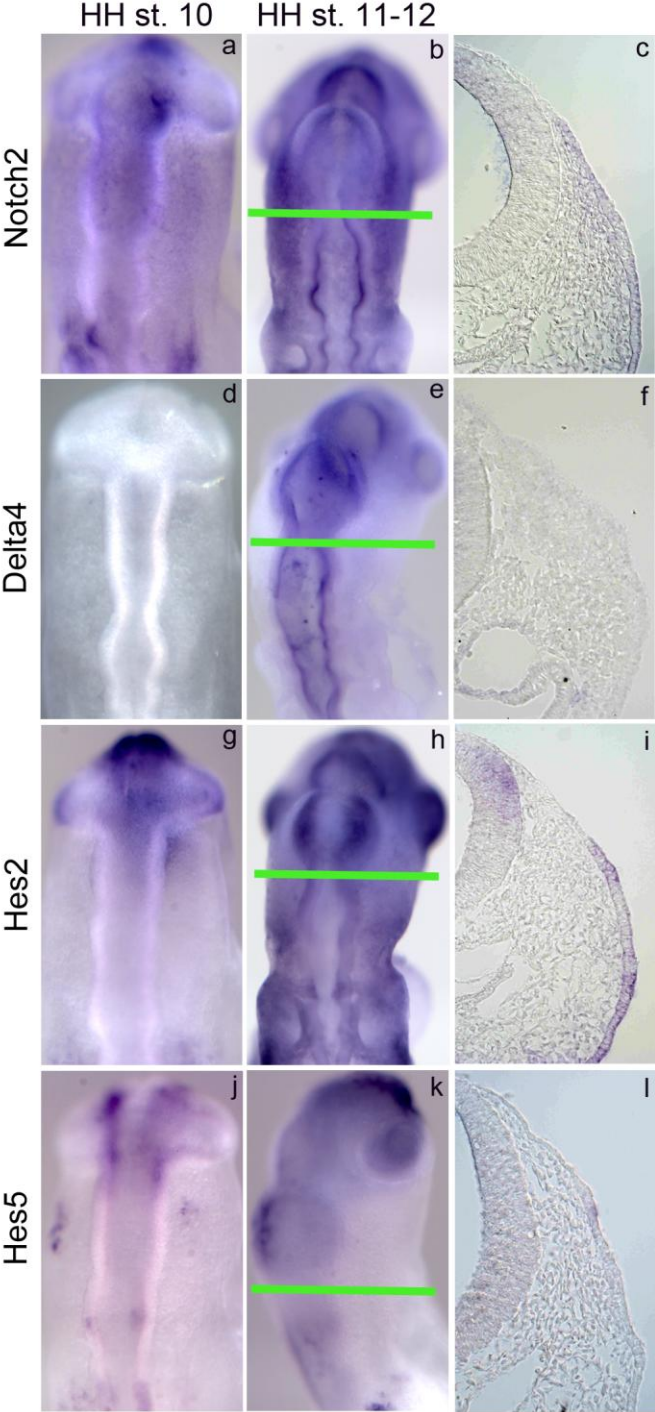


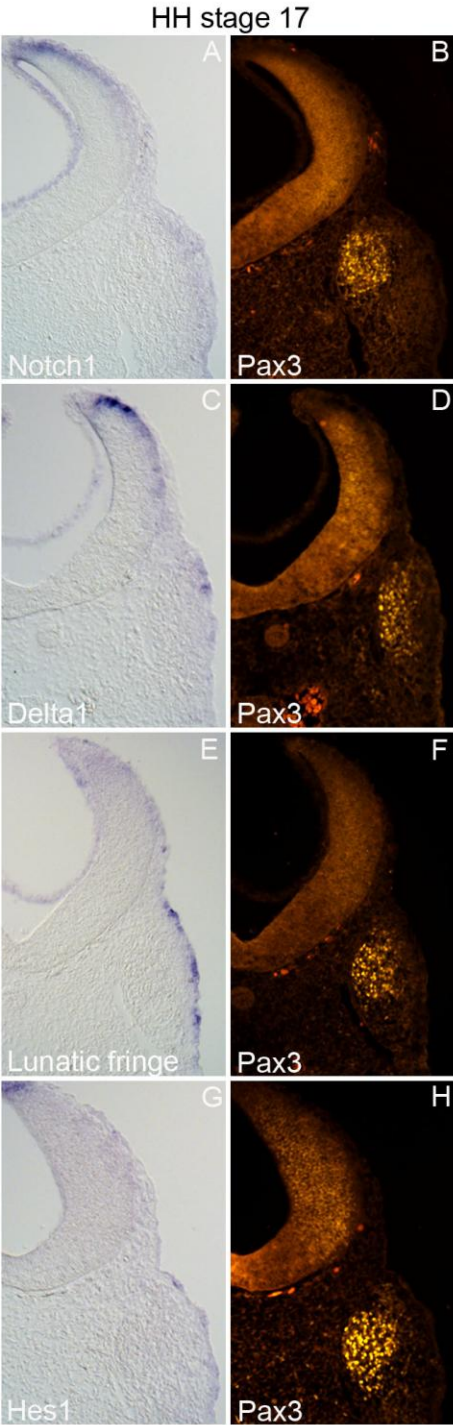
Supplemental Figure 1.



Supplemental Figure 1.

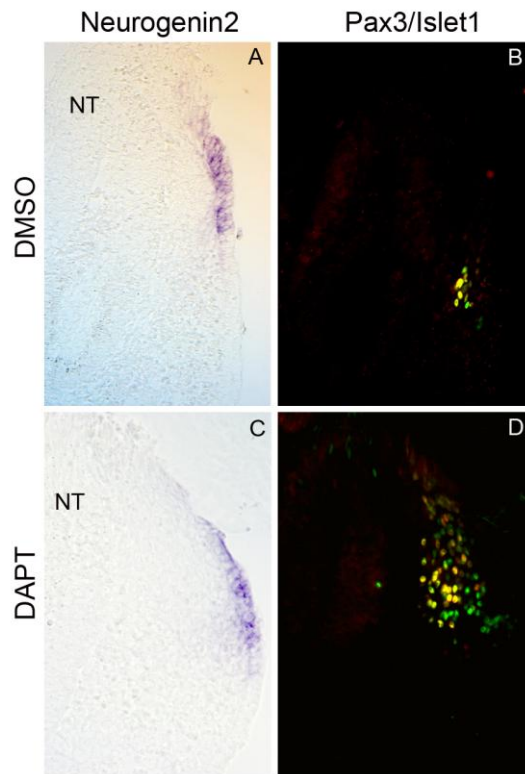
Notch signaling in the trigeminal placode. *Notch2* mRNA is expressed at low levels in ectodermal cells (c) of the midbrain region at HH stage 10 (a) and HH stage 11-12 (b). *Dll4* mRNA is not expressed in the midbrain ectoderm (f) at HH stage 10 (d) and HH stage 11-12 (e). *Hes2* mRNA was not detected in the midbrain ectoderm at HH stage 10 (g), though low levels of expression was detected in the midbrain ectoderm (i) at HH stage 11-12 (h). *Hes5* mRNA is not expressed in the midbrain at HH stage 10 (j), but a few midbrain ectoderm cells (l) expressed low levels of *Hes5* mRNA at HH stage 11-12 (k-l).

Supplemental Figure 2.



Supplemental figure 2. Notch pathway gene expression remains in the ectoderm at a later developmental stage. At HH stage 17, residual low-level expression of *Notch1*, *Delta1*, *Lunatic fringe*, and *Hes1* (A,C,E,G) is shown in the ectoderm. No obvious expression of Notch pathway genes is in the formed ganglion (A,C,E,G). At this stage, most Pax3+ cells have left the ectoderm and are aggregating in the formed ganglion (B,D,F,H).

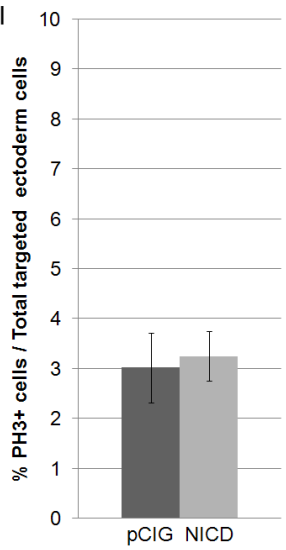
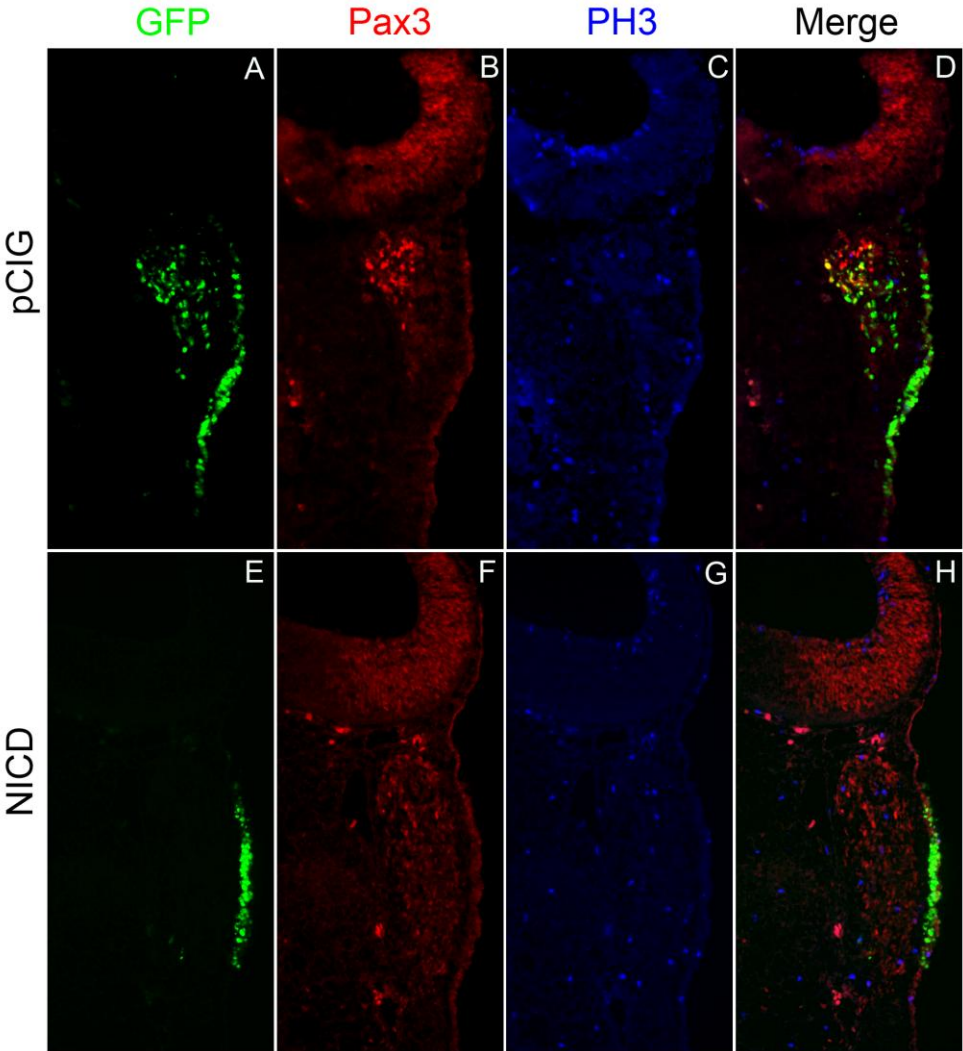
Supplemental Figure 3.



Supplemental Figure 3.

Notch inhibition does not expand or alter *Ngn2* expression in the opV domain. Transverse section through the opV region of a 30-32 ss embryo. Embryo heads were cultured in either DMSO or DAPT for 24 hr and then harvested before *in situ* hybridization was performed with a DIG-labeled *Ngn2* probe. The heads were then cryosectioned and immunostained for Pax3 and Islet1. The location of the placode was verified by expression of both Pax3 and Islet1, merge in yellow (B,D). The results of the hybridization did not reveal any significant difference of *Ngn2* expression between embryos treated in DAPT or DMSO (A,C).

Supplemental Figure 4.



Supplemental Figure 4. Activation of Notch signaling does not affect cell proliferation. Chick embryos were electroporated at 10-12 ss and incubated for 30 hr. Transverse sections showing cells targeted with the pCIG control construct in the ectoderm and ganglion, demonstrating normal development (A), whereas NICD electroporated embryos show targeted cells remaining in the ectoderm (E). Transverse sections through the opV placode were immunostained for Pax3 and PH3 (B,F, and C,G, respectively). NICD targeted cells did not show a statistically significant difference in PH3+ cells compared to pCIG control embryos ($p < 0.81$; I) Data was assessed as percentage of GFP+ cells expressing PH3.

Supplemental Table 1.

Genes	Stage 9	Stage 10-11	Stage 12-13	Stage 17
Notch1	Neural plate/tube	Neural plate/tube, otic placode, trigeminal placode , spinal cord, somites, paraxial mesoderm	Neural plate/tube, otic placode, trigeminal placode , olfactory placode, hindbrain, spinal cord, somites, paraxial mesoderm	Neural plate/tube, otic placode, trigeminal placode , olfactory placode, epibranchial placode, midbrain/hindbrain, spinal cord, somites, paraxial mesoderm
Notch2		Olfactory placode, trigeminal placode , otic placode	Olfactory placode, trigeminal placode , otic placode, lens placode, hindbrain	Olfactory placode, trigeminal placode , otic placode, lens placode, hindbrain
Delta1	Neural plate/tube, somites, paraxial mesoderm	Neural plate/tube, somites, paraxial mesoderm, olfactory placode, trigeminal placode , otic placode, ventral torso, spinal cord	Neural plate/tube, somites, paraxial mesoderm, olfactory placode, trigeminal placode , otic placode, epibranchial placodes, midbrain/hindbrain, head ectoderm, spinal cord	Neural plate/tube, somites, paraxial mesoderm, olfactory placode, trigeminal placode , otic placode, epibranchial placodes, midbrain/hindbrain, head ectoderm, spinal cord
Delta4	Paraxial mesoderm	Paraxial mesoderm	Paraxial mesoderm, midbrain ectoderm	Paraxial mesoderm, midbrain ectoderm
Serrate1	Hindbrain ectoderm	Hindbrain ectoderm, forebrain ectoderm, otic placode	Forebrain ectoderm, otic placode, lens placode	Forebrain ectoderm, otic placode, lens placode, olfactory placode, epibranchial placodes
Lunatic Fringe	Neural plate/tube	Neural plate/tube, trigeminal placode , spinal cord, paraxial mesoderm	Neural plate/tube, trigeminal placode , olfactory placode, otic placode, epibranchial placodes, midbrain/hindbrain, spinal cord, paraxial mesoderm	Neural plate/tube, trigeminal placode , olfactory placode, otic placode, epibranchial placodes, midbrain/hindbrain, spinal cord, paraxial mesoderm, somites
Hes1	Neural plate/tube, paraxial mesoderm	Neural plate/tube, olfactory placode, trigeminal placode , spinal cord, paraxial mesoderm	Neural plate/tube, olfactory placode, trigeminal placode , lens placode, otic placode, brain, spinal cord, paraxial mesoderm, somites	Neural plate/tube, olfactory placode, trigeminal placode , lens placode, otic placode, epibranchial placode, brain, spinal cord, paraxial mesoderm, somites
Hes2	Neural plate/tube, paraxial mesoderm	Neural plate/tube, trigeminal placode , otic placode, spinal cord, paraxial mesoderm	Neural plate/tube, otic placode, olfactory placode, lens placode, trigeminal placode , epibranchial placode, brain, somite, spinal cord, paraxial mesoderm	Neural plate/tube, otic placode, olfactory placode, lens placode, trigeminal placode , epibranchial placode, brain, somite, spinal cord, paraxial mesoderm
Hes5	Neural plate/tube	Neural plate/tube, head ectoderm	Neural plate/tube, olfactory placode, otic placode, midbrain/hindbrain, spinal cord, paraxial mesoderm	Neural plate/tube, olfactory placode, otic placode, epibranchial placode, midbrain/hindbrain, spinal cord, paraxial mesoderm
Neurogenin2	Midbrain	Midbrain, trigeminal placode	Midbrain, trigeminal placode , olfactory placode	Midbrain, trigeminal placode , olfactory placode, neural plate/tube, spinal cord