Culturing undifferentiated human ES cells

HES5::eGFP BAC transgenic human ES cells (H9; WA-09; Wicell) expressing GFP under the HES5 promoter were cultured on mitotically inactivated mouse embryonic fibroblasts (MEFs) (Globalstem). Undifferentiated ES cells were maintained as described previously[25] in medium containing DMEM/F12, 20% KSR, 1mM Glutamine, 1% Penicillin/Streptomycin, non essential amino acids and beta-mercaptoethanol. Undifferentiated ES cells were purified with pluripotency markers Alexa 647-conjugated Tra-1-60 and PE-conjugated SSEA-3 (BD Pharmingen).

Neural induction and long-term propagation of NPCs

Neural differentiation of ES cells was performed as described in refs[4,15,25]. Briefly, neuroepithelial cells were generated either by monolayer induction – with dissociated ES cells plated on Matrigel (BD biosciences), or by co-culture on MS5 stromal cells. In both cases neural fate was directed by dual SMAD inhibition protocol[15]. NE cells and Neural rosettes were harvested mechanically during all stages of differentiation and replated on culture dishes pre-coated with 15 µg/mL polyornithine (Sigma), 1 µg/mL Laminin (BD Biosciences) and 1ug/ml Fibronectin (BD Biosciences) (Po/Lam/FN) in N2 medium composed of DMEM/F12 and N2 supplement (Invitrogen). N2 supplement contained Insulin, Apo-transferin, Sodium Selenite, Putrecine and Progesterone. This medium was supplemented with SHH (30 ng/mL), FGF8 (100 ng/mL) and BDNF (20 ng/mL) (all from R&D Systems) to induce and maintain early anterior regionalization of the neural plate. These factors were gradually replaced by FGF2 (20 ng/mL) and EGF (20 ng/mL) in the following two weeks of differentiation in order to maintain a proliferative (FGF and EGF responsive) NPC state. NPCs from all stages were collected at indicated days and FACS purified for *HES5::GFP* (NE to LRG) or EGFR for LNPs to purify for the highest NPC state for each stage. NE cells were collected at day 12 of differentiation, ERG were collected at day 14, mid neurogenesis radial glial (MRG) cells were collected at day 35, late gliogenic radial glial (LRG) cells were collected at day 80, and long term NPCs (LNP) were collected at day 220. At each stage cells were either split for the next passage or subjected to FACS purification for *HES5::GFP* as

described. All replating was performed on Po/Lam/FN coated dishes. For generating mature differentiated populations, HES5+ sorted NPCs were seeded at high density and subjected to mitogen withdrawal differentiation medium for 17 days which included N2 supplemented with Ascorbic Acid (AA)/BDNF (neuronal; NEdN, ERGdN, MRGdN) or 5% Fetal Bovine Serum (FBS) (Invitrogen) (glial) (LRGdA). For additional details, see Edri et al.[4].

References:

4 Edri, R. *et al.* Analyzing human neural stem cell ontogeny by consecutive isolation of Notch active neural progenitors. *Under Review (Nature Comms.)* (2014).

15 Chambers, S. M. *et al.* Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol* **27**, 275-280, doi:10.1038/nbt.1529 (2009).

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