Inventory of Supplemental Material

The supplemental information includes the following items:

1. Supplemental experimental procedures
2. Supplemental results, including two Tables, and thirteen Figures and their legends
3. Supplemental reference

1. SUPPLEMENTAL EXPERIMENTAL PROCEDURES

List of Promoter Specific Primers for *SPARC:*

F1: CGCAAAAAGAGGAGGAAAGA, R1: GAGACAGGCAACAGGAAACC

F2: GGCTATGGGAGAAGGAGGAG, R2: GGGGGTCACACATACCTCAG

F3: TGCCTTTGTGGCAAATACAA, R3: TTGTTCAGAGGGCAAGTGAG)

Western Blot Antibodies

Actin (I-19) sc-1616 (Santa Cruz Biotechnology, Santa Cruz, CA);

SPARC (#5420; Cell Signaling Technology, Danvers, MA) and (SC-25574; Santa Cruz Biotechnology; for revision experiments)

GSK3β, AKT, P-GSK3β, P-AKT, p-β-catenin (Cell Signaling Technology),

MYC (Santa Cruz Biotechnology)

Sp1 (Santa Cruz Biotechnology)

P65 (EMD Millipore Corporation, Billerica, MA)

RUNX1(Calbiochem, San Diego, CA)

Chromatin Immunoprecipitation Antibodies

RUNX1 (Abcam) Sp1 and NFκB(p65) (Millipore, Billerica, MA).

Confocal Microscopy

For confocal microscopy analysis, cells (1×10^6) were washed twice with cold PBS, and then fixed in Cytofix/cytoperm buffer (500 µl per 1×10^6 cells) (BD Biosciences, Billerica, MA) by incubation for 20 min on ice. Cells were then washed twice with 1 ml of Perm/Wash buffer and blocked in 10% goat serum (diluted in PBS, pH 7.4) on a shaker for 2 hr. After washing twice with PBS, cells were resuspended in 100 µl of Perm/Wash buffer (1X) with 1 µl of β-catenin antibody (cell signaling) and incubated overnight on a shaker at 4°C. After washing, the samples were incubated with fluorochrome conjugated secondary antibody for 2 hr at room temperature. Nuclei were stained with 1 µl of Drag5. Ten microliters of suspension with stained cells were pipetted to a slide, air-dried for 3 min and coverslipped. Samples were analyzed by confocal microscope [The Ohio State University (OSU) Campus Microscopy & Imaging Facility].

Xenograft Transplantations

The acute myeloid leukemia (AML) patient blasts used for *ex vivo* experiments were obtained from apheresis blood samples collected from patients treated at OSU and stored in the OSU Leukemia Tissue Bank. Informed consent to use the tissue for laboratory studies was obtained from each patient according to OSU institutional guidelines. After thawing, $CD34^+$ cells were isolated using $CD34^+$ Microbeads according to the manufacturer's suggested protocol (Miltenyi, Ausburn, CA). Cells were pre-stimulated in RPMI supplemented with 10% (v/v) fetal calf serum (FCS), along with

cytokines supporting human stem/progenitor cells (StemSpan CC100, StemCell Technologies, Vancouver, BC) for 36 hr at 37°C, 5% CO_2. Retroviral transduction was performed in non-tissue culture-coated multi-well plates (BD Biosciences, San Jose, CA), which had been pre-coated with fibronectin CH296 fragment (Takara Bio Inc., Otsu, Japan) at 4 µg/cm (1). Thirty-six hr after the final exposure to viral particles, cells were harvested and then re-suspended in PBS, 2% FCS, 5 µg/ml 7-amino-actinomycin D (7-AAD) (Invitrogen, Carlsbad, CA), and viable GFP^+ cells were isolated by flow sorting (FACSAria). Cells were transduced with a multiplicity of infection (MOI) of 3 to obtain nearly equivalent transduction efficiencies between groups. One million GFP^+ isolated cells were resuspended into a volume of 250 µl of PBS and transplanted via tail-vein injection into NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ, Jackson Laboratory, Bar Harbor, ME) treated with 3 mg/kg of Busulfan 24 hr before transplantation.

Pathology Analysis

Eight weeks after tumor cell injection, selected tissues were fixed in neutral buffered 10% formalin and processed into paraffin. Tissue sections stained with hematoxylin and eosin (H&E) were evaluated using a coded ("blinded") scheme using a semi-quantitative scale based on the distribution of leukemic cells: no tumor infiltration (0% of section), or minimal (<5%), mild (6-25%), moderate (26-50%), marked (51-75%) or severe (>75%) tumor infiltration.

Luciferase Assays

HEK 293T cells were grown in culture to approximately 80% confluency. Prior to transfection, the medium was replaced with Opti-MEM I (Gibco, Grand Island, NY) and cells were incubated at 37ºC with 5% CO_2 for 24 hr. Cells were transfected with DNA

plasmid constructs and pRL-TK *Renilla* luciferase (control) using Lipofectamine™ 2000 (Invitrogen) followed by an incubation at 37ºC with 5% CO_2 for 24 hr. Following incubation, cells were collected, and cellular lysates were prepared according to the recommendations detailed in the Dual-Luciferase® Reporter Assay System (Promega, Madison, WI). Luciferase activity was assessed using a GloMax® 96 Microplate Luminometer (Promega). Both firefly and *Renilla* luciferase activities were assessed using the GloMax® 96 Microplate Luminometer (Promega). Values were corrected to account for differences in transfection efficiency by co-transfecting pRL-TK *Renilla* luciferase plasmid and adjusting luciferase values accordingly.

Bortezomib *in vivo* study

NSG mice were injected with $0.6X10^6$ cells CG-SH; CG-SH is a CN-AML cell line characterized by RUNX1 mutation and expressing relatively high level of SPARC. One week later, mice were treated with either liposomal bortezomib 1 mg/kg (n=3) or vehicle (n=2) twice a week for 4 weeks.

2. SUPPLEMENTAL RESULTS

Table S1. Comparison of clinical and molecular characteristics of Low vs. High *SPARC* younger patients

n=153	Low† *SPARC* (n=77)	High† *SPARC* (n=76)	P§ (Median cut)	P£ (Continuous)
Protocol, no. (%)			1.00	.72
9621	38 (49)	37 (49)		
19808	39 (51)	39 (51)		
Age (years)			.30	.43
Median	45	47		
Range	(18, 59)	(20, 59)		
Sex, no. (%)			.75	.73
Male	38 (49)	35 (46)		
Female	39 (51)	41 (54)		
Race, no. (%)			.45	.32
White	70 (91)	66 (87)		
Non-White	7 (9)	10 (13)		
Hemoglobin (g/dL)			**.003**	**<.001**
Median	9.8	9.0		
Range	(4.8, 13.3)	(4.6, 13.4)		
Platelet count (x10^9/L)			.83	.80
Median	60	64		
Range	(11, 277)	(8, 445)		
WBC count (x10^9/L)			**.03**	**.003**
Median	35.9	24.4		
Range	(1.5, 295.0)	(0.9, 172.8)		
%Blood Blasts			.34	**.03**
Median	65	56		
Range	(0, 97)	(0, 97)		
%Bone Marrow Blasts			**.04**	.12
Median	71	64		
Range	(10, 94)	(26, 95)		
FAB, no. (%)	*15 unknown*	*14 unknown*	**.01**	**.02**
M0	0 (0)	3 (5)		
M1	19 (30)	16 (26)		
M2	14 (23)	22 (35)		
M4	15 (24)	17 (27)		
M5	14 (23)	3 (5)		
M6	0 (0)	1 (2)		
M7	0 (0)	0 (0)		
Extramedullary Involvement, no. (%)	28 (37)	15 (20)	**.02**	**.003**
CNS	0 (0)	1 (1)	1.00	.72
Hepatomegaly	3 (4)	2 (3)	.68	.45
Splenomegaly	4 (5)	1 (1)	.21	.17
Lymphadenopathy	11 (14)	7 (9)	.45	.06
Skin Infiltrates	8 (10)	5 (7)	.56	.22
Gum Hypertrophy	12 (16)	4 (5)	.06	.24
Mediastinal Mass	0 (0)	1 (1)	.50	.10
Induction Treatment, no. (%)			.62	.51
ADE	49 (64)	45 (59)		
ADEP	28 (36)	31 (41)		

† Cases with *SPARC* expression in the lower half are defined as Low *SPARC*; cases with *SPARC* expression in the upper half are defined as High *SPARC*. Medians were computed separately by age.

§P-values for categorical variables are from Fisher's exact test, P-values for continuous variables are from Wilcoxon rank sum test.

£P-values are from the one-way analysis of variance overall F-test, evaluating the presence of any linear relationship between the CE score and the variable tested.

Comparison of clinical and molecular characteristics of Low vs. High *SPARC* younger patients (continued)

n=153	Low† *SPARC* (n=77)	High† *SPARC* (n=76)	P§ (Median cut)	P£ (Continuous)
NPM1, no. (%)			**<.001**	**<.001**
Mutated	61 (79)	38 (50)		
Wild-Type	16 (21)	38 (50)		
FLT3-ITD, no. (%)			.25	.54
ITD positive	26 (34)	33 (43)		
No ITD	51 (66)	43 (57)		
CEBPA, no. (%)			.23	.68
Mutated	12 (16)	18 (24)		
Single mutated	2	9		
Double mutated	10	9		
Wild-Type	65 (84)	58 (76)		
ELN Risk Group*, no. (%)			**.006**	**.002**
Favorable	49 (64)	31 (41)		
Intermediate-I	28 (36)	45 (59)		
FLT3-TKD, no. (%)			**.009**	**.05**
TKD positive	12 (16)	2 (3)		
No TKD	65 (84)	74 (97)		
WT1, no. (%)			**.04**	**.03**
Mutated	6 (8)	15 (20)		
Wild-Type	71 (92)	61 (80)		
TET2, no. (%)			.38	.94
Mutated	15 (19)	10 (13)		
Wild-Type	62 (81)	66 (87)		
MLL-PTD, no. (%)			.13	.07
Present	3 (4)	8 (11)		
Absent	74 (96)	68 (89)		
IDH1, no. (%)	*2 unknown*	*1 unknown*	1.00	.67
R132	7 (9)	8 (11)		
V71i	1 (1)	0 (0)		
Wild-Type	67 (90)	67 (89)		
IDH2, no. (%)	*2 unknown*	*1 unknown*	1.00	.68
IDH2	9 (12)	8 (11)		
R140	9	5		
R172	0	3		
Wild-Type	66 (88)	67 (89)		
RUNX1, no. (%)	*10 unknown*	*4 unknown*	**.047**	**.008**
Mutated	3 (4)	11 (15)		
Wild-Type	64 (96)	61 (85)		
ASXL1, no. (%)	*2 unknown*		.21	**.03**
Mutated	1 (1)	5 (7)		
Wild-Type	74 (99)	71 (93)		
DNMT3A	*5 unknown*	*2 unknown*	.09	.33
Mutated	32 (44)	22 (30)		
R882	22	19		
Non-R882	10	3		
Wild-Type	40 (56)	52 (70)		
ERG expression group			**<.001**	**<.001**
Median	-3.31	-2.61		
Range	(-5.63, -0.95)	(-4.70, -0.51)		
BAALC expression group			**<.001**	**<.001**
Median	-2.55	-0.39		
Range	(-6.53, 1.29)	(-5.66, 4.35)		
MN1 expression group (median cut), no. (%)	*32 unknown*	*32 unknown*	**<.001**	**<.001**
High	15 (33)	33 (75)		
Low	30 (67)	11 (25)		

† Cases with *SPARC* expression in the lower half are defined as Low *SPARC*; cases with *SPARC* expression in the upper half are defined as High *SPARC*. Medians were computed separately by age.

* AML management recommendations by ELN. Döhner et al., Blood 2010, 115(3):453-474

§ P-values for categorical variables are from Fisher's exact test, P-values for continuous variables are from Wilcoxon rank sum test.

£ P-values are from the one-way analysis of variance overall F-test, evaluating the presence of any linear relationship between the CE score and the variable tested.

Table S2. Cytogenetic and Molecular Data of Patients with AML whose Samples Were Used in *in vitro* and *in vivo* Experiments

Patient no.	Cytogenetics	Mutation analyses
1	inv(16)(p13.1q22)	ND
2	Normal	FLT3-ITD / NPM1-mut
3	Normal	FLT3-ITD / NPM1-wt
4	Normal	FLT3-ITD / NPM1-mut
5	inv(16)(p13.1q22)	ND
6	Normal	FLT3-ITD / NPM1-mut
7	inv(16)(p13.1q22)	ND
8	Normal	FLT3-wt
9	Normal	FLT3-wt / NPM1-mut
10	Normal	ND

* *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; mut, mutated; ND, not done; wt, wild-type.

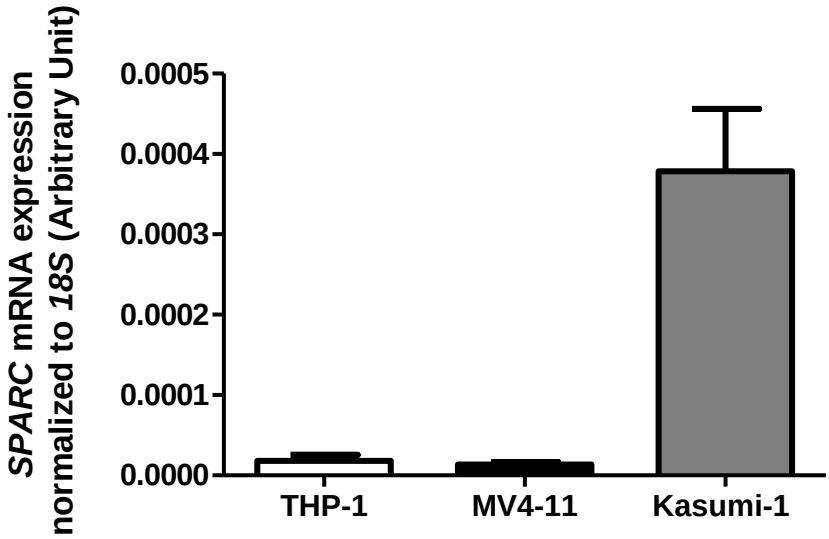


Figure S1. Endogenous Expression Levels of *SPARC* mRNA in AML Cell Lines.

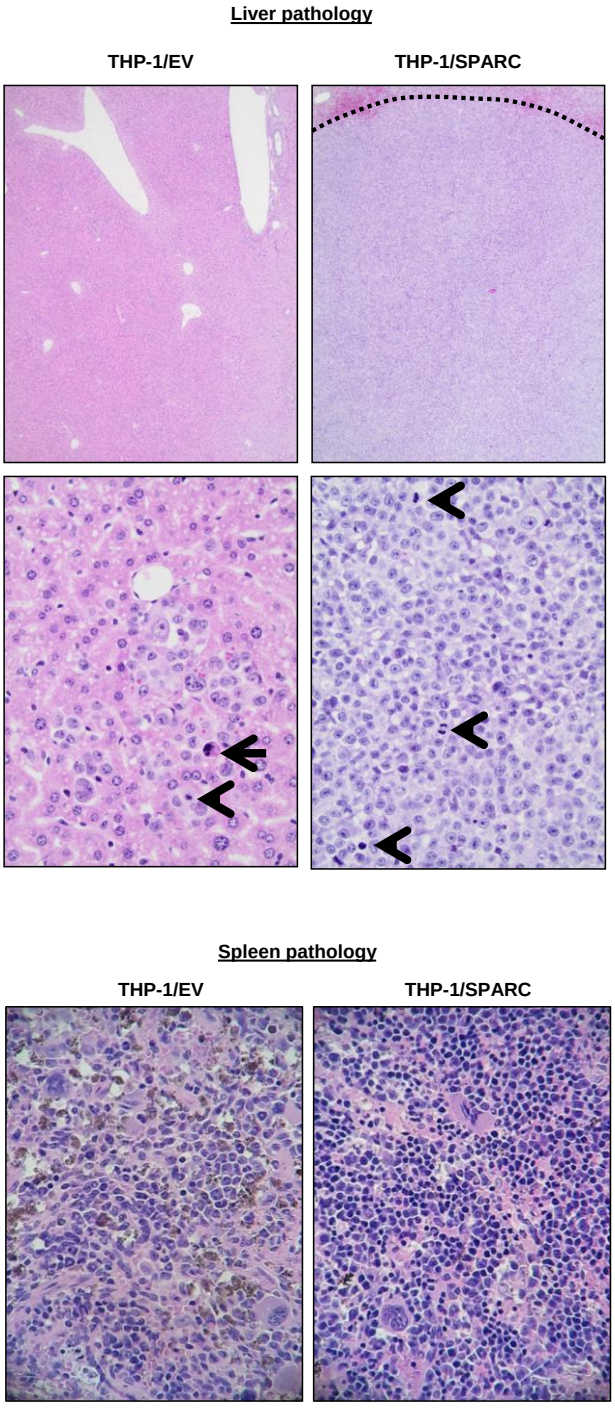


Figure S2. **SPARC overexpression correlates with increase proliferation *in vivo*.**

Liver and spleen histology; Leukemic cells were relatively rare in the livers of THP-1/EV mice (left panels). In contrast, mice injected with THP-1/SPARC cells, developed large

tumors (right panel, below the dotted line) that obliterated almost all of the normal liver parenchyma. In the middle panels, leukemia cells are large with pale basophilic to gray cytoplasm. Scattered apoptotic bodies (an arrow) were evident in the EV cells but mitotic figures were rare, whereas SPARC cells displayed mitotic figures (arrow heads) and had fewer apoptotic bodies. Stain: H&E; magnification, 40x (upper) and 400x (middle and lower). Spleen histology showed increase leukemia cells and destruction of splenic architecture in mice injected with THP-1/SPARC cells.

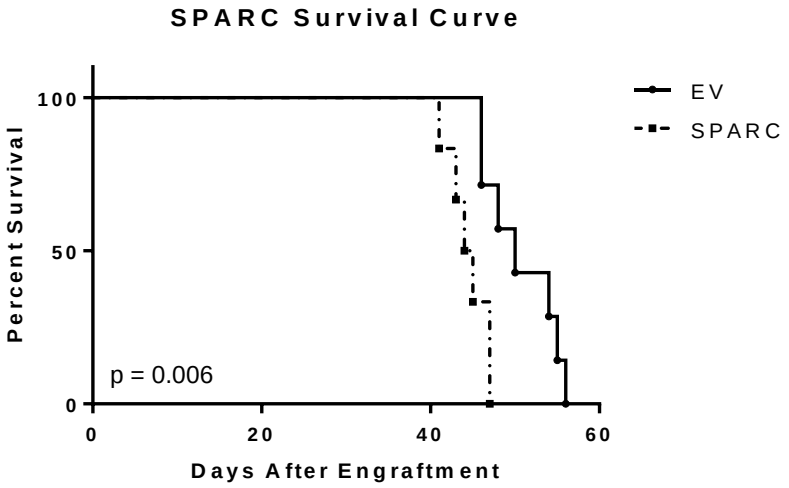


Figure S3. **SPARC Promotes Aggressive Disease *in vivo*.** Survival of THP-1/EV mice (n=7) compared with that of THP-1/SPARC mice (n=6).

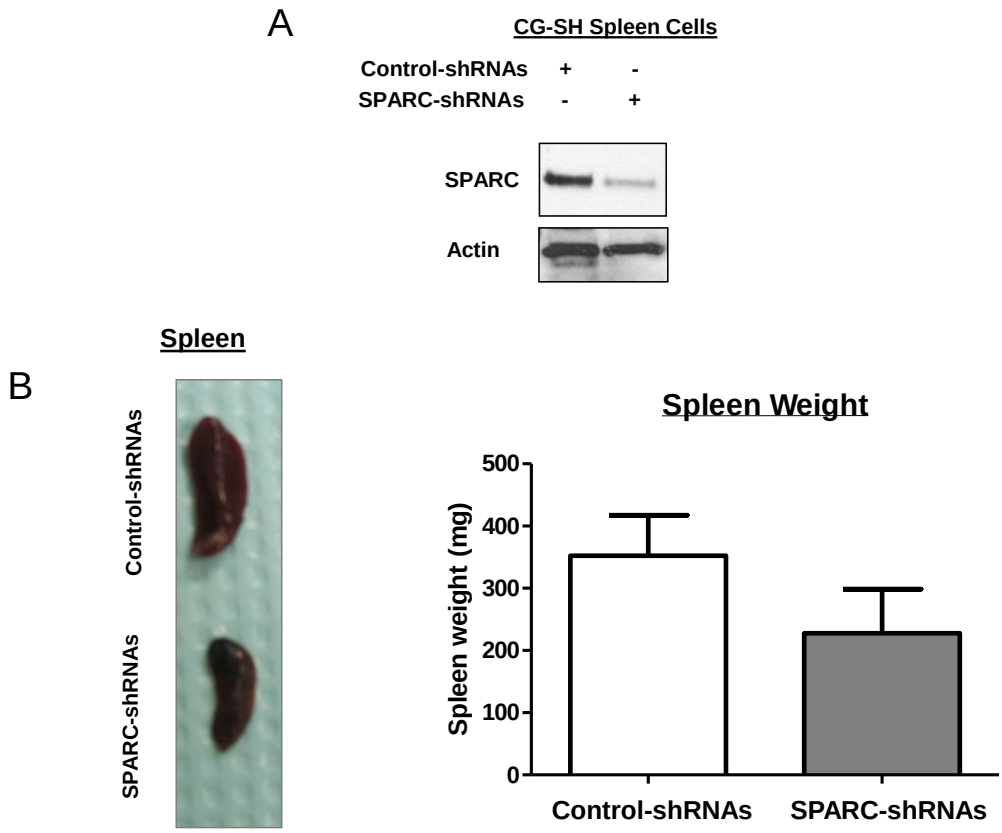


Figure S4. **SPARC knock-down results in decreased leukemia growth *in vivo*.** (A) Western blot showing SPARC knockdown in CG-SH spleen adapted AML cells infected with SPARC-shRNAs compared with their respective controls; (B) spleen from mice IV-injected with CG-SH spleen adapted SPARC-shRNAs cells compared with spleen from respective control and quantitative analysis for spleen weight (n=3).

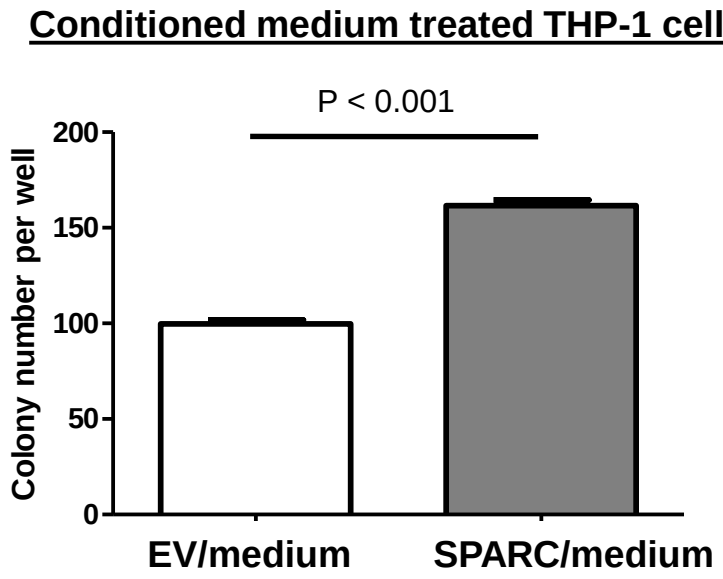


Figure S5. **Secreted SPARC promotes colony formation in THP-1 cells.**

THP-1 cells were incubated with medium collected from THP-1/EV cells or medium collected from THP-1/SPARC cells. Colony formation assay were performed and scored 10 days later.

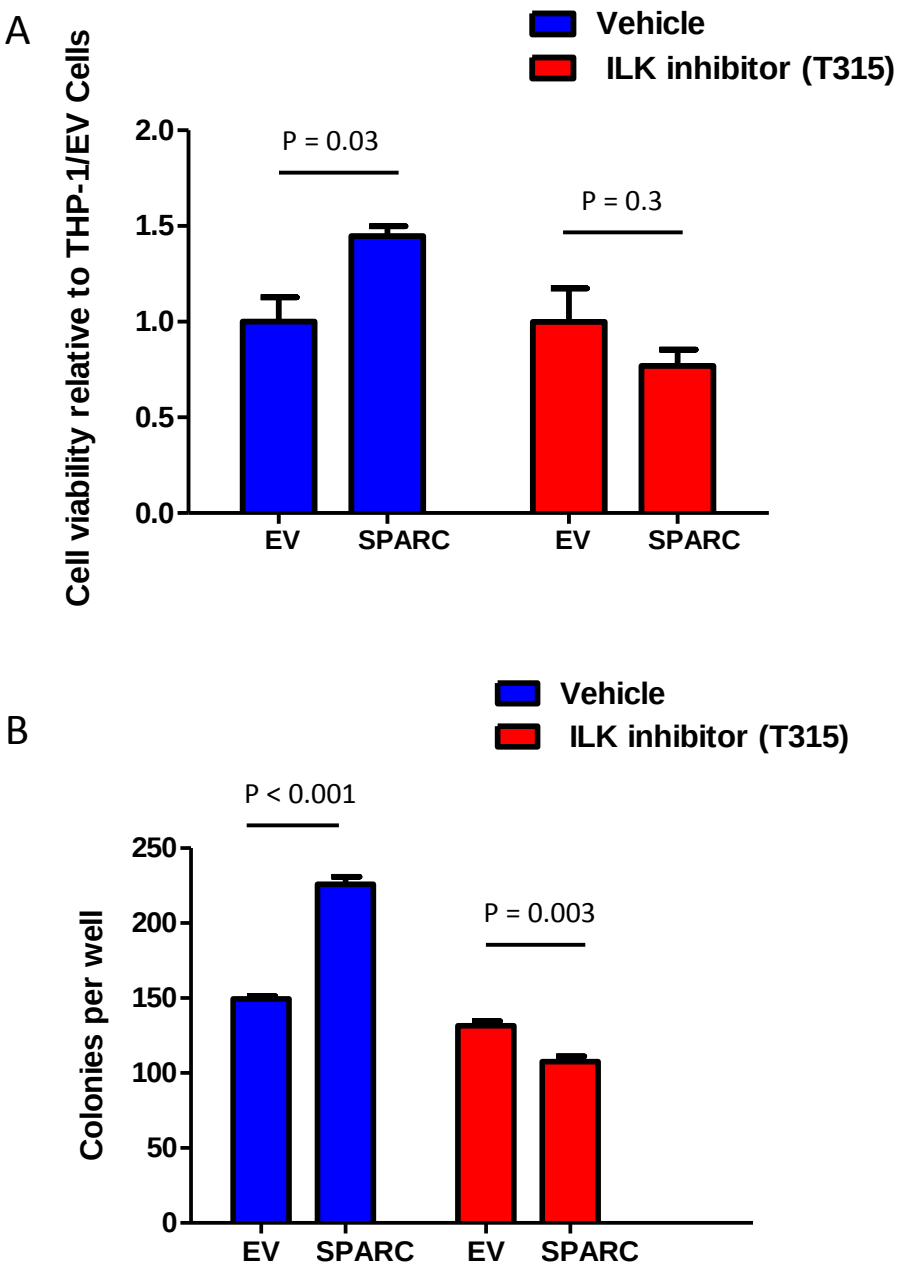


Figure S6. ILK inhibitor abrogates SPARC proliferative effect in THP-1 cells.

THP-1/EV and THP-1/SPARC cells were treated with an ILK inhibitor (T315), (A) cell viability measured by MTS assay and (B) colony formation were assessed on day 4 and 10 respectively.

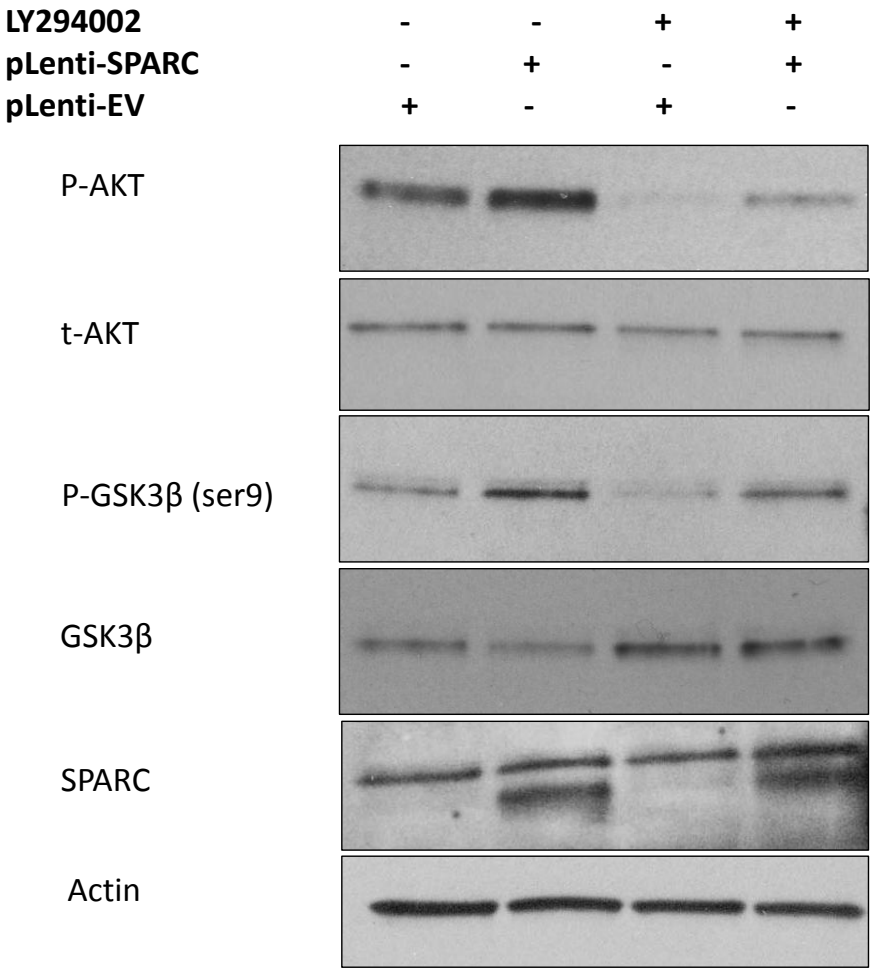


Figure S7. PI3K inhibitor partially abrogates SPARC effect in THP-1 cells.

THP-1/EV and THP-1/SPARC cells were treated with 50μM of the PI3K inhibitor LY294002; GSK3β and AKT phosphorylation were assessed in THP-1/EV cells compared to THP-1/SPARC cells 6 hours following treatment (SPARC upper band is likely to represent a glycosylated form of SPARC protein, SPARC antibody used is SC-25574; Santa Cruz Biotechnology).

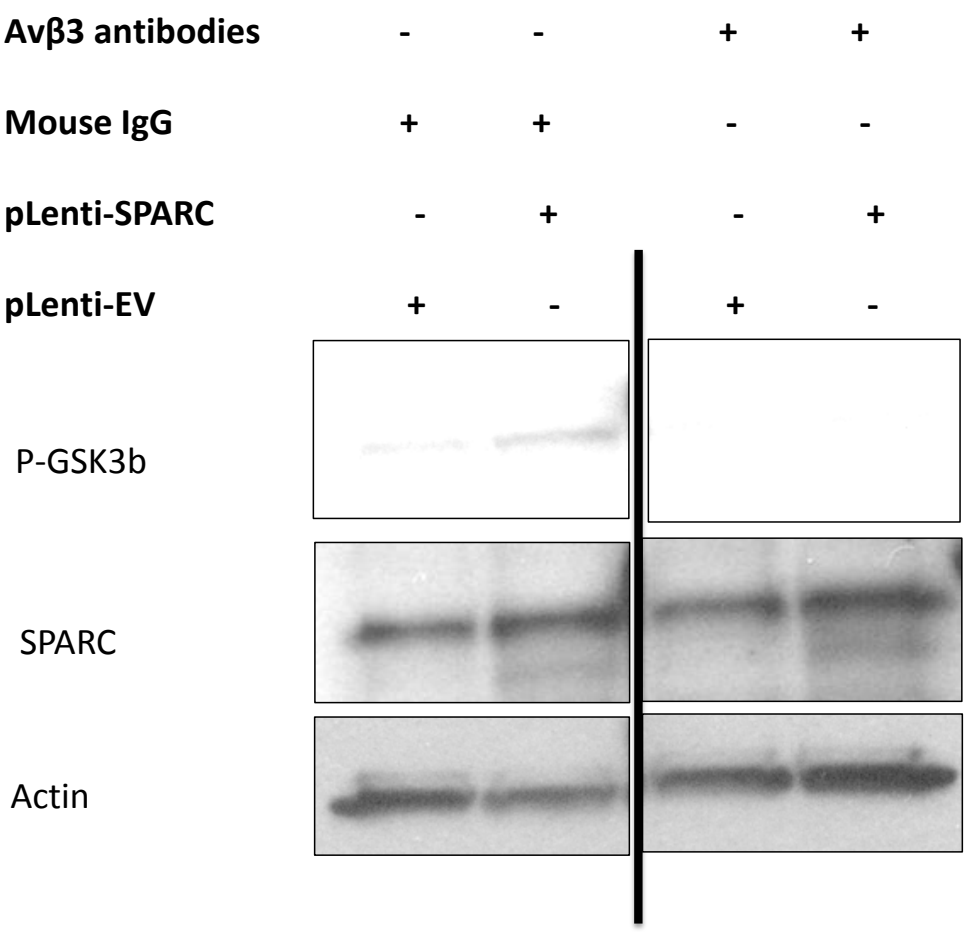


Figure S8. αvβ3 antibodies abrogates SPARC effect in THP-1 cells.

THP-1/EV and THP-1/SPARC cells were incubated with αvβ3 antibodies for 20min, GSK3β phosphorylation were assessed in THP-1/EV cells compared to THP-1/SPARC cells 24 hours later.

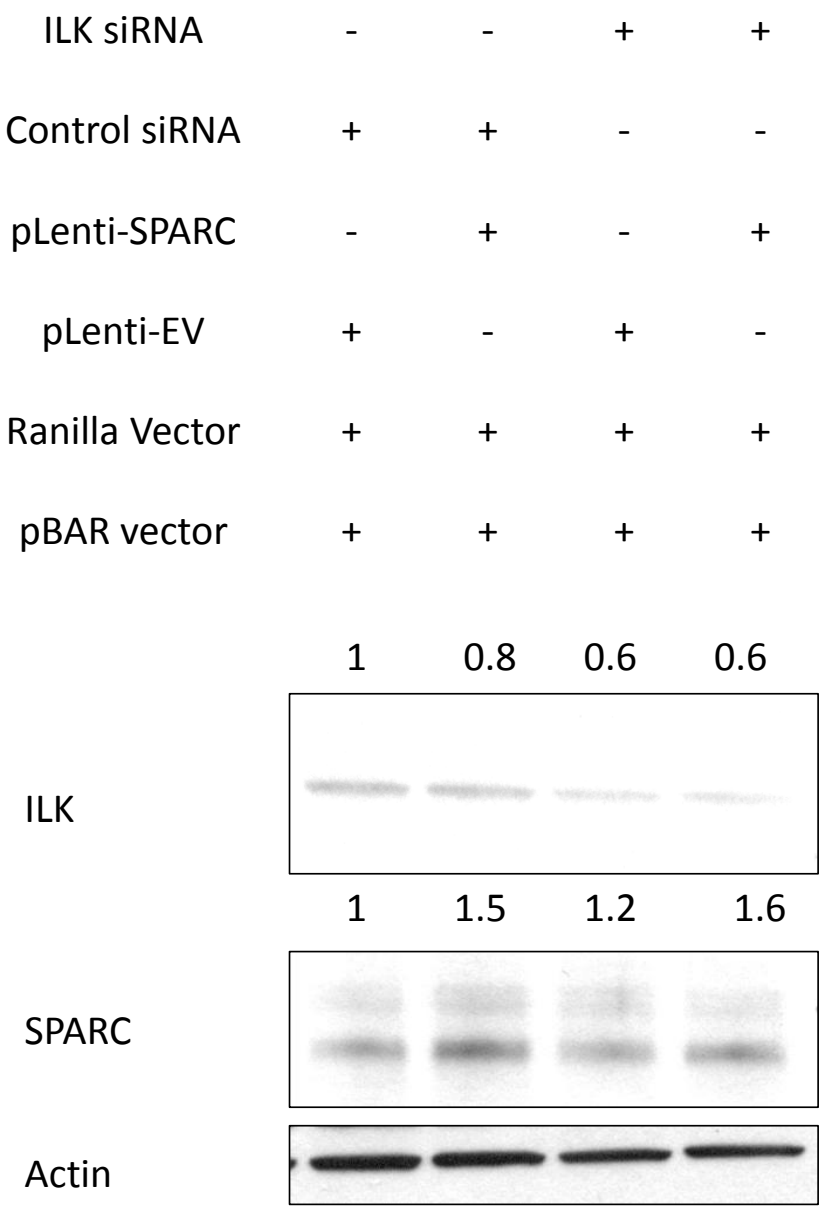


Figure S9. SPARC and ILK protein levels in 293T cells.

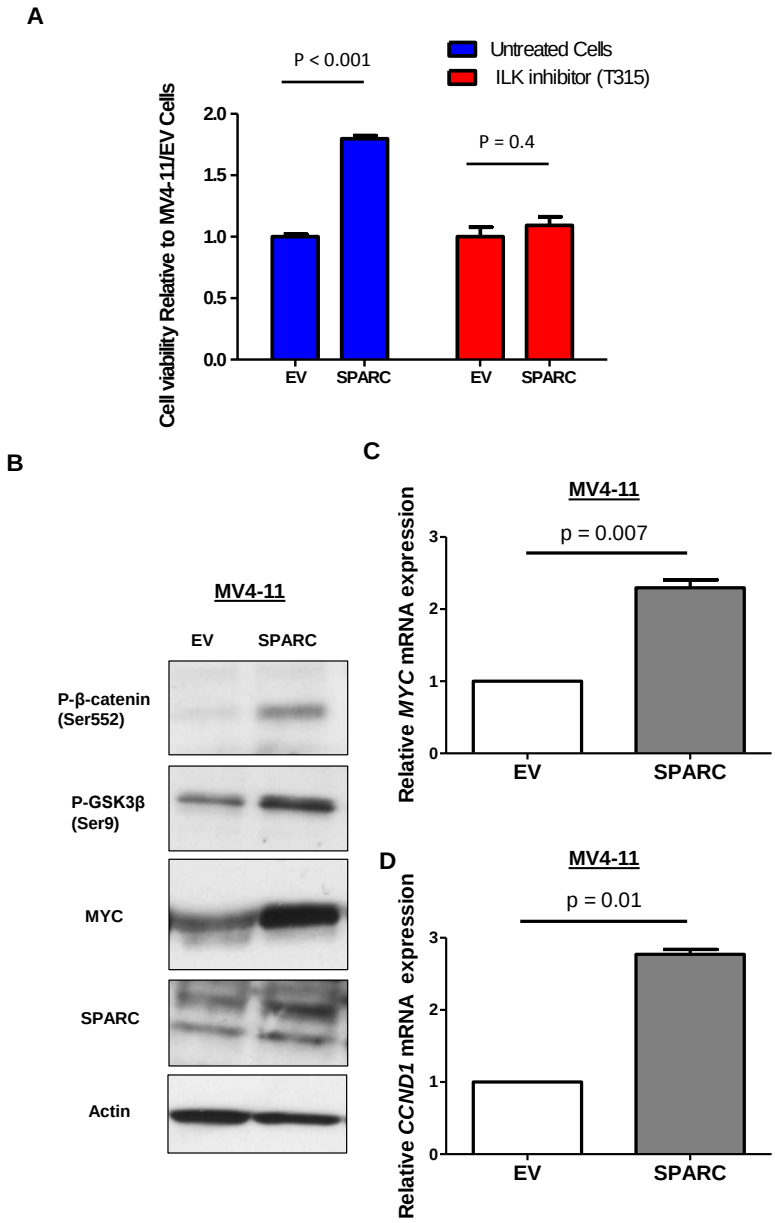
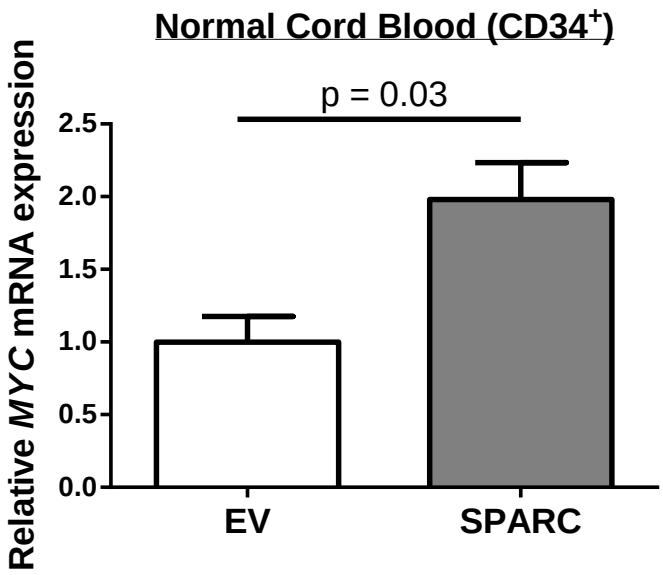


Figure S10. **Validation of SPARC Signaling Pathway in MV4-11 Cells**. (A) cell viability of MV4-11/EV and MV4-11/SPARC cells treated with ILK inhibitor (T315) measured by MTS assay; (B) Western blot analysis showing p-β-catenin(Ser552) and p-GSK3β(ser9) and MYC protein levels in MV4-11 cells ectopically expressing SPARC. Changes in (C) *MYC* mRNA levels and (D) *CCND1* mRNA levels measured in MV4-11 cells transfected with pLenti-SPARC vector compared with cells transfected with pLenti-EV.

A



B

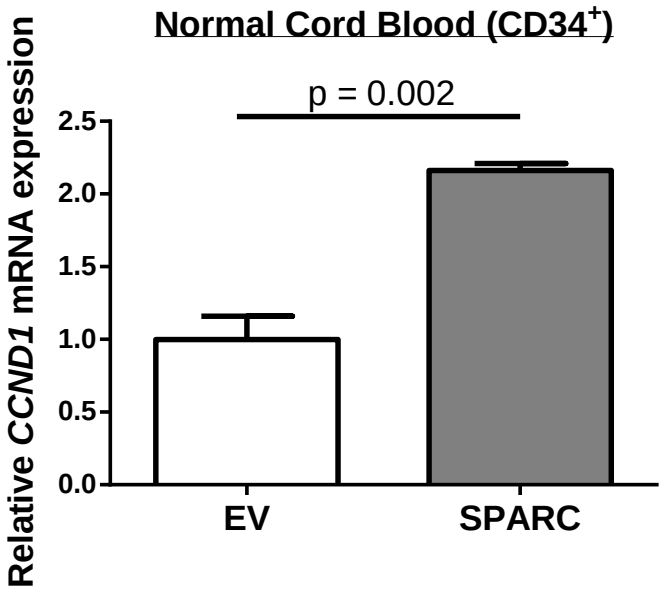


Figure S11. Validation of SPARC Signaling Pathway in CD34$^+$ Cord Blood Cells. Changes in (A) *MYC* mRNA levels and (B) *CCND1* mRNA levels measured in CD34$^+$ cord blood cells transfected with pLenti-SPARC vector compared with cells transfected with pLenti-EV.

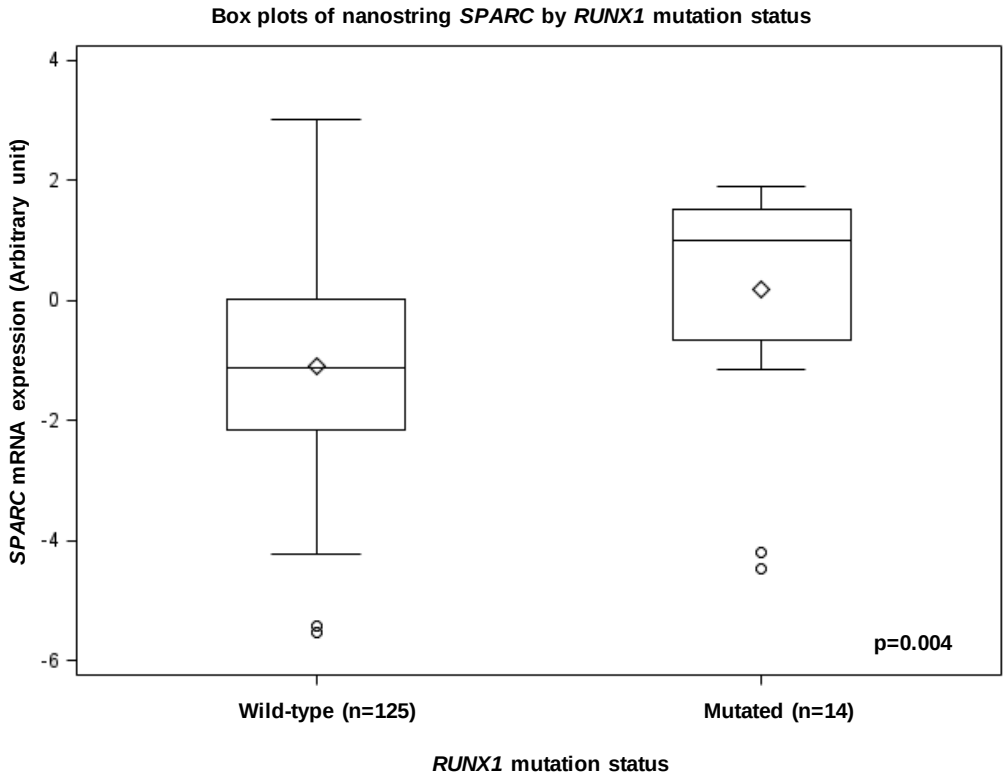


Figure S12. SPARC Expression According to *RUNX1* Mutational Status in Patients with CN-AML.

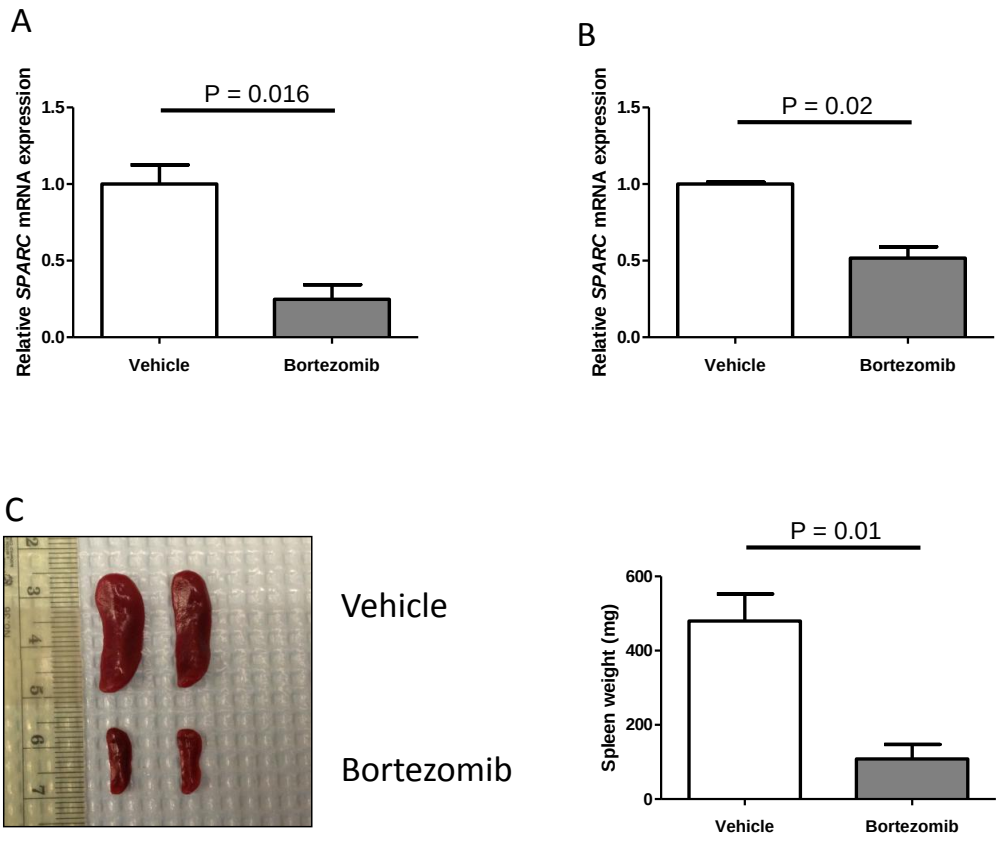


Figure S13. Bortezomib downregulates *SPARC* mRNA expression *in vivo.* *SPARC* mRNA downregulation in (A) blood and (B) spleen tissues obtained from CG-SH spleen adapted cells engraft NSG mice treated with bortezomib compared with vehicle treated mice. (C) Spleens of bortezomib treated mice compared with vehicle treated mice and quantification of spleen weights (mean±SEM).

3. SUPPLEMENTAL REFERENCE

1. Cancelas JA, Lee AW, Prabhakar R, Stringer KF, Zheng Y, Williams DA . Rac GTPases differentially integrate signals regulating hematopoietic stem cell localization. *Nat. Med*. 2005;11(*8*):886-891.