

Development of a 3D pharmacological SH-SY5Y neurosphere model to study pathological mechanisms underlying cognitive impairment and its application in drug screening

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Introduction

- Cognitive impairment is a major hallmark of schizophrenia.
- 2D models provide valuable insight but cannot fully reproduce 3D organization.
- Advanced 3D neuronal models provide a more predictive platform for studying disease mechanisms and evaluating drug responses.

Aims

To establish and characterize a 3D neuronal spheroid model from SH-SY5Y cells as a pharmacological tool to study cognitive impairment and to support drug discovery in schizophrenia.

- To analyze spheroid morphology during differentiation.
- To analyze spheroid morphology under schizophrenia-related insults (MK-801, dopamine, combined exposure).
- To evaluate neuronal functionality by calcium mobilization assays in response to depolarization.
- To assess the effect of pharmacological insults on neuronal excitability.

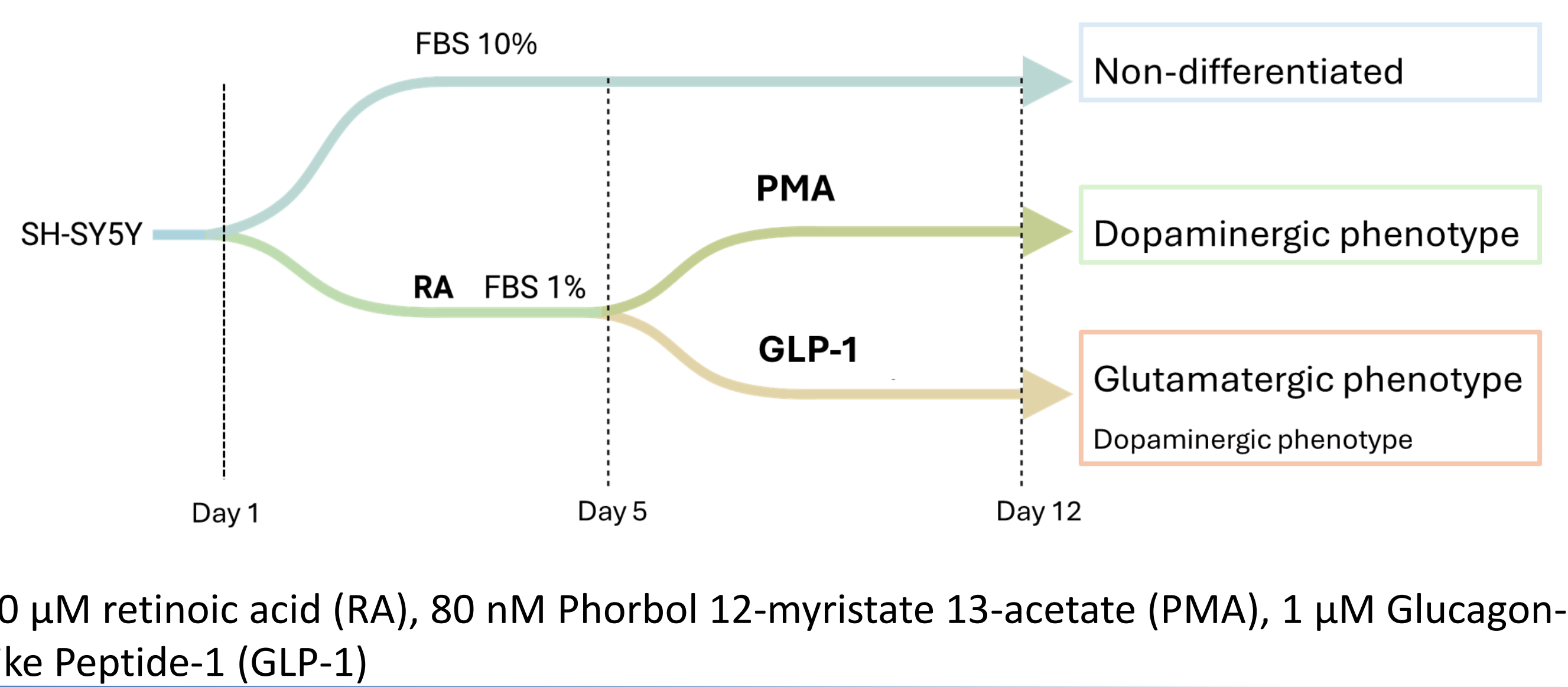
Materials & Methods

SH-SY5Y culture

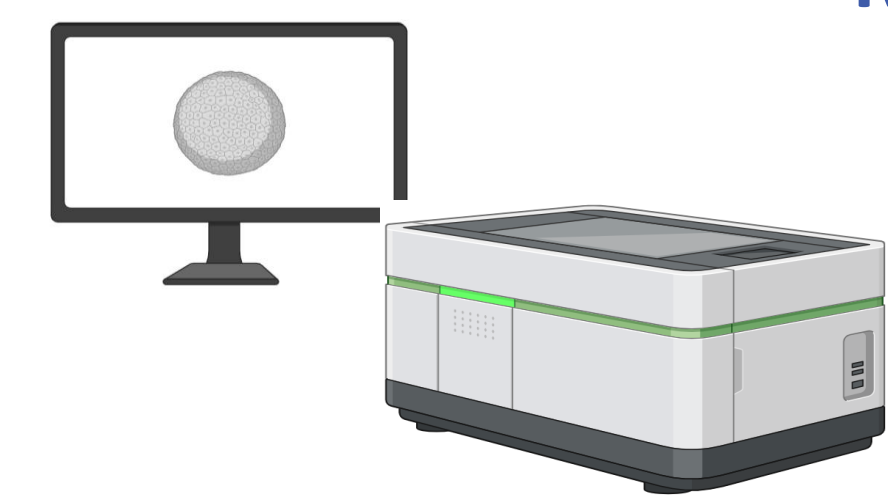


Methylcellulose-based matrix

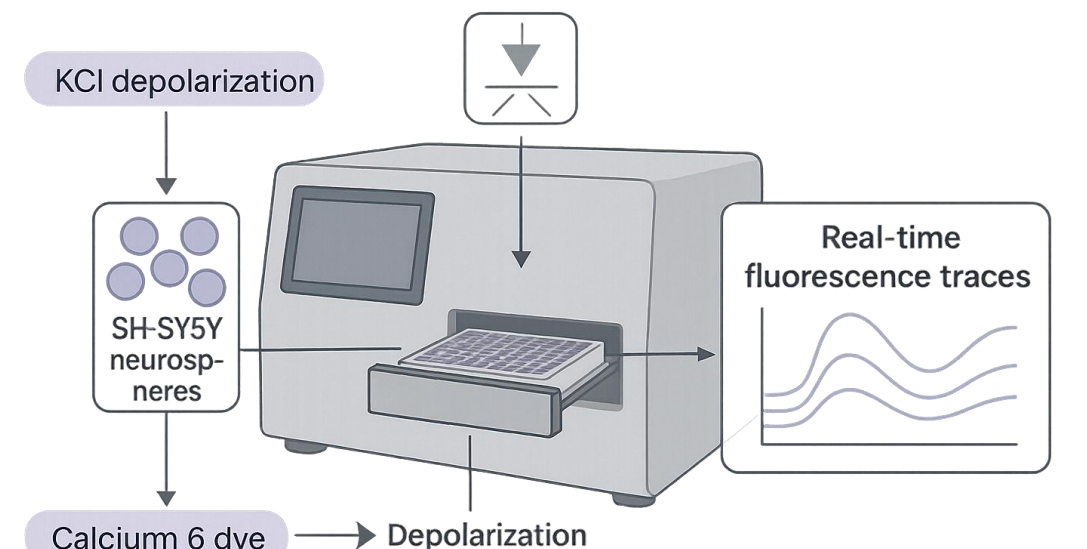
Experimental timeline



Readouts



Morphology analysis
PerkinElmer Operetta CLS™
High-Content Analysis System

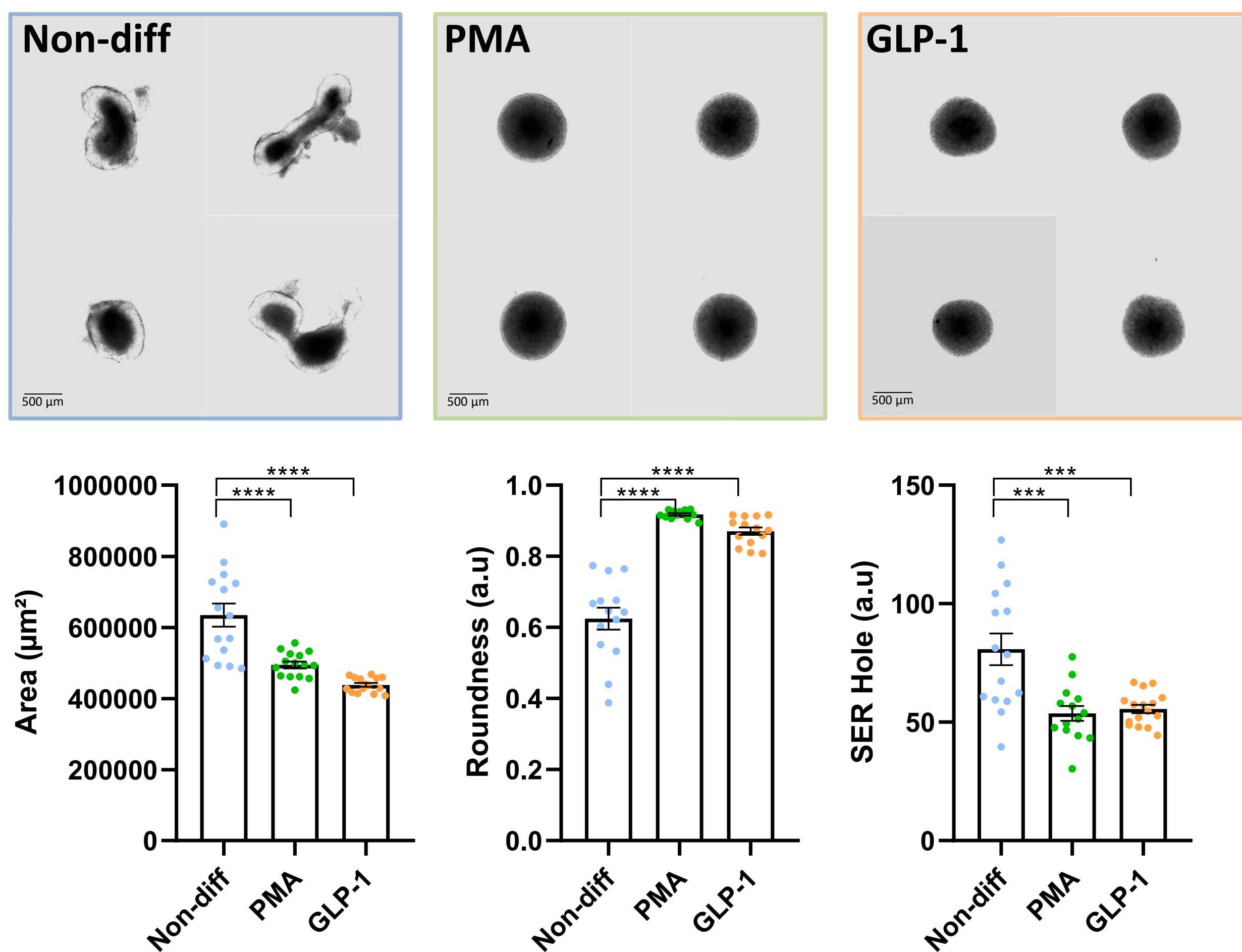


Functional analysis
FDSS/μCELL Kinetic Plate Imager
Hamamatsu Photonics

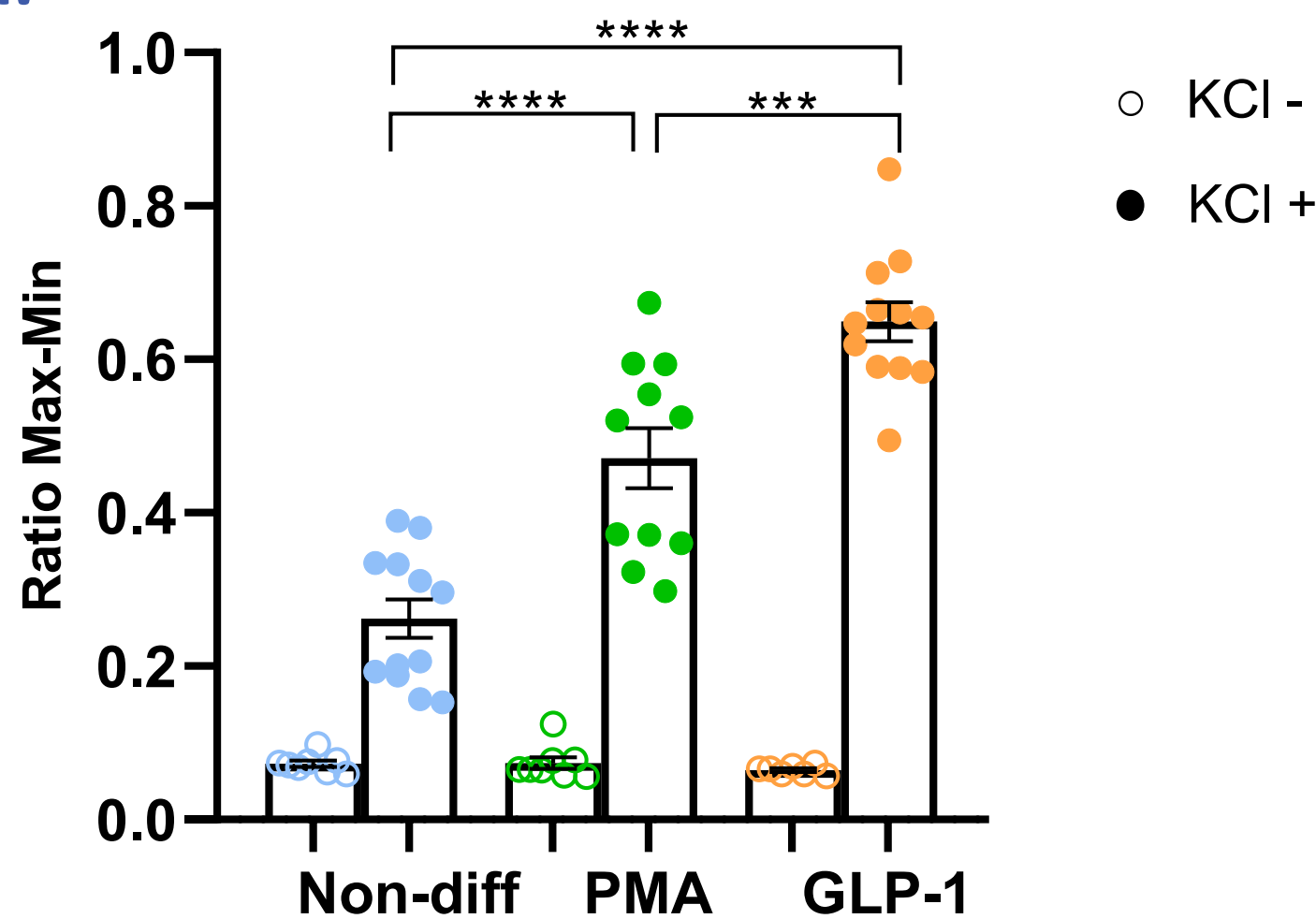
One-way ANOVA
p < 0,0001 ****
p < 0,001 ***

Results & Discussion

Structural maturation of neurospheres after differentiation.

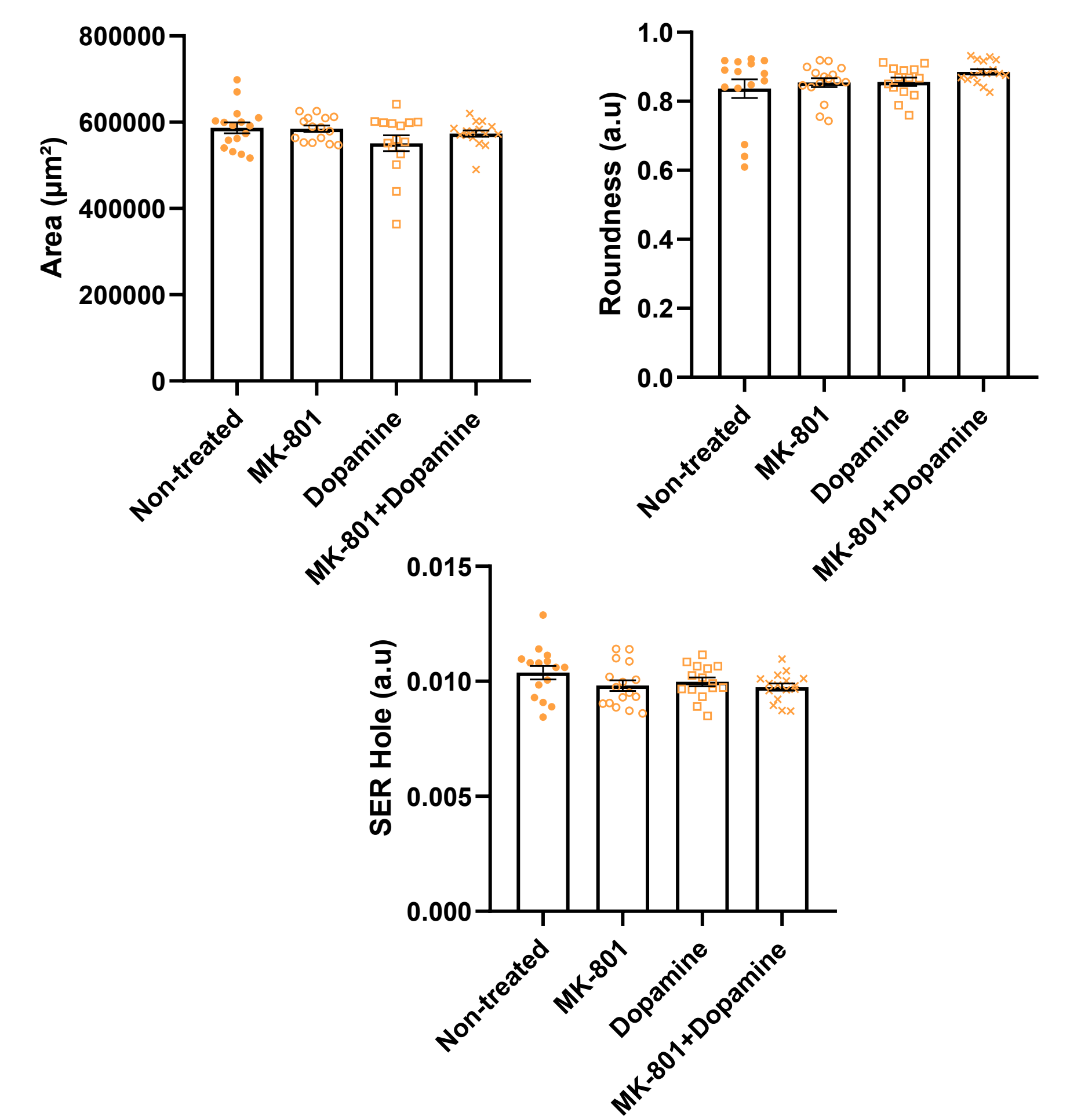
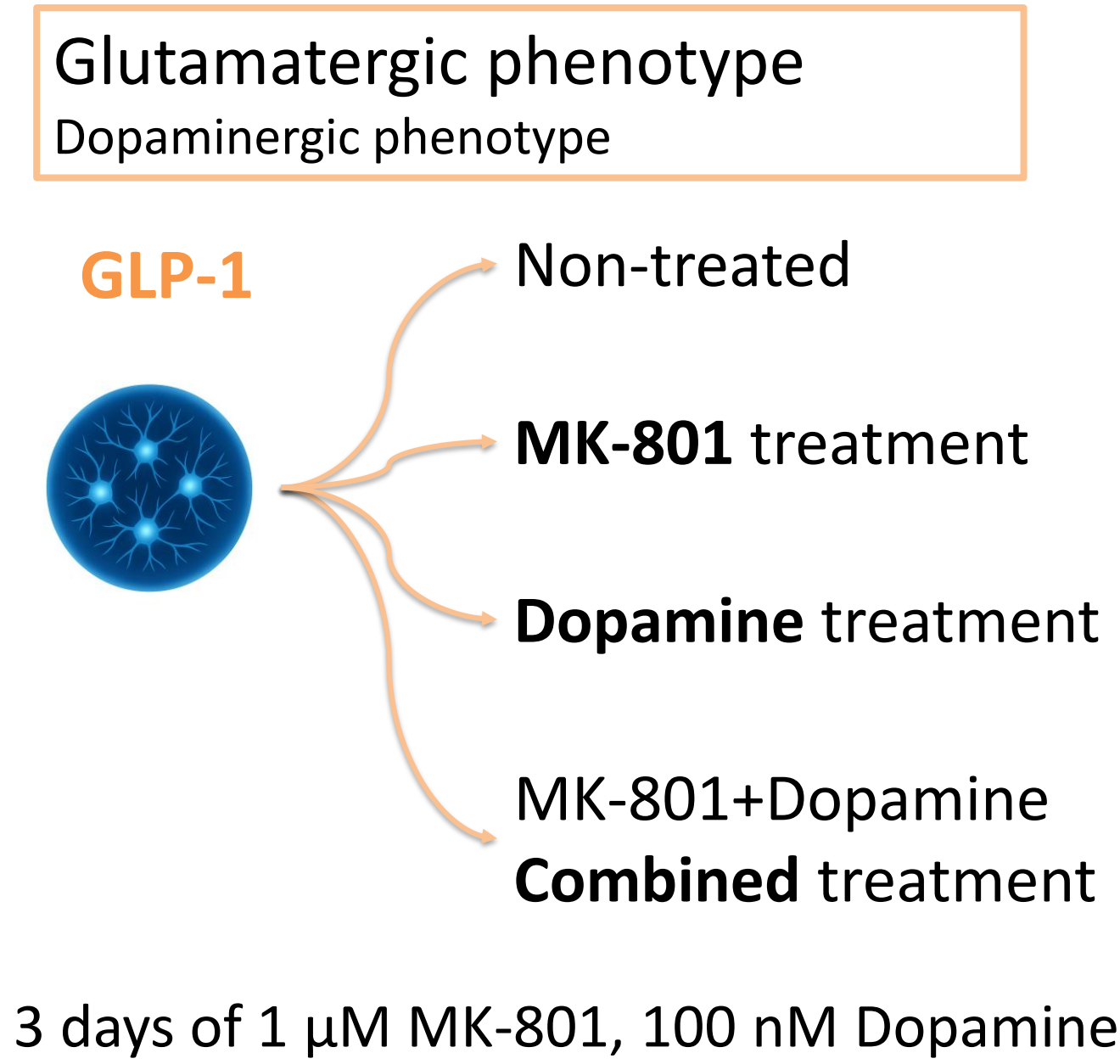


Differentiation enhances neuronal excitability through increased Ca²⁺ mobilization.

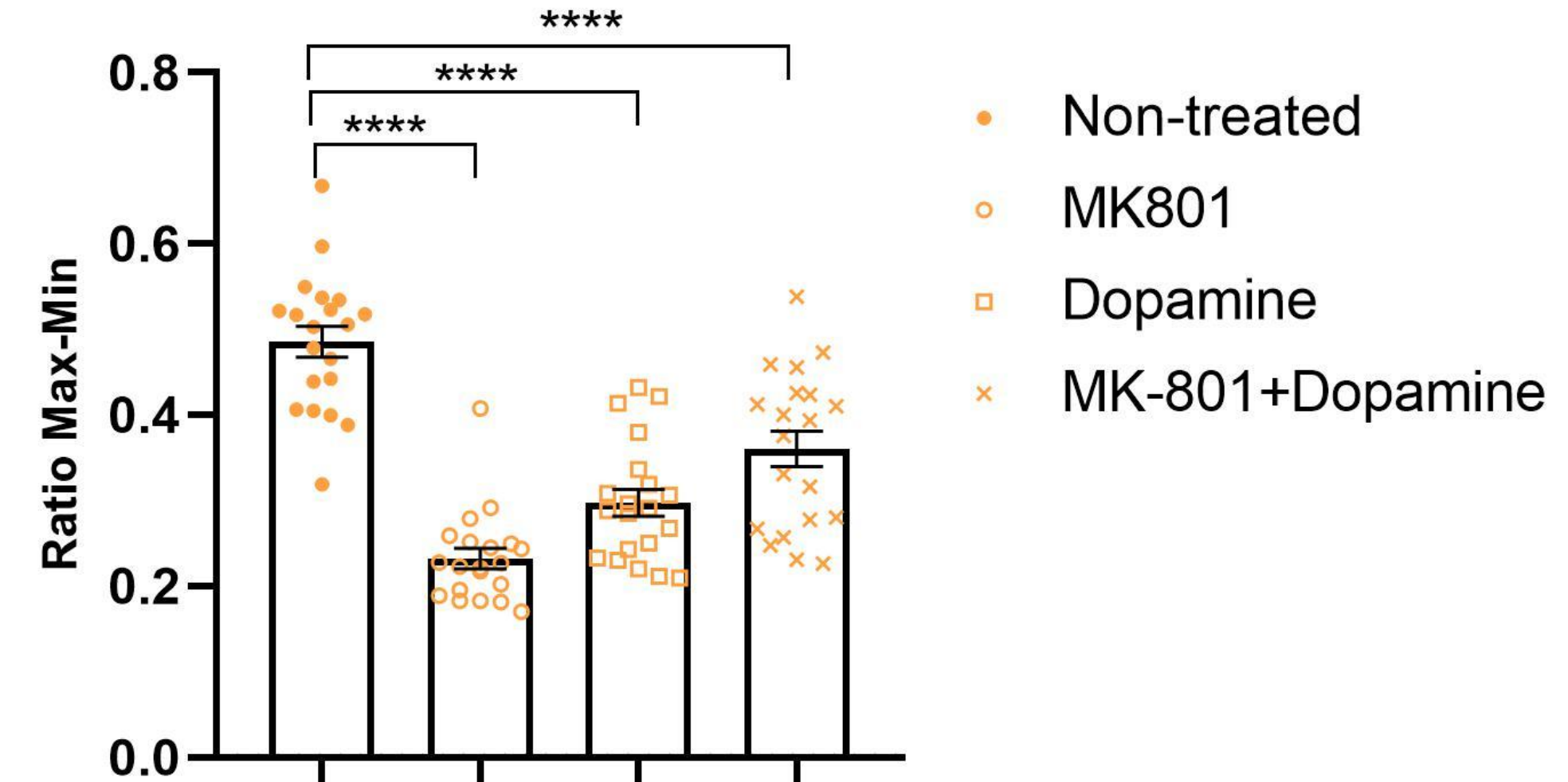


- PMA and GLP-1 induced compact, rounded neurospheres.
- Non-differentiated aggregates were irregular and less stable.
- GLP-1 neurospheres showed enhanced Ca²⁺ response to KCl.

Structural stability of GLP-1 neurospheres after pharmacological treatments.



Altered intracellular Ca²⁺ dynamics in response to MK-801 and dopamine in GLP-1 neurospheres.



- No significant morphological alterations were observed after any treatment.
- MK-801 and dopamine treatments reduced the depolarization-induced Ca²⁺ response compared with non-treated neurospheres.

Conclusions

This 3D pharmacological model bridges traditional 2D assays and *in vivo* systems, enabling reproducible, high-content analysis of cognitive deficit-related mechanisms.

Acknowledgments

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