Plants as non-mobile organisms constantly integrate varying environmental signals to flexibly adapt their growth and development. Local fluctuations in water and nutrient availability, sudden changes in temperature or other abiotic and biotic stresses can trigger changes in the growth of plant organs. Multiple mutually interconnected hormonal signaling cascades act as essential endogenous translators of these exogenous signals in the adaptive responses of plants. Although the molecular backbones of hormone transduction pathways have been identified, the mechanisms underlying their interactions are largely unknown. Here, using genome wide transcriptome profiling we identify an auxin and cytokinin cross-talk component; SYNERGISTIC ON AUXIN AND CYTOKININ 1 (SYAC1), whose expression in roots is strictly dependent on both of these hormonal pathways. We show that SYAC1 is a regulator of secretory pathway, whose enhanced activity interferes with deposition of cell wall components and can fine-tune organ growth and sensitivity to soil pathogens.
plants are sessile organisms, so throughout evolution, this lack of mobility has been compensated for by a unique survival strategy—an exceptional developmental plasticity of the plant body. Plants are able to rapidly modulate their growth and whole architecture in order to efficiently use local resources and adapt to fluctuating environmental conditions. Plant hormones are essential mediators and endogenous transducers of these environmental inputs. Hormonal pathways connected via multiple levels of interactions form powerful regulatory networks that sensitively react to changes in the environment and drive the relevant adaptive responses. Currently, a hormonal network formed by the two developmentally essential hormones, auxin and cytokinin, is among the best characterized endogenous regulatory systems1,2. Antagonistic inputs of auxin and cytokinin balance proliferation and differentiation of meristematic cells to maintain the root and shoot apical meristem activity3,4, as well as defining the branching pattern of both roots and shoots5,6. Conversely, cell division and growth in plant tissue cultures, or rapid elongation growth of roots, is under the synergistic control of cytokinin and auxin.8. The auxin pathway is a guarantor of plant developmental plasticity and coordination of plant development is obvious. Such a hormonal network, the complexity of the mechanisms underlying the interactions form powerful regulatory networks these environmental inputs. Hormonal pathways connected via cytokinin crosstalk, we performed genome wide transcriptome analysis performed on 5-day-old seedlings exposed to auxin (1 μM 1-naphthaleneacetic acid; NAA), cytokinin (10 μM N6-benzyladenine), and both hormones simultaneously for 3 h. As the original focus of the project was on genes involved in root branching, the transcriptome profiling was performed on pericycle tissue after sorting cells expressing a green fluorescent protein (GFP) reporter in J1201 reporter lines. SYNERGISTIC ON AUXIN AND CYTOKININ 1 (SYAC1, AT1G15600), which encodes a protein of unknown function, was among the top candidate genes selected for their expression being synergistically upregulated by simultaneous hormonal treatment when compared with the expected additive effect of both hormones applied separately. After a 3 h of treatment with either auxin or cytokinin increased SYAC1 expression (2.47- and 1.53-fold, respectively, n = 3 each) was detected, whereas application of both hormones simultaneously resulted in 16.36-fold (n = 3) higher expression compared with the untreated control (Supplementary Fig. 1a). The SYAC1 expression profile in roots was further validated by quantitative real-time (RT-qPCR) (Fig. 1a). Further to this, we found a significant increase of SYAC1 transcription only 30 min after application of both hormones when compared with untreated roots (Fig. 1b), thus indicating that SYAC1 is among the early response genes rapidly induced by auxin and cytokinin. Lack of either cytokinin or auxin perception mediated through CRE1-12/AHK4, AHK3 and TIR1, AFB2 receptors, respectively, severely attenuated transcription of SYAC1 in response to dual auxin and cytokinin treatment (Supplementary Fig. 1b); suggesting that both cytokinin and auxin signaling cascades contribute to synergistic regulation of SYAC1 transcription.

To examine the spatio-temporal pattern of SYAC1 expression and its responsiveness to hormones in roots, the SYAC1 promoter was cloned with either β-Glucuronidase (GUS), nuclear localized GFP reporters, or SYAC1 genomic coding sequence fused to GFP. The basal expression of pSYAC1:GUS, pSYAC1:nlsGFP, and pSYAC1:gSYAC1-GFP reporters was under the threshold of detection, however, exposure to cytokinin for 6 h enhanced reporter signal in the quiescent center (QC) and columella initials (CI) (Fig. 1c; Supplementary Fig. 1c–e). When treated with auxin the activity of pSYAC1 in the QC and provascular of the root apical meristem could be detected (Fig. 1c; Supplementary Fig. 1c). The promoter activity of pSYAC1 was substantially enhanced by the simultaneous application of both hormones, and remarkably, a strong reporter signal was detected in the differentiation and rapid elongation zone, a pattern not observed in roots exposed to either of the hormones separately (Fig. 1c and Supplementary Fig. 1c–e). As the initial concentrations of auxin (1 μM) and cytokinin (10 μM) used for transcriptome profiling within 3 h were relatively high, we tested the sensitivity of the pSYAC1 reporter line to varying concentrations of both hormones. We confirmed that pSYAC1 sensitively responded to simultaneous application of both hormones, and that their application at concentrations of 0.25 μM each is sufficient to trigger reporter expression in the root differentiation and elongation zones (Supplementary Fig. 1f). These results demonstrate that the SYAC1 promoter is under the tight control of the combined and synergistic action of auxin and cytokinin. When each hormone was applied individually, SYAC1 expression was activated in cells known to exhibit either auxin or cytokinin response maxima, such as QC/CI27 or cells of the provascular28, respectively. Intriguingly, SYAC1 transcription in the root differentiation and elongation zone was fully dependent on the simultaneous enhancement of both auxin and cytokinin.

As application of cytokinin and auxin might lead to the dysregulation of other hormonal pathways, in particular that of ethylene29, we also examined the sensitivity of SYAC1 to this
hormone. No enhancement of pSYAC1:GUS expression was detected in roots treated with either 1-aminocyclopropane-1-carboxylic acid (ACC, a precursor of ethylene biosynthesis) only or in combination with either cytokinin, auxin, or both hormones together (Supplementary Fig. 1g). Likewise, treatment with other hormones such as abscisic acid (ABA), methyl jasmonate (MeJA), brassinosteroids (BR), gibberellins (GA 3), or inhibitor of GA biosynthesis paclobutrazol (PAC) did not trigger or interfere with SYAC1 expression (Supplementary Fig. 1h). Taken together, the expression analysis confirms SYAC1 as a common target of the auxin and cytokinin pathways acting in roots.

Spatio-temporal pattern of SYAC1 expression in planta. To explore the growth and developmental processes in which SYAC1 might be involved, we monitored its expression at different stages of Arabidopsis thaliana development. Strong SYAC1 promoter activity was detected in the embryonic hypocotyl and cotyledons, but not in the embryonic root (Fig. 1d). During germination, in 2-day-old seedlings, the pSYAC1:GUS activity remained strong in cotyledons and the upper part of the hypocotyl, however, in the lower hypocotyl its expression ceased almost completely with commencing rapid elongation growth (Fig. 1e). As growth of hypocotyl and cotyledons progressed, in 3- to 4-day-old seedlings, SYAC1 promoter activity in these organs gradually attenuated (Fig. 1f, g) and in ~3-week-old seedlings expression was under detection limit (Supplementary Fig. 1i). In adult 8-week-old Arabidopsis plants, pSYAC1:GUS signal was observed in the upper part of the stem and the abscission zone of siliques (Fig.1h; Supplementary Fig. 1j). In etiolated seedlings, the SYAC1 promoter activity was concentrated in short cells at the inner (concave) side of the apical hook, whereas no signal was detected in expanded cells at outer side of the hook (Fig. 1i). Based on these data, SYAC1 function seems not to be limited to plant roots and its expression pattern spatio-temporally largely correlates with processes involving the control of elongation growth.

SYAC1 impacts on elongation growth of plant organs. To gain insights into the developmental function of SYAC1, we performed a detailed phenotypic analysis of plants with either a reduced or an enhanced activity of this gene. In the available mutant alleles,
the T-DNA is inserted either in the middle of the 3′ untranslated region (syac1-1, syac1-2, and syac1-3) or in the middle of the second intron (syac1-4) (Supplementary Fig. 2a). As in the syac1-3 allele expression of the gene is not fully suppressed (Supplementary Fig. 2b), we obtained an additional syac1-5 mutant line using the CRISPR/Cas9 approach. In the syac1-5 plant, the CRISPR/Cas9 cassette introduces an extra thymine at 90 bps after the ATG, which results in a STOP codon after 33 amino acids in the SYAC1 coding sequence (Supplementary Fig. 2a). In addition, to investigate the impact of increased SYAC1 expression on plant development, the transgenic lines SYAC1-HAox, HA-SYAC1ox, SYAC1-GFPox, and GFP-SYAC1ox carrying SYAC1 fused to either the -HA tag or a GFP reporter under the control of the 35S promoter were generated and enhanced expression was confirmed using RT-qPCR and western blot approaches (Supplementary Fig. 2c, d).

Given the observed pattern of SYAC1 expression, we focused on growth processes involving the tightly controlled cell expansion, such as apical hook development, hypocotyl elongation, and primary root growth. Specific expression at the concave side of the apical hook prompted us to more closely investigate SYAC1 function in this developmental process. In control Arabidopsis seedlings, shortly after germination, a progressive opening of the hook occurred around 180° (formation phase, F). This bend was stabilized cotyl progressively bent to establish an apical hook with an angle syac1-3 fa

**SYAC1 localizes to secretory pathway compartments.** To explore cellular function of SYAC1, we next compared its subcellular localization in Arabidopsis root cells with specific reporters for cellular compartments. In the estradiol inducible line, 6 h after induction SYAC1-GFP is restricted to small compartments in the cell interior. Measurement of Pearson correlation coefficient revealed a high SYAC1 colocalization pattern with Golgi and trans-Golgi (TGN) compartments labeled by the anti-SEC21 (0.57 ± 0.01; n = 24) and anti-ECH (0.51 ± 0.02; n = 36) antibody, respectively. This subcellular localization was further confirmed by colocalization with anti-ARF1 (0.45 ± 0.02; n = 34) and anti-SYP61 (0.49 ± 0.02; n = 34) antibodies, which label both Golgi and TGN. A significant colocalization was also observed with the pre-vacuolar/endosomal compartments (PVC), labeled with a mixture of anti-ARA7 and anti-RHA1 (0.52 ± 0.02; n = 33), or anti-VSR (0.34 ± 0.03; n = 13) antibodies. In contrast, almost no colocalization was observed between SYAC1 and anti-BIP2 (0.04 ± 0.04; n = 12) and anti-PIN2 (0.03 ± 0.04; n = 21) antibodies, which label the ER and the plasma membrane, respectively (Fig. 3a, b).

Accordingly, the SYAC1-GFP signal in SYAC1-GFPox line exhibited strong colocalization with markers for Golgi (anti-SEC21; 0.55 ± 0.02; n = 34), TGN (anti-ECH; 0.60 ± 0.02; n = 41), both of them (anti-ARF1; 0.55 ± 0.02; n = 41 and anti-SYP61; 0.40 ± 0.02; n = 40) and PVC (anti-ARA7/anti-RHA1; 0.44 ± 0.02; n = 39; anti-VSR; 0.47 ± 0.02; n = 31) but almost no colocalization with markers for ER (anti-BIP2; 0.01 ± 0.03; n = 22) and the plasma membrane (anti-PIN2; 0.02 ± 0.02; n = 35) (Supplementary Fig. 3a, b). To further validate the immunocolocalization results, we crossed the GFP-SYAC1ox line with the multicolor “Wave” marker set35 for analysis of plant endomembrane compartments. We confirmed colocalization of SYAC1 with markers for Golgi (wave 18R; 0.53 ± 0.03; n = 16 and wave 127R; 0.42 ± 0.02; n = 15), Golgi and endosomes (wave 25R; 0.69 ± 0.03; n = 20, and wave 29R; 0.35 ± 0.03; n = 12), Golgi and TGN (SYP61/SYP61-CFP; 0.45 ± 0.02; n = 28), TGN and early endosomes (wave 13R; 0.27 ± 0.06; n = 6) as well as for endosomes/recycling endosomes (wave 34R; 0.31 ± 0.05; n = 9 and wave 129R; 0.33 ± 0.02; n = 18). In agreement with immunocolocalization, SYAC1 displayed only minor colocalization with markers for ER/plasma membrane (wave 6R; 0.06 ± 0.02; n = 9), plasma membrane (wave 131R; 0.02 ± 0.02; n = 21 and wave 138R; 0.02 ± 0.03; n = 12) and vacuoles (wave 9R; 0.03 ± 0.02; n = 12) (Supplementary Fig. 3c, d). These results strongly support that SYAC1 largely resides in the Golgi, TGN, and endosomal and PVC compartments.
SYAC1 is a component of the ECHIDNA/Yip complex. To further assess molecular function of SYAC1, we identified its molecular interactors using a tandem affinity purification (TAP) assay with SYAC1 used as bait. Proteins including the integral membrane YIP1 family protein (YIP5b; At3g05280), β-ketoacyl reductase 1 (KCR1; At1g67730), an ubiquitin receptor protein (DSK2; At2g17200), and prohibitin 4 (PHB4; At3g27280) were recovered by this approach (Supplementary Data 1 and 2). As YIP5b is a member of the YIP (for YPT/RAB GTPase Interacting Protein) family in Arabidopsis thaliana that forms a TGN-localized complex with YIP4a (At1g18840) and YIP4b (At4g30260) homologs and Echidna (ECH; At1g09330) integral membrane protein34,35, we included them in our subsequent detailed interaction studies. A Yeast two-hybrid assay (Y2H) revealed a strong bimolecular fluorescence complementation (BiFC) assay. SYAC1 interacted strongly with KCR1 and not at all with the DSK2 and PHB4 proteins (Fig. 4a). The Y2H results were further validated in planta using a bimolecular fluorescence complementation (BiFC) assay. SYAC1 tagged with the C-terminus of Enhanced Yellow Fluorescent
Protein (EYFP), and YIP5b, YIP4a, YIP4b, ECH, KCR1, DSK2, and PHB4 tagged with the N-terminus of EYFP, were transiently expressed in an *Arabidopsis* root suspension culture. Yellow fluorescence was detected in protoplasts overexpressing SYAC1 in combination with YIP5b, YIP4a, YIP4b, and ECH, indicating the close physical proximity of these proteins in vivo. By contrast, no EYFP reconstitution was detected in cells overexpressing SYAC1 with KCR1, and PHB4 (Fig. 4b), respectively, in agreement with the result of the Y2H assay. Finally, the interaction between SYAC1 and YIP4a and between SYAC1 and ECH was also confirmed by a co-immunoprecipitation (Co-IP) assay (Fig. 4c). The results from TAP, BiFC, and Co-IP assays revealed SYAC1 has

**Fig. 3 SYAC1 colocalizes with Golgi/TGN/endosomal/PVC markers.** Co-immunolocalization of SYAC1-GFP with markers for different subcellular compartments (a) and quantification of colocalization using Pearson correlation coefficient (b). Five-day-old pEST:SYAC1-GFP seedlings grown on mock medium treated with 5 μM estradiol for 6 h used in co-immunolocalization experiments (*n* = 10 roots with 1–5 epidermal cells each). Co-immunolocalization performed using antibodies against anti-GFP and subcellular markers. In the boxplots, center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by Origin software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, individual data points are represented by dots. ER Endoplasmic reticulum, TGN trans-Golgi network, PVC prevacuolar compartment, PM plasma membrane. Scale bar 5 μm.
interactions with YIP5b, YIP4a, YIP4b, and ECH protein, and suggest it may function in the protein complex involved in maintaining the functionality of the secretory pathway.

SYAC1 affects activity of subcellular trafficking machinery. SYAC1 localization in Golgi/TGN/endosomal/PVC compartments and the interaction with ECH/YIPs pointed toward a potential function in the secretory pathway. The secretory pathway is of vital importance for all eukaryotic cells, since it manufactures, stores and distributes macromolecules, lipids and proteins as cargo to intracellular and extracellular locations. To assess the involvement of SYAC1 in the regulation of secretion, we performed a transient expression assays in Arabidopsis mesophyll protoplasts and evaluated the impact of SYAC1-HAox with ECH-HAox on the secretory index of the α-Amylase reporter. Amy is a protein that without any intrinsic sorting signal is secreted extracellularly and can be detected by its endogenous enzymatic activity. The secretion index (SI) was determined by quantifying the ratio of the α-Amylase activity in the medium and in the cells. The expression of the SYAC1 protein decreased the SI from 0.70 ± 0.04 (n = 4) in control sample to 0.55 ± 0.02 (SYAC1-HAox; n = 4) and 0.45 ± 0.01 (HA-SYAC1ox; n = 4), which suggests a function of SYAC1 as a negative regulator of the anterograde secretory route to the cell surface (Fig. 5a). Due to the colocalization of SYAC1 with marker for PVC compartments, we decided to explore the involvement of SYAC1 in transport to the vacuoles. To do this, an α-Amylase with a vacuolar sorting signal (Amy-Spo) was co-transfected with either SYAC1-HA or HA-SYAC1 encoding plasmids. The SI of α-Amylase (Amy-Spo) was increased from 0.07 ± 0.01 (n = 4) in the control sample to 0.29 ± 0.01 (SYAC1-HAox; n = 4) and 0.28 ± 0.03 (HA-SYAC1ox; n = 4), which suggests that SYAC1 might also interfere with transport to vacuoles leading to more α-Amylase secretion out of the cells (Fig. 5a). The effect of SYAC1 on α-Amylase containing an ER retention signal (Amy-HDEL) was tested. Co-transfection of SYAC1 significantly decreased the SI in protoplasts with leaky retention of α-Amylase from 0.34 ± 0.01 (n = 4) in the control sample to 0.24 ± 0.01 (SYAC1-HAox; n = 4) and 0.26 ± 0.04 (HA-SYAC1ox; n = 4) (Fig. 5a). Altogether these results support the conclusion that SYAC1 coordinates cargo trafficking toward the extracellular space and vacuoles.
SYAC1 fine tunes the cell wall composition and mechanics. In plants, new cell wall components such as pectins and hemicellulose are proposed to be delivered to the extracellular matrix via the secretory pathway. SYAC1 reduction of α-Amylase secretion, along with its Golgi/TGN/endosomal localization and interaction with YIPs and Echidna proteins, a previously found regulatory components of secretory pathway, motivated us to explore the role of SYAC1 in the control of soluble cell wall polysaccharides (pectin and hemicellulose) secretion. Investigating the seed coat epidermis, whose TGN is highly specialized for pectic mucilage...
Fig. 5 SYAC1 regulates composition of cell wall and alters its physical properties. a SYAC1 affects α-Amylase secretion index determined by quantifying ratio of the α-Amylase activity in the medium and in the cells. Transient co-expression of SYAC1 with α-Amylase (Amy) and its derivatives carrying different C-terminal sorting motifs, including vacuolar sorting (Amy-spo) and ER retention (Amy-HDEL) motif. (n = 4 biological and 2 technical replicates; average ± SE). Expression of SYAC1 fusion constructs confirmed by western blot analysis (insets) using anti-HA specific antibodies. b Ruthenium red-stained seed coat mucilage after imbibition of wild-type, SYAC1-GFPox, syac1-3, and syac1-5 seeds. Representative images shown (~100 seeds stained per line). Scale bar 200 μm. c FT-IR measurements in 4-day-old etiolated hypocotyls show alterations in cell wall composition in SYAC1-HAox lines. d The amount of galacturonic acid extracted from 4-day-old etiolated hypocotyls (n = 4–6, average ± SE). Wild type I represents respective control to SYAC1-GFPox and GFP-SYAC1ox. Wild type II was isolated from syac1-5 heterozygote population. e The apparent Young modulus measured by atomic force microscopy (AFM) in 4-day-old etiolated hypocotyls of wild type and SYAC1-GFPox. In the boxplots, center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by Origin software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, individual datapoints are represented by dots, n = 10–14. Significant differences are indicated as *P < 0.05, **P < 0.01, and ***P < 0.001 (t-test).

secretion39, using ruthenium red staining assay revealed that mucilage release from mature seeds was greatly reduced in SYAC1-GFPox seeds, relative to wild type (Fig. 5b, Supplementary Fig. 4a). This is in accordance with the anticipated function of SYAC1 as an inhibitor of polysaccharide secretion and with the previously described ech-1 and yip4a/yip4b mutants35. In syac1-3 or syac1-5 mutants, no dramatic change in mucilage secretion could be detected, presumably due to the lack of SYAC1 expression in seed coat epidermal cells (Supplementary Fig. 4b).

The delivery of new cell wall components during pollen tube growth is a particularly active process, and the main secreted cell wall component in the pollen tube apex is pectin. SYAC1 expression in tobacco pollen tubes severely affected the apical accumulation of pectin (Supplementary Fig. 4c–d) and resulted in an increased proportion of pollen tubes with growth defects (Supplementary Fig. 4i–k). This observation supports a role for SYAC1 in modulating pectin distribution, presumably by influencing the activity of the secretory pathway. As in Arabidopsis root cells the localization of plasma membrane proteins such as PIN1 and PIN2 was not affected, we conclude that SYAC1 might preferentially regulate specific branches of the secretory pathway (Supplementary Fig. 5a). Taken together, the data from altered pectin mucilage and pectin secretion in seeds and pollen tubes overexpressing SYAC1, respectively, support a function of SYAC1 in modulating the delivery of cell wall matrix polysaccharides.

To further assess the impact of SYAC1 on cell wall composition, hypocotyls of etiolated seedlings were inspected using Fourier transform-infrared (FT-IR) microspectroscopy. FT-IR analysis revealed that enhanced SYAC1 expression in plant cells substantially alters the composition of cell walls, which is manifested by a significantly reduced proportion of carbohydrates (Fig. 5c; Supplementary Fig. 5b). To further dissect the qualitative changes in the cell wall, analyses of pectin content and xyloglucans, two major components of cell walls, with modified SYAC1 expression were performed using hypocotyls of 4-day-old seedlings40. A quantitative analysis of galacturonic acid, a key structural component of pectin, did not reveal any significant changes in the hypocotyls of the syac1 loss of function mutant when compared with controls, consistent with only modest defects on syac1 hypocotyl growth (cf. Fig. 2b). By contrast, the amount of galacturonic acid extracted from hypocotyls of seedlings with enhanced expression of SYAC1 was significantly reduced when compared with controls (Fig. 5d). The pectin polysaccharides are secreted in a methylsterified form41. In correlation with a reduced amount of pectin in the cell wall, methanol content was decreased in hypocotyls of seedlings overexpressing SYAC1 (Supplementary Fig. 5c). Unlike pectin, no changes in the amount of fucose, galactose, and xylose, which are indicators of xyloglukan content in the cell wall, were detected in seedlings with either loss or enhanced expression of SYAC1 (Supplementary Fig. 5d). Together these results suggest that SYAC1 is involved in the control of pectin allocation to the cell wall.

Alterations in cell wall composition might ultimately result in changes in cell wall physical properties. Analyses of etiolated hypocotyls using atomic force microscopy (AFM) revealed a significantly reduced apparent Young modulus in the extra-cellular matrix of SYAC1 overexpressor line when compared with control (Fig. 5e). The observed reduction of stiffness of cell walls might affect the elasticity of hypocotyl tissues with increased expression of SYAC1, and thus impact on cell expansion, susceptibility of plant tissues to shearing, breaking, but also to attack of pathogens42–44. These results support the conclusion that SYAC1 might act as a regulator of pectin distribution, presumably through control of the TGN-mediated trafficking, and ultimately affect the composition and physical properties of cell walls.

SYAC1 might act as a modulator of YIP/ECH complex activity. Our screen for interacting partners revealed that SYAC1 can interact with YIPz and ECH, components of a protein complex required for the proper operation of the secretory pathway35. Intriguingly, compromised functionality of the YIP/ECH complex leads to cellular and developmental defects reminiscent of those caused by enhanced activity of SYAC1. Similarly to STAC1ox, yip4a, yip4b, and ech loss of function mutants displayed a deficiency in the secretion of pectins, as well as defects in the growth of roots and hypocotyls, and in apical hook development45. To explore SYAC1 function as a potential attenuator of YIP/ECH complex activity, we tested whether relief of the SYAC1 mediated inhibition of the secretory pathway might alleviate growth defects caused by defects of the YIP/ECH complex. To test this hypothesis, the syac1-3 allele was introduced into the yip4a, yip4b mutant background, and the resulting combined genotype was phenotyped. Importantly, the syac1-3 yip4a yip4b triple mutant displayed significantly improved growth of hypocotyls and shoot organs when compared with the yip4a, yip4b double mutant, indicating that SYAC1 might indeed act as a negative regulator of the YIP/ECH complex (Supplementary Fig. 6a–c). Based on these observations we conclude (i) that the constitutively expressed YIPz and ECH35 act as generic factors required for the secretion of cell wall components to maintain cell expansion and (ii) that the spatio-temporally controlled expression pattern of SYAC1 serves as a developmentally specific regulator of the YIP/ECH complex to fine tune secretory pathway activity and thereby plant organ growth.

SYAC1 impacts on root sensitivity to soil pathogens. In our experimental conditions the basal expression of SYAC1 in roots is very low and its activation requires simultaneous activation of the auxin and cytokinin pathways, thus raising a question about the function of SYAC1 in this organ. A possible explanation concerns the heterogeneous soil environment, where roots are exposed to a large variety of biotic and abiotic factors. Rhizospheric microbes
are among prominent biotic factors which have developed different strategies, including the modification of phytohormone responses, to penetrate, colonize, and hijack nutrients from host plants. Importantly, the early steps of pathogen infection can be associated with modulation of auxin and cytokinin levels in roots, for instance for the pathogenic protist *Plasmodiophora brassicae*, the causal agent of clubroot disease in cruciferous plants. To examine whether SYAC1, which we identified as an auxin–cytokinin crosstalk component, might be involved in host-protist interaction, we analyzed effects of the pathogen on SYAC1 expression, and tested the susceptibility of plants with altered SYAC1 expression to the pathogen.

**Fig. 6 Loss of SYAC1 activity increases tolerance to *Plasmodiophora brassicae* infection.** a syac1-3 and syac1-5 mutant alleles exhibited reduced sensitivity to fungal infection when compared with wild-type control. In contrast, the overexpression of SYAC1ox results in oversensitivity to the pathogen. Five-scale classification was used to evaluate disease severity: 0 (no symptoms), 1 (very small galls mainly on lateral roots and that do not impair the main root), 2 (small galls covering the main root and few lateral roots), 3 (medium to large galls, also including the main root; plant growth might be impaired), and 4 (severe galls on lateral root, main root, or rosette; fine roots completely destroyed; plant growth is reduced). Inoculation was performed with 10^4, 10^5, and 10^6 spore concentration. Significant differences between datasets are indicated by different letters. Data were statistically analyzed using the Kruskal–Wallis test.
patterns determined in roots were also apparent for above-ground tissues, with rosettes of syac1-3 and syac1-5 being less affected compared with wild type at higher spore concentrations, and SYAC1 overexpressors displaying symptoms of hypersensitivity (Supplementary Fig. 7c). Accordingly, the fresh weight of SYA Clox plants at 10^3 and 10^4 spores mL^-1 concentrations was reduced when compared with wild type. In contrast, infected syac1-3 and syac1-5 plants were affected less in their fresh weights compared with the overexpressor plants, but similar as wild type (Supplementary Fig. 7d). Together these results indicate that SYAC1-mediated control of cell wall composition in roots might also be involved in root–pathogen interaction.

Discussion

Auxin and cytokinin play important regulatory roles in various aspects of plant development. The current largely accepted view is that auxin acts mostly antagonistically with cytokinin to control developmental processes3,51. In root development, this antagonism is based on the competition between auxin as a promoter of cell division, and cytokinin as a promoter of cell differentiation, with both inputs contributing to the regulation of root meristem size3,7,52. In addition, to specify the root stem-cell niche during embryogenesis, auxin represses cytokinin action51. However, this antagonistic interaction between auxin and cytokinin does not occur in all developmental contexts: for instance in the control of cell division in plant suspension cultures, or in the shoot apical meristems, auxin acts synergistically with cytokinin47. Hence, the concept of yin–yang is probably more accurate, as auxin and cytokinin act together dynamically, with roles that can be paradoxically antagonistic or supportive, to provide robustness to developmental processes3. Recently, molecular principles of hormone perception and signal transduction have been deciphered9-11,13,33-35. However, the identity of factors and pathways that integrate and transduce inputs between signaling cascades into a proper developmental output is still largely unknown.

Here, we identified a molecular component of the auxin–cytokinin crosstalk SYNERGISTIC ON AUXIN AND CYTOKI NIN 1 (SYAC1), whose expression in roots is tightly controlled by the auxin–cytokinin balance. Under our experimental conditions, expression of SYAC1 in roots is suppressed and its activation requires levels of both hormones above a certain minimal threshold. Hence, in cells of the root meristem which typically exhibit a higher endogenous activity of either auxin37,59 or cytokinin5 supplementation of their respective hormonal counterpart is sufficient to stimulate gene expression. Intriguingly, in the zone of differentiation and rapid elongation, SYAC1 expression is dependent on the simultaneous action of both hormonal pathways. Enhanced transcription detected rapidly after only 30 min of the provision of both hormones and the contribution of auxin and cytokinin receptors to synergistic regulation of SYAC1 transcription, indicate that the gene might be among early common targets of both hormonal pathways. Unlike in roots, in the hypocotyl and cotyledons of germinating seedlings SYAC1 transcript can be detected without the need for exogenously added hormones. Different requirements for SYAC1 expression between roots and shoots might reflect distinct configurations of auxin and cytokinin hormonal pathways in these two organs. As a consequence, factors required for SYAC1 expression might be under suppression in the roots while remaining active in the shoots. In support of such a scenario, transcriptome profiling revealed differences in root and shoot responses to cytokinin, and prolonged exposure of roots to increased cytokinin levels led to an activation of gene clusters typically active only in the shoot40.

Plant cells are surrounded by complex cell walls, which must remain highly dynamic and adapt to the changing requirements of plants during growth while still providing mechanical support. Furthermore, as a direct contact with the extracellular environment, cell walls serve an important protective function61. Pectins, as one of the essential structural components, determine biochemical cell wall properties52 thus have a significant impact on fundamental plant processes such as elongation growth of plant organs and their adaptation to mechanical stress, abscission of leaves, fruits, seeds or flower organs, as well as a protective role during pathogen infection63-67. Intriguingly, recent studies have revealed that pectins are often localized in spatially distinct patterns and these nonuniform pectin distributions might contribute to important aspects of their regulatory function62.

The expression and functional characterization of SYAC1 suggests that it might be an important regulatory component in the determination of spatio-temporal patterns of pectin distribution and can thus steer the growth and the development of plant organs. A reduced proportion of carbohydrates detected by FT-IR and a decreased amount of galacturonic acid, but unaffected levels of xyloglucans in cell walls of seedlings with enhanced expression of SYAC1 support a potential function of this protein as a regulator of pectin allocation to the cell wall.

A distinct pattern of pectin distribution in plant cell walls is the result of cell and tissue specific regulation of pectin biosynthesis, its delivery to the cell wall, the control of methyl-esterification and acetylation status of pectin, or its degradation65,68. The subcellular localization of SYAC1 and its physical interaction with the ECH/YIP complex, previously linked with the transport of components to the cell wall40 points toward the SYAC1 involvement in the secretory pathway. A significant decrease of the α-Amylase SI by SYAC1 together with detailed in planta observations that reveal negative impact of SYAC1 on release of pectic mucilage from the seed coat, and defective accumulation of pectin at the tip of pollen tubes expressing SYAC1 support such a scenario. However, taking into account the complexity of the regulatory networks shaping plant cell walls in different tissues and organs, the possibility cannot be excluded that SYAC1 feedbacks onto the activity of factors involved in other processes than the delivery of components to the cell wall. Further studies on SYAC1’s potential role in regulating the activity and subcellular localization of proteins involved in the biosynthesis of polysaccharides that comprise pectin, as well as their processing by methylesterification, acetylation or degradation44,62,69 need to be performed.

Importantly, the pattern of SYAC1 expression and phenotypic alterations observed in Arabidopsis seedlings with modulated activity of SYAC1 support a function of SYAC1 as a regulatory component that might contribute to fine-tuning of pectin allocation to cell walls in a developmentally controlled and tissue-specific manner. SYAC1 expression is high in the embryonic hypocotyls and in cotyledons, but steadily decreases as seedlings start to germinate. In hypocotyls, the SYAC1 reporter signal remains strong in short cells close to the apex and is eliminated from elongated cells toward the hypocotyl base. Discrete pattern of the SYAC1 expression in the upper part of the stem and in the abscission zone of silique was detected in adult Arabidopsis plants. In etiolated seedlings SYAC1 expression appears concentrated in short cells at the inner and excluded from elongated cells at the outer side of the apical hook. Growth defects such as altered elongation of hypocotyls and roots, or the apical hook phenotype as result of modulated SYAC1 activity accords well with the expression pattern and cellular function of the gene as a regulatory component of pectin allocation to the cell wall66,67,70.

We propose that SYAC1 contributes to determining a zone of reduced cell expansion by modulating cell wall composition and thereby fine tunes the overall pattern of organ growth kinetics. We hypothesize that constitutively expressed YIPs and ECH
might act as generic factors required for the secretion of cell wall components to maintain cell expansion, and that SYAC1 influences the YIP/ECH complex as a developmentally specific regulator to fine tune pectin distribution pattern and thereby steer plant organ growth. Why the expression of SYAC1 in roots is normally strongly suppressed and can be unlocked only by the extraneous addition of hormones permitting the synergistic interaction of auxin and cytokinin remains an intriguing question. Restricting the expression of SYAC1 in embryonic roots when compared with embryonic hypocotyls and cotyledons might be part of a developmental mechanism, which coordinates the typical pattern of early germination, namely the emergence of roots prior to the outgrowth of shoot organs.

In roots of germinating seedlings, SYAC1 expression remains low, but can increase when the levels of auxin and cytokinin rise. This indicates that under optimal conditions, the expression of SYAC1 is suppressed and thus does not limit root growth. However, in heterogeneous soil environments roots might be challenged by various abiotic stresses such as excess of aluminum8 and oxygen13, 18, 25, 29, 34, 129, 137, 138, 35. SYAC1 expression of cytokinin remains an intriguing question. Restricting the expression of SYAC1 in embryonic roots when compared with embryonic hypocotyls and cotyledons might be part of a developmental mechanism, which coordinates the typical pattern of early germination, namely the emergence of roots prior to the outgrowth of shoot organs.

Cloning and generation of transgenic lines. All cloning procedure was conducted by using Gateway™ (Invitrogen) technology; with the sequences of all used vectors available online (https://gateway.psb.ugent.be/). For promoter analysis of SYAC1, the Biocytogen primers have been described elsewhere46, 48, 49, and were used by introducing the pDONR-P4-P1R entry vector. Then transcriptional lines (pSYAC1:GUS, pSYAC1:nlsGFP) were created: for pSYAC1:GUS, an LR reaction with SYAC1 promoter in pDONOR-P4-P1R, pen-L1-S-L20, and pk7mA24GWO, 0 vectors was performed. For pSYAC1:nlsGFP line, an LR reaction with SYAC1 promoter in pDONOR-P4-P1R, pen-L1-S-L20, and pk7mA24GWO, 0 vectors was performed. To generate overexpressor and inducible lines (SYAC1-GFPox, SYAC1-HAox, HA-SYAC1lox, pES:SYAC1-GFP, pES:SYAC1-GUS), SYAC1 ORF sequence with or without STOP codon was amplified and fused through a linker (four glycines and one alanine) to GFP or HA tag. The fragments were first introduced into pDONR221, and then into pbGW70 (overexpression lines), pGWO7 (protoplast expression assays), pMDC7 (estradiol inducible lines). For GFP-SYAC1 transgenic line SYAC1 ORF was amplified, introduced into pDONR221 and to the pbGW70.20 destination vector. To generate translational fusion line pSYAC1:SYAC1-GFP, SYAC1 promoter was amplified together with the genetic fragment of the SYAC1 gene, cloned into pSP74-PSP74-P1 with pEN-L1-S-L20, and then into pbGW70.3. Cloning primers are listed in Supplementary Table 1. All transgenic plants were generated by the floral dip method76 in Columbia (Col-0) background and transformants were selected on plates with appropriate antibiotic. Generation of CRISPR/Cas9 line. Design of the gRNA for SYAC1 gene, molecular cloning and plant transformation was done in collaboration with VBCF Protein Technologies Facility (www.vbcf.ac.at). Design, specificity and activity of gRNA: GATGGTCAGCAACCACACGA was performed using online available tools: http://chbz. hau.edu.cn/cgi-bin/CRISPR and http://www.broadinstitute.org/rma/public/analysis-tools/sgrna-design. gRNA was cloned into pGPG2003 CRISPR/Cas9 destination vector. Transformants resistant to BASTA antibiotic were selected, genomic sequence of SYAC1 amplified with CRISPR/Fw and Rv primers (see Supplementary Table 1 below) and sequenced. Individual mutant lines with single base pair insertion in coding sequence (90 bps after the ATG at the place of gRNA binding) were selected. These plants were then propagated to the next generation to obtain homozygote lines. Lines outcrossed of CRISPR/Cas9 cassette were confirmed by PCR for loss of BASTA coding sequence with specific primers (see Supplementary Table 1 below). Only plants without BASTA gene (part of CRISPR/Cas9 vector) were propagated to the next generation. Sensitivity of selected plants to BASTA was confirmed and plants were resequenced to confirm the point mutation. Identification of SYAC1 by transcriptome profiling. SYAC1 was recovered from transcriptome profiling aiming at identification of genes involved in regulation of root branching by auxin and cytokinin. Seven-day-old Arabidopsis seedlings of Col-GFP enhancer trap line J0121, a marker for xylen pale pole12, were treated with either auxin (1 µM NAA), 10 µM cytokinin (N6-benzyladenine) or both hormones applied simultaneously for 3 h. Fluorescence activated cell sorting (FACS) was performed according to ref. 80. Approximately 5000 J0121 seeds (per replicate) were sterilized and plated on high growth rate media (0.087% MS medium, 4.5% sucrose) in 16-h light/8-h dark photoperiod at 21 °C. To allow rapid harvesting, seeds were arranged in rows on square plates at a density of ~500 seeds per row on top of nylon mesh (Nitek 03 100/47, Sefar America, Briarcliff Manor, New York). The mesh with 7-day-old seedlings was transferred on high growth rate media containing the hormonal concentrations indicated. After 3 h, roots were cut off about 1 cm from their tip. Dissected roots were placed in supplemented solution B [Solution B = (Solution A + 1.5% cellulose, 0.1% pectolyase) inside 70 µm cell strainers placed in small petri dishes and incubated for 1 h at room temperature

Methods Plant material and growth conditions. The syac1-3 (GABI-KAT 760F05, Col-0, SUL3) T-DNA insertion line was obtained from the GABI KAT seed collection. Genotypic primers are listed in Supplementary Table 1. The syac1-5 CRISPR line was prepared in collaboration with the VBCF Protein Technologies Facility (www.vbcf.ac.at) (see below). The transgenic fluorescent-protein marker lines in Col-0 background have been described elsewhere36, 37, 50, 51, 54. C. elegans CRISPR1-12ahk2-2;cre1-12ahk3-3;ahk2-ahk3-3–3; tir1-1; tir1-1abf3-1; abf3-1

Seeds of Arabidopsis were plated and grown on square plates with solid half strength Murashige and Skoog (MS) medium (Duchefa), supplemented with 0.5 g L−1 MES, 10 g L−1 Sucrose, 1% agar, and pH adjusted to 5.9. The plates were incubated at 4 °C for 48 h to synchronize seed germination, and then vertically grown under a 16:8 h day/night cycle photoperiod at 21 °C. Cytokinin and auxin responses of SYAC1 mutants were performed with the N6-benzyladenine cytokinin derivative (Sigma, B3408) and NAA (Sigma, N6O40), respectively. Short treatments (6 h) for GUS/GFP expression were performed with 10 µM cytokinin and 1 µM auxin (unless indicated differently). For root growth transient assay 0.1 µM cytokinin and 0.05 µM auxin was used. Gibberellin treatment was performed with 10 µM Gibberellic acid (GA3) (Sigma, G7643). MeA treatment with 10 mM MeA (Sigma, 39277). ABA treatment with 1 µM ABA (Supid A1649) and BR treatment with 1 µM Epibrassinolide (Sigma, E1641). Overall, 10 µM PAC (Sigma, 46046) was used as a gibberellin biosynthesis inhibitor. Estradiol treatment was performed with β-Estradiol (Sigma, E8875). All experiments were performed 2–3 times.
with agitation. Protoplasts were collected from Petri dishes and concentrated by spinning down (at –800 RCF). The supernatant was aspirated and the cell pellet was resuspended in 1.5 mL of Solution A (600 mM mannitol, 2 mM MgCl₂, 0.1% BSA 2 mM CaCl₂ 2 mM MES, 10 mM KCl, pH 5.5). The cell suspension was then filtered through a 40 μm cell strainer. GFP expressing cells were isolated on a FACSVantage (Becton Dickinson FACSVantage) fit with a 100 μm nozzle at a rate of 2000–5000 events per second. We normally used a fluid pressure of 30 psi. Protoplasts from non-GFP expressing Columbia wild-type plants were used as a negative control for establishing sorting criteria based on the following cell properties: (i) a cluster of live protoplasts with intact membranes was selected based on a high forward to side scatter ratio; (ii) GFP expressing cells were sorted by their emission intensity in the green channel (~350 nm) above negative controls. Cells were sorted directly into lysis buffer (Quagen RLT buffer), mixed and immediately frozen at –80 °C for later RNA extraction. An autofluorescence filter was established by eliminating cells that fluoresced at equal intensity in the green and orange (~575 nm) channels. Standard Affymetrix protocols were then used for amplifying, labeling and hybridizing RNA samples. RNA was extracted using the Nucleospin plant kit (Macherey-Nagel). A DNA treatment with the RNase-free DNase Set (Quagen) was carried out for 15 min at 25°C. Total RNA concentration was determined using a Nanodrop ND-1000 spectrophotometer. All RNA samples were rejected if they did not reach a minimum concentration of 100 ng μL⁻¹, a 260/280 nm ratio above 1.8 ± 0.2, and an RNA integrity number superior to 7.5, with an Agilent 2100 Bioanalyzer (Agilent, USA). Arabidopsis Tiling 10 R arrays (Affymetrix) were hybridized at the VIB Nucleomics Core (www.nucleomics.be) according to the manufacturer’s instructions. Data were normalized from CEL files using the Robust multivariate average algorithm implemented in the Bioconductor package Affy (v1.24.23). The probe annotation was obtained from ath1g1drc1drc2d2c. Differential expression analysis was determined using the empirical Bayes (etwayes) function from the Limma package (v2.14.0) in R v2.8.0. P values were calculated and then transformed into false discovery rates, or Q values according to the method described by Storey and Tibshirani as implemented in the R package qvalue.

RNA extraction, RT, and qPCR. Total RNA was extracted from roots of 5-day-old plants under all conditions (untreated, 1 μM auxin, 10 μM cytokinin and both together for 3 h) using the RNeasy Plant Mini kit (Qiagen). Overall, 1 μg total mRNA was used to generate cDNA using the iScript cDNA Synthesis Kit (BioRad). SYACI expression was quantified with specific primer pair (see Supplementary Table 1 below). Three hundred and eighty-four-well plates (Roche) were loaded using a Robot Master Workstar (PerkinElmer, USA) with 2.5 μL reagent containing 2.5 μL Luma Universal QPCR Master Mix (New England Biolabs), qCPRs were performed using the LightCycler 480 (Roche). Samples (n = 3) were measured in technical triplicates, and the expression of PIN1 and PIN2 expression was monitored by differential interference contrast microscopy (Olympus BX53).

Phenotypic analysis. For root and hypocotyl length analyses, seedlings were photographed and length was measured with Image software version 1.52 (https://image.nih.gov/ij/). About 10–30 seedlings were processed and three independent experiments were performed. t-test was used for statistics.

Analysis and statistics of the apical hook development. Development of hook was monitored by using confocal microscopy (LSM 700, Zeiss) and quantified by the scatter ratio. Images were acquired with a 40× oil immersion objective (LSM 700, Zeiss). Images were analyzed using the Zeiss Image Browser software. For our analysis, one cell was considered as one ROI; in every root ~1–5 cells (five ROIs) were measured and a minimum of ten roots were used. Colocalization plug-in using an automatic threshold was used to obtain Rcoloc value, which represent Pearson’s correlation coefficient.

Phylogenetic analysis. Sequences from the Arabidopsis thaliana SYACI protein family (AT1G15590, AT1G15600, AT1G15610, AT1G15620, AT1G15625, AT1G15630, AT1G15640, and AT1G44010) were aligned using ClustalW2. Phylogenetic tree was generated with Mega7 (Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets; www.megasoftware.net) using Neighbor-joining method with a complete deletion model. Bootstrap tests were performed with 1000 replications. Poisson correction was used and sites were set as uniform.

Confocal imaging and image analysis. Zeiss LSM 700 confocal scanning microscope using either x20 or x40 (water immersion) objectives were employed to monitor expression of fluororescent reporters. GFP (YFP) and Cy3 signals were detected either at 488 nm excitation/507 nm emission or 550 nm excitation/570 nm emission wavelength, respectively. Quantification of immunodetected PIN1 and PIN2 expression in root meristems was performed by measurement of membrane signal in cortex and epidermal cells, respectively. Signal in approximately ten cells in a minimum of ten roots was measured using ImageJ software version 1.52. For cell size measurement, roots of 5-day-old wild-type and SyACI+ plants were stained for 5 min with FM4-64 dye (Thermo Fisher, T13320) followed by image acquisition. Cell size was measured by ImageJ software version 1.52. Significant differences were evaluated using t-test; n = 15–20 roots, two biological replicates.

Transient expression in root suspension culture protoplasts. The transient expression assays were performed on 4-day-old Arabidopsis root suspension culture protoplasts incubated in B5 medium with a mannitol solution (1% cellulose (Serva), 0.2% maceroyzime (Yakult), in B5—0.34 M glucose-mannitol solution (2.2 g MS with vitamins, 15.25 g sucrose, 15.25 g mannitol, pH to 5.5 with KOH) with slight shaking for 3–4 h, and afterward centrifuged at 800 × g for 5 min. The pellet was washed and resuspended in B5 glucose-mannitol solution to a final concentration of 4 × 10⁶ protoplasts/mL. DNA samples were gently mixed together with 50 µL of protoplast suspension and 60 µL of PE solution (0.1 M Ca(NO₃)₂, 0.45 M mannitol, 25% PEG 6000) and incubated in the dark for 30 min. Then 140 µL of 0.275 M Ca(NO₃)₂ solution was added to wash off PEG, wait for sedimentation of protoplasts and remove 240 µL of supernatant. The protoplast pellet was resuspended in 200 µL of B5 glucose–mannitol solution and incubated for 16 h in the dark at room temperature. Transfected protoplasts were mounted on the slides and viewed with Zeiss LSM 700 confocal scanning microscope.

Transient expression in Arabidopsis mesophyll protoplasts. Mesophyll protoplasts were isolated from rosette leaves of 4-week-old Arabidopsis plants grown in soil under controlled environmental conditions in a 16 h light/dark cycle at 21 C. Leaves were cutted with razor and protoplasts were isolated in enzyme solution (1.5% cellulose (Serva), 0.2% maceroyzime (Yakult), in MES-mannitol solution (0.4 M mannitol, 20 mM KCl, 20 mM MES, 10 mM CaCl₂, pH to 5.7 with KOH) with slight shaking for 3–4 h, and afterward centrifuged at 800 × g for 5 min. The pellet was washed in W5 solution (154 mM NaCl, 152 mM CaCl₂, 5 mM KCl, 2 mM MgSO₄) and resuspended to a final concentration of 4 × 10⁶ protoplasts/mL. DNA samples were gently mixed together with 50 µL of protoplast suspension and 60 µL of PE solution (0.1 M CaCl₂, 0.4 M mannitol, 25% PEG 6000) and incubated in the dark for 10 min. Then 140 µL of 0.275 M Ca(NO₃)₂ solution was added to wash off PEG, wait for sedimentation of protoplasts and remove 240 µL of supernatant. The protoplast pellet was resuspended in 200 µL of B5 glucose–mannitol solution and incubated for 16 h in the dark at room temperature. More details are described in ref. 93.
Characterization of SYAC1 effects in tobacco pollen tubes. Pollen was harvested from 8-week-old tobacco (Nicotiana tabacum) plants and resuspended in growth medium for 5 min. A fluorescent pollen suspension (15% w/v sucrose, 12.5% w/v casein hydrolysate, 15 mM MES-KOH pH 5.9, 1 mM CaCl2, 1 mM KCl, 0.8 mM MgSO4, 1.6 mM H2BO3, 30 µM CuSO4, 10 µg/mL rifampicin) and filtered onto cellulose acetate filters (Sartorius, Göttingen, Germany). The filters were placed on filter paper (Whatman, Maidstone, UK) moistened with growth medium. Within 5–10 min, pollen individuals were identified and pollen were washed away. DNA was isolated from the pollen with plasmid coated 1 µm gold particles using a helium-driven particle accelerator (PDS-1000/He; BIO-RAD, Munich, Germany) using 1350 psi rupture discs and a vacuum of 28 in. of mercury. Gold particles (1.25 µg) were coated with 3–7 µg of plasmid DNA, and then the DNA was precipitated with the DNA and 16 µM spermidine, and washing the gold particles three times with 95% (v/v) ethanol. STACI ORF sequence without STOP codon was amplified and fused through a linker (four glycines and one alanine) to mCherry tag. The fragment was first introduced into pDONR211 and then into platoGiv (Invitrogen) plasmid. Cloning primers are listed in Supplementary Table 1. After bombardment pollen was resuspended in growth medium and grown for 5–8 h in small droplets of media directly on microscope slides as previously described[84]. Fluorescent pollen tubes were analyzed for apical pectin deposition by staining with ruthenium red (Sigma) at a final concentration of 0.01% (w/v) and imaged under light microscopy within 5–10 min after addition of the dye, as previously described[85]. Microscopic examination was performed using an Axiovert200 epifluorescence microscope fitted with an LED Achromplan ×63/0.75 Korr Ph2 (DICIII) objective and an Axioscan HR color camera, and using Axios Vision Rel 4.6 Software (all Carl Zeiss Jena, Germany). Staining patterns were manually examined for deposition thickness, or automatically for the apical occupancy of red dye using Fiji software version 1.52.[https://fiji.sc/] The subcellular localization of SYAC1-mCherry in tobacco pollen tubes was performed using an LSM 880 Airyscan confocal microscope (Carl Zeiss Jena, Germany) and Zen blue software as previously described[89]. Pollen tube morphologies were assessed by scoring the incidence of normal, stunted or growth-arrested (nongerminating) cells among fluorescent pollen tubes[86].

Yeast two-hybrid assays. Y2H assay was performed using the GAL4-based two-hybrid system (Clontech). Full-length SYAC1 and YIP4a, YIP4b, YIP5b, ECH, KCR1, DSK2, and PHB4 ORFs were cloned into pDEST-DEST-GADT7 and pDEST-GGBK7 (Clontech) to generate the constructs AD-SYAC1 and BD-YIP4a (YIP4b, YIP5b, ECH, KCR1, DSK2, PHB4). The constructs were transformed into the yeast strain PJ69-4A with the lithium acetate method:100 µL of competent cells resuspended in TE/LiAc solution (100 mM LiAc, 10 mM Tris-HCl, 1 mM EDTA, pH 7.5) were mixed with DNA. Following bombardment, yeast were resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and plated on selective media (LEU/-Trp/-His or with increasing concentration of 3-Amino-1,2,4-triazole) to test the protein interactions.

α-Amylase enzymatic assay. α-Amylase assays and calculation of the SI were performed as described[87]. Briefly, α-amylase expression constructs were kindly provided by P. Pimpl and transformations were performed in Arabidopsis mesophyll protoplasts (see above). α-Amylase was extracted with extraction buffer (50 mM C5H12O4, 50 mM NaCl, pH 7.4, 0.05% Na2EDTA, pH 5.2) and activity was measured with a kit Ceralpha (Megazyme). The reaction was performed in a microtiter plate at 37°C with 30 µl of extract and 30 µl of substrate. The reaction was stopped by the addition of 150 µL of stop buffer. The absorbance was measured at a wavelength of 405 nm. Experiment was performed four times including two technical replicates with one additional control sample (water). The α-Amylase activity in medium (secreted to the apoplast) and in cells (retained in the inner part of the cell) was measured in two to three time points and SI (ratio between α-Amylase in medium in an cells) as a normalized parameter, which allows to compare the different replicates and neutralizes the variability, was calculated. Protoplasts transformed without any plasmid were used as blank measurement.

AFM measurements and apparent Young’s modulus calculations. The AFM data were collected and analyzed as described elsewhere with minor changes[66]. To examine extracellular matrix properties the turgor pressure was suppressed by seedlings immersion in a hypertonic solution (0.55 M mannitol) for at least 30 min before examination. 4-day old seedlings grown in darkness were placed on petri dishes filled with 1% agarose and 10% mannitol and immobilized by low melting agarose (0.7% with 10% mannitol). The focus was set on the anticlinal (perpendicular to the organ surface) cell walls and its extracellular matrix. To ensure proper indentations (especially in the bottom of the dome shape between two adjacent cells regions), cantilevers with long pyramidal tip (14–16 µm of pyramidal height, AppNano ACST-10), with a spring constant of 7.8 N/m were used. The instrument used was a JPK Nano-Wizard 3 and indentations were kept to <10% of cell height (typically indentations of 500 nN force). Three scan maps per sample were taken over an intermediate region of the hypocotyls, using a square area of 25 × 25 µm, with 16 × 16 measurements, resulting in 1792 force–indentation experiments per sample. The lateral deflection of the cantilever was monitored and in case of any abnormal increase the entire dataset was discarded. The apparent Young’s modulus (EA) for each force–indentation experiment was calculated using the approach curve (to avoid any adhesion interference) with the JPK Data Processing software (JPK Instruments AG, Germany). To calculate the average EA for each anticlinal wall, the total length of the extracellular region was measured using masks with Gwyddion 2.45 software (at least 20 points were taken in account). The pixels corresponding to the extracellular matrix were chosen based on the topography map. For topographical reconstructions, the height of each point was determined by the point of contact from the force-indentation curve. A total of 12–14 samples were analyzed. A standard t-test was applied to test for differences between genotypes.

Ruthenium red staining of seeds. Mature seeds were incubated in 0.01% (w/v) aqueous solution of Ruthenium red for 1 h while gently shaking. Seeds were washed with water, mounted in water and viewed using a DIC Olympus BX53 microscope. Approximately 100 seeds from each genotype were stained with ruthenium red and 30 representative images were recorded.

Tandem affinity purification (TAP). TAP assay was performed in Arabidopsis cell suspension culture as described[88] with minor modifications. Briefly, SYAC1 was produced as N-terminally tagged GST-EYFP fusion in PSB-D cell culture. Proteins were extracted for 1 h in TAP extraction buffer in which 0.1% NP40 was replaced by 1% digitonin. After 30 min of extraction, 0.1% benzonase was added to the mixture. The remaining complexes were washed with 0.1% digitonin for 1 h and nonspecific proteins were washed away. IgG elution was done with TEV protease, and the eluted fraction was bound to streptavidin resin. All wash steps and TEV elution was done in TEV elution buffer in which 0.1% NP40 was replaced by 0.2% digitonin. After the final streptavidin wash step, one additional wash step was applied with 0.2% digitonin detergent buffer (dissolved from the streptavidin resin with NuPAGE sample buffer containing 20 mM Des- thiobiotin. Eluted proteins were subjected to a short NuPAGE run and in-gel
digested with Trypsin. Protein interactors were identified by mass spectrometry using an LTQ Orbitrap Velos mass spectrometer. Proteins with at least two matched high confidence peptides were retained. Background proteins were filtered out based on frequency of occurrence of the copurified proteins in a large dataset containing 543 TAP experiments using 115 different baits.

Cell wall analyses. Analyses were performed on 4-day-old dark grown hypocotyls using an alcohol-insoluble residue (AIR) prepare as follows. Seeds of Arabidopsis were plated and grown on square plates with Millieu Arabidopsis medium (Duchefa) supplemented with 0.32 g·L\(^{-1}\) Ca(NO\(_3\))\(_2\), 10 g·L\(^{-1}\) suc, 0.8% agar and pH adjusted to 5.7. The plates were incubated at 4°C for 48 h to synchronize seed germination, and then vertically grown in darkness at 18°C. Freshly collected samples were submerged into 96% ethanol, graded and incubated for 30 min at 70°C. The pellet was then washed twice with 96% ethanol and once with acetone. The remaining pellet of AIR was dried in a fume hood overnight at room temperature.

Fourier transform-infrared spectroscopy (FT-IR). Spectra were recorded for the 4-day-old dark grown hypocotyl sections in transmission mode on a Bruker Tensor 27 spectrometer equipped with a Hyperion 3000 microscopy accessory and a liquid N\(_2\) cooled 64 × 64 mercury cadmium telluride focal plane array (FPA) detector. The entire setup was placed on a vibration-proof table. Spectra were recorded in the range 4000−700 cm\(^{-1}\), with 128 scans co-added in double sided, forward-backward mode. FPA frame rate was 3773 Hz and integration time 0.104 ms, with offset and gain optimized for each sample. Following separation conditions were applied: an isocratic gradient of 4 mM NaOH from 0 to 6 min followed by a linear gradient of 4 mM NaOH to 1 mM NaOH from 6 to 19 min. At 19.1 min, the gradient was increased to 450 mM NaOH to elute the acidic sugars.

SYAC expression analysis after infection by P. brassicae. Fourteen-day-old pSYAC1:GUS transgenic Arabidopsis seedlings were inoculated with the spore concentration 10\(^5\) as described above and GUS expression detected in roots at 20 and 28 dai. As a positive control 34-day-old plants were treated with 1 μM auxin (NAA) and 10 μM cytokinin (N\(_6\)-benzyladenine) for 6 h. GUS staining performed as described above (Histochemical and histological analysis).

Data availability

The authors declare that the data supporting the findings of this study are available within the paper, and its Supplementary Information file. A reporting summary for this Article is available as a Supplementary Information file. The datasets generated and analyzed during the current study are available from the corresponding author upon request. The source data underlying Figs. 1a, b; 2a and b; Supplementary Figs. 1a, b; 2b and c; 3a; 4c, a, d, e; 5d, e; 6a; 7d, e, as well as Supplementary Figs. 1a, b; 2b–d; 3b; 4c, d; 5c, d; 6a, b; 7a, b are provided as a Source Data file. Transcriptome profile data associated with this study has been deposited in the NCBI Sequence Read Archive under accession number GSE146778. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD001819 [10.6019/PXD001819].

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Author contributions

A.H. and E.B. conceived the project; A.H. performed most of experiments related to functional and phenotype analyses of SYACI; C.C. contributed to generation of constructs and transgenic lines; N.C. performed expression analysis of SYACI in wild-type and mutant background in response to hormones; K.O. performed Co-IP experiments; J. D. carried out transcriptome profiling; L.D. supported by E.S. and A.H. performed Y2H and BIFC analyses; J.C.M. contributed to a manuscript assay; M.G. analyzed cell wall mechanical properties; T.R. and H.S. contributed to co-immunolocalisation and phenotype analyses; I.S. and I.H. designed and carried out SYACI expression and phenotype analyses in pollen tubes; R.B. contributed to analyses of SYACI interaction with ECHI/YIP complex; A.G. and A.H. performed FT-IR measurements; J.S. and G.M. analyzed composition of cell walle; G.P. and G.J.S. identified SYACI interactors; E.B and J.L-M. tested tolerance of plants to Plasmodiophora brassicae infection. A.H. and E.B. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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