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Supplemental information

Somatic Dnmt3a inactivation leads

to slow, canonical DNA methylation

loss in murine hematopoietic cells

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Supplemental Figures for: Somatic *Dnmt3a* inactivation leads to slow, canonical DNA methylation loss in murine hematopoietic cells

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Week 22 Dynamic DMRs

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10 -log10(FDR)

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Week 22 Dynamic DMBs



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Supplementary Figure 1. GO annotations for dynamic and non-dynamic DMRs located within or near genes (related to Figure 4 and Supplemental Table 3)

Gene ontology enrichment analysis, using genes associated with DMRs (within a gene promoter, gene body, or within 10 kb of a gene). Terms with FDR < 0.001 are shown. a) Enriched terms from DMRs with dynamic (rapid) methylation changes (as defined in the text) at week 1 in the takeaway samples. b) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 1 in the takeaway samples a) Enriched terms from DMRs with dynamic (rapid) methylation changes at week 22 in the takeaway samples. b) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. b) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. B) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. B) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. B) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. B) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. GO enrichment analysis was performed with PANTHER (<u>http://pantherdb.org</u>, PMID: 30804569) using Fisher's Exact Test and FDR correction.



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b Week 1 Non-dynamic DMRs at promoter regions



С Week 22 Dynamic DMRs at promoter regions







Supplementary Figure 2

Supplementary Figure 2. GO annotations for dynamic and non-dynamic DMRs associated with gene promoters only (related to Figure 4 and Supplemental Table 3)

Gene ontology enrichment analysis, using genes associated with DMRs, requiring the DMR to intersect a gene promoter. Terms with FDR < 0.001 are shown. a) Enriched terms from DMRs with dynamic (rapid) methylation changes (as defined in the text) at week 1 in the takeaway samples. b) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 1 in the takeaway samples a) Enriched terms from DMRs with dynamic (rapid) methylation changes at week 22 in the takeaway samples. b) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. b) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. B) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. B) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. B) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. B) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. B) Enriched terms from DMRs with non-dynamic (slow/absent) methylation changes at week 22 in the takeaway samples. GO enrichment analysis was performed as described for Supplemental Figure 1.



Supplementary Figure 3. Differential methylation and expression signatures in hematopoietic progenitors and mature lymphoid cells (related to Figure 5)

- a) Heatmap of mean methylation levels for DMRs identified by comparing progenitors (KLS, MEP, CMP and GMP) and B-cells (CD19+B220+) enriched by FACS sorting. Methylation values for those DMRs in myeloid, T-cells and whole bone marrow are passively plotted.
- b) Venn diagram showing overlap of the 33,519 DMRs defined in *Dnmt3a^{-/-}* bone marrow (pink) vs. DMRs defined for the 2,031 *Dnmt3a^{+/+}* B-cells (green).
- c) Heatmap of mean methylation levels for DMRs identified by comparing progenitors (KLS, MEP, CMP and GMP) vs. T-cells (CD3e+) enriched by FACS sorting. Methylation values for those DMRs in myeloid, B-cells and whole bone marrow are passively plotted.
- d) Venn diagram showing overlap of the 33,519 DMRs defined in *Dnmt3a^{-/-}* bone marrow (pink) vs. DMRs defined for the 2,271 *Dnmt3a^{+/+}* T-cells (green).
- e) Heatmap of mean methylation levels for 33,519 Dnmt3a^{-/-} dependent DMRs with passive plotting for progenitors (KLS, MEP, CMP and GMP), mature myeloid cells (CD11b+/Gr1+), B-cells (CD19+B220+), T-cells (CD3e+) and whole bone marrow (WBM).



Supplementary Figure 4

Supplementary Figure 4. DMRs defined in early progenitor populations persist in terminally differentiated bone marrow cells (related to Figure 5)

Heatmap of mean methylation values for 6,435 DMRs identified by comparing WT vs *Dnmt3a* KO flow-purified progenitor cell populations (KLS and CMP; pooled for this analysis). Methylation values for the same DMRs are passively plotted in both WT and *Dnmt3* KO samples, from whole bone marrow and more committed progenitor cell populations (GMP and MEP).