SUPPLEMENTARY APPENDIX

The IL1-IL1RAP axis plays an important role in the inflammatory leukemic niche that favors acute myeloid leukemia proliferation over normal hematopoiesis

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 doi:10.3324/haematol.2020.254987

Received: April 9, 2020.

Accepted: October 9, 2020.

Pre-published: October 29, 2020.

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Supplemental figures, table and methods

The IL1-IL1RAP axis plays an important role in the inflammatory leukemic niche that favors acute myeloid leukemia survival over normal hematopoiesis.

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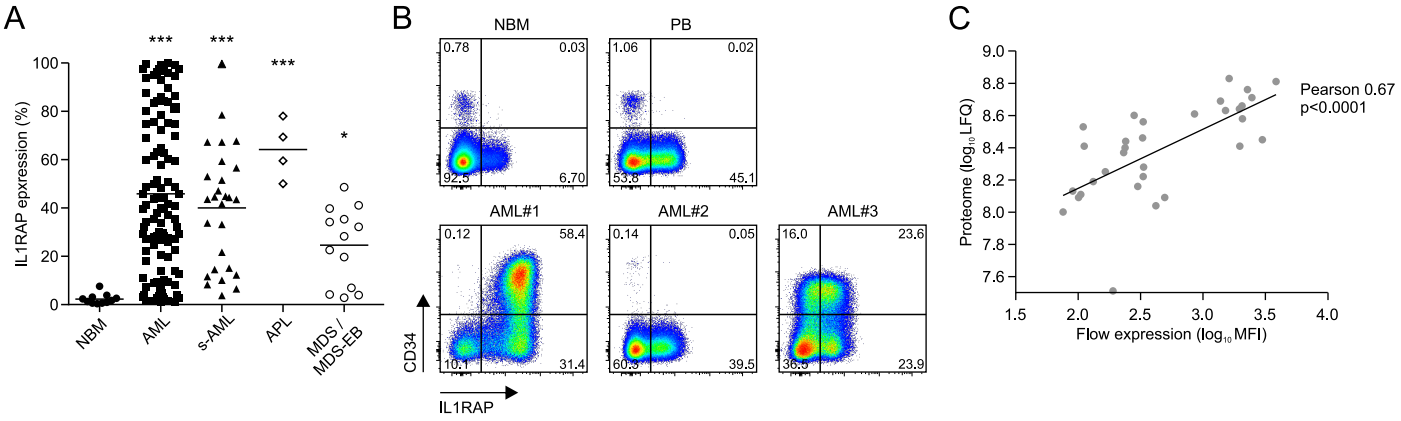
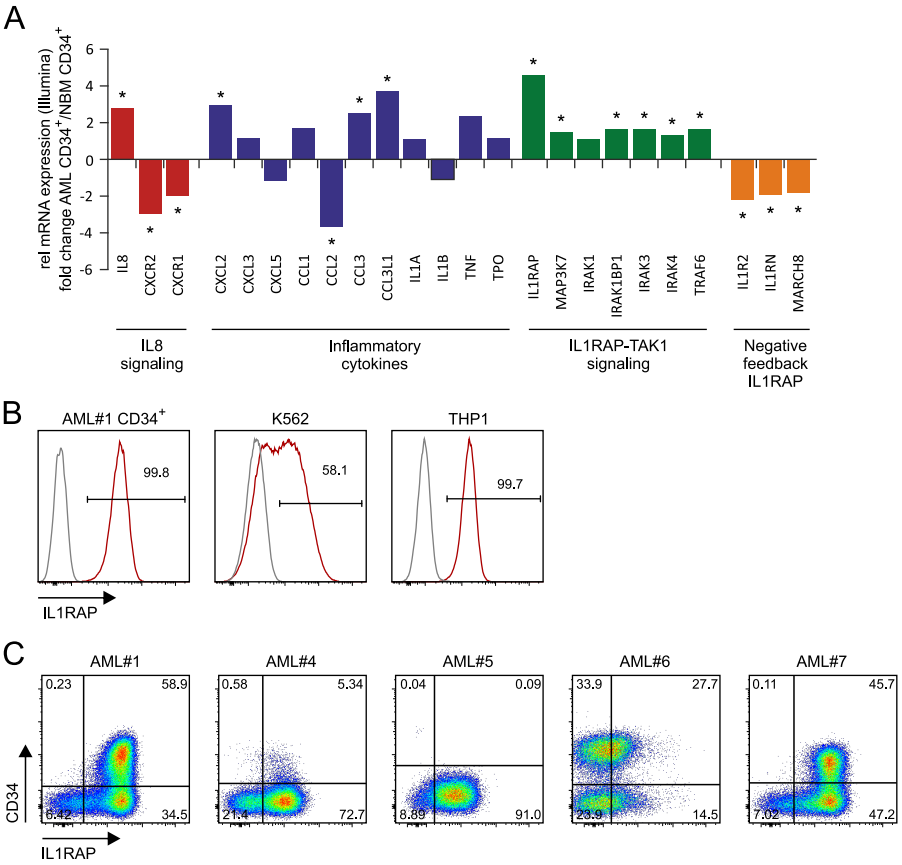
Supplemental Figure 1



Supplemental Figure 1. IL1RAP is a bona fide leukemia-specific PM protein.

(A) IL1RAP expression (%) measured by flow cytometry in NBM (n=11), *de novo* AML (n=110), sAML (n=27), APL (n=4) and MDS/MDS-RAEB (n=13). Statistical analysis was performed using a Kruskal-Wallis test. (B) Examples of IL1RAP expression in NBM, PB and 3 primary AML patient samples measured by flow cytometry. (C) Pearson correlation of IL1RAP PM expression (MFI) with quantitative proteome expression of IL1RAP (n=31).

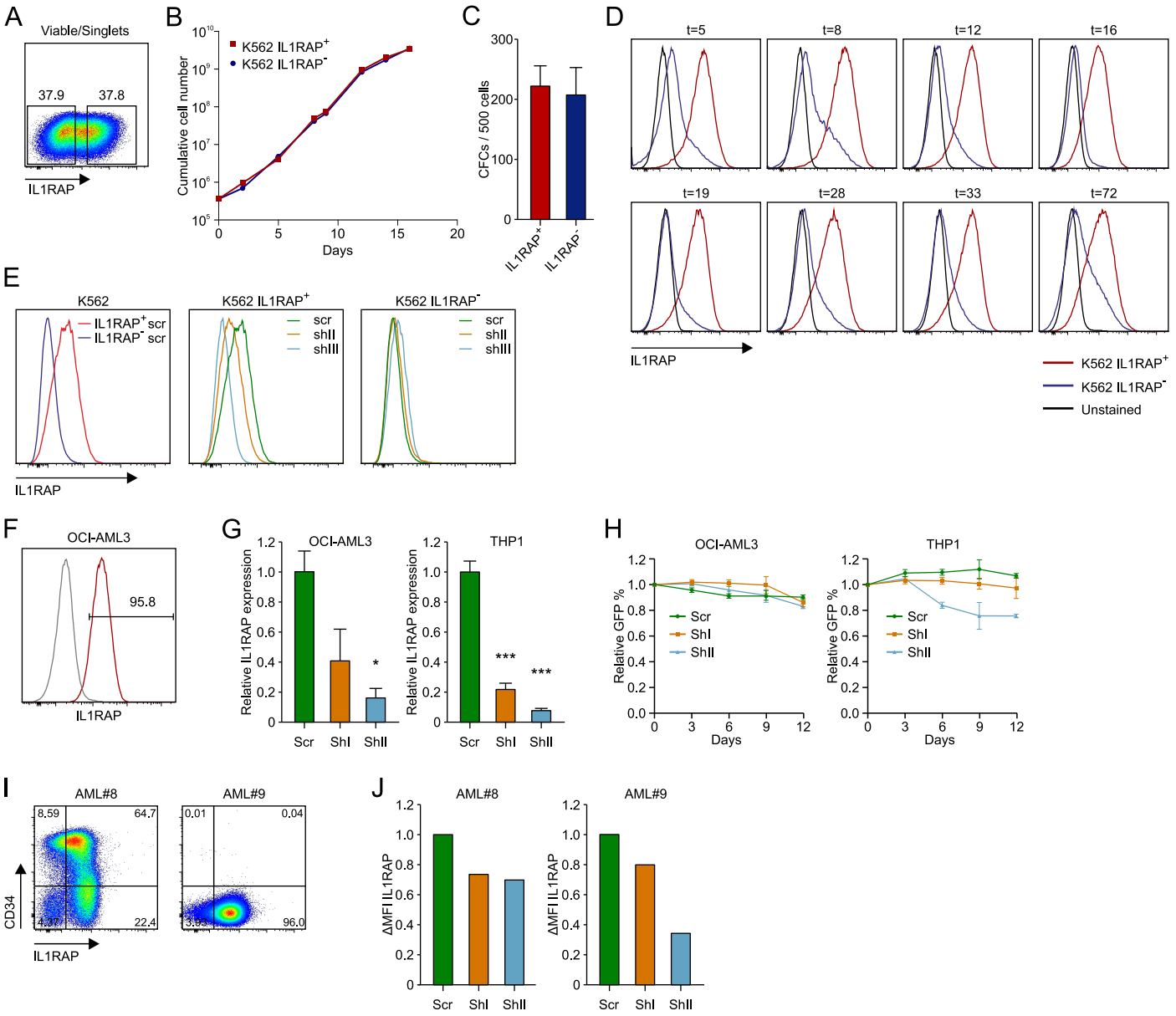
Supplemental Figure 2



Supplemental Figure 2. Genes associated with the IL1RAP signaling pathway are upregulated in AML.

(A) Transcriptome data of AML CD34$^+$ samples (n=60) and 40 NBM CD34$^+$ samples (n=40).[33] Fold change of the average of AML over the average of NBM was calculated for genes associated with the IL1RAP pathway and inflammatory cytokines. Statistical analysis was performed using a Student's t test. (B) Expression of IL1RAP in AML#1, K562, and THP1 cells measured by flow cytometry. (C) Expression of IL1RAP and CD34 in 5 primary AML patients measured by flow cytometry. * $p<0.05$.

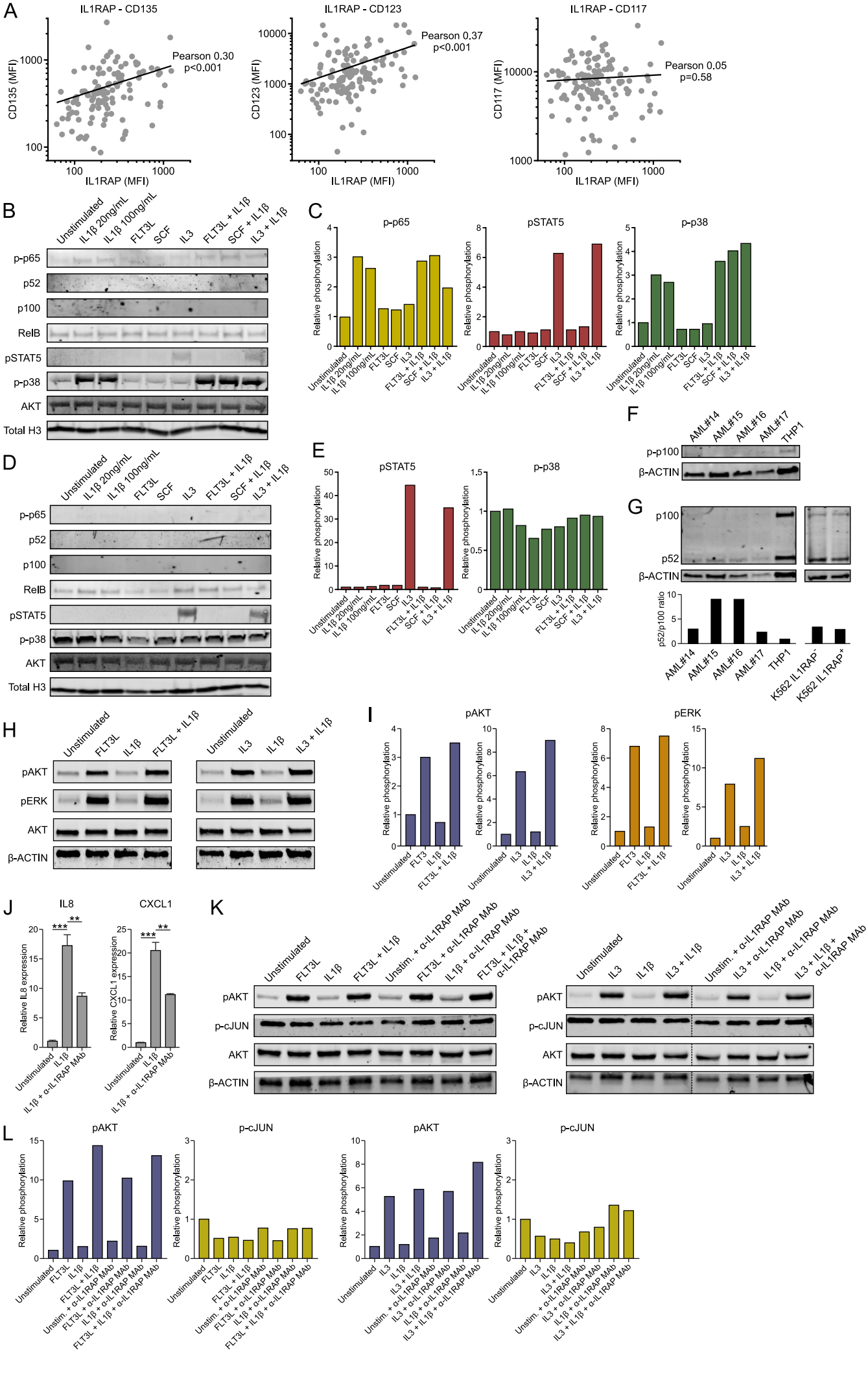
Supplemental Figure 3



Supplemental Figure 3. K562 IL1RAP$^+$ and IL1RAP$^-$ cells are stable in time.

(A) IL1RAP expression on K562 measured by flow cytometry. (B) Growth curve of K562 IL1RAP$^+$ and IL1RAP$^-$ sorted cells (n=3). (C) CFC output of K562 IL1RAP$^+$ and IL1RAP$^-$ sorted cells. Bars indicate mean ±SD of biological duplicates. (D) IL1RAP expression of sorted K562 IL1RAP$^+$ and IL1RAP$^-$ cells measured by flow cytometry in time. (E) IL1RAP plasma membrane protein expression measured by flow cytometry in K562 IL1RAP$^+$ and IL1RAP$^-$ cells transduced with shRNA's including a non-targeting control (scr) and shRNA's targeting IL1RAP (shI and shII). Expression was measured 26 days after transduction. (F) IL1RAP expression in OCI-AML3 cells measured by flow cytometry. (G) Knockdown efficiency in OCI-AML3 and THP1 cells measured by qRT-PCR. Bars indicate mean ±SD of technical triplicates. Statistical analysis was performed using a Student's t.test. (H) GFP percentage relative to day 0 of scrambled (scr) and shRNA's targeting IL1RAP (shI/II) transduced OCI-AML3 and THP1 cells measured in time. (I) IL1RAP and CD34 expression of 2 primary AML cells used for CFC analysis in Figure 3G. (J) IL1RAP plasma membrane protein expression (MFI) in AML#8 and #9 transduced with a non-targeting scrambled control (scr) and shRNA's targeting IL1RAP (shI and shII). Data are plotted relative to scr control. * $p<0.05$, *** $p<0.001$.

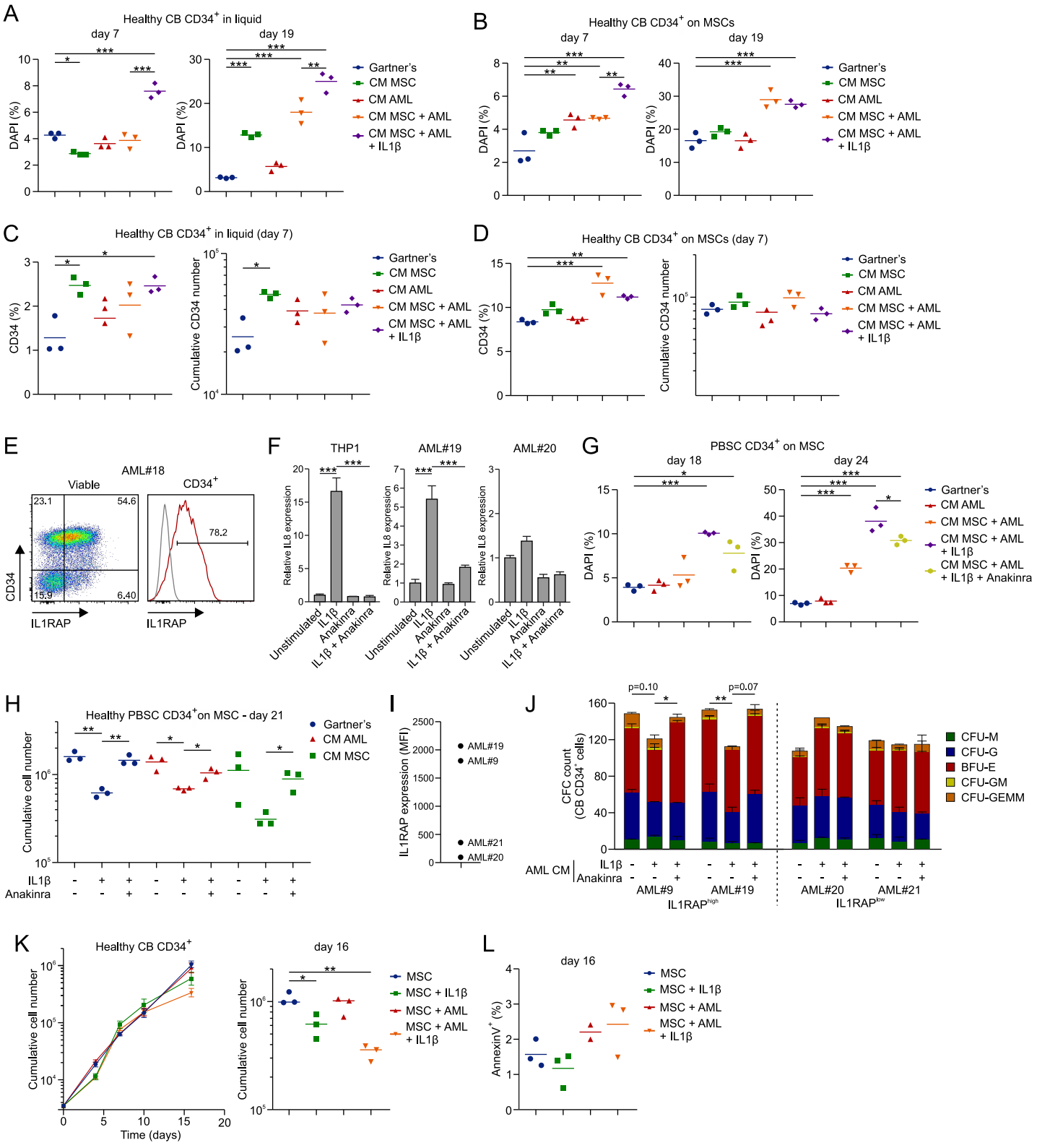
Supplemental Figure 4



Supplemental Figure 4. Synergism of the IL1-IL1RAP signaling with other active signaling pathways in AML.

(A) Pearson correlation of IL1RAP with CD135, CD123 and CD117 in blasts of 124 primary AML patients, related to Figure 4C. (B) Western blot of primary patient $CD34^+$ blasts with low CD135 expression but positive for IL1RAP, CD117 and CD123. Cells were stimulated with IL1β, FLT3L, SCF, IL3 or their combination. (C) Quantification of Western blot in panel B, p-p65 was normalized to total H3, pSTAT5 and p-p38 were normalized on AKT. (D) Western blot of primary patient $CD34^+$ blasts with low IL1RAP expression but positive for CD135, CD117 and CD123. Cells were stimulated with IL1β, FLT3L, SCF, IL3 or their combination. (E) Quantification of Western blot in panel D, pSTAT5 and p-p38 were normalized on AKT. (F) Western blot for p-p100 in primary patient $CD34^+$ blasts and THP1 cells (G) Western blot for p52/p100 in primary patient $CD34^+$ blasts, THP1 cells, K562 $IL1RAP^-$ cells, and K562 $IL1RAP^+$ cells. Relative p52/p100 levels are plotted as bar graph. (H) Western blot of THP1 cells stimulated with IL1β (50ng/ml), FLT3L, IL3 or their combination. (I) Quantification of Western blot in panel H, pAKT was normalized on total AKT and pERK was normalized on β-ACTIN. (J) qRT-PCR analysis of IL8 (left) and CXCL1 (right) in THP1 cells that were stimulated with IL1β after incubation with or without α-IL1RAP MAb. Bars indicate mean ±SD of technical triplicates. Statistical analysis was performed by a Student's t.test. (K) Western blot of THP1 cells stimulated with IL1β, FLT3L, IL3 or their combination after incubation with or without α-IL1RAP MAb. (L) Quantification of Western blot in panel a, pAKT was normalized on total AKT and p-cJUN was normalized on β-ACTIN. ** $p<0.01$ *** $p<0.001$

Supplemental Figure 5



Supplemental Figure 5. Effect of treatment with CM of AML (co-)cultures on the hematopoietic output.

(A) DAPI percentage at day 7 and 19 of CB $CD34^+$ liquid culture treated with different CM. (B) DAPI percentage at day 7 and 19 of CB $CD34^+$ MSC co-culture treated with different CM. (C) CD34 percentage and cumulative cell number at day 7 of CB $CD34^+$ liquid culture treated with different CM. (D) CD34 percentage and cumulative cell number at day 7 of CB $CD34^+$ MSC co-culture treated with different CM. (E) IL1RAP and CD34 expression of AML#18 as measured by flow cytometry. (F) qRT-PCR analysis of IL8 in THP1 cells, AML#19, and AML#20 that were stimulated with IL1β after incubation with or without Anakinra. Bars indicate mean ±SD of technical triplicates. (G) DAPI percentage at day 18 and 24 of PBSC $CD34^+$ MSC co-culture treated with different CM. (H) Cumulative cell number on day 21 of $CD34^+$ PBSCs on a stromal layer of MSCs with or without addition of IL1β and Anakinra. (I) IL1RAP plasma membrane protein expression measured by flow cytometry in AML#9, #19-21. (J) Representative example out of two independent CFC assays with CM-treated CB $CD34^+$ cells related to Figure 5G. Data of two technical duplicates are shown. (K) Growth curve (left) and cumulative cell number on day 16 (right) of CB $CD34^+$ cells in triple co-culture with MSCs and AML#22 $CD34^+$ cells ±IL1β (L) Percentage of $AnnexinV^+$ cells at day 16 of triple co-culture with MSCs, AML#22 $CD34^+$ cells and CB $CD34^+$ cells. Statistical analysis in all panels was performed by a Student's t.test. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Supplementary Table 1

Patient characteristics

AML #number	BM/PB	FLT3	NPM1	karyotype	Illumin Trusight Seq (VAF, %)	Used in Figure
1	BM/PB	ITD	wt	47,XY,t(3;5)(q23;q33),+8[10], IDH1 R132H	IDH1 (34.8), FLT3 (73.3), NRASG12V (3)	Fig. 1A, Fig. 2A-D, Fig. 5B-E, Supl. Fig. 1B, Supl. Fig. 2B-C, Supl. Fig. 5A-D
2	PB	wt	cyt	NK	IDH1 R132H	Supl. Fig. 1B
3	PB	ITD	wt	NK	NA	Supl. Fig. 1B
4	BM	wt	cyt	NK	IDH1 (33.6), NPM1 (42.2)	Fig. 2D, Supl. Fig. 2C
5	BM	wt	cyt	NK	IDH1 R132H	Fig. 2D, Supl. Fig. 2C
6	PB	wt	wt	NK	NA	Fig. 2D, Supl. Fig. 2C
7	BM	wt	cyt	NK	DNMT3A (49.8), NPM1 (42.2), WT1 (8.6), WT1 (26.5)	Fig. 2D, Supl. Fig. 2C
8	PB	wt	cyt	NK	NA	Fig. 3G, Supl. Fig. 3I-J
9	PB	ITD	wt	46,XY,t(7;11)(p15;p15)	NA	Fig. 3G, Fig. 5G, Supl. Fig. 3I-J, Supl. Fig. 5I-J
10	BM	ITD	wt	+8	NA	Fig. 4A, 4D-E
11	BM	wt	cyt	NK	EZH2, TET2, SFR3B	Fig. 4A, 4F-G
12	BM	wt	cyt	NK	NA	Fig. 4A, Supl. Fig. 4D-E
13	PB	ITD	cyt	NK	DNMT3A, IDH2	Fig. 4A, Supl. Fig. 4B-C
14	PB	ITD	cyt	NK	IDH1 (33.9), NPM1 (42.8), FLT3 (3.2), FLT3-ITD (51)	Supl. Fig. 4F-G
15	BM	ITD	cyt	NK	NA	Supl. Fig. 4F-G
16	PB	wt	wt	NK	EZH2 (97.4), ASXL1 (49.5), RUNX1 (97.7)	Fig. 5J, Supl. Fig. 4F-G
17	PB	wt	wt	NK	DNMT3A (48.8), IDH2 (46.9), SRSF2 (43.9)	Supl. Fig. 4F-G
18	PB	wt	wt	45,XY,-7, del(12)(p11p12)[7]/46,XY[3]	DNMT3A (54); SRSF2 (50); SETBP1 (42); PTPN11 (10); NRAS (9); KRAS (6)	Fig. 5F, Supl. Fig. 5E, 5G-H
19	BM	ITD	cyt	NK	DNMT3A, IDH2, NPM, TCF3	Fig. 5G, Supl. Fig. 5F, 5I-J
20	PB	wt	wt	inv16	NA	Fig. 5G, Supl. Fig. 5F, 5I-J
21	BM	wt	cyt	NK	NA	Fig. 5G, Supl. Fig. 5I-J
22	PB	NA	NA	NK	NA	Fig. 5K, Supl. Fig. 5K-L

Supplementary Table 2

Genome-wide transcriptome analysis in $CD34^{+}$ cells of AML#1, THP1 cells, and K562 cells that were stimulated with IL1β for 1 hr

Supplemental Methods

Patient samples

Neonatal cord blood (CB) was harvested from healthy full-term pregnancy placentas, healthy mobilized peripheral blood stem cells (PBSCs) were obtained from left overs of transplant material, normal bone marrow (NBM) was obtained after hip surgery of healthy individuals. Cells from acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) patients were obtained from bone marrow (BM) or peripheral blood (PB) at diagnosis. Primary mesenchymal stromal cells (MSCs) were retrieved from a healthy donor that underwent hip surgery. MSCs were expanded in αMEM with 200mM glutamine (Lonza), 10Units/mL heparin, 5% human platelet lysate (Sanquin) and 1% p/s and frozen at low passage until further use. Mononuclear cells (MNCs) were isolated using a ficoll gradient separation (LymphoprepTM) and either freshly used or frozen in liquid nitrogen. Cryopreserved MNCs of AML patients and mobilized PB were thawed, resuspended in new born calf serum (NCS) supplemented with DNase I (20Units/mL), 4μM $MgSO_4$ and heparin (5Units/mL) and incubated on 37˚C for 15 min. $CD34^+$ cells of CB, mobilized PB and AML MNCs were isolated on the autoMACS Pro Separator using a magnetically activated cell-sorting progenitor kit (Miltenyi Biotech). All healthy individuals and AML patients gave an informed consent in accordance with the Declaration of Helsinki at the University Medical Centre Groningen (UMCG) and Martini Hospital Groningen, the Netherlands. All protocols were approved by the Medical Ethical Committee of the UMCG.

Cell (co-/triple-)cultures

Leukemic human cell lines were cultured in RPMI 1640 medium with 200mM glutamine (Lonza) supplemented with 10% fetal calf serum (FCS) (Lonza) and 1% penicillin-streptomycin (p/s) (PAA laboratories), at 37°C. In case of serum depletion (Figure 3H), cells were treated with 10%, 1% and 0.5% FCS ± 10ng/mL IL1β (Sigma-Aldrich). Fresh medium and IL1β was added after each demi-population. For co-culture experiments, cryopreserved MSCs of the same donor were thawed, resuspended in Gartner's medium consisting of αMEM with 200mM glutamine supplemented with 12.5% FCS, 12.5% horse serum (Invitrogen), 1% p/s, 57,2μM β-mercaptoethanol and 1μM hydrocortisone (both Sigma-Aldrich). MSCs were plated in 0.1% gelatin coated 96-wells plates and grown confluent. Subsequently, $1x10^5$ AML $CD34^+$ cells or between $5x10^3$-$15x10^3$ PBSC/CB $CD34^+$ cells were plated into Gartner's medium with the addition of 20ng/mL granulocyte colony-stimulating factor (G-CSF), Nplate and IL3. Inhibition of the IL1-signaling pathway was established by the addition of 500ng/mL Anakinra (Swedish Orphan Biovitrum BVBA). MSC/AML/CB/PBSC (co-)cultures were grown at 37˚C and 5% CO_2 and demi-populated every 3-5 days, replacing 50% of the volume with fresh Gartner's medium or conditioned medium (CM). CM was added in a 1:1 ratio (v/v) to CB CD34+

cultures after 4 days and subsequently every time they were demi-populated (t=7, t=10, and t=14) (Figure 5C-E). In case of PBSC $CD34^+$ cultures, CM was added at day 0 and every following demi-population (t=4, t=11, t=15, t=18) (Figure 5F, and 5J). In case of triple co-cultures, CB $CD34^+$ cells were lentivirally transduced with a pLKO eGFP vector in order to distinguish them from AML cells. CB $CD34^+GFP^+$ cells were plated on a confluent layer of MSCs in a 1:15 – 1:20 ratio with AML $CD34^+$ cells and demi-populated every 3-5 days replacing 50% of the volume with fresh Gartner's medium. All cultures were performed in triplicate. Suspension cells were retrieved by shaking on a plate shaker, stained with CD34, CD38, CD45, AnnexinV, and DAPI (Thermo Scientific) and analyzed on the MacsQuant Analyzer 10 (Mylteni) or CytoFLEX (Beckman Coulter) flow cytometer.

Flow Cytometry

Cryopreserved MNCs of AML and MDS patients and NBM samples were thawed as described in the "patient samples" section. In general, for flow cytometry analysis and cell sorting, cells were treated with human FcR blocking reagent (Miltenyi Biotech) prior to antibody staining. Subsequently, cells were incubated with the appropriate antibody cocktail and incubated at 4˚C for 30 min. Immediately before analysis, DAPI (Thermo Scientific) was added as viability stain. Flow cytometry analysis in the diagnostic research lab, as shown in Figure 4A-B, Supplemental Figure 1A-B, and Supplemental Figure 4A, was performed according to the Euroflow protocol [1]. Here, freshly obtained whole BM aspirated samples were used. After ammonia lysis of erythrocytes, the isolated BM cells were FcR blocked with 50mg/mL human IgG (Sanquin) and incubated with different antibodies. After incubation, the cells were fixed with FACS lysing solution (BD Biosciences) and washed twice in PBS before flow cytometric measurements. Fluorescence was measured on the MacsQuant Analyzer 10 (Miltenyi Biotech) or in case of the routine diagnostics on the FACSCanto II ™ flow cytometer (BD Biosciences). Cell sorting was performed on a MoFlo XDP (Beckman Coulter). Data were analyzed using Flow Jo (Tree Star, Inc) in case of Supplemental Figure 1A-B. Co-expression of IL1RAP with FLT3, CD117 and CD123 in case of Figure 4A-B, and Supplemental Figure 4A was analyzed with Infinicyt™ (Cytognos) in order to merge expression on the basis of a common backbone followed by analysis in Flow Jo (Tree Star, Inc). Antibodies used in this study: CD34 (581), CD45 (HI30), FLT3 (4G8), CD38 (HIT2), AnnexinV 550475 (all BD Biosciences), IL1RAP (#89412) (R&D), CD123 (6H6) (Biolegend), CD34 (581) (Thermo Scientific), and CD117 (104D2D1) (Beckman Coulter).

Colony-forming-cell (CFC) assay

AML#9, #19-21 were cultured on a stromal layer of MSCs and treated with or without IL1β and Anakinra for 7 days. 900µL CM was harvested and mixed with 1600µL methylcellulose (Stem Cell

Technologies), and supplemented with 20ng/mL IL3, IL6, SCF, G-CSF, and TPO. CB CD34+ cells were sorted directly into the CFC-mixture and plated in two cell culture dishes as technical duplicate. Colonies were scored after 2 weeks of incubation at 37°C.

Lentiviral transfection

HEK 293T cells were transfected with 3µg of the pLKO eGFP construct, containing short hairpins against IL1RAP sh1: 5'-TGGCCTTACTCTGATCTGGTATTGGACTA-3' , sh2: 5'-CGGGCATTAATTGATTTCCTACTATATTC-3'[2] or scrambled control 5'-TTCTCCGAACGTGTCACGTT-3', together with 3µg of the packaging construct pCMV-dR8.91 (Delta 8.9) containing gag, pol and rev genes, 0,7µg of glycoprotein VSV-G expressing envelope plasmid and 21µl Fugene transfection reagent (Roche). In case of AMLs, prior to transduction the virus was concentrated 10 times and 24 wells plates were coated with retronectin. $0.75x10^6$-$1x10^6$ AML cells per well were transduced in 400 µL of Gartner's medium supplemented with 20ng/ml G-CSF, N-plate, and IL3, and 8µg/ml polybrene (Sigma-Aldrich) with 200µL of the virus. After 24 hour (hr), the cells were washed 3 times with PBS + 2% FCS and a second round of transduction (24 hr) was performed. Cells were washed 3 times and plated on mouse stromal 5 (MS5) cells in Gartner's medium supplemented with 20ng/ml G-CSF, N-plate, and IL3 until sorting. Viral particles were collected in αMEM and stored at -80°C until further use. For transduction, AML cell lines were transduced in 2 rounds with 1:1 ratio virus to medium in the presence of 8µg/mL Polybrene. Cells were washed 3 times with PBS + 2% FSC and sorted for GFP^+DAPI^- on a MoFlow Astrios cell sorter (Beckman Coulter).

Immunofluorescent microscopy

$5x10^4$ cells were spun down on slides with a cytospin and immediately fixated in 4% paraformaldehyde (PFA), permeabilized with PBS + 0,1% Triton X-100 and washed once with PBS^+ (blocking solution containing 0,5% bovine serum albumin (BSA) and 0,15% glycine). Slides were incubated with primary antibodies Rabbit anti-IL1RAP (Thermo Scientific) in a moist environment at room temperature, rinsed with PBS + 0,1% Triton X-100 and PBS^+ and incubated with secondary antibodies FITC-Goat anti-Rabbit (Life Technologies). At the final step, DAPI solution (Thermo Scientific) was added, cells were washed with PBS and preserved in the mounting medium (Vector Laboratories). Images were taken with a DM6000B fluorescent microscope (Leica).

Western blotting

For the detection of phosphorylated proteins, $5x10^4$ primary AML $CD34^+$ cells were serum-starved for 1 hr, stimulated with 20 or 100ng/mL IL1β for 10 min, spun down in a pre-cooled centrifuge at 4°C and

directly boiled in Laemmli-SDS-PAGE sample buffer. In case of K562 IL1RAP$^{+/-}$ cells (Figure 3B), cells were incubated with or without 2µM or 10µM IKK inhibitor (BMS-345541, Sigma Aldrich) for 4 hr, and subsequently stimulated with 20ng/mL IL1β for 15 minutes. Evaluation of non-canonical NFκB in AML#14-17 was performed on the mononuclear cell fraction. For co-stimulation analysis, AML cells were stimulated for 10 min either alone with 50ng/mL FLT3L, SCF or IL3 or in combination with 20ng/mL IL1β. In case of co-stimulation analysis in THP1 cells, 5x104 cells were serum-starved for 3 hr and subsequently stimulated for 10 min with 10ng/mL or 50ng/mL IL1β alone or in combination with either 50ng/mL FLT3L or 50ng/mL IL3. IL1-signaling was blocked using 10µg/mL anti-IL1RAP MAb (R&D) for 24 hr prior to stimulation. Samples were loaded onto a Mini-PROTEAN® TGX™ Precast Protein Gel (Bio-Rad) and run in electrophoresis buffer (25mM, 152mM glycine, 0.07% SDS). The PageRuler Plus Prestained Protein Ladder (Thermo Scientific) was used to determine protein size. Subsequently, the gel was blotted onto a methanol activated PVDF FL-membrane (Immobilion) using a Trans-Blot Turbo™ Blotting System (Bio-Rad) according to the manufacturer's protocol. The membrane was blocked with Odyssey Blocking Buffer in PBS (Li-Cor), followed by blocking in milk in case of p52/p100, and incubated overnight with primary antibodies including phospho-p38 Thr180/Tyr182, phospho-AKT Ser473, phospho-ERK 1/2 T202/Y204 (CST), p-cJUN S73(CST), p-p65 Ser536, p-p100 Ser866/870, p100/p52 (#4882), RelB (#4922) (all Cell Signaling Technology, CST), and phospho-STAT5 pY694 (BD Biosciences). Total AKT (pan) (CST), total H3 (CST) and β-ACTIN (Santa Cruz) were used as loading controls. Blots were washed with TBST (TBS with 0,1% Tween 20) and incubated for 2 hr with secondary antibodies including anti-mouse Alexa Fluor 800 and anti-rabbit Alexa Fluor 680 (Invitrogen). The membranes were visualized on the Odyssey CLx Near-Infrared Fluorescence Imaging device (Li-Cor) and analyzed with Image Studio Lite 5.2 software.

Transcriptome analysis

2x10^5 K562, THP1 and AML CD34$^+$ cells were stimulated in 10ng/mL IL1β (Sigma-Aldrich) for 1 hr. Cells were harvested and RNA was isolated using the RNeasy mini kit (Qiagen) according to the manufacturer's protocol. Genome-wide expression analysis was performed on Illumina (Illumina, Inc) BeadChip Arrays (Illumina Human HT-12 v4 Expression Beadchips). 0.75µg of cRNA combined was used in labelling reactions and hybridization with the arrays according to the manufacturer's instructions. Data was analyzed using GenomeStudio V2011.1 Gene Expression Module v1.9.0 (Illumina, Inc.) and Genespring (Agilent). A quantile log2 normalization of the data was performed. Gene ontology (GO) analysis and gene set enrichment analysis (GSEA) was performed on a ranked gene list using Gorilla and GSEA 4.0.3 software, respectively [3,4]. In case of IL1RAP^{high} and IL1RAP^{low} protein expression, proteins annotated in at least 10 AML samples were ranked on Pearson correlation coefficient with

IL1RAP expression (n=42). GSEA was performed with respect to MsigDB C2 and C5 gene sets (version 7) or gene sets generated from selected publications as shown in the relevant figures.

qRT-PCR

$2x10^5$ primary AML $CD34^+$ cells, human MSCs, K562 $IL1RAP^{+/-}$ or THP1 cells were incubated with 10ng/mL IL1β for 1 hr. IL1-signaling was blocked using 10µg/mL anti-IL1RAP MAb (R&D) or 500ng/mL Anakinra for 24 hr prior to stimulation. RNA was isolated using the RNeasy Mini Kit (QIAGEN) according to the manufacturer's protocol. cDNA was synthesized with iScript cDNA Synthesis Kit (Bio-Rad) and qRT-PCR was performed using SsoAdvanced™ Universal SYBR Green Supermix on the CFX Connect Real-Time instrument (Bio-Rad). Expression of the ribosomal protein like (RPL) 27 gene was used to calculate relative expression levels. In case of inhibitory experiment in Figure 3A, cells were pre-treated with TAK1 inhibitor (100nM 5z-7-oxozeaenol), NFκB inhibitor (2µM BMS-345541), P38 inhibitor (5µM SB203580) or MEK/ERK inhibitor (10µM U0126) (all Sigma-Aldrich) for 2 hr prior to stimulation with 10ng/mL IL1β for 1 hr.

Statistical analysis

Data of growth curves and qRT-PCR are presented as mean ± SD. Significance was determined by either a Kruskal-Wallis test or Student's t.test using Graphpad Prism software as indicated in the figure legends. p values are indicated in the figure legends. FDR q-values and NES scores were used as indication for significance of GSEA plots as indicated in the figure legends.

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