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Supplemental information

X chromosome inactivation in the human placenta is patchy and distinct from adult tissues

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Supplementary Tables

Table S1. Number of heterozygous variants on the X chromosome and chromosome 8

For each sample, we reported the number of heterozygous variants after variant genotyping using GATK and after filtering using VQSR.

SampleID	X chromosome	Chromosome 8
OBG0044	951	1691
OBG0068	1468	2218
OBG0111	832	1650
OBG0115	964	1568
OBG0120	824	1674
OBG0133	1316	2134
OBG0156	997	1600
OBG0170	1390	1979
OBG0174	1426	2158
OBG0175	607	1594
OBG0178	1157	1906
OBG0166	804	1620
OBG0022	1125	1964
OBG0024	1206	2152
OBG0026	1907	2912
OBG0028	1706	2730
OBG0030	1842	3126
OBG0039	1086	2359
OBG0050	1786	2553
OBG0051	1107	2154
OBG0066	2010	2906
OBG0121	1805	2747
OBG0138	1044	2268
OBG0180	1110	2257
OBG0188	1940	2874
OBG0201	1757	2741
OBG0205	1275	2393
OBG0289	2020	2805
OBG0338	1049	2093
OBG0342	1169	2139

Table S2. Mapped reads at each extraction site for the whole transcriptome and for the X chromosome.

For each sample, we used samtools stats to obtain the number of reads that mapped.

Sample ID	Site A		Site B	
	Whole transcriptome	Chr X	Whole transcriptome	Chr X
OBG0044	124821361	2570404	104951941	1956346
OBG0068	96468961	2028381	72630017	1632320
OBG0111	105360653	2210820	87310785	1746585
OBG0115	115047845	2233186	81406062	1794380
OBG0120	109637796	2360408	78369660	1653373
OBG0133	106542634	2158139	75940154	1709600
OBG0156	123238340	2617278	79648267	1954931
OBG0170	104502826	2539731	101090503	2263802
OBG0174	29178948	589785	95550124	1787220
OBG0175	98337411	2134510	85986263	2061137
OBG0178	114938081	2447404	86675194	1916321
OBG0166	114474479	2532057	93337543	2043997
OBG0022	79624620	1846538	77386563	2048223
OBG0024	56525905	1464827	136862205	3467075
OBG0026	59648434	1014174	86201051	1772717
OBG0028	69962192	1894168	42201549	964035
OBG0030	75533477	1797735	72423761	1799896
OBG0039	81923032	1986227	60580683	1631386
OBG0050	72749515	1711200	62546230	1564591
OBG0051	78292464	1885179	61467811	1513737
OBG0066	74368314	1535224	80264229	2054109
OBG0121	95299259	2477900	40005508	1132595
OBG0138	217429809	5692793	73761980	1578129
OBG0180	72664680	1974885	29697974	837063
OBG0188	21675595	617351	79407643	2295587
OBG0201	88301001	2190583	80598634	2085968
OBG0205	49073152	1316484	40796256	1070053
OBG0289	72176230	1850324	78471721	1954236
OBG0338	75400996	1972344	57209598	1381726
OBG0342	80308862	1916568	85785465	2074156

Table S3. Number of heterozygous and expressed variants on the non-pseudoautosomal regions of the X chromosome and chromosome 8

After running GATK ASEReadCounter, we tabulated the number of heterozygous variants that are expressed (where total RNA read count is greater than 10).

Sample ID	Chromosome X		Chromosome 8	
	Site A	Site B	Site A	Site B
OBG0044	97	87	219	214
OBG0068	100	94	251	258
OBG0111	74	65	170	161
OBG0115	76	68	166	151
OBG0120	74	57	241	197
OBG0133	103	94	239	211
OBG0156	100	93	218	222
OBG0170	120	124	270	268
OBG0174	62	120	116	239
OBG0175	61	87	167	182
OBG0178	89	87	223	219
OBG0166	89	67	193	176
OBG0022	119	112	234	244
OBG0024	100	212	177	344
OBG0026	109	125	262	273
OBG0028	92	159	153	253
OBG0030	165	168	362	373
OBG0039	68	94	225	297
OBG0050	160	148	256	274
OBG0051	100	91	249	214
OBG0066	160	127	422	355
OBG0121	186	95	338	176
OBG0138	191	77	534	212
OBG0180	92	43	262	133
OBG0188	59	183	111	336
OBG0201	125	131	304	328
OBG0205	71	61	169	144
OBG0289	161	171	319	316
OBG0338	87	63	244	173
OBG0342	92	96	255	288

Table S4. Number of samples for each adult GTEx tissue.

The number of samples for each adult GTEx tissue used in this study (column 2), and the number of skewed samples (median allele balance greater than 0.8) (column 3).

GTEx tissues	Number of samples	Number of skewed samples
Adipose_Subcutaneous	194	19
Adipose_Visceral_Omentum	149	11
Adrenal_Gland	94	18
Artery_Aorta	138	7
Artery_Coronary	84	11
Artery_Tibial	187	14
Brain_Amygdala	37	2
Brain_Anterior_cingulate_cortex_BA24	42	2
Brain_Caudate_basal_ganglia	52	3
Brain_Cerebellar_Hemisphere	51	5
Brain_Cerebellum	58	4
Brain_Cortex	64	4
Brain_Frontal_Cortex_BA9	48	2
Brain_Hippocampus	49	2
Brain_Hypothalamus	47	1
Brain_Nucleus_accumbens_basal_ganglia	55	4
Brain_Putamen_basal_ganglia	42	3
Brain_Spinal_cord_cervical_c-1	48	1
Brain_Substantia_nigra	33	2
Breast_Mammary_Tissue	151	20
Colon_Sigmoid	113	3
Colon_Transverse	136	15
Esophagus_Gastroesophageal_Junction	110	3
Esophagus_Mucosa	176	32
Esophagus_Muscularis	162	9
Heart_Atrial_Appendage	119	9
Heart_Left_Ventricle	122	21
Kidney_Cortex	18	0
Liver	62	12
Lung	166	13
Minor_Salivary_Gland	40	4
Muscle_Skeletal	237	17
Nerve_Tibial	177	13
Ovary	167	21
Pancreas	116	23
Pituitary	71	4
Skin_Not_Sun_Exposed_Suprapubic	169	28
Skin_Sun_Exposed_Lower_leg	208	35
Small_Intestine_Terminal_Ileum	63	5
Spleen	86	4
Stomach	122	15
Thyroid	196	14
Uterus	129	10
Vagina	141	20
Whole_Blood	229	60

Table S5. Number of heterozygous sites identified in nonPARs in XY males.

Sample	Number of called variants (AC > 0)	Number of heterozygous variants (AC = 1)	Number of heterozygous & expressed variants in site A	Number of heterozygous & expressed variant in site B
OBG0112	1,479	213	27	32
OBG0116	2,018	174	18	18
OBG0117	1,343	149	31	27
OBG0118	1,484	180	24	25
OBG0122	1,413	126	15	11
OBG0123	1,762	149	22	23
OBG0126	1,898	171	20	20
OBG0130	1,566	197	23	28
OBG0132	1,946	182	20	18
OBG0158	1,620	223	28	27
YPOPS0006	1,510	180	26	27
OBG0053	1,532	172	23	30

Table S6. Inactivation status for X chromosome genes in the placenta.

Inactivation status for X chromosome genes in the placenta assigned to criteria outlined in Supplemental Note 1 (in alphabetical order). Columns are defined as Gene (Gene Name of 198 X chromosome genes), Category (calculations for which category proportions are being calculated; either inactivated or escape), N Samples (number of samples fitting this category), N Total (total number of samples examined), Prop (proportion of the total samples fitting this category), and Gene Status (conclusion reached for this gene outlined in Supplemental Note 1).

Found in the accompanied Excel spreadsheet: "TableS6.xlsx"

Table S7. Paternal X chromosome is preferentially silenced.

The fourth column denotes the number of heterozygous and expressed variants that are skewed (allele balance ≥ 0.8 or ≤ 0.2). The fifth column denotes the number of variants that are heterozygous, expressed, and skewed in the placenta samples but are homozygous in the decidua samples. The sixth column denotes the number of variants where the biased allele from the placenta samples is the same as the decidua samples, which suggest that the biased allele is maternal in origin. The last column is our best guess of whether the maternal or the paternal X is silenced. If most of the heterozygous, expressed, and skewed variants are maternal in origin, we determined that the maternal X is active and the paternal X is silenced. For each placenta sample where the number of variants that are heterozygous in the placenta and homozygous in the decidua is less than or equal to 3 is also annotated uncertain.

Category	Sample	Site	Heterozygous, skewed, expressed, variants	Homozygous decidua variants	Matched biased allele in decidua & placenta	Maternal/Paternal silenced
Both sites are skewed towards the same haplotype	OBG0338	A/B	43	16	15	Paternal
		B	31	12	12	Paternal
	OBG0342	A	47	2	2	Paternal/Uncertain
		B	63	3	2	Paternal/Uncertain
	OBG0024	A	53	5	5	Paternal
		B	120	10	10	Paternal
	OBG0188	A	25	0	0	Unknown
		B	92	5	4	Paternal
	OBG0030	A	93	45	45	Paternal
		B	72	35	35	Paternal
	OBG0205	A	22	3	2	Paternal/Uncertain
		B	28	3	2	Paternal/Uncertain
	OBG0289	A	69	3	1	Paternal/Uncertain
		B	89	3	2	Paternal/Uncertain
	OBG0026	A	33	0	0	Unknown
		B	50	0	0	Unknown
Site A is skewed towards haplotype 1 but site B is biallelic	OBG0066	A	66	6	6	Paternal
		B	12	1	1	Paternal/Uncertain
	OBG0051	A	68	5	5	Paternal
		B	9	3	3	Paternal/Uncertain
	OBG0039	A	46	2	2	Paternal/Uncertain
		B	5	0	0	Unknown
Both sites are skewed towards opposite haplotypes	OBG0121	A	99	11	0	Maternal
		B	31	8	8	Paternal
	OBG0022	A	48	21	4	Maternal
		B	29	15	15	Paternal
	OBG0050	A	75	12	12	Paternal
		B	51	8	0	Maternal
	OBG0138	A	114	13	11	Paternal
		B	37	3	2	Paternal/Uncertain
	OBG0201	A	79	4	0	Maternal
		B	60	1	1	Paternal/Uncertain
	OBG0028	A	66	4	2	Uncertain
		B	46	2	2	Paternal/Uncertain
	OBG0180	A	39	15	4	Maternal
		B	19	4	4	Paternal

Supplementary Figures

Figure S1. Whole exome post-trimming FastQC Mean Quality Scores.

We used fastQC to check for quality of reads after trimming and used multiQC to aggregate results (A) 12 placenta samples from batch 1. (B) 18 placenta samples from batch 2.

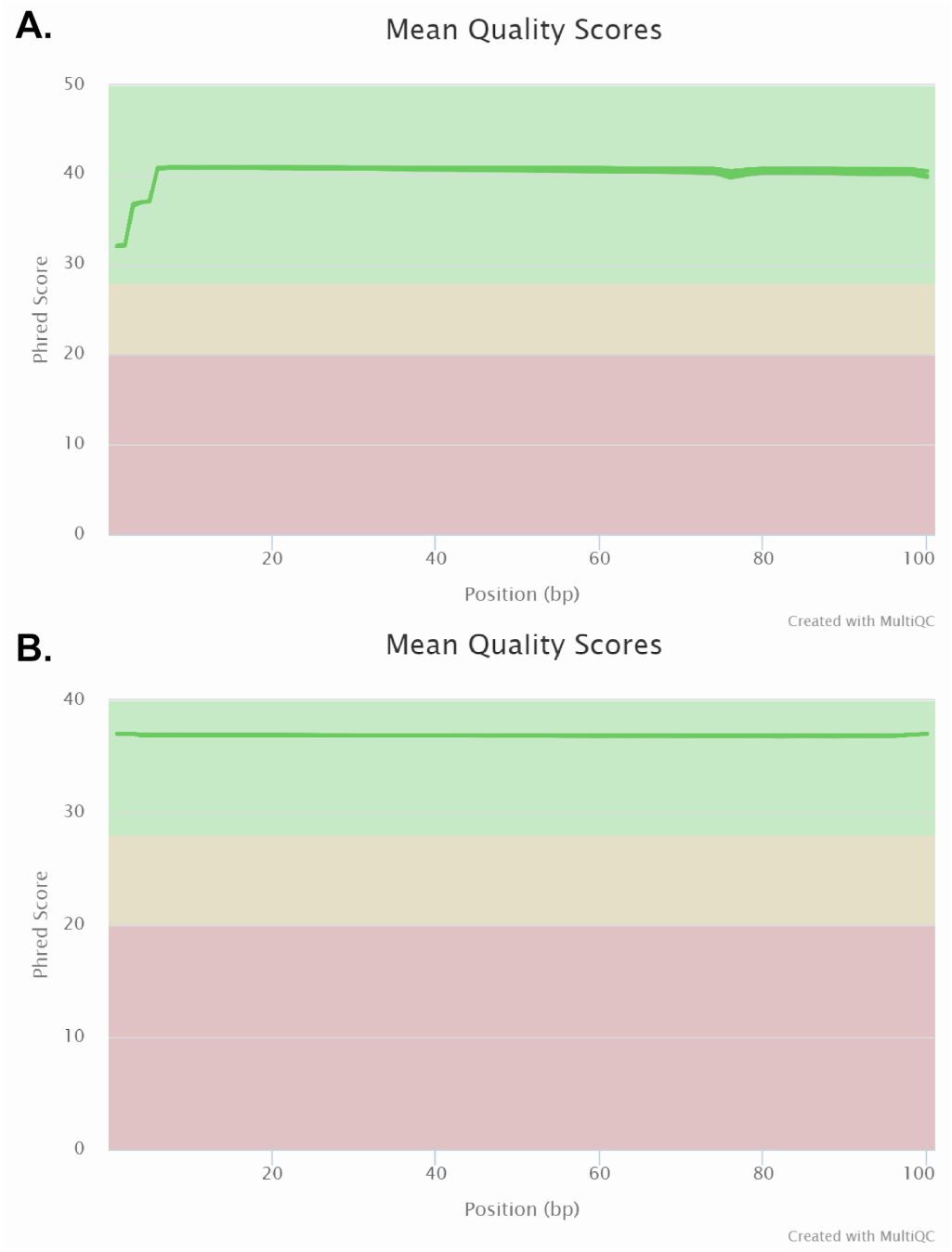


Figure S2. Reads mapped ratio.

The number of reads mapped to the X chromosome, the Y chromosome, and chromosome 19 was obtained by running samtools stats. Ratios in reads mapped were plotted for between the X chromosome and chromosome 19 (chrX/chr19), between the Y chromosome and chromosome 19 (chrY/chr19), and between the Y chromosome and the X chromosome (chrY/chrX). We observed that the ratio of reads mapped ratio between the X chromosome and chromosome 19 is much lower for OBG0175 than all other samples, suggesting that this sample is not genetic XX sample. Therefore, we removed this sample from further analyses.

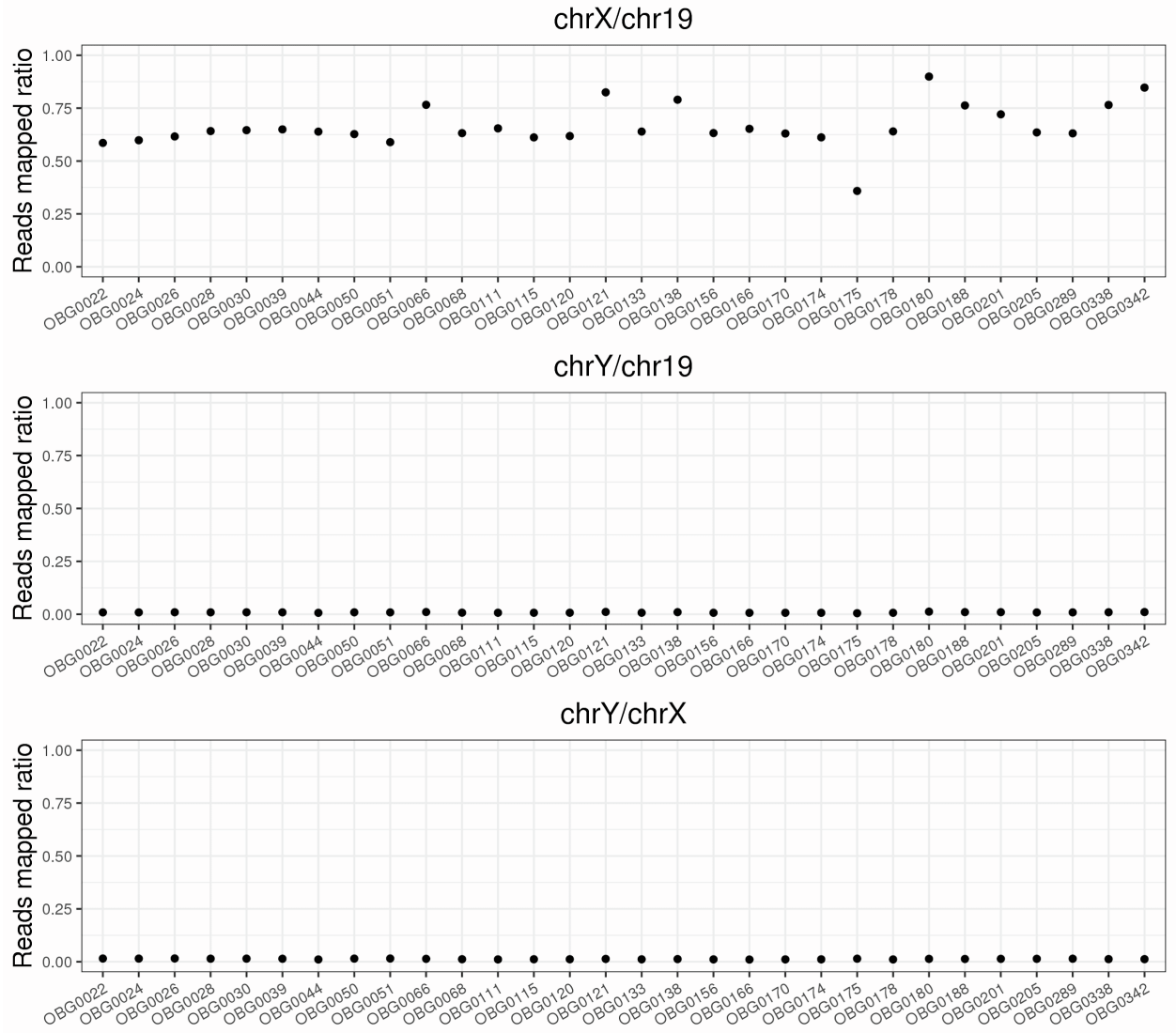


Figure S3. Principal component analysis for placenta samples.

Principal component for principal component 1 and 2 for the X chromosome (left panel) and for chromosome 8 (right panel) using the exome data from batch 1 and batch 2. We observed no clear separation by batches between these samples. The batch 1 samples are: OBG0044, OBG0068, OBG0111, OBG0115, OBG0120, OBG0133, OBG0156, OBG0170, OBG0174, OBG0175, OBG0178, and OBG0166. The batch 2 samples are: OBG0022, OBG0024, OBG0026, OBG0028, OBG0030, OBG0039, OBG0050, OBG0051, OBG0066, OBG0121, OBG0138, OBG0180, OBG0188, OBG0201, OBG0205, OBG0289, OBG0338, and OBG0342.

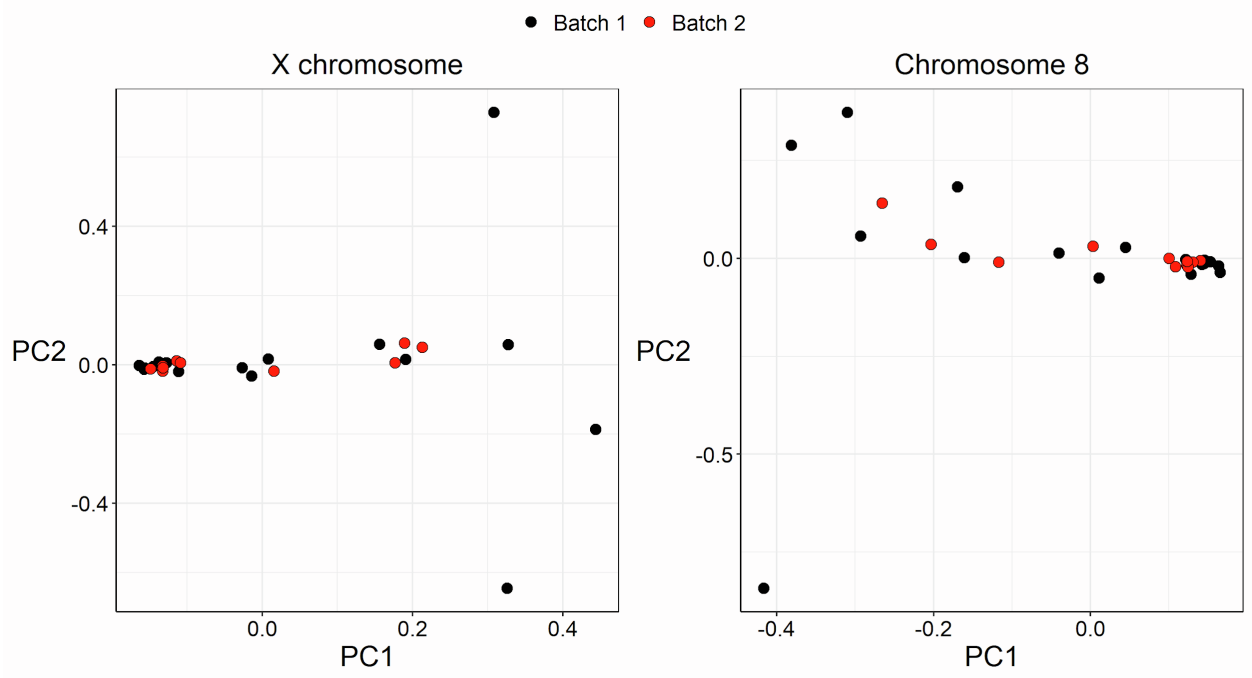


Figure S4. Whole transcriptome post-trimming FastQC Mean Quality Scores.

We used fastQC to check for quality of reads after trimming and used multiQC to aggregate results (A) 12 placenta samples from batch 1. (B) 18 placenta samples from batch 2.

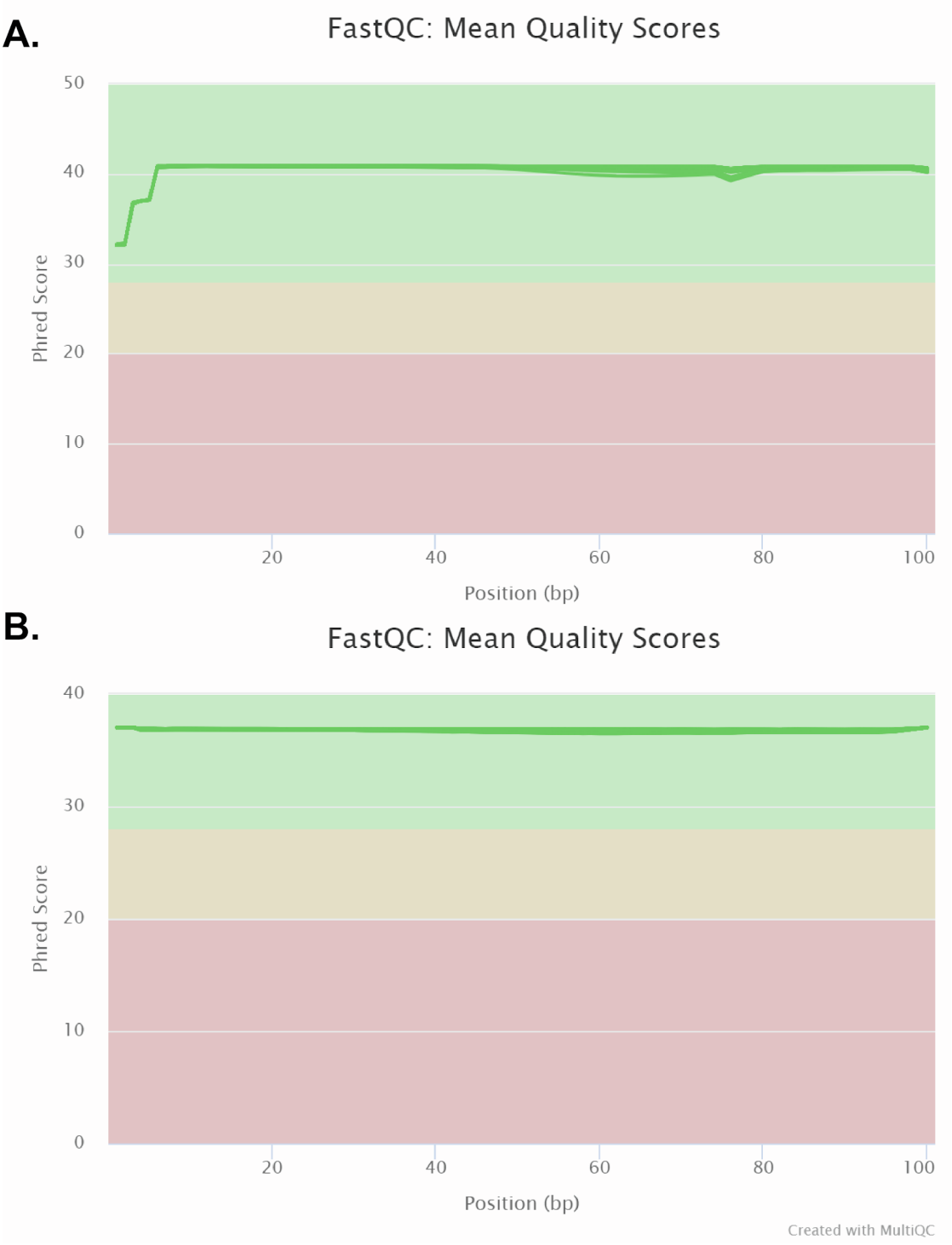
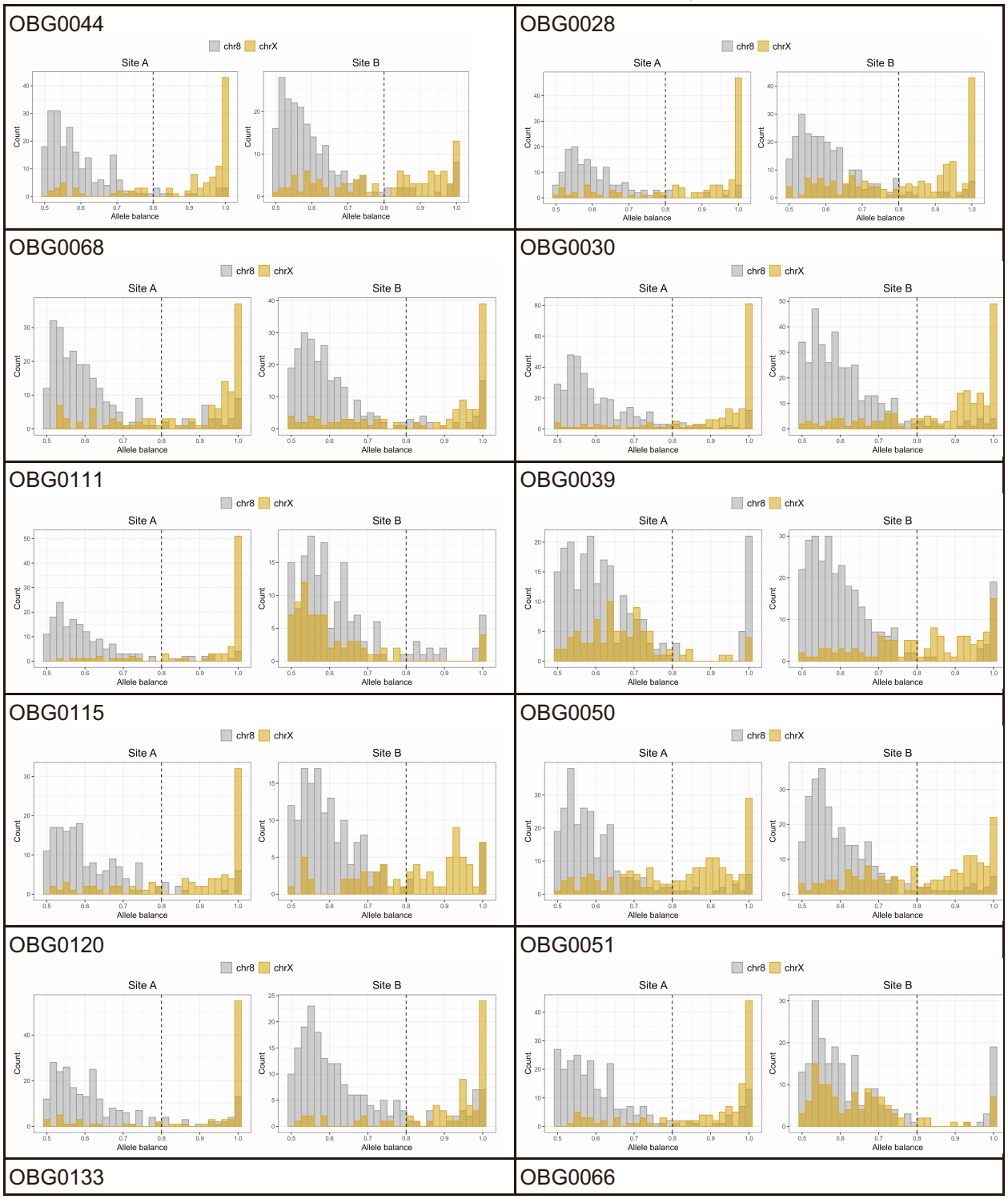
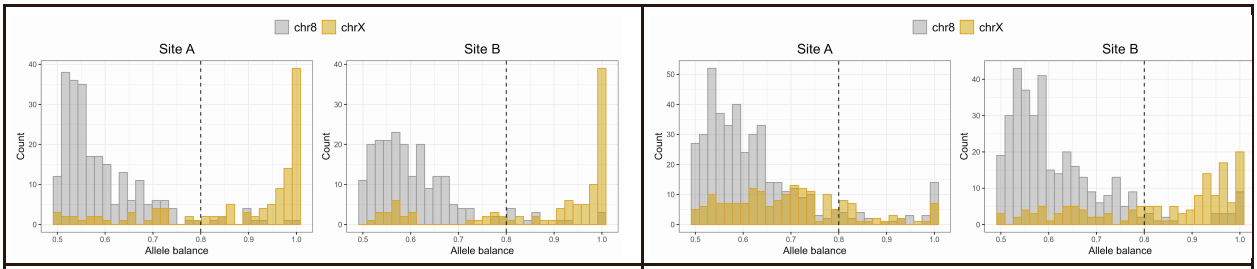


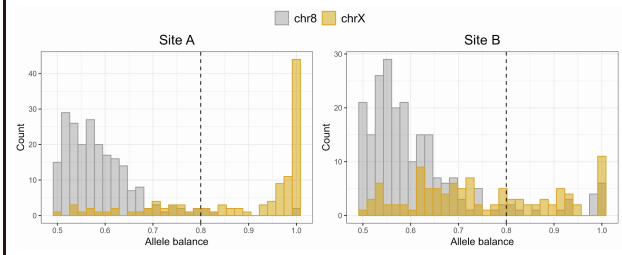
Figure S5. Determining threshold for skewed inactivation.

Each plot is a histogram of the allele balance for chromosome 8 (gray bars) and the X chromosome (yellow bars) for site A (left) and site B (right). Dotted lines denote allele balance of 0.8. We observed that the allele balance of most variants on chromosome 8 is less than 0.8 while the allele balance of most variants on the X chromosome is greater than 0.8.

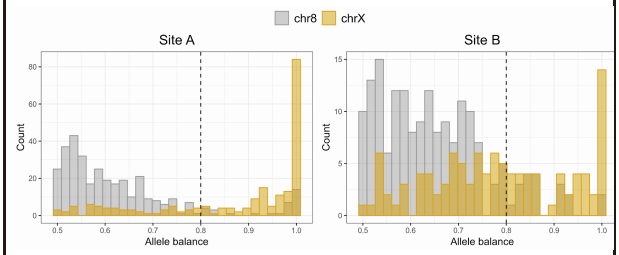




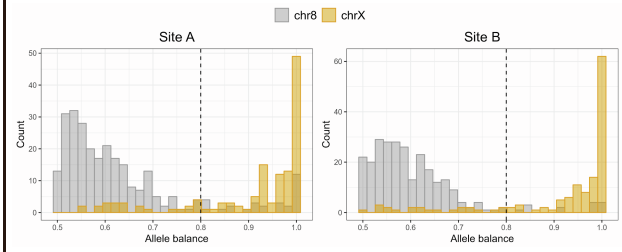
OBG0156



OBG0121



OBG0170



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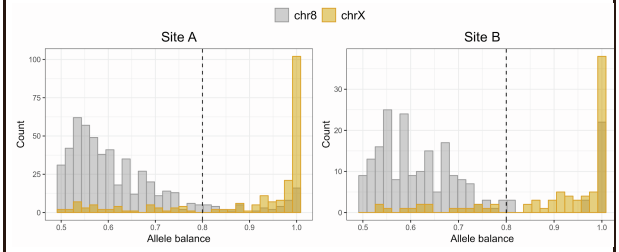

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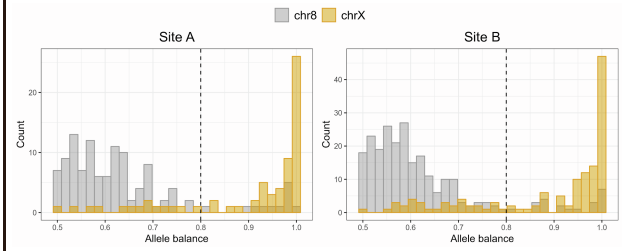
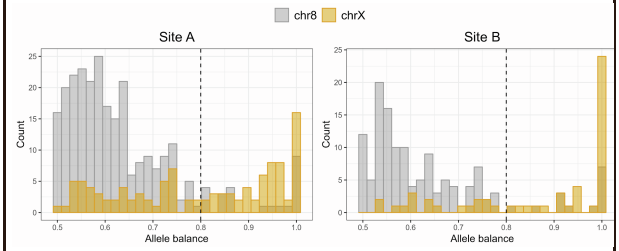
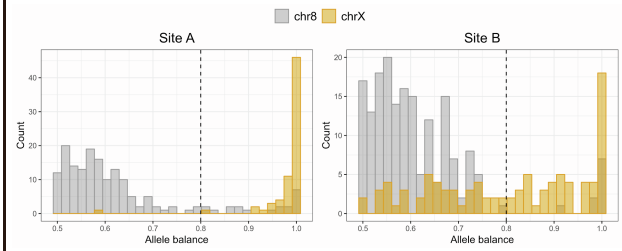


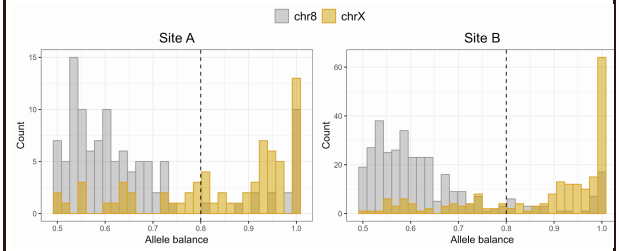
OBG0180



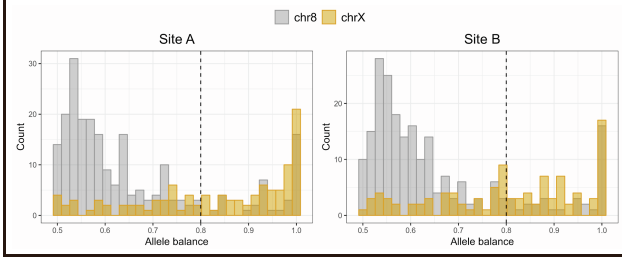
OBG0175



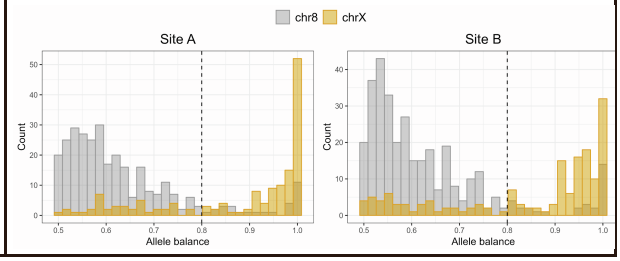
OBG0188



OBG0178



OBG0201



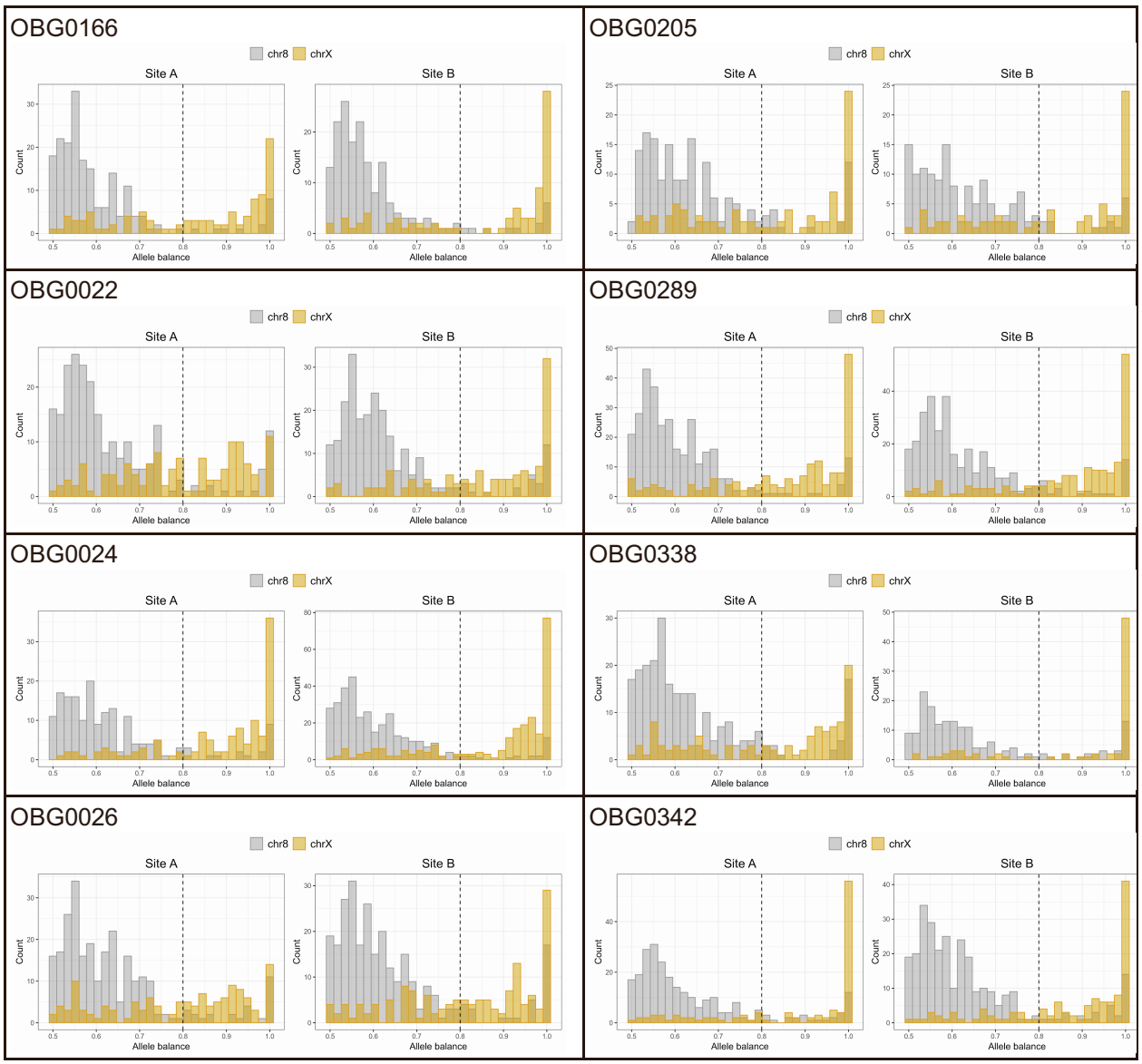

OBG0166
OBG0205
OBG0022
OBG0289
OBG0024
OBG0338
OBG0026
OBG0342
chr8
chrX
Site A
Site B
Count
Allele balance

Figure S6. Most variants of chrX non-PARs in XY males are skewed.

Histogram of allele balance in nonPARs in male XY samples called as diploid. We joint-called genotypes on 12 XY placentas (see **Methods**). Expression of variants on the nonPARs of the X chromosome should be completely biased towards one allele because there is only one X chromosome. However, even if we called the nonPARs as diploid, we wrongly identified only a small number of variants to be heterozygous (**Table S5**).

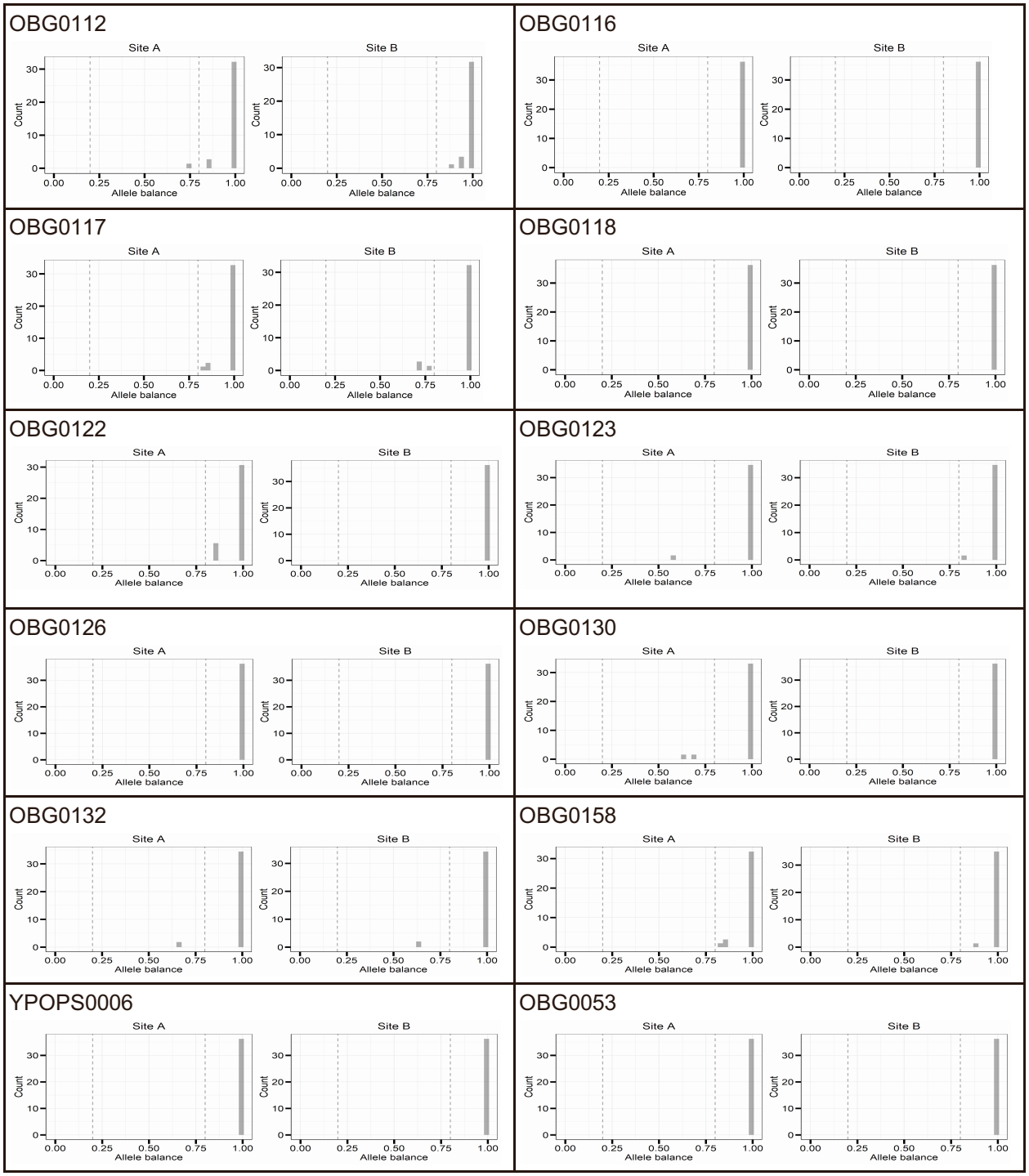
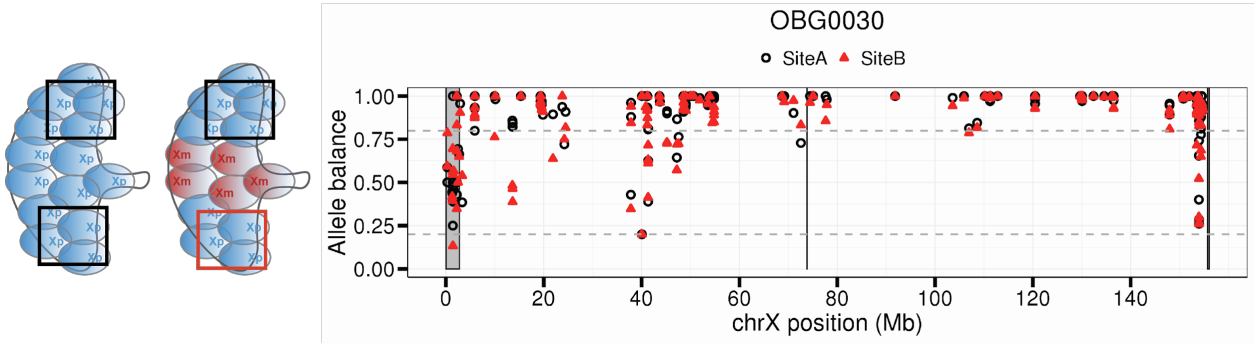


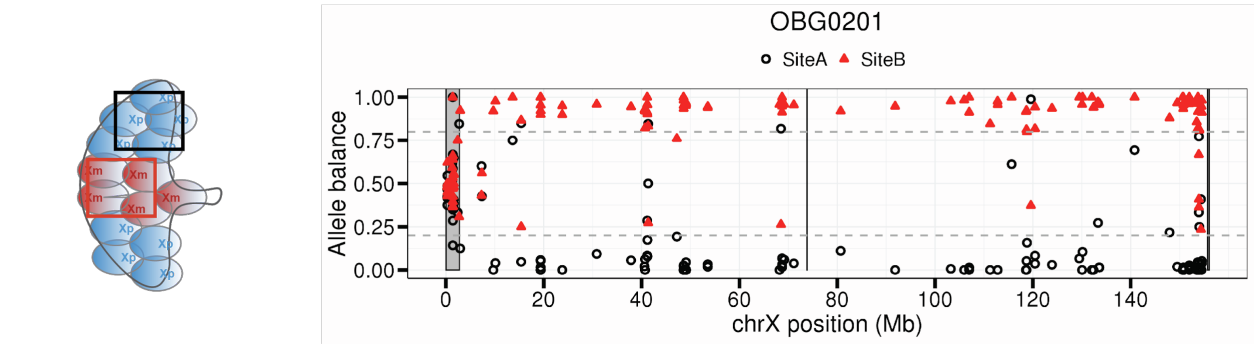
Figure S7. Patterns of X-inactivation across the entire X chromosome.

In each plot, allele balance at each heterozygous and expressed variant is plotted as a function of the position on the X chromosome. Open black circles denote variants on extraction site A. Filled red triangles denote variants on extraction site B. Gray boxes denote the pseudoautosomal regions and XIST.

A. Both extraction sites show the same X chromosome being inactivated



B. Each site shows a different X chromosome being inactivated



C. One extraction site shows skewed X-inactivation and the other shows both X chromosome being expressed

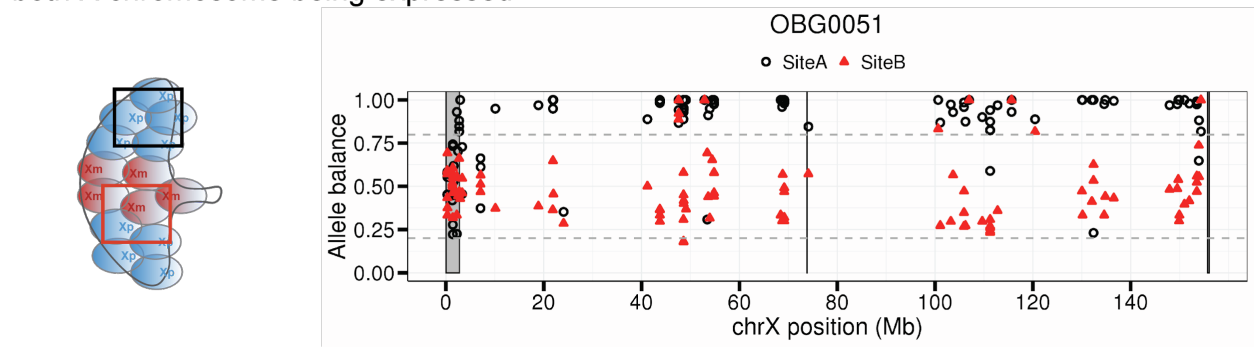


Figure S8. Chromosome 8 shows biallelic expression in placenta and adult tissues.

Unphased median allele balance is plotted for the placenta in this study (purple) and for 45 adult tissues in the GTEx dataset (blue) on chromosome 8. Each point of the violin plot is the median allele balance for each sample. Because there are no multiple site samplings for the GTEx data, unphased allele balance was computed.

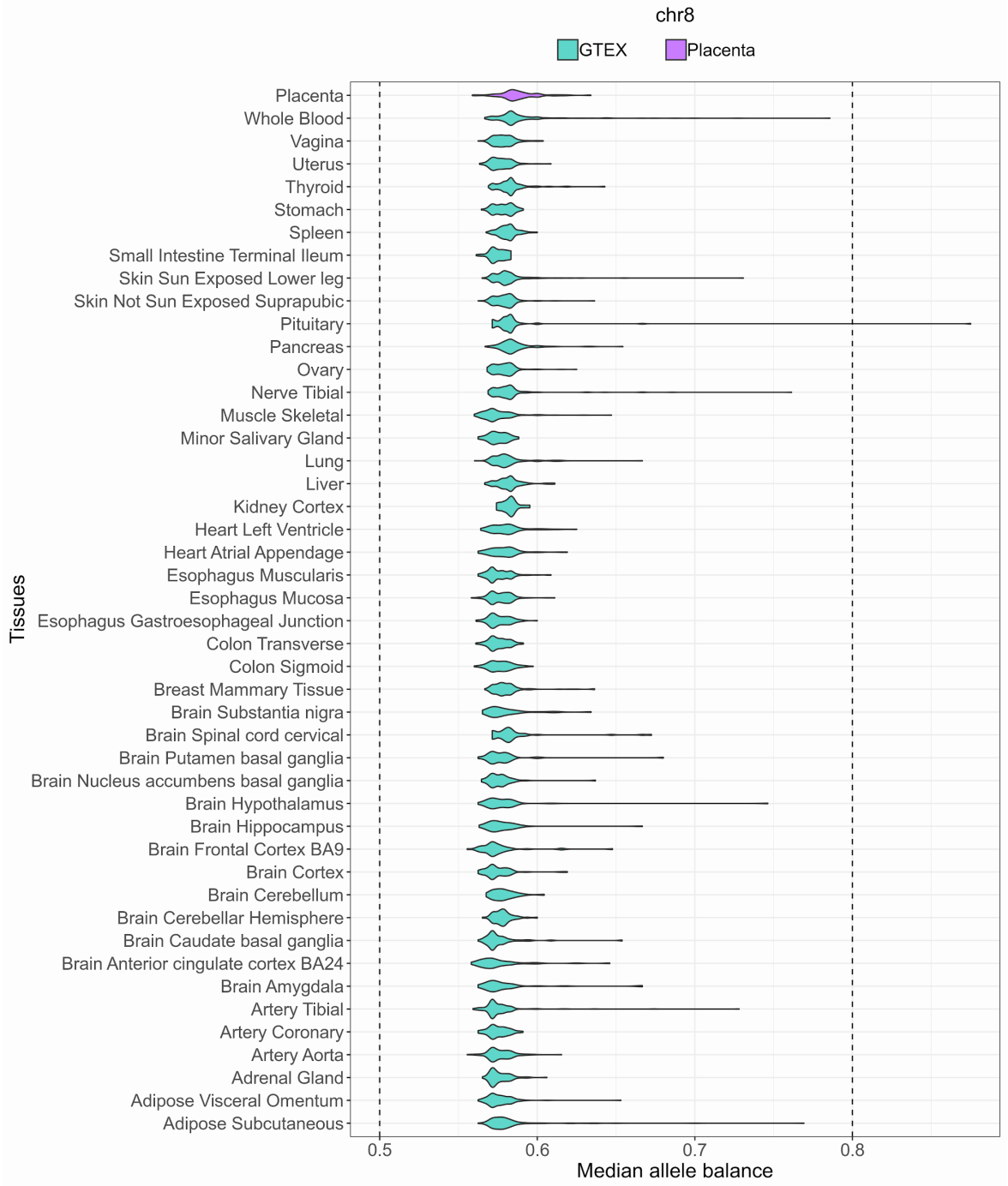


Figure S9. Heterogeneity in proportion of samples per gene that escape X-inactivation or are silenced.

For each gene, the dark blue bar denotes the proportion of samples that show evidence for that gene escaping XCI. The yellow bar denotes the proportion of samples that show evidence for that gene being silenced.

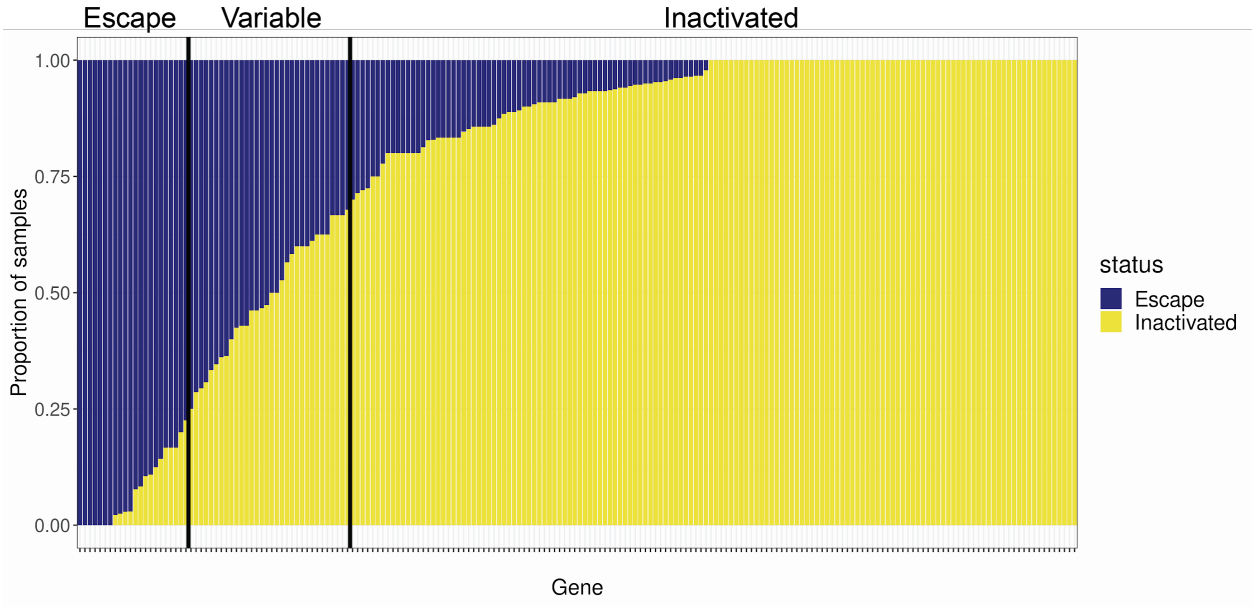


Figure S10. Heterogeneity in escape from X chromosome inactivation across and within placentas.

For each gene on the X chromosome, inactivation status is shown for each extraction site for each sample: dark blue (genes that escape XCI) and yellow (genes that are inactivated).

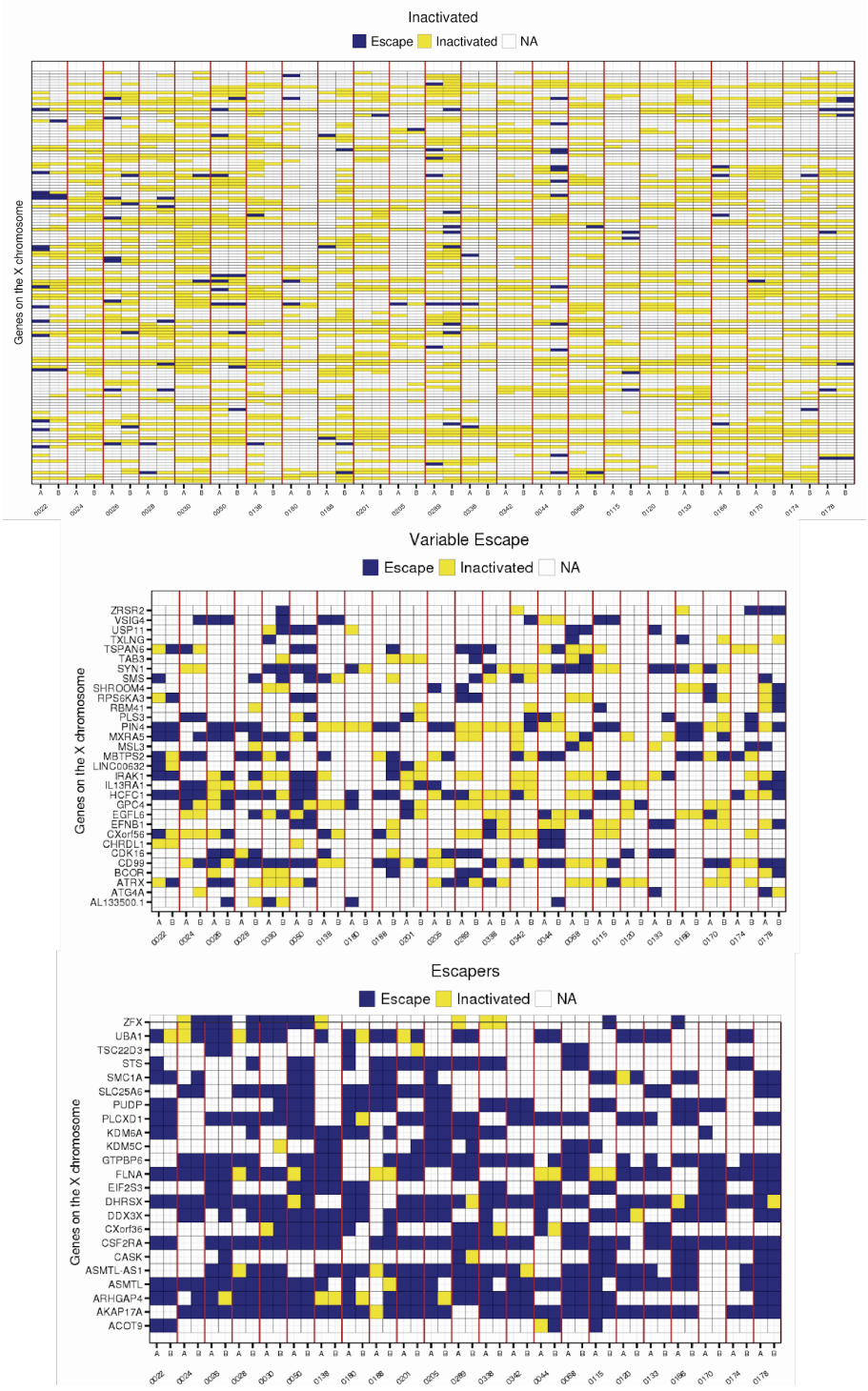


Figure S11. Higher gene expression in female does not necessarily equate to escape gene

Female to male $\log_2$ ratio (calculated as $\log_2(\text{female}_{CPM}/\text{male}_{CPM})$ in gene expression was computed for genes categorized as inactivated, escape, or variable in the placenta. The female_{CPM} and male_{CPM} were obtained from Olney et al. (unpublished data).

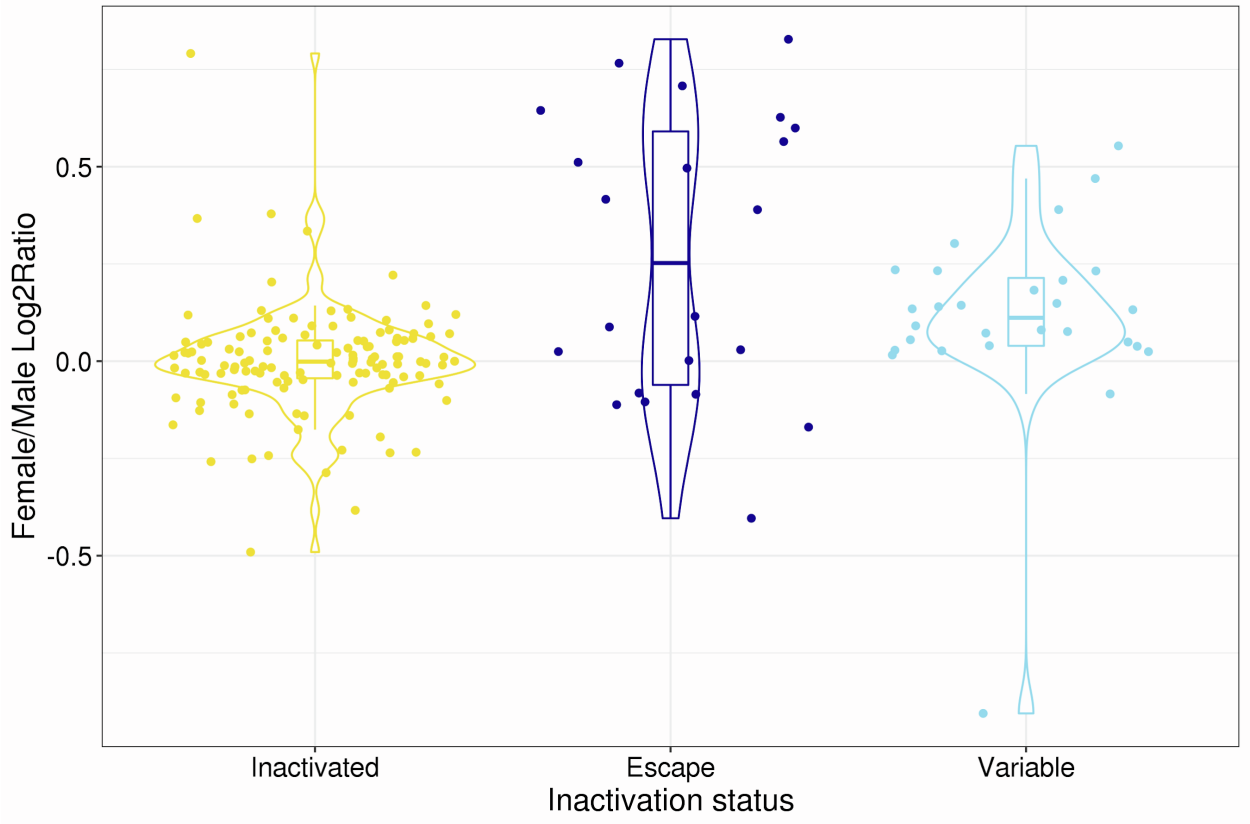
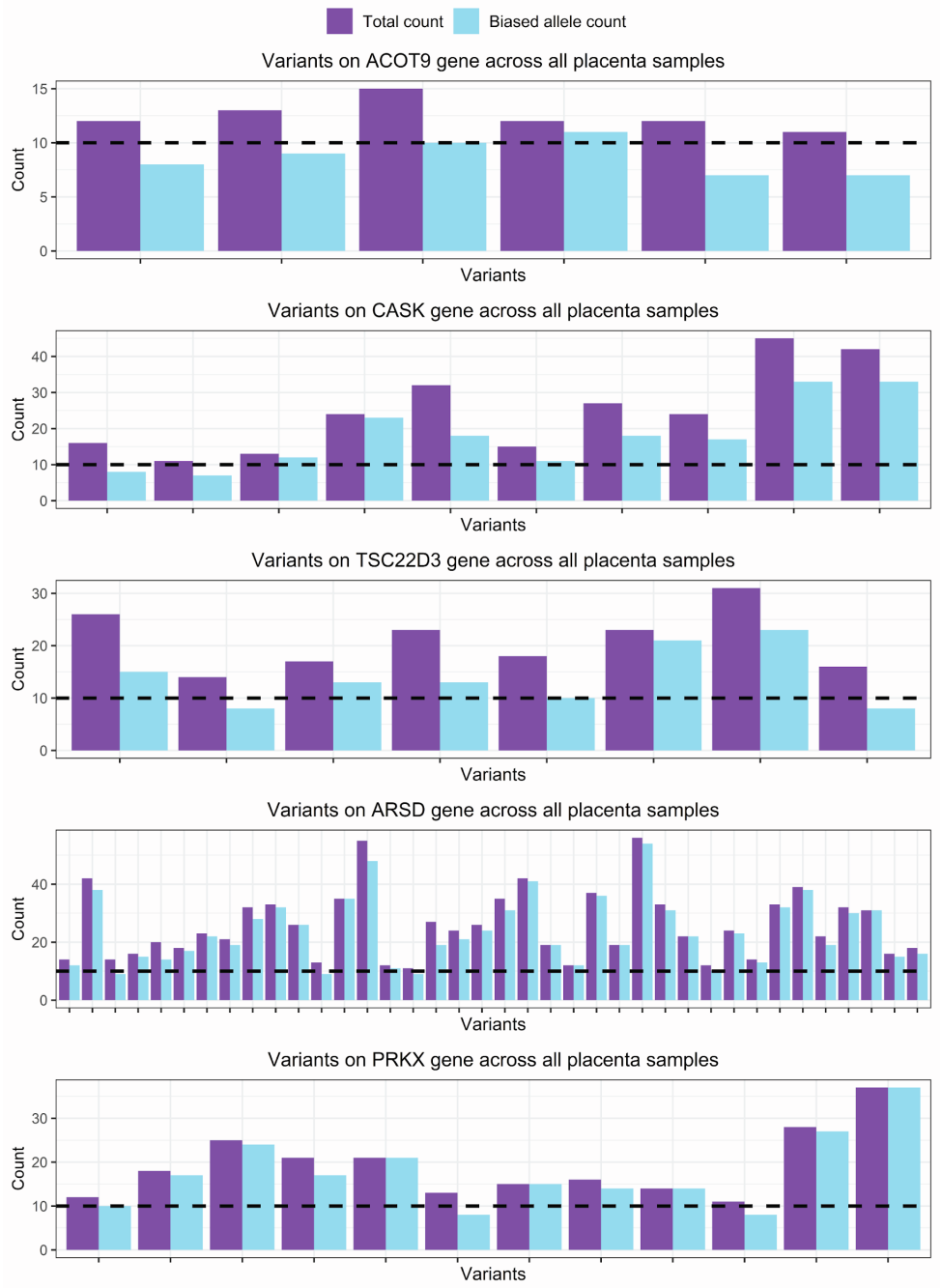


Figure S12. Total count and biased allele count for variants on genes that show opposite XCI patterns between the placenta and adult GTEx tissues and between the placenta.

For each gene, for each heterozygous and expressed variants, purple bars represent total RNA read count and light blue bars represent RNA read count of the biased allele. We observed that the total RNA read count for these variants are all greater than 10, suggesting that the patterns observed in Figure 4 is not due to technical artifacts.



Supplementary Notes

Note 1. Method to classify genes into genes that are inactivated, genes that escape XCI, and genes that show variable escape