SUPPLEMENTARY APPENDIX

MicroRNA-34b promoter hypermethylation induces CREB overexpression and contributes to myeloid transformation

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Online Supplementary Design and Methods

Patients

The patients included children with newly diagnosed acute myeloid leukemia (AML), aged from 0 to 18 years (mean 8.47±5.34), with written informed consent from their parents. Patients with granulocytic sarcoma, secondary AML, secondary myelodysplastic syndrome (MDS), or Down syndrome, as well as patients with pretreatment lasting longer than 14 days or patients with acute promyelocytic leukemia were excluded from the present analysis.[1] The initial diagnosis of AML was established according to the FAB classification and flow cytometry. Smears obtained at the time of diagnosis were centrally reviewed in the Laboratory of Pediatric Hematology at the University Hospital in Padova and subjected to a specific panel review in controversial cases. The presence of t(8;21), inv(16), MLL rearrangements, FLT3-ITD or related molecular transcripts was also evaluated. Pediatric patients with MDS or juvenile myelomonocytic leukemia (JMML) were enrolled in centers belonging to the Italian Association of Oncohematology Pediatric (AIEOP) and accrued over more than 10 years since these are rare diseases of childhood. Bone marrow $CD34^+$ cells from MDS patients and healthy individuals were sorted on a BD FACSAria cell sorter (BD Biosciences).

Methylation analysis

The primer sequences used were: MSP-34b/c-ms TTTAGTTACGCGTGTTGTGC, MSP-34b/c-mas ACTACAACTCCCGAACGATC, MSP-34b/c-us TGGTTTAGTTATGTGTGTTGTGT, MSP-34b/c-uas CAACTACAACTCCCAAACAATCC.[2] The product from the first reaction negative for methylation-specific polymerase chain reaction (PCR), underwent a second run PCR (nested) to confirm the unmethylated status.

Gene expression analysis

CEL files of paired MDS and AML samples can be found at the GEO repository (*http://www.ncbi.nlm.nih.gov/geo/*; Series Accession Number GSE25300), the data for *de novo* AML and MDS at diagnosis have already been published.[3,4] CEL files were normalized using the robust multiarray averaging (RMA) expression measure implemented in Partek Genomic Suite software (*http://www.partek.com*). A paired t-test was applied to identify differentially expressed genes between sample pairs. Multiplicity corrections were used to control false discovery rate (FDR) using the FDR q-value; probes with adjusted *P*-values less than 0.05 were declared significant. Clusters were generated using Partek Genomic Suite. We integrated genes significantly differentially expressed with the ChIP-Chip data of CREB binding in human tissues (Table S4, *http://natural.salk.edu/CREB/*) to search for direct CREB target genes.

Gene set enrichment analysis

We compared the gene expression signatures of patients with miR-34b hypermethylated promoter with those of patients with unmethylated promoters, using the signal-to-noise metric and 1,000 permutations of the genes. Enrichment of specific genes/pathways in the two groups of patients were considered statistically significant for *P*-values <0.05 and a FDR <0.1. For help with interpreting the gene set enrichment analysis go to *http://www.broadinstitute.org/gsea/doc/GSEAUserGuideFrame.html?%20_Interpreting_GSEA_Results*.

Constructs

Lentiviral constructs for miR-34b expression (FUGW-34b) were obtained by cloning approximately 250 base pairs of the human genomic sequence for miR-34b into FUGW.[5] Lentivirus for miR-34b knockdown (Fugw-miR-target) or the negative control (Fugw-SCR) were constructed as previously described by Gentner *et al.*[6] To generate VSV-G-pseudotyped lentiviruses, 2×10^6 293T cells were transfected with pGag-Pol, pVSV-G, and either Fugw-EV, Fugw-miR-34b, Fugw-miR-target, Fugw-miR-Neg or Fucwr-LUC After 48 h, viral supernatant was harvested and used to spin-infect 7.5×10^5 HL60 or K562 cells or 10^6 fetal liver cells (16-20 weeks old) for 1 h at 2500 rpm. Stably transduced cells were sorted with a BD FACSaria II.

Primary cell culture and transfection

The cells were cultured in RPMI (Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and the following recombinant human (rh) cytokines: interleukin-3 (rhIL-3; 20 ng/mL), interleukin-6 (rhIL-6; 20ng/mL), stem cell factor (rhSCF; 50 ng/mL), thrombopoietin (rhTPO; 50 ng/mL) and fms-like tyrosine kinase-3 ligand (rhFLT-3 ligand; 50 ng/mL). Healthy bone marrow cells were transfected with 3.5 μg of pEGFP-N1-ΔGFP-CREB plasmid or an empty vector (pEGFP-N1-ΔGFP-EV) used as controls. Fetal livers were obtained without identification information under federal and state regulations from the UCLA CFAR Gene and Cellular Therapy Core Laboratory and UCLA OB-GYN.

Cell cycle analysis

Primary AML cells were transfected with miR-34b or miR-Neg oligonucleotide. After 24 h, 5×10^5 cells were incubated with 50 μg/mL propidium iodide in 1 mL phosphate-buffered saline overnight at 4°C. Cells were run on a Cytomics FC500 (Beckman Coulter) and cell cycle was analyzed with Multicycle Wincycle software (Phoenix Flow Systems).

Colony assays

Living cells ($2\text{x}10^3$) after oligonucleotide transfection or LV transduction were counted and seeded in duplicate in 1 mL of methylcellulose semisolid medium supplemented with nutrients and cytokines (rhSCF, rhGM-CSF, rhIL-3, rhIL-6, rhG-CSF, rhEPO; StemCell Technologies). Colony evaluation was done *in situ* by light microscopy. Colonies were then collected from methylcellulose, washed in phosphate-buffered saline twice and stained with fluorochrome-linked antibodies to CD33 and CD15.

References

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Online Supplementary Table S1. Genes expression results. A. Genes differentially expressed between MDS and their evolution by GeneChip Human Genome U133 Plus 2.0. B. List of CREB target genes (by ChIP-chip database[6]) differentially expressed between the MDS samples and samples from patients after evolution to MDS (see Venn diagram Figure 2B). C. List of genes given by the overlap between genes differentially expressed between MDS and their evolution, between AML and healthy bone marrow and CREB targets by the ChIP-chip database (see Venn diagram Figure 3H).

A

Differently expressed genes in paired samples from patients with MDS and after evolution to AML.

Probeset ID	Gene Symbol	p-value	Mean(evolvedAML)	Mean(MDS)
205837_s_at	*GYPA /// GYPB*	0,0154529	4,30256	8,0168
205268_s_at	*ADD2*	0,00321165	3,34659	7,00302
207802_at	*CRISP3*	0,0472946	4,9766	8,60857
219672_at	*AHSP*	0,0269338	7,19135	10,8193
205950_s_at	*CA1*	0,0287966	8,62367	12,2389
206145_at	*RHAG*	0,0181125	5,19677	8,6699
211821_x_at	*GYPA*	0,027159	5,45517	8,86697
210746_s_at	*EPB42*	0,0320731	4,9991	8,36374
235102_x_at	*NA*	0,0266832	6,90408	10,1739
205592_at	*SLC4A1*	0,0441826	7,36832	10,5714
211820_x_at	*GYPA /// GYPB*	0,0210552	5,74035	8,87091
226682_at	*RORA*	0,0168465	3,26237	6,36328
234980_at	*TMEM56*	0,00944968	3,40187	6,45985
210426_x_at	*RORA*	0,0183272	3,29349	6,35069
205838_at	*GYPA /// GYPB*	0,00710907	4,13029	7,12779
214433_s_at	*SELENBP1*	0,0308919	5,1549	8,13633
211560_s_at	*ALAS2*	0,0436809	6,47417	9,43573
206698_at	*XK*	0,0279764	4,60752	7,49207
203936_s_at	*MMP9*	0,0179209	6,83678	9,71002
210479_s_at	*RORA*	0,017939	4,29018	7,13437
206515_at	*CYP4F3*	0,0425015	3,31601	6,1316
209047_at	*AQP1*	0,0294695	4,42017	7,20608
206937_at	*SPTA1*	0,0220541	4,90567	7,67688
226218_at	*IL7R*	0,00681063	4,50591	7,25548
215819_s_at	*RHCE /// RHD*	0,0360449	4,51676	7,24934
214407_x_at	*GYPB*	0,0207691	6,10421	8,72923
212531_at	*LCN2*	0,0448405	7,02196	9,63649
211339_s_at	*ITK*	0,00607457	4,47313	7,03149
202018_s_at	*LTF*	0,0456808	9,00537	11,4896
205389_s_at	*ANK1*	0,0276452	3,85779	6,32605
239206_at	*CR1L*	0,0247893	3,41037	5,87228
210429_at	*RHD*	0,0221161	3,83493	6,28228
205590_at	*RASGRP1*	0,0187959	4,55047	6,99708
214470_at	*KLRB1*	0,04189	5,48479	7,91778
210586_x_at	*RHD*	0,0313065	4,40625	6,82921
210036_s_at	*KCNH2*	0,00420226	5,19187	7,6133
219528_s_at	*BCL11B*	0,0177332	3,55211	5,89615
210504_at	*KLF1*	0,0249197	4,90135	7,24272
207459_x_at	*GYPB*	0,0281599	5,90099	8,22555
237515_at	*TMEM56*	0,00132444	2,35768	4,68163
201131_s_at	*CDH1*	0,0443855	2,51111	4,8191
223669_at	*HEMGN*	0,00965091	5,89949	8,20624
212372_at	*MYH10*	0,00863929	4,05511	6,35525
202219_at	*SLC6A8*	0,0423568	4,56707	6,85175
221558_s_at	*LEF1*	0,0324898	5,1177	7,38074
228376_at	*GGTA1*	0,0441999	4,85143	7,07387
239205_s_at	*CR1 /// CR1L*	0,0196757	5,10436	7,3116
223670_s_at	*HEMGN*	0,00717088	5,89861	8,09344
206337_at	*CCR7*	0,031964	4,99138	7,18431
203413_at	*NELL2*	0,0261533	4,59139	6,74185
211254_x_at	*RHAG*	0,0248647	5,00349	7,11667
221778_at	*JHDM1D*	0,0210068	5,09044	7,19932
216833_x_at	*GYPB*	0,0483263	5,99846	8,1008
202201_at	*BLVRB*	0,0222743	8,31132	10,4078
209604_s_at	*GATA3*	0,0345875	5,11366	7,20791
210395_x_at	*MYL4*	0,040726	6,35049	8,41562
230690_at	*TUBB1*	0,0448787	5,29633	7,35697
228390_at	*NA*	0,0175716	2,65291	4,70844
239142_at	*RFESD*	0,000502439	5,04856	7,0925
218793_s_at	*SCML1*	0,0163998	3,34639	5,34832
210430_x_at	*RHD*	0,0396489	5,92241	7,89421
221627_at	*TRIM10*	0,0120992	4,97563	6,94312
226489_at	*TMCC3*	0,0287354	3,90238	5,86398
209585_s_at	*MINPP1*	0,00356979	4,59874	6,55314
206522_at	*MGAM*	0,00640475	6,24225	8,18201
204466_s_at	*SNCA*	0,0260138	7,75699	9,68937
210088_x_at	*MYL4*	0,0302283	6,13949	8,06719
228220_at	*FCHO2*	0,00966679	4,10621	6,02133
216317_x_at	*RHCE*	0,0343992	5,60742	7,48126
219148_at	*PBK*	0,0104463	3,42578	5,2547

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203116_s_at	*FECH*	0,0420987	6,98384	8,79821
204976_s_at	*AMMECR1*	0,00182818	5,47627	7,28327
226751_at	*CNRIP1*	0,00466919	4,51021	6,308
226421_at	*AMMECR1*	0,000489275	5,29045	7,08128
235490_at	*TMEM107*	0,0363859	4,15534	5,93646
201839_s_at	*EPCAM*	0,0158616	3,05273	4,82641
223405_at	*NPL*	0,0333666	5,33192	7,09701
205831_at	*CD2*	0,013832	4,66492	6,42991
202587_s_at	*AK1*	0,016482	5,37449	7,13227
205821_at	*KLRK1*	0,0055517	5,30921	7,06167
205758_at	*CD8A*	0,0107182	6,27046	8,00955
203040_s_at	*HMBS*	0,037121	7,54259	9,26442
204467_s_at	*SNCA*	0,0470946	5,82245	7,53905
230988_at	*ADD2*	0,0132595	3,70442	5,40573
239370_at	*NA*	0,00848246	3,32455	5,01667
221011_s_at	*LBH*	0,00900268	4,41457	6,09531
205863_at	*S100A12*	0,00236858	9,2427	10,9199
213193_x_at	*TRBC1*	0,00960489	7,20487	8,88107
210031_at	*CD247*	0,0347575	5,24777	6,92316
217022_s_at	*/ IGHA1 /// IGHA2 /// LOC10*	0,0350888	7,42768	9,09319
232027_at	*SYNE1*	0,0410147	3,42506	5,07649
1562529_s_at	*NA*	0,0239225	3,93942	5,57993
213843_x_at	*SLC6A8*	0,0357347	4,82005	6,46019
204172_at	*CPOX*	0,0139635	5,79429	7,43358
220528_at	*VNN3*	0,0292523	4,7902	6,40804
207329_at	*MMP8*	0,00227517	6,4066	8,02135
216398_at	*GYPB*	0,0168644	3,68309	5,29766
204720_s_at	*DNAJC6*	0,00282506	4,35191	5,94115
213958_at	*CD6*	0,0424063	6,1758	7,74153
213539_at	*CD3D*	0,0453017	6,94453	8,49764
217591_at	*NA*	0,0464676	5,16333	6,70367
210915_x_at	*TRBC1*	0,0292279	6,80188	8,30995
204891_s_at	*LCK*	0,0157949	5,44306	6,94853
207854_at	*GYPE*	0,0340396	3,67857	5,17997
224496_s_at	*TMEM107*	0,0397747	5,62209	7,11944
231688_at	*MMP8*	0,0176629	7,81069	9,30078
244235_at	*IVNS1ABP*	0,0491204	3,65916	5,13638
211796_s_at	*TRBC1*	0,0265871	7,02189	8,4579
214677_x_at	*CYAT1 /// IGLV1-44*	0,00712254	8,91478	10,3452
209138_x_at	*IGL@*	0,00483327	8,44474	9,86714
233369_at	*NA*	0,00289498	6,37384	7,78853
236081_at	*SNCA*	0,00775485	3,88327	5,27777
206146_s_at	*RHAG*	0,0166621	4,76919	6,14411
215121_x_at	*CYAT1 /// IGLV1-44*	0,0121164	8,32313	9,64594
1554334_a_at	*DNAJA4*	0,00774629	5,94381	7,26068
218456_at	*CAPRIN2*	0,039328	5,64818	6,95692
232030_at	*KIAA1632*	0,0204249	3,99489	5,24056
1556499_s_at	*COL1A1*	0,0197153	2,49038	3,71288
205922_at	*VNN2*	0,0175127	6,48432	7,70499
223754_at	*C2orf88*	0,033031	5,26122	6,47162
238595_at	*NA*	0,0454579	4,49003	5,69912
210215_at	*TFR2*	0,00721659	5,87709	7,07802
1569652_at	*MLLT3*	0,00233691	3,03352	4,22257
225878_at	*KIF1B*	0,00583744	5,01807	6,18945
243509_at	*NA*	0,0347147	6,36092	7,45432
201362_at	*IVNS1ABP*	0,0408222	6,59815	7,67
239946_at	*NA*	0,0225537	5,30932	6,37098
210423_s_at	*SLC11A1*	0,0179046	6,256	7,28968
202388_at	*RGS2*	0,0330438	9,66241	10,6261
205681_at	*BCL2A1*	0,028085	9,53485	10,4472
238079_at	*TPM3*	0,0100257	3,45395	4,3295
242197_x_at	*CD36*	0,0014076	3,2754	4,10955
211806_s_at	*KCNJ15*	0,0124363	4,78845	5,59701
209369_at	*ANXA3*	0,00937685	7,7074	8,47943
204470_at	*CXCL1*	0,0410398	5,21987	5,86308
222934_s_at	*CLEC4E*	0,0375116	6,35133	6,95162
228378_at	*C12orf29*	0,0300768	6,87786	7,43988
206917_at	*GNA13*	0,0294782	5,26721	5,73932
204917_s_at	*MLLT3*	0,034959	4,5419	4,98536
230760_at	*ZFY*	0,0171075	4,6477	5,07505
223539_s_at	*SERF1A /// SERF1B*	0,0318013	7,24979	6,74637
214450_at	*CTSW*	0,0187014	10,0467	9,53788

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224763_at	*RPL37*	0,0102577	6,98522	6,41766
201559_s_at	*CLIC4*	0,0253322	5,34821	4,75998
233011_at	*ANXA1*	0,0414361	4,43069	3,84081
234394_at	*ZNF124*	0,0442278	3,96507	3,36808
205624_at	*CPA3*	0,0271575	8,47873	7,82363
239791_at	*LOC404266*	0,00243211	5,97584	5,30611
209905_at	*HOXA9*	0,026299	8,98919	8,31694
204049_s_at	*PHACTR2*	0,0383177	7,11691	6,41069
203647_s_at	*FDX1*	0,0196463	8,99046	8,2454
208740_at	*SAP18*	0,00157359	7,95218	7,20031
214651_s_at	*HOXA9*	0,0188247	10,0928	9,33843
224391_s_at	*SIAE*	0,0147845	5,15656	4,40162
221004_s_at	*ITM2C*	0,0238278	9,20718	8,44211
238365_s_at	*C1orf228*	0,0262278	8,71385	7,94138
222457_s_at	*LIMA1*	0,0309434	4,44797	3,63662
1562230_at	*NA*	0,0294703	4,48261	3,67086
233970_s_at	*TRMT6*	0,00201982	6,88525	6,03743
239963_at	*NA*	0,00991411	5,96676	5,04567
1560112_at	*WDFY2*	0,0451594	4,56402	3,62757
223457_at	*COPG2*	0,0404076	5,7392	4,78963
227556_at	*NME7*	0,0247774	4,75044	3,76174
210084_x_at	*TPSAB1*	0,0438335	6,62758	5,63861
230348_at	*LATS2*	0,0134483	6,82714	5,81581
236395_at	*NA*	0,0326183	5,24675	4,23018
235385_at	*MARCH1*	0,0306578	4,79051	3,76934
227792_at	*ITPRIPL2*	0,0298533	5,76496	4,7198
213668_s_at	*SOX4*	0,0241398	7,01683	5,9687
239580_at	*GUCY1A3*	0,0442871	4,66419	3,58754
201417_at	*SOX4*	0,00933044	9,44863	8,35143
230177_at	*GTF2H2B*	0,0213913	7,2341	6,09993
213537_at	*HLA-DPA1*	0,01126	7,94376	6,79794
224535_s_at	*MRP63*	0,0198997	7,77508	6,61691
207134_x_at	*TPSB2*	0,0299677	7,11309	5,92399
242260_at	*MATR3*	0,0127137	5,0266	3,83381
227860_at	*CPXM1*	0,000758343	8,6512	7,44653
211031_s_at	*CLIP2*	0,00750869	6,60448	5,39332
208325_s_at	*AKAP13*	0,0201288	6,84593	5,62406
223075_s_at	*AIF1L*	0,0425693	5,39472	4,17114
242471_at	*NA*	0,000276153	5,69468	4,46643
229380_at	*NA*	0,0391229	4,54625	3,30217
242520_s_at	*C1orf228*	0,00804156	7,37573	6,12961
221942_s_at	*GUCY1A3*	0,0115077	6,87498	5,61117
1558508_a_at	*C1orf53*	0,0459934	4,35754	3,089
204875_s_at	*GMDS*	0,014265	7,41101	6,1302
200890_s_at	*SSR1*	0,0310792	7,49214	6,20406
235753_at	*HOXA7*	0,0232557	7,46356	6,16513
218700_s_at	*RAB7L1*	0,00673104	5,42614	4,12396
235287_at	*CDK6*	0,00853707	5,22096	3,90391
207610_s_at	*EMR2*	0,0410016	6,54959	5,23206
235900_at	*SPNS3*	0,0263585	8,32038	6,99864
203139_at	*DAPK1*	0,0132484	7,32523	5,99487
214895_s_at	*ADAM10*	0,0368001	5,97296	4,57709
242794_at	*MAML3*	0,00886397	5,88643	4,48962
224839_s_at	*GPT2*	0,0382918	6,21508	4,81741
206272_at	*RAB4A /// SPHAR*	0,0197386	6,8711	5,46739
202068_s_at	*LDLR*	0,00616243	8,18854	6,76213
228408_s_at	*SDAD1*	0,0191732	6,78387	5,35055
223888_s_at	*LARS*	0,00586224	6,97908	5,54425
243000_at	*CDK6*	0,0278207	6,64788	5,18381
207836_s_at	*RBPMS*	0,0476944	5,65406	4,15995
202742_s_at	*PRKACB*	0,0293335	8,04061	6,53679
217974_at	*TM7SF3*	0,0156261	6,81356	5,30129
217853_at	*TNS3*	0,0109287	7,31077	5,73168
230866_at	*CYSLTR1*	0,0149026	6,56454	4,95852
229530_at	*GUCY1A3*	0,0301617	5,78178	4,14376
1555905_a_at	*C3orf23*	0,0406474	5,50067	3,83403
205608_s_at	*ANGPT1*	0,0156816	6,793	5,0535
219789_at	*NPR3*	0,0376205	5,79092	4,03919
209982_s_at	*NRXN2*	0,0262859	5,81351	4,06119
203920_at	*NR1H3*	0,00188873	6,40953	4,64698
214545_s_at	*PROSC*	0,0420814	5,81785	4,0227
243092_at	*LOC100288730*	0,0430186	6,47953	4,6273

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235391_at	*FAM92A1*	0,0256298	7,12851	5,26422
229309_at	*ADRB1*	0,0264561	4,63316	2,71805
1554876_a_at	*S100Z*	0,0264327	6,85447	4,93395
239237_at	*NA*	0,0148718	7,59952	5,55023
201324_at	*EMP1*	0,0406934	6,7641	4,70622
200762_at	*DPYSL2*	0,0167982	7,8256	5,76184
205609_at	*ANGPT1*	0,00192015	6,24972	4,10937
204030_s_at	*SCHIP1*	0,0375821	8,44707	6,26091
225285_at	*BCAT1*	0,0267124	8,14755	5,90159
226517_at	*BCAT1*	0,0223213	8,05383	5,5332

B

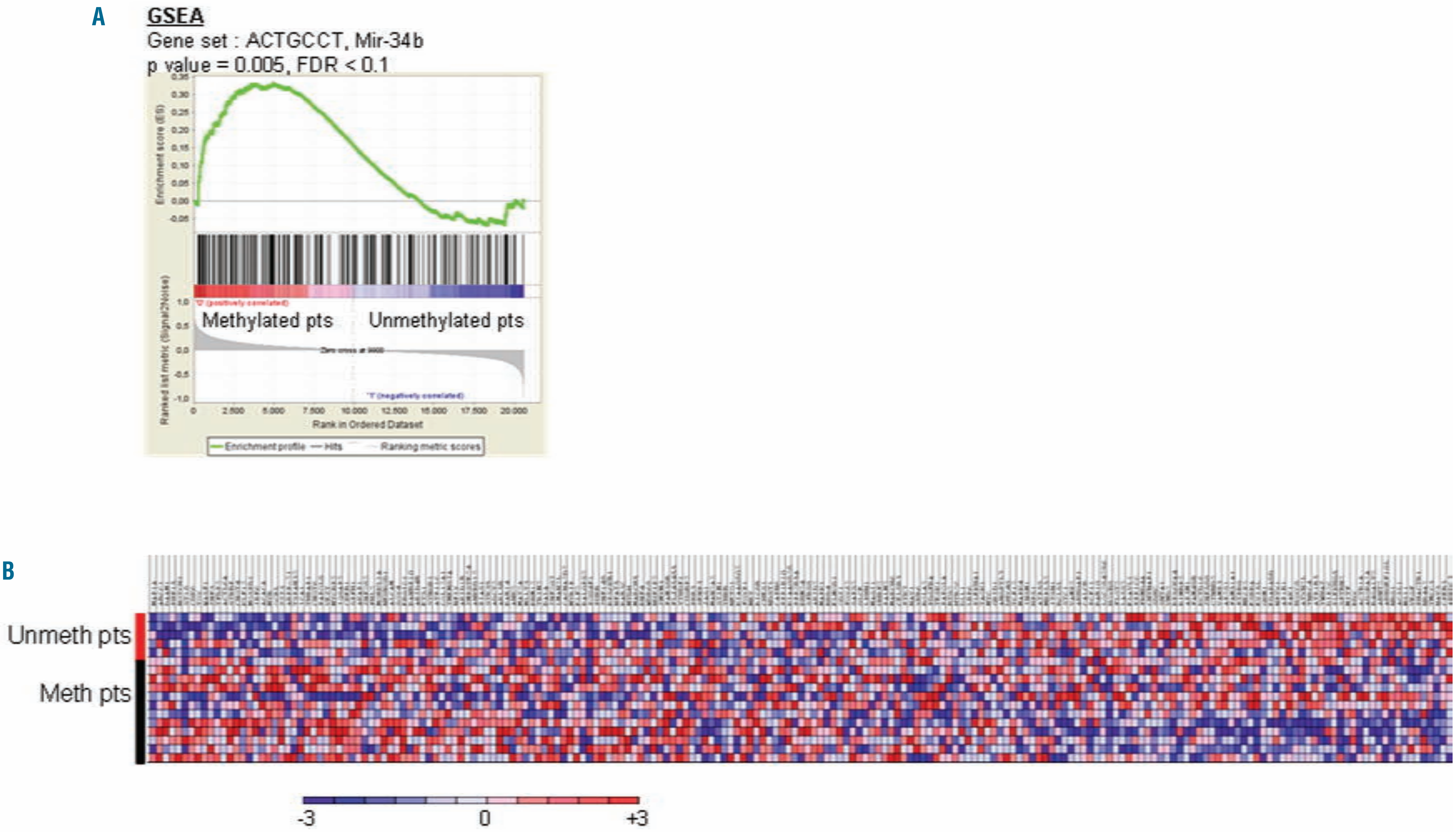
103 CREB Target Genes differently expressed in paired MDS and AML evolution patients

ADAM10
ADD2
ADRB1
AK1
AKAP13
AMMECR1
ANGPT1
ANK1
ANXA1
ANXA3
AQP1
BCAT1
BCL11B
BCL2A1
BLVRB
CA1
CCR7
CD2
CD36
CD3D
CD6
CD8A
CDH1
CDK6
COL1A1
COPG2
CPA3
CTSW
CYP4F3
DAPK1
DNAJA4
DNAJC6
DPYSL2
EMP1
EMR2
EPB42
FDX1
FECH
GATA3
GMDS
GNA13
GPT2
GUCY1A3
GYPB
GYPE
HMBS
HOXA7
IL7R
ITK
KCNH2
KCNJ15
KIF1B
KLF1
KLRB1
LATS2
LCK
LCN2
LDLR
LEF1
LTF
MGAM
MINPP1
MLLT3
MMP8
MMP9
MRP63
MYL4
NELL2
NME7
NPR3
NR1H3
NRXN2
PRKACB
PROSC
RAB7L1
RASGRP1
RBPMS
RGS2
RHAG
RHCE
RHD
RORA
RPL37
S100A12
S100Z
SAP18
SCHIP1
SCML1
SELENBP1
SLC11A1
SLC4A1
SLC6A8
SNCA
SOX4
SPTA1
TFR2
TPSB2
TUBB1
VNN2
VNN3
XK
ZFY
ZNF124

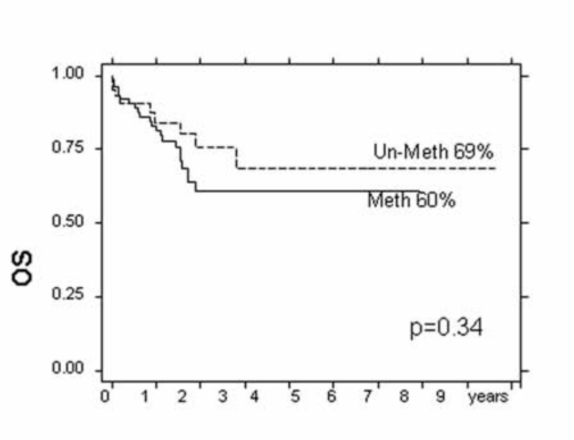
C

Common CREB target genes involved in myeloid transformation

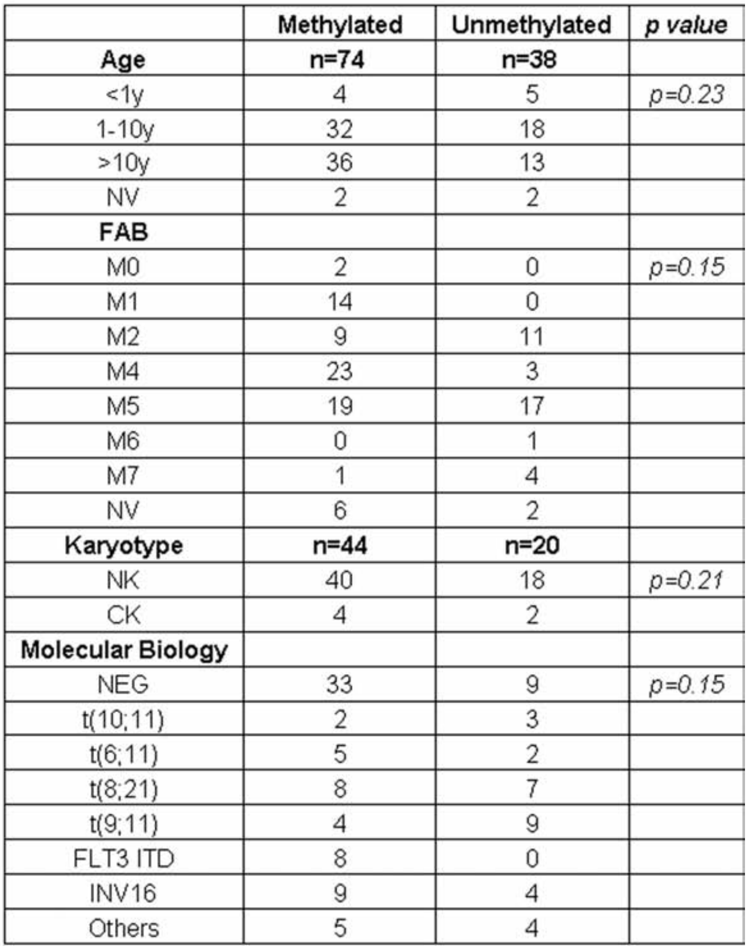
- *ADD2*
- *ADRB1*
- *AMMECR1*
- *ANGPT1*
- *ANK1*
- *ANXA1*
- *ANXA3*
- *AQP1*
- *BCAT1*
- *BCL11B*
- *BCL2A1*
- *BLVRB*
- *CA1*
- *CD2*
- *CD36*
- *CDH1*
- *CDK6*
- *COL1A1*
- *CPA3*
- *CTSW*
- *CYP4F3*
- *DNAJA4*
- *DNAJC6*
- *EMP1*
- *EPB42*
- *FECH*
- *GATA3*
- *GMDS*
- *GUCY1A3*
- *GYPB*
- *HOXA7*
- *IL7R*
- *ITK*
- *KCNH2*
- *KCNJ15*
- *KIF1B*
- *KLF1*
- *KLRB1*
- *LCK*
- *LCN2*
- *LDLR*
- *LEF1*
- *LTF*
- *MGAM*
- *MINPP1*
- *MLLT3*
- *MMP8*
- *MMP9*
- *MYL4*
- *NME7*
- *NPR3*
- *NR1H3*
- *NRXN2*
- *PRKACB*
- *PROSC*
- *RAB7L1*
- *RASGRP1*
- *RBPMS*
- *RGS2*
- *RHAG*
- *RHCE*
- *RHD*
- *RORA*
- *S100A12*
- *S100Z*
- *SCML1*
- *SELENBP1*
- *SLC11A1*
- *SLC4A1*
- *SNCA*
- *SOX4*
- *SPTA1*
- *TPSB2*
- *TUBB1*
- *VNN2*
- *VNN3*
- *XK*
- *ZFY*



Online Supplementary Figure S1. (A) Gene set enrichment analysis (GSEA) in AML patients (pts) with methylated or unmethylated miR-34b promoter. Positive enrichment of the genes containing the ACTGCCT motif (miR-34b consensus region) in patients with miR-34b hypermethylated promoter (*P*=0.005, false discovery < 0.1). (B) The heat map of the enrichment genes in methylated and unmethylated miR-34b promoters in AML patients (pts) containing the ACTGCCT motif (miR-34b consensus region). The columns in the heatmap show the genes and the rows each individual sample. The bar on the left is depicted in red for the unmethylated patients (n=15), and in black for the methylated ones (n=5). The scale bar shows the color-coded differential expression from the mean in standard deviation units, with red indicating higher expression and blue lower expression.



Online Supplementary Figure S2. Methylation impact on survival of 112 AML patients at diagnosis. Kaplan-Meier overall survival (OS) curves of AML patients with methylated miR-34b promoter (n= 74, thick line) and unmethylated miR-34b promoter (n= 38, broken line) determined by MS-PCR. AML patients with unmethylated promoter have a better overall survival (69%) compared to methylated (60%) patients (*P*=0.34). The table shows the main biological and clinical features of AML patients enrolled in the study. Multivariate analyses along with AML clinical and biological features such as FAB-morphology, karyotype (normal or complex if with more than three cytogenetic aberrations), age at diagnosis (< 1 year; > 1 year < 10 years; > 10 years) and recurrent genetic abnormalities [such as t(8;21)AML1-ETO, inv(16)CBFB-MYH11, t(9;11)MLL-AF9, t(10;11)MLL-AF10, FLT3ITD, t(6;11)MLL-AF6] did not show significant results.



	Methylated	Unmethylated	*p value*
Age	**n=74**	**n=38**	
<1y	4	5	*p=0.23*
1-10y	32	18	
>10y	36	13	
NV	2	2	
FAB			
M0	2	0	*p=0.15*
M1	14	0	
M2	9	11	
M4	23	3	
M5	19	17	
M6	0	1	
M7	1	4	
NV	6	2	
Karyotype	**n=44**	**n=20**	
NK	40	18	*p=0.21*
CK	4	2	
Molecular Biology			
NEG	33	9	*p=0.15*
t(10;11)	2	3	
t(6;11)	5	2	
t(8;21)	8	7	
t(9;11)	4	9	
FLT3 ITD	8	0	
INV16	9	4	
Others	5	4	

y: year; NV: not evaluable; FAB: French–American–British; NK: normal karyotype; CK: complex karyotype; NEG: negative for common molecular biology markers.