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Figure Legends

Figure S1 – Concentration-dependent Hedgehog signaling inhibition in vitro. Shh LIGHTII cells were treated \pm Shh peptide and graded concentrations of cyclopamine or AZ75. Shh stimulation caused a 10-fold induction of reporter activity (not shown) with percent inhibition by each compound plotted on this graph. AZ75 and cyclopamine demonstrated approximate EC₅₀ values of 25 and 100 nM, respectively. Values represent the mean \pm SEM of four replicate experiments.

Figure 1 – Hh signaling antagonist-induced CLP- and HPE- associated dysmorphology. GD16.5 fetuses exposed to vehicle (A), cyclopamine (B, F-H), or the cyclopamine analog, AZ75 (C-E). Cyclopamine exposure induced unilateral and bilateral CLP (B). AZ75 exposure predominantly induced severe holoprosencephalic phenotypes within the range shown in D and E. An intermediate CLP phenotype presenting with severely deficient medial nasal prominence derived tissue (C, I) was induced infrequently by both drug administration regimens. The phenotypic range produced by cyclopamine exposure included unilateral CLP (F), bilateral CLP with graded amounts of medial nasal prominence derived tissue (G,H), and median CL with cleft palate (I). Correlate human phenotypes are shown below. Images are presented courtesy of M. Muenke (J,L,M) and K. Sulik (K).

Figure 2 – Embryonic morphogenesis of cyclopamine-induced CLP. Bright field and scanning electron microscopy images of GD11.25 and 14.0 embryos exposed to vehicle, cyclopamine, or AZ75. At GD11.25, the medial nasal (M), lateral nasal (L), and maxillary (M_x) prominences have united in normal