# Supplemental Data

**Interaction of the brain-selective sulfotransferase SULT4A1 with other cytosolic sulfotransferases: effects on protein expression and function.**

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1. Yeast-2-hybrid assay

*Cloning*

The generation of the DNA binding domain fusion vector pGBKT7-SULT4A1 and the activating domain fusion vector pGADT7-SULT4A1 have been described previously (Mitchell and Minchin, 2009). To generate the GST-SULT4A1 and 1A1 constructs the SULT4A1 and 1A1 coding sequences were PCR amplified using the following forward and reverse primers containing BamH1 (SULT4A1) or EcoR1 (SULT1A1) together with Sal1 restriction enzyme sites (underlined); (4A1 forward) 5'-GATCGGATCCATGGCGGAGAGCGAG-3', (4A1 reverse) 5'-CTAGGTCGACTTATAAATAAAAGTCAAACGTGAGGTC-3', (1A1 forward) 5'-GATCGGAATTCCCATGGAGCTGATCCAGGA-3' and (1A1 reverse) 5'-GATCGTCGACTCACAGCTCAGAGCGGAAG-3'. The digested PCR fragments were then inserted into the appropriate restriction sites of pGEX6P (GE Healthcare Bio-Sciences). To make the pGBKT7 and pGADT7 SULT1A1 or SULT1A3 constructs, the coding sequence of human SULT1A1 or SULT1A3 was PCR amplified using the following primers in which the Nde1 and EcoR1 sites were integrated (underlined) for cloning into the appropriate sites in pGBKT7 and pGADT7; 5'-GATCGACATATGATGGAGCTGATCCAGGACAC-3' and 5'-GATCGAGAATTCTCACAGCTCAGAGCGGAAGC-3'.

*Yeast Two-Hybrid SULT Interaction Screen*

The pGBKT7 cloned SULT bait proteins were transformed into the AH109 yeast strain and mated with the Y187 yeast transformed pGADT7 cloned prey proteins as per the manufacturer's instructions (Clontech, CA, USA). Protein interactions were selected for on $Leu^-$, $Trp^-$, $His^-$, and $Ade^-$ solid media. Specific interactions were validated by co-expressing SULT proteins with Lamin to confirm reporter activation specificity. To investigate the involvement of flanking sequences and fusion proteins in reporter activation, SULT proteins were tested in both bait and prey constructs.

Results:

**Supplemental Table 1: Interaction of the cytosolic aryl sulfotransferases by yeast-2-hybrid assay**

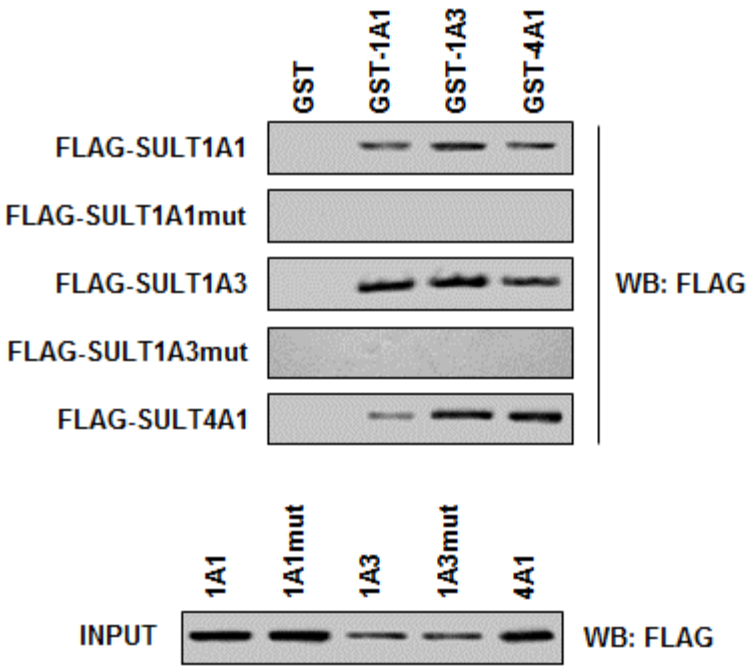
| Prey: | 1A1 | 1A2 | 1A3 | 1B1 | 1C1 | 1E1 | 2A1 | 4A1 | Lamin |
|---|---|---|---|---|---|---|---|---|---|
| 4A1 | +++ | +++ | +++ | +/- | - | +/- | +++ | +++ | - |

+++ = robust interaction, numerous large colonies
+/- = slight interact, few small colonies
- = no interact, no colony formation

2. GST pulldown

For GST pulldowns, recombinant GST-tagged proteins were expressed in *E. coli* strain BL21-Codon Plus (DE3)-RIL (Stratagene, La Jolla, CA) with 0.1 mM Isopropyl β-D-1-thiogalactopyranoside (Sigma) at 37°C. Bacterial cells were harvested and lysed in NP-40 lysis buffer on ice for 15 min. Lysates were cleared by centrifugation at 14,000 × *g* for 10 min at 4°C. Expressed GST or GST-SULT was purified using glutathione Sepharose 4B (GE Healthcare Bio-Sciences). FLAG-SULTs were purified from transiently transfected HeLa cells as follows: cells were washed in phosphate buffered saline (PBS) and whole cell extracts were prepared by incubation in NP-40 lysis buffer (1% Nonidet P-40, 50 mM Tris [pH 8.0], 150 mM NaCl, 5 mM NaF, 1 mM $Na_3VO_4$, 10 mM β-glycerophosphate, 1mM EDTA and protease inhibitor cocktail (Roche; Basel, Switzerland) for 15 min on ice. Lysates were cleared by centrifugation at 14,000 × *g* for 10 min at 4°C. Cell lysates were incubated with 25 μg of GST or GST-SULT on glutathione Sepharose for 4 h at 4°C. Beads were washed 3 × with wash buffer (0.5% Nonidet P-40, 50 mM Tris [pH 8.0], 150 mM NaCl) followed by suspension in Laemmli buffer. Samples were separated using SDS-PAGE, transferred to nitrocellulose membrane and immunoblotted with mouse anti-FLAG M2 horseradish peroxidase (HRP) conjugated antibody (Sigma) to detect FLAG-SULT binding.

Results:




**Supplemental Figure 1:** *In vitro* interaction of SULT4A1, 1A1 and 1A3 by GST-pulldown assays. Cell lysates from HeLa cells expressing exogenous FLAG-tagged SULTs (wild-type and dimerization mutants) were incubated with the different recombinant GST-SULT fusion proteins on glutathione sepharose beads. Interaction between FLAG-tagged SULTs and GST-SULT fusion proteins was assessed by FLAG Western blot. A FLAG Western blot of input samples is shown (lower panel) to confirm expression of the FLAG tagged SULT proteins.

Mitchell DJ, Minchin RF. Cytosolic Aryl sulfotransferase 4A1 interacts with the peptidyl prolyl cis-trans isomerase Pin1. Mol Pharmacol 2009;76:388-95.