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Supplemental Files
Drug Metabolism and Disposition

Down-regulation of sulfotransferase expression and activity in diseased human livers

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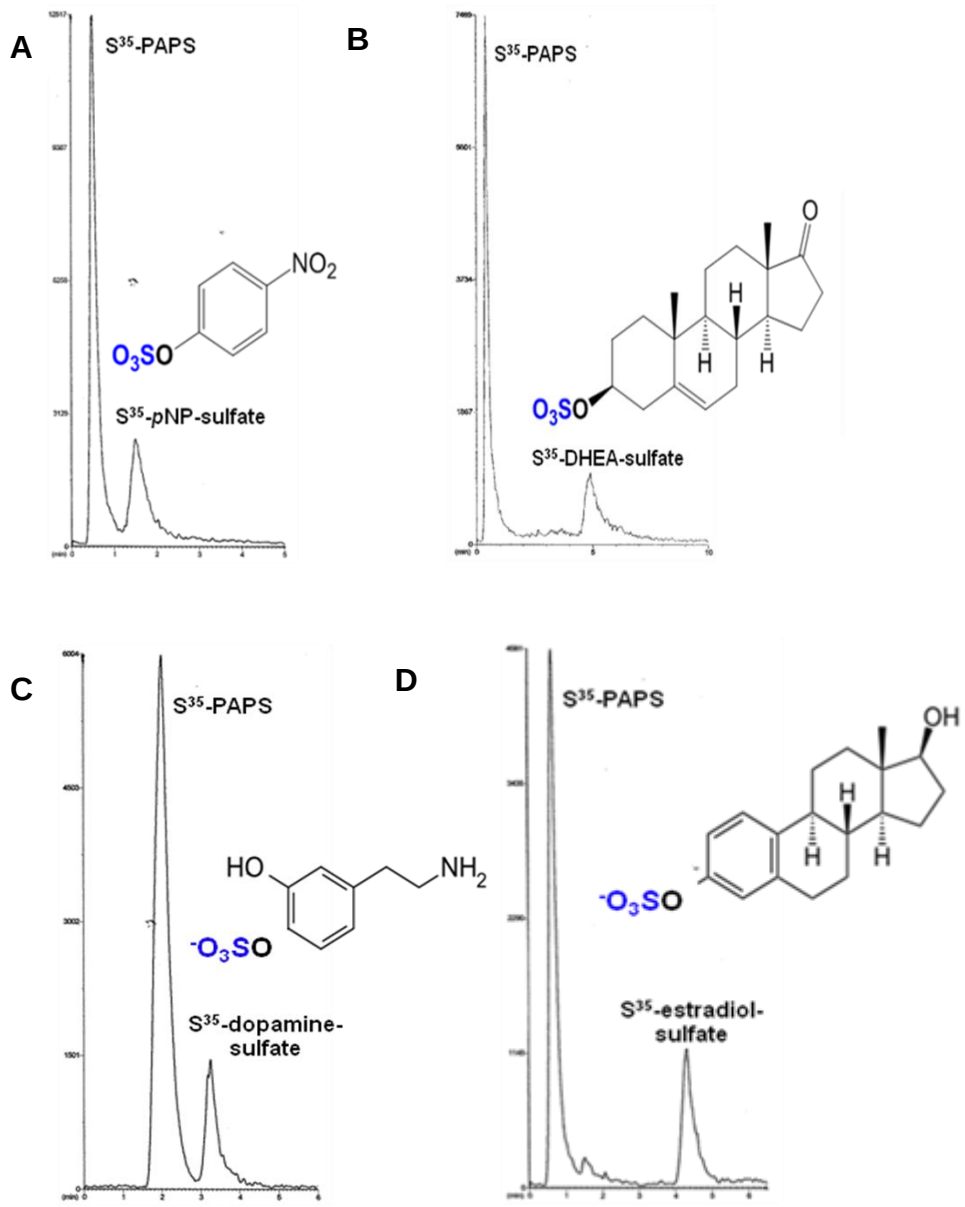
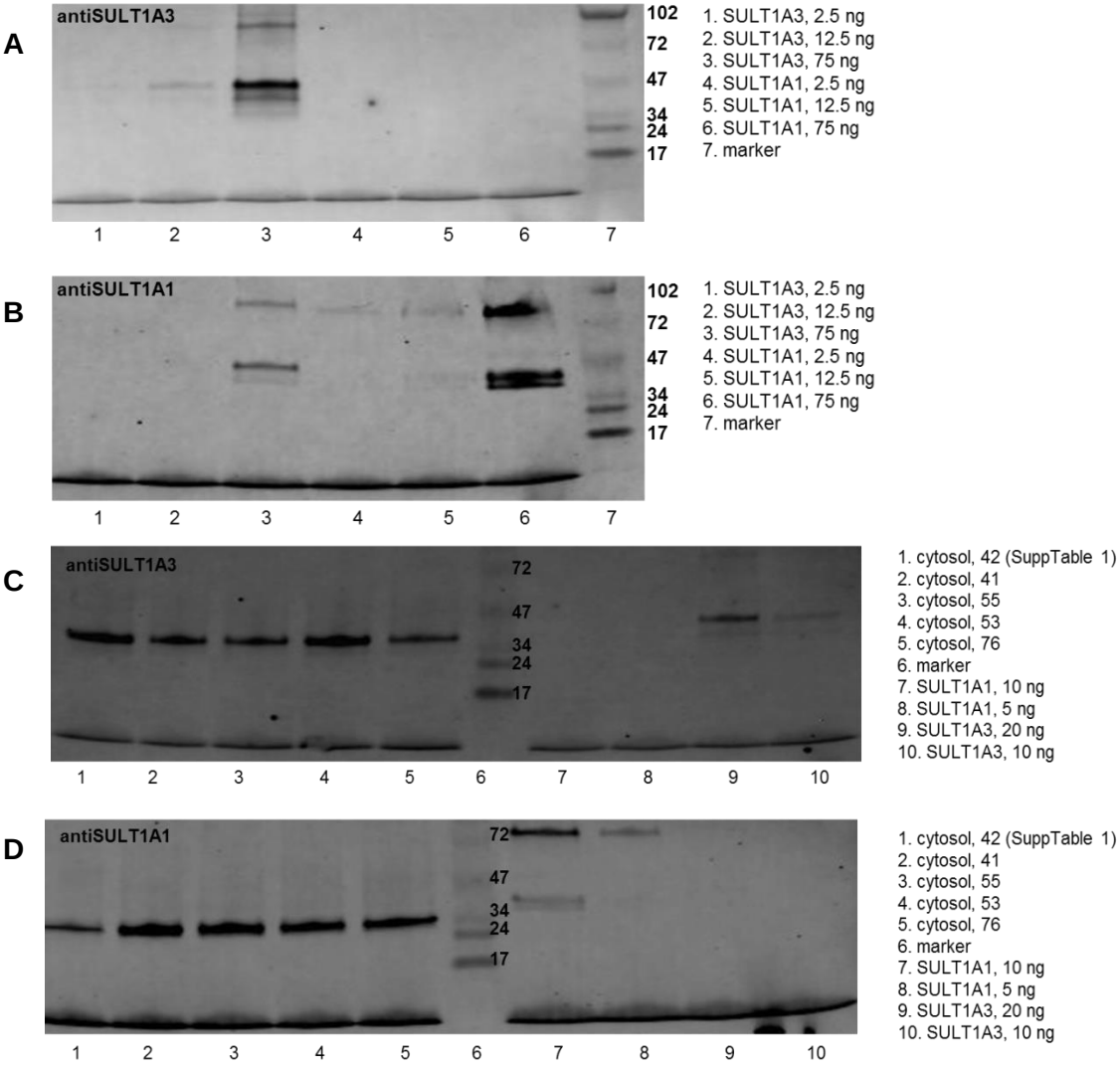


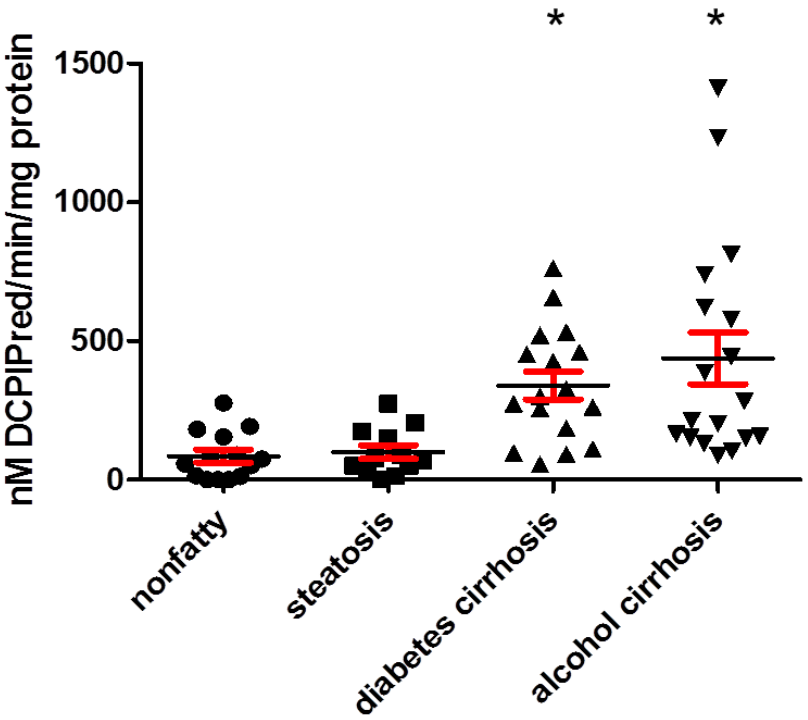
Supplemental Data, Figure 1. HPLC separation and radio detection of S-35 labeled metabolites (A) 35S-pNP-sulfate, (B) 35S-DHEA-sulfate, (C) 35S-dopamine-sulfate, and (D) 35S-estradiol-sulfate.

Supplemental Data, Figure 2



Supplemental Data, Figure 2. The specificities of SULT1A1 and SULT1A3 antibodies. The specificity of SULT1A1 antibody (Aviva Systems Biology) and SULT1A3 antibody (Abcam) against recombinant human SULT1A1 and SULT1A3 (Origene) and human liver cytosol protein were determined by Western blot using the same conditions (antibody source, dilution, and probing time) as for the original data. **(A)** Anti-SULT1A3 antibody recognized the monomer and dimer form of recombinant SULT1A3 (lanes 2,3) and did not cross-react with recombinant SULT1A1 even at very high loading (75 ng, lane 6). **(B)** Anti-SULT1A1 antibody recognized the monomer and dimer form of SULT1A1 recombinant protein (lanes 4,5,6), and also faintly detected a high loading amount of recombinant SULT1A3 (75 ng, lane 3). However, this limited amount of cross-reactivity would affect our results ONLY if SULT1A3 were present in the human liver cytosols at substantially higher concentrations than SULT1A1 (compare band intensity of lane 3 to lane 6, equal loading). **(C and D)** A subset of five of the 76 human liver samples were run next to recombinant SULT1A1 and recombinant SULT1A3, and were detected with anti-SULT1A3 **(C)** and anti-SULT1A1 **(D).** A single band at the expected molecular weight was detected for each liver sample on the blot, and the band intensity of individual samples was relative to previous blots. The recombinant proteins provided from Origene contain C-terminal MYC/DDK tag, thus their molecular weight is slightly higher than the native protein in human liver cytosol.

Supplemental Data, Figure 3



* differences between non-fatty and diseased livers ($p < 0.05$)

Supplemental Data, Figure 3. NAD(P)H:quinone oxidoreductase 1 (NQO1) activity assay in non-fatty (control) and diseased human liver. NQO1 activity was determined by measuring colorimetric reduction of Dichlorophenolindophenol (DCPIP) at 600 nm for an interval of 3 min. The reaction was performed in triplicate in 96 well plate containing 200 µM NAD(P)H, 40 µM DCPIP, and liver cytosol. Inhibition reactions were performed with the addition of 20 µM of dicumarol to the reaction mixture. The activity was determined by the difference in absorbance rates of the uninhibited and inhibited reaction rates, normalized to total cytosolic protein content, and expressed as nmole DCPIP reduced/min/mg protein. $P<0.05$ was considered statistically significant.

Supplemental Data, Table 1. Human Liver Donor Information

Sample ID	LTCDS code	DIAGNOSIS	GENDER	ETHNICITY	AGE	SGOT	AP
1	HH1038	NON-FATTY	M	CAUCASIAN	47	227	NA
2	HH991-4740	NON-FATTY	M	HISPANIC	47	125	NA
3	HH995-5640	NON-FATTY	M	CAUCASIAN	56	22	NA
4	HH1014-5640	NON-FATTY	M	CAUCASIAN	56	36	NA
5	HH997-4340	NON-FATTY	M	BLACK	43	26	NA
39	D386	NON-FATTY	M	NA	45	NA	NA
40	D587	NON-FATTY	M	NA	49	NA	NA
41	HH987-5740	NON-FATTY	M	CAUCASIAN	57	54	NA
42	HH996-5540	NON-FATTY	M	CAUCASIAN	55	47	NA
43	HH969-4640	NON-FATTY	M	CAUCASIAN	46	13	NA
6	HH985-5740	NON-FATTY	F	BLACK	57	257	NA
7	HH978-4140	NON-FATTY	F	CAUCASIAN	41	0	NA
8	HH968-5940	NON-FATTY	F	CAUCASIAN	59	13	NA
9	HH979-5440	NON-FATTY	F	ASIAN	54	0	NA
10	HH1013-5340	NON-FATTY	F	CAUCASIAN	53	58	NA
44	HH999-5740	NON-FATTY	F	CAUCASIAN	57	104	NA
45	HH1009-4940	NON-FATTY	F	HISPANIC	49	92	NA
46	HH989-5440	NON-FATTY	F	CAUCASIAN	54	38	NA
47	HH976-5040	NON-FATTY	F	CAUCASIAN	50	63	NA
48	HH982-5040	NON-FATTY	F	CAUCASIAN	50	60	NA
16	918	DIABETES	F	NA	55	528	154
17	935	DIABETES	F	0-WHITE	60	30	170
18	1202	DIABETES	F	2-ASIAN	42	405	115
19	1073	DIABETES	M	0-WHITE	57	35	124

11	HH883	STEATOSIS	M	CAUCASIAN	46	19	NA
12	HH870	STEATOSIS	M	CAUCASIAN	37	75	NA
13	HH930	STEATOSIS	M	CAUCASIAN	31	93	NA
14	HH1124	STEATOSIS	M	CAUCASIAN	40	230	NA
15	HH1020	STEATOSIS	M	CAUCASIAN	45	146	NA
49	HH969	STEATOSIS	M	CAUCASIAN	46	13	NA
50	HH894	STEATOSIS	M	CAUCASIAN	40	34	NA
51	HH1125	STEATOSIS	F	NA	49	59	NA
52	HH978	STEATOSIS	F	NA	41	0	NA
53	HH993	STEATOSIS	F	NA	46	52	NA
54	HH967	STEATOSIS	F	CAUCASIAN	48	80	NA
55	HH977	STEATOSIS	F	CAUCASIAN	38	23	NA
56	HH990	STEATOSIS	F	NA	37	239	NA
77	HH1085	STEATOSIS	M	CAUCASIAN	46	186	NA
20	1018	DIABETES CIRRHOSIS	M	NA	56	3930	226
21	1146	DIABETES CIRRHOSIS	M	0-WHITE	65	68	118
22	1101	DIABETES CIRRHOSIS	M	0-WHITE	36	144	222
23	1019	DIABETES CIRRHOSIS	M	0-WHITE	52	30	173
24	1003	DIABETES CIRRHOSIS	M	0-WHITE	62	71	79
25	989	DIABETES CIRRHOSIS	F	NA		34	180
26	992	DIABETES CIRRHOSIS	F	2-ASIAN	62	91	1168
27	1054	DIABETES CIRRHOSIS	F	0-WHITE	62	43	224
28	1006	DIABETES CIRRHOSIS	F	0-WHITE	58		69
29	970	DIABETES CIRRHOSIS	F	0-WHITE	62	25	88
57	1288	DIABETES	M	0-WHITE	54	97	142

		CIRRHOSIS					
58	1138	DIABETES, CIRRHOSIS CRYPTOGENIC	M	NA		52	105
59	1015	DIABETES CIRRHOSIS	M	0-WHITE	39	1720	178
60	1141	DIABETES TYPE-1, CIRRHOSIS CRYPTOGENIC	M	0-WHITE	51	31	96
61	1278	DIABETES CIRRHOSIS	M	0-WHITE	49	57	189
62	1232	DIABETES CIRRHOSIS	M	0-WHITE	50	200	259
63	1107	DIABETES, CIRRHOSIS CRYPTOGENIC	M	0-WHITE	72	36	216
64	1144	DIABETES, CIRRHOSIS CRYPTOGENIC	M	1-BLACK	33	43	330
65	1090	DIABETES TYPE-1, CIRRHOSIS	M	2-ASIAN	39	283	229
66	1188	DIABETES, CIRRHOSIS CRYPTOGENIC	M	NA	66	52	415
67	1198	DIABETES TYPE-1, CIRRHOSIS	F	0-WHITE	43	62	1763
68	1172	DIABETES CIRRHOSIS PRIMARY BILIARY	F	0-WHITE	53	36	163
30	769	ALCOHOL CIRRHOSIS	F	0-WHITE	36	41	NA
31	788	ALCOHOL CIRRHOSIS	M	0-WHITE	55	29	82
32	1079	ALCOHOL CIRRHOSIS	M	0-WHITE	63	102	258
33	1232	ALCOHOL CIRRHOSIS	M	0-WHITE	50	200	259

34	1177	ALCOHOL CIRRHOSIS	M	0-WHITE	57	36	184
35	815	ALCOHOL CIRRHOSIS	F	0-WHITE	44	240	145
36	869	ALCOHOL CIRRHOSIS	F	0-WHITE	47	553	285
37	1108	ALCOHOL CIRRHOSIS	F	NA	48	36	116
38	1126	ALCOHOL CIRRHOSIS	F	0-WHITE	47	50	140
69	1265	ALCOHOL CIRRHOSIS	M	0-WHITE	47	80	191
70	994	ALCOHOL CIRRHOSIS	M	NA	33	501	51
71	1048	ALCOHOL CIRRHOSIS	M	NA	42	28	305
72	1075	ALCOHOL CIRRHOSIS	M	0-WHITE	56	77	121
73	1251	ALCOHOL CIRRHOSIS	M	0-WHITE	48	54	261
74	1106	ALCOHOL CIRRHOSIS	M	0-WHITE	54	48	147
75	661	ALCOHOL CIRRHOSIS	F	NA	52	43	165
76	961	ALCOHOL CIRRHOSIS	F	NA	51	27	97
78	931	ALCOHOL CIRRHOSIS	F	NA	33	681	56
79	1125	ALCOHOL CIRRHOSIS	M	0-WHITE	48	37	174
80	1244	ALCOHOL CIRRHOSIS	M	0-WHITE	52	1425	114
81	1217	ALCOHOL CIRRHOSIS	M	0-WHITE	44	115	122
82	965	ALCOHOL CIRRHOSIS	M	NA	46	47	159
83	1280	ALCOHOL CIRRHOSIS	M	0-WHITE	55	52	178
84	1286	ALCOHOL CIRRHOSIS	M	0-WHITE	59	32	91

Supplemental Data, Table 2. Summary of western blot conditions for detection and quantification of sulfotransferase isoforms.

	SULT1A1	**SULT2A1**	**SULT1E1**	**SULT1A3**
Immunogen for 1º Antibody	ELIQDTSRP PLEYVKGVP LIKYFAEALG PLQSFQARP DDLLISTYPK SGT Aviva System Biology®	KLH conjugated synthetic peptide selected from C-terminal Abcam®	LMVAGHPN PGSFPEFVE KFMQGQVP YGSWYKHV KSWWEKGK SPRVLFLFY Aviva System Biology®	Synthetic peptide corresponds to residues in human SULT1A3 Abcam®
host	rabbit	rabbit	rabbit	rabbit
purification	affinity purified	protein G purified	affinity purified	tissue culture supernatant
concentration	250 ng/ml	25 ng/ml	1000 ng/ml	10 µl in 10 ml TBST
incubation time	overnight	1 hour	overnight	overnight
2º Antibody dilution	1:10,000	1:10,000	1:10,000	1:10,000