

Additional File 3. PCR and pyrosequencing primers for *LEP* gene proximal promoter CpG island amplification and pyrosequencing.

Target CpGs	Target sequence (5' to 3')	Chromosomal Region ^a	Target sequence after bisulfite conversion (5' to 3')	PCR and pyrosequencing primers (5' to 3')	Length (bp)
CpG3 to CpG7	<u>TC</u> ⁷ GTAGGAAT <u>C</u> ⁶ GCAG <u>C</u> ⁵ GC CAR <u>C</u> ⁴ GGTTGCAAGGTAAGG CCCC <u>G</u> ³ GGC ² GC ¹ G	chr7:127 881 330 to chr7:127 881 374	<u>TC</u> ⁷ GTAGGAAT <u>C</u> ⁶ GTAG <u>C</u> ⁵ GT TAR <u>C</u> ⁴ GGTTGTAAAGGTAAGG TTT <u>C</u> ³ GGC ² GC ¹ G	F: GGGGAGGGTAGGTATGGAG R: CCCTACATCCCTCCTAACT seq: GGGTAGGTATGGAGT	162
CpG11 to CpG17	<u>C</u> ¹⁷ GGGG <u>C</u> ¹⁶ GGGAGCTGGC ¹⁵ GCTAGAAATGC ¹⁴ GCC ¹³ GGG GCCTGC ¹² GGGGCAGTTGC ¹¹ G CAAGTTGTGATC ¹⁰ GGGCC ⁹ G CTATAAGWGGGGC ⁸ G	chr7:127 881 230 to chr7:127 881 316	<u>C</u> ¹⁷ GGGG <u>C</u> ¹⁶ GGGAGTTGGC ¹⁵ GTTAGAAATGC ¹⁴ GT <u>C</u> ¹³ GGGG TTTGC ¹² GGGGTAGTTGC ¹¹ GT AAGTTGTGATC ¹⁰ GGGTC ⁹ GT TATAAGWGGGGC ⁸ G	F:ATTTTAGGGAGGTATTTAAGGGTG R:ACCATTCCCTACCAAACCTCCATACCTACCC seq: GGTAAGTAGTTATTTTGAGGG	239
CpG23 to CpG31	<u>GC</u> ³¹ <u>GC</u> ³⁰ <u>GC</u> ²⁹ GTGGCTCCTGG <u>M</u> ²⁸ <u>GC</u> ²⁷ <u>GCC</u> ²⁶ GAGGCCCTCCC <u>TC</u> ²⁵ GAGGCC <u>CC</u> ²⁴ <u>GC</u> ²³ GAG	chr7:127 881 125 to chr7:127 881 173	<u>GC</u> ³¹ <u>GC</u> ³⁰ <u>GC</u> ²⁹ GTGGTTTTGG <u>M</u> ²⁸ <u>GC</u> ²⁷ GT <u>C</u> ²⁶ GAGGTTTTTT <u>TC</u> ²⁵ GAGGTTT <u>C</u> ²⁴ <u>GC</u> ²³ GAG	F:GAGTTTTTGGAGGGATATTAAGGA R:CAACTCCCCCCCCCCCCCTCAAAAATAA seq: GGGAGGTATTTAAGGG	194

The CpG sites are numbered according to Bouchard *et al.* 2010 [23]. The underlined CpG sites were epigenotyped in the current study

T in red font are cytosines that have been converted to thymine after Nabis treatment of DNA

F; Forward. R; Reverse. Seq; Sequencing

The primers were designed from bisulfite converted sequence using Pyromark Assay Design software (version 2.0.1.15; Qiagen).

^aUCSC Genome Browser (Human Feb. 2009: NM_000230)