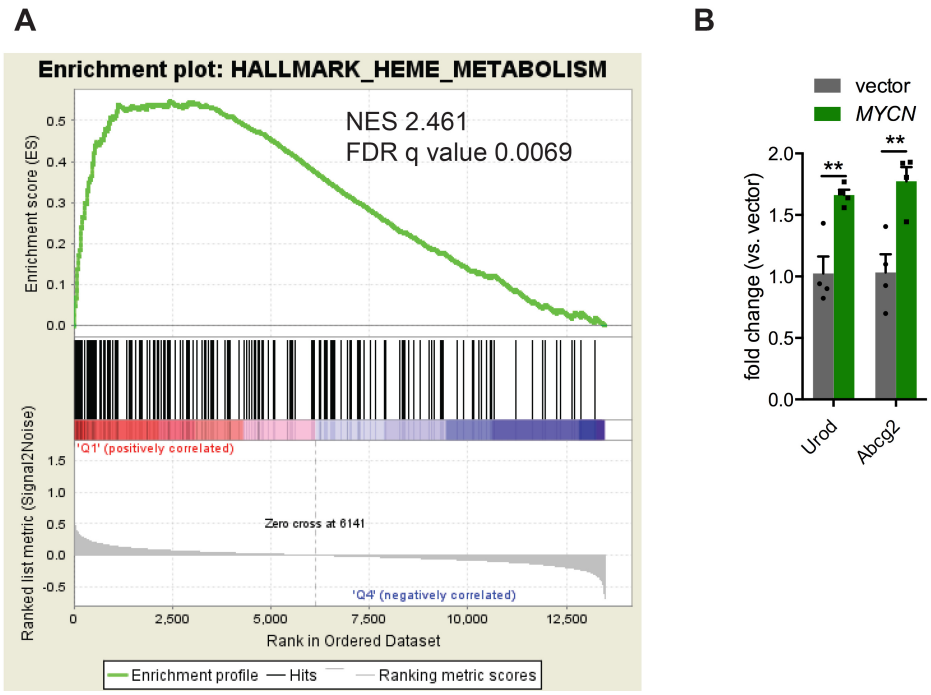
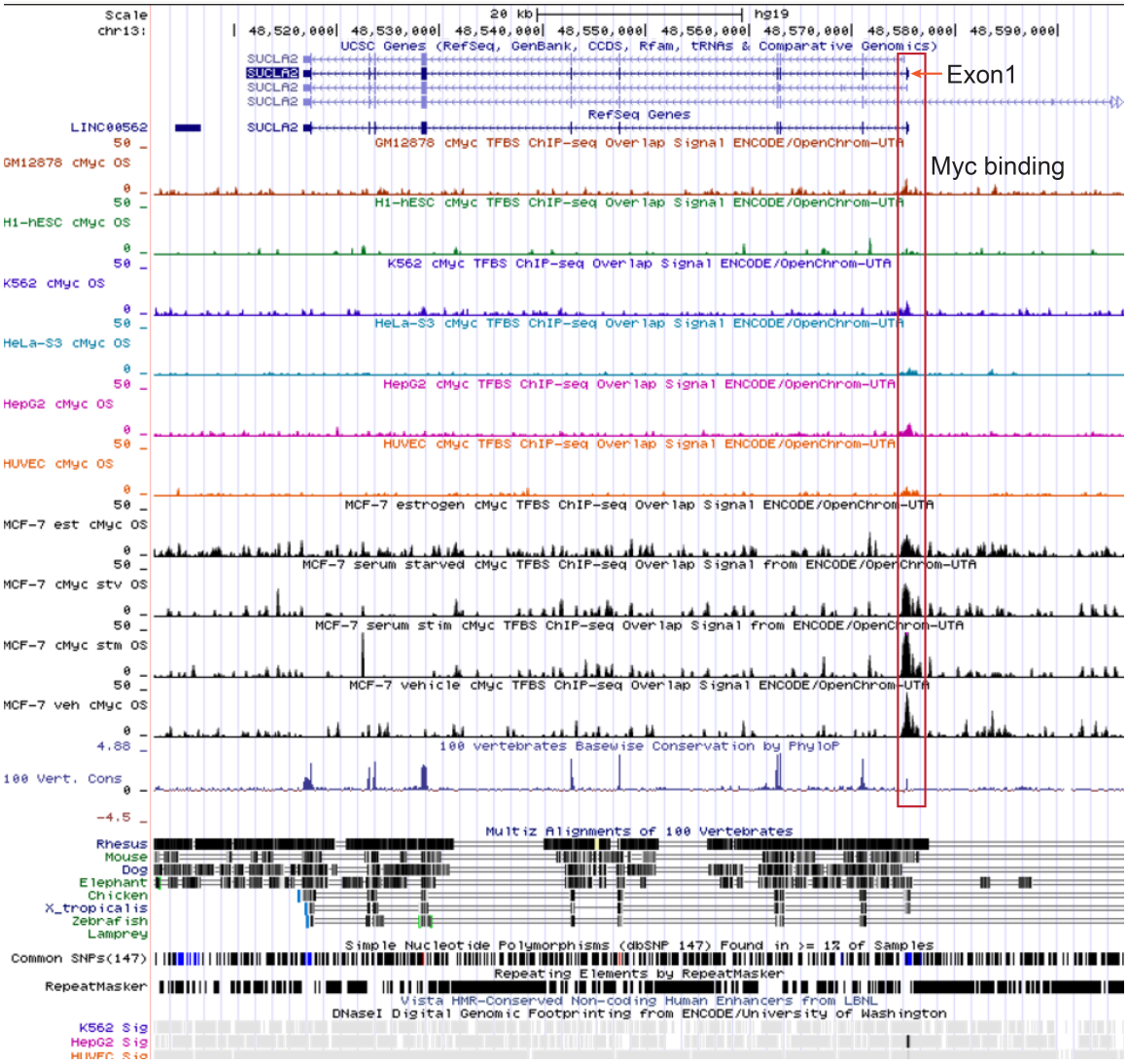
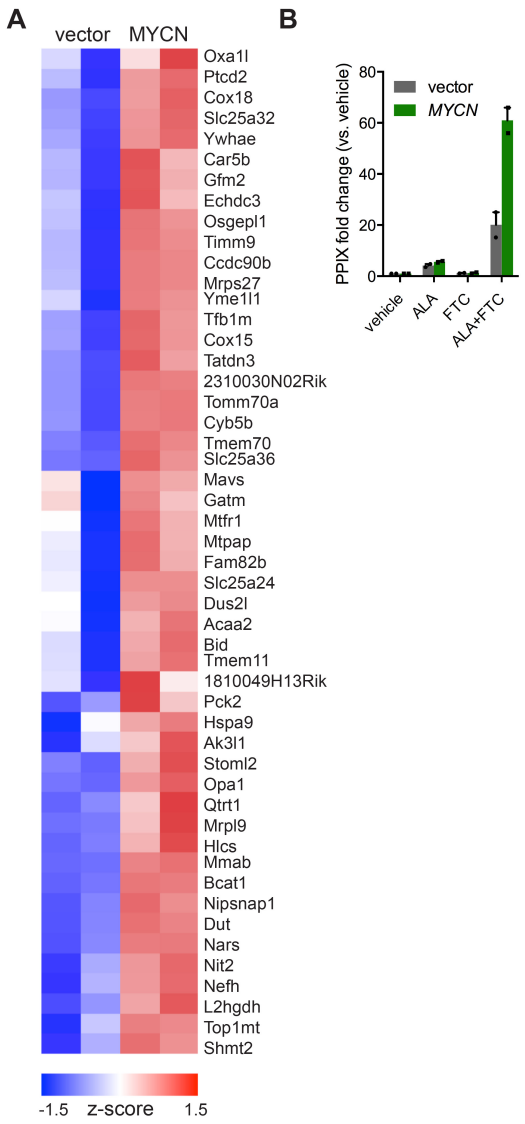
SUPPLEMENTAL FIGURES



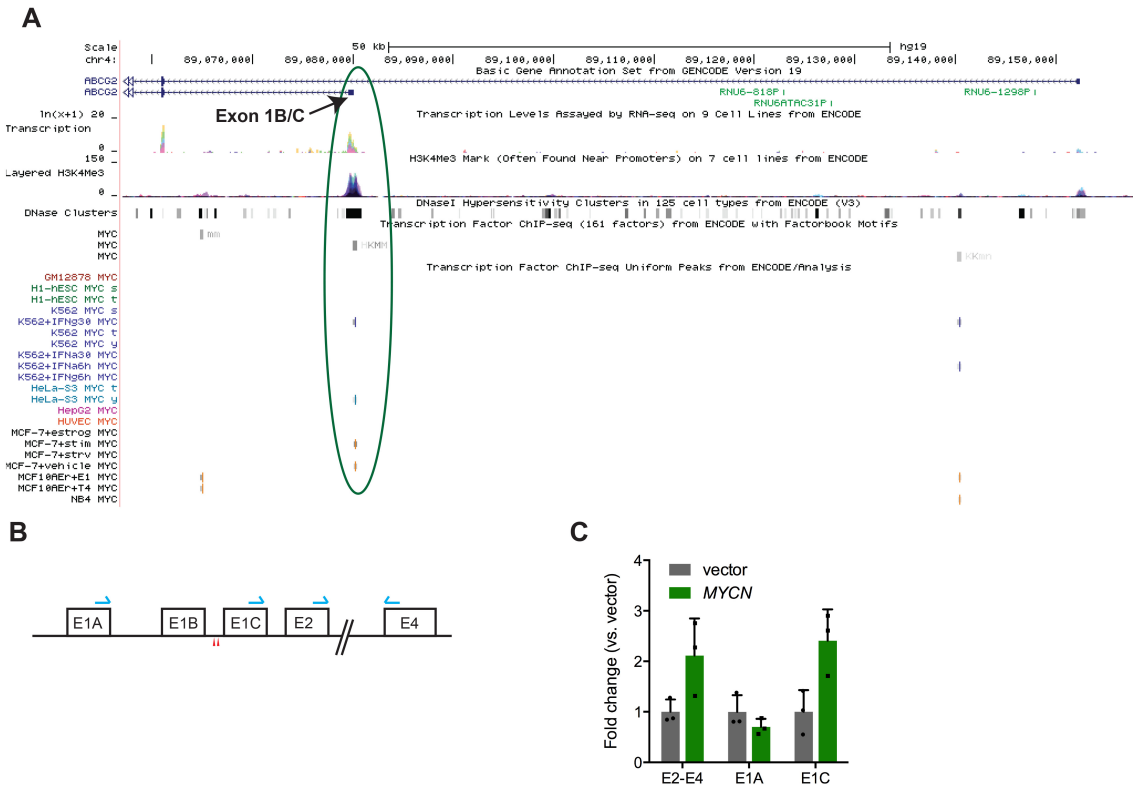
Supplemental Figure 1. Heme biosynthesis and mitochondrial genes are upregulated by MYCN (related to Figures 1 and 2). A) Geneset enrichment analysis comparing genes upregulated in high *MYCN* expression (first quartile) vs. rest from the SJ AML patient cohort shows enrichment of heme metabolism genes in patients with high *MYCN* expression. **B)** *Urod* and *Abcg2* mRNA levels were determined by qPCR. 2-Way ANOVA was used (**, $p<0.01$)



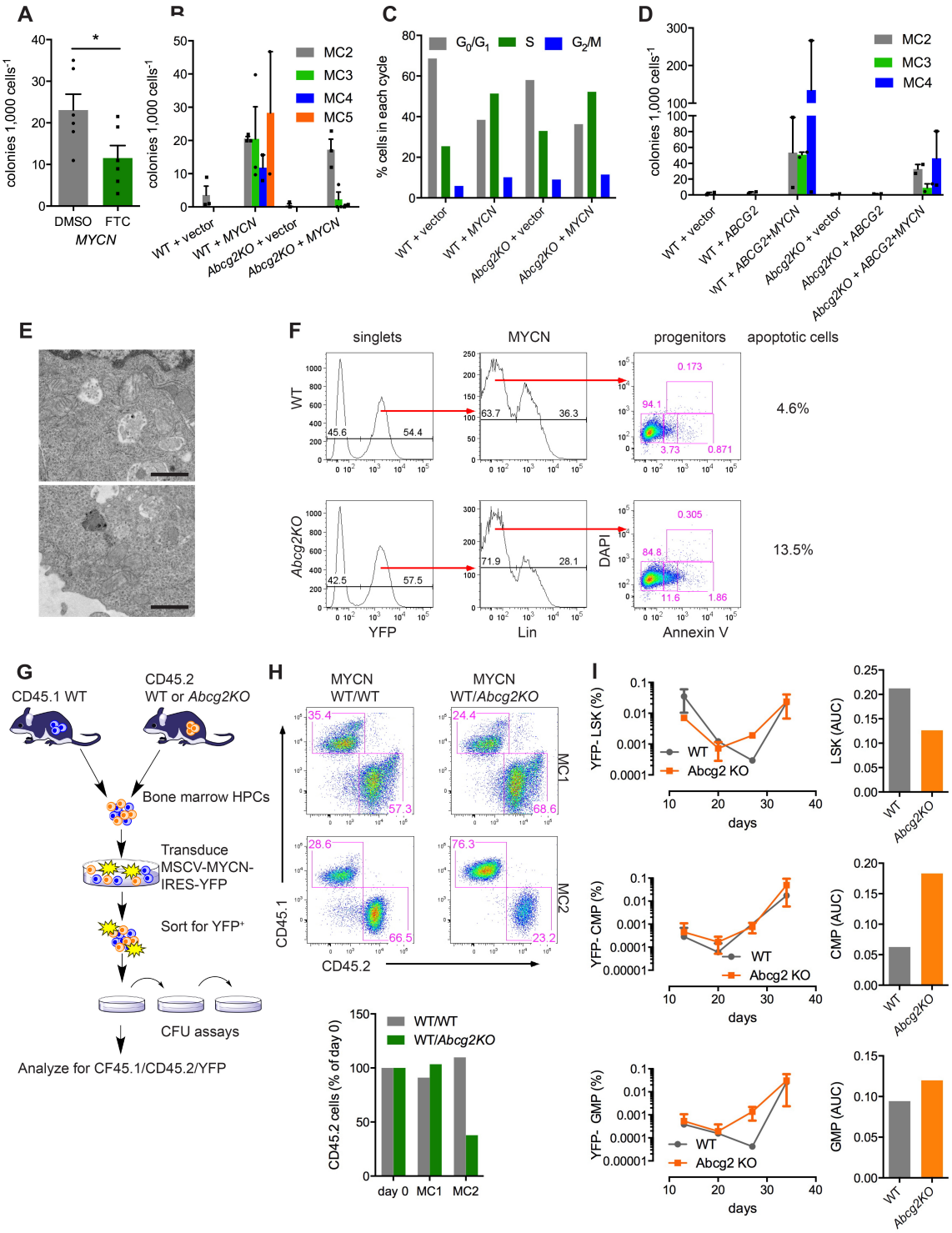
Supplemental Figure 2. SUCLA2 promoter is bound by MYC (related to Figure 2). A) Promoter and MYCN binding site analysis using ENCODE data from UCSC Genome Browser. The promoter is bound by MYC (shown in the red box) as demonstrated by ChIP-seq data.



Supplemental Figure 3. Mitochondrial genes are upregulated by MYCN (related to Figure 2). A) Heatmap from Fig. 2g is enlarged to include gene names. **B)** Vector- or MYCN transudced HPCs were incubated with vehicle, ALA, and FTC as indicated and intracellular PPIX measured by FACS.

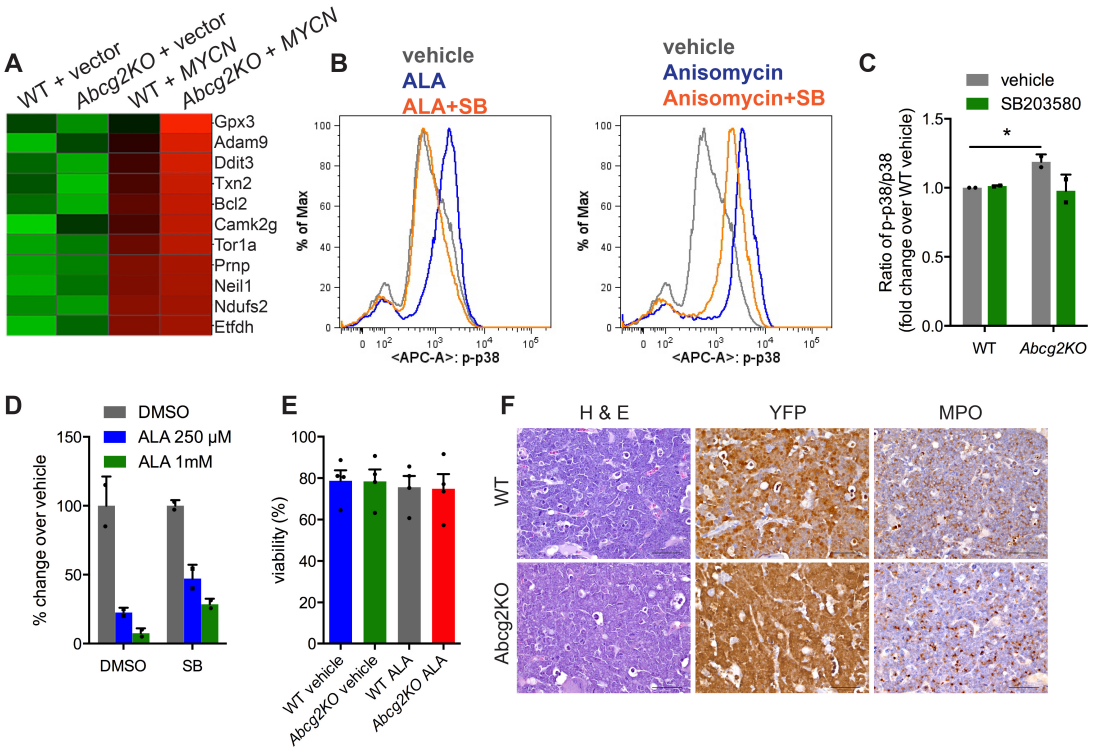


Supplemental Figure 4. Specific *Abcg2* variant is upregulated by MYCN (related to Figure 3). A) Promoter and MYCN binding site analysis using ENCODE data from UCSC Genome Browser. **B)** Schematic diagram of *Abcg2* promoter with putative MYCN-binding sites shown with red triangles. Primers used for qPCR is shown with blue arrows. Not drawn to scale. **C)** Levels of different splice variants were measured by qPCR. E2-E4 represents total *Abcg2* transcript.



Supplemental Figure 5. Progenitor self-renewal requires ABCG2 (related to Figure 3). A) MYCN-transduced HPCs were plated using methocult medium in the absence or presence of FTC. **B)** HPCs isolated from WT or *Abcg2KO* mice were transduced with vector or MYCN and serially replated on methocult to assess self-renewal. **C)** HPCs isolated from WT or *Abcg2KO* mice were transduced with vector or MYCN and cell cycle was measured by FACS. **D)** HPCs isolated from WT or *Abcg2KO* mice were transduced with vector or MYCN in combination with ABCG2 and serially replated on methocult to assess self-renewal. HPCs isolated from **E)** WT or *Abcg2KO* mice were transduced with MYCN, fixed and

processed for EM. **F)** Progenitors from methocult (MC1) culture were analyzed for apoptosis by FACS. **G)** Schematic drawing of the experiment to assess whether the defect in *Abcg2KO* MYCN HPCs are intrinsic or extrinsic. **H)** HPCs harvested from CD45.1 WT and CD45.2 WT or Abcg2KO were transduced with MYCN and serially replated on methocult. Colonies from methocult cultures were harvested and analyzed by FACS for CD45.1 and CD45.2 antigen. Frequency of CD45. 1 and CD45.2 cells was normalized to the value from day 0. **I)** WT or *Abcg2KO* HPCs were transduced with MYCN and transplanted into lethally irradiated congenic recipient mice. Bone marrow cells were harvested at indicated time points and analyzed for hematopoietic progenitors using FACS. YFP^- ($MYCN^-$) LSK, CMP and GMP populations are shown.



Supplemental Figure 6. PPIX toxicity is partially mediated by ROS and p38 (related to Figure 4). A) *Abcg2KO* MYCN-MC1 cells exhibit upregulation in ROS genes. **B)** MYCN-HPCs were incubated with ALA, p38 inhibitor, SB203580, or anisomycin as indicated. The cells were fixed, permeabilized, stained for phospho-p38 and analyzed by FACS. **C)** WT or *Abcg2KO* MYCN-HPCs were fixed, permeabilized, stained for total and phospho-p38 and analyzed by FACS. **D)** p38 inhibition by SB203580 partially rescued ALA toxicity in CFU-C assays. **E)** WT or *Abcg2KO* HPCs were transduced with MYCN, incubated with vehicle or ALA overnight and analyzed for viability by FACS. **F)** WT or Abcg2KO HPCs were transduced with MYCN and transplanted into lethally irradiated congenic recipient mice. Mice succumbing to leukemia were fixed in formalin, paraffin-embedded and histology slides prepared. Slides were stained with H&E, or antibodies to GFP (which also recognizes YFP) and myeloperoxidase (MPO).

Supplemental Table 1. Analysis of Patient characteristics with UROD expression

Response to therapy	Favorable(1)	Favorable	Favorable	Intermediate	Poor	Normal Karyotype
Lesion	t(15;17)	t(8;21)	PML-RARa	MLL	Ch7loss	
UROD>median	6	3	7	2	4	23
UROD<median	4	6	5	5	6	17

Supplemental Table 2. Hematopoietic stem cell genes in WT or *Abcg2KO*-vector, MYCN HPCs (related to Figure 3). Last four columns highlighted in blue are log ratio.

Probe set 430v2 PM	Gene symbol	KO vec avg	KO MYCN avg	WT vec avg	WT MYCN avg	MYCN WT vs KO (>2-fold with FDR<0.05)	LPE vec KO vs. WT	LPE MYCN KO vs. WT	LPE KO vec vs. MYCN	LPE WT vec vs. MYCN
1428816_PM_a_at	Gata2	8.77	9.53	8.64	9.70	Increased	0.14	-0.17	0.76	1.06
1450333_PM_a_at	Gata2	8.83	9.57	9.10	9.50	Not significant	-0.27	0.07	0.74	0.41
1417679_PM_at	Gfi1	4.69	5.87	5.43	5.44	Not significant	-0.74	0.43	1.19	0.02
1420399_PM_at	Gfi1b	5.34	4.67	5.06	5.07	Not significant	0.29	-0.40	-0.68	0.01
1448733_PM_at	Bmi1	5.73	5.19	5.73	5.58	Not significant	-0.01	-0.40	-0.54	-0.15
1417493_PM_at	Bmi1	7.67	7.06	7.41	7.35	Not significant	0.26	-0.30	-0.61	-0.06
1454086_PM_a_at	Lmo2	9.17	8.49	9.06	8.36	Not significant	0.11	0.13	-0.68	-0.70
1440878_PM_at	Runx1	7.78	7.26	7.51	7.35	Not significant	0.27	-0.10	-0.52	-0.16
1422864_PM_at	Runx1	9.01	8.75	8.88	8.91	Not significant	0.14	-0.16	-0.27	0.03
1422865_PM_at	Runx1	6.06	6.15	5.99	6.54	Not significant	0.08	-0.39	0.09	0.55
1427650_PM_a_at	Runx1	7.09	8.18	7.11	7.92	Not significant	-0.02	0.27	1.09	0.81
1452530_PM_a_at	Runx1	4.61	5.34	4.63	5.30	Not significant	-0.03	0.05	0.74	0.67
1452531_PM_at	Runx1	4.02	4.00	4.44	4.27	Not significant	-0.42	-0.27	-0.02	-0.18
1439107_PM_a_at	Mll5	8.19	7.70	8.07	7.92	Not significant	0.12	-0.22	-0.49	-0.15
1439108_PM_at	Mll5	8.72	8.13	8.45	8.24	Not significant	0.27	-0.11	-0.59	-0.21
1457193_PM_at	Mll3	5.64	4.44	5.65	4.67	Not significant	-0.01	-0.24	-1.21	-0.98
1427150_PM_at	Mll3	6.96	5.86	7.00	6.06	Not significant	-0.04	-0.20	-1.10	-0.94
1427236_PM_a_at	Mll5	8.66	8.18	8.45	8.17	Not significant	0.21	0.02	-0.48	-0.28
1427283_PM_at	Mll1	6.09	5.58	6.39	5.44	Not significant	-0.30	0.14	-0.51	-0.95
1427555_PM_at	Mll2	3.40	3.29	3.68	3.26	Not significant	-0.28	0.03	-0.11	-0.42
1434704_PM_at	Mll5	8.42	8.13	8.23	8.13	Not significant	0.19	0.00	-0.29	-0.10
1452377_PM_at	Mll1	5.65	4.89	4.86	4.87	Not significant	0.79	0.02	-0.76	0.01
1432601_PM_at	Mll5	3.08	2.87	3.12	2.82	Not significant	-0.04	0.06	-0.21	-0.30
1434178_PM_at	Mll3	8.03	7.21	7.95	7.28	Not significant	0.08	-0.07	-0.82	-0.68
1434179_PM_at	Mll3	7.07	6.57	6.70	6.55	Not significant	0.37	0.02	-0.50	-0.16
1416880_PM_at	Mcl1	9.80	9.62	9.52	9.59	Not significant	0.29	0.03	-0.18	0.08
1416881_PM_at	Mcl1	10.00	9.99	9.70	9.99	Not significant	0.30	-0.01	-0.01	0.29
1437527_PM_x_at	Mcl1	11.20	10.80	11.03	10.74	Not significant	0.17	0.06	-0.40	-0.29
1448503_PM_at	Mcl1	11.44	11.21	11.31	11.25	Not significant	0.13	-0.04	-0.23	-0.06
1456243_PM_x_at	Mcl1	10.62	10.29	10.46	10.15	Not significant	0.17	0.14	-0.33	-0.31
1456381_PM_x_at	Mcl1	10.08	9.51	10.08	9.61	Not significant	0.01	-0.10	-0.57	-0.47
1449389_PM_at	Tal1	8.30	8.20	8.39	8.16	Not significant	-0.09	0.04	-0.10	-0.23
1450517_PM_at	Tal2	3.76	2.74	3.37	2.62	Not significant	0.39	0.12	-1.02	-0.75

Reference

1. Ferrara F, and Schiffer CA. Acute myeloid leukaemia in adults. *Lancet.* 2013;381(9865):484-95.