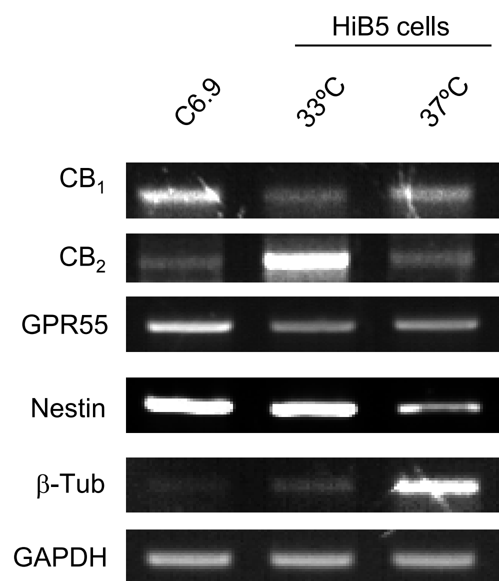
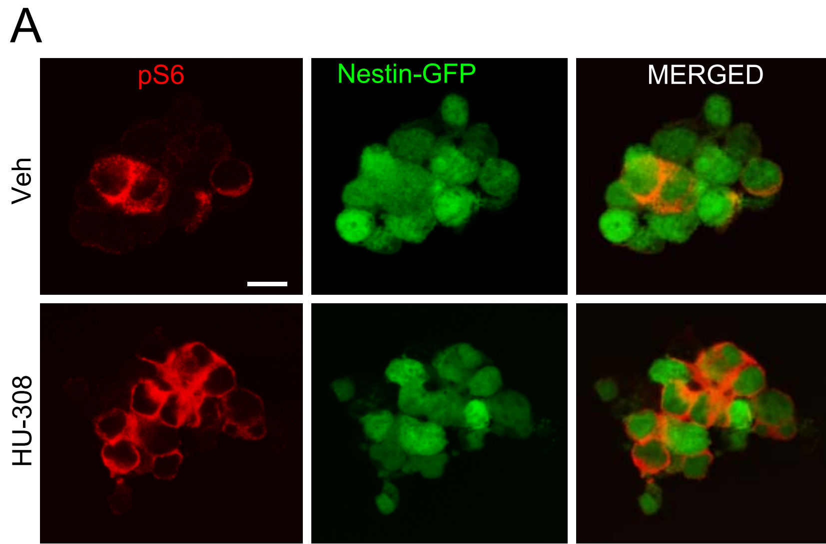
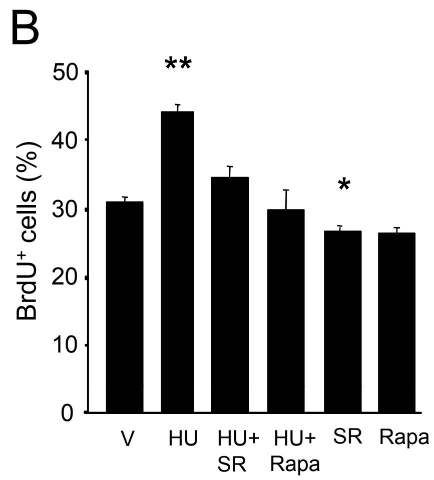
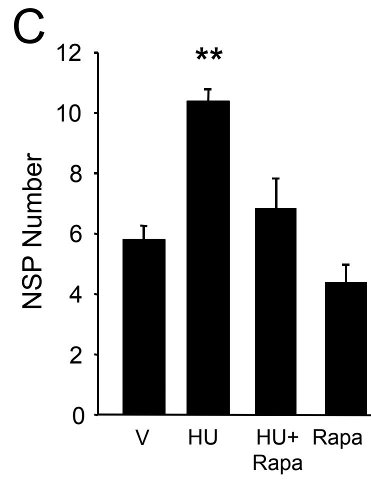


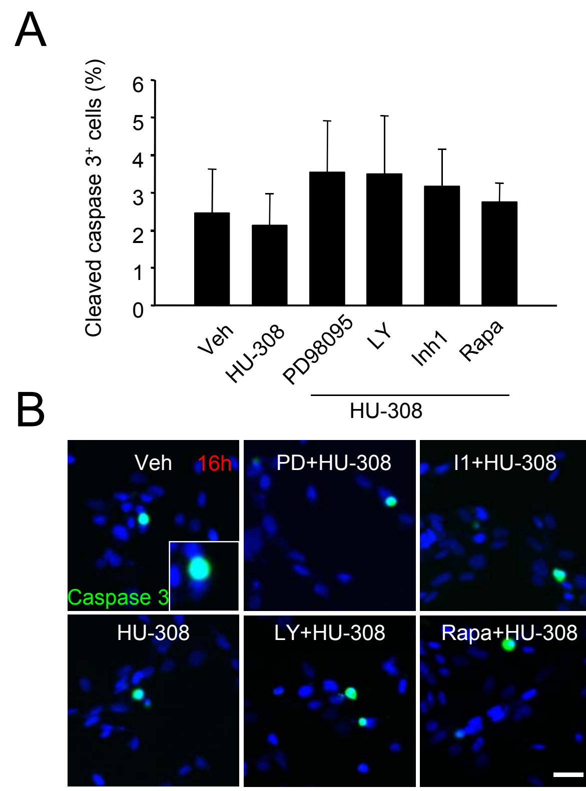
Suppl. Figure 1



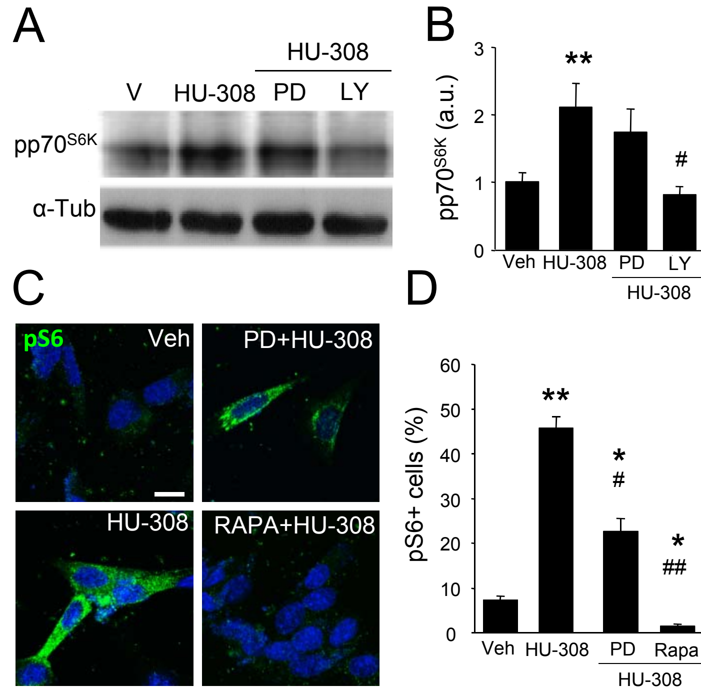




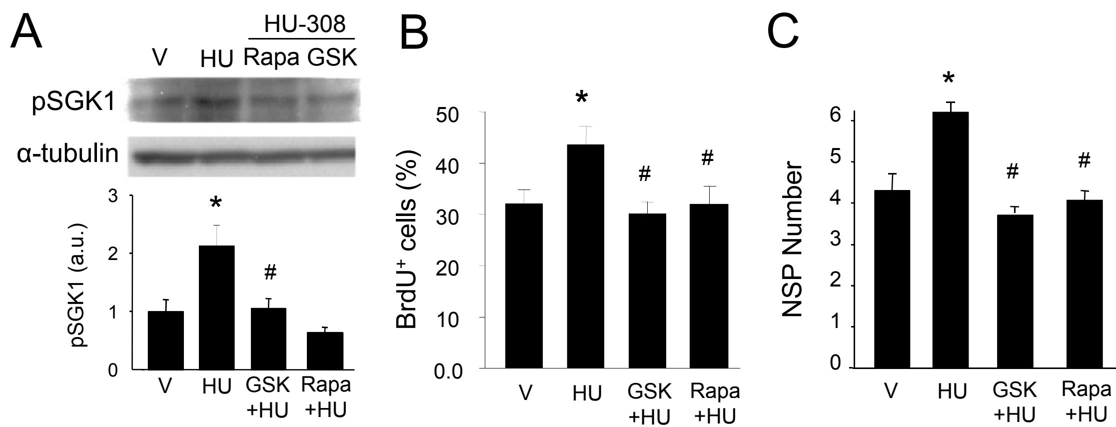
SUPPL. FIGURE 2



SUPPL. FIGURE 3



SUPPL. FIGURE 4



SUPPL. FIGURE 5

SUPPLEMENTAL Fig. 1. **HiB5 neural progenitor cells express cannabinoid receptors.** Reverse transcription-PCR analysis of CB_1, CB_2 and GPR55 receptors was performed in HiB5 cells grown at proliferating (33º C) and differentiating conditions (3 days in vitro at 37º C) using the C6.9 clone of the glioma C6 cell line as control. Nestin and β-tubulin III transcripts were analyzed to monitor cell differentiation. Loading control was performed with GAPDH probe.

SUPPLEMENTAL Fig. 2. **CB_2 cannabinoid receptors signal through the PI3K/Akt /mTORC1 axis on neural progenitors and neurospheres.** A, Immunofluorescence analysis of nestin-GFP neural progenitors with the anti-phospho-S6 antibody after HU-308 treatment for 30 min. B, Embryonic (E14.5) neural progenitor proliferation was determined by quantification of $BrdU^+$ cells after 16 h of incubation with HU-308 as above, alone or in the presence of SR144528 and rapamycin. The fraction of $BrdU^+$ cells is referred to the total cell number as determined by Hoechst 33342 counterstaining. Results correspond to 4 independent experiments. C, Neurosphere generation was determined in clonal-density progenitor cultures after 3 days of incubation with HU-308 in the absence or presence of rapamycin. Results correspond to 4 independent experiments. Scale bars, 10 μm. *, $p < 0.05$; **, $p < 0.01$ versus vehicle-treated cells.

SUPPLEMENTAL Fig. 3. **Lack of cell death in HiB5 progenitor cultures.** A, HiB5 cells were treated with HU-308 (50 nM) for 16 h in the absence or presence of PD-8059 (10 μM), LY-294,002 (5 μM), Akt Inhibitor 1 (5 μM) and rapamycin (50 nM) and cell death was quantified after immunofluorescence with a selective antibody against active caspase 3. Total cells were counterstained with Hoechst 33342. B, Representative immunofluorescence images are shown. Scale bar, 40 μm.

SUPPLEMENTAL Fig 4. **ERK does not play a major role in HU-308-induced mTORC1 activation in HiB5 cells.** A and B, HiB5 cells were incubated with HU-308 (50 nM) for 30 min in the absence or presence of PD98059 (10 μM) and LY-294,002 (5 μM). Levels of phospho-p70S6K were quantified and referred to loading control performed with anti-α-tubulin antibody. C and D, The number of phospho-$S6^+$ HiB5 cells was quantified after incubation with HU-308 (50 nM) for 30 min in the absence or presence of PD98059 (10 μM) and rapamycin (Rapa; 50 nM) and immunofluorescence. Phospho-$S6^+$ cells were normalized to total cell number as identified by Hoechst 33342 counterstaining. Representative images are shown for each condition. Scale bar, 10 μm. *, $p < 0.05$; **, $p < 0.01$ versus vehicle-treated cells; # $p < 0.05$; ## $p < 0.01$ versus HU-308-treated cells.

SUPPLEMENTAL Fig. 5**. SGK1 participates in CB_2 receptor/mTORC1-mediated HiB5 cell progenitor proliferation.** A, HiB5 cells were treated with HU-308 alone or in the presence of rapamycin (Rapa; 50 nM) and GSK-650394 (75 nM) and Western blot and immunofluoresce analyses were performed. Levels of phospho-SGK1 were quantified and referred to loading control performed with anti-α-tubulin antibody and given in arbitrary units (a.u.). Representative luminograms and immunofluorescence images are shown. B, HiB5 cells were treated as above for 16 h and BrdU incorporation was quantified. C, Neurosphere (NSP) generation was quantified in clonal-density conditions and the number of NSPs determined after 3 days of HU-308 treatment alone or in the presence of GSK-650394 and rapamycin (as above). Scale bars, B and D, 50 and 10 μm, respectively. *, $p < 0.05$, versus vehicle-treated cells; # $p < 0.05$, versus HU-308-treated cells.