

## Riluzole: A potential therapeutic intervention in human brain tumor stem-like cells

### Supplementary Materials

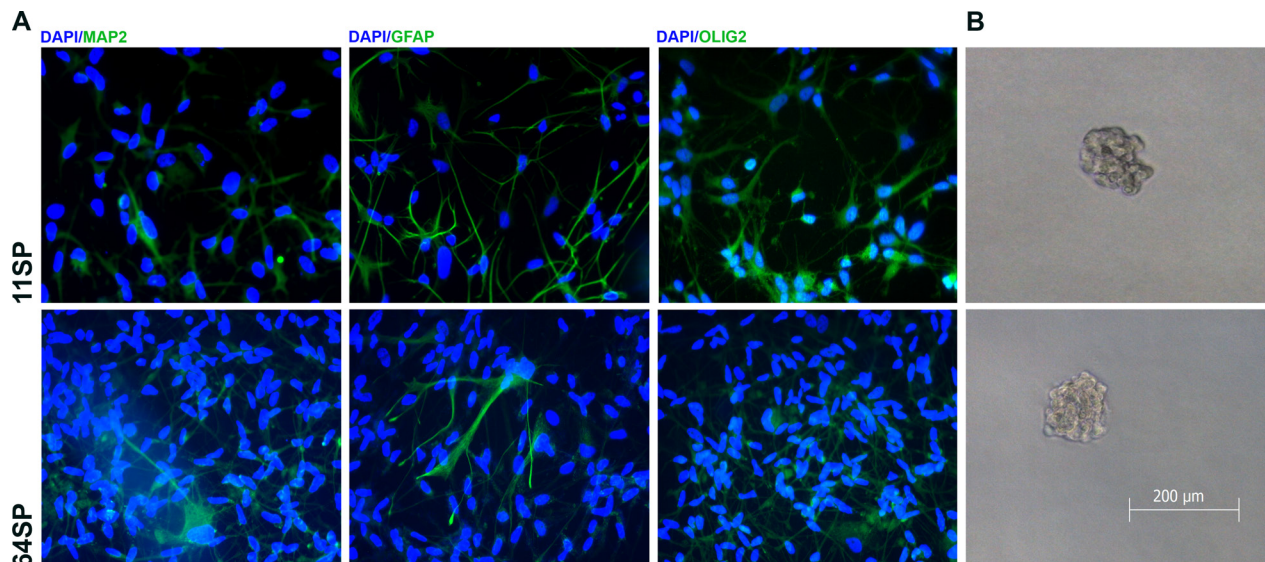
#### Differentiation of BITCs and immunocytochemistry

In order to identify differentiation potential, neurospheres were dissociated and seeded on coverslips coated with poly-D-Lysine (Sigma-Aldrich, St. Louis, MO, USA) in differentiation medium (culture medium with 10% FCS). After 10 days cells were fixed in 4% PFA, and incubated overnight with the following primary antibodies: anti-MAP2 (Millipore, Billerica, USA; 1:50), anti-GFAP (Leica, Wetzlar, Germany; 1:50), anti-OLIG2 (Millipore, Billerica, USA; 1:50). After washing, cells were incubated with secondary antibodies: biotinylated anti-mouse or anti-rabbit (Jackson ImmunoResearch Laboratories, West Grove, PA, USA; 1:500) and Streptavidin Alexa 488 (Life Technologies, Waltham, USA; 1:500) at room temperature.

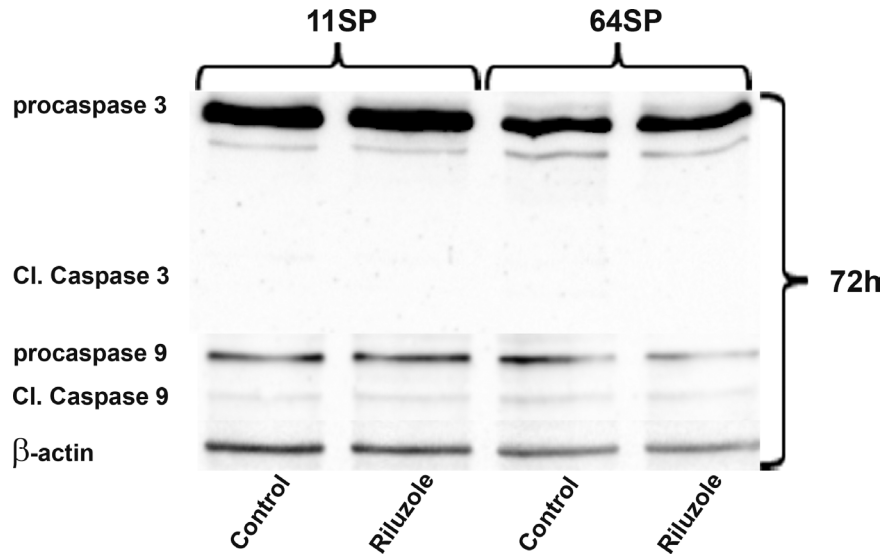
Nuclei were counterstained with DAPI. Image acquisition was done with 40 $\times$ .

#### Soft agar assay

Single-cell suspension of  $4 \times 10^3$  cells was plated in medium containing 0.4% agar noble (Difco, Becton Dickinson and Company, Franklin Lakes, New Jersey, USA), and after that seeded in 24-well plates already containing 1, 2% agar. Cells were cultured for 3–4 weeks. The number of wells with tumorspheres was scored after 28 days by counting the colonies.



**Supplementary Figure 1: Characterization of BTSCs.** (A) Immunofluorescent staining of differentiated 11SP and 64SP BTSCs with neural (MAP2), astrocytes (GFAP), and oligodendrocytes (OLIG2) marker. (B) Representative picture of neurosphere formation from 11SP and 64SP in soft agar assay after 28 days.



**Supplementary Figure 2: Caspase 3 and Caspase 9 expression analysis after riluzole treatment.** Whole cell lysates of 11SP and 64SP BTSCs treated with riluzole (50  $\mu$ M) for indicated time was collected and analyzed by western blot. Activation of Caspase 3 and Caspase 9 was not detected.  $\beta$ -actin was used as loading control.