Supplemental data

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Comprehensive Characterization of Mouse UDP-Glucuronosyltransferase (Ugt) belonging to the Ugt2b Subfamily: Identification of Ugt2b36 as the Predominant Isoform Involved in Morphine Glucuronidation

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Supplemental Table 1 Condition of PCR

Isoforms	Round	Methods (two or three cycles)	Annealing temperature (°C)
Ugt1a1	1	two	68
	2	two	68
Ugt2a3	1	three	54
	2	two	68
Ugt2b1	1	two	68
	2	two	68
Ugt2b5	1	three	57
	2	two	68
Ugt2b34	1	three	57
	2	two	68
Ugt2b35	1	three	57
	2	two	68
Ugt2b36	1	three	57
	2	two	68
Ugt2b37	1	three	57
	2	two	68
Ugt2b38	1	two	68

2	two	68

PCR conditions to generate the Ugts are listed. PCR methods were selected using the Tm value of the primers. When both of the primers had a Tm higher than 63 °C, we carried out the PCRs by the two cycle method (Table S2). When one of the primers had a lower Tm than 63 °C or we failed to obtain a PCR product using the two cycle method, the three cycle method was selected. In the two cycle method (Table S2), the extension temperature was fixed at 68 °C. Primers used in the PCR are shown in Table 1.

Supplement Table 2 PCR methods used in supplemental Table 1.

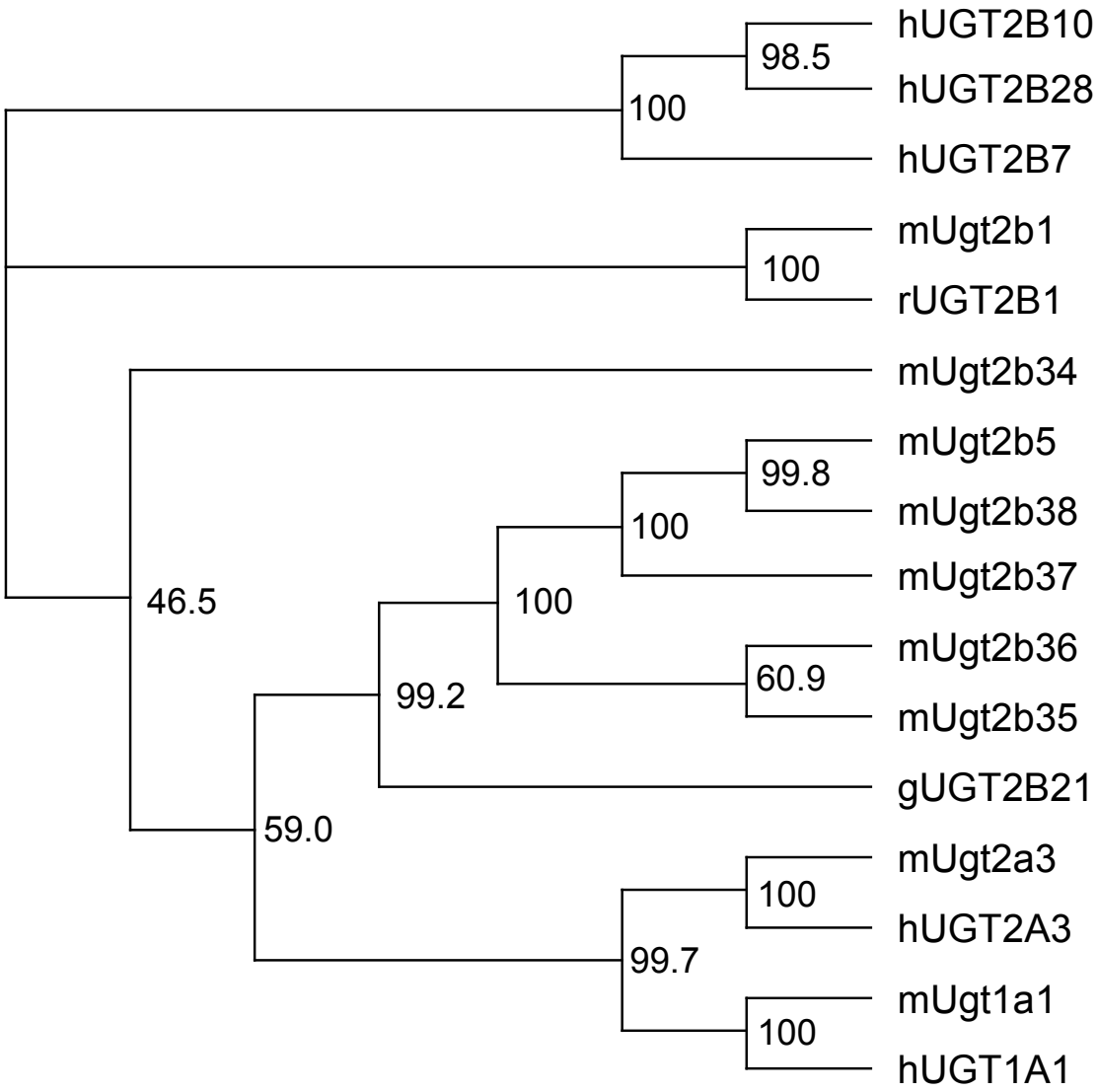
Two cycle method	Number of cycles	Temperature (°C)	Duration
Predenature	1	94	2 min
Denature	30	98	10 s
Extension		68	30 s/kb
Preservation	1	4	∞

Three cycle method	Number of cycles	Temperature (°C)	Duration
Predenature	1	94	2 min
Denature	30	98	10 s
Annealing		Tm- 3	30 s
Extension		68	30 s/kb
Preservation	1	4	∞

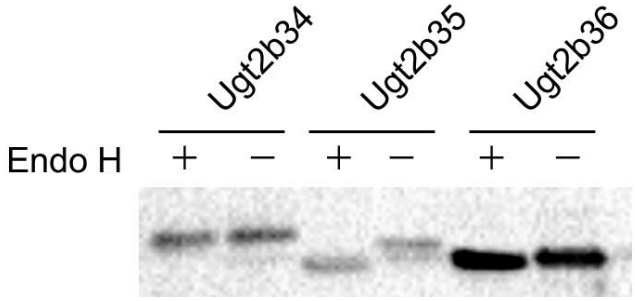
Supplemental Table 3 Multiple alignment of amino acid sequences between the antigen peptide (human UGT2B7) to produce anti-UGT2B antibody and the corresponding residues in mouse Ugt isoforms

Ugt isoform	Amino acid sequences	Residues
Antigen peptide (UGT2B7)		
Common in human UGT2B	**KWIPQNDLLGHPK**	355-367
Ugt1a1	**KWLPQNDLLGHPK**	355-367
Ugt2a3	**NWIPQNDLLGHPK**	355-367
Ugt2b1	**KWIPQNDLLGHPK**	356-368
Ugt2b5	**KWLPQNDLLGHPK**	356-368
Ugt2b34	**KWIPQNDLLGHSK**	358-370
Ugt2b35	**KWLPQNDLLGHPK**	355-367
Ugt2b36	**KWLPQNDLLGHPK**	356-368
Ugt2b37	**KWLPQNDLLGHPK**	356-368
Ugt2b38	**KWLPQNDLLGHPK**	356-368

Different amino acids are shown in gray background.


hUGT2B10
98.5
hUGT2B28
100
hUGT2B7
mUgt2b1
100
rUGT2B1
mUgt2b34
mUgt2b5
99.8
mUgt2b38
100
mUgt2b37
46.5
100
mUgt2b36
60.9
99.2
mUgt2b35
gUGT2B21
59.0
mUgt2a3
100
hUGT2A3
99.7
mUgt1a1
100
hUGT1A1

Supplemental Figure S1. Phylogenetic tree of the selected UGT isoforms from some animals using the Neighbor-joining (NJ) method. Their UGT amino acid sequences were obtained from the National Center for Biotechnology Information (NCBI) Gene. The following GenBank accession-numbers were used: human UGT1A1 (AAI28415)(Ritter et al, 1991), human UGT2A3 (AAI30534)(Court et al, 2008), human UGT2B7 (AAH30974)(Ritter et al, 1990), human UGT2B10 (AAI13650)(Jin et al, 1994), human UGT2B17 (EAW55622)(Beaulieu et al, 1996), human UGT2B28 (NP_001064)(Lévesque et al, 2001), rat UGT2B1 (NP_775417)(Mackenzie, 1988), guinea pig UGT2B21 (BAB82476)(Ishii et al, 2001). Robustness of bifurcation was estimated by bootstrap analysis with 1000 replicates. The numbers in the tree indicate the bootstrap values as a percentage. Annotation: m (mouse), h (human), r (rat), and g (guinea pig). A distance-based neighbor-joining phylogenetic tree was generated (Saitou and Nei, 1987). The reliability of the phylogeny was tested by bootstrap analysis with 1000 replicates. The bootstrap values are indicated in the neighbor-joining consensus tree. The phylogenetic tree was constructed by ClustalW (http://clustalw.ddbj.nig.ac.jp/).



Supplemental Figure S2 Deglycosylation of Ugt2b24, Ugt2b35 and Ugt2b36 with endoglycosidase H Deglycosylation of Ugt was carried out according to Nakamura et al (2016) with slight modifications. Ugt2b34, 2b35 or Ugt2b36-expressed baculosomes (20 μg protein) were treated with or without Endo H (250U) at 37°C for 1h. Resulting sample (10 μg protein) was subjected to the SDS-PAGE. Western blotting was performed with anti-mouse low p*I* UGT antibody (Mackenzie et al, 1984) as the primary antibody.