**Additional file 4**. We have also applied 10% and 25% cut-off levels while having min per group = 6. In addition using tile function, we applied 300bp window size, 300bp sliding window size to determine differentially methylated regions (DMR). Only regions with at least 4 Cs were included in the analysis.

In brief, regardless of the type criteria used, the main conclusions remained the same, which are:

* Majority of DMCs or DMRs are hypomethylated.
* By increasing the DFI both the total number and the number of hypomethylated DMCs/ DMRs increases.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | min per group | Methylation cut off | Number of DMCs/DMRsHypo | Number of DMCs/DMRsHyper |
| LM | LH | LM | LH |
| DMCs | 4(presented in MS) | 25 | 200 | 820 | 75 | 97 |
| 6 | 25 | 18 | 17 | 7 | 4 |
| 10 | 176 | 236 | 92 | 37 |
| DMRs(300bp window,min 4 mutual Cs / window) | 6 | 25 | 2 | 2 | 0 | 0 |
| 10 | 25 | 37 | 8 | 1 |