Supplemental Fig. 1
Supplemental Fig. 2
A.

<table>
<thead>
<tr>
<th>Competitor</th>
<th>DNA fragments with +30.3 kb TREs</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRβ-RXRα</td>
<td>mut</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

B.

<table>
<thead>
<tr>
<th>Competitor</th>
<th>DNA fragments +49.3 kb TRE oligo for +49.3 kb region</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRβ-RXRα</td>
<td>No</td>
</tr>
<tr>
<td>-</td>
<td>WT mut</td>
</tr>
<tr>
<td>+</td>
<td>WT mut TRE</td>
</tr>
</tbody>
</table>

Supplemental Fig. 3
A.

TTACAGGGGCGCCTGTGG AAGTCA GCTG AGGTCA CGGGCAGGGAAATGCCC

B.

Normalized Dnmt3a mRNA

Wildtype | Mutant line AB2

<table>
<thead>
<tr>
<th>T3 Dose (nM)</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wildtype</td>
<td></td>
<td>b</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Mutant line</td>
<td>C</td>
<td>B</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>

C.

Normalized Dnmt3a mRNA

Wildtype | Mutant line A1

<table>
<thead>
<tr>
<th>T3 Dose (nM)</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wildtype</td>
<td></td>
<td>b</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Mutant line</td>
<td>C</td>
<td>B</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>

Normalized Kif9 mRNA

Supplemental Fig. 4
Supplemental Figure Legends

Supplemental Fig. 1. Developmental increases in *Dnmt3a* mRNA and protein in neonatal mouse brain correlate with increases in circulating plasma [T₃] and [T₄]. (A.) *Dnmt3a* mRNA and protein levels peak at PND14 in neonatal mouse frontal cortex. The mRNA was measured by RNA-seq (FPKM, fragments per kilobase of exon per million fragments mapped) and protein by Western blotting (normalized to actin) (1). (B.) Like *Dnmt3a* mRNA and protein, circulating plasma [T₃] and [T₄] also reach their highest level at PND14 in mouse as measured by radioimmunoassay. Points represent the mean ± SEM (n ≥ 4) (2). Both figures were modified with permission from the authors.

Supplemental Fig. 2. DNA sequence of putative TREs located +30.3 kb from the transcription start site (TSS) of the mouse *Dnmt3a* gene. Shown is the DNA sequence of 500 bp centered at +30.3 kb from the TSS of the mouse *Dnmt3a* gene. We identified seven putative TREs (underlined) using sequence analysis within this region. Duplex oligonucleotides (Supplemental Table 2) for competitive electrophoretic mobility shift assay shown in Fig. 4A were designed to encompass these putative TREs. Mutation of predicted TREs #2, 3 and 4 caused partial reduction in T₃-dependent transactivation of the DNA fragment, while mutation of predicted TRE#6 nearly abolished transactivation (Fig. 4B).
Supplemental Fig. 3. TRβ-RXRα bind in vitro to predicted TREs associated with the mouse Dnmt3a locus. We conducted competitive electrophoretic mobility shift assay (EMSA) using a [32P]-labeled duplex oligonucleotide probe corresponding to a TRE within the X. tropicalis dnmtn3agene that we have characterized (Y. Kyono and R.J. Denver, unpublished data; oligonucleotide sequences given in Supplemental Table 2). Before gel electrophoresis we incubated the probe with in vitro synthesized TRβ and RXRα with or without the indicated radioinert oligonucleotides (1 μM) or PCR-generated DNA fragments (~20 nM); the DNA fragments included the predicted TREs located in regions +30.3 or +49.3 kb relative to the transcription start site of the mouse Dnmt3a gene. Plasmids containing wild type or mutant predicted TREs (used for transient-transfection-reporter assays; see Fig. 4 B&D) were used as templates for PCR to generate DNA fragments for competitive EMSA (see Supplemental Table 2 for oligonucleotide primer sequences.) The arrows indicate the location of shifted bands due to TRβ-RXRα binding to the probe. (A.) A radioinert DNA fragment (277 bp) corresponding to a sequence located within a 500 bp genomic region centered at +30.3 kb from the transcription start site (TSS) of the mouse Dnmt3a gene that encompassed predicted TREs 2, 3, 4, 5 and 6 was used as competitor in EMSA (wild type – WT; see Supplemental Fig. 2 for the location of these putative TREs and Supplemental Table 2 for oligonucleotide primer sequences that we used to generate DNA fragments by PCR). Same-size DNA fragments containing single base pair mutations (mut) at each of the two half sites of one of the TREs #2, 3, 4 or 6 were also used as competitor DNAs (Supplemental Table 3; predicted TRE #5 was not mutated because a duplex oligonucleotide corresponding to this sequence did not compete for TRβ-RXRα binding.
to the probe; see Fig. 4A). The radioinert WT DNA fragment, and fragments with mutations in TREs #2, 3, or 4 completely displaced TRβ-RXRα binding to the probe; whereas, the DNA fragment that contained the mutant TRE#6 sequence did not compete for binding. (B.) A radioinert duplex oligonucleotide corresponding to the sequence of the wild type (WT) TRE located +49.3 kb from the TSS of the mouse Dnmt3a gene sequence completely displaced TRβ-RXRα binding to the probe, while an oligonucleotide with single base mutations in the two half sites of this TRE (mut) exhibited reduced competition for binding. A radioinert DNA fragment (180 bp) that encompasses the predicted TRE sequence located at +49.3 kb completely displaced TRβ-RXRα binding to the probe (WT), and single base pair mutations introduced at each of the two half sites of this TRE (see Supplemental Table 3) slightly reduced the ability of this DNA fragment to compete for binding. This suggests that TRβ-RXRα may bind additional sequences in vitro in this region that our bioinformatics analysis did not identify; mutation of the predicted TRE within this DNA fragment reduced but did not abolish T3-dependent transactivation in transient transfection assays (Fig. 4D). A DNA fragment that did not contain a predicted TRE (immediately 3’ to the +49.3 kb TRE) was used as a negative control.

**Supplemental Fig. 4. Deletion of the Dnmt3a +30.3 kb DR+4 TRE in Neuro2a[TRβ1] cells strongly reduced the response to T3.** We used CRISPR/Cas9 genome editing in Neuro2a[TRβ1] cells to generate bi-allelic mutations at the Dnmt3a DR+4 TRE located +30.3 kb from the transcription start site. We transfected cells with two overlapping guide RNA/Cas9 plasmid constructs, alone (A or B) or in combination (AB); the locations of the two gRNAs relative to the +30.3 TRE are shown in panel A. The mutant
DNA sequences are given in Supplemental Table 5 for mutant lines AB2 and A1. B. Treatment of with T₃ caused a dose-dependent increase in Dnmt3a mRNA in the wild type and in mutant cells, but the magnitude of the responses in mutant lines AB2 and A1 was strongly reduced. The mRNA response to T₃ of the direct T₃-response gene Krüppel-like factor 9 (Klf9) was unaffected by mutation at the Dnmt3a locus. Bars represent the means ± SEM, and letters above the means indicate significant differences among treatments within a genotype; means with the same letter are not significantly different (P<0.05 Fisher’s LSD test). Left panel: Dnmt3a mRNA, wildtype F(3,15)= 40.888, p<0.001; mutant line AB2: F(3,16)=18.956, p<0.0001; Klf9 mRNA, wildtype F(3,15)=20.528, p<0.0001; mutant line AB2: F(3,16)= 166.654, p<0.0001. Right panel: Dnmt3a mRNA, wildtype F(3,16)= 37.513, p<0.001; mutant line A1: F(3,16)=32.403, p=0.431; Klf9 mRNA, wildtype F(3,16)=95.762, p<0.0001; mutant line A1: F(3,16)= 70.082, p<0.0001.
Reference


Supplemental Table 1. Oligonucleotide sequences used for quantitative PCR analysis of gene expression (RTqPCR) or chromatin immunoprecipitation (ChIP) assay.

**For SYBR Green RTqPCR**

*Dnmt3a* mRNA  
Forward: 5’ CCT GTG GTG CAC TGA AAT GG 3’  
Reverse: 5’ TCG CCA AGC GGC TCA T 3’

*Klf9* mRNA  
Forward: 5’ TCC TCC CAT CTT AAA GCC CAT 3’  
Reverse: 5’ CCG AGC GCG AGA ACT TT 3’

*GAPDH* mRNA  
Forward: 5’ TGT GTC CGT CGT GGA TCT GA 3’  
Reverse: 5’ CTT CAC CAC CTT GAT GTC ATC 3’

**For ChIP assays**

*Dnmt3a* +30.3 kb TSS  
Forward: 5’ CAG AGG AGG GAA CAC TGT ATA A 3’  
Reverse: 5’ CAA ACA CAA GCC CAG ATG TC 3’

*Dnmt3a* +39.0 kb TSS (negative control region)  
Forward: 5’ AGT GAG GCA GGA CTT TCT AGG TAT G 3’  
Reverse: 5’ ACC TCT GCT TTG CTC CTA CTC AA 3’

*Dnmt3a* +49.3 kb TSS  
Forward: 5’ CCA ACT TCC TCC AGG GTT ATT T 3’  
Reverse: 5’ GCC CTT GCT GGG TTA TTC T 3’
Supplemental Table 2. Oligonucleotides used to generate DNA fragments for plasmid constructs used in transfection-reporter assays, electrophoretic mobility shift assay (EMSA) and CRISPR/Cas9 genome editing.

Sequences of primers used to amplify genomic fragments by PCR for subcloning into pGL4.23/pCpGL luciferase vectors (restriction sites are underlined)

\[ \text{Dnmt3a} +30.3 \text{ kb TSS (pCpGL)} \]
Forward: 5’ CCC AGA TCT TGG GAC AAA CTC CTC TCA TC 3’
Reverse: 5’ GCC CAA GCT TGC TCT AGC CTT TGT CTA ACC 3’

\[ \text{Dnmt3a} +49.3 \text{ kb TSS (pGL4.23)} \]
Forward: 5’ AGC GGT ACC GGG ATT TAT ACC CAC CAA CT 3’
Reverse: 5’ TGA CGA GCT CCA GCT TGC TTC TGG AAG GCC A 3’

Sequences of primers used for site-directed mutagenesis of pGL4.23/pCpGL luciferase vectors (lowercase letters indicate mutated nucleotides; shading indicates TRE half sites)

\[ \text{Dnmt3a} +30.3 \text{ kb TSS TRE #2} \]
Forward: 5’ CTC CCT CTT CAG GtA GAT GTG ACT aCA GGA CAT CTC GG 3’
Reverse: 5’ CCC AGA TGT CCT GtA GTC TCT aCC TGA AGA GGC AG 3’

\[ \text{Dnmt3a} +30.3 \text{ kb TSS TRE #3} \]
Forward: 5’ GCC TCT TCA GGG AGA TaT GAC TTC AGa ACA TCT GGG CTT G 3’
Reverse: 5’ CAA GCC CAG ATG TiC TGA AGT CAT ATC TCC CTG AAG AGC C 3’

\[ \text{Dnmt3a} +30.3 \text{ kb TSS TRE #4} \]
Forward: 5’ GGA GAT GTG ACT TCA GaA CAT CTG GaC TTG TGT TTG CTC AGC 3’
Reverse: 5’ GCT GAG CAA ACA CAA GtC CAG ATG TiC TGA AGT CAC ATC TCC 3’

\[ \text{Dnmt3a} +30.3 \text{ kb TSS TRE #6} \]
Forward: 5’ GCC TGT GGA AGc CAG CTG AGa TCA CGG CCA GG 3’
Reverse: 5’ CCT GCC CTG GtA CTC AGC TiG CTT CCA CAG GC 3’

\[ \text{Dnmt3a} +49.3 \text{ kb TSS TRE} \]
Forward: 5’ GGG TGC ATT CTG aGG cCA CTC TTG aTA AGA ATA ACC CAG C 3’
Reverse: 5’ GCT GGG TTA TTG TTA tCA AGA GTG GcC TCA GAA TGC ACC C 3’

Oligonucleotides used for EMSA (lowercase letters indicate non-native nucleotides added to the 5’ ends for [\(^{32}\)P] labeling by Klenow fill-in)

\[ \text{Xenopus tropicalis dnmt3a TRE} \]
Forward: 5’ gat cTG CAG CGC AGA GTC AAA CCA GGT AAC CCA CTG GG 3’
Reverse: 5’ gat cCC CAG TGG GTT ACC TGG TTT GAC TCT GCG CTC GA 3’

\[ \text{Dnmt3a} +30.3 \text{ kb TSS TRE #1} \]
Forward: 5’ gat cCG CTT TGA AGC AGA GGA GGG AAC ACT GTA T 3’
Reverse: 5’ gat cAT ACA GTG TTC CCT CTG CTT CAA AGC G 3’

\[ \text{Dnmt3a} +30.3 \text{ kb TSS TRE #2-4} \]
Forward: 5’ gat cCT TCA GGG AGA TGT GAC TTC AGG ACA TCT GGG CTT GTG TTT 3’
Reverse: 5’ gat cAA ACA CAA GCC CAG ATG TCC TGA AGT CAC ATC TCC CTG AAG 3’

*Dnmt3a* +30.3 kb TSS TRE #5
Forward: 5’ gat cGT AAC TCT AGC CTT GCC TGT CTT ACA GGG C 3’
Reverse: 5’ gat cGC CCT GTA AGA CAG GCA AGG CTA GAG TTA C 3’

*Dnmt3a* +30.3 kb TSS TRE #6
Forward: 5’ gat cCC TGT GGA AGT CAG CTG AGG TCA CGG GCA G 3’
Reverse: 5’ gat cCT GCC GTT GAC CTC AGC TGA CTT CCA CAG G 3’

*Dnmt3a* +30.3 kb TSS TRE #7
Forward: 5’ gat cAG ATT CTG GGA CTG ATA GAG TGA GCG TAT T 3’
Reverse: 5’ gat cAA TAC GCT CAC TCT ATC AGT CCC AGA ATC T 3’

*Oligonucleotide primers used to generate double stranded DNA fragments for competitive EMSA*

*Dnmt3a* +30.3 kb TSS with TRE (wild-type, and mutant #2, 3, 4 and 6)
Forward: 5’ CAG AGG AGG GAA CAC TGT ATA A 3’
Reverse: 5’ CCC TCA TAA TTG GAC CAG AC 3’

*Dnmt3a* +49.3 kb TSS with TRE (wild-type and mutant)
Forward: 5’ AGC GGT ACC GGG ATT TAT ACC CAC CAA CT 3’
Reverse: 5’ GGC TGC TGT GGA GTA AGT A 3’

*Dnmt3a* +49.3 kb TSS with no TRE
Forward: 5’ TAC TTA CTC CAC AGC AGC C 3’
Reverse: 5’ CCA AAT GGC TCT TAG ACT GG 3’

*Guide RNA sequences used for CRISPR/Cas9 genome editing in Neuro2a[TRB1] cells*
gRNA-A 5’ GCGCCTGTTGGAAAGTCAGCTG 3’
gRNA-B 5’ GTGGAAGTCAGCTGAGGTCA 3’

*Oligonucleotide primers used to PCR amplify the Dnmt3a +30.3 kb TRE genomic region*
Forward: 5’ CCC AGA TCT TGG GAC AAA CTC CTC TCA TC 3’
Reverse: 5’ GCC CAA GCT TGC TCT AGC TTA TGT CTA ACC 3’
Supplemental Table 3. List of native and mutant TRE sequences within the mouse Dnmt3a locus. Half sites are underlined. Mutated nucleotides are in lower case and highlighted.

+30.3 kb TRE#2
Native: CTGCCCTCTTCA GGGAGA TGTG ACTTCA GGACATCTGGG
Mutant: CTGCCCTCTTCA GgAGA TGTG ACTaCA GGACATCTGGG

+30.3 kb TRE#3
Native: GCCTCTTCAGGGAG ATGTGA CTTC AGGACA TCTGGGCTTGG
Mutant: GCCTCTTCAGGGAG ATaTGA CTTC AGaACA TCTGGGCTTGG

+30.3 kb TRE#4
Native: GGAGATGTGA CTTC AGGACA TCTGGGCTTGG
Mutant: GGAGATGTGA CTTC AGaACA TCTGGGCTTGG

+30.3 kb TRE#6
Native: GCCTGTGG AAGTCA GCTG AGGTCA CGGGCAGG
Mutant: GCCTGTGG AAGaCA GCTG AGTCA CGGGCAGG

+49.3 kb TRE
Native: GGGTGCAATTCTG AGGTCA CTCT TGCTAA GAATAACCCAGC
Mutant: GGGTGCAATTCTG AGGaCA CTCT TGaTAA GAATAACCCAGC
Supplemental Table 4. Genomic coordinates for TR binding sites associated with the mouse *Dnmt3a* locus identified by ChIP-seq analyses

<table>
<thead>
<tr>
<th>Peak</th>
<th>Coordinates (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>chr12: 3,806,012-3,806,644</td>
</tr>
<tr>
<td>2</td>
<td>chr12: 3,807,928-3,809,079</td>
</tr>
<tr>
<td>3</td>
<td>chr12: 3,812,247-3,813,164</td>
</tr>
<tr>
<td>4</td>
<td>chr12: 3,837,246-3,837,346</td>
</tr>
<tr>
<td>5</td>
<td>chr12: 3,841,058-3,841,690</td>
</tr>
<tr>
<td>6</td>
<td>chr12: 3,859,292-3,860,559</td>
</tr>
<tr>
<td>7</td>
<td>chr12: 3,866,399-3,867,232</td>
</tr>
<tr>
<td>8</td>
<td>chr12: 3,868,339-3,869,565</td>
</tr>
<tr>
<td>9</td>
<td>chr12: 3,889,702-3,890,300</td>
</tr>
<tr>
<td>10</td>
<td>chr12: 3,894,480-3,895,087</td>
</tr>
<tr>
<td>11</td>
<td>chr12: 3,911,787-3,912,497</td>
</tr>
<tr>
<td>12</td>
<td>chr12: 3,919,268-3,919,956</td>
</tr>
</tbody>
</table>

**TR ChIP-seq conducted on mouse liver (1)**

**TR ChIP-seq conducted on the mouse cerebellar-derived cell line C17.2 (2)**

Peak 13 (+49.3 kb TSS): chr12: 3,855,855-3,856,520

(1) Ramadoss et al. (24) Coordinates are based on the Mm9 genome build.
(2) Chatonett et al. (25) Coordinates are based on the Mm9 genome build.
Supplemental Table 5. Neuro2a[TR\(\beta\)1] deletion mutants at the Dnmt3a +30.3 TRE created by CRISPR/Cas9 genome editing.

<table>
<thead>
<tr>
<th>Wildtype</th>
<th>Dnmt3a +30.3 TRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGTCAGAGTCAAGGGCAAGGAAGTGCCCTGCAG</td>
<td></td>
</tr>
<tr>
<td>Mutant AB2</td>
<td></td>
</tr>
<tr>
<td>5 bp deletion</td>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGT---------GAGGTCACGGGCAGGGAAGTGCCCTGCAG</td>
</tr>
<tr>
<td>24 bp deletion</td>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGT---------------------GGCAGGGAAGTGCCCTGCAG</td>
</tr>
<tr>
<td>Mutant A1</td>
<td></td>
</tr>
<tr>
<td>10 bp deletion</td>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGTCA------------GAGGTCACGGGCAGGGAAGTGCCCTGCAG</td>
</tr>
<tr>
<td>13 bp deletion</td>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGTCAAGCTG-------------CAGGGAAGTGCCCTGCAG</td>
</tr>
<tr>
<td>31 bp deletion</td>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGTCAAGCTGAGG-----------------------------AG</td>
</tr>
<tr>
<td>6 bp deletion</td>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGTCAAGCTGAGG--------GCAGGGAAGTGCCCTGCAG</td>
</tr>
<tr>
<td>2 bp deletion</td>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGTCAAGCTGAGG--------ACGAGGGAAGTGCCCTGCAG</td>
</tr>
<tr>
<td>10 bp deletion</td>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGTCAAGCTGAGG---------GGGAAAGTGCCCTGCAG</td>
</tr>
<tr>
<td>11 bp deletion</td>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGTCAAGCTGAGG---------GAAGTGCCCTGCAG</td>
</tr>
<tr>
<td>10 bp deletion</td>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGTCAAGCTGAGG---------CGCAGGGAAGTGCCCTGCAG</td>
</tr>
<tr>
<td>31 bp deletion</td>
<td>TTGCGCTGTCTTTACAGGGCGGCTGTGGAAGTCAAGCTGAGG---------AG</td>
</tr>
</tbody>
</table>

The two half sites of the DR+4 TRE located +30.3 kb upstream of the mouse Dnmt3a transcription start site are shaded in gray in the wild type sequence. The nucleotides targeted by the gRNAs are shown in Supplemental Fig. 4A.