Molecular Pharmacology
Supplemental Materials

Activation of Cryptic Donor Splice Sites Within the UGT1A First-Exon Region Generates Variant Transcripts That Encode UGT1A Proteins With Truncated Aglycone-binding Domains

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Legends of Supplemental Figures

Supplemental Fig. 1. Sequences of variant UGT1A first exons. Shown are the sequences for eight variant UGT1A first exons that are generated using cryptic donor sites within the UGT1A first-exon region, including *1A1E1v* (A), *1A3E1v1* (B), *1A3E1v2* (C), *1A4E1v* (D), *1A5E1v* (E), *1A8E1v* (F), *1A9E1v* (G), *1A10E1v* (H). The nucleotide sequences of variant first exons (BLUE) are positioned at the right according to the human GRCh38/hg38 genome assembly. The start ATG codons and the dinucleotide GT splice signals of the novel cryptic donor splice sites are also indicated.

Supplemental Fig. 2. **Predicted sequences for variant UGT1A proteins**. Shown are the sequences for eight predicted UGT1A variant proteins, including 1A1_in1 (A), 1A3_in3 (B), 1A3_in4 (C), 1A4_in4 (D), 1A5_in1 (E), 1A8_in2 (F), 1A9_in2 (G), and 1A10_in7 (H). The sequences encoded by the first exons and exons 2-5 are indicated in BLUE and RED, respectively. 1A3_in3 has a novel 77-aa C-terminal peptide (GREEN) but lacks the sequence encoded by exons 2-5.

Supplemental Figure 3. Sequence reads for variant UGT1A transcripts. Shown are the sequence reads (100 nucleotides) for variant transcripts 1A4_n4 (A), 1A8_n2 (B) and 1A9_n2 (C) identified from the UGT-enriched CaptureSeq datasets (GSE80463) using transcript-specific probes and the Sequence Read Archive (SRA) platform. The transcript-specific splice junctions are indicated by a vertical RED line. The nucleotide positions of the 3' ends of the three variant first exons (1A4E1v, 1A8E1v, 1A9E1v) are also indicated.

Supplemental Fig. 4. **Expression of variant transcripts 1A8_n2 and 1A9_n2 in normal and drug-metabolizing tissues**. Using transcript-specific probes and the Sequence Read Archive (SRA) platform, the sequence reads of canonical (1A8_v1, 1A9_v1) and variant (1A8_n2, 1A9_n2) transcripts were identified in fifteen CaptureSeq samples (GSE80463) generated from normal and cancerous drug-metabolizing tissues as indicated. The number of sequence reads for each transcript was normalized using the number of total sequence reads in the same sample and then presented as the relative reads of this transcript per 10^9 reads of the total sequence reads. Shown are the expression level of 1A8_n2 (A), 1A9_n2 (C), and the expression ratio for 1A8_n2/1A8_v1 (B), or 1A9_n2/1A9_v1 (D) in fifteen CaptureSeq samples.

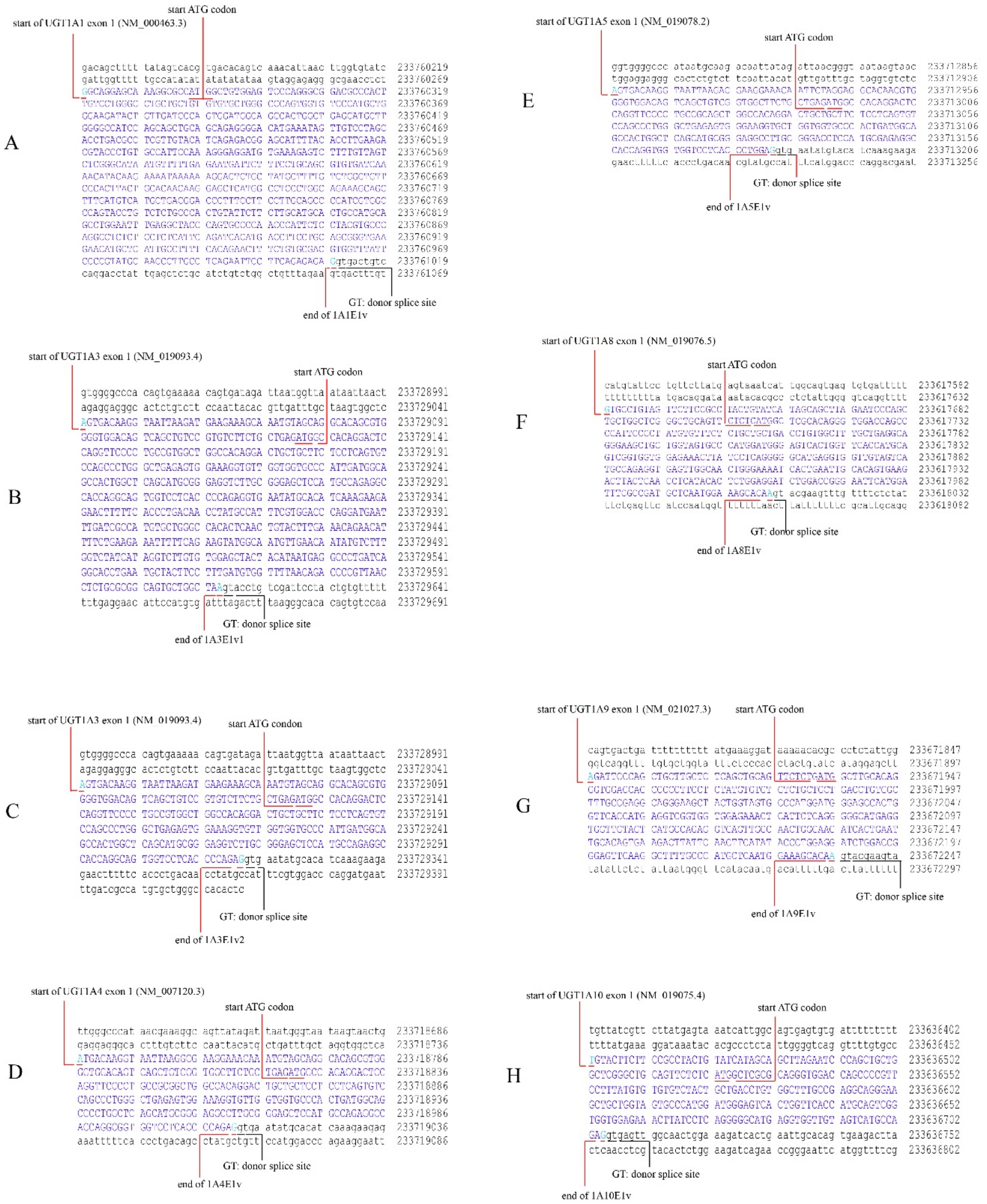
Supplemental Figure 5. Variant UGT1A transcripts and proteins. (A) RT-PCR was conducted using cDNA samples of colorectal cancer HT-29 cells and primers to clone the full coding sequence of canonical UGT1A8 mRNA (1A8_v1) or UGT1A10 mRNA (1A10_v1). The resultant amplicons were run on an ethidium-bromide-stained agarose gel and imaged using UV-illumination. (B) HEK293T cells were transfected with constructs expressing no UGT protein (control), wildtype (1A8_i1) and variant (1A8_i3) UGT1A8 proteins alone and in combination (1A8_i1 + 1A8_i3) as indicated. Lysates of transfected cells were subjected to standard Western blotting assays using a pan-UGT1A antibody and imaged using chemiluminescent agents as described in *Materials and Methods*. (C) Glucuronidation of HEK293T lysates transfected with vectors expression 1A8_i1 or 1A8_i3 alone and in combination were conducted using HPLC assays. The activity of 4MU glucuronidation was normalized to the band intensities of western blots obtained using equal amounts of HEK293T lysates of the same samples used for 4MU activity assays as described in *Materials and Methods*. Shown is the mean plus SD of 4MU-glucuronidation activity of 1A8_i1/_i3-transfected cells normalized to that of 1A8_i1-transfected cells (set as a value of 100%) from three independent experiments. Student’s t-test, $p < 0.05$ is considered statistically significant.

Supplemental Fig. 6. **Sequencing results identified the 1A8_n2 transcript in HT-29 cells**. The 1A8_n2 transcript has a variant first exon (*1A8E1v*) and common UGT1A exons 2-5. Shown are the sequencing chromatograms of a cloned pEF_IRESpuro6 construct from the HT-29 cell line that contains the 1A8_n2 transcript-specific splice junction (1A8E1v/1AE2) and all other four UGT1A common splice junctions [1AE2/1AE3, 1AE3/1AE4, 1AE4/1AE5]. All splice junctions are indicated by a vertical line. nt: nucleotide; AA: amino acid.

Supplemental Fig. 7. **Sequencing results identified the 1A10_n7 transcript in HT-29 cells**. The 1A10_n7 transcript has a variant first exon (*1A10E1v*) and common UGT1A exons 2-5. Shown are the sequencing chromatograms of a cloned pEF_IRESpuro6 construct from the HT-29 cell line that contain the 1A10_n7 transcript-specific splice junction (1A10E1v/1AE2) and all UGT1A common splice junctions [1AE2/1AE3, 1AE3/1AE4, 1AE4/1AE5]. All splice junctions are indicated by a vertical line. nt: nucleotide; AA: amino acid

Supplemental Fig. 8. **Variant transcripts UGT1A8_n2 and UGT1A10_n7 generated using novel cryptic donor splice sites within UGT1A first exons in colorectal cancer HT-29 cells**. (A) Shown are the exon structures of the UGT1A8 pre-mRNA (Aa), mRNA (1A8_v1) (Ab), and variant transcript (1A8_n2). Pre-mRNA splicing using the 1A8 canonical and cryptic donor splice sites generates 1A8 mRNA (1A8_v1) (Ab) and variant 1A8_n2 (Ac), respectively. (B)

Shown are the exon structures of the UGT1A10 pre-mRNA (Ba), mRNA (1A10_v1) (Bb), and variant transcript (1A10_n7). Pre-mRNA splicing using the 1A10 canonical and cryptic donor splice sites generates 1A10 mRNA (1A10_v1) (Bb) and variant 1A10_n7 (Bc), respectively. The sequencing results covering the 1A8_n2 (Ad) or 1A10_n7 (Bd) novel splice junction are also shown. The donor and acceptor splice signal dinucleotides are indicated GT and AG, respectively.



Supplemental Fig. 1

A

Protein predicted from transcript 1A1_n1 (487 aa)

```
MAVESQGGRPLVLGLLLCVLGPVVSHAGKILLIPVDGSHWLSMLGAIQQLQQRGHEIVVLAPDASLYIRDGAFYTLKTYPVPFQREDVKE
SFVSLGHNVFENDSFLQRVIKTYKKIKKDSAMLLSGCSHLLHNKELMASLAESSFDVMLTDPFLPCSPIVAQYLSLPTVFFLHALPCSLE
FEATQCPNPFSYVPRPLSSHSDHMTFLQRVKNMLIAFSQNFLCDVVYSPYATLASEFLQREEFEAYINASGEHGIVVFSLGSMVSEIPEK
KAMAIADALGKIPQTVLWRYTGTRPSNLANNTILVKWLPQNDLLGHPMTRAFITHAGSHGVYESICNGVPMVMMPLFGDQMDNAKRMETK
GAGVTLNVLEMTSEDLENALKAVINDKSYKENIMRLSSLHKDRPVEPLDLAVFWVEFVMRHKGAPHLRPAAHDLTWYQYHSLDVIGFLLA
VVLTVAFITFKCCAYGYRKCLGKKGRVKKAHKSKTH
```

B

Protein predicted from transcript 1A3_n3 (239 aa)

```
MATGLQVPLPWLATGLLLLLSVQPWAESGKVLVVPIDGSHWLSMREVLRELHARGHQAVVLTPEVNMHIKEENFFTLTTYAISWTQDEFD
RHVLGHTQLYFETEHFLKKFFRSMAMLNNMSLVYHRSCVELLHNEALIRHLNATSFDVVLTDPVNLCAAVLAKNLKPTLMLLENMELWFS
LWDQWSQKFQRRKLWQLLMLWAKSLRQSCGGTLEPDHRILRTTRYLLSGYPKTICLVTR
```

C

Predicted protein from transcript 1A3_n4 (309 aa)

```
MATGLQVPLPWLATGLLLLLSVQPWAESGKVLVVPIDGSHWLSMREVLRELHARGHQAVVLTPEEFEAYINASGEHGIVVFSLGSMVSEI
PEKKAMAIADALGKIPQTVLWRYTGTRPSNLANNTILVKWLPQNDLLGHPMTRAFITHAGSHGVYESICNGVPMVMMPLFGDQMDNAKRM
ETKGAGVTLNVLEMTSEDLENALKAVINDKSYKENIMRLSSLHKDRPVEPLDLAVFWVEFVMRHKGAPHLRPAAHDLTWYQYHSLDVIGF
LLAVVLTVAFITFKCCAYGYRKCLGKKGRVKKAHKSKTH
```

D

Predicted protein from transcript 1A4_n4 (309 aa)

```
 MARGLQVPLPRLATGLLLLLSVQPWAESGKVLVVPTDGSPWLSMREALRELHARGHQAVVLTPEEFEAYINASGEHGIVVFSLGSMVSEI
PEKKAMAIADALGKIPQTVLWRYTGTRPSNLANNTILVKWLPQNDLLGHPMTRAFITHAGSHGVYESICNGVPMVMMPLFGDQMDNAKRM
ETKGAGVTLNVLEMTSEDLENALKAVINDKSYKENIMRLSSLHKDRPVEPLDLAVFWVEFVMRHKGAPHLRPAAHDLTWYQYHSLDVIGF
LLAVVLTVAFITFKCCAYGYRKCLGKKGRVKKAHKSKTH
```

E

Predicted protein from transcript 1A5_n1 (309 aa)

```
MATGLQVPLPQLATGLLLLLSVQPWAESGKVLVVPTDGSHWLSMREALRDLHARGHQVVVLTLEEFEAYINASGEHGIVVFSLGSMVSEI
PEKKAMAIADALGKIPQTVLWRYTGTRPSNLANNTILVKWLPQNDLLGHPMTRAFITHAGSHGVYESICNGVPMVMMPLFGDQMDNAKRM
ETKGAGVTLNVLEMTSEDLENALKAVINDKSYKENIMRLSSLHKDRPVEPLDLAVFWVEFVMRHKGAPHLRPAAHDLTWYQYHSLDVIGF
LLAVVLTVAFITFKCCAYGYRKCLGKKGRVKKAHKSKTH
```

F

Predicted proetin (termed 1A8_i3)from transcript 1A8_n2 (346 aa)

```
MARTGWTSPIPLCVSLLLTCGFAEAGKLLVVPMDGSHWFTMQSVVEKLILRGHEVVVVMPEVSWQLGKSLNCTVKTYSTSYTLEDLDREF
MDFADAQWKAQEFEAYINASGEHGIVVFSLGSMVSEIPEKKAMAIADALGKIPQTVLWRYTGTRPSNLANNTILVKWLPQNDLLGHPMTR
AFITHAGSHGVYESICNGVPMVMMPLFGDQMDNAKRMETKGAGVTLNVLEMTSEDLENALKAVINDKSYKENIMRLSSLHKDRPVEPLDL
AVFWVEFVMRHKGAPHLRPAAHDLTWYQYHSLDVIGFLLAVVLTVAFITFKCCAYGYRKCLGKKGRVKKAHKSKTH
```

G

Predicted protein from transacript 1A9_n2 (346 aa)

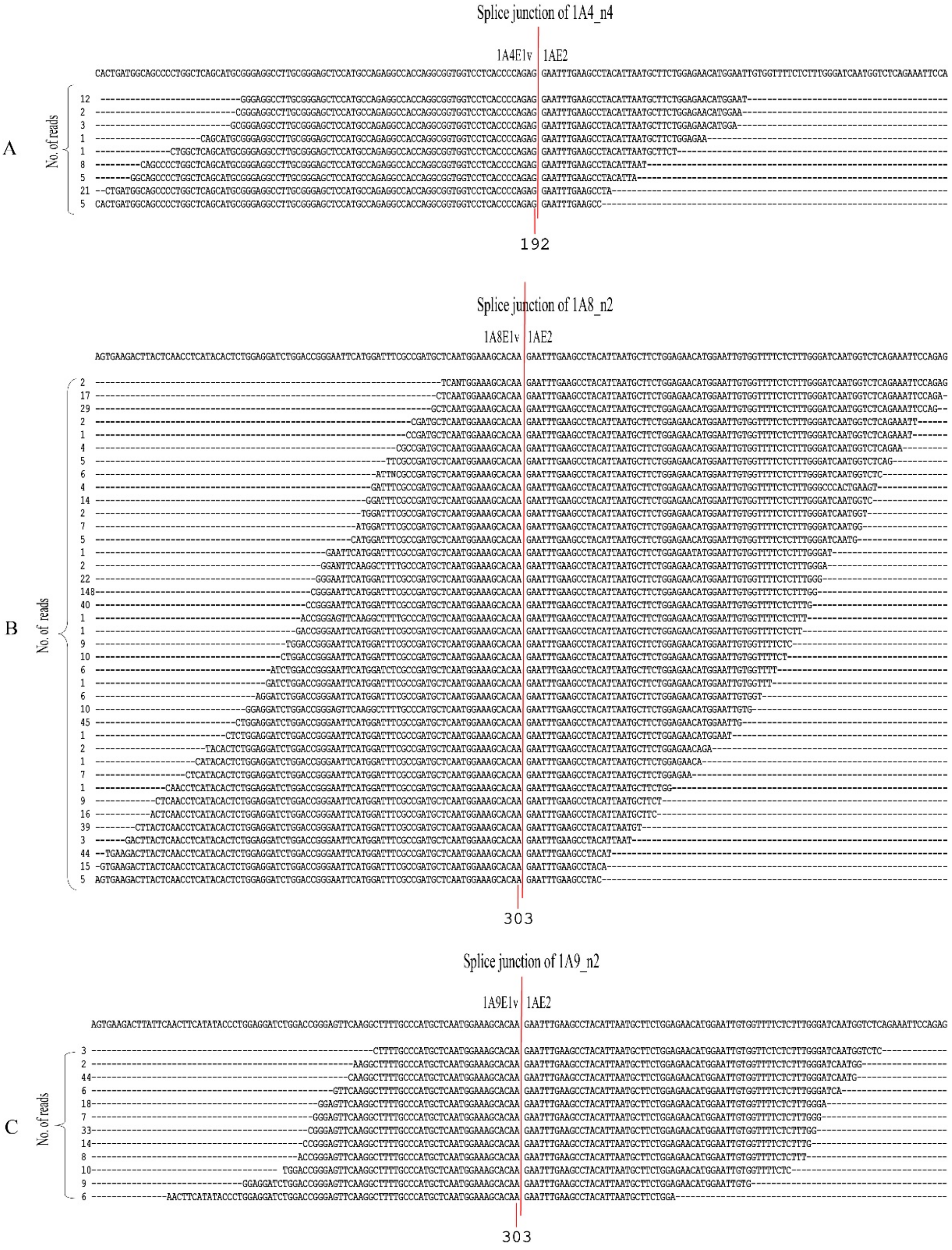
```
MACTGWTSPLPLCVCLLLTCGFAEAGKLLVVPMDGSHWFTMRSVVEKLILRGHEVVVVMPEVSWQLGRSLNCTVKTYSTSYTLEDLDREF
KAFAHAQWKAQEFEAYINASGEHGIVVFSLGSMVSEIPEKKAMAIADALGKIPQTVLWRYTGTRPSNLANNTILVKWLPQNDLLGHPMTR
AFITHAGSHGVYESICNGVPMVMMPLFGDQMDNAKRMETKGAGVTLNVLEMTSEDLENALKAVINDKSYKENIMRLSSLHKDRPVEPLDL
AVFWVEFVMRHKGAPHLRPAAHDLTWYQYHSLDVIGFLLAVVLTVAFITFKCCAYGYRKCLGKKGRVKKAHKSKTH
```

H

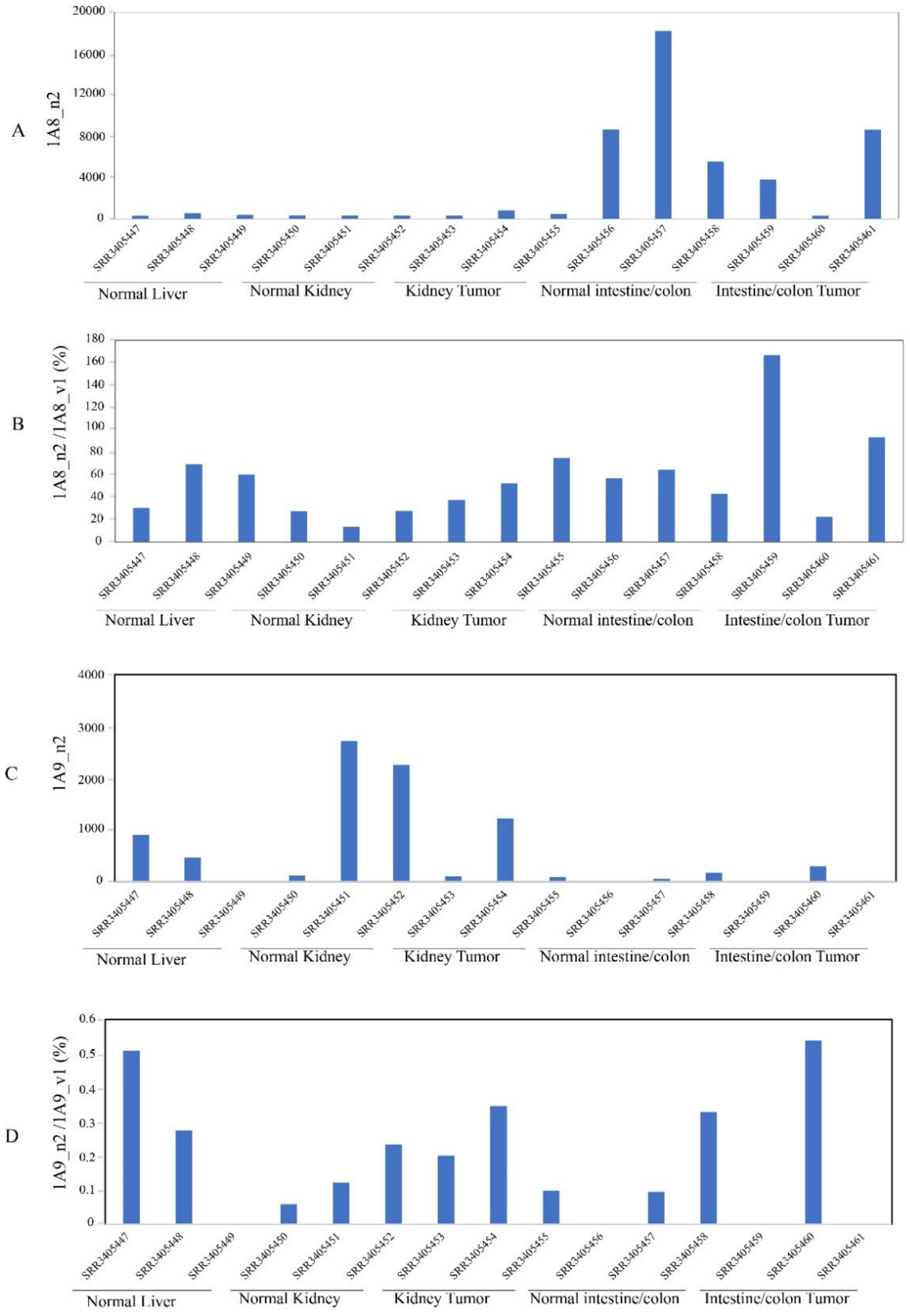
Predicted protein(termed 1A10_i3)from transcript 1A10_n7(306 aa)

```
MARAGWTSPVPLCVCLLLTCGFAEAGKLLVVPMDGSHWFTMQSVVEKLILRGHEVVVVMPEEFEAYINASGEHGIVVFSLGSMVSEIPEK
KAMAIADALGKIPQTVLWRYTGTRPSNLANNTILVKWLPQNDLLGHPMTRAFITHAGSHGVYESICNGVPMVMMPLFGDQMDNAKRMETK
GAGVTLNVLEMTSEDLENALKAVINDKSYKENIMRLSSLHKDRPVEPLDLAVFWVEFVMRHKGAPHLRPAAHDLTWYQYHSLDVIGFLLA
VVLTVAFITFKCCAYGYRKCLGKKGRVKKAHKSKTH
```

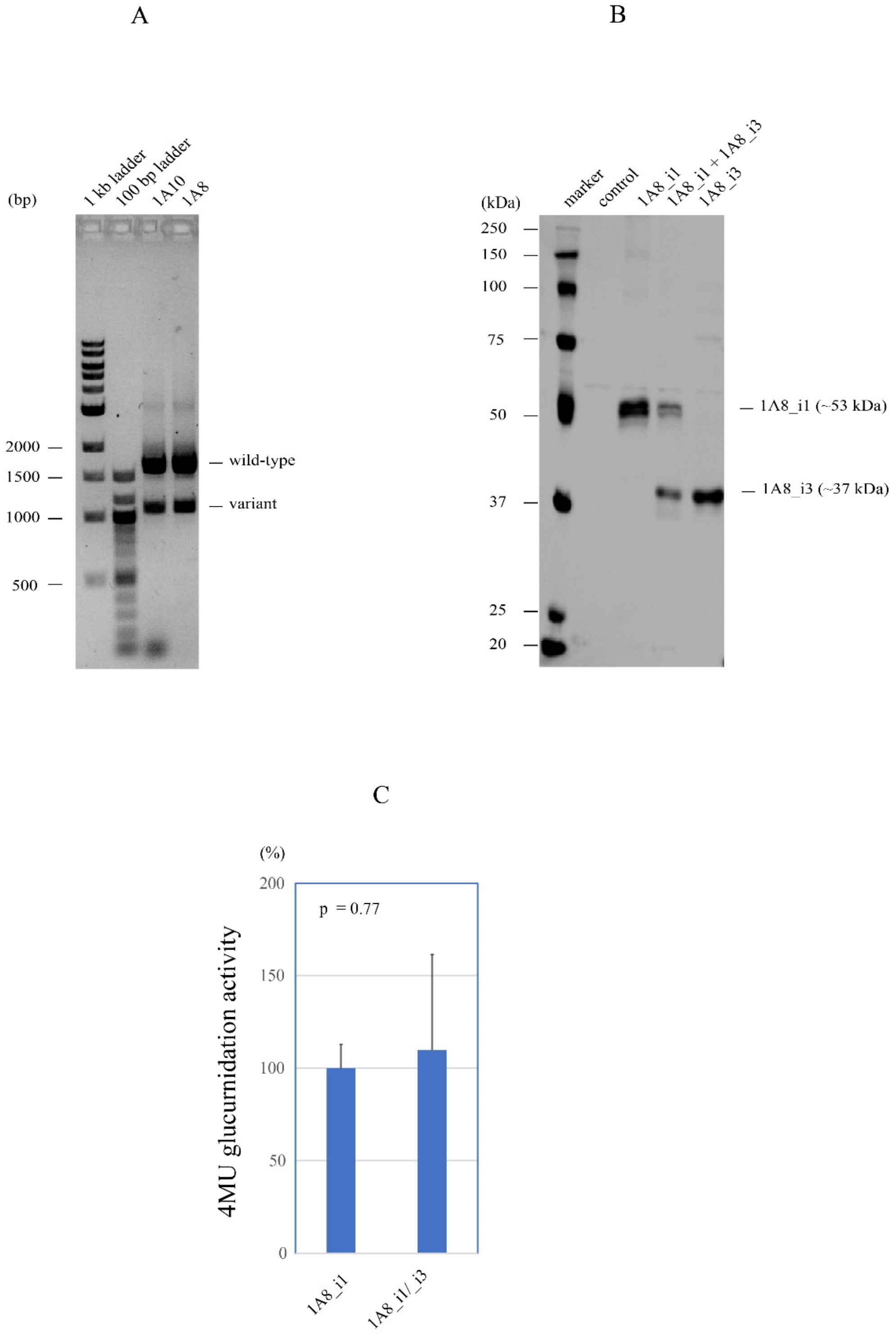
Supplemental Fig. 2



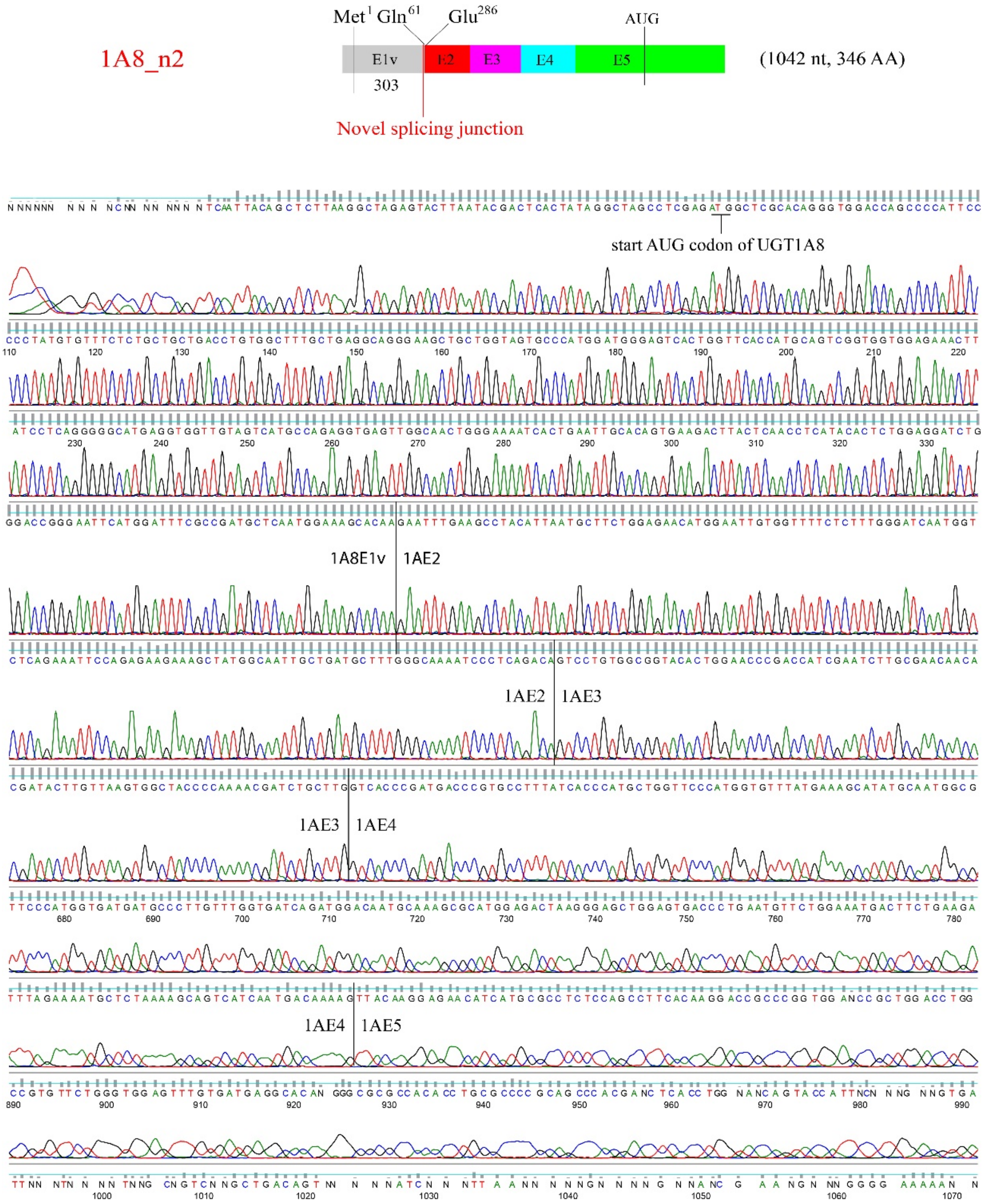
Supplemental Fig. 3.



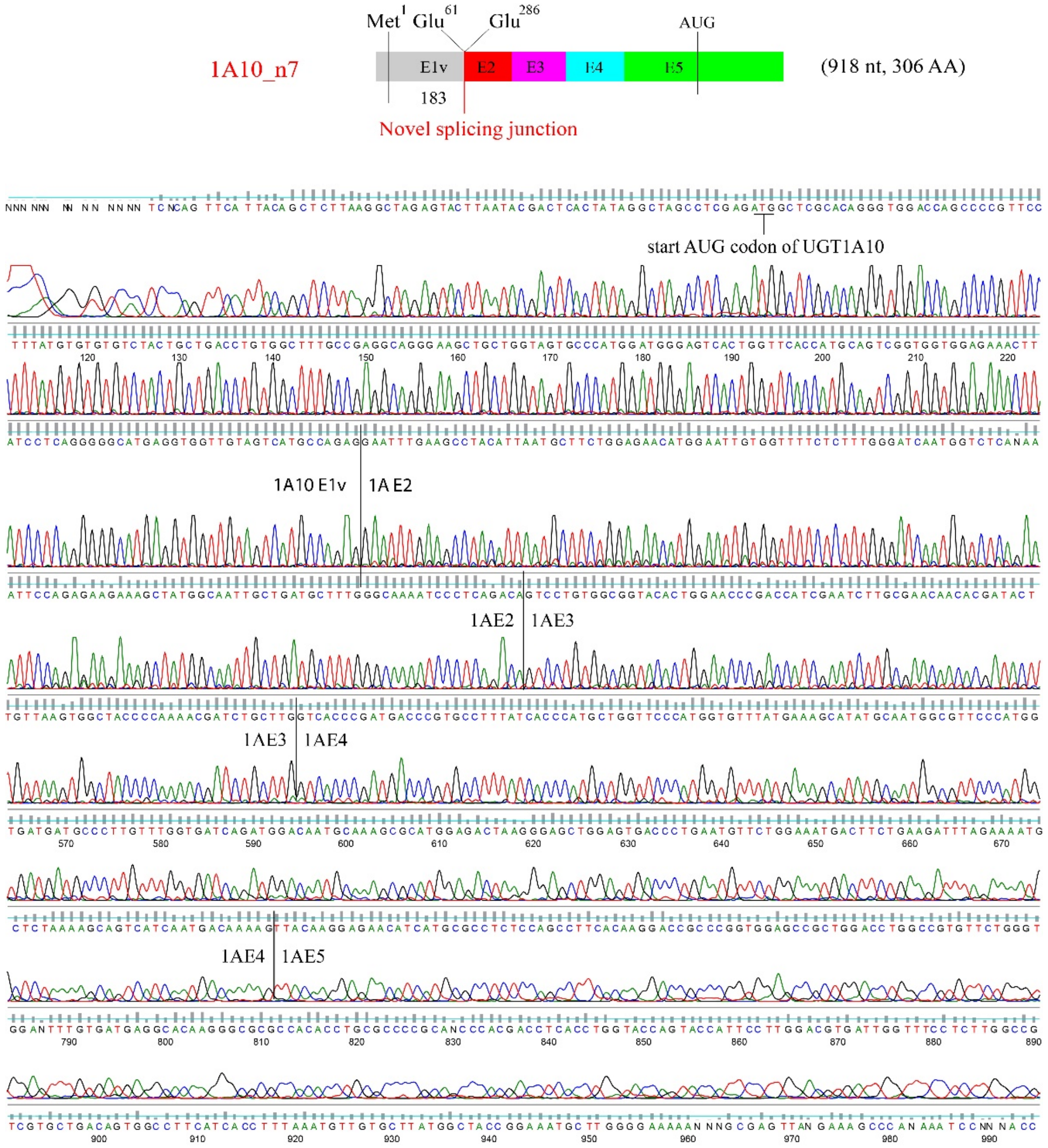
Supplemental Fig. 4.



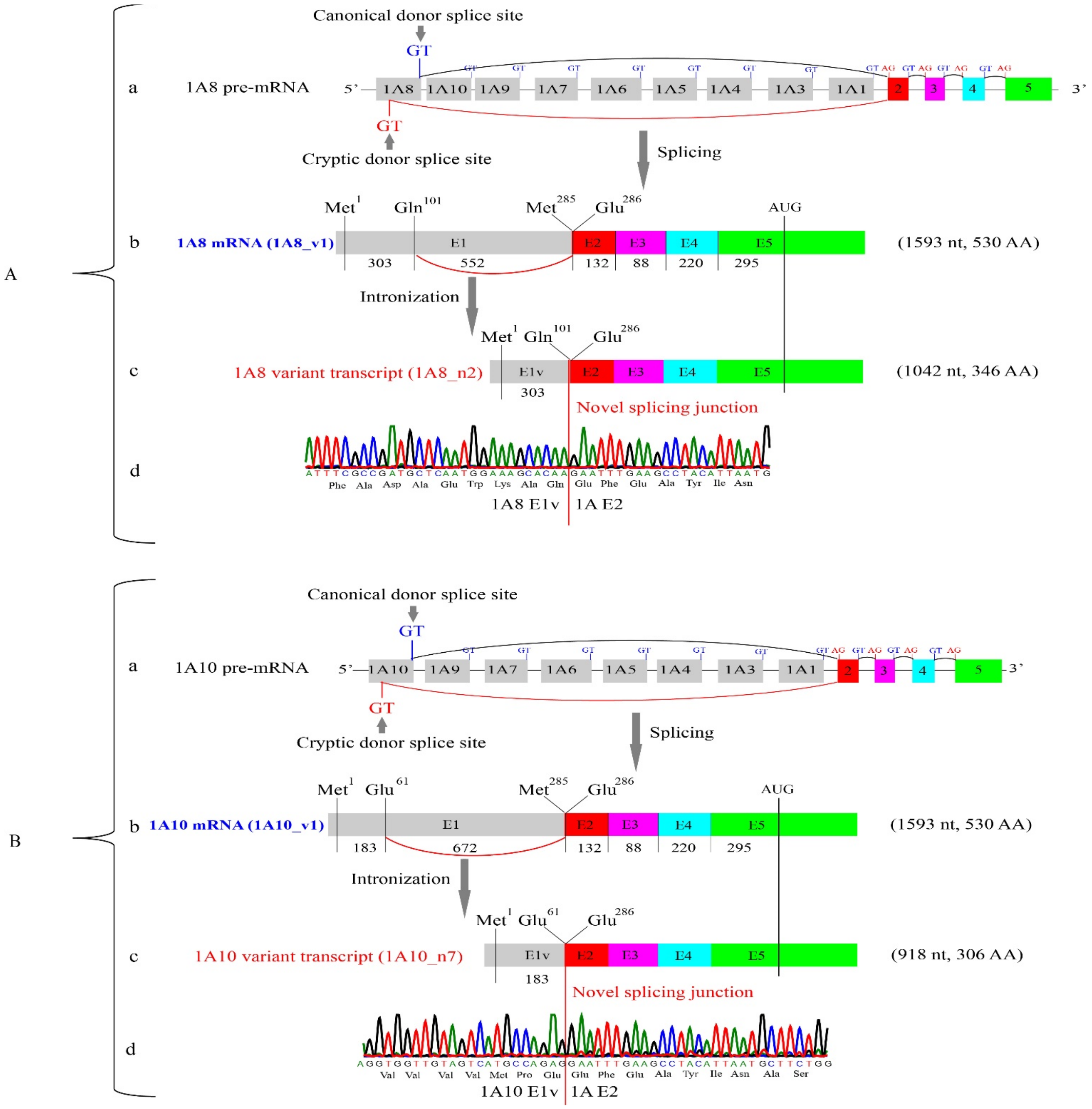
Supplemental Fig. 5



Supplemental Fig. 6



Supplemental Fig. 7



Supplemental Fig. 8

Supplemental Table 7: Known UGT1A transcripts and novel UGT1A variant transcripts identified in this study that are named using the current UGT1A nomenclature (Tourancheau A et al 2016)

	NCBI RefSeq (mRNAs)	NCBI RefSeq (proteins)	Experimental validation of transcript expression					
			Predicted proteins (aa)	RT-PCR	Cloning	CaptureSeq with UGT-enrichment	RNAseq without UGT enrichment	Western blotting
UGT1A1								
UGT1A1_v1	NM_000463.3	NP_000454 (533 aa)		√	√	√	√	√
UGT1A1_v2				√	√	√	√	√
UGT1A1_v3				√	√	√	√	√
UGT1A1_n1*			487			√	√	
UGT1A1_n2						√		
UGT1A1_n3						√		
UGT1A2P								
UGT1A2P_n1						√		
UGT1A2P_n2						√		
UGT1A2P_n3						√		
UGT1A2P_n4						√		
UGT1A2P_n5						√		
UGT1A2P_n6						√		
UGT1A2P_n7						√		
UGT1A2P_n8						√		
UGT1A2P_n9						√		
UGT1A2P_n10							√	
UGT1A2P_n11							√	
UGT1A3								
UGT1A3_v1	NM_019093.4	NP_061966 (534 aa)		√	√	√		√
UGT1A3_v2				√	√	√		√
UGT1A3_v3				√	√	√		√
UGT1A3_n1						√		
UGT1A3_n2						√		
UGT1A3_n3			239				√	
UGT1A3_n4			309				√	
UGT1A4								
UGT1A4_v1	NM_007120.3	NP_009051 (534 aa)		√	√	√		√
UGT1A4_v2				√	√	√		√
UGT1A4_v3				√	√	√		√
UGT1A4_n1						√		
UGT1A4_n2						√		
UGT1A4_n3						√		
UGT1A4_n4			309				√	
UGT1A5								
UGT1A5_v1	NM_019078.2	NP_061951 (534 aa)		√	√	√		√
UGT1A5_v2				√	√	√		√
UGT1A5_v3				√	√	√		√
UGT1A5_n1			309				√	
UGT1A6								
UGT1A6_v1	NM_001072.4	NP_001063 (532 aa)		√	√	√		√
UGT1A6_v2				√	√	√		√
UGT1A6_v3				√	√	√		√
UGT1A6_n1						√		
UGT1A6_n2						√		
UGT1A6_n3						√		
UGT1A6_n4						√		
UGT1A7								
UGT1A7_v1	NM_019077.3	NP_061950 (530 aa)		√	√	√		√
UGT1A7_v2				√	√	√		√
UGT1A7_v3				√	√	√		√
UGT1A7_n1						√		

UGT1A9								
UGT1A9_v1	NM_021027.3	NP_066307 (530 aa)		√	√	√		√
UGT1A9_v2				√	√	√		√
UGT1A9_v3				√	√	√		√
UGT1A9_n1						√		
UGT1A9_n2			346				√	
UGT1A10								
UGT1A10_v1	NM_019075.4	NP_061948 (530 aa)		√	√	√		√
UGT1A10_v2				√	√	√		√
UGT1A10_v3				√	√	√		√
UGT1A10_n4						√		
UGT1A10_n5						√		
UGT1A10_n6						√		
UGT1A10_n7			306	√	√		√	
UGT1A8								
UGT1A8_v1	NM_019076.5	NP_061949 (530 aa)		√	√	√		√
UGT1A8_v2				√	√	√		√
UGT1A8_v3				√	√	√		√
UGT1A8_n1						√		
UGT1A8_n2			346	√	√		√	√
Other UGT1A								
UGT1A_n1						√		
UGT1A_n2						√		
UGT1A_n3						√		
UGT1A_n4						√		
UGT1A_n5						√		
UGT1A_n6						√		
UGT1A_n7						√		
UGT1A_n8						√		
UGT1A_n9							√	
UGT1A_n10							√	
UGT1A_n11							√	
UGT1A_n12							√	
UGT1A_n13							√	
UGT1A_n14							√	
UGT1A_n15							√	
UGT1A_n16							√	
UGT1A_n17							√	
UGT1A_n18							√	
UGT1A_n19							√	
UGT1A_n20							√	
UGT1A_n21							√	
UGT1A_n22							√	

UGT1A transcripts highlighted in bold are reported in the present study and all others are reported by Tourancheau et al 2016 and several other studies (e.g. Levesque E et al 2007 and Giard H et al 2007). Also listed are the evidence for the synthesis of these transcripts in human tissues and cell lines from one or multiple experimental approaches, such as RT-PCR, Cloning, CaptureSeq, RNA-seq and Western Blotting assays. * This variant was also previously described in Tourancheau et al 2016.