Supplemental Figures

Regulation of pectin remodeling and ectomycorrhiza development by cell wall localized proanthocyanidins in hybrid aspen roots

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Supplemental figures (in this file)

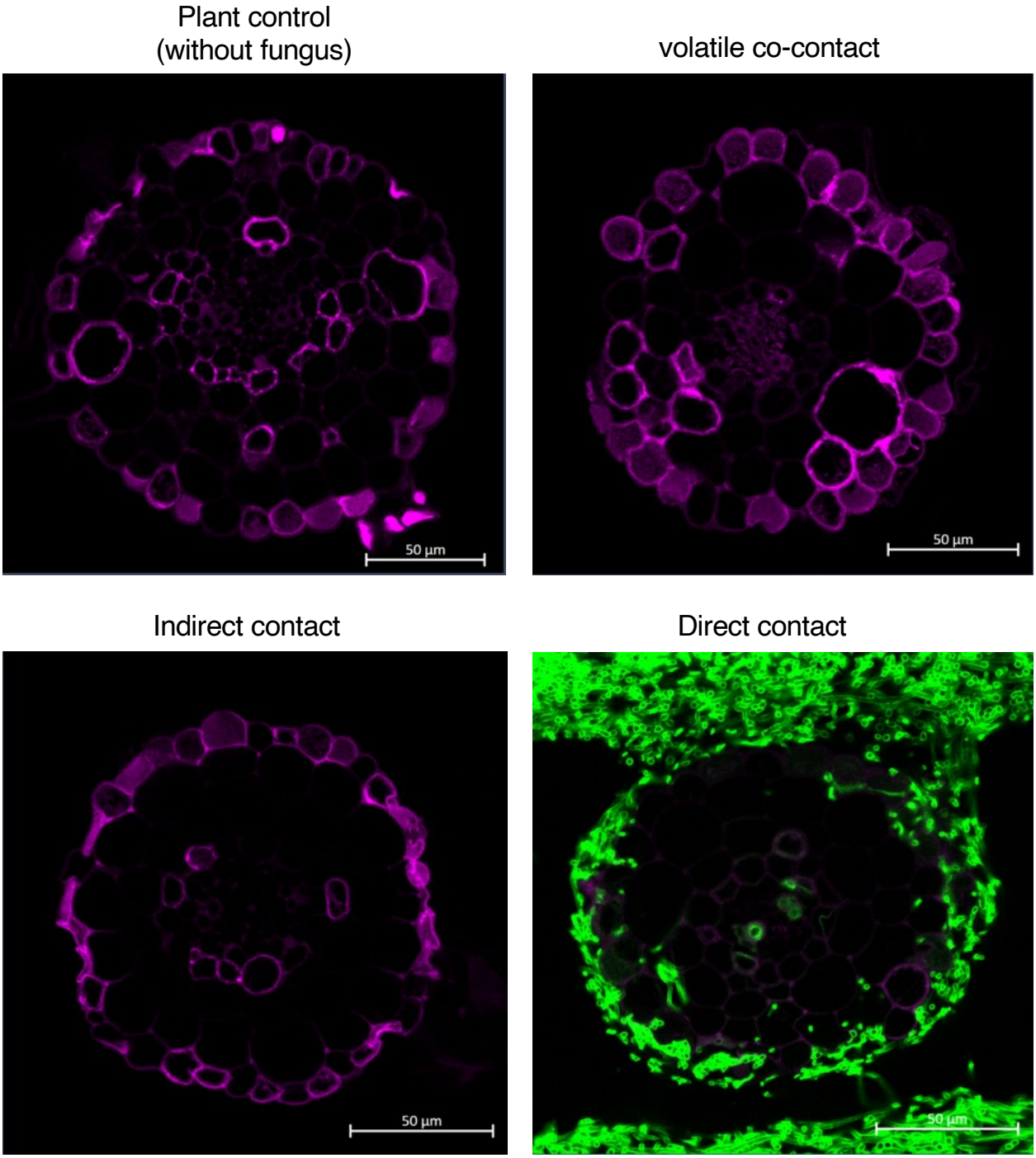

Figure S1. Confocal micrographs showing PA localization in hybrid aspen ECM root cross sections (70 *µ*m thickness) of agarose-embedded lateral roots of T89 plants from different plant-fungal culture systems. Control = Plant roots without fungal contact, Volatile co-contact = Roots from plants kept spatially separated from *Laccaria bicolor* culturing in bi-compartmented Petri dish allowing for gas exchange but no colonization nor solute exchange, Indirect contact = Roots from plants separated from *Laccaria bicolor* mycelia through a cellophane membrane allowing exchange of soluble and volatile signals but prohibiting colonization. Direct contact = Plant roots colonized by *Laccaria bicolor* mycelia. Sections were stained with PA-specific fluorescent dye DMACA (magenta) to observe PA localization. All observations are carried out at 7 days of contact or exposure. All sections were double stained with wheat germ agglutinin (WGA, green) conjugated with Alexa Fluor 488 to visualize the fungus *Laccaria bicolor* of ectomycorrhiza samples. Scale bar = 50 *µ*m. Refers to Figure 1.

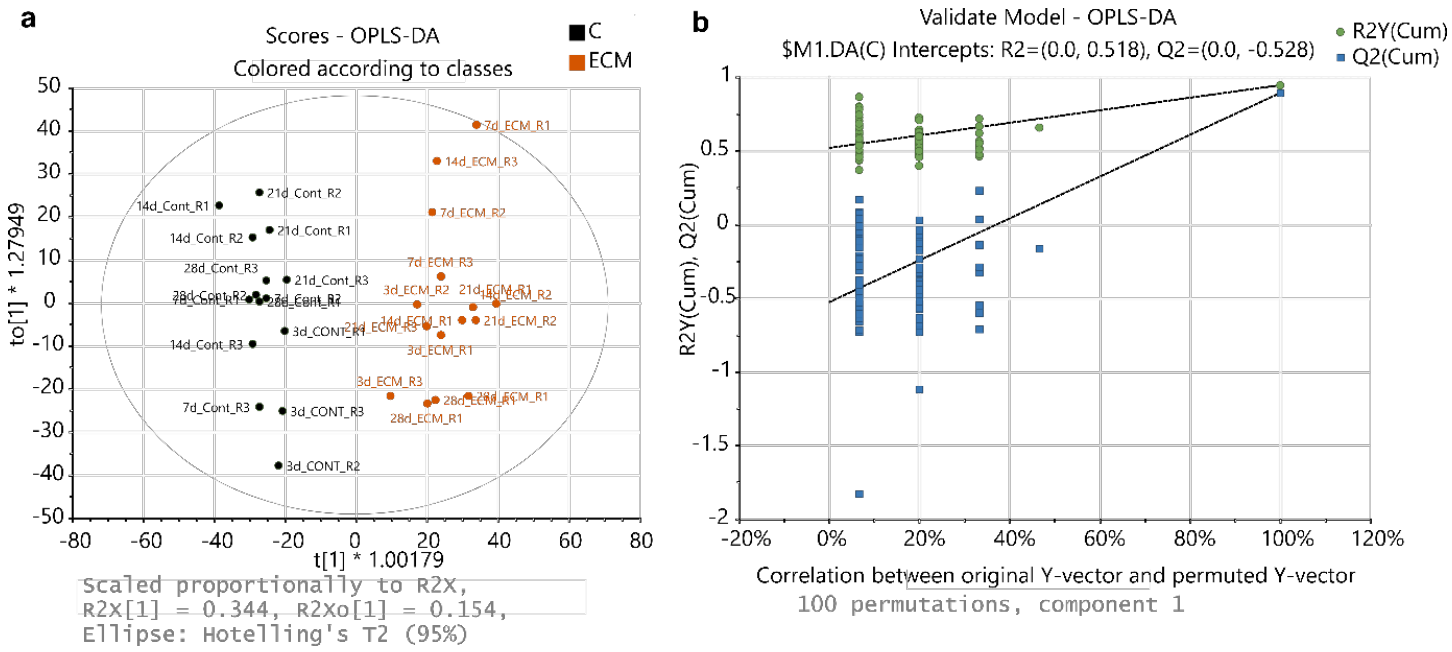

Figure S2. OPLS-DA (Orthogonal projections to latent structures) model scores plot (a) and model validation with 100 permutation tests (b). Data available in Supplementary table S4. Refers to Figure 1.

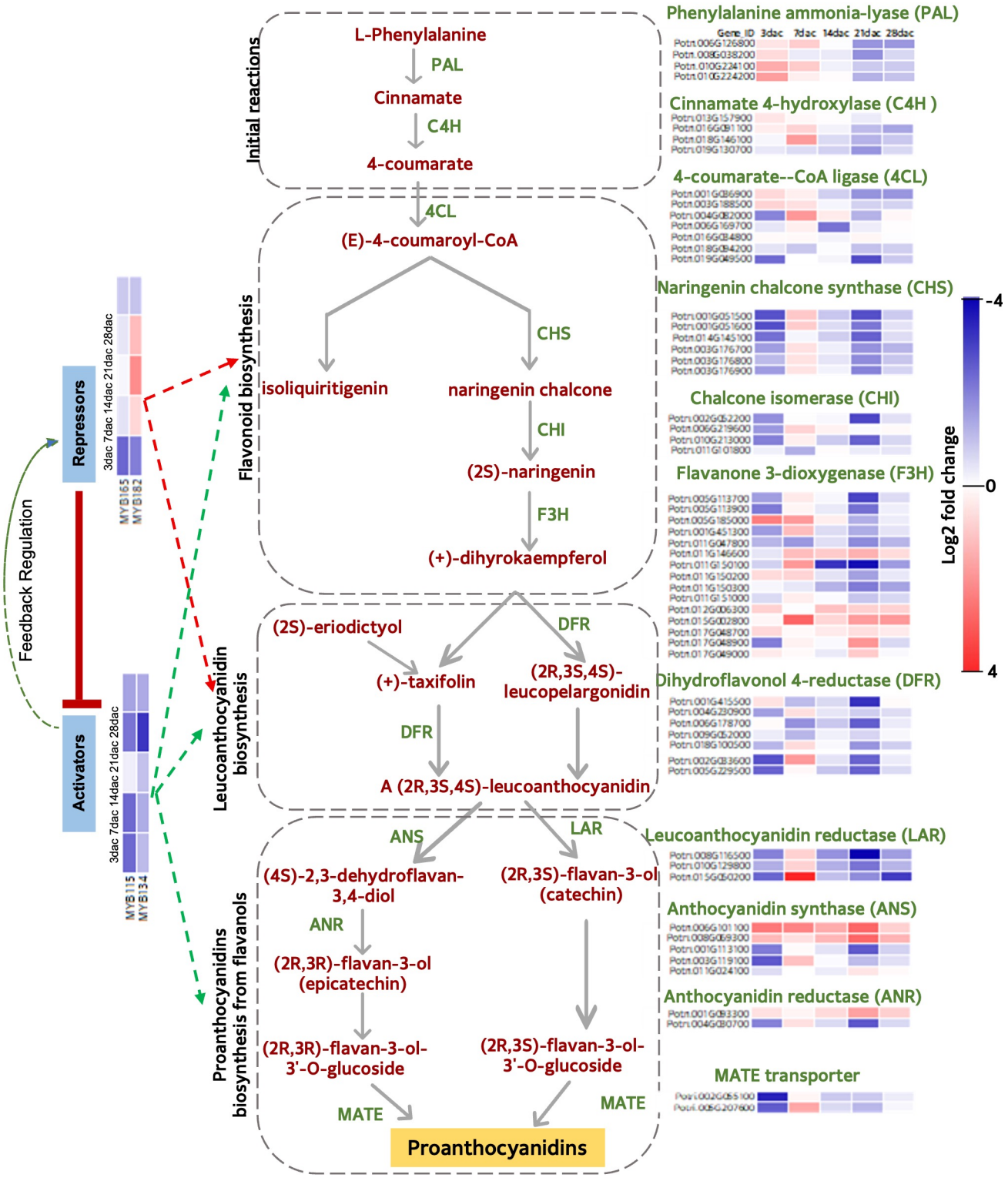

Figure S3. Expression of Proanthocyanidin (PA) biosynthesis genes during ECM development in hybrid aspen roots. The schematic diagram illustrates the key enzymatic steps in the PA biosynthesis pathway, from initial phenylalanine conversion through flavonoid biosynthesis to the final production of proanthocyanidins. Genes associated with each enzymatic step are indicated, with corresponding heat maps showing the log2 fold change in gene expression during ECM development across different time points (3 days after colonization (dac) to 28 dac). The heat maps indicate downregulation (blue) or upregulation (red) of gene expression in mycorrhized roots compared to non-mycorrhized controls. The pathway and genes were selected from "Plant metabolic network (PMN)" and gene expression data was extracted from a previously published RNAseq study (Chowdhury et al. 2022a). Refers to Figure 1.

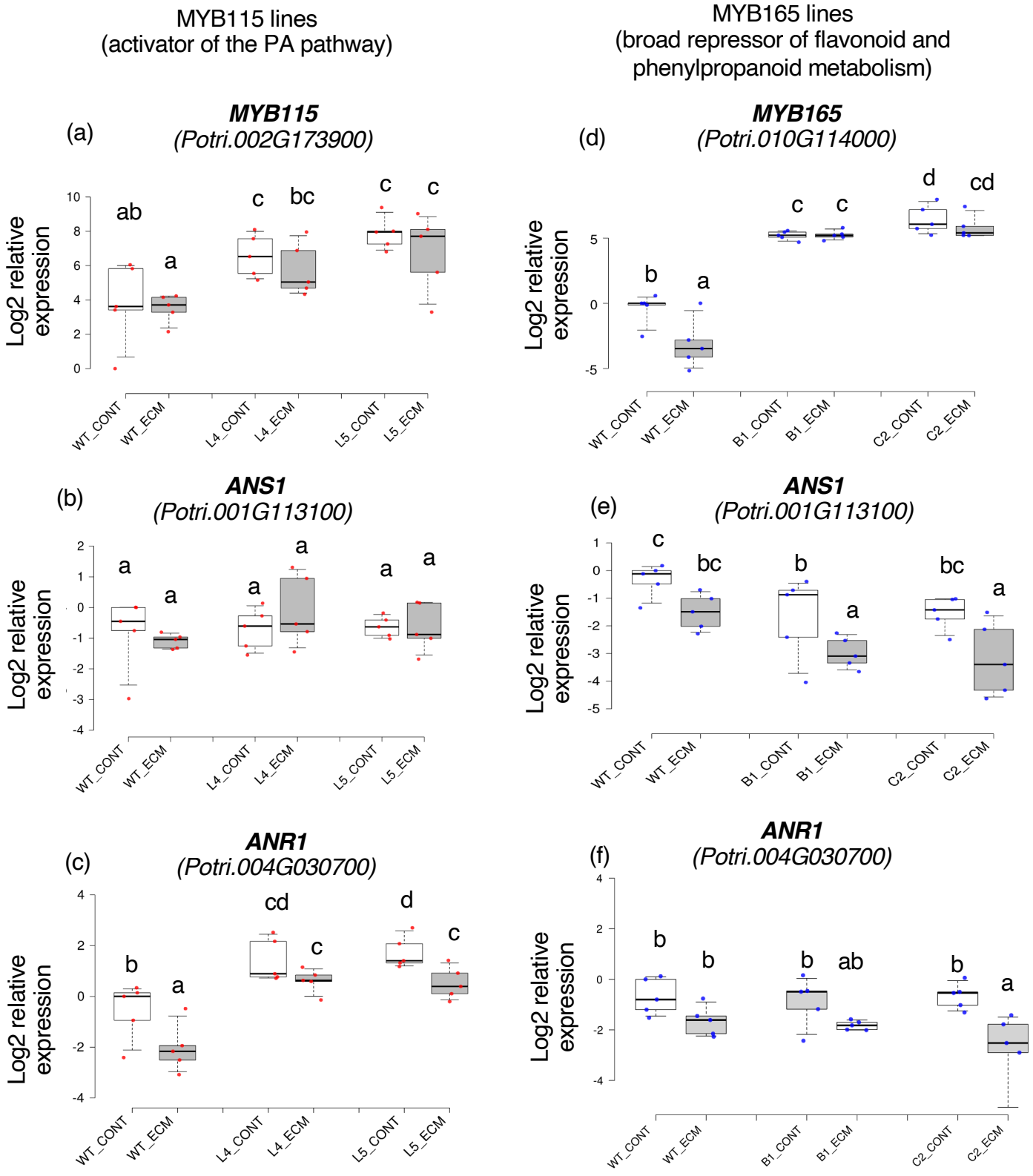

Figure S4: Gene expression of PA biosynthesis genes in roots and ECM of wildtype and PA activator (MYB115 Line L4 and L5), and PA repressor (MYB165 Line B1 and C2) hybrid aspen lines, respectively, determined using RT-qPCR. Boxplots depict the distribution data points with median (line in the box), interquartile range (box), and whiskers indicating the range of the data. Each treatment condition included five biological replicates (one biological replicate comprised with 3-5 lateral roots per plant pooled. Statistical significance was assessed via ANOVA with Fisher's LSD post hoc (Infostat) where values with unique letters indicate significant difference (*P*-value <0.05). Experimental time point was at 21 days after plant-fungal contact (dac). Refers to Figure 2.

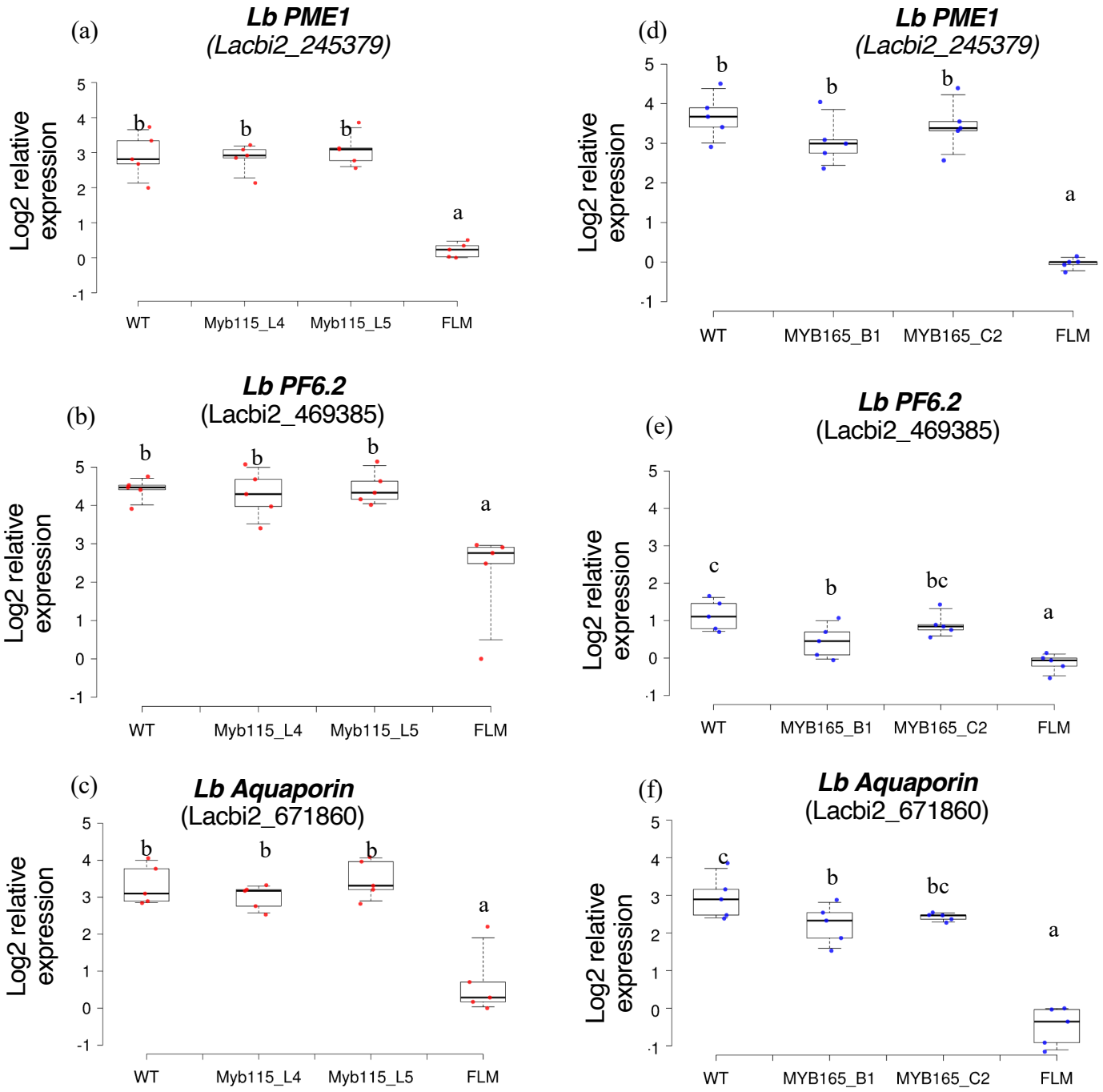

Figure S5: Fungal ECM marker gene expression in wildtype and PA-activator (MYB115 OE Line L4 and L5) and repressor lines (MYB165 OE Line B1 and C2) as well as free-living mycelium (FLM) determined using qRT-PCR. Boxplots depict the distribution data points with median (line in the box), interquartile range (box), and whiskers indicating the range of the data. Each experimental group consisted five biologically separate plant roots. Statistical significance was assessed via ANOVA with Fisher's LSD post hoc (Infostat) where values with unique letters indicate significant difference (*P*-value <0.05). Experimental time point was at 21 days after plant-fungal contact (dac). Refers to Figure 2.

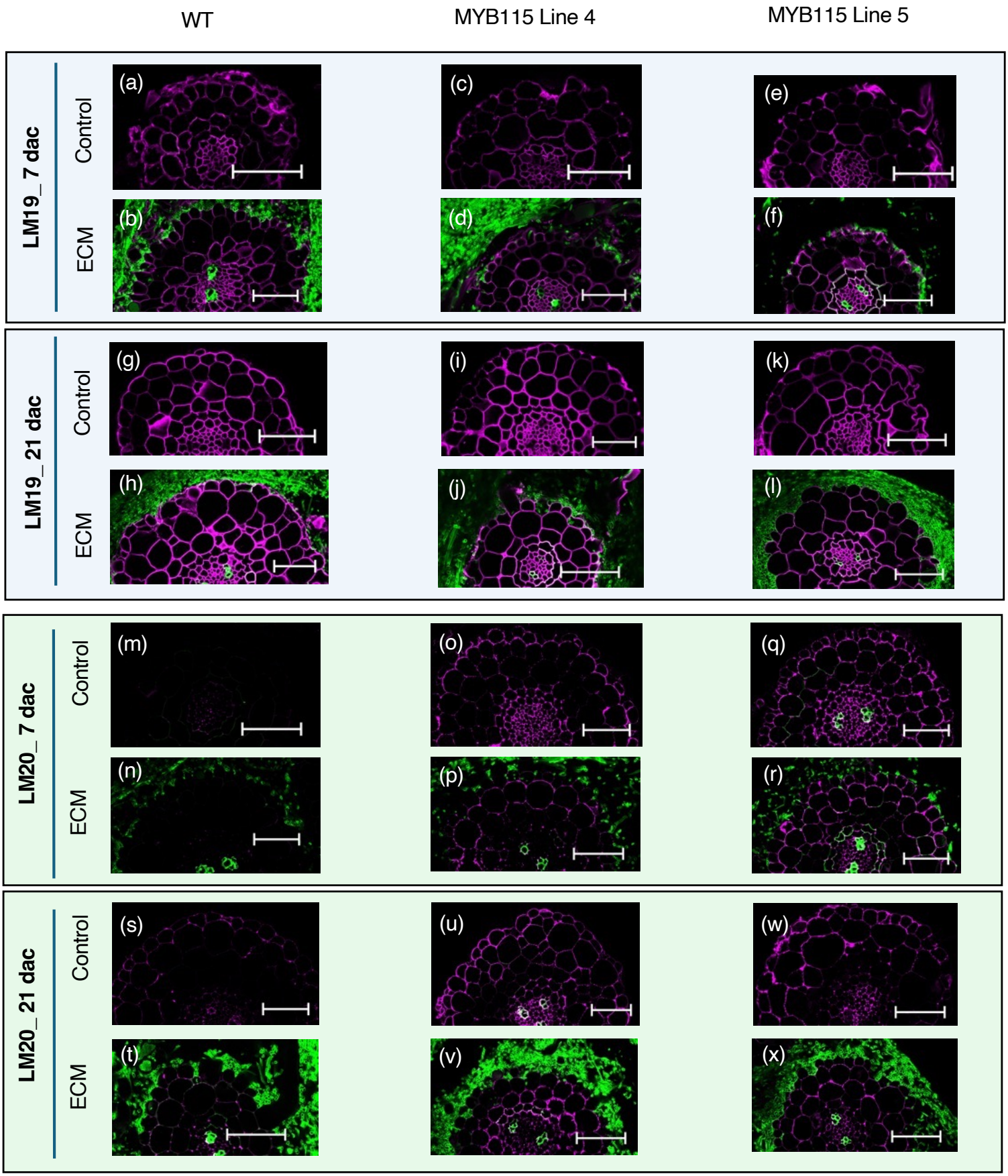

Figure S6. Confocal micrographs of immunolocalization on lateral root cross sections of PA-altered MYB115 overexpressed lines and wildtype plants. Non-colonized roots and ECM cross sections (70 μm thick) embedded in agarose were immunolabelled with pectin antibodies **LM19** (recognising de-esterified pectins at 7dac (a-f) and 21 dac (g-l)) and **LM20** (recognising highly methyl-esterified pectins at 7dac (m-r) and 21 dac (s-x)). *Laccaria bicolor* is visualised with wheat germ agglutinin (WGA) conjugated to Alexa Fluor 488 (in green); and plant cell-wall pectin is visualised with fluorescence of Cy5 (in magenta) conjugate with secondary antibody that binds with LM19 and LM20. The scale bar represents 50 μm. Refers to Figure 5.