Supplemental Data

Paradoxical enhancement of leukemogenesis in acute myeloid leukemia with moderately-attenuated RUNX1 expressions.

Supplemental Tables

PCR primers used for RT-qPCR	Forward (5' → 3')	Reverse (5' → 3')
RUNX1	CTGCTCCGTGCTGCCTAC	AGCCATCACAGTGACCAGAGT
RUNX2	GGTTAATCTCCGCAGGTCACT	CACTGTGCTGAAGAGGCTGTT
RUNX3	CAGAAGCTGGAGGACCAGAC	GTCGGAGAATGGGTTCAGTT
GSTA2	ATGTTCCAGCAAGTGCCAATG	TGAGAATGGCTCTGGTCTGCA
GAPDH	CATGTTCGTCATGGGGTGAACCA	AGTGATGGCATGGACTGTGGTCAT
Pan_*RUNX* (*RUNX1* + *RUNX2* + *RUNX3*)	GCACCGACAGCCCCAACTT	GTCTTGTTGCAGCGCCAGTG

Table S1
List of primers used for RT-qPCR experiments in this study.

PCR primers used for ChIP	Forward (5’ → 3’)	Reverse (5’ → 3’)
GSTA2 P1	ATGCCTATGACCAGATTTAGTTAAAA	GCAGAATGTCAGAAGTAAATTTCTATACA
GSTA2 P2	GCGTGTTTGGATTAGAAGTGATT	TCTTGGTATATCTGGATAGGCCC

Table S2
List of primers used for ChIP assay in this study.

Target sequences for shRNA knockdown experiments.	5’ → 3’
sh_ *RUNX1_profound* #1	AGCTTCACTCTGACCATCA
sh_ *RUNX1_profound* #2	AACCTCGAAGACATCGGCA
sh_ *RUNX1_moderate* #1	ATGCTACCGCAGCCATGAA
sh_ *RUNX1_moderate* #2	ACTTTCCAGTCGACTCTCA
sh_ *Luc.*	CGTACGCGGAATACTTCGA
sh_ *GSTA2* #1	AGGAGAAAGCCCTGATTGAT
sh_ *GSTA2* #2	AGGAACAAGATGCCAAGCT

Table S3
List of target sequences for shRNA-mediated knockdown experiments in this study.

RUNX1 expression	High	Medium	Low
Total number of patients included	62	63	62
Sex			
Female	34	29	23
Male	28	34	39
Age at diagnosis (average, years old)	58.2 ± 1.9	55.4 ± 1.8	52.3 ± 2.3
Cytogenetic risk category			
Favorable	9	15	11
Intermediate	34	35	41
Poor	18	11	9
Not available	1	1	1

Table S4

Clinical background of AML patients from TCGA clinical datasets (n = 187). Patients were divided into 3 groups according to their *RUNX1* expressions (*RUNX1* high; n = 62, *RUNX1* intermediate; n = 63, *RUNX1* low; n = 62). Cytogenetic risk is determined according to the WHO classification guidelines.

Supplemental Figures

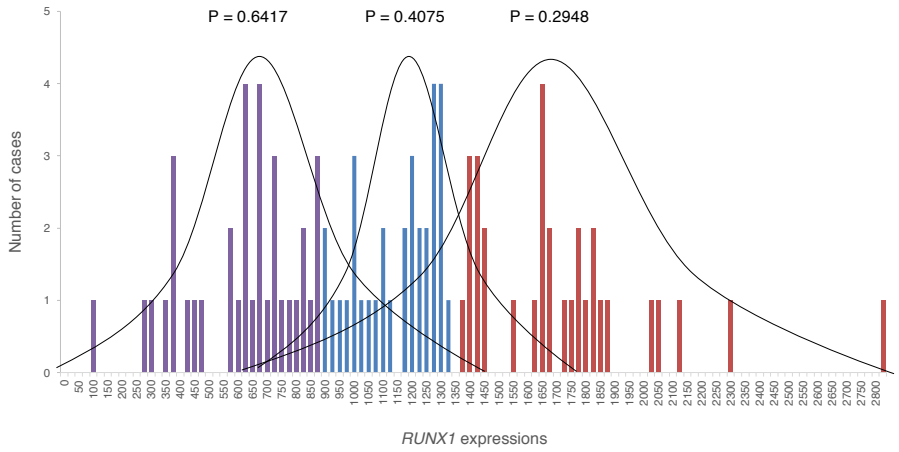

Figure S1
Histogram showing the distribution of RUNX1 expressions in AML patients from TCGA clinical datasets (n = 187). Patients were divided into 3 groups according to their *RUNX1* expressions (*RUNX1* high; n = 62, *RUNX1* intermediate; n = 63, *RUNX1* low; n = 62). Kolmogorov-Smirnov test was used to examine whether each peak follows normal (Gaussian) distribution. $P < 0.05$ was considered statistically significant.

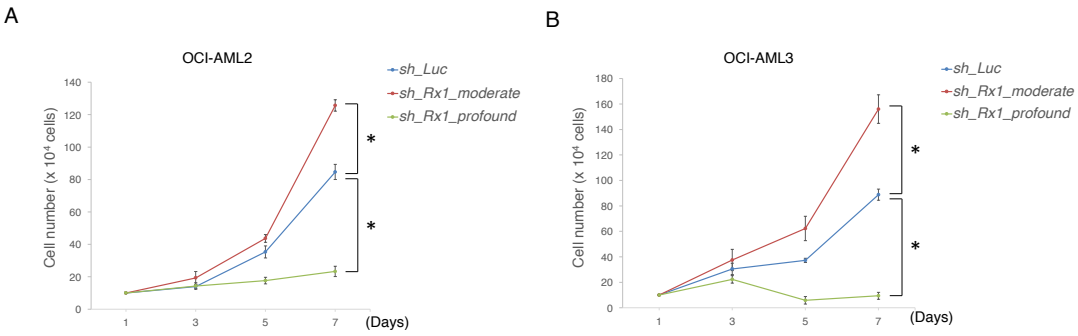

Figure S2
(A and B) Growth curves of OCI-AML2 (A) and OCI-AML3 (B). Cells were transduced with control (sh_*Luc.*) or with RUNX1 shRNAs (sh_*Rx1*_moderate #1 or sh_*Rx1*_profound #1), then cultured in the presence of 3 µM of doxycycline (n = 3). Data are mean±SEM values. * $P < 0.05$, by two-tailed Student's t test.

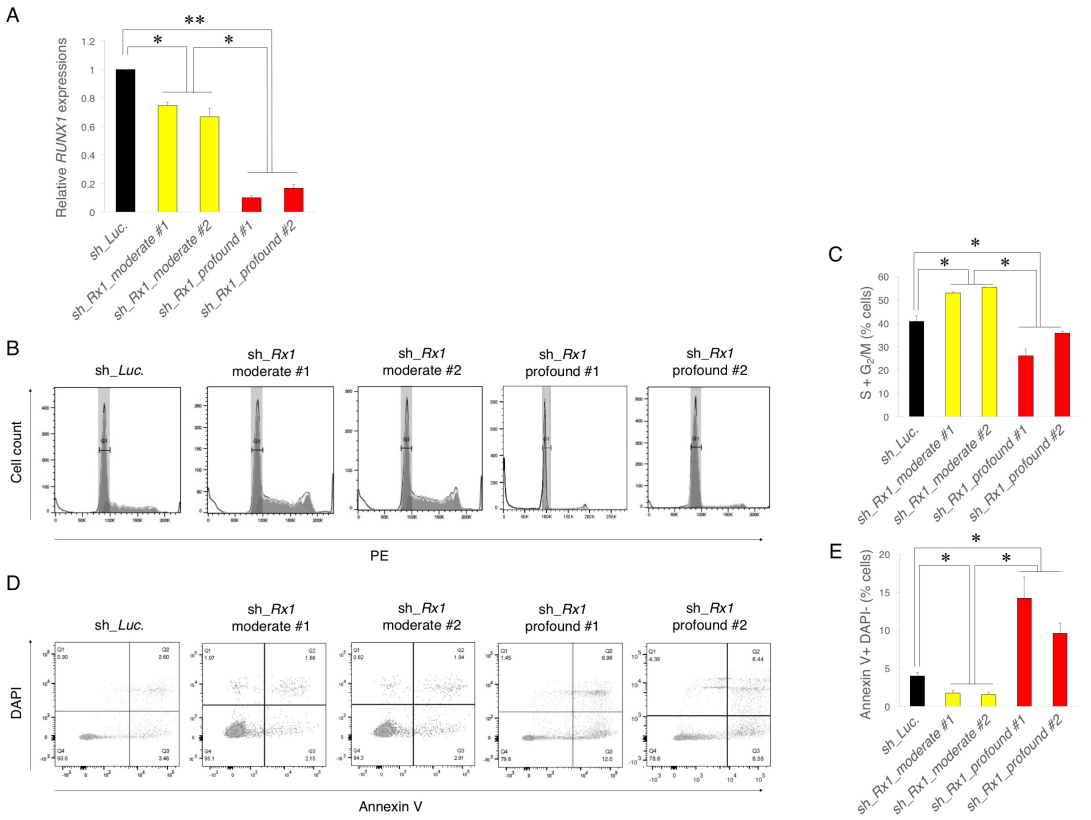

Figure S3

(A) Efficacy of shRNAs targeting *RUNX1*. MV4-11 cells were transduced with lentivirus encoding shRNA targeting *Luciferase* (sh_*Luc.*) or shRNAs against *RUNX1* (sh_*Rx1*_moderate #1, #2 or sh_*Rx1*_profound #1, #2) and incubated with 3μM doxycycline for 48 hours, then total RNA was prepared and analyzed by real-time RT-PCR. Values are normalized to that of control vector-transduced cells (n = 3).
(B and C) *RUNX1* depletion-mediated change in the cell cycle status was determined in MV4-11 cells used in (A). (B) Representative figures of cell cycle status.
(C) Cumulative data of the number of cells with S + G_2/M phase DNA content (n = 3).
(D and E) Frequency of early apoptotic cell death induced by RUNX1 silencing. MV4-11 cells transduced with control (sh_*Luc.*) or with *RUNX1* shRNAs (sh_*Rx1*_moderate #1, #2 or sh_*Rx1*_profound #1, #2) were treated as in (A), and the early apoptotic cells (Annexin V^+ $DAPI^-$) were scored by flow cytometric analysis. (D) Representative flow cytometry plots. (E) Cumulative data in (F) (n = 3).
Data are mean±SEM values. * $P < 0.05$, ** $P < 0.01$, by two-tailed Student's *t* test.

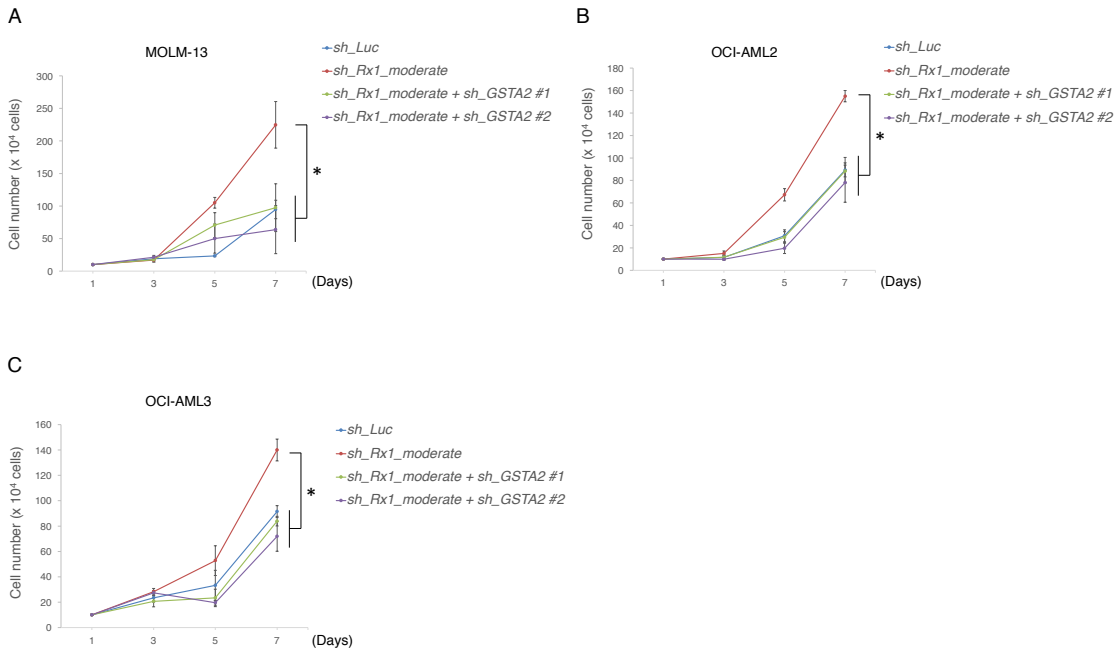

Figure S4

(A-C) Growth curves of MOLM-13 (A), OCI-AML2 (B) and OCI-AML3 (C). Cells were transduced with lentivirus encoding shRNA targeting *Luciferase* (sh_*Luc.*) or shRNAs against *GSTA2* (sh_*GSTA2* #1 or sh_*GSTA2* #2) with or without simultaneous transduction of shRNA targeting *RUNX1* (sh_*Rx1*_moderate), then cultured in the presence of 3 μM doxycycline (n = 3).

Data are mean±SEM values. * $P < 0.05$, by two-tailed Student's t test.