Supplementary Figures:

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Suppl. Figure 1:

Cluster analysis of all samples. Cluster analysis was performed for primary small cell lung tumors (T1-18), SCLC cell lines (DMS53, H1688, SW1271, H1447, H1836), normal lung tissue (N1, N2, N3, N5, and N6) and normal human bronchial epithelial cells (HBEC). Spearman correlation was used to derive the dendrograms.

Suppl. Figure 2:

Examples of tumor-specific methylation in SCLC.

Data are shown for the *PROX1*, *CCDC140*, *PAX3*, and *SIM1* genes. The top of the Figure indicates the chromosomal coordinates according to the UCSC Genome browser hg19. Gene names and direction of transcription are shown at the bottom of the Figure. The Nimblegen array data (methylated fraction versus input) are shown for three normal lung tissues (N, red) and five primary SCLC tumors (T, green). The methylation signal is shown plotted along the chromosome as a *P* value score. Therefore, the minimum number on the y-axis is 0 (when $P = 1$). The *P* value score was obtained by NimbleScan software and is derived from the Kolmogorov-Smirnov test comparing the log2 ratios (MIRA versus input) within a 750 bp window centered at each probe and the rest of the data on the array.

Suppl. Figure 3:

Tumor-specific methylation at the *HOXD* cluster in SCLC.

The top of the Figure indicates the chromosomal coordinates according to the UCSC Genome browser hg19. Gene names and direction of transcription are shown at the bottom of the Figure. The Nimblegen array data (methylated fraction versus input) are shown for three normal lung tissues (N, red) and five primary SCLC tumors (T, green). The methylation signal is shown plotted along the chromosome as a *P* value

score. The *P* value score was obtained by NimbleScan software and is derived from the Kolmogorov-Smirnov test comparing the log2 ratios (MIRA versus input) within a 750 bp window centered at each probe and the rest of the data on the array.

Suppl. Figure 4:

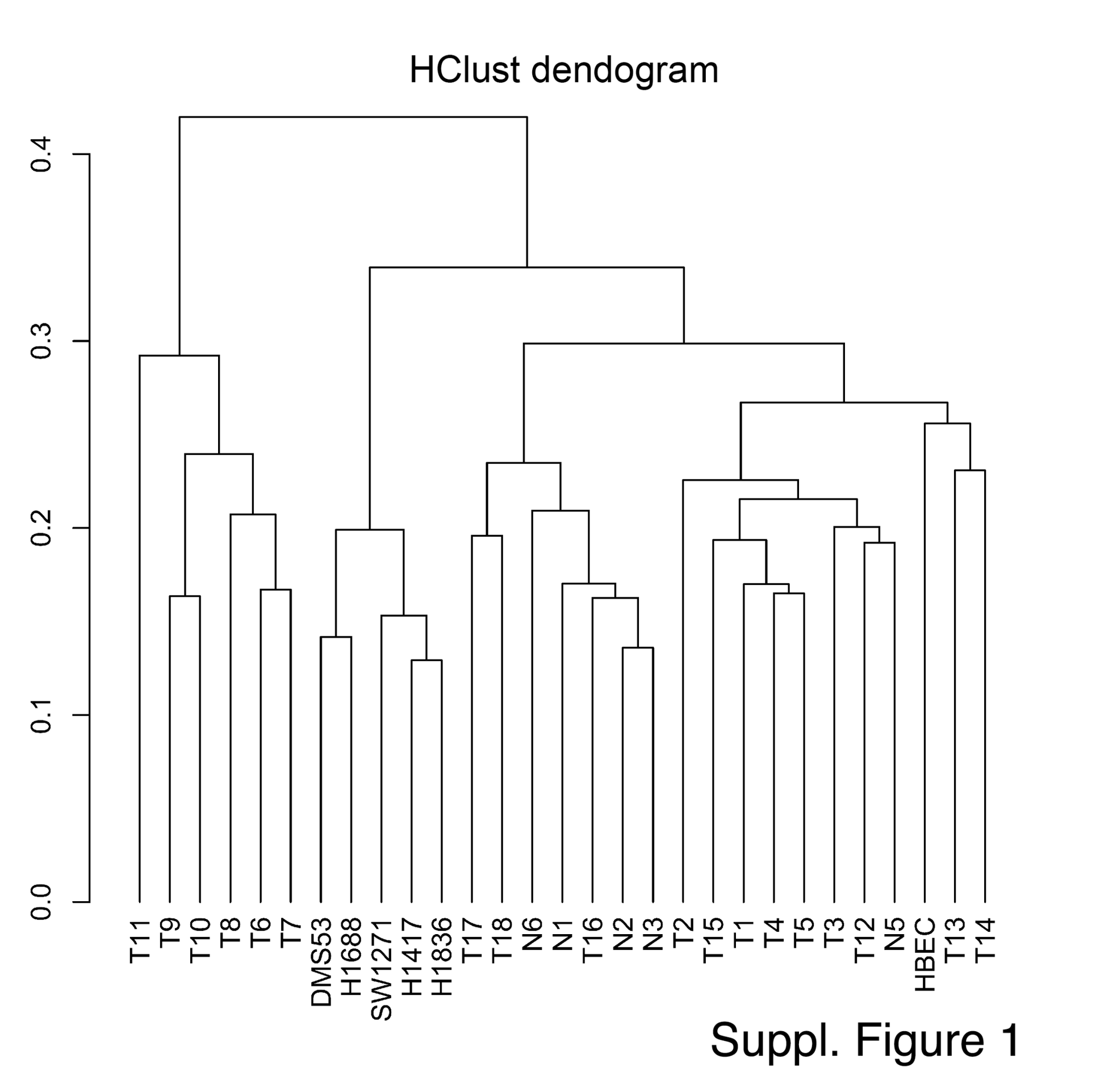
Validation of gene-specific methylation in SCLC by COBRA assays and by sodium bisulfite sequencing. A. COBRA analysis of the *DMRTA2*, *GALNTL1* and *MIR129-2* genes in normal lung (N) and primary SCLC (T). Matched tumor-normal sample pairs were N1 and T16, N2 and T17, and N3 and T18. **B.** Bisulfite sequencing analysis of the *DMRTA2* and *GALNTL1* genes. Two normal lung and four SCLCs were analyzed. Open circles indicate unmethylated CpG sites; closed circles show methylated CpG sites.

Suppl. Figure 5:

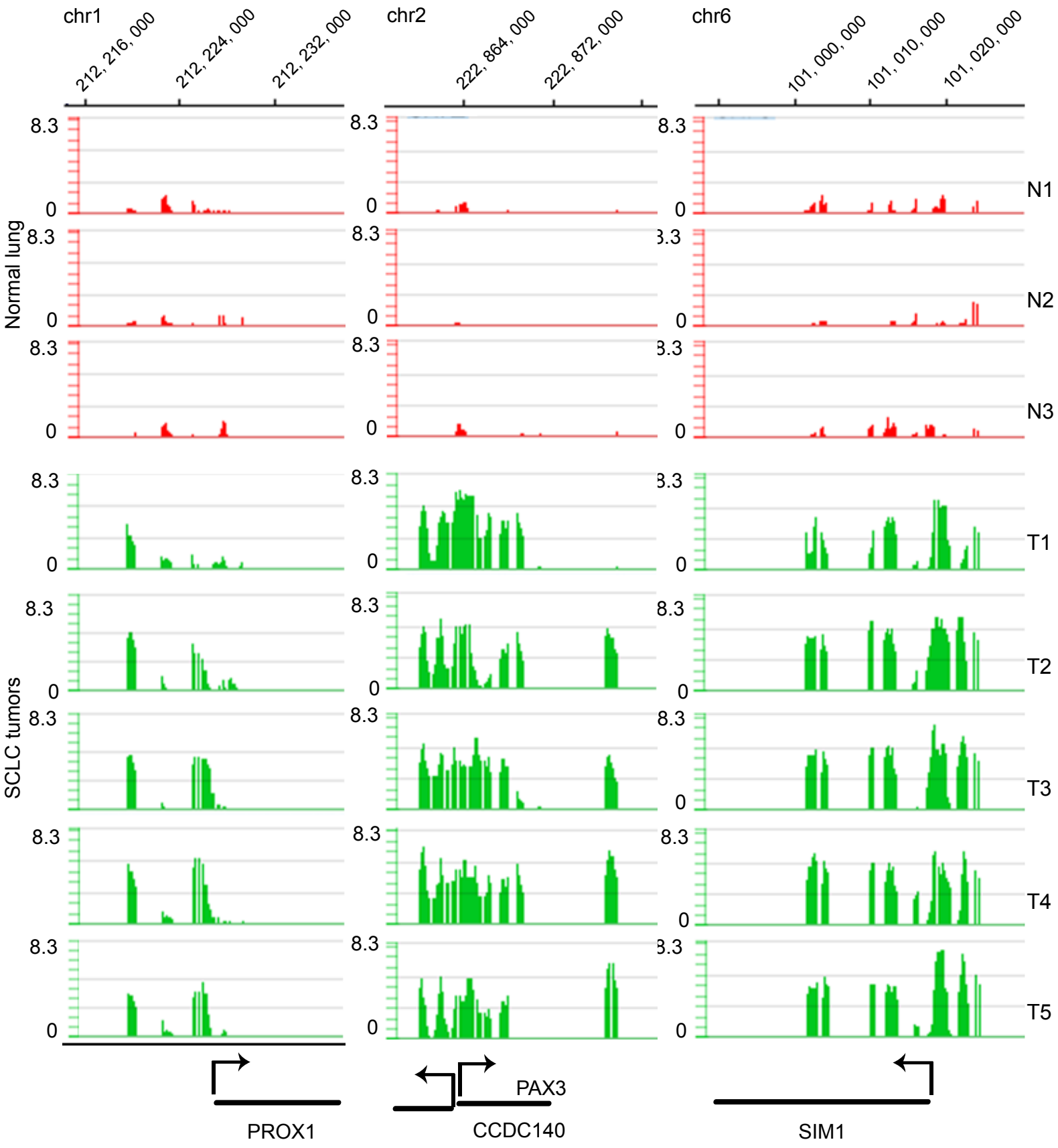
mRNA expression of *NUEROD1* in SCLC cell lines. SCLC and HBEC cell lines were cultured to ~90% confluence in 35-mm dishes. Total RNA was isolated, and mRNA expression of *NEUROD1* was determined by real-time RT-PCR, normalized to 18s RNA and expressed relative to HBEC levels. One of the SCLC cell lines (DMS53) was transiently transfected with a *NEUROD1* expression plasmid to validate the *NEUROD1* RT-PCR method. Expression of *NEUROD1* was extremely low in all samples, except for the one with NEUROD1 overexpression, as indicated by ct values of $\geq$40. Values are the averages of three independent experiments.

Suppl. Figure 6:

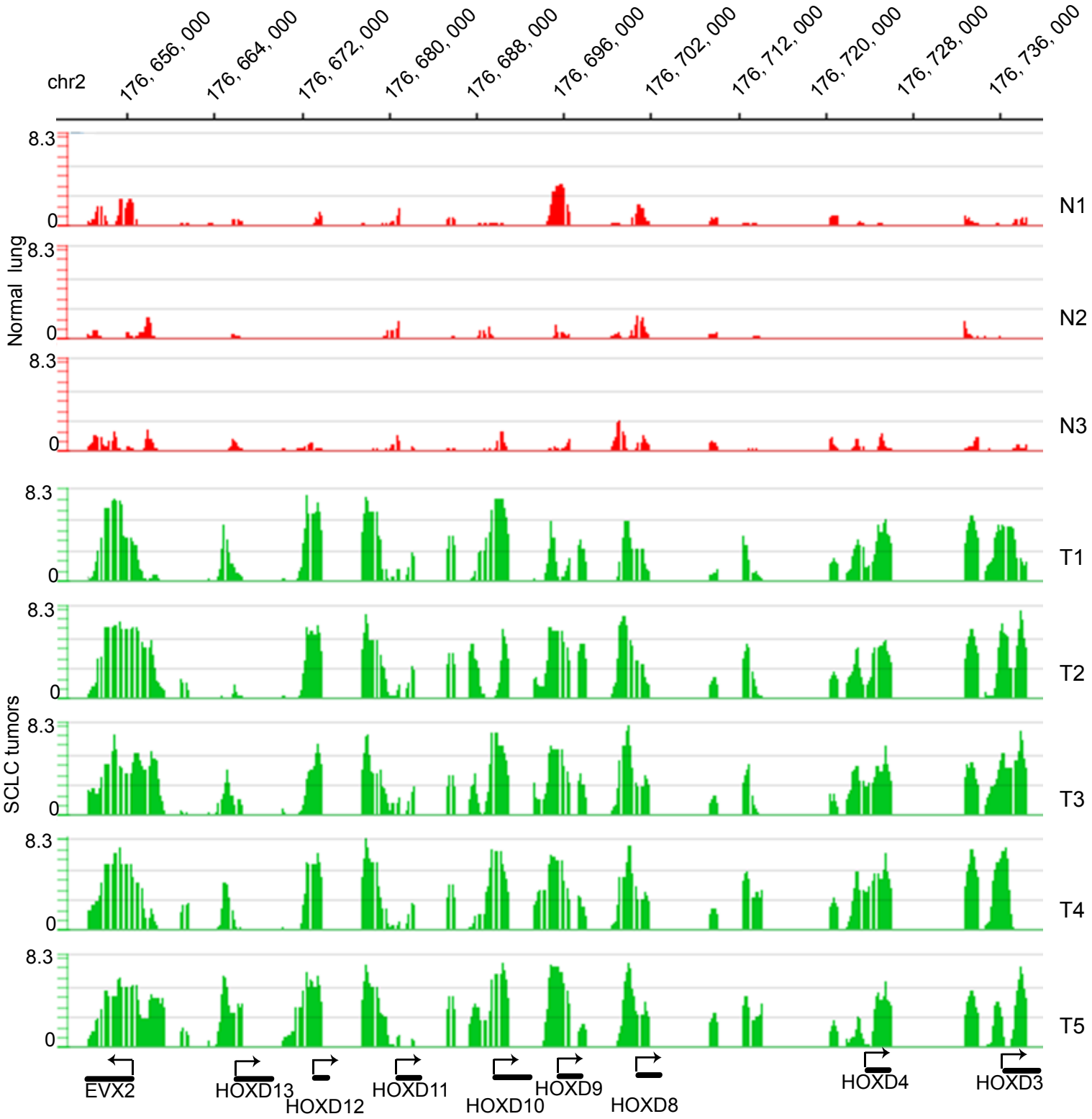
Methylation at the promoters of NEUROD1, HAND1, REST, and ZNF423 in SCLC. Methylation data are shown for normal lung and primary tumors (A) and HBEC and SCLC cell lines (B). The position of the transcription start sites and the direction of transcription are indicated by arrows.



Suppl. Figure 1

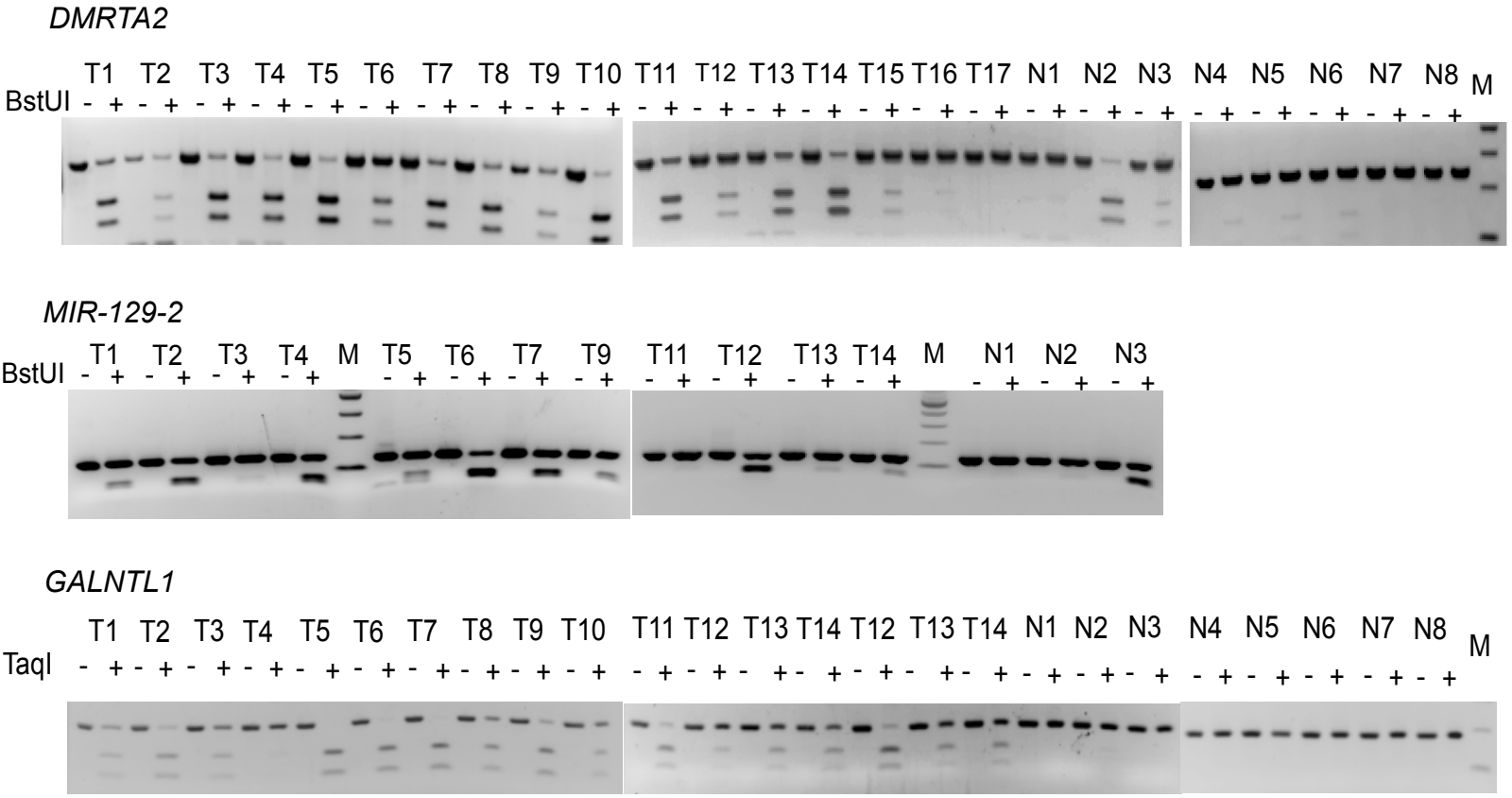


Suppl. Figure 2



Suppl. Figure 3

A



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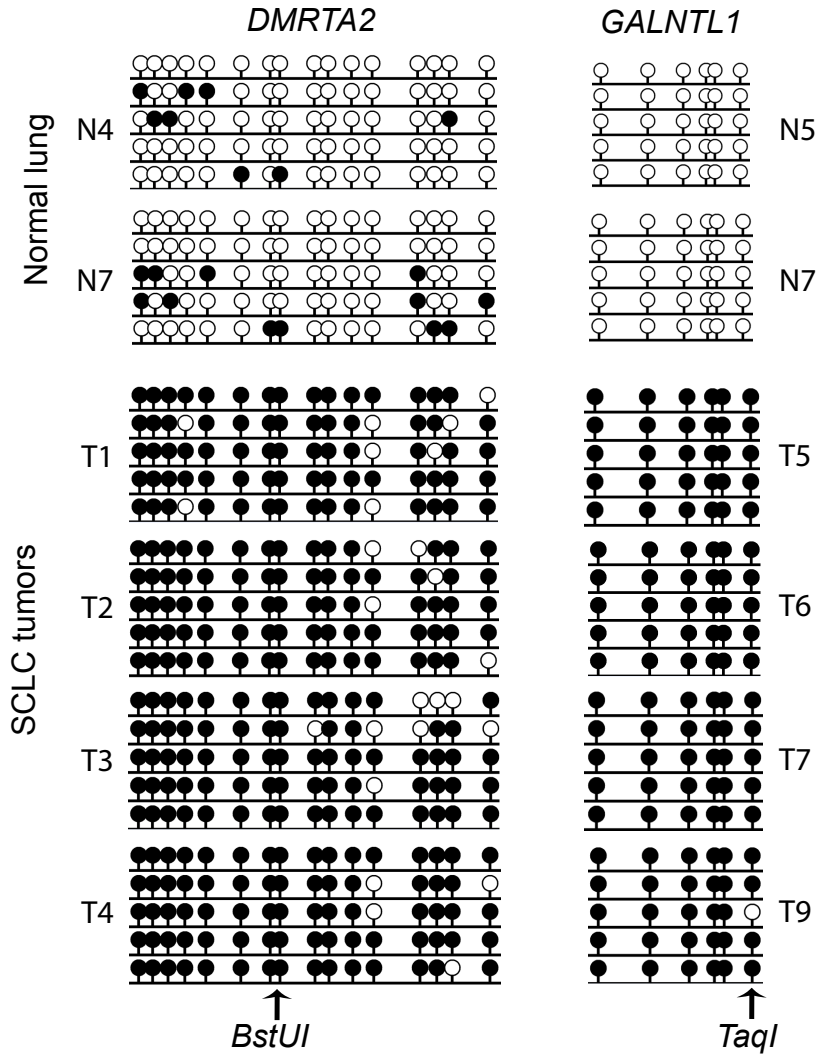
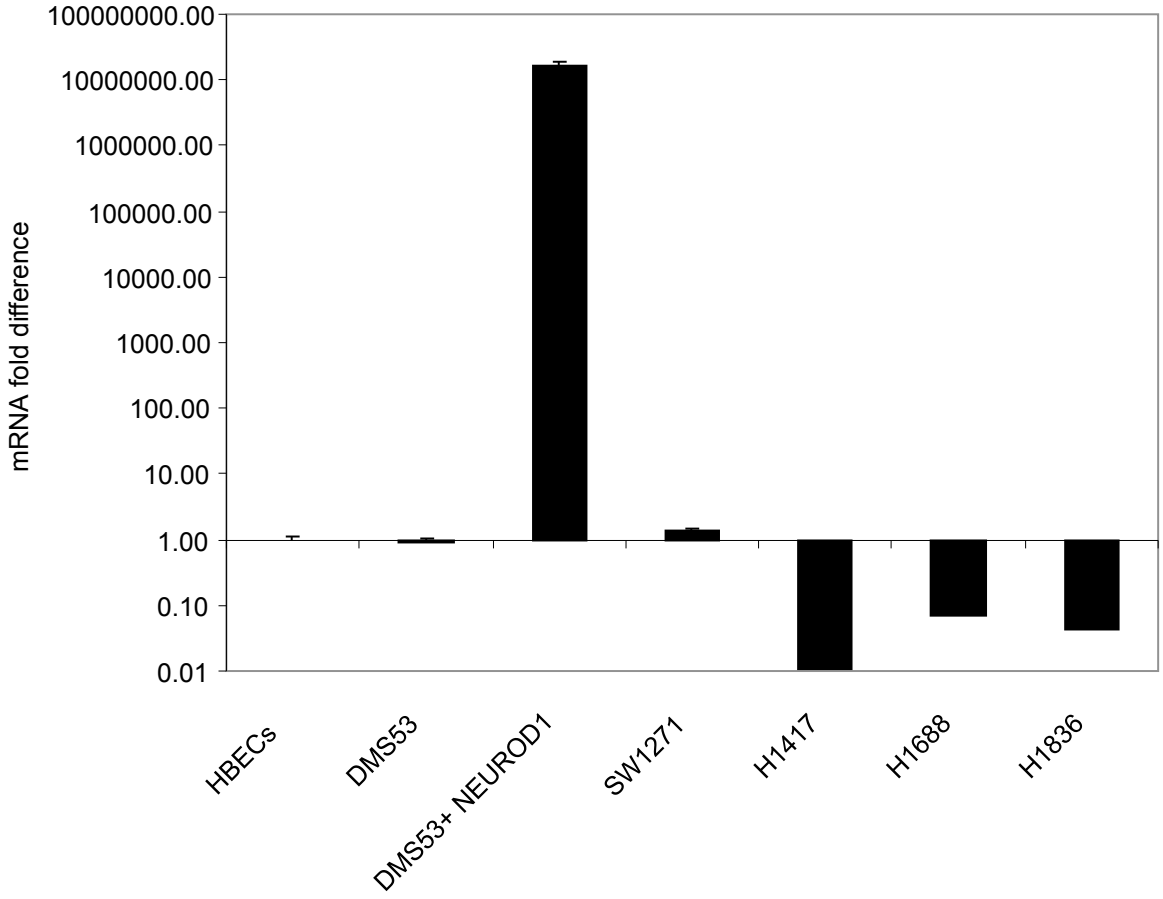
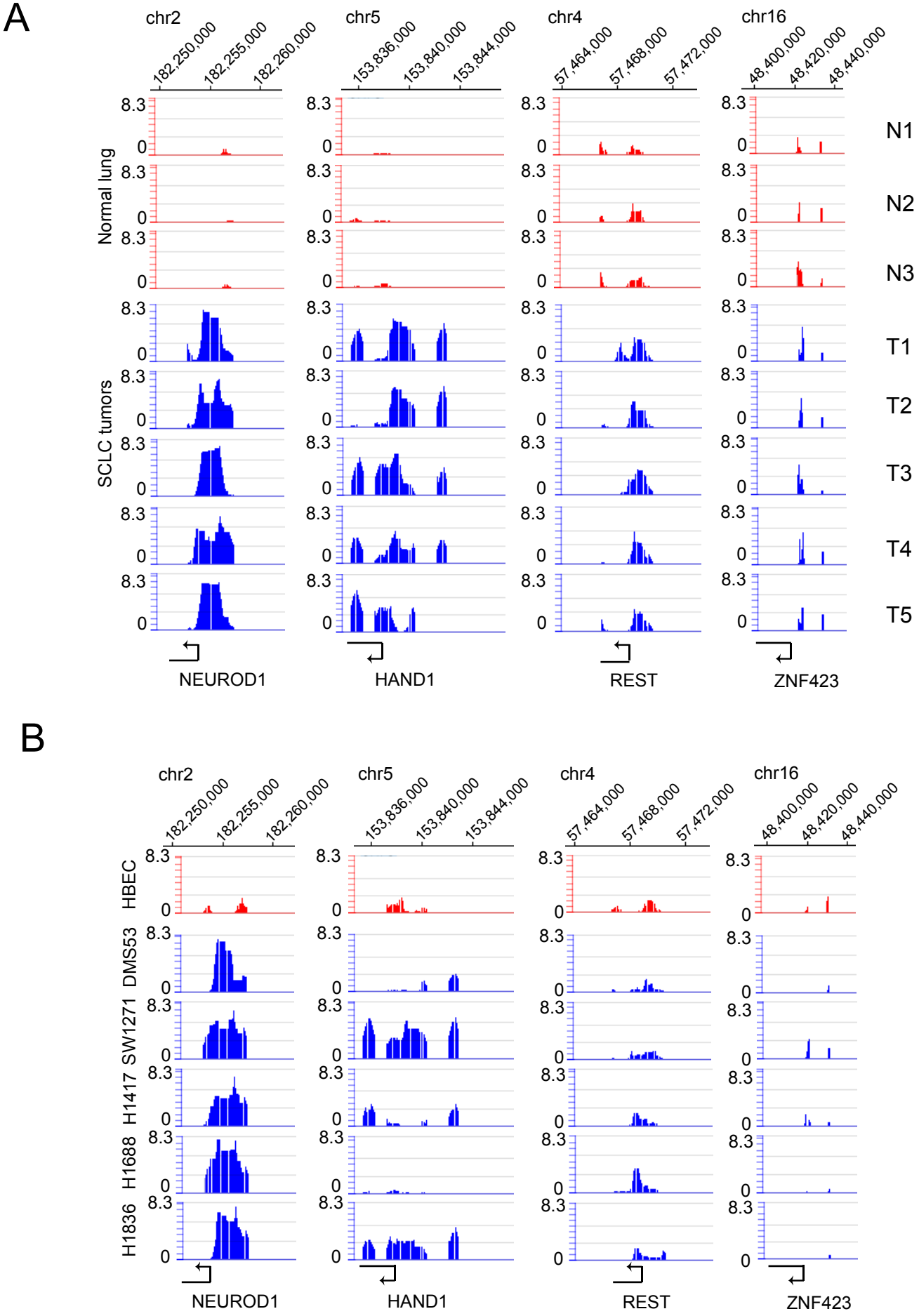


Suppl. Figure 4



Suppl. Figure 5



Suppl. Figure 6