Drug Metabolism & Disposition

Xenobiotic Metabolism in Mice Lacking the UDP-Glucuronosyltransferase 2 Family

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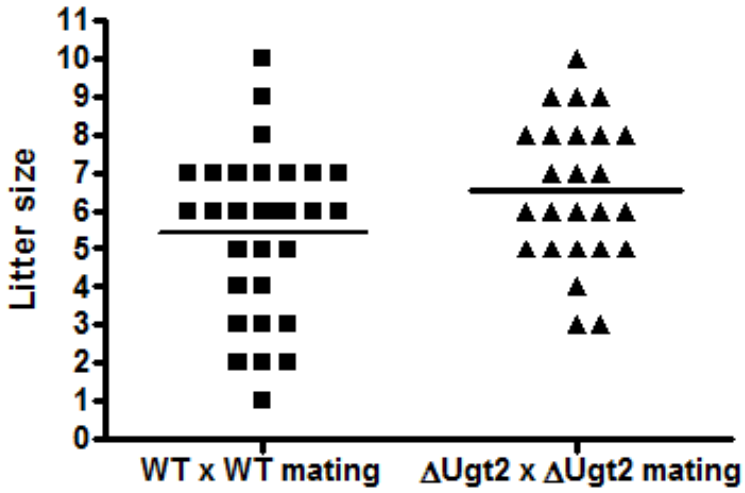
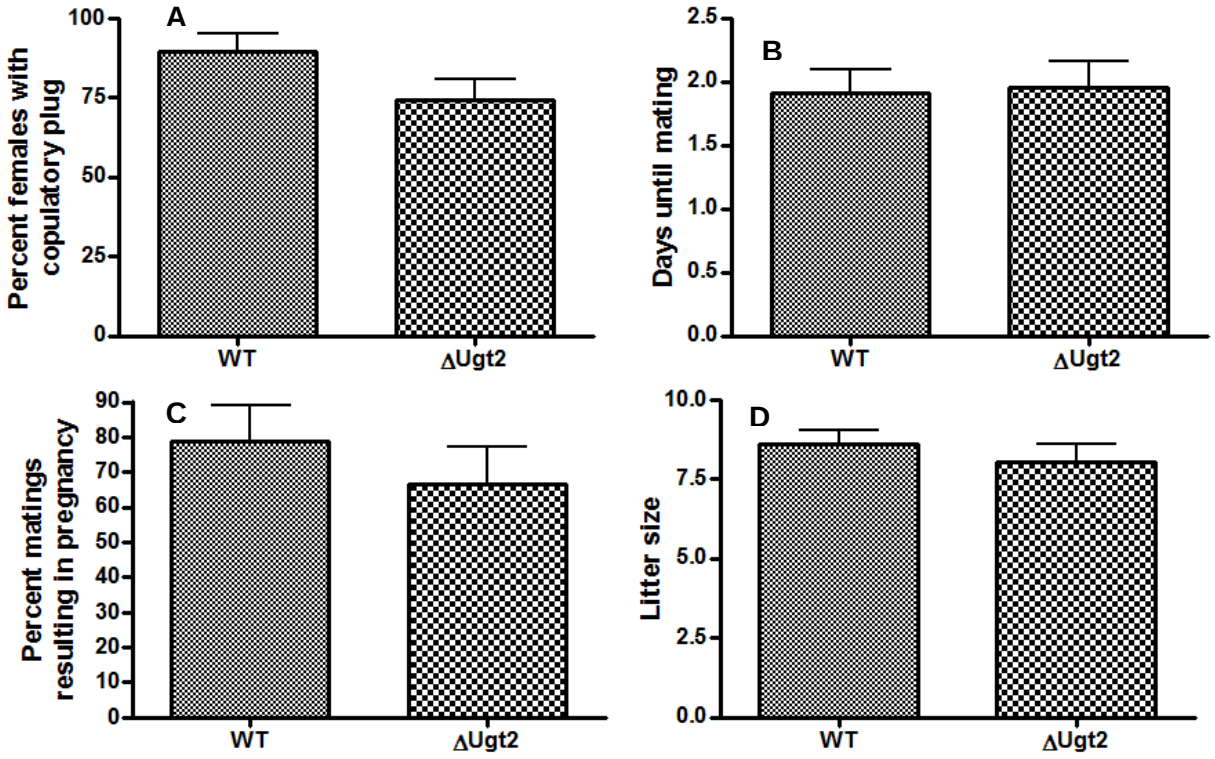
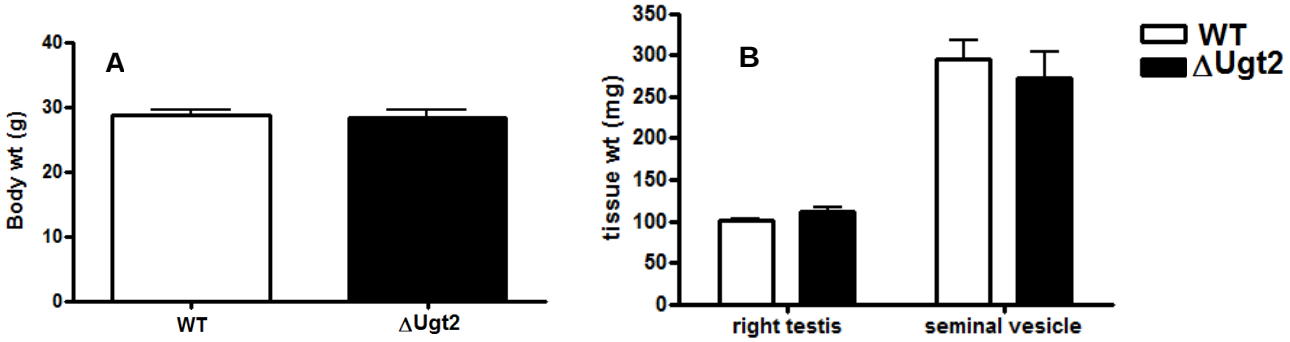
Supplemental Results



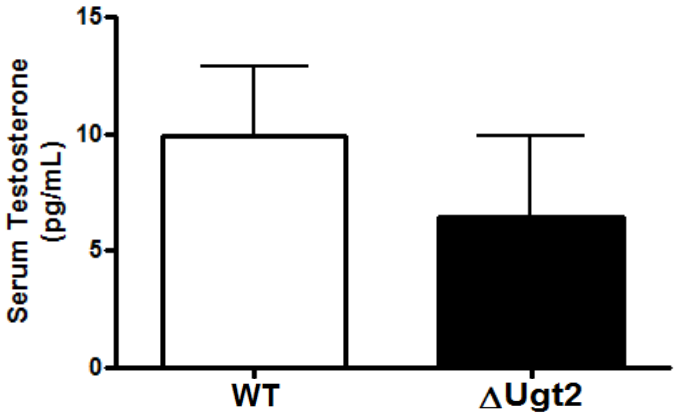
Supplemental Figure 1. Litter size in homozygous WT x WT mating versus homozygous *ΔUgt2* x *ΔUgt2* mating. 6 pairs of wild-type and 6 pairs of *ΔUgt2* mice were co-housed over a period of approximately 6 months. Each pair was housed individually. Sizes of all litters produced were recorded. All mice were 2.5 to 5 months old at the time of co-housing. Average litter sizes were not significantly different in an unpaired Student's *t* test.



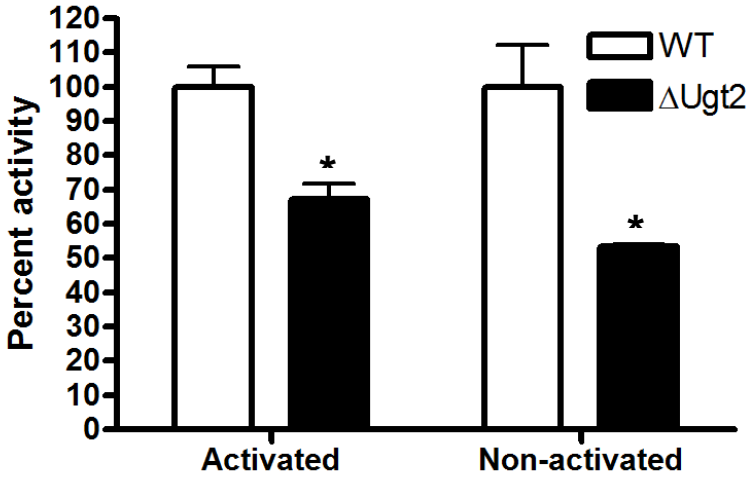
Supplemental Figure 2. Reproductive studies on WT and *ΔUgt2* males mated with WT females. 11 WT and 11 *ΔUgt2* males were housed individually. Two to three WT females were introduced into each cage and checked for copulation pugs over the following 5 days. Females that had mated were removed into individual cages. The number of days of co-housing before copulation, the number of matings, and the number of pups obtained from each mating were recorded. Compared to WT males, *ΔUgt2* males mated with a slightly lower percentage of cohoused females (**A**), had similar numbers of days until copulation plugs were observed (**B**), had a slightly lower percentage of matings resulting in pregnancy (**C**), and produced slightly smaller litters (**D**) on average. Overall, *ΔUgt2* males appeared to exhibit slightly lower fertility/fecundity, but no parameter examined was significantly different in an unpaired Student's *t* test. Error bars represent S.E.M., n=11 males of each genotype.



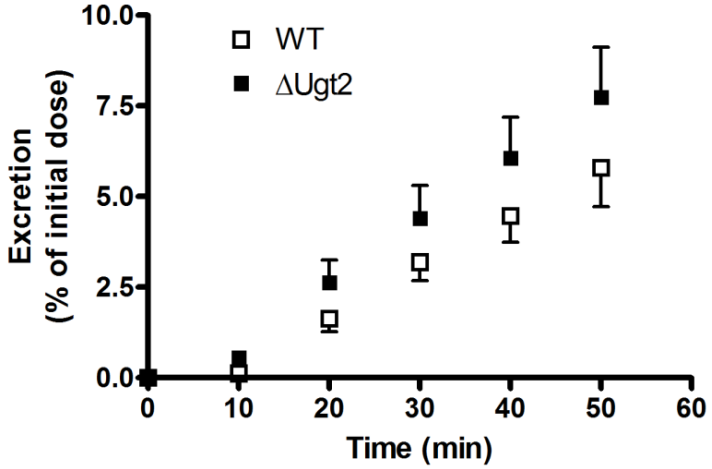
Supplemental Figure 3. Body and reproductive organ weights of WT and *ΔUgt2* male mice. A. Body weight of WT and *ΔUgt2* mice. **B.** Right testis and seminal vesicle from both groups were removed and weighed. There was no significant difference in body weight, testis weight and seminal vesicle weight. Error bars represent S.E.M., n=6.



Supplemental Figure 4. Serum testosterone levels in WT and *ΔUgt2* male mice. Testosterone levels were measured by ELISA in mature (2-5 months old) male mice. No Significant difference in serum testosterone concentration was observed between the groups in an unpaired Student's *t* test. Error bars represent S.E.M., n=6.



Supplemental Figure 5. Effect of microsomal activation with alamethicin on BPA glucuronidation. Reaction mixtures contained 0.1M Tris buffer (pH 7.5), 100 µM BPA, 4 mM magnesium chloride, 5 mM UDPGA, and microsomal protein. Activated reaction mixtures contained 25 µg/mL alamethicin. Activities were normalized to microsomal protein concentration and expressed as percentage of average WT activity for each experimental condition. Both in the presence and absence alamethicin, activities differed significantly between WT and *ΔUgt2* microsomes ($p<0.05$ in both cases) in Student's *t* tests. Error bars represent S.E.M., n=3.



Supplemental Figure 6. *In vivo* glucuronidation of BPA in WT and *ΔUgt2* female mice. Age- and weight-matched WT and *ΔUgt2* mice were dosed with 2 mg BPA/kg bodyweight and bile was collected by the same method described for males. Biliary excretion rates were not significantly different in a Student's *t* test. Error bars represent S.E.M., n=3.

Supplemental Methods

SYBR green qPCR primers

Common primer	AGGCGTTGACATAGGCTTCAAATT
Ugt1a1	TCATGCCCAACATGGTTTTTATTG
Ugt1a7c	ACCTGTGATGCCCAATGTGATCTA
Ugt1a9	AGGCCTGTGATGCCTAACATGGTC

***ΔUgt2* genotyping primers**

Common primer	CACAAATTTGTATGTGTTTGGACT
Endogenous locus	CTGCACTCAGGTGCATTTCTACAC
Deleted locus (GPRA Neo)	AAATGCCTGCTCTTTACTGAAGG

Southern blotting probes

Deletion targeting probe	
Upper	ATTCATCTATTCCAAATCCTTATC
Lower	GAAAGGGGTAAGAGGAGAAGAGTA
Universal binding probe	
Upper	TCCCCACAGGAAGCAACAGGATCT
Lower	GTGTGGCCGATGGAATTCAGTCAT

β-Glucuronidase protocol. Glucuronides were cleaved by addition of 20 uL of 5 mg/mL β-Glucuronidase (Sigma) to microsomal reaction products or bile samples. Microsomal incubations were acidified to pH 5.2 by addition of HCl prior to β-glucuronidase treatment. Bile samples did not need pH adjustment as biliary pH is 5 to 6. After incubation with β-glucuronidase for 4 hours at 37°C, cleavage reactions were terminated by addition of ice-cold 4% acetic acid in methanol and vortex mixing. Precipitated proteins were removed by centrifugation (10 min, 10,000 x *g*) and supernatants were then analyzed by HPLC.