Sensitive enzymatic quantification of 5-hydroxymethylcytosine in genomic DNA

Aleksandra Szwagierczak, Sebastian Bultmann, Christine Schmidt, Fabio Spada and Heinrich Leonhardt

Ludwig Maximilians University Munich, Department of Biology, Center for Integrated Protein Science Munich (CIPS^M), 82152 Planegg-Martinsried, Germany.



SUPPLEMENTARY FIGURE S1

Quantification of genomic hmC and Tet transcripts in undifferentiated E14 embryonic stem cells (ESCs) and embryoid bodies (EBs). (A) hmC glucosylation assay. The percentage of hmC over total cytosine is calculated from the incorporation of $[^{3}H]$ glucose using a calibration curve from the reference fragment (see Fig. 1C) and corrected for the difference in cytosine abundance between the latter (35%) and mouse genome (42%). Shown are values from one assay, where each sample was measured in duplicate. (B) Real time RT-PCR analysis for Tet transcript levels. Shown are average values and error bars from two independent cDNA synthesis reactions. In every PCR reaction each sample was measured in triplicate. Genomic DNA and RNA samples used in A and B, respectively, were isolated from the very same ESC and EB lysates.