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 ± 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\(^a\)

| Wild type | Amount in µg g\(^{-1}\) fresh weight\(^a\) | C.I. in µg g\(^{-1}\) fresh weight\(^a\) | ugdc2,3 mutant | Amount in µg g\(^{-1}\) fresh weight\(^a\) | C.I. in µg g\(^{-1}\) fresh weight\(^a\) |
|-----------|--------------------------------------------|------------------------------------------|-----------------|--------------------------------------------|------------------------------------------|
| 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                                     |

\(^a\)Average concentrations and confidence intervals (N=3, P=95\%) were calculated from triplicate measurements.
Table S2. Single factor ANOVA analysis results$^a$)

| Groups         | Count | Sum   | Average | Variance |
|----------------|-------|-------|---------|----------|
| UDP-Ara WT     | 4     | 3.34  | 0.83    | 0.05     |
| UDP-Ara ugdl2,3| 4     | 2.05  | 0.51    | 0.01     |
| ANOVA          |       |       |         |          |
| $F$            | 7.29  | 5.99  | 0.04    |          |

| Groups         | Count | Sum   | Average | Variance |
|----------------|-------|-------|---------|----------|
| UDP-Glc WT     | 4     | 106.04| 26.51   | 64.43    |
| UDP-Glc ugdl2,3| 4     | 140.05| 35.01   | 8.51     |
| ANOVA          |       |       |         |          |
| $F$            | 3.96  | 5.99  | 0.09    |          |

| Groups         | Count | Sum   | Average | Variance |
|----------------|-------|-------|---------|----------|
| UDP-Xyl WT     | 4     | 2.73  | 0.68    | 0.04     |
| UDP-Xyl ugdl2,3| 4     | 1.69  | 0.42    | 0.00     |
| ANOVA          |       |       |         |          |
| $F$            | 5.83  | 5.99  | 0.05    |          |

| Groups         | Count | Sum   | Average | Variance |
|----------------|-------|-------|---------|----------|
| UDP-Gal WT     | 4     | 15.07 | 3.77    | 1.40     |
| UDP-Gal ugdl2,3| 4     | 23.08 | 5.77    | 0.32     |
| ANOVA          |       |       |         |          |
| $F$            | 9.35  | 5.99  | 0.02    |          |

| Groups         | Count | Sum   | Average | Variance |
|----------------|-------|-------|---------|----------|
| UDP-GlcA WT    | 4     | 5.17  | 1.29    | 0.18     |
| UDP-GlcA ugdl2,3| 4     | 1.93  | 0.48    | 0.00     |
| ANOVA          |       |       |         |          |
| $F$            | 14.62 | 5.99  | 0.01    |          |

$^a$) Wild type and ugdl2,3 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.