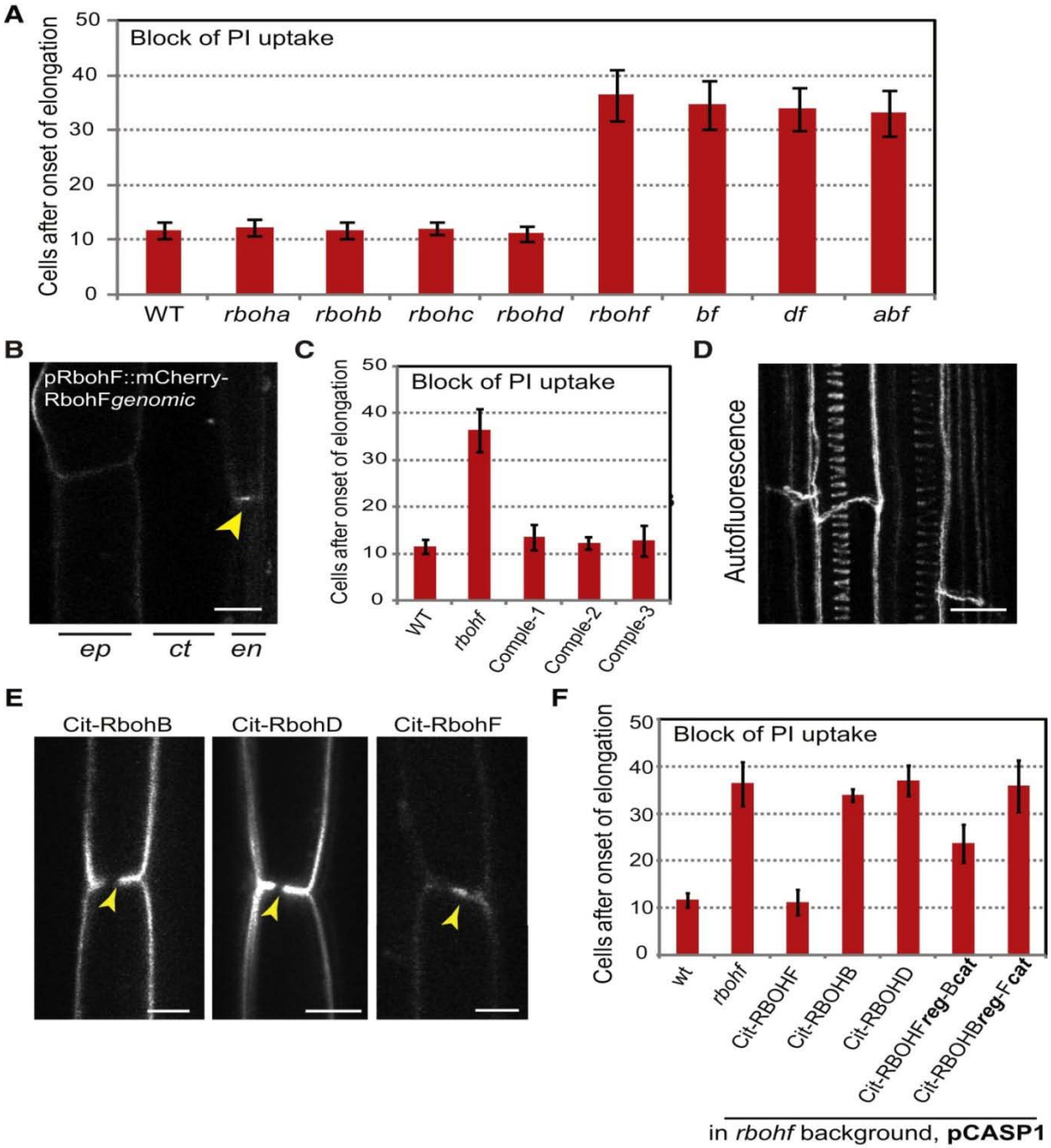
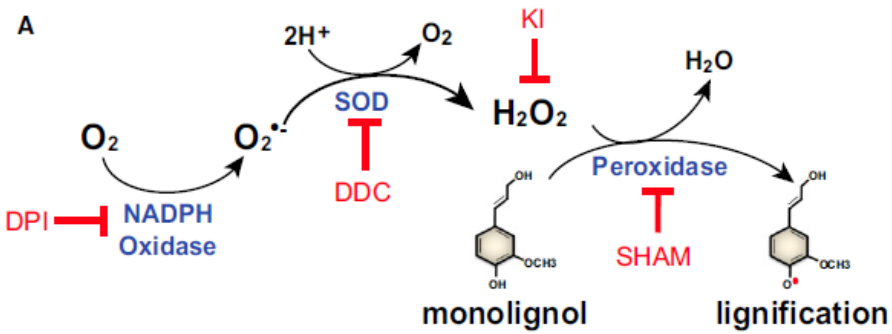


Figure 3. CSD Localization and Regulatory Input Determine Specificity of RBOHF Action in Casparian Strip Formation

Peroxidases Use Localized Superoxide-Derived H202 for Casparian Strip Formation



Diphenyleneiodonium (DPI, NADPH oxidase inhibitor),
Diethyldithiocarbamic acid (DDC, superoxide dismutase inhibito
Potassium iodide (KI, H_2O_2 scavenger),
Salicylhydroxamic acid (SHAM, peroxidase inhibitor).

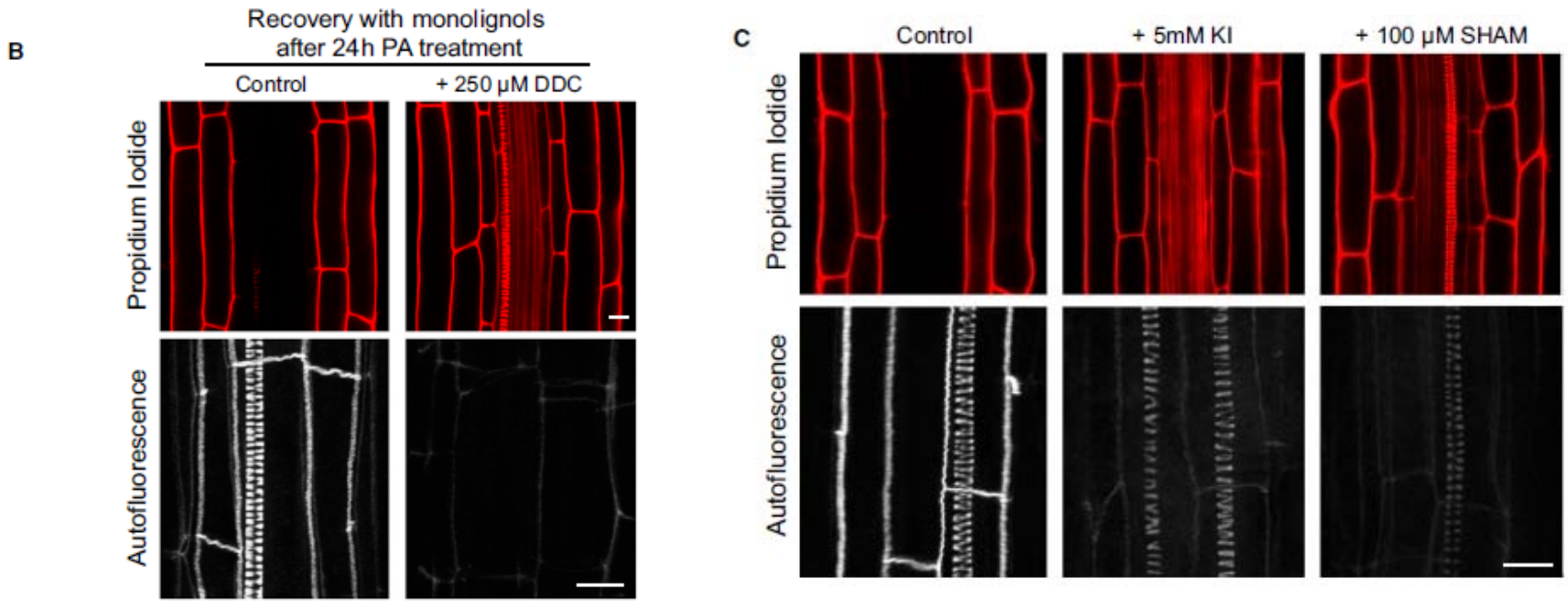


Figure 4. NADPH Oxidase Drives H_2O_2 Production for Peroxidase-Mediated Lignin Polymerization in Casparian Strips.
(A) Schematic representation of the required steps between NADPH oxidase activity and lignin polymerization (B) When 250 µM DDC is applied together with monolignols after 24 hr of PA treatment, as in Figure 2A, Casparian strip reconstitution does not occur
(C) When 5 mM KI and 100 µM SHAM is applied for 24 hr, root growth continues, but Casparian strip formation is inhibited in the newly grown root zone.

Specific, Endodermis-Expressed Peroxidases Are Required for Casparian Strip Formation

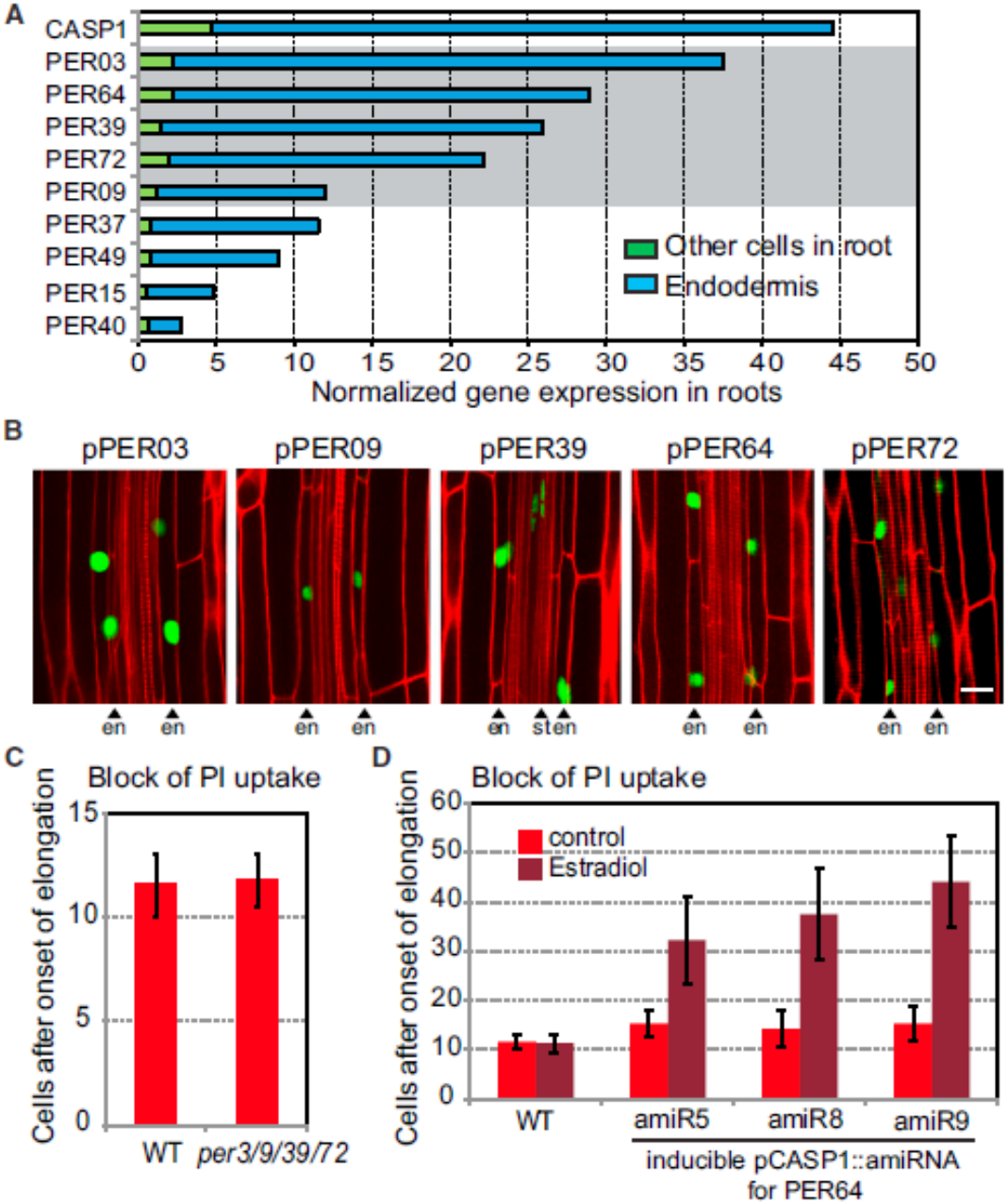


Figure 5. Peroxidases Are Involved in the Casparian Strip Formation

(A) Microarray data showing endodermis-enriched gene expression of CASP1 and peroxidases in roots (data from Bimbaum et al., 2003; Brady et al., 2007).

(B) Promoter gene expression analysis of peroxidases with nuclear-localized GFP-GUS as reporter confirms endodermis-specific/enriched gene expression in the endodermis. Arrowheads point to cell layers showing expression. en, endodermis; st, stele (see also Table S4). Scale bar, 20 μm.

(C) Establishment of a functional diffusion barrier, visualized by PI, is not affected in the quadruple mutant of *per3 per9 per39 per72* ($p > 0.5$, mean ± SD, $25 < n < 29$) (see also Table S2).

(D) Establishment of a functional diffusion barrier is delayed in mutants expressing inducible artificial microRNA for PER64 germinated in 1/2 MS plates including 10 μM estradiol ($p < 0.001$, mean ± SD, $16 < n < 27$) (see also Table S5).

PER64 has a more specific localization in casparian strip than other Peroxidases.

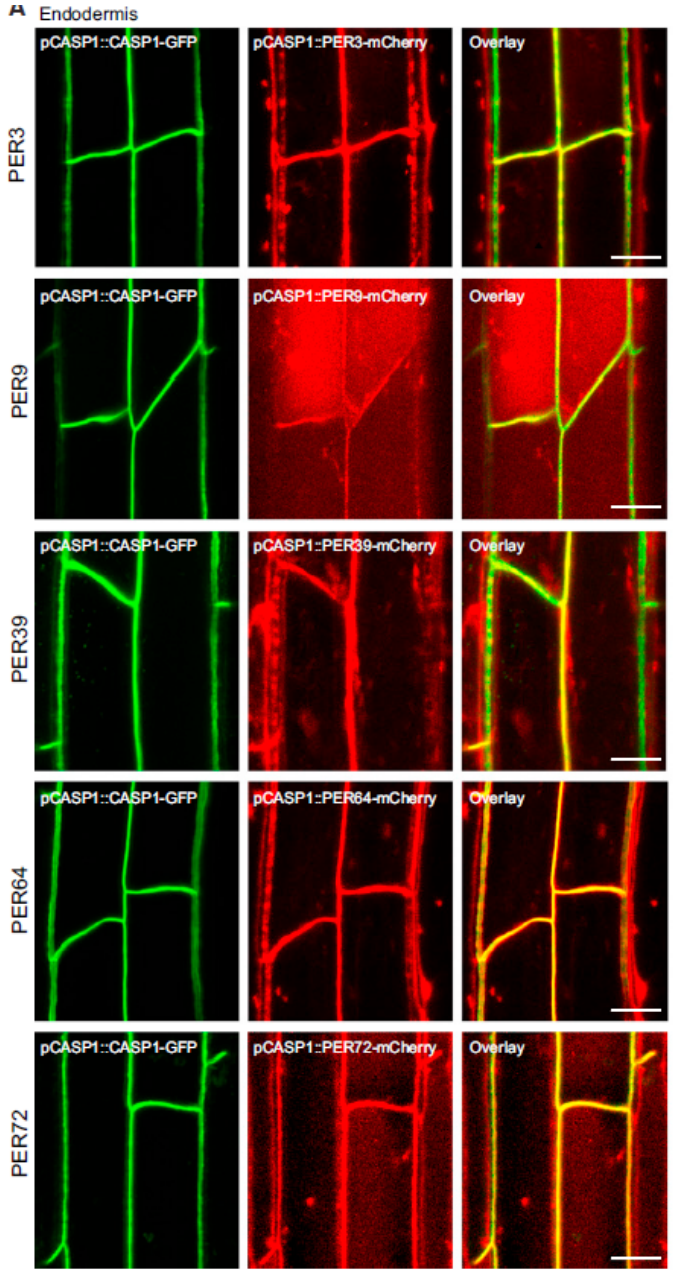
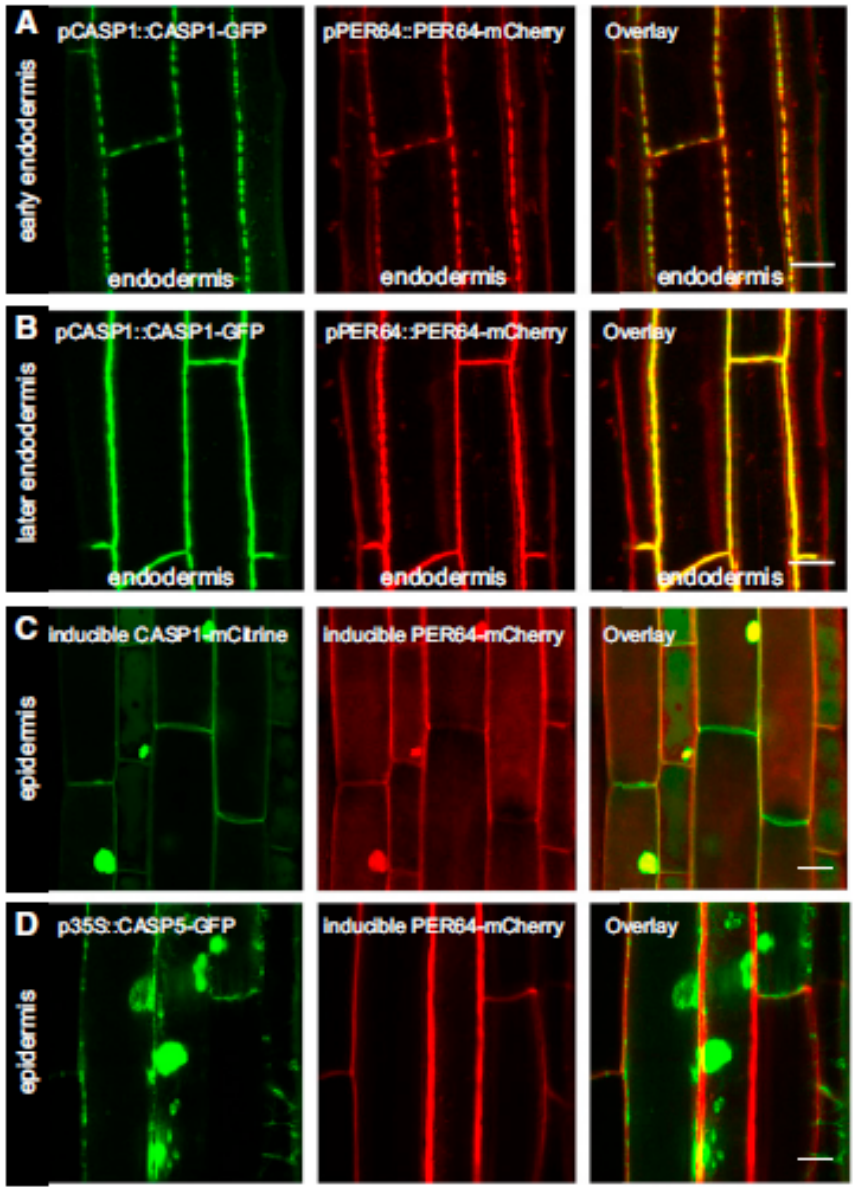


Figure S6. Localization of Peroxidases, Related to Figure 6

CASPs Determine Localization of an Endodermal Peroxidase to the Casparian Strips



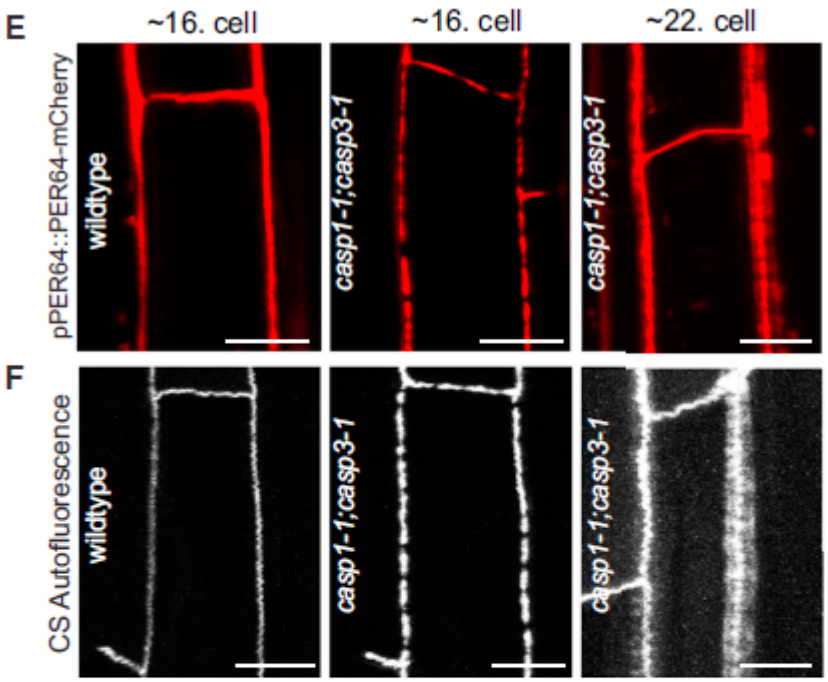


Figure 6. CASP1-GFP Determines PER64-mCherry Localization

(A and B) PER64-mCherry driven by own promoter colocalizes with CASP1-GFP in early endodermal cells (A) and later stage of endodermal cells (B) (see also Figures S6A and S6B and Table S5).

(C and D) Ectopically expressed PER64-mCherry, CASP1-mCitrine, and CASP5-GFP. PER64-mCherry colocalizes at the CASP1-mCitrine aberrant ER structures (C), but not at the CASP5-GFP aberrant ER structures (D) (see also Figures S6C–S6F).

(E) pPER64::PER64-mCherry in *casp1-1;casp3-1* mutant shows irregular localization of PER64, which is correlated with the pattern of CS auto-fluorescence in double mutant.

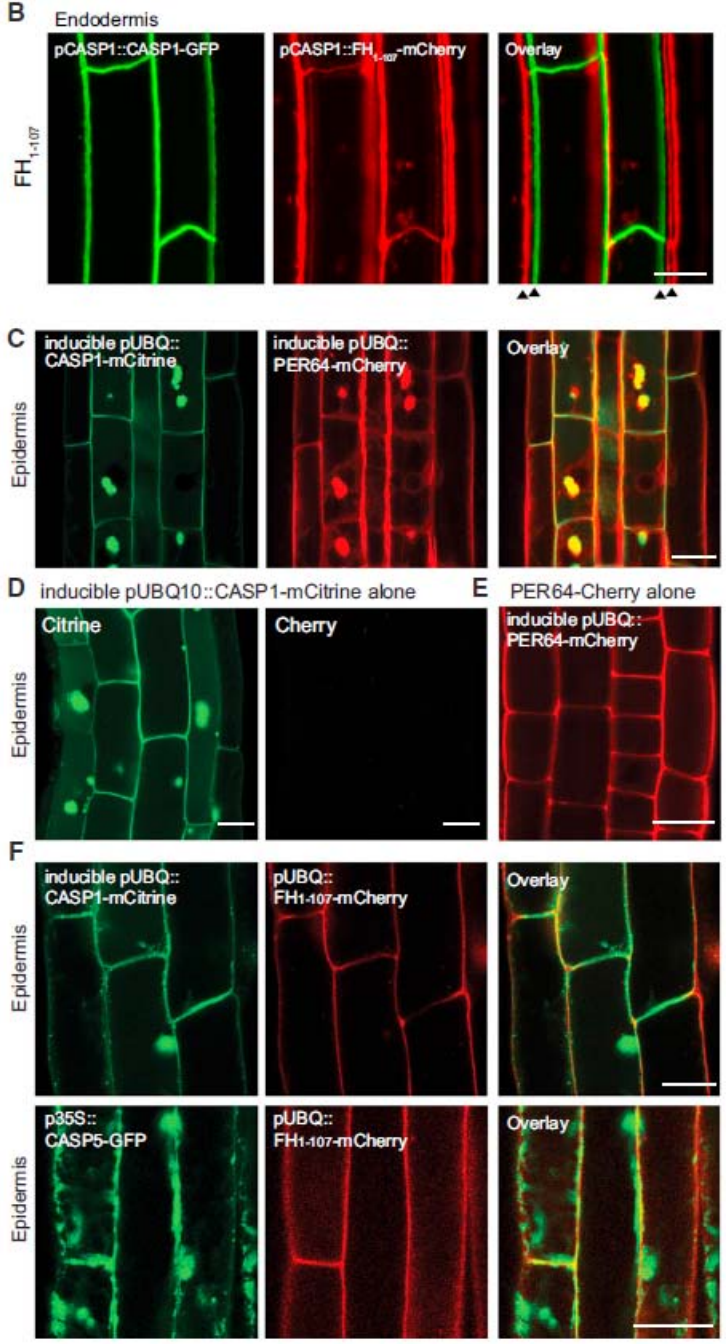
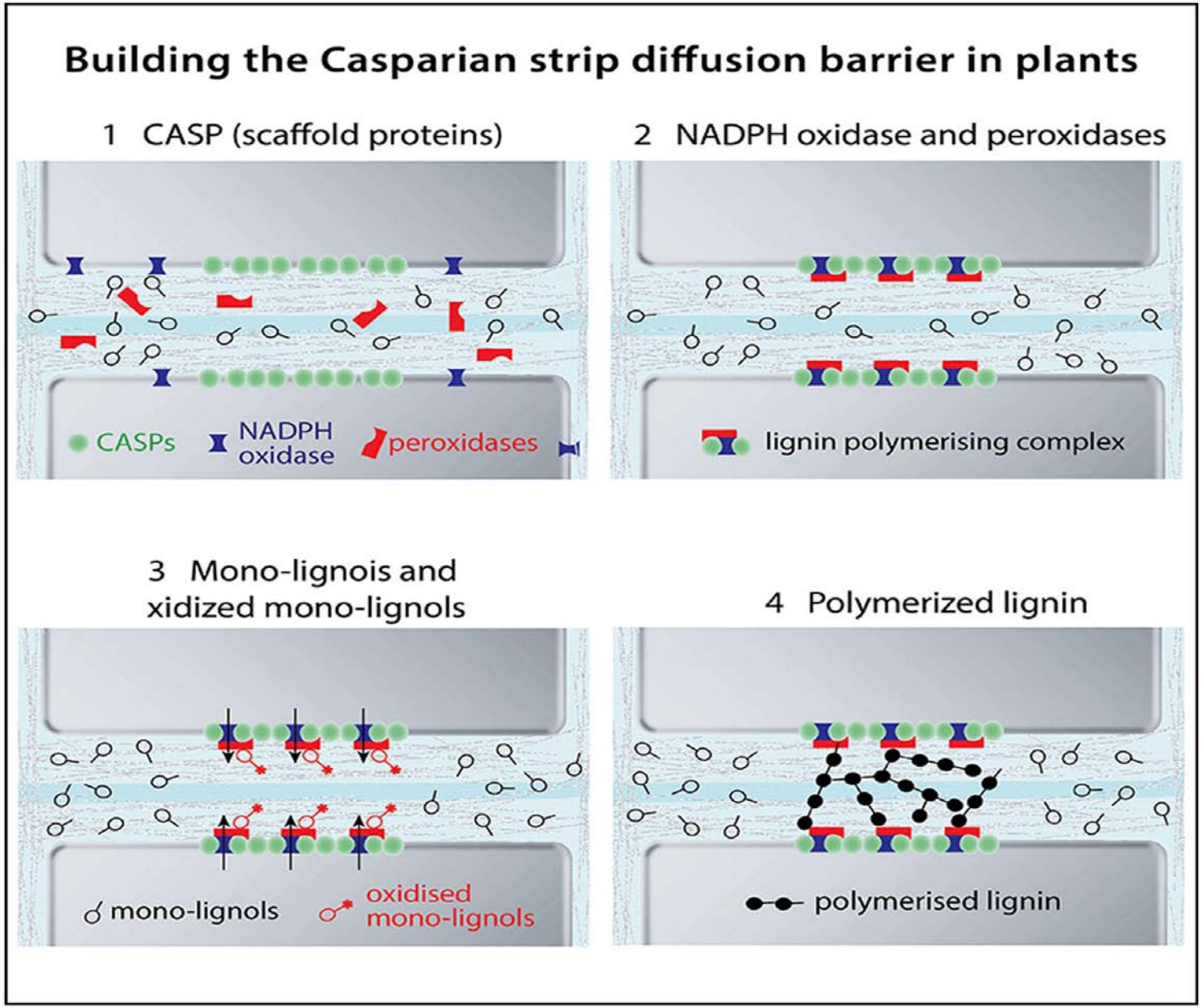


Figure S6. Localization of Peroxidases, Related to Figure 6

Working Model



Conclusion

1) RBOHF is a versatile signaling module involved in diverse cellular responses, but its specific function may depend on its subcellular localization.

2) CASPs are organizers of cell-wall-modifying proteins like RBOHF or PER64.

3) Scaffolding of NADPH oxidase or peroxidase by CASP-like protein might be a widespread mechanism.

More to explore…

1) It remains to be tested whether the CASP proteins have direct interaction with NADPH oxidase and peroxidase.

2)It is worthwhile testing if laccase also requires a scaffolding protein like CASP1 for its specific localization and function.

3)How are the Lignin monomers located to the place for lignification.

Take-Home Message

This work provide a good Model for a localized lignification in specific cell type like Endodermis. Similar mechanism might be applied to other cell types. Identifying other CASP-like proteins might be a good way to understand liginifcation processes in other cell types.

Thank you for your attention and questions!