In vitro differentiation of mES cells

PA6 and MS5 were propagated in alpha minimum essential medium (α-MEM, Invitrogen), 10% FBS (Sigma-Aldrich) and 2mM L-glutamine. For both PA6 and MS5 based protocols, the full differentiation time is 14 days. For differentiation on PA6, mES cells were seeded at a density of 500-1,000 cells/cm^2 onto either contact-inhibited or mitomycin-treated PA6 in differentiation media consisting of Glasgow Minimum Essential medium (G-MEM, Invitrogen), 10 % Knockout serum replacement (KSR, Invitrogen), 1x NEAA, 1mM sodium pyruvate (Invitrogen), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen) and 0.1 mM 2-mercaptoethanol (Invitrogen). Media was changed as needed. Days 8-14 of differentiation were continued in N2 media consisting of G-MEM, 1x N2-supplement, 0.1mM NEAA, 1 MM sodium pyruvate and 0.1 mM β-mercaptoethanol. For differentiation on MS5, mES cells were seeded at a density of 500-1,000 cells/cm^2 onto either contact-inhibited or mitomycin-treated MS5 in serum replacement media (SRM) [3] consisting of DMEM, 15 % fetal bovine serum (FBS, HyClone; Logan, UT, http://www.hyclone.com), 2mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen) and 10 μM β-mercaptoethanol. On day 5 of differentiation 100 ng/ml Fibroblast Growth Factor 8 (FGF8b, R&D Systems) and 200 ng/ml Sonic Hedgehog (Shh, R&D Systems) were added to the SRM media. Between days 8-11, cells were differentiated in N2 medium consisting of DMEM/F12 and 1x N2, supplemented with 10ng/ml bFGF, 100 ng/ml FGF-8 and 100 ng/ml Shh. Days 11-14, the N2 medium was supplemented with 200 μM ascorbic acid and 20 ng/ml Brain-Derived Neurotrophic Factor (BDNF, Sigma-Aldrich).

Primary antibodies used for immunofluorescent stainings

The following primary antibodies were used: rabbit anti-GFP (1:1,000; Abcam, Cambridge, MA, http://www.abcam.com; 1:1,000 Molecular Probes, Invitrogen), mouse anti-GFP (1:750) and sheep anti-aromatic L-amino acid decarboxylase (AADC) (1:200; Chemicon), sheep anti-TH (1:300; Pel-Freeze, Rogers, AR, http://www.dynalbiotech.com), rabbit anti-βIII-tubulin (TuJ1) (1:500) and rabbit anti-paired box gene 2 (Pax2) (1:100; Covance, http://www.covance.com), rabbit anti-dopamine β-hydroxylase (DBH) (1:1,000; gift from Dr Eipper), rabbit anti-paired-like homeodomain transcription factor 3 (Pitx3) (1:500, gift from Drs Burbach and Smidt; 1:150, Zymed, Invitrogen) rabbit anti-G protein-activated inwardly rectifying potassium channel 2 (GIRK2) (1:80; Alomone Labs, Israel, http://www.alomone.com), rabbit anti-glial fibrillary acidic protein (GFAP) (1:500) and rabbit anti-cytokeratin (1:400; Dako, Carpinteria. CA, http://www.dako.com), mouse anti-myosin (clone MF20; 1 μg/ml) and mouse anti-SSEA-1 (clone MC-480; 1 μg/ml; DSHB), rabbit anti-Ki67 (1:2,000; Novocastra; http://www.novocastra.co.uk), mouse anti-nestin (Rat-401, 1:50; Chemicon), mouse anti-Villin (ready-to-use, Lab Vision) and mouse anti-Oct-4 (C-10, 1:100, Santa Cruz Biotechnology, Santa Cruz, CA, http://www.scbt.com.

Karyotype analysis

Karyotype analysis was performed on D3, EV-1 and 9 kb TH-eGFP mES cells at the cytogenetics core facility at Dana-Farber/Harvard Cancer Center (http://www.dfhcc.harvard.edu/core-facilities/pathology/cytogenetics/) using standard

methods. G-banding was carried out to identify chromosomes. Karyotype was acquired from one cell of each sample and ten metaphase cells counted per sample.