SUPPLEMENTAL INFORMATION

Supplemental Methods:

Immunohistochemistry and western blots with preadsorbed anti-SHH. 1 pmol of IgG (anti-SHH) was mixed with a 10-fold molar excess of SHH-N or Bovine Serum Albumin (BSA) in block buffer and preincubated for one hour at room temperature with rocking. Preadsorbed antibody was then applied to sections pre-treated with block buffer or to western blots at the same concentration as non-adsorbed anti-SHH.

Supplemental Figure 1: sqRTPCR optimization curves. Graphs depict the normalized pixel density peak areas (y-axis) of PCR products plotted over PCR cycle number (x-axis). PCR products were run through agarose and stained with ethidium bromide. Images were captured with a Biorad XRS gel documentation system and all band images were captured equivalently in the non-saturated range. Peak areas were calculated using ImageJ software.

Supplemental Figure 2: Controls for specificity of anti-SHH immunoreactivity. (A) Western blots of P0 wild type retinal lysates probed with anti-SHH (lane 1) or anti-SHH preadsorbed with SHH-N (lane 2). Blots were reprobed with mouse anti-ACTB to indicate protein loading. (B) Western blots of purified SHH-N protein probed with anti-SHH (lane 1), anti-SHH preadsorbed with SHH-N (lane 2), or anti-SHH preadsorbed with BSA (lane 3). (C-F) Immunohistochemistry with anti-SHH (C,E) or anti-SHH preadsorbed with SHH-N (D,F) on P0 retina (C,D) and E10.5 spinal cord (E,F). FP, floor plate; N, notochord.