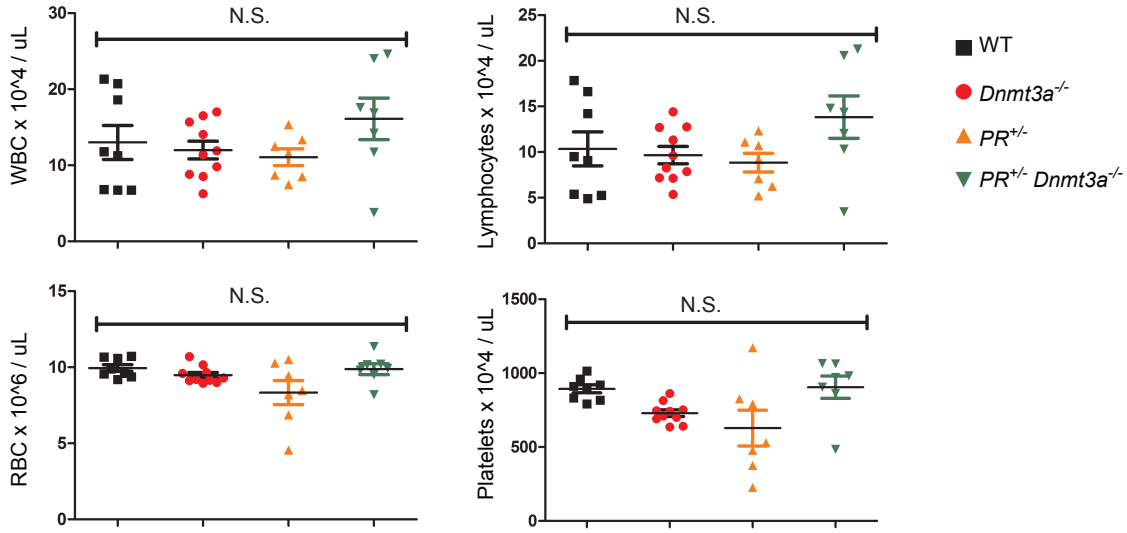
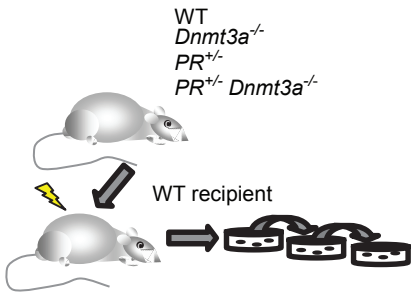


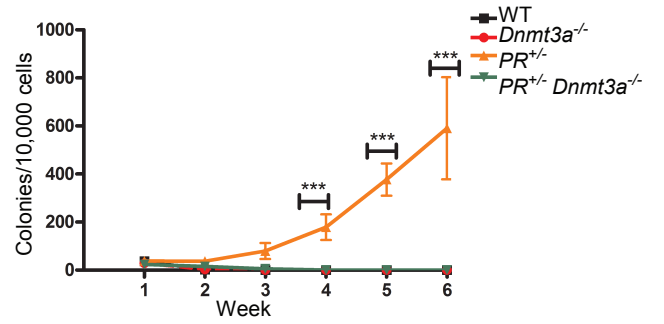
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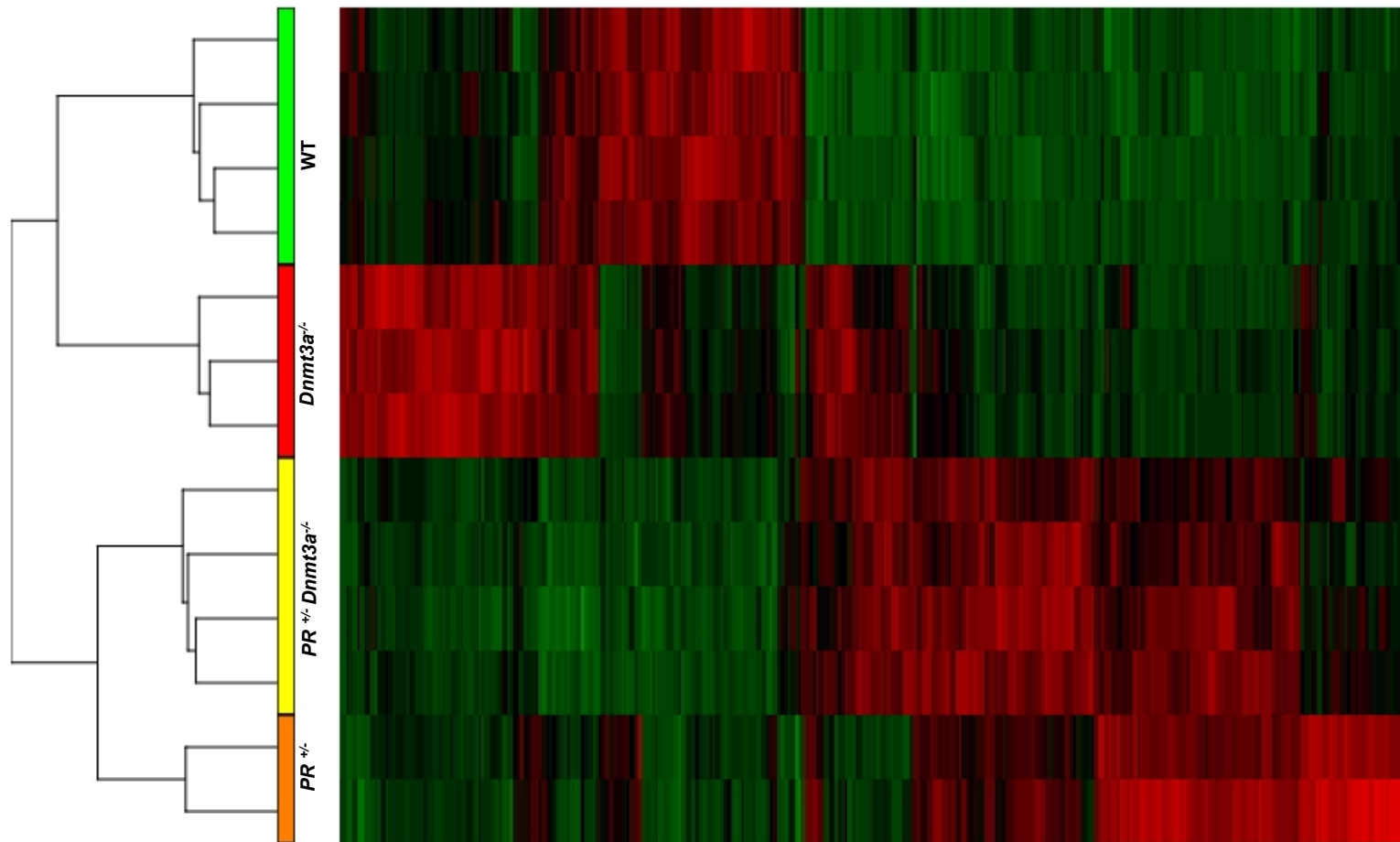
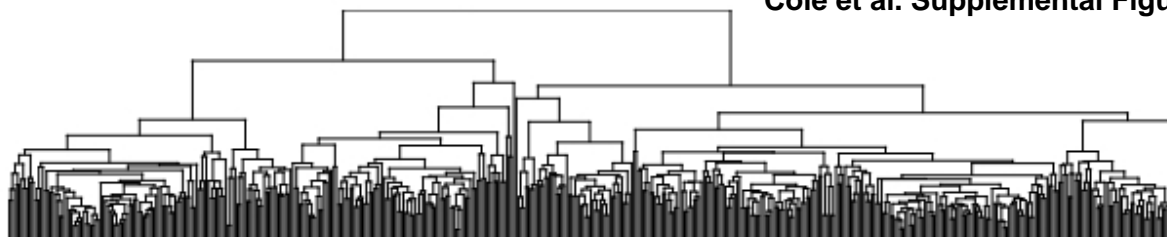


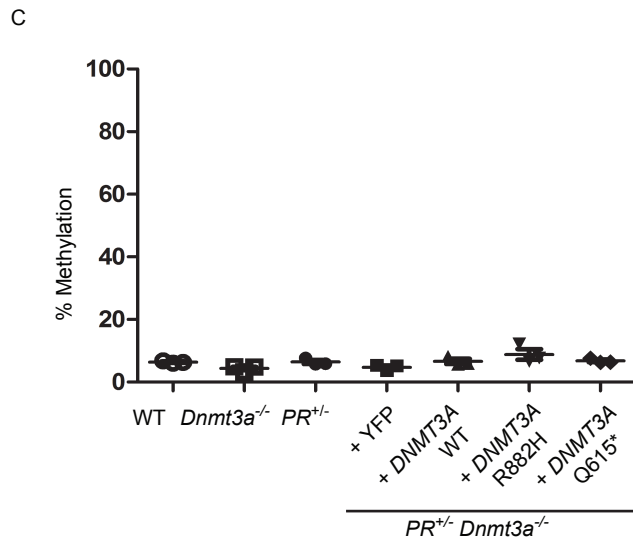
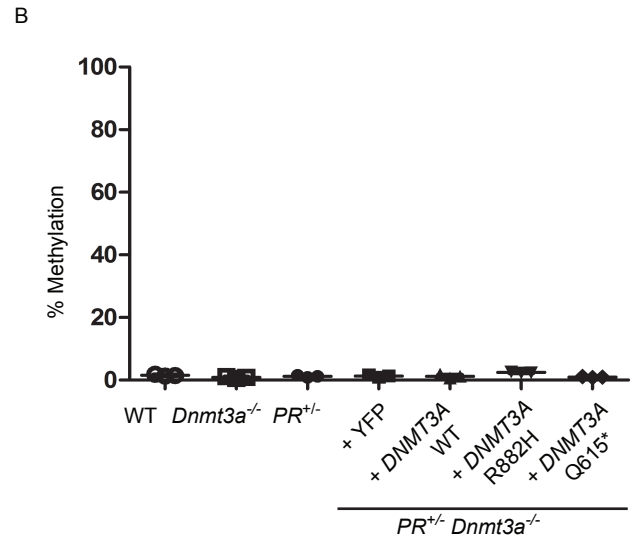
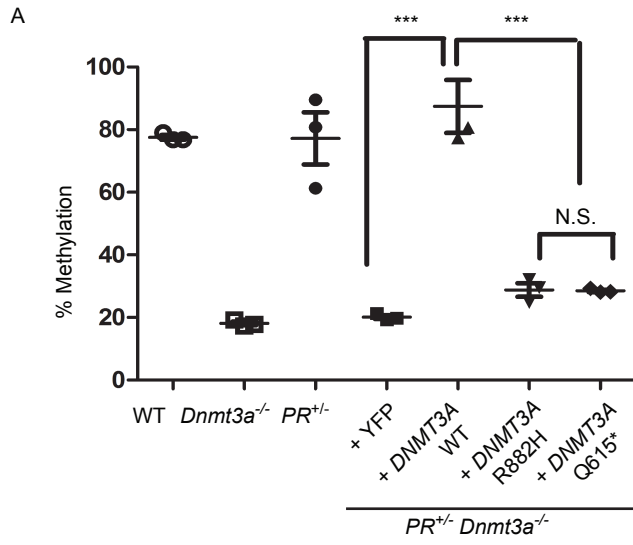
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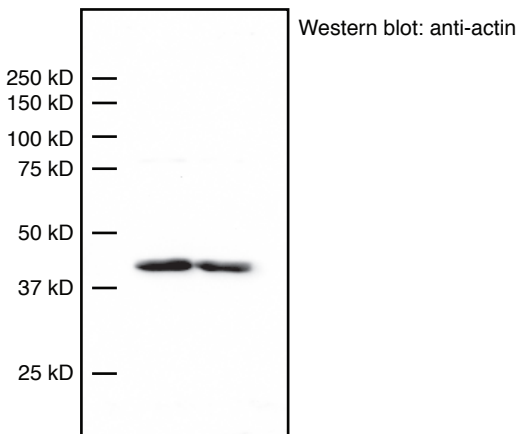
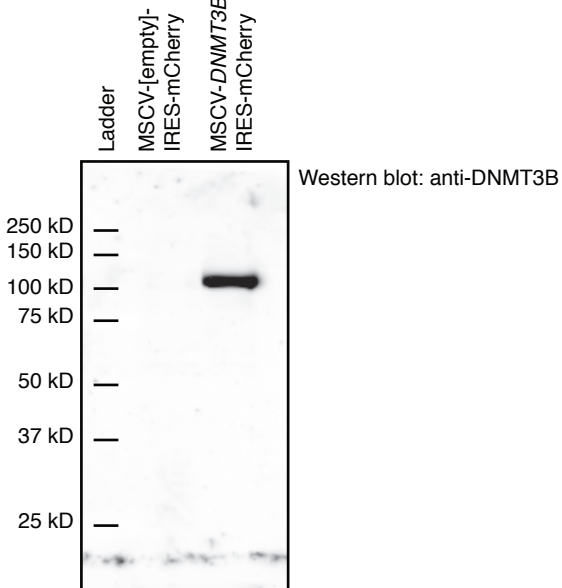
C







# Cole et al. Supplemental Figure S5



### Supplemental Figure S1

(A) Western blot of total protein extracts from E18.5 embryos using an antibody directed against the N-terminus. *Dnmt3a* genotypes are indicated. (B) Total bone marrow cells from 18 day old WT or *Dnmt3a* null mice was harvested and transplanted into lethally transplanted recipient mice. Bone marrow from three independent recipients was then harvested 8 weeks later, and DNA was prepared for CpG capture and bisulfite sequencing. Genome wide distribution of DNA methylation demonstrates a decrease in the fraction of methylated CpGs (green arrow) and a corresponding increase in unmethylated CpGs (red arrow) in *Dnmt3a*<sup>-/-</sup> bone marrow cells. (C) Plot of percent methylation of individual CpGs in wild-type vs *Dnmt3a*<sup>-/-</sup> bone marrow cells. The data represents the average values from three independent mice. CpGs with differential methylation between the two genotypes (adjusted p-value <.05) are highlighted in red. Of the differentially methylated CpGs, 240,000 were significantly hypomethylated in *Dnmt3a* null bone marrow, and 5,400 were more methylated compared to wild-type cells. (D) Specific CpGs are recurrently hypomethylated in *Dnmt3a* null marrow. Unsupervised clustering of the 10,000 most differentially methylated CpGs demonstrates that nearly all are hypomethylated in all three *Dnmt3a* null samples relative to all wild-type samples. (E) Example of the *Runx1* locus on chromosome 16 containing two regions that are entirely dependent on *Dnmt3a* for their methylation as well as additional regions that are *Dnmt3a*-independent. Schematic of *Runx1* exons is shown below the graph. Numbers 1-4 denote regions interrogated in methylation assay in Figure 4F and Supplemental Figure S3.

### Supplemental Figure S2

(A) Bone marrow from 2-2.5 week old WT and *Dnmt3a*<sup>-/-</sup> mice was transplanted into wildtype recipients and allowed to engraft for 16 weeks to assess the ability of *Dnmt3a*-null marrow to engraft and contribute to peripheral blood cells of all lineages. At 16 weeks post-transplant, no differences were observed in numbers of leukocytes, lymphocytes, red blood cells, or platelets, regardless of the presence or absence of *Dnmt3a*. (B) Design of experiment in (C). Bone marrow from 2-2.5 week old mice of the indicated genotypes was transplanted into lethally irradiated wildtype recipients and allowed to engraft for 10 weeks before being harvested and plated in methocult media as in Figure 3. (C) Quantification of colony numbers from transplanted bone marrow demonstrates that the replating deficit in *PR*<sup>+/-</sup> *Dnmt3a*<sup>-/-</sup> bone marrow cells is intrinsic to the bone marrow compartment. \*\*\*  $P < 0.001$  for *PR*<sup>+/-</sup> vs all other genotypes by two-way ANOVA.

### Supplemental Figure S3

Unsupervised hierarchical clustering of differentially expressed genes in sorted GMPs from stably engrafted recipients of WT, *PR*<sup>+/-</sup>, *PR*<sup>+/-</sup> *Dnmt3a*<sup>-/-</sup>, and *Dnmt3a*<sup>-/-</sup> bone marrow. The list of genes with significant variation in expression level was generated by using a fold change of 2 and a 0.05 FDR criterion as a significant cutoff as described in the Methods.

#### Supplemental Figure S4

**(A-C)** Bone marrow from WT, *Dnmt3a*<sup>-/-</sup>, *PR*<sup>+/-</sup>, and *PR*<sup>+/-</sup>, *Dnmt3a*<sup>-/-</sup> mice was harvested and transduced with control YFP vectors, *DNMT3A*, or *DNMT3A* mutants from AML patients before methocult plating and HpaII methylation assay was performed as in Figure 4F. Locations of CpGs 2,3, and 4 in *Runx1* are shown in Figure 1E. **(A)** Region 2 is hypomethylated in *Dnmt3a*<sup>-/-</sup> and *PR*<sup>+/-</sup>, *Dnmt3a*<sup>-/-</sup> cells and methylation is restored to *PR*<sup>+/-</sup>, *Dnmt3a*<sup>-/-</sup> cells by expression of *DNMT3A* but not methylation-deficient mutants R882H or Q615\*. **(B)** Methylation canyon at region 3 is largely unmethylated in all genotype and virus combinations, demonstrating the specificity of *DNMT3A* methylation activity and ability of HpaII to digest the unmethylated DNA. **(C)** Methylation canyon at region 4 likewise demonstrates specificity of *DNMT3A* methylation and effectiveness of HpaII digest. \*\*\* *P*<0.001.

#### Supplemental Figure S5

Western blot demonstrating overexpression of human DNMT3B protein in mouse bone marrow cells. Wildtype bone marrow was transduced with MSCV-[empty]-IRES-mCherry or MSCV-DNMT3B1-mCherry and cells were harvested 48 hours post-transduction and sorted by FACS.

Supplemental Table 1: Regions Targeted for Methylation Sequencing

Site Classification	Number of Targets	Total Bases Covered
CpG Islands	16,027	10,512,276
Tissue-specific DMRs	33,456	10,452,692
-CpG shores and shelves (+/- 4kb)		
-DNase I hypersensitive sites		
-Histone modifications		
-Transcription factor binding sites		
Ensembl Regulatory Features	171,796	91,799,015
-Promoters		
-Enhancers		
-Transcription factor binding sites		
-Regulatory polymorphisms		
Open Regulatory Annotation (OREgAnno)	14,951	9,983,957
Total Merged Intervals	201,788	109,147,819



Supplemental Table S2: Methylation Capture Sequencing Coverage Statistics

	Total reads	Mapped	Unmapped	Unique	Duplicates	Evaluable CpGs (covg ≥10x)		
			WT					
Sample 1	287,531,796	262,118,600	25,413,196	201,034,895	61,083,705	1,905,480		
Sample 2	254,844,017	234,214,689	20,629,328	148,123,163	86,091,526	1,968,501		
Sample 3	296,499,642	235,445,004	61,054,638	196,443,171	39,001,833	1,417,412		
			Dnmt3a <sup>-/-</sup>					
Sample 1	315,543,260	283,235,229	32,308,031	84,657,623	198,577,606	1,948,089		
Sample 2	289,287,027	265,512,324	23,774,703	166,570,302	98,942,022	1,570,078		
Sample 3	278,063,657	248,994,906	29,068,751	72,220,154	176,774,752	1,477,859		





















5058098	chr11	109441031	109441073	-	Wipi1	14.7294	59.3448	57.286	70.4299	278.027	236.184	291.647	223.36	272.795	333.332	130.443	181.101	176.415	50.447525	257.1055	280.2835	162.653	4.62554
4347714	chr6	91442851	91442994	-	Xpc	235.165	230.212	230.23	236.517	636.369	567.573	434.582	417.332	490.147	478.99	305.823	338.952	523.054	233.031	601.971	455.26275	389.2763333	2.06912
5101774	chr18	5766866	5767931	+	Zeb1	166.378	231.73	188.406	217.628	210.499	271.397	353.823	397.434	353.915	426.471	376.99	390.462	335.527	201.0355	240.948	382.91075	367.6596667	1.64402
4810972	chr13	64243530	64243642	-	Zip367	458.457	497.628	432.61	421.846	243.402	264.846	279.036	256.758	295.628	269.628	355.457	326.462	261.24	452.63525	254.124	275.2625	314.3863333	-1.60933
4477660	chr6	42300373	42300465	+	Zyx	235.833	207.888	212.051	125.202	567.675	457.203	394.452	353.999	407.471	369.573	291.499	281.304	378.517	195.2435	512.439	381.37375	317.1066667	2.06736
4777904	chr3	152109803	152109830	+	Zzz3	520.277	461.702	413.161	448.962	289.795	264.3	253.633	296.499	251.476	291.74	374.628	328.499	324.832	461.0255	277.0475	273.337	342.653	-1.54873

Supplemental Table S5: Amplicons for HpaII Methylation Assay

Amplicon Number	Amplicon Name	Chromosome	Start (mm9)	End (mm9)	Primer F	Primer R
1	RB2A1	16	92699722	92699919	AGCTAGACTCCCCTCAAATGC	CCAACGCGATTCTTTAATC
2	RB2A2	16	92700069	92700262	AGT TCG AAG CTC GAT CAG GAT	GGTGCCCTCGGTAGAGT
3	Canyon F5R5	16	92698431	92698688	AGCGGCTGGAGAGGAGATAG	AGGAAGGAGACCCTTAAAGC
4	Canyon F2R2	16	92697248	92697448	GGAACACTTTCTCCCGCAGTTG	CACCCTTGCGGCTCTTTCTAG

**Supplemental Table S6: Flow Markers for Stem Cell and Progenitor Stains**

<b>Population</b>	<b>Cell Phenotype</b>
Lin-neg	B220- CD3e- Gr-1- Ter-119-
HSC	Lin- Sca-1+ c-Kit+ CD34- Flk2-
MPP	Lin- Sca-1+ c-Kit+ CD34+ Flk2+
GMP	Sca-1- c-Kit+ CD34+ CD16/32-
CMP	Sca-1- c-Kit+ CD34+ CD16/32+
MEP	Sca-1- c-Kit+ CD34- CD16/32-