Blood-brain barrier/glioblastoma interplay 3D *in vitro* model for (nano-)therapeutic screening

Cláudia Martins, PhD

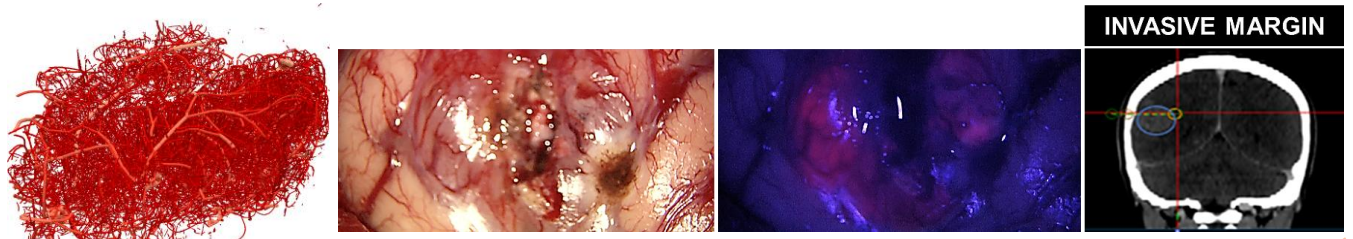
Junior Postdoctoral Researcher
i3S – University of Porto, Portugal

CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Glioblastoma (GBM)

The challenging rate of drug failures

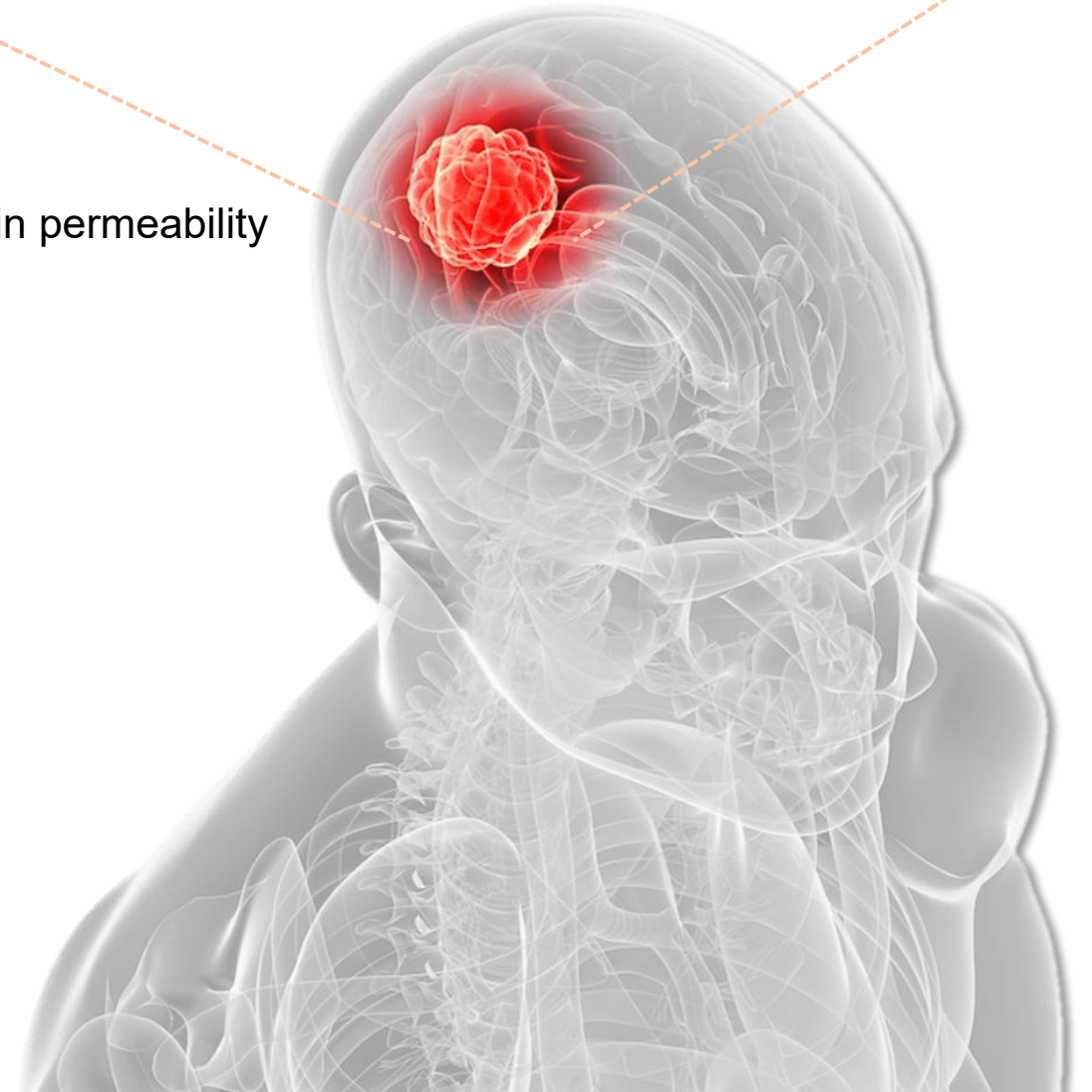


Martins, Smith, Rahman, Sarmento *et al.* Small. 2023, 19, 2300029

- **Deadliest adult brain cancer**
- **Average survival time** of **15 months**
- **Therapeutic access restricted by the blood-brain barrier (BBB)**

✕ **>90%** of anti-GBM drug candidates fail in clinical trials

✕ **>85%** of brain therapeutics fail in demonstrating sufficient blood-to-brain permeability



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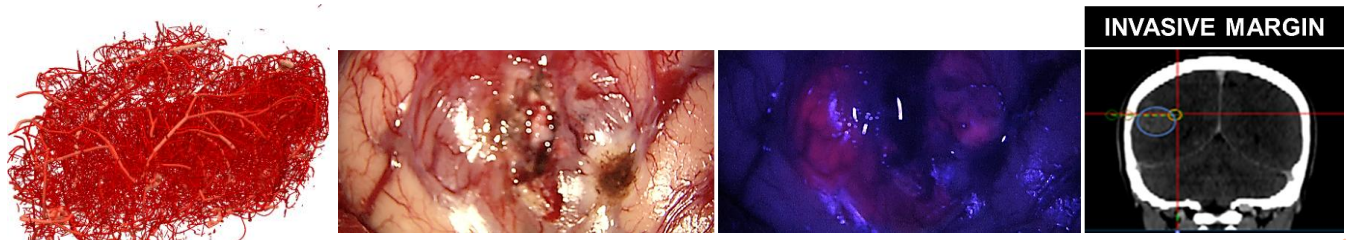

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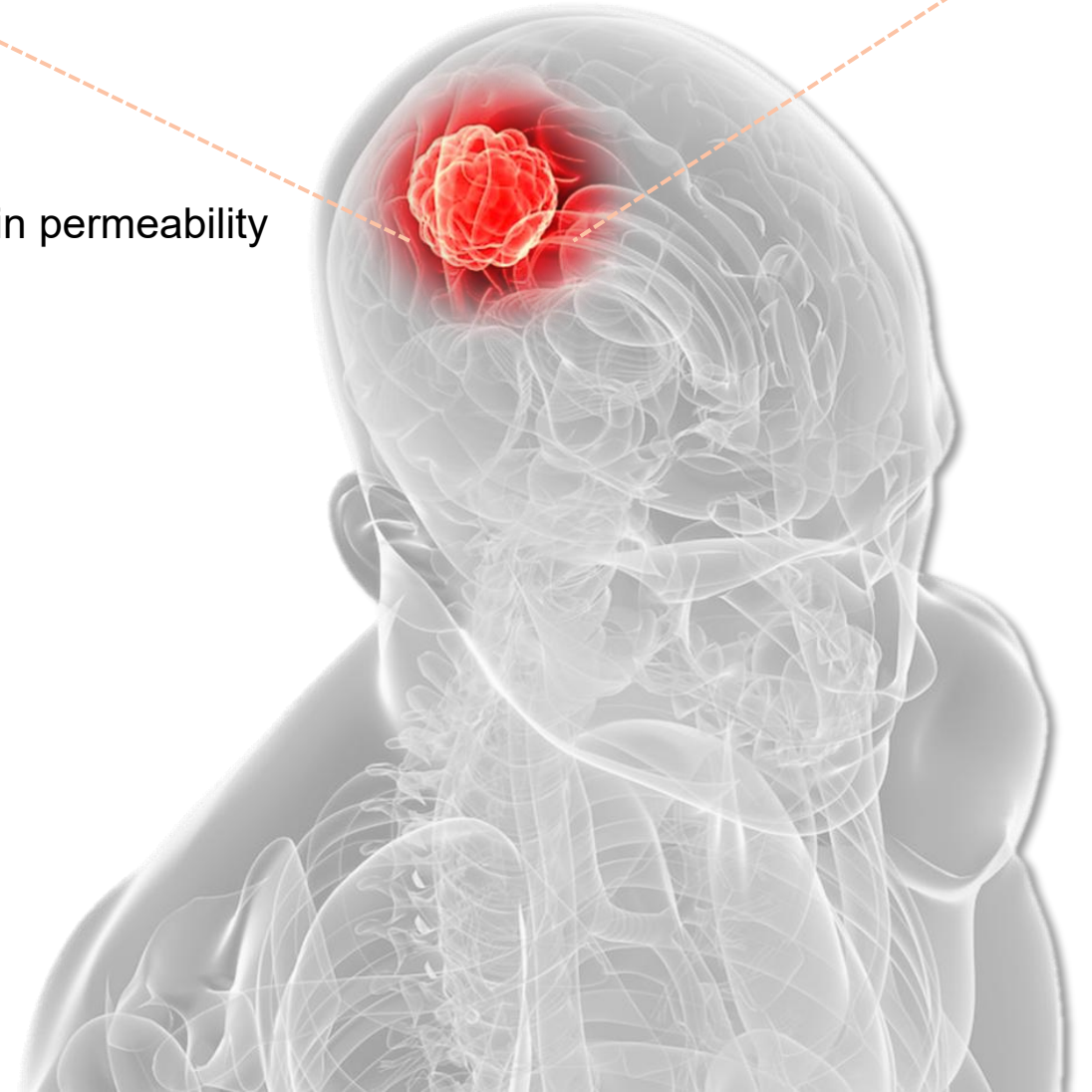
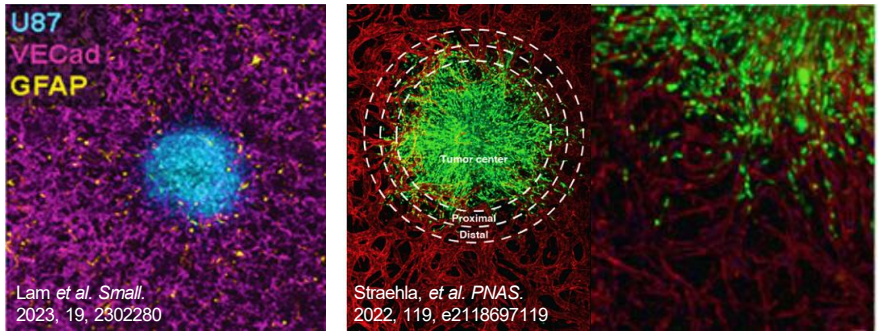


LANDSCAPE OF CLOSE-TO-NATIVE *IN VITRO* MODELS

Lack of predictive models that accurately recapitulate the blood-BBB-GBM layered bioarchitecture

Tumor neovascularization



Lam *et al. Small.* 2023, 19, 2302280

Straehla, *et al. PNAS.* 2022, 119, e2118697119

Abnormal, leaky, disorganized endothelial layers

BBB barrier interface function

Intact BBB

Glioblastoma (GBM)

The challenging rate of drug failures and OUR SOLUTION

- **Deadliest** adult brain cancer
- Average survival time of **15 months**
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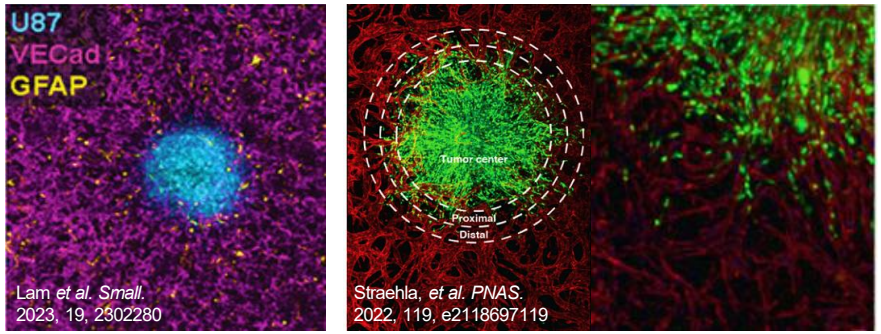

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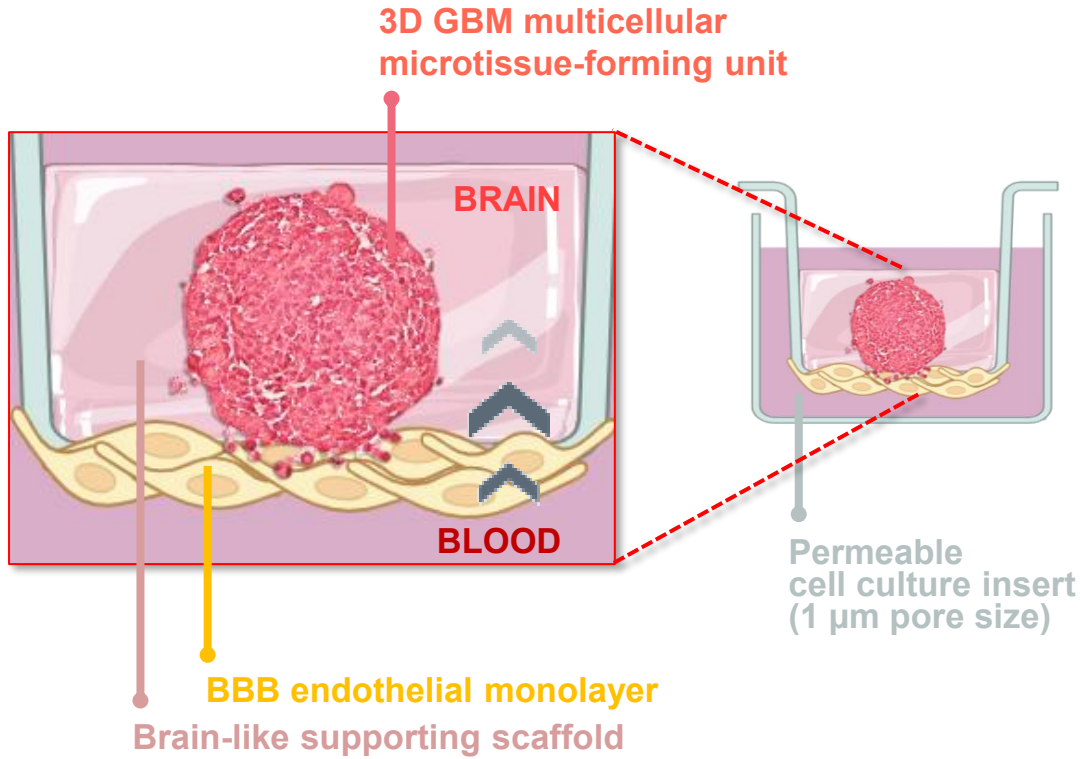
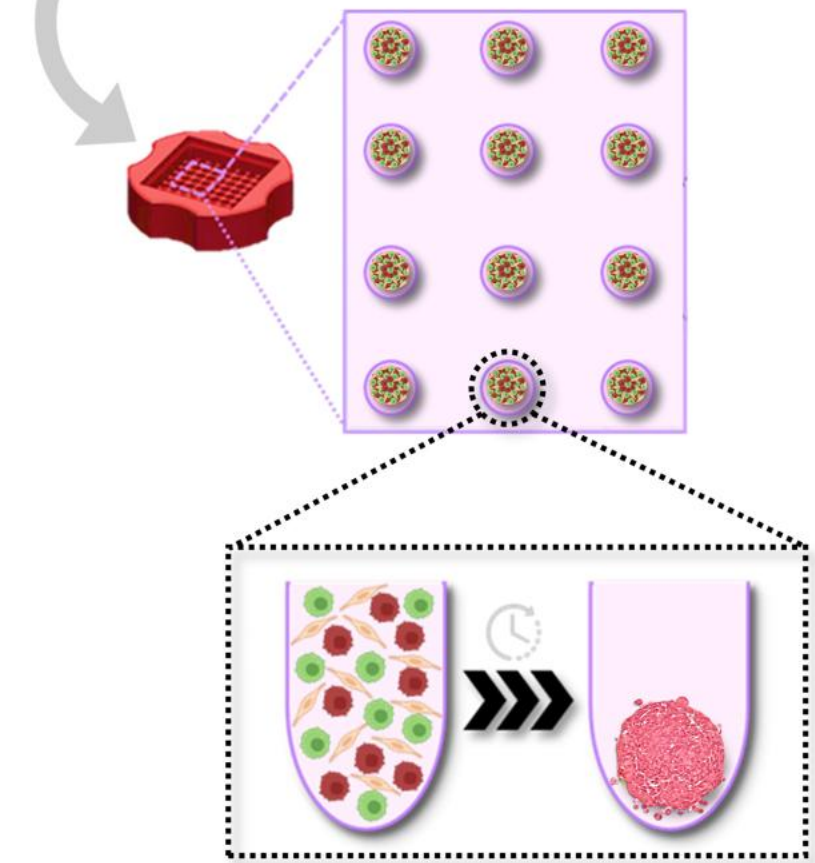


Graphical representations not to scale

Blood-brain barrier/glioblastoma interplay *in vitro* model

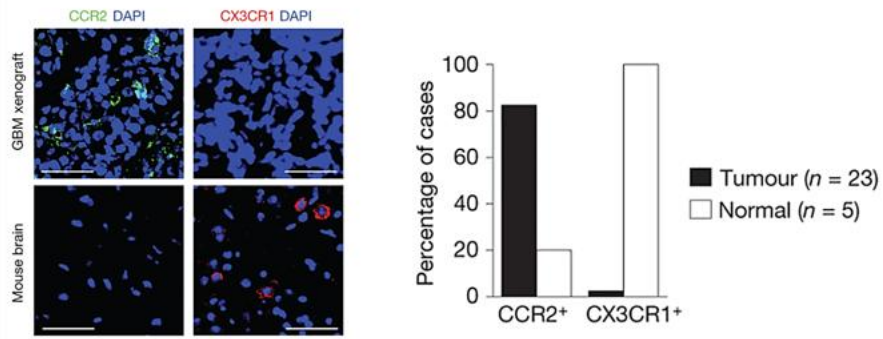
3D GBM multicellular microtissue-forming unit

- **Tumor cells**
- **Blood-derived peripheral monocytes**
- **Brain microvascular endothelial cells**

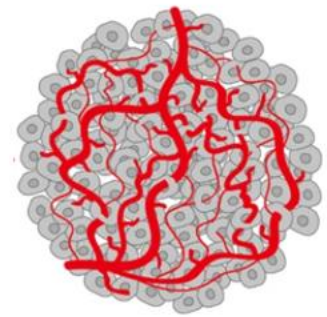


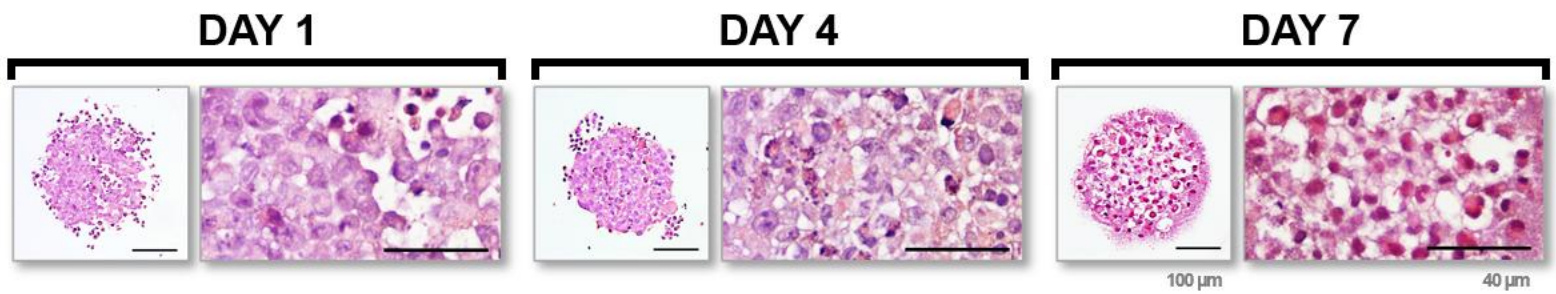
Blood-derived infiltrating macrophages:
Majority of GBM tumor-associated macrophages

Zhou *et al. Nat Cell Biol*. 2015, 17, 170



Endothelial cells:
GBM maintenance and growth, specially at the perivascular and invasive GBM niches





Martins *et al*. J Control Release. 2023, 353, 77

Blood-brain barrier/glioblastoma interplay *in vitro* model

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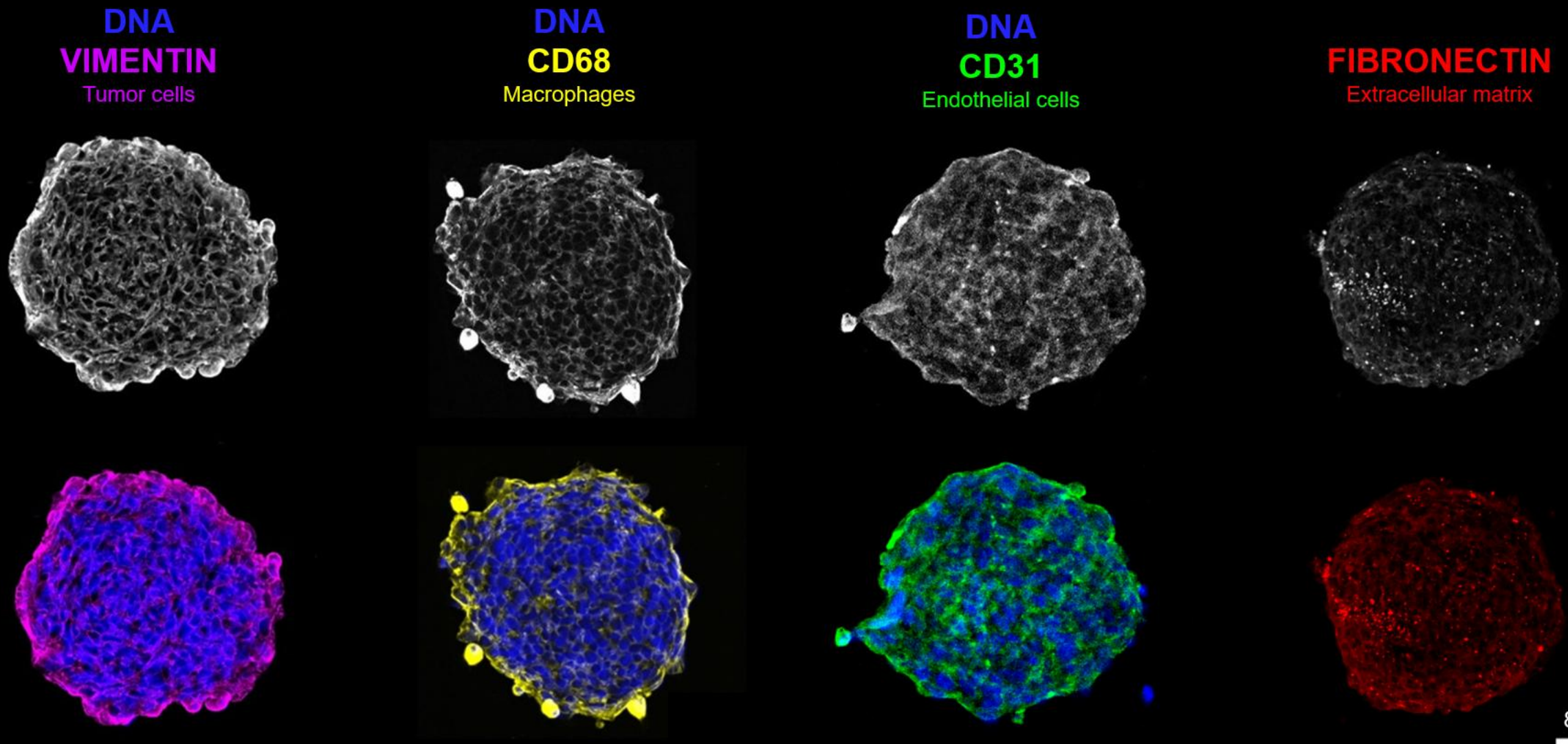

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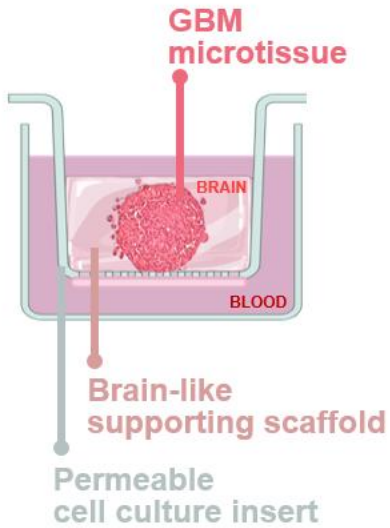
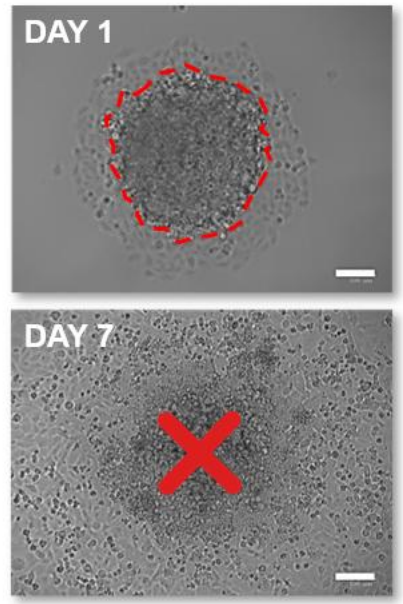
1

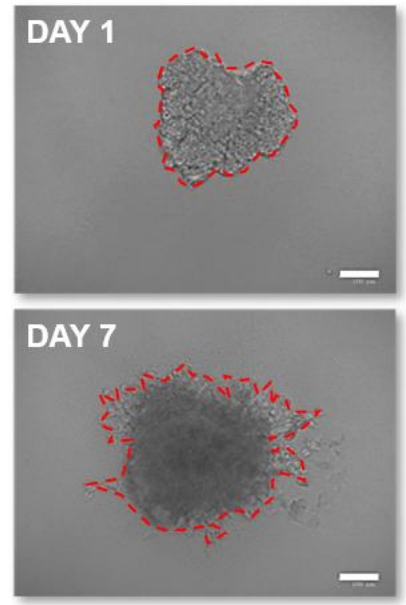
Optimization of brain-like supporting scaffold



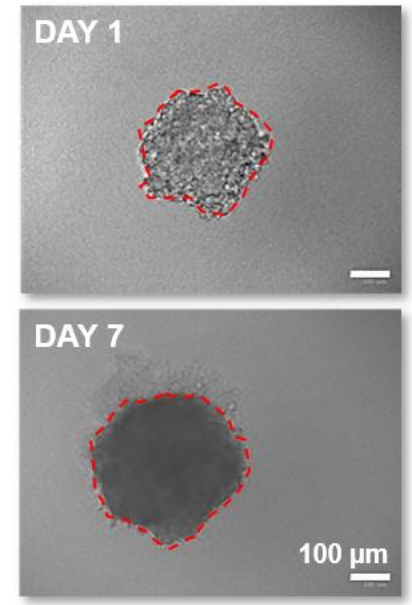
4.5 mg mL^{-1} Fibrin

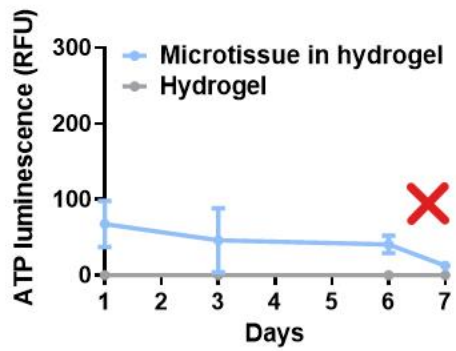


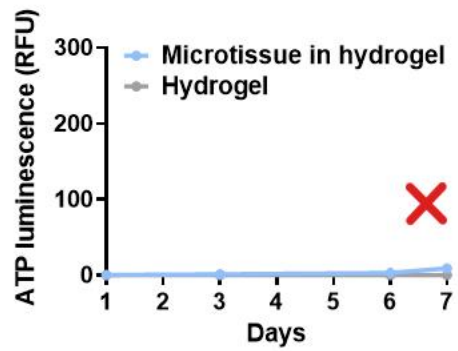
4.5 mg mL^{-1} Collagen I

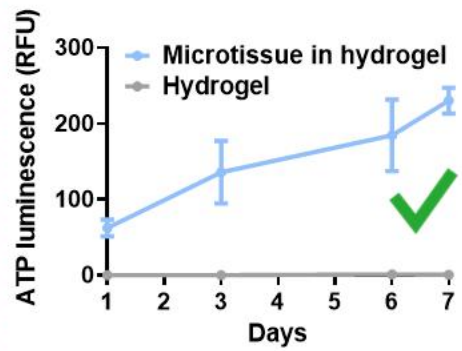


1.5 mg mL^{-1} Collagen I



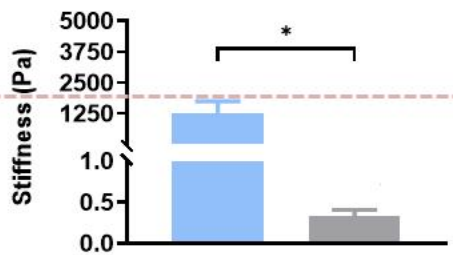


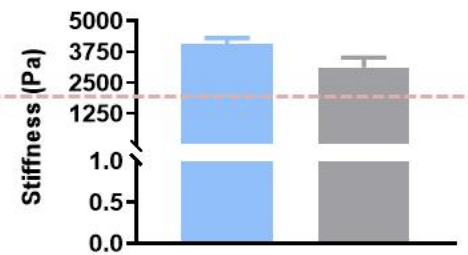


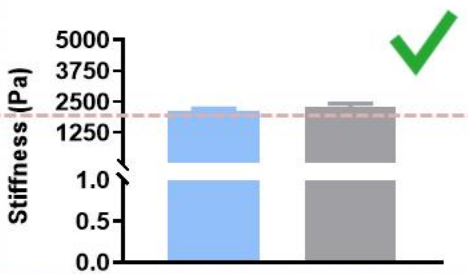


Human brain

2 kPa







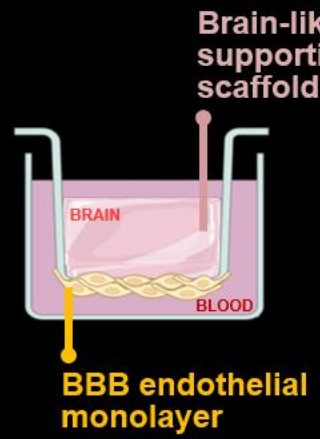
Microtissue in hydrogel Hydrogel

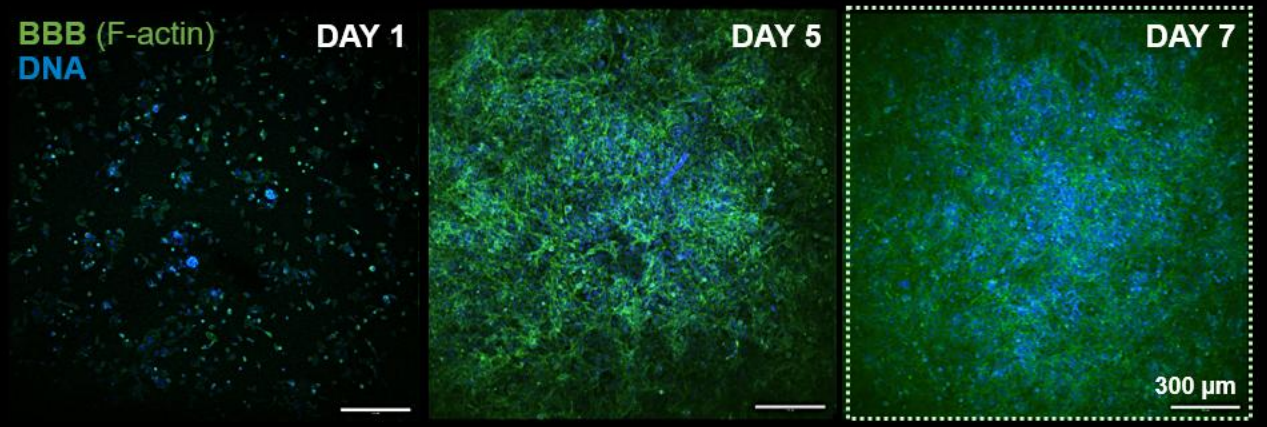
Unpublished data. Graphical representations not to scale

1 Optimization of **brain-like supporting scaffold**

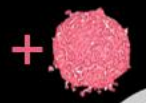
2 Recapitulation of the **intact BBB** at the **GBM invasive margin**

BBB (no microtissue)

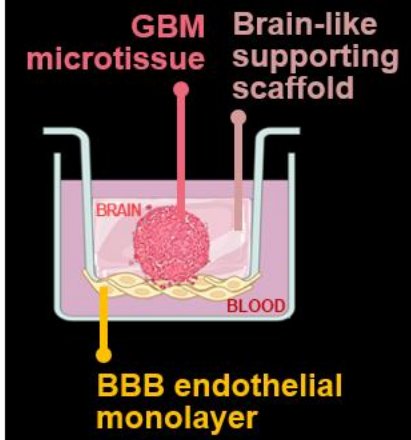


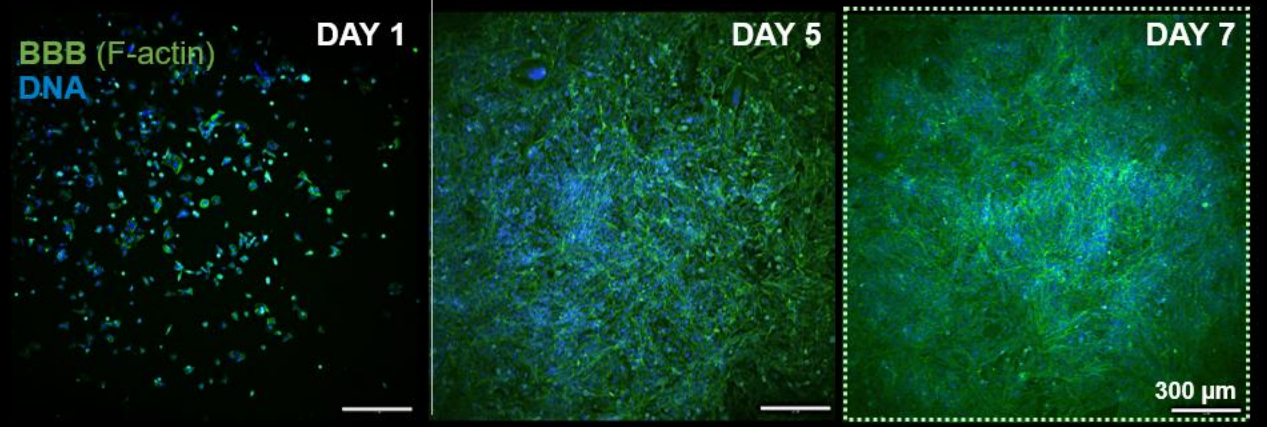


GBM microtissue co-cultured BBB



DAY 3 (embedding)





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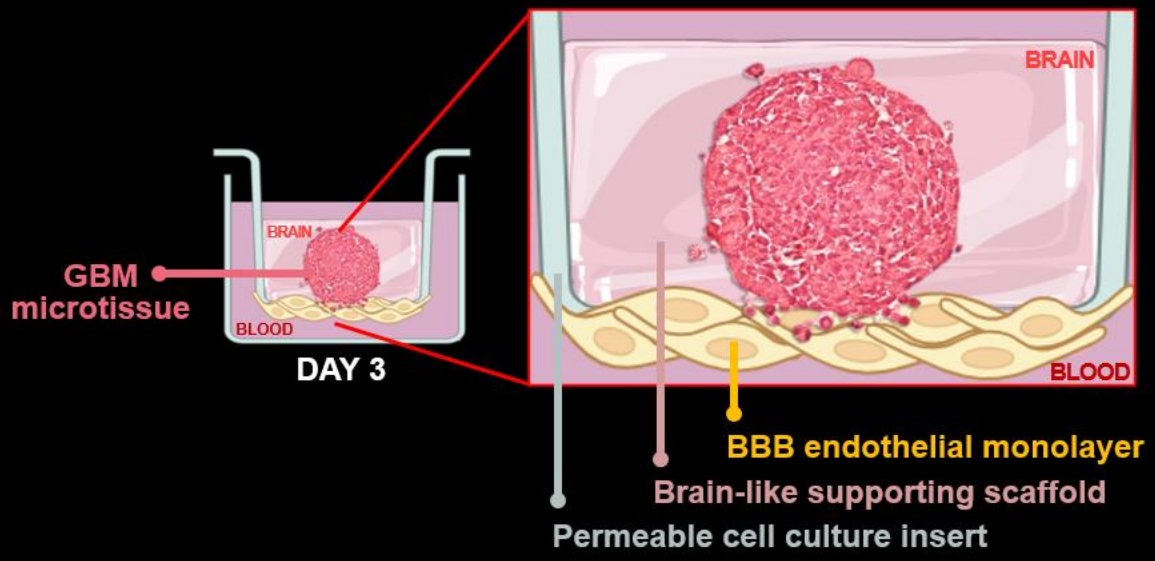
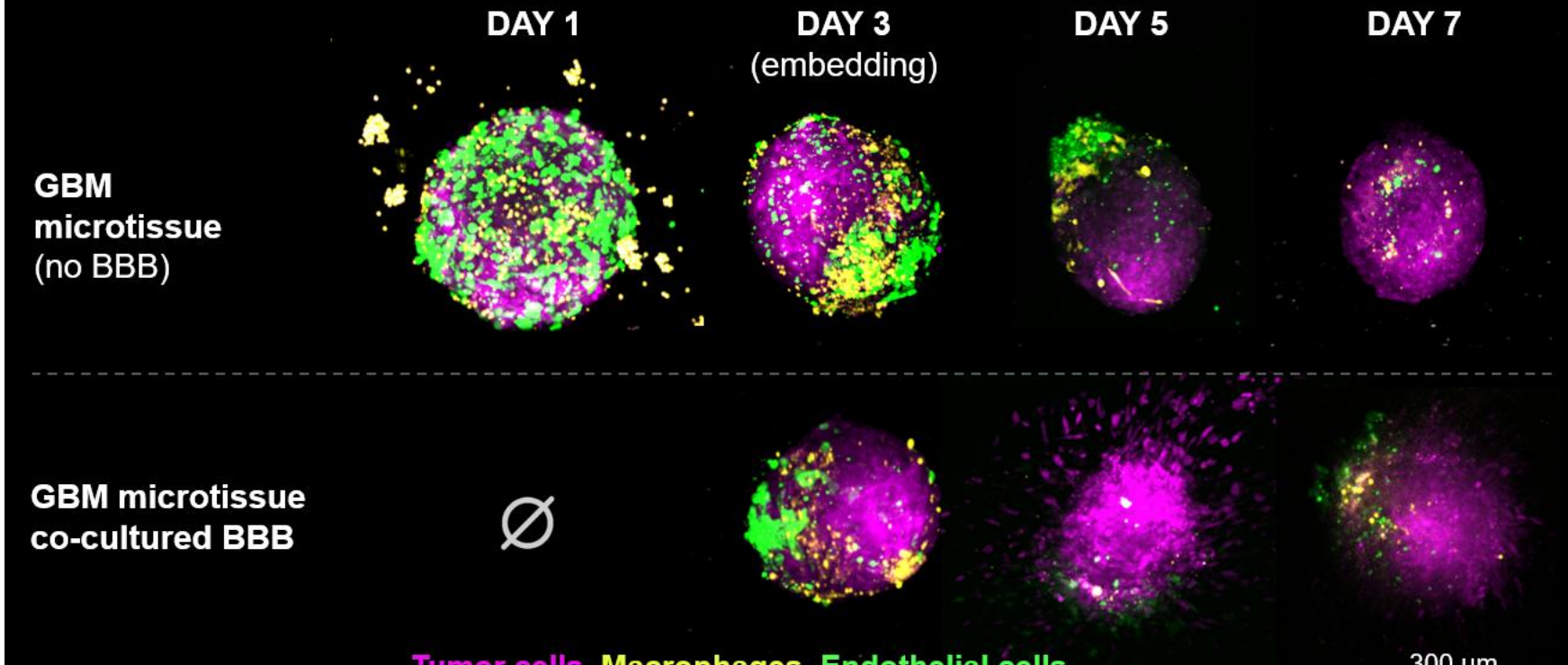
1 Optimization of **brain-like supporting scaffold**

2 Recapitulation of the **intact BBB** at the **GBM invasive margin**

3 Maintenance of **GBM microtissue integrity**





Unpublished data. Graphical representations not to scale

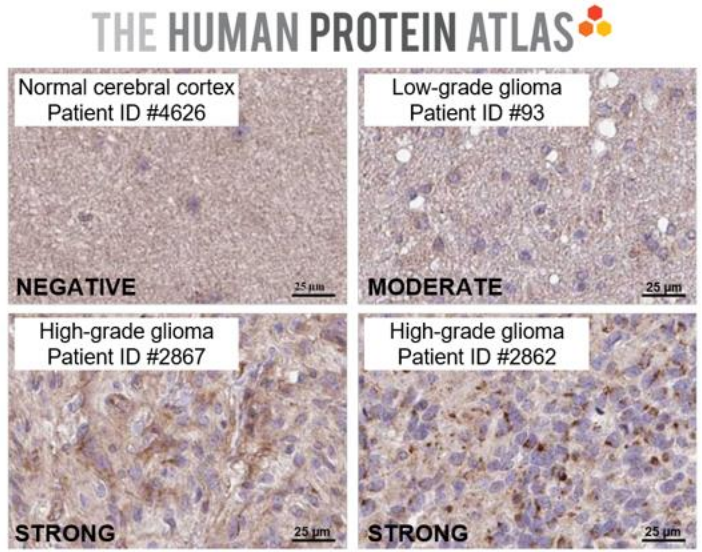
Is this model functional?

Blood-brain barrier/glioblastoma interplay *in vitro* model

Nano-vehicles targeted to GBM-overexpressed L-amino acid transporters

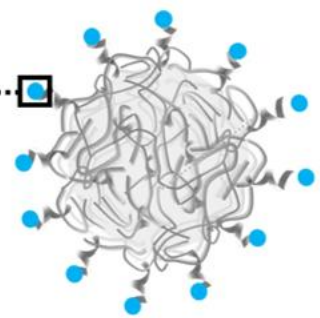
L-type amino acid transporter 1 (LAT1)

- **GBM cell overexpression**
- Basal expression in normal cerebral cortex



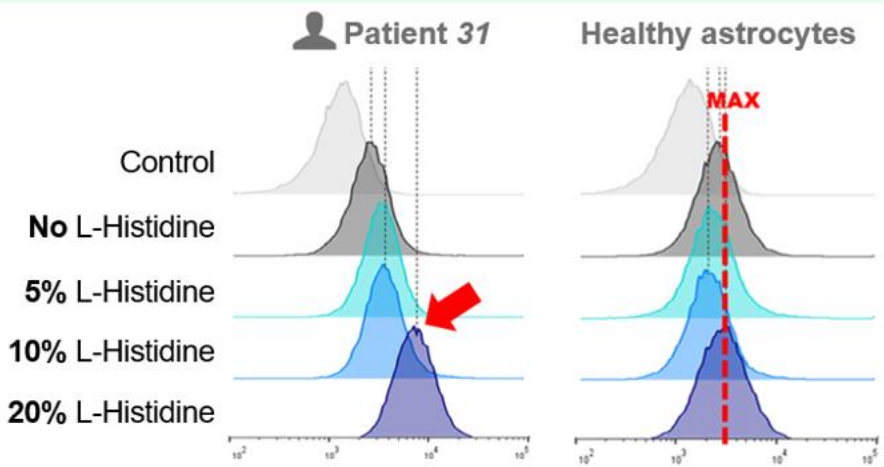
SLC7A5 immunohistochemistry in patient cohorts HPA052673

L-Histidine surface moiety
↑ **GBM uptake** via LAT1

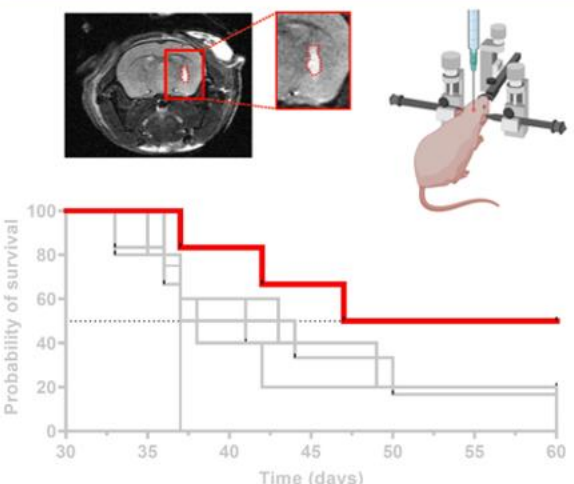


Poly(lactic-co-glycolic acid) (PLGA) and polyethylene glycol (PEG) nanoparticles
Controlled release & biocompatibility

✓ 12-fold increased cell uptake (tumor *vs* healthy)



✓ Up to 50% increased survival



Martins *et al.* Small. 2023, 19:2300029 | Martins *et al.* J Control Release. 2023, 353, 77

Blood-brain barrier/glioblastoma interplay *in vitro* model

Nano-vehicles targeted to GBM-overexpressed L-amino acid transporters

ELSEVIER

Pharmacology & Therapeutics

Volume 230, February 2022, 107964

Amino acid transporter LAT1 (SLC7A5) as a molecular target for cancer diagnosis and therapeutics

Yoshikatsu Kanai

Department of Bio-system Pharmacology, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Substrate	Km (μM) *Affinity for the substrate*
L-Histidine	**12.7**
L-Phenylalanine	14.2
L-Methionine	20.2
L-Isoleucine	25.1

Lower Km (Michaelis constant)
Higher affinity
Improved GBM uptake?

Blood-brain barrier/glioblastoma interplay *in vitro* model

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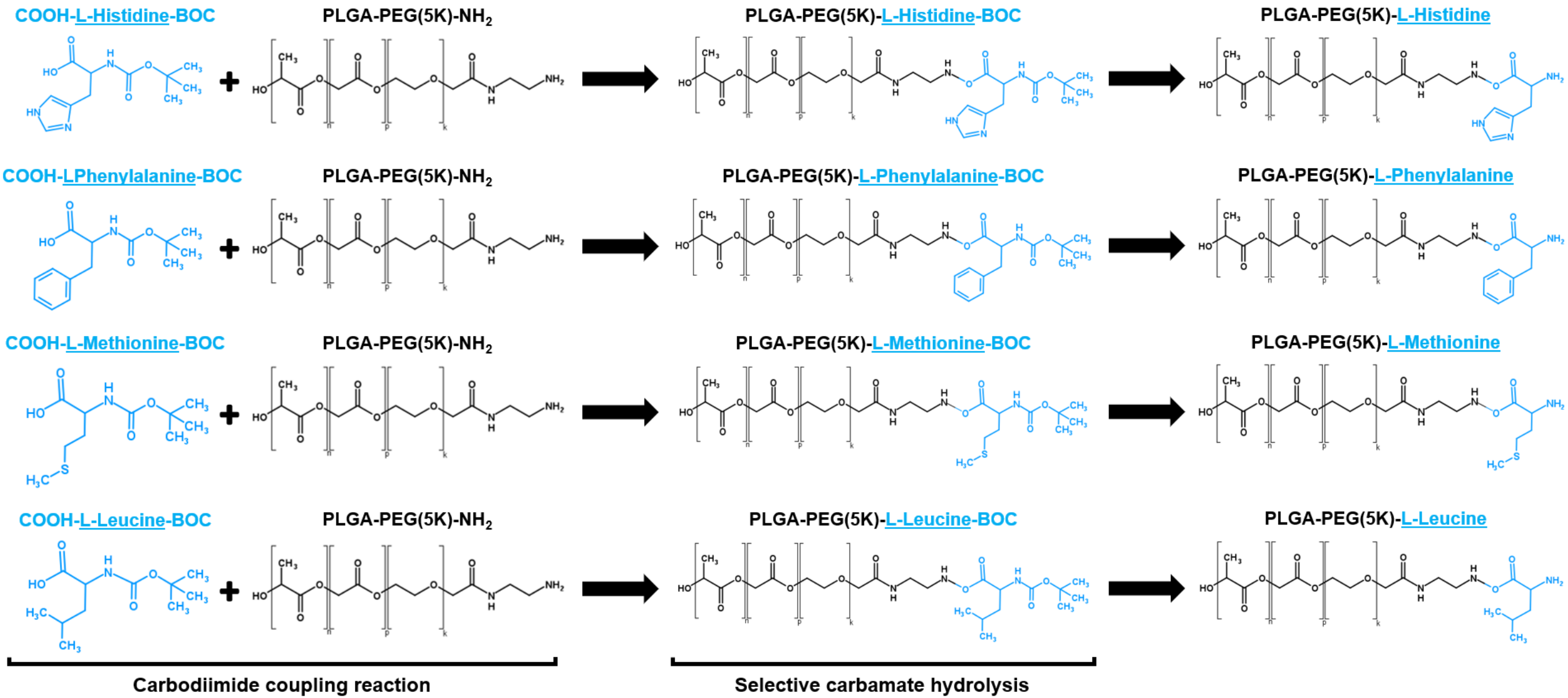

Unpublished data

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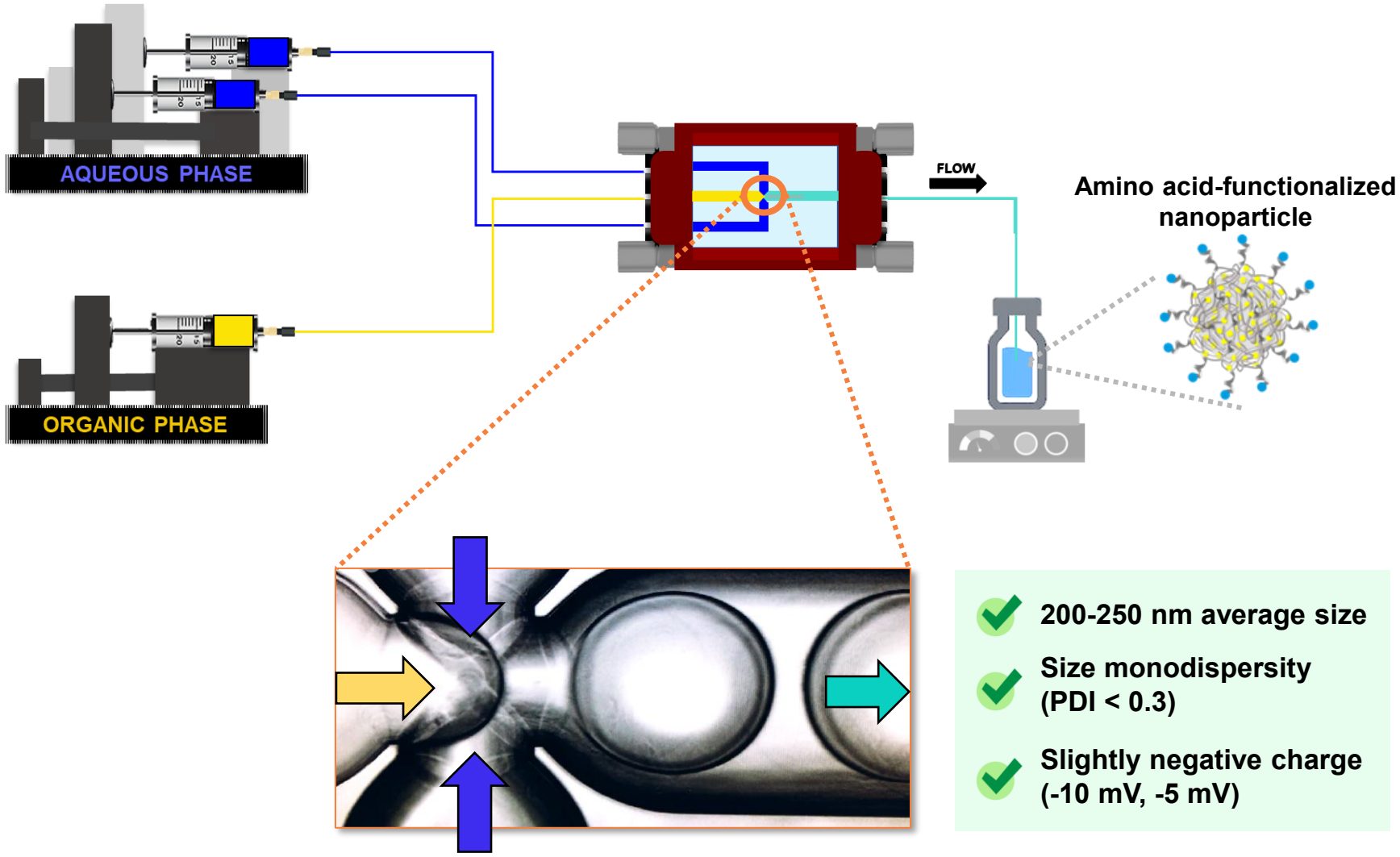


PLGA-PEG(5K)-Amino acid
0% | 5% | 10% | 20%

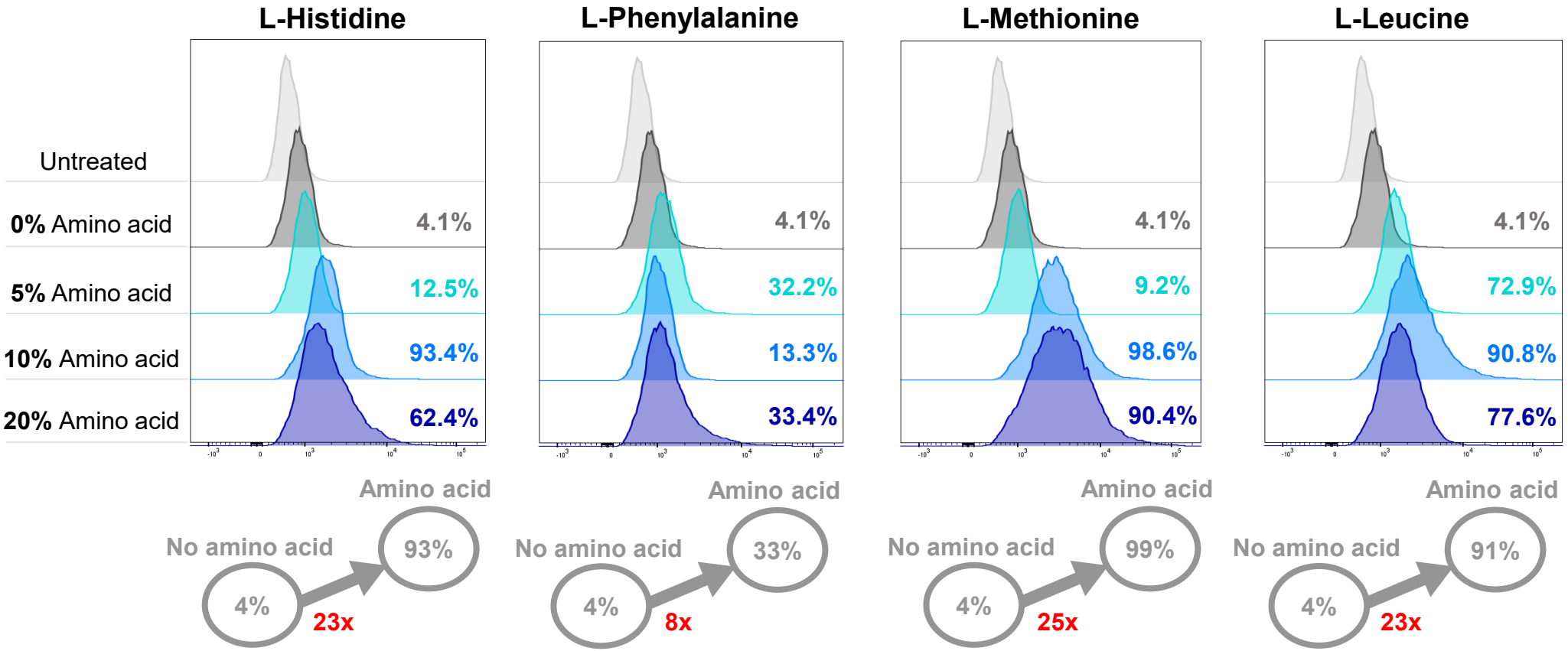
- **200-250 nm average size**
- **Size monodispersity (PDI < 0.3)**
- **Slightly negative charge (-10 mV, -5 mV)**

Unpublished data

Blood-brain barrier/glioblastoma interplay *in vitro* model

Nano-vehicles targeted to GBM-overexpressed L-amino acid transporters

2D GBM cells
U-251 MG



Unpublished data

Blood-brain barrier/glioblastoma interplay *in vitro* model

Nano-vehicles targeted to GBM-overexpressed L-amino acid transporters

2D GBM cells U-251 MG

2D "Normal" human astrocytes

	L-Histidine	L-Phenylalanine	L-Methionine	L-Leucine
Untreated	4.1%	4.1%	4.1%	4.1%
0% Amino acid	12.5%	32.2%	9.2%	72.9%
5% Amino acid	93.4%	13.3%	98.6%	90.8%
10% Amino acid	62.4%	33.4%	90.4%	77.6%
20% Amino acid				
Untreated	2.7%	2.7%	2.7%	2.7%
0% Amino acid	6.0%	30.2%	0.5%	4.4%
5% Amino acid	39.0%	4.2%	79.7%	69.0%
10% Amino acid	51.2%	15.5%	75.7%	34.6%
20% Amino acid				

Unpublished data

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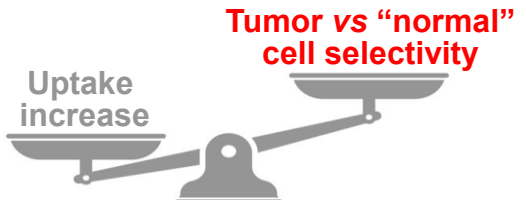
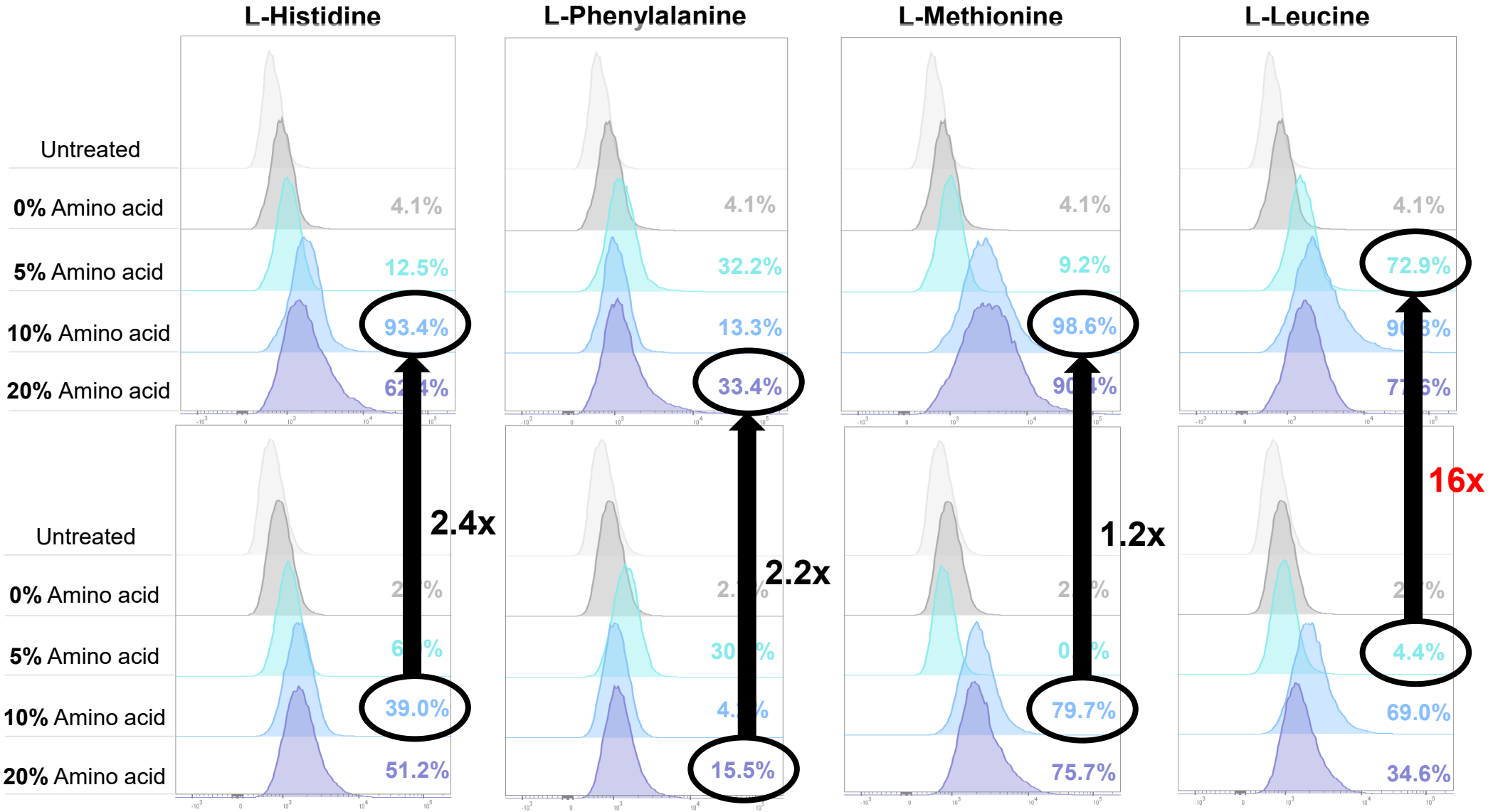


2D GBM cells U-251 MG

2D "Normal" human astrocytes



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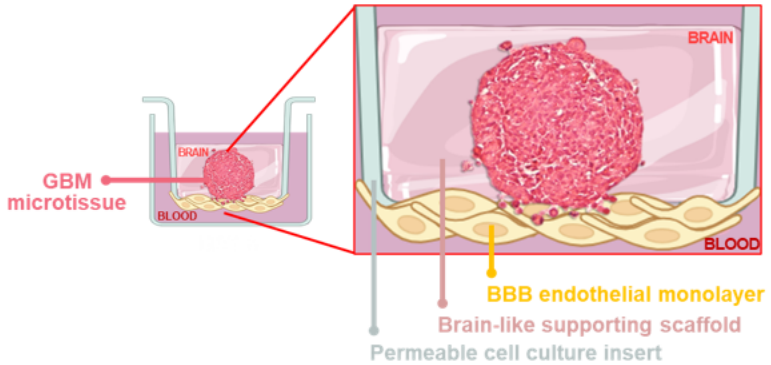

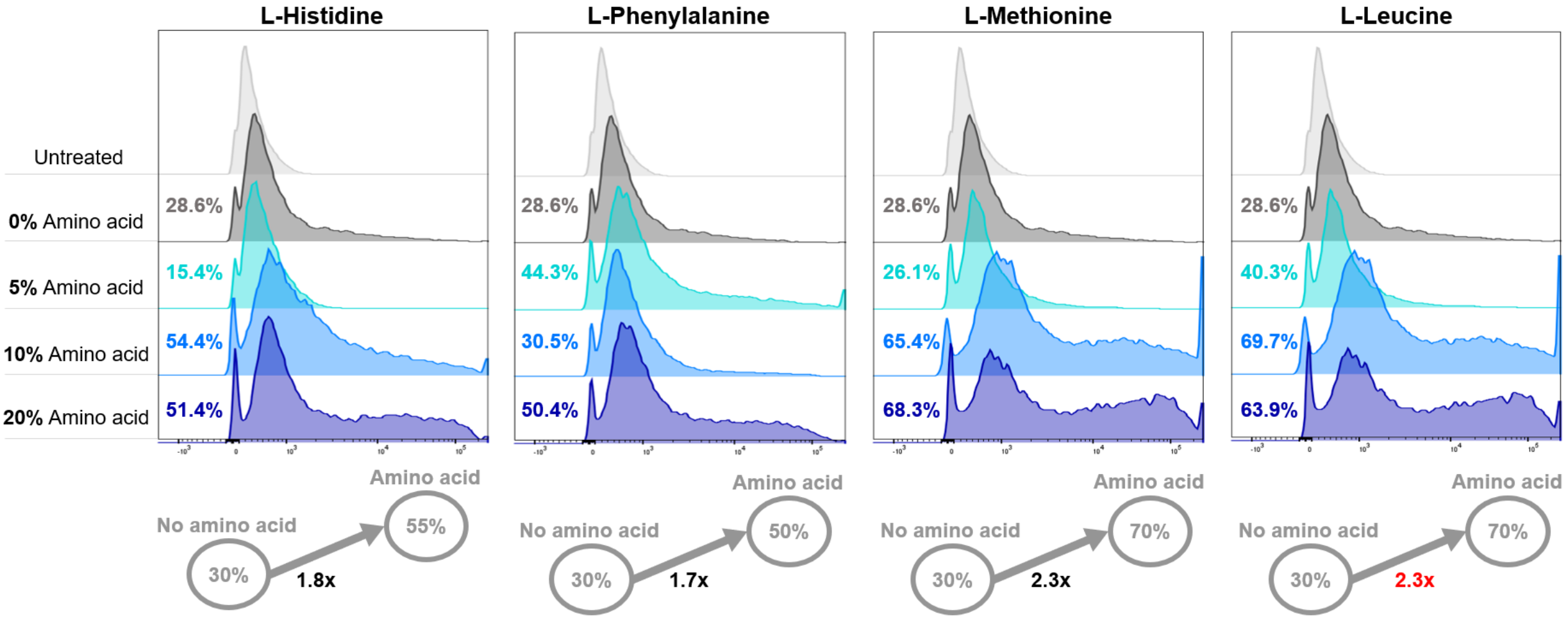

Unpublished data

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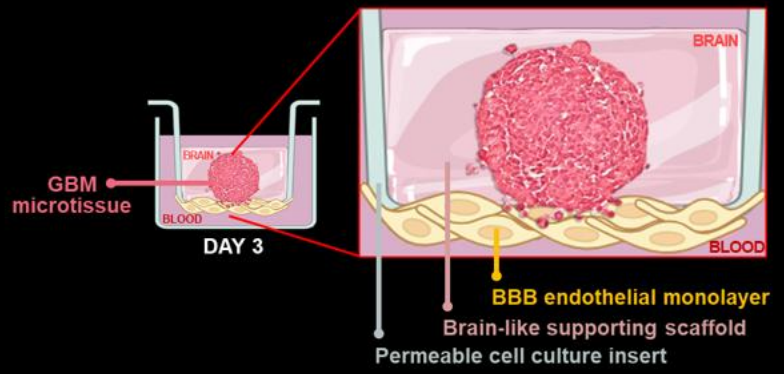

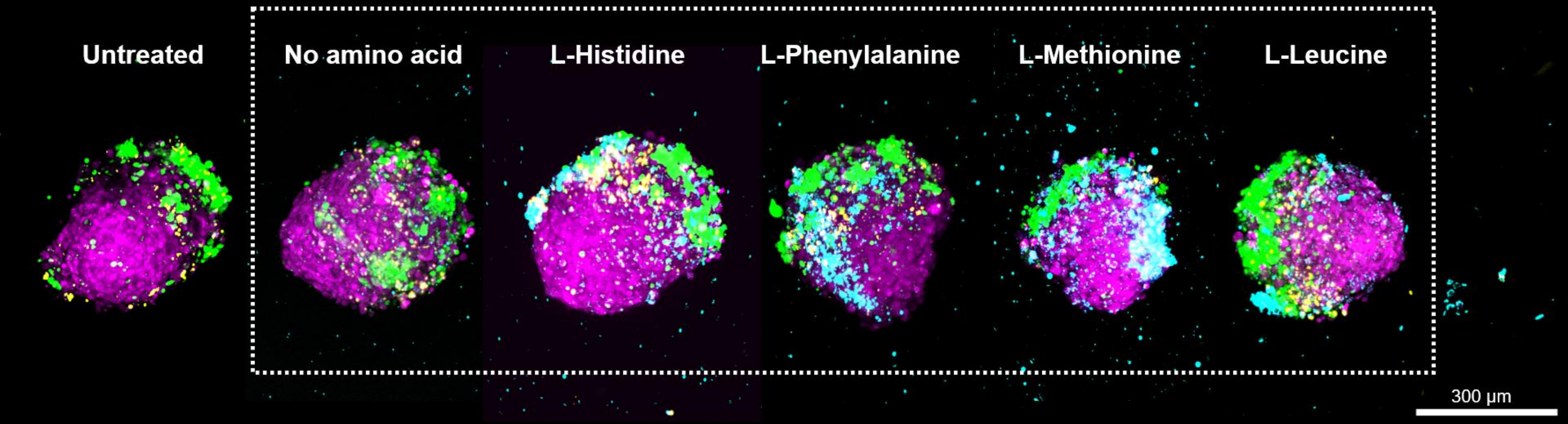

Tumor cells Macrophages Endothelial cells Nanoparticles

Unpublished data

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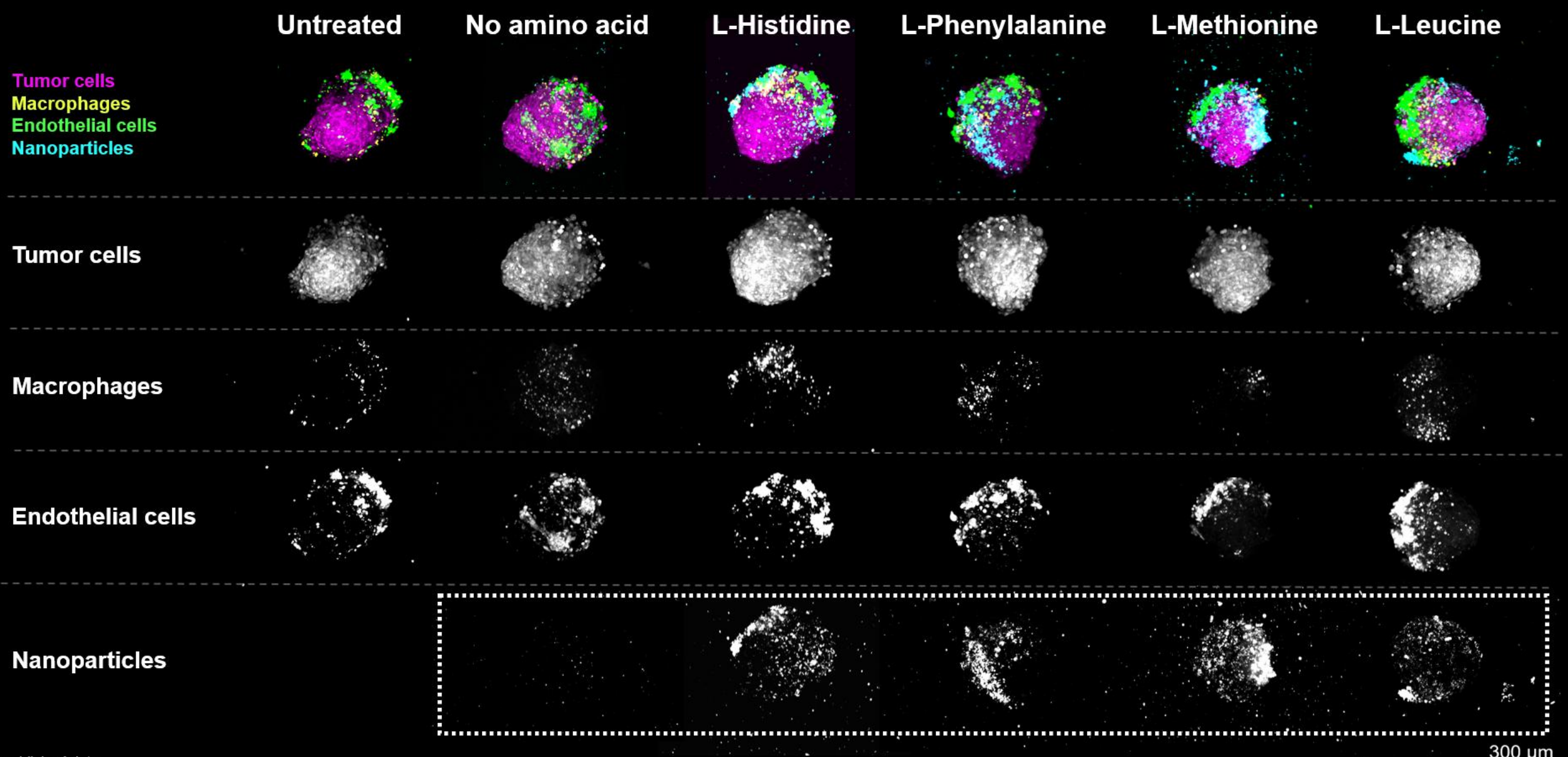
Nano-vehicles targeted to GBM-overexpressed L-amino acid transporters



Unpublished data

Take-home message

This **scalable 3D *in vitro* model** has potential to significantly impact **anti-GBM drug screening** and **optimize animal use**

L-Leucine: GBM targeting ligand demonstrating **superior tumor uptake** (18-times) and **selectivity** (16-times)

Ongoing work

BBB permeability studies

***In vitro* screening of anti-cancer (nano-)therapeutics**

Proof of concept of *in vivo* correlation

NIH ANNOUNCES END TO FUNDING FOR ANIMAL-ONLY STUDIES

The NIH has announced it will no longer fund new research proposals that rely solely on animal testing. Thus, researchers are now expected to incorporate New Approach Methodologies (NAMs), such as AI, organ-on-chip, and human tissue-based models.

This shift reflects growing recognition of the animal models' limitations, with ~90% of drugs that pass animal testing failing in humans. The policy marks a significant step toward more ethical and scientifically reliable methods in biomedical research.

This information was shared today during a webinar co-hosted by the U.S. Food and Drug Administration.

Jul 8, 2025

Adapted from linkedin.com/in/metatissue

Future work

Exploiting opportunities for integration into high-throughput on-chip and bioreactor platforms

Acknowledgments

claudia.martins@i3s.up.pt

Cecília Ferreira
MSc Student

Prof Bruno Sarmento
Group Leader

INSTITUTO DE INVESTIGAÇÃO E INOVAÇÃO EM SAÚDE
UNIVERSIDADE DO PORTO

NANOMEDICINES & TRANSLATIONAL DRUG DELIVERY

Marina Velogianni
Irena Ževrnja
Seem Awad
All current and former group members

Scientific Platforms @ i3S
André Maia
Maria Azevedo
Emília Cardoso, Catarina Meireles
Nuno Mendes, Rossana Correia, Cláudia Machado
Maria Lázaro, Ricardo Vidal
Frederico Silva

TMI @ i3S
Ângela Magalhães

ICVS-UM Prof Bruno Costa

CEMUP Mariana Andrade

CHSJ MD Paulo Linhares

QUEEN'S UNIVERSITY BELFAST
Dr Dimitrios Lamprou

Children's Brain Tumour Research Centre
The University of Nottingham
Porf Ruman Rahman
MD Stuart J. Smith
Prof Jonathan W. Aylott

UCLouvain BRUXELLES
Prof Véronique Préat
Dr Alessio Malfanti
Bernard Ucakar

ETH zürich
Prof Andreas Hierlemann
Dr Mario Modena
Dr Wei Wei

MyBiotech
PharmJet GmbH
Dr Nazende Günday-Türeli
Cemre Günday

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Blood-brain barrier/glioblastoma interplay 3D *in vitro* model for (nano-)therapeutic screening

Cláudia Martins, PhD

Junior Postdoctoral Researcher
i3S – University of Porto, Portugal

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