SUPPLEMENTARY MATERIALS AND METHODS

siRNA sequences, expression constructs and in situ hybridization probes

The siRNA oligos used for in ovo electroporation were designed and ordered from Ambion (Silencer Select Oligos). anti-MYCN siRNA #1 5’- UACUCUUAAUCAUGAAUATT-3’, siRNA #2 5’- GCAAGGAGGUCAACCUGGATT-3’; anti-c-MYC siRNA #3 5’- CCAAGGUGUUCAGGAATT-3’, siRNA #4 5’- AGAUCAGCAACAAACCGAAATT-3’ and “Silencer Select Negative Control siRNAs #1 and #2” (Ambion, Cat # 4390843 and # 4390846). In ovo overexpression of cDNAs were driven by the CMV enhancer/chick β-actin promoter in pCAeGFP [1]. All constructs were verified by DNA-sequencing. The chicken c-myc cDNA (Gene ID: 420332, NP_001026123) was subcloned from pRCAN-cmyc received as a kind gift from Professor Andrei Tikhonenko, Fred Hutchinson Cancer Research Center, Seattle, WA, USA. We cloned the chick mycn (Gene ID: 421948, NP_0010262.1) from a chicken embryo collected at embryonic day 4 (E4). The c-MYCΔC and MYCNΔC constructs were generated by deletion of the sequences encoding the C-terminal bHLH-Zip; aa 332-416 from c-MYC and aa 356-441 from MYCN, respectively. The sequences for in situ hybridization probes to the complete open reading frames for chicken cash1, neurod1, neurod2 and inscuteable were synthesized by Epoch Biolabs, Sugar Land TX, USA.

Plasmids for in ovo overexpression of NICD1 and dnCSL and riboprobe vectors for NICD1, Hes1, and Hes5 were kind gifts from Dr. Johan Holmberg, Karolinska Institutet, described in [2]. Full length ORFs of chick mycn and c-myc were cloned as a cDNA in pCRII-TOPO and used as templates for the corresponding riboprobe synthesis. Empty pCA vector (derived from pCAeGFP by deletion of the GFP open reading frame) was used as a control.
In ovo Electroporation and EdU Incorporation

Fertilized chicken eggs were incubated at 38°C for 48 hours prior to electroporation. The embryos were injected with a DNA solution into the lumen of the neural tubes at Hamburger-Hamilton developmental stages 12-14 (corresponding to embryonic day 2 (E2), 48 hours). The electrodes were spaced 4 mm apart allowing the embryo to fit in between, the electric pulses were conducted 5 times for 25 ms and 50 V each. The DNA solution contained 0.025% of fast green, the experimental plasmid DNA or siRNA in a concentration 1.5 ug/ul with 0.5 ug/ul of pCAeGFP or pCEeRFP plasmid for visualizing electroporated cells. MYC constructs were traced with mouse or rabbit anti c-MYC antibodies or by co-transfection with GFP or RFP plasmids. In control condition, experimental DNA was substituted with the same amount of empty vector. For EdU labeling 2 ml of EdU-containing PBS (100 uM) was added to the chick embryos in ovo followed by incubation for 2 hours at 38°C before collection.

Immunohistochemistry and in situ hybridization

Embryos were collected and fixed with 4% formaldehyde overnight at +4°C, cryoprotected in 30% sucrose solution, mounted in OCT media (Tissue-Tek) on dry ice and cryosectioned. The thickness of the sections was 14 μm. In situ hybridization (ISH) and immunochemistry experiments were performed as previously described [1]. The following antibodies were used: mouse anti-βIII tubulin (Tuj1) (Promega); mouse anti-Isl1 (39.4D5, Developmental Studies Hybridoma Bank DSHB, Iowa City, Iowa, USA); rabbit anti-Sox2 (Abcam ab97959); rabbit anti-PH3 and mouse anti-NeuN (Chemicon); mouse anti-c-MYC C33, rabbit anti-c-MYC N262 (Santa Cruz Biotechnologies), mouse anti-Brn3a (Chemicon), and guinea pig anti-NeuroM,
kindly provided by Professor Jonas Muhr, Karolinska Institutet. EdU incorporation was detected using Click-iT EdU-Alexa Fluor 647 Flow Cytometry Assay (Invitrogen, C10085). Apoptotic signals were detected with Click-iT TUNEL Alexa Fluor Imaging Assay kit (Invitrogen). Tunel positive cell counts were normalized to the non-electroporated halves of the neural tubes on transversal sections.

**In vitro culture of NPG cells isolated from developing chick neural tube and FACS analysis**

Chick embryos were electroporated with different MYC constructs together with a GFP expression vector at embryonic day 2 (E2) and incubated at 38°C for 24 hours. The neural tubes together with some remaining adjacent tissues were dissected out under an UV-light source. Single suspensions were obtained by incubating the isolated neural tubes in 1x Trypsin-EDTA (Sigma T4174)/PBS-solution during 15 min at 37°C. Cells were seeded in 12-well tissue culture plates at 5.0 x10^5 cells per well in DMEM/F12 media (Invitrogen, #21041) with 10% FBS (Hyclone). The cells were incubated *in vitro* during 24 hours at 37°C. For cell cycle analysis cells were pulsed with 1µM EdU (Invitrogen) for 2 hours, harvested and stained with Click-iT EdU Alexa Fluor 647 Flow Cytometry Assay Kit (C10424, Invitrogen), 100ug/ml propidium iodide solution (Sigma, P4864), and 200 ug/ml RNaseA solution (Sigma-Aldrich). Samples were analyzed on a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems) and data were analyzed by FlowJo 7.2.2 Data Analysis Software.

**qPCR**

Transformed cells from dissociated embryos were isolated using a FACS Vantage/Diva instrument using selection for GFP positive cells at +4°C, 12 psi with a 90um nozzle. The cDNA
library was generated with Power SYBR Green Cell-to-Ct kit (Ambion) and qPCR was performed with Power SYBR Green Master Mix (Ambion) using the following PCR-primers: *notch1*: 5'-GGCAACCTCAACATTCCCTACA-3' and 5'-TGAAGGCAAGCAAGACCAGTG-3', and *gapdh* as the internal control: 5'-GGCTGAGAAGGGAAACTTGT-3' and 5'ACTCCCAAACATACTCAGCACC-3'.

**Live imaging of chick neural tube slices**

For time-lapse imaging of acute slices, E3 chick embryos were dissected out in ice-cold PBS, embedded in warm (~45°C) 4% low melting point agarose (Cambrex, ME, USA) in PBS and rapidly cooled on ice. Spinal cord coronal slices (400 µm) were cut with a Vibratome (Leica VT1000S, Leica, Germany). The temperature in the cutting chamber was kept at 1°C using a combined water cooler bath and chamber (Huber CC1, Germany). Slices selected for high GFP expression were mounted on coverslips and embedded in BD Matrigel™ (BD Biosciences), which was left to harden 30 min in an incubator. Coverslips were then mounted on a heated (37°C) and computer-controlled stage bolted to a Zeiss LSM510 META NLO laser scanning microscope equipped with a 40x/1.0 dipping lens (Carl Zeiss, Germany), a Ti:Sapphire Chameleon Ultra2 laser (Coherent, CA, USA) tuned to 910 nm and Non-Descanned Detectors, and continuously perfused with oxygenated Artificial Cerebrospinal Fluid (ACSF, 125mM NaCl, 25mM NaHCO₃, 1.25mM NaH₂PO₄, 20mM glucose, 5mM KCl, 2mM CaCl₂, 1 mM MgCl₂). Stacks of ~50 µm were acquired every 3-5 min for 4-6 hours.

**Statistical Analysis**
Experimental data plotted on graphs are presented as the mean +/- the standard error of the mean (SEM). Data were analyzed for statistical significance with Student's t tests. Differences were considered to be significant at P< 0.05 (*), P< 0.01 (**), and P< 0.001 (***)

SUPPLEMENTARY REFERENCES