

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

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## 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 Squibb and genetically engineered to constitutively express mCherry as a cellular biomarker.

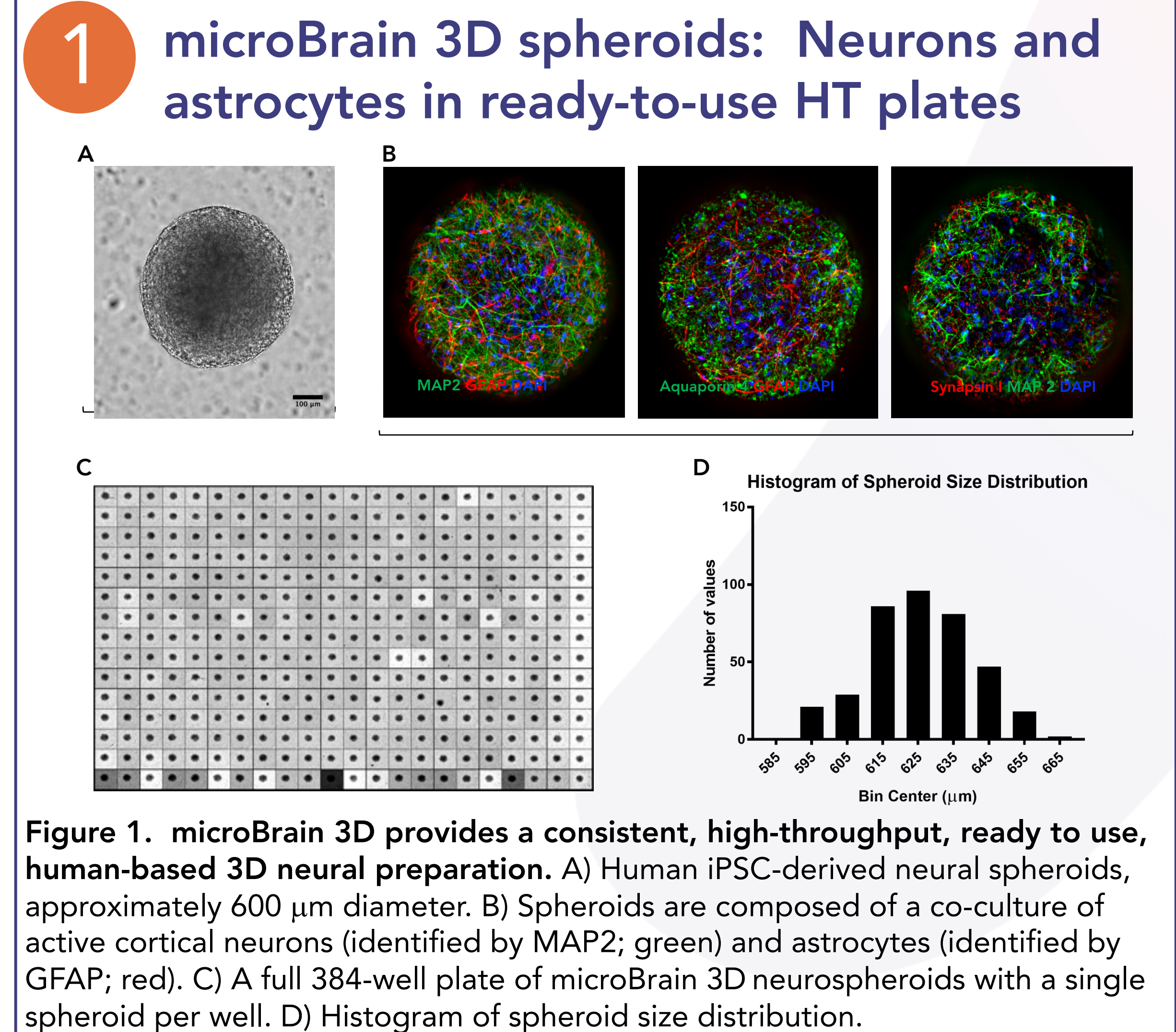
**Co-culture, Treatment, and Imaging**

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.

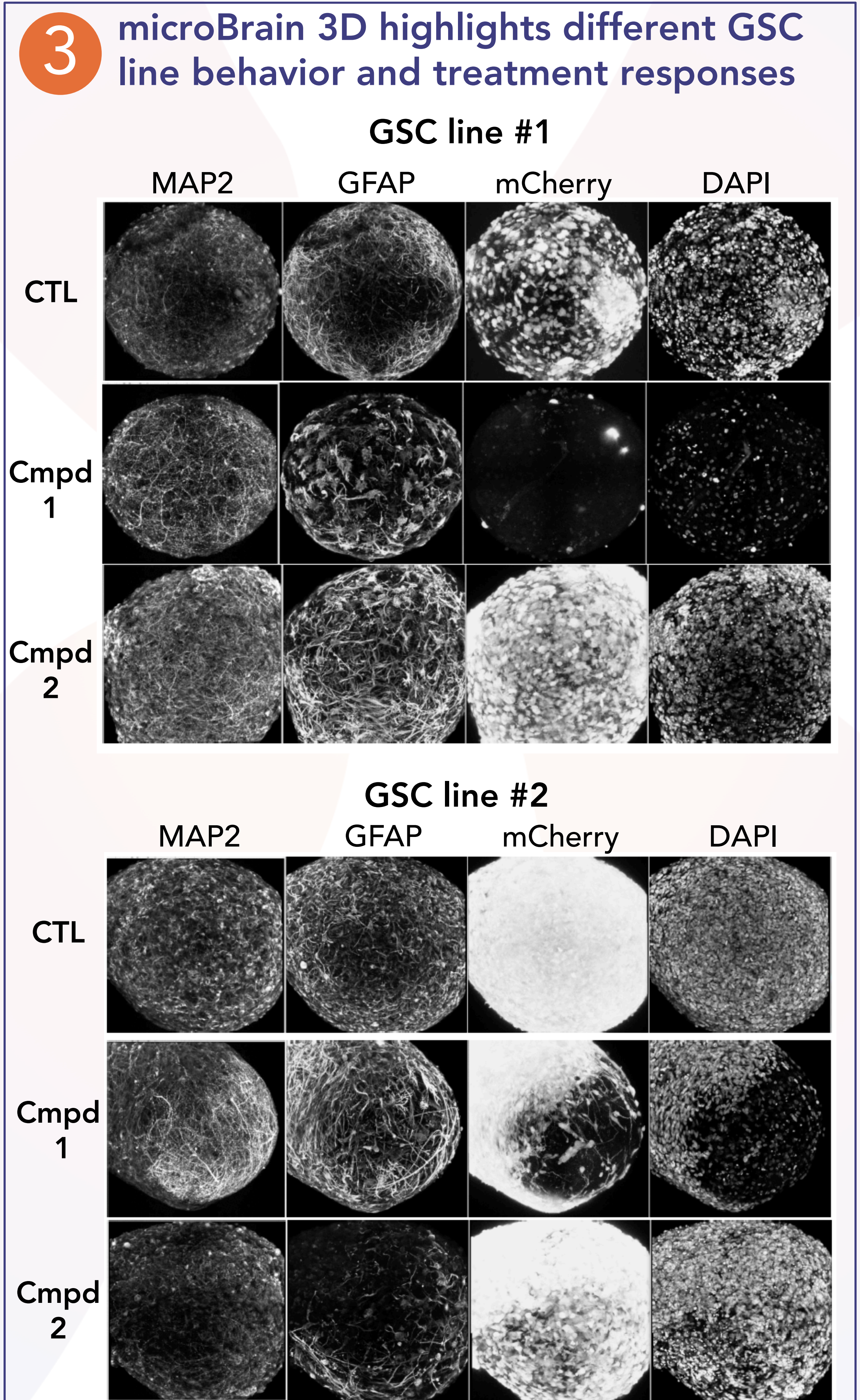
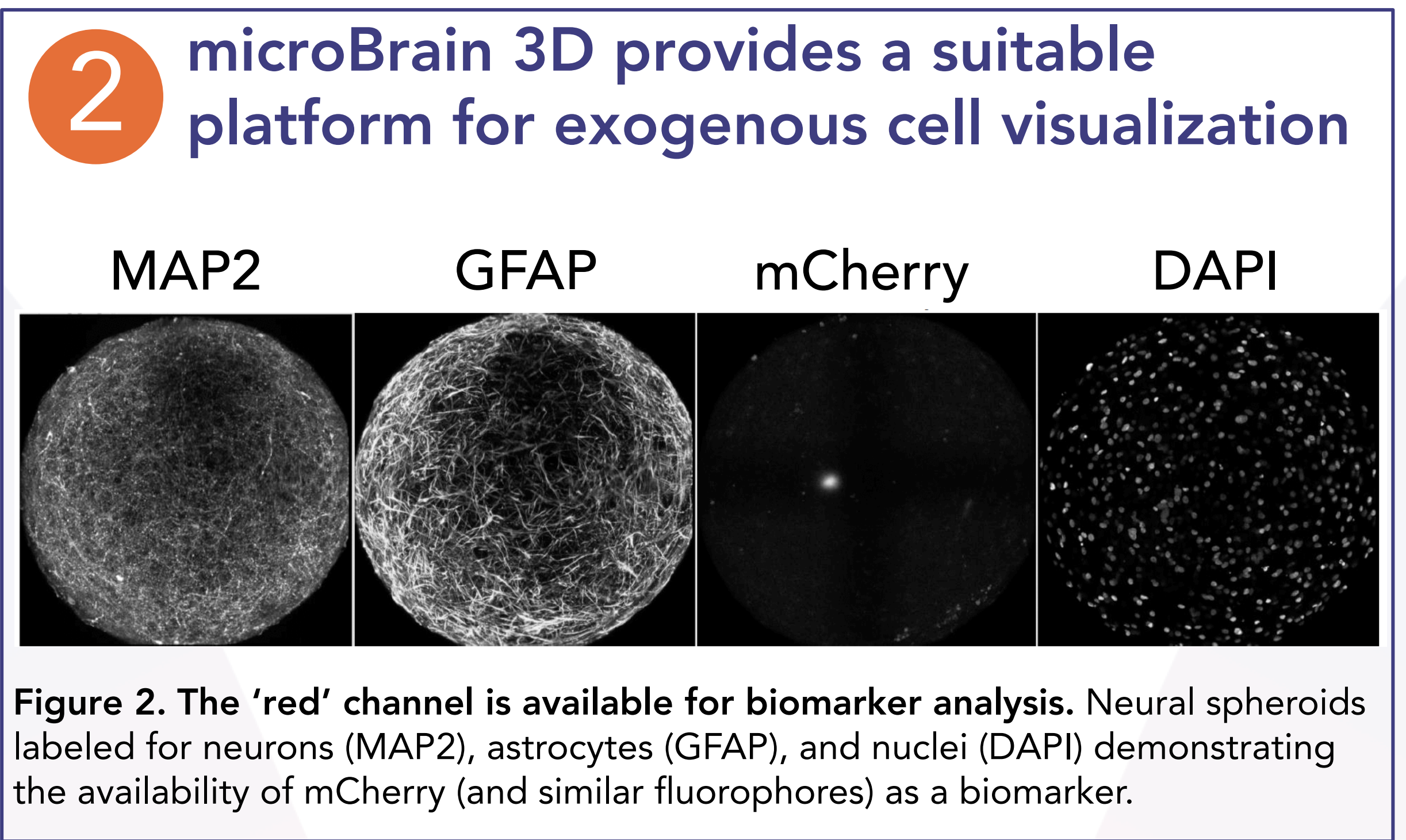
Confocal slices were obtained through approximately ½ of the spheroid diameter with representative images shown as maximum intensity projections for each imaging channel.

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

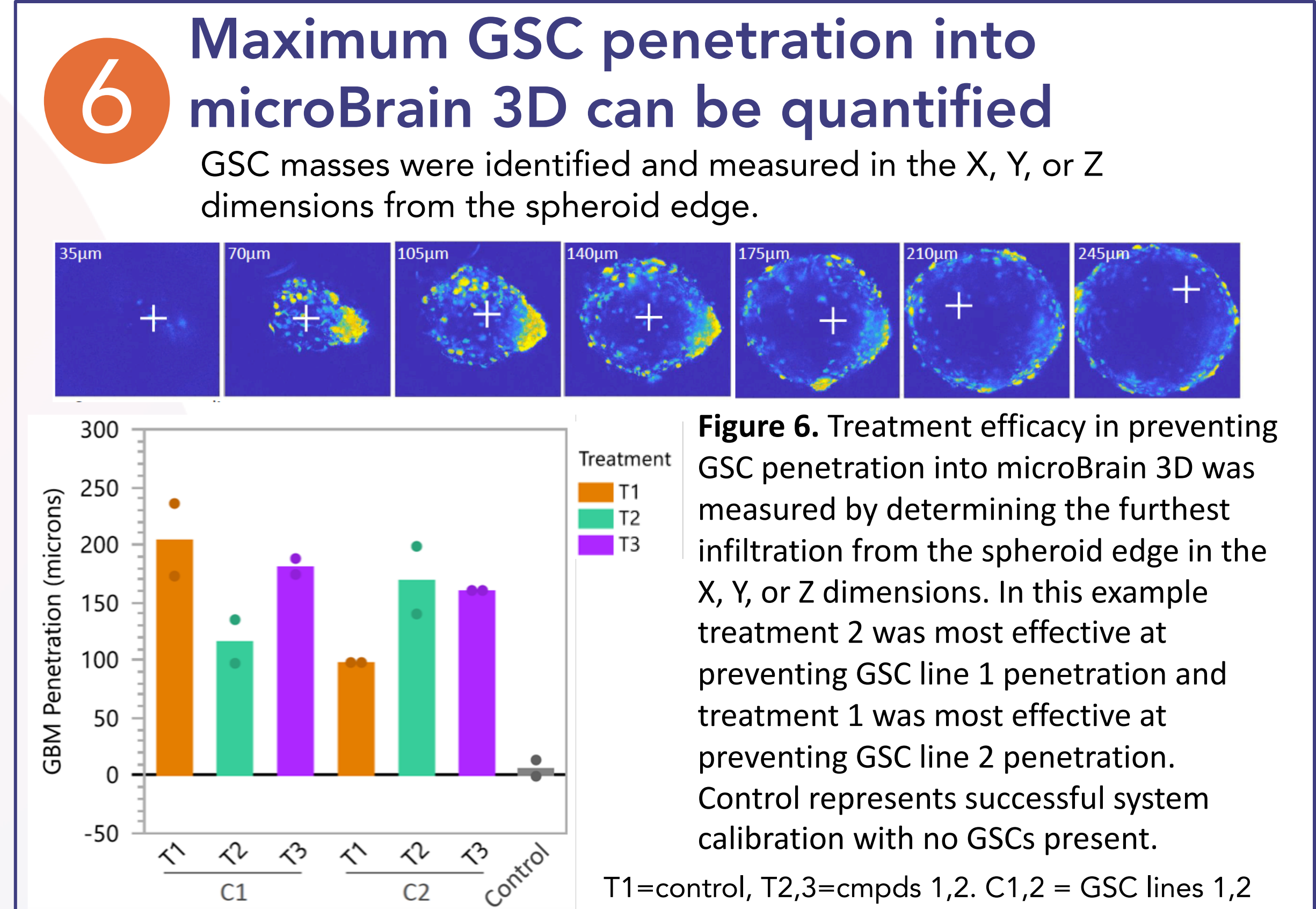
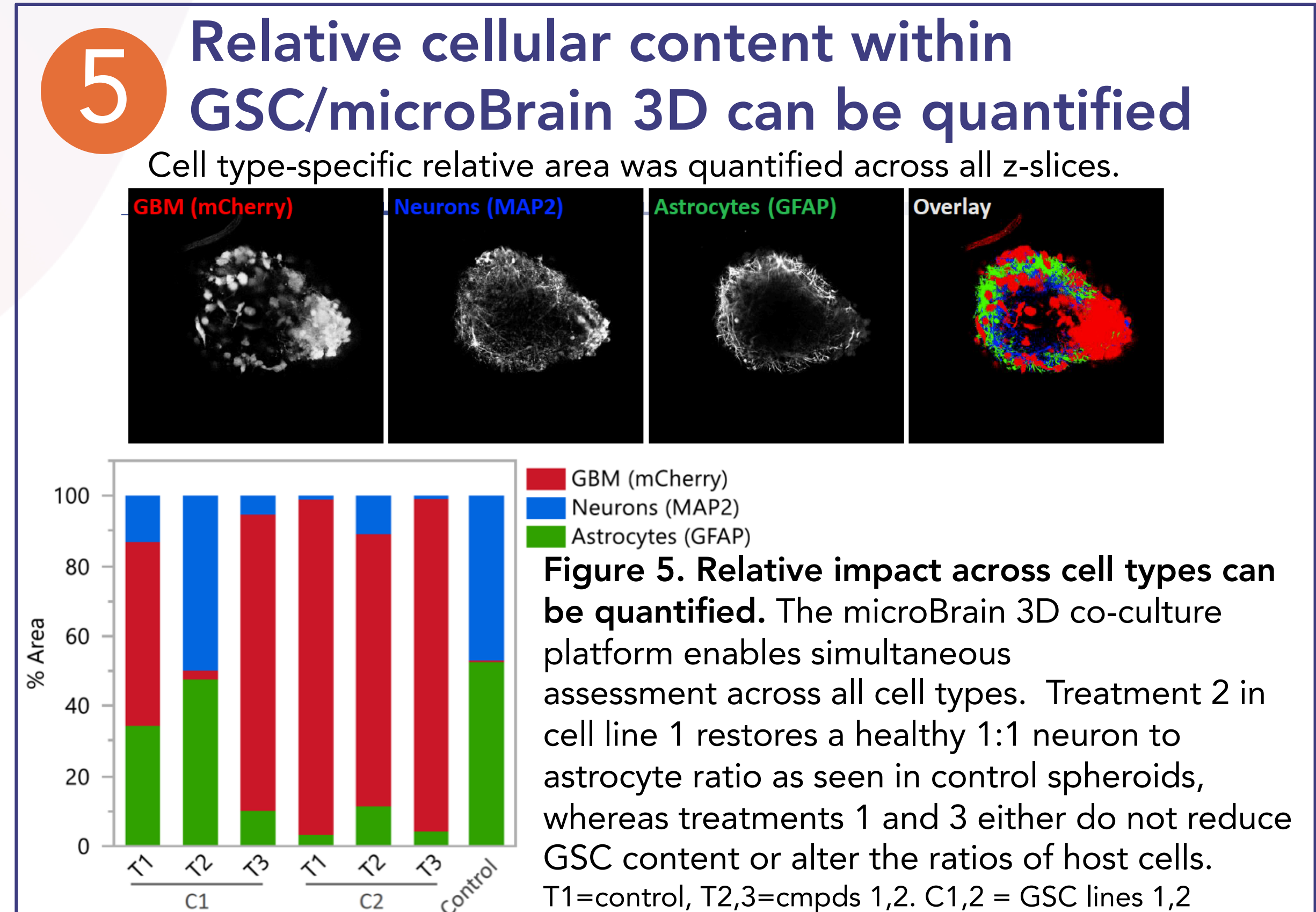
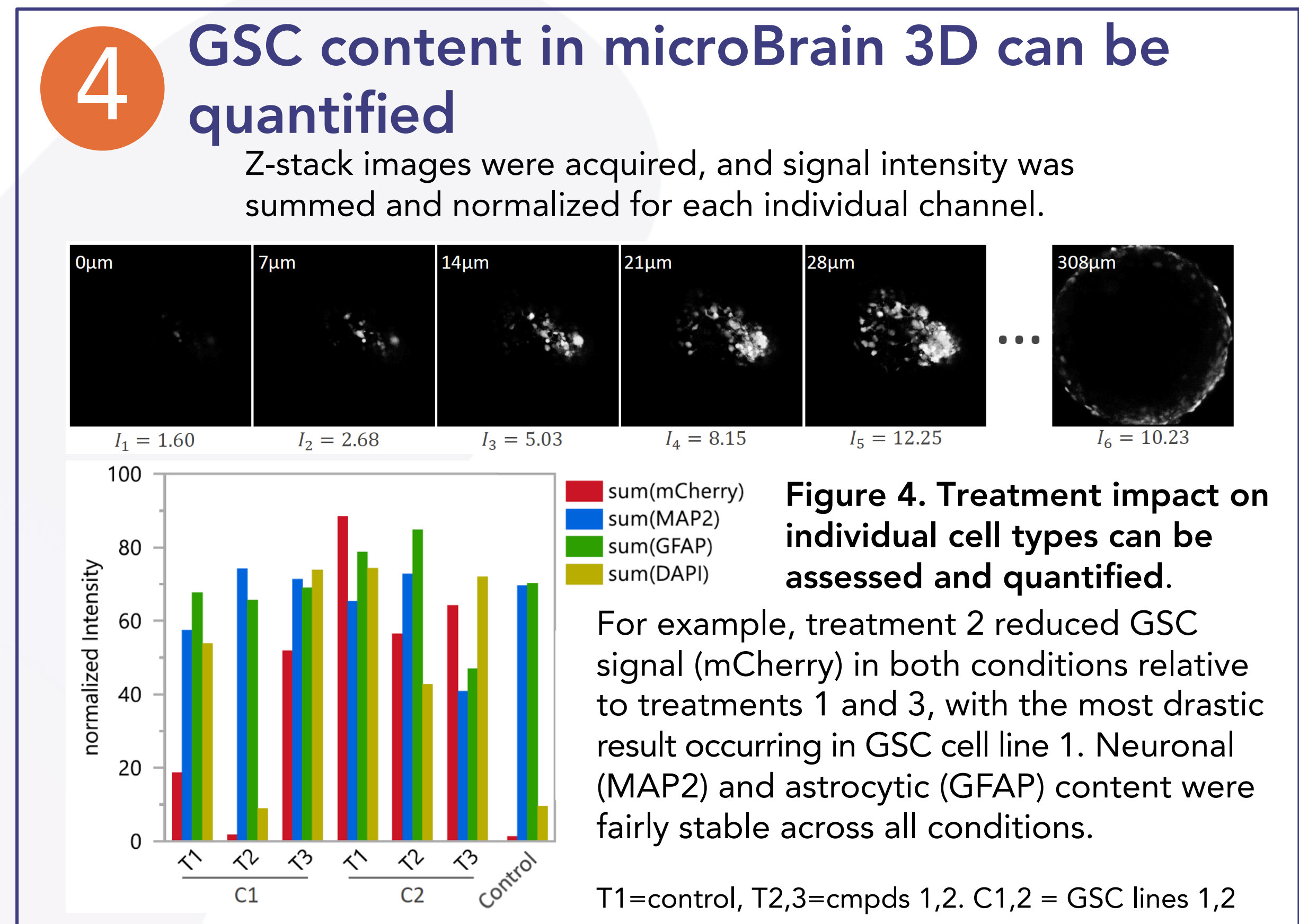
## Results



## RESULTS



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## 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**