Supporting Figure Legends

Supporting Figure S1. Haplotype structure and haplotype frequency in the (a) *TMD case-control* and (b) OPPERA cohorts. Haplotypes were constructed with SNPs situated within COMT gene haploblock 2.

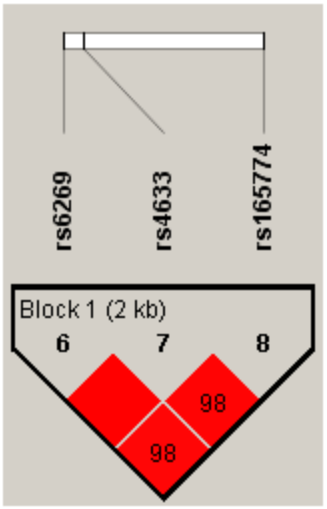
Supporting Figure S2. Conservation of alternative COMT protein variants in primates. (a) Multiple alignment of the COMT alternative protein regions (marked in red) from human, chimp and gorilla showing unambigous similarity. Stop codons are marked by @. (b) Maximum-likelihood tree of alternative regions of (a)-COMT isoforms based on the multiple alignments of different mammals.

Supporting Figure S3. Prediction of potential miRNA targets in the 3'UTR of (a)-COMT isoform, specifically in the rs165774 (G>A) vicinity. Positions of rs165774 are shown in red; G-C pairs, which could influence the difference between G and A variants, are shown in bold.

Supporting Figure S4. Three-dimensional representation of the active site of (a) S-COMT or (b) (a)S-COMT. Interaction between the enzyme's residues, enzymatic co-factor (orange sphere), and catechol substrate (red).

Supporting Figure 5. Predicted docking poses of DHBA, norepinephrine, and epinephrine. Zoomed view of best DHBA, norepinephrine, or epinephrine docking solutions for S-COMT and (a)S-COMT (Carbon atoms of ligands, SAM, and (a)S-COMT residues are represented in green, grey and white, respectively. Catalytic Mg^{2+} ion and Mg-coordinating conserved water molecule are represented as a pink and red sphere, respectively; see Supporting Table S7 for chemical structures of catecholamines and docking energy values).

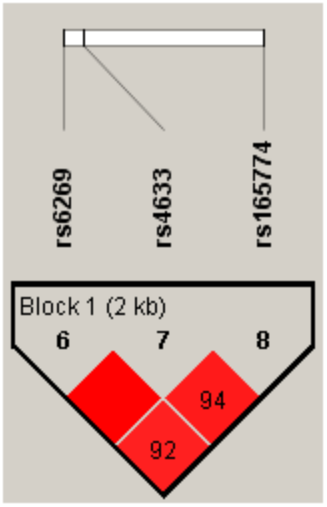
a



Genotype	Haplotype	Frquency
CCG	LPS-G	41.7%
ATA	APS-A	31.3%
ATG	APS-G	19.1%
ACG	HPS-G	7.7%

D': 0.985/r^2: 0.323

b



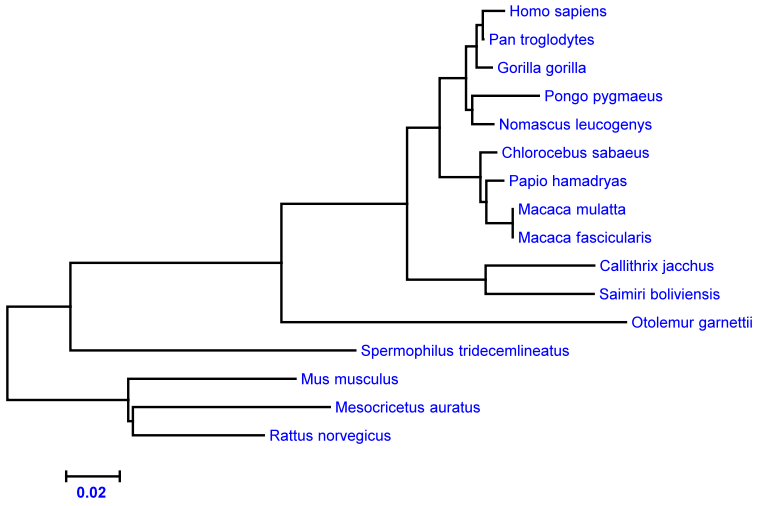
Genotype	Haplotype	Frquency
CCG	LPS-G	37.7%
ATA	APS-A	26.5%
ATG	APS-G	17.6%
ACG	HPS-G	17.4%

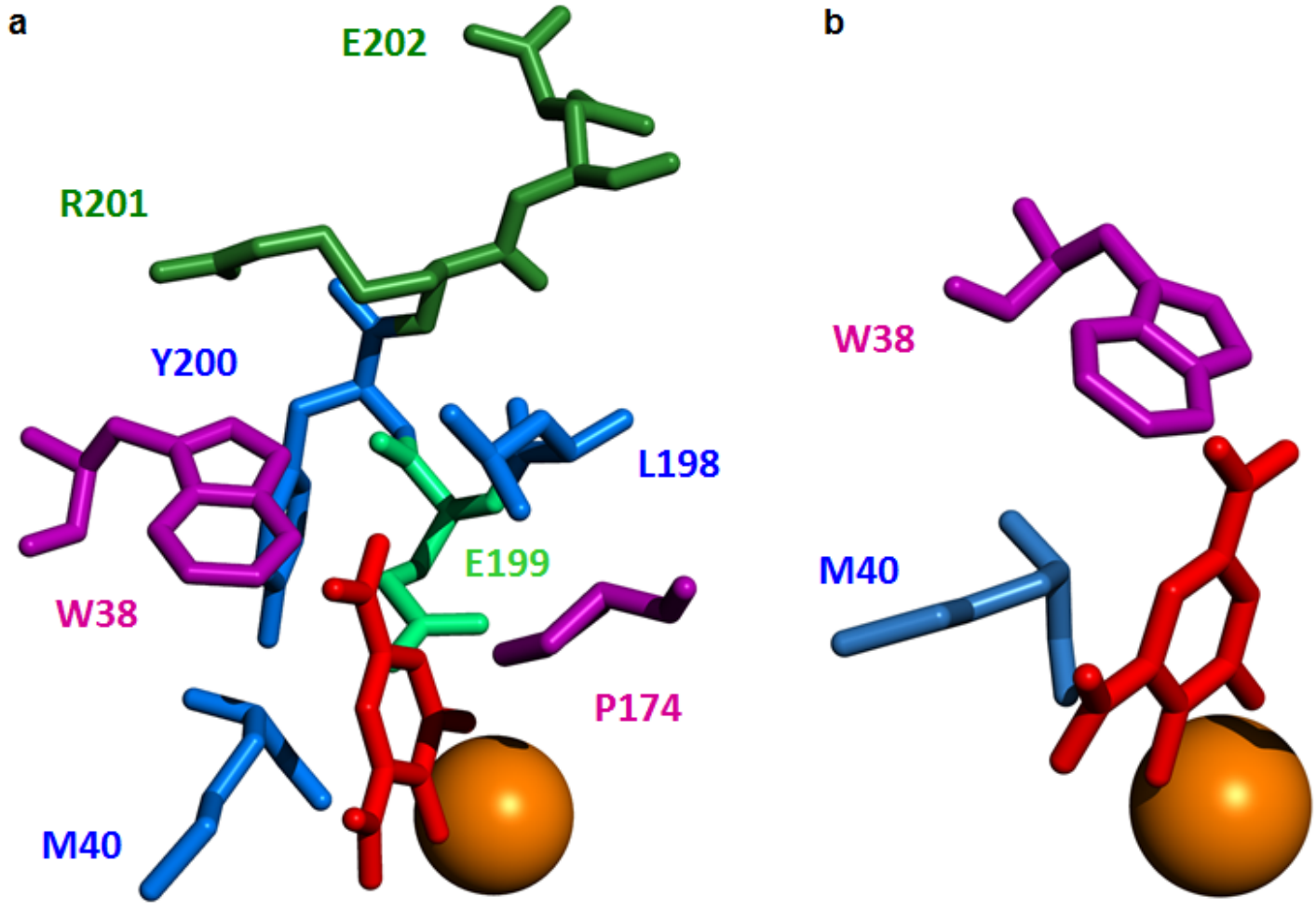
D':0.927 /r^2:0.204

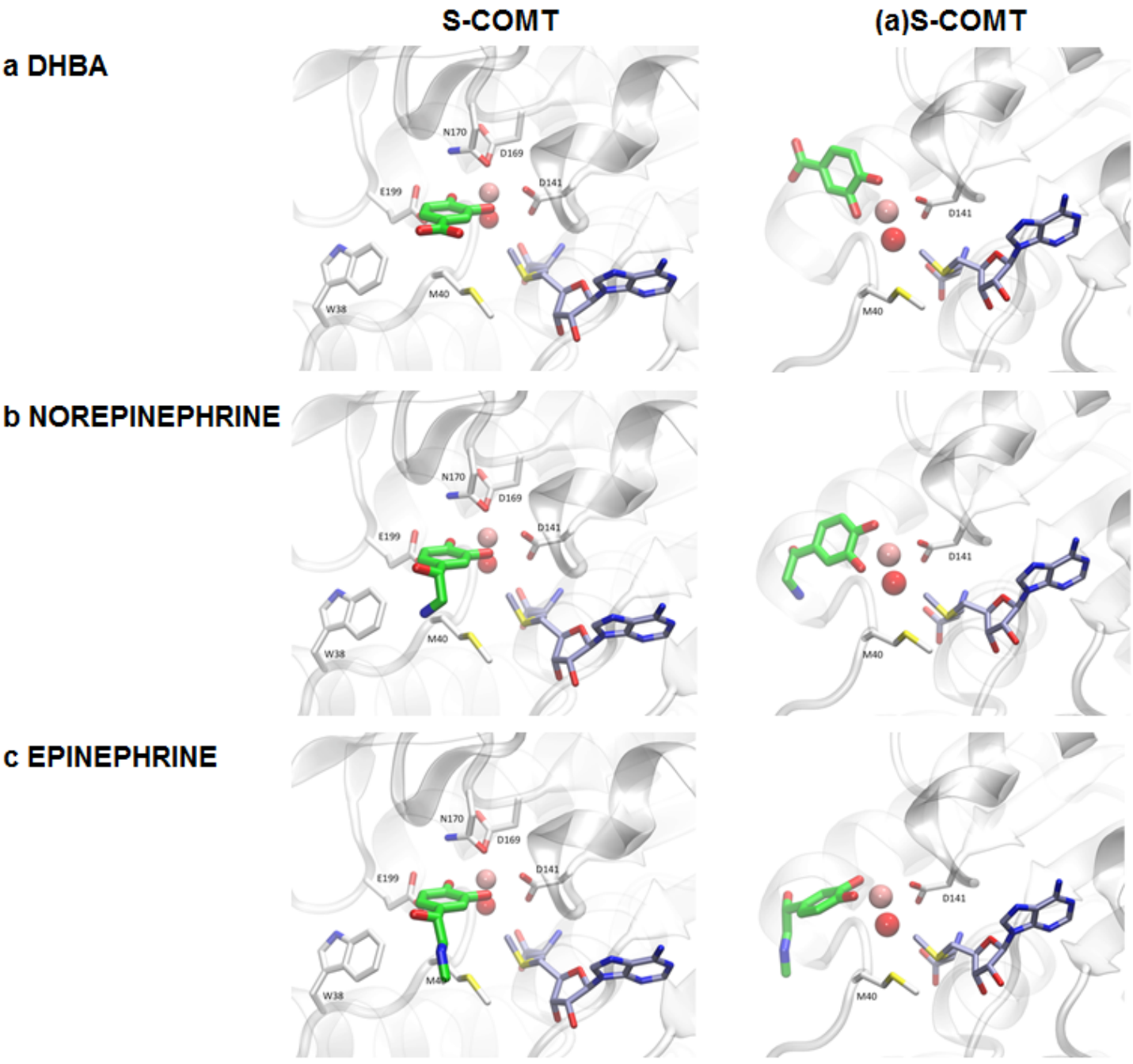
a

```
human           MPEAPPLLLAAVLLGLVLLVVLLLLLRHWGWGLCLIGWNEFILQPIHNLLMGDTKEQRIL
chimp           MLEAPPLLLAAVLLGLVLLVVLLLLLRHWGWGLCLIGWNEFVLQPIHNLLMGDTKEQRIL
gorilla         MLEAPPLLLASVLLGLVLLVVLLLLLRHWGWGLCLIGWNEFVLQPIHNLLMGDTKEQRIL
                * ********:******************************:******************

human           NHVLQHAEPGNAQSVLEAIDTYCEQKEWAMNVGDKKGKIVDAVIQEHQPSVLLELGAYCG
chimp           NHVLQHAEPGNAQSVLEAIDTYCEQKEWAMNVGDKKGKIVDAVIQEHQPSVLLELGAYCG
gorilla         NHVLQHAEPGNAQSVLEAIDTYCEQKEWAMNVGDKKGKIVDAVIQEHQPSVLLELGAYCG
                ************************************************************

human           YSAVRMARLLSPGARLITIEINPDCAAITQRMVDFAGVKDKVTLVVGASQDIIPQLKKKY
chimp           YSAVRMARLLSPGARLITIEINPDCAAITQRMVDFAGVKDKVTLVVGASQDIIPQLKKKY
gorilla         YSAVRMARLLSPGARLITIEINPDCAAITQRMVDFAGVQDKVTLVVGASQDIIPQLKKKY
                ************************************:***********************

human           DVDTLDMVFLDHWKDRYLPDTLLLEVSPNQDGIRASCCPEPIQSASASPKSQAFQ@
chimp           DVDTLDMVFLDHWKDRYLPDTLLLEVSPNQDGICASCCPEPIQSASASPKSQAFQ@
gorilla         DVDTLDMVFLDHWKDRYLPDTLLLEVSPNQDGICASCCPGPIQSASASPKSQAFQ@
                ************************************** ***** ***************
```

b




a
E202
R201
Y200
L198
E199
W38
P174
M40
b
W38
M40


S-COMT
(a)S-COMT
a DHBA
b NOREPINEPHRINE
c EPINEPHRINE
N170
D169
E199
D141
W38
M40

Supporting Table S1. Logistic regression analyses between TMD and SNP rs165774 assuming additive effects, for *TMD case-control* cohort.

	SNP	A1	N	OR	SE	L95	U95	STAT	*P*
	rs2020917	T	384	1.088	0.159	0.796	1.486	0.527	0.598
	rs737865	G	393	1.124	0.156	0.828	1.527	0.750	0.453
	rs1544325	A	392	1.081	0.151	0.804	1.454	0.517	0.606
	rs6269	G	392	1.262	0.149	0.943	1.690	1.562	0.118
TMD	rs4633	C	391	1.383	0.152	1.027	1.861	2.138	0.033
	rs165774	**A**	**392**	**0.621**	**0.162**	**0.452**	**0.852**	**-2.951**	**0.003**
	rs174697	A	394	1.812	0.399	0.829	3.962	1.490	0.136
	rs9332381	G	394	1.812	0.418	0.798	4.114	1.421	0.155
	rs165599	G	383	1.404	0.163	1.021	1.931	2.089	0.037

Results are for single-nucleotide polymorphisms (SNPs) situated within *COMT* gene, located on chromosome 22. (SNPs that failed quality checks have been removed). A1 = minor allele; N = number of subjects with non-missing data; OR = odds ratio; SE = standard error; L95 = lower bound of 95%confidence interval; U95 = upper bound of 95% confidence interval; STAT = Coefficient t-statistic; *P* = asymptotic p-value for t-statistic. *P* value lower than threshold of significance after correction for multiple testing using the method of spectral decomposition[15] is shown in bold.

Supporting Table S2. Association between each COMT haplotype against the others and TMD, and association between each COMT haplotype enriched for SNP rs165774 against the others and TMD, in the *TMD case-control* cohort.

	Haplotype	**OR**	**STAT**	***P***	**Freq_cases**	**Freq_controls**
	LPS	1.27	2.61	0.106	0.4304	0.3897
COMT haplotype	APS	0.724	4.5	**0.0339**	0.482	0.5359
	HPS	1.27	0.777	0.378	0.08763	0.07436
	LPS-G	1.250	2.230	0.135	0.392	0.407
Enriched	APS-G	1.200	0.981	0.322	0.179	0.196
COMT haplotype	APS-A	0.614	8.980	**0.003**	0.357	0.315
	HPS-G	1.310	0.967	0.325	0.072	0.079

OR = odds ratio; STAT = Test statistic (T from Wald test); *P* = Asymptotic p-value; Freq_cases = frequency of the haplotype in cases; Freq_controls = frequency of the haplotype in controls.

Supporting Table S3. Linear regression analyses between SNP rs165774 and QST phenotypes in the *TMD case-control* cohort, assuming additive effects.

	A1	N	BETA	SE	L95	U95	STAT	*P*	z-score rs165774G	z-score rs165774A
Pressure Pain	A	290	-1.712	0.546	-2.782	-0.641	-3.133	**0.002**	0.279	-1.519
Heat Pain	A	289	0.082	0.127	-0.167	0.330	0.644	0.520	0.006	-0.035
Heat Pain-First Pulse	A	287	0.055	0.239	-0.414	0.523	0.229	0.819	-0.028	0.155
Heat Pain-Rate of Rise	A	287	-0.031	0.228	-0.477	0.416	-0.134	0.893	0.046	-0.256
Heat Pain-Sum	A	287	-0.055	0.244	-0.533	0.422	-0.228	0.820	0.027	-0.148

A1 = minor allele; N = number of subjects with non-missing data; BETA = regression coefficient; SE = standard error; L95 = lower bound of 95% confidence interval; U95 = upper bound of 95% confidence interval; STAT = Coefficient t-statistic; *P* = Asymptotic p-value for t-statistic; z-score rs165774G = average z-score for subjects carrying one or two copies of the major allele (GG and GA); z-score rs165774A = average z-score for subjects carrying two copies of the minor allele (AA).

Supporting Table S4. Logistic (TMD) or linear (QST) regression analyses between SNP rs165774 and risk of TMD or SNP rs165774 and QST phenotypes in the subset of Caucasian females from the *OPPERA* cohort, assuming additive effects.

	Allele	N	OR	SE	L95	U95	STAT	*P*	Frequency rs165774G	Frequency rs165774A
TMD	A	964	0.899	0.101	0.737	1.095	-1.061	0.289	0.2746	0.2751
	Allele	**N**	**BETA**	**SE**	**L95**	**U95**	**STAT**	***P***	**z-score rs165774G**	**z-score rs165774A**
Pressure Pain	A	964	-0.624	0.303	-1.217	-0.030	-2.059	**0.040**	0.084	-0.840
Heat Pain	A	964	0.006	0.064	-0.119	0.131	0.097	0.923	0.009	-0.092
Heat Pain-First Pulse	A	964	-0.347	0.185	-0.709	0.015	-1.880	0.060	0.019	-0.185
Heat Pain-Rate of Rise	A	964	0.287	0.167	-0.041	0.614	1.717	0.086	0.017	-0.171
Heat Pain-Sum	A	964	-0.138	0.195	-0.519	0.244	-0.707	0.480	0.017	-0.165
Heat Pain-After Sensation	A	964	-0.840	0.349	-1.524	-0.156	-2.408	**0.016**	0.113	-1.122
Mechanical Pain-Threshold	A	964	-0.056	0.075	-0.204	0.092	-0.739	0.460	0.010	-0.105
Mechanical Pain-Single Stimulus	A	964	-0.252	0.115	-0.476	-0.027	-2.196	**0.028**	0.040	-0.398
Mechanical Pain-Windup	A	964	-0.109	0.118	-0.340	0.122	-0.922	0.357	0.021	-0.212
Mechanical Pain-After Sensation	A	964	-0.682	0.237	-1.146	-0.217	-2.874	**0.004**	0.112	-1.113

A1 = minor allele; N = number of subjects with non-missing data; OR = odds ratio; BETA = regression coefficient; SE = standard error; L95 = lower bound of 95% confidence interval; U95 = upper bound of 95% confidence interval; STAT = Coefficient t-statistic; *P* = Asymptotic p-value for t-statistic; Frequency rs165774G = frequency of major allele *G* among cases; frequency rs165774A = frequency of minor allele *A* among cases; z-score rs165774G = average z-score for subjects carrying one or two copies of the major allele (GG and GA); z-score rs165774A = average z-score for subjects carrying two copies of the minor allele (AA).

Supporting Table S5. Meta-analysis of the association between SNP rs165774 and TMD or different QST, combining subjects from the *TMD case-control* cohort and Caucasian females from the *OPPERA* cohort.

	OR	**SE**	**L95**	**U95**	***P***
TMD	0.810	0.086	0.685	0.958	**0.014**
	BETA	**SE**	**L95**	**U95**	***P***
Pressure Pain	-0.880	0.265	-1.399	-0.360	**0.001**
Heat Pain	0.021	0.057	-0.090	0.133	0.707
Heat Pain-First Pulse	-0.197	0.146	-0.483	0.089	0.178
Heat Pain-Rate of Rise	0.176	0.135	-0.088	0.440	0.192
Heat Pain-Sum	-0.106	0.152	-0.404	0.193	0.487

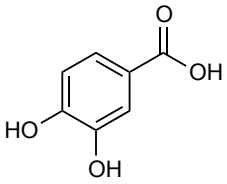
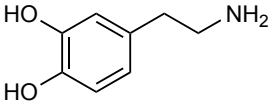
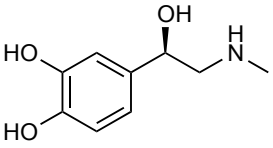
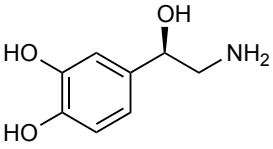
OR = odds ratio; BETA = regression coefficient; SE = standard error; L95 = lower bound of 95% confidence interval; U95 = upper bound of 95% confidence interval; *P* = combined p-value.

Supporting Table S6. Relative expression level of (a)-COMT versus reference MB-COMT (Ratio) in different tissues from human, chimp, and macaca individuals.

Human	Ratio	Chimp	Ratio	Gorilla	Ratio
hsa br F 1	0.018	ptr br F 1	0.020	ggo br F 1	0.012
hsa br M 3	0.014	ptr br M 1	0.014	ggo br M 1	0.008
hsa br M 1	0.010	ptr br M 2	0.013	ggo cb F 1	0.078
hsa br M 2	0.004	ptr br M 3	0.011	ggo cb M 1	0.016
hsa br M 4	0.008	ptr br M 4	0.007	ggo ht F 1	0.048
hsa br M 5	0.020	ptr br M 5	0.010	ggo ht M 1	0.005
hsa cb F 1	0.132	ptr cb F 1	0.043	ggo kd F 1	0.016
hsa cb M 1	0.060	ptr cb M 1	0.112	ggo kd M 1	0.008
hsa ht F 1	0.008	ptr ht F 1	0.036	ggo lv F 1	0.011
hsa ht M 1	0.022	ptr ht M 1	0.015	ggo lv M 1	0.004
hsa ht M 2	0.011	ptr kd F 1	0.023	ggo ts M 1	0.016
hsa kd F 1	0.021	ptr kd M 1	0.014		
hsa kd M 1	0.011	ptr lv F 1	0.014		
hsa kd M 2	0.015	ptr lv M 1	0.011		
hsa lv M 1	0.006	ptr ts M 1	0.129		
hsa lv M 2	0.020				
hsa ts M 1	0.000				
hsa ts M 2	0.070				

M = male; F = female; br = brain without cerebellum; cb = cerebellum; ht = heart; kd = kidney; lv – liver; ts = testis

Supporting Table S7. Chemical structures and docking scores of COMT substrates.

Molecule	Structure	S-COMT		(a)S-COMT	
		conformers[a]	Gscore[b]	conformers[a]	Gscore[b]
DHBA		2	-8.45 ± 0.08	7	-6.47 ± 0.18
Dopamine		5	-8.01 ± 0.09	2	-5.64 ± 0.1
Epinephrine		2	-7.86 ± 0.17	2	-5.76 ± 0.24
norepinephrine		5	-8.18 ± 0.06	2	-5.32 ± 0.22

Notes: **a**) Number of top energy-ranked poses with crystallographic-like distances between catechol hydroxyl groups and catalytic Mg^{2+} ion; **b**) Gscore expressed as average and standard deviation of retrieved conformer scores.

Supporting Table S8. Enzyme kinetic values of alternative COMT isoforms (a)MB-COMT and (a)S-COMT and their reference counterparts for catechol substrates DHBA, dopamine, norepinephrine and epinephrine, generated using nonlinear fitting from representative substrate-velocity curves. 95% confidence limits are shown in parenthesis**.** We want to point out that, in contrast to DHBA and its products, natural catecholamines and their O-methylated metabolites are not as stable under physiological conditions. Particularly 3-MT is easily destructed and may increase variation when using dopamine as a substrate. However, we think that these results are reliable for comparison of the performance of reference and alternative COMT isoforms.

	DHBA		**DOPAMINE**		**NOREPINEPHRINE**		**EPINEPHRINE**	
$\mathbf{V_{max}}$ (pmol x min-1 x mg protein-1)	**MB-COMT**	**S-COMT**	**MB-COMT**	**S-COMT**	**MB-COMT**	**S-COMT**	**MB-COMT**	**S-COMT**
Reference	8.1 (4.9–11.3)	73.1 (70.7–76.9)	6.5 (9.6–22.7)	55.3 (44.0–66.7)	12.6 (10.7–14.6)	78.3 (55.6–100.9)	8.4 (6.9–9.9)	52.6 (50.1–55.1)
Alternative	7.7 (7.3–8.1)	8.2 (6.7–9.7)	8.7 (4.9–12.6)	11 (2.9–19.1)	5.1 (3.5–6.6)	14.8 (12.9–16.6)	0	0
$\mathbf{K_m}$ (μM)								
Reference	0.12 (0–0.27)	0.04 (0.03–0.05)	0.12 (0–0.85)	0.13 (0.04–0.22)	0.44 (0.42–0.67)	0.59 (0.52–0.66)	0.26 (0.11–0.41)	0.26 (0.21–0.3)
Alternative	0.63 (0.57–0.7)	0.38 (0.14–0.61)	0.19 (0–0.45)	0.25 (0–0.87)	0.52 (0.13–0.91)	0.63 (0.42–0.83)	0	0