

Supplemental Figure 1. Work flow of transcriptome assembly and analysis. Reads from the 16 transcriptomes, including four biological replicates for four time points, were filtered using Bowtie2 (version 2.2.6). Un-mapped, reads were then trimmed using Trimmomatic (version 0.30). Overlapping paired-end reads were then merged into single-ended reads using BBMerge (version 34.92). Merged reads together with un-merged paired-end reads were assembled *de novo* with Velvet (version 1.2.10) and Oases (version 0.2.8) to produce the assembly. Contigs were clustered at 99% sequence identity level using CD-HIT-EST (version 4.6.1). Predicted peptides were generated from TransDecoder (version 2.0.1). Salmon (version 0.3.0) was used to quantify results from CD-HIT-EST before differential expression analysis with voom / limma.

A

```

PgUGT5  MQPCELMGMS  RKP HVA I F P S  AGM GHL I P S A  E F A K R L S A D H  G F T V T F I T C K  W M F S G F R L Q Q  A Y S E R I A S L R  G F D V R F V Q L P  H V E I E E E A Q H
PgUGT5b  -----G-----V-----L-----G S-----L S L-----P-----L A-----N M-----S-----I N-----I-----E-----G-----
PgUGT5c  -----R-----L-----F-----L I-----D H-----L S-----S-----P-----M A-----S-----S-----L-----I T-----E-----D G-----E-----
PgUGT5  LEK S K G F V E S  A L T S L Q I D D S  F S P L S A F I T D  F F C S T M F D V T  A K L H I P T Y L F  F T S P A S L L S V  M L C L P K L A S E  T Q V S F K D A D F  S I E V A G V P P I
PgUGT5b  F-----S-----N-----R-----V-----A-----V-----A-----R-----V-----A-----V-----L-----S-----I V-----I P I-----P-----P-----T-----
PgUGT5c  -----D S-----V-----R-----L-----A P-----I-----S-----A-----E-----G-----V-----T-----F-----S-----F-----V-----I P I-----T E-----P-----I P-----L-----
PgUGT5  D R S D E V F Y W F  V H H S R L R E A  T G I L L N T F E E  L E S E Q I K A L R  E G K V - N P S D P  R R M P H I Y P V G  P L I S S S P V E Y  E-----A-----D C L K W L D N Q A
PgUGT5b  -----A-----Q-----L-----W K V-----F-----V-----D-----P-----A-----M-----I S K-----K-----I-----D-----S-----L-----H-----N D K L V E D G R-----P-----
PgUGT5c  -----N-----A-----A-----Q-----C-----C-----W K V-----R-----I-----P-----T-----V-----I S-----T T E A-----D-----V-----R-----F-----V-----L-----N D K H V E D G R-----P-----
PgUGT5  S G V V M S R E Q I  T E L A L G L D A S  G H R F L W V L R S  P S S T F L S I N D  S D V S E L L P Q G  F E D R C K D R G V  V V P S W A P Q I P  I L S H P S T R G F  L S H C G W N S S L
PgUGT5b  ..T G L P . A . V-----E-----S-----E E-----T E L F Q-----E-----Y Q N-----T Q-----Q-----L-----A-----S-----V-----G-----T-----
PgUGT5c  ..A S L P S A . V-----V-----E-----L-----S-----E E-----T E L-----Q V-----E-----N-----T R-----L-----A-----V-----G-----I-----T-----
PgUGT5  A W P V F A E Q K M  N K I L L V N D F K  V A L E A K M D S D  G F V K R E E V E R  A V R E L M E G E A  G M A V R E R T R E  L K E K A A S A L A  E G G S S Y K A M A  D A V S D W - - G K
PgUGT5b  C . . L . . . . . . R . . . . E . . . . I A . . . . E . . . . E . . R . G . . . . T . . . . E . S . . . . R . . A . V . . . . V . . . . E . . . . . . A . . . E . T - - A
PgUGT5c  C . . L . . . . R . . . . K L . . G I A . . . . E . . . . G . . . . . . . . . . G D . R R . . A . M . . . . V . . V . . . . E . . . . . . A . . . E . T T N A

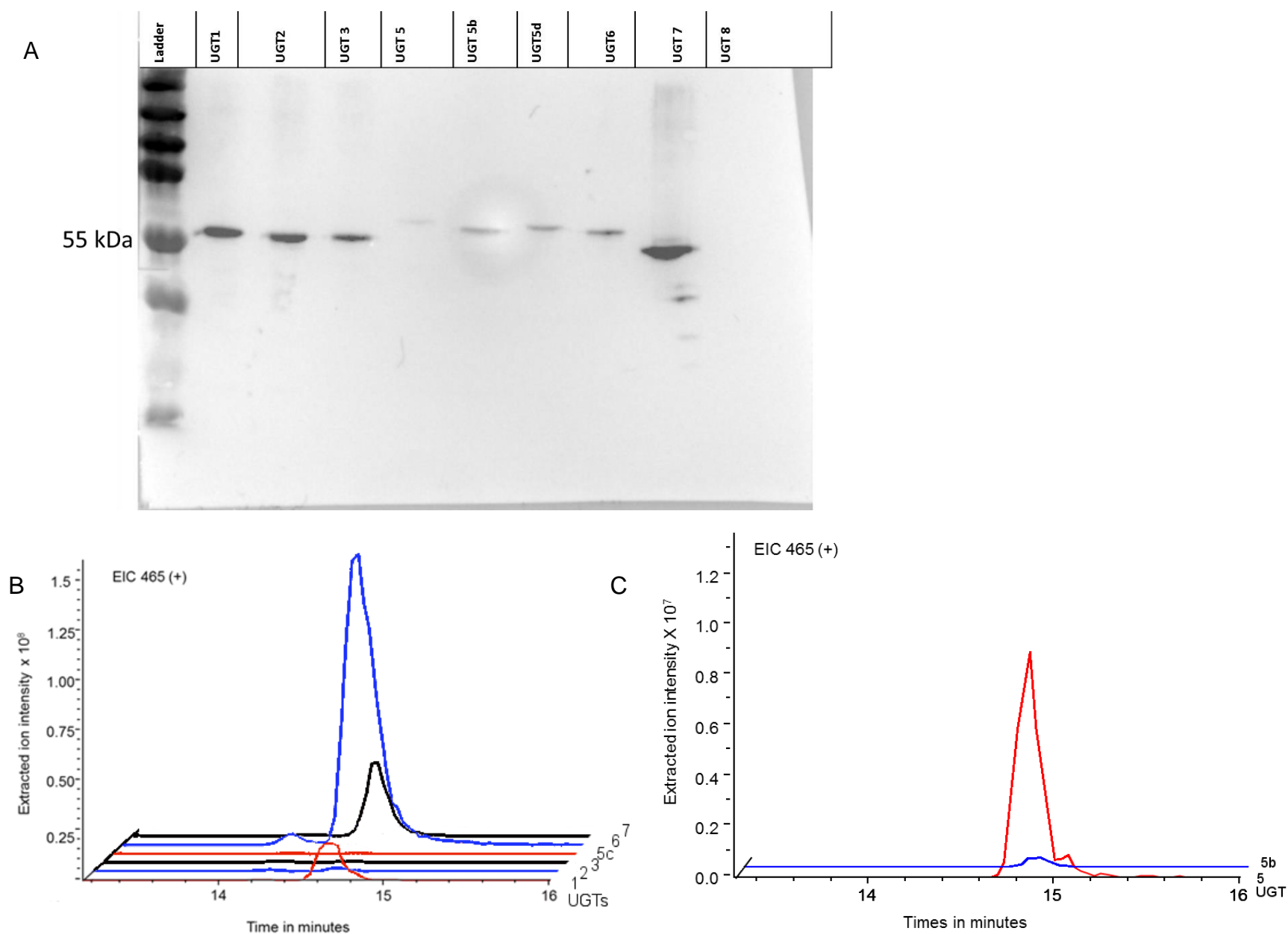
PgUGT5  DT* 492
PgUGT5b  --- 485
PgUGT5c  HI 491

```

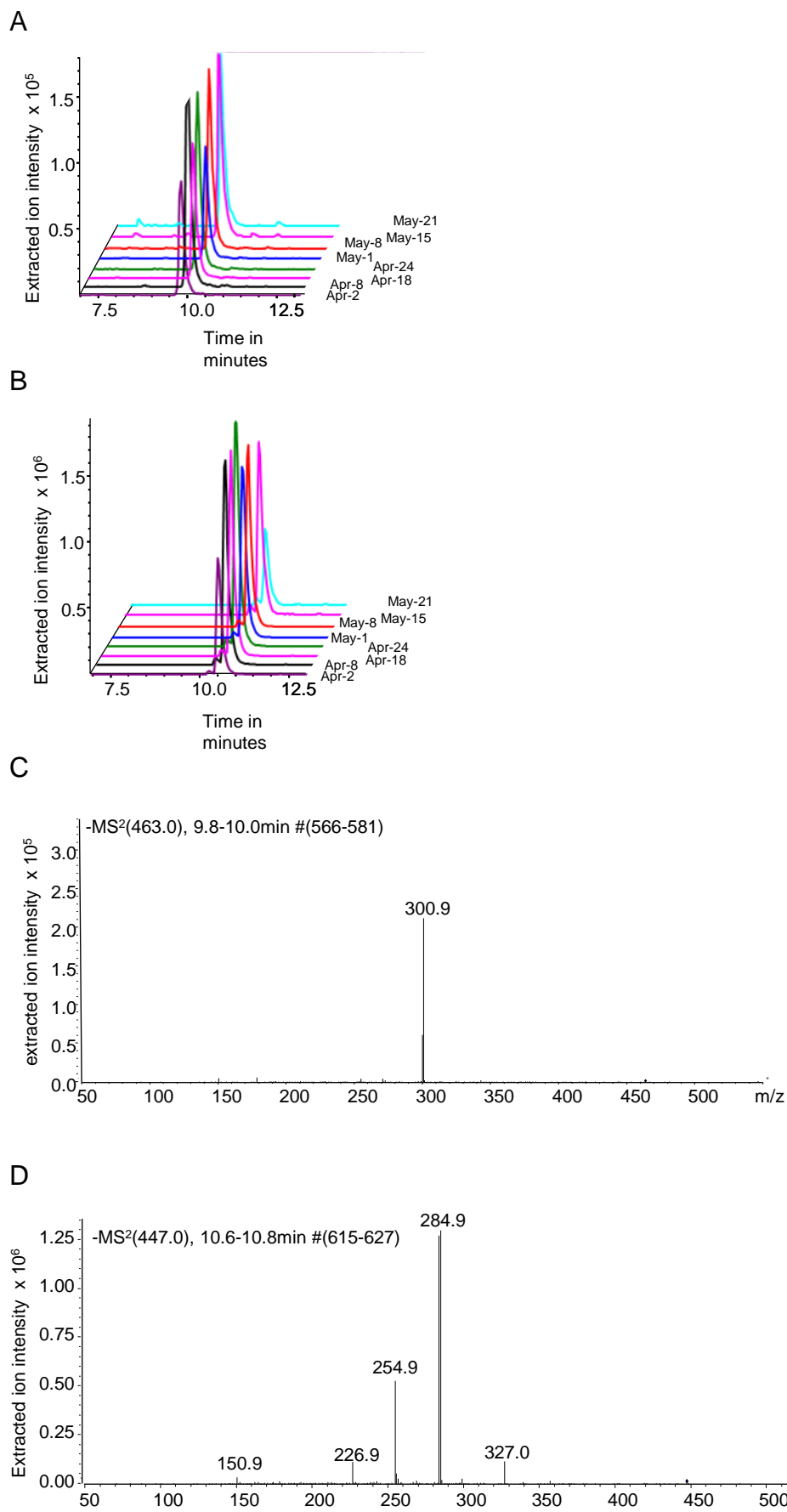
B

		1	2	3
PgUGT5	1		76.7	75.0
PgUGT5b	2	69.6		82.5
PgUGT5d	3	66.6	77.9	

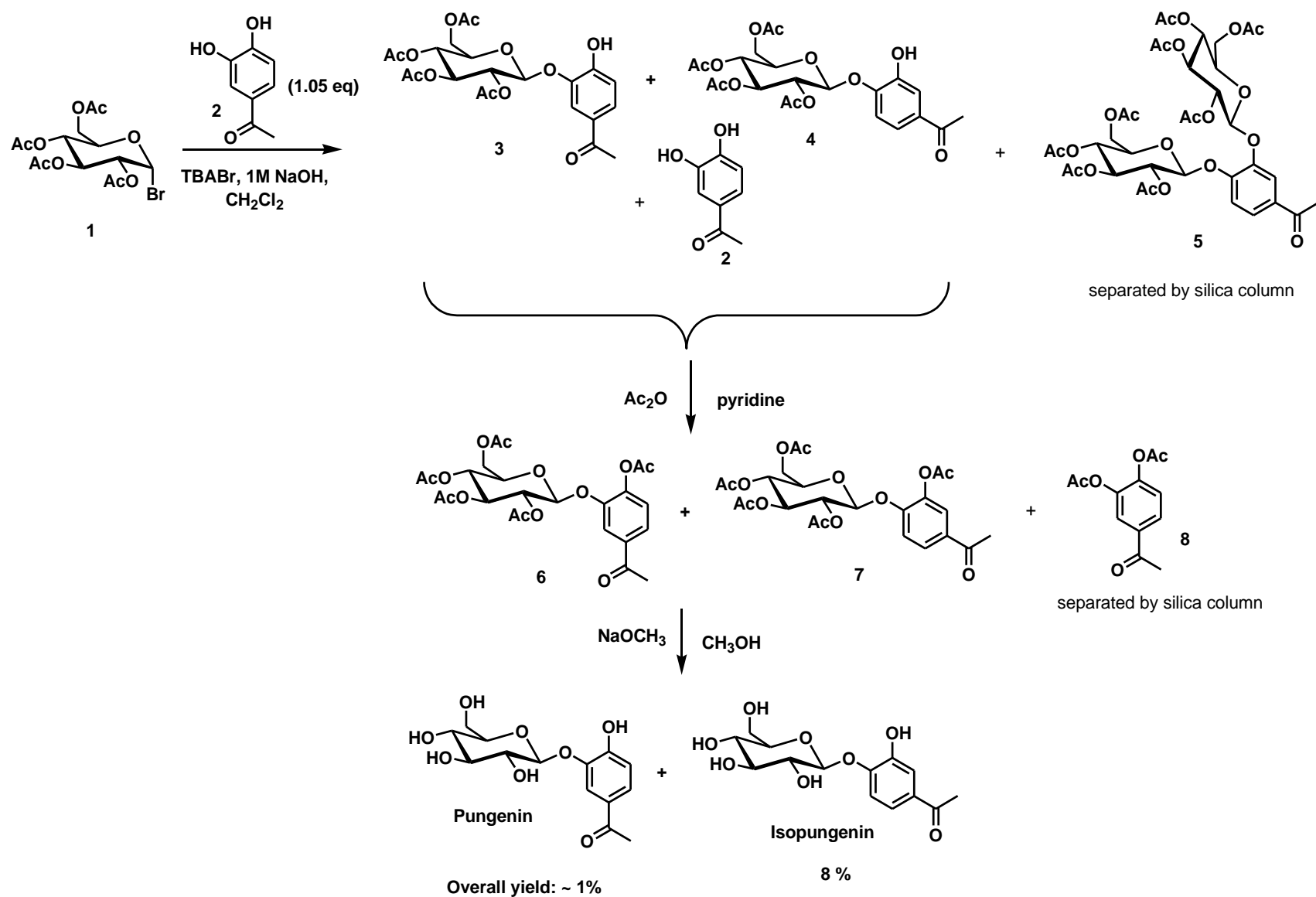
Supplemental Figure 2. Sequence comparison of PgUGT5, PgUGT5b, and PgUGT5c. A, Amino acid alignment of PgUGT5, PgUGT5b, and PgUGT5c. Matching residues are shown as dots. The highly conserved PSPG motif is highlighted by the gray bar. B, Pairwise comparison was used to determine the percent identity (blue) and the percent similarity (red) distance between two sequences.



Supplemental Figure 3. Expression and activity of PgUGT candidates on quercetin demonstrating activities of other UGT candidates. A, Purified histidine tagged-protein was analyzed by SDS-PAGE and Western blot for verification of candidate acetophenone PgUGTs. The estimated molecular weight for the PgUGT candidates was 55 kDa. No protein could be detected for PgUGT8. B, Purified PgUGTs were tested for activity with quercetin. The extracted ion chromatograms (+465) for the parent mass of quercetin glucoside show that candidate PgUGTs that do not have activity on pungenol are still functional glycosyltransferases, except for PgUGT8 which did not express in *E. coli*. C, The extracted ion chromatograms (+465) for the parent mass of quercetin glucoside show the activity of PgUGTs 5 and 5b with the substrate quercetin.



Supplemental Figure 4. Flavonoid glycoside presence over the time course of early shoot development does not follow the same pattern as acetophenones or *PgUGT5b* expression. Tissue was collected over the time course of shoot development beginning with bud burst until full shoot expansion. Metabolites were extracted with methanol and analyzed by LC-MS. A, Quercetin glucoside was detected at all time points sampled (EIC -463). B, Kaempferol glucoside was also detected at all time points sampled (EIC -447). C, MS² of quercetin glucoside showing daughter ion for quercetin aglycon. D, MS² of kaempferol glucoside showing daughter ion for kaempferol aglycon.



Supplemental Figure 5. Schematic of isopungenin and pungenin chemical synthesis. (1) 2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl bromide (2) 3,4-dihydroxyacetophenone (3) acetylated pungenin (4) acetylated isopungenin (5) diglucosidated acetophenone (6) peracetylated pungenin (7) peracetylated isopungenin (8) 3,4-di-*O*-acetyl-3,4-dihydroxyacetophenone