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Supplemental Information

Development Refractoriness of MLL-Rearranged Human B Cell Acute Leukemias to Reprogramming into Pluripotency

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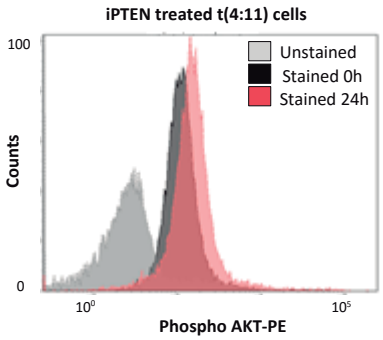
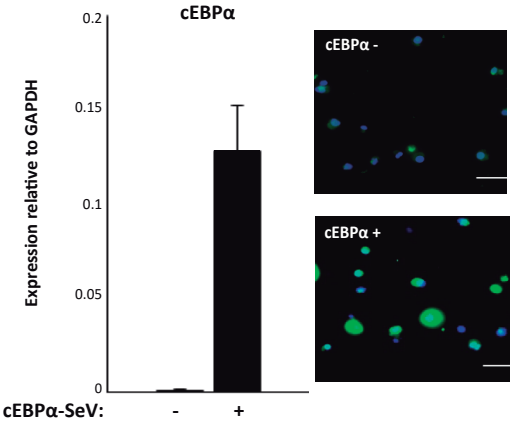
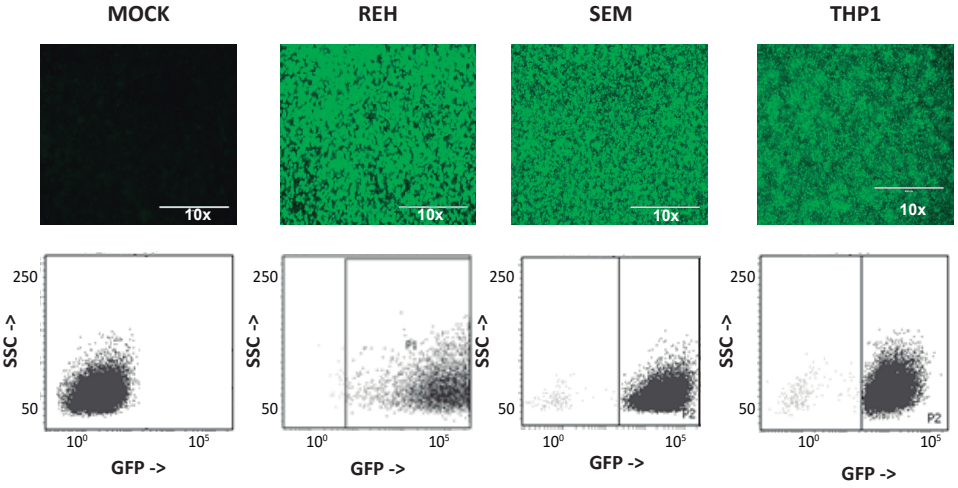
A


iPTEN treated t(4:11) cells
Unstained
Stained 0h
Stained 24h
Counts
Phospho AKT-PE

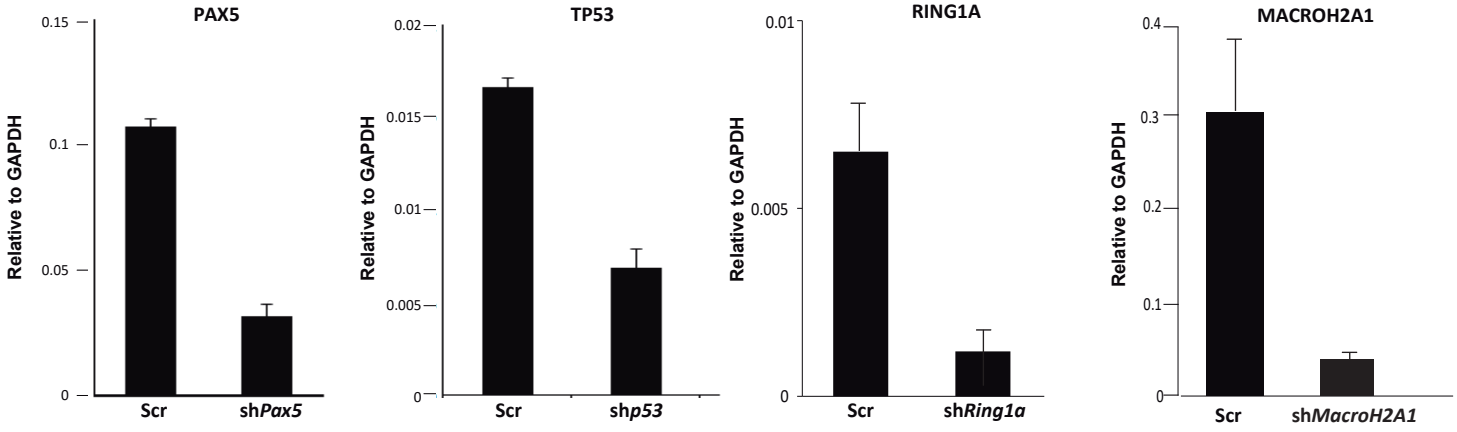
B


cEBPα
Expression relative to GAPDH
cEBPα -
cEBPα +
cEBPα-SeV:

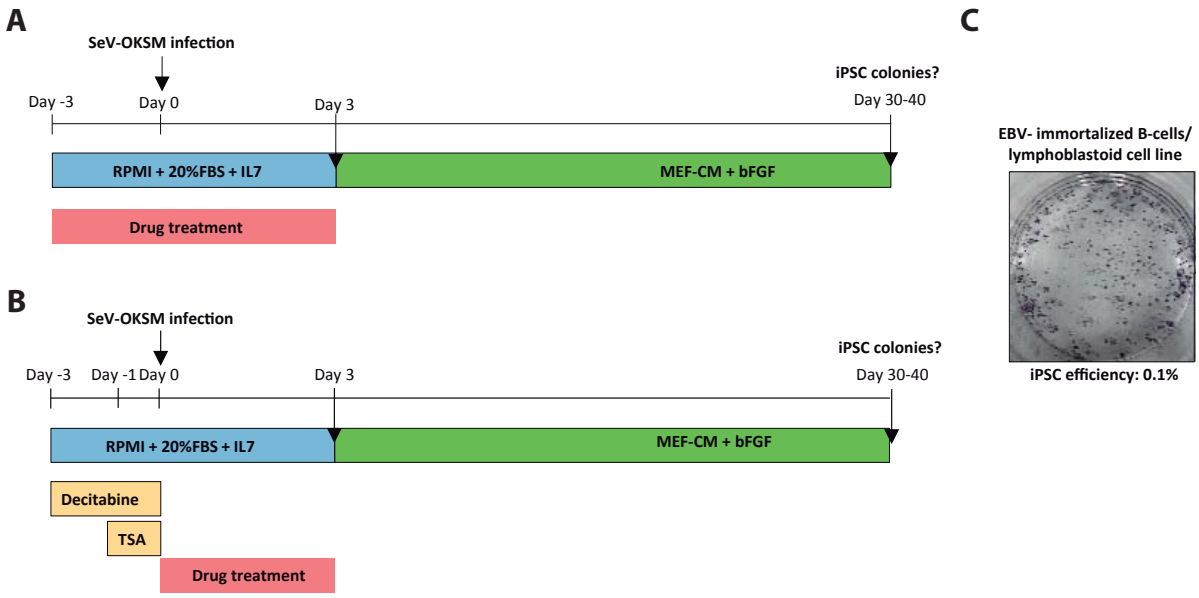
C


MOCK
REH
SEM
THP1
10x
SSC ->
GFP ->
P1
P2

D


PAX5
TP53
RING1A
MACROH2A1
Relative to GAPDH
Scr
shPax5
shp53
shRing1a
shMacroH2A1

Muñoz-Lopez A et al. Figure 2S



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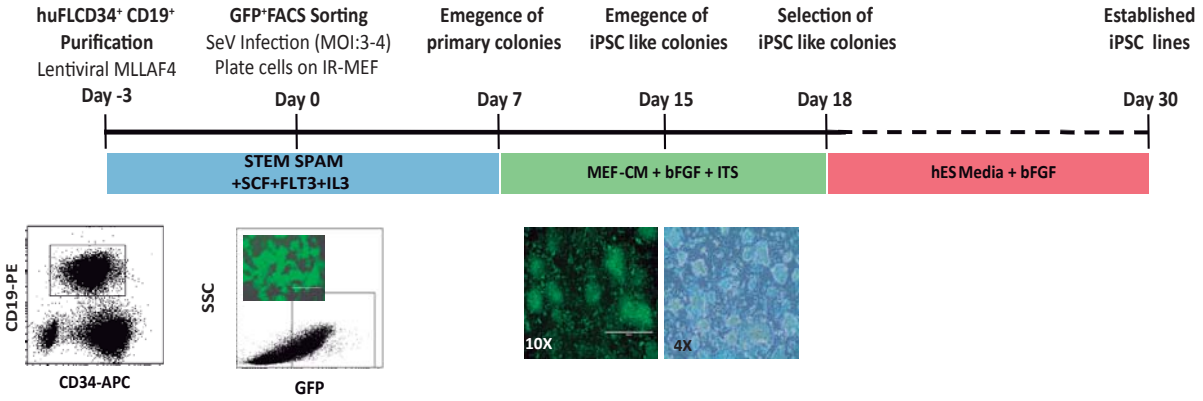
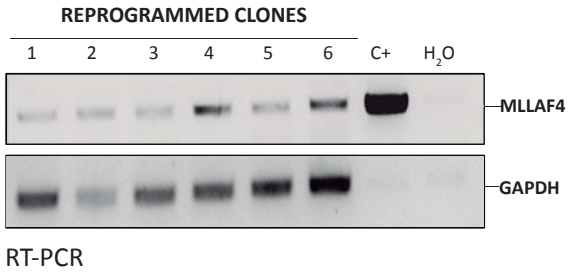
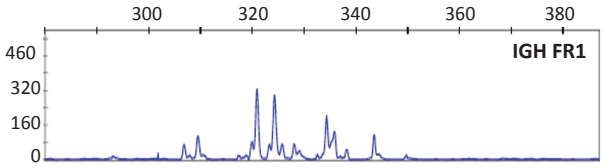

huFLCD34+ CD19+
Purification
Lentiviral MLLAF4
Day -3
GFP+FACS Sorting
SeV Infection (MOI:3-4)
Plate cells on IR-MEF
Day 0
Emegence of
primary colonies
Day 7
Emegence of
iPSC like colonies
Day 15
Selection of
iPSC like colonies
Day 18
Established
iPSC lines
Day 30
STEM SPAM
+SCF+FLT3+IL3
MEF-CM + bFGF + ITS
hES Media + bFGF
CD19-PE
CD34-APC
SSC
GFP
10X
4X

B


REPROGRAMMED CLONES
1
2
3
4
5
6
C+
H2O
MLLAF4
GAPDH

RT-PCR

C


300
320
340
360
380
460
320
160
0
IGH FR1

LEGENDS TO SUPPLEMENTAL FIGURES

Figure S1: Functional validation of PTEN chemical inhibition, cEBPα infection, PAX5, TP53, RING1A and MACROH2A1 shRNA knocks-down. (A) Flow cytometry confirming AKT phosphorylation upon PTEN inhibition (bpV-HOPic). **(B)** qRT-PCR and immunofluorescence confirming cEBPα expression (left) and SeV infection (right), respectively. **(C)** Representative fluorescence images (top) and flow cytometry (bottom) of the indicated ALL cell lines stably transduced with shRNA lentiviral vectors for PAX5 and TP53. **(D)** qRT-PCR showing 60-85% downregulation of *Pax5*, *p53*, *Ring1a* and *macroH2A1* transcripts in ALL cell lines (n=3 independent experiments).

Figure S2: SeV-OSKM-mediated reprogramming of immortalized B-cell lines. (A, B) Schemes of the reprogramming strategy for immortalized B-cell ALL cell lines in the presence of individual treatments (A) or individual treatments after a decitabine+TSA priming (B). **(C)** TRA-1-60 staining revealing efficient iPSC generation from EBV-immortalized normal B lymphocytes.

Figure S3: Efficient reprogramming of MLL-AF4-expressnig CD34+CD19+ B-cell progenitors. (A) MLL-AF4 lentiviral transduction of CD34+CD19+ B-cell progenitors and reprogramming strategy. **(B)** The majority of the iPSC clones expressed MLL-AF4 by RT-PCR. **(C)** MLL-AF4-expressing iPSCs carry VDJH Imunoglobulin gene rearrangements, confirming the B-cell identity.

Table S1: Human iPSC lines reported to date from hematological cancer cells

Disease	Hematologic Lineage Affected	Reprogramming Method	Reference
JAK2-V617F+ Myeloproliferative disorder (PV/PMF)	Erythroid	Retrovirus OKSM	Ye *et al*. 2009
BCR-ABL+ Chronic myeloid leukemia	Myeloid	Retrovirus OSKM	Carette *et al.* 2010
		Episomal vector OSLN**	Hu *et al*. 2011
		Retrovirus OSKM	Kumano *et al*. 2012
		SIN-lentivectors OSK1 & Mshp5	Bedel A *et al.* 2013
Juvenile myelomonocytic leukemia	Myeloid	Lentivirus OKSM	Gandre-Babbe *et al*. 2013
8p11 Myeloproliferative syndrome	Myeloid	Retrovirus OKSM	Yamamoto S *et al.* 2015

OKSM: Oct4, Klf4, Sox2, cMyc; OSLN: Oct4, Sox2, Lin28, Nanog; PV: Polycytemia Vera; PMF: Primary Myelofibrosis

Table S2: Primer sequences and antibodies used in this study for PCR and flow cytometry.

CD45	FITC-conjugated (555482), Becton Dickinson	**Antibodies**
CD45	APC-conjugated (130-080-202), Miltentyi	
CD34	APC-conjugated (130-081-001), Miltentyi	
CD19	PE-conjugated (130-091-328), Miltentyi	
CD10	PE-Cy5-conjugated (555376), Becton Dickinson	
HLA-ABC	FITC-conjugated (555552), Becton Dickinson	
Phospho AKT	PE-conjugated (560378), Becton Dickinson	
SSEA-3	PE-conjugated, (560237) Becton Dickinson	
SSEA-4	V450-conjugated (561156), Becton Dickinson	
Tra-1-60	BV510-conjugated (563188), Becton Dickinson	
AF4-MLL Fw	genomic: Patient-specific breakpoint*	**Primers**
AF4-MLL Rv	genomic: Patient-specific breakpoint*	
ETV6-RUNX1 Fw	genomic: Patient-specific breakpoint*	
ETV6-RUNX1 Fw	genomic: Patient-specific breakpoint*	
MLL-AF4 Fw	RNA: 5'-CAGAGCAAACAGAAAAAAGTG-3'	
MLL-AF4 Rv	RNA: 5'-GTTCTGGAAGGGACTGTGGA-3'	
PAX5 Fw	RNA: 5'-CCTACTATTATAGCGCTGCCG-3'	
PAX5 Rv	RNA: 5'-TCAGTGACGGTCATAGGCAGT-3'	
P53 Fw	RNA: 5'-CACAGCACATGACGGAGGTT-3'	
P53 Rv	RNA: 5'-TCCTTCCACTCGGATAAGATG-3'	
OCT4 Fw	RNA 5'-GGGTTTTTGGGATTAAGTTCTTCA-3'	
OCT4 Rv	RNA: 5'-GCCCCCACCCTTTGTGTT-3'	
SOX2 Fw	RNA: 5'-CAAAAATGGCCATGCAGGTT-3'	
SOX2 Rv	RNA: 5'-AGTTGGGATCGAACAAAAGCTATT-3'	
DNMT3B Fw	RNA: 5'-GCTCACAGGGCCCGATACTT-3'	
DNMT3B Rv	RNA: 5'-GCAGTCCTGCAGCTCGAGTTTA-3'	
CRIPTO Fw	RNA: 5'-CGGAACTGTGAGCACGATGT-3'	
CRIPTO Rv	RNA: 5'-GGGCAGCCAGGTGTCATG-3'	
NANOG Fw	RNA: 5'-ACAACTGGCCGAAGAATAGCA-3'	
NANOG Rv	RNA: 5'-GGTTCCCAGTCGGGTTCAC-3'	
REX1 Fw	RNA: 5'-CCTGCAGGCGGAAATAGAAC-3'	
REX1 Rv	RNA: 5'-GCACACATAGCCATCACATAAGG-3'	
SeV Fw	RNA: 5'-GGATCACTAGGTGATATCGAGC-3'	
SeV Rv	RNA: 5'-ACCAGACAAGAGTTTAAGAGATATGTATC-3'	
Hu GAPDH Fw	genomic: 5'-GAAGGTGAAGGTCGGAGTC-3'	
Hu GAPDH Rv	genomic:5'-GAAGATGGTGATGGGATTTC-3'	
Hu B-actin Fw	genomic: 5'-GATGGCCACGGCTGCTT-3'	
Hu B-actin Rv	genomic: 5'-AGGACTCCATGCCCAGGAA-3'	
shPAX5	5'-CCGGTGATGTAGACAATAATT-3'	**shRNA seq**
shTP53	5'-GACTCCAGTGGTAATCTACT-3'	
shRING1a	5'-CCCAGAAGAAGCCTGTATCTAA-3'	
shMacroH2A1	5'-CCCAGAAGAAGCCTGTATCTAA-3'	

*Please see Meyer et al. Leukemia 2013.

Table S3: Source and concentration of chemicals used in this study

Chemical	Expected Function in blasts	Concentration / Source
Ascorbic acid	Senescence inhibitor - p53 repressor, promotes DNA demethylation through TET1 activity	50µg/mL / Selleckchem
Sodium Butyrate	HDAC inhibitor, acetylation in a permissive histone mark that enhances reprogramming	0.5mM / Selleckchem
Valproic Acid	HDAC inhibitor, acetylation in a permissive histone mark that enhances reprogramming	2mM / Selleckchem
LiCl	GSK3 inhibitor / Wnt signaling activator, upregulate and enhances the transcriptional activity of NANOG and downregulates LSD1	5mM / Selleckchem
Sodium Salicylate	NF-kB & Dot1L inhibition, depletion of senescence barriers for reprogramming	0.2 mM / Selleckchem
Decitabine	Demethylating agent, genome-wide demethylation is associated with permissive chromatin status and therefore with reprogramming efficiency increase	20µM / Selleckchem
SGC0946	Dot1L inhibition, loss of H3K79me2 downregulates the expression of MLL fusions	0.1µM / Selleckchem
EPZ004777	Dot1L inhibition, loss of H3K79me2 downregulates the expression of MLL fusions	3.3µM / Epizyme
MI2	Menin-MLL inhibition, disruption of the expression and epigenetic leukemic pattern	1.5µM / Selleckchem
bVp(OH)pic	PTEN inhibitor, promotes cell proliferation an B-cell survival	10µM / Selleckchem
Trichostatin A	HDAC inhibitor, acetylation in a permissive histone mark that enhances reprogramming	2µM / Sigma Aldrich
Chaetocin	EZH1 inhibitor, regulates H3K27me3 at lineage-specific genes	0.3µM / Selleckchem
GSK126	EZH2 inhibitor, regulates H3K27me3 at lineage-specific genes	5µM / BioVision
DZNep	EZH2 inhibitor, regulates H3K27me3 at lineage-specific genes	5µM / Selleckchem
JQ1	BRD4 inhibitor, disruption of the leukemic cell identity by transcriptional pause induction	0.1µM / BPS Bioscience
Flavopiridol	CDK-P-TEFb inhibitor, induces transcriptional pause disrupting the leukemic transcriptome	0.1µM / Sigma Aldrich
Octyl-α-ketoglutarate	TET1 & TET2 modulator, promotes DNA demethylation enhancing reprogramming	0.1µM / CaymanChem

SUPPLEMENTARY METHODS

Gene expression profiling

Profiling was performed on FACS-purified (purity>98%) MLL-AF4+ blasts from infant B-ALL (n=3), CB-derived CD34+CD38-CD19-CD33- HSC (n=2), CD34+CD19+CD33- B-cell HPCs (n=2) and CD34+CD33+CD19- myeloid HPCs (n=2). Technical duplicates were performed for each independent sample. Total RNA was extracted using TRIzol reagent (Invitrogen) and quantified on a Nanodrop ND1000 spectrophotometer (Isogen). The integrity of the RNA was assessed on an Agilent 2100 Bioanalyzer (Agilent). High-quality RNA was reverse transcribed using T7-linked oligo-dT primers, and the obtained cDNA was used as a template to synthesize biotinylated cDNA. Labeled cDNA was then fragmented and hybridized as duplicates/triplicates to HG-U133 Plus2.0 GeneChips (Affymetrix) according to the manufacturer's guidelines. Microarray data has been deposited in NCBI Gene Expression Omnibus (GSE79450). Raw array data was normalized by the robust multiarray average (RMA) method using the Affymetrix package.[29] Differential expression was statistically evaluated using the SAM package, which computes a statistic for each gene, measuring the strength of the relationship between gene expression and the response variable.[30] All statistical analyses were performed in the statistical environment R using bioconductor packages. Hierarchical clustering of genes, heatmaps and graphical representations were also produced using R packages. A gene was considered differentially expressed when it was >2-fold regulated (up or down) relative to the control. Statistical significance was set as $p<0.01$. Genes differentially expressed were submitted to the GOrilla application for analysis of gene functions and canonical pathways significantly altered between experimental conditions.

Bisulfite pyrosequencing, Human Methylation 450 BeadChip array and data analysis

Differences in global DNA methylation were compared between MLL-AF4+ blasts and healthy CD34+CD19+ B-cell HPCs and in B-cell lines before and after decitabine treatment by bisulfite

pyrosequencing of LINE-1 elements using the EZ DNA Methylation-Gold kit (Zymo Research). PCR amplification of modified DNA was performed using a set of primers reported previously.[26,31,32] After PCR amplification, pyrosequencing was performed using PyroMark Q24 reagents, equipment and software (Qiagen). We used the methylation level of the first three LINE-1 CpG sites as a surrogate marker of the global DNA methylation.

Microarray-based DNA methylation profiling was performed with the Illumina Infinium HumanMethylation450 BeadChip.[33] Bisulfite conversion of DNA was performed as above, with the modifications described in the Infinium assay methylation protocol guide. Processed DNA samples were then hybridized to the BeadChip following the Illumina Infinium HD methylation protocol. Genotyping services were provided by the Centro Nacional de Genotipado (CEGEN-ISCIII, www.cegen.org). IDAT files from the HumanMethylation450 BeadChip were processed further using the R/Bioconductor package (R package version 1.14.0). For adjustment of the distinct probe design types present in the Methylation450 BeadChip architecture, red and green signals from the IDAT files were corrected using a Functional Normalization algorithm[34] preceded by a Noob background correction step. [35] Probes found in at least two samples with detection p-values >0.01 were filtered out. Both beta-values and M-values were computed and employed across the analysis pipeline.[36] M-values were used for all the statistical analyses assuming homoscedasticity, while beta-values were mostly used for the intuitive interpretation and visualization of results. Probes located in the X/Y chromosomes were removed from the data set. We used the information from the SNP137Common track from the UCSC Genome Browser for removal of probes containing SNPs.[37] Differences in methylation between MLL-AF4+ blasts and healthy CD34+CD19+ B-cell HPCs and/or MLL-AF4-expressing CD34+ cells were obtained using a cell-means linear model and two different contrasts for each microarray probe. Significant methylation was determined by the moderated t-test implemented in the R/Bioconductor package limma.[38] A linear model with

methylation level as response and the sample group as the only predictor was used. P-values were corrected for multiple testing using the Benjamini-Hochberg method for controlling the false discovery rate (FDR). A significance level of 0.05 was employed to determine differentially methylated probes. An additional threshold on effect size was applied, meaning that only those probes with the strongest differences between groups (beta difference >0.5) were selected.

For gene ontology (GO) analysis and annotation, probe sets were converted to gene sets using the annotation information present in the R/Bioconductor package. A probe was assigned to a gene if the probe was contained inside the union of all the genomic regions represented by the different transcripts belonging to that gene, or in a 2 kb region upstream of the corresponding transcription start site. Probes converted this way can be assigned to zero (intergenic probes) or more genes. After gene conversion, each subset of interest was analyzed using the HOMER software tool.[39] The software was configured to use the whole set of genes represented in the HumanMethylation450 architecture as a background.