

Title	Sulfotransferases (SULTs), enzymatic and genetic variation in Carnivora : Limited sulfation capacity in pinnipeds
Author(s)	Kondo, Mitsuki; Ikenaka, Yoshinori; Nakayama, Shouta M. M.; Kawai, Yusuke K.; Mizukawa, Hazuki; Mitani, Yoko; Nomyama, Kei; Tanabe, Shinsuke; Ishizuka, Mayumi
Citation	Comparative biochemistry and physiology Part C: Toxicology & pharmacology, 263, 109476 https://doi.org/10.1016/j.cbpc.2022.109476
Issue Date	2023-01
Doc URL	http://hdl.handle.net/2115/91043
Rights	© 2023. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	http://creativecommons.org/licenses/by-nc-nd/4.0/
Туре	article (author version)
File Information	SULT papers Kondo et al CBP reviced 2nd clear.pdf



1	Sulfotransferases (SULTs), enzymatic and genetic variation in Carnivora:
2	Limited sulfation capacity in pinnipeds.
3	
4	Mitsuki Kondo1, Yoshinori Ikenaka1,2,3,4, Shouta M.M. Nakayama1,5, Yusuke K.
5	Kawai6, Hazuki Mizukawa7, Yoko Mitani8, Kei Nomyama9, Shinsuke Tanabe9, and
6	Mayumi Ishizuka1*
7	
8	1: Laboratory of Toxicology, Department of Environmental Veterinary Science, Faculty
9	of Veterinary Medicine, Hokkaido University, N18, W9, Kita-ku, Sapporo, 060-0818,
10	Japan
11	http://tox.vetmed.hokudai.ac.jp/en/
12	
13	2: Water Research Group, Unit for Environmental Sciences and Management, North-
14	West University, Potchefstroom, South Africa
15	
16	3: Translational Research Unit, Veterinary Teaching Hospital, Faculty of Veterinary
17	Medicine, Hokkaido University
18	
19	4: One Health Research Center, Hokkaido University
20	
21	5: Biomedical Sciences Department, School of Veterinary Medicine, The University of
22	Zambia, P.O. Box 32379, Lusaka 10101, Zambia
23	
24	6:Laboratory of Toxicology, Department of Veterinary Medicine, Obihiro University of
25	Agriculture and Veterinary Medicine, Obihiro, 080-8555, Japan

- 26 http://www.obihiro.ac.jp/~toxicology/index.html
- 27
- 28 7: Department of Science and Technology for Biological Resources and Environment,
- 29 Graduate School of Agriculture, Ehime University, Tarumi 3-5-7, Matsuyama 790-8566,
- 30 Japan
- 31
- 32 8: Field Science Center for Northern Biosphere, Hokkaido University, N11, W10, Kita-
- 33 ku, Sapporo, 060-0811, Japan
- 34 <u>https://www.fsc.hokudai.ac.jp/home\_en/</u>
- 35 Current address: Wildlife Research Center, Kyoto University, 2-24,
- 36 Tanaka-Sekiden-cho, Sakyo-ku, Kyoto, 606-8203, Japan
- 37
- 38 9 : Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho
- 39 2-5, Matsuyama, 790-8577, Japan
- 40 <u>http://www.cmes.ehime-u.ac.jp/en/index.html</u>
- 41
- 42 \* Corresponding author and address
- 43 Mayumi Ishizuka
- 44 Laboratory of Toxicology, Department of Environmental Veterinary Science, Faculty of
- 45 Veterinary Medicine, Hokkaido University, N18 W9, Kita-ku, Sapporo, 060-0818,
- 46 Japan
- 47 Tel: +81-11-706-6949
- 48 Fax: +81-11-706-5105
- 49 <u>ishizum@vetmed.hokudai.ac.jp</u>
- 50

#### 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 is a significant chemical defense system from xenobiotics because it degrades the 78 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 genetic properties of SULTs in a wide range of mammals, focusing on carnivorans, 85 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

#### 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

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

128 Wild mammals are continuously exposed to a vast variety of environmental chemicals, such as polyhalogenated aromatic hydrocarbons (PAHs) and organochlorine 129 130 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

genetic and enzymatic features of SULTs in wild mammals including pinnipeds andfelines.

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

157

#### 158 2. Materials and methods

#### 159 **2-1. Chemicals**

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

167

#### 168 2-2. Animals

Details about the animals used for liver cytosol preparations are provided in Supplementary Table S1. Liver samples were collected from northern fur seals (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 accidently captured by fishing nets and drowned. Northern fur seal 174 livers were provided by the Environmental Specimen Bank (es-BANK: http://esbank-175 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  $(23^{\circ}C \pm 1^{\circ}C)$  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 ± 1°C with 35 ± 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 KCI 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 × g at 4°C for 20 199 minutes. The supernatants were further centrifuged at 105,000 × 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 (Pierce, Rockford, IL, USA). 203

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 MgCl2, 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 × 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-guadrupole 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  $\beta$ -estradiol-3-sulfate was 351 > 271.

229

230 2-3-4. Data analysis

All kinetic parameters, including maximum velocity (Vmax), Michaelis–Menten constant (Km), and Vmax/Km ratio, were determined using the Michaelis–Menten equation and GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). Statistical analyses were performed using JMP® 12 (SAS Institute, Inc., Cary, NC, USA). Tukey's HSD test was used for the Vmax/Km of each substrate for each species; differences of P < 0.05 were considered statistically significant in all 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 was used. All positions containing gaps and missing data were eliminated, and total 257 258 924 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 phylogenic 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/gdv/) 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.

#### 285 **3. Results**

#### **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.

#### 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 sealsm, 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 Vmax, Km, and Vmax/Km 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 Vmax/Km 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**

The SULT1B1 isoform was known to be highly conserved in mammals. Surprisingly, in our further investigation, even platypus and marsupials had orthologs of SULT1B1 (Supplementary Figure S2). Avian SULT1B1 and xenopus SULT1B isoforms 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 systems, yet affinity for their exogenous substrate is also low. 389

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 evolutional 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 unestablished. In rats, several isoforms in the SULT1C2 clade were observed while 469 470 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; Meinl et al., 2008; Sakakibara et al., 1998; Stanley et al., 2005).

476 In humans, SULT1Cs 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 SULT1Cs 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 SULT1C4 481 has been cloned and characterized as a phenol-preferring SULT (Kurogi et al., 2010). 482 Furthermore, Kurogi et al. revealed that SULT1C4 was expressed in the kidneys, 483 stomach, testes, ovaries, and thyroid glands but not in the liver, suggesting a 484 significant role of SULT1C4-mediated detoxification in non-liver organs in adult dogs 485 and possibly other carnivorans.

486 Interestingly, SULT1C1s were detected as pseudogenes or low-quality protein coding 487 genes in Hawaiian monk seals, southern elephant seals, and Weddell seals, indicating 488 that SULT1C1s in these species may not be functionally expressed. These species 489 are classified as Monachinae (southern seals) (Berta et al., 2018), suggesting low 490 SULT activity in this group of animals. Moreover, several variations of SULT1C2s were 491 found in carnivorans. Many species had nonsense mutations in SULT1C2s, including 492 pinnipeds, lions, and leopards (Panthera genus). Overall, SULT1Cs 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 SULTs**

496 Many chemicals have been shown to be simultaneously glucuronidated and sulfated, 497 suggesting that UGTs and SULTs 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 (Kakehi 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**

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

#### 518 **References**

- 519 Allali-Hassani, A., Pan, P.W., Dombrovski, L., Najmanovich, R., Tempel, W., Dong,
- 520 A., Loppnau, P., Martin, F., Thonton, J., Edwards, A.M., Bochkarev, A.,
- 521 Plotnikov, A.N., Vedadi, M., Arrowsmith, C.H., 2007. Structural and Chemical
- 522 Profiling of the Human Cytosolic Sulfotransferases. PLOS Biol. 5, e97.
- 523 https://doi.org/10.1371/JOURNAL.PBIO.0050097
- 524 Almazroo, O.A., Miah, M.K., Venkataramanan, R., 2017. Drug Metabolism in the
- 525 Liver, Clinics in Liver Disease. W.B. Saunders.
- 526 https://doi.org/10.1016/j.cld.2016.08.001
- 527 Berta, A., Churchill, M., Boessenecker, R.W., 2018. The Origin and Evolutionary
- 528 Biology of Pinnipeds: Seals, Sea Lions, and Walruses. Annu. Rev. Earth Planet.
- 529 Sci. 46, 203–228. https://doi.org/10.1146/annurev-earth-082517-010009
- 530 Blanchard, R.L., Freimuth, R.R., Buck, J., Weinshilboum, R.M., Coughtrie,
- 531 M.W.W.H., 2004. A proposed nomenclature system for the cytosolic
- 532 sulfotransferase (SULT) superfamily [WWW Document]. Pharmacogenetics.
- 533 https://doi.org/10.1097/00008571-200403000-00009
- Böhmdorfer, M., Szakmary, A., Schiestl, R., Vaquero, J., Riha, J., Brenner, S.,
- 535 Thalhammer, T., Szekeres, T., Jäger, W., 2017. Involvement of UDP-
- 536 Glucuronosyltransferases and Sulfotransferases in the Excretion and Tissue
- 537 Distribution of Resveratrol in Mice. Nutrients 9, 1347.
- 538 https://doi.org/10.3390/nu9121347
- 539 Browne, P., Conley, A.J., Spraker, T., Ream, R.R., Lasley, B.L., 2006. Sex steroid
- 540 concentrations and localization of steroidogenic enzyme expression in free-

- 541 ranging female northern fur seals (Callorhinus ursinus). Gen. Comp. Endocrinol.
- 542 147, 175–183. https://doi.org/10.1016/j.ygcen.2005.12.019
- 543 Coughtrie, M.W.H., 2016. Function and organization of the human cytosolic
- 544 sulfotransferase (SULT) family. Chem. Biol. Interact. 259, 2–7.
- 545 https://doi.org/10.1016/j.cbi.2016.05.005
- 546 Dajani, R., Hood, A.M., Coughtrie, M.W.H., 1998. A single amino acid, Glu146,
- 547 governs the substrate specificity of a human dopamine sulfotransferase,
- 548 SULT1A3. Mol. Pharmacol. 54, 942–948. https://doi.org/10.1124/mol.54.6.942
- 549 Dajani, R., Sharp, S., Graham, S., Bethell, S.S., Cooke, R.M., Jamieson, D.J.,
- 550 Coughtrie, M.W.H., 1999. Kinetic properties of human dopamine
- 551 sulfotransferase (SULT1A3) expressed in prokaryotic and eukaryotic systems:
- 552 Comparison with the recombinant enzyme purified from Escherichia coli. Protein

553 Expr. Purif. 16, 11–18. https://doi.org/10.1006/prep.1999.1030

- 554 Falany, C.N., 1991. Molecular enzymology of human liver cytosolic
- 555 sulfotransferases. Trends Pharmacol. Sci. https://doi.org/10.1016/0165-
- 556 6147(91)90566-B
- 557 Gamage, N., Barnett, A., Hempel, N., Duggleby, R.G., Windmill, K.F., Martin, J.L.,
- 558 McManus, M.E., 2006. Human sulfotransferases and their role in chemical
- 559 metabolism. Toxicol. Sci. https://doi.org/10.1093/toxsci/kfj061
- 560 Gershon, E., Hourvitz, A., Reikhav, S., Maman, E., Dekel, N., 2007. Low expression
- of COX-2, reduced cumulus expansion, and impaired ovulation in SULT1E1-
- 562 deficient mice. FASEB J. 21, 1893–1901. https://doi.org/10.1096/FJ.06-
- 563 7688COM

- 564 Grimm, F.A., Lehmler, H.-J., Koh, W.X., DeWall, J., Teesch, L.M., Hornbuckle, K.C.,
- 565 Thorne, P.S., Robertson, L.W., Duffel, M.W., 2017. Identification of a sulfate
- 566 metabolite of PCB 11 in human serum. Environ. Int. 98, 120–128.
- 567 https://doi.org/10.1016/j.envint.2016.10.023
- 568 Huang, A.C., Nelson, C., Elliott, J.E., Guertin, D.A., Ritland, C., Drouillard, K.,
- 569 Cheng, K.M., Schwantje, H.M., 2018. River otters (Lontra canadensis) "trapped"
- 570 in a coastal environment contaminated with persistent organic pollutants:
- 571 Demographic and physiological consequences. Environ. Pollut. 238, 306–316.
- 572 https://doi.org/10.1016/j.envpol.2018.03.035
- 573 Jancova, P., Anzenbacher, P., Anzenbacherova, E., 2010. PHASE II DRUG
- 574 METABOLIZING ENZYMES.
- 575 Johnson, A.C., 2019. The necessity for wildlife population studies to assess real
- 576 chemical impacts. Curr. Opin. Environ. Sci. Heal.
- 577 https://doi.org/10.1016/j.coesh.2019.10.005
- 578 Kakehi, M., Ikenaka, Y., Nakayama, S.M.M., Kawai, Y.K., Watanabe, K.P.,
- 579 Mizukawa, H., Nomiyama, K., Tanabe, S., Ishizuka, M., 2015. Uridine
- 580 Diphosphate-Glucuronosyltransferase (UGT) Xenobiotic Metabolizing Activity
- and Genetic Evolution in Pinniped Species. Toxicol. Sci. 147, 360–369.
- 582 https://doi.org/10.1093/toxsci/kfv144
- 583 Kester, M.H.A., Kaptein, E., Roest, T.J., Van Dijk, C.H., Tibboel, D., Meinl, W., Glatt,
- 584 H., Coughtrie, M.W.H., Visser, T.J., 2003. Characterization of rat iodothyronine
- 585 sulfotransferases. Am. J. Physiol. Endocrinol. Metab. 285.
- 586 https://doi.org/10.1152/ajpendo.00046.2003

- 587 Kodama, S., Negishi, M., 2013. Sulfotransferase genes: Regulation by nuclear
- 588 receptors in response to xeno/endo-biotics. Drug Metab. Rev.
- 589 https://doi.org/10.3109/03602532.2013.835630
- 590 Kondo, T., Ikenaka, Y., Nakayama, S.M.M., Kawai, Y.K., Mizukawa, H., Mitani, Y.,
- 591 Nomiyama, K., Tanabe, S., Ishizuka, M., 2017. Uridine Diphosphate-
- 592 Glucuronosyltransferase (UGT) 2B subfamily interspecies differences in
- carnivores. Toxicol. Sci. 158, 90–100. https://doi.org/10.1093/toxsci/kfx072
- 594 Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular
- 595 evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35,
- 596 1547–1549. https://doi.org/10.1093/molbev/msy096
- 597 Kurogi, K., Sakakibara, Y., Yasuda, S., Liu, M.-C., Suiko, M., 2010. Molecular
- 598 Cloning and Characterization of a Novel Canine Sulfotransferase, in: Basic and
- 599 Applied Aspects. Springer Netherlands, pp. 221–229.
- 600 https://doi.org/10.1007/978-90-481-3892-0\_36
- 601 Kurogi, K., Shimohira, T., Kouriki-Nagatomo, H., Zhang, G., Miller, E.R., Sakakibara,
- 602 Y., Suiko, M., Liu, M.-C., 2017. Human Cytosolic Sulphotransferase SULT1C3:
- 603 genomic analysis and functional characterization of splice variant SULT1C3a
- and SULT1C3d. https://doi.org/10.1093/jb/mvx044
- Liu, M.C., Sakakibara, Y., Liu, C.C., 1999. Bacterial expression, purification, and
- 606 characterization of a novel mouse sulfotransferase that catalyzes the sulfation of
- 607 eicosanoids. Biochem. Biophys. Res. Commun. 254, 65–69.
- 608 https://doi.org/10.1006/bbrc.1998.9872
- Lu, H., Gunewardena, S., Cui, J.Y., Yoo, B., Zhong, X.B., Klaassen, C.D., 2013.
- 610 RNA-sequencing quantification of hepatic ontogeny and tissue distribution of

- 611 mRNAs of phase II enzymes in mice. Drug Metab. Dispos. 41, 844–857.
- 612 https://doi.org/10.1124/dmd.112.050211
- 613 Meinl, W., Donath, C., Schneider, H., Sommer, Y., Glatt, H., 2008. SULT1C3, an
- orphan sequence of the human genome, encodes an enzyme activating various
- 615 promutagens. Food Chem. Toxicol. 46, 1249–1256.
- 616 https://doi.org/10.1016/j.fct.2007.08.040
- 617 Nagata, K., Ozawa, S., Miyata, M., Shimada, M., Gong, D.W., Yamazoe, Y., Kato,
- 618 R., 1993. Isolation and expression of a cDNA encoding a male-specific rat
- 619 sulfotransferase that catalyzes activation of N-hydroxy-2-acetylaminofluorene. J.
- 620 Biol. Chem. 268, 24720–24725. https://doi.org/10.1016/s0021-9258(19)74524-4
- Nomiyama, K., Kanbara, C., Ochiai, M., Eguchi, A., Mizukawa, H., Isobe, T.,
- Matsuishi, T., Yamada, T.K., Tanabe, S., 2014. Halogenated phenolic
- 623 contaminants in the blood of marine mammals from Japanese coastal waters.
- 624 Mar. Environ. Res. 93, 15–22. https://doi.org/10.1016/j.marenvres.2013.08.016
- Nowell, S., Green, B., Yong, M.T., Wiese, R., Kadlubar, F.F., 2005. Examination of
- 626 human tissue cytosols for expression of sulfotransferase isoform 1A2
- 627 (SULT1A2) using a SULT1A2-specific antibody. Mol. Pharmacol. 67, 394–399.
- 628 https://doi.org/10.1124/mol.104.006171
- Noyes, P.D., Lema, S.C., 2015. Forecasting the impacts of chemical pollution and
- 630 climate change interactions on the health of wildlife. Curr. Zool. 61, 669–689.
- 631 Oda, S., Fukami, T., Yokoi, T., Nakajima, M., 2015. A comprehensive review of
- 632 UDP-glucuronosyltransferase and esterases for drug development. Drug Metab.
- 633 Pharmacokinet. 30, 30–51. https://doi.org/10.1016/j.dmpk.2014.12.001

- 634 Omura, T., Sato, R., 1964. The carbon monoxide-bindng pigent of liver microsomes.
- 635 I. Evidence for its hemoprotein nature. J. Biol. Chem. 239, 2370–8.
- 636 Paitz, R.T., Angles, R., Cagney, E., 2020. In ovo metabolism of estradiol to estrone
- 637 sulfate in chicken eggs: Implications for how yolk estradiol influences embryonic
- 638 development. Gen. Comp. Endocrinol. 287, 113320.
- 639 https://doi.org/10.1016/j.ygcen.2019.113320
- 640 Paitz, R.T., Bowden, R.M., 2013. Sulfonation of Maternal Steroids is a Conserved
- 641 Metabolic Pathway in Vertebrates. Integr. Comp. Biol. 53, 895–901.
- 642 https://doi.org/10.1093/icb/ict027
- Riches, Z., Stanley, E.L., Bloomer, J.C., Coughtrie, M.W.H., 2009. Quantitative
- 644 evaluation of the expression and activity of five major sulfotransferases (SULTs)
- in human tissues: The SULT "pie." Drug Metab. Dispos. 37, 2255–2261.
- 646 https://doi.org/10.1124/dmd.109.028399
- 647 Rodríguez-Estival, J., Mateo, R., 2019. Exposure to anthropogenic chemicals in wild
- 648 carnivores: a silent conservation threat demanding long-term surveillance. Curr.
- Opin. Environ. Sci. Heal. https://doi.org/10.1016/j.coesh.2019.06.002
- 650 Runge-Morris, M., Kocarek, T.A., 2013. Expression of the sulfotransferase 1C family:
- 651 Implications for xenobiotic toxicity. Drug Metab. Rev.
- 652 https://doi.org/10.3109/03602532.2013.835634
- 653 Saengtienchai, A., Ikenaka, Y., Nakayama, S.M.M., Mizukawa, H., Kakehi, M.,
- Bortey-Sam, N., Darwish, W.S., Tsubota, T., Terasaki, M., Poapolathep, A.,
- 655 Ishizuka, M., 2014. Identification of interspecific differences in phase II
- 656 reactions: Determination of metabolites in the urine of 16 mammalian species

- 657 exposed to environmental pyrene. Environ. Toxicol. Chem. 33, 2062–2069.
- 658 https://doi.org/10.1002/etc.2656
- 659 Sakakibara, Y., Yanagisawa, K., Katafuchi, J., Ringer, D.P., Takami, Y., Nakayama,
- 660 T., Suiko, M., Liu, M.C., 1998. Molecular cloning, expression, and
- 661 characterization of novel human SULTIC sulfotransferases that catalyze the
- 662 sulfonation of N-hydroxy-2- acetylaminofluorene. J. Biol. Chem. 273, 33929–
- 663 33935. https://doi.org/10.1074/jbc.273.51.33929
- 664 Shimada, M., Terazawa, R., Kamiyama, Y., Honma, W., Nagata, K., Yamazoe, Y.,
- 665 2004. Unique properties of a renal sulfotransferase, St1d1, in dopamine
- 666 metabolism. J. Pharmacol. Exp. Ther. 310, 808–814.
- 667 https://doi.org/10.1124/jpet.104.065532
- 668 Shrestha, B., Reed, J.M., Starks, P.T., Kaufman, G.E., Goldstone, J. V., Roelke,
- 669 M.E., O'Brien, S.J., Koepfli, K.P., Frank, L.G., Court, M.H., 2011. Evolution of a
- 670 major drug metabolizing enzyme defect in the domestic cat and other Felidae:
- 671 Phylogenetic timing and the role of hypercarnivory. PLoS One 6, 221–237.
- 672 https://doi.org/10.1371/journal.pone.0018046
- 673 Stanley, E.L., Hume, R., Coughtrie, M.W.H., 2005. Expression profiling of human
- 674 fetal cytosolic sulfotransferases involved in steroid and thyroid hormone
- 675 metabolism and in detoxification. Mol. Cell. Endocrinol. 240, 32–42.
- 676 https://doi.org/10.1016/j.mce.2005.06.003
- 677 Suiko, M., Kurogi, K., Hashiguchi, T., Sakakibara, Y., Liu, M.C., 2017. Updated
- 678 perspectives on the cytosolic sulfotransferases (SULTs) and SULT-mediated
- 679 sulfation. Biosci. Biotechnol. Biochem.
- 680 https://doi.org/10.1080/09168451.2016.1222266

- Suiko, M., Sakakibara, Y., Liu, M.-Y., Yang, Y.-S., Liu, M.-C., 2005. Cytosolic
- 682 Sulfotransferases and Environmental Estrogenic Chemicals. J. Pestic. Sci. 30,
- 683 345–353. https://doi.org/10.1584/jpestics.30.345
- 684 Teramoto, T., Sakakibara, Y., Inada, K., Kurogi, K., Liu, M.C., Suiko, M., Kimura, M.,
- 685 Kakuta, Y., 2008. Crystal structure of mSULT1D1, a mouse catecholamine
- sulfotransferase. FEBS Lett. 582, 3909–3914.
- 687 https://doi.org/10.1016/j.febslet.2008.10.035
- 688 Tong, M.H., Jiang, H., Liu, P., Lawson, J.A., Brass, L.F., Song, W.-C.C., 2005.
- 689 Spontaneous fetal loss caused by placental thrombosis in estrogen
- sulfotransferase-deficient mice. Nat. Med. 11, 153–9.
- 691 https://doi.org/10.1038/nm1184
- 592 Tsoi, Carrie, Falany, C.N., Morgenstern, R., Swedmark, S., 2001. Molecular cloning,
- 693 expression, and characterization of a canine sulfotransferase that is a human
- 694 ST1B2 ortholog. Arch. Biochem. Biophys. 390, 87–92.
- 695 https://doi.org/10.1006/abbi.2001.2373
- 596 Tsoi, C., Falany, C.N., Morgenstern, R., Swedmark, S., 2001. Identification of a new
- 697 subfamily of sulphotransferases: Cloning and characterization of canine
- 698 SULT1D1. Biochem. J. 356, 891–897. https://doi.org/10.1042/0264-
- 699 6021:3560891
- 700 Tsoi, C., Morgenstern, R., Swedmark, S., 2002. Canine sulfotransferase SULT1A1:
- 701 Molecular cloning, expression, and characterization. Arch. Biochem. Biophys.
- 702 401, 125–133. https://doi.org/10.1016/S0003-9861(02)00021-8
- 703 Wilson, L.A., Reyns, G.E., Darras, V.M., Coughtrie, M.W.H., 2004. cDNA cloning,
- functional expression, and characterization of chicken sulfotransferases

- belonging to the SULT1B and SULT1C families. Arch. Biochem. Biophys. 428,
- 706 64–72. https://doi.org/10.1016/j.abb.2004.05.008
- 707 Wu, B., Basu, S., Meng, S., Wang, X., Zhang, S., Hu, M., 2011. Regioselective
- 708 Sulfation and Glucuronidation of Phenolics: Insights into the Structural Basis of
- 709 Conjugation. Curr. Drug Metab. 12, 900.
- 710 Yamauchi, K., Katsumata, S., Ozaki, M., 2019. A prototype of the mammalian
- sulfotransferase 1 (SULT1) family in <i&gt;Xenopus laevis&lt;/i&gt;: molecular
- and enzymatic properties of <i&gt;Xl&lt;/i&gt;SULT1B.S. Genes Genet. Syst.
- 713 94, 207–217. https://doi.org/10.1266/ggs.19-00026
- Yi, M., Negishi, M., Lee, S.-J., 2021. Estrogen Sulfotransferase (SULT1E1): Its
- 715 Molecular Regulation, Polymorphisms, and Clinical Perspectives. J. Pers. Med.
- 716 11, 194. https://doi.org/10.3390/jpm11030194

# Graphical abstract



## Tables

	Rat	Cat	Steller Sea Lion	Harbor Seal
Vmax/Km	5 17 + 0 77 0	562 ± 27 1 b		
(µl/min/mg)	5.17 ± 0.77 a	505 ± 57.4 D	N.D.	N.D.
Vmax	54 3 + 6 03	<i>1</i> 67±583		
(pmol/min/mg)	54.5 ± 0.95	40.7 ± 5.65	N.D.	N.D.
Km (uM)	10 5 + 2 23 a	0.0829 ± 0.0390	ND	ND
	10.0 ± 2.20 a	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. Vmax/Km values that were significantly different (P < 0.05) within a substrate, based on Tukey's HSD tests for each Vmax/Km, 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

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

# Supplementary Table 1. Animal liver samples used in this study

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

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

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

Canidae

			SULT1C4		XM038680150.1
			SULT1D1		XM038685809.1
			SULT1E1		XM038685811.1
	Red fox	Vulpes	LOC112929198 (SULT1	A1)	XM026011091
			SULT1B1		XM026003470
			SULT1C2		XM026011035
			SULT1C4		XM026010893
			SULT1D1-like:	LOC112923601	XM026002460
			(SULT1D1)		71020003409
			LOC112923635 (SULT1	E1)	XM026003484
Mustelidae	North American river otter	Lontra canadensis	LOC116858063 (SULT1	A1)	XM032842614
			SULT1B1		XM032845783
			LOC116857948 (SULT1	C1)	XM032842428
			SULT1C2		XM032842417.1

		SULT1C4-like:	LOC116857940	XM032842416
		(SULT1C4)		XIII032042410
		SULT1D1-like:	LOC116859864	XM022845501
		(SULT1D1)		XIVI032645591
		SULT1E1-like:	LOC116859692	XM000045400
		(SULT1E1)		XIVIU32845460
Sea otter	Enhydra lutris kenyoni	SULT1A1: LOC111162234 (SULT1A1) SULT1B1: LOC111149182 (SULT1B1) SULT1C1-like: LOC111155904		XM022526502.1
				XM022506124
				N/1 10005 10000 1
		(SULT1C1)		XIVIU22516369.1
		SULT1C4-like:	LOC111155885	XM0005400444
		(SULT1C4) SULT1D1-like: LOC111149106		XM022516341.1
				XM000500000
		(SULT1D1)		XMU22506029
		SULT1E1: LOC1111491	78 (SULT1E1)	XM022506117

Ermine	Mustela erminea	SULT1A1: LOC116581510 (SULT1A1)		XM032328707
		SULT1B1	SULT1B1	
		SULT1C1: LOC116595393 (SULT1C1) SULT1C2: LOC116595392 (SULT1C2)		XM032351657
				XM032351655
		SULT1C4-like:	LOC116595391	VM000054050
		(SULT1C4)		XMU32351652
	SULT1D1: LOC116585153 (SULT1D1)		153 (SULT1D1)	XM032334485
		SULT1E1-like:	LOC116585151	XM000004404
		(SULT1E1)		XMU32334461
Domestic ferret	Mustela putorius furo	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:       LOC11326902         (SULT1C1)       SULT1C2: LOC113267746 (SULT1C2)         SULT1C4       SULT1D1: LOC113262555 (SULT1D1)		XM026517600
					XIVI020517099
					XM026515916
					XM026515929
					XM026509011
			SULT1E1		XM026509014
	Polar bear	Ursus maritimus	SULT1A1: LOC1036578	25 (SULT1A1)	XM008685238.2
			SULT1B1		XM008683684
			SULT1C1:LOC1036706	21 (SULT1C1)	XM040622914.2
			SULT1C2		XM008699075.2
			SULT1C4		XM 008699073.2
			SULT1D1: LOC1036563	319 (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:		Lentenvehetee weddellij		VM006722020 2
Monachinae		Leptonychotes weddellii	SULTAT: LUC 102733278 (SULTAT)	XIVIUU0733938.2
			SULT1B1: LOC102727575 (SULT1B1)	XM006729321.2
			SULT1C1-like: LOC102725762	2
			(SULT1C1)	XMU31022251.1
			SULT1C2: LOC102747532 (SULT1C2)	XM006728933
			SULT1C4: LOC102747824 (SULT1C4)	XM006728934.2

Southern seal	elephant	Mirounga leonina	SULT1A1: LOC117999643 (SULT1A1)		XM034988959
			SULT1B1: LOC1180104	78 (SULT1B1)	XM035004869
			SULT1C1-like:	LOC118008308	XM025001042 1
			(SULT1C1)		XM035001943.1
			SULT1C2		XM035001944.1
			SULT1C4-like:	LOC118008292	XM035001000 1
			(SULT1C4)		XIM033001900.1
Hawaiian m	nonk seal	Neomonachus schauinslandi	SULT1A1: LOC1105898	44 (SULT1A1)	XM021700477.1
			SULT1B1: LOC1105914	81 (SULT1B1)	XM021702403
			SULT1C1-like:	LOC110572024	
			(SULT1C1P)		
			SULT1C2		XM021680275.1
			SULT1C4		XM021680274.1

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

				SULT1C4-like: LOC113	937589	XM027622118.2
	Stellar sea lion	Eumetopias jub	atus	SULT1A1: LOC1142144	38 (SULT1A1)	XM028110090
				SULT1B1: LOC1142068	00 (SULT1B1)	XM028100710
				SULT1C1: LOC1141993	885 (SULT1C1)	XM028091278.1
				SULT1C2-like:	LOC114199388	XM028001270
				(SULT1C2)		XW028091279
				SULT1C4		XM028091284
	Northern fur seal	Callorhinus ursi	nus	SULT1A1: LOC1128104	57 (SULT1A1)	XM025854081.1
				SULT1B1: LOC1128359	01 (SULT1B1)	XM025887547
				SULT1C1: LOC1128215	508 (SULT1C1)	XM025868772
				SULT1C2		XM025869098.1
				SULT1C4-like:	LOC112821718	XM025860000 1
				(SULT1C4)		7.0023009099.1
Odobenidae	Pacific walrus	863 (SI    T1A1)	XM012561271 1			
Cuopeniuae	r acine wairus	divergens		50LTIAT. 2001013000		AWO 1230 127 1.1

			SULT1B1		XM004392889.1
			SULT1C1-like:	LOC101376188	VM012567211 1
			(SULT1C1)		XINU 12307311.1
			SULT1C2		XM004414460.2
			SULT1C4		XM004414459.1
Falidae	Domestic cat	Felis catus	SULT1A1		XM019820729.1
			SULT1B1		XM023253069
			SULT1C3 (SULT1C1)		XM003983864.5
			SULT1C2		XM011281074
			SULT1C4		XM003983869.5
			SULT1D1-like:	LOC101086557	XM022252066 1
			(SULT1D1)		AM023233000.1
			SULT1E1		XM003985305.3
	Cheetah	Acinonyx jubatus	SULT1A1: LOC106966	152 (SULT1A1)	XM027053118.1
			SULT1B1		XM015074496.2

		SULT1C1-like:	LOC106977307	VM016074701 0
		(SULT1C1)		XIVI015074791.2
		SULT1C2: LOC1069772	290 (SULT1C2)	XM027069399.1
		SULT1C4: LOC1069773	305 (SULT1C4)	XM015074790.2
		SULT1D1-like:	LOC106977024	XM027064250 1
		(SULT1D1)		XIVIU27004330.1
		SULT1E1		XM015074488.2
Puma	Puma concolor	SULT1A1: LOC1128559	996 (SULT1A1)	XM025919525.1
Puma	Puma concolor	SULT1A1: LOC1128559 SULT1B1	996 (SULT1A1)	XM025919525.1 XM025921642.1
Puma	Puma concolor	SULT1A1: LOC1128559 SULT1B1 SULT1C1-like:	996 (SULT1A1) LOC112868851	XM025919525.1 XM025921642.1
Puma	Puma concolor	SULT1A1: LOC1128559 SULT1B1 SULT1C1-like: (SULT1C1)	996 (SULT1A1) LOC112868851	XM025919525.1 XM025921642.1 XM025931831.1
Puma	Puma concolor	SULT1A1: LOC1128559 SULT1B1 SULT1C1-like: (SULT1C1) SULT1C2	996 (SULT1A1) LOC112868851	XM025919525.1 XM025921642.1 XM025931831.1 XM025932749.1
Puma	Puma concolor	SULT1A1: LOC1128559 SULT1B1 SULT1C1-like: (SULT1C1) SULT1C2 SULT1C4-like:	996 (SULT1A1) LOC112868851 LOC112869068	XM025919525.1 XM025921642.1 XM025931831.1 XM025932749.1

\_

		SULT1D1-like:	LOC112858721	VM005000407.4
		(SULT1D1)		XINU25922137.1
		SULT1E1-like:	LOC112858407	XM005004005
		(SULT1E1)		XINIU25921805
Leopard	Panthera pardus	SULT1A1: LOC1092	256521 (SULT1A1)	XM019431898
		SULT1B1: LOC1092	278167 (SULT1B1)	XM019468233
		SULT1C1-like:	LOC109258064	VM040424645 4
		SULT1C1-like: (SULT1C1) SULT1C2-like:		XIVIU 194340 15. I
		SULT1C2-like:	LOC109258069	VM0404246474
		(SULT1C2)		XIVIU 19434017.1
		SULT1C4		XM019434612.1
		SULT1D1-like:	LOC109278146	XM040469226 4
		(SULT1D1)		XIVIU 19408220. I
		SULT1E1		XM019468175.1
Tiger	Panthera tigris	SULT1A1: LOC1029	972472 (SULT1A1)	XM007084062.3

			SULT1B1		XM007086908.3
			SULT1C1: LOC1029523	35 (SULT1C1)	XM007097761.3
			SULT1C2: LOC1029520	42 (SULT1C2)	XM042979753.1
			SULT1C4		XM007097759.3
			SULT1D1: LOC1029699	65 (SULT1D1)	XM007086913.3
			SULT1E1		XM007086910.3
	Lynx	Lynx canadensis	SULT1A1: LOC1155045	63 (SULT1A1)	XM030301602
			SULT1B1		XM030313745
			SULT1C1: LOC1155104	37 (SULT1C1)	XM030310292.2
			SULT1C2: LOC1155096	92 (SULT1C2)	XM030309021.1
			SULT1C4		XM030310291
			SULT1D1-like:	LOC115512581	VM020212742
			(SULT1D1)		XIM030313743
			SULT1E1		XM030313739
Hyaenidae	Striped hyena	Hyaena hyaena	SULT1A1: LOC1202422	77 (SULT1A1)	XM039247942.1

			SULT1B1		XM039251765.1
			SULT1C1-like:	LOC120227542	XM020225020 4
			(SULT1C1)		XM039225639.1
			SULT1C4		XM039222195
			SULT1D1-like:	LOC120244963	XM039251783 1
			(SULT1D1)		////000201/00.1
			SULT1E1-like:	LOC120244962	VM020251792 1
			(SULT1E1)		XIVI039251762.1
Herpestidae	Meerkat	Suricata suricatta	SULT1A1: LOC1152	297986 (SULT1A1)	XM029946269
			SULT1B1		XM029931052.1
			SULT1C1-like:	LOC115288947	XM020026407.4
			(SULT1C1)		XIII029930197.1
			SULT1C4		XM029937066

			SULT1D1-like:	LOC115301877	VM0200540824
			(SULT1D1)		XM029951883.1
			estrogen	sufotransferase-like:	XM020045225 1
			LOC115296737 (SU	JLT1E1)	AM029943223.1
Monotremata:					
Ornithorhynchid	Platypus	Ornithorhynchus anatinus	SULT1A		NM001127619
ae					
			SULT1B1		XM007669210
			SULT1C1-like: LOC	100090962	XM029048649
			SULT1C4-like:	LOC114809133	XM020058204
			(SULT1C4)		AW029030204
			SULT1E1		XM029073465

# Supplementary Figures

## a.

number of aligned nucleotide	142	143	144	145	5 146	147	148	3 149	150	151	1 152	2 15	3 15	4 155	5 156	6 157	158	159	160	161	162	163 1	64 16	65 16	6 16	7 168	169	9 170	171	172	173 1	74 1	75 176
translated amino acid from human SULT1C2		Ρ			Κ			Α			G	i		Т			Т			W			I		Q			Е			V		
H. sapiens SULT1C2 NM 001056	С	С	Т	А	А	А	G	С	А	G	G	G	А	С	А	А	С	G	Т	G	G	A T	Т	С	А	G	G	А	А	A	т т	G	Т
R. norvegicus Sult1c2a NM 001013177	С	С	Т	Α	Α	А	Т	С	А	G	G	G	А	С	А	А	С	А	Т	G	G	A T	Т	С	Α	А	G	А	А	A	т т	G	Т
R. norvegicus SULT1C2-like LOC100910526 XM 006244254	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	А	А	С	A	Т	G	G	A T	Т	С	А	А	G	А	А	A	т т	G	Т
R. norvegicus SULT1C2 XM 039084411 RGD1559960	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	А	А	А	A	Т	G	G	A T	Т	С	А	А	G	А	А	A	ΓA	۰ G	Т
R. norvegicus Sult1c2 NM 133547.4	С	С	Т	А	Α	А	Т	С	А	G	G	G	Α	С	Α	А	С	A	Т	G	G	A T	Т	С	Α	Α	G	Α	А	A	т т	G	Т
M. musculus Sult1c2 NM 026935.4	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	А	А	С	A	Т	G	G	A T	Т	С	Α	А	G	А	А	A	т т	G	Т
C. lupus familiaris SULT1C2 XM 038680149.1	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	Т	А	С	A	Т	G	G	A T	Т	С	А	G	G	А	А	A	т т	G	Т
V. vulpes SULT1C2 XM 026011035	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	Т	А	С	A	Т	G	G	A T	Т	С	А	G	G	А	А	A	т т	G	Т
V. lagopus SULT1C2 XM 041754093 LOC121490422	С	С	Т	Α	Α	А	Т	С	А	G	G	G	Α	С	Т	А	С	А	Т	G	G	A T	Т	С	Α	G	G	А	А	A	т т	G	Т
U. arctos horribilis SULT1C2 XM 026515916	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	A	Т	G	G	A T	Т	С	Α	G	G	А	G	A	т т	G	Т
U. maritimus SULT1C2 XM 008699075.1 LOC103670620	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	А	Т	G	G	A T	Т	С	А	G	G	А	G	A	т т	G	Т
A. melanoleuca SULT1C2 XM 002930286.4 LOC100467202	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	A	Т	G	G	A T	Т	С	А	G	G	А	G	A	т т	G	Т
M. putorius furo SULT1C2 x3 XM 004764352.2	С	С	Т	А	А	А	Т	С	А	G	G	С	А	С	С	А	С	A	Т	G	G	A T	Т	С	Α	G	G	А	А	A	т т	G	Т
N. vison SULT1C2 XM 044262435 LOC122915683	С	С	Т	Α	А	А	Т	С	А	G	G	С	А	С	С	А	С	А	Т	G	G	A T	Т	С	Α	G	G	А	А	A	т т	G	Т
M. erminea SULT1C2 XM 032351655 LOC116595392	С	С	Т	А	А	А	Т	С	А	G	G	С	А	С	С	А	С	A	Т	G	G	A T	Т	С	А	G	G	А	А	A	т т	G	Т
F. catus SULT1C2 x1 XM 011281074.3	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	G	Т	G	G	A T	Т	С	А	G	G	А	А	A	т т	G	Т
L. canadensis Lynx SULT1C2 XM 030309021 LOC115509692	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	G	Т	G	G	A T	Т	С	Α	G	G	Α	Α	A	т т	G	Т
P. yagouaroundi SULT1C2-like XM 040454146 LOC121016479	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	G	Т	G	G	A T	Т	С	А	G	G	А	А	A	г т	G	Т
P. bengalensis SULT1C2 XM 043553279 LOC122466971	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	G	Т	G	G	A T	Т	С	Α	G	G	А	А	A	г т	G	Т
P. tigris SULT1C2 XM 042979753 LOC102952042	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	G	Т	G	G	A T	Т	С	Α	G	G	А	А	A	г т	G	Т
P. pardus SULT1C2-like XM 019434617.1 LOC109258069	С	С	Т	А	Α	А	Т	С	А	G	G	G	А	С	С	А	С	G	Т	G	G	A T	Т	С	Α	G	G	А	А	A	г т	G	Т
P. leo SULT1C2-like XM 042930191 LOC122214989	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	G	Т	G	G	A T	Т	С	А	G	G	А	А	A	т т	G	Т
O. rosmarus divergens SULT1C2 XM 004414460.2	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	А	Т	G	G	A T	Т	С	Α	G	G	А	А	A	т т	A	Т
Z. californianus SULT1C2 XM 027622119	С	С	Т	А	Α	А	Т	С	А	G	G	G	А	С	С	А	С	A	Т	G	G	A T	Т	С	Α	G	G	Α	А	A	т т	A	Т
E. jubatus SULT1C2-like XM 028091279 LOC114199388	С	С	Т	Α	А	А	Т	С	А	G	G	G	А	С	С	А	С	А	Т	G	G	A T	Т	С	Α	G	G	А	А	A	т т	A	Т
C. ursinus SULT1C2 XM 025869098	С	С	Т	А	Α	А	Т	С	А	G	G	G	А	С	С	А	С	А	Т	G	G	A T	Т	С	Α	G	G	А	А	A	т т	A	Т
P. vitulina SULT1C2 XM 032419671	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	А	T.	A	G	A T	Т	С	А	G	G	А	А	Α	гτ	G	Т
H. grypus SULT1C2 XM 036120462	С	С	Т	А	А	А	Т	С	А	G	G	G	А	С	С	А	С	А	T.	A	G	A T	Т	С	А	G	G	А	А	A	гτ	G	Т
N. schauinslandi SULT1C2 XM 021680275.1	С	С	т	А	А	А	т	С	А	G	G	G	А	С	С	А	С	A	т.	A	G	A T	т	С	А	G	G	А	А	A	т т	G	Т

b.

number of aligned nucleotide	379	38	38	1 382	2 38	3 384	385	386	6 387	7 388	3 389	390	391	1 392	2 39	3 39	4 39	5 396	397	398	3 399	400	401	402	403	404	405	i 406	407	408
translated amino acid from human SULT1C2		L			Y			V			Α			R			N			Α			Κ			D			С	
H. sapiens SULT1C2 NM 001056	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	Т
R. norvegicus Sult1c2a NM 001013177	С	Т	Т	Т	А	Т	G	Т	G	G	С	Т	С	G	А	А	А	С	G	С	С	А	А	А	G	А	С	Т	G	С
R. norvegicus SULT1C2-like LOC100910526 XM 006244254	С	Т	Т	Т	Α	Т	G	Т	G	G	С	Т	С	G	А	А	Α	Т	G	С	С	Α	А	А	G	А	С	Т	G	С
R. norvegicus SULT1C2 XM 039084411 RGD1559960	С	Т	Т	Т	А	Т	G	Т	G	G	С	Т	С	G	А	А	Α	С	G	С	С	Α	А	А	G	А	С	Т	G	С
R. norvegicus Sult1c2 NM 133547.4	С	Т	Т	Т	А	Т	G	Т	G	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
M. musculus Sult1c2 NM 026935.4	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	Т	А	А	А	G	А	С	Т	G	С
C. lupus familiaris SULT1C2 XM 038680149.1	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
V. vulpes SULT1C2 XM 026011035	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
V. lagopus SULT1C2 XM 041754093 LOC121490422	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
U. arctos horribilis SULT1C2 XM 026515916	С	Т	Т	Т	А	Т	G	G	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
U. maritimus SULT1C2 XM 008699075.1 LOC103670620	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	А	Т	G	С	С	А	А	А	G	А	С	Т	G	С
A. melanoleuca SULT1C2 XM 002930286.4 LOC100467202	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
M. putorius furo SULT1C2 x3 XM 004764352.2	С	Т	Т	Т	Α	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
N. vison SULT1C2 XM 044262435 LOC122915683	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
M. erminea SULT1C2 XM 032351655 LOC116595392	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
F. catus SULT1C2 x1 XM 011281074.3	С	Т	Т	Т	А	Т	G	Т	G	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
L. canadensis Lynx SULT1C2 XM 030309021 LOC115509692	С	Т	Т	Т	Α	Т	G	Т	G	G	С	Т	С	А	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
P. yagouaroundi SULT1C2-like XM 040454146 LOC121016479	С	Т	Т	Т	А	Т	G	Т	G	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
P. bengalensis SULT1C2 XM 043553279 LOC122466971	С	Т	Т	Т	А	Т	G	Т	G	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
P. tigris SULT1C2 XM 042979753 LOC102952042	С	Т	Т	Т	Α	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
P. pardus SULT1C2-like XM 019434617.1 LOC109258069	С	Т	Т	Т	А	Т	G	т	А	G	С	Т	т	G	А	Α	Α	Т	G	С	С	А	А	А	G	А	С	т	G	С
P. leo SULT1C2-like XM 042930191 LOC122214989	С	Т	Т	Т	А	Т	G	т	А	G	С	Т	т	G	А	Α	Α	Т	G	С	С	А	А	А	G	А	С	т	G	С
O. rosmarus divergens SULT1C2 XM 004414460.2	С	Т	Т	Т	А	Т	G	т	А	G	С	Т	т	G	А	Α	Α	Т	G	С	С	А	А	А	G	А	С	т	G	С
Z. californianus SULT1C2 XM 027622119	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
E. jubatus SULT1C2-like XM 028091279 LOC114199388	С	Т	Т	Т	Α	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
C. ursinus SULT1C2 XM 025869098	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
P. vitulina SULT1C2 XM 032419671	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
H. grypus SULT1C2 XM 036120462	С	Т	Т	Т	Α	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С
N. schauinslandi SULT1C2 XM 021680275.1	С	Т	Т	Т	А	Т	G	Т	А	G	С	Т	С	G	А	А	Α	Т	G	С	С	А	А	А	G	А	С	Т	G	С

#### C.

number of aligned nucleotide	781	782	783	784	785	786	6 787	788	3 789	790	791	792	2 793	3 794	4 795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813
translated amino acid from human SULT1C2		G			Т			V			G			D			W			Κ			Ν			Н			F			Т	
H. sapiens SULT1C2 NM 001056	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	т	G	G	А	A	A	A	А	С	С	А	С	Т	Т	С	А	С	Т
R. norvegicus Sult1c2a NM 001013177	G	G	А	А	Т	Т	G	Т	G	G	G	Т	G	Α	Т	т	G	G	А	А	А	А	А	С	С	А	С	Т	Т	Т	Α	С	Т
R. norvegicus SULT1C2-like LOC100910526 XM 006244254	G	G	А	А	С	Т	G	Т	G	G	G	Т	G	А	Т	Т	G	G	А	A	А	А	А	С	С	А	С	Т	Т	Т	А	С	Т
R. norvegicus SULT1C2 XM 039084411 RGD1559960	G	G	А	А	Т	Т	G	Т	G	G	G	Т	G	А	Т	Т	G	G	А	A	А	А	А	С	С	А	С	Т	Т	Т	А	С	Т
R. norvegicus Sult1c2 NM 133547.4	G	G	А	А	С	Т	G	Т	G	G	G	Т	G	А	Т	Т	G	G	А	A	А	А	А	С	С	А	С	Т	Т	Т	А	С	Т
M. musculus Sult1c2 NM 026935.4	G	G	А	А	С	Т	G	Т	G	G	G	Т	G	Α	Т	Т	G	G	А	Α	A	A	А	С	С	А	С	Т	Т	Т	Α	С	Т
C. lupus familiaris SULT1C2 XM 038680149.1	G	G	А	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	G	G	А	A	A	A	А	С	С	А	С	Т	Т	С	А	С	Т
V. vulpes SULT1C2 XM 026011035	G	G	А	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	G	G	А	A	А	А	А	С	С	А	С	Т	Т	С	А	С	Т
V. lagopus SULT1C2 XM 041754093 LOC121490422	G	G	А	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	G	G	А	A	А	А	А	С	С	А	С	Т	Т	С	А	С	Т
U. arctos horribilis SULT1C2 XM 026515916	G	G	А	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	G	G	А	А	А	А	А	С	С	А	С	Т	Т	С	А	С	С
U. maritimus SULT1C2 XM 008699075.1 LOC103670620	G	G	А	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	G	G	А	A	А	А	А	С	С	А	С	Т	Т	С	А	С	С
A. melanoleuca SULT1C2 XM 002930286.4 LOC100467202	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	Т	G	G	А	Α	A	A	А	С	С	А	С	Т	Т	С	Α	С	С
M. putorius furo SULT1C2 x3 XM 004764352.2	G	G	А	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	G	G	А	A	A	A	А	С	С	А	С	Т	Т	С	А	С	Т
N. vison SULT1C2 XM 044262435 LOC122915683	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	Т	G	G	А	A	A	G	А	С	С	А	С	Т	Т	С	Α	С	Т
M. erminea SULT1C2 XM 032351655 LOC116595392	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	Т	G	G	А	Α	A	A	А	С	С	Α	С	Т	Т	С	А	С	Т
F. catus SULT1C2 x1 XM 011281074.3	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	Т	G	G	А	Α	A	A	А	С	С	Α	С	Т	Т	С	Α	С	Т
L. canadensis Lynx SULT1C2 XM 030309021 LOC115509692	G	G	А	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	G	G	А	A	А	А	А	С	С	А	С	Т	Т	С	А	С	Т
P. yagouaroundi SULT1C2-like XM 040454146 LOC121016479	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	Т	G	G	А	Α	A	A	А	С	С	Α	С	Т	Т	С	А	С	Т
P. bengalensis SULT1C2 XM 043553279 LOC122466971	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	Т	G	G	А	Α	A	A	А	С	С	А	С	Т	Т	С	Α	С	Т
P. tigris SULT1C2 XM 042979753 LOC102952042	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	Т	G	G	А	A	A	A	А	С	С	А	С	Т	Т	С	Α	С	Т
P. pardus SULT1C2-like XM 019434617.1 LOC109258069	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	Т	G	G	А	A	A	A	А	С	С	Α	С	Т	Т	С	А	С	Т
P. leo SULT1C2-like XM 042930191 LOC122214989	G	G	А	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	G	G	А	A	А	A	А	С	С	А	С	Т	Т	С	А	С	Т
O. rosmarus divergens SULT1C2 XM 004414460.2	G	G	А	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	Α	G	А	А	А	А	А	С	С	А	С	Т	Т	С	А	С	Т
Z. californianus SULT1C2 XM 027622119	А	G	Т	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	Α	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. jubatus SULT1C2-like XM 028091279 LOC114199388	А	G	Т	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	Α	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. ursinus SULT1C2 XM 025869098	G	G	Т	А	С	Т	G	Т	G	G	G	G	G	А	Т	Т	Α	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. vitulina SULT1C2 XM 032419671	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	Т	G	G	А	A	A	А	А	С	С	Α	С	Т	Т	С	А	С	Т
H. grypus SULT1C2 XM 036120462	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	Т	G	G	А	A	A	A	А	С	С	А	С	Т	Т	С	А	С	Т
N. schauinslandi SULT1C2 XM 021680275.1	G	G	А	А	С	Т	G	Т	G	G	G	G	G	Α	Т	Т	G	G	А	A	А	A	А	С	С	А	С	Т	Т	С	Α	С	Т

## 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 scorfa, 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.

#### FUNDING

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan awarded to M. Ishizuka (No. 21H04919), Y. Ikenaka (No. 26304043, 15H0282505, 15K1221305), S. Nakayama (17KK0009 and 20K20633), the foundations of Sumitomo and JSPS Core to Core Program (AA Science Platforms) and Bilateral Joint Research Project (PG36150002 and PG36150003). We also acknowledge the financial support by the Soroptimist Japan Foundation, the Nakajima Foundation, JST AJ-CORE, Hokkaido University Sosei Tokutei Research and the Inui Memorial Trust for Research on Animal Science.

#### ACKNOWLEDGMENTS

We would like to express our appreciation to Dr. Kaoru Hattori (Hokkaido National Fisheries Research Institute, Fisheries Research Agency), Dr. Akihiko Wada (Hokkaido Research Organization, Central Fisheries Research Institute), and Lecturer Kentaro Q Sakamoto (Laboratory of Physiology, Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University) for the provision of samples. We are grateful to Takahiro Ichise for his technical support.