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SUPPLEMENTARY INFORMATION

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Complete substitution of a secondary cell wall with a primary cell wall in *Arabidopsis*

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Supplementary Methods

Plasmid constructions. For the screening, a mixture of ca. 30 TF Gateway entry clone was transferred into the pDEST_NST3p_VP16_HSP_GWB5 plasmid[22] by a Gateway LR reaction to prepare one pool. Most binary vectors used in this study are based on pGWB vector[38]. Most gene constructs in this study include *Arabidopsis* HSP18.2 terminator which is very efficient to terminate transcription[39]. We produced 10 pools which include ca. 300 TFs. For transient gene expression in protoplast, CDS fragments of ERFs in pDONR207 (entry clones) were transferred by the LR reaction into each of pDEST35SHSP and pDEST35SVP16HSP[40,41] and each coding region was inserted into SmaI-site of p35SSRDXG[42] for the expression of ERF035 fused with SRDX peptide to act as a strong repressor[18]. For overexpression of ERFs, each entry clone was transferred by the LR reaction into pDEST_35S_HSP_GWB5 binary vector including CaMV35S promoter and Arabidopsis HSP18.2 terminator. For the expression of ERFs without fusion of VP16, each entry clone was transferred by the LR reaction to pDEST_NST3p_ HSP_GWB5 vector which was prepared from pDEST_HSP_GWB5 vector containing Arabidopsis HSP18.2 terminator by inserting *NST3* promoter. For the phenotypic analysis of chimeric repressor of ERF035, ca. 3 kb *ERF035* promoter was amplified using gene

specific primers listed in Table S6 and cloned into pDONR_P4P1R vector by Gateway BP reaction. The resultant plasmid and entry clone of ERF035 were assembled and transferred into R4pGWB5_SRDX_HSP vector[43] by multisite Gateway LR reaction. For promoter analysis, cloned ERFs promoter into pDONR_P4P1R using primers listed in Supplemental Table 6 and pENTR^{TM}-gus (Thermo Fisher Inc.) were assembled and transferred into R4pGWB5_SRDX_HSP vector. *CESA1, 3, 6* promoters were cloned into pDONR_P4P1R vector by the BP reaction and transferred into R4L1pDEST_GLHSP[43] by the LR reaction for reporter constructs. For the yeast one-hybrid assay, approximately 1.2 kb of the *CESA1* were amplified by the primers described in Supplementary Table 6, cloned into pENTR-5´-TA TOPO vector (Thermo Fisher Inc.) and then recombined with the pMW2 and pMW3 vectors to generate HIS3 and LACZ reporters.

Phenotypic screening of the *nst1 nst3* transgenic plants. The *nst1-1 nst3-1* double mutant[12] of *Arabidopsis thaliana* ecotype Columbia-0 plants were grown for two months in soil at 22 °C under a 16 h/8 h (light/dark) photoperiod. We prepared 10 sets of approximately 30 transcription factor (TF) genes (total ca. 300 genes) which represented at least one of each clade of all TF families in the *Arabidopsis* genome. Each plasmid mixture was transformed into Arabidopsis plants using the floral dip method[44]. For

phenotypic screening, transgenic T1 plants selected on Murashige and Skoog agar media containing hygromycin were transferred to soil and grown for 5 weeks. we grew 153 *NST3pro:TF nst1 nst3* transgenic plants for each set, checked stem brittleness via a bending test and examined cross-sections of inflorescence stem of all plants. In those plants that deviated from wild-type characteristics, the transgene was identified by PCR followed by sequencing. To confirm that the identified gene induced altered cell walls, the individual *NST3pro:TF-VP16* constructs were introduced into the *nst1 nst3* plants and phenotypes were examined in multiple T1 plants. All individual transgenic plants were selected and grown in same manner as described above.

Cell wall analysis. Fine powder of AIR purified from inflorescence stem of 8-week-old plants was treated with α-amylase and was subjected to cell wall analysis including monosaccharide composition analysis, lignin analysis, FT-IR analysis, and saccharification analysis. Cell wall hydrolysis and monosaccharide analysis were performed as described previously[45]. Saccharification analyses were conducted as previously described[22]. In brief, cellulose in AIR powder (2-3 mg) was digested in 1 mL cellulose solution containing Celluclast 1.5L (0.2FPU) and Novozyme 188 (0.4CBU) (Novozymes Inc.) in 5mM citrate buffer (containing 0.02% w/v sodium azide) at 50°C

for 24hrs with shaking at 210 rpm. After heating cellulose at 95˚C for 10 min, released glucose was quantified with a commercial glucose test kit (Glucose CII-Test kit, Wako Inc.) by following manufacturer's instructions. Acid insoluble lignin was determined gravimetrically as the residue remaining after secondary acid hydrolyzation with sulfuric acid, as previously described[22]. FT-IR analysis was performed using a IRAffinity-1S (Shimadzu Inc., Japan). For each sample, 40 scans were performed to increase signal-to-noise ratio. Maximum and minimum absorbance of spectrum in the range from 900 nm to 1800 nm were normalized to 100 and 0, respectively. Quantitative polysaccharide analysis using antibodies was performed as described in Leroux et al.[46] Crystalline cellulose was determined as the glucose amount in TFA insoluble residue based on Updegraff method[47].

Tensile test. A rehydrated explant was stretched at speed of 2 mm/min, and terminated when the final force reached 100 g. The force-extension curve was automatically recorded and used to calculate the stiffness as follows. The mass (M) per unit length (*l*) of each dried specimen (M/*l*, mg/mm) was measured after the tensile test. The stiffness (the ratio of force to extension) was obtained by the application of Cleland's method for the total compliance calculation as follows[49,50]; Stiffness = F/ΔL. F is force (N). ΔL is $L_{1N} - L_{0N,}$

where L_{1N} is the distance between upper and lower clump fixing the inflorescence tissue segment at 1 N of force and L_{0N} is the initial distance of these clumps at 0 N. Initial distance is obtained by extending the slope of the force-extension curve back to the abscissa.

Immunolabelling and cellulose staining. Briefly, micro-sliced stem sections were fixed in FAA solution containing 50% ethanol, 3% formaldehyde, and 5% acetic acid and stored until use. The fixed stem sections without treatments for the selective removal of the homogalacturonan epitope, which potentially masks the effect of immunolabelling, were then rinsed with TBS, containing 20 mM Tris-HCl and 150 mM NaCl, and then co-incubated with carbohydrate specific antibody or binding protein (CBM3a, Plant probes Inc.). Signals were detected by the two-step detection method with Alexa Fluor® 488-fused Anti-Rat IgG (Thermo Fisher Scientific Inc.), Anti-Mouse IgG (Thermo Fisher Scientific Inc.), or Anti-His tag (Medical & Biological Laboratories Inc.) as secondary antibodies and were visualized by a fluorescent microscope. For cellulose visualization, the sections were stained with Calcofluor-White, Congo-Red solution[29], and Pontamine Fast Scarlet 4B (S4B)[30] and visualized with emission at 365 nm and excitation at 445/50

nm (for Calcofluor-White) or emission at 470/40 nm and excitation at 525/50 nm (for Congo-Red and S4B).

Details of TEM observation. Stems were fixed with 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M cacodylate buffer (pH7.4) at 4 °C overnight. After washing, the samples were post-fixed with 2% osmium tetroxide for 3 hr. Following the dehydration process, the samples were embedded in Quetol-651 resin (Nisshin EM Inc., Japan). 80 nm thick ultra-thin sections were prepared and stained with 2% uranyl acetate at room temperature for 15 min. The sections were secondary stained with lead stain solution (Sigma-Aldrich Inc.) at room temperature for 3 min after washing. Section-mounted grids were observed by a TEM (JEM-1400Plus, JEOL Inc., Japan) at an acceleration voltage of 80 kV.

Microscopic observation of root hair. Arabidopsis T2 plants (*35Spro:ERF035:HSPter* and vector control *35Spro:HSPter*), vertically grown for 2 weeks on MS agar media, were fixed in FAA solution and stained with 0.0002% (w/v) Calcofluor-white in 20 mM $NaPO_4$ buffer for 20 min. Stained roots were mounted in ClearSee Solution[51] [10% (w/v) Xylitol,

15% (w/v) Sodium Deoxycholate, 25% (w/v) Urea] and observed via confocal laser-scanning microscopy (LSM700, Carl Zeiss Inc.) equipped with Diode laser (Diode 405-5) and split filter (Main Beam Splitter 405/488/555/639). The integrated signal intensity from stained root cell wall of each plant was calculated with average curve of 100 individual data sets using Simpson's rule method.

Transient protoplast assays. Protoplasts were isolated from mesophyll cells grown for 4–5 weeks and transient transformation was performed as described previously[35]. For the reporter-effector analyses, effector, reporter, and internal control plasmids were co-transformed via the PEG/Ca^{2+} method, and the relative reporter activity was calculated as the value of firefly luciferase activity divided with the Renilla luciferase activity, which served as internal reference. The reporter activity value when the negative control effector was employed was set to 1. For cell wall regeneration analysis, protoplasts were transformed with plasmids as described above and incubated for 18 hrs in the buffer containing Gamborg's B5 basal salt (Nihon Pharmaceutical Inc.); Gamborg's B5 vitamin (Sigma Inc.), 250 mM mannitol, 200 mM glucose, 1.0 mg/L 2,4-D, 0.022 mg/L thidiazuron, 200 mg/L ampicillin, and 1.0 mg/L fungizone (Amphotericin B; Sigma Inc.), and 0.1% (v/v) EtOH. After the incubation, the protoplasts were washed with W5 buffer

125 (150mM NaCl, 125mM $CaCl_2$, 5mM KCl, 5mM MES, pH5.7) and cell wall regeneration
126 was visualized using a fluorescence microscope (Axioskop2 plus system; Zeiss Inc.) with
127 the filter set with the following specifications: excitation filter - 365 nm short pass;
128 dichroic mirror - 395 nm; emission filter - 400 nm long pass.

129

130 **Yeast one hybrid analyses.** The *CESA1* promoters followed by HIS3 and LacZ reporters
131 in, pMW2 and pMW3 respectively, were transformed into YM4271 and autoactivation
132 analysis as well as genotyping were carried out as described in Gaudinier et al.[16]. Yeast
133 one hybrid assays were performed by the mating-based method using the set of ERF
134 Group IIId and e TFs described in Li et al.[52] and performed as described previously[16].

135

136 **Electron Mobility Shift Assay (EMSA).** The coding sequence of ERF035 was inserted
137 into pMAL vector and fusion protein with maltose binding protein (MBP) was produced
138 in *E.coli* and purified. IRDye-labelled synthetic oligonucleotides were annealed and were
139 used for the DNA probe. A DNA–protein binding reaction was performed using the
140 Odyssey infrared EMSA Kit (LI-COR Inc.). The reaction contained 0.5 ng DNA, 10 mM
141 Tris, 50 mM KCl, 3.5 mM DTT, 0.25% Tween-20, 1 µg Poly dI-dC and 5% glycerol, and

142 unlabelled competitor. The reactions were incubated at room temperature for 20 min in
143 the dark and subjected to electrophoresis in a 5% native polyacrylamide gel containing
144 2.5% glycerol and Tris-borate-EDTA buffer. The signals were detected by the Odyssey
145 infrared imaging system (LI-COR Inc.).

146

147 **Cellulose analysis.** In order to determine the crystallinity and degree of polymerization
148 of the synthesized cellulose, 12-week-old aerial plant tissue was harvested (Main shoot
149 was harvested at 8 weeks so that the harvested samples at 12 weeks are secondary shoot),
150 air-dried, and ground to pass the #40 mesh (0.425 mm) on a Wiley Mini Mill (Thomas
151 Wiley). The ground tissue was then used to generate Alcohol Insoluble Residue (AIR) by
152 first suspending the ground tissue for 12 hours in 80% ethanol at room temperature with
153 constant shaking, and then recovering the tissue by centrifugation at 4000 × g for 15
154 minutes. The pellet was then re-suspended in absolute ethanol, vortexed vigorously and
155 then centrifuged at 4000 × g for 15 minutes. This was followed by two sequential washes
156 in acetone, where the pellet was re-suspended mixed thoroughly, and then collected by
157 centrifugation 4000 × g for 15 minutes. The final AIR pellet was then dried in a 50°C for
158 24 hours. The AIR tissue was then subject to a series of extractions, as previously
159 described[53,54]. Briefly, the AIR was subject to three sequential 1-hour 200mM sodium

chlorite extraction in a 70°C water bath in a fume hood. Following each reaction, a pellet was collected by centrifugation at 6000 × g for 15 minutes, while the supernatant was decanted and discarded. The pellet was then re-suspended in nanopure water and re-pelleted as above. This wash was repeated four times, discarding the supernatant after each wash. The pellet was then re-suspended in excess 50 mM sodium oxalate, and shaken on rotary shaker (100 rpm) at room temperature for 24 hours. The tissue was then concentrated by centrifugation at 6000 × g for 15 minutes, and the supernatant discarded. The pellet was then washed with nanopure water, pelleted as per above, re-suspended in 50mM sodium carbonate, and maintained on a rotary shaker at 100 rpm at room temperature for 24 hours. Again, the tissue was then concentrated by centrifugation at 6000 × g for 15 minutes, and the supernatant discarded. The pellet was then re-suspended in excess 4M KOH, and shaken on rotary shaker (100 rpm) at room temperature for 24 hours. The tissue was then concentrated by centrifugation at 6000 × g for 15 minutes, and the supernatant discarded, and then subject to three consecutive washes with nanopure water. The isolated alpha cellulose was then allowed to dry for 24 hours in a 50°C oven. The molecular weight distributions of the isolate cellulose derived from the wild-type, mutant and transgenic plant lines (four biological replicates, each with two technical replicates) were measured using gel permeation chromatography (GPC) coupled to a

multi angle light scattering detector (MALS; Dawn Helos-II, Wyatt Technologies, Santa Barbara, CA, USA). Roughly 5 mg of isolated cellulose was individually weighed into glass vials. Prior to dissolution, solvent exchange from water (Nanopure), to anhydrous ethanol to *N*,*N*-Dimethylacetamide (DMAC) was performed in series. In brief, the isolated cellulose was stirred for three days in water, followed by two days in ethanol and, finally two days in DMAC. At each solvent change, the solvent was removed by centrifugation for 20 minutes at 6000 × g. The final pellet of cellulose was then re-suspended in a 9% (w/v) lithium chloride in DMAC solution (LiCl/DMAC) at a ratio of 5 mg of cellulose per 1 mL of solvent. This suspension was then stirred for three days at room temperature, and stored at 4°C prior to GPC analysis. The cellulose in LiCl/DMAC was diluted 1:12 with 0.9% (w/v) lithium chloride in DMAC. Samples were filtered through a 0.45 µm nylon filter and run on an Agilent 1100 series GPC instrument equipped with two Styragel columns in series (Water Inc.; 5E, 4E) maintained at room temperature with an isocratic flow rate of 0.5mL/min of 0.9% LiCl/DMAC. Following separation, cellulose polymer size was estimated by multi-angle light scattering and processed to determine the average molecular weights using the ASTRA program (Wyatt Technologies Inc.). The crystallinity of the isolated cellulose was determined on a Bruker D8 Discover X-ray diffraction unit (configured in a theta-theta orientation) using wide-

196 angle diffraction in the transmission mode, where measurements were made with CuKα1
197 radiation (λ = 1.54Å), the X-Ray source fit with a 0.5mm collimator, and the scattered
198 photons collected by a general area array (GADDS) detector. The X-ray source was set
199 to a theta angle of 0°, while the GADDS detector was set to a theta angle of 17°, and the
200 diffraction intensities were counted at 0.1° increments between 4 and 40° Bragg angle.
201 Crystallinity was determined mathematically by fitting the diffraction data using the
202 method of Vonk[55].
203
204 **Observation of root growth.** Arabidopsis T2 plants (*ERF035pro::GUS* for control and
205 *ERF035pro::ERF035-SRDX*) were vertically grown for 3 weeks on MS medium
206 supplemented with 0.5% (w/v) or 4.0% (w/v) sucrose and hygromycin under 16h light/8h
207 dark condition. Similar experiments were performed on *cesa* mutants, cesa*1* (*rsw1-1*[19])
208 and *cesa3* (*ixr1-1* and *ixr1-2*[20]), whose ABRC stock numbers are CS6554, CS6201, and
209 CS6202, respectively, and compared to wild-type plant on MS medium without
210 hygromycin. Root length was measured with ImageJ software.
211
212 **Histochemical GUS staining.** Transgenic plants expressing GUS reporter gene
213 constructs were grown for 3 weeks on solid agar plates with hygromycin. These plants

were stained with solutions containing 20% (v/v) methanol, 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, 0.3% (v/v) Triton-X, and 0.5 mg/ml X-Gluc in 100 mM potassium phosphate buffer. The samples were briefly vacuum-infiltrated, and then incubated for 4 hours at 37°C. Stained plants were rinsed with 80% EtOH and observed using a SZ61 stereo microscope (Olympus Inc.) and a BZ9000 Generation II Biorevo microscope (Keyence Inc.)

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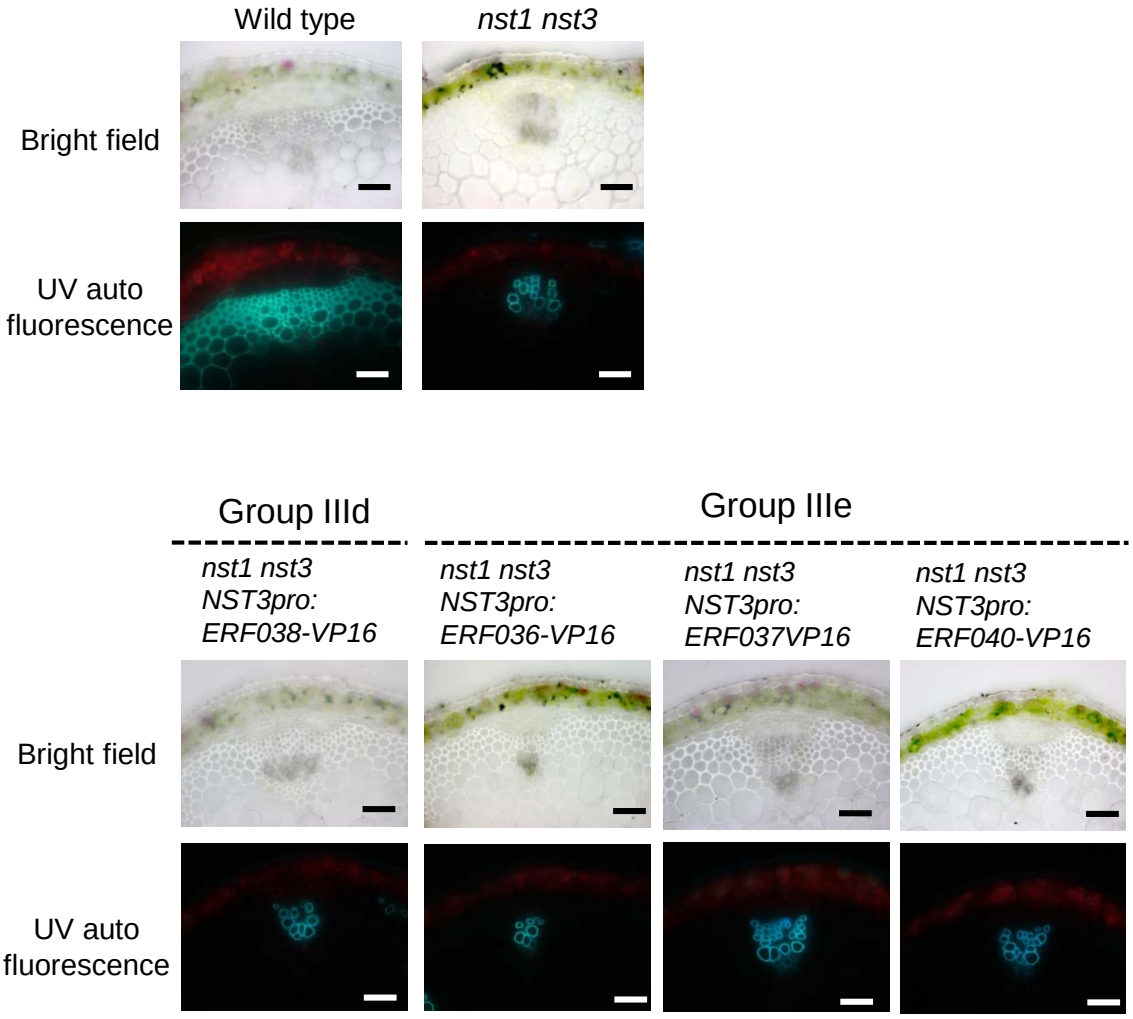
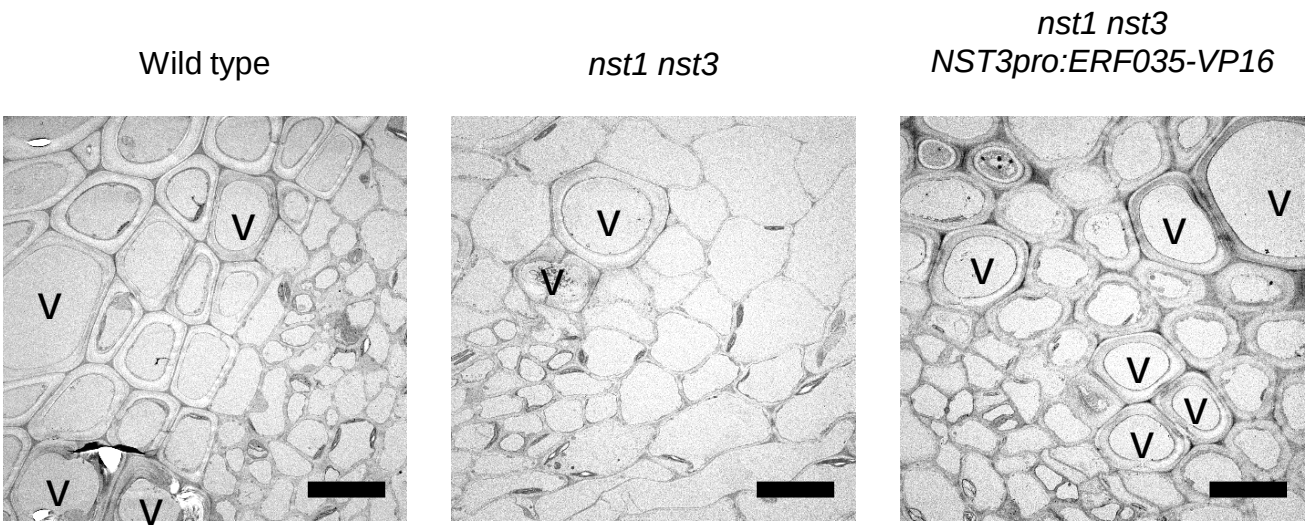
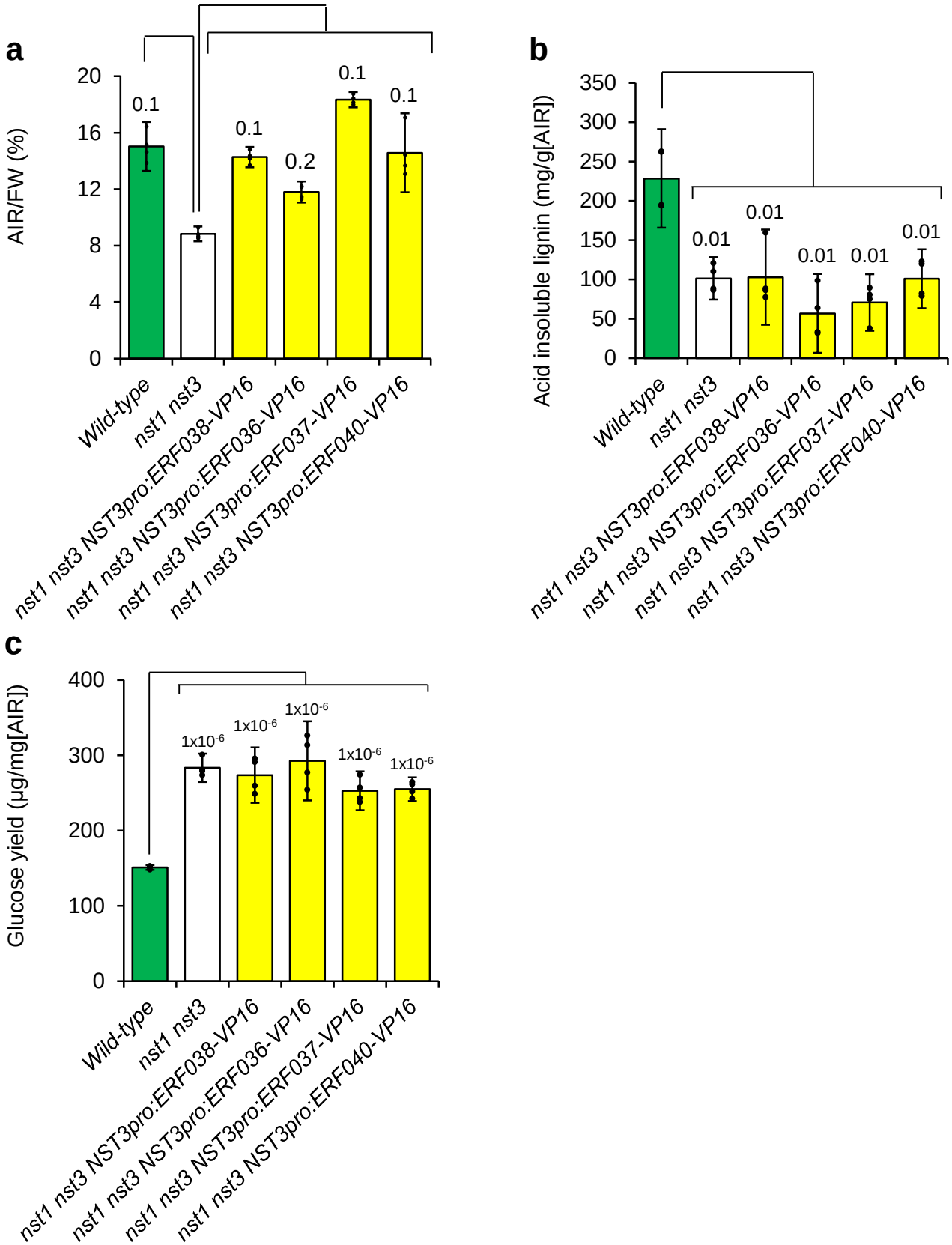


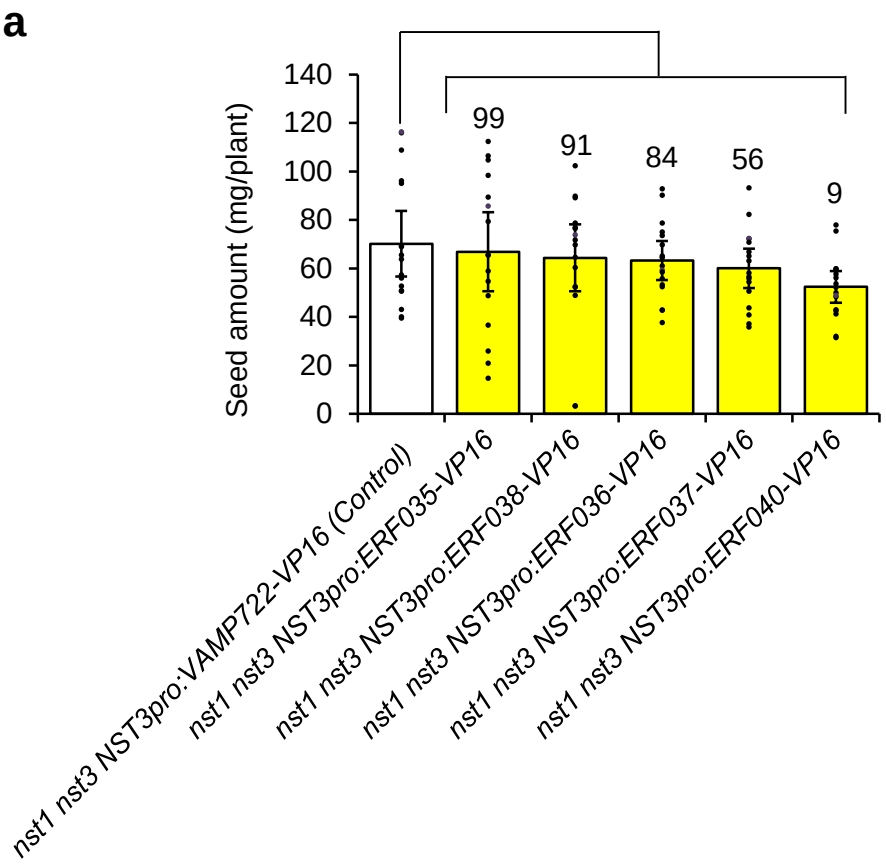
Supplementary Figure 1 | Accumulation of lignin-deficient cell walls in fiber cells of *nst1 nst3* plant by AP2/ERF group IIId and e family genes fused with VP16 supporting Figs 1a-f. Sections are as those described in Figure 1a-f. Scale bars = 50 μm. Transgenic/mutant plants in this figure were cultivated only once but cross sections were taken from 17 independent plants which showed similar results. Many pictures were taken and representative one picture is shown in this figure.



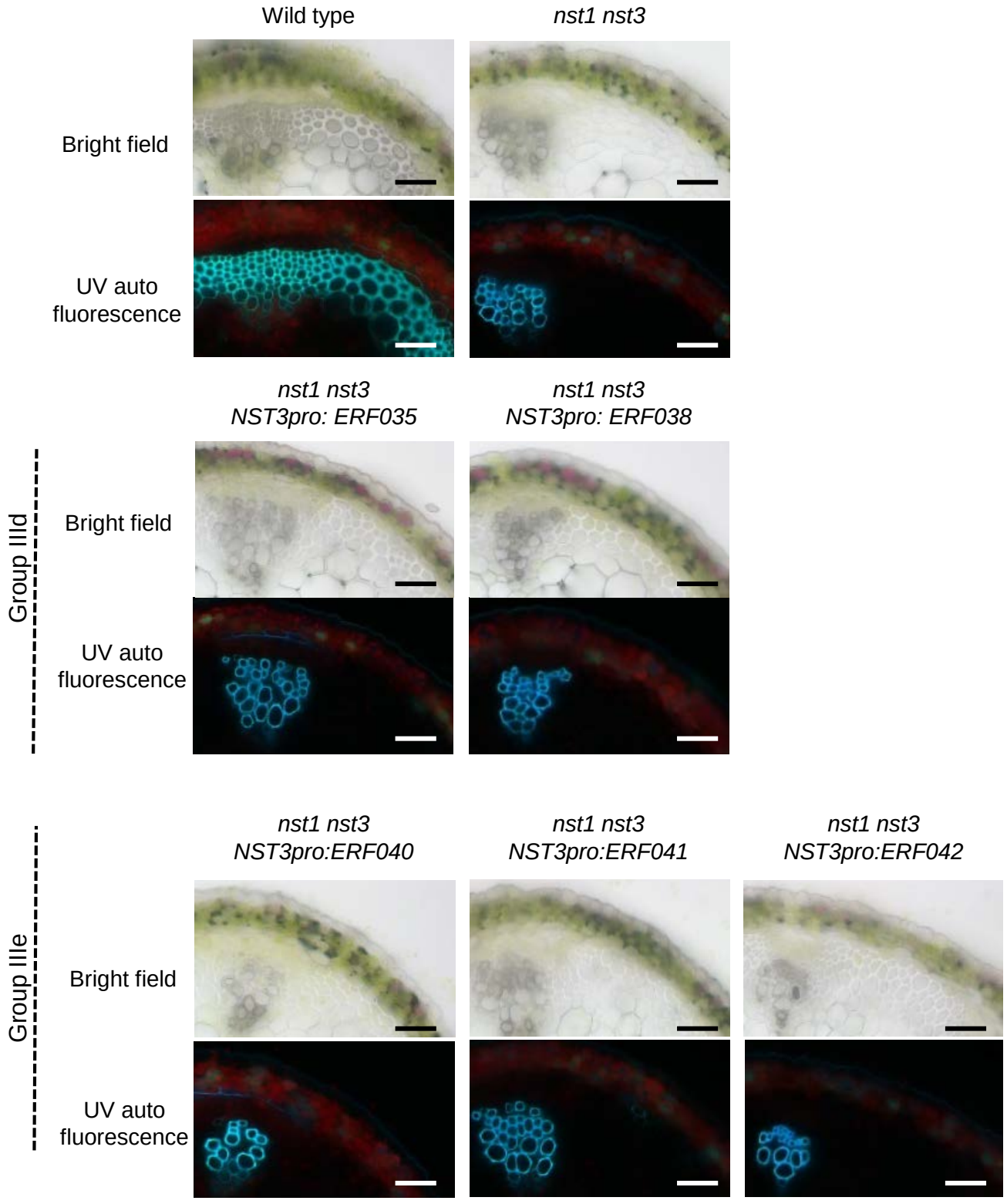
Supplementary Figure 2 | No visible change was found in xylem vessels of *nst1 nst3 NST3pro:ERF035*-VP16 plants, supporting Figs 1g-i. Inflorescence stems were taken from 8-week-old plants. "v" indicates vascular vessels. Scale bars represent 10 μm. Three plants of each line were selected for this experiment. TEM imaging was performed twice from different plants. Similar result was obtained from each set of imaging.



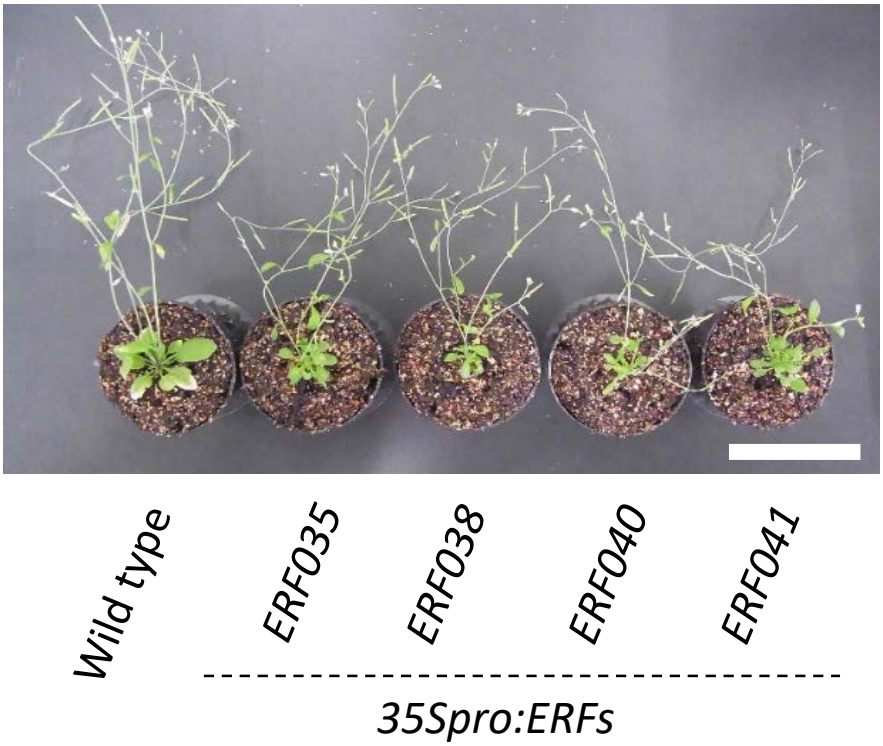
Supplementary Figure 3 | Cell wall property in terms of glycan productivity supporting Figs 1m-o. Quantitative analysis of AIR/FW ratio of cell walls (a), acid insoluble lignin (b), glucose yield by cellulose (c) induced by AP2/ERF group IIId and e family genes. Analyses are further outlined in Figure 1. Error bars indicate 95% confidence interval for the mean (n = 4 biologically independent samples). Each dot represents individual data point. Numbers in top of each graph indicate 100-fold of *P* value calculated by the Dunnett's test (two-sided). Analysis was performed only once but with four independent replicates.



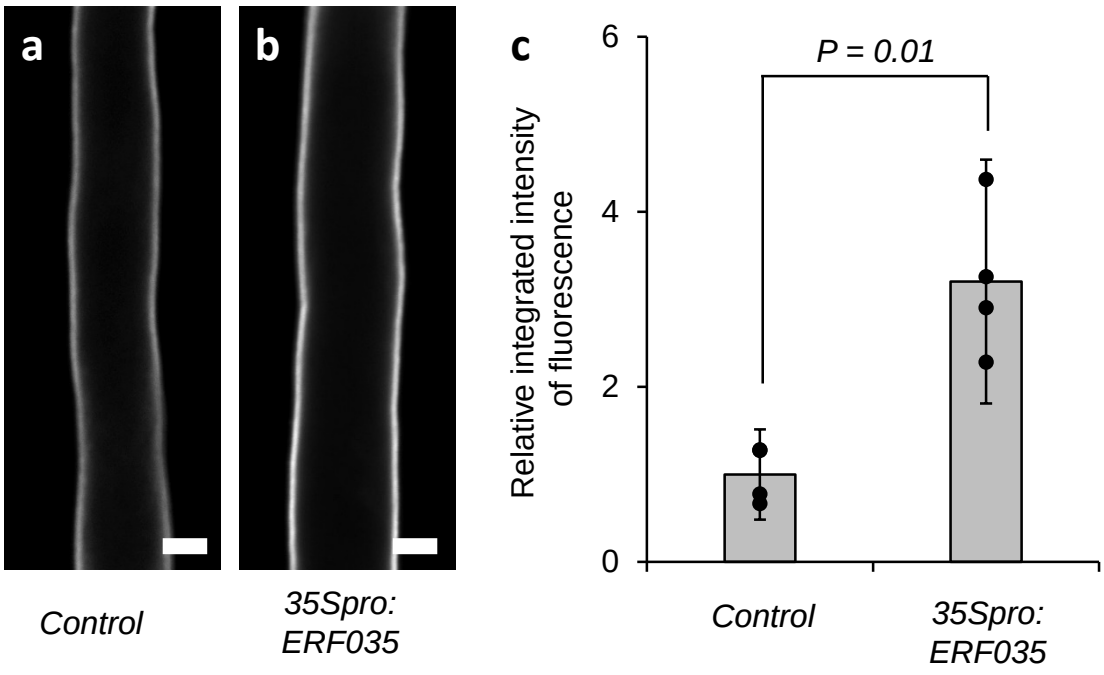
Supplementary Figure 4 | Growth characteristics of *nst1 nst3 NST3pro:ERFs-VP16* plants supporting Figs. 1m and 1o. Seed amount was measured for each line. Error bars indicate 95% confidence interval for the mean (n = 17 biologically independent samples). Each dot represents individual data point. Numbers in top of each graph indicate 100-fold of *P* value calculated by the Dunnett's test (two-sided). Analysis was performed only once but with many independent replicates.



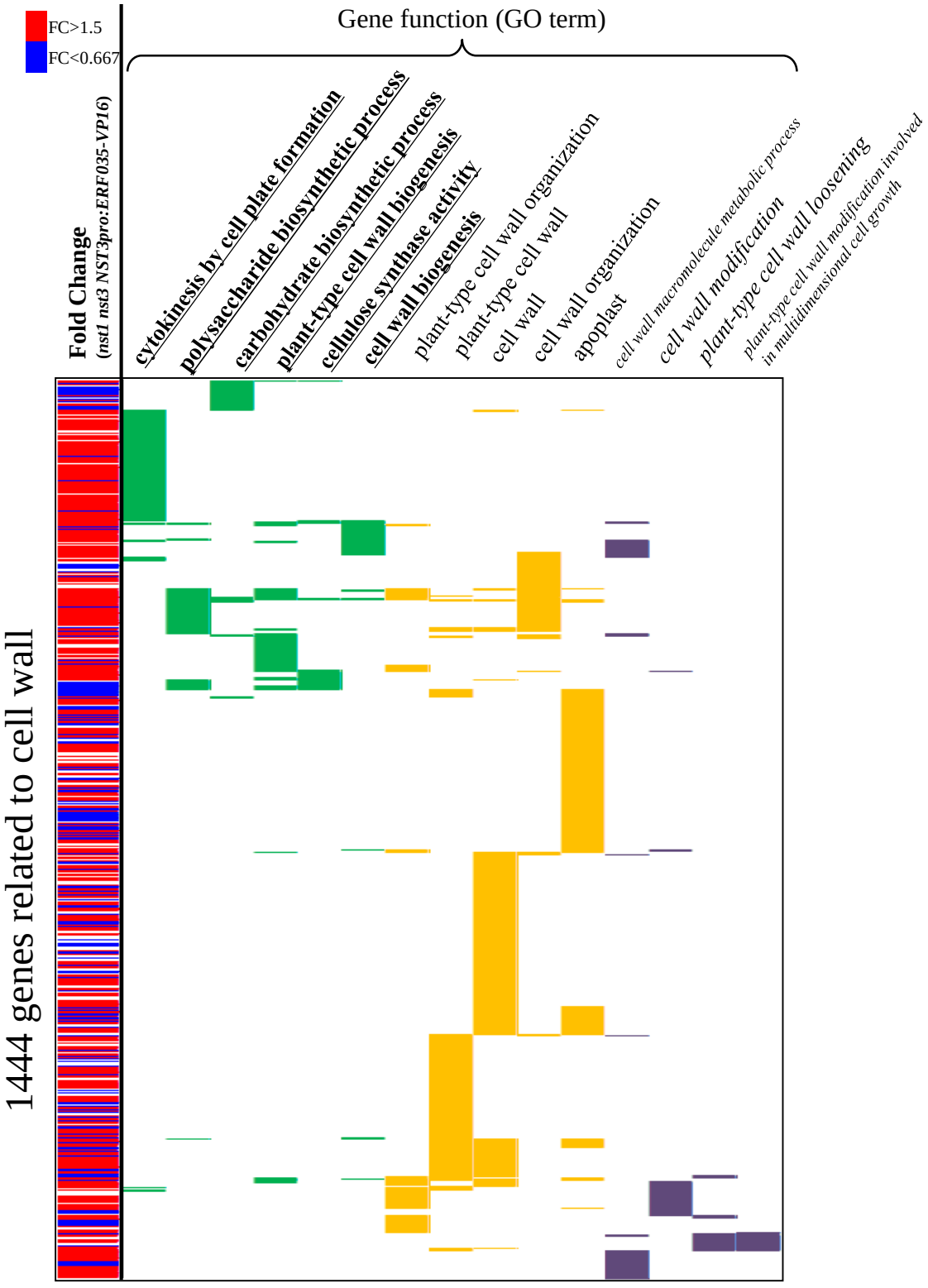
Supplementary Figure 5 | Accumulation of cell walls devoid of lignin in fiber cells of *nst1 nst3* plants by AP2/ERF group IIId and e family genes without fusion of VP16 supporting Figs 1a-f. Sections are as those described in Figure 1a-f. Scale bars = 50 μm. Transgenic/mutant plants in this figure were cultivated only once but cross sections were taken from at 12 or 13 biologically independent plants which showed similar results. Representative one picture is shown in this figure.



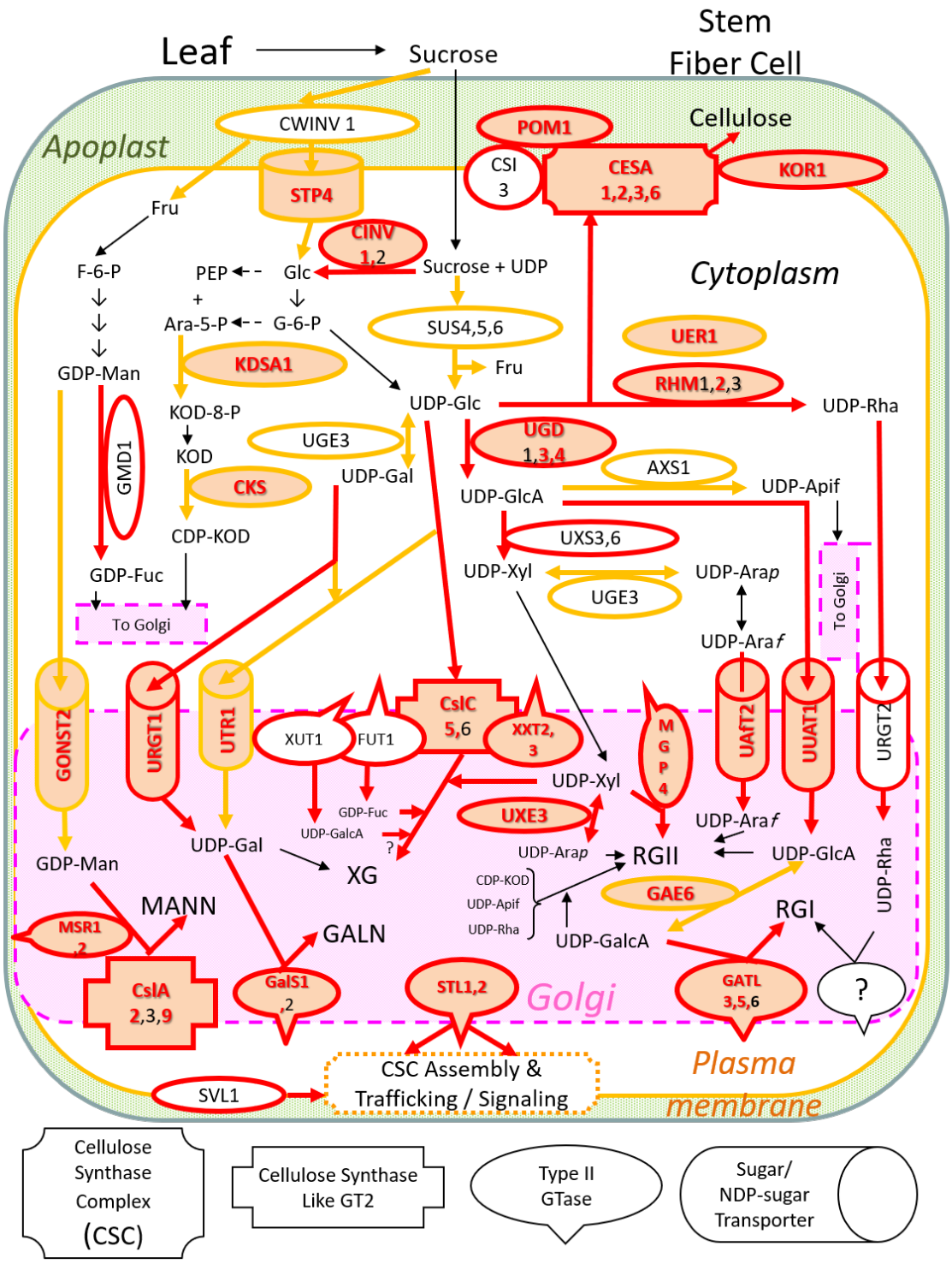
Supplementary Figure 6 | Soil-grown wild-type and *35Spro:ERF* plants supporting Fig 1p-s.
6-week-old plants. Scale bar indicates 5 cm. Transgenic plants in this figure were cultivated only once but 10 biologically independent plants were grown separately and all plants showed similar phenotype.



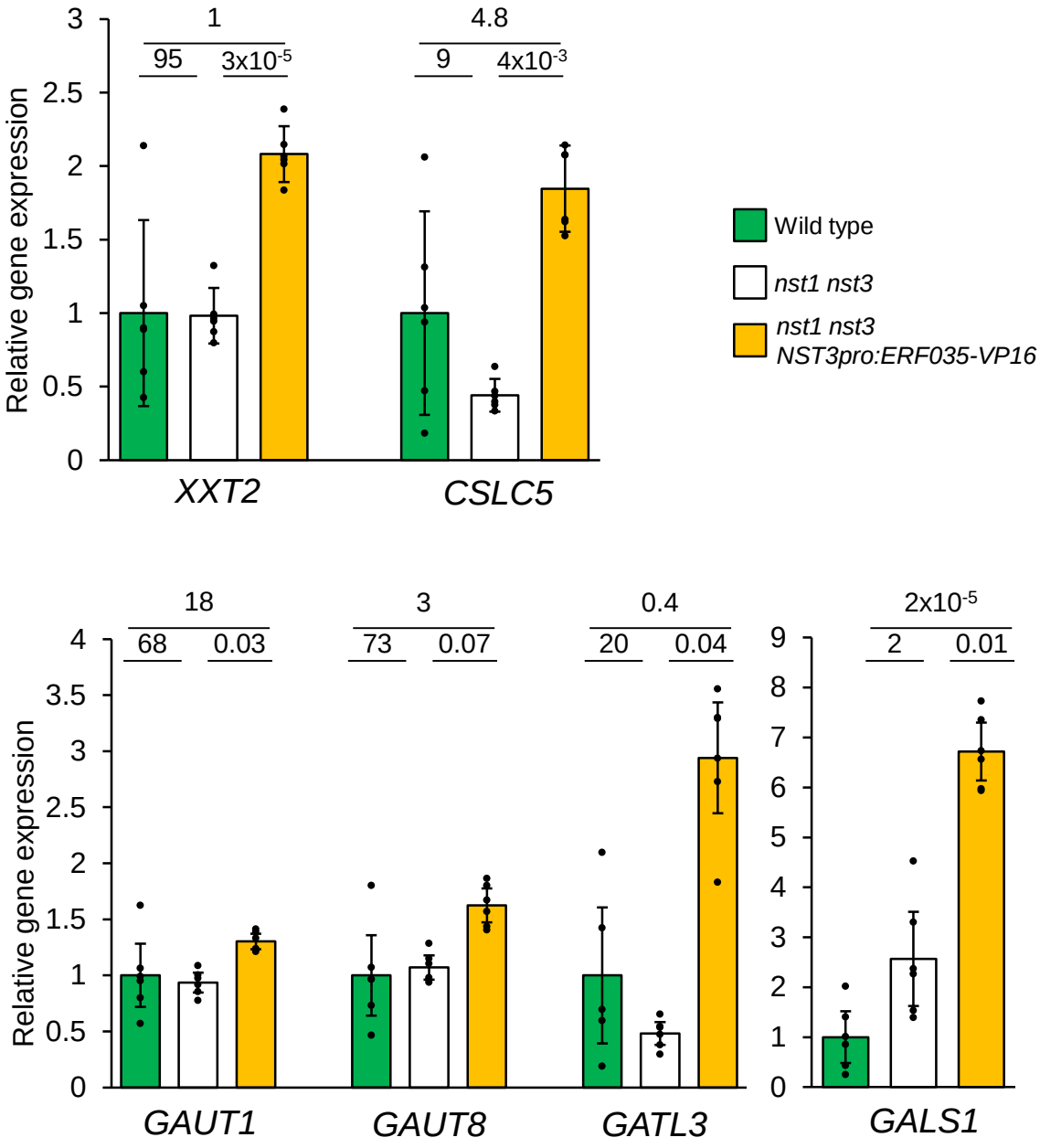
Supplementary Figure 7 | Root hair cell wall of wild type and *35Spro::ERF035* plants
(**a, b**) Root hair of 2-week-old plants grown on solid MS media stained with Calcofluor-white and photographed by confocal microscopy. Pictures were taken from four biologically independent plants which showed similar phenotype and representative one is shown in this figure. Bars indicate 10 μm. (**c**) Relative integrated signal intensity of fluorescence was calculated from normalized peak curve from 100 data sets with Simpson's rule. Score of signal intensity in control was set to 1. Error bars indicate 95% confidence interval for the mean (n = 4 biological replicates from individual T2 transgenic lines). Each dot represents individual data point of average score of 3 technical replicates. *P* value was calculated by the Welch's t-test (two-sided). Analysis was performed only once.



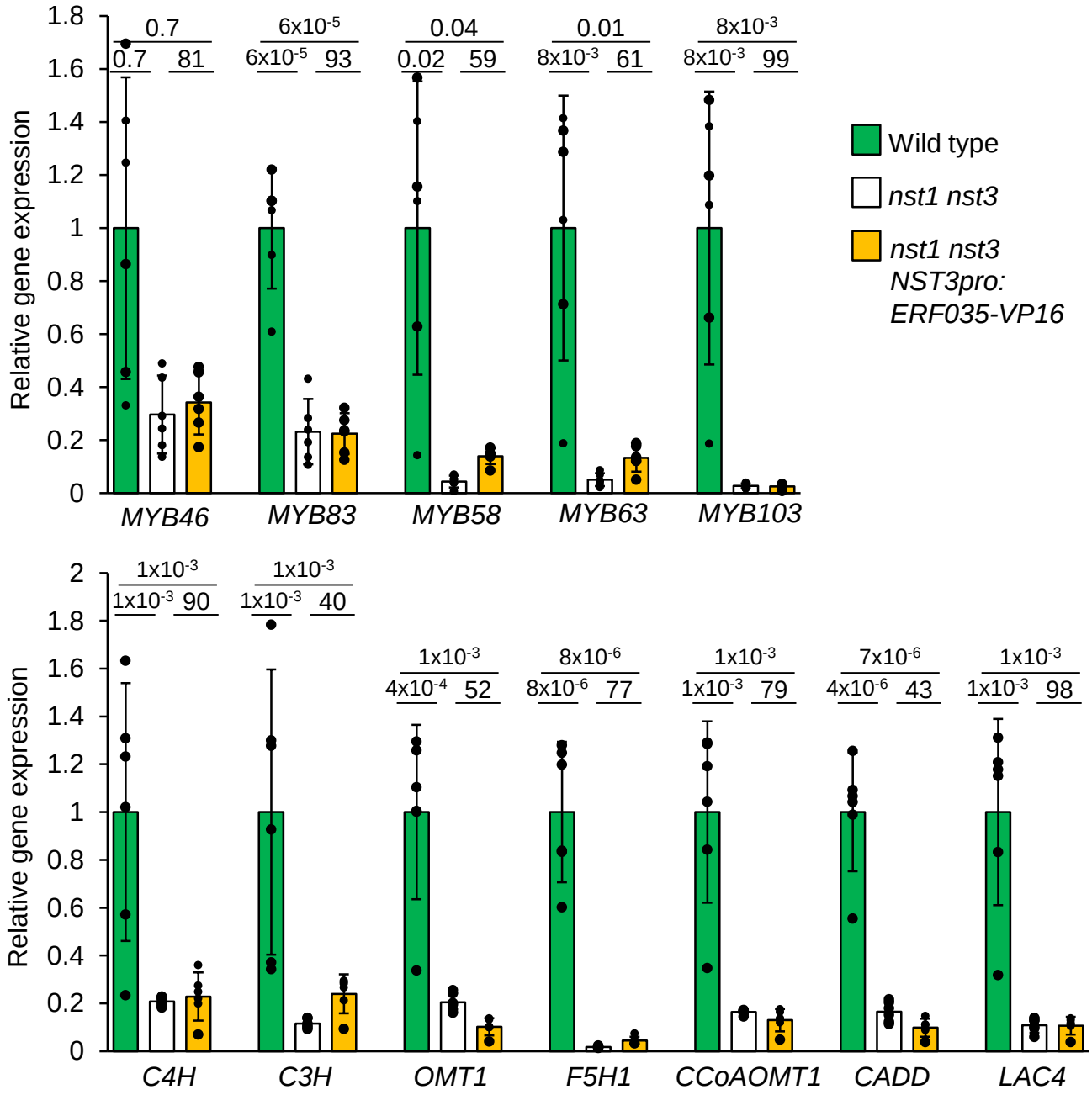
Supplementary Figure 8 | Expression analysis of cell-wall related genes based on GO annotation supporting Fig 2a. Cell-wall related genes are sorted by GO annotation. Red and blue color represents fold change (>1.5-fold or <0.67-fold, respectively) of gene expression in *nst1 nst3 NST3pro:ERF035-VP16* compared to *nst1 nst3*. Green, yellow, and purple color below GO terms indicate that the gene belongs to each GO term, that is, GO term related to cell wall biosynthesis (bold underlined), general cell wall processes, or cell wall degradation/modification (italicized), respectively, which is also shown in Table S1.



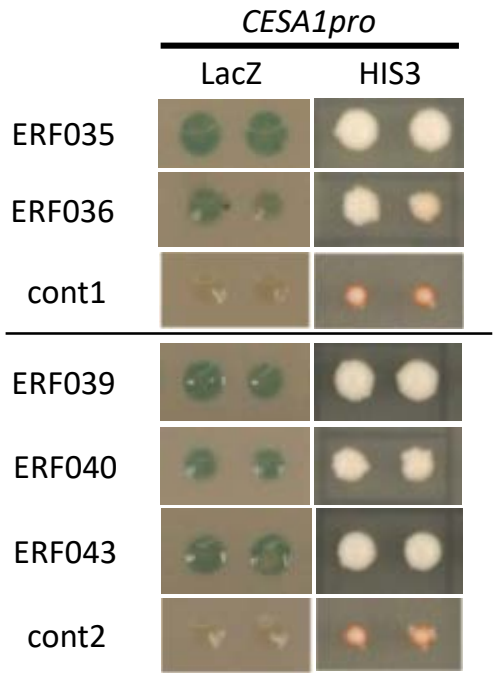
Supplementary Figure 9 | Schematic diagram of primary wall biosynthesis showing genes upregulated in the ERF035-VP16 lines, which supports Fig 2a. Orange and red colors represent upregulated genes which are involved in primary wall biosynthesis with biochemical or genetic evidence, respectively. The biochemical evidence means there is a publication about enzymatic activity of the recombinant protein. The genetic evidence means there is a publication that the knockout or overexpression induced reduction or increase of primary wall component, respectively. If each feature is filled with orange color, it means the gene is a putative direct target of grouop IIId and IIIe ERFs which has at least four DAP-seq/ampDAP-seq peak(s) out of eight group IIId amd IIIe ERFs in the DNA between -3000 and +500 from transcriptional start site.



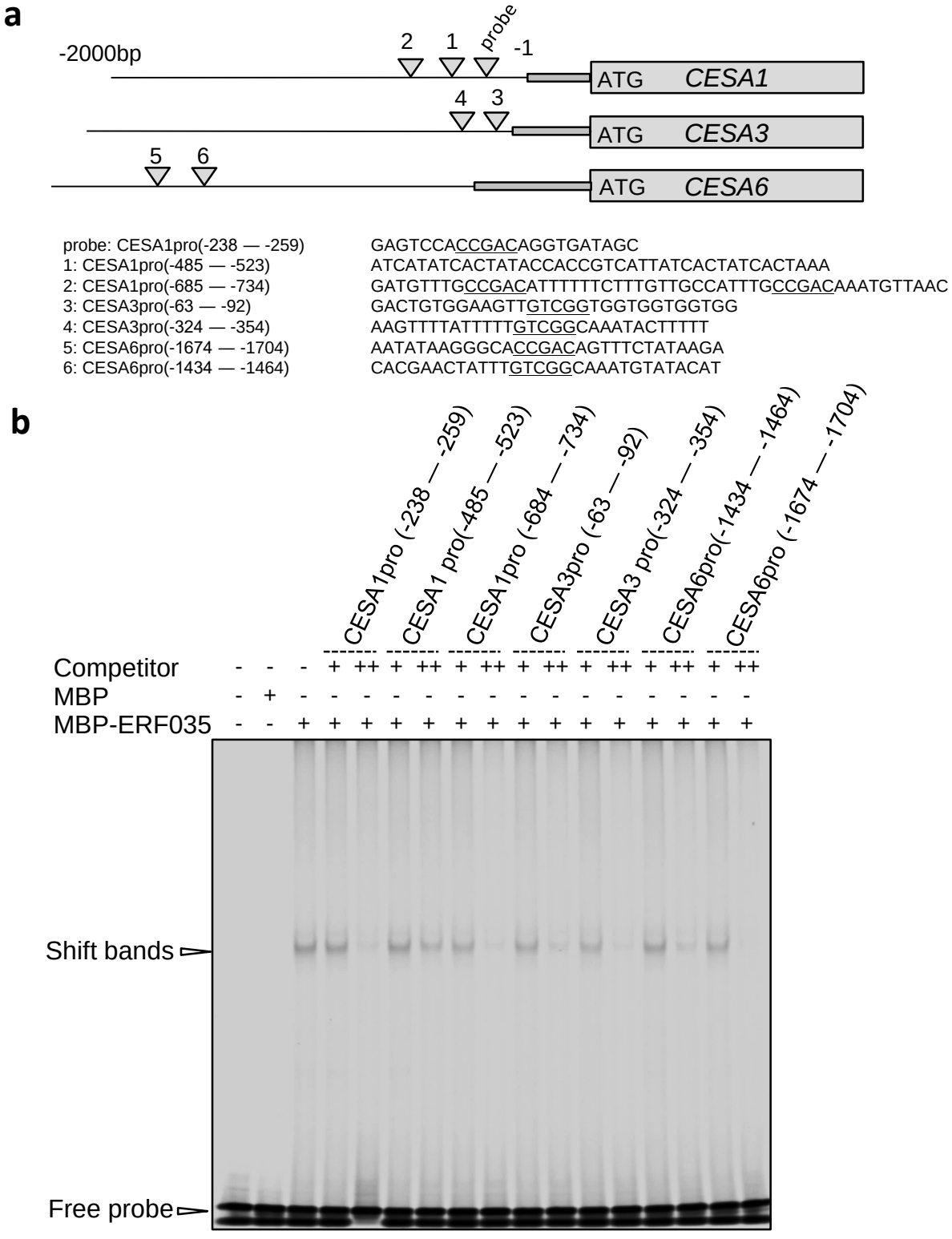
Supplementary Figure 10 | Gene expression analysis of xyloglucan and pectin biosynthetic enzymes supporting Fig 2b. Expression levels of each gene in wild-type were set to 1. Error bars indicate 95% confidence interval for the mean (n = 6 biologically independent plants). Each dot represents individual data point. Numbers in top of each graph indicate 100-fold of *P* value calculated by the Welch's t-test (two-sided) with Bonferroni-Holm correction. RT-PCR analysis was performed at least twice and similar data was obtained each time.



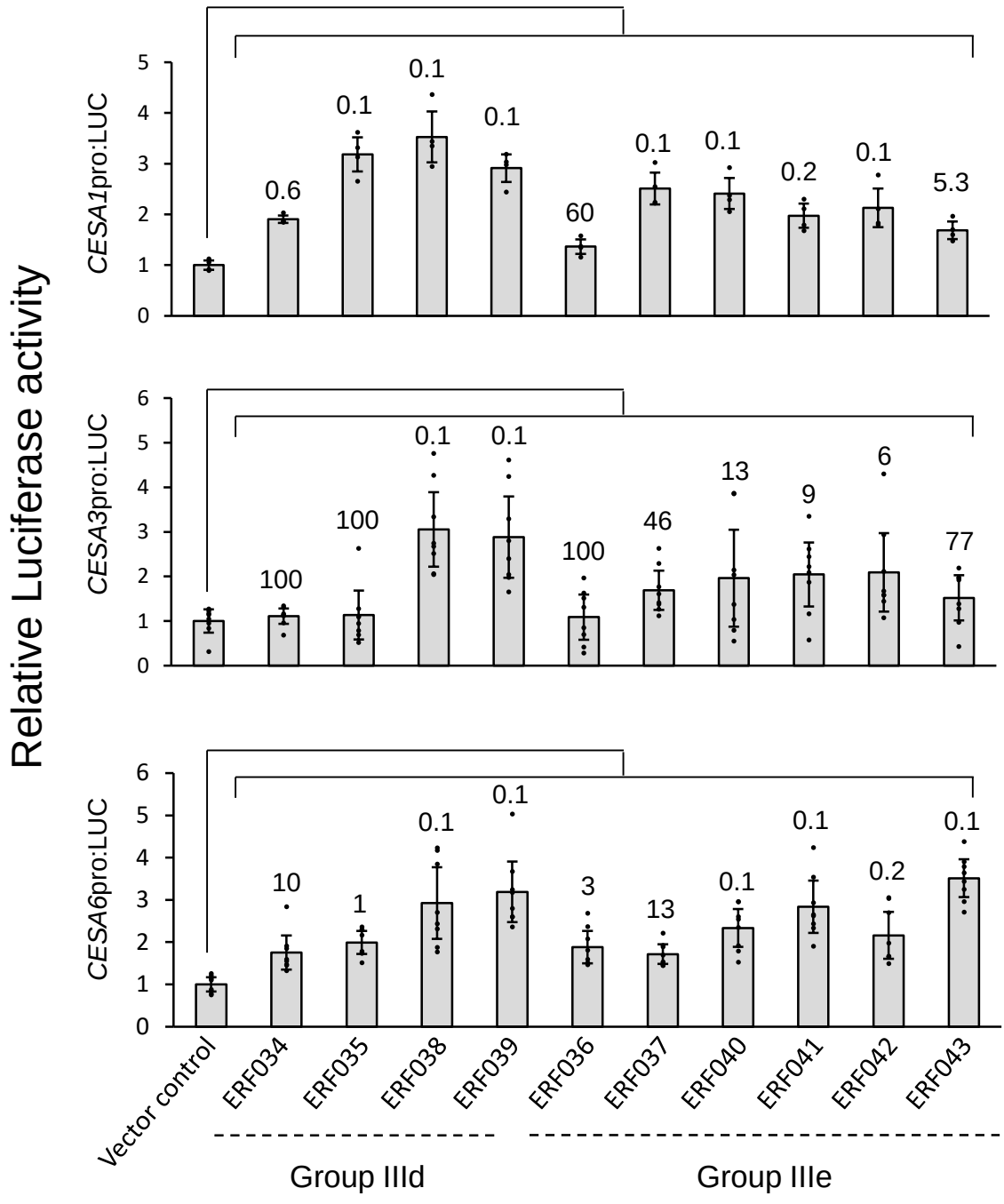
Supplementary Figure 11 | Expression analysis of genes related to lignin biosynthesis supporting Fig 2b. Expression level of each gene in wild type was set to 1. Error bars indicate 95% confidence interval for the mean (n = 6 biologically independent plants). Each dot represents individual data point. Numbers in top of each graph indicate 100-fold of *P* value calculated by the Welch's t-test (two-sided) with Bonferroni-Holm correction. RT-PCR analysis was performed at least twice and similar data was obtained in each time.



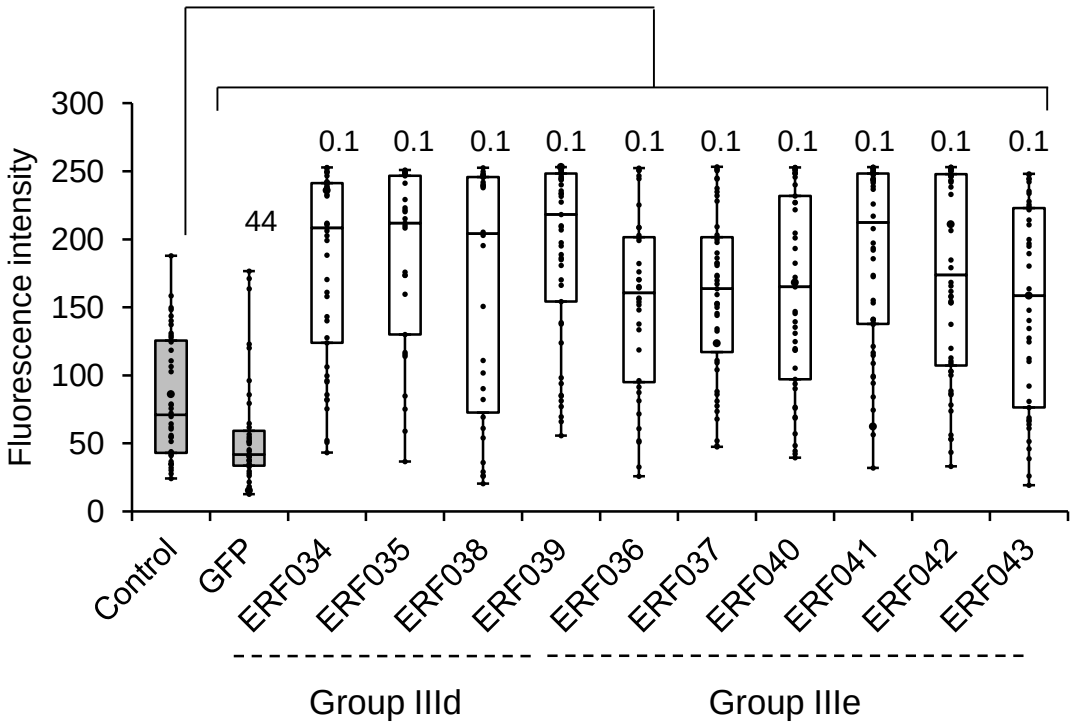
Supplementary Figure 12 | AP2-ERF group IIId and e TFs can bind to *CESA1* promoter in yeast supporting Fig 2b. Yeast one-hybrid assay employing LacZ and HIS3 reporters demonstrates that some of the AP2-ERF group IIId and e TFs can bind to *CESA1* promoter in yeast. Blue color and colony growth on LacZ and HIS3, respectively, represent the binding of each TF to the promoter. Upper and lower panels were in different plates having different control spots. Experiment was performed once but duplicated as shown in the pictures.



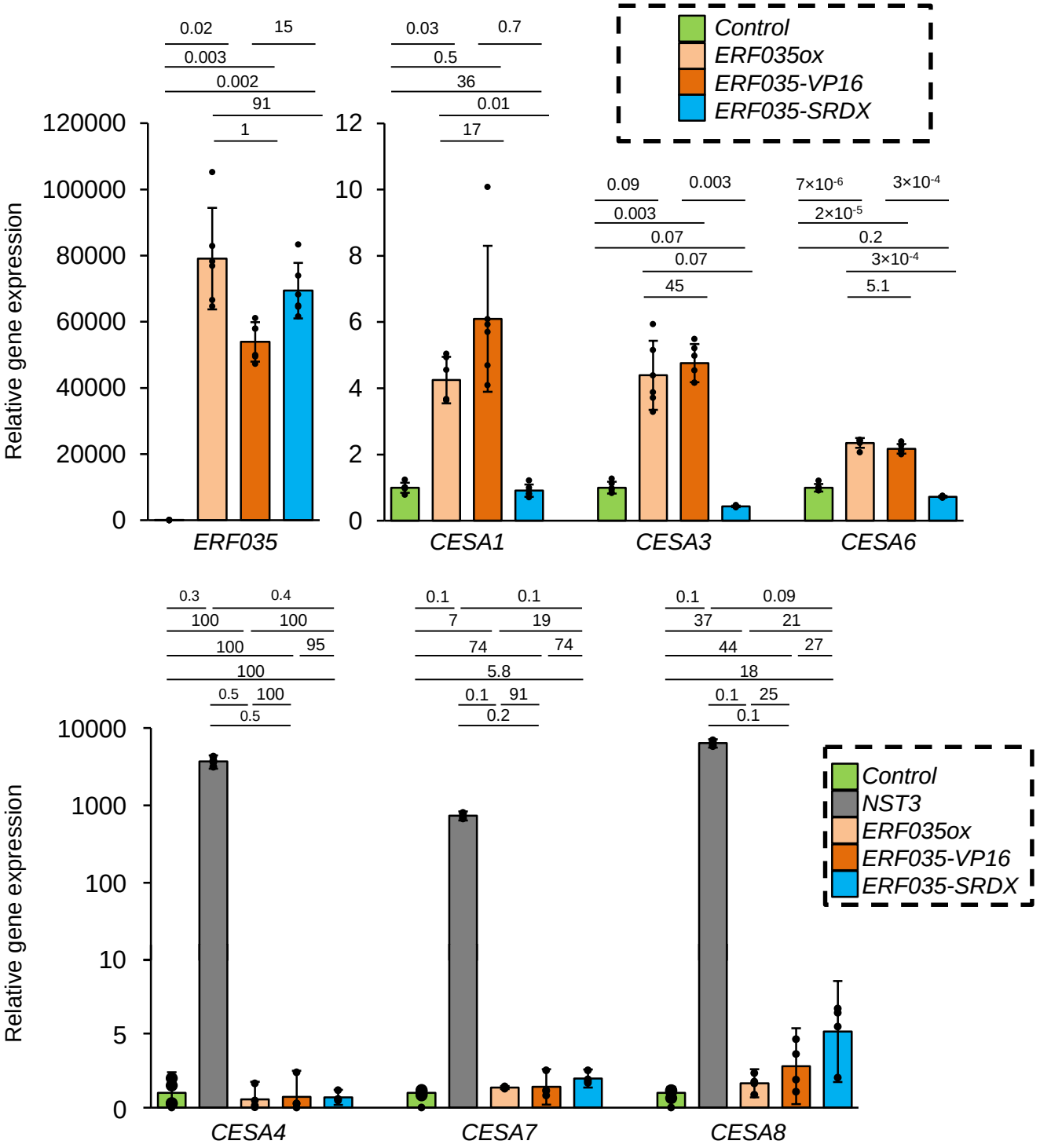
Supplementary Figure 13 | ERF035 binds to primary wall-related *CESA* gene promoters, supporting Fig 2b. (a) Schematic diagram of *CESA1*, *CESA3*, and *CESA6* promoters. Triangles represent potential binding sites of ERF transcription factors; sequences shown below. These sequences are same as those of probe or competitors in (**b**). Thinner box left to "ATG" represents 5'UTR. "-1" indicates putative transcriptional start site. (**b**) Recombinantly expressed ERF035 fused with MBP protein in *E.coli* was used for EMSA assay. Short fragment of *CESA1*promoter (-238 - -259; sequence is shown in [**a**]) was used for probe. Various short fragments of *CESA* promoters were employed for cold competitors (sequences are shown in [**a**]). Experiment was performed three times and similar result was obtained from each time.



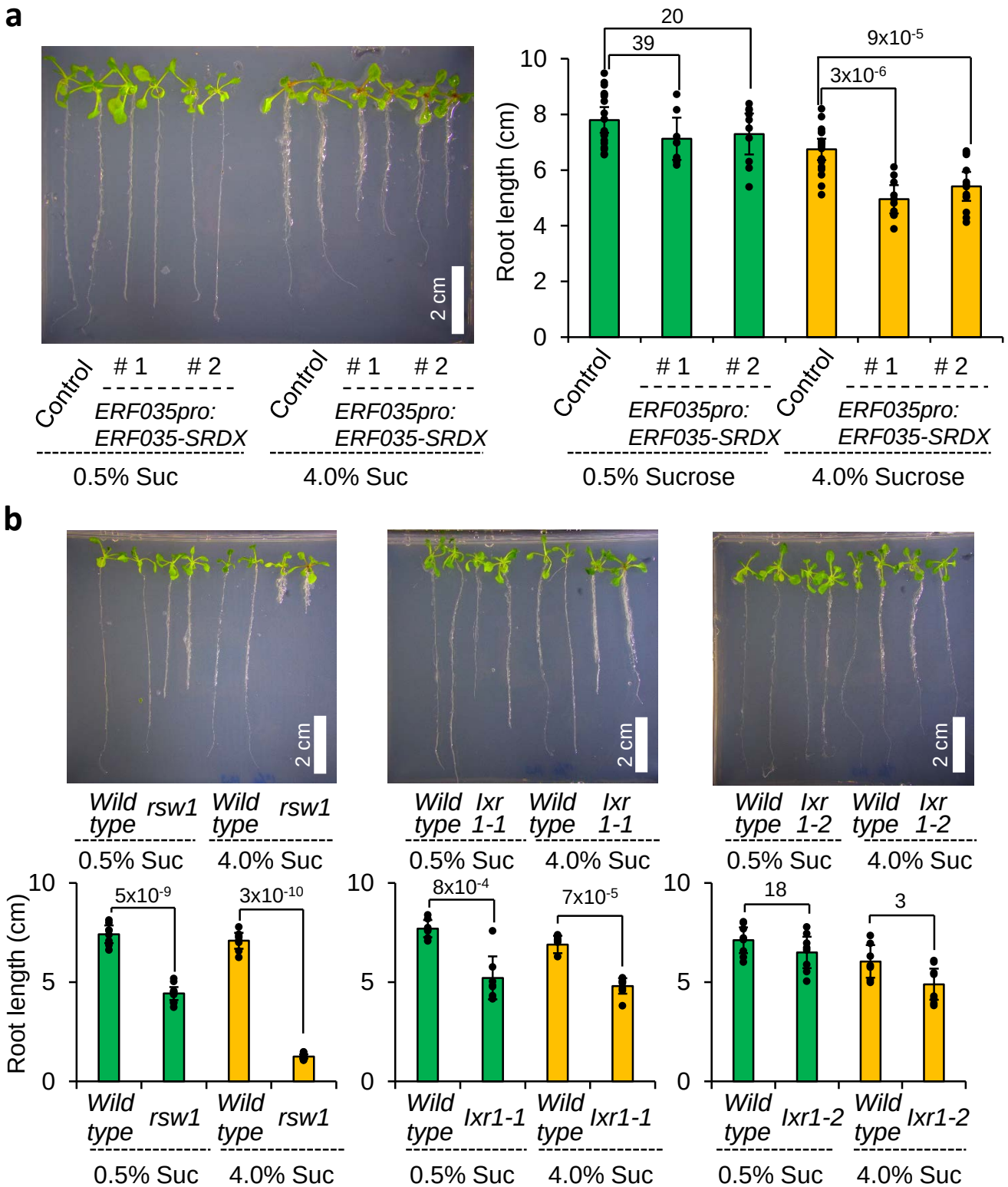
Supplementary Figure 14 | AP2-ERF group IIId and e genes affect primary-wall related *CESA* gene expression, supporting Fig 2b. Transient effector-reporter assay using Arabidopsis mesophyll protoplasts. The effector construct consisted of a TF gene's coding region fused to the VP16 activation domain, and the reporter construct consisted of the firefly luciferase gene driven by the *CESA1*, *CESA3* and *CESA6* promoter. The relative reporter value was calculated by dividing the reporter value with the reference value of *Renilla* luciferase. Bars indicate 95% confidence interval for the mean (n = 4 for *CESA1*, 8 for *CESA3* and *CESA6*, biologically independent samples). Each dot represents individual data point. Numbers in top of each graph indicate 100-fold of *P* value calculated by the Dunnett's test (two-sided). Experiment was performed at least twice.



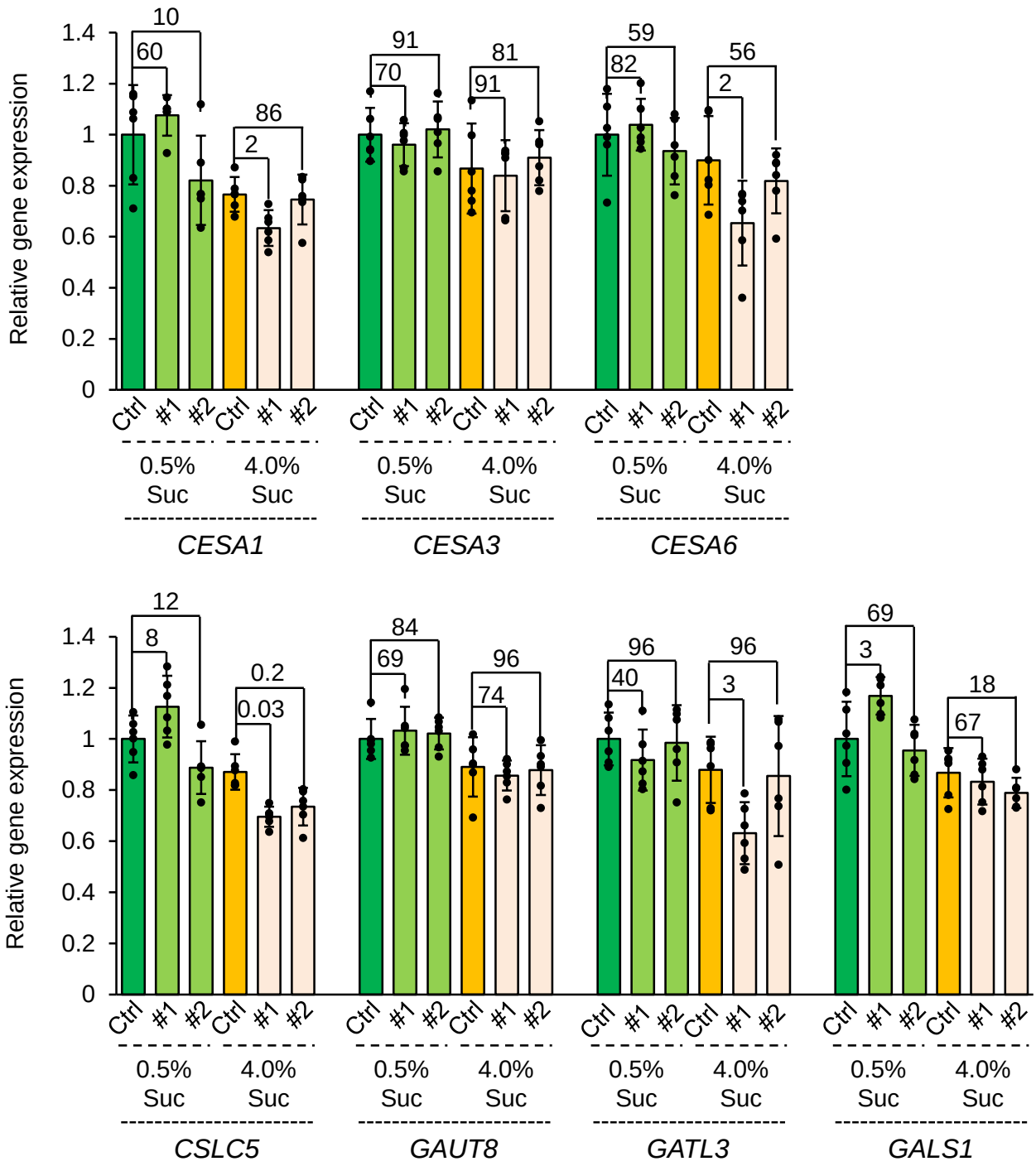
Supplementary Figure 15 | Enhancement of cell wall regeneration by the chimeric activator of AP2-ERF group IIId and e genes supporting Figs 2c and 2d. Cell wall regeneration from protoplast transiently expressing the chimeric activator fusions of AP2-ERF transcription factor Group III d and e genes. Cell walls were observed using Calucofluor white similar to described in Figure 2. Boxplots represent a range of values. Horizontal bar in the box indicates the median of the reported values. Upper and lower hinges of box indicate 75%- and 25%-ranges of reported values, respectively. The upper and lower extreme bars indicate maximum (upper) and minimum (lower) of reported values, respectively. The number of examined cells and the 95% confidence interval for the mean are n = 40; 68.8 to 96.6 (Control), n = 39; 43.4 to 69.7 (GFP), n = 45; 161.2 to 201.2 (ERF034), n = 29; 161.7 to 210.0 (ERF035), n = 30; 130.5 to 195.5 (ERF038), n = 49; 175.2 to 211.5 (ERF039), n = 36; 131.1 to 174.1 (ERF036), n = 47; 142.8 to 177.5 (ERF037), n = 45; 138.7 to 181.1 (ERF040), n = 54; 168.6 to 205.2 (ERF041), n = 52; 154.1 to 194.3 (ERF042), n = 41; 127.9 to 173.7 (ERF043). Numbers in top of each graph indicate 100-fold of *P* value calculated by the Dunnett's test (two-sided). Experiment was performed at least twice.



Supplementary Figure 16 | Gene expression in protoplasts transiently expressing ERF035, or ERF035 fused to a chimeric activator (VP16) or repressor (SRDX) supporting Figs 2b-d. Expression of *ERF035*, *CESA1*, *CESA3*, *CESA6*, *CESA4*, *CESA7*, and *CESA8* was measured by quantitative RT-PCR analysis in protoplast expressing *ERF035*, *ERF035-VP16*, or *ERF035-SRDX* from introduced plasmids. *NST3* was used as the positive control of the upregulation of secondary cell wall related-*CESA* genes because expression of them was very low in protoplast. Error bars represent 95% confidence interval for the mean (n = 6 [in upper panel] or n = 4 [in lower panel] biologically independent cell lines). Each dot represents individual data point. Numbers in top of each graph indicate 100-fold of *P* value calculated by the Welch's t-test (two-sided) with Bonferroni-Holm correction. Experiment was performed at least twice.

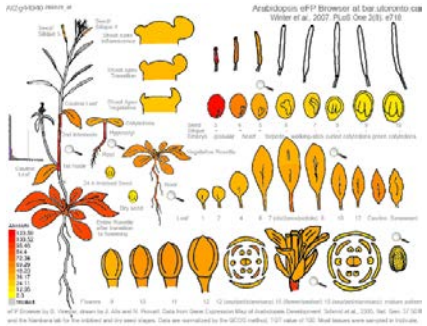


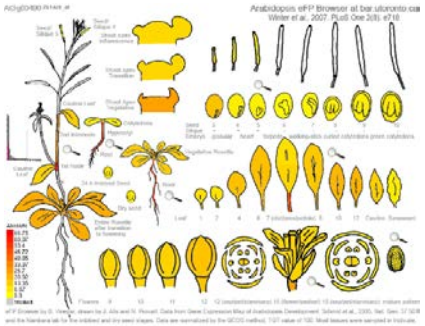
Supplementary Figure 17 | ERF035 chimeric repressor line showed similar short-root phenotype under high sucrose condition to the mild loss-of-function mutants of primary cell wall-related *CESA*s supporting Fig 2. Primary root length of *ERF035pro:ERF035-SRDX* line (a) and mild loss-of-function mutants of *CESA* genes (b; *rsw1*, *ixr1-1*, *ixr1-2*) grown on MS solid media containing normal or high concentration of sucrose. *ERF035pro:GUS* line was used as a control for *ERF035pro:ERF035-SRDX* line. Error bars indicate 95% confidence interval for the mean (**a;** from left to right; n = 19, 8 10, 19, 10, and 13 biologically individual transgenic plants grown on MS solid medium with hygromycin, and **b;** from left to right; n = 8, 10, 9, 10, 7, 7, 10, 8, 8, 8, 7, and 8 biologically individual plants grown on MS solid medium without antibiotics) Each dot represents individual data point. *P* value was calculated by the Dunnett's test (two-sided, **a**) or Welch's t-test (two-sided, **b**). Experiment was performed three times and similar results were obtained from all experiments.

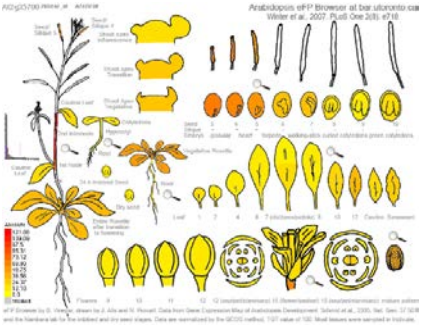


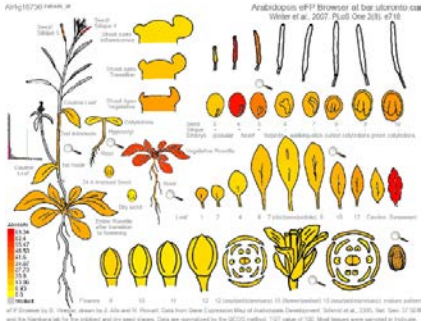
Supplementary Figure 18 | Gene expression analysis of xyloglucan and pectin biosynthetic gene genes supporting Fig 2.

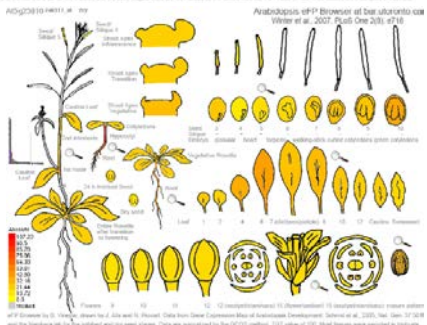
Expression of xyloglucan and pectin biosynthesis-related genes in *ERF035pro::ERF035-SRDX* lines (described as "#1" or "#2" in graph) grown on MS solid media with 0.5% sucrose or 4.0% sucrose. *ERF035pro:GUS* line (expressed as "Ctrl" in graph) was used as a control. Error bars indicate 95% confidence interval for the mean (n = 6 biologically independent sample). Each dot represents individual data point. *P* value was calculated by the Dunnett's test (two-sided). Experiment was performed three times.

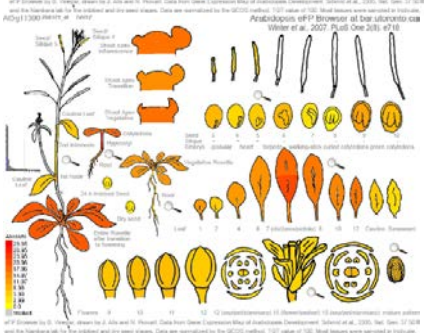


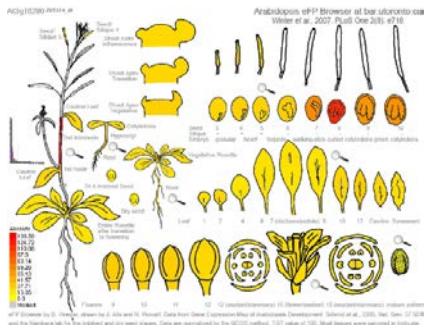


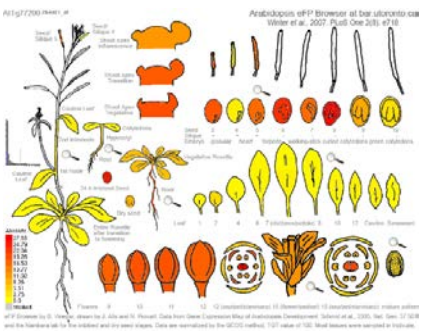


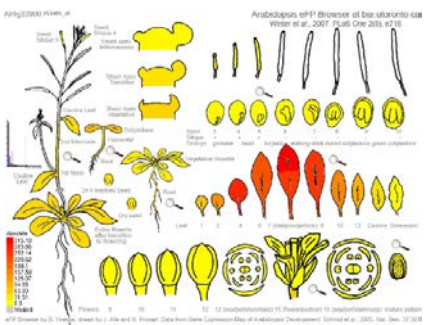


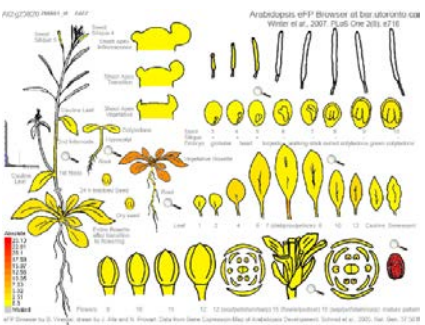




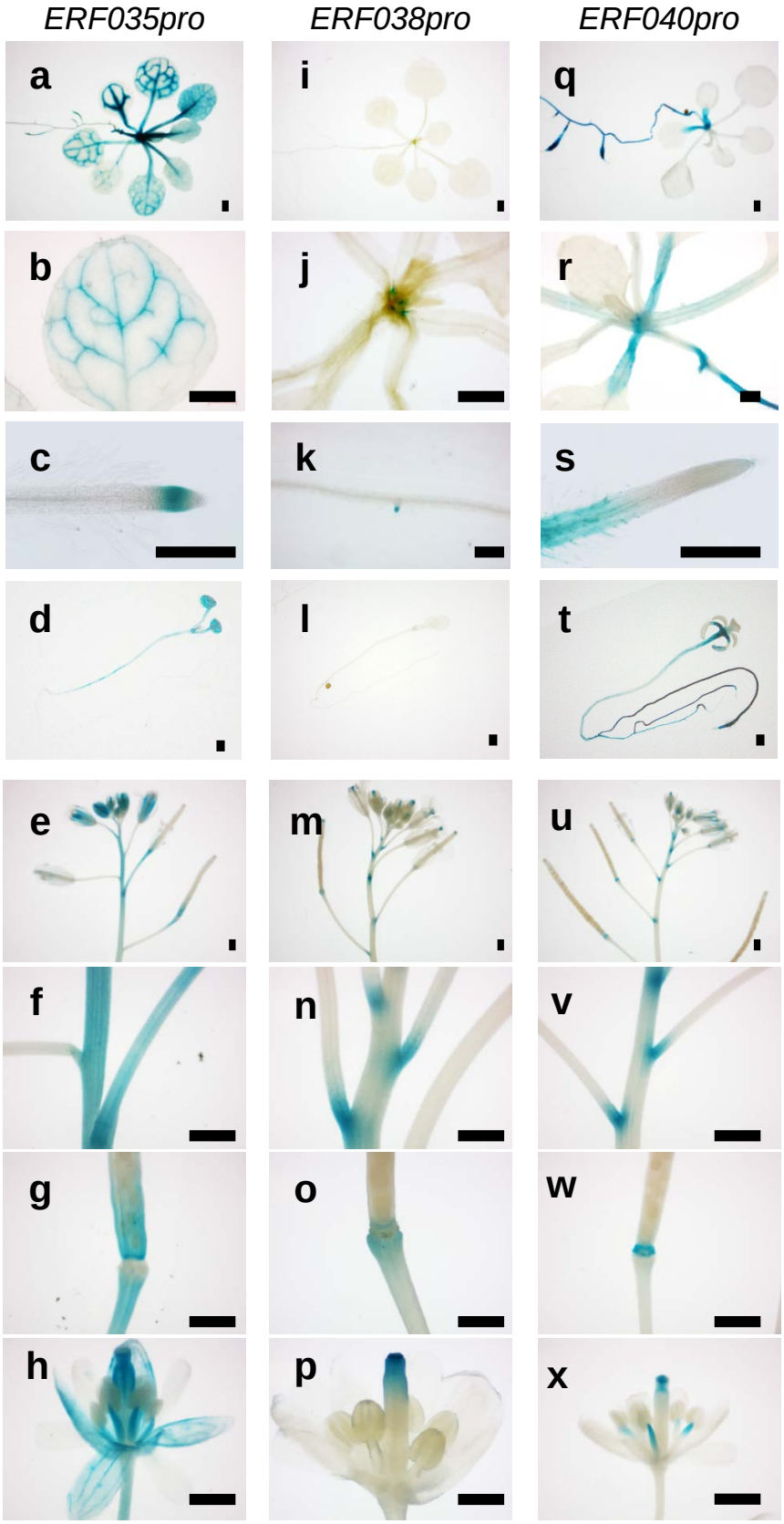




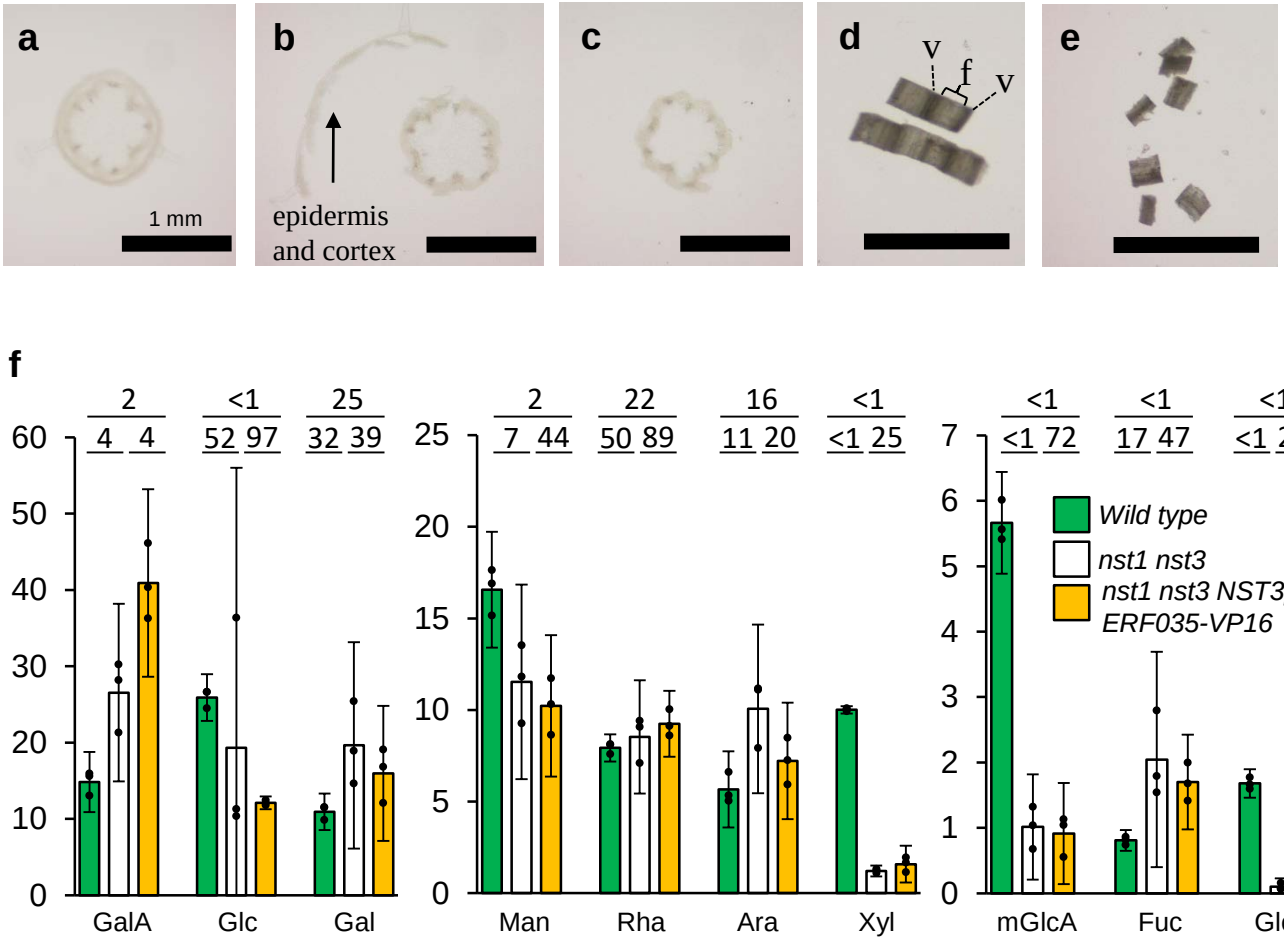




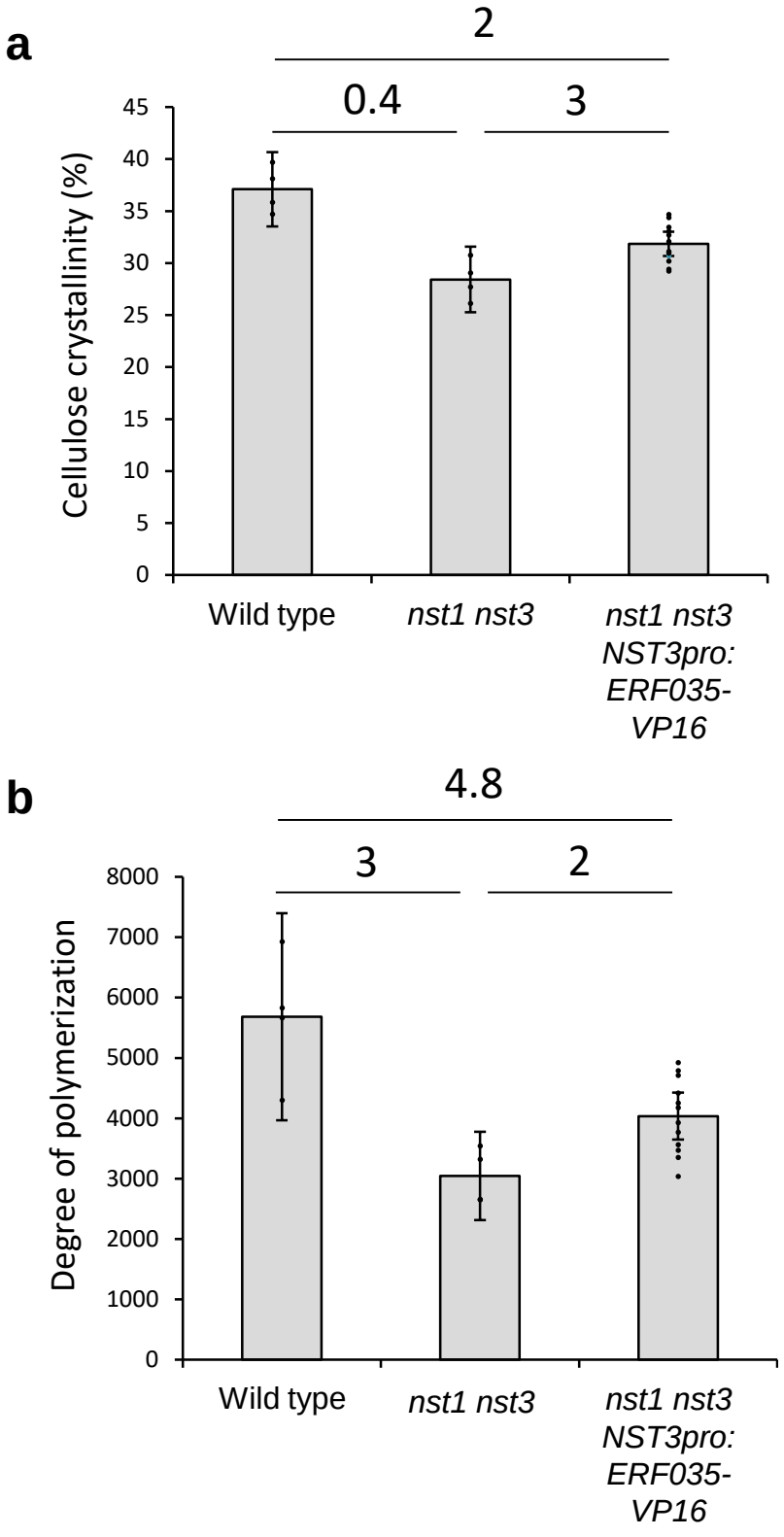
Supplementary Figure 19 | Expression data obtained from the eFP browser[56] for AP2-ERF group IIId and IIIe genes.



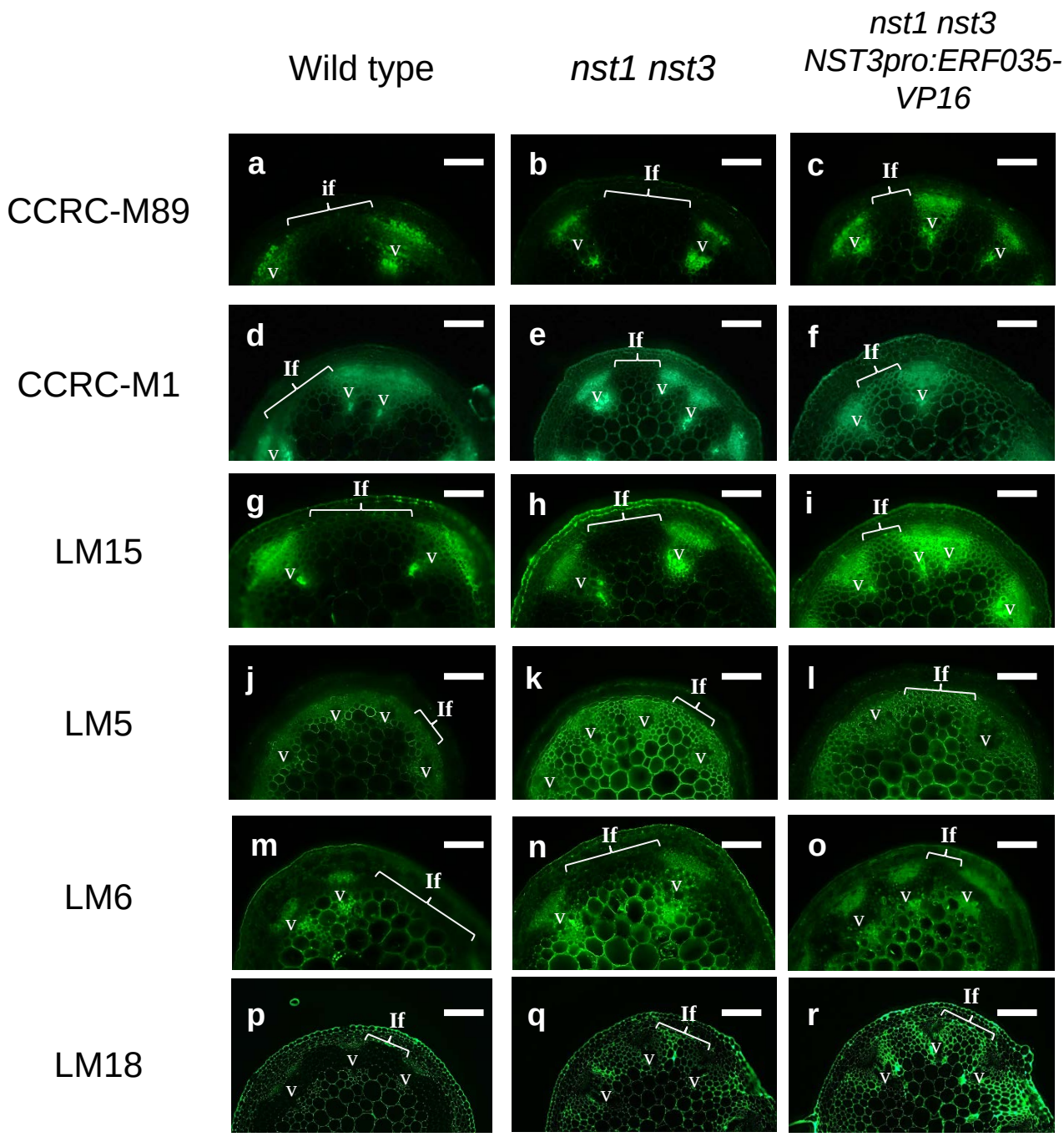
Supplementary Figure 20 | *ERF035*, *ERF038* and *ERF040* promoter activities as assessed by GUS reporter activity. GUS staining patterns of transgenic plants expressing *GUS* driven by the promoter of *ERF035* (**a**–**h**), *ERF038* (**i**–**p**), or *ERF040* (**q**–**x**) in young seedlings grown for 3 weeks on agar plates (**a, b, c, i, j, k, q, r, s**), etiolated seedling (**d, l, t**), and inflorescence tissues grown for 8 weeks on soil (**d, e, f, g, k, l, m, n, r, s, t, u**). Scale bars indicate 500 μm. Staining was performed only once but similar results were obtained from five biologically independent plants.



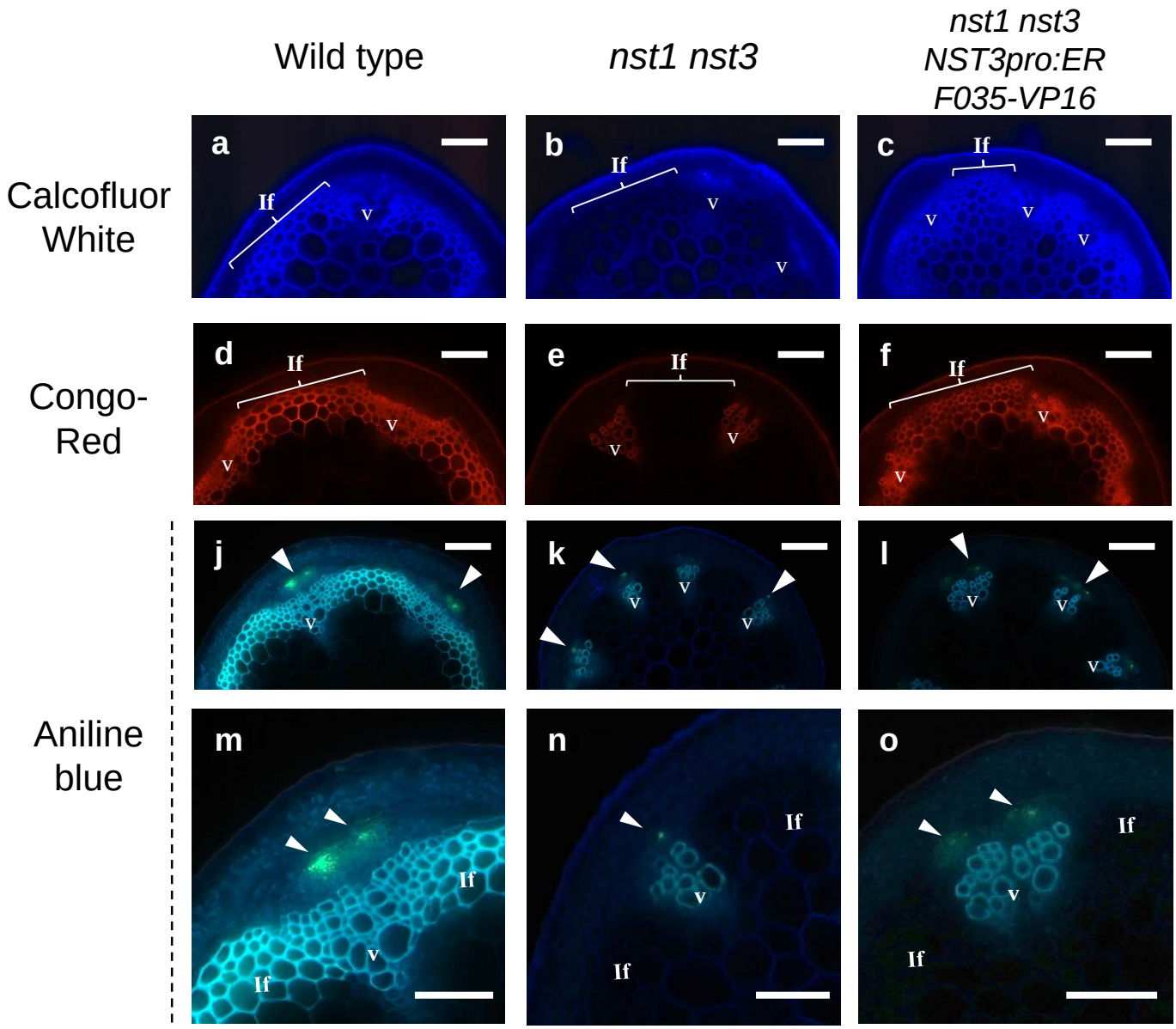
Supplementary Figure 21 | Monosaccharide composition of cell walls in fiber cells dissected from cross section supporting Fig. 3d. Fiber cells were dissected manually from 150 μm-thick cross sections (**a**) taken from 8-week-old plants. First, epidermis and cortex were removed (**b**) and then pith was removed (**c**). Fiber cells and vasculature were put vertically (**d**) and then only portions enriched with fiber cells were isolated (**e**). Several pieces enriched with fiber cells were subjected to AIR purification followed by α-amylase treatment and monosaccharide composition was analyzed (**f**). The scale bars indicate 1 mm. v; vasculature, f; fiber cells. Error bars represent 95% confident interval for the mean (n = 3 biologically independen samples). Each dot represents individual data point. Numbers in top of each graph indicate 100-fold of *P* value calculated by the Welch's t-test (two-sided) with Bonferroni-Holm correction. Experiment was performed once but employed three biologically independent samples.



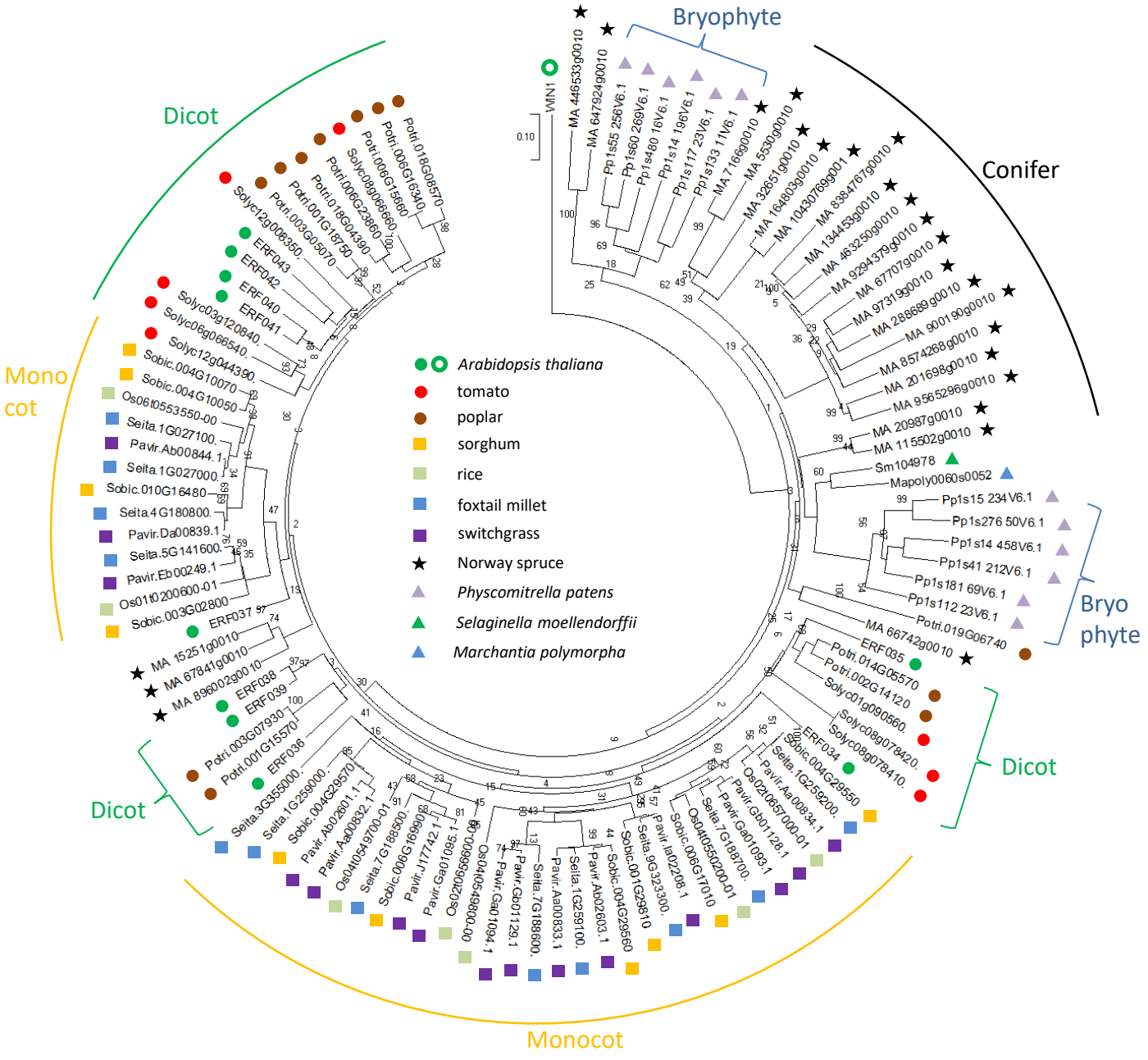
Supplementary Figure 22 | Cellulose properties of wild type, *nst1 nst3* and *nst1 nst3 NST3pro:ERF035-VP16* supporting Fig. 3f. Cellulose crystallinity (a) and degree of cellulose polymerization (b) were determined. Error bars indicate 95% confidence interval for the mean (n = 4 [*Wild-type* and *nst1 nst3*] or 12 [*nst1 nst3 NST3pro:ERF035-VP16*] biologically individual samples). Each dot represents individual data point. Numbers in top of each graph indicate 100-fold of *P* value calculated by the Welch's t-test (two-sided) with Bonferroni-Holm correction. Experiment was performed twice.



Supplementary Figure 23 | Immuno-labelled cross sections of *nst1 nst3 NST3pro:ERF035-VP16* plants show distinct patterns supporting Fig. 4. Cross sections of inflorescence stems of 8-week-old wild-type (a, d, g, j, m, p), *nst1 nst3* double mutant (b, e, h, k, n, q), and *nst1 nst3 NST3pro:ERF035-VP16* (c, f, i, l, o, r) plants decorated with CCRCM89, CCRCM1, LM15 that binds to xyloglucans (a–i), LM5 to galactan (j-l) and LM6 to arabinan (m–o), or LM18 to homogalacturonan (p–r). If, interfascicular fiber; V, vascular bundle. The scale bar indicates 100 μm. Transgenic/mutant plants in this figure were cultivated three times and cross sections were taken from five independent plants which showed similar results



Supplementary Figure 24 | Cellulose is deposited in interfascicular fiber cells of *nst1 nst3 NST3pro:ERF035-VP16* lines, supporting Fig. 4m-o. Cross sections of 8-week-old inflorescence stem were stained with fluorescent dyes in the inflorescence stem of wild type (**a, d, j, m**), *nst1 nst3* (**b, e, k, n**), and *nst1 nst3 NST3pro:ERF035-VP16* lines **(c, f, i, o**). Panels (**m-o**) are magnified view of panels (**j-l**). If, interfascicular fiber; V, vascular bundle. Arrow heads in (j-o) indicate staining of Aniline blue which indicates callose deposition. Note that most light-blue fluorescence represents autofluorescence of lignin. Scale bars = 100 μm. Transgenic/mutant plants in this figure were cultivated three times and cross sections were taken from 5 independent plants which showed similar results.



Supplementary Figure 25 | Phylogenetic tree of group IIId and IIIe ERF genes from various plant species, supporting Fig. 1. Group IIId and IIIe ERF genes were collected from Arabidopsis, tomato, poplar, rice, sorghum, foxtail millet, switchgrass, Norway spruce, *Physcomitrella patens*, *Marchantia polymorpha* and *Selaginella moellendorffii* and phylogenetic tree was prepared by Neighbor-Joining method by using conserved domain. Arabidopsis WIN1 was employed as an outgroup. All sequence information is listed in Supplementary Table 8.