Figure S1. The scheme for the opossum crosses and tissue dissection.

(A-B) Reciprocal F1 crosses between LL1 and LL2 animals. (C-D) Parental crosses of LL1 and LL2 animals. In the four crosses, three LL1 animals and three LL2 animals were used. LL1 individuals: A0579 (female) and A0580 (female) are full sibs; LL2 individuals: A0571 (female), A0572 (female) and A0573 (male) are full sibs. A (C/T) SNP was shown (LL1: T and LL2: C). (E-F) 12.5 d.p.c. – 13.0 d.p.c fetus and extra-embryonic membranes (EEM). (E) Fetus and EEM; (F) Fetus with EEM removed. Solid white line is the approximate incision line used to isolate fetal brain. The arrow shows the tissue used for the EEM analysis.

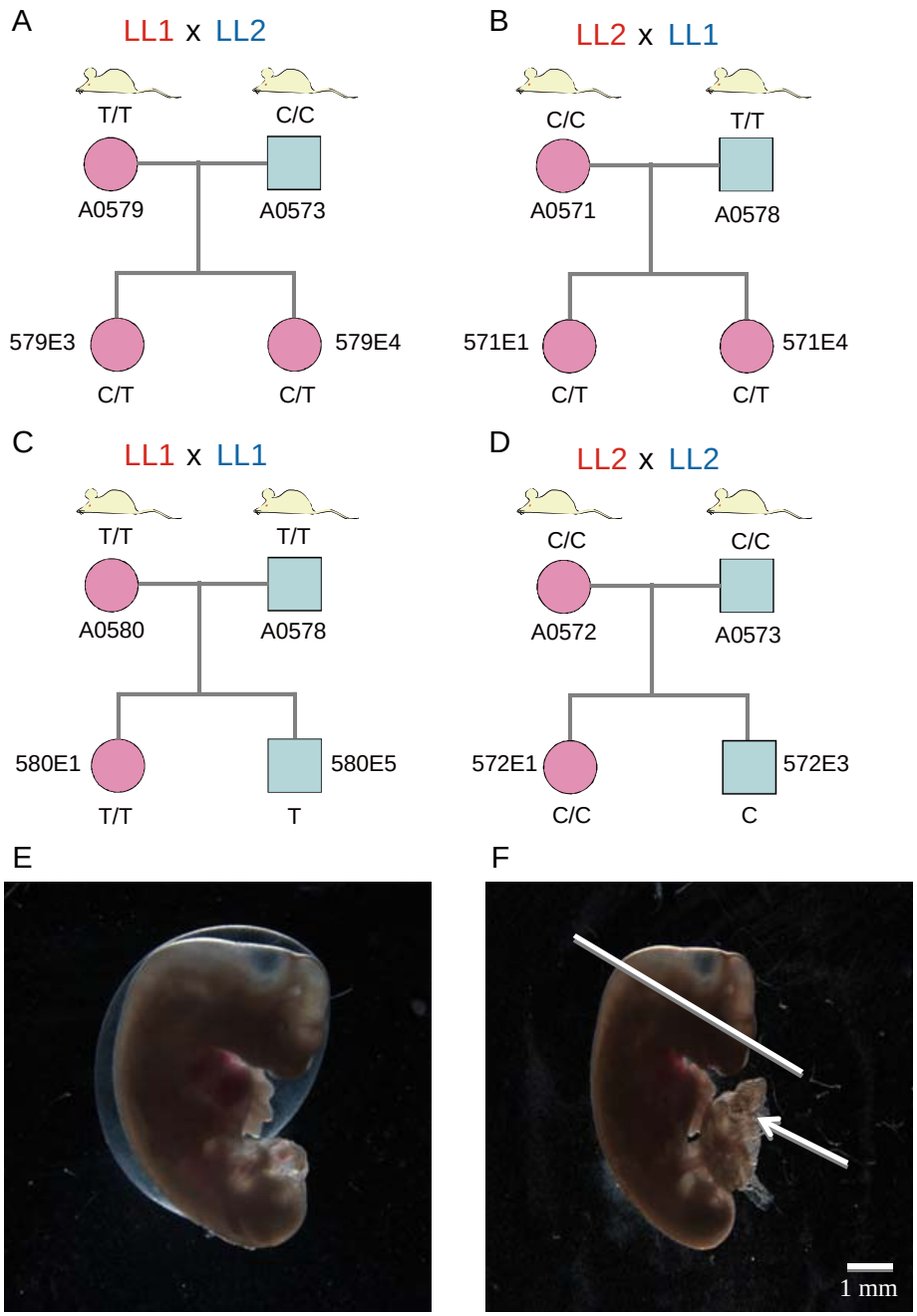


Figure S2. Sex genotyping results for opossum embryos.

*: samples selected for Illumina RNA-seq.

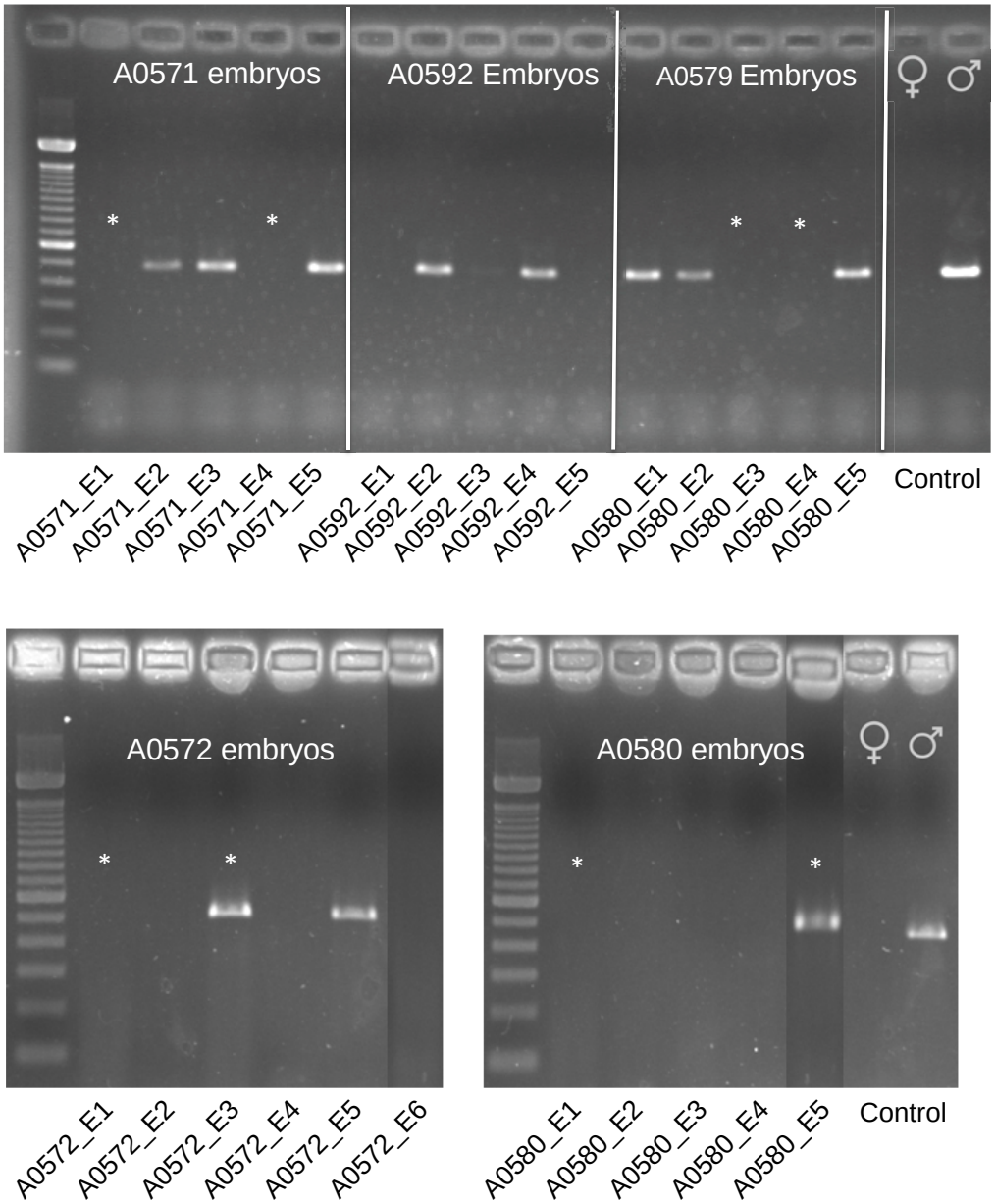


Figure S3. M-A plot for brain and EEM expression levels before and after normalization.

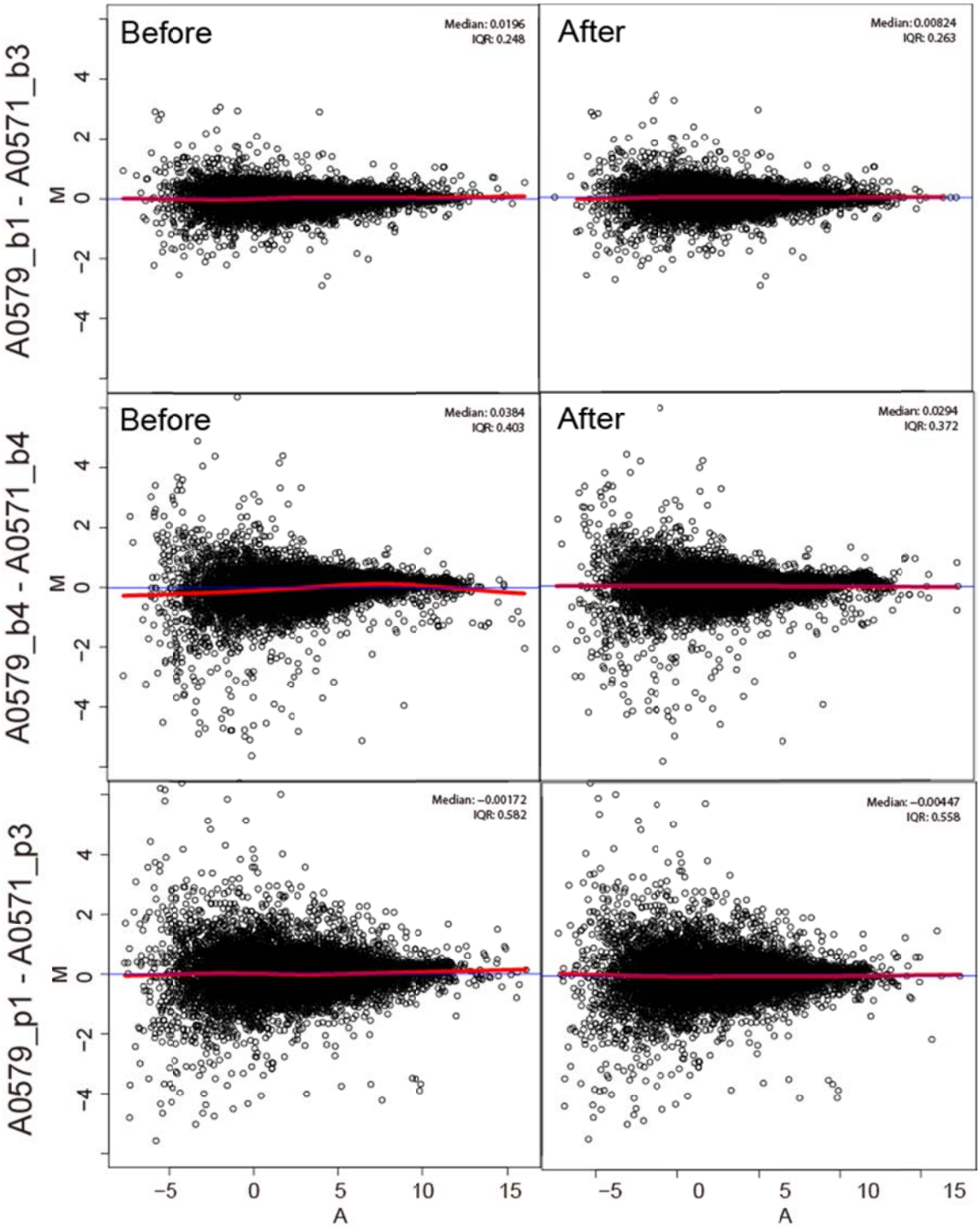
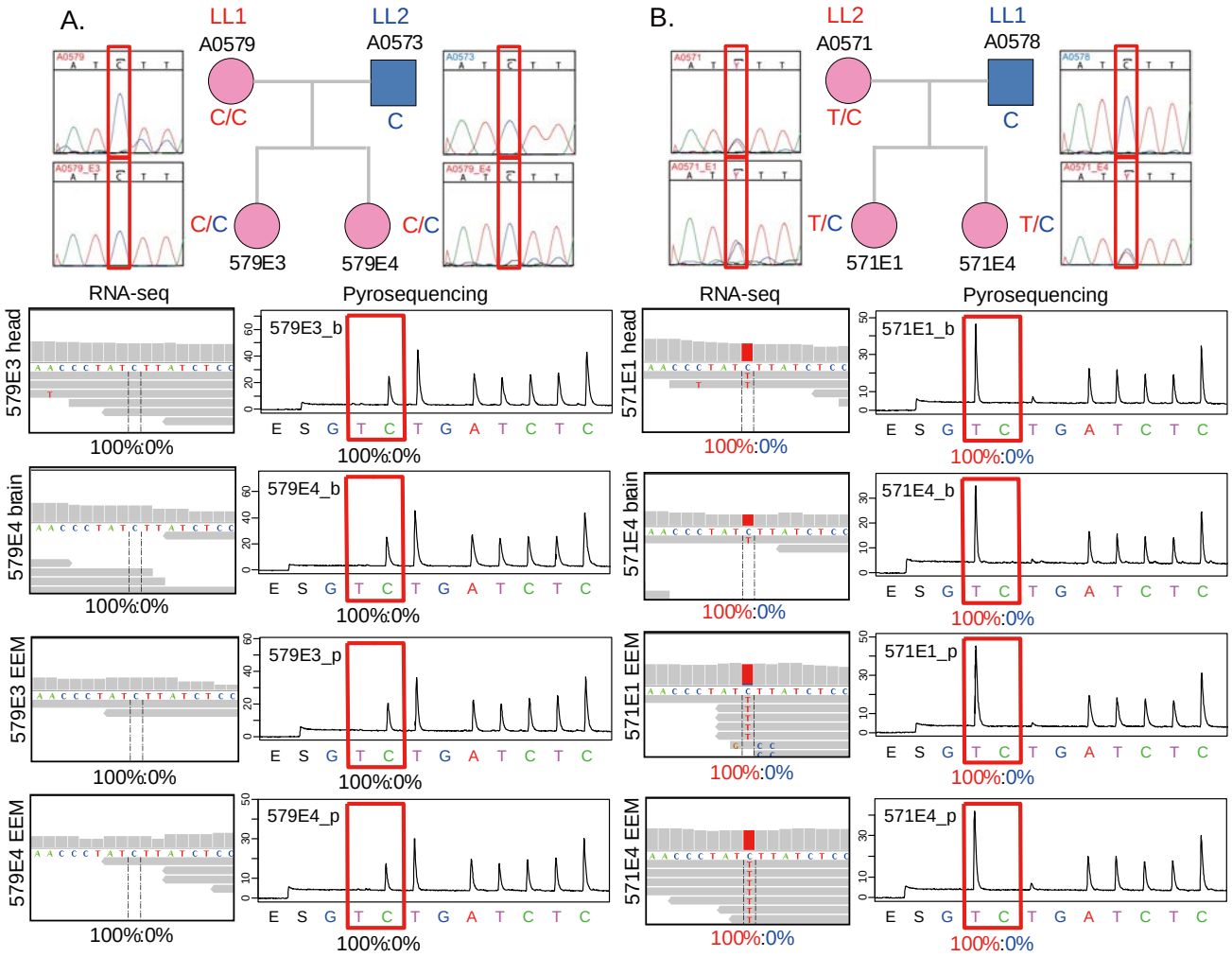
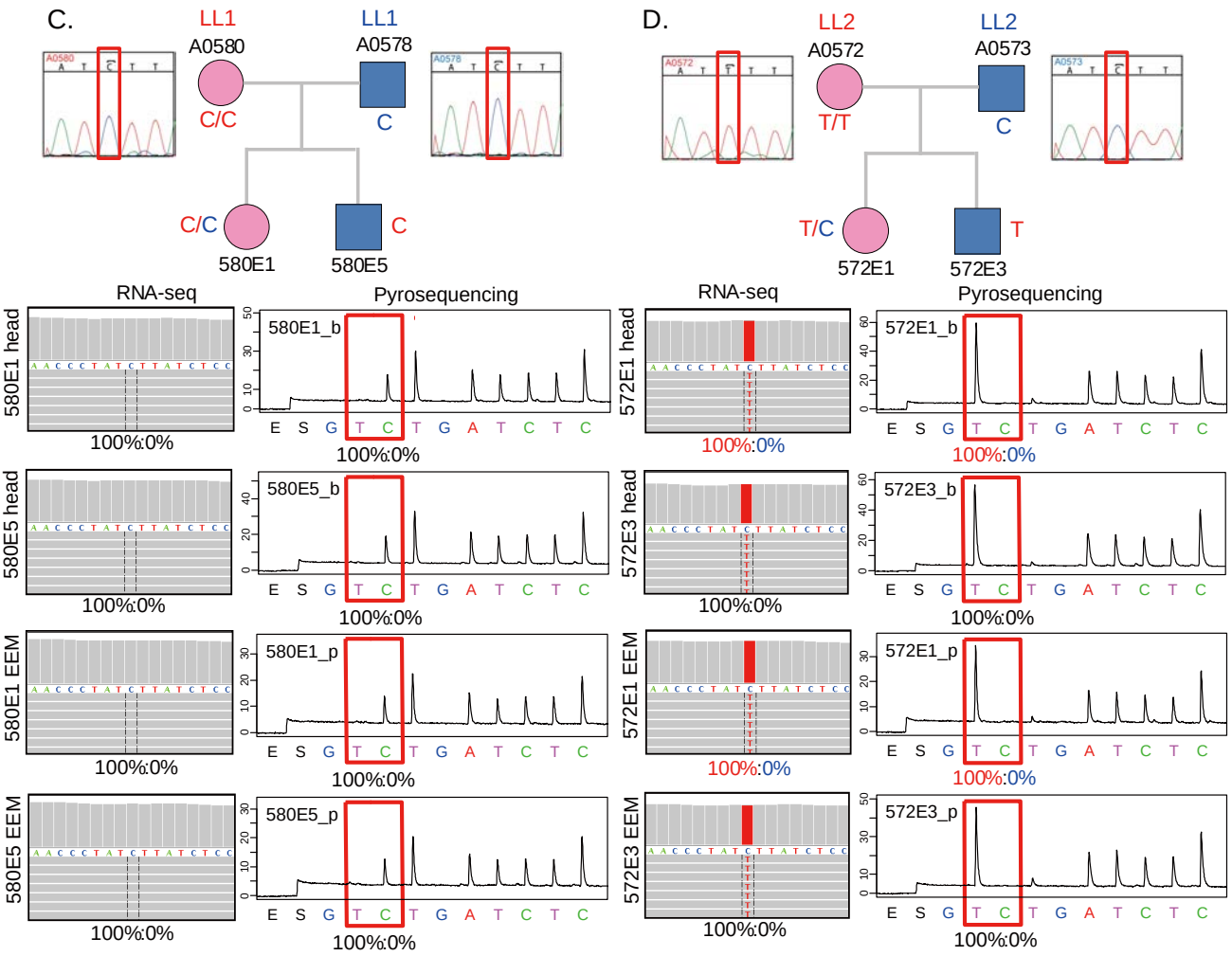


Figure S4. RNA-seq, SNP genotyping and pyrosequencing verification results for non-escaper gene *HPRT1* in opossum fetal brain and EEM samples.

(A) F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0222971) is informative in three embryos (571E1, 571E4 and 572E1). In brain/head and EEM tissues of all three individuals, 100% maternal expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *HPRT1* is subject to imprinted XCI with zero paternal leakage in both tissues. The target sequence for pyrosequencing is (T/C)TTATCTCC.



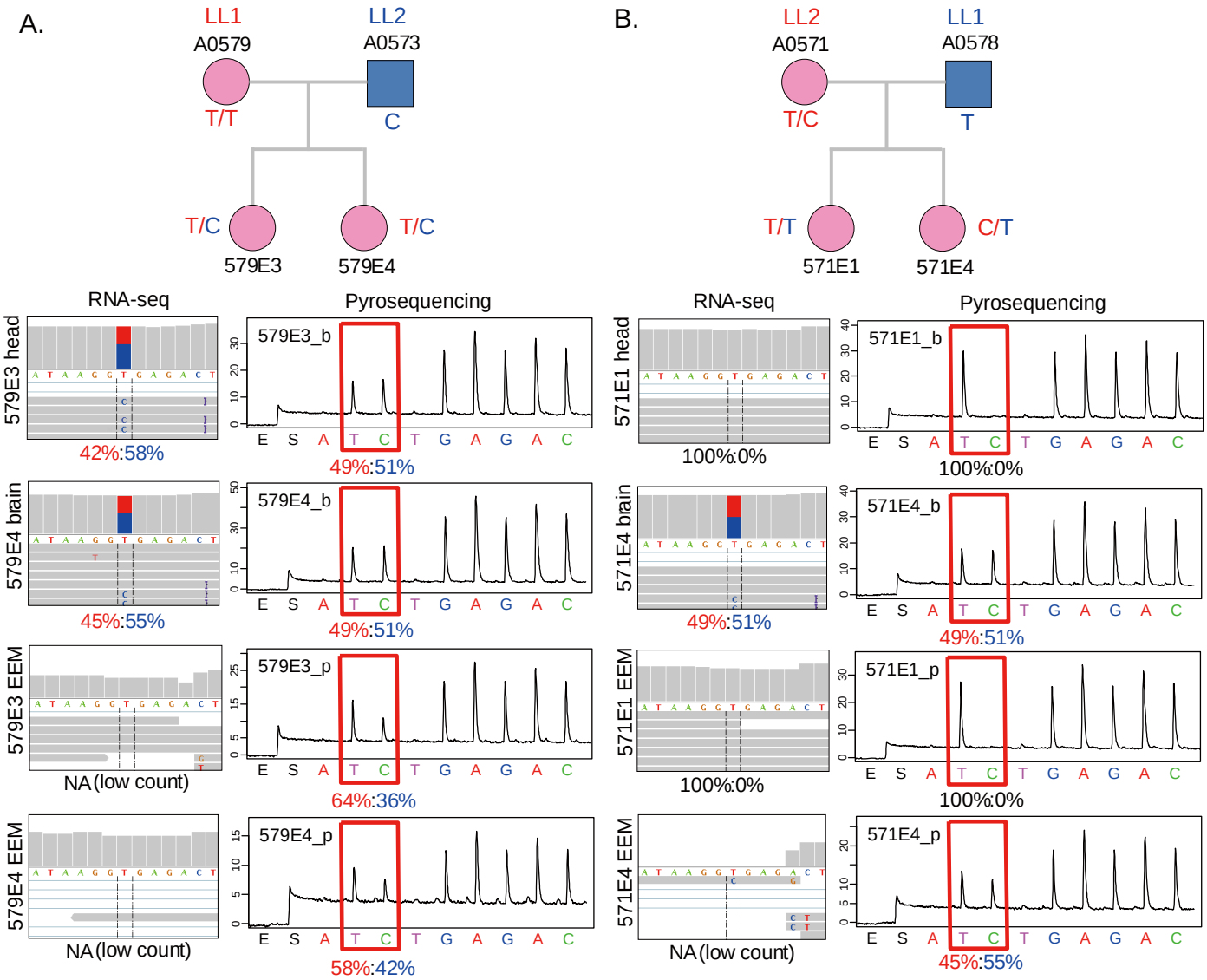
HPRT1 (SNP ID: OMSNP0222971)

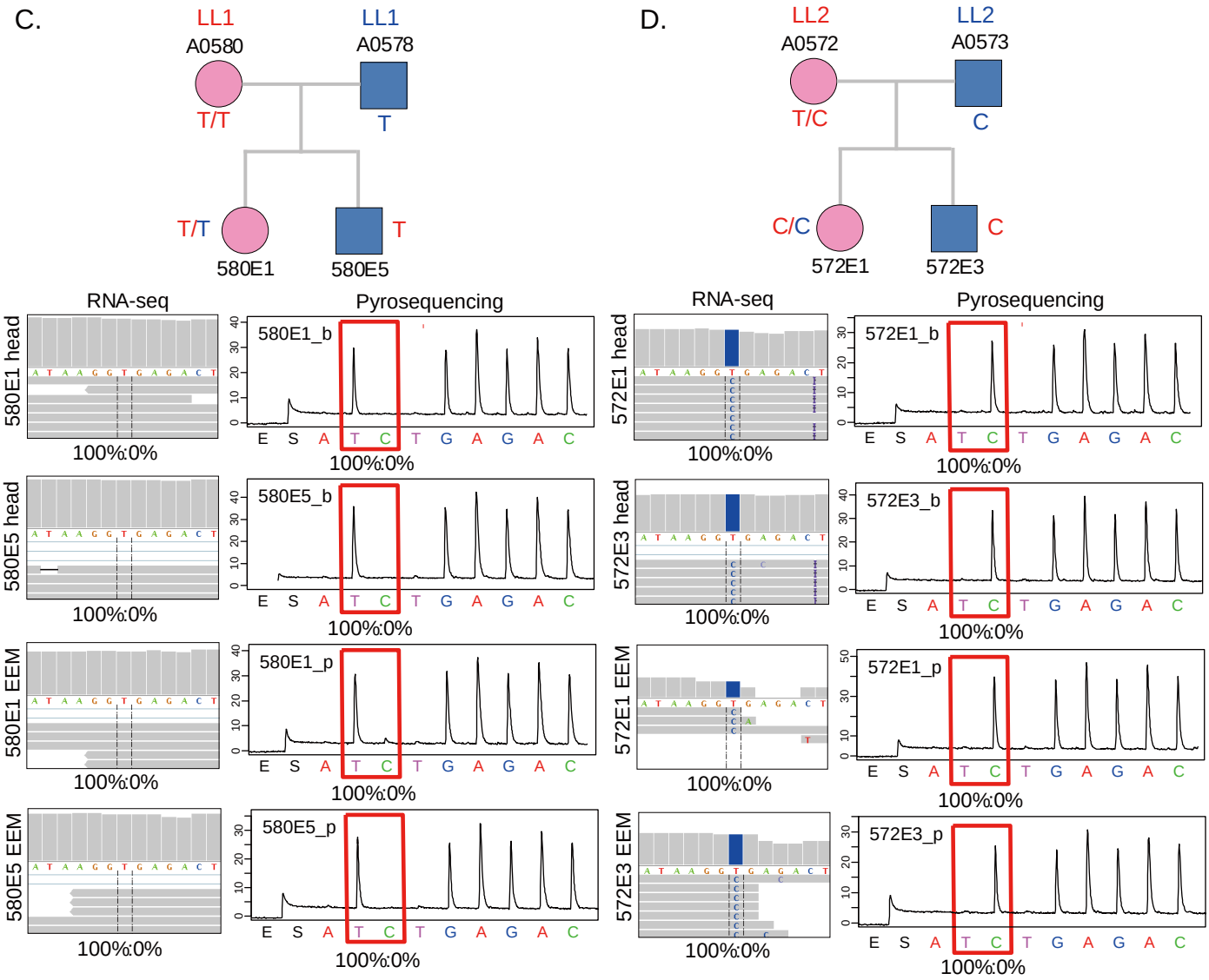


HPRT1 (SNP ID: OMSNP0222971)

Figure S5. RNA-seq, SNP genotyping and pyrosequencing verification results for autosomal control gene *GPM6B* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. *GPM6B* is an autosomal gene in opossum on chromosome 7. From the Sanger sequencing genotyping results, the SNP (chr7_27283330) is informative in three embryos (579E3, 579E4 and 571E4). In brain/head and EEM tissues of all three individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification, which is expected for autosomal genes with two parental alleles. The target sequence for pyrosequencing is (T/C)GAGACT. The Sanger sequencing traces were not shown here because an indel polymorphism in the amplicon shifted the trace, but the genotypes could be determined by the software.

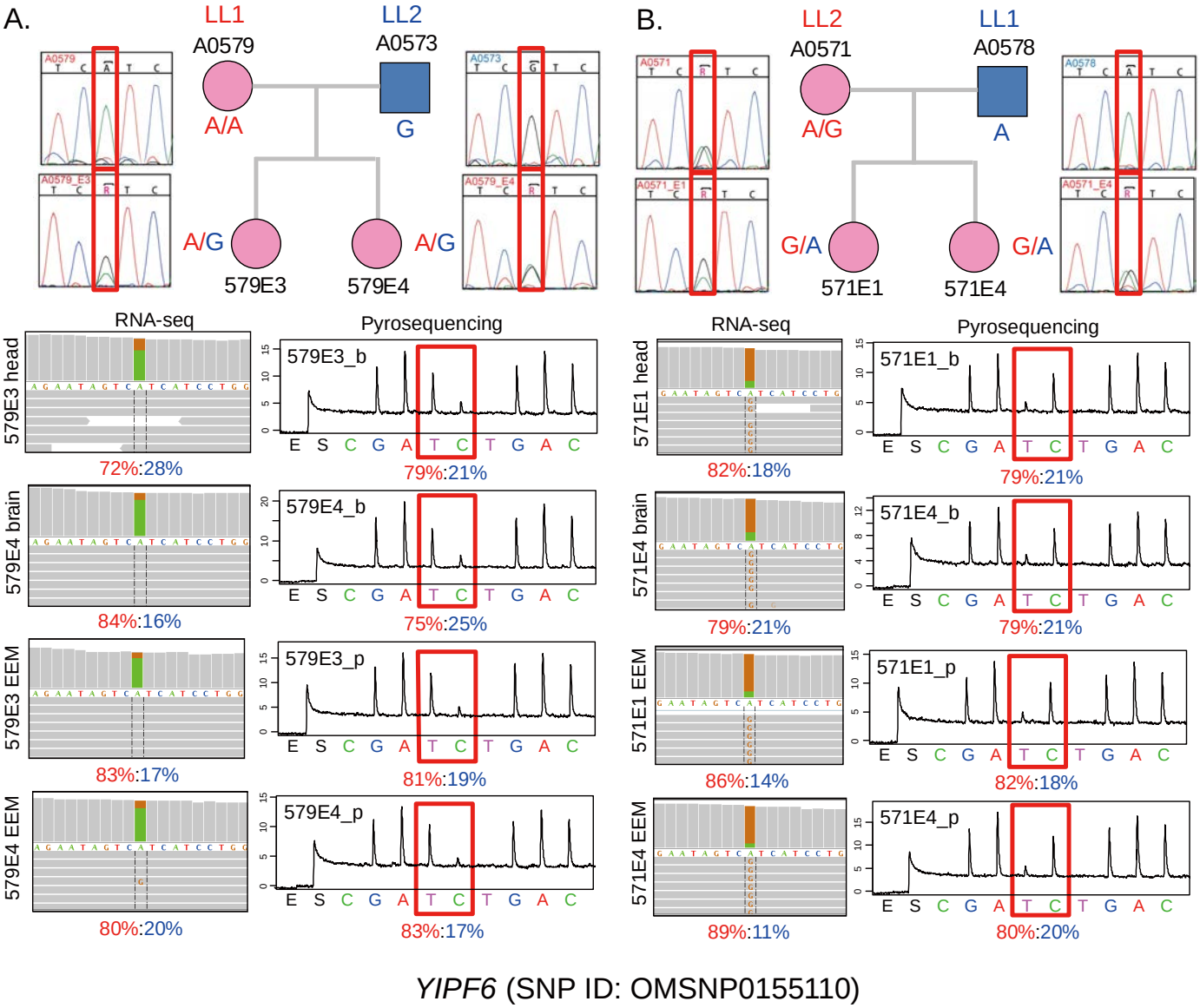


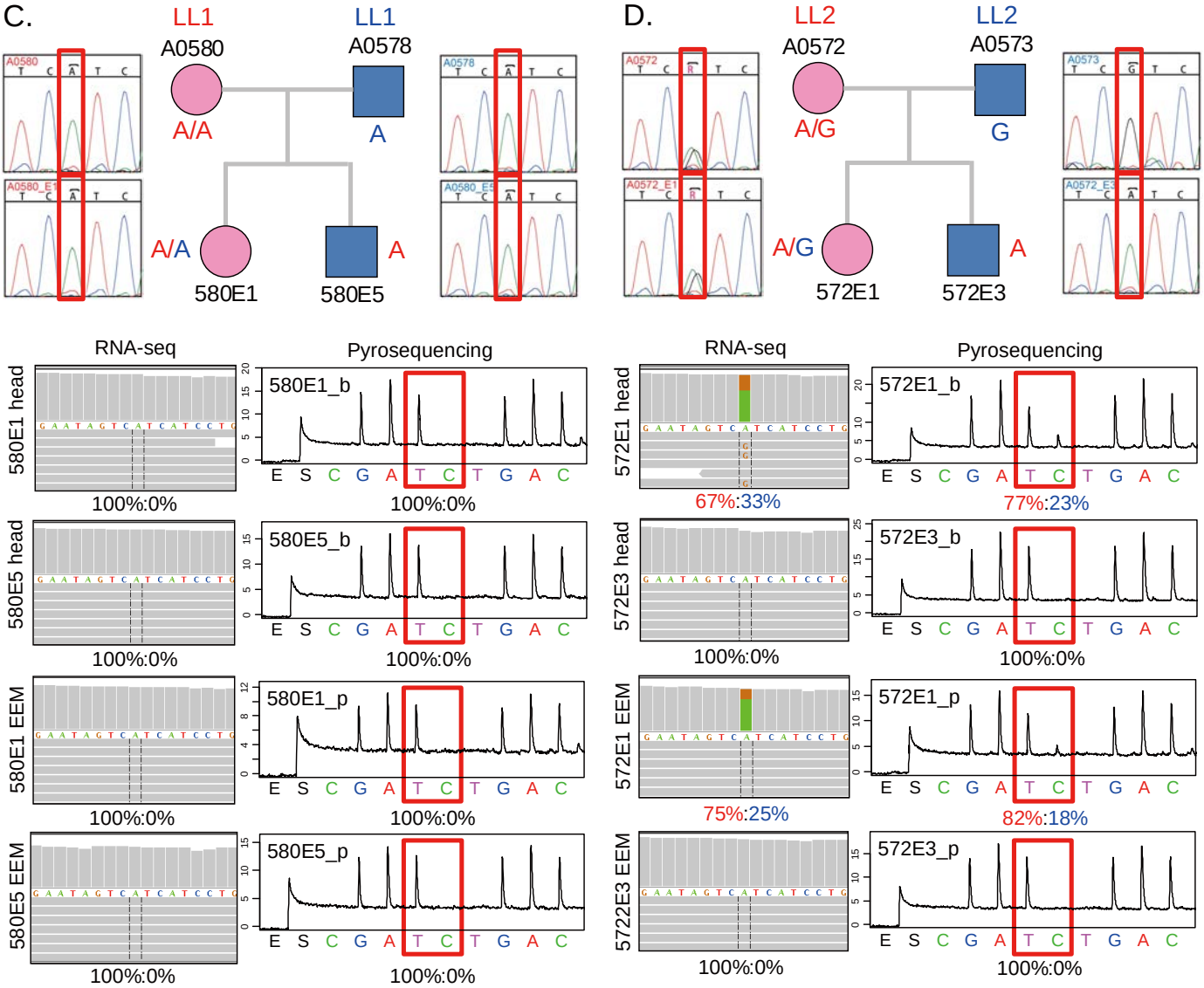


GPM6B (SNP ID: chr7_27283330)

Figure S6. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *YIPF6* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0155110) is informative in five embryos (579E3, 579E4, 571E1, 571E4 and 572E1). In brain/head and EEM tissues of all five individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *YIPF6* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is GA(T/C)GACTA (on the opposite strand).

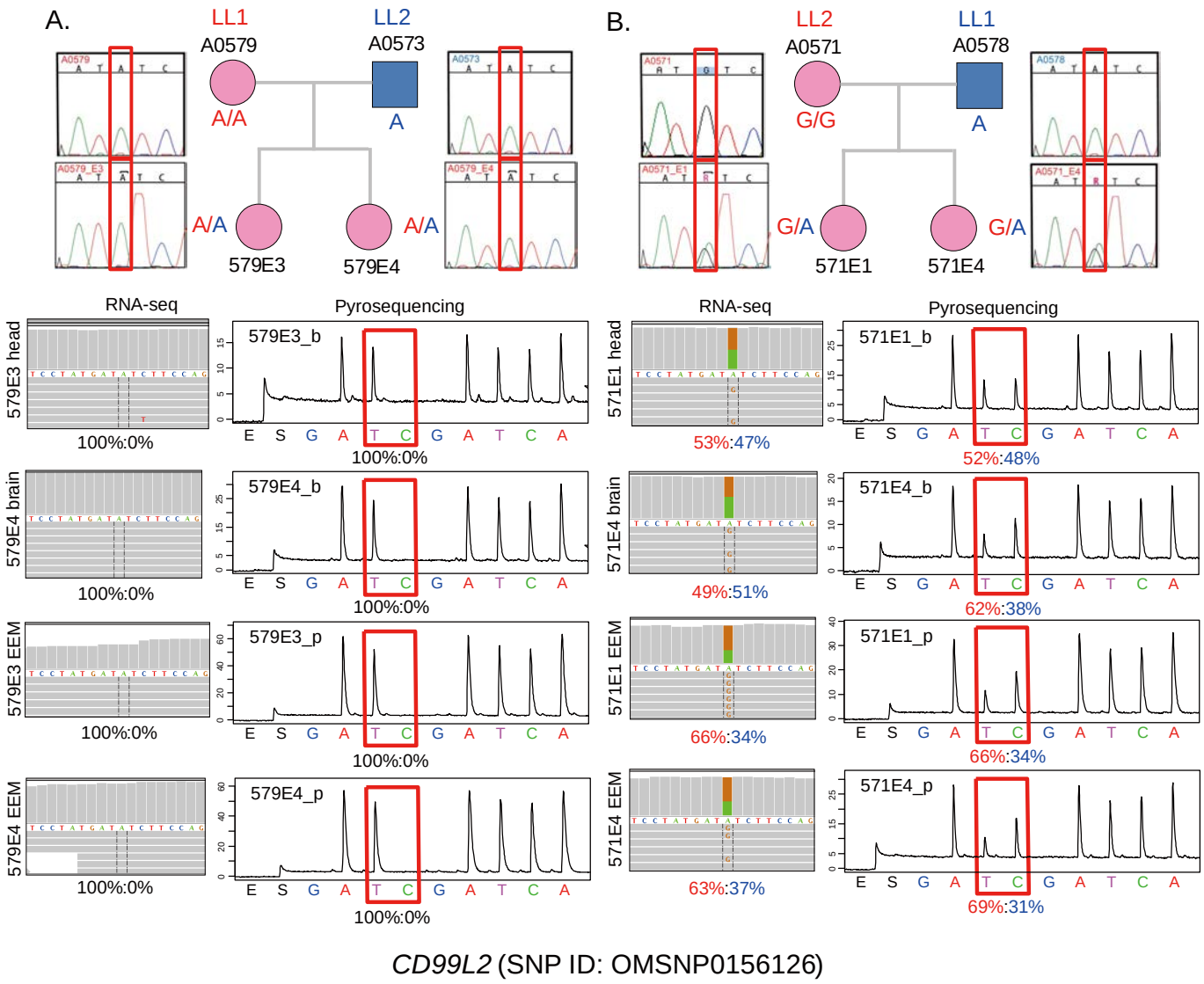


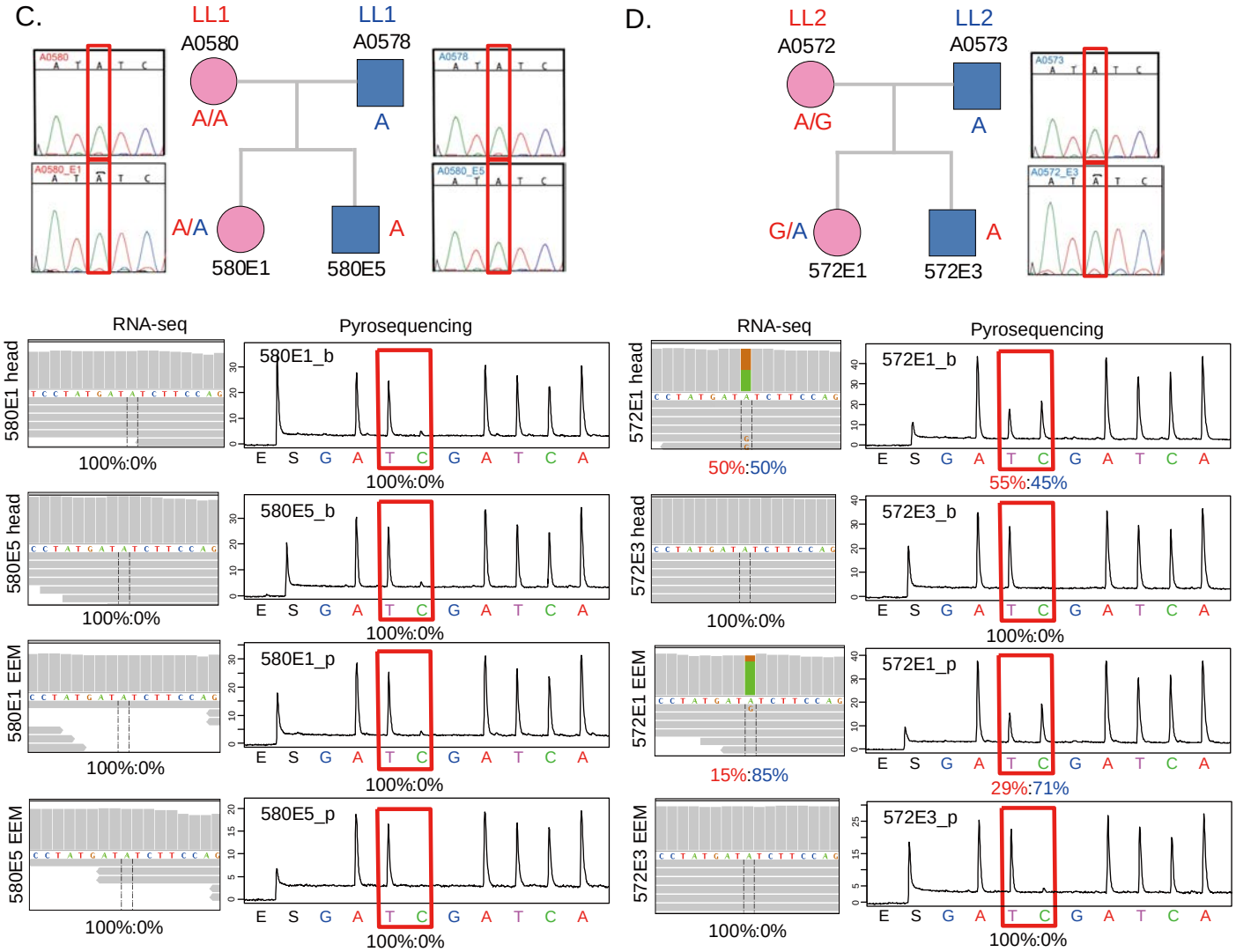


YIPF6 (SNP ID: OMSNP0155110)

Figure S7. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *CD99L2* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0156126) is informative in three embryos (571E1, 571E4 and 572E1). In brain/head and EEM tissues of all three individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *CD99L2* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is A(T/C)ATCATAG (on the opposite strand).

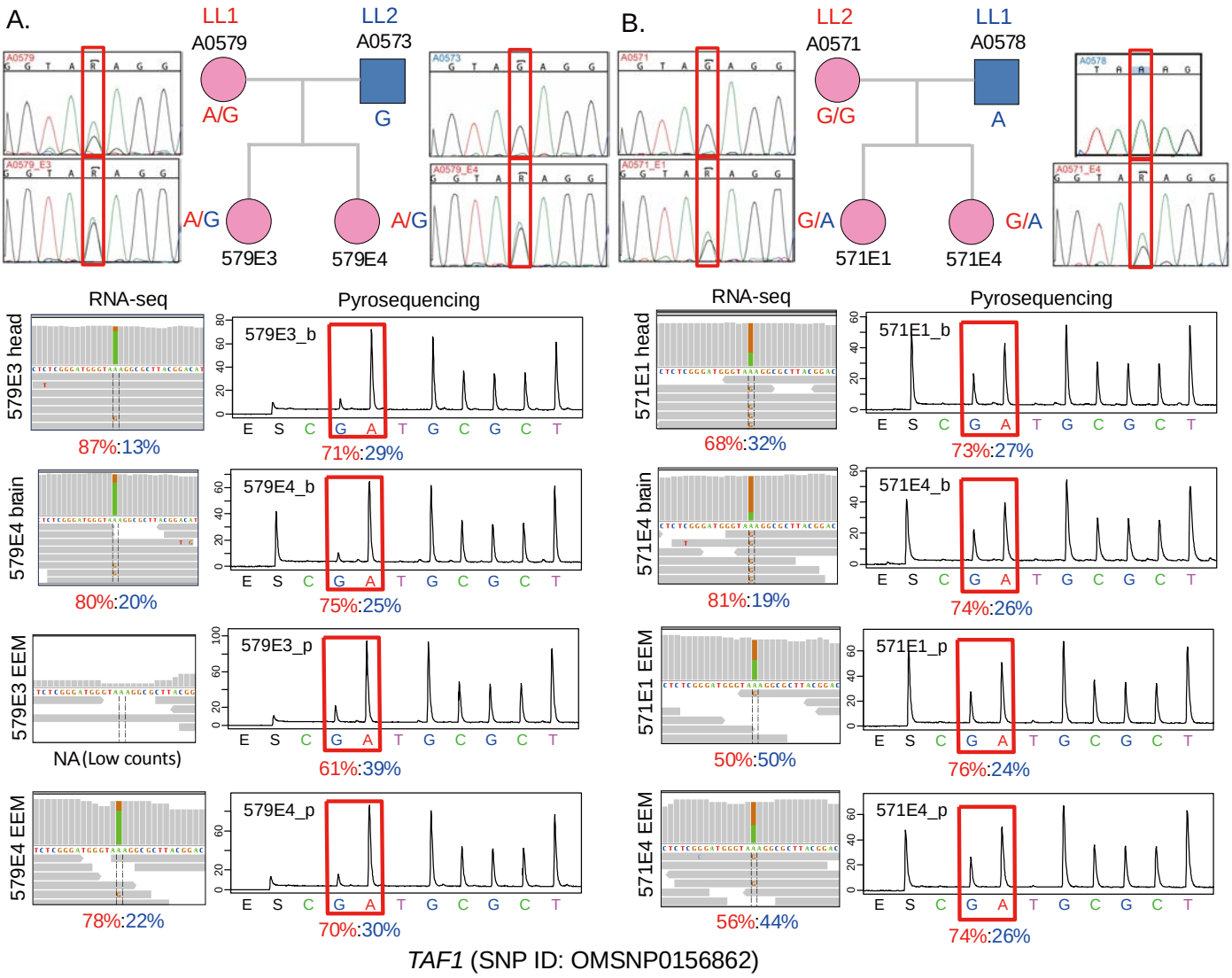




CD99L2 (SNP ID: OMSNP0156126)

Figure S8. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *TAF1* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0156862) is informative in five embryos (579E3, 579E4, 571E1, 571E4 and 580E1). In brain/head and EEM tissues of all five individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *TAF1* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is (A/G)AGGCGCTTA.



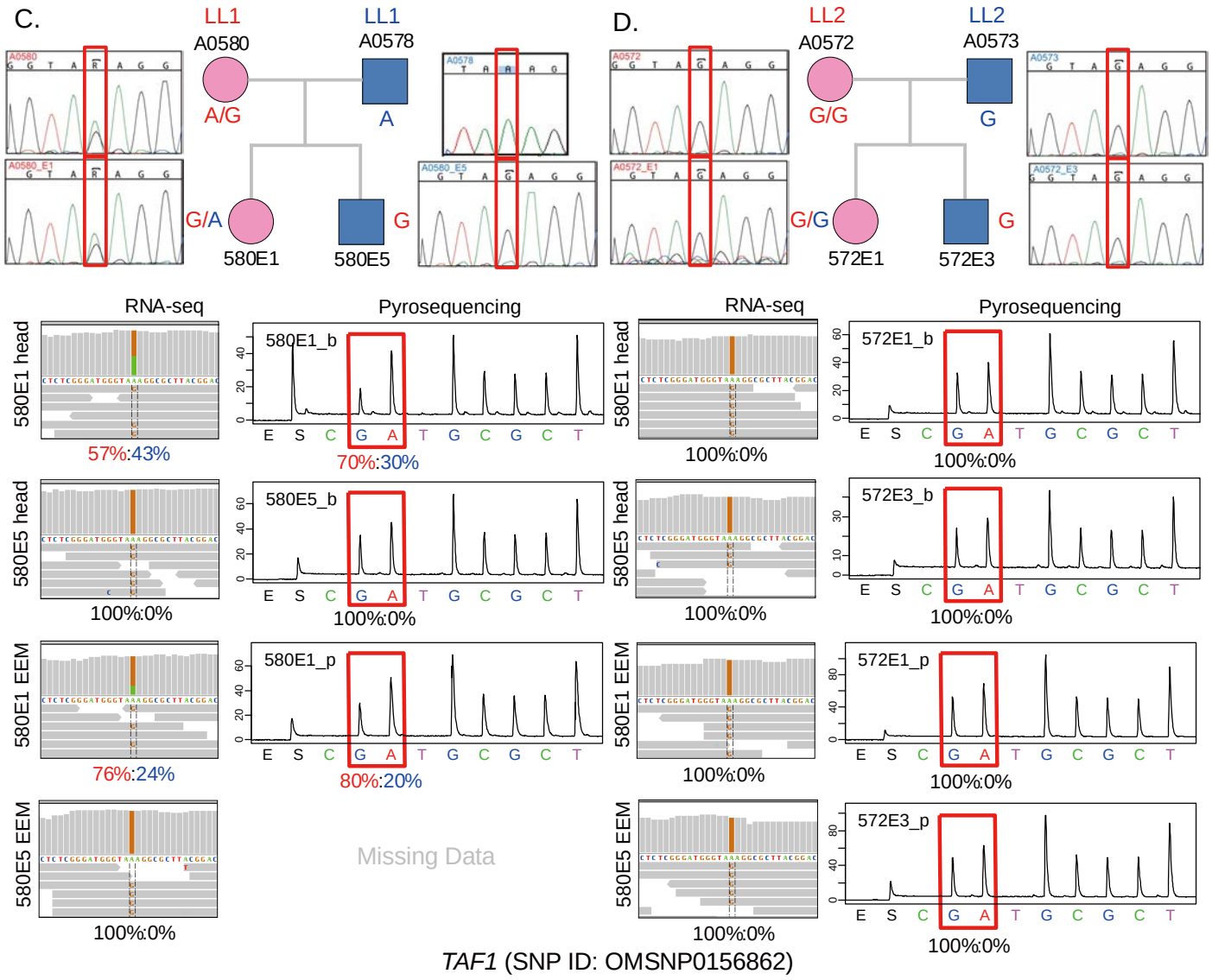
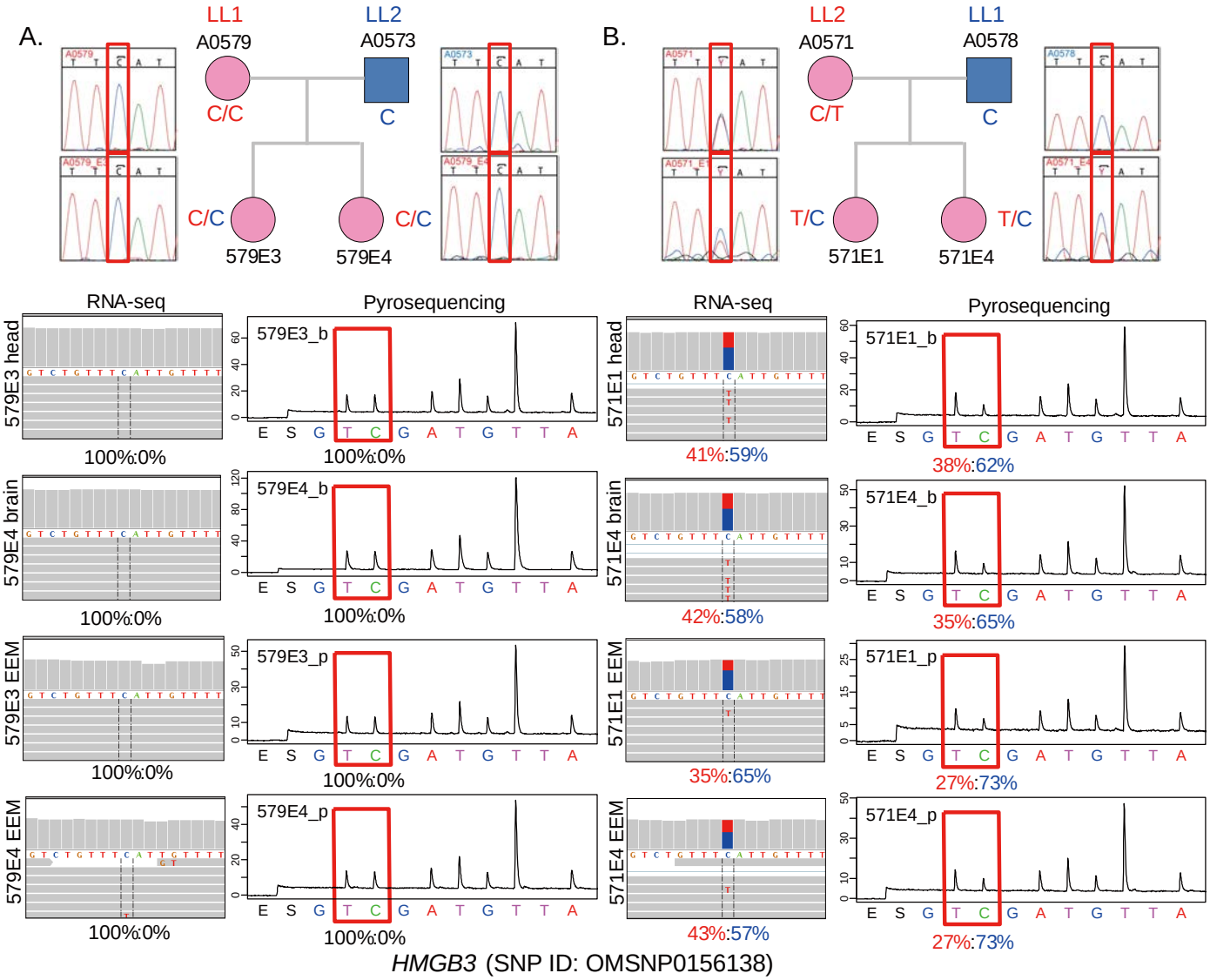

C.
LL1
A0580
LL1
A0578
A/G
A
G/A
G
580E1
580E5
D.
LL2
A0572
LL2
A0573
G/G
G
G/G
G
572E1
572E3
RNA-seq
Pyrosequencing
580E1 head
580E1_b
57%:43%
70%:30%
100%:0%
572E1_b
100%:0%
580E5 head
580E5_b
100%:0%
100%:0%
572E3_b
100%:0%
100%:0%
580E1 EEM
580E1_p
76%:24%
80%:20%
100%:0%
572E1_p
100%:0%
580E5 EEM
Missing Data
100%:0%
572E3_p
100%:0%
E S C G A T G C G C T
TAF1 (SNP ID: OMSNP0156862)

Figure S9. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *HMGB3* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0156138) is informative in three embryos (571E1, 571E4 and 572E1). In brain/head and EEM tissues of all three individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *HMGB3* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is T(C/T)ATTGTTTTTTACC.



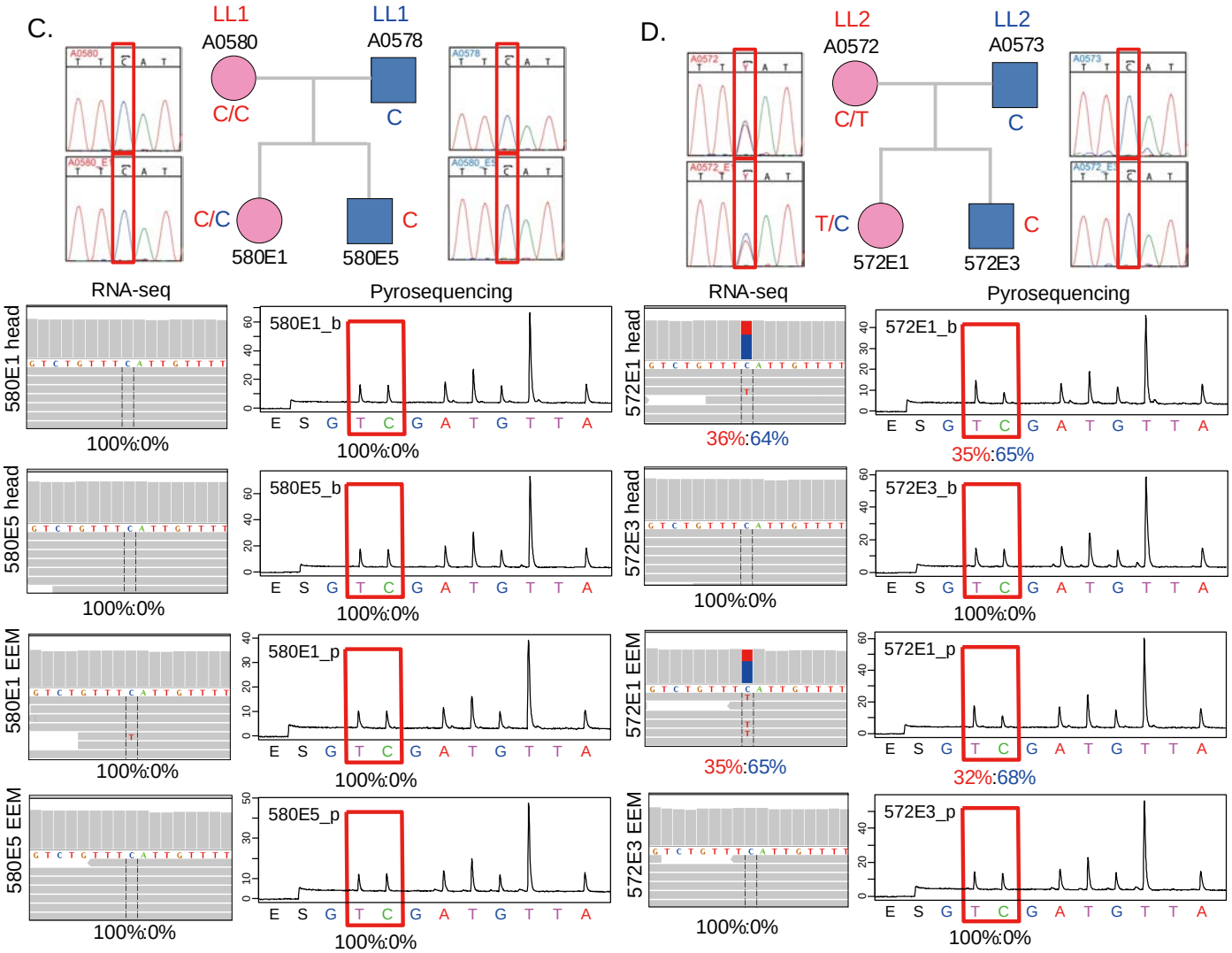
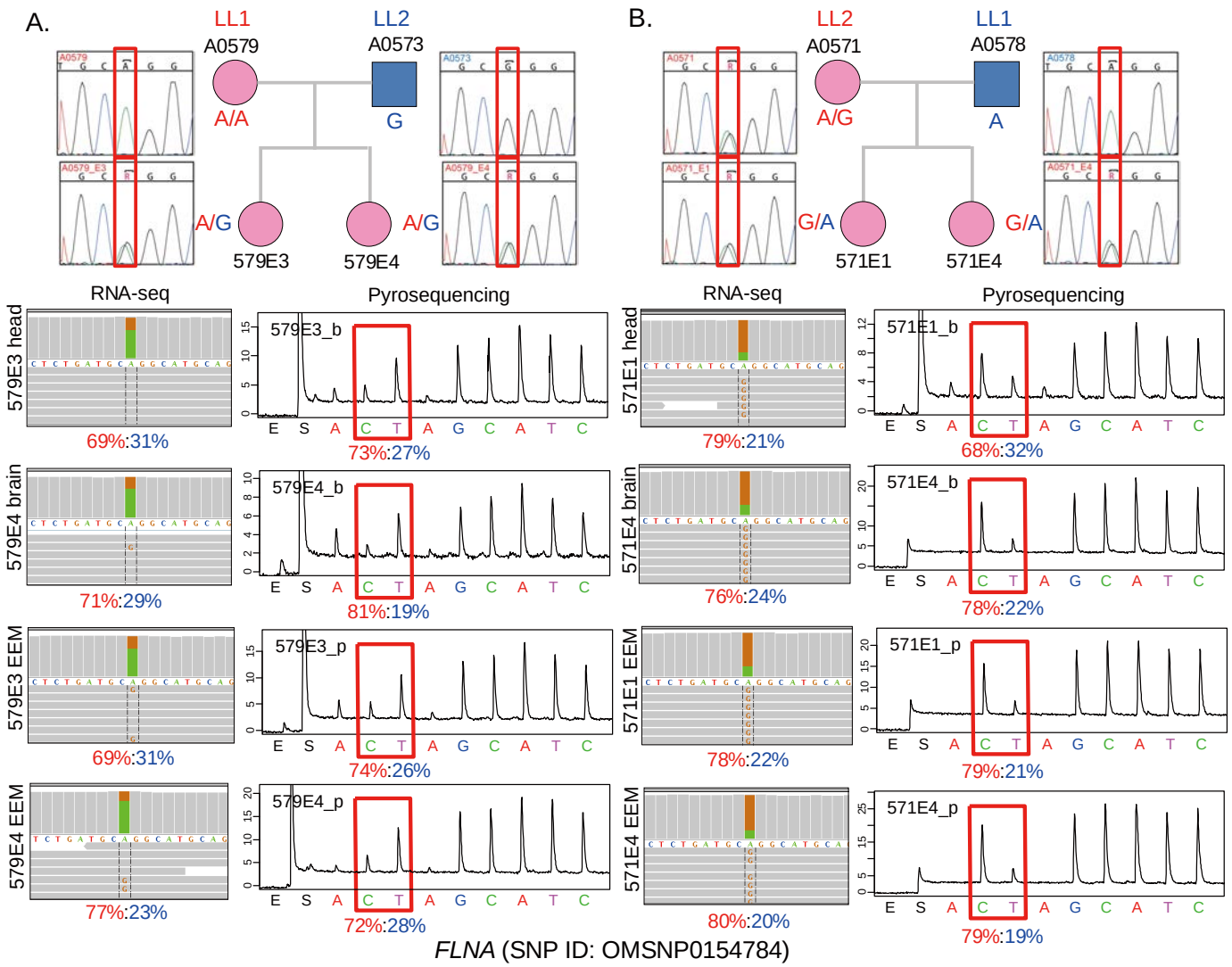
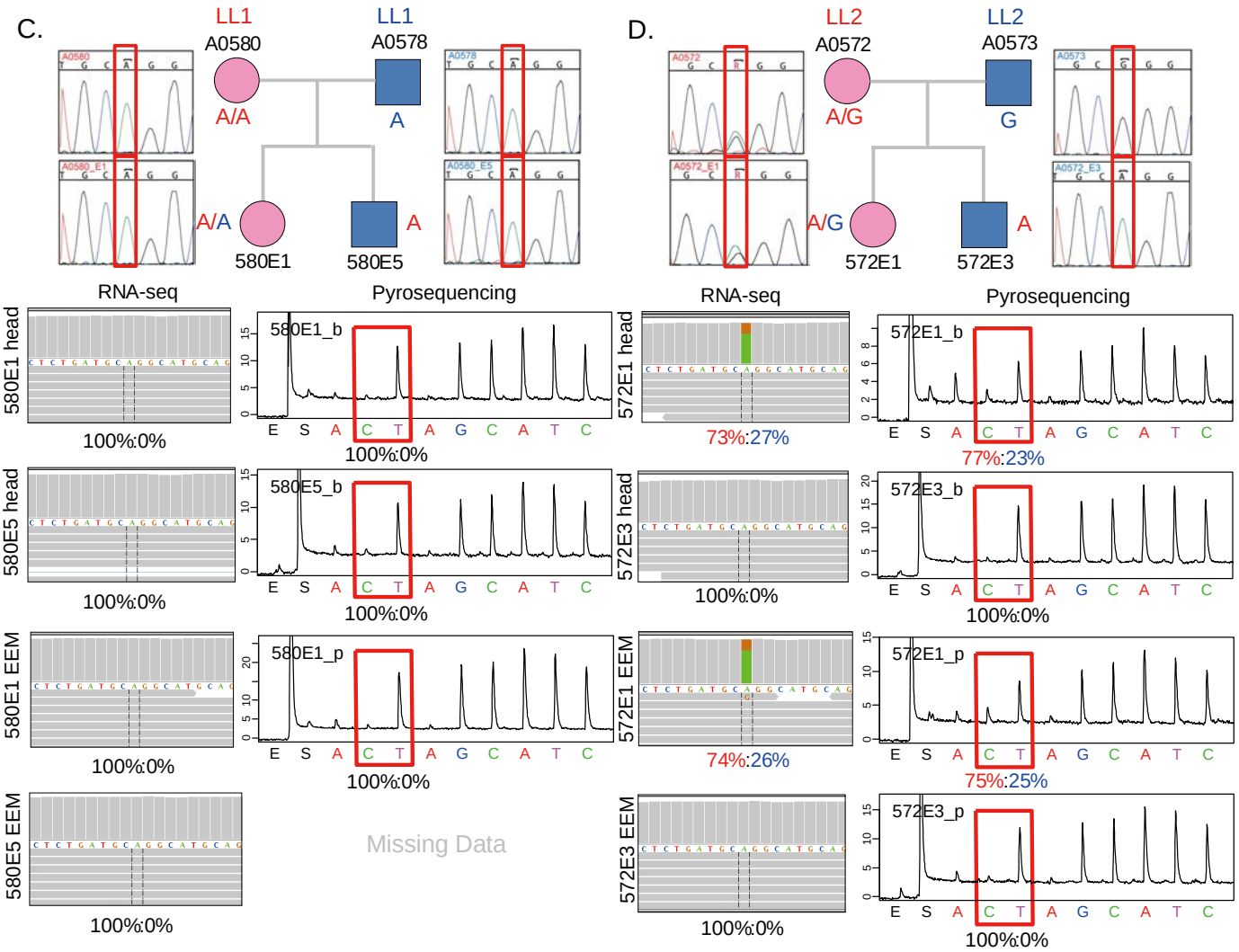

C.
LL1
A0580
LL1
A0578
C/C
C
C/C
C
580E1
580E5
D.
LL2
A0572
LL2
A0573
C/T
C
T/C
C
572E1
572E3
RNA-seq
Pyrosequencing
RNA-seq
Pyrosequencing
580E1 head
580E1_b
100%:0%
100%:0%
572E1 head
572E1_b
36%:64%
35%:65%
580E5 head
580E5_b
100%:0%
100%:0%
572E3 head
572E3_b
100%:0%
100%:0%
580E1 EEM
580E1_p
100%:0%
100%:0%
572E1 EEM
572E1_p
35%:65%
32%:68%
580E5 EEM
580E5_p
100%:0%
100%:0%
572E3 EEM
572E3_p
100%:0%
100%:0%
E S G T C G A T G T T A
HMGB3 (SNP ID: OMSNP0156138)

Figure S10. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *FLNA* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0154784) is informative in five embryos (579E3, 579E4, 571E1, 571E4 and 572E1). In brain/head and EEM tissues of all five individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *FLNA* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is (C/T)GCATCAGA (on the minus strand).

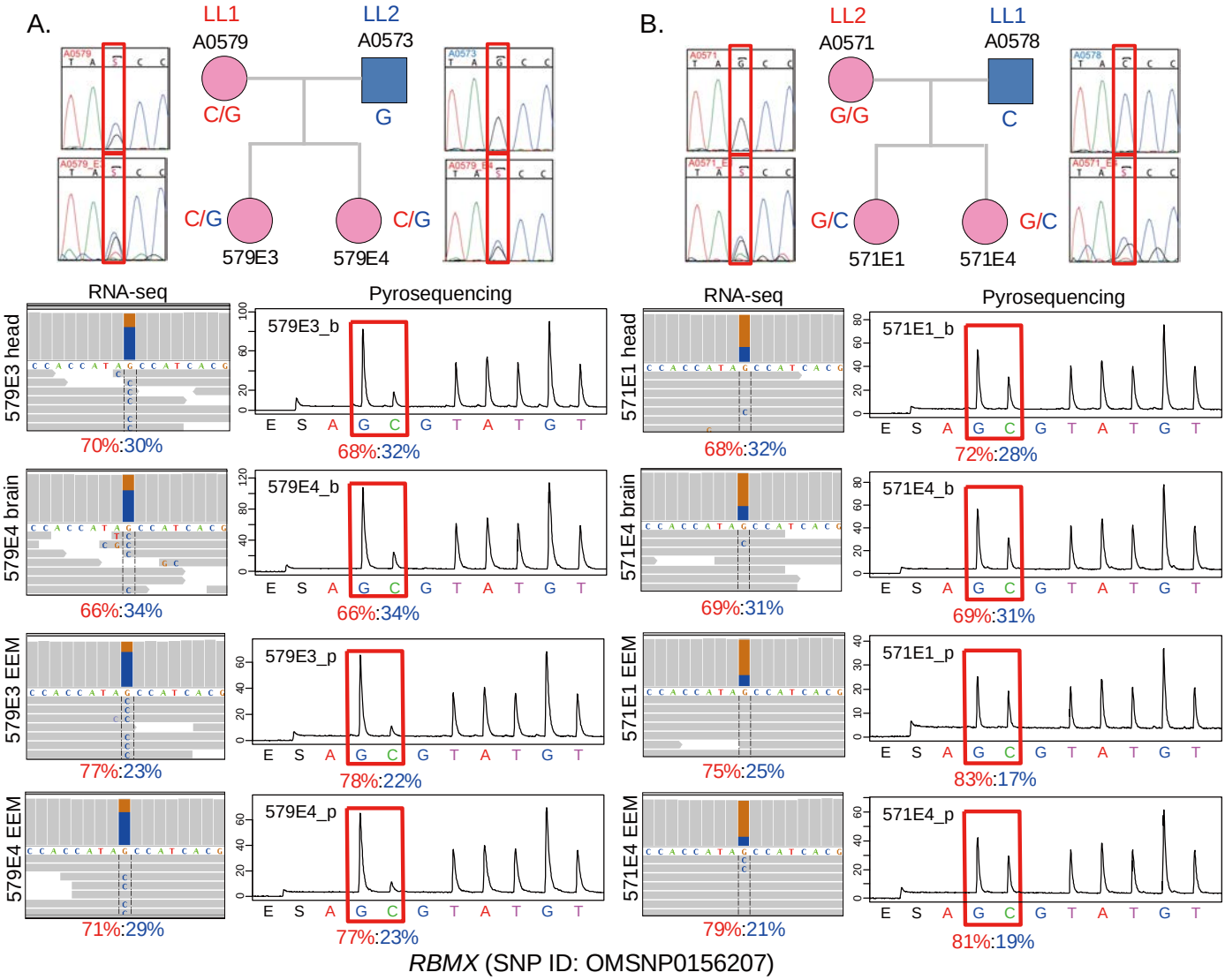


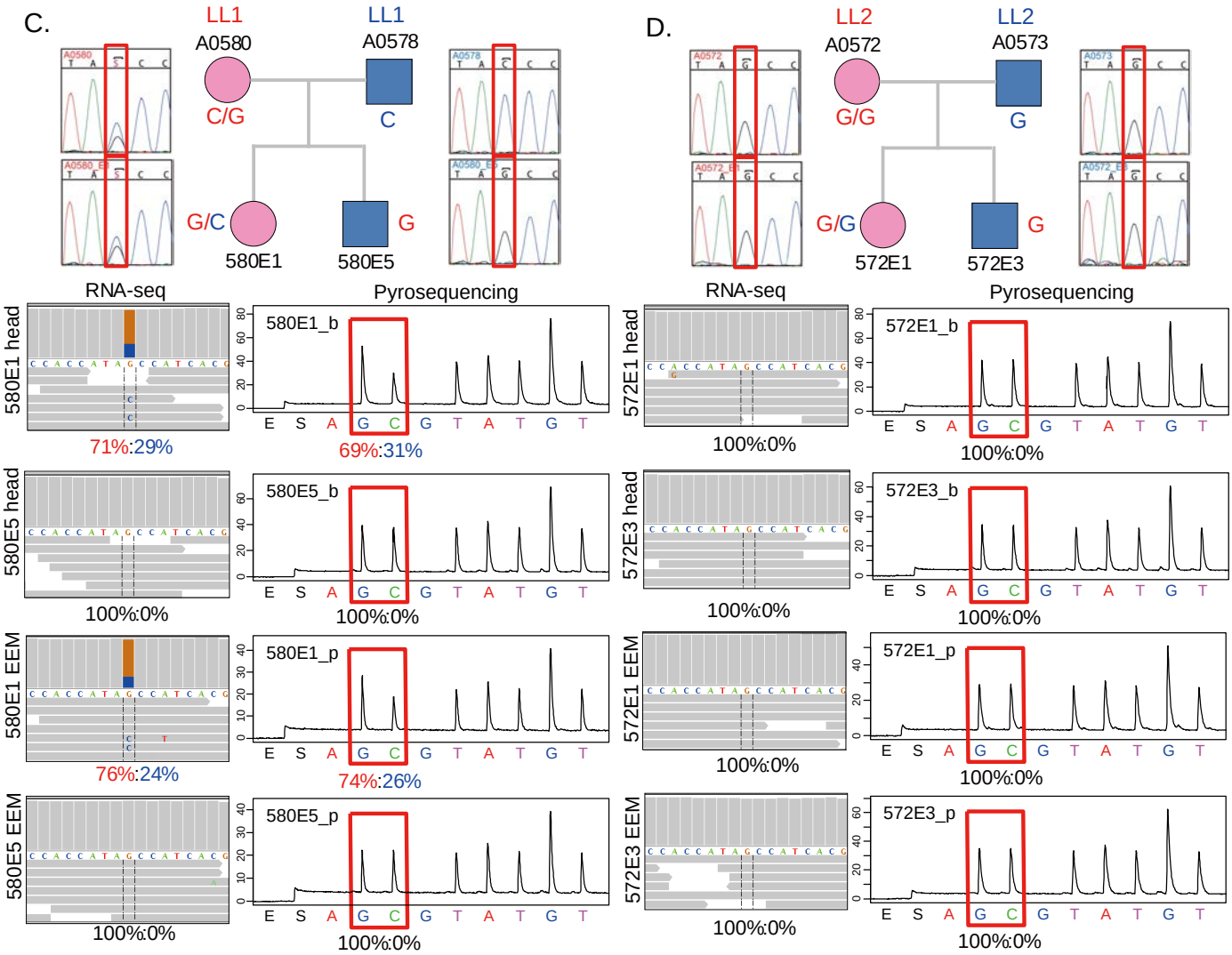


FLNA (SNP ID: OMSNP0154784)

Figure S11. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *RBMX* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0156027) is informative in five embryos (579E3, 579E4, 571E1, 571E4 and 580E1). In brain/head and EEM tissues of all five individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *RBMX* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is G(C/G)TATGGTGGT (on the minus strand).

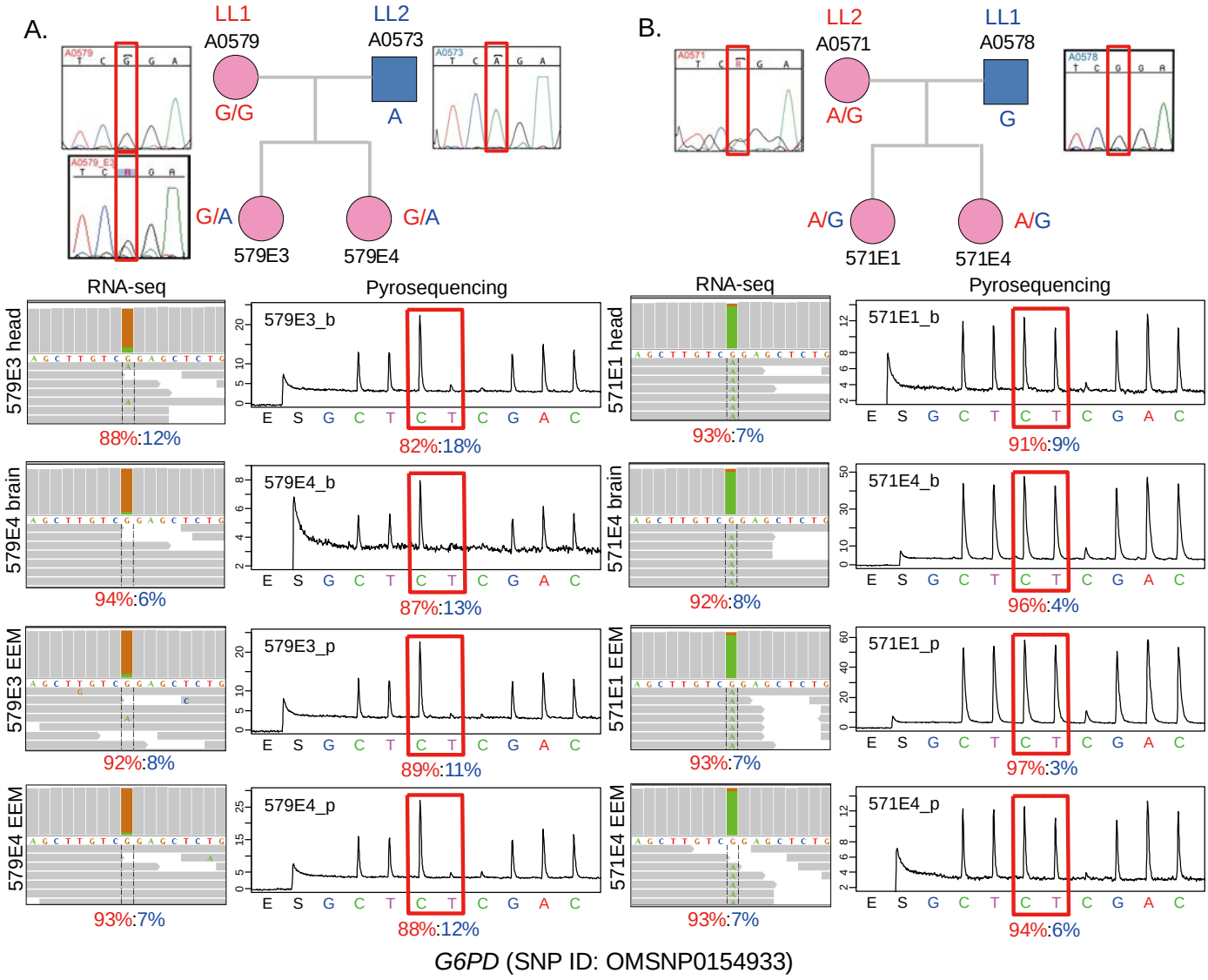


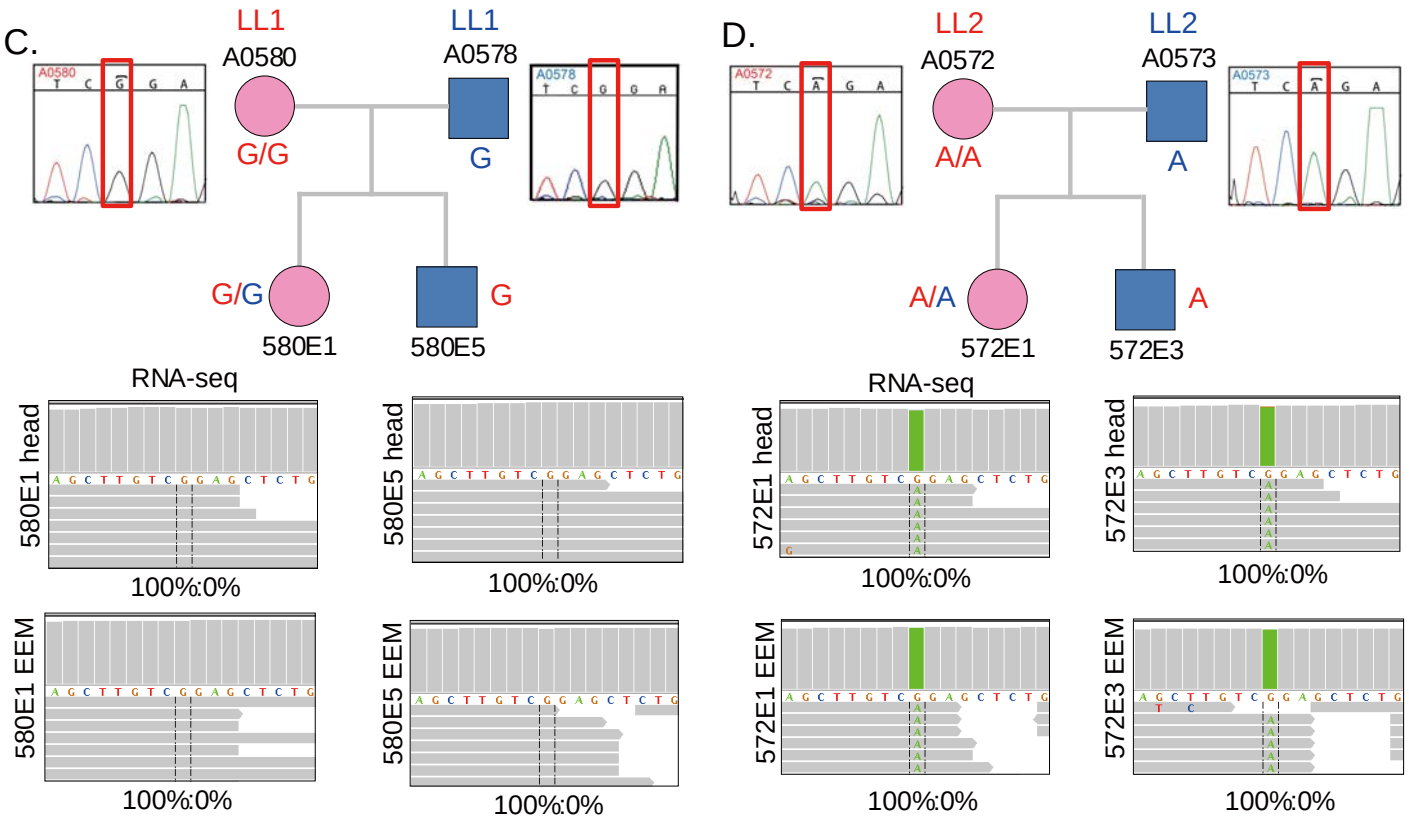


RBMX (SNP ID: OMSNP0156207)

Figure S12. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *G6PD* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0154933) is informative in four embryos (579E3, 579E4, 571E1 and 571E4). In brain/head and EEM tissues of all four individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *G6PD* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is CTC(C/T)GACAAG (on the minus strand). Pyrosequencing was not performed for parental crosses because they are not informative (C and D).

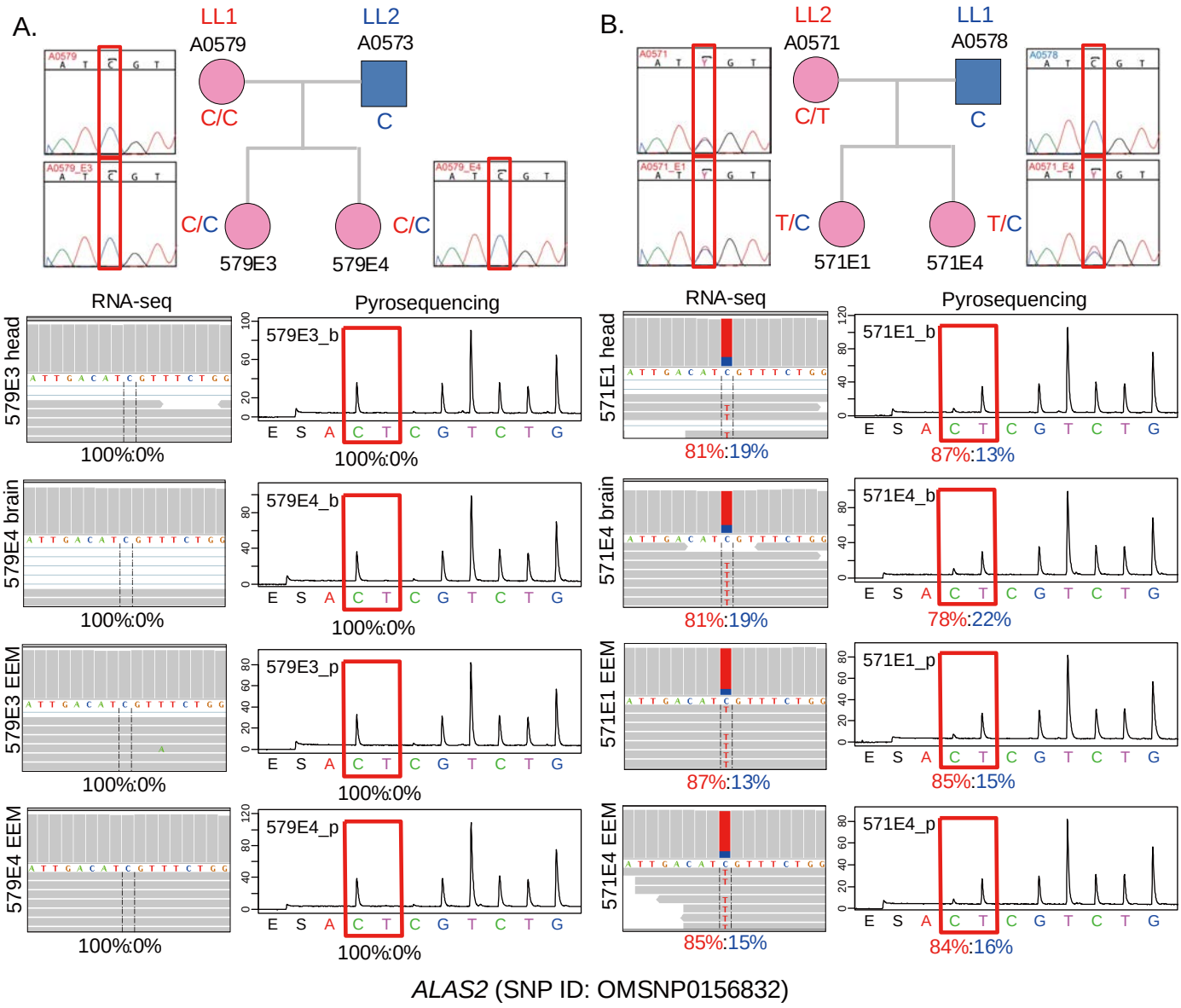


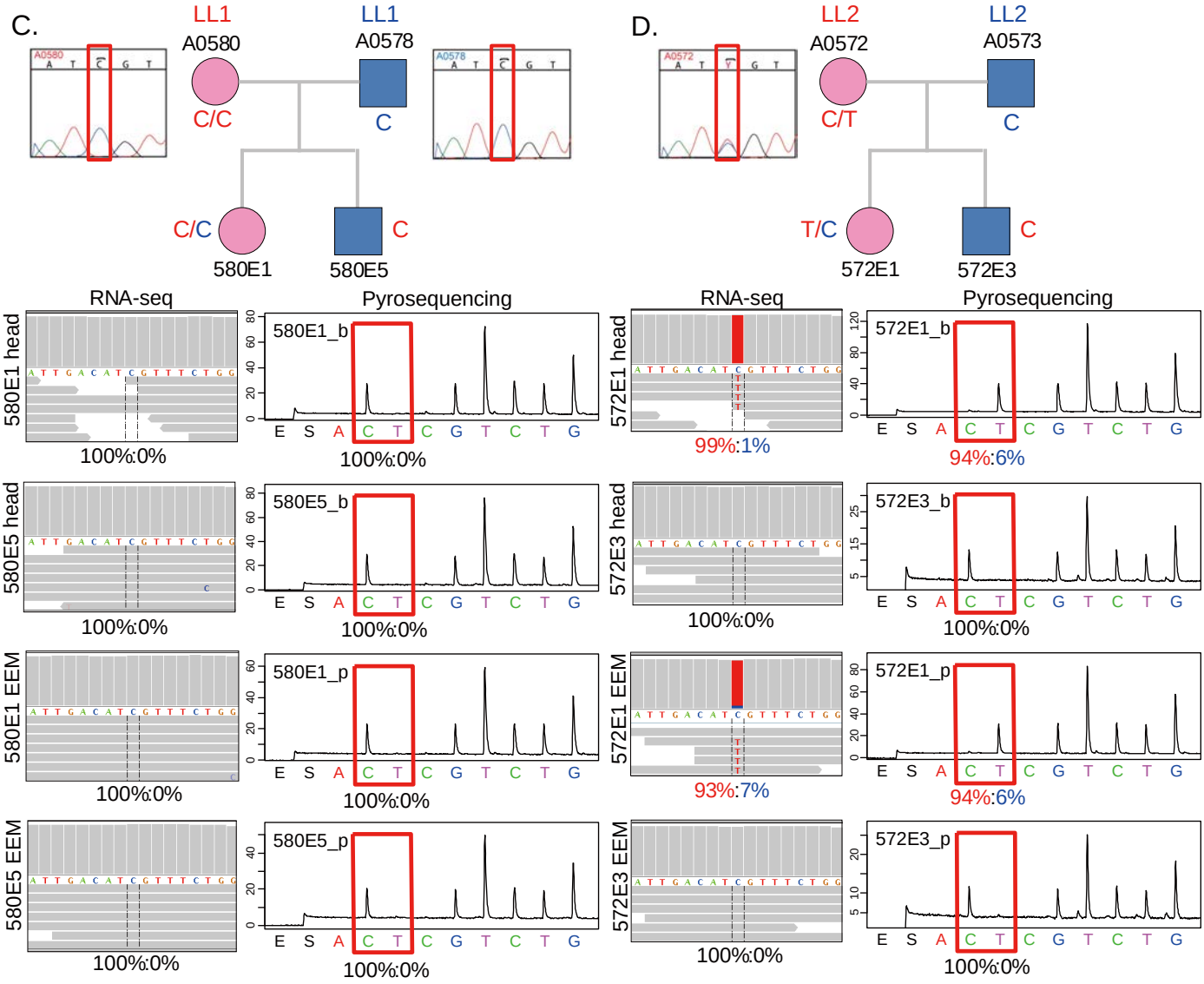


G6PD (SNP ID: OMSNP0154933)

Figure S13. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *ALAS2* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0156832) is informative in three embryos (571E1, 571E4 and 572E1). In brain/head and EEM tissues of all three individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification (572E1 has less than 10% paternal expression). Therefore, *ALAS2* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is (C/T)GTTTCTGGAA.

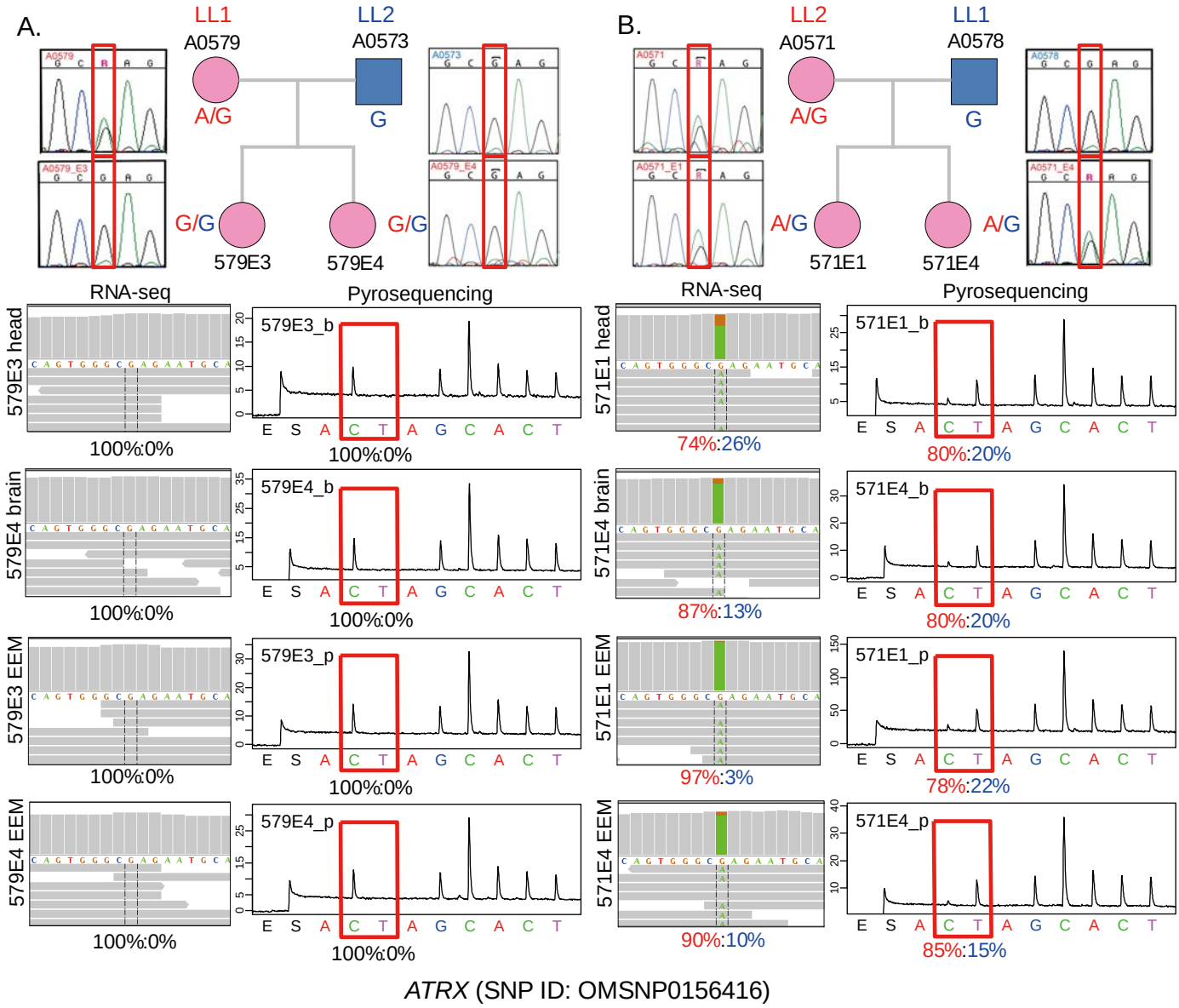


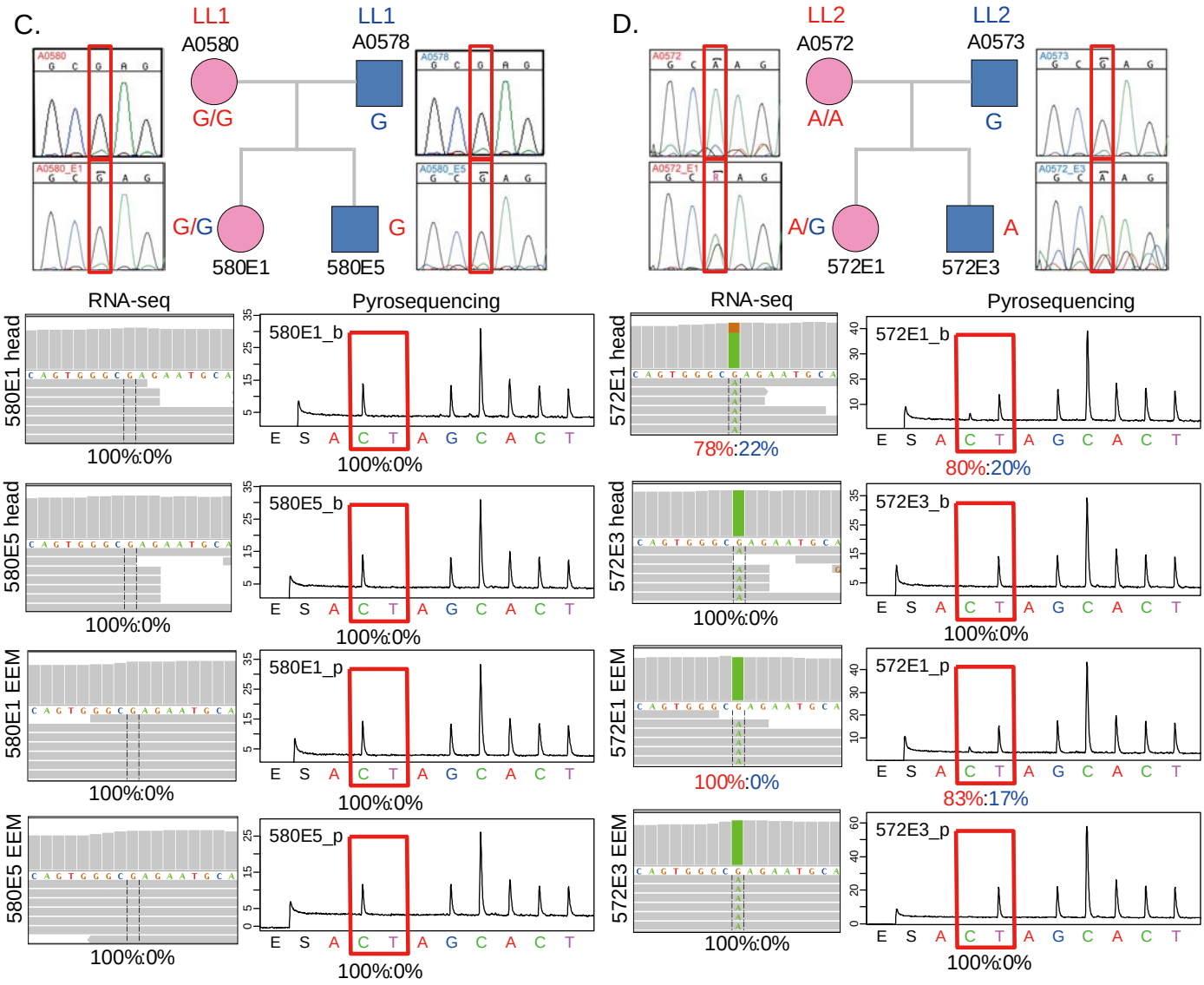


ALAS2 (SNP ID: OMSNP0156832)

Figure S14. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *ATRX* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0156416) is informative in three embryos (571E1, 571E4 and 572E1). In brain/head and EEM tissues of all three individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *ATRX* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is (C/T)GCCCACTGC (on the minus strand).

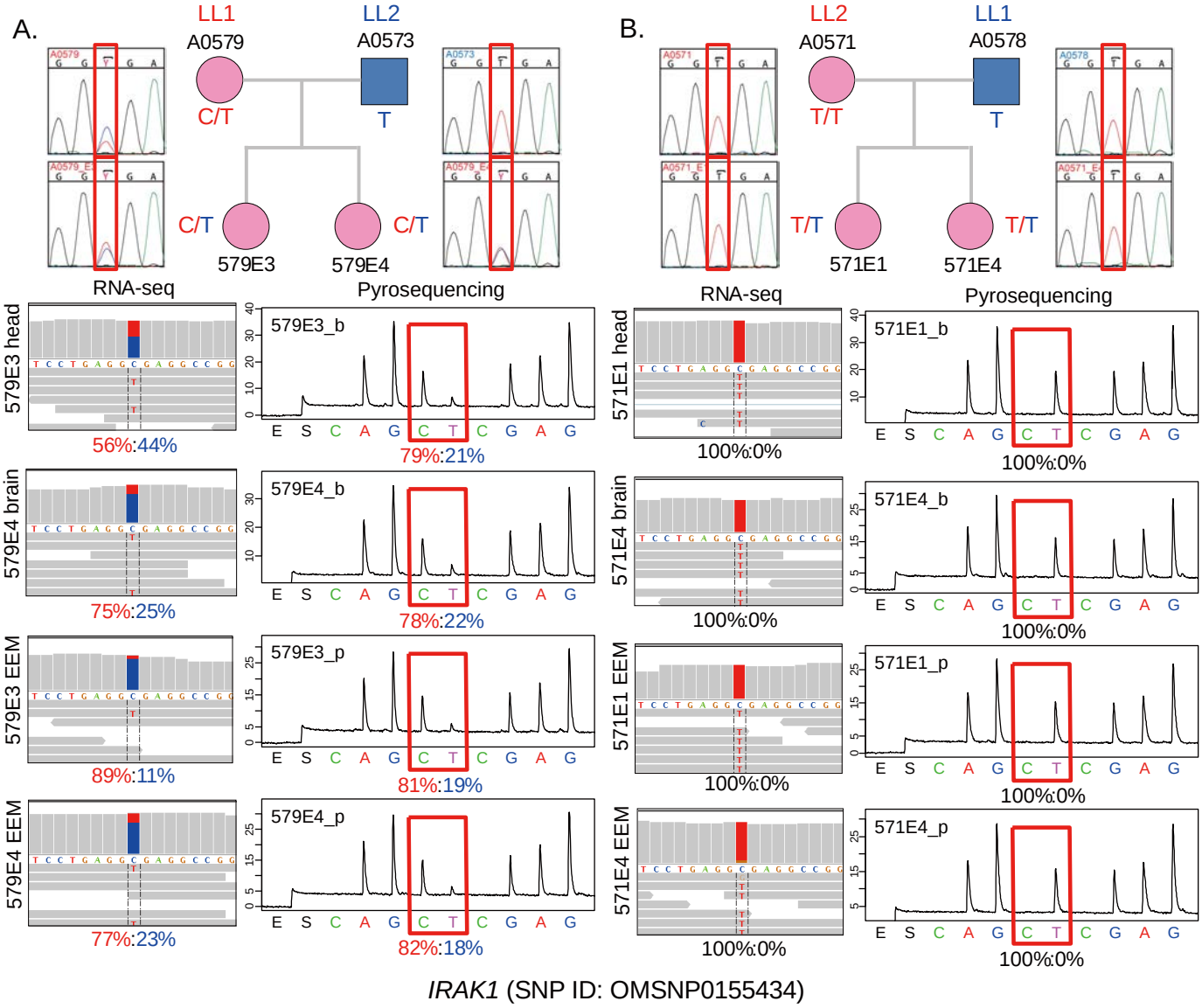


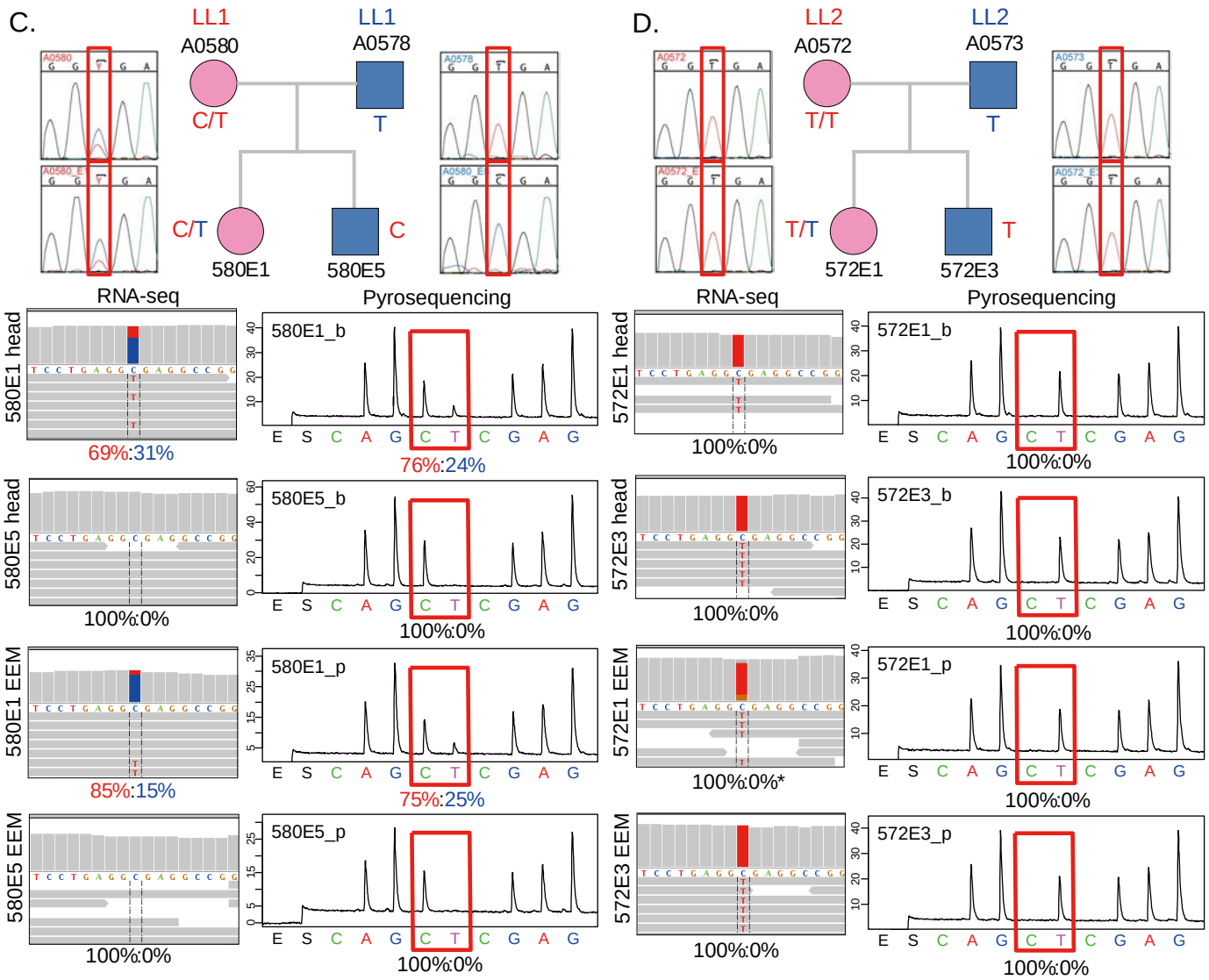


ATRX (SNP ID: OMSNP0156416)

Figure S15. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *IRAK1* in opossum fetal brain and EEM samples.

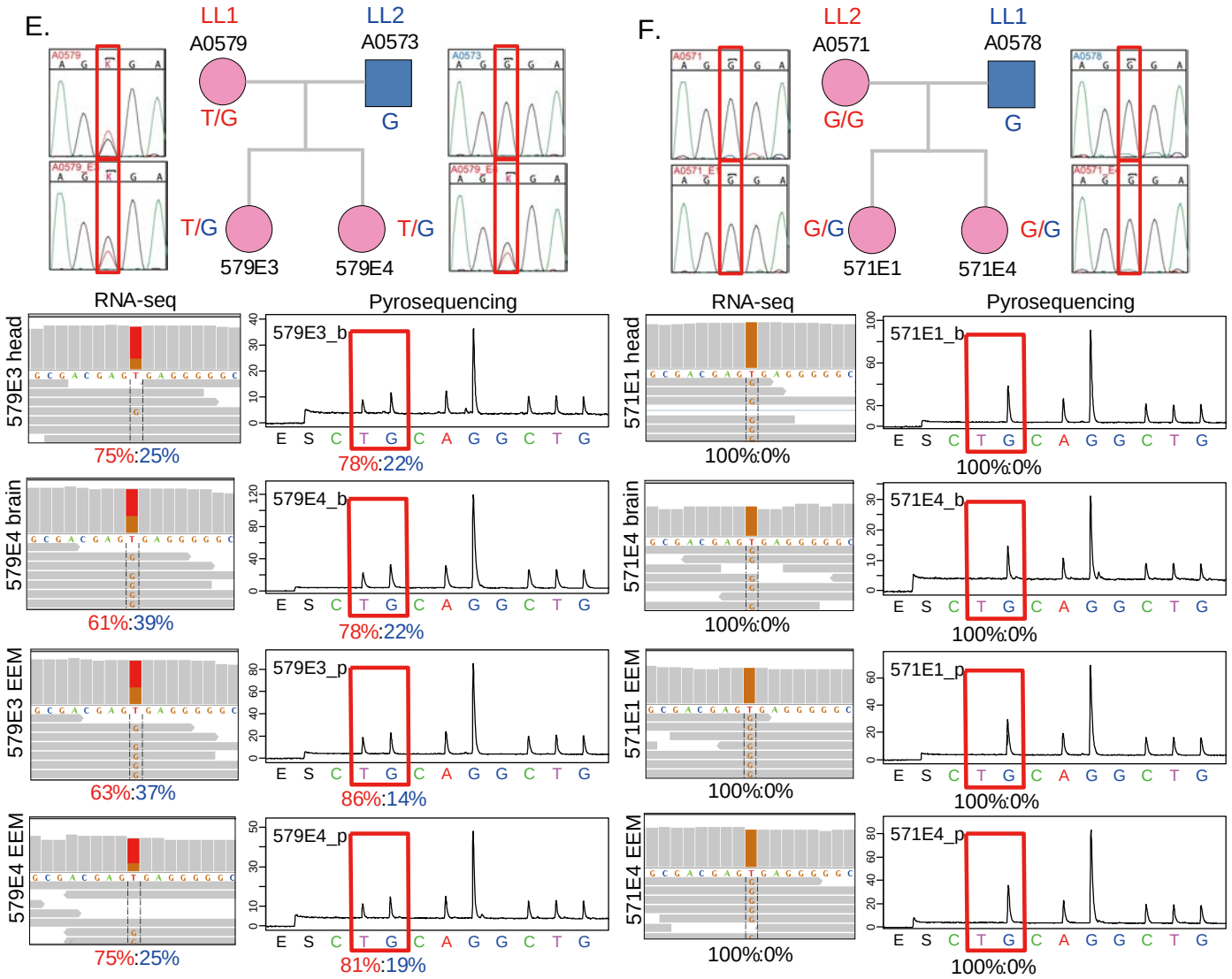
(A)-(D). Results for SNP OMSNP0155434. (A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. (E)-(H). Results for SNP OMSNP0155433. (E). F1 cross of LL1 (mother) x LL2 (father). (F) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (G). LL1 parental cross. (H). LL2 parental cross. From the Sanger sequencing genotyping results, two SNPs (OMSNP0155434 and OMSNP0155433, 101bp apart) are informative in three embryos (579E3, 579E4 and 580E1). In brain/head and EEM tissues of all three individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification, and the allelic expression percentages agreed well at the two SNP positions. Therefore, *IRAK1* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is AGG(C/T)GAGGCC at SNP OMSNP0155434. The target sequence for pyrosequencing is (G/T)GAGGGGGCTGCCGA at SNP OMSNP0155433.



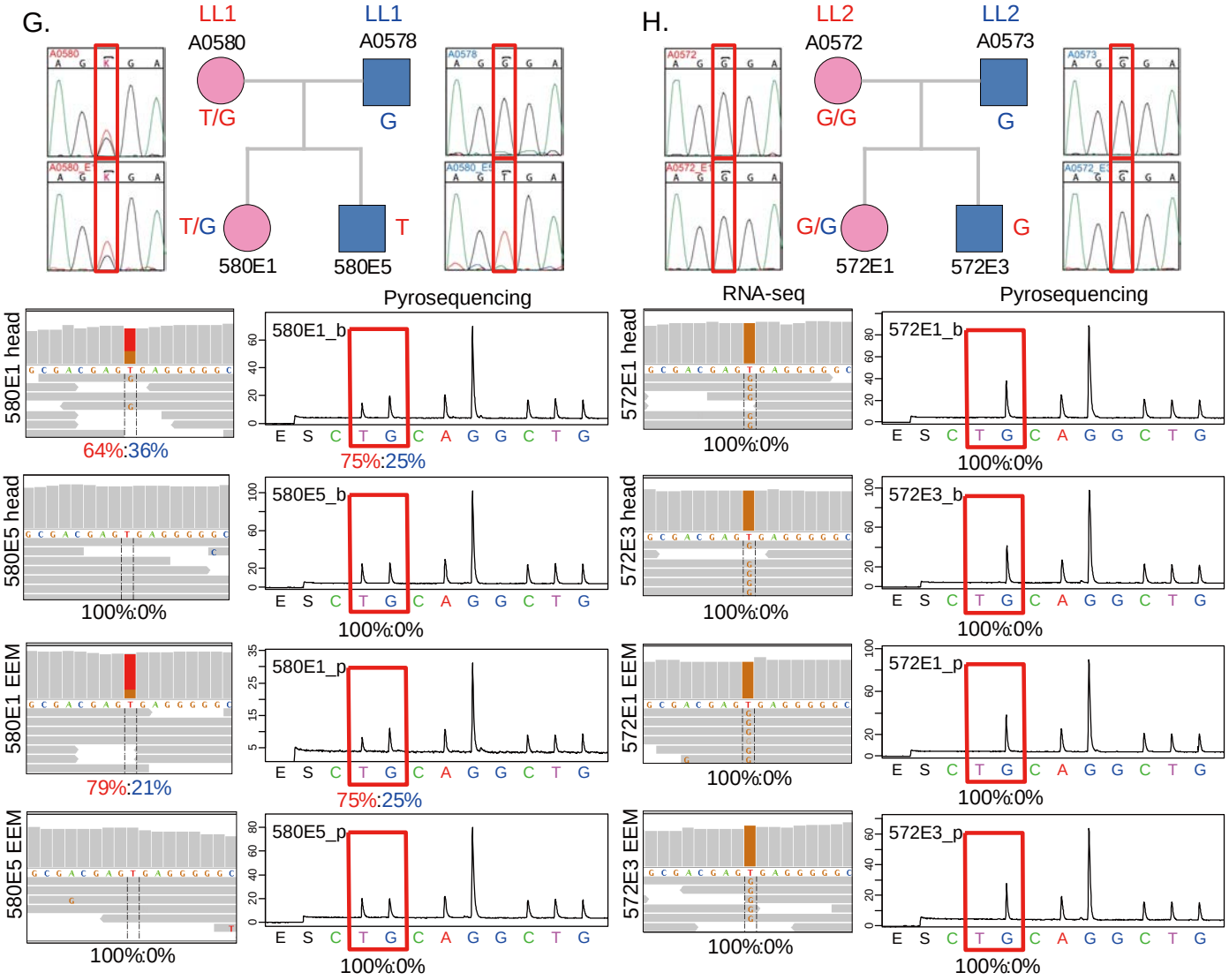


IRAK1 (SNP ID: OMSNP0155434)

*: There are two reads containing G allele at this SNP position caused by Illumina sequencing error (Q-score<=2), and they were excluded from the analysis.



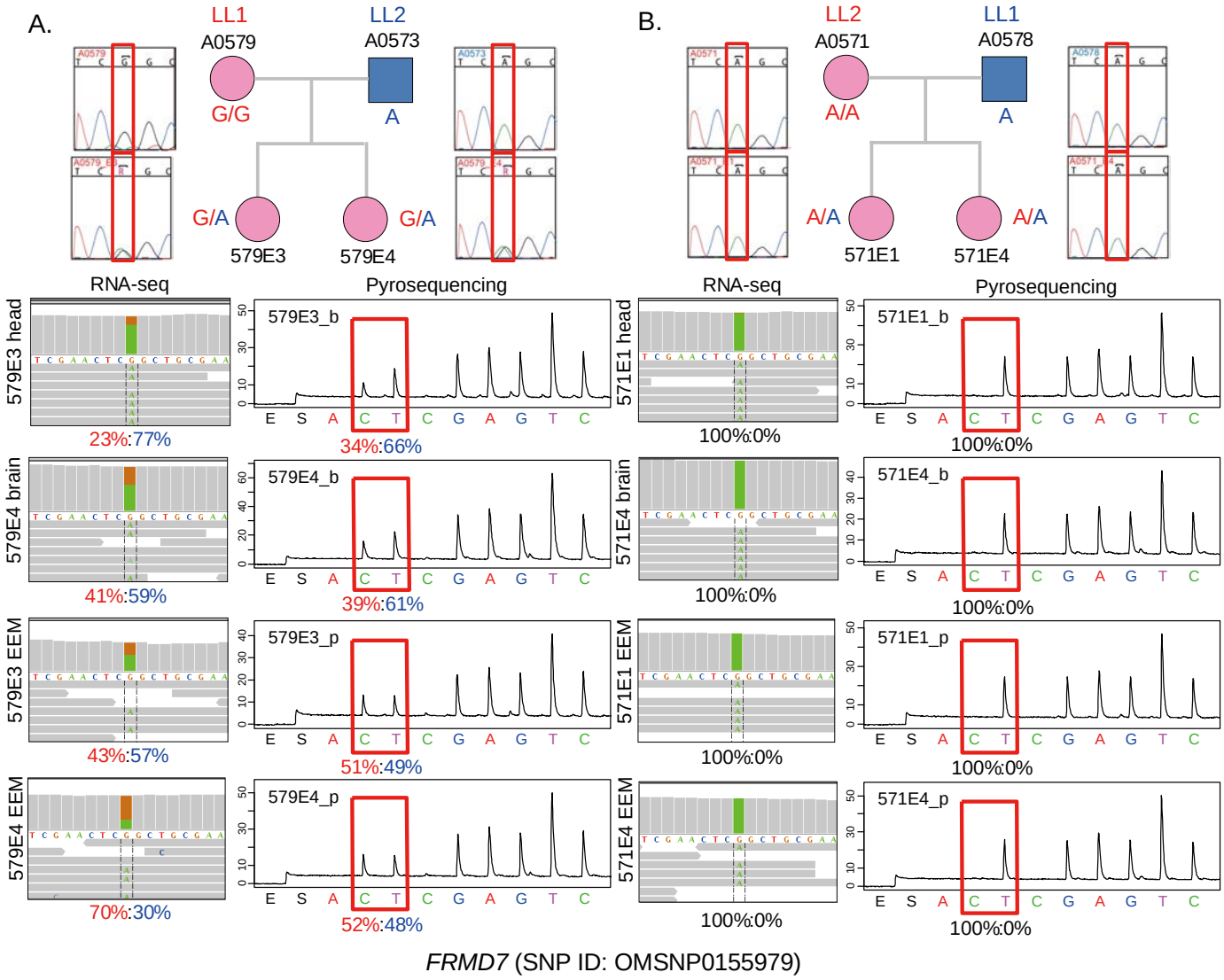
IRAK1 (SNP ID: OMSNP0155433)

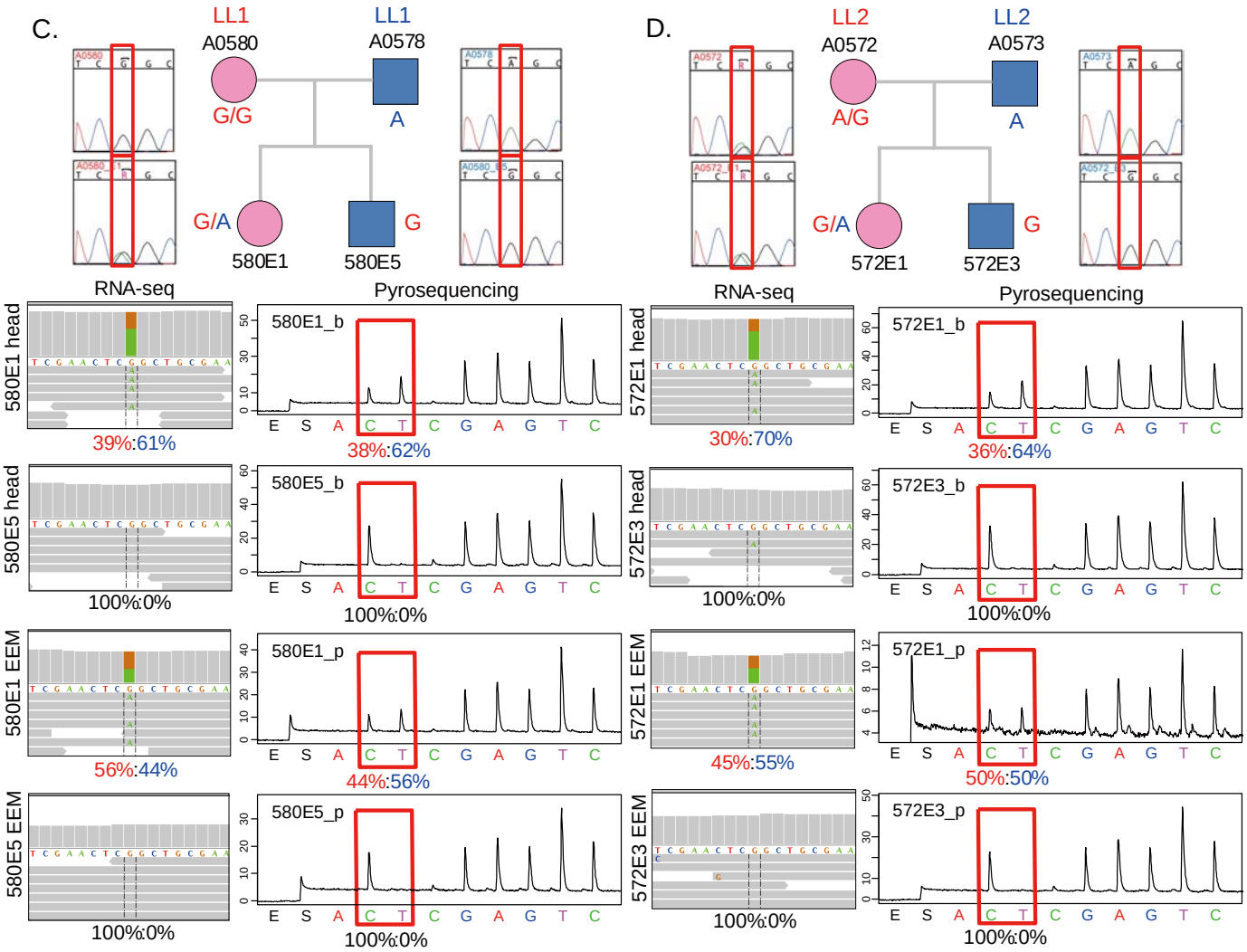


IRAK1 (SNP ID: OMSNP0155433)

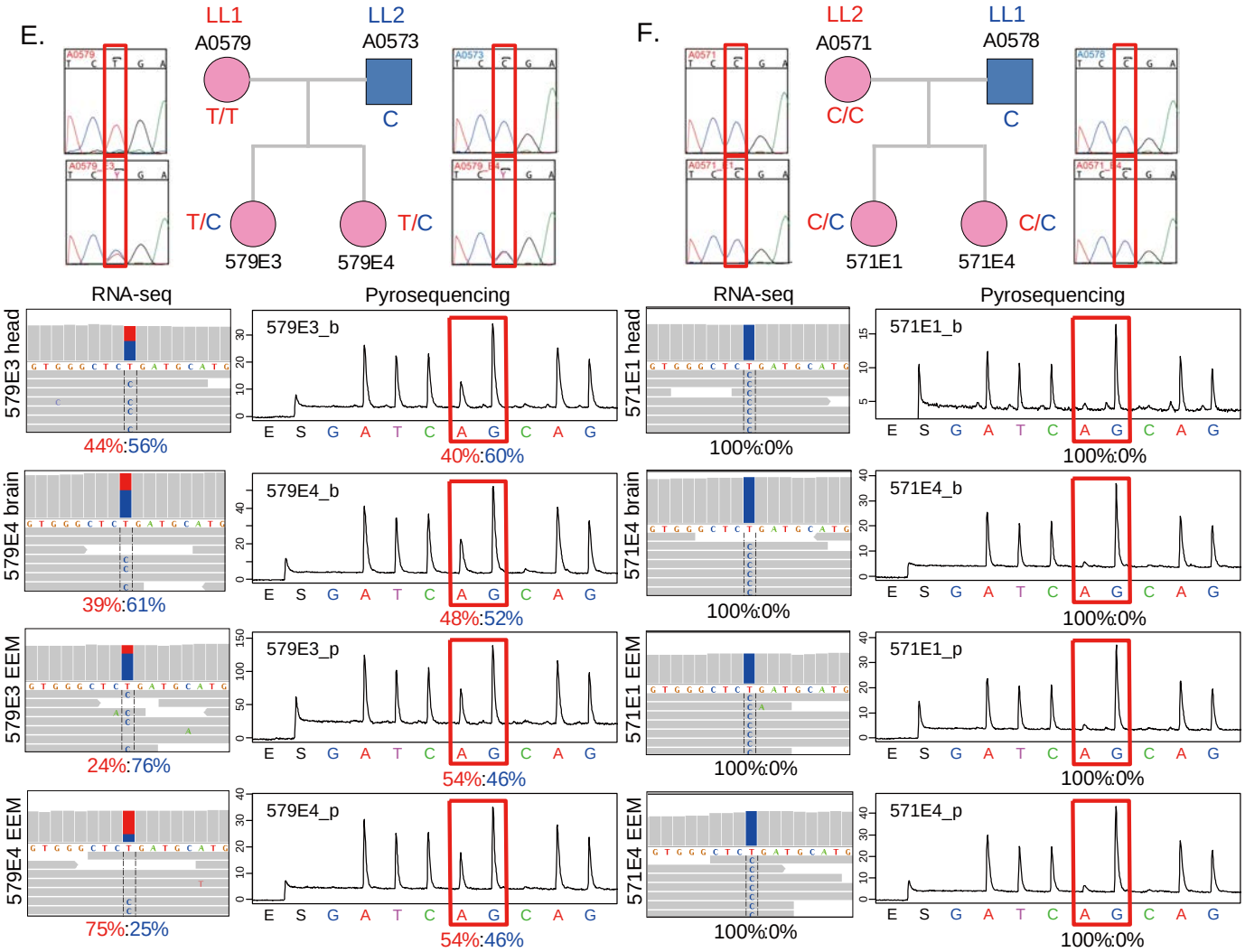
Figure S16. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *FRMD7* in opossum fetal brain and EEM samples.

(A)-(D). Results for SNP OMSNP0155979. (A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. (E)-(H). Results for SNP OMSNP0155980. (E). F1 cross of LL1 (mother) x LL2 (father). (F) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (G). LL1 parental cross. (H). LL2 parental cross. From the Sanger sequencing genotyping results, two SNPs (OMSNP0155979 and OMSNP0155980, 57bp apart) are informative in four embryos (579E3, 579E4, 572E1 and 580E1). In brain/head and EEM tissues of all four individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification, and the allelic expression percentages agreed well at the two SNP positions. Therefore, *FRMD7* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is (C/T)GAGTTCGA at SNP OMSNP0155979 (on the minus strand). The target sequence for pyrosequencing is ATC(A/G)GAGCCCA at SNP OMSNP0155980 (on the minus strand).

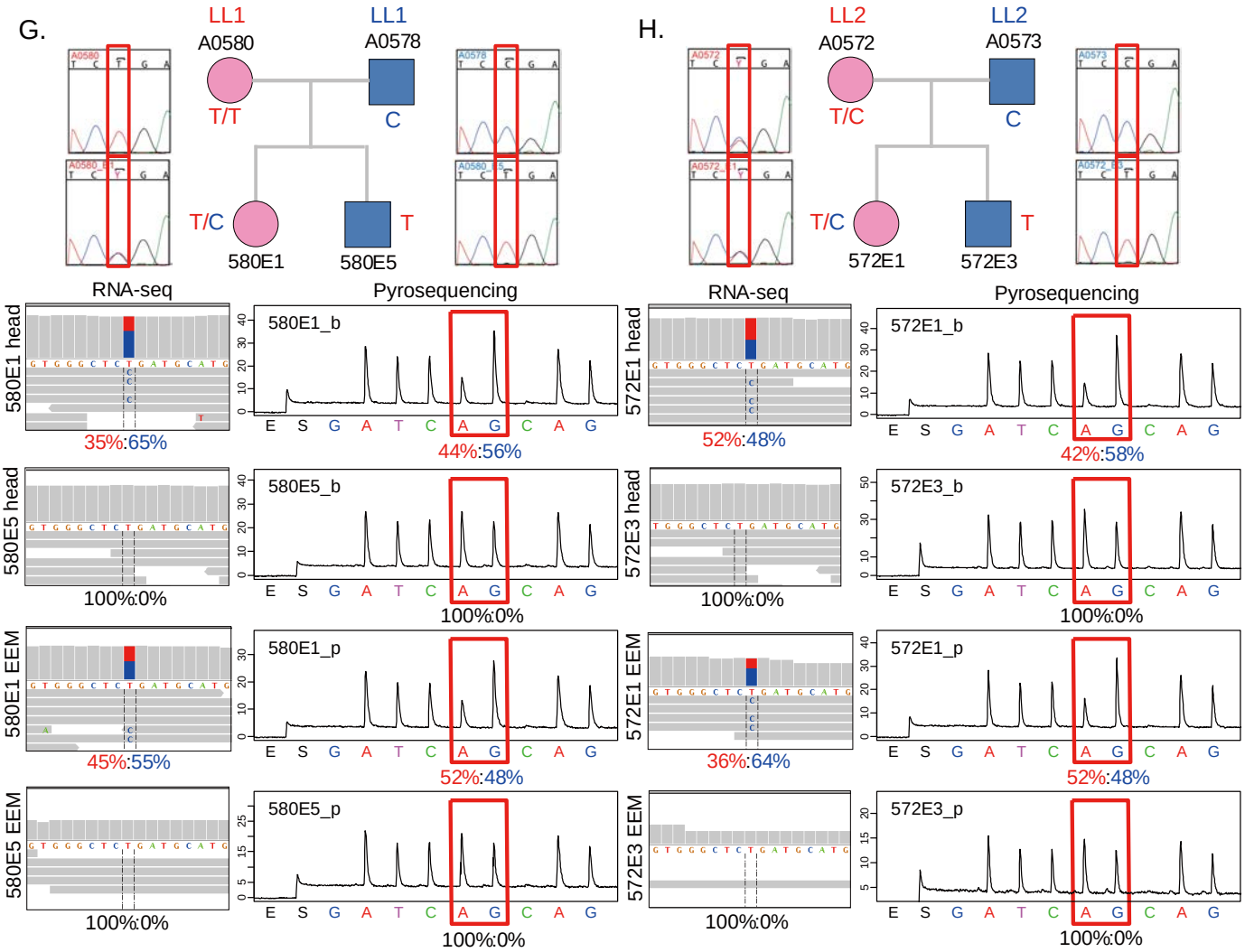




FRMD7 (SNP ID: OMSNP0155979)



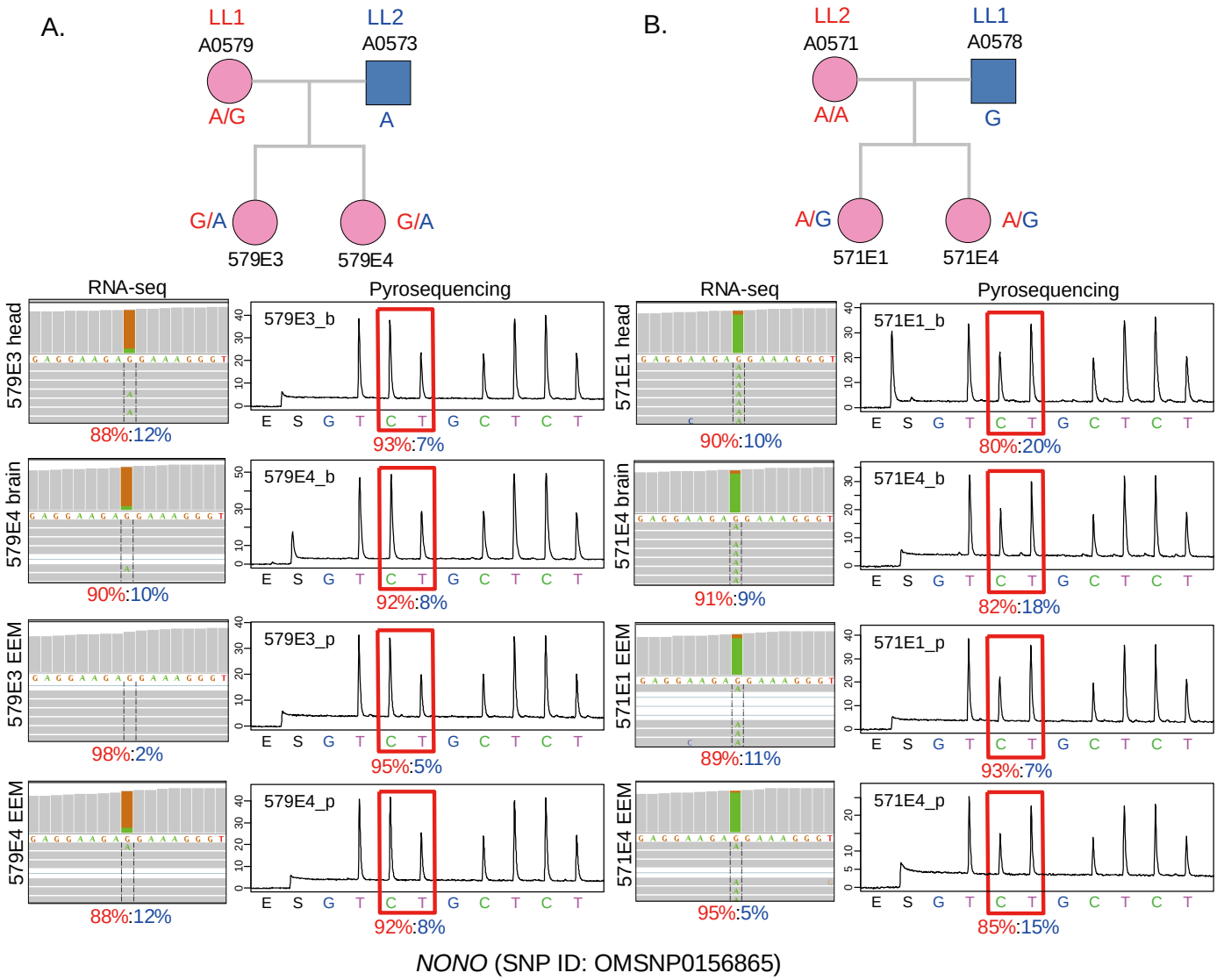
FRMD7 (SNP ID: OMSNP0155980)

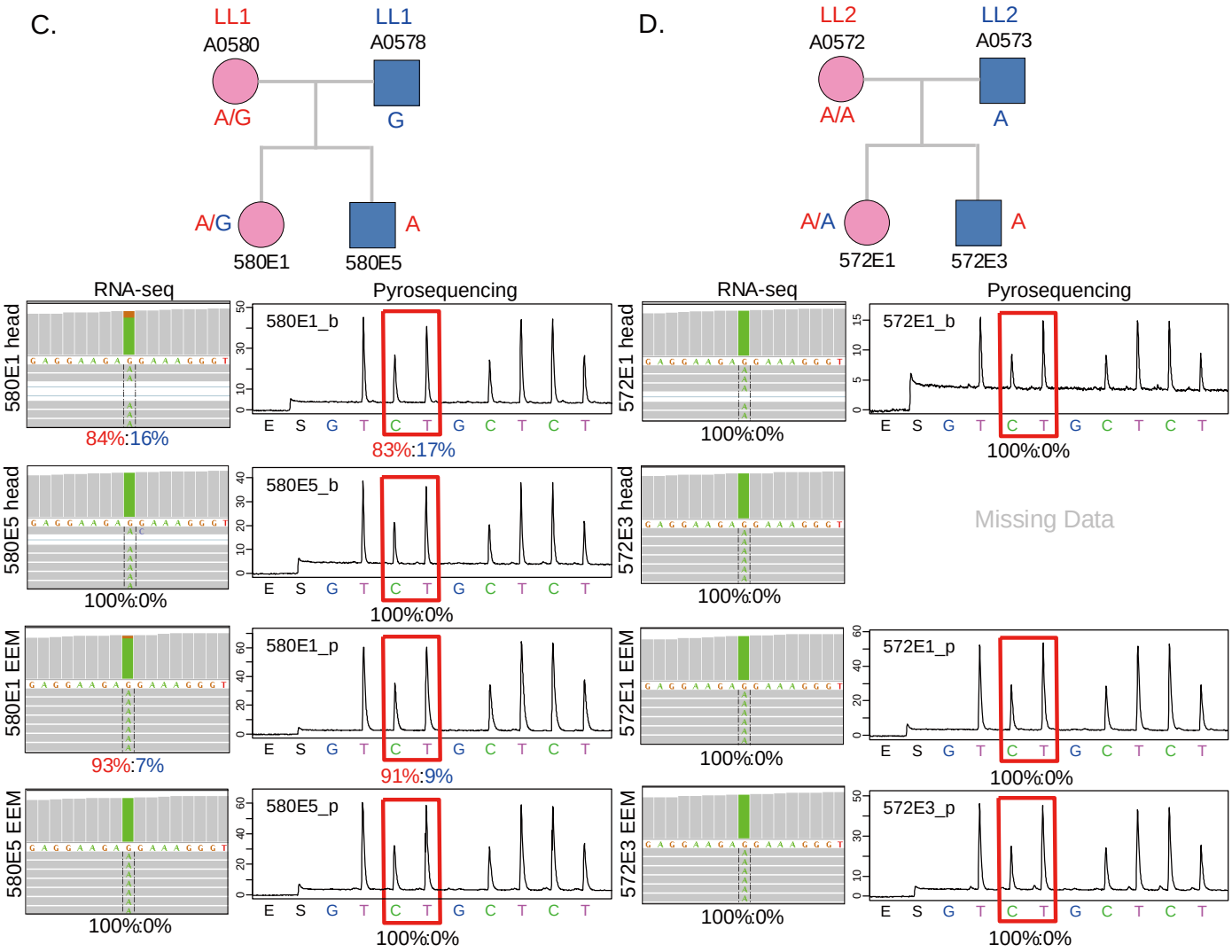


FRMD7 (SNP ID: OMSNP0155980)

Figure S17. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *NONO* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0156865) is informative in five embryos (579E3, 579E4, 571E1, 571E4 and 580E1). In brain/head and EEM tissues of all five individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *NONO* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is TTC(C/T)TCTTCCTC (on the minus strand). The Sanger sequencing traces were not shown here because an indel polymorphism in the amplicon shifted the traces, but the genotypes could be determined by the software.

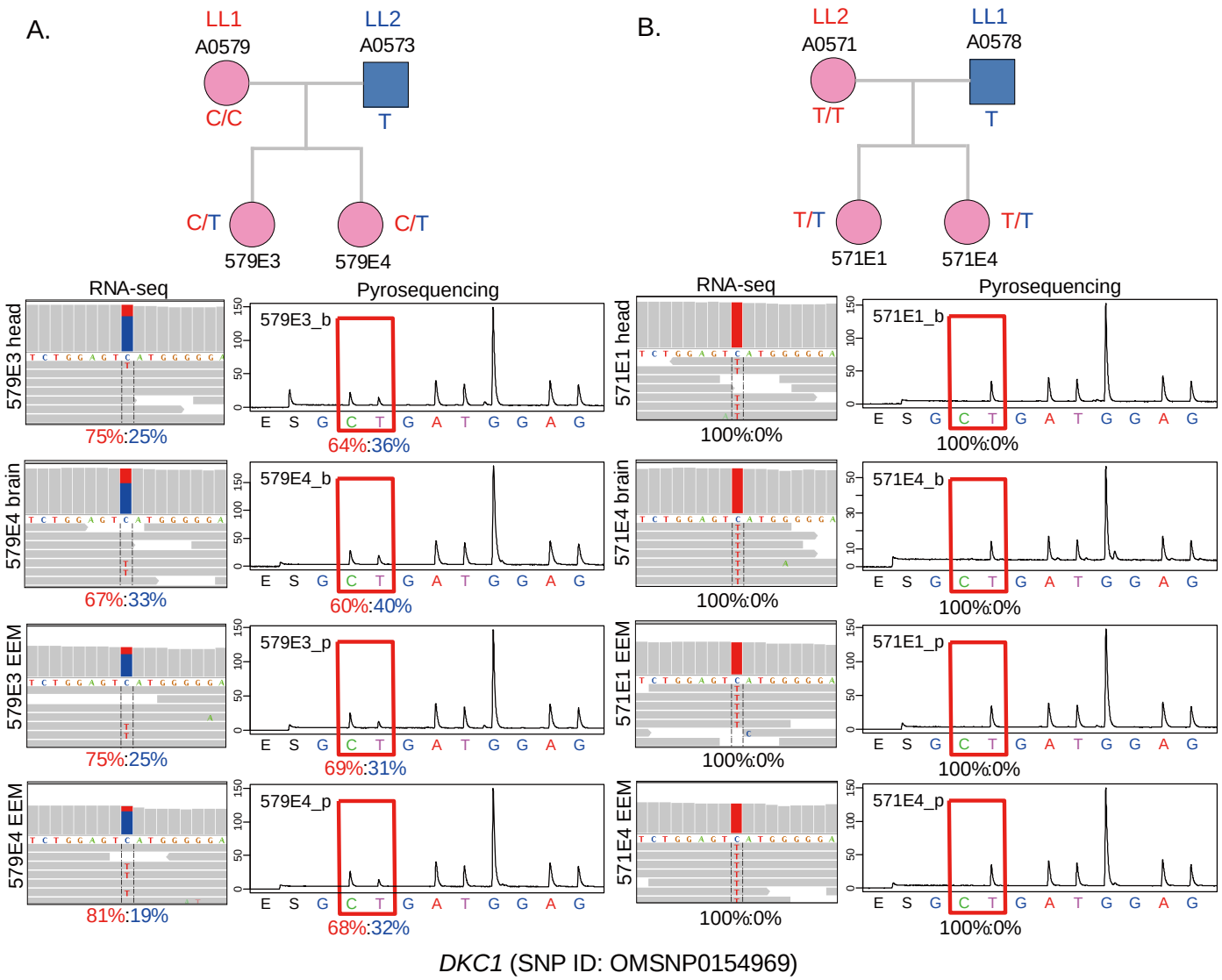


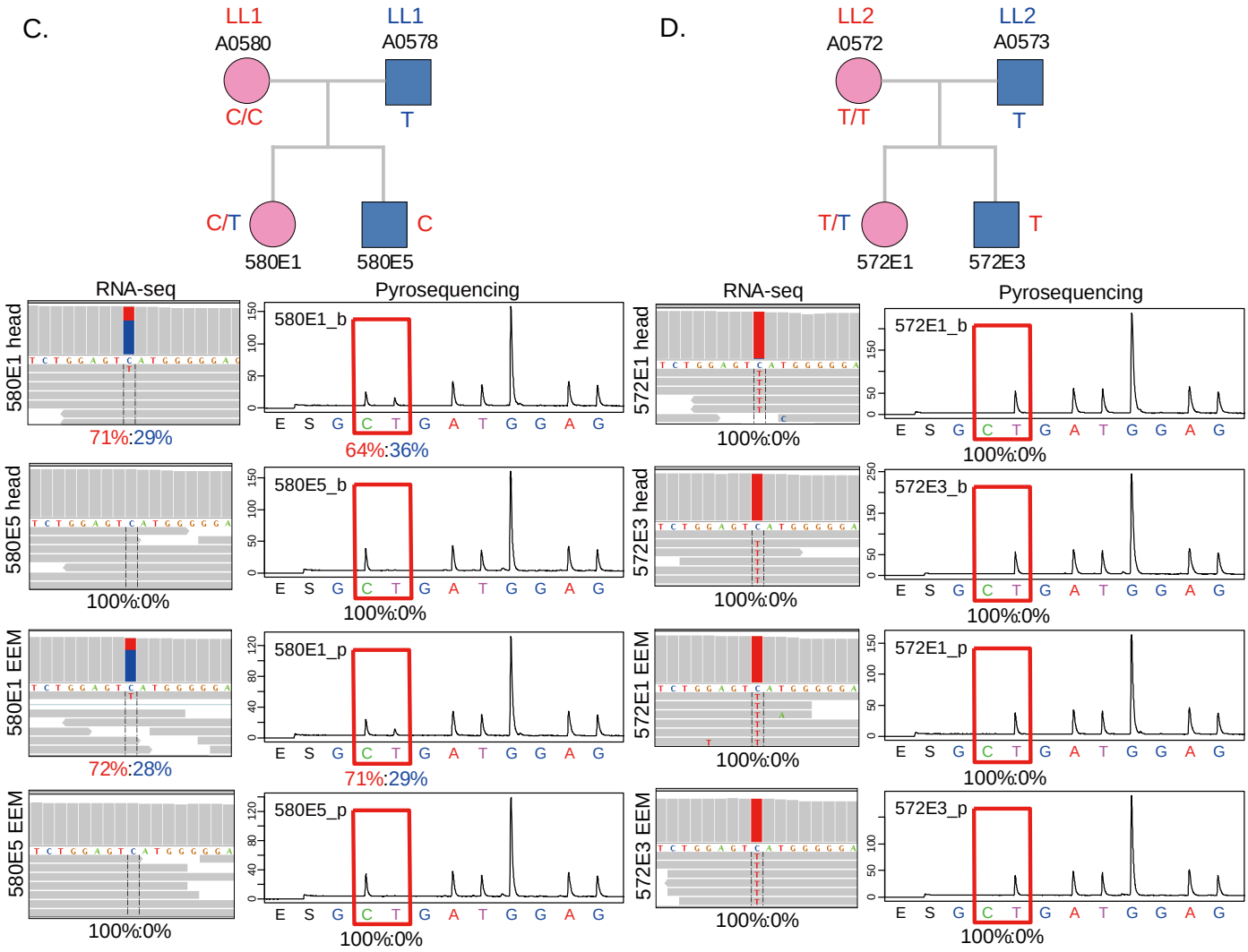


NONO (SNP ID: OMSNP0156865)

Figure S18. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *DKC1* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0154969) is informative in three embryos (579E3, 579E4 and 580E1). In brain/head and EEM tissues of all three individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *DKC1* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is (C/T)ATGGGGGAGAAG.

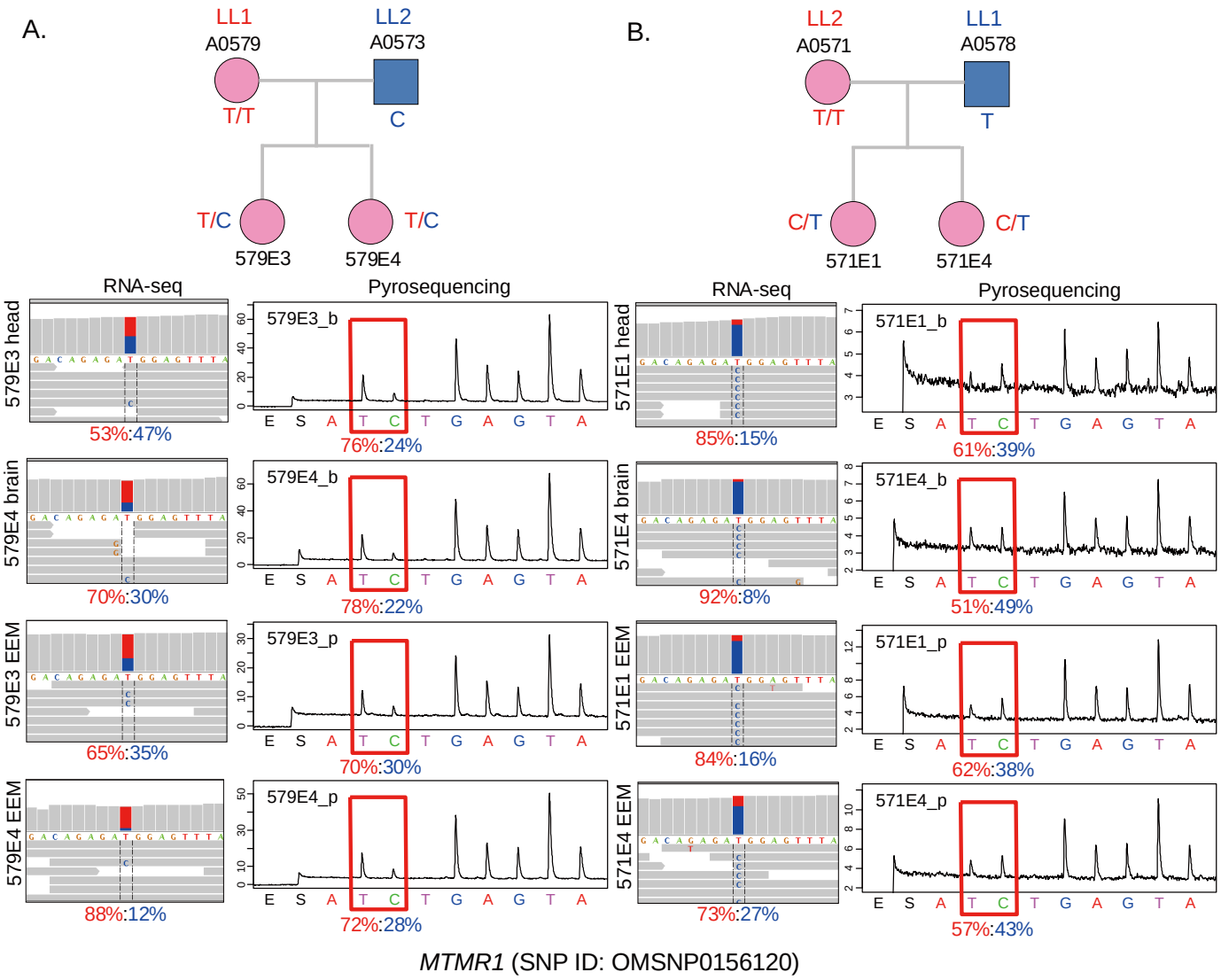


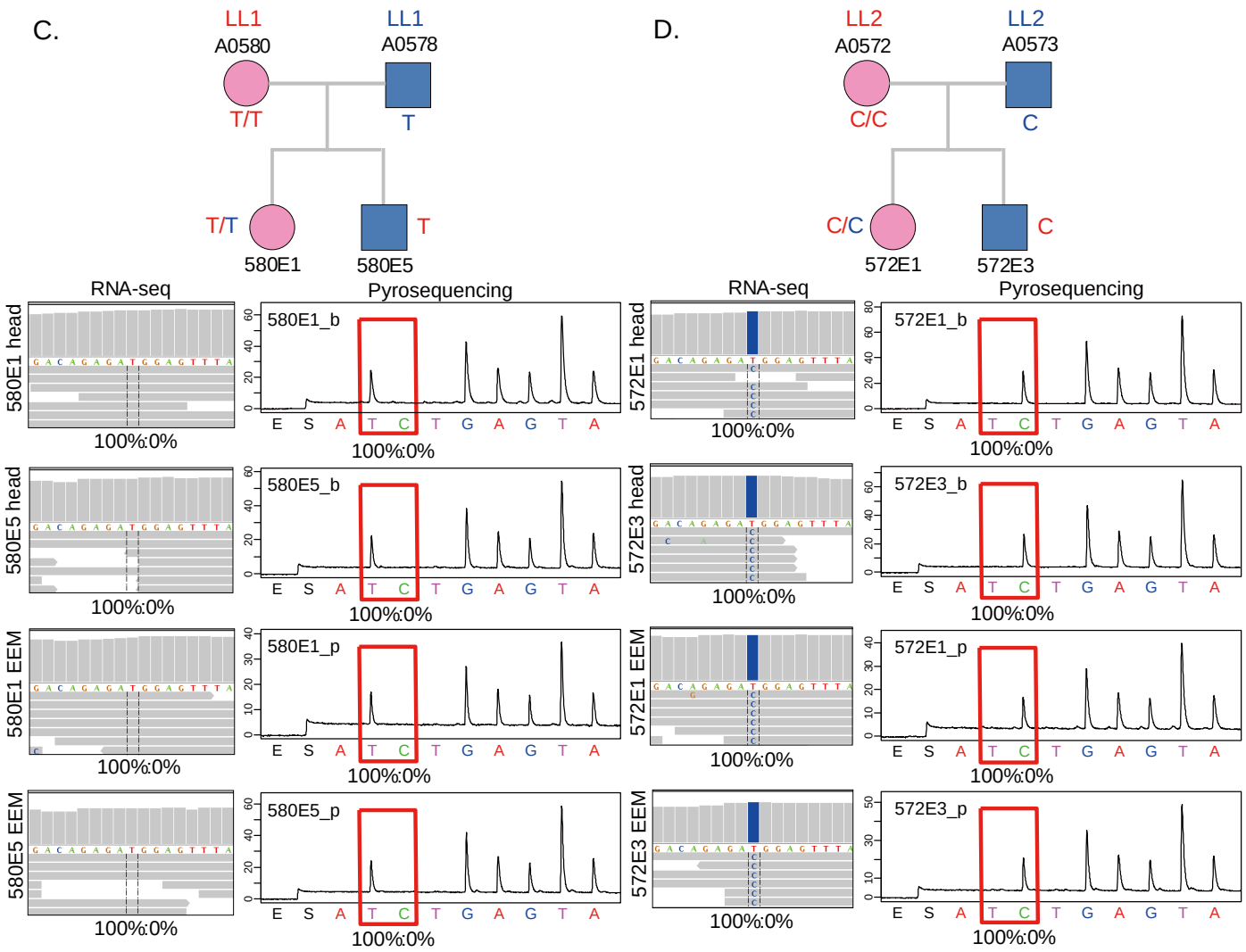


DKC1 (SNP ID: OMSNP0154969)

Figure S19. RNA-seq, SNP genotyping and pyrosequencing verification results for escaper gene *MTMR1* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. From the Sanger sequencing genotyping results, the SNP (OMSNP0156120) is informative in four embryos (579E3, 579E4, 571E1 and 571E4). In brain/head and EEM tissues of all four individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *MTMR1* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is (T/C)GGAGTTTACA.

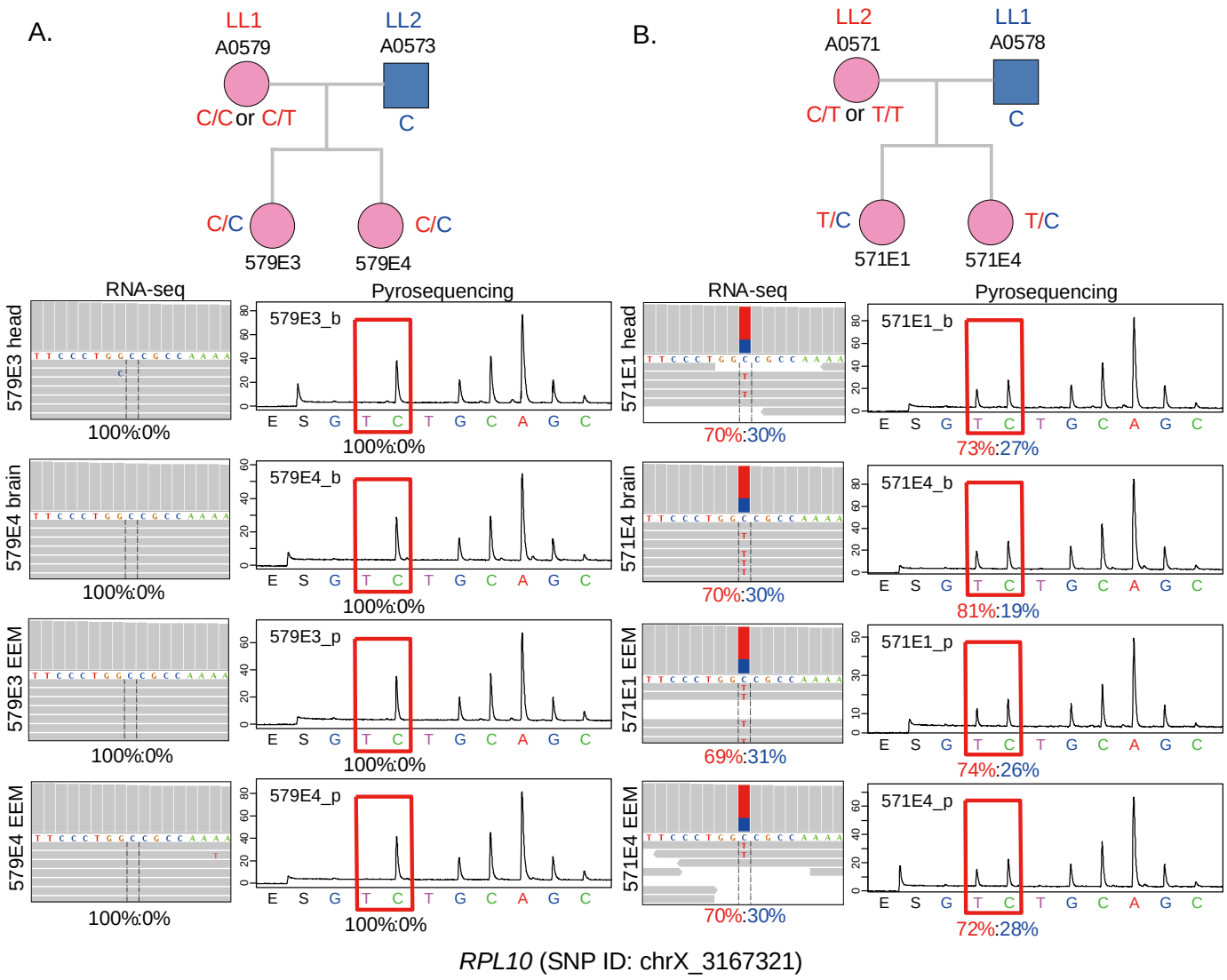


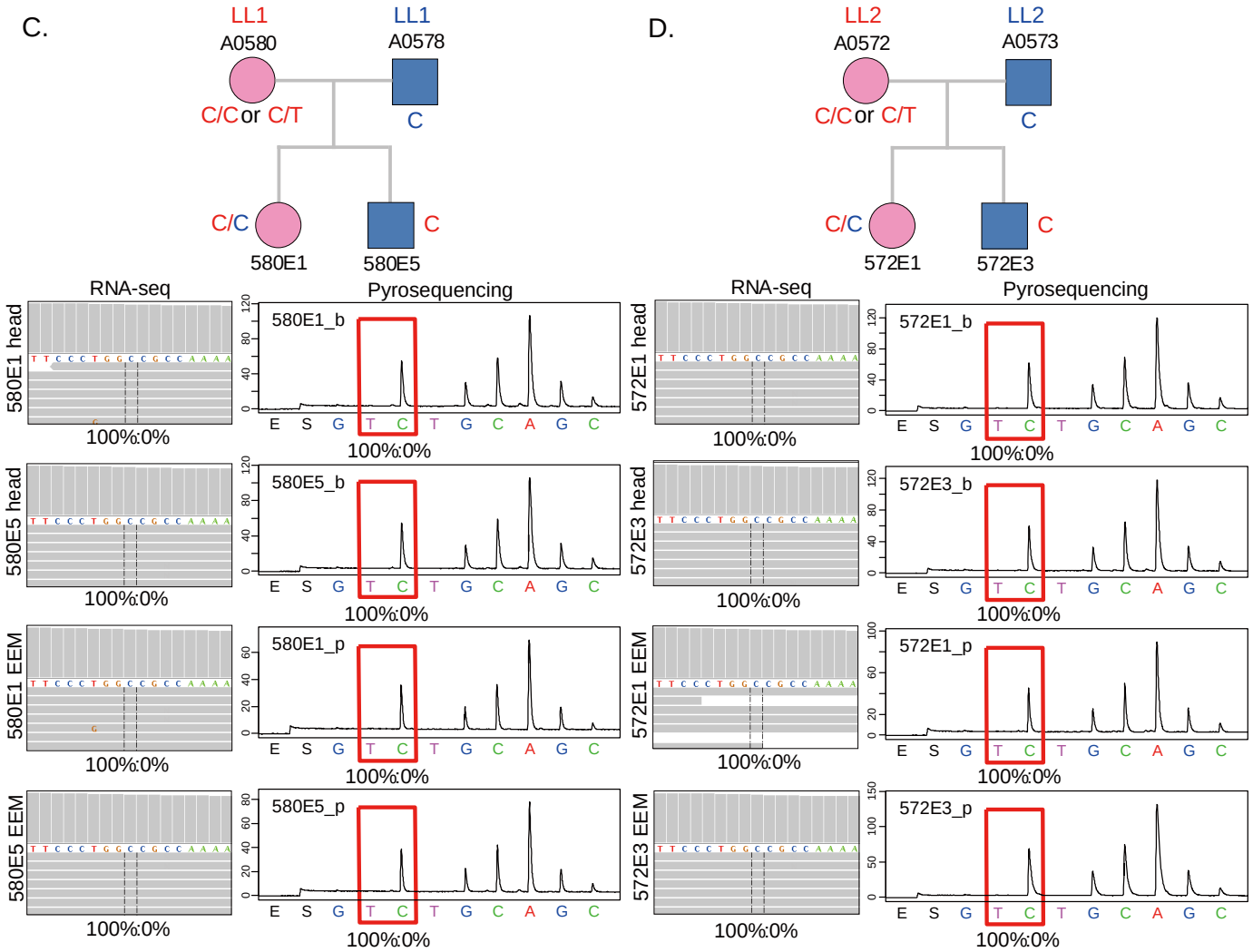


MTMR1 (SNP ID: OMSNP0156120)

Figure S20. RNA-seq and pyrosequencing verification results for escaper gene *RPL10* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. The SNP (chrX_3167321) is informative in two embryos (571E1 and 571E4). In brain/head and EEM tissues of both individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *RTL10* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is (C/T)CGCCAAAAGCGG. The SNP genotypes were called from the RNA-seq data and inferred based on the pedigree information. The parental transmission directions were unambiguously determined.

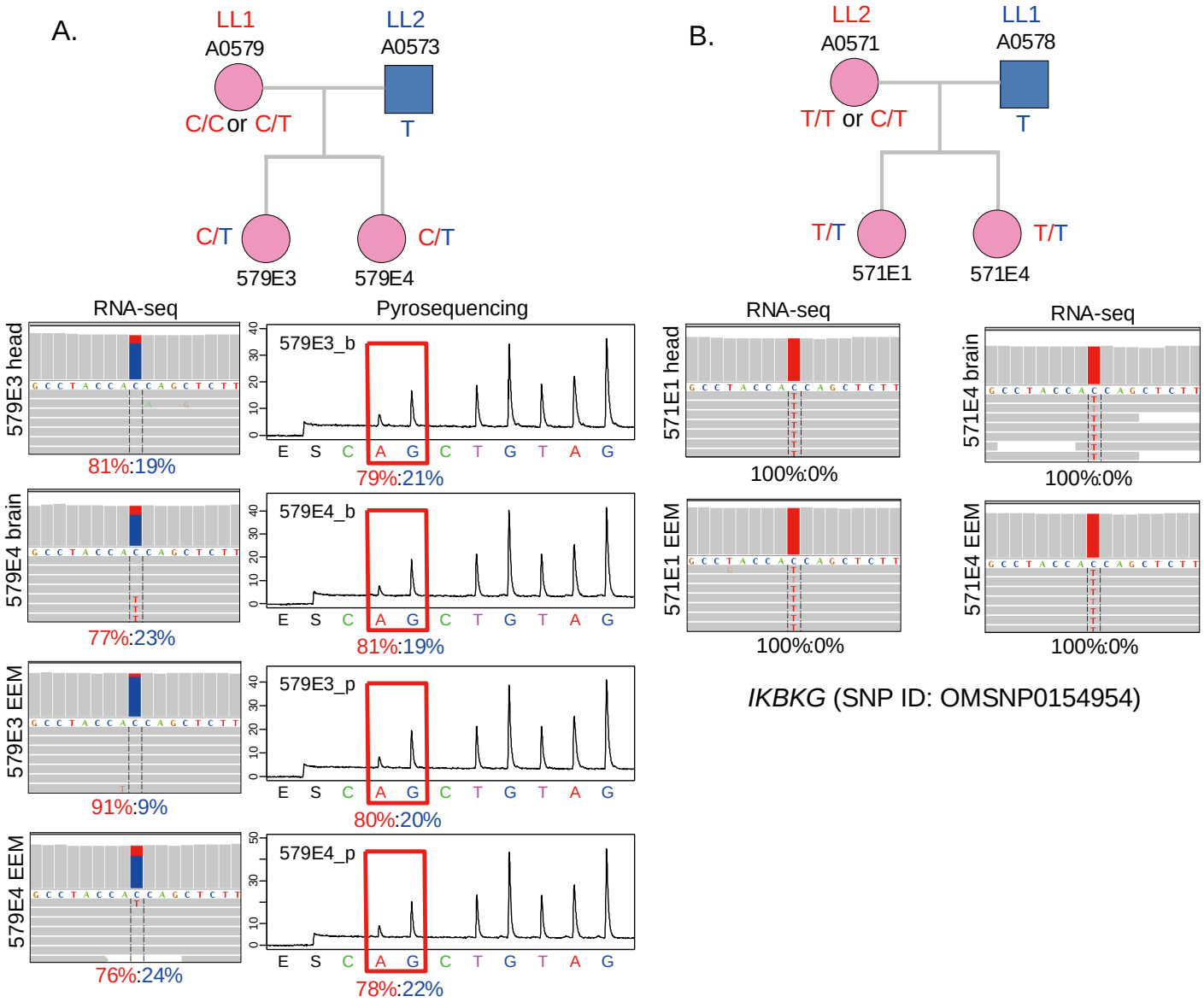




RPL10 (SNP ID: chrX_3167321)

Figure S21. RNA-seq and pyrosequencing verification results for escaper gene *IKBKG* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. The SNP (OMSNP0154954) is informative in three embryos (579E3, 579E4 and 580E1). In brain/head and EEM tissues of all three individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *IKBKG* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is (A/G)TGGTAGGCT (on the minus strand). Pyrosequencing was not performed for two of the four crosses because they are not informative (B and D). The SNP genotypes were called from the RNA-seq data and inferred based on the pedigree information. The parental transmission directions were unambiguously determined.



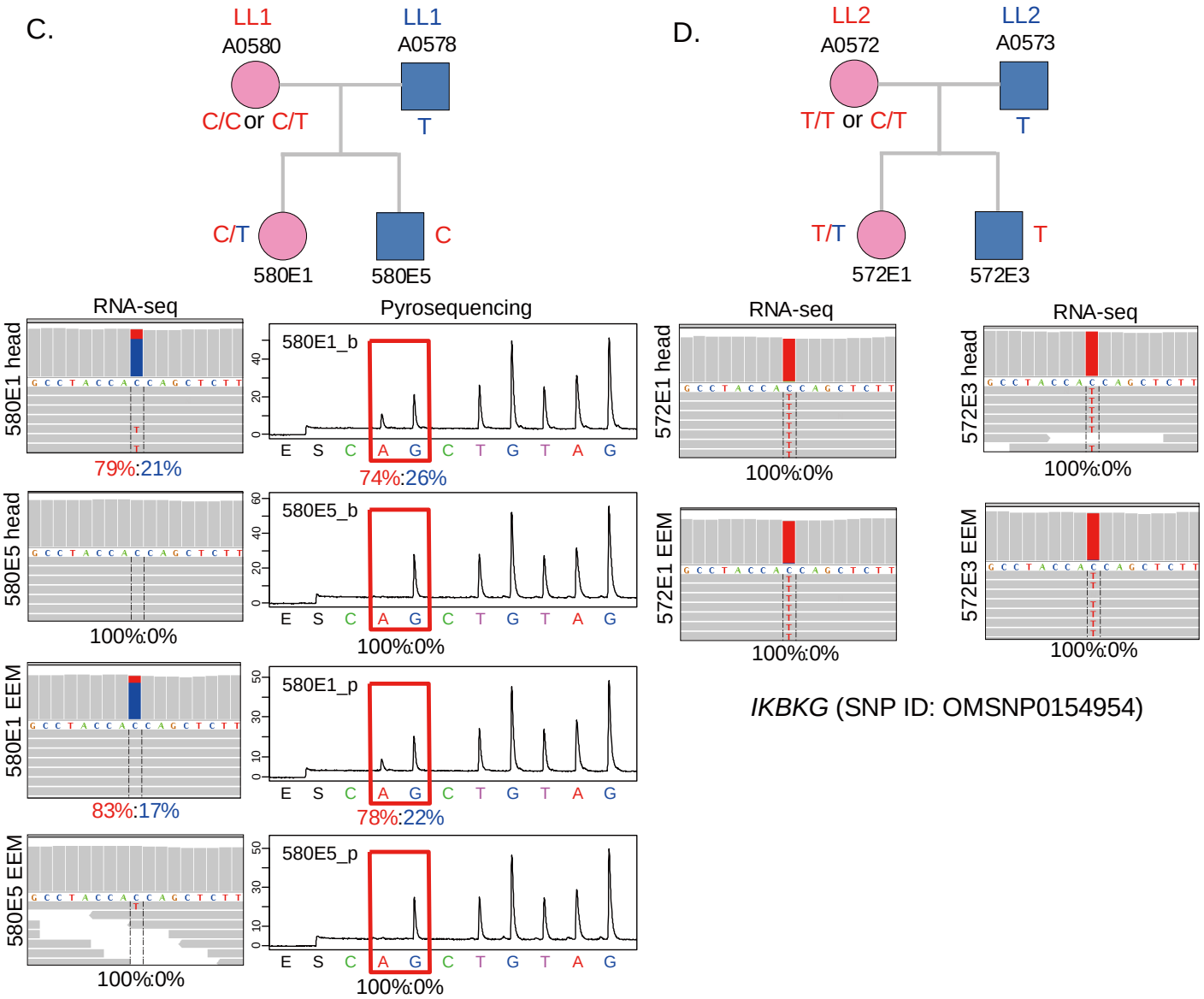
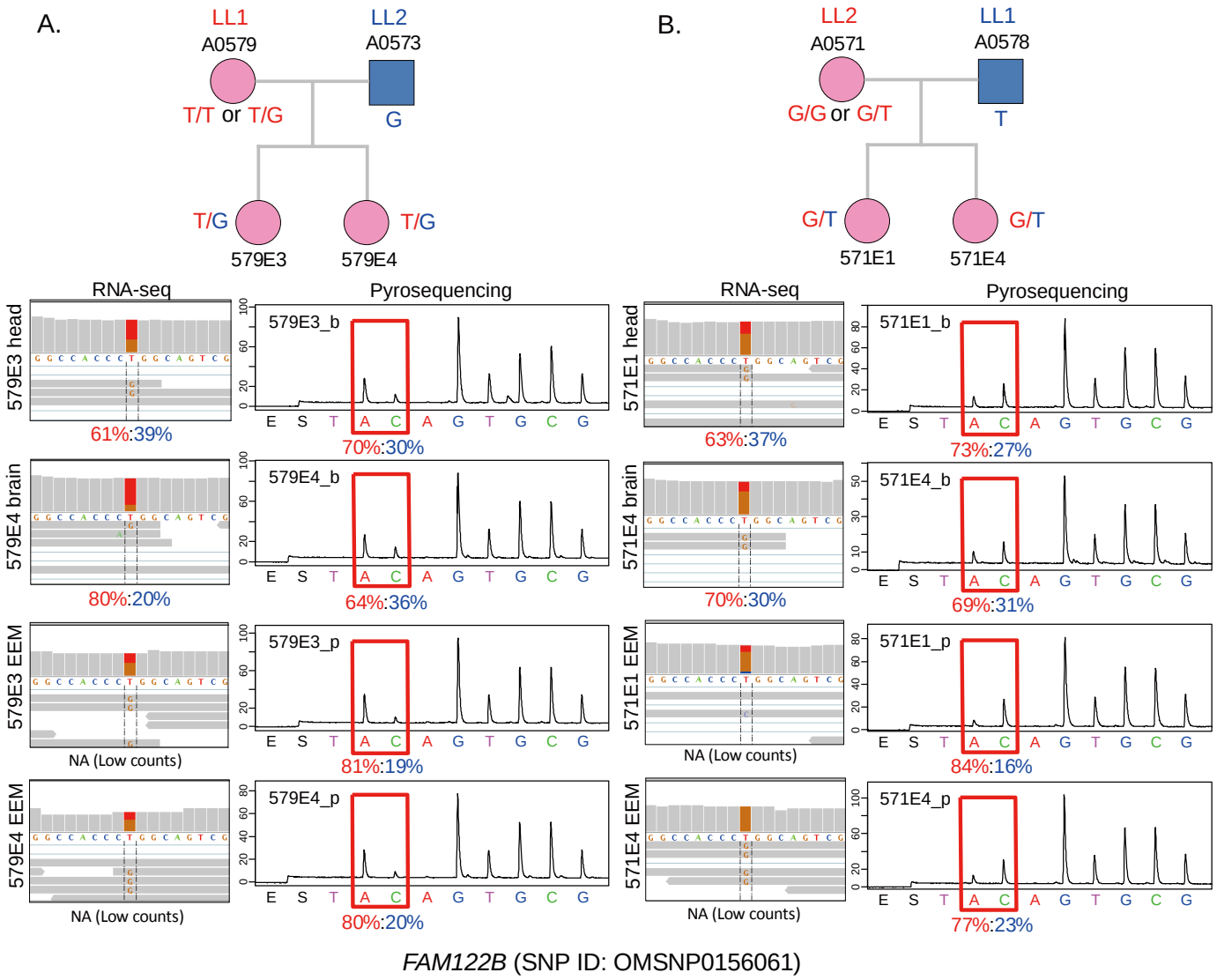
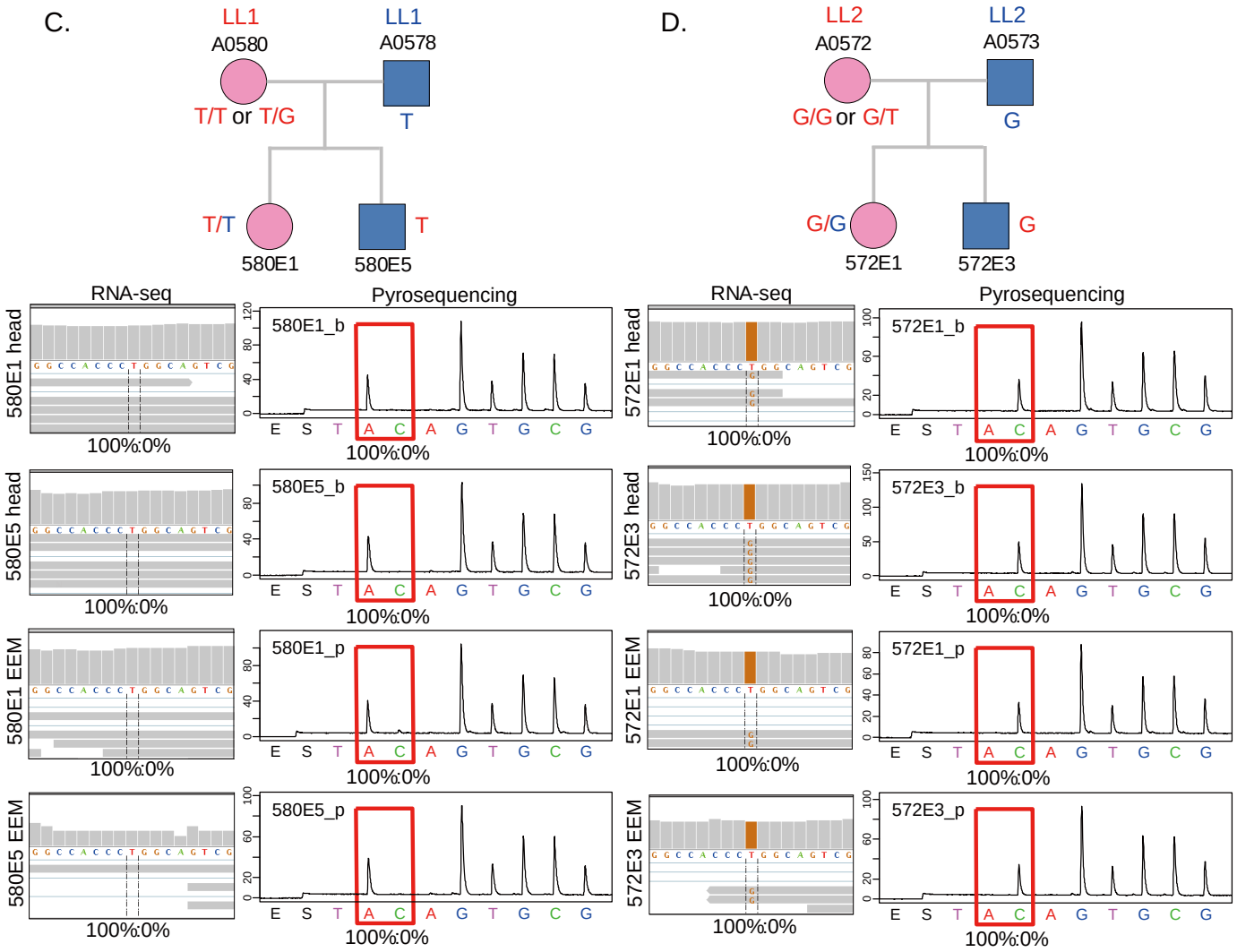

C.
LL1
A0580
LL1
A0578
C/C or C/T
T
C/T
C
580E1
580E5
D.
LL2
A0572
LL2
A0573
T/T or C/T
T
T/T
T
572E1
572E3
RNA-seq
Pyrosequencing
580E1 head
580E5 head
580E1 EEM
580E5 EEM
580E1_b
580E5_b
580E1_p
580E5_p
E S C A G C T G T A G
79%:21%
74%:26%
100%:0%
100%:0%
83%:17%
78%:22%
100%:0%
100%:0%
572E1 head
572E3 head
572E1 EEM
572E3 EEM
100%:0%
100%:0%
100%:0%
100%:0%
IKBKG (SNP ID: OMSNP0154954)

Figure S22. RNA-seq and pyrosequencing verification results for escaper gene *FAM122B* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. The SNP (OMSNP0156061) is informative in four embryos (579E3, 579E4, 571E1 and 571E4). In brain/head and EEM tissues of all four individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *FAM122B* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is (A/C)GGGTGGCCGCG (on the minus strand). The SNP genotypes were called from the RNA-seq data and inferred based on the pedigree information. The parental transmission directions were unambiguously determined. This SNP is located in an alternatively spliced exon.

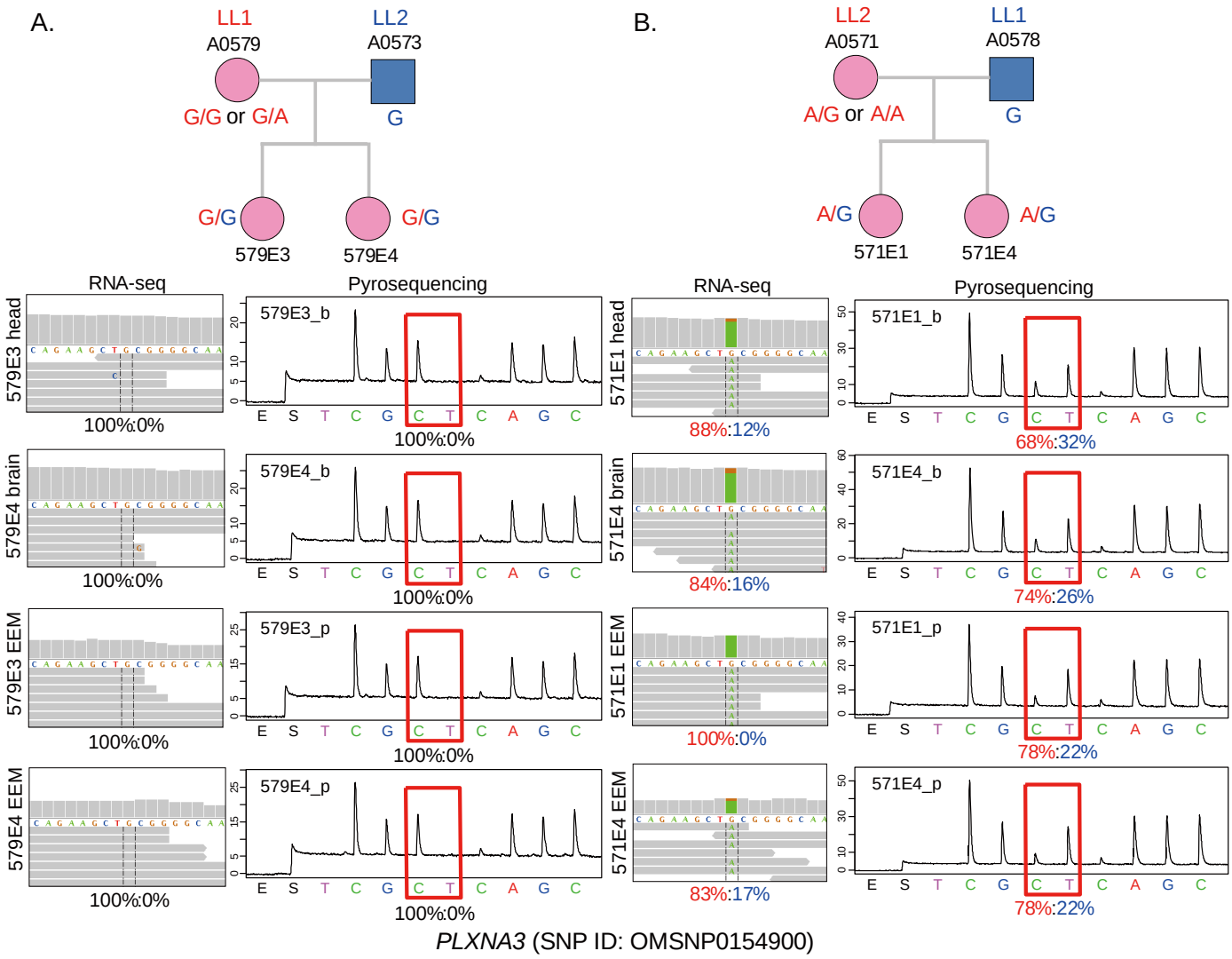


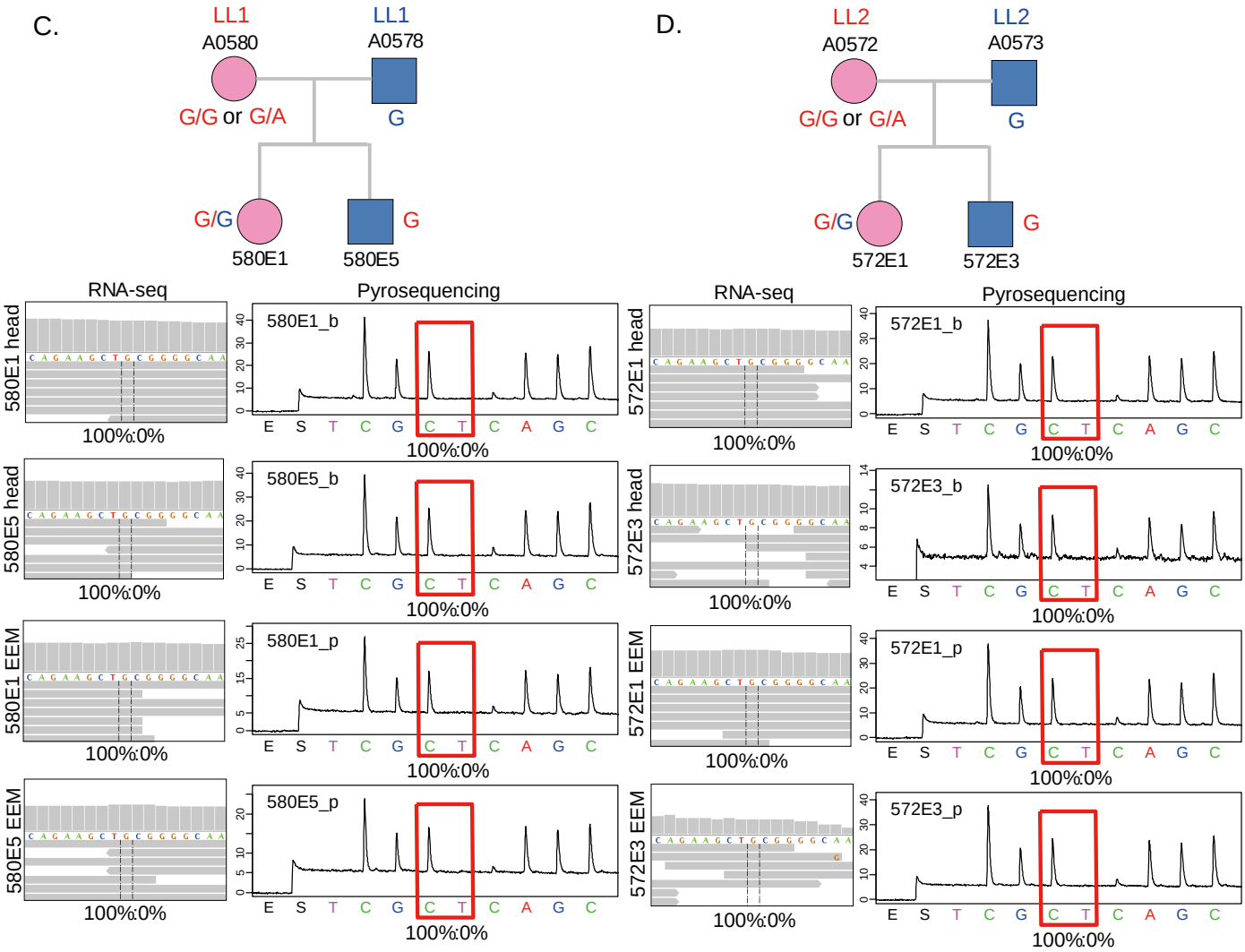


FAM122B (SNP ID: OMSNP0156061)

Figure S23. RNA-seq and pyrosequencing verification results for escaper gene *PLXNA3* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. The SNP (OMSNP0154900) is informative in two embryos (571E1 and 571E4). In brain/head and EEM tissues of both individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *PLXNA3* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is CCG(C/T)AGCTTCT (on the minus strand). The SNP genotypes were called from the RNA-seq data and inferred based on the pedigree information. The parental transmission directions were unambiguously determined.

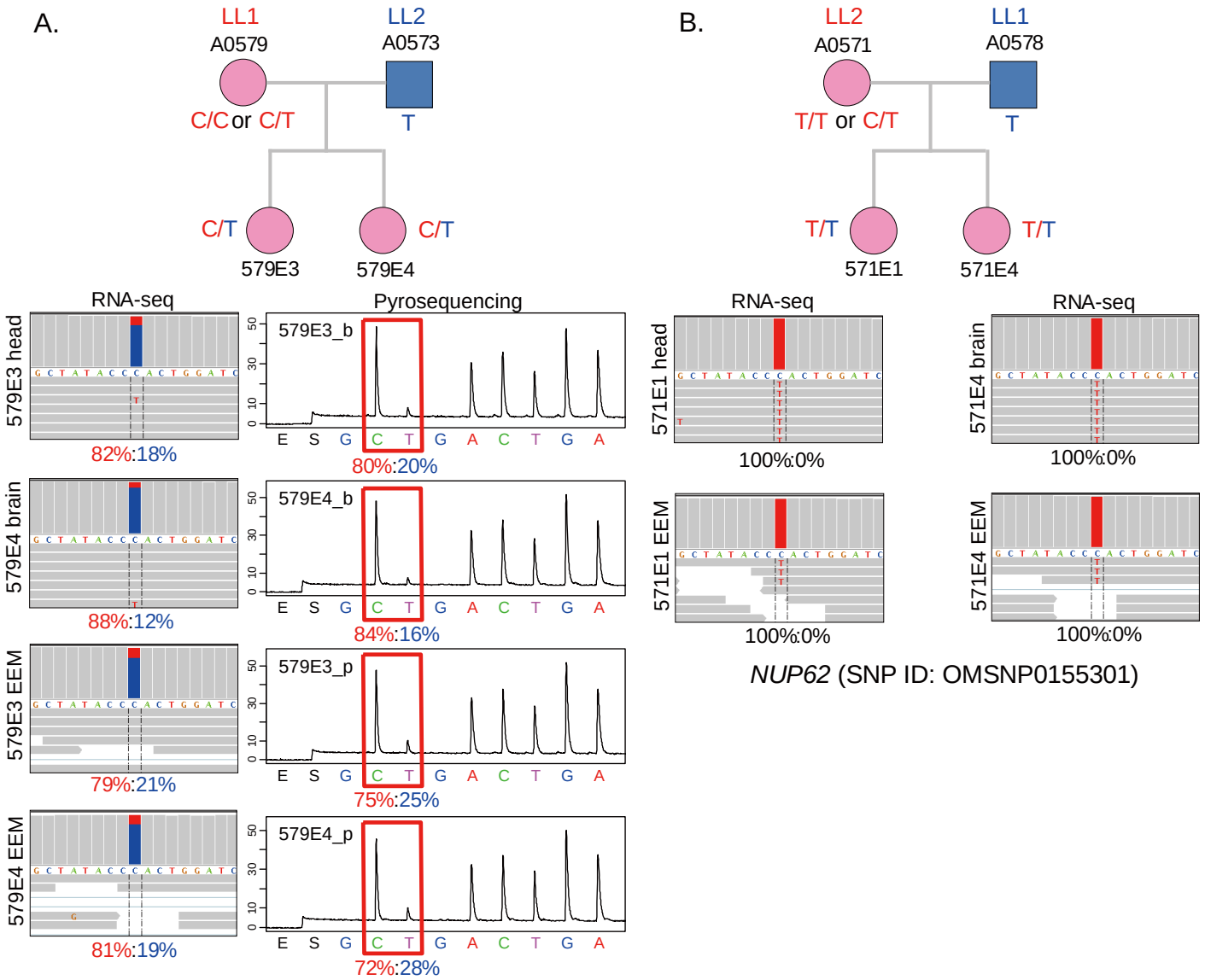




PLXNA3 (SNP ID: OMSNP0154900)

Figure S24. RNA-seq and pyrosequencing verification results for escaper gene *NUP62* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. The SNP (OMSNP0155301) is informative in three embryos (579E3, 579E4 and 580E1). In brain/head and EEM tissues of all three individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *NUP62* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is C(C/T)ACTGGATC. Pyrosequencing was not performed for two of the four crosses because they are not informative (B and D). The SNP genotypes were called from the RNA-seq data and inferred based on the pedigree information. The parental transmission directions were unambiguously determined.



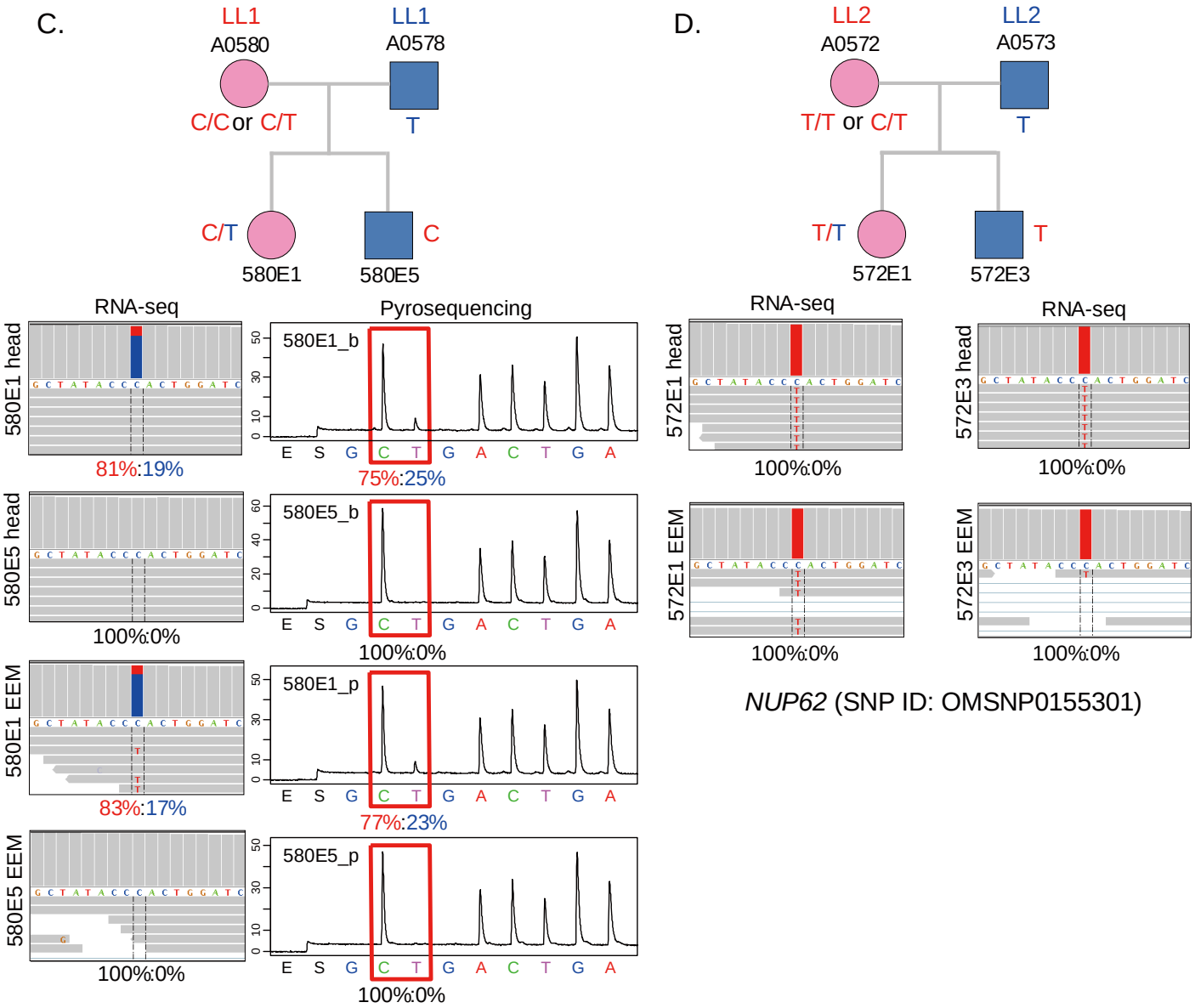
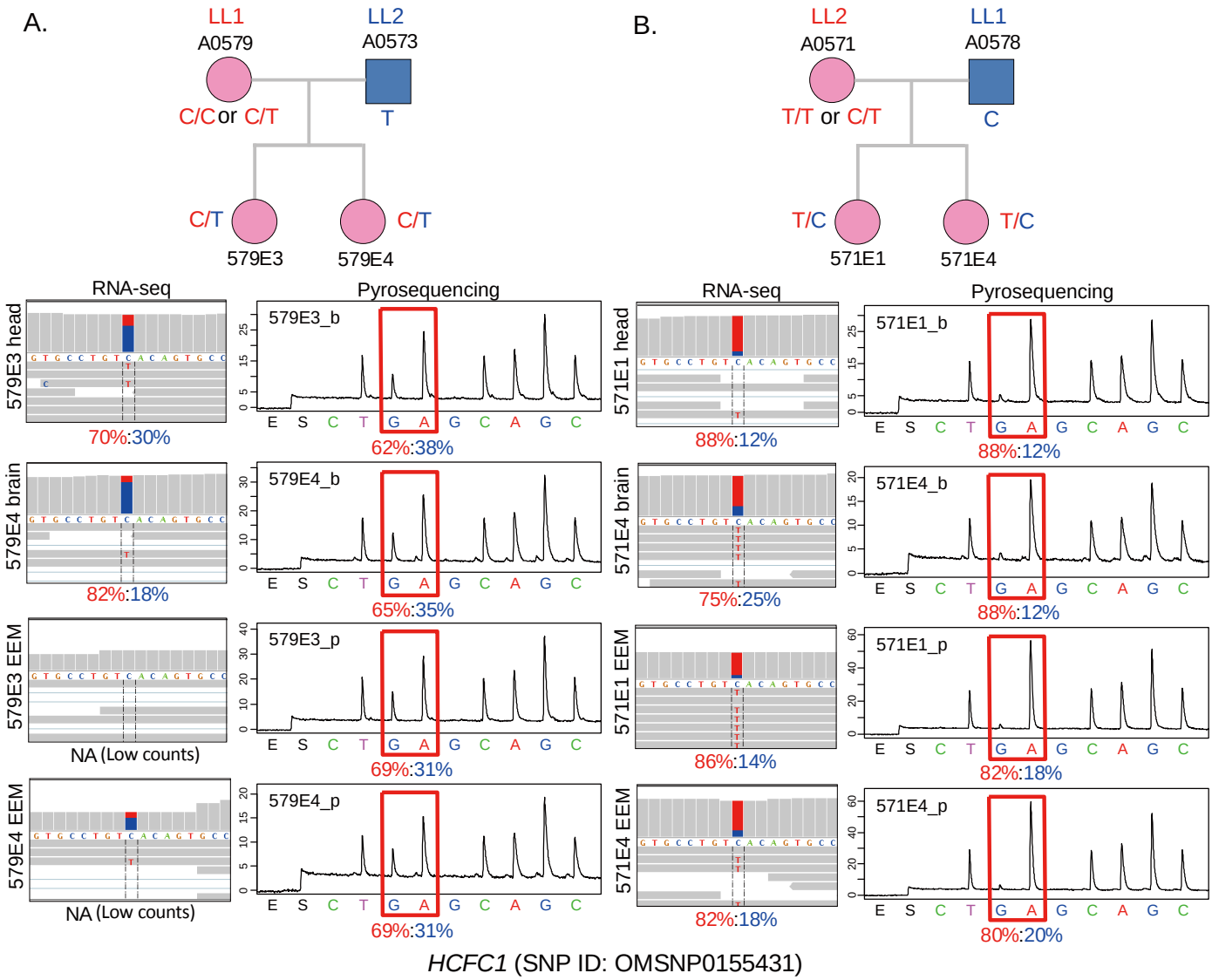
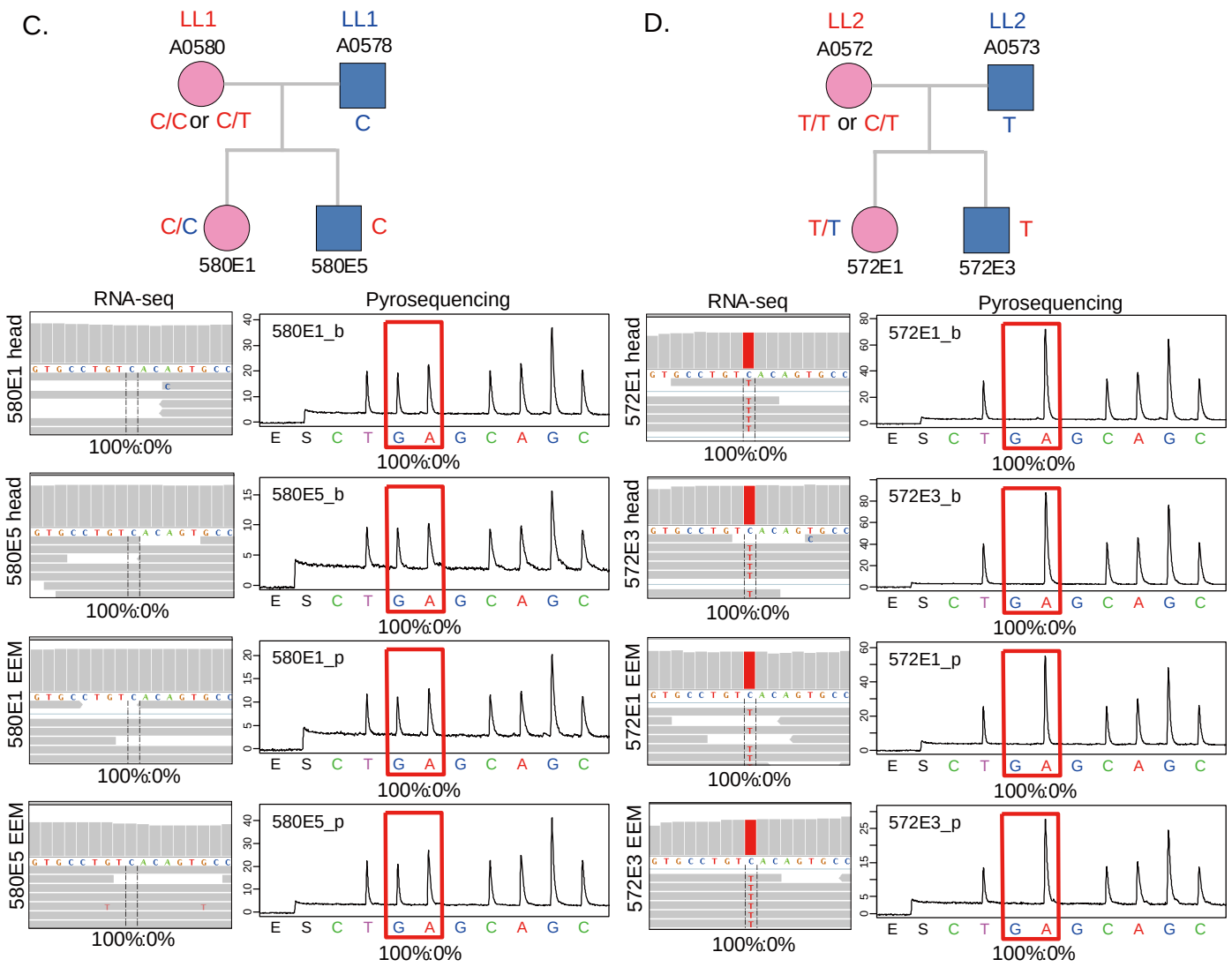

C.
LL1
A0580
LL1
A0578
C/C or C/T
T
C/T
C
580E1
580E5
RNA-seq
Pyrosequencing
580E1 head
580E1_b
81%:19%
75%:25%
580E5 head
580E5_b
100%:0%
100%:0%
580E1 EEM
580E1_p
83%:17%
77%:23%
580E5 EEM
580E5_p
100%:0%
100%:0%
E S G C T G A C T G A
D.
LL2
A0572
LL2
A0573
T/T or C/T
T
T/T
T
572E1
572E3
RNA-seq
RNA-seq
572E1 head
572E3 head
100%:0%
100%:0%
572E1 EEM
572E3 EEM
100%:0%
100%:0%
NUP62 (SNP ID: OMSNP0155301)

Figure S25. RNA-seq and pyrosequencing verification results for escaper gene *HCFC1* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. The SNP (OMSNP0155431) is informative in four embryos (579E3, 579E4, 571E1 and 571E4). In brain/head and EEM tissues of all four individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *HCFC1* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is T(G/A)ACAGGCAC. The SNP genotypes were called from the RNA-seq data and inferred based on the pedigree information. The parental transmission directions were unambiguously determined.

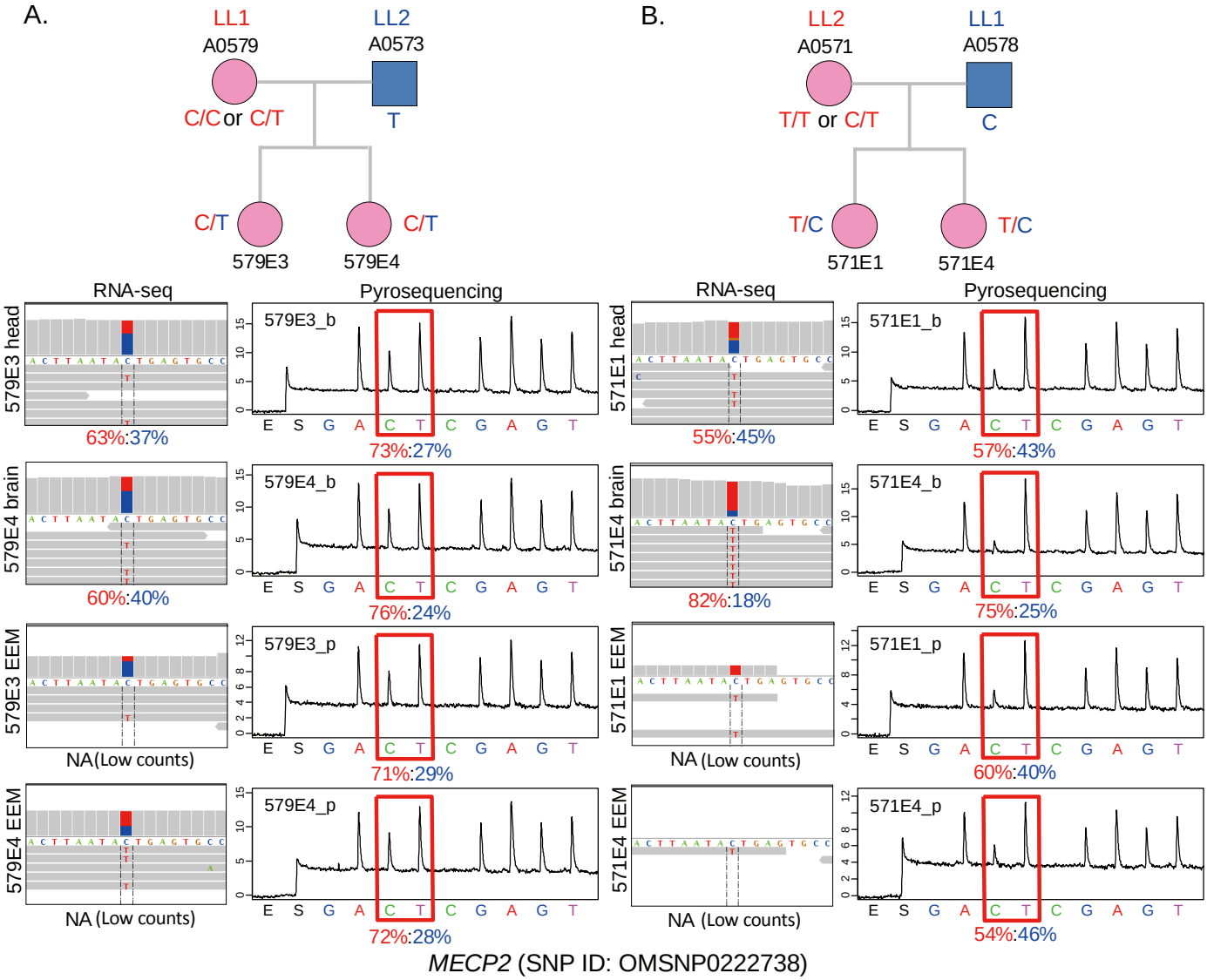


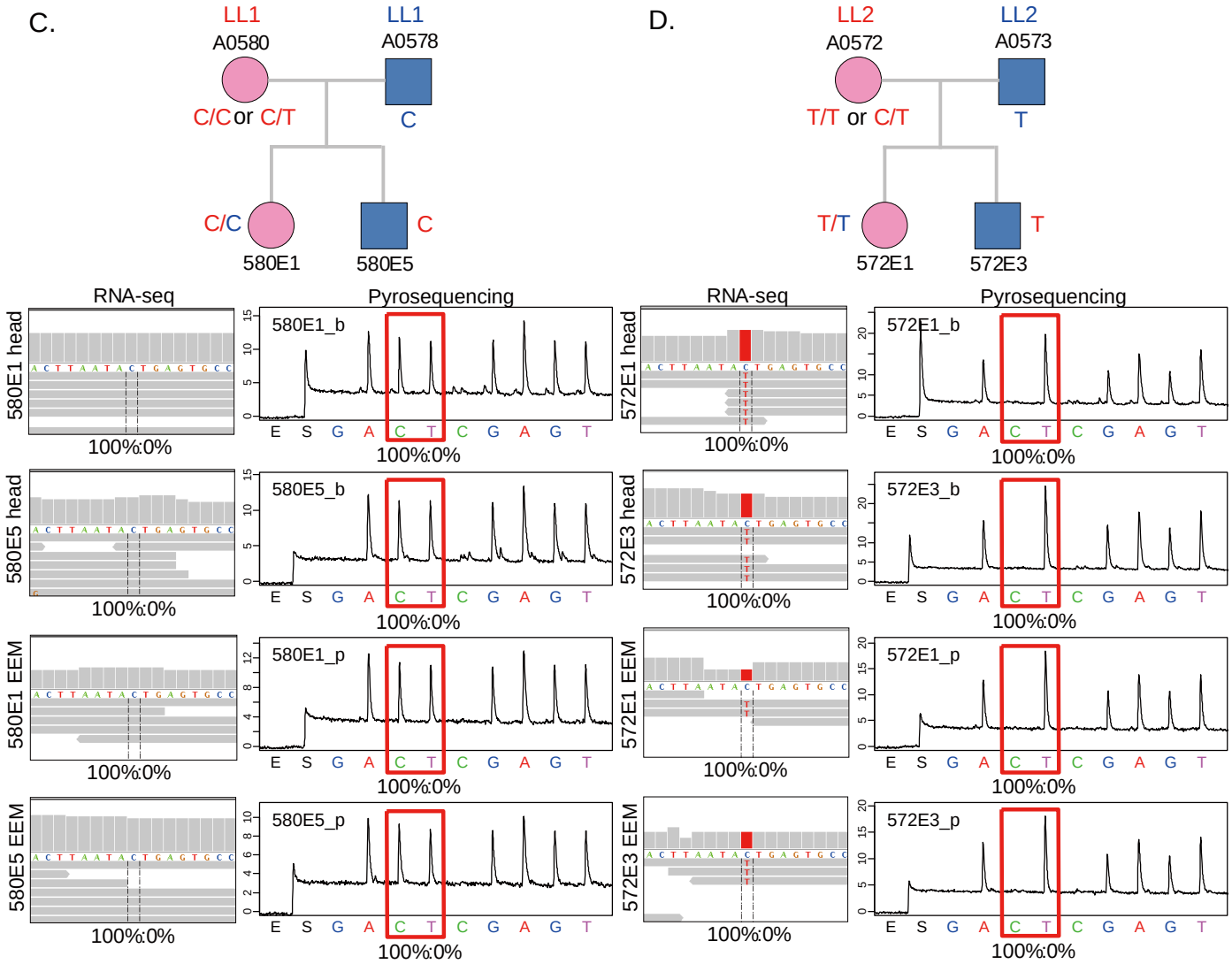


HCFC1 (SNP ID: OMSNP0155431)

Figure S26. RNA-seq and pyrosequencing verification results for escaper gene *MECP2* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. The SNP (OMSNP0222738) is informative in four embryos (579E3, 579E4, 571E1 and 571E4). In brain/head and EEM tissues of all four individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *MECP2* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is A(C/T)TGAGTGCCC. The SNP genotypes were called from the RNA-seq data and inferred based on the pedigree information. The parental transmission directions were unambiguously determined. This SNP is located in an alternatively spliced exon.

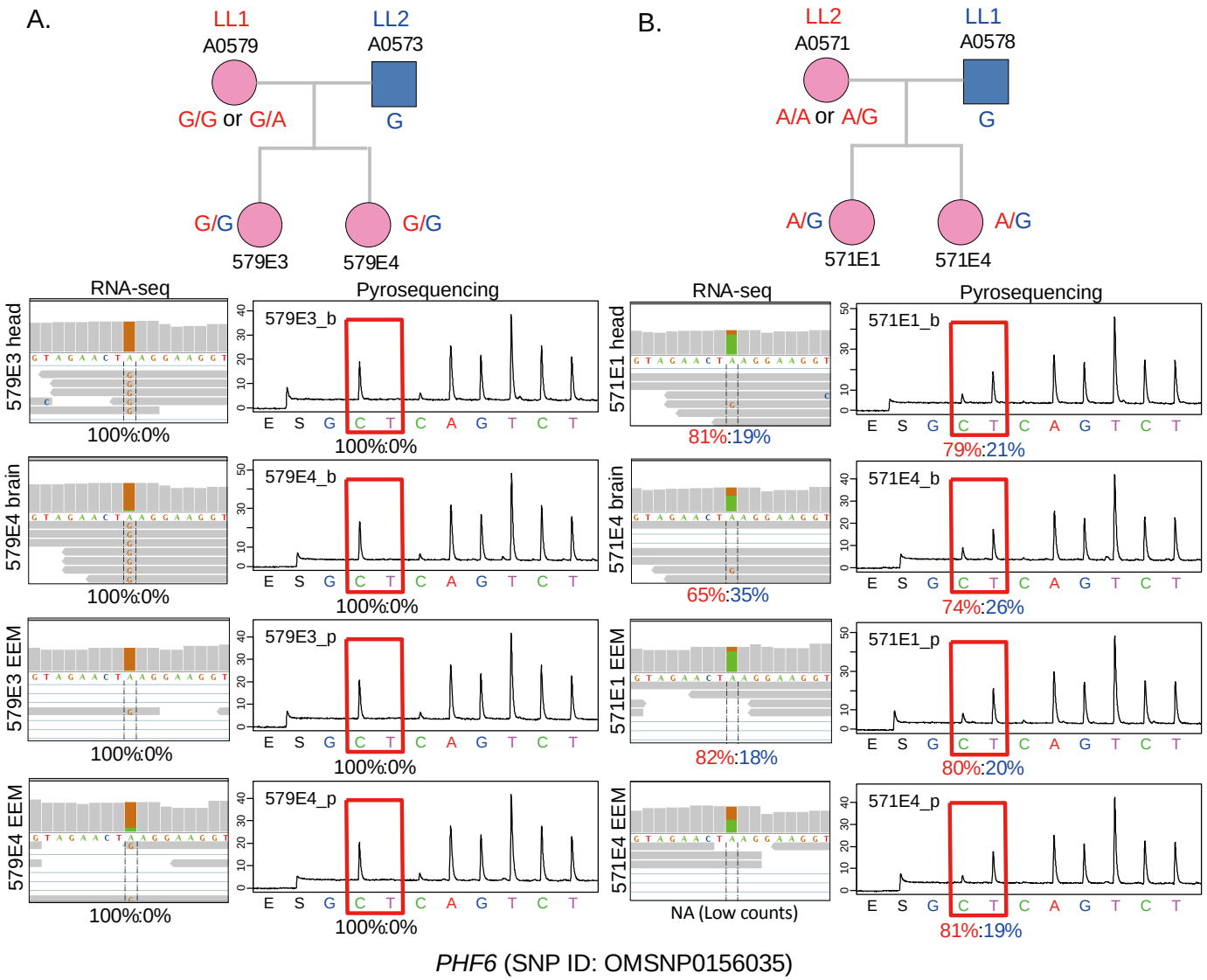


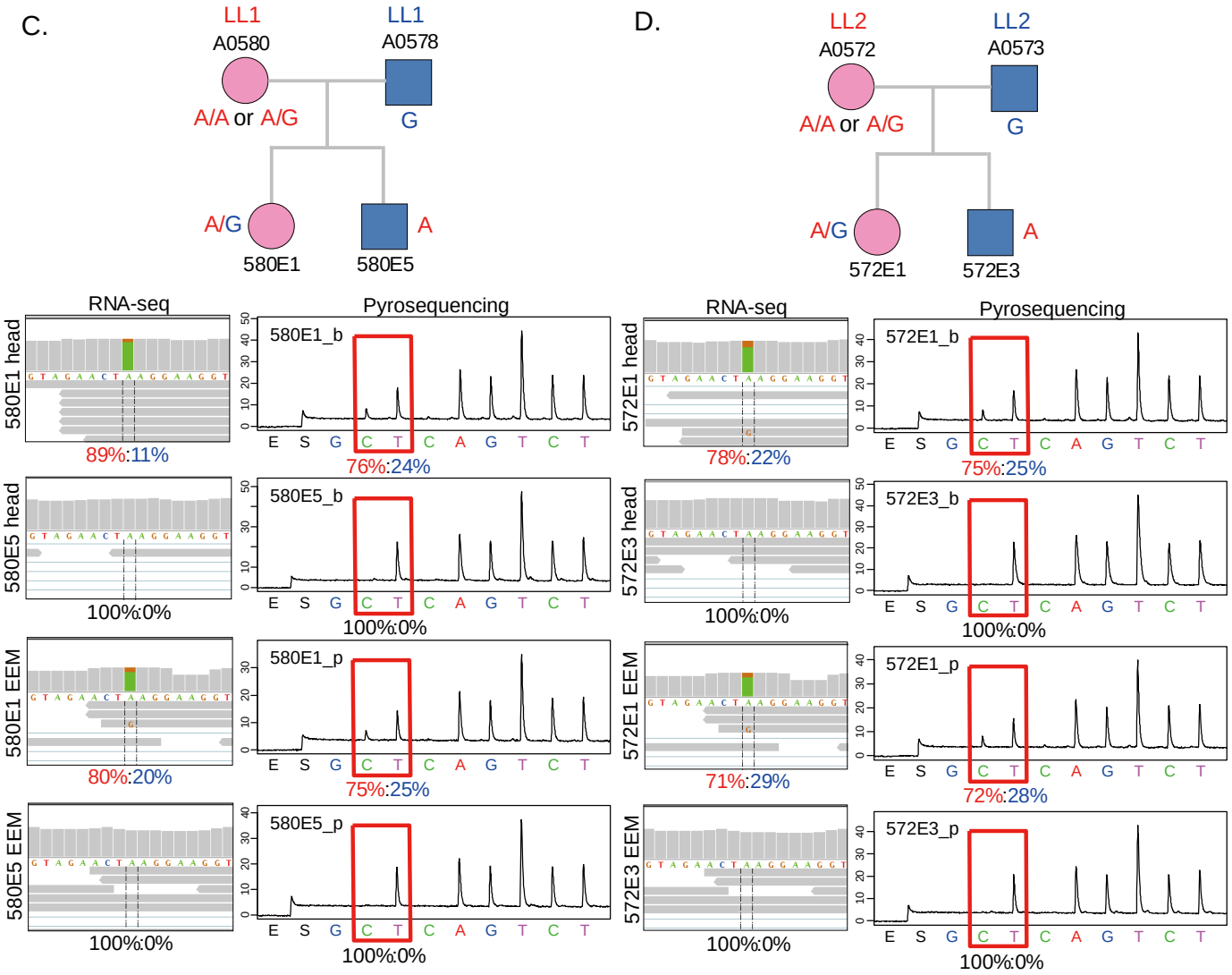


MECP2 (SNP ID: OMSNP0222738)

Figure S27. RNA-seq and pyrosequencing verification results for escaper gene *PHF6* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. The SNP (OMSNP0156035) is informative in four embryos (571E1, 571E4, 580E1 and 572E1). In brain/head and EEM tissues of all four individuals, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *PHF6* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is (C/T)AGTTCTAC. The SNP genotypes were called from the RNA-seq data and inferred based on the pedigree information. The parental transmission directions were unambiguously determined. This SNP is located in an alternatively spliced exon.

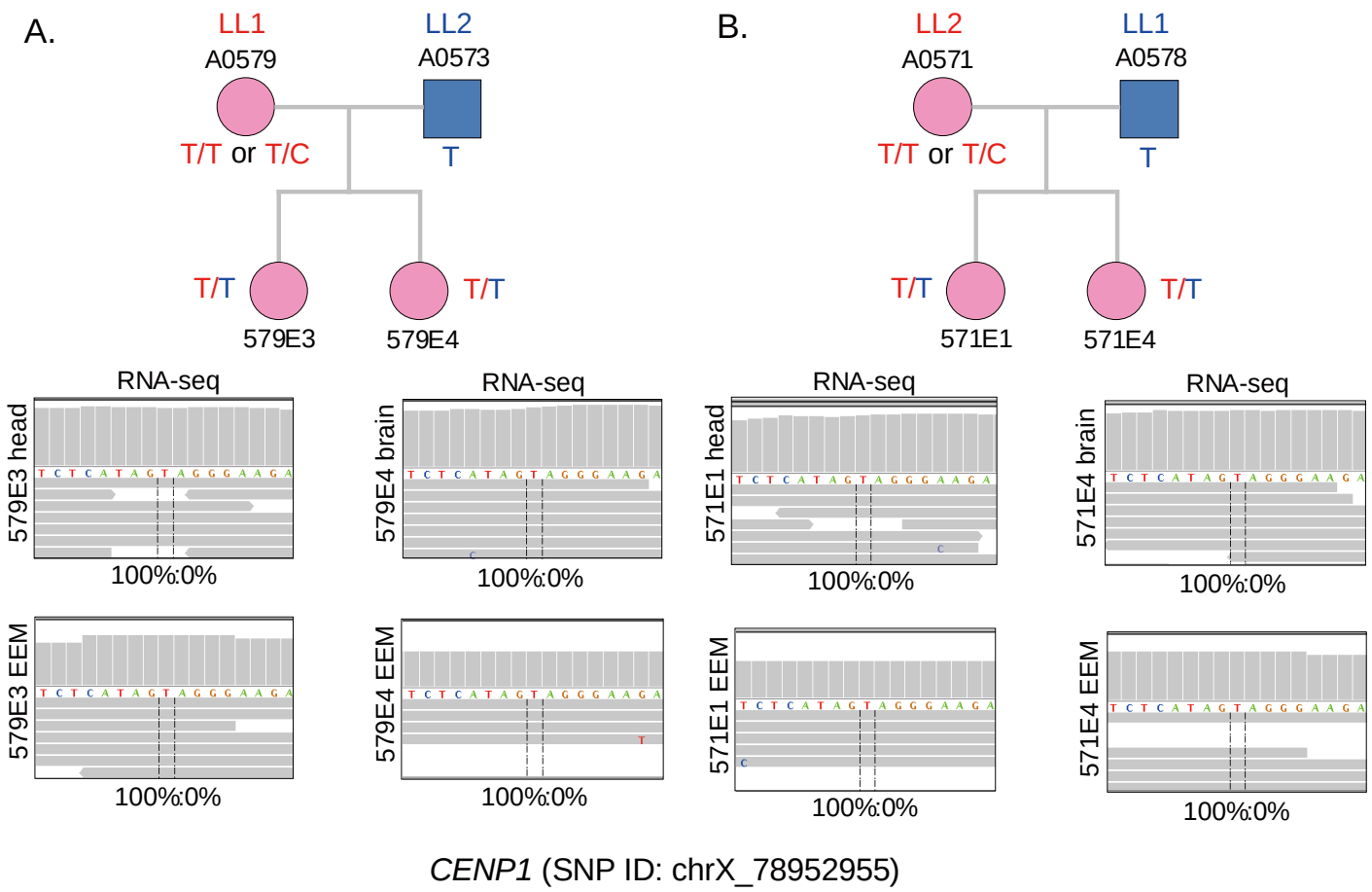




PHF6 (SNP ID: OMSNP0156035)

Figure S28. RNA-seq and pyrosequencing verification results for escaper gene *CENPI* in opossum fetal brain and EEM samples.

(A). F1 cross of LL1 (mother) x LL2 (father). (B) Reciprocal F1 cross of LL2 (mother) x LL1 (father). (C). LL1 parental cross. (D). LL2 parental cross. The SNP (chrX_78952955) is informative in embryo 580E1. In brain/head and EEM tissues of this individual, biallelic expression was observed from both RNA-seq and allele-specific pyrosequencing verification. Therefore, *CENPI* is an escaper of imprinted XCI in both tissues. The target sequence for pyrosequencing is CT(A/G)CTATG (on the minus strand). Pyrosequencing was not performed for two of the four crosses because they are not informative (A and B). The SNP genotypes were called from the RNA-seq data and inferred based on the pedigree information. The parental transmission directions were unambiguously determined.



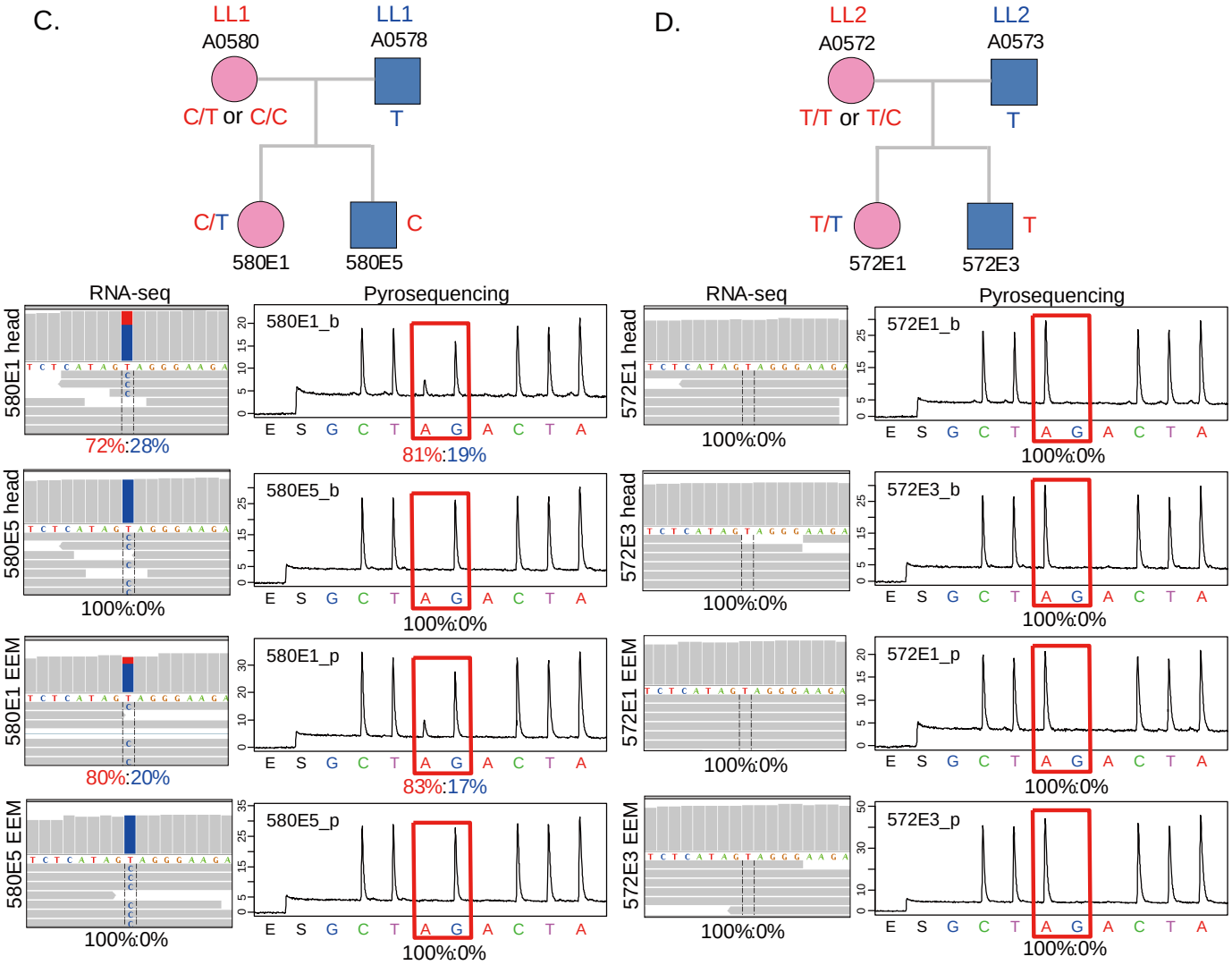

C.
LL1
A0580
LL1
A0578
C/T or C/C
T
C/T
C
580E1
580E5
D.
LL2
A0572
LL2
A0573
T/T or T/C
T
T/T
T
572E1
572E3
RNA-seq
Pyrosequencing
580E1 head
580E1_b
72%:28%
81%:19%
580E5 head
580E5_b
100%:0%
100%:0%
580E1 EEM
580E1_p
80%:20%
83%:17%
580E5 EEM
580E5_p
100%:0%
100%:0%
572E1 head
572E1_b
100%:0%
100%:0%
572E3 head
572E3_b
100%:0%
100%:0%
572E1 EEM
572E1_p
100%:0%
100%:0%
572E3 EEM
572E3_p
100%:0%
100%:0%
E S G C T A G A C T A
CENP1 (SNP ID: chrX_78952955)

Figure S29. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper genes G6PD and IKBKG in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the two escaper genes (*G6PD* and *IKBKG*), the H3K4me3 marks were present at promoter CpG islands, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. For the two nonescapers (*FAM3A* and *SEPHS2*), the H3K4me3 marks were present and the H3K27me3 peaks covered the entire gene body, consistent with monoallelic expression. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 marks were present and H3K27me3 were absent for all expressed genes in this region.

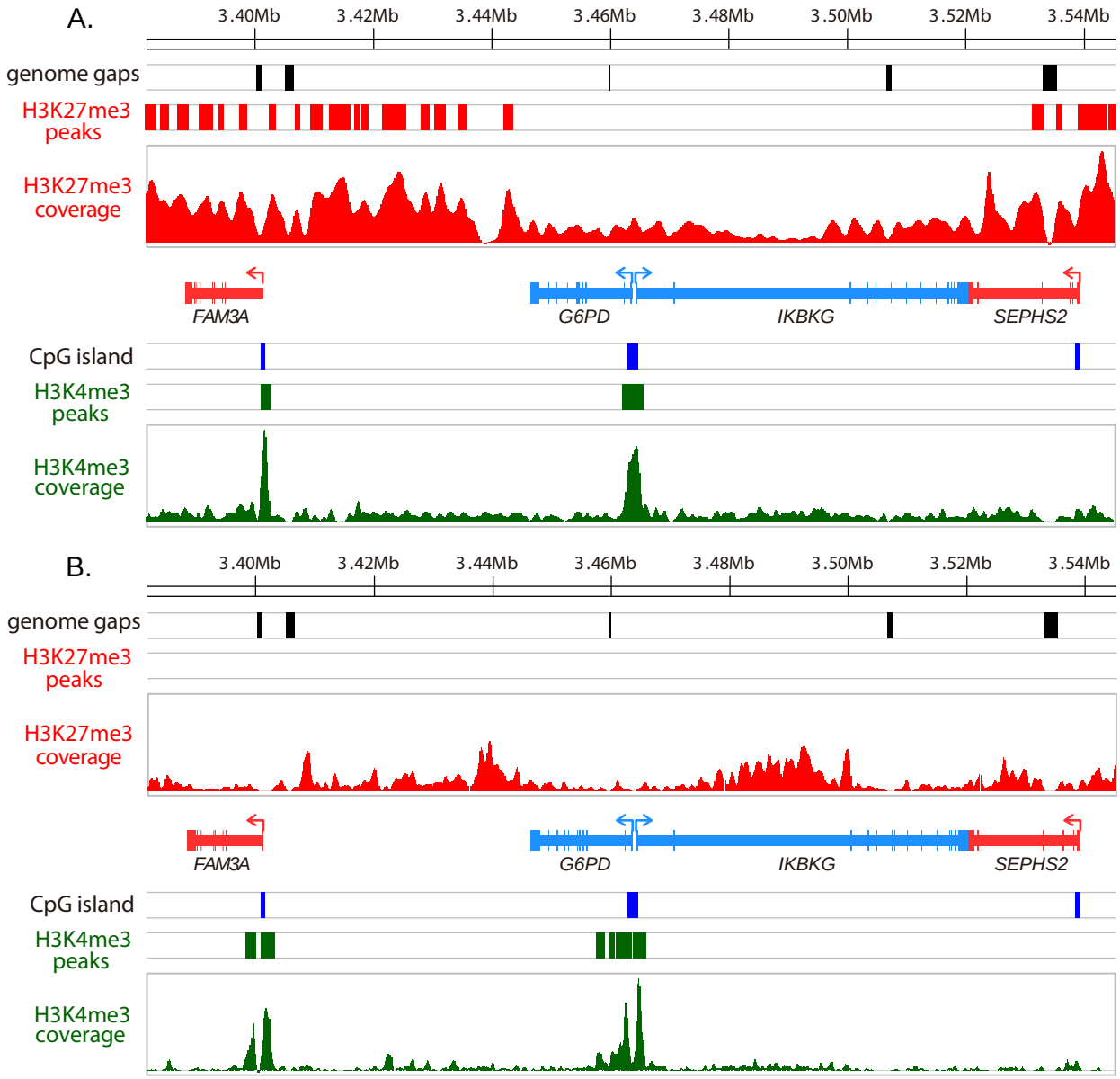


Figure S30. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper genes *FLNA* and *RPL10* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the two escaper genes (*FLNA* and *RPL10*), the H3K4me3 marks were present at promoter CpG islands, suggesting active transcription. The H3K27me3 were depleted across the gene body, consistent with biallelic expression. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 marks were present and H3K27me3 were absent for all expressed genes in this region.

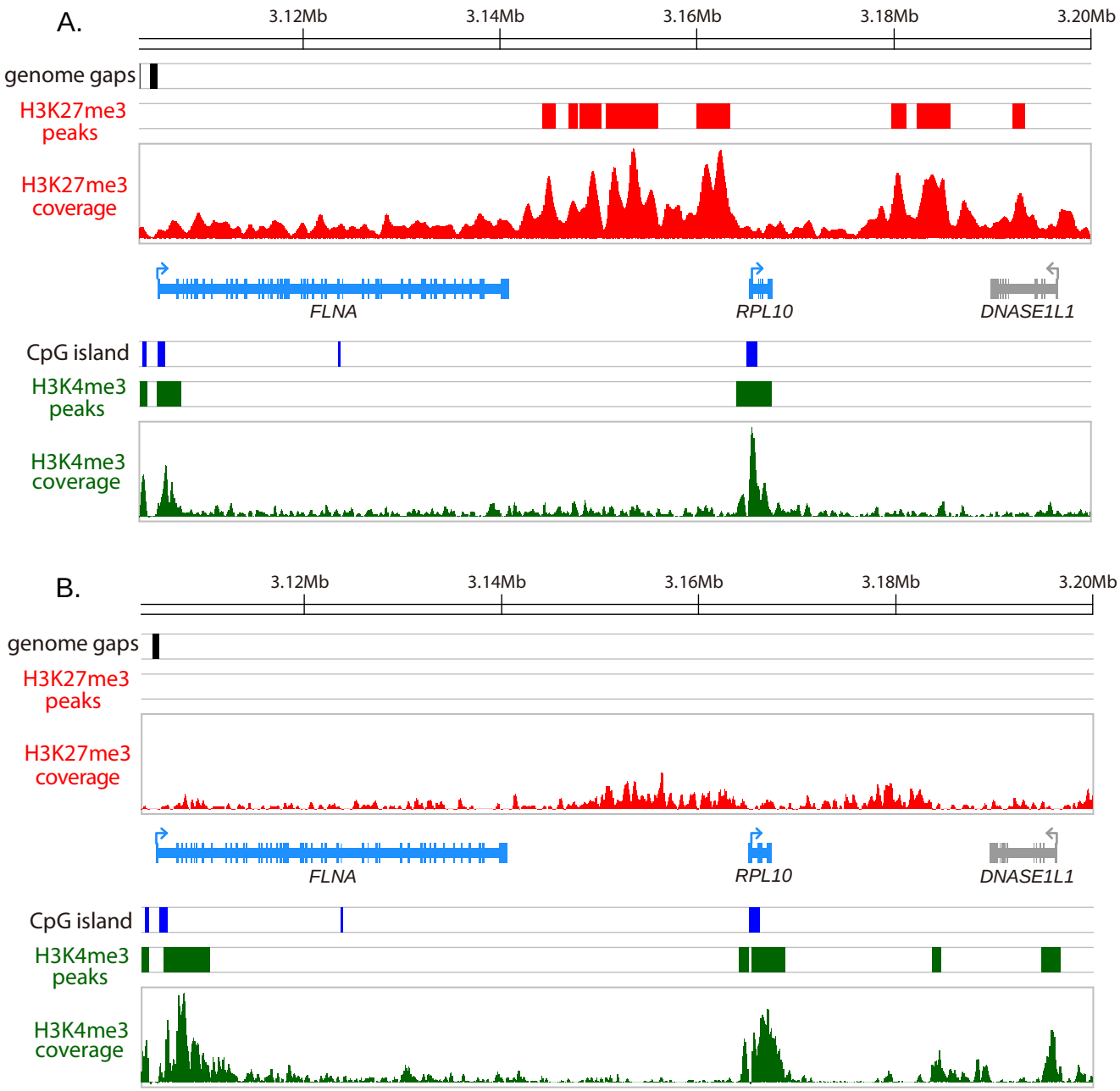


Figure S31. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper gene *PLXNA3* and candidate escaper gene *UBL4A* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the escaper gene *PLXNA3*, the H3K4me3 mark was present at promoter CpG islands, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. The downstream gene *UBL4A* does not have informative SNPs, but its histone modification profile suggests it is a candidate escaper. The other three non-informative genes (*ATP6AP1*, *GDI1* and *SLC10A3*) were covered with H3K27me3 peaks across the entire gene body, consistent with nonescaper status. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 marks were present and H3K27me3 were absent for all five expressed genes in this region.

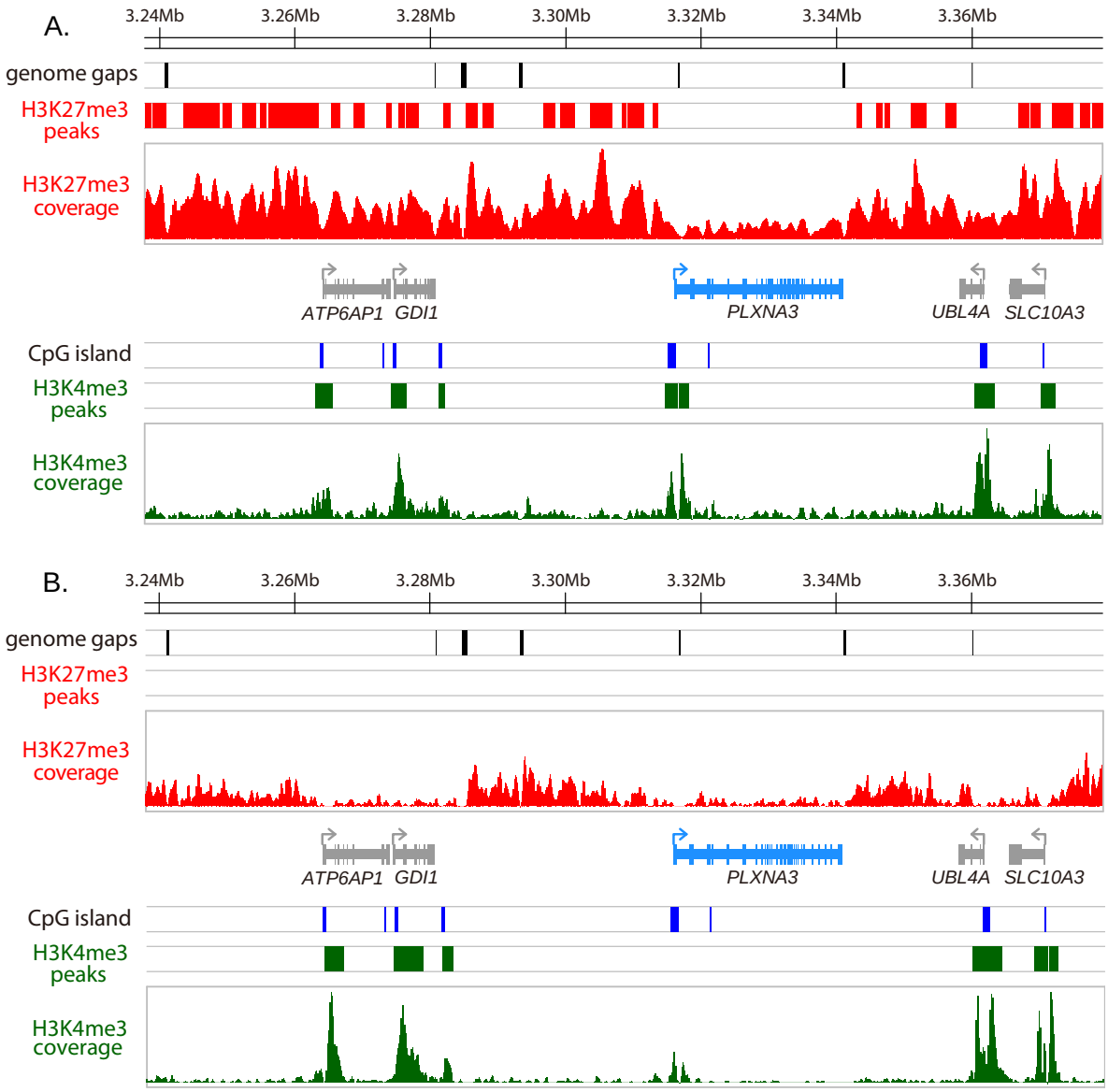


Figure S32. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper gene *DKC1* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the escaper gene *DKC1*, the H3K4me3 marks were present at promoter CpG islands, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. For the nonescaper gene *MPP1*, the H3K27me3 peaks covered the entire gene body, consistent with monoallelic expression. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 marks were present and H3K27me3 marks were absent for all expressed genes in this region.

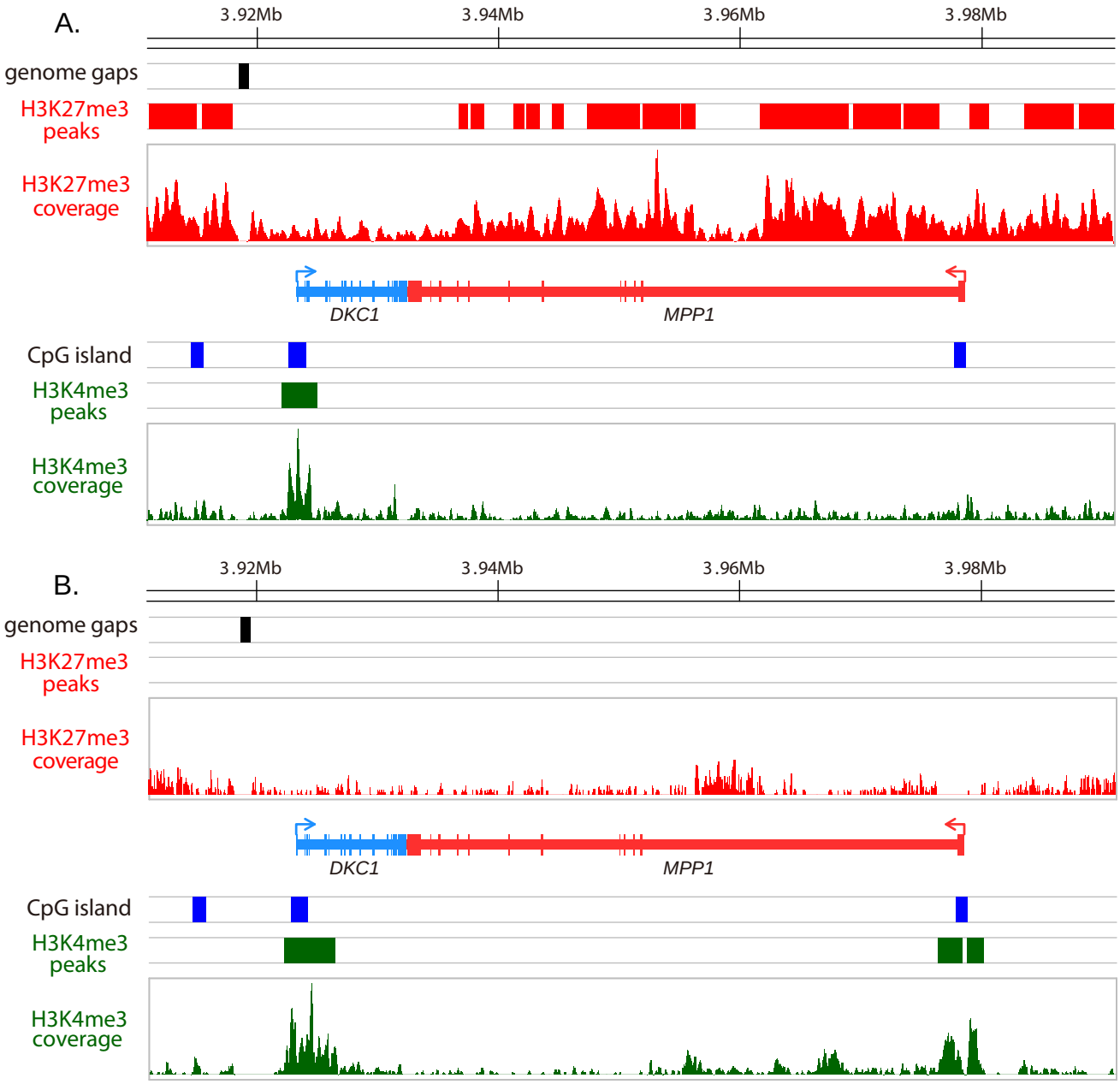


Figure S33. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper gene *YIPF6* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the escaper gene *YIPF6*, the H3K4me3 mark was present at the promoter CpG island, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 mark was present and H3K27me3 was absent for *YIPF6*.

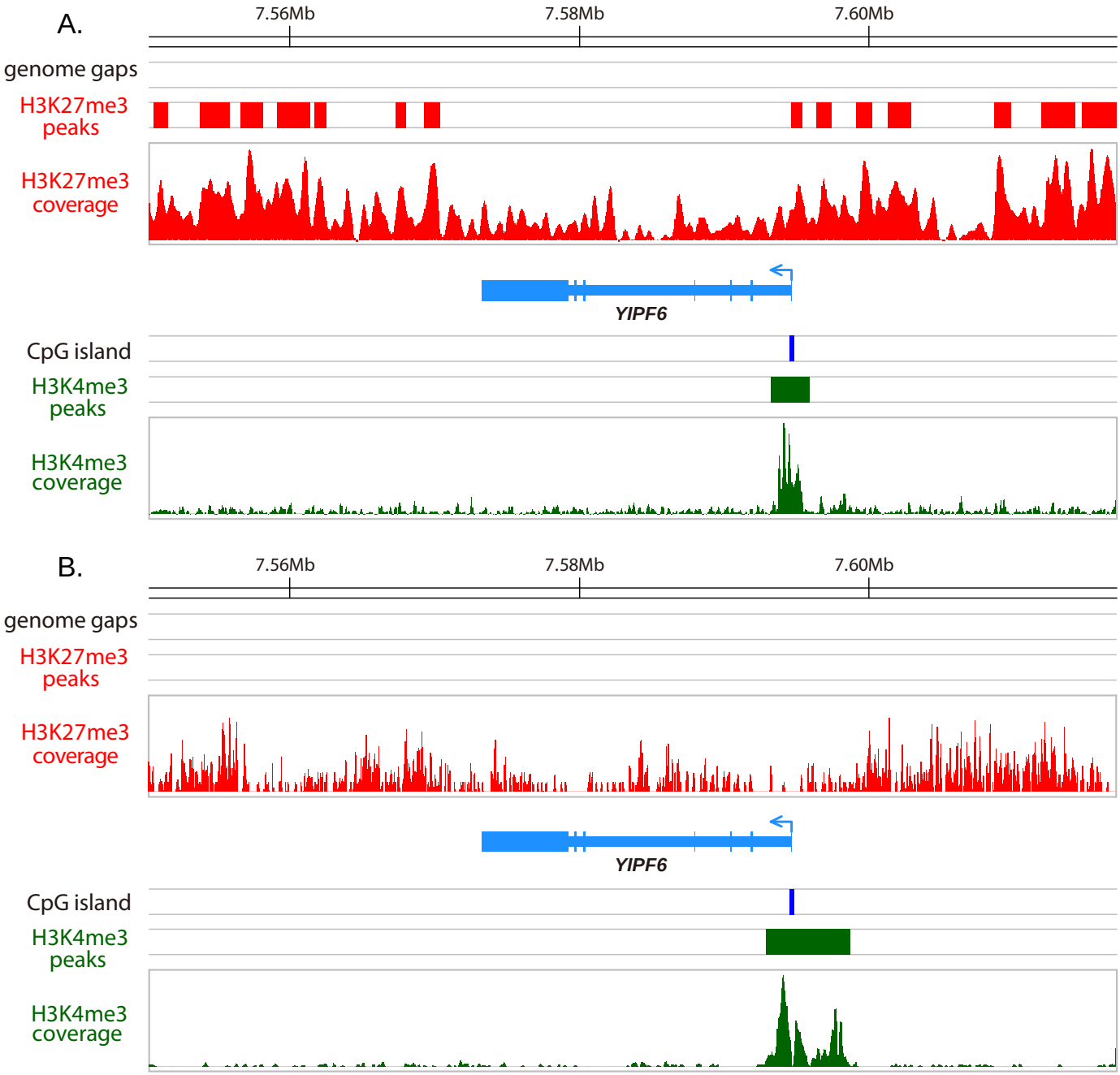


Figure S34. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper gene *NUP62* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the escaper gene *NUP62*, the H3K4me3 mark was present at the promoter CpG island, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. For the nonescaper gene *RBM41*, the H3K4me3 peak was present and the H3K27me3 peaks covered the entire gene body, consistent with monoallelic expression. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 mark was present and H3K27me3 marks were absent for all expressed genes in this region.

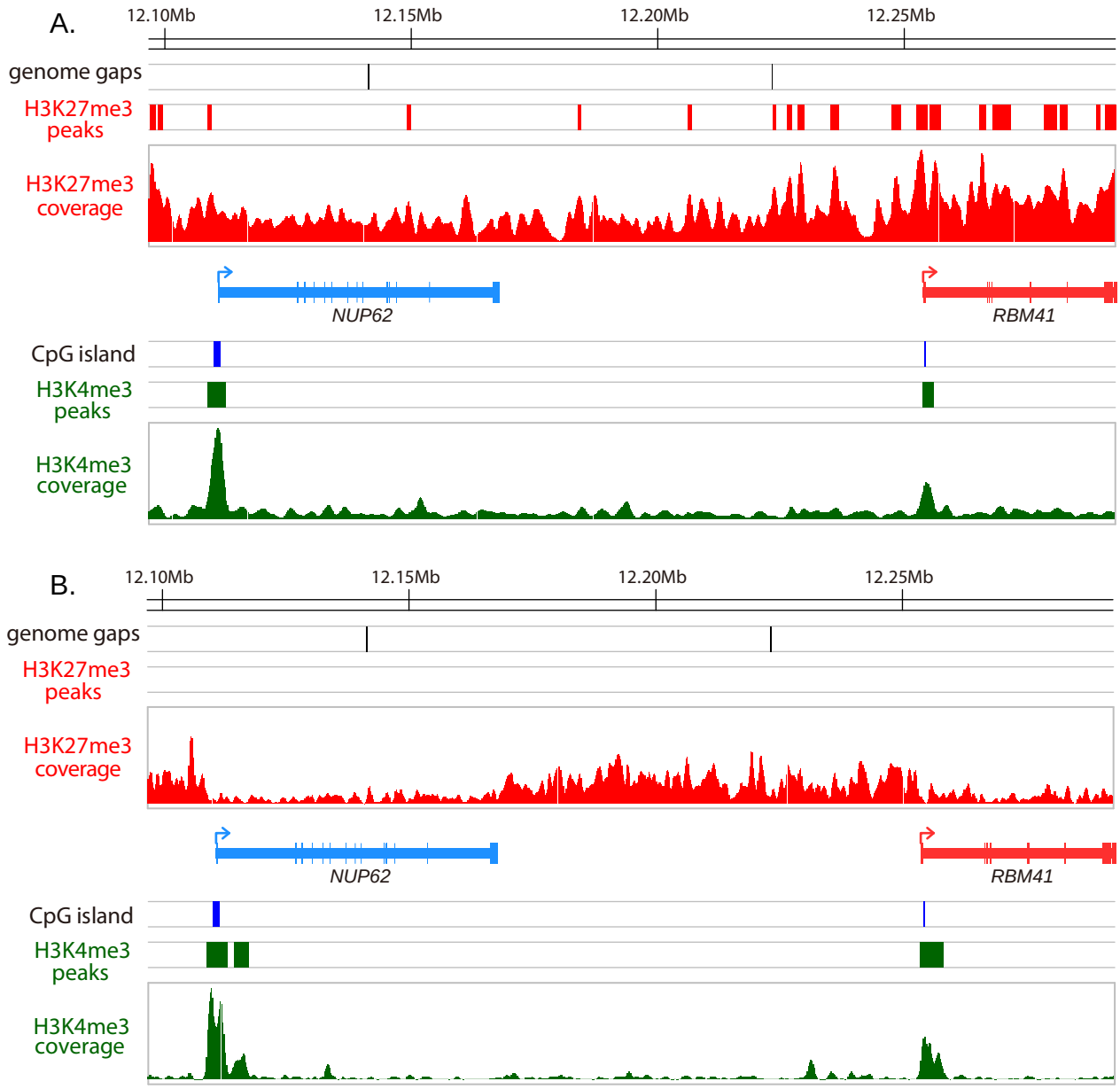


Figure S35. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper genes *HCFC1*, *IRAK1* and *MECP2* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the three escaper genes (*HCFC1*, *IRAK1* and *MECP2*), the H3K4me3 marks were present at promoter CpG islands, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. For the two nonescapers (*TKTL1* and *LOC10002972*), the H3K4me3 marks were present and the H3K27me3 peaks covered the entire gene body, consistent with monoallelic expression. For the rest four genes, there is no informative SNP to infer the XCI status from RNA-seq data, but the histone modification profile of *NAA10* is consistent with escaper status. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 marks were present and H3K27me3 were absent for all expressed genes in this region.

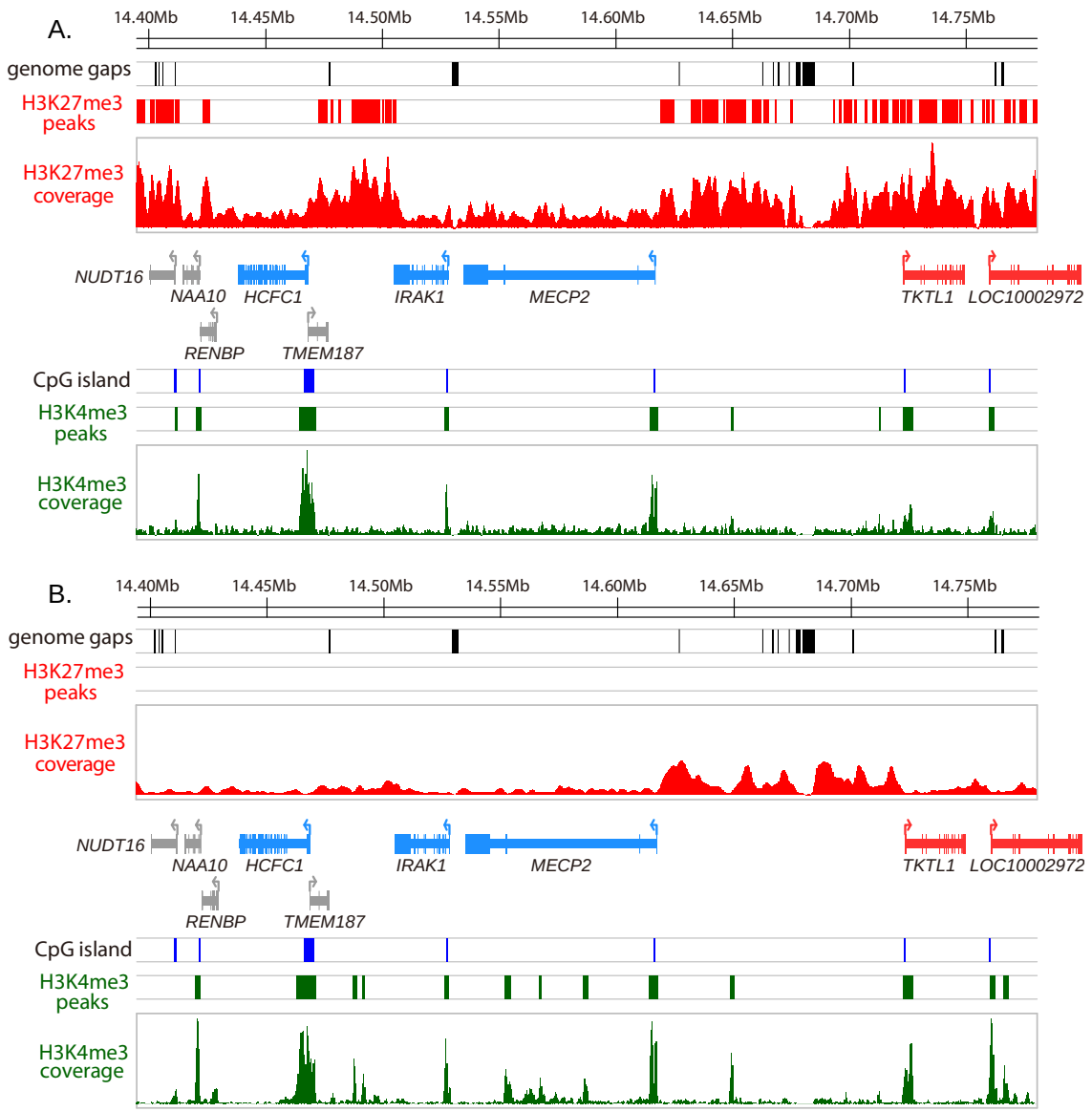


Figure S36. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper gene *FRMD7* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the escaper gene *FRMD7*, the H3K4me3 peak was present at promoter CpG islands, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. For the nonescaper gene *RAP2C*, the H3K4me3 peak was present and the H3K27me3 peak covered the entire gene body, consistent with monoallelic expression. The histone modification profile of the upstream non-informative gene *MST4* is consist with non-escaping status. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 marks were present and H3K27me3 were absent for all expressed genes in this region.

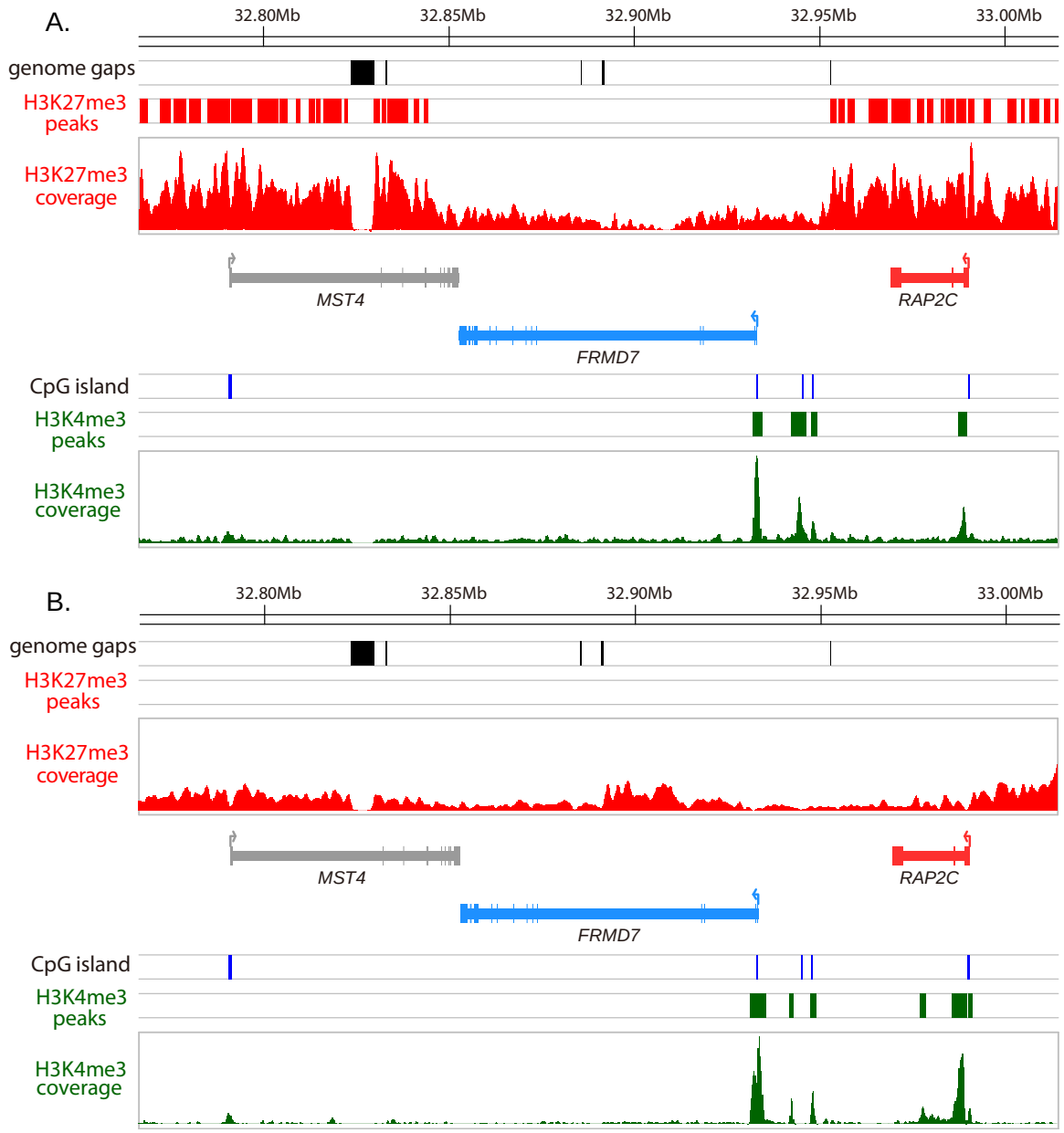


Figure S37. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper genes *PHF6, FAM122B* and *FAM122A* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the three escaper genes (*PHF6*, *FAM122B* and *FAM122A*), the H3K4me3 marks were present at promoter CpG islands, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. For the two nonescapers (*HPRT1* and *MOSPD1*), the H3K4me3 marks were present and the H3K27me3 peaks covered the entire gene body, consistent with monoallelic expression. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 marks were present and H3K27me3 were absent for all expressed genes in this region.

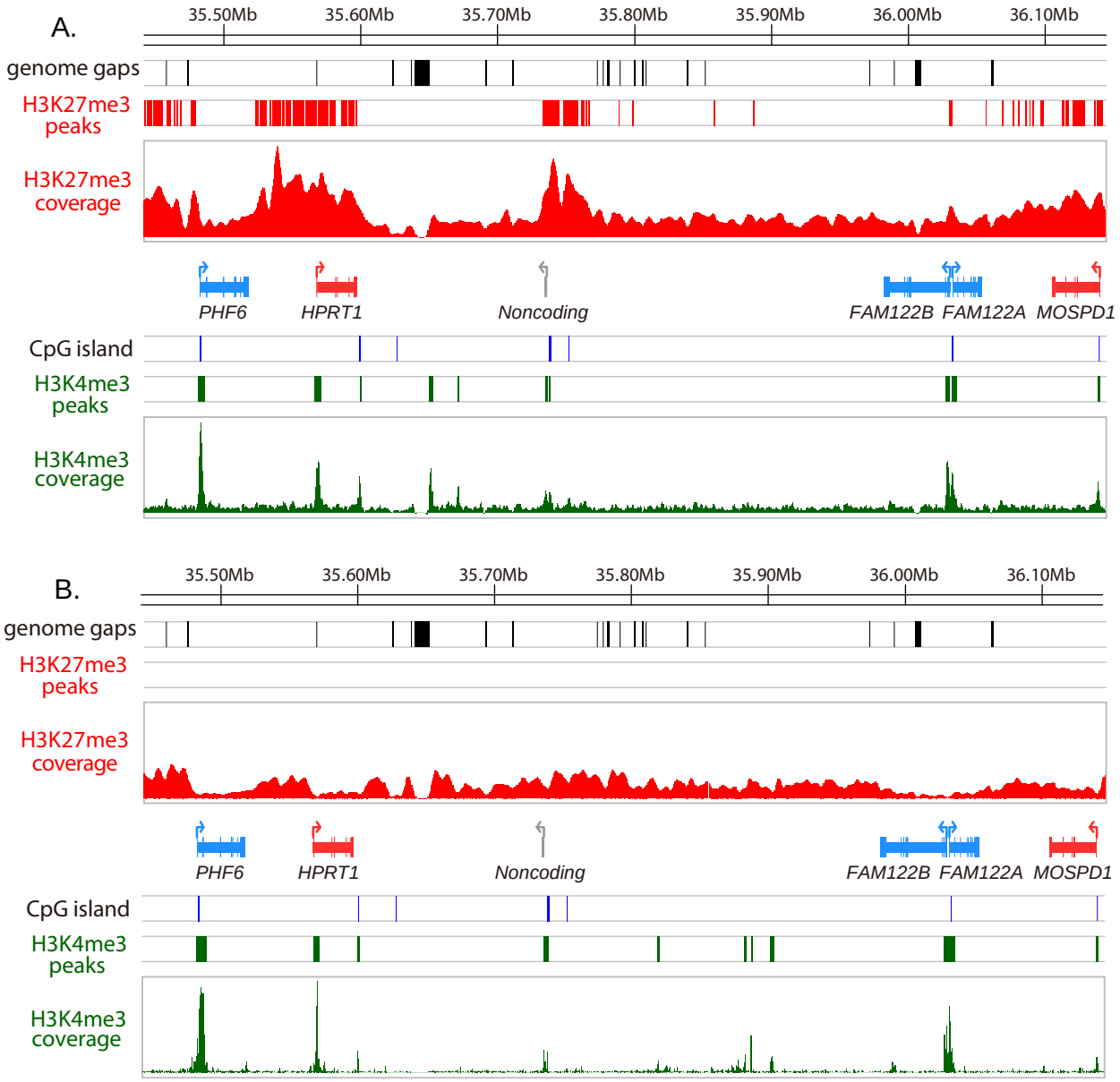


Figure S38. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper genes *MTMR1*, *CD99L2* and *HMGB3* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the three escaper genes (*MTMR1*, *CD99L2* and *HMGB3*), the H3K4me3 marks were present at promoter CpG islands, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. For the two nonescapers (*MTM1* and *GPR50*), the H3K27me3 peaks covered the entire gene body, consistent with monoallelic expression. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 marks were present and H3K27me3 were absent for all expressed genes in this region.

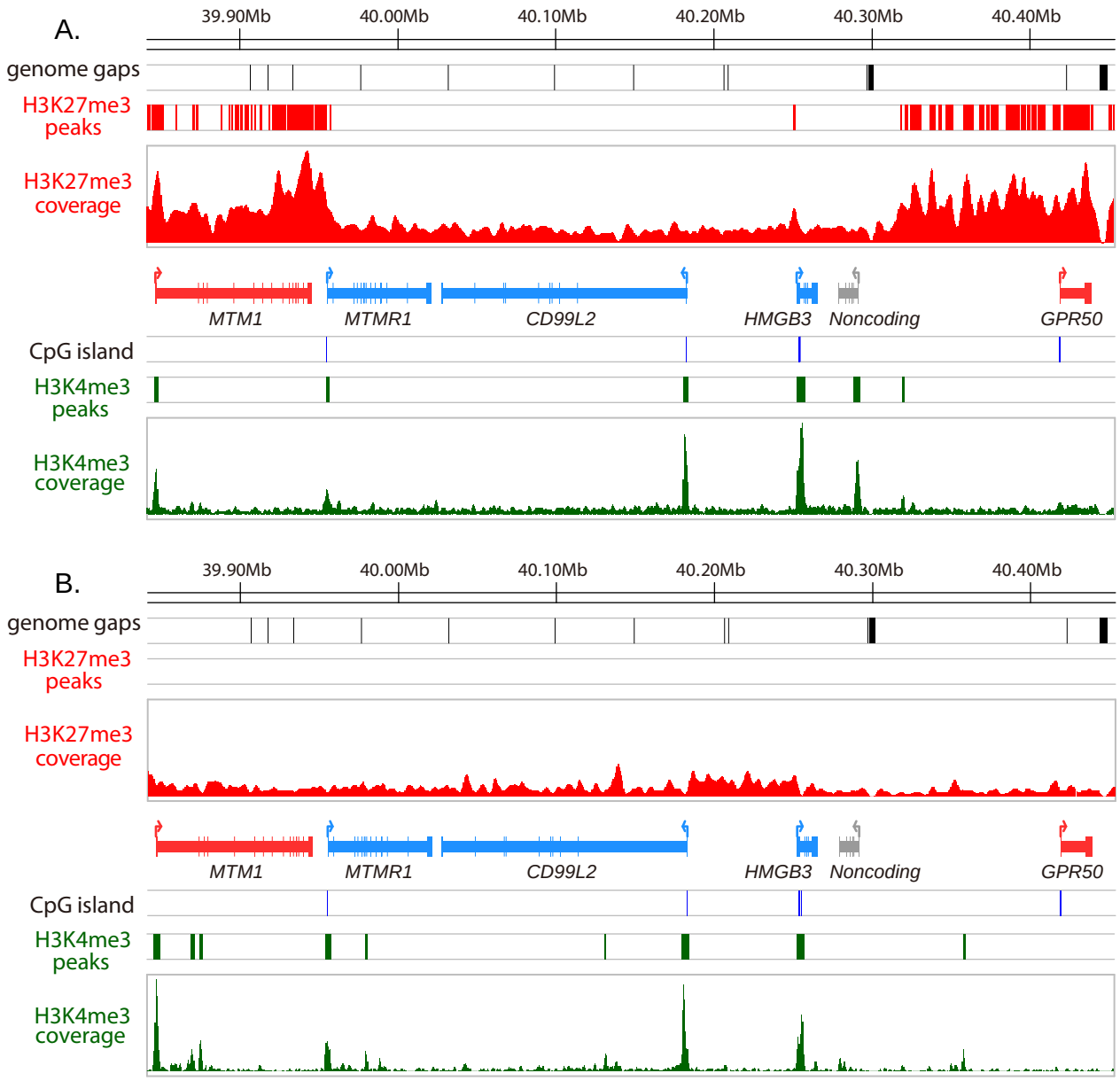


Figure S39. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper gene *RBMX* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the escaper gene *RBMX*, the H3K4me3 mark was present at the promoter CpG island, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. For the two nonescapers (*ARHGEF6* and *TM9SF2*), the H3K4me3 marks were present and the H3K27me3 peaks covered the entire gene body, consistent with monoallelic expression. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 mark was present and H3K27me3 marks were absent for all expressed genes in this region.

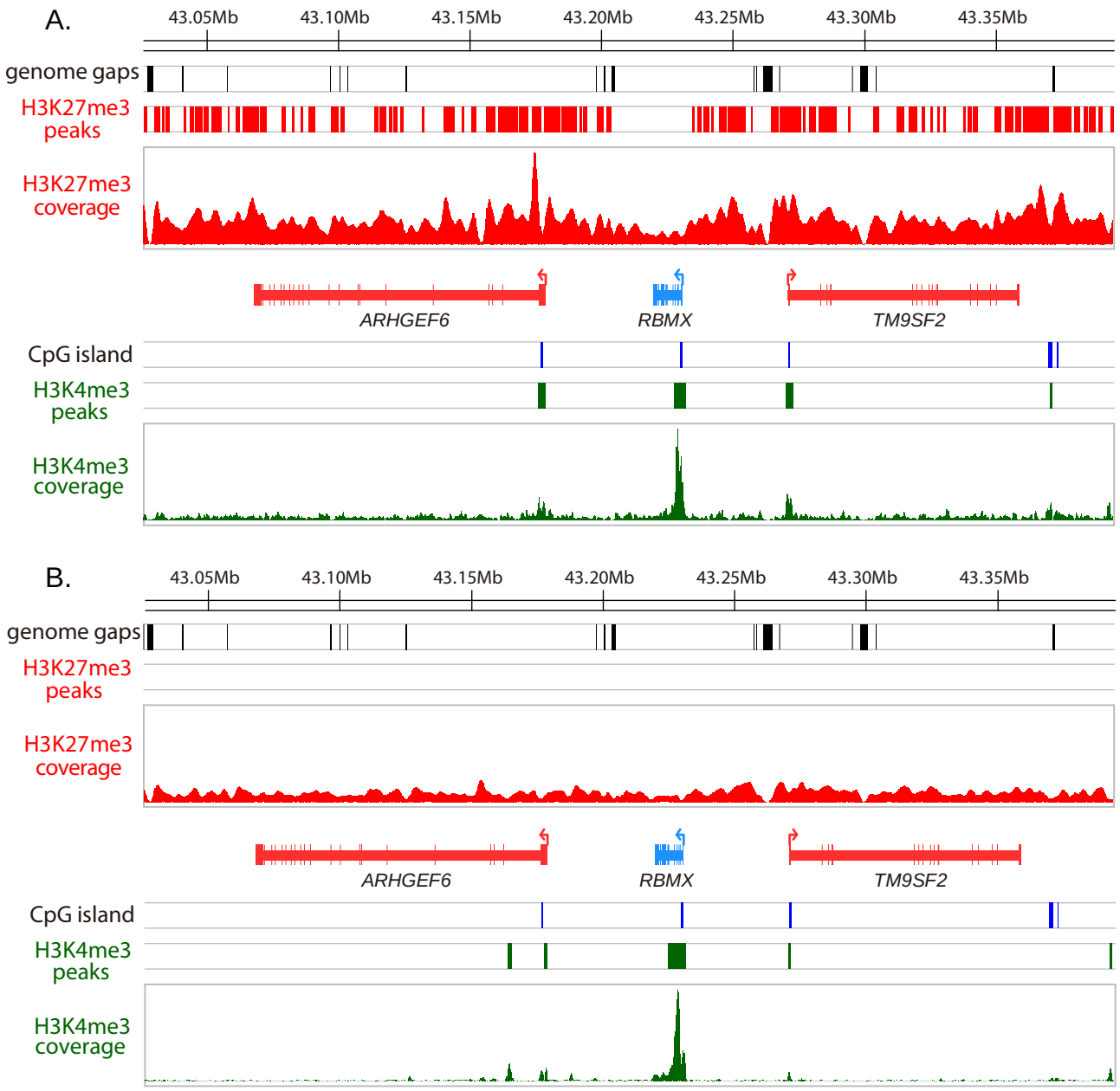


Figure S40. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper gene *ATRX* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the escaper gene *ATRX*, the H3K4me3 mark was present at the promoter CpG island, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. For the two nonescapers (*MAGT1* and *COX7B*), the H3K4me3 marks were present and the H3K27me3 peaks covered the entire gene body, consistent with monoallelic expression. The non-informative upstream gene *FGF16* was covered with H3K27me3 peaks across the entire gene body, consistent with nonescaper status. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 mark was present and H3K27me3 marks were absent for all expressed genes in this region.

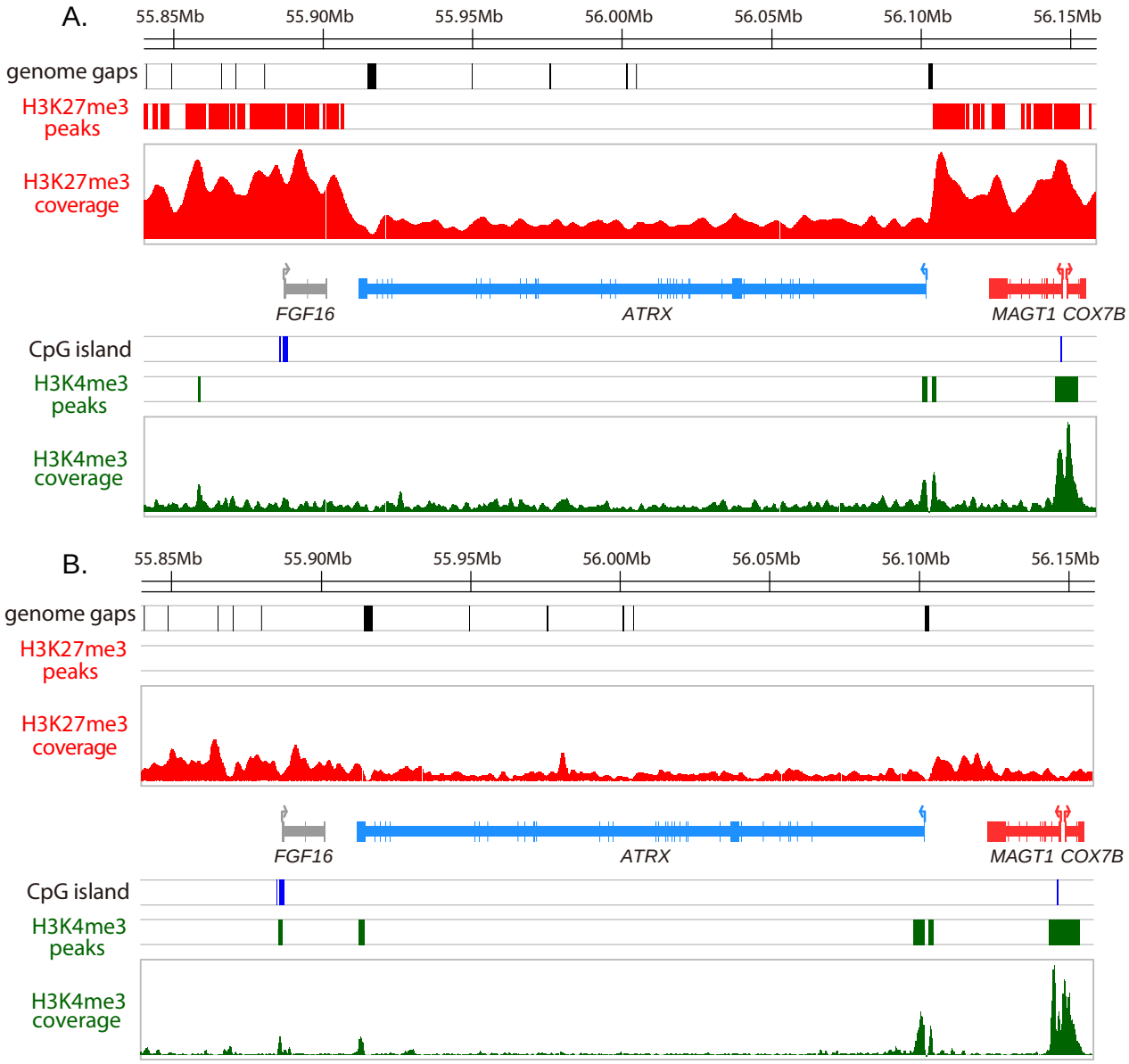


Figure S41. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper genes *TAF1* and *NONO* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the two escaper genes (*TAF1* and *NONO*), the H3K4me3 marks were present at promoter CpG islands, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. For the three nonescapers (*APEX2*, *ZMYM3* and *NLGN3*), the H3K27me3 peaks covered the entire gene body, consistent with monoallelic expression. Three non-informative genes (*OGT*, *RHOG* and *ITGB1BP2*) in the H3K27me3 depleted region are consistent with escaper status. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 marks were present and H3K27me3 were absent for all expressed genes in this region.

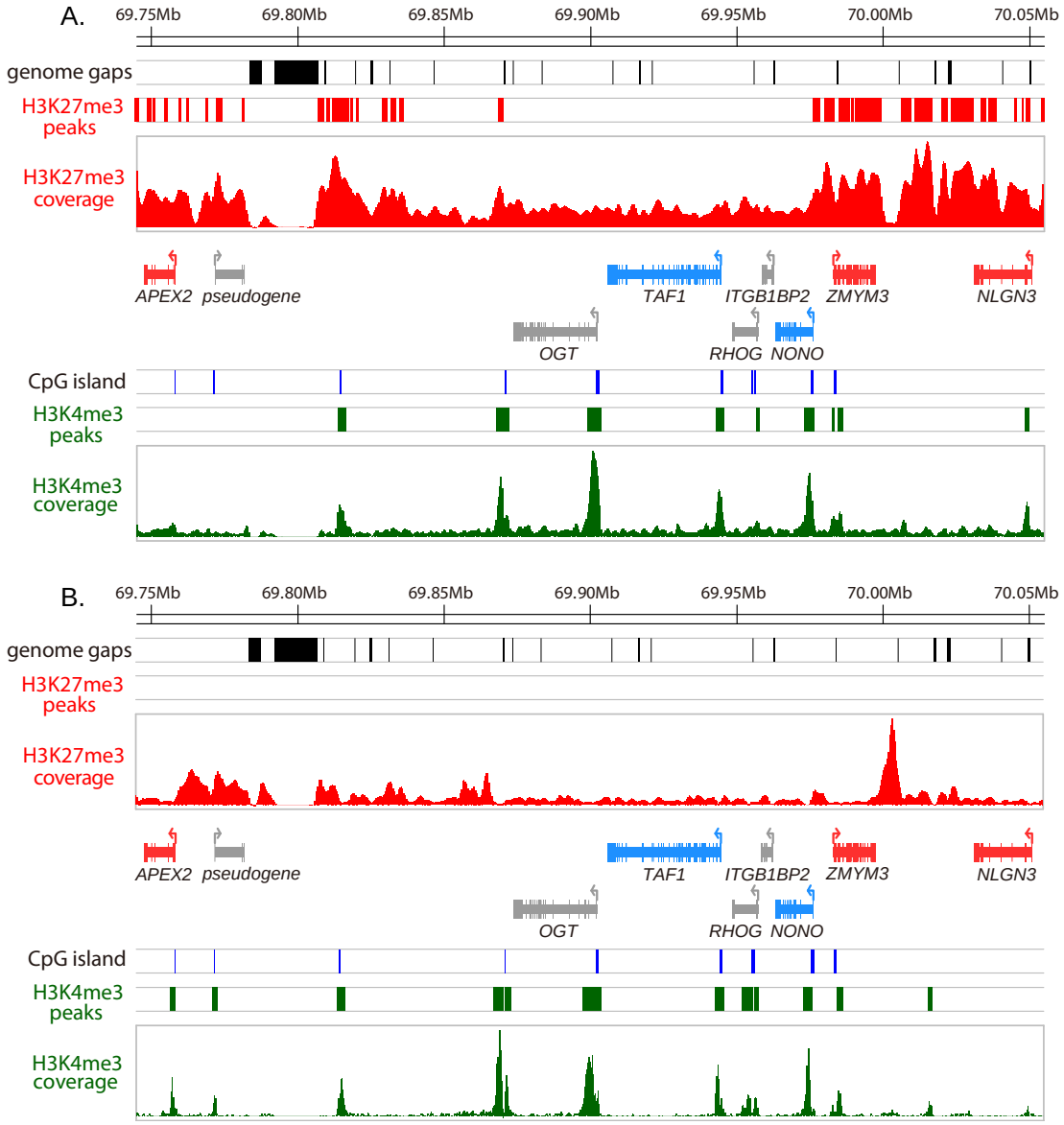


Figure S42. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing escaper gene *CENPI* and candidate escaper gene *CSTF2* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the escaper gene *CENPI*, the H3K4me3 mark was present at promoter CpG islands, suggesting active transcription. The H3K27me3 marks were depleted across the gene body, consistent with biallelic expression. For the nonescaper *SYTL4*, the H3K27me3 peaks covered the entire gene body, consistent with monoallelic expression. The downstream gene *CSTF2* does not have informative SNPs, but its histone modification profile suggests it is a candidate escaper. The other two non-informative genes (*TMEM35* and *XKRX*) were covered with H3K27me3 peaks across the entire gene body, consistent with nonescaper status. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 marks were present and H3K27me3 were absent for all expressed genes in this region.

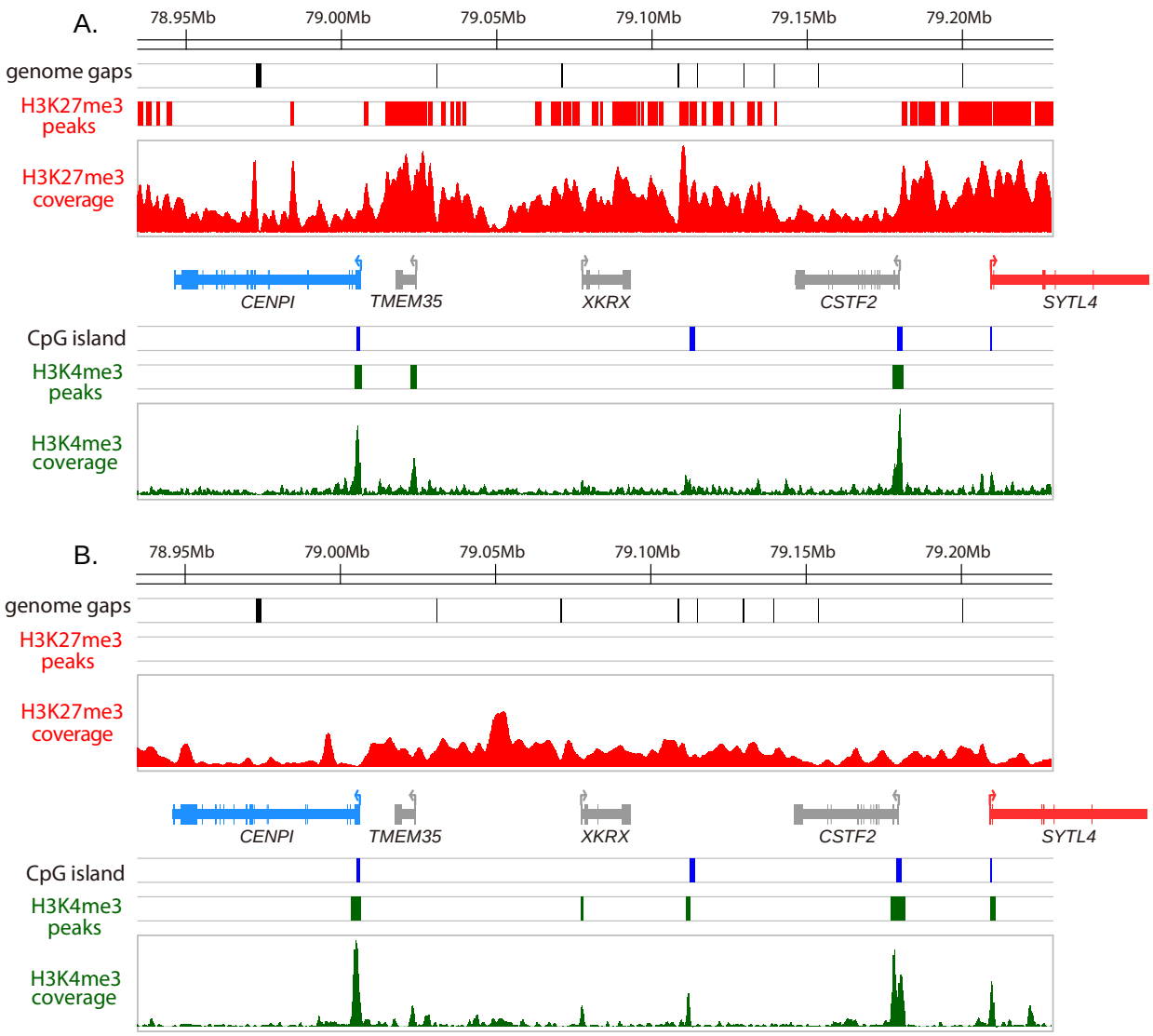


Figure S43. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing candidate escaper gene *ZFP347L* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. In females for the non-informative candidate escaper gene *ZFP347L*, the H3K4me3 mark was present at the promoter CpG island, indicating active transcription. The H3K27me3 marks were depleted across the gene body, suggesting biallelic expression. For the nonescaper gene *CENPBD1*, the H3K4me3 peak was present and the H3K27me3 peaks covered the entire gene body, consistent with monoallelic expression. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 mark was present and H3K27me3 marks were absent for all expressed genes in this region.

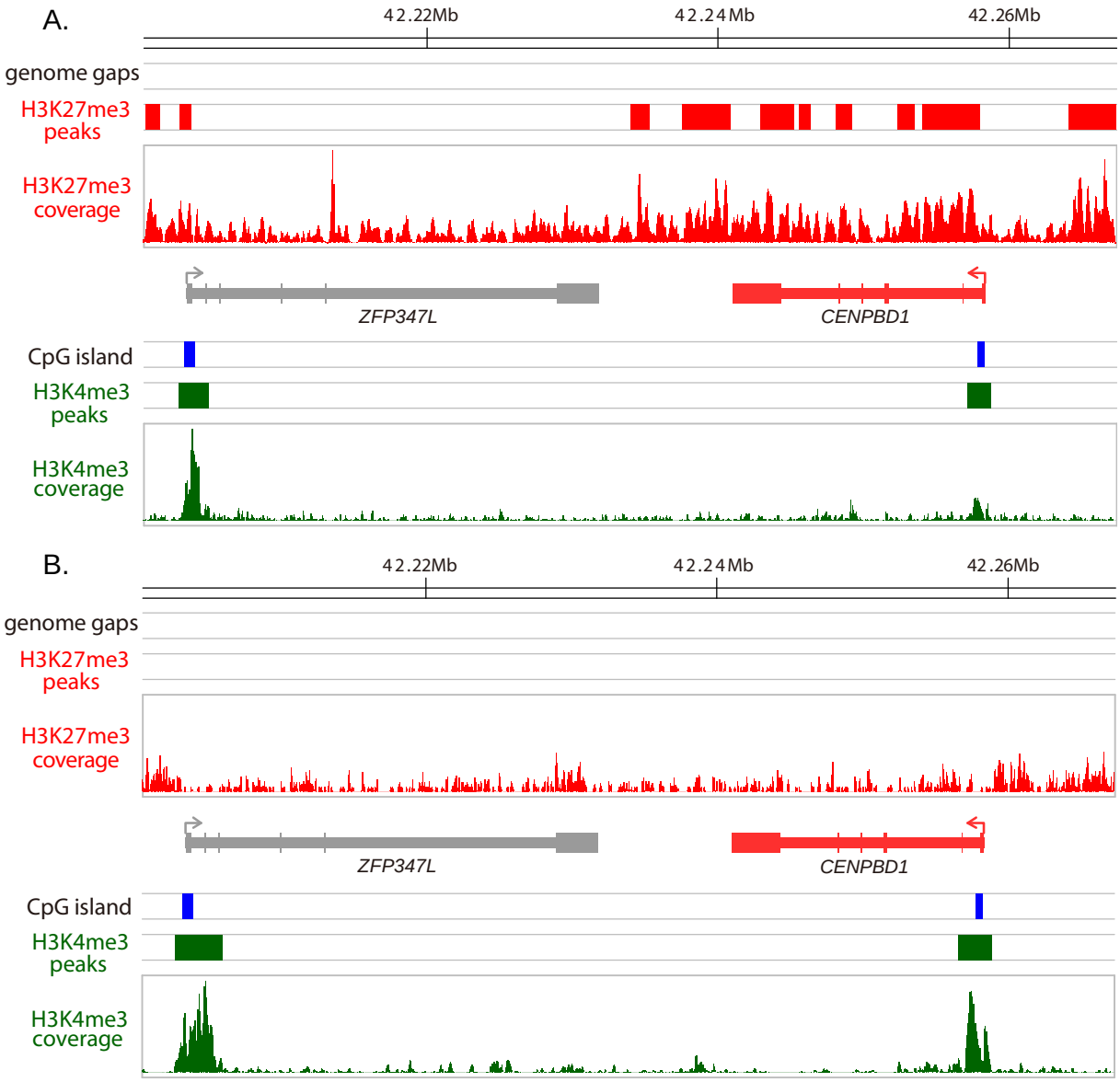


Figure S44. Histone modification H3K4me3 and H3K27me3 peaks and coverage profile for X chromosome region containing candidate escaper gene *CCDC22* and *KDM5C* in female head and control male fibroblasts from ChIP-seq data. (A). ChIP-seq results from female fetal head sample. (B) ChIP-seq results from control male fibroblasts. In each figure, plotted in the top panel are the genome gap locations and the H3K27me3 peaks and coverage. Gene models are shown in the middle panel, color-coded based on their imprinted XCI status (blue: escapers; red: non-escapers; gray: not known due to lack of informative SNPs). In the bottom panel are the CpG island locations and the H3K4me3 peaks and coverage profile. There are four non-informative genes in this region. *PPP1R3F* was expressed at low level. In females for *CCDC22* and *KDM5C*, the H3K4me3 mark was present at the promoter CpG islands and H3K27me3 marks were depleted across the gene body, suggesting escaper status. *GPR173* was partly covered with H3K27me3 peak, which is a potential non-escaper. In the control male sample, there is only one copy of X-linked genes, therefore the H3K4me3 mark was present and H3K27me3 marks were absent for all expressed genes in this region.

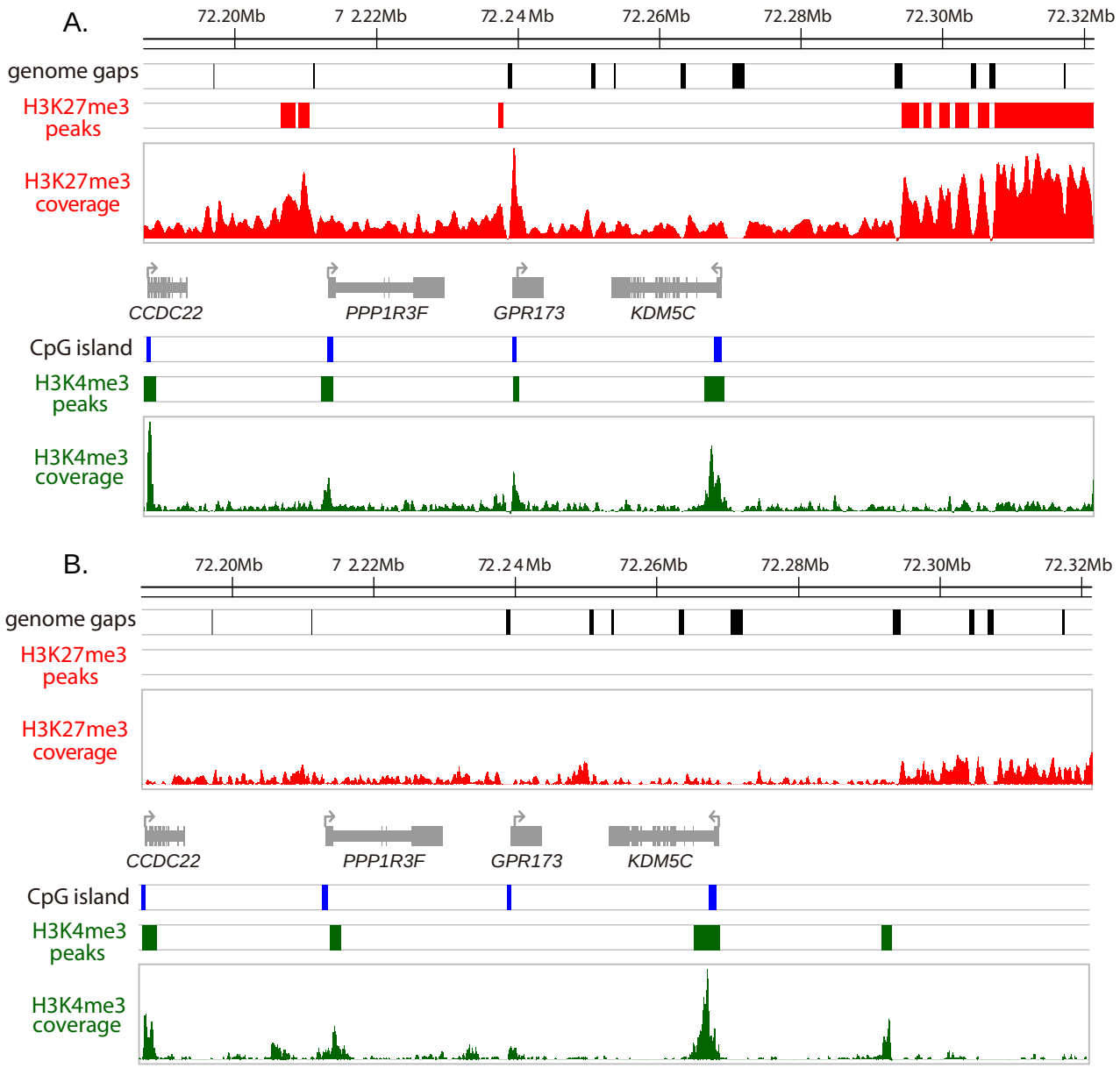


Figure S45. Allele-specific histone modification H3K4me3 for escaper gene *YIPF6* in female head ChIP-seq data from LL1 x LL2 cross. (A). The 5'-end gene model, CpG island location and the H3K4me3 peak/coverage profile for an escaper gene *YIPF6*. There is one SNP (chrX_7594487) under the H3K4me3 peak with enough coverage to infer allele-specific histone modification. (B). Sanger sequencing genotyping results in the two embryos (579E10 and 579E11, used for ChIP-seq experiments) and their parents confirmed that the SNP (chrX_7594487) is informative in both embryos. (C). From the ChIP-seq data, we observed 64% of the H3K4me3 reads from the maternal allele and 36% from the paternal allele at chrX_7594487, suggesting both parental alleles are active. This is consistent with allele-specific expression profile at SNP OMSNP0155108 in the RNA-seq data and SNP OMSNP0155110 from the allele-specific pyrosequencing results.

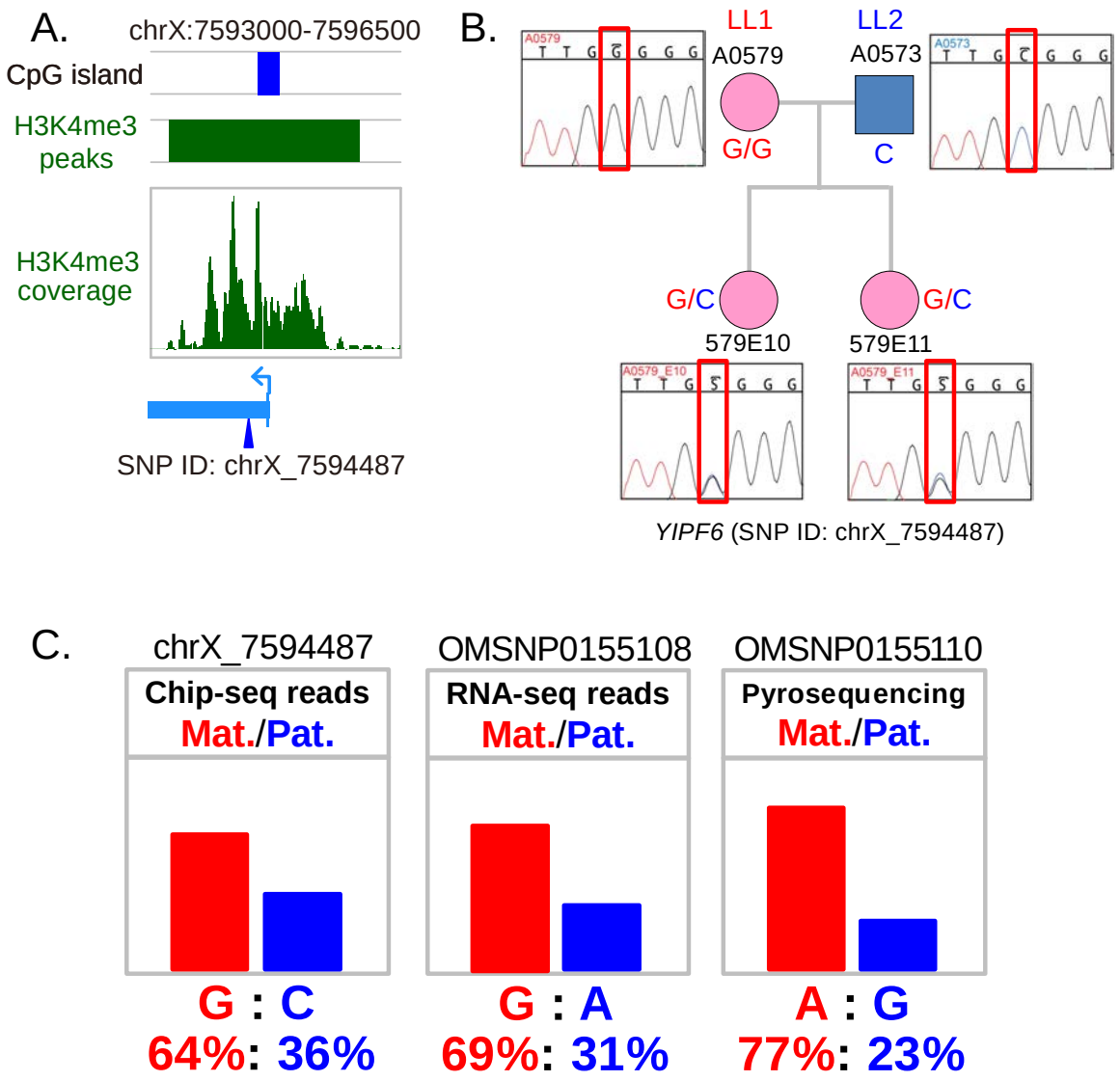


Figure S46. Allele-specific histone modification H3K4me3 for escaper gene *FAM122B* in female head ChIP-seq data from LL1 x LL2 cross. (A). The 5'-end gene model, CpG island location and the H3K4me3 peak/coverage profile for an escaper gene *FAM122B*. There is one SNP (OMSNP0156061) under the H3K4me3 peak with enough coverage to infer allele-specific histone modification. (B). Sanger sequencing genotyping results in the two embryos (579E10 and 579E11, used for ChIP-seq experiments) and their mother confirmed that the SNP (OMSNP0156061) is informative in both embryos. (C). From the ChIP-seq data, we observed the H3K4me3 reads from both parental alleles at OMSNP0156061, suggesting both parental alleles are active. This is consistent with biallelic expression from the allele-specific pyrosequencing results.

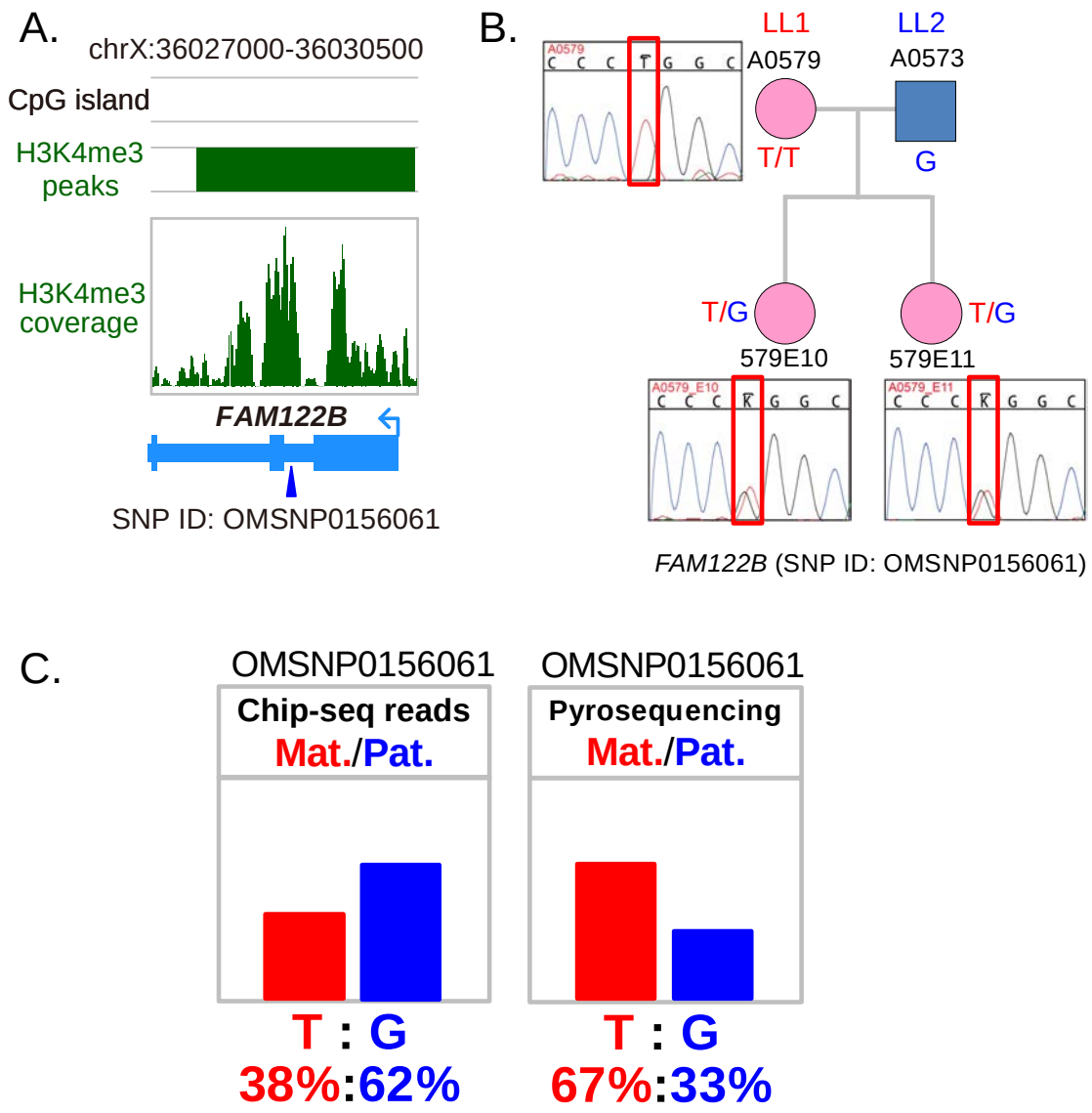


Figure S47. Allele-specific histone modification H3K4me3 for nonescaper gene *PNCK* in female head ChIP-seq data from LL1 x LL2 cross. (A). The 5'-end gene model, CpG island location and the H3K4me3 peak/coverage profile for a nonescaper gene *PNCK*. There is one SNP (OMSNP0155237) under the H3K4me3 peak with enough coverage to infer allele-specific histone modification. (B). Sanger sequencing genotyping results in the two embryos (579E10 and 579E11, used for ChIP-seq experiments) and their parents confirmed that the SNP (OMSNP0155237) is informative in both embryos. (C). From the ChIP-seq data, we observed 100% of the H3K4me3 reads from the maternal allele at OMSNP0155237, suggesting the on-mark is only present at the maternal allele. This is consistent with maternal-specific expression at OMSNP0155237 and OMSNP0155219 in the RNA-seq data.

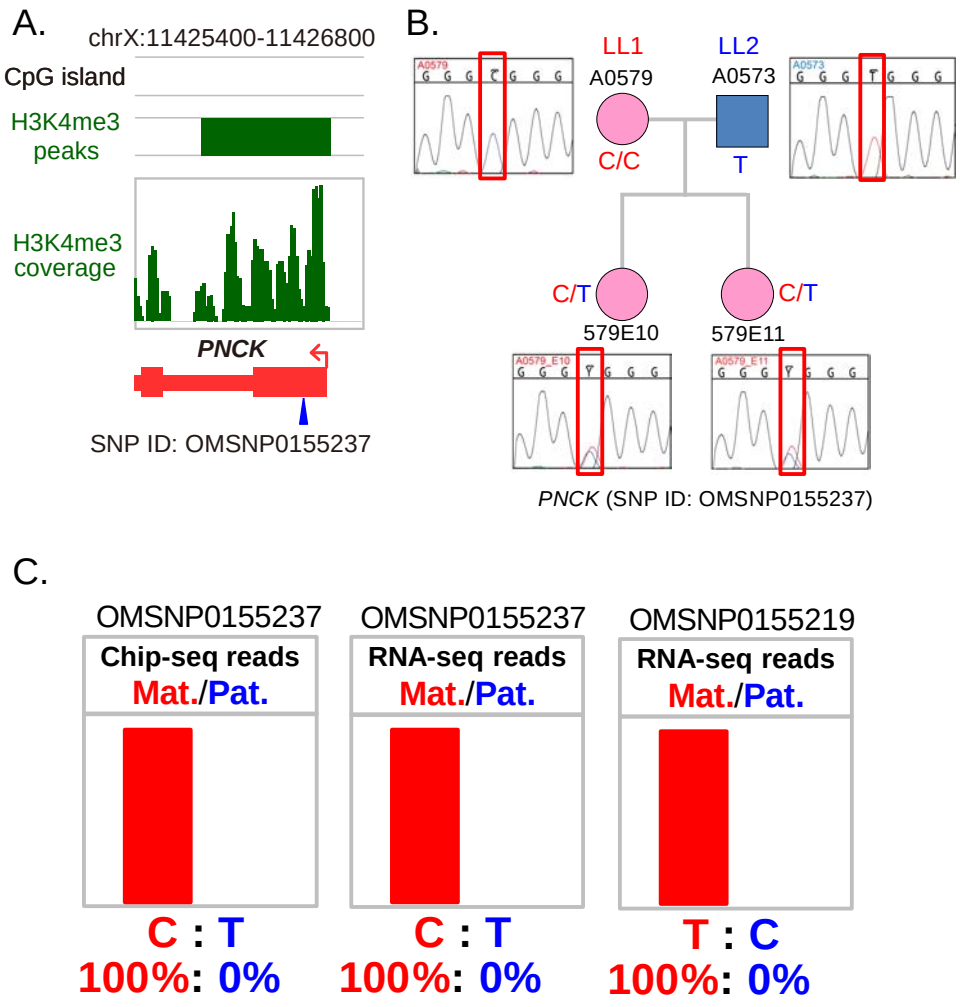


Figure S48. Allele-specific histone modification H3K4me3 for nonescaper gene *GPC4* in female head ChIP-seq data from LL1 x LL2 cross. (A). The 5'-end gene model, CpG island location and the H3K4me3 peak/coverage profile for a nonescaper gene *GPC4*. There is one SNP (OMSNP0156005) under the H3K4me3 peak with enough coverage to infer allele-specific histone modification. (B). Sanger sequencing genotyping results in the two embryos (579E10 and 579E11, used for ChIP-seq experiments) and their parents confirmed that the SNP (OMSNP0156005) is informative in both embryos. (C). From the ChIP-seq data, we observed 100% of the H3K4me3 reads from the maternal allele at OMSNP0156005, suggesting the on-mark is only present at the maternal allele. This is consistent with maternal-specific expression at OMSNP0156005 and OMSNP0156006 in the RNA-seq data.

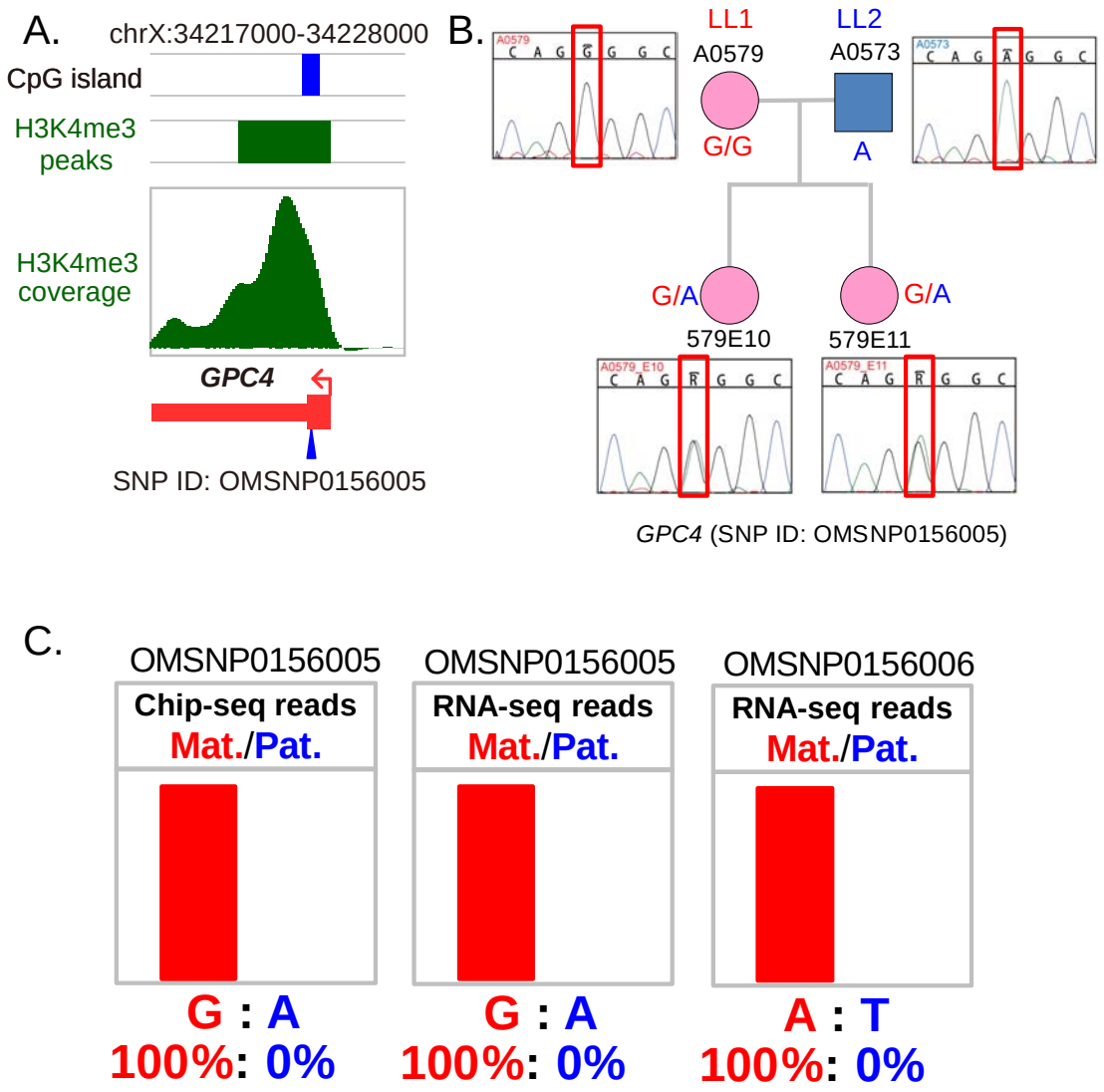


Figure S49. Allele-specific histone modification H3K4me3 for nonescaper gene *ITM2A* in female head ChIP-seq data from LL1 x LL2 cross. (A). The 5'-end gene model, CpG island location and the H3K4me3 peak/coverage profile for a nonescaper gene *ITM2A*. There is one SNP (OMSNP0156531) under the H3K4me3 peak with enough coverage to infer allele-specific histone modification. (B). Sanger sequencing genotyping results in the two embryos (579E10 and 579E11, used for ChIP-seq experiments) and their parents confirmed that the SNP (OMSNP0156531) is informative in both embryos. (C). From the ChIP-seq data, we observed 100% of the H3K4me3 reads from the maternal allele at OMSNP0156531, suggesting the on-mark is only present at the maternal allele. This is consistent with maternal-specific expression at OMSNP0156531 in the RNA-seq data.

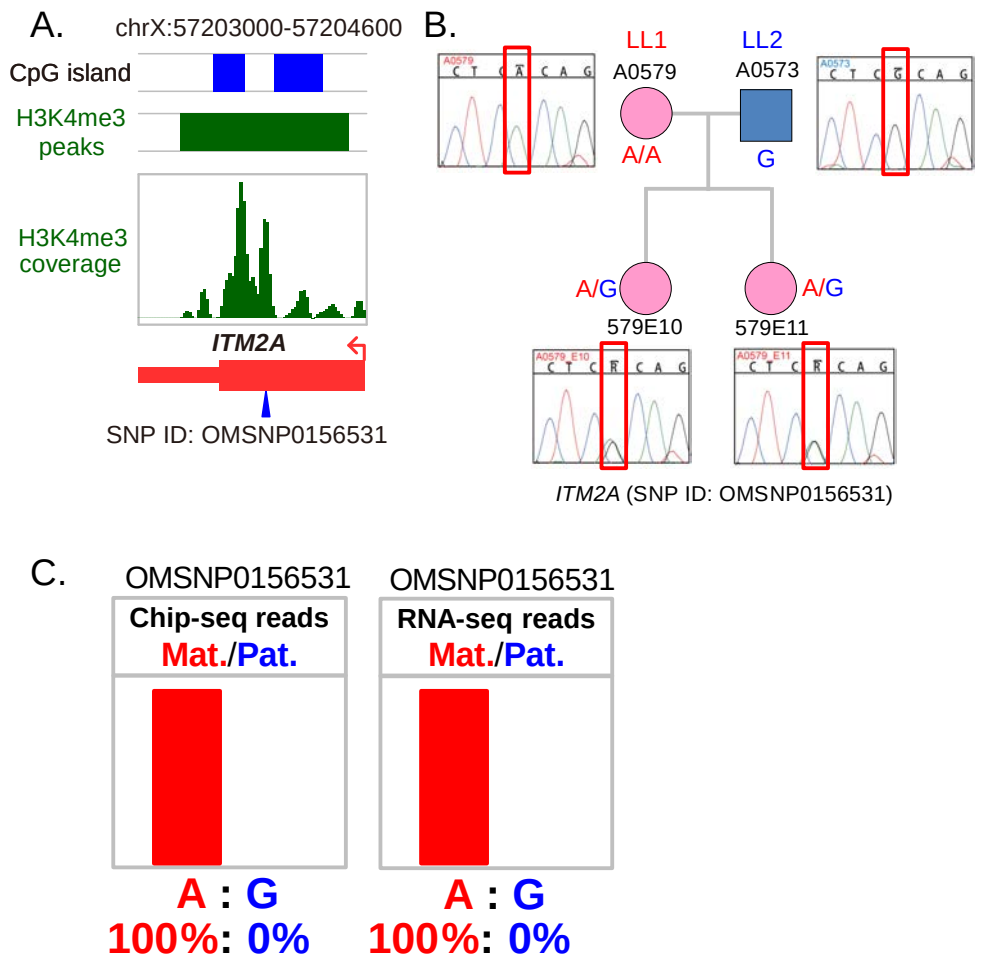


Figure S50. Allele-specific histone modification H3K4me3 for nonescaper gene *PDZD11* in female head ChIP-seq data from LL1 x LL2 cross. (A). The 5'-end gene model, CpG island location and the H3K4me3 peak/coverage profile for two X-linked genes, *PDZD11* (+ strand) and *KIF4A* (- strand). They are organized in head-to-tail orientation, and they share one CpG island and one H3K4me3 peak. *PDZD11* is a nonescaper gene (colored in red) and the escaping status for KIF4A (colored in gray) is unknown due to lack of informative exonic SNPs. There is one SNP (OMSNP0223343) under the H3K4me3 peak with enough coverage to infer allele-specific histone modification. (B). Sanger sequencing genotyping results in the two embryos (579E10 and 579E11, used for ChIP-seq experiments) and their parents confirmed that the SNP (OMSNP0223343) is informative in both embryos. (C). From the ChIP-seq data, we observed 100% of the H3K4me3 reads from the maternal allele at OMSNP0223343, suggesting the on-mark is only present at the maternal allele. This is consistent with maternal-specific expression of *PDZD11* at OMSNP0223343 and OMSNP0156925 in the RNA-seq data.

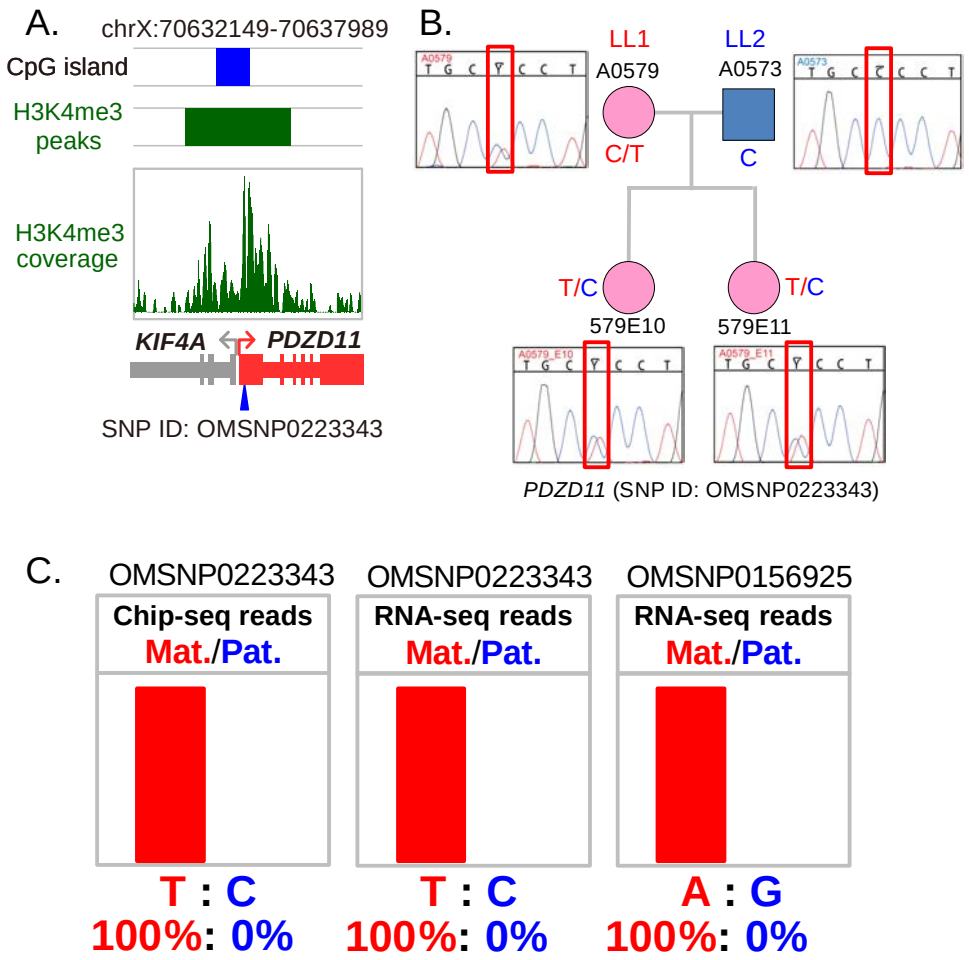
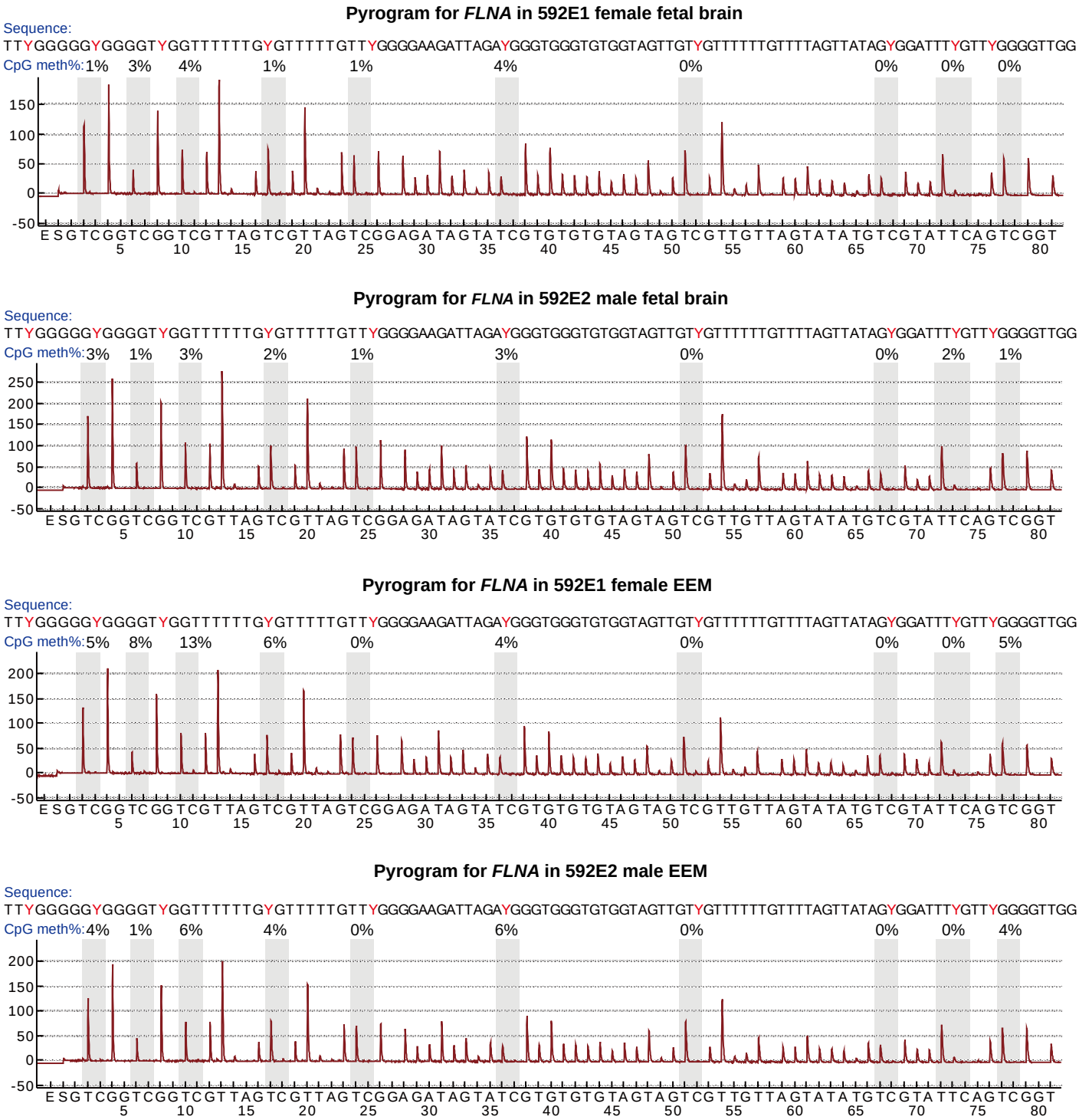
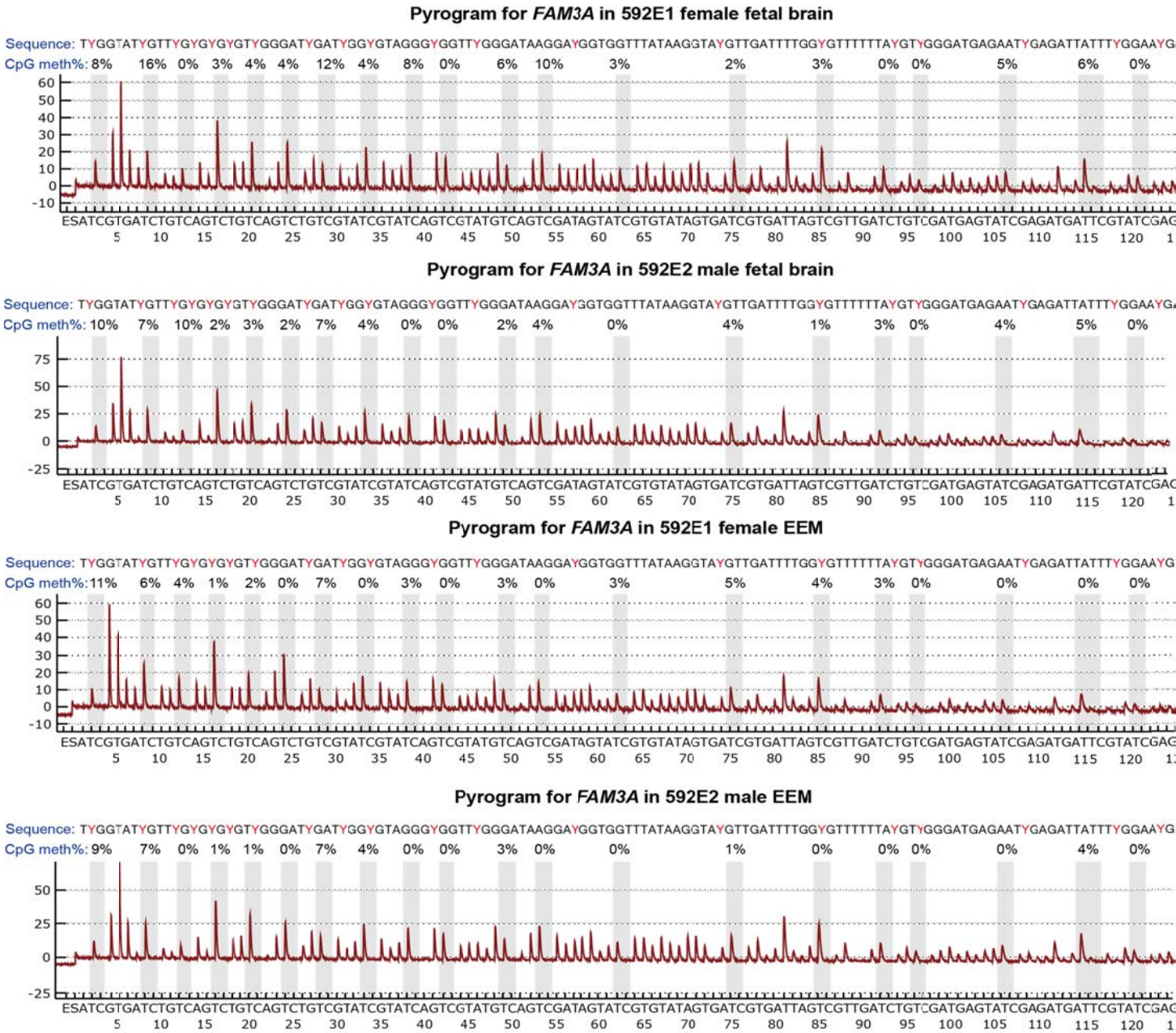


Figure S51. Raw pyrograms from the PyroMark assays for DNA methylation quantification.

(A). Pyrograms for PyroMark analysis of the methylation profile at *FLNA* promoter CpG island. The methylation percentages for 10 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

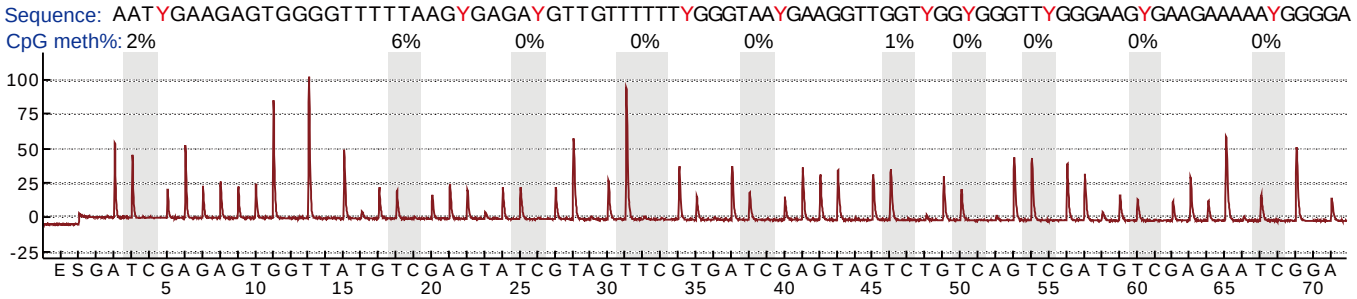


(B). Pyrograms for PyroMark analysis of the methylation profile at *FAM3A* promoter CpG island. The methylation percentages for 20 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

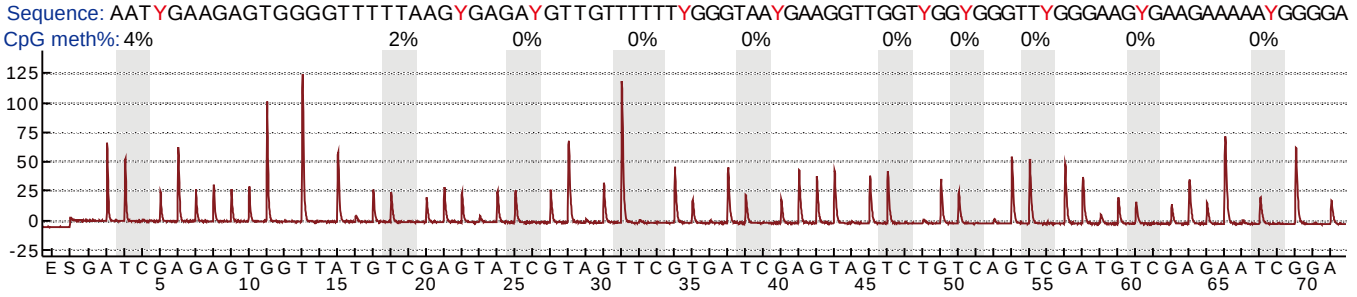


(C). Pyrograms for PyroMark analysis of the methylation profile at *AMMECR1* promoter CpG island. The methylation percentages for 10 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

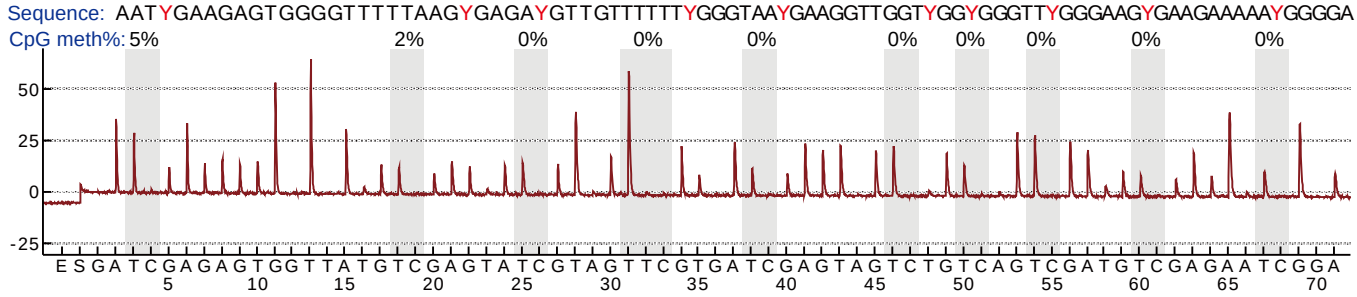
Pyrogram for *AMMECR1* in 592E1 female fetal brain



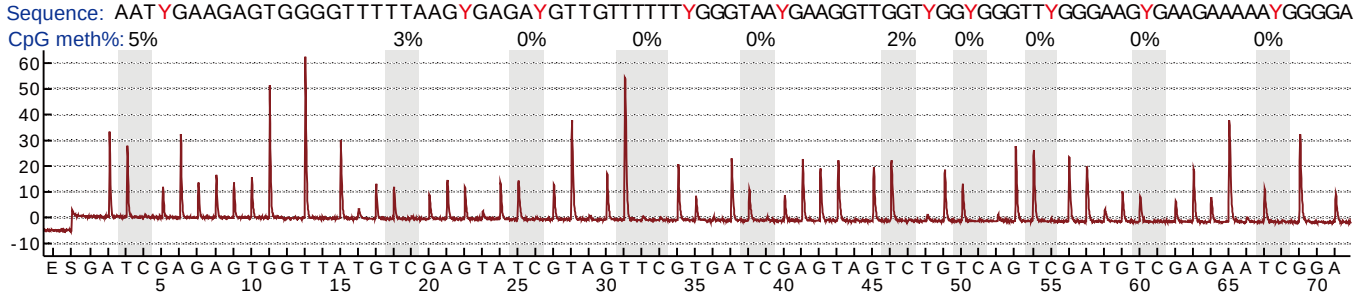
Pyrogram for *AMMECR1* in 592E2 male fetal brain



Pyrogram for *AMMECR1* in 592E1 female EEM

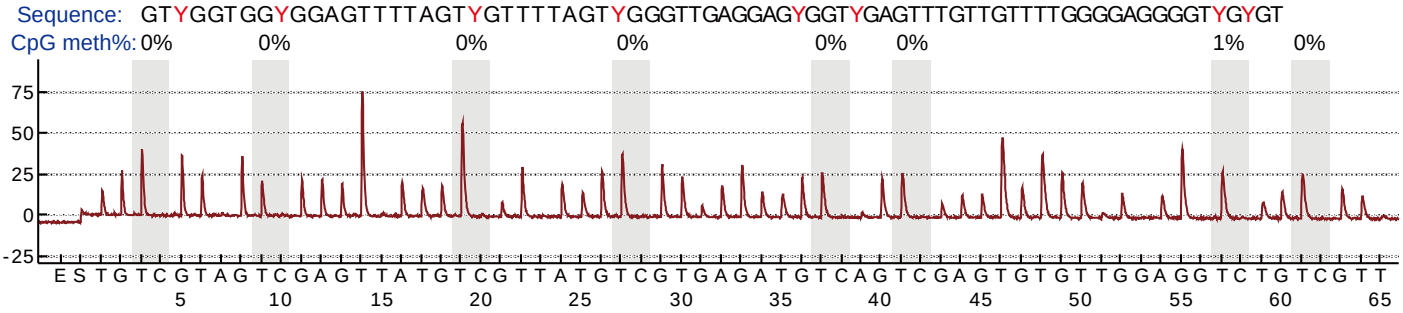


Pyrogram for *AMMECR1* in 592E2 male EEM

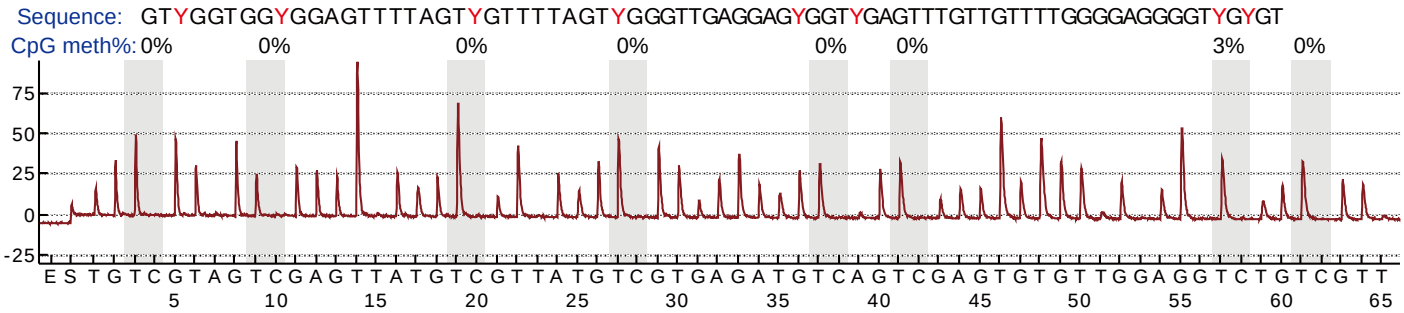


(D). Pyrograms for PyroMark analysis of the methylation profile at *BCAP31* promoter CpG island. The methylation percentages for 8 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

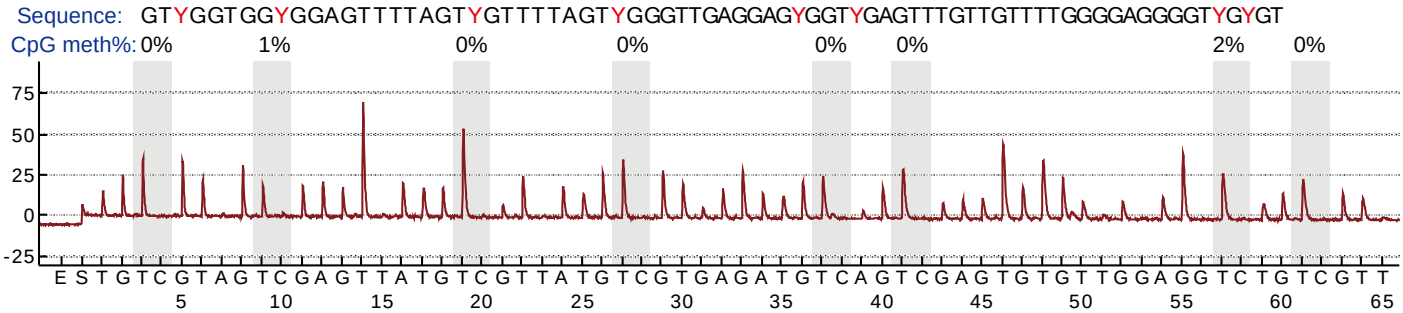
Pyrogram for *BCAP31* in 592E1 female fetal brain



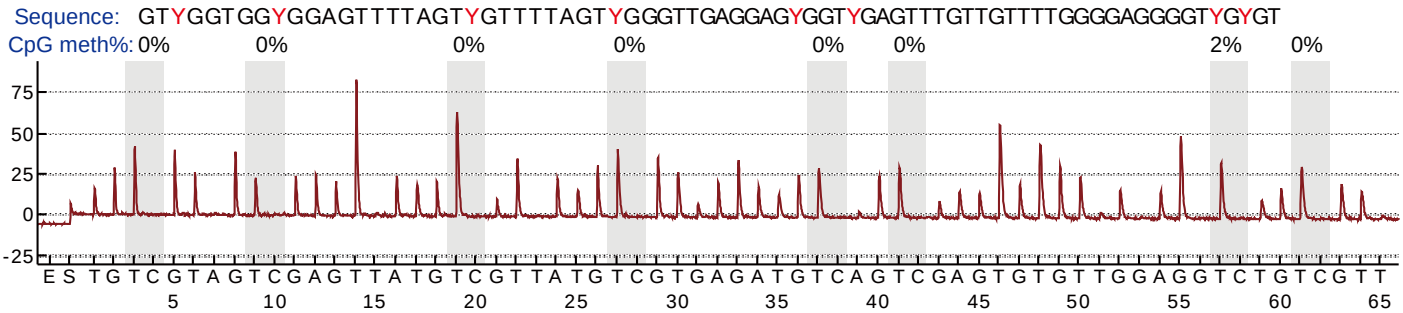
Pyrogram for *BCAP31* in 592E2 male fetal brain



Pyrogram for *BCAP31* in 592E1 female EEM

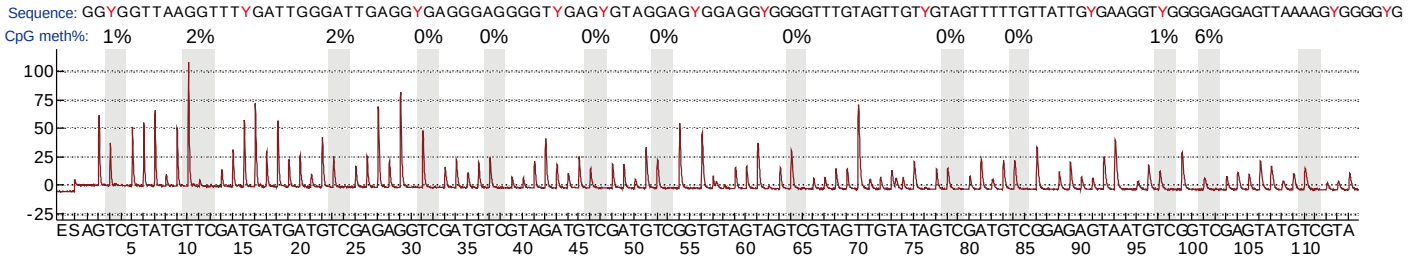


Pyrogram for *BCAP31* in 592E2 male EEM

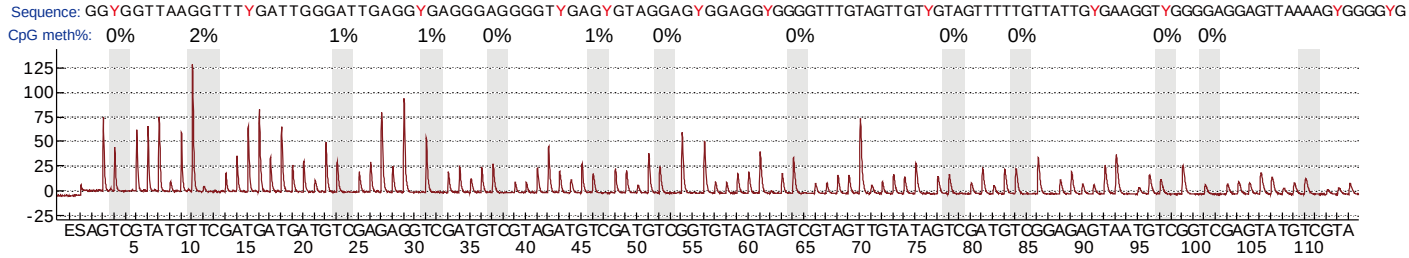


(E). Pyrograms for PyroMark analysis of the methylation profile at *LAS1L* promoter CpG island. The methylation percentages for 13 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

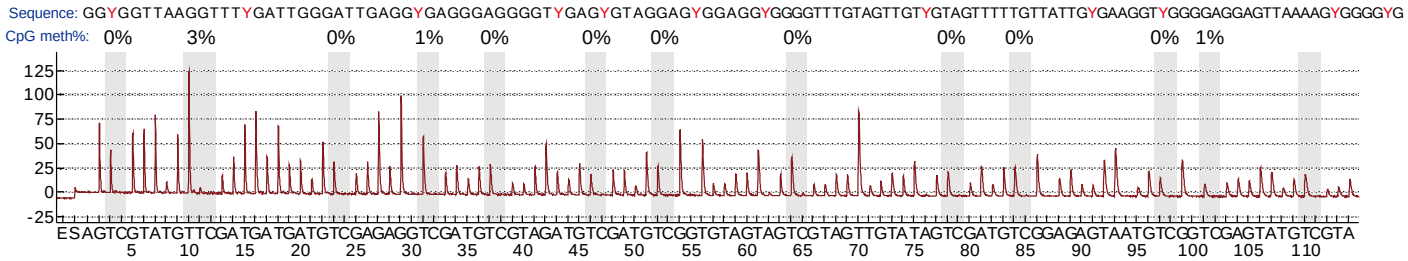
Pyrogram for *LAS1L* in 592E1 female fetal brain



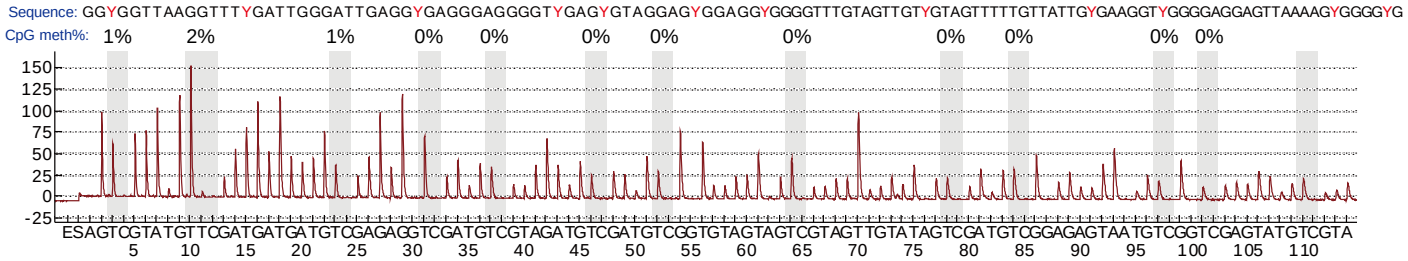
Pyrogram for *LAS1L* in 592E2 male fetal brain



Pyrogram for *LAS1L* in 592E1 female EEM

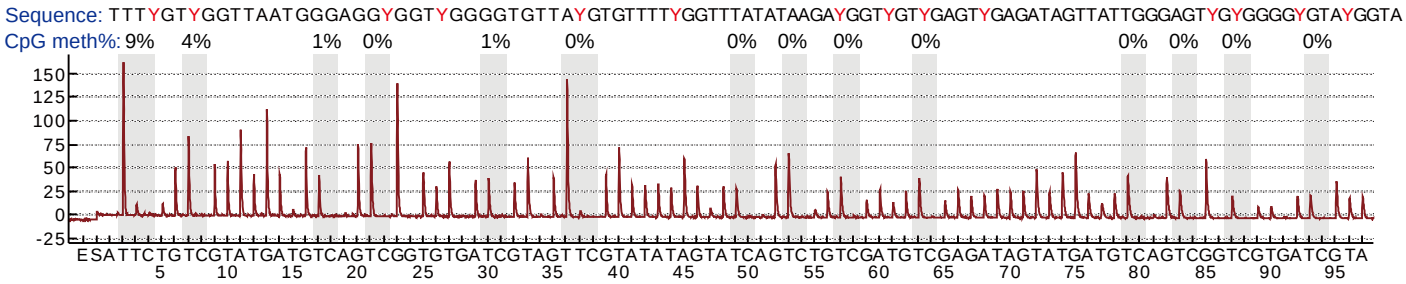


Pyrogram for *LAS1L* in 592E2 male EEM

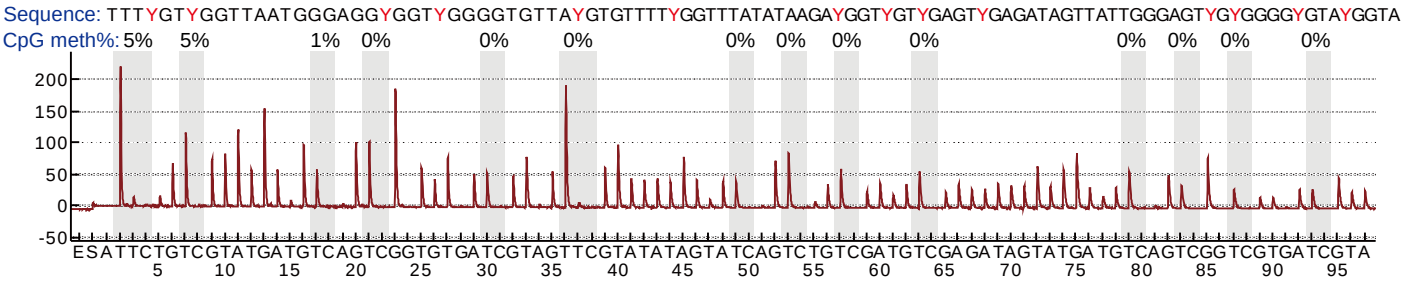


(F). Pyrograms for PyroMark analysis of the methylation profile at *LONRF3* promoter CpG island. The methylation percentages for 14 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

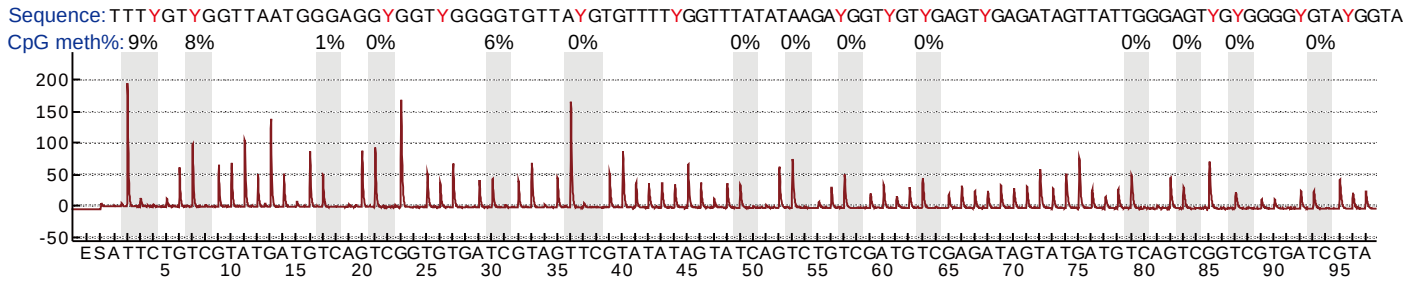
Pyrogram for *LONRF3* in 592E1 female fetal brain



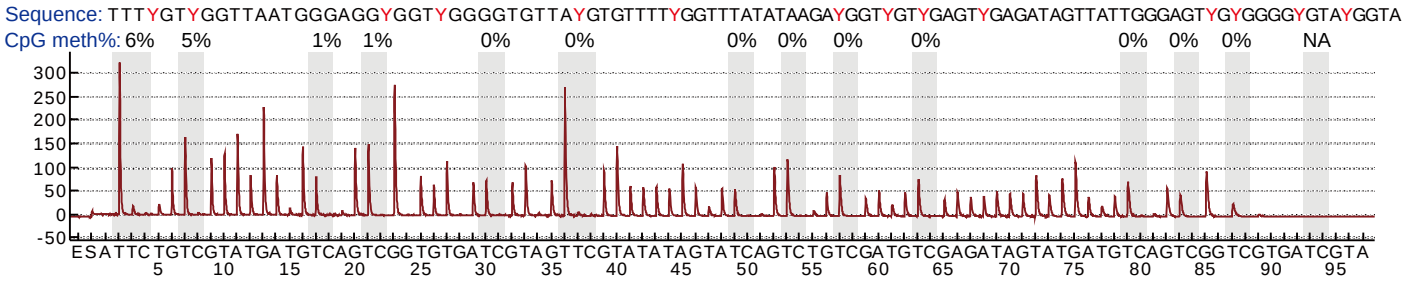
Pyrogram for *LONRF3* in 592E2 male fetal brain



Pyrogram for *LONRF3* in 592E1 female EEM

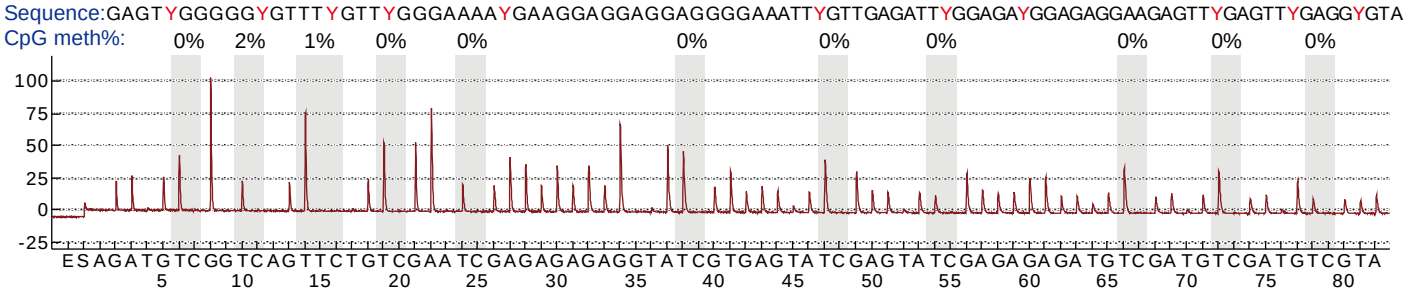


Pyrogram for *LONRF3* in 592E2 male EEM

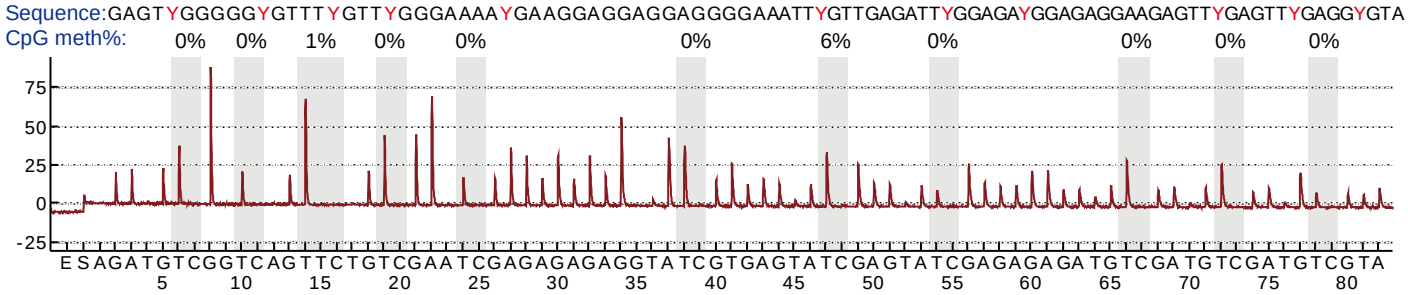


(G). Pyrograms for PyroMark analysis of the methylation profile at *ELF4* promoter CpG island. The methylation percentages for 11 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

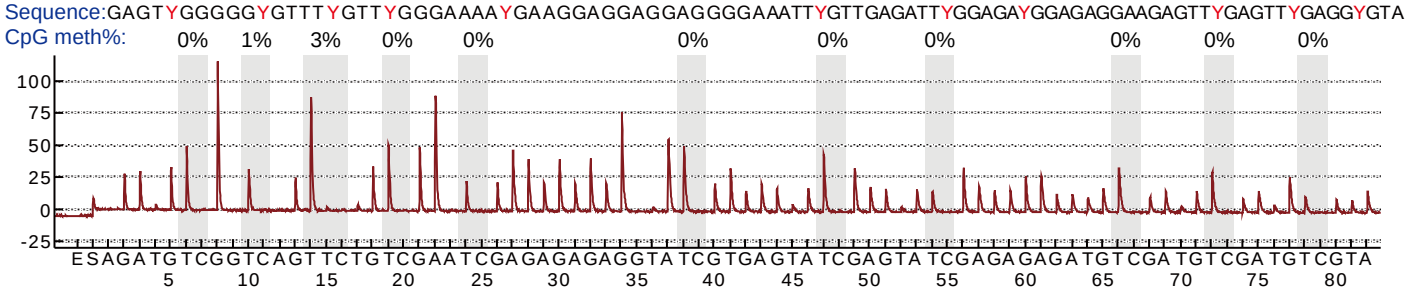
Pyrogram for *ELF4* in 592E1 female fetal brain



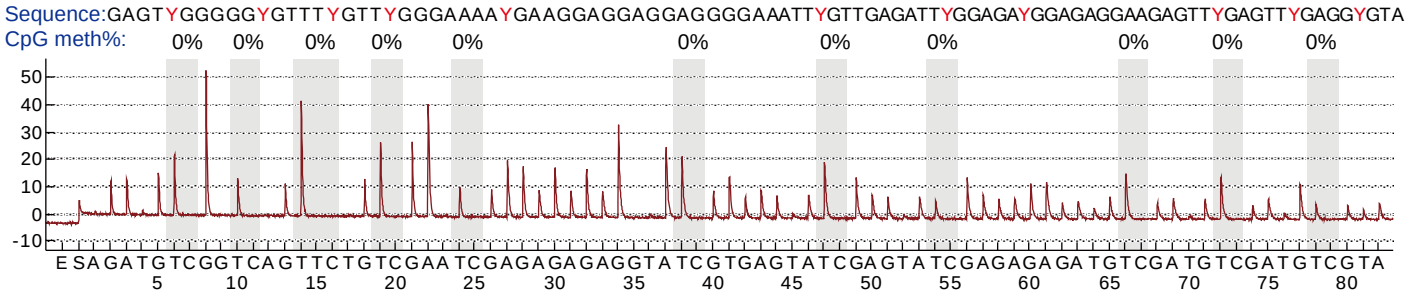
Pyrogram for *ELF4* in 592E2 male fetal brain



Pyrogram for *ELF4* in 592E1 female EEM

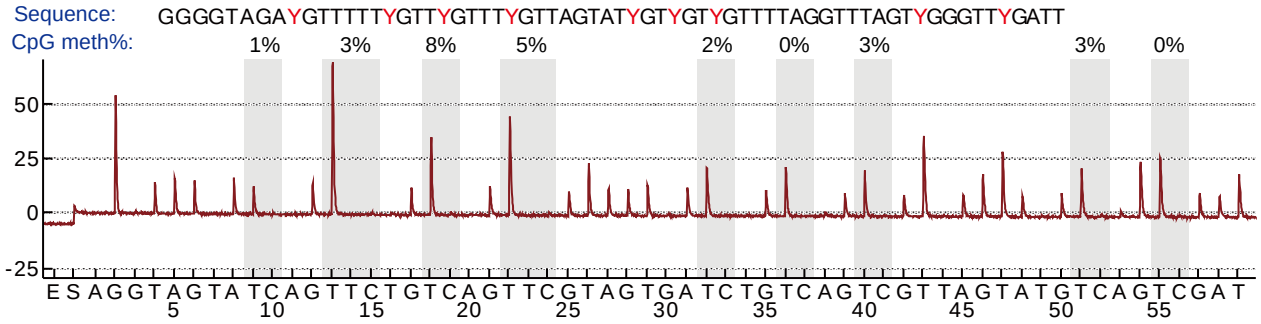


Pyrogram for *ELF4* in 592E2 male EEM

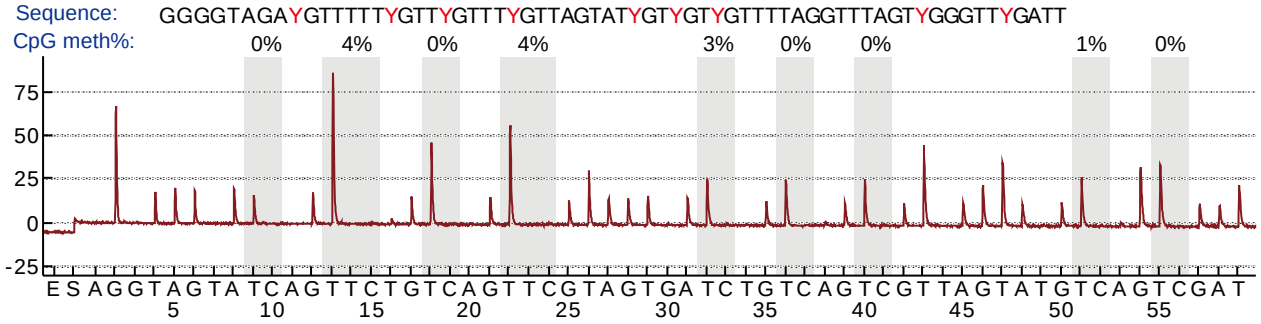


(H). Pyrograms for PyroMark analysis of the methylation profile at *RAP2C* promoter CpG island. The methylation percentages for 9 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

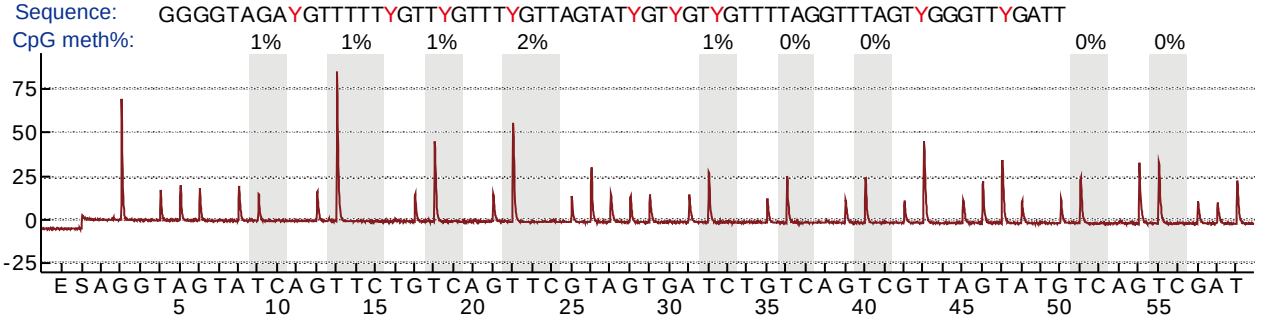
Pyrogram for *RAP2C* in 592E1 female fetal brain



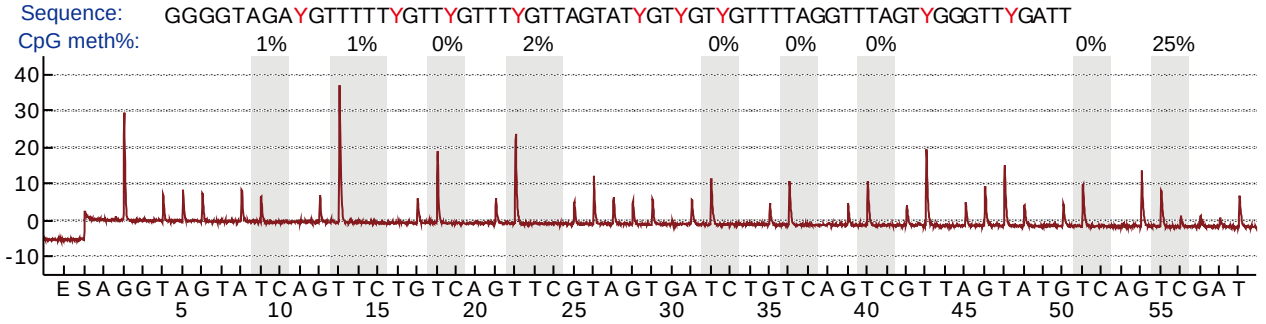
Pyrogram for *RAP2C* in 592E2 male fetal brain



Pyrogram for *RAP2C* in 592E1 female EEM

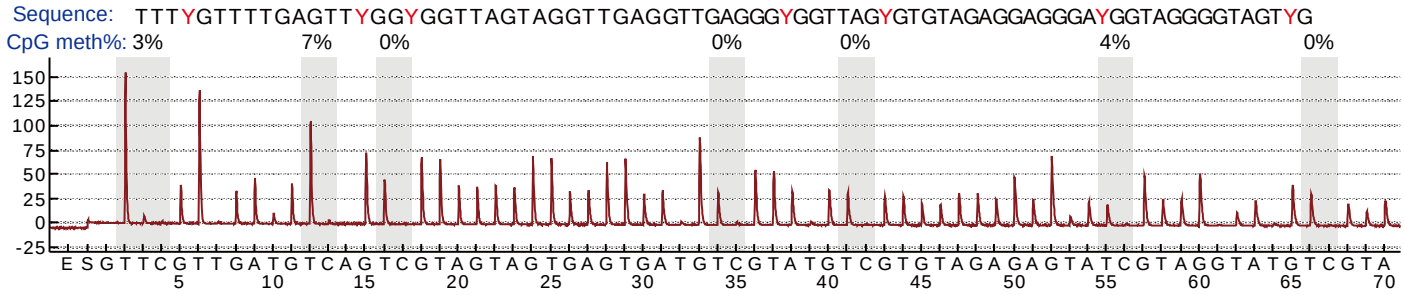


Pyrogram for *RAP2C* in 592E2 male EEM

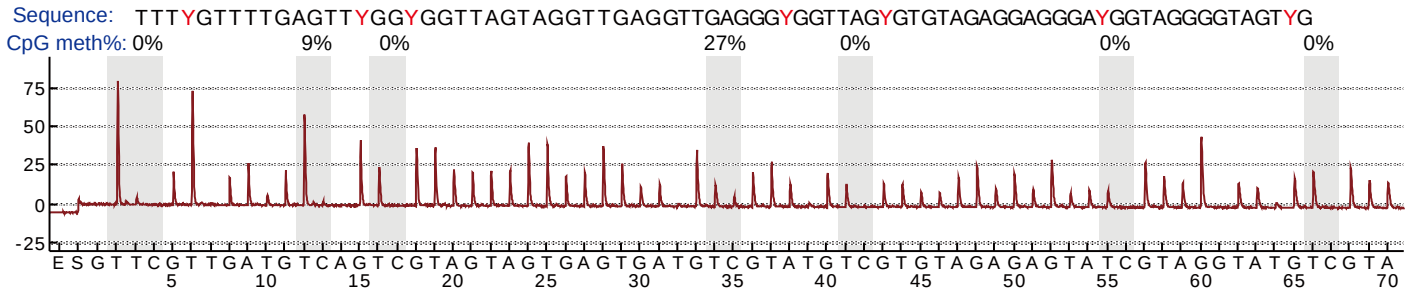


(I). Pyrograms for PyroMark analysis of the methylation profile at *GPC4* promoter CpG island. The methylation percentages for 7 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

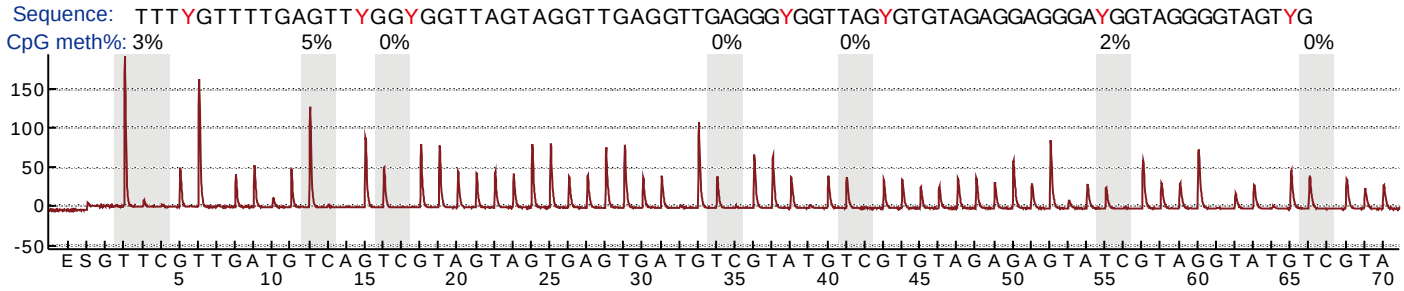
Pyrogram for *GPC4* in 592E1 female fetal brain



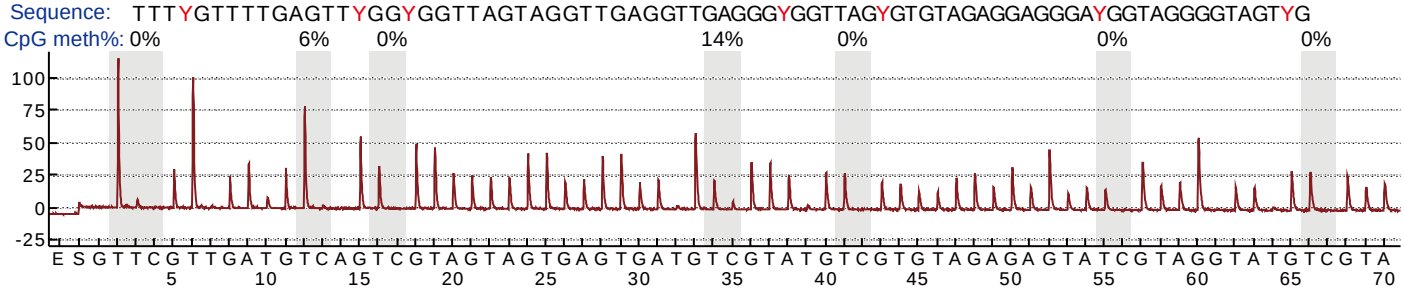
Pyrogram for *GPC4* in 592E2 male fetal brain



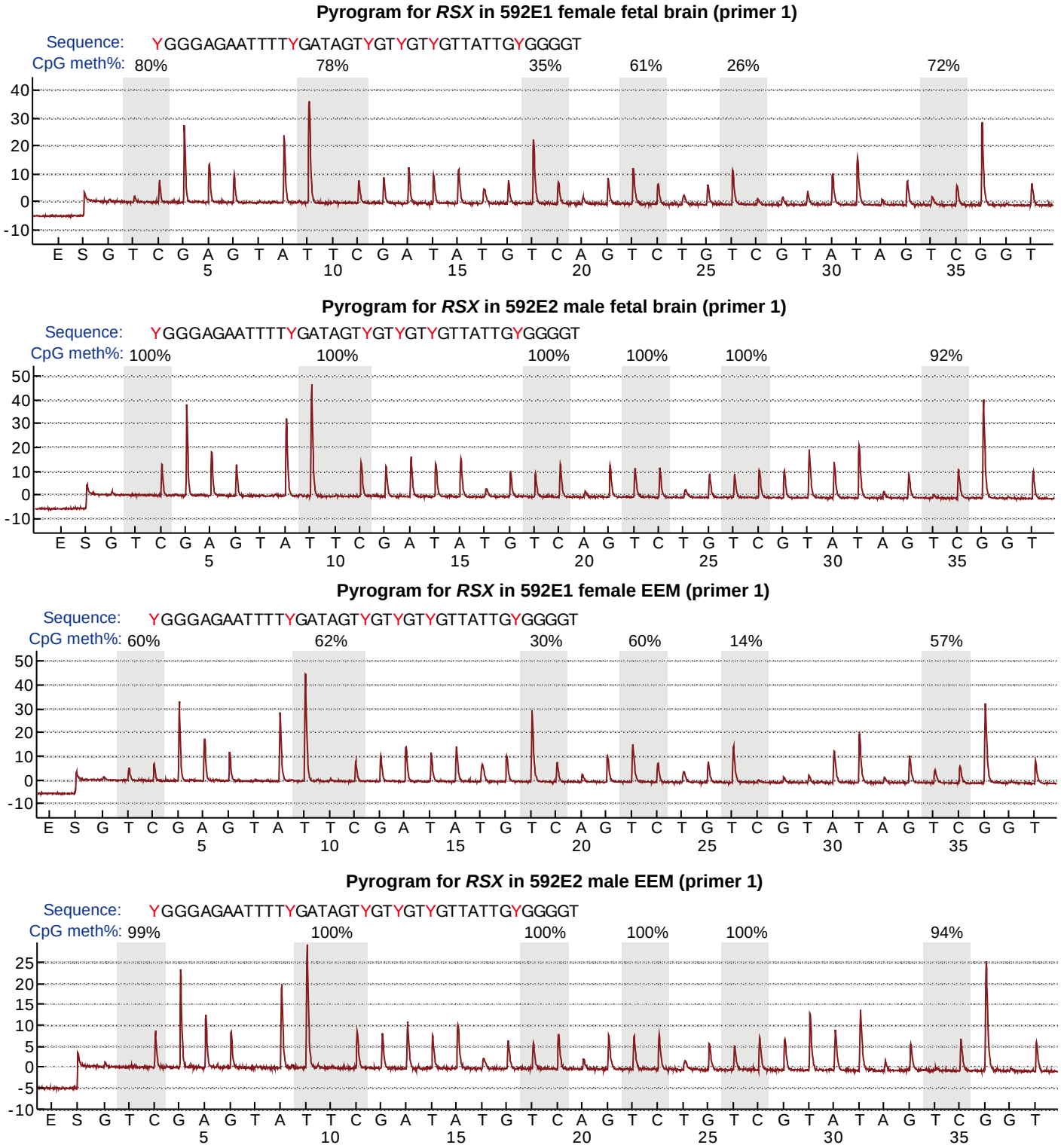
Pyrogram for *GPC4* in 592E1 female EEM



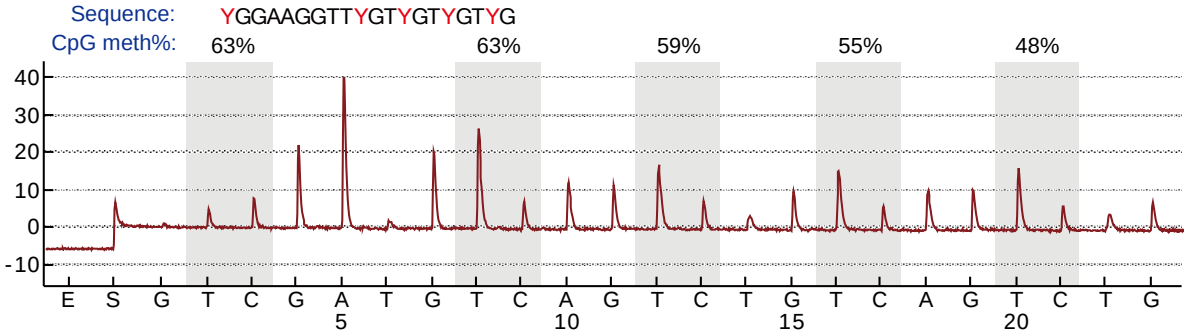
Pyrogram for *GPC4* in 592E2 male EEM



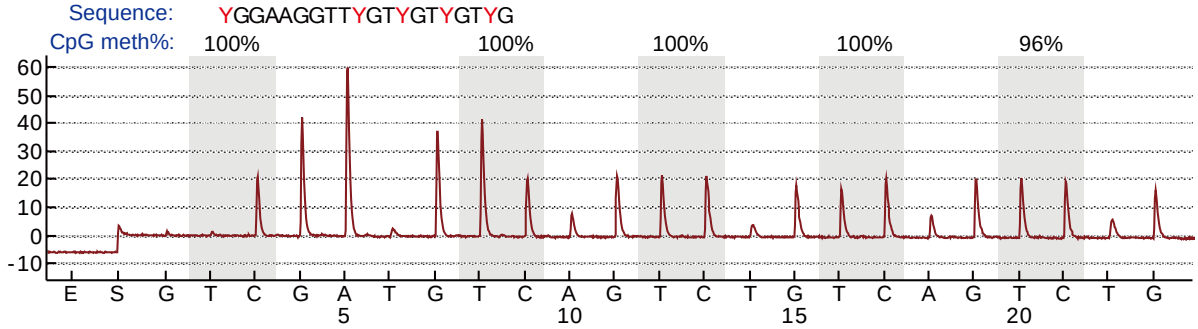
(J). Pyrograms for PyroMark analysis of the methylation profile at *RSX* promoter CpG island. The methylation percentages for a total of 23 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM) using four pyrosequencing primers (primer 1-4).



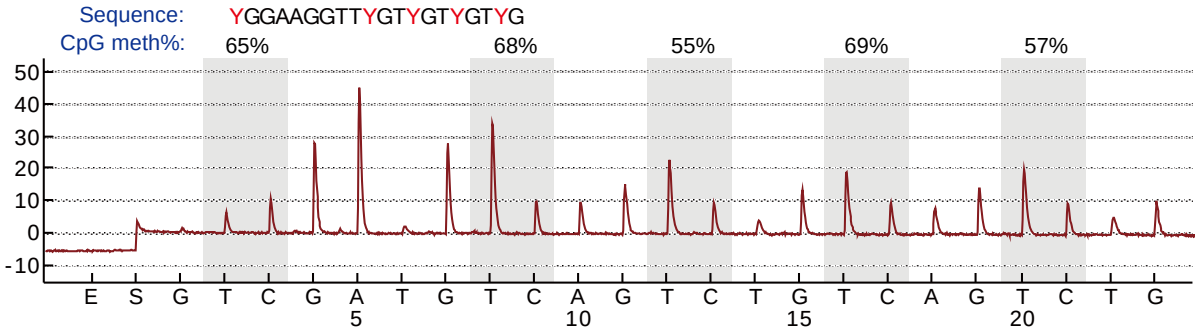
Pyrogram for *RSX* in 592E1 female fetal brain (primer 2)



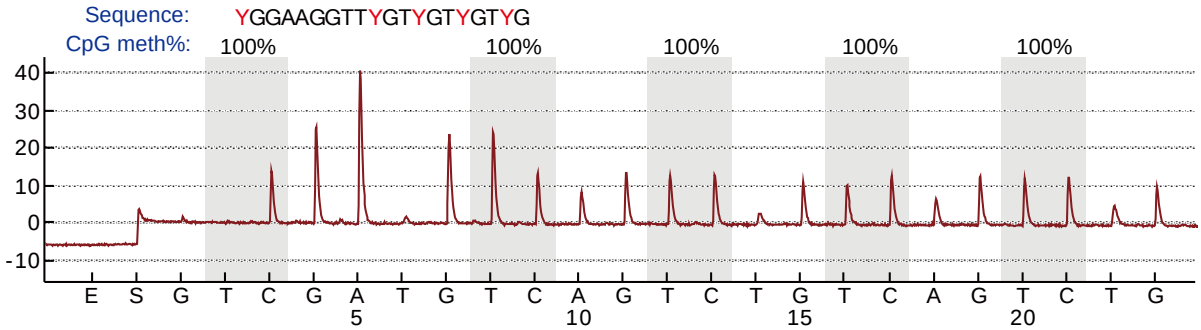
Pyrogram for *RSX* in 592E2 male fetal brain (primer 2)



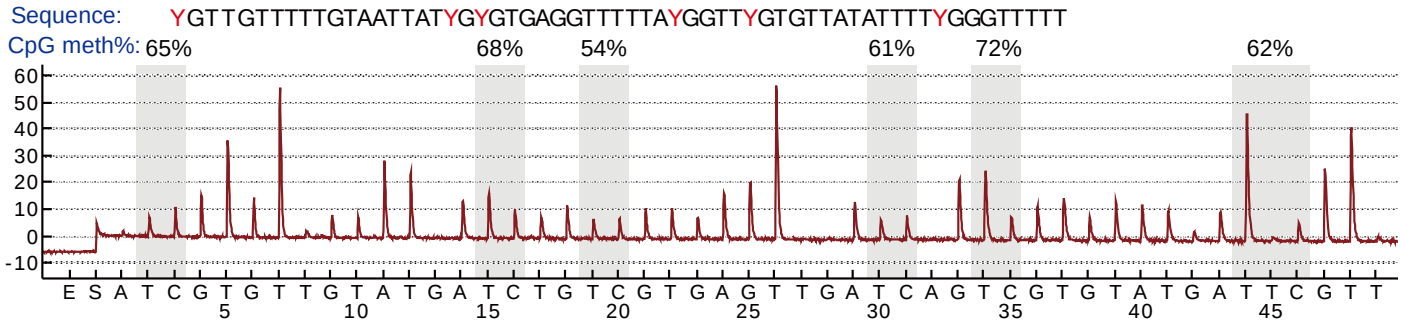
Pyrogram for *RSX* in 592E1 female EEM (primer 2)



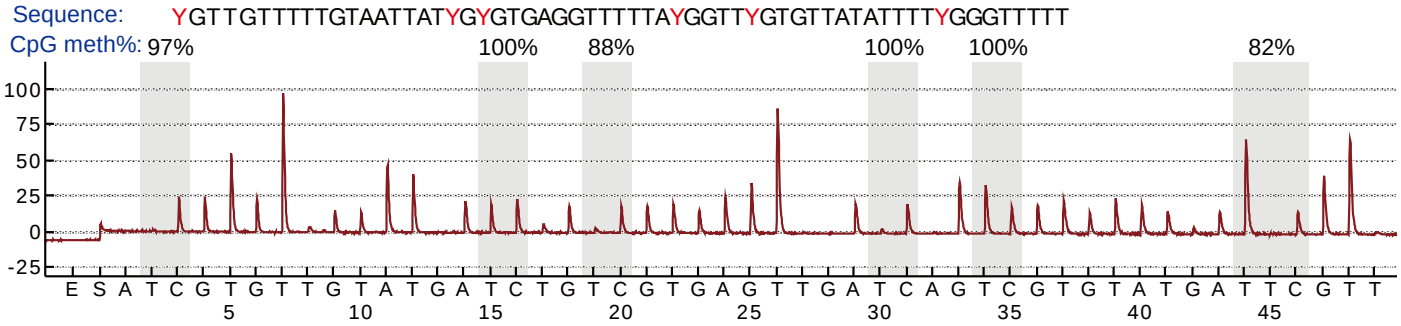
Pyrogram for *RSX* in 592E2 male EEM (primer 2)



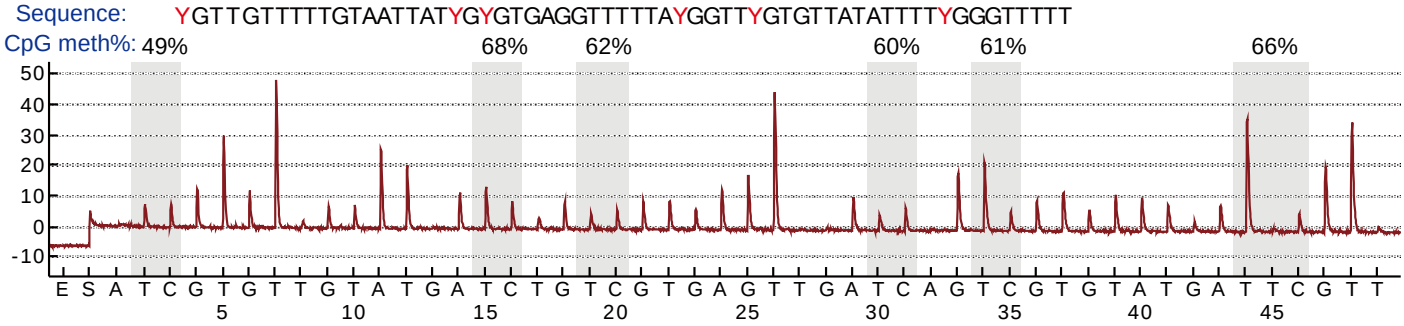
Pyrogram for *RSX* in 592E1 female fetal brain (primer 3)



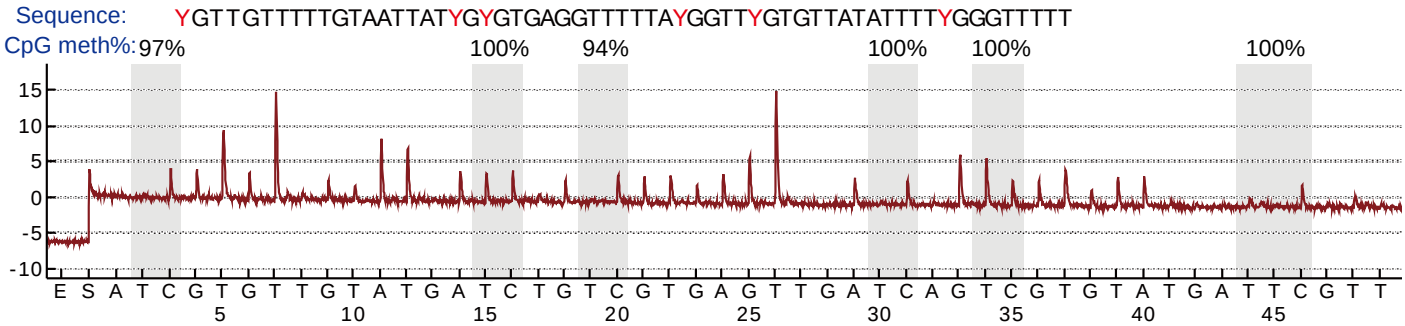
Pyrogram for *RSX* in 592E2 male fetal brain (primer 3)



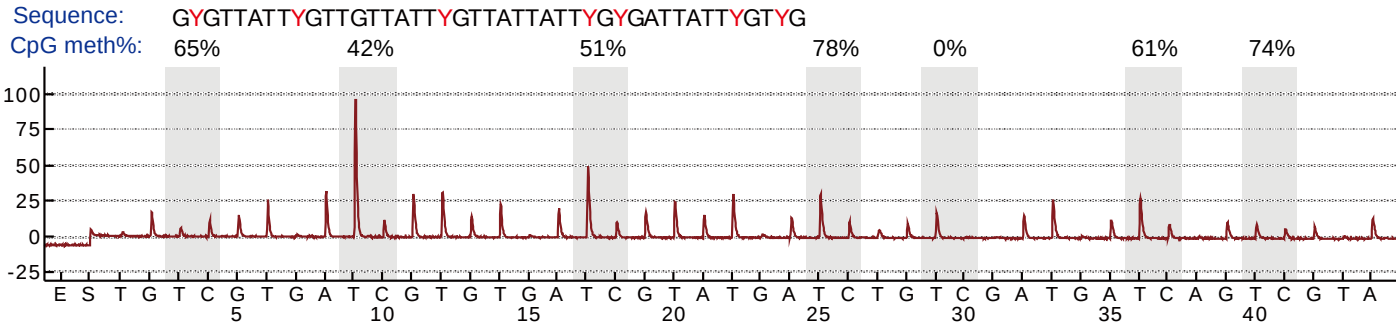
Pyrogram for *RSX* in 592E1 female EEM (primer 3)



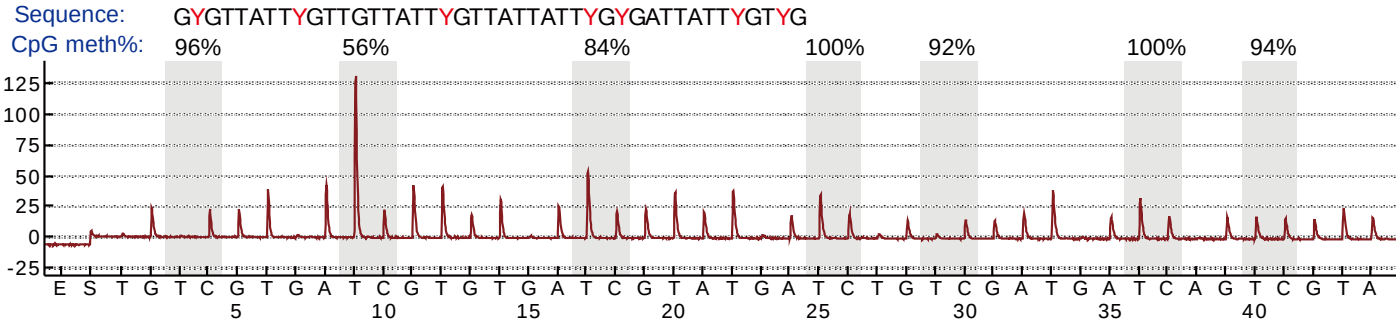
Pyrogram for *RSX* in 592E2 male EEM (primer 3)



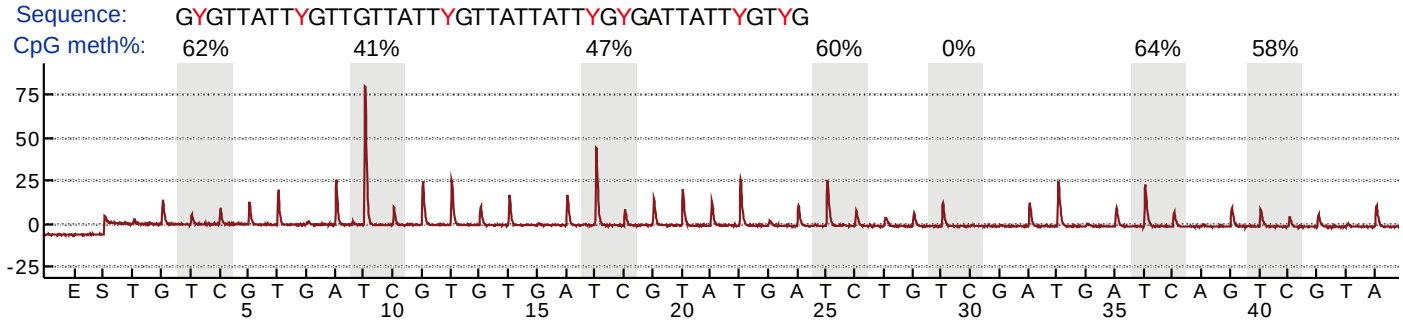
Pyrogram for *RSX* in 592E1 female fetal brain (primer 4)



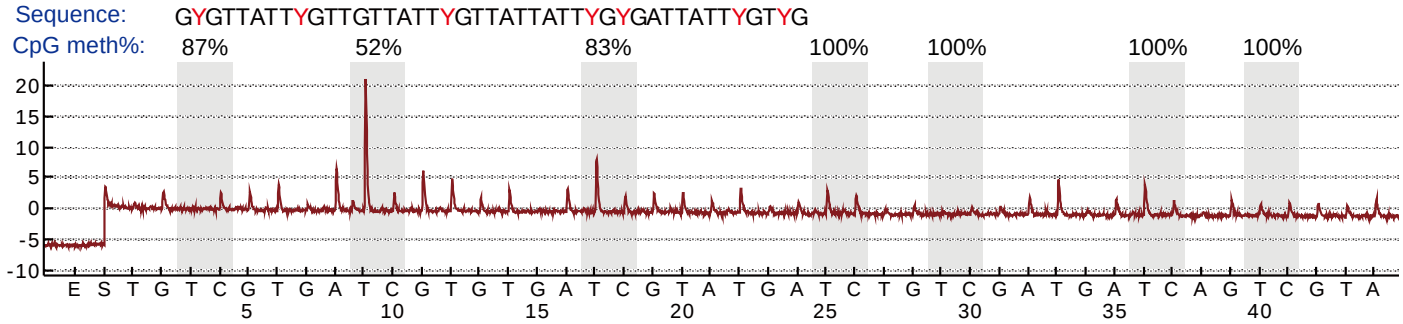
Pyrogram for *RSX* in 592E2 male fetal brain (primer 4)



Pyrogram for *RSX* in 592E1 female EEM (primer 4)

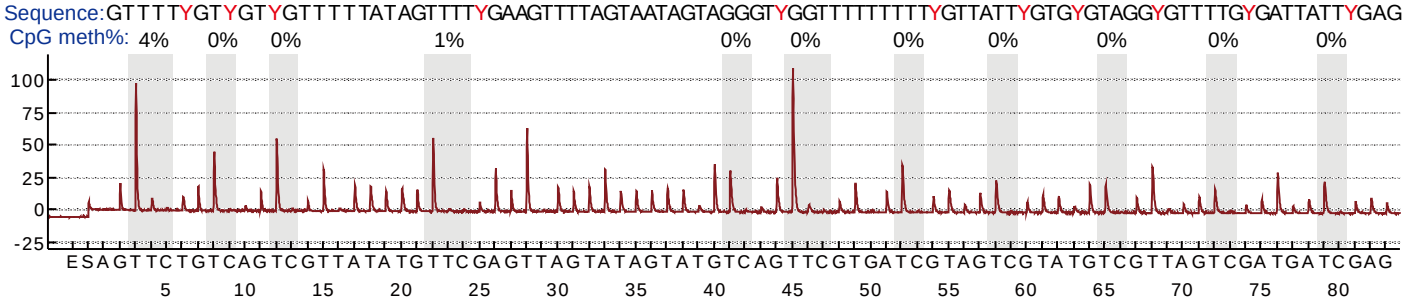


Pyrogram for *RSX* in 592E2 male EEM (primer 4)

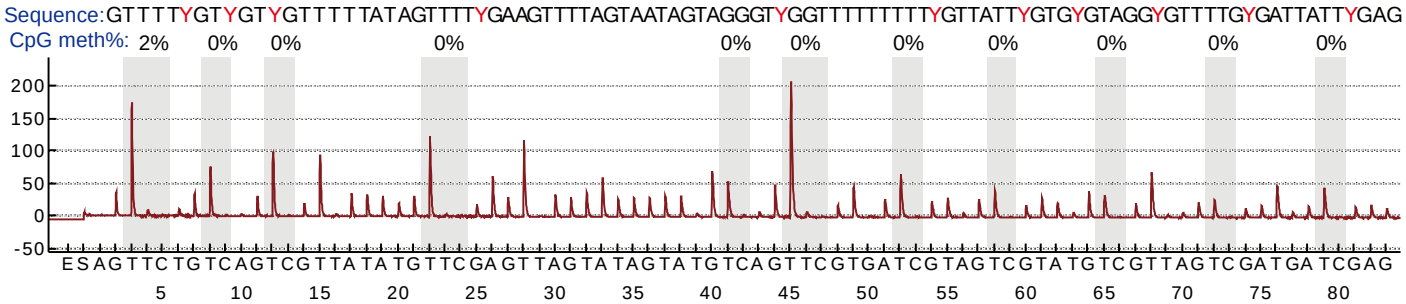


(K). Pyrograms for PyroMark analysis of the methylation profile at *FAM122B* promoter CpG island. The methylation percentages for 8 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

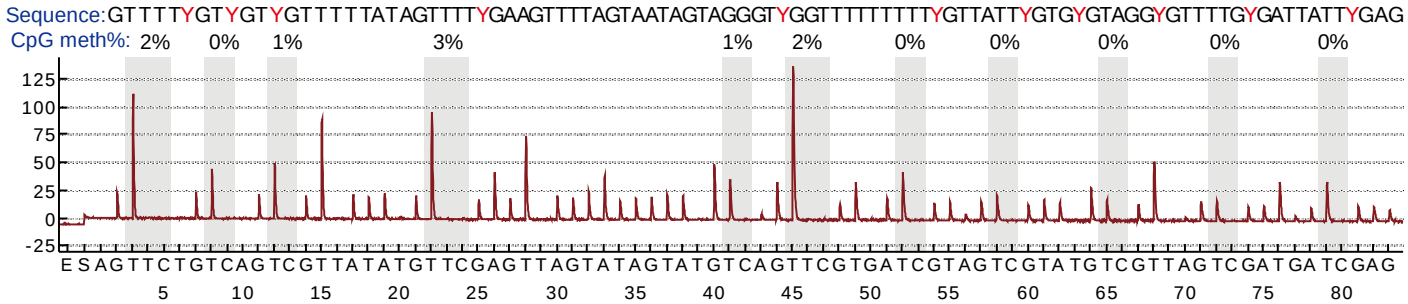
Pyrogram for *FAM122B* in 592E1 female fetal brain



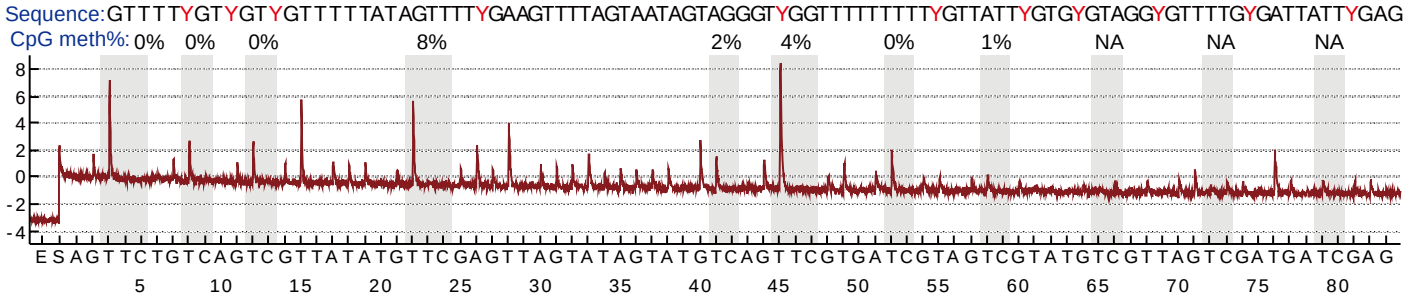
Pyrogram for *FAM122B* in 592E2 male fetal brain



Pyrogram for *FAM122B* in 592E1 female EEM

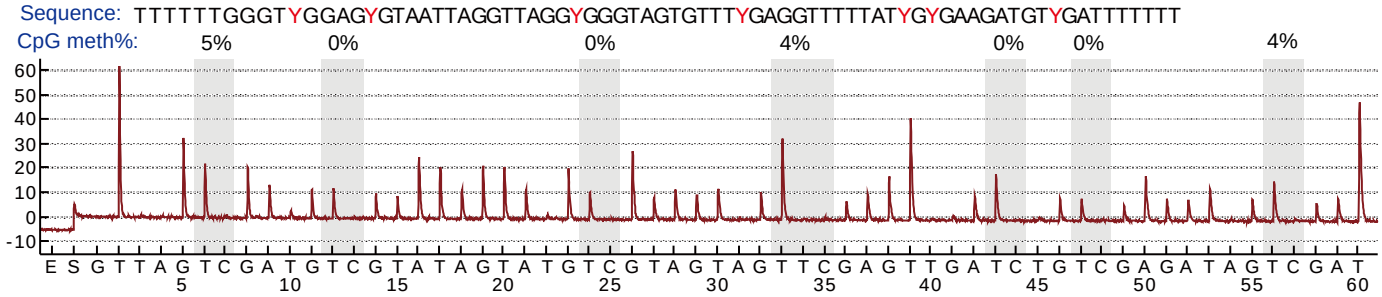


Pyrogram for *FAM122B* in 592E2 male EEM

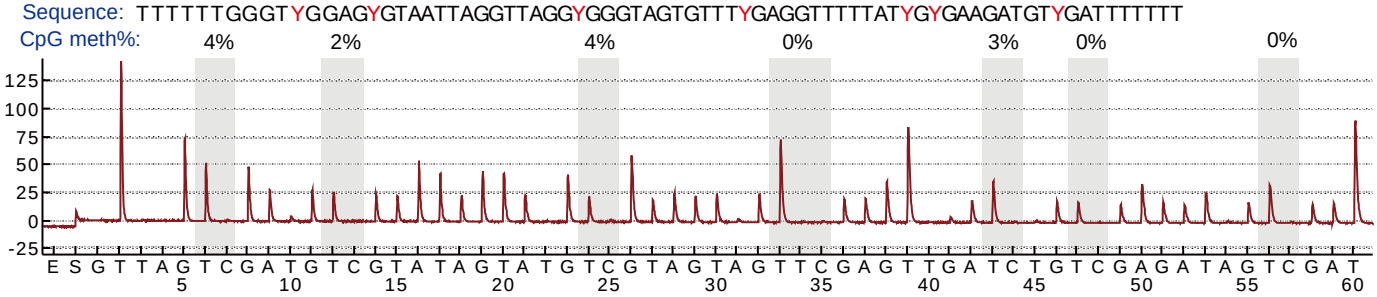


(L). Pyrograms for PyroMark analysis of the methylation profile at *DDX26B* promoter CpG island. The methylation percentages for 7 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

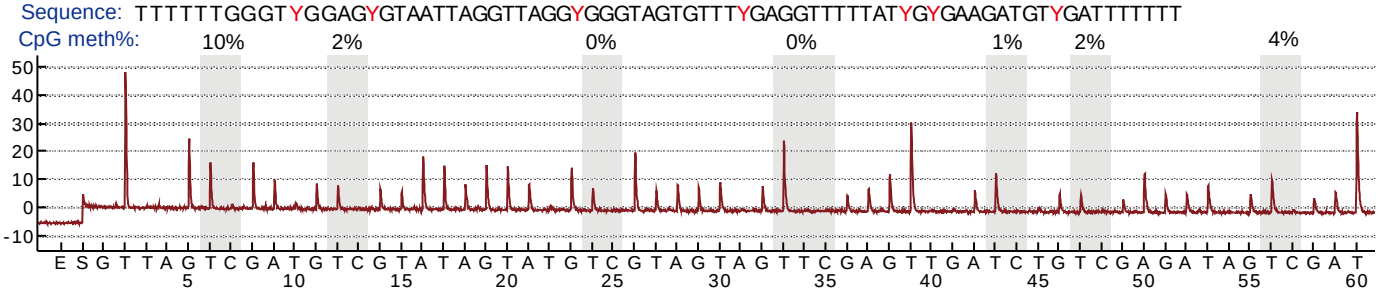
Pyrogram for *DDX26B* in 592E1 female fetal brain



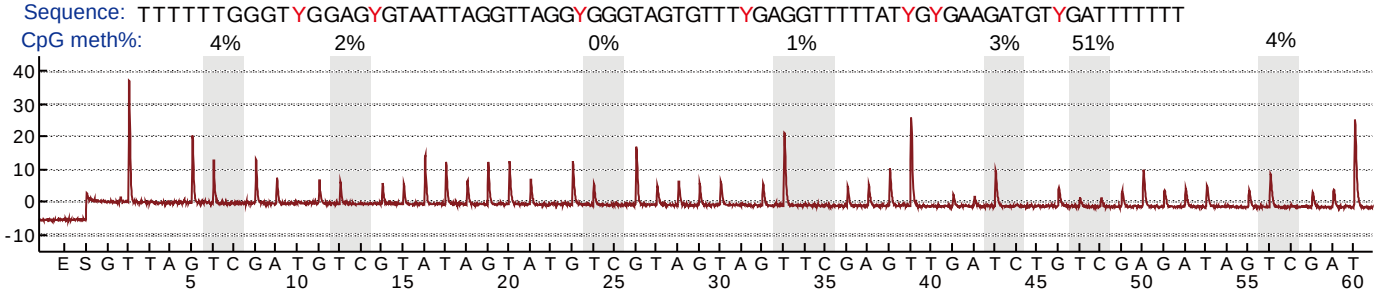
Pyrogram for *DDX26B* in 592E2 male fetal brain



Pyrogram for *DDX26B* in 592E1 female EEM

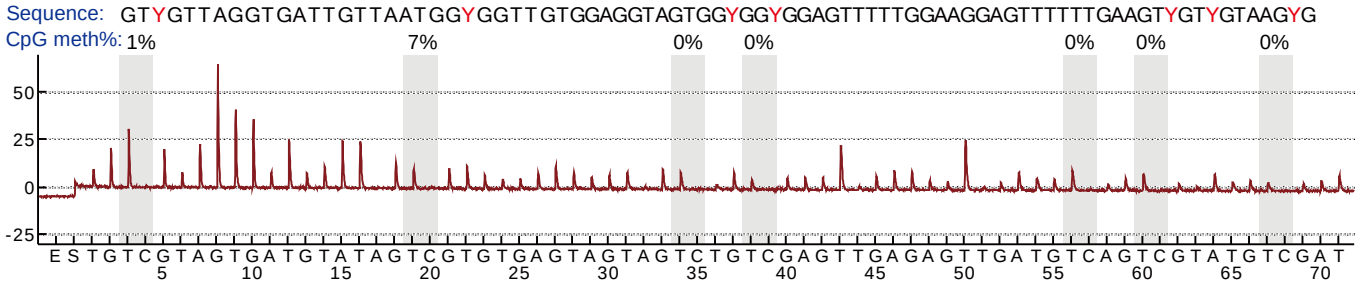


Pyrogram for *DDX26B* in 592E2 male EEM

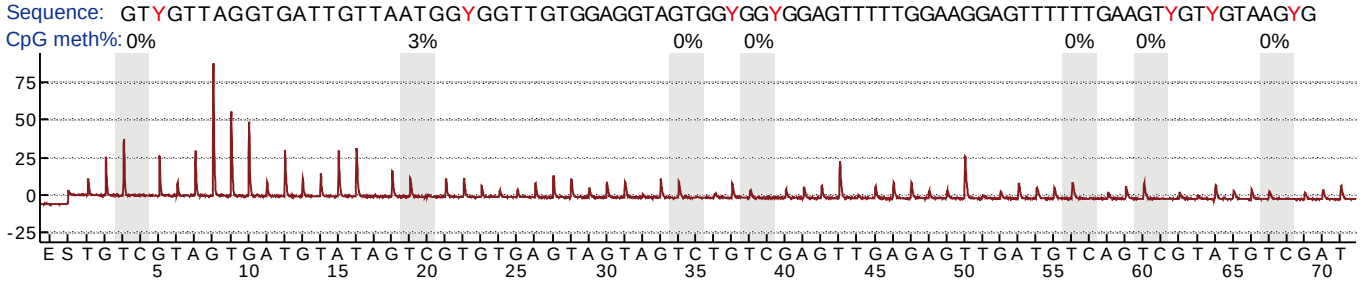


(M). Pyrograms for PyroMark analysis of the methylation profile at *MTMR1* promoter CpG island. The methylation percentages for 7 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

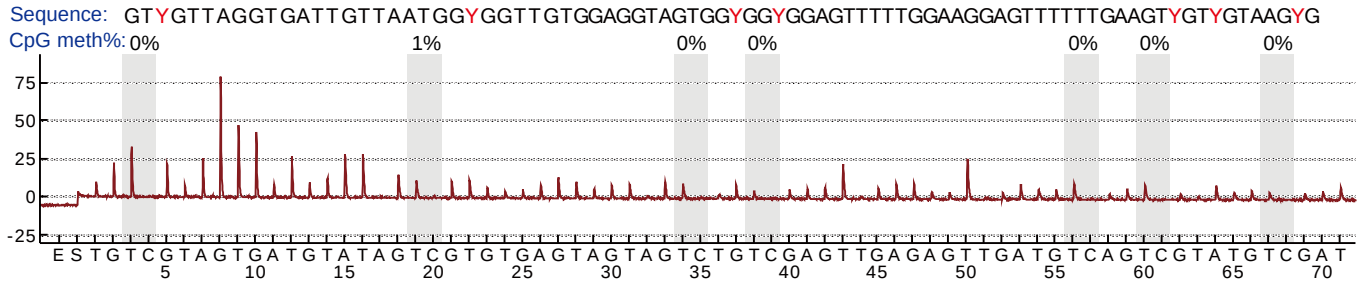
Pyrogram for *MTMR1* in 592E1 female fetal brain



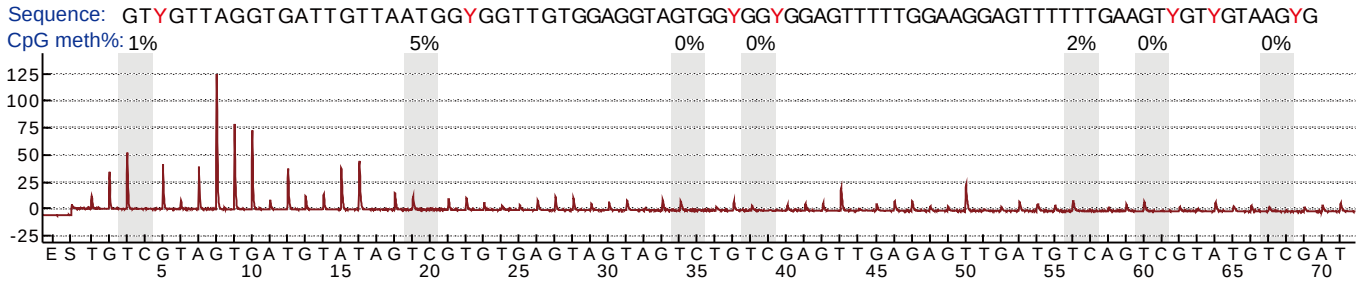
Pyrogram for *MTMR1* in 592E2 male fetal brain



Pyrogram for *MTMR1* in 592E1 female EEM

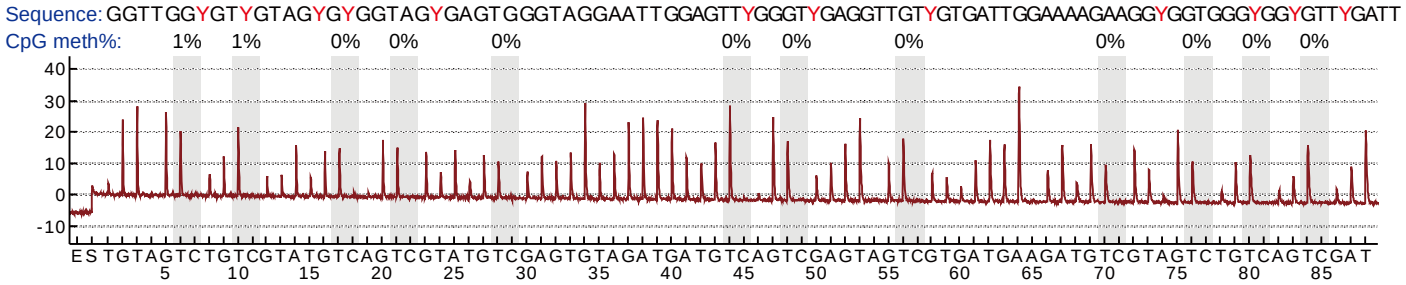


Pyrogram for *MTMR1* in 592E2 male EEM

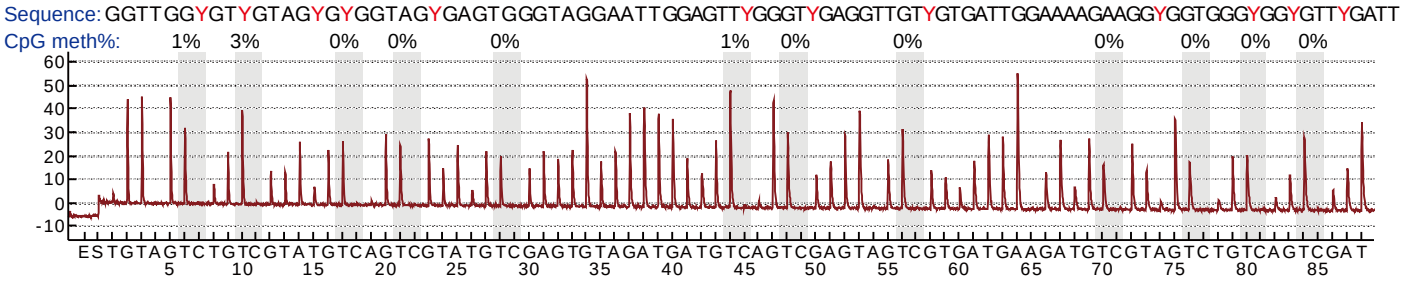


(N). Pyrograms for PyroMark analysis of the methylation profile at *CD99L2* promoter CpG island. The methylation percentages for 12 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

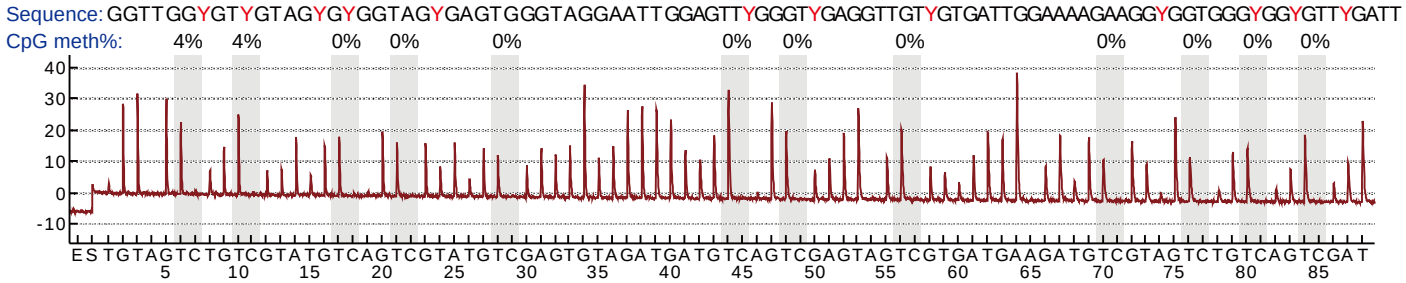
Pyrogram for *CD99L2* in 592E1 female fetal brain



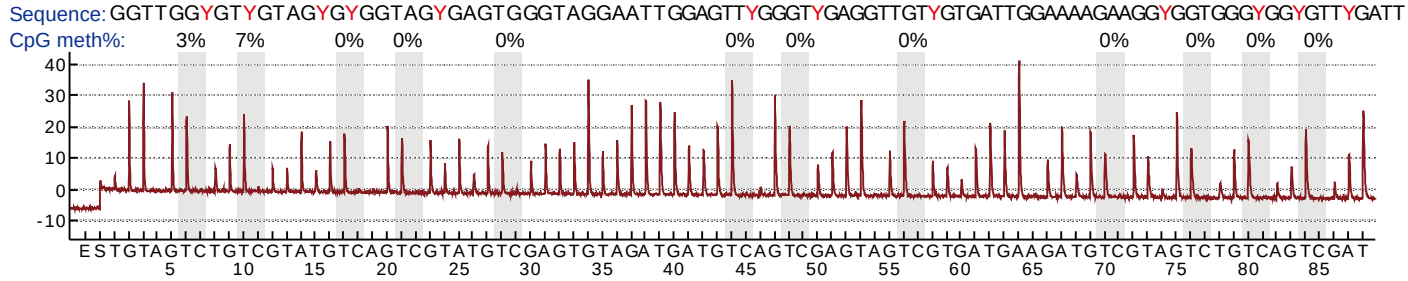
Pyrogram for *CD99L2* in 592E2 male fetal brain



Pyrogram for *CD99L2* in 592E1 female EEM

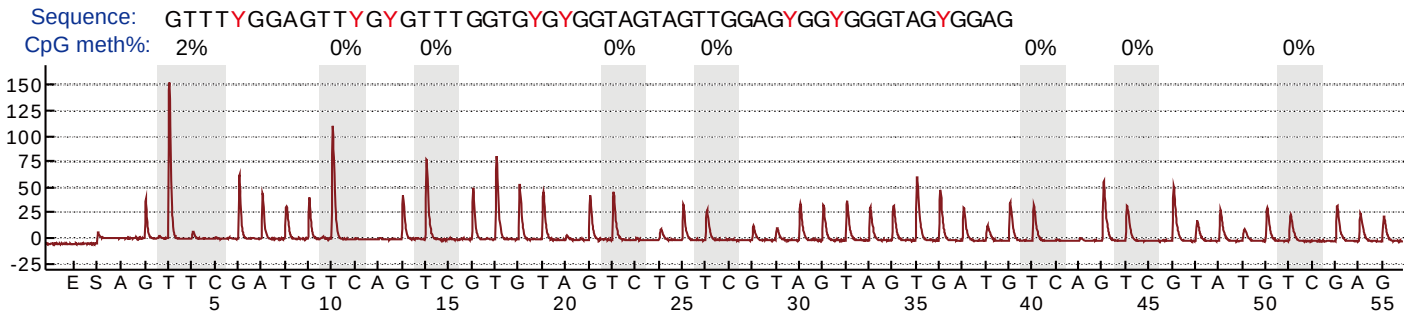


Pyrogram for *CD99L2* in 592E2 male EEM

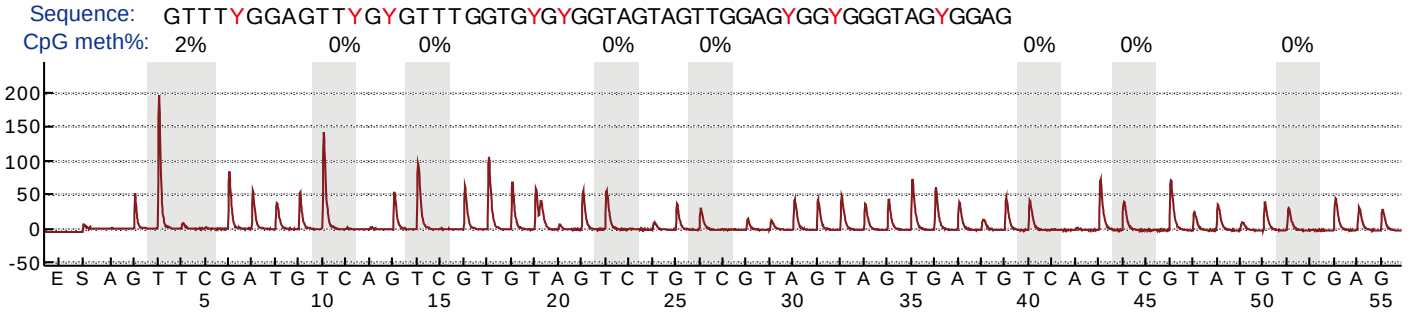


(O). Pyrograms for PyroMark analysis of the methylation profile at *GPR50* promoter CpG island. The methylation percentages for 8 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

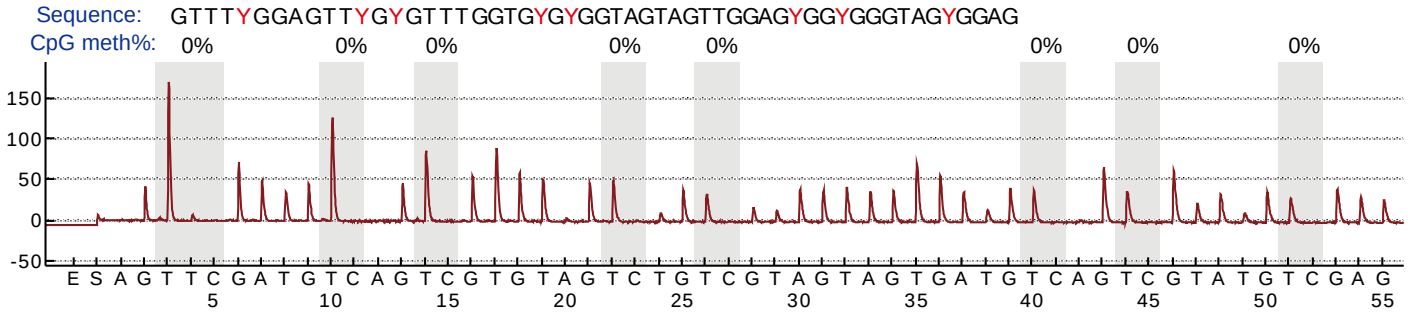
Pyrogram for *GPR50* in 592E1 female fetal brain



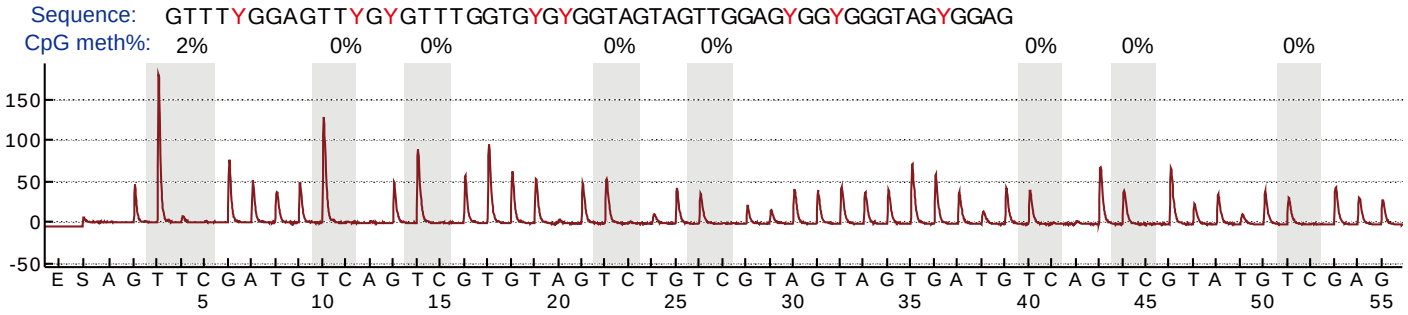
Pyrogram for *GPR50* in 592E2 male fetal brain



Pyrogram for *GPR50* in 592E1 female EEM

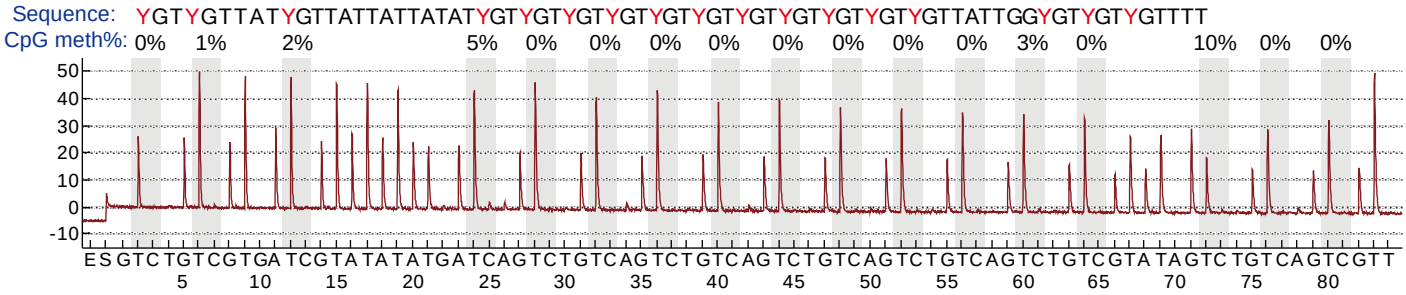


Pyrogram for *GPR50* in 592E2 male EEM

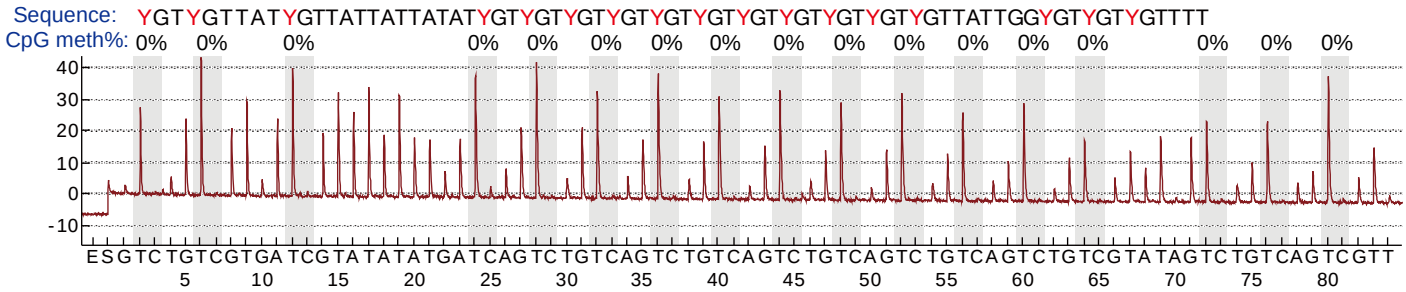


(P). Pyrograms for PyroMark analysis of the methylation profile at *ATP7A* promoter CpG island. The methylation percentages for 17 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

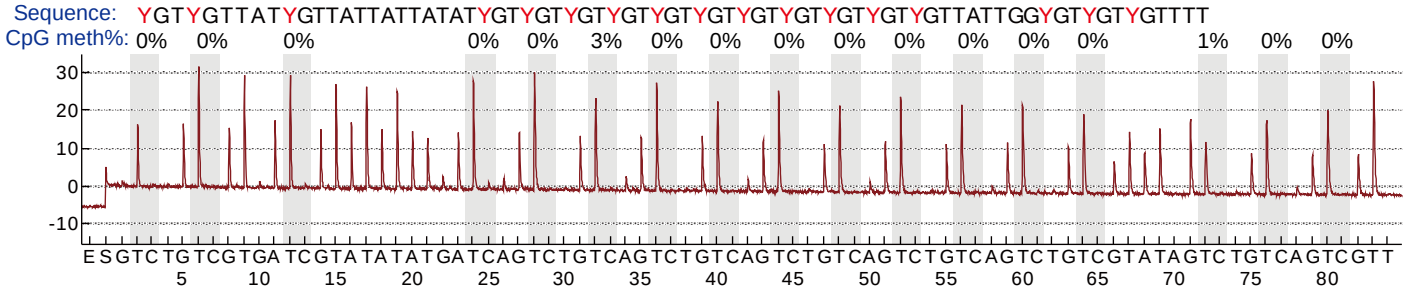
Pyrogram for *ATP7A* in 592E1 female fetal brain



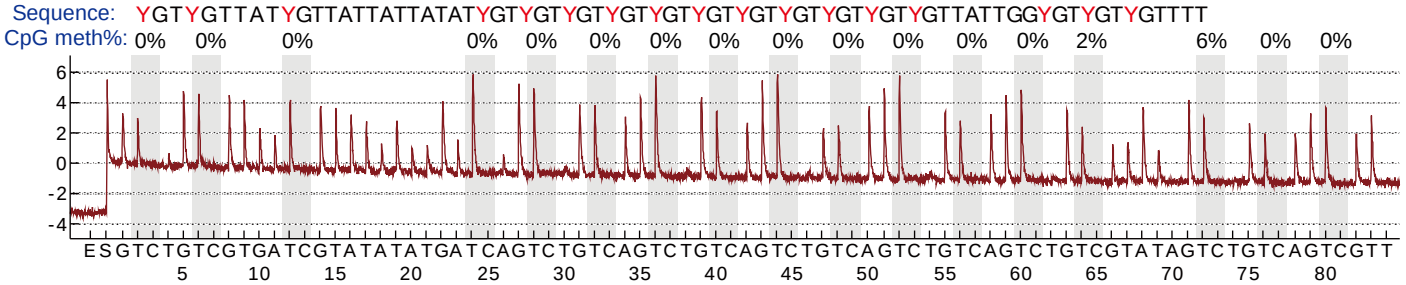
Pyrogram for *ATP7A* in 592E2 male fetal brain



Pyrogram for *ATP7A* in 592E1 female EEM

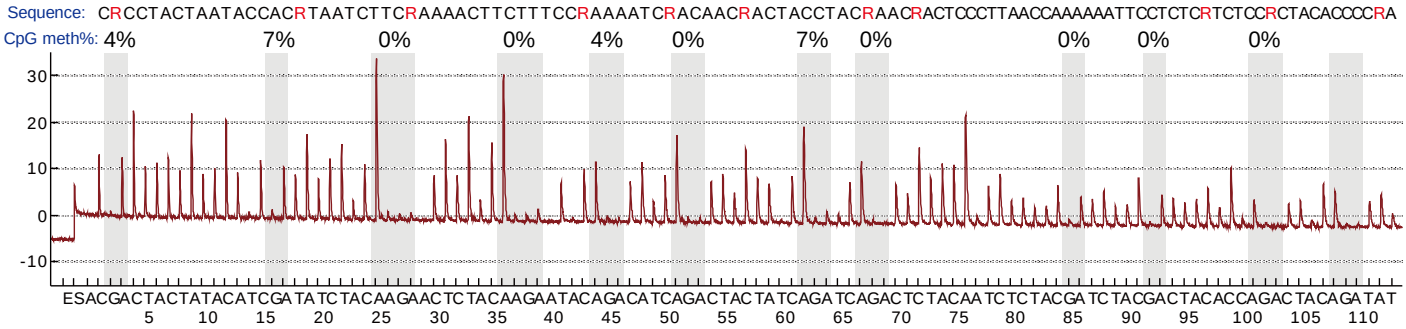


Pyrogram for *ATP7A* in 592E2 male EEM

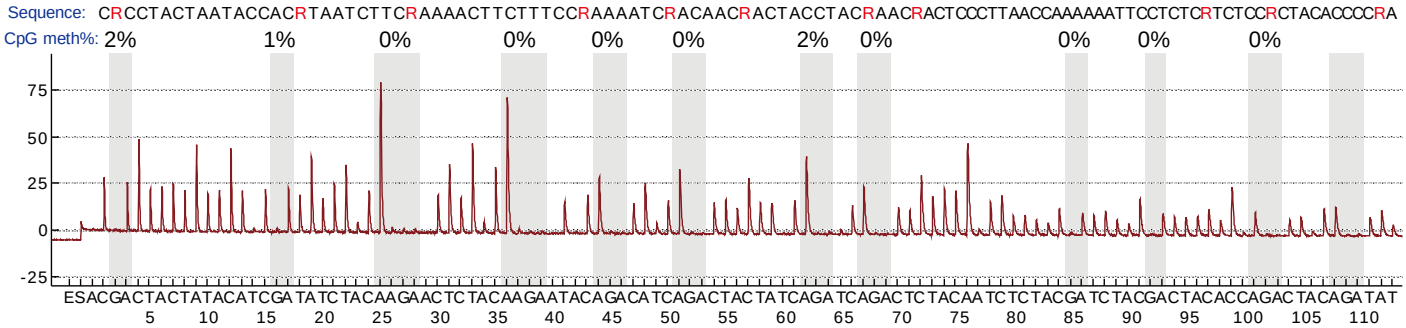


(Q). Pyrograms for PyroMark analysis of the methylation profile at *FNDC3C1* promoter CpG island. The methylation percentages for 11 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM). The PyroMark primers target the minus strand.

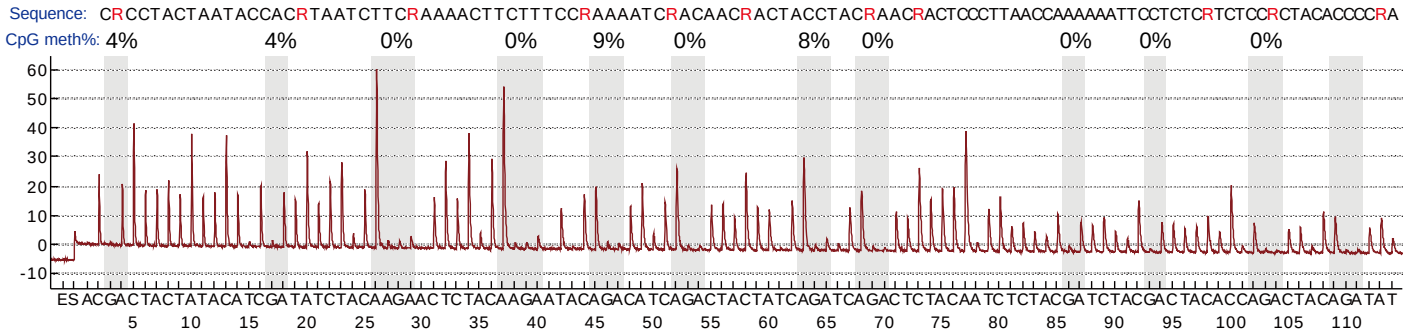
Pyrogram for *FNDC3C1* in 592E1 female fetal brain



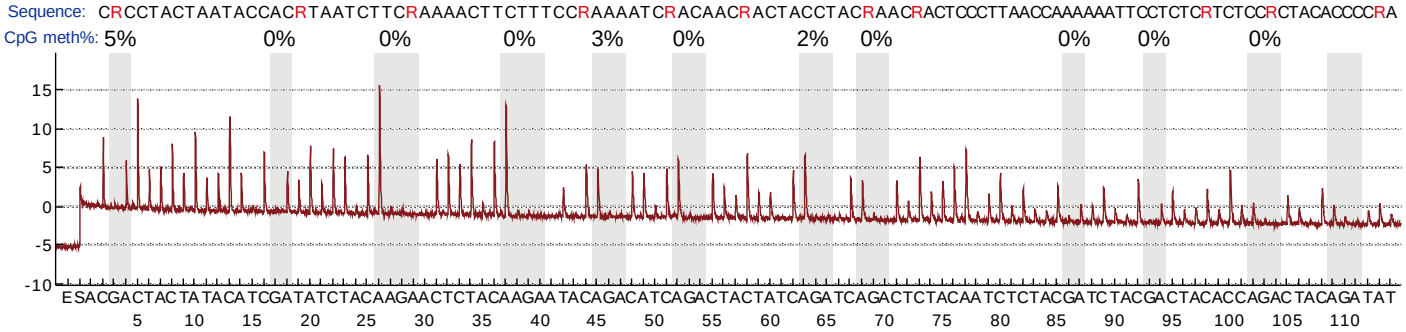
Pyrogram for *FNDC3C1* in 592E2 male fetal brain



Pyrogram for *FNDC3C1* in 592E1 female EEM

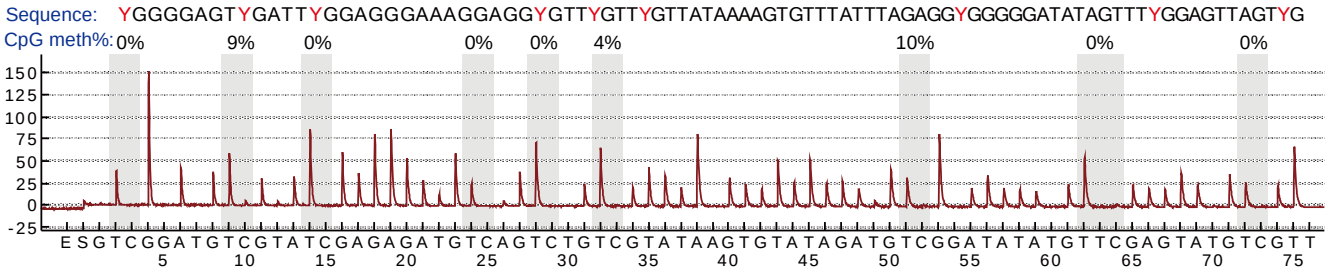


Pyrogram for *FNDC3C1* in 592E2 male EEM

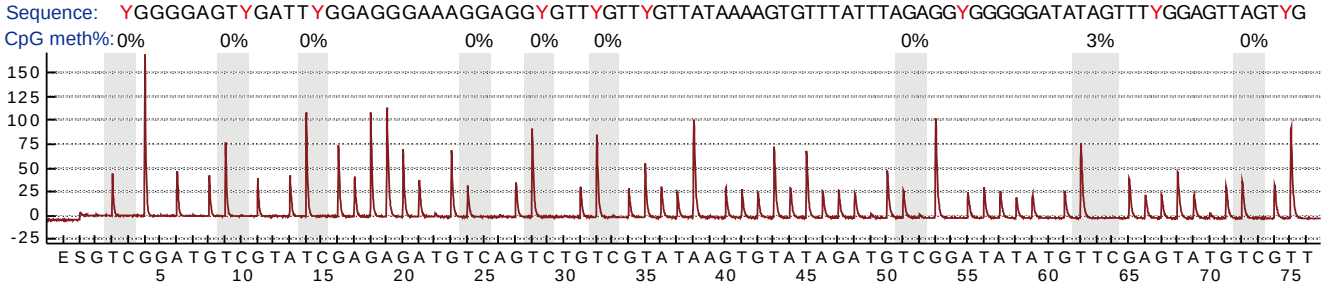


(R). Pyrograms for PyroMark analysis of the methylation profile at *PLS3* promoter CpG island. The methylation percentages for 9 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

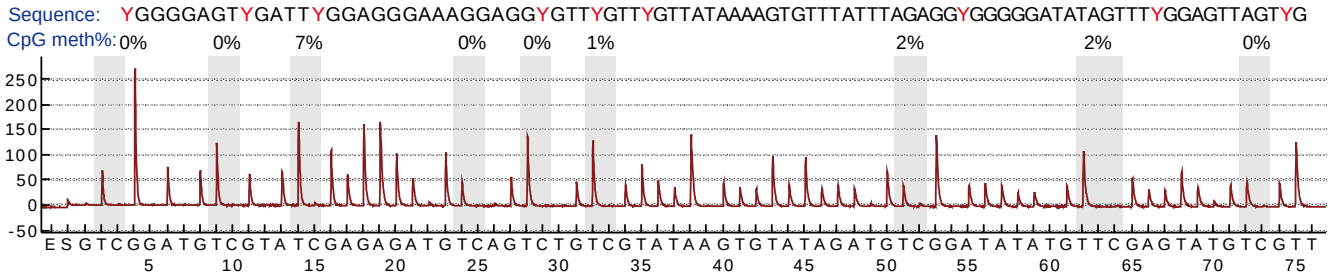
Pyrogram for *PLS3* in 592E1 female fetal brain



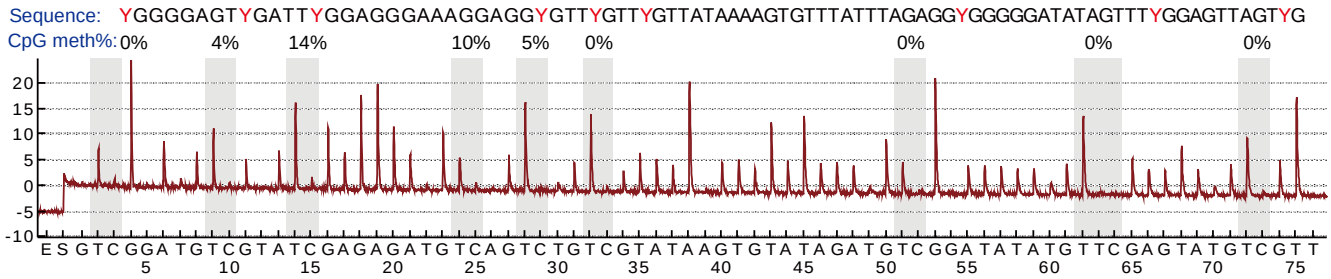
Pyrogram for *PLS3* in 592E2 male fetal brain



Pyrogram for *PLS3* in 592E1 female EEM

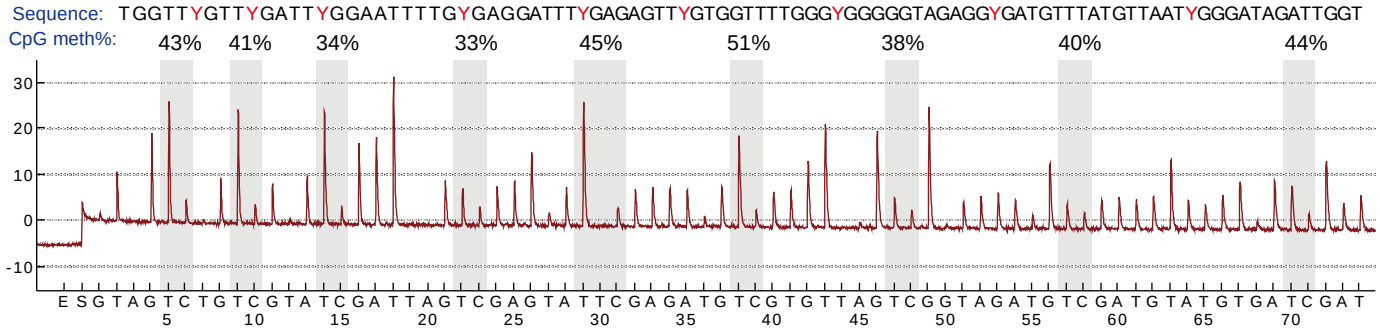


Pyrogram for *PLS3* in 592E2 male EEM

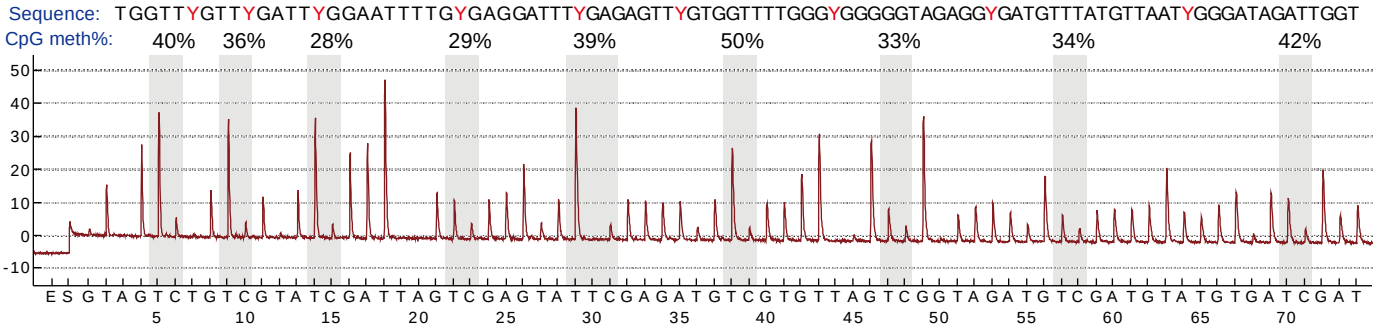


(S). Pyrograms for PyroMark analysis of the methylation profile at *AMOT* promoter CpG island. The methylation percentages for 9 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

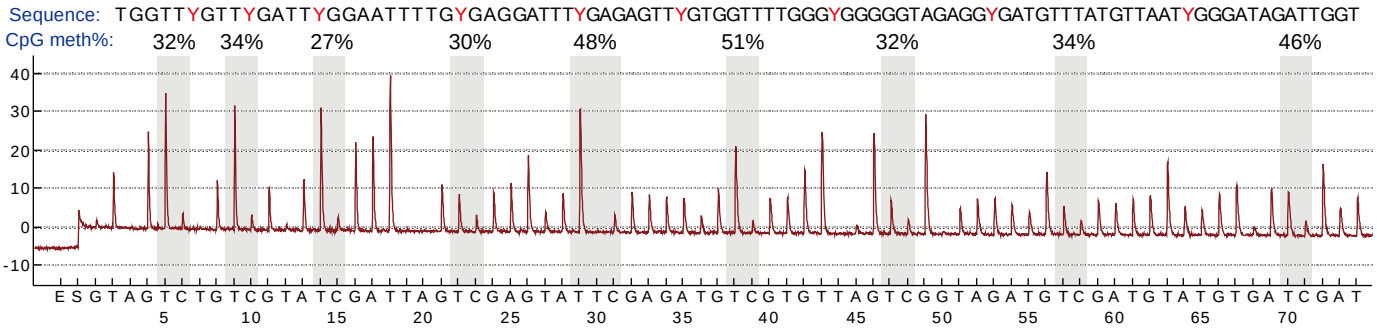
Pyrogram for *AMOT* in 592E1 female fetal brain



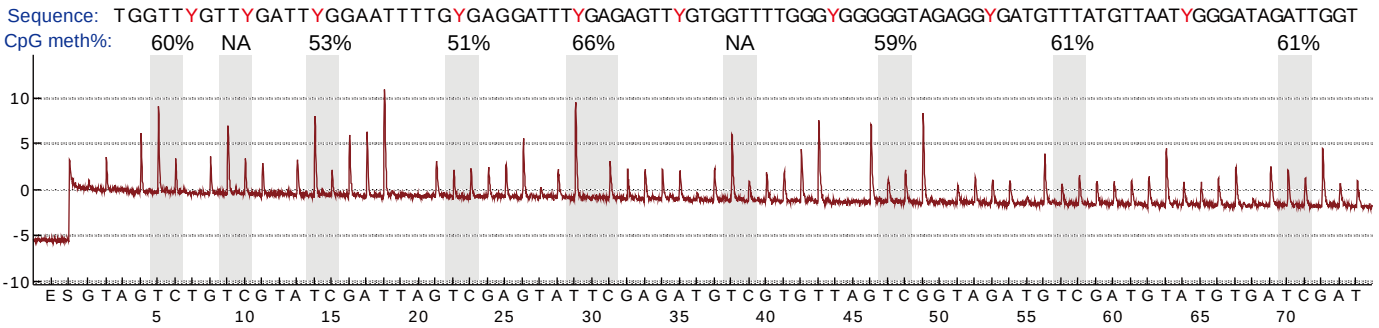
Pyrogram for *AMOT* in 592E2 male fetal brain



Pyrogram for *AMOT* in 592E1 female EEM

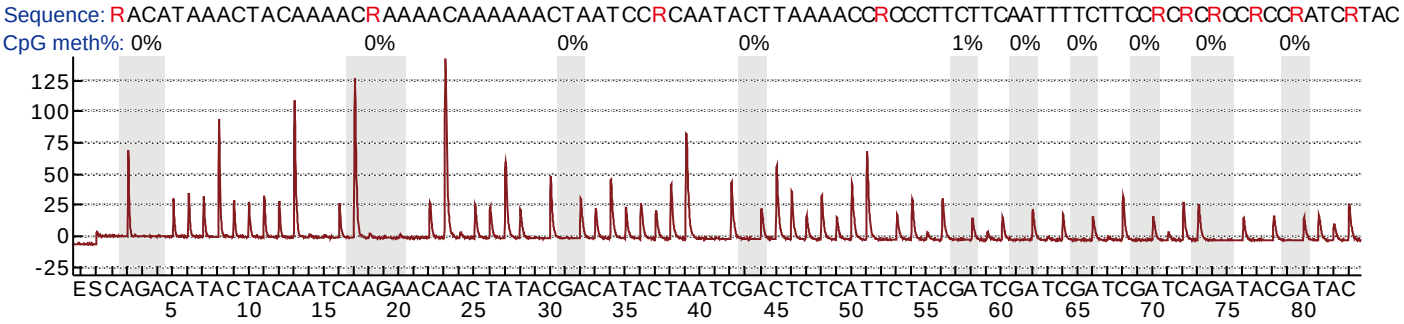


Pyrogram for *AMOT* in 592E2 male EEM

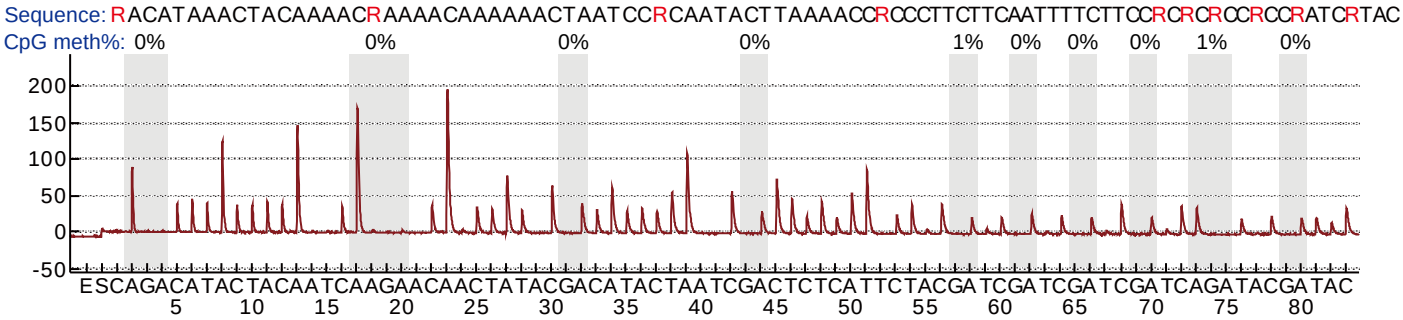


(T). Pyrograms for PyroMark analysis of the methylation profile at *TAF1* promoter CpG island. The methylation percentages for 10 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM). The PyroMark primers target the minus strand.

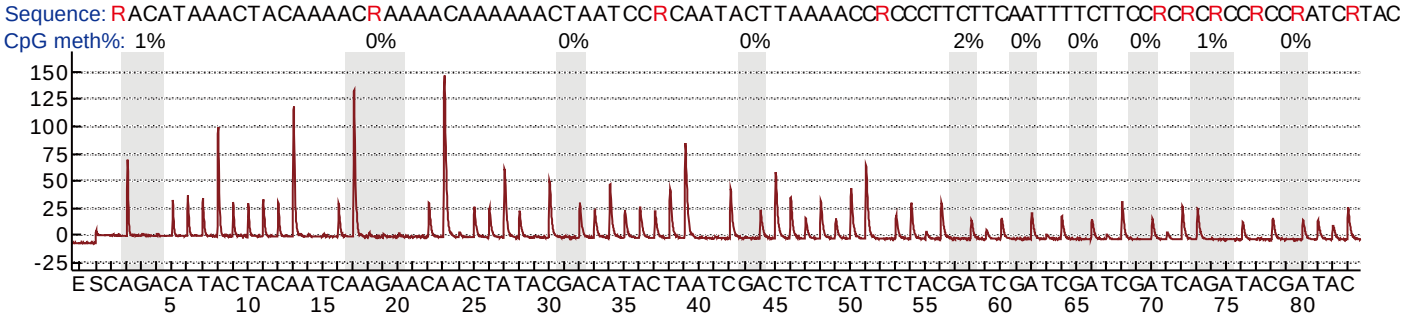
Pyrogram for *TAF1* in 592E1 female fetal brain



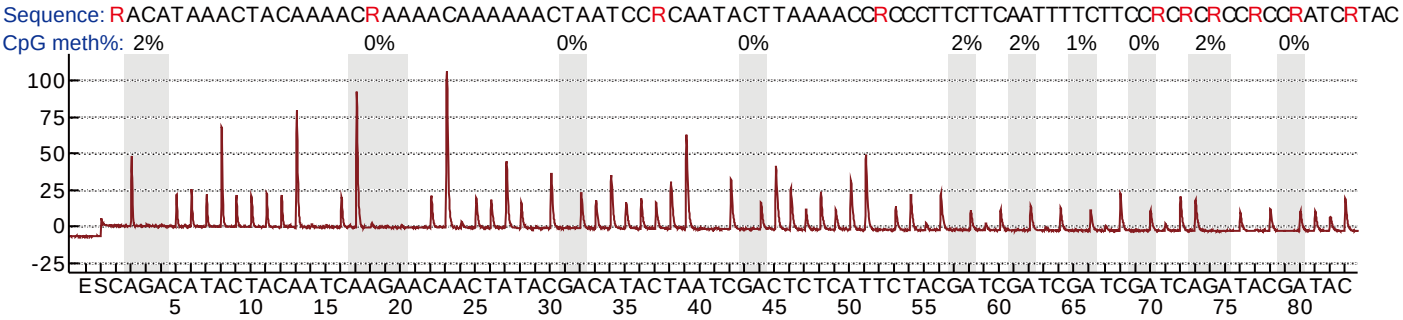
Pyrogram for *TAF1* in 592E2 male fetal brain



Pyrogram for *TAF1* in 592E1 female EEM

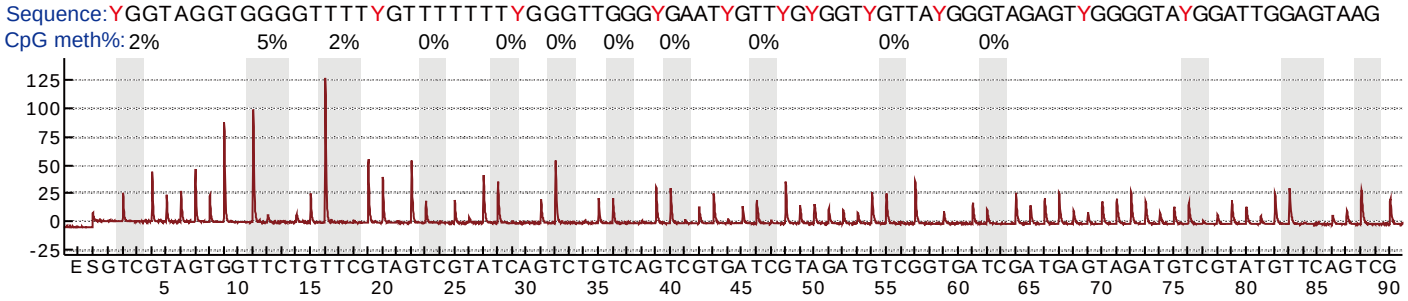


Pyrogram for *TAF1* in 592E2 male EEM

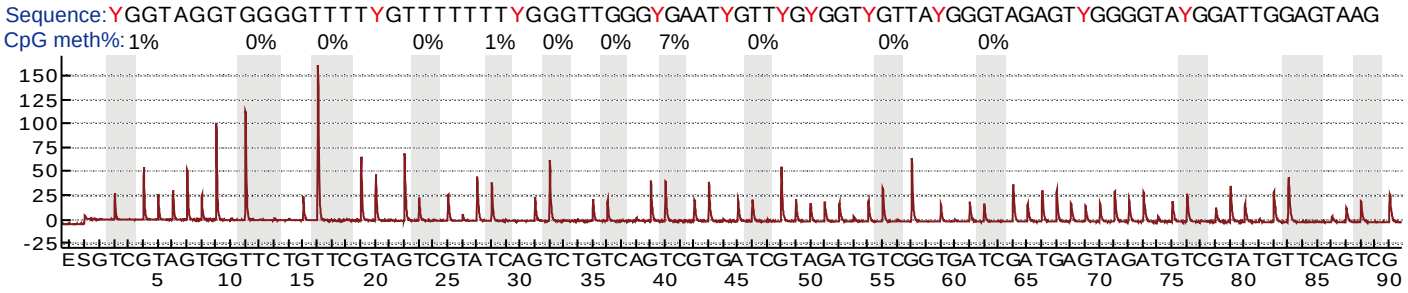


(U). Pyrograms for PyroMark analysis of the methylation profile at *KCTD12B* promoter CpG island. The methylation percentages for 11 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

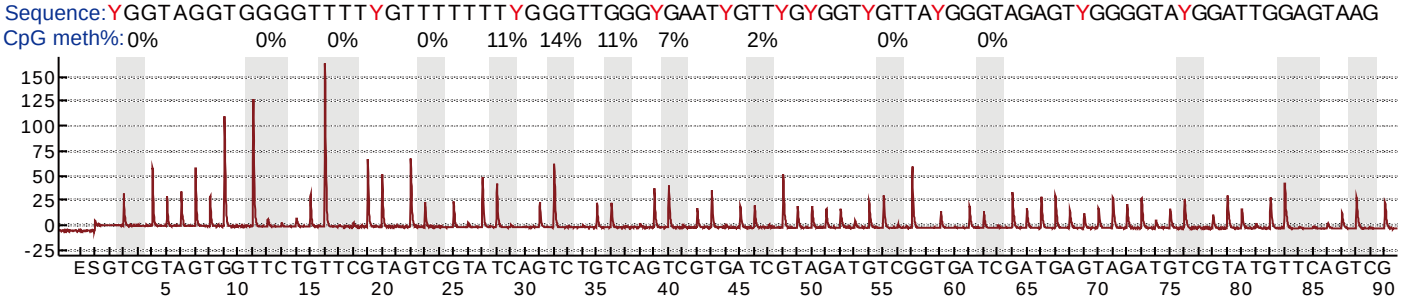
Pyrogram for *KCTD12B* in 592E1 female fetal brain



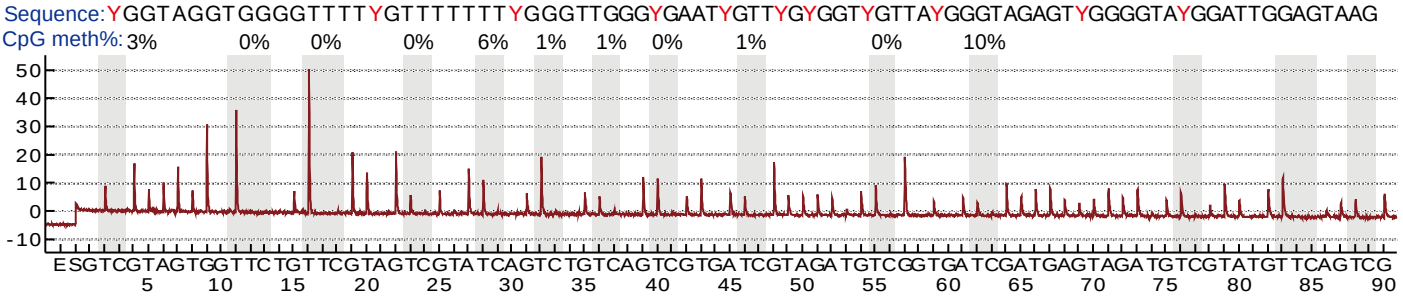
Pyrogram for *KCTD12B* in 592E2 male fetal brain



Pyrogram for *KCTD12B* in 592E1 female EEM

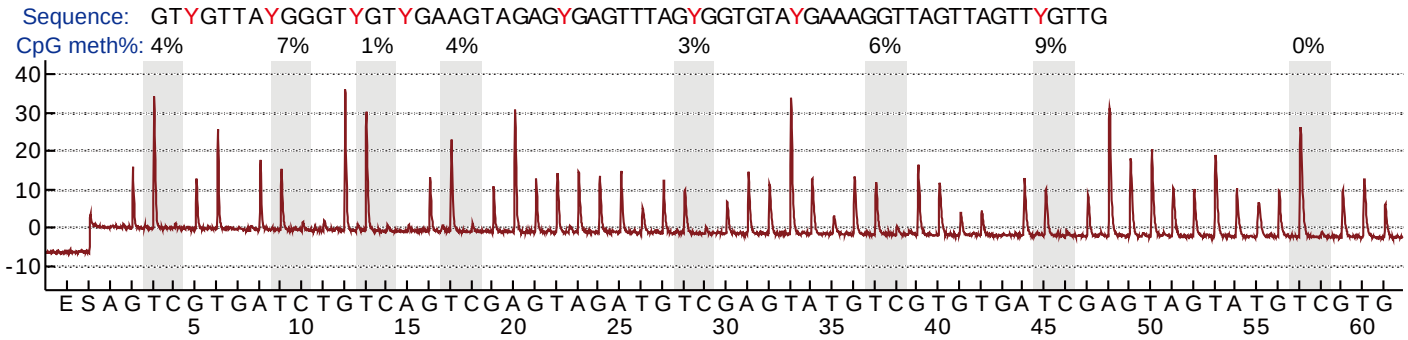


Pyrogram for *KCTD12B* in 592E2 male EEM

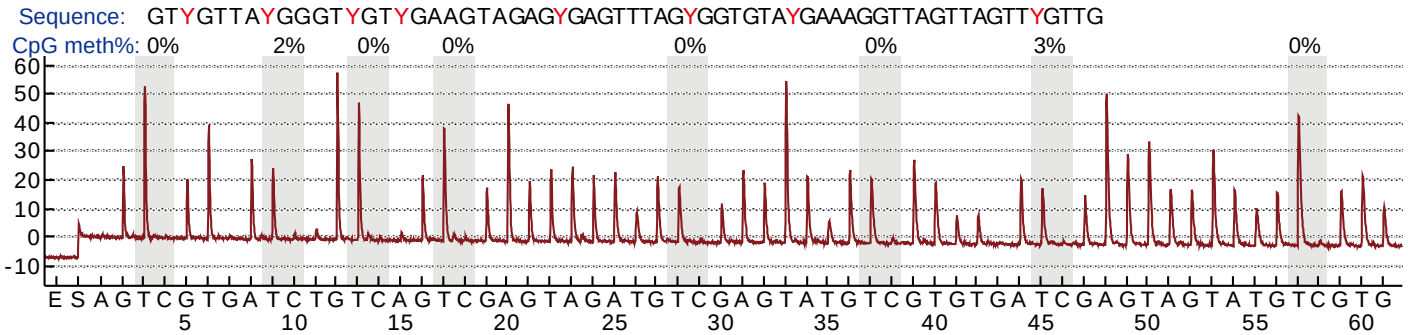


(V). Pyrograms for PyroMark analysis of the methylation profile at *KLF8* promoter CpG island. The methylation percentages for 8 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

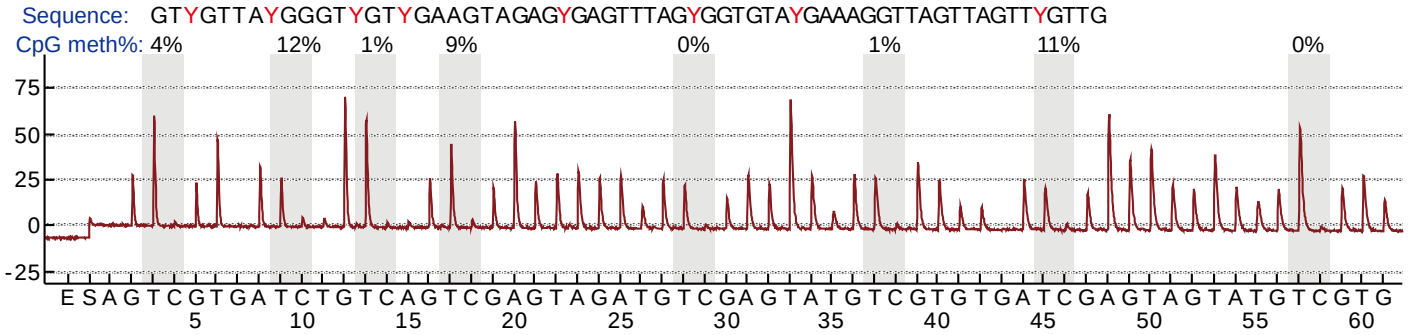
Pyrogram for *KLF8* in 592E1 female fetal brain



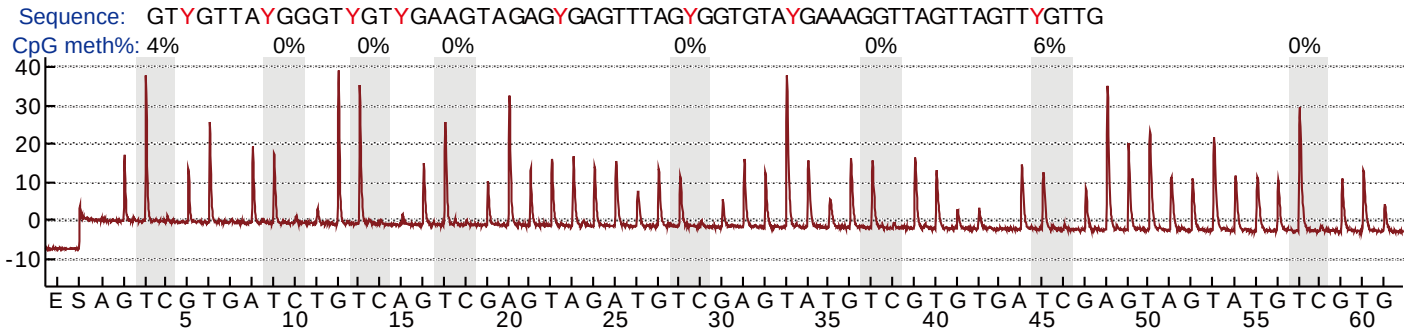
Pyrogram for *KLF8* in 592E2 male fetal brain



Pyrogram for *KLF8* in 592E1 female EEM

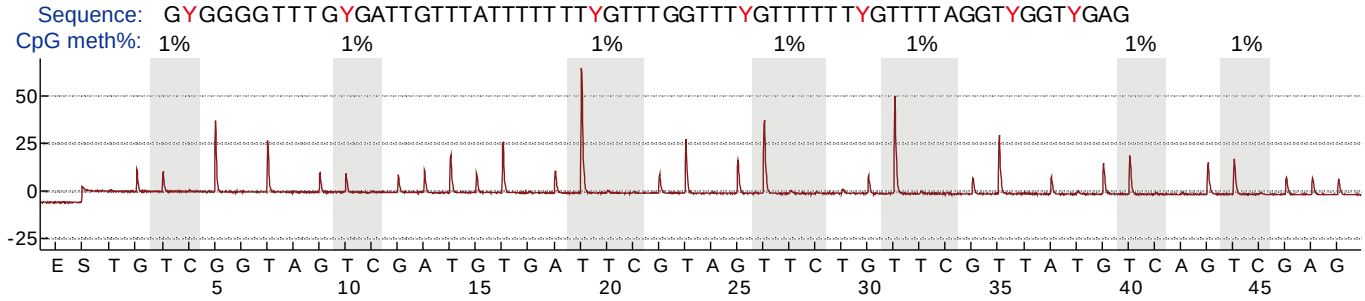


Pyrogram for *KLF8* in 592E2 male EEM

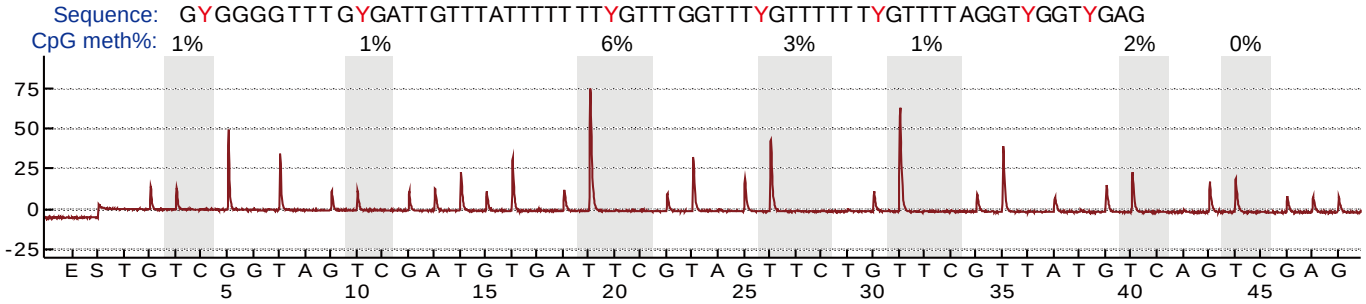


(W). Pyrograms for PyroMark analysis of the methylation profile at *IL13RA1* promoter CpG island. The methylation percentages for 7 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

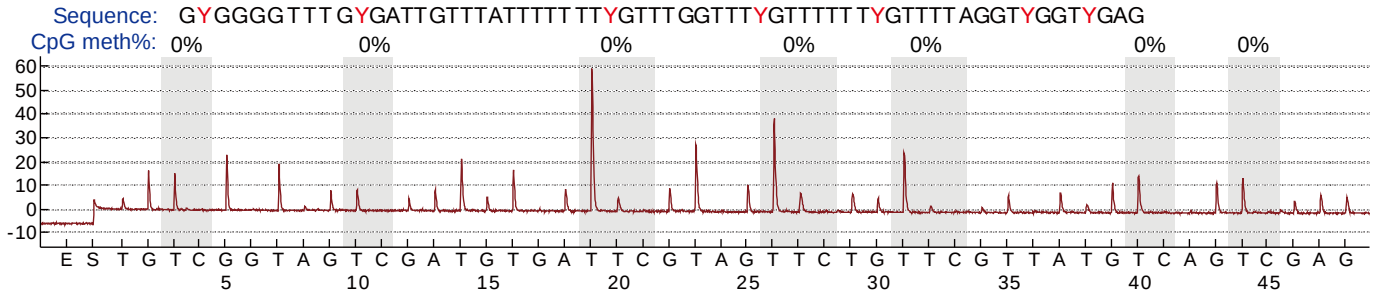
Pyrogram for *IL13RA1* in 592E1 female fetal brain



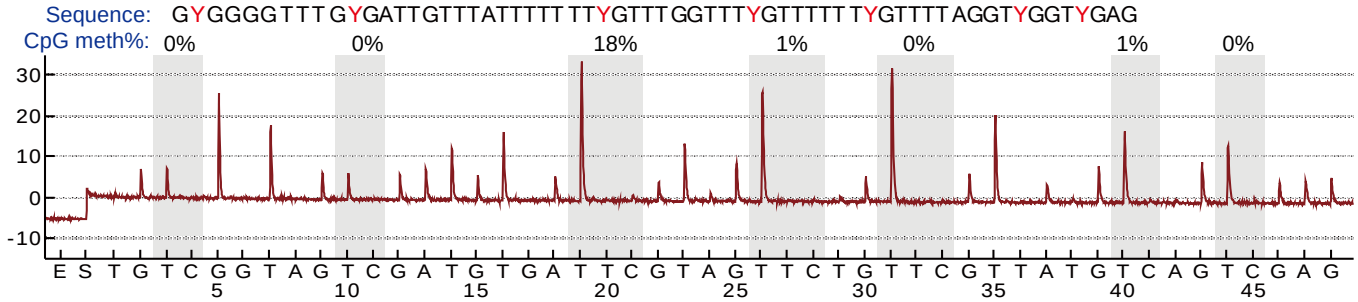
Pyrogram for *IL13RA1* in 592E2 male fetal brain



Pyrogram for *IL13RA1* in 592E1 female EEM

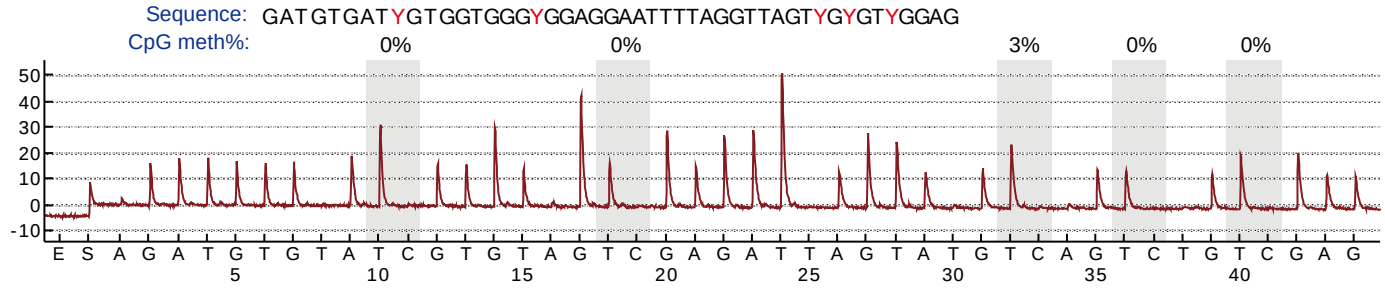


Pyrogram for *IL13RA1* in 592E2 male EEM

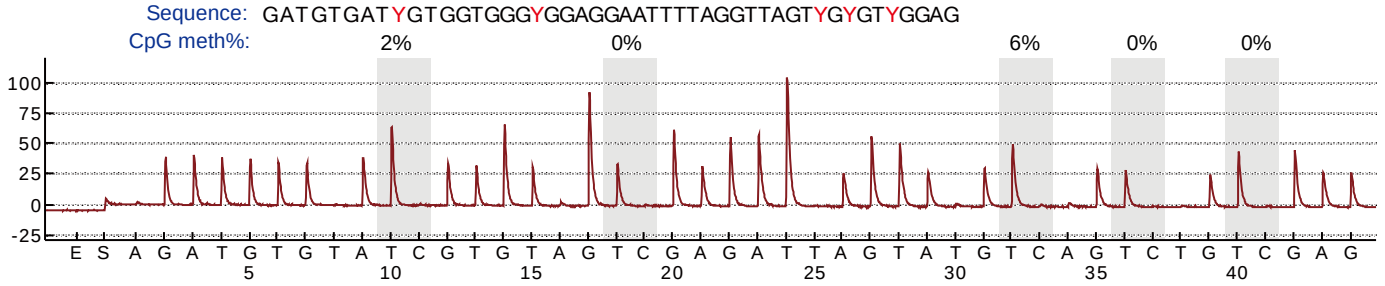


(X). Pyrograms for PyroMark analysis of the methylation profile at *MAOA* promoter CpG island. The methylation percentages for 5 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

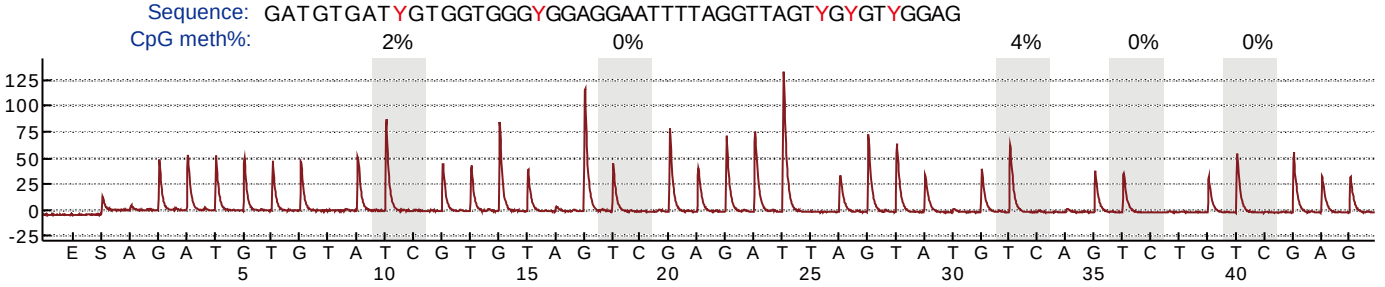
Pyrogram for *MAOA* in 592E1 female fetal brain



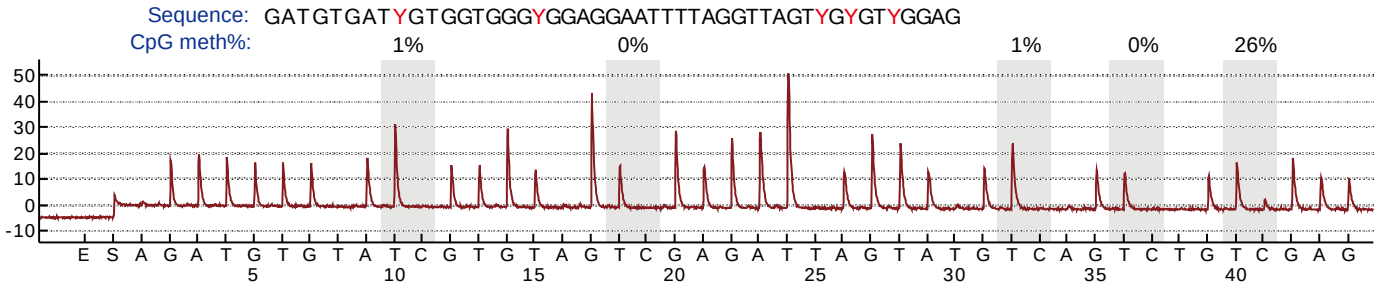
Pyrogram for *MAOA* in 592E2 male fetal brain



Pyrogram for *MAOA* in 592E1 female EEM

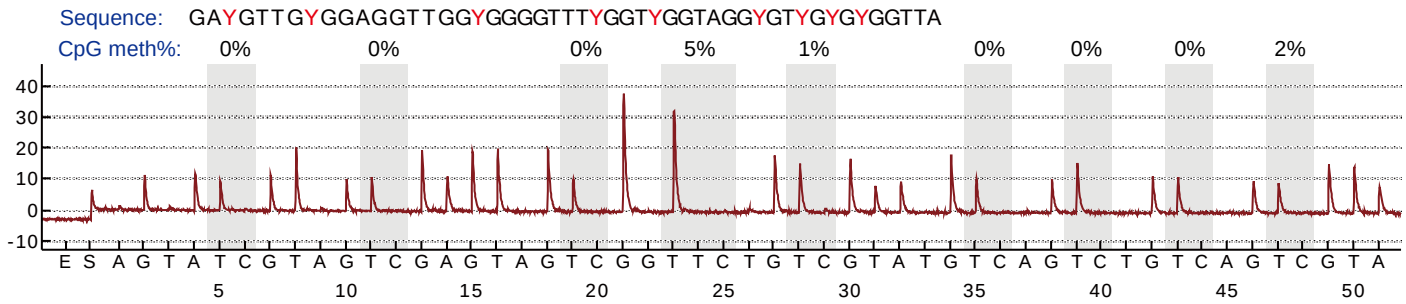


Pyrogram for *MAOA* in 592E2 male EEM

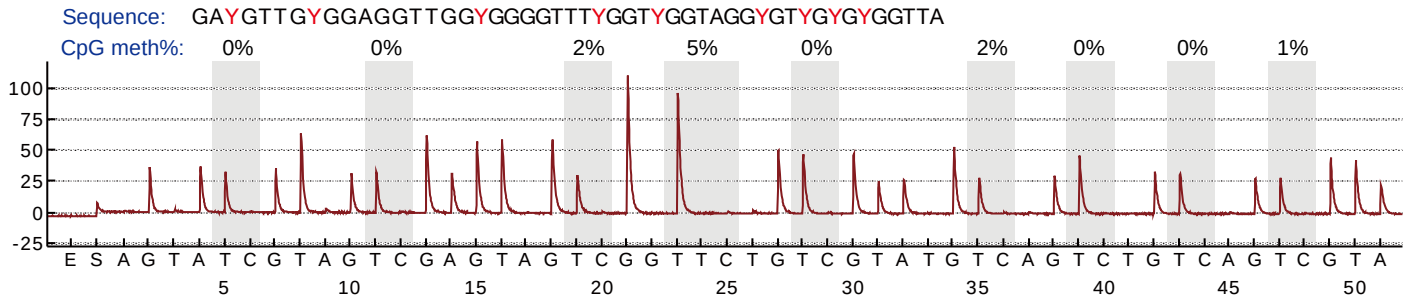


(Y). Pyrograms for PyroMark analysis of the methylation profile at *RBBP7* promoter CpG island. The methylation percentages for 9 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

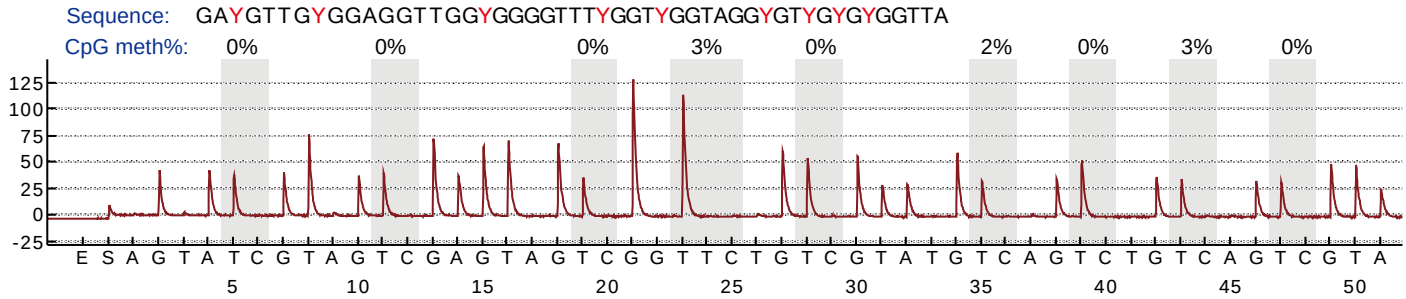
Pyrogram for *RBBP7* in 592E1 female fetal brain



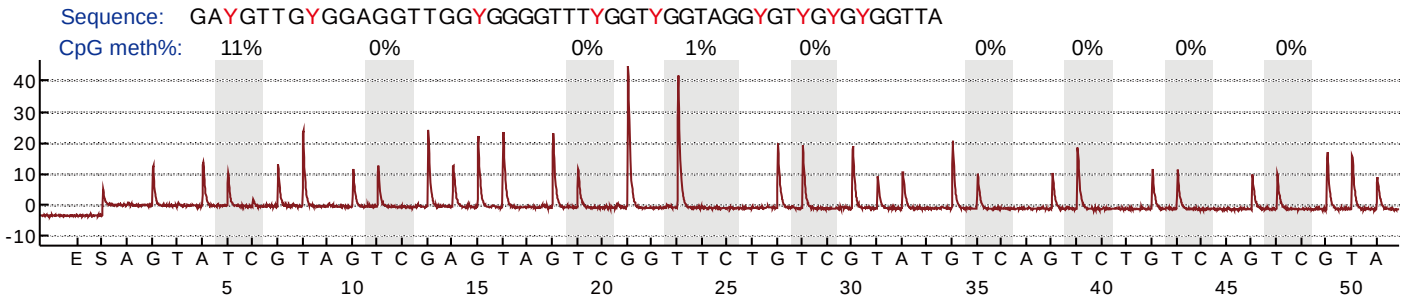
Pyrogram for *RBBP7* in 592E2 male fetal brain



Pyrogram for *RBBP7* in 592E1 female EEM

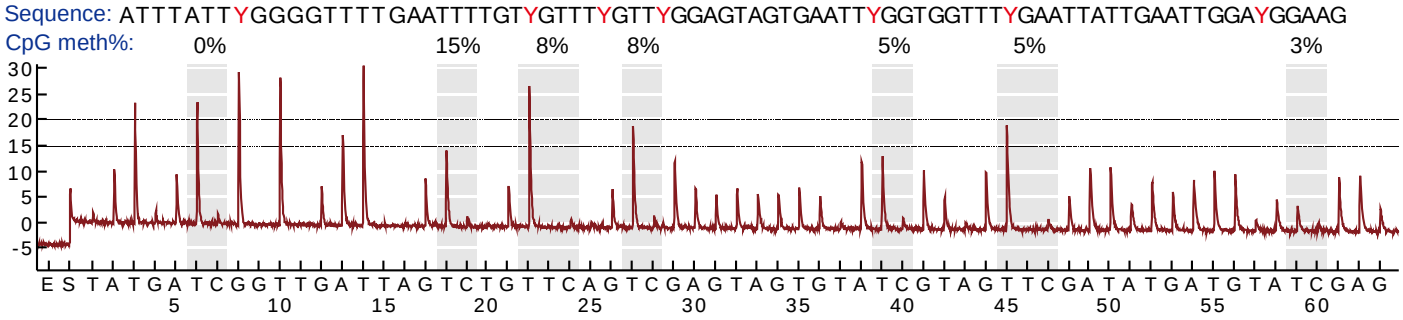


Pyrogram for *RBBP7* in 592E2 male EEM

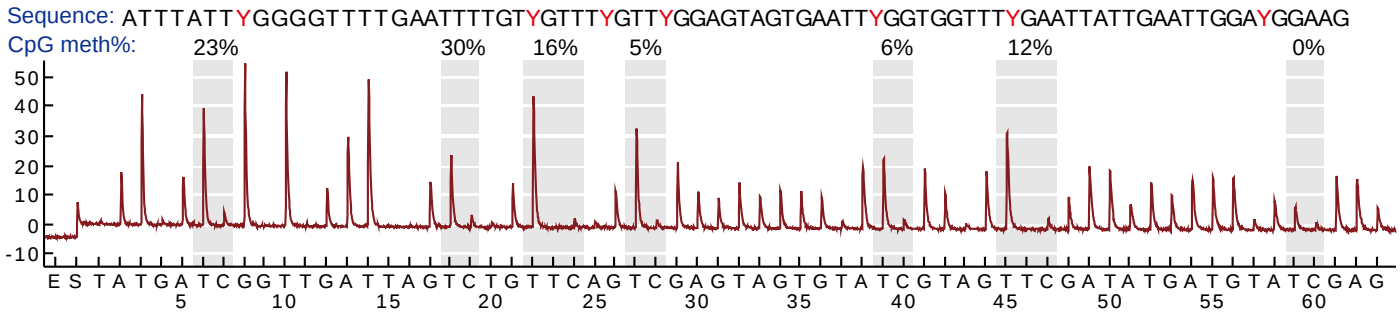


(Z). Pyrograms for PyroMark analysis of the methylation profile at *ACE2* promoter CpG island. The methylation percentages for 7 CpGs were quantified in female (592E1) and male (592E2) fetal brain and extra-embryonic membranes (EEM).

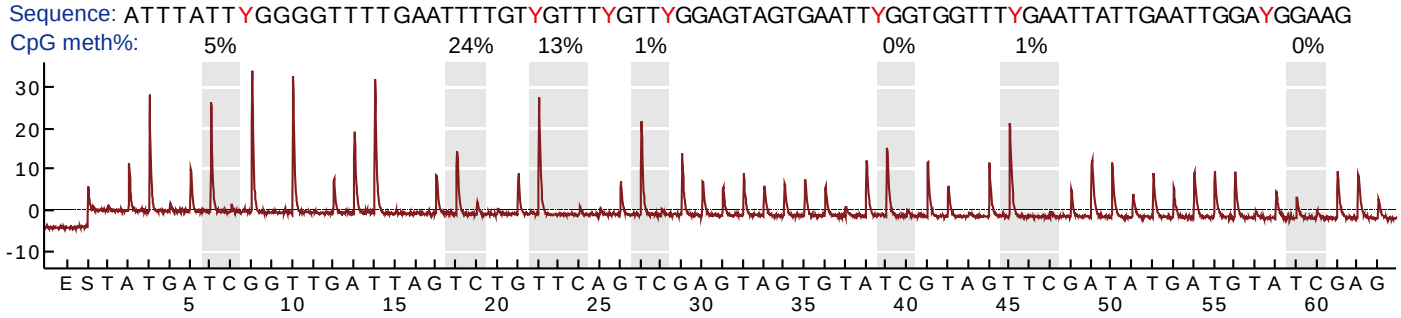
Pyrogram for *ACE2* in 592E1 female fetal brain



Pyrogram for *ACE2* in 592E2 male fetal brain



Pyrogram for *ACE2* in 592E1 female EEM



Pyrogram for *ACE2* in 592E2 male EEM

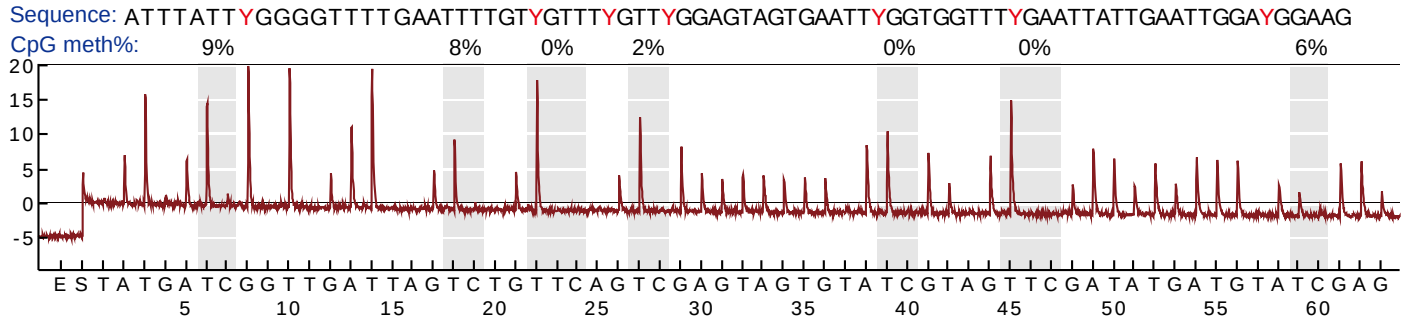


Figure S52. DNA methylation profile at promoter CpG island of nonescaper gene IDS in female head and EEM samples. Bisulfite sequencing in fetal brain and EEM shows that the *IDS* CpG island is unmethylated in both tissues. 12 CpG sites were targeted and 15 clones were sequenced for each tissue. Each line represents results from one clone. Yellow boxes depict methylated CpGs and blue boxes are unmethylated CpGs.