Supplementary Materials and Methods

Immunocytochemistry

Cells and brain sections were rinsed in PBS and fixed in 3.7% PFA for 15 min. FOXG1 antibody staining was performed with methanol-acetone fixation. Cells were permeabilised via three washes in PBS containing 0.3% Triton X-100 (PBST) and then blocked in PBST containing 1% BSA and 3% normal donkey serum. Primary antibodies were added in blocking solution for 2 hours at ambient temperature or overnight at 4ºC. The cells were washed in PBST three times before being incubated for 1 hour in the dark in Alexa-Fluor secondary antibodies, 1:200 (Invitrogen). Three PBST washes were then performed that included one with DAPI, 1:1000 (Molecular Probes). The primary antibodies used in this study were: Calbindin (Swant, CB-38a, 1:500), CTIP2 (Abcam, 25B6, 1:500), DARPP32 (Santa Cruz, sc 11365, 1:200), DLX2 (Millipore, ab5726, 1:300), ENK (Immunostar, 20065, 1:400), SubP (Immunostar, 20064, 1:400), Forse1 (DSHB, 1:100), FOXP2 (Abcam, ab58599, 1:100), FOXG1 (Abcam, ab5274,1:250), GABA (Sigma, A2052, 1:500), GAD65/67 (Sigma, G5163, 1:1000), GSX2 (Millipore, abn162, 1:1000), HuNu (Millipore, mab1281, 1:250), hNCAM (Santa Cruz, sc-106, 1:200), MAP2 (Sigma, M1406, 1:250), Nestin (BD, 611659, 1:300), NeuN (Millipore, mab377, 1:250), NKX2.1 (Abcam, ab40880, 1:1000), OTX2 (Millipore, ab9566, 1:300), PAX6 (DSHB, 1:1000), PSD95 (Thermo Scientific, 6G6-1C9, 1:200), TH (Pelfreez, P40101, 1:500). Images were taken on a Leica TCS SP5 confocal microscope. Quantification of markers was carried out manually by examining randomly selected fields from at least three independent experiments and presented as mean ± sem. Statistical significance was determined using two-tailed Student's t-test.

Quantitative PCR

Total RNA was extracted using TRI Reagent (Sigma) according to the manufacturer's instructions. Reverse transcription was performed using SS RTIII (Invitrogen). qPCR was carried out using SYBR MESA Green and a Chromo4 machine (BioRad). All data are relative to three reference genes (*GAPDH, b-ACTIN, CYCLOPHILIN*) and normalised to the basal conditions. The sequence information for all PCR primers can be found in Table S2. All qPCR data are presented as mean ± s.e.m. of biological duplicates or triplicates.

Electrophysiology

H7 hESCs were differentiated following the scheme illustrated in Fig. 3A but the maintenance was extended for up to 93 days. On the day of recording, neurons were placed on a recording chamber and viewed using an Olympus BX51WI microscope with a 40x water immersion lens and DIC (differential interference contrast) optics. Cells were bathed in a solution containing (in mM): 140 NaCl, 3.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES. For whole-cell electrophysiological recordings, low resistance recording pipettes (10-15 MΩ) were pulled from capillary glass (Harvard Apparatus) and coated with ski wax to reduce pipette capacitance. Recording pipettes were filled with a solution containing (in mM): 140 K-gluconate, 5 NaCl, 2 Mg-ATP, 0.5 LiGTP, 0.1 $CaCl_2$, 1 $MgCl_2$, 1 ethylene glycol-bis (b-aminoethyl ether) -N,N,N,N-tetraacetic acid (EGTA), and 10 HEPES. Osmolarity and pH of both solutions were adjusted before recordings. For mIPSCs, pipettes were filled with a high-chloride solution containing (in mM): 135 CsCl, 4 NaCl, 2 ATP, 0.3 GTP, 0.5 $MgCl_2$, 2 EGTA, and 10 HEPES. TTX (1 µM) was added to the external solution. Data were acquired at room temperature using an Axon Multiclamp 700B amplifier and a Digidata 1440a acquisition system, with pClamp 10 software (Molecular Devices). Data analysis was carried out using Clampfit 10.2 software (Axon Instruments), OriginPro 8.1 (OriginLab Corporation), and Spike2v5 software (Cambridge Electronic Design).

Transplantation

All animal experiments were performed in full compliance with the UK Animals (Scientific Procedures) Act 1986 and approved by local ethical review. Adult female Sprague-Dawley rats (Harlan, UK) weighing 200-250 g at the start of the experiment were used. Rats received unilateral injections of 45 nmol quinolinic acid to the right striatum, at stereotaxic coordinates: +0.4/+1.4 mm anterior (A) of bregma, -3.2/-2.4 mm lateral (L) of bregma, and -5.0/-4.5 mm below dura, as described previously (Kelly et al., 2007). Rats received unilateral transplants of activin-treated H7 hESC-derived neural progenitors harvested at day 20. $4x10^5$ cells were injected in a final volume of 2 µl into the lesioned striatum at stereotaxic coordinates, from bregma: +0.8 mm A, -2.8 mm L and -5.0/-4.5 mm below dura. Transplanted adult rats received immunosuppression in the form of daily cyclosporin A (CsA, Sandimmun, 10 mg/kg, i.p.) commencing the day prior to transplantation and continuing for the

duration of the experiment. Rats were sacrificed at 4, 8 and 16 weeks post transplantation, by barbiturate overdose and transcardial perfusion, as described previously (Kelly et al., 2011). Brains were sectioned coronally at 40 µm, collected in Tris-buffer with azide and were processed for immunohistochemistry.

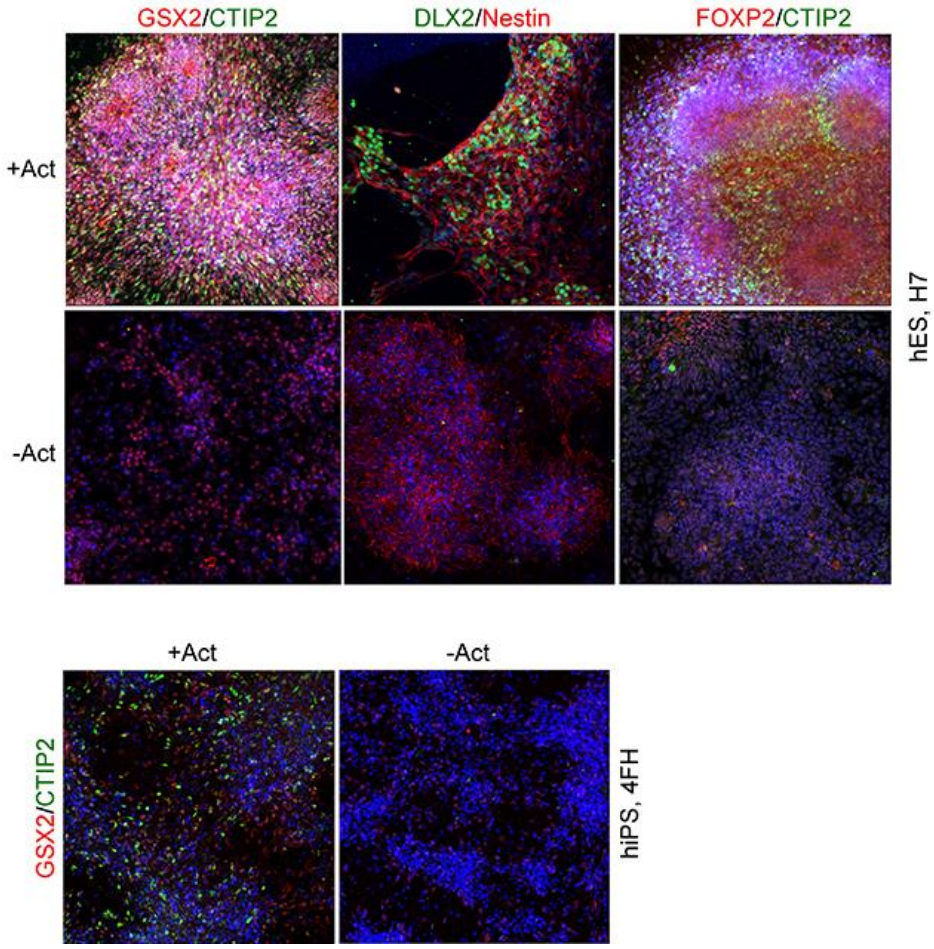

Fig. S1. Activin induces LGE progenitor characteristics and striatal differentiation of hPSCs.

The same as Fig. 1G with DAPI counterstain in blue. Day 22 cultures of H7 hESCs and day 18 cultures of 4FH hiPSCs exposed to activin from day 9 were double immunostained for GSX2 and CTIP2; DLX2 and Nestin; and FOXP2 and CTIP2.

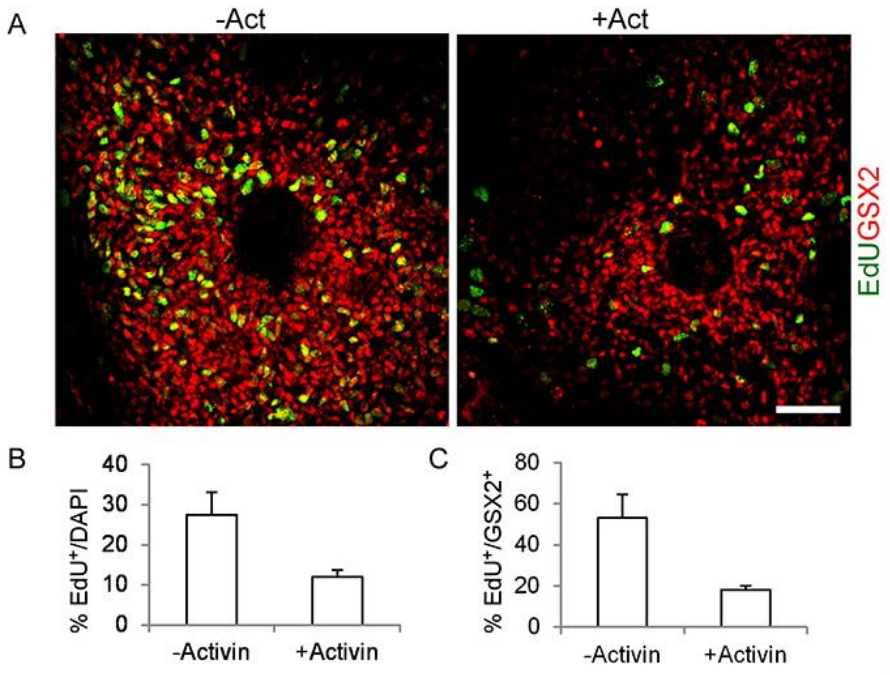

Fig.S2. Activin does not induce preferential proliferation of GSX2$^+$ progenitor characteristics.

A, Chemical and immunostaining of day16 cultures for EdU and GSX2, respectively.

B-C, Quantification of the above illustrating the percentage of cells expressing EdU.

Scale bar: 50 μm.

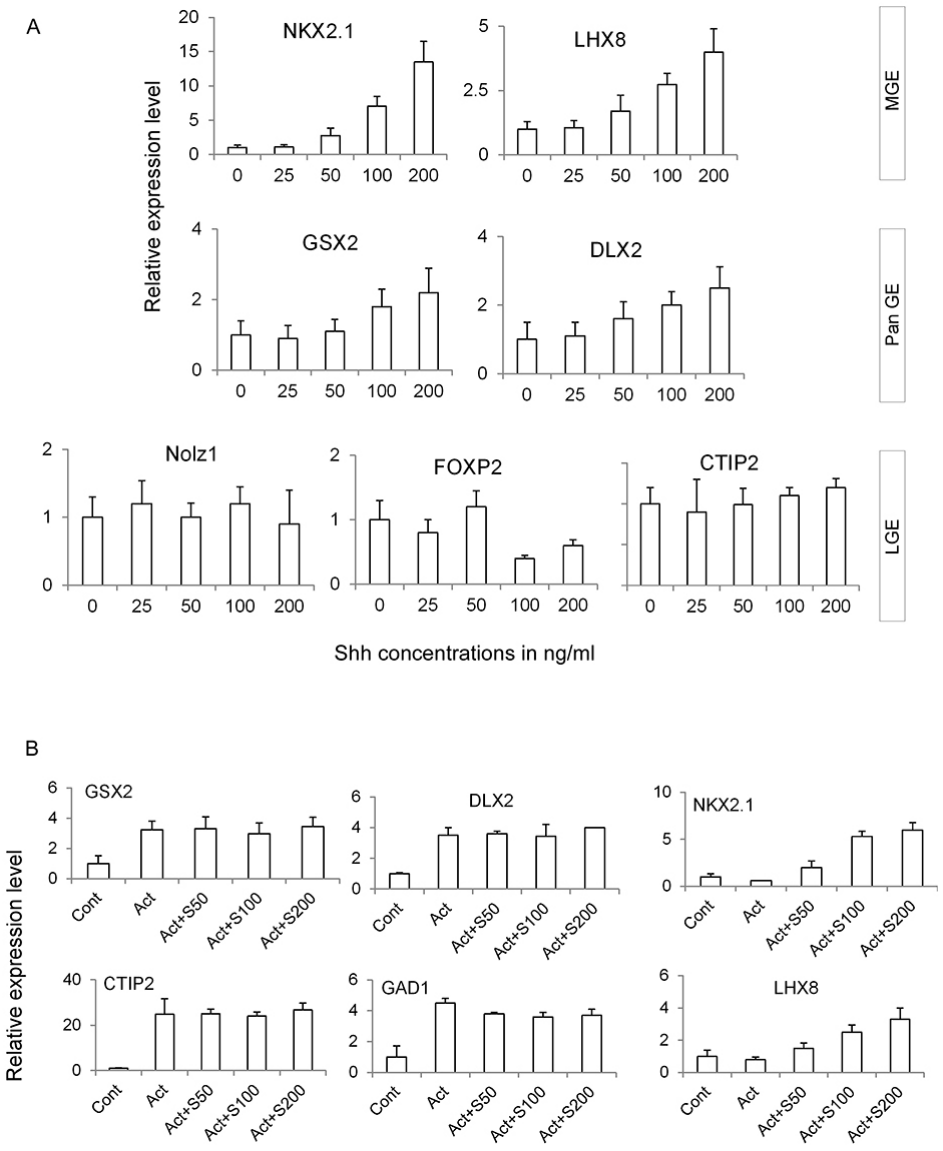

Fig. S3. Activin-induced striatal fate does not involve SHH signalling.

A, Day 9 cultures were treated with increasing concentrations of SHH for 4 days. Cultures were then processed for qPCR analysis to examine the effect of SHH on MGE and LGE expressed genes.

B, Day 9 cultures were treated with activin (Act) with or without increasing concentrations of SHH. Cultures were harvested 4 days later for qPCR.

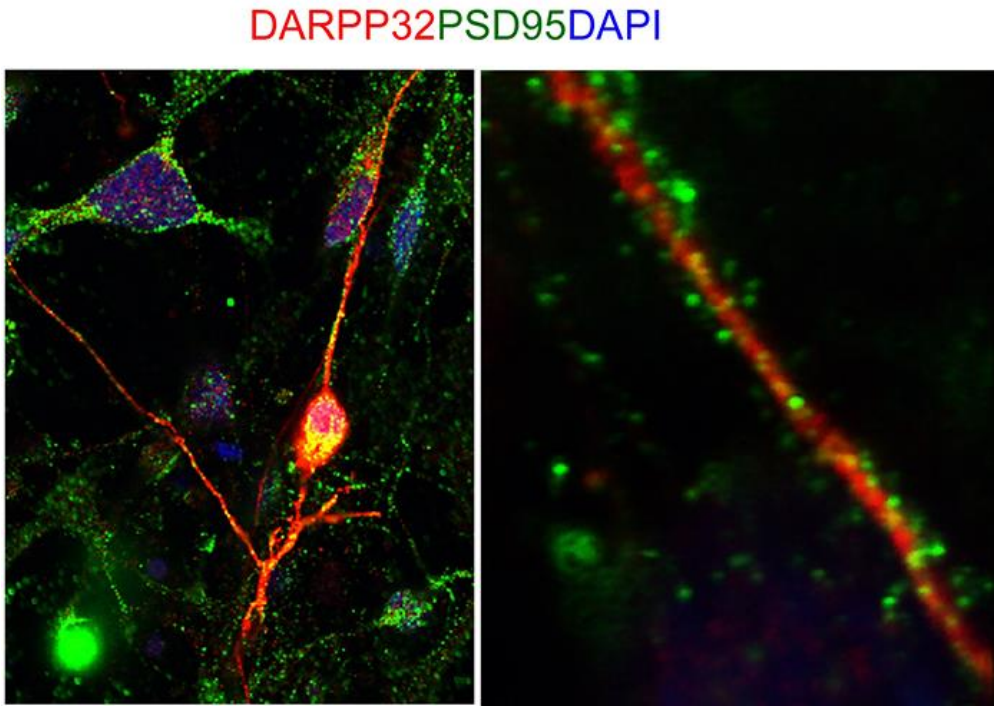

Fig. S4. Expression of post-synaptic protein PSD95 by hESC-derived striatal neurons.

Day 35 cultures treated with activin from day 9 were double stained for DARPP32 and PSD95. The right panel shows accumulation of PSD95 on spines and dendrites of DARPP32^{+} neurons.

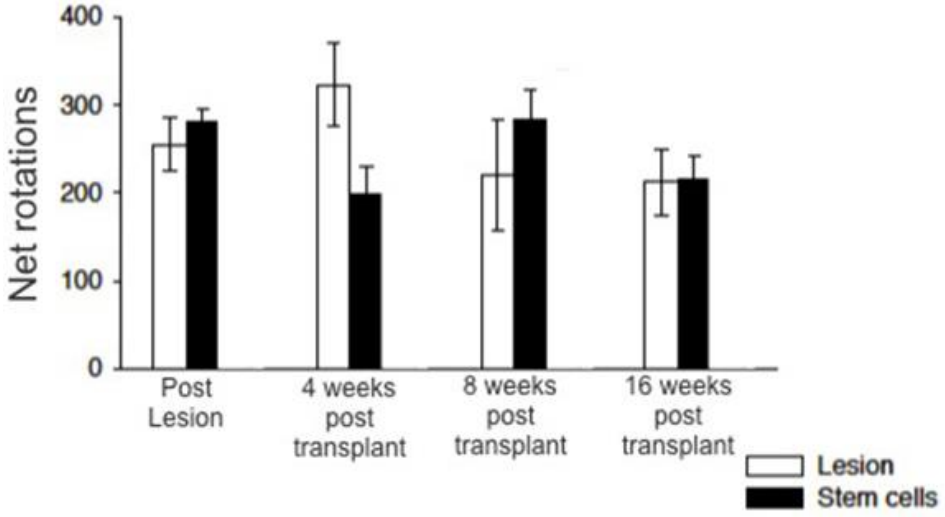

Fig. S5. Functional assessment of the hESC grafts transplantation into rat striatum.

Apomorphine-induced rotations rotation test at 4, 8 and 16 weeks after transplantation. No improvement was observed.

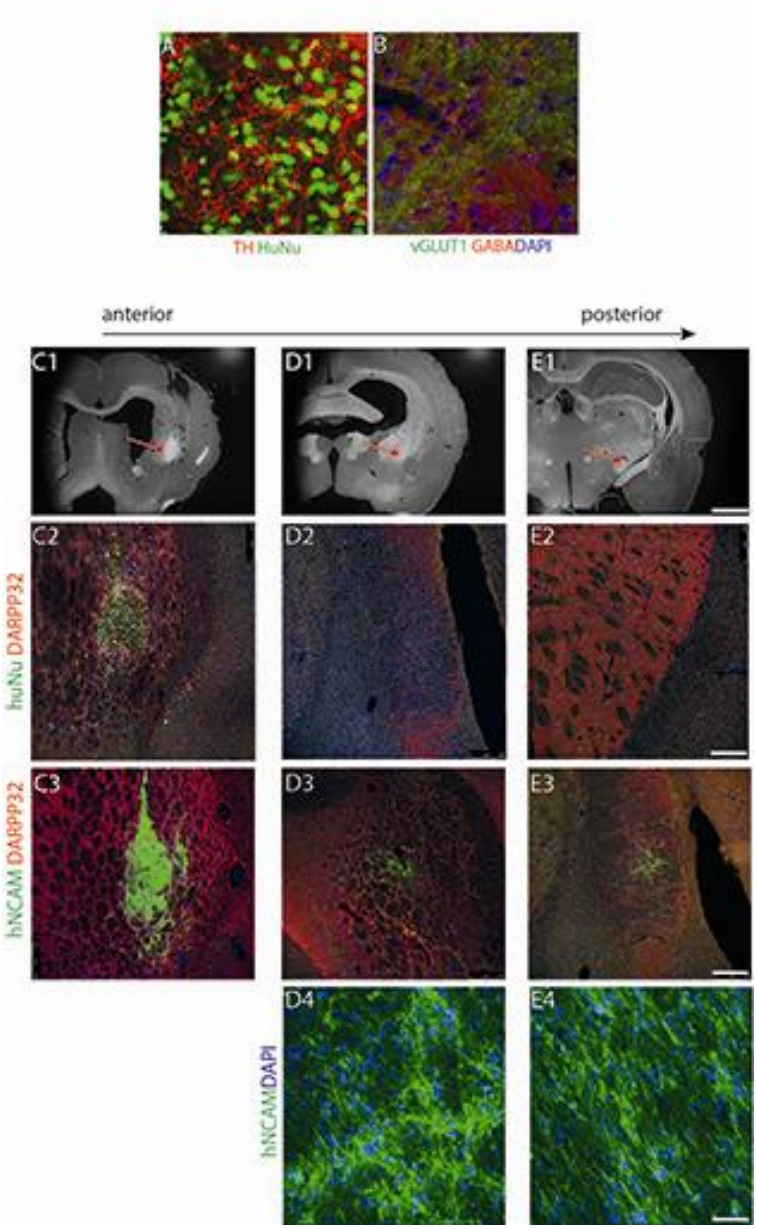

Fig S6. Analysis of graft cell integration in the host brain.

A-B, a section crossing the centre of a 16 week old graft double stained for TH and HuNu in (A), and vGLUT1 and GABA with DAPI counter stain in (B). C. Shown are coronal sections of rat brains 16 weeks post-transplant through the forebrain and striatum (C1-4), globus pallidus (D1-4) and the substantia nigra in the midbrain (E1-4). Arrows mark the location of the high power images below. Immunostaining for DARPP32, HuNu and hNCAM reveal human nuclei in the striatum (graft location,C2) and hNCAM projecting caudally towards the globus pallidus (D3-4) and midbrain (E3-4). Scale bars: 3mm for C1, D1, E1; 400μm for C2-3, D2-3 and E2-3; 60 μm for D4 and E4.

Table S1. Electrophysiological properties of neurons during differentiation

Group:	**d30-49**	**d50-69**	**>d70**	**ALL**
N=	12	21	8	41
Resting Membrane Potential (mV)	-41.2 ± 3.57	-44.8 ± 2.36	-47.7 ± 2.98	-44.3 ± 1.7
N=	17	28	15	60
Capacitance (pF)	20 ± 1.94	22.4 ± 2.02	29.3 ± 3.95	23.7 ± 1.54
Input Resistance (mW)	686 ± 66.9	942 ± 255	502 ± 81.4	760 ± 123
N=	17	28	13	58
Series Resistance (mW)	15.6 ± 1.56	16.1 ± 1.22	16.6 ± 1.29	16.0 ± 0.79

Table S2. PCR primers

ARPP21	GGAAGCTGGTTGACGATGTGTC	GGCTTCTGTCGTTCTACGCC
βActin	TCACCACCACGGCCGAGCG	TCTCCTTCTGCATCCTGTCG
Calbindin	ATCAGGACGGCAATGGATAC	TAAGAGCAAGATCCGTTCGG
CTIP2	CTCCGAGCTCAGGAAAGTGTC	TCATCTTTACCTGCAATGTTCTCC
Cyclophilin	GGCAAATGCTGGACCAAACAC	TTCCTGGACCCAAAACGCTC
DARPP32	TTGGAAAATCCAGAAAACCG	CTGGTAGAAGCCGGTGAGAG
DLX2	ACTACCCCTGGTACCACCAGAC	TCTGCTCTCAGTCTCTGGCGAGTTCTC
DRD2	CTGAGGGCTCCACTAAAGGAG	CATTCTTCTCTGGTTTGGCG
EBF1	AATGTAAGCAAGGTGGACGC	TCAAGGTCTAAGCCGGACAC
FOXP2	AATGTGGGAGCCATACGAAG	GCCTGCCTTATGAGAGTTGC
GAD67	CGTCTTCGACCCCATCTTCGT	CGCAGATCTTGAGCCCCAGTT
GAPDH	ATGACATCAAGAAGGTGGTG	CATACCAGGAAATGAGCTTG
GLI1	TGAGGCCCTTCAAAGCCC	GTATGACTTCCGGCACCCTTC
GSX2	TCACTAGCACGCAACTCCTG	TTTTCACCTGCTTCTCCGAC
NKX2.1	CGCATCCAATCTCAAGGAAT	TGTGCCCAGAGTGAAGTTTG
NOLZ1	ACATTTTGCACCCCGAGTAC	GGAGTACGGCTTGAAACTCG
PAX6	AACAGACACAGCCCTCACAAACA	CGGGAACTTGAACTGGAACTGAC
PENK	GCTGTCCAAACCAGAGCTTC	TCTGGCTCCATGGGATAAAG
PTCH1	TTCGCTCTGGAGCAGATTTCCAAG	GCTTTTAATCCCACCGCGAAG
TAC1	TGGGGTTGAAAATTCAAAAAG	GGAGTTTCCTTCCTTTTCCG
TH	GAGTACACCGCCGAGGAGATTG	GCGGATATACTGGGTGCACTGG
vGLUT1	AGTTCGCAACGATGATGGCA	CTGCACCCCAGCATCTCTGA