

Figure S1. Gene expression in early larvae of *Branchiostoma floridae* in controls and in larvae treated with the Hh inhibitor cyclopamine (B,D,G,I) at 1.5 μ M and the nodal inhibitor SB505124 (E,J) at 15 μ M from the early gastrula through the early larva. With both treatments the pharynx is considerably reduced with no gill slits and pharyngeal markers have much smaller, anteriorized domains.

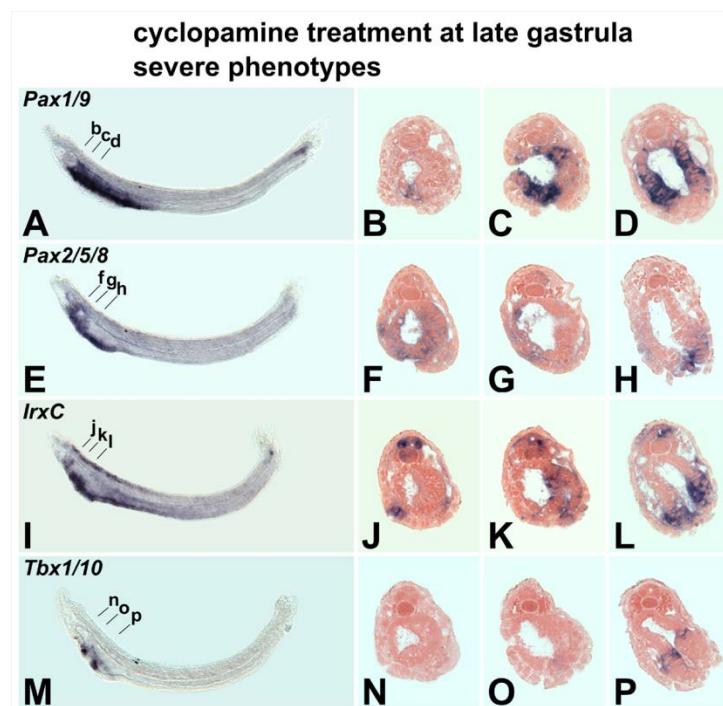


Figure S2. Severe phenotype of larvae treated with 1.75 μ M cyclopamine for 20 min at the late gastrula stage. The gill slits are impenetrant, but markers of the gill slit primordia are expressed as in control larvae.

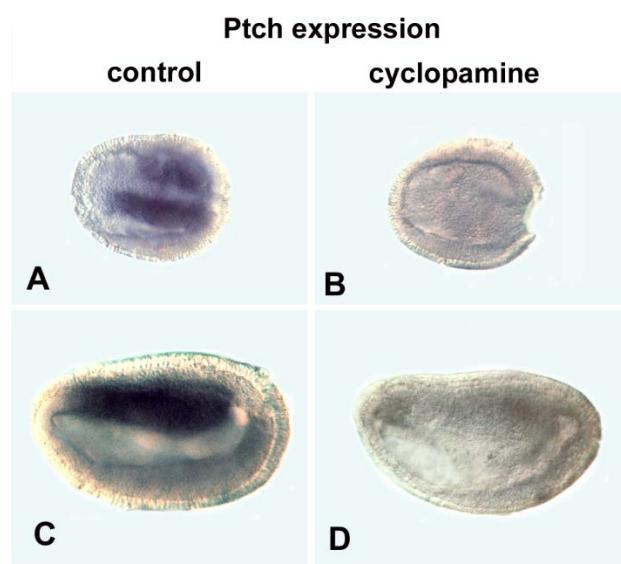


Figure S3. Application of 1.75 μM cyclopamine at the early gastrula stage inhibits expression of the Hh target Patched (Ptch). A, B. Late gastrula/very early neurula, dorsal views. C.D. Early neurula, side view. Anterior to the left. Cyclopamine treatment inhibits expression of the Hh signaling pathway target Ptch in muscular somites.

Time of treatment	Newly hatched gastrula				Early neurula				Early mid-neurula			
concentration	DMSO	1 µM	10 µM	50 µM	DMSO	1 µM	10 µM	50 µM	DMSO	1 µM	10 µM	50 µM
Large gill slits	+++++	NA	----	All dead	+++++	++++	--	--	++++	++	--	--
Medium gill slits	-	NA	+++	All dead	--	+	+++	--	--	+	++	--
Small gill slits	-	NA	+	All dead	--	--	+	++	--	--	+	--
No gill slits	-	NA	+	All dead	--	--	+	+++	--	--	+	+++
Time of treatment	Mid-neurula				Mid/late neurula				Late neurula			
Large gill slits	+++++	++++	++	--	+++++	++	--	--	++++	++	--	--
Medium gill slits	-	++	++	--	--	++	++++	--	--	++	++	--
Small gill slits	-	-	+	--	--	+	+	--	--	+	+	--
No gill slits	-	-	--	++++	--	--	--	++++	--	--	--	+++
cyclopamine												
Time of treatment	Newly-hatched late gastrula					Early neurula						
concentration	ETOH	1.5 µM	1.75 µM	2.0 µM	4.0 µM	ETOH	1.5 µM	1.75 µM	2.0 µM	4.0 µM		
Large gill slits	+++++	--	--	--	--	+++++	--	--	--	--	--	--
Medium gill slits	--	+++	++	--	--	--	+++	+	--	--	--	--
Small gill slits	--	++	--	+++	--	--	+	+++	++++	++++	--	--
No gill slits	--	--	+	++	++++	--	--	+	+	+	+	++++
Time of treatment	Early mid-neurula					Mid-neurula						
Large gill slits	+++++	--	--	--	--	+++++	--	--	--	--	--	--
Medium gill slits	--	+++	++	--	--	--	++++	++	--	--	--	--
Small gill slits	--	++	+++	+++++	--	--	+	++	+++++	++++	--	--
No gill slits	--	--	--	--	+++++	--	--	+	--	--	+++++	--

Table S1. Time and concentration dependent effects of the nodal inhibitor SB505124 and the Hh inhibitor cyclopamine on pharyngeal development of *Branchiostoma floridae*. – indicates no larvae demonstrate the phenotype. + to ++++ indicates approximate proportion of larvae demonstrating the listed phenotype.