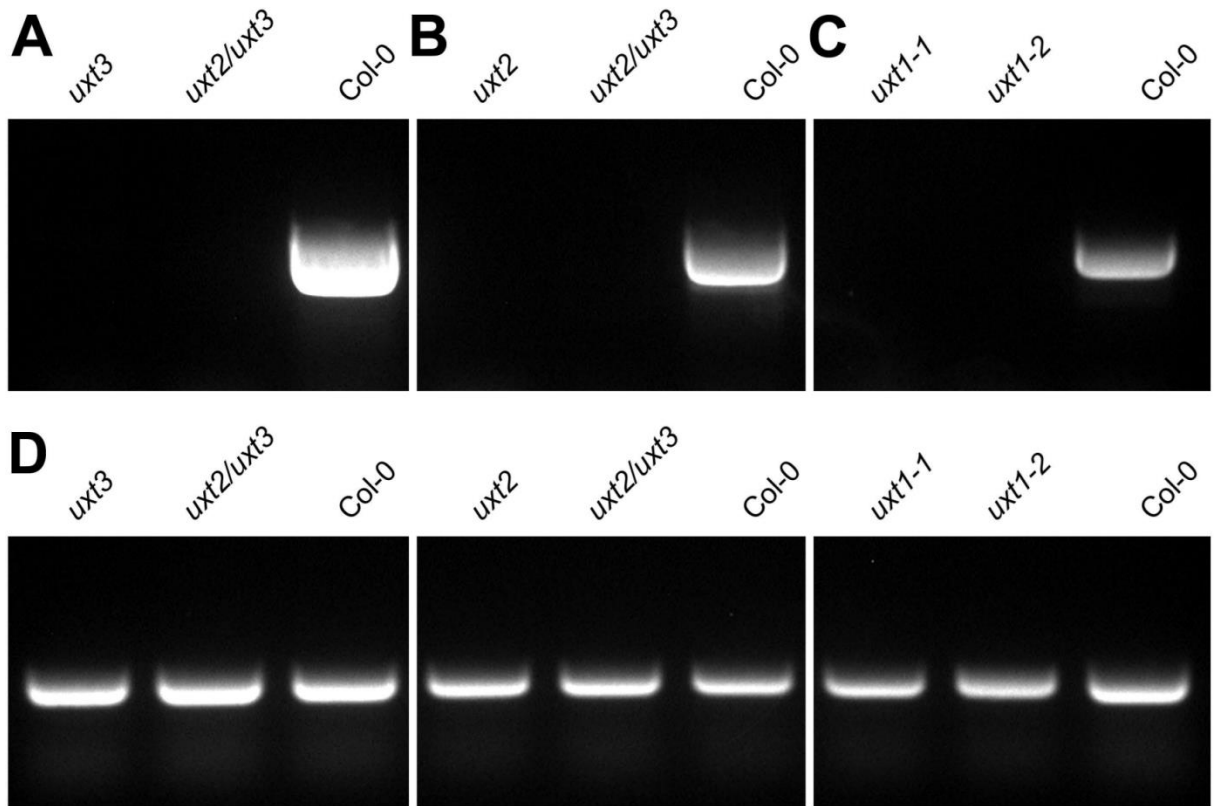
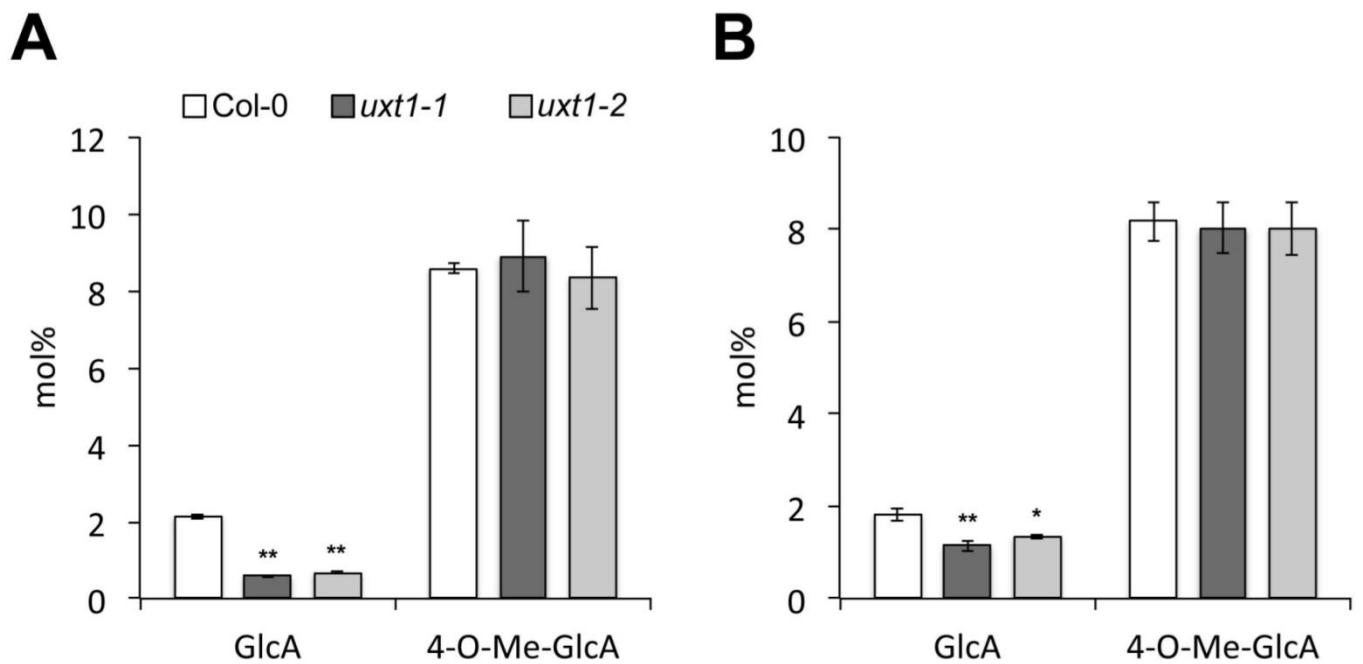


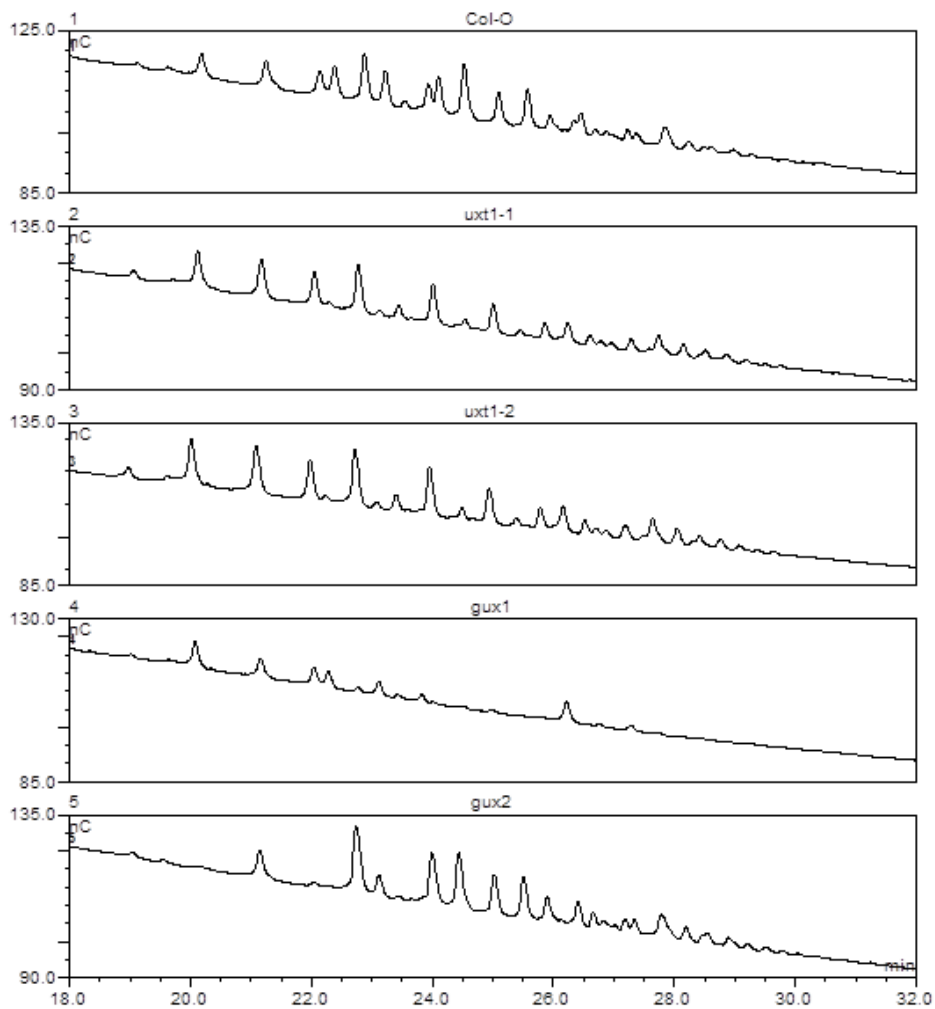
Supplemental Figure 1. Subcellular localization of UXT1-YFP co-expressed with the ER-ck marker in tobacco leaves demonstrating ER localization pattern.



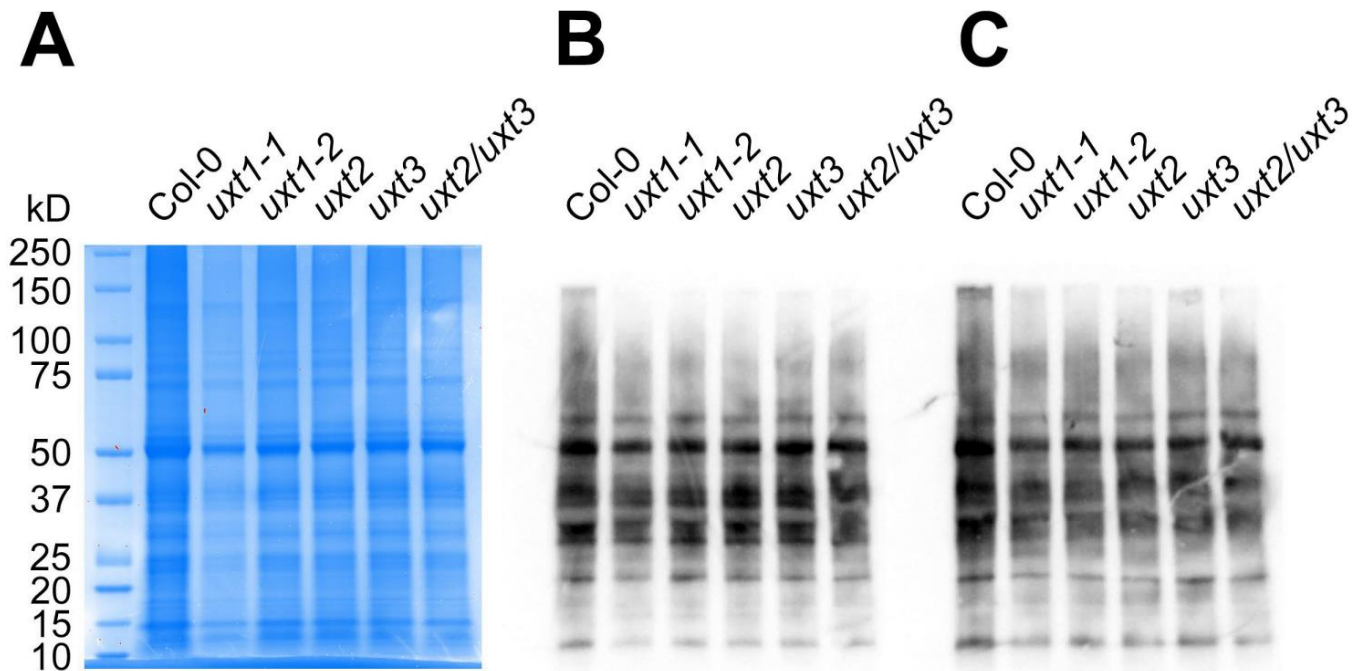
Supplemental Figure 2. Assessment of UXT transcripts by RT-PCR in the of *uxt* mutant backgrounds (A) *UXT3* (B) *UXT2* (C) *UXT1* (D) *ACTIN2* (At3g18780).



Supplemental Figure 3. The 4-O-Methyl-D-glucuronic acid (4-O-Me-GlcA) content of pooled stem material from Col-0, *uxt1-1* and *uxt1-2* measured by HPAEC. (A) Lower stem (B) upper stem. No significant difference could be detected for 4-O-Me-GlcA. Significant differences are (*) $p < 0.05$ and (**) $p < 0.001$. Data are technical replicates ($n=5$).



Supplemental Figure 4. Xylan profiling of *uxt1* mutants. AIR from Col-0, *uxt1-1*, *uxt1-2*, *gux-1* and *gux-2* was digested with the glucuronoxylanase XynC and analyzed by HPAEC. The analysis was conducted in triplicate, representative results are shown.



Supplemental Figure 5. Protein glycosylation profiling of *UXT* mutants. Total protein from stem material was separated by SDS-PAGE and stained using **(A)** Coomassie. Protein was transferred to nitrocellulose and probed using antibodies raised against **(B)** β -(1,2)-Xyl and **(C)** α -(1,3)-Fuc. No major differences were observed.

Supplemental Table 1. Calculations of UXT protein contents in reconstituted proteo-liposomes used for transport assays. The molecular weight is the estimated monoisotopic mass including the V5-tag and 6-His tag estimated 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) of a shared C-terminal peptide (SRGPFEGKPIPPELLGLDSTR) with a synthesized standard. Samples are $n=2 \pm$ (STDEV).

Expressed Protein	Molecular Weight (Da)	fmol (STDEV) (in 5 μg)	ng (STDEV) (in 5 μg)	% Total protein
UXT1	42317.8	131.7 (14.7)	5.6 (0.6)	0.11 (0.01)
UXT2	43703.2	36.2 (7.6)	1.6 (0.3)	0.03 (0.01)
UXT3	43911.6	9.1 (2.5)	0.4 (0.1)	0.01 (0.00)

Supplemental Table 2. Monosaccharide composition of *UXT* mutant cell wall preparations derived from different *Arabidopsis* organs.

Sugar composition of *uxt1-1*, *uxt1-2*, *uxt2*, *uxt3*, *uxt2uxt3* and wild-type (Col-0) plants. The amounts are presented as mol %. For leaf samples $n=8 \pm$ STDEV, all other tissues are from pooled plant material and $n=5$ technical replicates were analyzed. (n.d. = not determined). The data were analyzed with ANOVA and Duncan's test for multiple comparisons. Flowers and leaves showed no significant differences in the monosaccharide composition between any of the mutants and Col-0 ($p>0.05$). Stem data showed a significant difference in the monosaccharide composition of *uxt1-1* and *uxt1-2* compared with Col-0 ($p<0.05$) whereas all other mutants did not show difference from Col-0 ($p>0.05$). In the *uxt1-1* and *uxt1-2* mutants, only Xyl and GlcA were significantly decreased compared to Col-0 ($p<0.05$).

Plant Line	Fuc	Rha	Ara	Gal	Xyl	GalA	GlcA
Col-0							
Flowers	0.87 ± 0.08	3.78 ± 0.33	17.32 ± 0.73	13.21 ± 0.64	9.17 ± 0.41	50.96 ± 1.99	4.70 ± 0.30
Leaves	1.83 ± 0.14	7.59 ± 0.55	12.06 ± 0.85	18.37 ± 0.97	11.04 ± 0.84	47.15 ± 1.90	1.95 ± 0.34
Lower stem	0.70 ± 0.03	3.69 ± 0.12	9.09 ± 0.24	8.69 ± 0.15	55.42 ± 0.43	20.07 ± 0.07	2.34 ± 0.08
Upper stem	0.89 ± 0.02	3.78 ± 0.08	10.33 ± 0.26	7.51 ± 0.19	43.19 ± 1.09	32.32 ± 1.43	1.98 ± 0.16
<i>uxt1-1</i>							
Flowers	0.95 ± 0.04	4.22 ± 0.14	19.37 ± 0.42	14.41 ± 0.21	9.57 ± 0.26	47.64 ± 0.91	3.8 ± 0.13
Leaves	1.67 ± 0.07	7.11 ± 0.30	12.64 ± 0.75	18.77 ± 0.64	9.49 ± 0.29	48.74 ± 1.33	1.56 ± 0.17
Lower stem	1.05 ± 0.12	5.08 ± 0.29	12.11 ± 1.25	11.12 ± 0.75	46.49 ± 3.91*	23.51 ± 1.52	0.64 ± 0.03*
Upper stem	1.17 ± 0.05	4.77 ± 0.26	13.10 ± 0.31	9.93 ± 0.40	30.75 ± 1.25*	39.03 ± 2.08	1.25 ± 0.13*
<i>uxt1-2</i>							
Flowers	0.91 ± 0.05	3.95 ± 0.18	18.97 ± 0.86	14.24 ± 0.57	9.51 ± 0.37	48.24 ± 1.40	4.18 ± 0.65
Leaves	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lower stem	1.00 ± 0.08	4.99 ± 0.33	11.20 ± 0.85	10.66 ± 0.47	46.48 ± 2.48*	24.94 ± 1.54	0.73 ± 0.01*
Upper stem	1.20 ± 0.04	5.15 ± 0.08	13.40 ± 0.39	10.26 ± 0.21	28.44 ± 0.98*	40.05 ± 0.80	1.50 ± 0.07*
<i>uxt2</i>							
Flowers	0.88 ± 0.10	3.74 ± 0.41	18.14 ± 0.99	13.95 ± 0.63	9.46 ± 0.59	49.78 ± 2.36	4.05 ± 0.39
Leaves	1.72 ± 0.07	7.33 ± 0.36	11.25 ± 0.36	17.92 ± 0.42	11.40 ± 0.31	48.53 ± 0.81	1.85 ± 0.25
Lower stem	0.72 ± 0.07	3.92 ± 0.11	9.47 ± 1.10	9.80 ± 0.81	55.92 ± 4.17	18.03 ± 2.47	2.15 ± 0.22
Upper stem	0.74 ± 0.03	4.21 ± 0.11	9.16 ± 0.31	7.04 ± 0.22	55.44 ± 0.52	21.38 ± 0.59	2.03 ± 0.15
<i>uxt3</i>							
Flowers	0.96 ± 0.05	4.05 ± 0.17	17.53 ± 0.51	14.62 ± 0.39	9.51 ± 0.33	50.48 ± 0.40	2.85 ± 1.33
Leaves	1.77 ± 0.11	7.60 ± 0.31	11.42 ± 0.24	18.64 ± 1.11	11.49 ± 0.64	47.34 ± 1.58	1.74 ± 0.14
Lower stem	0.67 ± 0.08	3.85 ± 0.19	7.67 ± 0.76	8.80 ± 0.82	59.32 ± 2.70	17.17 ± 1.22	2.51 ± 0.36
Upper stem	0.86 ± 0.05	3.98 ± 0.35	8.87 ± 0.24	7.89 ± 0.35	47.50 ± 0.73	28.70 ± 1.55	2.19 ± 0.14
<i>uxt2/uxt3</i>							
Flowers	0.98 ± 0.02	4.24 ± 0.12	18.41 ± 0.23	15.20 ± 0.25	10.62 ± 0.20	46.73 ± 0.73	3.81 ± 0.11
Leaves	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lower stem	0.61 ± 0.02	3.54 ± 0.02	7.21 ± 0.24	8.38 ± 0.16	60.81 ± 1.65	16.88 ± 1.21	2.56 ± 0.27
Upper stem	0.97 ± 0.05	4.24 ± 0.13	9.29 ± 0.45	8.83 ± 0.29	47.17 ± 0.72	27.47 ± 0.59	2.03 ± 0.05

Supplemental Table 3. Primer list.

gene	forward primer	reverse primer
cloning		
<i>UXT1</i>	CACCATGGGAGAGATGAAGAGTATGCAAATGG	TACTGGAGAATGCTTGTCTAATTTC
<i>UXT2</i>	CACCATGAGCGATGCCCAGAAGTTCC	CGCTTGAAAATCTTTGTTTGAA
<i>UXT3</i>	CACCATGAGCGAGGGCCAGAAGTTCC	GGCTTGAAAATCTTTATTTGAGTTCCA
T-DNA insertion lines		
<i>uxt1-1</i>	AATGGGTGTGATTGGAGCAT	GTGTAACCAAATGCGAGCAC
<i>uxt1-2</i>	AATGGGTGTGATTGGAGCAT	GTGTAACCAAATGCGAGCAC
<i>uxt2-1</i>	TAATGGAAGTATGGAACCAAGAAA	AGCGAGCAAATATGAGCGATG
<i>uxt3-1</i>	TCAGTCTCCCCTACTTCTCTGC	GTTCTGTCTTGTGCGCTGCTG
transcript abundance		
<i>UXT1</i>	ATGGGAGAGATGAAGAGTATGCAAATGG	TACTGGAGAATGCTTGTCTAATTTC
<i>UXT2</i>	ATGAGCGATGCCCAGAAGTTCC	CGCTTGAAAATCTTTGTTTGAA
<i>UXT3</i>	ATGAGCGAGGGCCAGAAGTTCC	GGCTTGAAAATCTTTATTTGAGTTCCA
qRT-PCR		
<i>UXT1</i>	CGGCCAAATTCTAACAAACA	TGAGGCTAGTGAGGTATTTGTGCG
<i>UXT2</i>	AAGACGATGAAGAACACAACCTGA	TAAGGTTTCATCCACCCAACCT
<i>UXT3</i>	TCGCTCTCTTTCATCTGAGG	CATTCTCGGTATTCTAGTGGCTGT
control genes		
<i>ACTIN-2</i>	CTCAAAGACCAGCTCTTCCATC	GCCTTTGATCTTGAGAGCTTAG
<i>UBI10</i>	GGCCTTGTATAATCCCTGATGAATAAG	AAAGAGATAACAGGAACGGAAACATAGT
<i>PP2A</i>	TAACGTGGCCAAAATGATGC	GTTCTCCACAACCGCTTGGT
<i>SAND family (MON1)</i>	AACTCTATGCAGCATTGATCCACT	TGATTGCATATCTTTATCGCCATC