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Supplemental material

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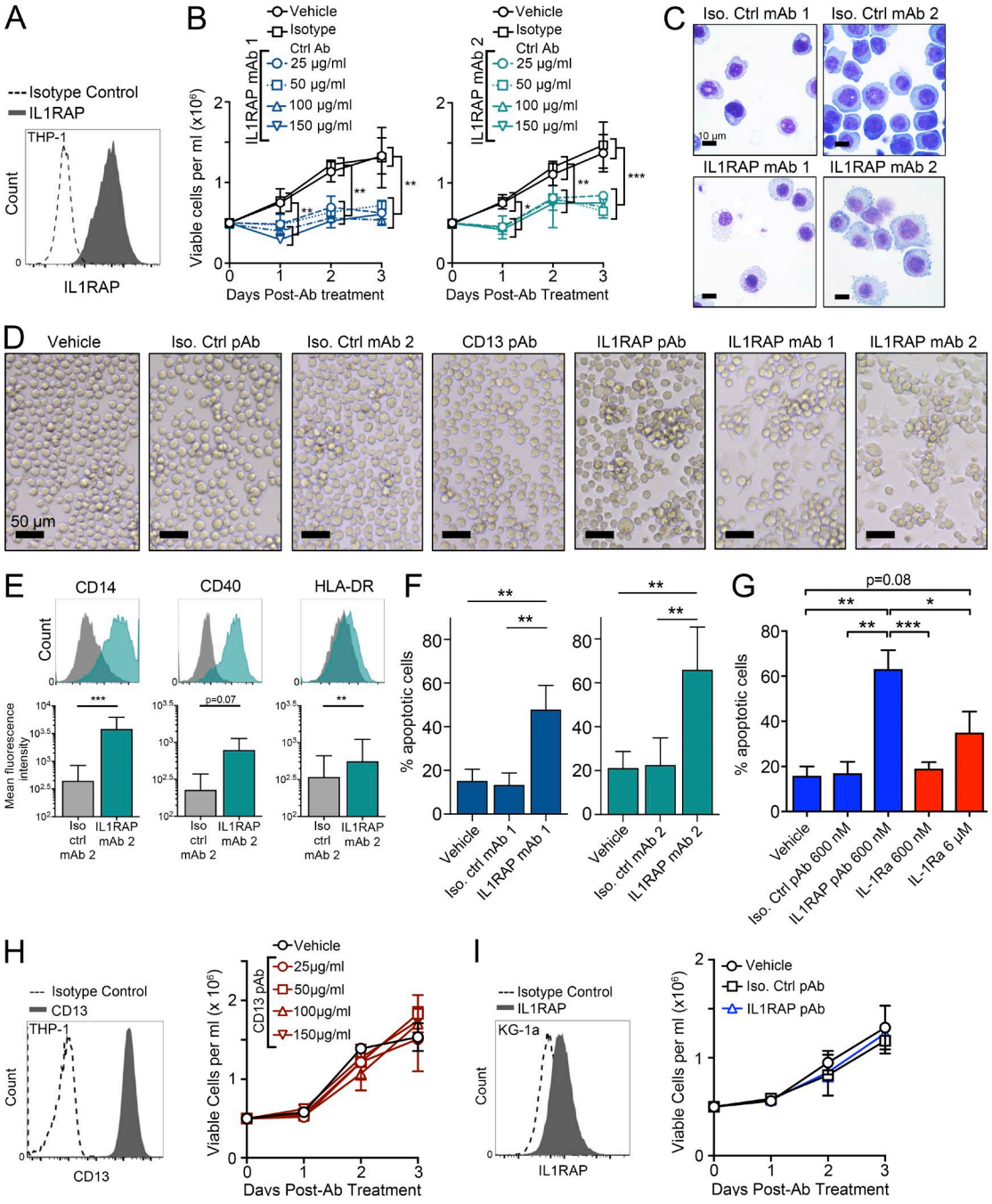

Figure S1. **Targeting of IL1RAP with antibodies reduces growth of AML cells by inducing differentiation and apoptosis. (A)** IL1RAP expression by flow cytometry in THP-1 cells using a fluorescently conjugated IL1RAP antibody. One representative experiment is shown. **(B)** Cell proliferation of THP-1 cells treated with different doses of two IL1RAP monoclonal antibodies. Data represent the mean ± SD of two independent experiments. P-values were calculated using unpaired two-tailed *t* tests and multiple comparisons were corrected for using the Holm-Sidak method. **(C)** Morphology of THP-1 cells after treatment with 150 µg/ml IL1RAP mAbs or isotype control mAbs for 24 hrs. 100× magnification, 1.25 NA. **(D)** Photos of THP-1 cells in liquid culture after treatment with IL1RAP antibodies. 10×, 0.25 NA. **(E)** Expression of macrophage differentiation markers in THP-1 cells by flow cytometry 24 h after addition of 150 µg/ml IL-1RAP mAb 2. Representative histograms are shown (top). Bar graphs (bottom) represent the mean ± SD of four independent experiments. P-values were calculated using ratio-paired two-tailed *t* tests comparing raw MFI values of isotype control mAb 2 treated cells versus IL1RAP mAb 2 treated cells. **(F)** Percentage of apoptotic (annexinV⁺) THP-1 cells after treatment with 150 µg/ml IL1RAP mAb 1 or mAb 2 for 72 hrs. Data represent the mean ± SD of four independent experiments. P-values were calculated using unpaired two-tailed *t* tests. **(G)** Percentage of apoptotic (annexinV⁺) THP-1 cells after treatment with 600 nM IL1RAP pAb, 600 nM IL-1Ra, or 6 µM IL-1Ra for 72 h. Data represent the mean ± SD of three independent experiments. P-values were calculated using unpaired two-tailed *t* tests. **(H)** CD13 expression by flow cytometry in THP-1 cells using a fluorescently conjugated CD13 antibody. One representative experiment is shown (left). Cell proliferation of THP-1 cells treated with a CD13 pAb. Data represent the mean ± SD of two technical replicates (right). **(I)** IL1RAP expression by flow cytometry in KG-1a cells using a fluorescently conjugated IL1RAP antibody. One representative experiment is shown (left). Cell proliferation of KG-1a cells treated with 150 µg/ml IL1RAP pAb. Data represent the mean ± SD of two independent experiments (right). *, P < 0.05; **, P < 0.01; *** P < 0.001.

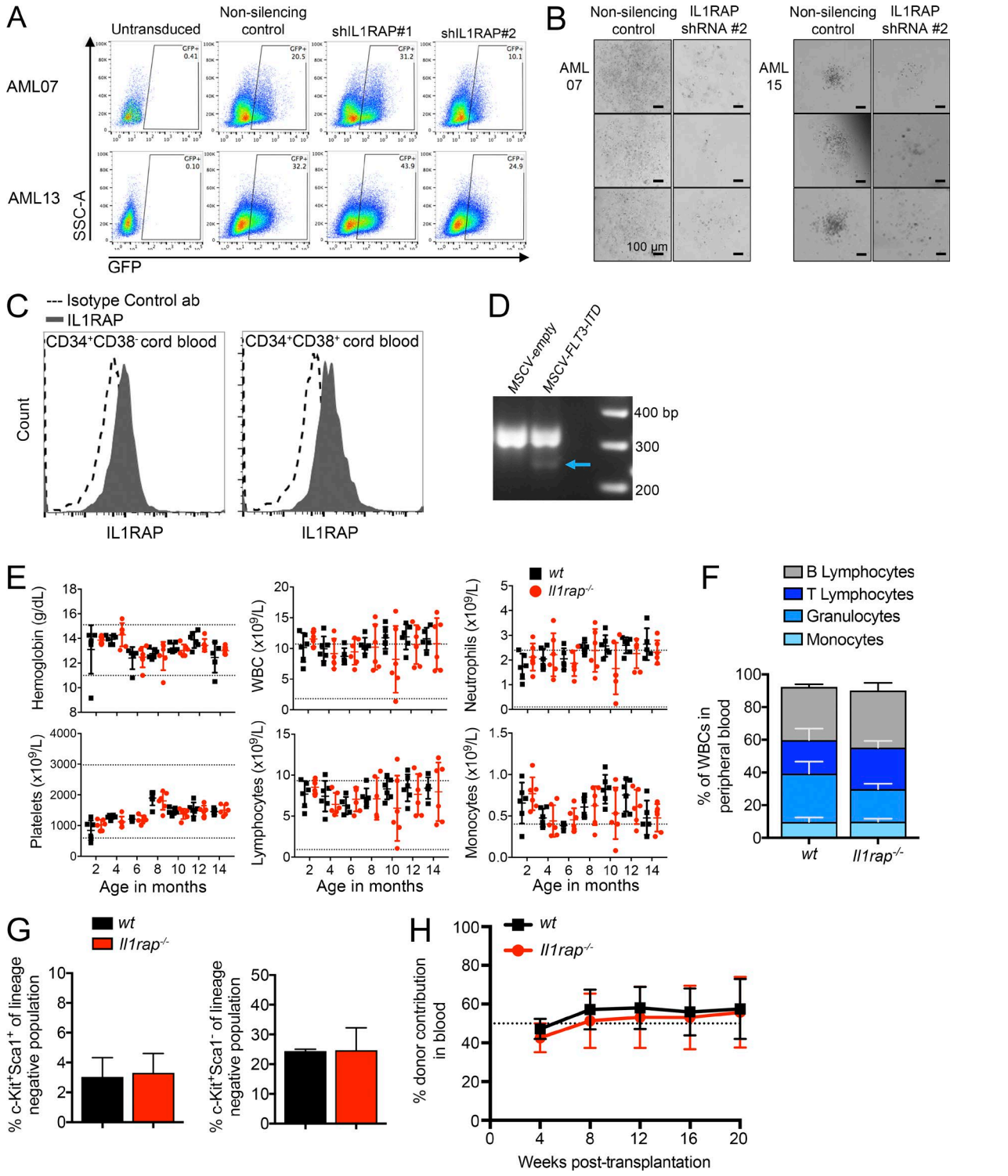

Figure S2. **Targeting of IL1RAP reduces growth of primary human AML cells without affecting healthy hematopoietic cells. (A)** IL1RAP shRNA transduction efficiencies of 2/5 primary AML samples used in Fig. 1 F. **(B)** Photos of individual colonies from primary AML colony assays in Fig. 1 F. 10×, 0.25 NA. **(C)** IL1RAP expression by flow cytometry in $CD34^+CD38^-$ and $CD34^+CD38^+$ cells from a cord blood specimen using a fluorescently conjugated IL1RAP antibody. One representative experiment is shown. **(D)** PCR amplification of the *FLT3* gene on genomic DNA isolated from THP-1 cells transduced with MSCV-GFP empty control or MSCV-FLT3-ITD-GFP. The WT allele gives a 329-bp fragment, while the transgene (which includes the *FLT3*-ITD coding sequence only) gives a 239-bp sequence (indicated by blue arrow). **(E)** PB counts of *wt* and *Il1rap*$^{-/-}$ mice every 2 mo until 14 mo of age. WBC, white blood cells. Dotted lines indicate normal range. **(F)** Flow cytometry analysis of PB of *wt* and *Il1rap*$^{-/-}$ mice at 7.5 mo of age. B lymphocytes were identified as $CD11b^-Gr1^-B220^+$; T lymphocytes, $CD11b^-Gr1^-CD3^+$; granulocytes, $CD11b^+Gr1^+$; monocytes, $CD11b^+Gr1^-$. Data represent the mean ± SD of six mice per group. **(G)** Percentage of c-Kit^+Sca1^+ and c-Kit^+Sca1^- cells in the $Lineage^-$ population of BM of *wt* and *Il1rap*$^{-/-}$ mice by flow cytometry. Data represent the mean ± SD of three mice per group. **(H)** Percentage of donor cells in the PB of mice transplanted *wt* or *Il1rap*$^{-/-}$ cells and *wt* competitor cells. Data represent the mean ± SD of 13 mice transplanted with *wt* versus *wt* cells and 15 mice transplanted with *Il1rap*$^{-/-}$ versus *wt* cells.

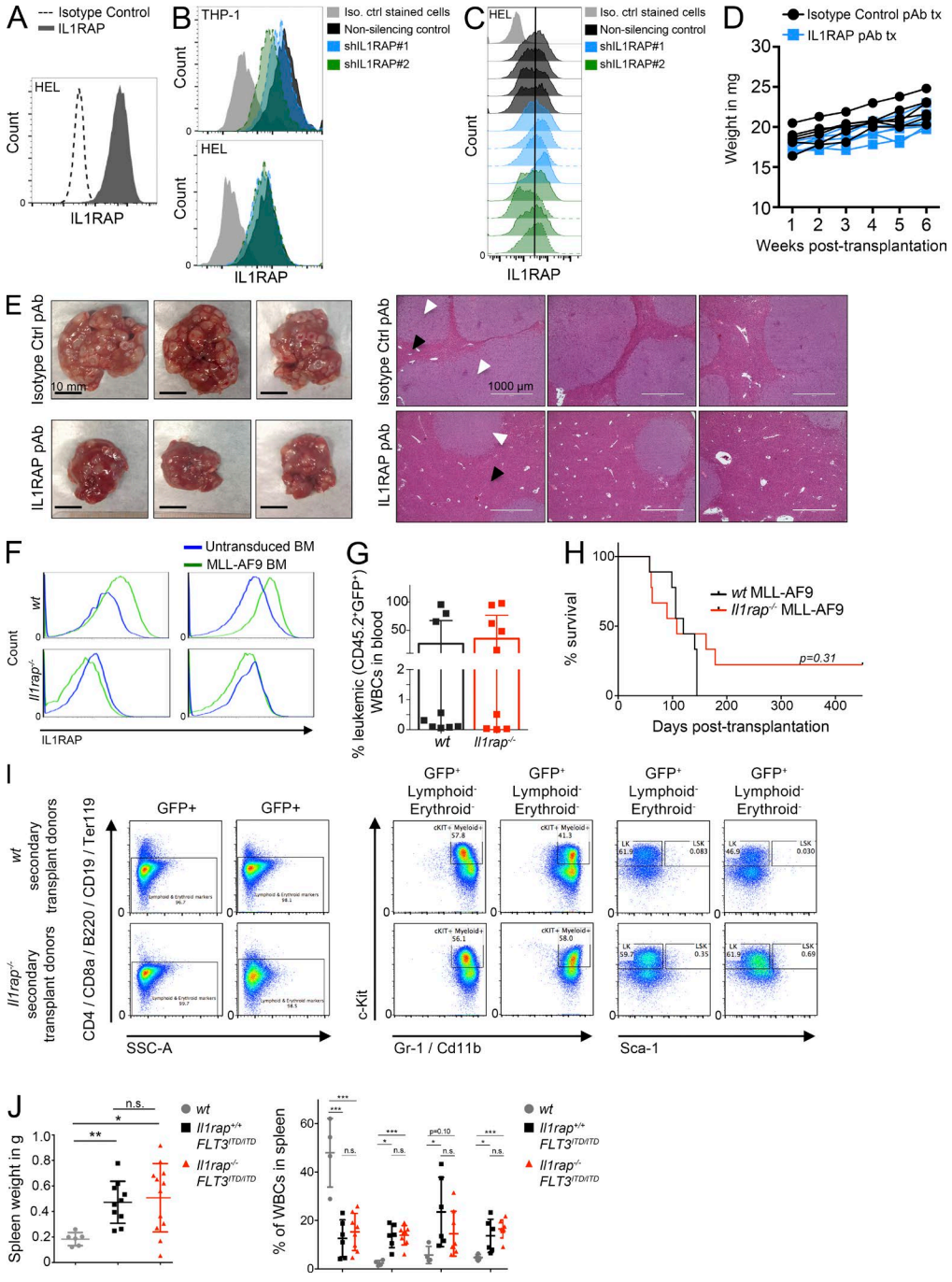

Figure S3. **Down-regulation, antibody targeting, or genetic deletion of IL1RAP inhibits AML pathogenesis in vivo. (A)** IL1RAP expression by flow cytometry in HEL cells using a fluorescently conjugated IL1RAP antibody. One representative experiment is shown. **(B)** Flow cytometry histograms showing IL1RAP protein knockdown achieved by IL1RAP shRNAs in cell lines used for xenografts in Fig. 2 B. **(C)** IL1RAP expression was measured in IL1RAP shRNA-transduced HEL cells isolated from BM of xenografted mice (Fig. 2 B) at day 30. HEL cells were identified by lack of mouse CD45.1 expression and presence of GFP (contained in the shRNA vectors). **(D)** Total body weights of antibody-treated THP-1 xenograft mice over the time course of the experiment in Fig. 2 C. tx, treatment. **(E)** Representative images of livers from antibody-treated THP-1 xenograft mice (left). H&E staining of liver sections from antibody-treated THP-1 xenograft mice (right). Each photo represents an individual mouse. THP-1 cell infiltration (white arrowheads) and residual normal mouse liver (black arrowheads) are indicated. 4×, 0.13 NA. **(F)** IL1RAP expression in BM aspirates from MLL-AF9 leukemic mice by flow cytometry. MLL-AF9–transduced BM cells (GFP^+, green histograms) and residual nonleukemic BM (GFP^-, blue histograms) are shown. **(G)** Leukemic cell chimerism in BM of MLL-AF9 primary recipient mice 4 wk after transplant (experimental scheme is shown in Fig. 2 F). Data represent the mean ± SD of nine mice per group. **(H)** Kaplan-Meier plot showing survival of primary recipient mice transplanted with *wt* or *Il1rap*$^{-/-}$ MLL-AF9 cells. P-value was calculated using log-rank (Mantel-Cox) test. **(I)** Immunophenotypes of *wt* and *Il1rap*$^{-/-}$ MLL-AF9 donor cells used for secondary transplant in Fig. 2 (G and H). **(J)** Left, spleen weights of *wt*, homozygous *FLT3*-ITD knock-in mice (*Il1rap*$^{+/+}$*FLT3*$^{ITD/ITD}$) and *Il1rap*$^{-/-}$*FLT3*$^{ITD/ITD}$ mice at 12 mo of age. Right, flow cytometric analysis of spleen cell populations in *wt*, *Il1rap*$^{+/+}$*FLT3*$^{ITD/ITD}$, and *Il1rap*$^{-/-}$*FLT3*$^{ITD/ITD}$ mice at 12 mo of age. P-values were calculated using unpaired two-tailed t-tests. *, P < 0.05; **, P < 0.01; ***, P <0.001. n.s., not significant.

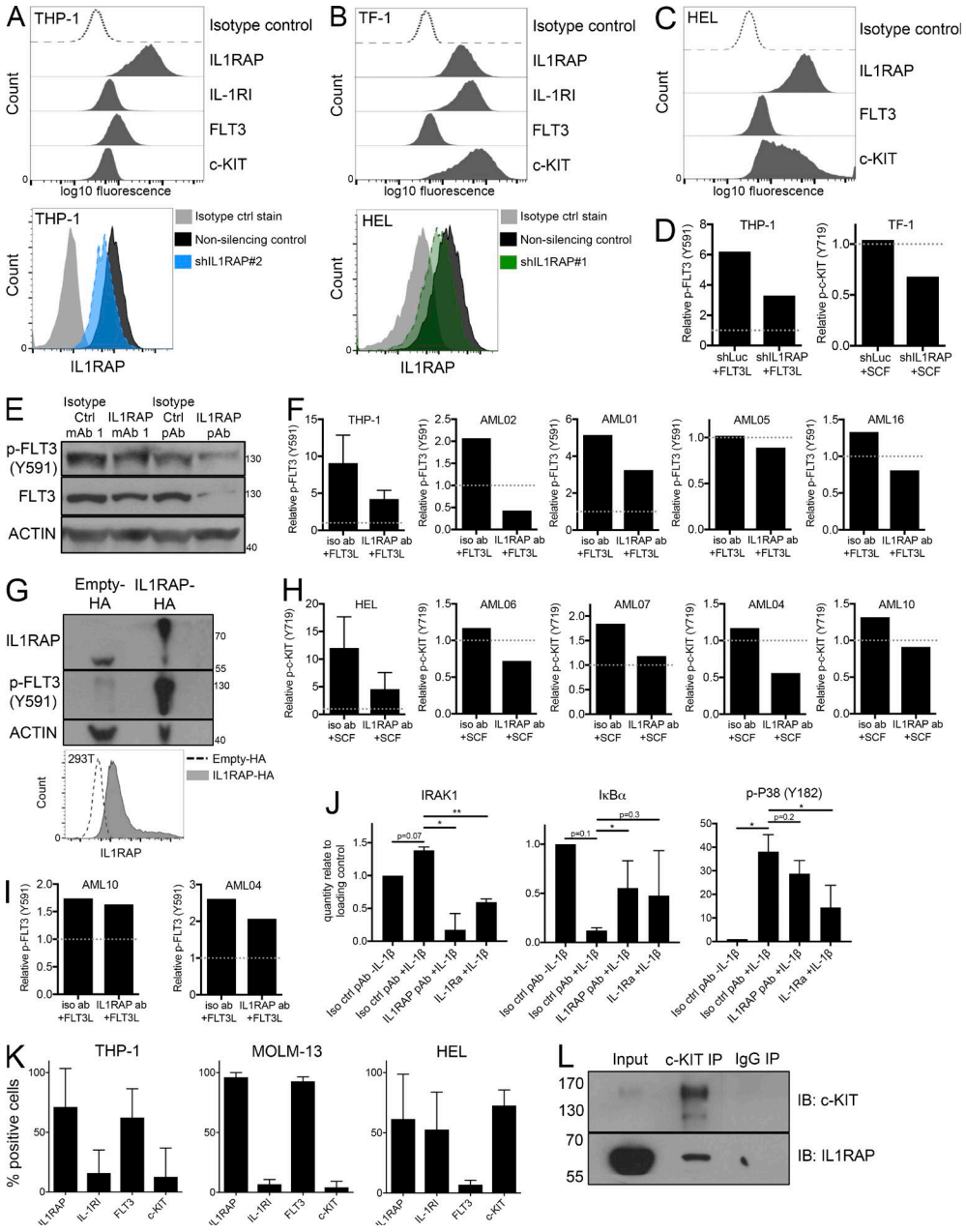

Figure S4. **Down-regulation, antibody targeting, or genetic deletion of IL1RAP inhibits FLT3 ligand and SCF-mediated signaling and growth, and IL1RAP interacts with their respective receptors. (A)** Top, surface expression of IL1RAP, IL-1RI, FLT3, and c-KIT by flow cytometry in THP-1 cells. One representative experiment is shown. Bottom, flow cytometry histograms showing IL1RAP protein knockdown achieved by IL1RAP shRNA#2 in THP-1 cells used for western blots in Fig. 3 A. **(B)** Top, surface expression of IL1RAP, IL-1RI, FLT3, and c-KIT by flow cytometry in TF-1 cells. One representative experiment is shown. Bottom, flow cytometry histograms showing IL1RAP protein knockdown achieved by IL1RAP shRNA#1 in TF-1 cells used for western blot in Fig. 3 F. **(C)** Surface expression of IL1RAP, FLT3, and c-KIT by flow cytometry in HEL cells. One representative experiment is shown. **(D)** Left, quantification of p-FLT3 (Y591) from Western blot in Fig. 3 A. Right, quantification of p-c-KIT (Y719) from western blot in Fig. 3 F. Dotted line indicates unstimulated, control shRNA-transduced cells. Protein levels relative to actin are shown. **(E)** THP-1 cells were treated with IL1RAP mAb, IL1RAP pAb, or isotype controls (100 µg/ml) for 30 minutes. Protein lysates were examined for p-FLT3 (Y591) and total FLT3 by immunoblotting. **(F)** Quantification of p-FLT3 (Y591) from Western blots in Fig. 3 (B and C). Dotted line indicates unstimulated, isotype control antibody treated cells. Protein levels relative to actin are shown. Error bars represent the mean ± SD of independent experiments. **(G)** 293T cells were transfected with HA-tagged IL1RAP and examined for IL1RAP and p-FLT3 (Y591) 18 h later by Western blot (top). Surface overexpression of IL1RAP in 293T cells by flow cytometry (bottom). **(H)** Quantification of p-c-KIT (Y719) from Western blots in Fig. 3 (G and H). Dotted line indicates unstimulated, isotype control antibody treated cells. Protein levels relative to actin are shown. Error bars represent the mean ± SD of independent experiments. **(I)** Quantification of p-FLT3 (Y591) from western blots in Fig. 3 D. Dotted line indicates unstimulated, isotype control antibody treated cells. Protein levels relative to ACTIN are shown. **(J)** Quantification of Western blot in Fig. 3 I. Protein levels relative to ACTIN are shown. Data represent the mean ± SD of two independent experiments. P-values were calculated using unpaired two-tailed *t* tests. **(K)** Surface expression of IL1RAP, IL-1RI, FLT3, and c-KIT by flow cytometry in AML cell lines used in Fig. 4 (A–G). "Positive" gates were set using a fluorescent isotype control antibody. Data represent the mean ± SD of two or more independent experiments. **(L)** Coimmunoprecipitation of endogenous c-KIT in protein lysates from THP-1 cells using an independent c-KIT antibody. Immunoblot was performed for c-KIT and IL1RAP. *, P < 0.05; **, P < 0.01.

Table S1. **Absolute colony number output for IL1RAP shRNA-transduced primary AML samples used in Fig. 1 F**

	Control shRNA	IL1RAP shRNA#1	IL1RAP shRNA#2
MDS01	48	14	13
MDS02	20	8	0
AML03	182	124	57
AML07	5538	3268	2320
AML13	10	7	5
AML15	46		26

Table S2. **Flow cytometer configuration used for determination of FRET in Fig. 4 (C and G)**

	Photomultiplier tube	Dichroic mirror	Band pass filter	Channel
Blue laser, 488 nm	A	735LP	780/60	PE-Cy7
	B	635LP	660/20	FRET
	C	595LP	610/20	PE-Texas red
	D	556/LP	575/26	PE
	E	502LP	530/30	FITC/GFP/Alexa488
	F		488/10	SSC
Red laser, 633 nm	A	755LP	780/60	APC-Cy7
	B	690LP	730/45	Alexa700
	C		660/20	APC/Alexa647
Violet laser, 407 nm	A	505LP	530/30	AmCyan/Pacific orange
	B		450/50	Pacific blue/eFluro450/BV421/CFP/Dapi