



Title	Sulfotransferases (SULTs), enzymatic and genetic variation in Carnivora : Limited sulfation capacity in pinnipeds
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1 **Sulfotransferases (SULTs), enzymatic and genetic variation in Carnivora:**
2 **Limited sulfation capacity in pinnipeds.**

3

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51 **Abbreviations**

52 AAALAC: Association for Assessment and Accreditation of Laboratory Animal Care

53 CE: collision energies

54 DW: distilled water

55 GSTs: glutathione-S transferase

56 HPLC: high-performance liquid chromatography

57 KO: knockout

58 KPB: potassium phosphate buffer

59 LC/MS: liquid chromatography/mass spectrometry

60 MEGA: Molecular Evolutionary Genetics Analysis

61 MUSCLE: Multiple Sequence Comparison by Log-Expectation

62 NATs: N-acetyltransferases

63 NCBI: National Center for Biotechnology Information

64 OCPs: organochlorine pesticides

65 PAHs: polyhalogenated aromatic hydrocarbons

66 PAPS: 3'-phosphoadenosine 5'-phosphosulfate

67 PBDE: polybrominated diphenyl ether

68 PCB: polychlorinated Biphenyl

69 SGF29: SAGA complex associated factor 29

70 SNP: single nucleotide polymorphism

71 STS: steroid sulfatase

72 SULTs: sulfotransferases

73 UGTs: UDP-glucuronosyltransferases

74 **Abstract**

75 Wild carnivorans are one of the most important species due to their high positions in
76 the food chain. They are also highly affected by numerous environmental
77 contaminants through bioaccumulation and biomagnification. Xenobiotic metabolism
78 is a significant chemical defense system from xenobiotics because it degrades the
79 activity of a wide range of chemicals, generally into less active forms, resulting in their
80 deactivation. Sulfotransferases (SULTs) are one of the most important xenobiotic
81 metabolic enzymes, which catalyze the sulfonation of a variety of endogenous and
82 exogenous chemicals, such as hormones, neurotransmitters, and a wide range of
83 xenobiotic compounds. Although SULTs are of such high importance, little research
84 has focused on these enzymes in wild carnivorans. In this study, we clarified the
85 genetic properties of SULTs in a wide range of mammals, focusing on carnivorans,
86 using *in silico* genetic analyses. We found genetic deficiencies of SULT1E1 and
87 SULT1D1 isoforms in all pinnipeds analyzed and nonsense mutations in SULT1Cs in
88 several carnivorans including pinnipeds. We further investigated the enzymatic activity
89 of SULT1E1 *in vitro* using liver cytosols from pinnipeds. Using a SULT1E1 probe
90 substrate, we found highly limited estradiol sulfonation in pinnipeds, whereas other
91 mammals had relatively high sulfation. These results suggest that pinnipeds have
92 severely or completely absent SULT1E1 activity, which importantly catalyzes the
93 metabolism of estrogens, drugs, and environmental toxins. This further implies a high
94 susceptibility to a wide range of xenobiotics in these carnivorans, which are constantly
95 exposed to environmental chemicals throughout their lifetime.

96

97 Keywords: Wildlife, xenobiotic metabolism, in silico analysis, genome database, phase

98 II metabolism

99

100 **1. Introduction**

101 Cytosolic sulfotransferases (SULTs) are an essential metabolic enzyme superfamily
102 that catalyzes sulfate conjugation for various endogenous and exogenous compounds
103 including neurotransmitters, hormones, drugs, and environmental toxins (Falany
104 1991; Blanchard et al. 2004; Gamage et al. 2006; Coughtrie 2016; Suiko et al. 2017).
105 Using 3'-phosphoadenosine 5'-phosphosulfate (PAPS) as a sulfonate donor, SULTs
106 transfer sulfuric moieties to acceptor compounds and alter their bioactivity, typically
107 towards less active and more water-soluble forms, thus accelerating their excretion.
108 SULTs are primarily major phase II xenobiotic detoxification enzymes, which catalyze
109 conjugations after phase I reactions (oxidation, reduction, and hydrolysis), together
110 with UDP-glucuronosyltransferases (UGTs), N-acetyltransferases (NATs), and
111 glutathione-S transferase (GSTs) (Almazroo et al., 2017; Jancova et al., 2010; Oda et
112 al., 2015).

113 The mammalian SULT superfamily consists of at least seven families, SULTs 1–7.
114 The SULT1 family, also known as phenol-SULTs, is well characterized and is
115 responsible for the metabolism of xenobiotics and a variety of endogenous chemicals
116 (Blanchard et al. 2004, Coughtrie 2016). The SULT1 family is further divided into five
117 different subfamilies including SULT1A, 1B, 1C, 1D, and 1E. Each subfamily has
118 distinct substrate specificities, although some overlap exists. The substrate
119 specificities of the SULT subfamilies are generally considered to be as follows:
120 SULT1A members for simple phenols, 1B members for thyroid hormones, 1C
121 members for hydroxyaryl amines, 1D members for catecholamines, and 1E members
122 for estrogens (Kester et al., 2003; Sakakibara et al., 1998; Shimada et al., 2004; Suiko
123 et al., 2017; Carrie Tsoi et al., 2001). Although SULT1 isoforms and their function have

124 been well characterized in humans, rodents, and a few other experimental animal
125 models (Liu et al., 1999; Teramoto et al., 2008; Carrie Tsoi et al., 2001; Tsoi et al.,
126 2002; Wilson et al., 2004), information is still limited in other mammalian species
127 including wild mammals.

128 Wild mammals are continuously exposed to a vast variety of environmental chemicals,
129 such as polyhalogenated aromatic hydrocarbons (PAHs) and organochlorine
130 pesticides (OCPs) (Nomiyama et al., 2014; Noyes and Lema, 2015). Due to their high
131 position in their respective food chains, carnivorans (the order of the placental
132 mammals includes dogs, pinnipeds, weasels, racoons, bears, felines, mongooses,
133 and hyenas) accumulate severe amounts of lipophilic environmental contaminants
134 more so than other species (Huang et al., 2018; Johnson, 2019; Rodríguez-Estival
135 and Mateo, 2019). Therefore, the toxicological assessment of carnivorans is critically
136 needed. However, xenobiotic metabolic enzymes in wild mammals are not clearly
137 understood. We recently reported the genetic deficiencies and in vitro enzymatic
138 dysfunction of some UGTs (UGT1A6s and 2B31s) in feline and pinniped species,
139 which suggest that these species may poorly metabolize chemical compounds (Kakehi
140 et al. 2015; Kondo et al. 2017). Since UGTs and SULTs are known to have similar
141 substrate specificities, and some excreted polyphenols and chemicals are
142 glucuronide-sulfate double conjugated, UGTs and SULTs may play concerted roles in
143 xenobiotic metabolism (Böhmdorfer et al., 2017; Suiko et al., 2017). Considering the
144 synergistic actions of UGTs and SULTs, information about SULTs in wildlife carnivorous
145 species should be elucidated to facilitate a comprehensive understanding of xenobiotic
146 metabolism in these mammals. The importance of the SULT1 family in xenobiotic
147 metabolism and the lack of information about its function have led us to investigate the

148 genetic and enzymatic features of SULTs in wild mammals including pinnipeds and
149 felines.

150 In this study, the genetic information of the SULT1 isozymes of various carnivorans
151 including pinnipeds and Felidae were collected from the NCBI GenBank data, and in
152 silico phylogenetic analyses were conducted. In addition, gene loci coding SULT
153 isoforms in these species were investigated and compared to understand the
154 evolutionary background of each isoform. Furthermore, the in vitro SULT activities of
155 cats, rats, and pinnipeds (northern fur seal, harbor seal, stellar sea lion) were
156 measured using liver cytosolic fractions.

157

158 **2. Materials and methods**

159 **2-1. Chemicals**

160 β -Estradiol and PAPS were obtained from Sigma-Aldrich (St. Louis, MO, USA).
161 Acetonitrile, formic acid, sodium phosphate, and potassium dichromate were
162 purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). β -Estradiol 3-
163 (β -d-sulfate) sodium salt was obtained from Santa Cruz Biotechnology, Inc. (Santa
164 Cruz, CA, USA). All chemicals used for high-performance liquid chromatography
165 (HPLC) and mass spectrometry (MS) were HPLC or MS grade and were obtained from
166 Kanto Chemical Co., Inc. (Tokyo, Japan).

167

168 **2-2. Animals**

169 Details about the animals used for liver cytosol preparations are provided in
170 Supplementary Table S1. Liver samples were collected from northern fur seals
171 (*Callorhinus ursinus*), harbor seals (*Phoca vitulina*), cats (*Felis catus*), and rats (*Rattus*

172 norvegicus; Sprague–Dawley strain). Harbor seal livers from Erimo were collected
173 from individuals accidentally captured by fishing nets and drowned. Northern fur seal
174 livers were provided by the Environmental Specimen Bank (es-BANK: [http://esbank-](http://esbank-ehime.com/)
175 [ehime.com/](http://esbank-ehime.com/)) of Ehime University. Eight-week-old rats were used as controls.
176 Sprague–Dawley rats were purchased from Sankyo Labo Service Corporation, Inc.
177 (Tokyo, Japan). Cats (Narc: Catus, 24–28 months old, male, weight: 1 kg) were
178 purchased from Kitayama Labes Co., Inc. (Nagano, Japan). Seven-week-old rats were
179 housed at a constant temperature ($23^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and constant humidity ($55\% \pm 5\%$)
180 with automatically controlled lighting (lights on from 07:00–19:00) and were given food
181 and water ad libitum for one week prior to sacrifice. Rats and cats were kept in a 12-
182 hour light/dark cycle (7:00–19:00 light, 19:00–7:00 dark) at $20 \pm 1^{\circ}\text{C}$ with $35 \pm 5\%$
183 humidity. Food (Royal Canin, Japan) and water were given appropriately twice a
184 day. Cat livers were collected following anesthesia with pentobarbital and euthanasia
185 by KCl injection. Dissections were performed by a qualified veterinarian. Liver samples
186 from all five species were immediately frozen in liquid nitrogen and stored at -80°C
187 until further use. All experiments and animal care for rats and cats were performed in
188 accordance with the guidelines of the Association for Assessment and Accreditation of
189 Laboratory Animal Care International (AAALAC) and under the supervision and with
190 the approval of the Institutional Animal Care and Use Committee of Hokkaido
191 University (no. 13-0213, no. 14-0054).

192

193 **2-3. Measurements of in vitro SULT activity using carnivore liver cytosols**

194 *2-3-1. Preparation of liver cytosols*

195 Liver cytosolic fractions were prepared as previously shown by Omura and Sato
196 (1964). Briefly, approximately 5 g of liver tissue from each of the six species were
197 homogenized in 15 mL of potassium phosphate buffer (KPB: 0.1 M, pH 7.4).
198 Homogenates were transferred into tubes and centrifuged at $9,000 \times g$ at 4°C for 20
199 minutes. The supernatants were further centrifuged at $105,000 \times g$ for 70 minutes to
200 separate microsomal and cytosolic fractions. The cytosolic fraction (supernatant) was
201 transferred and stored at -80°C until further analysis. Protein concentrations in the
202 cytosol were measured using a BCA (Bicinchoninic acid) protein assay reagent kit
203 (Pierce, Rockford, IL, USA).

204

205 *2-3-2. In vitro sulfation assay*

206 SULT activities for each of the five substrates were assessed. First, 25 μL of hepatic
207 cytosolic solution was mixed with 22.5 μL of KPB (0.1 M, pH 7.4). The cytosol
208 preparation was mixed with 2.5 μL of 1% sodium cholate solution and incubated on
209 ice for 30 minutes. 50 μL of cytosolic solution was mixed with KPB (0.1 M, pH 7.4), 5
210 μL of 100 mM MgCl_2 , and estradiol dissolved in methanol, resulting in a final
211 concentration of 1.25% in a total volume of 195 μL . Final substrate concentrations
212 varied from 12.5 μM to 400 μM for estradiol. Samples were preincubated at 37°C for
213 5 minutes, and the sulfation reaction was initiated by adding 5 μL of 100 mM PAPS.
214 Samples were incubated for 15 minutes, and the reaction was stopped by adding 200
215 μL of ice-cold methanol. Reaction samples were then placed on ice for 15 minutes
216 before centrifugation at $750 \times g$ for 10 minutes. The resultant supernatants were
217 injected into a liquid chromatography/mass spectrometry (LC/MS) system.

218

219 *2-3-3. Analysis of sulfate metabolite by LC/MS/MS*

220 An HPLC system coupled with electrospray ionization ion-trap triple-quadrupole
221 mass spectrometry (ESI/MS/MS, LC-8030, Shimadzu, Kyoto, Japan) was equipped
222 with a Wakopak® Ultra C18-3 column (2.0 mm × 100 mm; Wako Pure Chemical
223 Industries, Ltd., Osaka, Japan). Mobile phase A consisted of 0.1% formic acid in
224 distilled water (DW), and phase B consisted of 0.1% formic acid in acetonitrile in all
225 analyses. The percentage of mobile phase B was changed linearly as follows: 2 min,
226 30%; 25 min, 70%; 26 min, 90%; 28 min, 90%; and 30 min, 30%. The injection volume
227 was 5 µL, the flow rate was 0.2 mL/min, and the column temperature was 40°C.
228 The m/z of β-estradiol-3-sulfate was 351 > 271.

229

230 *2-3-4. Data analysis*

231 All kinetic parameters, including maximum velocity (V_{max}), Michaelis–Menten
232 constant (K_m), and V_{max}/K_m ratio, were determined using the Michaelis–Menten
233 equation and GraphPad Prism version 5.0 for Windows (GraphPad Software, San
234 Diego, CA, USA). Statistical analyses were performed using JMP® 12 (SAS Institute,
235 Inc., Cary, NC, USA). Tukey's HSD test was used for the V_{max}/K_m of each substrate
236 for each species; differences of P < 0.05 were considered statistically significant in all
237 analyses.

238

239 **2-4. In silico genetic analysis of SULTs in carnivores**

240 *2-4-1. Phylogenetic analysis of SULT genes*

241 Phylogenetic analyses were performed on the SULT1 genes (SULT1As, 1B1, 1Cs,
242 1D1, 1E1) of human, rat, mouse, dog, red fox, domestic ferret, ermine, American river

243 otter, sea otter, polar bear, giant panda, brown bear, meerkat, striped hyena, cat, Amur
244 tiger, cheetah, puma, Canada lynx, leopard, Weddell seal, harbor seal, gray seal,
245 Hawaiian monk seal, northern fur seal, southern elephant seal, Stellar sea lion,
246 California sea lion, and Pacific walrus origins. Sequences were retrieved using
247 National Center for Biotechnology Information (NCBI) BLAST searches using human
248 and dog SULT1A1, 1B1, 1C1, 1C2, 1C3, 1E1 and SULT1D1 as the query sequence.
249 BLAST searches have been conducted for database Nucleotide collection (nr/nt) for
250 each species using Blastn (Optimize for somewhat similar sequences). The gene
251 sequences used are listed in Supplementary Table S2, and the protein coding region
252 of each isozyme was analyzed. The deduced amino acid sequences were aligned
253 using MUSCLE (Multiple Sequence Comparison by Log-Expectation) and were used
254 for model selection (model showing minimal set of BIC and AICc were chosen) and
255 construction of maximum likelihood trees (bootstrapping = 100) using MEGA X
256 (Molecular Evolutionary Genetics Analysis) (Kumar et al., 2018). The JTT+G model
257 was used. All positions containing gaps and missing data were eliminated, and total
258 924 bp length of protein-coding sequence alignment are used for phylogenetic
259 analysis. The results of phylogenetic analyses for human, mouse, rat, and dog SULT1
260 genes were examined in reference to the phylogenetic analysis of published papers (C.
261 Tsoi et al. 2001; Blanchard et al. 2004) to ensure that the analysis was conducted
262 successfully.

263

264 *2-4-2. Synteny analysis of SULT1 genes*

265 Sequence data from genome projects are freely available. NCBI's genome data
266 viewer (<https://www.ncbi.nlm.nih.gov/genome/gdvl/>) was used to visualize the

267 chromosomal synteny maps for each species. The following latest genome assemblies
268 were used: human Annotation Release 106, rat Annotation Release 105, mouse
269 Annotation Release 105, dog Annotation Release 103, cat Annotation Release 102,
270 Weddell seal Annotation Release 100, red fox Annotation Release 100, domestic ferret
271 Annotation Release 101, ermine Annotation Release 100, American river otter
272 Annotation Release 100, sea otter Annotation Release 100, polar bear Annotation
273 Release 101, giant panda Annotation Release 103, brown bear Annotation Release
274 101, meerkat Annotation Release 100, striped hyena Annotation Release 100, Amur
275 tiger Annotation Release 100, cheetah Annotation Release 101, puma Annotation
276 Release 100, Canada lynx Annotation Release 102, leopard Annotation Release 100,
277 harbor seal Annotation Release 100, gray seal Annotation Release 100, Hawaiian
278 monk seal Annotation Release 100, northern fur seal Annotation Release 100,
279 southern elephant seal Annotation Release 100, Stellar sea lion Annotation Release
280 100, California sea lion Annotation Release 100, and Pacific walrus Annotation
281 Release 101. UCSC (University of California, Santa Cruz) BLAT (BLAST-like
282 alignment tool) (<http://genome.ucsc.edu/index.html>) was used for the additional
283 confirmation of missing genes.

284

285 3. Results

286 3-1. In silico genetic analysis of the SULT1 family in carnivores

287 3-1-1. SULT1 family in carnivorans and phylogenetic analysis of SULT1s

288 Potential SULT1 family isoforms in carnivorans were retrieved using BLAST searches,
289 and candidate isoforms equivalent to UGT1A1, 1B1, 1C1, 1C2, 1C3, 1C4, 1D1, and
290 1E1 were found in almost all carnivorans analyzed. Several genes were automatically
291 annotated, making their identification and naming confusing. Phylogenetic analyses
292 were conducted to clarify SULT isoforms in carnivorans and were tentatively renamed
293 based on their phylogeny. As shown in Figure 1, carnivoran SULT1A1s were in the
294 same clade as human and rodent SULT1A. Although humans had several SULT1A
295 isoforms (SULT1A1, 1A2, 1A3/4), carnivorans only had one isoform in the SULT1A
296 family (SULT1A1). Carnivoran SULT1B, 1D, and 1E genes were also in the same
297 clades as rodents and humans, respectively, and all mammals analyzed had either
298 one or no isoforms of SULT1B1, 1D1, or 1E1, with some pseudogenes, such as human
299 SULT1D1. Moreover, carnivoran SULT1Cs were also grouped into the same clade as
300 human and rodent SULT1Cs. Carnivoran SULT1C2s and 1C4s were classified into the
301 same clades as human or rodent SULT1C2s and human SULT1C4, respectively.
302 SULT1C1s in carnivorans were in the same clade as rat SULT1C3 and mouse
303 SULT1C1, whereas human SULT1C3 was not in the same clade as carnivorans and
304 rodents. According to the review by Coughtrie (2016), SULT1C3s are only present in
305 primates, which suggests that rat SULT1C3, mouse 1C1, and carnivoran SULT1C1s
306 are not orthologs of human SULT1C3 and are tentatively named SULT1C1s in this
307 article.

308

309 *3-1-2. SULT1 coding loci in mammals*

310 SULT1 coding loci in rodents, humans, and carnivorans were analyzed and
311 compared (Figure 2). SULT1A coding loci were highly conserved among Mammalia,
312 and almost all isozymes were coded next to SGF29 (SAGA Complex Associated
313 Factor 29) (data not shown). SULT1B1, 1D1, and 1E1 coding loci were also conserved,
314 and SULT1B1, 1D1, 1E1 were coded in the same loci between UGT2A1 (UDP
315 Glucuronosyltransferase Family 2 Member A1 Complex Locus) and CSN1S1 (Casein
316 Alpha S1) or CSN2 (Casein 2). Despite most mammals having the same genetic loci,
317 pinnipeds displayed different features. Almost all pinnipeds had SULT1D1
318 pseudogenes like the human SULT1D1 pseudogene. Some pinnipeds, such as
319 Weddell seals and harbor seals, had SULT1D1 protein coding genes. However, these
320 genes coded very short and low-quality proteins, suggesting that they encoded
321 dysfunctional SULTs. Moreover, SULT1E1s were not registered in any analyzed
322 pinnipeds (Weddell seal, harbor seal, gray seal, Hawaiian monk seal, northern fur seal,
323 southern elephant seal, Stellar sea lion, California sea lion, and Pacific walrus). To
324 investigate the existence of SULT1E1 in these species further, BLAST searches were
325 conducted using a human SULT1E1 query sequence (NM_005420.3) with datasets
326 from the Refseq Genome Database. No potential SULT1E1 sequences were observed
327 in any pinnipeds. All SULT1C isoforms were coded on conserved regions between
328 SLC5A7 (Solute Carrier Family 5 Member 7) and GCC2 (GRIP and coiled-coil domain
329 containing 2) in humans and carnivorans or SLC5A7 and SGOL1 (Shugoshin like 1)
330 in rodents. Rats had six isoforms equivalent to SULT1C1 and 1C2s (five isoforms),
331 whereas mice had two isoforms (SULT1C1 and 1C2). Carnivorans and humans had
332 three SULT1Cs isoforms each, whereas carnivorans had SULT1C1s, 1C2s, and 1C4s,

333 and humans had 1C3, 1C2, and 1C4 (Figures 1 and 2). In addition, phocids like the
334 Hawaiian monk seal, southern elephant seal, and Weddell seal had pseudogenes or
335 low-quality protein coding SULT1C1 isoforms. The low-quality protein coding genes
336 had stop codons within their sequences, suggesting dysfunctional genes, although
337 there were several gaps of scaffolded assembly in this locus in Weddell seals. Further
338 variations were observed in SULT1C2s in carnivorans, such as nonsense mutations
339 in residues 54, 131, and 264 (a PAPS binding site) of SULT1C2s in the Panthera genus
340 and in some pinnipeds. These mutations were present in residue 54 for Hawaiian
341 monk seals, gray seals, and harbor seals (Phocidae clade); residue 131 for lions and
342 leopards, but not tigers (Panthera) and walruses (Odobenidae); and residue 264 for
343 California sea lions, Stellar sea lions, northern fur seals, and walruses (Otariidae and
344 Odobenidae) (Supplementary Figure S2).

345

346 **3-2. In vitro activity of SULTs in the liver cytosols of pinnipeds**

347 Enzymatic properties including V_{max} , K_m , and V_{max}/K_m of estradiol sulfation are
348 shown in Table 1, and a Michaelis-Menten plot of estradiol sulfation activity is shown
349 in Figure 3. In vitro analysis of cat liver cytosols revealed a relatively high V_{max}/K_m
350 compared to that of rats and pinnipeds. Data obtained from cat liver cytosols were fit
351 for a substrate-inhibition model in a high dose range, which is commonly observed for
352 SULT activity. Estradiol-sulfate metabolites in Stellar sea lion and Harbor seal liver
353 cytosols were not detected. We detected UGT activity or CYP450 concentration using
354 same liver samples of these pinniped animals, and we detected certain amount of their
355 activity to make sure their liver samples were not degraded.

356

357 **4. Discussion**

358 **4-1. SULT1As are highly conserved in mammals**

359 In this study, we analyzed the phylogeny of SULT1 family members and found that
360 most isoforms were highly conserved in mammals. SULT1As in carnivorans were all
361 named SULT1A1 (or 1A1-like). Based on phylogenetic analyses, these isoforms
362 appeared to be orthologs of rodent SULT1A1s. Humans have two other isoforms of
363 SULT1A, SULT1A2, and 1A3/4. Like SULT1A1, human SULT1A2 is known to catalyze
364 the sulfation of simple and neutral phenols like nitrophenol. However, previous studies
365 have shown that SULT1A2 transcripts have a splicing defect and may not be translated.
366 No protein has been detected in any human tissues with a SULT1A2 antibody (Nowell
367 et al., 2005). Therefore, it is unlikely that this isoform is functionally active or that it
368 affects differences in xenobiotic metabolism between humans and carnivorans.
369 SULT1A3/4 shows catecholamine sulfation activity, is highly expressed in the
370 intestines of humans, cynomolgus macaques, and common marmosets, and plays
371 important roles in neurotransmitter biosynthesis and metabolism in the intestines
372 (Riches et al., 2009). To date, these isoforms have only been found in higher-order
373 primates (New World monkeys, Old World monkeys, apes, and humans), suggesting
374 that they were originally duplicated and diverted during primate evolution. This may
375 explain the lack of SULT1A3/4 orthologs in carnivorans.

376 **4-2. SULT1Bs are highly conserved in carnivorans**

377 The SULT1B1 isoform was known to be highly conserved in mammals. Surprisingly,
378 in our further investigation, even platypus and marsupials had orthologs of SULT1B1
379 (Supplementary Figure S2). Avian SULT1B1 and xenopus SULT1B isoforms
380 equivalent to mammalian SULT1B1 have also been characterized. SULT1B1 has a

381 similar substrate specificity as SULT1A1 but with lower affinity (for simple phenols and
382 thyroid hormones). Selective probe substrates for 1B1 still remain to be elucidated.
383 Interestingly, no endogenous substrate for xenopus 1B1 has been found, and it does
384 not catalyze the sulfation of thyroid hormones, which is a common substrate for
385 mammalian and avian SULT1B1s (Wilson et al., 2004; Yamauchi et al., 2019).
386 Therefore, the physiological functions of SULT1B1 isoforms are unclear, even though
387 these isoforms are highly conserved in tetrapods. Together with SULT1A1s, SULT1B1
388 may have evolved for exogenous metabolism and important xenobiotic defense
389 systems, yet affinity for their exogenous substrate is also low.

390 **4-3. SULT1D1 defects in pinnipeds suggest unique catecholamine metabolism**

391 SULT1D1 is another isoform that showed interspecies differences. Like humans, all
392 pinnipeds had SULT1D1s pseudogenes. However, carnivorans, rodents, and avian
393 species had orthologous SULT1D1 isoforms in conserved regions. Canine and mouse
394 SULT1D1 was cloned and characterized and had a high affinity for dopamine,
395 naphtha-1-ol, and PNP (Liu et al., 1999; Carrie Tsoi et al., 2001). Previous studies
396 using immunoblots have found that canine SULT1D1 was highly expressed in the
397 intestines and kidneys but lowly expressed in the liver (C. Tsoi et al., 2001). In rats,
398 SULT1D1 mRNA was highly expressed in the kidneys, followed by the intestines and
399 lungs, and lowly expressed in the liver. Thus, SULT1D1s were suggested to play
400 significant roles in sulfating catecholamines in the kidneys rather than in the liver.
401 Some reports have suggested that primate SULT1A3 could compensate for
402 catecholamine sulfation (Dajani et al., 1999, 1998) and may explain the presence of
403 the SULT1D1 pseudogene in primates. BLAST searches have suggested that the
404 SULT1D1 gene was only present in Strepsirrhini and Tarsiidae but not in higher

405 primates (data not shown), which is consistent with SULT1A3 expression in these
406 species. However, in pinnipeds, both SULT1A3 and 1D1 were missing from the
407 genome, indicating that sulfation of catecholamine in these animals may be limited.
408 Catecholamine sulfates are mainly found in the blood and may be precursors of active
409 molecules that are later deconjugated by sulfatases in peripheral tissues. SULTs may
410 be essential to regulate catecholamine function in other mammals. From our findings,
411 pinnipeds might have completely different pathways to regulate neurotransmitter
412 function and estrogen metabolism (this will be discussed later).

413 **4-4. Physiological significance of 1E1 defects in pinnipeds**

414 Surprisingly, one of the most important and well-characterized isoforms, SULT1E1,
415 was completely absent in pinnipeds including Phocidae, Otariidae, and Odobenidae.
416 In vitro enzymatic analysis also suggested remarkably limited SULT1E1 activity in the
417 liver of harbor seals, northern fur seals, and Stellar sea lions. This is the first report of
418 innate SULT1E1 deficiency in placental mammals. SULT1E1s are critically important
419 for the metabolism of sulfate estrogens (estradiol and estrone) and have a very high
420 affinity for a vast range of xenobiotics including some environmental pollutants, such
421 as hydroxylated-polychlorinated biphenyls (OH-PCBs) and hydroxylated-
422 polybrominated diphenyl ether (OH-PBDEs). A previous report by Tong et al. (2005)
423 suggested that SULT1E1 ablation in mice caused severe thrombosis in the placenta,
424 resulting in fetal loss in the knock out (KO) mice because of the excessive estrogen
425 levels in the placenta. Moreover, Gershon et al. (2007) showed that excessive
426 estrogen resulted in the low expression of COX-2, reduced cumulus expansion, and
427 impaired ovulation in SULT1E1 KO mice. In addition, single nucleotide polymorphisms
428 (SNP) in human SULT1E1 may be a risk factor for breast or endometrial cancer

429 development (Yi et al., 2021). Like catecholamine, estrogen sulfates are also mainly
430 found in the blood and are precursors of active steroids, utilizing steroid sulfatase
431 (STS) to resume their actions in peripheral tissues. Hence, SULT1E1 is an essential
432 estrogen-modulating factor in mammals. The detailed mechanism of estrogen
433 modulation in pinnipeds has not yet been described but pinnipeds may not utilize
434 estrogen sulfation to modulate estrogenic activity. Currently, SULT1E1 orthologs have
435 only been discovered in mammals including placental mammals and platypuses, but
436 not in marsupials or other vertebrates, indicating that SULT1E1 diverged after the
437 evolutionary emergence of mammals (Coughtrie, 2016). However, in chicken and turtle
438 eggs, biosynthesis of estrogen sulfate was observed, suggesting the existence of
439 estrogen-sulfotransferases in these species (Paitz et al., 2020; Paitz and Bowden,
440 2013), despite there being no SULT1E1 orthologs in reptiles or birds in our analyses.
441 Phylogenetic analysis of avian SULTs showed one clade of avian SULTs (tentatively
442 named SULT1D/1E), which was located closely to mammalian 1E1 and 1D1 groups
443 in the phylogenetic tree, suggesting that avian SULT1E/1D may have similar substrate
444 specificity as mammalian SULT1D1 or 1E1. In addition, since SULTs have a vast
445 overlap in their substrate specificities, other SULTs could also catalyze estrogen
446 conjugation with lower affinity, suggesting a possible role for these isoforms. However,
447 these reactions were not observed in vitro using pinniped liver cytosols. Previously,
448 Browne et al. (2006) reported the detection of estrone-sulfate in the blood of some
449 pinnipeds using radioimmunoassays followed by HPLC separation, indicating that
450 estrogen sulfation may not be completely absent in pinnipeds. However, the
451 involvement of other SULT isoforms or activity in other organs is still unclear.

452 This in vitro analysis has limitation and didn't completely reflect the SULT1E1 activity
453 because we didn't investigate substrate specificity for other isoforms in Carnivorans
454 and studies using recombinant SULTTs in carnivora is highly important for further
455 discussion. Also in this in vitro analysis, we utilized environmental samples and we
456 didn't conduct chemicals analysis to detect environmental pollutants in these
457 specimens. Thus, some contaminants such as persistent organic pollutants (POPs)
458 might have effect on SULT expression or activity (Kodama and Negishi, 2013).
459 Although this in vitro analysis had such limitation, we considered the result in this study
460 suggested important species-differences of SULT activity in Carnivora.

461

462 **4-5. SULT1Cs in carnivorans and genetic deficiency**

463 Along with other SULT isoforms, SULT1Cs were highly conserved, with some
464 differences between rodents, human, and carnivorans. Phylogenetic analysis revealed
465 that SULT1C1, 1C2, and 1C4 in carnivorans were in the same clade of the
466 phylogenetic tree as rodent SULT1C1, 1C2s and human SULT1C4, respectively. Rat
467 SULT1C3 is considered to be an ortholog of mouse SULT1C1 and not an equivalent
468 of human SULT1C3, suggesting that a comprehensive nomenclature system remains
469 unestablished. In rats, several isoforms in the SULT1C2 clade were observed while
470 mice, humans, and carnivorans had only one isoform in this clade. Human and rodent
471 SULT1Cs are known to conjugate xenobiotics, such as p-nitrophenol, 1-naphthol, 2-
472 ethylphenol, 2-n-propylphenol, and 2-sec-butylphenol (Allali-Hassani et al., 2007);
473 they also conjugate procarcinogen hydroxyaryl amines, such as N-hydroxy-2-
474 acetylaminofluorene, resulting in the metabolic activation of their carcinogenicity
475 (Kurogi et al., 2017; Meini et al., 2008; Sakakibara et al., 1998; Stanley et al., 2005).

476 In humans, SUL1Cs were mainly detected in fetal tissues and were thought to play a
477 possible role in terminating several signaling pathways during fetal development
478 (Runge-Morris and Kocarek, 2013; Stanley et al., 2005), whereas rat SUL1Cs were
479 still detected in adults and played important roles in xenobiotic metabolism into
480 adulthood (Lu et al., 2013; Nagata et al., 1993). In Carnivora, only canine SUL1C4
481 has been cloned and characterized as a phenol-preferring SUL1 (Kurogi et al., 2010).
482 Furthermore, Kurogi et al. revealed that SUL1C4 was expressed in the kidneys,
483 stomach, testes, ovaries, and thyroid glands but not in the liver, suggesting a
484 significant role of SUL1C4-mediated detoxification in non-liver organs in adult dogs
485 and possibly other carnivorans.

486 Interestingly, SUL1C1s were detected as pseudogenes or low-quality protein coding
487 genes in Hawaiian monk seals, southern elephant seals, and Weddell seals, indicating
488 that SUL1C1s in these species may not be functionally expressed. These species
489 are classified as Monachinae (southern seals) (Berta et al., 2018), suggesting low
490 SUL1 activity in this group of animals. Moreover, several variations of SUL1C2s were
491 found in carnivorans. Many species had nonsense mutations in SUL1C2s, including
492 pinnipeds, lions, and leopards (*Panthera* genus). Overall, SUL1Cs are highly diverse,
493 and some 1Cs, like 1C1 and 1C2, were absent in pinnipeds and some carnivoran
494 species, indicating a possible lack of sulfation for some xenobiotics in these animals.

495 **4-6. Balance between UGTs and SUL1s**

496 Many chemicals have been shown to be simultaneously glucuronidated and sulfated,
497 suggesting that UGTs and SUL1s may compensate for each other, with some
498 regioselective differences (Böhmdorfer et al., 2017; Saengtienchai et al., 2014; Wu et
499 al., 2011). Previous reports have shown very limited function for UGTs in felines and

500 pinnipeds, suggesting the compensatory activity of SULTs in these species (Takechi et
501 al., 2015; Kondo et al., 2017). Our present in vitro analysis suggests that feline livers
502 have high SULT activity towards estrogens compared to rats. Limited or no SULT
503 activity was detected in pinnipeds. These findings indicate that SULTs may
504 compensate for limited activity of UGTs in felines, but not in pinniped species. Together
505 with low UGT activity, our present findings suggest that pinniped species have very
506 limited phase II metabolic processes, resulting in poor degradation of numerous
507 chemicals including environmental estrogens, such as Bisphenol A, 4-n-octylphenol,
508 4-n-nonylphenol, and OH-PCBs (Grimm et al., 2017; Suiko et al., 2005).

509 **Conclusion**

510 This is the first comprehensive report of the genetic characteristics of SULT isoforms
511 in wild, non-laboratory mammals. In this study, we found that some pinnipeds may
512 have an extremely limited capacity to sulfonate both exogenous and endogenous
513 chemicals, such as estrogens, medicines, and environmental chemicals. These
514 findings improve our knowledge of the genetic variation of SULT genes in carnivorans
515 and, importantly, improve our understanding of xenobiotic metabolism as carnivorans'
516 defense system for numerous anthropogenic chemicals.

517

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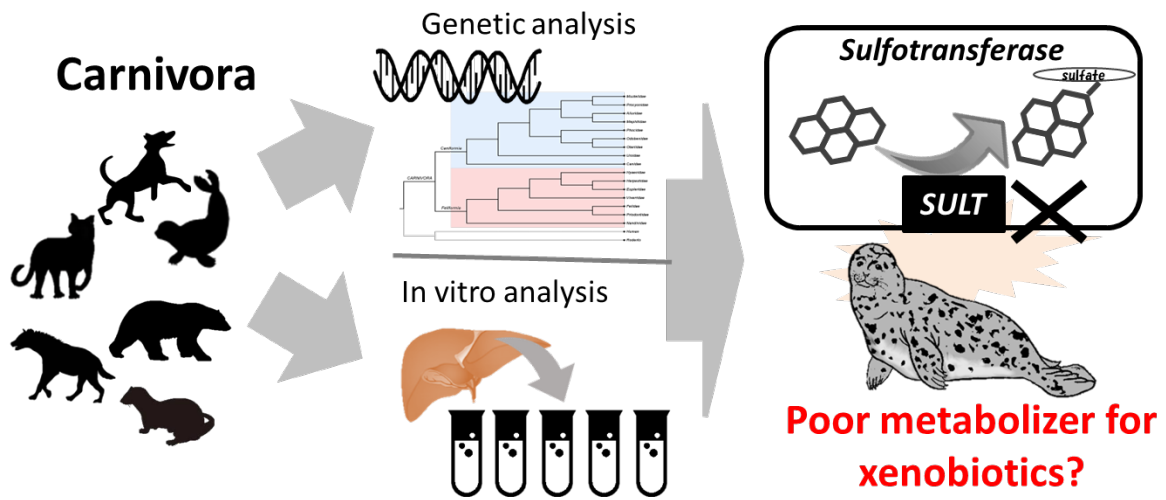
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717

Graphical abstract



Tables

	Rat	Cat	Steller Sea Lion	Harbor Seal
V _{max} /K _m (μ l/min/mg)	5.17 \pm 0.77 a	563 \pm 37.4 b	N.D.	N.D.
V _{max} (pmol/min/mg)	54.3 \pm 6.93	46.7 \pm 5.83	N.D.	N.D.
K _m (μ M)	10.5 \pm 2.23 a	0.0829 \pm 0.0390 b	N.D.	N.D.

Table 1. Kinetic parameters of the SULT estradiol activity for each species

Data presented for rats, cats, and pinnipeds as means \pm SD. V_{max}/K_m values that were significantly different ($P < 0.05$) within a substrate, based on Tukey's HSD tests for each V_{max}/K_m, are indicated by "a" and "b".

N.D.: not determined.

Figures

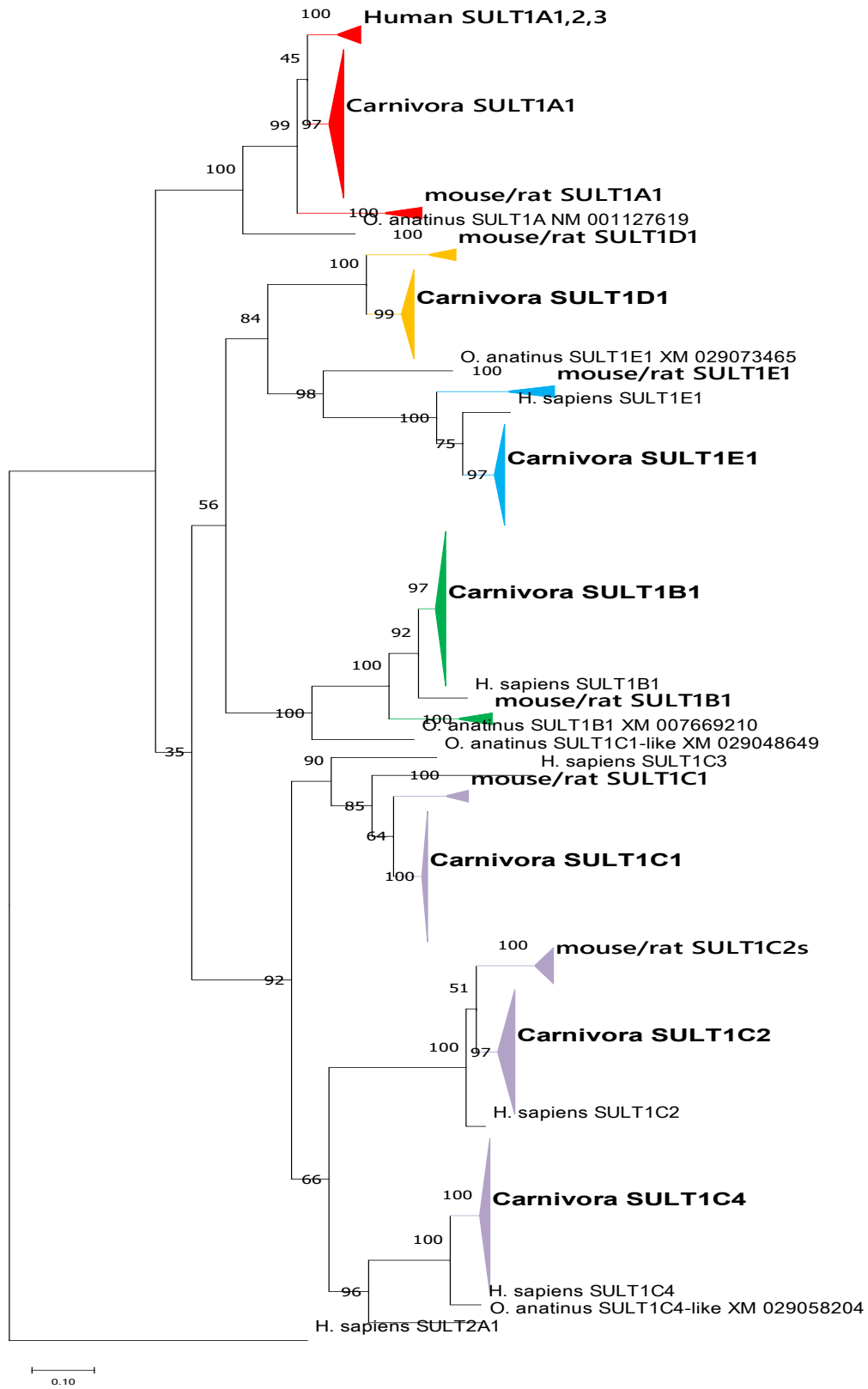


Figure 1. Phylogenetic tree of SULT1s in mammals including carnivorans.

Phylogenetic tree of SULT1 amino acid sequences in humans, mice, rats, platypuses, and carnivorans. Gene sequences of protein-coding regions for each isozyme were analyzed. The JTT + G model was used. The numbers next to the branches indicate the number of occurrences per 100 bootstrap replicates. Gene names and clade names are tentatively named for carnivoran SULTs in this article along with their phylogeny. Clades of carnivorans, mouse, and rat SULTs in the phylogenetic tree are shown as triangles with the following colors: red for SULT1As, green for SULT1B1s, pale purple for SULT1Cs, yellow for SULT1D1s, and light blue for SULT1E1s. Human SULT2A1 is shown as an outgroup of SULT1s.

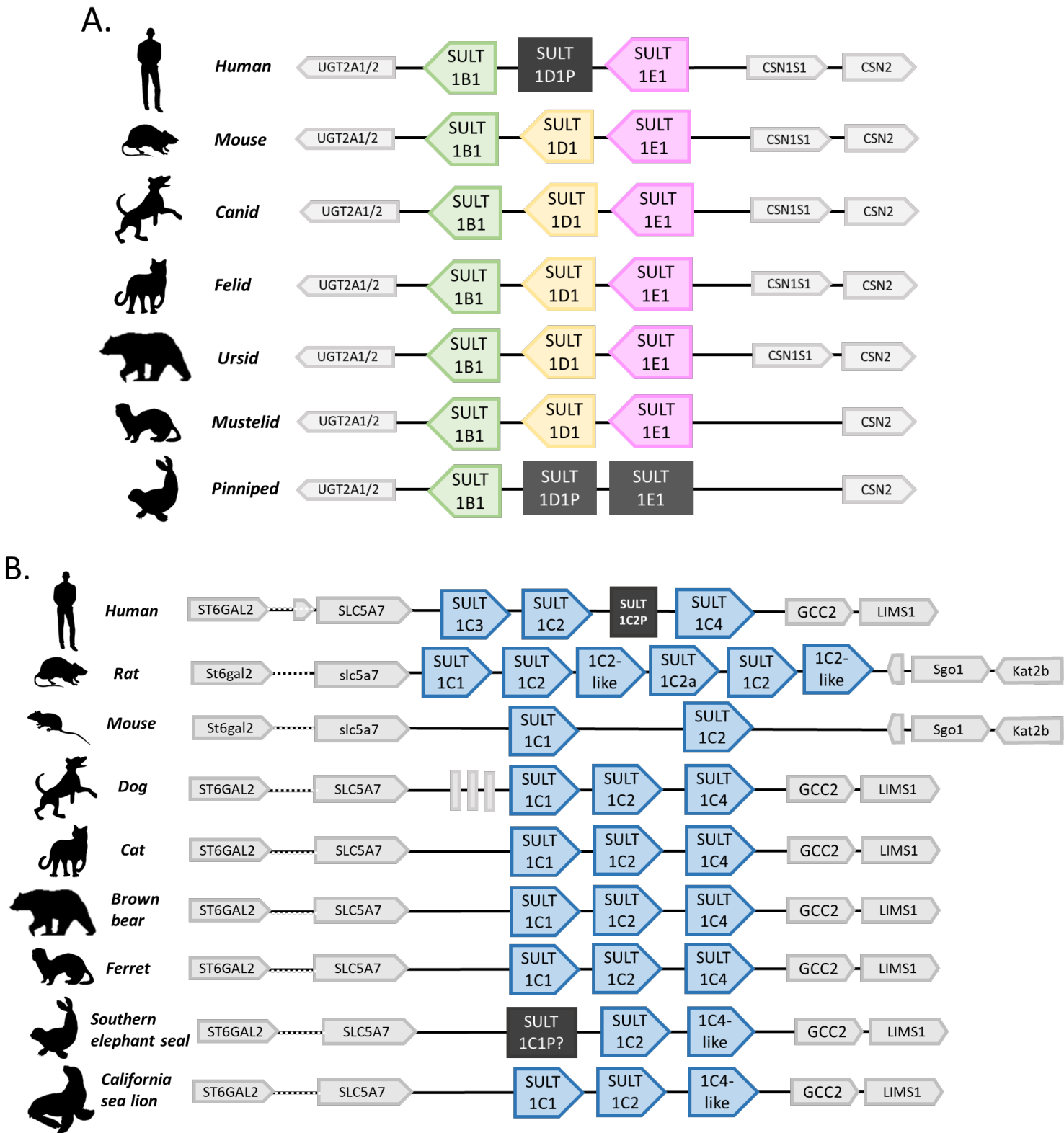


Figure 2. Genetic loci of SULTs in mammals.

A. Gene loci of SULT1B1s, 1D1s, and 1E1s in humans, mice, and carnivorans are described. B. Gene loci of SULT1Cs in mammals are shown. Black blocks indicate pseudogenes. Gray blocks show other non-SULT genes. Dotted lines represent long omitted gene loci. P stands for Pseudogene.

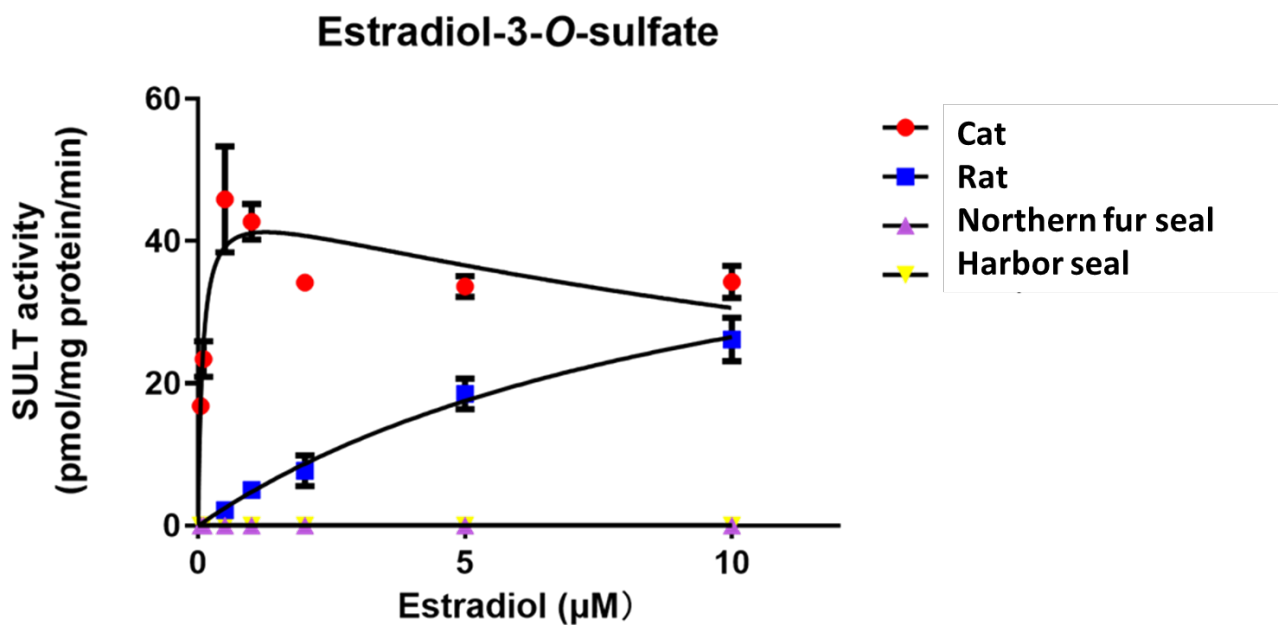


Figure 3. Michaelis-Menten plot for the in vitro SULT activity of estradiol.

In vitro SULT enzymatic activity is shown in the Michaelis-Menten plot. Cats (circle), rats (square), northern fur seal (triangle), and Harbor seal (reverse triangle) cytosols and estradiol substrates were used for in vitro analyses. Cat data were fit for a substrate-inhibition model.

Supplementary Data

Supplementary Table 1. Animal liver samples used in this study

Species	Steller Sea Lion	Harbor Seal	Cat	SD Rat
Scientific name	<i>Eumetopias jubatus</i>	<i>Phoca vitulina</i>	<i>Felis catus</i>	<i>Rattus norvegicus</i>
Number	4	4	3	4
Gender	Male	Male	Male	Male
Sampling year	2003	2016	2017	2014
Location	Rausu (Japan)	Erimo (Japan)	Kitayama Labes Co., Inc	Sankyo Labo Service Corporation, Inc.
Age class	Mature	Mature	24–28 months	8 weeks

Supplementary Table 2. SULT genes used in the phylogenetic analysis and sequence comparative analysis.

Family	Common name	Species name	Gene name (with tentative name)	Accession number
Hominidae	Human	Homo sapiens	SULT1A1	NM001055.3
			SULT1A2	NM001054
			SULT1A4	NM001017390
			SULT1B1	NM014465
			SULT1C2	NM001056
			SULT1C3	NM001008743.3
			SULT1C4	NM001321770.2
			SULT1E1	Y11195.1
			SULT2A1 as outer group	NM003167
Muridae	Rat	Rattus norvegicus	sult1a1	AF394783.1
			ST1b1 (sult1b1)	D89375.1
			RGD1559960 (sult1c2)	XM039084411.1
			sult1c2a	NM001013177.2

			sult1c2	NM133547.5
			LOC120093086 (sult1c2-like)	XM039084404.1
			sult1c3 (sult1c1)	NM031732.2
			sult1d1	NM021769.1
			sult1e1	NM012883.1
			<hr/>	
	Mouse	Mus musculus	sult1a1	NM133670
			sult1b1	BC024361
			sult1c1	NM018751.2
			sult1c2	NM026935.4
			sult1d1	NM016771.3
			sult1e1	BC034891.1
			<hr/>	
Canidae	Dog	Canis lupus familiaris	SULT1A1	AY069922.1
			SULT1B1	NM001195835.2
			SULT1C3 (SULT1C1)	XM038680151.1
			SULT1C2	XM038680149.1

			SULT1C4	XM038680150.1
			SULT1D1	XM038685809.1
			SULT1E1	XM038685811.1
			<hr/>	
	Red fox	Vulpes	LOC112929198 (SULT1A1)	XM026011091
			SULT1B1	XM026003470
			SULT1C2	XM026011035
			SULT1C4	XM026010893
			SULT1D1-like: LOC112923601	
			(SULT1D1)	XM026003469
			LOC112923635 (SULT1E1)	XM026003484
			<hr/>	
Mustelidae	North American river otter	Lontra canadensis	LOC116858063 (SULT1A1)	XM032842614
			SULT1B1	XM032845783
			LOC116857948 (SULT1C1)	XM032842428
			SULT1C2	XM032842417.1

		SULT1C4-like: (SULT1C4)	LOC116857940	XM032842416
		SULT1D1-like: (SULT1D1)	LOC116859864	XM032845591
		SULT1E1-like: (SULT1E1)	LOC116859692	XM032845460
Sea otter	<i>Enhydra lutris kenyoni</i>	SULT1A1:	LOC111162234 (SULT1A1)	XM022526502.1
		SULT1B1:	LOC111149182 (SULT1B1)	XM022506124
		SULT1C1-like: (SULT1C1)	LOC111155904	XM022516369.1
		SULT1C4-like: (SULT1C4)	LOC111155885	XM022516341.1
		SULT1D1-like: (SULT1D1)	LOC111149106	XM022506029
		SULT1E1:	LOC111149178 (SULT1E1)	XM022506117

Ermine	<i>Mustela erminea</i>	SULT1A1: LOC116581510 (SULT1A1)	XM032328707
		SULT1B1	XM032334482
		SULT1C1: LOC116595393 (SULT1C1)	XM032351657
		SULT1C2: LOC116595392 (SULT1C2)	XM032351655
		SULT1C4-like: LOC116595391 (SULT1C4)	XM032351652
		SULT1D1: LOC116585153 (SULT1D1)	XM032334485
		SULT1E1-like: LOC116585151 (SULT1E1)	XM032334481
		<hr/>	
Domestic ferret	<i>Mustela putorius furo</i>	SULT1A1	XM 004773964
		SULT1B1	XM013063435.1
		SULT1C2	XM004764352.2
		SULT1C3	XM004764354.2
		SULT1C4	XM004764349.2
		SULT1D1	XM013063434.1

			SULT1E1	XM004766333.2
Ursidae	Brown bear	Ursus arctos horribilis	SULT1A1	XM026515715
			SULT1B1	XM026509010
			SULT1C1-like: LOC113269027 (SULT1C1)	XM026517699
			SULT1C2: LOC113267746 (SULT1C2)	XM026515916
			SULT1C4	XM026515929
			SULT1D1: LOC113262555 (SULT1D1)	XM026509011
			SULT1E1	XM026509014
	Polar bear	Ursus maritimus	SULT1A1: LOC103657825 (SULT1A1)	XM008685238.2
			SULT1B1	XM008683684
			SULT1C1:LOC103670621 (SULT1C1)	XM040622914.2
			SULT1C2	XM008699075.2
			SULT1C4	XM 008699073.2
			SULT1D1: LOC103656319 (SULT1D1)	XM008683686

			SULT1E1: LOC103656321 (SULT1E1)	XM008683687.1
Giant panda	Ailuropoda melanoleuca		LOC100479867: SULT1A1 (SULT1A1)	XM002927364.4
			SULT1B1	XM002929461.4
			SULT1C1: LOC100466952 (SULT1C1)	XM002930285.4
			SULT1C2: LOC100467202 (SULT1C2)	XM002930286.4
			SULT1C4	XM002929382.4
			SULT1D1: LOC100468789 (SULT1D1)	XM011237499.3
			SULT1E1: LOC100469041 (SULT1E1)	XM019810143.2
Phocidae:				
Monachinae	Weddell seal	Leptonychotes weddellii	SULT1A1: LOC102733278 (SULT1A1)	XM006733938.2
			SULT1B1: LOC102727575 (SULT1B1)	XM006729321.2
			SULT1C1-like: LOC102725762	XM031022251.1
			(SULT1C1)	
			SULT1C2: LOC102747532 (SULT1C2)	XM006728933
			SULT1C4: LOC102747824 (SULT1C4)	XM006728934.2

Southern elephant seal	Mirounga leonina	SULT1A1: LOC117999643 (SULT1A1)	XM034988959
		SULT1B1: LOC118010478 (SULT1B1)	XM035004869
		SULT1C1-like: LOC118008308 (SULT1C1)	XM035001943.1
		SULT1C2	XM035001944.1
		SULT1C4-like: LOC118008292 (SULT1C4)	XM035001900.1
<hr/>			
Hawaiian monk seal	Neomonachus schauinslandi	SULT1A1: LOC110589844 (SULT1A1)	XM021700477.1
		SULT1B1: LOC110591481 (SULT1B1)	XM021702403
		SULT1C1-like: LOC110572024 (SULT1C1P)	
		SULT1C2	XM021680275.1
		SULT1C4	XM021680274.1

Phocidae:	Harbor seal	<i>Phoca vitulina</i>	SULT1A1: LOC116648763 (SULT1A1)	XM032431006.1
Phocinae			SULT1B1: LOC116628089 (SULT1B1)	XM032398193.1
			SULT1C1: LOC116641753 (SULT1C1)	XM032419668
			SULT1C2	XM032419671
			SULT1C4: LOC116641754 (SULT1C4)	XM032419669.1
	Gray seal	<i>Halichoerus grypus</i>	SULT1A1: LOC118535031 (SULT1A1)	XM036091215.1
			SULT1B1: LOC118525390 (SULT1B1)	XM036076112
			SULT1C1: LOC118553409 (SULT1C1)	XM036120449
			SULT1C2	XM036120462.1
			SULT1C4	XM036087224
Otariidae	California sea lion	<i>Zalophus californianus</i>	SULT1A1: LOC113933049 (SULT1A1)	XM027612717
			SULT1B1: LOC113924953 (SULT1B1)	XM027599339.2
			SULT1C1: LOC113937879 (SULT1C1)	XM027622796.1
			SULT1C2: LOC113937590 (SULT1C2)	XM027622119.1

			SULT1C4-like: LOC113937589	XM027622118.2
Stellar sea lion	Eumetopias jubatus		SULT1A1: LOC114214438 (SULT1A1)	XM028110090
			SULT1B1: LOC114206800 (SULT1B1)	XM028100710
			SULT1C1: LOC114199385 (SULT1C1)	XM028091278.1
			SULT1C2-like: LOC114199388	
			(SULT1C2)	XM028091279
			SULT1C4	XM028091284
Northern fur seal	Callorhinus ursinus		SULT1A1: LOC112810457 (SULT1A1)	XM025854081.1
			SULT1B1: LOC112835901 (SULT1B1)	XM025887547
			SULT1C1: LOC112821508 (SULT1C1)	XM025868772
			SULT1C2	XM025869098.1
			SULT1C4-like: LOC112821718	
			(SULT1C4)	XM025869099.1
Odobenidae	Pacific walrus	Odobenus divergens	rosmarus	SULT1A1: LOC101380863 (SULT1A1)
				XM012561271.1

			SULT1B1	XM004392889.1
			SULT1C1-like: (SULT1C1)	LOC101376188 XM012567311.1
			SULT1C2	XM004414460.2
			SULT1C4	XM004414459.1
<hr/>				
Falidae	Domestic cat	Felis catus	SULT1A1	XM019820729.1
			SULT1B1	XM023253069
			SULT1C3 (SULT1C1)	XM003983864.5
			SULT1C2	XM011281074
			SULT1C4	XM003983869.5
			SULT1D1-like: (SULT1D1)	LOC101086557 XM023253066.1
			SULT1E1	XM003985305.3
<hr/>				
	Cheetah	Acinonyx jubatus	SULT1A1: LOC106966152 (SULT1A1)	XM027053118.1
			SULT1B1	XM015074496.2

		SULT1C1-like: LOC106977307 (SULT1C1)	XM015074791.2
		SULT1C2: LOC106977290 (SULT1C2)	XM027069399.1
		SULT1C4: LOC106977305 (SULT1C4)	XM015074790.2
		SULT1D1-like: LOC106977024 (SULT1D1)	XM027064350.1
		SULT1E1	XM015074488.2
<hr/>			
Puma	Puma concolor	SULT1A1: LOC112855996 (SULT1A1)	XM025919525.1
		SULT1B1	XM025921642.1
		SULT1C1-like: LOC112868851 (SULT1C1)	XM025931831.1
		SULT1C2	XM025932749.1
		SULT1C4-like: LOC112869068 (SULT1C4)	XM025932133

		SULT1D1-like: (SULT1D1)	LOC112858721 XM025922137.1
		SULT1E1-like: (SULT1E1)	LOC112858407 XM025921805
Leopard	<i>Panthera pardus</i>	SULT1A1: LOC109256521 (SULT1A1)	XM019431898
		SULT1B1: LOC109278167 (SULT1B1)	XM019468233
		SULT1C1-like: (SULT1C1)	LOC109258064 XM019434615.1
		SULT1C2-like: (SULT1C2)	LOC109258069 XM019434617.1
		SULT1C4	XM019434612.1
		SULT1D1-like: (SULT1D1)	LOC109278146 XM019468226.1
		SULT1E1	XM019468175.1
Tiger	<i>Panthera tigris</i>	SULT1A1: LOC102972472 (SULT1A1)	XM007084062.3

			SULT1B1	XM007086908.3
			SULT1C1: LOC102952335 (SULT1C1)	XM007097761.3
			SULT1C2: LOC102952042 (SULT1C2)	XM042979753.1
			SULT1C4	XM007097759.3
			SULT1D1: LOC102969965 (SULT1D1)	XM007086913.3
			SULT1E1	XM007086910.3
Lynx	Lynx canadensis		SULT1A1: LOC115504563 (SULT1A1)	XM030301602
			SULT1B1	XM030313745
			SULT1C1: LOC115510437 (SULT1C1)	XM030310292.2
			SULT1C2: LOC115509692 (SULT1C2)	XM030309021.1
			SULT1C4	XM030310291
			SULT1D1-like: LOC115512581 (SULT1D1)	XM030313743
			SULT1E1	XM030313739
Hyaenidae	Striped hyena	Hyaena hyaena	SULT1A1: LOC120242277 (SULT1A1)	XM039247942.1

SULT1B1		XM039251765.1
SULT1C1-like:	LOC120227542	
(SULT1C1)		XM039225639.1
SULT1C4		XM039222195
SULT1D1-like:	LOC120244963	
(SULT1D1)		XM039251783.1
SULT1E1-like:	LOC120244962	
(SULT1E1)		XM039251782.1

Herpestidae	Meerkat	Suricata suricatta	SULT1A1: LOC115297986 (SULT1A1)	XM029946269
			SULT1B1	XM029931052.1
			SULT1C1-like:	LOC115288947
			(SULT1C1)	XM029936197.1
			SULT1C4	XM029937066

SULT1D1-like:	LOC115301877	
(SULT1D1)		XM029951883.1
estrogen	sufotransferase-like:	
LOC115296737 (SULT1E1)		XM029945225.1

Monotremata:

Ornithorhynchid Platypus

Ornithorhynchus anatinus

SULT1A

NM001127619

ae

SULT1B1

XM007669210

SULT1C1-like: LOC100090962

XM029048649

SULT1C4-like: LOC114809133

XM029058204

(SULT1C4)

SULT1E1

XM029073465

Supplementary Figures

a.

number of aligned nucleotide	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176
translated amino acid from human SULT1C2	P			K			A			G			T			T			W			I			Q			E			V				
H. sapiens SULT1C2 NM 001056	C	C	T	A	A	A	G	C	A	G	G	G	A	C	A	A	C	G	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
R. norvegicus Sult1c2a NM 001013177	C	C	T	A	A	A	T	C	A	G	G	G	A	C	A	A	C	A	T	G	G	A	T	T	C	A	A	G	A	A	A	T	T	G	T
R. norvegicus SULT1C2-like LOC100910526 XM 006244254	C	C	T	A	A	A	T	C	A	G	G	G	A	C	A	A	C	A	T	G	G	A	T	T	C	A	A	G	A	A	A	T	T	G	T
R. norvegicus SULT1C2 XM 039084411 RGD1559960	C	C	T	A	A	A	T	C	A	G	G	G	A	C	A	A	A	A	T	G	G	A	T	T	C	A	A	G	A	A	A	T	A	G	T
R. norvegicus Sult1c2 NM 133547.4	C	C	T	A	A	A	T	C	A	G	G	G	A	C	A	A	C	A	T	G	G	A	T	T	C	A	A	G	A	A	A	T	T	G	T
M. musculus Sult1c2 NM 026935.4	C	C	T	A	A	A	T	C	A	G	G	G	A	C	A	A	C	A	T	G	G	A	T	T	C	A	A	G	A	A	A	T	T	G	T
C. lupus familiaris SULT1C2 XM 038680149.1	C	C	T	A	A	A	T	C	A	G	G	G	A	C	T	A	C	A	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
V. vulpes SULT1C2 XM 026011035	C	C	T	A	A	A	T	C	A	G	G	G	A	C	T	A	C	A	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
V. lagopus SULT1C2 XM 041754093 LOC121490422	C	C	T	A	A	A	T	C	A	G	G	G	A	C	T	A	C	A	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
U. arctos horribilis SULT1C2 XM 026515916	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	A	T	G	G	A	T	T	C	A	G	G	A	G	A	T	T	G	T
U. maritimus SULT1C2 XM 008699075.1 LOC103670620	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	A	T	G	G	A	T	T	C	A	G	G	A	G	A	T	T	G	T
A. melanoleuca SULT1C2 XM 002930286.4 LOC100467202	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	A	T	G	G	A	T	T	C	A	G	G	A	G	A	T	T	G	T
M. putorius furo SULT1C2 x3 XM 004764352.2	C	C	T	A	A	A	T	C	A	G	G	C	A	C	C	A	C	A	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
N. vison SULT1C2 XM 044262435 LOC122915683	C	C	T	A	A	A	T	C	A	G	G	C	A	C	C	A	C	A	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
M. erminea SULT1C2 XM 032351655 LOC116595392	C	C	T	A	A	A	T	C	A	G	G	C	A	C	C	A	C	A	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
F. catus SULT1C2 x1 XM 011281074.3	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	G	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
L. canadensis Lynx SULT1C2 XM 030309021 LOC115509692	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	G	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
P. yagouaroundi SULT1C2-like XM 040454146 LOC121016479	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	G	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
P. bengalensis SULT1C2 XM 043553279 LOC122466971	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	G	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
P. tigris SULT1C2 XM 042979753 LOC102952042	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	G	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
P. pardus SULT1C2-like XM 019434617.1 LOC109258069	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	G	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
P. leo SULT1C2-like XM 042930191 LOC122214989	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	G	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
O. rosmarus divergens SULT1C2 XM 004414460.2	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	A	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	A	T
Z. californianus SULT1C2 XM 027622119	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	A	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	A	T
E. jubatus SULT1C2-like XM 028091279 LOC114199388	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	A	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	A	T
C. ursinus SULT1C2 XM 025869098	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	A	T	G	G	A	T	T	C	A	G	G	A	A	A	T	T	A	T
P. vitulina SULT1C2 XM 032419671	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	A	T	A	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
H. grypus SULT1C2 XM 036120462	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	A	T	A	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T
N. schauinslandi SULT1C2 XM 021680275.1	C	C	T	A	A	A	T	C	A	G	G	G	A	C	C	A	C	A	T	A	G	A	T	T	C	A	G	G	A	A	A	T	T	G	T

b.

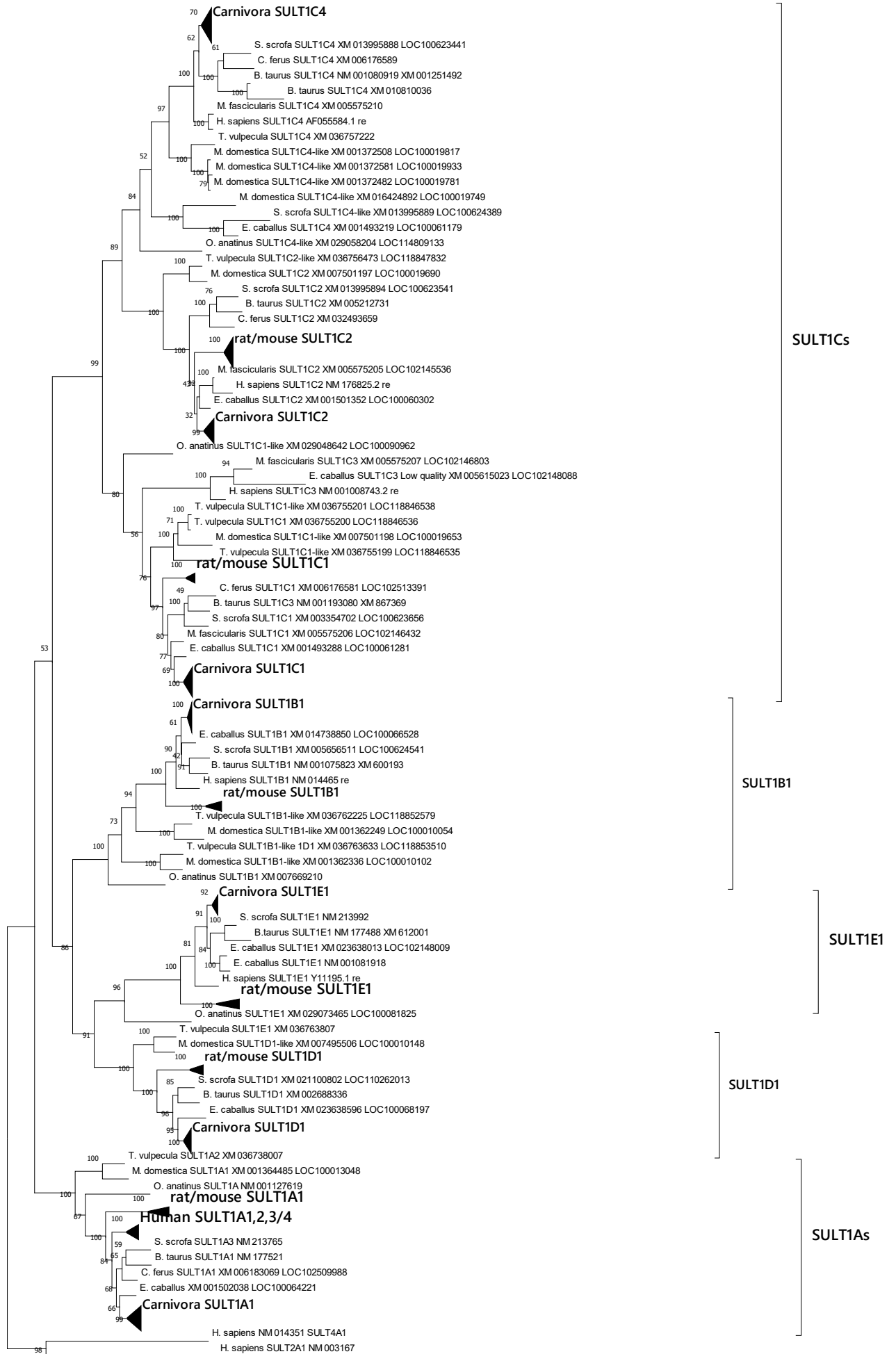
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translated amino acid from human SULT1C2	L			Y			V			A			R			N			A			K			D			C		
H. sapiens SULT1C2 NM 001056	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	T
R. norvegicus Sult1c2a NM 001013177	C	T	T	T	A	T	G	T	G	G	C	T	C	G	A	A	A	C	G	C	C	A	A	A	G	A	C	T	G	C
R. norvegicus SULT1C2-like LOC100910526 XM 006244254	C	T	T	T	A	T	G	T	G	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
R. norvegicus SULT1C2 XM 039084411 RGD1559960	C	T	T	T	A	T	G	T	G	G	C	T	C	G	A	A	A	C	G	C	C	A	A	A	G	A	C	T	G	C
R. norvegicus Sult1c2 NM 133547.4	C	T	T	T	A	T	G	T	G	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
M. musculus Sult1c2 NM 026935.4	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	T	A	A	A	G	A	C	T	G	C
C. lupus familiaris SULT1C2 XM 038680149.1	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
V. vulpes SULT1C2 XM 026011035	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
V. lagopus SULT1C2 XM 041754093 LOC121490422	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
U. arctos horribilis SULT1C2 XM 026515916	C	T	T	T	A	T	G	G	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
U. maritimus SULT1C2 XM 008699075.1 LOC103670620	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
A. melanoleuca SULT1C2 XM 002930286.4 LOC100467202	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
M. putorius furo SULT1C2 x3 XM 004764352.2	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
N. vison SULT1C2 XM 044262435 LOC122915683	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
M. erminea SULT1C2 XM 032351655 LOC116595392	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
F. catus SULT1C2 x1 XM 011281074.3	C	T	T	T	A	T	G	T	G	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
L. canadensis Lynx SULT1C2 XM 030309021 LOC115509692	C	T	T	T	A	T	G	T	G	G	C	T	C	A	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
P. yagouaroundi SULT1C2-like XM 040454146 LOC121016479	C	T	T	T	A	T	G	T	G	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
P. bengalensis SULT1C2 XM 043553279 LOC122466971	C	T	T	T	A	T	G	T	G	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
P. tigris SULT1C2 XM 042979753 LOC102952042	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
P. pardus SULT1C2-like XM 019434617.1 LOC109258069	C	T	T	T	A	T	G	T	A	G	C	T	T	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
P. leo SULT1C2-like XM 042930191 LOC122214989	C	T	T	T	A	T	G	T	A	G	C	T	T	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
O. rosmarus divergens SULT1C2 XM 004414460.2	C	T	T	T	A	T	G	T	A	G	C	T	T	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
Z. californianus SULT1C2 XM 027622119	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
E. jubatus SULT1C2-like XM 028091279 LOC114199388	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
C. ursinus SULT1C2 XM 025869098	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
P. vitulina SULT1C2 XM 032419671	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
H. grypus SULT1C2 XM 036120462	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C
N. schauinslandi SULT1C2 XM 021680275.1	C	T	T	T	A	T	G	T	A	G	C	T	C	G	A	A	A	T	G	C	C	A	A	A	G	A	C	T	G	C

C.

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R. norvegicus Sult1c2a NM 001013177	G	G	A	A	T	T	G	T	G	G	G	T	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	T	A	C	T
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M. musculus Sult1c2 NM 026935.4	G	G	A	A	C	T	G	T	G	G	G	T	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	T	A	C	T
C. lupus familiaris SULT1C2 XM 038680149.1	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
V. vulpes SULT1C2 XM 026011035	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
V. lagopus SULT1C2 XM 041754093 LOC121490422	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
U. arctos horribilis SULT1C2 XM 026515916	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	C
U. maritimus SULT1C2 XM 008699075.1 LOC103670620	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	C
A. melanoleuca SULT1C2 XM 002930286.4 LOC100467202	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	C
M. putorius furo SULT1C2 x3 XM 004764352.2	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
N. vison SULT1C2 XM 044262435 LOC122915683	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	G	A	C	C	A	C	T	T	C	A	C	T
M. erminea SULT1C2 XM 032351655 LOC116595392	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
F. catus SULT1C2 x1 XM 011281074.3	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
L. canadensis Lynx SULT1C2 XM 030309021 LOC115509692	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
P. yagouaroundi SULT1C2-like XM 040454146 LOC121016479	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
P. bengalensis SULT1C2 XM 043553279 LOC122466971	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
P. tigris SULT1C2 XM 042979753 LOC102952042	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
P. pardus SULT1C2-like XM 019434617.1 LOC109258069	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
P. leo SULT1C2-like XM 042930191 LOC122214989	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
O. rosmarus divergens SULT1C2 XM 004414460.2	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T A G	A	A	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
Z. californianus SULT1C2 XM 027622119	A	G	T	A	C	T	G	T	G	G	G	G	G	A	T	T A G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. jubatus SULT1C2-like XM 028091279 LOC114199388	A	G	T	A	C	T	G	T	G	G	G	G	G	A	T	T A G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. ursinus SULT1C2 XM 025869098	G	G	T	A	C	T	G	T	G	G	G	G	G	A	T	T A G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. vitulina SULT1C2 XM 032419671	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
H. grypus SULT1C2 XM 036120462	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T
N. schauinslandi SULT1C2 XM 021680275.1	G	G	A	A	C	T	G	T	G	G	G	G	G	A	T	T	G	G	A	A	A	A	A	C	C	A	C	T	T	C	A	C	T

Supplementary Figure S1. Several SULT1C2 nonsense mutations in pinnipeds and Panthera lineage

The figures show a: mutation at residue 55 for Phocidae, b: mutation at residue 131 in lions, leopards, and walruses, and c: mutation at residue 264 in Odobenidae and Otariidae.



Supplementary Figure S2. Phylogeny of SULT isoforms in mammals and marsupials

Gene sequences of SULT isoforms in several mammals (cow: *Bos taurus*, horse: *Equus caballus*, pig: *Sus scrofa*, camel: *Camelus ferus*, human, rat, mouse, and several Carnivora), marsupials (gray short-tailed opossum: *Monodelphis domestica* and common brushtail: *Trichosurus vulpecula*), and platypus were added for additional phylogenetic analysis.

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