Phosphatase PRL2 promotes AML1-ETO-induced acute myeloid leukemia

Supplemental Methods

Mice

Wild type C57BL/6 ($CD45.2^+$) and B6.SJL ($CD45.1^+$) mice were purchased from the Jackson Laboratories. $Prl2^{+/+}$ and $Prl2^{-/-}$ mice were maintained in the Indiana University Animal Facility and kept in Thorensten units with filtered germ-free air. All mice were 10-12 weeks of age at the time of analysis. Both male and female mice were utilized in the experiments. The Institutional Animal Care and Use Committee (IACUC) of the Indiana University School of Medicine approved all experimental procedures.

Human AML cell lines, primary AML samples, and cord blood cells

Human AML cell lines, including K562, Kasumi-1, KG1a, HL-60, MV4-11, and MO7e were obtained from ATCC. All cell lines were authenticated by SRT profiling and tested for mycoplasma contamination. Primary AML samples and normal human cord blood samples were obtained after informed consent following the guidelines of the institutional review board of the Indiana University School of Medicine.

Production of Lentivirus

Lentiviral shRNA plasmid (pLB) was purchased from Addgene (11619). Oligonuculeotides targeting control (Luciferase) and human PRL2 cDNAs were cloned into the pLB plasmid. Oligonuculeotide sequences are available upon request. Lentiviral particles were generated by standard method using the third generation packaging system (pMDL, pMD2.G, and pRSV-Rev). Human AML cell lines were infected with high-titer lentiviral suspensions. Twenty-four hours after infection, GFP-positive cells were sorted by FACS. The reduction of PRL2 proteins was determined by immunoblot analysis.

Immunoblotting analysis

Cells were washed with ice-cold PBS, and lysed on ice for 30 min in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, and 10% glycerol) supplemented with a Complete Protease Inhibitor tablet (Roche Applied Science). Cell lysates were cleared by centrifugation at 15,000 rpm for 10 min. The lysate protein concentration was estimated using a BCA protein assay kit (Pierce). The protein samples were boiled with sample buffer, separated by SDS-PAGE, transferred electrophoretically to a nitrocellulose membrane, and immunoblotted with appropriate antibodies, followed by incubation with horseradish peroxidase-conjugated secondary antibodies. The blots were developed by the enhanced chemiluminescence technique (ECL kit, GE Healthcare). Representative results from at least two independent experiments are shown. Antibodies were purchased from Cell Signaling (ERK1/2, pERK1/2, Actin and GAPDH). PRL2 antibody is a generous gift from Dr. Qi Zeng.

Apoptosis assays

Human AML cell lines were treated with DMSO or different concentration of PRL inhibitor (PRLi). 24 hours later, cell viability was evaluated by PI/Annexin V staining. Apoptotic cells were defined as PI^- Annexin V^+.

Production of Retrovirus

Retroviral particles were produced by transfection of Phoenix E cells with the MSCV-IRES-GFP or MSCV-AML1-ETO9a-IRES-GFP plasmids, according to standard protocols. Mouse hematopoietic progenitor cells were transduced on retronectin (Takara)-coated non-tissue culture plates with high-titer retroviral suspensions. Twenty-four hours after infection, GFP-positive cells were sorted by FACS. Transduced cells were then transplanted into lethally irradiated recipient mice. The presence of GFP^+ cells in the peripheral blood was measured by flow cytometry analysis.

Statistical Analysis

The animal sample size was based on previous studies evaluating the roles of AML1-ETP9a in leukemia and POWER analysis.[15] Using Chi-Square analysis, 5 mice per group will provide 80% POWER in detecting difference with 95% difference. Gehan-Breslow-Wilcoxon test was used for Kaplan-Meier survival curves. The other data were analyzed by paired or unpaired t test using GraphPad Prizm 5. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, not significant. All experiments were repeated at least once.

Supplementary Figure Legends

Figure S1. (**a**) PRL2 is highly expressed in several human AML cell lines compared to mononuclear cells from human peripheral blood and cord blood. (**b**) K562 cells were transduced with lentiviruses expressing control shRNA (Sh-Luc) or PRL2 shRNAs (PRL2-shRNA-1 and PRL2-shRNA-2). The level of PRL2 proteins was determined by western blot analysis. (**c**) Kasumi-1 cells were transduced with retroviruses expressing dominant-negative PRL2 (PRL2-CSDA) or GFP. The proliferation of transduced cells (GFP^+) was measured over time (**$p<0.01$, n=3). (**d**) Human cord blood $CD34^+$ cells expressing AML1-ETO were transduced with lentiviruses expressing control or PRL2 shRNA. The proliferation of transduced cells was measured over time (**$p<0.01$, n=3). (**e**) Human cord blood $CD34^+$ cells expressing AML1-ETO were transduced with retroviruses expressing WT or dominant-negative PRL2 (PRL2-CSDA). Myeloid progenitors were quantified by using methylcellulose culture (*$p<0.05$, n=3).

Figure S2. (**a**) Loss of PRL2 decreased the proliferation of AML1-ETO9a$^+$ progenitor cells (**$p<0.01$, n = 3). (**b**) Lin^- cells isolated from WT and *Prl2* null mice were transduced with retroviruses expressing AML1-ETO9a (AE9a). Equal number of transduced cells were seeded in methylcellulose culture. One week later, total cell number was measured from both groups (**$p<0.01$, n= 3). (**c**) Frequency of GFP^+ bone marrow cells in recipient mice repopulated with WT or *Prl2* null cells expressing AML1-ETO9a at 20 weeks post transplantation (**$p<0.01$, n= 5). (**d**) Frequency of GFP^+ cells in the peripheral blood of secondary recipient mice at 40 days after transplantation ($p>0.01$, n= 5). (**e**) Wild type recipient mice show

splenomegaly compared to *Prl2* null recipients. (**f**) Wild type recipient mice show thin and lethargic phenotype compared to *Prl2* null recipients.

Figure S3. (**a**) PRL2 mRNA was upregulated in mouse HSPCs expressing AML1-ETO9a (*p<0.05, n=3). (**b**) Ectopic expression of AML1-ETO9a increased the levels of PRL2 protein in mouse HSPCs.

Figure S1

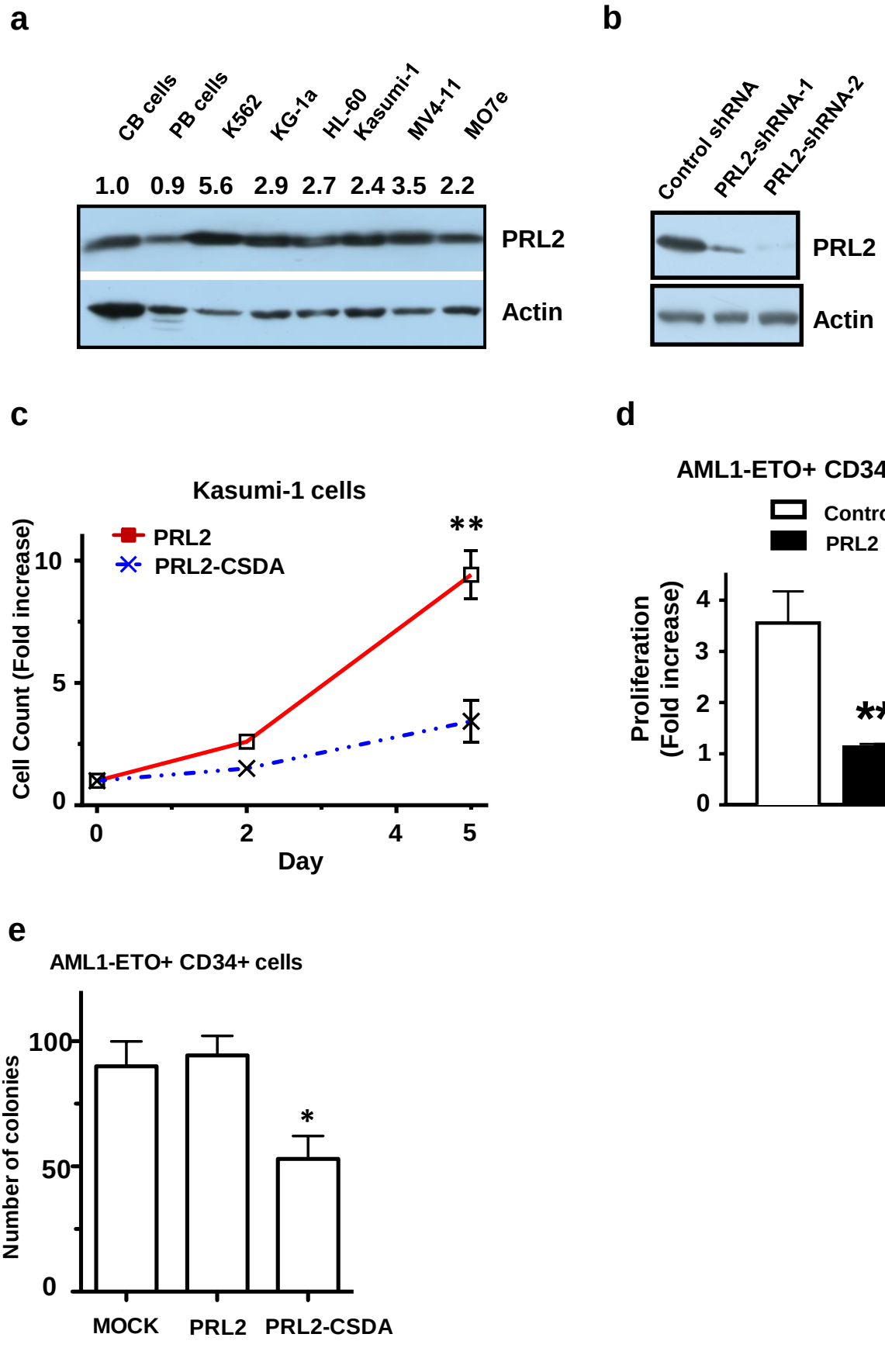

a
CB cells
PB cells
K562
KG-1a
HL-60
Kasumi-1
MV4-11
MO7e
1.0 0.9 5.6 2.9 2.7 2.4 3.5 2.2
PRL2
Actin
b
Control shRNA
PRL2-shRNA-1
PRL2-shRNA-2
PRL2
Actin
c
Kasumi-1 cells
PRL2
PRL2-CSDA
**
Cell Count (Fold increase)
10
5
0
0
2
4
5
Day
d
AML1-ETO+ CD34+ cells
Control shRNA
PRL2 shRNA
Proliferation (Fold increase)
4
3
2
1
0
**
e
AML1-ETO+ CD34+ cells
Number of colonies
100
50
0
*
MOCK
PRL2
PRL2-CSDA

Figure S2

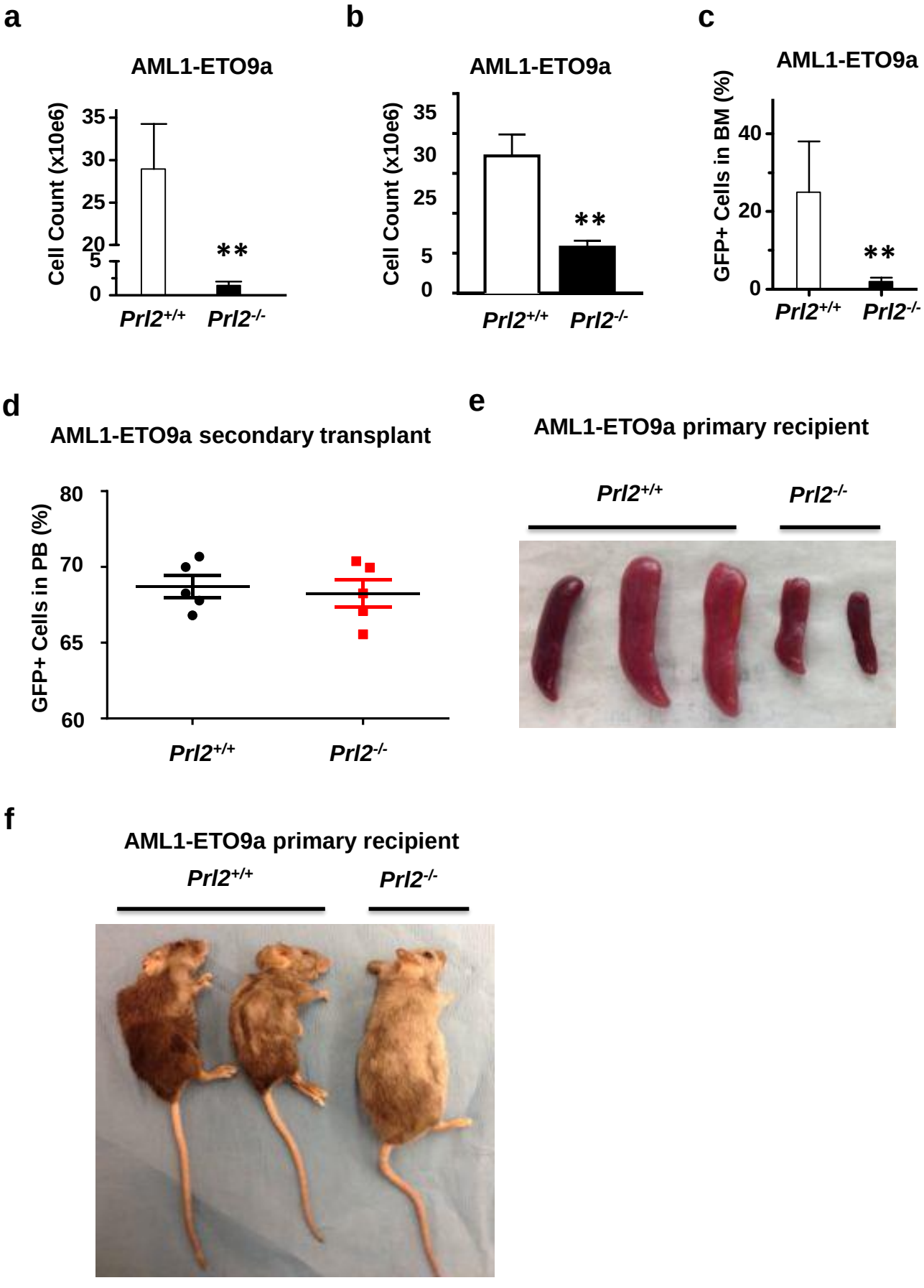
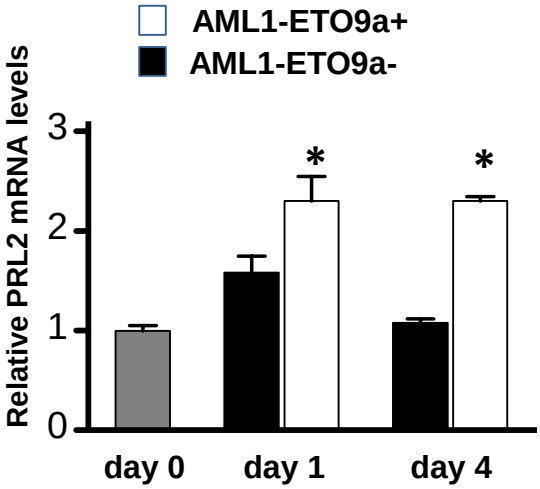
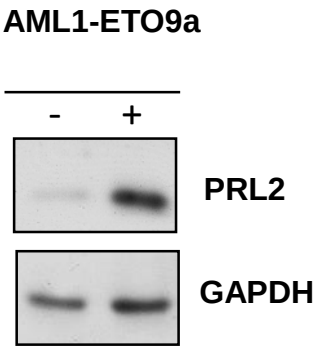

a
AML1-ETO9a
Cell Count (x10e6)
35
30
25
20
5
0
**
Prl2+/+
Prl2-/-
b
AML1-ETO9a
Cell Count (x10e6)
35
30
25
5
0
**
Prl2+/+
Prl2-/-
c
AML1-ETO9a
GFP+ Cells in BM (%)
40
20
0
**
Prl2+/+
Prl2-/-
d
AML1-ETO9a secondary transplant
GFP+ Cells in PB (%)
80
70
65
60
Prl2+/+
Prl2-/-
e
AML1-ETO9a primary recipient
Prl2+/+
Prl2-/-
f
AML1-ETO9a primary recipient
Prl2+/+
Prl2-/-

Figure S3

a


AML1-ETO9a+
AML1-ETO9a-
Relative PRL2 mRNA levels
3
2
1
0
*
*
day 0
day 1
day 4

b


AML1-ETO9a
-
+
PRL2
GAPDH