The chromatin-remodeling factor CHD4 is required for maintenance of childhood acute myeloid leukemia

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Supplementary information to:

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Supplementary Methods

Vectors, molecular cloning and knockout analysis

The inducible shRNA and gRNA vectors were generated as previously described [1]. Optimized CRISPR Design - MIT (http://crispr.mit.edu) provided by the Zhang laboratory was used to design the gRNAs and that were subsequently cloned into the inducible vector pRSITEP-U6Tet-(sh)-EF1-TetRep-2A-Puro. To produce stable cell lines expressing Cas9, the constitutive lentiviral-based Cas9-mCherry vector was used, kindly provided by Marco Herold [2]. Cas9-mCherry positive cells were sorted on BD Fusion BSL2 and thereafter the cells were transduced with gRNA vectors expressing either iRFP670 or BFP. Real time PCR was used to determine the level of CRISPR-Cas9 knockout efficiency. Either forward or reverse PCR primers were designed to specifically anneal at the gRNA target sites. Primer pair specific for a non-coding locus upstream of the *CHD4* gene was used as a normalization control. Genomic DNA from the CRISPR-Cas9 cells was isolated using QIAgen DNA extraction kit and 40 ug of DNA was used for the real time PCR. Primer sequences are available in Supplementary Table 3.

Cell culture, cell lines and primary cells

The human AML cell lines THP-1, NOMO-1, MV4-11, K-562 HL-60 and NB-4 were purchased from DSMZ and the cells were cultured in RPMI with 10% FBS, 100 U/mL penicillin and 100 ug/mL streptomycin at 37 °C, 5% CO2. The murine *MLL-AF9* cells were cultured in RPMI with 10% FBS, 10 ng/mL rmIL-3 (R&D systems) and penicillin-streptomycin antibiotics at 37 °C, 5% CO2. Normal murine cKit$^+$ cells were cultured in SFEMII media (Stemcell technology Inc.) including; rhFlt3/Flk-2 ligand (Stemcell technology Inc.), rhTPO (Stemcell technology Inc.), rhIL-6 (R&D system), rmIL-3 (R&D systems) and rmSCF (R&D systems) at a concentration of 20 ng/mL. All cell lines were routinely tested for mycoplasma contamination. The mononuclear cell fraction was isolated from the UCBs by using Lymphoprep solution (Invitrogen) and the CD34$^+$ cells were enriched by using CD34 microbead kit (Miltenyi Biotec). Isolated UCBs were cultured in SFEMII media supplemented with; rhIL-6, rhIL-3 (R&D systems), rhFl3/Flk-2 ligand, rhTPO and rhSCF all in a final concentration of 20 ng/mL (R&D systems), for 14 days.

Isolation of murine cKit$^+$ cells

To isolate cKit$^+$ cells, BM cells were collected from femur and tibias of C57BL/6 mice and lineage depletion of cells with committed lineages was achieved with Dynabeads (Invitrogen) by staining cells with purified antibodies for Ter119, B220, Gr1, CD3, Nk1.1, and CD11b (Biolegend). The fluorochrome-conjugated CD117 (cKit) antibody was used to sort cKit$^+$ cells by FACS Aria III (BD) (Supplementary Table 4). The dead cells were excluded by PI (Invitrogen).

Ethics Statement

Investigation has been conducted in accordance with the ethical standards and according to the Declaration of Helsinki and according to national and international guidelines and has been approved by the authors' institutional review board. The

primary AML childhood BM samples were obtained from Uppsala biobank (reg.nr 827) after approval from NOPHO - Nordic Society of Paediatric Haematolgy and Oncology and the local ethical committee at Stockholm (ethical permit: 2017-1059-32). All procedures relating to animal experiments were approved by the local ethics committee in Linköping, Sweden and the Swedish board of agriculture (Ethical permit number; ID 530). UCB samples were obtained from the Karolinska Hospital (Stockholm, Sweden) with informed consent of the parents.

Transfection and Transduction

Transfection and transduction was performed as previously described [3].

Large-scale shRNA screens

To ensure a recommended coverage >1000 transduced cells/shRNA, approximately 3×10^8 cells (THP-1, NOMO-1, FDCP-mix, murine AML) were cultured in concentration between 250,000 to 1,000,000 cells/mL and transduced with pooled barcoded lentiviral shRNA libraries targeting disease-associated genes (Module 2, Cellecta Inc). The transduction efficiency was determined by flow cytometry and kept below 25% to minimize the number of cells with multiple integrations of the vectors. 48 hours after selection with puromycin (2 μg/mL) (Invitrogen) when the selection control cells were dead, half of the cells were collected for an initial input control (T0). The remaining cells were propagated for 10 cell divisions (T10, to equalize for different cell division rates between the cell types). Thereafter, the genomic DNA was extracted by QIAgene DNA extraction kit according to the manufacturer's instructions, and the barcodes were amplified using nested PCR according to Cellecta's protocols. After purification using the QIAgene PCR purification kit, the barcodes were used for next generation sequencing (HiSeq 2000, Illumina). The barcodes were deconvoluted using Cellecta's software and the number of reads for the barcodes was normalized between all samples. The ratios of the barcodes from the two screens were calculated by first dividing the normalized abundance of each barcode after T10, the abundance at the initial time point (T0) separately for each cell line. For calculating the ratios between the murine AML cells and the FDCP-mix cells the relative abundance of each barcode in the murine AML cells was divided with the corresponding barcode in the FDCP-mix control cells.

RNA extraction and quantitative real time PCR (qPCR)

RNA from sorted mouse hematopoietic cell fractions was extracted using RNeasy Micro Kit (Qiagen) and DNase, following the manufactures instructions. RNA concentration and quantity was determined by DNA/RNA/Protein analyzer (NanoDrop). cDNA synthesis was performed in 20 uL reaction buffer containing 500 ng of RNA by using cDNA synthesis kit (Fermentas). qPCR was performed using ABI Thermal Cycler and qPCR primers were purchased from Eurofins company (primers are presented in Supplementary Table 3).

Mice studies

All the mice were kept in a pathogen-free animal facility in Karolinska Institutet, Huddinge, Sweden. The C57BL/6 wild type mice and the NOD-scid IL2Rgnull-

3/GM/SF, NSG-SGM3 mice were obtained from The Jackson Laboratory. All of the transplanted mice were investigated daily for symptoms of leukemogenesis and disease progression was monitored by complete blood tests.

The murine MLL-AF9 induced AML cells and mouse model was generated as previously described 43. In short, CD45.1 BM cKit+ cells from wild type C57BL/6 mice aged 6–8 weeks were transduced with a retroviral vector expressing the human MLL-AF9 fusion oncogene and serially re-plated in semisolid medium. Thereafter, individual colonies were isolated and expanded. After isolation of individual clones, the retroviral transduced cells (2x106) were transplanted via tail vein injection into sub-lethally radiated (600 cGy) C57BL/6 wild type mice. The transplanted mice were investigated daily for symptoms of leukemogenesis and disease progression was monitored by complete blood tests. After disease onset, the MLL-AF9 induced AML cells were harvested from femur and tibias of euthanized primary recipient mice and leukemic cells were analyzed and sorted by flow cytometry and expanded in RPMI media in presence of IL3 (10ng/mL) and 10% FBS. The generated MLL-AF9 AML cell lines were subsequently used for further transplantation experiments or in vitro experiments.

For the Kaplan-Meier survival analysis, wild type CD45.2 C57BL/6 mice aged six-12 weeks from The Jackson Laboratory were used for transplantation of murine MLL-AF9 AML cells. AML cells transduced with lentiviral shRNA vectors were selected with puromycin (2 μg/mL) and 250,000 AML cells were intravenously transplanted into non-irradiated recipient mice. After disease onset, the mice were euthanized.
The experiments using humanized NSG-SGM3 mice were performed on animals aged six to ten weeks. Recipient mice were subjected to sub-lethal irradiation (220 Gy) and six to 12 hours post irradiation the primary childhood AML patient BM samples were transplanted via intra-femur injection using doses of 100,000-500,000 cells/mouse. BM samples were collected from the bones of euthanized primary recipient mice 6-8 weeks post transplantation and the primary leukemic cells were analyzed with flow cytometric analysis for donor cell engraftment (See Supplementary Table 4 for antibodies).

Apoptosis analysis

Briefly, THP-1 cells were washed with PBS and stained with Live/Dead Fixable Near IR dead cells stain (Thermo Fisher) for 20 minutes on ice. After washing the cells, they were washed once with Annexin-binding buffer (BioLegend) and then stained with Annexin V FITC (BioLegend) in Annexin-binding buffer for 20 minutes at 4 degrees. Washing, cells were resuspended in Annexin-binding buffer and analyzed by flow cytometric analysis.

Cell cycle analysis

THP-1 cells were washed with PBS and stained with Live/Dead Fixable Near IR dead cells stain for 20 minutes at 4 degrees and then washed with cold PBS. Then they were resuspended in 250 μL of fixation/permeabilization solution (BD) for 30 minutes at 4 degrees in dark. Then cells were washed with 1x wash buffer (BD) two times and pelleted cells were incubated in 10 μL of Ki67-FITC for 2 hours on ice in dark. After washing in cold PBS with 10% FCS, the cells were resuspended in DAPI

at the concentration of 0.5 μg/mL in PBS+10% FCS for 20 minutes at room temperature, before being analyzed with FACS Fortessa LSRII (BD).

RNA-Sequencing

Total RNA was extracted from 2 x 103 FACS-sorted THP-1 cells by RNeasy Micro Kit (Qiagen), 72 hours after transduction of the cells, or by selection with puromycin. TotalScriptTM RNA-seq kit (Epicentre, Madison, WI) was used to prepare strand specific pair-end RNA libraries according to the manufacturer's instructions. Libraries were pair-end sequenced using the Illumina platform HiSeq2000 or Nextseq500. RNA-Seq reads were mapped to the Ensembl Homo_sapiens.GRCh38 reference genome using the STAR aligner. Gene assignment was performed using featureCounts. Normalization and the sample group comparison was performed using DESeq2. The RNA-Seq data (GSE106392) are available at:
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE106392

Western Blotting

Total cellular proteins were extracted from cultured cells using RIPA Buffer, including Protease Inhibitor Cocktail (sc24948, Santa Cruz Biotechnology). 15-20 ug protein was prepared in NuPAGE reducing agent (NP0007, Invitrogen). Proteins were separated in 4-15% Tris Glycine gels (456-1084, Biorad) and transferred to nitrocellulose membranes (1704158, Biorad) by using Trans Blot Turbo Blotting System (170-4155, Biorad). Precision Plus Protein™ Dual Color Standards (1610374, Biorad) was used for molecular weight comparison of protein. Nitrocellulose membranes were blocked with 5% nonfat dried milk (170-6404, Biorad) dissolved in 1X PBS (D8537, Sigma) containing 0.2% Tween 20 (P9416, Sigma) at room temperature for 1 h and probed with primary antibodies against Mi-2 β/CHD4 (MABE 915 Milipore, 06-1306, Milipore) and Beta Actin (A5441, Sigma) overnight at 4^{0C}. Blots were incubated in secondary antibodies of goat anti-rabbit and anti-mouse IgG (H+L) horseradish peroxidase conjugated for 1 hour at room temperature (G21234, 31430, Thermo Scientific). The detection of proteins was performed by using enhanced chemiluminescence kit (32106, Thermo Scientific) and the image was captured by a LI-COR Odyssey Fc Imager system. Quantification of the relative intensity of the bands were performed by using Image Studio Lite (LI-COR Biosciences).

Statistical analysis

Statistic analysis was done with GraphPad Prism version 6 and P-values of <0.05 were considered statistically significant. t-tests were used to determine the statistical differences between groups. GSEA analysis was done using log2 transformed expression values and the Molecular Signature Data Base, MSigDB (http://software.broadinstitute.org/gsea/msigdb/annotate.jsp), applying "Compute Overlaps" tool and "Hallmark gene sets" and "Reactome gene sets".

Supplemental References

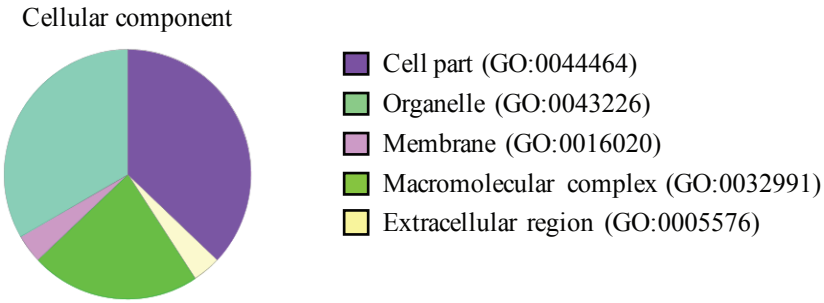
1. Eshtad S, Mavajian Z, Rudd SG, et al. hMYH and hMTH1 cooperate for survival in mismatch repair defective T-cell acute lymphoblastic leukemia. Oncogenesis. 2016;5(12):e275.
2. Aubrey BJ, Kelly GL, Kueh AJ, et al. An inducible lentiviral guide RNA platform enables the identification of tumor-essential genes and tumor-promoting mutations in vivo. Cell Rep. 2015;10(8):1422-1432.
3. Sadeghi B, Heshmati Y, Khoein B, et al. Xeno-immunosuppressive properties of human decidual stromal cells in mouse models of alloreactivity in vitro and in vivo. Cytotherapy. 2015;17(12):1732-1745.

Supplemental Data Information

Supplementary Table 2: shRNA screening data, and Supplementary Table 4: shRNA screening data, are supplemented as excel files**.**

Supplementary Figure 1

A



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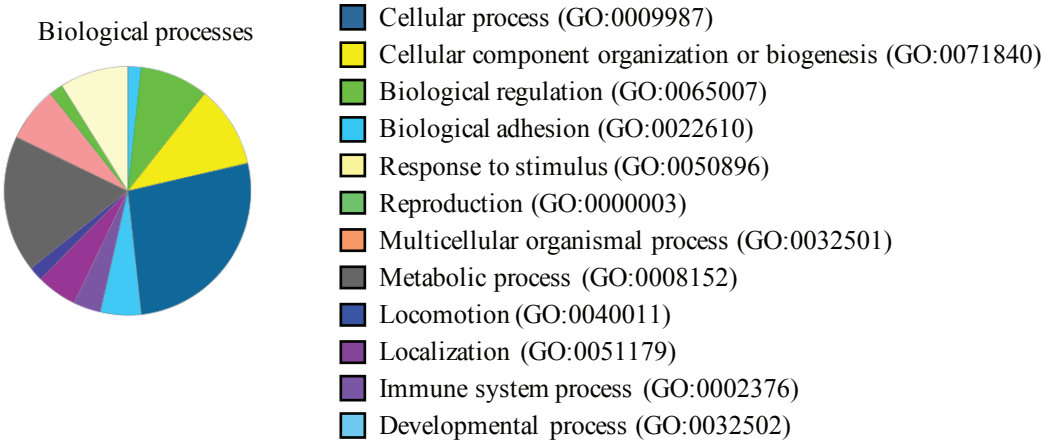

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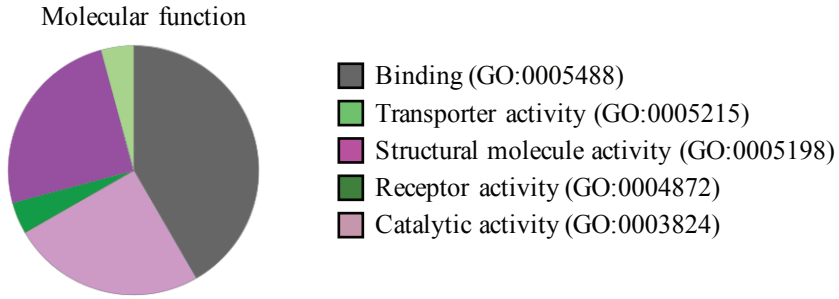

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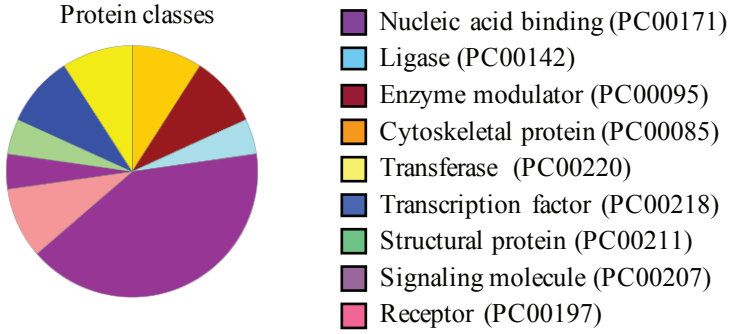
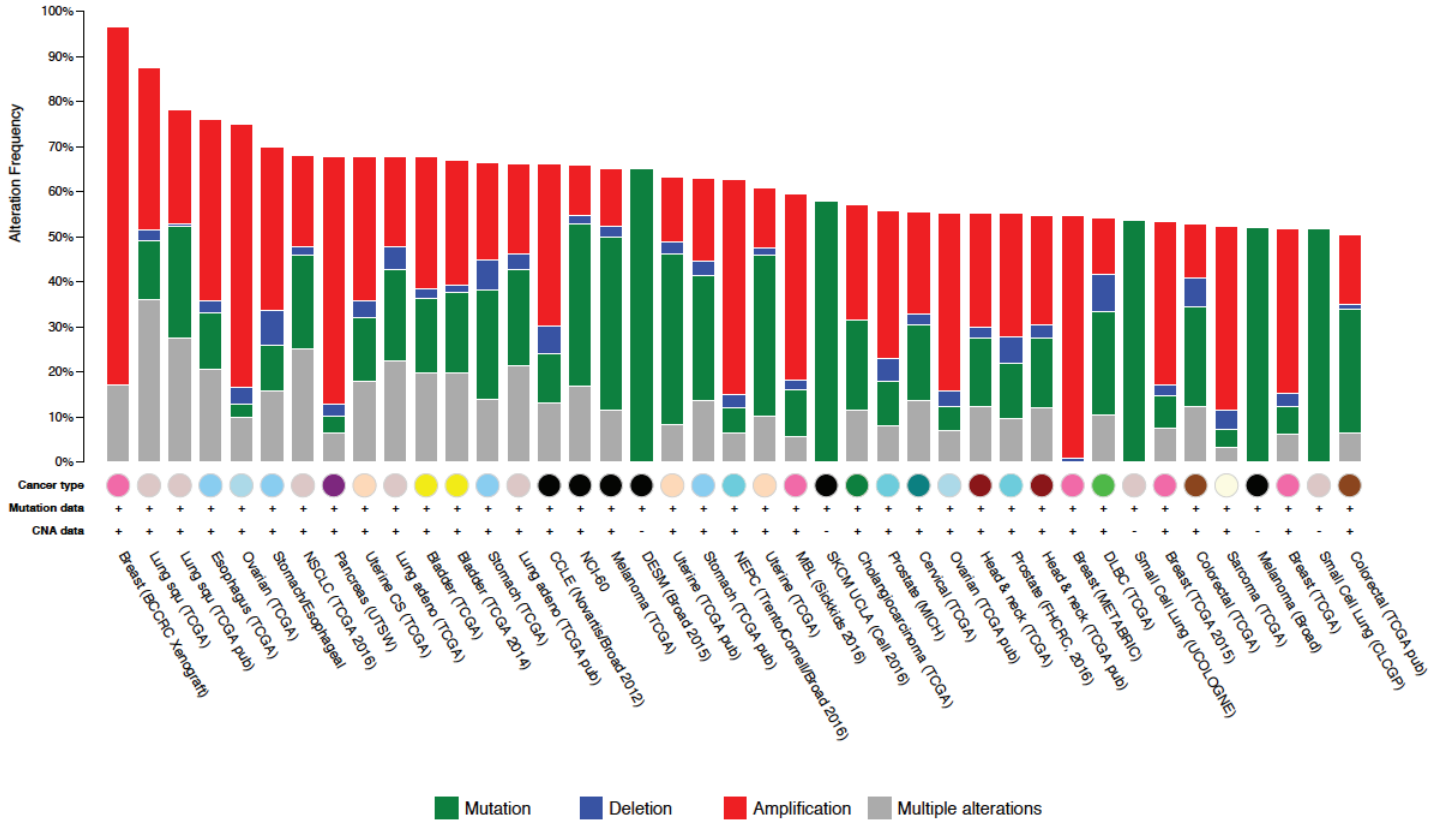


Supplementary Figure 1. Gene ontology analysis of the 34 target genes identified from the shRNA screens

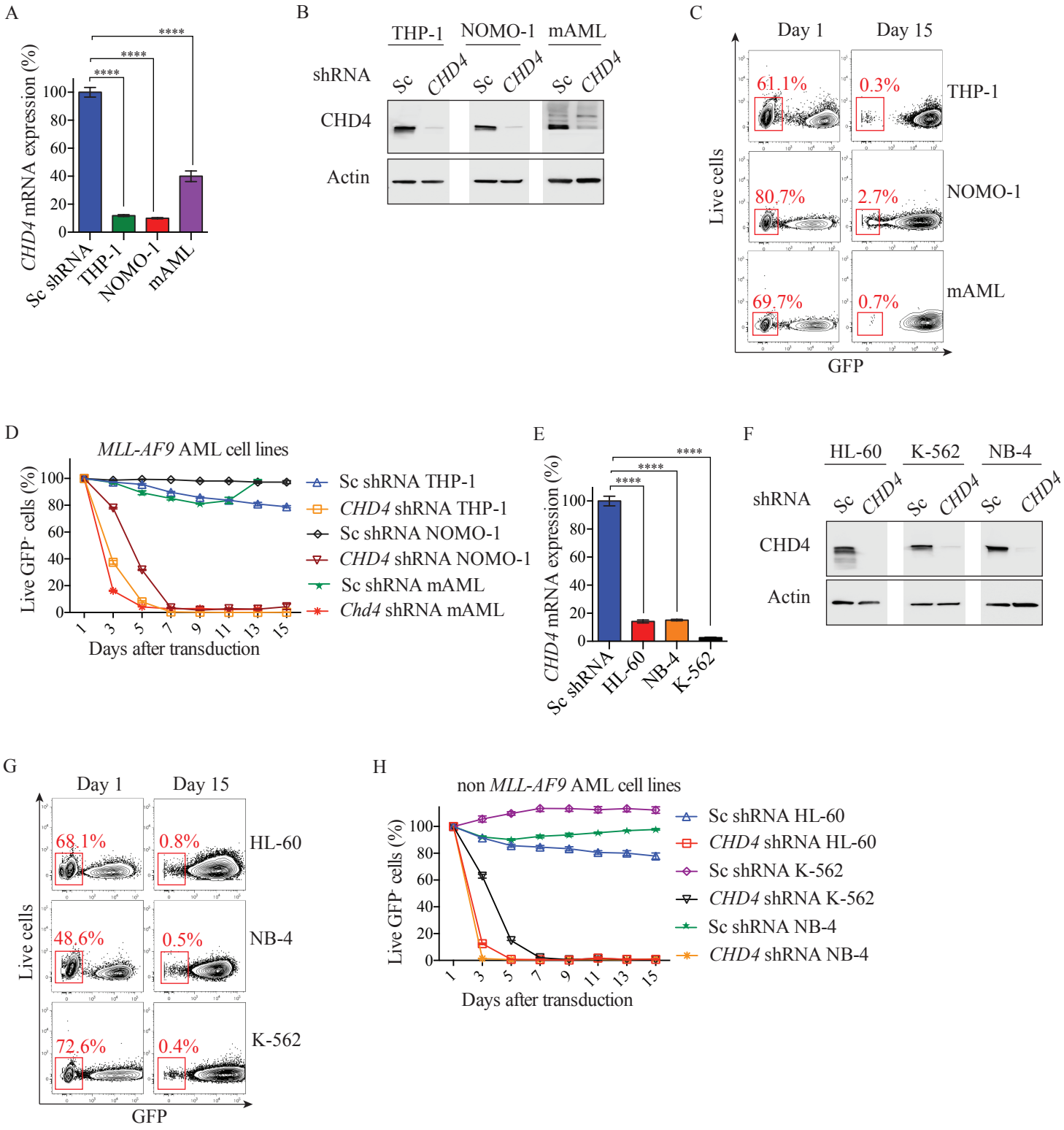
Pie charts showing GO term distribution of the 34 target genes in **A.** cellular components, **B.** biological processes, **C.** molecular functions, and **D.** protein classes, based on gene expression changes at 72 hours after inhibition of *CHD4* in THP-1 cells.



Supplementary Figure 2. Cross cancer alteration analysis of the 34 target genes identified from the shRNA screens

Bar chart representing the frequency of alterations in percentage of the 34 target genes identified from the shRNA screens in various cancer. The cancer type, and type of mutations are indicated in the chart.

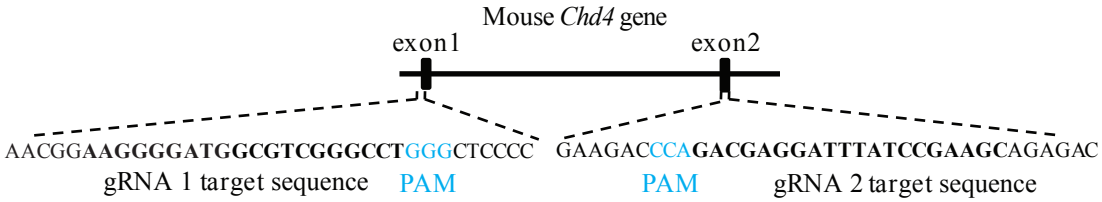
Supplementary Figure 3



Supplementary Figure 3. *CHD4* inhibition impairs cell growth of leukemic cell lines.
A and **E.** Real time PCR analysis of mRNA levels after shRNA-based knockdown of *CHD4* relative to control cells, in the indicated leukemia cells, at 72 hours post transduction. Transduced cells were used for flow cytometry and growth competition assays (Supplementary Figure 3C, D, G and H). mRNA levels were normalized to UBC. The data is represented as the mean ±S.E.M., ****p<0.001 (unpaired t-test), n=3. **B** and **F**. Western blot analyses of the endogenous *CHD4* and *Actin* levels, with and without knockdown of *CHD4* in the indicated cells. **C** and **G**. Representative flow cytometry plots showing percentage of live *CHD4* knockdown cells (live cells) relative to GFP$^+$ control cells (GFP), at the indicated days after transduction. **D** and **H**. Line charts depicting the percentage of live cells normalized to 100% at the initial time point (Day one), determined by flow cytometry analysis at the indicated time points.

Supplementary Figure 4

A



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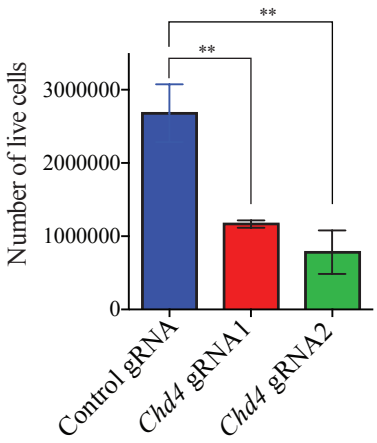
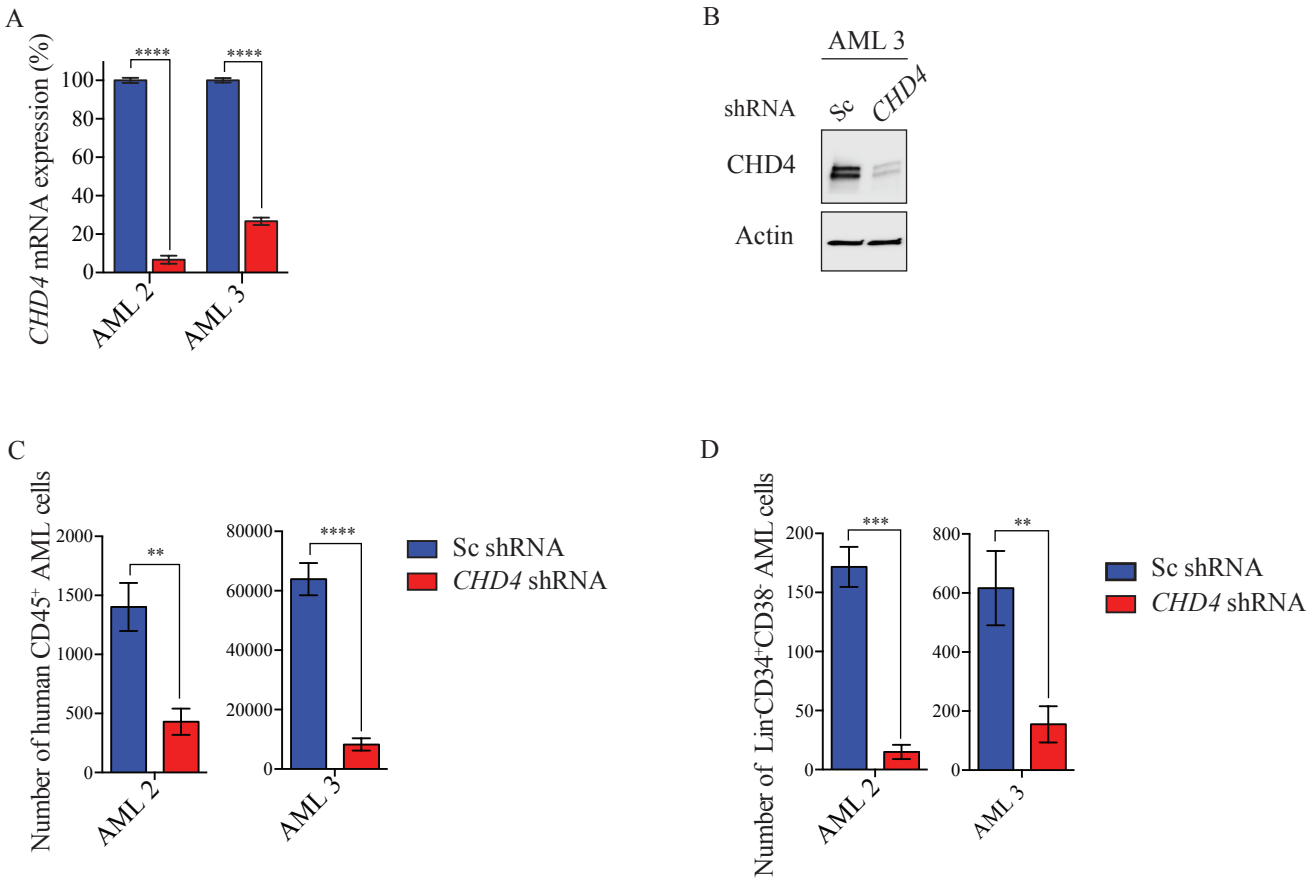


Supplementary Figure 4. CRISPR-Cas9 mediated knockout of *Chd4* prevents growth of mouse AML cells.

A. Schematic image depicting the homology of the gRNAs used for targeting of the coding region of the mouse *Chd4* gene. **B**. Bar charts show the total number of live mouse MLL-AF9 AML cells transduced with gRNA vectors against *Chd4* relative to cells transduced with negative control vectors (control gRNA), after 10 days of cell growth. The data is represented as the mean ±S.E.M., **p<0.01 (unpaired t-test), n=3.

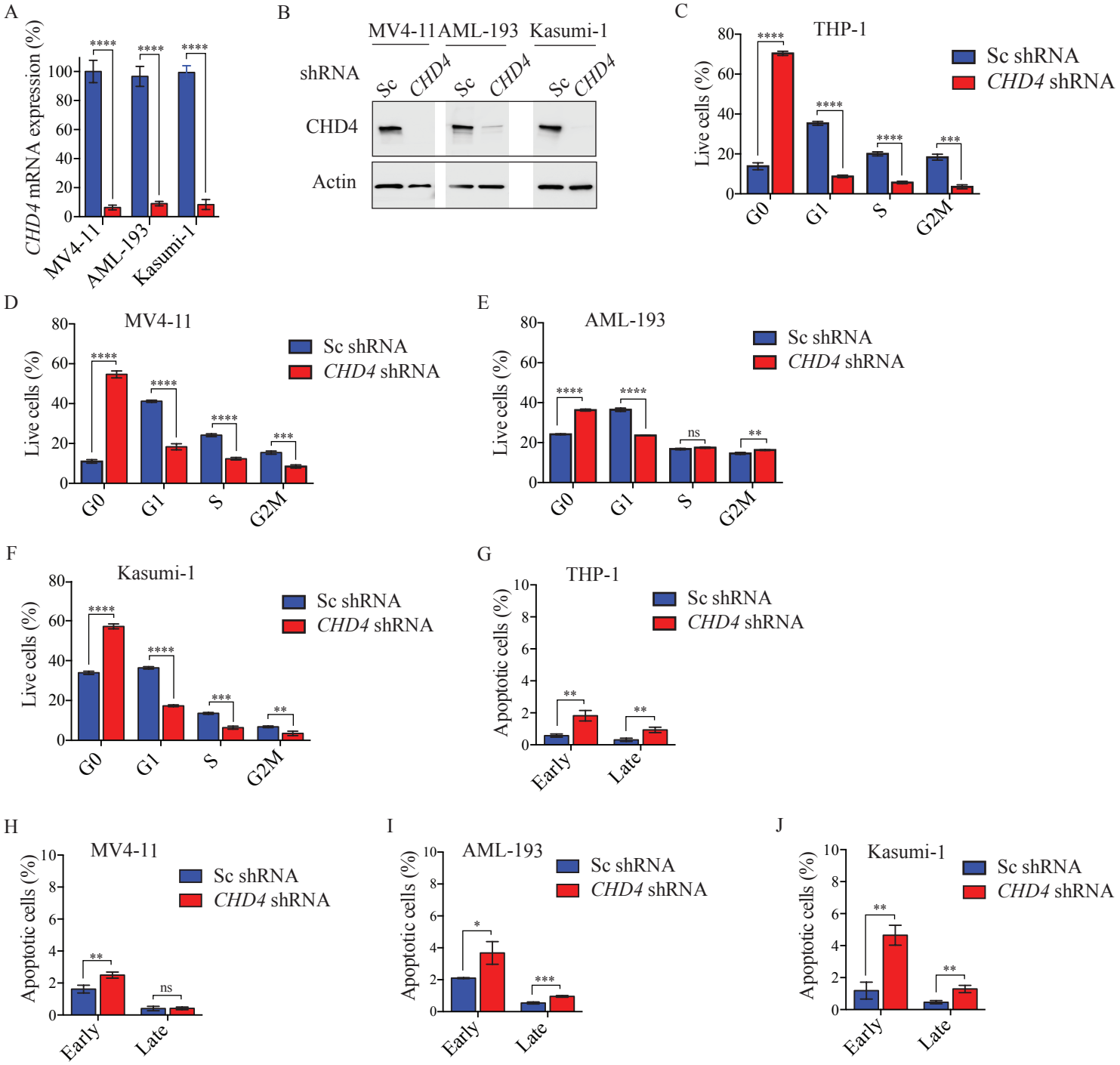
Supplementary Figure 5



Supplementary Figure 5. *CHD4* inhibition prevents growth of patient-derived childhood AML cells ex vivo and in vivo.

A. Real time PCR analysis of mRNA levels after shRNA-based knockdown of *CHD4,* as compared to control cells, of the indicated primary childhood AML samples used in the cell growth assays (Supplementary Figure 5C and D), at 72 hours post transduction. mRNA levels were normalized to UBC. The data is represented as the mean ±S.E.M., ****p<0.001 (unpaired t-test), n=3. **B**. Western blot analyses of the CHD4 and Actin levels, with and without knockdown of CHD4, in primary childhood cells. **C** and **D**. Bar chart of total numbers of live $CD45^+$ (C) and $Lin^-CD34^+CD38^-$ (D) primary childhood AML cells transduced with *CHD4* shRNA, or a control vector, as indicated. **p<0.01, ***p<0.005, ****p<0.001 (unpaired t-test), n=3.

Supplementary Figure 6

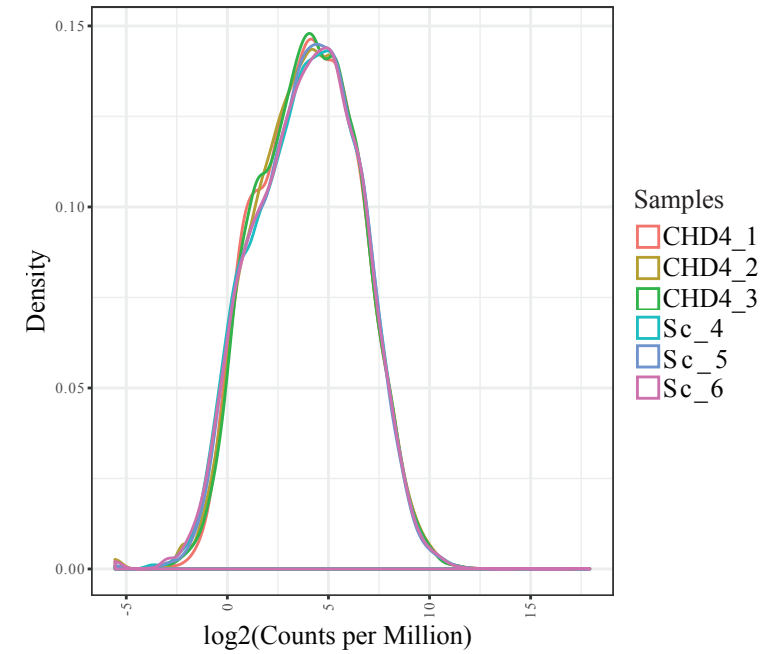


Supplementary Figure 6. *CHD4* inhibition in AML cells causes an arrest in the G0 phase of the cell cycle.

A. Real time PCR analysis of mRNA levels after shRNA-based knockdown of *CHD4* in MV4-11, AML-193 and Kasumi-1 (knockdown of mRNA levels of *CHD4* in THP-1 cells used in this assay is depicted in Supplementary Figure 3A), relative to control samples transduced with negative control vectors, at 72 hours post transduction. mRNA levels were normalized to *UBC*. The data is represented as the mean ±S.E.M., ****p<0.001 (unpaired t-test), n=3. **B**. Western blot analysis of CHD4 and Actin levels in the indicated cells, with and without knockdown of *CHD4* (knockdown of protein levels of CHD4 in THP-1 cells used in this assay is depicted in Supplementary Figure 3B). *C-F*. Flow cytometric quantification as the percentage of cells in each population of the indicated cell lines transduced with *CHD4* shRNA or a control vector, at 72 hours post transduction. The data is presented as mean ±S.E.M., ns = non significant, **p<0.01, ***p<0.005, ****p<0.001 (unpaired t-test), n=3. **G-J**. Bar graphs illustrating flow cytometric quantification as the percentage of cells in each population of the indicated cell lines transduced with CHD4 shRNA or a control vector, at 72 hours post transduction. The data is presented as mean ±S.E.M., ns = non significant, *p<0.05, **p<0.01, ***p<0.005, (unpaired t-test), n=3.

Supplementary Figure 7

A



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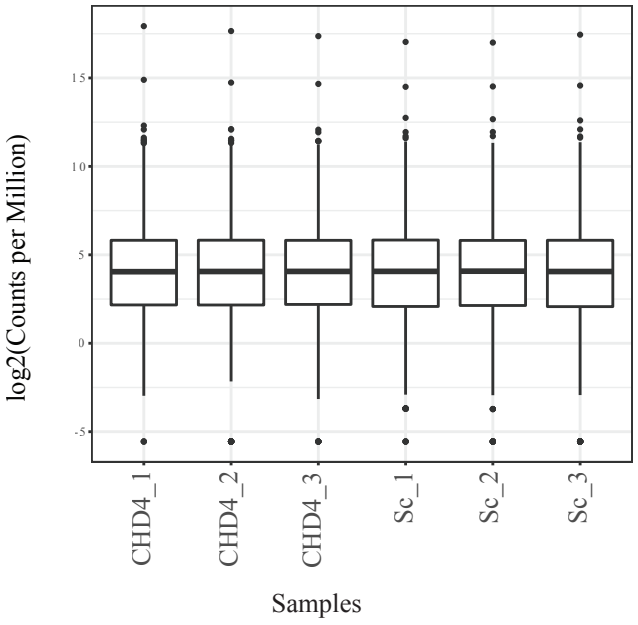
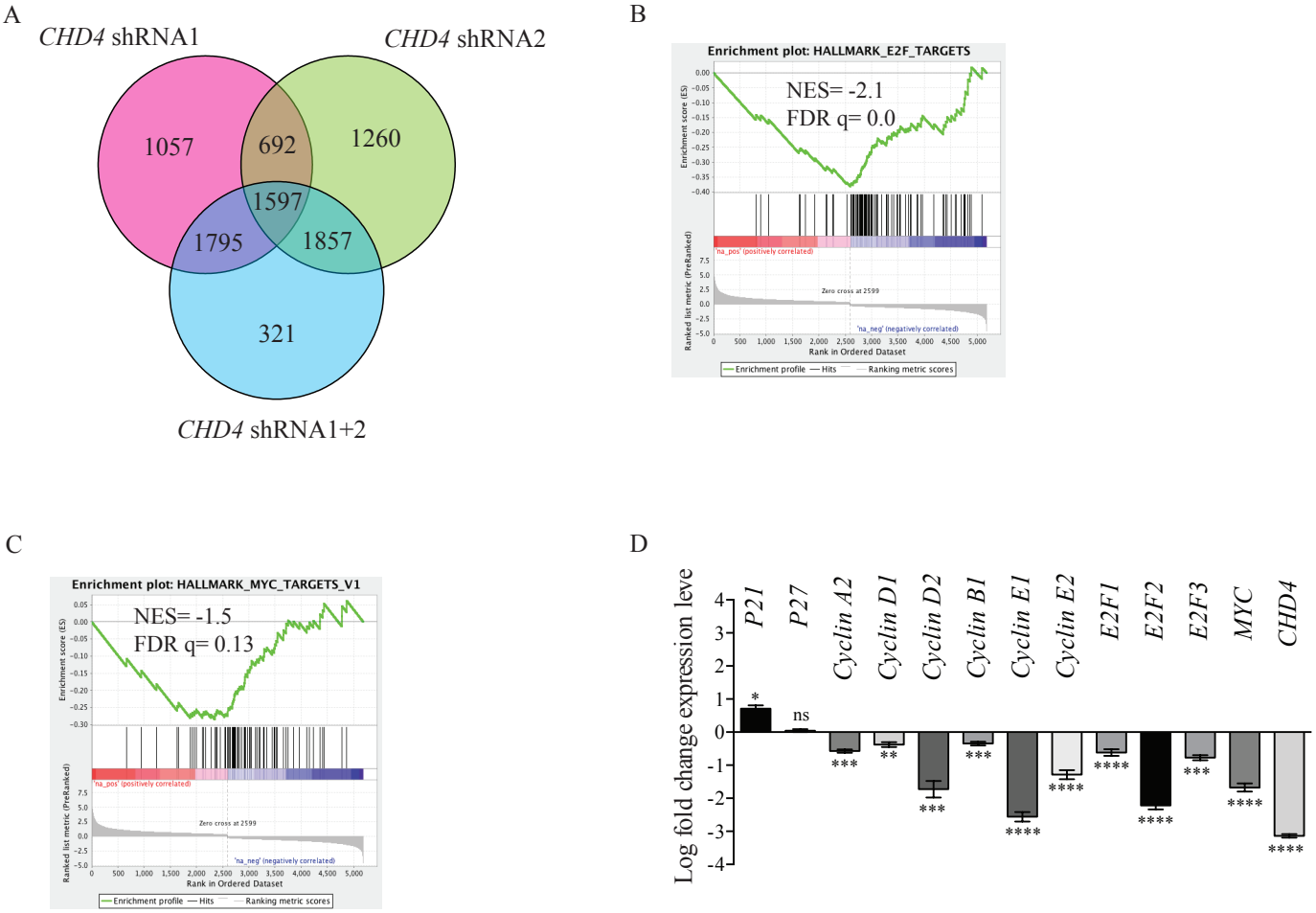


Supplemental Figure 7. Density plots and boxplot of RNA sequencing data show a very even distribution across the samples.

A and **B**. Density plot (A) and boxplot (B) of the samples after normalization (counts per million [CPM] + weighted trimmed mean of M-values [TMM]) show the distribution of the tag counts between all the samples. Only genes that had more than 1 CPM in 3 or more experiments were further used in the analysis.

Supplementary Figure 8



Supplementary Figure 8. *CHD4* inhibition causes downregulation of *MYC* and its target genes involved in cell cycle progression.

A. Venn diagram showing the overlap between significant changes in gene expression (FDR <0.05), using two independent shRNAs against *CHD4* compared to control cells (Sc) (*CHD4* shRNA1 and *CHD4* shRNA 2), and pooled data of the two experiments ([*CHD4* shRNA 1 + 2 = [*CHD4* shRNA 1 + 2] / [Sc 1 + Sc 2]), in THP-1 cells. **B** and **C**. Enrichment plots of gene set enrichment analysis (GSEA) of mRNA gene expression profiling in response to *CHD4* knockdown in THP-1 cells, depicting normalized enrichment score (NES) and FDRq values of *E2F* and *MYC* targets **C.** Real time PCR analysis of mRNA levels in THP-1 cells, after shRNA-based knockdown of *CHD4* (shRNA 2), relative to control cells transduced with vectors expressing scrambled shRNA, at 72 hours post transduction. mRNA levels were normalized to *UBC*. The data is represented as the mean ±S.E.M., ns = non significant, **p<0.01, ***p<0.005, ****p<0.001, (unpaired t-test), n=3. **D.** Bar charts of real time PCR analysis of mRNA levels of genes showing changes in RNA-Seq of THP-1 cells, after shRNA-based knockdown of *CHD4*, relative to control cells transduced with vectors expressing scrambled shRNA, at 72 hours post transduction. mRNA levels were normalized to *UBC*. The data is represented as the mean ±S.E.M., ns = non-significant, *p<0.05, **p<0.01, ***p<0.005, ****p<0.001 (unpaired t-test), n=3.

Supplementary Table 1

Antibody	Conjugated	Clone	Source
Anti-mouse TER-119	Purified	TER-119	Biolegend
Anti-mouse CD3	Purified	17A2	Biolegend
Anti-mouse/Human CD45R/B220	Purified	RA3-6B2	Biolegend
Anti-mouse Ly-6G/Ly-6C (Gr-1)	Purified	RB6-8C5	Biolegend
Anti-mouse NK1.1	Purified	PK136	Biolegend
Anti-mouse CD11B	Purified	M1/70	Biolegend
Anti-mouse CD16/32	Purified	93	Biolegend
Anti-mouse CD117 (c-kit)	APC-Cy7	2B8	Biolegend
Ki-67	FITC	B56	BD
Anti-human CD20	PE-Cy5	2H7	Biolegend
Anti-human CD4	PE-Cy5	RPA-T4	Biolegend
Anti-human CD8	PE-Cy5	RPA-T8	Biolegend
Anti-human CD2	PE-Cy5	RPA-2.10	Biolegend
Anti-human CD56	PE-Cy5	HCD56	Biolegend
Anti-human CD235a	PE-Cy5	HIR2	Biolegend
Anti-human CD3	PE-Cy5	HIT3a	Biolegend
Anti-human CD19	PE-Cy5	HIB19	Biolegend
Anti-human CD34	APC	581	Biolegend
Anti-human CD38	PE-Cy7	HB7	BD
Anti-human CD45	BV786	HI30	BD
Anti-Mi-2β	Purified	2G8	Milipore
Anti-Mi-2β (CHD4)	Unpurified		Milipore
Beta-Actin	Purified	AC-15	Sigma
Rabbit IgG (H+L) secondary	HRP		ThermoScientific
Mouse IgG (H+L) secondary	HRP		ThermoScientific

Supplementary Table 3

Sample	ID	MLL Translocation	Experiment	Figure
AML 1	07-148	Non MLL	*Ex vivo*	5A, C and D
AML 2	06-200	Non MLL	Ex vivo and vivo	5A, C, D, E and F
AML 3	07-171	Non MLL	*Ex vivo*	5A, C and D
AML 4	06-21	Non MLL	*Ex vivo*	5A, C and D
AML 5	07-226	Non MLL	*Ex vivo*	5A, C and D
AML 6	07-174	MLL	*Ex vivo*	5C and D
AML 7	06-013	Non MLL	*In vivo*	5E and F

Supplementary Table 5

Target site	Application	Forward oligo (5´>3´)	Reverse oligo (5´>3´)
m*Chd4*	qPCR	CCACCCCATCCAGAAAATGA	CTTTGGAGTCTCTGCTTCGGATA
h*Chd4*	qPCR	AACCTCGGGACCCTAAAATCC	GGCATAAGAGCATACGCTCCTT
h*UBC*	qPCR	CTGGAAGATGGTCGTACCCTG	GGTCTTGCCAGTGAGTGTCT
h*P27*	qPCR	CCTGCAACCGACGATTCTTC	CGTCTGAAACATTTTCTTCTGTTCTG
h*P21*	qPCR	CCTGTCACTGTCTTGTACCCTTGT	TTTGGAGTGGTAGAAATCTGTCATG
h*Cyclin A2*	qPCR	GAAGACGAGACGGGTTGCA	GAACGGTGACATGCTCATCATT
h*Cyclin D1*	qPCR	CCCCAACAACTTCCTGTCCTACT	GCAGTCCGGGTCACACTTG
h*Cyclin D2*	qPCR	TGCCACCGACTTTAAGTTTGC	TGCTCCCACACTTCCAGTTG
h*Cyclin B1*	qPCR	TTGTTGCAGGAGACCATGTACAT	GGCACACAATTATTCTGCATGAA
h*Cyclin E2*	qPCR	CTTCCAAACTTGAGGAAATCTATGC	CTTCACTGCAAGCACCATCAG
h*Cyclin E1*	qPCR	TCGGATTATTGCACCATCCA	TCTCTATTTGCCCAGCTCAGTACA
h*E2F1*	qPCR	TCCAGGAAAAGGTGTGAAATCC	GCTTGGTGGTCAGATTCAGTGA
h*E2F2*	qPCR	CAAGAGGCTGGCCTATGTGACT	CAATCACTGTCTGCTCCTTAAAGTTG
h*E2F3*	qPCR	CCCAAGGGCAAAGGAAGAG	GGAGATTTTGGAGTTTTTGGACTATC
h*MYC*	qPCR	AGCGACTCTGAGGAGGAACAAG	CCTGCCTCTTTTCCACAGAAA
m*Hprt1*	qPCR	GAAAAGGACCTCTCGAAGTGTTG	CACTAATGACACAAACGTGATTCAAA
h*GAPDH*	qPCR	AAGGCTGAGAACGGGAAGCT	GGATCTCGCTCCTGGAAGATG
dHTS	1st PCR barcodes	TCTCTGGCAAGCAAAAGACGGCATA	TGCCATTTGTCTCGAGGTCGAGAA
dGex	2nd PCR barcodes	CAAGCAGAAGACGGCATACGAGA	AATGATACGGCGACCACCGAGA
GexSeqN	HT seq. barcodes	ACAGTCCGAAACCCCAAACGCACGAA	
h*CHD4* g1	CRISPR gRNA	ACCGGGGAGCGGAAGGGGATGGCGT	AAACACGCCATCCCCTTCCGCTCCC
h*CHD4* g2	CRISPR gRNA	ACCGGGAGTACCTGGATGCTACAGG	AAACCCTGTAGCATCCAGGTACTCC
m*Chd4* g1	CRISPR gRNA	ACCGGAAGGGGATGGCGTCGGGCCT	AAACAGGCCCGACGCCATCCCCTTC
m*Chd4* g2	CRISPR gRNA	ACCGGGCTTCGGATAAATCCTCGTC	AAACGACGAGGATTTATCCGAAGCC
h*CHD* g1	qPCR KO test	GAGCGGAAGGGGATGG	GGCTGTTGTTCAAAAGTGCAT
h*CHD* g2	qPCR KO test	AGCCAGAGTACCTGGATGCTACA	TCTCATCAGCCAAGATGGTGTC
h*CHD* control	qPCR KO test	GGTTGGAGTTGGTTGAGGTT	CTTAAAGGGCCTGAAACACC

Supplementary Table Legends

Supplementary Table 1. All antibodies used in this study. Table of all antibodies used in this study, including for flow cytometry and western blot analysis.

Supplementary Table 2. **shRNA screening data.** The table is related to Figure 1. The excel file contain three work sheets with screening data of all shRNAs and their target genes that have a five-fold lower abundance in mouse AML vs FDCP-mix cell cells (sheet 1), and the corresponding shRNA-targeted human genes that are five-fold depleted after 10 cell divisions in NOMO-1 cells (sheet 2) and THP-1 cells (sheet 3), using Cellecta ´s genome wide shRNA libraries against mouse or human genes

Supplementary Table 3. Childhood patient samples used in this study. Table listing all childhood AML patient samples used in this study and in which experiment the samples were used.

Supplementary Table 4. **RNA sequencing data**. The table is related to Figure 7. The RNA-Seq data was generated using two independent shRNAs against *CHD4* in THP-1 cells, at 72 hours after transduction.

Supplementary Table 5. All oligos used in this study. Table of all PCR primers, sequencing primers and gRNAs that were used in this study.