Figure S1

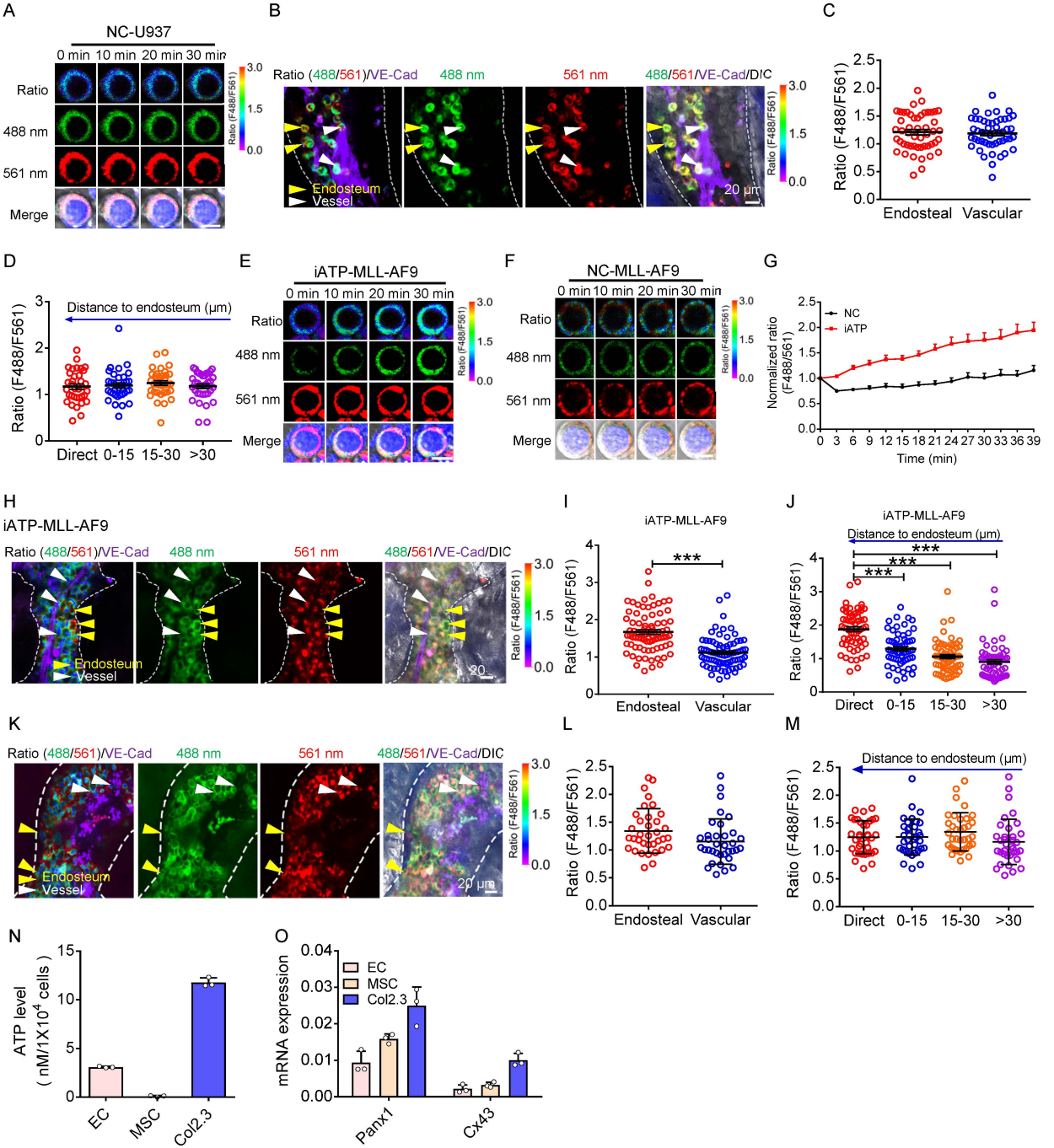
A
NC-U937
0 min 10 min 20 min 30 min
Ratio
488 nm
561 nm
Merge
Ratio (F488/F561)
3.0
1.5
0.0
B
Ratio (488/561)/VE-Cad
488 nm
561 nm
488/561/VE-Cad/DIC
Endosteum
Vessel
20 μm
C
Ratio (F488/F561)
Endosteal Vascular
D
Distance to endosteum (μm)
Ratio (F488/F561)
Direct 0-15 15-30 >30
E
iATP-MLL-AF9
F
NC-MLL-AF9
G
NC
iATP
Normalized ratio (F488/561)
Time (min)
H
iATP-MLL-AF9
I
iATP-MLL-AF9
***
Endosteal Vascular
J
iATP-MLL-AF9
Distance to endosteum (μm)
Direct 0-15 15-30 >30
K
L
M
N
ATP level (nM/1X10^4 cells)
EC
MSC
Col2.3
O
mRNA expression
Panx1
Cx43

Figure S1, related to Figure 1. The endosteal niche has a much higher ATP level than the vascular niche. A) Representative images of the control sensor (mCherry-cpSFGFP, NC) fluorescence ratio at excitation wavelengths of 488 nm and 561 nm (F488/F561 nm) in NC-U937 cells at the indicated time points upon the ATP stimulation. B) Representative images of the localization of NC-U937 cells expressing the control sensor in the endosteal niche (dashed lines) or the vascular niche and their ratios of fluorescence (488/561 nm) are shown. C) Quantification of the fluorescence ratio of the control sensor in panel B (n=approximately 50 cells/group from 3 biological replicates). D) Quantification of the fluorescence ratio of NC-U937 cells expressing the control sensor and their relationships to the distance to the endosteum in panel B (n=approximately 35 cells/group from 3 biological replicates). E-F) Representative images of iATPSnFR (E) and the control sensor (F) fluorescence ratio at excitation wavelengths of 488 nm and 561 nm (F488/F561 nm) in MLL-AF9^{+} murine AML cells at the indicated time points upon ATP stimulation. G) Quantification of the fluorescence ratio of the iATPSnFR and control sensor in murine AML cells (n=25 cells/group). H) Representative images of the localization of iATPSnFR-AML cells in the endosteal niche (dashed lines) and the vascular niche and their fluorescence ratios. Scale bar, 10 μm. I) Quantification of the iATPSnFR fluorescence ratio in murine iATPSnFR-AML cells in the endosteal and the vascular niche in panel H (n=approximately 80 cells/group from 4 biological replicates, ***P<0.001, one-way ANOVA with Tukey's multiple comparison test). J) Quantification of the iATPSnFR fluorescence ratio in murine iATPSnFR-AML cells and their relationships to the

distance to the endosteum in panel H (n=approximately 60 cells/group from 4 biological replicates, ***P<0.001, one-way ANOVA with Tukey's multiple comparison test). K) Representative images of the localization of MLL-AF9^{+} murine AML cells expressing the control sensor in the endosteal niche (dashed lines) or the vascular niche and their ratios of fluorescence (488/561 nm) are shown. Scale bar, 10 μm. L) Quantification of the fluorescence ratio of the control sensor in panel K (n=approximately 50 cells/group from 3 biological replicates). M) Quantification of the fluorescence ratio of MLL-AF9^{+} murine AML cells expressing the control sensor and their relationships to the distance to the endosteum in panel K (n=approximately 35 cells/group from 3 biological replicates). N) The ATP levels were determined in the supernatant of several key types of niche cells in the BM, including CD31^{-}CD45^{-}Ter119^{-}Lepr^{+} mesenchymal stem cells (MSC), CD31^{+}CD45^{-}Ter119^{-} endothelial cells (EC) and Col2.3^{+} osteoblasts (Col2.3), 24 h after culture in vitro. O) The mRNA levels of Panx1 and Cx43 were measured in several niche cells, including CD31^{-}CD45^{-}Ter119^{-}Lepr^{+} mesenchymal stem cells (MSC), CD31^{+}CD45^{-}Ter119^{-} endothelial cells (EC) and Col2.3^{+} osteoblasts (Col2.3), by quantitative RT-PCR (n=3).

Figure S2

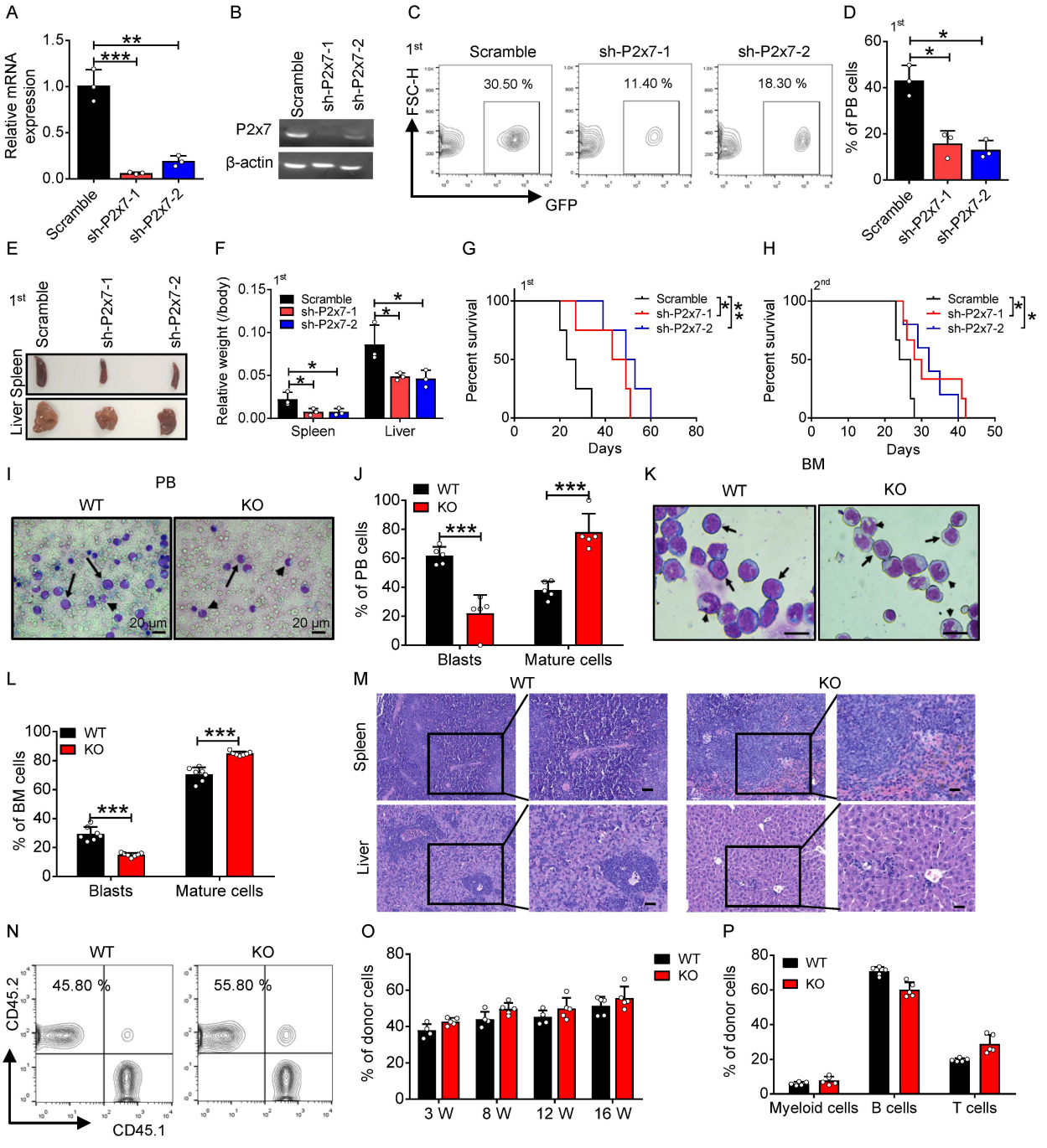
A
Relative mRNA expression
Scramble
sh-P2x7-1
sh-P2x7-2
B
P2x7
β-actin
C
1st
FSC-H
GFP
30.50 %
11.40 %
18.30 %
D
% of PB cells
E
Liver
Spleen
F
Relative weight (/body)
Spleen
Liver
G
Percent survival
Days
H
2nd
I
PB
WT
KO
20 μm
J
% of PB cells
Blasts
Mature cells
K
BM
L
% of BM cells
M
Spleen
Liver
N
45.80 %
55.80 %
CD45.2
CD45.1
O
% of donor cells
3 W
8 W
12 W
16 W
P
Myeloid cells
B cells
T cells

Figure S2, related to Figure 2. P2X7 is highly expressed in LICs and promotes AML development. A-B) The knockdown efficiency of shRNAs (sh-P2x7-1 and -2) targeting murine P2x1 was determined by quantitative RT-PCR (A, n=3). Representative eletrophoresis images of the RT-PCR products are shown (B). C) Representative flow cytometric analysis of GFP^+ AML cells in the peripheral blood of recipient mice receiving the transplants of P2x7-knockdown MLL-$AF9^+$ BM cells or the scrambled control upon primary transplantation. D) Quantification of the frequency of the AML cells in panel C (n=5; *, $P<0.05$; one-way ANOVA with Tukey's multiple comparison test). E-F) Representative images of the size of the spleens and livers of recipient mice in panel C (E) and quantification of the weight of spleens and livers are shown (F, n=3; ***, $P<0.001$; two-way ANOVA with Sidak's multiple comparison test). G-H) The overall survival of the recipient mice transplanted with the P2x7-knockdown MLL-$AF9^+$ BM cells or the scrambled control upon primary (G) and secondary transplantation (H) (n=5; *, $P<0.05$, **, $P<0.01$; log-rank test). I) Representative images of the Giemsa-Wright staining for WT and P2x7-KO AML cells in the peripheral blood upon secondary transplantation. J) Quantification of the frequency of the blast cells in panel I (n=5-6; ***, $P<0.001$; two-way ANOVA with Sidak's multiple comparison test). K) Representative images of the Giemsa-Wright staining for WT and P2x7-KO AML cells in the BM upon 21 days after transplantation. L) Quantification of the frequency of the blast cells in panel K (n=5-6; ***, $P<0.001$; two-way ANOVA with Sidak's multiple comparison test). M) Histological HE staining of the livers and spleens of the recipients transplanted with WT and P2x7-KO

AML cells upon primary transplantation. N-O) Representative flow cytometric analysis of donor cells (CD45.2) in the peripheral blood of recipient mice upon the competitive BM transplantation is shown (N). The frequency WT and P2x7-KO donor cells was evaluated 3, 8, 12 and 16 weeks after transplantation (O). P) Multilineages of donor cells in the recipient mice transplanted with WT and P2x7-KO normal BM cells 16 weeks post-transplantation (n=5). Scale bar, 20 μm.

Figure S3

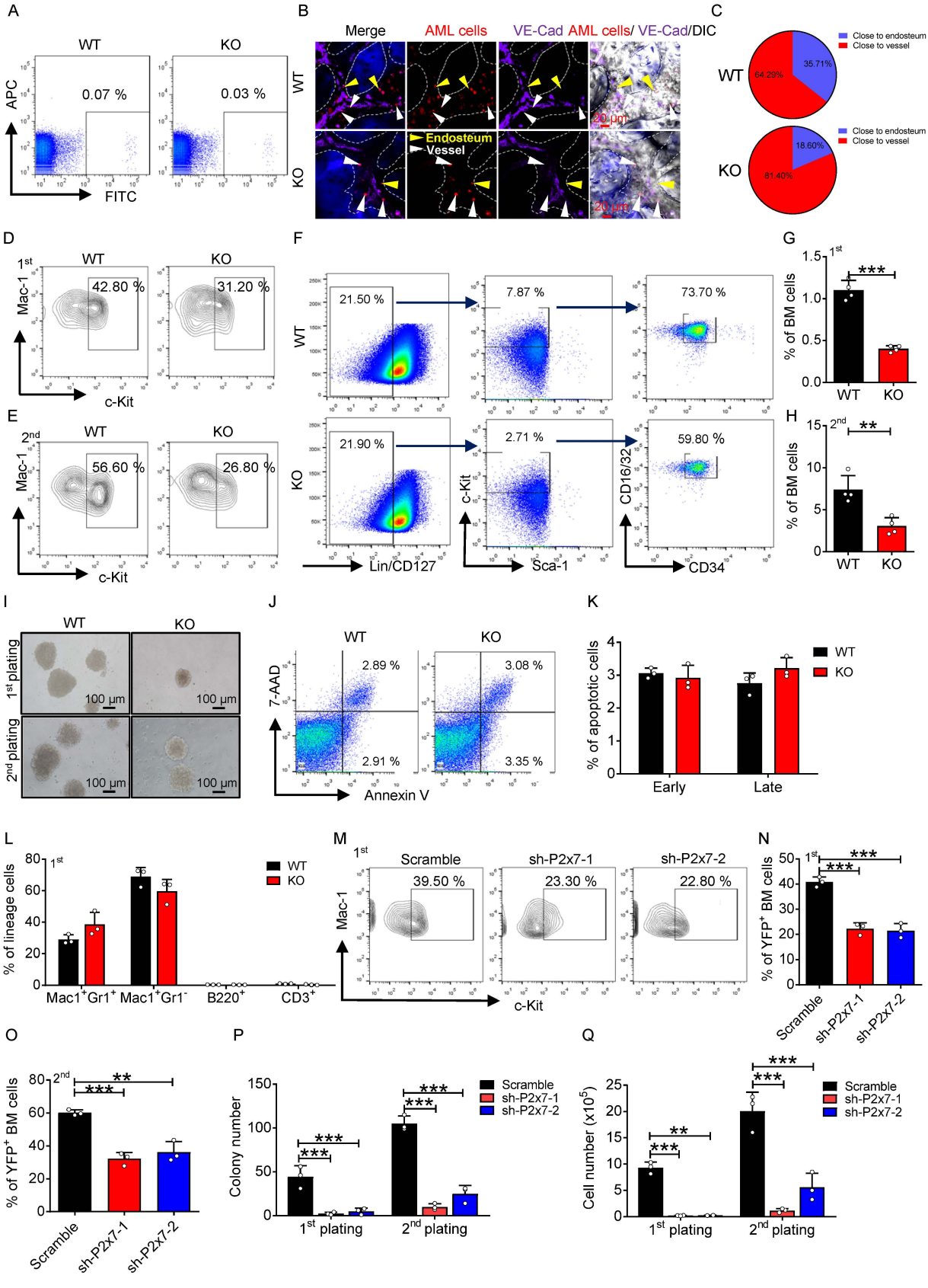
A
WT
KO
0.07 %
0.03 %
APC
FITC
B
Merge
AML cells
VE-Cad AML cells/ VE-Cad/DIC
WT
KO
Endosteum
Vessel
20 μm
C
WT
64.29%
35.71%
Close to endosteum
Close to vessel
KO
81.40%
18.60%
D
1st
Mac-1
c-Kit
42.80 %
31.20 %
E
2nd
56.60 %
26.80 %
F
21.50 %
7.87 %
73.70 %
21.90 %
2.71 %
59.80 %
Lin/CD127
Sca-1
CD16/32
CD34
G
% of BM cells
***
H
**
I
1st plating
2nd plating
100 μm
J
7-AAD
Annexin V
2.89 %
3.08 %
2.91 %
3.35 %
K
% of apoptotic cells
Early
Late
L
% of lineage cells
Mac1+Gr1+
Mac1+Gr1-
B220+
CD3+
M
Scramble
sh-P2x7-1
sh-P2x7-2
39.50 %
23.30 %
22.80 %
N
% of YFP+ BM cells
O
P
Colony number
1st plating
2nd plating
Q
Cell number (x10^5)

Figure S3, related to Figure 3. ATP-P2X7 signaling maintains the homing and self-renewal abilities of LICs. A) Representative flow cytometric analysis for CFSE-labeled WT and P2x7-KO leukemia cells in the BM of the recipients 16 h after transplantation. B) Representative images of the localizations of AML cells (red) in BM niches. The vascular niche (vessel) was labeled with the anti-VE-Cadherin antibody (purple) and the endosteal niche (endosteum) was indicated by the dashed lines. C) Percentages of AML cells attached to the endosteal niche or the vascular niche in panel B were calculated (n=approximately 43 cells/group from 3 biological replicates). D-E) Representative flow cytometric analysis for WT and P2x7-KO Mac-1$^+$c-Kit$^+$LICs of the recipients upon primary (D) and secondary (E) transplantation. F) Representative flow cytometric analysis for WT and KO L-GMPs (Lin$^-$Sca-1$^+$c-Kit$^+$CD34$^+$CD16/CD32$^+$) of the recipients upon primary transplantation. G-H) Quantification of the frequency of L-GMPs of the recipients in panel F upon primary (G) and secondary transplantation (H) (n=4; **, P<0.01, ***, P<0.001; Student's t test). I) Representative images of colony formation of WT and P2x7-KO YFP$^+$ AML cells during the 1st and 2nd plating. J) Representative flow cytometric analysis of apoptosis of WT or P2x7-KO Mac-1$^+$c-Kit$^+$ LICs. K) Quantification of data in panel J (n=3). L) Quantitative results of the differentiation status of BM cells in the recipient mice as determined with myeloid cell markers (Mac-1/Gr-1), B cell markers (B220) and T cell markers (CD3e) upon primary transplantation (n=5). M) Representative flow cytometric analysis for the Mac-1$^+$c-Kit$^+$ LICs upon the P2x7 knockdown (sh-P2x7-1 and -2) from the recipients upon primary transplantation. N-O) Quantification of the

frequency of LICs in BM of the recipients in panel M upon primary (N) and secondary (O) transplantation (n=3; **, P<0.01, ***, P<0.001; one-way ANOVA with Tukey's multiple comparison test). P-Q) The colony numbers (P) and derived total cell counts (Q) of the AML cells upon the P2x7 knockdown (sh-P2x7-1 and -2) during the 1st and 2nd plating (n=3; **, P<0.01, ***, P<0.001; two-way ANOVA with Sidak's multiple comparison test).

Figure S4

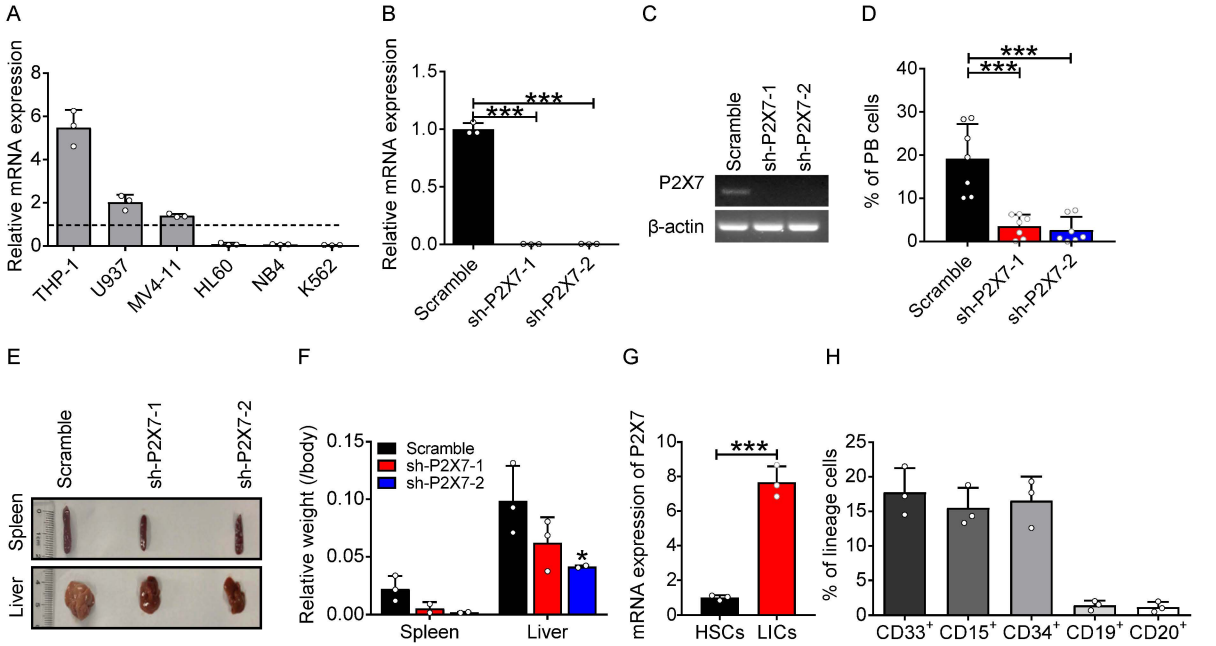
A
Relative mRNA expression
8
6
4
2
0
THP-1
U937
MV4-11
HL60
NB4
K562
B
Relative mRNA expression
1.5
1.0
0.5
0.0
***
***
Scramble
sh-P2X7-1
sh-P2X7-2
C
Scramble
sh-P2X7-1
sh-P2X7-2
P2X7
β-actin
D
% of PB cells
40
30
20
10
0
***
***
Scramble
sh-P2X7-1
sh-P2X7-2
E
Scramble
sh-P2X7-1
sh-P2X7-2
Spleen
Liver
F
Relative weight (/body)
0.15
0.10
0.05
0.00
Scramble
sh-P2X7-1
sh-P2X7-2
*
Spleen
Liver
G
mRNA expression of P2X7
10
8
6
4
2
0
***
HSCs
LICs
H
% of lineage cells
25
20
15
10
5
0
CD33+
CD15+
CD34+
CD19+
CD20+

Figure S4, related to Figure 4. P2X7 is required for the proliferation of human AML cells. A) The mRNA levels of P2X7 in THP-1, U937, MV4-11, HL60, NB4 and K562 cells was measured by quantitative RT-PCR (n=3). B-C) The knockdown efficiency of P2X7 was measured in THP-1 cells targeted by the shRNAs (sh-P2X7-1 and -2) by quantitative RT-PCR (n=3; ***, $P<0.001$; one-way ANOVA with Tukey's multiple comparison test). Representative electrophoresis images of RT-PCR products are shown in panel C. D) Quantification of the frequency of the AML cells in the peripheral blood of recipient mice receiving the transplants of P2X7-knockdown THP-1 cells or the scrambled control upon transplantation. (n=7; ***, $P<0.001$; one-way ANOVA with Tukey's multiple comparison test). E) Representative images of the spleens and livers of the recipient mice transplanted with THP-1 cells infected with shRNAs targeting P2X7 (sh-P2X7-1 and -2) and the scrambled controls. F) Quantification of the weight of spleens and livers in panel E (n=3; *, $P<0.05$; two-way ANOVA with Sidak's multiple comparison test). G) The mRNA levels of P2X7 in human $Lin^-CD34^+CD38^-CD90^+CD45RA^-$ HSCs and $Lin^-CD34^+CD38^-CD90^-CD45RA^+$ LICs were measured by quantitative RT-PCR (n=3; ***, $P<0.001$; Student's t test). H) The frequency of lineage cells in NOD-SCID mice transplanted with the patient's AML cells (AML #1).

Figure S5

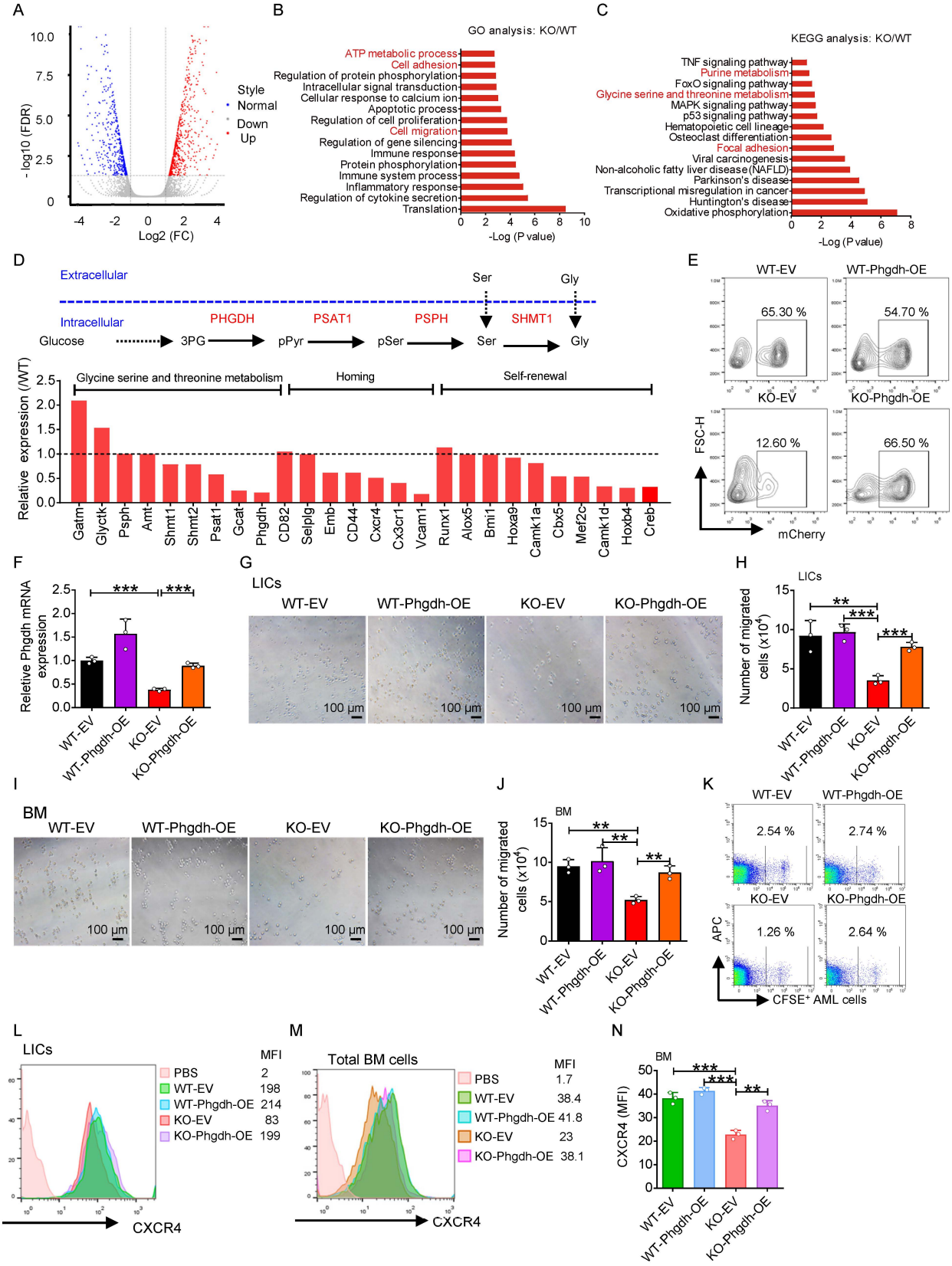
A
10.0
7.5
5.0
2.5
0
- log10 (FDR)
-4
-2
0
2
4
Log2 (FC)
Style
Normal
Down
Up
B
GO analysis: KO/WT
ATP metabolic process
Cell adhesion
Regulation of protein phosphorylation
Intracellular signal transduction
Cellular response to calcium ion
Apoptotic process
Regulation of cell proliferation
Cell migration
Regulation of gene silencing
Immune response
Protein phosphorylation
Immune system process
Inflammatory response
Regulation of cytokine secretion
Translation
0 2 4 6 8 10
-Log (P value)
C
KEGG analysis: KO/WT
TNF signaling pathway
Purine metabolism
FoxO signaling pathway
Glycine serine and threonine metabolism
MAPK signaling pathway
p53 signaling pathway
Hematopoietic cell lineage
Osteoclast differentiation
Focal adhesion
Viral carcinogenesis
Non-alcoholic fatty liver disease (NAFLD)
Parkinson's disease
Transcriptional misregulation in cancer
Huntington's disease
Oxidative phosphorylation
0 2 4 6 8
-Log (P value)
D
Extracellular
Intracellular
Ser
Gly
Glucose
3PG
PHGDH
pPyr
PSAT1
pSer
PSPH
Ser
SHMT1
Gly
Relative expression (/WT)
Glycine serine and threonine metabolism
Homing
Self-renewal
Gatm
Glyctk
Psph
Amt
Shmt1
Shmt2
Psat1
Gcat
Phgdh
CD82
Selplg
Emb
CD44
Cxcr4
Cx3cr1
Vcam1
Runx1
Alox5
Bmi1
Hoxa9
Camk1a
Cbx5
Mef2c
Camk1d
Hoxb4
Creb
E
WT-EV
WT-Phgdh-OE
65.30 %
54.70 %
KO-EV
KO-Phgdh-OE
12.60 %
66.50 %
FSC-H
mCherry
F
Reletive Phgdh mRNA expression
***
***
WT-EV
WT-Phgdh-OE
KO-EV
KO-Phgdh-OE
G
LICs
WT-EV
WT-Phgdh-OE
KO-EV
KO-Phgdh-OE
100 μm
H
LICs
Number of migrated cells (x10⁴)
**
***
***
WT-EV
WT-Phgdh-OE
KO-EV
KO-Phgdh-OE
I
BM
WT-EV
WT-Phgdh-OE
KO-EV
KO-Phgdh-OE
100 μm
J
BM
Number of migrated cells (x10⁴)
**
**
**
WT-EV
WT-Phgdh-OE
KO-EV
KO-Phgdh-OE
K
WT-EV
WT-Phgdh-OE
2.54 %
2.74 %
KO-EV
KO-Phgdh-OE
1.26 %
2.64 %
APC
CFSE⁺ AML cells
L
LICs
MFI
PBS 2
WT-EV 198
WT-Phgdh-OE 214
KO-EV 83
KO-Phgdh-OE 199
CXCR4
M
Total BM cells
MFI
PBS 1.7
WT-EV 38.4
WT-Phgdh-OE 41.8
KO-EV 23
KO-Phgdh-OE 38.1
CXCR4
N
BM
CXCR4 (MFI)
***
***
**
WT-EV
WT-Phgdh-OE
KO-EV
KO-Phgdh-OE

Figure S5, related to Figure 5. ATP-P2X7 signaling maintains LIC activities by activating the PHGDH pathway. A) Volcano plots show differentially expressed genes in WT and P2x7-KO Mac-1$^+$c-Kit$^+$ LICs. B-C) GO (biological process) and KEGG (pathway) analyses were performed with mRNA-sequencing data of WT or P2x7-KO Mac-1$^+$c-Kit$^+$ LICs (n=3 mice/group for three independent experiments and representative data from one experiment are shown). D) Candidate genes related to the glycine serine and threonine metabolism, self-renewal and homing were analyzed in the RNA-sequencing data of WT and P2x7-KO Mac-1$^+$c-Kit$^+$ LICs. E) Representative flow cytometric analysis of leukemia cells (YFP$^+$ mCherry$^+$) in the peripheral blood of recipient mice transplanted with WT, P2x7-KO, Phgdh-overexpressing WT or P2x7-KO leukemia cells. F) The mRNA levels of Phgdh in BM cells of recipient mice transplanted with WT, P2x7-KO, Phgdh-overexpressing WT or P2x7-KO AML cells were measured by quantitative RT-PCR (n=3; ***, P<0.001; one-way ANOVA with Tukey's multiple comparison test). G-H) A transwell assay was used to test the migration abilities of WT, P2x7-KO, Phgdh-overexpressing WT or P2x7-KO Mac-1$^+$c-Kit$^+$ LICs. Panel G is the representative images of migrated cells and panel H is the quantification data in panel G (n=3; **, P<0.01, ***, P<0.001; one-way ANOVA with Tukey's multiple comparison test). I) The representative images of migrated bulk leukemia cells from the groups of WT, P2x7-KO, Phgdh-overexpressing WT or P2x7-KO recipients by a transwell assay. J) Quantification of the numbers in panel I (n=3; **, P<0.01; one-way ANOVA with Tukey's multiple comparison test). K) Representative flow cytometric analysis for CFSE-labeled WT, P2x7-KO, Phgdh-

overexpressing WT or P2x7-KO leukemia cells in the BM of the recipients 16 h after transplantation. L) The expression levels of CXCR4 in WT, P2x7-KO, Phgdh-overexpressing WT or P2x7-KO Mac-1^{+}c-Kit^{+} LICs were analyzed by flow cytometry. M) The expression levels of CXCR4 in WT, P2x7-KO, Phgdh-overexpressing WT or P2x7-KO AML cells were analyzed by flow cytometry. N) Quantification of the mean fluorescence intensities (MFI) in panel M (n=3; **, $P<0.01$, ***, $P<0.001$; one-way ANOVA with Tukey's multiple comparison test).

Figure S6

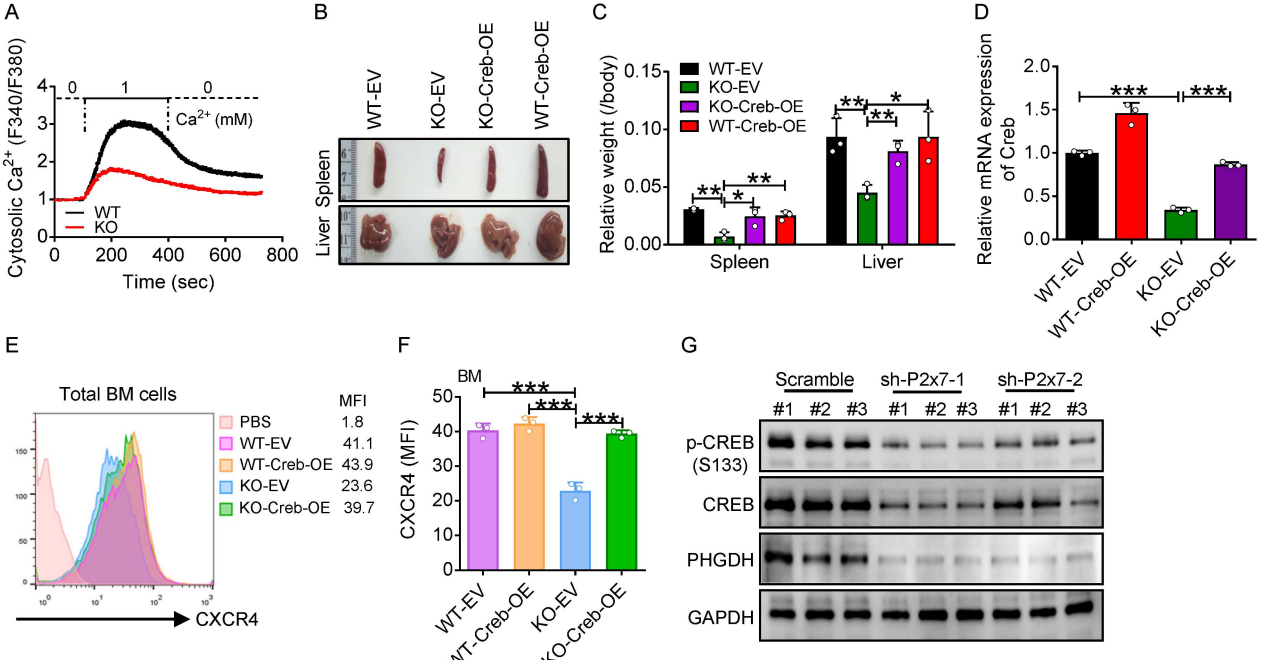
A
Cytosolic Ca2+ (F340/F380)
0
1
0
Ca2+ (mM)
WT
KO
Time (sec)
B
WT-EV
KO-EV
KO-Creb-OE
WT-Creb-OE
Liver
Spleen
C
Relative weight (/body)
WT-EV
KO-EV
KO-Creb-OE
WT-Creb-OE
Spleen
Liver
D
Relative mRNA expression of Creb
WT-EV
WT-Creb-OE
KO-EV
KO-Creb-OE
E
Total BM cells
MFI
PBS 1.8
WT-EV 41.1
WT-Creb-OE 43.9
KO-EV 23.6
KO-Creb-OE 39.7
CXCR4
F
BM
CXCR4 (MFI)
WT-EV
WT-Creb-OE
KO-EV
KO-Creb-OE
G
Scramble
sh-P2x7-1
sh-P2x7-2
#1 #2 #3 #1 #2 #3 #1 #2 #3
p-CREB (S133)
CREB
PHGDH
GAPDH

Figure S6, related to Figure 6. CREB signaling maintains PHGDH levels to enhance leukemogenesis. A) Constitutive calcium influx was measured in WT and P2x7-KO bulk AML cells using Fura-2 AM (0: Ca^{2+}-free solution;1: solution with 1 mM Ca^{2+}). B) Representative images of the spleens and livers of the recipient mice transplanted with WT, P2x7-KO, Creb-overexpressing WT or P2x7-KO AML cells. C) Quantification of the weight of spleens and livers in panel B (n=3; *, P<0.05, **, P<0.01; two-way ANOVA with Sidak's multiple comparison test). D) mRNA levels of Creb in BM cells of the recipient mice transplanted with WT, P2x7-KO, Creb-overexpressing WT or P2x7-KO leukemia cells were measured by quantitative RT-PCR (n=3; ***, P<0.001; one-way ANOVA with Tukey's multiple comparison test). E) The protein expression levels of CXCR4 in WT, P2x7-KO, Creb-overexpressing WT or P2x7-KO AML cells was analyzed by flow cytometry. F) Quantification of the MFI in panel E (n=3; ***, P<0.001; one-way ANOVA with Tukey's multiple comparison test). G) The protein levels of phospho-CREB (S133), CREB and PHGDH were determined in Mac-1^{+}c-Kit^{+} LICs upon the knockdown of P2x7 (sh-P2x7-1 and -2) by western blot.

Figure S7

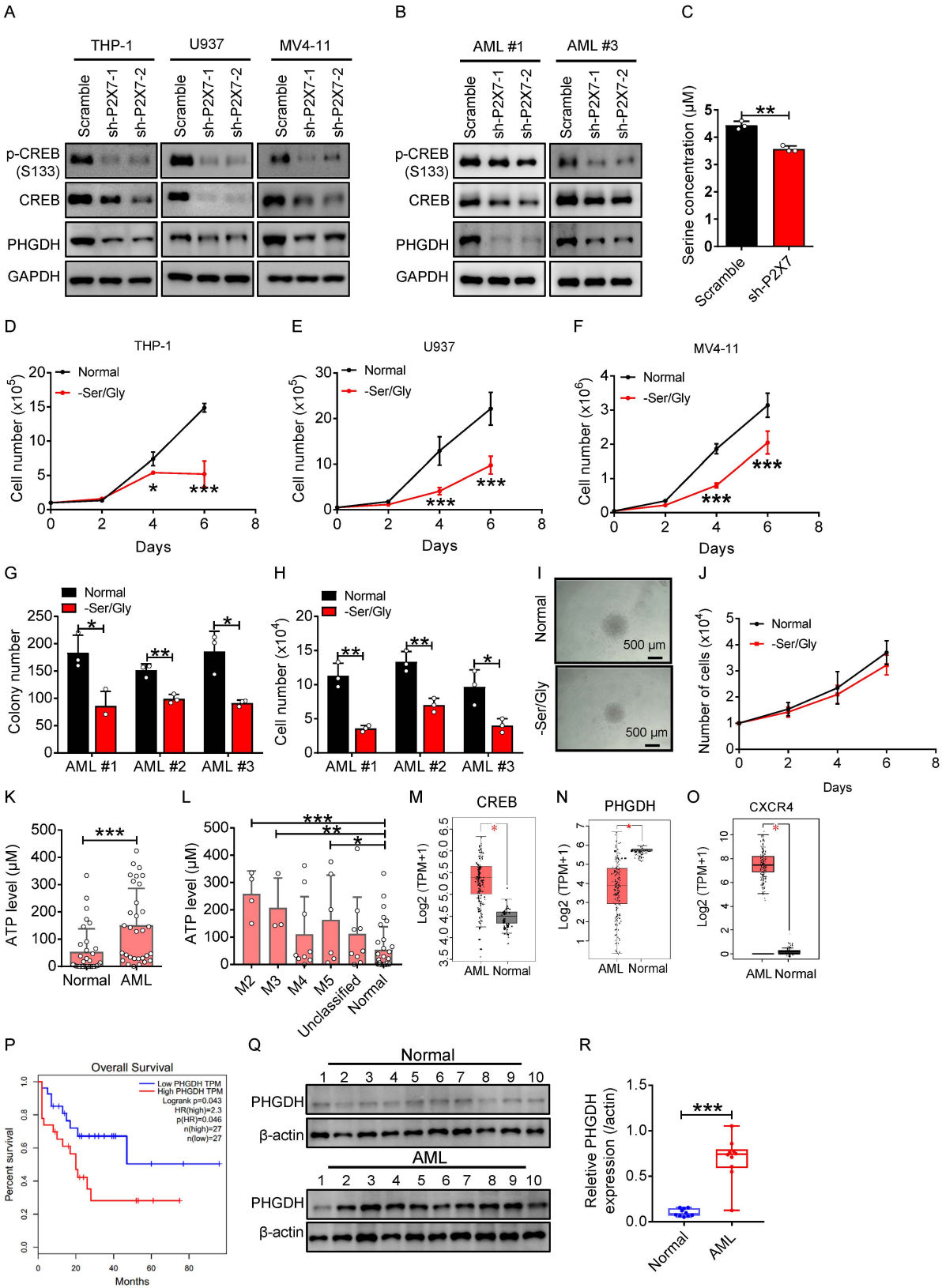
A
THP-1
U937
MV4-11
Scramble
sh-P2X7-1
sh-P2X7-2
p-CREB (S133)
CREB
PHGDH
GAPDH
B
AML #1
AML #3
C
Serine concentration (μM)
Scramble
sh-P2X7
D
THP-1
E
U937
F
MV4-11
Normal
-Ser/Gly
Cell number (x10^5)
Cell number (x10^6)
Days
G
Colony number
AML #1
AML #2
AML #3
H
Cell number (x10^4)
I
500 μm
J
Number of cells (x10^4)
K
ATP level (μM)
Normal
AML
L
M2
M3
M4
M5
Unclassified
M
CREB
N
PHGDH
O
CXCR4
Log2 (TPM+1)
AML Normal
P
Overall Survival
Low PHGDH TPM
High PHGDH TPM
Logrank p=0.043
HR(high)=2.3
p(HR)=0.046
n(high)=27
n(low)=27
Percent survival
Months
Q
PHGDH
β-actin
R
Reletive PHGDH expression (/actin)

Figure S7, related to Figure 6. ATP-P2X7 signaling maintains human LIC activities by activating the CREB/PHGDH pathway. A) The protein levels of phospho-CREB (S133), CREB and PHGDH were determined in the P2X7-knockdown THP-1, U937 and MV4-11 cells (sh-P2X7-1 or sh-P2X7-2) or the scrambled controls by western blot. B) The protein levels of phospho-CREB (S133), CREB and PHGDH were determined in the P2X7-knockdown human $CD45^{+}GFP^{+}$ AML cells (sh-P2X7-1 or sh-P2X7-2, AML#1 and AML#3) from the recipient mice by western blot. C) Serine levels were measured in human AML cells upon the knockdown of P2X7 (sh-P2X7) and the control group by LC-MS/MS (n=3; **, P<0.01; Student's t test). D-F) The numbers of THP-1, U937 and MV4-11 cells were determined at the indicated days after cultured in the presence or absence of the serine and glycine (Normal or -Ser/Gly) (n=3; *, P<0.05, ***, P<0.001; two-way ANOVA with Sidak's multiple comparison test). G-H) The colony formation abilities of human AML cells upon the depletion of the serine and glycine (Normal or -Ser/Gly) were compared to that of the control group. The colony numbers (G) and derived total cell counts (H) were calculated (n=3; *, P< 0.05, **, P< 0.01; two-way ANOVA with Sidak's multiple comparison test). I-J) The $CD34^{+}$ cord blood cells were cultured in the presence or absence of the serine and glycine (Normal or -Ser/Gly) and cell numbers were determined at the indicated days after culture (n=3). K-L) Biochemical analysis of ATP levels in BM fluid of healthy controls (Normal) and human AML samples (AML, K), as well as of different AML subtypes. (Normal, n=33; AML, n=31; M2, n=4; M3, n=3; M4, n=9; M5, n=6; Unclassified, n=9; *, P<0.05; **, P<0.01; ***P<0.001, Student's t test). M-O) The

mRNA expression levels of CREB (M), PHGDH (N) and CXCR4 (O) were analyzed in AML cells and normal BM cells from the TCGA database (AML, n=173; Normal, n=70; *, P<0.05; Student's t test). P) The relationship between the PHGDH expression level and the overall survival in AML patients from the TCGA database. Q) The protein levels of PHGDH were determined in the BM cells of healthy control (normal) and human AML samples by western blot. R) Quantification of the protein level of PHGDH in panel Q (n=10, ***, P<0.001; Student's t test).

Figure S8

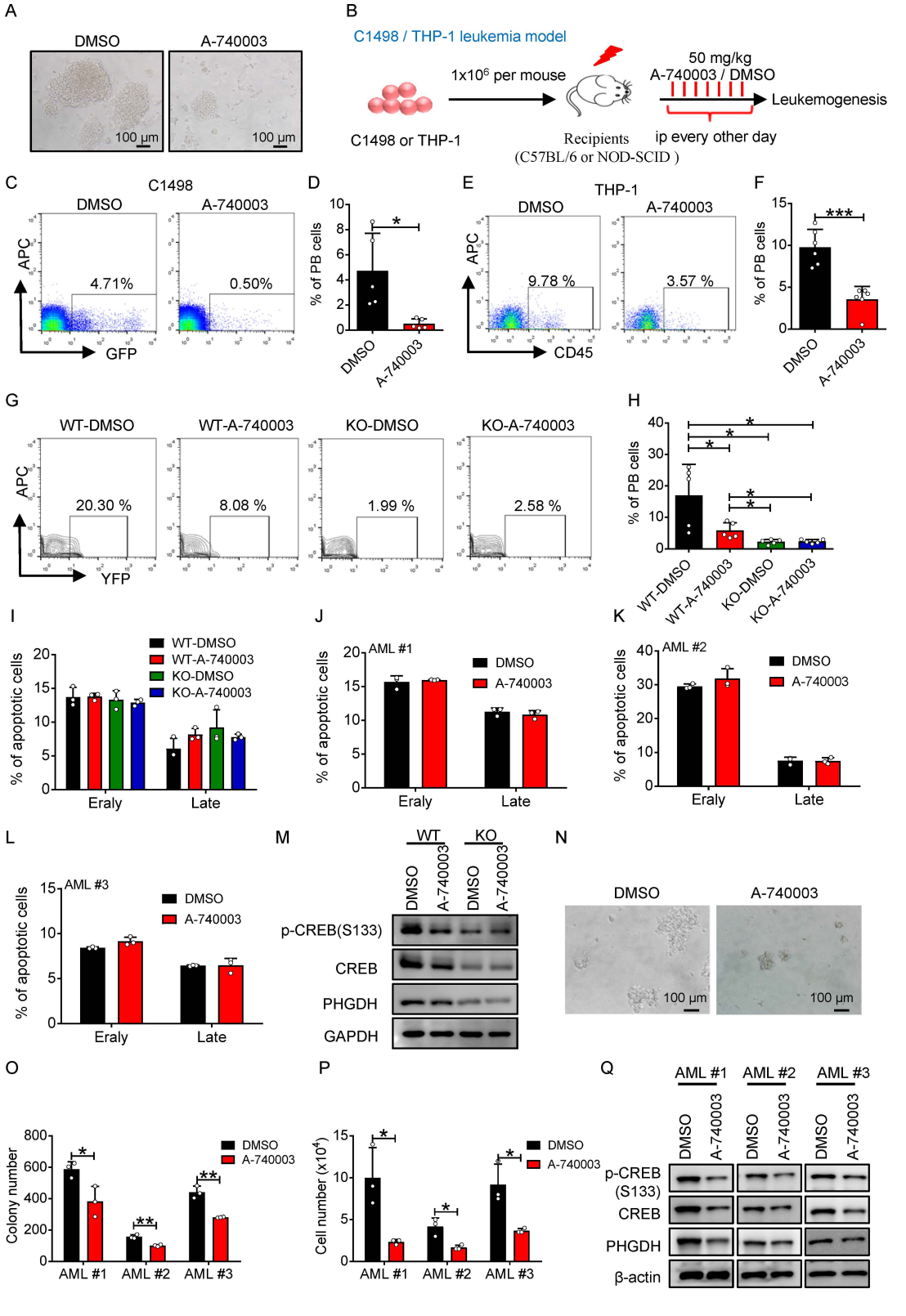
A
DMSO
A-740003
100 μm
100 μm
B
C1498 / THP-1 leukemia model
1x10^6 per mouse
50 mg/kg
A-740003 / DMSO
Leukemogenesis
C1498 or THP-1
Recipients
(C57BL/6 or NOD-SCID)
ip every other day
C
C1498
DMSO
A-740003
APC
4.71%
0.50%
GFP
D
% of PB cells
DMSO
A-740003
E
THP-1
DMSO
A-740003
APC
9.78 %
3.57 %
CD45
F
% of PB cells
***
DMSO
A-740003
G
WT-DMSO
WT-A-740003
KO-DMSO
KO-A-740003
APC
20.30 %
8.08 %
1.99 %
2.58 %
YFP
H
% of PB cells
WT-DMSO
WT-A-740003
KO-DMSO
KO-A-740003
I
% of apoptotic cells
WT-DMSO
WT-A-740003
KO-DMSO
KO-A-740003
Eraly
Late
J
AML #1
% of apoptotic cells
DMSO
A-740003
Eraly
Late
K
AML #2
% of apoptotic cells
DMSO
A-740003
Eraly
Late
L
AML #3
% of apoptotic cells
DMSO
A-740003
Eraly
Late
M
WT
KO
DMSO
A-740003
DMSO
A-740003
p-CREB(S133)
CREB
PHGDH
GAPDH
N
DMSO
A-740003
100 μm
100 μm
O
Colony number
DMSO
A-740003
AML #1
AML #2
AML #3
P
Cell number (x10^4)
DMSO
A-740003
AML #1
AML #2
AML #3
Q
AML #1
AML #2
AML #3
DMSO
A-740003
DMSO
A-740003
DMSO
A-740003
p-CREB (S133)
CREB
PHGDH
β-actin

Figure S8, related to Figure 7. Targeting leukemia development by suppressing ATP-P2X7 signaling. A) Representative images of the colonies of THP-1 cells treated with A-740003 (10 μM) and DMSO are shown. B) Schematic diagram of the C1498 cell or THP-1 cell leukemia model for the in vivo treatment with A-74003. The mice were transplanted with murine C1498 cells or THP-1 cells pretreated with A-740003 (10 μM) or DMSO in vitro, followed by the treatment with A-740003 or DMSO (50 mg/kg every other day for 2 weeks) 1 day after transplantation. C-D) Representative flow cytometric analysis of leukemia cells in the peripheral blood of recipients transplanted with C1498 cells 3 weeks after transplantation is shown (C). Quantification data are shown in panel D (n=5; *, $P<0.05$; Student's t test). E-F) Representative flow cytometric analysis of leukemia cells in the peripheral blood of recipients transplanted with THP-1 cells (E) and quantification of the frequency of leukemia cells in panel E are shown (F) (n=5; ***, $P<0.001$; Student's t test). G-H) WT and P2x7-KO AML cells were transplanted into the recipient mice, followed by the intraperitoneal injection with A-740003 7 days after transplantation (50 mg/kg every other day for 2 weeks). Shown are the representative flow cytometric analysis of AML cells in the peripheral blood (G) and quantification data (H) 3 weeks after transplantation (n=5; *, $P<0.05$; one-way ANOVA with Tukey's multiple comparison test). I-L) Murine (I) and human primary AML cells (J-L) were treated with A-740003 or DMSO for 48 h, followed by the determination of the apoptotic status by flow cytometric analysis (n=3). M) WT and P2x7-KO AML cells were treated with A-740003 or DMSO for 48 h and the protein levels of phospho-CREB (S133), CREB and

PHGDH were determined by western blot. N-P) Representative images of colonies derived from patient's AML cells treated with A-740003 and DMSO are shown (N). The colony numbers (O) and derived total cell counts (P) in panel K were calculated. A total of three human primary samples were evaluated (AML #1-#3) (n=3; *, $P<0.05$, **, $P<0.01$; two-way ANOVA with Sidak's multiple comparison test). Q) Human $CD45^+$ AML cells were treated with A-740003 or DMSO for 48 h and the protein levels of phospho-CREB (S133), CREB and PHGDH were determined by western blot.

Figure S9

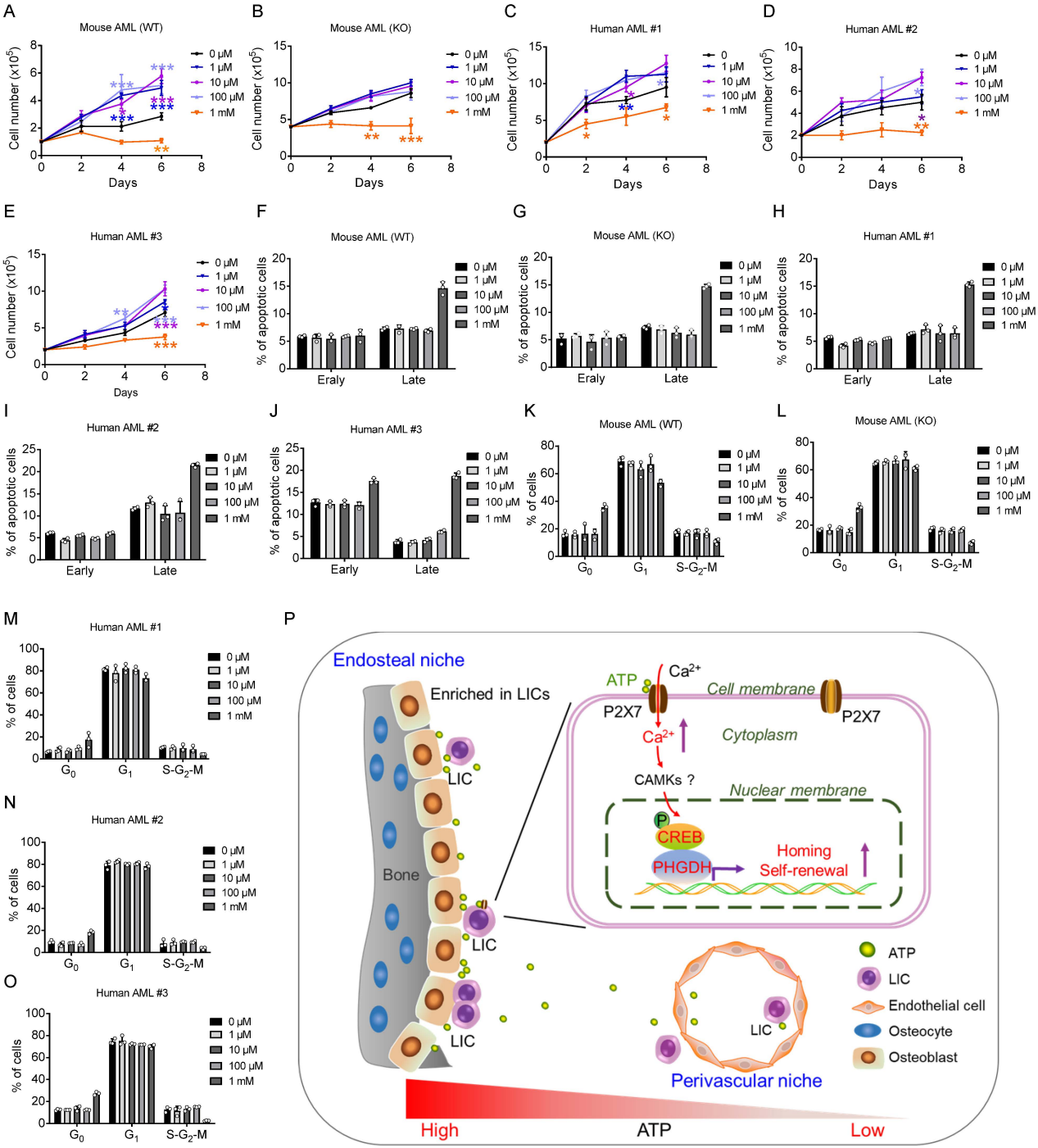
A
Mouse AML (WT)
B
Mouse AML (KO)
C
Human AML #1
D
Human AML #2
E
Human AML #3
F
Mouse AML (WT)
G
Mouse AML (KO)
H
Human AML #1
I
Human AML #2
J
Human AML #3
K
Mouse AML (WT)
L
Mouse AML (KO)
M
Human AML #1
N
Human AML #2
O
Human AML #3
Cell number (x10^5)
Days
% of apoptotic cells
% of cells
0 μM
1 μM
10 μM
100 μM
1 mM
Eraly
Early
Late
G0
G1
S-G2-M
P
Endosteal niche
Enriched in LICs
LIC
Bone
ATP
Ca2+
P2X7
Cell membrane
Cytoplasm
CAMKs ?
Nuclear membrane
CREB
PHGDH
Homing
Self-renewal
Endothelial cell
Osteocyte
Osteoblast
Perivascular niche
High
ATP
Low

Figure S9, related to Figure 7. Effects of ATP treatment in vitro in both murine and human primary AML cells. A-E) WT and P2x7-null murine primary AML (A-B) and human primary AML cells (C-E) were treated with the indicated doses of ATP and the numbers were calculated at indicated time points (n=4, *, P<0.05; **, P<0.01; ***P<0.001, one-way ANOVA with Tukey's multiple comparison test). F-J) WT and P2x7-null murine (F-G) and human AML cells (H-J) were treated with the indicated doses of ATP for 48 h and their apoptosis was determined (n=3). K-O) WT and P2x7-null murine (K-L) and human AML cells (M-O) were treated with the indicated doses of ATP for 48 h and their cell cycle was analyzed (n=3). P) Working model for the functions of ATP-P2X7 signaling in the determination of the fates of AML-LICs.

Supplemental Methods

Animals

The CD45.1 mice were provided by Dr. Jiang Zhu at Ruijin Hospital, Shanghai, China. The P2X7-knockout (KO) mice with a C57BL/6 background were generated at the Animal Core Facility at the School of Basic Medicine, Shanghai Jiao Tong University School of Medicine. Six-eight-week old C57BL/6 CD45.2 and NOD-SCID mice were ordered from the Shanghai SLAC Laboratory Animal Company and maintained at the Animal Core Facility at Shanghai Jiao Tong University School of Medicine. All animal experiments were approved by our hospital and conducted under the guidance of animal care at Shanghai Jiao Tong University School of Medicine.

P2x7 knockout generation

The sgRNA was designed to target the sequence of exon 2 of P2x7 (MGI:1339957) gene. The pCD-CAS plasmid (Biotechnologies) was used as the template for PCR with the primer-sgRNA and T7R1. The PCR products were purified and used for in vitro RNA synthesis with a MEGASHORT T7 high yield Transcription Kit (Ambion). The sgRNA was purified with a MEGAclear-96 Purification of Transcription Reactions Kit (Ambion). The pT7-cas9 plasmid (Biotechnologies) was linearized by Xba I and transcribed into mRNA in vitro using a mMESSAGE mMACHINE™ T7 ULTRA Transcription Kit (Ambion). Approximately 5 pL of the mixture of the Cas9 mRNA (20 ng/μL) and sgRNA (10 ng/μL) was injected into the cytoplasm of C57BL/6 zygote with a 3 μm diameter pipette using a Piezo instrument (Pmm 4G, Prime Tech). After microinjection, zygotes were cultured in KSOM medium in a humidified atmosphere containing 5% CO_2 and 95% air at 37°C for 24 h. The embryos at the 2-cell stage were surgically transferred to both the oviducts of recipient ICR mice. Genomic DNA was

extracted from the toe tissues of one-week old mice by using a TIANGEN Genomic DNA kit (TIANGEN). The fragments were amplified from genomic DNA by PCR using the primers. Three F0 mice were born and a messy peak appeared near the sgRNA2 in one of the mouse sequencing results. F1 mice were further obtained and one mouse line carrying 7bp deletion was subjected to the current studies.

Cloning of iATPSnFR

The iATPSnFR$^{1.0}$ (45) sensor gene was synthesized by GenScript Biotech Co., Ltd. For the ratiometric measurement, the red fluorescent protein mCherry was fused to the N-terminus of iATPSnFR using overlap PCR, with an NheI restriction site between mCherry and iATPSnFR coding sequence. For imaging cell surface ATP, the sequence mCherry-iATPSnFR with N-terminal secretion signal and the C-terminal transmembrane anchoring domain of platelet-derived growth factor receptor (PDGFR) was cloned into the pLVX-IRES-Puro plasmid with the same restriction site EcoRI and NotI. The control sensor mCherry-cpSFGFP was performed by the same construction method as the mCherry-iATPSnFR.

Imaging of iATPSnFR-expressing AML cells in vitro and in vivo

To evaluate whether the cell-surface anchored iATPSnFR sensor can sensitively indicate the dynamic changes of the extracellular ATP levels, a human AML cell line of U937 or MLL-AF9^{+} murine primary AML cells were overexpressed with iATPSnFR sensor and the control sensor (mCherry-cpSFGFP, NC), plated onto a 35 mm glass-bottom dish (Cellvis) precoated with 100 μg/mL Poly-D-lysine hydrobromide and maintained in the serum-free RPMI-1640 medium before imaging. iATPSnFR signaling was determined using the NiKon A1 confocal microscope at the excitation wavelengths

of 488 nm (to measure the ATP level) and 561 nm (to normalize the iATP sensor protein level in individual cells) and acquired at the emission wavelengths of 525 nm and 585 nm, respectively. To monitor the dynamic ratio changes of iATPSnFR fluorescence upon the ATP stimulation, 2 mM ATP or PBS was added to the medium upon the first image was taken. iATPSnFR signaling was measured every 1.5 min for a total of 40 min. The iATPSnFR fluorescence ratios and pseudocolor images were evaluated by ImageJ software. The dynamic changes of iATPSnFR fluorescence ratio were normalized to that in the first image.

To label the vascular endothelial cells in vivo, 10 μg eFluor660-conjugated anti-VE-cadherin antibodies (BV13, eBiosciences) in 200 μL of PBS were injected into recipient mice by intravenous injection. For the ex vivo imaging of the BM niches in the cranium, mice were sacrificed 10 min after the injection of VE-cadherin antibodies, followed by the isolation of the cranium. The bone surface of the cranium was gently scraped with a surgical blade without damaging the BM to facilitate the subsequent imaging. The skived cranium was immersed in a 35 mm glass bottom dish with the RPMI-1640 medium containing 2% FBS and subjected to imaging with the confocal microscope. iATPSnFR or its control sensor (NC) signaling was measured using NiKon A1 confocal microscope at excitation wavelength of 488 nm and 561 nm and emission wavelengths of 525 nm and 585 nm, respectively. The fluorescence of VE-cadherin-eFluor660 labeled blood vessels in the BM was also measured. Differential interference contrast (DIC) was used to show the structure of the BM and bones. The iATPSnFR fluorescence ratio or the control fluorescence ratio and pseudocolor images were analyzed by ImageJ software.

Establishment of the AML model and BM competitive transplantation

To establish the murine AML model, an MSCV-MLL-AF9-IRES-YFP-encoding plasmid (50) and a pCL-ECO packaging plasmid (2:1) were co-transfected into 293T cells to produce retroviruses. Retroviruses were used for the infection of isolated WT or P2x7-KO Lin^- fetal liver cells through two rounds of spinning infection in the presence of 4 μg/mL polybrene. Infected cells ($1\text{-}3\times10^5$) were transplanted into the lethally irradiated C57BL/6 wild-type (WT) recipient mice by retroorbital injection, followed by the analysis for the frequency of leukemia cells in the peripheral blood and the overall survival of the leukemic mice. Serial transplantations were performed with the same number of the purified YFP^+ or GFP^+ BM AML cells. To exclude the homing effect, $1x10^4$ WT and P2x7-null YFP^+ BM leukemia cells were transplanted into the lethally irradiated C57BL/6 recipient mice by intratibial injection, followed by the analysis for the frequency of leukemia cells in the peripheral blood and the overall survival of the leukemic mice. The limiting dilution assay was performed with the indicated YFP^+ BM leukemia cells (30, 100 and 500), followed by the calculation of the frequency of the functional LICs according to the overall survival of recipients using the L-Calc software (Stemcell Technologies). In some cases, the shRNA plasmids (pLKO.1-IRES-GFP) specifically targeting murine or human P2x7 or P2X7 were co-transfected with pSPAX2 and pMD2G packaging plasmids (4:3:1) into 293T cells. Lentiviruses were used for the infection of MLL-AF9-YFP^+ BM AML cells, THP-1 cells (ATCC) or patients' primary AML cells (Table S3). FACS-purified GFP^+ BM leukemia cells (5,000-10,000), GFP^+ THP-1 cells (5×10^6) or 2×10^6 human primary AML cells, were injected into the lethally irradiated C57BL/6 or the sublethally irradiated NOD-SCID mice, followed by the analysis of leukemia development.

For the rescue experiments, the retroviral plasmid XZ201-Creb-HA-mCherry or pMIGR1-Phgdh-T2A-mCherry were mixed with pCL-ECO packaging plasmid at a

ratio of 2:1 and transfected into 293T cells. Virus-containing supernatant was collected for the infection with WT and P2x7-KO BM bulk leukemia cells, followed by the transplantation into the recipient mice. The expression levels of Creb and Phgdh were further measured in WT, P2x7-KO, Creb- or Phgdh-overexpressing WT or P2x7-KO leukemia cells by quantitative RT-PCR.

For the competitive reconstitution analysis, a total number of 3×10^5 WT or P2x7-KO CD45.2 donor BM cells were mixed with 3×10^5 CD45.1 competitor BM cells and transplanted into the lethally irradiated CD45.1 recipient mice by retroorbital injection. The repopulated donor cells in the peripheral blood were analyzed at 3, 8, and 16 weeks after transplantation. Multilineages of the hematologic cells in the peripheral blood were also evaluated 16 weeks after transplantation.

Flow cytometry

Flow cytometric analyses were performed as previously described (48). Briefly, the WT or P2x7-KO/-knockdown immunophenotypic Mac-1$^+$c-Kit$^+$ LICs, myeloid or lymphoid lineages were stained with the monoclonal antibodies (eBioscience) of anti-Mac-1-APC (or PE), anti-Gr-1-PE, anti-CD3-APC, anti-B220-PE and anti-c-Kit-PE (or APC). Murine Lin$^-$CD127$^-$Sca-1$^-$c-Kit$^+$CD16/32$^+$CD34$^+$ L-GMP cells were stained with the biotinylated antibodies of anti-CD127, anti-Gr-1, anti-B220, anti-CD3, anti-CD8 and anti-Ter119, followed by staining with the antibodies of streptavidin-PE/Cy5.5, Sca-1-PE/Cy7, c-Kit-APC, CD16/32-eflour450 and CD34-PE (eBioscience). Anti-CXCR4-PE antibody (eBioscience) was used for the determination of the CXCR4 expression levels of WT and P2x7-KO Mac-1$^+$c-Kit$^+$ LICs or bulk leukemia cells. The repopulation and its multilineages were evaluated in WT and P2x7-KO donor HSCs with the antibodies of anti-CD45.1-PE, anti-CD45.2-APC, anti-Mac-1-APC, anti-Gr-

1-PE, anti-CD3-APC and anti-B220-PE. In some cases, human AML cell lines and primary AML cells were stained with the antibodies of anti-CD45-PE (or FITC), anti-CD33-PE, anti-CD15-FITC, anti-CD34-PE, anti-CD19-APC and anti-CD20-PE. Detailed antibody information is listed in Supplemental Table 5

Quantitative RT-PCR

The candidate genes were further validated in Mac-1$^+$c-Kit$^+$ LICs by quantitative RT-PCR. Murine P2xs and human P2Xs were also measured in murine Mac-1$^+$c-Kit$^+$ LICs, Lin$^-$CD127$^-$Sca-1$^-$c-Kit$^+$CD16/32$^+$CD34$^+$ L-GMP cells, bulk leukemia cells, Lin$^-$Sca-1$^+$c-Kit$^+$CD34$^-$Flk2$^-$ HSCs, human AML cell lines, human Lin$^-$CD34$^+$CD38$^-$CD90$^+$CD45RA$^-$ HSCs or human Lin$^-$CD34$^+$CD38$^-$CD90$^-$CD45RA$^+$ LICs, respectively. Briefly, first-strand cDNA was reversely transcribed using AMV reverse transcriptase (TakaRa). PCR reactions were performed according to the manufacturer's protocol with the Applied Biosystems 7900HT. The mRNA levels were normalized to the level of β-actin RNA transcripts. The primer sequences used are shown in Table S2.

In vitro colony forming unit assay and cell proliferation analysis

Three thousand murine BM AML cells were seeded in the methylcellulose medium (M3534, Stem Cell Technologies) according to the manufacturer's information. The numbers of colonies and derived cell counts were determined 5-8 days after plating. Then the same numbers of AML cells from primary plating were subjected to secondary plating. In another case, 10 μM of P2X7 antagonist (A-740003, TOCRIS) or DMSO were added to the methylcellulose medium and colony formation abilities were determined. For colony forming assay of human primary AML cells, P2X7-knockdown (sh-P2X7-1 and -2) and the scrambled AML cells were seeded to the methylcellulose

medium (H4436, Stemcell Technologies) according to the manufacturer's information, followed by the calculation of the colony numbers and derived total cell counts 7-10 days after plating. In some cases, the methylcellulose medium in the presence (Normal) or absence of serine and glycine (-Ser/Gly) was used for the evaluation of the colony formation capacities of murine and human primary AML cells at the indicated days after plating; or the in vitro pretreated murine AML cells were further subjected to the transplantation into the recipient mice and their overall survival was evaluated.

Human AML cell lines, such as THP-1 (ATCC), U937 (ATCC) and MV4-11 (ATCC), were cultured in the RPMI-1640 medium containing 10% fetal bovine serum (FBS) and subjected to the analysis of the changes in proliferation upon P2X7 knockdown (sh-P2X7-1, -2 or scrambled) at the indicated time points. In some experiments, the RPMI-1640 medium in the presence or absence serine and glycine was used for the evaluation of the proliferation abilities of human AML cell lines including THP-1, U937 and MV4-11 cells. For analyze the proliferation abilities of human cord blood $CD34^+$ cells, the cells were cultured in the RPMI-1640 medium in the presence or absence serine and glycine medium supplemented with cytokines including 10 ng/mL of SCF, 10 ng/mL TPO, 10 ng/mL FLT3-L, 10 ng/mL of IL-3 and 10 ng/mL of IL-6. Cell numbers were calculated at indicated time points.

For ATP treatment in vitro, murine BM AML cells or human primary AML cells (Table S3) were cultured in basic medium (Stemcell Technologies) supplemented with 10 ng/mL of SCF, 10 ng/mL of IL-3 and 10 ng/mL of IL-6 (Peprotech). Different doses of ATP (0 μM, 10 μM, 100 μM and 1 mM) were added to the medium for the analysis of the cell proliferation at the indicated time points.

Giemsa-Wright staining and H&E staining

Wright-Giemsa staining was performed with murine AML cells in the blood smear of the peripheral blood and the BM from the recipient mice, and the frequency of the blast cells and differentiated leukemia cells were calculated according to the typical morphologies. In some cases, the livers and spleens of the leukemic mice were fixed with 4% paraformaldehyde and embedded in paraffin. The liver and spleen paraffin sections were stained with H&E and evaluated for the infiltration of AML cells.

Homing assay

Homing assays were performed as previously described (14). Briefly, a total number of 2-5x10^6 WT, P2x7-KO, Phgdh-overexpressing WT or P2x7-KO BM AML cells were labeled with 5(6)-carboxy fluorescein diacetate succinimidyl ester (CFSE) and transplanted into the lethally irradiated recipient mice. The frequency of the $CFSE^+$ cells were measured in the BM 16 h after transplantation by flow cytometric analysis.

Transwell assay

A total number of 3x10^5 WT, P2x7-KO, Phgdh-overexpressing WT or P2x7-KO Mac-1^+c-Kit^+ LICs or YFP^+ bulk AML cells were purified, washed once with serum-free PBS, resuspended in the culture medium (RPMI-1640+5% BSA) and seeded in the upper chamber of a transwell. Six hundred microliter of the RPMI-1640 medium containing 5% BSA and 160 ng/mL SDF-1α were added to the lower chamber. The numbers of Mac-1^+c-Kit^+ LICs or YFP^+ bulk AML cells that migrated to the lower chamber were calculated 4-6 h after culture.

Immunofluorescence staining

Immunofluorescence staining was performed as previously described (14). In brief, the

fresh long bones were isolated from the leukemic mice transplanted with WT or P2x7-KO AML cells and fixed in 4% paraformaldehyde solution at 4°C for overnight. The fixed bones were then decalcified in 15% EDTA at 4 °C for 3 days. The decalcified long bones were then embedded in OCT (Fisher) and sectioned longitudinally using a cryostat (Leica). Intact half-bone sections were washed with PBS to remove OCT, followed by the incubation with the indicated primary antibodies at 4°C overnight. The sections were further washed with PBS, stained with a secondary antibody and subjected to the evaluation of the changes of the localizations of LICs in the BM. Images were obtained with a NiKonA1 confocal microscope. The following antibodies are used in the current study: anti-c-Kit (R&D Systems, AF1356), anti-Laminin (Abcam, ab11575), anti-goat secondary antibody conjugated with Alexa Fluor647 and anti-rabbit secondary antibody conjugated with Alexa Fluor555 (Thermo Fisher Scientific, A-31572 and A-21447).

Calcium imaging

WT and P2x7-KO Mac-1$^+$c-Kit$^+$ LICs or YFP$^+$ bulk AML cells were seeded on the glass coverslips pretreated with poly-D-lysine (100 μg/mL, Sigma). Cells were then incubated with Fura-2-AM (2 μM, Thermo) and Pluronic F127 (0.02% (w/v), Sigma) for 30 min at 37 °C. Fluorescent intensities at excitation wavelength of 340 nm and 380 nm were measured using NIS-Elements Soft every 2 sec. For the detection of the constitutive Ca^{2+} influx, leukemia cells were incubated in the Ca^{2+}-free buffer for 2 min, followed by the switch to the buffer with 1 mM Ca^{2+} for 2 min and the Ca^{2+}-free medium later on. The ratios of fluorescence at excitation wavelength of 340 nm (F340) and 380 nm (F380) were recorded every 2 sec and calculated.

Whole-cell patch-clamp electrophysiological recording

The whole-cell patch-clamp recordings were performed using Axon 200B (Axon Instruments, Foster City, CA) with a voltage clamp. The recording electrodes were filled with a pipette solution. Patch electrodes (3-5 MΩ) were filled with intracellular solution containing 30 Mm NaCl, 120 mM KCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM EGTA and 10 mM HEPES (pH 7.2). Indicated cells were incubated in the standard extracellular solution with 150 mM NaCl, 10 mM glucose, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$ and 10 mM HEPES (pH 7.4). $CaCl_2$ and $MgCl_2$ were substituted with 5 mM EGTA in the Ca^{2+}-free solution. Then the membrane current signals were amplified by using Axon 200B. Data were sampled at 10 kHz and filtered at 2 kHz. CHO cell electrophysiology was recorded 24-48 h after transfection. Murine Mac-1^+c-Kit^+ LICs or human $Lin^-CD34^+CD38^-CD90^-CD45RA^+$ primary LICs were seeded on the glass coverslips pretreated with poly-D-lysine (100 μg/mL, Sigma) before imaging. The antagonist of P2X7, A-740003, was used for the inhibition after ATP stimulation. Data were analyzed using a Digi data 1440 interface and a computer with the Clampex and Clampfit 10.0 software (Molecular Devices). All currents were sampled and analyzed.

Luciferase reporter assay

A luciferase reporter vector pGL4.27 containing a Phgdh promoter was constructed to identify the transcriptional activation of Phgdh by Creb. The indicated doses of XZ201-HA-Creb-mCherry (or negative control vector) plasmid and the pGL4.27-Phgdh promoter vector were co-transfected into 293T cells. Twenty-four hours after transfection, luciferase activities were measured using a luciferase reporting system (GloMax® Multi Instrument). The luciferase measurement is the ratio of firefly luciferase units to Renilla luciferase units.

Chromatin immunoprecipitation (ChIP) assays

ChIP assays were performed using the ChIP Assay Kit (Beyotime, P2078). 293T cells were co-transfected with XZ201-HA-Creb-mCherry and pGL4.27-Phgdh-promoter plasmids, crosslinked with 1% formaldehyde (Sigma) at 37°C for 10 min, followed by the incubation with anti-CREB antibodies (Abway) or rabbit control IgG (CST) at 4°C overnight. For the sample input, 1% of the sonicated precleared DNA was purified at the same time with the precipitated immune complex. The ChIP samples were purified by the Gel and PCR-clean up Kit (Nucleospin). The Creb-binding sequence was amplified by the semi-quantitative PCR using primers specific for Phgdh promoter region as listed in Table S2.

In vivo treatment with the P2X7 antagonist of A-740003

To test the therapeutic effect of the P2X7 antagonist of A-740003 (TOCRIS) in vivo, a total number of 1×10^6 C1498 cells (a murine AML cell line) and THP-1 cells (a human AML cell line) were transplanted into the C57BL/6 or NOD-SCID mice, respectively, followed by the intraperitoneal administration of A-740003 (50 mg/kg) and DMSO 1 day after transplantation (every other day for 2 weeks). For the MLL-AF9-induced murine leukemia model, a total number of 1×10^4 WT and P2x7-KO AML cells with 2×10^5 normal BM cells were co-transplanted into the lethally irradiated mice by retroorbital injection, followed by the intraperitoneal administration of A-740003 (50 mg/kg) and DMSO 7 days after transplantation (every other day for 2 weeks). The percentages of leukemia cells in the peripheral blood and the overall survival of recipients were compared.

Analyses for the ATP levels and the intracellular serine concentration

The BM of control mice transplanted with BM cells expressing an empty vector four months after injection or AML mice was flushed out with 1 mL PBS and the supernatant was collected for the measurement of ATP levels using ATP Bioluminescence Assay Kit HS II (Roche) according to the manufacturer's protocol. In some cases, the bone marrow fluid of healthy donors or AML patients (Table S4) was collected and ATP levels were measured with ATP Bioluminescence Assay Kit HS II accordingly. Intracellular serine measurement was performed as previously described (13). In brief, 1×10^6 WT, P2x7-KO murine YFP^+ bulk AML cells, P2X7-knockdown human primary AML cells were collected and processed for the measurement of serine levels using an aTRAQ® assay kit (AB Sciex). The collected AML cells were dissolved in 40 μL of methanol, sonicated, and precipitated with 10 μL of 10% sulfosalicylic acid and 400 mM norleucine. Then 10 μL of supernatant was further mixed with 40 μL of norvaline-containing labeling buffer, and 10 μL of the mixture was further mixed with 5 μL aTRAQΔ8 reagent, followed by the addition of 5 μL of hydroxylamine, 32 μL of hydroxylamine and aTRAQ internal standard solution. The derivatized contents were loaded onto 4000QTRAP LC-MS/MS linear (AB Sciex) using the Agilent 1200 LC system. All data collection and processing were performed using Analyst 1.5.1 software (AB Sciex).

Western blotting

Cell lysates of FACS-purified WT, P2x7-KO Mac-1^+c-Kit^+ LICs, bulk leukemia cells, P2X7-knockdown THP-1 cells, U937 cells, MV4-11 cells, healthy donor BM cells or human primary AML cells were electrophoresed on 10% SDS polyacrylamide gels and transferred onto nitrocellulose membranes (Millipore). The membranes were blocked

with 5% nonfat milk and then incubated with primary antibodies of anti-P2X7 (NOVUS, NBP2-41300), anti-phospho-CREB (S133) (Abways, CY5043), anti-CREB (Abways, CY5426), and anti-PHGDH (ABclonal, WH126491), followed by the incubation with HRP-conjugated secondary antibodies. In some cases, relative protein levels were quantified by measuring the signaling intensity and normalizing to the actin level by using ImageJ software.

In silico analysis for clinical data

For the analysis of P2X7, CREB, PHGDH and CXCR4 expression in AML patients, data were extracted from the curated GEPIA database (http://gepia.cancer-pku.cn). For the analysis of P2Xs expression in AML patients, data were extracted from the TCGA database (https://www.cancer.gov/tcga). For the analysis of the relationship between the P2X7 expression levels and the overall survival of AML patients, the available survival data from 150 patients were obtained from the OncoLnc (http://www.oncolnc.org/). AML patients were subdivided into two subgroups according to the low or high P2X7 expression levels and the overall survival were compared between the P2X7-low and high subgroups.

Statistical analysis

Statistical analysis was performed using GraphPad software (Prism 7.0). Data are represented as Mean ± SD unless indicated elsewhere. All the experiments are conducted independently for at least 3 times. Data were analyzed with a Student's t test (two-tailed), one-way ANOVA with Tukey's multiple comparison test, or two-way ANOVA with Sidak's multiple comparison test accordingly and statistical significance was set at $P<0.05$ (*, $P < 0.05$; **, $P<0.01$; ***, $P<0.001$).

Table S1. Limiting dilution assays for frequencies of WT and P2x7-KO LICs

Quantification of CRUs	Survival ratio	
Transplanted cells	WT	KO
30	2/5	2/5
100	0/5	2/5
500	0/5	1/5
Frequency of leukemia-initiating cells	1:28	1:145
95% confidence interval	(11-68)	(62-341)

Table S2. List of primers and shRNA target sequence

Genotyping Primers	Sequences
***P2x7*-JD581-F**	ACTCACCCAGAGCAGAT
***P2x7*-JD581-R**	TCCAGCCTTGATCTCC

q-PCR Primers	Sequences
mouse *P2x1*-F	ACTGGGAGTGTGACCTGGAC
mouse *P2x1*-R	TCCCAAACACCTTGAAGAGG
mouse *P2x2*-F	GCGTTCTGGGACTACGAGAC
mouse *P2x2*-R	CACTTTGTGTTCCGACATGG
mouse *P2x3*-F	AAAGCTGGACCATTGGGATCA
mouse *P2x3*-R	CGTGTCCCGCACTTGGTAG
mouse *P2x4*-F	GCGTCTGTGAAGACCTGTGA
mouse *P2x4*-R	GATTTGGCCAAGACGGAATA
mouse *P2x5*-F	GGGGTTCGTGTTGTCTCTGT
mouse *P2x5*-R	CACTCTGCAGGGAAGTGTCA
mouse *P2x6*-F	GGGGTTTCTGGATTACAAGACG
mouse *P2x6*-R	CCTATCACGTAGACTACCACTGC
mouse *P2x7*-F	TGTGTGCATTGACTTGCTCA
mouse *P2x7*-R	CTTGCAGACTTTTCCCAAGC
mouse *Amt*-F	CAGGCACAACCCTTGGTCC
mouse *Amt*-R	TGCAGGTGTGAATCAACATGAC
mouse *Gatm*-F	GCTTCCTCCCGAAATTCCTGT
mouse *Gatm*-R	CCTCTAAAGGGTCCCATTCGT
mouse *Shmt1*-F	CAGGGCTCTGTCTGATGCAC

mouse *Shmt1*-R	CGTAACGCGCTCTTGTCAC
mouse *Psph*-F	AGGAAGCTCTTCTGTTCAGCG
mouse *Psph*-R	GAGCCTCTGGACTTGATCCC
mouse *Shmt2*-F	TGGCAAGAGATACTACGGAGG
mouse *Shmt2*-R	GCAGGTCCAACCCCATGAT
mouse *Gcat*-F	GGACAGCGAACTGGAAGGG
mouse *Gcat*-R	AGTTATTGGCACAGAAGTTGAGG
mouse *Psat1*-F	AAGCCACCAAGCAAGTGGTTA
mouse *Psat1*-R	GATGCCGAGTCCTCTGTAGTC
mouse *Phgdh*-F	ATGGCCTTCGCAAATCTGC
mouse *Phgdh*-R	AGTTCAGCTATCAGCTCCTCC
mouse *Hoxa9*-F	AAAACACCAGACGCTGGAAC
mouse *Hoxa9*-R	TCTTTTGCTCGGTCCTTGTT
mouse *Bmi1*-F	ATCCCCACTTAATGTGTGTCCT
mouse *Bmi1*-R	CTTGCTGGTCTCCAAGTAACG
mouse *Camk1d*-F	ACTGGGGCCTTTTCTGAAGT
mouse *Camk1d*-R	CCGATCGAAGAGTTCTCCAC
mouse *Camk4*-F	CTTCGAGGTGGAGTCAGAGC
mouse *Camk4*-R	TCAAGGACCAGGCTGATTTC
mouse *Hoxb4*-F	CGTGAGCACGGTAAACCCC
mouse *Hoxb4*-R	GTGTTGGGCAACTTGTGGTC
mouse *Runx1*-F	GATGGCACTCTGGTCACCG
mouse *Runx1*-R	GCCGCTCGGAAAAGGACAA
mouse *Alox5*-F	CTACGATGTCACCGTGGATG
mouse *Alox5*-R	GTGCTGCTTGAGGATGTGAA

mouse *Arb2*-F	AAGTCGAGCCCTAACTGCAA
mouse *Arb2*-R	GGAAAGACAGGCCCAGTACA
mouse *Creb*-F	AGCAGCTCATGCAACATCATC
mouse *Creb*-R	AGTCCTTACAGGAAGACTGAACT
mouse *Cxcr4*-F	GACTGGCATAGTCGGCAATG
mouse *Cxcr4*-R	AGAAGGGGAGTGTGATGACAAA
mouse *CD44*-F	CACCATTGCCTCAACTGTGC
mouse *CD44*-R	TTGTGGGCTCCTGAGTCTGA
mouse *CD82*-F	TTCGGGGTGTGGATTCTTGC
mouse *CD82*-R	AGGAAGCCCATCACTATGGTG
mouse *Selplg*-F	GAAAGGGCTGATTGTGACCCC
mouse *Selplg*-R	AGTAGTTCCGCACTGGGTACA
mouse *Panx1*-F	GCTGCACAAGTTCTTCCCCTA
mouse *Panx1*-R	CGCGGTTGTAGACTTTGTCAAG
mouse *Cx43*-F	GGATCGCGTGAAGGGAAGAAG
mouse *Cx43*-R	TTGCGGCAGGAGGAATTGTTT
mouse β-*actin*-F	GGCTGTATTCCCCTCCATCG
mouse β-*actin*-R	CCAGTTGGTAACAATGCCATGT
human P2X7-F	AAGCTGTACCAGCGGAAAGA
human P2X7-R	GCTCTTGGCCTTCTGTTTTG
human ACTIN-F	AGAGCTACGAGCTGCCTGAC
human ACTIN-R	AGCACTGTGTTGGCGTACAG

shRNAs	**Target sequences**
Scramble	CCTAAGGTTAAGTCGCCCTCG
mouse shP2x7-#1	CCCGGCTACAACTTCAGATAT

mouse shP2x7-#2	GCCACAACTATACCACGAGAA
human shP2X7-#1	CCGAGAAACAGGCGATAATTT
human shP2X7-#2	GCATGAATTATGGCACCATTA

Cloning Primers	**Sequences**
mouse *Creb*-XhoI-F	CCGCTCGAGCCAGCAGCTCATGCAAC
mouse *Creb*-EcoRI-R	CCGGAATTCTTAATCTGATTTGTGGCAGT
mouse *Phgdh*-BamHI-F	CGCGGATCCGATCCATGGCCTTCGCAAATCTG
mouse *Phgdh*-NotI-R	ATAAGAATGCGGCCGCTCAGAAGCAGAACTGGAA

Luciferase primers	**Sequences**
mouse *Phgdh*-promoter-F	CCGCTCGAGAGCGCAAAGGTACGACGACCT
mouse *Phgdh*-promoter-R	CCGGAATTCTTACATGTAGTGCCACTGCC
***Phgdh*-F (for ChIP)**	GCTTTACTTCTCACTGTG
***Phgdh*-R (for ChIP)**	TCCTTCCTTTCCCTTCTC

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Table S3. AML patient samples used for functional analysis

Sample	Age	Gender	Cytogenetics	Subtype	Survival (month)
AML #1	73	Female	47, XX, 8[6]/46, XX	M5	Alive
AML #2	36	Female	46, XX, MLL-AF9 fusion	M5	Alive
AML #3	62	Male	Normal karyotype M2	M2	15

Table S4. AML patient samples used for ATP level detection

Sample	Age	Gender	Cytogenetics	Subtype	Survival (month)
AML #4	50	Male	46, XY, FLT3-ITD^{+}	M4	Alive
AML #5	54	Female	46, XX	M4	Alive
AML #6	32	Male	46, XY, t (15;17) (q24; q21)	M3	Alive
AML #7	41	Male	46, XY	Unclassified	Alive
AML #8	21	Female	46, XY, t (15;17) (q24; q21)	M3	Alive
AML #9	59	Male	46, XY	M4	Alive
AML #10	67	Female	46, XX	M4	Alive
AML #11	62	Male	46, XY	Unclassified	Alive
AML #12	67	Female	46, XX	M4	Alive
AML #13	30	Female	46, XX	M4	alive
AML #14	20	Male	46, XY, FLT3-ITD+	M5	Alive
AML #15	68	Male	46, XY	Unclassified	Alive
AML #16	59	Male	46, XY	M2	Alive
AML #17	57	Male	46, XY	M5	Alive
AML #18	57	Male	46, XY	Unclassified	Alive
AML #19	81	Male	Unknown	M2	3.2
AML #20	32	Female	46, XX, t (15;17) (q22; q31)	M3	Alive
AML #21	54	Male	46, XY	M5	Alive
AML #22	71	Male	46, XY, t (16;16) (p13.1; q22)	M4	0.2
AML #23	64	Female	45, XX [7]/46, XX [13]	Unclassified	Alive
AML #24	36	Female	46, XX	Unclassified	Alive
AML #25	54	Female	46, XX	M4	13
AML #26	46	Female	46, XX	M4	Alive
AML #27	67	Male	46, XY	M5	9
AML #28	34	Male	46, XY, t (8;21) (q22; q22)	M2	202

AML #29	34	Male	46, XY	M2	Alive
AML #30	20	Male	46, XY, FLT3-ITD^{+}	M5	Alive
AML #31	55	Male	46, XY	Unclassified	Alive
AML #32	26	Male	46, XY	Unclassified	Alive
AML #33	56	Female	46, XX, WT1^{+}, FLT3^{+}	M5	Alive
AML #34	57	Male	46, XY	Unclassified	Alive

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Table S5. List of antibodies for flow cytometric analysis

Antibodies	Source	Catalog Number
Anti-mouse c-Kit-APC	eBioscience	17-1171-82
Anti-Mouse-Sca-1-FITC	eBioscience	130-102-831
Anti-mouse Gr-1-PE	eBioscience	12-5931-83
Anti-mouse Mac-1-APC	eBioscience	17-0112-83
Anti-mouse CD34-PE	eBioscience	12-0349-42
Anti-mouse Sca-1-PEcy7	eBioscience	25-5981-82
Anti-mouse CD16/32-eFlour 450	eBioscience	48-0161-82
Streptavidin PerCP-Cyanine5.5	eBioscience	45-4317-82
Anti-mouse Gr-1- Biotin	eBioscience	13-5931-85
Anti-mouse CD127-Biotin	eBioscience	13-1271-82
Anti-mouse Ter 119-Biotin	eBioscience	13-5921-85
Anti-mouse Cxcr4-Biotin	eBioscience	13-9991-82
Anti-mouse CD3e Biotin	eBioscience	13-0031-85
Anti-mouse CD45R(B220)-Biotin	Thermo Fisher Scientific	13-0452-85
Anti-mouse CD3e-APC	eBioscience	17-0031-82
Anti-Mouse-B220-PE	eBioscience	12-0452-85
Anti-Mouse-CD45.2-APC	eBioscience	17-0454-82
Anti-Mouse-CD45.1-PE	eBioscience	12-0453-82
Anti-Human-CD34-FITC	eBioscience	11-0349
Anti-Human-CD33-PE	Mitenyi	130-098-896
Anti-Human-CD45RA-PE	eBioscience	85-12-0458-42
Anti-Human-CD19-PE	Mitenyi	130-098-068
PE Mouse Anti-Human Ki-67 Set	BD Pharmingen	BD556027
AnnexinV-PE Apoptosis Detection Kit	BD Pharmingen	BD559763