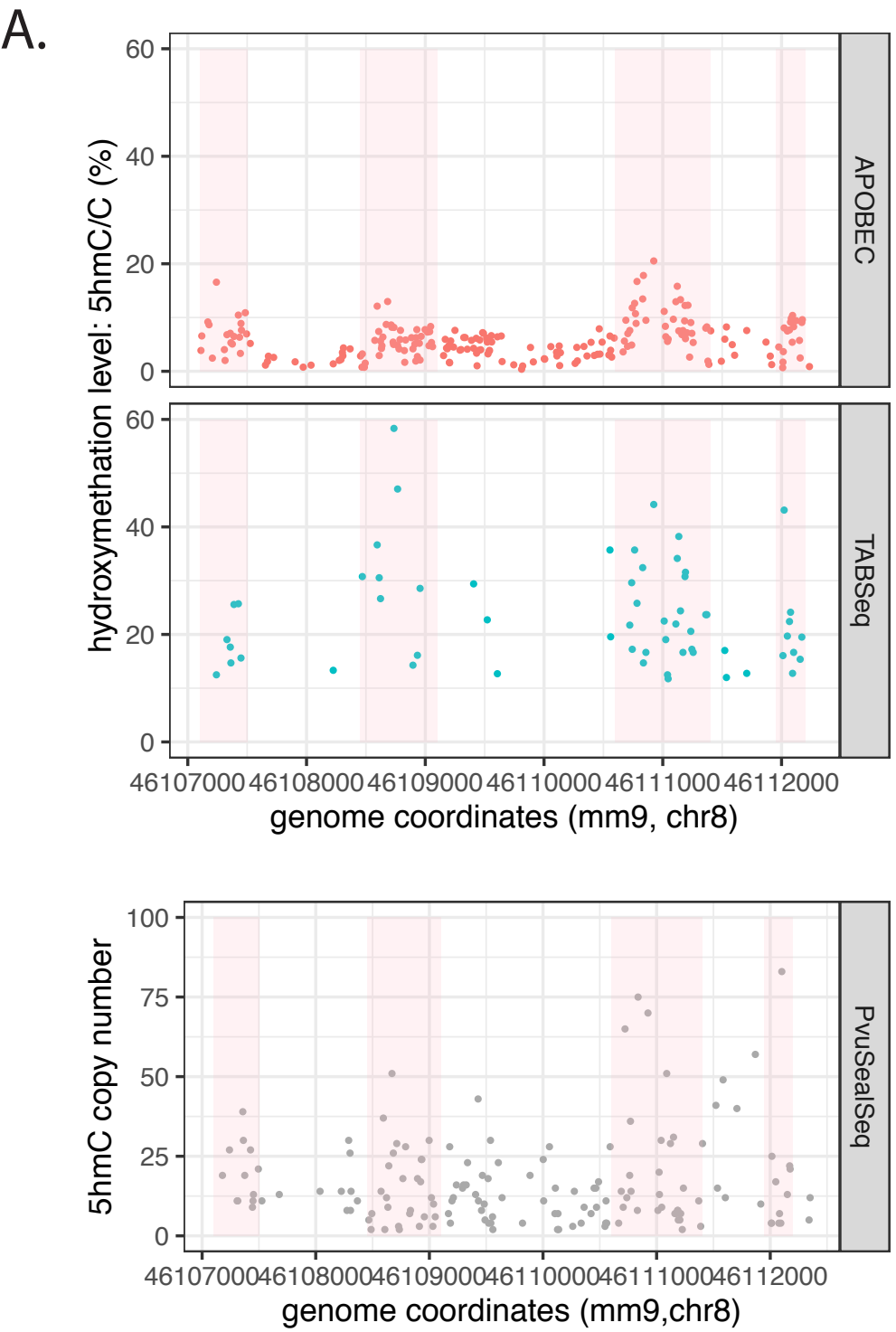

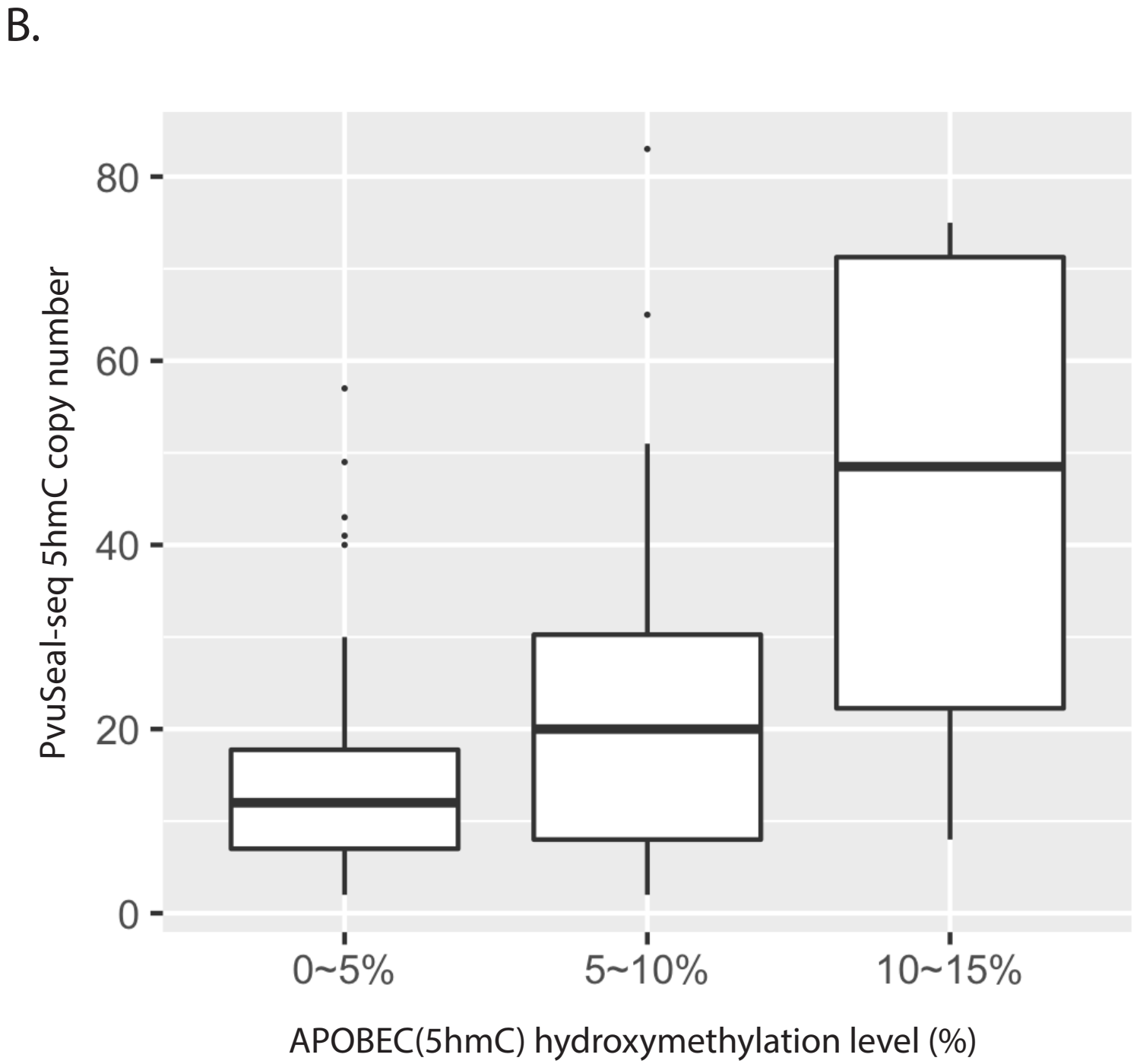

Supplemental Figure 4

Hydroxymethylation analysis of a 5078 bp region in the mouse E14 cells **(A)** Single-base hydroxymethylation level at CpG sites calculated from 3 methods: LR-EM-seq and TAB-seq (top panel) and Pvu-Seal-seq (bottom panel). In the case of Pvu-Seal-seq, the y-axis shows the number of captured modified copies, which is a relative measurement of hydroxymethylation level. In the cases of LR-EM-seq and TAB-seq, the y-axis shows the percentage of hydroxymethylated copies among all the sequenced copies (modified + unmodified). Due to large discrepancy in sequencing coverage (17X for TAB-seq on average and over 1000X for LR-EM-seq) which has a profound effect on statistical power and accuracy for 5hmC estimation, the calculated 5hmC values also differ between the two methods. Nevertheless, four hydroxymethylation-rich regions are consistently detected by all the 3 methods and are highlighted by the pink shaded areas on the plots.**(B)** LR-EM-seq hydroxymethylation measurements are in good agreement with Pvu-Seal-seq results. CpG sites were divided into 3 subgroups based on LR-EM-seq measured hydroxymethylation level. For each subgroup, hydroxymethylation level reported by Pvu-Seal-seq method (represented by sequencing copy number as a relative quantification) was depicted by boxplot.