SUPPLEMENTARY INFORMATION

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Materials and Methods

Plasmids and reagents

The shRNAs against human/mouse *VEGF* (V2LHS_8150, TRCN0000003344, V2LHS_171929) and its scrambled constructs (pLKO.1 or pGIPZ) were purchased from BMGC RNAi (University of Minnesota, Minneapolis, MN). On-target^{plus} Smart pool siRNAs containing a mixture of 4 oligonucleotides against human/mouse *DNMT1* (L-004605-00, 056796-01) and its scrambled oligos (D-001810-10) were obtained from Thermo Scientific (Waltham, MA). The FABP4 inhibitor (BMS309403, 341310) was obtained from Calbiochem (San Diego, CA). The stearic acid (S4751) and palmitic acid (P0500) were from Sigma Aldrich (St. Louis, MO). The mouse/human VEGF proteins (PMG0114, PHC9394) were from Invitrogen (Carlsbad, CA). Solutions of stearic and palmitic acid were prepared in dimethyl sulfoxide (DMSO) at 50 mM, then diluted in phosphate buffered saline (PBS) with 1% BSA at 5 mM. BMS309403 was prepared at 100 mM in ethanol for pre-clinical testing and in DMSO for cell culture experiments.

Cell culture and transfection

The 293T, MV4-11, Kasumi-1, THP-1 and C1498 cell lines were obtained from American Type Culture Collection. The SKNO-1 cell line was kindly provided by Dr. Clara Nervi (University of Rome, Rome, Italy). The 293T and C1498 cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM), and the others, including mouse macrophages (*FABP4*$^{+/+}$ and *FABP4*$^{-/-}$), were grown in RPMI 1640, supplemented with 5% (macrophages), 20% (Kasumi-1, SKNO-1) or 10% (others) FBS at 37°C under 5% CO_2. Cell lines were newly purchased with no further testing for mycoplasma. Cells (1×10^6) were seeded into 6-well plates overnight before transfection. The siRNA oligos (100 nM), expression or shRNA vectors (2 μg) and their controls were introduced

into cells using Lipofectamine™ RNAiMAX or Lipofectamine™ 2000 (13778-150, 11668-019, Life Technologies, Carlsbad, CA) as previously described.[1, 2] Three shRNAs for *VEGF* were tested, respectively, and the one achieving the most reduction was utilized for further investigations.

Cytospin/Wright-Giemsa staining

The AML cells (0.1×10^6) were harvested and placed in the Shandon EZ Single Cytofunnel (Thermo Electron Corporation). Samples were centrifuged at 1,000 rpm for 8 min. The slides were air-dried and stained with Hema-3 Kit (22-122-911, Fisher Scientific, Hampton, NH) as previously described.[1, 3] 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 (original magnification ×200).

Cell differentiation assays

Approximately 5×10^5 treated cells were washed once in 1 ml of staining buffer (1 × PBS with 1% FBS) for 5 min and incubated in 100 μl of staining buffer with the CD11b antibody (553310, Clone M1/70, 1:1000, BD Biosciences PharMingen, San Jose, CA) for 15 min. The stained cells were washed twice, resuspended in 300 μl of staining buffer and analyzed in a FACsCalibur flow cytometer. Data were analyzed using Flowjo (Tristar).

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

H&E and IHC staining were performed as previously described.[1-4] Briefly, tissues collected from the animal studies were fixed in 10% paraformaldehyde/PBS. The paraffin-embedded samples

were stained with H&E (H9627, HT100180, Sigma) or the Ki-67 antibody (ab15580, Abcam, 1:1000). 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 using MethoCult® mixture (03434, Stem Cell Technologies, Vancouver, Canada) and MTS assays using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (G5421, Promega, Madison, WI) as previously described.[1-4] Colonies were scored at 7–10 days.

Wound-healing assays

Mouse macrophages (*FABP4*$^{+/+}$ and *FABP4*$^{-/-}$) or 293T cells were treated as indicated, grown to confluence and the wound-healing assays were conducted as previously described.[4, 5] The migration of cells towards the wound was photographed under a light microscope and CorelDRAWX5 Software was used to determine the migration distance.

Bodipy staining

Cells were fixed in 3.7% formaldehyde (w/v) in 0.1 M phosphate buffer (pH 7.4) for 10 min, then permeabilized with 0.1% Saponin in 1 × PBS for 10 min at room temperature. The treated cells were stained with Bodipy (Invitrogen, 1000 × dilution of stock saturated in 100% ethanol) plus DAPI (100 × dilution of 28 μM) for 10 min, mounted in a drop of Fluoromount on a glass slide and visualized by confocal microcopy.

Animal studies

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Minnesota. For survival studies, mice were sacrificed when they showed any signs of distress (i.e., breathing disorders, weight loss or immobility). The AML1/ETO9a (AE9a) mouse model:[6] t(8;21), resulting in a chimeric protein AML1/ETO, represents one of the most common chromosomal abnormalities associated with about 40% of M2 AML cases. AE9a is a C-terminal truncated form of AML1/ETO and highly leukemogenic in mice. To establish the AE9a AML model in our study, C57BL/6 mice (male, 4–6 weeks) were engrafted with ~0.5×10^6 AE9a primary cells, and were monitored for signs of disease by using the white blood cell (WBC) counts. All of these mice developed leukemia within 8 to 10 weeks of transplantation. Then bone marrow (BM) cells were isolated for further investigations. For preclinical testing of BMS309403: C1498 cells (0.5×10^6) were injected into C57BL/6 mice (male, 4–6 weeks) through the tail-vein for the development of leukemic disease. BMS309403 was prepared just prior to administration by dissolving in ethanol to provide a clear solution and then diluting with PEG400 and saline (ratio 15:38:47). We designed two methods to administer BMS309403: 1) Because the C57BL/6 mice have baseline FABP4 (25 ng/ml), which alters the potential of leukemia cell growth,[3] we administered intraperitoneally one dose of BMS309403 (5 mg/kg) at 12 hours prior to leukemia cell engraftment. This treatment was followed by one dose at 5 mg/kg and two doses at 10 mg/kg. 2) When the average of WBC counts is significantly higher in all leukemia cell-engrafted mice than in normal mice, the leukemia-bearing mice were randomized and administrated BMS309403 intraperitoneally every three days at 5 mg/kg for four times in total and at 10 mg/kg for two times in total. The administration of vehicle (PEG400 and saline, 15:38:47) was used as a negative control. The survival time was analyzed by Kaplan–Meier

estimate, and comparison between groups was analyzed by log-rank tests. The survival time was from the start of leukemia cell injection. For the histopathological and molecular characterization, the mice were sacrificed in 3 weeks after cell injections. Cytospin preparations of BM cells were processed for Giemsa staining. Lungs, spleens and livers were harvested and immediately fixed in 10% neutral-buffered formalin and stained with H&E (Thermo-Scientific). For WBC counting, 2 μl of blood from mouse tail-vein was mixed with 38 μl of Turk blood dilution fluid (R8850000, Ricca Chemical, Pleasant Prairie, WI) and the WBCs were counted under a microscope.

AML patient samples

The bone marrow (BM) diagnosis of AML was performed according to the criteria of the 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[1, 3] for subsequent 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 before entering the study.

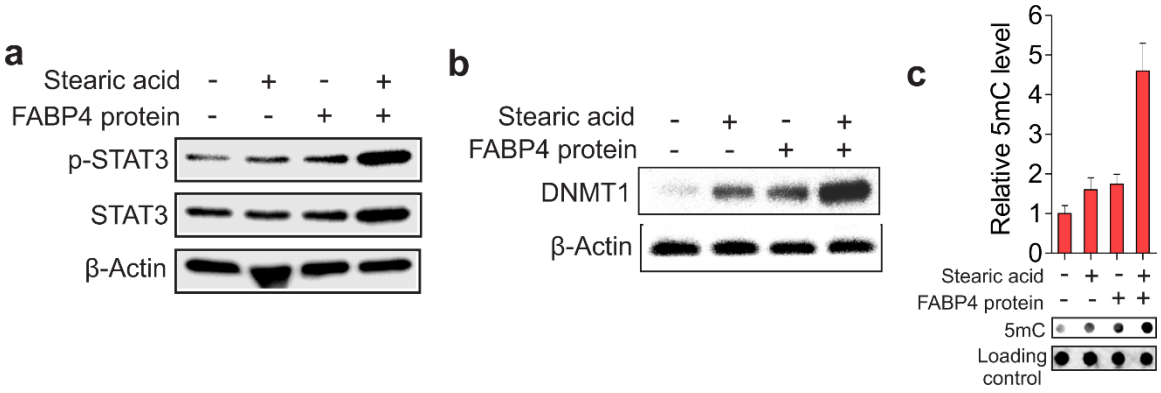
GEO (Gene Expression Omnibus) analysis

Public GEO datasets (GSE12417, platform GPL570 and GPL96, containing 242 AML patients) were analyzed for the mRNA expression of *FABP4*, *VEGF*, *DNMT1*, *DNMT3a* and *DNMT3b*, 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.[7, 8] These samples were normalized, managed and analyzed by GraphPad Prism 5 Software using Spearman correlation coefficients. For the survival assays, the expression

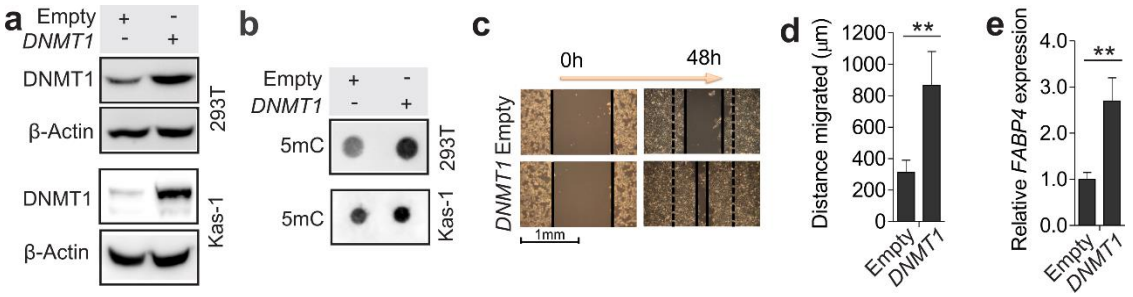
of *FABP4*, *VEGF* and *DNMTs* was defined as high if mRNA level is ⩾ 75th percentile of mean expression in the cohort and as low if < 75th percentile of mean expression in the cohort.

Statistical analysis

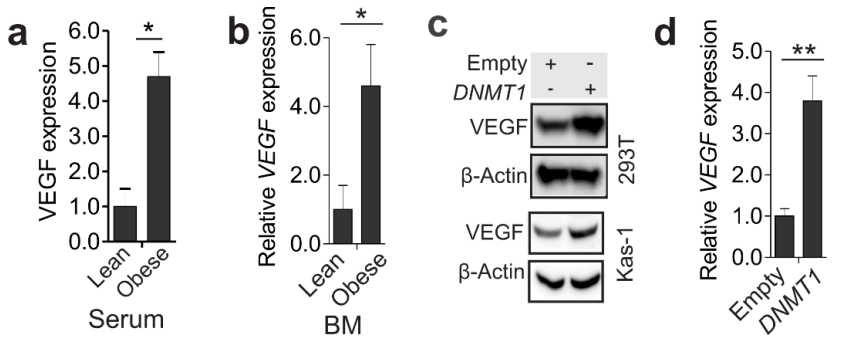
All graphs were generated using the Student's t test, but the Kaplan-Meier survival curves were generated by the log-rank test. Correlation data were generated using the Pearson correlation coefficients. All analyses were conducted using the GraphPad Prism 5 Software program and $P < 0.05$ was considered statistically significant. All *P* values were two-tailed. *In vitro* experiments were routinely repeated three times unless indicated otherwise in figure legends or main text. 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. The statistical analysis was conducted using the Student's t test. No samples or animals were excluded from the analysis. All criteria were pre-established. No randomization was used in our studies and no blinding for all experiments. Variations were compatible between groups. The statistical tests were justified as appropriate for every figure.



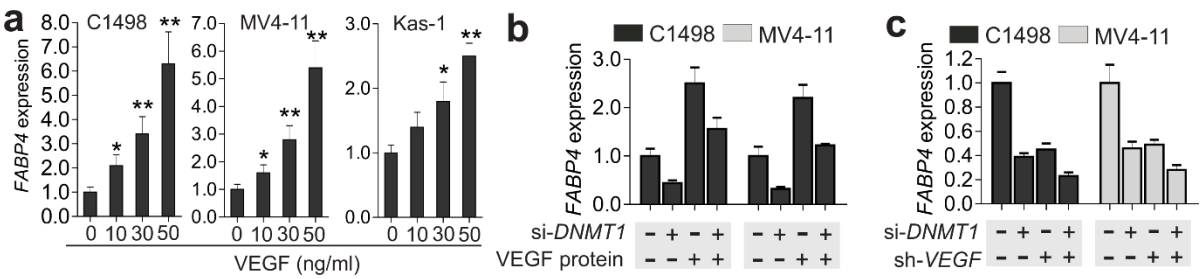
Supplementary Figure 1. Stearic acid upregulates DNMT1 expression and increases global DNA methylation. MV4-11 cells were treated with stearic acid (50 μM) or/and the FABP4 protein (30 ng/ml) for 24 hours and subjected to Western blotting (**a**, **b**) or dotblotting analysis (**c**). Graphs show the quantification of dot intensity. Data represent three independent experiments.



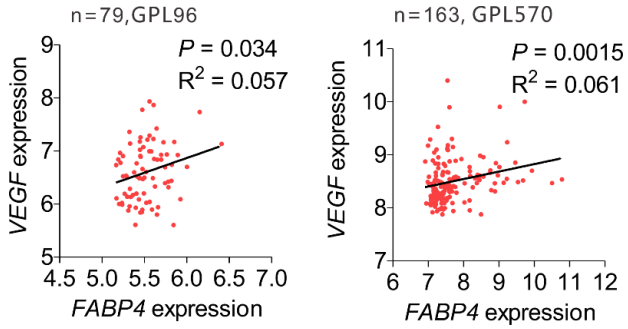
Supplementary Figure 2. DNMT1 expression levels are implicated in DNA methylation, cell migration and FABP4 regulation. (**a** and **b**) Western blotting for DNMT1 protein expression (**a**) and Dotblotting for global DNA methylation (**b**) in 293T or Kasumi-1 cells transfected with *DNMT1* expression vectors for 48 hours. (**c** and **d**) 293T cells were transfected with *DNMT1* expression vectors and subjected to wound-healing assays. Pictured (**c**) are representative images and the graph (**d**) shows measurement of the migrated distance. (**e**) 293T cells were transfected with *DNMT1* expression vectors and subjected to qPCR. The experiments were performed three times independently; Data are shown as mean values ± S.D., $^{**}P < 0.01$; scale bars, 1 mm.



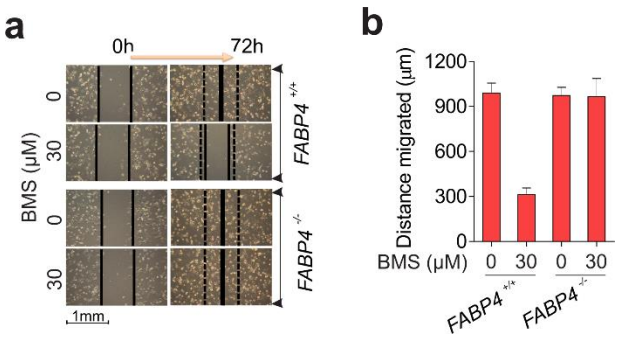
Supplementary Figure 3. DNMT1 is involved in *VEGF* regulation. (**a**) Graph shows the quantification of ELISA of sera from lean and obese mice (n = 5). (**b**) qPCR of BM cells from leukemia-bearing lean and obese mice (n = 3). **P* < 0.05. (**c** and **d**) Western blotting (**c**) or qPCR (**d**) of 293T or Kasumi-1 cells transfected with *DNMT1* expression vectors for 48 hours. The experiments were performed 3 times independently; Data are shown as mean values ± S.D., ***P* < 0.01.



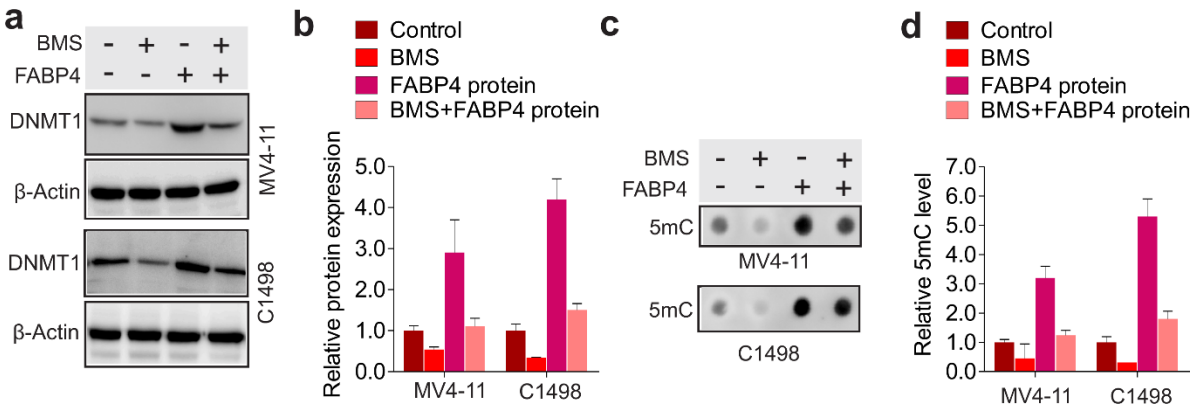
Supplementary Figure 4. VEGF acts as a molecular link between DNMT1 and FABP4 abnormality in leukemia cells. (**a**) qPCR of C1498, MV4-11 or Kasumi-1 cells treated with VEGF recombinant protein (30 ng/ml). (**b** and **c**) C1498 and MV4-11 cells were transfected with *DNMT1* shRNA for 24 hours followed by VEGF protein (30 ng/ml) treatment (**b**) or shRNA transfection (**c**) for an additional 24 hours. FABP4 expression was assessed by qPCR. The experiments were performed 3 times independently. Data are shown as mean values ± S.D., **P* < 0.05, ***P* < 0.01; All treatments are 48 hours unless otherwise indicated; si, siRNA; sh, shRNA; Kas-1, Kasumi-1.



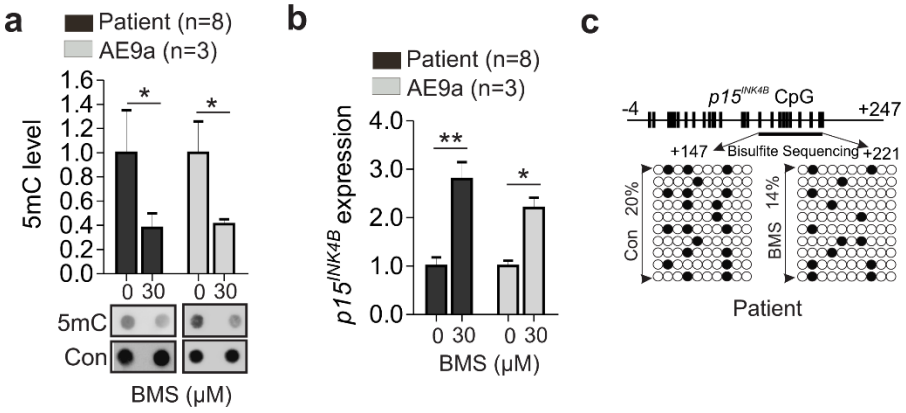
Supplementary Figure 5. The expression levels of *VEGF* and *FABP4* genes are positively correlated. The analysis of GEO dataset GSE12417 series, platform GPL570 and GPL96 (AML, n = 242), shows the correlation between *VEGF* and *FABP4* expression, which was assessed by Spearman correlation.



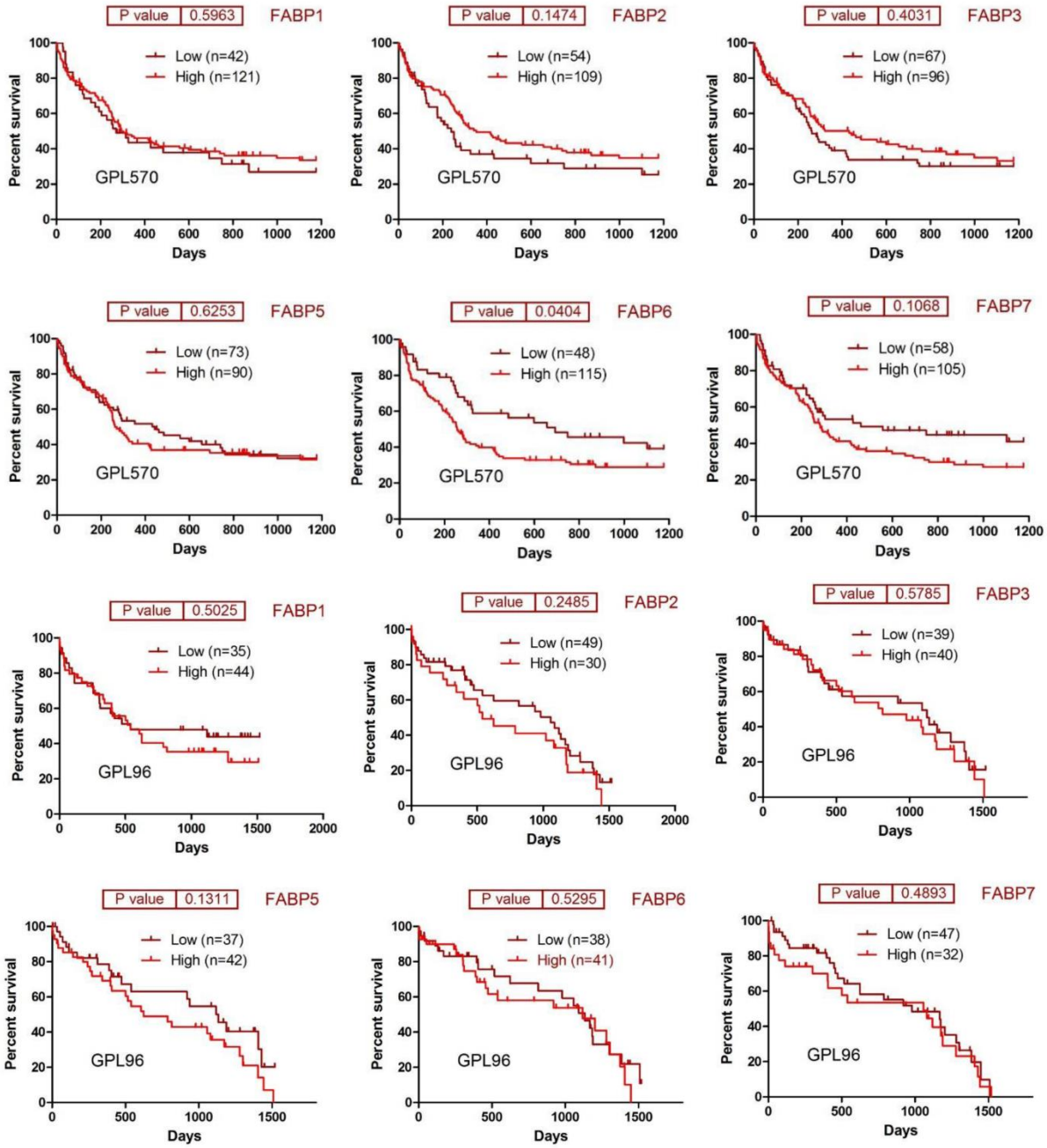
Supplementary Figure 6. FABP4 is required for the inhibitory effects of BMS on cell proliferation. (**a**) Pictured are representative images of wound-healing assays in *FABP4*$^{+/+}$ and *FABP4*$^{-/-}$ macrophages treated with BMS (scale bar = 1 mm). (**b**) Graph shows the quantification of the migrated distance from (**a**). The experiments were performed 3 times independently; BMS, BMS309403.



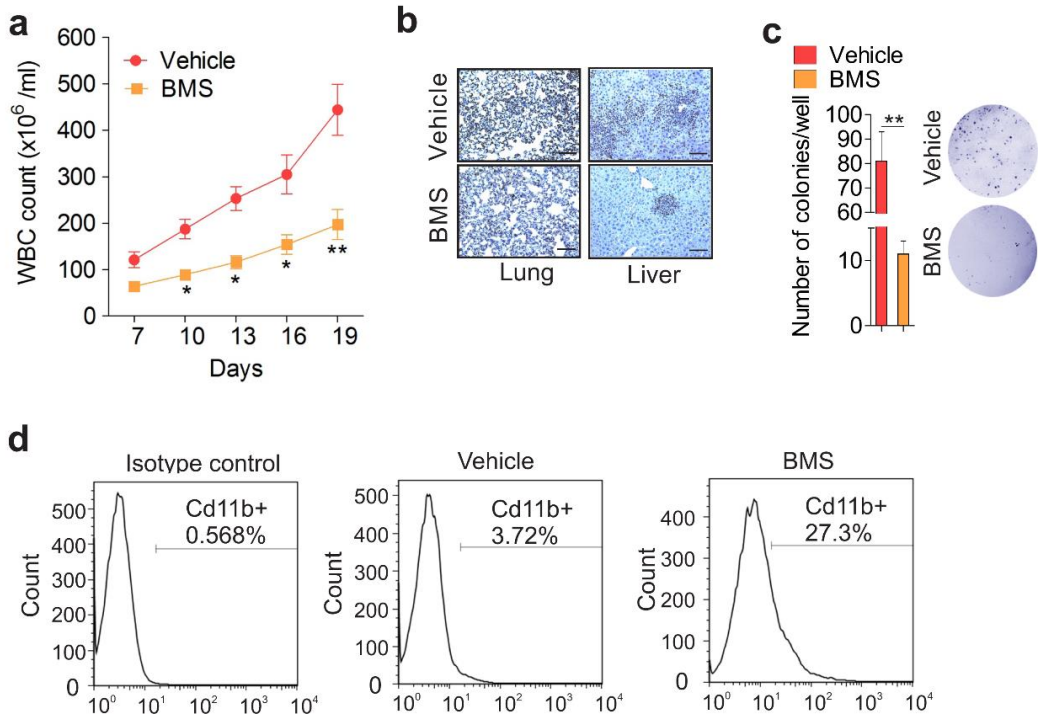
Supplementary Figure 7. BMS decreases DNMT1 expression and induces DNA demethylation in a FABP4-dependent manner. (**a** and **b**) Western blotting (**a**) showing changes in DNMT1 in MV4-11 and C1498 cells treated with 30 μM BMS alone or plus 200 ng/ml of the FABP4 protein. The graph (**b**) shows the quantification of band intensities. (**c** and **d**) Dotblotting (**c**) showing changes in DNA methylation in MV4-11 and C1498 cells treated with 30 μM of BMS alone or plus 200 ng/ml of the FABP4 protein. The graph (**d**) shows the quantification of dot intensity. Representative data are shown from 3 independent experiments. All treatments are 48 hours unless otherwise indicated; BMS, BMS309403.



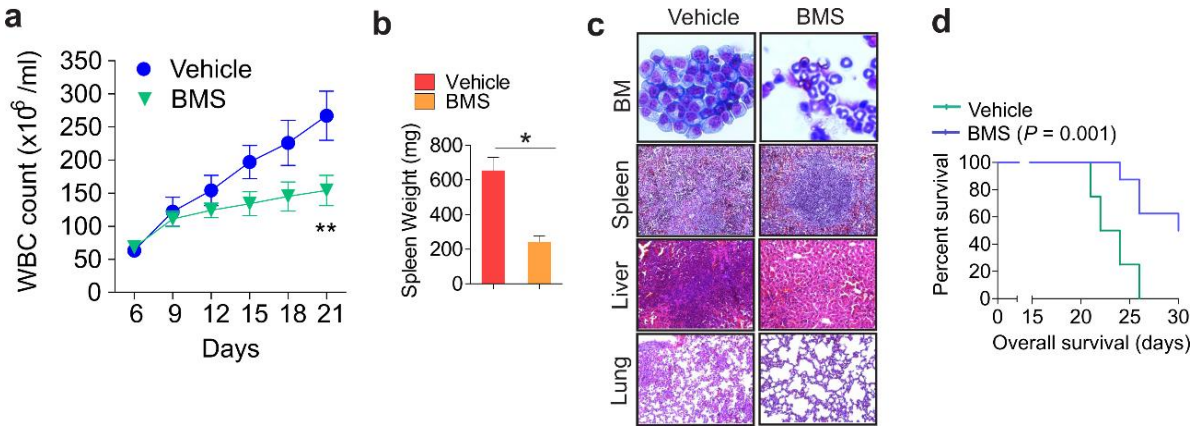
Supplementary Figure 8. BMS treatment induces DNA demethylation and upregulates *p15*^{INK4B} in primary cells. (**a** and **b**) Dotblotting (**a**) or qPCR (**b**) of human patient (n = 8) or mouse (n = 3) AML primary cells treated with BMS. (**c**) Bisulfite sequencing of the *p15*^{INK4B} promoter in AML patient cells treated with BMS. Data are shown as mean values ± S.D., **P* < 0.05, ***P* < 0.01; In **c**, results of 10 clones are presented. Vertical bars indicate CpG locations; arrows indicate the bisulfite sequencing region; open circles show unmethylated CpG; and solid circles show methylated CpGs. All treatments are 48 hours unless otherwise indicated; Con, loading control; BMS, BMS309403.



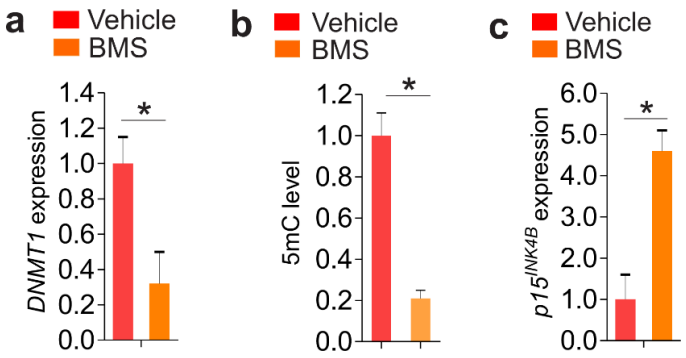
Supplementary Figure 9. The correlation of the expression of FABP family members with the survival of AML patients, which were reported in GSE12417 and determined by the Kaplan-Meier estimate.



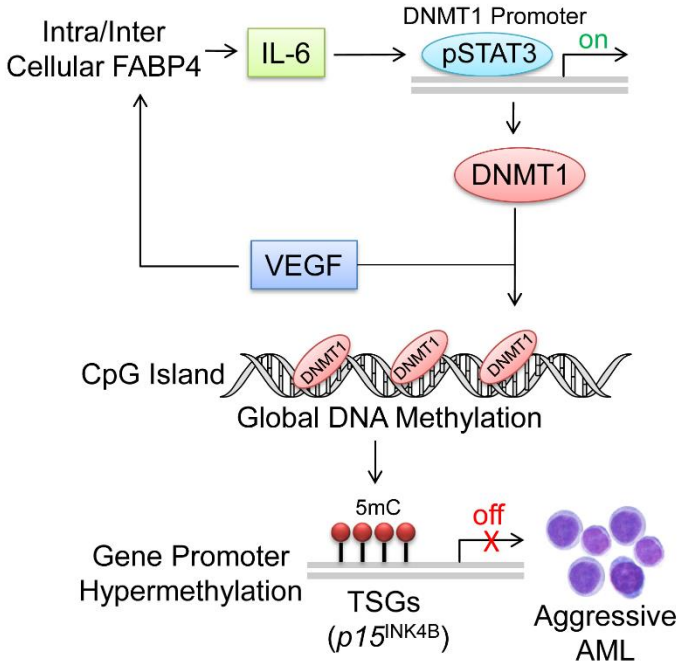
Supplementary Figure 10. BMS treatment reduces leukemic burden in mice. C1498 cells (0.5×10^6) were intravenously injected into 4–6 weeks old C57BL/6 mice (n = 6). Twelve hours prior to cell injection, one dose of BMS (5 mg/kg) was administered. (**a**) The white blood cell (WBC) count began at day 7 after cancer cell engraftment. Data are shown as mean values ± S.D., **P* < 0.05, ***P* < 0.01. (**b**) A Ki-67 antibody was used for IHC staining of lung or liver sections (boxed areas in Figure 6b) from leukemic mice (n = 3) receiving BMS or vehicle (original magnification ×200). (**c**) Colony-forming assays of BM cells from leukemia mice (n = 3) receiving BMS or vehicle. (**d**) Flow cytometry assays for CD11b+ expression in BM cells isolated from leukemic mice (n = 3) receiving BMS or vehicle. Data are shown as mean values ± S.D., **P* < 0.01, ***P* < 0.01.



Supplementary Figure 11. BMS therapy induces leukemia regression. C1498 cells were injected into C57BL/6 mice (n = 8) through the tail-vein. When leukemic disease became apparent, BMS was administered twice a week at 5 mg/kg for two weeks, then 10 mg/kg was administered two times in the third week. The WBC count began at day 6 after cancer cell engraftment. (**a**) Graph shows the WBC number. Data are shown as mean values ± S.D., $^{**}P < 0.01$. (**b**) Graph shows the spleen weight. Data are represented as mean values ± S.D., $^{*}P < 0.05$. (**c**) Wright-Giemsa-stained BM (original magnification ×400) and H&E-stained spleen, liver and lung sections (original magnification ×200). (**d**) Survival curve was measured by Kaplan-Meier estimate (log-rank test). BMS, BMS309403.



Supplementary Figure 12. The antileukemic effects of BMS309403 *in vivo* are mediated by DNA hypomethylating activities. (**a**-**c**) qPCR for the expression of *DNMT1* (**a**) and $p15^{INK4B}$ (**c**) or Dotblotting for DNA methylation (**b**) in BM cells of leukemia-bearing mice (n = 3) reported in Figure 6. BMS, BMS309403. Data are shown as mean values ± S.D., **P* < 0.05.



Supplementary Figure 13. Schematic of the FABP4/DNMT1 vicious circle in aggressive AML leukemia. (**a**) Overproduction of intra/inter cellular FABP4 enhances IL-6 expression, leading to STAT3-dependent DNMT1 upregulation. (**b**) DNMT1 overexpression feeds back to promote FABP4 levels through VEGF signaling. (**c**) The FABP4/DNMT1 regulatory loop induces DNA hypermethylation and epigenetically silences TSGs (e.g., $p15^{INK4B}$), resulting in aggressive AML growth.

Supplemental Table. Sequences of primers used in experiments

	Name		Oligo sequences (5' to 3')
Bisulfite sequencing	*p15*^{INK4B} promoter region	forward	GGTTGGTTTTTTATTTTGTTAGAG
		reverse	ACCTAAACTCAACTTCATTACCCTC
qPCR	*hp15*^{INK4B}	forward	CCAGATGAGGACAATGAG
		reverse	AGCAAGACAACCATAATCA
	mp15^{INK4B}	forward	TTGATGCCTGGATTCTGA
		reverse	CATAGAGTAACCTGCTACCA
	hFABP4	forward	CGCATTGAACTCTACAACAT
		reverse	ACTAAGAATACATCATAAGCACAA
	mFABP4	forward	TGGTGGAATGTGTTATGAA
		reverse	TGCTTGCTTATTAGTGGAA
	m18S	forward	ACAGGATTGACAGATTGA
		reverse	TATCGGAATTAACCAGACA
	h18S	forward	ACAGGATTGACAGATTGA
		reverse	TATCGGAATTAACCAGACA
	hVEGF	forward	CTAATGTTATTGGTGTCTTCA
		reverse	TCTCATCTCCTCCTCTTC
	mVEGF	forward	GACTATTCAGCGGACTCA
		reverse	AAGAACCAACCTCCTCAA
	hIL-6	forward	ACCTCAGATTGTTGTTGT
		reverse	GTCCTAACGCTCATACTT
	mIL-6	forward	ACCTGTCTATACCACTTC
		reverse	GCATCATCGTTGTTCATA

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