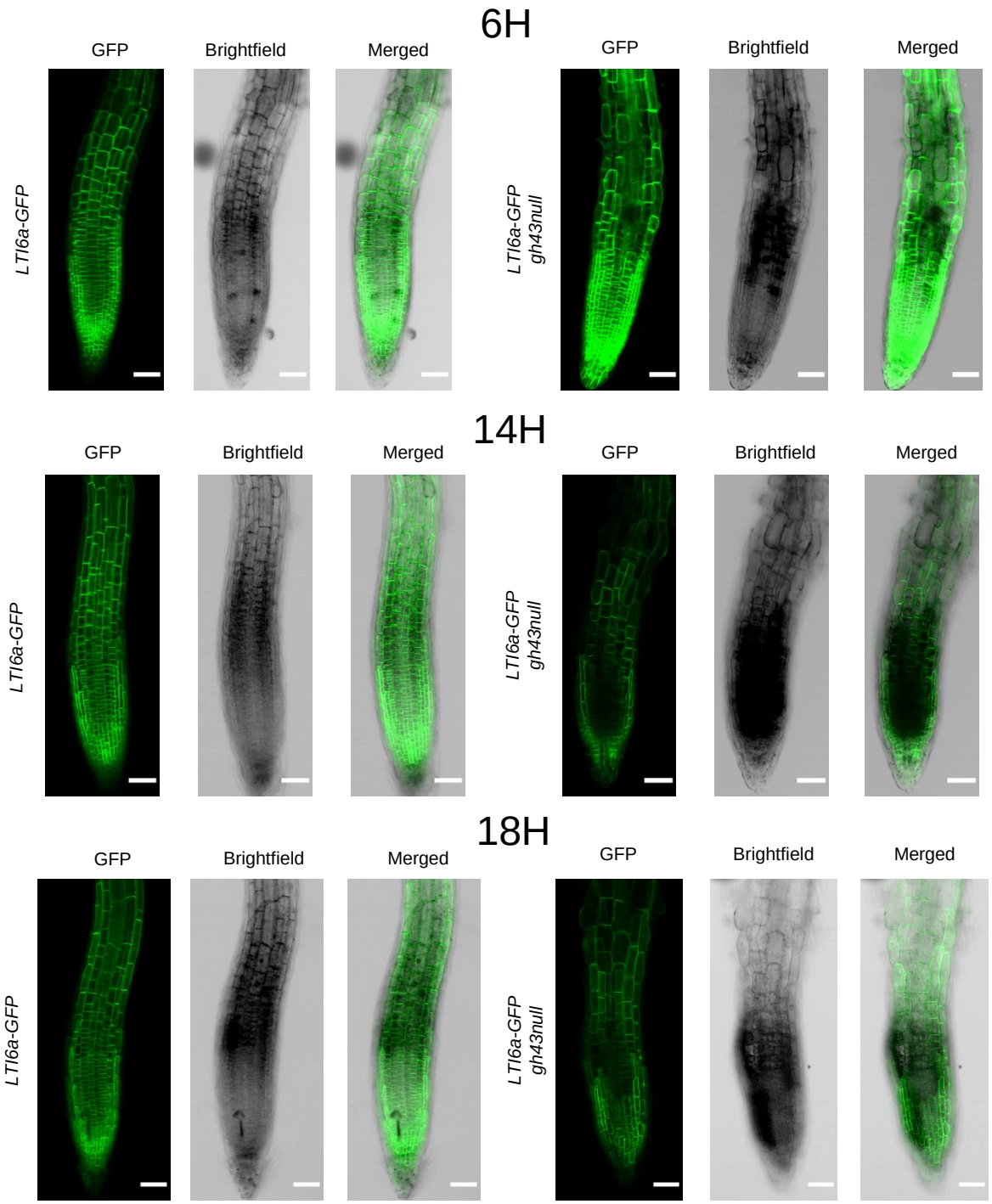


Figure S1. Confocal laser scanning microscope images of wild type and *gh43null* mutant roots expressing the cell membrane marker LTI6a-GFP. Seedlings were first grown for 4 days on ½ MS medium and then moved to ½ MS medium containing 4.5% glucose. Images were taken every 4 hours after seedlings were moved to sugar plates (scale bar: 100 µm).

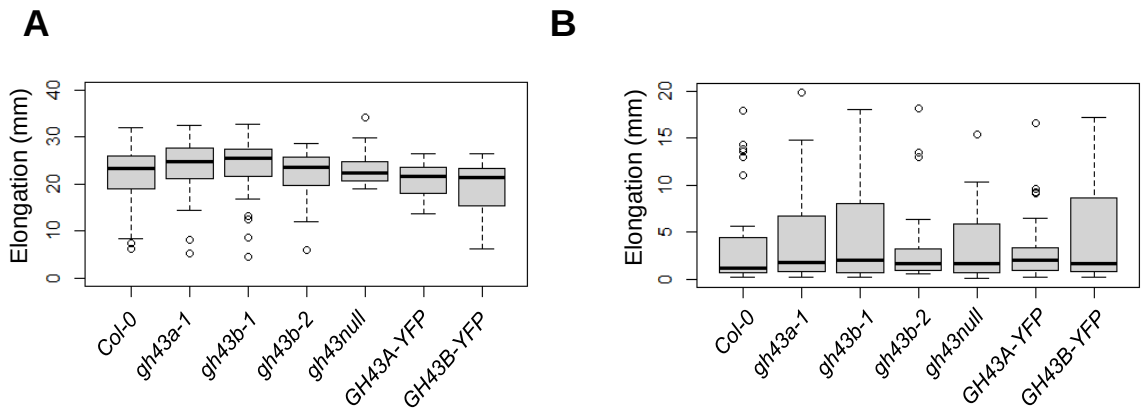
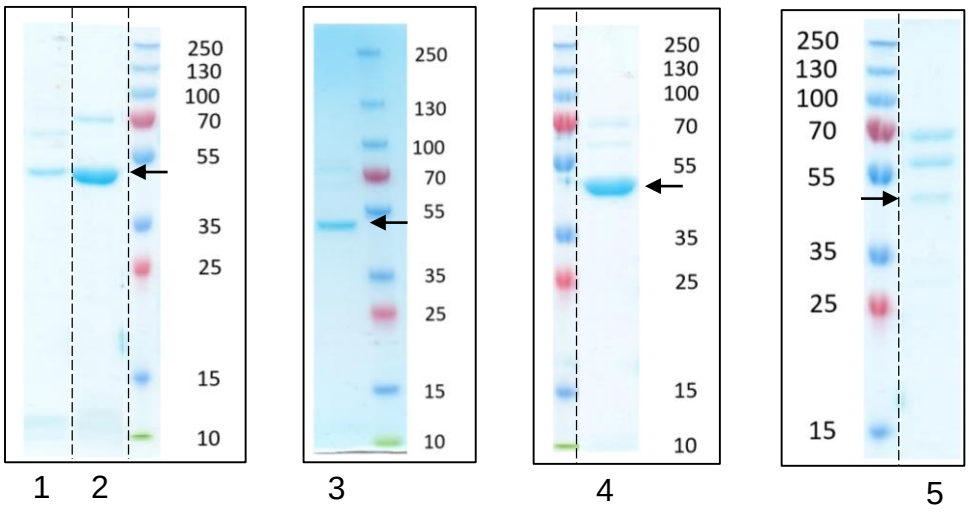


Figure S2. Root elongation in *gh43* mutants grown on (A) 4.5% sorbitol or (B) 100mM NaCl. Seedlings were first grown for 4 days on ½ MS and then moved to 4.5% sorbitol or to 100 mM NaCl containing ½ MS plates. Root elongation was quantified 6 days after seedlings were moved. The boxplot's dark horizontal lines represent the median, the two grey boxes the 25th and 75th percentile, the whiskers the 1.5 interquartile limits and the dots the outliers. *n* = 29-32 biological replicates.

```
GH43A      -MKKNNKYNKKSTSLHCNDAGGCRYSLLTIVWTVVGFFLVAHLISLYSRKDNNIHQQVSS 59
GH43B      MRVMKNKHNKKATFLRCSP--------FGLVSTVVGCVFMIHLTMLYSRSYSVD------ 46
               :**:***:* *:*.         : :* **** .:: **  ****. .

GH43A      DQLQVVHHLAHPIVRELIRVEEEVLRMPPPRKRSPRTSKRRSRKPIPLVEEFLDDKSPIR 119
GH43B      LDLSPQLLIHHPIVRELERVEEENIHMPPPRKRSPRAIKRKPKTPTTLVEEFLDENSQIR 106
            :*.    : ******* ***** ::**********: **: :.*  *******::* **

GH43A      HLFFPGIKTAAFGPTK-DMGNETSYYFPGKIWMDTQGNPIQAHGGGILLDVKSNTYYWYG 178
GH43B      HLFFPDMKSA-FGPTKEDTNDTSHYYFPGRIWTDTEGNPIQAHGGGILFDDISKVYYWYG 165
           *****.:*:* ***** * .: : *****:** **:************:*  *:.*****

GH43A      EYKDGPTYHAHKKGPARVDIIGVGCYSSKDLWTWKNEGIVLGAEETNKTHDLHKSNVLER 238
GH43B      EYKDGPTYLSHKKGAARVDIIGVGCYSSKDLWTWKNEGVVLAAEETDETHDLHKSNVLER 225
           ******** :**** ***********************:**.****::************

GH43A      PKVIYNEKTEKYVMWMHIDDANYTKASVGVAISNSPTGPFEYLYSKRPHGFDSRDMTVFK 298
GH43B      PKVIYNSDTGKYVMWMHIDDANYTKASVGVAISDNPTGPFDYLYSRSPHGFDSRDMTVYK 285
           ******..* ***********************:.*****:****: ***********:*

GH43A      DDDGVAYLIYSSEVNSVLHIGPLTEDYLDVTPVMKRVMVGQHREAPAIFKHQNIYYMVTS 358
GH43B      DDDNVAYLIYSSEDNSVLHIGPLTENYLDVKPVMKRIMVGQHREAPAIFKHQNTYYMITS 345
           ***.********* ***********:****.*****:**************** ***:**

GH43A      WCTGWAPNEALAHAAESIMGPWEKLGNPCIGGNKVFRLTTFFAQSTYVIPLPGVPGAFIF 418
GH43B      GCTGWAPNEALAHAAESIMGPWETLGNPCVGGNSIFRSTTFFAQSTFVIPLPGVPGVFIF 405
            **********************.*****:***.:** ********:*********.***

GH43A      MADRWNPADLRDSRYVWLPLVIGGPADQPLEFNFGFPSWSRVSIYWHSKWRLP------- 471
GH43B      MADRWNPADLRDSRYLWLPLIVGGPADRPLEYSFGFPMWSRVSVYWHRQWRLPSAREKKI 465
           ***************:****::*****:***:.**** *****:*** :****

GH43A      -    471
GH43B      A    466
```

Figure S3. Protein sequence alignment of GH43A (AT5G67540.1) and GH43B (AT3G49880) performed with ClustalW. The GH43A and GH43B proteins are more than 90% similar in their predicted enzymatic domain, which in GH43A corresponds to amino acids 145-425, and in GH43B to amino acids 158-440aa.

A



B

```
Ct1,3Gal43A      SNL-----------FLGVSCYRSKDLVNWEYRGEVLSRNSAPEL----NHCNIERPKVMY        118
AtGH43A          PTYHAHKKGPARVDIIGVGCYSSKDLWTWKNEGIVLGAEETNKTHDLHKSNVLERPKVIY        238
AtGH43B          PTYLSHKKGAARVDIIGVGCYSSKDLWTWKNEGVVLAAEETDETHDLHKSNVLERPKVIY        230
                  .            ::**.** **** .*: .* **. :.: :     :   :*****:*

Ct1,3Gal43A      NASTGEFVMWMHWENGINYGQARAAVAYSKTPDGKFTYIRSFRPMQDTGVMDHGLPGYMS        178
AtGH43A          NEKTEKYVMWMHIDD-ANYTKASVGVAISNSPTGPFEYLYSKRP-----------HGFDS        286
AtGH43B          NSDTGKYVMWMHIDD-ANYTKASVGVAISDNPTGPFDYLYSRSP-----------HGFDS        278
                 * .* ::***** ::  ** :* ..** *..* * * *: *  *            *: *

Ct1,3Gal43A      RDCNVFVDTDGKGYFISAANENMDLHLYELTPDYKNIASLKAKLFVGQQRAPCLIKRNG        238
AtGH43A          RDMTVFKDDDGVAYLIYSSEVNSVLHIGPLTEDYLDVTPVMKRVMVGQHREAPAIFKHQN        346
AtGH43B          RDMTVYKDDDNVAYLIYSSEDNSVLHIGPLTENYLDVKPVMKRIMVGQHREAPAIFKHQN        338
                 ** .*: * *. .*:* ::: *  **:  ** :* ::  :  :::***:****.::*::.
```

C

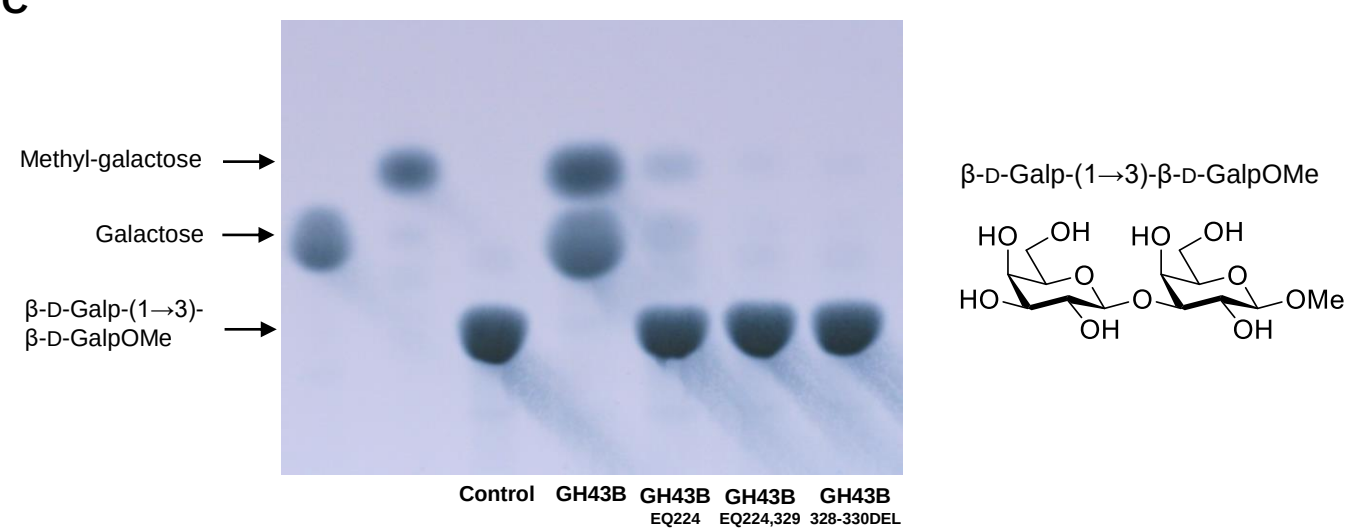


Figure S4. Testing the actívity of heterologously expressed GH43B proteins with mutations in predicted active sites

(A) Heterologoulsy expressed GH43 proteins. Dotted lines indicate splicing between gels.

(B) Predicted active sites highlighted in bold and yellow based on the crystal structure of 1,3Gal43A from *Clostridium thermocellum* (Jiang et al. 2012).

(C) GH43B with mutations/deletions in putative active site(s), lose activity towards Methyl β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside .

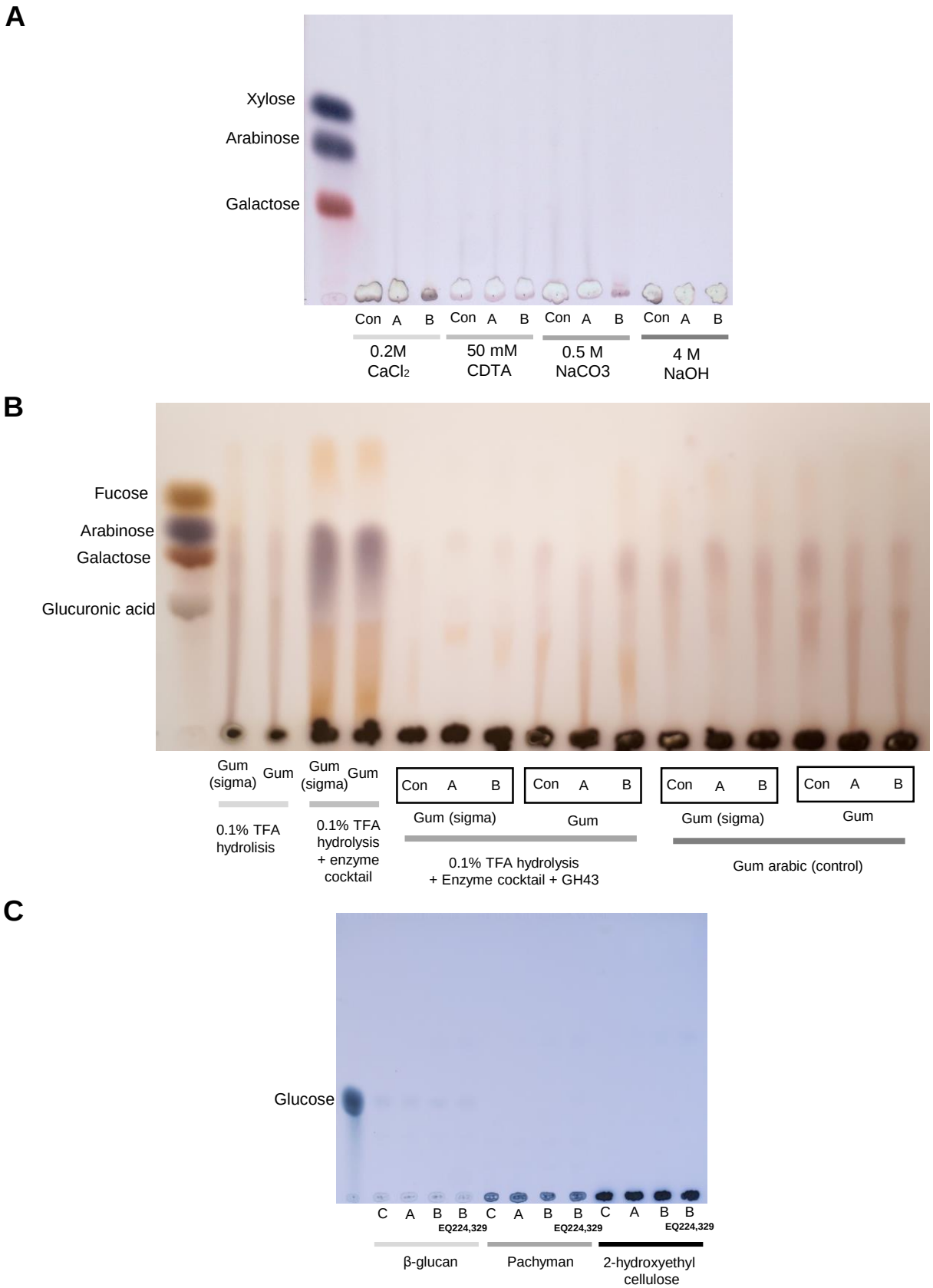


Figure S5. Activity assay of the heterologously expressed GH43s on Gum Arabic, partially digested Gum Arabic, sequentially extracted cell wall material and β1,3 or β1,4 glucan substrates. (A) No activity on sequentially extracted cell wall material isolated from wild type Col-0 rosette leaves. **(B)** No activity on Gum Arabic or partially trimmed Gum Arabic, which was first partially hydrolysed in 0.1M TFA, then digested with an enzyme cocktail to remove most side chains of AGP glycosylation. The digestion products were buffer changed and then hydrolized with the GH43 enzymes. **(C)** No activity on β-glucan, pachyman or 2-hydroxyethyl cellulose**.** C/Con=control, A= GH43A, B=GH43B and $B^{EQ224,229}$= $GH43B^{EQ224,229}$

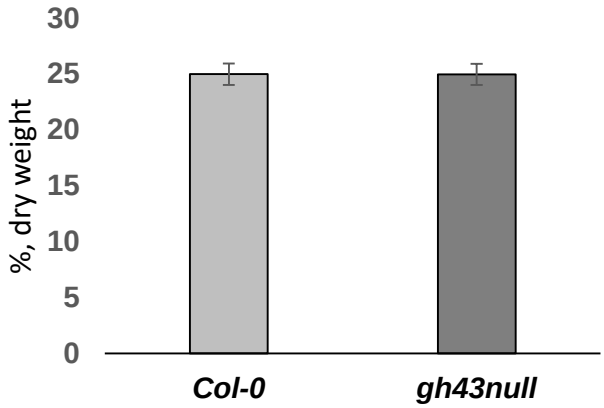


Figure S6. Crystalline cellulose content of 7 day old seedlings grown on ½ MS plates. Crystalline cellulose was quantified from AIR1 and AIR2 treated 7 day old cell wall material from *Arabidopsis* seedlings. *n* = 5 biological replicate seedlings pools.

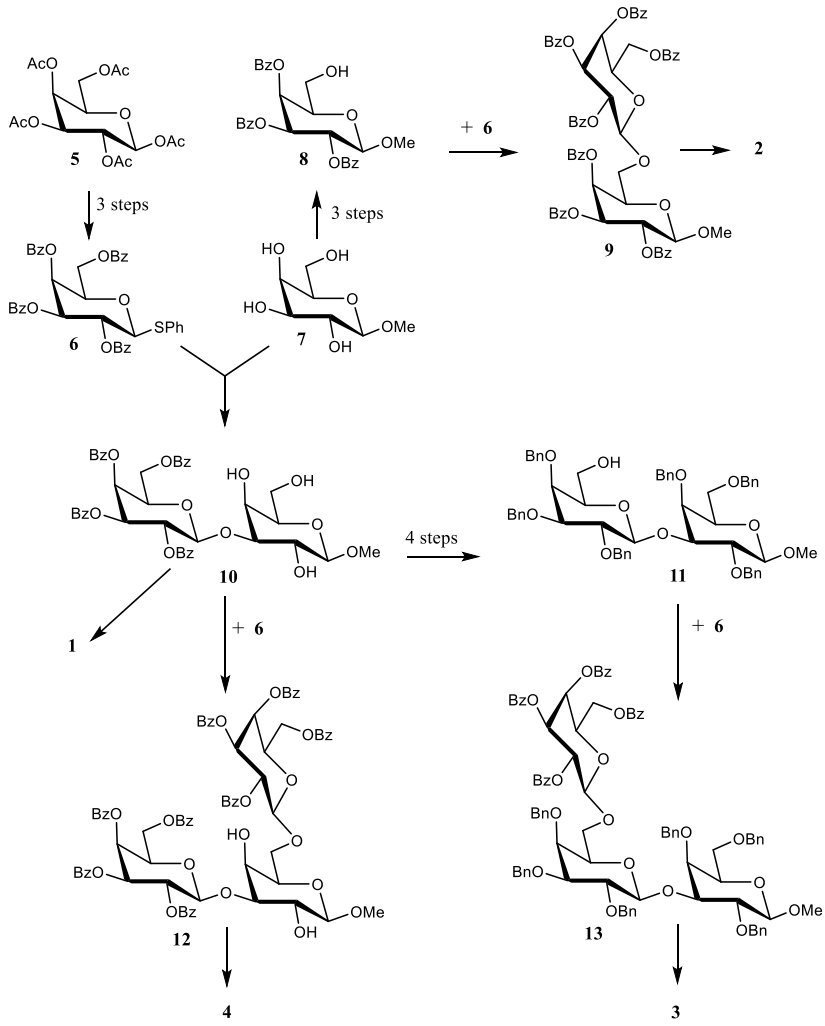


Figure S7. The strategy for chemical synthesis of methyl β-galactopyranosides 1-4.

In this strategy, commercially available 1,2,3,4,6-penta-O-acetyl-β-D-galactopyranose (**5**) and methyl β-D-galactopyranoside (**7**) were used as the starting materials. Compound **5** was converted into phenyl 2,3,4,6- tetra-O-benzoyl-1-thio-β-D-galactopyranoside (**6**) and compound **7** was converted into methyl 2,3,4,-tri-O-benzoyl-β-D-galactopyranoside (**8**) (Nashed and Glaudemans 1987) as described by literature. Glycosidation reaction between the phenylthio galactopyranoside derivative **5** as a glycosyl donor using the methyl galactopyranoside **7** as the glycosyl acceptor with the activator NIS–TfOH system (Kaji et al., 2010) according to the reported procedure provided the corresponding disaccharide derivatives **10**. Removal of the protecting groups from compounds **10** afforded the desired disaccharide **1**. Further glycosidation of compound **10** afforded the target trisaccharide **4** as described (Kaji et al., 2010). On the other hand, compound **10** was manipulated and converted into methyl 2,4,6-tri-O-benzyl-3-O-(2,3,4-tri-O-benzyl-β-D-galactopyranosyl)-β-D-galactopranoside (**11**) in four steps using conventional procedures. Similarly, glycosidation reaction between the glycosyl donor **6** and the glycosyl acceptors **8** and/or **11** with the activator NIS–TfOH system (Kaji et al., 2010) provided both the corresponding disaccharide derivative **9** and/or the trisaccharide derivative **13**. Removal of the protecting groups from both compounds **9** and **13** afforded the desired disaccharide **2** and the trisaccharide **3**. Detailed experimental data will be published elsewhere.

Kaji E, Nishino T, Ishige K, Ohya Y, Shirai Y. 2010. Regioselective glycosylation of fully unprotected methyl hexopyranosides by means of transient masking of hydroxy groups with arylboronic acids. Tetrahedron Letters 51(12): 1570-1573.

Nashed EM, Glaudemans CP. 1987. Selective silylation of. beta.-D-galactosides. A new approach to the synthesis of (1. fwdarw. 6)-. beta.-D-galactopyranooligosaccharides. The Journal of Organic Chemistry 52(23): 5255-5260.