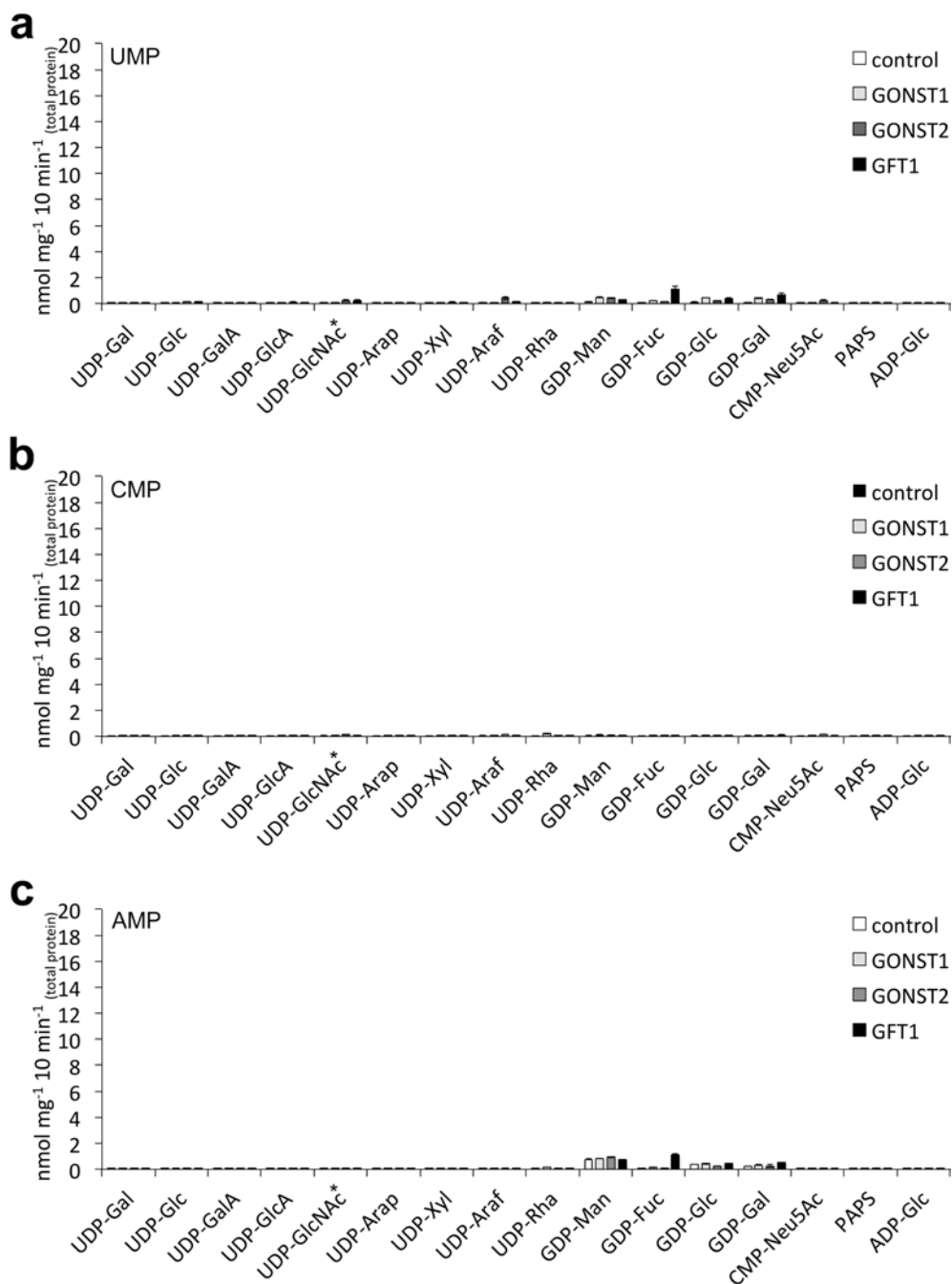


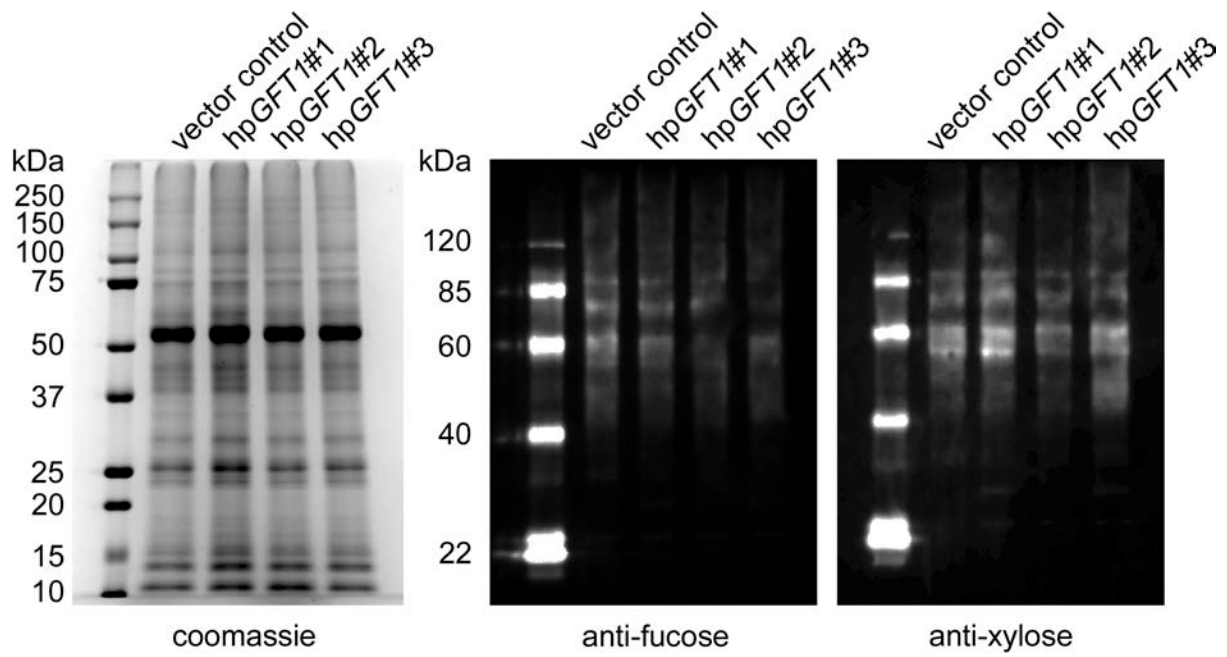
The Arabidopsis Golgi-localized GDP-L-fucose transporter is required for plant development

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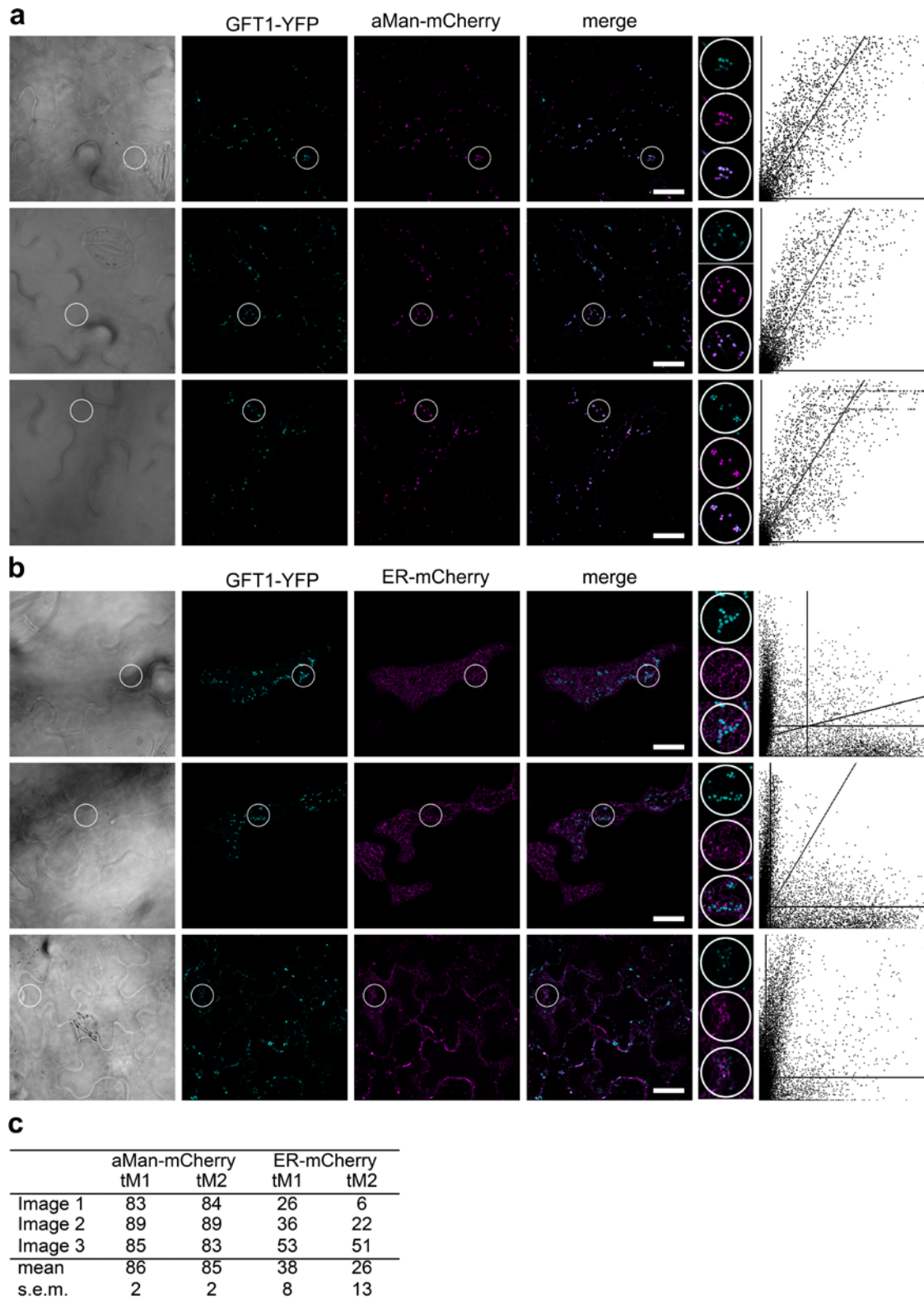


Supplementary Figure 1. Exchange substrate specificities of GFT1, GONST1 and GONST2.

Proteoliposomes derived from yeast transformed with the empty vector (control) or yeast expressing GONST1, GONST2, or GFT1 were pre-loaded with (a) 10 mM UMP, (b) 10 mM CMP, or (c) 10 mM AMP and incubated with 16 nucleotide sugar substrates. Only minor transport was observed when compared to proteoliposomes pre-loaded with 10 mM GMP (Figure 3).



Supplementary Figure 2. Immunoblot analysis and Coomassie stained gel of total protein extracted from hpGFT1 cohorts probed with antibodies against *N*-glycan xylosyl and fucosyl epitopes.



Supplementary Figure 3. Confirmed localization of GFT1.

Transient sub-cellular co-localization of the GFT1-YFP fusion protein with the **(a)** G-rk (aMan-mCherry) Golgi marker or the **(b)** ER-rk (ER-mCherry) ER marker in *N. benthamiana* leaves. Regions of interest (circles) were used to assess the overlap of pixels in each channel to generate scatter plots. The x-axis represents channel 1 (YFP) and the y-axis channel 2 (mCherry). Three independently transformed cells are shown for each experiment. Scale bars = 25 μ m. **(c)** Quantification of fluorescent signal overlap in the region of interest (circles) between the GFT1-YFP signal and the organelle marker signals using the Colocalization Threshold tool in ImageJ to calculate the Manders' tM1 and tM2 overlap coefficients.

Supplementary Table 1. Calculations for amount of expressed protein in proteo-liposomes used for transport assays.

| NST | Molecular Mass (Da) | fmol (in 10 μ g) | ng (in 10 μ g) | Total protein (%) |
|--------|------------------------|-------------------------|-----------------------|----------------------|
| GFT1 | 42,259 | 764.7 \pm 36.0 | 32.3 \pm 1.5 | 0.32 \pm 0.02 |
| GONST1 | 41,713 | 1123.9 \pm 49.3 | 46.9 \pm 2.1 | 0.47 \pm 0.02 |

The molecular mass is the estimated monoisotopic mass including the V5-tag and 6-His tag using the Compute pI/Mw tool at ExPASy (<http://web.expasy.org/>). The amount (fmol) in the sample was estimated using LC-MS/MS (MRM) quantitation of a shared C-terminal peptide (SRGPFEGKPIP NPLLGLDSTR). Results are mean ($n=2$) \pm s.e.m. from the proteo-liposome preparations used for the transporter assay.

Supplementary Table 2. Nucleotide sugar content of hpGFTI plants and empty vector control plants.

| compound | vector control | hpGFT1#1 | hpGFT1#2 | hpGFT1#3 | hpGFT1#4 |
|-------------------------|----------------|----------------|----------------|----------------|----------------|
| UDP- α -D-Glc | 61.0 \pm 1.5 | 59.8 \pm 0.2 | 59.8 \pm 1.5 | 57.7 \pm 0.8 | 58.6 \pm 2.3 |
| UDP- α -D-Gal | 15.1 \pm 0.8 | 15.0 \pm 0.2 | 15.0 \pm 0.7 | 15.9 \pm 0.7 | 14.8 \pm 1.2 |
| UDP- β -L-Rha | 3.3 \pm 0.2 | 3.1 \pm 0.1 | 3.1 \pm 0.3 | 3.4 \pm 0.2 | 3.3 \pm 0.2 |
| UDP- α -D-GlcA | 4.7 \pm 0.7 | 5.6 \pm 0.2 | 5.6 \pm 0.5 | 5.2 \pm 0.4 | 5.0 \pm 0.6 |
| UDP- α -D-GalA | 3.1 \pm 0.2 | 3.2 \pm 0.1 | 3.2 \pm 0.1 | 2.8 \pm 0.0 | 3.2 \pm 0.0 |
| UDP- α -D-Xyl | 3.4 \pm 0.2 | 3.9 \pm 0.4 | 3.9 \pm 0.3 | 4.1 \pm 0.2 | 4.0 \pm 0.1 |
| UDP- β -L-Arap | 2.2 \pm 0.2 | 2.8 \pm 0.3 | 2.8 \pm 0.0 | 3.5 \pm 0.1 | 3.3 \pm 0.1 |
| UDP- β -L-Araf | 0.7 \pm 0.0 | 0.7 \pm 0.2 | 0.7 \pm 0.0 | 1.0 \pm 0.2 | 1.0 \pm 0.1 |
| UDP- α -D-GlcNAc | 3.5 \pm 0.3 | 3.2 \pm 0.3 | 3.2 \pm 0.4 | 3.4 \pm 0.4 | 4.0 \pm 0.5 |
| GDP- β -L-Fuc | 0.3 \pm 0.1 | 0.2 \pm 0.0 | 0.2 \pm 0.0 | 0.3 \pm 0.0 | 0.3 \pm 0.0 |
| GDP- α -D-Glc | trace | trace | trace | trace | trace |
| GDP- α -L-Gal | trace | trace | trace | trace | trace |
| GDP- α -D-Man | 2.8 \pm 0.5 | 2.5 \pm 0.2 | 2.5 \pm 0.3 | 2.6 \pm 0.1 | 2.5 \pm 0.3 |

Values are given in mol% and represent the mean \pm s.d. of each hpGFTI cohort analyzed in triplicate. No significant differences were observed.

Supplementary Table 3. Monosaccharide composition of sequentially extracted cell wall material from control and hpGFTI lines.

| Fraction | Fuc Avg mol% | Rha Avg mol% | Ara Avg mol% | Gal Avg mol% | Xyl Avg mol% | GalA Avg mol% | GlcA Avg mol% |
|-------------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|
| CDTA | | | | | | | |
| control | 0.67 (0.11) | 5.19 (0.88) | 12.53 (1.83) | 9.34 (0.08) | 4.11 (0.67) | 67.70 (3.71) | 0.46 (0.47) |
| hpGFTI#1 | 0.35 | 4.61 | 9.99 | 9.43 | 2.83 | 72.65 | 0.14 |
| hpGFTI#2 | 0.33 | 5.56 | 12.07 | 8.61 | 3.86 | 69.52 | 0.07 |
| hpGFTI#3 | 0.31 | 5.33 | 11.76 | 8.36 | 4.03 | 70.11 | 0.10 |
| hpGFTI#4 | 0.16 | 4.95 | 11.92 | 8.62 | 3.38 | 70.83 | 0.15 |
| average | 0.29 (0.09)* | 5.11 (0.42) | 11.43 (0.97) | 8.75 (0.47) | 3.52 (0.54) | 70.78 (1.36) | 0.12 (0.04) |
| <i>p</i> -value | 0.001 | 0.87 | 0.29 | 0.09 | 0.19 | 0.14 | 0.18 |
| Na₂CO₃ | | | | | | | |
| control | 0.98 (0.12) | 9.75 (0.78) | 20.54 (3.73) | 14.43 (1.58) | 6.39 (1.33) | 46.56 (7.13) | 1.32 (0.27) |
| hpGFTI#1 | 0.42 | 9.32 | 16.77 | 13.60 | 5.08 | 53.55 | 1.24 |
| hpGFTI#2 | 0.34 | 10.4 | 21.32 | 13.08 | 5.78 | 48.15 | 0.93 |
| hpGFTI#3 | 0.31 | 9.53 | 20.94 | 13.05 | 6.07 | 49.35 | 0.75 |
| hpGFTI#4 | 0.16 | 11.29 | 27.05 | 13.79 | 6.01 | 40.92 | 0.77 |
| average | 0.31 (0.1)* | 10.13 (0.9) | 21.51 (4.22) | 13.37 (0.37) | 5.73 (0.45) | 47.99 (5.25) | 0.92 (0.22) |
| <i>p</i> -value | 0.00006 | 0.52 | 0.73 | 0.21 | 0.35 | 0.74 | 0.05 |
| 1N KOH | | | | | | | |
| control | 2.01 (0.26) | 1.32 (0.32) | 12.13 (3.84) | 11.55 (1.05) | 35.64 (4.32) | 27.8 (4.05) | 9.51 (7.98) |
| hpGFTI#1 | 1.01 | 1.25 | 8.08 | 11.10 | 29.40 | 32.26 | 16.90 |
| hpGFTI#2 | 0.70 | 2.15 | 10.98 | 12.37 | 35.92 | 27.86 | 10.01 |
| hpGFTI#3 | 0.76 | 2.04 | 11.55 | 12.79 | 38.95 | 24.13 | 9.77 |
| hpGFTI#4 | 0.20 | 1.54 | 10.71 | 13.01 | 47.19 | 19.40 | 7.95 |
| average | 0.66 (0.33)* | 1.74 (0.42) | 10.33 (1.54) | 12.31 (0.85) | 37.86 (7.38) | 25.91 (5.46) | 11.15 (3.93) |
| <i>p</i> -value | 0.0007 | 0.15 | 0.38 | 0.27 | 0.62 | 0.59 | 0.70 |
| 4N KOH | | | | | | | |
| control | 4.89 (1.23) | 1.32 (1.18) | 4.52 (1.55) | 15.2 (0.57) | 54.62 (8.43) | 17.65 (6.64) | 1.76 (0.52) |
| hpGFTI#1 | 2.33 | 0.58 | 4.21 | 15.60 | 60.93 | 15.43 | 0.93 |
| hpGFTI#2 | 1.27 | 1.49 | 7.38 | 17.12 | 51.26 | 20.47 | 1.01 |
| hpGFTI#3 | 1.32 | 1.02 | 6.26 | 17.67 | 54.86 | 15.61 | 3.27 |
| hpGFTI#4 | 0.00 | 0.70 | 5.40 | 16.81 | 59.96 | 16.19 | 0.94 |
| average | 1.22 (0.95)* | 0.94 (0.4) | 5.81 (1.34) | 16.79 (0.87)* | 56.75 (4.52) | 16.92 (2.38) | 1.53 (1.15) |
| <i>p</i> -value | 0.002 | 0.53 | 0.23 | 0.03 | 0.64 | 0.83 | 0.73 |

Values are shown as mole percent (mol%) of evaluated sugars. The control values are mean (s.d.) of five biological replicates ($n=5$). The data for hpGFTI lines are mean values of each cohort analyzed in triplicate. The average is the mean (s.d.) of the data from the four ($n=4$) hpGFTI lines. The *p*-values between the control and average were calculated using a Student's t-test. The significant differences ($p<0.05$) are marked (*). The Glc values were omitted as a de-starching step was not included in the extraction.

Supplementary Table 4. Relative abundance of xyloglucan oligosaccharides from hpGFTI wall material determined by oligosaccharide mass profiling (OLIMP).

| Oligosaccharide | vector control | hpGFTI#1 | hpGFTI#2 | hpGFTI#3 | hpGFTI#4 |
|-----------------|----------------|-------------|-------------|-------------|-------------|
| XXXG | 29.9 (0.9) | 28.2 (2.1) | 28.2 (2.7) | 27.1 (3.2) | 26.7 (1.8) |
| XXLG/XLXG | 8.0 (0.4) | 22.1 (1.2)* | 29.5 (1.2)* | 35.8 (0.7)* | 38.1 (0.5)* |
| XXLG | 1.3 (0.4) | 0.9 (0.0) | 0.8 (0.1) | 0.7 (0.2) | 0.6 (0.2) |
| XXFG | 10.0 (0.9) | 5.1 (0.6)* | 2.7 (0.3)* | 1.2 (0.1)* | 0.5 (0.1)* |
| XLLG/XXJG/XXFG+ | 2.7 (0.8) | 14.1 (1.6)* | 21.0 (1.8)* | 27.2 (3.1)* | 29.5 (2.0)* |
| XXFG | 11.5 (2.1) | 7.1 (0.8)* | 4.1 (0.5)* | 1.4 (0.3)* | 0.7 (0.1)* |
| XLLG/XXJG/XXFG+ | 1.9 (0.9) | 2.0 (0.6) | 2.2 (0.2) | 2.2 (0.2) | 2.2 (0.5) |
| XLFG | 14.6 (2.3) | 7.7 (0.8)* | 4.3 (0.6)* | 1.7 (0.5)* | 0.7 (0.1)* |
| XLJG/XLFG+ | trace | trace | trace | trace | trace |
| XLFG | 20.2 (1.4) | 12.9 (1.1)* | 7.3 (0.5)* | 2.7 (0.4)* | 0.9 (0.1)* |
| XLJG/XLFG+ | trace | trace | trace | trace | trace |
| fucosylated XyG | 56.2 (2.4) | 32.7 (1.4)* | 18.3 (0.4)* | 7.1 (0.6)* | 2.9 (0.1)* |

All xyloglucan oligosaccharide masses are $[M + Na^+]$, except those labelled (+) which indicates $[M + K^+]$. One letter code nomenclature of oligosaccharides according to (Fry et al. 1993). Suggested likely oligosaccharide structure is based on the m/z of the ion. “Fucosylated XyG” represents the sum of the abundance of XXFG, XXFG, XLFG, and XLFG. Data are mean \pm (s.e.m.) of each hpGFTI cohort analyzed in triplicate, Student’s *t*-test $p < 0.05$ (*).