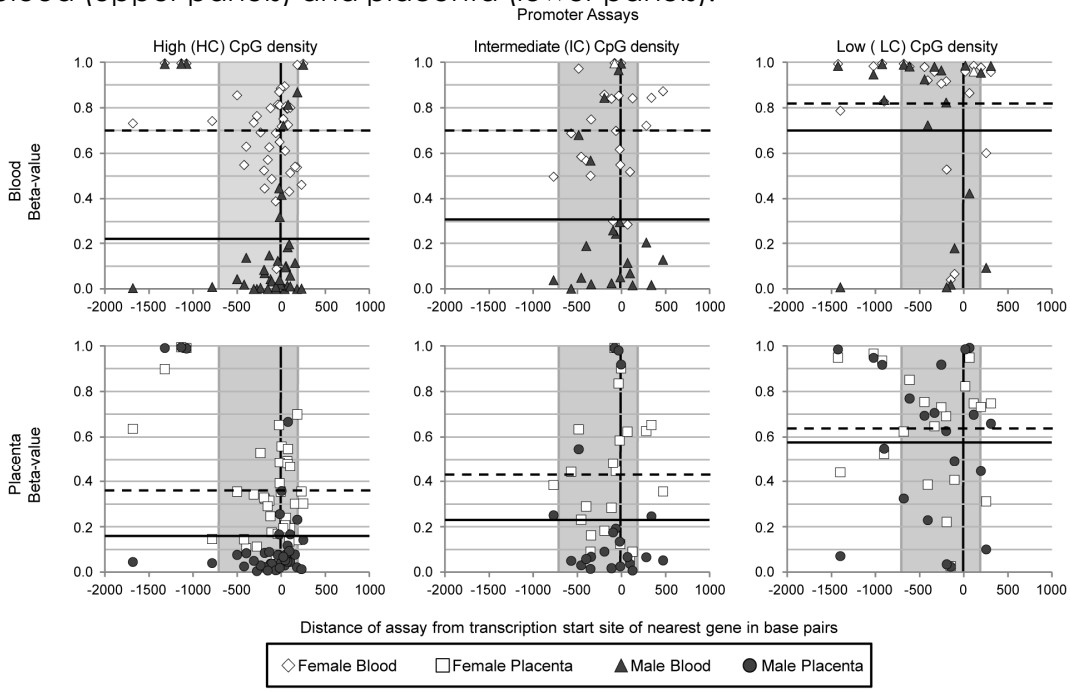
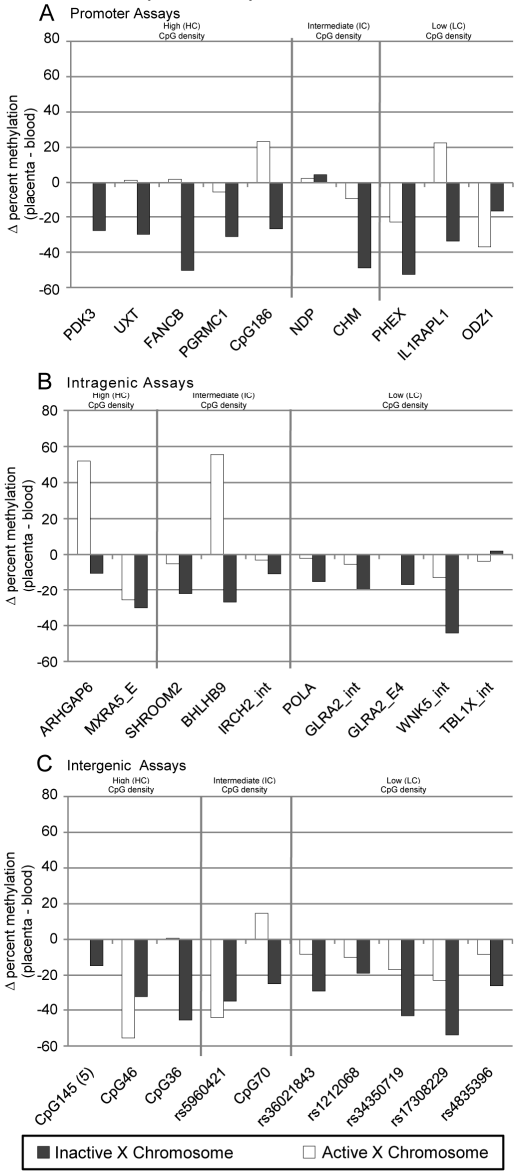
Supplementary Figure 1: Effect of distance from transcription start site on methylation. Beta-values for female blood (white diamond), female placenta (white square), male blood (black triangle) and male placenta (black circle) versus distance from the TSS for each X-linked assay present on Illumina GoldenGate panel. The average beta-value for each sex and tissue is shown as a dashed horizontal line for females and a solid horizontal line for males. Black vertical line marks the TSS (0 bp) and the grey area contains the promoter region as defined by Weber *et al.* (700 bp upstream to 200 bp downstream of the TSS) (10). Assays were separated based on the CpG density (HC, IC and LC) of the 500 bp around each assay with MeXiP being observed in HC and IC in both blood (upper panels) and placenta (lower panels).



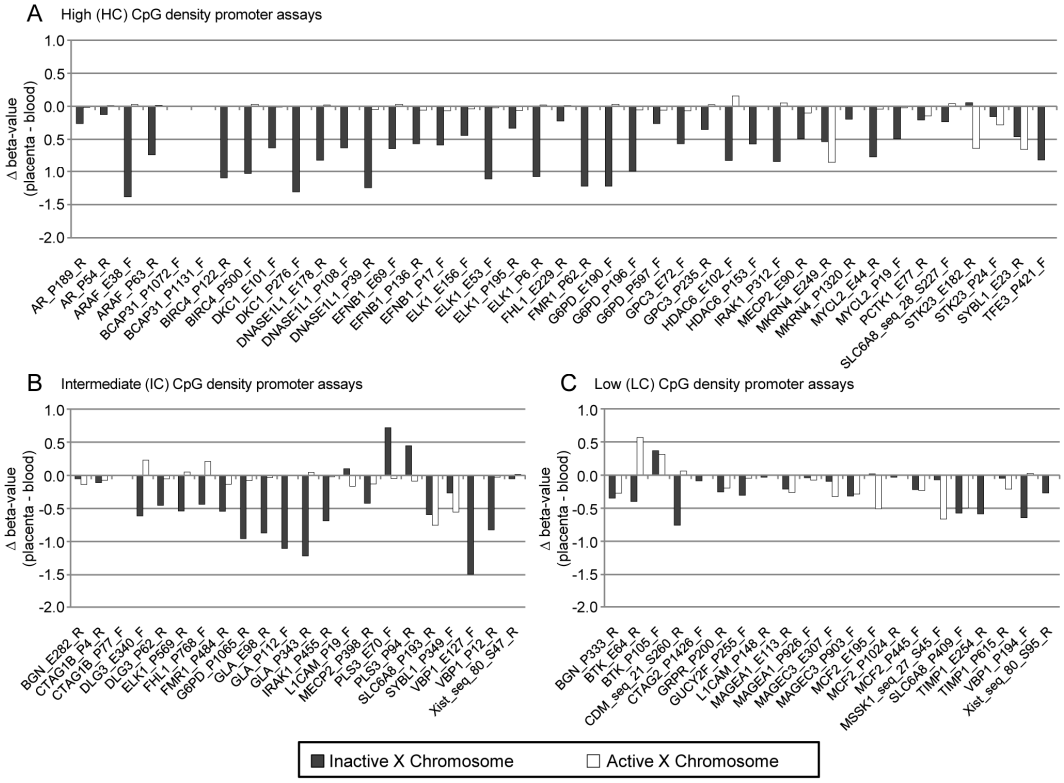
Supplementary Figure 2: Heatmap illustrating the percent methylation as determined by pyrosequencing at all 145 sites examined in the 31 pyrosequencing assays in 6 female blood, 6 female placentas (2 sites each), 6 male blood and 6 male placenta (2 sites each). DNA methylation levels represented as a gradient from blue (high methylation) to yellow (low methylation). Specific samples which failed for a particular assay were replaced with another sample and are marked in red with the replacement sample listed at the end of the figure.

Submitted as separate Excel file "Cotton Sup Fig 2.xls"

Supplementary Figure 3: The Xi shows less placental methylation compared with blood than the Xa at the majority of regions examined across the X chromosome. Percent methylation change from blood to placenta for Xa (white) and Xi (black) at 30 pyrosequencing assays. Negative percent change methylation indicates that blood is more methylated than placenta while positive shows that placenta is more methylated than blood. Assays are separated into CpG density classes, HC, IC and LC, by vertical lines. (A) promoter assays (B) intragenic assays (C) intergenic assays. Xa value is the level of methylation observed in male, Xi value is calculated by subtracting the Xa from the female methylation level multiplied by two.



Supplementary Figure 4: The Xi shows less placental methylation compared with blood than the Xa at the majority of promoters examined on the X chromosome. Beta-value methylation change from blood to placenta for Xa (white) and Xi (black) at Illumina GoldenGate panel assays. Negative percent change methylation indicates that blood is more methylated than placenta while positive shows that placenta is more methylated than blood. Assays are separated into CpG density classes, HC, IC and LC, by vertical lines. (A) promoter assays (B) intragenic assays (C) intergenic assays. Xa value is the level of methylation observed in male, Xi value is calculated by subtracting the Xa from the female methylation level multiplied by two.



Supplementary Table 1: Primer sequences and cycling conditions used in pyrosequencing assays. PCR product size, assay class and CpG class of each assay also listed.

Name [a]	Sequence (5' to 3')	Annealing Temperature	PCR product size	Assay Class	CpG class
PDK3_78_F1 PDK3_78_R1_B PDK3_78_S1	GGTTGTAAAATTTAAGTGTTAGGA /Biotin/AACCCAACCCAACAAATACAA AAAATTTAAGTGTTAGGATG	57°C	211	Promoter	HC
UXT_89_F1 UXT_89_R1B UXT_89_S1	GTTAATGGGGGATTGTAAAAG /Biotin/TCACTTCCTCTACCTCCACCTAT ATGGGGGATTGTAAAA	57°C	130	Promoter	HC
FANCB_93_F1B FANCB_93_R1 FANCB_93_S1	/Biotin/TTTGGGGAGTGTTGTGAAAGTA AACCAAACCCTCAACCTAAATC CCTCAACCTAAATCCCAT	57°C	167	Promoter	HC
PGRMC1_95_F1 PGRMC1_95_R1_B PGRMC1_95_S1	GGGGAAGGGTTATTAAGGAGAG /Biotin/CCCATTCTAAAACCCCTCATCT GGGAAGGGTTATTAAGGA	57°C	164	Promoter	HC
CpG186_89_F1_B CpG186_89_R1 CpG186_89_S1	/Biotin/TGTAGTTTGGATATTTTGATGGG AACCAATCCTTACCTTACAACCT TCCTTACCTTACAACCTTT	57°C	220	Promoter	HC
NDP_F1 NDP_R1 NDP_S1	AGAGAGAGAATGTTAAATGGAAAAGTGTTA /Biotin/ATTTAACCTCTTATTAATTCCATAATACCA AGAGAATGTTAAATGGAAAA	57°C	255	Promoter	IC
CHM_F1 CHM_R1 CHM_S1	GTGGGAGATTTGGATATTTTTTGAT /Biotin/AAATAAAAATCTCCTTTATTCACAAAAC GATAATATTGAAGTAAAATTGTTAG	57°C	111	Promoter	IC
PHEX_F1 PHEX_R1 PHEX_S1	AGTTTTTTAAAGTGTTGGGATTATAGG /Biotin/ACTTCAACAAATTCCCCAAAATAAA AAAGTGTTGGGATTATAGG	57°C	93	Promoter	LC

Il1RAPL1_F1 Il1RAPL1_R1 Il1RAPL1_S1	/Biotin/TTGGGGAGATAGTGATGGG CACACTCTTAATAACCTCCTTTTCATC ATCTCTTCTCTTTAAAACAAAT	55°C	91	Promoter	LC
ODZ1_F1 ODZ1_R1 ODZ1_S1	GTATTAAGGATTAAGTTGGAGGTTGTAGT /Biotin/TTATACTCCTCACCACTTTCAAATCTAAT ATAGTTTTTAAAAATATTTGTATTG	57°C	193	Promoter	LC
ARHGAP6_F1 ARHGAP6_R41 ARHGAP6_S1	/Biotin/ATTTGATTGAAGGTTGAATGAG CCAACCCTAAATTCAATATTTCTT CAATATTTCTTTACCCCA	64.5°C	149	Within Gene	HC
MXA5_E_F1 MXA5_E_R1 MXA5_E_S1	TTTTTTTGATGGAAAGGGTT /Biotin/TCTTCCCTAACAAAAAAATATAACAAACT TTTTTTTGATGGAAAGG	57°C	90	Within Gene	HC
ShROOM2_F1 SHROOM2_R1 SHROOM2_S1	GGTGGAGAATGTTTTTAATAATTTG /Biotin/CCCCCATTTCCAAATCAA GGTGGAGAATGTTTTTA	53°C	86	Within Gene	IC
BHLHB9_F2 BHLHB9_R2 BHLHB9_S2	/Biotin/GGGGTTTTTTTGAGGTAGTTTGGTGT CCCCTCTCAAACCCACCTTAATT TCTCAAACCCACCTTAAT	57°C	102	Within Gene	IC
IRCH2_int_F1 IRCH2_int_R1 IRCH2_int_S1	GAGTAGGAGGTTATTATGAGGAGAA /Biotin/ACTAAAACTACTATAACCCCCACTATAAAT GGAGGTTATTATGAGGAGA	57°C	101	Within Gene	IC
POLA_F3 POLA_R2	/Biotin/GGGGGGTAGTGTTTTATGTATATTAAAAT ACCACATAAAACCCACACATATAAT	57°C	115	Within Gene	LC

POLA_S5	ATAAACTAACTTTTCCTATC				
GLRA2_Int_F3 GLRA2_Int_R4 GLRA2_Int_S2	/Biotin/GAATTTTTTGATGGATTGGATATGG CCTTCTATTAACTCCACACTCCTATATCA ATCTCATAACTATCTACATTAACC	57°C	183	Within Gene	LC
GLRA2_E4_F3 GLRA2_E4_R1 GLRA2_E4_S3	TGTAAATAGAATTTTTGTGTTAGGGTAAT /Biotin/ATAGAATTTTTGTGTTAGGG ATAGAATTTTTGTGTTAGGG	57°C	135	Within Gene	LC
WNK5_Int_F1 WNK5_Int_R1 WNK5_Int_S1	/Biotin/TAAAAATTAGTTGGGAGTGGTGGTAGG CTCATTTACATTTTCCTCCCTCATCA CCAAATTAAAATACAATAACACA	57°C	217	Within Gene	LC
TBL1X_int_F1 TBL1X_int_R1 TBL1X_int_S1	TGTGTTAAGTTTGGATTGTAGAAATGAAT /Biotin/CCCTAAATAATAATCTCAATTTTCCTCATA GTAGAAATGAATTTGAAGAAG	55°C	147	Within Gene	LC
CpG145_89_F1 CpG145_89_R1_B CpG145_89_S1	TTGGATTTGTTTGTTTAGGATTG /Biotin/CAAACCCAACTACTTCAATAACCT GGATTTGTTTGTTTAGGAT	57°C	182	Between Genes	HC
CpG46_F1 CpG46_R1 CpG46_S1	/Biotin/GGTTTTAGTGGTTTTTGATTTTATAGAGT CTCCTCTTACTAAAAACAACCTACC TCCTCTTACTAAAAACAACCT	57°C	108	Between Genes	HC
CpG36_F1 CpG36_R20 CpG36_S18	GGAAAGGAAAAGGGAGAATT /Biotin/CCCTCACCACTAAACAATTAA GGAAAGGAAAAGGGAGAAT	57°C	80	Between Genes	HC

rs5960421_1_F1 rs5960421_1_R1 rs5960421_1_S1	GGTTTGTAGAGTGTTTGGTAGAGG /Biotin/CCCTCCCACCAAAATCAAAT AGTGTTTGGTAGAGGTGTT	57°C	143	Between Genes	IC
CpG70_F1 CpG70_R1 CpG70_S1	GTTTGAAGTAGGAGGTTTGGATGTA /Biotin/CTAAACTCCTATTTCTCCAATTTATACAAC GAAGTAGGAGGTTTGGAT	55°C	169	Between Genes	IC
rs36021843_F1 rs36021843_R1 rs36021843_S1	/Biotin/ATGGTTGGTTTATATGGTTATTTAGAGTT CCCTAAAAAATAACCTCCTACTTAACTAT AATAATATTCCACCTCCC	57°C	185	Between Genes	LC
rs1212068_1_F1 rs1212068_1_R1 rs1212068_1_S1	/Biotin/TGAGAGATGAGTGTTATGGAGAAA CAAAAAACAAACTCTCCAAATTCA TCTCCAAATTCAAATCAAT	64.5°C	183	Between Genes	LC
rs34350719_F1 rs34350719_R1 rs34350719_S1	GTTTTGGGTTTGGAAAAATTAGAGT /Biotin/CCCATAAAATTCAAAAAACTTCTTACCT TGGGTTTGGAAAAATTAG	57°C	79	Between Genes	LC
rs17308229_F1 rs17308229_R1 rs17308229_S1	GGGTTTTTTATTTTTTTGAGATTTGTTAG /Biotin/AACCACTCAAACTATATCTACAAACAACTA TATTTATAAGTTATTGTATTTAGGG	64°C	214	Between Genes	LC
rs4825396_F1 rs4825396_R1 rs4825396_S1	/Biotin/TTTTTTGATGGGGGAGAAGGGT CCCATCCTAATCTTCCTATTTTCTTATCC TTCCTATTTTCTTATCCACA	57°C	113	Between Genes	LC

a) F: Forward primers, R: Reverse primer, S: Sequencing primers

Supplementary Table 2: Methylation assays for island (HC and IC) promoters failing to show MeXiP or containing featured believed to interfere with MeXiP or showing discordant methylation results.

Assay name	Average Methylation				Features interfering with MeXiP [a]				Discordant Assay Methylation Patterns [d]	Previous methylation data [e]	Evaluation
	Female Blood	Female Placenta	Male Blood	Male Placenta	CpG Density	Repetitive Element [b]	Escapes X inactivation	Distance to TSS (bp) [c]			
AR_P54_R	0.09	0.04	0.00	0.01	HC	-	0/6	-54	Yes	(1)	The 2 Illumina assays for AR are discordant and P189 does not match previous methylation results.
AR_P189_R	0.45	0.33	0.07	0.10	HC	-	0/6	-189			
BCAP31_P1072_F	0.99	0.99	0.99	0.99	HC	-	3/9	*-1074*	-	(2)	Also near a duplication on chr 16 [f].
BCAP31_P1131_F	0.99	0.99	0.99	0.99	HC	-	3/9	*-1133*			
BGN_E282_R	0.72	0.59	0.20	0.13	IC	-	0/5	282	Yes	-	The 2 Illumina assays for BGN are discordant however the other assay is an LC.
CTAG1B_P4_R	1.00	0.90	0.99	0.92	IC	-	-	-4	-	(3,4)	CT gene family highly methylated, additionally many other CTs on the array were LCs [g].
CTAG1B_P77_F	1.00	0.99	1.00	0.99	IC	-	-	-77			
DLG3_E340_F	0.84	0.67	0.02	0.27	IC	-	-	340	-	-	Shows MeXiP despite distance to TSS.
FHL1_E229_R	0.46	0.34	0.00	0.05	HC	-	1/9	229	-	-	Shows MeXiP despite distance to TSS and/or presence of a LINE.
FHL1_P768_F	0.50	0.39	0.04	0.26	IC	LINE	1/9	-768			

FMR1_P484_R	0.97	0.60	0.68	0.57	IC	-	1/9	-484	Yes	(5,6,7)	The 2 Illumina assays for FMR1 are discordant and P484 does not match previous methylation results.
FMR1_P62_R	0.69	0.08	0.01	0.01	HC	-	1/9	-62			
G6PD_E190_F	0.74	0.15	0.01	0.04	HC	-	0/5	-783	-	(8, 9)	Shows MeXiP despite distance to TSS and/or presence of a LINE.
G6PD_P1065_R	0.87	0.37	0.13	0.07	IC	LINE	0/5	472			
L1CAM_P19_F	0.62	0.56	0.30	0.18	IC	-	-	-19	Yes	-	The 2 Illumina assays for L1CAM are discordant however the other assay is an LC.
MKRN4_E249_R	0.99	0.31	0.99	0.15	HC	-	-	249	Yes	-	Pseudogene
MKRN4_P1320_R	0.99	0.90	0.99	0.98	HC	-	-	-1320	Yes		
NDP	13%	16%	6%	9%	IC	-	-	-59	-	-	undetermined
PCTK1_E77_R	0.72	0.54	0.81	0.66	HC	-	6/6	77	-	(10)	Assay does not match previous methylation results.
PLS3_E70_F [i]	0.29	0.61	0.11	0.11	IC	-	5/9	70	-	(11)	Previous methylation results only examined methylation in males.
PLS3_P94_R	0.30	0.49	0.26	0.20	IC	-	5/9	-94			
SLC6A8_P193_R	0.86	0.17	0.84	0.10	IC	-	-	-193	Yes	(2)	The 3 Illumina assays for SLC6A8 are discordant however the other assay is an LC. Previous methylation results only examined methylation in males and there is also has a pseudogene on chr 16 [f].
SLC6A8_seq_28_S227_F	0.73	0.63	0.00	0.09	HC	-	-	*-1681*			
STK23_E182_R	0.99	0.72	0.87	0.26	HC	-	-	182	Yes	(2)	The 2 Illumina assays for STK are discordant and Previous methylation results only examined methylation in males.
STK23_P24_F	0.87	0.63	0.45	0.21	HC	-	-	-24			

SYBL1_E23_R	0.75	0.21	0.72	0.07	HC	-	0/5	23	-	(12,8)	Silent on both X and Y chromosomes. P349 shows MeXiP despite presence of a LINE.
SYBL1_P349_F	0.50	0.11	0.57	0.01	IC	LINE	0/5	-349	-		
Xist_seq_80_S47_R	0.85	0.83	0.97	0.97	IC	-	-	-31	Yes	(13,14,15)	The 2 Illumina assays for XIST are discordant however the other assay is an LC. Is also expressed only from the Xi [h].

a) Grey shading represents possible features which may interfere with MeXiP.
b) Three LC assays (CTAG2_P1426_F, MAGEC3_P903_F, TIMP1_P615_R) were also located within repetitive elements
c) Seven LC assays (CDM_seq_21_S260_R, CTAG2_P1426_F, MAGEA1_P926_F, MAGEC3_E307_F, MAGEC3_P903_F, MCF2_P1024_R, TIMP1_E254_R) were also beyond 700 bp upstream or 200 bp downstream
d) Grey shading indicates a gene with multiple Illumina assays which show different methylation patterns. Three genes (BTK, MCF2, TIMP1) had only LC assays and also were discordant between assays within the same gene.
e) Grey shading indicates that Illumina methylation results conflict with previous methylation results.
f) Recent genome-wide studies suggest a hypermethylation of pseudogenes and duplicated regions thus it is possible that the presence of a tandem duplication or pseudogene may predispose genes to hypermethylation which may explain the high methylation seen for *BCAP* and *SLC6A8* [(2),(16)].
g) Members of cancer-testis (CT) antigen family of genes are often found in palindromic repeats as multicopy genes and pseudogenes and have typically been shown to be highly methylated in all tissues except the germline – a pattern generally found for genes with germline-specfic expression [(3),(2),(17)]. Consistent with high levels of methylation in all tissues other than testis, all MAGEs and CTAGs showed hypermethylation in blood and placenta regardless of CpG density emphasizing that gene function as well as CpG density is important in determining methylation status [(3),(2)].
h) Both XIST assays on the Illumina GoldenGate panel showed nearly 100% methylation in males however females showed methylation levels up to 95%. While the trend of these methylation levels was as expected the level of methylation in females appears to have been substantially overestimated by the Illumina assay.

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