

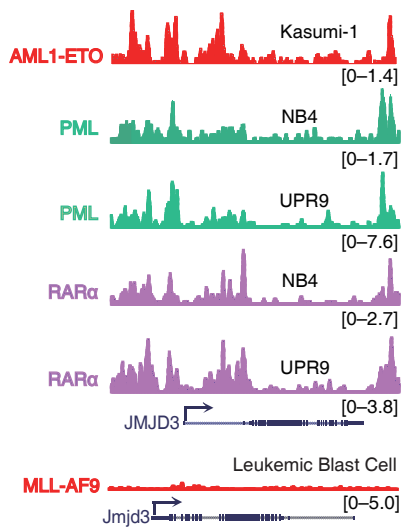
**Supplementary Information**

**JMJD3 facilitates C/EBP $\beta$ -centered transcriptional program to exert  
oncorepressor activity in AML**

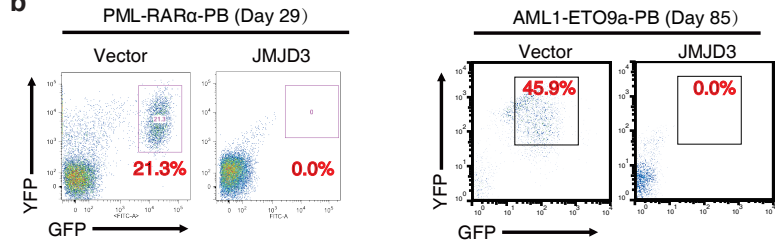
Yu et al.

# Supplementary Figure 1

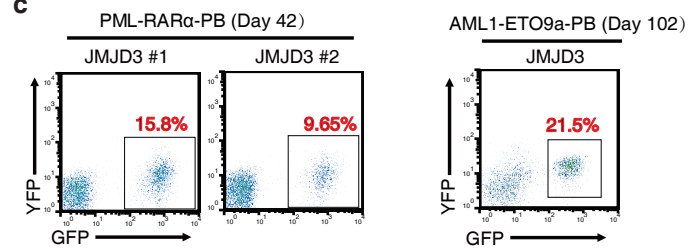
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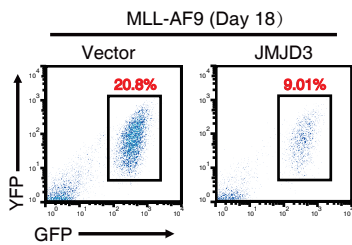
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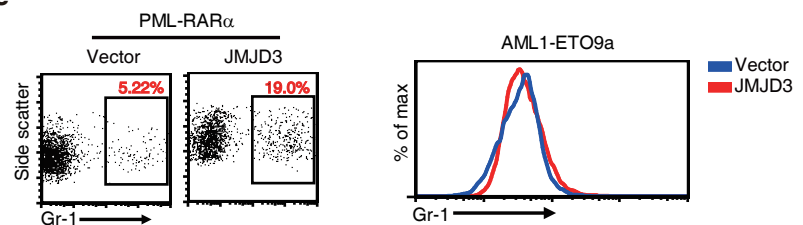
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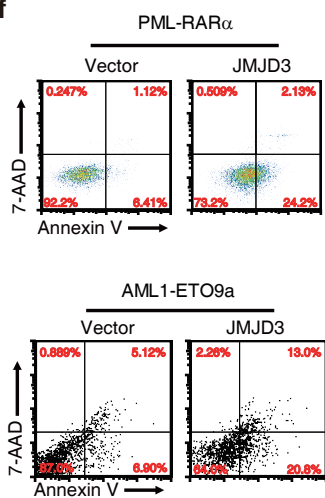
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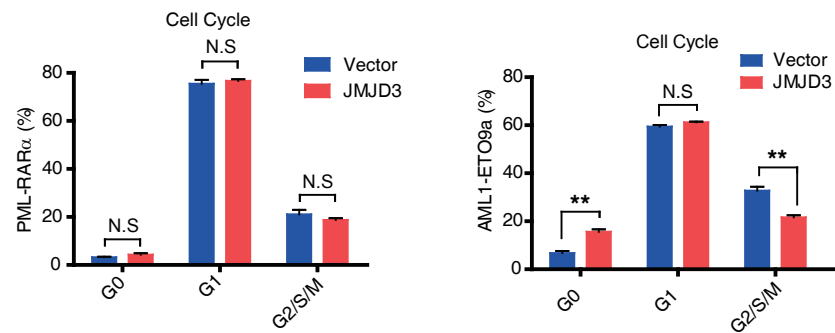
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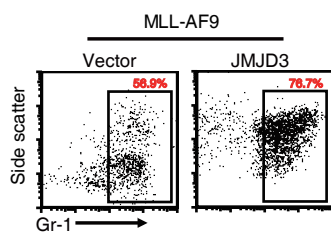
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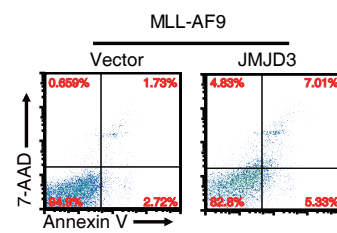
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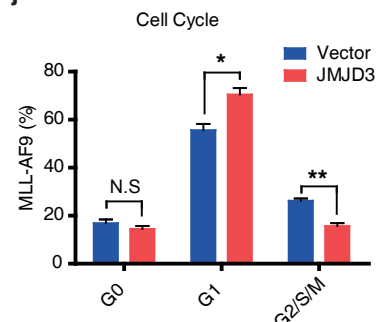
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**i**



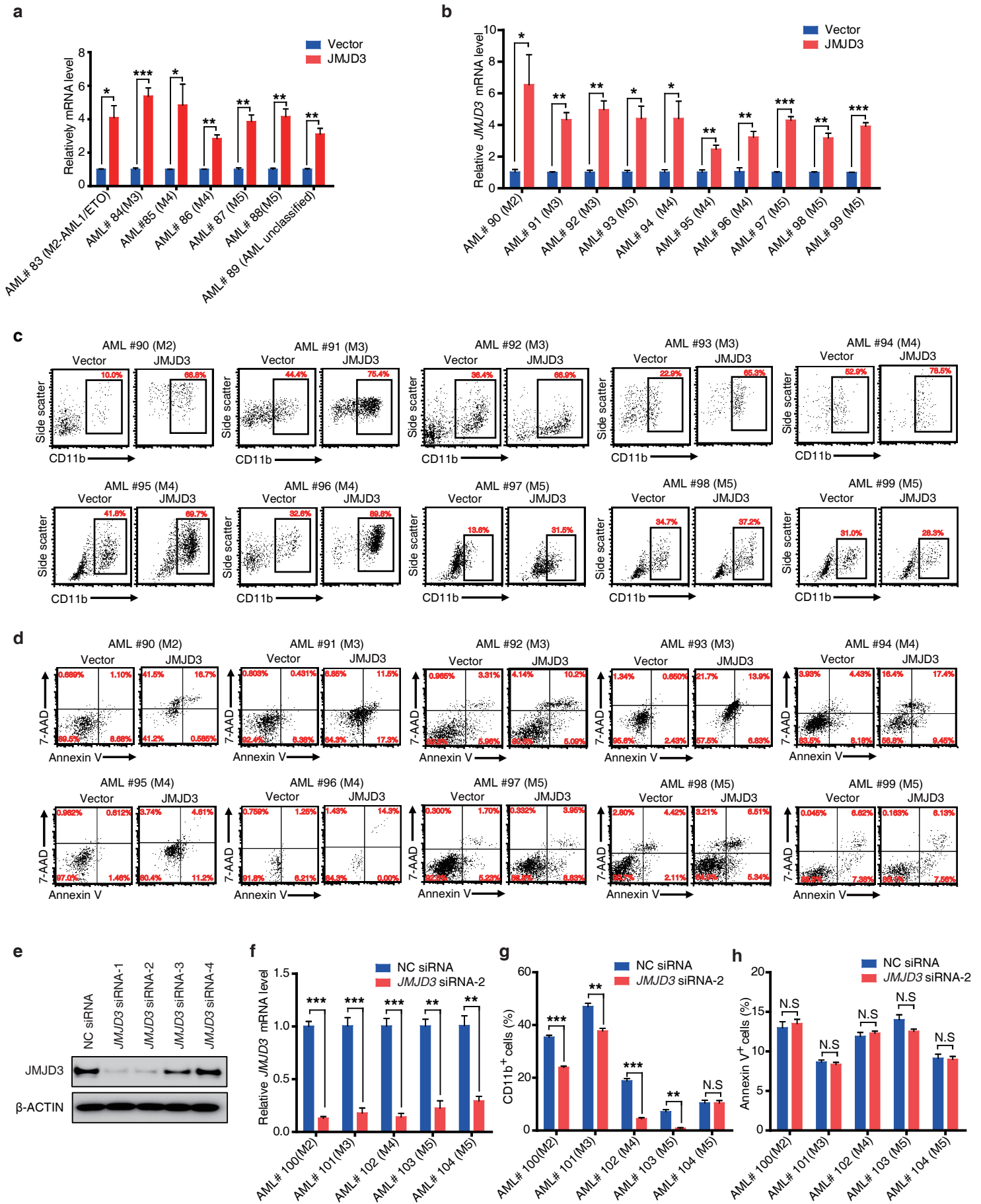
**j**



**Supplementary Figure 1. Characterization of JMJD3 oncorepressor activity in three mouse AML models, related to Figure 2.**

(a) Genome browser tracks representing the binding sites of AML1-ETO, PML, RAR $\alpha$  or MLL-AF9 at the *JMJD3* gene locus in the AML cells as indicated. (b) The representative flow cytometric analyses on the percentages of PML-RAR $\alpha^+$  or AML1-ETO9a $^+$  GFP $^+$ YFP $^+$  cells in the peripheral blood of the recipients after they had been transduced with empty vector or JMJD3-expressing vector. (c) The percentages of PML-RAR $\alpha^+$  (left panel) or AML1-ETO9a $^+$  (right panel) GFP $^+$  leukemia cells in the peripheral blood of syngeneic recipients. (d) The percentages of MLL-AF9 $^+$ GFP $^+$ YFP $^+$  cells in the peripheral blood of syngeneic mice after the leukemia cells had been transduced with empty vector or JMJD3-expressing vector. (e-g) Flow cytometric assay of Gr-1 expression (e), Annexin V/7-AAD staining (f) or cell-cycle status (g) on PML-RAR $\alpha^+$  or AML1-ETO9a $^+$  leukemia BM cells transduced with YFP $^+$  empty vector or JMJD3-expressing vector. (h-j) Flow cytometric analyses of CD11b expression (h), Annexin V/7-AAD staining (i) and cell cycles (j) on MLL-AF9 $^+$ GFP $^+$ YFP $^+$  cells in the BM of syngeneic recipients after the leukemia cells had been transduced with empty vector or JMJD3-expressing vector. Data are shown as the mean  $\pm$  SEM. \* $p$  < 0.05, \*\* $p$  < 0.01.

# Supplementary Figure 2

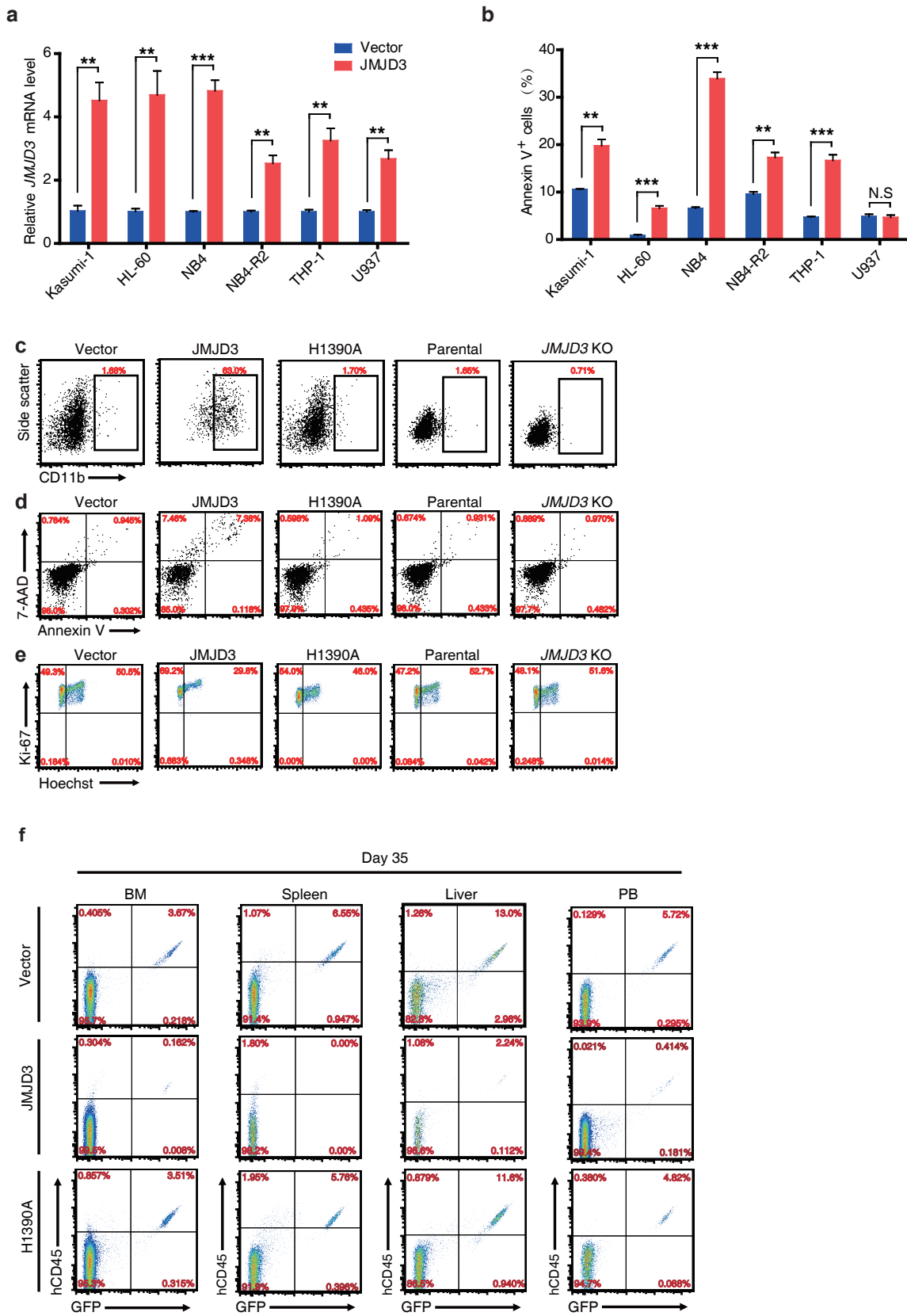




**Supplementary Figure 2. JMJD3 exhibits oncorepressor activity in the primary leukemic blasts, related to Figure 3.**

(a) qRT-PCR expression analysis of *JMJD3* mRNA level on 7 primary AML blasts transduced with control or JMJD3-expressing vector. (b) qRT-PCR expression analysis of *JMJD3* mRNA level on 10 primary AML blasts transduced with control or JMJD3-expressing vector. (c-d) Analyses of CD11b expression (c) or Annexin V/7-AAD staining (d) on 10 primary AML blasts transduced with control or JMJD3-expressing vector using flow cytometric assay. (e) Western blotting assay on JMJD3 protein level in 293T cells transduced with NC siRNA or *JMJD3* siRNA. (f) qRT-PCR expression analysis of *JMJD3* mRNA level on 5 primary AML blasts transduced with NC siRNA or *JMJD3* siRNA. (g-h) Analyses of CD11b expression (g) or Annexin V staining (h) on 5 primary AML blasts transduced with NC siRNA or *JMJD3* siRNA. Data are shown as the mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

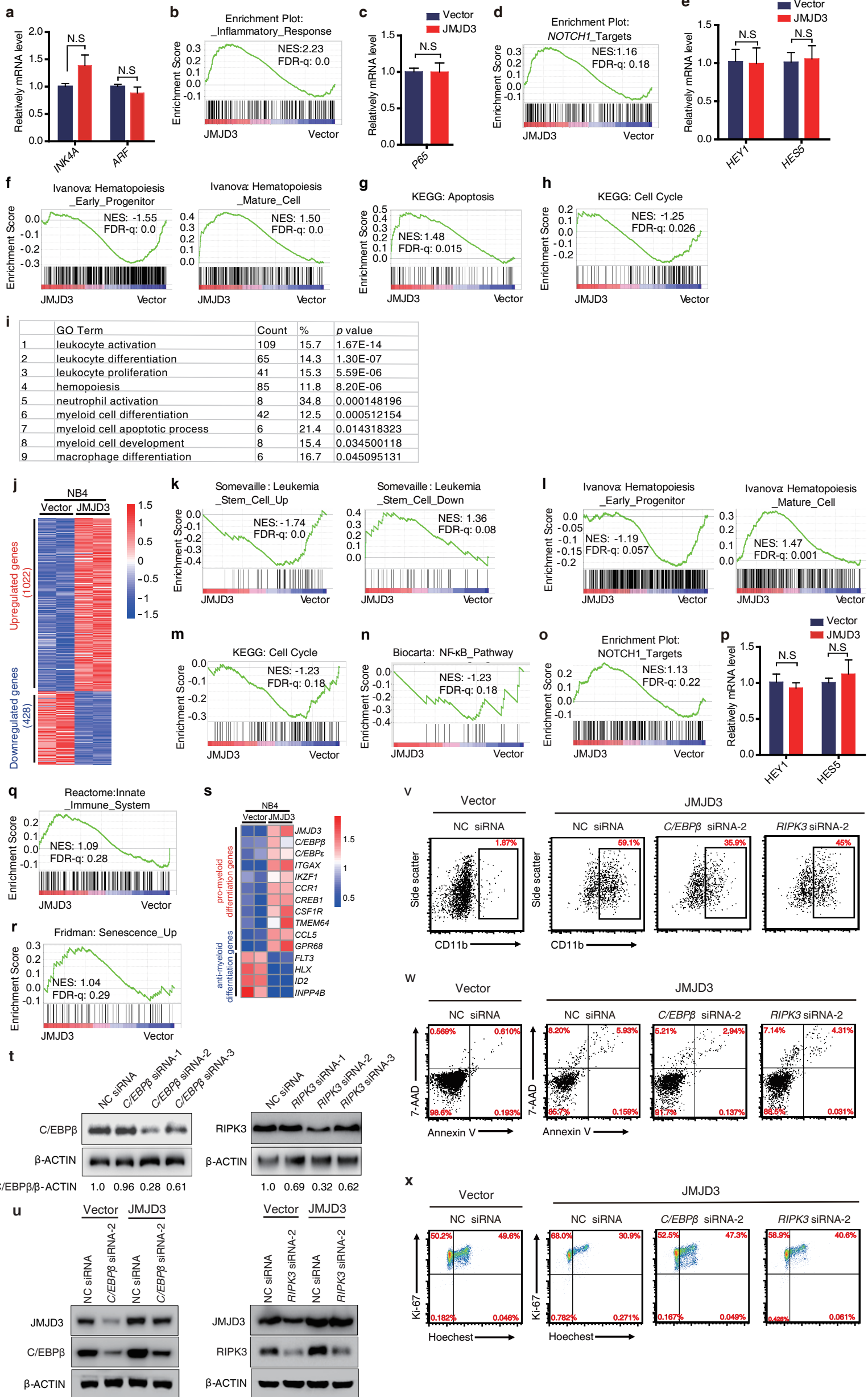
# Supplementary Figure 3



**Supplementary Figure 3. JMJD3 exhibits oncorepressor activity in the established human AML cell lines, related to Figure 3.**

(a) qRT-PCR analyses of *JMJD3* mRNA level on the established AML cells transduced with control or JMJD3-expressing vector. (b) Flow cytometric analyses of Annexin V staining on the established AML cells transduced with control or JMJD3-expressing vector. (c-e) Flow cytometric assay of CD11b expression (c), Annexin V/7-AAD staining (d) or Hoechst/KI-67 staining (e) on HL-60 cells transduced with vector control, JMJD3- or H1390A mutant-expressing vector, or in parental or *JMJD3* knockout HL-60 cells. (f) Flow cytometry plots indicating BM, Spleen, Liver and PB engraftment of HL-60 cells transduced with vector control, JMJD3- or H1390A mutant-expressing vector on the day 35 after inoculation. The co-staining with anti-human CD45 confirmed that the engraftment was from transplanted human leukemia cells. Data are shown as the mean  $\pm$  SEM. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

# Supplementary Figure 4

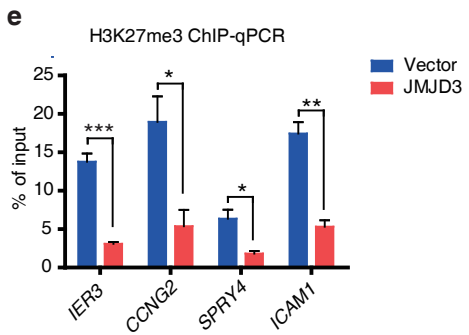
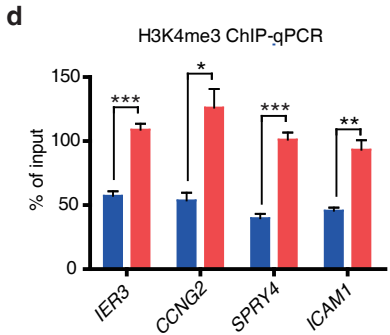
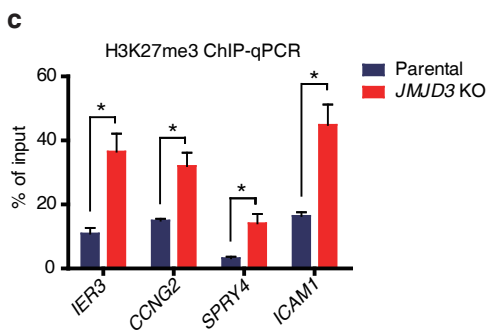
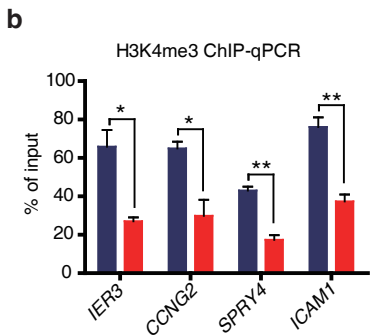
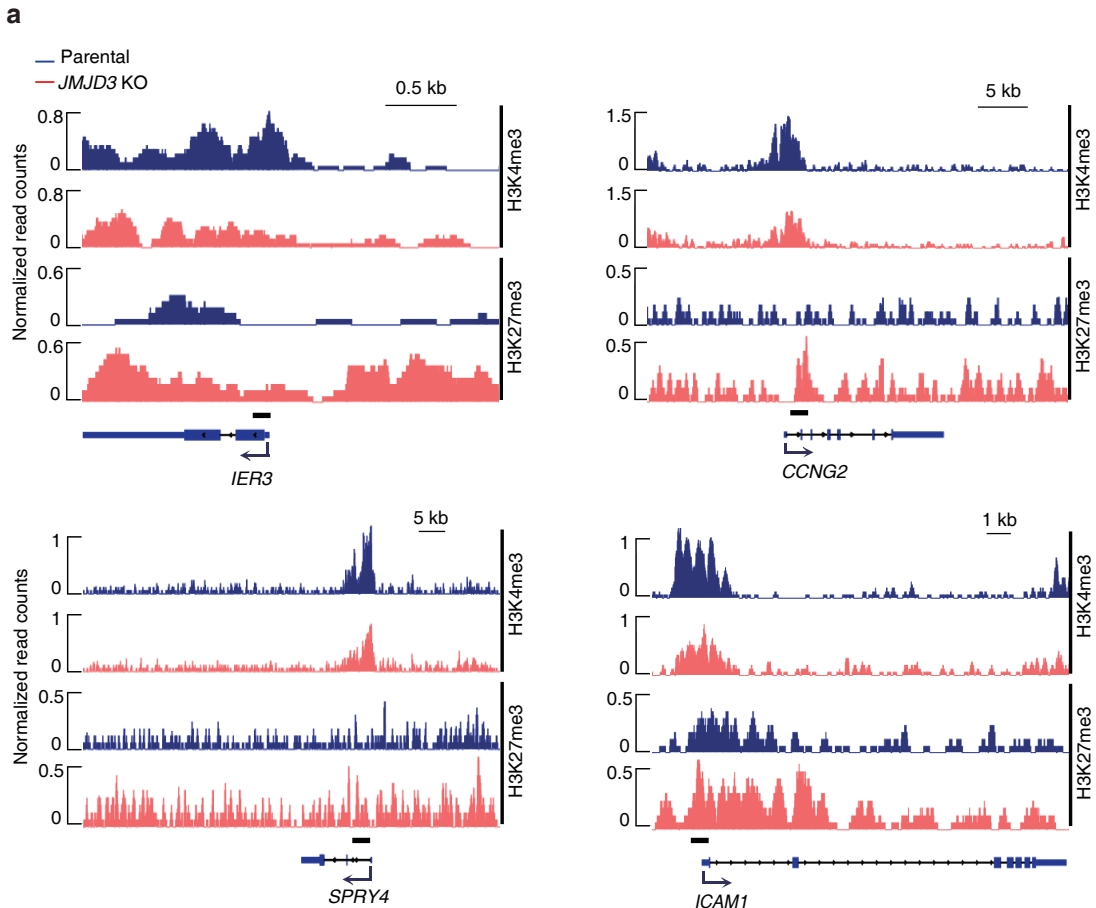


**Supplementary Figure 4. JMJD3 modulated the expression of key myelopoietic genes, related to Figure 4.**

(a) qRT-PCR expression analysis of *INK4A* and *ARF* mRNA level on HL-60 cells transduced with control or JMJD3-expressing vector. (b) GSEA of the expressing profile of HL-60 cells transduced with control or JMJD3-expressing vector using an inflammatory response-associated signature. (c) qRT-PCR expression analysis of the mRNA level of the *P65* subunit of NF- $\kappa$ B on HL-60 cells transduced with control or JMJD3-expressing vector. (d) GSEA of the expressing profile of HL-60 cells transduced with control or JMJD3-expressing vector using a NOTCH1 targets-associated signature. (e) qRT-PCR expression analysis of *HEY1* and *HES5* mRNA level on HL-60 cells transduced with control or JMJD3-expressing vector. (f-h) GSEA of the expressing profile of HL-60 cells transduced with control or JMJD3-expressing vector using a hematopoietic early progenitor-associated signature (f, left panel), a hematopoietic mature cell-associated signature (f, right panel), an apoptosis-associated signature (g) and a cell cycle-associated signature (h). (i) Summary of the top functional categories of genes significantly enriched in HL-60 cells transduced with JMJD3-expressing vector. Analyses were performed on the upregulated genes in HL-60 cells by JMJD3 overexpression using DAVID. (j) Heatmap showing the differentially expressed genes between NB4 cells transduced with control or JMJD3-expressing vector (fold change  $\geq 2$ ,  $p < 0.05$ ). (k-o) GSEA of the expressing profile of NB4 cells transduced with control or JMJD3-expressing

vector using a leukemic stem cell (LSC)-associated upregulated signature (k, left panel), a LSC-associated downregulated signature (k, right panel), a hematopoietic early progenitor-associated signature (l, left panel), a hematopoietic mature cell-associated signature (l, right panel), a cell cycle-associated signature (m), a NF- $\kappa$ B pathway-associated signature (n) and a NOTCH1 targets-associated signature (o). (p) qRT-PCR expression analysis of *HEY1* and *HES5* mRNA level on NB4 cells transduced with control or JMJD3-expressing vector. (q-r) GSEA of the expressing profile of NB4 cells transduced with control or JMJD3-expressing vector using an innate immunity-associated signature (q) and a senescence-associated signature (r). (s) Heatmap showing the differentially expressed genes between NB4 cells transduced with control or JMJD3-expressing vector (fold change  $\geq 1.5$ ,  $p < 0.05$ ) on a number of pro-myeloid differentiation genes and anti-myeloid differentiation genes. (t) Western blotting assay on C/EBP $\beta$  or RIPK3 protein in HL-60 cells transduced with NC siRNA, C/EBP $\beta$  siRNA or RIPK3 siRNA. (u) Western blotting assay on JMJD3 and C/EBP $\beta$  protein for C/EBP $\beta$  knockdown experiments in HL-60 cells transduced with control or JMJD3-expressing vector (left panel), western blotting assay on JMJD3 and RIPK3 protein for RIPK3 knockdown experiments in HL-60 cells transduced with control or JMJD3-expressing vector (right panel). (v-x) Flow cytometric assay of CD11b expression (v), Annexin V/7-AAD staining (w) and Hoechst/KI-67 staining (x) for C/EBP $\beta$  or RIPK3 knockdown experiments in HL-60 cells transduced with control or JMJD3-expressing vector.

**Supplementary Figure 5**

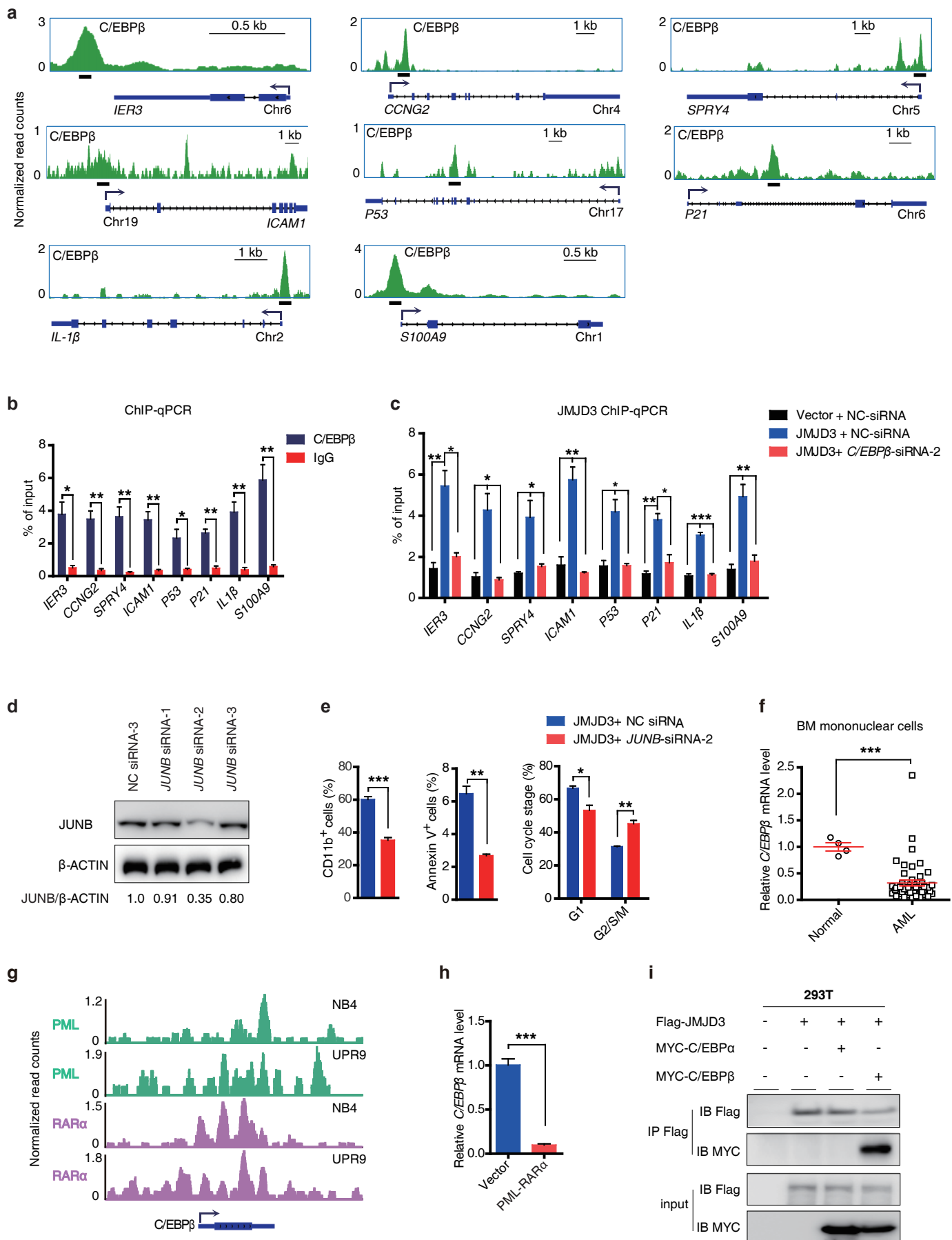


**Supplementary Figure 5. JMJD3 modulated the H3K4/K27 methylation in the promoter of myelopoietic regulators, related to Figure 5.**

(a) Genome browser tracks representing the binding sites of H3K4me3 and H3K27me3 at four representative gene locus in parental and *JMJD3* knockout HL-60 cells. (b-c) ChIP-qPCR assay for H3K4me3 (b) and H3K27me3 (c) at four representative gene loci in parental and *JMJD3* knockout HL-60 cells. (d-e) ChIP-qPCR assay for H3K4me3 (d) and H3K27me3 (e) at four representative gene loci in HL-60 cells transduced with control or JMJD3-expressing vector. Data are shown as the mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



# Supplementary Figure 6



**Supplementary Figure 6. JMJD3 couples with C/EBP $\beta$  program, related to**

**Figure 6.**

(a) Genome browser tracks representing the binding sites of C/EBP $\beta$  at 8 representative gene loci. (b) ChIP-qPCR assay for C/EBP $\beta$  occupancy at 8 representative gene loci in HL-60 cells transduced with C/EBP $\beta$ -expressing vector. (c) ChIP-qPCR assay for JMJD3 occupancy at the 8 representative gene loci for C/EBP $\beta$  or NC knockdown experiments in HL-60 cells transduced with control or JMJD3-overexpressing vector. (d) Western blotting assay on JUNB protein in HL-60 cells transduced with NC siRNA or *JUNB* siRNA. (e) HL-60 cells transduced with control or JMJD3-expressing vector were further treated with NC siRNA or *JUNB* siRNA. Analyses of CD11b expression (left panel), Annexin V staining (middle panel) and cell cycle status (right panel) were performed using flow cytometric assay. (f) C/EBP $\beta$  mRNA level in the AML blasts-enriched primary BM mononuclear cells (n=49) or normal BM mononuclear cells (n=4) were assayed by qRT-PCR. (g) Genome browser tracks representing the binding sites of PML or RAR $\alpha$  at the C/EBP $\beta$  gene locus in the AML cells as indicated. (h) PML-RAR $\alpha$  was transduced into normal c-Kit<sup>+</sup> BM cells by retrovirus infection, and the mRNA levels of C/EBP $\beta$  were measured by qRT-PCR. (i) 293T cells were transfected with human Flag-JMJD3 and MYC-C/EBP $\alpha$  or MYC-C/EBP $\beta$  constructs. Cell lysates were immunoprecipitated with anti-Flag beads, followed by immunoblot with an anti-MYC antibody. Data are shown as the mean  $\pm$  SEM. \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001.

Figure 1d

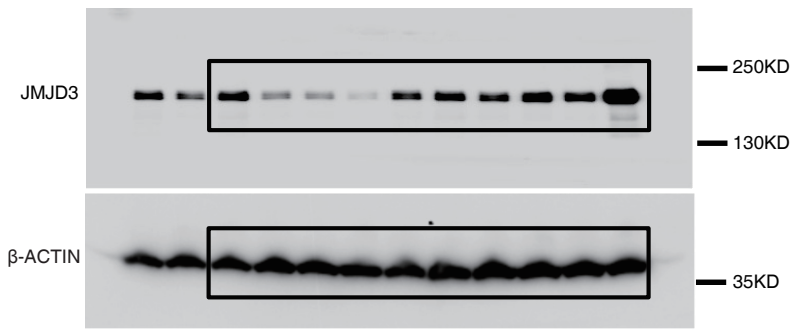


Figure 1e

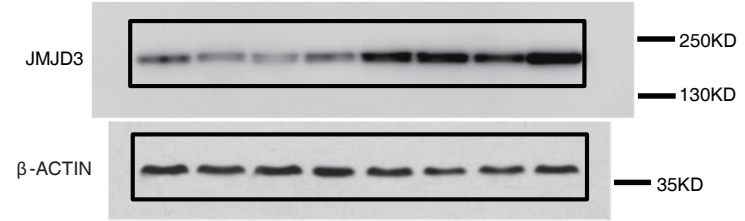


Figure 2a

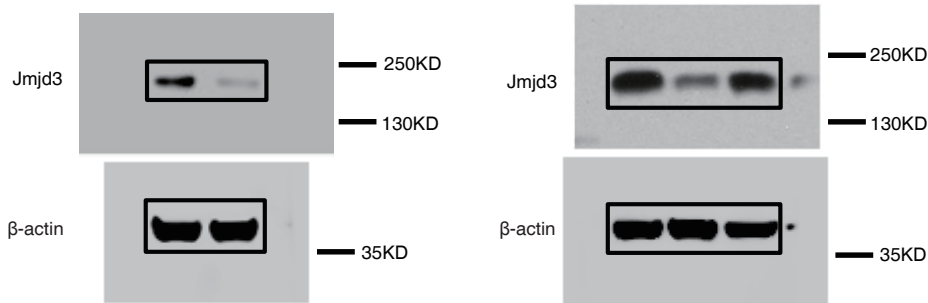


Figure 3e

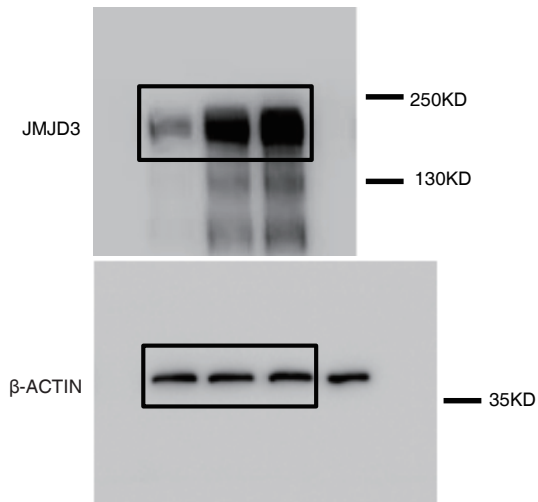


Figure 6m

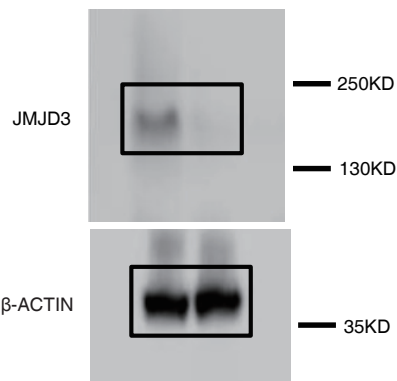
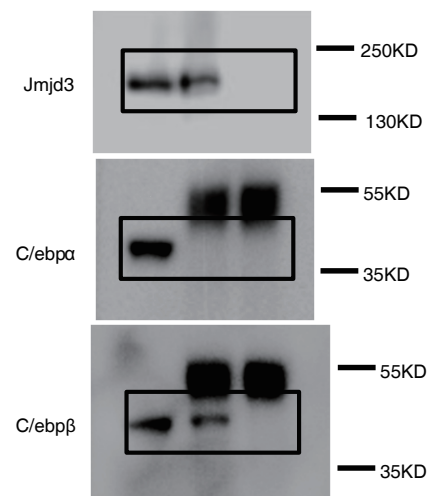


Figure 7a

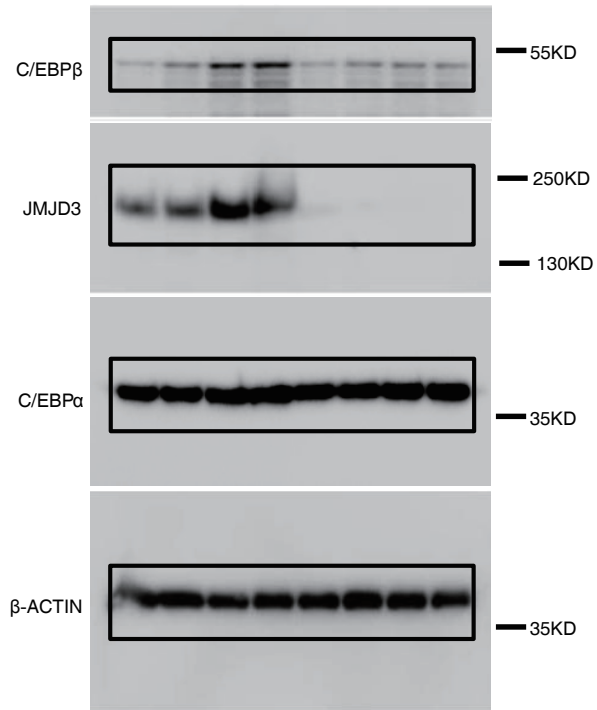
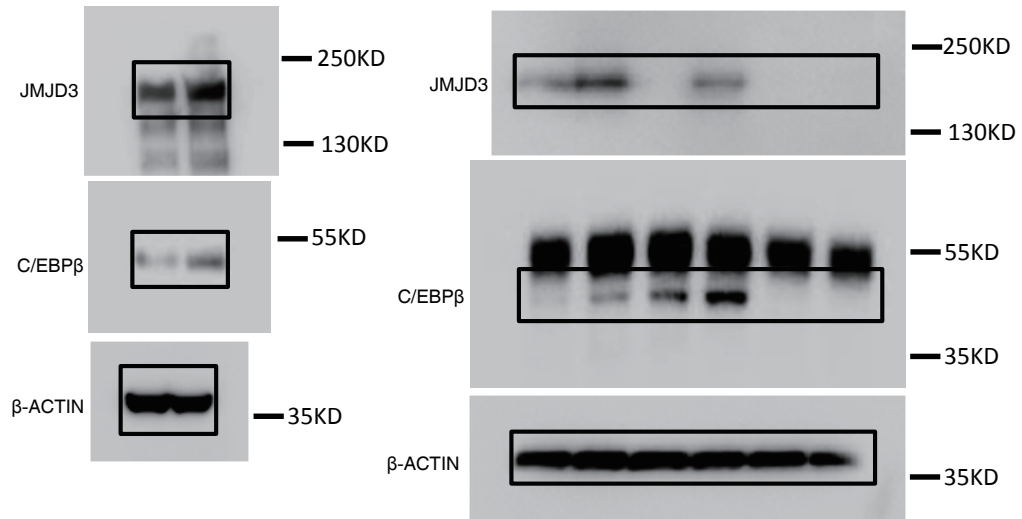
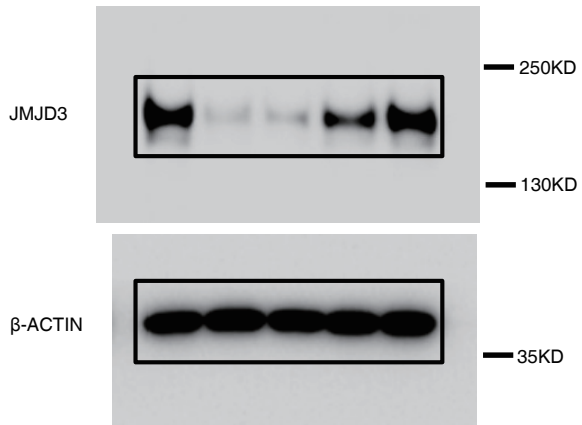


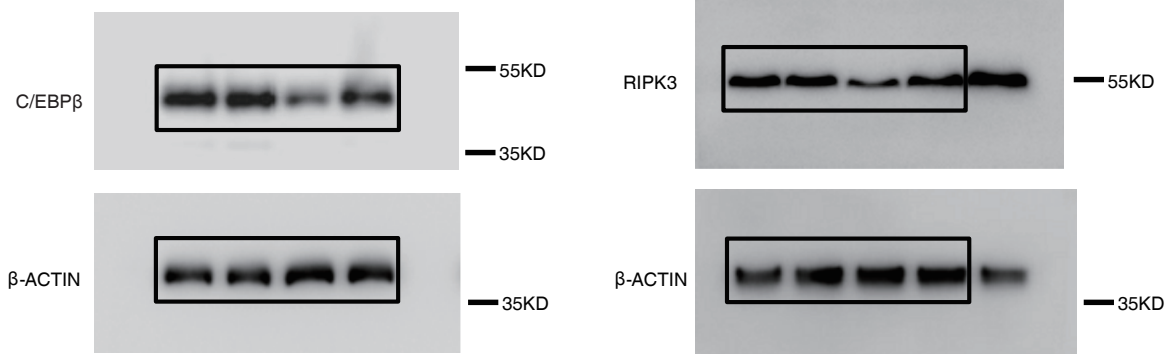
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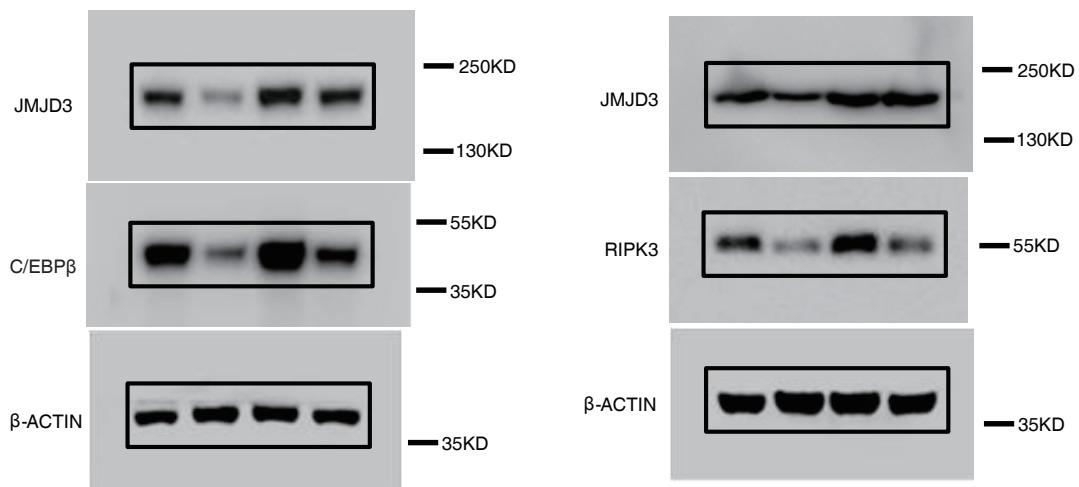
Supplementary Figure 2e



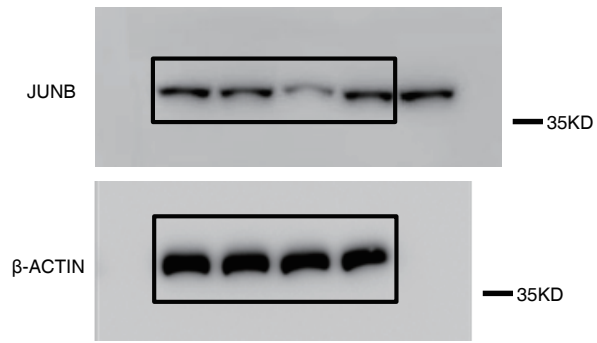
Supplementary Figure 4t



Supplementary Figure 4u



Supplementary Figure 6d



Supplementary Figure 6i

