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A cell surface *O*-glycosylated peptide, AGP21, acts on the brassinosteroid pathway and modulates root hair cell fate

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Word count 2,738

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Highlights

- **Perturbation of AGPs and the loss of AGP21 peptide trigger an abnormal RH cell fate.**
- **AGP21-mediated repression of *GL2* expression activates the expression of RSL4 and EXP7 root hair proteins.**
- **AGP21 peptide acts in both a BR-dependent and BR-independent manner, with both pathways converging on a BIN2 downstream signalling cascade that controls *GL2* expression.**

Summary

Root hairs (RHs) develop from specialized epidermal cells called trichoblasts, whereas epidermal cells that lack RHs are known as atrichoblasts. The mechanism controlling root epidermal cell fate is only partially understood. Root epidermis cell fate is regulated by a transcription factor complex that promotes the expression of the homeodomain protein GLABRA 2 (GL2), which blocks RH development by inhibiting ROOT HAIR DEFECTIVE 6 (RHD6). Suppression of GL2 expression activates RHD6, a series of downstream TFs including ROOT HAIR DEFECTIVE 6 LIKE-4 (RSL4 [1] and their target genes, and causes epidermal cells to develop into RHs. Brassinosteroids (BRs) influence root epidermis cell fate. In the absence of BRs, phosphorylated BIN2 (a Type-II GSK3-like kinase) inhibits a protein complex that directly downregulates GL2 [2]. Here, we demonstrate that the genetic and pharmacological perturbation of the arabinogalactan protein (AGP) AGP21 in *Arabidopsis thaliana*, triggers aberrant RH development, similar to that observed in plants with defective BR signaling. We reveal that an *O*-glycosylated AGP21 peptide, which is positively regulated by BZR1, a transcription factor activated by BR signaling, affects RH cell fate by altering *GL2* expression in a BIN2-dependent manner. These results indicate that perturbation of a cell surface AGP disrupts BR perception and inhibits the downstream effect of BIN2 on the RH repressor GL2 in root epidermal cells. In addition, AGP21 also acts in a BR-independent, AGP-dependent mode that together with BIN2 signalling cascade controls RH cell fate.

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Results and Discussion

AGP perturbation influences root hair (RH) cell fate programming

Plant cell surface proteoglycans known as arabinogalactan proteins (AGPs) function in cell proliferation, cell expansion, organ extension, and somatic embryogenesis [3,4]. The precise mechanisms underlying AGP action are unknown. *O*-glycosylated AGPs interact with and modulate the activity of other plasma membrane (PM) proteins. To determine whether *O*-glycosylated AGPs regulate specific RH developmental processes, we exposed roots of *Arabidopsis thaliana* to β-glucosyl Yariv (β-Glc-Y), which specifically binds structures in the *O*-glycans of AGPs: oligosaccharides with at least 5–7 units of 3-linked *O*-galactoses [5,6]. β-Glc-Y–linked AGP complexes on the cell surface induce AGP aggregation and disrupt native protein distribution, triggering developmental reprogramming [7,8]. α-mannosyl Yariv (α-Man-Y), an analog that does not bind to AGPs, served as the control. While α-Man-Y treatment did not affect RH cell fate (≈2–5% of total RHs that are contiguous), β-Glc-Y treatment increased contiguous RH development (≈40%) (**Figure S1A**), suggesting that *O*-glycosylated AGPs influence RH formation.

To test whether *O*-glycans on hydroxyproline-rich glycoproteins (HRGPs) alter RH cell fate, we blocked proline 4-hydroxylase enzymes (P4Hs) that catalyse proline (Pro)-hydroxylation into hydroxyl-proline units (Hyp), the subsequent step of HRGP *O*-glycosylation. Two P4H inhibitors, α,α-dipyridyl (DP) and ethyl-3,4-dihydroxybenzoate (EDHB), prevent Pro-hydroxylation [9,10]; both increased contiguous RH development to ≈15–20% (**Figure S1B**). Additionally, *p4h5* (a key P4H in roots [11,12]) and four glycosyltransferase mutants defective in AGP *O*-glycosylation (*hpgt* triple mutant*; ray1*, *galt29A*, and *fut4 fut6*) (see **Table S1**) showed significantly increased (≈8–20%) ectopic RH development (**Figure 1A**), substantiating the previous report that the triple mutant *hpgt* mutant has an increased RH density [13]. These mutants were mostly insensitive to β-Glc-Y; however, the treatment increased the number of contiguous RHs in *fut4 fut6,* although to a lesser extent than in the wild type (**Figure 1B**). β-Glc-Y inhibits root cell expansion [15,16]. Glycosyltransferase (GT) mutations affecting extensin (EXTs) O-glycosylation (e.g. *rra3* and *sgt1 rra3*; **Table S1**) drastically affect RH cell elongation [14]. Intriguingly, (see) these mutations did not affect RH cell fate, and β-Glc-Y stimulated ectopic RH development, indicating that EXT O-glycosylation does not function in RH cell fate reprogramming (**Table S1**, **Figure 1C**), and specifically *O*-glycans attached to AGPs. *P4H5* and *AGP-related GTs (e.g. RAY1, GALT29A, HPGT1-HPGT3* and *FUT4/FUT6),* are expressed in the root epidermis elongation and differentiation zones (**Supplementary Item 1**). Under-arabinosylated AGPs in *ray1* and under–*O*-fucosylated AGPs in *fut4 fut6* show similar root growth inhibition [17,18], highlighting a key role for AGP O-glycans in regulating root cell development, albeit by unknown mechanisms.

The BR–BZR1 pathway regulates AGP21 expression

Brassinosteroid (BR) signaling regulates RH cell patterning [2]. The BR-insensitive mutant, *bri1-116*, developed many (≈20%) contiguous RH cells (**Supplementary Item 2A**), resembling plants subjected to β-Glc-Y and DP/EDHB treatments (**Figure S1**). The *agp21*, *p4h5*, *hpgt* triple

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mutant, *ray1-1*, *galt29A*, and *fut4 fut6* mutants exhibited similar phenotypes, suggesting that an interplay between cell surface AGPs and BR signaling determines RH cell fate. As chromatin-immunoprecipitation (ChIP)-sequencing and RNA-sequencing indicate that BZR1 directly upregulates *AGP* expression, most predominantly *AGP21* [19], we investigated how root epidermal BR signaling regulates *AGP21* expression. Since the *AGP21* regulatory region contains one BZR1 binding motif (E-BOX, CATGTG at -279 bp relative to ATG start codon), we tested whether BR directly modulates *AGP21* expression. Compared with no treatment, 100 nM BL (brassinolide, BR's most active form) enhanced of both AGP21p::GFP (transcriptional reporter) and AGP21p::V-AGP21 (V= Venus tag; translational reporter) expression (**Supplementary Item 2B–C**). Expression of *AGP21p::GFP* in *bri1-116* resulted in lower *AGP21* expression than in untreated wild type (**Supplementary Item 2B**), confirming that BR-mediated BZR1 controls *AGP21* expression in the root.

Trichoblasts and atrichoblasts expressed V-AGP21 peptide in a discontinuous pattern (**Figure S1C**), indicating that some root epidermal cells lacked AGP21. Treatment with β-Glc-Y—but not α-Man-Y—resulted in excess AGP21p::Venus-AGP21 at transverse cell walls (**Figure S1C**). We used the BZRp::BZR1-YFP reporter to test whether disrupting PM AGPs with β-Glc-Y would downregulate the response to BL (**Supplementary Item 3A**). Treatment with 100 nM BL induced *BZR1* expression, whereas exposure to β-Glc-Y or β-Glc-Y followed by 100 nM BL suppressed *BZR1* expression, suggesting that AGP disruption affects BR perception and downstream BZR1-mediated signaling. However, global BR–BZR1/BES1-mediated transcriptional responses are not involved in the anomalous RH cell fate phenotype, because lines constitutively expressing BZR1 (BZR1-D) and BES1 (BES1-D), overexpressing BZR1 and BES1 lines, and CRISPR-CAS9 null *bzr1* and *bes1* mutants showed no anomalous phenotypes (**Supplementary Item 3B)**.

_O_-glycosylated AGP21 peptide influences RH cell fate

The molecular link to BR–BRZ1 signaling suggested that AGP21 function in RH cell fate determination. AGP peptides are post-translationally modified in the ER-Golgi (**Figure 2A**), undergoing signal peptide (SP) removal, proline-hydroxylation/Hyp-*O*-glycosylation, and C-terminal GPI anchor signal (GPI-AS) addition [20]. Processed mature AGP-peptides are 10–13 amino acids long and bear three putative *O*-glycosylation sites (*O*-AG). Three prolines in the AGP21 peptide are hydroxylated *in vivo* as Hyp (Hyp=O), suggesting that AGP21 peptides are *O*-glycosylated at maturity [20]. The AGP21 deficient mutant *agp21* (**Supplementary Item 4A–B**), exhibited ectopic contiguous RHs (**Figure 2B**). Both *AGP21* expression under its endogenous promoter (*AGP21p::V-AGP21/agp21*) and overexpression (*35Sp::V-AGP21/agp21*) restored a wild type RH phenotype and patterning to *agp21* (**Figure 2B**), confirming that deficient *AGP21* expression causes contiguous RH development. Furthermore, while β-Glc-Y treatment triggered up to ≈40% of contiguous RH (vs. ≈2–5% induced by α-Man-Y) in the wild type (**Figure S1**), it induced no additional anomalous RH in *agp21* (vs. α-Man-Y treatment or untreated roots) (**Figure 2B**). We tested whether the closely related BZR1-induced peptide AGP15 functions with AGP21. *agp15* (**Supplementary Item 4C–D**) exhibited a milder phenotype than *agp21*, and the

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double *agp15 agp21* double mutant had no additional effects to *agp21* (**Supplementary Item 4E**). Together, these results confirm that β-Glc-Y acts through *O*-glycosylated AGP21 to stimulate contiguous RH development.

RH cell fate determination requires *O*-glycosylation of the AGP21 peptide

To determine whether functional AGP21 requires *O*-glycosylation, three putative *O*-glycosylation sites were mutated (Pro→Ala) (**Figure 2A**) and driven by the endogenous *AGP21* promoter in *agp21* (*AGP21p::V-AGP21*^{ALA}*/agp21*). Mass spectrometry had detected that all three proline units (Pro/P) within the AGP21 sequence ATVEAPAPSPTS can be hydroxylated as ATVEAOAOSOTS (Hyp= O) [20], indicating likely sites for *O*-glycosylation. Even though AGP21^{ALA} protein was detected in root epidermal cells (**Figure S2B**), AGP21^{ALA} failed to rescue the *agp21* RH phenotype (**Figure 2B–C**), confirming that Hyp-linked *O*-glycans in AGP21 are required for its function in RH cell fate. Moreover, β-Glc-Y treatment did not induce anomalous RH cell fate in AGP21^{ALA} plants demonstrating that β-Glc-Y requires *O*-glycans to alter RH development.

To localize AGP21 within cells, we transiently expressed *V-AGP21* in *Nicotiana benthamiana* and induced plasmolysis in epidermal cells with sorbitol (80 mM). Although some signal remained within cells, most V-AGP21 signal was secreted to the apoplast (i.e., between the cell wall and the PM) (**Figure 2C**). When transiently expressed at high levels, AGPs with GPI-AS typically follow this pattern [21]. Under its endogenous promoter, most AGP21 signal localized to the cell surface (**Figure S2A**). V-AGP21^{ALA}, however, never reached the cell surface; retention in the secretory pathway could indicate that *O*-glycans direct AGP to the PM–cell surface (**Figure S2A–B**). These data corroborate previous reports of a requirement for *O*-glycans in the secretion and targeting of AGPs and related fasciclin-like AGPs [22,23].

We tested the hypothesis that AGP21 is processed and modified during its synthesis along the secretory pathway. Using immunoblot analysis, we examined the apparent molecular weight of AGP21 peptide in transient AGP21-overexpressing plants and in AGP21p::V-AGP21 plants (**Figure 2D**). In the overexpressing plants, most AGP21 peptide was detected as a strong broad band around ≈100–120 kDa with minor bands at ≈80 and ≈55 kDa, whereas endogenously driven AGP21 produced a stronger band at ≈80 kDa and lacked the band at ≈55 kDa, suggesting that, in both cases, AGP21 peptide was present in a tri-*O*-glycosylated form. Mature peptide with no posttranslational modifications is approximately 30 kDa; the extra bands could be intermediate single- and di-*O*-glycosylated forms of AGP21 peptide. An apparent molecular shift of ≈25–30 kDa for each putative *O*-glycosylation site in AGP21 accords with AGP14 peptide, whose protein sequence is highly similar [13], and with the electrophoretic migration of an AGP-xylogen molecule that contains two arabinogalactan-*O*-Hyp sites [24]. V-AGP21^{ALA}, which lacks *O*-glycans, is not targeted to the cell surface, formed puncta (**Figure S2B**), showed one band close to ~55 kDa suggesting the presence a dimer (**Figure 2D**). The lack of *O*-glycans V-AGP21^{ALA}'s may cause to interact with itself. A detailed analysis is required to characterize *O*-glycosylation in AGP21.

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_O_-glycans stabilize AGP21 peptide's functional conformation

To address the effect of *O*-glycan on the conformation and stability of AGP21 peptide, we modeled a minimal, 15-sugar Hyp-*O*-linked arabinogalactan (AG) structure ([ATVEAP(O)AP(O)SP(O)TS], **Supplementary Item 5A–B**). This is the simplest carbohydrate structure characterized for a single AGP synthetic peptide [25], although complex, 150 residues structures exist for several AGPs [6]. To assess the conformation of AGP21 peptide and the effect of *O*-glycosylation, molecular dynamics (MD) simulations considered three nonglycosylated peptides (with alanines [nG-Ala], prolines [nG-Pro], or hydroxyprolines residues [nG-Hyp], respectively) and one *O*-glycosylated peptide with three Hyp-*O*-glycans (**Supplementary Item 5C**). In the MD simulations, the root mean square deviation (RMSD) varied up to ≈6 Å (**Supplementary Item 5D**), indicating that peptide structure may have deviated from the starting type-II polyproline helix. By contrast, larger conformational stabilization effects were observed in the *O*-glycosylated peptide (**Supplementary Item 5E**). Individual residue RMSF analysis indicated that the peptide's stiffer region depended on the MD conditions applied (**Supplementary Item 5F**). To characterize conformational profiles, we measured the angle formed by four consecutive alpha carbon atoms (ζ angle) (**Table S3**). The ζ angle of a type-II polyproline helix is **–**110 ± 15°. In this context, the *O*-glycosylated AOAOSOTS peptide structure is slightly extended between Pro2**–**Thr7, as observed by ζ angles 2**–**4 closer to 180° (**Table S3**). Our analysis suggests that *O*-linked glycans affect the conformation and stability of AGP21 peptide. This conformational change could explain, at least in part, the failure of $AGP21^{ALA}$ to complement *agp21*. How this conformational change in mature AGP21 peptide without *O*-glycans affects its function in RH cell determination remains unclear.

AGP21 acts in a BRI1–BIN2-dependent pathway to define RH cell fate

BR signaling controls RH cell fate by inhibiting BIN2 phosphorylation activity to modulate *GL2* expression [1]. In atrichoblasts, BIN2 phosphorylates TRANSPARENT TESTA GLABRA1 (TTG1), controlling protein complex TTG1-WER-GL3/EGL3 (WEREWOLF-GLABRA3/ENHANCER OF GLABRA3) activity, and stimulating *GL2* expression [1]. We hypothesized that disrupting AGPs activity with β-Glc-Y, a mutation (i.e., *agp21*), or abnormal glycosylation, would interfere with BR perception and *GL2* expression. We treated the triple mutant *gsk* (*gsk triple*: *bin2-3 bil1 bil2*; BIL1, BIN2-like 1 and BIL2, BIN2-like 2), which almost completely lacks RH cells [1], with 5 µM β-Glc-Y treatment. *Gsk triple* exhibited few contiguous RH cells (**Figure 3**), suggesting that β-Glc-Y requires BIN2-BIL1-BIL2 to alter cell fate. Interestingly, β-Glc-Y induced ≈60% contiguous RHs (**Figure 3**) in the constitutively active mutant *bin2-1* [26]. Furthermore, β-Glc-Y induced ≈60% contiguous RHs in *bri1-116* (which lacks BR signaling and has high BIN2 activity) and little response in *bri1-301* (a weak BRI1 mutant that retains some BR signaling and partial BIN2 repression). These data suggest that BR interferes with AGP-mediated RH cell fate reprogramming (**Figure 3A**), confirm that active BIN2, BIL1, and BIL2 are required for this reprogramming, and indicates the existence of an BR-independent response related AGPs perturbation with GSK proteins that induces RH cell fate.

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As *BRI1* expression is similar in trichoblasts and atrichoblasts (Fridman et al., 2014), we sought to determine whether BRI1 acts differently in these cell types during RH cell fate determination (**Figures 3B**). We examined the effect of cell type-specific *BRI1* expression on the percentage of contiguous RHs in three plant lines, all in the *bri1-116* background: trichoblast-only (*COBL9p::BRI1/bri1-116*), atrichoblast-only (*GL2p::BRI1-GFP/bri1-116*), and expression in both cell types (*GL2p::BRI1* + *COBL9p::BRI1/bri1-116*) (Hacham et al., 2011; Fridman et al., 2014). BRI1 expression in atrichoblasts only did not rescue *bri1-116* (plants showed abundant contiguous RHs), lines that expressed BRI1 in trichoblasts or in both cell types were similar to wild type (**Figure 3B**). Additionally, only *COBL9p::BRI1/bri1-116* was completely insensitive to β-Glc-Y while the other two lines exhibited more contiguous RHs. These data imply that only the BR pathway in atrichoblasts is linked to AGP disruption and ectopic RH development.

Disturbance or absence of AGP21 blocks *GL2* expression

A complex of transcription factors [27,28] promotes GL2 expression, which inhibits RHD6 and thereby blocks the RH pathway. In trichoblasts, a second transcription factor complex suppresses GL2 expression [27], forcing cells to enter the RH cell fate program via concomitant RHD6 activation and downstream TFs, including RSL4, and RH genes [2]. We tracked epidermal cell fate and analyzed β-Glc-Y and α-Man-Y's translational effects on several markers: an early RH marker (RHD6p::RHD6-GFP), a downstream transcription factor (RSL4p::RSL4-GFP), a late RH marker (EXP7p::EXP7-GFP), and an atrichoblast marker GL2 (GL2p::GL2-GFP) (**Figure 4A–D**). β-Glc-Y, not α-Man-Y, repressed GL2 expression and enhanced RHD6, RSL4 and EXP7 expression in contiguous epidermal cells (**Figure 4A–E**). This corroborates the effects of both β-Glc-Y and deficiencies in the AGP *O*-glycosylation pathway on contiguous epidermis cell development. When we expressed RSL4p::RSL4-GFP in *agp21*, two contiguous epidermis cells showed GFP expression, while this rarely occurred in wild type roots; GL2p::GFP/*agp21* showed discontinuous RH patterning similar to β-Glc-Y treatment (**Figure 4B** and **4D**). This result implies feedback between aberrant AGP21, GL2 repression, and RHD6-RSL4 and EXP7 upregulation in contiguous epidermal cell development (**Figure 4E**). Constitutively active *bin2-1* phenocopies *agp21* and β-Glc-Y treatment: it represses GL2 expression in some epidermal cells and enhances EXP7-GFP in contiguous epidermal cells, stimulating contiguous RH development (**Figure 4F–G**). This suggests that AGP21 acts on GL2 in BIN2-dependent manner and affects BR perception at the cell surface.

AGP21 influences cell surface BR perception, modifying RH cell fate

To test whether AGP21 (and AGPs in general), affect BR perception, we treated roots with 100 nM BL. Wild type roots exhibited repressed RH development as previously reported [1]; *agp21* and three glycosyltransferase mutants (*triple hpgt, ray1* and *galt29A*) defective in AGP *O*-glycosylation were unaffected (**Figure S2C**), suggesting that *O*-glycosylated AGP21 promotes BR perception and signaling. We hypothesized that AGP21 would closely associate with BRI1–BAK1 receptors, possibly affecting BR perception and BIN2 signaling to influence RH cell fate. BRI1–BAK1 proteins form hetero-oligomers in specific microdomains at the PM [29] where the

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environment restricts lateral diffusion [30]. We examined whether AGP21 expressed in *Nicotiana benthamiana* colocalized with the BRI1 coreceptor BAK1 (**Figure S3A–B**). V-AGP21 partially colocalized with BAK1-mRFP protein, suggesting they exist in close to the PM (**Figure S3A**). When epidermal cells were plasmolyzed, most AGP21 signal localized to the apoplast but some remained close to the PM (**Figure S3B**), implying that AGP21 lies close to BAK1 and influences BR perception and BIN2-mediated RH cell fate programming in atrichoblasts. Immunoprecipitation failed to detect an interaction between V-AGP21 and BAK1-mRFP in a transient expression system (results not shown). Nonetheless, measuring direct physical interactions between *O*-glycosylated AGP21 and BRI1–BAK1 in the apoplast–PM space could support a direct interaction and would corroborate for the first time a role for an AGP peptide in BR perception on the plant cell surface.

Conclusions

In root epidermal cells, atrichoblast fate is the default, while environmental cues inhibit *GL2* expression in specific atrichoblasts to produce trichoblasts [1]. In the absence of BRs, active P-BIN2 represses *GL2* expression and *RHD6* and *RSL4* expression proceeds, triggering RH development in atrichoblasts and producing contiguous RHs. Abnormal AGPs at the cell surface stimulate ectopic RH development similar to that observed in BR mutants. BZR1 regulates *AGP21* expression and the *O*-glycosylated cell surface peptide AGP21 modulates RH cell fate. We propose a model in which the *O*-glycosylated AGP21 peptide localizes close to BAK1 (and potentially also BRI1) and thereby influences BR perception and BIN2 (and BIL1-BIL2)-mediated responses, controlling root epidermal cell fate (**Figure S4**). In addition, an BR-independent response links AGP21 peptide (and AGPs) to a downstream BIN2 component that also promotes RH cell development via an unknown molecular connection. These results imply an interesting parallel between plant AGPs and animal heparin sulfate proteoglycans (HSPGs), which are important coreceptors in signaling pathways mediated by growth factors, including members of Wnt/Wingless, Hedgehog, transforming growth factor-β, and fibroblast growth factor family members [31]. The molecular mechanisms by which *O*-glycosylated AGP21 peptide affects BR perception by BRI1–BAK1 remain unclear. Future work should investigate the roles of AGP21 peptide and *O*-glycans in BR perception by root epidermal cells.

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Acknowledgements

We thank ABRC (Ohio State University) for providing T-DNA lines seed lines. We would like to thank Dr. Sigal Savaldi-Goldstein for providing Gl2p::BRI1-GFP and COBL9p::BRI1-GFP reporter lines, Dr. Paul Dupree for providing *fut4* and *fut6* mutant lines, Dr. Santiago Mora García for providing *bzr1* and *bes1* mutant lines, Dr. Ana Caño Delgado for providing seeds of BRI1-GFP, *bin2-1* and BZR-YFP, and Dr. Gustavo Gudesblat for providing *gsk triple* mutant seeds. Prof. Malcolm Bennet and Prof. Liam Dolan for the RHD6-GFP and RSL4-GFP lines. This work was supported by grants from ANPCyT (PICT2014-0504, PICT2016-0132 and PICT2017-0066) and ICGEB CRP/ARG16-03 to J.M.E.

Author Contribution

C.B, J.G.D and M.M.R performed most of the experiments, analysed the data and wrote the paper. L.P.F and H.V. performed molecular dynamics simulations and analysed this data. M.C.S analysed the phenotype of glucosyl transferase mutants and BRI1-GFP reporters. B. V. and analysed the molecular dynamics simulations data. M.C synthesized the α-Man-Y and β-Glc-Y reagents. G. S. commented on the project, read the manuscript and commented on the results. S.M. and E.M. analysed the data and commented on the results. J.M.P., D.R.R.M., Y.R., and S.M.V commented on the results. J.M.E. designed research, supervised the project, and wrote the paper. This manuscript has not been published and is not under consideration for publication elsewhere. All the authors have read the manuscript and have approved this submission.

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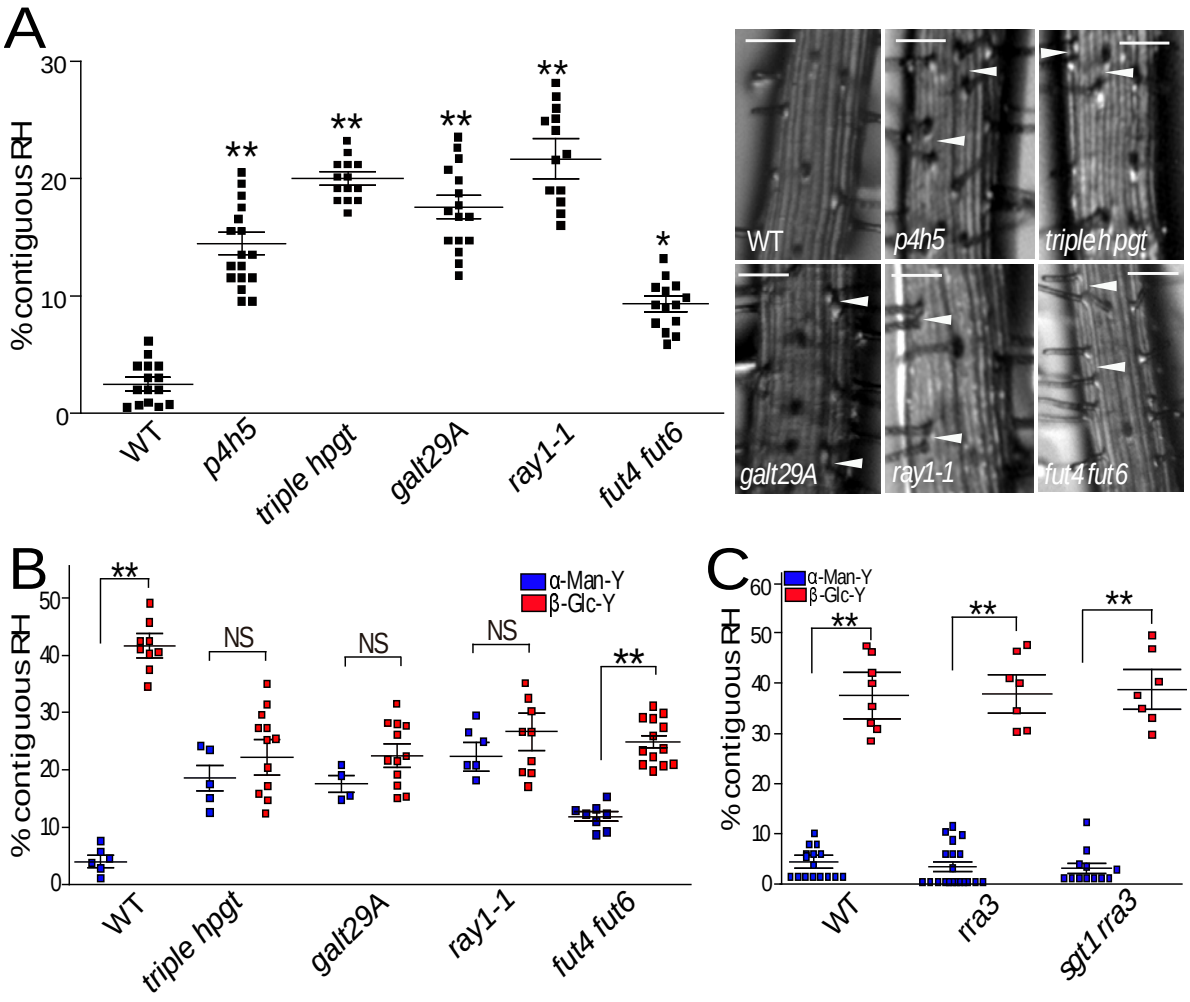

Figure 1. Contiguous RH phenotype in *O*-underglycosylated AGPs phenocopy BR mutants.

(A) RH phenotype in the *p4h5* mutant and in four glycosyltransferase mutants (*triple hpgt*, *ray1*, *galt29A*, and *fut4 fut6*) that act specifically on AGP *O*-glycosylation. Right, selected pictures. Arrowheads indicated two contiguous RHs. Scale bar= 50 µm.

(B) RH phenotype in three glycosyltransferase mutants (*triple hpgt*, *ray1*, *galt29A* and *fut4 fut6*) that act specifically on AGP *O*-glycosylation. Effect on contiguous RH phenotype in roots treated with 5µM α-Mannosyl Yariv (α-Man-Y) or 5µM β-Glucosyl Yariv (β-Glc-Y).

(C) RH phenotype in two glycosyltransferase mutants (*rra3* and *rra3 sgt1*) that act specifically on EXT *O*-glycosylation. Effect on contiguous RH phenotype in roots treated with 5µM α-Mannosyl Yariv (α-Man-Y) or β-Glucosyl Yariv (β-Glc-Y).

(A-C) *P*-value of one-way ANOVA, (**) P<0.001, (*) P<0.01. NS= not significant different. Error bars indicate ±SD from biological replicates.

See also Figure S1 and Supplementary Item 1.

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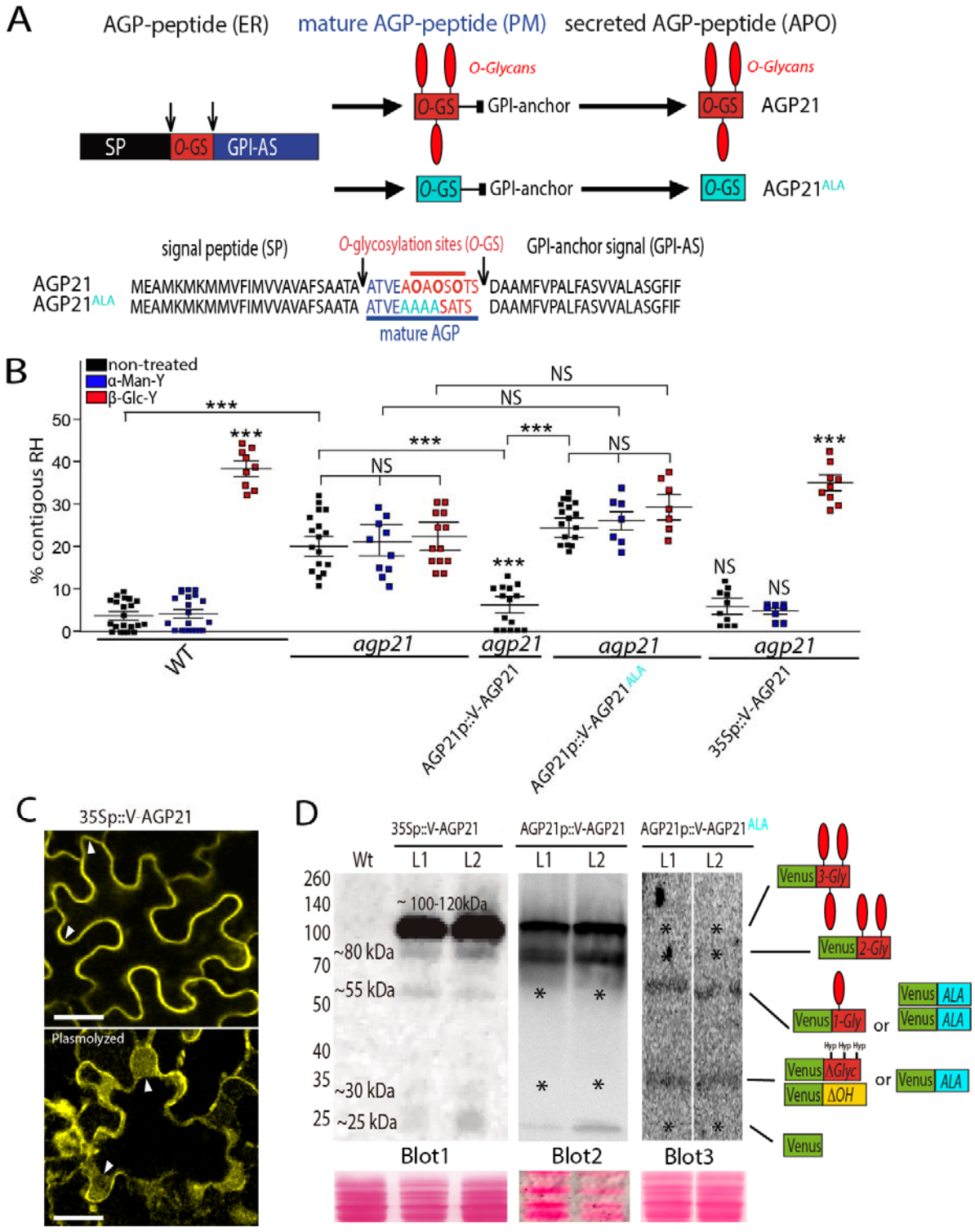

Figure 2. *O*-glycosylated AGP21 peptide at the cell surface modulates RH cell fate.

(A) Identified AGP21 peptide acting on root epidermis development. AGP21 peptide sequence and its posttranslational modifications carried out in the secretory pathway. The mature AGP21 peptide contains only 10-13 aa in length. APO= Apoplast. ER=Endoplasmic Reticulum. GPI anchor= GlycosylPhosphatidylInositol (GPI) anchor. PM=Plasma membrane.

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(B) Contiguous RH phenotype in *agp21*, complemented *agp21* mutant with AGP21p::V-AGP21 and with 35Sp::V-AGP21 constructs as well as AGP21p::V-AGP21^{ALA} expression in *agp21*. Only one line is shown. *P*-value of one-way ANOVA, (**) P<0.001, (*) P<0.01. NS= not significant differences. Error bars indicate ±SD from biological replicates.

(C) Subcellular localization of 35Sp::V-AGP21 transiently expressed in *Nicotiana benthamiana* (on the left) or in *Arabidopsis thaliana* (on the right). Plasmolysis (P+) induced with a Mannitol (800 mM) treatment (bottom pictures) showed a secretion outside the plasma membrane and in the plasma membrane of AGP21 in *N. benthamiana* or only plasma membrane AGP21 localization in *A. thaliana*. Arrowheads indicate plasma membrane located AGP21. Scale bar= 50 µm.

(D) Immunoblot analysis of two stable lines expressing 35Sp::V-AGP21 (L1-L2) and two lines expressing AGP21p::V-AGP21 (L1-L2) and two lines expressing AGP21p::V-AGP21^{ALA} (L1-L2). Each blot is an independent experiment. Putative Venus-AGP21 structures are indicated on the right. *O*-glycans are indicated as red elongated balloons. ΔOH = non-hydroxylated. ΔGly = without *O*-glycans. 1-Gly to 3-Gly = 1 to 3 sites with Hyp-*O*-glycosylation. Asterisk indicates missing AGP21 glycoforms or lack of Venus protein.

See also Figure S2 and Supplementary Items 2-5.

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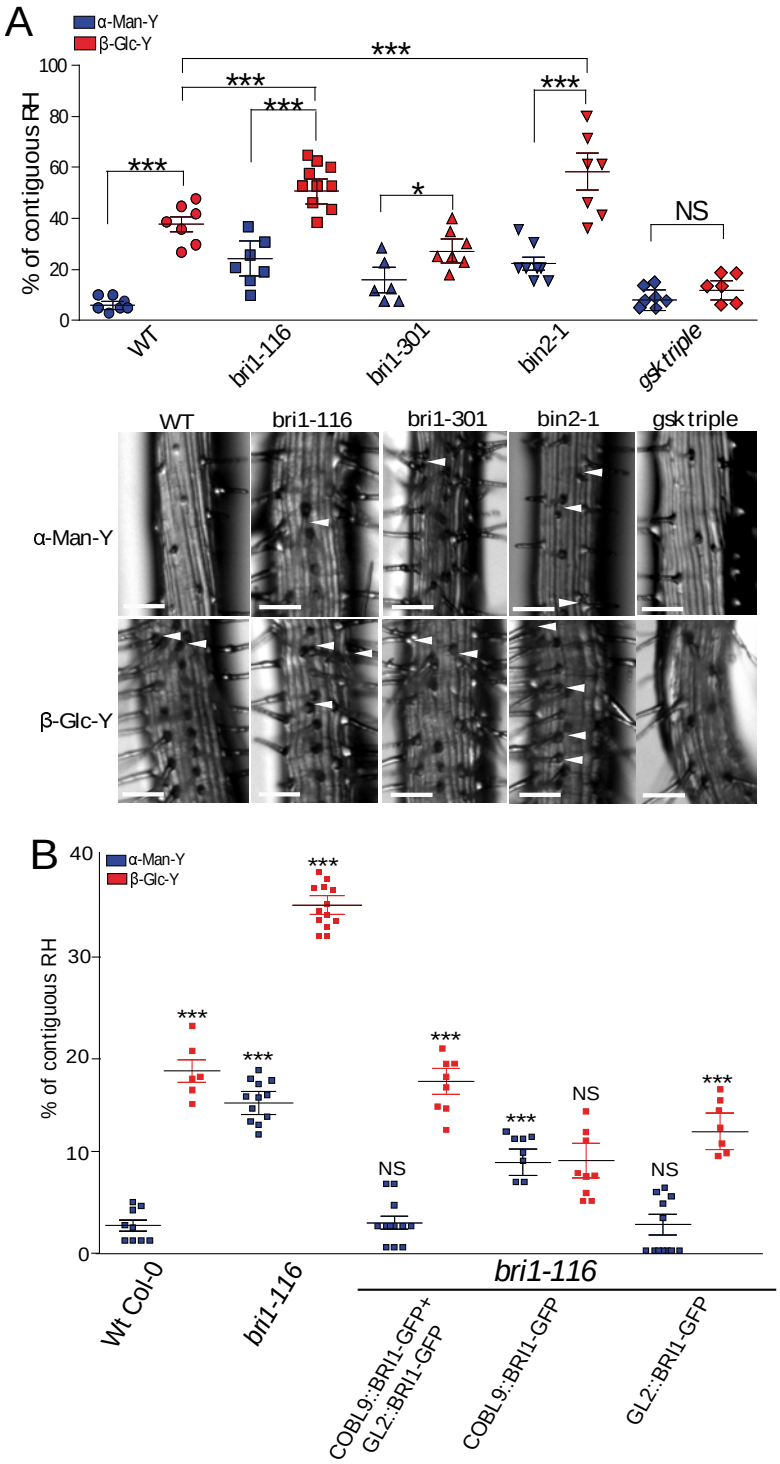

Figure 3. Perturbation of AGPs requires active BRI1 expression in atrichoblast cells and downstream BIN2-BIL1-2 proteins to triggers changes in RH cell fate.

(A) Contiguous RH phenotype in roots treated with 5µM β-Glucosyl Yariv (β-Glc-Y) or 5µM α-Mannosyl Yariv (α-Man-Y). Scale bar= 20 µm. *P*-value of one-way ANOVA, (***) P<0.001, (*) P<0.05. NS= not significant differences. Error bars indicate ±SD from biological replicates. Arrowheads indicated two contiguous RHs.

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(B) Effect of the BRI1 differential expression on the development of contiguous RH. BRI1 is active when expressed in atrichoblast cells (under GL2 promoter).

See also Figure S3.

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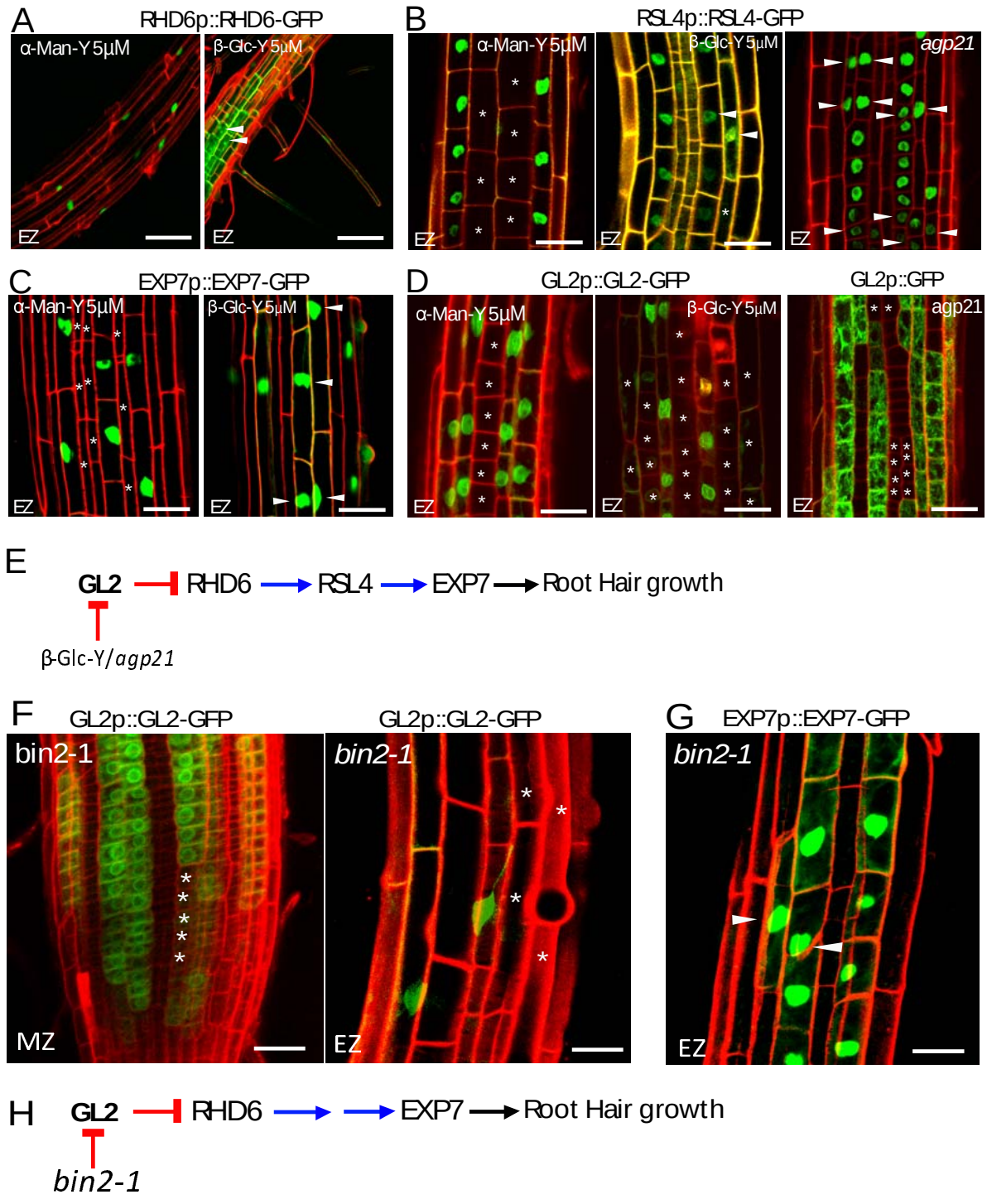

Figure 4. AGPs disruption, the lack of AGP21, and *bin2-1* block the RH repressor GLABRA2 (GL2) and triggers RHD6-RSL4-EXP7 expression in some atrichoblast cells.

The effect of β-Glucosyl Yariv (β-Glc-Y), α-Mannosyl Yariv (α-Man-Y), and the absence of AGP21 peptide were monitored on several markers to study epidermis cell fate.

(A) RHD6 (RHD6p::RHD6-GFP) as an early RH marker.

(B) A downstream RHD6 factor RSL4 (RSL4p::RSL4-GFP).

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(C) The RSL4-gene target EXP7 (EXP7p::EXP7-GFP).

(D) The main RH repressor GL2 (GL2p::GL2-GFP). (A-D) Arrowheads indicate expression of a given marker in two contiguous epidermis cell lines. Asterisks indicate absence of expression. Scale bar= 20 µm.

(E) Proposed sequence of events triggered by β-Glucosyl Yariv (β-Glc-Y) or the lack of AGP21 peptide that leads to abnormal RH development.

(F) GL2 expression in the *bin2-1* background in the Meristematic Zone (MZ) and Elongation Zone (EZ) of the root.

(G) The RH marker EXP7 expression in the *bin2-1* background in the Elongation Zone (EZ) of the root. (F-G) Arrowheads indicate expression of a given marker in two contiguous epidermal cell lines. Asterisks indicated absence of expression. Scale bar= 10 µm.

(H) Proposed sequence of events triggered by *bin2-1* that leads to abnormal RH development.

See also Figure S4.