



Supplemental Fig. S1. Experimental design and quality control analysis for profiling gene expression changes induced by T3 and BPA during microarray analysis. (A) Tadpoles of similar sizes and stages (stages 54) were divided into 4 treatment groups. Each group consisted of 10 animals. Groups 1 represents tadpoles treated with vehicle only and therefore represent the control group. Group 2 were treated with 10 mM BPA, Group 3 were treated with 2 nM T3 and Group 4 were treated with 2nM T3 and 10 mM BPA. Animals were treated for 4 days. For microarray analysis, the intestine from each treatment group was pooled, and each treatment group was performed in triplicate. Total RNA was isolated and Cy3-CTP-labeled cRNA was prepared. The universal reference mRNA was isolated from whole tadpoles at stages throughout metamorphosis (stages 50 – 66) and was labeled with Cy5-CTP. A two-color reference design, where each RNA sample is hybridized with the same reference sample to the array was used to make the data directly comparable among different experiments. (B) The dendrogram of the 4 treatment groups revealed that the three replicates within each treatment group were most similar to each other and that control and BPA treatments were more similar to each other than to the T3 only and T3+BPA combined groups.