Supplementary Methods

In ovo electroporation and plasmids

Fertilized chicken (Gallus gallus domesticus) eggs were obtained from Winter Egg Farm (Hertfordshire - Royston SG8 7RF, UK) and incubated at 38°C to the desired stages as defined by Hamburger and Hamilton [1]. Spinal cords were electroporated with 0.1-0.5μg/μl plasmid DNA. GFP-*Insc* (pCAGGS-GFP-Insc) contained the mouse *Inscuteable* ORF (NM_173767) cloned downstream of GFP, and cloned into the pCAGGS vector from an original construct, K14-mINSC-GFP, provided by Elaine Fuchs [2]. The *Inscuteable* ORF was amplified from GFP-*Insc* using primers InscF: 5' GGCGGGAATTCATGATGGCACTGCCTGGAGGCCGCC 3' and InscR: 5' GGCGGCCCGGGTACACAAAACTCTCCTCCATATTG 3' and cloned into the *Eco*RI and *Sma*I sites of pCIG (pCAGGS-IRES-nucGFP) to generate pCIG-*Insc.* The *Inscuteable* ORF was excised from pCIG-*Insc* on an *Xho*I/*Sma*I fragment and cloned into the *Xho*I/*Sma*I sites of pCAGGS-IRES-H2B RFP (a kind gift from Celia Shiau) to generate pCAGGS-Inscuteable-IRES-H2B RFP. The pHes5-1 VNP construct consists of the chicken Hes5-1 promoter region around 2Kbp upstream of the coding sequence driving expression of the fast folding YFP variant Venus with a nuclear localisation sequence (NLS). This is followed by 3'UTR of chicken Hes5-1 and the rabbit *β-globin* polyadenylation signal [3].

Immunofluorescence and imaging

Primary antibodies were used at the following dilutions: HuC/D (Molecular Probes) 1:500, p27 (BD Biosciences) 1:200, BrDU (Sigma) 1:500, Sox2 (Millipore) 1:2000, pH3 (Millipore) 1:1000, Par3 (Millipore) 1:1000, aPKC (Santa Cruz) 1:200, N-Cadherin (DSHB) 1:500. All secondary antibodies used were Alexa Fluor conjugates (Invitrogen) diluted at 1:500. For BrDU labeling, sections were treated with 45

units/ml Dnase 1 (Sigma) in TBS (50 mM Tris pH 7.5, 150 mM NaCl) with 10 mM $MgCl_2$ and 10 Mm $MnCl_2$ for 60 min at 37°C to reveal the epitope. Images were acquired using a 20x, 40x or 60x objective on a Deltavision Core microscope system (Applied Precision LLC, Issaquah, WA).

Embryo slice culture and time-lapse imaging

Slices were imaged as previously using a Deltavision Core microscope system in a WeatherStation environmental chamber maintained at 37^0C. The slice culture medium was buffered with a 5% CO_2/95% air mix and maintained in a humid environment. Images were acquired using an Olympus 40x 1.30 NA objective using a Xenon light source and a CoolSnap HQ2 cooled CCD camera (Photometrics). Thirty-forty optical sections (exposure time= 10 – 50 milliseconds for each channel, 512x512 pixels, 2x2 binning) spaced 1.5μm apart were imaged for each slice at 7-minute intervals for up to 60 hours. The culture medium was replaced every 24 hours.

Clonal Analysis

Single cells transfected with KiKGR (green) (MBL International) [4] were targeted with a 405nm laser (10x50 millisecond pulses, 50-100% laser power) using an Olympus 60x 1.4NA objective on a Deltavision Core microscope system equipped with a quantitative laser module. This generated single cells labelled with photoconverted KiKGR (red), which we were able to detect up to 24 hours later.

Angle measurements

To measure cleavage plane orientations in anaphase and telophase cells, images were acquired using a 40x or 60x objective on 20μm thick cyosections, or spinal cord slices cultured in collagen. Stacks were spaced 1.5μm apart and only anaphase cells where both chromosome pairs were visible within 3-4 consecutive sections were chosen for measurement. Measurements were made using the angle measurement tool in the

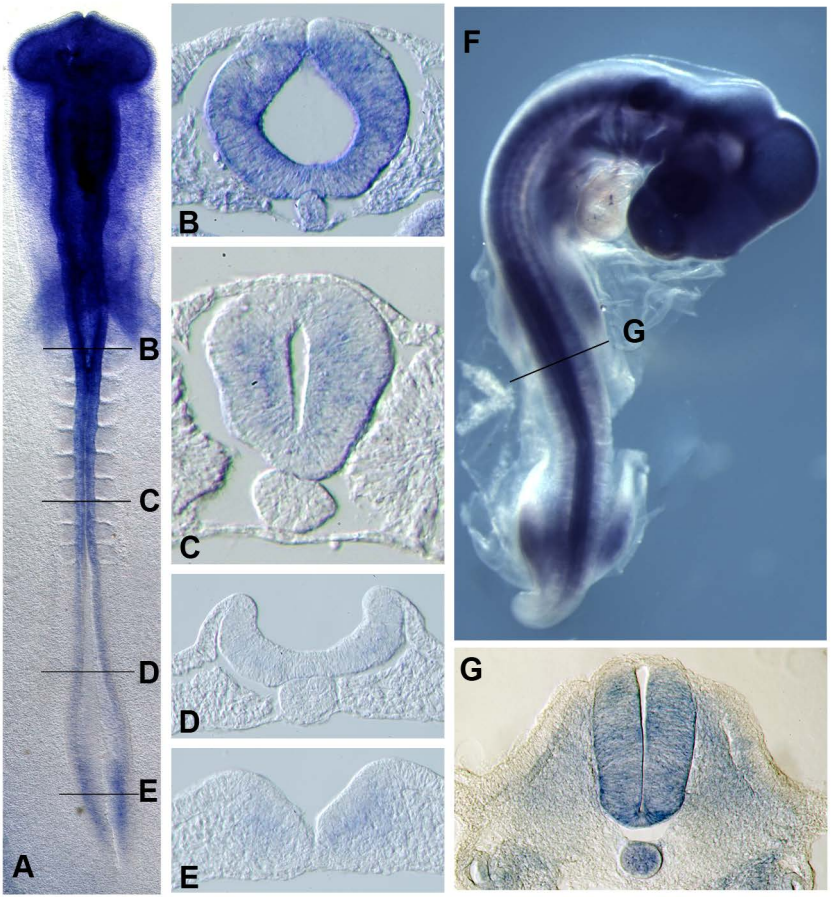
SoftWorx image analysis suite (Applied Precision LLC, Issaquah, WA), using the apical surface as a baseline. If cells divided with a skewed orientation or if the apical surface was not perpendicular to the imaged surface, 3D reconstructions were made using the volume view tool of SoftWorkx to accurately measure cleavage plane orientations.

In situ hybridisation

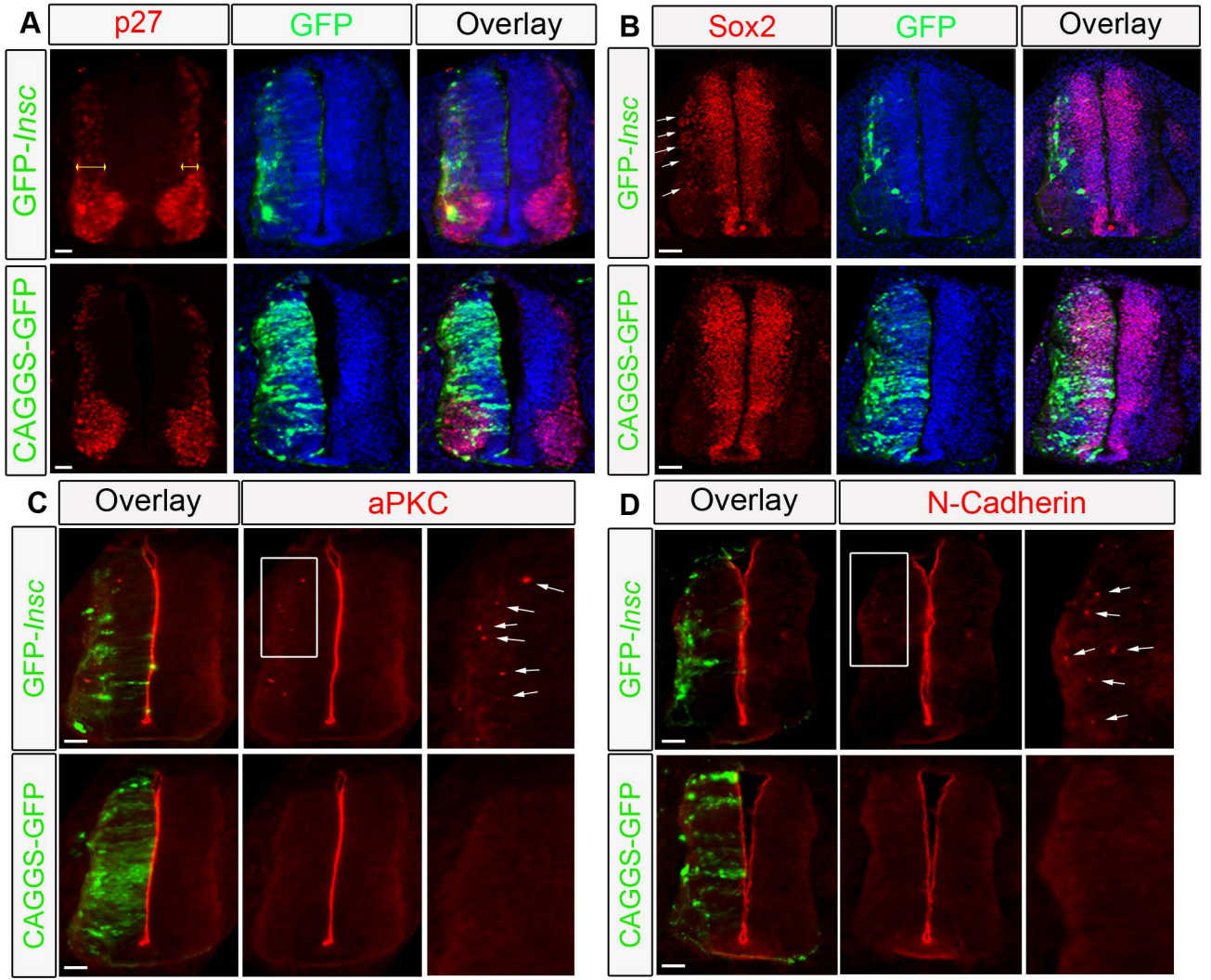
Standard methods for whole-mount *in situ* hybridisation were used.

References

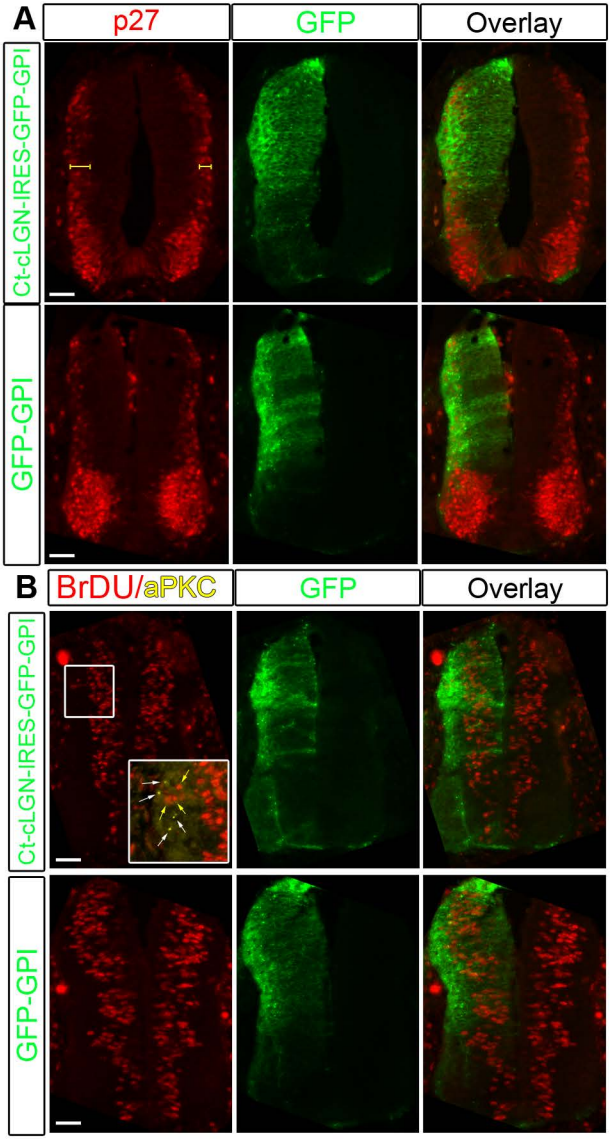
1. Hamburger V, Hamilton HL (1951) A series of normal stages in the development of the chick embryo. *Journal of Morphology* **88:** 49-92
2. Lechler T, Fuchs E (2005) Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature* **437:** 275-280
3. Vilas-Boas F, Fior R, Swedlow J, Storey K, Henrique D (2011) A novel reporter of notch signalling indicates regulated and random notch activation during vertebrate neurogenesis. *BMC Biology* **9:** 58
4. Stark DA, Kulesa PM (2007) An in vivo comparison of photoactivatable fluorescent proteins in an avian embryo model. *Developmental Dynamics* **236:** 1583-1594

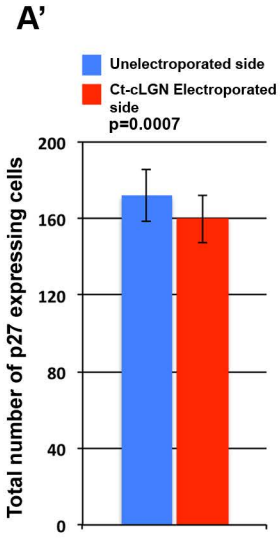


Das and Storey Figure S1

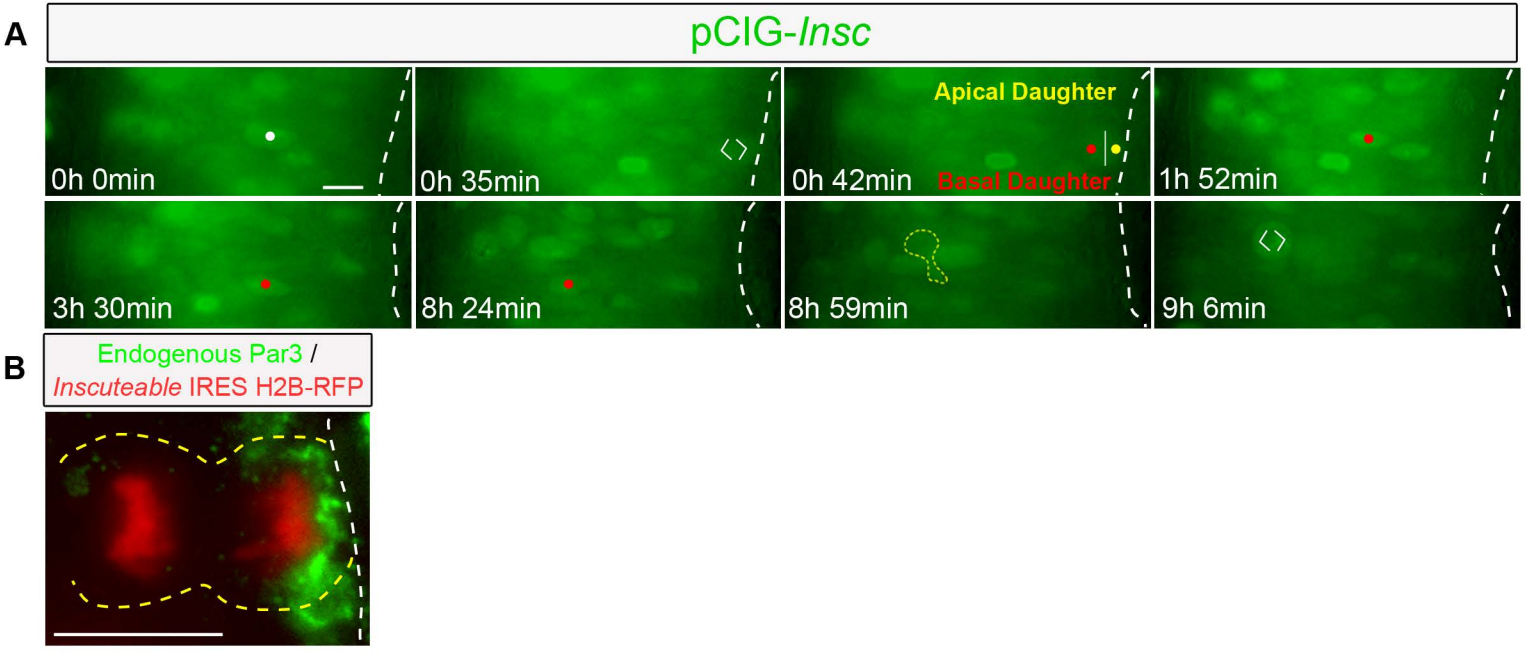


Das and Storey Figure S2

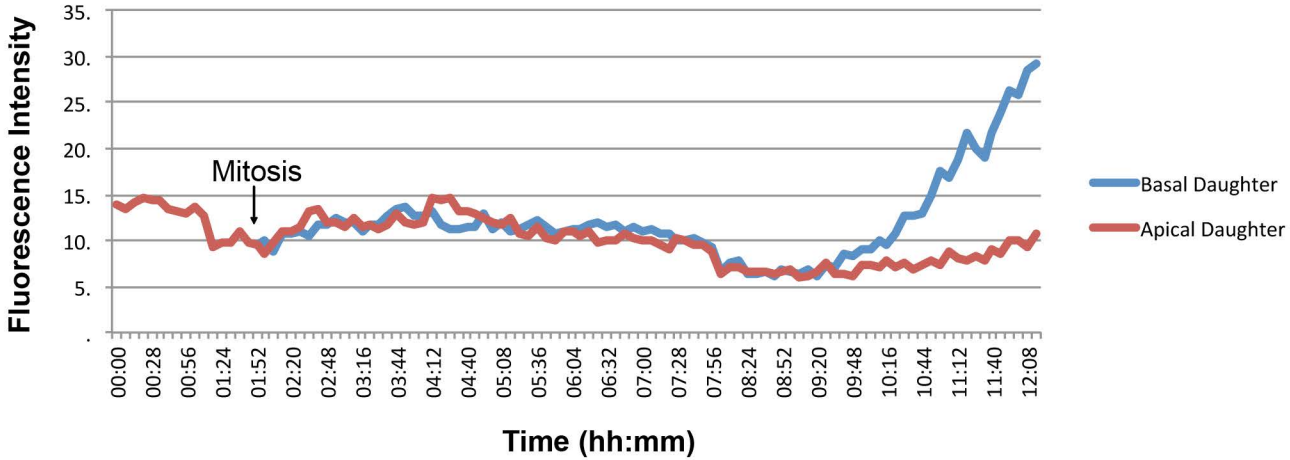




Das and Storey Figure S3



Das and Storey Figure S4



Das and Storey Figure S5

Figure S1. *Inscuteable* expression in the chick spinal cord

(**A**) Wholemount view of a HH10 embryo showing *Inscuteable* mRNA expression. *Inscuteable* is expressed throughout the nervous system, with expression strongest in the brain ventricles. (**B, C, D, E**) Cross sections of regions indicated in **A. (F)** Wholemount view of *Inscuteable* expression in an HH18 embryo. (**G**) Cross section through the spinal cord indicated in **F.**

Figure S2. GFP-*Insc* mis-expression induces cell cycle exit and mis-localises progenitors in the mantle zone

(A) The p27 expression domain is expanded in spinal cords transfected with GFP-*Insc* compared to untransfected sides. **(B)** GFP-*Insc* mis-expression generates a population of Sox2 expressing progenitors in the mantle zone (white arrows). **(C,D)** Patches of ectopic aPKC and N-Cadherin expression in the mantle zone induced by GFP-*Insc* mis-expression (white arrows). Scale bars=40μm

Figure S3. Ct-cLGN mis-expression increases neuron numbers and mis-localises polarised progenitors in the mantle zone

A truncated version of the G-protein regulator LGN (Ct-cLGN) that interferes dominantly with endogenous LGN, was used to assess the role of spindle orientation (**A)** Spinal cords transfected with Ct-cLGN-IRES-GFP-GPI have a broader domain of p27 expression compared to the unelectroporated side 48 hours after transfection. (**A'**) Quantification of p27 expressing cells on the electroporated side versus the unelectroporated side reveals a slight increase in postmitotic cells following Ct-cLGN mis-expression (5 embryos, 5 sections per embryo, paired t-test, Error bars represent SEM). (**B**) Ct-cLGN mis-expression also generated an ectopic population of BrDU

incorporating cells in the mantle zone with associated ectopic aPKC expression (high magnification image in top left panel) (2/5 embryos). Scale bars=40μm

Figure S4. Cell behaviour following apico-basal divisions and during normal neurogenesis

(A) Cell indicated with a white dot undergoes an apico-basally orientated division (t=0h 35min) and the resulting basal sister cell (red dot) fails to migrate back to the apical surface and instead divides again in the mantle zone (t=8h 59min). (**B**) A fixed spinal cord progenitor cell expressing *Inscuteable*-IRES-H2B-RFP undergoes an apico-basally oriented division during which the majority of endogenous Par3 is inherited by the apical daughter. Scale bars=10μm

Figure S5. Quantification of notch reporter activity following an apico-basal division.

Measurement of VNP fluorescence intensities revealed a three-fold increase of notch activity in the basal daughter (blue line) compared to the apical daughter (red line) within 7-10 hours of mitosis. Notch activity remains relatively constant in the apical daughter. This example is a measurement of the lineage shown in **Figure 3C**. The precise time at which increased Notch activity is detected reflects, to some extent, the amount of plasmid transfected into the mother cell.

Supplementary Movie Legends

Movie S1. Robust re-polarisation of cells following apico-basal division

Cell transfected with pCIG-*Insc* (green) and mApple-Farn (red) undergoes an apico-basal division following which the apical daughter inherits the original apical process and the basal daughter inherits the original basal process. The apical daughter extends

a new basal process and the apical daughter extends a new apical process and re-establishes contact with the apical surface.

Movie S2. Cell transfected with GFP-GPI and pCIG-*Insc* (white dot) undergoes an apico-basal division to generate an apical daughter cell (yellow dot) that differentiates into a neuron while the basal daughter cell (red dot) remains as a progenitor and divides again.

Movie S3. Cell transfected with pCIG-*Insc* (white dot) undergoes an apico-basal division to generate a basal daughter (red dot) that divides ectopically away from the apical surface.

Movie S4. Cell transfected with pCAGGS-*Inscuteable*-IRES-H2B RFP (red) and Par3-GFP (green) undergoes an apico-basal division where the apical daughter inherits Par3.

Movie S5. During normal neurogenesis, a cell transfected with mApple-Farn (red) and Par3-GFP (green) undergoes a division with a perpendicular cleavage plane, but the majority of the Par3 is inherited by one of the daughter cells.

Movie S6. Cell transfected with GFP-Tubulin (green) undergoes division during which a short apical process is retained by the daughter cell (red dot) that goes on to differentiate. The other daughter cell forms a new apical process and divides again.

Movie S7. Cell transfected with pCAGGS-*Inscuteable*-IRES-H2B RFP (red) and pHes5-1-VNP (green) undergoes an apico-basal division following which the basal daughter shows strong onset of VNP expression.