# hiPSC-derived neurospheroids support assessment of glioblastoma cancer stem cell behavior and compound responses

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## StemoniX

#### **INTRODUCTION** and **METHODS**

Glioblastoma Multiforme (GBM) is an aggressive and incurable brain tumor with limited therapeutic options.

Human iPSC-derived 3D neurospheroids (microBrain® 3D) were used to evaluate two distinct GBM cancer stem cells (GSCs) and three possible treatments.

GSCs cells were transduced with an mCherry reporter, co-cultured with microBrain 3D and treated with potential anti-neoplastic compounds.

The resulting impact on GSCs and host cells (neurons and astrocytes) was measured via quantitative fluorescence confocal microscopy.

The system was used to quantify, discriminate, and assess

- 1) Line-specific GSC behaviors of proliferation and infiltration.
- 2) Potential therapeutic impact of compounds.
- 3) Possible adverse effects on healthy host neurons and astrocytes.

Collectively, our results show that microBrain 3D neurospheroids together with fluorescent imaging can be used to stratify GSC-specific behavior and treatment response, thus establishing a foundation to identify and personalize GSC treatment.

#### Instrument

Nikon A1R upright confocal microscope with 25x, 1.15NA water-dipping objective

Channels used: DAPI (ex. 405 nm), GFAP-AlexaFluor488 (ex. 488 nm), mCherry (ex. 561 nm), MAP2-AlexaFluor647 (ex. 637 nm).

#### **Cell Cultures**

Neural spheroids: microBrain 3D Assay Ready 96-well and 384-well plates are off-the-shelf products from StemoniX®, Inc. Each well contained a single, uniformly sized human iPSC-derived cortical neural spheroid matured 8-9 weeks.

GSC lines were obtained by Bristol-Myers Squib and genetically engineered to constitutively express mCherry as a cellular biomarker.

#### Co-culture, Treatment, and Imaging

Transduced GSCs were added to six-week-old microBrain 3D neurospheroids, and compound treatment was started 72 hours later with either vehicle control or one of two different compounds. Treatment continued for 5 weeks; media and compounds were replenished every Monday. At the end of the 5 week, spheroids were fixed with 4% paraformaldehyde, permeabilized in Triton-X100, exposed to 1° and 2° antibodies, and optically cleared for better visualization.

Confocal slices were obtained through approximately ½ of the spheroid diameter with representative images shown as maximum intensity projections for each imaging channel.

Relative amounts of each cell population were determined from their respective staining patterns GSC=mCherry(+), Neurons=MAP2(+), Astrocytes=GFAP(+) and mCherry(-).

#### Results

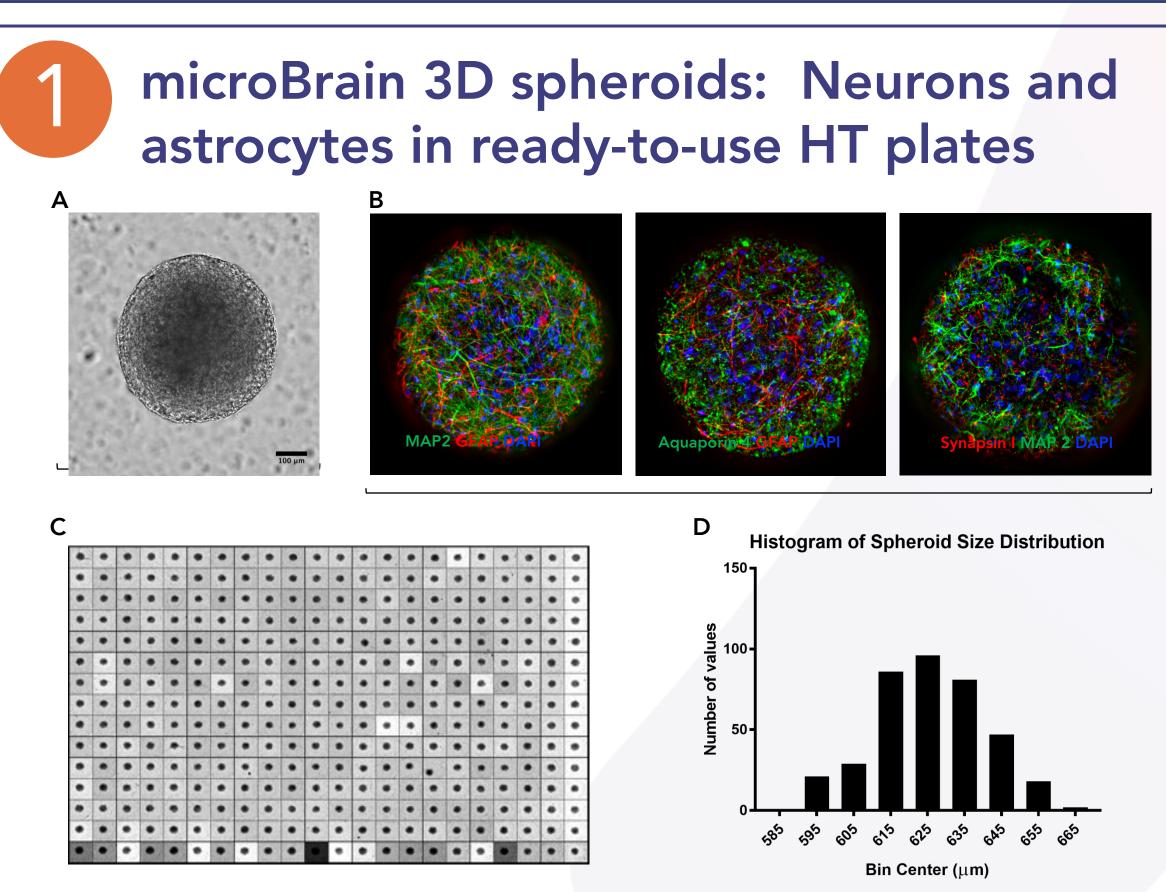


Figure 1. microBrain 3D provides a consistent, high-throughput, ready to use, human-based 3D neural preparation. A) Human iPSC-derived neural spheroids, approximately 600 μm diameter. B) Spheroids are composed of a co-culture of active cortical neurons (identified by MAP2; green) and astrocytes (identified by GFAP; red). C) A full 384-well plate of microBrain 3D neurospheroids with a single spheroid per well. D) Histogram of spheroid size distribution.

#### RESULTS

microBrain 3D provides a suitable platform for exogenous cell visualization

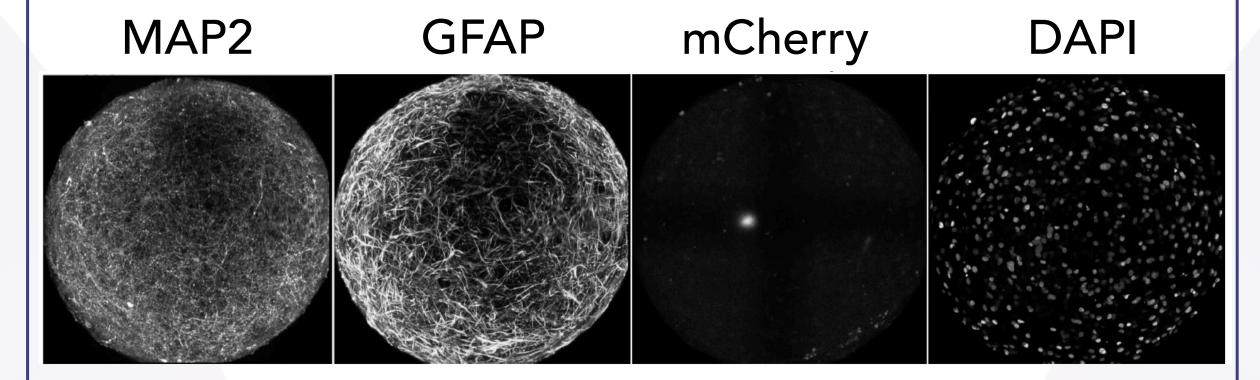
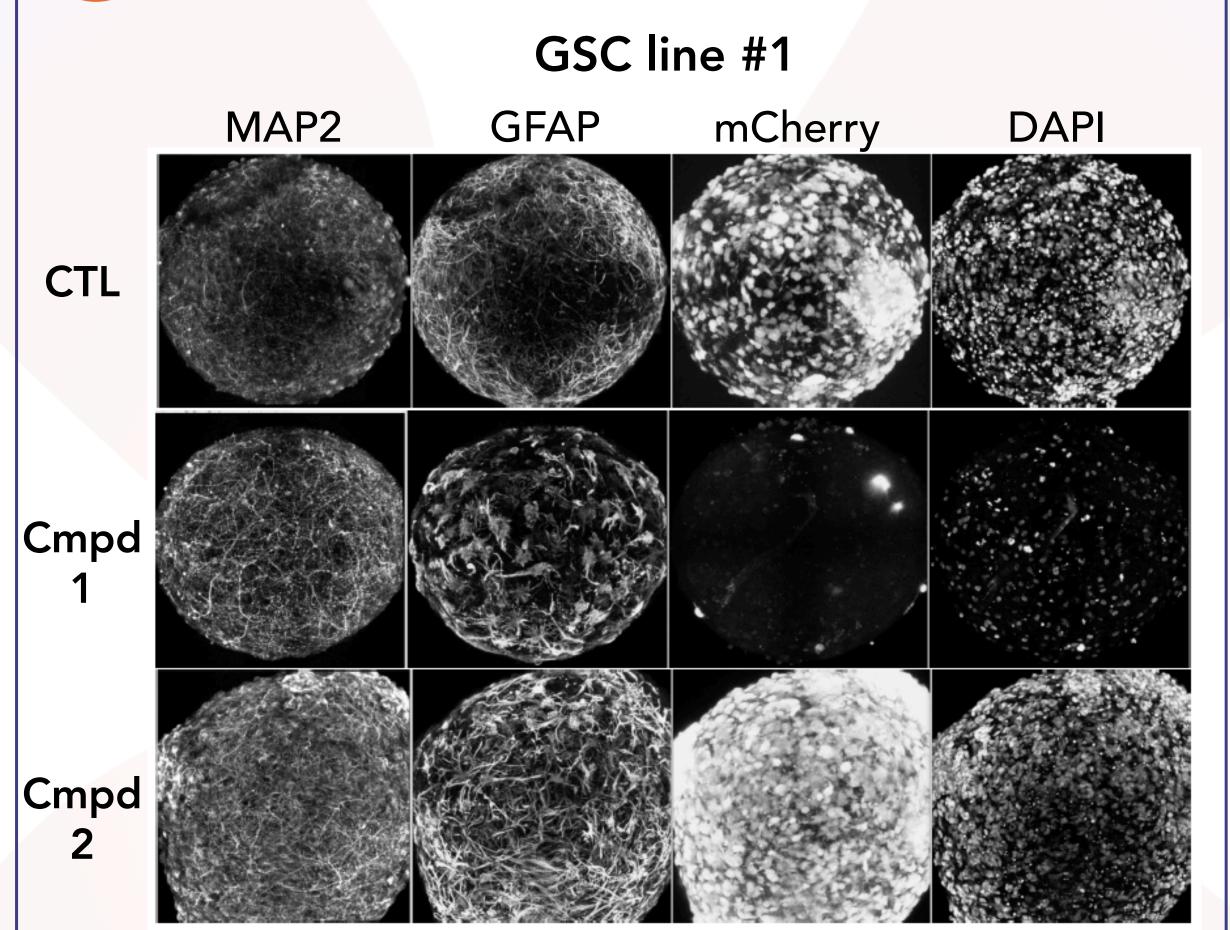


Figure 2. The 'red' channel is available for biomarker analysis. Neural spheroids labeled for neurons (MAP2), astrocytes (GFAP), and nuclei (DAPI) demonstrating the availability of mCherry (and similar fluorophores) as a biomarker.

### microBrain 3D highlights different GSC line behavior and treatment responses



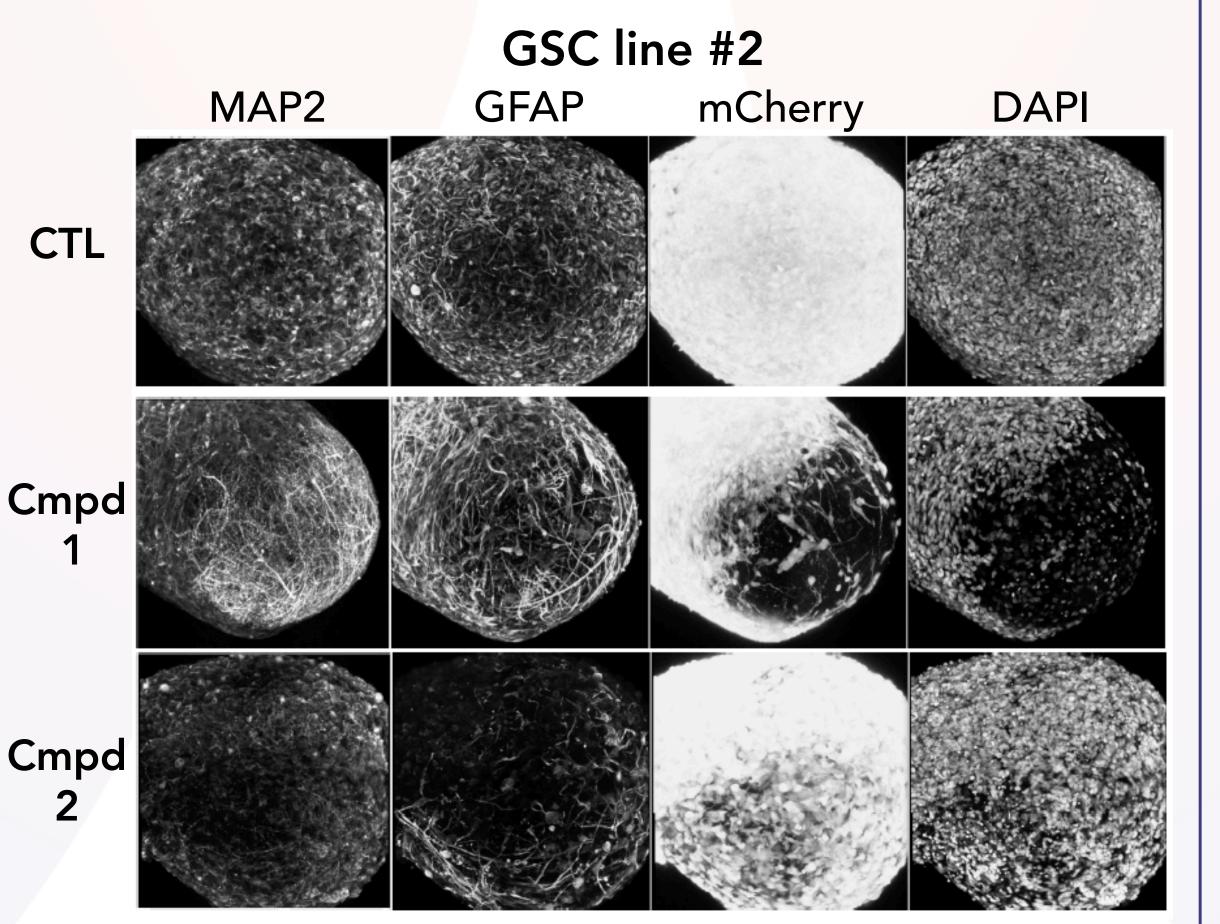


Figure 3. microBrain infiltration by GSC cells and their response to treatment was line specific. ICC staining of microBrain 3D neurospheroids co-cultured with two different GSC lines and exposed to three different compound treatments.

Phenotypic differences: GSC lines show different phenotypes when co-cultured with microBrain 3D. GSC line #2 appears to be more 'aggressive' than GSC cell line as judged by the increased mCherry labelling under control (CTL) conditions.

<u>Treatment differences</u>: Treatment impact can be line-specific. Compound 1

appears to have the largest relative impact on GSC line 1 versus GSC line 2.

#### RESULTS

### GSC content in microBrain 3D can be quantified

Z-stack images were acquired, and signal intensity was summed and normalized for each individual channel.

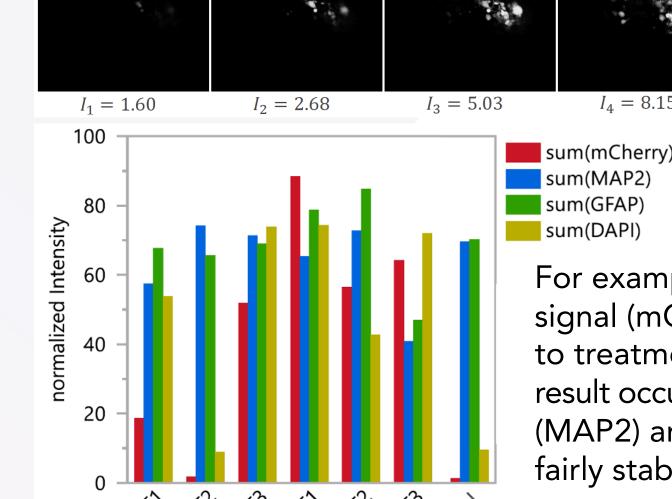


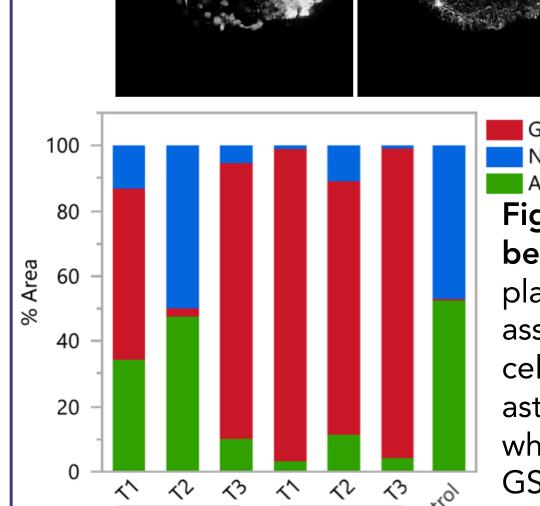
Figure 4. Treatment impact on individual cell types can be assessed and quantified.

For example, treatment 2 reduced GSC signal (mCherry) in both conditions relative to treatments 1 and 3, with the most drastic result occurring in GSC cell line 1. Neuronal (MAP2) and astrocytic (GFAP) content were fairly stable across all conditions.

T1=control, T2,3=cmpds 1,2. C1,2 = GSC lines 1,2

### Relative cellular content within GSC/microBrain 3D can be quantified

Cell type-specific relative area was quantified across all z-slices.



GBM (mCherry)
Neurons (MAP2)
Astrocytes (GFAP)
Figure 5. Relative impact across cell types can

be quantified. The microBrain 3D co-culture platform enables simultaneous assessment across all cell types. Treatment 2 in cell line 1 restores a healthy 1:1 neuron to astrocyte ratio as seen in control spheroids, whereas treatments 1 and 3 either do not reduce GSC content or alter the ratios of host cells. T1=control, T2,3=cmpds 1,2. C1,2 = GSC lines 1,2

### Maximum GSC penetration into microBrain 3D can be quantified

GSC masses were identified and measured in the X, Y, or Z dimensions from the spheroid edge.

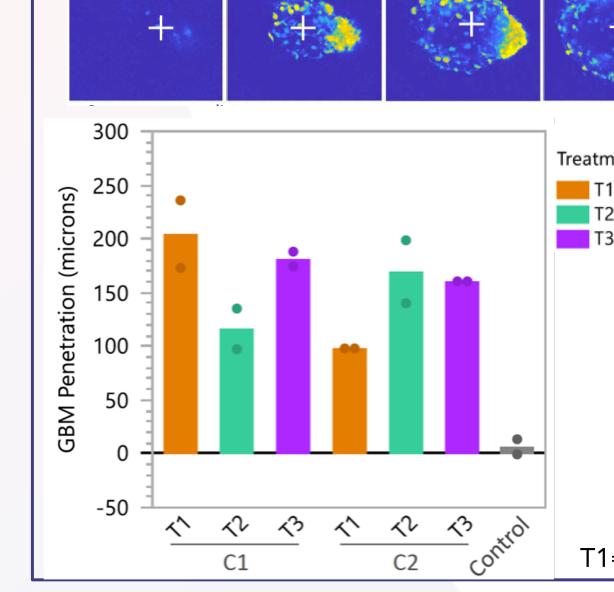


Figure 6. Treatment efficacy in preventing GSC penetration into microBrain 3D was measured by determining the furthest infiltration from the spheroid edge in the X, Y, or Z dimensions. In this example treatment 2 was most effective at preventing GSC line 1 penetration and

calibration with no GSCs present.
T1=control, T2,3=cmpds 1,2. C1,2 = GSC lines 1,2

treatment 1 was most effective at

preventing GSC line 2 penetration.

Control represents successful system

#### CONCLUSIONS

We developed methods and assessed the feasibility of using iPSC-derived StemoniX microBrain 3D Assay Ready neural cultures as an assay platform for studying GSC behavior and as a substrate for drug discovery.

- The data demonstrate that microBrain 3D neurospheroids
- 1) Provide a suitable host cell population for GSC co-culture.
- 2) Enable specific GSC line behavior to be assessed.
- 3) Stratify compound impact on GSC growth and penetration.
- 4) Facilitate simultaneous assessment of compound impact on healthy cells.

microBrain 3D provides a suitable, HT, ready-to-use platform for GSC-based drug discovery