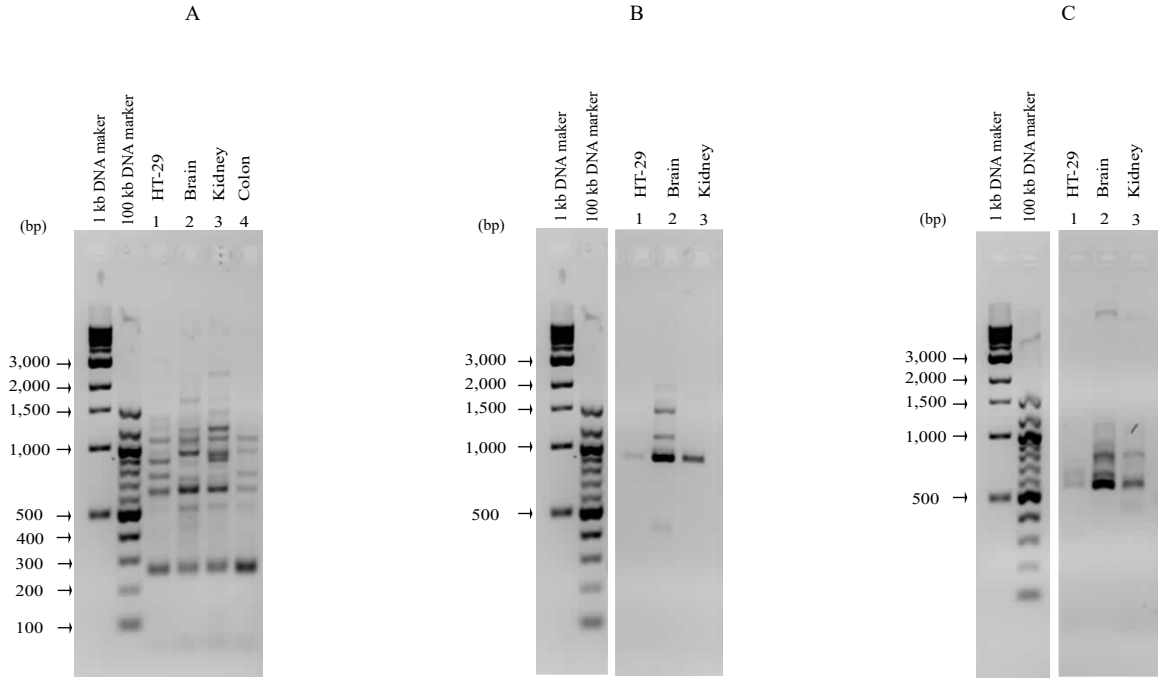
MOLPHARM-AR-000225

Molecular Pharmacology

Supplemental Materials

Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech



Supplemental Fig. 1

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Molecular Pharmacology
Supplemental Materials

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Supplemental Fig. 2

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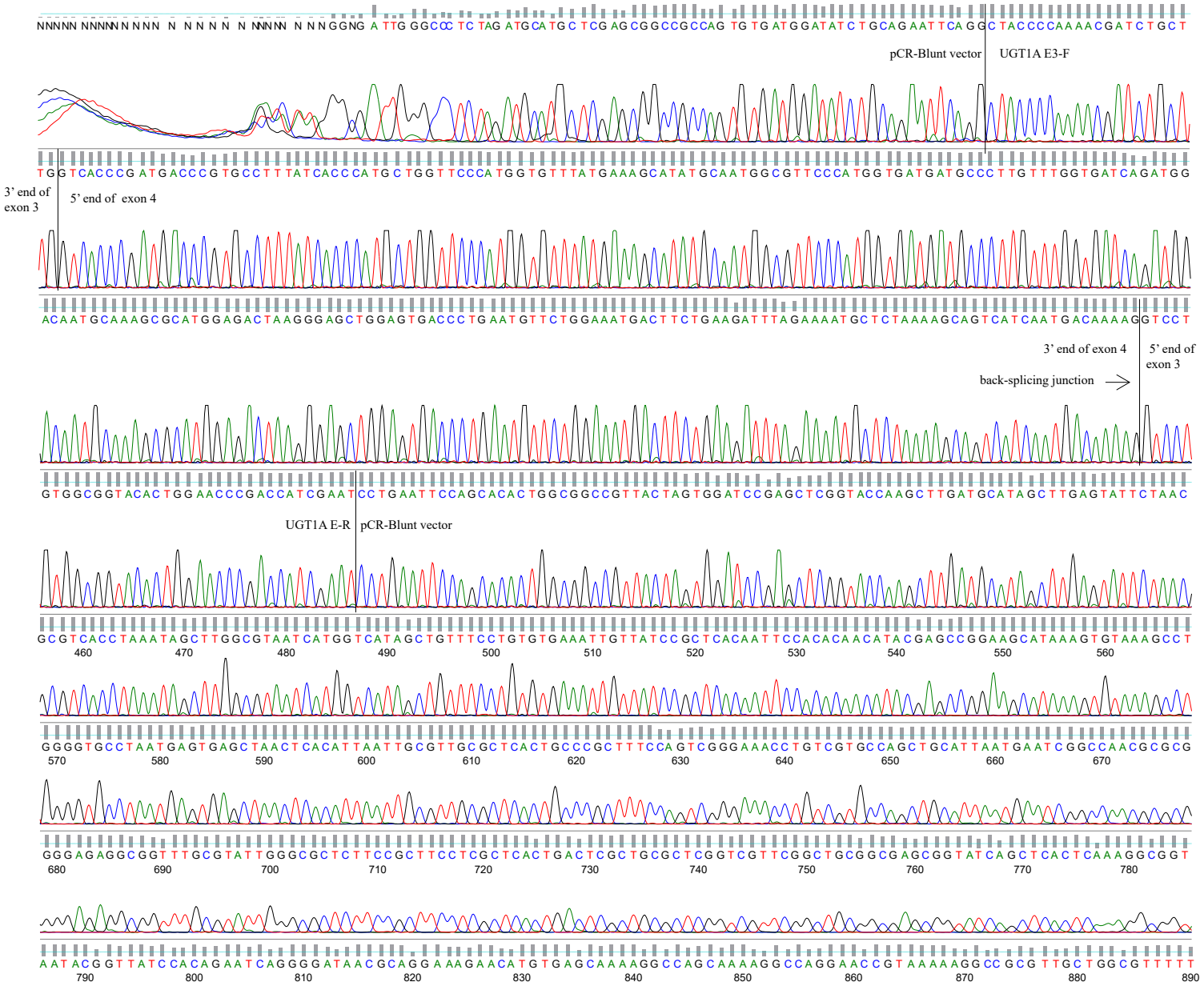

Supplemental Fig. 3

MOLPHARM-AR-000225
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Supplemental Materials

Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

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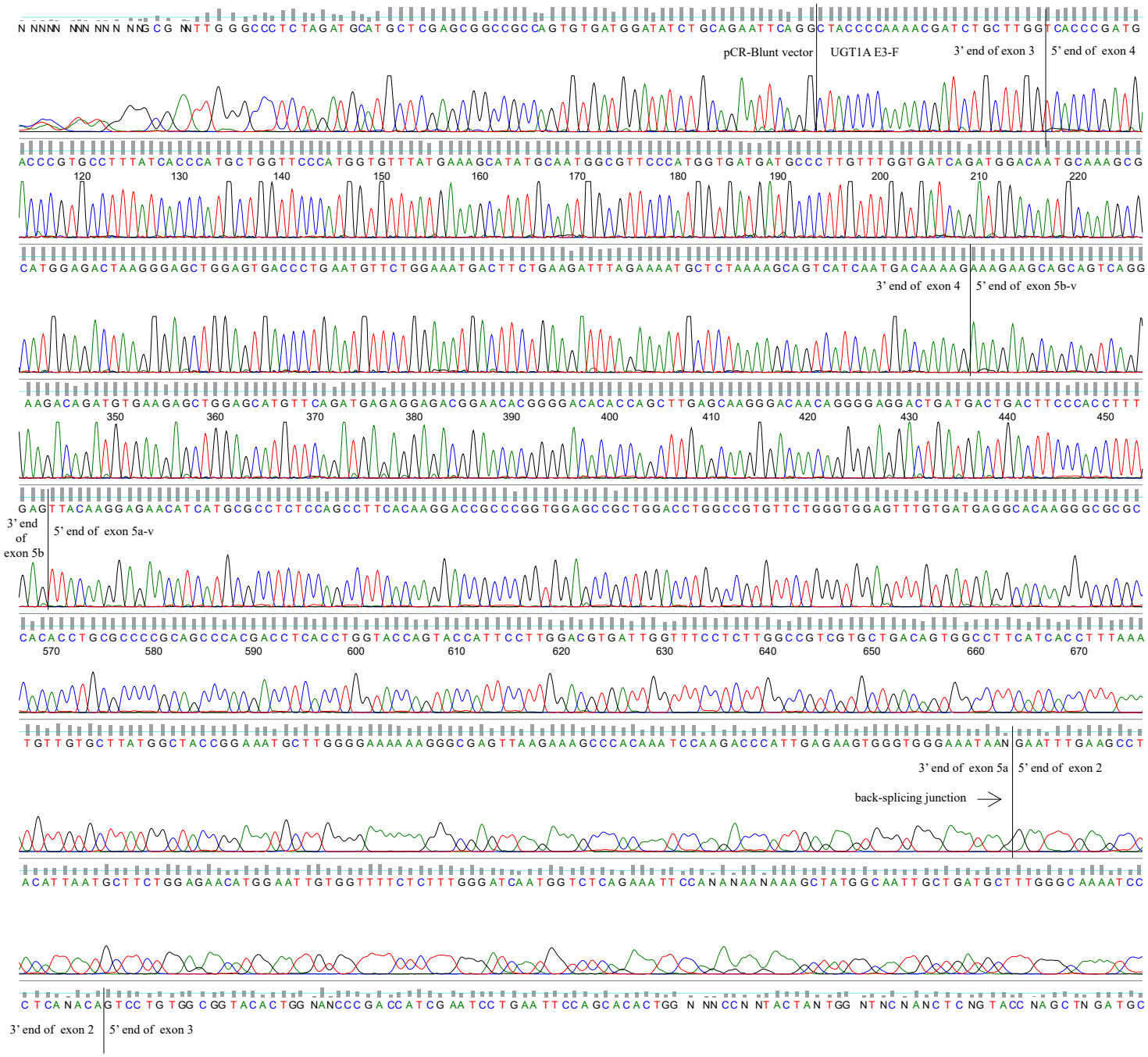

Supplemental Fig. 4

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Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

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Supplemental Fig. 5

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Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

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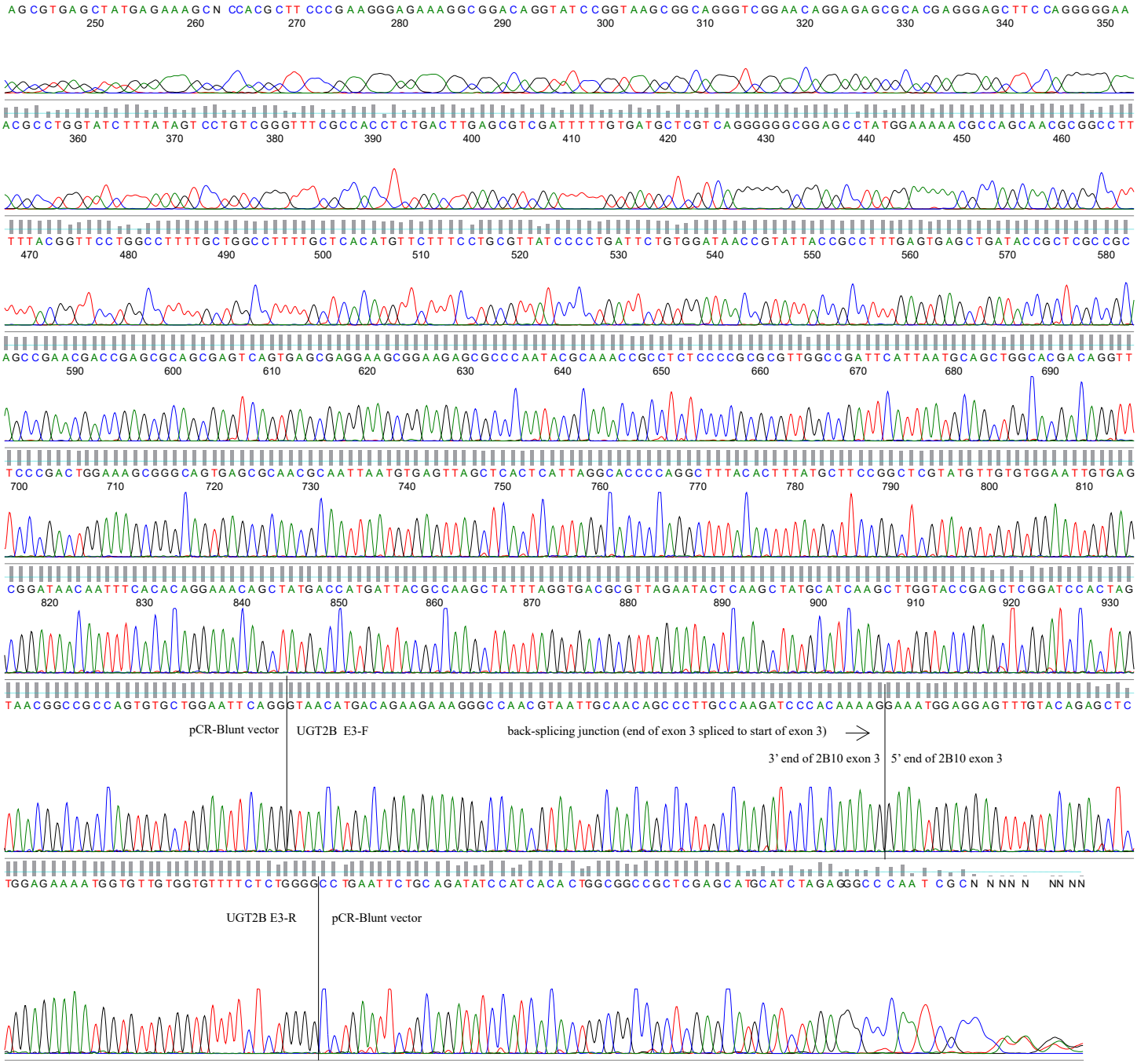

Supplemental Fig. 6

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Supplemental Materials

Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech

Supplemental Fig. 7

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Supplemental Materials

Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech

Supplemental Fig. 8

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Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech

Supplemental Fig. 9

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Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech

Supplemental Fig. 10

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Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech

Supplemental Fig. 11

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Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech

Supplemental Fig. 12

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Supplemental Materials

Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

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Supplemental Fig. 13

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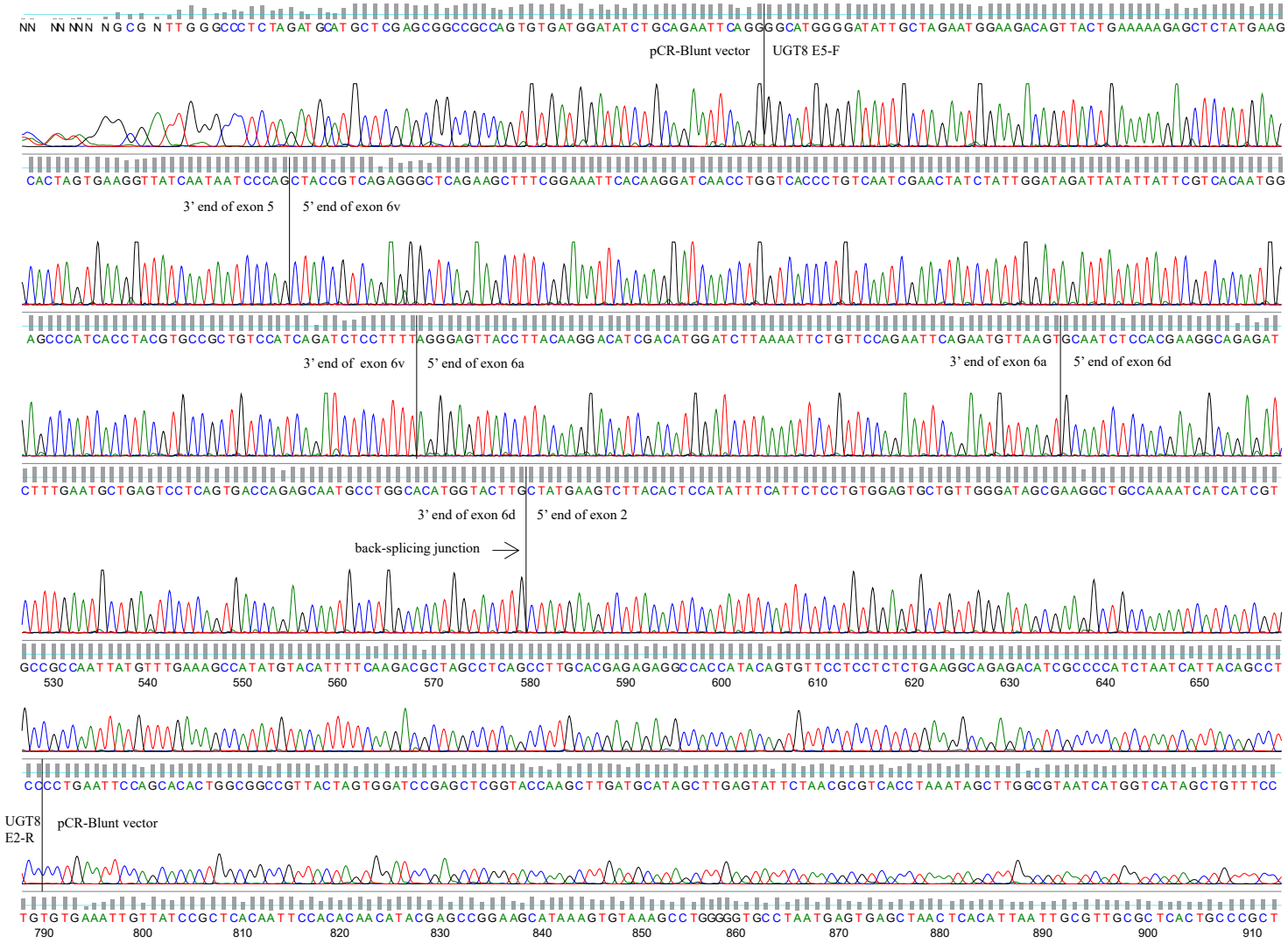
Supplemental Materials

Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech

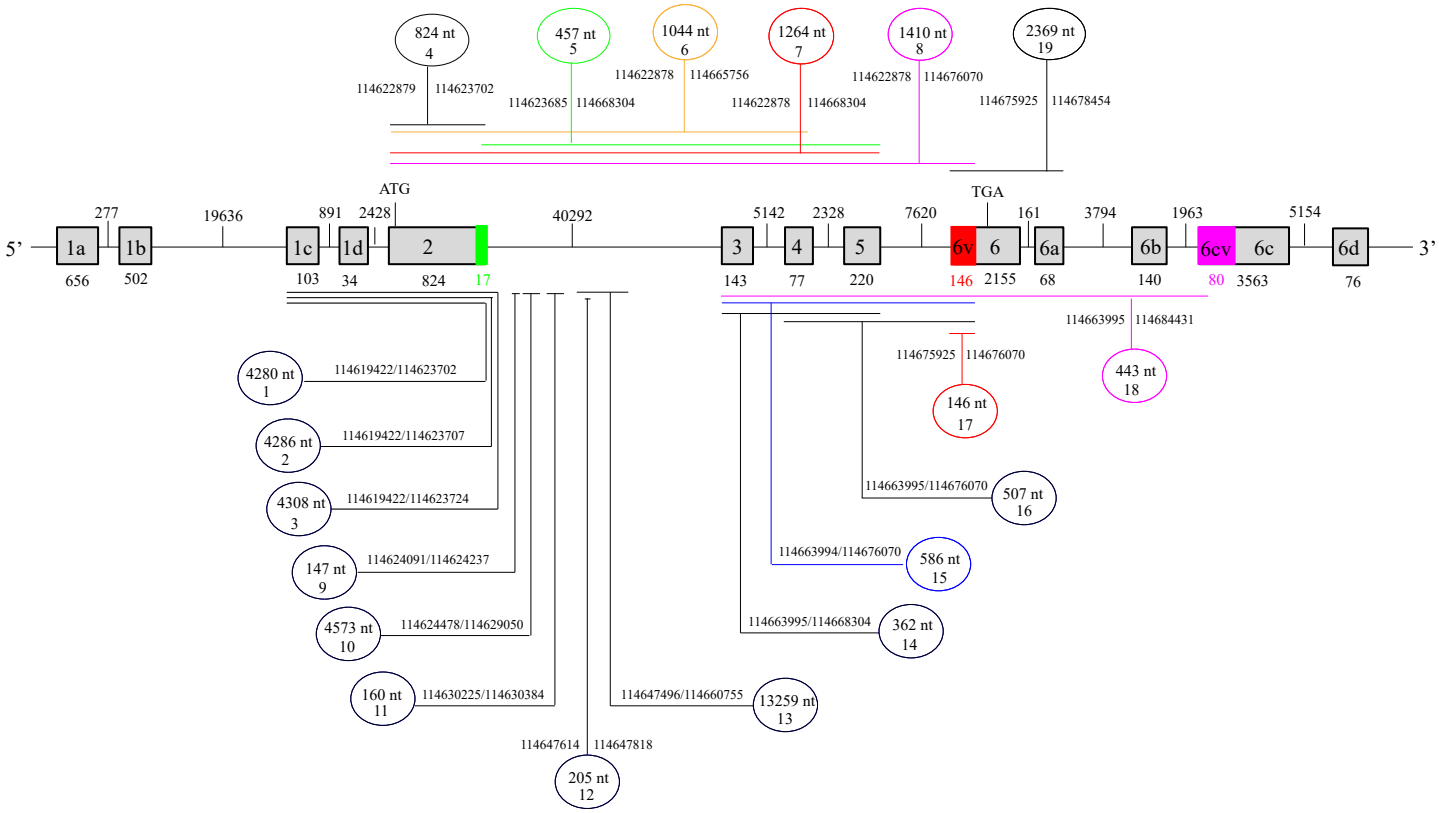


Supplemental Fig. 14

MOLPHARM-AR-000225
Molecular Pharmacology
Supplemental Materials

Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

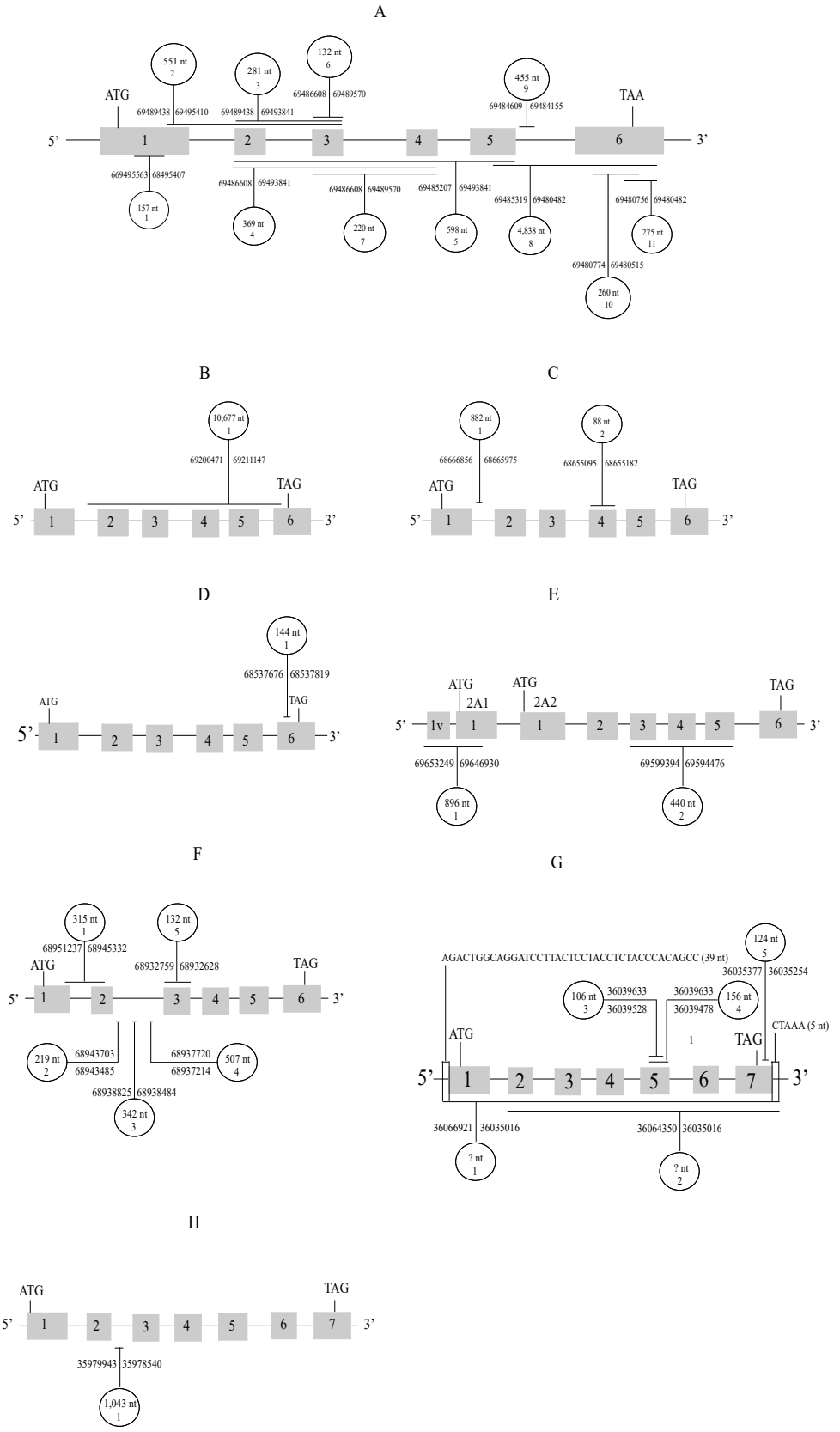
Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech



Supplemental Fig.15

Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech



Supplemental Fig. 16

Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech

A

start codon ATG of UGT1A6
donor splice site (Circ_UGT1A-4)
acceptor splice site (Circ_UGT1A-5)
acceptor splice site (Circ_UGT1A-4)
start of UGT1A6 exon 1

```
gctgacacgg ccatagttgg ttcatattaa ccatgtgatt aaaatggtta 233692839
aatattaatt tgggttctta catatcaaag ggtaaaattc agagcaaggg 233692889
AGAGGTAGAC AGGACCTGTG AAAAGCAGTG GTTAGTTTAG GGAAAATACC 233692939
TAGGAGCCCT GTGATTTGGA GAGTGAAAAC TCTTTATTAC CGTTGTTACT 233692989
TTAACTCTTT CCAGGATGGC CTGCCTCCTT CGCTCATTTC AGAGAATTTC 233693039
TGCAGGGGTT TTCTTCTTAG CACTTTGGGG CATGGTTGTA GGTGACAAGC 233693089
TGCTGGTGGT CCCTCAGGAC GGAAGCCACT GGCTTAGTAT GAAGGATATA 233693139
GTTGAGGTTC TCAGTGACCG GGGTCATGAG ATTGTAGTGG TGGTGCCTGA 233693189
AGTTAATTTG CTTTTGAAAG AATCCAAATA CTACACAAGA AAAATCTATC 233693239
CAGTGCCGTA TGACCAAGAA GAGCTGAAGA ACCGTTACCA ATCATTTGGA 233693289
AACAATCACT TTGCTGAGCG ATCATTCCTA ACTGCTCCTC AGACAGAGTA 233693339
CAGGAATAAC ATGATTGTTA TTGGCCTGTA CTTCATCAAC TGCCAGAGCC 233693389
TCCTGCAGGA CAGGGACACC CTGAACTTCT TTAAGGAGAG CAAGTTTGAT 233693439
GCTCTTTTCA CAGACCCAGC CTTACCCTGT GGGGTGATCC TGGCTGAGTA 233693489
TTTGGGCCTA CCATCTGTGT ACCTCTTCAG GGGTTTTCCG TGTTCCCTGG 233693539
AGCATACATT CAGCAGAAGC CCAGACCCTG TGTCCTACAT TCCCAGGTGC 233693589
TACACAAAGT TTTCAGACCA CATGACTTTT TCCCAACGAG TGGCCAACTT 233693639
CCTTGTTAAT TTGTTGGAGC CCTATCTATT TTATTGTCTG TTTTCAAAGT 233693689
ATGAAGAACT CGCATCAGCT GTCCTCAAGA GAGATGTGGA TATAATCACC 233693739
TTATATCAGA AGGTCTCTGT TTGGCTGTTA AGATATGACT TTGTGCTTGA 233693789
ATATCCTAGG CCGGTCATGC CCAACATGGT CTTCATTGGA GGTATCAACT 233693839
GTAAGAAGAG GAAAGACTTG TCTCAGgttg gtgggtttat ttcttttgga 233693889
ctgccttgtt tcttccaggc tctgtcctcc ctcactcatt tggctccttg 233693939
agccgactgt cccttggagg atttcctgga gaaacggtgg ggggaagtga 233693989
```

acceptor splice site (Circ_UGT1A-8)
donor splice site (Circ_UGT1A-5)
donor splice site (Circ_UGT1A-8)
acceptor splice site (Circ_UGT1A-7)
acceptor splice site (Circ_UGT1A-6)
donor splice site (Circ_UGT1A-6)
donor splice site (Circ_UGT1A-7)

B

start of Exon 5b and Exon 5b-v of UGT1A
stop codon of Exon 5b and Exon 5b-v of UGT1A

```
tcatgtgtgg gtgcacacgt gtgcattcat atgcgtgtgt gtgtgtgtgc 233769480
gtgtgtttat gagagtgtcc attgctttct cccatggtta cctcctttag 233769530
AAAGAAGCAG CAGTCAGGAA GACAGATGTG AAGAGCTGGA GCATGTTCAG 233769580
ATGAGAGGAG ACGGAACACG GGGACACACC AGCTTGAGCA AGGGACAACA 233769630
GGGGAGGACT GATGACTGAC TTCCCACCTT TGAGgtgcta atgtgtgtgt 233769680
ggtggcactg gataaaagat caatgttggc taggcaccat ggcacacgcc 233769730
tgtagtccca gccactctgg aggctaaggc ggga
```

donor splice site (Circ_UGT1A1-29, -30)

C

start of Exon 5a and Exon 5a-v of UGT1A
stop codon of Exon 5a of UGT1A

```
ttggaaaatc tggtagtctt cttaagcagc catgagcata aagagaggat 233772211
tgttcatacc acaggtgttc caggcataac gaaactgtct ttgtgtttag 233772261
TTACAAGGAG AACATCATGC GCCTCTCCAG CCTTCACAAG GACCGCCCGG 233772311
TGGAGCCGCT GGACCTGGCC GTGTTCTGGG TGGAGTTTGT GATGAGGCAC 233772361
AAGGGCGCGC CACACCTGCG CCCCGCAGCC CACGACCTCA CCTGGTACCA 233772411
GTACCATTCC TTGGACGTGA TTGGTTTCCT CTTGGCCGTC GTGCTGACAG 233772461
TGGCCTTCAT CACCTTTAAA TGTTGTGCTT ATGGCTACCG GAAATGCTTG 233772511
GGGAAAAAAG GGCGAGTTAA GAAAGCCCAC AAATCCAAGA CCCATTGAGA 233772561
AGTGGGTGGG AAATAAGgta aaattttgaa ccattcccta gtcatttcca 233772611
aacttgaaaa cagaatcagt gttaaattca ttttattctt attaaggaaa 233772661
tactttgcat aaattaa
```

donor splice site (Circ_UGT1A1-31, -33)

Supplemental Fig. 17

MOLPHARM-AR-000225
Molecular Pharmacology
Supplemental Materials

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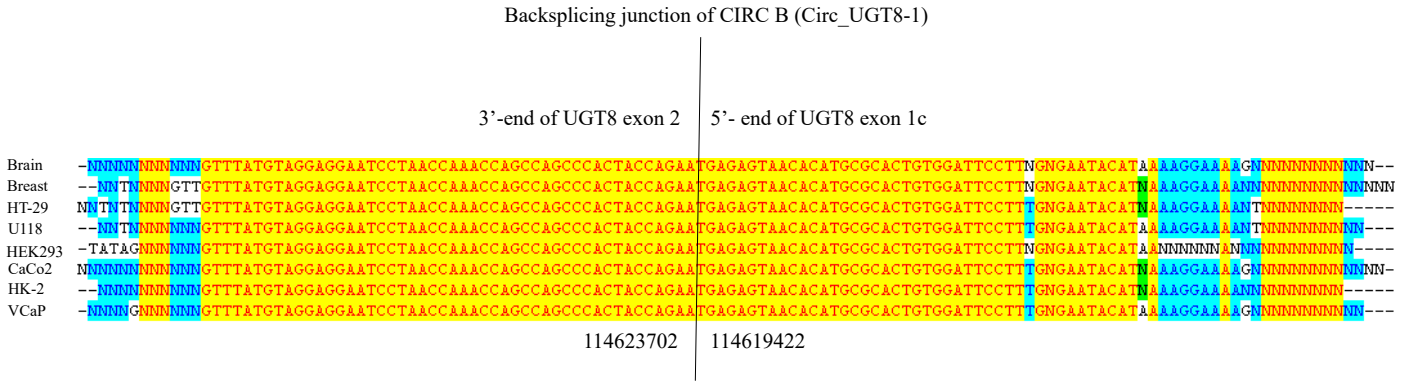
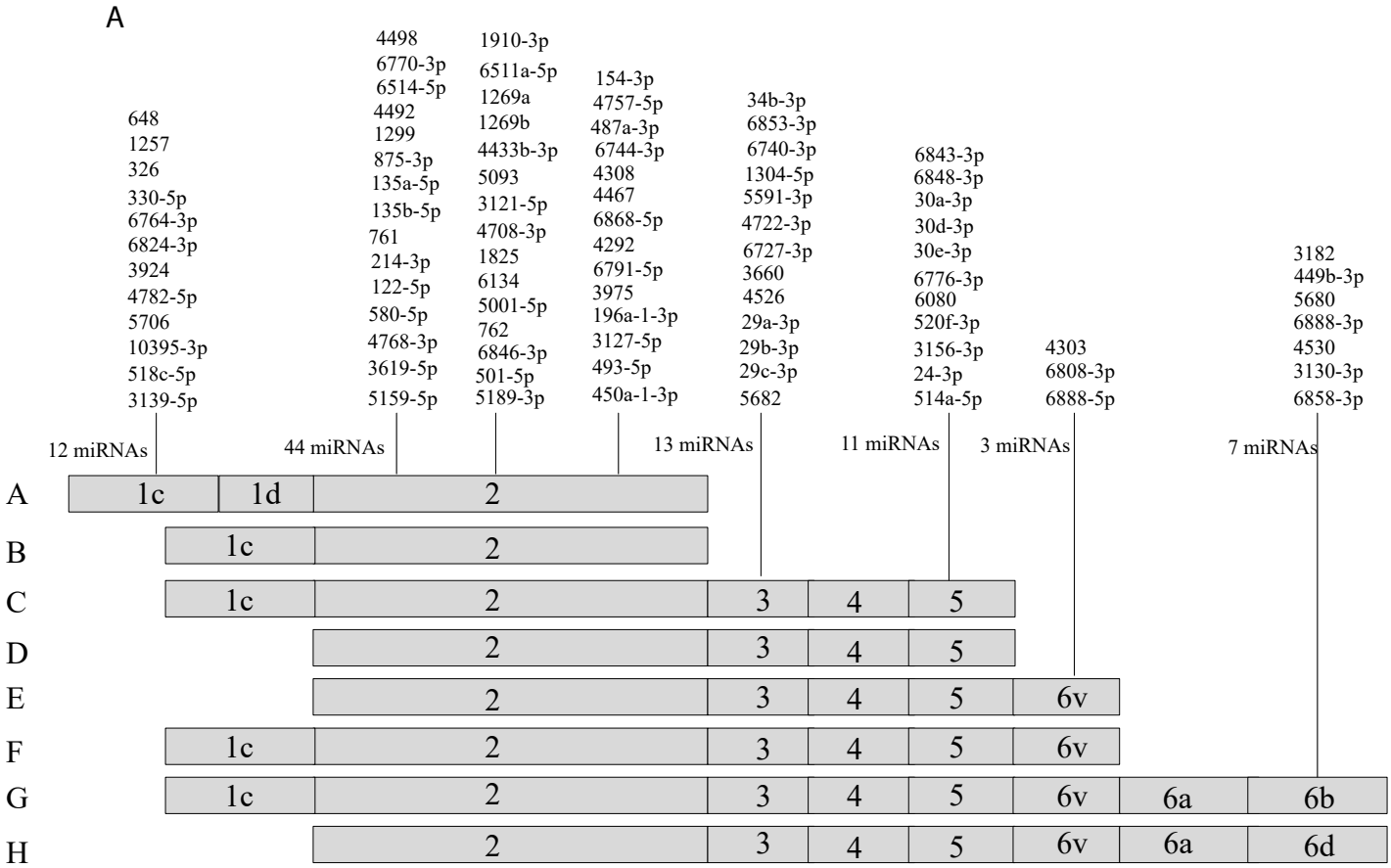


Supplemental Fig. 18

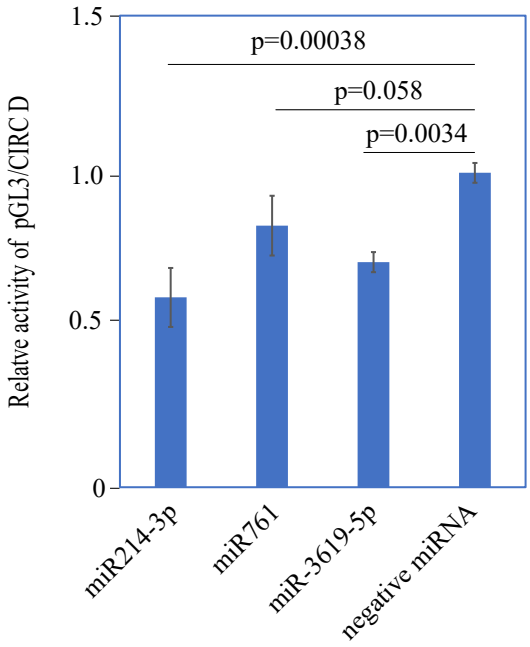
Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech

A



B



Supplemental Fig. 19

MOLPHARM-AR-000225
Molecular Pharmacology
Supplemental Materials

Circular RNAs of UDP-Glycosyltransferase (UGT) genes expand the complexity and diversity of the UGT transcriptome

Dong Gui Hu, Peter I. Mackenzie, Julie-Ann Hulin, Ross A. McKinnon, and Robyn Meech

A (exon 2)

start codon ATG of UGT8

```
tgatttttt tttagacaaa gtattagatc agatattttt ggggaaaaat  114622806
gctttgtgat tgcttgtttt gaatggtgag cattgtattt tgttttaagt  114622856
tgttttctgg ttgttattac aaCTATGAAG TCTTACACTC CATATTTCAT  114622906
TCTCCTGTGG AGTGCTGTTG GGATAGCGAA GGCTGCCAAA ATCATCATCG  114622956
TGCCGCCAAT TATGTTTGAA AGCCATATGT ACATTTTCAA GACGCTAGCC  114623006
TCAGCCTTGC ACGAGAGAGG CCACCATACA GTGTTCCTCC TCTCTGAAGG  114623056
CAGAGACATC GCCCCATCTA ATCATTACAG CCTCCAGCGC TACCCAGGGA  114623106
TCTTTAACAG TACCACCTCA GATGCTTTCC TACAGTCCAA GATGCGGAAT  114623156
ATTTTCTCTG GGAGATTGAC AGCAATCGAA CTGTTTGACA TACTGGATCA  114623206
CTATACTAAG AACTGTGACC TGATGGTTGG CAACCATGCC CTGATCCAGG  114623256
GTCTGAAGAA AGAAAAATTT GACCTGCTGC TGGTGGACCC TAATGATATG  114623306
TGTGGATTTG TGATAGCTCA TCTTTTAGGG GTTAAATATG CTGTATTTTC  114623356
AACTGGCCTT TGGTATCCTG CTGAAGTGGG TGCTCCTGCT CCATTAGCAT  114623406
ACGTCCCAGA GTTTAACTCA CTCCTCACAG ACCGCATGAA CTTGCTGCAA  114623456
AGGATGAAAA ATACCGGTGT TTACCTCATT TCCAGATTAG GGGTCAGCTT  114623506
TCTGGTTCTT CCCAAATATG AAAGGATAAT GCAGAAGTAC AACCTGCTGC  114623556
CAGAGAAGTC CATGTATGAT TTGGTTCATG GGTCCAGCCT GTGGATGCTG  114623606
TGTACTGACG TAGCACTGGA ATTCCCAAGA CCCACTCTGC CTAATGTTGT  114623656
TTATGTAGGA GGAATCCTAA CCAAACCAGC CAGCCCACTA CCAGAAgtaa  114623706
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tacactaaaa ggtgattagg acatggggcc tccaaagtcc ctttatgtgg  114623856
gtccaccact tccctgggaa acattcctgc tttgaatgag catttctgta  114623906
atactgaaaa gaccactatg ccatatggtc aatagactgc ctgggaagta  114623956
```

Donor splice site (Circ_UGT8-2)

Donor splice site (E2, Circ_UGT8-1)

acceptor splice site (Circ_UGT8-5)

Donor splice site (Circ_UGT8-3)

B (exon 6)

stop codon TGA of UGT8

Donor splice site (Circ_UGT8-15, -16)

```
atagttgttt taattatttc ccctttttaa atgaagatat gcataaatat  114675874
agaactttat tataagcatc atcattctgt gttttgtccc ctctccatag  114675924
CTACCGTCAG AGGGCTCAGA AGCTTTCGGA AATTCACAAG GATCAACCTG  114675974
GTCACCCTGT CAATCGAACT ATCTATTGGA TAGATTATAT TATTCGTCAC  114676024
AATGGAGCCC ATCACCTACG TGCCGCTGTC CATCAGATCT CCTTTTGTCA  114676074
GTATTTTTTA CTGGATATTG CCTTTGTGCT TTTGCTTGGT GCTGCCTTGT  114676124
TATACTTTCT CTTGTCTTGG GTGACAAAAT TTATCTACAG AAAAATCAAA  114676174
AGTCTGTGGT CTAGAAATAA GCATAGCACA GTTAATGGAC ATTACCACAA  114676224
TGGAATCCTC AATGGCAAGT ACAAAAGAAA TGGCCATATT AAACATGAAA  114676274
AGAAAGTGAA ATGAGCCAAC AGCCCAGGTG ATAGAAATAA ATTGGTTCAC  114676324
TCATTGAATT TTTATTGCTA TTATTTAGTC TAACAGCTAC TAAAAGTAAA  114676374
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AATCTTGATA TGTGCGTGTC CCGGATCAGG AATGGTTTCA TTTTTCTTAA  114676624
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AAAGAACACT TCCCCCCAAA ACTGGATGCA GAGGAAGAAA AAAAAAAAAG  114676874
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TGTTTGTATT GTTTTTCTTT TCTGATAGAG TATGGCCACT TCTGGGGGAA  114676974
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CCCCAGCAGC CTTTTCCGGT CATTGTAGCT GACATGGAGC AGTGACAGTA  114677574
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TCTGTTTACT TAGCACTTGC ACTACCCTTG TTGGTTGAGT GTATGCTTTA  114677724
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CAACATTTAC CTAATGTCAT TCACTAACAT GGAAGAGTTG TGAAAATTCT  114677824
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GGAATTTGAT TAAGTTGACA GTAGGCACTG ATTGGATGAT TAAACATAAG  114678174
TTAATCTCCA CTGTGATAAA AACTTAAATA ATAAACATGA TTTAAAATAG  114678224
Agaagtgttg ttggaataaa atttttattc agattttatt tcataatatt  114678274
atatatattc attttaaaca tgtttctaag acaggtctcc caacttttgt  114678324
```

C (exon 1d, 34 bp)

```
gcagtgagca gtagctgctg gaatacctac ctgttttggc attagtctta  114620366
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ATTTGTGAGA AGGCAACATT GGAGAGACAC ATAGgtgagg cagtacagta  114620466
gaataaacat ttggaaggtt tttctaagat cagctcctta attttctttc  114620516
ttcactgatc ttgtaattgt agtcaacttt acta
```

D (exon 6v, 146 bp)

```
atagttgttt taattatttc ccctttttaa atgaagatat gcataaatat  114675874
agaactttat tataagcatc atcattctgt gttttgtccc ctctccatag  114675924
CTACCGTCAG AGGGCTCAGA AGCTTTCGGA AATTCACAAG GATCAACCTG  114675974
GTCACCCTGT CAATCGAACT ATCTATTGGA TAGATTATAT TATTCGTCAC  114676024
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tatactttct cttgtcttgg gtgacaaaat ttatctacag aaaaat
```

E (exon 6a, 68 bp)

```
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GAATTCAGAA TGTTAAGTgt aggtatttac tgatcattgt tgtttttttt  114678486
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ttaaattttc cacacttg
```

F (exon 6b, 140 bp)

```
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atctataggc atatgggcca cattatgtca tacatgttct attgatgcag  114682248
ATTTCAAGAT AAATGGGTGT CCTACCTGAG AATGTGGCTA TACCACTGGA  114682298
AGTGTAGGTG GCATCAACTC ACATGTTTGT GCATATCAAG ACAGAAGGTG  114682348
CAGAACATCT CTCTGCTGGC TATCATTTCT TATATCAGAG gtaaatgaca  114682398
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gtgacatgga attttatttt atagttgtgt gttctattta
```

G (exon 6cv, 80 bp)

```
taatattcct ttgtcagtat tcttattatt ctcttgatct cattgcttta  114684301
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TGCCAGGCTC TGTACGTAAT ACTGGAAATG CTGGGATGAA GAAGCCATAG  114684401
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aaggaatata tattgtaata taggtacaga caatgtgcca ttgaaacaga  114684501
ggaaagtgag agaagtttcc acctggggat
```

H (exon 6d, 76 bp)

```
cggatcaccc tttcttaata actctcacta ttatctaaaa tcatgttact  114693018
tatgtatttc tttctttact tgttttgtgt ttacttctct caatgagaag  114693068
GCAATCTCCA CGAAGGCAGA GATCTTTGAA TGCTGAGTCC TCAGTGACCA  114693118
GAGCAATGCC TGGCACATGG TACTTGgtaa gtaaatattg ttgaataagt  114693168
aaatgtttaa gaaggcatat tcccaataaa gtgtgagagc aagccagggc  114693218
aattcatata tcctctctgt cggtaa
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Supplemental Fig. 20

Legends of Supplemental figures

Supplemental Fig 1. Amplicons generated by RT-PCR with divergent primers in human tissues and cell lines. Total RNA extraction and RT-PCR was performed as described in *Materials and Methods.* All amplicons were resolved on 1% Agarose gels. Images show amplicons generated using divergent primers targeting (A) *UGT1A* exon 3 in HT-29 cells (lane 1), brain (lane 2), kidney (lane 3), and colon (lane 4); (B) *UGT8* exon 2 in HT-29 cells (lane 1), brain (lane 2), and kidney (lane 3); (C) *UGT8* exons 2 and 5 from HT-29 cells (lane 1), brain (lane 2), and kidney (lane 3). Panels (A) and (B) were derived from the same gel and thus have the same DNA markers. Spaces in panels (B) and (C) indicate that DNA markers and PCR products were run on the same gel but at different parts of the gel.

Supplemental Figs 2-14. The sequencing chromatograms showing the backsplicing junctions (indicated by a vertical line and positioned according to GRC38/hg38 coordinates) for the thirteen circRNAs identified by the present study, including 1) three from *UGT1A* [circ-UGT1A-26 (Suppl Fig. 2), Circ_UGT1A-28 (Suppl Fig. 3), Circ_UGT1A-33 (Suppl Fig. 4)], one from *UGT2B7* (Circ_UGT2B7-3, Suppl Fig. 5), one from *UGT2B10* (Circ-UGT2B10-2, Suppl Fig. 6), eight from *UGT8* [Circ_UGT8-1 (Suppl Fig. 7), Circ_UGT8-20 (Suppl Fig. 8), Circ_UGT8-21 (Suppl Fig. 9), Circ-UGT8-7 (Suppl Fig. 10), Circ_UGT8-8 (Suppl Fig. 11), Circ_UGT8-22 (Suppl Fig. 12), Circ_UGT8-23 (Suppl Fig. 13), Circ_UGT8-24 (Suppl Fig. 14)].

Supplemental Fig. 15. CircRNAs of the *UGT8* gene identified by circRNA databases. A schematic diagram shows the *UGT8* gene structure, the locations and genomic coordinates (GRCh38/hg38) of nineteen UGT8 circRNAs identified in circRNA databases. The *UGT8* gene contains 13 exons (lengths given in nt); however, partial sequences of three exons [the last 17

nts of exon 2 (E2v, green), the first 146 nts of exon 6 (E6v, red), the first 80 nts of exon 6c (E6cv, purple)] were found backspliced within circRNAs through conserved exonic cryptic splice sites.

Supplemental Fig. 16. UGT CircRNAs identified in circRNA databases. Schematic diagrams showing the locations and backsplicing junctions (indicated by a vertical line and positioned according to GRC38/hg38 coordinates) for circRNAs found in circRNA databases for eight *UGT* genes, including *UGT2B4* (A), *UGT2B11* (B), *UGT2B15* (C), *UGT2B17* (D), *UGT2A1/2* (E), *UGT2A3* (F), *UGT3A2* (G), *UGT3A1* (H).

Supplemental Fig. 17. The donor/acceptor splice sites for *UGT1A* circRNAs conform to the splicing AG/GT rule. The sequences of three *UGT1* exons are shown, namely *UGT1A6* exon 1 (A), exon 5b-v (B), and exon 5a-v (C). All three exons have conserved acceptor/donor splicing sites with the splicing signal dinucleotide AG/dinucleotide GT. Of note, the *UGT1A6* exon 1 are involved in the formation of five circRNAs (Circ_UGT1A-4, -5, -6, -7, -8) using conserved acceptor/donor splice sites (GRC38/hg38).

Supplemental Fig. 18. DNA sequencing verified the identity of CIRC B RT-PCR products. RT-PCR products (154 bp) of UGT8 circRNA B (CIRC B) were generated using divergent primers targeting *UGT8* exon 2 (UGT8 qPCP E2-F) and exon 1c (UGT8 qPCR E1c-R) and cDNA from human tissues (brain, breast) and cell lines (HT-29, U118, HEK293, CaCo2, HK-2, VCaP), purified and then sequenced. Sequence alignment verified that all amplicons had the same sequence containing the backsplicing junction (E2/E1c) of CIRC B (indicated by a vertical line and positioned according to GRC38/hg38 coordinates).

Supplemental Fig. 19. miRNAs predicted to bind to UGT8 circRNAs. A: schematic diagrams showing the exon structure of eight UGT8 circRNAs (A-H) and the miRDB-predicted miRNAs that bind to six of the ten exons implicated in UGT8 circRNAs. B: MDA-MB-453 cells were transfected with luciferase reporters (either pGL3/CIRC D vector or empty pGL3 vector) and the indicated miRNA mimics; luciferase activities were measured after 48 hours and normalised as described in the Methods. pGL3/CIRC D activities in cells cotransfected with mimics are presented relative to that in cells cotransfected with the negative control miRNA (set as a value of 100%). Data are the means ± 1 S.D. from three independent measurements. The difference in means were assessed using one-way analysis of variance followed by Bonferroni's multiple comparison tests. A p value of less than 0.05 was considered statistically significant.

Supplemental Fig. 20. The donor/acceptor splice sites of *UGT8* circRNAs conform to the splicing AG/GT rule. The sequences of eight *UGT8* exons are shown, including exon 2 (A), exon 6 (B), exon 1d (C), exon 6v (D), exon 6a (E), exon 6b (F), exon 6cv (G), and exon 6d (H). All exons have conserved acceptor/donor splicing sites with the splicing signal dinucleotide AG/dinucleotide GT (GRC38/hg38).