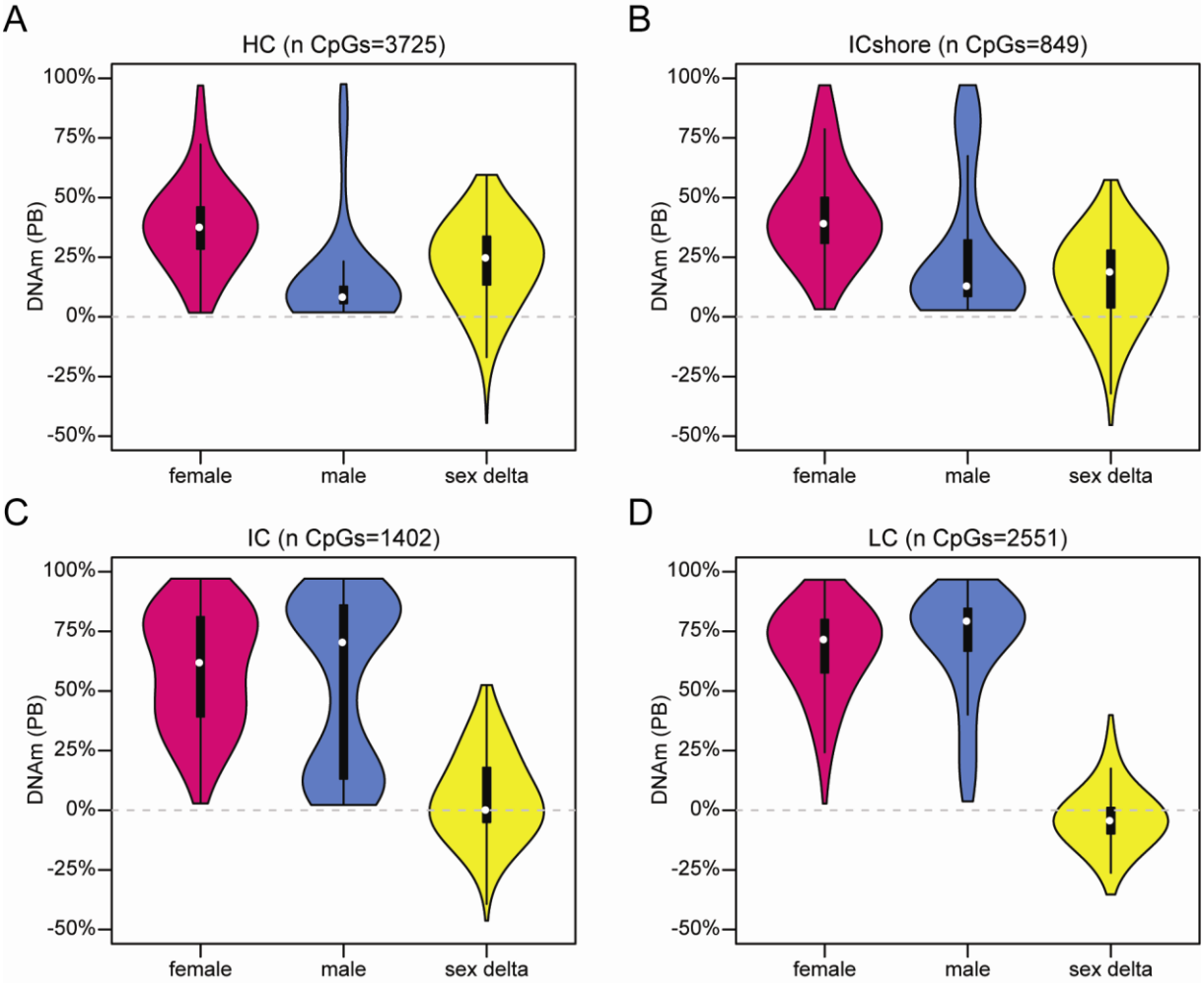
Supplementary Figure 1: X-linked DNAm differed the most between males and females at CpG islands. The distribution of DNAm in females (pink), males (blue) and the sex delta (female DNAm – male DNAm) (yellow) for each HIL (reference 22) CpG density (A: HC, B: ICshore, C: IC, D: LC). Within each violin plot, the mean DNAm is represented by the white dot while quartile boundaries are shown as thick black lines. ICs which flanked an HC (the ICshore of reference 22) had a sex DNAm delta very similar to HCs and thus for subsequent analysis, HC and ICshores were combined into a single CpG class called HIC.

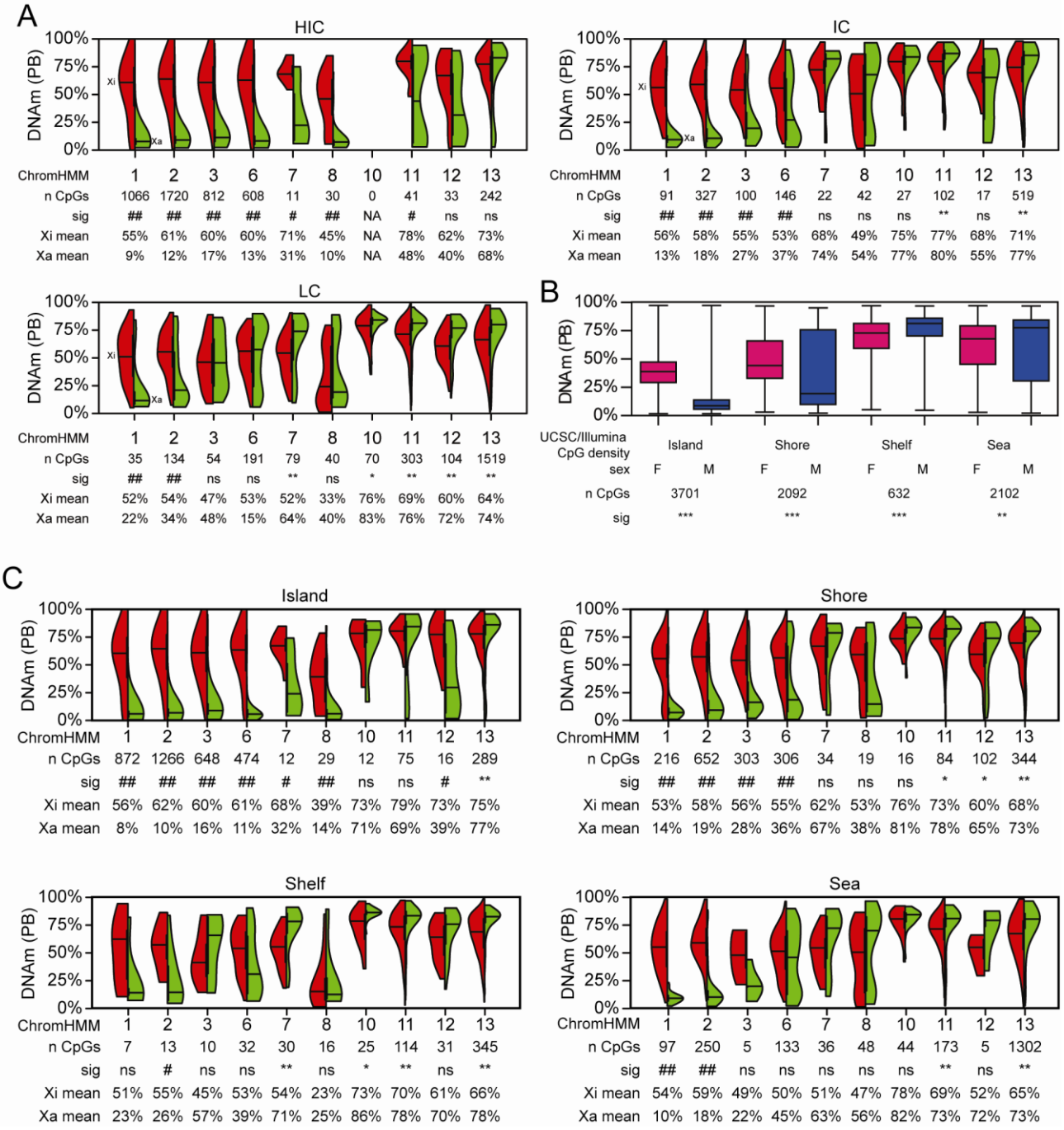
Supplementary Figure 2: DNAm landscape of the X chromosome by CpG density and chromatin state. A) The distribution of DNAm on the Xi (red) and the Xa (green) for each combination of HIL CpG density (reference 22) and ChromHMM chromatin state (reference 20). Chromatin states 4, 5, 9, 14 and 15 were excluded from our analysis as each had fewer than 20 X-linked CpGs on the 450K array. The Xa and Xi DNAm of the remaining ten states were compared within each HIL CpG density class and resulted in 29 HIL/ChromHMM state combinations. 13 of the 29 HIL/ChromHMM states had significantly (p-value<0.05, pound signs) more DNAm on the Xi than the Xa, including active and weak promoters (states 1 and 2) of all CpG densities. At these 13 Xi>Xa states, the average difference in Xi and Xa DNAm was 36%. In contrast, Xa DNAm was significantly (p-value<0.05, asterisks) higher than Xi DNAm at seven HIL/ChromHMM states; however, the difference in DNAm between the Xa and Xi was smaller with an average of only 9%. Horizontal black lines represent the median DNAm while the mean DNAm is given below along with the number of CpGs within each HIL/ChromHMM state. Significance was assessed using a Wilcox test comparison of means (#/* p-values 0.05 to 0.001, ##/** p-values 0.001 to 2.2 E-16 and ###/*** p-values < 2.2 E-16). B) Box and whisker plots of the average female (pink) and male (blue) DNAm based on the Illumina/UCSC CpG density. Significance based on a Wilcox test comparison of means is as follow: * p-values 0.05 to 0.001, ** p-values 0.001 to 2.2 E-16 and *** p-values < 2.2 E-16). C) The distribution of DNAm on the Xi (red) and the Xa (green) for each Illumina/ChromHMM state combination. Horizontal black lines represent the median DNAm while the mean DNAm is given below along with the number of CpGs within each Illumina/ChromHMM state. Significance based on a Wilcox test comparison of means is denoted in pound (#) signs when the mean Xi DNAm > Xa and asterisks (*) when the mean Xi DNAm < Xa (#/* p-values 0.05 to 0.001, ##/** p-values 0.001 to 2.2 E-16 and ###/*** p-values < 2.2 E-16).

Supplementary Figure 3: CpGs which map to multiple sex chromosome locations and/or repetitive elements show the same patterns of DNAm as CpGs which do not. A) PB DNAm differences between females and males (sex DNAm delta) distributions in HIL/ChromHMM states that show a significant difference (p-value<0.05) between tier1 and tier2 CpGs. Within each violin plot the horizontal black lines represent the median DNAm while the mean DNAm is given below along with the number of CpGs. Although statistically significant, the average difference in DNAm between CpGs was only 4% making it unlikely that there was a large biological effect of the potential ancestral repetitive origin of these CpGs. B) PB Xi (left side) and Xa (right side) DNAm distributions in HIL/ChromHMM states which show a significant difference (p-value<0.05) between CpGs that map to a single unique location on the X chromosome but are not in a repetitive element (unique chrX) and all other CpGs (multi chrX: CpGs that map to multiple sex chromosome locations but are not in a repetitive element, unique repetitive element chrX: CpGs that map to a single unique location on the X chromosome and are in a repetitive element and multi repetieve element XY: CpGs that map to multiple sex chromosome locations and are in a repetitive element). Significance levels (sig) from a Wilcox test comparison of means (* p-values 0.05 to 0.001, ** p-values 0.001 to 2.2 E-16 and *** p-values < 2.2 E-16).

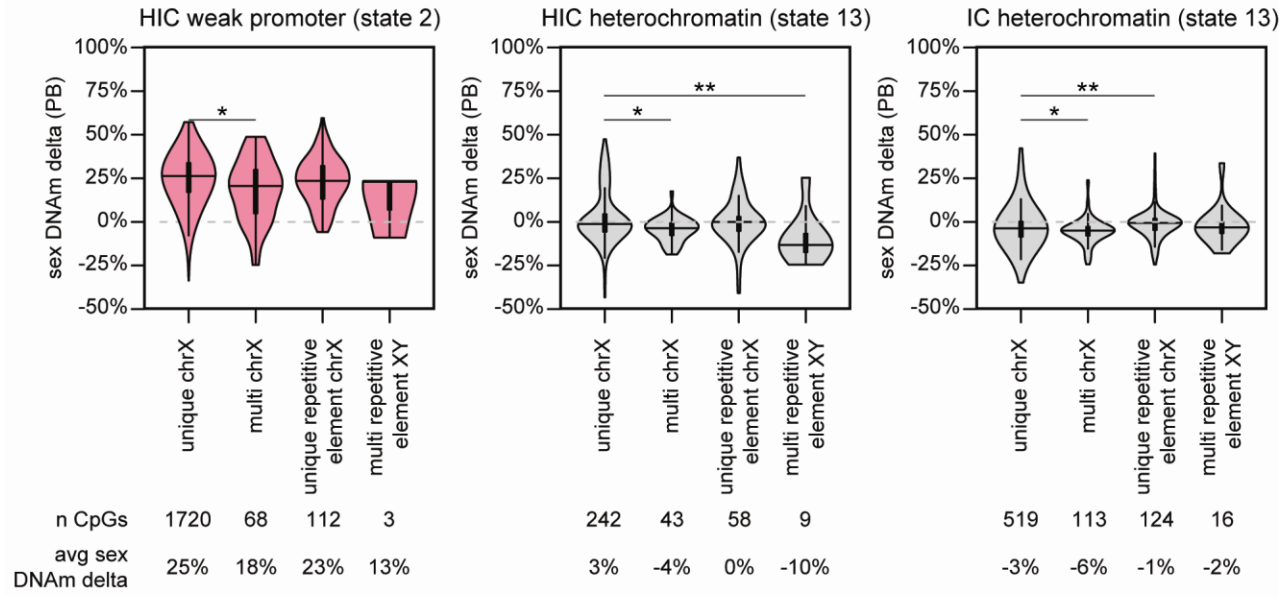
Supplementary Figure 4: DNAm at subject TSSes is significantly higher than at escape TSSes in females but not males. A) Box and whisker plots of female DNAm, male DNAm and the difference between female and male DNAm (sex DNAm delta) for all CpGs in the subject (n=2682 CpGs) and escape (n=160 CpGs) training sets. The mean DNAm and the significance levels from a Wilcox test comparison of means are given below each plot. B) Moving averages (solid lines) of the sex DNAm delta calculated in the subject (n=2398 CpGs) and escape (n=148 CpGs) training sets. Dotted lines represent moving averages calculated with less than the target number of CpGs. The grey shaded region (-500 to 1000 basepairs) indicates the target basepair region used in further analysis. C) Comparison of average TSS DNAm between the subject (n=364 TSSes) and escape (n=26 TSSes) training sets for female DNAm, male DNAm and the sex DNAm delta. Only CpGs in which the male DNAm was less than 25% were included. The mean DNAm and the significance levels (sig) from a Wilcox test comparison of means is given below each plot (p-values 0.05 to 0.001, ** p-values 0.001 to 2.2 E-16 and *** p-values < 2.2 E-16).

Supplementary Figure 5: Age and X-linked DNAm in WB show no relationship. DNAm and percent escape analysis in BC males (n=24) and females (n=566). Age (in years) of samples was plotted versus: the average DNAm at the XIST IC promoter in males (squares, upper row); the average DNAm at the XIST IC promoter in females (circles, second row); the average promoter DNAm of all variably escaping TSSes (diamonds, third row); and the percent escape from XCI at only the variably escaping TSSes (green triangles, fourth row). No significant correlation between age and any of these four variables was observed.


A
HC (n CpGs=3725)
B
ICshore (n CpGs=849)
C
IC (n CpGs=1402)
D
LC (n CpGs=2551)
DNAm (PB)
100%
75%
50%
25%
0%
-25%
-50%
female
male
sex delta


A
HIC
IC
LC
DNAm (PB)
100%
75%
50%
25%
0%
Xi
Xa
ChromHMM 1 2 3 6 7 8 10 11 12 13
n CpGs 1066 1720 812 608 11 30 0 41 33 242
sig ## ## ## ## # ## NA # ns ns
Xi mean 55% 61% 60% 60% 71% 45% NA 78% 62% 73%
Xa mean 9% 12% 17% 13% 31% 10% NA 48% 40% 68%
n CpGs 91 327 100 146 22 42 27 102 17 519
sig ## ## ## ## ns ns ns ** ns **
Xi mean 56% 58% 55% 53% 68% 49% 75% 77% 68% 71%
Xa mean 13% 18% 27% 37% 74% 54% 77% 80% 55% 77%
n CpGs 35 134 54 191 79 40 70 303 104 1519
sig ## ## ns ns ** ns * ** ** **
Xi mean 52% 54% 47% 53% 52% 33% 76% 69% 60% 64%
Xa mean 22% 34% 48% 15% 64% 40% 83% 76% 72% 74%
B
UCSC/Illumina CpG density
Island
Shore
Shelf
Sea
sex F M F M F M F M
n CpGs 3701 2092 632 2102
sig *** *** *** **
C
Island
Shore
Shelf
Sea
n CpGs 872 1266 648 474 12 29 12 75 16 289
sig ## ## ## ## # ## ns ns # **
Xi mean 56% 62% 60% 61% 68% 39% 73% 79% 73% 75%
Xa mean 8% 10% 16% 11% 32% 14% 71% 69% 39% 77%
n CpGs 216 652 303 306 34 19 16 84 102 344
sig ## ## ## ## ns ns ns * * **
Xi mean 53% 58% 56% 55% 62% 53% 76% 73% 60% 68%
Xa mean 14% 19% 28% 36% 67% 38% 81% 78% 65% 73%
n CpGs 7 13 10 32 30 16 25 114 31 345
sig ns # ns ns ** ns * ** ns **
Xi mean 51% 55% 45% 53% 54% 23% 73% 70% 61% 66%
Xa mean 23% 26% 57% 39% 71% 25% 86% 78% 70% 78%
n CpGs 97 250 5 133 36 48 44 173 5 1302
sig ## ## ns ns ns ns ns ** ns **
Xi mean 54% 59% 49% 50% 51% 47% 78% 69% 52% 65%
Xa mean 10% 18% 22% 45% 63% 56% 82% 73% 72% 73%

A

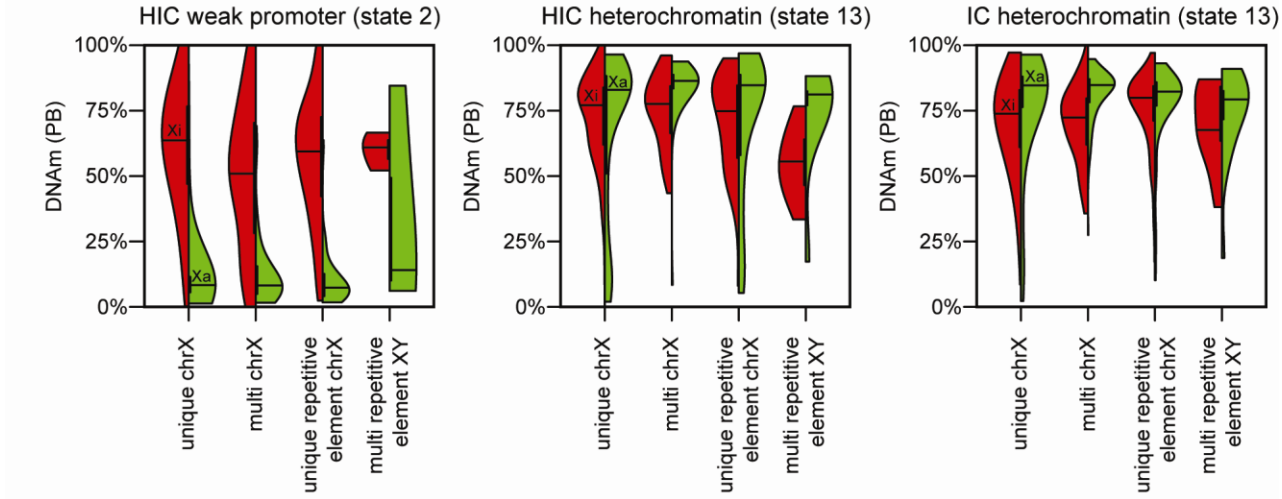


	unique chrX	multi chrX	unique repetitive element chrX	multi repetitive element XY
n CpGs	1720	68	112	3
avg sex DNAm delta	25%	18%	23%	13%

	unique chrX	multi chrX	unique repetitive element chrX	multi repetitive element XY
n CpGs	242	43	58	9
avg sex DNAm delta	3%	-4%	0%	-10%

	unique chrX	multi chrX	unique repetitive element chrX	multi repetitive element XY
n CpGs	519	113	124	16
avg sex DNAm delta	-3%	-6%	-1%	-2%

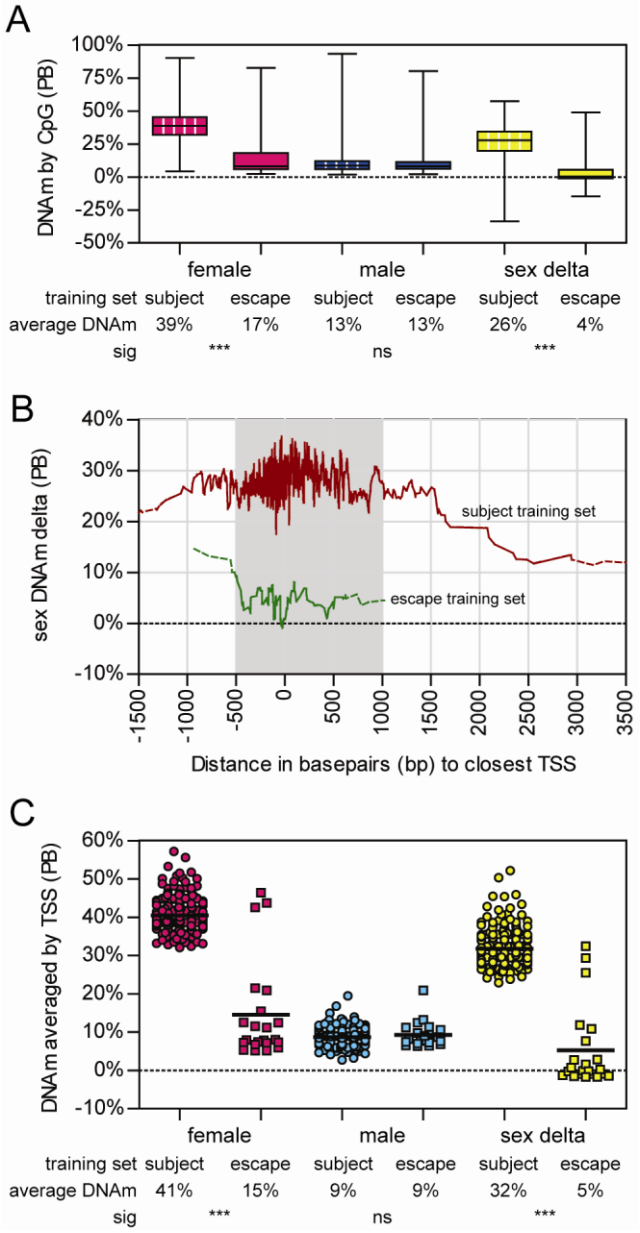
B



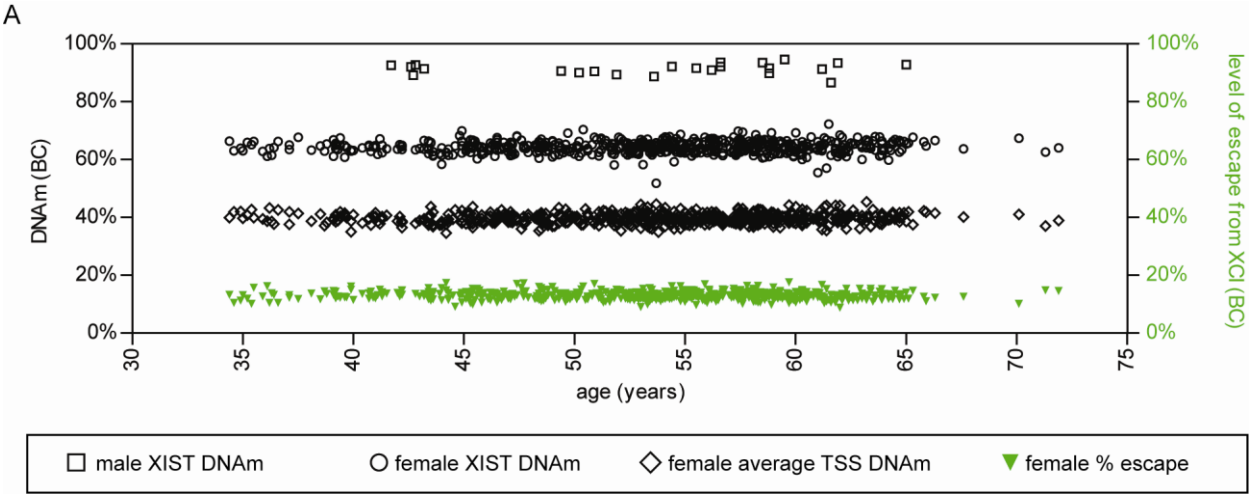
	unique chrX	multi chrX	unique repetitive element chrX	multi repetitive element XY
sig	***	**	***	ns
Xi mean	61%	50%	58%	61%
Xa mean	12%	13%	11%	36%

	unique chrX	multi chrX	unique repetitive element chrX	multi repetitive element XY
sig	ns	**	ns	*
Xi mean	73%	76%	71%	55%
Xa mean	71%	84%	71%	75%

	unique chrX	multi chrX	unique repetitive element chrX	multi repetitive element XY
sig	***	**	*	ns
Xi mean	71%	71%	76%	70%
Xa mean	77%	82%	78%	75%


A
100%
75%
50%
25%
0%
-25%
-50%
DNAm by CpG (PB)
female
male
sex delta
training set subject escape subject escape subject escape
average DNAm 39% 17% 13% 13% 26% 4%
sig *** ns ***
B
40%
30%
20%
10%
0%
-10%
sex DNAm delta (PB)
subject training set
escape training set
-1500
-1000
-500
0
500
1000
1500
2000
2500
3000
3500
Distance in basepairs (bp) to closest TSS
C
60%
50%
40%
30%
20%
10%
0%
-10%
DNAm averaged by TSS (PB)
female
male
sex delta
training set subject escape subject escape subject escape
average DNAm 41% 15% 9% 9% 32% 5%
sig *** ns ***

A


100%
80%
60%
40%
20%
0%
DNAm (BC)
30
35
40
45
50
55
60
65
70
75
age (years)
level of escape from XCI (BC)
male XIST DNAm
female XIST DNAm
female average TSS DNAm
female % escape