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Supplemental Material to:

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The epigenome of AML stem and progenitor cells

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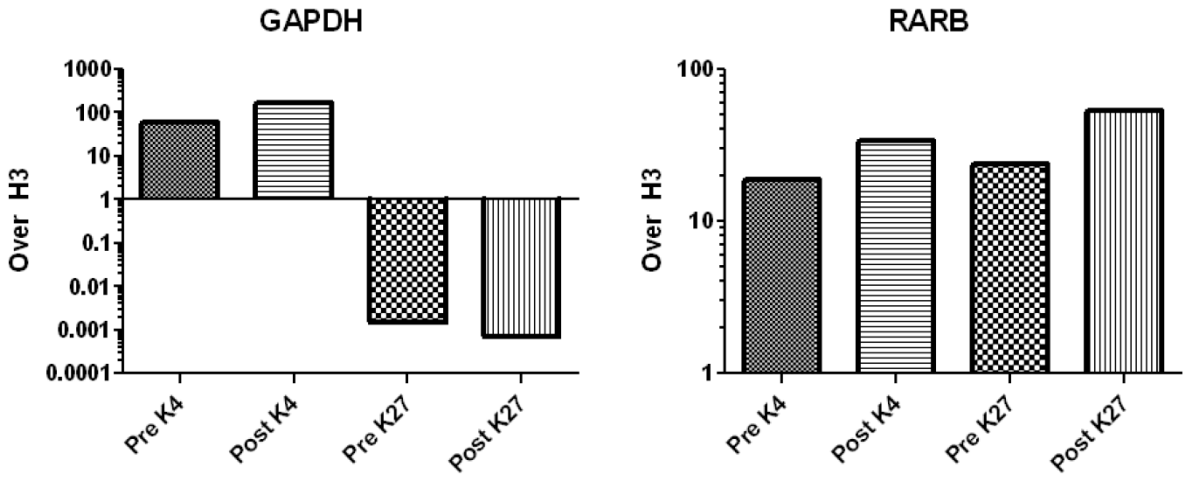
Supplemental Figure 1

Confirmation of library preparation with small numbers of cells for ChIP-seq. qPCR was performed for GAPDH and RARB for their enrichment of K4 and K27 before (Pre) and after (Post) library preparation. Representative result from P10 is shown.

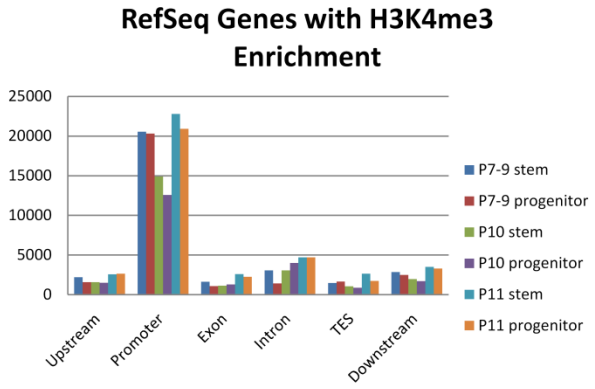


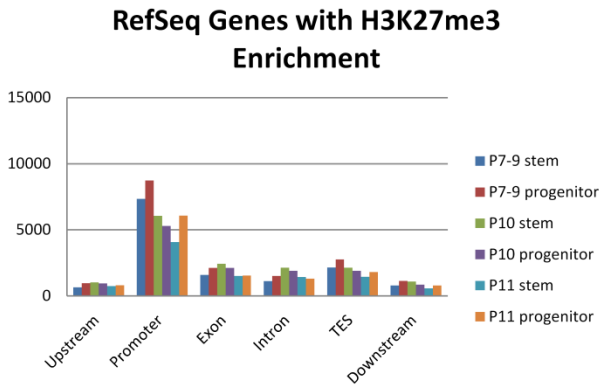
Supplemental Figure 2

The number of peaks for K4/K27 marks according to their locations. Note that peaks generated from these tags revealed that K4 is concentrated at promoter regions whereas K27 is broadly distributed from transcription start sites to transcription end sites covering the entire genes.

# of RefSeq Genes with H3K4me3/H3K27me3 Enrichment at Specific Locations across the Genome

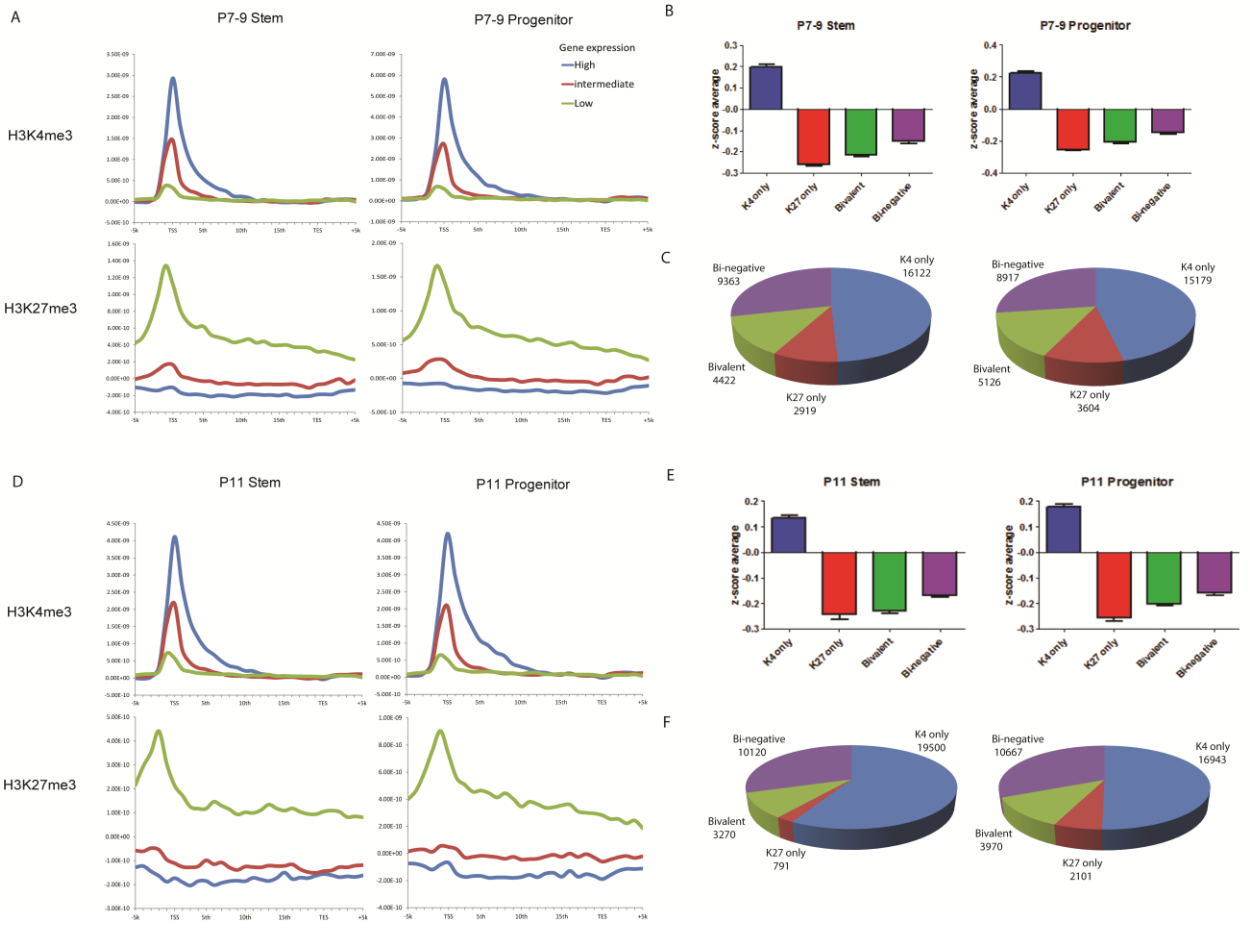
# of RefSeq Genes	Peak type	Upstream	Promoter	Exon	Intron	TES	Downstream
P7-9 stem	H3K4me3	2204	20546	1610	3048	1465	2843
P7-9 progenitor		1560	20312	1073	1399	1652	2480
P10 stem		1555	14891	1128	3056	1033	1967
P10 progenitor		1482	12562	1270	3979	856	1690
P11 stem		2548	22770	2586	4703	2629	3497
P11 progenitor		2633	20913	2235	4692	1731	3281
P7-9 stem	H3K27me3	636	7341	1584	1119	2146	783
P7-9 progenitor		956	8730	2109	1499	2760	1133
P10 stem		1013	6050	2419	2132	2127	1081
P10 progenitor		939	5294	2117	1895	1894	848
P11 stem		742	4061	1497	1432	1434	563
P11 progenitor		797	6071	1532	1297	1806	781





Supplemental Figure 3

Histone mark enrichment by gene expression patterns in P7-9 (A) and P11 (D). Gene expression patterns were divided into high (top 2000 genes), intermediate (medium 2000 genes), and low (bottom 2000 genes). Categories of promoter histone marks (K4 only, K27 only, bivalent and none) and associated gene expression levels in P7-9 (B) and P11 (E). (C) Numbers of the genes with respective histone status in P7-9 (C) and P11 (F). Gene expression data was taken from GEO data set GSE24006.

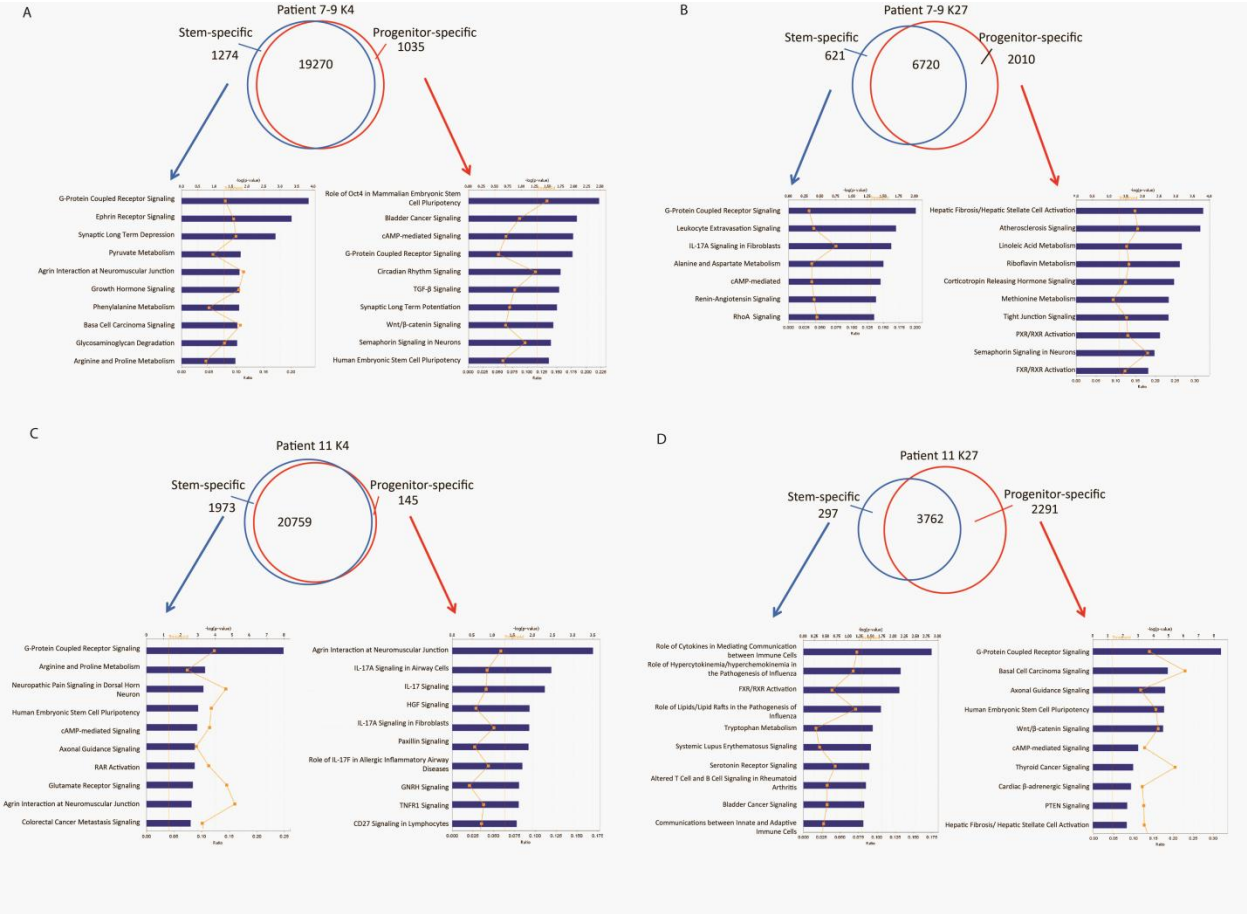


Supplemental Figure 4

Pathway analyses from the gene enriched with either K4 or K27 in stem (Left) and progenitor (Right) in P10 (A), P7-9 (B), and P11 (C). . Ingenuity pathway analysis was performed with the exclusively enriched genes in stem or progenitor cells. Bars show p-values in –log (p-value) for each pathway. Rectangles on each bar show ratio calculated by the number of molecules in a given pathway divided by total number of molecules that make up that pathway. Pathways are listed in order of rank from the most associated pathway.

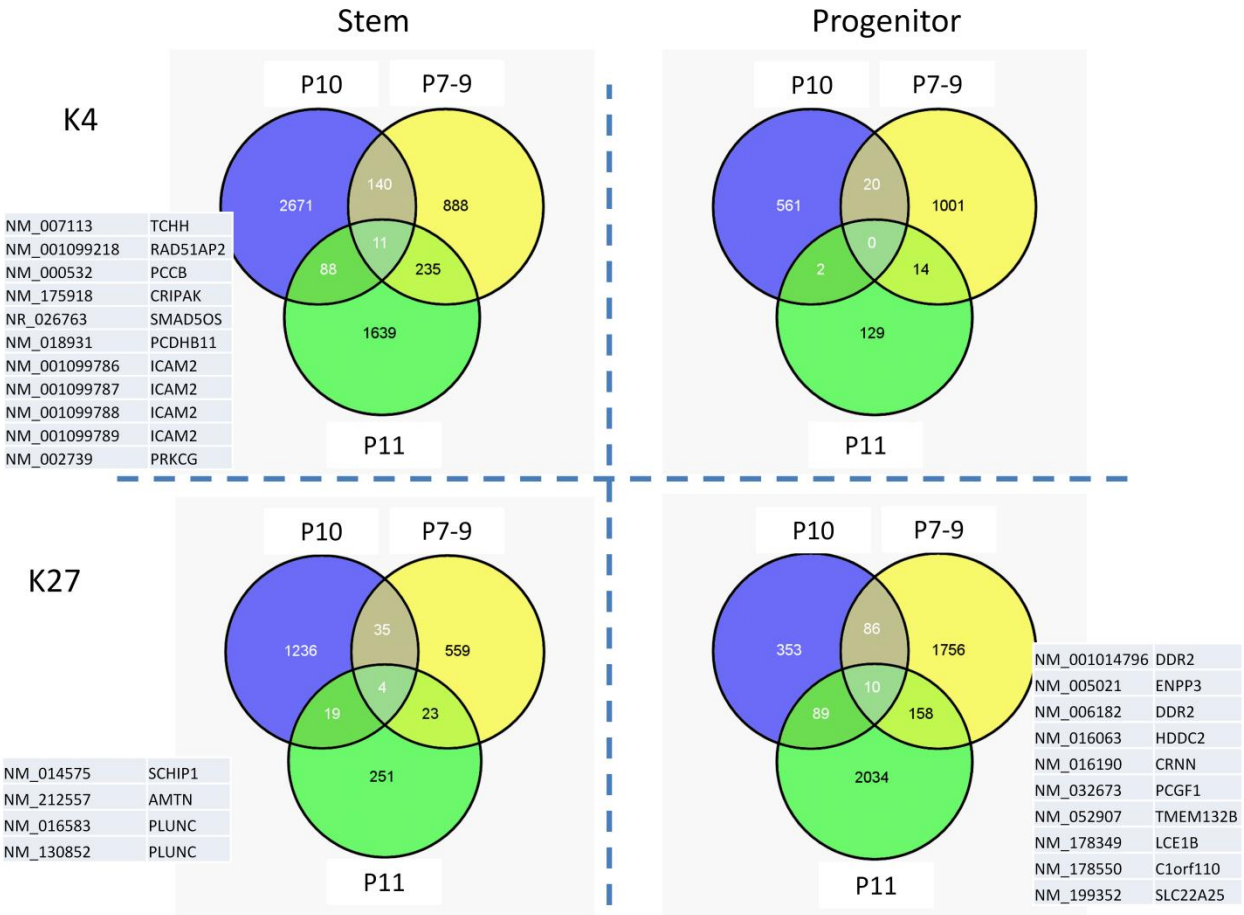
Supplemental Figure 5

Pathway analysis for genes enriched in K4 or K27 in P7-9 (A and B) and P11 (C and D). The Venn diagram show overlap in genes marked by K4 or K27 in their promoters. Ingenuity pathway analysis was performed with the exclusively enriched genes in stem or progenitor cells. Bars show p-values in –log (p-value) for each pathway. Rectangles on each bar show ratio calculated by the number of molecules in a given pathway divided by total number of molecules that make up that pathway. Pathways are listed in order of rank from the most associated pathway.



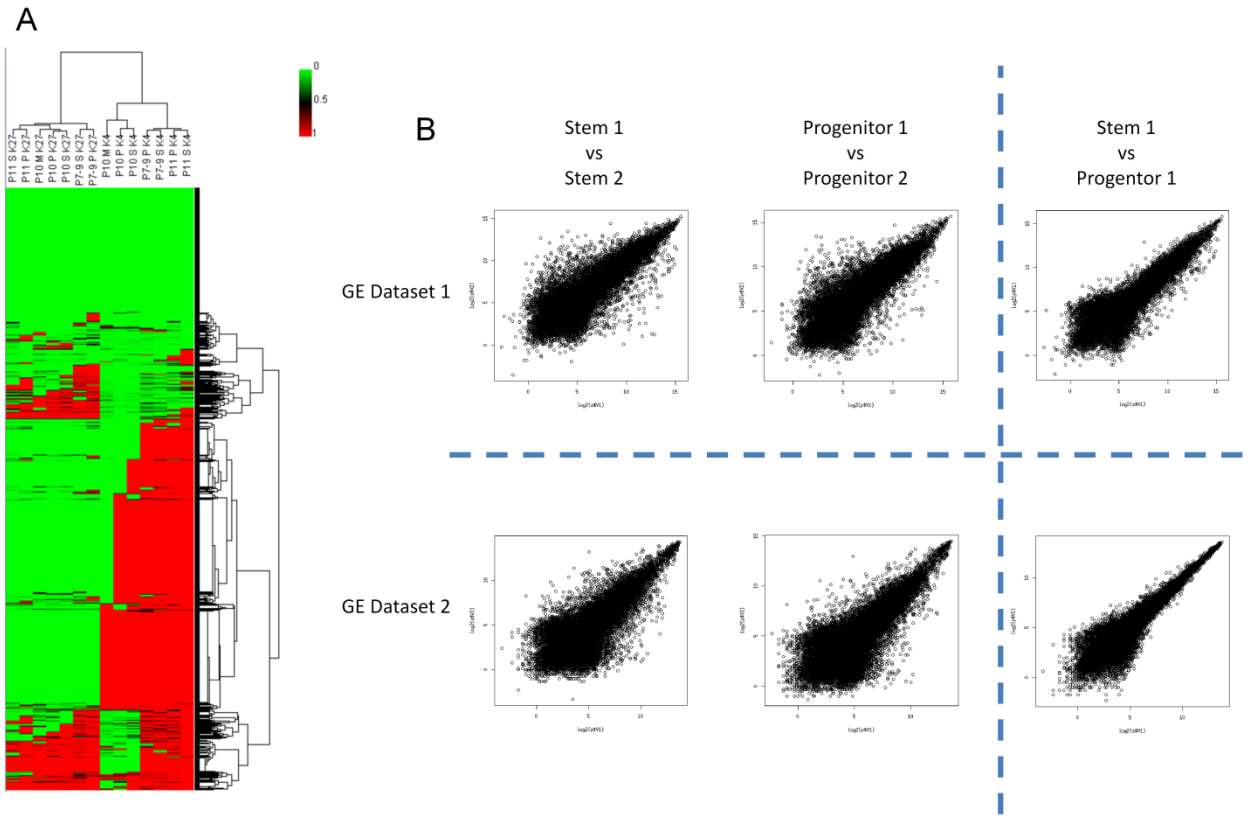
Supplemental Figure 6

Differentially marked genes with K4/K27 in stem or progenitor cells common in P10, P11, and P7-9. Refseq numbers and gene names are shown in each corner.



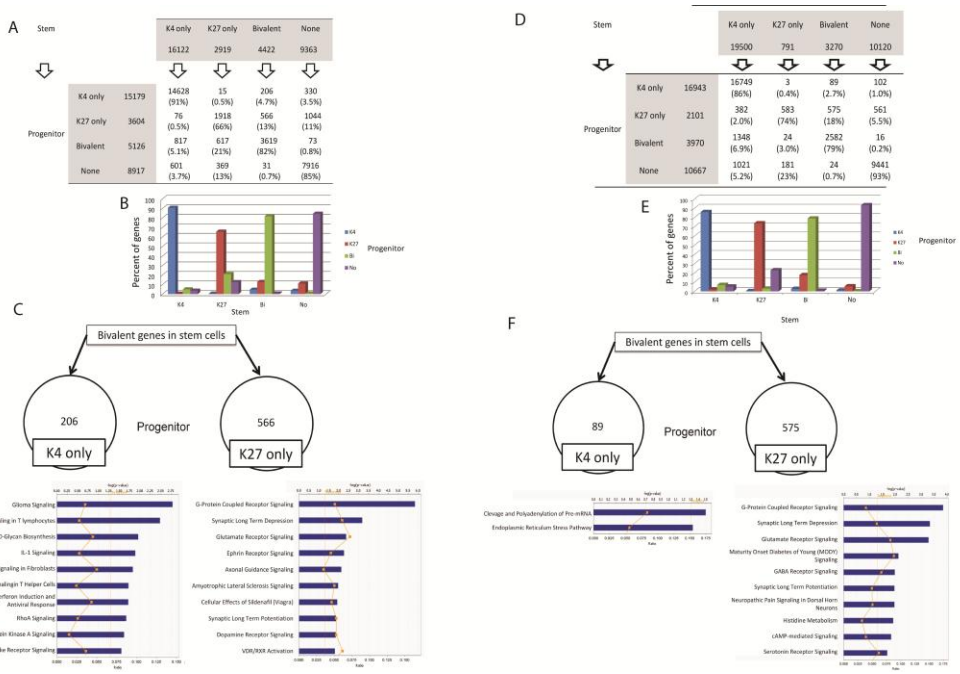
Supplemental Figure 7

(A) Unsupervised hierarchical analysis for K4/K27 marks with samples analyzed in ChIP-seq. Note that the stem and progenitor profiles clustered intra-patient rather than by cell subtype. (B) Representative correlations in gene expression between different patients of the same cell type (Left; Stem 1 vs Stem2 and Progenitor 1 versus Progenitor 2) and correlation between stem and progenitor in the same patient (Right; Stem 1 versus Progenitor 1). These analyses were done in two separate gene expression datasets (GE Dataset 1 and 2; GEO Accession No. GSE24006 and 34044, respectively)



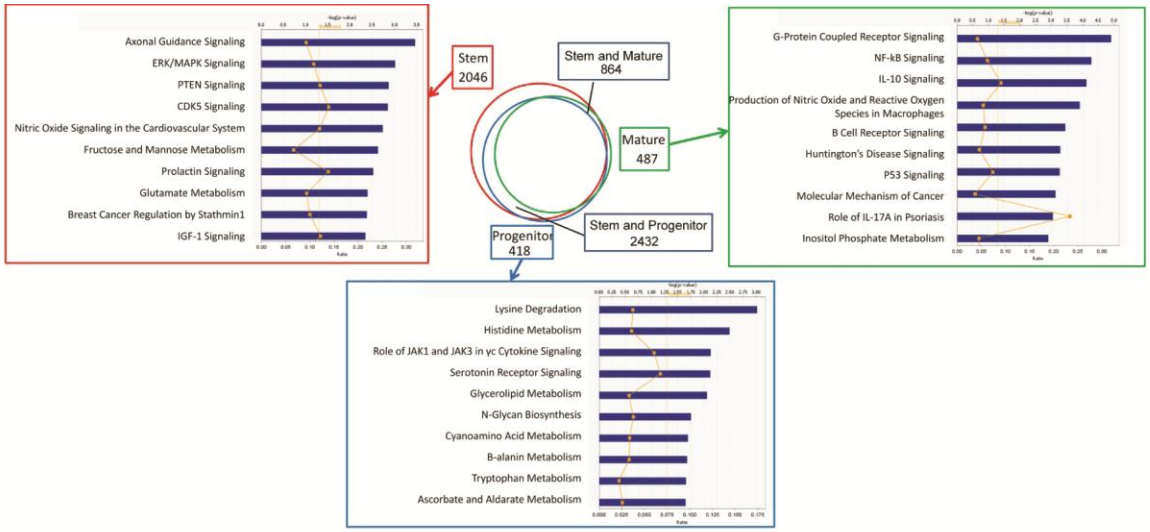
Supplemental Figure 8

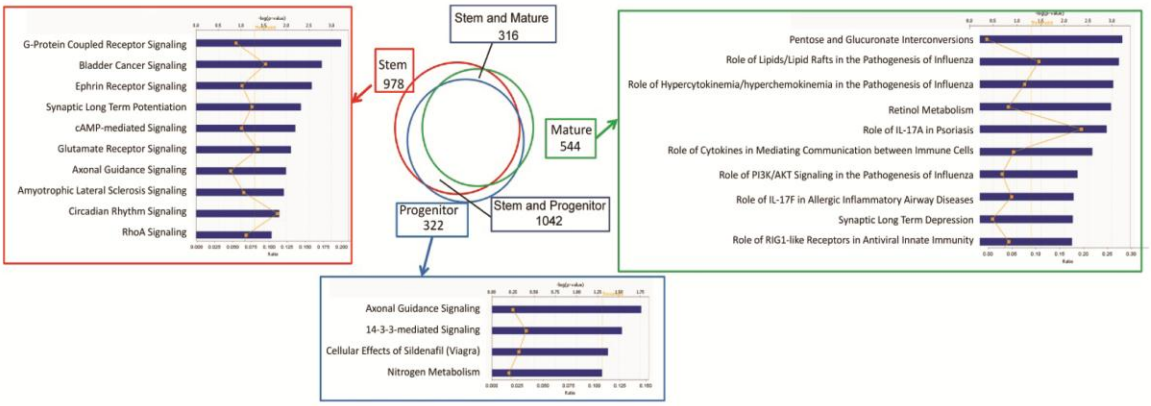
Histone switches in AML stem versus progenitor cells. (A and D) Number of genes in each histone category in stem and progenitor AML cells. Arrows point to the histone status in progenitor cells by baseline histone status in stem cells in P7-9 (A) and P11 (D). (B and E) Graphical representation of data shown in (B and E). (C and F) Pathway analysis for the genes that are bivalent in stem cells and switch to K4 only or K27 only in progenitor cells in P7-9 (C) and P11 (F).



Supplemental Figure 9

Pathway analysis from the gene enriched with K4 (Upper) or K27 (Lower) marks in P10. The numbers of the genes enriched only in stem cells (red), in progenitor cells (blue), and in mature cells (green). Pathway analyses were performed with the exclusively enriched genes each population.





Supplemental Table 1. Patient samples studied.

Patient	Stem (CD34+CD38-)	Progenitor (CD34+CD38+)	Mature (CD34-)	Source	Blasts
Patient 1	MCAM&Pyro	MCAM&Pyro	Pyro	PB	91%
Patient 2	MCAM&Pyro	MCAM&Pyro	N/A	PB	70%
Patient 3	MCAM&Pyro	MCAM&Pyro	N/A	PB	72%
Patient 4	MCAM&Pyro	MCAM&Pyro	Pyro	PB	85%
Patient 5	Pyro	Pyro	Pyro	BM	94%
Patient 6	Pyro	Pyro	Pyro	PB	91%
Patient 7	ChIP-seq	ChIP-seq	N/A	BM	53%
Patient 8				BM	95%
Patient 9				BM	43%
Patient 10	ChIP-seq	ChIP-seq	N/A	BM	86%
Patient 11	ChIP-seq	ChIP-seq	N/A	BM	94%

Pyro; Bisulfite-pyrosequencing, MCAM; Methylated CpG island Amplification followed by Microarray, PB; peripheral blood, BM; bone marrow

Supplemental Table 2. Primer sequences PCR conditions

Gene	Step	Annealing Temp. (°C)	Sequence 5’ to 3’	5’-modified
OCT4	1	53	GGGTTAGAGGTTAAGGTTAGTG AAATCCCAAACCAAATATC	
	2	54	GGGTTAGAGGTTAAGGTTAGTG GGGACACCGCTGATCGTTTAAAATCCCAAACCAAATATC GGGACACCGCTGATCGTTTA	Biotin
	S		GTAAGTTTTTATTTTATTAGG	
SOX2	1	53	GGGAGGTTTAGTTTTTTTTTATGTAAAATT CCAAAACTATCAAAAAATAAATAAATTTCT	
	2	54	GGGAGGTTTAGTTTTTTTTTATGTAAAATT GGGACACCGCTGATCGTTTACCAAAACTATCAAAAAATAAATAAATTTCT GGGACACCGCTGATCGTTTA	Biotin
	S		GGGTTAGAGTGGAGGAGT	
MYC	1	55	GTGGGGGAAAAGAAAAAAGATT CCTCTAAAAAACCCTACCCTTCTC	
	2	56	GTGGGGGAAAAGAAAAAAGATT GGGACACCGCTGATCGTTTACCTCTAAAAAACCCTACCCTTCTC GGGACACCGCTGATCGTTTA	Biotin
	S		TTTAGTTTATAGGTTTTTTATAATG	
HOXB4	1	55	GTGGGGTTAGGGTGAGTAGATTTTT TCCCAAAACCCTCCTACTTACTATC	
	2	56	GTGGGGTTAGGGTGAGTAGATTTTT GGGACACCGCTGATCGTTTATCCCAAAACCCTCCTACTTACTATC GGGACACCGCTGATCGTTTA	Biotin
	S		AGGGGAGGTTATTGGT	
KLF4	1	52	GGGAGGTGTAGTTAGGTGAGATTG CCCCCTCCACACAACTCA	
	2	54	GGGAGGTGTAGTTAGGTGAGATTG GGGACACCGCTGATCGTTTACCCCCTCCACACAACTCA GGGACACCGCTGATCGTTTA	Biotin
	S		GAGGTGTAGTTAGGTGAGATT	
CDH13	1	60	TTTGGGAAGTTGGTTGGTTG ACAACCCCTCTTCCCTACCT	
	2	56	AGTTTGGTTTTTAAGGAAAATATGTTTAGT AACCAAATTCTCCACTACATTTTATCC	Biotin
	S		AGGAAAATATGTTTAGTGTA	
OLIG2	1	53	TTTTAAAGGTGAGGATGTTTATTAT AAAAATCCAAACCCCCTATAT GGGACACCGCTGATCGTTTACTCCCTCCCAAAAACCTCAA	
	2	56	TTTTAAAGGTGAGGATGTTTATTAT GGGACACCGCTGATCGTTTA	Biotin
	S		AGGTGAGGATGTTTATTATA	
PGRB	1	60	TGTGGGTGGTATTTTTAATGAGA CCCCCTCACTAAAACCCTAAA	
	2	56	GAGAATTAGTTTTATTTGTTATTTGAGTGA CAACCCATTCCCAAAAAAAATC	Biotin
	S		GGGATTTGAGATTTT	

PGRA	1	60	GGAGGAGAAAAGGGGAGTTTA AAATCCTATCCCTAACAAAA GGGACACCGCTGATCGTTTAAATCCTATCCCTAACAAAA	
	2	56	ATTGAGTTGAAGGTAAAGGGTTT GGGACACCGCTGATCGTTTA	Biotin
	S		GGATTTTTATTGTTGTGT	
PMF1	1	53	TTTTTTTAGGAGTGGGGAGTT CCATAATATATATATACCACATTAAAACC	
	2	54	TTTTTTTAGGAGTGGGGAGTT GGGACACCGCTGATCGTTTACCATAATATATATATACCACATTAAAACC GGGACACCGCTGATCGTTTA	Biotin
	S		AGTGGGGAGTTGGTTA	
FANK1	1	53	GTTTTATTAGAGGGTAGGTTTAT CCCACTAAAAACACTACTCCT	
	2	54	GTTTTATTAGAGGGTAGGTTTAT GGGACACCGCTGATCGTTTACCCACTAAAAACACTACTCCT GGGACACCGCTGATCGTTTA	Biotin
	S		GGGGTAGTTAGGGAGG	
KLHL34	1	58	TGGGTTTTAAATTGAGTAGGTAGAAATG AAACATCCCCAAACACAACAC	
	2	60	TGGGTTTTAAATTGAGTAGGTAGAAATG GGGACACCGCTGATCGTTTAAAACATCCCCAAACACAACAC GGGACACCGCTGATCGTTTA	Biotin
	S		TTAGGGGTTTTGTGGT	
ALPPL2	1	49	GGAAAAGGTTTAGGTTATGTGTTTAAG TTAACACCCTAATCCCCCTCAA	
	2	50	GGAAAAGGTTTAGGTTATGTGTTTAAG GGGACACCGCTGATCGTTTATTAACACCCTAATCCCCCTCAA GGGACACCGCTGATCGTTTA	Biotin
	S		GGTTTAGGTTATGTGTTTAAG	
RLN3R1	1	58	GAGGAAAAGGATAAAGGTATTTGAAAG CCCCCCATCACTAAAACACTAA	
	2	60	GAGGAAAAGGATAAAGGTATTTGAAAG GGGACACCGCTGATCGTTTACCCCCCATCACTAAAACACTAA GGGACACCGCTGATCGTTTA	Biotin
	S		AAGGATAAAGGTATTTGAAAG	
GMPPA	1	58	GGAGTAAGGAAGGTAAGGAGTTA TACCACCACCTCAAAATCCTC	
	2	60	GGAGTAAGGAAGGTAAGGAGTTA GGGACACCGCTGATCGTTTATACCACCACCTCAAAATCCTC GGGACACCGCTGATCGTTTA	Biotin
	S		AGTTAAGTGAAGGTTTTAAGTAT	
ECE2	1	58	GGTGGAGGGGTGATAGAGAGAT TACCCACCACCCTACCCCAATA	
	2	60	GGTGGAGGGGTGATAGAGAGAT GGGACACCGCTGATCGTTTATACCCACCACCCTACCCCAATA GGGACACCGCTGATCGTTTA	Biotin
	S		AGGGAAGGGTTTTTTTA	

Supplemental Table 3. Number of unique usable tags obtained from ChIP-sequencing

Patient sample	Stem: CD34+CD38-			Progenitor: CD34+CD38+		
	H3	H3K4me3	H3K27me3	H3	H3K4me3	H3K27me3
Patients 7-9	22,604,491	15,838,286	7,428,134	21,956,111	7,128,653	6,675,346
Patient 10	7,733,952	13,582,925	16,134,237	13,198,472	14,289,082	15,454,622
Patient 11	25,501,731	12,213,414	8,189,862	11,307,037	9,124,941	6,363,660