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1 **Reduced PPAR $\gamma$ 2 expression in adipose tissue of male rat offspring from obese dams is**  
2 **associated with epigenetic modifications**

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18

19 **Short title:** epigenetic modifications and PPAR $\gamma$ 2 programming

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27 **Abbreviations**

28

29 AdipoQ: adiponectin

30 ACOX1: Peroxisomal acyl-coenzyme A oxidase 1

31 CD36: fatty acid translocase

32 ChIP: Chromatin immunoprecipitation

33 C/EBP $\alpha$ : CCAAT/enhancer binding protein alpha

34 DNMT: DNA methyltransferase

35 DOHaD: Developmental Origin of Health and Disease

36 DGAT2: diglyceride acyltransferase

37 FABP4: fatty acid binding protein 4

38 FAS: fatty acid synthase

39 GC: glucocorticoid

40 GLUT4: Glucose transporter type 4

41 HAT: histone acetyltransferase

42 HDAC: histone deacetylase

43 KAT2: lysine acetyltransferase 2A

44 LPL: lipoprotein lipase

45 MBD: methyl-binding domain

46 5hmC: 5-hydroxymethylcytosine

47 5mC: 5-methylcytosine

48 MECP2: methyl CpG binding protein 2

49 P300: histone acetyltransferase P300

50 PGC1- $\alpha$ : PPAR $\gamma$  coactivator 1- $\alpha$

51 PND: postnatal days

52 PPAR: peroxisome proliferator-activated receptor

53 SIRT1: sirtuine 1

54 SREBP1: sterol regulatory element-binding protein 1

55 TET: Ten-eleven translocation methylcytosine dioxygenase

56 WAT: white adipose tissue

57 iWAT: subcutaneous inguinal

58 pWAT: visceral perirenal

59 SVF: stromal vascular fraction

60

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64

65 **Abstract**

66 According to the Developmental Origin of Health and Disease (DOHaD) concept,  
67 maternal obesity and accelerated growth in neonates program obesity later in life. White  
68 adipose tissue (WAT) has been the focus of developmental programming events, although  
69 underlying mechanisms remain elusive. In rodents, WAT development primarily occurs  
70 during lactation. We previously reported that adult rat offspring from high-fat diet-fed dams  
71 (called HF) showed fat accumulation and decreased PPAR $\gamma$  mRNA levels in WAT. We  
72 hypothesized that PPAR $\gamma$  downregulation occurs via epigenetic malprogramming which takes  
73 place during adipogenesis. We therefore examined epigenetic modifications in the PPAR $\gamma$ 1  
74 and PPAR $\gamma$ 2 promoters in perirenal (pWAT) and inguinal (iWAT) fat pads of HF offspring at  
75 weaning (postnatal days 21 (PND21) and in adulthood. PND21 is a period characterized by  
76 active epigenomic remodeling in the PPAR $\gamma$ 2 promoter (DNA hypermethylation and  
77 depletion in active histone modification H3ac and H3K4me3) in pWAT, consistent with  
78 increased DNMT and DNA methylation activities. Adult HF offspring exhibited sustained  
79 hypermethylation and histone modification H3ac of the PPAR $\gamma$ 2 promoter in both deposits,  
80 correlated with persistent decreased PPAR $\gamma$ 2 mRNA levels. Consistent with the DOHaD  
81 hypothesis, retained epigenetic marks provide a mechanistic basis for the cellular “memory”  
82 linking maternal obesity to a predisposition for later adiposity.

83

84 **Key words:** perinatal programming, Developmental Origin of Health and Disease, epigenetic  
85 mechanisms, gene expression, fat expansion.

86

87

88

89 **Introduction**

90 Epidemiological studies demonstrated that adult-onset metabolic disorders may  
91 originate from events taking place during fetal and postnatal development (1). These  
92 observations led to the Developmental Origin of Health and Disease (DOHaD) hypothesis  
93 also called “developmental programming” (1). In particular, maternal obesity and accelerated  
94 growth in neonates program obesity later in life. Numerous studies confirmed that maternal  
95 obesity is correlated with increased offspring's adiposity in rodents (2–7) and in human  
96 neonates (8). Thus, white adipose tissue (WAT) has been the focus of developmental  
97 programming events in a sex- and depot-specific manner (2). In rodents, adipose tissue  
98 development occurs mainly during lactation (9–11), although adipose tissue remains to some  
99 extent expandable throughout life (12, 13). Adipocyte progenitors are highly sensitive to  
100 maternal factors. Suboptimal nutritional and hormonal milieu during the perinatal period may  
101 affect adipogenesis, resulting in modified growth and persistent changes in the structure and  
102 function of WAT (14–16).

103 However, the mechanisms underlying developmental programming are poorly  
104 understood. Because the consequences of the events occurring in early life are maintained  
105 throughout adult life, epigenetic modifications are likely to play an important role in this  
106 process (17). Epigenetics is defined as heritable changes in gene expression that do not  
107 involve changes to the underlying DNA sequence. Epigenetics relate to chromatin  
108 modifications, including DNA methylation and histone modifications (18). DNA methylation  
109 results from the transfer by DNA methyltransferase (DNMT) of a methyl group to the  
110 cytosine residue within CpG dinucleotides to form 5-methylcytosine (5mC) and serves to  
111 establish gene silencing (19). 5mC both positively and negatively modulates binding of  
112 numerous DNA binding proteins including methyl-binding domain (MBD) proteins (MBD2,  
113 MeCP2). MBD can recruit protein and chromatin remodeling complexes known to promote

114 the formation of a condensed chromatin structure (20). 5-hydroxymethylcytosine (5hmC) is  
115 another important cytosine modification catalyzed by the enzymes of the Ten-eleven  
116 translocation methylcytosine dioxygenase (TET) family that serves as an intermediate for  
117 demethylation. It can also promote chromatin opening at transcriptional regulatory regions  
118 (21). Chromatin structure and accessibility are also controlled by histone posttranslational  
119 modifications (18). For example, acetylation of histone H3 lysine residues (H3ac) and  
120 methylation of H3K4 (H3K4me3) are associated with active transcription while methylation  
121 of H3K9 (H3K9me3) generally indicates silenced chromatin (18). These histone  
122 posttranslational modifications are catalyzed by various enzymes including histone  
123 acetyltransferase (HAT) such as KAT2 and P300 as well as histone deacetylase (HDAC) such  
124 as SIRT1. Histone-modifying enzyme activity is dependent on intermediary metabolites and  
125 hormonal responses (22). Editing of epigenetic marks during critical developmental windows  
126 in early life may serve as a memory of exposure to potential environment insults (1). The  
127 persistence of these marks throughout life may program permanent changes in gene  
128 expression (1).

129 We previously showed that adult male rat offspring from high-fat diet-fed dams (called  
130 HF) displayed higher adiposity (2), hyperleptinemia and elevated leptin gene expression in  
131 WAT (25). Persistent leptin upregulation occurs via epigenetic malprogramming during  
132 lactation in a depot-specific manner (23). We also showed that adult HF offspring exhibited a  
133 decrease in PPAR $\gamma$  gene expression in perirenal (pWAT) fat pad (2). PPAR $\gamma$  is considered the  
134 master regulator of adipose tissue promoting all processes involved in adipocyte function and  
135 WAT expansion. PPAR $\gamma$  is present in two isoforms, PPAR $\gamma$ 1 and PPAR $\gamma$ 2, generated by  
136 alternative promoter usage (24). PPAR $\gamma$ 1 is expressed at low level in many cell types  
137 including cells from stromal vascular fraction (SVF) of adipose tissue, whereas the expression  
138 of PPAR $\gamma$ 2 is mainly restricted to adipocyte (24). Although several studies reported

139 dysregulation of PPAR $\gamma$  expression in offspring from obese dams (3–5, 7, 25, 26), scarce data  
140 is available about programming mechanisms.

141 Here, we hypothesized that maternal obesity programs persistent PPAR $\gamma$   
142 downregulation in HF offspring via epigenetic mechanisms malprogramming which take  
143 places during WAT development. Thus, we examined epigenetic modifications (i.e., DNA  
144 and histone modifications) focusing on both PPAR $\gamma$  promoters as well as activity and  
145 expression of chromatin modifying enzymes in pWAT and inguinal (iWAT) fat pads of HF  
146 offspring at weaning and 9 months of age.

147

## 148 **Materials and methods**

149

### 150 *Animals*

151 Animal use accreditation by the French Ministry of Agriculture (No. 04860) has been  
152 granted to our laboratory for experimentation with rats. Experiments were conducted in  
153 accordance with the principles of laboratory animal care (European Communities Council  
154 Directive of 1986, 86/609/EEC) as previously described (2, 25). One-month-old virgin female  
155 Wistar rats were purchased from Charles River Laboratories (L'Arbresle, France) and were  
156 housed in individual cages in a humidity-controlled room with a 12:12-h light-dark cycle.  
157 Food and water were available *ad libitum*. After two weeks of acclimatization on a control (C)  
158 diet (3.85 kcal/g with 10% of total calories as fat consisting of soybean oil (5.6%) and lard  
159 (4.4%), 70% as carbohydrate and 20% as protein; D12450J, Research Diets, New Brunswick,  
160 NJ, USA), female rats were fed either a high-fat (HF) diet (5.24 kcal/g with 60% of total  
161 calories as fat consisting of soybean oil (5.6%) and lard (54.4%), 20% as carbohydrate and  
162 20% as protein; D12492B, Research Diets, New Brunswick, NJ, USA) or a C diet for 16  
163 weeks (n=30 per group). After mating with a male rat fed a C diet, 22-week-old pregnant

164 females were transferred into individual cages with free access to water and to their respective  
165 diets (C or HF diet) throughout gestation and lactation. At parturition, pups were weighed and  
166 sexed. Litter size was adjusted to 8 pups per dam (four males and four females). Body weight  
167 of pups was assessed on postnatal days 21 (PND21). Only male offspring have been used for  
168 further analysis. After weaning, male offspring from C or HF dams were housed individually  
169 with free access to water and C diet. Body weight of animals was measured weekly until 9  
170 months of age. Male offspring were sacrificed at 2 different stages: PND21 and 9 months of  
171 age (n=10 from C or HF dams). To obviate any litter effects, animals used for each  
172 experiment were randomly chosen in different litters and only a limited number of animals  
173 (n= 1 to 2) was used from each litter.

174

#### 175 *Plasma and tissue collections*

176 21-day-old male neonates were separated from their dams and 9-month-old (9M) male  
177 rats were fasted 16 hours before they were sacrificed by decapitation (between 9 and 10 a.m.).  
178 Trunk blood samples were collected into prechilled tubes containing EDTA (20 µl of a 5%  
179 solution) and centrifuged at 4000 g for 10 min at 4°C. Plasma was stored at -20°C. Fat pads  
180 were weighed, frozen in liquid nitrogen and stored at -80°C.

181

#### 182 *Quantification of endocrine parameters*

183 Plasma hormone levels were evaluated in PND21 and 9M male offspring. Blood  
184 glucose was determined using a glucometer (Glucotrend 2; Roche Diagnostics, France).  
185 Plasma corticosterone levels were determined by a competitive enzyme immunoassay  
186 (Immunodiagnostic Systems Ltd, Boldon, U.K). Plasma insulin concentrations were measured  
187 by ELISA (DRG, Internatlukaional, Inc. USA). The assay sensitivity was 0.07 ng/ml  
188 (insulin), 0.55 ng/ml (corticosterone) and the intra-and inter-assay coefficients of variation

189 were 4% and 9.1% (insulin), 4.9% and 7.8% (corticosterone), respectively. Assay kits were  
190 applied to determine the contents of plasma triglycerides (61238 Triglyceride Enzymatique  
191 PAP100).

192

### 193 ***Western blot analysis***

194 Immunoblotting analysis were carried out as described previously (23). Monoclonal  
195 mouse antibody against PPAR $\gamma$  (catalogue no. sc-7273) was purchased from Santa Cruz  
196 Biotech (Dallas, TX, USA) and was diluted 1:200. Actin antibody (catalogue no. A2228) was  
197 purchased from Sigma Aldrich (Saint Quentin Fallavier, France) and was diluted 1:1000.  
198 Anti-mouse IgG, HRP-linked Antibody (catalogue no. 7076) secondary antibodies were  
199 purchased from Cell Signalling (Danvers, MA, USA) and were diluted 1:10,000.

200

### 201 ***Pyrosequencing analysis***

202 Genomic DNA was extracted from frozen WAT using a DNA extraction kit (DNeasy  
203 blood and tissue kit, Qiagen, Courtaboeuf, France) and modified with sodium bisulfite using  
204 the EpiTect Fast Bisulfite Conversion kit (Qiagen, Courtaboeuf, France) according to the  
205 manufacturer's protocol. The percentage of cytosine methylation was determined by  
206 pyrosequencing bisulfite converted DNA using Pyro-Mark Q24 (Qiagen, Courtaboeuf,  
207 France). Pyrosequencing primers were designed to amplify PPAR $\gamma$ 1 and PPAR $\gamma$ 2 CpG sites  
208 (Table 1). PCR was performed with 20  $\mu$ l final reaction volumes with 1.5 ml of bisulfite DNA  
209 (10 ng), 10  $\mu$ l of QuantiTect EurobioGreen PCR Mix Hi-ROX (Eurobio, Les Ulis, France),  
210 7.7  $\mu$ l of H<sub>2</sub>O, and 0.4  $\mu$ l of each primer set (10 mM). Sequencing reactions used 10  $\mu$ l of  
211 PCR product and 20  $\mu$ l of 0.375 mM sequencing primer (Qiagen, Courtaboeuf, France).  
212 Pyrosequencing assays were validated with a DNA methylation scale (0%, 5%, 25%, 50%,

213 75% and 100%) (EpigenDX, Hopkinton, USA). Each assay included a bisulfite conversion  
214 check to verify full conversion of the DNA.

215

### 216 ***Chromatin immunoprecipitation***

217 ChIP assays were performed as previously described (27) with antibodies against  
218 H3K4me3, H3ac and H3K9me3 (Abcam, Cambridge, UK, ab8580, ab47915 and ab1773) and  
219 IgG (Cell Signaling Technology, Danvers, USA, #2729) as a negative control. H3ac,  
220 H3K4me3 and H3K9me3 levels were analyzed using immunoprecipitated and input DNA as  
221 control.

222

### 223 ***RNA isolation and real-time PCR***

224 PPAR $\gamma$  isoforms (PPAR $\gamma$ 1, PPAR $\gamma$ 2), PPAR $\gamma$  targets (C/EBP $\alpha$ , SREBP1, FAS,  
225 DGAT2, LPL, CD36, FABP4, GLUT4, ACOX1, AdipoQ, Resistin), enzymes of the  
226 epigenetic machinery (DNMT1, MBD2, MECP2, TET2, TET3, KAT2, P300, HDAC1,  
227 SIRT1, LSD1, SUV39) mRNA levels were determined by RT-qPCR using SyberGreen-based  
228 chemistry as previously described (2). Cyclophilin A (PpiA) and Ribosomal Protein Lateral  
229 Stalk Subunit P1 (RPLP1) were used as reference genes for RT-qPCR. Primer sequences are  
230 listed in Table 1.

231

### 232 ***Global DNA methylation and hydroxymethylation***

233 Relative levels of global DNA methylation were assessed by me-CpG ELISA using  
234 the MethylFlash<sup>TM</sup> Methylated DNA Quantification Kit (Cat # P-1034; Epigentek,  
235 Mundolsheim, France). Global DNA hydroxymethylation were measured using the Quest 5-  
236 hmC<sup>TM</sup> DNA ELISA Kit (Zymo Research, Irvine, CA, USA) according to manufacturer's

237 instructions. The amount of DNA methylation and DNA hydroxymethylation was calculated  
238 based on a standard curve generated using the kit controls.

239

#### 240 *Enzyme activity*

241 DNMT, TET, HAT and HDAC activities were studied using the EpiQuick DNMT  
242 Assay kit (Cat # P-3001-2; Epigentek, Mundolsheim, France), TET Hydroxylase Activity  
243 Quantification kit (Cat # ab156912; Abcam, Cambridge, UK), EpiQuick HAT Assay kit (Cat  
244 # P-4003-96; Epigentek, Mundolsheim, France) and EpiQuick HDAT Assay kit (Cat # P-  
245 4002-96; Epigentek, Mundolsheim, France). These assays were performed according to  
246 manufacturer's instructions.

247

#### 248 *Statistical analysis*

249 All data are expressed as means  $\pm$  standard error of the mean (SEM). Statistical analysis  
250 was carried out using GraphPad Prism6. A direct comparison between a pair of groups was  
251 made using an unpaired Student's t test. *P* values  $< 0.05$  was considered statistically  
252 significant.

253

## 254 **Results**

255

### 256 **Effects of maternal obesity on HF offspring metabolic parameters**

257 Given that metabolic signals including hormonal signaling pathways play critical roles  
258 in determining chromatin structure (28), we assessed the plasma metabolic profile in HF  
259 offspring. We previously reported that weaned and adult HF offspring exhibited  
260 hyperleptinemia (2, 23). Here, we show that serum triacylglycerol and insulin levels were  
261 elevated in HF male offspring at PND21 (Table 2). At 9 months of age, HF male offspring

262 still displayed hyperinsulinemia and had hypercorticosteronemia (2). No difference in plasma  
263 glucose concentration was observed at both stages (Table 2, (2)).

264

265 **Offspring from obese dams show persistent decreased PPAR $\gamma$  expression levels in**  
266 **adipose tissue**

267 At PND21, HF offspring showed a decrease in PPAR $\gamma$  mRNA and protein expression  
268 in pWAT and iWAT, consistent with global reduction in PPAR $\gamma$ 1 and PPAR $\gamma$ 2 expression  
269 (Fig 1A-D). At 9M, among PPAR $\gamma$ 1 and PPAR $\gamma$ 2, only the latter showed a persistent  
270 downregulation in both fat pads. The impact of maternal obesity on HF offspring's PPAR $\gamma$   
271 target genes was more pronounced in pWAT at both stages, suggesting a depot-specific  
272 programming of adipogenesis and lipogenesis (Fig 1E, F).

273

274 **Persistent lower PPAR $\gamma$  expression is associated with CpG hypermethylation in the**  
275 **PPAR $\gamma$ 2 promoter of HF offspring**

276 We first assessed whether maternal obesity affects CpG methylation status of the  
277 PPAR $\gamma$ 1 and PPAR $\gamma$ 2 proximal promoter regions (Fig 2A) in offspring. To do this, we  
278 analyzed single CpG sites by bisulfite pyrosequencing. For the PPAR $\gamma$ 1 promoter, at PND21,  
279 higher 5mC levels with hypermethylation at two out of seven CpG sites analyzed (-364/-346)  
280 were observed in pWAT of HF offspring (Fig 2B). Only hypermethylation of CpG -346  
281 persisted in HF offspring at 9 months of age (Fig 2B). No variation in DNA methylation  
282 levels were detected in iWAT (Fig 2C). For the PPAR $\gamma$ 2 promoter, higher 5mC levels were  
283 measured in both fat pads and stages in HF offspring. Two out of four CpG dinucleotides (-  
284 215/-199) were similarly affected by maternal obesity. Both CpG were hypermethylated at  
285 PND21 whereas only the CpG -199 remained hypermethylated at 9M (Fig 2D and E).

286 Consistently, CpG hypermethylation were correlated with PPAR $\gamma$  mRNA levels at PND21  
287 and 9M in both deposits.

288

### 289 **Weaned offspring from obese dams display higher DNMT activity and global DNA** 290 **methylation in pWAT**

291 We then assessed whether maternal obesity affects the epigenetic machinery activity in  
292 offspring. At PND21, DNMT activity (Fig 2F) and global DNA methylation (Fig 2G) were  
293 higher in pWAT, consistent with the CpG hypermethylation observed in the PPAR $\gamma$   
294 promoters of HF offspring. No variations in TET activity (Fig 2J) and global  
295 hydroxymethylation (Fig 2K) were observed. No changes of DNMT1, MBD2, MECP2, TET2  
296 and TET3 mRNA levels were detected (Fig S1). No gross modifications were observed in  
297 iWAT at PND21 and in both fat pads at 9M for all parameters (Fig S1).

298

### 299 **Persistent lower PPAR $\gamma$ expression is associated with depletion in H3ac/H3K4me3 in the** 300 **PPAR $\gamma$ 2 promoter of HF offspring**

301 Finally, we assessed whether maternal obesity affects histone modifications in both  
302 promoters of offspring. For the PPAR $\gamma$ 1 promoter, at PND21, ChIP-qPCR revealed less  
303 enrichment of H3ac and H3K4me3 (active marks) in both fat pads of HF offspring (Fig 3A-  
304 F). These differences were no longer observable at 9 months of age (Fig 3A-F). For the  
305 PPAR $\gamma$ 2 promoter, at PND21, a reduction of active marks H3ac and H3K4me3 and a  
306 tendency towards enrichment of inactive mark H3K9me3 were evidenced only in pWAT of  
307 HF offspring (Fig 3A-F). At 9 months of age, persistent reduction of active mark H3ac was  
308 measured in both fat pads of HF offspring (Fig 3 A-F). No changes in global histone acetyl  
309 transferase and histone deacetylase activities were measured (Fig G and J). No variations of  
310 KAT2, P300, HDAC1, SIRT1, LSD1 and SUV39 mRNA levels were evidenced (Fig S1).

311 **Discussion**

312 Here, we show, for the first time, that persistent decreased mRNA PPAR $\gamma$ 2 levels in  
313 adult HF offspring's WAT may occur via epigenomic remodeling in the PPAR $\gamma$ 2 promoter  
314 which take place during lactation.

315 We extended our previous work (2) reporting that obesity-prone 9-month-old HF  
316 offspring had lower PPAR $\gamma$ 2 mRNA expression levels in both pWAT and iWAT fat pads.  
317 Given that PPAR $\gamma$ 2 promotes adipocyte differentiation and triglyceride storage in WAT (24),  
318 it may appear paradoxical that maternal obesity reduces PPAR $\gamma$ 2 expression in WAT of HF  
319 offspring. However, others have also shown that obesity is associated with a decline in the  
320 PPAR $\gamma$ 2 activity and expression (29–32). These modifications appear to be strongly  
321 associated with the pathogenesis of metabolic syndrome (30–32). The decrease in PPAR $\gamma$ 2  
322 gene expression might be seen as an adaptive mechanism to prevent further fat accumulation  
323 in WAT (33). It may, in turn, durably change the expression of PPAR $\gamma$  target genes.  
324 Consistent with this hypothesis, recent findings have demonstrated that PPAR $\gamma$   
325 downregulation in obese mice is associated with a decrease in genome-wide association  
326 occupancy of PPAR $\gamma$  (31).

327 We previously reported that HF neonates exhibited a rapid weight gain during lactation.  
328 At PND21, HF male offspring displayed higher iWAT and pWAT deposit weights with  
329 increased leptin mRNA levels, marked adipocyte hypertrophy and hyperplasia. HF 9-month-  
330 old male rats showed persistent increased pWAT mass with elevated leptin mRNA levels  
331 whereas this "expandable" phenotype was no longer observed in iWAT (2, 23). By contrast,  
332 both fat pads had persistent lower PPAR $\gamma$  expression. These varying outcomes may reflect  
333 difference in postnatal and depot-specific programming. Depot-specific adipogenesis is  
334 regulated by the anatomic location, the unique signature of developmental gene expression  
335 and the hormonal microenvironment of WAT (35). PPAR $\gamma$  governs biological processes by

336 modifying the expression of a large number of target genes. As already reported (2, 25), gene  
337 expression profiling revealed differences in adipogenic and lipogenic activities between  
338 iWAT and pWAT of adult HF offspring, suggesting a depot-specific programming. It may  
339 appear paradoxical that persistent reduced PPAR $\gamma$ 2 expression was correlated with an  
340 increase in expression of genes associated with fat accumulation (i.e., FAS, DGAT2 and LPL)  
341 in pWAT. One possible explanation is that regulation of lipogenic genes in adult HF  
342 offspring's WAT may occur, at least in part, via additional PPAR $\gamma$ -independent signaling  
343 pathways. Consistent with this hypothesis, HF adult male offspring exhibited  
344 hyperinsulinemia with elevated SREBP-1c mRNA levels within pWAT (2) that might be due  
345 for activation of insulin signaling. Indeed, offspring from obese dams showed greater AKT  
346 phosphorylation in response to insulin challenge associated with up-regulation of lipogenic  
347 pathways in WAT (5).

348 The underlying molecular mechanisms regulating PPAR $\gamma$  gene expression remain  
349 unclear. However, decreased PPAR $\gamma$  expression via modifications of epigenetic mechanisms  
350 may play a pivotal role in the development of obesity and diabetes (29, 30, 32, 35–37). Few  
351 studies have shown that DNA methylation and histone modifications regulate the expression  
352 of PPAR $\gamma$  in WAT (32, 35, 36, 38–40). First, during adipogenesis, DNA demethylation (33)  
353 as well as enrichment of active marks H3ac (41) and H3K4me3 (42, 43) were observed in the  
354 PPAR $\gamma$  promoter region that positively regulates gene expression. Second, luciferase reporter  
355 assays demonstrated that PPAR $\gamma$  promoter methylation may repress reporter gene expression  
356 (33). Third, hypermethylation of the PPAR $\gamma$ 2 promoter, which was negatively associated with  
357 PPAR $\gamma$ 2 expression, was evidenced in visceral WAT of obese animals (32, 36, 38, 40).  
358 However, to our knowledge, no data are available regarding PPAR $\gamma$  programming  
359 mechanisms in offspring from obese dams.

360 We showed that PND21 is a period characterized by active epigenomic remodeling (i.e.,  
361 higher DNA methylation and lower active histone modification H3ac and H3K4me3) of the  
362 PPAR $\gamma$ 2 promoter in pWAT, consistent with decreased PPAR $\gamma$ 2 mRNA expression levels.  
363 Higher DNMT activity parallels the increase in global DNA methylation and CpGs  
364 methylation in the PPAR $\gamma$ 2 promoter. During lactation, adipocyte stem cells are plastic and  
365 highly sensitive to maternal factors (44). Thus, maternal obesity and modified milk  
366 composition may influence offspring's nutritional and hormonal status (45, 46). Indeed,  
367 epigenetic enzyme activities are particularly sensitive to intracellular fluctuation in energy as  
368 well as metabolic intermediates (22). In line with this notion, weaned HF offspring exhibited  
369 higher plasma leptin levels (2) as well as insulin and triglycerides concentrations. Consistent  
370 with epigenomic remodeling, global enzyme activity modifications were observed at PND21.  
371 However, the increase in DNMT and DNA methylation activities were restricted to pWAT in  
372 weaned HF offspring. It may rely on spatiotemporal developmental differences that occur  
373 between depots. The DNMT activity is maximal during adipogenesis (47). In rodents, iWAT  
374 depots begin to develop during embryogenesis and the progenitor compartment is established  
375 before the first few days of life whereas pWAT depots mainly develop postnatally during  
376 lactation which coincides with the period of maximum adipogenesis (11, 48). As already  
377 suggested (2, 25), offspring's pWAT seems to be much more sensitive to maternal obesity.

378 Some of these epigenetic modifications were still observable in the PPAR $\gamma$ 2 promoter in  
379 adult HF offspring. Sustained epigenetic modifications (i.e., higher DNA methylation and  
380 lower active histone modification H3ac) were observed in both fat pads of adult HF offspring,  
381 consistent with persistent decreased PPAR $\gamma$  gene expression. 5mC is the only known  
382 modification that targets the DNA itself. In most cases, the extent of 5mC of a promoter is  
383 inversely correlated with the activity of the respective gene (19). We also showed that the  
384 global 5mC level of the PPAR $\gamma$ 2 promoter in adult HF offspring was lower than weaned HF

385 pups, suggesting that a demethylation process occurs during development of WAT. The  
386 enrichment of H3ac is characteristic of promoter activation (43). A limitation of our work lies  
387 in the fact that DNA analysis was performed on entire tissue without distinguishing adipocyte  
388 and SVF fractions. Extracts may contain different cell type depending on the stage of  
389 development, tissue expansion and physiological states. However, gene expression profiling  
390 using several cell markers suggests that the composition of HF offspring's WAT is not  
391 modified regardless of the stage of development.

392 Our results provide compelling evidence that downregulation of PPAR $\gamma$  expression may  
393 have developmental origins through epigenetic mechanisms. Thus, editing of early-life  
394 epigenetic markings that occurs during development of WAT may persist throughout life.  
395 Consistent with the DOHaD hypothesis, retained marks may provide a mechanistic basis for  
396 the cellular "memory" linking maternal obesity to a predisposition for later adiposity.

397

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399 data and contributed to discussion and manuscript. C.P., L.B., L.M. performed the  
400 experiments. C.L., C.G., J.L., D.V. contributed to discussion.

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575

576 **Table 1**

577 Primers used for RT-qPCR and pyrosequencing. Primer sequences are indicated (see text for  
578 definitions).

579

RT-qPCR primer sequences		Targeted mRNA	Pyrosequencing primer sequences		Targeted regions	
Forward	TTGGCCATATTTATAGCTGTCATTATT	<i>PPAR<math>\gamma</math></i>	Forward	ATGTTGGTTAAATGAGTTTATTTAGTG	<i>PPAR<math>\gamma</math>2 promoter #1</i>	
Reverse	TGCTCTGGATGGGCTTCA		Reverse	<i>Biotin</i> -ACACTATCCTAATTAACCAACTA		
Forward	GCACTGCCTATGAGCATTTC		Sequence	ATTGAATGTGTGGG		
Reverse	GTGGCCTGTTGTAGAGTTGG	<i>PPAR<math>\gamma</math>1</i>	Forward	TTGTTTAGTTTTGTGGAATGTGGAGAGTAG	<i>PPAR<math>\gamma</math>2 promoter #2</i>	
Forward	GCTGCAGCGCTAAATTCATC		Reverse	<i>Biotin</i> -ACTATCTCCCAATAACCCACACATTCA		
Reverse	GCTGATTCCGAAGTTGGTGG		Sequence	TGTTGAATA-AATTATTTTAGAAGT		
Forward	CCAATCACGCAATAGTTCTGG	<i>Acox</i>	Forward	GGGGGATTATTGTTATGTGATATGTT	<i>PPAR<math>\gamma</math>2 promoter #3</i>	
Reverse	CGCTGTATCGTATGGCGAT		Reverse	<i>Biotin</i> -ATAAAAAAATTAACCCCTCCCTAAAAAT		
Forward	CAGGAGATGCTGGAATGACA		Sequence	ATTAGGTTATTTATGTGATAAGG		
Reverse	CTACGCTGAATGCTGAGTGAT	<i>AdipoQ</i>	Forward	AACTATCACCAATCCACACAAT	<i>PPAR<math>\gamma</math>1 promoter #1</i>	
Forward	TGCATGAATTAGTTGAACCGCCA		Reverse	<i>Biotin</i> -CCCAAAACAATCTAACCAACCATAA		
Reverse	CCACAGTTCCGATCGCAGCCC		Sequence 1	GGTTTTTTTAGAAGGTGT		
Forward	AGTTGACCACTGACAATGACCG	<i>C/EBP<math>\alpha</math></i>	Sequence 2	GTGAGAGTTAGGAGTAGGGTA		
Reverse	TCAGGCAGCTGGCGGAAG		Sequence 3	TTTGATAGGAAAGTTAGGT		
Forward	AGGCCTTGATGGTTTCTATCCA		<b>ChIP-qPCR primer sequences</b>			<b>Targeted regions</b>
Reverse	GCTGCCCTTCCCAATTAAC	<i>DGAT2</i>	Forward	ACAGTTTCAGAGAGGGCT	<i>PPAR<math>\gamma</math>1 promoter</i>	
Forward	TAGTTCGGTGCTACGAGGA		Reverse	CGACAGTGACCCCATTTTCC		
Reverse	CGTTTAGCGGGACCCCTGAA		<i>DNMT1</i>	Forward		ACAGTTCACACCCTCACAA
Forward	GACCTGGAAGCTCGTCTCCA	Reverse		TGGCACTGTCTGATTGAGA		
Reverse	CATACCGGCCACTTTCCTGG	<i>FABP4</i>		Forward	AGAGCTGAAAGAGGGCAAGA	Control A
Forward	GGACATGGTCACAGACGATGAC		Reverse	GTCTCTCCTGCCCTCAAAC		
Reverse	GTCGAACTTGGACAGATCCTTCA		<i>FAS</i>	Forward	CAGGCTGTTGAGTGGGTAGA	
Forward	AGGCACCCTACTACCCITT	Reverse		GGGCCACTGTTGCCATTTTA		
Reverse	ATAGCCCTTTTCCCTCCCAA	<i>GLUT4</i>				
Forward	CCAGAAAGCCAAAGGGTCAA		<i>HDAC1</i>			
Reverse	TGCGCTGGTCCCTATCTAGT					
Forward	ACTCCATCTTCAGTCCTTC	<i>KAT2A</i>				
Reverse	CTTCTCCTCTCTCTGGC					
Forward	AAGGTCATCTTCTGTGCCA		<i>LPL</i>			
Reverse	CAGCCGACTTCTCAGAGA					
Forward	GGCAAGAGCGATGTCTACTA	<i>MBD2</i>				
Reverse	CTGGACCGACTCCTTGAAGA					
Forward	CAGCTCCAACAGGATCCATGGT		<i>MeCP2</i>			
Reverse	TGATGTCTCTGCTTTGCCTGCCT					
Forward	AGATTACAGAGGCAGCAGAGAC	<i>P300</i>				
Reverse	GCCATAGGAGGTGGGTTCATAC					
Forward	AGACTTCAGCTCCCTACTG		<i>Resistin</i>			
Reverse	CTAGTGACGGTTGTGCCTT					
Forward	CTGTTTCTGTGGGATACCTGACT	<i>Sirt1</i>				
Reverse	ATCGAATGGCTTGAGGATC T					
Forward	CATCGACTACATCCGTTCTTACA		<i>SREBP1</i>			
Reverse	GTCTTTTCACTGATTTGCTTTTGTGA					
Forward	AATCGCCTTCGGATTGAGACTC	<i>TET2</i>				
Reverse	CTTGACCTCCGATACACCCAT T					
Forward	TCAGCAACACCTTCATCACAA		<i>TET3</i>			
Reverse	TTTTCTTGGGTGGTTTGTCA					
Forward	ATTCATGTCCAGGGTGGT	<i>Ppia</i>				
Reverse	GATGCCAGGACTGTATGCT					
Forward	GACGGTCACGGAGGATAAGATC		<i>Rplp1</i>			
Reverse	GCAAACAAGCCAGGCCAGAAA					

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585 **Table 2**

586 Plasma parameters of weaned male offspring from obese dams fed either a control (C) or a  
 587 high-fat (HF) diet

588

Variable	Male Offspring PND21		
	C	HF	<i>Maternal diet effect p value</i>
Glucose (dg/ml)	168.22 ± 5.49	171.14 ± 2.54	ns
Triacylglycerol(mg/dl)	210.9 ± 11.28	281.9 ± 13.10	*
Insulin (μU/ml)	34.35 ± 5.94	60.99 ± 5.49	*
Corticosterone (ng/ml)	221.21 ± 16.87	216.63 ± 20.84	ns

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591 All data are presented as means +/- SEM. Data were analyzed using Student's *t* test. Effects of  
 592 maternal obesity at PND21 (\*P < 0.05). n = 20 per group.

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603 **Figure legends**

604

605 **Figure 1: Maternal obesity induces persistent lower PPAR $\gamma$  expression in adipose tissue**  
606 **of offspring**

607 PPAR $\gamma$  expression was assessed in male offspring from dams fed a control diet (called  
608 C) or high-fat diet-fed dams (called HF) on postnatal days 21 (PND21) and 9 months of age  
609 (9M). PPAR $\gamma$ , PPAR $\gamma$ 1 and PPAR $\gamma$ 2 mRNA levels were determined in pWAT (**A**) and iWAT  
610 (**B**) by RT-qPCR. Representative Western blot of PPAR $\gamma$  (55 KDa) and  $\beta$ -Actin (43 KDa)  
611 were indicated in pWAT (**C**) and iWAT (**D**). mRNA levels of PPAR $\gamma$  target genes were  
612 determined in pWAT (**E**) and iWAT (**F**) by RT-qPCR. Relative gene expression in the C  
613 group at PND21 was set to 1. Ppia and Rplp1 were used as standard genes. All data are  
614 presented as means  $\pm$  SEM. Data were analyzed using Student's *t* test. Effects of maternal  
615 obesity at PND21 (\**P* < 0.05) and at 9M (#*P* < 0.05). n = 5-10 per group.

616

617 **Figure 2: Persistent lower PPAR $\gamma$  expression is associated with CpG hypermethylation**  
618 **in the PPAR $\gamma$ 2 promoter of HF offspring**

619 Epigenetic modifications were assessed in male C and HF offspring in two fat pads  
620 (pWAT and iWAT) at PND21 and 9M. Schematic positions and sequences of rat PPAR $\gamma$ 1 and  
621 PPAR $\gamma$ 2 promoters were shown (**A**). CpG methylation in PPAR $\gamma$ 1 promoter (**B**, **C**) and  
622 PPAR $\gamma$ 2 promoter (**D**, **E**) were assessed by bisulfite pyrosequencing in pWAT (**B**, **D**) and  
623 iWAT (**C**, **E**). DNMT activity from nuclear protein (**F**) and global DNA methylation (**G**)  
624 were assessed in pWAT and iWAT. TET activity from nuclear protein (**H**) and global DNA  
625 hydroxymethylation (**I**) were assessed in pWAT and iWAT. All data are presented as means

626 +/- SEM. Data were analyzed using Student's *t* test. Effects of maternal obesity at PND21 (\*P  
627 < 0.05) and at 9M (#< 0.05). n = 5 per group.

628

629 **Figure 3: Persistent lower PPAR $\gamma$  expression is associated with depletion in**  
630 **H3ac/H3K4me3 in the PPAR $\gamma$ 2 promoter of HF offspring**

631 Epigenetic modifications were assessed in male C and HF offspring in two fat pads  
632 (pWAT and iWAT) at PND21 and 9M. Histone modifications were measured by ChIP assays  
633 with antibodies against H3ac (**A, B**), H3K4me3 (active mark) (**C, D**) and H3K9me3 (inactive  
634 mark) (**E, F**) in pWAT (**A, C, E**) and iWAT (**B, D, F**). HAT (**G**) and HDAC (**H**) activities  
635 were assessed from nuclear protein of pWAT and iWAT. All data are presented as means +/-  
636 SEM. Data were analyzed using Student's *t* test. Effects of maternal obesity at PND21 (\*P <  
637 0.05) and at 9M (#P < 0.05). n = 5 per group.

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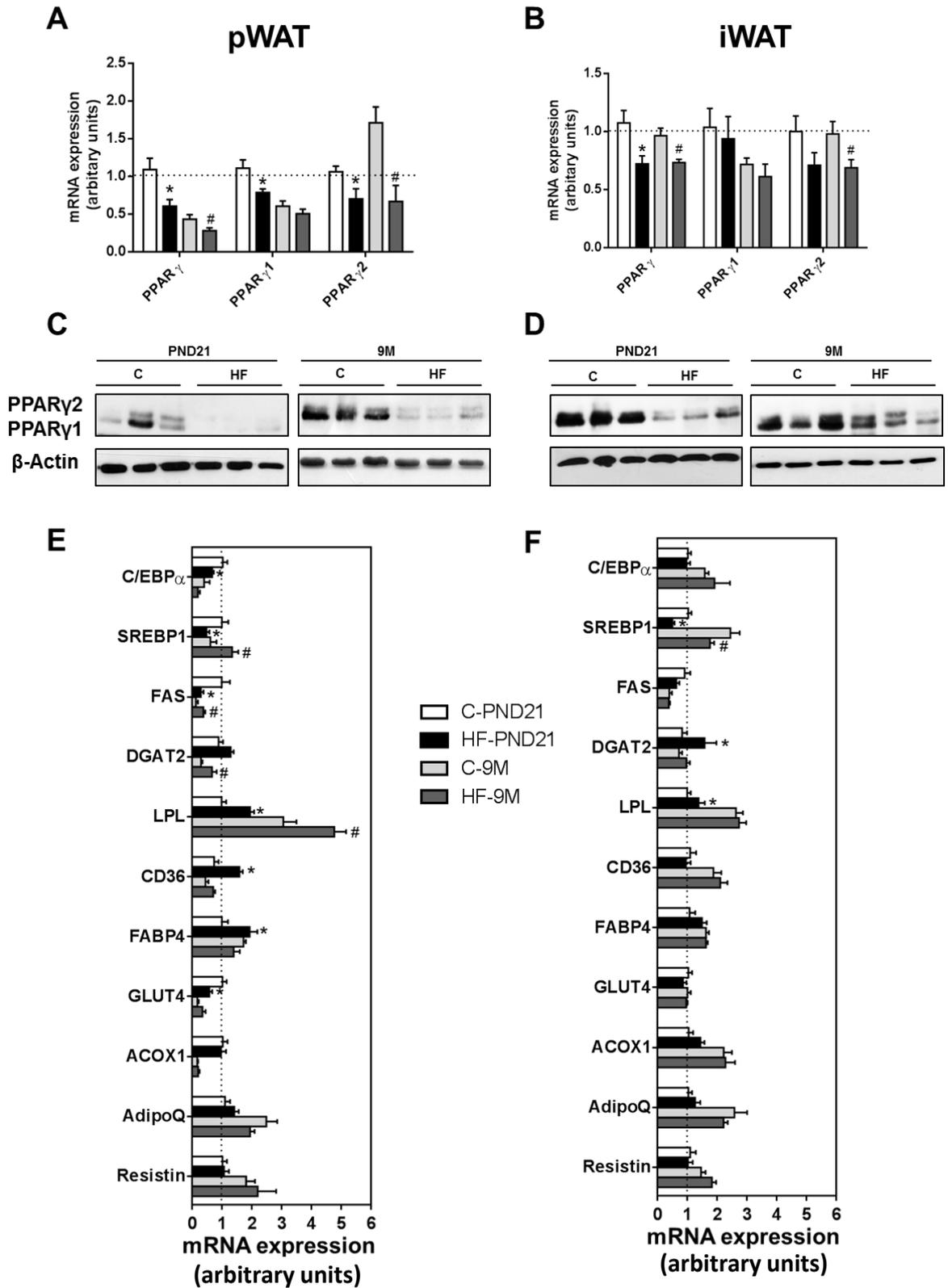
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**Figure 1**

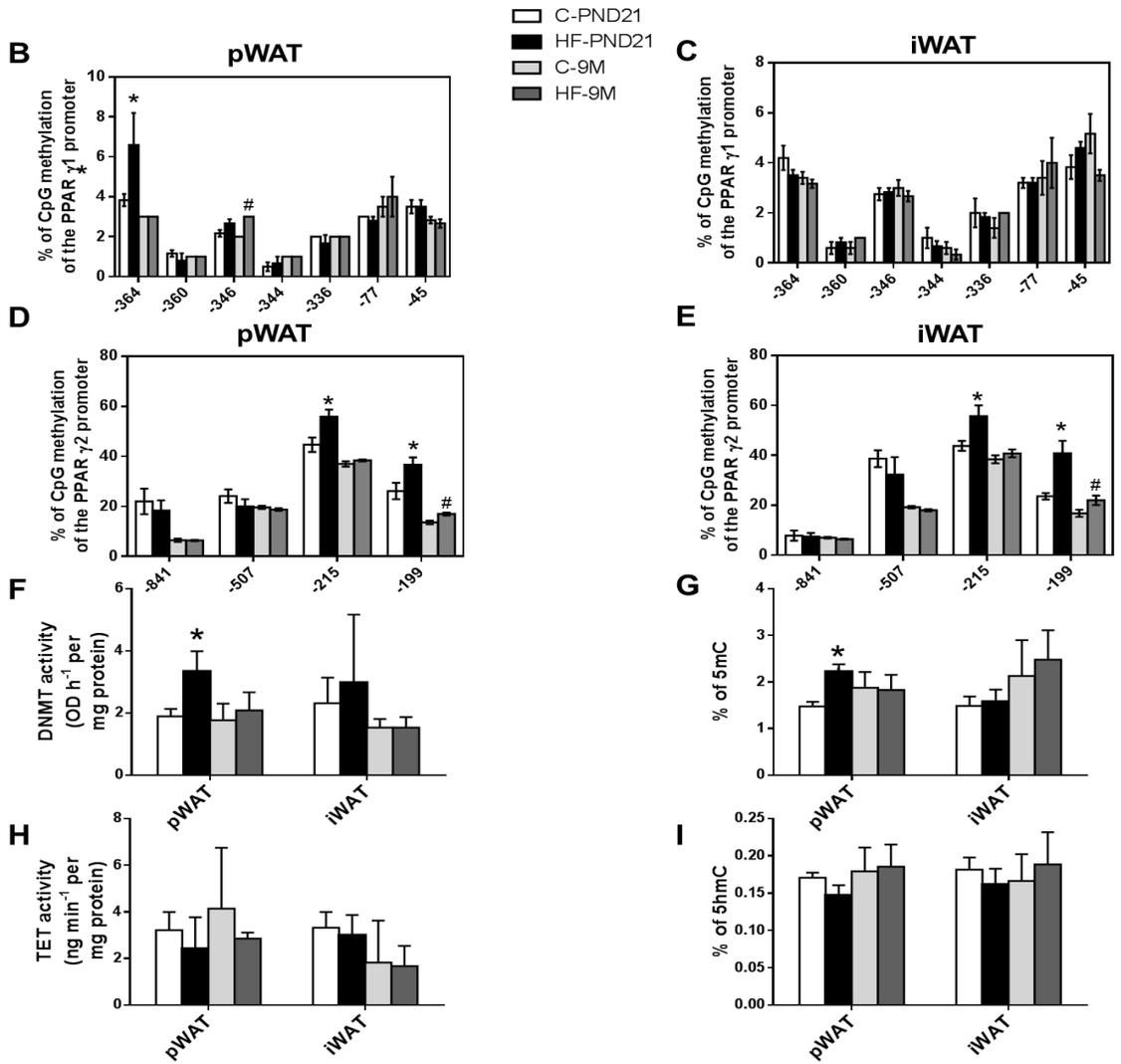
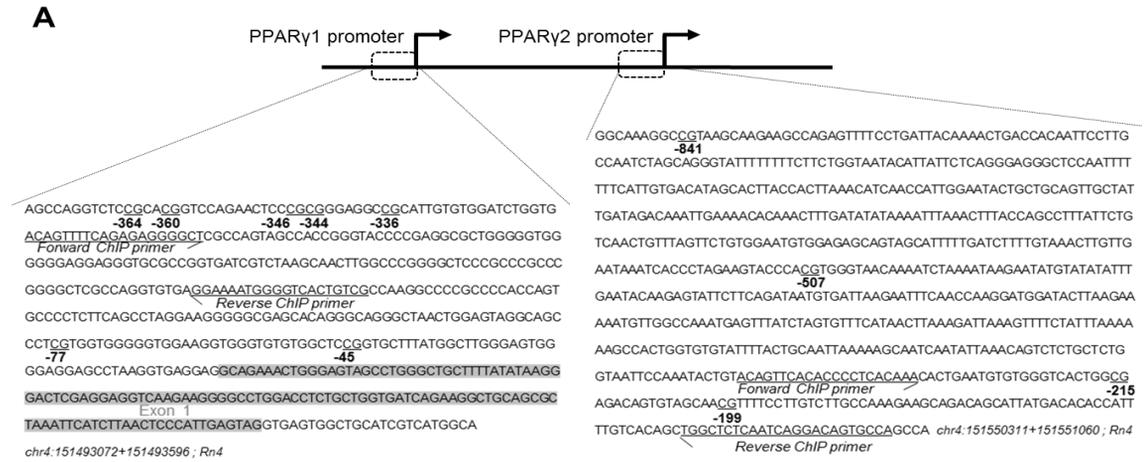


Figure 2

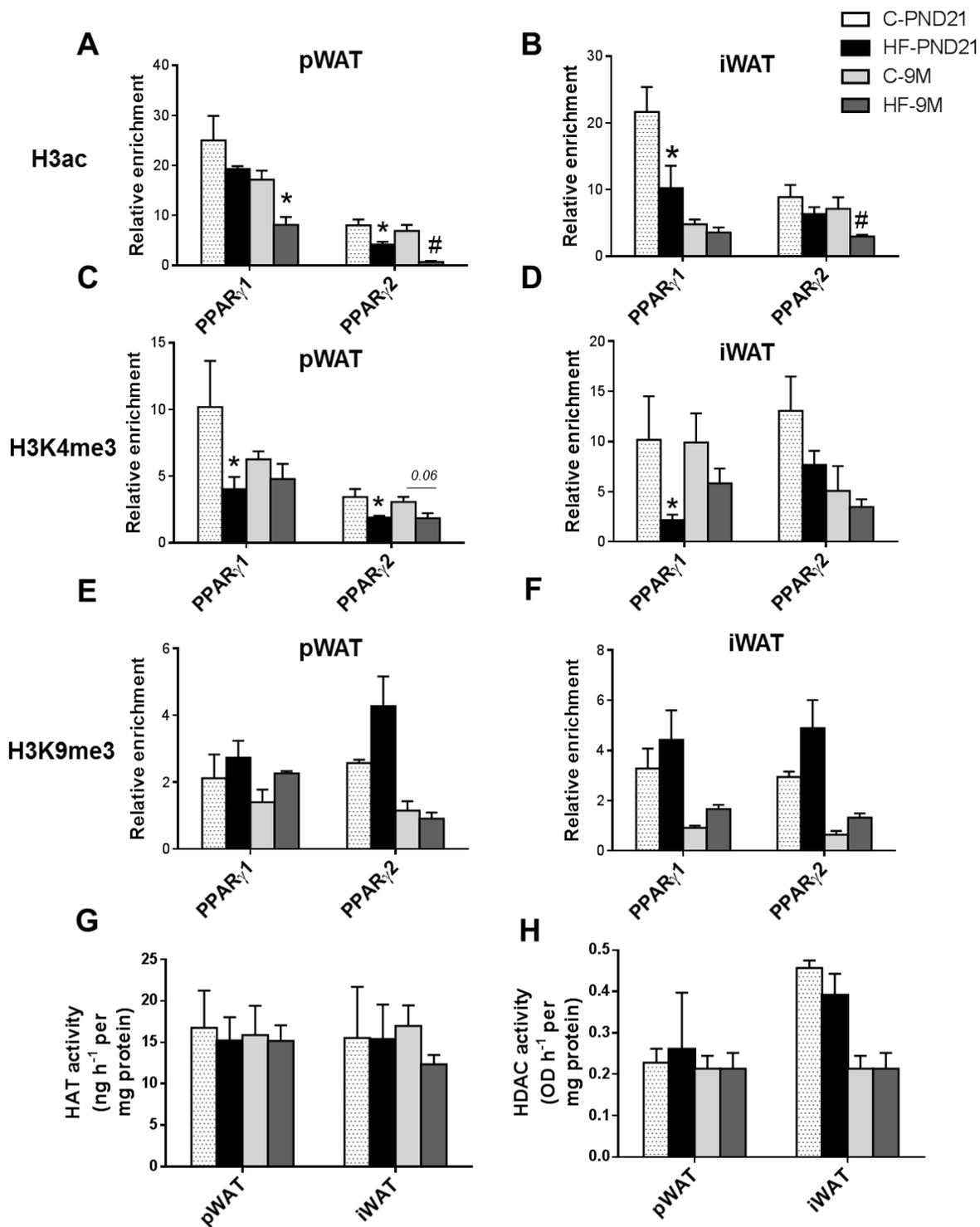


Figure 3