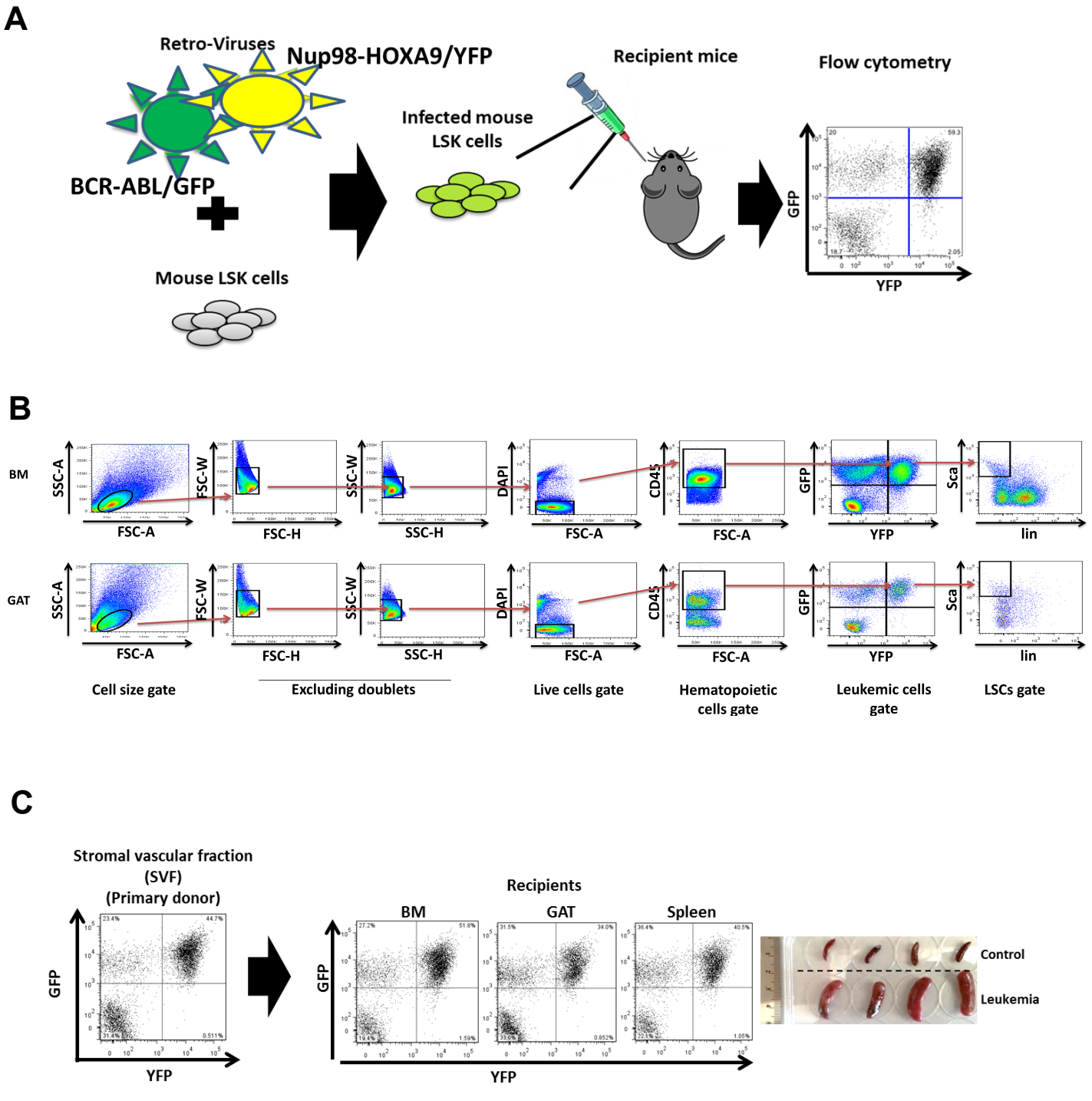
A
Retro-Viruses
Nup98-HOXA9/YFP
BCR-ABL/GFP
Mouse LSK cells
Infected mouse LSK cells
Recipient mice
Flow cytometry
GFP
YFP
B
BM
GAT
SSC-A
FSC-A
FSC-W
FSC-H
SSC-W
SSC-H
DAPI
CD45
GFP
YFP
Sca
lin
Cell size gate
Excluding doublets
Live cells gate
Hematopoietic cells gate
Leukemic cells gate
LSCs gate
C
Stromal vascular fraction (SVF) (Primary donor)
Recipients
BM
GAT
Spleen
GFP
YFP
Control
Leukemia

Figure S1. Gonadal adipose tissue functions as a reservoir for LSCs (related to Figure 1).

(A) A schematic diagram that outlines generation of the murine model of blast crisis CML used in this study. GFP and YFP double positive cells (GFP+/YFP+) identify the cells expressing both the BCR/ABL and Nup98/ HoxA9 translocations and are considered blast crisis CML cells. See also supplemental experimental procedures. (B) Gating strategy employed to isolate LSCs from BM and GAT. (C) Analysis of LSCs in GAT. The stromal vascular fraction (SVF) from leukemic mice was injected into recipients. Ten days later, BM, spleen and GAT cells from recipients were examined by flow cytometry. Spleens from transplanted animals are also shown.

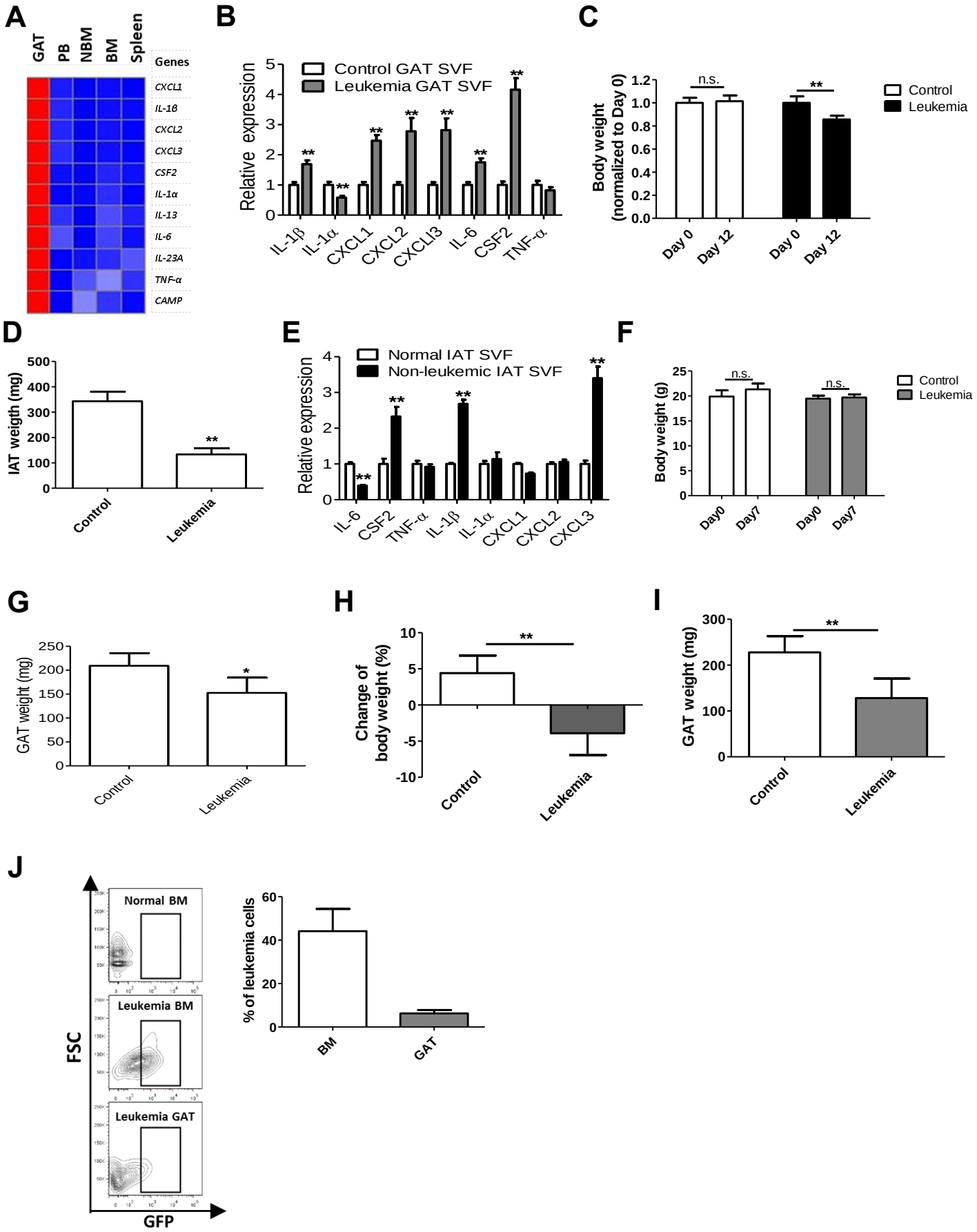
A
GAT
PB
NBM
BM
Spleen
Genes
CXCL1
IL-1β
CXCL2
CXCL3
CSF2
IL-1α
IL-13
IL-6
IL-23A
TNF-α
CAMP
B
Relative expression
Control GAT SVF
Leukemia GAT SVF
IL-1β
IL-1α
CXCL1
CXCL2
CXCLI3
IL-6
CSF2
TNF-α
C
Body weight (normalized to Day 0)
n.s.
Control
Leukemia
Day 0
Day 12
D
IAT weigth (mg)
Control
Leukemia
E
Relative expression
Normal IAT SVF
Non-leukemic IAT SVF
IL-6
CSF2
TNF-α
IL-1β
IL-1α
CXCL1
CXCL2
CXCL3
F
Body weight (g)
Control
Leukemia
Day0
Day7
G
GAT weight (mg)
Control
Leukemia
H
Change of body weight (%)
Control
Leukemia
I
GAT weight (mg)
Control
Leukemia
J
Normal BM
Leukemia BM
Leukemia GAT
FSC
GFP
% of leukemia cells
BM
GAT

Figure S2. Leukemia cells in adipose tissue are pro-inflammatory (related to Figure 2).

(A) Heat map shows the expression of inflammatory cytokines/chemokines in NBM and LSCs in different tissues (Red=up-regulated, Blue=down-regulated). (B) Expression of pro-inflammatory cytokines/chemokines genes in leukemic and control gonadal adipose tissue (GAT) stromal vascular fraction (SVF). Error bars show means ± S. D. from triplicates. ** $P<0.005$. (C and D) Change of body weight (C), inguinal adipose tissue (IAT) weight (D) in control (transplanted with normal BM hematopoietic cells) and leukemic mice. Weights were determined before transplantation and 12 days after transplantation. Error bars show means ± S. D. n=8, ** $P<0.005$. (E) Expression of pro-inflammatory cytokines in IAT SVF from normal mice and non-leukemic (GFP-/ YFP-) IAT SVF from leukemia mice. Error bars show means ± S. D. from triplicates. ** $P<0.005$. (F and G) Change of body weight (F) as well as GAT weight (G) during early stage development of leukemia (Day 7 after transplantation with leukemia cells). Error bars show means ± S. D. n=6, * $P<0.05$. (H and I) Change of body weight (H) as well as GAT weight (I) in an MLL-AF9 induced leukemia model. Mice were sacrificed at day 30 after transplantation with primary MLL-AF9 leukemia cells. Error bars show means ± S. D. n=5, * $P<0.05$. (J) Engraftment of MLL-AF9 leukemia cells in BM and GAT. Error bars show means ± S. D. n=5.

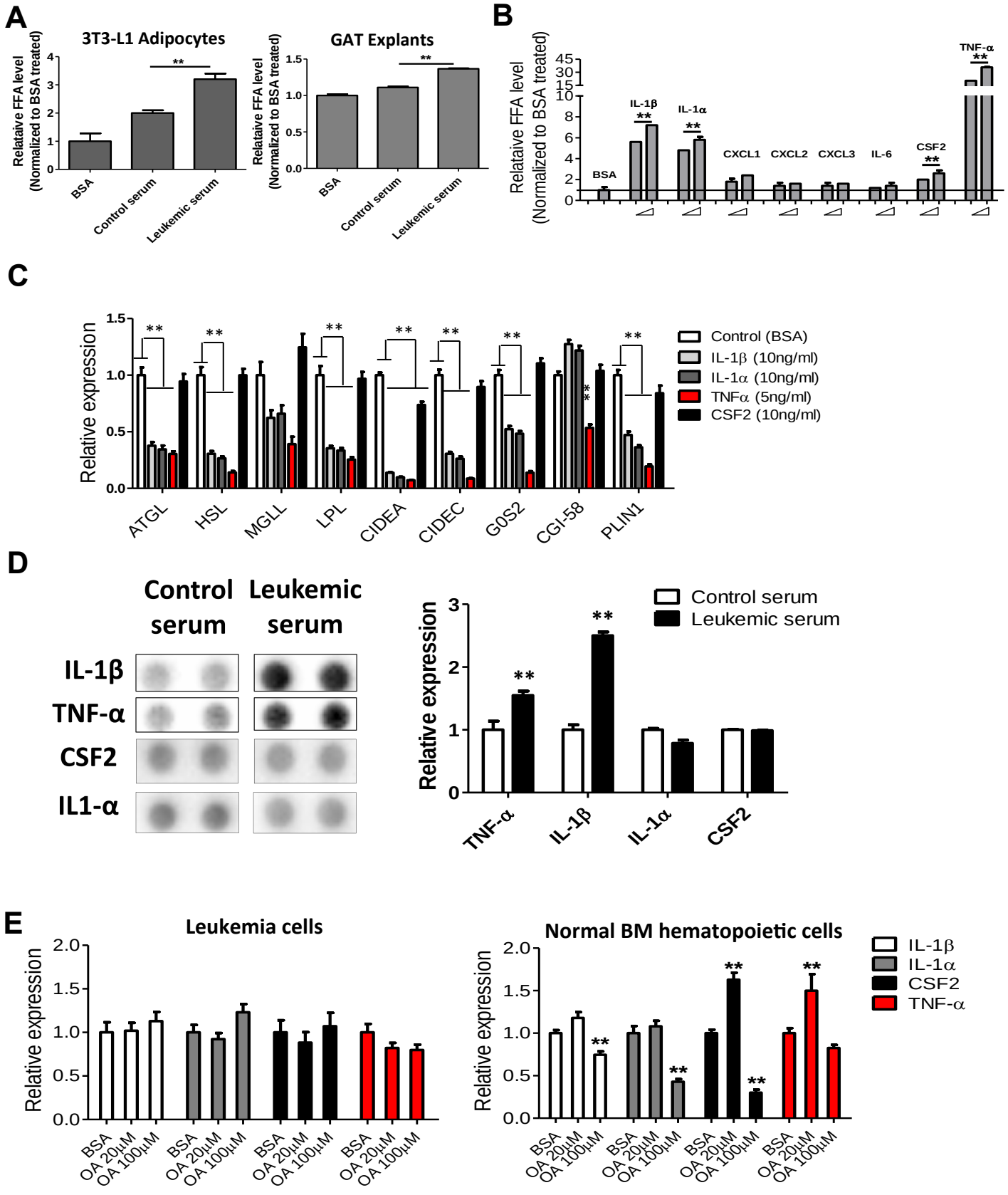
A
3T3-L1 Adipocytes
GAT Explants
Relataive FFA level (Normalized to BSA treated)
BSA
Control serum
Leukemic serum
B
Relataive FFA level (Normalized to BSA treated)
BSA
IL-1β
IL-1α
CXCL1
CXCL2
CXCL3
IL-6
CSF2
TNF-α
C
Relative expression
ATGL
HSL
MGLL
LPL
CIDEA
CIDEC
G0S2
CGI-58
PLIN1
Control (BSA)
IL-1β (10ng/ml)
IL-1α (10ng/ml)
TNFα (5ng/ml)
CSF2 (10ng/ml)
D
Control serum
Leukemic serum
IL-1β
TNF-α
CSF2
IL1-α
Relative expression
Control serum
Leukemic serum
TNF-α
IL-1β
IL-1α
CSF2
E
Leukemia cells
Normal BM hematopoietic cells
Relative expression
BSA
OA 20μM
OA 100μM
IL-1β
IL-1α
CSF2
TNF-α

Figure S3. Pro-inflammatory cytokines induce lipolysis (related to Figure 3).

(A) 3T3-L1 adipocytes and GAT explants were treated with BSA, and serum from control or leukemic mice, and FFA levels in culture medium was measured as described in supplemental experimental procedures. Error bars show means ± S. D. from triplicates. ** *P*<0.005. (B-C) 3T3-L1 adipocytes were cultured in DMEM containing 1% BSA and indicated cytokines (2.5 and 5ng/ml for TNF- α and 5 and 10ng/ml for others). FFA levels in culture medium (B) and expression of lipolysis related genes (C) were determined. Error bars show means ± S. D. from triplicates. ** *P*<0.005. (D) Serum from control and leukemic mice was subjected to cytokine arrays. Array results as well as quantification for protein levels of IL-1α, IL-1β, CSF2 and TNF-α are shown. Error bars show means ± S. D. from the duplicate dots for each cytokines. ** *P*<0.005. (E) BM leukemia cells and normal BM cells were treated with BSA or oleic acid (OA) (20 and 100μm) for 24h and then mRNA of pro-inflammatory cytokines/chemokines was determined. Error bars show means ± S. D. from triplicates. ** *P*<0.005.

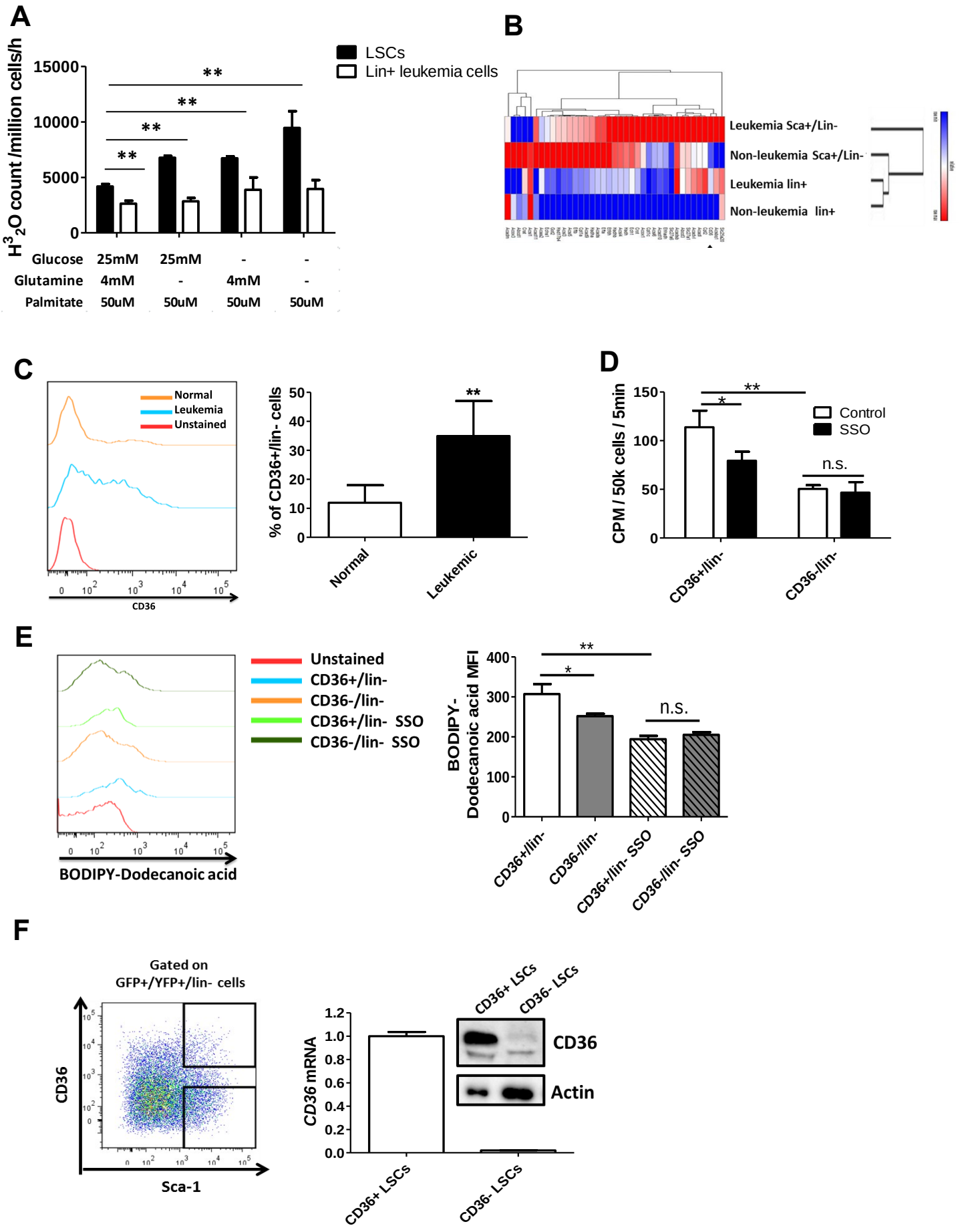
A
LSCs
Lin+ leukemia cells
H^3_2O count /million cells/h
15000
10000
5000
0
**
**
**
**
Glucose 25mM 25mM - -
Glutamine 4mM - 4mM -
Palmitate 50uM 50uM 50uM 50uM
B
Leukemia Sca+/Lin-
Non-leukemia Sca+/Lin-
Leukemia lin+
Non-leukemia lin+
C
Normal
Leukemia
Unstained
CD36
% of CD36+/lin- cells
50
40
30
20
10
0
**
Normal
Leukemic
D
CPM / 50k cells / 5min
150
100
50
0
**
*
n.s.
Control
SSO
CD36+/lin-
CD36-/lin-
E
Unstained
CD36+/lin-
CD36-/lin-
CD36+/lin- SSO
CD36-/lin- SSO
BODIPY-Dodecanoic acid
BODIPY-Dodecanoic acid MFI
400
300
200
100
0
**
*
n.s.
CD36+/lin-
CD36-/lin-
CD36+/lin- SSO
CD36-/lin- SSO
F
Gated on
GFP+/YFP+/lin- cells
CD36
Sca-1
CD36 mRNA
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CD36+ LSCs
CD36- LSCs
CD36
Actin
CD36+ LSCs
CD36- LSCs

Figure S4. Fatty acid oxidation is elevated in LSCs (related to Figure 4).

(A) FAO rates determined by release of tritiated water (^3H_2O) from tritium labeled palmitate for LSCs and Lin+ leukemia cells were determined under different nutritional conditions. Error bars show means ± S. D. from triplicates. ** *P*<0.005. (B) Heat map shows expression of FAO-related genes in BM LSCs, BM Lin+ leukemia cells and their counterparts from BM non-leukemic cells (Red=up-regulated, Blue=down-regulated). RNA from these four populations was isolated and real-time PCR was performed to determine the expression of FAO-related genes. (C) Surface expression of CD36 on LSCs and their non-leukemic counterparts was examined. Error bars show means ± S. D. n=5, ** *P*<0.005. (D) CD36+/lin- and CD36-/lin- leukemia cells were isolated and 5 minute fatty acid uptake was measured as described in supplemental experimental procedures. Error bars show means ± S. D. from triplicates. * *P*<0.05. (E) Fatty acid uptake in normal CD36+/ lin- and CD36-/lin- cells was determined by the fluorescent fatty acid analog, BODIPY-Dodecanoic acid. Cells were serum starved for 30 minutes and pretreated with or without SSO (50μM) for another 30 minutes, followed by incubation with 1μM BODIPY-Dodecanoic acid and labeling with surface marker antibodies. Fatty acid uptake was determined by the *mean fluorescence intensity* (MFI) of BODIPY-Dodecanoic acid by flow cytometry. Error bars show means ± S. D. from triplicates. * *P*<0.05, ** *P*<0.005. (F) CD36+ and CD36- LSCs were isolated and *CD36* mRNA as well as *CD36* protein was examined.

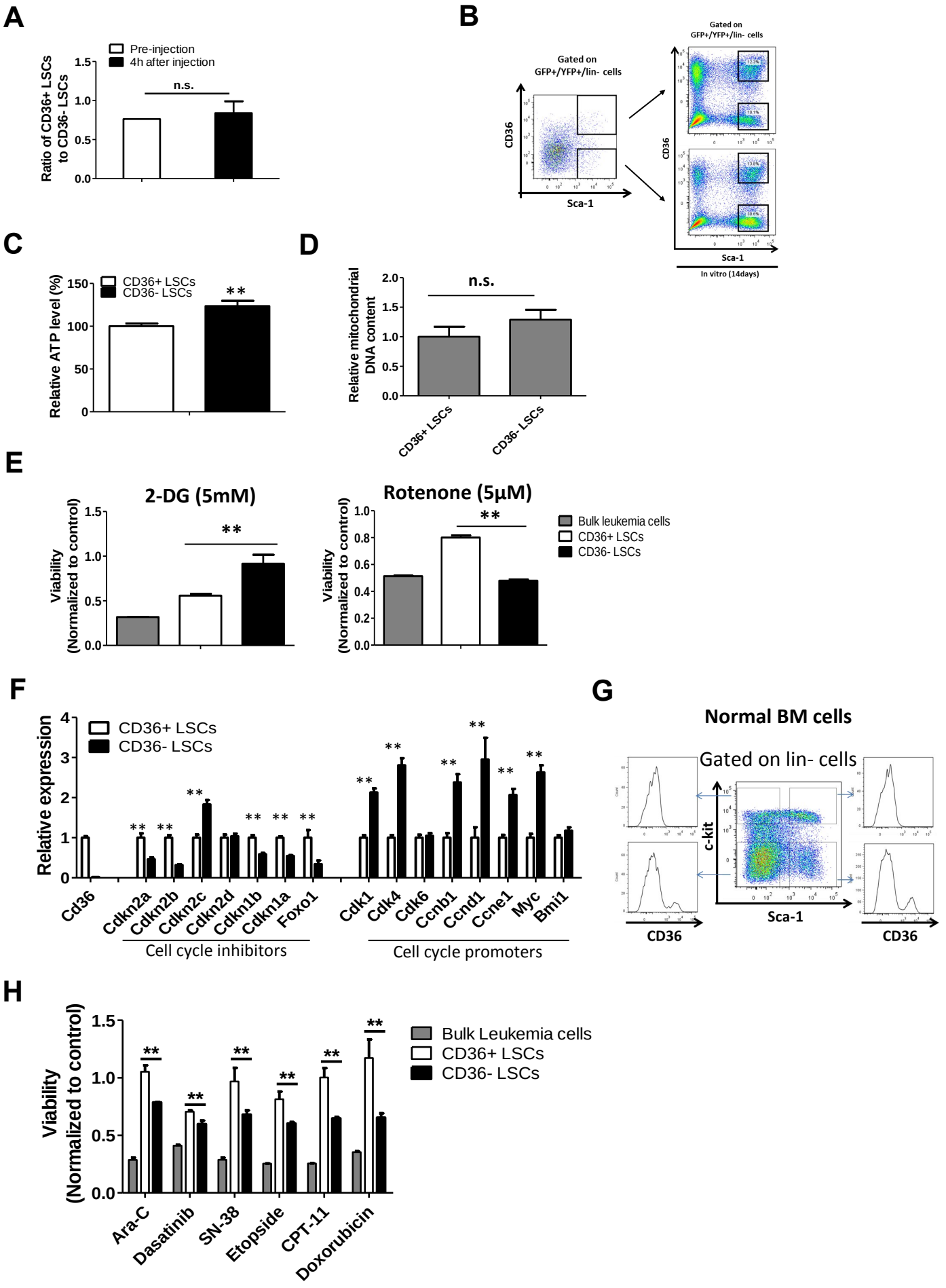
A
Pre-injection
4h after injection
Ratio of CD36+ LSCs to CD36- LSCs
n.s.
B
Gated on GFP+/YFP+/lin- cells
CD36
Sca-1
Gated on GFP+/YFP+/lin- cells
CD36
Sca-1
In vitro (14days)
C
CD36+ LSCs
CD36- LSCs
Relative ATP level (%)
**
D
Relative mitochondrial DNA content
n.s.
CD36+ LSCs
CD36- LSCs
E
2-DG (5mM)
Viability (Normalized to control)
**
Rotenone (5μM)
Viability (Normalized to control)
**
Bulk leukemia cells
CD36+ LSCs
CD36- LSCs
F
CD36+ LSCs
CD36- LSCs
Relative expression
Cd36
Cdkn2a
Cdkn2b
Cdkn2c
Cdkn2d
Cdkn1b
Cdkn1a
Foxo1
Cdk1
Cdk4
Cdk6
Ccnb1
Ccnd1
Ccne1
Myc
Bmi1
Cell cycle inhibitors
Cell cycle promoters
G
Normal BM cells
Gated on lin- cells
c-kit
Sca-1
CD36
CD36
H
Viability (Normalized to control)
Bulk Leukemia cells
CD36+ LSCs
CD36- LSCs
Ara-C
Dasatinib
SN-38
Etopside
CPT-11
Doxorubicin

Figure S5. CD36+ and CD36- LSCs are metabolically distinct (related to Figure 5).

(A) Homing ability of CD36+ and CD36- LSCs to BM. BM bulk leukemia cells were injected into recipient mice. Four hours later, BM cells from recipient mice were collected. Composition of leukemia cells localized to recipients' BM was examined and the ratio of CD36+ LSCs to CD36- LSCs was calculated before and after injection. Error bars show means ± S. D. n=3, n.s., not significant. (B) CD36+ and CD36- LSCs were isolated from primary leukemic bone marrow (populations shown by gates in left panel) and cultured for 14 days. The composition of resulting populations is shown in the right two panels. (C) Comparison of ATP level between CD36+ and CD36- LSCs. Error bars show means ± S. D. from triplicates. ** P<0.005. (D) Comparison of mitochondrial mass (represented by mitochondrial DNA copy number) between CD36+ and CD36- LSCs. Error bars show means ± S. D. from triplicates. (E) Leukemia cells were treated with 2-DG (2-deoxglucose) or rotenone at the indicated doses for 24h and viability of CD36+ LSCs, CD36- LSCs and bulk leukemia cells was determined as described in supplemental experimental procedures. Error bars show means ± S. D. from triplicates. ** P<0.005. (F) CD36+ and CD36- LSCs were isolated and expression of cell cycle related genes was examined by real-time PCR. Error bars show means ± S. D. from triplicates. ** P<0.005. (G) Expression of surface CD36 in normal BM cells. (H) Leukemia cells were treated with chemotherapeutic drugs including Ara-C (0.25μM), Dasatinib (5nM), Doxorubicin (1 μM), SN-38 (1 μM), Etoposide (25 μM) and CPT-11 (40uM) for 24h and viability of CD36+ LSCs, CD36- LSCs and bulk leukemia cells was determined. Error bars show means ± S. D. from triplicates. ** P<0.005.

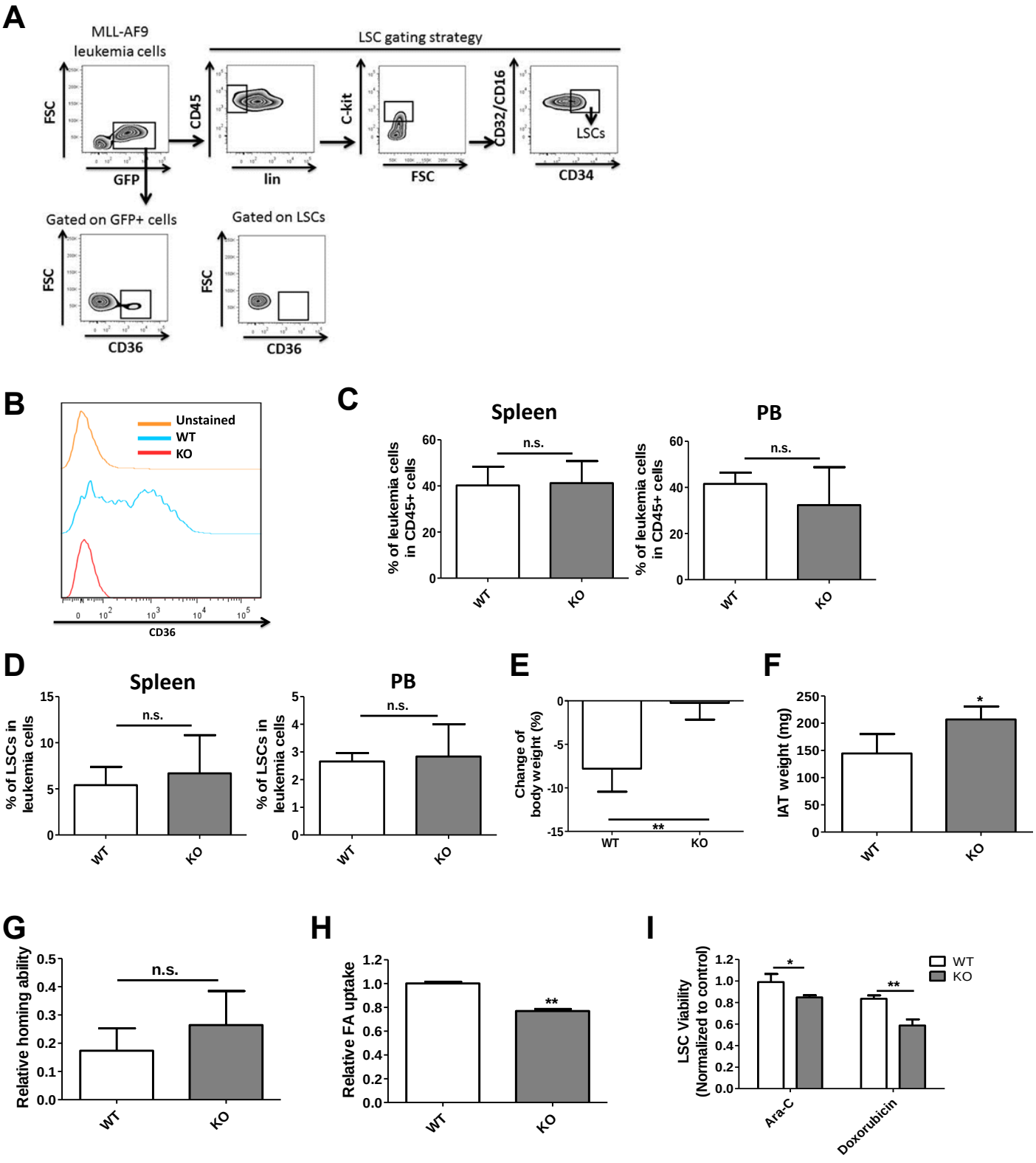
A
MLL-AF9 leukemia cells
LSC gating strategy
FSC
GFP
CD45
lin
C-kit
FSC
CD32/CD16
LSCs
CD34
Gated on GFP+ cells
Gated on LSCs
FSC
CD36
FSC
CD36
B
Unstained
WT
KO
CD36
C
Spleen
PB
% of leukemia cells in CD45+ cells
n.s.
n.s.
WT
KO
WT
KO
D
Spleen
PB
% of LSCs in leukemia cells
n.s.
n.s.
WT
KO
WT
KO
E
Change of body weight (%)
**
WT
KO
F
IAT weight (mg)
*
WT
KO
G
Relative homing ability
n.s.
WT
KO
H
Relative FA uptake
**
WT
KO
I
LSC Viability (Normalized to control)
*
**
WT
KO
Ara-C
Doxorubicin

Figure S6. Loss of CD36 decreases leukemic burden in adipose tissue and sensitizes LSCs to chemotherapy (related to Figure 6).

(A) Expression of CD36 in MLL-AF9 bulk leukemia cells (GFP+ cells) and LSCs. (B) Expression of CD36 in WT LSCs and CD36KO LSCs. (C) Leukemia burden in spleen and peripheral blood (PB) in WT and KO leukemia mice. Error bars show means ± S. D. n=5. (D) Percentage of LSCs in spleen and PB leukemia cells from WT and KO leukemia mice. Error bars show means ± S. D. n=5. (E and F) Change of body weight (E) as well as IAT weight (F) in WT and KO leukemia mice. Error bars show means ± S. D. n=5, * P<0.05, ** P<0.005. (G) Homing ability of WT and CD36 KO LSCs to BM. Homing ability of LSCs was determined by the ratio of the percentage of LSCs in the leukemia cells localized to BM to the percentage of LSCs in leukemia cells before injection. Error bars show means ± S. D. n=4. (H) Fatty acid uptake in lin- WT and CD36 KO leukemia cells. Error bars show means ± S. D. from triplicates. ** P<0.005. (I) Viability of LSCs after 24h treatment with Ara-C (0.25μM) or Doxorubicin (1μM). Error bars show means ± S. D. from triplicates. * P<0.05, ** P<0.005.

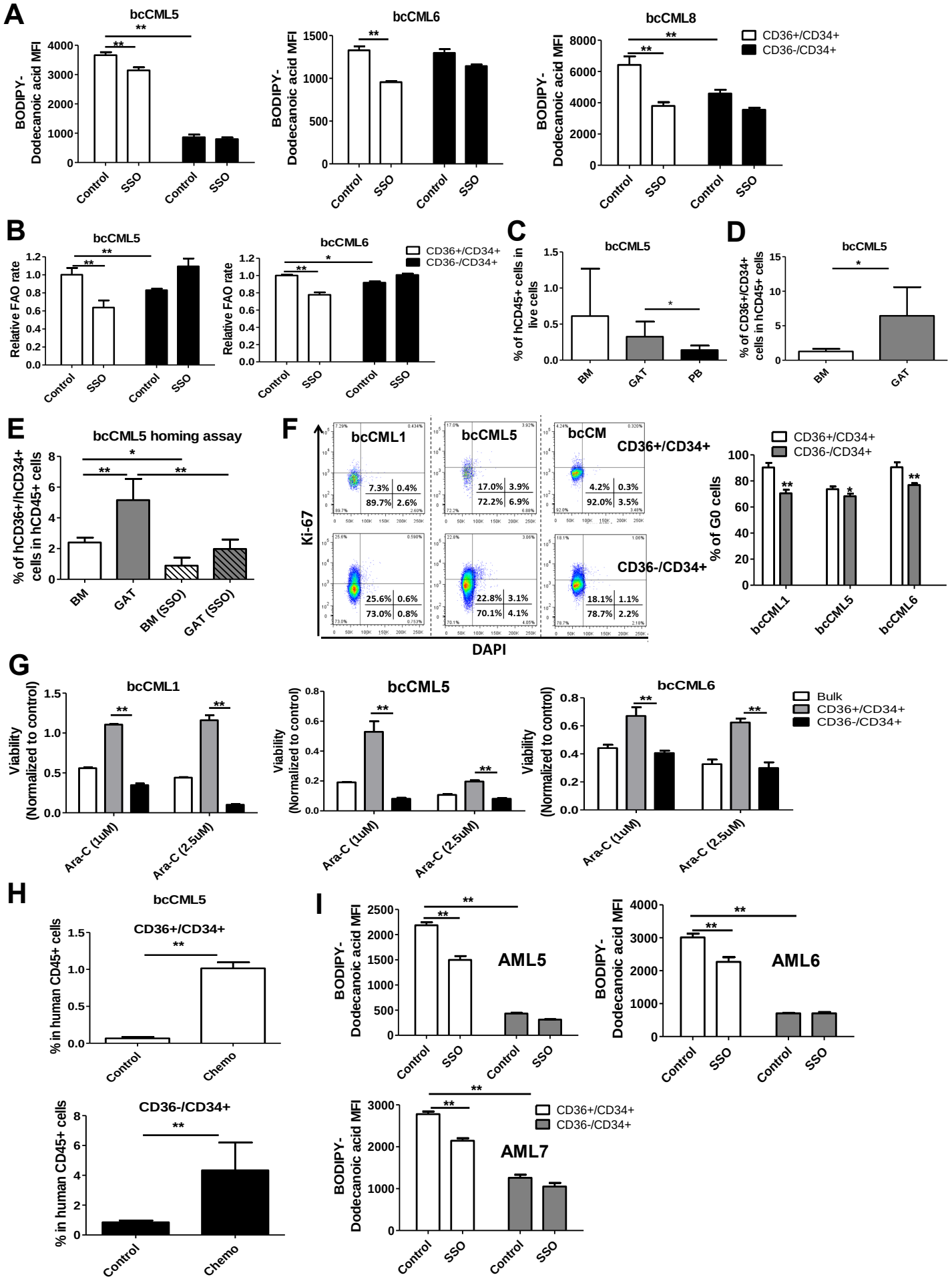
A
bcCML5
bcCML6
bcCML8
BODIPY-Dodecanoic acid MFI
CD36+/CD34+
CD36-/CD34+
Control
SSO
B
bcCML5
bcCML6
Relative FAO rate
C
bcCML5
% of hCD45+ cells in live cells
BM
GAT
PB
D
bcCML5
% of CD36+/CD34+ cells in hCD45+ cells
E
bcCML5 homing assay
% of hCD36+/hCD34+ cells in hCD45+ cells
BM
GAT
BM (SSO)
GAT (SSO)
F
bcCML1
bcCML5
bcCM
CD36+/CD34+
CD36-/CD34+
Ki-67
DAPI
7.3%
0.4%
89.7%
2.6%
17.0%
3.9%
72.2%
6.9%
4.2%
0.3%
92.0%
3.5%
25.6%
0.6%
73.0%
0.8%
22.8%
3.1%
70.1%
4.1%
18.1%
1.1%
78.7%
2.2%
% of G0 cells
bcCML1
bcCML5
bcCML6
G
bcCML1
bcCML5
bcCML6
Viability (Normalized to control)
Bulk
CD36+/CD34+
CD36-/CD34+
Ara-C (1uM)
Ara-C (2.5uM)
H
bcCML5
CD36+/CD34+
% in human CD45+ cells
Control
Chemo
CD36-/CD34+
I
AML5
AML6
AML7
BODIPY-Dodecanoic acid MFI
CD36+/CD34+
CD36-/CD34+

Figure S7. CD36 expression segregates primary human blast crisis CML and AML cells to two functionally distinct populations (related to Figure 7).

(A) Fatty acid uptake in bcCML cells isolated from 3 independent patients. Error bars show means ± S. D. from triplicates. ** *P*<0.005. (B) FAO rate in CD36+/CD34+ vs CD36-/CD34+ cells in the presence or absence of SSO (50 μM). Error bars show means ± S. D. from triplicates. * *P*<0.05, ** *P*<0.005. (C) Leukemia burden in BM, GAT and PB in NSG mice transplanted with human bcCML cells. Error bars show means ± S. D. n=5, **P*<0.05. (D) CD36+/CD34+ cells are enriched in GAT relative to BM. Error bars show means ± S. D. n=5, **P*<0.05. (E) Homing ability of CD36+/CD34+ cells to BM and GAT. Leukemia cells were pre-treated with or without SSO (50 μM) for 1h before injection. Error bars show means ± S. D. n=5, **P*<0.05, ** *P*<0.005. (F) Cell cycle analyses of CD36+/CD34+ vs. CD36-/CD34 leukemia cells. Ki67-/Dapi-low cells are considered as G0 cells. Error bars show means ± S. D. from triplicates. * *P*<0.05, ** *P*<0.005. (G) Human bcCML cells were treated with Ara-C at indicated doses for 48h and the viability of CD36+/CD34+ cells, CD36-/CD34+ cells, and bulk leukemia cells was examined for three independent patient specimens. Error bars show means ± S. D. from triplicates. ** *P*<0.005. (H) Drug resistance of CD36+/CD34+ vs. CD36-/CD34+ leukemia cells. NSG mice transplanted with bcCML cells were treated with Ara-C (100mg/kg/day) or saline (control) for 3 days. The composition of BM residual leukemia cells after chemotherapy was examined. Error bars show means ± S. D. n=4, ** *P*<0.005. (I) Fatty acid uptake in 3 independent AML specimens. Error bars show means ± S. D. from triplicates. ** *P*<0.005.

Table S1

Cytogenetics and mutations found in the eight human primary AML samples used for this study. N/A, not available (related to Figure 7).

Specimen	Cytogenetics	Mutation
AML1	MLL-AF9 , t(9;11)(p22;q23), 45,X,-Y	FLT3-ITD
AML2	N/A	NPM1, FLT3-ITD
AML3	del5q13, t(7;22), del4q21,, t(7;22), t(11;16), -13, -17, -18	FLT3-ITD
AML4	N/A	FLT3+, IDH2
AML5	Normal	NPM1
AML6	N/A	FLT3+
AML7	N/A	NPM1, FLT3-ITD
AML8	Monosomy 7	NPM1, FLT3-ITD, CEBPA

Supplementary Experimental Procedures

Generation of the MLL-AF9 Mouse Model

The mouse model was created as described previously (Somervaille and Cleary, 2006). Primary BM leukemia cells were used in this study to generate 2nd leukemia mice.

Immunofluorescence

Immunofluorescence (IF) of adipose tissue section was performed using anti-GFP antibody (Abcam), anti-CD31 antibody (R&D), anti-perilipin 1 (Plin) antibody (Abcam) and Tyramide signal amplification (TSA) kit (Invitrogen) per manufacturer's instructions.

Fatty acid uptake using BODIPY-Dodecanoic acid

BODIPY-Dodecanoic acid was purchased from Molecular Devices (QBT Fatty Acid Uptake Assay Kit). Briefly, cells (1 million/ml) were serum starved for half hour and pretreated with or without the CD36 inhibitor SSO (50μM) for another half hour in IMDM containing 1% BSA. BODIPY-Dodecanoic acid was added to the medium to a final concentration of 1 μM. After half hour incubation, cells were harvested and labeled with different surface markers. Quantification of fatty acid uptake by different populations was determined by the mean fluorescence intensity (MFI) of BODIPY-Dodecanoic acid.

IL-1α ELISA

Concentration of IL-1 α in culturing medium after fatty acid treatment was determined by Mouse IL-1 alpha ELISA kit (RayBiotech) per manufacturer's instructions.

Chemotherapeutic treatment in blast crisis mouse model

Chemotherapeutic treatment was performed as described previously (!!! INVALID CITATION !!!). Briefly, at day 8 after transplantation with leukemia cells, mice were treated with cytarabine (Ara-C, 100mg/kg/day) combined with doxorubicin (Dox, 3mg/kg/day) for 3 days followed by 2-day treatment of Ara-C (100mg/kg/day). Mice were sacrificed at day 13 after transplantation with leukemia cells.

Homing assay

For homing assays, bulk leukemia cells were injected into recipient mice (20 million cells/mouse) through the retro-orbital sinus. Four hours later, GAT and BM were harvested. BM cells and stromal vascular fraction (SVF) from GAT cells were collected to determine the presence and composition of leukemia cells.

ATP measurement

Measurement of ATP was performed using the ATP Bioluminescence Assay Kit HS II (Roche) per manufacturer's instructions.

In vivo BrdU labeling

BrdU incorporation experiments were performed as described previously (Neering et al., 2007). Briefly, animals were administered 2.0 mg/mouse BrdU via intra-peritoneal injection at 90 minutes prior to being killed. Cells from BM were collected, subjected to cell-surface labeling, then fixed and permeabilized and labeled using Becton Dickinson BrdU Flow kit per manufacturer's instructions. DAPI was used to replace 7-AAD.

Fatty Acid Oxidation

Fatty acid oxidation (FAO) assay was performed as described previously (Ito et al., 2012). Briefly, sorted cells were cultured in IMDM containing 10μM palmitate (Sigma) and 10% FBS (Thermo

Scientific) overnight and then equal numbers of cells were plated in 48-well plates supplemented with FAO assay medium (IMDM containing 2.5% FBS, 10μM palmitic acid, 1% fatty acid free BSA (Sigma), 500μM carnitine (Sigma)). Cells were pulsed for 4 hours with 0.5μCi [9,10-^{3}H(N)]-palmitic acid (Perkinelmer) and the medium was collected to analyze the released $^{3}H_2O$, formed during cellular oxidation of [^{3}H] palmitate. Briefly, medium was precipitated by 10% trichloroacetic acid (Sigma) and then supernatant was neutralized with 6N NaOH (Sigma) and loaded into ion exchange columns packed with DOWEX 1X2-400 resin (Sigma). The radioactive product was eluted with water and quantitated by liquid scintillation counting. For etomoxir (Ex) treatment, cells were incubated with 100μM etomoxir (Sigma) when pulsed with [^{3}H] palmitate. For SSO (Cayman) treatment, cells were pretreated with SSO (50μM) for half hour and then pulsed with [^{3}H] palmitate. For adipocytes CM treatment, after overnight culturing, cells were cultured in medium containing half FAO assay medium and half adipocytes CM or half DMEM containing 1% BSA (control) and then pulsed with [^{3}H] palmitate. For measurement of FAO rates under different nutritional conditions, cells were cultured IMDM containing 10% FBS overnight and then equal numbers of cells were plated in 48-well plates supplemented with IMDM containing 1% fatty acid free BSA (Sigma), 500μM carnitine (Sigma) and indicated concentrations of carbon sources) and then pulsed for 4 hours with 0.5μCi [9,10-^{3}H(N)]-palmitic acid.

Fatty acid uptake

Fatty acid (FA) uptake was performed as described previously (Stremmel and Berk, 1986) except a different fatty acid substrate was used. Briefly, sorted cells were cultured overnight and then equal numbers of cells were incubated for 5 min at 37 °C with [^{3}H] palmitate uptake solution (IMDM containing 10 μM palmitate, 1% BSA, 1μCi [9,10-^{3}H(N)]-palmitic acid (Perkinelmer)). After stopping and washing with ice-cold DPBS containing 1% BSA, cells were lysed with 1 N NaOH and quantitated by scintillation counting. For SSO treatment, cells were pretreated with 50 μM SSO for half hour.

Measurement of free fatty acid

Free fatty acid (FFA) levels in serum and medium were determined by Free fatty acids, half micro test kit (Roche) per manufacturer's instructions. For measurement of FFA level in 3T3-L1 adipocytes culturing medium, 3T3-L1 adipocytes were starved in DMEM containing 1% BSA for 4h before adding indicated cytokines or chemokines or serum (10 µl/ml). Cells were treated for 8h and washed twice with DMEM containing 1% BSA and then cultured in DMEM containing 1% BSA for another 16h. Culturing medium was span to remove residuals and supernatant was collected to determine FFA level. For measurement of FFA level in GAT explants culturing medium, equal amounts of GAT explants (100mg) were cultured in DMEM containing 1% BSA and then treated with indicated cytokines or chemokines or serum (10 µl/ml) for 24h. Culturing medium was then collected to determine FFA level.

Determination of mitochondrial DNA copy number

Determination of mitochondrial DNA copy number was performed as described previously (Tan et al., 2015). Briefly, total DNA was extracted using QIAamp DNA Mini Kit (Qiagen). The ratio of mitochondrial gene MITO1 to nuclear gene B2M1 was measured by real-time PCR to determine the relative mitochondrial DNA copy number.

Induction of adipogenesis in 3T3-L1 cells

Induction of adipogenesis in 3T3-L1 cells was performed as described previously (Lipert et al., 2014). Briefly, 3T3-L1 cells (ATCC) were seeded at 70-80% confluency. Adipogenesis was induced when cells reached full confluency (day 1). Cells were cultured in growth medium (10% FBS DMEM) containing 1 µg/ml insulin (Sigma), 1 µM dexamethasone (Sigma) and 0.5 mM isobutylmethylxanthine (Sigma). After 48h medium was replaced by growth medium supplemented with 1 µg/ml insulin (day 3). Medium was changed every 2 days up to day 9 when fully differentiated adipocytes had been developed. Differentiated adipocytes were starved in serum free DMEM containing 1% BSA for 4h before treatment with cytokines and chemokines.

Isolation of primary adipocytes and adipose tissue leukemia cells

Isolation of primary adipocytes from adipose tissue (AT) was done by enzymatic digestion. Briefly, AT was minced to small pieces and washed with DPBS and then digested in DPBS containing 1mg/ml Liberase TM (Roche) and 1% BSA in shaker (80rmp/min) (Thermo Scientific) at 37°C for 30min. After digestion, span the digested liquid at 1000 rpm to separate adipocytes (top layer) and stromal vascular fraction (SVF) (pellet). Adipocytes were carefully filtered through 100µm mesh strainer and washed with DPBS containing 1% BSA for 2 times. Leukemia cells were in the SVF fraction. Red blood cells in SVF were removed by red blood cell lysis buffer. And then SVF was filtered through 40µm mesh strainer and washed with DPBS containing 2% FBS.

Conditioned medium (CM) from adipocytes

Primary adipocytes were cultured in DMEM containing 1% BSA for 24h and culturing medium was collected and span to remove any residual cell in the medium. Supernatant was collected as CM.

Immunoblot analysis

Primary antibodies for LPL and CIDEA were purchased from Santa Cruz Biotechnology; Primary antibodies for ATGL, ACTIN, TUBULIN and CD36 were purchased from Cell Signaling Technologies, Sigma, Abcam and Novus Biologicals respectively. Immunoblot was performed as previously described (Guzman et al., 2005). HRP detection was performed using the automated Gel Doc XR+ system and the Image Lab Software (Bio-Rad Laboratories).

Cell cycle analysis of human blast crisis CML cells

Cell cycle status of human blast crisis CML cells was determined as described previously (Lagadinou et al., 2013).

Viability analysis

Leukemia cells were treated with indicated drugs and the number of living cells after treatment was determined. Composition of viable cells was determined by flow cytometry. Change of cell number of indicated populations compared to control group was calculated and presented as change of viability.

Isolation of RNA

RNA from adipocytes/adipose tissue (AT) was extracted using Tri Reagent Solution (Thermo Scientific) per manufacturer's instructions. RNA from leukemia cells was extracted using RNeasy Plus Mini Kit (Qiagen) or RNeasy Micro Kit (Qiagen) per manufacturer's instructions.

Limiting dilution analysis

Limiting dilution analysis was performed as described previously (Ashton et al., 2012).

Cytokine array

Cytokine array was performed using the Mouse Cytokine Antibody Array, Panel A (R&D systems) per manufacturer's instructions. For gonadal adipose tissue (GAT) explants conditioned medium (CM) cytokine array, GAT was minced to small pieces and washed with DPBS. GAT explants (0.3g) were then cultured in DMEM containing 1% BSA for 24h. Culturing medium was then collected for cytokine array.

Flow cytometry analysis

Flow cytometry was performed using the BD™ LSR II Flow Cytometer System. Following antibodies were used in this study: murine Sca-1 antibody and lineage markers antibodies (Gr-1, CD3, Ter-119 and B220) were purchased from Biolegend; murine CD45 and CD36 antibodies were purchased from BD; human CD45, CD34 and CD36 antibodies were purchased from BD.

RNA-seq

RNA-seq was performed as described previously (Lagadinou et al., 2013). Low Input RNA-seq library was prepared using Clontech SMARTer chemistry followed by Illumina NexteraXT library construction. Sequenced reads were cleaned according to a rigorous pre-processing workflow (Trimmomatic-0.32) before mapping them to the *Mus musculus* (mm10) with SHRiMP2.2.3 (http://compbio.cs.toronto.edu/shrimp/). Cufflinks2.0.2 (cuffdiff2 - Running Cuffdiff) was used to perform differential expression analysis with an FDR cutoff of 0.05 (95% confidence interval). Results were deposited to NCBI GEO with the GEO number GSE81842.

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