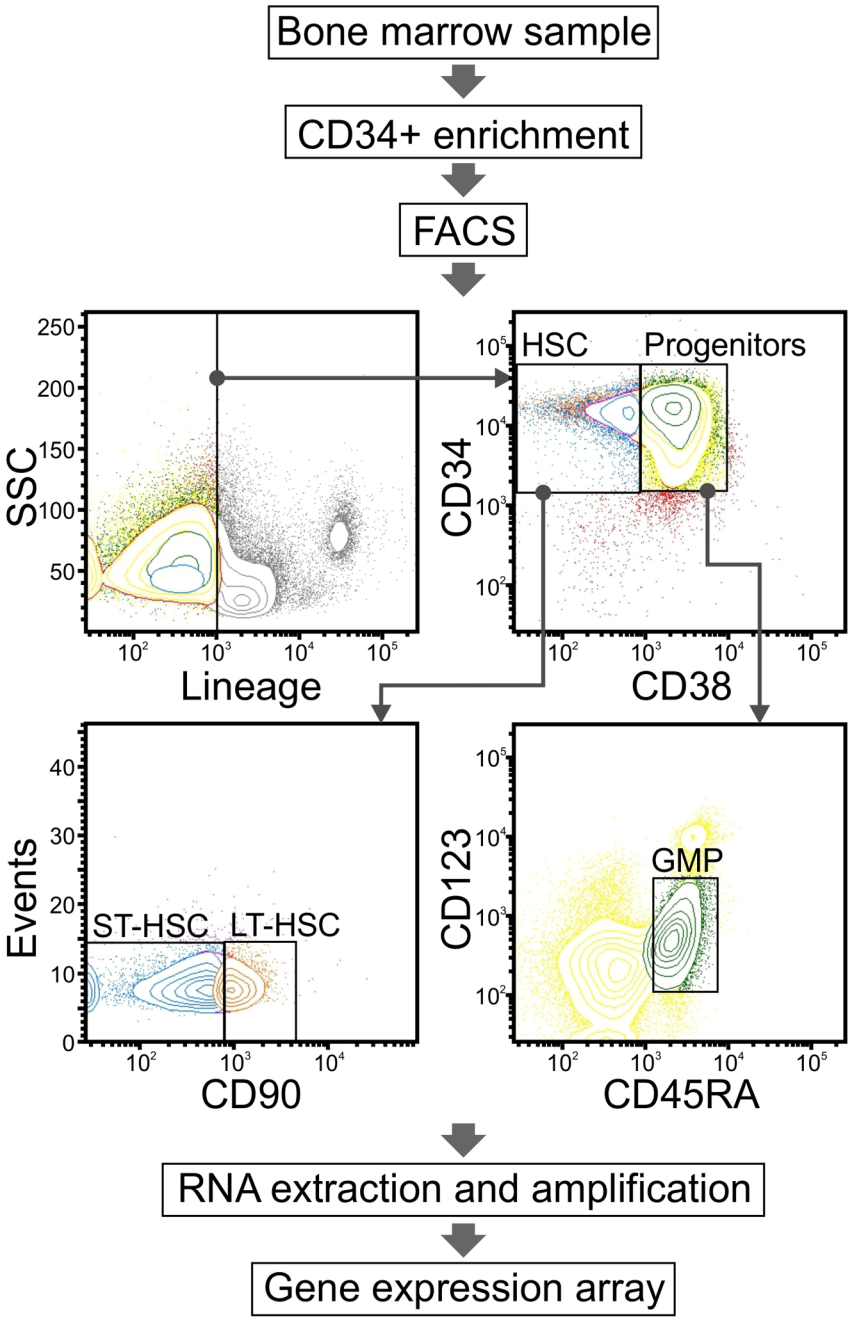
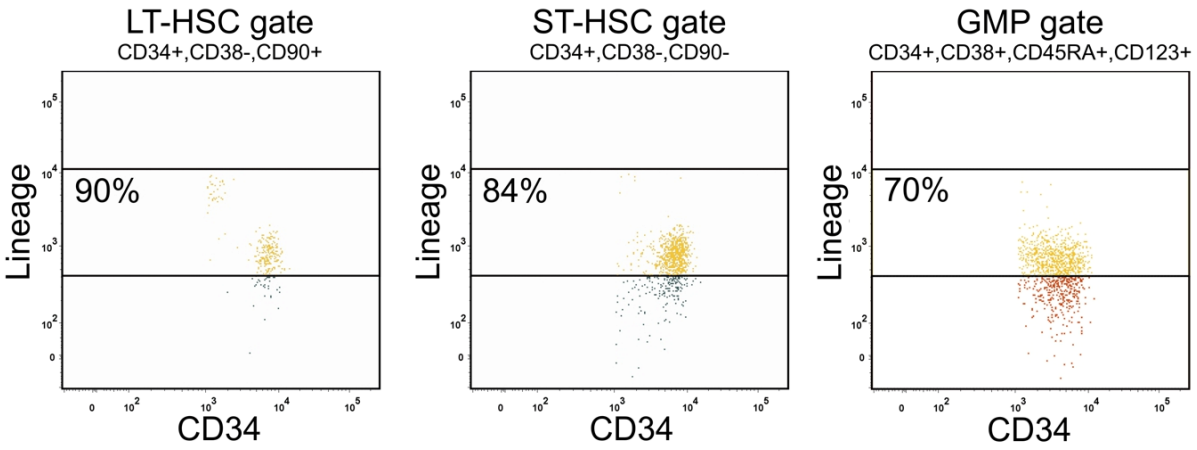
Supplementary figures and tables

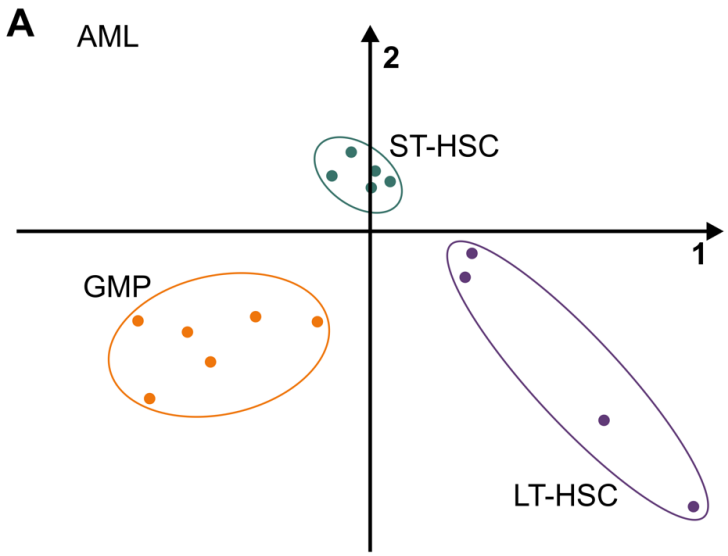
Suppl. Fig. S1. Sorting strategy for the isolation of phenotypically defined LT-HSC, ST-HSC and GMP. LT-HSC, ST-HSC and GMP were sorted for gene expression analysis as indicated.

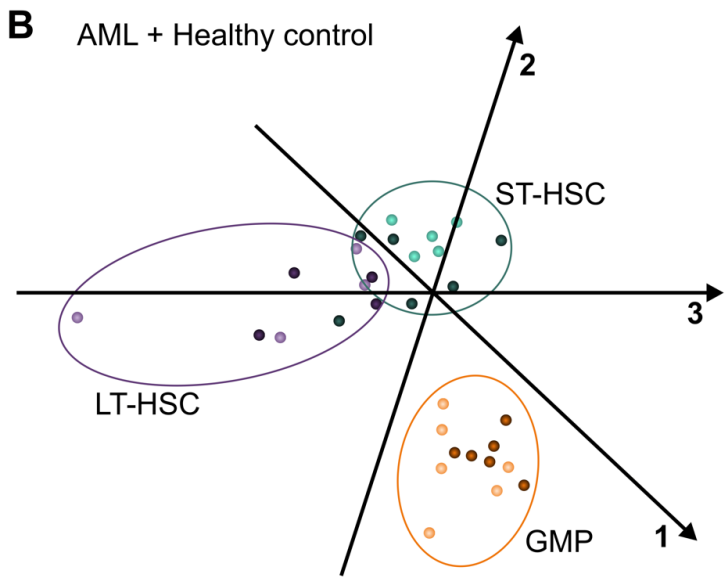


Suppl. Fig. S2. Lineage exclusion is critical for stem and progenitor sorting. Analysis of lineage positive cells in phenotypic stem (LT-HSC and ST-HSC) and granulocyte-macrophage progenitor (GMP) compartments from bone marrow of a healthy donor. Percentages indicate the percentage of lineage^{+} cells in each compartment. Surface markers are indicated.

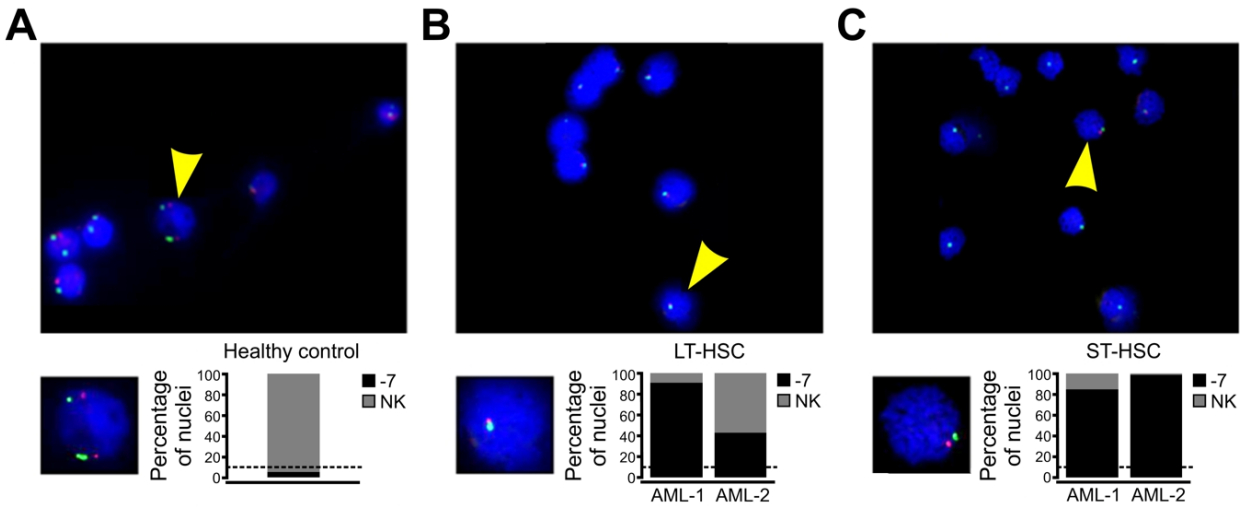


Suppl. Fig. S3. Principal component analysis separates phenotypic AML-LT-HSC, AML-ST-HSC, and AML-GMP and shows resemblance to the corresponding compartments in healthy controls. A) Principal component analysis of differentially expressed genes within AML with monosomy 7 separates phenotypically defined AML LT-HSC, ST-HSC and GMP. **B)** Phenotypically defined compartments in AML with monosomy 7 (lighter colors) cluster together with phenotypically defined LT-HSC, ST-HSC and GMP of healthy controls (shown in darker colors) in principal component analysis.

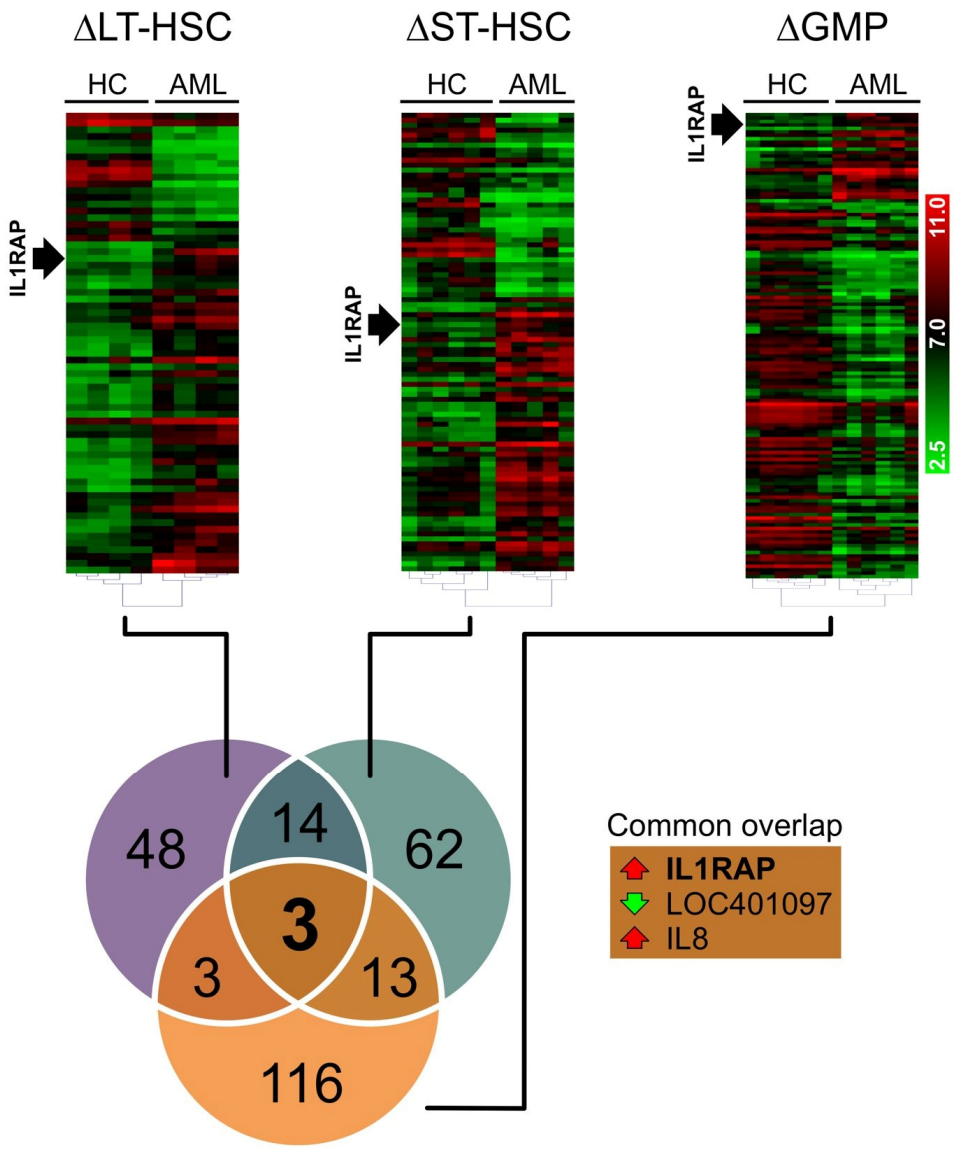




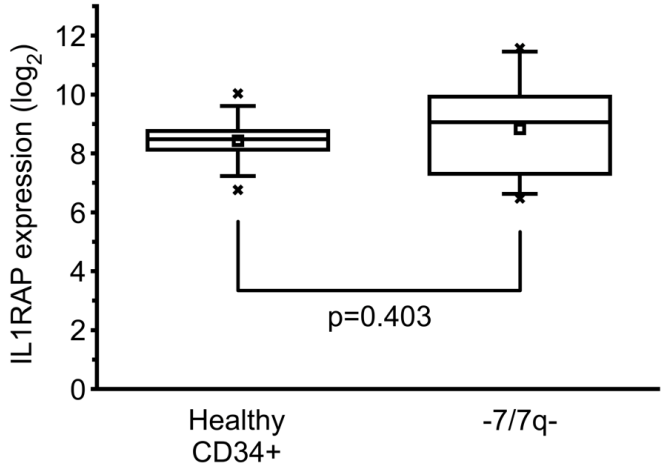
Suppl. Fig. S4. Chromosome 7 aberrations are detectable in the earliest HSC stages by fluorescence in-situ hybridization (FISH). Sorted hematopoietic stem cells of AML patients bearing monosomy 7 were hybridized with the Vysis LSI D7S486 (7q31) SpectrumOrange/ CEP 7 SpectrumGreen Probes. Standard magnification is shown in the upper panels. A higher magnification of the cells indicated with a yellow arrow in the upper panel, showing a representative FISH signal, is shown in the lower left panel. The lower right panel shows the percentage of nuclei counted bearing monosomy 7 (-7) or a normal karyotype (NK). **A)** Normal FISH pattern with two individual green (centromere chromosome 7) and orange (7q31) signals per nuclear section in healthy control LT-HSC. **B)** AML-LT-HSC. **C)** AML ST-HSC.



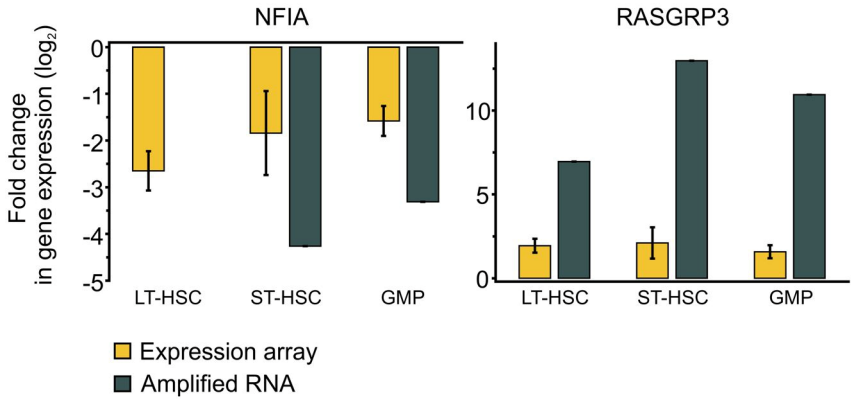
Suppl. Fig. S5. Overexpression of *IL1RAP* is highly significant in all hematopoietic stem and progenitor compartments studied. Venn diagram showing the three most significant genes commonly dysregulated in AML with monosomy 7 in phenotypically defined LT-HSC (purple), ST-HSC (blue), and GMP (orange) compartments. Heat maps show log2-transformed expression levels of all differentially expressed genes in LT-HSC, ST-HSC, and GMP of AML patients (AML) compared with healthy controls (HC). (Cutoff: fold: 1.5, $p < 0.016$).



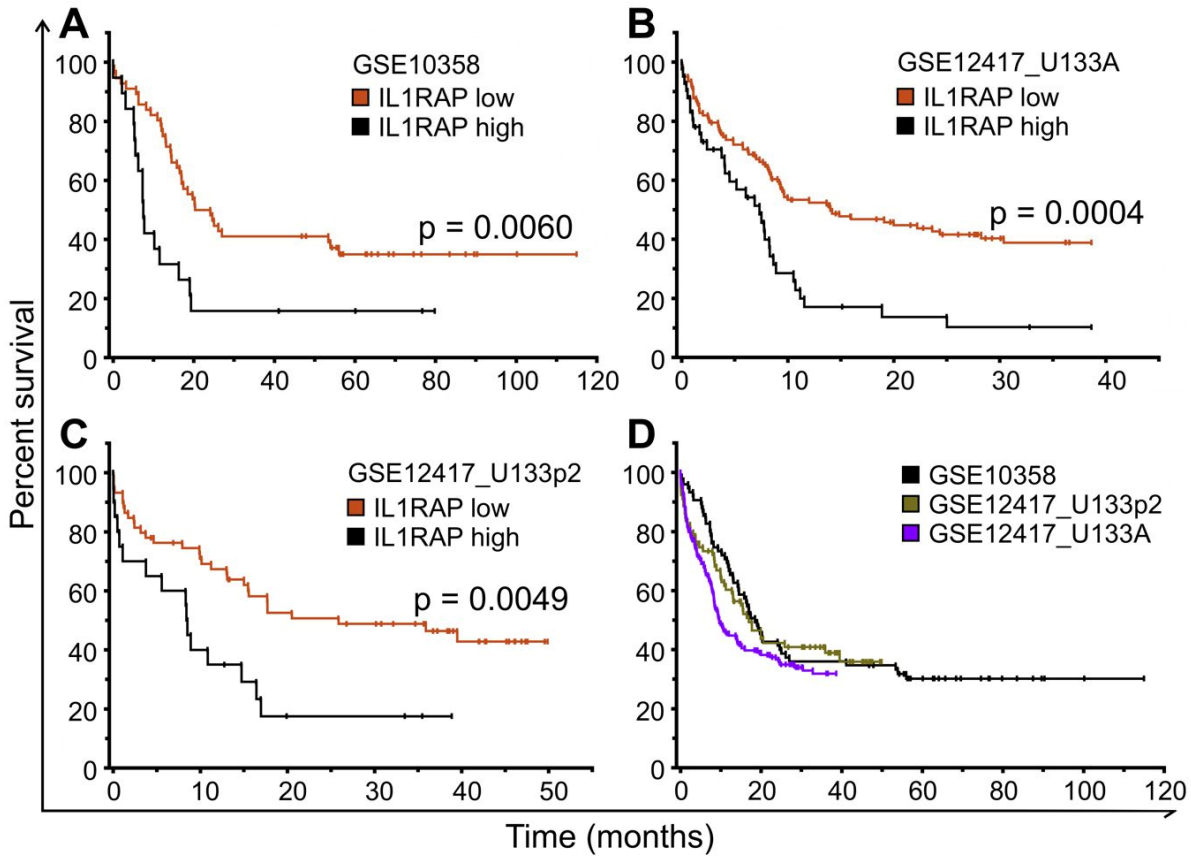
Suppl. Fig. S6. Unfractionated blasts of patients with -7 AML show similar levels of *IL1RAP* expression as healthy CD34$^+$ cells. Box plots showing *IL1RAP* expression levels in healthy CD34$^+$ cells (n=11) and in AML blasts with -7/7q chromosomal aberrations (n=18). The central box represents the values from the lower to the upper quartile (25th to 75th percentile). The middle square represents the mean and the median is indicated by the horizontal line. A line extends from the minimum to the maximum value.



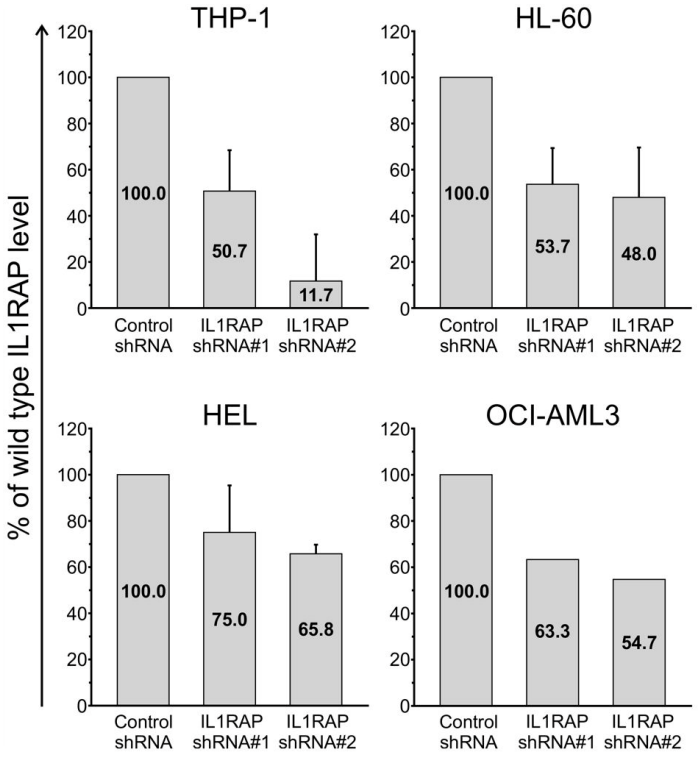
Suppl. Fig. S7. Validation of gene expression by RT-PCR of additional dysregulated genes in AML with monosomy 7.



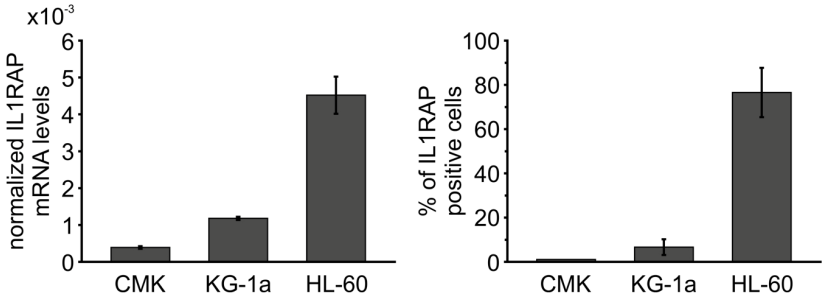
Suppl. Fig. S8. High *IL1RAP* expression levels are associated with poor overall survival in three different clinical cohorts of AML patients with normal karyotype. AML patients with normal karyotype are dichotomized using the 75th percentile as a cutoff for expression of *IL1RAP* in each of the published GEO datasets GSE12417 (U133plus2), GSE12417 (U133A), and GSE10358. **A)**, **B)**, **C)**: Kaplan-Meier plots of overall survival of patients with normal karyotype AML with high (black line) or low (red line) *IL1RAP* expression from the aforementioned datasets. Patients with high *IL1RAP* expression (cutoff at 75th percentile) show significantly inferior clinical outcome in each individual dataset. P-values (log-rank test) are indicated. **D)** Combined Kaplan-Meier plot of overall survival in all examined cohorts irrespective of IL1RAP status. The graph shows superimposable survival curves (p=0.1227, log-rank test), indicating that the patients have very similar overall survival in these datasets and can be combined for further analysis.



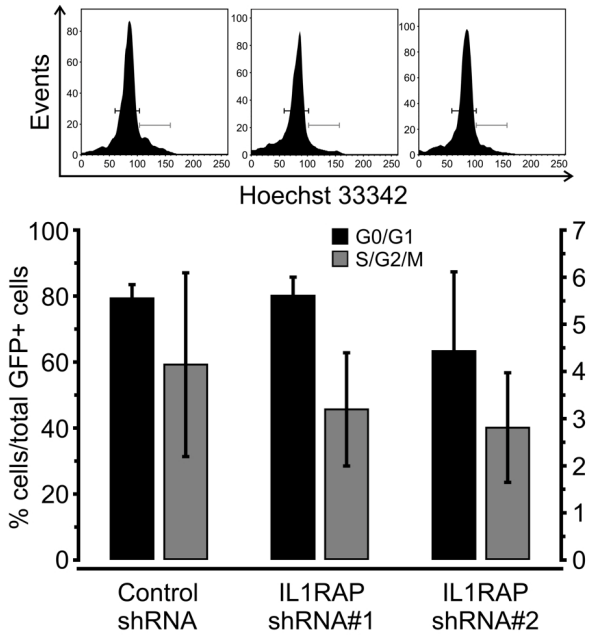
Suppl. Fig. S9. IL1RAP protein knockdown levels in AML cell lines. IL1RAP protein knockdown levels were determined by flow cytometry in THP-1, HL-60, HEL, OCI-AML3 cells infected with two different IL1RAP shRNA constructs. Relative expression levels normalized to a non-silencing control shRNA are shown. Mean values and standard deviation are shown.



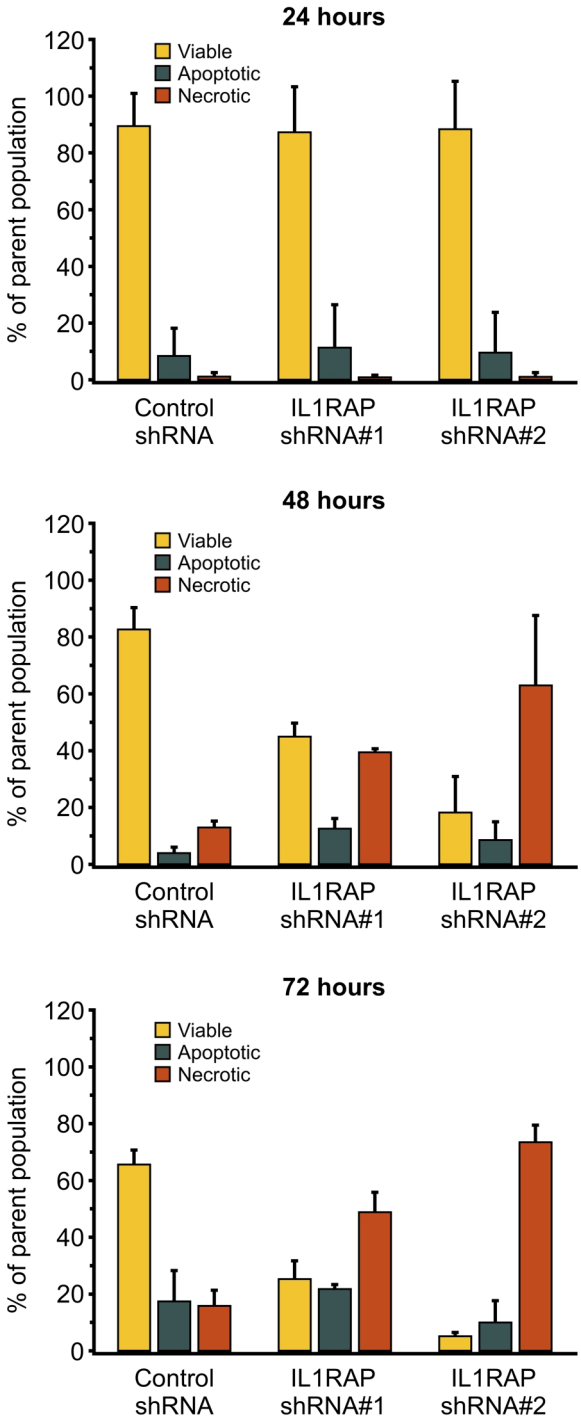
Suppl. Fig S10. Parallel detection of IL1RAP mRNA (left panel) and protein (right panel) in 3 AML cell lines with different expression levels. mRNA and protein levels of IL1RAP in 3 cell lines with different expression levels. *IL1RAP* mRNA was measured by quantitative RT-PCR and normalized to GAPDH mRNA. IL1RAP protein levels were measured by FACS. Mean and standard deviation are shown.



Suppl. Fig. S11. IL1RAP knockdown does not cause significant changes of the cell cycle profile in THP-1 cells. THP-1 cells were infected with a non-silencing or IL1RAP-specific shRNA constructs, and DNA content was analyzed using Hoechst 33342 staining. A representative histogram is shown in the upper panel. Mean and standard deviation of 3 independent experiments are plotted in the lower panel.



Suppl. Fig. S12. Time course of cell death in THP-1 cells. Analysis of apoptosis/necrosis with Annexin V/DAPI in THP-1 cells infected with control and IL1RAP shRNAs. Mean and standard deviation of 3 independent experiments are shown.



Supplementary tables

Table S1. AML patients characteristics

Specimen	Age at diagnosis/gender	Karyotype
AML-1	NA/F	45,XX,-7[3]/46,XX[1]
AML-2	58/M	45,XY,-7[4]/45,XY,-7,del(12)(p11p13)[16]
AML-3	78/M	46,XY,-7,+19[25]
AML-4	61/F	45,XX,-7[20]
AML-5	77/M	45,XY,-7[21]
AML-6	58/F	45,XX,-7[17]/45,XX,-7,add(15)(p15)[1]
AML-7	NA/F	45,XX,-7[5]/46,XX[15]
AML-8	60/M	45,XY,-7[20]
AML-9	26/F	45,XX,-7[6]
AML-10	62/F	45,XX,-7[15]
AML-11	80/F	45,XX,-7[23]
AML-12	50/M	46,XY[20] [a]
AML-13	55/M	46,XY[20] [a]
AML-14	56/M	46,XY[20] [a]
AML-15	56/M	46,XY[20] [a]
AML-16	60/F	46,XX[20] [a]

[a] Normal Karyotype is defined by 20 normal bone marrow metaphases, FISH negative for aberrations in chromosomes 5 and 7, and normal by PCR for BCR/ABL, PML/RARα, AML1/ETO, CBFβ/MYH11, and MLL-PTD. NA: not available

Table S2. Frequency of sorted populations in AML and healthy controls [% of total Lin^- cells]

	Specimen	Age/gender	Karyotype	%Lin^- $CD34^+CD38^-$ $CD90^+$	%Lin^- $CD34^+CD38^-$ $CD90^-$	%Lin^- $CD34^+CD38^+CD45RA^+$ $CD123^+$
AML	AML-5	77/M	45,XY,-7[21]	6.62	41.24	21.95
	AML-6	58/F	45,XX,-7[17]/45,XX,-7,add(15)(p15)[1]	0.13	0.95	71.54
	AML-8	60/M	45,XY,-7[20]	0.14	80.58	15.51
	AML-9	26/F	45,XX,-7[6]	0.08	92.47	4.79
	AML-10	62/F	45,XX,-7[15]	0.51	50.26	18.25
	AML-11	80/F	45,XX,-7[23]	1.10	6.17	73.46
HEALTHY	HBM-1	52/F		1.93	8.23	29.5
	HBM-2	40/M		1.64	14.55	25.3
	HBM-3	48/F		1.21	9.24	27.5
	HBM-4	47/M		1.47	11.09	31.4
	HBM-5	48/F		1.71	8.44	27.6
	HBM-6	47/M		1.71	8.87	25.5

Table S3. MDS patients characteristics

Specimen	Disease	% blasts	Karyotype
MDS-1	MDS (RAEB)/ AML	10	46,XX[20]
MDS-2	MDS (RAEB)	15	46,XY,del(5q),del(7q),del(20q)[6]
MDS-3	MDS (RAEB)	10-20	46,XY,del(7)(q21)[12]/46,XY,-7,+mar[8]
MDS-4	MDS (RCMD)	3	46,XX,del(11)(p11.2)[5]/46,XX[15]
MDS-5	MDS (RCMD)	<5	44,XX,del(5)(q13q33),-7,del(10)(q24), add(12)(p11.2),-20[20]
MDS-6	MDS(RCMD)	2	46,XY,del(5)(q13q33)x2,del(7)(q22),add(17)(p11.2)[14]/46,XY,t(3;14)(q21;q24),del(5)(q13q33),del(7)(q22),add(17)(p11.2),del(20)(q11.2)[6]
MDS-7	MDS/AML	80	47,XY,+8[20]

Table S4. Healthy control characteristics

Specimen	Age at diagnosis/gender	Karyotype
HBM-1	52/F	NA
HBM-2	40/M	NA
HBM-3	48/F	NA
HBM-4	47/M	NA
HBM-5	48/F	NA
HBM-6	47/M	NA
HBM-7	51/M	NA
HBM-8	43/F	NA
HBM-9	26/M	NA

NA: not available

Table S5. shRNA oligonucleotide sequences

shRNA oligonucleotide	Sequence
Luciferase Control shRNA	5'-gtgcgttgttagtactaatcctattt-3'
IL1RAP shRNA 1	5'- tggccttactctgatctggtattggacta-3'
IL1RAP shRNA 2	5'-cgggcattaattgatttcctactatattc-3'

Table S6. Differentially expressed genes in distinct compartments in AML with monosomy 7.

EXCEL SPREADSHEET, please see separately uploaded file