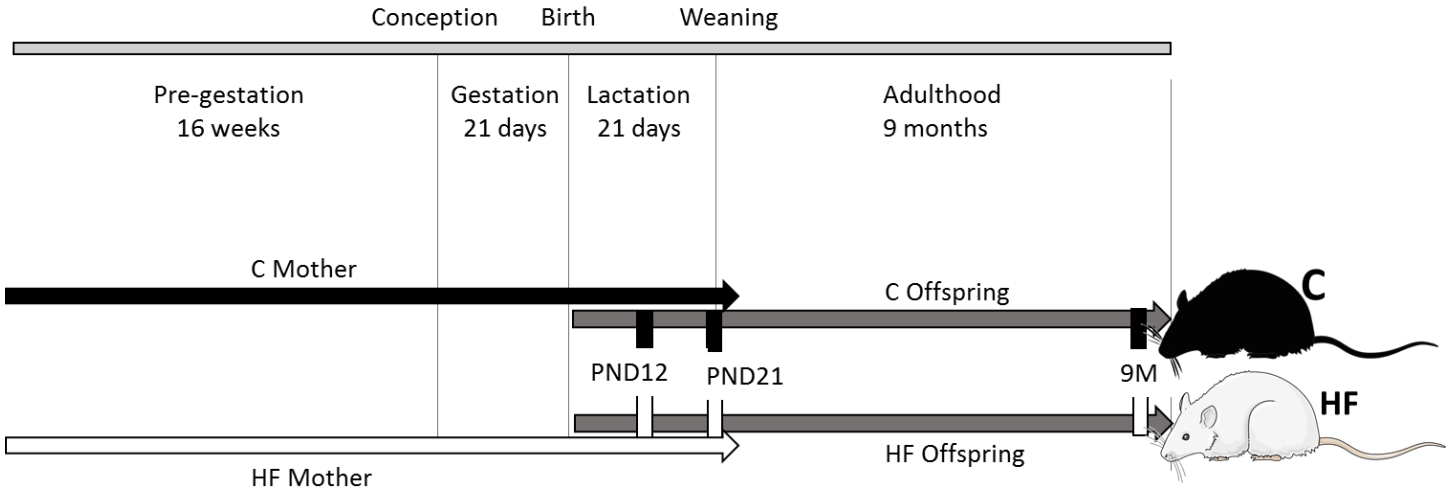
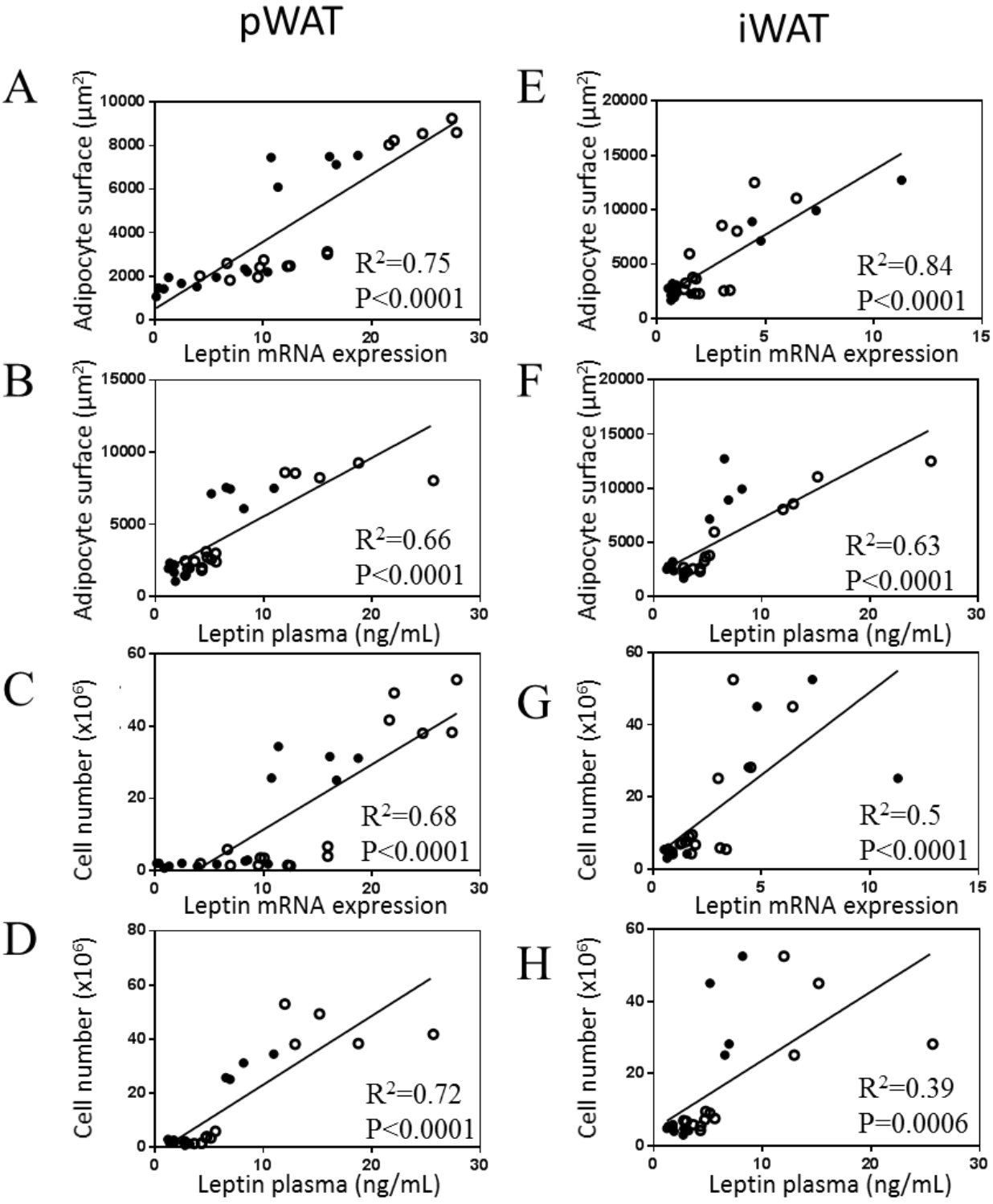
SUPPLEMENTARY DATA

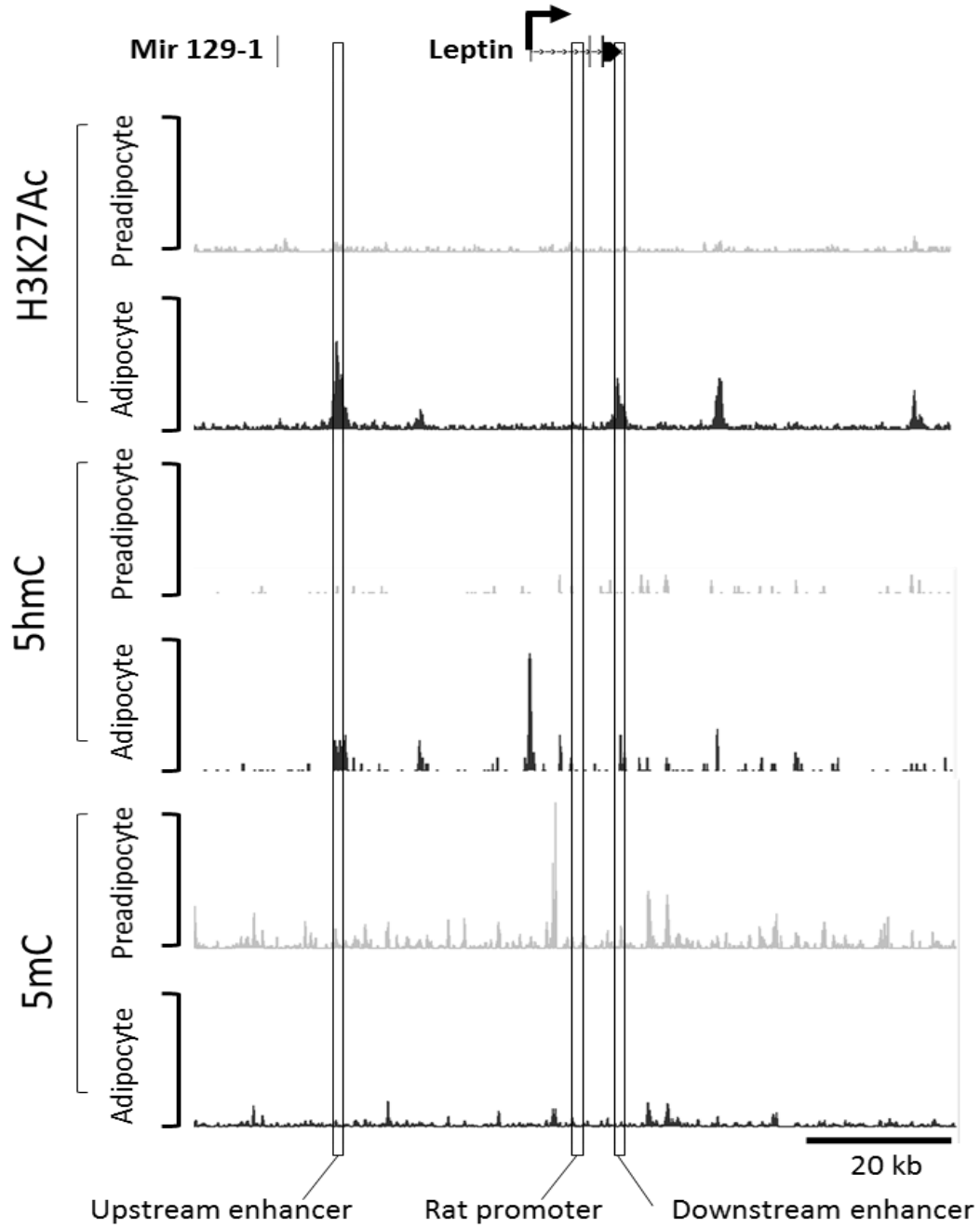
Supplementary Figure 1: Experimental protocol



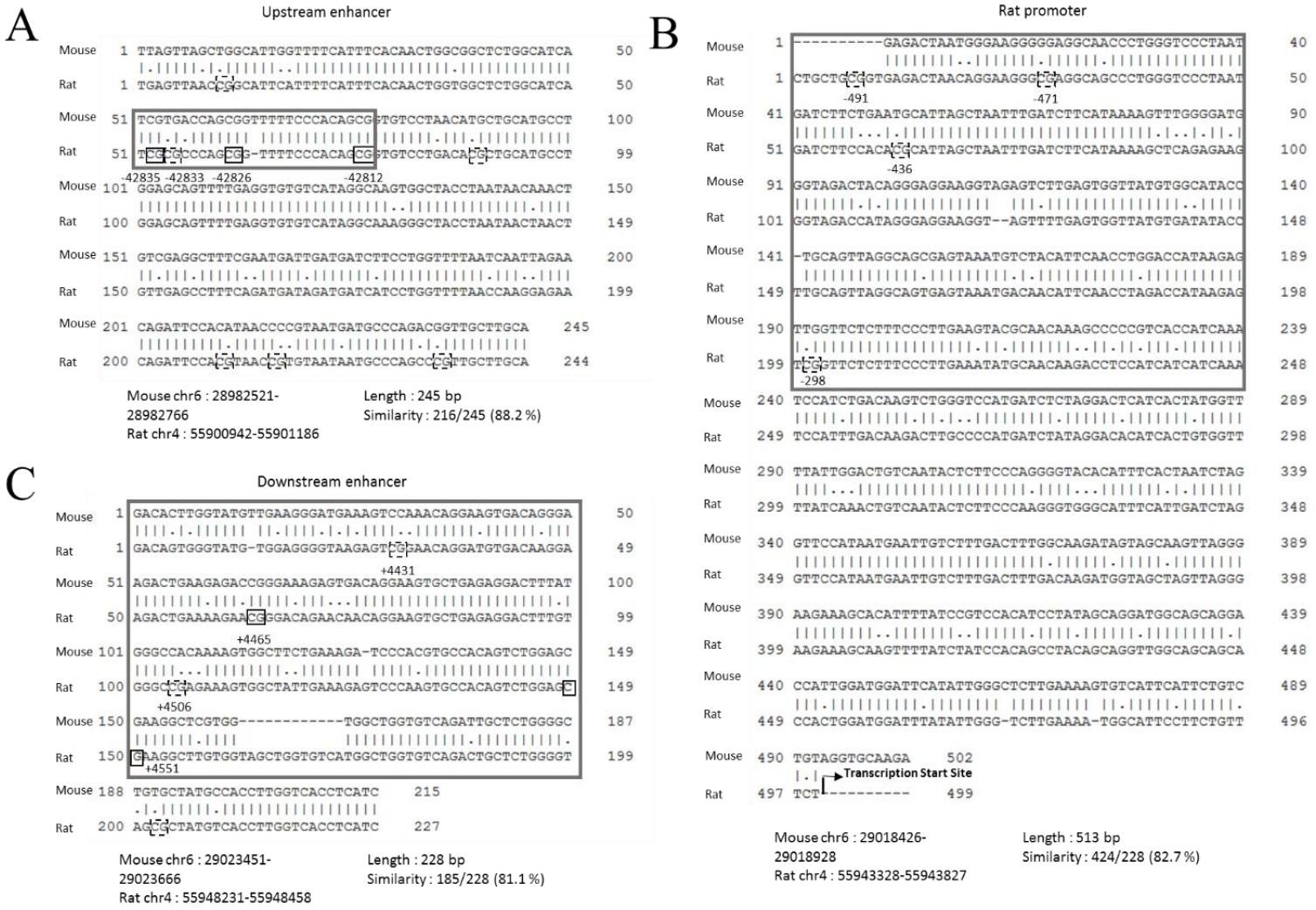
Supplementary Figure 2: Leptin mRNA levels and plasma concentration are correlated with average adipocyte area and total cell number in pWAT and iWAT. Pearson correlations were performed for each rat between average adipocyte area (A, B, E and F) or total cell number (C, D, G and H) and leptin mRNA expression (A, C, E and G) or plasma leptin concentration (B, D, F and H) in pWAT (A-D) and iWAT (E-H). Correlations were determined using the three stages. Black circles: C offspring. White circles: HF offspring.



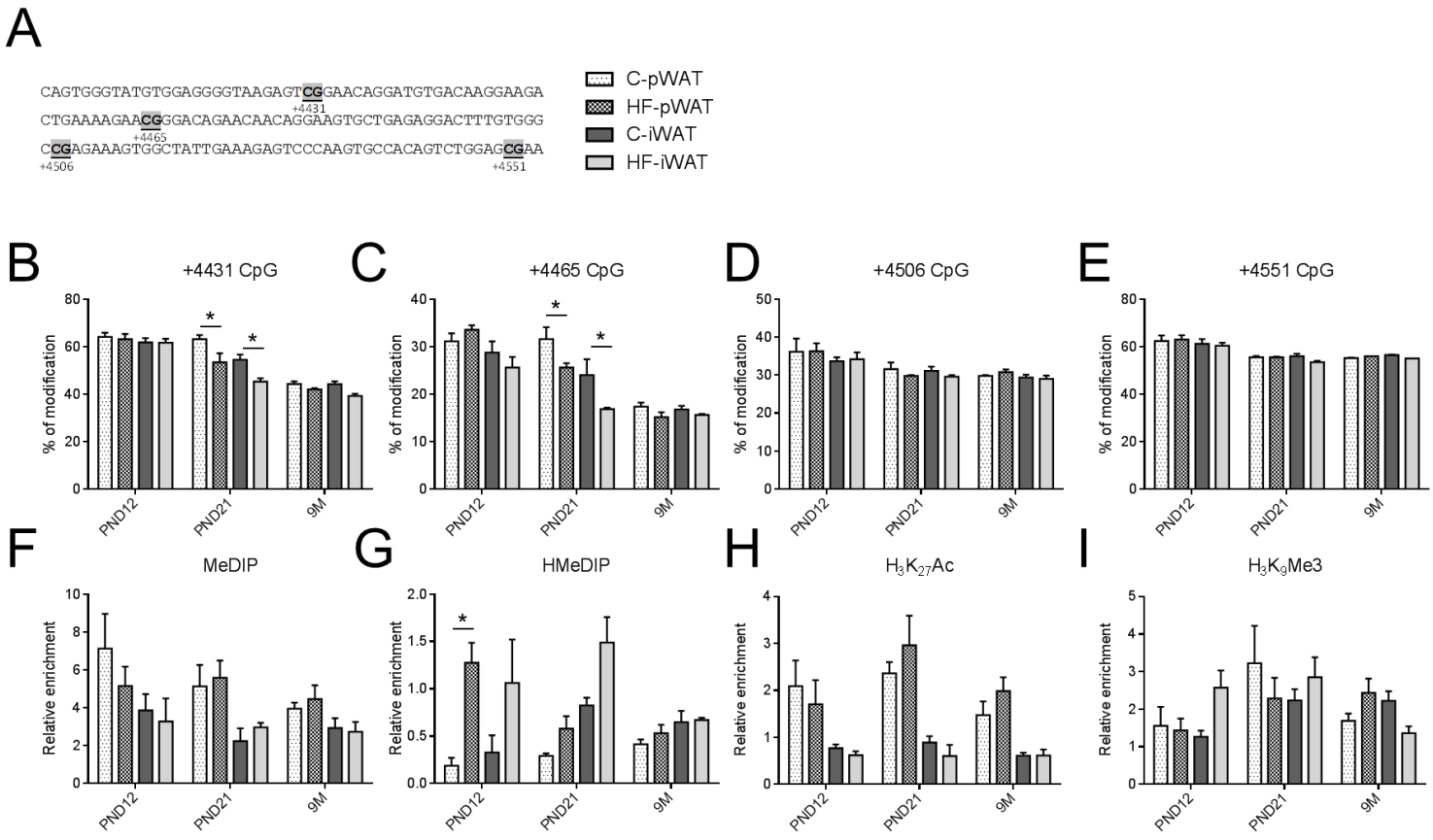
Supplementary Figure S3: Identification of two transcriptionally active regions (enhancers) during 3T3-L1 adipocyte differentiation. The Integrated Genome Browser [1] was used to visualize H3K27ac, 5mC and 5hmC signal intensities from 3T3-L1 preadipocytes and adipocytes (A). Potential leptin enhancers from 3T3-L1 were identified by an enrichment for both H3K27ac and 5hmC and depletion in 5mC during adipocyte differentiation [2,3]. Using this signature, we discriminated two novel potential leptin enhancers (upstream and dowstream) activated during adipocyte differentiation (i.e., activated enhancers gaining H3K27ac/5hmC during differentiation) from enhancers inactive in preadipocytes (i.e., genomic regions displaying 5mC in preadipocytes). As already described [4], the region corresponding to the rat leptin promoter is indicated on the mouse genome. The involvement of the two potential enhancers identified in the transcriptional regulation of leptin gene was validated by the CisMapper model [5]. Genes potentially regulated by the two regions and predicted by CisMapper have been listed in Table S2.



Supplementary Figure S4: Alignment of the two potential enhancers and the promoter between rat and mouse. Sequences of the two enhancers (A and C) and the promoter (B) were aligned between both species by the EMBOSS needle [6]. The genomic sequence tested was surrounded. We investigated zones containing the maximum of conserved CpG between the two species. Conserved CpG are indicated in box whereas not conserved CpG are indicated with dashed box.



Supplementary Figure S5: The downstream enhancer in both deposits of HF offspring shows depletion in 5mC at PND21 that is no longer visible in 9M. Epigenetic modifications of four CpG indicated in gray (A) and located in the downstream enhancer at +4431bp (B), +4465bp (C), +4506bp (D) and +4551bp (E) of the transcription start site were assessed in male C and HF offspring in two fat pads (pWAT and iWAT) at PND12, PND21 and 9 months. To discriminate the nature of DNA modifications, DNA extracted from both depots was immunoprecipitated with antibodies against DNA methylation (MeDIP) (F), DNA hydroxymethylation (HMeDIP) (G) and subject to qPCR using primers of targeted regions. Histone modifications were measured after chromatin immunoprecipitation with antibodies against H3K27Ac (active mark) (H) or H3K9me3 (inactive mark) (I) and qPCR using primers of targeted regions. Immunoprecipitation with normal rabbit IgG was used as a negative control. All data are presented as means ± SEM. Data were analysed using two-way ANOVA followed by Bonferroni post hoc test. * Effect of maternal obesity (**P*<0.05, ***P*<0.01 and ****P*<0.001). n = 4-6 per group.



Supplementary Table S1: Primers used in RT-qPCR, (H)MeDIP-qPCR and pyrosequencing

	RT-qPCR primer sequences	**Targeted gene**
Forward	GTTCCTGTGGCTTTGGTCCT	*Leptin*
Reverse	CTGGTGACAATGGTCTTGATGA	
Forward	ATTCATGTGCCAGGGTGGT	*Ppia*
Reverse	GATGCCAGGACCTGTATGCT	
Forward	GACGGTCACGGAGGATAAGATC	*Rplp1*
Reverse	GCAAACAAGCCAGGCCAGAAA	
	(H)MeDIP-qPCR and ChIP-qPCR primer sequences	**Targeted regions**
Forward	GACAAGACTTGCCCCATGAT	*Leptin promoter*
Reverse	ACCTGCTGTAGGCTGTGGAT	
Forward	ATCGATTCTTTGGCACGGGG	*Leptin upstream enhancer*
Reverse	ACACACCTCAAAACTGCTCCA	
Forward	GTGGAGGGGTAAGAGTCGGA	*Leptin downstream enhancer*
Reverse	CAGCCATGACACCAGCTACC	
Forward	CTCCCACAGACGCTATGGTG	*Control*
Reverse	GCCTGTAAATCCCACCCCAG	
	Pyrosequencing primer sequences	**Targeted regions**
Forward	TTGAGTGGTTATGTGATATATTTTGTAGT	*Leptin promoter #1*
Reverse	*Biotin-* AATCAATAAAATACCCACCCTTAAAA	
Sequence	AGATATAATAAGAGGTTGTTG	
Forward	GGAGAGGAAGGAAGTTATGGATTAGTA	*Leptin promoter #2*
Reverse	*Biotin-* CCCTATAATCTACCCTTCTCTAAACTTT	
Sequence	AGTTTTGGGTTTTTAATGATT	
Forward	GGGGTAGTTGAAATAATGAGTTAAT	*Leptin upstream enhancer*
Reverse	*Biotin* -ACCCTTTACCTATAACACACC	
Sequence 1	TGGTGGTTTTGGTATTAT	
Sequence 2	GTTTAGAGGTTTTTTTATAG	
Forward	TAGTGGGTATGTGGAGGGGTAAG	*Leptin downstream enhancer*
Reverse	*Biotin-* CCCAAAACAATCTAACACCAACCATAA	
Sequence 1	GTGGAGGGGTAAGAG	
Sequence 2	GGATGTGATAAGGAAGAT	
Sequence 3	AGAGGATTTTGTGGG	
Sequence 4	ATTGAAAGAGTTTTAAGTGTTATA	

Supplementary Table S2: Genes potentially regulated by the two enhancers as predicted by the CisMapper model

Target genes
Lep (NM_008493)
Rbm28 (NM_133925)
Mir129-1 (NR_029567)
Prrt4 (NM_001101443)
Impdh1 (NM_011829)
Lrrc4 (NM_138682)
Fam71f2 (NM_001101486)
Hilpda (NM_001190461)
Hilpda (NM_023516)
Fam71f1 (NM_207258)
Ccdc136 (NM_001201378)
Ccdc136 (NM_145574)
Flnc (NM_001081185)
Calu (NM_007594)
Calu (NM_184053)
Opn1sw (NM_007538)
Gm9047 (NM_001145360)
Atp6v1f (NM_025381)
Kcp (NM_001029985)
Irf5 (NM_012057)
Irf5 (NM_001252382)

References

[1] Nicol, J.W., Helt, G.A., Blanchard, S.G., Raja, A., Loraine, A.E., 2009. The Integrated Genome Browser: free software for distribution and exploration of genome-scale datasets. Bioinformatics (Oxford, England) 25(20): 2730–1.

[2] Oger, F., Dubois-Chevalier, J., Gheeraert, C., Avner, S., Durand, E., Froguel, P., et al., 2014. Peroxisome proliferator-activated receptor α regulates genes involved in insulin/insulin-like growth factor signaling and lipid metabolism during adipogenesis through functionally distinct enhancer classes. Journal of Biological Chemistry 289(2): 708–22.

[3] Mikkelsen, T.S., Xu, Z., Zhang, X., Wang, L., Gimble, J.M., Lander, E.S., et al., 2010. Comparative epigenomic analysis of murine and human adipogenesis. Cell 143(1): 156–69.

[4] Cordero, P., Campion, J., Milagro, F.I., Goyenechea, E., Steemburgo, T., Javierre, B.M., et al., 2011. Leptin and TNF-alpha promoter methylation levels measured by MSP could predict the response to a low-calorie diet. Journal of Physiology and Biochemistry 67(3): 463–70.

[5] O'Connor, T., Bodén, M., Bailey, T.L., 2016. CisMapper: predicting regulatory interactions from transcription factor ChIP-seq data. Nucleic Acids Research 45(4): gkw956.

[6] Li, W., Cowley, A., Uludag, M., Gur, T., McWilliam, H., Squizzato, S., et al., 2015. The EMBL-EBI bioinformatics web and programmatic tools framework. Nucleic Acids Research 43(W1): W580–4.