Acute erythroid leukemias have a distinct molecular hierarchy from non-erythroid acute myeloid leukemias

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Supplementary information

Supplementary material and methods

The 8 antibodies used for sorting CD34+ subpopulations were purchased from BD Biosciences: CD45-V500 (HI-30), CD34-PE-Cy7 (8G12), CD38-BV421 (HIT2), CD90-FITC (5E10), CD123-PE (9F5), CD45RA-APC-H7 (HI100), CD10-APC (HI 10a), and 7-Amino-Actinomycin D-PerCPCy5.5 (68981E) as a marker of cell viability. The antigens have been shown to be characteristic of the major types of hematopoietic stem and progenitor cells including CD34+CD38-CD90+CD45RA- hematopoietic stem cells (HSC), CD34+CD38-CD90-CD45RA- multipotent progenitors (MPP), CD34+CD38+CD10+CD45RA+ common lymphoid progenitor (CLP), CD34+CD38+CD123+CD45RA- common myeloid progenitors (CMP), CD34+CD38+CD123+CD45RA+ granulocyte-monocyte progenitors (GMP), CD34+CD38+CD123-CD45RA- megakaryocyte-erythrocyte progenitors (MEP).[7] We tried to discriminate leukemic stem cell (LSC) from HSC based on an hypothetical leukemic phenotype CD34+CD38-CD90+CD45+RA expression. CD45RA is a specific marker for leukemia stem cell sub-populations in AML and aberrant marker expression is a possibility to differentiate LSC from HSC (Kersten et al Bjh 2016).

Seven antibodies were used to sort CD34- subpopulations: CD45-V450 (2D1), CD3-PE (UCHT1), CD19-APC (SJ25C1), CD16-APC-H7 (3G8), CD33-PerCP-Cy5.5 (P67.6), CD235-FITC (11E4B-7-8), and the marker of viability Live/Dead fixable Aqua Stain-Amcyan, and this allowed the recovery of T-lymphocytes, B-lymphocytes, neutrophils/NK, immature granulocytes and erythroblasts, respectively.

Supplementary Table S1. Clinical, biological and molecular data of the 12 patients studied.

Supplementary Table S2.

The 227 genes studied by targeted next generation sequencing on Illumina Miseq using a custom made Hemato v14 panel (HaloPlex Design ID: 27066-1485800404, Agilent Technologies).

Supplementary Figure S1. FACS analysis.

A. Example of FACS analysis in bone marrow cells from an *NPM1*-mutated M6-AML patient.
    1. In the CD34+ fraction, HSC, LSC, MPP, LMPP, GMP, CMP, MEP, and CLP were sorted.
    2. In the CD34- fraction, erythrocytes, granulocytes, PNN, T-lymphocytes and B-lymphocytes were sorted.
B. Example of FACS analysis in bone marrow cells from a *TP53*-mutated M6-AML patient. 1 and 2 same as in A.

Supplementary Figure S2. Comparison of the number of cells in subpopulations of the cellular hierarchy in CD34+ (A) and CD34- (B) fractions. The percentage of living cells is depicted in four groups of patients: 3 *NPM1*-mutated M6-AMLs, 3 *TP53* mutated M6-AMLs, 3 *NPM1*-mutated non-M6-AMLs and 2 *TP53*-mutated non-M6-AMLs.

Supplementary Figure S3. aCGH profiles showing losses at regions of chromosome 17 (including *TP53* and *NF1*) and of chromosome 7 (including *EZH2*) in sorted subpopulations of M6 AMLs (A), and losses of chromosome 17 and chromosome 7 (left) or chromosome arm 7q (right) in sorted subpopulations but not in CD235+ cells of non-M6 AMLs (B). Dotted vertical line is the reference for absence of either loss or gain.

Supplementary Figure S4. Schematic structural organization of EPOR (A) and TRIM10 (B). The localization, type and variant allele frequency (VAF) of the mutations are indicated. In A, the left part shows the exonic organization of the *EPOR* gene and the right part the dimerization of the EPOR receptor bound to its EPO ligand and its JAK2 signal transducer. In B, domains of TRIM10 are indicated; the ring finger, B-box and coiled-coil regions are also present in the PML and RFP proteins, and are conserved in the truncated PML and RFP moieties found in the PML-RARA and RFP-RET oncogenic fusions.

Supplementary Table S1: clinical and biological data of the 12 patients studied by FACS.

WHO 2008	FAB	PATIENTS	Sex	Age (year)	Primary	Secondary	WBC (G/l)	Hemoglobin (g/dl)	Hematocrit (%)	MCV (fl)	reticulocyte count (G/l)	Neutrophils (G/l)	Platelets (G/l)	BLOOD BLASTS (%)	BONE MARROW BLASTS (%)	BONE MARROW ERYTHRO-BLASTS	Dysplasia	WHO 2016	GRAFT		KARYOTYPE	aCGH	aCGH 23S+	KARYOTYPE PROGNOSIS (R-IPSS or AML)	PROGNOSIS (R-IPSS)	PROGNOSIS (ELN 2017)	MOLECULAR CLASSIFICATION
																			AUTO	ALLO							
M6a-AEL	M6a-AEL	HD-2271	M	63	yes	-	1.2	8.2	24	107.5	22.6	0.19	72	0	28	54	MLD	AML-RGA	no	yes	47,XX,+8[2] / 46,XX[21]	normal-like	nd	intermediate	na	intermediate	NPM1 (C1)
	M6a-AEL	HD-2256	F	52	yes	-	2.6	9	26.3	104.3	59	2.05	47	2	13	56	DMK-DGP	MDS-EB-2	no	no	46,XX[20]	normal-like	nd	good	high	-	NPM1 (C1)
	M6a-AEL	HD-2295	F	36	yes	-	6.2	6.2	18.7	102.2	106.5	1.05	42	26	16	68	DMK	MDS-EB-2	no	yes	46,XX[20]	normal-like	nd	good	high	-	NPM1 (C1)
	M6a-AEL	HD-2180	M	68	yes	-	5.6	8.6	nd	97	nd	3.192	88	2	12	61	DGP-DEP	MDS-EB-2	no	no	47,XY,+14[18]/46,XY[2]	14+	nd	intermediate	very high	-	SECONDARY DISEASE (C3)
	M6a-AEL	HD-2170	M	67	yes	-	3	8.7	nd	82	nd	0.75	61	26	10	54	MLD	MDS-EB-2	no	no	46, XY, del(5)(q13q31) [1]/ 44 ~ 46, idem, del(7)(p22q36) [2], -7 [18], del(12)(p11p13), der(15)(?;15)(p11;p11) [16], der(17)(?;18)(q21;q11-12) [16], -18,+mar1 [11], + mar1 [3], + mar2 [3], 1~2 dmin [cp21]	multiple delations: 5q, 7q (EZH2), 12 (ETV6, SOCS2, BTG1), 17 (NF1), 18	nd	very poor	high	-	TP53 (C4)
	M6a-AEL	HD-2322*	M	71	-	MPN	10.1	6.2	19.4	80	228.6	5.45	147	12	20	57	DGP-DEP	AML-MRC	no	no	44,XY,-5,-7,add(16)(q24),-17,-18,-20,+3mar[7] / 45,sl,-8,+add(20)(q17),-mar[2] /44,sdl1,-8,ins(12;?)(q15;?),?del(15)(q12q24)[12]	multiple delations: 5q, 7q (EZH2), 12 (ETV6), 17 (TP53, NF1), 18, 20q	multiple delations: 5q, 7q (EZH2), 15, 16, 17 (TP53, NF1), 18, 20q	unfavorable/ secondary LA	unfavorable/ secondary LA	adverse	TP53 (C4)
	M6a-AEL	HD-2622*	F	75	yes	-	2.9	7.6	25.7	55.3	80.7	0.92	104	25	14	52	MLD	AML-MRC	no	yes	45,XX,?t(2;17)(q33;q25),add(3)(p14) or del(3)(q21;del(5)(q23q35),-12,-16,+mar[7] / 44,sl,-7[4] / 44,sdl1,i(8)(q10),+7add(12)(p24),-mar[6] / 45,sdl2,+i(8)(q10)[4]	multiple deletions: 5q, 7 (EZH2), 12p (ETV6), 16q, 8q+	multiple deletions: 5q, 7 (EZH2), 12p (ETV6), 16q, 8q+	unfavorable	-	adverse	TP53 (C4)
non-M6-AML	AML-M4	HB-1147	M	71	yes	-	5.8	11.7	32.6	104.8	64.8	2.8	88	5	77	6	DGP	AML-RGA	no	yes	46, XY[20]	normal-like	nd	intermediate	na	favorable	NPM1 (C1)
	AML-M2	HB-1289	M	42	yes	-	50.4	9.8	30.7	102.4	nd	3.5	79	84	75	1	DGP	AML-RGA	no	yes	46,XY[20]	normal-like	normal-like	intermediate	na	favorable	NPM1 (C1)
	AML-M1	HD-2683	F	79	yes	-	26.6	9.9	30.7	94	nd	1.3	177	76	69	6	no	AML-RGA	no	no	46,XX[24]	normal-like	nd	intermediate	na	favorable	NPM1 (C1)
	AML-M1	HB-1007	M	73	-	CMML	12.8	13.1	37.4	126	nd	0.3	71	67	82	5	no	AML-NOS	no	no	45, XY, -7, -16, +mar1[8] / 46, idem, +mar2 [12]	multiple deletions : 7q, 8q, 16	normal-like	unfavorable	na	adverse	TP53 (C4)
	AML-M4	HB-1317	M	56	yes	-	2.8	9.7	nd	nd	nd	0.1	30	32	61	16	DGP	AML-NOS	no	yes	44,XY,del(5)(q15q35),del(14)(q12q23),add(15)(p24) or dup(15)(q23q26),-17, -21,?add(21)(p11)[2] / 43,sl,-add(15),+15,+?add(15)(p11)+?der(17;21)(q10;p10), -20, -add(21),add(22)(p11)[2] / 44,sdl1,add(9)(p22?),add(11)(p15) or der(11)(?;11)(p15;q13)[7] / 44,sdl1,+mar[5] / 44,sdl2,del(4)(q31)[3] / 46,XY[1]	multiple deletions: 5q, 11p, 14q, 17, 19q (TSHZ3), 20p, 21; gains: 10q, 21q	deletions (low log ratio): 5q, 14q, 17p	unfavorable	na	adverse	TP53 (C4)

nd: no data

na: non applicable

MPN: Myeloproliferative Neoplasm

CMML: Chronic Myelomonocytic Leukemia

DMK: dymegakaryocytopoiesis defined as 50% or more of cells being dysplastic

DGP: dysgranulopoiesis defined as 50% or more of cells being dysplastic

DEP: dyserythrotopoiesis defined as 50% or more of cells being dysplastic

MLD : multilineage dysplasia represented by dysplasia in more 50% of cells off the three myeloid lineages

AML-MRC: acute myeloid leukemia with myelodysplasia-related changes defined by the presence of history of myelodysplastic syndrome (MDS) and/or multilineage dysplasia (MLD) and/or MDS-related cytogenetics.

AML-RGA: acute myeloid leukemia with recurring genetic abnormality

AML-NOS: Acute myeloid leukemia Not Otherwise Specified

MDS-EB-2: Myelodysplastic Syndromes with Excess of Blasts type II

HD-2322*: AML was supposed to be secondary to MPN because of the dystrophic megacaryocytes on bone marrows smears and of the mutation of *JAK2*

Supplementary Table S2: Genes studied by sequencing

HEMATO V14 used on Illumina Miseq_ HaloPlex Design ID: 27066-1485800404

ABCC9
ABL1
AKT1
ANKRD11
ANKRD26
APC
ARIH1
ARNTL
ASXL1
ASXL2
ASXL3
ATG2B
ATM
ATRX
BAP1
BARD1
BCL11B
BCOR
BCORL1
BCR
BMI1
BRAF
BRCC3
CALR
CBL
CBLB
CDC25C
CDH23
CDKN1B
CDKN2A
CDKN2B
CDYL
CEBPA
chr16:820,183-820,277
chr17:74,732,532-74,732,630
chr4:153,258,807-153,259,248
chr7:139,102,209-139,112,272
chr8:144,895,127-144,895,212
CLSTN1
COPA
CREBBP
CRNKL1
CSF3R
CSMD1
CSNK1A1

CTCF
CUX1
CXCR4
DAXX
DDX11
DDX3X
DDX41
DDX54
DHX29
DNAH2
DNMT1
DNMT3A
DOCK2
DOK1
DOK2
E2F2
EED
EGLN1
EP300
EPOR
ERG
ETNK1
ETNK2
ETS1
ETS2
ETV6
EZH2
FAT1
FAT4
FBXW7
FES
FLT3
FOXP1
GATA1
GATA2
GATA3
GFI1
GFI1B
GNAS
GNB1
GSKIP
HES1
HHEX
HUWE1
IDH1
IDH2
IDH3B
IKZF1
IL7R
IRF1

JAK1
JAK2
JAK3
JARID2
KANSL1
KANSL2
KANSL3
KDM3B
KDM5A
KDM5C
KDM6A
KIF17
KIT
KLF1
KLHL6
KMT2A
KMT2D
KRAS
LAMB4
LDB1
LEF1
LMO1
LMO2
LMO3
LUC7L2
LYL1
MAFK
MAML1
MAPK1
MDM2
MECOM
MED12
MEF2C
MEIS1
MFSD11
MLL3
MPL
MSI2
MYBL2
MYC
MYD88
NAMPT
NCSTN
NF1
NFE2
NFIA
NIPBL
NOTCH1
NOTCH2
NOTCH3

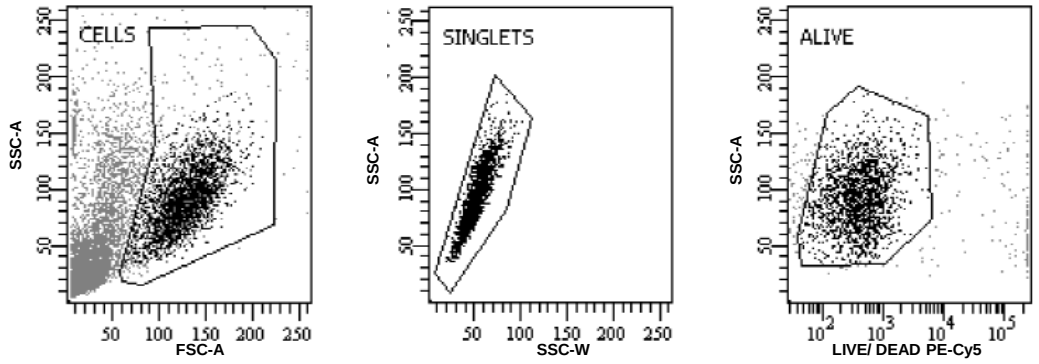
NOTCH4
NPM1
NRAS
NSD1
OBSCN
OGT
PAX5
PDGFRA
PDS5B
PHF6
PIAS2
PIK3R2
PIM1
PIM2
PIM3
PPM1D
PRDX2
PRMT5
PRPF8
PTCH1
PTEN
PTK2B
PTP4A3
PTPN1
PTPN11
PTPRT
RAC1
RAC2
RAD21
RB1
RBBP6
RBL1
RCOR1
RCOR2
RCOR3
RIT1
ROBO1
ROBO2
RRAS
RRAS2
RUNX1
SCRIB
SETBP1
SETD2
SF3B1
SH2B3
SIL1
SMC1A
SMC3
SOCS2

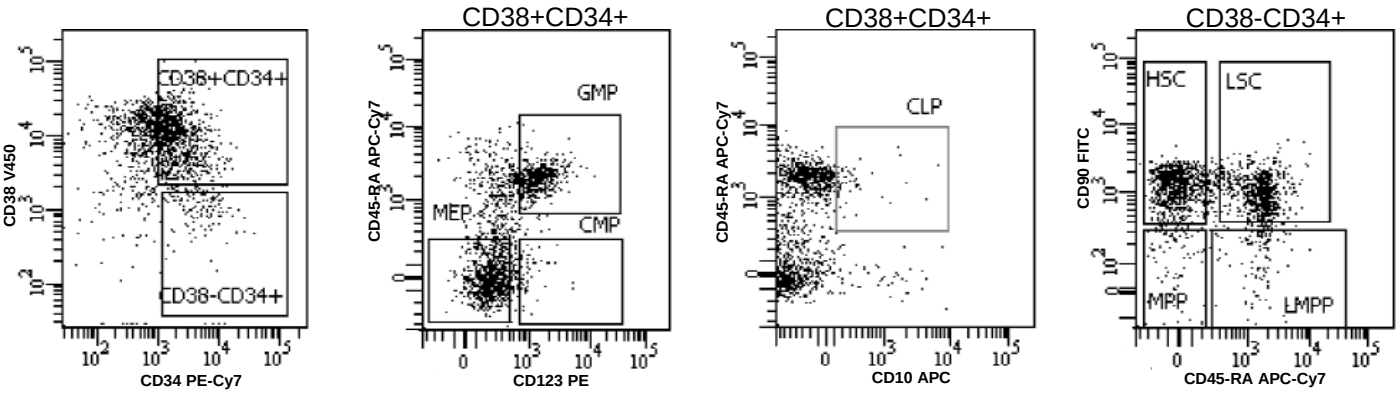
SPI1
SPIB
SRSF2
STAG1
STAG2
STAT3
STAT5A
STAT5B
SUZ12
TAL1
TAL2
TCF12
TCF3
TCF7
TET1
TET2
TET3
TP53
TRIM10
TRIM33
TYK2
U2AF1
USP9X
WDR5
WHSC1
WT1
ZMYM3
ZNF717
ZRSR2

Supplementary Figure S1: Sorting analysis

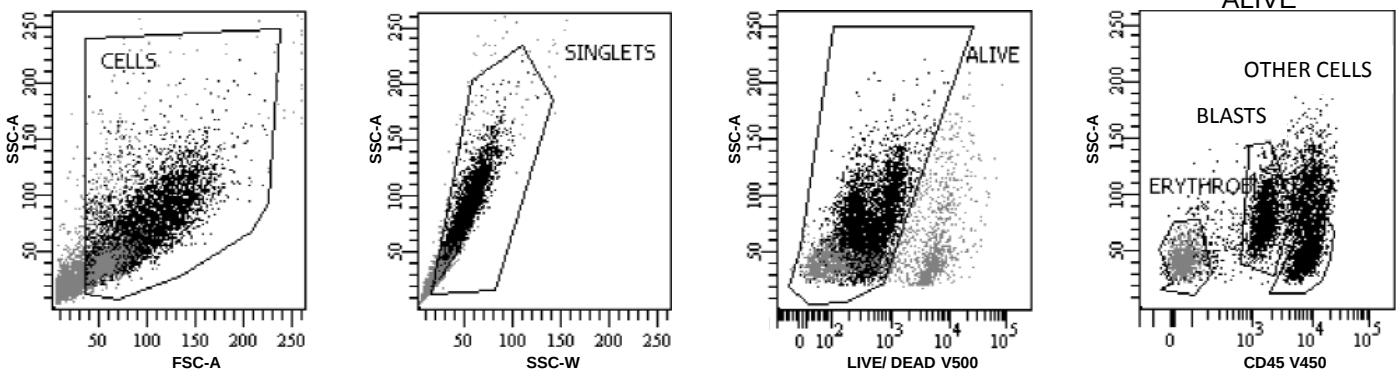
A. HD-2295: *NPM1*-mutated M6-AML

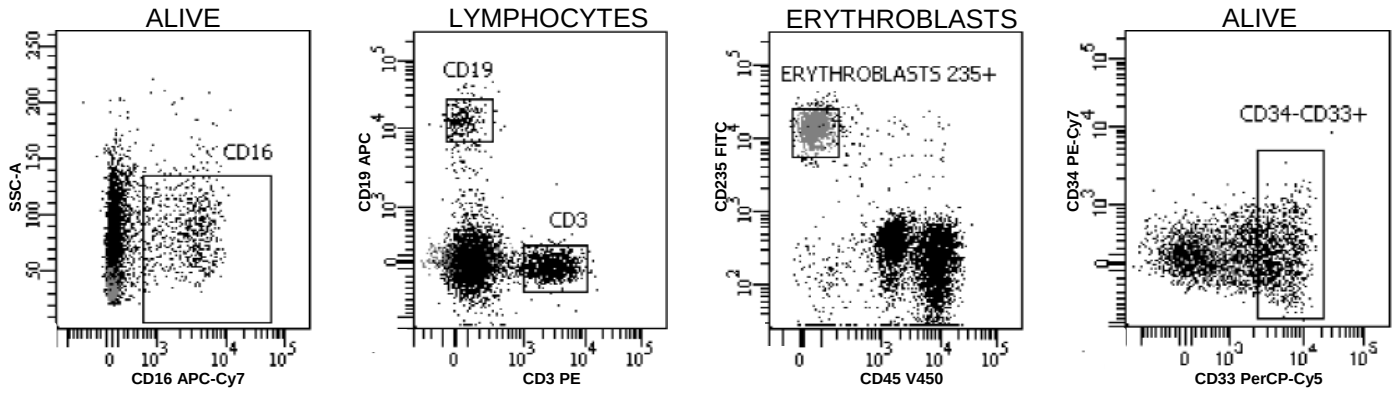
1. CD34+ fraction





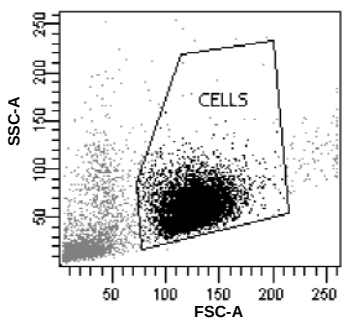
2. CD34- fraction

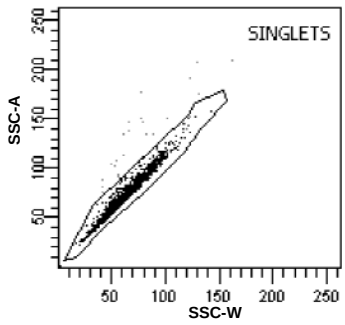


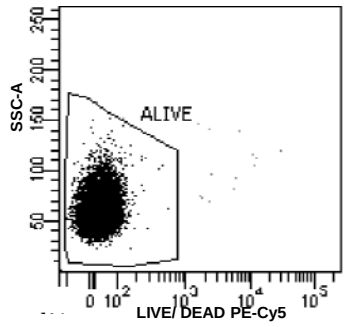


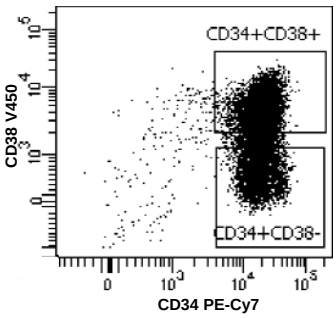
B. HD-2170: *TP53*-mutated M6-AML

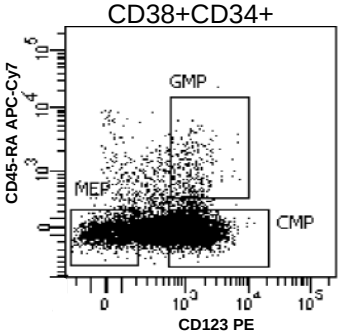
1. CD34+ fraction

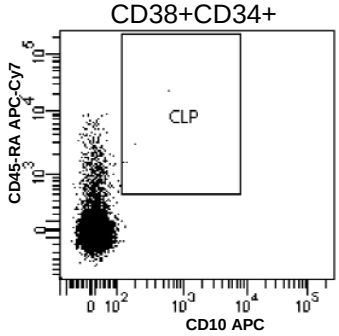


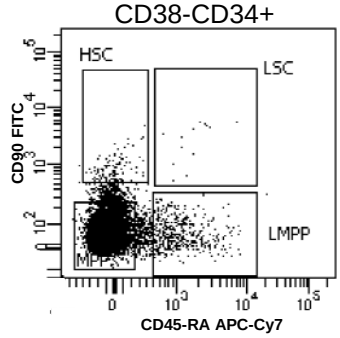




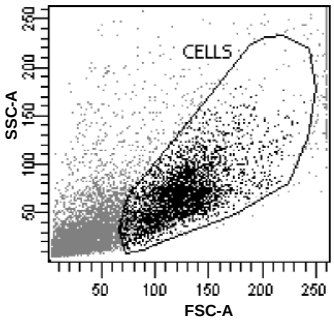


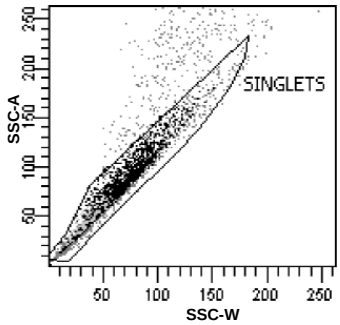


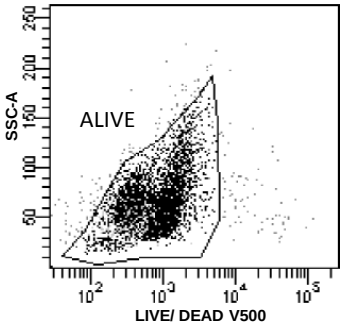


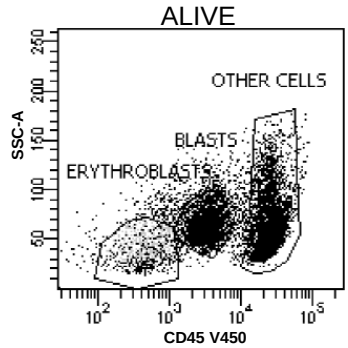


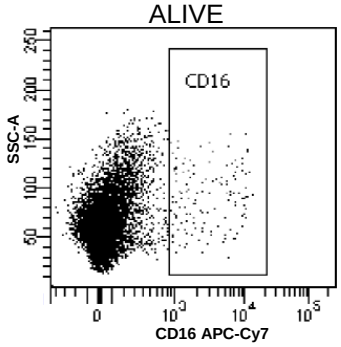
2. CD34- fraction

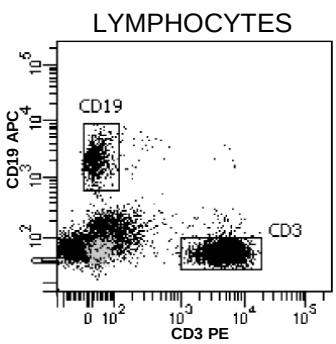


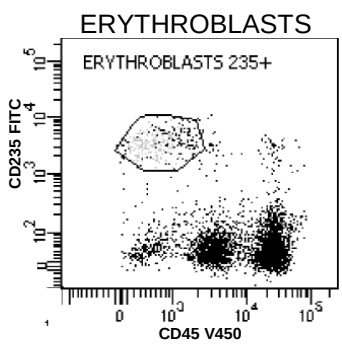


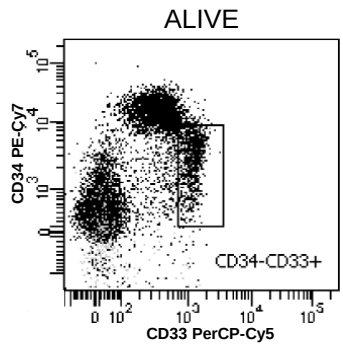








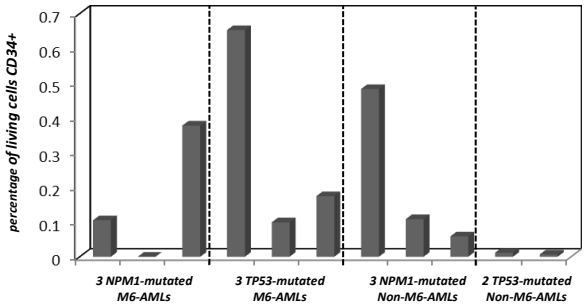




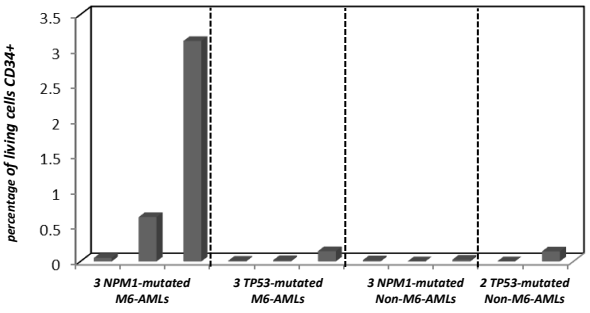
Supplementary Figure S2: Percentage of cells in each hematopoietic compartment

A. CD34+ fraction

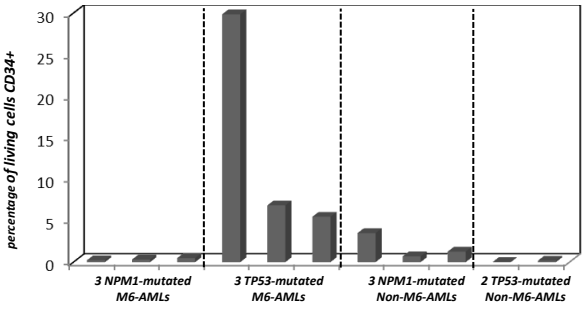
1. HSC



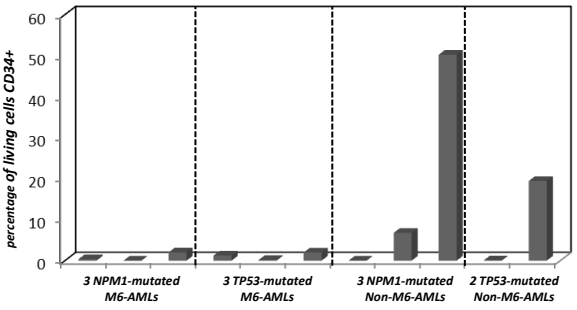
2. LSC



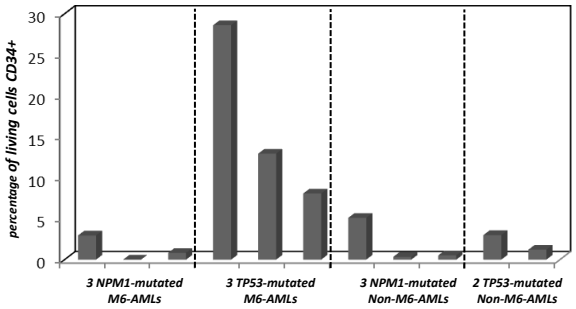
3. MPP



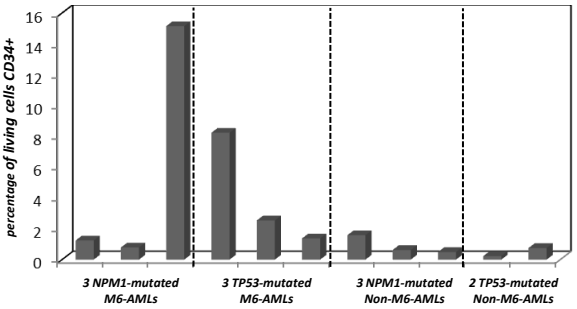
4. LMPP



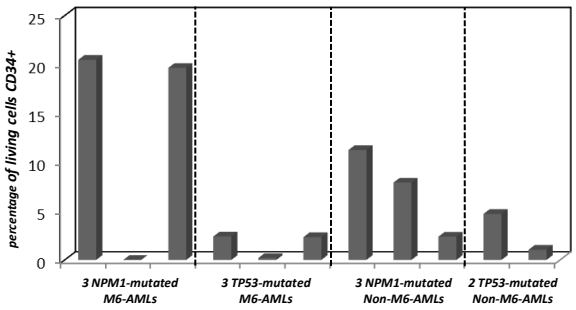
5. CMP



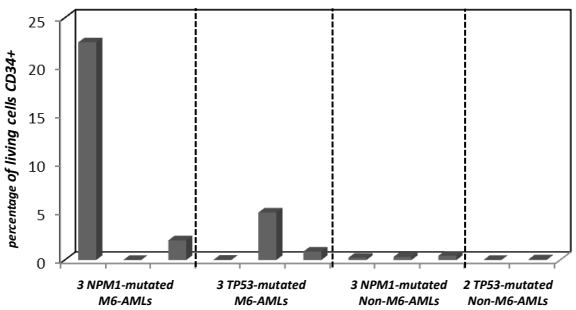
6. MEP



7. GMP

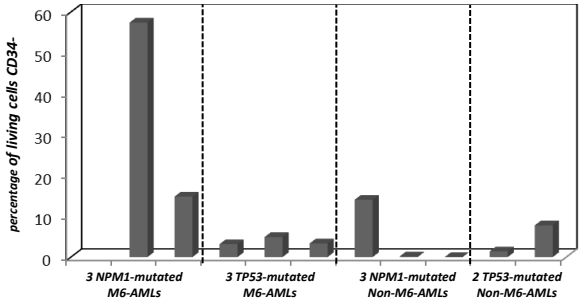


8. CLP

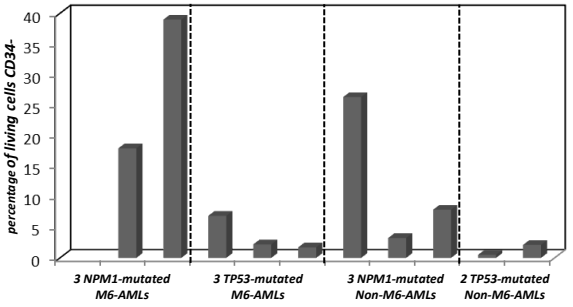


B. CD34- fraction

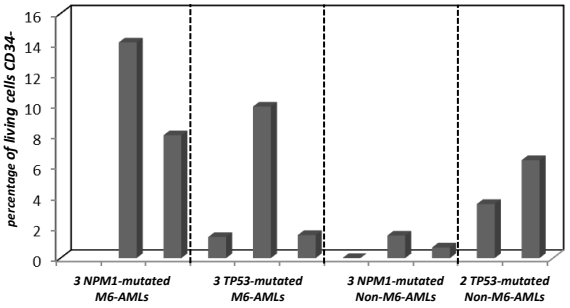
1. ERYTHROBLASTS



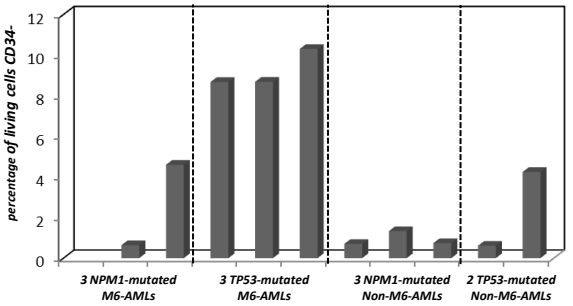
2. GRANULOCYTES



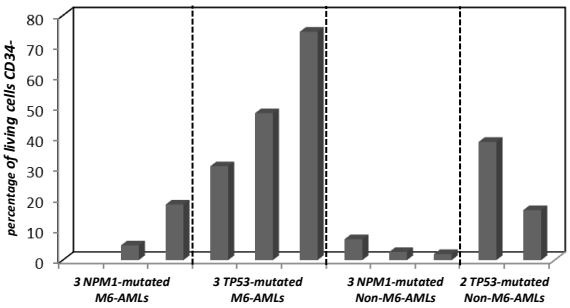
3. POLYNUCLEAR CELLS



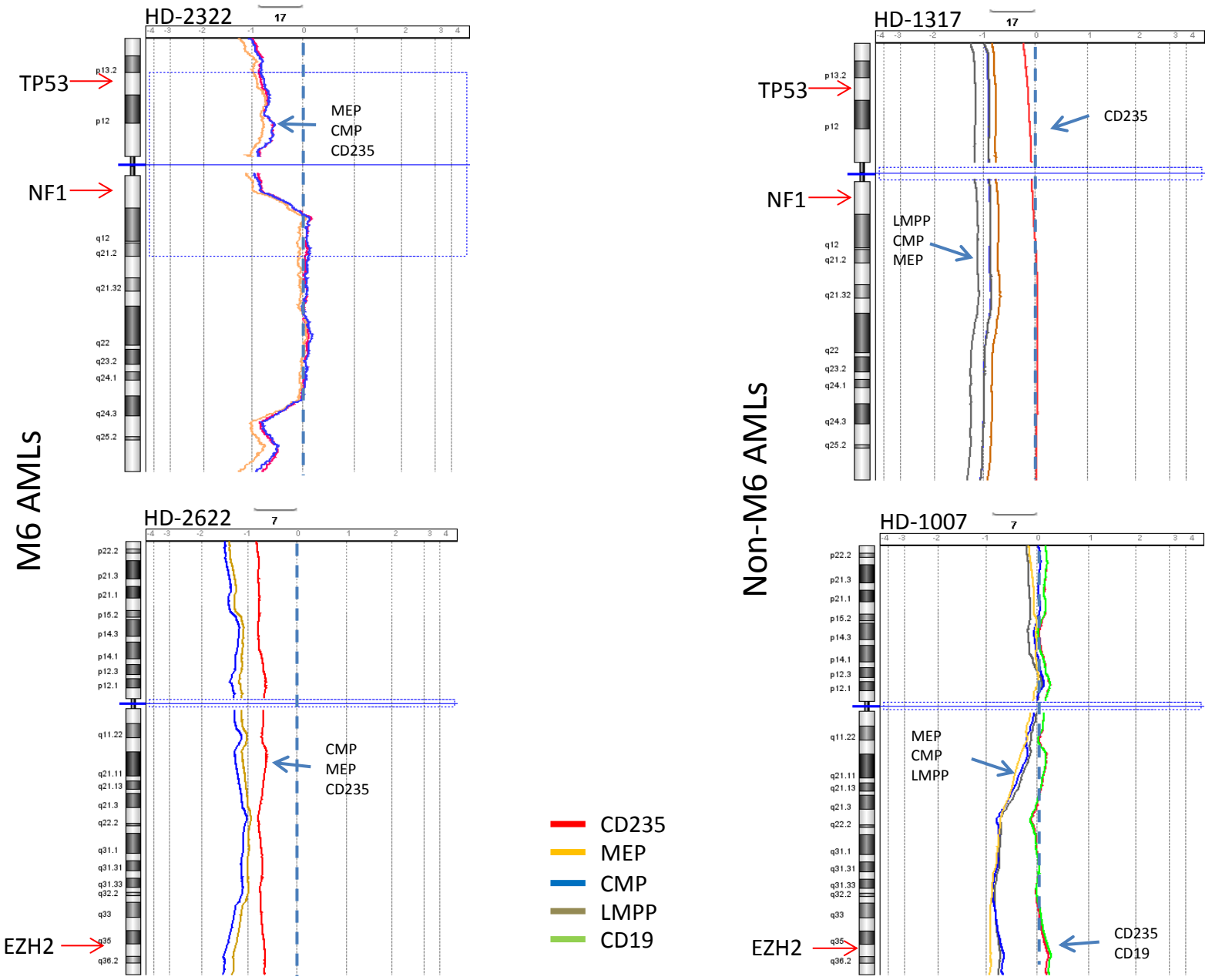
4. B-LYMPHOCYTES



5. T-LYMPHOCYTES



Supplementary Figure S3: aCGH profiles of chromosome 7 and chromosome 17 in sorted subpopulations of M6-AMLs and non-M6 AMLs.



Supplementary Figure S4