Rondini EA, Pant A, and Kocarek TA. Transcriptional regulation of cytosolic sulfotransferase (SULT)1C2 by intermediates of the cholesterol biosynthetic pathway in primary cultured rat hepatocytes. *The Journal of Pharmacology and Experimental Therapeutics.*

Supplemental Table 1. Primer pairs and corresponding gene sequence alignments used to generate the rat SULT1C2 promoter constructs.

Construct Name	Forward (5' → 3')[a]	Reverse (5' → 3')[a]	Fragment Size (bp)	RACE Alignment Reference	Gene Alignment Reference[b]
1C2-1	GCGGCTAGCCTGTCCTTTCTTCATATGTGT	GCGGCTAGCCCTCCAAAATGCTGCGATTCC	3092	-3140:-49	5686-8777
1C2-2	GCGGGTACCAGCCAGTATTTTAGTTTCTCC	GCGCTCGAGCCTCCAAAATGCTGCGATTCC	2727	-2775:-49	6051-8777
1C2-2d1	GCGGGTACCTTAGACTCGTGTTCTGAAATG	GCGCTCGAGCCTCCAAAATGCTGCGATTCC	2616	-2664:-49	6162-8777
1C2-2d2	GCGGGTACCGTTTACTTAGTGAATCCTGGT	GCGCTCGAGCCTCCAAAATGCTGCGATTCC	2531	-2579:-49	6247-8777
1C2-2d3	GCGGGTACCATCACGAGTCTTAAAAGGTAA	GCGCTCGAGCCTCCAAAATGCTGCGATTCC	2476	-2524:-49	6302-8777
1C2-2d4	GCGGGTACCCAGCTTTTGAGTTATTTTTCG	GCGCTCGAGCCTCCAAAATGCTGCGATTCC	2354	-2402:-49	6424-8777
1C2-3	GCGGCTAGCCTCTGTGCATTTCTCTAAACA	GCGGCTAGCCCTCCAAAATGCTGCGATTCC	2172	-2220:-49	6606-8777
1C2-4	GCGGGTACCCATCTCAACTTAATGACATCT	GCGCTCGAGCCTCCAAAATGCTGCGATTCC	233	-281:-49	8545-8777
1C2-4d1	GCGGGTACCTTAAAGTAAGAAGATTAGAGC	GCGCTCGAGCCTCCAAAATGCTGCGATTCC	184	-232:-49	8594-8777
1C2-4d2	GCGGGTACCGTCCATCATATTCACCAATGC	GCGCTCGAGCCTCCAAAATGCTGCGATTCC	132	-180:-49	8646-8777
1C2-4d3	GCGGGTACCCAAATAGTTTGCTGTTGCTCA	GCGCTCGAGCCTCCAAAATGCTGCGATTCC	91	-139:-49	8687-8777
1C2-5	GCGGGTACCTGCAACAAGATGTGGTAGTAC	GCGCTCGAGCCTCCAAAATGCTGCGATTCC	48	-96:-49	8730-8777

a. All sequences are presented as 5'→3'. Underscored nucleotides represent restriction sites used for cloning into the pGL4.10 promoterless firefly reporter plasmid.

b. Alignments correspond to the nucleotide positions on the rat SULT1C2 gene, chromosome regions 4654278- 4685416 (reverse complement) of the NCBI reference sequence NC_005108.4 located on rat chromosome 9.

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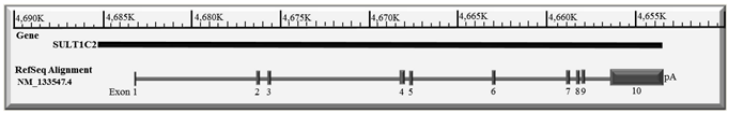
A.



B.

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Pr1_Cl_1      GAGAGGCAGAGGCAGAGGCAGAGGCAGTGGCAGAGGCAGAGGCAGAGGTAGAGGCAGAGG  60
Pr1_Cl_2      GAGAGGCAGAGGCAGAGGCAGAGGCAGTGGCAGAGGCAGAGGCAGAGGCAGAGGCAGAGG  60
Pr1_Cl_3      GAGAGGCAGAGGCAGAGGCAGAGGCAGTGGCAGAGGCAGAGGCAGAGGCAGAGGCAGAGG  60
Pr1_Cl_4      -AGAGGCAGAGGCAGAGGCAGAGGCAGTGGCAGAGGCAGAGGCAGAGGCAGAGGCAGAGG  59
Pr2_Cl_1      ----------------------------------------------------CAGAGG  6
Pr2_Cl_2      -GGAGGCAGAGGCAGAGGCAGAGGCAGTGGCAGAGGCAGAGGCAGAGGCAGAGGCAGAGG  59
Pr2_Cl_3      -GGAGGCAGAGGCAGAGGCAGAGGCAGTGGCAGAGGCAGAGGCAGAGGCAGAGGCAGAGG  59
Pr2_Cl_4      GAGAGGCAGAGGCAGAGGCAGAGGCAGTGGCAGAGGCAGAGGCAGAGGCAGAGGCAGAGG  60
                                                                  ******

Pr1_Cl_1      CAGAGGCAGAGGCAGAGGCAGAGGCAGAGACAGAGGCAGAGGCAGAGGGATACTTGCAAT  120
Pr1_Cl_2      CAGAGGCAGAGGCAGAGGCAGAGGCAGAGACAGAGGCAGAGGCAGAGGGATACTTGCAAT  120
Pr1_Cl_3      CAGAGGCAGAGGCAGAGGCAGAGGCAGAGACAGAGGCAGAGGCAGAGGGATACTTGCAAT  120
Pr1_Cl_4      CAGAGGCAGAGGCAGAGGCAGAGGCAGAGACAGAGGCAGAGGCAGAGGGATACTTGCAAT  119
Pr2_Cl_1      CAGAGGCAGAGGCAGAGGCAGAGGCAGAGACAGAGGCAGAGGCAGAGGGATACTTGCAAT  66
Pr2_Cl_2      CAGAGGCAGAGGCAGAGGCAGAGGCAGAGACAGAGGCAGAGGCAGAGGGATACTTGCAAT  119
Pr2_Cl_3      CAGAGGCAGAGGCAGAGGCAGAGGCAGAGACAGAGGCAGAGGCAGAGGGATACTTGCAAT  119
Pr2_Cl_4      CAGAGGCAGAGGCAGAGGCAGAGGCAGAGACAGAGGCAGAGGCAGAGGGATACTTGCAAT  120
              ************************************************************

Pr1_Cl_1      TTTGTTTAGAAAAACAAAGGATGAATTAATTAACTCAAATTTTTTCCTTTATTCTTATTT  180
Pr1_Cl_2      TTTGTTTAGAAAAACAAAGGATGAATTAATTAACTCAAATTTTTTCCTTTATTCTTATTT  180
Pr1_Cl_3      TTTGTTTAGAAAAACAAAGGATGAATTAATTAACTCAAATTTTTTCCTTTATTCTTATTT  180
Pr1_Cl_4      TTTGTTTAGAAAAACAAAGGATGAATTAATTAACTCAAATTTTTTCCTTTATTCTTATTT  179
Pr2_Cl_1      TTTGTTTAGAAAAACAAAGGATGAATTAATTAACTCAAATTTTTTCCTTTATTCTTATTT  126
Pr2_Cl_2      TTTGTTTAGAAAAACAAAGGATGAATTAATTAACTCAAATTTTTTCCTTTATTCTTATTT  179
Pr2_Cl_3      TTTGTTTAGAAAAACAAAGGATGAATTAATTAACTCAAATTTTTTCCTTTATTCTTATTT  179
Pr2_Cl_4      TTTGTTTAGAAAAACAAAGGATGAATTAATTAACTCAAATTTTTTCCTTTATTCTTATTT  180
              ************************************************************

Pr1_Cl_1      TGTTTTGTGCCAGCCTAGGTCTGCTTCTGCCCTTGAGGTATCCAGGTACCATTGTGCACT  240
Pr1_Cl_2      TGTTTTGTGCCAGCCTAGGTCTGCTTCTGCCCTTGAGGTATCCAGGCACCATTGTGCACT  240
Pr1_Cl_3      TGTTTTGTGCCAGCCTAGGTCTGCTTCTGCCCTTGAGGTATCCAGGTACCATTGTGCACT  240
Pr1_Cl_4      TGTTTTGTGCCAGCCTAGGTCTGCTTCTGCCCTTGAGGTATCCAGGTACCATTGTGCACT  239
Pr2_Cl_1      TGTTTTGTGCCAGCCTAGGTCTGCTTCTGCCCTTGAGGTATCCAGGTACCATTGTGCACT  186
Pr2_Cl_2      TGTTTTGTGCCAGCCTAGGTCTGCTTCTGCCCTTGAGGTATCCAGGTACCATTGTGCACT  239
Pr2_Cl_3      TGTTTTGTGCCAGCCTAGGTCTGCTTCTGCCCTTGAGGTATCCCGGTACCATTGTGCACT  239
Pr2_Cl_4      TGTTTTGTGCCAGCCTAGGTCTGCTTCTGCCCTTGAGGTATCCAGGTACCATTGTGCACT  240
              ****************************************** ** **************

Pr1_Cl_1      GAGCAGGACTGAAAAGAAAAAAGTTTATTCTGAAGTTTGAGCAGATTACAGCCGCTGGGT  300
Pr1_Cl_2      GAGCAGGACTGAAAAGAAAAAAGTTTATTCTGAAGTTTGAGCAGATTACAGCCGCTGGGT  300
Pr1_Cl_3      GAGCAGGACTGAAAAGAAAAAAGTTTATTCTGAAGTTTGAGCAGATTACAGCCGCTGGGT  300
Pr1_Cl_4      GAGCAGGACTGAAAAGAAAAAAGTTTATTCTGAAGTTTGAGCAGATTACAGCCGCTGGGT  299
Pr2_Cl_1      GAGCAGGACTGAAAAGAAAAAAGTTTATTCTGAAGTTTGAGCAGATTACAGCCGCTGGGT  246
Pr2_Cl_2      GAGCAGGACTGAAAAGAAAAAAGTTTATTCTGAAGTTTGAGCAGATTACAGCCGCTGGGT  299
Pr2_Cl_3      GAGCAGGACTGAAAAGAAAAAAGTTTATTCT---GTTTGAGCAGATTACAGCCGCTGGGT  296
Pr2_Cl_4      GAGCAGGACTGAAAAGAAAAAAGTTTATTCTGAAGTTTGAGCAGATTACAGCCGCTGGGT  300
              ******************************   **************************

Pr1_Cl_1      GAAATGAAGTTTTGCACACCAAGAGGCTAGGGGAAAGCATCCCTCCTCAGTTCATCCGCT  360
Pr1_Cl_2      GAAATGAAGTTTTGCACACCAAGAGGCTAGGGGAAAGCATCCCTCCTCAGTTCATCTGCT  360
Pr1_Cl_3      GAAATGAAGTTTTGCACACCAAGAGGCTAGGGGAAAGCATCCCTCCTCAGTTCATCTGCT  360
Pr1_Cl_4      GAAATGAAGTTTTGCACACCAAGAGGCTAGGGGAAAGCATCCCTCCTCAGTTCATCTGCT  359
Pr2_Cl_1      GAAATGAAGTTTTGCACACCAAGAGGCTAGGGGAAAGCATCCCTCC--------------  292
Pr2_Cl_2      GAAATGAAGTTTTGCACACCAAGAGGCTAGGGGAAAGCATCCCTCC--------------  345
Pr2_Cl_3      GAAATGAAGTTTTGCACACCAAGAGGCTAGGGGAAAGCATCCCTCC--------------  342
Pr2_Cl_4      GAAATGAAGTTTTGCACACCAAGAGGCTAGGGGAAAGCATGCCTCC--------------  346
              ************************************** *****

Pr1_Cl_1      GAACATCAGCCTTCTGTTCTGTTGTGCAGGG  391
Pr1_Cl_2      GAACATCAGCCTTCTGTTCTGTTGTGCAGGG  391
Pr1_Cl_3      GAACATCAGCCTTCTGTTCTGTTGTGTAGGG  391
Pr1_Cl_4      GAACATCAGCCTTCTGTTCTGTTGTGCAGGG  390
Pr2_Cl_1      -------------------------------  292
Pr2_Cl_2      -------------------------------  345
Pr2_Cl_3      -------------------------------  342
Pr2_Cl_4      -------------------------------  346
```

Supplemental Figure 1. Sequence of the 5' region of rat SULT1C2 mRNA determined using rapid amplification of cDNA ends (RACE). The 5' mRNA sequence for rat SULT1C2 was determined using the SMARTer RACE cDNA Amplification Kit and gene-specific reverse primers targeting exon 3 of the reference sequence mRNA (NM_133547.4) as described in Methods. Following amplification, gel-purified PCR products were ligated into the pGEM-T Easy plasmid and sequenced. Sequences were aligned using Clustal Omega. (A) SULT1C2 reference sequence mRNA (NM_133547.4) aligned to the predicted *SULT1C2* locus on the chromosome 9 reference sequence (nt 4654278-4685416 of NC_005108.4, reverse orientation). Small vertical bars represent exons in the reference sequence mRNA. The lack of alignment of the 5'-end of NM_133547.4 with the predicted 5'-end of the *SULT1C2* gene reflects the fact that several computationally predicted mRNA variants have exon 1 sequences that align to the chromosome at upstream locations. (B) Alignment of SULT1C2 cDNA sequences obtained from 5' RACE. Shown are 4 clones each from 2 different reverse primers targeting exon 3 of the reference sequence mRNA as described in the Methods section.

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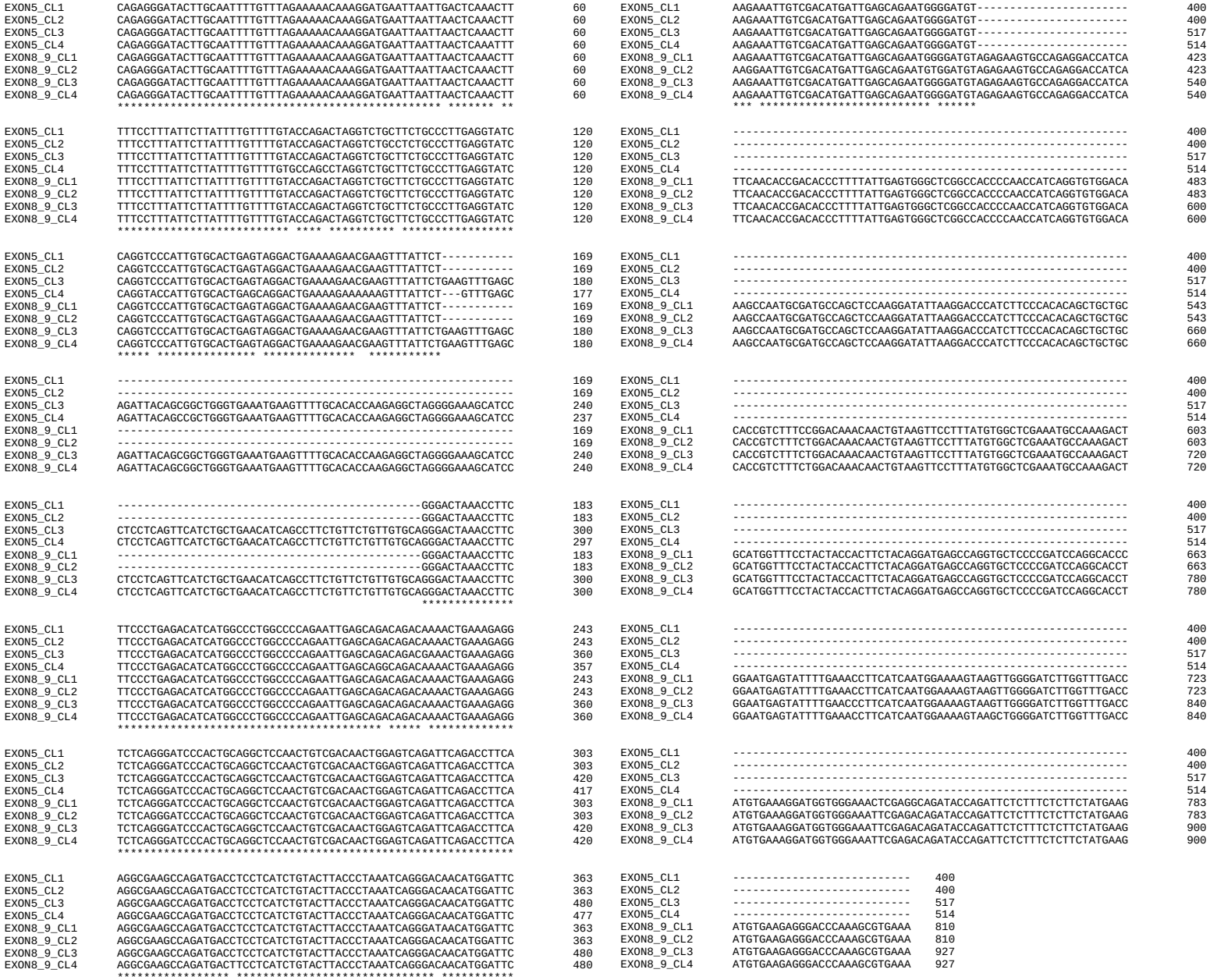

Supplemental Figure 2. Sequence and alignment of clones obtained through PCR validation of rat SULT1C2 mRNA as determined by RACE. Rat hepatic SULT1C2 cDNA was PCR amplified using a forward primer targeting nt 103-123 on exon 2 of the rat SULT1C2 mRNA identified through 5'-RACE and reverse primers targeting either exon 5 or exons 8-9 as described in the Methods. PCR purified bands were gel purified and cloned into the pGEM-T Easy plasmid for sequencing. Shown are the aligned sequences of individual cones (n=4/primer) determined using the Clustal Omega program.

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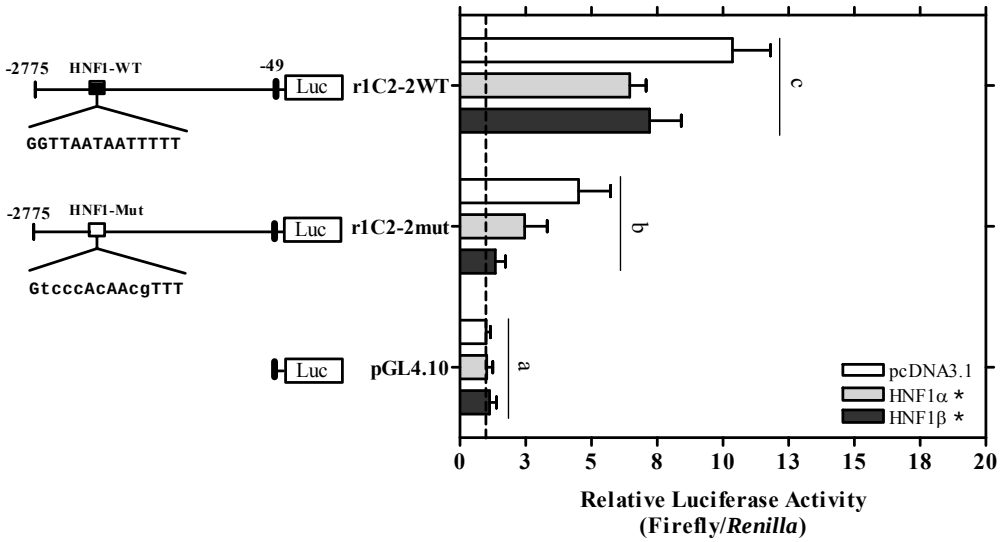

Supplemental Figure 3. Effect of overexpressing HNF1α or HNF1β on rat SULT1C2 promoter activity in primary cultured rat hepatocytes. Primary rat hepatocytes were freshly isolated and plated onto 12 well plates. Twenty four hours following transfection, hepatocytes were transiently transfected with 50 ng of an HNF1α or HNF1β expression plasmid or pcDNA3.1 control plasmid together with the r1C2-2 reporter, the r1C2-2 reporter containing a mutated HNF1 site (r1C2-2mut), or with an empty vector control (pGL4.10) as described in the Methods. Forty eight hours after transfection, cells were harvested for the measurement of luciferase activity. Bars represent the mean ± S.E.M. of luciferase measurements (Firefly/*Renilla*) normalized to the empty vector, pcDNA3.1 controls. Results are combined from 2 independent experiments (n=3 wells/treatment/experiment; each experiment represents one hepatocyte preparation). Significant effects of construct type and expression plasmid were detected. *, significant effect of the HNF1 expression plasmid compared to the pcDNA3.1 controls ($P<0.05$). Different letters denote significant differences among the individual constructs on luciferase reporter activity ($P<0.05$). See manuscript text for additional details.