Negative Regulation of the UGT2B7 Enzyme by C-Terminally Truncated Proteins Derived from Alternative Splicing

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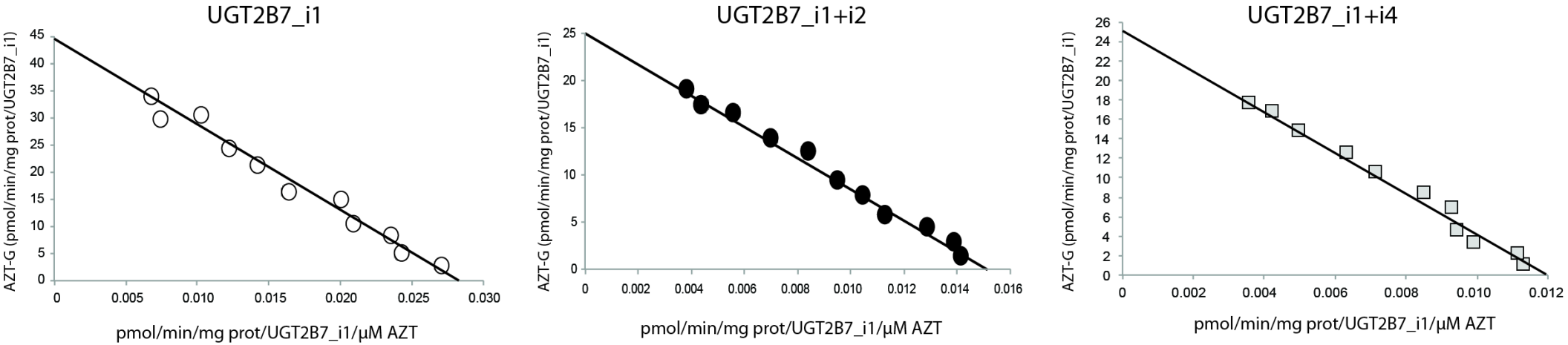
Supplemental Figure 1



Supplemental Figure 1 - Eadie-Hofstee plots of UGT2B7 glucuronidation assays.
Glucuronidation reaction assays using different concentrations of the substrate (AZT) were performed (see Fig. 6A for the graphical representation of the results). Here, data were transformed to plot the AZT-G production (in pmol/min/mg prot/ UGT2B7_i1) with the AZT-G production/AZT concentration, thus producing Eadie-Hofstee plots. Coupled with the aspect of the glucuronidation kinetic curves, the linearity and the aspect of the curves obtained further prove that the enzymatic reaction is well fitted by the Michaelis-Menten equation.

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SUPPLEMENTAL TABLE 1

Amplification strategies for RT-PCRs and quantitative real-time PCRs of UGT2B7.

Target	Primer	Sense	Sequence (5'-3')	Final primer concentration (nM)	Amplicon length (bp)
UGT2B7 – RT-PCR					
v1	1568	F	GTGCTTTACTTTGACTTTTGGTTCG	1000	791
	2700	R	TAAACACCTTCGGGTTGCAGC	1000	
v5	1568	F	GTGCTTTACTTTGACTTTTGGTTCG	1000	736
	2745	R	GAAGAACTGCTAAACTGTCTTCC	1000	
v7	1568	F	GTGCTTTACTTTGACTTTTGGTTCG	1000	461
	2467	R	CTTCTAGATATAAAGAGAATGTTATG	1000	
UGT2B7 – qRT-PCR					
ex4-ex6a	3766	F	CTGCCTAAGGAAATGGAAGAC	800	248
	2467	R	CTTCTAGATATAAAGAGAATGTTATG	800	
ex5-ex6b	3137	F	CAGACTTGCTGAATGCATTGAAGAG	450	79
	3421	R	GTGGAATTGCTGCTTCATGTGG	300	
ex5-ex6a	2699	F	CATTGCTCACATGAAGGCCAG	300	234
	2700	R	TAAACACCTTCGGGTTGCAGC	300	
36B4 (RPLP0) – qRT-PCR					
ex4-ex5	75	F	CCCATGTGAAGTCACTGTGC	125	251
	76	R	CAATGGCAGCATCTACAACC	125	

Ex: Exon; F: forward; R: reverse; RPLP0: Ribosomal Protein Large P0