

Supplementary Information

Supplementary Table S1; Oligo sequences

Supplementary Figures S1-S6

Supplementary Data S1; Sample and read alignment information, excel sheet

Supplementary Data S2; Liver cluster assignments, excel sheet

Supplementary Data S3; DMR annotations, excel sheet

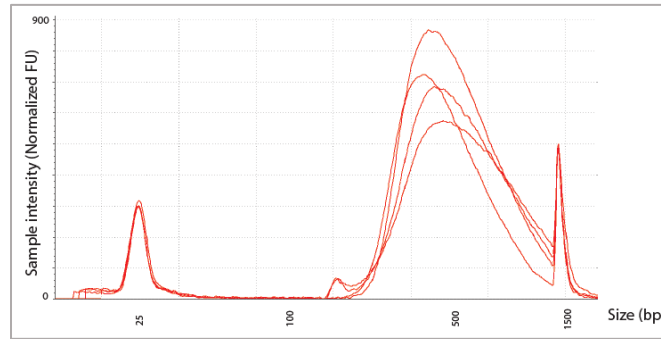
Supplementary Data S4; GO:BP intersections, excel sheet

Supplementary Data S5; DMR-marker overlaps, excel sheet

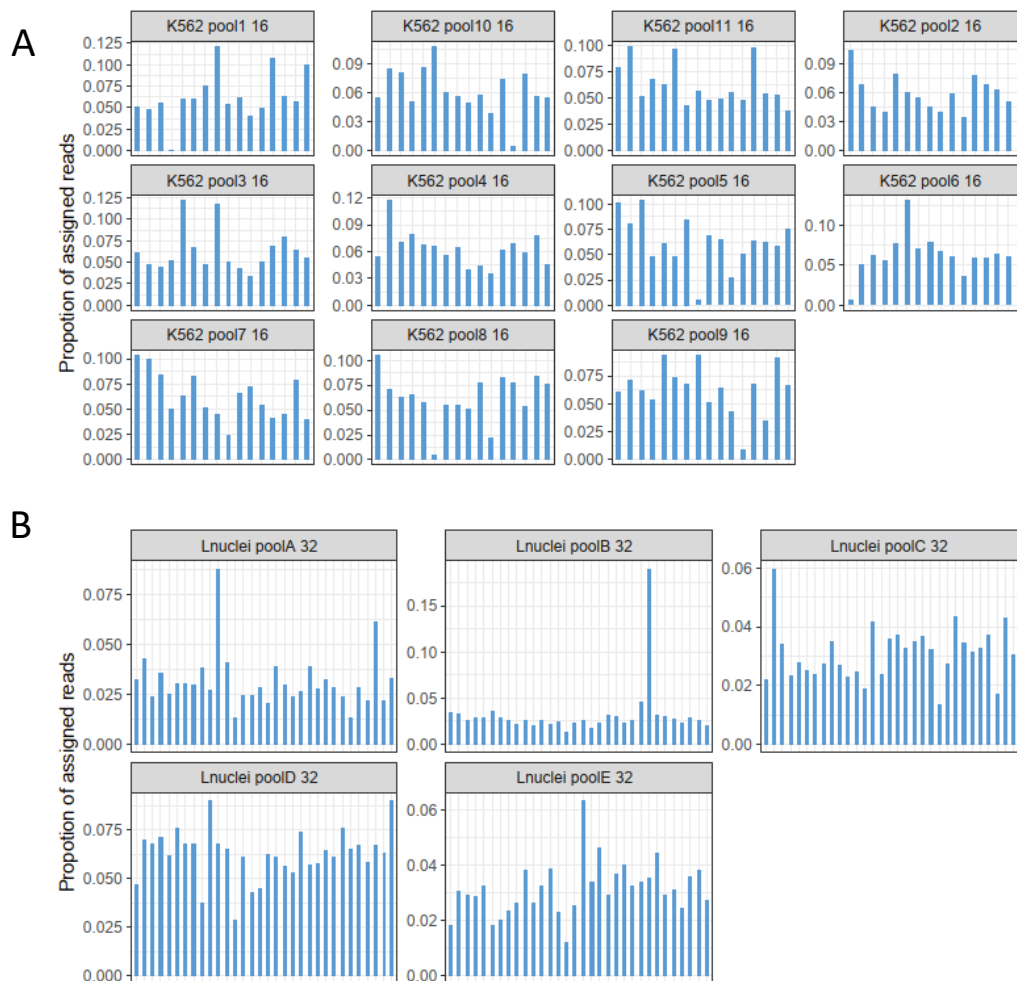
Table S1. Oligo sequences

Oligo name	Sequence
SSO_H_1	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTTAGCTTHHHHHHHHH
SSO_H_2	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTACTTGAHHHHHHHHH
SSO_H_3	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTGCCAATHHHHHHHHH
SSO_H_4	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTCGATGTHHHHHHHHH
SSO_H_5	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTATCACGHHHHHHHHH
SSO_H_6	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTTGACCAHHHHHHHHH
SSO_H_7	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTCAGATCHHHHHHHHH
SSO_H_8	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTCTTGTAAHHHHHHHH
SSO_H_9	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTGACGAGHHHHHHHHH
SSO_H_10	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTAAGGTCHHHHHHHHH
SSO_H_11	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTTGTAGCHHHHHHHHH
SSO_H_12	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTTTGGCAHHHHHHHHH
SSO_H_13	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTCTACTGHHHHHHHHH
SSO_H_14	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTCATATGHHHHHHHHH
SSO_H_15	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTGTATACHHHHHHHHH
SSO_H_16	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTACCTTCHHHHHHHHH
SSO_H_17	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTCTTCCAHHHHHHHHH
SSO_H_18	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTCGAATGHHHHHHHHH
SSO_H_19	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTAGCATCHHHHHHHHH
SSO_H_20	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTTACCGAHHHHHHHHH
SSO_H_21	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTCAAGGAHHHHHHHHH
SSO_H_22	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTCCGTAHHHHHHHHH
SSO_H_23	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTATAGACHHHHHHHHH
SSO_H_24	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTAGGTCTHHHHHHHHH
SSO_H_25	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTGGAATGHHHHHHHHH
SSO_H_26	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTGCTTCAHHHHHHHHH
SSO_H_27	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTTCTATHHHHHHHHH
SSO_H_28	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTTAAGGCHHHHHHHHH
SSO_H_29	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTAACCTGHHHHHHHHH
SSO_H_30	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTTTCCCGHHHHHHHHH
SSO_H_31	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTGATCATHHHHHHHHH
SSO_H_32	5AmMC6/TTCCCTACACGACGCTCTTCCGATCTGCACTTHHHHHHHHH
ss1a	5'AmMC6/GACGTGTGCTCTTCCGATCTNNNNNN/3'AmMo
ss1b	5'Phos/AGATCGGAAGAGCACACGTC/3'AmMo

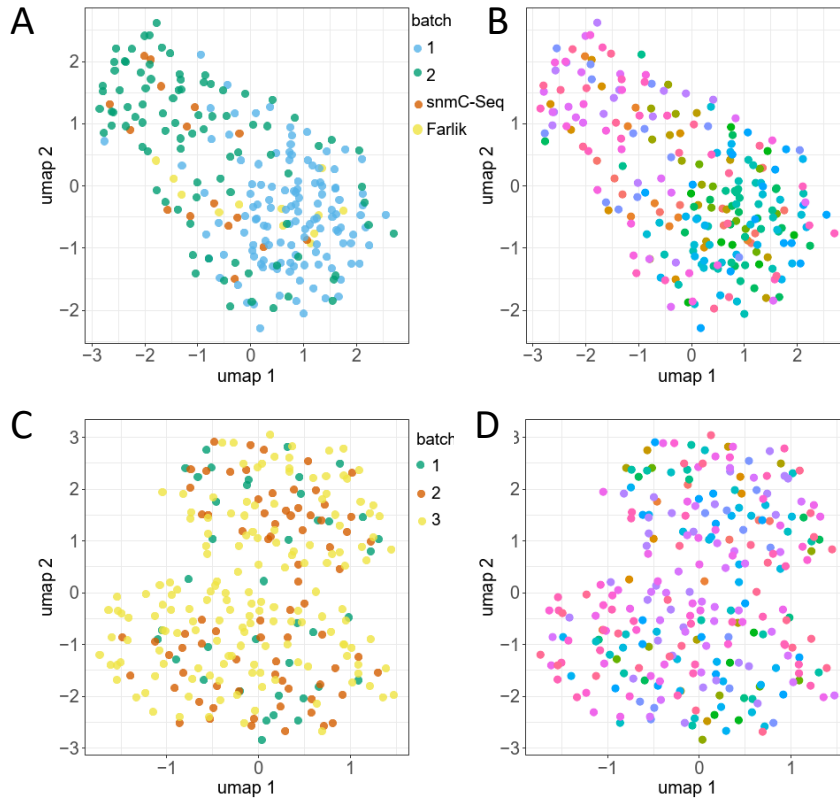
Supplementary Fig. S1 Examples of TapeStation profiles. Library size profiles for pooled scSPLAT libraries



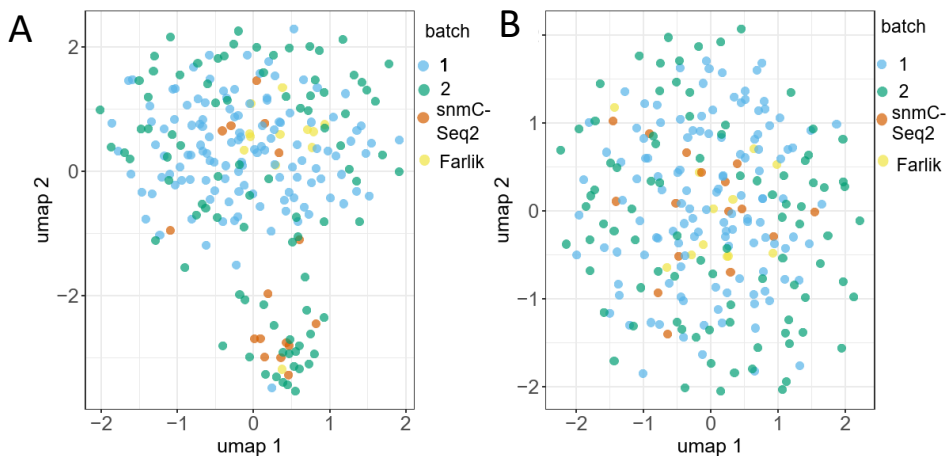
Supplementary Fig. S2 Demultiplexing profiles. A) K562 16-cell pools and B) liver nuclei 32-cell pools. The bars represent the proportion of reads assigned to each cell in the pool.



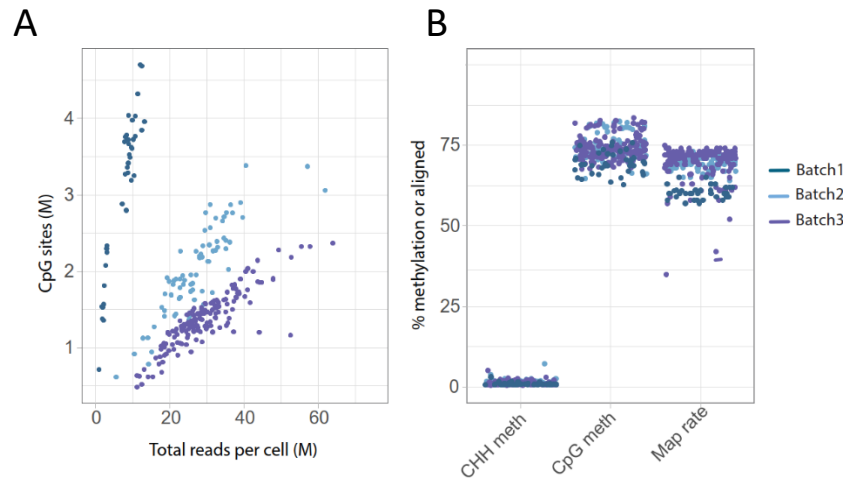
Supplementary Fig S3 K562 and liver nuclei EpiClomal Region clustering based on methylation in CpG islands. A) K562 cells colored by batch. B) K562 cells colored pool-wise. C) Liver nuclei colored by batch. D) Liver nuclei colored pool-wise.



Supplementary Fig. S4 Alternative clustering of K562 cells. EpiClomal Region assigned all K562 cells to a single cluster irrespective of whether the clustering was performed based on methylation in CpG islands, gene bodies or TSS regions. A) K562 EpiClomal Region clustering based on methylation in gene bodies. Cells colored by batch. Although it appears like a small number of cells form a ‘subcluster’ in the UMAP plot, they were all assigned to one single cluster by EpiClomal. B) K562 EpiClomal Region clustering based on methylation in regions of 1000 bp +/- of TSS. Cells colored by batch.



Supplementary Fig. S5 Liver nuclei QC metrics A) Number of detected CpG sites per cell vs sequencing depth B) Global methylation levels and read alignment efficiency per nuclei.



Supplementary Fig. S6 Reclustering of liver nuclei cluster 1. Clustering was performed on: CpG island methylation, gene body methylation and methylation across TSS regions. With the two latter EpiClomal finds one cluster only. In contrast, with CpG-island methylation EpiClomal assigns the cells to two clusters that were were intermingled in the UMAP visualization.

