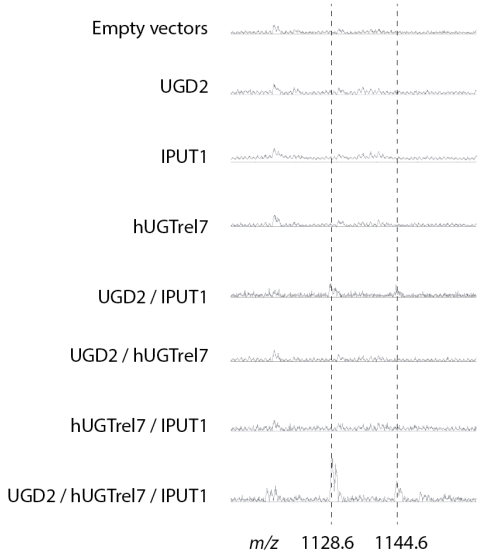
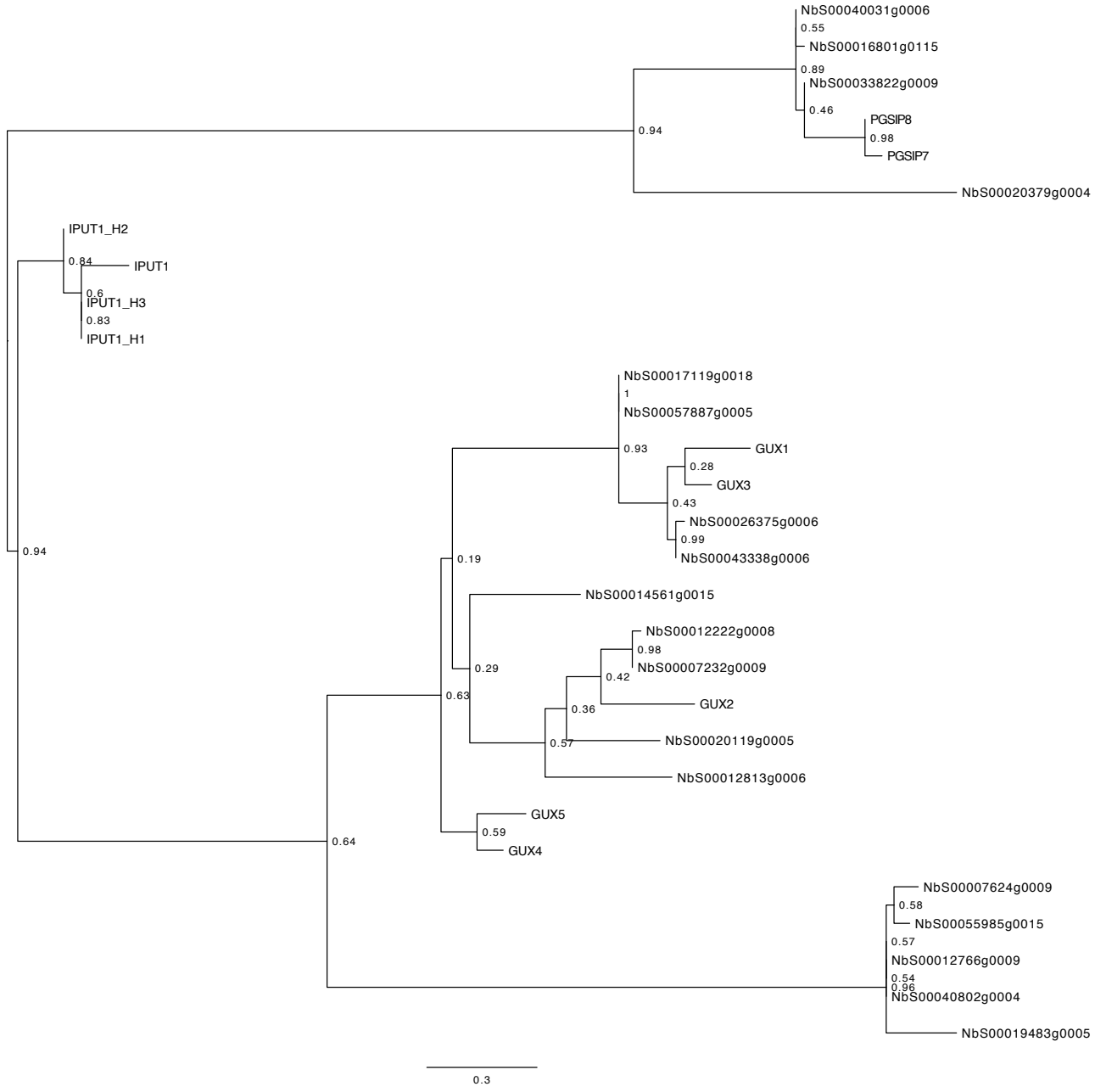
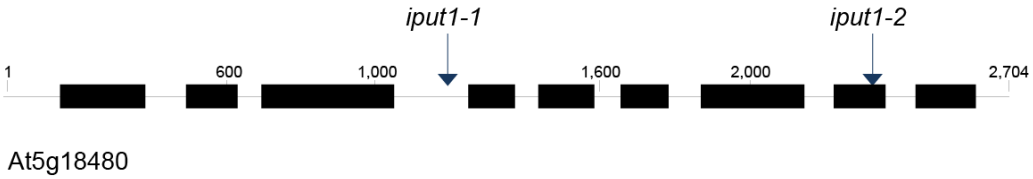


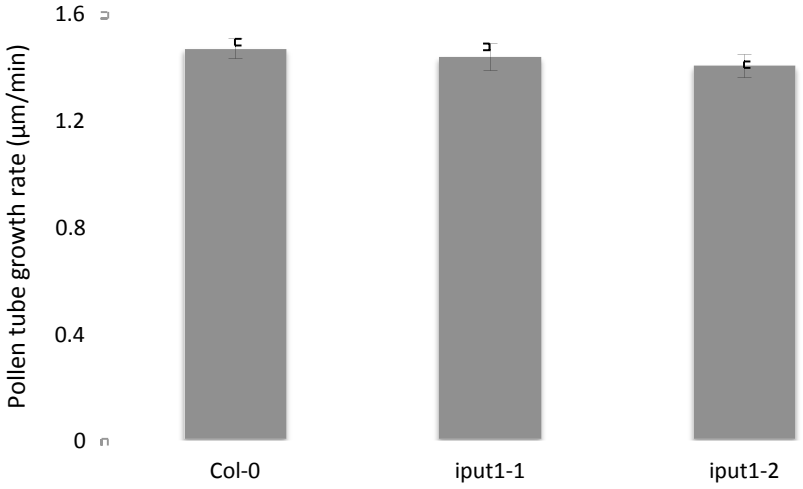
Supplemental Figure 1. Production of GlcA-IPC in sur1Δ yeast expressing UGD2, hUGTrel7, and IPUT1. All yeast contain the Δsur1 knockout mutation and vectors with Leu, His, and Ura markers. Vectors are either empty (contain a small non-coding DNA fragment in the Gateway site) or include UGD2, hUGTrel7, and/or IPUT1 cDNA. Mass spectrum of lipids from each yeast transformant showing GlcA-IPC peaks at m/z 1128.6 (t18:0/h26:0 ceramide) and 1144.6 (t18:0/d26:0 ceramide).



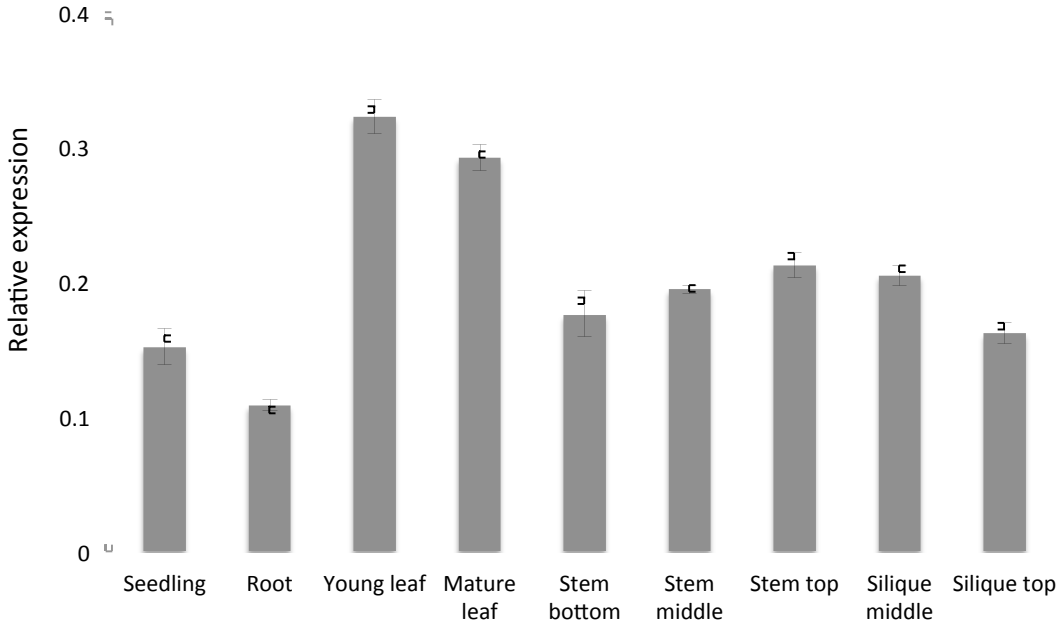
Supplemental Figure 2. Homologs of GUX, PGSIP, and IPUT1 proteins in *N. benthamiana.*In order to silence homologs of IPUT1, we identified members of the GUX/ PGSIP/IPUT clade in *N. benthamiana.* IPUT1 has three closely related members in *N. benthamiana.* Bootstrap values are shown at nodes. Bar represents number of substitutions per 100 amino acids. Alignments used to generate the phylogeny can be found in Supplemental Dataset 1 online.



Supplemental Figure 3. Schematic representation of the At5g18480 gene locus without promoter region. Black boxes represent exons; line represents 5' and 3'UTR and introns. Numbers indicate the bp position starting from the 5'UTR. Arrows indicate T-DNA insertion sites in the alleles *iput1-1* (SALK_131322) and *iput1-2* (GK_856G03).



Supplemental Figure 4. Pollen tube growth rates of pollen from *iput1-1/+* and *iput1-2/+* Arabidopsis plants. Pollen was collected from 10 plants of each genotype. Values shown are mean ± SE, n = 318.



Supplemental Figure 5. Expression of *IPUT1* throughout Arabidopsis. Quantitative, real-time PCR showing expression of *IPUT1* cDNA in Arabidopsis tissues. Values are expressed as the ratio to geometric mean of three reference genes (UBI, ACT2 and EF1α) and are the mean ± SE for three technical replicates.

Primer sequence (forward/reverse)	Purpose
CAGGCTTCACCATGGCAAACTCTCCCGCTGCTCC/ AAAGCTGGGTCCAAGTTATGGCCGGGAAGTGATG	Gene-specific primers for cloning GUX1
CAGGCTTCACCATGGTGAGACTCAAGACGAGTCT/ AAAGCTGGGTCACAGAGGAAACATAGGGAATTTG	Gene-specific primers for cloning IPUT1
GGGGACAAGTTTGTACAACCCAGCAGGCTTCACC/ GGGGACCACTTTGTACAAGAAAGCTGGGTC	Gateway attB-specific primers
GATGAATTCTTGCTTGGGGTTAGGG/ CATTCTAGAAGTCTCTCAGAATGTTTTAAATTT	Cloning N.benthamiana IPUT1 fragment for VIGS
GCGAGCAGGTGGGTCTTGT/ CCGCGAGGTGCTCTGAAG	qPCR primers for Arabidopsis IPUT1
GGCCTTGTATAATCCCTGATGAATAAG/ AAAGAGATAACAGGAACGGAAACATAGT	qPCR primers for Arabidopsis UBQ10
TGAGCACGCTCTTCTTGCTTTCA/ GGTGGTGGCATCCATCTTGTTACA	qPCR primers for Arabidopsis EF1a
CTTGCACCAAGCAGCATGAA/ CCGATCCAGACACTGTACTTCCTT	qPCR primers for Arabidopsis ACT2
ATTTGCACTTGGTGTATCATTGG/ ATGATTGTTGCCGCTGATGAC	qPCR primers for N. benthamiana IPUT1-H1
AAAAATCCTAGAGATGAACTTCTTGT/ TCACACAATGACCGGGTCTTT	qPCR primers for N. benthamiana IPUT1-H2
TGTCATGAAGGATGCCAAAATAAG/GATTAGGGATGGCATTTCTTGC	qPCR primers for N. benthamiana IPUT1-H3
CACTGGTCACTTGATCTACAAG/GTCAATAATCAGGACAGCACAG	qPCR primers for N. benthamiana EF1a
TTGAGACTTTTAATACCCCAGC/AACATGTAACCACGCTCGGTAA	qPCR primers for N. benthamiana ACT2
GCCGATTACAACATCCAGAAGG/TGAAGTACAGCGAGCTTAACC	qPCR primers for N. benthamiana UBI3
TGATTATAAGAAAGTTGTG/ TCATCAACCATCCACTTAC	Amplifying IPUT1 from pollen
AGCTCCCTTTCCAGAGGCTA/ TCCAAGTCTCCTACACCCAAA	Amplifying Histone H3 from pollen
TCTTCTCCTTGCCCTCGTTTC/ GGGAATTTGATCTCGTCGTGTC	Gene-specific primers for genotyping iput1-1
TTTGGTTTTCGAGAAAATTGAGA/ CTCATCGGAGAGGTTGAGTGA	Gene-specific primers for genotyping iput1-2
ATATTGACCATCATACTCATTGC	GABI-KAT T-DNA left border
GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC	SAIL T-DNA left border

Supplemental Table 1. Primer sequences.

Long-chain base	Fatty acid	[M+H] (m/z)	Product ion (m/z)	Dwell time (ms)	DP (V)	CE (V)
t18:0	h16:0	814.6	554.5	30	45	45
t18:0	h18:0	842.6	582.5	30	45	45
t18:0	h20:0	870.7	610.6	30	45	45
t18:0	h20:1	868.7	608.6	30	45	45
t18:0	h22:0	898.7	638.6	30	45	45
t18:0	h22:1	896.7	636.6	30	45	45
t18:0	h24:0	926.7	666.6	30	45	45
t18:0	h24:1	924.7	664.6	30	45	45
t18:0	h26:0	954.8	694.7	30	45	45
t18:0	h26:1	952.8	692.7	30	45	45
t18:1	h16:0	812.6	552.5	30	45	45
t18:1	h18:0	840.6	580.5	30	45	45
t18:1	h20:0	868.7	608.6	30	45	45
t18:1	h20:1	866.7	606.6	30	45	45
t18:1	h22:0	896.7	636.6	30	45	45
t18:1	h22:1	894.7	634.6	30	45	45
t18:1	h24:0	924.7	664.6	30	45	45
t18:1	h24:1	922.7	662.6	30	45	45
t18:1	h26:0	952.8	692.7	30	45	45
t18:1	h26:1	950.8	690.7	30	45	45
d18:0	h16:0	798.6	538.5	30	45	45
d18:0	h18:0	826.7	566.6	30	45	45
d18:0	h20:0	854.7	594.6	30	45	45
d18:0	h20:1	852.7	592.6	30	45	45
d18:0	h22:0	882.7	622.6	30	45	45
d18:0	h22:1	880.7	620.7	30	45	45
d18:0	h24:0	910.7	650.6	30	45	45
d18:0	h24:1	908.8	648.6	30	45	45
d18:0	h26:0	938.8	678.7	30	45	45
d18:0	h26:1	936.8	676.7	30	45	45
d18:1	h16:0	796.6	536.5	30	45	45
d18:1	h18:0	824.6	564.5	30	45	45
d18:1	h20:0	852.7	592.6	30	45	45
d18:1	h20:1	850.7	590.6	30	45	45
d18:1	h22:0	880.7	620.6	30	45	45
d18:1	h22:1	878.7	618.6	30	45	45
d18:1	h24:0	908.7	648.6	30	45	45
d18:1	h24:1	906.7	646.6	30	45	45
d18:1	h26:0	936.7	676.6	30	45	45
d18:1	h26:1	934.7	674.6	30	45	45
d18:2	h16:0	794.8	534.7	30	45	45
d18:2	h18:0	822.8	562.7	30	45	45
d18:2	h20:0	850.8	590.7	30	45	45
d18:2	h20:1	848.8	588.7	30	45	45
d18:2	h22:0	878.7	618.7	30	45	45
d18:2	h22:1	876.7	616.7	30	45	45
d18:2	h24:0	906.7	646.7	30	45	45
d18:2	h24:1	904.7	644.7	30	45	45
d18:2	h26:0	934.7	674.7	30	45	45
d18:2	h26:1	932.7	672.7	30	45	45

Supplemental Table 2. Parameters for MRM detection of IPCs in positive ion mode. Masses for precursor (M+H, Q1) and product ions (Q3) were based on observed [M+H] ions detected by precursor ion scans for the t18:1/h24:0 hydroxyceramide backbone (*m/z* 664.6) and known fragmentation patterns. MRMs were calculated based on known IPC structures. Declustering potential (DP) and collision energy (CE) values were chosen based on average values used for GlcOH-GlcA-IPCs.