

Table S1. Opossum crosses and individual IDs.

No.	Sample_ID	Sex	mother ID	father ID	mother line	father line	Developmental Stage	Tissue
1	A0571_p1	female	A0571	A0578	LL2	LL1	E13.0*	EEM**
2	A0571_p4	female	A0571	A0578	LL2	LL1	E13.0	EEM
3	A0579_p3	female	A0579	A0573	LL1	LL2	E13.0	EEM
4	A0579_p4	female	A0579	A0573	LL1	LL2	E13.0	EEM
5	A0571_b1	female	A0571	A0578	LL2	LL1	E13.0	embryonic head
6	A0571_b4	female	A0571	A0578	LL2	LL1	E13.0	embryonic brain
7	A0579_b3	female	A0579	A0573	LL1	LL2	E13.0	embryonic head
8	A0579_b4	female	A0579	A0573	LL1	LL2	E13.0	embryonic brain
9	A0580_p1	female	A0580	A0578	LL1	LL1	E12.5	EEM
10	A0580_p5	male	A0580	A0578	LL1	LL1	E12.5	EEM
11	A0572_p1	female	A0572	A0573	LL2	LL2	E12.5	EEM
12	A0572_p3	male	A0572	A0573	LL2	LL2	E12.5	EEM
13	A0580_b1	female	A0580	A0578	LL1	LL1	E12.5	embryonic head
14	A0580_b5	male	A0580	A0578	LL1	LL1	E12.5	embryonic head
15	A0572_b1	female	A0572	A0573	LL2	LL2	E12.5	embryonic head
16	A0572_b3	male	A0572	A0573	LL2	LL2	E12.5	embryonic head

*: embryonic day 13.

**: extra-embryonic membranes.

Table S2. RNA-seq summary and alignment percentages.

No.	Sample_ID	Illumina RNA-seq	read length	multiplexing	# of lanes	total number of reads	uniquely mapped reads	mapping percentage
1	A0571_p1	HiSeq2000	51bp	1-8 8-plex	4	122,984,871	96,491,161	78.46%
2	A0571_p4	HiSeq2000	51bp	1-8 8-plex	4	102,676,227	80,265,550	78.17%
3	A0579_p3	HiSeq2000	51bp	1-8 8-plex	4	97,126,305	75,901,042	78.15%
4	A0579_p4	HiSeq2000	51bp	1-8 8-plex	4	93,176,519	70,214,242	75.36%
5	A0571_b1	HiSeq2000	51bp	1-8 8-plex	4	111,441,595	92,237,141	82.77%
6	A0571_b4	HiSeq2000	51bp	1-8 8-plex	4	104,401,730	87,413,805	83.73%
7	A0579_b3	HiSeq2000	51bp	1-8 8-plex	4	123,279,342	102,502,500	83.15%
8	A0579_b4	HiSeq2000	51bp	1-8 8-plex	4	107,001,858	88,789,694	82.98%
9	A0580_p1	HiSeq2000	51bp	9-16 8-plex	4	89,170,950	73,057,075	81.93%
10	A0580_p5	HiSeq2000	51bp	9-16 8-plex	4	80,905,285	66,088,617	81.69%
11	A0572_p1	HiSeq2000	51bp	9-16 8-plex	4	57,906,830	47,408,285	81.87%
12	A0572_p3	HiSeq2000	51bp	9-16 8-plex	4	76,082,440	62,107,048	81.63%
13	A0580_b1	HiSeq2000	51bp	9-16 8-plex	4	78,348,683	66,295,368	84.62%
14	A0580_b5	HiSeq2000	51bp	9-16 8-plex	4	111,042,946	93,760,306	84.44%
15	A0572_b1	HiSeq2000	51bp	9-16 8-plex	4	75,672,460	63,848,790	84.38%
16	A0572_b3	HiSeq2000	51bp	9-16 8-plex	4	78,107,923	65,288,060	83.59%

Table S3. X-linked genes on the unmapped scaffold covered in RNA-seq data.

No.	Scaffold ID	Coordinates on chrUn	Gene name
1	scaffold_217	chrUn:5892577-6515230	<i>ABCB7</i>
2	scaffold_217	chrUn:5892577-6515230	<i>KIAA2022</i>
3	scaffold_217	chrUn:5892577-6515230	<i>RLIM</i>
4	scaffold_217	chrUn:5892577-6515230	<i>SLC16A2</i>
5	scaffold_251	chrUn:23882535-24256940	<i>RNF170-like</i>
6	scaffold_251	chrUn:23882535-24256940	<i>Rab-1B-like</i>
7	scaffold_251	chrUn:23882535-24256940	<i>KLHL4</i>
8	scaffold_251	chrUn:23882535-24256940	<i>ENSMODG00000022796</i>
9	scaffold_261	chrUn:27708438-28006343	<i>MTMR8</i>
10	scaffold_261	chrUn:27708438-28006343	<i>XM_001378411.1</i>
11	scaffold_298	chrUn:37707632-37883514	<i>FUNDC2</i>
12	scaffold_298	chrUn:37707632-37883514	<i>MTCP1</i>
13	scaffold_298	chrUn:37707632-37883514	<i>BRCC3</i>
14	scaffold_298	chrUn:37707632-37883514	<i>PICALM-like</i>
15	scaffold_298	chrUn:37707632-37883514	<i>VBPI</i>
16	scaffold_298	chrUn:37707632-37883514	<i>RAB39B</i>
17	scaffold_352	chrUn:45973239-46059762	<i>SLC9A6</i>
18	scaffold_352	chrUn:45973239-46059762	<i>MMGT1-like</i>
19	scaffold_1524	chrUn:75514428-75525886	<i>EMD</i>

Table S4. Retrotransposed X-linked genes excluded from the analysis.

No.	Ensembl trans ID	Ensembl gene ID	Gene name
1	ENSMODT00000009535	ENSMODG00000007539	<i>HAUS7</i>
2	ENSMODT00000039334	ENSMODG00000025336	<i>HSP90AB1-transposed</i>
3	ENSMODT00000039297	ENSMODG00000025324	<i>LUC7L2-transposed</i>
4	ENSMODT00000008835	ENSMODG00000006990	<i>ENSMODG00000006990</i>
5	ENSMODT000000021722	ENSMODG00000017105	<i>PABPC1L2B</i>
6	-	-	<i>chrX.288</i>
7	ENSMODT00000009094	ENSMODG00000007195	<i>RNASET2-transposed</i>
8	ENSMODT00000026898	ENSMODG00000021142	<i>TAX1BP1-transposed</i>
11	ENSMODT00000017947	ENSMODG00000014093	<i>SSU72-transposed</i>
13	ENSMODT00000022195	ENSMODG00000017486	<i>SET-transposed</i>
14	ENSMODT00000011525	ENSMODG00000009064	<i>KDM2A-transposed</i>

Table S5. Informative SNP classes in non-escaper genes.

Class*	Counts	With parental crosses support	Refer allele	Segregating within other LL1 individuals?	Class description
1	75	YES	LL1	NO	best "fixed" SNPs
2	9	YES	LL2	NO	best "fixed" SNPs
3	49	YES	LL1	unknown	2nd best "fixed" SNPs
4	2	YES	LL2	unknown	2nd best "fixed" SNPs
5	173	NO	LL1	NO	2nd best "fixed" SNPs, with less support from the parental cross
6	24	NO	LL2	NO	2nd best "fixed" SNPs, with less support from the parental cross

*: SNPs outside these six classes belong to class 0.

Table S6. Average promoter CpG methylation percentages for 5 escaper and 17 non-escaper genes in female and male fetal brain and EEM from PyroMark sequencing results.

Gene name	chr	CGI_start	CGI_end	pXCI status	# of CpGs	Average CpG methylation percentage			
						female brain	male brain	female EEM	male EEM
<i>FLNA</i>	chrX	3105210	3105995	Escaper	10	1.43%	1.58%	4.08%	2.46%
<i>FAM3A</i>	chrX	3400962	3401678	Nonescaper	20	4.45%	3.23%	2.40%	1.80%
<i>AMMECRI</i>	chrX	5988670	5989919	Nonescaper	10	0.85%	0.56%	0.69%	0.98%
<i>BCAP31</i>	chrX	11514655	11515266	Nonescaper	8	0.12%	0.37%	0.40%	0.30%
<i>LASIL</i>	chrX	13387573	13387813	Nonescaper	13	1.10%	0.43%	0.49%	0.35%
<i>LONRF3</i>	chrX	27458981	27459730	Nonescaper	14	1.03%	0.79%	1.64%	0.91%
<i>ELF4</i>	chrX	31571699	31572038	Nonescaper	11	0.29%	0.61%	0.35%	0.03%
<i>RAP2C</i>	chrX	32989986	32990510	Nonescaper	9	2.85%	1.20%	0.72%	3.20%
<i>GPC4</i>	chrX	34223543	34224297	Nonescaper	7	1.97%	5.25%	1.43%	2.91%
<i>RSX</i>	chrX	35651221	35651426	RSX	23	60.57%	95.03%	56.34%	95.92%
<i>FAM122B</i>	chrX	36029151	36029318	Escaper	8	0.63%	0.26%	1.07%	1.87%
<i>DDX26B</i>	chrX	36362206	36362830	Nonescaper	7	1.89%	1.95%	2.69%	9.17%
<i>MTMR1</i>	chrX	39955076	39955459	Escaper	7	1.14%	0.47%	0.19%	1.21%
<i>CD99L2</i>	chrX	40182295	40182801	Escaper	12	0.17%	0.41%	0.65%	0.87%
<i>GPR50</i>	chrX	40418664	40419488	Nonescaper	8	0.23%	0.29%	0.09%	0.28%
<i>ATP7A</i>	chrX	56159948	56160563	Nonescaper	17	1.29%	0.00%	0.22%	0.47%
<i>FNDC3C1</i>	chrX	56480924	56481174	Nonescaper	11	1.99%	0.52%	2.20%	0.92%
<i>PLS3</i>	chrX	67094850	67095761	Nonescaper	9	2.55%	0.39%	1.41%	3.70%
<i>AMOT</i>	chrX	68740642	68741518	Nonescaper	9	40.98%	36.72%	37.15%	58.91%
<i>TAF1</i>	chrX	69944348	69945053	Escaper	10	0.14%	0.25%	0.35%	0.84%
<i>KCTD12B</i>	chrX	71564312	71565156	Nonescaper	11	0.88%	0.86%	4.13%	1.98%
<i>KLF8</i>	chrX	71604414	71607119	Nonescaper	8	4.30%	0.66%	4.71%	1.16%
<i>IL13RA1</i>	chrX	78514312	78514793	Nonescaper	7	0.89%	2.08%	0.00%	2.83%

Table S7. Candidate escaper genes without informative SNPs to quantify the allele-specific expression.

No.	gene ID	gene name	H3K4me3 fold-enrichment	% of H3K27me3 coverage	Female/male expression ratio
1	ENSMODG00000008997	<i>UBL4A</i>	13.21	0.00%	1.26
2	ENSMODG00000008581	<i>SSR4</i>	18.5	0.00%	1.15
3	ENSMODG00000011547	<i>NAA10</i>	12.77	4.98%	1.37
4	ENSMODG00000014984	<i>THOC2</i>	10.13	0.00%	1.07
5	ENSMODG00000003985	<i>ZFP347L</i>	16.3	1.03%	1.23
6	ENSMODG00000004615	<i>OGT</i>	17.18	0.00%	1.19
7	ENSMODG00000004864	<i>KIF4A</i>	12.33	4.85%	1.09
8	ENSMODG00000009860	<i>CCDC22</i>	13.65	0.00%	1.34
9	ENSMODG00000009765	<i>KDM5C</i>	13.21	0.00%	1.34
10	ENSMODG00000011070	<i>RPS4X</i>	8.37	0.00%	1.11
11	ENSMODG00000011785	<i>CSTF2</i>	18.5	0.00%	1.26

Table S8. Opossum imprinted XCI escapers and the random XCI status for their human/mouse ortholog genes.

Gene name	Opossum escaping status	Human random XCI status	Mouse random XCI status
<i>FLNA</i>	Escaper	Non-escaper	Non-escaper
<i>RPL10</i>	Escaper	Non-escaper	Non-escaper
<i>PLXNA3</i>	Escaper	Non-escaper	Non-escaper
<i>G6PD</i>	Escaper	Non-escaper	Non-escaper
<i>IKBKG</i>	Escaper	Escaper	Non-escaper
<i>DKC1</i>	Escaper	Non-escaper	Non-escaper
<i>YIPF6</i>	Escaper	Non-escaper	Non-escaper
<i>NUP62CL</i>	Escaper	NA	Non-escaper
<i>HCFC1</i>	Escaper	Non-escaper	Non-escaper
<i>IRAK1</i>	Escaper	Non-escaper	Non-escaper
<i>MECP2</i>	Escaper	Non-escaper	Non-escaper
<i>FRMD7</i>	Escaper	NA	Non-escaper
<i>PHF6</i>	Escaper	Non-escaper	Non-escaper
<i>FAM122B</i>	Escaper	Non-escaper	Non-escaper
<i>FAM122A</i>	Escaper	NA	Non-escaper
<i>MTMR1</i>	Escaper	Non-escaper	Non-escaper
<i>CD99L2</i>	Escaper	Non-escaper	Non-escaper
<i>HMGB3</i>	Escaper	Non-escaper	Non-escaper
<i>RBMX</i>	Escaper	Non-escaper	Non-escaper
<i>ATRX</i>	Escaper	Non-escaper	Non-escaper
<i>ALAS2</i>	Escaper	Non-escaper	Non-escaper
<i>TAF1</i>	Escaper	Non-escaper	Non-escaper
<i>NONO</i>	Escaper	Non-escaper	Non-escaper
<i>CENPI</i>	Escaper	Non-escaper	Non-escaper