Table W1. Patient Characteristics.

Patient ID	Classification	World Health Organization	Phenotype	Age at Diagnosis (Years)	Sex	Sample	Engraftment
Fi-IEO	AML	M0	$CD45^+/CD13^+/CD11c^+/CD14^-/CD15^-/CD33^+/CD34^+/CD38^+/CD117^+/CD133^+/CD2^-/CD3^-/CD19^-MPO^+$	66	F	BM	+
Lo-IEO	AML	M1	$CD45^+/CD13^+/CD33^+/CD34^+/CD117^+/CD14^-/CD15^-$	68	M	PB	–
Bo-IEO	AML	M1	$CD45^+/CD13^-/CD117^+/CD14^+/CD15^+/MPO^+$	58	F	BM	–
Ga-IEO	AML	M1	$CD45^+/CD3^-/CD4^-/CD7^-/CD8^-/CD19^-/CD56^-/CD14^-/CD64^-/CD33^+/CD13^+/CD34^\pm/CD15^\pm/CD117^+/CD11b^-/MPO^+/Tdt^-$	39	F	BM	+
Col-IEO	AML	M1	$CD45^+/CD3^-/CD19^-/CD14^-/CD15^-/CD33^+/CD13^+/CD34^+/CD133^+/CD117^+/MPO^+/Tdt^-$	70	F	BM	–
Co-IEO	AML	M1	$CD45^+/CD33^+/CD13^+/CD34^+/CD117^+/CD2^-/CD3^-/CD4^-/CD5^-/CD8^-/CD19^-/CD20^-/CD22^-/MPO^+$	47	M	BM	+
Ti-IEO	AML	M2	$CD45^+/CD33^+/CD13^+/CD34^+/CD117^+/CD56^+/CD14^-/CD15^-/CD2^-/CD7^-/CD3^-/CD192^-/CD16^-/CD11b/MPO^+/Tdt^-$	57	F	BM	+
Ca-IEO	AML	M2	$CD45^+/CD13^-/CD33^+/CD34^\pm/MPO^+$	73	M	BM	–
Q-IEO	AML	M4	$CD45^+/CD33^+/CD13^+/CD34^-/CD4^+/CD11c^+/CD14^+/CD15^+/CD2^-/CD79^-/MPO^+/Tdt^-$	42	F	BM and PB	+
Pet-IEO	AML	M4	$CD45^+/CD33^+/CD13^+/CD34^-/CD4^-/CD117^+/CD14^-/CD15^-/CD20^-/CD3^-/CD16^-/CD19^-/CD8^-/MPO^+/Tdt^-$	51	F	BM	+
Ot-IEO	AML	M4	$CD45^+/CD33^+/CD13^+/CD34^\pm/CD117^+/CD14^-/CD15^-/CD133^-/MPO^+/Tdt^-$	69	M	BM	+
So-IEO	AML	M5	$CD45^+/CD33^+/CD13^+/CD34^-/CD117^-/CD14^+/CD11b^-/CD133^-/CD19^-/MPO^+$	57	M	BM	+
Pu-IEO	AML	M5	$CD45^+/CD64^+/CD11c^+/CD34^-/CD117^-/CD14^+/CD15^+/CD133^-/MPO^+$	44	M	BM	+
Pa-IEO	AML	M5	$CD45^+/CD33^+/CD13^+/CD66b^-/CD14^\pm$	69	F	BM	+
Vla-IEO	ALL-B		$CD45^+/CD19^+/CD10^+/CD34^-/CD20^-/CD22^+/CD38^+/CD133^-/CD117^-/Tdt^-$	28	F	BM	+
Cro-IEO	ALL-ProB		$CD45^+/CD19^+/CD79^+/CD34^+/CD20^-/CD15^+/CD38^+/CD133^-/Tdt^+$	41	F	BM	+
Ge-IEO	ALL-ProB		$CD45^+/CD19^+/CD10^+/CD34^+/CD20^\pm/CD3(+)/CD22^+/CD15^+/CD38^+/CD133^-/CD9^+Tdt^+$	26	M	BM and PB	+
Cos-IEO	ALL-T		$CD45^+/CD19^+/CD10^+/CD34^+/CD20^-/CD3^+/Tdt^+$	32	M	BM	+
Za-IEO	ALL-T		$CD45^+/CD4^+/CD8^-/CD5^+/CD7^-/CD3^+$	32	F	BM	+

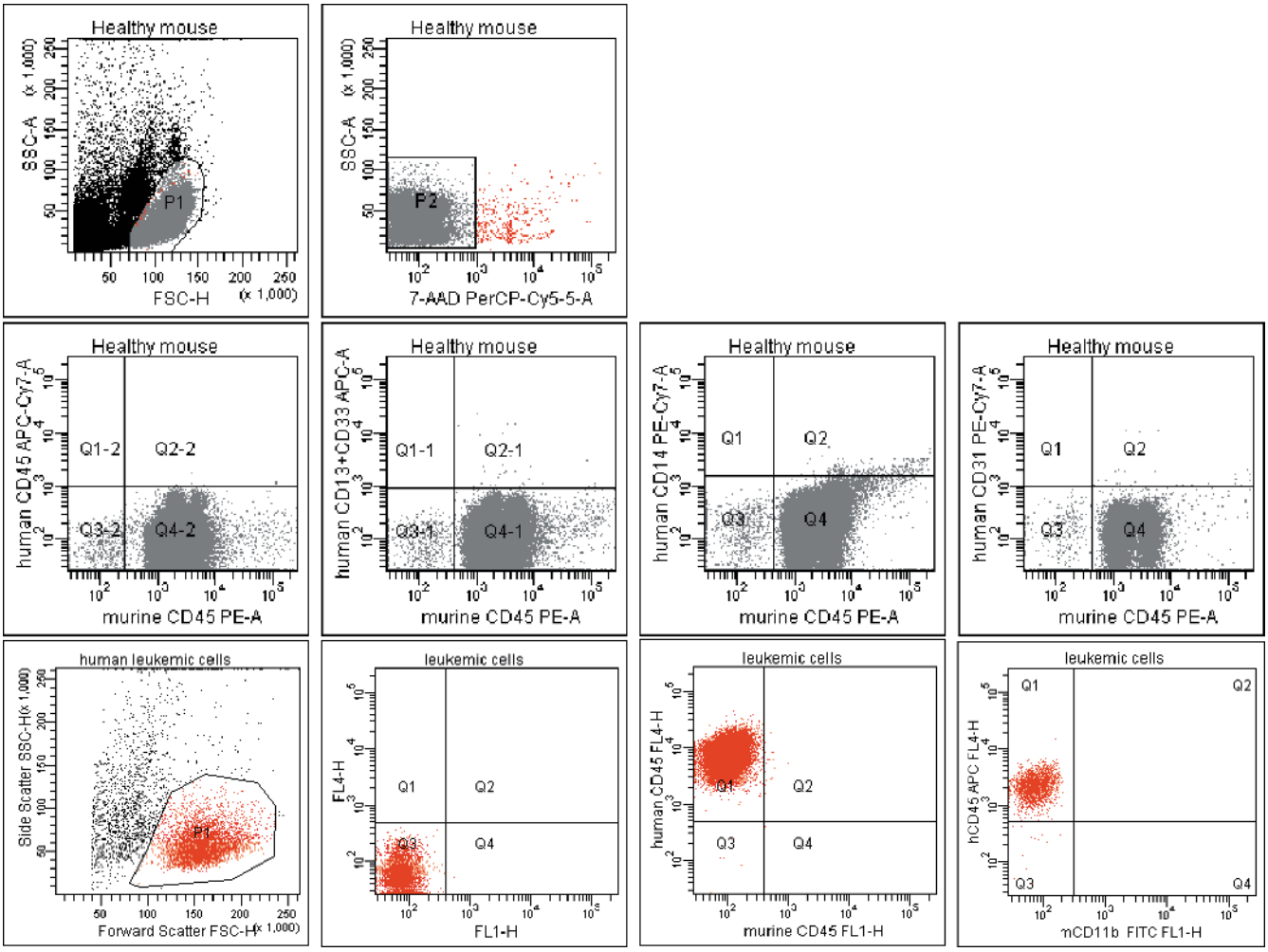

Figure W1. Monoclonal antibodies used in this study were unambiguously species specific. mAbs to human CD45, CD13, CD33, CD14, and CD31 do not bind mouse PB cells. mAbs to mouse CD45 and CD11b do not bind human leukemic cells.

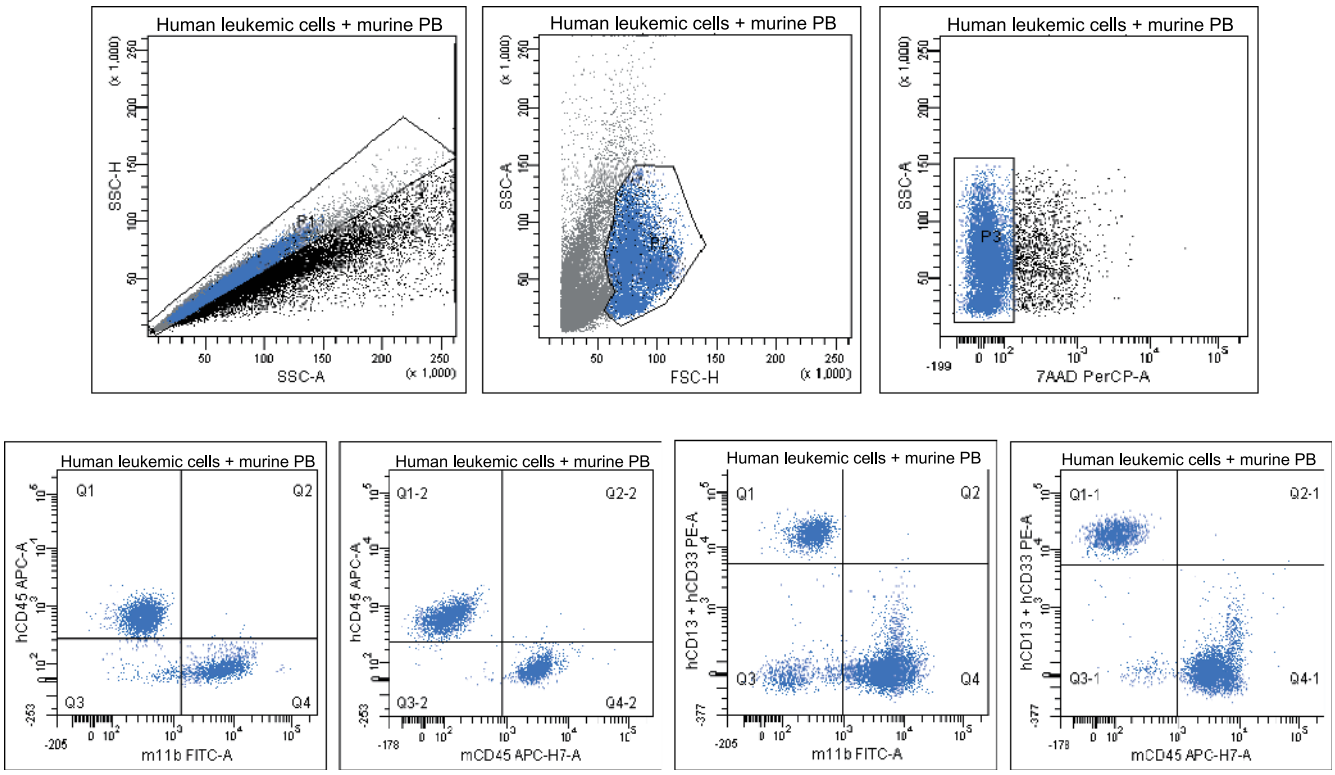

Figure W2. Doublets can be excluded by physical parameters and mouse CD45 and CD11b and human CD13, CD33, and CD45 recognize mouse PB cells and human leukemic cells, respectively.

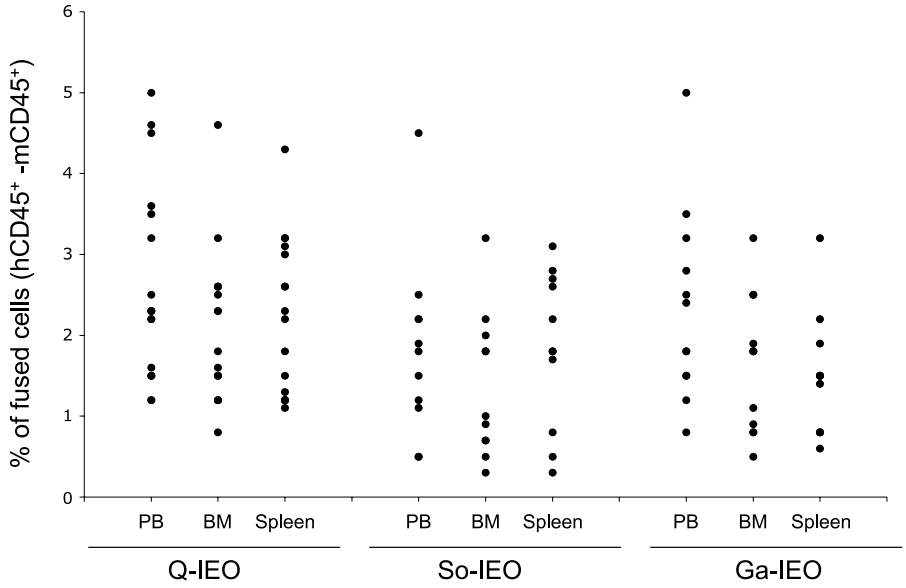

Figure W3. Hybrid human CD45^{+}–mouse CD45^{+} cells are detected in PB, BM, and spleen of leukemic mice after human AML transplant of three patients (Q-IEO, $n = 17$; So-IEO, $n = 11$; Ga-IEO, $n = 12$).

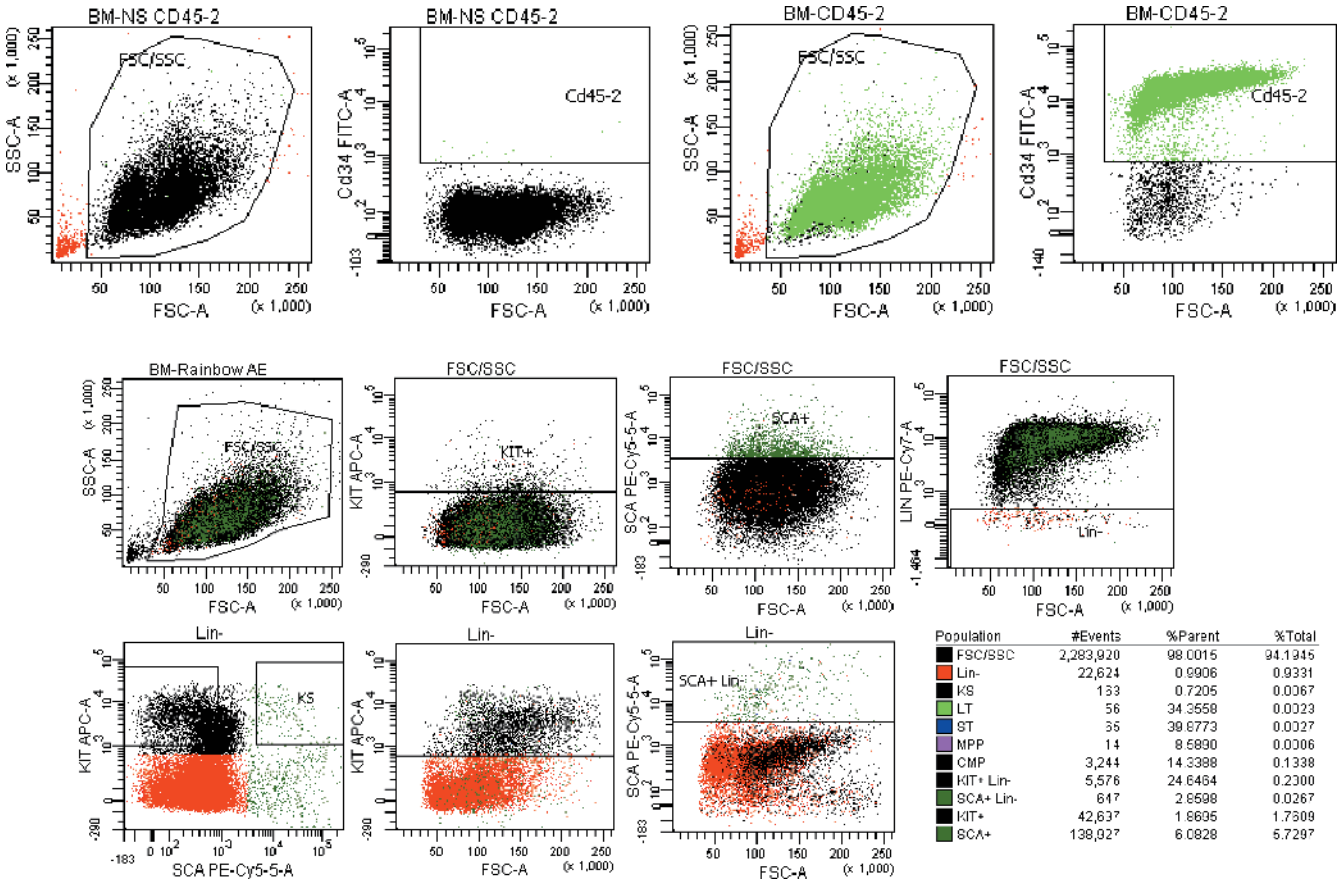

Figure W4. Cytofluorimetric analysis of c-Kit expression on a cell population of Lin^{-}Sca-1^{+} cells of a spleen of a mouse with AML1-ETO–induced leukemia.

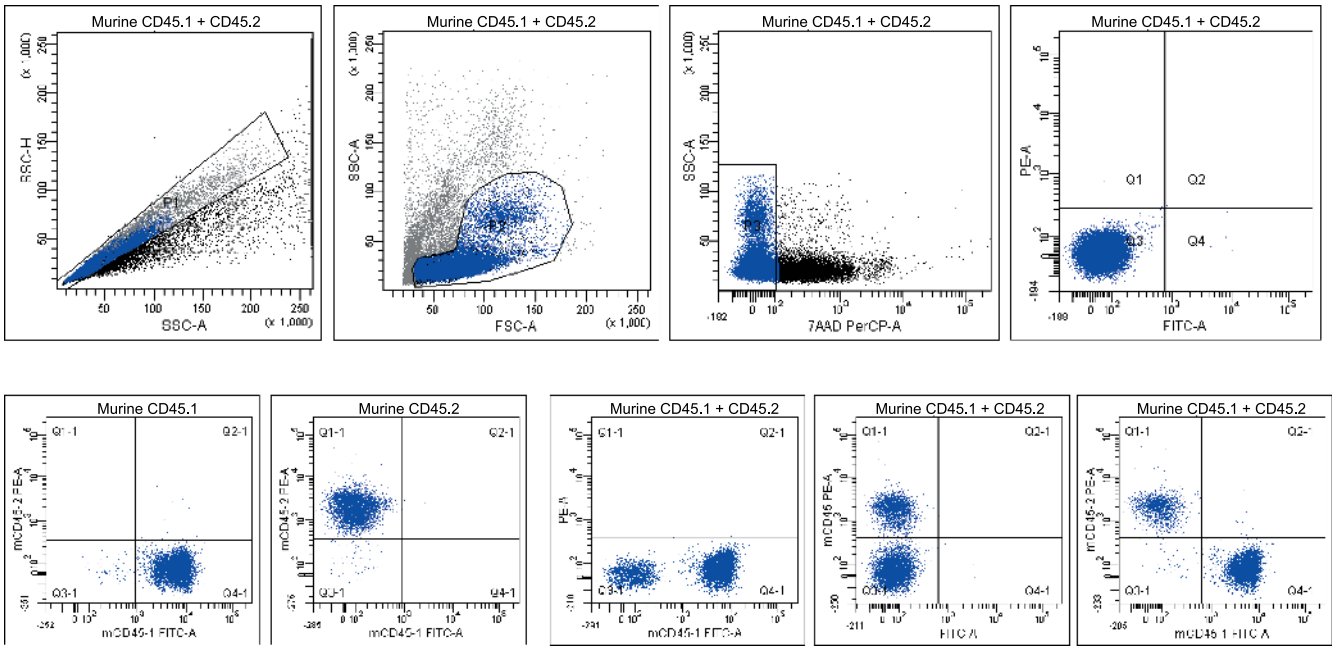

Figure W5. Doublets can be excluded by physical parameters and antibodies to mouse CD45.1 and CD45.2 selectively recognize two different cell populations in a mixed sample of PB from C57-CD45.1 and C57 CD45.2 mice.

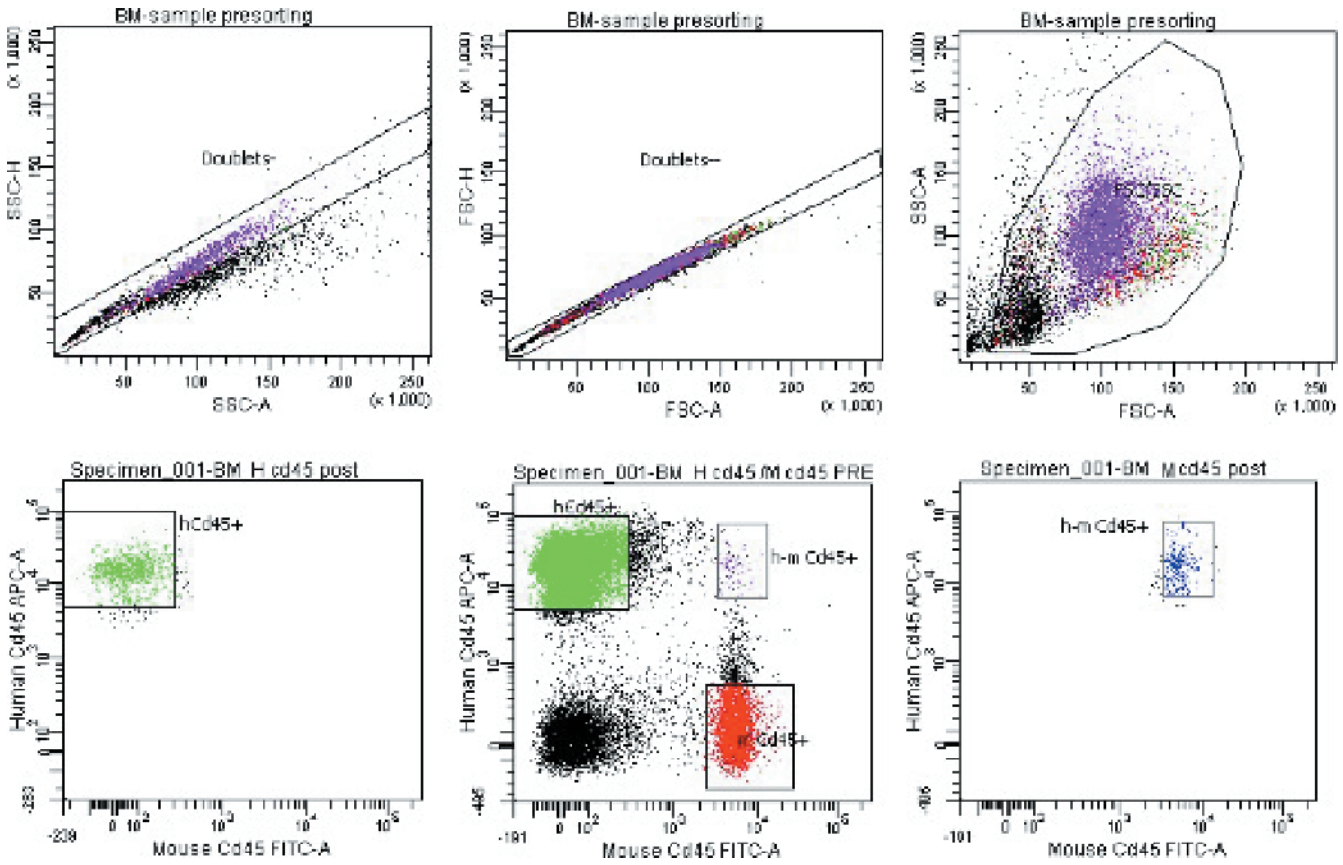

Figure W6. Sorting strategy to isolate human CD45– and mouse CD45–positive cells. An appropriate gate to exclude putative doublet formation allows us to sort only hybrid cells expressing hCD45 and mCD45.

A

Cell line	Cell type	NSB				NSG				NS			
		% Engraftment hCD45+			mice with fusion events/ total injected	% Engraftment hCD45+			mice with fusion events/ total injected	% Engraftment hCD45+			mice with fusion events/ total injected
		PB	BM	Spleen		PB	BM	Spleen		PB	BM	Spleen	
HL-60	AML	5,31	1,42	1,84	2/3	6,73	5,84	5,27	3/4	10,12	1,58	1,40	1/3
KG-1	AML	1,09	0,02	0,09	0/3	3,23	0,31	0,19	1/3	5,91	1,14	0,21	1/3
MOLT-16	ALL	21,47	23,60	27,06	2/3	0,1	0,1	17,5	2/3	1,86	0,17	1,08	1/3
697	ALL	26,70	13,90	19,25	2/2	27,44	35,47	31,33	2/3	11,89	6,77	7,64	1/2

B

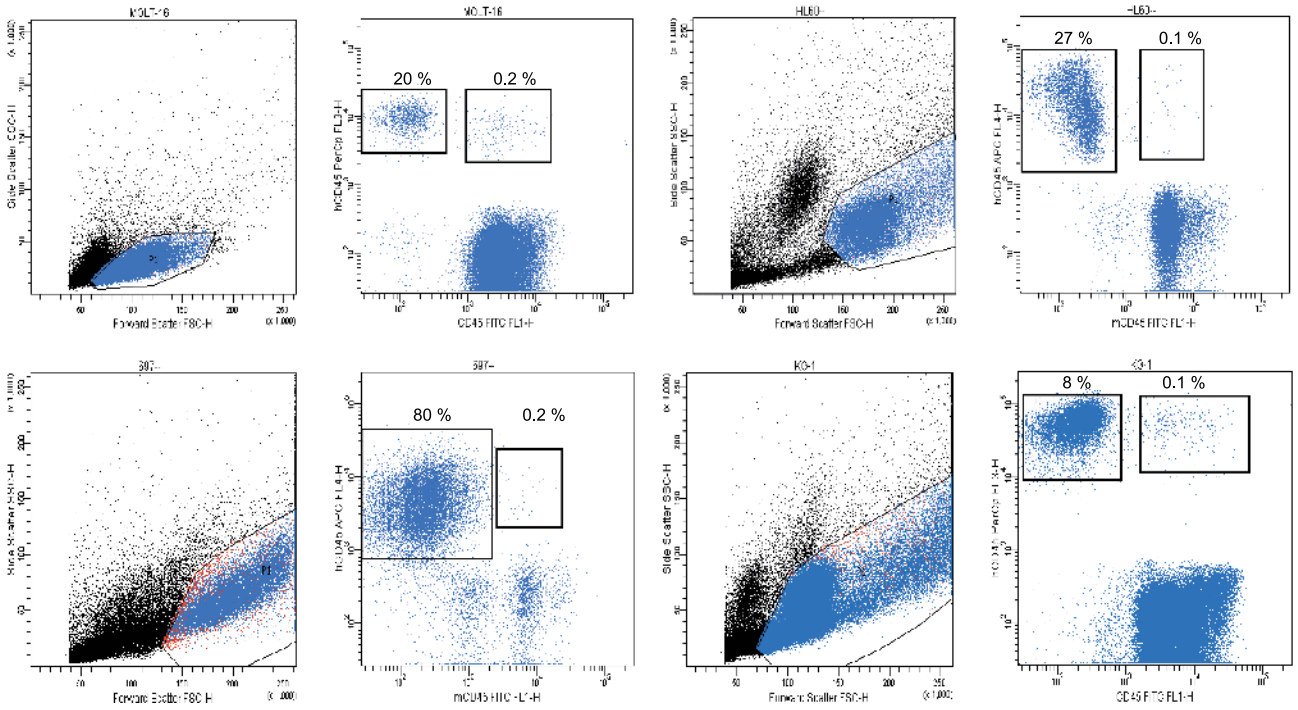

Figure W7. Cell fusion is a common event in different mouse strains during AML development and is not restricted to NSB mice. (A) Evaluation of engraftment (percent of hCD45^{+} cells) and frequency of fusion events of AML and ALL cell lines on NS, NSB, and NSG mice. (B) Flow cytometric analysis of human CD45 and mouse CD45 expression of BM samples from mice injected with HL60, KG-1, MOLT-16, and 697 AML cell lines into NSB mice.

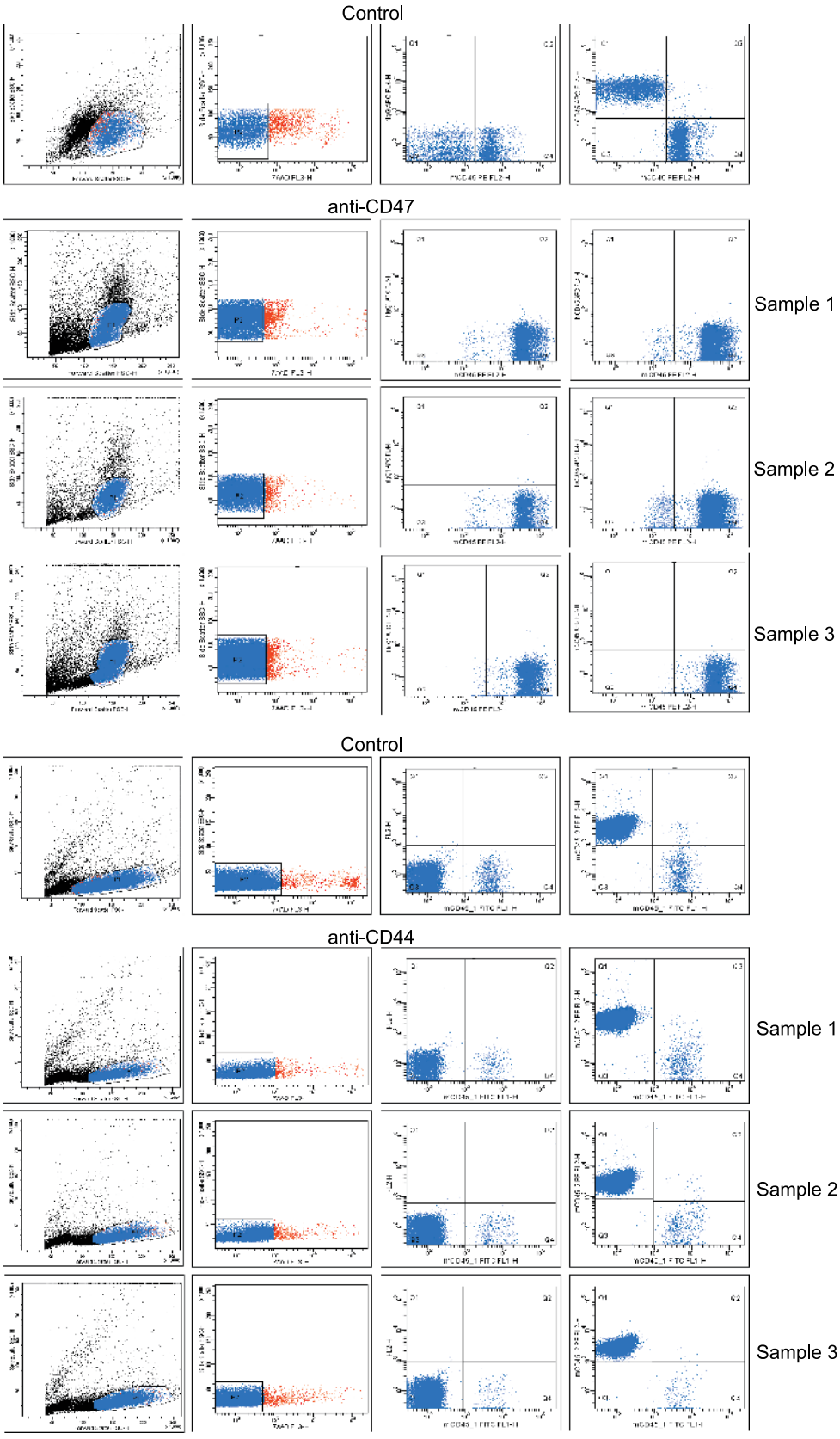

Figure W8. Targeting CD44 or CD47 does not inhibit specifically AML cell fusion. Engraftment evaluation by cytofluorimetric analysis of hCD45 staining. Mice ($n = 3$), samples 1 to 3, treated with anti-human CD47 antibody (50 μg, ip, daily for 4 weeks) do not show presence of human leukemic cells in comparison with mice treated with isotypic matching IgG1 (control, upper panels). Engraftment evaluation by cytofluorimetric analysis of CD45.2 staining. Mice treated with isotypic matching IgG2b (control, upper panels) and mice (samples 1–3) treated with F(ab')2 fragments of anti-CD44 (500 μg, ip, three times a week, for 4 weeks) do not present differences in terms of percentage engraftment and cell fusion events.

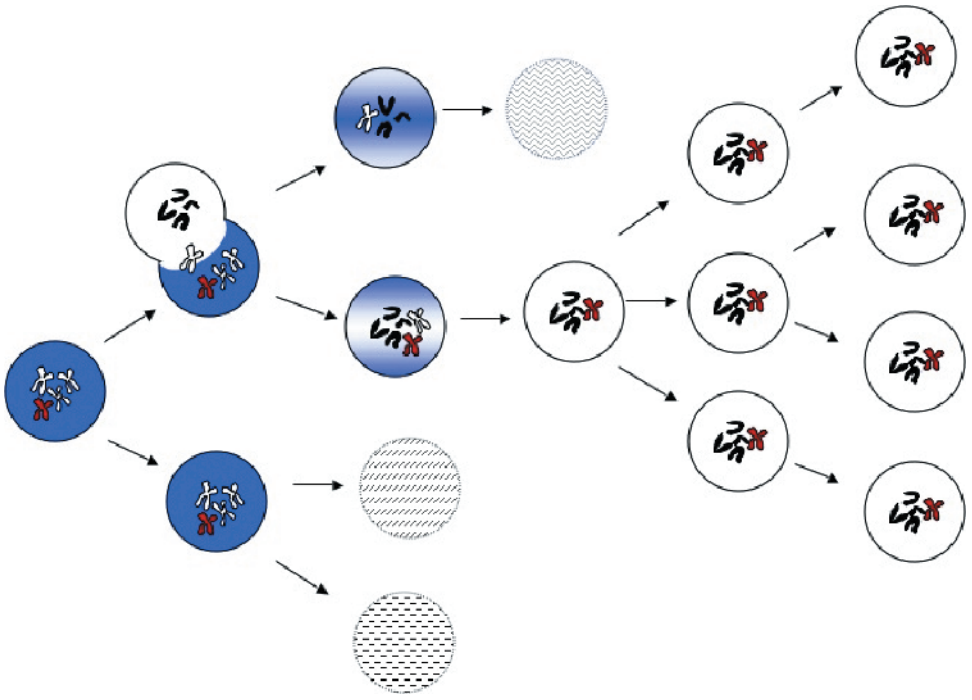

Figure W9. Our working hypothesis on AML cell fusion (schema depicting the potential mechanism by which gene transfer by cell fusion can play a role during leukemogenesis): AML cells (blue cells, containing leukemic DNA represented as a red chromosome) fuse with host cells (white cells) generating hybrid cells with different genomic content. These hybrid cells might represent a reservoir to develop leukemia.