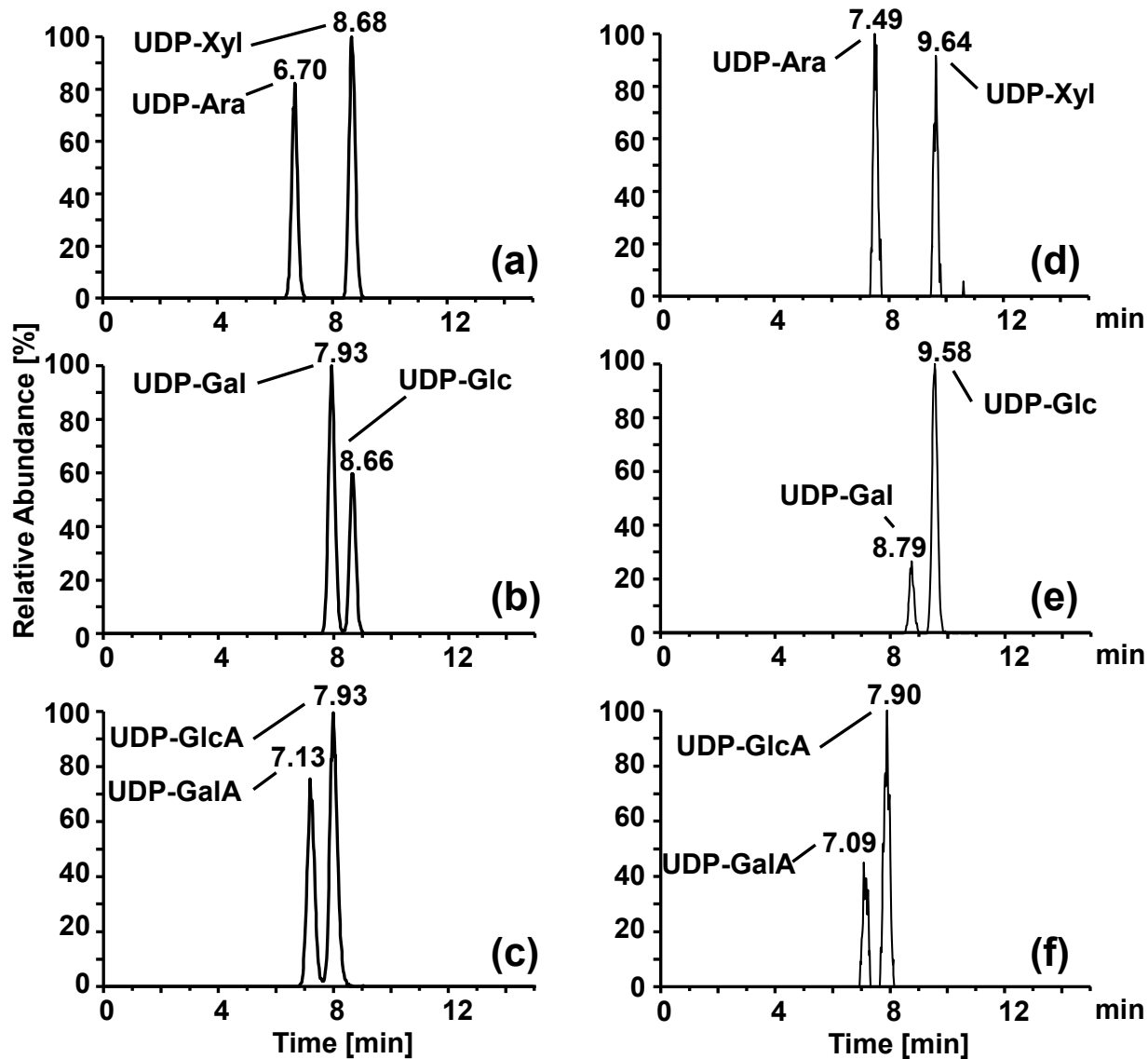


Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

**Quantitative HPLC-MS analysis of nucleotide sugars in plant cells following off-line SPE sample preparation**

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**Fig. S1.** Extracted ion current chromatograms (EICC) of UDP-Xyl and UDP-Ara ( $m/z$  535.0371) (a), UDP-Gal and UDP-Glc ( $m/z$  565.0477) (b), and UDP-GalA and UDP-GlcA ( $m/z$  579.0270) (c). Chromatographic and column regeneration conditions are given in the materials and methods section. EICCs were extracted with a  $\pm 10$  ppm mass window., sample, UDP-sugar standards (a-c) and UDP-sugars extracted from wild type Arabidopsis plants (d-f)

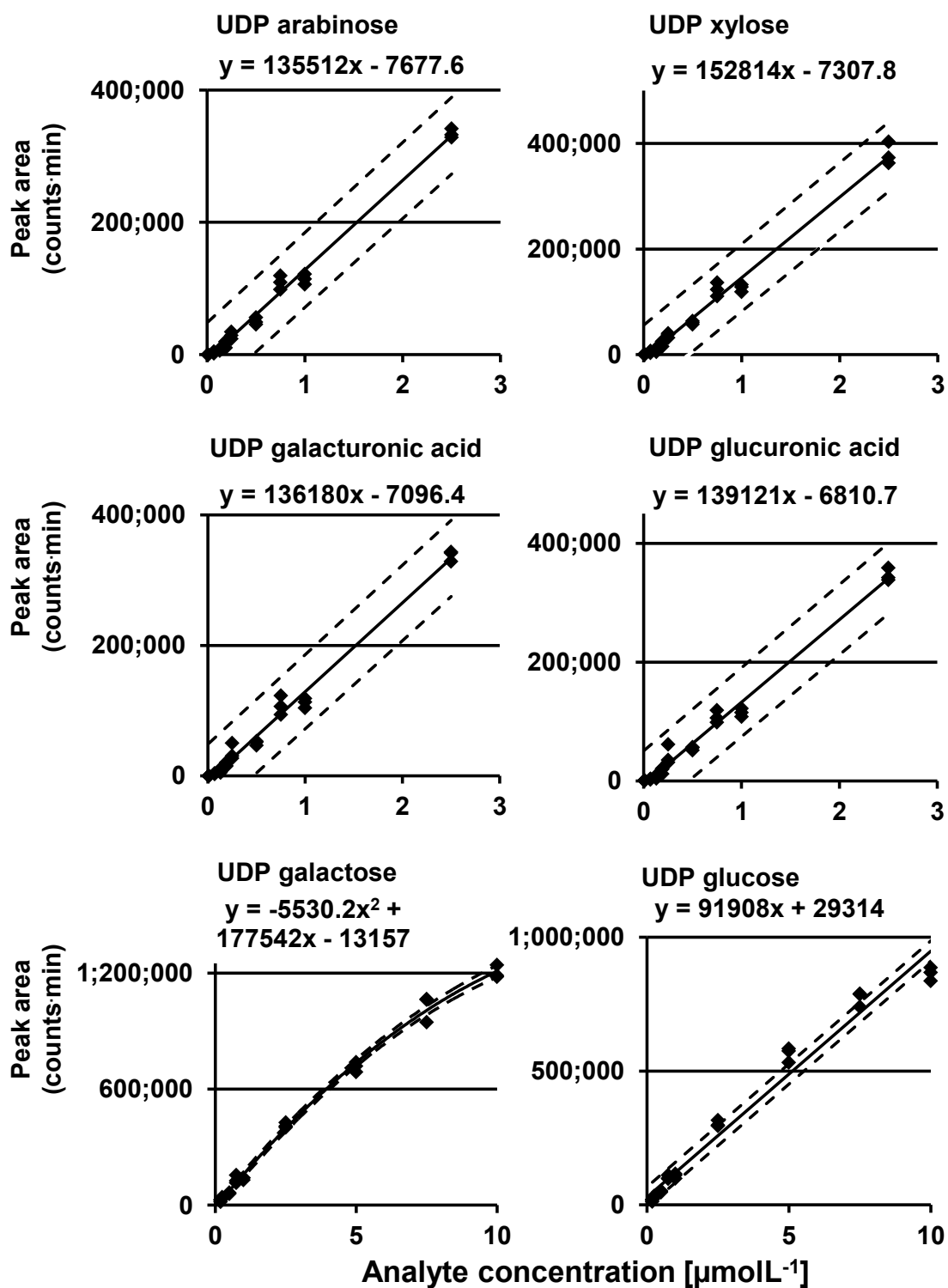


Fig. S2 Calibration graphs of six UDP-sugars. Parameters of the regression functions are given in Table 1

**Table S1.** Concentration of each UDP sugar and its confidence interval for each biological replicate<sup>a)</sup>

Wild type	Amount in $\mu\text{g g}^{-1}$ fresh weight <sup>a)</sup>	C.I. in $\mu\text{g g}^{-1}$ fresh weight <sup>a)</sup>	<i>ugd2,3</i> mutant	Amount in $\mu\text{g g}^{-1}$ fresh weight <sup>a)</sup>	C.I. in $\mu\text{g g}^{-1}$ fresh weight <sup>a)</sup>
UDP-Ara 1	0.84	0.28	UDP-Ara 1	0.44	0.28
UDP-Ara 2	0.76	0.28	UDP-Ara 2	0.60	0.28
UDP-Ara 3	0.60	0.28	UDP-Ara 3	0.46	0.28
UDP-Ara 4	1.14	0.28	UDP-Ara 4	0.56	0.28
UDP-Xyl 1	0.65	0.31	UDP-Xyl 1	0.38	0.31
UDP-Xyl 2	0.65	0.31	UDP-Xyl 2	0.52	0.31
UDP-Xyl 3	0.47	0.31	UDP-Xyl 3	0.38	0.31
UDP-Xyl 4	0.96	0.31	UDP-Xyl 4	0.42	0.31
UDP-Gal 1	3.22	0.71	UDP-Gal 1	5.49	0.75
UDP-Gal 2	3.58	0.72	UDP-Gal 2	5.88	0.76
UDP-Gal 3	2.79	0.70	UDP-Gal 3	5.20	0.75
UDP-Gal 4	5.47	0.75	UDP-Gal 4	6.50	0.78
UDP-Glc 1	22.69	2.24	UDP-Glc 1	32.24	2.30
UDP-Glc 2	26.22	2.25	UDP-Glc 2	36.27	2.37
UDP-Glc 3	19.35	2.23	UDP-Glc 3	33.03	2.31
UDP-Glc 4	37.79	2.40	UDP-Glc 4	38.52	2.42
UDP-GalA 1	0.64	0.34	UDP-GalA 1	0.32	0.34
UDP-GalA 2	0.58	0.34	UDP-GalA 2	0.46	0.34
UDP-GalA 3	0.41	0.34	UDP-GalA 3	0.41	0.34
UDP-GalA 4	0.75	0.34	UDP-GalA 4	0.41	0.34
UDP-GlcA 1	1.40	0.35	UDP-GlcA 1	0.50	0.35
UDP-GlcA 2	1.21	0.35	UDP-GlcA 2	0.56	0.35
UDP-GlcA 3	0.96	0.35	UDP-GlcA 3	0.47	0.35
UDP-GlcA 4	2.03	0.35	UDP-GlcA 4	0.55	0.35

<sup>a)</sup>Average concentrations and confidence intervals (N=3, P=95%) were calculated from triplicate measurements.

**Table S2.** Single factor ANOVA analysis results<sup>a)</sup>

SUMMARY for UDP arabinose					SUMMARY for UDP glucose				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>	<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
UDP-Ara WT	4	3.34	0.83	0.05	UDP-Glc WT	4	106.04	26.51	64.43
UDP-Ara <i>ugd2,3</i>	4	2.05	0.51	0.01	UDP-Glc <i>ugd2,3</i>	4	140.05	35.01	8.51
ANOVA					ANOVA				
<i>F</i>	<i>F crit</i>	<i>P-value</i>			<i>F</i>	<i>F crit</i>	<i>P-value</i>		
7.29	5.99	0.04			3.96	5.99	0.09		
SUMMARY for UDP xylose					SUMMARY for UDP galacturonic acid				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>	<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
UDP-Xyl WT	4	2.73	0.68	0.04	UDP-GalA WT	4	2.20	0.55	0.02
UDP-Xyl <i>ugd2,3</i>	4	1.69	0.42	0.00	UDP-GalA <i>ugd2,3</i>	4	1.48	0.37	0.00
ANOVA					ANOVA				
<i>F</i>	<i>F crit</i>	<i>P-value</i>			<i>F</i>	<i>F crit</i>	<i>P-value</i>		
5.83	5.99	0.05			6.42	5.99	0.04		
SUMMARY for UDP galactose					SUMMARY for UDP glucuronic acid				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>	<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
UDP-Gal WT	4	15.07	3.77	1.40	UDP-GlcA WT	4	5.17	1.29	0.18
UDP-Gal <i>ugd2,3</i>	4	23.08	5.77	0.32	UDP-GlcA <i>ugd2,3</i>	4	1.93	0.48	0.00
ANOVA					ANOVA				
<i>F</i>	<i>F crit</i>	<i>P-value</i>			<i>F</i>	<i>F crit</i>	<i>P-value</i>		
9.35	5.99	0.02			14.62	5.99	0.01		

<sup>a)</sup> Wild type and *ugd2,3* data for each UDP sugar were compared for the four biological replicates via ANOVA analysis. The ANOVA analysis was performed by means of Microsoft Excel using an alpha value of 0.05. The F values are highlighted in red.