Supplementary Methods

Identification of berbamine target

1× 10^8 K562 Cells were lysed with lysis buffer (50mM Tris-HCl, pH7.2; containing 25mM MOPS/15mM EGTA/15mM $MgCl_2$/2mMDTT/1mM sodium vanadate/1mM sodium fluoride/ 1mM $CaCl_2$/1mM PMSF/0.1% NP-40, plus protease inhibitors for 10 min on ice and then sonicated for 10 min on ice. Cell lysate supernatant were collected by centrifugation for 15 min at 4℃ and incubated with berbamine affinity matrix overnight at 4℃. After washing five times with lysis buffer, cellular proteins bound berbamine affinity matrix were eluted with 100μl of 50μM BBM solution as competitor for 1h at 4℃. After heat shock for 10 min at 95 °C, eluted samples were loaded and separated on a 10% SDS-PAGE, and stained with a Silver Stain Plus Kit (Invitrogen). The bands were cut, destained, and analyzed with LC/MS. Eluted samples were also used for western blot analysis with goat anti-CaMKII-γ and rabbit anti-phosphospecific CaMKII (Thr^{286} and Thr^{287})(1:200; Santa Cruz Biotechnology).

Construction of pEGFP-C1-CaMKII γ plasmid

The pEGFP-C1-CaMKII γ plasmid was constructed as following. CaMKII γ was amplified by reverse transcription PCR (RT-PCR) using messenger RNA (mRNA) from human K562 leukemia cells, with the forward primer 5'-AAGCTTCGATGGCCACCA CCGCCACCTG-3' and reverse primer 5'- GGATCCTCACTGCAGCG GTCCGGCAG -3' containing HindIII and BamH I sites, respectively. The PCR product was cloned into pEGFP-C1 Vector to generate pEGFP-C1-CaMKII γ plasmid.

Transient transfections

293T cells were transiently transfected with pEGFP-C_1-CAMKIIγ plasmid. 6×10^5 cells were plated in 2ml of growth medium without antibiotics in 6-well-culture plates one day before transfection. 4μg DNA was diluted in 250 μl of Opti-MEM® I Reduced Serum Medium. 10μl lipofectmine™2000 were diluted in 250μl of Opti-MEM® I Reduced Serum Medium and incubated for 5 minutes at room temperature. After incubation, the diluted DNA and lipofectmine™2000 were mixed gently and

incubated for 20 minutes at room temperature. 500μl complexes were added to each well and the medium was changed after 5 hours. The cells were incubated at 37^{o}C in a CO_2 incubator for 24-48 hours prior to transgene expression testing.

Colocalization of berbamine and EGFP-CAMKII γ

Biotin-labeled berbamine was added to the 293T cells after transient transfection. 48 hours later, cells were fixed by freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 minutes at room temperature and then permeabilized with 0.2% Triton X-100 in PBS for 20 minutes. The slide were washed with PBS twice, and incubated with 4μg/ml Rhodamine conjugated streptavidin working solution in PBS for 30 minutes in a humid environment at 37℃. After 3 times washes with PBS, the cells were incubated with DAPI (5μg/ml) for 5 minutes followed by observation under Zeiss Confocal Laser Scanning Microscope 710.

Construction of CaMKII γ mutant plasmids, transfection and Co-IP

We constructed two mutants of CaMKII γ using site-directed mutagenesis. One is mutated Lys^{43} of this kinase to methionine to generate a kinase-deficient CaMKII γ mutant labeled 43kd-MT. The other is a deletion mutated Asp^{157} of the kinase labeled 157del-MT. We next transfected 293T cells with these mutant CaMKII γ for 48 hours and then extracted total cellular proteins for co-immunoprecipitation using biotinylated berbamine.

Immunofluorescence staining

Cells (3×10^{3}) were deposited on glass slides by cytocentrifugation, fixed in cold 4% paraformaldehyde and permeabilized in PBS containing 0.3% BSA, 0.5% Triton X-100. Slides were blocked using antibody dilution buffer (3% BSA, 0.1% Tween20/PBS) for 30 minutes, incubated with primary antibody at room temperature for 2 hours, washed in PBS and with anti-mouse IgG-Texas Red for 1 hour. Following additional washes, coverslips were mounted on glass slides in Anti-fade containing DAPI

(Invitrogen). Images were obtained using a Zeiss AxioImager microscope and Zeiss Upright LSM310 Laser Scanning Confocal Microscope.

Establishment of stable cells with CaMKII γ overexpression and treatment

CaMKII γ sequence was cloned into the retroviral vector pRetroX-Tight-puro (Clontech). The vector was used to package retrovirus with doxycycline-inducible expression of CaMKII γ using a standard HBS-based retrovirus preparation as suggested by the manual of Retro-X™ Tet-On® Advanced Inducible Expression System. Briefly, K562 cells were transduced with pRetroX-Tet-on virus and selected by G418. The selected cells were then transduced with pRetroX-Tight-puro virus expressing CaMKII γ. The stable cells were treated with doxycyclin for 2 days before BBM treatment. After another 2 days, MTS assay (Promega) was used to measure the viability of K562 cells with the doxycylin treatment versus the cells without the treatment.

CaMKIIγ RNA interference constructs

Four different synthetic human oligos designed to generate short hairpin RNA (shRNA) targeting human CaMKIIγ were constructed into the lentiviral vector pLKO.1. The following targets in the coding sequences were selected for the design of shRNAs: GGATATGTCGACTTCTGAAAC, GGAGCCTATGATTTCCCATCA, GCCACAAACCACTGTGGTACA, GCATCCATGATGCATCGTCAGGA.

Establishment of leukemia xenograft with CaMKII γ overexpression in NOD-SCID mice and treatment

For leukemia xenograft with CaMKII γ overexpression in NOD-SCID mice, K562 cells were transduced with the retroviral vector pMSCV-puro (Addgene 24828) using MOI of 5 and selected by 3 ng/ml puromycin for 1 week. 1 X 10^7 cells with CaMKII γ expression or control were injected s.c. to NOD-SCID mice. 24 hours after injection, 50 mg/kg, 100 mg/kg, or vehicle only was only to the mice orally 3 times a day for 10 days. The mice were sacrificed 25 days after the injection. Tumor volume was measured at Day 15 and Day 25. The volume = 1/2 * Length * width^2. Tumor weight

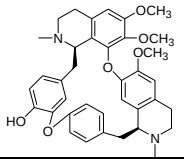
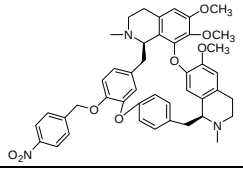
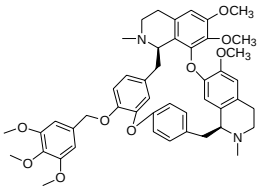
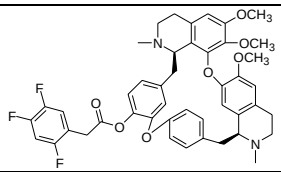
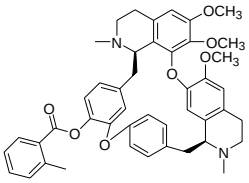
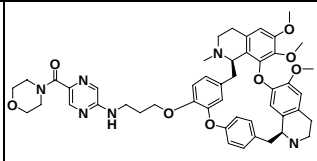
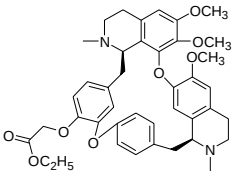
was measured at Day 25. The protein lysate from the leukemia xenografts was used for analysis of in vivo signaling pathways with western bloting.

Immunoprecipitation

For immunoprecipitation, cell extracts composed of 6.0 x 10^6 cells were prepared by solubilization in 400µl cell lysis buffer (1% Triton X-100, 150mM NaCl,20mM Tris-Cl (pH7.4), 1mM EDTA, 1mM EGTA, 1mM Na_3VO_4, 2.5mM pyrophosphate, 1mM glycerol phosphate, and protease inhibitor cocktail for 10 min at 4℃. After brief sonication, the lysates were cleared by centrifygation at 15,000 x g for 10 min at 4℃, and the cell extract was immunoprecipitated with 4µg antibodies against CaMKIIγ1 and incubated with 60µl of protein G plus protein A agarose for 16 h at 4℃ by continuous inversion. Immunocomplexes were pelleted and washed three times. The precipitated immunocomplexes were then boiled in Laemmli buffer and subjected to western blot analysis using anti-β-catenin and Stat3 antibodies.

Supplementary Table1

Table 1 Bioactivities of berbamine and its analogs

		IC_{50} (μ g/ml)	
Entry	Structure	CaMKII γ activity	K562 proliferation
Berbamine (BBM)		6.0	5.43
O-(4-nitrobenzyl) berbamine (BBD3)		0.50	0.27
O-(3,4,5-trimethoxybenzyloxy) berbamine (BBD12)		1.50	1.20
O-(2,4,5-trifluorophenylacetyl) berbamine (BBD15)		2.0	2.36
2-methylbenzoyl berbamine (BBD24)		0.45	0.50
O-(3-(5-(morpholine-4-carbonyl) pyrazin-2-ylmino)propyl)-berbamine (CP15)		8.0	8.34
O-(ethoxycabonylmethyl)berbamine (XBA05, inactive berbamine analog)		>32	>32

Supplementary Figure Legends

Supplementary Figure 1. The inhibitory effect of BBM on CaMKIIγ activity is overcome by increasing the concentrations of ATP.

Supplementary Figure 2 ShRNA-mediated knockdown of CaMKIIγ inhibits the proliferation of K562 and KCL22 cells

K562 and KCL22 cells were stably transduced with lentiviral vectors harboring CaMKIIγ shRNAs or scramble shRNA as the control. The expression of CaMKIIγ protein in CaMKIIγ shRNA–transduced K562 (A) and KCL22 (B) cells were detected by western blotting. The inhibitory effect of CaMKIIγ knockdown on K562 (A) and KCL22 (B) cells was measured by using the MTT assay. *P<0.01.

Supplementary Figure 3 Partial knockdown of CaMKIIγ sensitizes K562 and KCL22 cells to the effect of berbamine

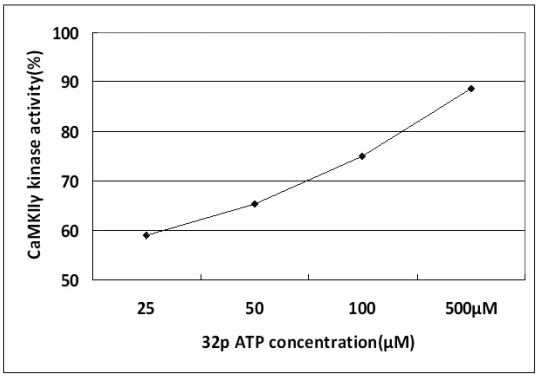
K562 and KCL22 cells were transiently infected with lentiviral vectors harboring CaMKIIγ shRNAs or scramble shRNA as the control for 24h. The expression of CaMKIIγ protein in CaMKIIγ shRNA–transduced KCL22 and K562 cells were verified by western blotting. After 24h tranduction, cells were treated with different concentrations of berbamine up to 72 hours. Cell viability was measured using the MTT assay and IC_{50} values were calculated. *P<0.01.

Supplementary Figure 4. Pathological observations of CML xenografts in NOD/SCID mice. Xenograft tumors were removed from the untreated or BBM-treated mice at 50 mg/kg and 100mg/kg, respectively.

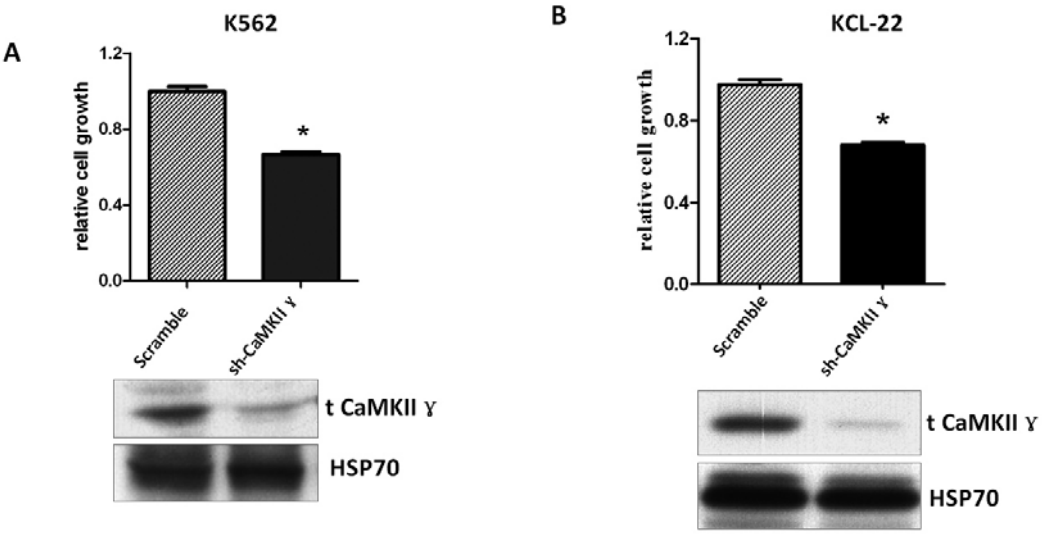
Supplementary Figure 5 Berbamine inhibits CaMKIIγ-dependent downstream signaling pathways in mouse xenograft

Lysates of xenograft tumors from NOD-SCID mice dosed with Vehicle, BBM (50mg/kg) and BBM (100mg/kg) were subjected to western blotting using the indicated antibodies. Control: empty vector group. pMSCV-CaMKIIγ: CaMKIIγ overexpression group.

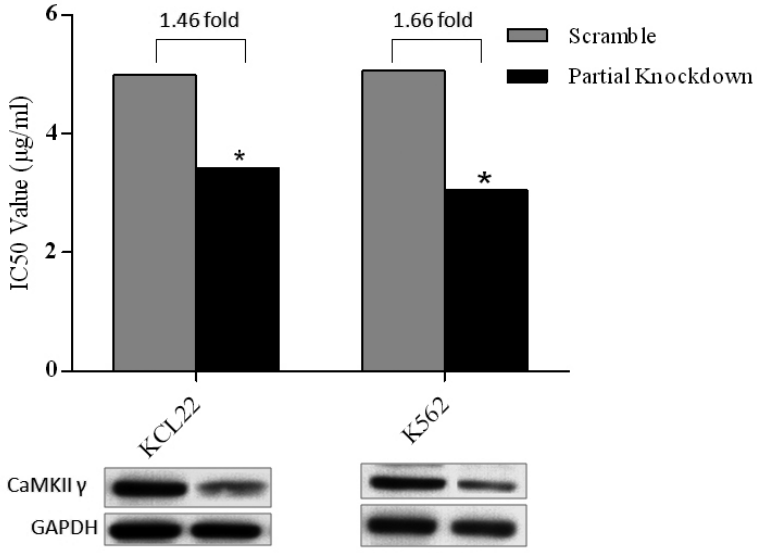
Supplementary Figure 1



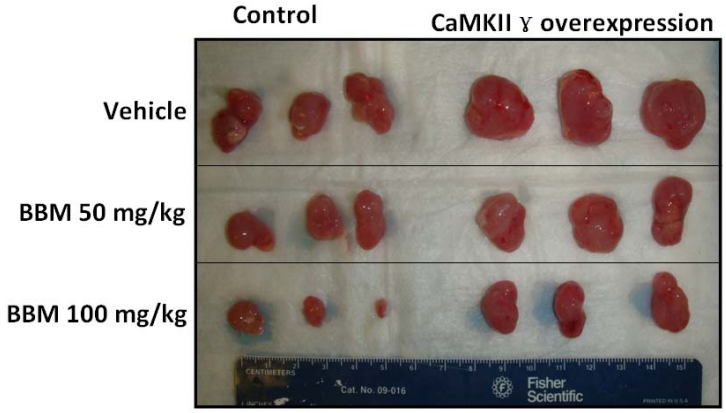
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5