Supplemental Figure 1, related to Figure 1: Activation and inhibition of Hh signaling narrow the head.

(A-C) Dorsal views of stage 45 tadpoles. (A) Control treated tadpole. (B) 250 μM cyclopamine treated from the 2-cell stage. Eyes (black) are close-set and head is narrow compared to controls. (C) Tadpole treated with 100 μM purmorphamine from the 2-cell stage. Eyes (black) are close-set and head is narrower than controls. (D) Graph showing total width of head at the level of the eye (blue) and the distance between eyes (yellow) in stage 45 tadpoles. According to both measurements cyclopamine and purmorphamine treament significantly decreased the width of tadpole heads compared to control treated tadpoles (p<0.001, cyclopamine treated compared to control, and p<0.001, purmorphamine compared to control). Diagram indicates measurement planes. (E) Graph indicating head length from the anterior-most extent of the head to either the posterior (green) or anterior extent of the eye (red). According to both measurements cyclopamine and purmorphamine treatment significantly decreased head length compared to controls (p<0.001, cyclopamine treated compared to control, and p<0.001, purmorphamine compared to control). Diagram indicates measurement planes.

Supplemental Figure 2, related to Figure 1: Hh signaling exhibits dose dependent regulation of primary mouth size.
(A-G) Frontal view of stage 45 tadpoles incubated from the 2-cell stage with 250 μM cyclopamine (A), 50 μM cyclopamine (B), or 5 μM cyclopamine (C) (A, n=35, B, n=35, C, n=150). (D) Control tadpole incubated with 0.7% DMSO from the 2-cell stage. (E-G) Tadpoles incubated with 2 μM purmorphamine (E), 20 μM purmorphamine (F) or 100 μM purmorphamine (G). Primary mouth is indicated by red arrowheads. Cyclopamine caused a loss of the primary mouth perforation. A dose dependent increase in mouth size was observed with increasing concentrations of purmorphamine (E, n=35, F, n=35, G, n=140). Red gradient indicates Hh activation, and green bar indicates primary mouth size relative to Hh activation.