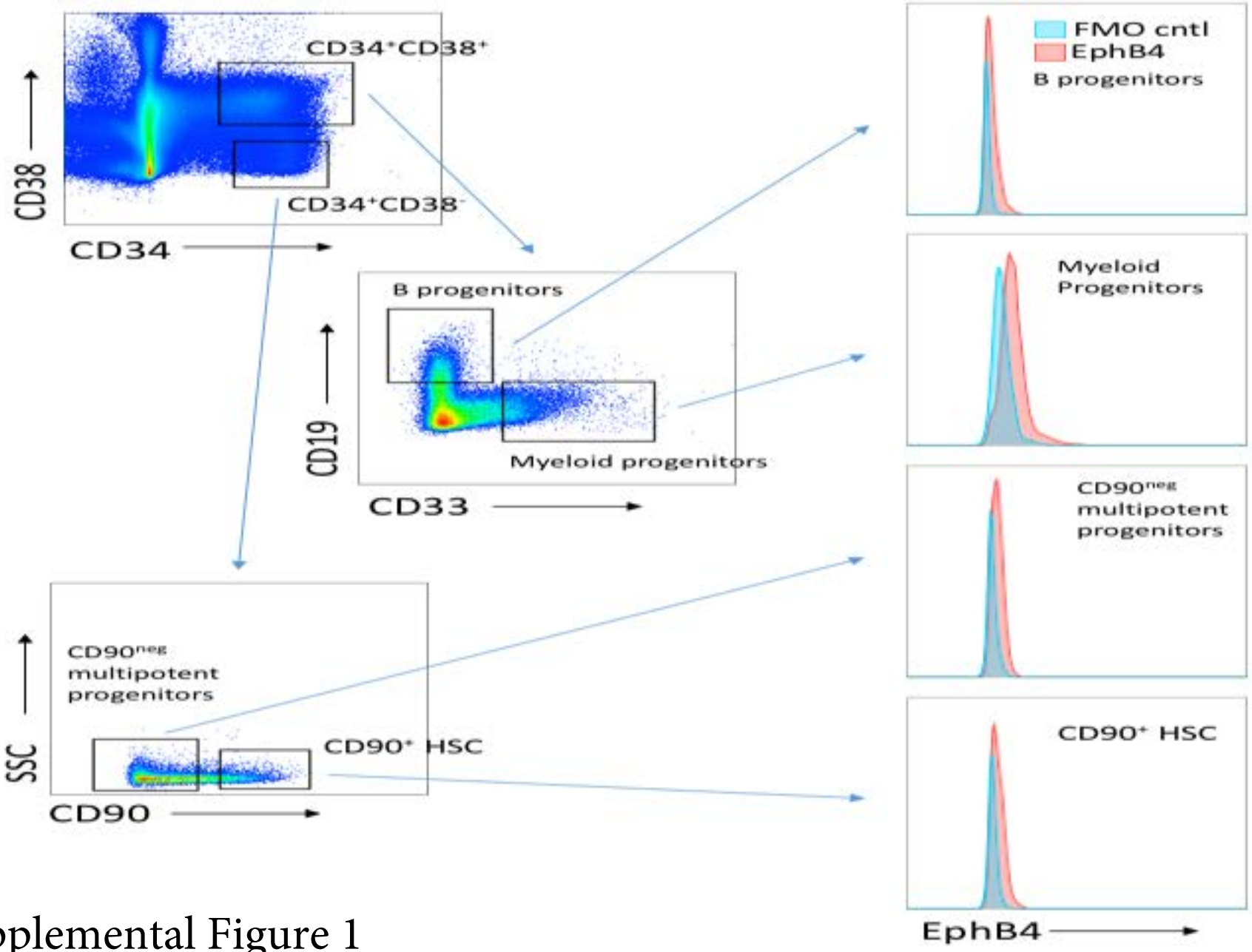
Supplemental Figure Legends

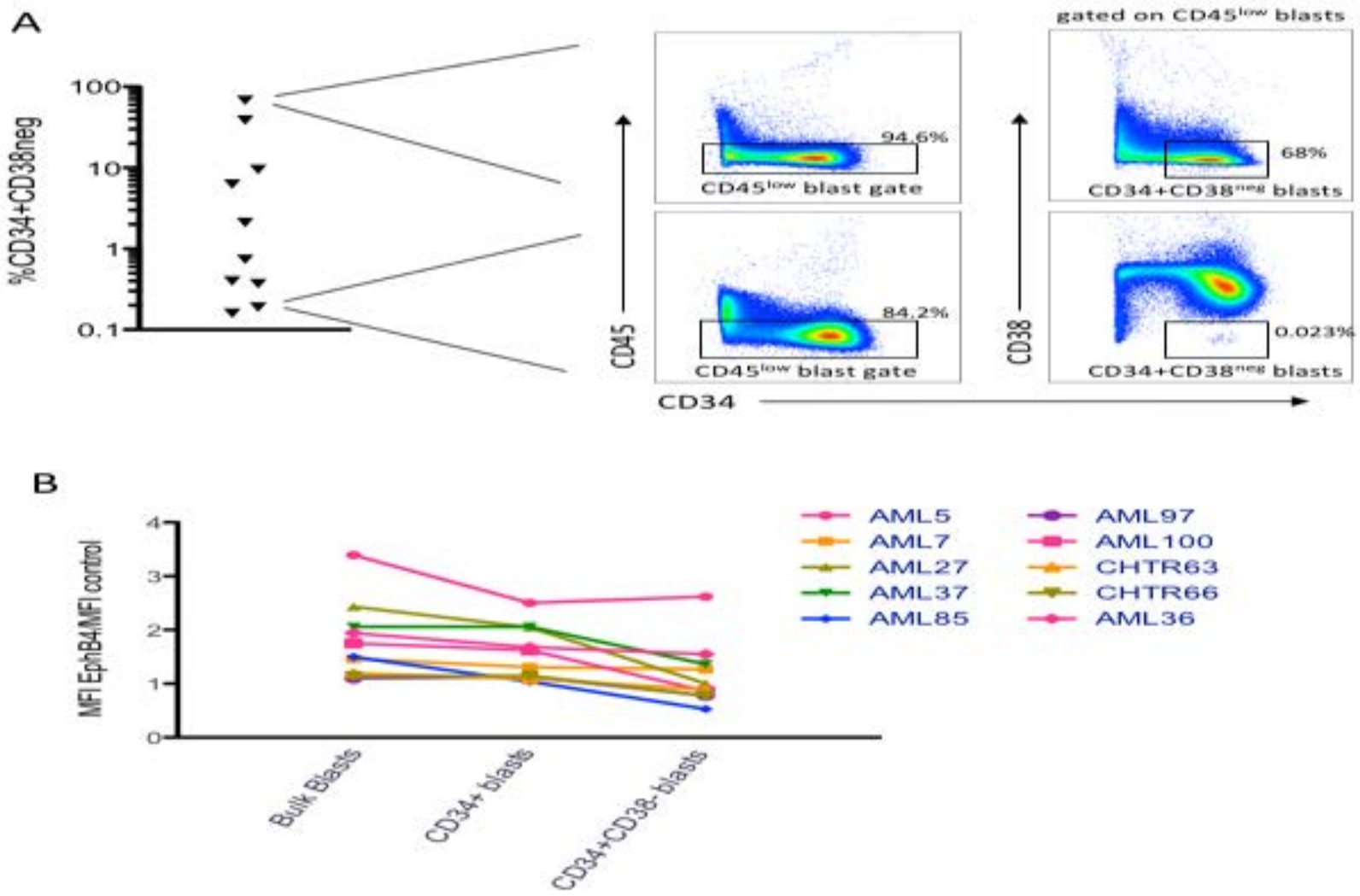
Supplemental Figure 1. Representative flow plots from normal bone marrow demonstrating a lack of EphB4 expression on the CD34 compartment. CD34+CD38+ lineage specific progenitors (CD19 B cell and CD33 Myeloid cells) are negative for EphB4. The CD34+CD38- compartment is further divided into $CD90^{neg}$ multipotent progenitors as well as CD90+ hematopoietic stem cells (HSC). All compartments show staining similar to fluorescence minus one (FMO) controls.

Supplemental Figure 2. Analysis of EphB4 expression on $CD34+CD38^{neg}$ putative leukemia stem cell populations. 10 AML samples were labeled for CD45/CD34/CD38/EphB4 and analyzed for differential expression of EphB4 on bulk blasts verses leukemia stem cell populations. A) Samples show a wide range of blasts that fall into the stem cell gate. $CD34+CD38^{neg}$ cells were expressed as a percentage of total $CD45^{low}$ blasts and ranged from 0.012-68%. Gating from two representative samples at the high and low range are shown. B) EphB4 expression in bulk vs CD34+ vs $CD34+CD38^{neg}$ cells is shown. In most EphB4+ AML, EphB4 is expressed in the $CD34+CD38^{neg}$ fraction of the leukemia.

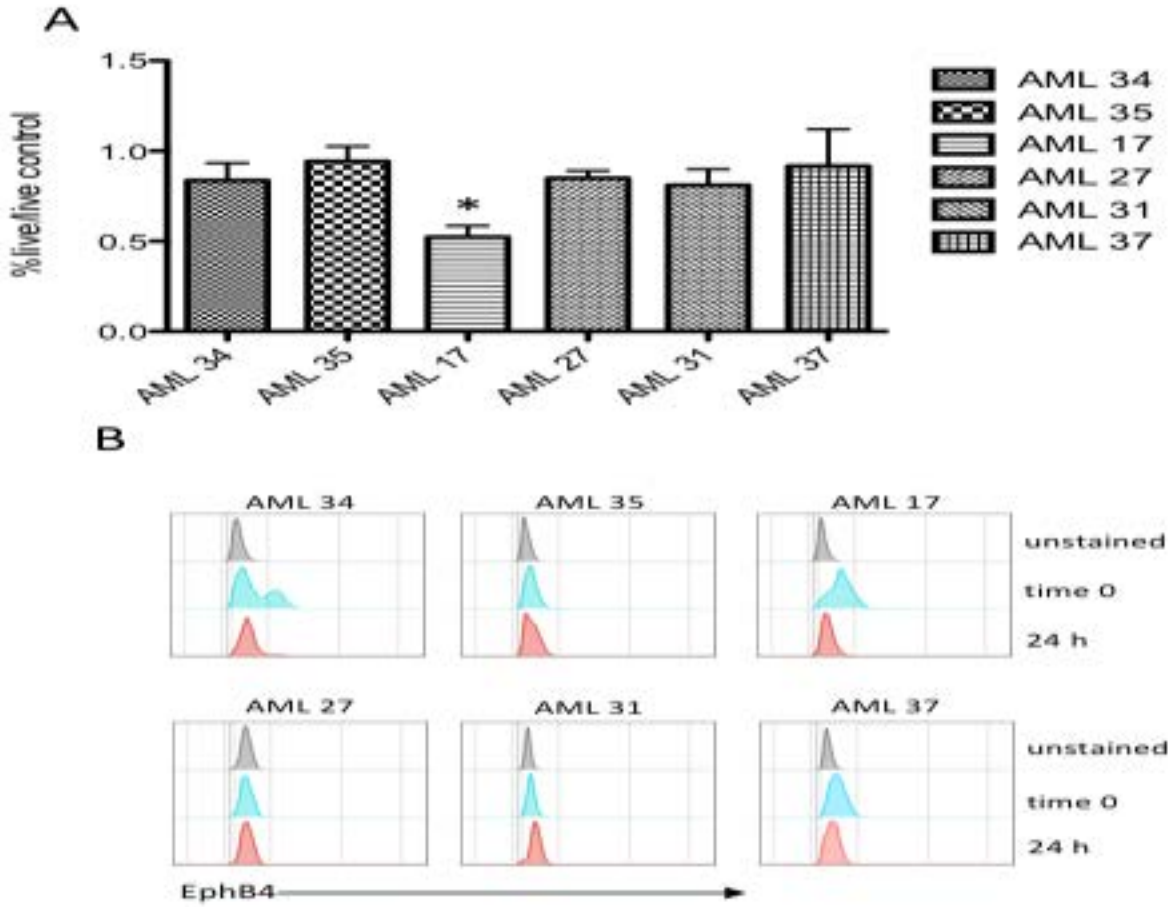
Supplemental Figure 3. In vitro treatment of primary AML blasts with MAb131. Results of samples with high expression of EphB4 were presented in Figure 2E-F. Data for an additional 6 samples are presented here. A) Primary AML blasts (not selected for EphB4 expression) were treated with MAb131 at 10ng/ul in triplicate. Live cells were counted on a hemacytometer by trypan blue exclusion at 48 hours and normalized to untreated controls. Treatment with MAb131 lead to a significant response in 1 out of 6 samples, AML 17. *=$p<0.05$. B) In this unselected pool of primary samples, most samples show very low EphB4 expression with the exception of AML 34 which showed partial expression, AML 17 which showed moderate expression and AML 37 which showed low expression. Treatment with MAb131 caused down regulation of EphB4 in samples with expression as detected by flow cytometry at 24 hours. AML 17 was the only sample which responded to MAb131 treatment and had the highest level of EphB4 expression among the samples tested.



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3

Supplemental Table 1. Clinical characteristics and raw MFI value of EphB4 expression for clinical samples. NL=normal cytogenetics, N/A=not available

ID	Age	Sex	Cytogenetics	Molecular	MFI EphB4/ MFI Control
AML2	47	M	NL	FLT3-ITD+, NPM1+	5.6
AML3	40	F	ins(20)	FLT3-ITDneg, NPM1neg	3.2
AML4	65	M	Tri8	FLT3-ITDneg, NPM1neg	2.4
AML5	67	F	N/A	N/A	60.8
AML6	60	F	NL	FLT3-ITD+, NPM1+	7.3
AML7	47	F	NL	FLT3-ITDneg, NPM1neg	6.5
AML9	22	F	NL	FLT3-ITD+, FLT3-TKD+, NPM1+	6.1
AML10	38	M	NL	FLT3-ITD+, NPM1neg	5.9
AML11	60	M	N/A	N/A	4.7
AML16	54	M	Mono 21	FLT3-ITDneg, NPM1+	1.2
AML18	36	M	NL	CEBPAmut, NPM1+	27.1
AML20	41	F	NL	FLT3-ITDneg, NPM1+	84.9
AML21	60	M	NL	N/A	3.1
AML26	N/A	N/A	N/A	N/A	7.7
AML27	59	F	Tri8	FLT3-ITDneg, NPM1neg	3.1
AML30	45	M	N/A	N/A	15.8
AML33	58	F	Mono 7	FLT3-ITDneg, NPM1neg, DNMT3A+, IDH1+	14.0
AML37	63	M	MLL	N/A	174.4
CHTR61	52	M	t(15;17)	PML/RARA+, FLT3-ITD+	1.8
CHTR63	58	M	iso 17q	FLT3-ITDneg, NPM1neg	1.3
CHTR10	46	F	NL	FLT3-ITD+, NPM1neg	2.3
CHTR57	52	F	t(15;17),del9	PML/RARA+	4.2
CHTR38	54	M	Tri8	FLT3-ITDneg, NPM1neg	6.3
CHTR82	44	F	NL	FLT3-ITD+, NPM1neg	2.7
CHTR25	33	M	NL	CEBPA bi-allelic mutation, FLT3-ITDneg, NPM1neg	9.8