# Phosphatase PRL2 promotes oncogenic NOTCH1-induced T cell 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 T-ALL cell lines and cord blood cells

Human T-ALL cell lines, including HPB-ALL, Loucy, CEM, Tail-7, Molt4, and Supt1, were obtained from ATCC. All cell lines were authenticated by SRT profiling and tested for mycoplasma contamination. 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 mRNAs 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 T-ALL 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 (ERK, pERK, c-Kit, and Actin) and Abcam (Histone H3).

**Apoptosis assays**

Human T-ALL 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- NOTCH1-ICN1-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 $CD4^+CD8^+$ double positive (DP) leukemic cells in the peripheral blood was measured by flow cytometry analysis.

**Real-time PCR**

For quantitative reverse-transcription polymerase chain reaction (RT-PCR), total RNA was isolated using the RNeasy Plus Micro Kit (QIAGEN), and then subjected to reverse transcription with random hexamers (SuperScript III kit; Invitrogen). Quantitative real-time PCR was performed with SYBR Green (Qiagen) on an ABI PRISM 7500 system. Gene specific primers were designed to flank introns so that products from cDNA could be distinguished from possible genomic DNA contamination. Primer sequences are available upon request.

**Statistical Analysis**

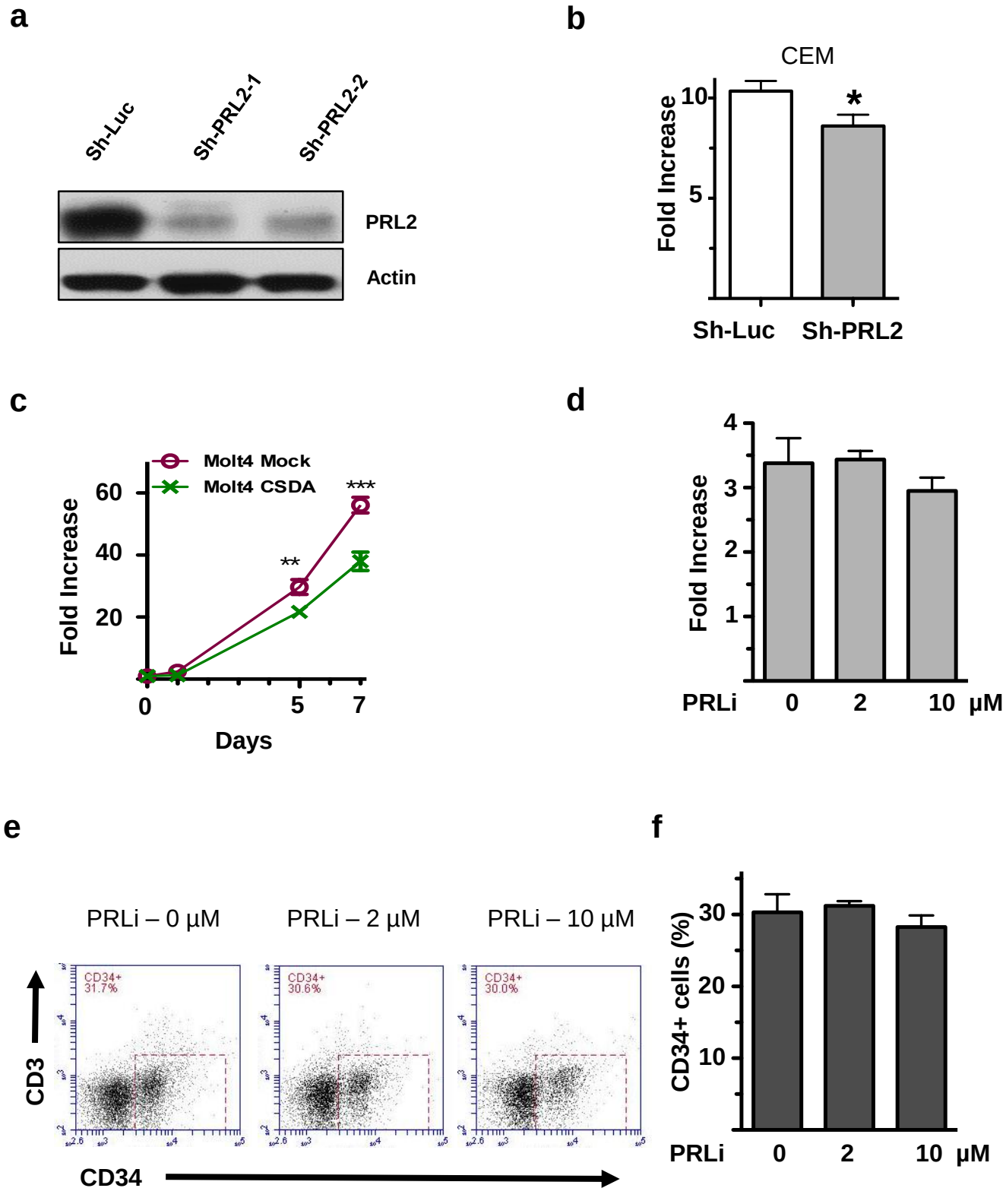
The animal sample size was based on previous studies evaluating the roles of oncogenic Notch in leukemia and POWER analysis. Using Chi-Square analysis, 5 mice per group will provide 80% POWER in detecting difference with 95% difference. Gehan-Breslow-Wilcoxon test was used for Kaplan-Meier survival curves. The other data were analyzed by paired or unpaired t test using GraphPad Prizm 5. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; ns, not significant. All experiments were repeated at least once.

**Supplementary Figure Legends**

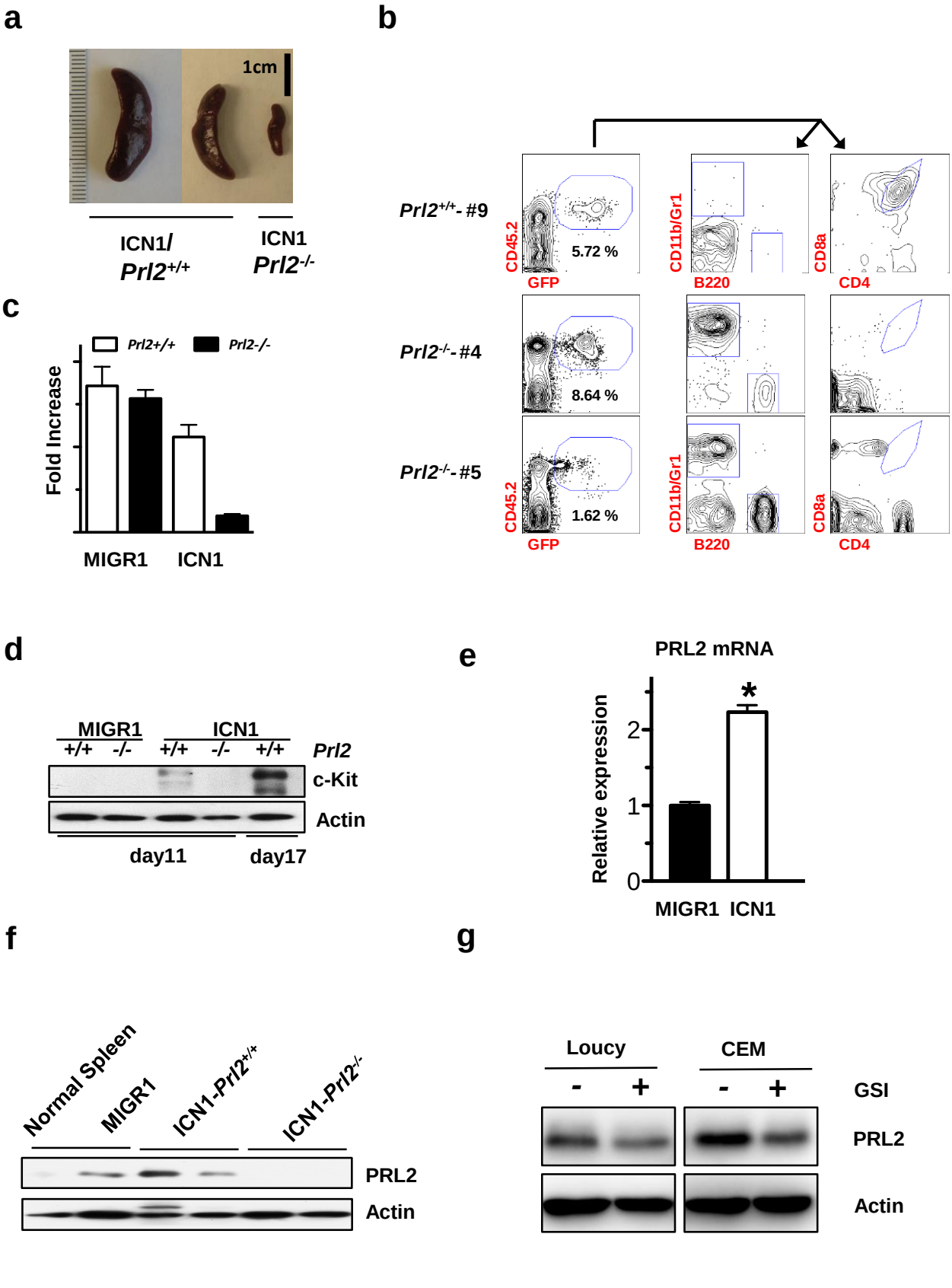
**Figure S1.** (**a**) Tail-7 cells were transduced with lentiviruses expressing control (Sh-Luc) or PRL2 shRNAs (Sh-PRL2-1 and Sh-PRL2-2). The level of PRL2 proteins was determined by western blot analysis. (**b**) CEM cells were transduced with lentiviruses expressing control or PRL2 shRNA. The proliferation of transduced cells ($GFP^+$) was measured over time (*p<0.05, n=3). (**c**) Molt4 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**) PRL2 inhibitor (PRLi) treatment did not affect the viability of human cord blood mononuclear cells (p<0.1, n = 3). (**e**) The Frequency of $CD34^+$ cells in human cord blood mononuclear cells was determined by flow cytometry analysis. Representative flow cytometry plots were shown. (**f**) PRL2 inhibitor (PRLi) treatment did not affect the viability of human cord blood $CD34^+$ cells (p<0.1, n = 3).

**Figure S2**. (**a**) Representative pictures for spleen isolated from recipient mice repopulated with *Prl2*$^{+/+}$ or *Prl2*$^{-/-}$ cells expressing NOTCH1-ICN1. (**b**) Representative flow cytometry plots for analyzing myeloid cell, B cell, CD4$^{+}$ and CD8$^{+}$ T cell engraftment in the surviving recipients. (**c**) Lin$^{-}$Sca1$^{+}$ bone marrow cells isolated from *Prl2*$^{+/+}$ and *Prl2*$^{-/-}$ mice were transduced with retroviruses expressing NOTCH1-ICN1 or GFP. Transduced cells (GFP$^{+}$) were cultured in liquid medium in the presence of SCF, FLT3 ligand, and IL-7. Cell proliferation was measured at day 11. (**d**) Lin$^{-}$Sca1$^{+}$ bone marrow cells isolated from *Prl2*$^{+/+}$ and *Prl2*$^{-/-}$ mice were transduced with retroviruses expressing NOTCH1-ICN1 or GFP. Expression of c-Kit in transduced cells (GFP$^{+}$) was determined by western blot analysis at day11 and day17. *Prl2*$^{-/-}$ cells (GFP$^{+}$) failed to growth at day17. (**e**) The level of PRL2 mRNA in spleenocytes isolated from recipient mice repopulated with *Prl2*$^{+/+}$ cells expressing NOTCH1-ICN1 was determined by quantitative real-time PCR analysis (*$p<0.05$, n=3). (**f**) The level of PRL2 proteins in spleenocytes isolated from recipient mice repopulated with *Prl2*$^{+/+}$ and *Prl2*$^{-/-}$ cells expressing NOTCH1-ICN1 was determined by western blot analysis. Splenocytes from *Prl2*$^{+/+}$ mice or recipients repopulated with *Prl2*$^{+/+}$ bone marrow cells were used as controls. (**g**) γ-secretase inhibitor (GSI) treatment decreased the level of PRL2 proteins in some human T-ALL cell lines.

# Figure S1


a
Sh-Luc
Sh-PRL2-1
Sh-PRL2-2
PRL2
Actin
b
CEM
Fold Increase
10
5
*
Sh-Luc
Sh-PRL2
c
Molt4 Mock
Molt4 CSDA
Fold Increase
60
40
20
**
***
0
5
7
Days
d
Fold Increase
4
3
2
1
PRLi
0
2
10 µM
e
PRLi – 0 µM
PRLi – 2 µM
PRLi – 10 µM
CD34+
31.7%
CD34+
30.6%
CD34+
30.0%
CD3
CD34
f
CD34+ cells (%)
30
20
10
PRLi
0
2
10 µM

# Figure S2


a
1cm
ICN1/
Prl2+/+
ICN1
Prl2-/-
b
Prl2+/+- #9
Prl2-/- - #4
Prl2-/- - #5
CD45.2
GFP
5.72 %
8.64 %
1.62 %
CD11b/Gr1
B220
CD8a
CD4
c
Prl2+/+
Prl2-/-
Fold Increase
MIGR1
ICN1
d
MIGR1
ICN1
+/+
-/-
+/+
-/-
+/+
Prl2
c-Kit
Actin
day11
day17
e
PRL2 mRNA
Relative expression
*
2
1
0
MIGR1
ICN1
f
Normal Spleen
MIGR1
ICN1-Prl2+/+
ICN1-Prl2-/-
PRL2
Actin
g
Loucy
CEM
-
+
-
+
GSI
PRL2
Actin