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Supplemental Information

**Uclacyanin Proteins Are Required
for Lignified Nanodomain Formation
within Casparian Strips**

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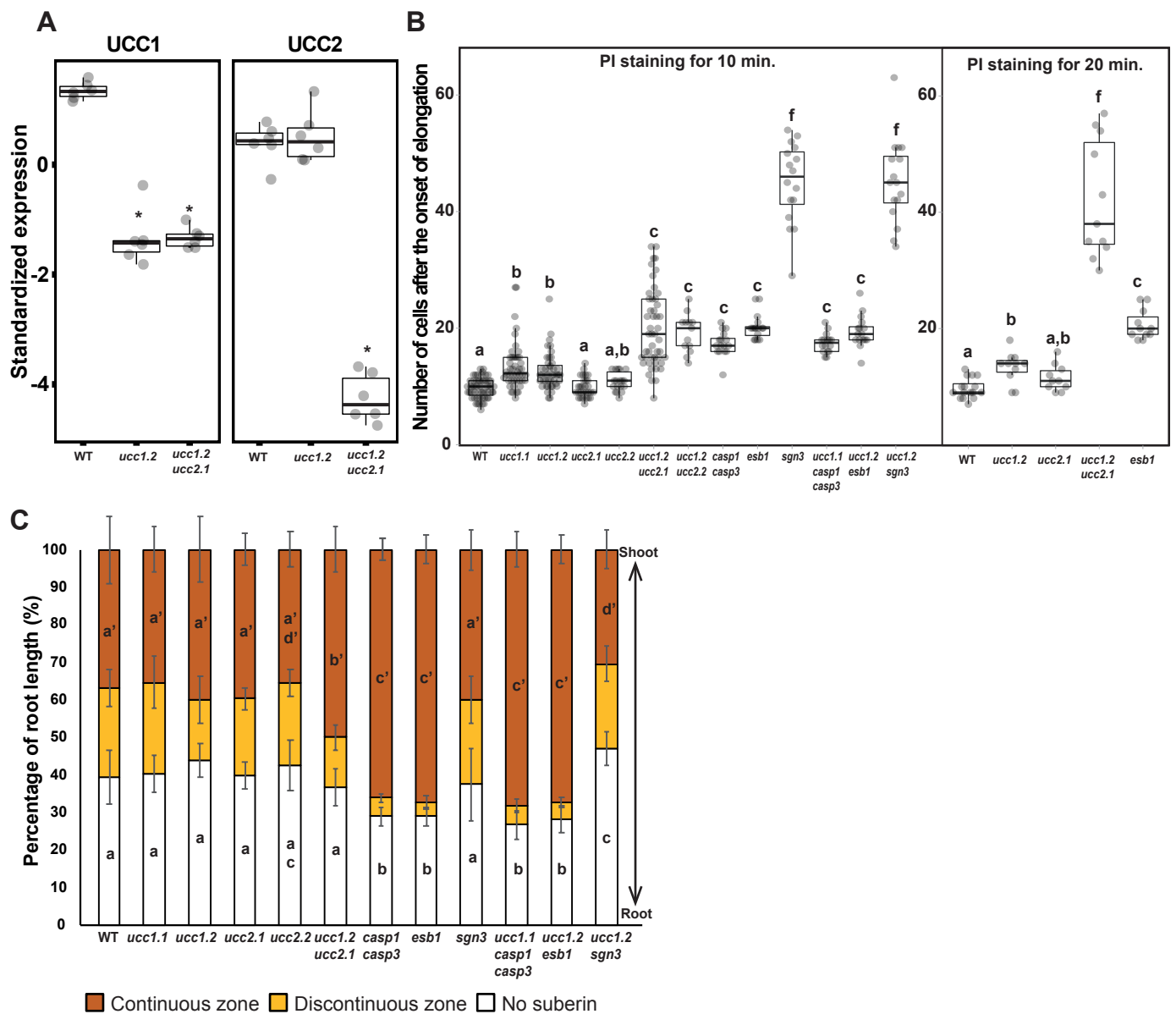


Figure S1. Uclacyanins UCC1 and UCC2 are required for the correct function of the Casparian strips. Related to Figure 1.

A. Boxplot showing the standardized expression of UCC1 and UCC2 in roots of wild type (WT), *ucc1.2* and *ucc1.2 ucc2.1*. Asterisk indicates a significant difference with WT using a t.test ($p < 0.01$; $n=6$). **B.** Boxplot showing the number of cells, from the onset of elongation, permeable to propidium iodide (PI) after 10 min or 20 min of root staining in wild type plants (WT) and a collection of mutants (*ucc1.1*, *ucc1.2*, *ucc2.1*, *ucc2.2*, *ucc1.2 ucc2.1*, *ucc1.2 ucc2.2*, *caspl casp3*, *esb1*, *sgn3*, *ucc1.1 caspl casp3*, *ucc1.2 esb1*, *ucc1.2 sgn3*). The number of plants analysed was $n \geq 13$ and $n \geq 10$ for the 10 min and 20 min of PI exposition, respectively. Different letters represent significant statistical differences between genotypes using one-way ANOVA and Tukey's test ($p < 0.01$). **C.** Quantification of the endodermal suberization using the suberin specific staining Fluorol yellow 088 in the genotypes from B (except *ucc1.2 ucc2.2*). The results (Percentage of root length (%)) represent the percentage of the root that remains unsuberised (white), discontinuously suberised (yellow) or continuously suberised (orange). In all cases the number of plants analysed was $n \geq 15$, error bars mean standards deviation (SD). Different letters represent significant differences between genotypes using a Mann-Whitney test ($p < 0.01$).

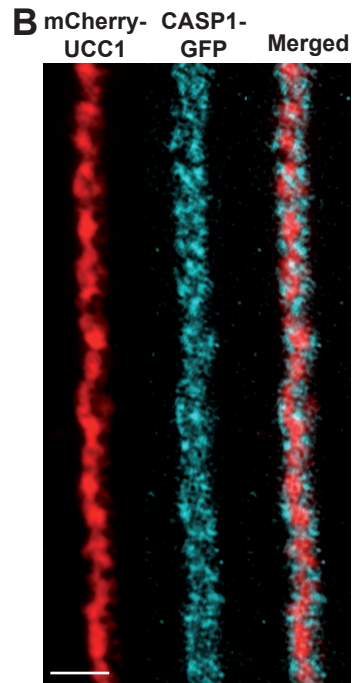
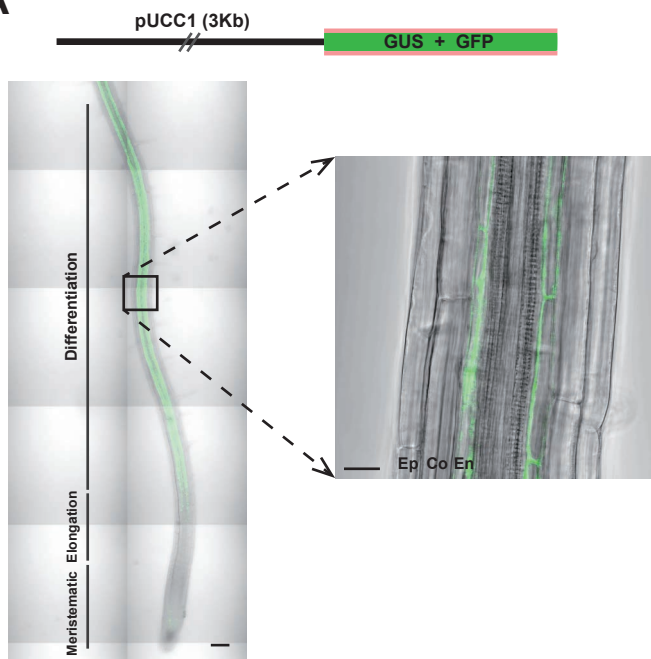
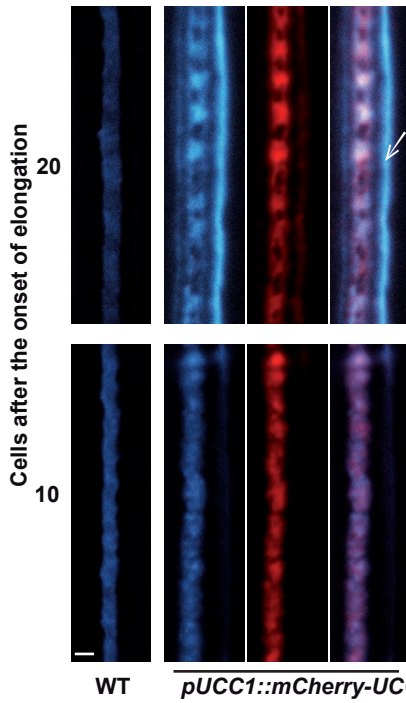
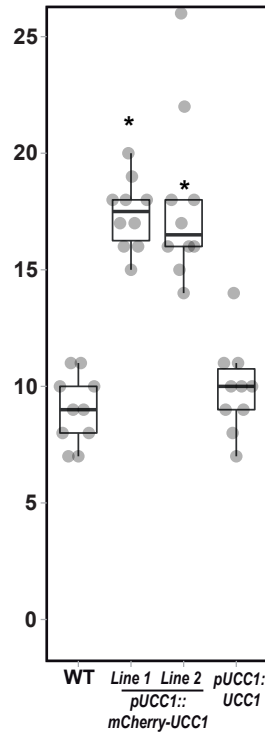
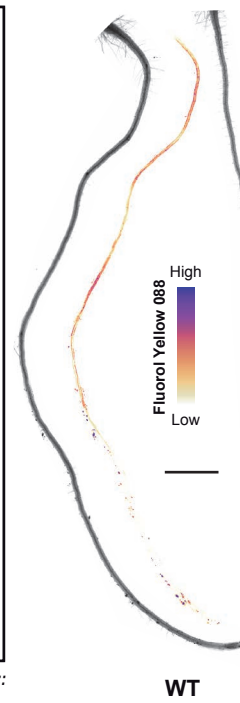
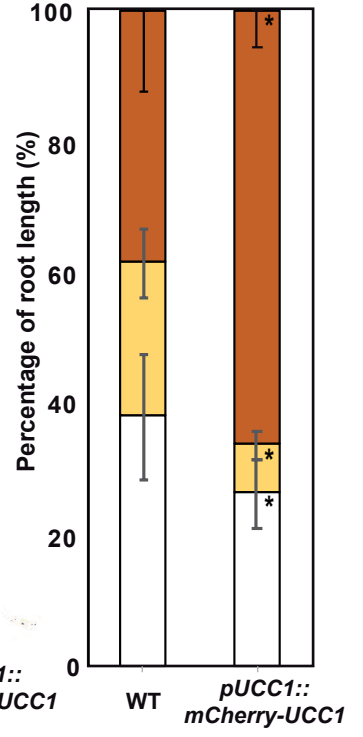
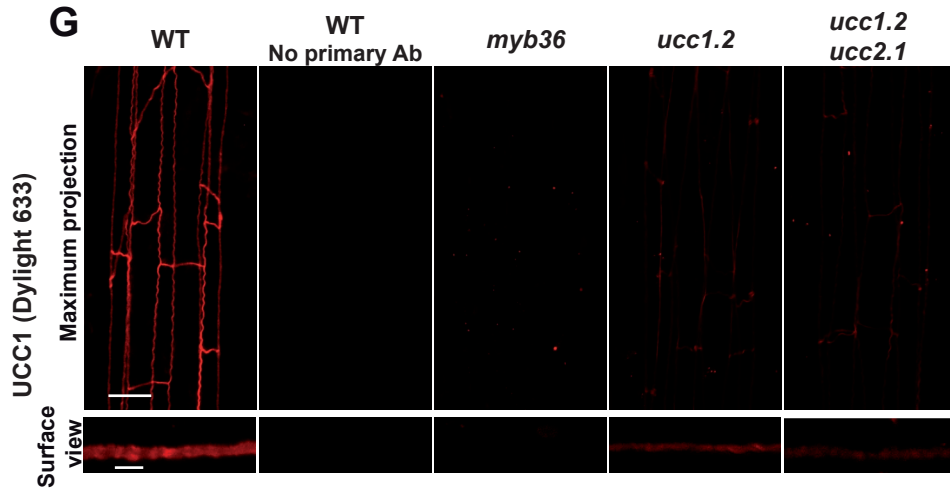
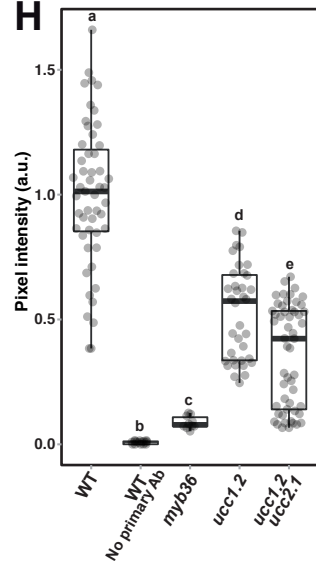
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Figure S2. UCC1 accumulates in the root endodermis and defines a central sub-domain at the Casparian strip. Related to Figure 2.

A. (Top) Schematic representation of the construct used to study the expression of *UCC1* in the root. Approximately 3 Kb of the promoter region of the gene *UCC1* (pUCC1 (3Kb)) was used to drive the expression of the β -glucuronidase (GUS) and the green fluorescent protein (GFP). (Bottom) Pictures showing the GFP accumulation in the root of plants expressing the construct *pUCC1::GUS-GFP*. The fluorescence (GFP, green) and brightfield (grey) pictures are merged. The different zones of the root (meristematic, elongation and differentiation) are highlighted in the figure. Scale bars = 100 μ m (left) and 20 μ m (right). (Ep: Epidermis; Co: Cortex; En: Endodermis). **B.** Structured illumination microscopy of a surface view of plants expressing CASP1-GFP (cyan) and mCherry-UCC1 (red) at the 10th endodermal cell after the onset of elongation in cleared root. Scale bar = 2 μ m. **C.** Surface view of Casparian strip lignin stained with Auramine-O (cyan) in wild type plants (WT) and in a line expressing the construct *pUCC1::mCherry-UCC1* (red) in cleared roots at 10th and 20th cells after the onset of elongation. In the figure the white arrow shows ectopic lignification. Scale bar = 2 μ m. **D.** Boxplot showing the number of the cells from the onset of elongation permeable to propidium iodide in wild type plants (WT), two lines expressing *pUCC1::mCherry-UCC1* and a line expressing *pUCC1::UCC1* all in a WT background. For both genotypes 10 plants were analysed. Asterisk indicates a significant difference with WT using a Mann-Whitney test ($p < 0.01$). **E.** Brightfield and suberin staining using Fluorol yellow 088 of roots in WT and a line expressing *pUCC1::mCherry-UCC1*. Scale bar = 2 mm. **F.** Quantification of suberin staining with Fluorol yellow 088 along the root. The results are expressed in percentage of root length that is unsuberised (white), discontinuously suberised (yellow), and continuously suberised (orange). In all cases $n \geq 10$ plants were used, error bars: SD. Asterisks indicates significant differences in comparison with WT for the same zone using a Mann-Whitney test ($p < 0.01$). **G.** The anti UCC1 antibody generated in this work is functional and specifically recognises the UCC1 protein. Maximum intensity projection and surface view of UCC1 immunolocalization (red) in wild type plants (WT), the mutants *myb36*, *ucc1.2*, *ucc1.2 ucc2.1* in the root at the 10th cell after the onset of elongation. As a control the primary polyclonal antibody anti UCC1 was used or not (“No primary Ab”) in combination with a secondary antibody conjugated with Dylight 633. Scale bar = 20 μ m and 2 μ m for maximum intensity projection and surface view. **H.** Pixel intensity quantification of UCC1 immunolocalization on pictures similar to G. Pixel intensity quantification was specifically performed along a 1.2 μ m-wide line following the CS (defined as Dylight 633 signal) in WT, *ucc1.2* and *ucc1.2 ucc2.1*. In “No primary Ab” control and *myb36*, pixel intensity was not specifically measured at the CS due to the absence of signal. However, pixel intensity was quantified in endodermal cells. Measurements were performed on 8 plants for WT, 3 plants for “No primary Ab”, 2 plants for *myb36*, 5 plants for *ucc1.2* and 7 plants for *ucc1.2 ucc2.1*. Different letters represent significant differences between genotypes using a Mann-Whitney test ($p < 0.01$).

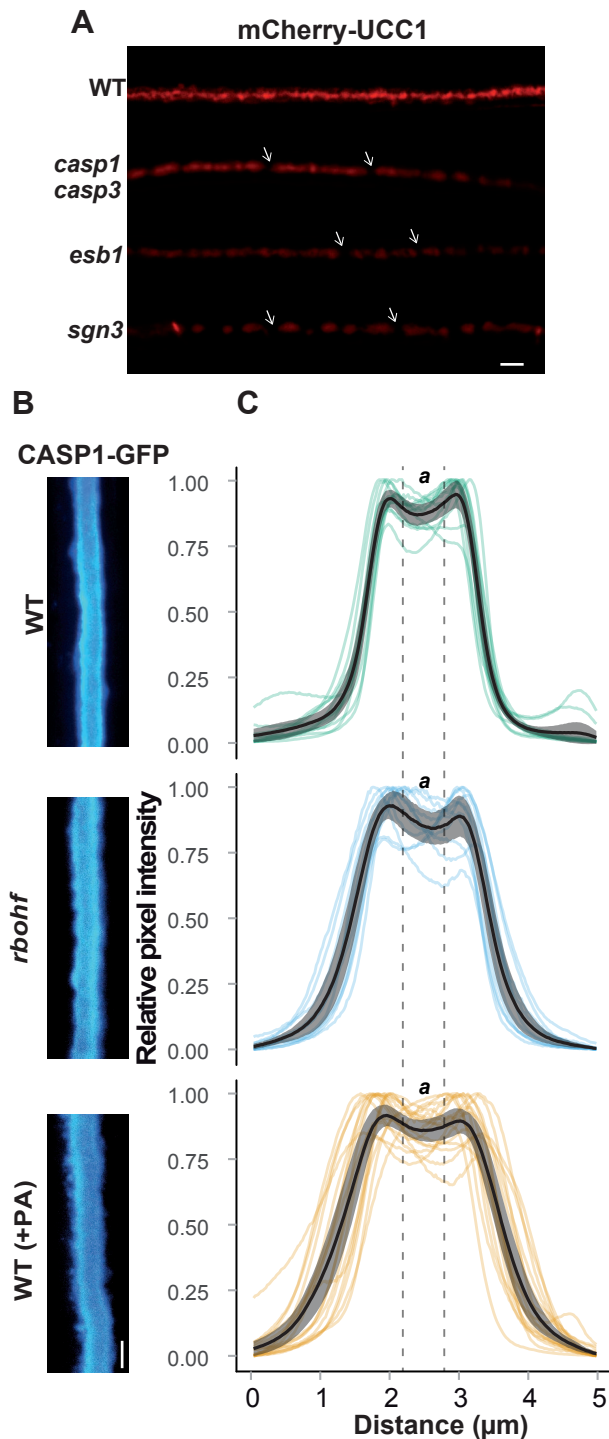


Figure S3. Relations between UCC1 positioning and other components of the Casparian strips machinery. Related to Figure 3.

A. Surface view of mCherry-UCC1 at the 15th cell after the onset of elongation in cleared root of WT, *casp1 casp3*, *esb1* and *sgn3*. White arrows represent discontinuous mCherry-UCC1 localization. Scale bar = 2 μm . **B.** Surface view of the localization of CASP1-GFP in root of wild type plants (WT), *rbohF*, and WT treated with piperonylic acid (+PA, 10 μM). Scale bar = 2 μm . **C.** Quantification of normalised pixels intensity (Relative Pixel Intensity; 0 to 1) across the Casparian strip in plants expressing CASP1-GFP. The plots are showing the intensity profile for individual replicates ($n \geq 11$), the mean value (black line) and the 95% confidence interval (grey interval). Each replicate corresponds to the quantification of one picture containing a Casparian strip segment of approximately 45 μm long. The pictures were generated at the 15th cells after the onset of elongation from at least 6 individual plants per genotype or treatment. Letters indicate statistically significant differences between genotypes for the intensity values comprised between the dashed lines using an ANOVA and Tukey's test as *post hoc* analysis ($p < 0.01$).

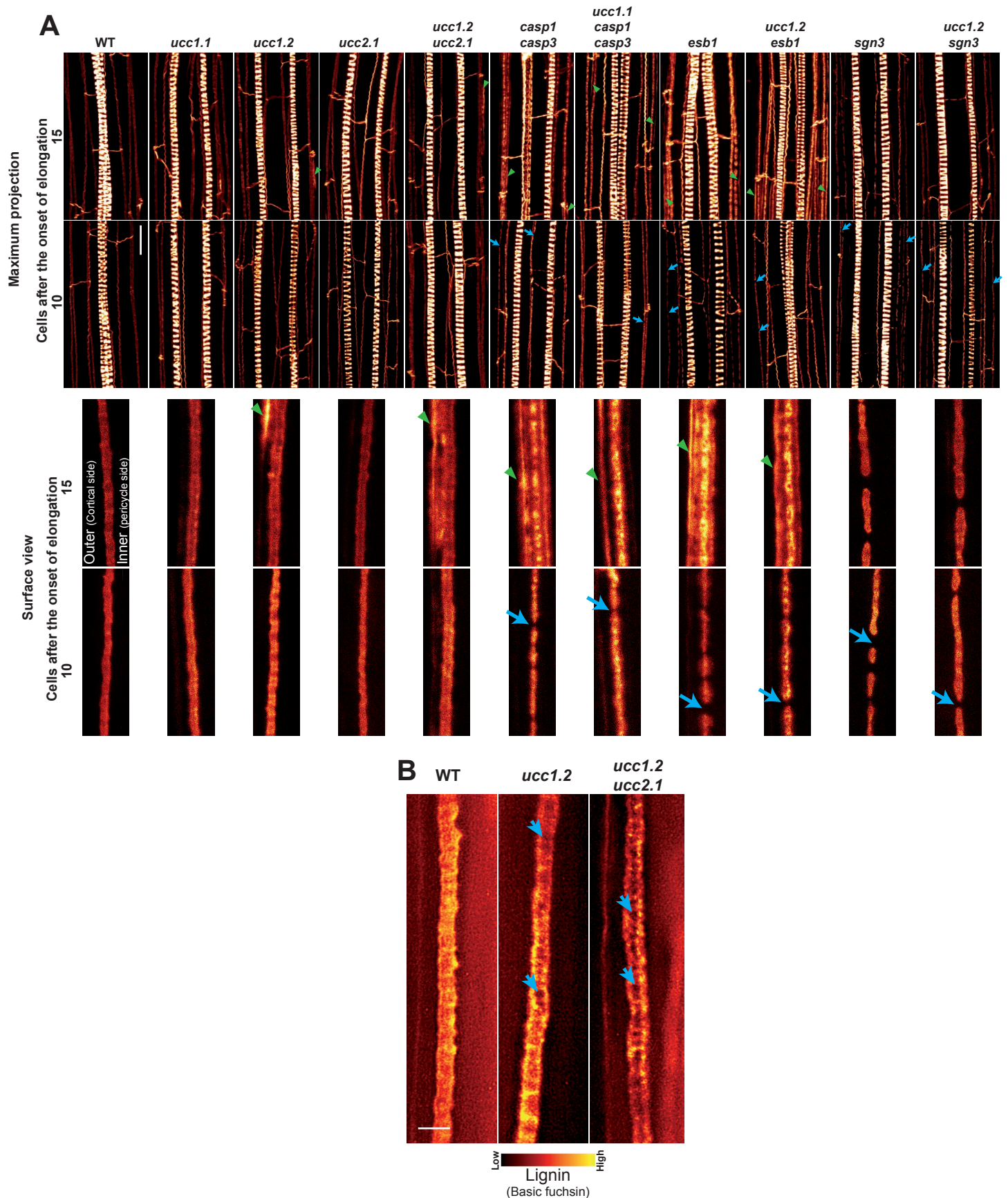


Figure S4. UCC1 and UCC2 are necessary for the central lignification of the Casparian strip. Related to Figure 4.

A. Figures show the maximum intensity projection (upper panel) of confocal sections of plant roots and surface views of CS (lower panel) stained with basic fuchsin for visualising lignin. Pictures were taken using cleared roots at the 10th and 15th cells after the onset of elongation of wild type plants (WT), and a collections of mutants: *ucc1.1*, *ucc1.2*, *ucc2.1*, *ucc1.2 ucc2.1*, *caspl caspl*, *ucc1.1 caspl caspl*, *esb1*, *ucc1.2 esb1*, *sgn3* and *ucc1.2 sgn3*. Spiral structures in the centre of the root are the xylem. Blue arrows indicate gaps in the lignin deposition at the Casparian strip. Green triangles show ectopic lignification. Scale bar = 20 μ m for maximum projections. Scale bar = 2 μ m for surface views.

B. Structured illumination microscopy of a surface view of Casparian strip lignin stained with basic fuchsin in cleared roots of WT, *ucc1.2* and *ucc1.2 ucc2.1*. Blue arrows show lack of lignification in the central domain of the Casparian strip. Scale bar = 2 μ m.

Element	WT	esb1			sgn3			ucc2.1			ucc1.1			ucc1.2			ucc1.1sgn3			ucc1.2sgn3.3			ucc1.1esb1			ucc1.2esb1			ucc1.2ucc2.1		
		Mean ± SD	Mean ± SD	p-value	Mean ± SD	Mean ± SD	p-value	Mean ± SD	Mean ± SD	p-value	Mean ± SD	Mean ± SD	p-value	Mean ± SD	Mean ± SD	p-value	Mean ± SD	Mean ± SD	p-value	Mean ± SD	Mean ± SD	p-value	Mean ± SD	Mean ± SD	p-value	Mean ± SD	Mean ± SD	p-value			
Li	0.11 ± 0.01	0.14 ± 0.01	2.1E-08	0.15 ± 0.02	1.1E-07		0.11 ± 0.01	3.3E-01		0.13 ± 0.01	9.3E-01		0.12 ± 0.01	4.2E-04		0.14 ± 0.02	7.6E-06		0.15 ± 0.01	1.9E-09		0.14 ± 0.01	1.3E-08		0.14 ± 0.01	6.8E-07		0.13 ± 0.02	6.5E-03		
B	65.14 ± 3.30	72.04 ± 4.41	9.1E-04	64.03 ± 5.53	5.9E-01		69.12 ± 4.24	3.1E-02		74.75 ± 5.00	7.9E-05		67.98 ± 4.30	1.2E-01		71.03 ± 10.05	9.6E-02		68.56 ± 7.14	1.2E-01		63.75 ± 3.61	3.8E-01		59.28 ± 4.33	3.2E-03		61.56 ± 5.00	7.5E-02		
Na	7018.04 ± 584.97	9015.89 ± 767.67	3.8E-06	9078.37 ± 799.61	3.5E-06		7081.35 ± 801.72	8.4E-01		7619.27 ± 836.58	7.9E-02		7437.08 ± 653.06	1.5E-01		10049.03 ± 1600.53	2.5E-05		8594.43 ± 628.46	2.0E-06		8629.91 ± 436.91	1.6E-06		9222.20 ± 463.35	2.5E-08		8669.27 ± 865.04	9.3E-05		
Mg	2979.44 ± 57.30	3111.87 ± 76.53	3.6E-04	2982.05 ± 58.66	9.2E-01		3161.74 ± 115.59	6.8E-01		3144.32 ± 89.25	1.1E-04		3002.79 ± 85.41	4.8E-01		2966.03 ± 77.54	6.7E-01		3101.96 ± 72.32	9.2E-04		3026.98 ± 79.52	1.4E-01		2951.54 ± 105.05	4.7E-01		3044.64 ± 96.97	3.6E-03		
P	21520.59 ± 864.21	21936.86 ± 1167.83	3.8E-01	21399.07 ± 1676.84	8.4E-01		23074.83 ± 657.36	2.6E-04		23630.50 ± 1472.38	1.0E-03		24688.07 ± 1533.57	2.1E-05		22676.96 ± 1654.24	6.6E-02		22440.81 ± 1244.45	9.2E-02		24186.78 ± 1679.71	3.0E-04		24679.85 ± 1483.26	1.6E-05		24867.09 ± 768.28	3.4E-08		
S	15553.96 ± 833.67	16932.37 ± 1109.73	4.2E-07	17309.21 ± 588.08	6.6E-05		16883.46 ± 409.35	2.6E-04		16785.15 ± 600.05	1.3E-03		16625.63 ± 629.99	4.5E-03		17857.27 ± 480.26	5.3E-07		17510.79 ± 800.07	2.4E-05		19755.62 ± 581.02	1.3E-10		18657.41 ± 555.65	1.2E-08		17377.82 ± 829.98	1.1E-04		
K	77759.28 ± 2915.45	87147.58 ± 4155.35	3.3E-06	64064.70 ± 2636.41	2.0E-09		78925.57 ± 4475.87	5.0E-01		80474.03 ± 3742.59	8.7E-02		81133.74 ± 3002.28	2.0E-02		67342.75 ± 3476.22	9.5E-07		72141.51 ± 3123.34	1.1E-03		69933.09 ± 2848.20	1.2E-07		63052.01 ± 4192.43	3.7E-08		72225.48 ± 2545.79	2.6E-04		
Ca	8059.54 ± 299.72	7425.74 ± 219.58	4.0E-05	8114.52 ± 562.32	7.9E-01		8176.90 ± 361.28	4.4E-01		8362.83 ± 350.28	5.2E-02		8150.55 ± 362.33	5.5E-01		8211.02 ± 603.54	4.9E-01		8505.45 ± 506.64	6.1E-04		7167.76 ± 323.14	6.7E-06		7229.80 ± 322.73	1.2E-05		7773.89 ± 382.88	8.0E-02		
Mn	349.57 ± 8.84	356.24 ± 8.80	1.1E-01	385.08 ± 19.76	6.2E-05		353.17 ± 14.01	5.0E-01		364.31 ± 13.59	1.0E-02		360.03 ± 7.64	1.1E-02		381.73 ± 30.27	4.7E-03		398.98 ± 24.44	5.9E-06		347.75 ± 11.61	7.0E-01		338.40 ± 15.67	6.5E-02		346.29 ± 16.67	5.9E-01		
Fe	178.05 ± 12.58	160.21 ± 6.48	8.7E-04	177.32 ± 12.87	9.0E-01		167.57 ± 16.13	1.2E-01		155.96 ± 10.16	4.1E-04		155.43 ± 9.05	2.1E-04		159.33 ± 13.61	5.0E-03		154.35 ± 9.94	3.1E-04		148.86 ± 5.81	3.0E-06		157.73 ± 17.15	7.4E-03		159.83 ± 15.88	1.1E-02		
Co	0.26 ± 0.04	0.23 ± 0.04	9.6E-02	0.22 ± 0.03	2.6E-02		0.20 ± 0.03	1.4E-03		0.19 ± 0.03	3.4E-04		0.20 ± 0.04	2.0E-03		0.17 ± 0.03	5.3E-05		0.20 ± 0.04	3.1E-04		0.25 ± 0.02	4.8E-01		0.23 ± 0.03	6.8E-02		0.15 ± 0.01	1.8E-07		
Ni	0.10 ± 0.02	0.20 ± 0.09	2.6E-03	0.20 ± 0.10	3.9E-03		0.16 ± 0.17	2.3E-01		0.17 ± 0.09	2.1E-02		0.20 ± 0.09	1.8E-03		0.16 ± 0.09	5.1E-02		0.15 ± 0.08	5.4E-02		0.16 ± 0.05	7.4E-04		0.13 ± 0.03	6.8E-03		0.19 ± 0.16	2.3E-02		
Cu	2.09 ± 0.17	2.21 ± 0.10	7.1E-02	2.31 ± 0.14	5.9E-03		2.25 ± 0.21	8.0E-02		2.22 ± 0.16	1.0E-01		2.21 ± 0.06	5.5E-02		2.31 ± 0.26	4.2E-02		2.40 ± 0.12	1.3E-04		2.24 ± 0.11	3.1E-02		2.35 ± 0.34	4.9E-02		2.12 ± 0.15	7.2E-01		
Zn	293.02 ± 27.65	252.98 ± 25.86	3.6E-03	268.22 ± 24.79	4.9E-02		284.04 ± 25.48	4.6E-01		282.03 ± 24.62	3.6E-01		282.40 ± 24.47	3.8E-01		252.59 ± 37.79	1.4E-02		246.48 ± 26.43	5.5E-04		278.17 ± 19.99	1.9E-01		301.20 ± 30.13	5.3E-01		264.13 ± 23.52	2.2E-02		
As	0.30 ± 0.02	0.45 ± 0.18	1.6E-02	0.51 ± 0.16	7.0E-04		0.34 ± 0.08	2.6E-01		0.33 ± 0.06	1.3E-01		0.35 ± 0.05	1.6E-03		0.58 ± 0.25	2.6E-03		0.37 ± 0.03	1.2E-04		0.40 ± 0.07	8.6E-04		0.41 ± 0.05	1.0E-05		0.41 ± 0.05	2.2E-05		
Se	0.02 ± 1.02	0.03 ± 1.73	9.9E-01	-0.03 ± 0.92	9.0E-01		0.19 ± 0.38	6.3E-01		0.01 ± 0.82	9.7E-01		0.00 ± 0.26	9.4E-01		-0.15 ± 0.34	6.2E-01		-0.12 ± 1.13	8.1E-01		0.15 ± 0.34	7.2E-01		0.23 ± 0.73	6.2E-01		-0.31 ± 0.54	3.7E-01		
Rb	1.42 ± 0.07	1.24 ± 0.06	1.1E-05	1.16 ± 0.03	7.1E-09		1.41 ± 0.07	8.9E-01		1.42 ± 0.06	7.6E-01		1.43 ± 0.04	6.9E-01		1.22 ± 0.06	5.3E-06		1.26 ± 0.03	1.4E-05		1.22 ± 0.04	8.0E-07		1.17 ± 0.07	3.6E-07		1.36 ± 0.10	2.0E-02		
Sr	4.11 ± 0.15	4.00 ± 0.16	1.4E-01	4.52 ± 0.08	4.5E-07		4.08 ± 0.15	6.1E-01		4.16 ± 0.13	4.5E-01		4.12 ± 0.17	9.3E-01		4.63 ± 0.24	1.7E-05		4.40 ± 0.20	1.1E-05		3.82 ± 0.18	1.0E-03		3.95 ± 0.09	9.1E-03		4.28 ± 0.14	1.7E-02		
Mo	3.61 ± 0.33	4.61 ± 0.52	7.4E-05	3.84 ± 0.31	1.4E-01		3.85 ± 0.40	1.7E-01		3.60 ± 0.57	9.3E-01		3.85 ± 0.34	1.3E-01		4.59 ± 1.13	1.7E-02		3.76 ± 0.28	2.8E-01		4.20 ± 0.36	4.0E-04		4.31 ± 0.27	6.7E-05		5.07 ± 0.49	3.7E-07		
Cd	0.07 ± 0.01	0.05 ± 0.01	3.1E-05	0.04 ± 0.005	5.5E-06		0.07 ± 0.01	9.3E-01		0.07 ± 0.01	1.2E-01		0.07 ± 0.01	4.5E-01		0.10 ± 0.13	4.3E-01		0.06 ± 0.04	8.9E-01		0.07 ± 0.02	9.9E-01		0.07 ± 0.02	7.1E-01		0.06 ± 0.01	3.3E-01		

Table S1. UCC1 and UCC2 are required for maintaining ion homeostasis in the shoot. Related to Figure 1.

Elemental content in shoot of *ucc2.1*, *ucc1.1*, *ucc1.2*, *ucc1.2 ucc2.1*, *esb1*, *ucc1.1 esb1*, *ucc1.2 esb1*, *sgn3*, *ucc1.2 sgn3* and *ucc1.1 sgn3* mutants compared to WT grown in agar plates (long day, n=10). Elements concentration were determined by ICP-MS. Data are presented as mean ± standard deviation (SD). *t-tests* were performed to determine the significant differences to WT and the corresponding *p*-values are presented.