

Figure S1. Exposure to xMLTK-MO, thioridazine, ethanol, and ICI 118,551 produces consistent craniofacial phenotypes. Typical range of craniofacial phenotypes observed with these perturbations. For experimental groups the phenotypes are arranged from most abnormal on the left to least abnormal on the right. Morpholino-based knock-down of xMLTK and finite pharmacological (e.g. thioridazine, ethanol, and ICI 118,551) exposures during neurula stages produce abnormal phenotypes with little variation, relative to most teratogens. White asterisks denote the injected side of xMLTK-MO tadpole. Scale bars: 500 μ m.

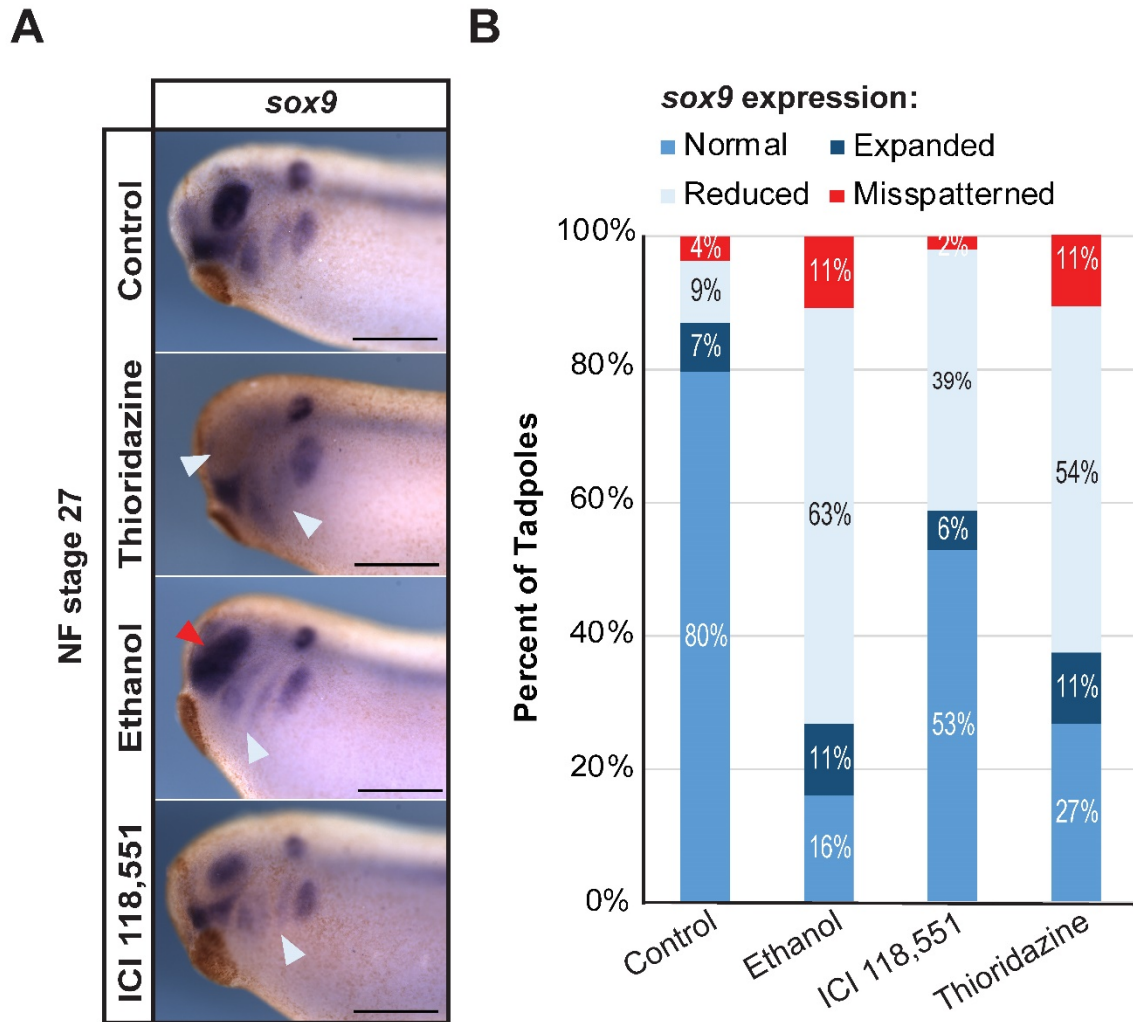


Figure S2. Whole-mount *in situ* hybridization for *sox9* on embryos following exposure to ethanol, ICI 118,551, and thioridazine highlight their effects on neural crest cell localization. A) Representative images showing the range of *sox9* spatial patterning in control and experimental tadpoles. Grey arrowheads point to regions of reduced *sox9* expression, red arrowheads point to mispatterned *sox9* expression. B) Quantification of scored *sox9* phenotypes in control and experimental tadpoles. N = 2, n = 25-29, for a total n of 54 for controls, 56 for ethanol, 51 for ICI 118,551, and 56 for thioridazine. Scale bars: 500µm.

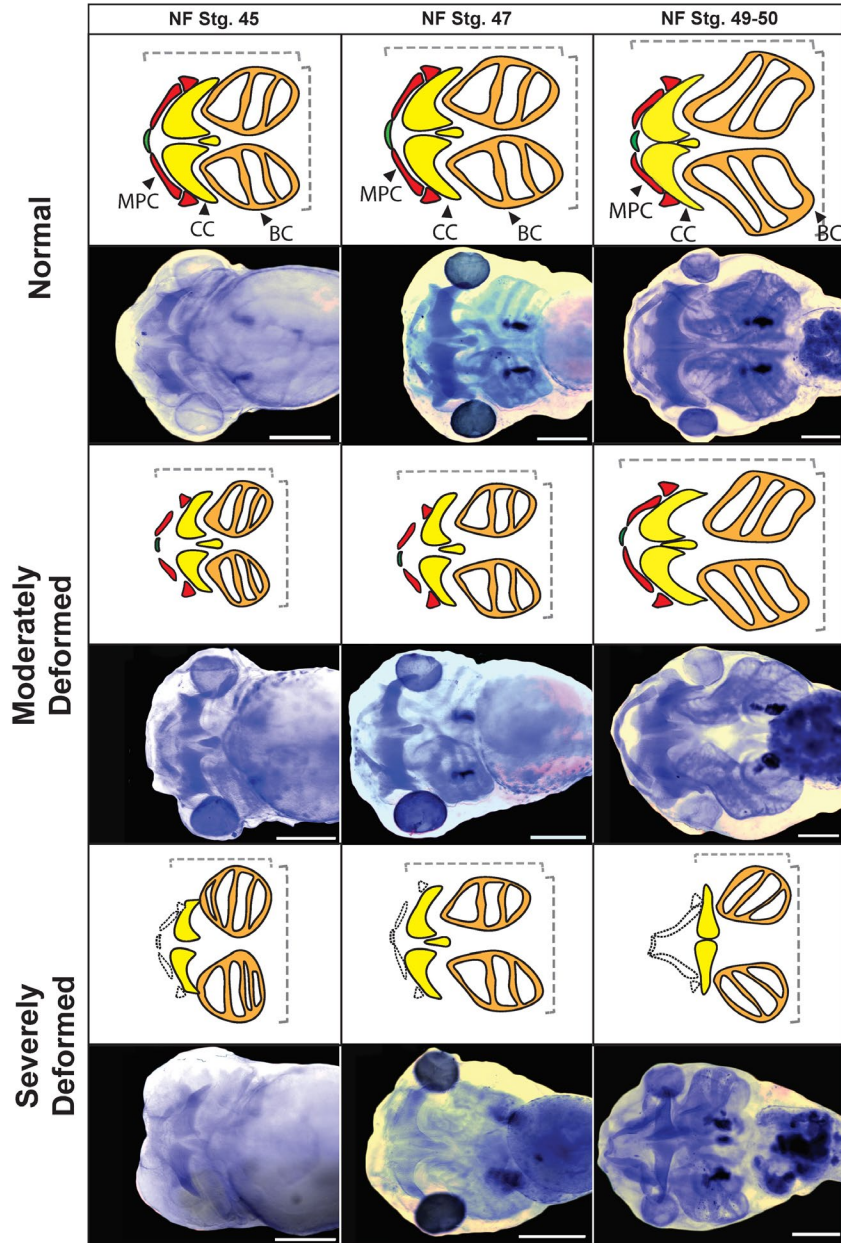


Figure S3. Schematics for cartilage phenotype scoring categories, based on Alcian blue staining. Bleached and Alcian blue stained NF stg. 45-50 specimen, with corresponding WT cartilage schematics. Craniofacial cartilage phenotypes were qualitatively scored as normal, moderate, or severe based on the shape and presence of these major craniofacial cartilaginous features. MPC = Meckel's and Palatoquadrate cartilage, CC = Ceratohyal cartilage, BC = Branchial cartilage. Schematics for severe phenotypes leave uncolored, but outlined, the features commonly missing entirely from the most abnormal specimen. Scale bars: 500 μ m.

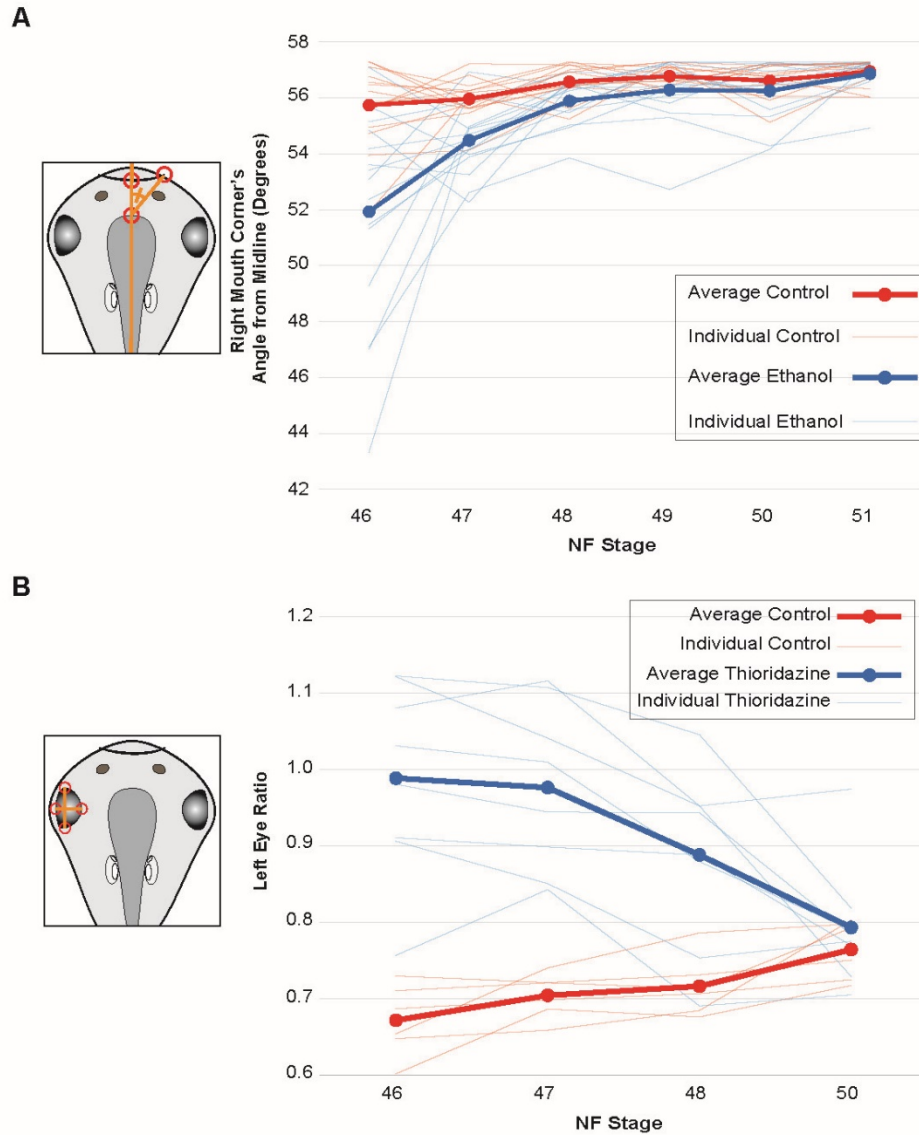


Figure S4. Geometric morphometric analysis in Control, thioridazine, and ethanol tadpoles at pre-metamorphic stages 46-50/51. Morphological metrics (e.g. left eye ratio and right mouth corner-midline angle) were quantified at NF stg 45 through 50 for wild-type control and thioridazine tadpoles. Measurements were taken from Individually tracked tadpoles across several stages, light blue and red lines represent individual experimental tadpoles and control tadpoles, respectively. N = 1 biological replicate, n = 8-15 tadpoles. Opaque blue and red lines represent experimental and control group means, no error bars or statistical tests were applied because data are from a single biological replicate.

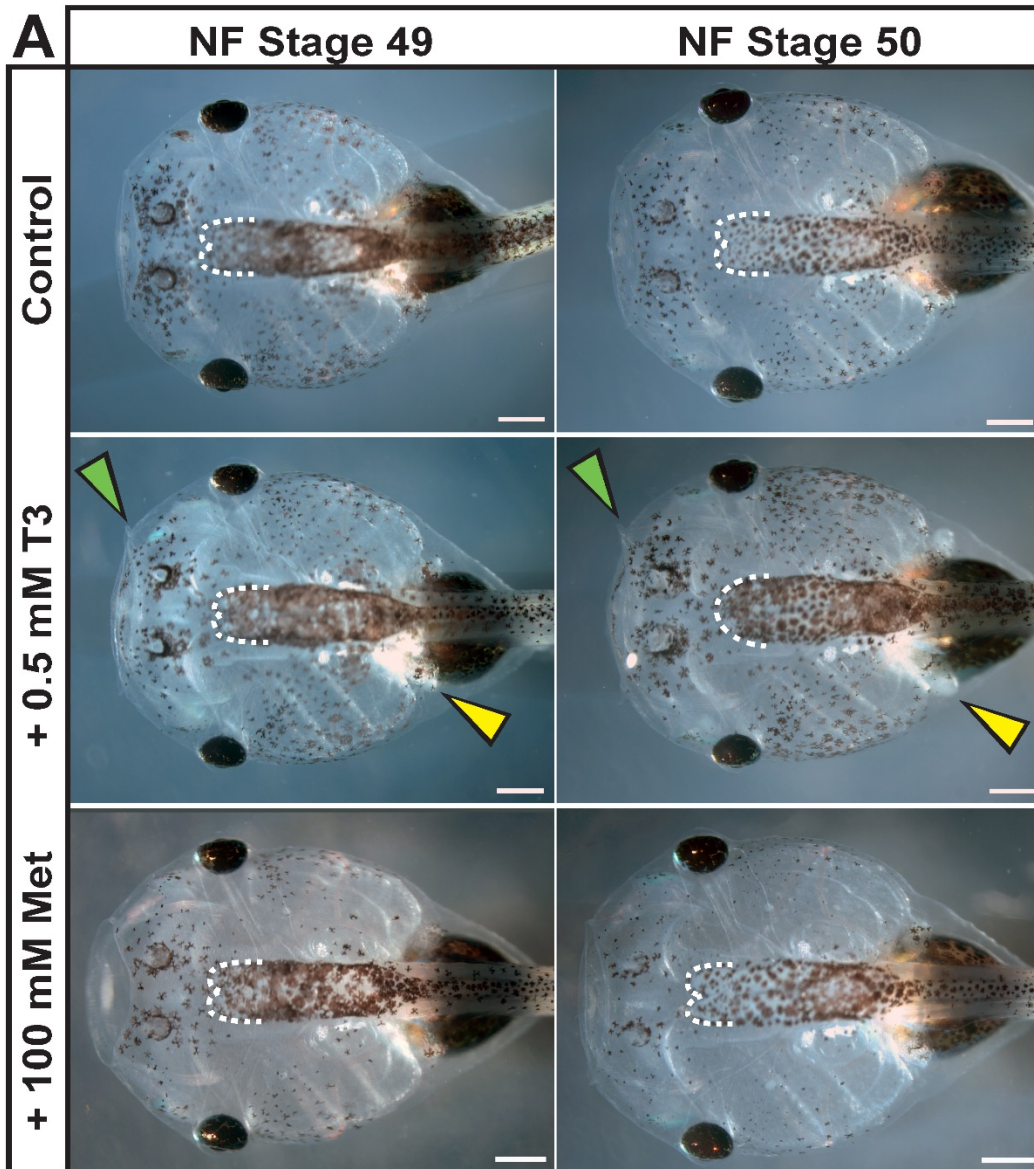


Figure S5. T3 exposure leads to precocious limb bud and barbel growth by NF stages 49 and 50. Representative images of control, +T3 (thyroid hormone), and +Met (methimazole) tadpoles at NF stage 49 and 50. The yellow and green arrow heads point to precocious growth of barbels and limb buds, respectively. The white dashed outline highlights premature or accelerated fusion of the olfactory bulbs in +T3 tadpoles. Scale bars: 500 μ m.

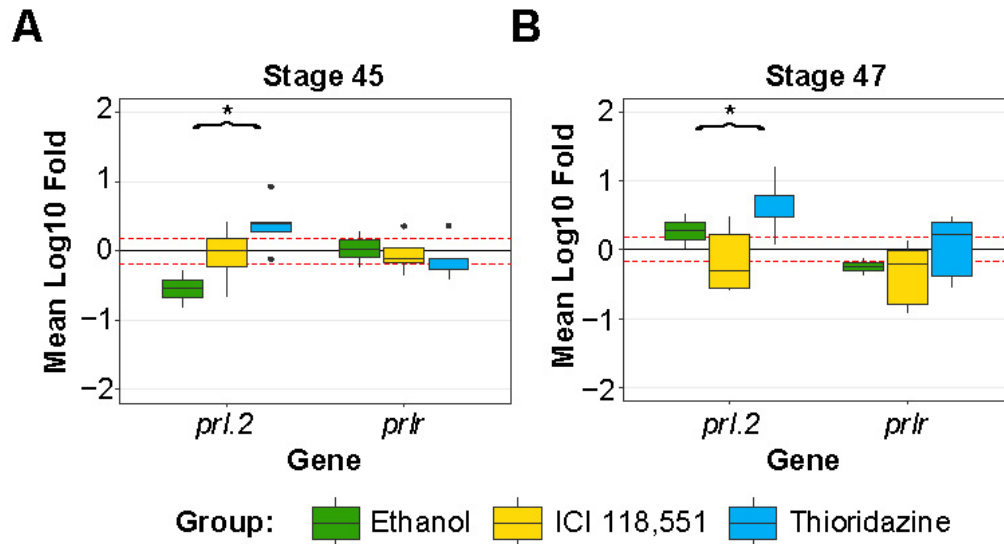


Figure S6. *Prolactin.2.S* is differentially expressed in ethanol and thioridazine tadpoles at NF stage 45 and 47. A,B) Expression of *prolactin.2.S* and *prolactin receptor* was quantified in ethanol, thioridazine, and ICI 118,551 tadpoles at NF stage 45 and 47, respectively. Mean log₁₀ fold candidate gene expression is relative to the WT control group (control log₁₀ fold change = 0); mean gene expression levels for controls and experimental tadpoles were first normalized to the eukaryotic elongation factor 1a (*eef1a*) gene. The red dashed line denotes a conservative biological significance threshold (1.5x fold change). N = 2-3 biological replicates, n = 10 tadpoles per group per stage. * = KW test, p < 0.05.

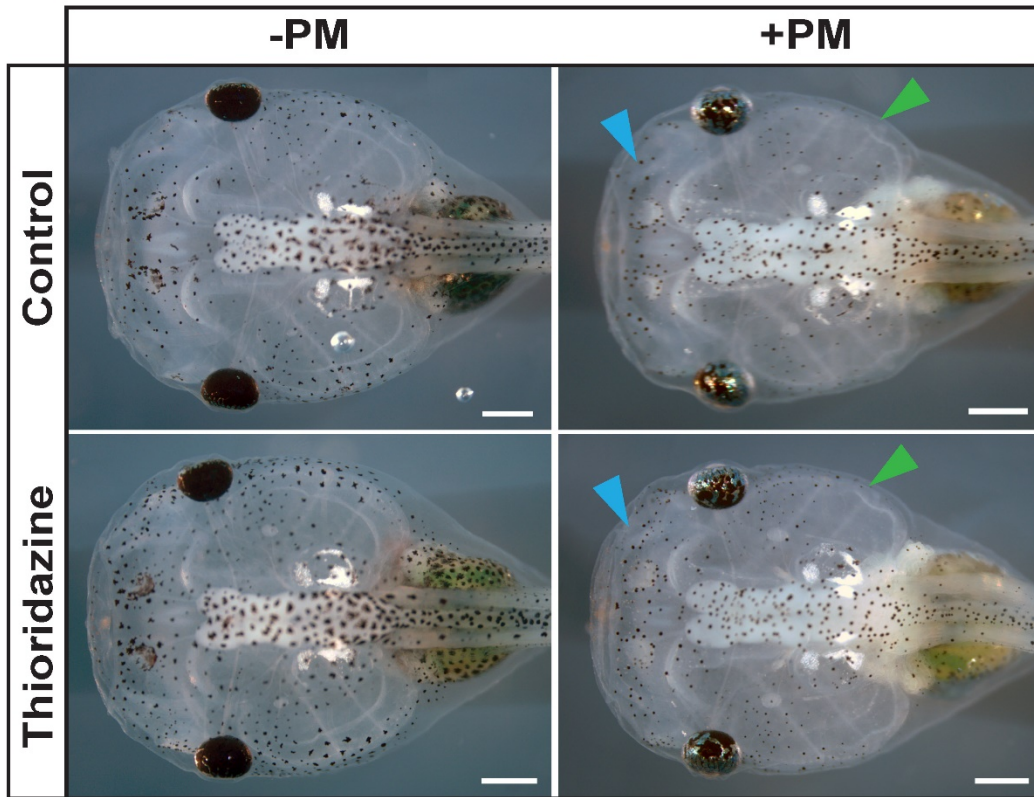


Figure S7. Pergolide mesylate exposure affects the growth and morphology of anterior craniofacial structures and branchial arches. Representative images of wild-type control and thioridazine tadpoles at NF stg 50 that were either left in untreated media (-PM) or exposed to pergolide mesylate (+PM) from NF stg 45 to 50. Pergolide mesylate exposure, reduces branchial arches and anterior CF features, altering the overall head morphology of +PM specimen. Blue arrowhead denotes underdeveloped anterior craniofacial region and green arrowhead denotes reduced branchial arch width, relative to -PM control. Scale bars: 500 μ m.

Table S1. Differential gene expression in head tissue of tadpoles with thioridazine-induced craniofacial defects at NF stage 45 (Excel file).

[Click here to download Table S1](#)

Table S2. Differential gene expression in head tissue of tadpoles with thioridazine-induced craniofacial defects at NF stage 47 (Excel file).

[Click here to download Table S2](#)

Table S3. RT-qPCR Primer sequences.

Gene	Gene Symbol	NCBI Gene ID	Forward & Reverse Primers (5'-3')
<i>thra</i>	<i>thra.L</i>	397942	F: AGAAGCTGCCCATGTTCTCT R: ACCTCCGTTCTTAAGCTGCT
<i>thrβ</i>	<i>thrb.L</i>	779054	F: CATAGTTAATGCGCCCGAGG R: TGTCACTGCCATCTCACCAT
<i>rxra</i>	<i>rxra.L</i>	378685	F: AAGTACCCATGCATCCCTCC R: GTCTTTGCTATCCCTGCACG
<i>rara</i>	<i>rara.L</i>	399081	F: AGGAGCGAGTTTCTCTGGAC R: GCATCTGAGTGCGGTTTAGG
<i>mmp2</i>	<i>mmp2.S</i>	380389	F: ATTCTGGTCGCTCAGATGGC R: GTTCCGGACAGAAGCCGTA
<i>prl.2</i>	<i>prl.2.S</i>	108697263	F: CCGTCAGATTTTAGGGAAAGCC R: CTGGTTCCATGAGCGCAGTA
<i>prlr</i>	<i>prlr.L</i>	373618	F: ATATGGGCATTGCTGCACGA R: CCAGGTAGAGACTGCGCGTT
<i>mmp1</i>	<i>mmp1.S</i>	495287	F: AAAGAATTGATGCGGCTGTTCA R: GAGCTTGGGGTCCGTCTTATT
<i>mmp13</i>	<i>mmp13.S</i>	379564	F: TCCTCCAGACGAGCAGACAT R: CATGGGCAGCAACAAGGAAC
<i>mmp7</i>	<i>mmp7.S</i>	379369	F: GCCCCAACCTGAAGATCCTATG R: CCGAGGGGTCTTCATCATCTT