Reversible switching between epigenetic states in honeybee behavioral subcastes

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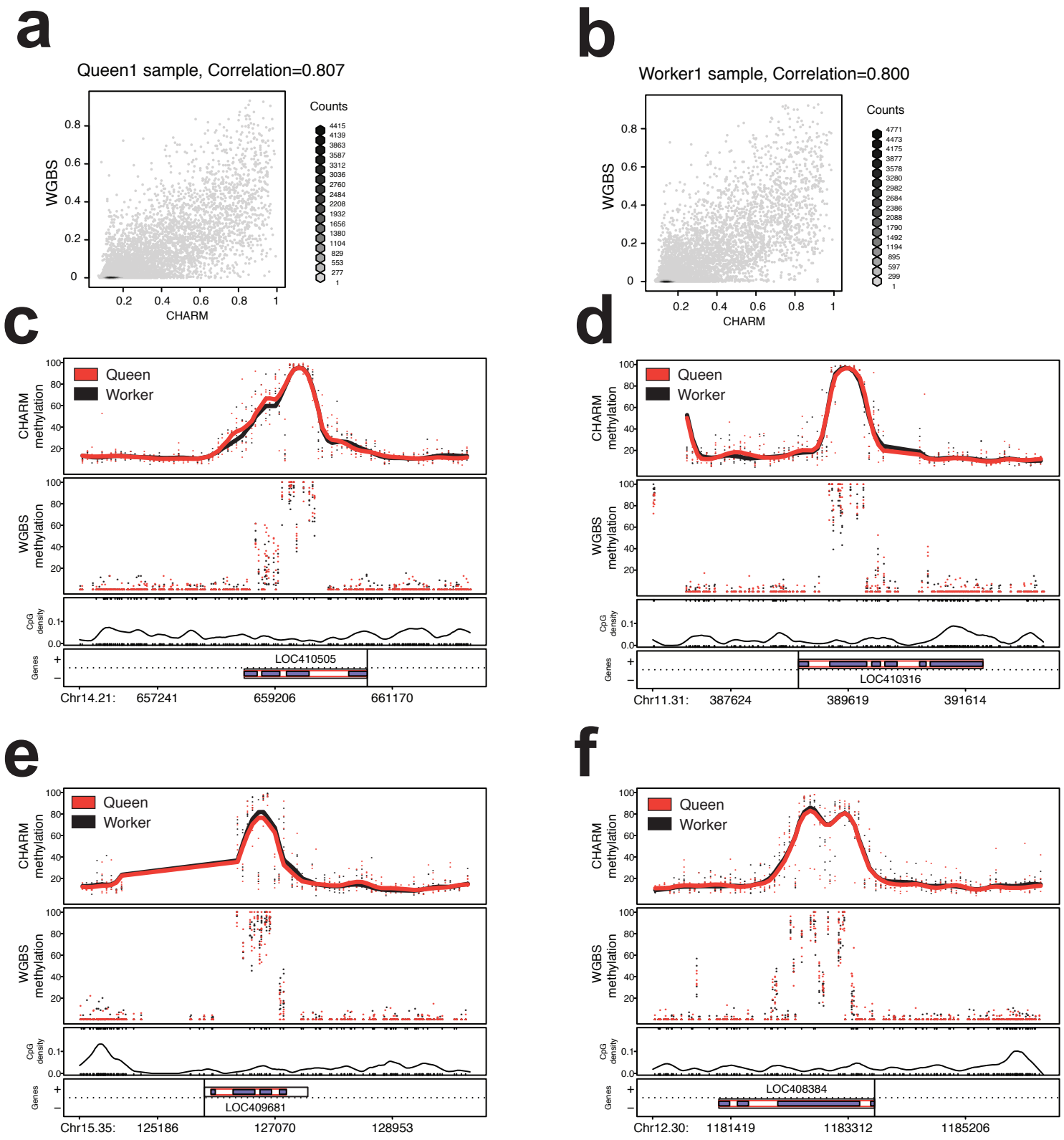
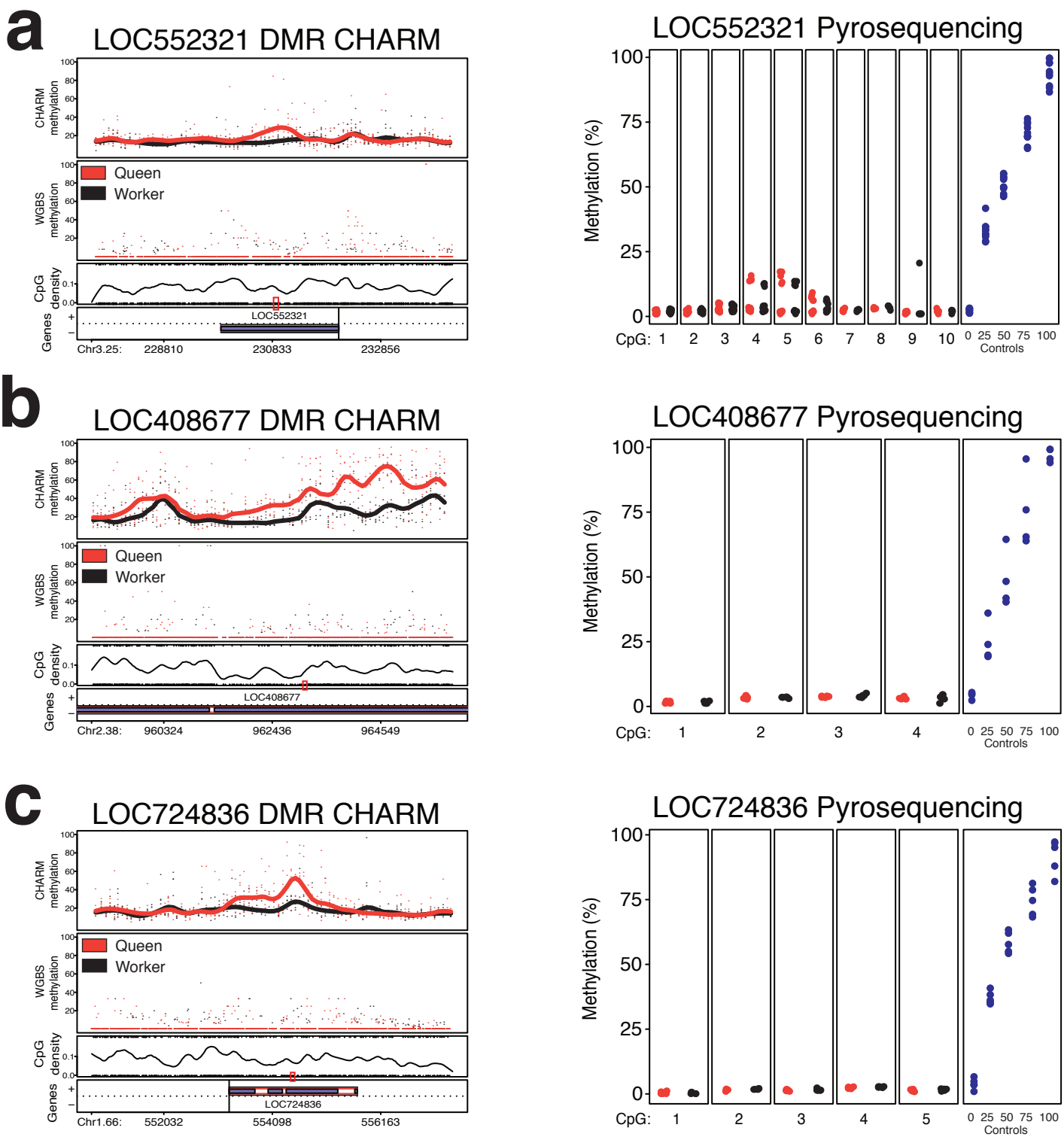
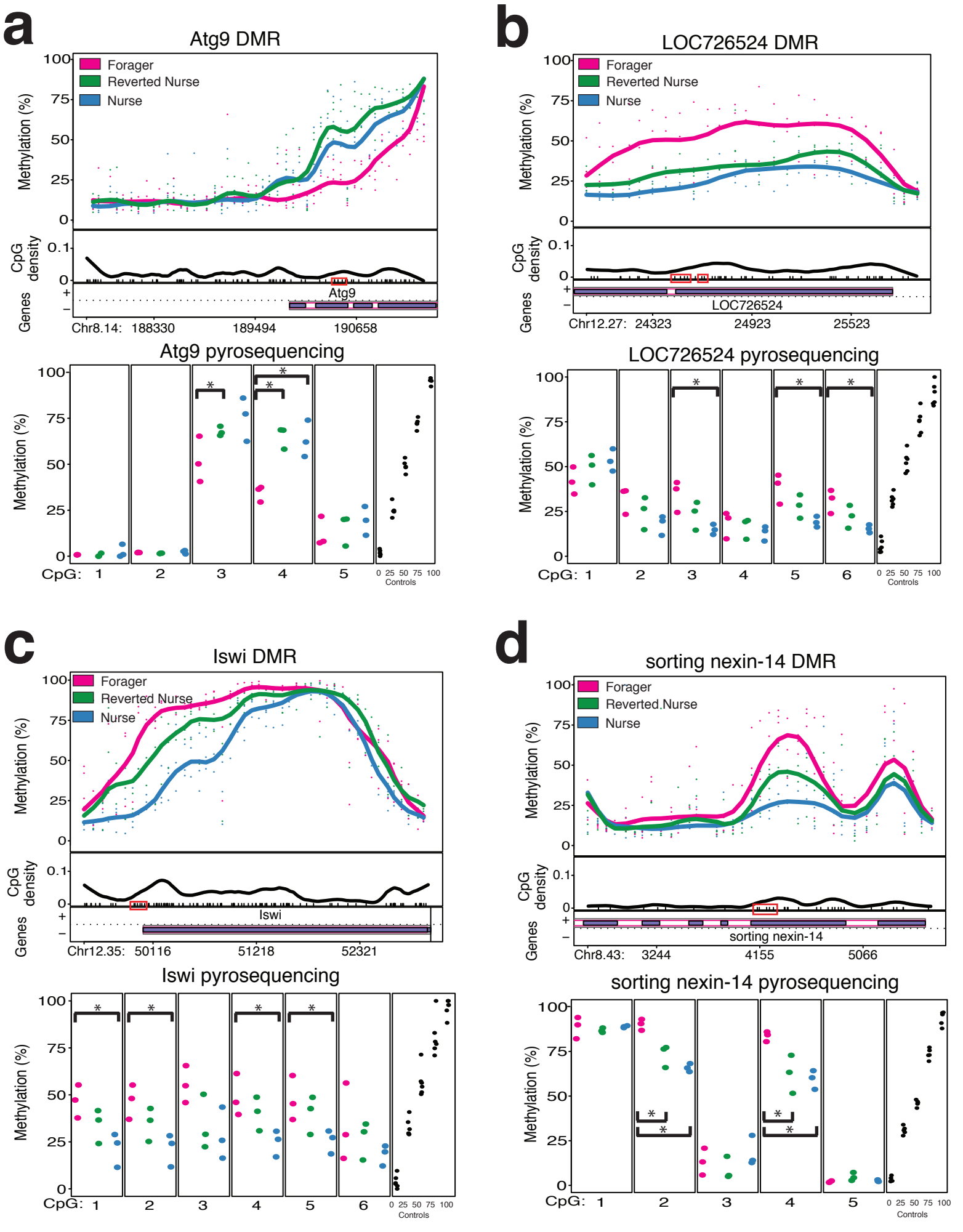
Supplementary Figures and Legends

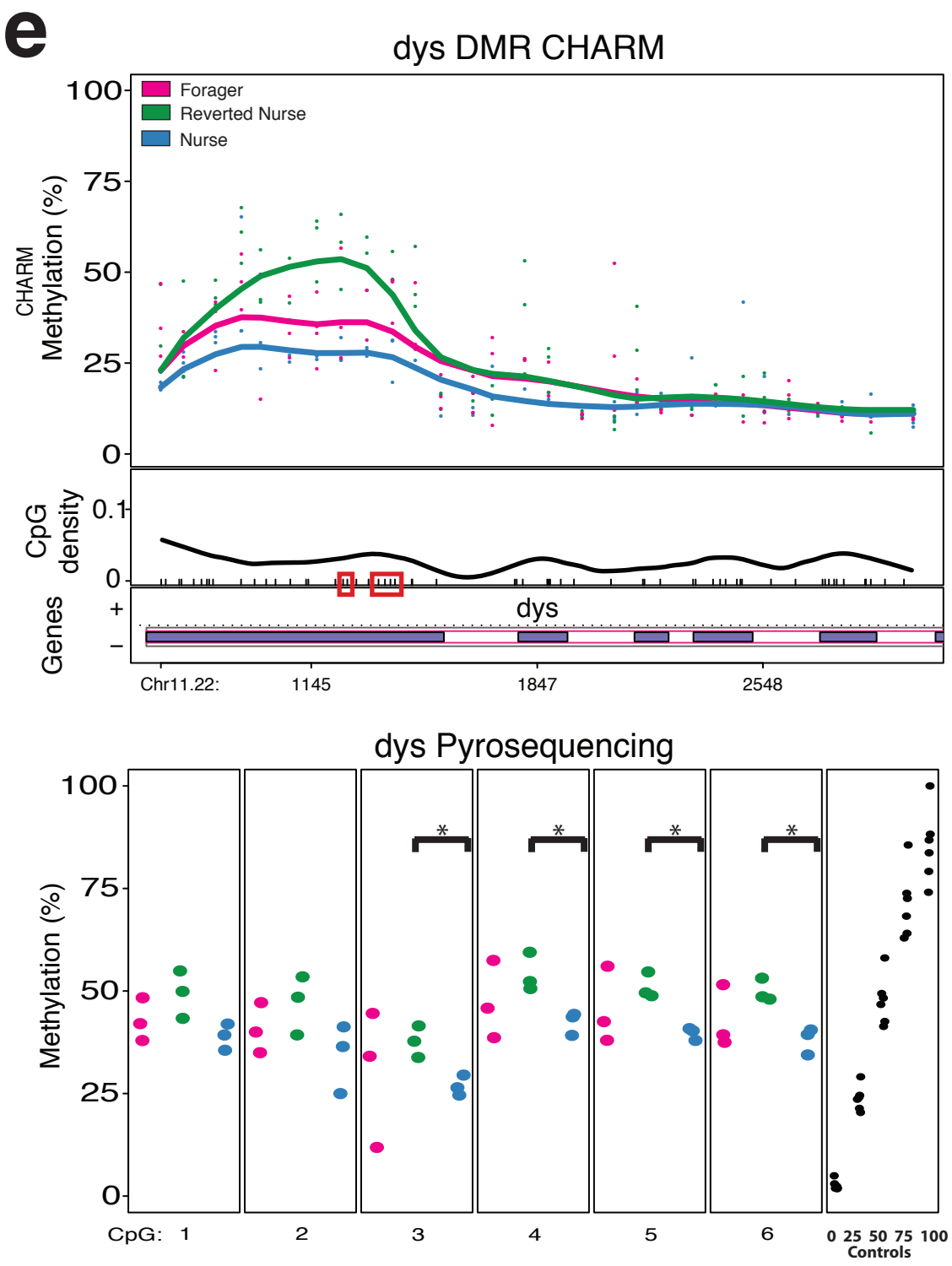


Supplementary Figure 1. CHARM correlates with WGBS.
a-b Genome-wide DNA methylation levels for queen and worker brain samples were determined by both CHARM and WGBS analysis. To determine the correlation between the two techniques, genes were broken into1000 bp windows and the average methylation for each window was calculated for each technique. Each window was required to contain at least 4 CHARM probes and 8 CpG's. Sample and correlation value is indicated above each plot. **c-f** Since methylation is sparse throughout the bee genome, it is important that genome-wide methods are able to identify regions of methylation. Both CHARM and WGBS are able to determine the location of clusters of methylation, and correctly determine the boundaries of these clusters. These four plots show the CHARM methylation in the top panel, with the same profile detected by WGBS in the second panel. CpG density and gene position information is displayed in the bottom two panels.

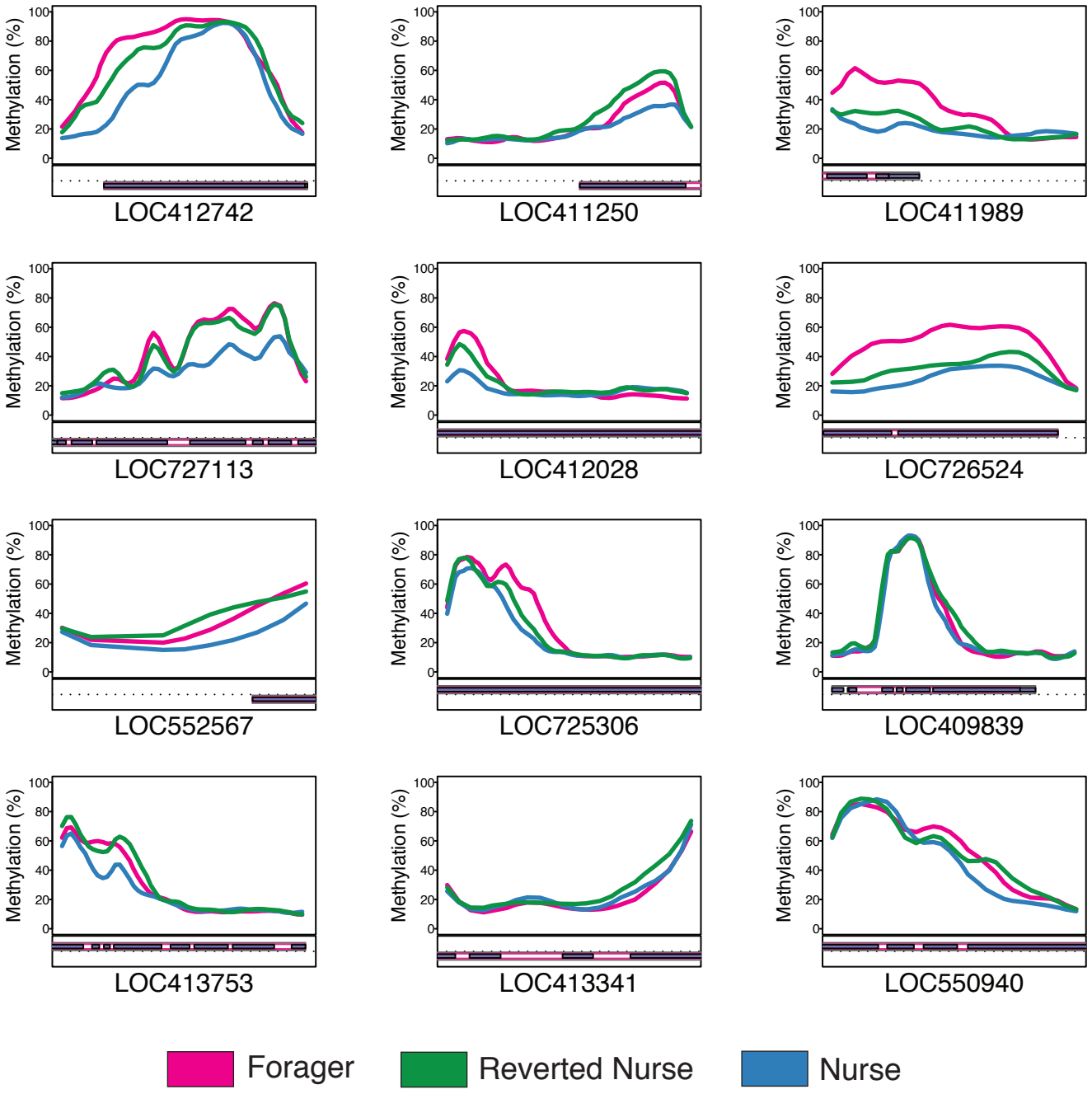


Supplementary Figure 2. Confirmation of negative CHARM result.
Newly emerged queens and workers were compared by CHARM, and no differences in methylation were found (FDR cutoff of 0.05). To confirm this negative result, we tested three regions by bisulfite pyrosequencing and compared to WGBS and found no difference in methylation between queens and workers. **a-c,** The left panel depicts the CHARM and WGBS data, where solid lines indicate the smoothed methylation levels over the region. The right panels show bisulfite pyrosequencing data of the CpGs in the CHARM plots indicated by a red box. 0%, 25%, 50%, 75%, 100% methylated controls are shown in right panel. Bisulfite Pyrosequencing data shows no evidence of caste-specific methylation.

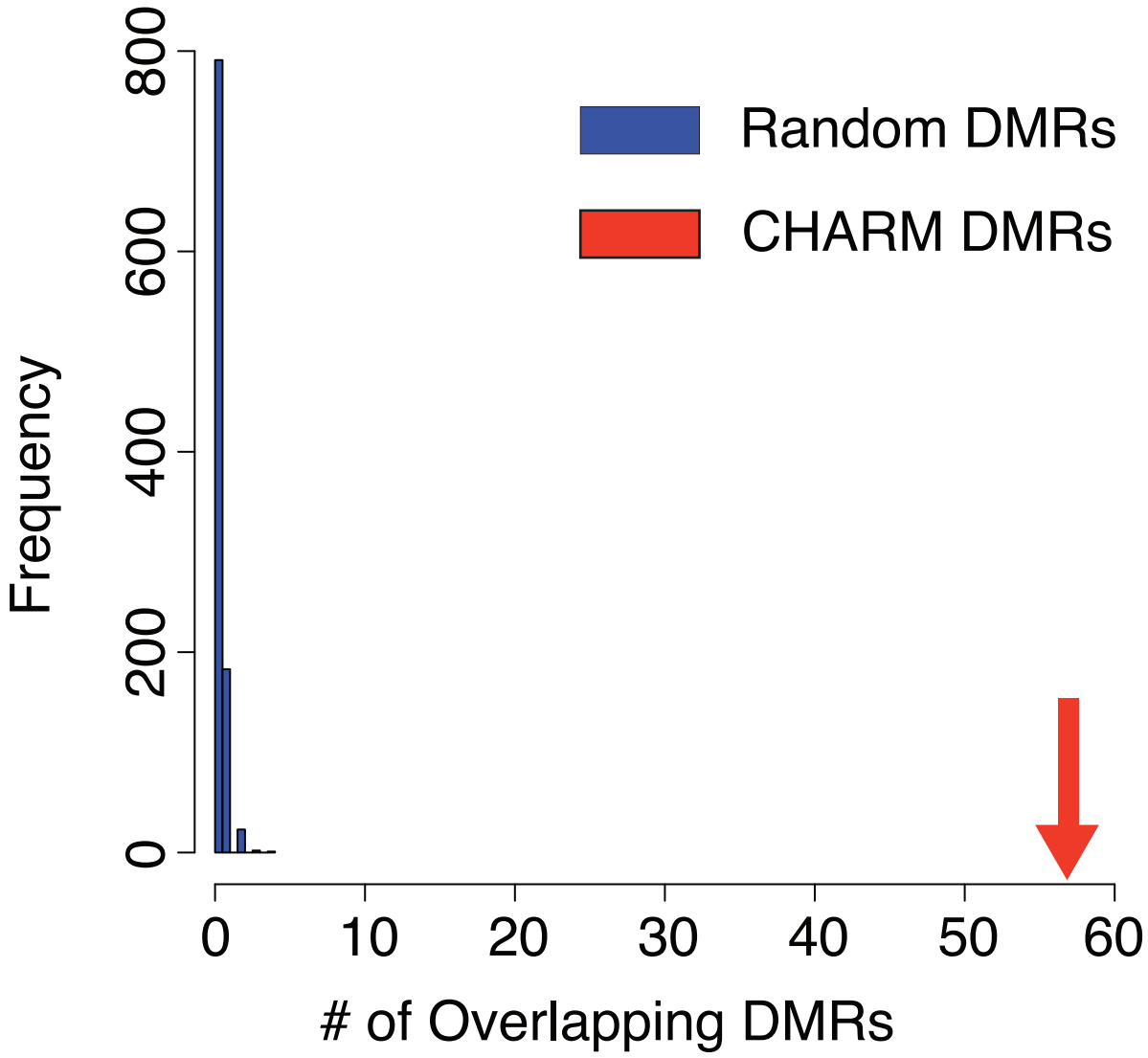

a
Atg9 DMR
Forager
Reverted Nurse
Nurse
Methylation (%)
CpG density
Genes
Atg9
Chr8.14: 188330 189494 190658
Atg9 pyrosequencing
CpG: 1 2 3 4 5
Controls
b
LOC726524 DMR
Forager
Reverted Nurse
Nurse
Methylation (%)
CpG density
Genes
LOC726524
Chr12.27: 24323 24923 25523
LOC726524 pyrosequencing
CpG: 1 2 3 4 5 6
Controls
c
Iswi DMR
Forager
Reverted Nurse
Nurse
Methylation (%)
CpG density
Genes
Iswi
Chr12.35: 50116 51218 52321
Iswi pyrosequencing
CpG: 1 2 3 4 5 6
Controls
d
sorting nexin-14 DMR
Forager
Reverted Nurse
Nurse
Methylation (%)
CpG density
Genes
sorting nexin-14
Chr8.43: 3244 4155 5066
sorting nexin-14 pyrosequencing
CpG: 1 2 3 4 5
Controls



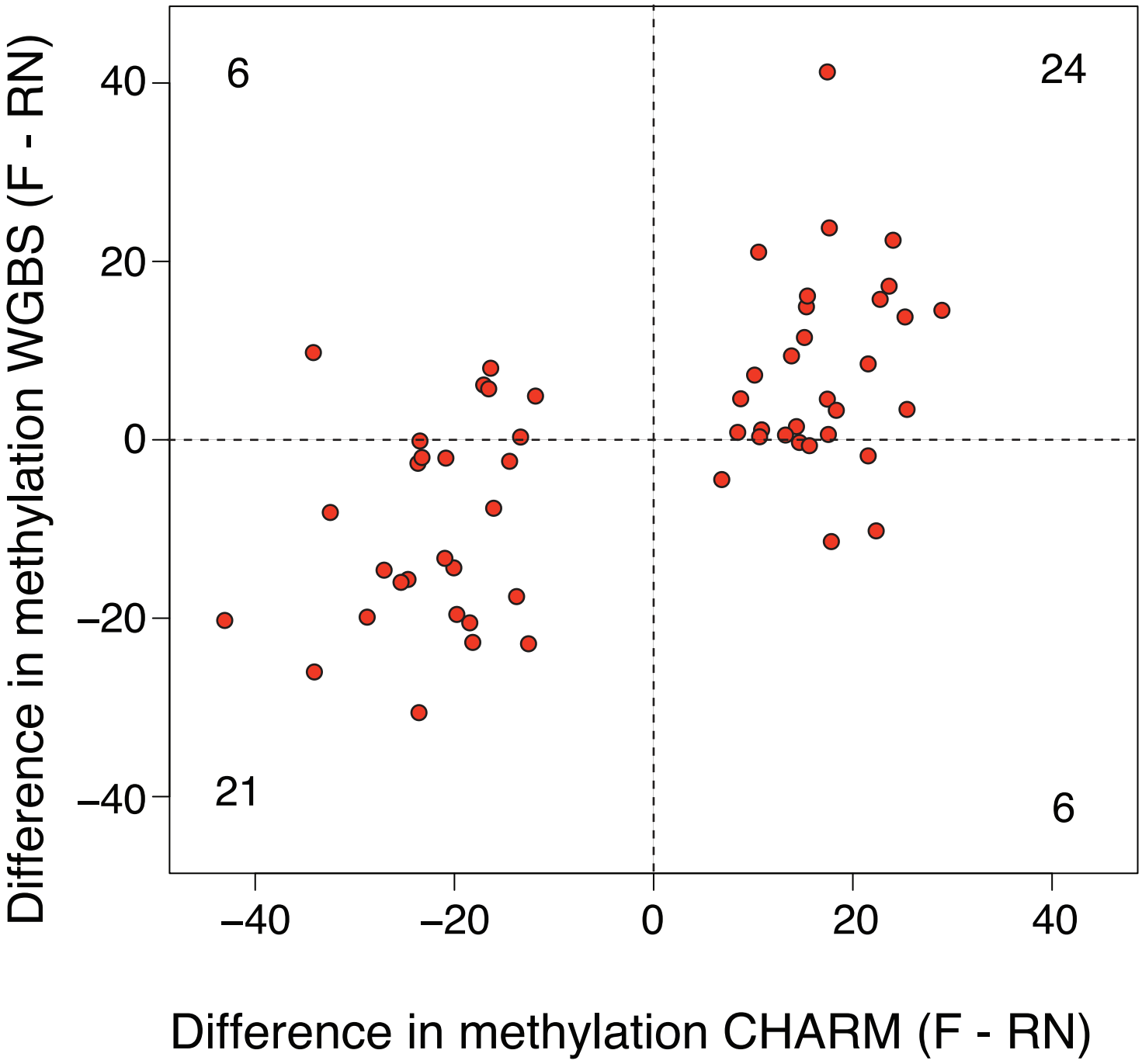
Supplementary Figure 3. Bisulfite Pyrosequencing confirmation of DMRs. Top panel depicts the percent DNA methylation, as assessed by CHARM, in 5 genes associated with CHARM DMRs. Individual biological replicates are represented by colored points, and average methylation for each phenotype is shown as a solid colored line. Average CpG dinucleotide density is calculated by using a standard density estimator, and individual CpGs are represented by tick marks at the bottom of the CpG density plot. The gene information is shown at the bottom of the DMR plot, with purple boxes representing exons, and the direction of the gene indicated by the + or - strand. Bisulfite pyrosequencing data is shown in the bottom panel depicts the percent methylation of individual CpGs. Asterisks indicate *P*-value < 0.05 by student's t-test. Individual CpGs correspond to CpGs in the top panel outlined by the red boxes. 0%, 25%, 50%, 75%, 100% methylated controls are shown in right panel.



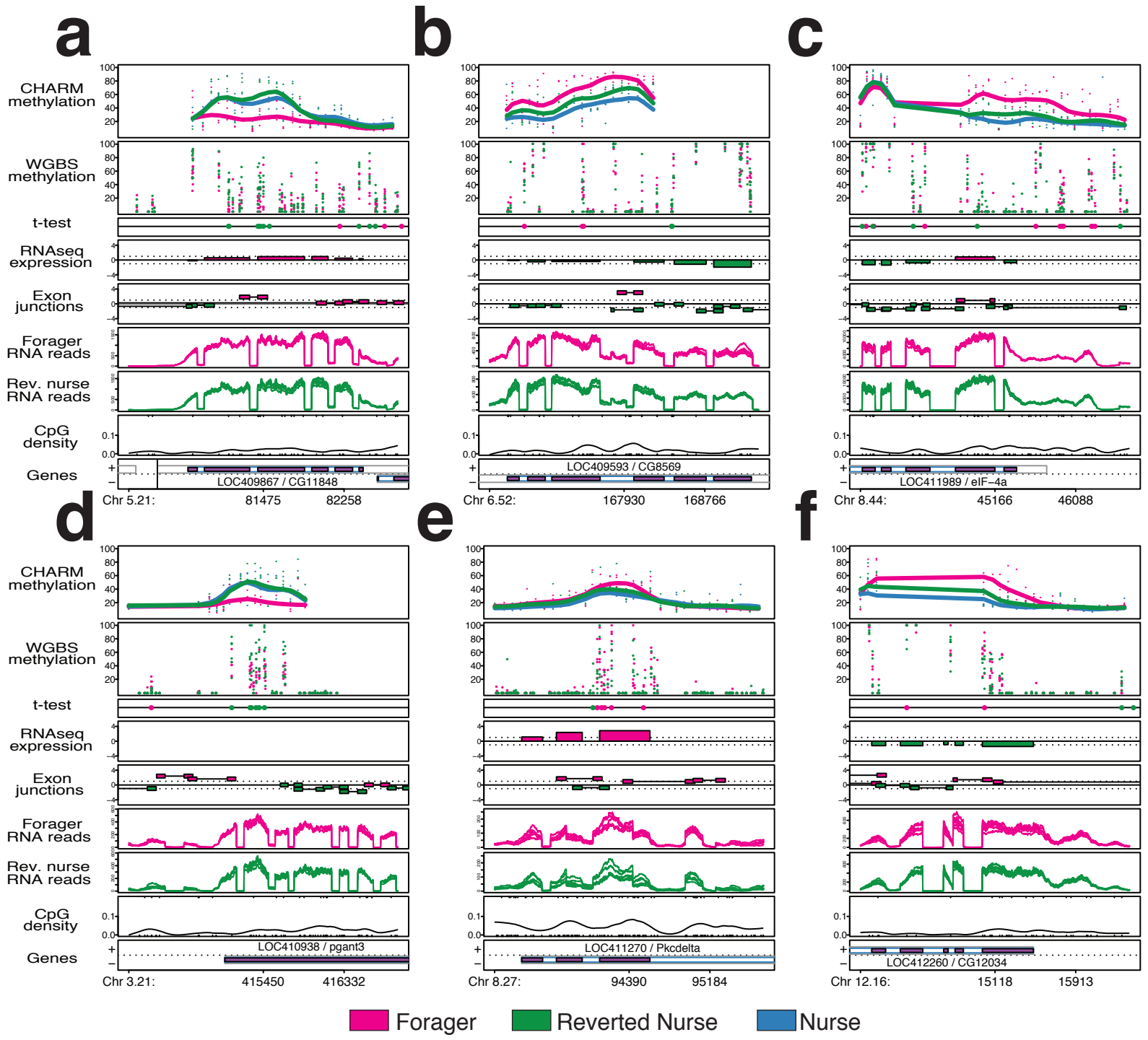
Supplementary Figure 4. Genes that contain DEAD-like and helicase domains exhibit similar patterns in methylation. Genes that contain DEAD-like and helicase domains play a role in RNA processing and gene regulation, which may assist the nurse to forager transition. These twelve plots show methylation relative to gene position(gene shown in purple). In all cases, DMRs are located over genes, and methylation increases from nurse to forager.



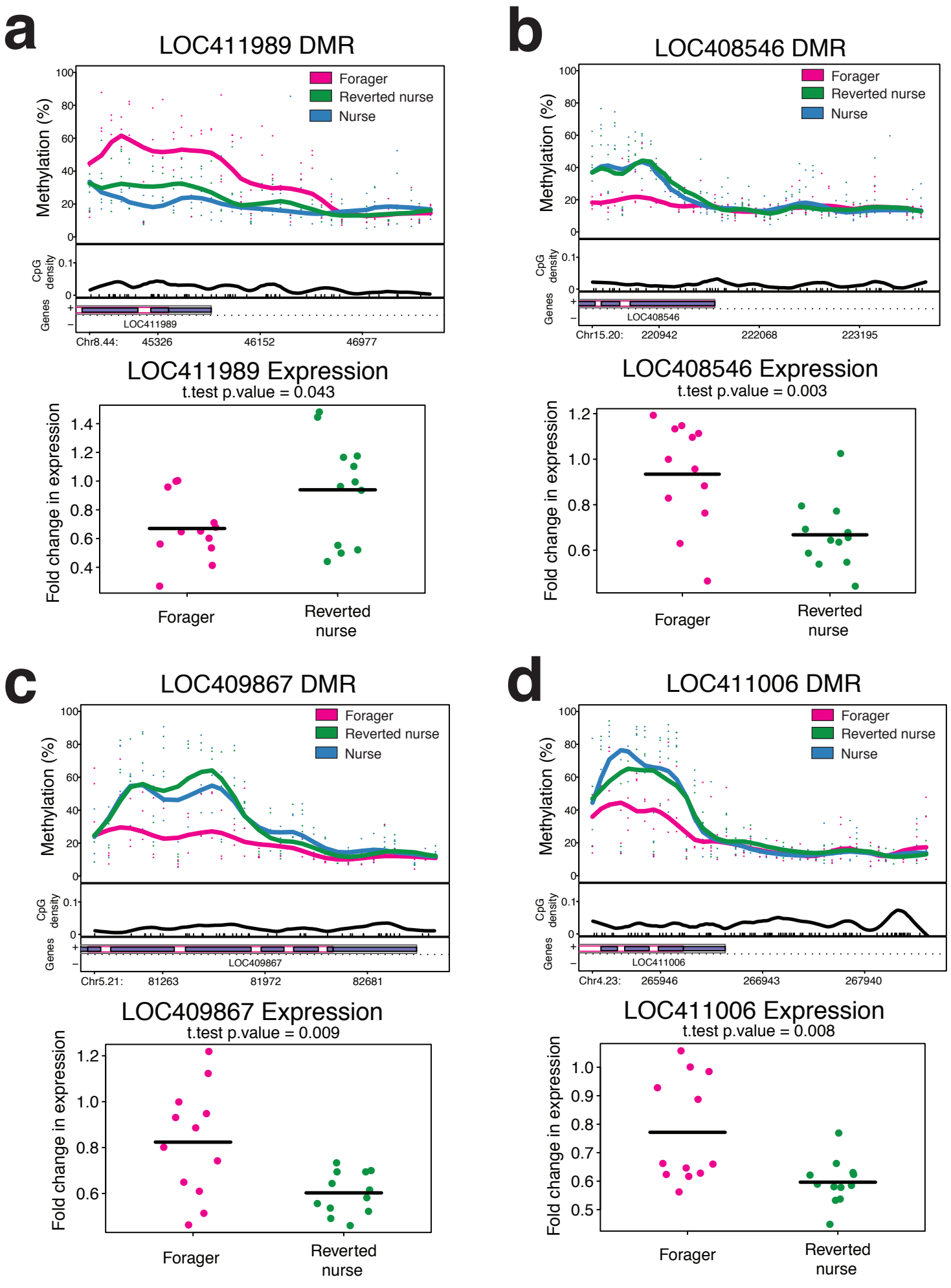
Supplementary Figure 5. Reversion DMRs are present at a higher frequency than expected by chance. To determine if the methylation changes that occur during the nurse to forager transition co-localize with those that occur during the forager to nurse transition, we performed a permutation test. We created random DMRs that were the same size, GC content and quantity as the nurse to forager transition DMRs and quantified how many overlapped with the forager to nurse transition DMRs. 1000 such permutations are depicted by the histogram in blue and are all much less than the observed 57 Reversion DMRs shown in red.

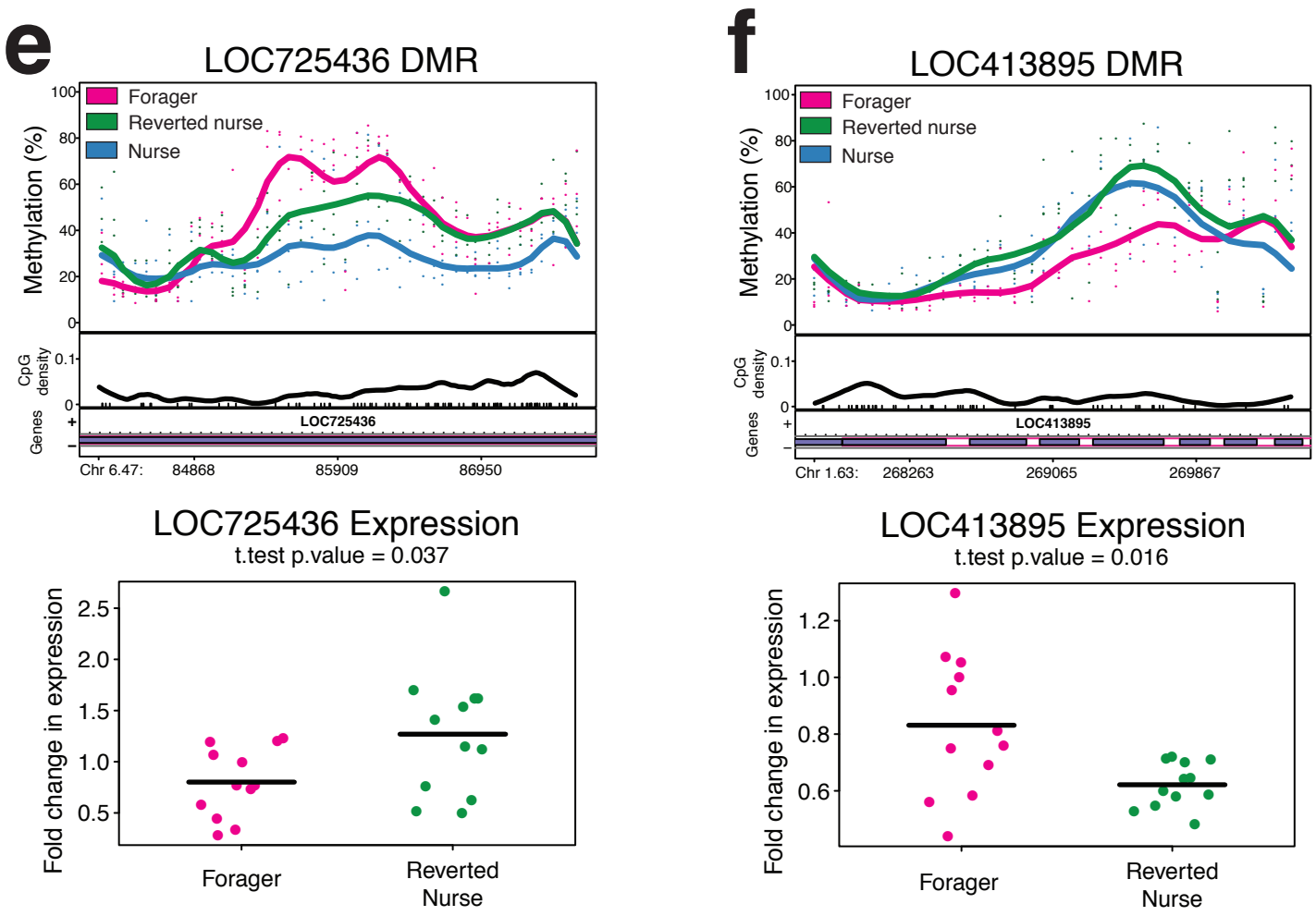


Supplementary Figure 6. CHARM DMRs correlate with WGBS DMRs.
WGBS was performed on 6 additional pools each of forager and reverted nurse brains. The CHARM DMRs were expanded by 500 bp on either side, and the top three WGBS CpGs by t-test within the CHARM DMRs were averaged, and then compared to average methylation in CHARM. 45/57 reversion DMRs change in the same direction in both data sets, correlation = 0.643 and $r^2 = 0.414$. Numbers in graph indicate the number of points in each quadrant.

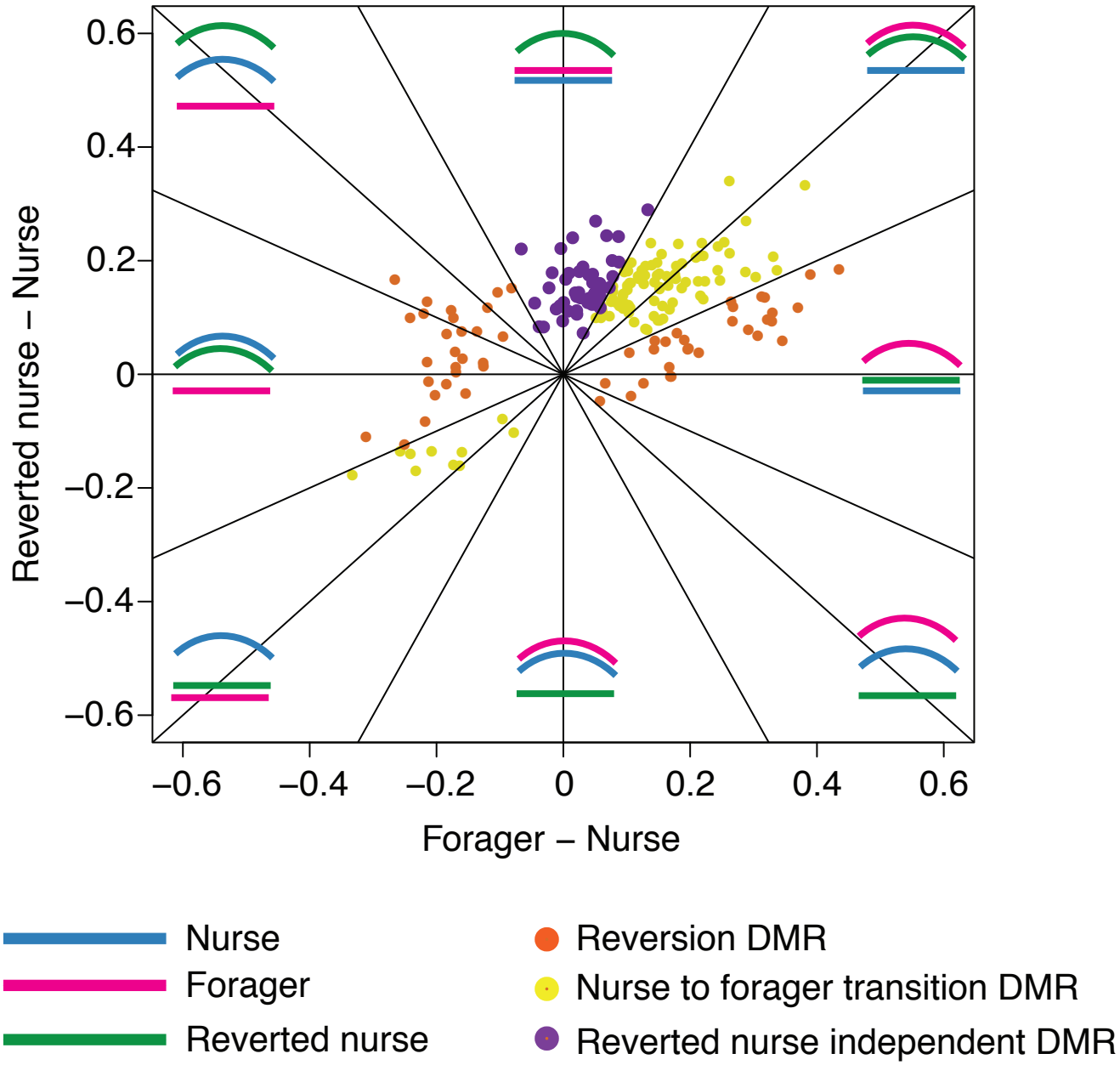


Supplementary Figure 7. Alternative splicing events colocalize with DMRs .
Six examples of alternative splicing occurring in DMRs **a-f**, Top panels show percent methylation for both CHARM and WGBS data sets, with points representing individual samples, and the smoothed lines representing the average for the phenotype. The t-test panel displays the top 1% differentially methylated CpGs by t-test. Color of point indicates which phenotype has greater methylation at that CpG. The RNAseq expression panel is a t-statistic based on the number or reads detected within the annotated exons, with the color indicating the higher expressed phenotype. The Exon junctions panel is a t-statistic based on the number or reads detected spanning the exon junctions, as predicted by the TopHat program, with the color indicating the higher expressed phenotype. Switching between higher expressed nurse and forager exon junctions is indicative of alternative splicing events. The RNA reads panels indicate the number of reads per phenotype as compiled by TopHat program. The bottom two panels show the CpG density, and the relative position of the gene.


a
LOC411989 DMR
Forager
Reverted nurse
Nurse
Methylation (%)
CpG density
Genes
LOC411989
Chr8.44:
45326
46152
46977
LOC411989 Expression
t.test p.value = 0.043
Fold change in expression
Forager
Reverted nurse
b
LOC408546 DMR
LOC408546
Chr15.20:
220942
222068
223195
LOC408546 Expression
t.test p.value = 0.003
c
LOC409867 DMR
LOC409867
Chr5.21:
81263
81972
82681
LOC409867 Expression
t.test p.value = 0.009
d
LOC411006 DMR
LOC411006
Chr4.23:
265946
266943
267940
LOC411006 Expression
t.test p.value = 0.008



Supplementary Figure 8. DNA methylation represses gene expression. Six examples (a-f) of genes that contain DMRs and exhibit differential gene expression. Top panel depicts CHARM data, and bottom panel shows real time analysis comparing twelve individual brains from continuous foragers and reverted nurses. In all cases, higher levels of methylation correspond to lower levels of gene expression.



Supplementary Figure 9. Classifying DMRs by methylation profile. DMRs cluster into three classes; Reversion, Nurse to forager transition, and Reverted nurse independent. Methylation differences relative to continuous nurses were calculated for reverted nurses and continuous foragers to determine the relative methylation of all samples at each DMR. DMRs were grouped based on the methylation profile for each DMR. Cartoon representations of DMR profiles are depicted at the edge of the graph. 57 Reversion DMRs show similar methylation levels for both nurse types, with foragers showing hypo and hypermethylation in 27 and 30 DMRs respectively. 98 DMRs show a change in methylation only during the nurse to forager transition, and the majority (88), involve an increase in methylation during this transition. 50 DMRs show an unique profile for reverted nurses, and in all cases, reverted nurses are hypermethylated.