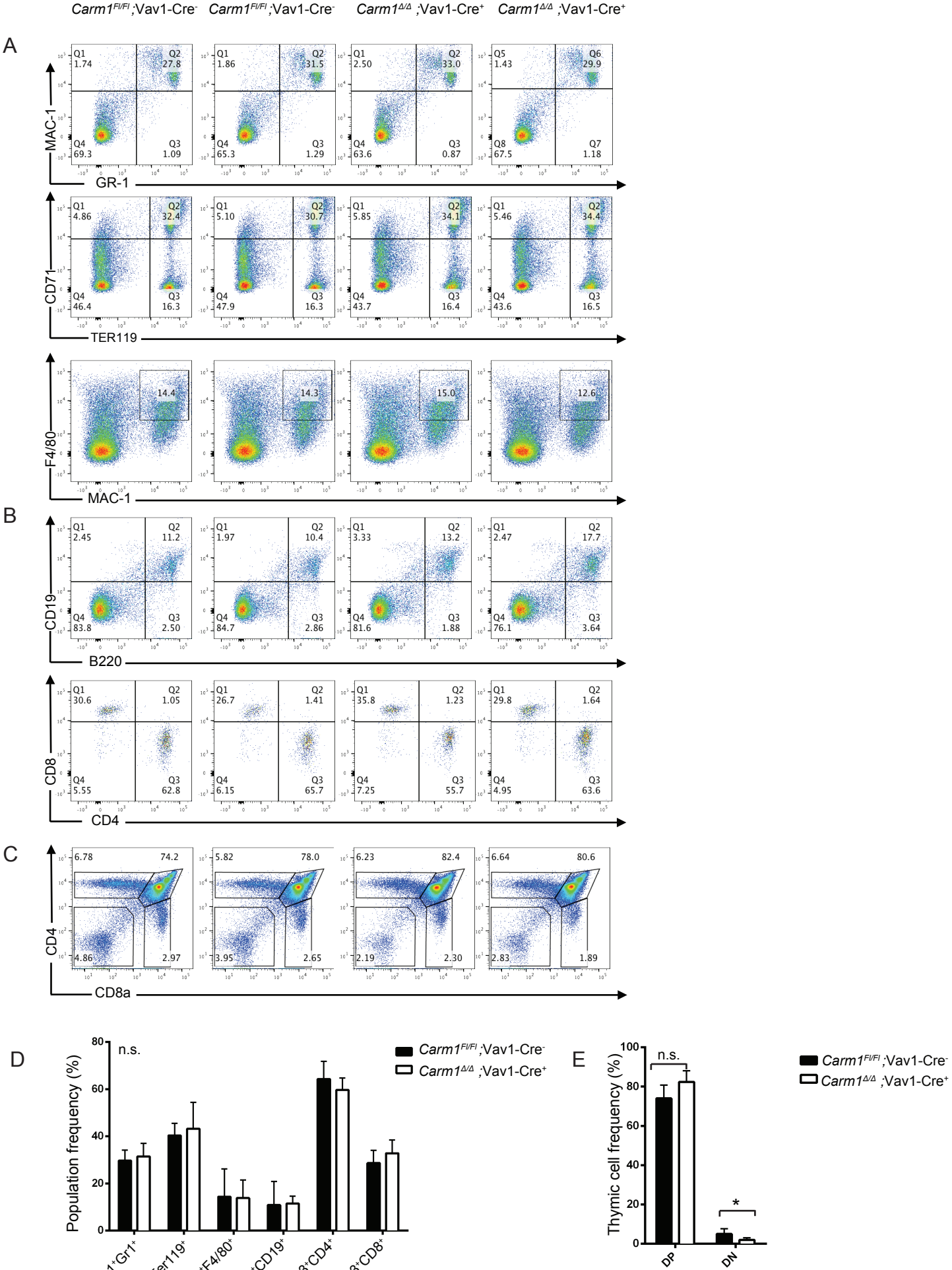
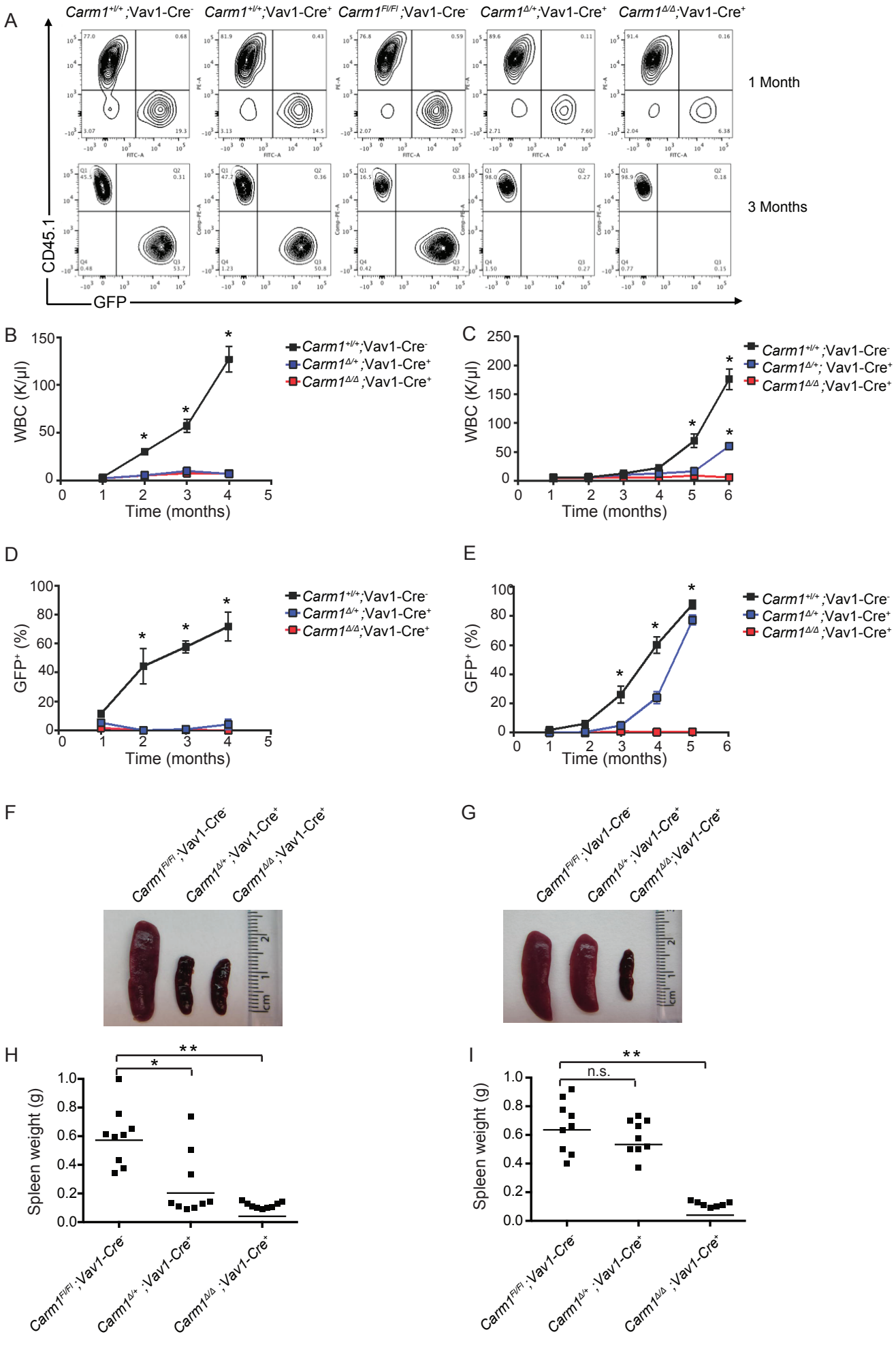

Carm1Fl/Fl;Vav1-Cre-
Carm1Fl/Fl;Vav1-Cre-
Carm1Δ/Δ;Vav1-Cre+
Carm1Δ/Δ;Vav1-Cre+
A
MAC-1
GR-1
Q1 1.74
Q2 27.8
Q4 69.3
Q3 1.09
Q1 1.86
Q2 31.5
Q4 65.3
Q3 1.29
Q1 2.50
Q2 33.0
Q4 63.6
Q3 0.87
Q5 1.43
Q6 29.9
Q8 67.5
Q7 1.18
CD71
TER119
Q1 4.86
Q2 32.4
Q4 46.4
Q3 16.3
Q1 5.10
Q2 30.7
Q4 47.9
Q3 16.3
Q1 5.85
Q2 34.1
Q4 43.7
Q3 16.4
Q1 5.46
Q2 34.4
Q4 43.6
Q3 16.5
F4/80
MAC-1
14.4
14.3
15.0
12.6
B
CD19
B220
Q1 2.45
Q2 11.2
Q4 83.8
Q3 2.50
Q1 1.97
Q2 10.4
Q4 84.7
Q3 2.86
Q1 3.33
Q2 13.2
Q4 81.6
Q3 1.88
Q1 2.47
Q2 17.7
Q4 76.1
Q3 3.64
CD8
CD4
Q1 30.6
Q2 1.05
Q4 5.55
Q3 62.8
Q1 26.7
Q2 1.41
Q4 6.15
Q3 65.7
Q1 35.8
Q2 1.23
Q4 7.25
Q3 55.7
Q1 29.8
Q2 1.64
Q4 4.95
Q3 63.6
C
CD4
CD8a
6.78
74.2
4.86
2.97
5.82
78.0
3.95
2.65
6.23
82.4
2.19
2.30
6.64
80.6
2.83
1.89
D
n.s.
Carm1Fl/Fl;Vav1-Cre-
Carm1Δ/Δ;Vav1-Cre+
Population frequency (%)
0
20
40
60
80
Mac1+Gr1+
Ter119+
Mac1+F4/80+
B220+CD19+
CD3+CD4+
CD3+CD8+
E
n.s.
*
Thymic cell frequency (%)
0
20
40
60
80
100
DP
DN
Carm1Fl/Fl;Vav1-Cre-
Carm1Δ/Δ;Vav1-Cre+

Figure S1: Flow cytometry analysis of mature bone marrow, spleen, and thymic hematopoietic populations, Related to Figure 2.

A) Representative flow cytometry analysis of mature myeloid, erythroid, and macrophage populations in the bone marrow of 1-year-old mice.
B) Representative flow cytometry analysis of mature B cell and T cell populations in the spleen of 1-year-old mice.
C) Representative flow cytometry analysis of T cell populations in the thymus of 1-year-old mice.
D) Percentages of *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^{+} and *Carm1*$^{Fl/Fl}$;Vav1-Cre^{-} age-matched mice for mature bone marrow and spleen populations. n=5, n.s.= no significant differences.
E) Average percentages of *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^{+} and *Carm1*$^{Fl/Fl}$;Vav1-Cre^{-} age-matched mice for thymic T-cell populations. n=5, n.s.= no significant difference. *p< 0.05. Bar graphs represent the mean ± SD. Statistics represent a Student's t-test for samples of unequal variance.


A
Carm1+l/+;Vav1-Cre-
Carm1+l/+;Vav1-Cre+
Carm1Fl/Fl;Vav1-Cre-
Carm1Δ/+;Vav1-Cre+
Carm1Δ/Δ;Vav1-Cre+
1 Month
3 Months
CD45.1
GFP
B
WBC (K/μl)
Time (months)
Carm1+l/+;Vav1-Cre-
Carm1Δ/+;Vav1-Cre+
Carm1Δ/Δ;Vav1-Cre+
C
WBC (K/μl)
Time (months)
Carm1+l/+;Vav1-Cre-
Carm1Δ/+; Vav1-Cre+
Carm1Δ/Δ;Vav1-Cre+
D
GFP+ (%)
Time (months)
Carm1+l/+;Vav1-Cre-
Carm1Δ/+;Vav1-Cre+
Carm1Δ/Δ;Vav1-Cre+
E
GFP+ (%)
Time (months)
Carm1+l/+;Vav1-Cre-
Carm1Δ/+;Vav1-Cre+
Carm1Δ/Δ;Vav1-Cre+
F
Carm1Fl/Fl;Vav1-Cre-
Carm1Δ/+;Vav1-Cre+
Carm1Δ/Δ;Vav1-Cre+
G
Carm1Fl/Fl;Vav1-Cre-
Carm1Δ/+;Vav1-Cre+
Carm1Δ/Δ;Vav1-Cre+
H
Spleen weight (g)
Carm1Fl/Fl;Vav1-Cre-
Carm1Δ/+;Vav1-Cre+
Carm1Δ/Δ;Vav1-Cre+
I
Spleen weight (g)
n.s.
Carm1Fl/Fl;Vav1-Cre-
Carm1Δ/+;Vav1-Cre+
Carm1Δ/Δ;Vav1-Cre+

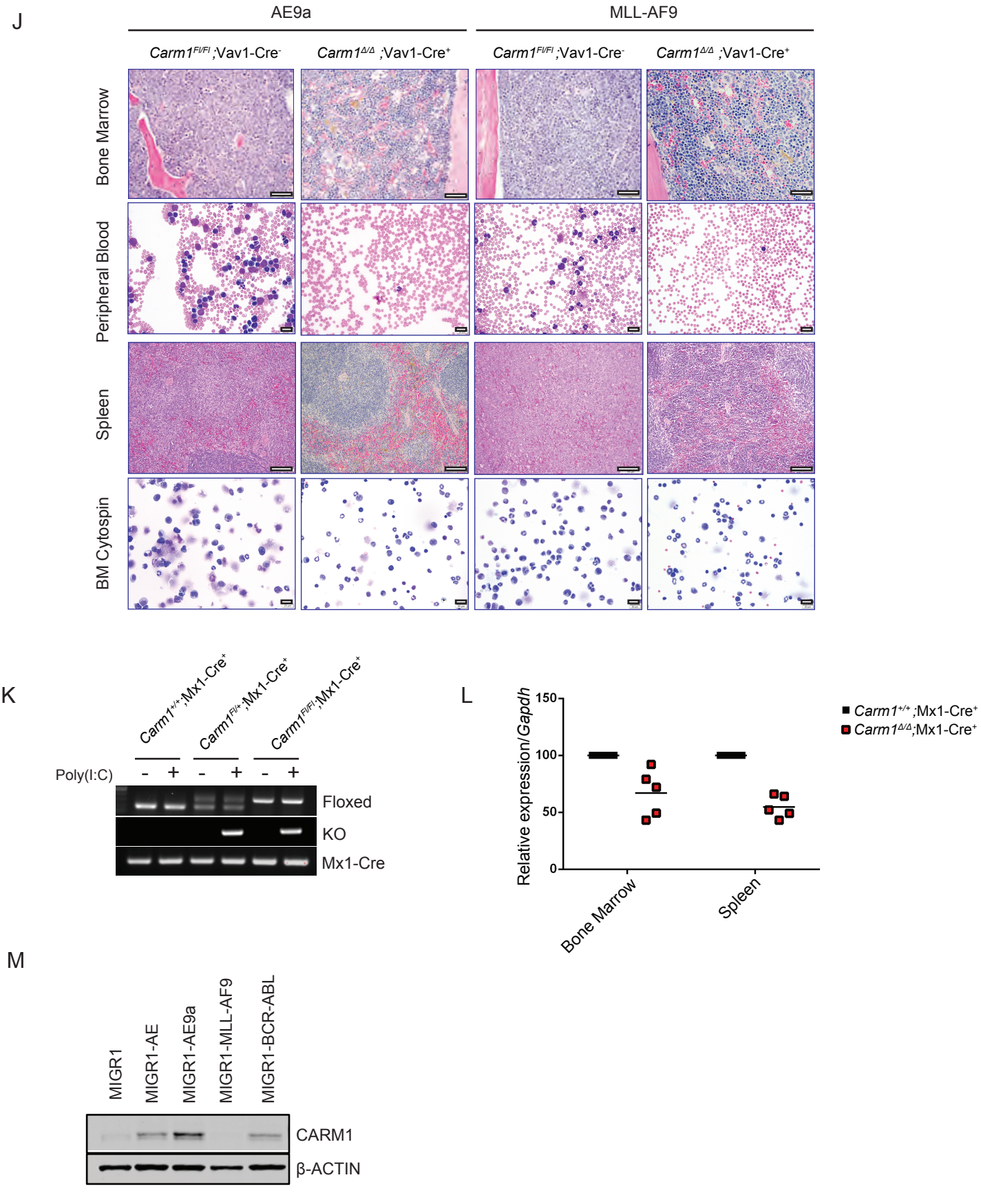

J
AE9a
MLL-AF9
Carm1Fl/Fl;Vav1-Cre-
Carm1Δ/Δ;Vav1-Cre+
Carm1Fl/Fl;Vav1-Cre-
Carm1Δ/Δ;Vav1-Cre+
Bone Marrow
Peripheral Blood
Spleen
BM Cytospin
K
Carm1+/+;Mx1-Cre+
Carm1Fl/+;Mx1-Cre+
Carm1Fl/Fl;Mx1-Cre+
Poly(I:C)
- + - + - +
Floxed
KO
Mx1-Cre
L
Relative expression/Gapdh
150
100
50
0
Bone Marrow
Spleen
Carm1+/+;Mx1-Cre+
Carm1Δ/Δ;Mx1-Cre+
M
MIGR1
MIGR1-AE
MIGR1-AE9a
MIGR1-MLL-AF9
MIGR1-BCR-ABL
CARM1
β-ACTIN

Figure S2: Characterization of AE9a and MLL-AF9 mice by flow cytometry, organ histology, and blood counts, Related to Figure 3.

A) Representative flow cytometry plot of GFP^+ and $CD45.1^+$ peripheral blood engraftment over time in the AE9a recipient initiation experiments.
B) Average AE9a C*arm1*$^{Fl/Fl}$;Vav1-Cre^-, *Carm1*$^{\Delta/+}$;Vav1-Cre^+, and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^+ recipient white blood cell count (WBC) over time. n = 10, *p<0.01
C) Average MLL-AF9 C*arm1*$^{Fl/Fl}$;Vav1-Cre^-, *Carm1*$^{\Delta/+}$;Vav1-Cre^+, and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^+ recipient white blood cell count (WBC) over time. n = 10, *p<0.01
D) Average AE9a C*arm1*$^{Fl/Fl}$;Vav1-Cre^-, *Carm1*$^{\Delta/+}$;Vav1-Cre^+, and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^+ recipient peripheral blood GFP^+ percentage over time. n = 10, *p<0.01
E) Average AE9a C*arm1*$^{Fl/Fl}$;Vav1-Cre^-, *Carm1*$^{\Delta/+}$;Vav1-Cre^+, and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^+ recipient peripheral blood GFP^+ percentage over time. n = 10, *p<0.01
F) Representative spleen size for AE9a C*arm1*$^{Fl/Fl}$;Vav1-Cre^-, *Carm1*$^{\Delta/+}$;Vav1-Cre^+, and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^+ recipient mice, three months post-transplant.
G) Representative spleen size for MLL-AF9 C*arm1*$^{Fl/Fl}$;Vav1-Cre^-, *Carm1*$^{\Delta/+}$;Vav1-Cre^+, and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^+ recipient mice, three months post-transplant.
H) Average spleen weight for AE9a C*arm1*$^{Fl/Fl}$;Vav1-Cre^-, *Carm1*$^{\Delta/+}$;Vav1-Cre^+, and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^+ recipient mice, three months post-transplant. n = 10, *p<0.01, **p<0.001
I) Average spleen weight for MLL-AF9 C*arm1*$^{Fl/Fl}$;Vav1-Cre^-, *Carm1*$^{\Delta/+}$;Vav1-Cre^+, and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^+ recipient mice, three months post-transplant. n =10, **p<0.001, n.s. = no significant differences
J) Histological analysis of C*arm1*$^{Fl/Fl}$;Vav1-Cre^- and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^+ recipient mice. Scale bars represent 20 μm for the bone marrow, peripheral blood smears and bone marrow cytospins. Scale bars for the spleen represents 100 μm (spleen and bone marrow) or 20 μm (peripheral blood smears and bone marrow cytospins)
K) PCR for the knockout of *Carm1* post poly(I:C) induction in C*arm1*$^{+/+}$;Mx1-Cre^+, *Carm1*$^{\Delta/+}$;Mx1-Cre^+, and *Carm1*$^{\Delta/\Delta}$;Mx1-Cre^+ maintenance recipient mice pre and post induction of Cre by poly(I:C).
L) Expression of *Carm1* in *Carm1*$^{\Delta/\Delta}$;Mx1-Cre^+ maintenance recipient mice normalized to *Gapdh* and compared to *Carm1*$^{+/+}$;Mx1-Cre^+ recipient mice.
M) Comparison of CARM1 protein expression in fetal liver cells transduced with the MIGR1 vector alone, or MIGR1 AML1-ETO (AE), AML1-ETO9a (AE9a), MLL-AF9, or BCR-ABL. β-ACTIN is shown as a loading control.

All error bars represent the mean ± SD. Statistics represent a Student's t-test for samples of unequal variance.

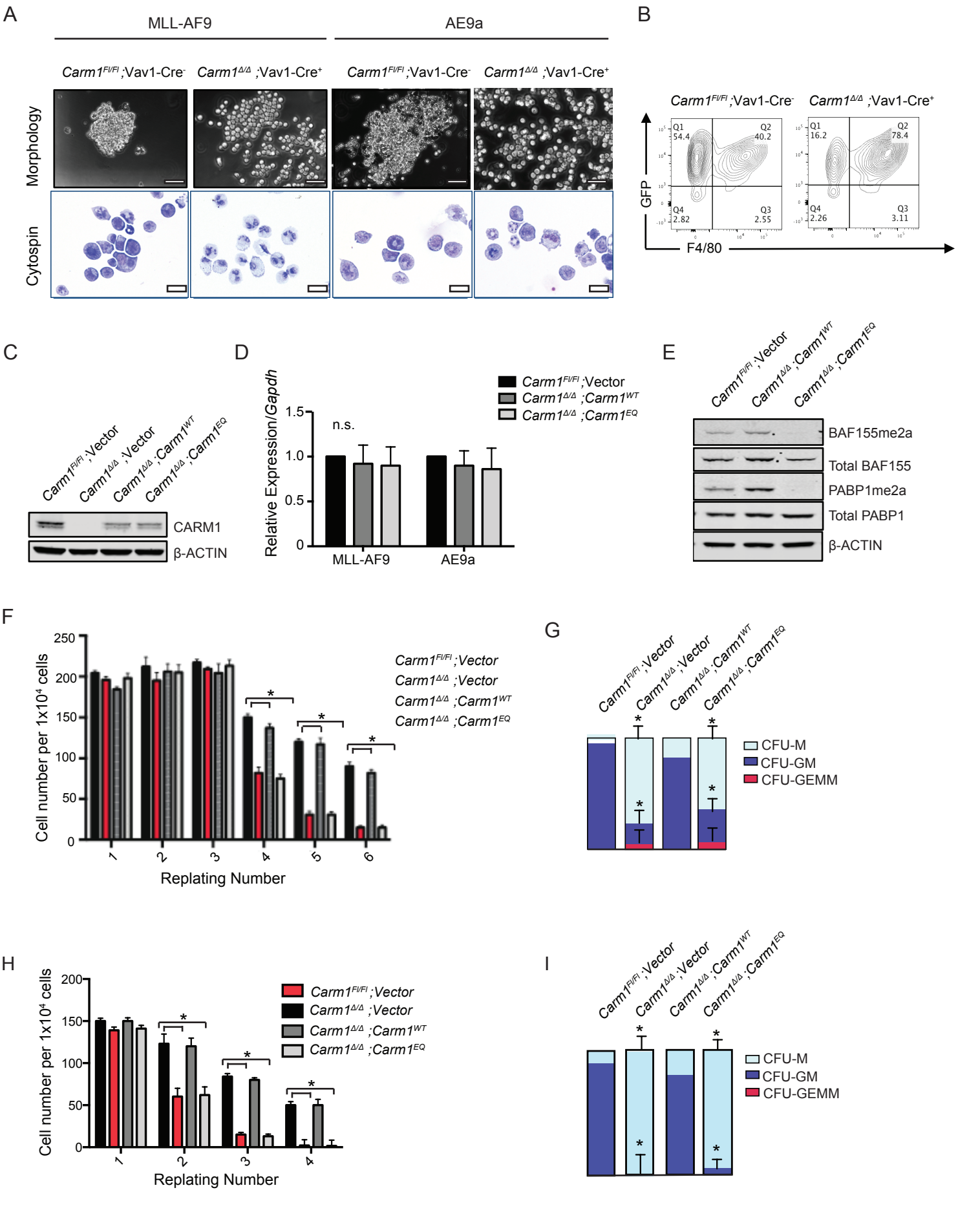

A
MLL-AF9
AE9a
Carm1FI/FI;Vav1-Cre-
Carm1Δ/Δ;Vav1-Cre+
Carm1FI/FI;Vav1-Cre-
Carm1Δ/Δ;Vav1-Cre+
Morphology
Cytospin
B
Carm1FI/FI;Vav1-Cre-
Carm1Δ/Δ;Vav1-Cre+
Q1 54.4
Q2 40.2
Q4 2.82
Q3 2.55
Q1 16.2
Q2 78.4
Q4 2.26
Q3 3.11
GFP
F4/80
C
Carm1FI/FI;Vector
Carm1Δ/Δ;Vector
Carm1Δ/Δ;Carm1WT
Carm1Δ/Δ;Carm1EQ
CARM1
β-ACTIN
D
Carm1FI/FI;Vector
Carm1Δ/Δ;Carm1WT
Carm1Δ/Δ;Carm1EQ
Relative Expression/Gapdh
1.5
1.0
0.5
0
n.s.
MLL-AF9
AE9a
E
Carm1FI/FI;Vector
Carm1Δ/Δ;Carm1WT
Carm1Δ/Δ;Carm1EQ
BAF155me2a
Total BAF155
PABP1me2a
Total PABP1
β-ACTIN
F
Cell number per 1x10⁴ cells
250
200
150
100
50
0
Carm1FI/FI;Vector
Carm1Δ/Δ;Vector
Carm1Δ/Δ;Carm1WT
Carm1Δ/Δ;Carm1EQ
1
2
3
4
5
6
Replating Number
G
Carm1FI/FI;Vector
Carm1Δ/Δ;Vector
Carm1Δ/Δ;Carm1WT
Carm1Δ/Δ;Carm1EQ
CFU-M
CFU-GM
CFU-GEMM
H
Cell number per 1x10⁴ cells
200
150
100
50
0
Carm1FI/FI;Vector
Carm1Δ/Δ;Vector
Carm1Δ/Δ;Carm1WT
Carm1Δ/Δ;Carm1EQ
1
2
3
4
Replating Number
I
Carm1FI/FI;Vector
Carm1Δ/Δ;Vector
Carm1Δ/Δ;Carm1WT
Carm1Δ/Δ;Carm1EQ
CFU-M
CFU-GM
CFU-GEMM

Figure S3: In vitro rescue of CARM1, Related to Figure 3.

A) Morphological analysis of colony formation (top) and colony cytospins (bottom) from MLL-AF9 and AE9a C*arm1*$^{Fl/Fl}$;Vav1-Cre^{-} and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^{+} fetal liver cells cultured in M3434 methylcellulose. Scale bar (top) = 50 μm, Scale bar (bottom)= 20 μm

B) Representative flow cytometry plot for GFP and the macrophage cell differentiation marker F4/80 in the AE9a C*arm1*$^{Fl/Fl}$;Vav1-Cre^{-} and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^{+} fetal liver cells after the second replating.

C) CARM1 protein levels following tranduction of C*arm1*$^{Fl/Fl}$;Vav1-Cre^{-} and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^{+} fetal liver cells with either vector alone, *Carm1*^{WT} or *Carm1*^{EQ}. β-ACTIN is shown as a loading control.

D) *Carm1* mRNA levels following transduction of C*arm1*$^{Fl/Fl}$;Vav1-Cre^{-} and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^{+} fetal liver cells with either vector alone, *Carm1*^{WT} or *Carm1*^{EQ}, normalized to *Gapdh.* n.s.= no significant differences

E) Western blot analysis of CARM1 enzymatic activity following transduction of C*arm1*$^{Fl/Fl}$;Vav1-Cre^{-} and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^{+} fetal liver cells with either vector alone, *Carm1*^{WT} or *Carm1*^{EQ}. β-ACTIN is shown as a loading control.

F) CFU replating assay of MLL-AF9 expressing C*arm1*$^{Fl/Fl}$;Vav1-Cre^{-} and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^{+} fetal liver cells transduced with either vector alone, *Carm1*^{WT} or *Carm1*^{EQ}. *p< 0.01

G) Average colony percentage of MLL-AF9 expressing C*arm1*$^{Fl/Fl}$;Vav1-Cre^{-} and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^{+} fetal liver cells transduced with either vector alone, *Carm1*^{WT} or *Carm1*^{EQ}, scoring CFU-M, CFU-GM, and CFU-GEMM from triplicate CFU experiments after replating 4. *p< 0.01

H) CFU replating assay of AE9a expressing C*arm1*$^{Fl/Fl}$;Vav1-Cre^{-} and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^{+} fetal liver cells transduced with either vector alone, *Carm1*^{WT} or *Carm1*^{EQ}. Error bars represent the mean ± SD for three independent experiments.*p< 0.01

I) Average colony percentage of AE9a expressing C*arm1*$^{Fl/Fl}$;Vav1-Cre^{-} and *Carm1*$^{\Delta/\Delta}$;Vav1-Cre^{+} fetal liver cells transduced with either vector alone, *Carm1*^{WT} or *Carm1*^{EQ} scoring CFU-M, CFU-GM, and CFU-GEMM from triplicate CFU experiments after replating 4. *p< 0.01

All error bars represent the mean ± SD. Statistics represent a Student's t-test for samples of unequal variance.

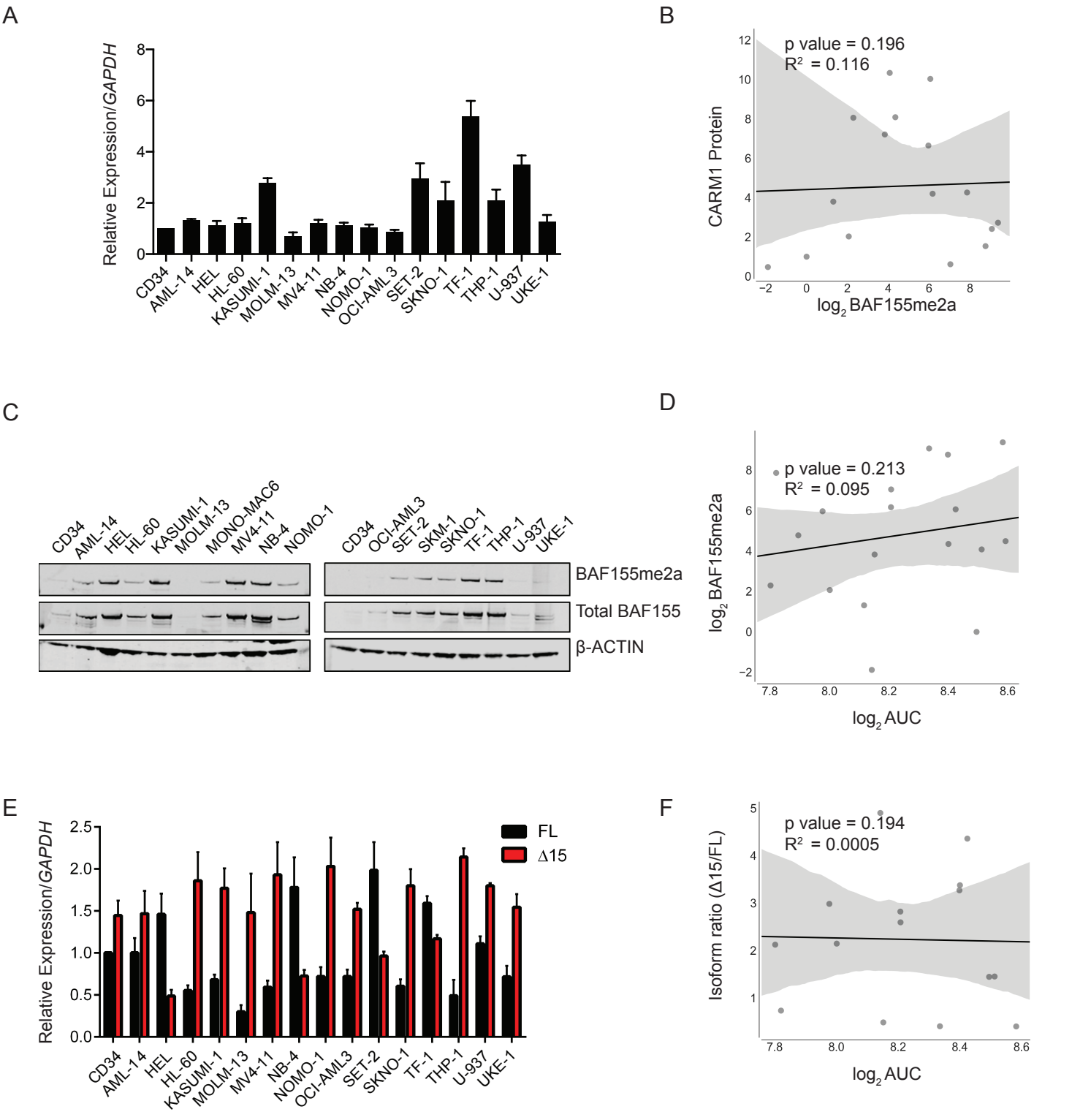

A
Relative Expression/GAPDH
CD34
AML-14
HEL
HL-60
KASUMI-1
MOLM-13
MV4-11
NB-4
NOMO-1
OCI-AML3
SET-2
SKNO-1
TF-1
THP-1
U-937
UKE-1
B
p value = 0.196
R² = 0.116
CARM1 Protein
log2 BAF155me2a
C
MONO-MAC6
SKM-1
BAF155me2a
Total BAF155
β-ACTIN
D
p value = 0.213
R² = 0.095
log2 BAF155me2a
log2 AUC
E
FL
Δ15
Relative Expression/GAPDH
F
p value = 0.194
R² = 0.0005
Isoform ratio (Δ15/FL)
log2 AUC

Figure S4. Comparison of CARM1 mRNA, isoform abundance, and target methylation in AML cell lines, Related to Figure 5.

A) Quantitative RT-PCR for *CARM1* mRNA expression in 16 AML cell lines and $CD34^+$ cells. Error bars represent the mean ± SD of three independent experiments. Statistics represent a Student's t-test for samples of unequal variance.

B) Quantification of $\log_2$ BAF155me2a compared to CARM1 protein expression in 16 AML cell lines and $CD34^+$ cells. Spearman correlation and linear regression: p value = 0.196, $R^2 = 0.116$

C) Representative western blot showing levels of methylated BAF155 and total BAF155 in 18 AML cells lines and $CD34^+$ cells. β-ACTIN is shown as a loading control.

D) Comparison of the $\log_2$ AUC for 18 cell lines and $CD34^+$ cells treated with EPZ025654 for 10 days vs. $\log_2$ BAF155me2a. Spearman correlation and linear regression: p value = 0.213, $R^2 = 0.1095$

E) Quantification of *CARM1* full length (FL) and *CARM1* lacking exon 15 (Δ15) by semi quantitative RT-PCR. Error bars represent the mean ± SD from three independent samples from each cell line. Statistics represent a Student's t-test for samples of unequal variance.

F) Comparison of the $\log_2$ AUC for 18 cell lines and $CD34^+$ cells treated with EPZ025654 for 10 days vs. isoform ratios. Spearman correlation and linear regression: p value = 0.194, $R^2 = 0.0005$

Table S2. Top 100 differentially regulated genes in AML cell lines, Related to Figure 5.

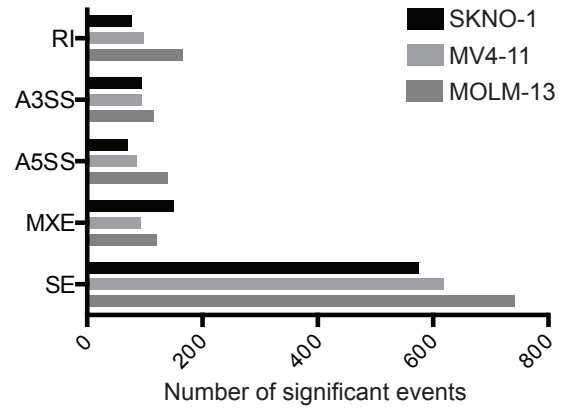
Gene ID	HGNC symbol	p value	Adjusted p value	log_2FoldChange
ENSG00000166803_KIAA0101	*KIAA0101*	2.84E-09	9.99E-07	-2.34
ENSG00000124635_HIST1H2BJ	*HIST1H2BJ*	6.63E-10	2.88E-07	-2.21
ENSG00000142453_CARM1	*CARM1*	2.23E-25	5.09E-21	-2.21
ENSG00000196584_XRCC2	*XRCC2*	3.26E-18	1.86E-14	-2.07
ENSG00000065328_MCM10	*MCM10*	2.87E-08	6.78E-06	-2.01
ENSG00000159259_CHAF1B	*CHAF1B*	1.00E-08	2.85E-06	-1.95
ENSG00000128408_RIBC2	*RIBC2*	2.45E-17	1.12E-13	-1.89
ENSG00000276043_UHRF1	*UHRF1*	2.54E-12	2.23E-09	-1.88
ENSG00000127564_PKMYT1	*PKMYT1*	1.06E-12	1.05E-09	-1.87
ENSG00000256663_RP11-424C20.2	*RP11-424C20.2*	4.49E-10	2.14E-07	-1.84
ENSG00000171320_ESCO2	*ESCO2*	6.83E-10	2.89E-07	-1.83
ENSG00000121211_MND1	*MND1*	2.35E-13	2.99E-10	-1.83
ENSG00000215784_FAM72D	*FAM72D*	1.68E-13	2.37E-10	-1.81
ENSG00000092470_WDR76	*WDR76*	1.87E-08	4.70E-06	-1.80
ENSG00000159055_MIS18A	*MIS18A*	3.61E-14	6.35E-11	-1.77
ENSG00000165490_DDIAS	*DDIAS*	3.38E-08	7.74E-06	-1.73
ENSG00000168078_PBK	*PBK*	8.20E-09	2.44E-06	-1.71
ENSG00000104738_MCM4	*MCM4*	8.81E-22	1.01E-17	-1.71
ENSG00000119969_HELLS	*HELLS*	2.41E-15	6.13E-12	-1.71
ENSG00000188610_FAM72B	*FAM72B*	5.32E-10	2.48E-07	-1.63
ENSG00000105011_ASF1B	*ASF1B*	3.70E-09	1.26E-06	-1.59
ENSG00000076003_MCM6	*MCM6*	4.81E-16	1.83E-12	-1.57
ENSG00000137812_KNL1	*KNL1*	9.85E-10	3.69E-07	-1.56
ENSG00000164109_MAD2L1	*MAD2L1*	5.34E-11	3.13E-08	-1.56
ENSG00000060982_BCAT1	*BCAT1*	2.57E-11	1.73E-08	-1.56
ENSG00000123219_CENPK	*CENPK*	1.86E-08	4.70E-06	-1.54
ENSG00000132436_FIGNL1	*FIGNL1*	4.86E-15	1.01E-11	-1.51
ENSG00000277224_HIST1H2BF	*HIST1H2BF*	1.05E-08	2.93E-06	-1.50
ENSG00000175279_CENPS	*CENPS*	1.21E-08	3.29E-06	-1.47
ENSG00000091651_ORC6	*ORC6*	3.87E-09	1.30E-06	-1.45
ENSG00000196550_FAM72A	*FAM72A*	6.14E-13	6.39E-10	-1.44
ENSG00000012048_BRCA1	*BRCA1*	6.42E-15	1.22E-11	-1.44
ENSG00000111445_RFC5	*RFC5*	1.76E-13	2.37E-10	-1.44
ENSG00000198056_PRIM1	*PRIM1*	3.06E-10	1.52E-07	-1.43
ENSG00000146918_NCAPG2	*NCAPG2*	2.80E-12	2.37E-09	-1.43
ENSG00000162062_C16orf59	*C16orf59*	1.61E-08	4.24E-06	-1.39
ENSG00000122952_ZWINT	*ZWINT*	5.36E-09	1.78E-06	-1.35
ENSG00000164087_POC1A	*POC1A*	2.93E-13	3.53E-10	-1.33
ENSG00000167325_RRM1	*RRM1*	9.71E-16	2.77E-12	-1.31
ENSG00000058804_NDC1	*NDC1*	1.11E-11	8.74E-09	-1.31
ENSG00000109881_CCDC34	*CCDC34*	1.03E-11	8.38E-09	-1.30
ENSG00000112118_MCM3	*MCM3*	6.06E-09	1.90E-06	-1.30
ENSG00000177602_GSG2	*GSG2*	9.96E-09	2.85E-06	-1.29
ENSG00000097046_CDC7	*CDC7*	1.83E-11	1.27E-08	-1.28
ENSG00000153044_CENPH	*CENPH*	2.47E-10	1.25E-07	-1.26

Gene ID	HGNC symbol	p value	Adjusted p value	log_2FoldChange
ENSG00000156802_ATAD2	*ATAD2*	1.64E-10	8.52E-08	-1.25
ENSG00000149636_DSN1	*DSN1*	3.05E-15	6.98E-12	-1.24
ENSG00000112029_FBXO5	*FBXO5*	1.33E-08	3.54E-06	-1.22
ENSG00000213585_VDAC1	*VDAC1*	1.13E-09	4.09E-07	-1.21
ENSG00000197299_BLM	*BLM*	1.51E-11	1.12E-08	-1.21
ENSG00000104889_RNASEH2A	*RNASEH2A*	9.14E-10	3.52E-07	-1.21
ENSG00000112312_GMNN	*GMNN*	2.07E-12	1.97E-09	-1.20
ENSG00000158169_FANCC	*FANCC*	5.23E-14	8.54E-11	-1.19
ENSG00000040275_SPDL1	*SPDL1*	9.24E-10	3.52E-07	-1.18
ENSG00000146263_MMS22L	*MMS22L*	1.01E-08	2.85E-06	-1.18
ENSG00000144554_FANCD2	*FANCD2*	1.84E-08	4.70E-06	-1.13
ENSG00000136824_SMC2	*SMC2*	1.73E-11	1.24E-08	-1.13
ENSG00000176974_SHMT1	*SHMT1*	4.30E-11	2.66E-08	-1.10
ENSG00000077514_POLD3	*POLD3*	3.82E-19	2.91E-15	-1.09
ENSG00000178966_RMI1	*RMI1*	7.55E-16	2.47E-12	-1.08
ENSG00000166881_NEMP1	*NEMP1*	5.87E-09	1.88E-06	-1.06
ENSG00000120802_TMPO	*TMPO*	6.06E-11	3.47E-08	-1.05
ENSG00000156876_SASS6	*SASS6*	8.69E-10	3.52E-07	-1.05
ENSG00000106399_RPA3	*RPA3*	1.20E-10	6.36E-08	-1.04
ENSG00000075131_TIPIN	*TIPIN*	2.67E-11	1.75E-08	-1.01
ENSG00000080839_RBL1	*RBL1*	1.22E-13	1.86E-10	-0.99
ENSG00000172009_THOP1	*THOP1*	1.26E-08	3.40E-06	-0.98
ENSG00000101868_POLA1	*POLA1*	6.16E-10	2.76E-07	-0.97
ENSG00000198826_ARHGAP11A	*ARHGAP11A*	8.96E-10	3.52E-07	-0.96
ENSG00000184445_KNTC1	*KNTC1*	3.60E-10	1.75E-07	-0.96
ENSG00000106462_EZH2	*EZH2*	4.53E-11	2.73E-08	-0.95
ENSG00000204899_MZT1	*MZT1*	2.10E-08	5.16E-06	-0.93
ENSG00000014138_POLA2	*POLA2*	3.55E-11	2.26E-08	-0.91
ENSG00000213390_ARHGAP19	*ARHGAP19*	6.19E-09	1.91E-06	-0.88
ENSG00000166483_WEE1	*WEE1*	2.97E-08	6.93E-06	-0.85
ENSG00000138780_GSTCD	*GSTCD*	2.36E-12	2.16E-09	-0.84
ENSG00000105486_LIG1	*LIG1*	1.19E-10	6.36E-08	-0.83
ENSG00000171208_NETO2	*NETO2*	1.92E-08	4.77E-06	-0.81
ENSG00000079616_KIF22	*KIF22*	3.45E-09	1.19E-06	-0.79
ENSG00000166147_FBN1	*FBN1*	1.82E-09	6.50E-07	-0.79
ENSG00000163781_TOPBP1	*TOPBP1*	3.89E-13	4.45E-10	-0.77
ENSG00000111775_COX6A1	*COX6A1*	5.51E-10	2.52E-07	-0.71
ENSG00000120539_MASTL	*MASTL*	2.24E-08	5.38E-06	-0.69
ENSG00000198924_DCLRE1A	*DCLRE1A*	1.14E-08	3.14E-06	-0.64
ENSG00000129484_PARP2	*PARP2*	5.93E-09	1.88E-06	-0.64
ENSG00000232653_GOLGA8N	*GOLGA8N*	6.92E-09	2.11E-06	-0.57
ENSG00000143315_PIGM	*PIGM*	2.16E-08	5.25E-06	-0.44
ENSG00000142687_KIAA0319L	*KIAA0319L*	3.22E-08	7.43E-06	0.50
ENSG00000060971_ACAA1	*ACAA1*	2.30E-08	5.48E-06	0.57
ENSG00000112679_DUSP22	*DUSP22*	5.16E-13	5.62E-10	0.70

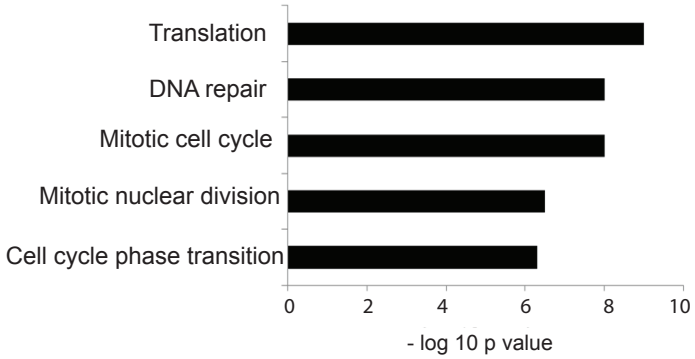
Gene ID	HGNC symbol	p value	Adjusted p value	log_2FoldChange
ENSG00000243749_TMEM35B	*TMEM35B*	8.82E-10	3.52E-07	0.87
ENSG00000163956_LRPAP1	*LRPAP1*	8.43E-09	2.47E-06	0.88
ENSG00000161011_SQSTM1	*SQSTM1*	1.11E-09	4.09E-07	0.89
ENSG00000167996_FTH1	*FTH1*	8.49E-10	3.52E-07	1.41
ENSG00000177606_JUN	*JUN*	6.68E-10	2.88E-07	1.58
ENSG00000185909_KLHDC8B	*KLHDC8B*	7.14E-09	2.15E-06	2.04
ENSG00000100292_HMOX1	*HMOX1*	5.92E-09	1.88E-06	2.33
ENSG00000244734_HBB	*HBB*	1.26E-11	9.57E-09	6.09

* Red font represents gene associated with Gene Ontology Term ‘Cell Cycle Progression’*

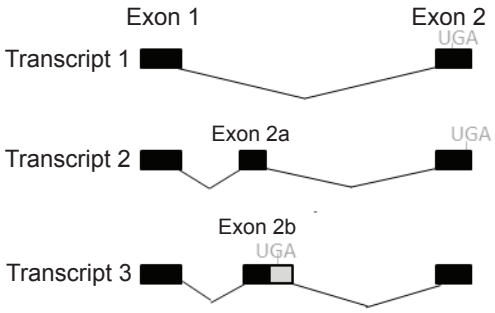
A


SKNO-1
MV4-11
MOLM-13
RI
A3SS
A5SS
MXE
SE
0
200
400
600
800
Number of significant events

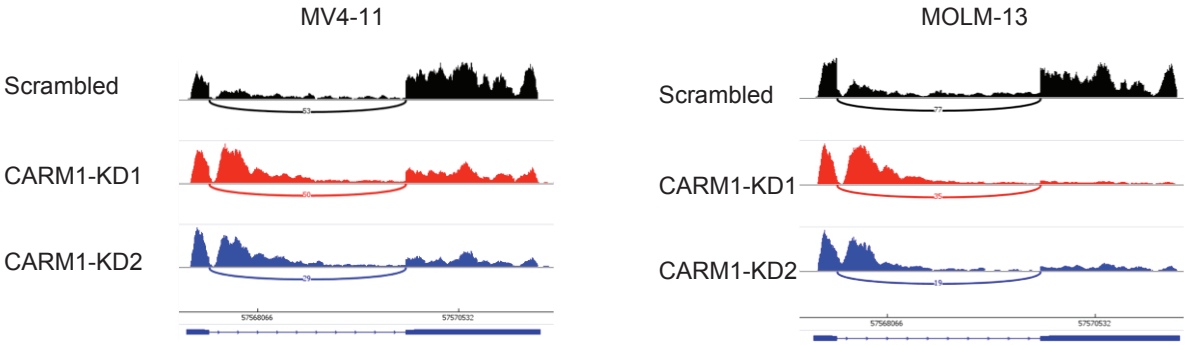
B


Translation
DNA repair
Mitotic cell cycle
Mitotic nuclear division
Cell cycle phase transition
0
2
4
6
8
10
- log 10 p value

C


Exon 1
Exon 2
UGA
Transcript 1
Exon 2a
UGA
Transcript 2
Exon 2b
UGA
Transcript 3

D


MV4-11
MOLM-13
Scrambled
CARM1-KD1
CARM1-KD2
Scrambled
CARM1-KD1
CARM1-KD2

E

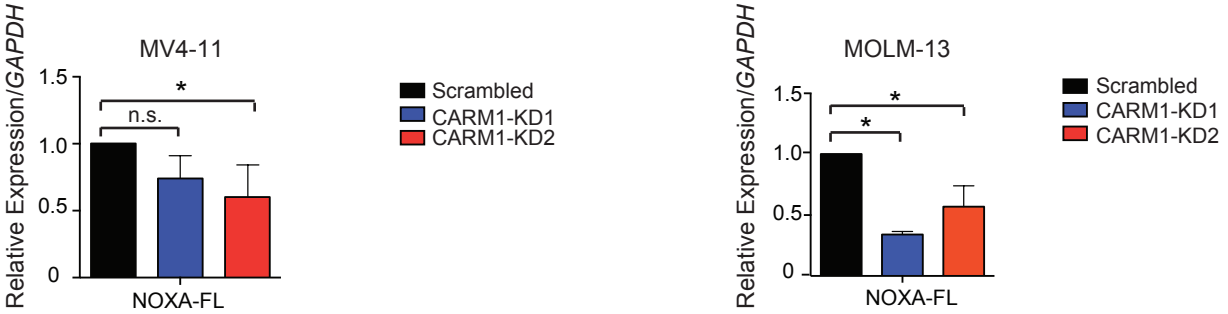
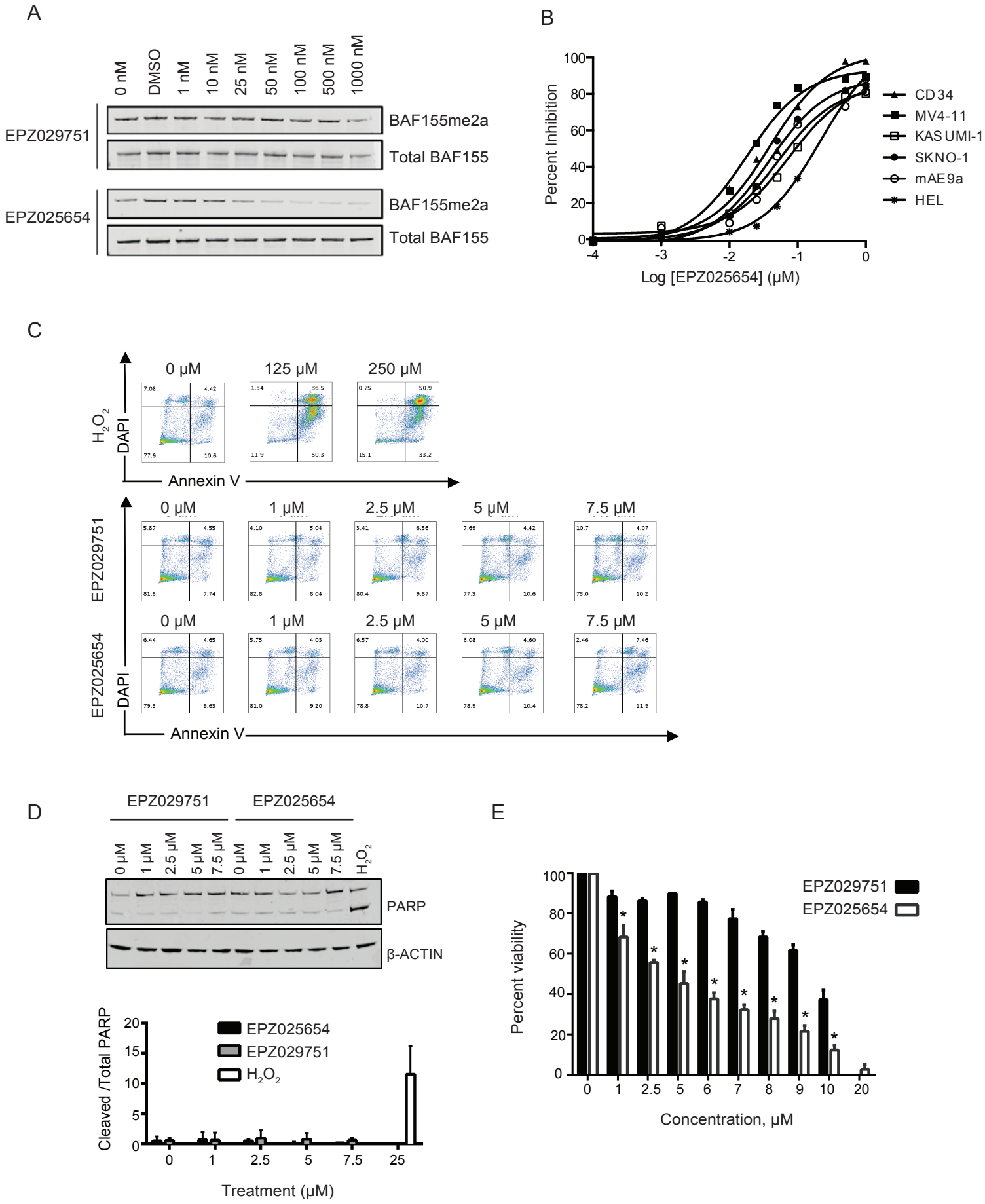
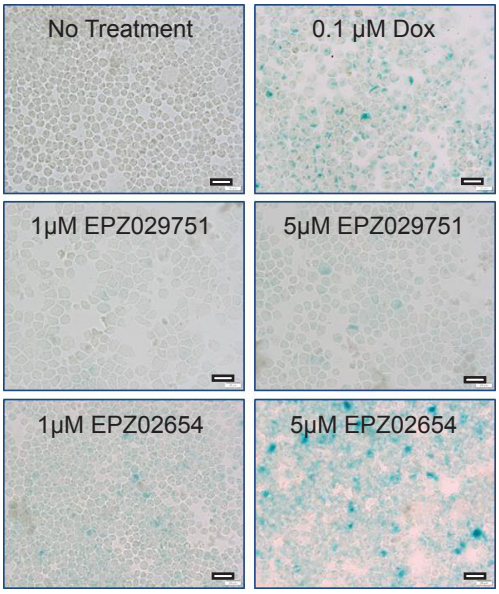

MV4-11
Relative Expression/GAPDH
1.5
1.0
0.5
0
n.s.
*
NOXA-FL
Scrambled
CARM1-KD1
CARM1-KD2
MOLM-13
Relative Expression/GAPDH
1.5
1.0
0.5
0
*
*
NOXA-FL
Scrambled
CARM1-KD1
CARM1-KD2

Figure S5: Characterization of alternative splicing in leukemia cell lines in response to CARM1 knockdown, Related to Figure 5.

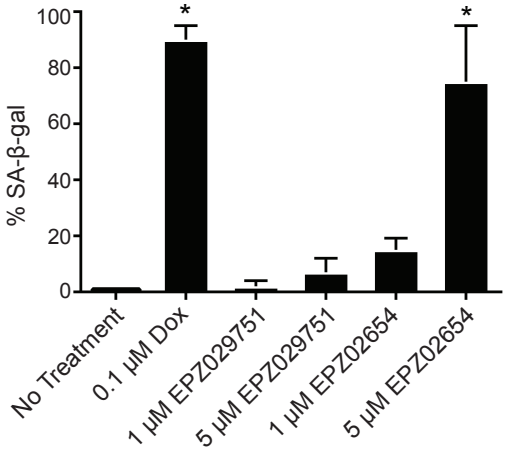
A) Number of significant alternative splicing events for the SKNO-1, MV4-11, and MOLM-13 cells lines. Events are annotated as Retained Intron (RI), Alternative 3' Splice Site (A3SS), Alternative 5' Splice Site (A5SS), Mutually Exclusive Exons (MXE), or Skipped Exon (SE). Events were considered significant if present in both CARM1 KD samples and not the scrambled control for each cell line.
B) Gene ontology (GO) analysis of significant skipped exon events common to all three cell lines. Bars represent the –log10 p value.
C) Schematic of PMAIP transcripts.
D) Sashimi plot for PMAIP1/NOXA in Scrambled, CARM1-KD1, or CARM1-KD2 MV4-11 (left) and MOLM-13 (right) cells.
E) Full length *PMAIP/NOXA* mRNA expression in Scrambled, CARM1-KD1, or CARM1-KD2 sorted MV4-11 (left) and MOLM-13 (right) cells, normalized to *GAPDH*. Results represent the mean ± SD of three independent experiments. n=3, *p<0.05 Statistics represent a Student's t-test for samples of unequal variance.


A
0 nM
DMSO
1 nM
10 nM
25 nM
50 nM
100 nM
500 nM
1000 nM
EPZ029751
BAF155me2a
Total BAF155
EPZ025654
BAF155me2a
Total BAF155
B
Percent Inhibition
Log [EPZ025654] (μM)
CD34
MV4-11
KASUMI-1
SKNO-1
mAE9a
HEL
C
0 μM
125 μM
250 μM
H2O2
DAPI
Annexin V
EPZ029751
0 μM
1 μM
2.5 μM
5 μM
7.5 μM
EPZ025654
DAPI
Annexin V
D
EPZ029751
EPZ025654
0 μM
1 μM
2.5 μM
5 μM
7.5 μM
H2O2
PARP
β-ACTIN
Cleaved /Total PARP
EPZ025654
EPZ029751
H2O2
Treatment (μM)
E
Percent viability
EPZ029751
EPZ025654
Concentration, μM

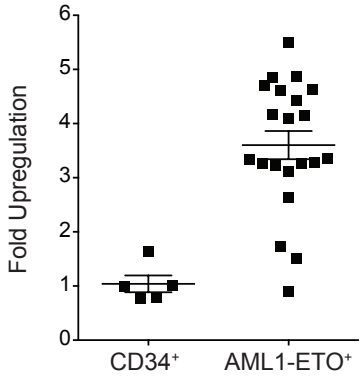
F


No Treatment
0.1 μM Dox
1μM EPZ029751
5μM EPZ029751
1μM EPZ02654
5μM EPZ02654

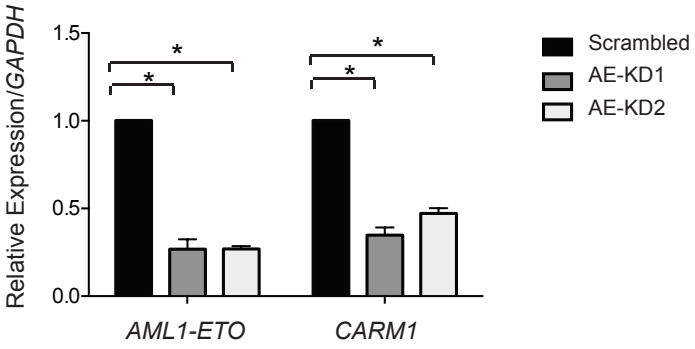
G


% SA-β-gal
100
80
60
40
20
0
*
*
No Treatment
0.1 μM Dox
1 μM EPZ029751
5 μM EPZ029751
1 μM EPZ02654
5 μM EPZ02654

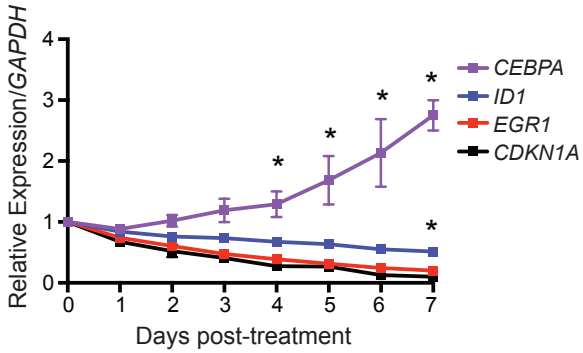
H


Fold Upregulation
6
5
4
3
2
1
0
CD34+
AML1-ETO+

I


Relative Expression/GAPDH
1.5
1.0
0.5
0.0
*
*
*
*
AML1-ETO
CARM1
Scrambled
AE-KD1
AE-KD2

J


Relative Expression/GAPDH
4
3
2
1
0
0
1
2
3
4
5
6
7
Days post-treatment
*
*
*
*
*
CEBPA
ID1
EGR1
CDKN1A

K

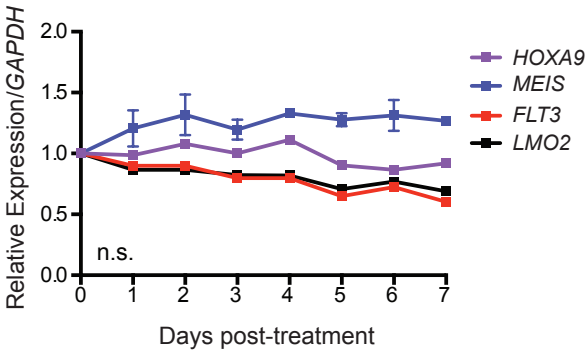

Relative Expression/GAPDH
2.0
1.5
1.0
0.5
0.0
n.s.
0
1
2
3
4
5
6
7
Days post-treatment
HOXA9
MEIS
FLT3
LMO2

Figure S6. Biological comparison of pharmacological inhibition of EPZ025654 and EPZ029751, Related to Figure 6.

A) Quantification of immunoblots showing the percent inhibition of BAF155me2a vs. BAF155 total protein expression in SKNO-1 cells after 48 hours of treatment with increasing doses of the active CARM1 inhibitor (EPZ025654) and control compound(EPZ029751).

B) Log[EPZ05654] compared to the percent inhibition of the asymmetric methylation of BAF155 in $CD34^+$, MV4-11, KASUMI-1, SKNO-1, HEL, or mouse AE9a cells treated for 48 hours. Data points represent the quantification of BAF155me2a and total BAF155 by western blot. Lines represent the non-linear regression fit with variable slope.

C) Comparison of the effects of EPZ025654 or EZP029751 on Annexin V/DAPI positivity after 7 days of treatment. Cells treated with 125 μM or 250 μM of H_2O_2 are shown as a positive control for apoptosis.

D) Western blot comparing the effects of EPZ025654 or EZP029751 on PARP cleavage in SKNO-1 cells after 7 days of treatment (top). Cells treated with 125 μM of H_2O_2 are shown as a positive control. β-ACTIN is shown as a loading control. Quantification of Cleaved/Total PARP for three independent experiments (bottom).

E) Comparison of the effects of EPZ025654 or EZP029751 on cell viability after 7 days of treatment as measured by Cell Titer Glo. n = 3, *p <0.01

F) IHC for senescence associated β-galactosidase in SKNO-1 cells treated with 1 μM or 5 μM of EPZ025654 for 6 days. Cells with no treatment, identical doses of the control compound EPZ02975, or Doxorubicin (Dox) are shown for comparison. Scale bar = 20 μm

G) Quantification of percentage senescence associated β-galactosidase positive SKNO-1 cells treated with EPZ025654 or EPZ029751 for 6 days. Doxorubicin (Dox) is shown as a positive control for senescence. Scale bar = 20 μm, n=2, *p< 0.01.

H) Comparison of *CARM1* expression in AML1-ETO^+ AML patients in the Eastern Cooperative Oncology Group (ECOG) cohort compared to normal $CD34^+$ controls.

I) Comparison of *CARM1* expression following the KD of AML1-ETO in the t(8;21) positive cell line, Kasumi-1. n=3 , *p<0.01

J) Evaluation of AML1-ETO specific gene regulation (*CEBPA*, *ID1*, *EGR1*, and *CDKN1A*) over time in SKNO-1 cells treated with 5 μM EPZ025654 for 7 days. Expression is relative to *GAPDH*. n=3 , *p<0.01

K) Evaluation of MLL-AF9 specific gene regulation (*HOXA9*, *MEIS1*, *FLT3*, and *LMO2*) over time in MV4-11 cells treated with 5 μM EPZ025654 for 7 days. Expression is relative to *GAPDH*. n=3 , n.s. = no significant differences

For all bar graphs, error bars represent the mean ± SD. Statistics represent a Student's t-test for samples of unequal variance.

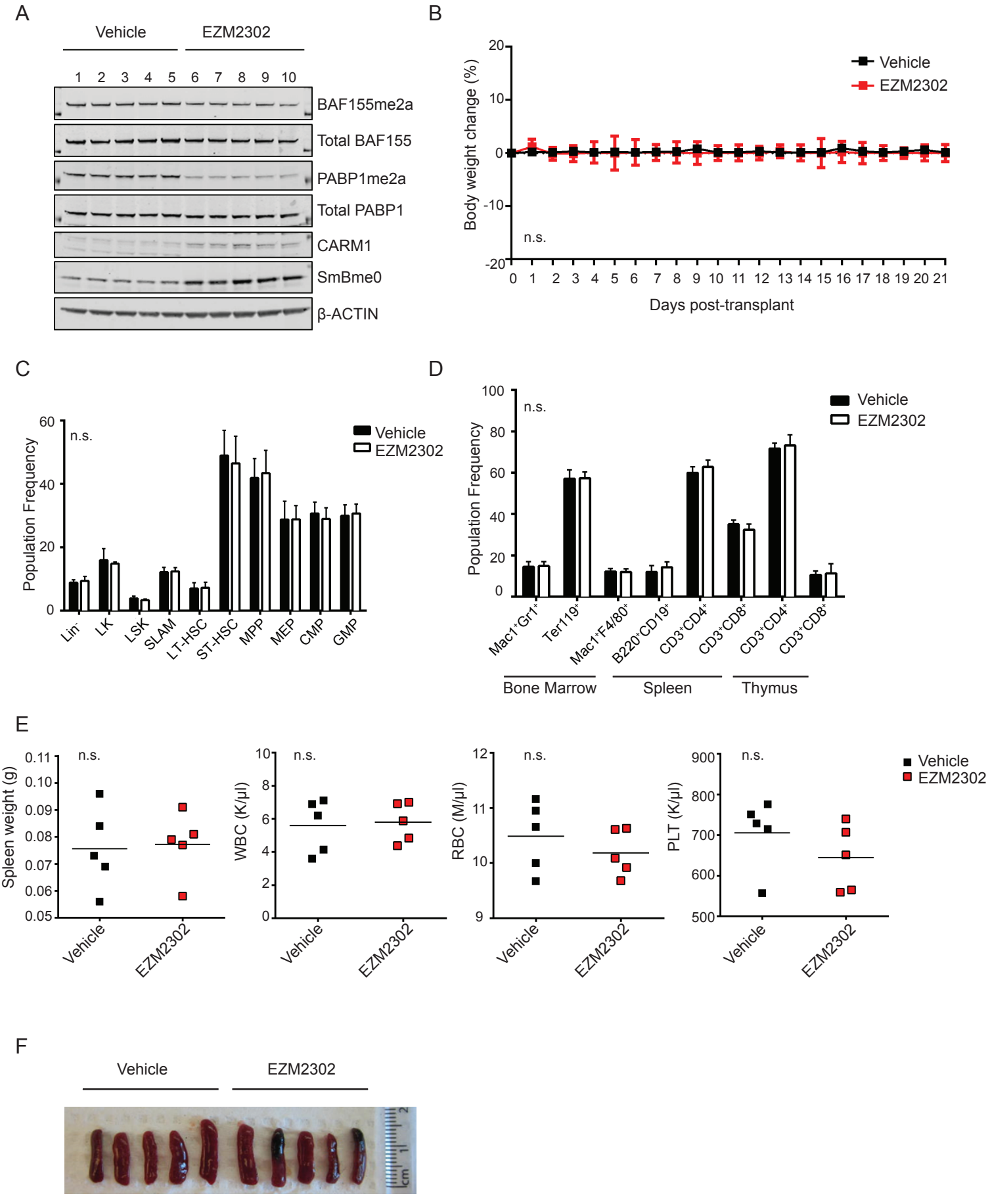

A
Vehicle
EZM2302
1 2 3 4 5 6 7 8 9 10
BAF155me2a
Total BAF155
PABP1me2a
Total PABP1
CARM1
SmBme0
β-ACTIN
B
Body weight change (%)
Vehicle
EZM2302
n.s.
Days post-transplant
C
Population Frequency
n.s.
Vehicle
EZM2302
Lin-
LK
LSK
SLAM
LT-HSC
ST-HSC
MPP
MEP
CMP
GMP
D
Population Frequency
n.s.
Vehicle
EZM2302
Mac1+Gr1+
Ter119+
Mac1+F4/80+
B220+CD19+
CD3+CD4+
CD3+CD8+
CD3+CD4+
CD3+CD8+
Bone Marrow
Spleen
Thymus
E
Spleen weight (g)
WBC (K/μl)
RBC (M/μl)
PLT (K/μl)
n.s.
Vehicle
EZM2302
F
Vehicle
EZM2302

Figure S7. Evaluation of in vivo CARM1 inhibitor treatment, Related to Figure 7.
A) Western blot analysis for BAF155 and PABP1 asymmetric methylation in the spleen of mice treated with either vehicle or 100 mg/kg EZM2302 for three weeks. β-ACTIN is shown as a loading control. n = 5
B) Daily percentage body weight change for mice treated with either vehicle or 100 mg/kg EZM2302 for three weeks. n = 5, n.s.= not statistically significant
C) Frequency of hematopoietic stem and progenitor populations from mice treated with either vehicle or 100 mg/kg EZM2302 for three weeks. n = 5, n.s. = not statistically significant
D) Frequency of mature hematopoietic populations in the bone marrow, spleen, and thymus, from mice treated with either vehicle or 100 mg/kg EZM2302 for three weeks. n= 5, n.s. = not statistically significant
E) Comparison of spleen weights, peripheral blood white blood cell (WBC), red blood cell (RBC), and platelet (PLT) counts from mice treated with either vehicle or 100 mg/kg EZM2302 for three weeks. n = 5, n.s. = not statistically significant
F) Image showing spleen morphology of mice treated with either vehicle or 100 mg/kg EZM2302 for three weeks. n = 5
All error bars represent the mean ± SD. Statistics represent a Student's t-test for samples of unequal variance.

Table S4. IC_{50} of leukemia cell lines treated with EPZ025654 for 10 days, Related to Figure 7.

Cell line	Viability EPZ025654	Media	Age	Sex	Reported mutations (CCLE/COSMIC/Tru Myeloid)	FAB[a]	Fusion Oncoprotein
$CD34^+$	>10 μM	X-vivo +20% BIT	N/A	N/A	N/A	N/A	N/A
AML-14	>10 μM	RPMI 10%	68	M	TP53, NRAS	M2	
HEL	>10 μM	RPMI 10%	30	M	JAK2V617F,TET2, KMT2D, TP53, EP300	M6	
HL-60	6.3 μM	IMDM 20%	36	F	CDKN2A, NRAS, SF3B1	M2	
KASUMI-1	1.4 μM	RPMI 20%	7	M	C-KIT, TP53, ASXL1, CBP, RAD21	M2	AML1-ETO
MOLM-13	1.1 μM	RPMI 10%	20	M	FLT3/ITD, CBL, KMT2A, KMT2C, NF1, SETD1A	M5	MLL-AF9
MONO-MAC6	>10 μM	RPMI 10%	64	M	FLT3/ITD, KAT6B, RUNX1, ARID2A, ASXL1, IDH1, U2AF1	M5	MLL-AF9
MV4-11	3.0 μM	IMDM 10%	10	M	FLT3/ITD, NPM1	M5	MLL-AF4
NB-4	2.3 μM	RPMI 10%	23	F	KRAS, TP53, ETO, EP300, MOZ, ASXL2	M3	PML-RARA
NOMO-1	1.1 μM	RPMI 10%	31	F	KRAS, ASXL1, EP300, SF3B1, TP53, CDKN1B, KMT2C	M5	MLL-AF9
OCI-AML3	0.1 μM	alpha-MEM 20% FBS	57	M	NPM1, NRAS, DNMT3A	M4	
SET-2	7.8 μM	RPMI 20%	71	F	JAK2V617F, TP53, UTX, DNMT3A	N.D.	
SKNO-1	0.4 μM	RPMI 10% +GM-CSF	22	M	TP53, KDM6B, KRAS, TET2, ASXL1	M2	AML1-ETO
SKM-1	2.2 μM	RPMI 20%	76	M	TP53, c-KIT	M5	
TF-1	0.2 μM	RPMI 10%+ IL-3	35	M	TP53, NRAS	M6	
THP-1	6.3 μM	RPMI 10%	1	M	CDKN2A, NRAS, TP53, ARID1A, UTX	M5	MLL-AF9
U-937	1.0 μM	RPMI 10%	37	M	TP53, WT1, PTEN, ETV6, PTPN11	M5	
UKE-1	3.5 uM	IMDM 10% +HS+HC	59	F	JAK2V617F, IKZF1, EZH2, ETV6, PTPN11, STAG2	N.D.	

[a]FAB: French-American-British classification system

Table S6. Primers used in study, Related to STAR methods

Probe	Source	Identifier
GAPDH (human)	Thermo Fisher	Hs02758991_g1
CARM1 (human)	Thermo Fisher	Hs00406354_m1
MEIS1 (human)	Thermo Fisher	Hs01017441_m1
HOXA9 (human)	Thermo Fisher	Hs00365956_m1
FLT3 (human)	Thermo Fisher	Hs00174690_m1
LMO2 (human)	Thermo Fisher	Hs00153473_m1
E2F1 (human)	Thermo Fisher	Hs00153451_m1
E2F2 (human)	Thermo Fisher	Hs00231667_m1
CENPA (human)	Thermo Fisher	Hs0015655_m1
CDC25A (human)	Thermo Fisher	Hs00947994_m1
MYBL2 (human)	Thermo Fisher	Hs00942543_m1
TOP2A (human)	Thermo Fisher	Hs01032137_m1
UHRF1 (human)	Thermo Fisher	Hs00380204_m1
E2F1 (human)	Thermo Fisher	Hs00153451_m1
E2F2 (human)	Thermo Fisher	Hs00231667_m1
E2F3 (human)	Thermo Fisher	Hs00605457_m1
E2F4 (human)	Thermo Fisher	Hs00608098_m1
E2F5 (human)	Thermo Fisher	Hs00231092_m1
E2F6 (human)	Thermo Fisher	Hs01034552_m1
E2F7 (human)	Thermo Fisher	Hs00403170_m1
E2F8 (human)	Thermo Fisher	Hs00226635_m1
TP53 (human)	Thermo Fisher	Hs01034249_m1
CDKN1A (human)	Thermo Fisher	Hs00355782_m1
Gapdh (mouse)	Thermo Fisher	Mm99999915_g1
Carm1 (mouse)	Thermo Fisher	Mm01171448_m1