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Materials and methods

Cultures/cells were fixed in 4% PFA/PBS, washed in PBS, and blocked with blocking solution (1% [wt/vol] of BSA; 0.15% [wt/vol] glycine, and 0.1% [vol/vol] triton X-100 in PBS) for 1 h at RT. Fixation was omitted for Ptc1 staining. Primary antibody incubation was performed at 4°C for 18 h. Secondary antibody incubation was performed for 1 h at RT. Stained cultures/cells were mounted with anti-fade mountant with DAPI (Vector Laboratories).

To analyze the cellular compositions of neurospheres, neurospheres were trypsinized (37°C; 10 min) and triturated into cells. The cell suspensions were resuspended in PBS. Neurosphere cells (10^5 cells) were cytospun onto TESPA-coated microscope glass. Slides were fixed for 1 h, washed in PBS, blocked, and incubated with primary antibody. After secondary antibody incubation and washing, slides were dehydrated, dried, and mounted with anti-fade mountant with DAPI.

For the staining of explants, explants with filters were fixed (20 min; RT). After washing with PBS, explants with filters were free from the agarose bed and blocked before primary antibody incubation. After secondary antibody incubation and washing, explants with filters were stained with DAPI solution. For the analysis of cell clumps of explants, explants were mildly triturated into suspension before being cytospun onto TESPA-coated microscope glasses. Slides were fixed for 1 h, washed with PBS, and dried. Triturated explants on slides were washed with PBS and blocked before primary antibody incubation. After secondary antibody incubation and washing, slides were dehydrated and dried before being mounted with anti-fade mountant with DAPI. Explants were also processed for paraffin sectioning. Processing of tissues and immunohistochemistry were performed as described previously (Fu et al., 2003).

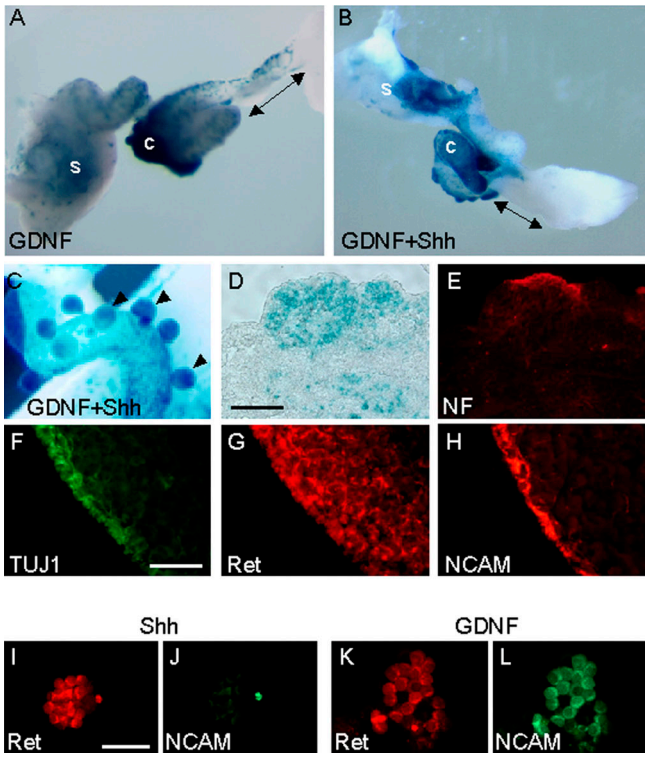

Figure S1. **E11.5 guts (from stomach to anus) of *b3-IIIa-LacZ* mice were cultured in GDNF without Shh (A) or with Shh (B) for 3 d.** (A) X-gal/IPTG staining revealed that NCCs (blue) migrated to the distal colon (↔) in GDNF culture. (B) In contrast, the distal colon (↔) of the GDNF Shh-cotreated explant was devoid of NCCs. NCC clumps (blue) were observed on the GDNF Shh-cotreated explant (C, arrowheads). (D) Sections of the cell clumps revealed that most of the cells expressed β-galactosidase. The sections were stained for neurofilament (E, NF), TUJ1 (F), Ret (G), and NCAM (H). Neurospheres cultured in different treatments were stained for Ret (I and K, red) and NCAM (J and L, green). s, stomach; c, cecum. Photos D and E were taken at the same magnification. F–H were taken at the same magnification. I–L were taken at the same magnification. Bars: (D and E) 25 μm; (F–H) 10 μm; (I–L) 50 μm.