Supporting Information

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SI Materials and Methods

Cell Culture, Reagents, and Antibodies. U937-mt-PLZF/RARα and U937-mt-P/R9 cells, which conditionally express promyelocytic leukemia zinc finger/retinoic acid receptor-α (PLZF/RARα) and promyelocytic leukemia/retinoic acid receptor-α (PML/RARα) fusion proteins, respectively, were kindly provided by P. G. Pelicci (University of Milano, Milan, Italy). In parallel, U937-mt-Bulk cells were used as parental control. All cell lines were maintained in RPMI 1640 supplemented with 10% (vol/vol) FBS (Gibco BRL) at 37 °C in a fully humidified atmosphere with 5% CO_2. COS-7 cells were maintained in DMEM supplemented with 10% (vol/vol) FBS. Primary murine leukemic cells were flushed from the tibia and femur of mice afflicted by full-blown myeloid leukemia, and cultured in DMEM containing 20% (vol/vol) FBS. All-*trans* retinoic acid (ATRA), TSA, PMA, DMSO, theophylline, H89 (Sigma-Aldrich), and 8-CPT-cAMP (BIOLOG Life Science) were prepared as described previously (1). The following antibodies were used: RARα (sc-551), Lamin B (sc-6216) (all from Santa Cruz), PLZF (ab39354), silencing mediator for retinoic acid and thyroid hormone receptors (SMRT; ab24551), nuclear receptor corepressor (NCoR1; ab24552), acetylated H3 (ab47915), trimethylated H3K4 (ab8580), and H3K27 (ab6002) (all from Abcam), acetylated H4 (06-866, Millipore), p-(Ser/Thr) PKA substrates (9621), p-Ser133-CREB (9198), CREB (9104) (all from Cell Signaling), and β-actin (a1978, Sigma).

Microarray Analysis. We performed a cDNA microarray analysis to profile the gene expression patterns in $CD34^+$ bone marrow (BM) cells from both *PLZF/RARα* (three PLZF-RARα and two PLZF/RARα-RARα/PLZF animals) and *PML/RARα* (four PML-RARα animals) transgenic mice with leukemia. Primary BM cells were harvested from the indicated transgenic mice with leukemia and subjected to immunomagnetic-positive selection using the CD34 monoclonal antibody (Miltenyi Bio-tech). Total RNA from the sorted cells was hybridized to the CodeLink UniSet Mouse I Bioarray chips (Amersham). Labeled chips were read by GenePix 4000B microarray scanner (Molecular Devices). Normalized data were filtered for minimal expression and then tested for gene-set enrichment using Gene Set Enrichment Analysis (GSEA) v2.0 (www.broad.mit.edu/gsea). GSEA enrichment results were filtered for statistical significance using a nominal *P*-value threshold of 0.05.

Immunophenotyping, Cytochemistry, and Histology. For immunophenotypic analysis, fresh 1×10^6 BM cells were stained with anti-mouse Mac-1-FITC, Gr-1-PerCP-cy5.5, c-Kit-APC, CD34-PE, Sca-1-PE antibodies, and antilineage kit (BD Pharmingen) separately or in combination, and then analyzed on an LSR II system (BD Biosciences). For morphological analysis, cytospin slides containing aliquots of 3–4 $\times 10^4$ cells of each sample were stained with Wright–Giemsa staining solution before microscopic inspection. For the nitroblue tetrazolium (NBT) reduction test, cells were incubated in PBS containing 0.1% NBT and 0.5 μg/mL PMA for 30 min at 37 °C. Cells were rinsed with PBS three times before being spun to slides for microscopic examination. For histopathological analysis, sections of selected organs were prepared and stained with H&E by using standard protocol, as previously described (2).

Luciferase Reporter Analysis. The pRARE-tk-Luc reporter plasmid, which was designed to drive the expression of firefly luciferase reporter under the control of a synthetic core consensus sequence derived from the retinoic acid response element (RARE) in the human *RARβ2* gene, has been described previously (3). pRL-SV40 was used as internal control for transfection efficiency evaluation (Promega). Transient transfection of U937 cells was carried out using GenePulser II electroporation system (Bio-Rad). Before electroporation, U937-mt-P/R9 and U937-mt-PLZF/RARα cells were cultured in medium containing 100 μM $ZnSO_4$ for 12 h to induce the expression of the PML/RARα and PLZF/RARα fusion protein, respectively. For electroporation, the complete medium was removed and 2×10^6 cells were resuspended in RPMI 1640 containing pRARE-tk-Luc (10 μg) and pRL-SV40 (2.5 μg). The electroporation parameters were 950 μF and 220 V at room temperature. Twelve hours after transfection, cells were treated with indicated materials for an additional 24 h. Then luciferase activities of each sample were detected with Dual-Luciferase Reporter Assay System (Promega) using Lumat LB9507 luminometer (Berthold).

Mammalian Two-Hybrid Analysis. Plasmids for mammalian two-hybrid analysis including $pGAL(RE)_5$-tk-Luc, pNLVP16, pVP16-PLZF/RARα, pGal4 empty vector, pGal4-SMRT and pGal4-NCoR1 were kindly provided by A. Zelent (Institute of Cancer Research, Sutton, United Kingdom) and R. N. Cohen (University of Chicago, Chicago, IL) (4, 5). The pVP16-PLZF/RARα-S765A mutant was constructed by site-directed mutagenesis kit (Stratagene). COS-7 cells maintained in 24-well plates were transiently transfected using SuperFect (Qiagen) with 50 ng pVP16-PLZF/RARα and 50 ng pGal4-SMRT or pGal4-NCoR1, in company with 600 ng $pGAL(RE)_5$-tk-Luc and 1.5 ng pRL-SV40. After 18 h, cells were treated with ATRA and/or 8-CPT-cAMP for additional 6 h. The activities of luciferase were detected as above.

ChIP. After $ZnSO_4$ induction, U937-mt-PLZF/RARα cells were treated with ATRA and/or 8-CPT-cAMP, as described above. Next, 2×10^7 cells of each sample were collected for ChIP assay, as previously described (6). Specific primer pairs spanning RARE sites of individual genes are listed below.

RT-PCR and Western Blot. Real-time RT-PCR assays were carried out by using SYBR Green PCR Master Mix reagents on ABI PRISM 7000 SDS (Applied Biosystems). For Western blot, 20-μg proteins of each sample were fractioned by denatured SDS/PAGE and then transferred onto PVDF membrane (GE Healthcare). Specific antibodies were detected by using an ECL detection kit (Millipore). PCR Primers and ChIP-PCR primers of human or murine genes are listed below.

Assays	Genes	Forward primers (5′ to 3′)	Reverse primers (5′ to 3′)
For gene expression	Human *RARα2*	GAACCGGGCCTGTTTGCTCCCAGA	GGATGCTGCGGCGGAAG
	Human *RARβ2*	GATCGTGGAGTTTGCTAAACG	CAGAGGACCAAATCCAGCAT
	Human *CEBPε*	AGCCGAGGCAGCTACAATC	TCACAGTGCAACTTTATTC
	Human *TGM2*	GCCACTTCATTTTGCTCTTCAA	TCCTCTTCCGAGTCCAGGTACA
	Mouse *RARα2*	AGACACGCAGACGGGTTG	GAGGATGCCACTCCCAGA
	Mouse *RARβ2*	CACCGGCATACTGCTCAA	CAAACGAAGCAGGGCTTG
	Mouse *CEBPε*	TCCCCTGCAGTACCAAGTG	GTGCCTTGAGAAGGGGACT
	Mouse *TGM2*	CTCACGTTCGGTGCTGTG	CTCACGTTCGGTGCTGTG
	Mouse *Cathepsin G*	CTGACTAAGCAACGGTTCTGG	GATTGTAATCAGGATGGCGG
	Mouse *Elastase2*	TGGAGGTCATTTCTGTGGTG	CTGCACTGACCGGAAATTTAG
	Mouse *MPO*	GGAAGGAGACCTAGAGGTTGG	TCCCCATCCACCATGTTTTA
	Mouse *Lactoferrin*	TATTTCTTGAGGCCCTTGGA	TCTCATCTCGTTCTGCCACC
	Mouse *MMP9*	ACGGTTGGTACTGGAAGTTCC	CCAACTTATCCAGACTCCTGG
	18S RNA	CGCGGTTCTATTTTGTTGGTTT	TTCGCTCTGGTCCGTCTTG
For ChIP	*hRARα2* promoter	GAGCTGCACAATGTCACACC	TCCTCCCCTTAACACACACC
	hRARβ2 promoter	TCCTGGGAGTTGGTGATGTCAG	AAACCCTGCTCGGATCGCTC
	hCEBPε promoter	GTGCCTCAAGAGCAGGTGG	GACCTACTACGAGTGTGAG

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4. Guidez F, et al. (1998) Reduced retinoic acid-sensitivities of nuclear receptor corepressor binding to PML- and PLZF-RARalpha underlie molecular pathogenesis and treatment of acute promyelocytic leukemia. *Blood* 91(8):2634–2642.
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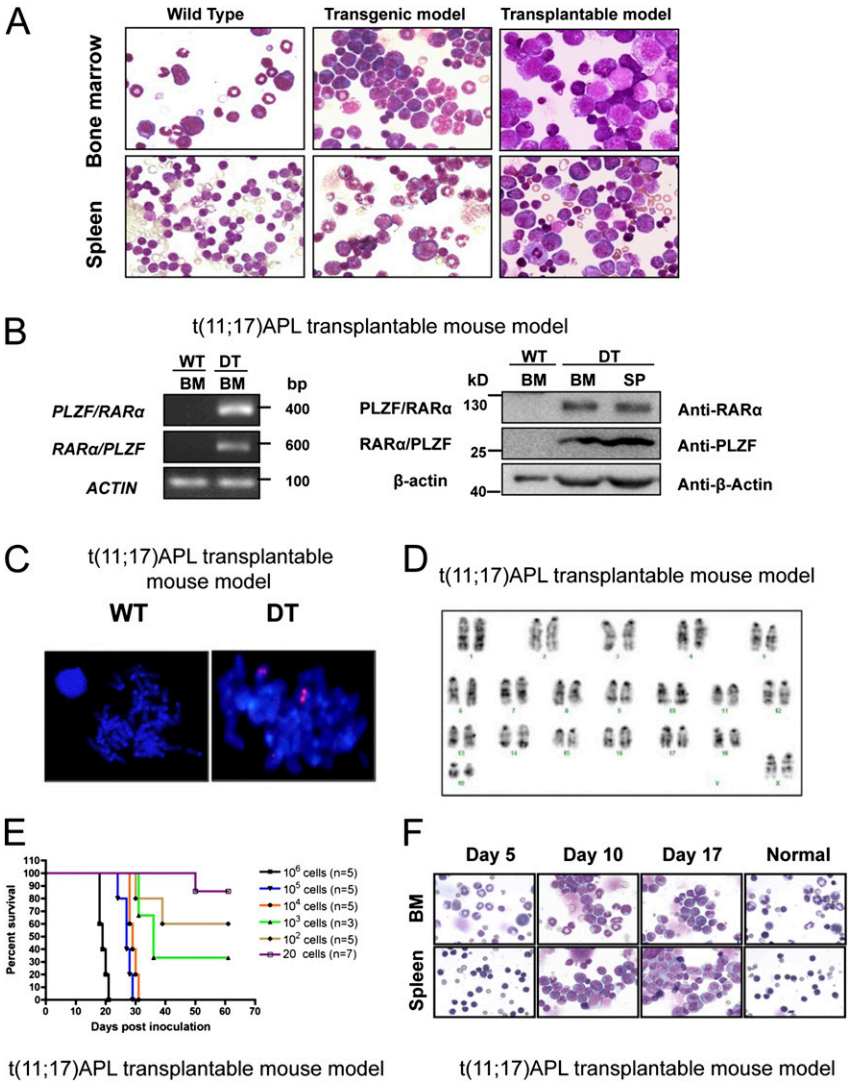
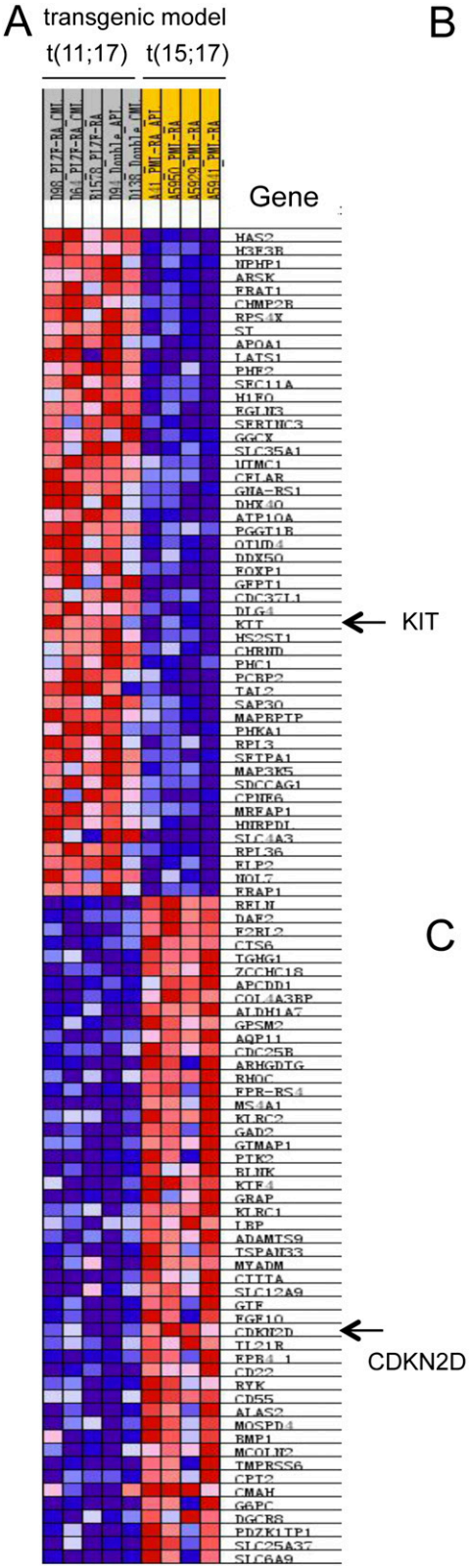


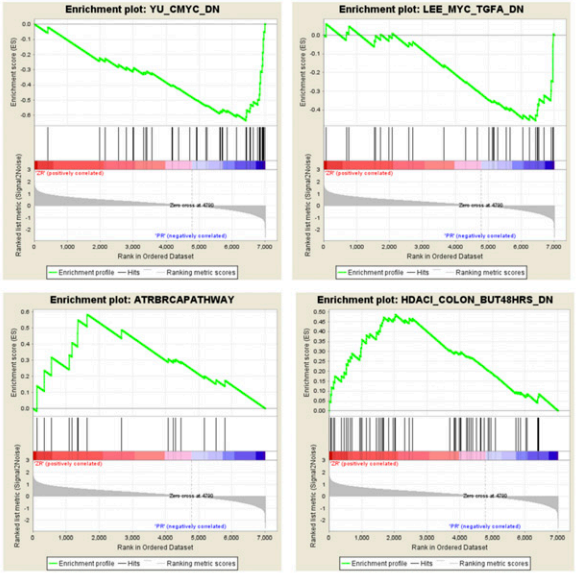
Fig. S1. Establishment of a unique *t*(11;17) acute promyelocytic leukemia (APL) transgenic and transportable murine model. (*A*) Morphological features of cells collected from bone marrow (*Upper*) and spleen (*Lower*) of wild-type C57/BL6J mouse (*Left*), transgenic leukemic mouse (*Center*), and recipient of leukemia transplantation (*Right*) (magnification, 1,000×). (*B*) RT-PCR and Western blot results confirmed expression of the *PLZF/RARα* and *RARα/PLZF* fusion genes at the mRNA and protein level in tissues from a transplantable mouse model. (WT, wild-type; DT, dual transgene; BM, bone marrow; SP, spleen). (*C*) FISH analysis displays the integration of exogenous transgenes in the genome of the transplantable model. Detection of both *PLZF/RARα* and *RARα/PLZF* fusion genes in leukemic cells from mouse BM (magnification, 1,000×). (*D*) Cytogenetic analysis of the leukemic blasts cannot detect any gross chromosomal abnormalities. Cytogenetic analysis of leukemic cell from transplantable model with both *PLZF/RARα* and *RARα/PLZF* fusion genes is shown. (*E*) Survival analysis of recipient mice inoculated with different doses of transplantable leukemic cells. Different doses (a dilution from 20 to 1×10^6 cells) of nucleated cells collected from spleens of mice with full-blown leukemia were injected into each recipient mouse via tail vein. (*F*) Wright–Giemsa staining of BM and spleen cells from the mice inoculated with 1×10^6 leukemic cells at days 5, 10, and 17 postinoculation, respectively (magnification, 1,000×). A normal C57BL/6J mouse was used as a wild-type control.

A



B

Gene Set	*Size (n)*	*NES*	*Nom P value*
Genes sets enriched in PML/RARα transgenic mice			
YU_CMYC_DN	44	-1.66	0.0001
LEE_MYC_TGFA_DN	33	-1.47	0.120
Genes sets enriched in PLZF/RARα transgenic mice			
ATRBRCAPATHWAY	16	1.73	0.0001
HDACI_COLON_BUT48HRS_DN	68	1.55	0.0001



C

Gene name	*Scores*
PLZF target genes up-regulated in PLZF/RARα transgenic mice	
KIT, c-KIT	1.500
Runx2, runt-related transcription factor 2	1.202
MYC, v-myc myelocytomatosis viral oncogene homolog (avian)	0.635
PLZF target genes down-regulated in PLZF/RARα transgenic mice	
CDKN2D, cyclin-dependent kinase inhibitor 2D	-1.268
TESK1, testis-specific kinase 1	-0.617
MYC target genes up-regulated in PLZF/RARα transgenic mice	
HAS2, hyaluronan synthase 2	2.748
H3F3B, H3 histone, family 3B (H3.3B)	1.881
BRD4, bromodomain containing 4	0.821
MYC target genes down-regulated in PLZF/RARα transgenic mice	
MS4A1, membrane-spanning 4-domains, subfamily A, member 1	-1.400
CMAH	-1.218
Myeliod leukemia cell differentiation related genes	
RXRA, retinoid X receptor, alpha	1.092
NCOR1, nuclear receptor co-repressor 1	1.122
SMRT, nuclear receptor co-repressor 2	0.921

Fig. S2. GSEA of the microarray data of two distinct APL transgenic mouse models. (*A*) Heat map representation of the top 50 up- and down-regulated genes in *t*(11;17) APL compared with *t*(15;17) APL. *KIT* and *CDKN2D* (arrow) is up- and down-regulated in *t*(11;17) APL, respectively. (*B*) Representative gene sets were listed by normalized enrichment score (NES) in each APL model. GSEA plots showed particular gene sets that enriched in PML/RARα APL (*Upper*) or PLZF/RARα APL (*Lower*), respectively. (*C*) Significant target genes were listed with normalized expression score.

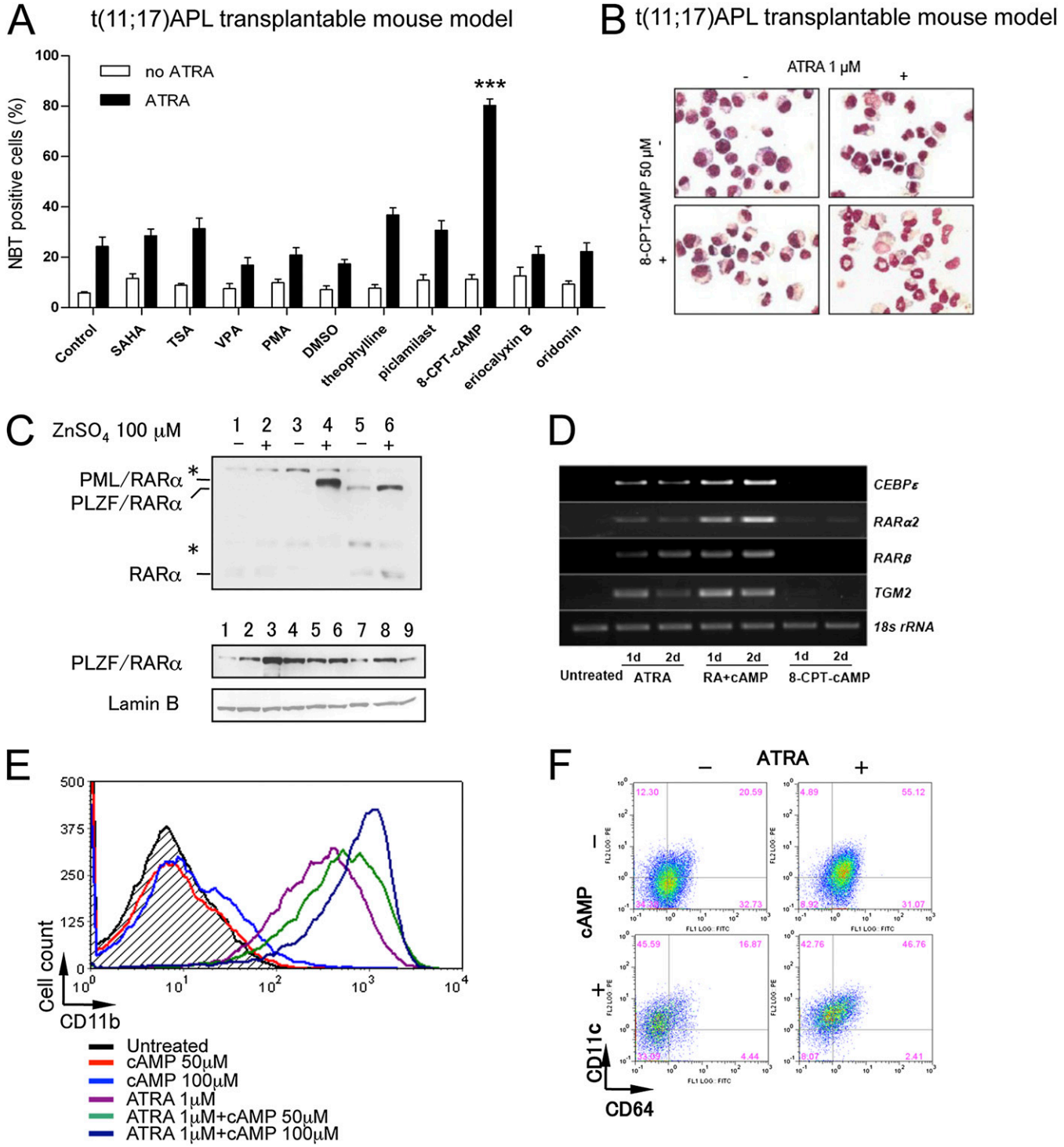


Fig. S3. 8-CPT-cAMP potentiates ATRA-mediated gene transactivation. (*A*) Results of NBT reduction assay of murine primary leukemic cells after 48-h treatment of differentiating agents [including 1 μM SAHA, 25 nM TSA, 0.5 μM VPA, 1 nM PMA, 1% (vol/vol) DMSO, 2 mM theophylline, 2 μM piclamilast, 50 μM 8-CPT-cAMP, 0.5 μM eriocalyxin B, 1 μM oridonin] with or without 1 μM ATRA. (*B*) Morphological analysis of primary mouse leukemia blasts from *t*(11;17) APL transplantable mouse model after 8-CPT-cAMP (50 μM) and/or ATRA (1 μM) treatment for 2 d (magnification, 1,000×). (*C*) Result of Western blot with anti-RARα antibody showed the expression of PML/RARα or PLZF/RARα fusion proteins in U937-mt-Bulk (lanes 1 and 2), U937-mt-P/R9 (lanes 3 and 4), and U937-mt-PLZF/RARα (lanes 5 and 6) cells, respectively, after $ZnSO_4$ exposure for 12 h (*Upper*, * denotes unspecific band), and the expression pattern of PLZF/RARα in U937-mt-PLZF/RARα cells (with lanes 1–9 representing samples after 0, 12, 24, 48, 72, 96, 120, 144, and 168 h of $ZnSO_4$ exposure, respectively). The amounts of Lamin B were used as internal control indicating equivalent loadings (*Lower*). (*D*) Semiquantitative RT-PCR results of *RARα2*, *RARβ2*, *CEBPε*, and *TGM2* genes in U937-mt-PLZF/RARα cells after 8-CPT-cAMP (100 μM) and/or ATRA (1 μM) for 1 and 2 d. 18s rRNA was used as an internal control. (*E* and *F*) The expression of CD11b, CD11c and CD64 on U937-mt-PLZF/RARα cells after 8-CPT-cAMP (50 or 100 μM) and/or ATRA (1 μM) for 72 h. Each typical result out of three independent assays was shown.

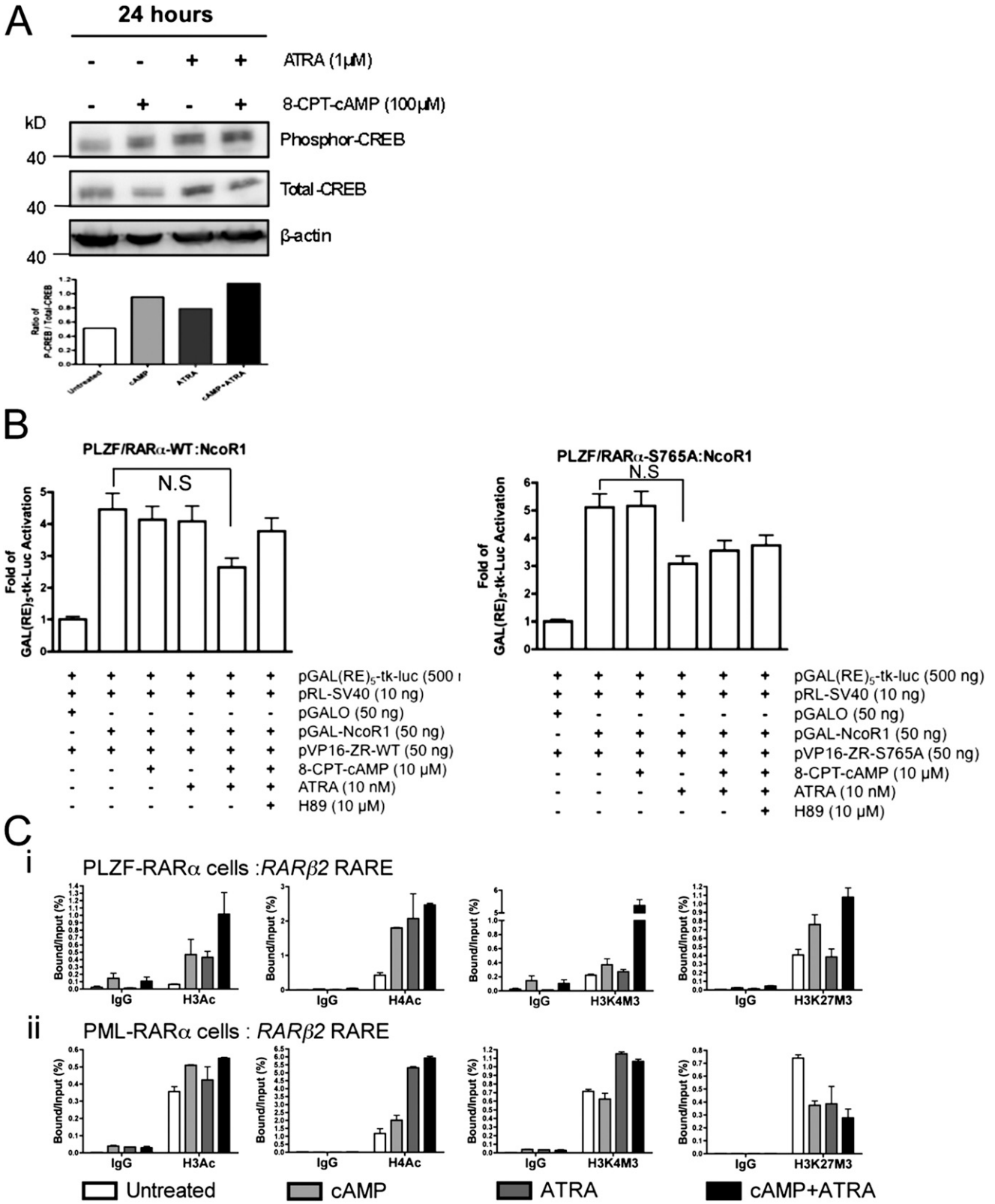


Fig. S4. 8-CPT-cAMP enhances ATRA-induced gene transactivation through its Ser765 phosphorylation. (*A*) Phosphorylated CREB level is elevated upon 8-CPT-cAMP addition. Both Ser133-phosporylated and total CREB proteins were detected by Western blot analysis. Loading was calibrated by β-actin (*Upper*). Quantitative analysis of the ratio of Ser133-phosphorylated and total CREB level was presented by calculating the band's integrated optical density. (*B*) 8-CPT-cAMP couldn't potentiate the ATRA-induced dissociation between NcoR1 and PLZF/RARα S765A mutant. A similar experiment was performed as above. Results are expressed as mean ± SD of two independent experiments. ChIP assays in U937-mt-PLZF/RARα (*C, i*) U937-mt-P/R9 (*C, ii*) cells after being treated with indicated agents. N.S. refers to "not significant" in comparison with Student *t* test.

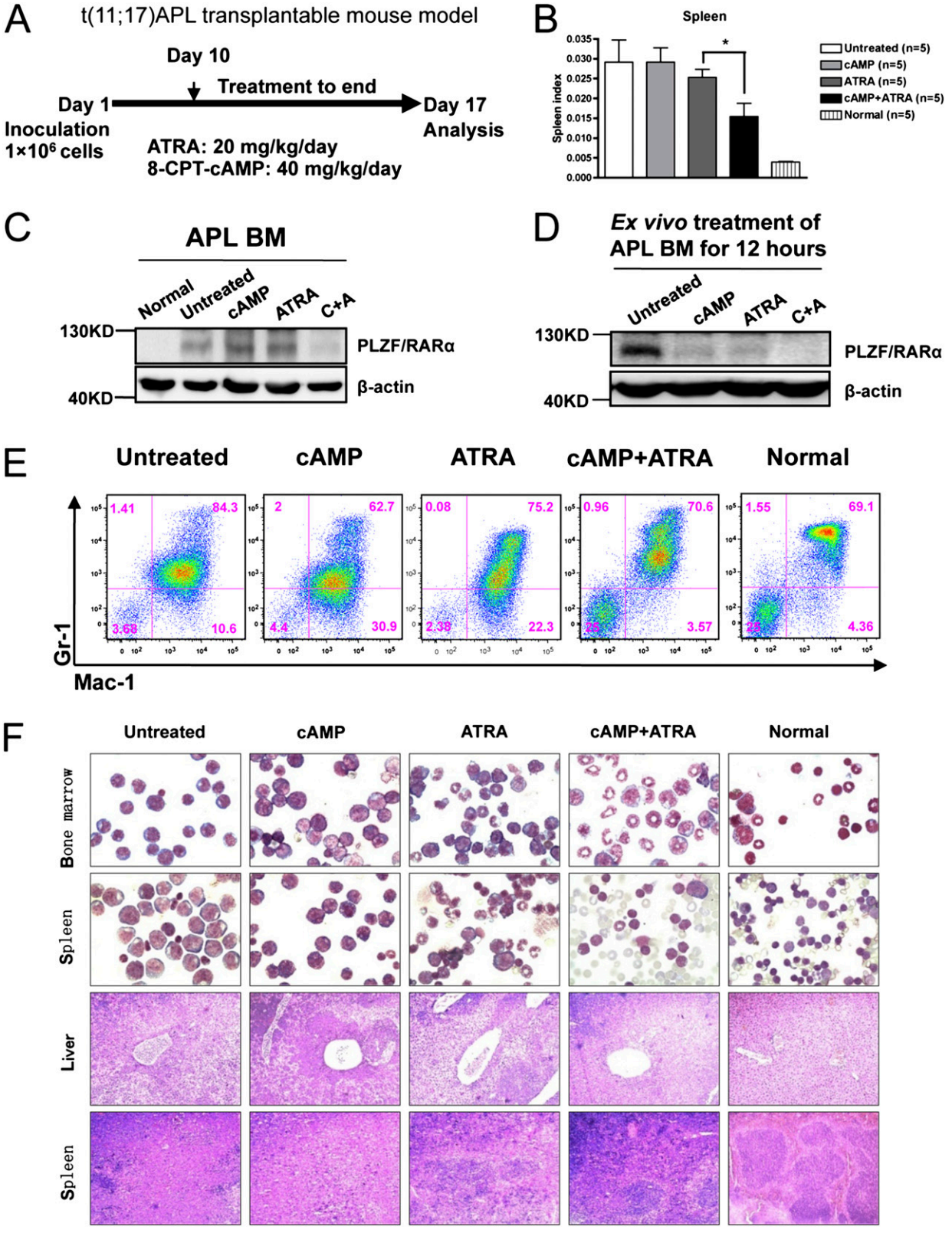


Fig. S5. Combined treatment with 8-CPT-cAMP and ATRA reduces the tumor burden in *t*(11;17) APL transplantable mouse model in vivo. (*A*) Scheme of the short-term high-dose drug treatment in vivo. (*B*) Spleen index of mice receiving drug treatment in vivo. (*C*) Western blot of PLZF/RARα protein in bone marrow cells from each treatment group was done by anti-RARα antibody (*Upper*). Equal loading was assessed by β-actin (*Lower*). (*D*) PLZF/RARα protein was degraded by combined therapy ex vivo. BM cells from *t*(11;17) APL mice were treated with 8-CPT-cAMP (100 μM) and/or ATRA (1 μM) ex vivo for 12 h. (*E*) Flow cytometry analysis of bone marrow cells from each treatment group by Mac-1 and Gr-1. Representative data of two independent experiments were shown. (*F*) Leukemic cell infiltrations of organs (BM, spleen, and liver) among mice receiving different treatments. Both cytospin (*Upper* two panels; magnification, 1,000×) and biopsy results (*Lower* two panels; magnification, 100×) of infiltration organs were presented.

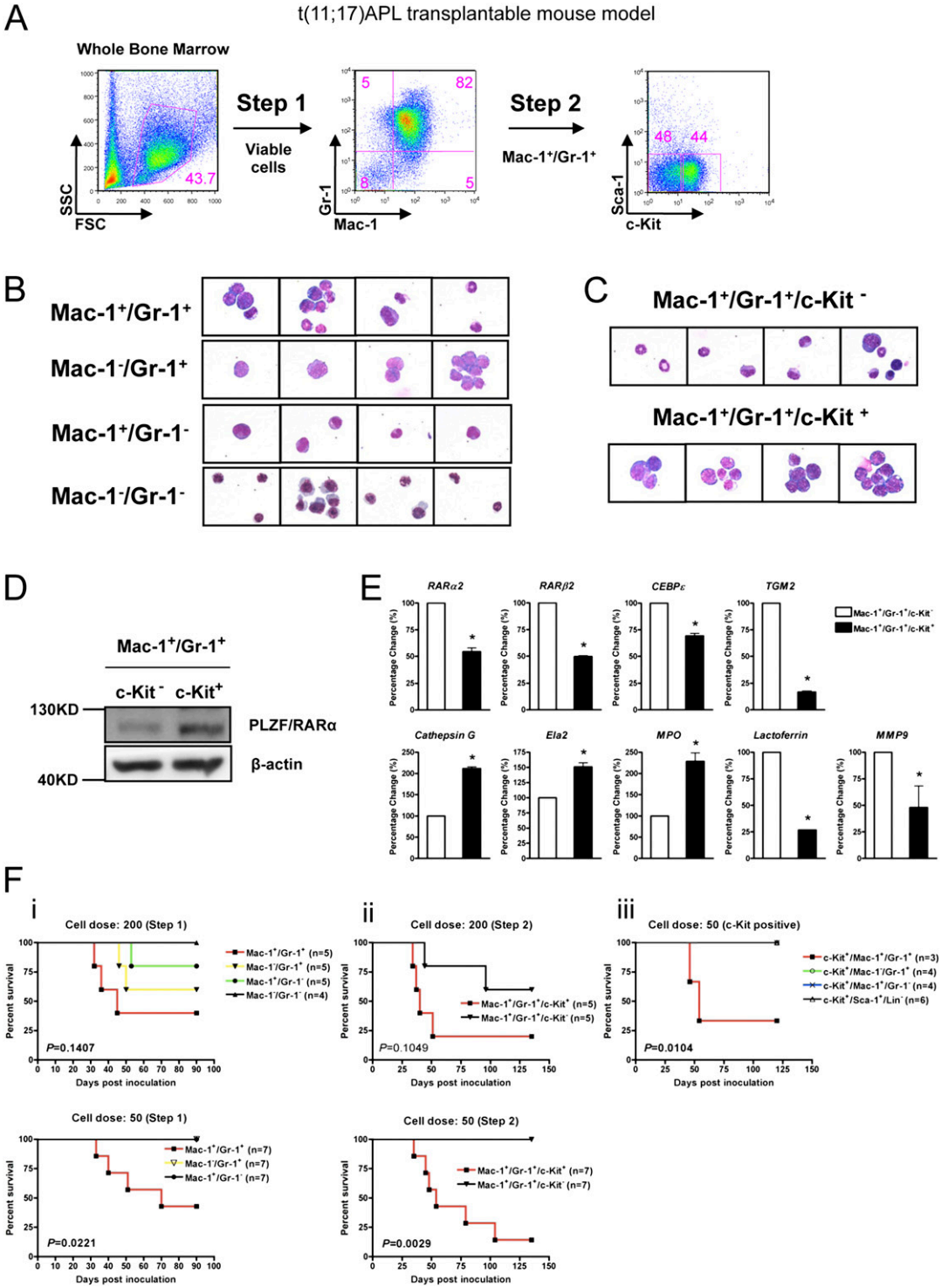


Fig. S6. Identification and characterization of leukemia-initiating cell (LIC) population in *t*(11;17) APL transplantable mouse model. (*A*) Illustration of the sorting strategy of LIC population in BM from *t*(11;17) APL transplantable mouse model. (*B*) Sorted cells in step 1 were stained by Wright–Giemsa staining, and four representative pictures of each population were taken by an Olympus BX51 microscope (original magnification, 1,000×). (*C*) Cells isolated from step 2 (Mac-1$^+$/Gr-1$^+$/c-Kit$^+$ and Mac-1$^+$/Gr-1$^+$/c-Kit$^-$) were stained by Wright–Giemsa method. Four representative pictures of each population were taken by Olympus BX51 microscope (magnification, 1,000×). (*D*) Expression level of PLZF/RARα protein in both c-Kit–positive and –negative subsets. (*E*) Qualitative real-time RT-PCR determined the expression of RA-response genes (*RARα2*, *RARβ2*, *CEBPε*, and *TGM2*) (*Upper*), and granulocyte-specific genes [*Cathepsin G*, *Elastase2* (*Ela2*), *Myeloperoxidase* (*MPO*), *Lactoferrin*, and *MMP9*] (*Lower*) in sorted cells from Mac-1$^+$/Gr-1$^+$/c-Kit$^+$ and Mac-1$^+$/Gr-1$^+$/c-Kit$^-$ subpopulations. Representative data of two independent experiments are shown. (*F*) Survival curves of secondary recipients inoculated by different sorted populations from indicated steps. (*i*) 200-cell and 50-cell doses of sorted populations were transplanted in step 1 isolation. (*ii*) 200-cell and 50-cell doses of sorted populations were transplanted in step 2 isolation. (*iii*) Four c-Kit$^+$ populations were isolated and transplanted at 50-cell dose.

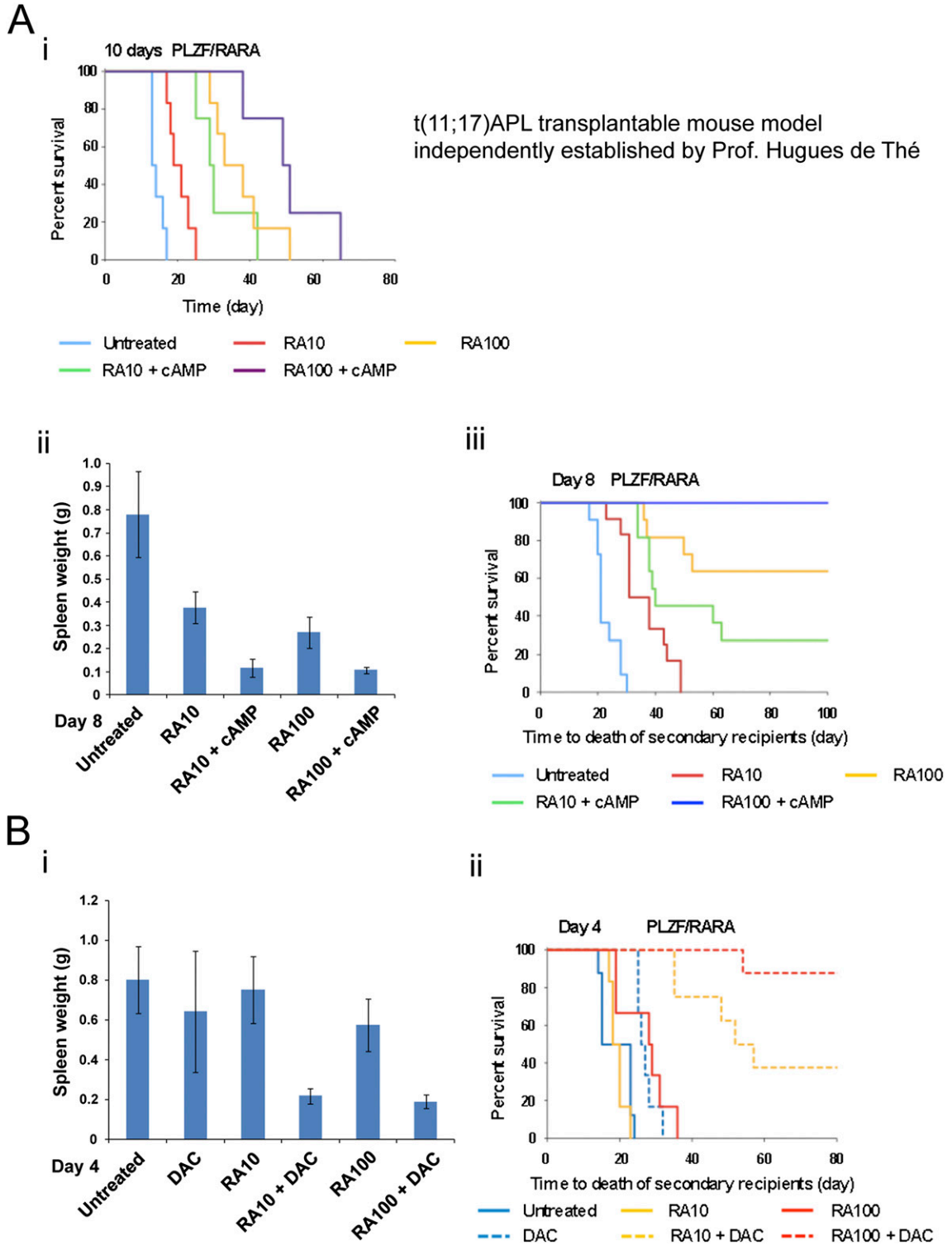


Fig. S7. In vivo combined treatment with 8-CPT-cAMP, 5-Aza-2-Deoxycytidine (DAC) and different doses of ATRA improves the survival of transplantable *t*(11;17) APL mice by promoting clearance of LICs. (*A*) A transplantable *t*(11;17) APL model was independently established and nude mice were treated as previously described (1), using APLs from PLZF/RARα-RARα/PLZF mice (2). (*i*) The survival curve of primary mice treated with indicated therapies for 10 d, 7 d after inoculation. (*ii*) Spleen weight of moribund mice killed at day 8. (*iii*) The survival curve of the secondary recipients inoculated with BM APL cells derived from treated mice at day 8. (*B*) Similar experiments were carried out using DAC (5 mg·kg·d), given intraperitoneally, and ATRA. (*i*) Spleen weight of mice killed at day 4. (*ii*) The survival curve of the secondary recipients of day 4 for LIC assessment. RA10, ATRA 10 mg·kg·d; RA100, ATRA 100 mg·kg·d.

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Table S1. Identification of LIC population by limited dilution assay in BM transplantation experiment

Cell-sorting strategy	Sorted population (donor *n*)	Cell doses	APL incidence of recipients*	LIC frequency (95% CI)	P value[†]
First sorting step[‡]	Mac-1$^+$/Gr-1$^+$ (n = 2)	1,000	3/3 (100%)	1 in 124 (54–283)	P = 0.0174
		200	3/5 (60%)		
		50	4/7 (57%)		
	Mac-1$^-$/Gr-1$^+$ (n = 2)	1,000	2/2 (100%)	1 in 668 (219–2041)	
		200	1/5 (20%)		
		50	0/7 (0%)		
	Mac-1$^+$/Gr-1$^-$ (n = 2)	1,000	2/3 (67%)	1 in 730 (259–2,059)	
		200	2/5 (40%)		
		50	0/7 (0%)		
	Mac-1$^-$/Gr-1$^-$ (n = 2)	5,000	4/4(100%)	1 in 2,745 (1,626–4,567)	
		1,000	0/3 (0%)		
		200	0/4 (0%)		
Second sorting step[§]	Mac-1$^+$/Gr-1$^+$/ c-Kit$^+$ (n = 2)	1,000	3/3 (100%)	1 in 59 (39–89)	P = 0.0164
		200	4/5 (80%)		
		50	6/7 (86%)		
	Mac-1$^+$/Gr-1$^+$/ c-Kit$^-$ (n = 2)	1,000	2/2 (100%)	1 in 432 (155–1207)	
		200	2/5 (40%)		
		50	0/6 (0%)		
c-Kit$^+$ populations[¶]	c-Kit$^+$ /Mac-1$^+$/Gr-1$^+$(n = 2)	50	2/3 (67%)		P = 0.0104
	c-Kit$^+$/Mac-1$^-$/Gr-1$^+$(n = 2)	50	0/4 (0%)	N.A.	
	c-Kit$^+$/Mac-1$^+$/Gr-1$^-$ (n = 2)	50	0/4 (0%)		
	c-Kit$^+$/Sca-1$^+$/Lin$^-$ (n = 2)	50	0/6 (0%)		

N.A., not available.

*APL incidence represented as "Recipients died from APL/Total recipients."

[†]Comparison between groups of Mac-1$^+$/Gr-1$^+$ and Mac-1$^-$/Gr-1$^+$ treatment in first BM transplantation (BMT) experiment. Comparison between groups of Mac-1$^+$/Gr-1$^+$/c-Kit$^+$ and Mac-1$^+$/Gr-1$^+$/c-Kit$^-$ treatment in second BMT experiment. Two-tailed P value was estimated by limited-dilution assay analysis (L-software; StemCell). Comparison of the differences of the survival curves of four groups. Two-tailed P value was calculated by Long-rank algorithm for survival analysis.

[‡]Follow-up time was up to 90 d.

[§]Follow-up time was up to 135 d.

[¶]Follow-up time was up to 120 d.

Table S2. 8-CPT-cAMP and ATRA combinatorial therapy can target LIC (Mac-1$^+$/Gr-1$^+$/c-Kit$^+$) and reduce LIC frequency and absolute number in *t*(11;17) APL model

BMT	Treatment (donor *n*)	Cell doses	APL incidence of recipients*	LIC frequency (95% CI)	P value†	Whole BM‡ (×10^6 cells)	Mac-1$^+$/Gr-1$^+$/ c-Kit$^+$ (%)	Mac-1$^+$/Gr-1$^+$/c-Kit$^+$ (×10^6 cells)	Absolute number of LIC§ (×10^6 cells)	P value¶
First BMT‖ (day 27)	Untreated (n = 2)	1,000	3/3 (100%)	1 in 26 (10–64)	P = 0.2625	18.25 ± 3.54	45.00 ± 1.84	8.18 ± 1.26	0.3146 ± 0.048	P = 0.3002
		200	5/5 (100%)							
		50	6/7 (86%)							
	8-CPT-cAMP (n = 2)	1,000	3/3 (100%)	1 in 72 (32–160)		22.50 ± 1.77	36.00 ± 4.95	8.06 ± 0.48	0.1119 ± 0.0066	
		200	4/5 (80%)							
		50	5/7 (71%)							
	ATRA (n = 2)	1,000	3/3 (100%)	1 in 417 (164–1063)		28.38 ± 0.88	19.70 ± 13.86	5.53 ± 3.76	0.013 ± 0.0090	
		200	2/5 (40%)							
		50	0/7 (0%)							
	cAMP+ATRA (n = 2)	1,000	2/2 (100%)	1 in 1047 (283–3875)		41.75 ± 5.30	11.00 ± 0.47	4.61 ± 0.78	0.004398 ± 0.00074	
		200	0/5 (0%)							
		50	0/5 (0%)							
	Ara-C (n = 2)	1,000	3/4 (75%)	1 in 331 (118–929)		43.25 ± 1.41	10.88 ± 1.41	4.69 ± 0.45	0.036 ± 0.0035	
		200	2/3 (67%)							
		50	2/3 (67%)							
Second BMT** (day 41)	ATRA (n = 4)	1,000	8/10 (80%)	1 in 428 (211–869)	P = 0.0054	39.67 ± 18.24	28.87 ± 17.99	8.99 ± 4.74	0.021 ± 0.011	P = 0.0374
		200	4/5 (80%)							
	cAMP+ATRA (n = 3)	1,000	2/10 (20%)	1 in 2483 (900–6844)		46.11 ± 14.63	16.98 ± 20.01	5.94 ± 5.17	0.0024 ± 0.0021	
		200	2/6 (33%)							

*APL incidence represented as "Recipients died from APL/total recipients."

†Comparison of LIC frequency between groups of ATRA and cAMP+ATRA treatment in each BMT experiment. Two-tailed *P* value was estimated by limited-dilution assay analysis (L-calc software; StemCell).

‡Data for LIC quantification were derived from two femurs and tibias.

§Absolute number of LICs of each group was calculated by multiplying absolute number of Mac-1$^+$/Gr-1$^+$/c-Kit$^+$ by LIC frequency.

¶Comparison of absolute number of LIC between groups of ATRA and cAMP+ATRA treatment in each BMT experiment. Two-tailed *P* value was estimated by Student *t* test.

‖Follow-up time was up to 120 d.

**Follow-up time was up to 105 d.