Supplemental Material to:

Hartung T, Zhang L, Kanwar R, Khrebtukova I, Reinhard M, Wang C, Therneau TM, Banck MS, Schroth GP, Beutler AS.
Diametrically opposite methylome-transcriptome relationships in high- and low-CpG promoter genes in postmitotic neural rat tissue
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Included here: Figure S1 – S3

Figure 1S. Cytosine methylation consensus motif

Cytosine methylation was detected primarily at the CpG consensus motif. CpG sites positioned within a MspI restriction enzyme recognition site (CCGG) were enriched as expected due to the use of MspI in the reduced representation bisulfite sequencing protocol employed. Methylation of cytosines at CpH sites (H=any base but C) was rare (<1% of CpH) yet specific with a preference for the CpA motif. CpA methylation was previously reported in embryonal tissues.[15, 30]

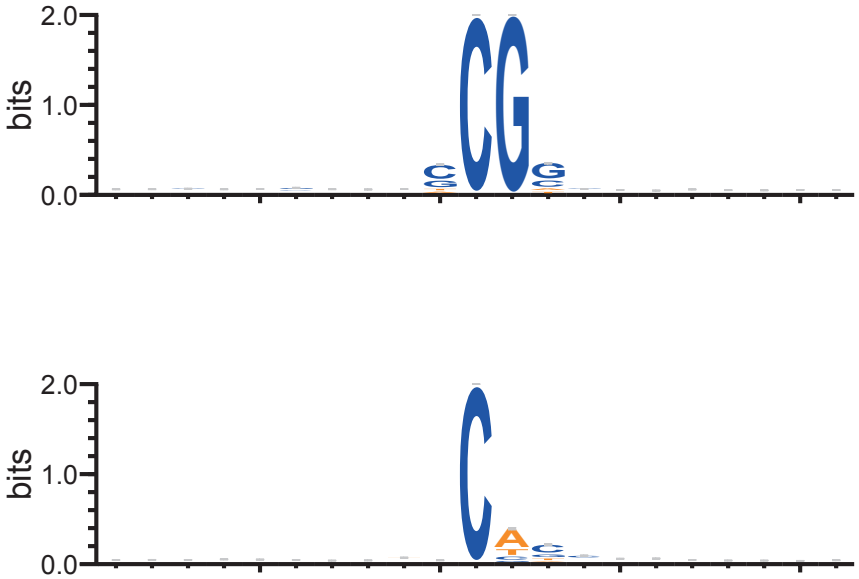

Figure S2. Global cytosine methylation in the rat peripheral nervous system.

CpG methylation levels were bimodally distributed. Peaks were seen at both extremes of methylation levels, unmethylated and fully methylated. Only a minority of CpG sites, 21.9%, was methylated at intermediate levels.

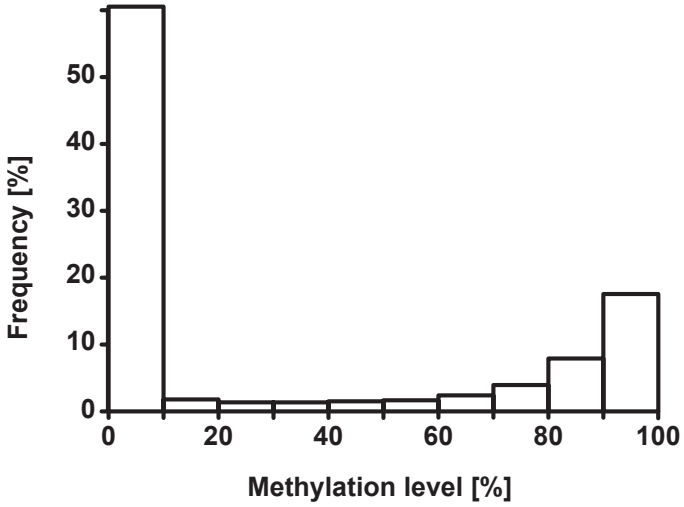

Figure S3. Indiscriminate methylome-transcriptome analysis

The LCP *versus* HCP gene distinction was found to be critical for understanding the methylome-transcriptome association. Furthermore, using the trimmed mean and percentile ranks was identified in the present study as effective descriptive statistics approach. Shown here is a graph that results when both of these methodological improvements are neglected. For this SI Figure LCP and HCP genes were pooled and the mean (average) methylation level was used for the graph. This results in the misleading impression that methylation levels differ only around the TSS coinciding with an overall symmetric valley in this region. This impression is misleading because it results from the overlaying of methylation levels from two distinct groups of genes, whereby the difference in methylation at the TSS is due to LCP genes and the "valley" from HCP genes. Outside of the TSS, methylation differences are obscured because the differences in each group are diluted or compensate for by differences occurring in the other group. Separate LCP and HCP analyses are shown in Fig. 2.