**Supplemental Fig. 1**

**A.**

![Graph showing normalized dnmt3a mRNA over time after T3 (hr).](image)

**B.**

![Images showing tissue sections with and without T3 treatment.](image)
Supplemental Fig. 2

<table>
<thead>
<tr>
<th>Competitor</th>
<th>$dnmt3a$ region A</th>
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Supplemental Figure Legends

**Supplemental Fig. 1.** Exogenous T3 induces *dnmt3a* mRNA in *X. laevis* tadpole brain. (A.) We treated early prometamorphic tadpoles (NF stages 53-54) with vehicle (0.0003% DMSO) or T3 (5 nM) for 0, 2, 4, 6, 8, 12, 24 and 48 hr and measured *dnmt3a* mRNA by RTqPCR. We normalized *dnmt3a* mRNA to *alpha-actinin* mRNA which did not change with T3 treatment (data not shown). Points represent the mean ± SEM (n=5-6/time point/treatment), and asterisks above the means indicate a statistically significant increase in *dnmt3a* mRNA compared to the 0 hr time point (1.6-fold at 24 hr; 2.1-fold at 48 hr; $F_{(6,34)} = 13.420, p < 0.001$; ANOVA). Vehicle treatment did not alter the mRNA level at any time point (data not shown). (B.) We treated early prometamorphic *X. laevis* tadpoles (NF stages 53-54) with vehicle (0.0003% DMSO) or T3 (5 nM) for 48 hr and conducted ISHH for *dnmt3a* mRNA (n=3-4/treatment). Shown are representative photomicrographs of two transverse sections at anatomical positions 1 and 3 shown in Fig. 1B. Scale bars = 160 µm.

**Supplemental Fig. 2.** Regions A and B of the *Xenopus tropicalis* *dnmt3a* gene possess sequences that competitively displace TRβ-RXRα binding to a radiolabeled probe *in vitro*. We conducted competitive electrophoretic mobility shift assays (EMSA) using a $[^{32}P]$-labeled duplex oligonucleotide probe corresponding to the T3RE-B2 sequence from the *X. tropicalis* *dnmt3a* locus (Supplemental Table 3). Before gel electrophoresis we incubated the probe with *in vitro* synthesized *Xenopus* TRβ and RXRα with or without the indicated radioinert PCR-generated DNA fragments (~20 nM each); the DNA fragments included one of the predicted TREs located in regions A or B of the *X. tropicalis* *dnmt3a* gene (TRE-A1 or -A2, TRE-B1, -B2...
or -B3; see Fig. 4 for the location of these predicted TRES). Plasmids containing wild type or
mutant predicted TRES (used for transient-transfection-reporter assays; see Fig. 5C) were used as
templates for PCR to generate DNA fragments for competitive EMSA (see Supplemental Table
3 for oligonucleotide primer sequences used to amplify the DNA). The arrow indicates the
location of shifted bands due to TRβ-RXRα binding to the probe.

Radioinert DNA fragments of the native region A, or region A with mutations introduced
into the TRE-A1 sequence both displaced TRβ-RXRα binding to the probe. This suggests that
TRβ-RXRα may bind additional sequences within this region in vitro that our bioinformatics
analysis did not identify. However, because mutations at TRE-A1 within this DNA fragment
completely abolished T3-dependent transactivation in transient transfection assays (Fig. 5C), it is
unlikely that these additional sequences represent functional TRES. The radioinert DNA
fragment containing only the predicted TRE-A2 sequence displaced TRβ-RXRα in vitro. Again,
mutations in TRE-A1 within the DNA fragment encompassing region A (which still contained
the native TRE-A2 sequence) completely abolished T3-dependent transactivation in transient
transfection assay (Fig. 5C).

Radioinert DNA fragments that encompass the wild type TRE-B2 sequence completely
displaced TRβ-RXRα binding to the probe, while those that contain T3RE-B1, T3RE-B3, or the
mutant T3RE-B2 sequences did not. A DNA fragment that did not contain a predicted T3RE
(immediately 5’ to the T3RE-B3) was used as a negative control.