UNIVERSAL MONITORING OF MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA

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Supplemental Material

(Supplemental Tables S3-S6; Supplemental Figures S1-S6)

Table S3. Genes overexpressed in AML "stem cells" according to previous studies and their overexpression in AML according to the present analysis

Gene overexpressed in AML stem cells according to Saito et al.(1)	Gene overexpressed in AML stem cells according to Kikushige et al.(2)	AML cases with overexpression in this study (%)[a]
WT1		84.7
CD32	CD32	76.4
DOK2		70.1
	CD96	66.9
HCK		65.6
CD86	CD86	64.3
	CD44	64.3
CD93		56.7
ITGB2, CD18	ITGB2, CD18	52.9
	CSF1R, CD115	51.0
IL2RA, CD25	IL2RA, CD25	47.8
LY86		43.3
IL7R, CD127		42.0
	CD99	42.0
	IL17R	39.5
CD97	CD97	37.6
CD33	CD33	36.9
	CD9	36.9
CD1C		35.7
AK5		33.1
BIK		31.2
	CD47	30.6
TNFRSF4, CD134		29.3
	CD84	29.3
IL2RG, CD132		27.4
	ITGB7	27.4
CEACAM6, CD66c		26.8
	FLT3	25.5
CD180		<25
CTSC		<25
PDE9A		<25
CD24		<25
	CD36	<25
	CD123	<25
	ITGAE	<25

LRG1		Not on HG-U133A array
SUCNR1		Not on HG-U133A array
TNFSF13B, CD257		Not on HG-U133A array
	CD366, HAVCR2, TIM-3	Not on HG-U133A array
	CD371, CLEC12A, CLL-1	Not on HG-U133A array

[a]Gene expression was studied by HG-U133A oligonucleotide microarrays in157 AML samples and 7 samples of normal CD34+ myeloid progenitors. Shown is the percentage of AML cases with expression signals higher than 2-fold of the highest value obtained among normal CD34+ myeloid cells

1. Saito Y, Kitamura H, Hijikata A, Tomizawa-Murasawa M, Tanaka S, Takagi S, et al. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. *Sci Transl Med.* 2010;2(17):17ra9.
2. Kikushige Y, Shima T, Takayanagi S, Urata S, Miyamoto T, Iwasaki H, et al. TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. *Cell Stem Cell.* 2010;7(6):708-17.

Table S4. Antibodies used in this study and cell types used to test their reactivity

Specificity	**Clone**	**Fluorochrome**	**Source**	**Catalog Number**	**Positive Control**	**Negative Control (negative or dim expression)**
CD9	M-L13	FITC	BD Biosciences	341646	Monocytes	Lymphoid subset
CD18/ ITGB2	MEM-48	PE	GeneTex	GTX79945	All leukocytes	K562 cell line
CD25/ IL2RA	2A3	PE	BD Biosciences	341010	Activated lymphocytes	Resting lymphocytes
CD32/ FCGR2A	2E1	PE	Beckman Coulter	IM1935	Monocytes	Lymphoid subset
CD44	G44-26 (C26)	V450	BD Biosciences	561292	All leukocytes	Jurkat cell line
CD47	B6H12	PE	BD Biosciences	556046	All leukocytes	Not known
CD52	CF1D12	FITC	Life Technologies	MHCD5201	Lymphoid	NK subset, neutrophils
CD54/ ICAM1	LB-2	PE	BD Biosciences	347977	Daudi cell line	Lymphoid subset
CD59	P282 (H19)	PE	BD Biosciences	555764	K562 cell line	Lymphocytes
CD64/ FCGR1A	10.1	V450	BD Biosciences	561202	Monocytes	Lymphocytes
CD68[1]	Y1/82A	PE	BD Biosciences	556078	Monocytes	Lymphocytes
CD86	2331 (FUN-1)	BV421	BD Biosciences	562432	Monocytes	Resting lymphocytes
CD93	VIMD2	PE	Biolegend	336108	Monocytes	Lymphocytes
CD96	6F9	PE	BD Biosciences	562379	Activated NK and T cells	Resting lymphocytes
CD97	VIM3b	FITC	BD Biosciences	555773	Monocytes	Resting lymphocytes

CD99	Tü12	PE	BD Biosciences	555689	T cell lymphoblastic leukemia cells	Granulocytes
CD115/ CSF1R	61708	PE	R&D Systems	FAB329P	Monocytes	Lymphocytes
CD123/ IL3RA	9F5	PE	BD Biosciences	340545	Lymphoid subset, basophils, eosinophils	Lymphoid subset
CD163	GHI/61	PE	BD Biosciences	556018	Monocytes	Lymphocytes
CD177/ PRV1	MEM-166	PE	Bio-Rad	MCA2045	Granulocytes	Lymphoid
CD200	MRC OX-104	V450	BD Biosciences	562126	B lymphocytes	Monocytes
CD209	DCN46	V450	BD Biosciences	561275	Peripheral blood dendritic cells	Lymphocytes
CD210/ IL10RA	3F9	PE	BD Biosciences	556013	Monocytes	Lymphoid subset
CD300a/c	E59.126	PE	Beckman Coulter	A22328	Monocytes, lymphoid subset	Lymphoid subset
CD366/ HAVCR2/ TIM3	F38-2E2	BV421	Biolegend	345008	Monocytes	Lymphoid subset
CD371/ CLEC12A	50C1	PE	Biolegend	353604	Monocytes	Lymphocytes
CX3CR1	2A9-1	PE	Medical & Biological Laboratories	D070-5	Monocytes	Lymphoid subset
CCL5/ Rantes[1]	21445	PE	R&D Systems	IC278P	Activated NK	Resting lymphocytes

[1] Requires membrane permeabilization

Table S5. Standard marker panel for MRD studies in AML

FITC	PE	PerCP	APC	PE-Cy7	APC-H7	BV421 or v450	BV510 or v500
CD13	CD133	CD34	CD117	CD33	CD45	CD38	HLA-Dr
CD15	CD56	CD34	CD117	CD33	CD45	CD19	CD4
CD7	NG2 (7.1)	CD34	CD117	CD33	CD45	CD11b	HLA-Dr
Mouse IgG2a	Mouse IgG1	CD34	CD117	CD33	CD45	CD41a	Mouse IgG1

Abbreviations: FITC, fluorescein isothiocyanate; PE, phycoerythrin; APC, allophycocyanin; BV, Brilliant Violet.

The following antibodies were used: CD13 (WM-47) from Merck; CD15 (MMA), CD56 (NCM16.2), CD34 (8G12), CD33 (P67.6), CD45 (2D1), CD41a (HIP8), CD19 (HIB19), anti-HLA-Dr (G46-6), Mouse IgG1 (X40), Mouse IgG2a (X39), Mouse IgG2a (G155-178), from BD Biosciences; CD38 (HIT2), CD11b (ICRF44), CD4 (OKT4) from Biolegend; CD133 (AC133/1), CD117 (A3C6E2) from Miltenyi Biotec; CD7 (4H9) from eBioscience; NG2 (7.1) from Beckman Coulter.

Table S6. Suggested marker panel for MRD studies in AML including new markers

FITC	PE	PerCP	APC	PE-Cy7	APC-H7	BV421	BV510
CD44	CD54	CD34	CD117	CD33	CD45	CD96	Reserved for the addition of standard or new markers*
CD97	CD18	CD34	CD117	CD33	CD45	CD99	
Mouse IgG1 or IgG2b	Mouse IgG1 or IgG2b	CD34	CD117	CD33	CD45	Mouse IgG1 or IgG2a	

Abbreviations: FITC, fluorescein isothiocyanate; PE, phycoerythrin; APC, allophycocyanin; BV, Brilliant Violet.

The antibodies used in our laboratory are: CD44 (G44-26/C26), CD97 (VIM3b), CD54 (LB-2), CD34 (8G12), CD33 (P67.6), CD45 (2D1), CD96 (6F9), CD99 (Tu12), (Mouse IgG1 X40), Mouse IgG2a (G155-178), Mouse IgG2b (27-35), from BD Biosciences; CD18 (MEM-48) from GeneTex; CD117 (A3C6E2) from Miltenyi Biotec.

*Alternatively, multiple antibodies conjugated to BV510 can be added simultaneously to identify and exclude irrelevant cells from the analysis.

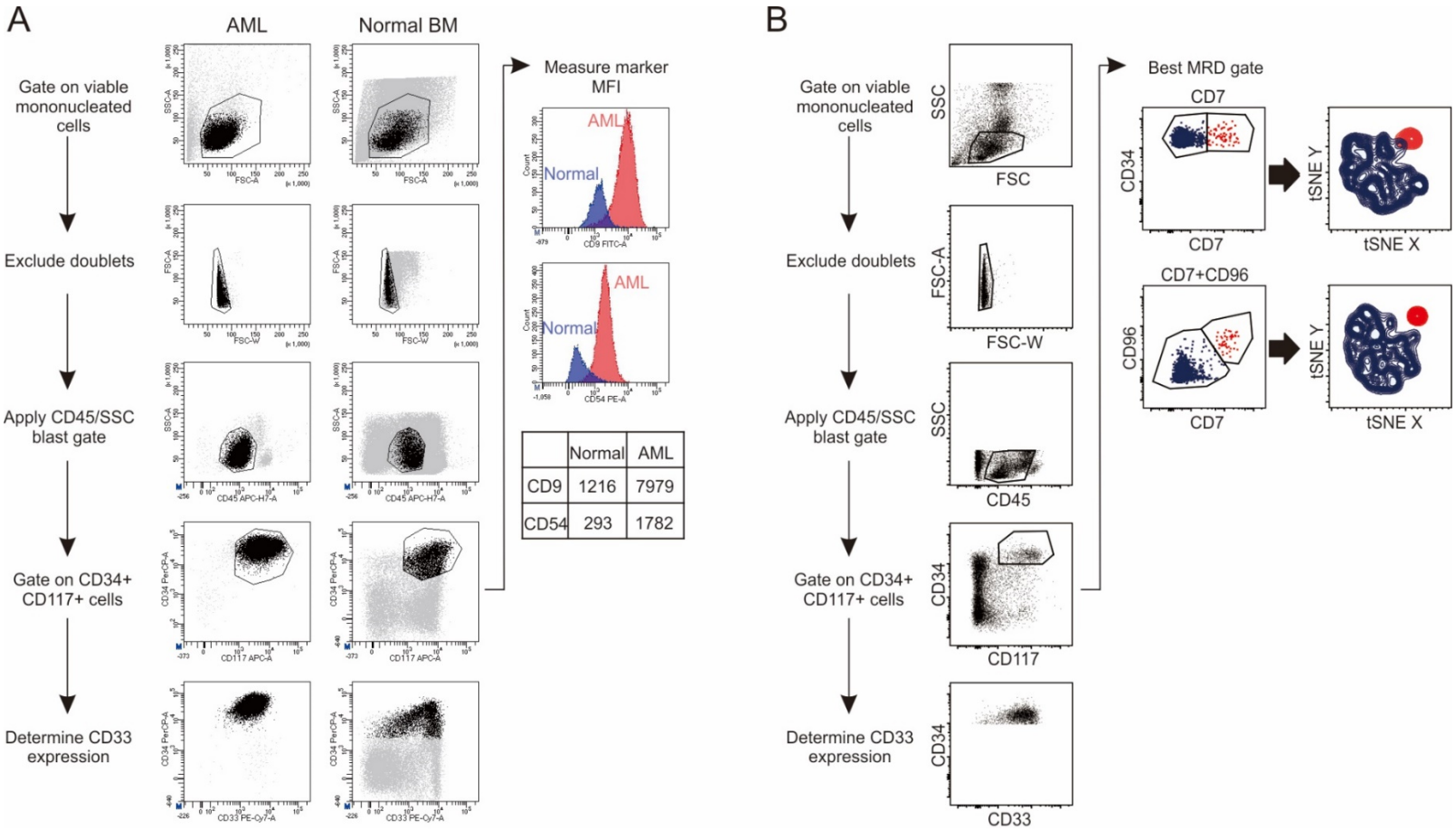

Figure S1. Examples of the flow cytometric analysis procedures used in this study. **A.** Representative comparison of the median fluorescence intensity (MFI) of CD9 and CD54 in one AML sample collected at diagnosis and a bone marrow (BM) sample from a healthy donor. Cells were labelled with CD34, CD117, CD45 and CD33, as well as CD9 and CD54. Analysis was done using DIVA software (BD Biosciences). Calculated MFIs are shown, and were included among those shown in Fig. 2. **B.** Flow cytometric analysis leading to the tSNE contour plots shown in Fig. 7C. A bone marrow sample was collected from a patient with AML after the second cycle of remission induction chemotherapy. Mononucleated cells were labelled with CD34, CD117, CD45 and CD33, in combination with CD7 (the best standard marker in this case) and the new marker CD96. Analysis was done with FlowJo (Tree Star, Ashland, OR).

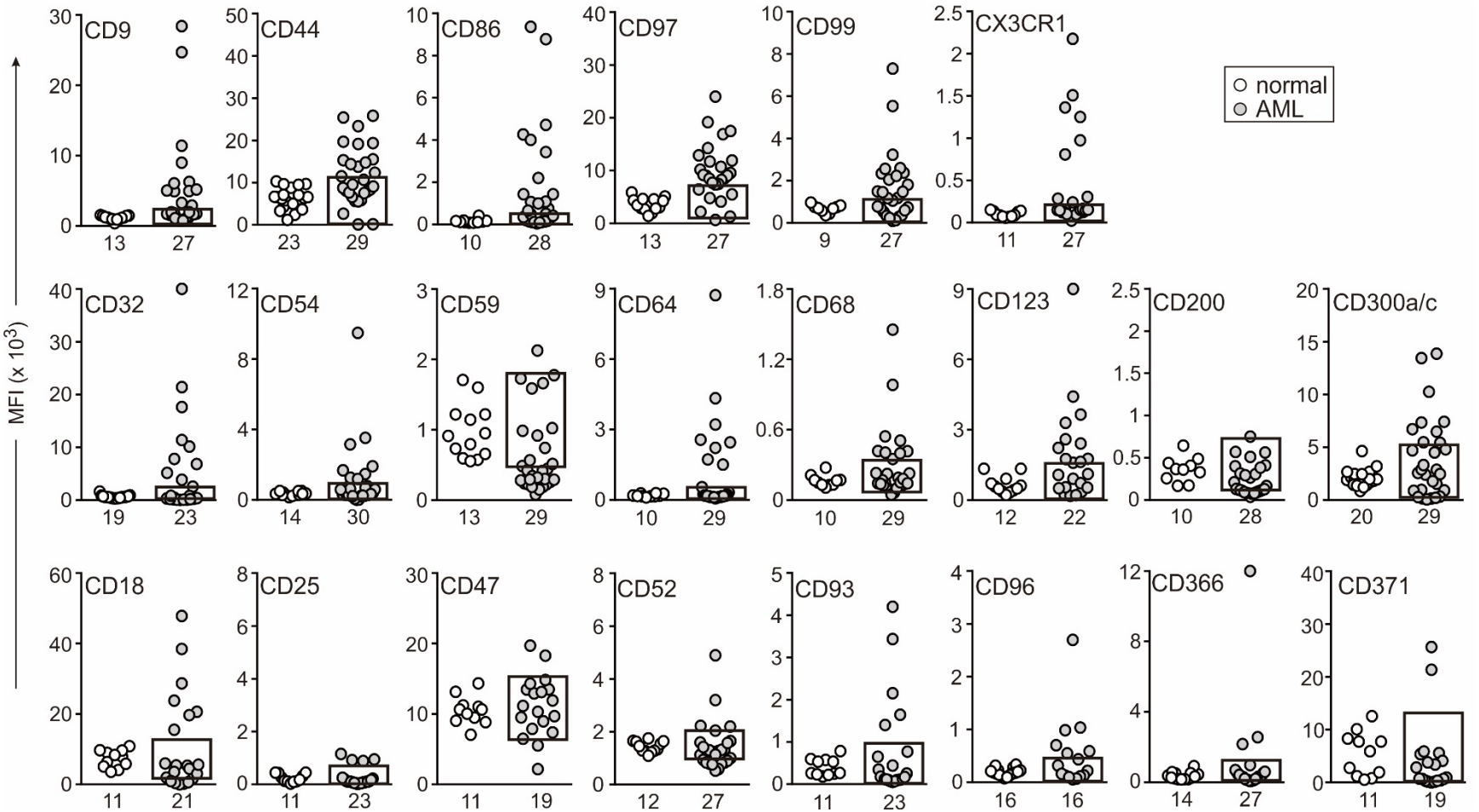

Figure S2. Marker expression in AML cases with <25% CD34+ cells. Markers were tested by flow cytometry in 34 cases of AML with low/absent CD34 and compared to that of CD117+ CD33+ cells from non-leukemic bone marrow samples, including maturing myeloid cells, monoblasts and erythroblasts, and excluding mature monocytes and granulocytes.Plots indicate median fluorescence intensity (MFI) of each marker in normal cells (white circles), and AML cells (gray circles). Boxes on the AML plots indicate upper and lower normal limits. Number of samples studied is shown under each plot. Top row, P <0.001; middle row, P <0.05 but >0.01; bottom row, P >0.05.

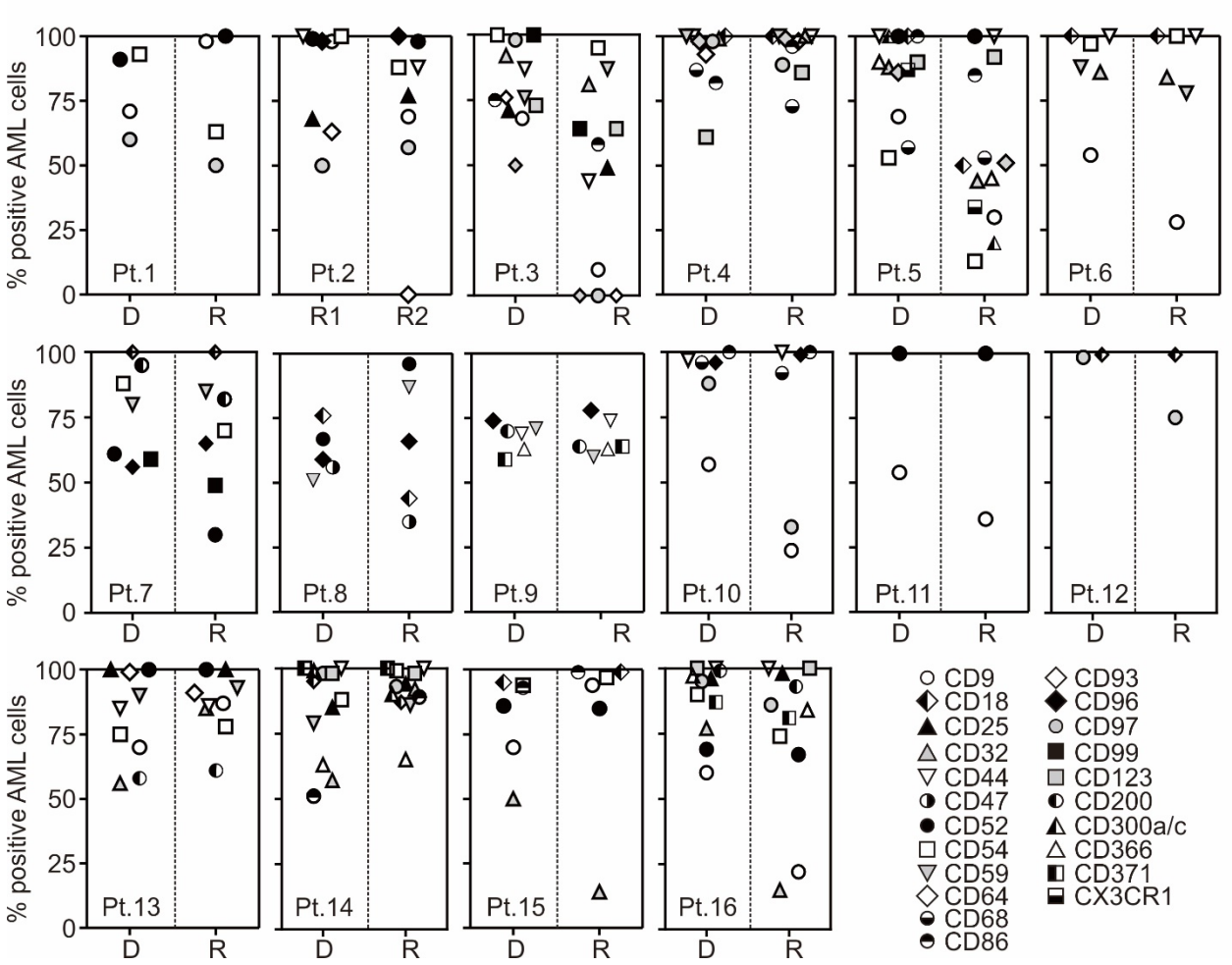

Figure S3. Expression of new markers at relapse. Plots show percentage of AML cells expressing the indicated marker at diagnosis ("D") and relapse ("R), or at first ("R1") and second relapse ("R2") in 16 patients with AML. Each marker studied is indicated by a symbol.

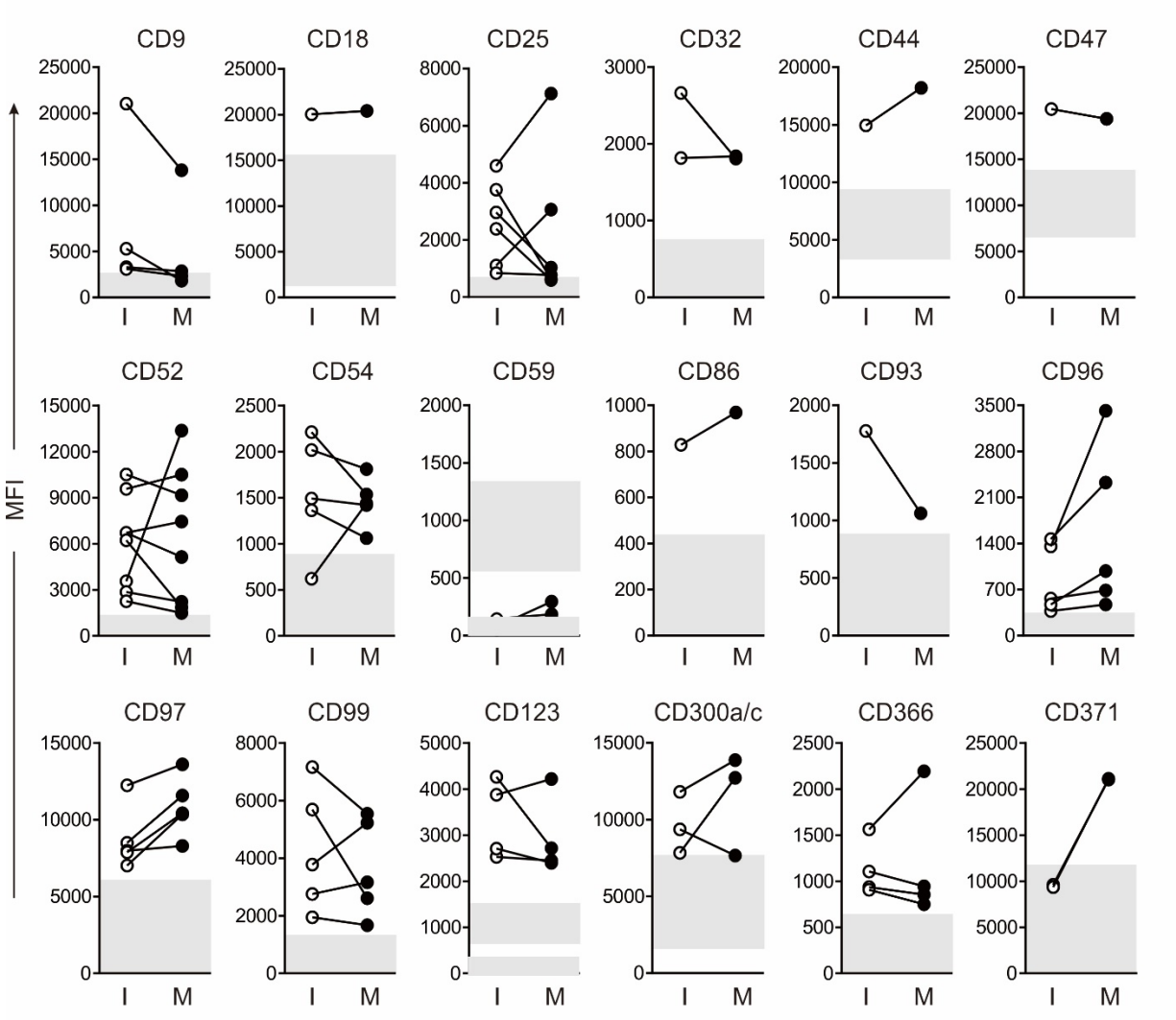

Figure S4. Expression of new markers in AML blasts with the CD34+ CD38 dim/neg immunophenotype ("I"), in comparison with the more mature CD38 bright cell population ("M"). Each symbol is the percent median fluorescence intensity (MFI) of a new marker in the "stem cells" relative to that of the more mature AML cells in the same sample. Gray areas indicate limits of expression in normal CD34+ CD13 and/or CD33+ cells.

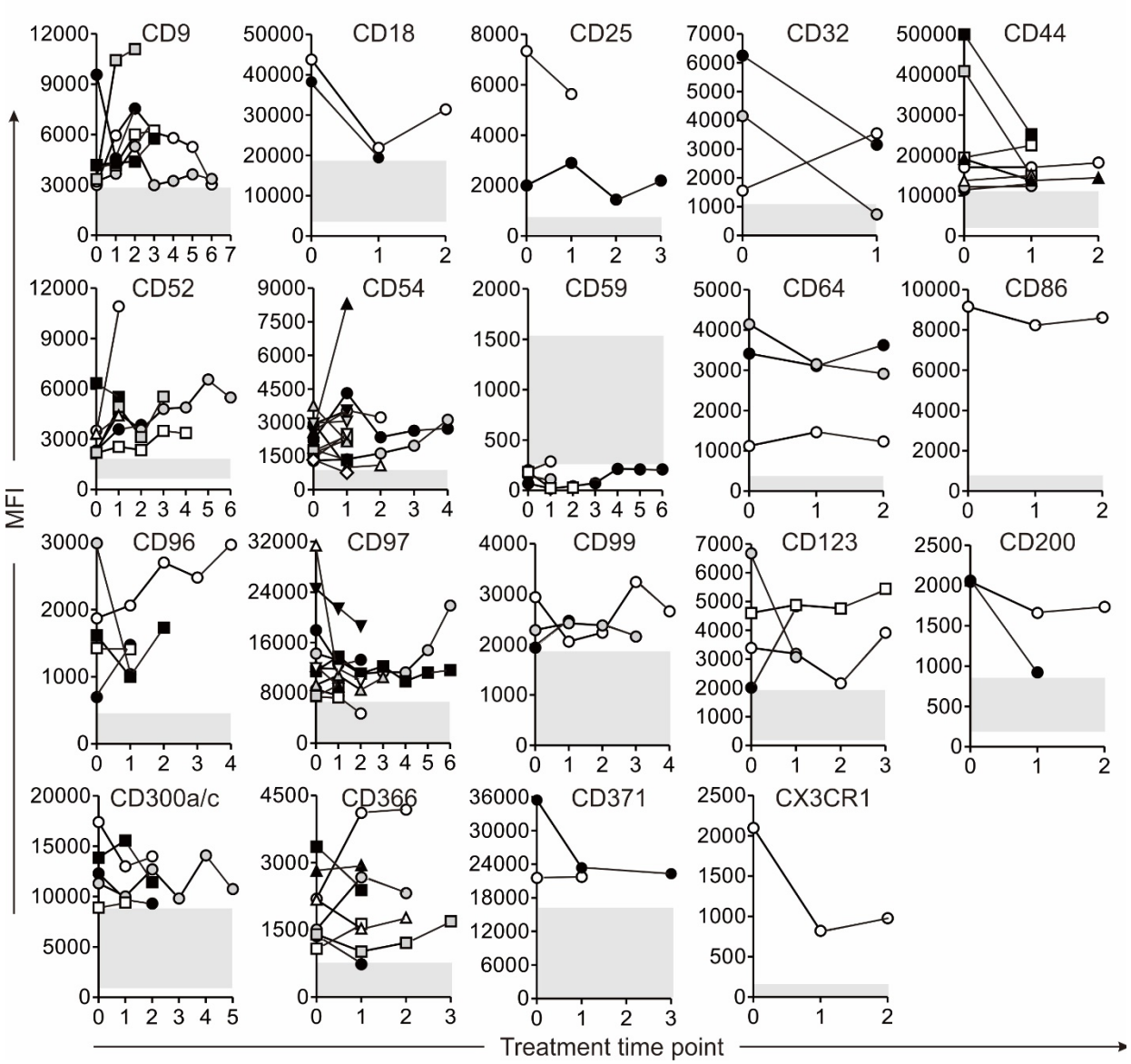

Figure S5. Expression of the new markers on residual AML cells during treatment. Median fluorescence intensity ("MFI") of the indicated new markers as measured at diagnosis ("0") and in subsequent follow-up samples ("1, 2, etc."). Each symbol represents results obtained in samples from one patient. Gray areas indicate limits of expression in normal CD34+ CD13 and/or CD33+ cells.

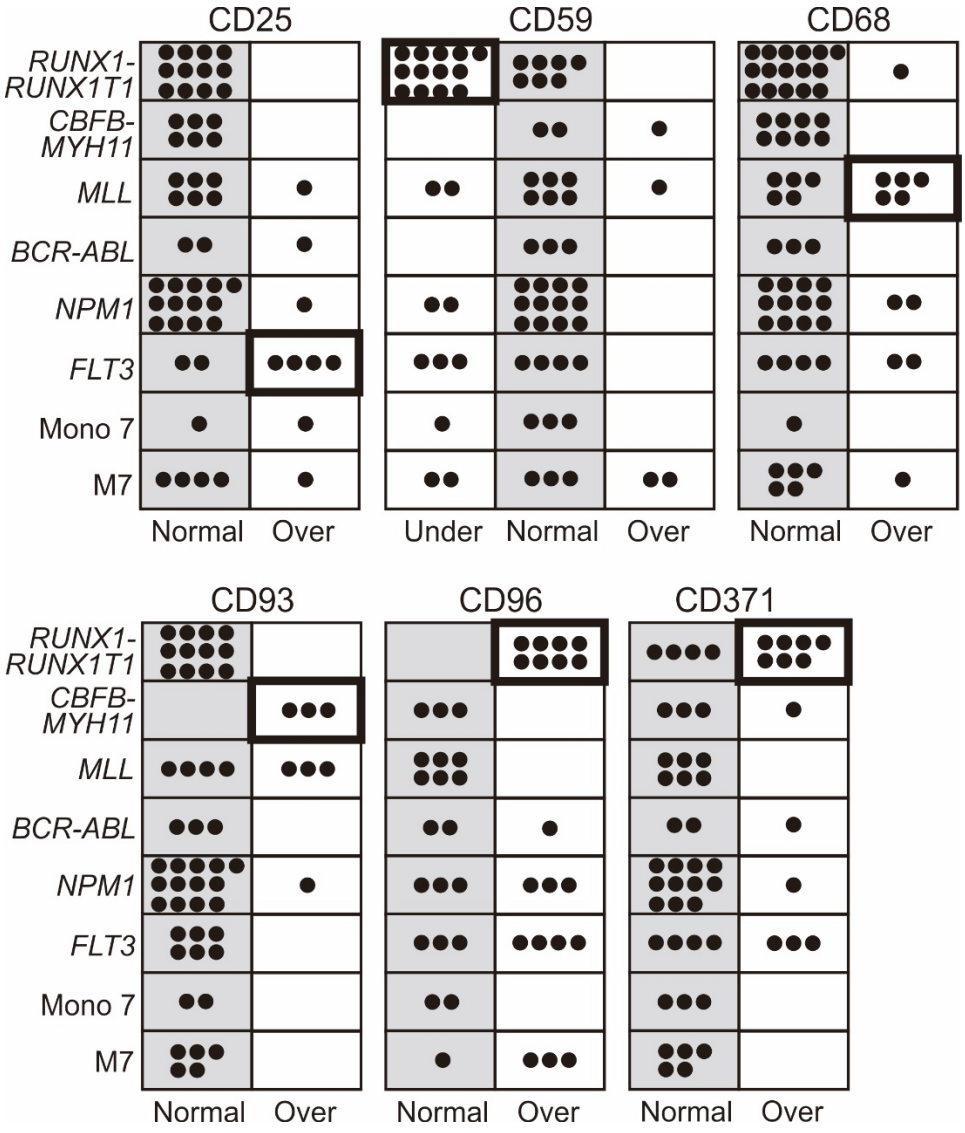

Figure S6. Preferential expression of the new markers in subgroups of AML. Each symbol correspond to an AML diagnostic sample studied with the indicated marker. Markers significantly over- or underexpressed are in boxes with a thicker frame (all P <0.001 by Fischer's exact test). Additional data shown in Fig. 5.