Supplemental Table 1: Characteristics of *Ctsg-PML-RARA* mice and murine APL samples. ND, not determined due to failure of colony formation; SF, sample failure.

Mouse	Gender	Age at death (mo)	Spleen (g)	WBC (K/uL)	%Jag1+	GSI responsive
3842	M	5	1.14	11.3	34.5	ND
13441	F	11.5	1.21	11.5	51.5	ND
13497	M	8	1.15	25.8	92.8	ND
13843	M	5	1.03	45.2	46.8	ND
2894	M	7	2.29	109.5	19.3	Yes
2972	F	11	1.02	94.4	19.3	No
3149	F	8	1.55	35.5	SF	Yes
3430	M	8	1.61	182.3	29.9	Yes
3673	M	5	0.97	224	SF	No
13346	F	8	1.7	17	36.1	No
13355	M	9	0.93	18.8	90.7	Yes
13499	M	6	1.23	69	SF	Yes
13659	M	7.5	0.75	29.8	15.5	Yes

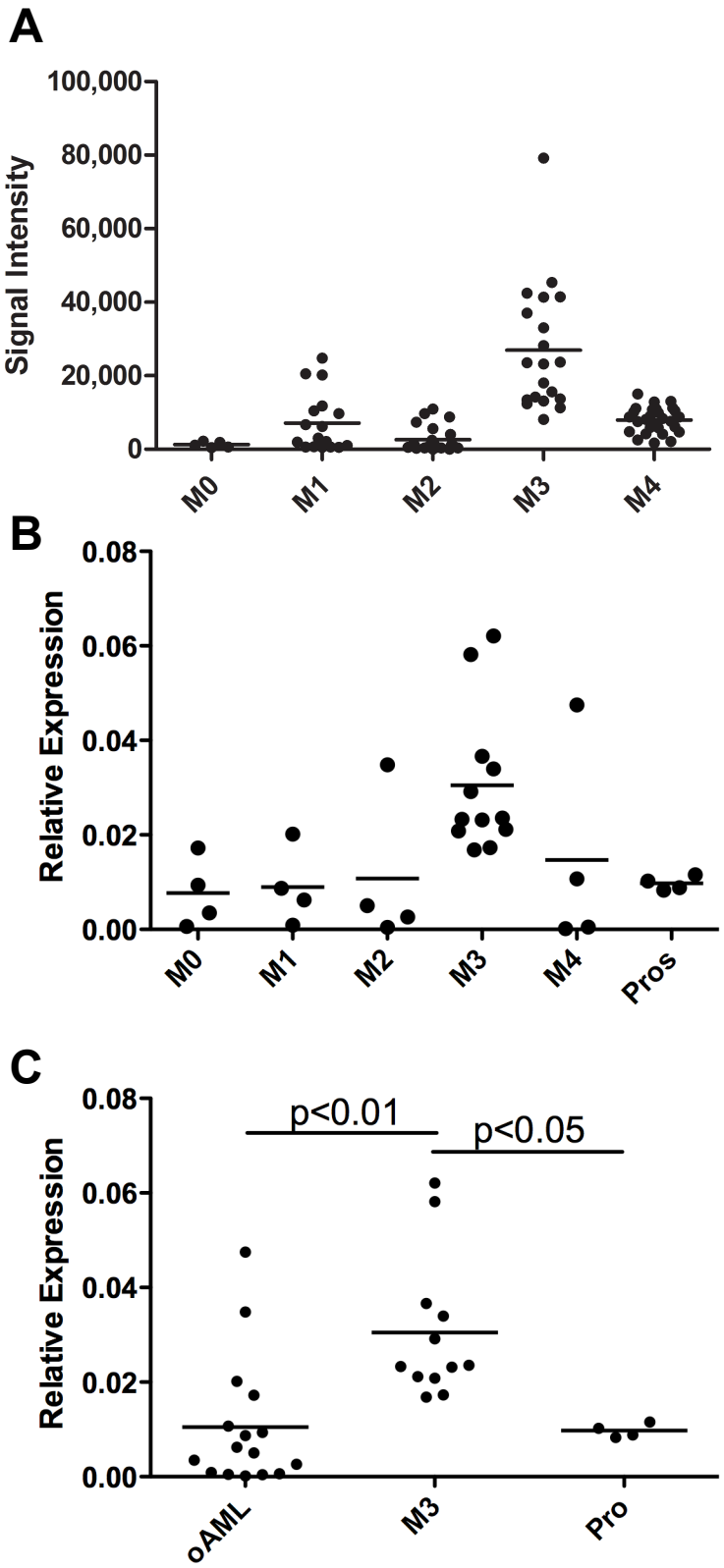


Figure S1: Validation of *JAG1* Expression in APL
A) JAG1 expression in the CALGB set of 93 de novo AML samples, showing the same probeset as in Figure 1A (probe set 209099). B) qRT-PCR measurement of *JAG1* expression in 11 APL, 12 other AML (4 samples each of M0, M1, M2, and M4 FAB subtypes) and 4 flow-sorted normal promyelocyte samples. Data are normalized to GAPDH expression. C) Summary of the data in B. Each data point represents one patient sample or one normal sample.

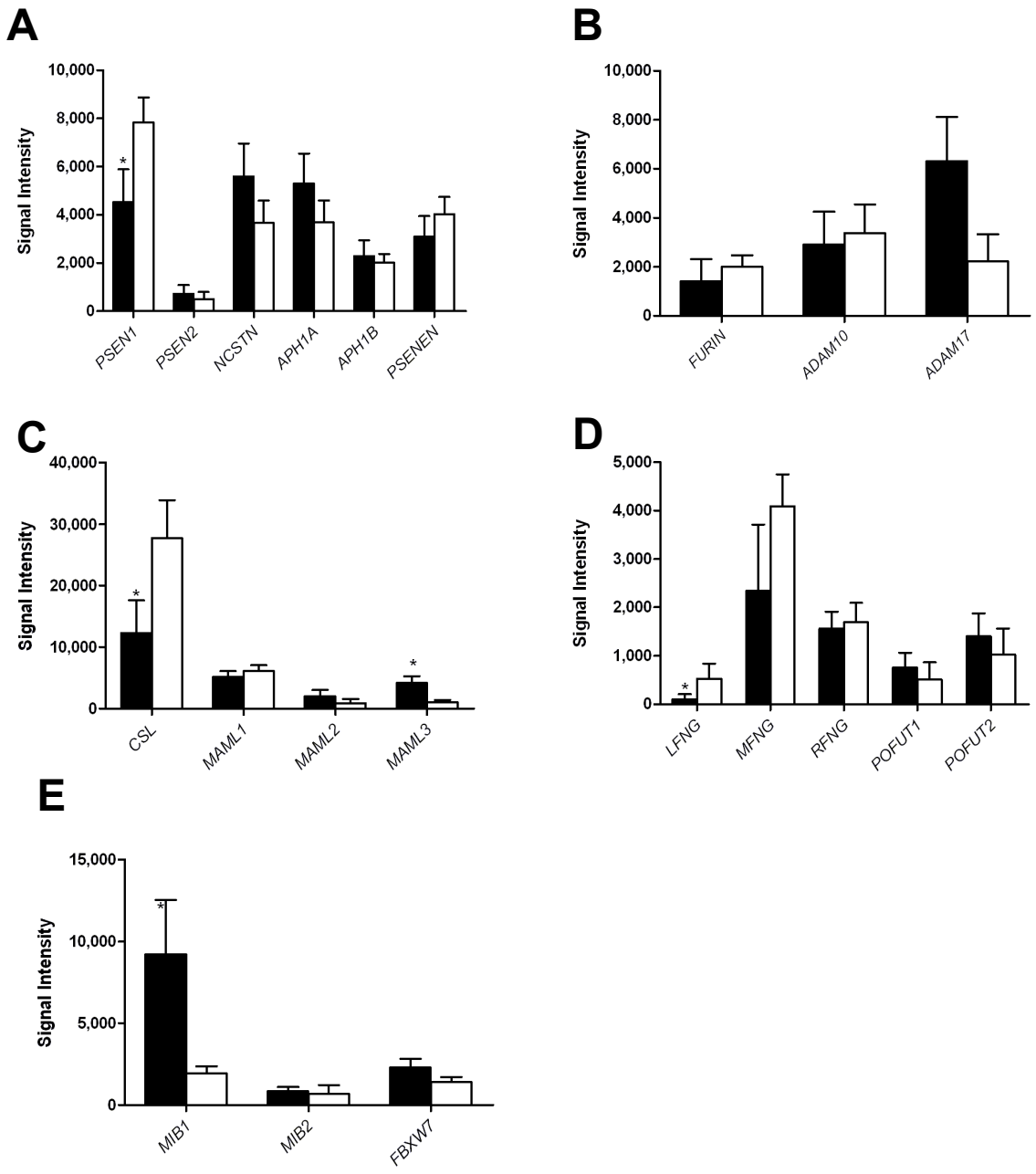
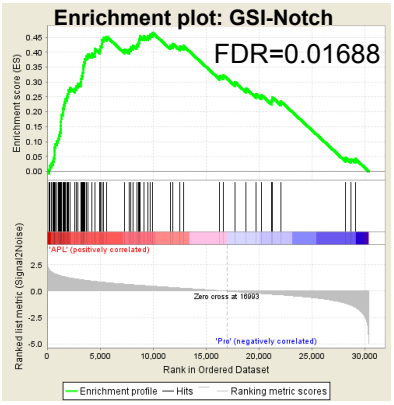
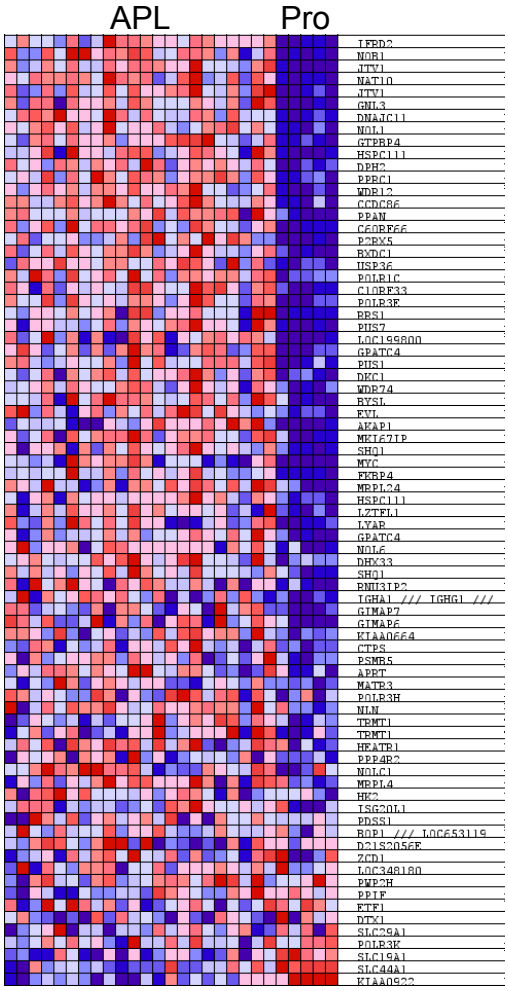


Figure S2. Expression of genes encoding Notch signaling components in APL (closed bars) and normal promyelocytes (open bars). (A). Gamma secretase components; B). Additional enzymes involved in Notch cleavage; C). Transcriptional cofactors of cleaved Notch; D). Notch modifying enzymes; E). E3 ubiquitin ligases involved in Notch signaling. Data are the mean +/- standard deviation of the probeset with the highest average signal intensity for 22 APL and 5 normal promyelocyte samples present in the Washington University *de novo* AML set. Genes present in the APL dysregulome are marked with an asterisk.

A





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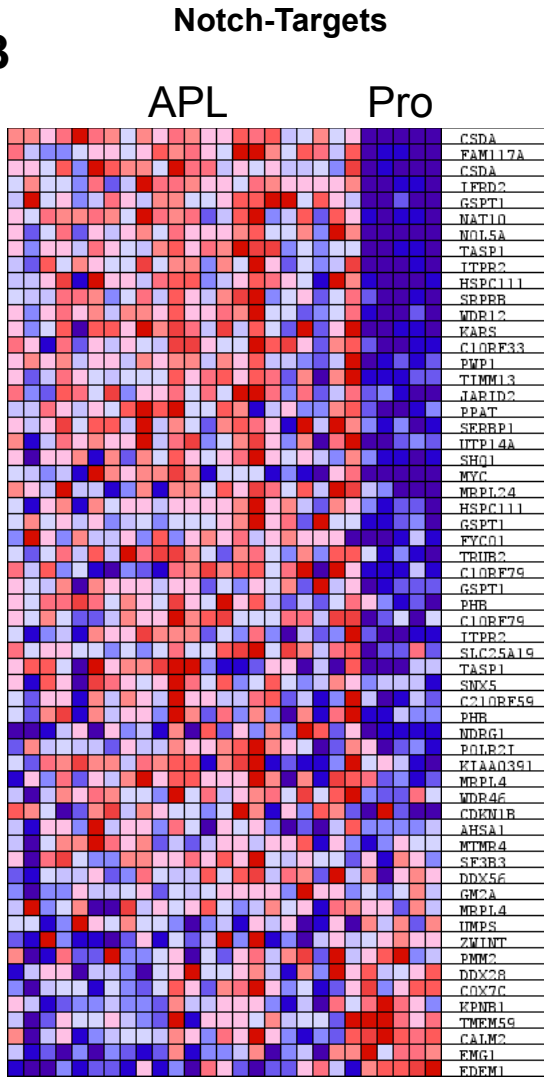


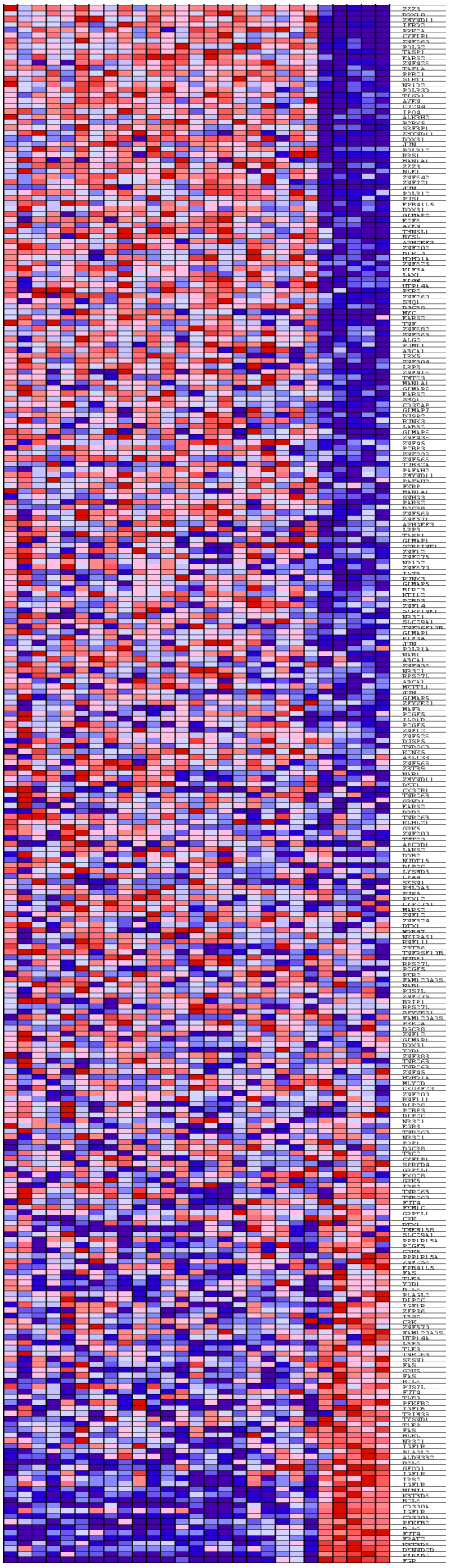
Figure S3: Expression of Notch signatures in APL

A) GSEA plot and heat map of 22 APL samples compared to 5 normal promyelocyte (Pro) samples demonstrates significant enrichment in APL of a previously published set of genes downregulated in T-ALL by gamma secretase inhibitor treatment[30]. Normalized enrichment score 1.8927.

Heatmaps of a (B)previously described set of Notch transcriptional targets in T-ALL[31] and (C) a previously published set of transcriptional targets of Notch whose expression is inhibited by both gamma secretase inhibitor treatment and DNMAML expression[32].

C **Notch-GSI-DNMAML**

APL Pro



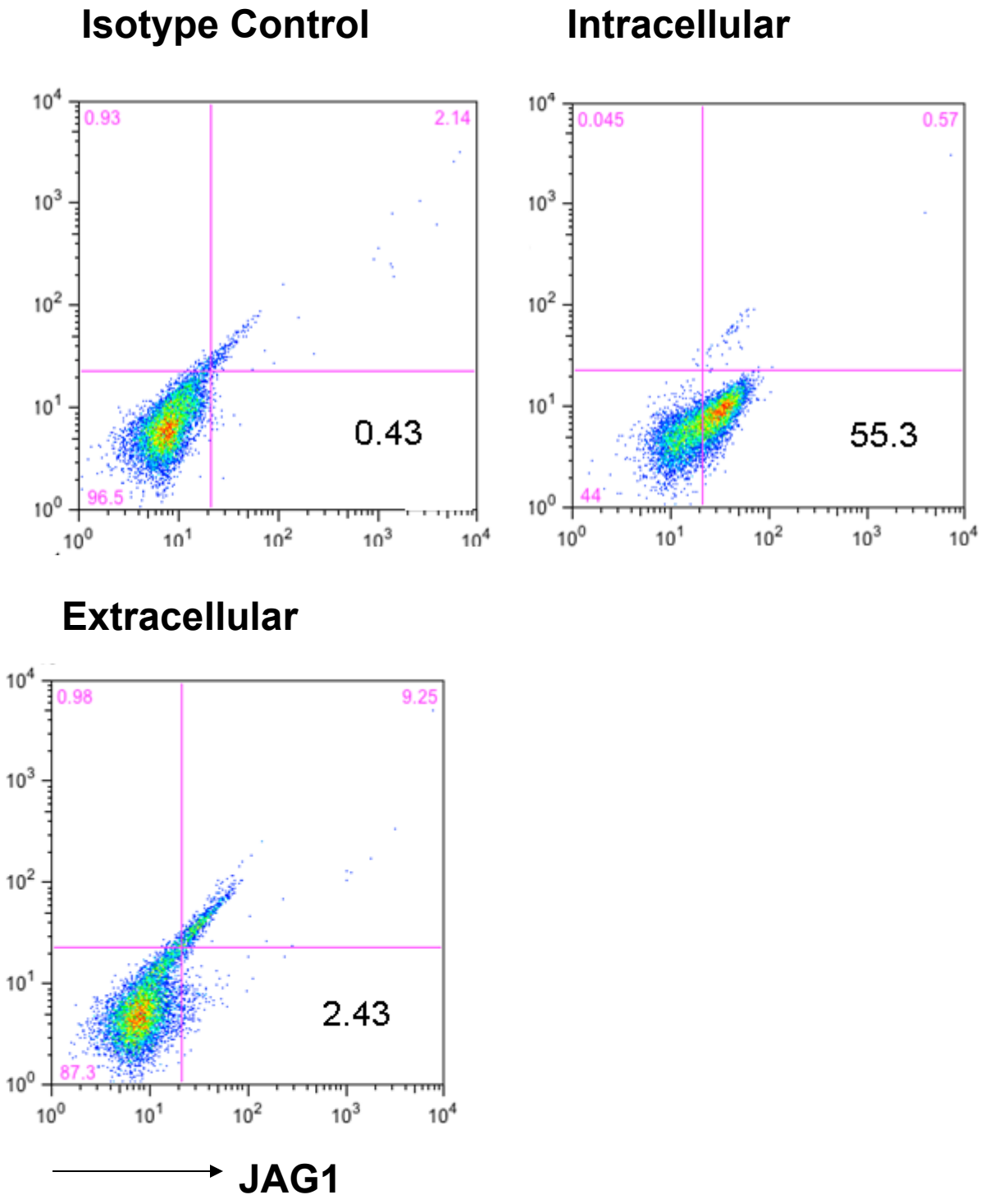


Figure S4: JAG1 protein is found in an intracellular compartment in PR-9 cells. Flow cytometry plots showing staining of Zn^{2+}-induced PR-9 cells with an isotype control antibody, JAG1 antibody by surface staining or JAG1 antibody by intracellular staining following fixation and permeabilization.

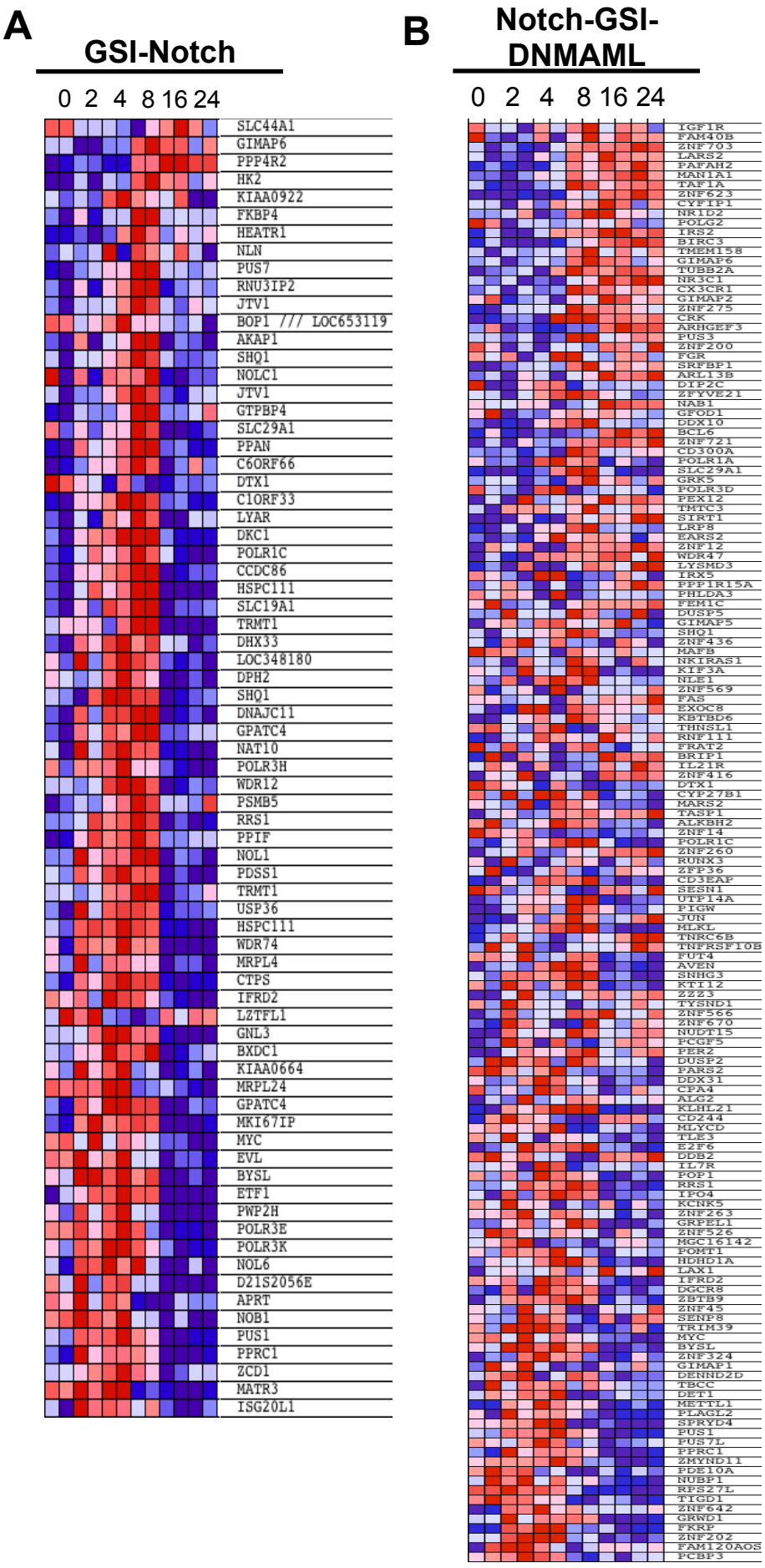


Figure S5: Expression of Notch signatures in induced PR-9 cells.

Heat maps showing expression of A) the GSI-Notch[30] and B) Notch-GSI-DNMAML[32] signatures in PR-9 cells 0, 2, 4, 8, 16 or 24 hours after Zn^{2+} induction of *PML-RARA* expression .

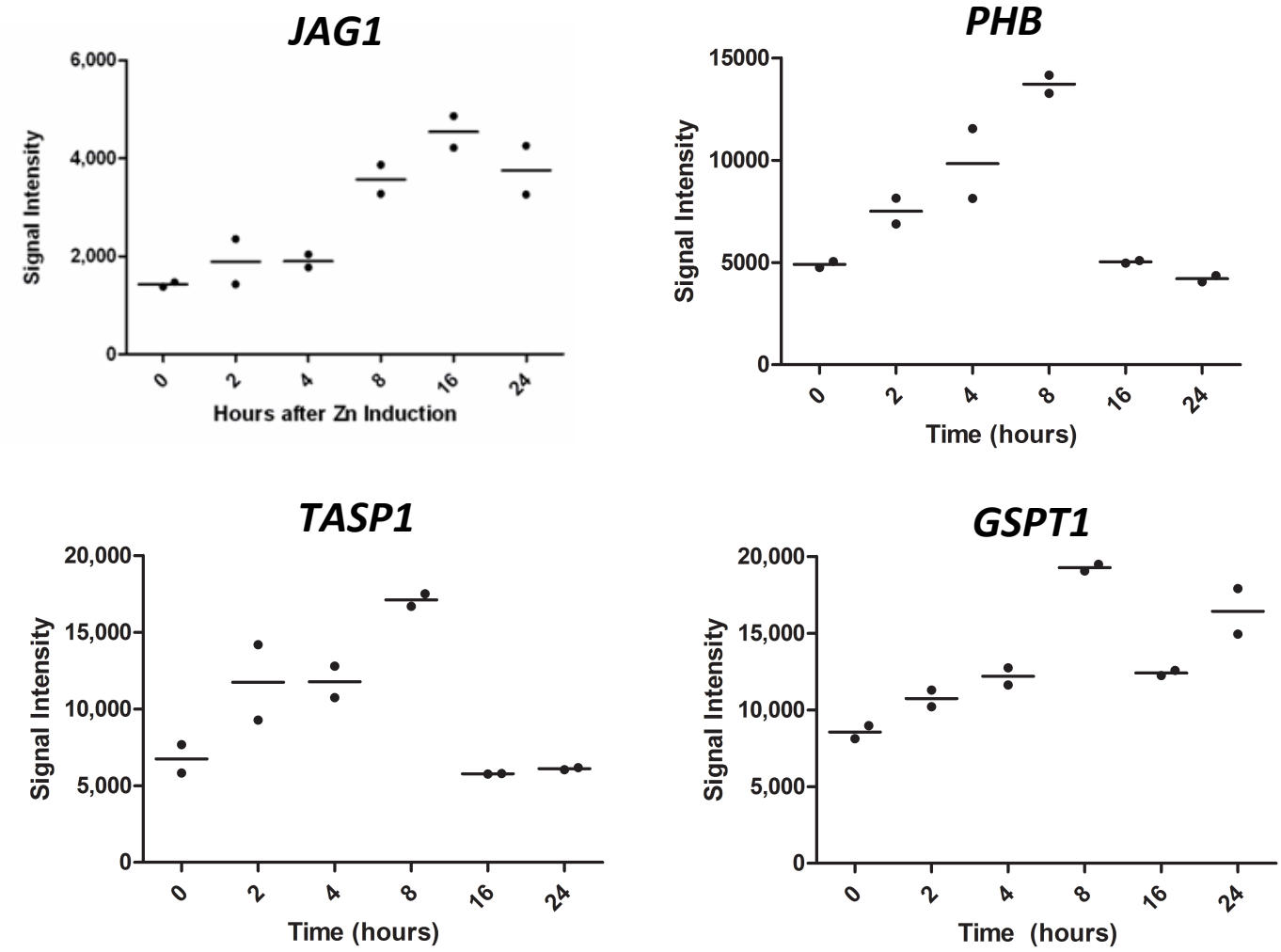


Figure S6: Expression of *JAG1* and Notch target genes in PR-9 cells
Microarray gene expression data for *JAG1* and three Notch target genes (*PHB, TASP1* and *GSPT1*) in PR-9 cells after Zn^{2+} induction of *PML-RARA* expression.

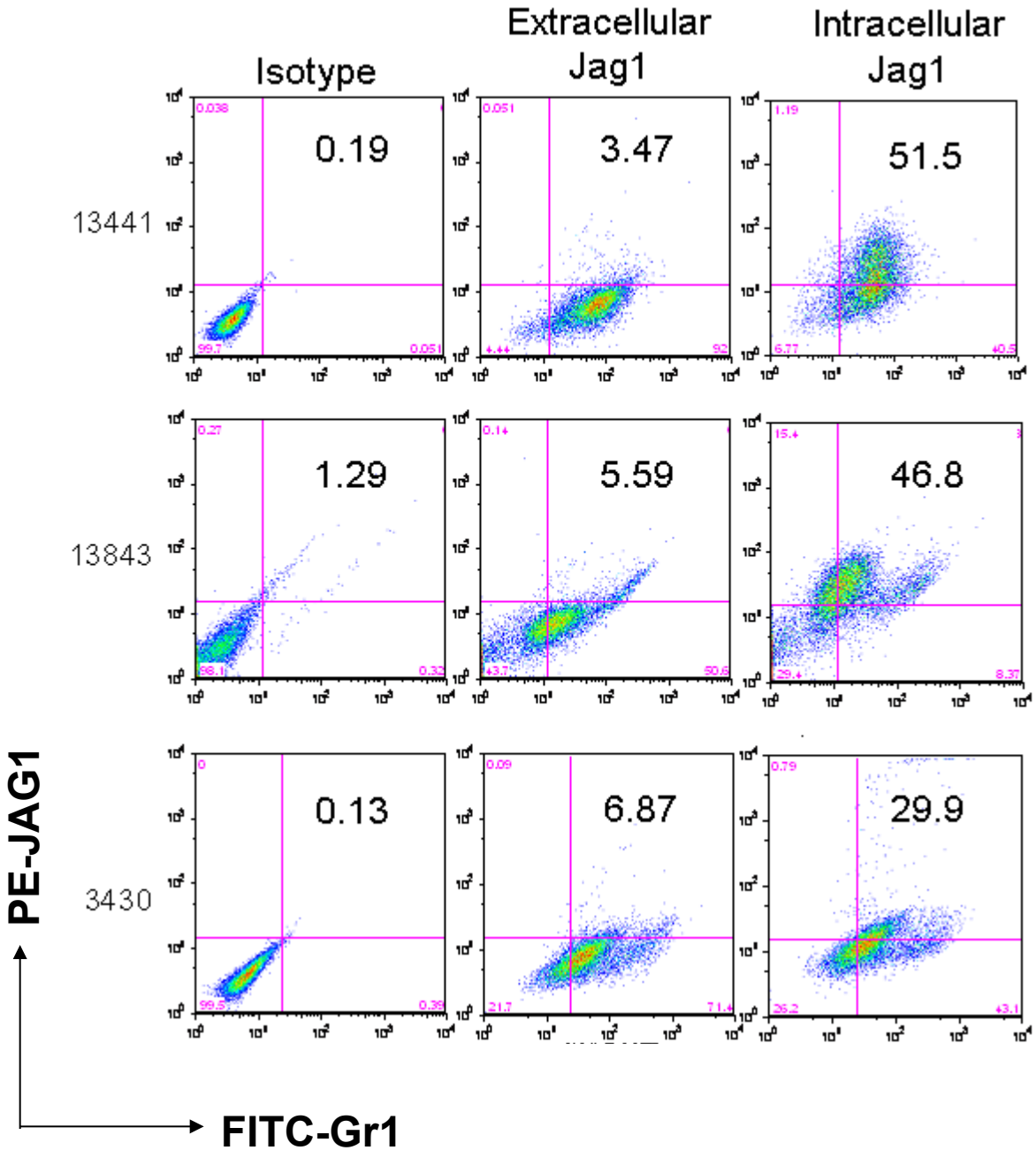


Figure S7: JAG1 protein is found in an intracellular compartment in murine APL. Flow cytometry plots showing staining of three independent murine APL samples with an isotype control antibody, JAG1 antibody by surface staining or JAG1 antibody by intracellular staining following fixation and permeabilization. Counterstaining with the myeloid marker Gr-1 is provided as a control.

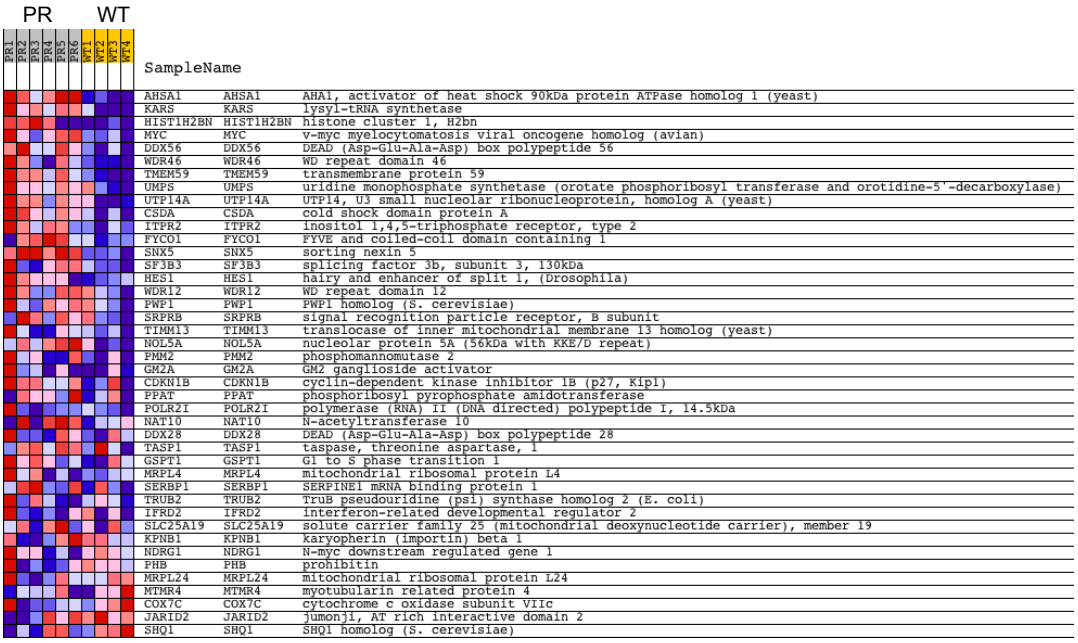


Figure S8: Expression of Notch signature in *Ctsg-PML-RARA* KLS cells

Heat map of 6 KLS samples sorted from preleukemic *Ctsg-PML-RARA* (PR) mice compared to 4 wildtype C57BL/6 KLS (WT) samples, demonstrating significant enrichment of the previously described Notch-Targets[31] signature in *Ctsg-PML-RARA* KLS cells.

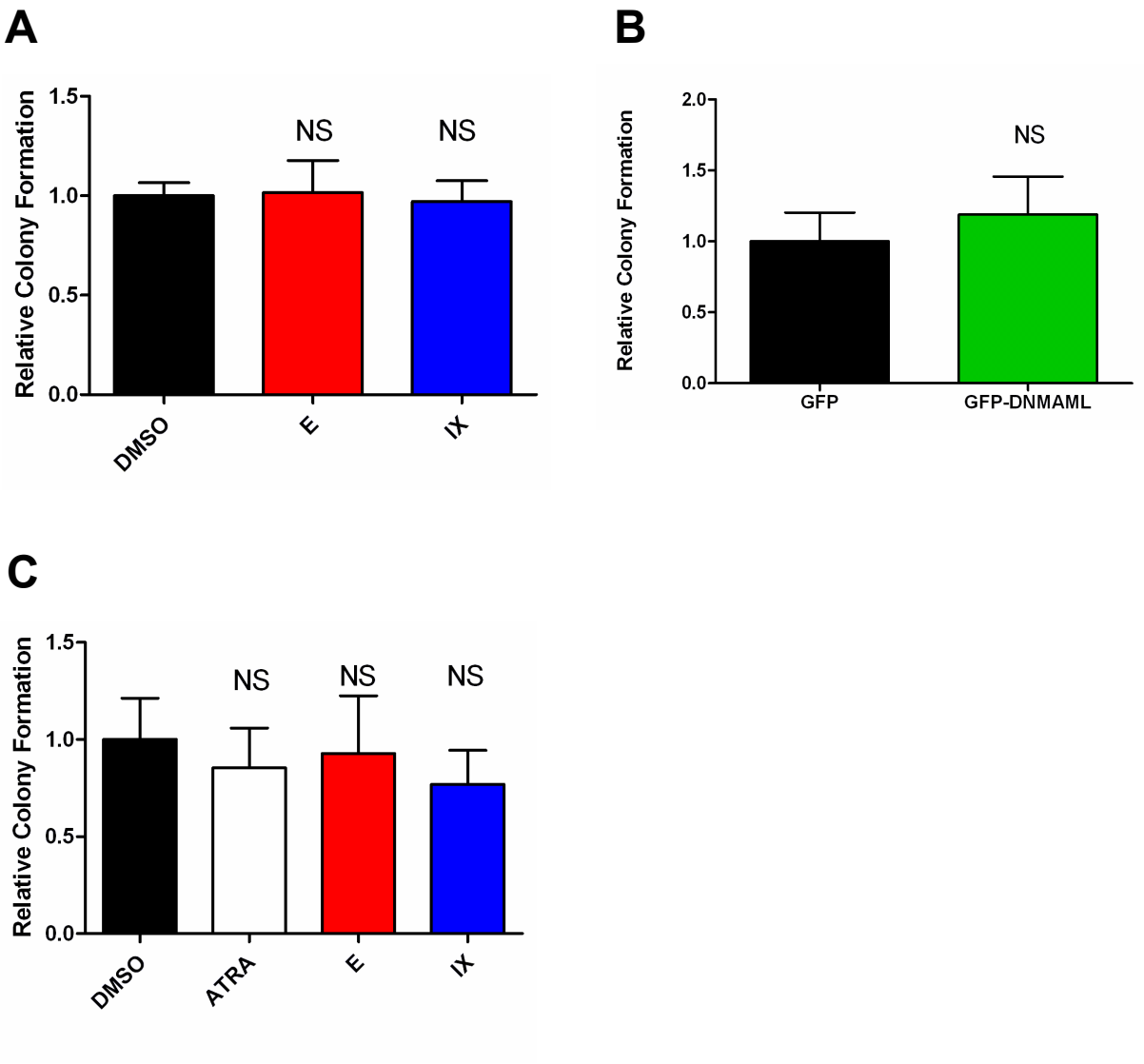


Figure S9: Inhibition of Notch signaling does not change colony formation by wildtype marrow cells.
A). Relative colony formation for 3 wildtype C57BL/6 marrow samples plated in Methocult (3534) after a two day treatment with DMSO, 2 μM compound E, or 25 μM compound IX. Data shown are mean +/- standard deviation. Data are normalized to the DMSO treated control colony counts. B). Relative colony formation for 3 wildtype C57BL/6 marrow samples transduced with either MSCV- DNMAML-GFP or GFP control, sorted for GFP+ cells and plated in methylcellulose media. Data shown are mean +/- standard deviation and are normalized to the GFP control. In both A and B, cells from each animal were plated in triplicate for all treatment conditions. C) Summarized data for 3 wildtype 129SvJ/B6 F1 marrow samples treated for 48 hours with 1 μM ATRA, 2 μM compound E, 25 μM compound IX or DMSO control, and then plated in methylcellulose media without drugs in triplicate. Data shown are mean +/- standard deviation and are normalized to the average colony formation for the DMSO control of each animal. NS=Not Significant.

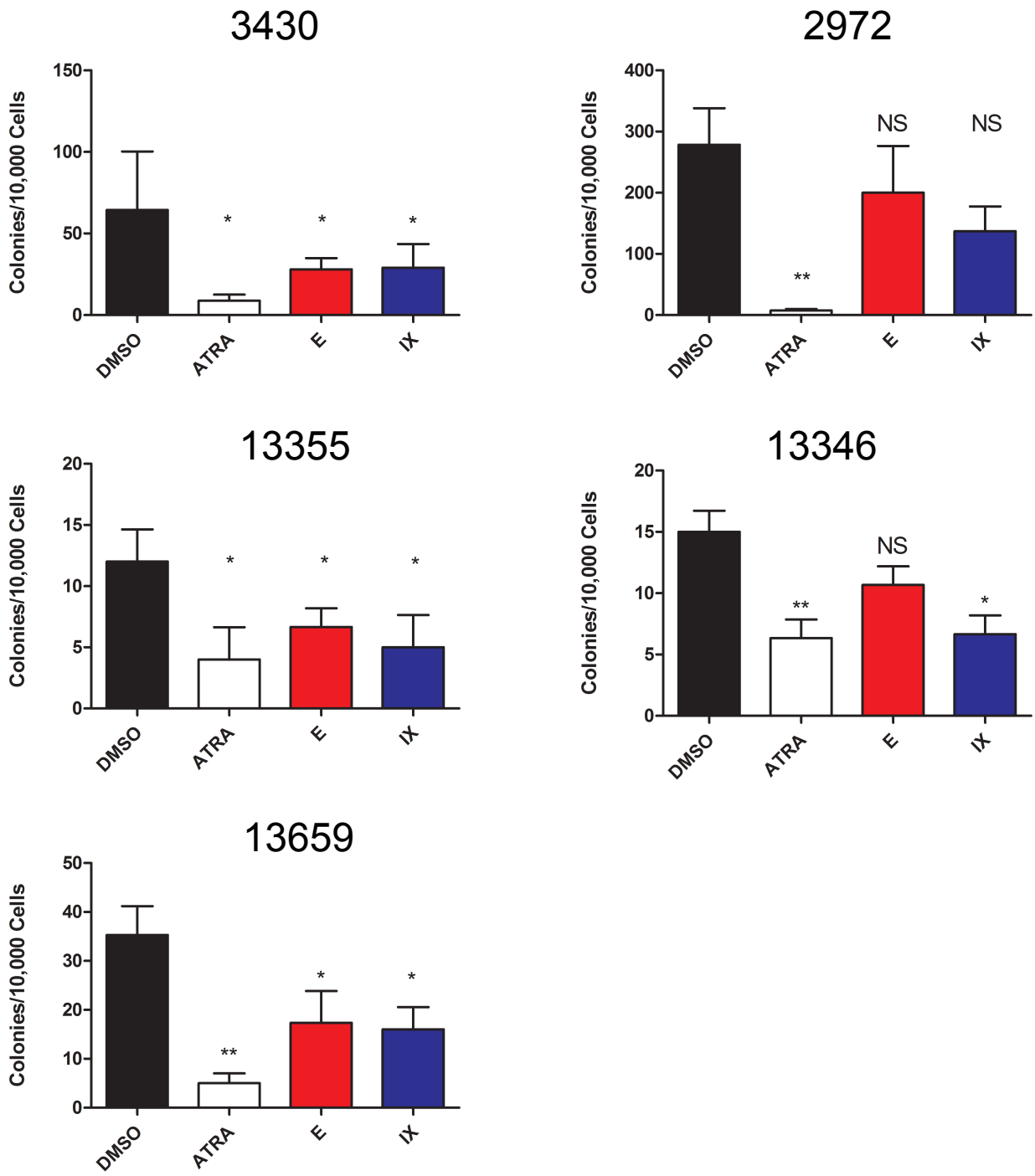
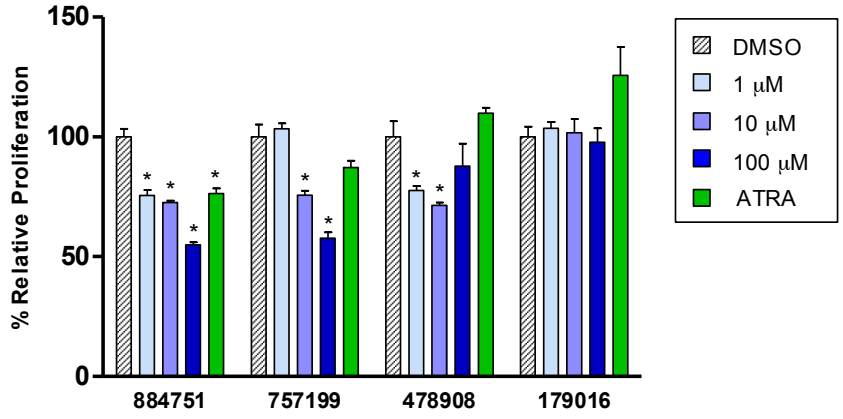


Figure S10: Response of Murine APL Cells to Inhibition of Notch Signaling. Colony formation data for 5 murine APL samples treated for 48 hours with 1 μM ATRA, 2 μM compound E, 25 μM compound IX, or DMSO vehicle control, and then plated in methylcellulose media supplemented with IL-3, IL-6 and SCF in triplicate. Data bars are the mean numbers of colonies per 10,000 cells plated. Error bars represent standard deviations. In all graphs, one asterisk (*) indicates $p<0.05$, two asterisks (**) indicates $p<0.01$ and three asterisks (***) indicates $p<0.001$. NS=Not Significant.

A



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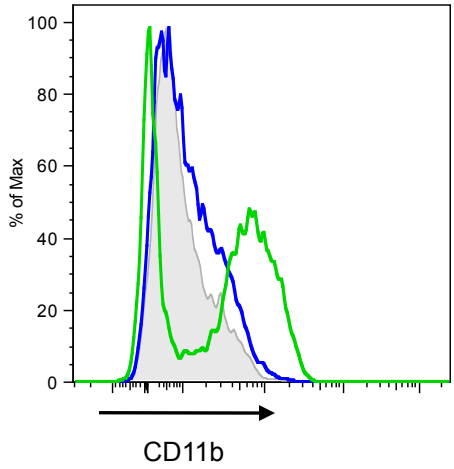

C

				% Blasts/Promyelocytes		
UPN	Age	Sex	FAB	PB	BM	Mutations
884751*	54	F	M3	70	86	***FLT3* D835**
757199	50	F	M3	0	90	***MT-ND1*, *CALR*, *OR11H12*, *PHIP*,** *FAM155A, MLLT4, SEPX1*
478908	50	M	M3	10	74	***FLT3-ITD*, *CACNA2D3*, *XIRP1*,** *ASTE1, DCT, RBKS*
179016	42	M	M3	2	88	***TTN*, *CALR*, *DCHS2*, *LRRC4*, *NMUR2*, *RBM16*,** *CTNNA3, DYRK4, EXD1, PCDH11X, TCL1A, TJAP1*

Figure S11. Variable responses of patient-derived APL samples to gamma-secretase inhibition. A) Graph depicting proliferation of 4 patient-derived APL samples relative to a DMSO control after 5 days of treatment with compound IX at indicated concentrations, or with 1 μM ATRA. Two patient samples responded in a dose-dependent manner (884751 and 757199). Cells were expanded using a stromal co-culture system, as described previously (Klco JM, et. al., *Blood, in press*). Error bars represent s.e.m. * indicates $p < 0.05$ using unpaired two-sided t test, n=3-4, technical replicates. **B)** Histogram of CD11b cell surface expression assessed by FACS analysis of cells from UPN 884751 from same experiment described in (a). The highest concentration of compound IX (100 μM) minimally altered CD11b expression compared to 1 μM ATRA, suggesting a minimal effect on differentiation. This result was representative of the other doses and samples analyzed. **C)** Table summarizing the clinical and pathological details of patient-derived APL samples. Recurrently mutated genes in bold. *All patient specimens underwent whole genome/exome sequencing except for 884751, which underwent standard clinical FLT3 and NPM1 testing. FAB, French-American-British classification; PB, peripheral blood; BM, bone marrow.