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.

Rat chr4 : 55943328-55943827

		Upstream enhancer		р
Mouse	1	TTAGTTAGCTGGCATTGGTTTTCATTTCACAACTGGCGGCTCTGGCATCA	50	В
Rat	1	TGAGTTAACCGCATTCATTTCATTTCACAACTGGTGGCTCTGGCATCA	50	
Mouse	51	TCGTGACCAGCGGTTTTTCCCACAGCG STGTCCTAACATGCTGCATGCCT	100	
Rat	51	TCCCCCACCGCTTTTCCCACACCCFTGTCCTGACACCCTGCATGCCT	99	
Mouse	101	12835 -42833 -42826 GGAGCAGTTTTGAGGTGTGTCATAGGCAAGTGGCTACCTAATAACAAACT	150	
Rat	100	GGAGCAGTTTTGAGGTGTGTCATAGGCAAAGGGCTACCTAATAACTAAC	149	
Mouse	151	GTCGAGGCTTTCGAATGATTGATGATCTTCCTGGTTTTAATCAATTAGAA	200	
Rat	150	GTTGAGCCTTTCAGATGATGATGATCATCCTGGTTTTAACCAAGGAGAA	199	
Mouse	201	CAGATTCCACATAACCCCGTAATGATGCCCAGACGGTTGCTTGC		
Rat	200	CAGATTCCACGTAACCGTGTAATAATGCCCAGCCCGTTGCTTGC		
	ł	Rat chr4 : 55900942-55901186 Downstream enhancer		
Mouse	1	GACACTTGGTATGTTGAAGGGATGAAAGTCCAAACAGGAAGTGACAGGGA	50	
Rat	1	GACAGTGGGTATG-TGGAGGGGTAAGAGTCGGACAAGGATGTGACAAGGA	49	
Mouse	51	+4431 AGACTGAAGAGACCGGGAAAGAGTGACAGGAAGTGCTGAGAGGACTTTAT	100	
Rat	50	AGACTGAAAAGAACGGGACAGAACAACAGGAAGTGCTGAGAGGACTTTGT	99	
Mouse	101	+4465 GGGCCACAAAAGTGGCTTCTGAAAGA-TCCCACGTGCCACAGTCTGGAGC	149	
Rat	100	gggccgagaaagtggctattgaaagagtcccaagtgccacagtctggagC	149	
Mouse	150	+4506 GAAGGCTCGTGGTGGCTGGTGTCAGATTGCTCTGGGGC	187	
Rat	150	AGGCTTGTGGTAGCTGGTGTCATGGCTGCTGTCAGACTGCTCTGGGGT	199	
Mouse	188	TGTGCTATGCCACCTTGGTCACCTCATC 215		
Rat	200	AGCCTATGTCACCTTGGTCACCTCATC 227		

Length : 228 bp

Similarity : 185/228 (81.1%)

Mouse chr6 : 29023451-

Rat chr4 : 55948231-55948458

29023666

А

		Rat promoter
Mouse	1	GAGACTAATGGGAAGGGGAGGCAACCCTGGGTCCCTAAT 40
Rat	1	CTGCTGCGGTGAGACTAACAGGAAGGGCGAGGCAGCCCTGGGTCCCTAAT 5(
Mouse	41	-491 -471 GATCTTCTGAATGCATTAGCTAATTTGATCTTCATAAAAGTTTGGGGGATG 90
Rat	51	GATCTTCCACACGCATTAGCTAATTTGATCTTCATAAAAGCTCAGAGAAG 10(
Mouse	91	-436 GGTAGACTACAGGGAAGGAAGGTAGAGTCTTGAGTGGTTATGTGGCATACC 14(
Rat	101	GGTAGACCATAGGGAGGAAGGTAGTTTTGAGTGGTATGTGATATACC 148
Mouse	141	-tgcagttaggcagcgagtaaatgtctacattcaacctggaccataagag 18
Rat	149	TTGCAGTTAGGCAGTGAGTAAATGACAACATTCAACCTAGACCATAAGAG 19
Mouse	190	TTGGTTCTCTTTCCCTTGAAGTACGCAACAAAGCCCCCGTCACCATCAAA 23
Rat	199	LUISETTCTCTTTCCCTTGAAATATGCAACAAGACCTCCATCATCAAA 24
Mouse	240	TCCATCTGACAAGTCTGGGTCCATGATCTCTAGGACTCATCACTATGGTT 28
Rat	249	TCCATTTGACAAGACTTGCCCCATGATCTATAGGACACATCACTGTGGTT 29
Mouse	290	TTATTGGACTGTCAATACTCTTCCCAGGGGTACACATTTCACTAATCTAG 333
Rat	299	TTATCAAACTGTCAATACTCTTCCCAAGGGTGGGCATTTCATTGATCTAG 34
Mouse	340	GTTCCATAATGAATTGTCTTTGACTTTGGCAAGATAGTAGCAAGTTAGGG 38
Rat	349	GTTCCATAATGAATTGTCTTTGACTTTGACAAGATGGTAGCTAGTAGGG 39
Mouse	390	AAGAAAGCACATTTTATCCGTCCACATCCTATAGCAGGATGGCAGCAGGA 43
Rat	399	AAGAAAGCAAGTTTTATCTATCCACAGCCTACAGCAGGTTGGCAGCAGCA 44
Mouse	440	CCATTGGATGGATTCATATTGGGCTCTTGAAAAGTGTCATTCAT
Rat	449	CCACTGGATGGATTATATTGGG-TCTTGAAAA-TGGCATTCCTTCTGTT 490
Mouse	490	TGTAGGTGCAAGA 502
Rat	497	TCTL 499
	M 29	ouse chr6 : 29018426- Length : 513 bp 018928 Similarity : 424/228 (82.7 %)

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 \pm 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	Lendin	
Reverse	CTGGTGACAATGGTCTTGATGA	Leptin	
Forward	ATTCATGTGCCAGGGTGGT	- Ppia	
Reverse	GATGCCAGGACCTGTATGCT		
Forward	GACGGTCACGGAGGATAAGATC	<i>Rplp1</i> Targeted regions	
Reverse	GCAAACAAGCCAGGCCAGAAA		
	(H)MeDIP-qPCR and ChIP-qPCR primer sequences		
Forward	GACAAGACTTGCCCCATGAT	– Leptin promoter	
Reverse	ACCTGCTGTAGGCTGTGGAT		
Forward	ATCGATTCTTTGGCACGGGG	Leptin upstream enhancer	
Reverse	ACACACCTCAAAACTGCTCCA		
Forward	GTGGAGGGGTAAGAGTCGGA	Leptin downstream	
Reverse	CAGCCATGACACCAGCTACC	enhancer	
Forward	CTCCCACAGACGCTATGGTG	Control	
Reverse	GCCTGTAAATCCCACCCCAG	Control	
	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		
Reverse	Biotin - ACCCTTTACCTATAACACACC Leptin upstream		
Sequence 1	TGGTGGTTTTGGTATTAT	enhancer	
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

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