SUPPLEMENTARY INFORMATION

Supplementary Materials and Methods

Supplementary References

Supplementary Figures

Supplementary Tables
Supplementary Materials and Methods

Plasmids and reagents

Human STAT3 was constructed by inserting the STAT3 (ID: 6774) sequence into the pCDNA4 vector. The human DNMT1 promoter fragment (−1048/+36) containing two STAT3 binding sites was subcloned into the NheI and HindIII sites of the pGL3-basic vector (Promega). The correct sequences of STAT3 and pGL3-DNMT1 were confirmed by sequencing. pCMV-Myc-DNMT1 expressing the Myc-tagged full-length DNMT1 was used. STAT3 or NFκB siRNAs and respective negative controls (Supplementary Table 3) were synthesized by Integrated DNA Technologies. The shRNAs against human/mouse FABP4, IL-6 and scrambled constructs (pLKO.1 or pGIPZ) were purchased from BMGC RNAi (University of Minnesota). On-target\textsuperscript{plus} Smart pool siRNAs containing a mixture of 4 oligonucleotides against human/mouse DNMT1, Fabp4 and their scrambled oligos were obtained from Thermo Scientific. The NSC74859 was obtained from SelleckBio, the mouse/human FABP4 proteins and Bay-11-7082 from Cayman Chemical, the mouse/human IL-6 protein from R&D systems, and the Decitabine from LC Laboratories.

Cell culture and transfection

The MV4-11, Kasumi-1, K562, THP-1, 293T, U937 and C1498 cell lines were obtained from American Type Culture Collection, but the NB4 cell line was from Deutsche Sammlung von Mikroorganismen und Zellkulturen. The SKNO-1 cells were kindly provided by Dr. Clara Nervi (University of Rome, Italy). 293T and C1498 cells were grown in DMEM, and the others, including mouse Fabp\textsuperscript{+/+} and Fabp4\textsuperscript{-/-} macrophages, were grown in RPMI1640, supplemented with 5% (macrophages), 20% (Kasumi-1, SKNO-1) or 10% (others) FBS. Cell lines were newly purchased with no further authentication and no further testing for mycoplasma. Cells (1×10\textsuperscript{6})
were seeded into 6-well plates overnight before transfection. The siRNA oligos (100 nM), expression or shRNA vectors (2 µg) and respective controls were introduced into cells using Lipofectamine™ RNAiMAX or Lipofectamine™ 2000 (Life Technologies).\textsuperscript{1,2} Three siRNAs for \textit{STAT3} and \textit{NFκB} (p65), and three shRNAs for \textit{FABP4} and \textit{IL-6}, were tested, and the one achieving the most reduction was utilized for further investigations.

**Cytospin/Wright-Giemsa staining**

At the designated time points, AML cells (0.1×10$^6$) were harvested and placed in Shandon EZ Single Cytofunnel (Thermo Electron Corporation). Samples were centrifuged at 1,000 rpm for 8 min. The slides were air-dried and stained using Hema-3 Kit (Fisher Scientific).\textsuperscript{1} Stained slides were viewed and photographed using a Leica microscope mounted with a high-resolution spot camera with Image-Pro Plus software. Morphologic differentiation was determined by calculating the percentage of post-mitotic cells containing metamyelocytes, bands and segmented neutrophils within six visual fields per slide.

**Hematoxylin and eosin (H&E) and immunohistochemistry (IHC) staining**

H&E and IHC staining were performed as previously described.\textsuperscript{2,3} Briefly, tissues collected from the animal studies were fixed in 10% paraformaldehyde/PBS. The paraffin-embedded samples were stained with H&E (Sigma) or the Ki-67 antibody (Abcam, ab15580). Stained slides were viewed and photographed with a Leica microscope mounted with a high-resolution spot camera and Image-Pro Plus software.

**Colony-forming and MTS assays**
Colony-forming assays were performed in MethoCult® mixture (Stem Cell Technologies) and MTS assays in CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega) as previously described. Colonies were scored at 7–10 days.

**DNA Dotblotting**

Genomic DNA was purified using the DNA Blood/Tissue Kit (Qiagen). About 2 μg DNA were denatured and subjected to Dotblotting using anti-5mC (39649, Active Motif). The DNA spotted membrane was stained with 0.02% methylene blue (Sigma) in 0.5 M sodium acetate (pH 5.0) for DNA loading control. Different amounts of C or 5mC (Zimo Research) were used as a standard.

**Bisulfite sequencing**

About 2 μg of DNA was converted and purified using the EpiTect Bisulfite Kit (Qiagen). The region (−4 to +247) within the $p15^{\text{INK4B}}$ promoter was PCR-amplified from bisulfite-treated DNA. The primers are listed in Supplementary Table 3. The PCR products were subcloned using the TA Cloning® Kit (Invitrogen) and sequenced in Genewiz.

**Cell differentiation assays**

Approximately 5×10⁵ treated AML cells were washed once in 1 ml staining buffer (1× PBS with 1% FBS) for 5 min and incubated in 100 μl staining buffer with CD11b antibody (BD Biosciences PharMingen, Clone M1/70, 553310) for 15 min. The stained cells were washed twice, resuspended in 300 μl staining buffer and analyzed in a Calibur flow cytometer. Data were analyzed using Flowjo (Tristar).
Immunosorbent analysis (ELISA)

The serum FABP4 levels were assessed using CircuLex Fabp4/A-Fabp ELISA Kit (MBLI) for mouse and FABP4 EIA Kit (Cayman Chemical) for human as previously described.  

Reporter assays

293T cells at 40–50% confluence were transfected with pGL3-\textit{DNMT1} alone plus \textit{STAT3} expression vectors or treated with \textit{STAT3} inhibitor NSC74859. The dual luciferase reporter assays were carried out using the Dual-Luciferase® Reporter Assay System (Promega) as previously described.  

Western blotting

After the various treatments, whole cellular lysates were prepared by harvesting the cells in 1× cell lysis buffer and Western blotting was performed as previously described.  

RNA isolation, cDNA preparation and qPCR
RNA was isolated using the miRNeasy Kit (Qiagen) and reverse transcription for cDNA was performed using the SuperScript® III First-Strand Synthesis System (Invitrogen). TaqMan qPCR was performed to measure \( \text{DNMT1} \) expression, but SYBR-Green qPCR was used for other genes. The primers are listed in Supplementary Table 3. The 18S levels were used for normalization and target expression was analyzed using the \( \Delta\text{CT} \) approach.

**Animal studies**

Mice were monitored daily for signs of deteriorating health as indicated by weight loss, slow movement or hunched posture etc.. All mice had free access to food and water throughout the study. Syngeneic mouse experiments were conducted in \( \text{Fabp4}^{-/-} \) and \( \text{Fabp4}^{+/+} \) (male, C57BL/6 background) littermates. For survival studies, mice were sacrificed when they showed any signs of distress (e.g., breathing disorders, weight loss or immobility). Spleen weight and number of metastatic nodules were determined at the end of each experiment. For the leukemia-bearing obese model, C57BL/6 mice (male, 5-7 weeks) were fed a high-fat diet (HFD, D12492, 60 Kcal% fat, Research Diets) and the control group (age-matched mice) was fed a standard diet (LFD, D12450B, 10 Kcal% fat, Research Diets) for 12 weeks. When the mice became obese, C1498 cells were injected into the mice through the tail-vein. For siRNA knockdown, C1498 cells were transfected with mouse \( \text{Fabp4} \) or scrambled siRNA, and at 6 hours after transfection when the transfected cells were not apoptotic, the transfected cells were injected into C57BL/6 mice (male, 4–6 weeks) through the tail-vein; The AML1/ETO9a (AE9a) mouse model:\(^5\) (t(8;21), resulting in a chimeric protein AML1/ETO (AE), is one of the most common chromosomal abnormalities associated with about 40% of M2 AML cases. AE9a is a C-terminal truncated form of AE and highly leukemogenic in mice. To establish the AE9a AML model, C57BL/6 mice (male, 4–6
weeks) were engrafted with AE9a primary cells, and monitored for signs of disease. All mice developed leukemia within 8 to 10 weeks of transplantation. Then BM cells were isolated for further investigations. For WBC counting, 2 μl of blood from the mouse tail-vein were mixed with 38 μl of Turk blood dilution fluid (Ricca Chemical) and the WBCs were counted under a microscope.

**AML patient samples**

The bone marrow diagnosis of AML was performed according to the criteria of World Health Organization. The primary blasts from AML patients (n=8) with >80% blasts were obtained from the Tumor Tissue/Biospecimen Bank of Mayo Clinic and cultured as previously described for molecular and pathological assays. The human study was approved by the Mayo Clinic Institutional Review Board (IRB14-005505). All patients signed an informed consent document approved by the Institutional Review Board before entering the study.

**GEO (Gene Expression Omnibus) analysis**

AML GEO datasets [GSE12417, platform GPL570 (n=79) and GPL96 (n=163), n=242 in total; GSE16432, n=222] were analyzed for the mRNA expression of *FABP4, IL-6* and *DNMT1*, which was assessed by gene-expression arrays. The patients reported in these GEO datasets are AML and cytogenetically normal. The detailed clinical characteristics of the patients were referred to the original reports. These samples were normalized, managed and analyzed by GraphPad Prism 5 Software using Spearman correlation coefficients. For the survival assays, the expression of *FABP4*, *IL-6* and *DNMT1* was defined as high if mRNA level is ≥75th percentile of mean expression in the cohort and defined as low if <75th percentile of mean expression in the cohort.
Because the public database only provided limited information for each patient, including WBC, cytogenetics, and mutation status, we were unable to address whether FABP4 expression was associated with these biological changes. Although the patients in GSE12417 had FLT3 ITD mutations (~41%) and NPM1 (~53%), we were unable to identify which patients were carrying such mutations; For GSE16432, the cohort reported very few mutations (~1%) that were not considered when performing the survival assays. Thus, the survival analysis was not corrected by mutations.

**Statistical analysis**

All graphs were generated using the Student’s t test, and the Kaplan-Meier survival curves were created by the log-rank test. Correlation data were acquired using the Pearson correlation coefficients. The sample sizes for each study were chosen to be sufficient to allow statistical analysis of the outcomes of the experimental versus control of the studies based on literature documentation of similar well-characterized experiments. In *vitro* experiments, such as qPCR, cell proliferation assays, dotblotting, and clonogenic assays, were routinely repeated three times unless indicated otherwise in figure legends or main text. The statistical analysis was conducted using the Student’s t test. All analyses were conducted using the GraphPad Prism 5 Software. *P*<0.05 was considered statistically significant. All *P* values were two-tailed. No samples or animals were excluded from the analysis. All criteria were pre-established. No randomization was used in our studies. No blinding for all experiments. Variations were compatible between groups. The statistical tests were justified as appropriate for every figure.
Supplementary References

1. Gao XN, Yan F, Lin J, et al. AML1/ETO cooperates with HIF1alpha to promote leukemogenesis through DNMT3a transactivation. *Leukemia*. 2015.

2. Shen N, Yan F, Pang J, et al. A nucleolin-DNMT1 regulatory axis in acute myeloid leukemogenesis. *Oncotarget*. 2014;5(14):5494-5509.

3. Yan F, Shen N, Pang J, et al. Restoration of miR-101 suppresses lung tumorigenesis through inhibition of DNMT3a-dependent DNA methylation. *Cell Death Dis*. 2014;5:e1413.

4. Zhang Y, Sun Y, Rao E, et al. Fatty acid binding protein E-FABP restricts tumor growth by promoting IFNbeta responses in tumor-associated macrophages. *Cancer Res*. 2014.

5. Yan M, Kanbe E, Peterson LF, et al. A previously unidentified alternatively spliced isoform of t(8;21) transcript promotes leukemogenesis. *Nat Med*. 2006;12(8):945-949.

6. Metzeler KH, Hummel M, Bloomfield CD, et al. An 86-probe-set gene-expression signature predicts survival in cytogenetically normal acute myeloid leukemia. *Blood*. 2008;112(10):4193-4201.

7. Kharas MG, Lengner CJ, Al-Shahrour F, et al. Musashi-2 regulates normal hematopoiesis and promotes aggressive myeloid leukemia. *Nat Med*. 2010;16(8):903-908.
Supplementary Figures

Supplementary Figure 1. Obesity is associated with aggressive AML leukemia. (a) C57BL/6 mice were fed a high-fat diet (obese) or a low-fat diet (lean) for 12 weeks. When the mice became obese, C1498 cells (2×10⁶) were injected into the mice through the tail-vein. After 4 weeks, bone marrow (BM), liver, lung and spleen from leukemia-bearing obese or lean mice were collected for a pathology study. Pictured are representative external views of the spleen and graph shows the quantification of spleen weight. Scale bars, 5.0 mm; Data are mean ±SD, *P<0.05. (b) Representative external views of the lung and the quantification of tumor nodules growing on lung surface. Data are mean ±SD, *P<0.05. (c) H&E-stained lung sections from boxed areas (original magnification ×200, ×400). (d) Wright-Giemsa-stained BM (original magnification ×400) and graph shows the count of post-mitotic cells. Data are mean ±SD, *P<0.05. (e) The Kaplan-Meier curve of leukemia-bearing obese and lean mice (n=6) (log-rank test). In a to d, n=3. (f) The Kaplan-
Meier curve of elderly AML patients. Forty-nine elderly patients with AML, age ≥60 and normal cytogenetics, were analyzed using Kaplan-Meier estimate. Note: Blue line represents obese patients (BMI ≥30, n=22), median overall survival is 14 months; Red line represents lean patients (BMI <30, n=27), median overall survival is 31 months. BMI, body mass index.
**Supplementary Figure 2. Host FABP4 contributes to leukemia growth in vivo.** (a-d) C1498 cells (2×10⁶) were injected intravenously into Fabp4+/+ or Fabp4−/− mice (n=3) and leukemia development was monitored by white blood cell count. Graphs show the quantification of the spleen weight (a), the metastatic foci on lung surfaces (b), the metastatic foci on liver surfaces (c), and the post-mitotic cells detected by Giemsa staining (d), from leukemia-bearing Fabp4+/+ or Fabp4−/− mice. Data are mean ±SD, *P<0.05.
Supplementary Figure 3. Knockdown of cellular FABP4 inhibits leukemia growth in vivo.

(a,b) qPCR or Western blotting showing FABP4 levels in macrophages, different types of human (a) and mouse (b) leukemia cell lines or BM from Fabp4−/− and Fabp4+/+ mice. (c-f) C1498 cells were transfected with Fabp4 siRNA or scramble. At 6 hours after transfection, cells (0.5×10⁶) were injected intravenously into 4–6 weeks old C57BL/6 mice (n=3). The graphs show the spleen weight (c), the metastatic foci growing on lung surfaces (d), the metastatic foci growing on liver surfaces (e) and post-mitotic cells in Geimsa-stained BM cytospins (f) from leukemia-bearing mice. Data are mean ±SD, *P<0.05; Si, siRNA.
Supplementary Figure 4. FABP4 regulates DNMT1 expression. (a) Western blotting of Fabp4-depleted macrophages. (b) Western blotting of SKNO-1 or THP-1 treated with the FABP4 protein. (c) Western blotting of C1498 or MV4-11 transfected with FABP4 shRNA.
Supplementary Figure 5. The FABP4-IL-6 axis governs DNMT1 gene expression. (a) Left: Graph shows the quantification of Western blotting of sera from Fabp4+/+ and Fabp4−/− mice (n=3); Right: qPCR of C1498 cells from leukemia-bearing Fabp4+/+ and Fabp4−/− mice. *P<0.05. (b) Kaplan-Meier estimate for overall survival (log-rank test) of AML patients. (c) Western blotting of C1498, MV4-11 or Kasumi-1 treated with the IL-6 protein for 24 hours or transfected with IL-6 shRNA for 48 hours. (d) qPCR of C1498 and MV4-11 transfected with IL-6 shRNA for 24 hours followed by the treatment with FABP4 protein (30 mg/mL) for another 24 hours.
Supplementary Figure 6. STAT3 links FABP4/IL-6 axis to DNMT1 expression. (a) Western blotting of BM from *Fabp4*+/+ and *Fabp4*−/− mice (n=3). (b,c) Western blotting of C1498, MV4-11 or Kasumi-1 transfected with FABP4 siRNA (b) or treated with the FABP4 protein (c). (d) Western blotting of MV4-11 treated with IL-6 (1 ng/ml). (e,f) Luciferase assays of 293T cells transfected with pGL3-DNMT1 plus STAT3 expression vectors (e) or plus NSC74859 treatment (f). (g,h)
Western blotting of C1498, MV4-11 or Kasumi-1 transfected with STAT3 expression vectors or siRNA. (i) Western blotting of C1498, MV4-11 or Kasumi-1 treated with NSC74859. (j) qPCR of C1498 and MV4-11 transfected with STAT3 shRNA for 24 hours followed by the treatment with FABP4 protein (30 mg/mL) for another 24 hours. Data are mean ±SD, *P<0.05, **P<0.01; All experiments were performed three times independently; All treatments are 48 hours unless otherwise indicated; si, siRNA; Kas-1, Kasumi-1.
Supplementary Figure 7. FABP4 regulates DNA methylation. (a) Dot-blotting was performed with different amounts of standard cytosine (C) or 5-methylcytosine (5mC) using a 5mC antibody. (b) Dot-blotting of SKNO-1 and THP-1 treated with the FABP4 protein. (c) Dot-blotting of C1498 cells from leukemia-bearing obese and lean or Fabp4+/+ and Fabp4−/− mice (n=3). (d) Dot-blotting of MV4-11 and C1498 transfected with FABP4 shRNA or scramble vectors. (e) Dot-blotting to assess the changes in DNA methylation in 293T transfected with STAT3 expression or empty vectors. (f) C1498 or MV4-11 were transfected with IL-6 shRNA or scrambled vectors and the genomic DNA was subjected to Dot-blotting. (g) Western blotting of MV4-11 transfected with DNMT1 siRNA or scramble. (h) MV4-11 cells were transfected with DNMT1 siRNA or scramble and the genomic DNA was subjected to Dot-blotting. (i) MV4-11 cells were transfected with DNMT1 siRNA or scramble. At 6 hours after transfection, ~500 cells were subjected to colony-
forming assays. The graph indicates the colony number from 3 independent experiments. Note: the experiments were performed 3 times independently; the graphs show the relative densitometric intensities expressed as the mean of the dot; Data are mean ±SD, *P<0.05, **P<0.01; All treatments are 48 hours unless otherwise indicated.
Supplementary Figure 8. FABP4 is involved in the regulation of p15INK4B gene. (a,b) qPCR of C1498, MV4-11 or Kasumi-1 (a) or human patient (n=8) and mouse (n=3) AML primary cells (b) treated with the FABP4 protein. (c) qPCR of p15INK4B expression in C1498 cells from leukemia-bearing obese and lean mice or Fabp4<sup>+/+</sup> and Fabp4<sup>−/−</sup> mice (n=3). (d) qPCR of C1498, MV4-11 or Kasumi-1 transfected with FABP4 siRNA. (e,f) Bisulfite sequencing of the p15INK4B promoter in MV4-11 treated with the FABP4 protein (100 ng/ml) (e) or transfected with FABP4 siRNA (f). Results of 10 clones are presented; vertical bars indicate CpG locations; arrows indicate the bisulfite sequencing region; open circles are unmethylated CpG sites; and filled circles are methylated CpG sites. The graphs from Dot-blotting show the quantification of dot intensities from
≥3 individual samples or 3 independent experiments; Data are mean ±SD, *P<0.05, **P<0.01; All treatments are 48 hours unless otherwise indicated.
Supplementary Figure 9. FABP4 induces aggressive AML phenotype through NFκB/STAT3/DNMT1 cascade. Colony-forming assays in MV4-11, Kasumi-1 (Kas-1) or C1498 cells treated with FABP4 protein (30 ng/ml) for 24 hours, and followed by transfection with siRNAs for NFκB, STAT3 or DNMT1 (a, c, d), or treatment with NFκB inhibitor Bay-11 (b, 1µM) or DNMT1 inhibitor Decitabine (e, 1 µM) for an additional 24 hours. Graphs indicate the colony number from three independent experiments. Data are mean ±SD.
Supplementary Figure 10. Schematic of FABP4/DNMT1 axis linking obesity to AML leukemia. (a) A high-fat diet induces obesity and subsequent FABP4 upregulation; (b) Leukemia cells also abundantly express FABP4; (c) Inter/intra cellular FABP4 activates NFkB/IL-6/STAT3 signaling that leads to an increase in DNMT1 expression; (d) TSGs are silenced by FABP4/DNMT1-dependent DNA hypermethylation resulting in aggressive AML. ?, unsolved issues.
Supplementary Tables

Supplementary Table 1

Table 1. Clinical characteristics of non-obese and obese AML patients

| Patients            | Non-obese       | Obese          |
|---------------------|-----------------|----------------|
| Number              | 27              | 22             |
| Age, year           | 74 (60-82)      | 70 (60-92)     |
| Males %             | 67              | 64             |
| Hemoglobin g/dL     | 9.4 (7.4-13.7)  | 9 (7-11.6)     |
| Platelets x10⁹      | 53 (11-215)     | 66 (17-146)    |
| WBC x10⁹            | 22.5 (0.8-95.3) | 19.1 (0.6-227) |
| Peripheral blood blasts % | 20 (0-91)      | 8 (0-95)       |
| Bone marrow blasts % | 68 (22-90)      | 29 (1-98)      |
| Complete Remission  | 74%             | 64%            |
| 60% days mortality  | 0               | 14%            |

Note: These patients were treated with the 7+3 regimen, and these data were collected through an IRB-approved retrospective study of AML patients. JMP software was used for analysis. Only elderly patients 60 or older with diploid cytogenetics were included in this study.
Supplementary Table 2

Table 2. Clinical characteristics of AML patients in GSE12417

| Characteristic                           | GPL570       | GPL96       |
|-----------------------------------------|--------------|-------------|
| Patient number                          | 163          | 79          |
| Median age, year (range)                | 58 (17-83)   | 62 (18-85)  |
| Sex (male/female)                       | 75/ 88       | 33/ 46      |
| AML type, number                        |              |             |
| De novo AML                             | 156          | 71          |
| s-AML                                   | 6            | 5           |
| t-AML                                   | 1            | 3           |
| FLT3 ITD status, number (%)             |              |             |
| FLT3 ITD                                | 86 (53)      | 57 (72)     |
| FLT3 ITD+                               | 77 (47)      | 22 (28)     |
| NPM1 status, number (%)                 |              |             |
| NPM1+                                   | 77 (47)      | 37 (47)     |
| NPM1*                                   | 86 (53)      | 42 (53)     |
| FAB subtypes, number (%)                |              |             |
| M0                                      | 5            | 1           |
| M1                                      | 45           | 23          |
| M2                                      | 45           | 34          |
| M4                                      | 42           | 11          |
| M5                                      | 19           | 6           |
| M6                                      | 6            | 3           |
| Median leukocyte count, 109/L (range)   | 36.9 (0.85-486) | 15.9 (1-440.3) |
| Median hemoglobin level, g/L (range)    | 91 (40-142)  | 93.5 (60-147) |
| Median platelet count, 109/L (range)    | 56 (6-471)   | 64 (9-239)  |
| Median BM blasts, % (range)             | 85 (17-100)  | 80 (11-97)  |
| Median follow-up for surviving patients, month (range) | 30 (1.6-79) | 39 (4.6-50) |
| Overall survival (OS)                   |              |             |
| Median, month                           | 9.7          | 17.7        |
| Estimated OS at 2 years, %              | 37           | 44          |
Supplementary Table 3

Table 3. Sequences of siRNAs and primers used in experiments

| Name            | Oligo sequences (5’ to 3’)                          |
|-----------------|-----------------------------------------------------|
| **siRNA**       |                                                     |
| STAT3 siRNA and scramble |                                                   |
| siRNA1          | GAAUCAAGCAGUUUCUUCAGAGCAG                            |
| siRNA2          | CACUGUAUCAGCAUAGCCUUUCUGT                            |
| siRNA3          | GCACCUUCCUGCUAAGUUCAGUGA                            |
| scramble        | GAUUCUCGAACGUGUCACGUTT                              |
| NFXB siRNA and scramble |                                                   |
| siRNA1          | CCAUCAAGAUAUUGCUACACAGG                             |
| siRNA2          | CAUCAUGAAGGAGGCUACACAGG                             |
| siRNA3          | AGAGGACAAGAGGAGGUAUUUCACG                           |
| scramble        | GAUUCUCGAACGUGUCACGUTT                              |
| **Vector**      |                                                     |
| STAT3 vector    | forward CCAAGCCTTGCCACATGGCCAATGGGAATCACGCT        |
| reverse         | CCGCTCGGATCTACATGGGAGGATGGCAACT                     |
| DNMT1 promoter vector |                                               |
| forward         | GGGGTACCTACCTGTCGCCCAGGAGCATCACGTGG                |
| reverse         | CCAAGCCTTGAGGGAGATGGGAAGGATGGGAACG                 |
| **Bisulfite sequencing** |                                               |
| p15INK4B promoter region |                                               |
| forward         | GGTGTTGTATTTTATTTGTTAGAG                           |
| reverse         | ACCCTAAACTCAACTCAACTCAACT                          |
| **qPCR**        |                                                     |
| hp15INK4B       | forward CCAGATGAGGACAATGAG                           |
| reverse         | AGCAAGAACAACAAATCA                                 |
| mp15INK4B       | forward TTAGCTGGATTTCTGAG                           |
| reverse         | CATAGAGTGACCTGCTACCA                                |
| hFABP4          | forward CGCATGGAACTCTCAACAT                         |
| reverse         | ACTAAAGAATACCATACATAGC                             |
| mFabp4          | forward TGGTGAAATGTGTATGAA                          |
| reverse         | TGCTTGCTTTATTAGGAA                                 |
| m18S            | forward ACAGATTGACAGATTG                            |
| reverse         | TACGGGAATTAACAGACA                                 |
| h18S            | forward ACAGATTGACAGATTG                            |
| reverse         | TACGGGAATTAACAGACA                                 |
| hIL-6           | forward ACCTCAGATTGTTGTTG                           |
| reverse         | GTCCAACGCTCATACCT                                  |
| mIL-6           | forward ACCTGCTATACACTTC                            |
| reverse         | GACATCAGCTGTTGTACATA                                |