Clinical and preclinical characterization of CD99 isoforms in acute myeloid leukemia

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Supplementary materials:

The supplemental materials include supplemental methods, tables and figures.

Supplementary Methods:

Patient Datasets and statistical analyses

For the TCGA dataset, 186 patients with previously untreated AML were studied, all of whom had been diagnosed and received treatment according to the National Comprehensive Cancer Network (NCCN) guidelines between November 2001 and March 2010 was downloaded from oncomine. The GSE3077 dataset had microarray data for cell populations sorted based on CD34 and CD38 cell markers obtained from 23 patients with AML. *CD99* transcripts analysis was performed using the GSE106291 dataset that consisted of 246 all of whom received intensive induction treatment. The cohort consists of an unselected patient population (mixed cytogenetics) and additional refractory patients (most with poor cytogenetics). The median age for the patients was 58 years old (range 18-74). M3 patients were excluded from this analysis. *CD99* transcript counts were extracted from GSE106291 annotated with GRCh38 release-96 build using Salmon (v0.9.1)[1]. Counts were normalized (TMM) using edgeR [2]and log2 transformed counts per million were extracted.

For gene expression analysis, patients were divided into two groups based on *CD99* median expression. To investigate the associations between *CD99* expression levels and the clinical and molecular characteristics, Student's *t*-test and univariate analysis were used in the hypothesis testing for categorical and continuous variables, respectively. The Mantel-Cox log-rank test was used to estimate the association between *CD99* expression and EFS and OS of the patients. Univariate and multivariable analysis were performed using STATA3. The statistical cutoff value was adjusted to p-value ≤0.05. All other analyses were performed using the GraphPad Prism software packages.

Cell lines and primary blasts

THP-1, MV4-11, KG-1, and Kasumi-1 were purchased from ATCC. MOLM 13, U937, KG-1A and NB4 cells were kindly provided by Dr. Wendy Stock's lab. All AML cell lines were authenticated at the USC Cell authentication Core.

Plasmids

The PLVX-*CD99-L*-AcGFP-C1 and PLVX-*CD99-S*-AcGFP-C1 was constructed by cloning the *CD99* cDNA from U937 into the Apa1 and Xho1 sites of the PLVX-Zsgreen-C1 (Clontech, CA, USA). Primers used to generate PLVX-*CD99-L*-Zsgreen-C1 are as follows: Forward: CGCTCTGGGCGCACC, Reverse: AACAAATTGAAGGGC. Primers used to generate PLVX-*CD99-S*-Zsgreen-C1 are as follows: Forward: CGCTCTGGGCGCACC, Reverse: TCAGCCATCATTTTC. For the PLVX- AcGFP -C1, the GFP is fused with *CD99* on the N-Terminal. For *CD99*-shRNA plasmid PLKO.1 backbone was used. The PLKO.1 CD99-sh RNA plasmid was generated by using the following target sequence: CCATCTCTAGCTTCATTGCTT. Primers used to generate this are as follows:

Forward:

CCGGCCATCTCTAGCTTCATTGCTTCTCGAGAAGCAATGAAGCTAGAGATGGTTTTTG,

Reverse:

AATTCAAAAACCATCTCTAGCTTCATTGCTTCTCGAGAAGCAATGAAGCTAGAGATGG.

Transient transfection and viral induction

Transient knockdown was performed using 10 nmol of siRNA per reaction using 200 ul of electroporation buffer in an electroporator. Lentivirus infection was performed by transfecting 293T cells with PLVX plasmids together with psPAX and MD2.G packing plasmids using Calcium Phosphate Transfection Kits (Clontech, CA, USA). Virus was collected 72 hours after transfection, filtered and concentrated using PEG reagent at 1:4 dilution, pelleted 24 hours

later and resuspended in RPMI supplemented with 10% FBS and 1% Antibiotics. $1X10^5$ suspension cells were infected with the virus and expanded after puromycin selection. Two separate batches of lenti-viral infection were performed for THP-1, U937, and MOLM-13 cells. For the CD99-shRNA experiments, CD99-shRNA plasmid was added to cells for 96 hours and cells were used for various assays.

Migration Assay

Migration assay was performed using the modified Boyden chambers (cat# 3436, Corning, New York, USA) that consisted of Transwell-coated matrigel membrane filter inserts with 8 µm pores in 24-well tissue culture plates. The assay was performed as previously described [3]. Briefly, $1X10^5$ cells in duplicates of THP-1, MOLM-13 and isolated healthy PBMCs were re-suspended in 100ul RPMI media containing 10% FBS and treated with 5µg/mL of mAbCD99 for 30mins. The cells were then seeded in the insert wells and 600 ul of RPMI media containing 10% FBS and SDF-1α were added to the lower chamber and incubated for 4 hours at 37°C. The experiment was performed twice. Following this, cells migrated towards SDF-1α were analyzed by capturing multiple images of the lower chamber for each well and analyzed using ImageJ software. Similarly, for the overexpression experiment, duplicate experiments from two separate batches of infection for THP-1, MOLM-13 and U937 cells stably overexpressing CD99-L, CD99-S or EV cells were seeded and analyzed.

Immunoblotting

For immunoblotting, cells were lysed in Pierce-Protease lysis buffer (cat# 8788, Thermo Fisher, MA, USA), supplemented with a protease inhibitor mix (cat# A32959, Thermo Fisher, MA, USA). Protein concentrations were determined using the BCA protein assay reagent (Pierce). 10 μg of total cellular lysates was added to each lane of SDS-PAGE gels. Trans-blot (BioRad) was used

to semi-dry transfer. Then lysates were resolved by SDS-PAGE and transferred to a PVDF membrane. Membranes were blocked with 5% non-fat milk or BSA and probed with indicated antibodies. Horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology) were used for detection. Anti-CD99 antibody used was 013 (Thermofisher, MA5-12287), Anti- H2Axɣ (Santacruz, sc-517348), MDM2 (SantaCruz, sc-965), SRC (Cell Signaling, cat# . 2123), P-SRC (Cell Signaling, cat# .6943), ERK (Cell Signaling, cat# .9102), P-ERK (Cell Signaling, cat# .9101), Actin (Cell Signaling, cat# .3700). Western blot band density was evaluated using ImageJ analysis. Immunodetection was achieved with the ECL super signal reagent and detected by a Bio-Rad ECL machine.

RNA extraction and RNA expression quantification

Total RNA was extracted using TriZOL reagent (Invitrogen, CA, USA) as previously described. cDNA was synthesized using SuperScript III reagents (Invitrogen, CA, USA) according to the manufacturer's instructions. Quantitative real-time RT-PCR (qRT-PCR) was performed using commercially available TaqMan Gene Expression Assay primers and probes. The expression levels were normalized to *B2M*. qPCR analysis for isoform analysis was performed using SYBR green assay. For CD99 transcript analysis of the two isoforms, the following primers were used forward (CD99-L and CD99-S): GTGATCCCCGGGATTGTG; CD99-L reverse: CTATTTCTCTAAAAGAGTACG; CD99-S reverse: CCTAGGTCTTCAGCCATC.

Wright-geimsa assay

THP-1 cells treated were treated with 2.5µg/mL of anti-CD99mAb for 3 days following which cells were washed and fixed onto a glass slide using a cytospin. Cells were then fixed with methanol and then stained with quick stain wright-geimsa stain for 1 min and imaged.

BrdU staining assay

For the BrdU staining assay, the APC BrdU Flow kit was used (act no. 552598, BD Biosciences, San Jose, USA). Cells were synchronized by starvation. Following this BrdU was added to the cells for 12 hours and the assay was performed as per the manufacturer's protocol.

Flow analysis

Cell surface expression of CD99 (cat#: 12-0997-42, eBiosceince, CA, USA), CD11b (cat# A18613), CD45 (cat# 25-0459-41, eBioscience, CA, USA) and Apoptosis detection kit APC (cat# 88-8007-74, eBioscience, CA, USA) were analyzed using the LSRII flow cytometer. Cells were stained with PE-conjugated anti-CD99 or anti-annexin V, APC conjugated CD11b or PE-Cy7-A conjugated anti-huCD45 for 15 mins analyzed. The data was processed using FloJo software

Viability and clonogenic assays

Cells were treated with anti-CD99 mAb clone H036-1.1 (Thermofisher), cells were seeded at $1X10^5$ cells/well in a 96-well plate with three replicates. 48-hours later, alamar blue assay was performed per the manufacturer's protocol (Invitrogen). For CD99 overexpression viability assay, stable cells expressing CD99-L, CD99-S or EV cells from two separate viral transductions. The experiment was performed in duplicates for each set of transduced cells. The number of live cells was counted at 24, 48 and 72-hours using trypan blue. For primary blasts overexpression, viability was determined 96 hours after infection with CD99-L, CD99-S or EV lenti-viral particles using trypan blue. Methylcellulose clonogenic assays were carried out by plating 5×10^4 primary blasts in MethoCult (StemCell Technologies) as previously described[4] and counted 14 days later.

Aggregation assay

Cells overexpressing CD99-L or CD99-S (or EV) were seeded at a concentration of $1X10^5$ cells /mL for 6 hours, then microscope images were taken.

ROS assay

ROS assay was performed using Cell ROX deep red reagent (Invitrogen, cat# C10422) according to the manufacturer's protocol and measured using flow cytometry.

In vivo studies

For the xenograft experiments, 4- to 6-week-old NOD-*scid* /Il2rg$^{-/-}$ (NSG) mice were used. For the THP-1 xenograft model, $2.5X10^6$ THP-1 cells were injected via tail vein injection. Two separate experiments were conducted and results were summarized together. For the first experiment mice were implanted with empty vector (n=3) and CD99-L (n=3). For the second experiment mice were implanted with empty vector (n=3), CD99-L (n=3) and CD99-S (n=3). For both the experiments, mice were sacrificed once the control mice were sick. For the MOLM-13 experiment, mice were implanted with $2.5X10^6$ cells of empty vector (n=3), CD99-L (n=3) or CD99-S (n=3) and were sacrificed only when sick. For the primary cell experiment, cells were transduced with CD99-L (or EV) for 96 hours. Prior to engraftment 4- to 6-week-old NOD-*scid* /Il2rg$^{-/-}$ (NSG) mice were irradiated using the X-ray irradiator at a dose of 250 cGy and 24 hours later, $1X10^6$ cells were engrafted via tail vein in empty vector (n=3) and CD99-L (n=3) mice. Mice were sacrificed four months after engraftment. For all the *in vivo* experiments, bone marrow, peripheral blood, liver and spleen tissues were stained for huCD45 and analyzed using flow cytometry.

Supplemental tables:

Tables S1: CD99 isoform Transcript ID and description

Name	Transcript ID	bp	Protein	Biotype	Isoform	CCDS	Uniprot
CD99-205	ENST00000381192.10	1129	185aa	Protein coding	Long Isoform	CCDS14119	P14209
CD99-210	ENST00000611428.5	1243	160aa	Protein coding	Short Isoform	CCDS75947	P14209
CD99-208	ENST00000482405.7	842	160aa	Protein coding	Short Isoform	CCDS75947	P14209
CD99-212	ENST00000624481.4	1089	184aa	Protein coding		CCDS83452	A0A096LP69
CD99-204	ENST00000381187.8	892	169aa	Protein coding		CCDS48071	P14209
CD99-203	ENST00000381184.6	918	177aa	Protein coding		-	A8MQT7
CD99-202	ENST00000381180.9	533	76aa	Protein coding		-	A6NJT9
CD99-211	ENST00000623253.4	573	160aa	Nonsense mediated decay		CCDS75947	P14209
CD99-201	ENST00000381177.7	756	22aa	Nonsense mediated decay		-	A6NGF6
CD99-206	ENST00000449611.6	604	No protein	Processed transcript		-	-
CD99-207	ENST00000482293.6	466	No protein	Processed transcript		-	-
CD99-214	ENST00000646103.1	1278	No protein	Retained intron		-	-
CD99-209	ENST00000497752.7	815	No protein	Retained intron		-	-
CD99-215	ENST00000647297.1	583	No protein	Retained intron		-	-
CD99-213	ENST00000645950.1	497	No protein	Retained intron		-	-

Table S2: Mutational characteristics of patients with AML in the TCGA dataset according to CD99 expression

		CD99-Low	CD99 High	p value (WT vs Mutated)	Fischer Exact Test
FLT3-ITD, no. (%)				p=0.002	P=0.0402
Present	37 (30)	12 (25.2)	24 (47.5)		
Absent	149 (70)	81 (74.7)	69 (70.3)		
IDH1, no. (%)				p=0.08	P=1
Mutated	17 (9.0)	8 (8.4)	9 (9.6)		
Wild type	169 (91.0)	85 (91.5)	84 (90.3)		
IDH2, no. (%)				p=0.11	P=0.3
Mutated	17 (9.5)	6 (6.3)	11 (12.7)		
Wild type	169 (91.5)	87 (93.6)	82 (87.2)		
RUNX1, no. (%)				p=0.50	P=1
Mutated	17 (8.5)	8 (8.4)	9 (10.4)		
Wild type	169 (91.5)	85 (91.5)	84 (89.5)		
TET2, no. (%)				p=0.98	P=0.6
Mutated	17 (9.0)	10 (10.6)	7 (7.4)		
Wild type	169 (91)	83 (89.4)	86 (92.5)		
NRAS, no. (%)				p=0.2	P=0.7
Mutated	14 (7.9)	8 (8.4)	6 (6.3)		
Wild type	172 (91.4)	85 (91.5)	87 (93.6)		
CEBPA, no. (%)				p=0.1	P=0.08
Mutated	13 (9.0)	3 (10.6)	10 (7.4)		
Wild type	173 (91)	90 (89.4)	83 (92.5)		
WT1, no. (%)				p=0.92	P=0.5
Mutated	11 (9.0)	4 (10.6)	7 (7.4)		
Wild type	175 (91)	89 (89.4)	86 (92.5)		
DNMT3A, no. (%)				p=0.9	P=0.31
Mutated	49 (26.0)	28 (29.7)	21 (22.3)		
Wild type	137(73.9)	65 (70.2)	72 (77.65)		
NMP1, no. (%)				p=0.24	P=0.4
Mutated	50 (26.0)	28 (29.7)	22 (22.3)		
Wild type	136(73.9)	65 (70.2)	71 (77.65)		
TP53					
Mutated	15	14 (93)	1 (6.6)	p=0.001	P=0.0006
Wild type	171	79 (46.2)	92 (53.8)		

Table S3: Clinical characteristics of the acute myeloid leukemia (AML) cohort in the TCGA dataset according to CD99 median expression.

	Total	CD99-Low	CD99 High	p value (CD99-Low vs High)	Fischer Exact
Sex, no. (%)					P=0.55
Female	85 (45.7)	45 (53)	40 (47)		
Male	101(55.3)	48 (47.5)	53 (52.5)		
Age, years (range)				p=0.017	
Median	57.5	57.82	52.1		
Mean	55	57.82 ± 1.555	52.17 ± 1.764		
WBC count				p=0.019	
Median	16.55	27.34946	45.84516		
Mean	36.59731	27.35 ± 3.885	45.85 ± 5.912		
PB blasts				p<0.0001	
Median	34	23.76667	50.76923		
Mean	37.34254	23.77 ± 2.858	50.77 ± 3.042		
BM blasts				p=0.024	
Median	73	66.21505	72.51613		
Mean	69.36559	66.22 ± 2.155	72.52 ± 1.749		
NCCN subtype, no					Vs Favorable
Favorable	34	8	26		
Intermediate	112	60	52		P=0.0029
Poor	40	25	15		P=0.001
FAB subtype, no					
M0	16	5	11		
M1	43	14	29		
M2	41	19	22		
M3	18	6	12		
M4	40	27	13		
M5	21	17	4		
M6	2	1	1		
M7	3	3			

Table S4: Multivariate analysis of overall survival of patients with AML.

Multivariate Analysis	**OS (categorical CD99)**	
Parameter	**p-value**	**Hazard Ratio (95% Conf. Interval)**
Age	0.033	1.016617 (1.001304, 1.032165)
Intermediate Risk	0.001	3.135137 (1.601362, 6.137954)
Poor Risk	<0.001	4.704015 (2.109915, 10.48751)
Transplant status	<0.001	0.4344322 (0.2781283, 0.6785766)
DNMT3A	0.108	1.396372 (0.9291901, 2.098445)
TP53	0.05	1.95511 (0.9791412, 3.903891)
CD99	0.36	0.839078 (0.546331, 1.22522)

Tables S5: Primer match list for CD99 primer set 1 and set 2 for all CD99 transcripts

Transcript	ID	AA	Primer Set-1 Forward	Primer Set-1 Reverse	Primer Set-2 Forward	Primer Set-2 Reverse
ENST00000381192.10	205	185	Y	Y	N	N
ENST00000381187.8	204	169	Y	Y	N	N
ENST00000381184.6	203	177	Y	N	N	N
ENST00000482405.7	208	160	Y	N	Y	Y
ENST00000611428.5	210	160	Y	Y	N	N
ENST00000624481.4	212	184	Y	Y	N	N
ENST00000381180.9	202	76	N		N	

Tables S6: Patient’s information of primary samples used in the functional studies

Patient ID	AML Status sample	FLT3 Mutation
AML 1	Diagnosis	NA
AML 2	Diagnosis	NA
AML 3	Diagnosis	NA
AML 4	Diagnosis	ITD
AML 5	Relapse	ITD
AML 6	Diagnosis	WT
AML 7	Diagnosis	WT
AML 8	Relapse	ITD
AML 9	Diagnosis	NA
AML 10	Diagnosis	NA
AML 11	Diagnosis	NA

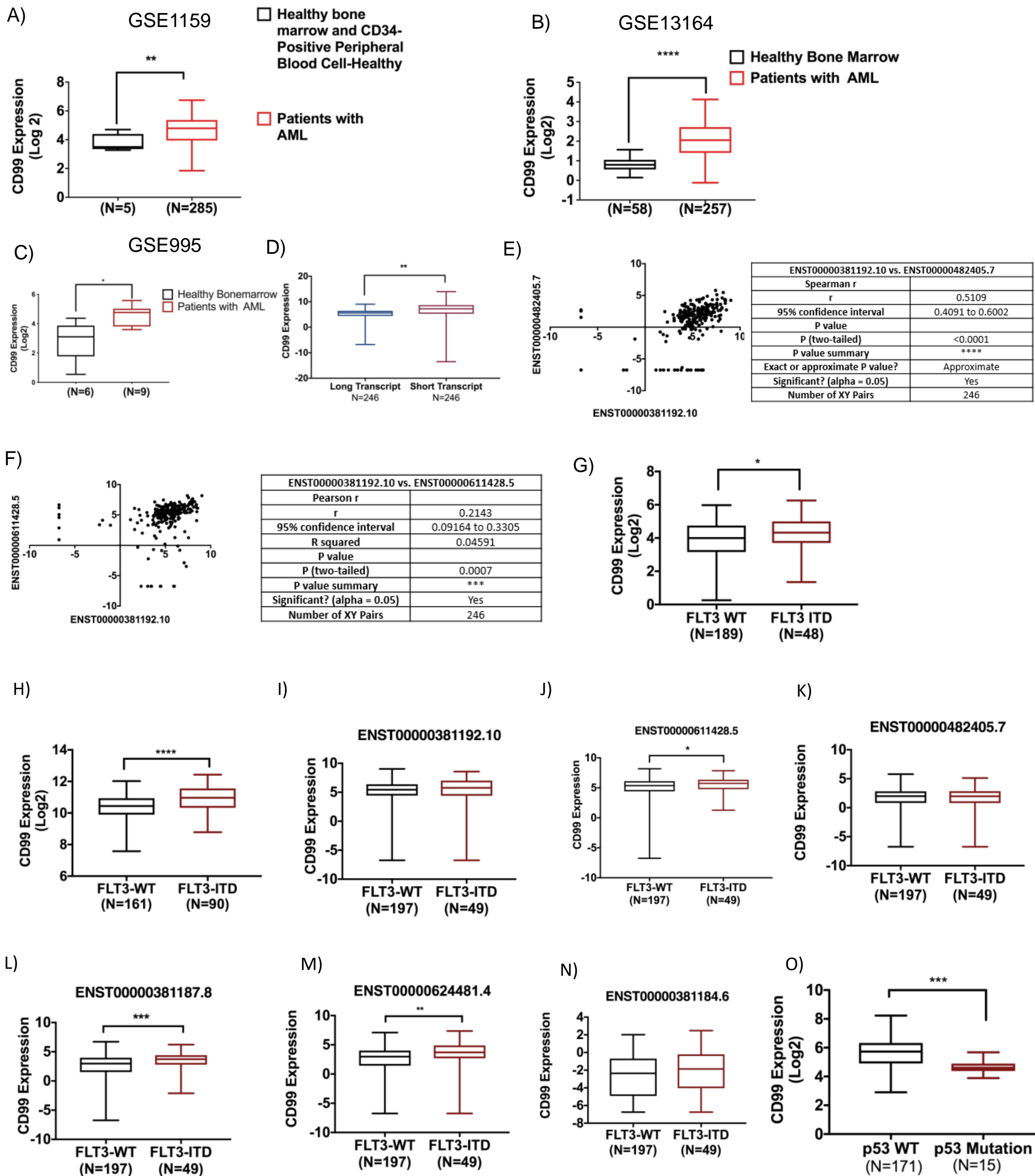


Figure S1: A) Relative expression of *CD99* in 285 AML cases compared with five healthy donors in the GSE71159 dataset. B) Relative expression of *CD99* in 257 AML cases compared with 58

healthy donors in the GSE13164 dataset. C) Relative expression of *CD99* in nine AML cases as compared with six healthy donors in the GSE995 dataset. E) Correlation between ENST00000482405.7 and ENST00000381192.10 *CD99* transcript. F) Correlation between ENST00000611428.5 and ENST00000381192.10 *CD99* transcript. G) Relative expression of *CD99* in 48 patients with *FLT3*-ITD as compared with 189 patients with *FLT3* WT in the GSE 17855 dataset. H) Relative expression of *CD99* in 90 patients with *FLT3*-ITD as compared with 161 patients with *FLT3* WT. I-N) Relative expression of various *CD99* transcripts in 49 patients with *FLT3*-ITD as compared with 197 patients with *FLT3* WT in the GSE106291 dataset. O) Relative expression of *CD99* in 15 patients with *TP53* mutation as compared with 171 patients with *TP53* wild type in the TCGA dataset. Differences among groups were analyzed using Student's T test. (* $p < 0.05$, ** <0.005, ***$p<0.001$, ****$p<0.0001$)).

Supplementary Figure 2:

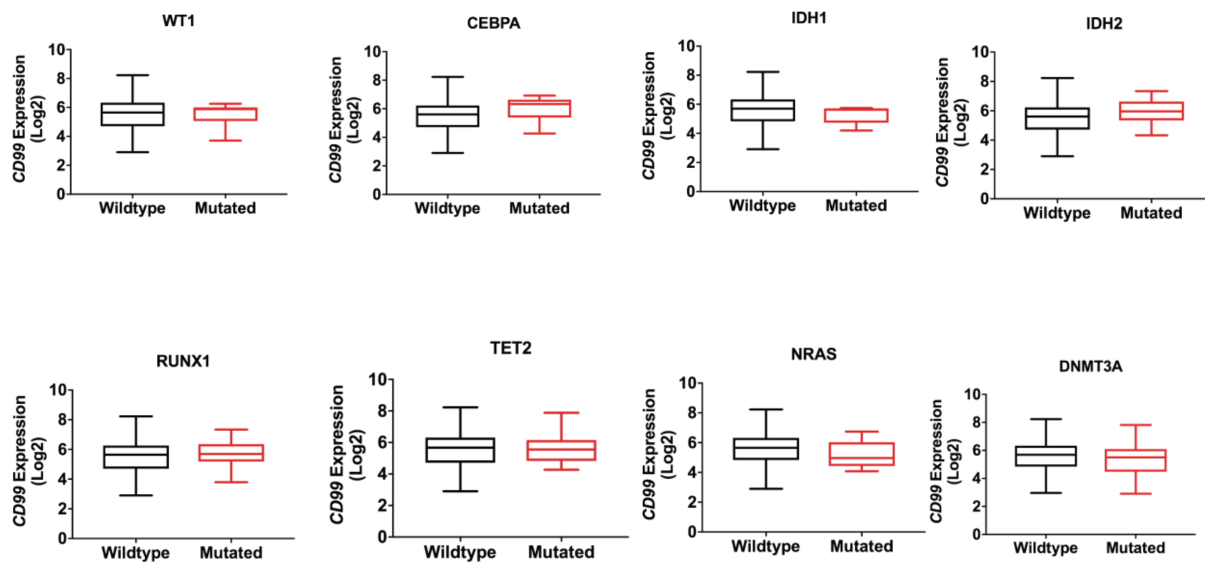
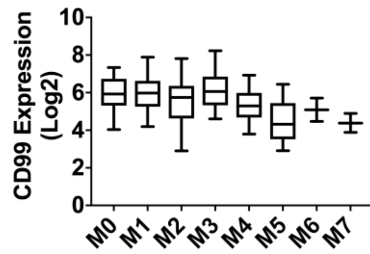


Figure S2: Relative expression of *CD99* in patients with *WT1*, *CEBPA*, *IDH1*, *IDH2*, *RUNX1*, *RUNX2*, *NRAS* and *DNMT3A* mutation compared with the wildtype.

Supplementary Figure 3

A)



B)

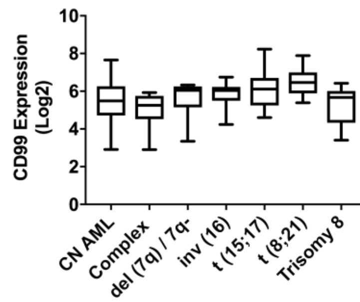


Figure S3: A) Relative expression of *CD99* in the different FAB subtypes in the TCGA dataset.

B) Relative expression of *CD99* based on different cytogenetics of patients in the TCGA dataset.

Supplementary Figure 4

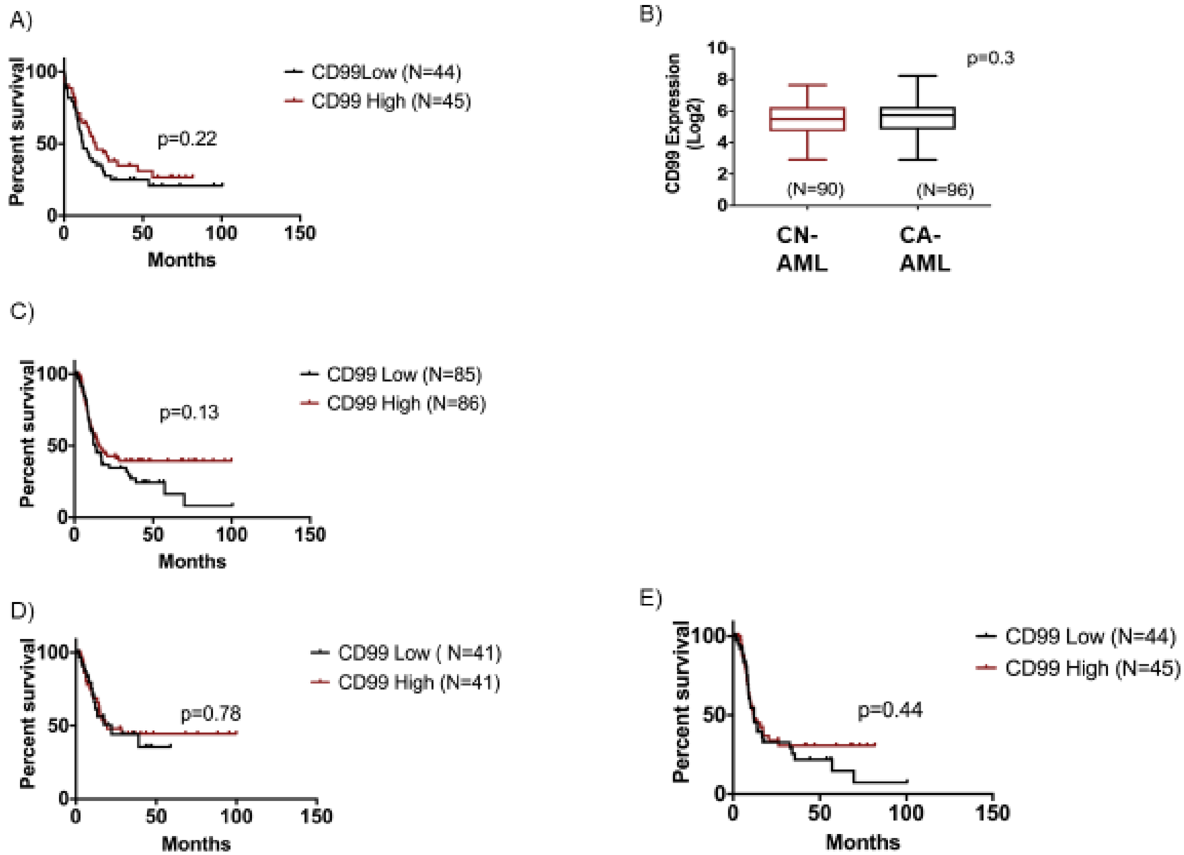


Figure S4: A) OS of cytogenetically normal (CN-AML) cases grouped based on *CD99* median expression into *CD99* high (N=45) and *CD99* Low (N=44) in the TCGA dataset. B) Relative *CD99* expression in CN-AML vs CA-AML groups in the TCGA dataset. Differences among groups were analyzed using Student's T-test. C) Event free survival (EFS) of 171 patients grouped based on *CD99* median expression into *CD99* high (N=86) and *CD99* low (N=85). D) EFS of cytogenetically abnormal (CA-AML) cases grouped based on *CD99* median expression into *CD99* high (N=41) and *CD99* low (N=41). E) EFS of cytogenetically normal (CN-AML) cases grouped based on *CD99* median expression into *CD99* high (N=45) and *CD99* low (N=44). Red line: *CD99* high group, black line: *CD99* low group.

Supplementary Figure 5

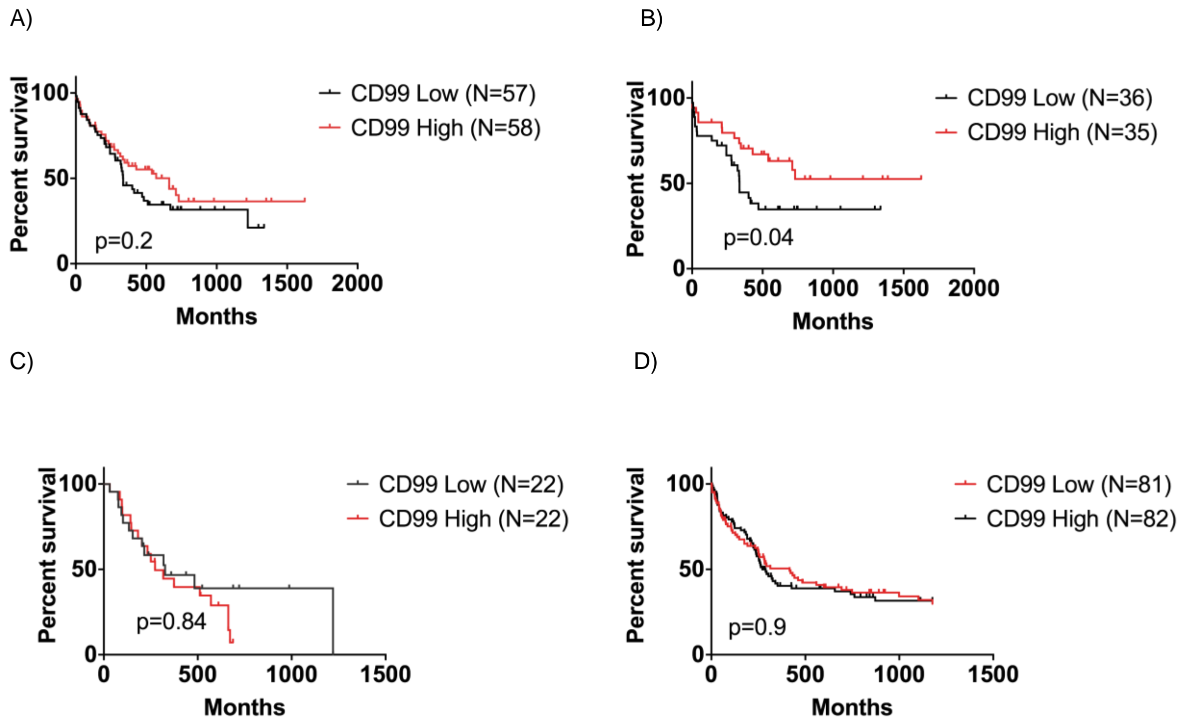


Figure S5: A) Overall survival (OS) of 115 patients grouped based on *CD99* median expression into *CD99* high (N=58) and *CD99* Low (N=57) from the GSE425 dataset. (B) OS of cytogenetically abnormal (CA-AML) cases grouped based on *CD99* median expression into *CD99* high (N=35) and *CD99* low (N=36). C) OS of cytogenetically normal (CN-AML) cases grouped based on *CD99* median expression into *CD99* high (N=22) and *CD99* low (N=22). D) OS of 163 cytogenetically normal (CN-AML) patients from the GSE12417 dataset grouped based on *CD99* median expression into *CD99* high (N=82) and *CD99* low (N=81). Red line: *CD99* high group, black line: *CD99* low group.

Supplementary Figure 6

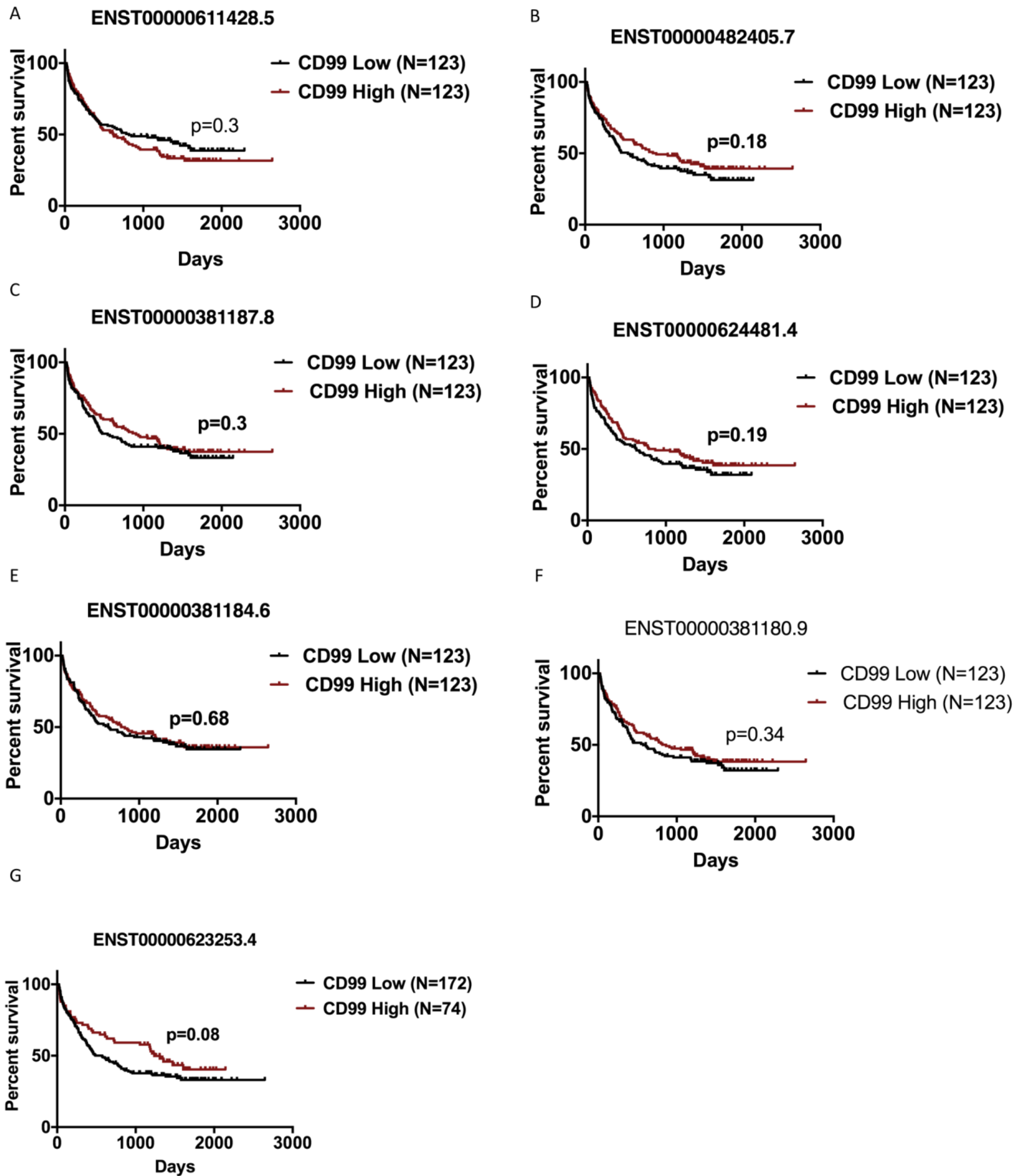


Figure S6: A-G) Overall survival (OS) of 246 patients grouped based on median *CD99* expression for various transcripts of *CD99* into high (N=123) and low (N=123) from the GSE106291 dataset.

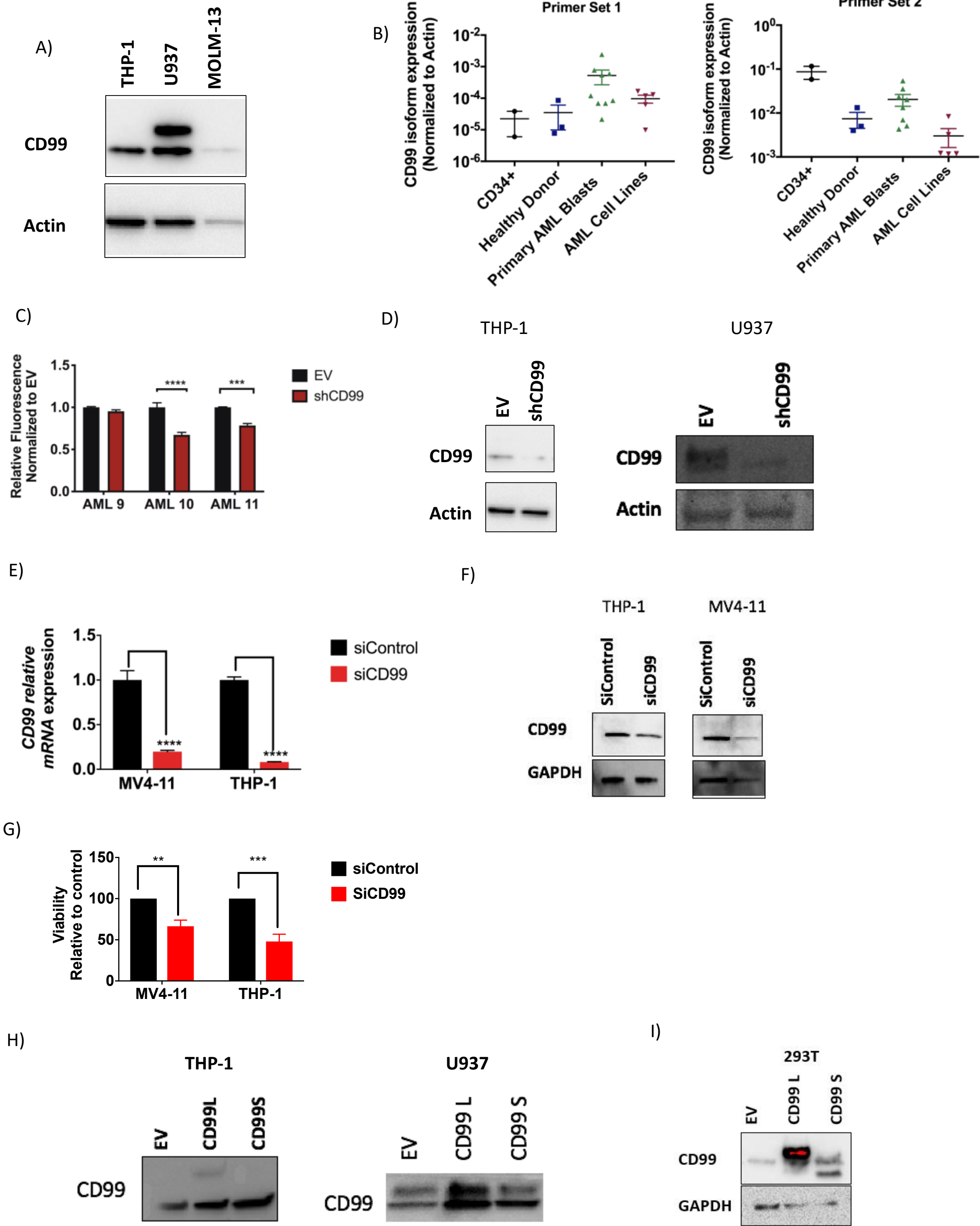


Figure S7: A) Western blot analysis for CD99 expression in THP-1, U937 and MOLM-13 AML cell lines. B) CD99 isoform analysis using two different primer sets for CD34+ cells, healthy donor

PBMCs, primary patient blasts and AML cell lines. C) Viability assay for primary AML blast (N=3) transfected with sh*CD99* plasmid (or EV) for 96 hours measured by trypan blue assay. Number of live cells were normalized to EV cells. D) Western blot analysis of CD99 expression in shD99 knockdown cells of THP-1 and U937 cells. E) Expression of *CD99* in siCD99 knockdown cells of THP-1 and MV4-11 normalized to siControl cells using Taqman assay (n=3). F) Western blot analysis of CD99 expression in si*CD99* knockdown cells of THP-1 and MV4-11 cells. G) Viability assay for si*CD99* knockdown cells of THP-1 and MV4-11 performed by alamar blue at 48 hours; Viability is normalized to control cells (n=3. H) Western blot analysis of CD99 overexpression in THP-1 and U937 cells transduced with EV, *CD99*-L and *CD99*-S virus. I) Western blot analysis of CD99 overexpression in 293T cells transfected with EV, CD99-L and CD99-S plasmid used to generate lentivirus particles.

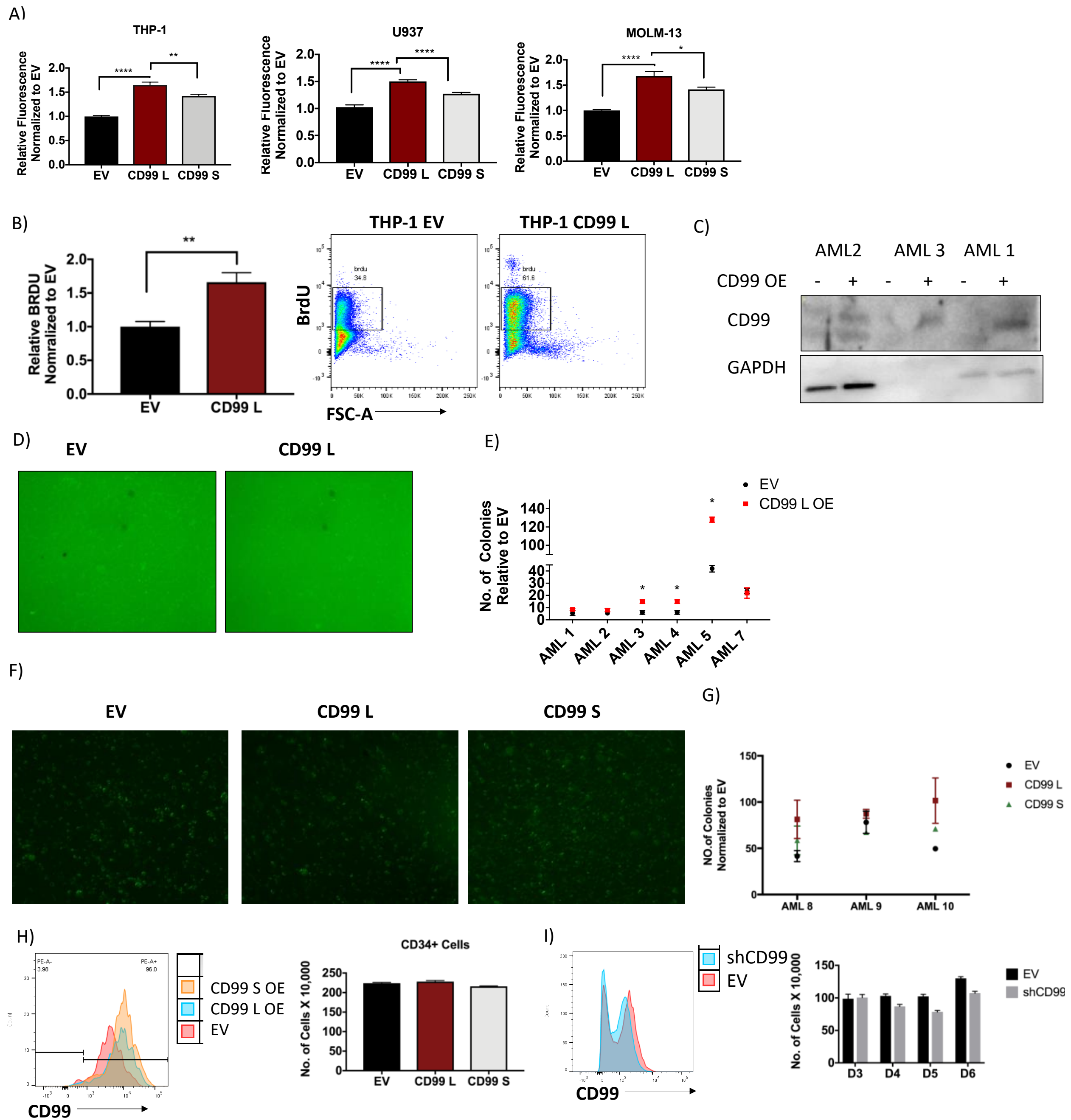


Figure S8: A) Relative Viability of THP-1, U937 and MOLM-13 cells stably expressing CD99-L or CD99-S isoform measured using alamar blue at 72 hours. Viability is normalized to EV cells (N=4). B) Proliferation assay using BrdU assay in THP-1 cells stably overexpressing CD99-L or

EV measured 24 hours after adding BrdU using flow cytometry. Data normalized to EV cells. C) Western blot analysis for CD99 expression of 3 primary patient blasts infected with *CD99*-L (or Empty vector) lentivirus for 5 days. D) Representative GFP images for CD99 expression (or Empty vector) in primary patient blasts infected with *CD99* (or Empty vector) lentivirus for 5 days. E) Relative number of colonies between AML blasts overexpressing CD99-L and EV blasts (n=6) measure on day 14. F) Representative GFP images for CD99-L, CD99-S (or Empty vector) in primary patient blasts infected with *CD99* (or Empty vector) lentivirus for 5 days. G) Relative number of colonies between AML blasts overexpressing CD99-L, CD99-S or EV blasts (n=3) measure on day 14. H) Flow analysis for CD99 overexpression and proliferation assay measured using trypan blue 96 hours after infecting $CD34^+$ healthy donor cells with EV, *CD99*-L and *CD99*-S lentivirus. H) Flow analysis for CD99 knockdown at day 6 and proliferation assay measured using trypan blue after infecting $CD34^+$ healthy donor cells with EV or sh*CD99-L*entivirus.

Supplementary Figure 9:

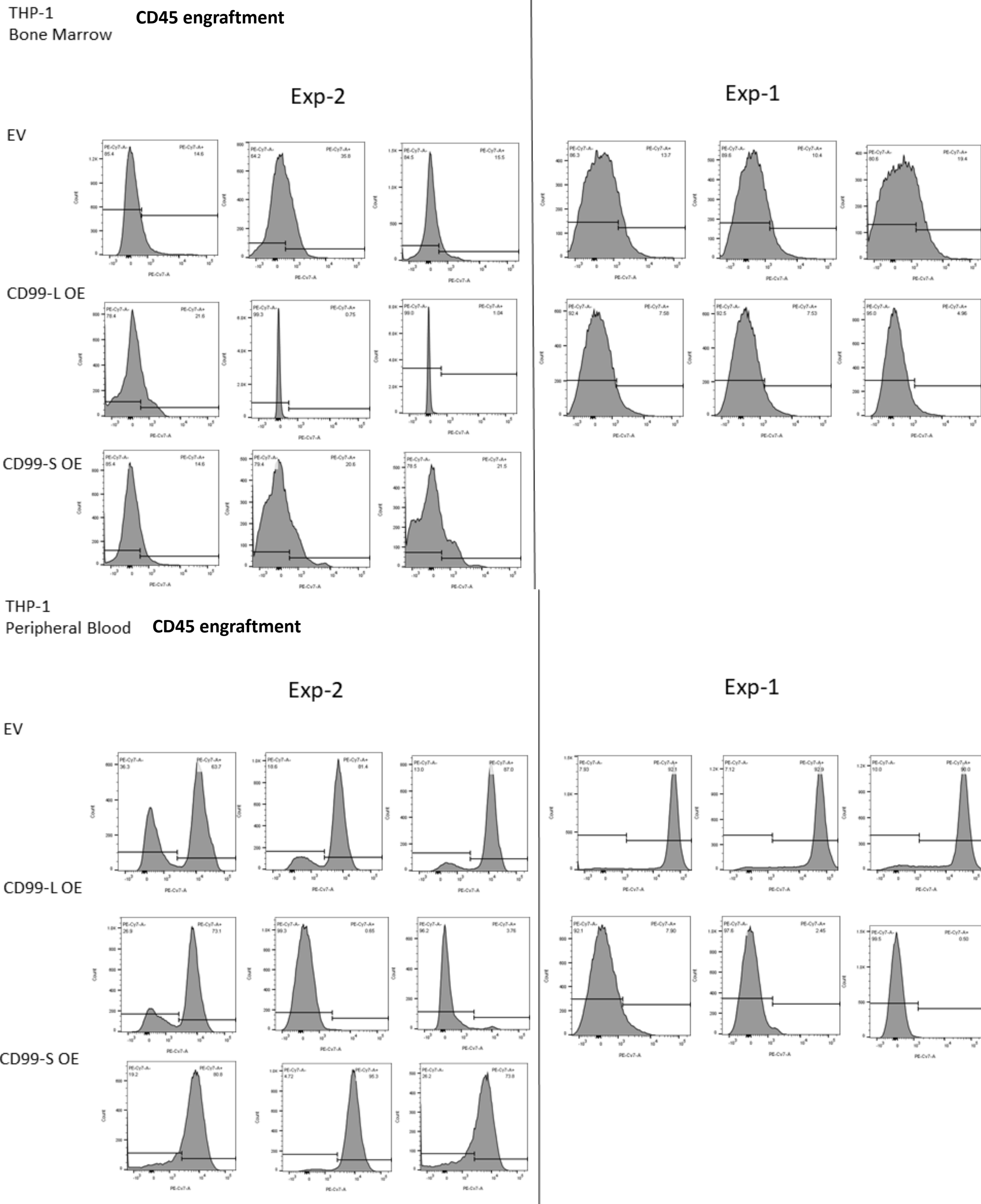


Figure S9: Flow cytometry plot data for huCD45 staining in the bone marrow and peripheral blood of mice engrafted with THP-1 cells expressing EV, *CD99*-L or *CD99*-S for two separate experiments.

Supplementary Figure 10

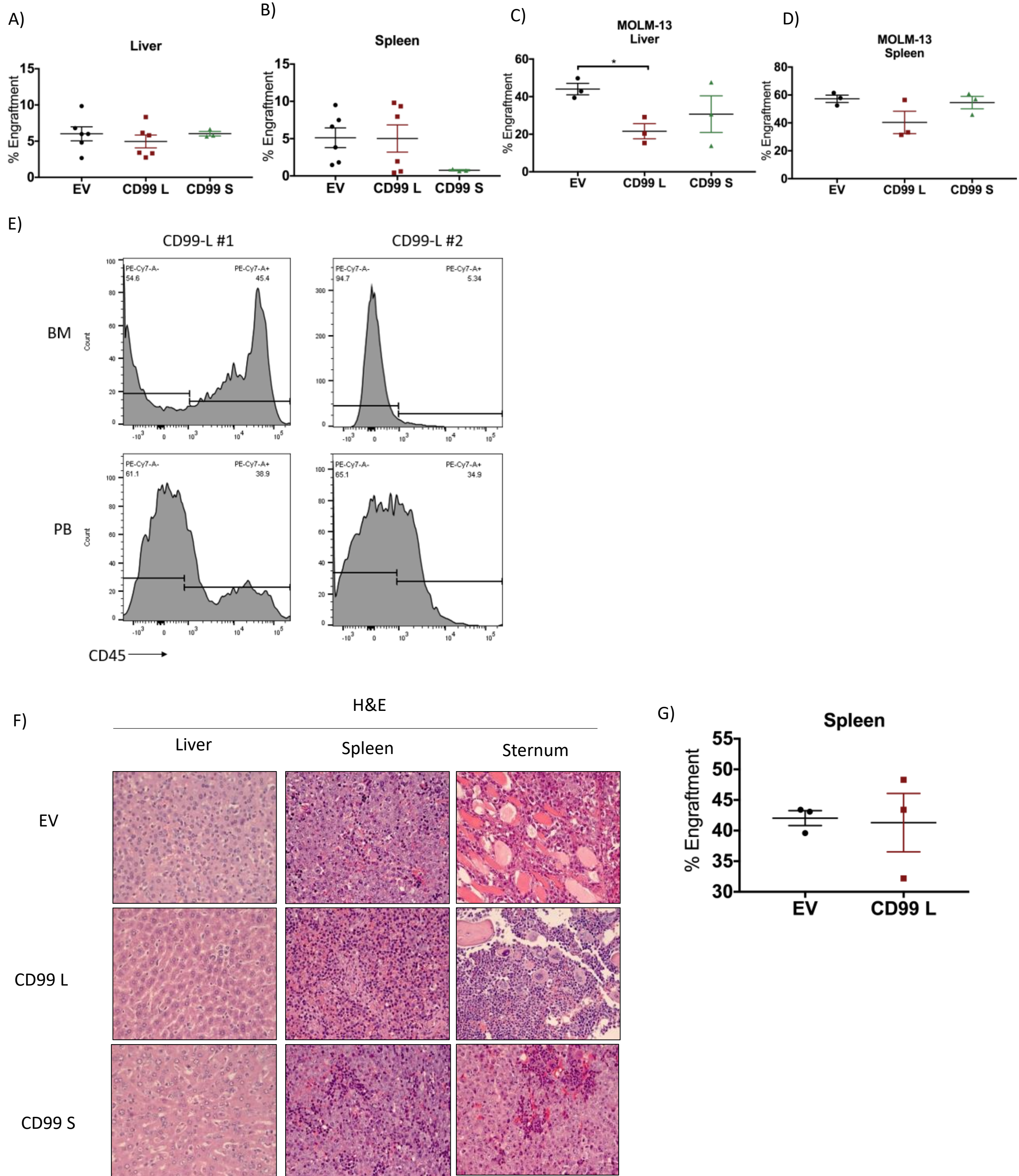


Figure S10: THP-1 (2.5X10^{6}) EV (n=6), CD99-L (n=6) or CD99-S (n=3) cells were implanted in mice and sacrificed when mice were sick. A-B) Liver and Spleen engraftment of THP-1 cells in

EV, CD99-L and CD99-S mice determined by quantitative analysis of huCD45$^+$ cells through flow cytometry. MOLM-13 ($2.5X10^6$) EV (n=3), CD99-L (n=3) or CD99-S (n=3) cells were implanted in mice and sacrificed when mice were sick. C-D) Liver and Spleen engraftment of MOLM-13 cells in EV, CD99-L and CD99-S mice determined by quantitative analysis of CD45$^+$ cells through flow cytometry. E) Flow cytometry analysis of huCD45 staining of peripheral blood and bone marrow cells in mice engrafted with MOLM-13 CD99-L cells and sacrificed on day 29 and 44 F) Representative H&E staining images of Liver, spleen and sternum of mice engrafted with MOLM-13 EV, CD99-L and CD99-S cells. (* p< 0.05, ** <0.005))

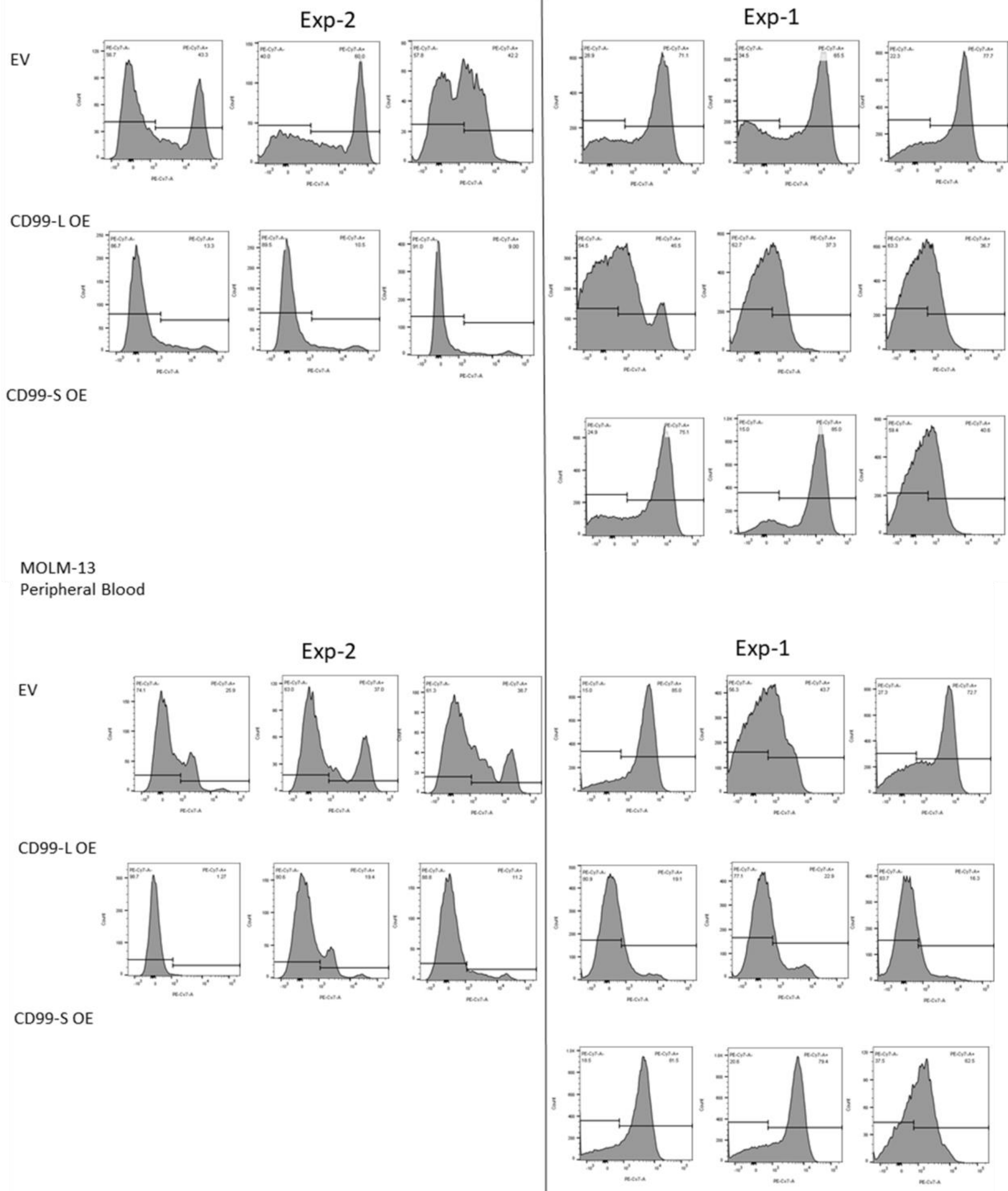


Figure S11. Flow cytometry plot data for huCD45 staining in the bone marrow and peripheral blood of mice engrafted with MOLM-13 EV, CD99-L or CD99-S for two separate experiments.

Supplementary Figure 12:

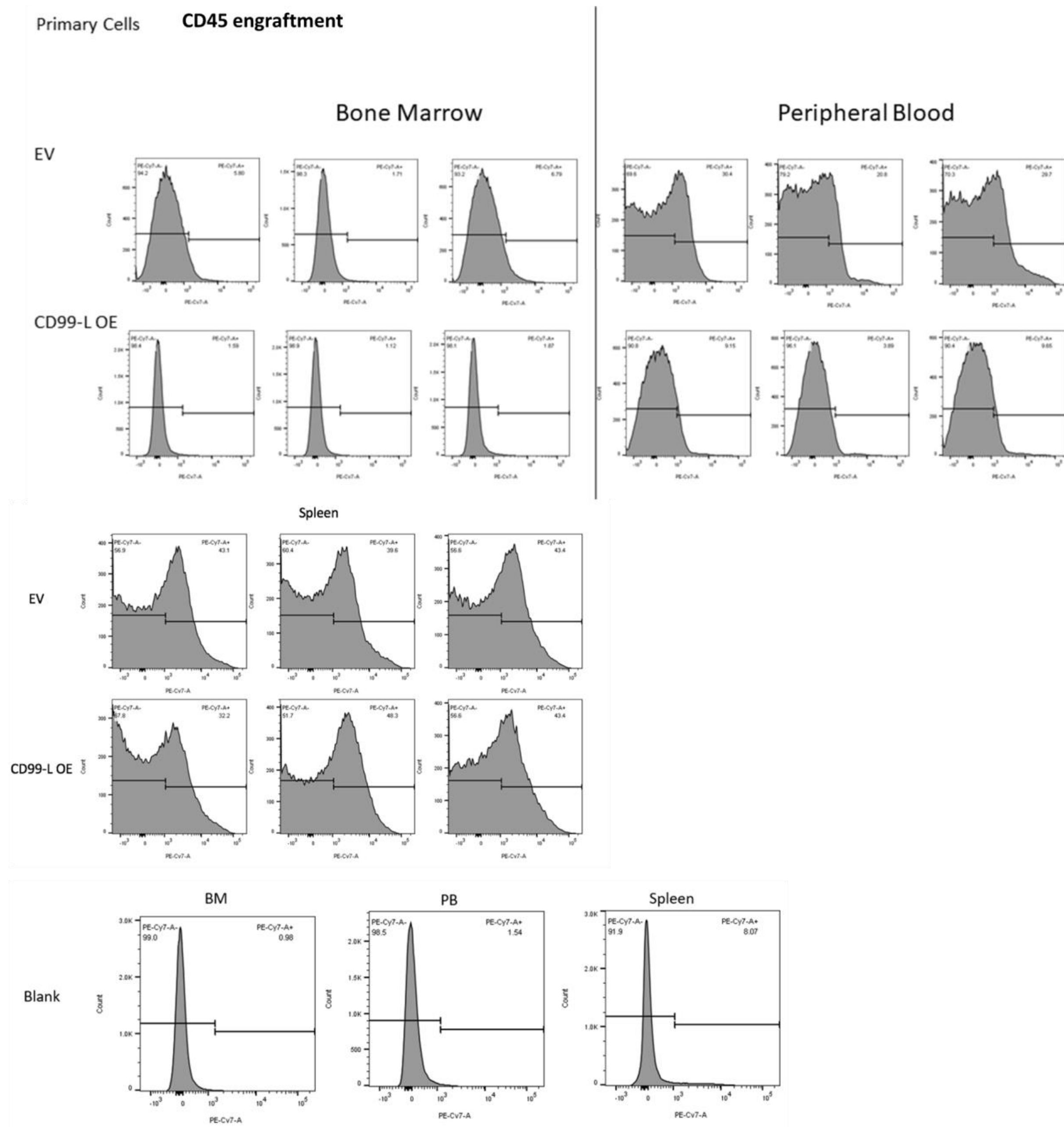


Figure S12. Flow cytometry plot data for huCD45 staining in the bone marrow, peripheral blood and spleen of mice engrafted with primary AML blasts infected with EV or CD99-L lentivirus. Blank mouse cells stained with huCD45 used as a negative control.

Supplementary Figure 13:

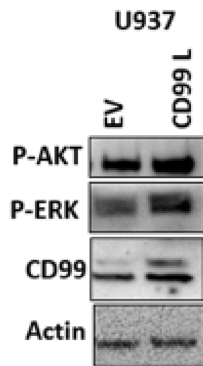


Figure S13. Western blot analysis of P-AKT and P-ERK in U937 cells infected with EV of CD99-L lentivirus.

References

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