Supplementary Figure 1. LINE-1 methylation assay. (Top) Example pyrogram of LINE-1 methylation assay. (Bottom) Methylation level of LINE-1 CpG sites. Four CpG sites in the LINE-1 consensus sequence (accession number: X58075) were measured. Note that no statistical differences in DNA methylation levels between samples were found. Values are mean ± SD.
Supplementary Figure 2. Illumina Golden Gate assay. (A) Two-way hierarchical clustering based on the DNA methylation levels of the 1505 CpG sites. (B) Hierarchical clustering of the CpG sites show large methylation changes between human and chimpanzee cortex. The CpG sites are also listed in Supplementary Table 3.
Supplementary Figure 3. Overview of tiling array experiments. (Left) Flowchart of tiling array experiments. After the amplification of methylated DNA fraction retrieved by MBD2-beads, the product was fragmented, labeled, and hybridized to the tiling array. (Right) Definition of the methylated region (MR) in this study. Among the identified individual MRs (illustrated by three pink and three light blue bars), we extracted overlapping regions. Red, blue, and green bars indicate neuronal, nonneuronal, and common MRs, respectively. The neuronal-specific and nonneuronal-specific MRs as well as common MRs were used for transcription factor binding-site analysis.
Supplementary Figure 4. Filtering procedure. (A) Summary of the results of MAT analysis before filtering. Average of the maximum, minimum, top 0.1%, and bottom
0.1% of the MAT scores are given. Values are mean ± SD. (B) Number of MRs before and after the filtering procedure. (C) Filtering procedure. In each candidate MR, restriction sites were searched 1 kb upstream and downstream from the identified MR. After that, an MR longer than 100 bp in length was selected. The number of CpGs included in the MR was then counted. The MR having four or fewer CpGs (case F1) was discarded, whereas the MR having five or more CpGs (case F2) was considered to be methylated of CpGs are plotted. (D) Effect of the filtering procedure.
Supplementary Figure 5. Example of methylation status of the imprinted genes and the results of the Nimblegen tiling array. Individual neuronal and nonneuronal MRs detected in the Affymetrix tiling array are shown with pink and blue bars, respectively. The location of probes in the Nimblegen tiling array and detected methylated peaks (Nimble common peaks) are also shown. Note that only the peaks detected in both NeuN+ and NeuN− samples are shown in the Nimble common peaks.
Supplementary Figure 6. Expression analysis of the genes associated with common MRs. The number of probe sets expressed or not expressed in the all control human brain samples ($N = 34$) (Iwamoto et al. 2005) is given.
Supplementary Figure 7. Bisulfite sequencing analysis of the neuronal activity-related genes. DNA methylation status examined by tiling array, bisulfite-sequenced region, and CpG island are illustrated. In the case of NRXN1, we successfully analyzed only one CpG site due to sequence difficulties.
Supplementary Figure 8. Transcription factor-binding site analysis in the MRs. 
(Top) List of enriched binding sites in the common MRs. Among the input sequences, 
the number of sequences containing binding sites (number of seq), the total number of 
binding sites (number of matches), expected match numbers in an equally sized sample 
of the human promoter background (expected), and Z-scores are calculated. The 
underlined sequences show high conservation, and capital letters indicate core 
sequences used by the software. (Bottom) List of enriched binding sites and associated 
RefSeq genes in the neuronal- and nonneuronal-specific MRs. In the case of V$PAX 
and V$HIFF, the number of associated RefSeq genes in neuronal and nonneuronal MRs, 
respectively, is shown.
Supplementary Figure 9. Variably methylated region (VMRs) in neuronal nuclei. (A) List of VMRs and associated genes. (B) Validation of arbitrary chosen three VMRs by Q-PCR. (C) Example of significant relationship between expression and methylation level at VMRs. Expression level of SMYD3 (218778_s_at) was derived from previous DNA microarray study, and derived from the same subject used in this study.
Reference: