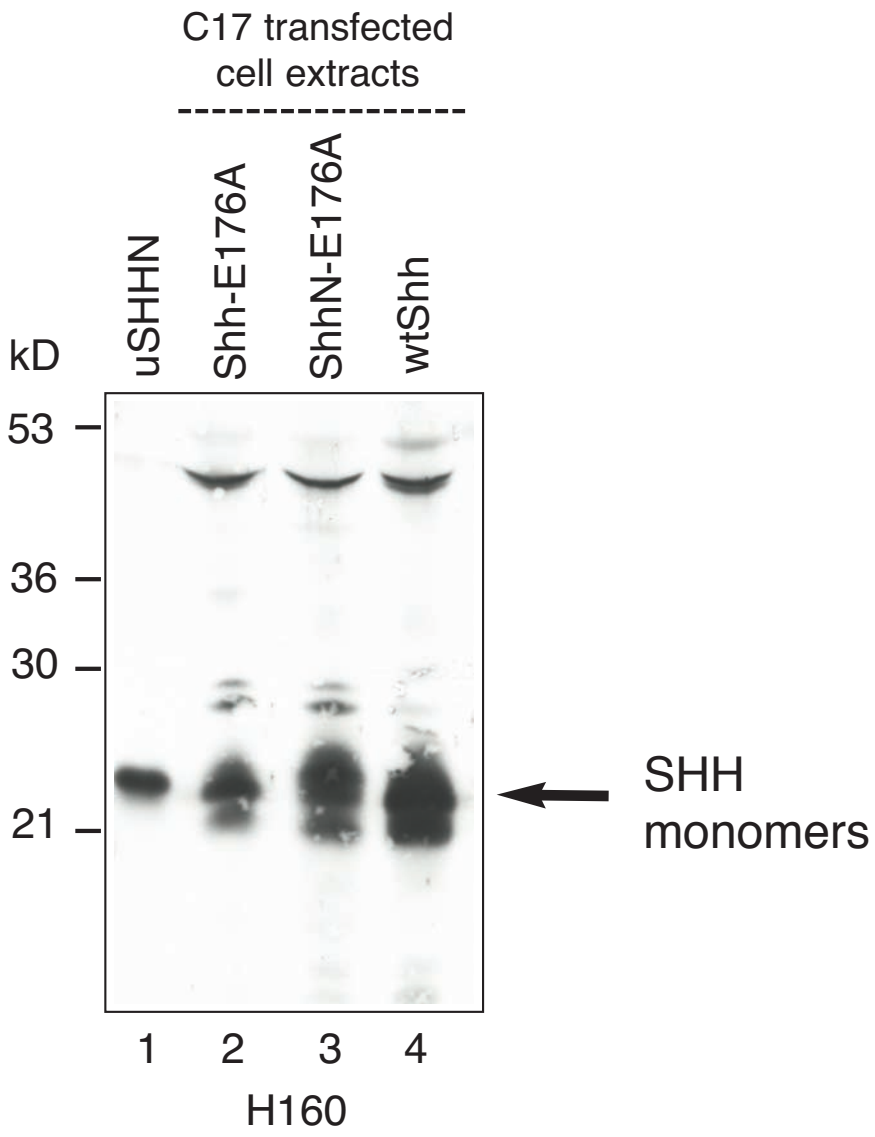
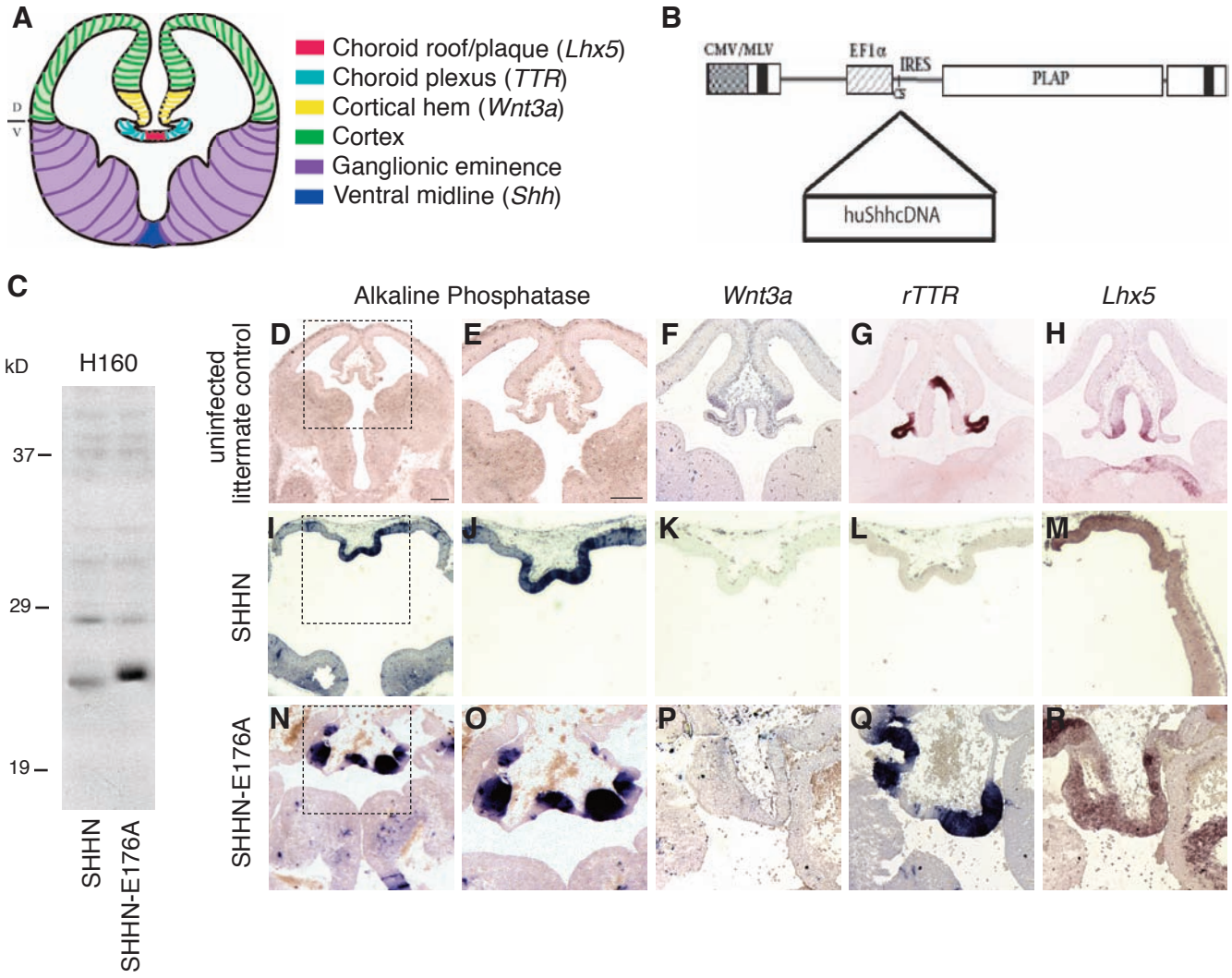


Supplementary Figure S1. H160 detects cell-associated monomeric SHH-E176A in transfected C17 cells. Western analysis of 1. uSHHN (recombinant, E.coli N-terminal protein), and whole cell lysates from C17 cells transfected with different SHH expressing constructs, 2. Shh-E176A, 3. ShhN-E176A, 4. wtShh. The H160 antibody detects cell associated, monomeric SHH-E176A and SHHN-E176A proteins. Comparison with the profile obtained from secreted SHH-E176A (Fig 3C), supports that while secreted SHH-E176A becomes cross-linked, and fails to be recognized by H160, cell associated SHH-E176A forms monomers that are recognized by H160.



Supplementary Figure S2. Virally expressed SHHN-E176A causes dorsal midline defects in mouse embryonic brain. (A) E12.5 forebrain schematic outlining regional boundaries and gene expression patterns relative to Shh. (B) Diagram of retroviral backbone (pCLE) used to express the human SHH protein. Placental alkaline phosphatase (PLAP) is bicistronic with the SHH cDNA, allowing detection of virally infected cells. (C) Lysates of C17 cells infected with SHHN or SHHN-E176A virus are Western-blotted and probed with H160. (D-R) Sections of E12.5 mouse brains 3 days after infection with SHHN- virus (I-M), SHHN-E176A virus (N-R), or uninfected littermate control (D-H). Infected viral clusters are visualized by alkaline phosphatase staining in the dorsal midline of the telencephalon (I,J,N,O). Uninfected littermate control does not contain alkaline phosphatase-expressing clusters of cells (D,E). In situ hybridization of adjacent sections probed for ectopic expression of Wnt3a (F,K,P), rTTR (G,L,Q), Lhx5 (H,M,R). Scale bars = 200µm (D,I,N) and 200µm (E-H, J-M, O-R). Dorsal midline defects are detected in SHHN (n=8/8), SHHN-E176A (n=8/8) infected embryos.