# SUPPLEMENTARY MATERIALS

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**SUPPLEMENTARY DATA INCLUDES:**

Supplementary figures and legends, supplementary table S1-S3, and supplementary table S4-S12 (see attached excel file).
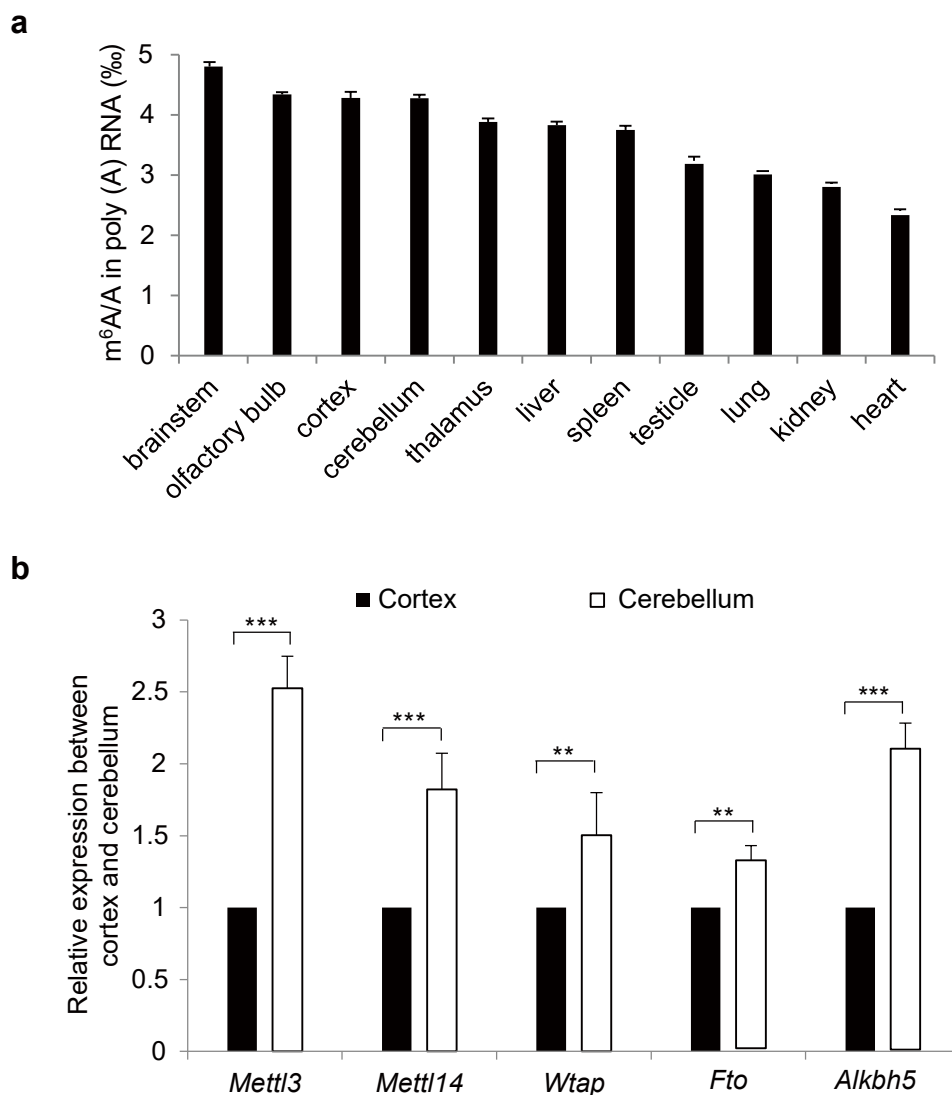
**SUPPLEMENTARY FIGURES**

**a**



**b**



**Figure S1, related to Figure 1. RNA methylation profiles across mouse tissues and RNA expression profiles of m⁶A writers and erasers.** (a) UHPLC-MS/MS analysis of poly(A) RNA in adult mouse tissues for m⁶A /A ratio. The experiments were repeated for three times using 10 male mice in total. Representative data was shown here as average values + SEM. (b) Real-time PCR analysis to compare the RNA expression of m⁶A writer and eraser genes in mouse cerebellum and cerebral cortex. The ratio of RNA expression between cerebellum and cortex was shown here as mean value + SEM from 3 male and 3 female 3-month-old mice. **, p value<0.01, ***, p value<0.001.
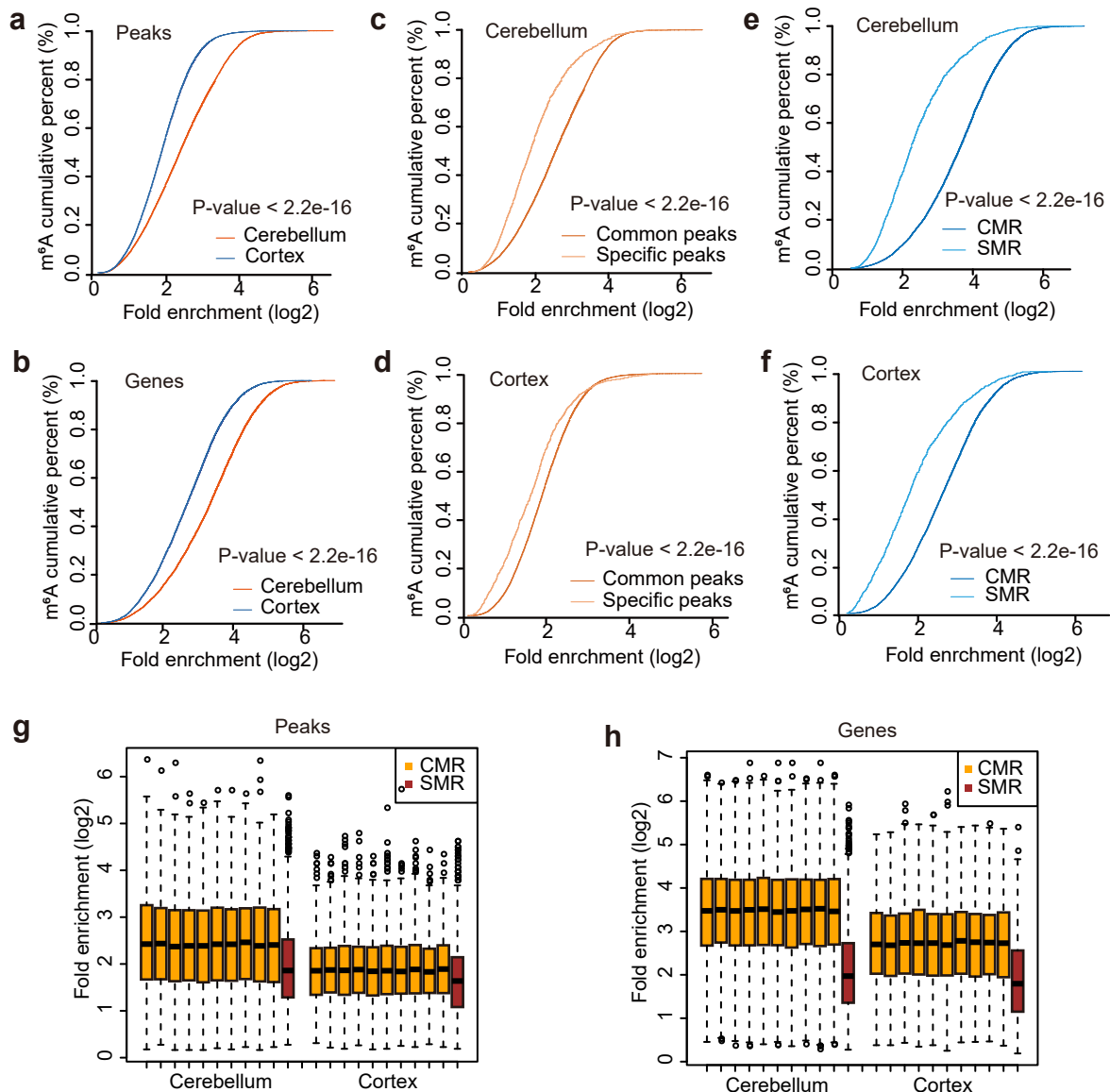
**Figure S2, related to Figure 2. Cumulative curves comparing the methylation levels between cerebellum and cortex, or common and specific methylation.** (a, b) Cumulative distribution function of log2-fold enrichment of m⁶A peaks to compare the methylation levels between cerebellar and cortical RNAs at peaks level (a) and genes level (b). (c, d) Cumulative distribution function of log2-fold enrichment of common and specific m⁶A peaks in mouse cerebellum (c) and cerebral cortex (d). (e, f) Cumulative distribution function of log2-fold enrichment of m⁶A peaks to compare the methylation levels between CMRs and SMRs in mouse cerebellum (e) and cerebral cortex (f). CMR, commonly methylated RNA. SMR, specifically methylated RNA. Wilcoxon test was performed for statistical analysis. (g, h) Box plots showing the methylation levels of CMRs and SMRs by comparing the fold enrichment at peaks level (g) or genes level (h). The same number of peaks or genes as those of SMRs were randomly extracted from CMRs, and compared with SMRs for 10 times in total.
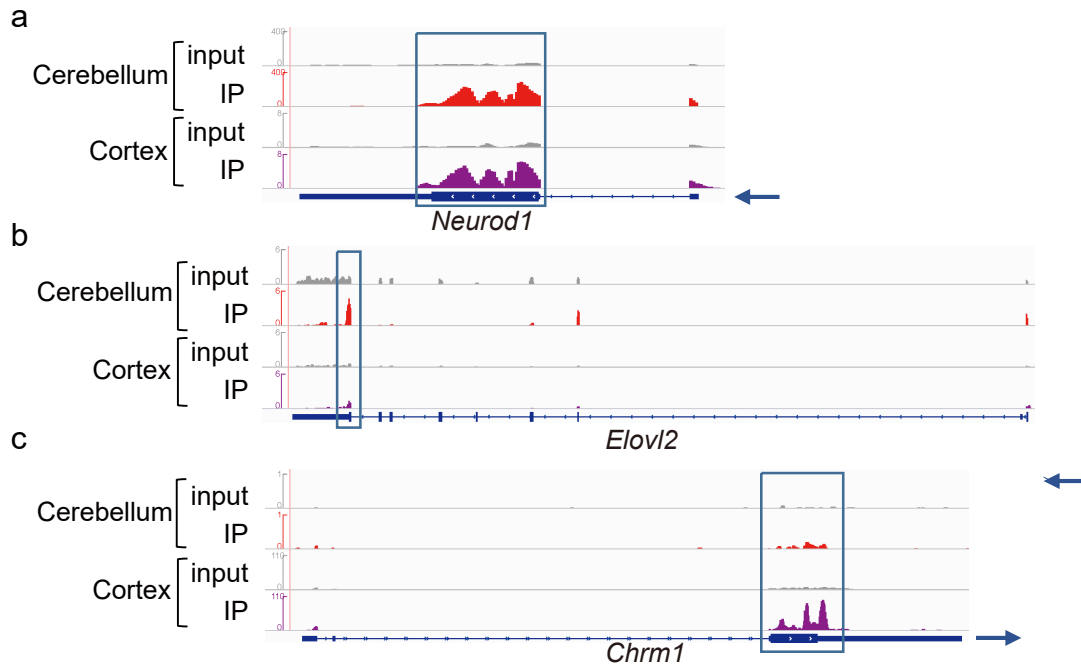
**Figure S3, related to Figure 2. IGV plots showing examples of common (a), cerebellar (b) and cortical specifically (c) methylated transcripts.** The grey reads are from non-IP control (input) libraries; red and purple reads are from m⁶A-IP libraries of mouse cerebellum and cerebral cortex, respectively. Arrows show the direction of transcription. Y-axis represents normalized numbers of reads count. Positions of m⁶A peaks are highlighted in blue box.

**Figure S4, related to Figure 2. Comparative analysis of m⁶A methylated lncRNAs between mouse cerebellum and cerebral cortex.** (a) Venn diagram showing the numbers of lncRNAs commonly or specifically expressed in mouse cerebellum and cerebral cortex. (b) Venn diagram showing the numbers of commonly or specifically methylated lncRNAs between cerebellum and cerebral cortex. (c) IGV plots showing examples of commonly (upper panel), cerebellar (middle panel) and cortical specifically (lower panel) methylated lncRNAs. The grey reads are from non-IP control (input) libraries; red and purple reads are from m⁶A-IP libraries of mouse cerebellum and cerebral cortex, respectively. Arrows show the direction of transcription. Y-axis represents normalized numbers of reads count. Positions of m⁶A peaks are highlighted in blue box.
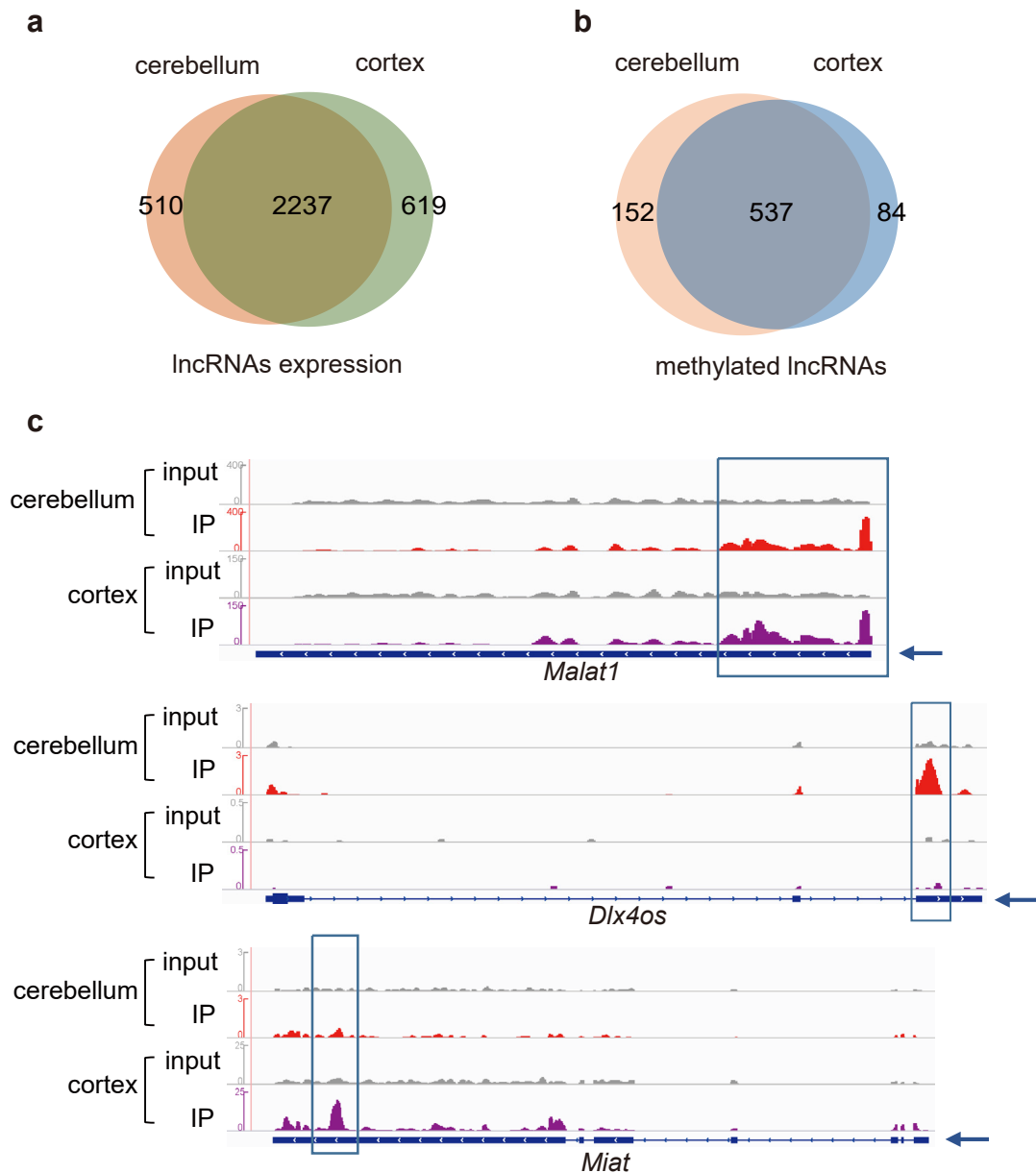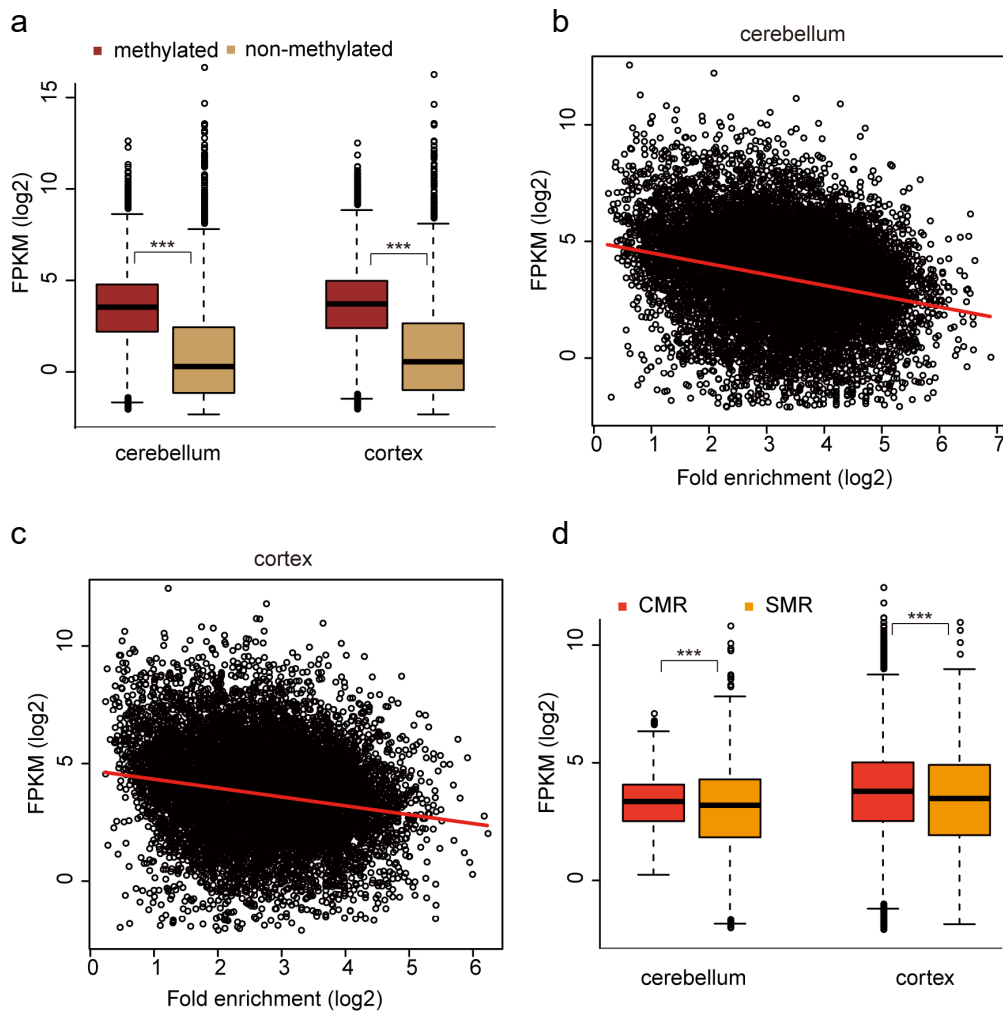
**Figure S5, related to Figure 2. Correlation analysis between RNA methylation and expression.** (a) Box plots showing the median expression levels of methylated genes and non-methylated genes in mouse cerebellum and cerebral cortex. (b, c) Scatter diagrams showing correlation between m⁶A peak enrichment (fold enrichment log2) and RNA abundance (FPKM log2) in mouse cerebellum (b) and cerebral cortex (c). (d) Box plot showing the relative expression levels of CMRs and SMRs in mouse cerebellum and cerebral cortex. CMR, commonly methylated RNA. SMR, specifically methylated RNA. *** indicates p value <2.2e-16. Wilcoxon test was performed for statistical analysis.

**a**



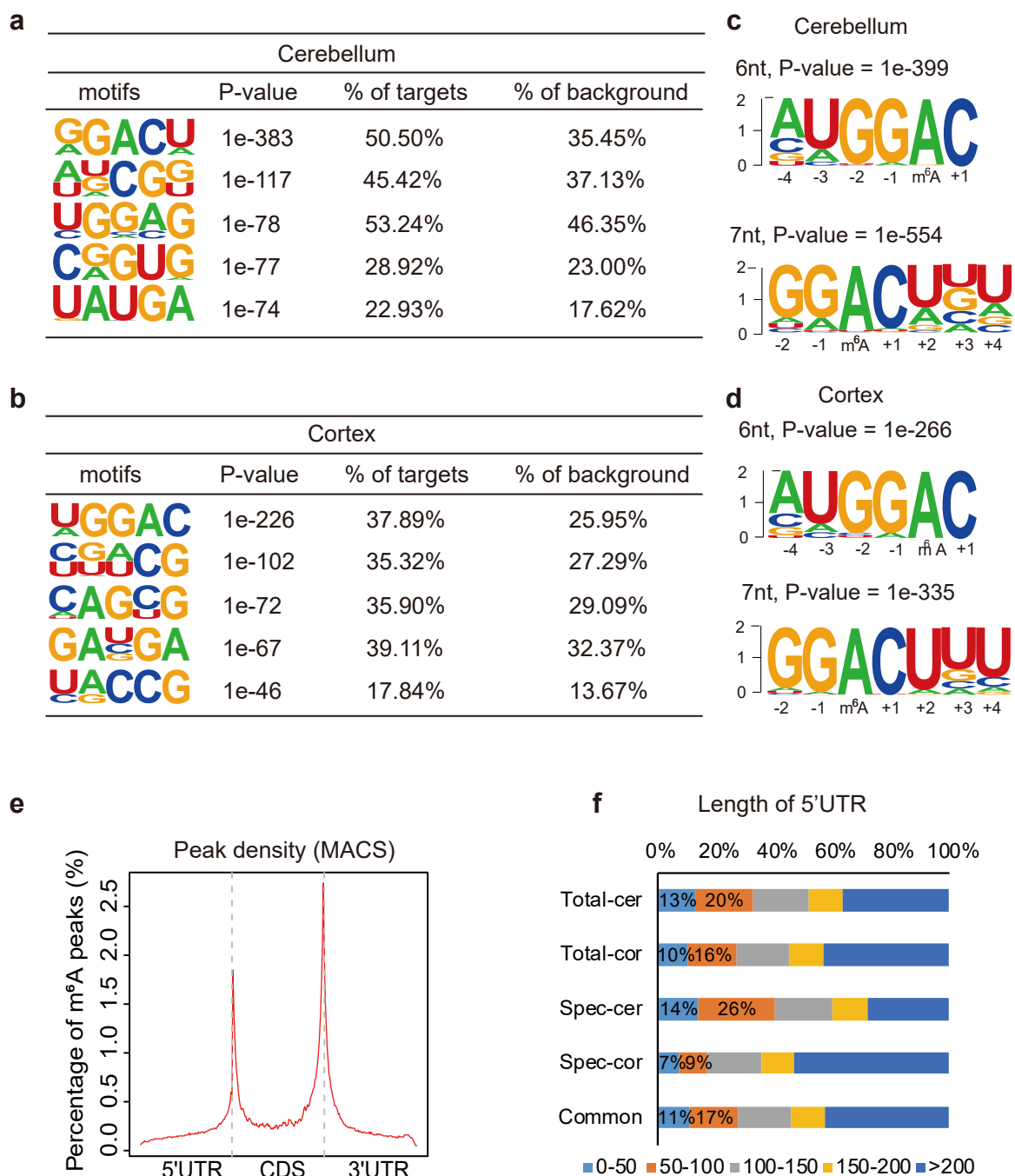| Cerebellum | | | |
|---|---|---|---|
| motifs | P-value | % of targets | % of background |
| GGACU | 1e-383 | 50.50% | 35.45% |
| AUCGG | 1e-117 | 45.42% | 37.13% |
| UGGAG | 1e-78 | 53.24% | 46.35% |
| CGGUG | 1e-77 | 28.92% | 23.00% |
| UAUGA | 1e-74 | 22.93% | 17.62% |

**b**



| Cortex | | | |
|---|---|---|---|
| motifs | P-value | % of targets | % of background |
| UGGAC | 1e-226 | 37.89% | 25.95% |
| CGACG | 1e-102 | 35.32% | 27.29% |
| CAGCG | 1e-72 | 35.90% | 29.09% |
| GAUGA | 1e-67 | 39.11% | 32.37% |
| UACCG | 1e-46 | 17.84% | 13.67% |

**c** Cerebellum

6nt, P-value = 1e-399



7nt, P-value = 1e-554



**d** Cortex

6nt, P-value = 1e-266



7nt, P-value = 1e-335



**e**



**f** Length of 5'UTR



**Figure S6, related to Figure 3. Consensus sequence and distribution of m⁶A peaks.** (a, b) Lists of the top 5 consensus sequence (5nt) identified in m⁶A peaks in mouse cerebellum (a) and cortex (b). (c, d) Consensus sequence (upper panel, 6nt) and (lower panel, 7nt) identified in m⁶A peaks in mouse cerebellum (c) and cortex (d). (e) Distribution of cerebellar m⁶A peaks density along the transcripts as analyzed using MACS software. (f) Statistics of 5'UTR length of the transcripts including m⁶A methylation sites surrounding start codons.
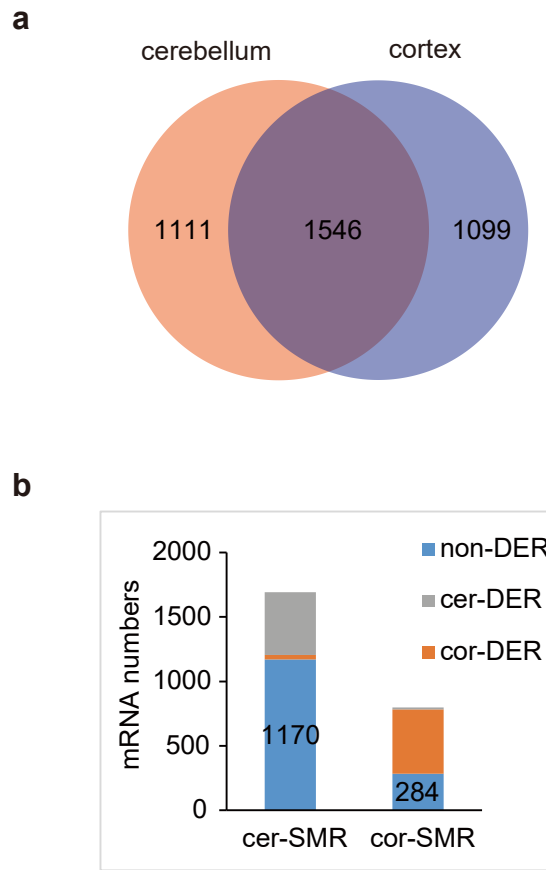
**a**



**b**



**Figure S7, related to Figure 5. Characterization of commonly and specifically methylated genes in mouse cerebellum and cerebral cortex.** (a) Venn diagram showing the overlap between the top 3000 m$^6$A peaks-containing, commonly methylated genes in cerebellum and cerebral cortex. (b) Status of differential expression of specifically methylated genes in cerebellum and cortex. cer-SMR, specifically methylated RNAs in cerebellum; cor-SMR, specifically methylated RNAs in cerebral cortex. non-DER, non-differentially expressed RNAs. cor-DER, differentially expressed RNAs with higher expression in cortex; cer-DER, differentially expressed RNAs with higher expression in cerebellum. $p < 0.05$ was set to judge the significance of differential expression.

# SUPPLEMENTARY TABLES

## Table S1. Information of $m^6A$-seq data quality and processing.

| | cerebellum_1 | | cerebellum_2 | | cortex_1 | | cortex_2 | |
|---|---|---|---|---|---|---|---|---|
| | INPUT | IP | INPUT | IP | INPUT | IP | INPUT | IP |
| Raw_data_reads | 81898124 | 59903174 | 46652031 | 62481982 | 65790997 | 62320609 | 57505666 | 56331522 |
| Clean reads | 74423860 | 53808924 | 42799584 | 57320663 | 59574371 | 56302825 | 52149919 | 50709485 |
| Ratio | 91% | 90% | 92% | 92% | 91% | 90% | 91% | 90% |
| length > 50bp | 70019400 | 50642244 | 40539120 | 54549366 | 56093974 | 53102625 | 49029708 | 47889156 |
| Ratio | 94% | 94% | 95% | 95% | 94% | 94% | 94% | 94% |
| #mapped_reads | 61013860 | 45966387 | 35225594 | 47868528 | 48727738 | 47488219 | 40713068 | 40681156 |
| Ratio | 87% | 91% | 87% | 88% | 87% | 89% | 83% | 85% |
| #unique_mapped reads | 58223489 | 44619904 | 33691142 | 46323848 | 46625042 | 45981248 | 38713635 | 39515641 |
| Ratio | 83% | 88% | 83% | 85% | 83% | 87% | 79% | 83% |

## Table S2. Statistics of RNA-seq data of mouse cerebellum and cerebral cortex.

| | Total | | Specific | | Common |
|---|---|---|---|---|---|
| samples | cerebellum | cortex | cerebellum | cortex | Cerebellum/cortex |
| Genes | 19657 | 19760 | 1656 | 1759 | 18001 |
| mRNA | 14438 | 14861 | 426 | 849 | 14012 |
| lncRNA | 2856 | 2747 | 619 | 510 | 2237 |
| others | 2363 | 2152 | 611 | 400 | 1752 |

## Table S3. Statistics of methylated peaks and genes in mouse cerebellum and cerebral cortex as analyzed from the two biological repeats of $m^6A$-seq.

| | Total | | Region-specific | | Common | |
|---|---|---|---|---|---|---|
| samples | cerebellum | cortex | cerebellum | cortex | cerebellum | cortex |
| genes | 11324 | 10381 | 1728 | 805 | 9519 | 9519 |
| peaks | 21814 | 19746 | 2018 | 1043 | 16576 | 16989 |
| peaks/gene | 1.93 | 1.90 | 1.17 | 1.30 | 1.74 | 1.78 |
| mRNA | 10453 | 9636 | 1559 | 712 | 8924 | 8924 |
| % methylation | 72.4% | 64.8% | ND | ND | ND | ND |
| peaks | 20648 | 18734 | 1826 | 932 | 15806 | 16209 |
| peaks/mRNA | 1.98 | 1.94 | 1.17 | 1.31 | 1.77 | 1.82 |
| lncRNA | 777 | 664 | 152 | 84 | 537 | 537 |
| % methylation | 27.2% | 24.2% | ND | ND | ND | ND |
| Peaks | 1045 | 916 | 175 | 101 | 703 | 712 |
| peaks/lncRNA | 1.34 | 1.38 | 1.15 | 1.20 | 1.31 | 1.33 |

(ND: no data)

**Supplemental Table S4-S12 are attached as excel files.**

Table S4. List of commonly methylated m$^6$Apeaks in mouse cerebellum.

Table S5. List of commonly methylated m$^6$A peaks in mouse cerebral cortex.

Table S6. List of specifically methylated m$^6$A peaks in mouse cerebellum.

Table S7. List of specifically methylated m$^6$A peaks in mouse cerebral cortex.

Table S8. GO analysis of commonly methylated genes in mouse cerebellum.

Table S9. GO analysis of commonly methylated genes in mouse cerebral cortex.

Table S10. GO analysis of specifically methylated genes in mouse cerebellum.

Table S11. GO analysis of specifically methylated genes in mouse cerebral cortex.

Table S12. Sequence of primers used for RT-qPCR.