UGT71C5, a major glucosyltransferase mediates ABA homeostasis in *Arabidopsis thaliana*

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Supporting Online Figures

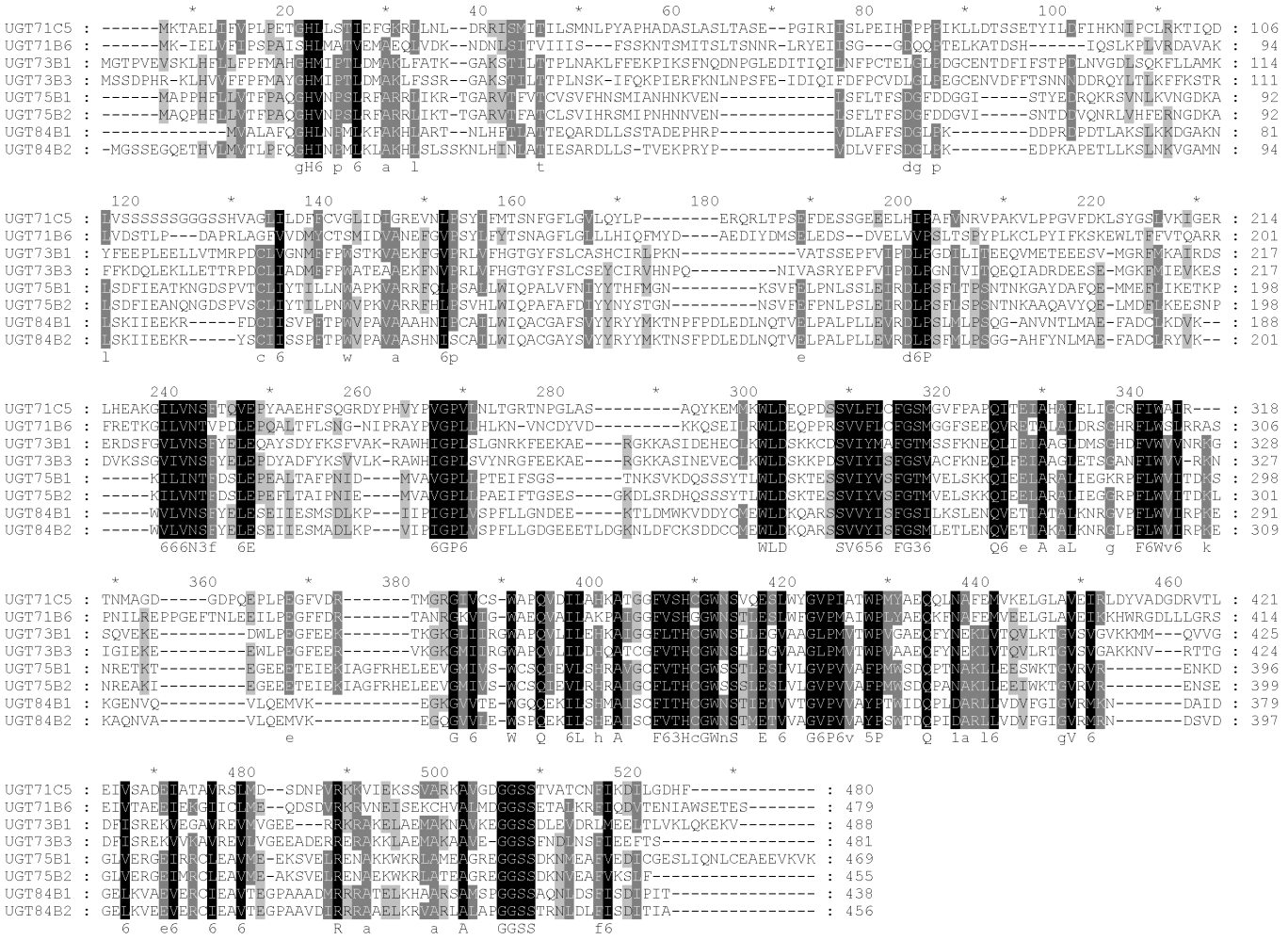

Figure S1. Sequence alignment of UGT homologs.
The alignment was made by using Clustal W2 sorftware (http://www.ebi.ac.uk/). Identical amino acid residues are shown in black, and those conserved at least in four sequences are shown in shaded color.

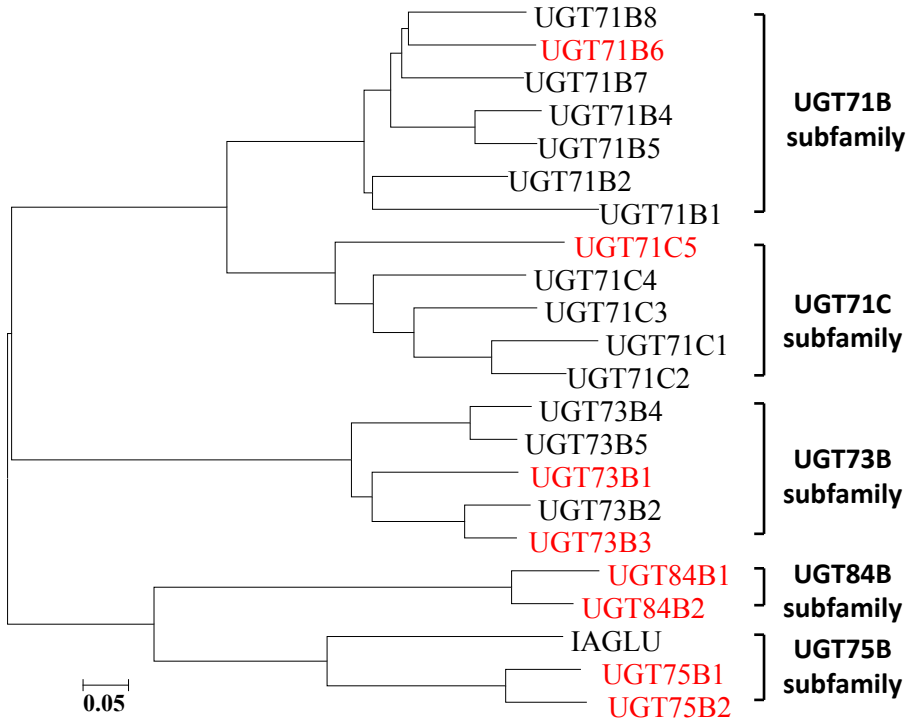

Figure S2. Phylogenetic analysis of the UGT homologs. The scale bar represents 0.5 substitutions per site. Phylogenetic analyses were conducted by using MEGA5.1 software.

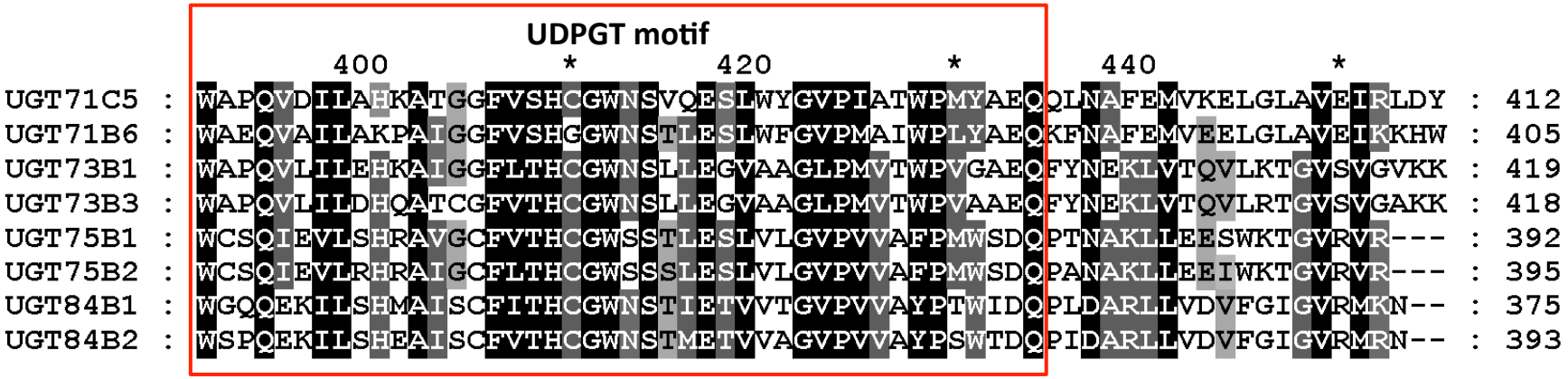

Figure S3. Partial amino acid sequence alignment of UGT71C5 and its homologs. The most conserved sequences in all UGTs are named as UDPGT (boxed). Identical amino acid residues are shown in black, and those conserved at least in four sequences are shown in shaded color.

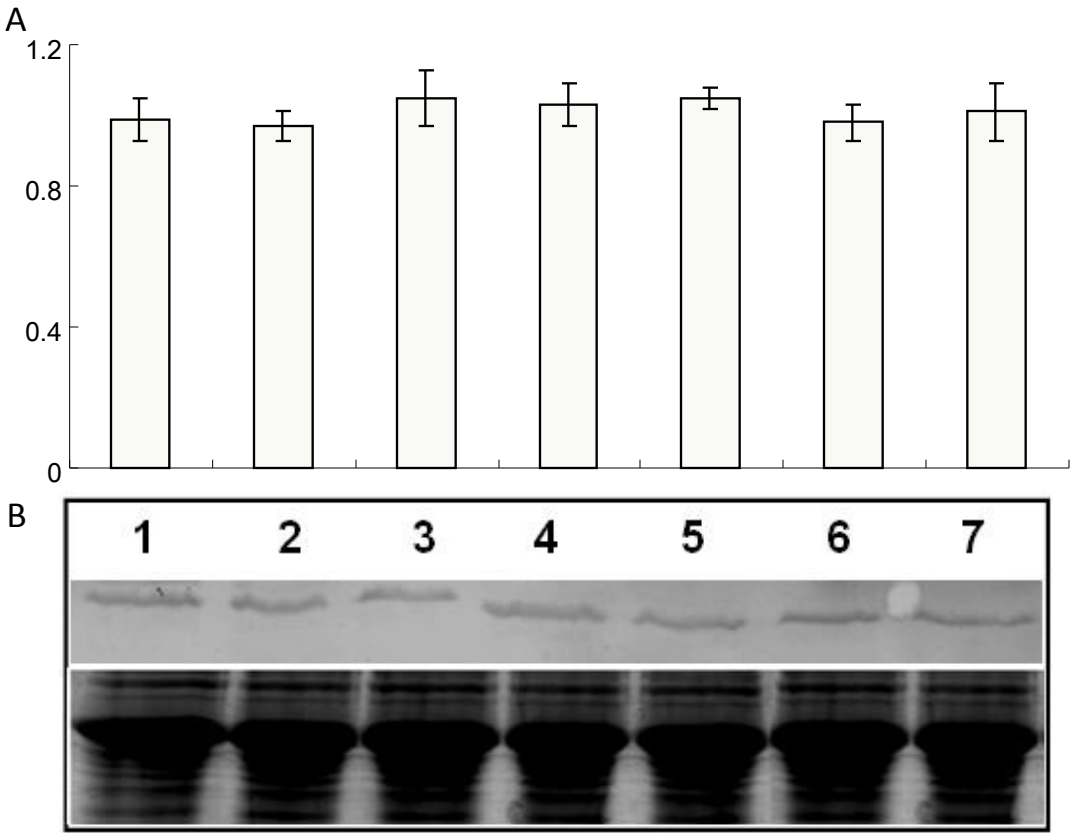

Figure S4. The expression pattern of UGT71C5 in different plant tissues. A, The expression was detected by qRT-PCR Actin2 is used as an internal control. B, Western blot with anti-UGT71C5 (B). 1, root (2 weeks); 2, leaf (2 weeks); 3, seeds; 4, leaf (45 days); 5, stem; 6, siliques; 7, flower.

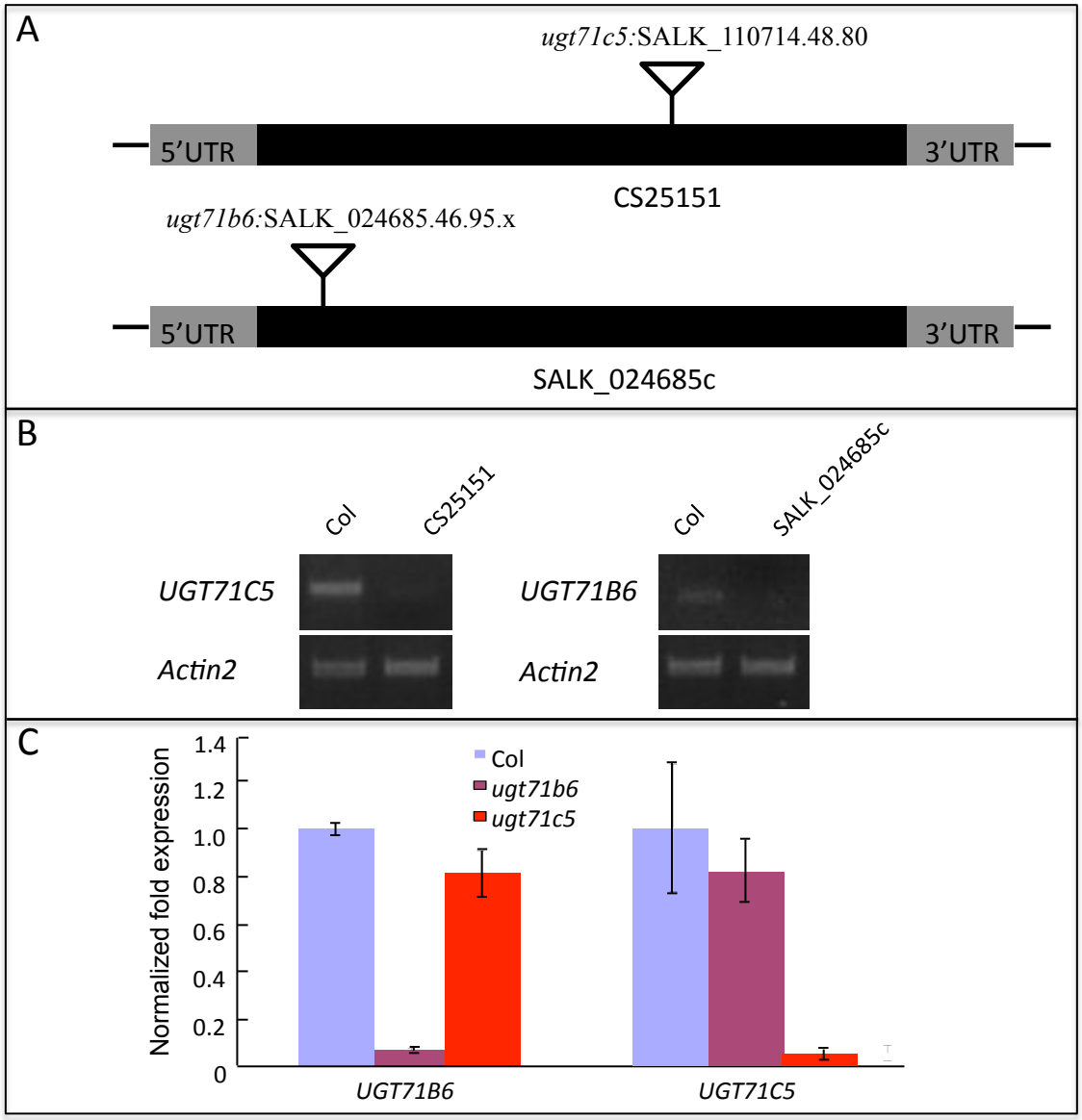

Figure S5. Confirmation of *ugt71c5* and *ugt71b6* knockout mutants. A. Schematic representation of the T-DNA insertion sites in *ugt71c5* and *ugt71b6* mutants. **B. C.** Semi-quantitative PCR (**B**) and qRT-PCR (**C**) analyses of *UGT71C5* and *UGT71B6* transcripts in wild-type and *ugt71c5* and *ugt71b6* mutant plants. CS25151: *ugt71c5* mutant; SALK_024685c: *ugt71b6* mutant; Col: wild-type. *UGT71C5* and *UGT71B6* specific primers for semi-quantitative PCR (B) and qRT-PCR (C) are listed in Supplemental table 1. The actin 2 was used as internal control.

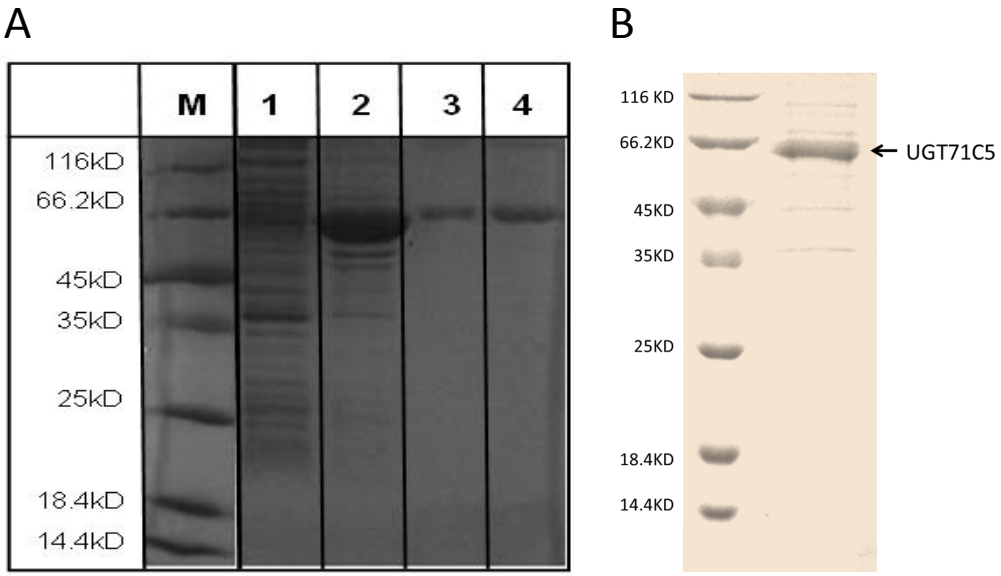

Figure S6. Preparation of UGT71C5 in a bacterial expression system and the use of Western blot analysis to test UGT71C5 antibody. A. Analysis of purified UGT71C5 in SDS-PAGE. M: protein molecular markers; 1: supernatant from bacterial cellular extracts; 2: sediment of bacterial cellular extracts; 3,4: purified UGT71C5 from bacterial expression system. **B.** Western blot analysis to test the quality of UGT71C5 antibody. The antibody could detect 10 ng of UGT71C5 in SDS-PAGE.

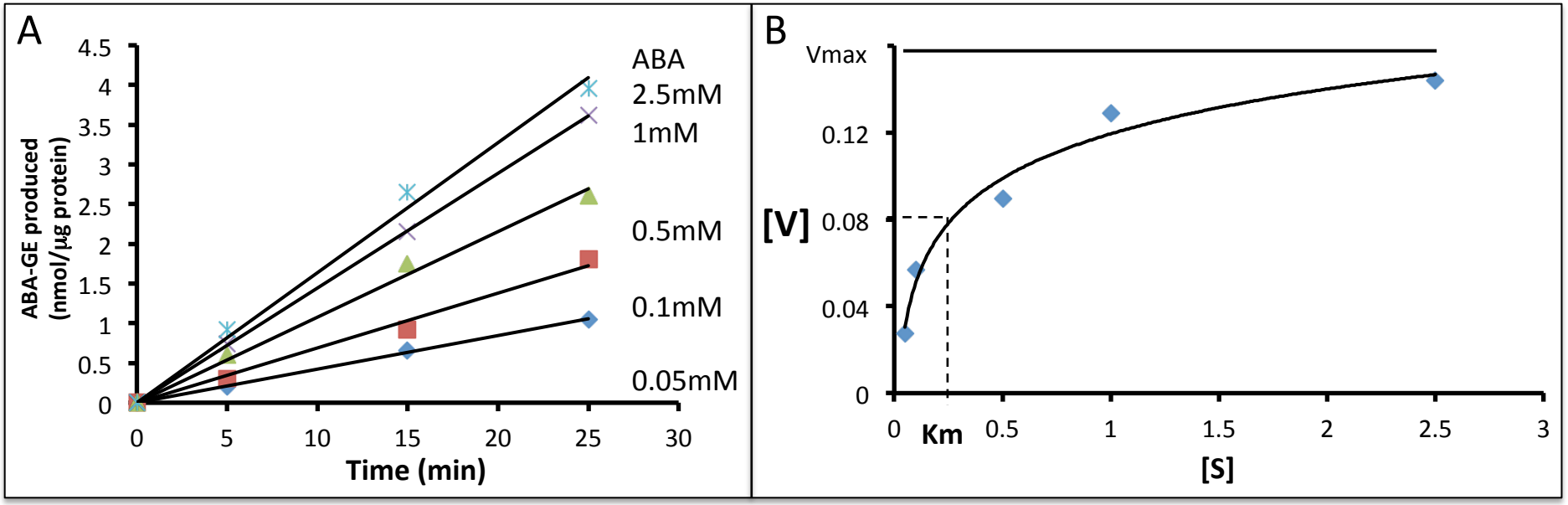

Figure S7. Kinetic analysis of UGT71C5. **A.** Measurement of the reaction rate of UGT71C5. Reaction mixture contained 1 μg UGT71C5 protein, 5 mM UDP-Glucose, 5 mM $MgCl_2$, 10 mM DTT. Concentrations of ABA used were 0, 0.05 mM, 0.1 mM, 0.5 mM, 1mM, and 2.5 mM, respectively. Times of reaction used were 0, 5min, 15min, and 25min. **B.** Determination of kinetic parameters, reaction velocity (V) and concentration of ABA (S) were obtained using the Michaelis-Menton equation.

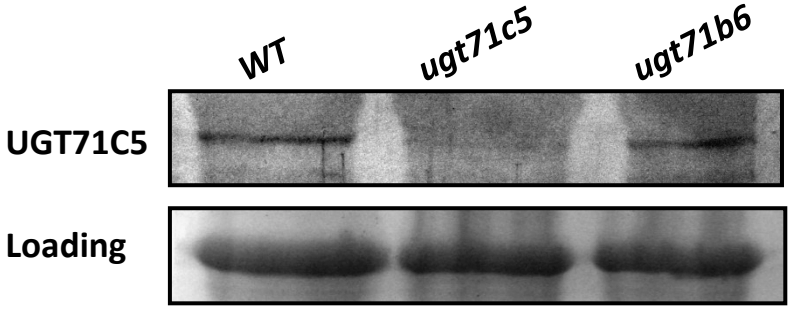

Figure S8. Analysis of the specificity of the UGT71C5 antibody. Equal amounts of plant cellular extracts (15 mg) were loaded in each lane and Coommassie blue staining was used as the protein loading control. WT: wild type (Col); *ugt71c5* and *ugt71b6*: knockout mutants.

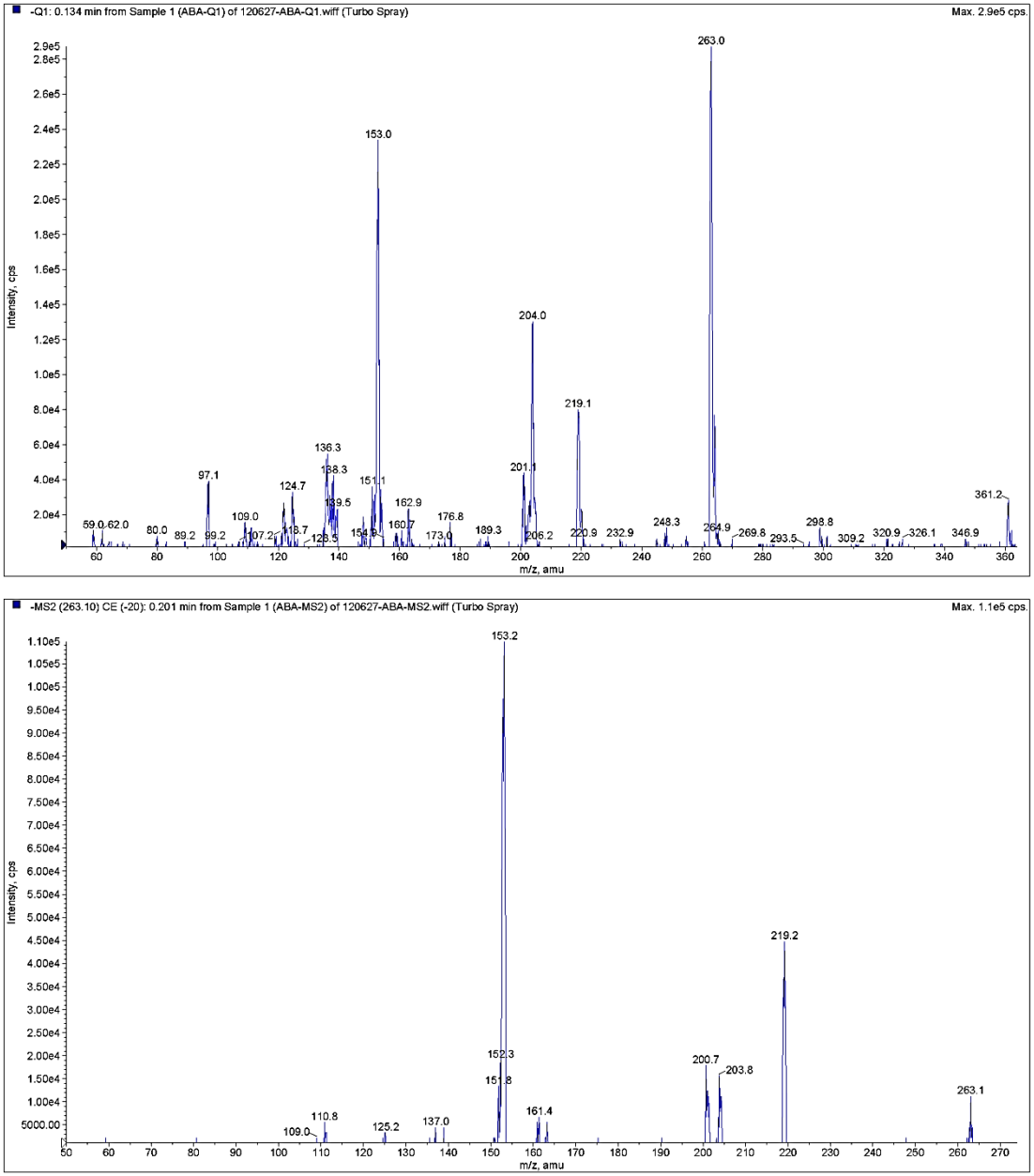

Figure S9. Fragmentation pattern of ABA standard. Precursor(upper pannel, m/z 263.1) and product (lower pannel, m/z 153.0) ions of ABA standard analyzed in negative-ion mode.

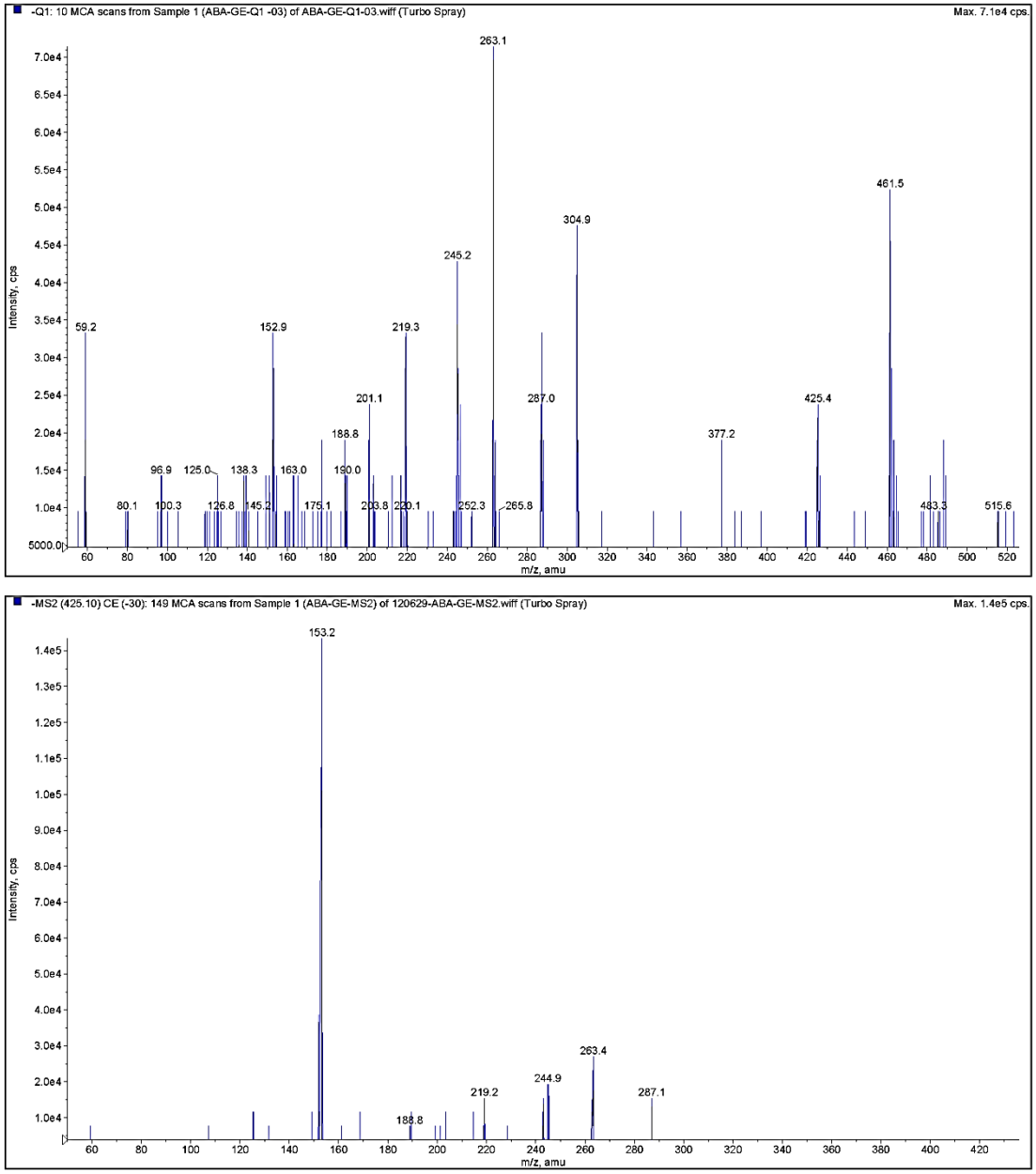

Figure S10. Fragmentation pattern of ABA-GE standard. Precursor (upper pannel, m/z 425.1) and product (down, m/z 263) ions of ABA-GE standard analyzed in negative-ion mode.

Supplemental Table 1. Primers used in this study

Transgenic plants construction
UGT71C5
F: 5'-CGCGGATCCTAGATGAAGACAGCAGAGCTCATATTCG-3'
R: 5‘-TCCCCCGGGTCAAAAGTGATCCCCAAGAATATCT-3’
Mutants identification
ugt71c5 (CS25151)
LP: 5'-TTCTCTTGCTTCGCTAACAGC-3'
RP: 5‘-ACTGCTAAGCCCAACTCCTTC-3’
ugt71b6 (SALK_024685c)
LP:5'-TAAACACGGTTCCTGACTTGG-3'
RP:5‘-TATGCACCCAAGAATGATGTG-3’
SALKBP:5‘-ATTTTGCCGATTTCGGAAC-3’
Mutants confirmation
UGT71C5 (Semi-quantitive PCR)
F: 5'-CTCTCTTACGGGTCTCTGGTCA-3'
R: 5’-TGACACGATCTCCAAAGTAACC-3’
UGT71B6 (Semi-quantitive PCR)
F: 5’-CTAACGAATTTGGCGTCCCT-3'
R: 5’-CACTGAACCCTCCCATGCTC-3’
UGT71C5 (qRT-PCR)
F: 5’-ATCCGGGTCTAGCTTCGG-3'
R: 5’-ATTCCACGGCCCATTGTT-3'
UGT71B6 (qRT-PCR)
F: 5’-TCGAGATGGTGGAAGAGC-3'
R: 5’-GTTTCCGACCAAGCAATA-3'
Actin2
F: 5’-TCTTCCTCACGCTATCCTCCG -3'
R: 5’-CGATGTTTCCATACAGATCCTTCC-3'

UGT71C5 subcellular location and expression
F: 5’-CTCCCCGGGATGAAGACAGCAGAGCTCATAT-3’
R: 5’-CCCGGATCCAAAGTGATCCCCAAGAATAT-3’
Expression of ABA responsive genes (qRT-PCR)
RD29A
F: 5’-CAGAGGAACCACCACTCAACACA-3’
R: 5’-CTCTAGGTTTACCTGTTACGCCTG-3’;
RD29B
F: 5’-ATGGAGTCACAGTTGACACGTCCT-3’
R: 5’-CTTCTGGGTCTTGCTCGTCATACT-3’
RAB18
F: 5’-ATGGCGTCTTACCAGAACCGTCCA-3’
R: 5’-ACCACCACTTTCCTTGTGGAGTTG-3’
NCED3
F: 5'-CCGGTGGTTTACGACAAGAA-3’
R: 5' -CCCAAGCGTTCCAGAGATG-3’
CYP707A2
F: 5’-CGTCTCTCACATCGAGCTCCTT-3’
R: 5’-CCAAAAGTCCATCAACACCCTC-3’
ABI5
F: 5’-TGGTAACTAGAGAAACGAAG-3’
R: 5’-TTACTACTACTACTACGTCC-3’
UGT71C5
F: 5'-GGAATTCCGTCCAAGAGAGTC-3'
R: 5'-TGACACGATCTCCAAAGTAACC-3’
Actin2
F: 5’-TTGACTACGAGCAGGAGATGG-3’
R: 5’-ACAAACGAGGGCTGGAACAAG-3’

F: forward primer; R: reverse primer