

Fig S1. Experimental design.

A) Secondary neurosphere formation assays. Upper panel. Glioblastoma stem cells (GSC) were dissociated and cultivated for one day in the presence of the drugs (RP, PP242 and PI103) and vehicle (DMSO). Primary neurospheres (1° neurosphere) were dissociated and secondary neurospheres (2° neurosphere) were counted 3 days later. Lower panel. GSC were dissociated, transfected with siRNA (sic, mTOR1, Raptor1 and Rictor1) and cultivated for 2 days. Primary neurospheres were dissociated and re-transfected with the siRNAs. Three days after, 2° neurospheres were counted. B) HEK293T and hCMEC/D3 were grown for 3 days in the presence of serum-containing DMEM and EBM2 media, respectively. Confluent cultures were then extensively washed in PBS and transferred into serum-free EBM2. Conditioned media (CM) from HEK293T and hCMEC/D3 were collected 3 days later, centrifuged and filtered before use.

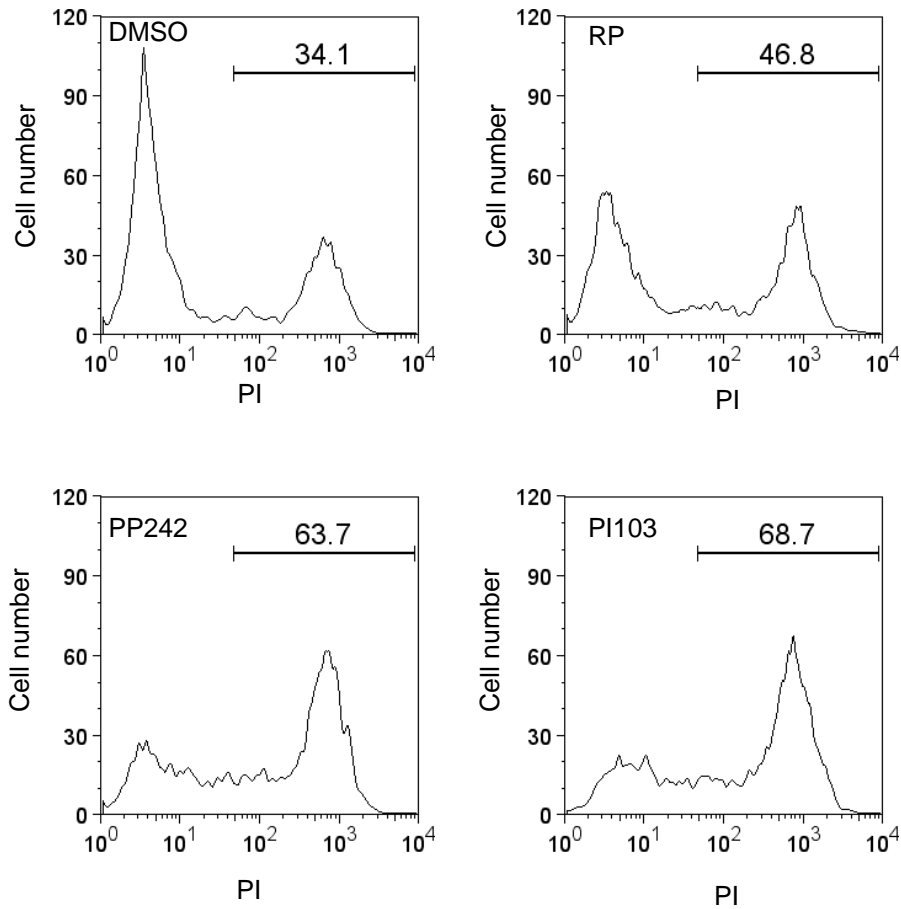


Fig S2. Effects of pharmacological inhibition of the mTOR pathway on cell survival.

TG16 cells were dissociated and cultivated for 3 days in the presence of the drugs (DMSO, RP, PP242 and PI103). Cell death was determined by propidium iodide (PI) staining using flow cytometry analysis. Right windows indicate percentage of dead cells.

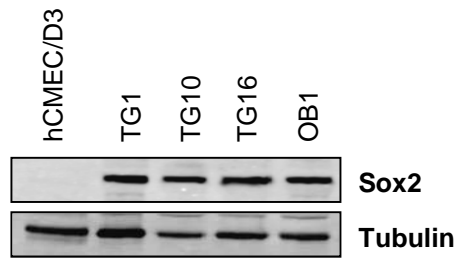


Fig S3. Sox2 expression is expressed in GSC.

50 μ g of total proteins were analysed for Sox2 expression by western blot in hCMEC/D3 endothelial cells and GSC (TG1, TG10, TG16 and OB1). Tubulin expression served as loading control.

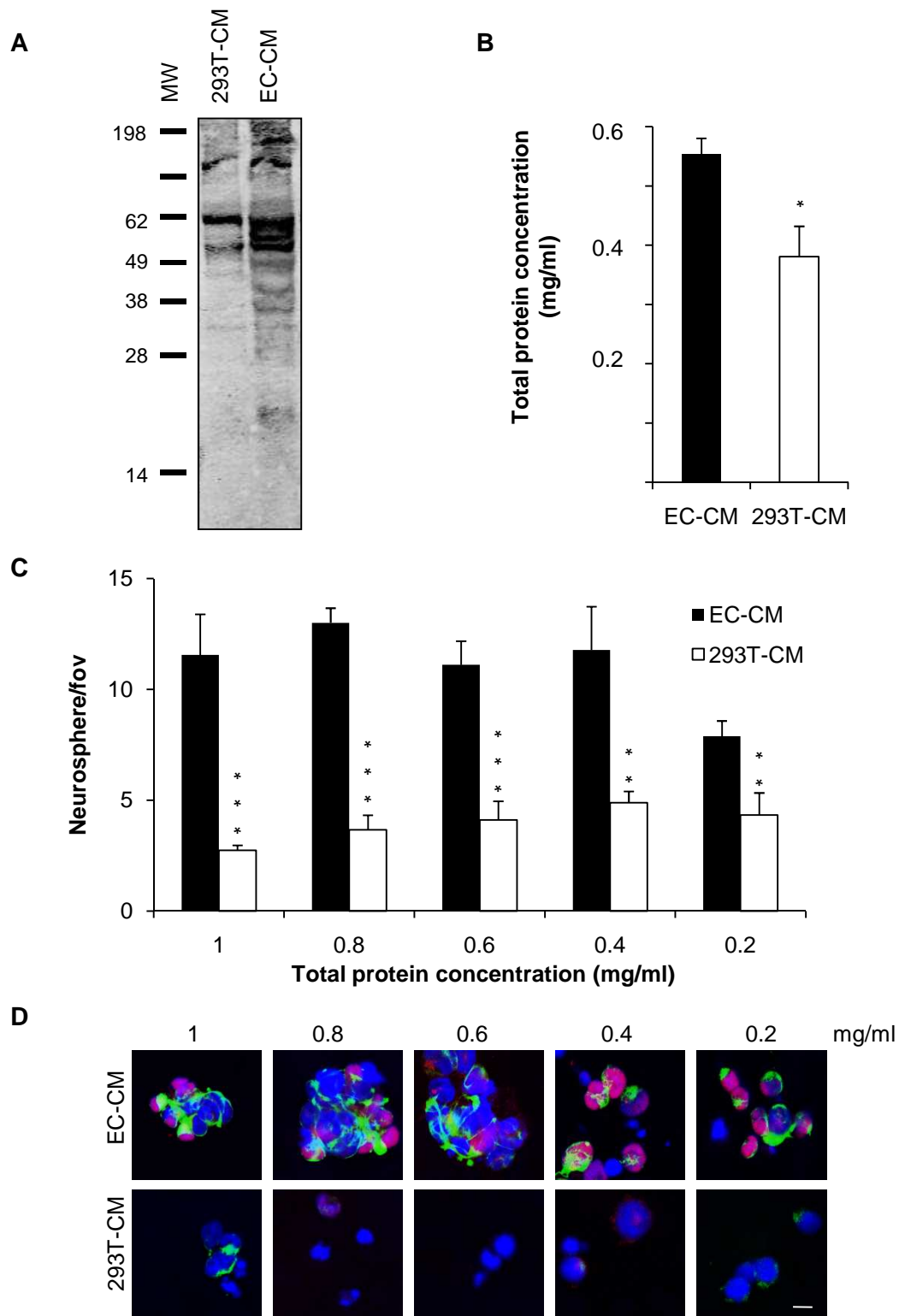


Fig S4. Protein characterisation of conditioned medium.

A) HEK-293T and hCMEC/D3 conditioned medium (293T-CM and EC-CM) were collected, 10-fold concentrated using centrifugal filter units (Millipore, 3K cut-off) and loaded onto 4-12% gradient NuPAGE (Invitrogen). After electrophoretic separation, gel was stained with Coomassie blue and scanned. B) Protein concentration was evaluated in each CM using micro-BCA kit (Pierce). C) CM were titrated from concentrations ranging between 1 to 0.2 mg/ml and neurosphere formation was evaluated in TG1. D) Labelling of the stemness markers Sox2 (red) and Nestin (green) in TG1 treated with the indicated protein concentrations of CM. Nuclei are counterstained with DAPI (blue). Scale bar: 10 μ m. Graphs represent mean + sem of 3 independent experiments. ANOVA test: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

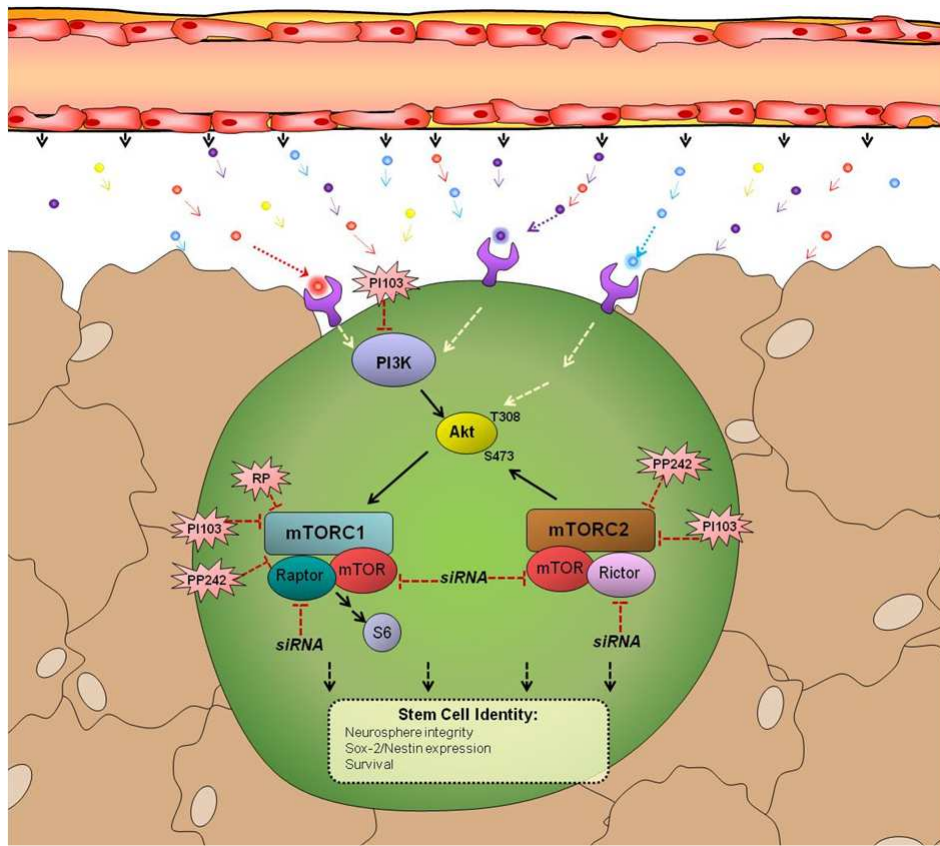


Fig S5. Schema for the mTOR-dependent expansion of cancer stem cells. Brain endothelial cells (red) secreted soluble factors that can act on cancer stem cells (green) within the tumour mass (brown background). Endothelial factors promote mTOR activation, which can be blocked by siRNA or pharmacological inhibitors as indicated. Ultimately, the mTOR signalling nexus contributes to the stem cell identity.