Supporting Information

SI Materials and Methods

Plant materials and growth conditions

All experiments were performed in Columbia (Col-0) accession of *Arabidopsis thaliana*. *UGT76F1OE* lines and *ugt76f1* mutant lines were prepared in this study. The crosses of *UGT76F1OE* with *35S:PIF4* and *ugt76f1* with *pif4* were prepared, and homozygous lines were selected based on analysis of kanamycin or hygromycin resistance and PCR testing. For all experiments, the seeds were surface-sterilized with 10% bleach for 10 min and then rinsed 3-4 times with distilled water. The sterilized seeds were then plated on Murashige and Skoog (MS) medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 1% sucrose and 0.8% agar (Sigma) and cultured vertically. For photomorphogenic experiments, the plates were cold-treated at 4°C in the dark for 3 days, and then transferred to continuous white light at 22°C for 8 h to induce germination. The plates were then transferred to dark or white light (70 μmol m^{-2} s^{-1}) or monochromatic light conditions: red light (35 μmol m^{-2} s^{-1}), blue light (10 μmol m^{-2} s^{-1}) and incubated at 22°C for the specified times. For thermomorphogenic experiments, the seedlings grew under continuous white light at 22°C and then shifted to 28°C for the specified times.

The preparation of *UGT76F1 Promoter:GUS* construct

Using *UGT76F1* specific promoter primers (*SI Appendix*, Table S3), sequences of about 1.8 kb upstream of the start site (ATG) of *UGT76F1* (At3g55710) were amplified from *Arabidopsis* genomic DNA by PCR. The amplified DNA fragment was inserted into the pBI121 vector to replace the CaMV35S promoter and generate the *UGT76F1Promoter:GUS* construct. The *UGT76F1Promoter:GUS* construct was introduced into *Arabidopsis* via the floral dip method (58) and independent transgenic lines were generated. Homozygous T3 progenies were used to analyze promoter activity.

β-Glucuronidase (GUS) staining

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For analyzing light induction of gene expression, 5-day-old *UGT76F1Promoter:GUS* transgenic seedlings were grown in the dark or transferred to white light for 5 h and used for histochemical staining of GUS activity. *UGT76F1Promoter:GUS* transgenic seedlings were incubated overnight at 37°C in 1 mg/l 5-bromo-4-chloro-3-indolylglucuronic acid, 5 mM potassium ferrocyanide, 0.03% Triton X-100 and 0.1 M sodium phosphate buffer (pH 7.0). The tissues were cleaned with 70% ethanol and then observed (59). These GUS staining data represented at least five independent transgenic lines. The determination of GUS activities was performed as described previously (60).

In order to detect the auxin signals, homozygous cross lines of *UGT76F1OE DR5:GUS* and *ugt76f1ko DR5:GUS* were first selected based on analysis of Basta resistance, and then stained for GUS activity.

Preparation for overexpression and knock-out lines of *UGT76F1*

The coding sequence of *UGT76F1* gene was amplified from cDNA of *Arabidopsis* using TransStart® FastPfu DNA Polymerase (TransGen), and then cloned into pBI121 binary vector to generate *CaMV 35S Promoter:UGT76F1* construct. The knock-out construct of *UGT76F1* was prepared by CRISPR/Cas9 strategy (Hangzhou Biogle Vector Construction Kit, http://www.biogle.cn/). Primers used for plasmid construction are shown in *SI Appendix,* Table S3. Those constructs were then transformed into *Agrobacterium tumefaciens* strain GV3101, which was used to introduce those constructs into *Arabidopsis* via the floral dip method (58). Independent T1 kanamycin-resistant lines were selected and subjected to PCR testing. Furthermore, the expression level of *UGT76F1* in overexpression lines was determined by RT-PCR, and *ACTIN2* was used as an internal control for RT-PCR analysis. Homozygous T3 progeny lines were isolated and used in all experiments.

For the *ugt76f1* mutants generated by CRISPR/Cas9 strategy, hygromycin was added to the MS medium to select resistant seedlings. Homozygous mutant lines were identified by DNA sequencing and then used in the experiments.

Phenotypic analyses

For the observation of hypocotyl elongation, the seedlings were cultured vertically or horizontally on MS plates for a specified time, and then photographed with a digital camera. For the observation of the cell length, the samples were observed and photographed with a scanning electron microscope. The hypocotyl length was measured using ImageJ software (http://rsbweb.nih.gov/ij/).

Scanning electron microscopy

The seedlings grew vertically in continuous white light at 22°C for 6 days or at 22°C for 4 days and then shifted to 28°C for another 48 h. These seedlings were fixed in 2.5% glutaraldehyde at 4°C for 12 h, then washed five times with 0.1 mol/l phosphate buffer, and afterwards dehydrated with gradient ethanol. Samples were bonded with a drying instrument on the HCP-2 critical point, then finally sprayed gold with an IB-v ion sputtering device and filmed with a scanning electron microscope (FEI Quanta250 FEG).

Yeast one-hybrid assay

For the yeast one-hybrid assay, the yeast one-hybrid kit was used in accordance with the manufacturer's protocol (www.clontech.com). The coding sequences of *PIF1*, *PIF3*, *PIF4* and *PIF5* were inserted separately into pGADT7 (Takara, USA), while the promoter sequence of UGT76F1 was inserted into the cloning site of pAbAi. Yeast cells were also transformed using the empty pGADT7 vector as a control. The primer sequences used for plasmid construction are given in *SI Appendix*, Table S3. The PEG/LiAc method was used to separately transfer each of the constructs (including an empty vector for control purposes) to the Y1HGold yeast competent cell. The background AbAr expression of the strains was tested. Yeast cells were grown in SD/-Ura/-Leu medium in the presence of 200 ng/ml of Aureobosidin A allowing for a highly stringent interaction screening. After culturing at 30°C for 3-5 days, the activation of the reporter gene was checked by cell growth.

Transient luciferase activity assay

The *PIF1*, *PIF3* and *PIF4* coding sequences were amplified, and the resulting

sequences were introduced into pBI221 to place them under the control of the CaMV 35S promoter. The *UGT76F1* promoter sequence was amplified and introduced into the pGreenII0800-LUC reporter vector. The two recombinant plasmids were then transferred into *A. thaliana* protoplasts. The activities of firefly luciferase (LUC) and renilla luciferase (REN) were measured using the Dual-Luciferase Reporter Assay System (www.promega.com). LUC activities were normalized against REN activities (61). Details of all primers used are given in *SI Appendix*, Table S3.

Chromatin immunoprecipitation (ChIP) assays

Two-week-old transgenic plants were used for the ChIP assay using the EpiQuik™ Plant ChIP Kit (EpiGentek, https://www.epigentek.com). RT-qPCR was used to detect the amount of precipitated DNA and input DNA. For the UGT76F1 promoter, primers were designed to amplify a fragment length ranging between ~80 –150 bp, and lying within the 2 kb of sequence upstream of the transcription start site. The relevant primers are given in *SI Appendix*, Table S3. Enrichment is calculated from the ratio of bound sequence to the input.

Purification of UGT76F1 enzyme and assays of glucosyltransferase activity

To construct a prokaryotic expression vector, the full-length cDNA of *UGT76F1* was cloned into a pGEX-4T vector fused to a glutathione S-transferase (GST) tag. The expression of soluble recombinant proteins was induced by isopropyl-b-D-thiogalactoside in *Escherichia coli* XL1-Blue. The fusion protein was purified according to the method described by (62). The glucosyltransferase activity assay of UGT76F1 was performed as previously described with some modifications (41). 2 μg of purified protein was added to the enzyme reaction mixture containing 100 mM Tris-HCl (pH 7.0), 2.5 mM $MgSO_4$, 10 mM KCl, 5 mM UDP-glucose, and 1 mM substrate. All reactions were performed at 30°C for 2-3 h. The kinetic data of the enzyme for IPyA glycosylation were measured in a range of 0-0.5 mM of IPyA substrates and in the presence of 1 μg of recombinant protein. Reactions were carried out at 30°C for 30 min due to the linearity of the reactions. All reaction products were analyzed on a reverse-

phase HPLC system (Shimadzu; http://www.shimadzu.com) equipped with a UV detector and a 5 μM C18 column (150×4.6 mm; Agilent; http:// www.agilent.com).

IPyA glucose conjugates were quantified with minor modifications to the previously described protocol (56, 62). The 7-day-old seedlings grown on the MS agar plates were transferred to MS liquid culture media without or with 100 μM IPyA and enclosed in a covered tube for 12 h, then the leaf tissues were frozen in liquid nitrogen, ground to a powder and homogenized in 80% (v/v) methanol at 4°C for 1 h. After centrifugation at 5000 g for 10 min, the supernatant was collected, concentrated in vacuo, and finally dissolved in 100 % (v/v) methanol. At the beginning in these assays, caffeic acid was used as a reference to monitor the recovery rate. A linear gradient was used to analyze IPyA glucose conjugates by HPLC with increasing acetonitrile (solvent A) against double-distilled H_2O (solvent B) at a flow rate of 1 ml/min over 35 min. Both solutions contained 0.1% formic acid. The detailed HPLC conditions were as follows: 0 min, 90% B; 22 min, 60% B; 25 min, 90% B; 35 min, stop. A photodiode array was used to detect UV-visible absorption at 306 nm. Because no standard sample of IPyA glucose conjugates is commercially available, we used the reaction products from the in vitro enzymatic reaction of UGT76F1 as a positive control. The IPyA glucose conjugates were further confirmed by the LC-MS system (Thermo Scientific, Waltham, MA, USA). The methods and mobile phases were the same as the HPLC conditions, and the mass spectrometer was operated in positive electrospray ionization mode.

LC-MS/MS Assay for IAA

Plant tissue auxin (IAA) content was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS 8030 plus, Shimadzu, Japan) as follows: 200 mg of fresh sample frozen by liquid nitrogen was well homogenized using a TissueLyser homogenizer (QIAGEN, Germany) with a small glass pestle in a 2 ml vial. After 1.0 ml of 80% methanol was added, the homogenates were thoroughly mixed in an ultrasonic bath, and then maintained overnight at 4°C. After being centrifuged at 15,200 g for 10 min, the supernatant was collected and vacuum-dried in a Jouan RCT-60 concentrator. The dried extract was dissolved in 200 μl of sodium phosphate solution

(0.1 mol /l, pH 7.8) and then passed through a Sep-Pak C18 Cartridge (Waters, USA). The cartridge was eluted with 1.5 ml of 80% methanol, and the eluate was again vacuumed to dryness. After dissolving it in 10 ml of 10% methanol, 5 µl of this solution was injected into the LC-MS/MS system. Liquid chromatography was performed using a 2.0 mm I.D.×75 mm Shim-pack XR-ODS column (2.2 µM, Shimadzu, Japan) at a column temperature of 40°C. The mobile phase comprising of solvent A (0.02% v/v aqueous acetic acid) and solvent B (100% v/v methanol) was employed in a gradient mode [time/A concentration/B concentration (min/%/%) for 0/90/10; 5/10/90; 6/10/90; 6.1/ 80/20] at an eluant flow rate of 0.3 ml/min. The mass system was set to multiple reaction monitoring (MRM) and negative ion mode using electrospray ionization (ESI). The operating conditions such as nebulizing gas flow, drying gas flow, desolvation temperature, and heat block temperature were respectively optimized. Deuterium-labeled IAA (Olchemim, Czech Republic) was used as internal standard. Collision energy of -16 eV and mass-to-charge ratio (m/z) of 174.2 was employed.

RNA isolation and quantitative real-time PCR (RT-qPCR)

Total RNA was isolated from plants using an RNAiso Plus (TaKaRa, Tokyo, Japan). Similarly, cDNA was synthesized from total RNA using PrimeScript RT reagent kit (TaKaRa, Tokyo, Japan). RT-qPCR was performed with a Bio-Rad thermal-cycling system (CFX Connect, Hercules, CA, USA) using a SYBR Green PCR Master Mix kit (TaKaRa, Tokyo, Japan). Expression levels were normalized with the reference gene *ACTIN2* using the ΔΔCt method. *SI Appendix*, Table S3 contains primer information for RT-qPCR assay.

Statistical analysis

All experiments were carried out with at least three independent biological replicates. Each measurement was performed in triplicate. Data are expressed as means ± SD. Data were statistically analyzed using the Student's *t*-test. Asterisks indicate significant differences relative to WT or control (*$P < 0.05$, **$P < 0.01$).

Accession numbers

Sequences of genes in this study can be found in the Arabidopsis Genome Initiative with the following locus identifiers: UGT76F1(AT3G55700), CRY1(AT4G08920), CRY2(AT1G04400), PHYA(AT1G09570), PHYB(AT2G18790), PIF1(AT2G20180), PIF3(AT1G09530), PIF4(AT2G43010), PIF5(AT3G59060), IAA19(AT3G15540), IAA29(AT4G32280), GH3.1(AT2G14960), GH3.6(AT5G54510), SAUR19(AT5G18010), SAUR24(AT5G18080), CAB2(AT1G29920), LHCA6(AT1G19150), ACTIN2 (AT3G18780).

Supplementary Figures and Supplementary Tables

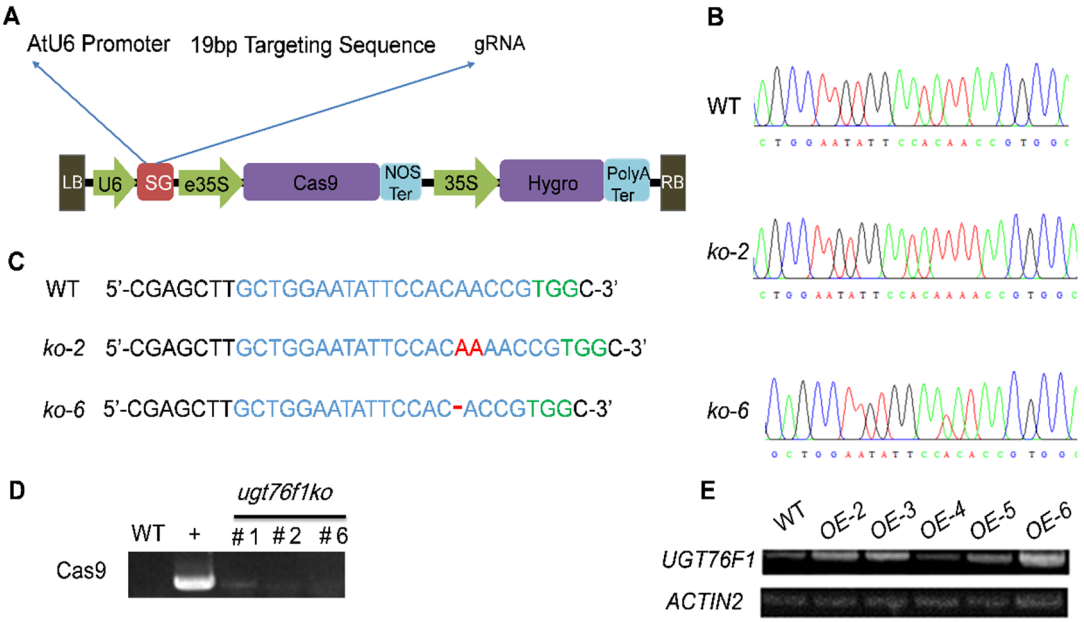

Fig S1. Generation and identification of *UGT76F1* mutant lines and overexpression lines. (A) Generation of *ugt76f1* mutant by CRISPR/Cas9-mediated gene editing. Hygromycin was used as a selection marker for successful transformation plants. (B) DNA sequencing showed that double base "A" were found to be inserted into the *UGT76F1* coding sequence of ko-2, a base "A" deletion occurs in target sites of mutant line ko-6. (C) Schematic diagram showing the mutation locations of *ugt76f1* mutant lines. The bases in green are the PAM sequence. (D) Confirmation of Cas9-free in *ugt76f1* mutant lines by RT-PCR. (E) Transcript levels of *UGT76F1* gene in wild-type (WT) and several overexpression lines determined by RT-PCR. *ACTIN2* was used as the internal control.

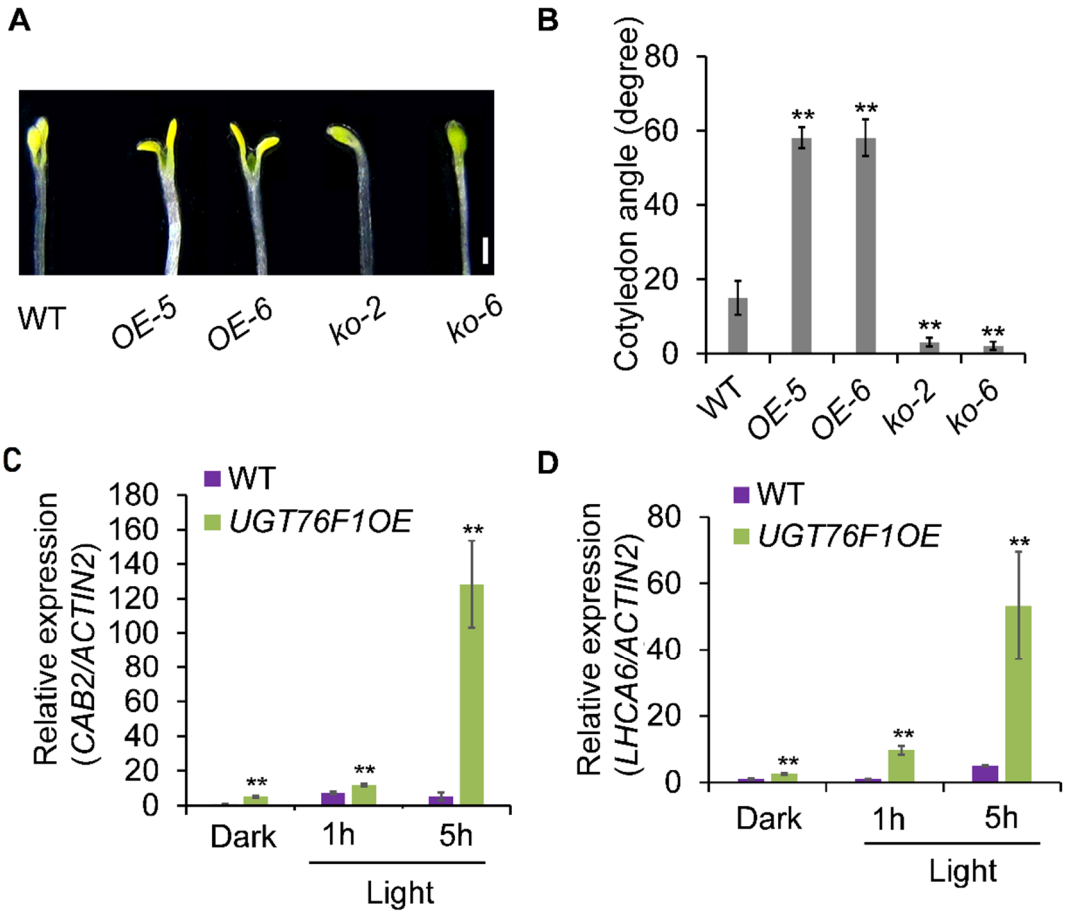

Fig S2. Phenotypic Characterization of *UGT76F1* mutants and overexpression lines. (A) Cotyledon phenotype of *UGT76F1* overexpression lines and mutants grown under continuous darkness for 10 days. Scale bar=1 mm. (B) Quantification of the cotyledon angles of 10-day-old seedlings in (A). Data are means±SD (n = 30). (C and D) Expression of Light-responsive genes *CAB2* (C) and *LHCA6* (D). WT and *UGT76F1OE* seedlings were grown in darkness for 4 days and then exposed to white light for various periods of time. Data are means ± SD, n = 3. Asterisks indicate significant differences compared to the corresponding WT (Student's *t*-test, **P < 0.01). Experiments were conducted for three biological replicates, yielding similar results.

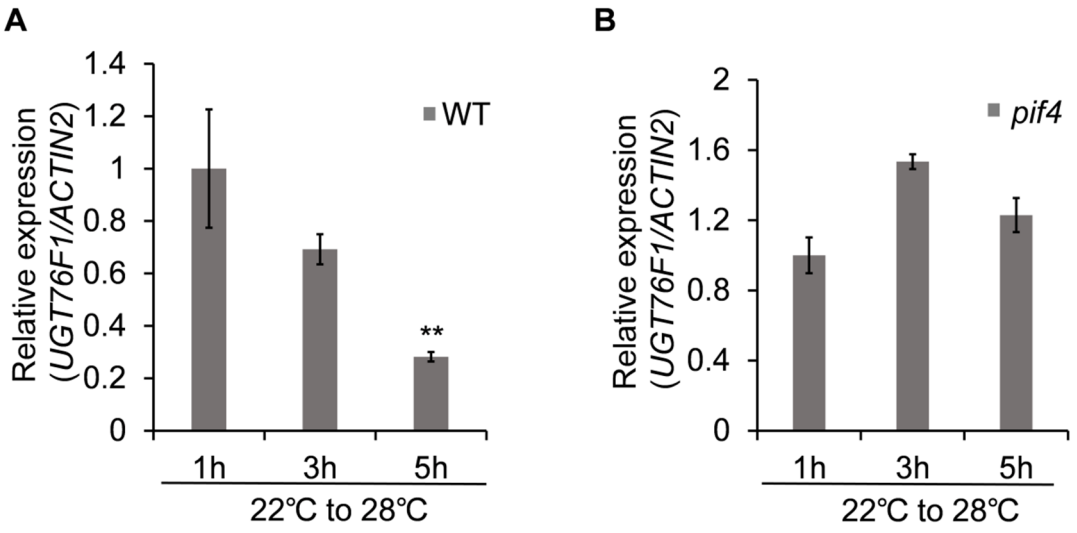

Fig S3. The temperature-mediated down-regulation of *UGT76F1* expression is inhibited in *pif4*.

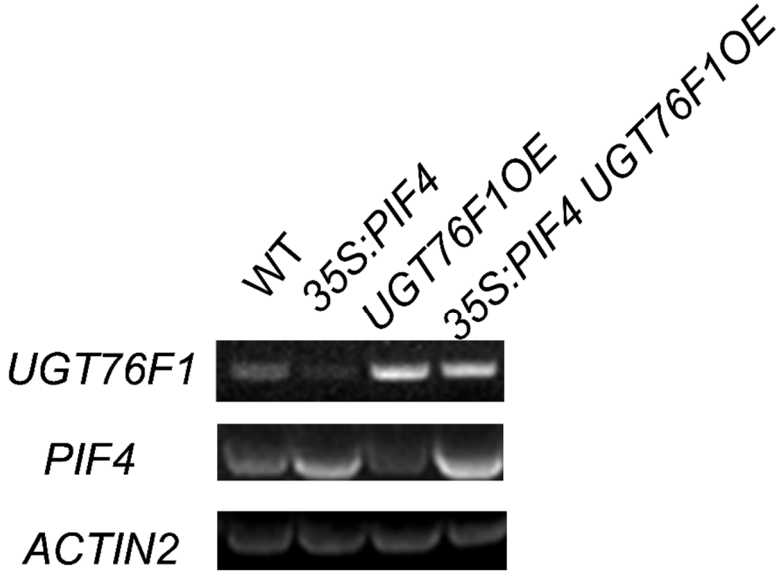

Fig S4. RT-PCR analysis of the expression of *UGT76F1* and *PIF4* in *35S:PIF4 UGT76F1OE* hybrid lines described in Figure 5D. *ACTIN2* was used as the internal control.

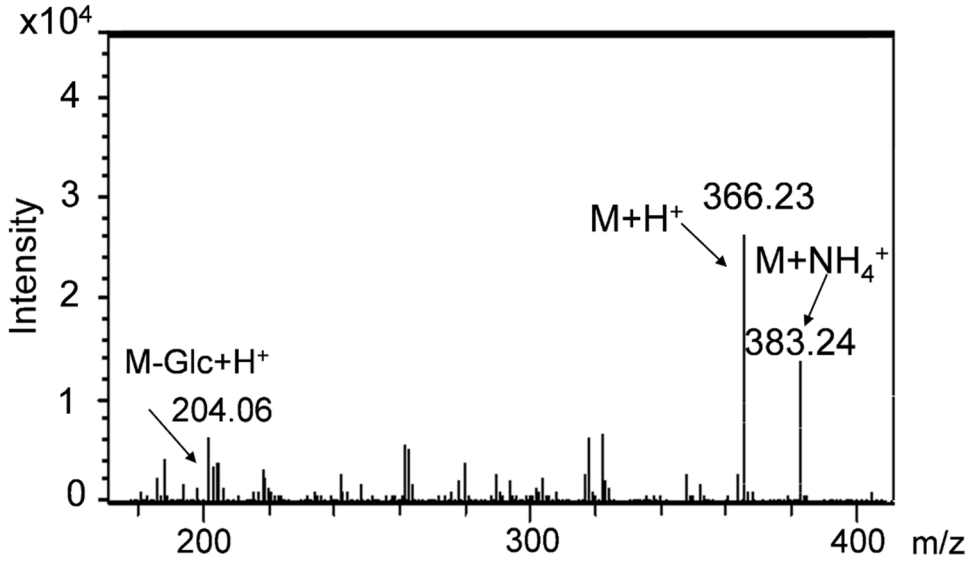

Fig S5. Ion peaks of IPyA glucose conjugates extracted from overexpression lines and mutant lines are consistent with that predicted under positive ion mode.

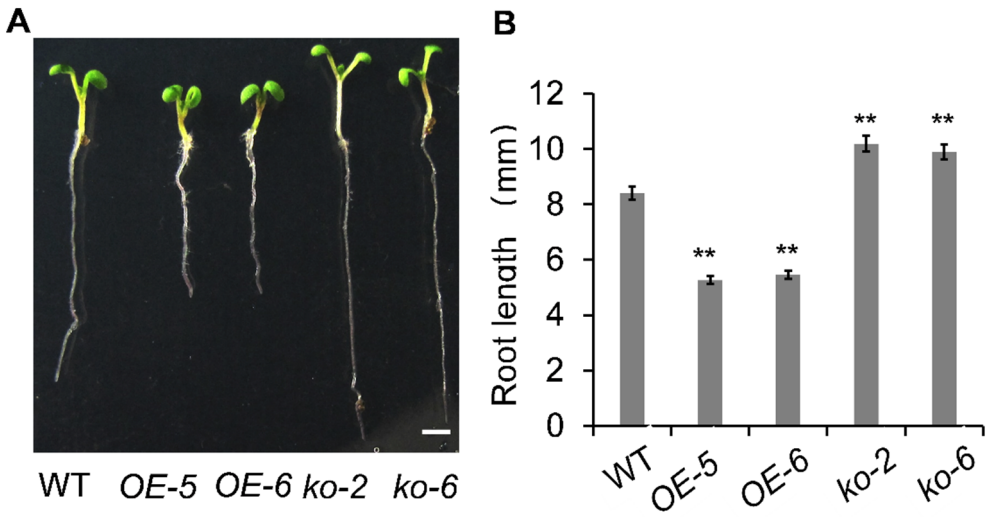

Fig S6. Phenotypic Characterization of the entire seedlings. (A) Phenotypes of the *UGT76F1* overexpression lines and mutants grown under continuous white light conditions for 5 days. Scale bar=1 mm. (B) Root length of seedlings shown in (A). Data are means ± SD, n = 20. Asterisks indicate significant differences from WT based on Student's *t*-test (*P < 0.05, **P < 0.01).

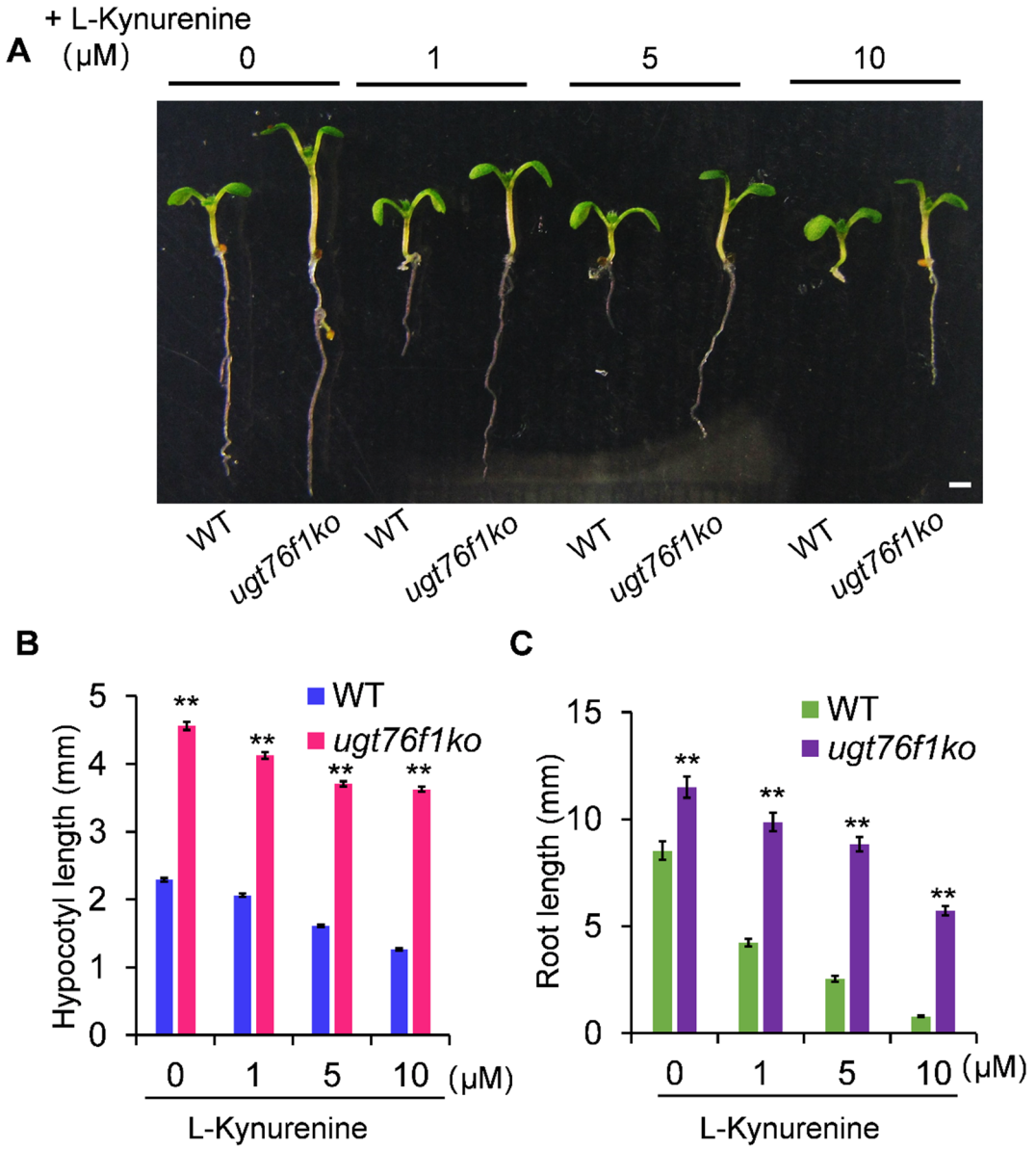

Fig S7. The phenotypes of *ugt76f1ko* plants can be suppressed by L-Kynurenine. (A) Phenotypes of 5-day-old WT and *ugt76f1ko* seedlings grown at 22℃ and exposed to 0-10 μM L-Kynurenine. Scale bar=1 mm. (B) Hypocotyl length of seedlings shown in (A). (C) Root length of seedlings shown in (A). Data are means ± SD, n = 30. Asterisks indicate significant differences from WT based on Student's *t*-test (**P < 0.01).

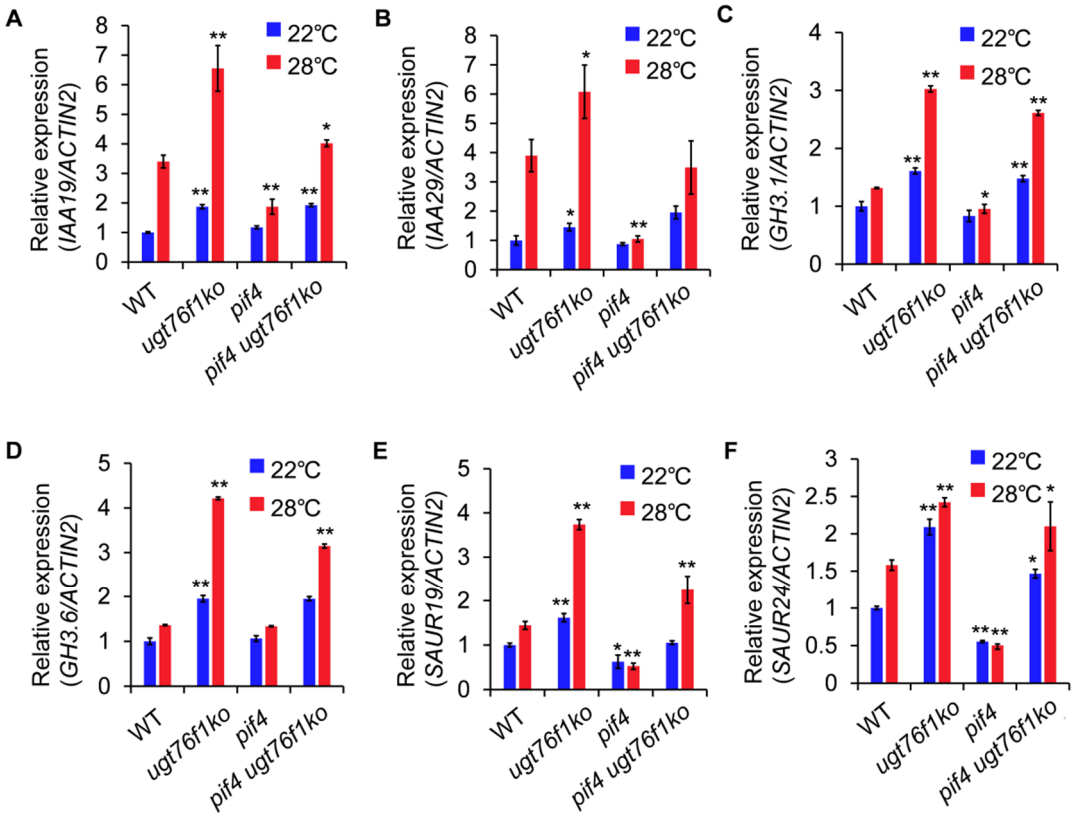

Fig S8. PIF4 regulates expression of auxin-responsive genes in a UGT76F1 dependent and contrary manner. (A-F) Relative transcript abundance of *IAA19*, *IAA29*, *GH3.1*, *GH3.6*, *SAUR19* and *SAUR24* in 5-day-old WT, *ugt76f1 ko*, *pif4*, *pif4 ugt76f1ko* seedlings grown at 22℃ or shifted to 28 ℃ for another 5 h. Expression was normalized against constitutively expressed *ACTIN2*. The value of wild-type plants under 22℃ condition was set at 1.0. Data shown are means ± SD of three biological replicates. n=3. Asterisks indicate significant differences relative to respective WT at 22℃ or 28℃ (Student's *t*-test, *P<0.05, **P<0.01).

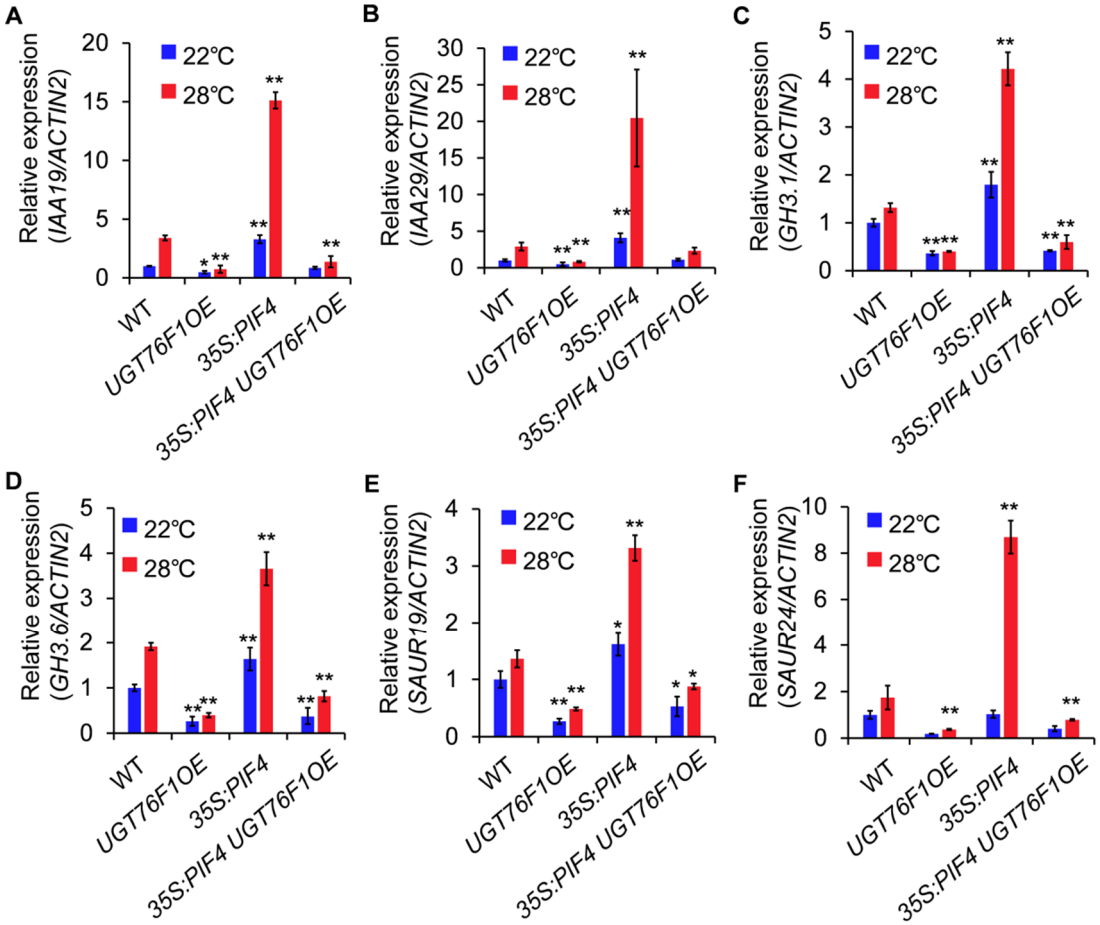

Fig S9. PIF4 regulates expression of auxin-responsive genes in a UGT76F1 dependent and contrary manner. (A-F) Relative transcript abundance of *IAA19*, *IAA29*, *GH3.1*, *GH3.6*, *SAUR19* and *SAUR24* in 5-day-old WT, *35S:PIF4*, *UGT76F1 OE*, *35S:PIF4 UGT76F1OE* seedlings grown at 22℃ or shifted to 28℃ for another 5 h. Expression was normalized against constitutively expressed *ACTIN2*. The value of wild-type plants under 22℃ condition was set at 1.0. Data shown are means ± SD of three biological replicates. n=3. Asterisks indicate significant differences relative to respective WT at 22℃ or 28℃ (Student's *t*-test, *P<0.05, **P<0.01).

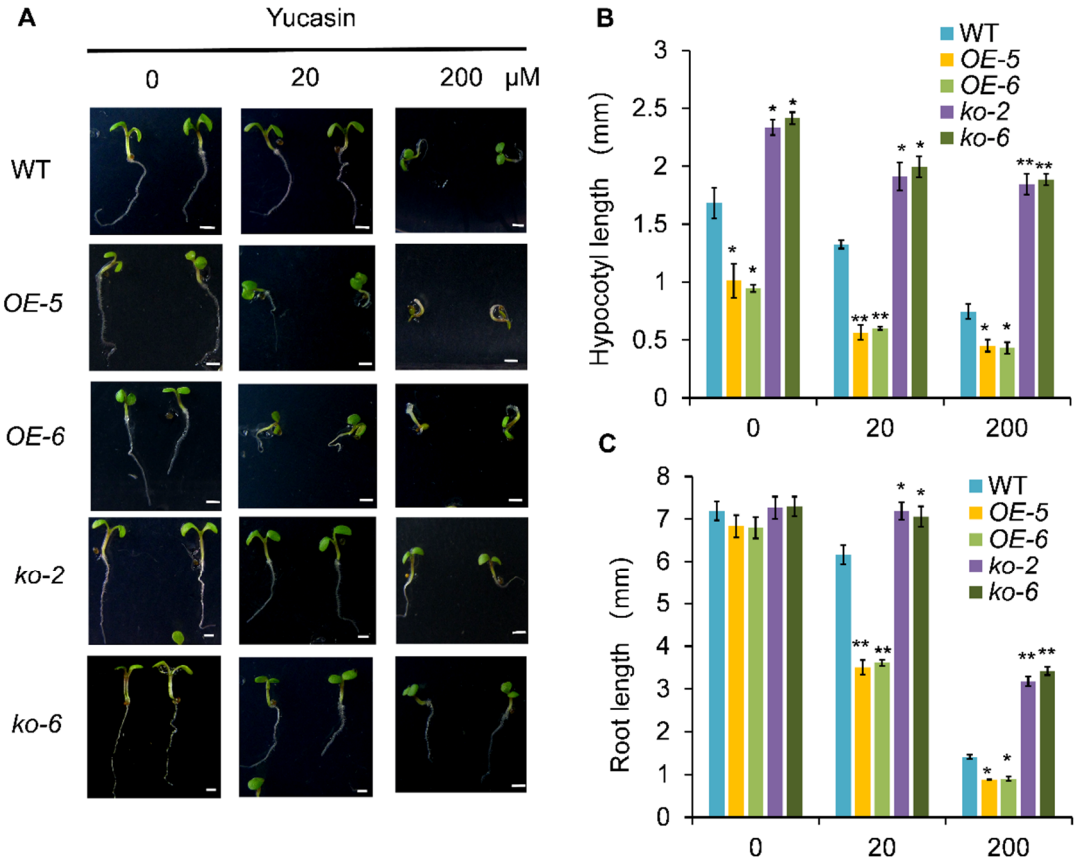

Fig S10. UGT76F1 antagonizes YUC in regulating auxin responses under light condition. (A) Effects of YUC inhibitor yucasin on hypocotyl length and root length of WT, *UGT76F1* overexpression lines and *ugt76f1* mutant lines under white light and 22℃ conditions for 4 days (Scale bar =1mm). (B and C) Hypocotyl and root length measured for seedlings in (A). Data shown are means ± SD. n=30. Asterisks represent significant difference relative to WT (Student's *t*-test, *P<0.05, **P<0.01).

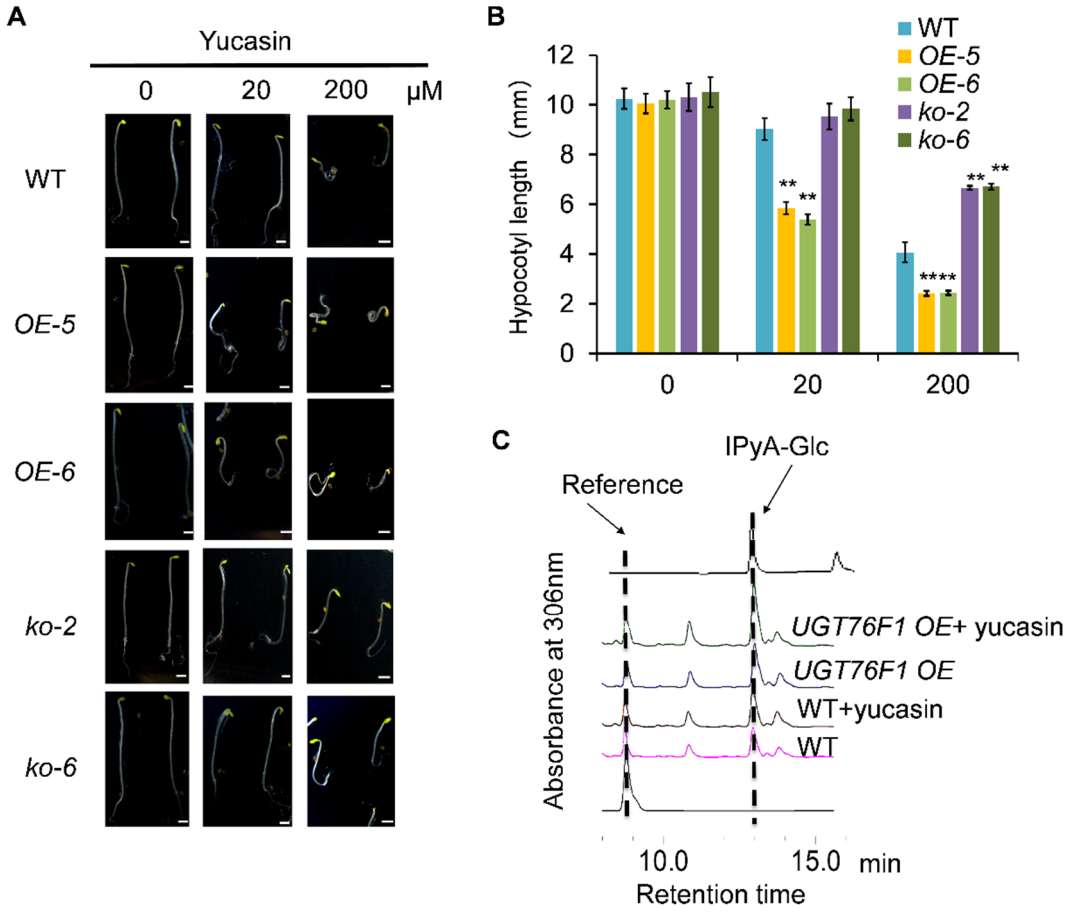

Fig S11. UGT76F1 antagonizes YUC in regulating auxin responses under dark condition. (A) Effects of YUC inhibitor yucasin on hypocotyl length of WT, *UGT76F1* overexpression lines and *ugt76f1* mutant lines under dark condition for seven days (Scale bar =1mm). (B) Hypocotyl length measured for seedlings in (A) after a seven-day exposure to 0 to 200 μM yucasin. Data shown are means ± SD. n=30. Asterisks represent significant difference relative to WT (Student's *t*-test, *P<0.05, **P<0.01). (C) HPLC profiling of IPyA sugar conjugates in the 5-day-old WT and *UGT76F1* overexpression lines treated with or without yucasin for another 24 h. Caffeic acid was used as an internal reference in these assays to monitor the recovery rate. The IPyA sugar conjugates formed by UGT76F1 catalysis were used as the glycoside standards. Three biological replicates were conducted, yielding similar results.

Table S1. Substrates screened for enzyme activity of UGT76F1

Classification	Substrates screened by UGT76F1	Enzyme activity (“ ● ” high activity, “ × ” no activity)
Phenylpropanoids	Cinnamic acid	×
	p-coumaric acid	×
	o-coumaric acid	×
	Caffeic acid	×
	Ferulic acid	×
	Sinapic acid	×
Flavonoids	Naringenin	×
	Quercetin	×
	Kaempferol	×
Anthocyanins	Cyanidin	×
SA and its Derivatives	Salicylic acid (SA)	×
	Methyl salicylate (MeSA)	×
	2,3-Dihydroxybenzoic acid	×
	2,5-Dihydroxybenzoic acid	×
Auxin and related compounds	Indole-3-acetic acid (IAA)	×
	Indole-3-butyric acid (IBA)	×
	Indole-3-pyruvic acid (IPyA)	●
	Indole-3-acetonitrile (IAN)	×
	Indole-3-acetamide (IAM)	×
Other hormones	Trans-zeatin (TZ)	×
	6-Benzylaminopurine (6-BA)	×
	Gibberellin A1 (GA1)	×
	Gibberellin A4 (GA4)	×
	Abscisic acid (ABA)	×

Table S2. Enzyme kinetics of UGT76F1

	Enzyme	Km (mM)	Vmax (nkat/mg)	Kcat (s^{-1})	Kcat/Km ($mM^{-1}s^{-1}$)
Indole-3-pyruvic acid	UGT76F1	0.28±0.02	1.10±0.05	0.87±0.04	3.12±0.08

Table S3. Primers used in this study

Primer name	Sequence (5'---3')
RT-qPCR primer	
ACT2-F	GCTCCTCTTAACCCAAAGGC
ACT2-R	CACACCATCACCAGAATCCAGC
IAA19-F	GCCAGGTGGTGGGGTGGCCACCG
IAA19-R	GGCCACACCGATGCCACGGAAAC
IAA29-F	CACGGCGATGAACAACAACATAT
IAA29-R	CTCTGTCGCAATCTTCATATTCG
SAUR19-F	CTTCAAGAGCTTCATAATAATTCAAACTT
SAUR19-R	GAAGGAAAAAATGTTGGATCATCTT
SAUR24-F	GAGATATTTGGTGCCTGTCTCATATTTAAACC
SAUR24-R	CAAGAAGAAAGAGGAAAAAGGGCTCATC
GH3.1-F	AACTTATGCCGACCATTAAAGAA
GH3.1-R	TCTAGACCCGGCACATACAA
GH3.6-F	CCTTGTTCCGTTTGATGCTT
GH3.6-R	CGTGTTACCGTTCAAGCAGA
76F1-F	TCCACCGAAGCCAAAGAACA
76F1-R	GGCAATGACTCAAGCCACTC
CAB2-F	TGGGACACCGCTGGACTTTC
CAB2-R	CATAGCCAATCTTCCGTTCTT
LHCA6-F	CTTGCTTTCCTTGGGTTCTG
LHCA6-R	AACGGCGAGTGACACTATACAA
RT-PCR primer	
76F1-F	GTGGTCTATGTGAGTTTTGGA
76F1-R	AAGAATGGTAGTTCGCTGTTT
PIF4-F	TCCGACCGGTTTGCTAGATACA
PIF4-R	AACATCTCCATCGGCTGCATCT
Dual-LUC construction primer	
76F1-P-F	CATAATACGTACGTCATAGT
76F1-P-R	ATTTAAGTTGTACCCATCAC
Yeast one-hybrid construction primer	
PIF1-F	ATGCATCATTTTGTCCCTGAC
PIF1-R	ACCTGTTGTGTGGTTTC
PIF3-F	ATGCCTCTGTTTGAGCTTTTC
PIF3-R	CGACGATCCACAAAACTG
PIF4-F	ATGGAACACCAAGGTTGGAG
PIF4-R	GTGGTCCAAACGAGAACCG
PIF5-F	ATGGAACAAGTGTTTGCTGAT
PIF5-R	GCCTATTTTACCCATATGAAGA

76F1-P-F	AAGCTTAGGATTAGTTTTGCTATCGGTTA
76F1-P-R	GGTACCTTTCTTCTTCTTATCTTTTTCTTTTC
ChIP-RT-qPCR primer	
P1-F	ATTGTTGCCATTTGCTAA
P1-R	GCAATAAAACTTACGAAT
P2-F	TATATGTTTTTAGAAGAA
P2-R	TTAGTTCATTTATATCAA
P3-F	GGAGCCAAGTCTTCTTCAGG
P3-R	ACGGTCGTTCACTGTTTCTT
P4-F	AGGGAAAAATCGTGAAAT
P4-R	TATCTCGCGTTVCACATGCTG
P5-F	GAAGGGAGATGGATTGAG
P5-R	TTTGGAACTTTTGGAGAG
P6-F	TTTTCCCTAATGTTTTAA
P6-R	AAGTCACTGTCTGTCCGC
Gene cloning primer	
76F1P-F	ATGGAAGAGAGAAAAGGGAGGAG
76F1P-R	CTTCTCTCTAACAACATCCCGAC
76F1-F	GGGACTCTAGAGGATCATGGAAGAGAGAAAAGTGAAG
76F1-R	GGCTTTTGCAAGTTAAAGCTCGAATTTCCCCGATCGTTC
76F1-F'	ATCGGATCTGGTTCCGCGTGATGGAAGAGAGAAAAGTGAAGAG
76F1-R'	CGCTCGAGTCGACCCGGGTTAACTTGCAAAAGCCGAGG