Supplementary Information

Dnmt3a is Essential for Hematopoietic Stem Cell Differentiation

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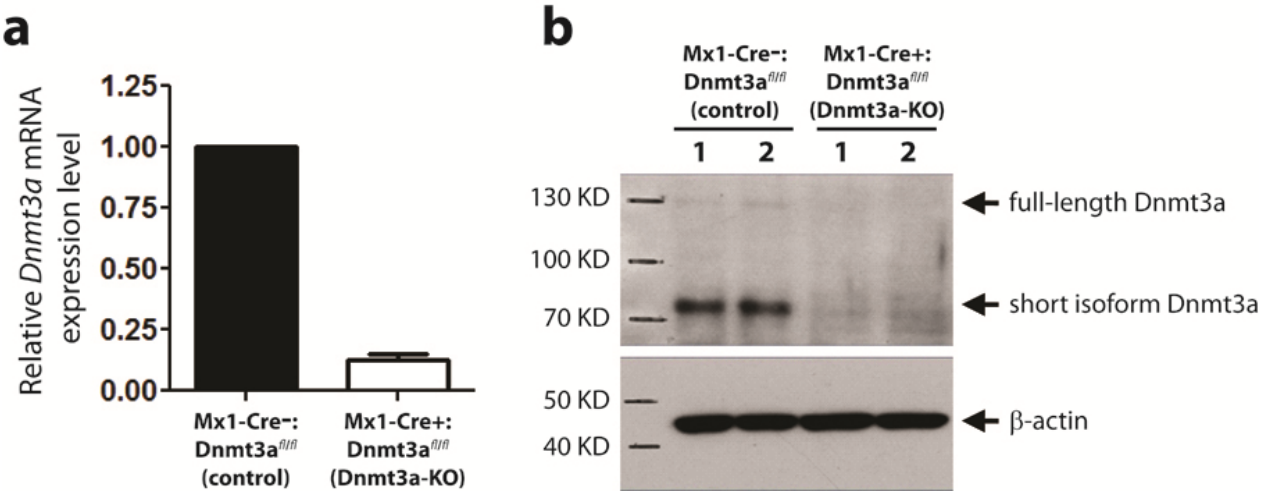
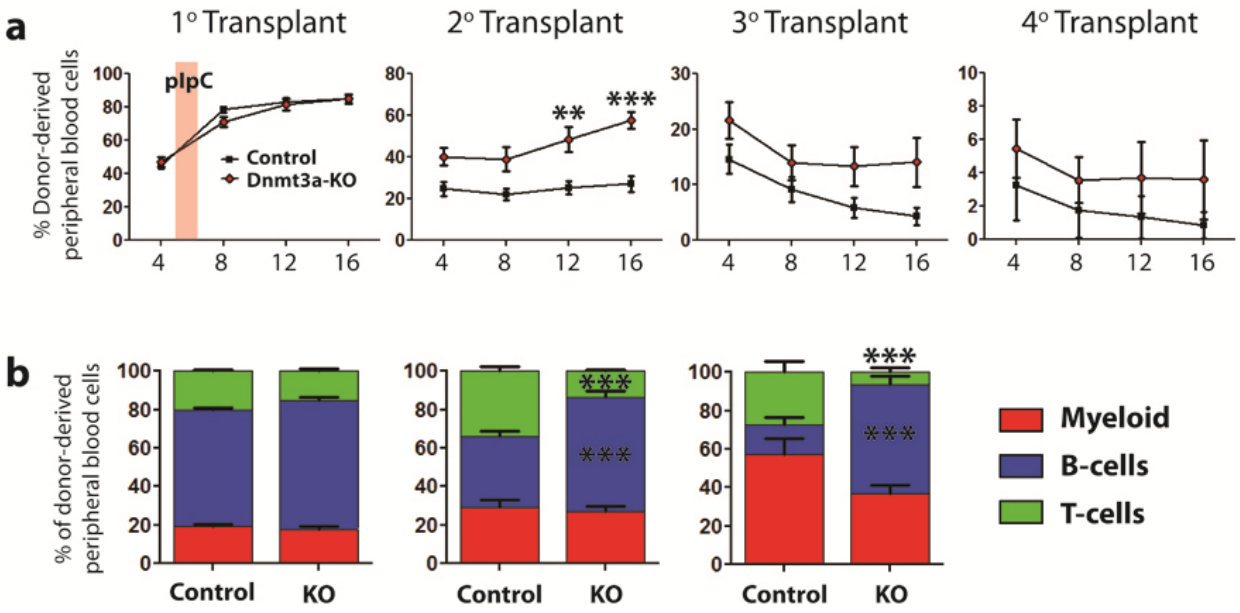
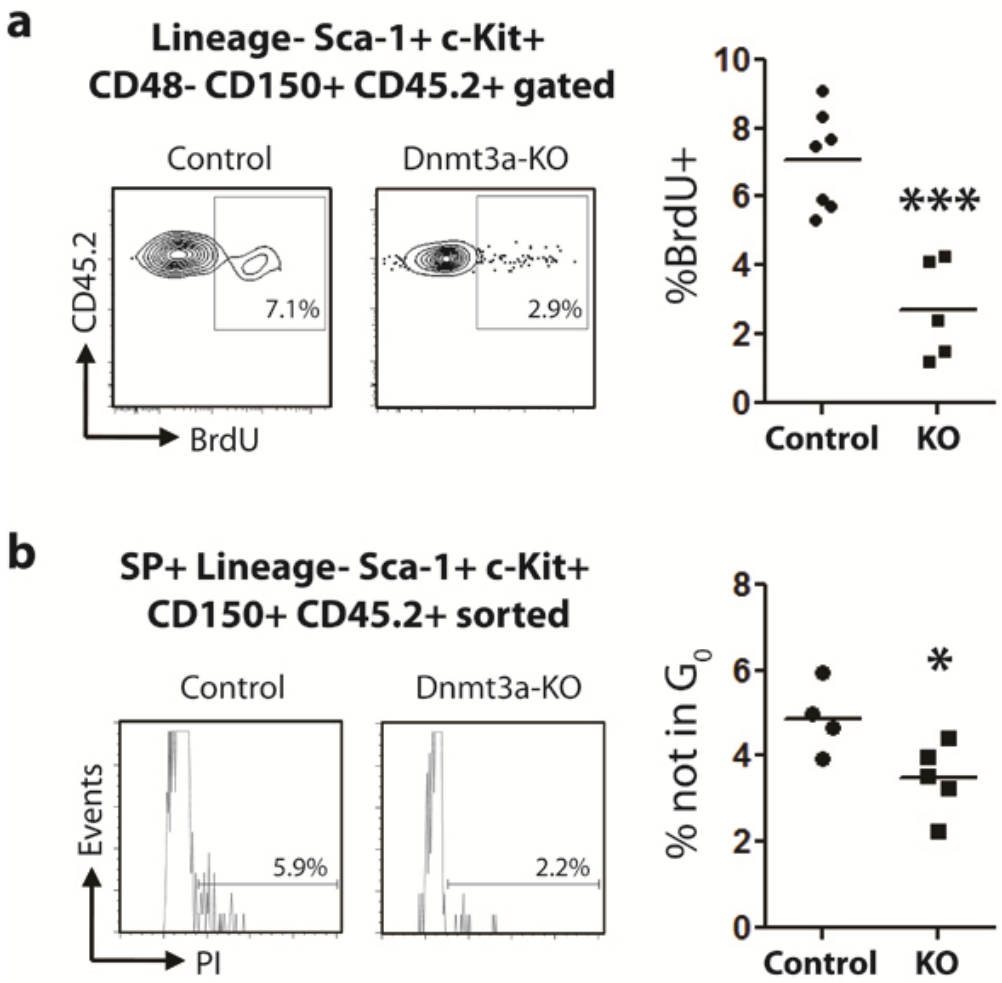


Supplementary Figure 1: Expression of Dnmt3a in donor cells following pIpC treatment. Donor-derived cells were purified from primary transplant recipient mice 12-weeks post-pIpC administration. (**a**) Expression of *Dnmt3a* mRNA in control (Mx1-Cre-:Dnmt3a$^{fl/fl}$) compared to *Dnmt3a*-KO (Mx1-Cre+:Dnmt3a$^{fl/fl}$) in purified HSCs (CD45.2^{+}SP^{KLS}) shows a significant reduction in gene expression following conditional deletion. Mean ± SEM values are shown of three biological replicates. (**b**) Dnmt3a protein levels in donor-derived whole bone marrow cells following pIpC administration shows no full-length or Dnmt3a short isoform expression in *Dnmt3a*-KO cells. The targeted allele used in this study contains the first 18 exons but we could find no evidence for a truncated protein using an N-terminal antibody. The full-length Dnmt3a protein is only weakly expressed in control bone marrow (see Figure 1a for mRNA expression comparison to HSCs), but we could not obtain sufficient HSCs to perform protein quantification.



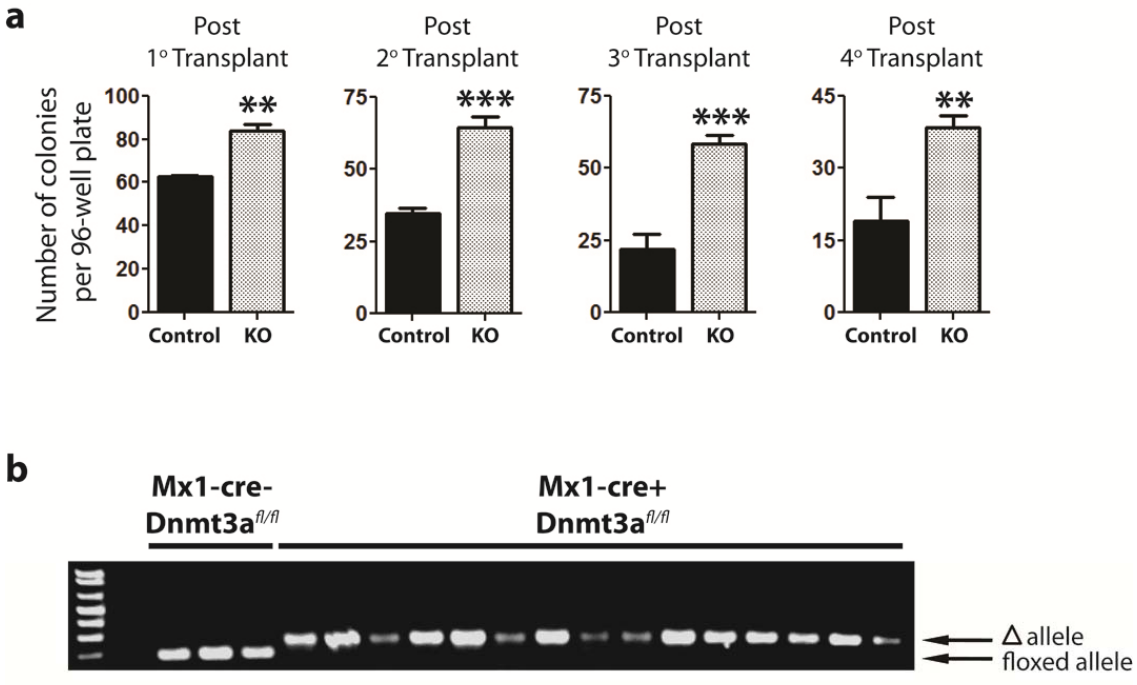
<u>Supplementary Figure 2:</u> Engraftment kinetics and multilineage differentiation of *Dnmt3a*-KO HSCs in primary recipients.

Two-hundred-fifty HSCs were purified from test (Mx1-Cre^{+}:Dnmt3a$^{fl/fl}$) and control (Mx1-Cre^{-}:Dnmt3a$^{fl/fl}$) mice and transplanted into recipients (CD45.1) along with 250x10^{3} WBM competitor cells from C57Bl/6-CD45.1 mice. (**a**) Contribution of test HSCs (CD45.2) to recipient mouse peripheral blood chimerism measured at monthly intervals. Grey rectangle indicates timeframe for injection of pIpC. (**b**) Analysis of HSC lineage differentiation. Shown is the percentage of cells of the indicated lineages within the donor-derived (CD45.2^{+}) peripheral blood cell compartment long-term post-transplant (16-weeks). Myeloid cells (Gr1^{+}or Mac1^{+}), B-cells (B220^{+}), T-cells (CD4^{+} or CD8^{+}).



<u>Supplementary Figure 3:</u> Proliferation and apoptosis analysis of control and *Dnmt3a*-KO HSCs from secondary-transplanted mice.

(**a**) BrdU staining of secondary-transplanted marrow gated KLS / $CD150^+$ / $CD48^-$ / $CD45.2^+$ for donor-derived control and *Dnmt3a*-KO HSCs following 12-hour BrdU exposure. (**b**) Donor-derived HSCs ($SP^{KLS}CD150^+CD45.2^+$) from control and *Dnmt3a*-KO HSC secondary-transplants were purified and subject to propidium iodide staining for cell cycle analysis. Gated areas show the percentage of HSCs not in G_0. Mean ± SEM values are shown, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

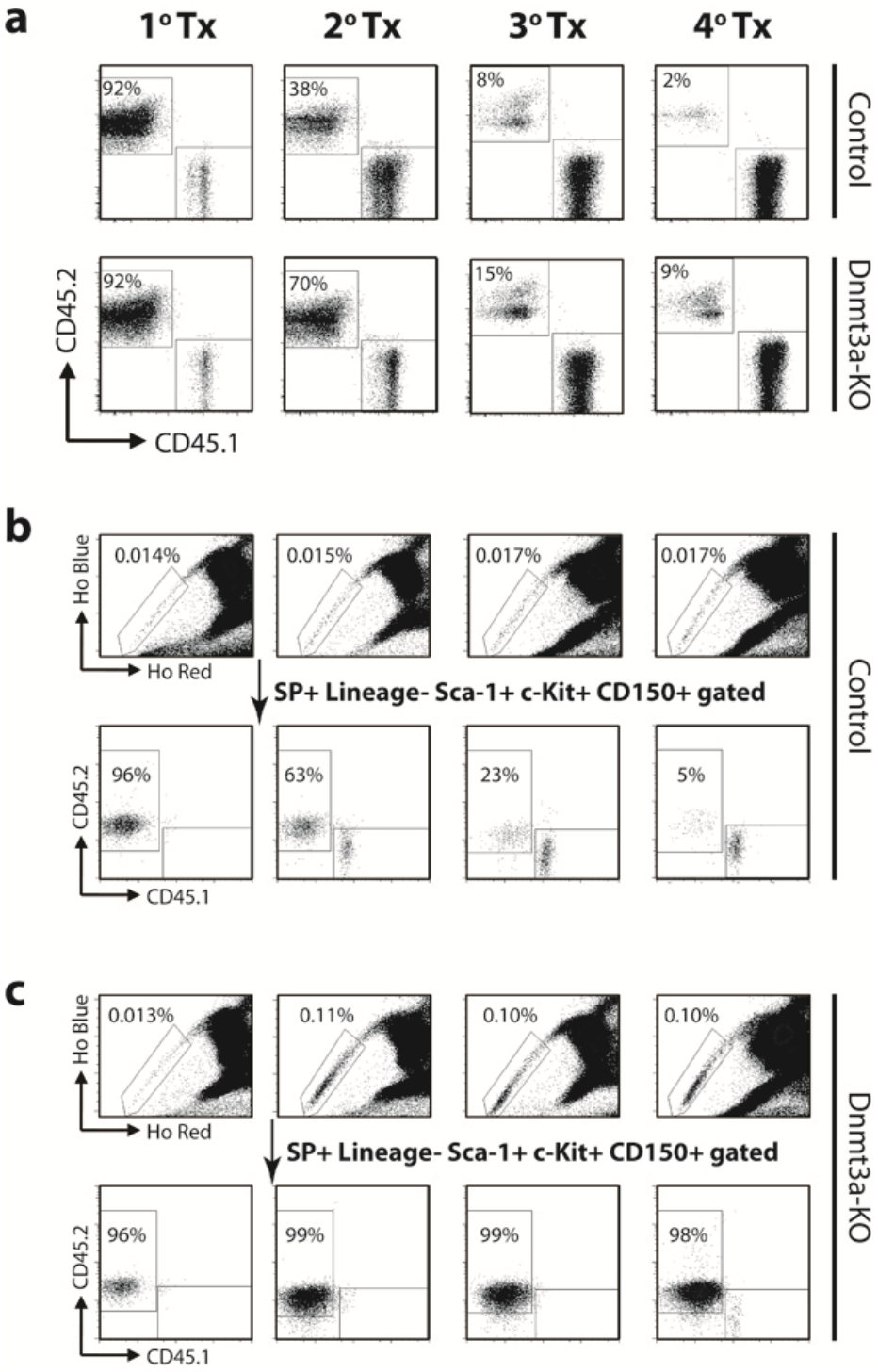


c

	Mx1-cre+:Dnmt3a$^{fl/fl}$		
	+ / +	+ / -	- / -
Post 1° Transplant	4 / 202	10 / 202	188 / 202 (93.1%)
Post 2° Transplant	1/129	0/129	128 / 129 (99.2%)
Post 3° Transplant	1/129	0/129	128 / 129 (99.2%)
Post 4° Transplant	1/130	0/130	129 / 130 (99.2%)

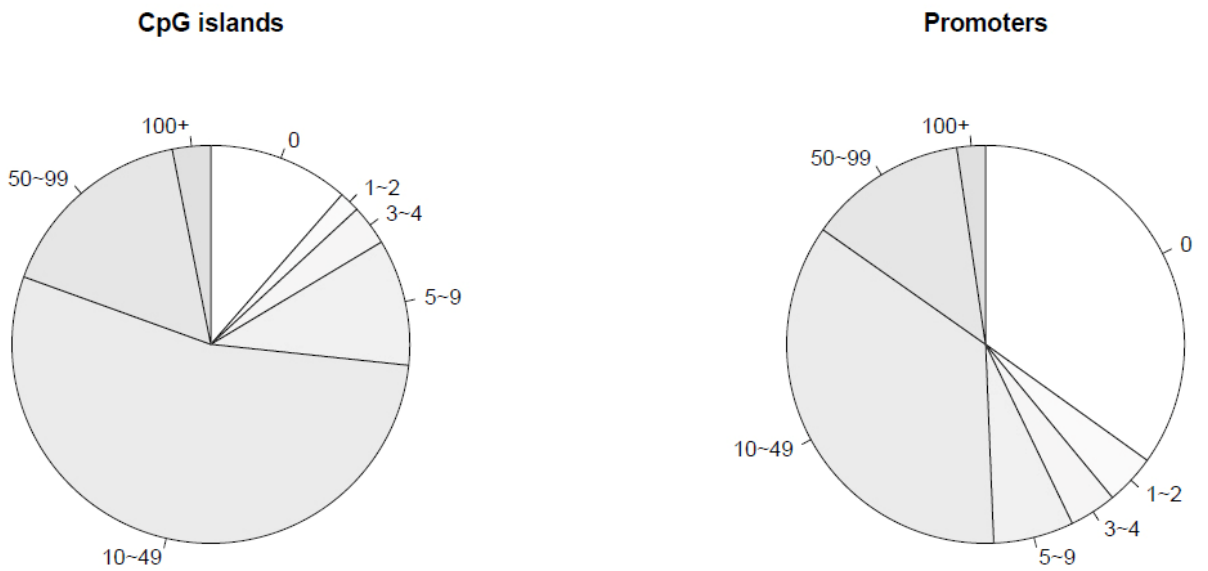
Supplementary Figure 4: Loss of Dnmt3a causes a cell-autonomous change in HSC functional potential.

(**a**) Single HSCs (CD45.2^{+}SP^{KLS}CD150^{+}) were sorted after transplantation into wells of a 96-well plate containing Methocult. The average number of hematopoietic colonies formed per plate is displayed, showing a significant increase in colony-forming activity on a per cell basis from *Dnmt3a*-KO HSCs. (**b**) Genomic DNA was prepared from individual hematopoietic colonies (each arising from a single HSC) and screened by PCR for *Dnmt3a* target allele deletion following pIpC treatment. Two weeks after plating, colonies were picked and subjected to PCR screening for excision of the floxed alleles. (**c**) Table showing the number of colonies with the indicated genotype over the total number screened. The total colonies screened represents 20-40 colonies from each of a number of separate transplant cohorts. Percentages of null HSCs for *Dnmt3a* target alleles are shown.



<u>Supplementary Figure 5:</u> Differentiation and self-renewal of donor cells through serial transplantation.

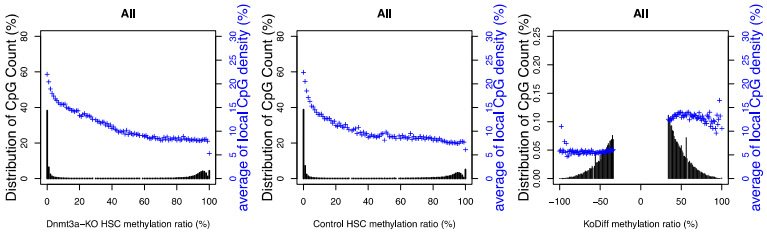
(**a**) Donor-cell contribution (%CD45.2+) to peripheral blood of representative recipient mice 16-weeks post-transplant. HSC frequency in bone marrow of representative control (**b**) and *Dnmt3a*-KO (**c**) transplanted mice 18-weeks after the indicated stage of serial transplantation shows the expansion of HSC compartment in *Dnmt3a*-KO transplanted mice. Gating of HSCs to a plot of CD45.1 (WT competitor) versus CD45.2 (donor) shows the decline in control HSC self-renewal over serial transplant while the expanded *Dnmt3a*-KO HSC pools are almost entirely composed of *Dnmt3a*-KO-derived HSCs.



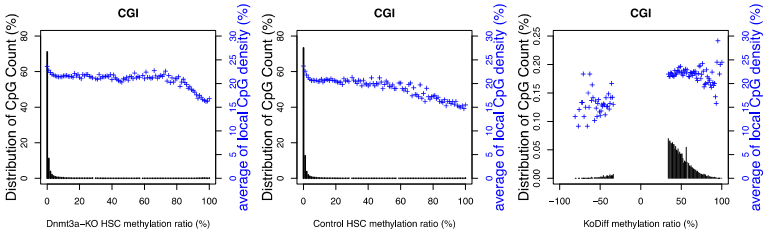
Supplementary Figure 6: RRBS data coverage of RefSeq gene promoters and CpG islands.
We obtained around 1 million CpGs with at least 10-fold coverage (covered CpGs) in both control and *Dmnt3a*-KO HSC samples. Pie charts represent the percent of CpG islands and promoters (2 kb regions centered at RefSeq transcription start sites) that have the specified range of covered CpGs.

Supplementary Figure 7: Genome-wide distribution of CpG methylation in control and *Dnmt3a*-KO HSCs. (following 4 pages).
Each row represents the distribution of CpG methylation within a given genomic feature. In each row, the left and middle panels have black histograms representing the distribution of basal methylation levels (%) of CpGs in the control and *Dnmt3a*-KO HSC samples respectively, and the blue dots denote the average local CpG densities (defined as % CpGs in a 200bp window centered at a given CpG) of all CpGs with the same methylation level (e.g. all CpGs with 0% methylation). In the right panel, the relationship between local CpG density (blue) and the distribution of hypo-methylated DMCs (defined as CpGs that are ≤33% less methylated in the *Dnmt3a*-KO HSCs) and hyper-methylated DMCs (defined as CpGs that are ≥33% more methylated in the *Dnmt3a*-KO HSCs) is shown. The genomic features are the same as those defined in Supplementary Table 4 and abbreviations can be found at the bottom of the figure.

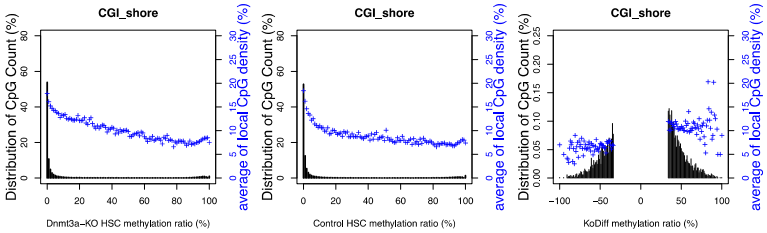
All



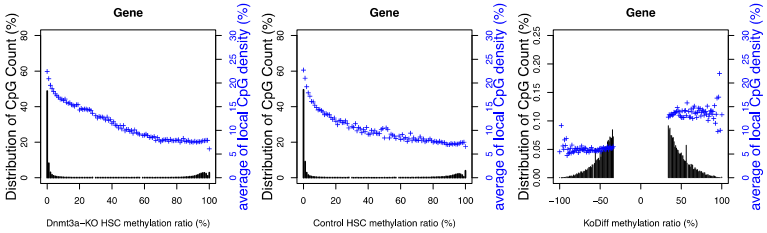
CGI



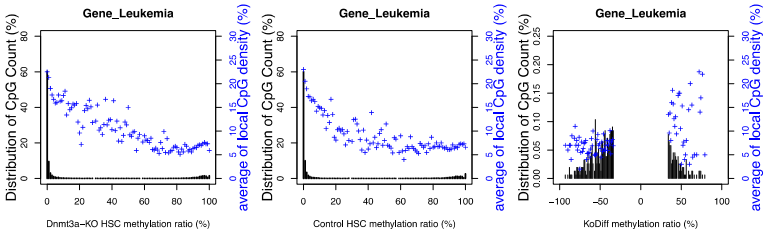
CGI_Shore



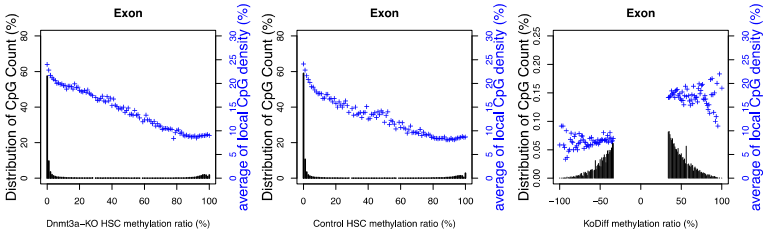
Gene



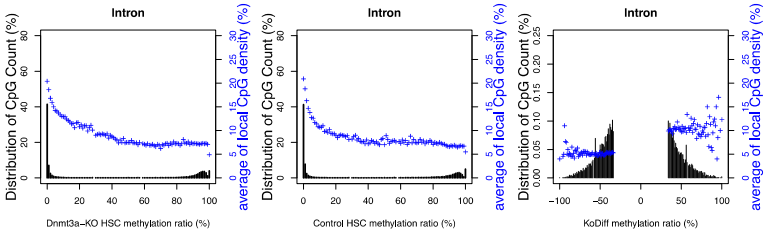
Gene_Leukemia



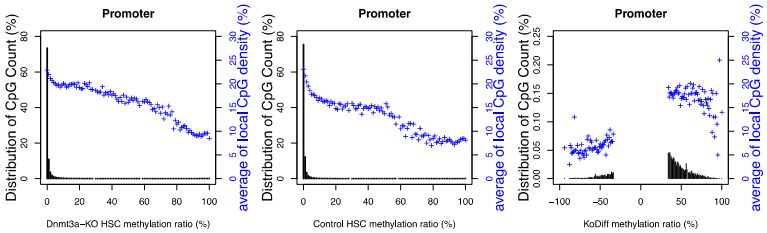
Exon



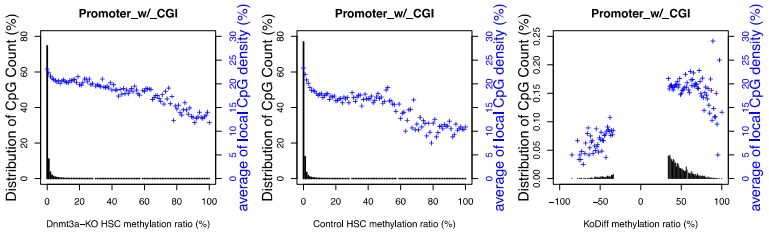
Intron



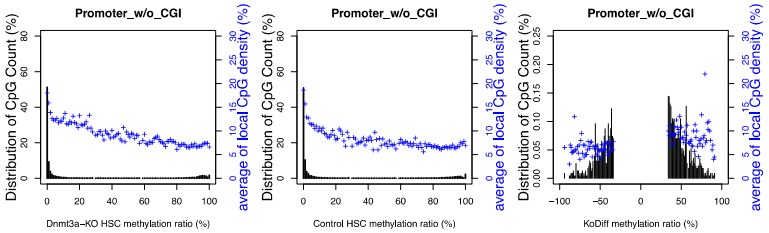
Promoter



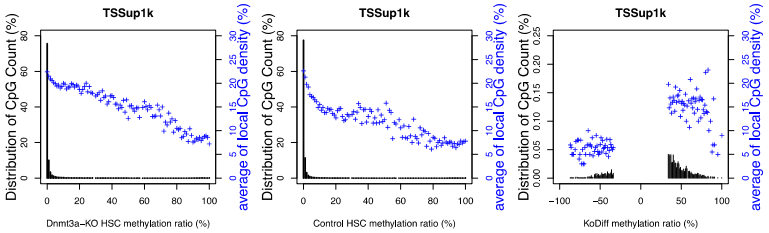
Promoter_w/_CGI



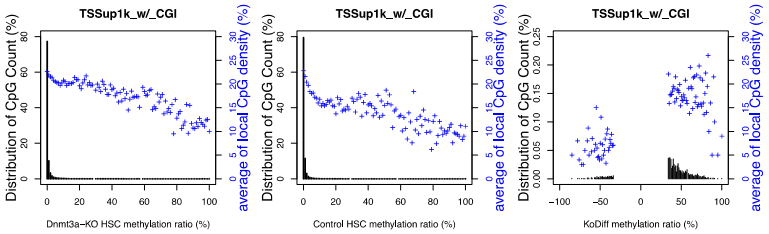
Promoter_w/o_CGI



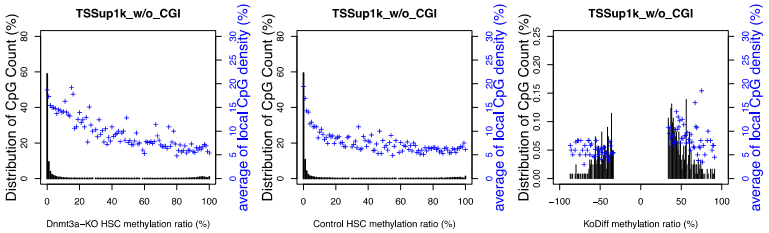
TSSup1k



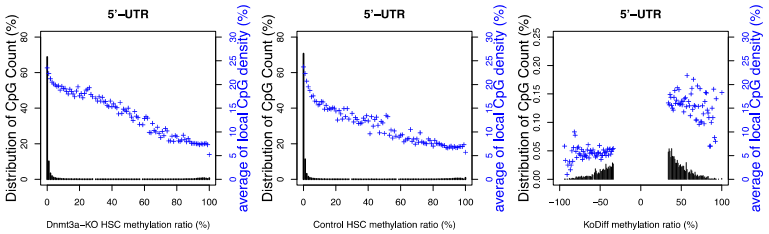
TSSup1k_w/_CGI



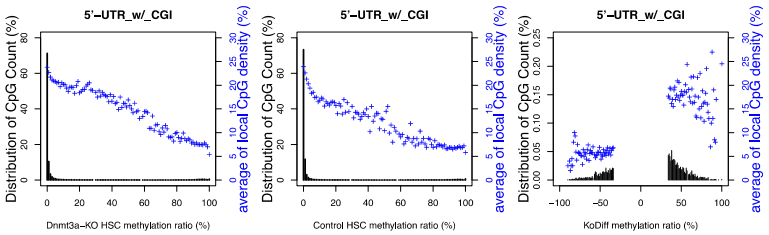
TSSup1k_w/o_CGI



5’-UTR



5’-UTR_w/_CGI



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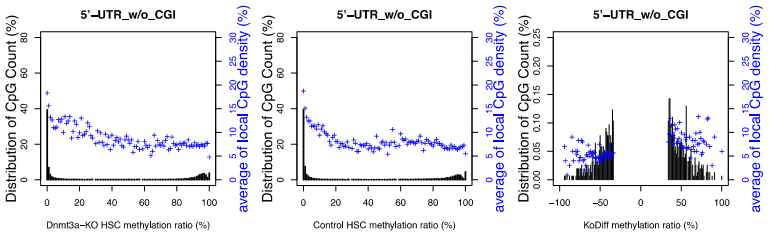
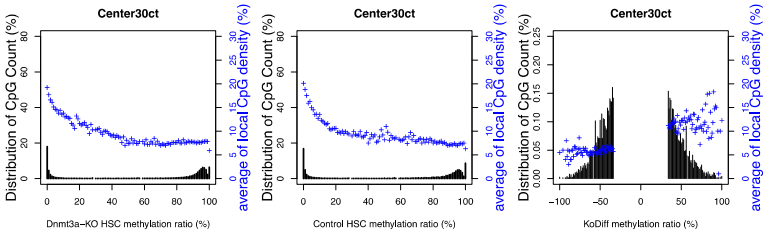
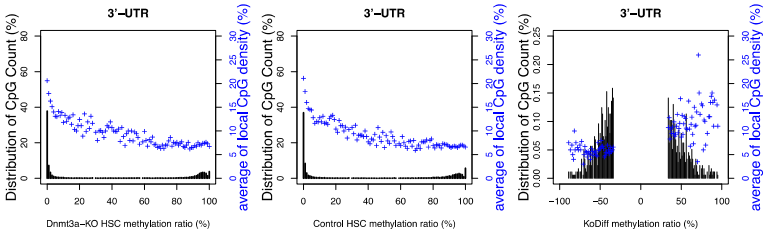


Center30ct



3’-UTR



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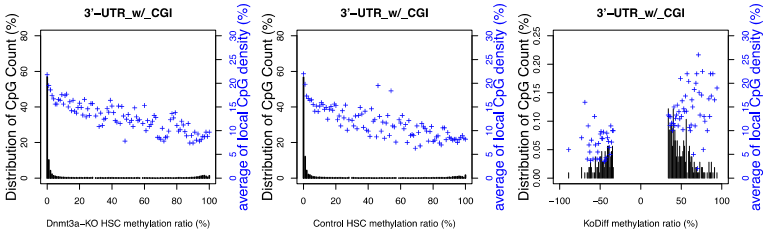

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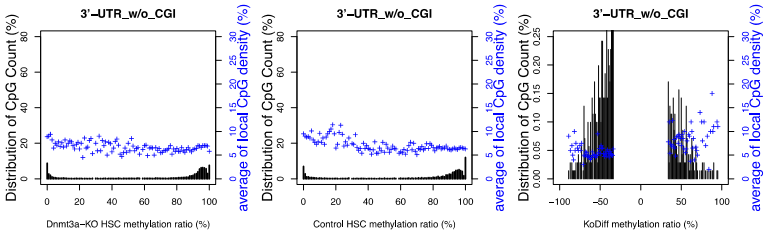
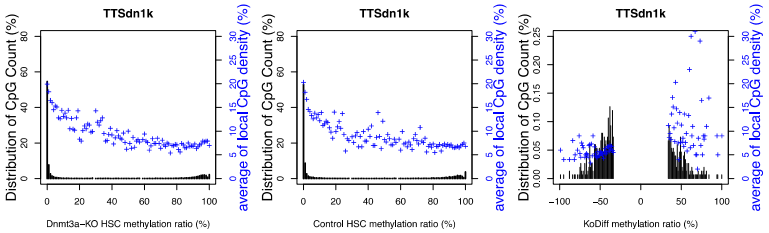


TSSdn1k



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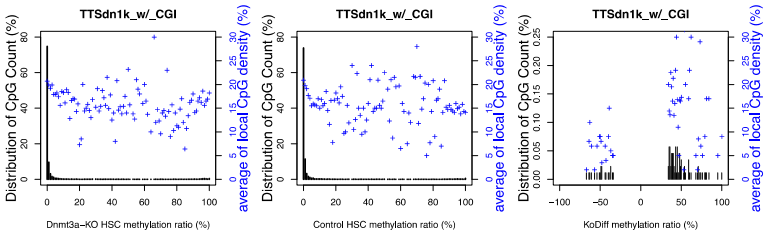

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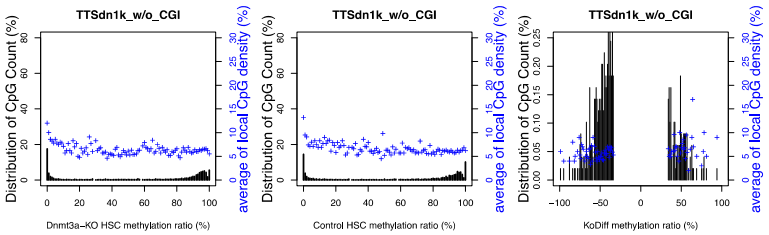
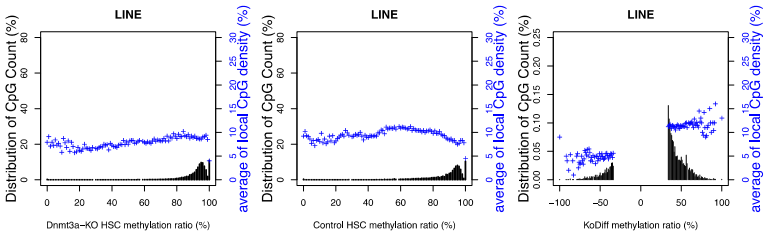


LINE



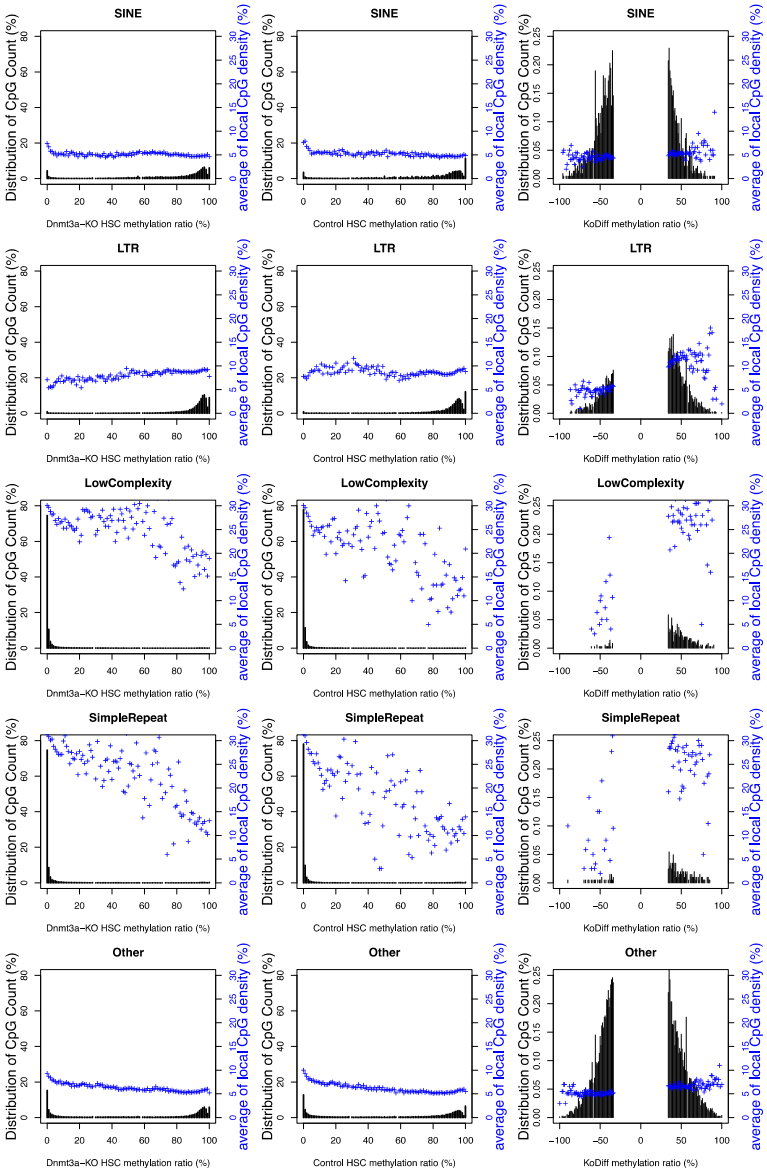
SINE

LTR

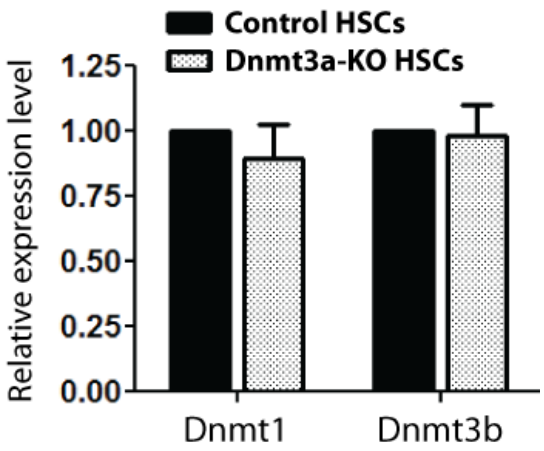
LowComplexity

SimpleRepeat

Other



All: All CpGs with at least 10-fold coverage in both control and Dmnt3a-KO samples.
CGI: CpG islands.
CGI_Shore: CpG island shores, defined as 2kb flanking regions of CpG islands.
Gene: All RefSeq gene bodies.
Gene_Leukemia: defined as from 1 kb upstream of transcription starting site (TSS) to 1 kb downstream of transcription termination site (TTS), of 262 leukemia RefSeq genes.
Exon (Intron): RefSeq exons (introns).
Promoter: 2 kb regions centered at RefSeq transcription starting sites.
TSSup1k (TTSdn1k): upstream (downstream) 1kb of transcription starting (termination) site of RefSeq genes.
5'-UTR (3'-UTR): 5'-UTR (3'-UTR) of RefSeq genes.
Center30ct: The middle 30% of RefSeq gene bodies.
LINE, SINE, LowComplexity, SimpleRepeat: Repeat subfamilies.
Other: All the remaining CpG sites not located in any above genomic features.



Supplementary Figure 8: Loss of Dnmt3a in HSCs does not alter expression levels of Dnmt1 or Dnmt3b.
Real-time PCR analysis of Dnmt1 and Dnmt3b expression in transplanted control and *Dnmt3a*-KO HSCs.

434 Dmnt3a-KO hypo-methylated genes

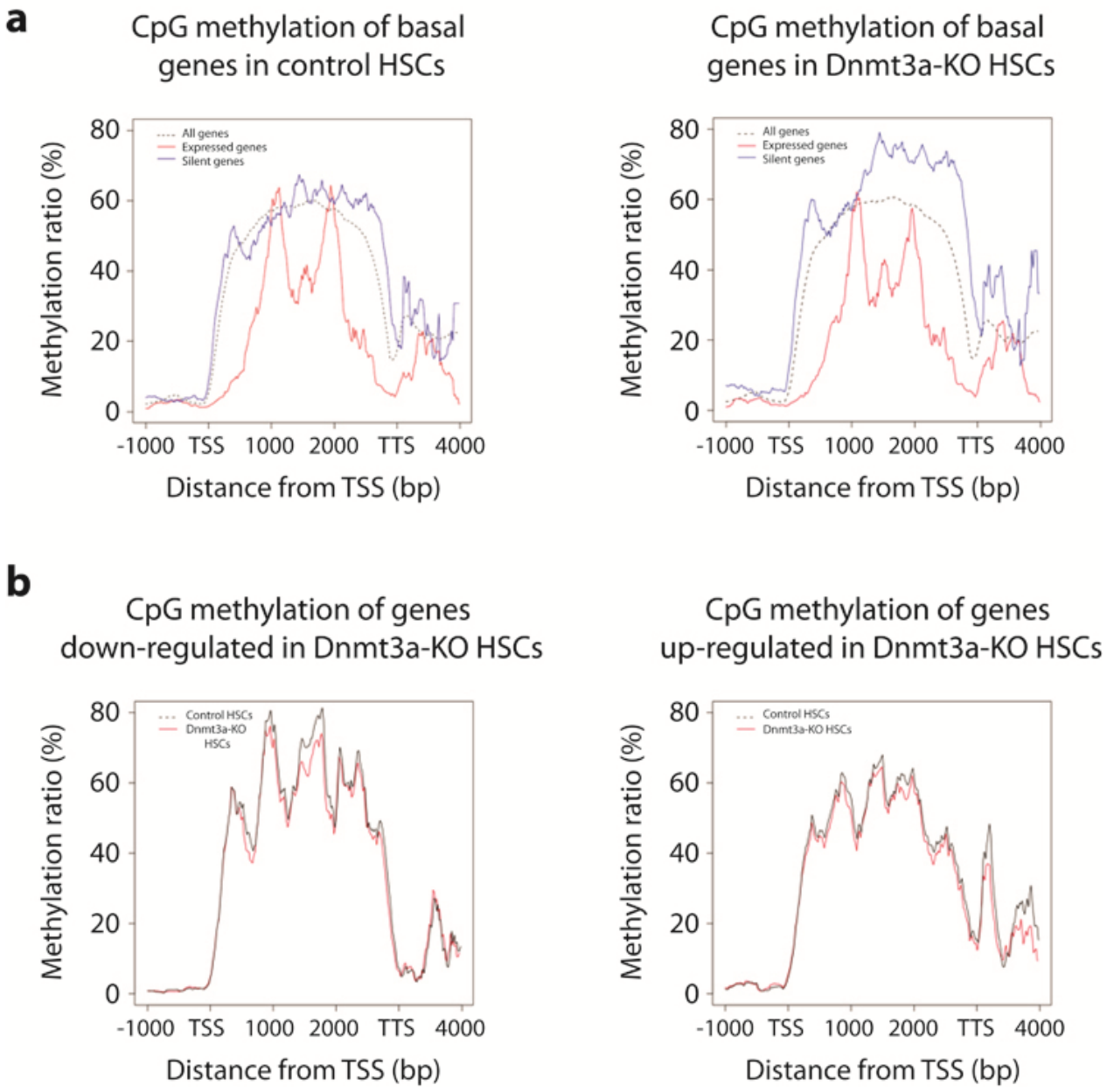
Oncomine Concepts	Overlapped Genes	adjusted p-value
Cancer Gene Census - all causal cancer genes	18	2.76E-05
Acute Myeloid Leukemia - CBFB-MYH11 Gene Fusion - Top 10% Over-expressed (Valk Leukemia)	58	5.25E-04
Acute Lymphoblastic Leukemia - BCR-ABL1 Gene Fusion - Top 1% Over-expressed (Ross Leukemia)	14	6.00E-03
B-Cell Acute Lymphoblastic Leukemia - Top 5% Over-expressed (Haferlach Leukemia)	51	2.67E-06
KEGG Pathways		
mmu05200:Pathways in cancer	21	1.50E-02

Aff3, Arhgef12, Bcl3, Brca2, CCND1, Col1a1, Epas1, Erg, Etv6, Fnbp1, Hip1, Mecom, Men1, Mn1, Msi2, Myc, Notch, Pdgfrb, Prdm16, Ptch1, Rbm15, Runx1, Smad3

534 Dmnt3a-KO hyper-methylated genes

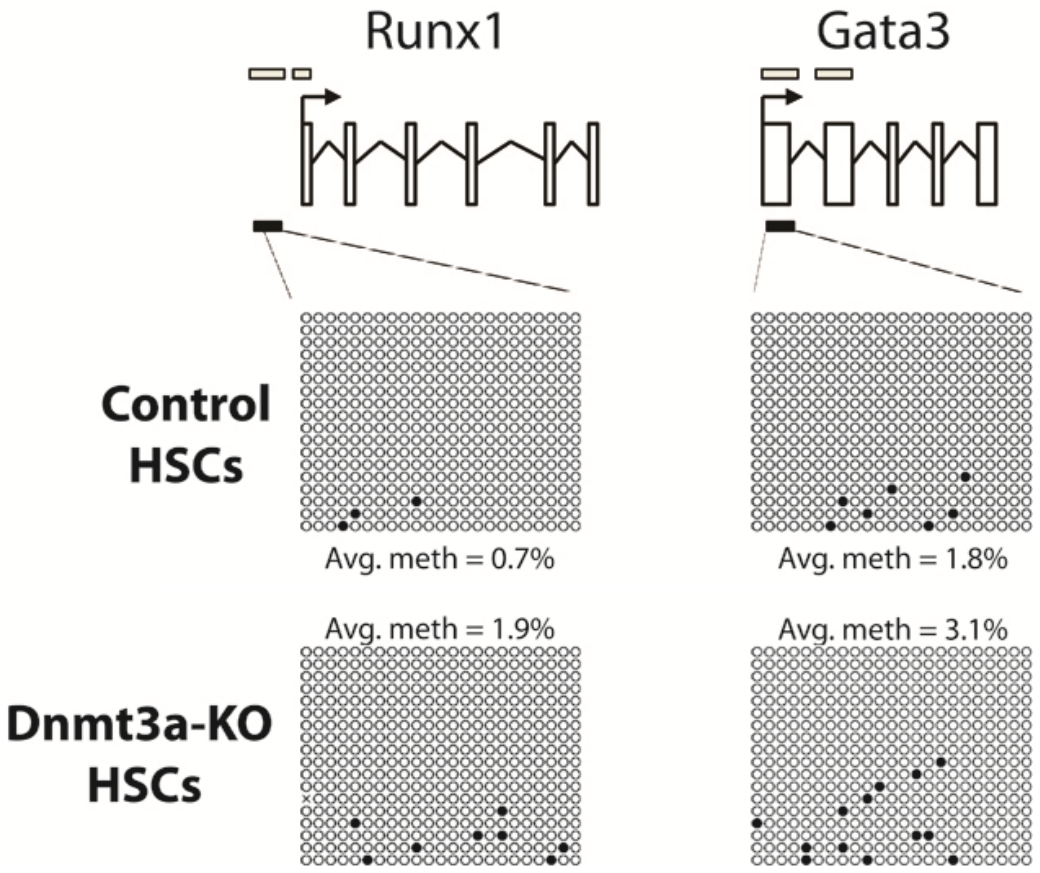
Oncomine Concepts		
Cancer Type: Leukemia - Top 10% Under-expressed (Wooster CellLine)	82	9.66E-05
Cancer Type: Leukemia - Top 5% Under-expressed (Ramaswamy Multi-cancer)	23	4.80E-02
Hypermethylated genes in cancer	24	1.18E-08
KEGG Pathways		
mmu04916:Melanogenesis	9	4.40E-02

Supplementary Figure 9: KEGG pathway and Oncomine concept analysis of DMRs in *Dnmt3a*-KO HSCs.
Analysis of hypo- and hyper-methylated DMRs with Oncomine and KEGG. Hypomethylated DMRs show a striking enrichment for cancer causal genes, and genes over-expressed in leukemias such as acute myeloid leukemia and acute lymphoblastic leukemia.



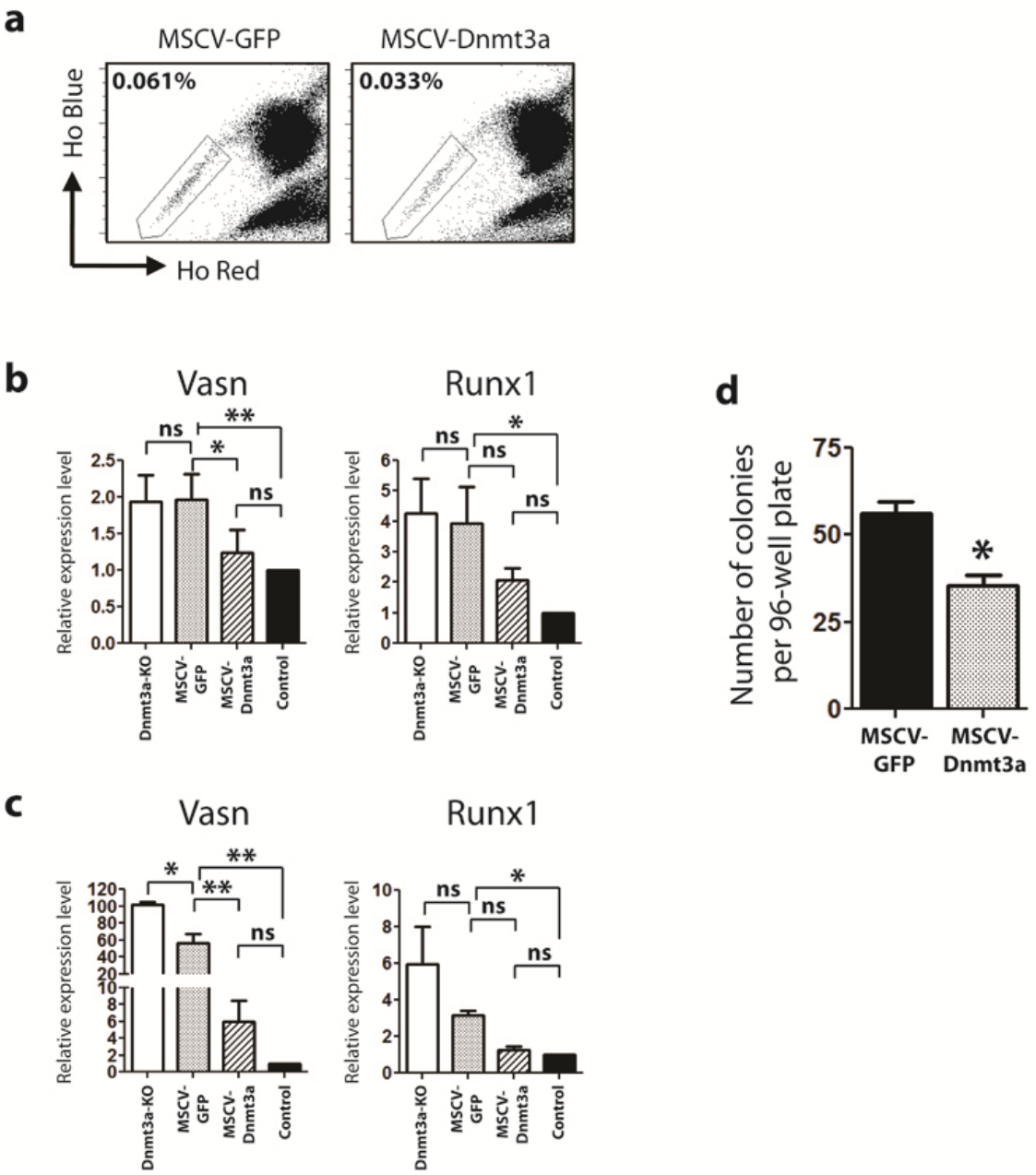
<u>Supplementary Figure 10:</u> CpG methylation profile along RefSeq genes.

(**a**) CpG methylation profile along the top 400 highly expressed genes, all genes and the bottom 400 silent genes in control and *Dnmt3a*-KO HSCs. (**b**) Two separate CpG methylation profiles from control and *Dnmt3a*-KO HSCs; CpG methylation for down-regulated and up-regulated genes in *Dnmt3a*-KO HSCs. Each gene is normalized to 3 KB long from transcription start site (TSS) to transcription termination site (TTS). Methylation correlates with gene expression within samples, but not in differentially expressed genes between samples.



Supplementary Figure 11: Promoter CGI methylation of *Runx1* and *Gata3* in control and *Dnmt3a*-KO HSCs.

Analysis of the CGIs associated with the promoter or *Runx1* and *Gata3* showed the promoters of both genes were unmethylated in control and *Dnmt3a*-KO HSCs. This is in contrast to the gene-body CGIs which were hypermethylated in *Dnmt3a*-KO HSCs.



<u>Supplementary Figure 12:</u> Exogenous Dnmt3a can restore function, gene expression and methylation patterns in *Dnmt3a*-KO HSCs and B-cells.

Sca-1$^+$ cells from mice secondarily-transplanted *Dnmt3a*-KO HSCs were transduced with either MSCV-Dnmt3a or empty vector control (MSCV-GFP) retrovirus and transplanted into tertiary recipients. (**a**) Hoechst staining of transduced tertiary-transplanted bone marrow 18-weeks post-transplant showed a reduction in SP frequency of *Dnmt3a*-KO cells transduced with MSCV-Dnmt3a retrovirus compared to control empty-vector (MSCV-GFP) transduced HSCs. Restoration of Dnmt3a expression in *Dnmt3a*-KO HSCs can restrain expression of *Runx1* and *Vasn* in HSCs (**b**) and B-cells (**c**). While MSCV-GFP transduced KO-HSCs and B-cells retained expression comparable to untransduced controls (Dnmt3a-KO), MSCV-Dnmt3a transduced KO-HSCs and B-cells showed expression levels comparable to transplant-matched control Cre– samples. (**d**) Dosage effect of Dnmt3a in HSCs. Control HSCs transduced with MSCV-Dnmt3a showed reduced colony-forming potential compared to control HSCs transduced with MSCV-GFP control virus.

Supplementary Table 1: Comparison of stem and progenitor cell frequencies in secondary transplant mice.

Cell Type	Cell Phenotype	Genotype	Frequency in BM	Donor Cell Chimerism
LT-HSC	SP+ Lin- Sca-1+ c-Kit+	Control (n= 16)	0.010 ± 0.00078%	63.2 ± 4.28%
		Dnmt3a-KO (n= 14)	0.063 ± 0.0055%	95.5 ± 0.98%
LT-HSC	Lin- Sca-1+ c-Kit+ CD34- Flk2-	Control (n= 11)	0.010 ± 0.0024%	46.1 ± 6.18%
		Dnmt3a-KO (n= 11)	0.050 ± 0.0078%	82.8 ± 2.93%
LT-HSC	Lin- Sca-1+ c-Kit+ CD48- CD150+	Control (n= 9)	0.0090 ± 0.0021%	56.9 ± 4.30%
		Dnmt3a-KO (n= 8)	0.045 ± 0.0094%	96.5 ± 1.44%
ST-HSC	Lin- Sca-1+ c-Kit+ CD34+ Flk2-	Control (n= 11)	0.11 ± 0.0088%	44.6 ± 6.96%
		Dnmt3a-KO (n= 11)	0.097 ± 0.016%	54.6 ± 11.2%
MPP	Lin- Sca-1+ c-Kit+ CD34+ Flk2+	Control (n= 11)	0.041 ± 0.0067%	49.5 ± 6.20%
		Dnmt3a-KO (n= 11)	0.040 ± 0.0073%	55.5 ± 9.29%
CLP	Lin- Il7rα+ Sca-1+ c-Kit+	Control (n= 11)	0.017 ± 0.0016%	31.8 ± 7.66%
		Dnmt3a-KO (n= 11)	0.019 ± 0.0022%	43.2 ± 10.26%
CMP	Lin- Il7rα– Sca-1- c-Kit+ CD34+ CD16/32-	Control (n= 10)	0.24 ± 0.024%	40.94 ± 10.08%
		Dnmt3a-KO (n= 10)	0.23 ± 0.029%	54.4 ± 9.94%
GMP	Lin- Il7rα– Sca-1- c-Kit+ CD34+ CD16/32+	Control (n= 10)	0.41 ± 0.041%	43.3 ± 11.17%
		Dnmt3a-KO (n= 10)	0.41 ± 0.043%	48.0 ± 11.55%
MEP	Lin- Il7rα– Sca-1- c-Kit+ CD34- CD16/32-	Control (n= 10)	0.10 ± 0.0093%	40.2 ± 8.49%
		Dnmt3a-KO (n= 10)	0.10 ± 0.0073%	52.7 ± 7.65%

Cell Type: LT-HSC (long-term HSC), ST-HSC (short-term-HSC), MPP (multipotent progenitor), CLP (common lymphoid progenitor), CMP (common myeloid progenitor), GMP (granulocyte-macrophage progenitor), MEP (megakaryocyte-erythroid progenitor). Cell Phenotype: Surface marker phenotype used for cell identification. Frequency in BM: Frequency of target cell in nucleated whole bone marrow cells. Donor Cell Chimerism: Percentage of donor-derived cells (CD45.2+) within the test cell population. There was no difference in absolute counts of whole bone marrow cells of control and *Dnmt3a*-KO transplanted mice.

Supplementary Table 2: Quantification of HSC self-renewal quotient and differentiation quotient over four rounds of transplantation.

	Genotype	# Donor HSCs	# Donor HSCs Post-Transplant	Amplification per HSC	%CD45.2 Blood	16-week CBC WBC	Differentiation per HSC
Primary Transplant	Control (n=36)	250	9.92×10^3 (92.0%)	39.7	85.3 ± 2.3%	11.6 ± 0.32	0.99
	Dnmt3a-KO (n=21)	250	1.09×10^4 (93.4%)	43.8	84.9 ± 2.8%	10.9 ± 0.25	0.84
Secondary Transplant	Control (n=39)	250	5.50×10^3 (63.2%)	22.0	27.1 ± 2.0%	11.4 ± 0.43	0.56
	Dnmt3a-KO (n=31)	250	6.76×10^4 (95.5%)	270.5	57.7 ± 4.6%	9.8 ± 0.61	0.084
Tertiary Transplant	Control (n=17)	250	1.24×10^3 (9.48%)	4.9	5.0 ± 3.1%	11.1 ± 0.31	0.39
	Dnmt3a-KO (n=26)	250	6.81×10^4 (94.5%)	272.5	15.1 ± 4.6%	8.9 ± 0.62	0.018
Quaternary Transplant	Control (n=15)	250	5.51×10^2 (2.77%)	2.2	1.2 ± 0.2%	11.2 ± 0.62	0.17
	Dnmt3a-KO (n=21)	250	6.53×10^4 (94.7%)	261.1	3.6 ± 2.2%	9.0 ± 0.74	0.0050

Donor HSCs: Number of donor HSCs transplanted into recipient mice. # Donor HSCs post-transplant: Average absolute number of donor-derived (CD45.2+) HSCs (SP-KLS) per mouse (iliac crests, tibias and femurs) 18 weeks post-transplant. This is derived by determining (% of bone marrow with the HSC phenotype)*(average number of extracted bone marrow cells)*(proportion of HSCs that were donor derived). In parentheses is the proportion of total HSCs that were donor derived (CD45.2+). Amplification per HSC: # donor HSCs post-transplant divided by input HSCs (always 250). %CD45.2 peripheral blood: proportion of nucleated PB cells generated from transplanted HSCs (CD45.2+). 16-week CBC WBC: complete white blood cell counts of transplanted recipients (average; K/Ml) at 16-weeks post-transplant. Differentiation per HSC: 16-week CBC x %CD45.2 PB / # donor HSCs post-transplant. While all recipient mice were analyzed for CBCs and donor cell contribution to the blood, not all recipient mice were analyzed for HSC content. However, within each group, results were highly consistent for bone marrow counts and proportion of HSCs.

Supplementary Table 3: Statistics of RRBS experiments.

We obtained high quality data with ~99% bisulfite conversion rate and around 35M raw reads per sample. After quality filtering, around 71% quality reads were mapped to the mouse genome.

Sample name	Total Reads	% High Quality Reads	Unique Cpg Sites	Total CpGs sequenced	Fold Coverage	% Bisulfite Conversion Rate*	% Mean Methylation
Control_r1	41,186,939	78.70	1,331,893	95,129,290	71.42	99.86	41.94
Control_r2	39,100,383	73.94	1,327,319	85,085,153	64.10	99.85	42.08
Dnmt3a-KO_r1	46,602,878	68.32	1,304,390	81,831,132	62.74	98.98	46.60
Dnmt3a-KO_r2	46,910,735	63.91	1,311,398	81,711,149	62.31	98.91	43.61

* Bisulfite Conversion Rate refers to the percentage of unconverted Cytosines in non-CpG context.

Supplementary Table 4: Global comparison of RRBS samples.

Percentage of Differentially Methylated CpGs (Pearson's correlation coefficient)	Control _r2	Dnmt3a_KO _r1	Dnmt3a_KO _r2
Control _r1	0.32 (0.98)	3.85 (0.96)	3.19 (0.96)
Control _r2		3.83 (0.96)	3.13 (0.96)
Dnmt3a_KO_r1			0.63 (0.98)

Supplementary Table 5: Genome-wide distribution of differentially methylated CpGs in *Dnmt3a*-KO HSCs.

Genomic Features	% CpGs	% Mean Methylation	% DMCs	% Hyper Methylation	% Mean Diff Methylation
All CpGs (1,020,508)	100.00	38.10	3.71	57.69	1.27
CGI	47.04	3.14	1.84	94.96	1.57
CGI_Shore	10.75	13.25	3.92	60.13	0.83
Gene	53.66	27.16	3.39	51.97	0.65
Gene_ Leukemia	1.58	23.43	3.90	31.59	-0.79
Exon	25.43	17.74	2.90	61.46	0.89
Intron	28.22	35.65	3.83	45.48	0.44
Promoter	44.01	2.81	1.23	84.73	1.00
Promoter_w/_CGI	41.70	1.90	1.04	91.34	1.00
Promoter_w/o_CGI	2.31	19.19	4.74	58.61	1.01
TSSup1k	16.53	2.74	1.14	81.13	0.87
TSSup1k_w/_CGI	15.03	1.78	0.89	90.24	0.85
TSSup1k_w/o_CGI	1.23	12.54	4.08	60.04	1.07
5'-UTR	20.37	8.13	1.67	66.31	0.86
5'-UTR_w/_CGI	18.84	5.98	1.45	69.80	0.85
5'-UTR_w/o_CGI	1.53	34.52	4.41	52.17	1.07
Center30ct	7.32	58.72	5.96	46.07	0.49
3'-UTR	1.77	36.86	5.36	44.02	-0.07
3'-UTR_w/_CGI	1.08	14.83	3.33	69.29	1.39
3'-UTR_w/o_CGI	0.69	71.38	8.53	28.57	-2.36
TTSdn1k	1.52	24.44	3.54	37.45	-0.49
TTSdn1k_w/_CGI	0.89	5.14	1.23	79.46	0.64
TTSdn1k_w/o_CGI	0.49	61.96	8.07	24.63	-3.03
LINE	16.71	86.83	1.93	71.45	3.52
SINE	2.25	76.80	6.99	37.62	-0.27
LTR	7.67	87.16	3.93	59.19	1.08
LowComplexity	3.78	2.14	1.41	95.05	1.31
SimpleRepeat	2.05	4.13	1.47	69.35	0.80
Other	7.12	62.49	10.36	46.32	0.44

% CpGs: percent of CpGs within a specific genomic feature, relative to all CpGs.

% Mean Methylation: mean methylation ratio of CpGs within a specific genomic feature in WT sample.

% DMCs: Percentage of CpGs differentially methylated by ≥ 33% within a specific genomic feature, relative to all CpGs within the same genomic feature. Percentages significantly higher (RED) or lower (YELLOW) than genomic background were highlighted.

% Hyper Methylation: percent of hyper-methylated DMCs in Dnmt3a-KO within a specific genomic feature, relative to all DMCs within the same genomic feature. Percentages

significantly higher (RED) or lower (YELLOW) than genomic background were highlighted.

% Mean Diff Methylation: mean methylation ratio difference between Dnmt3a-KO and WT samples within a specific genomic feature.

Genomic features were defined as the following:

All CpGs: All CpGs with at least 10-fold coverage in both control and Dmnt3a-KO samples.

CGI: CpG islands.

CGI_Shore: CpG island shores, defined as 2kb flanking regions of CpG islands.

Gene: All RefSeq gene bodies.

Gene_Leukemia: defined as from 1 kb upstream of transcription starting site (TSS) to 1 kb downstream of transcription termination site (TTS), of 262 leukemia RefSeq genes.

Exon (Intron): RefSeq exons (introns).

Promoter: 2 kb regions centered at RefSeq transcription starting sites.

TSSup1k (TTSdn1k): upstream (downstream) 1kb of transcription starting (termination) site of RefSeq genes.

5'-UTR (3'-UTR): 5'-UTR (3'-UTR) of RefSeq genes.

Center30ct: The middle 30% of RefSeq gene bodies.

LINE, SINE, LowComplexity, SimpleRepeat: Repeat subfamilies.

Other: All the remaining CpG sites not located in any above genomic features.

Supplementary Table 6: Annotation of differentially methylated regions (DMRs).
This is a multi-sheet Excel spreadsheet with information on the DMRs. Annotation of DMRs that are either hyper- or hypo-methylated in *Dnmt3a*-KO HSCs compared to control HSCs. This table combines information of the DMRs, the genes and genomic features associated with the DMR, and gene expression data.

Supplementary Table 7: Microarray transcriptional profiling comparison of secondarily-transplanted control and *Dnmt3a*-KO HSCs. Excel file with multiple sheets.

Supplementary Table 8: DREAM sequencing of secondary transplant control and *Dnmt3a*-KO B-cells. Excel file with multiple sheets.

Supplementary Table 9: PCR primer sequences.

ChIP

Gene	Forward Primer	Reverse Primers
Vasorin	GCAGAGACCAGCCTCTTACG	GCCTCAGTCCTTCACCTCTG
Gata3	GCAGCTGCACCTGATACTTG	CGGCTTCATCCTCTTCTCTG
Nr4a2	GAAGGTCTGCCCATCCACTA	TCGAGCAGAGGAAGACACCT
Runx1	TTAGCAACTGGCCGCTTAGT	TCCGGGACCGTTTGTAATAG

Bisulfite Sequencing PCR

Gene	PCR1 primers	PCR2 primers
Vasn	F: GGTGGGTGTGTATAGGTTTGG	F: GGTGGGTGTGTATAGGTTTGG
	R: CCCCTAAACACTCACCAAAAA	R: CCTCAATCCTTCACCTCTAACC
Mn1	F: GATTGTAATTGGTTTAGGTTAGTTAAGTT	F: TTTTTGGTATTTTTAGAGGTTTTTG
	R: CCACCAATCCCTACAACAACA	R: CAACCCAACCTAACCCAACT
Gata3	F: TAGGTTGTTGGGTGGGAAGA	F: TGGGTTGAGGATGAGGTTTT
	R: CAAAACCCTAAACAACCACCA	R: CCCTAAACAACCACCACACC
Pdxdc1	F: TGGAGTTGGGTTTTTGTAGTTT	F: TGGAGTTGGGTTTTTGTAGTTT
	R: CACTACTACTTCCAACTATCTCCTT	R: AACCATACTCTTCCCCCTAC
Basp1	F: TTTTAAGATGGGAGGTAAGTTGAG	F: TTTTAAGATGGGAGGTAAGTTGAGT
	R: AAAACCTCAATCTTTTTAACCTC	R: ACCTCCTTCTCCTCTACCTT
Wbscr17	F: TGTTTGATGGTTTTATTGAGGAGA	F: AGTGTTGTTGGTGTTGAATTTGA
	R: AATCCCAAACACAAAACAAAA	R: TCACCCTATACCCTACTTACACCA
Ccnd1	F: AGAAAGGAGAAAGATTAAGGAAAAA	F: GGGTGTTATTATTTGGTGGTTTT
	R: AAACAAAAACCCCCTCCATC	R: ACAAAAACCCCCTCCATCTC
Men1	F: TGTTGTAGGTGTTGGAGTTTGAG	F: TGTTGTAGGTGTTGGAGTTTGAG
	R: AAAAACCTATCCAAAACATAAAAACT	R: AATCCTCAACCTTTCACTTAACTTAC
Runx1	F: GATGGGTAGGGTTTTGTTGTAG	F: GATGGGTAGGGTTTTGTTGTAG
	R: AACTTTAAATTCTAATTACCCACTTTTT	R: TTTAATCTCCTACCCCCACA
Nr4a2	F: GGTGAGGGTATATTGTTGGGTTTT	F: GGTGAGGGTATATTGTTGGGTTTT
	R: AACCACCTACCCCCTCAATC	R: CCCTTCACAACTTCCACCAA
Gata3-promoter	F: GAGTGTGTTTGGTTTTAAGGATAT	F: TTGTTTTGTTTAGTTAGGGTTTTTG
	R: AAATAAACCACCATCACCCC	R: AAATAAACCACCATCACCCC
Runx1-promoter	F: AGTTATTTAAATAAGGTTAGTTATTGTTTT	F: GTTTTGGGTTTTAAGTATTTTTTTT
	R: ACAAAACCCAAAAAAAATAAAAAAC	R: ACAAAACCCAAAAAAAATAAAAAAC