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Supplementary Materials for

Endoreplication mediates cell size control via mechanochemical signaling from cell wall

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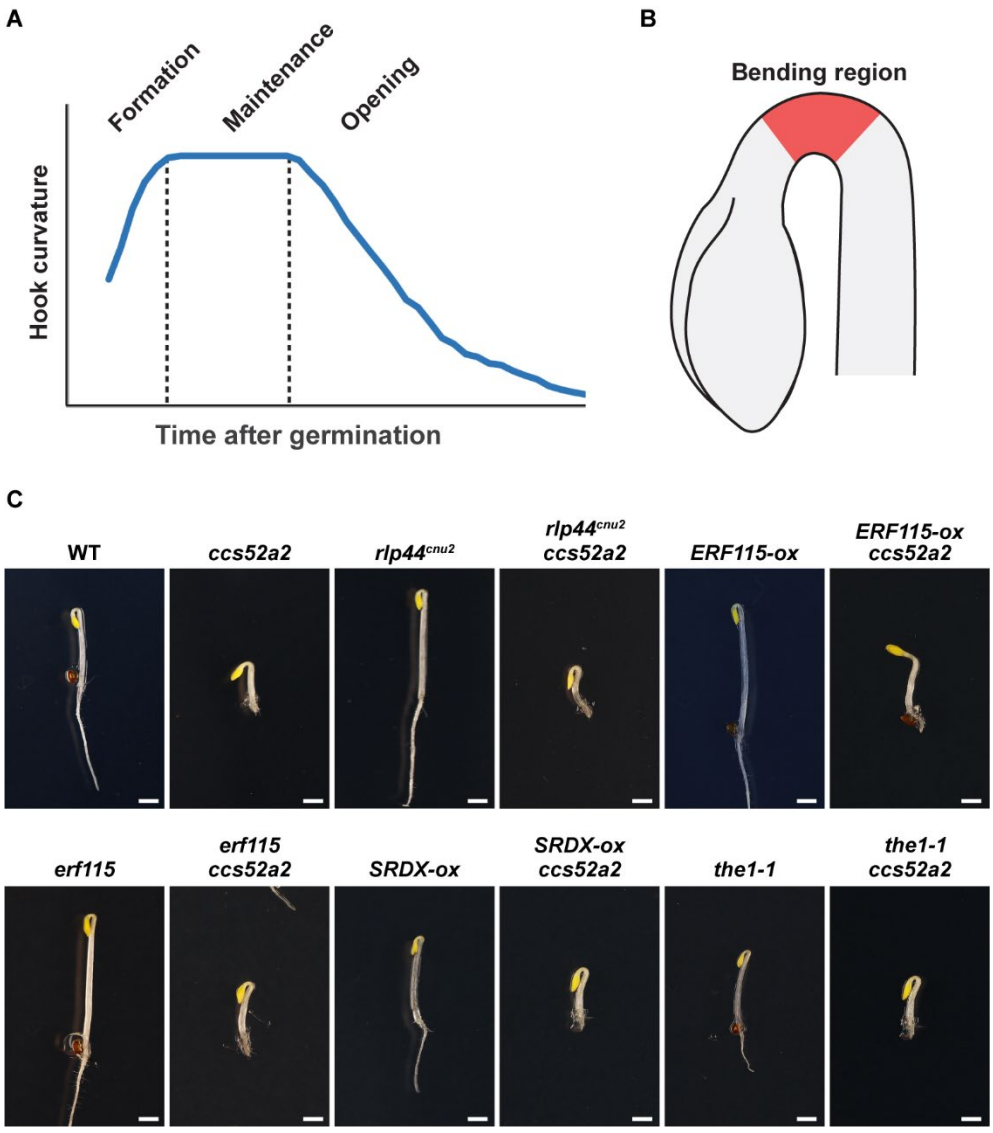

Fig. S1. (A) A schematic representation of phases of apical hook development showing formation, maintenance, and opening phase. **(B)** A schematic representation of apical hook in WT. The bent region with differential growth is highlighted. **(C)** Seedling phenotypes of different genotypes used in the study. Scale bars: 1 mm. Note that apical hook defects do not necessarily relate to global defects in hypocotyl growth and shape. For instance, *xxt1 xxt2* mutants have normal hypocotyl but abnormal hooks. Related to Fig. 1.

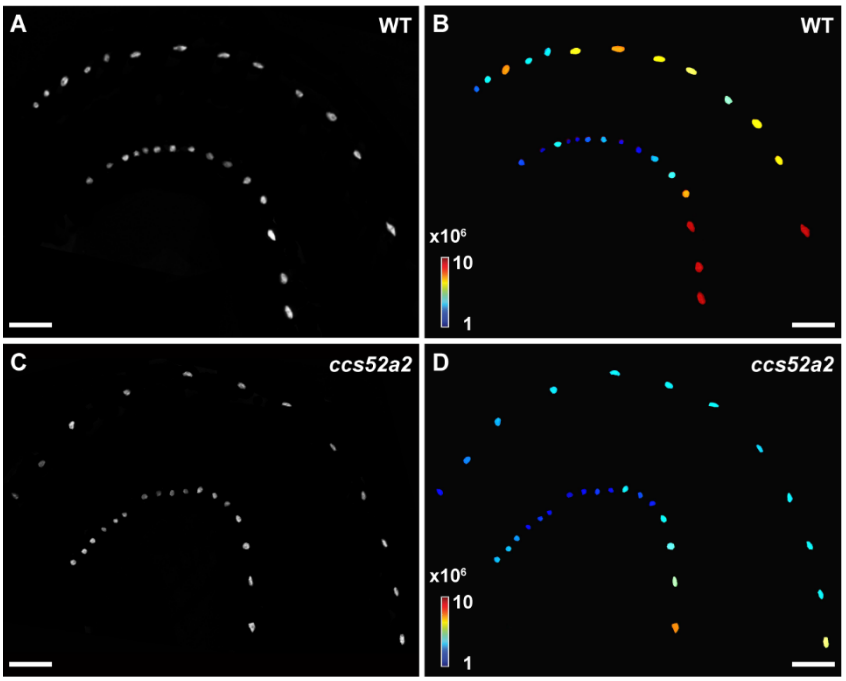

Fig. S2. *ccs52a2* mutant showed reduced endoreplication asymmetry at hook region. Related to Fig. 1.

(A) Representative DAPI stained nuclei on outer and inner side of the WT seedling. **(B)** Heatmap of DAPI stained nuclei showed in (A) based on intensity. **(C)** Representative DAPI stained nuclei on outer and inner side of the *ccs52a2* seedling. **(D)** Heatmap of DAPI stained nuclei showed in (C) based on intensity. Scale bar: 50 µm.

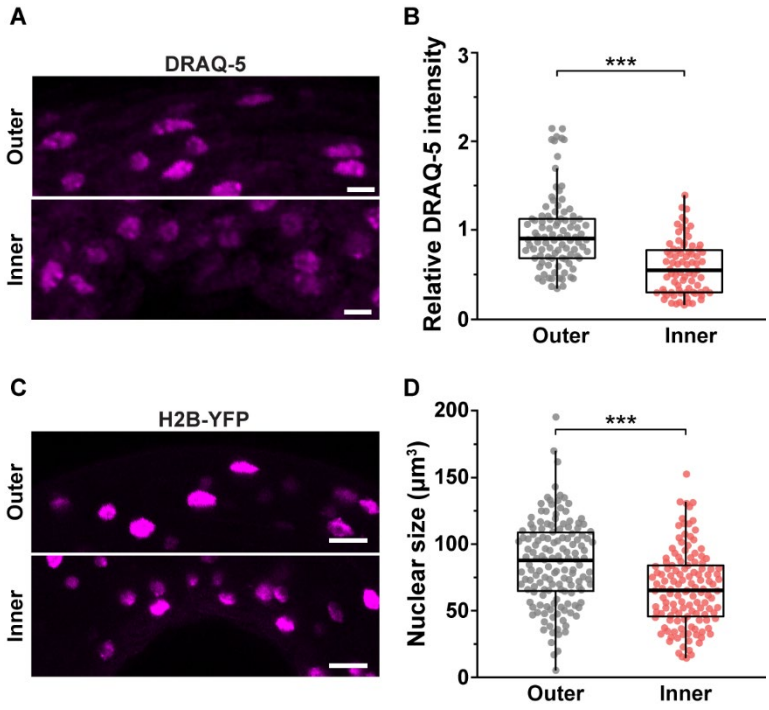

Fig. S3. Endoreplication asymmetry at hook region. Related to Fig. 1.
(A) Representative images of DRAQ5 stained epidermal cells on the inner and outer side overlapping the bent region of the apical hook in WT and **(B)** Corresponding plot of DRAQ5 intensity (n=7, outer: 96 nuclei, inner: 77 nuclei). Scale bar: 10 µm. **(C)** Representative images of nuclear size monitored by H2B-YFP signal in epidermal cells on the inner and outer side overlapping the bent region of the apical hook in WT and **(D)** Corresponding plot of nuclear size (n=11, outer: 146 nuclei, inner: 129 nuclei). Scale bar: 20 µm. Thick lines in the boxes indicate medians. Statistical significance was calculated using Student's t-test (unpaired, two-tailed), and is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

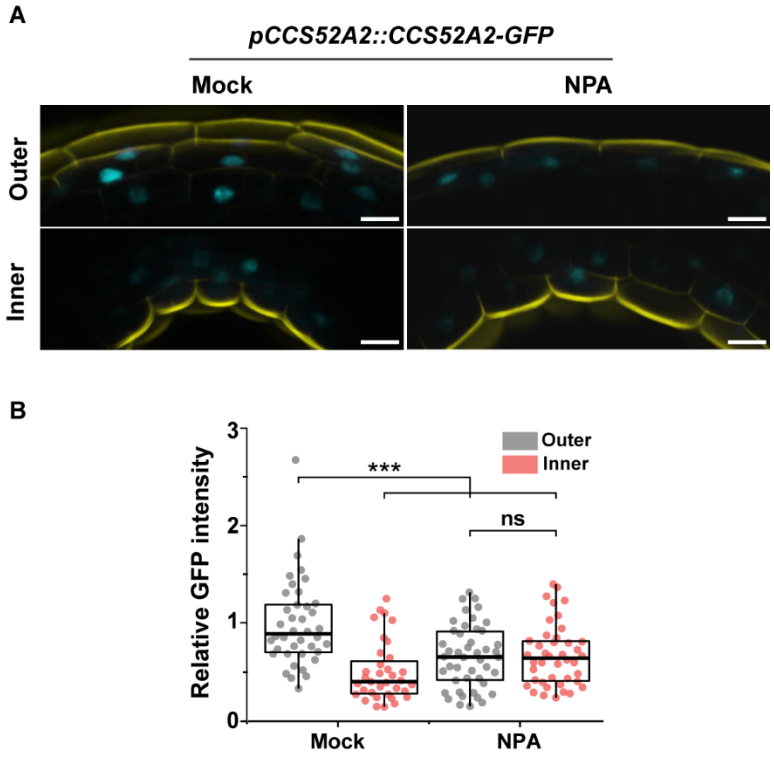

Fig. S4 NPA treatment abolished the asymmetric expression of CCS52A2.
(A) Representative confocal images of mock and NPA treated *pCCS52A2::CCS52A2-GFP* seedlings. Cyan signal represents CCS52A2-GFP. PI staining was performed to visualize the cell wall (yellow). Scale bars: 20 µm. **(B)** Quantitative analysis of the CCS52A2 accumulation in the outer and inner side of mock and NPA treated *pCCS52A2::CCS52A2-GFP* in the wild-type background (Mock: outer: n=40, inner: n=35; NPA: outer: n=46, inner: n=42). Thick lines in the boxes indicate medians. Statistical significance was calculated using Student's t-test (unpaired, two-tailed), and is indicated as follows: ns, not statistically significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

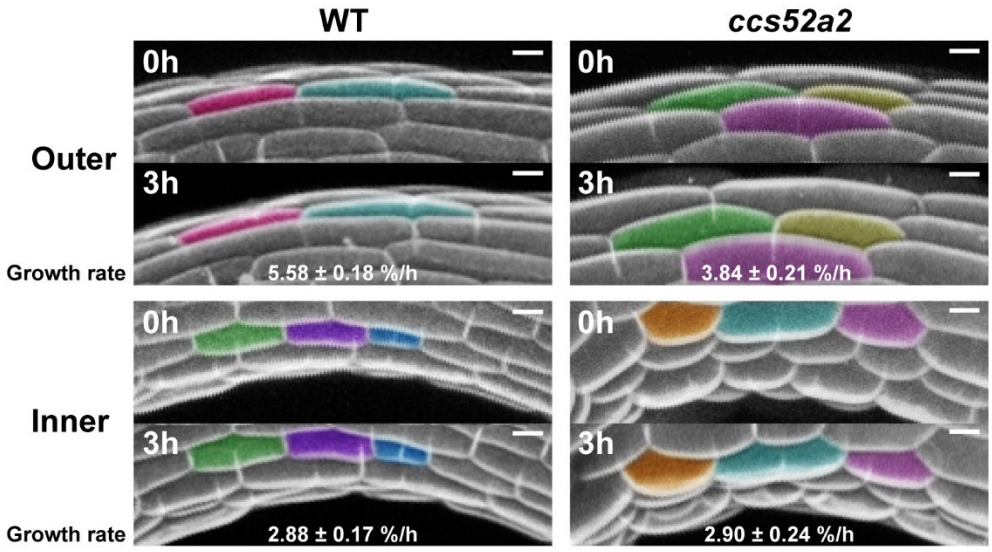

Fig. S5. Endoreplication mediates in control of differential cell elongation. Related to Fig. 2. Representative micrographs of the dark-grown seedlings of the genotypes used for calculating cell growth rate (in length, along the hypocotyl axis) on the outer and inner sides of the hook in Figure 2A. In total, cell lengths of ca 70-85 epidermal cells on outer and inner side (from 5-6 seedlings for each genotype) were measured at 0 and 3 hours (see Fig. 2A for details). This image shows representative cells at 0h and 3h (the same color indicates the same cell) and average growth rate reflecting percent elongation per hour is indicated. Scale bar: 10 µm.

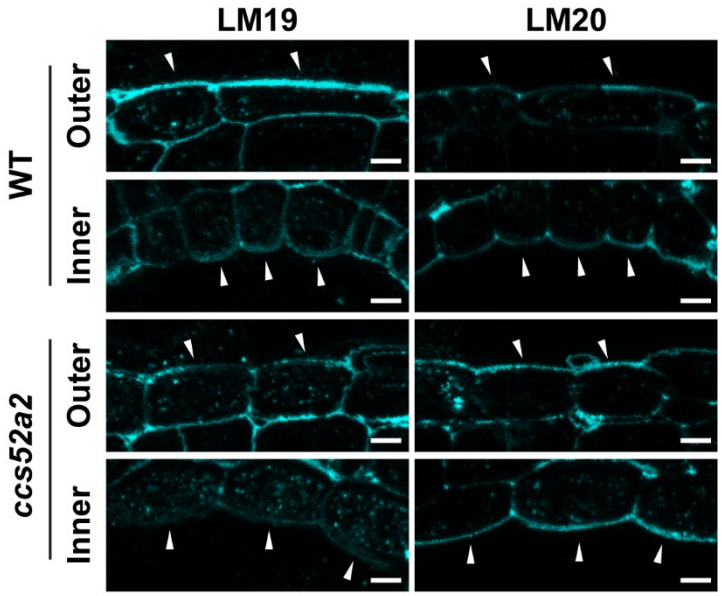

Fig. S6. Enhanced HG methylesterification underlies hook defects in endoreplication mutant *ccs52a2*. Related to Fig. 3.
Representative photographs of immunolabeled longitudinal epidermal cell walls with LM19 and LM20 of the lines used in Figure 3C. White arrowheads indicate the outer longitudinal walls of the representative cells. Scale bar: 10 μm.

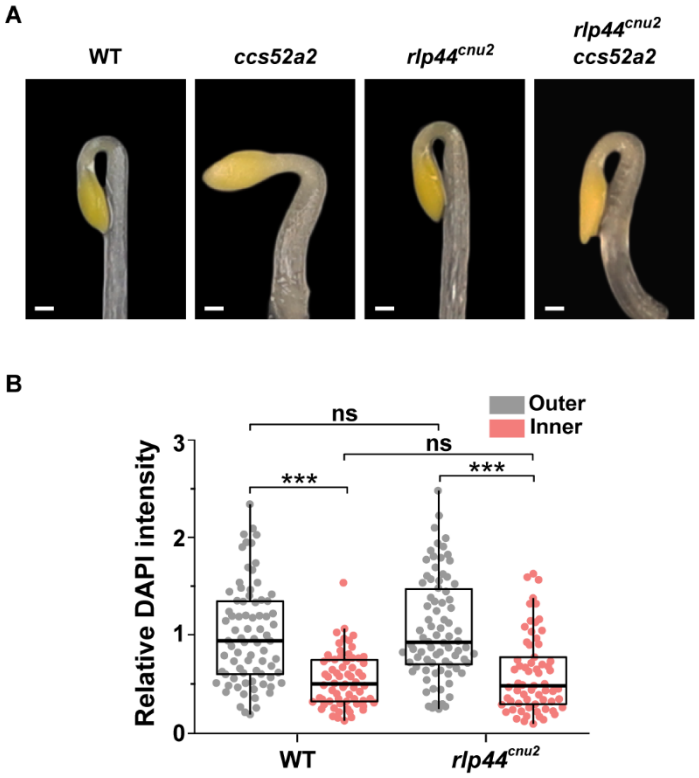

Fig. S7. Enhanced HG methylesterification underlies hook defects in endoreplication mutant *ccs52a2*. Related to Fig. 3.

(A) Representative photographs of the dark-grown seedlings of the lines used in Figure 3D. Scale bar: 200 µm. **(B)** DAPI intensity of epidermal cells on the inner and outer side overlapping the bent region of the apical hook in WT (n=6, outer: 78 nuclei, inner: 66 nuclei) and *rlp44*^{cnu2} (n=6, outer: 82 nuclei, inner: 65 nuclei). Thick lines in the boxes indicate medians. Statistical significance was calculated using Student's t-test (unpaired, two-tailed), and is indicated as follows: ns, not statistically significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

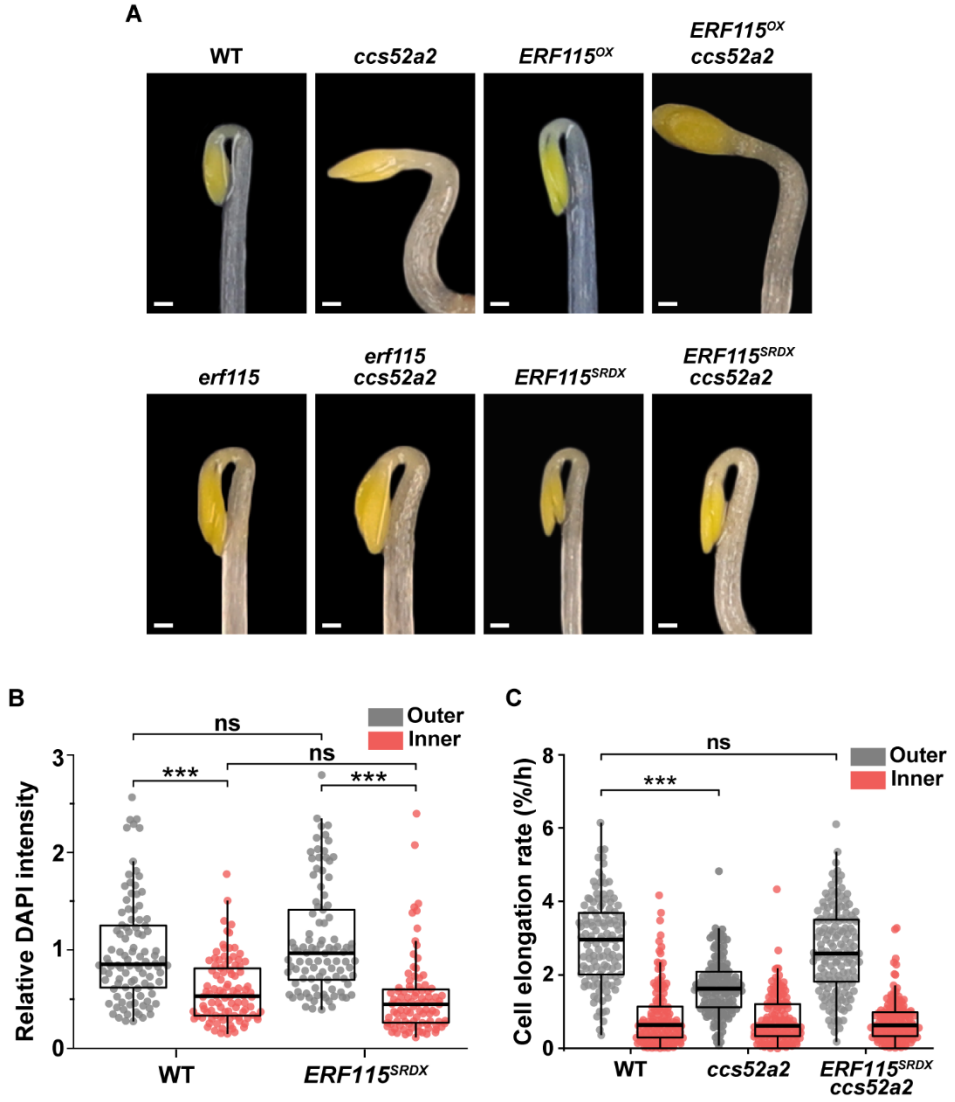

Fig. S8. Defects in endoreplication are mediated via *ERF115* transcription factor to modulate hook development. Related to Fig. 4.

(A) Representative photographs of dark-grown seedlings of the lines used in Figure 4. Scale bar: 200 µm. **(B)** DAPI intensity of epidermal cells on the inner and outer side overlapping the bent region of the apical hook in WT (n=6, outer: 100 nuclei, inner: 98 nuclei) and *ERF115*^{SRDX} (n=6, outer: 97 nuclei, inner: 93 nuclei). **(C)** Cell elongation rate of epidermal cells (%/h) at 0-400 µm from the shoot apex in the WT (n=5, outer: 120 cells, inner: 128 cells), *ccs52a2* (n=5, outer: 137 cells, inner: 136 cells) and *ERF115*^{SRDX} *ccs52a2* (n=5, outer: 156 cells, inner: 143 cells). Thick lines in the boxes indicate medians. Statistical significance was calculated using Student's t-test (unpaired, two-tailed), and is indicated as follows: ns, not statistically significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

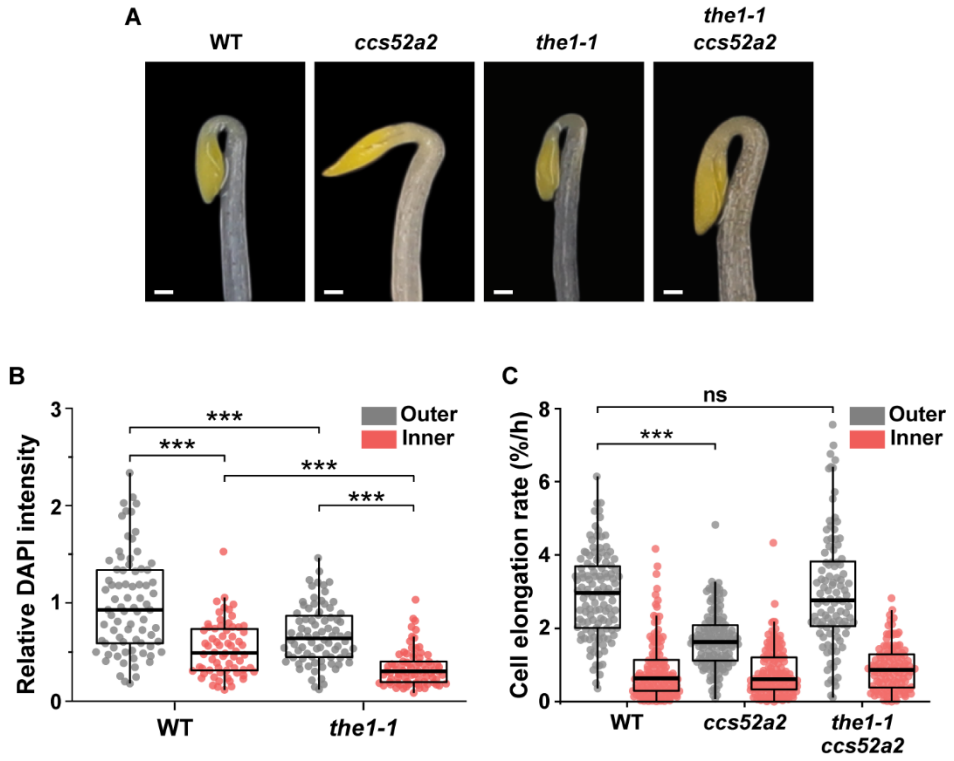

Fig. S9. THESEUS1 receptor kinase monitors cell wall changes in response to reduced endoreplication. Related to Fig. 5.

(A) Representative photographs of dark-grown seedlings of the lines used in Figure 5A. Scale bar: 200 µm. **(B)** DAPI intensity of epidermal cells on the inner and outer side overlapping the bent region of the apical hook in WT (n=6, outer: 78 nuclei, inner: 66 nuclei) and *the1-1* (n=7, outer: 82 nuclei, inner: 79 nuclei). The wild-type control is identical to Fig. S6B. **(C)** Cell elongation rate of epidermal cells (%/h) at 0-400 µm from the shoot apex in the WT (n=5, outer: 120 cells, inner: 128 cells), *ccs52a2* (n=5, outer: 137 cells, inner: 136 cells) and *the1-1 ccs52a2* (n=5, outer: 104 cells, inner: 118 cells). The wild-type control and *ccs52a2* data are identical to Fig. S8C. Thick lines in the boxes indicate medians. Statistical significance was calculated using Student's t-test (unpaired, two-tailed), and is indicated as follows: ns, not statistically significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table S1. Primers were used in this study.

Name	Sequence	Experiment
ERF108-EXP-F	CCGGTTCAGCTGTGACTAAAG	Expression
ERF108-EXP-R	CTGAGTTCCAACATTTTCGGG	Expression
ERF113-EXP-F	CAAGGCCCTACTACCACCACAA	Expression
ERF113-EXP-R	GGTCGAGGAGGAGGTGAGTTC	Expression
ERF114-EXP-F	AGAACTTGTTCCCGGTCTTCTCG	Expression
ERF114-EXP-R	AGTCAAGGCCGAGACCATAACAC	Expression
ERF115-EXP-F	GGAAACCAAAGCAGCTCTCA	Expression
ERF115-EXP-R	GCAGCTTCAGCAGTCTCAAA	Expression
UBQ10-EXP-F	ATCACCCTTGAAGTGGA	Expression
UBQ10-EXP-R	GAAACCACCACGAAGAC	Expression
ccs52a2-1_LP	GGCATGGAGCTCATCTGTTC	Genotyping
ccs52a2-1_RP	TCAGGTATTTCATTTCCGCTG	Genotyping
LBb1.3	ATTTTGCCGATTTCGGAAC	Genotyping
erf115_LP	CCAAATGCAAGGGTTACAAAC	Genotyping
erf115_RP	TGGCGAATTCAGGAAATTATG	Genotyping
rlp44^{cnu2}_F	AATCTACAAACTCTCACTCAC	Genotyping
rlp44^{cnu2}_R	CTGACCGGATAATTCGTTATC	Genotyping
the1-1_F	TGGTGTTCACAAAATCATTACTTG	Genotyping
the1-1_R	TGAAGGGTTTAACGCCAAAG	Genotyping
SRDX_R	CCCAAACGGAGTTCTAGATCCAG	Genotyping