Supplement figure S1: Blocking netrin function does not affect cell survival.

To assess the effect of blocking autocrine netrin function on cell survival, cells were grown in the presence of PN3 antibodies or the unc5H2/Fc chimera. C6 cells (15000/well) were plated in 24-well plates in triplicate in DMEM containing 1% Penicillin/Streptomycin and 0.2% BSA. Cells were allowed to settle for 2 hours at 37°C and then incubated for 48 hours in the presence of PN3 netrin function blocking antibodies (N_{FB}) or control IgG, at concentrations of 5, 10, 25, or 100 µg/ml; recombinant rat Unc5H2/Fc chimera (5 µg/ml; R&D Systems); or staurosporine (50 nM; Sigma). Cell viability was assessed by labelling F-actin with Alexa 488-conjugated phalloidin (diluted 1/500; Invitrogen) for 1 hour and nuclei with Hoechst dye (Invitrogen). Cells were counted using Image J software (NIH). Cell viability in the presence of netrin function blocking agents was comparable to control cells grown in the presence of normal IgG. Cell survival tended to increase gradually with increasing protein concentration (N_{FB} and IgG). In contrast, staurosporine, a positive control for cell toxicity, caused extensive cell death.