





**Figure S2. Western-blotting analysis of the 11 recombinant AtGT14 family genes expressed in *Nicotiana benthamiana*.** Lane GFP, 5 mg/mL MMs extracted from *N. benthamiana* expressing empty pEarley101 vector with GFP tag (27 kDa, 25  $\mu$ g total protein). Lanes 240-250, 25  $\mu$ g microsomal samples of the eleven recombinant AtGT14 family genes (240: AT1G03520, 241: AT1G53100, 242: AT1G71070, 243: AT2G37585, 244: AT3G03690, 245: AT3G15350, 246: AT3G24040, 247: AT4G03340, 248: AT4G27480, 249: AT5G15050, 250: AT5G39990).

**Table S1.** Selected examples of *Arabidopsis* cell wall biosynthetic GTs whose catalytic specificities have been analyzed by following expression in heterologous systems.

GT family	Gene	Biochemical function	Expression System	Reference
2	CESA	cellulose synthase	<i>Pichia pastoris</i>	Purushotham et al. (2016). Proc. Natl. Acad. Sci. U.S.A. [1]
2	CSLA	$\beta$ -1,4-mannan synthase	<i>Pichia pastoris</i>	Voiniciuc et al. (2018). Proc. Natl. Acad. Sci. U.S.A. [2]
2	CSLC	xyloglucan backbone $\beta$ -1,4-glucan synthase	<i>Pichia pastoris</i>	Cocuron et al. (2007). Proc. Natl. Acad. Sci. U.S.A. [3]
2	CSLD	(gluco)mannan synthase	<i>Nicotiana benthamiana</i>	Yin et al. (2011). Mol. Plant [4]
2	CSLD3	$\beta$ -1,4-glucan synthase	<i>Pichia pastoris</i>	Yang et al. (2020). Plant Cell [5]
8	GAUT	homogalacturonan $\alpha$ -1,4-galacturonosyltransferase	Human embryonic kidney cell line HEK293	Sterling et al. (2006). Proc. Natl. Acad. Sci. U.S.A. [6]
8	GUX1,2 and 4	xylan $\alpha$ -1,2-glucuronosyltransferase	<i>Nicotiana benthamiana</i>	Rennie et al.

				(2012). Plant Physiol. [7]
14	GLCAT14	AGP $\beta$ -1,6-/ $\beta$ -1,3-galactan $\beta$ -1,6-glucuronosyltransferase	<i>Pichia pastoris</i>	Knoch et al. (2013). Plant J. [8]
31	GALT	AGP $\beta$ -1,3-galactosyltransferase	<i>Nicotiana benthamiana</i>	Basu et al. (2015). BMC Plant Biol. [9]
34	XXT	xyloglucan $\alpha$ -1,6-xylosyltransferase	<i>Escherichia coli</i>	Urbanowicz et al. (2012). Proc. Natl. Acad. Sci. U.S.A. [10] Faik et al. (2002). Proc. Natl. Acad. Sci. U.S.A. [11]
34	GMGT	galactomannan $\alpha$ -1,6-galactosyltransferase	<i>Nicotiana benthamiana</i>	Voiniciuc et al. (2015). Plant Physiol. [12]
37	FUT1	xyloglucan $\alpha$ -1,2-fucosyltransferase	Mammalian COS cells	Perrin et al. (1999). Science [13]
37	FUT4/6	AGP $\alpha$ -1,2-fucosyltransferase	Tobacco BY2 cells	Wu et al. (2010). J. Biol. Chem. [14]
43	IRX9/14	xylan $\beta$ -1,4-xylosyltransferase	<i>Nicotiana benthamiana</i>	Zeng et al. (2016). Plant Physiol. [15]

47	IRX10	xylan $\beta$ -1,4-xylosyltransferase	<i>Pichia pastoris</i>	Jensen et al. (2014). Plant J. [16]
47	MUR3	xyloglucan $\beta$ -1,2-galactosyltransferase	<i>Pichia pastoris</i>	Madson et al. (2003). Plant Cell [17]
47	ARAD	arabinan $\alpha$ -1,5-arabinosyltransferase	<i>Nicotiana benthamiana</i>	Lampugnani et al. (2016). Plant Physiol. [18]
48	GSL	callose synthase	<i>Pichia pastoris</i>	Lars et al. (2002). Plant Mol. Biol. [19]
77	RGXT	rhamnogalacturonan-II $\alpha$ -1,3-xylosyltransferase	<i>Insect cells</i>	Egelund et al. (2006). Plant Cell [20]
92	GALS1,2 and 3	rhamnogalacturonan-I $\beta$ -1,4-galactan synthases	<i>Nicotiana benthamiana</i>	Ebert et al. (2018). Plant Cell Physiol. [21]
9	HPAT	hydroxyproline $\beta$ -arabinofuranosyltransferase	<i>Pichia pastoris</i>	Suzuki et al. (2017). Plant Physiol. [22]
106	RRT	rhamnogalacturonan-I rhamnosyltransferase	<i>Nicotiana benthamiana</i>	Takenaka et al. (2018). Nat. Plants [23]

## References

1. Purushotham, P.; Cho, S.H.; Díaz-Moreno, S.M.; Kumar, M.; Nixon, B.T.; Bulone, V.; Zimmer, J. A single heterologously expressed plant cellulose synthase isoform is sufficient for cellulose microfibril formation in vitro. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 11360, doi:10.1073/pnas.1606210113.
2. Voiniciuc, C.; Dama, M.; Gawenda, N.; Stritt, F.; Pauly, M. Mechanistic insights from plant heteromannan synthesis in yeast. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 522-527, doi:10.1073/pnas.1814003116.
3. Cocuron, J.C.; Lerouxel, O.; Drakakaki, G.; Alonso, A.P.; Liepman, A.H.; Keegstra, K.; Raikhel, N.; Wilkerson, C.G. A gene from the cellulose synthase-like C family encodes a  $\beta$ -1,4 glucan synthase. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 8550-8555, doi:10.1073/pnas.0703133104.
4. Yin, L.; Verherbruggen, Y.; Oikawa, A.; Manisseri, C.; Knierim, B.; Prak, L.; Jensen, J.K.; Knox, J.P.; Auer, M.; Willats, W.G., et al. The cooperative activities of CSLD2, CSLD3, and CSLD5 are required for normal *Arabidopsis* development. *Mol. Plant.* **2011**, *4*, 1024-1037, doi:10.1093/mp/ssr026.
5. Yang, J.; Bak, G.; Burgin, T.; Barnes, W.J.; Mayes, H.B.; Pena, M.J.; Urbanowicz, B.R.; Nielsen, E. Biochemical and genetic analysis identify CSLD3 as a beta-1,4-glucan synthase that functions during plant cell wall synthesis. *Plant Cell* **2020**, *32*, 1749-1767, doi:10.1105/tpc.19.00637.
6. Sterling, J.D.; Atmodjo, M.A.; Inwood, S.E.; Kumar Kolli, V.S.; Quigley, H.F.; Hahn, M.G.; Mohnen, D. Functional identification of an *Arabidopsis* pectin biosynthetic homogalacturonan galacturonosyltransferase. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 5236-5241, doi:10.1073/pnas.0600120103.
7. Rennie, E.A.; Hansen, S.F.; Baidoo, E.E.; Hadi, M.Z.; Keasling, J.D.; Scheller, H.V. Three members of the *Arabidopsis* glycosyltransferase family 8 are xylan glucuronosyltransferases. *Plant Physiol.* **2012**, *159*, 1408-1417, doi:10.1104/pp.112.200964.
8. Knoch, E.; Dilokpimol, A.; Tryfona, T.; Poulsen, C.P.; Xiong, G.; Harholt, J.; Petersen, B.L.; Ulvskov, P.; Hadi, M.Z.; Kotake, T., et al. A  $\beta$ -glucuronosyltransferase from *Arabidopsis thaliana* involved in biosynthesis of type II arabinogalactan has a role in cell elongation during seedling growth. *Plant J.* **2013**, *76*, 1016-1029, doi:10.1111/tpj.12353.
9. Basu, D.; Tian, L.; Wang, W.; Bobbs, S.; Herock, H.; Travers, A.; Showalter, A.M. A small multigene hydroxyproline-*O*-galactosyltransferase family functions in arabinogalactan-protein glycosylation, growth and development in *Arabidopsis*. *BMC Plant Biol.* **2015**, *15*, 295, doi:10.1186/s12870-015-0670-7.
10. Urbanowicz, B.R.; Pena, M.J.; Ratnaparkhe, S.; Avci, U.; Backe, J.; Steet, H.F.; Foston, M.; Li, H.; O'Neill, M.A.; Ragauskas, A.J., et al. 4-*O*-methylation of glucuronic acid in *Arabidopsis* glucuronoxylan is catalyzed by a domain of unknown function family 579 protein. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 14253-14258, doi:10.1073/pnas.1208097109.
11. Faik, A.; Price, N.J.; Raikhel, N.V.; Keegstra, K. An *Arabidopsis* gene encoding an  $\alpha$ -xylosyltransferase involved in xyloglucan biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.*

- 2002**, 99, 7797-7802, doi:10.1073/pnas.102644799.
12. Voiniciuc, C.; Schmidt, M.H.; Berger, A.; Yang, B.; Ebert, B.; Scheller, H.V.; North, H.M.; Usadel, B.; Gunl, M. MUCILAGE-RELATED10 produces galactoglucomannan that maintains pectin and cellulose architecture in *Arabidopsis* seed mucilage. *Plant Physiol.* **2015**, 169, 403-420, doi:10.1104/pp.15.00851.
  13. Perrin, R.M.; DeRocher, A.E.; Bar-Peled, M.; Zeng, W.; Norambuena, L.; Orellana, A.; Raikhel, N.V.; Keegstra, K. Xyloglucan fucosyltransferase, an enzyme involved in plant cell wall biosynthesis. *Science* **1999**, 284, 1976-1979, doi:10.1126/science.284.5422.1976.
  14. Wu, Y.; Williams, M.; Bernard, S.; Driouich, A.; Showalter, A.M.; Faik, A. Functional identification of two nonredundant *Arabidopsis*  $\alpha(1,2)$ fucosyltransferases specific to arabinogalactan proteins. *J. Biol. Chem.* **2010**, 285, 13638-13645, doi:10.1074/jbc.M110.102715.
  15. Zeng, W.; Lampugnani, E.R.; Picard, K.L.; Song, L.; Wu, A.M.; Farion, I.M.; Zhao, J.; Ford, K.; Doblin, M.S.; Bacic, A. Asparagus IRX9, IRX10, and IRX14A are components of an active xylan backbone synthase complex that forms in the Golgi apparatus. *Plant Physiol.* **2016**, 171, 93-109, doi:10.1104/pp.15.01919.
  16. Jensen, J.K.; Johnson, N.R.; Wilkerson, C.G. *Arabidopsis thaliana* IRX10 and two related proteins from psyllium and *Physcomitrella patens* are xylan xylosyltransferases. *Plant J.* **2014**, 80, 207-215, doi:10.1111/tpj.12641.
  17. Madson, M.; Dunand, C.; Li, X.; Verma, R.; Vanzin, G.F.; Caplan, J.; Shoue, D.A.; Carpita, N.C.; Reiter, W.D. The MUR3 gene of *Arabidopsis* encodes a xyloglucan galactosyltransferase that is evolutionarily related to animal exostosins. *Plant Cell* **2003**, 15, 1662-1670.
  18. Lampugnani, E.R.; Ho, Y.Y.; Moller, I.E.; Koh, P.L.; Golz, J.F.; Bacic, A.; Newbigin, E. A glycosyltransferase from *Nicotiana glauca* pollen mediates synthesis of a linear (1,5)- $\alpha$ -L-arabinan when expressed in *Arabidopsis*. *Plant Physiol.* **2016**, 170, 1962-1974, doi:10.1104/pp.15.02005.
  19. Lars, Ø.; Petersen, M.; Mattsson, O.; Mundy, J. An *Arabidopsis* callose synthase. *Plant Mol. Biol.* **2002**, 49, 559-566.
  20. Egelund, J.; Petersen, B.L.; Motawia, M.S.; Damager, I.; Faik, A.; Olsen, C.E.; Ishii, T.; Clausen, H.; Ulvskov, P.; Geshi, N. *Arabidopsis thaliana* RGXT1 and RGXT2 encode Golgi-localized (1,3)- $\alpha$ -D-xylosyltransferases involved in the synthesis of pectic rhamnogalacturonan-II. *Plant Cell* **2006**, 18, 2593-2607, doi:10.1105/tpc.105.036566.
  21. Ebert, B.; Birdseye, D.; Liwanag, A.J.M.; Laursen, T.; Rennie, E.A.; Guo, X.; Catena, M.; Rautengarten, C.; Stonebloom, S.H.; Gluza, P., et al. The three members of the *Arabidopsis* glycosyltransferase family 92 are functional  $\beta$ -1,4-galactan synthases. *Plant Cell Physiol.* **2018**, 59, 2624-2636, doi:10.1093/pcp/pcy180.
  22. Suzuki, T.; Narciso, J.O.; Zeng, W.; van de Meene, A.; Yasutomi, M.; Takemura, S.; Lampugnani, E.R.; Doblin, M.S.; Bacic, A.; Ishiguro, S. KNS4/UPEX1: a type II arabinogalactan  $\beta$ -(1,3)-galactosyltransferase required for pollen exine development. *Plant Physiol.* **2017**, 173, 183-205, doi:10.1104/pp.16.01385.
  23. Takenaka, Y.; Kato, K.; Ogawa-Ohnishi, M.; Tsuruhama, K.; Kajiura, H.; Yagyu, K.; Takeda, A.; Takeda, Y.; Kunieda, T.; Hara-Nishimura, I., et al. Pectin RG-I rhamnosyltransferases represent a novel plant-specific glycosyltransferase family. *Nat.*

*Plants* **2018**, *4*, 669-676, doi:10.1038/s41477-018-0217-7.