

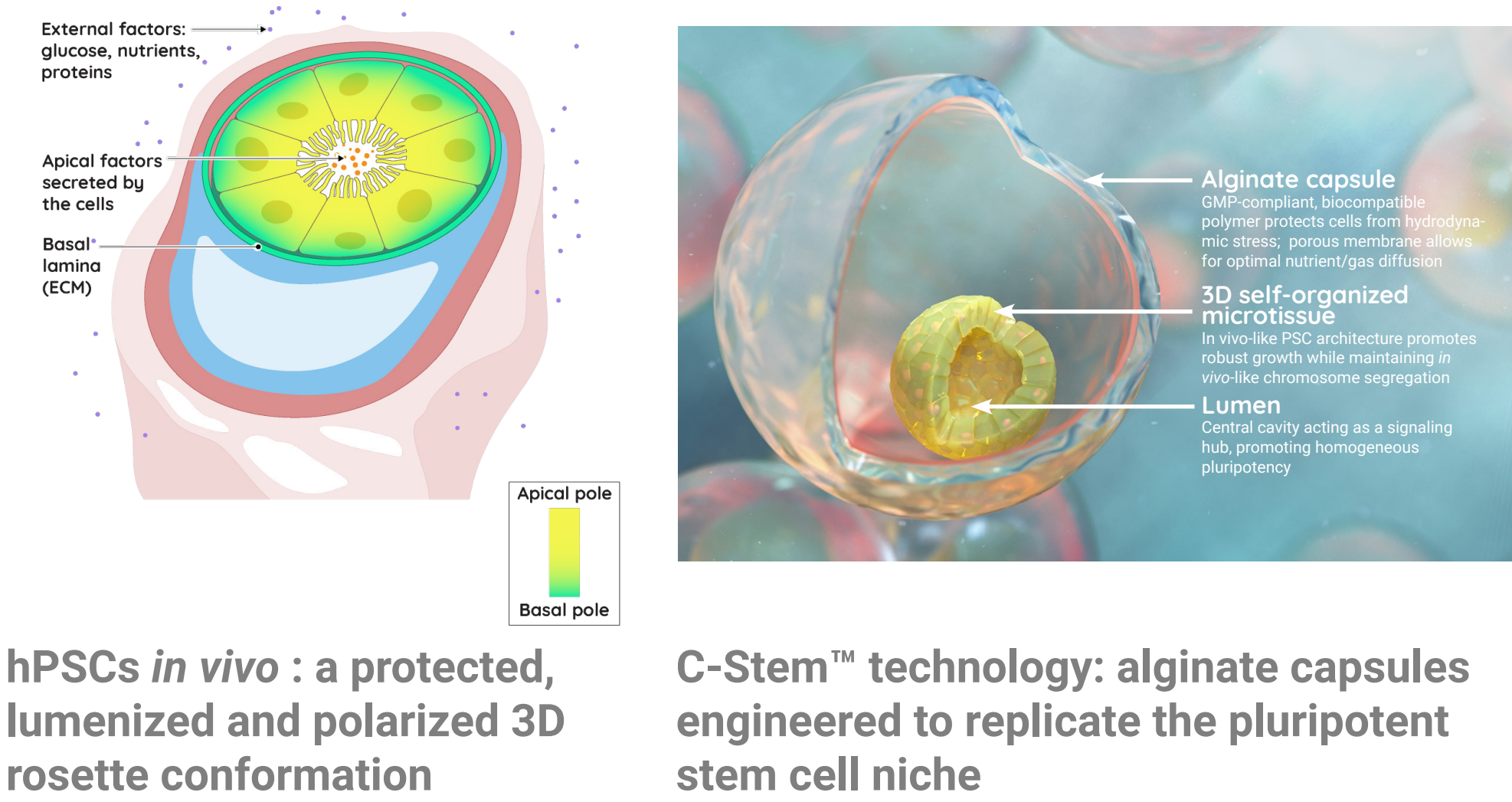
Scaling-up iPSC-based cell therapies: real-world processes with biomimetic C-Stem™ technology



Maxime FEYEUX, Co-founder and Chief Scientific Officer of TreeFrog Therapeutics.

C-Stem™: biomimetic cell culture at scale

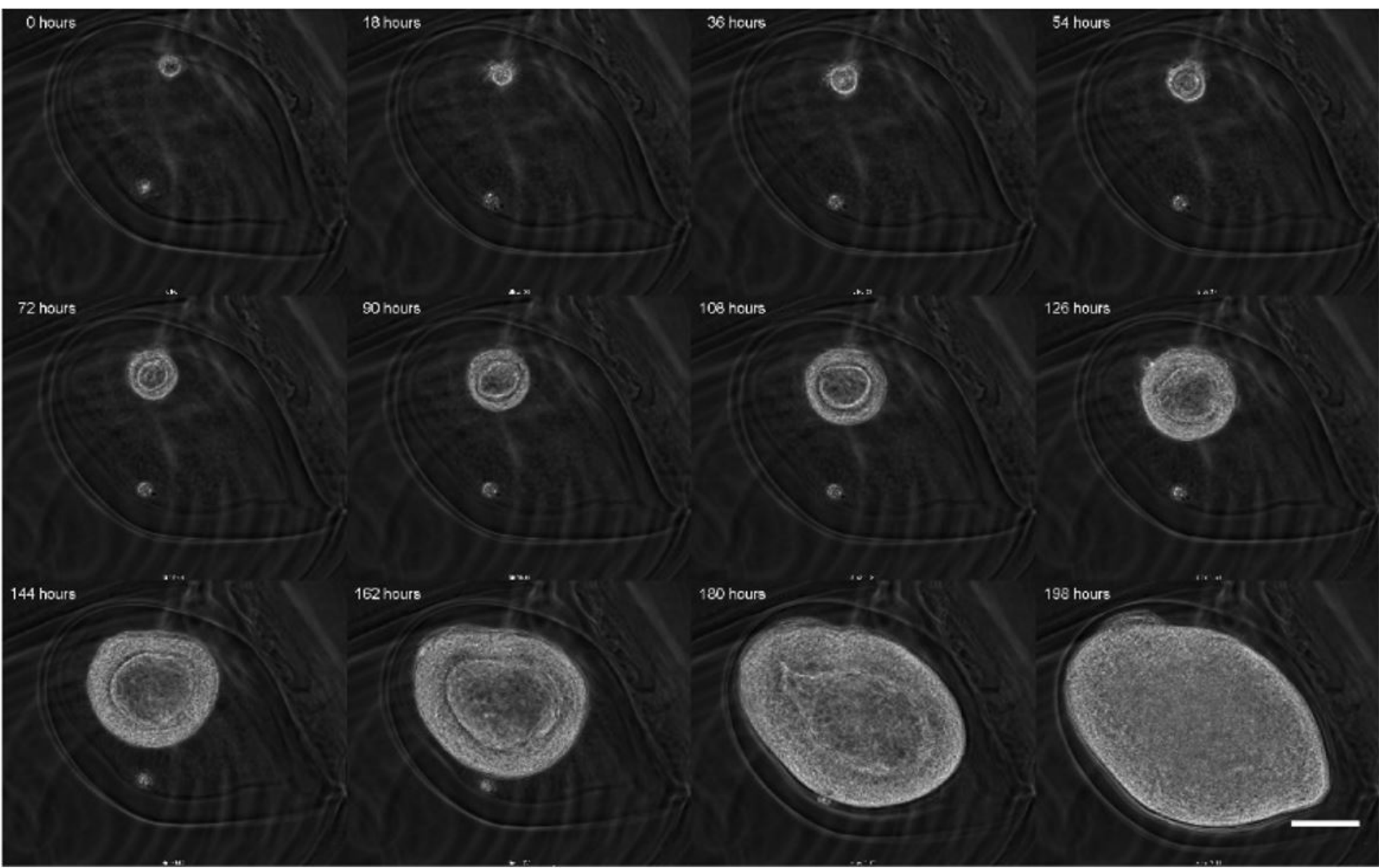
Mimicking the *in vivo* micro-environment of human pluripotent stem cells (hPSCs)



In vivo, hPSCs form a lumenized and polarized rosette architecture, enabling fast growth while maintaining robust quality. The central lumen is required for establishing critical signaling pathways, syncing cells and maintaining homogenous pluripotency. The rosette architecture promotes high-fidelity chromosome segregation and elimination of unfit cells.^{1,2,3}

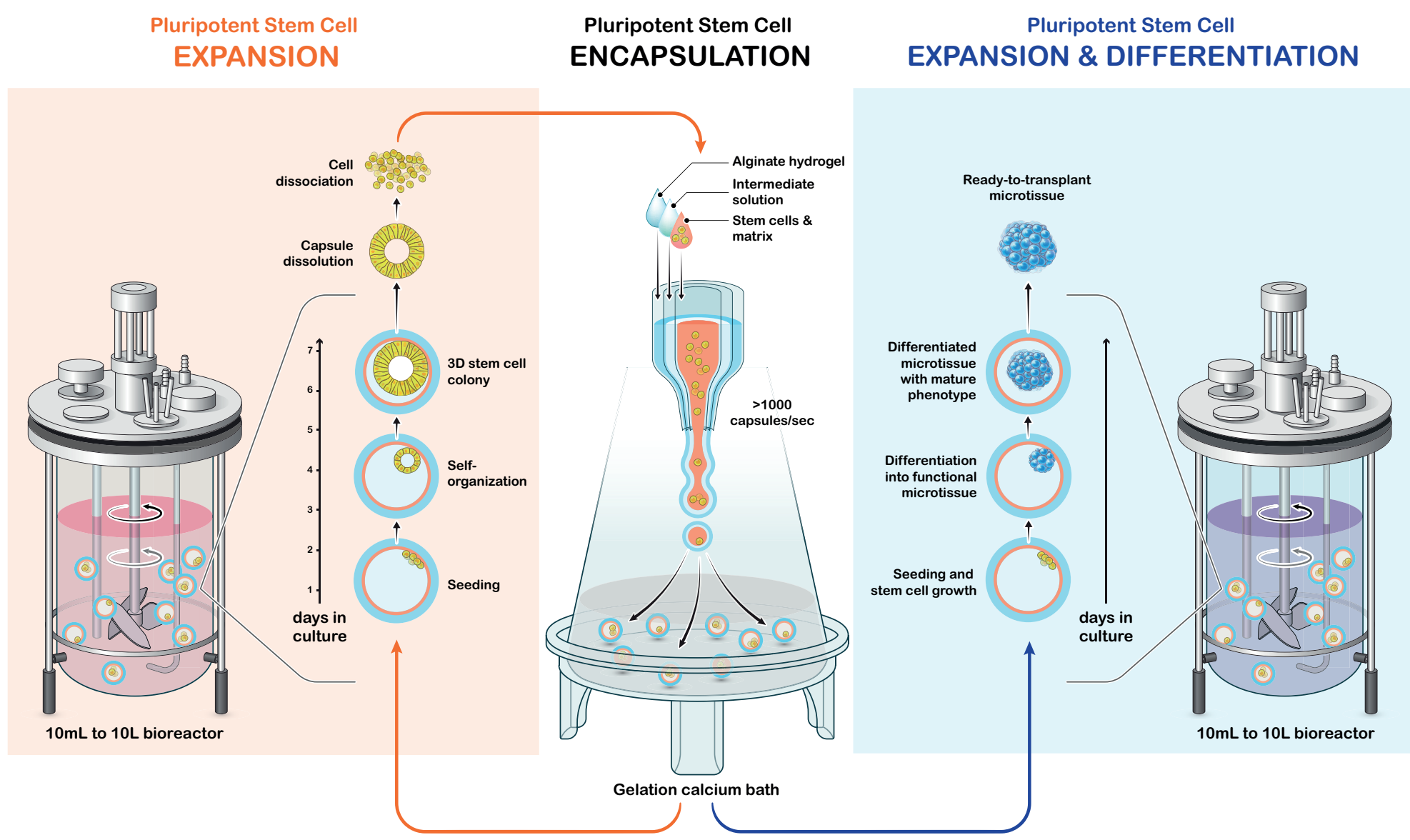
The C-Stem™ technology utilizes proprietary microfluidics^{4,5,6} to encapsulate hPSCs in hollow alginate shells at very high throughput (> 1000 capsules per second). The inner wall of the capsule is decorated with extracellular matrix, thus mimicking the basement membrane of the hPSC niche. In this biomimetic microenvironment, hPSCs spontaneously self-organize in 3D and form *in vivo*-like lumenized rosette structures. The size of the capsule (tunable from 100 to 800 µm diameter) and the porosity of the alginate allow for optimal diffusion of oxygen and nutrients, thus preventing the formation of a necrotic core. On the outside, the 30 µm thick wall of alginate constitutes a highly resistant shell, which protects PSCs from hydrodynamic stress.

Fast hiPSC growth in 3D lumenized rosette conformation in capsule



Sequence of phase-contrast microscopy images showing luminogenesis, growth and collapse of an encapsulated hiPSC colony. The time interval between successive images is 18h. Scale bar=100µm.⁶

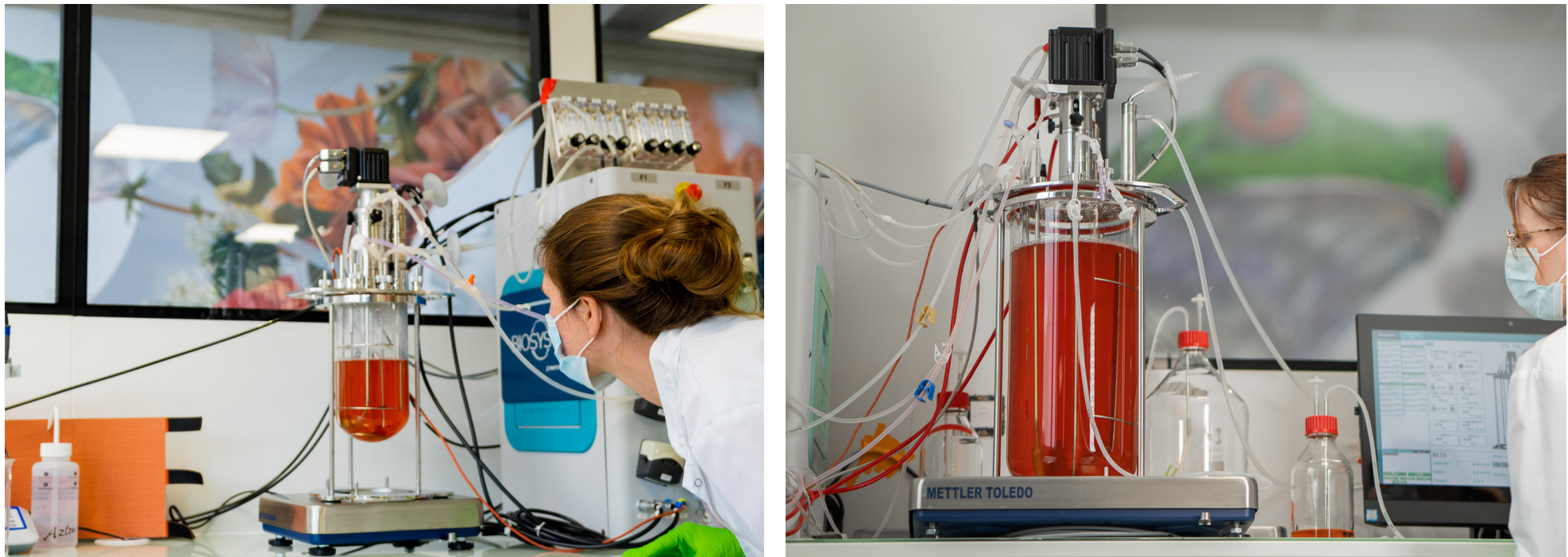
C-Stem™, a single platform for hiPSC amplification & differentiation in bioreactors



Following encapsulation using high-throughput proprietary microfluidics (middle panel), hiPSCs in matrix-laden capsules can be either amplified serially to create master and working cell banks, or differentiated into functional microtissues. In each case, the cellular content is easily harvested by dissolving the capsule with a calcium chelator.

hiPSC mass-production in bioreactors

GMP platform combining encapsulation device & standard stirred-tank bioreactors (up to 10L)

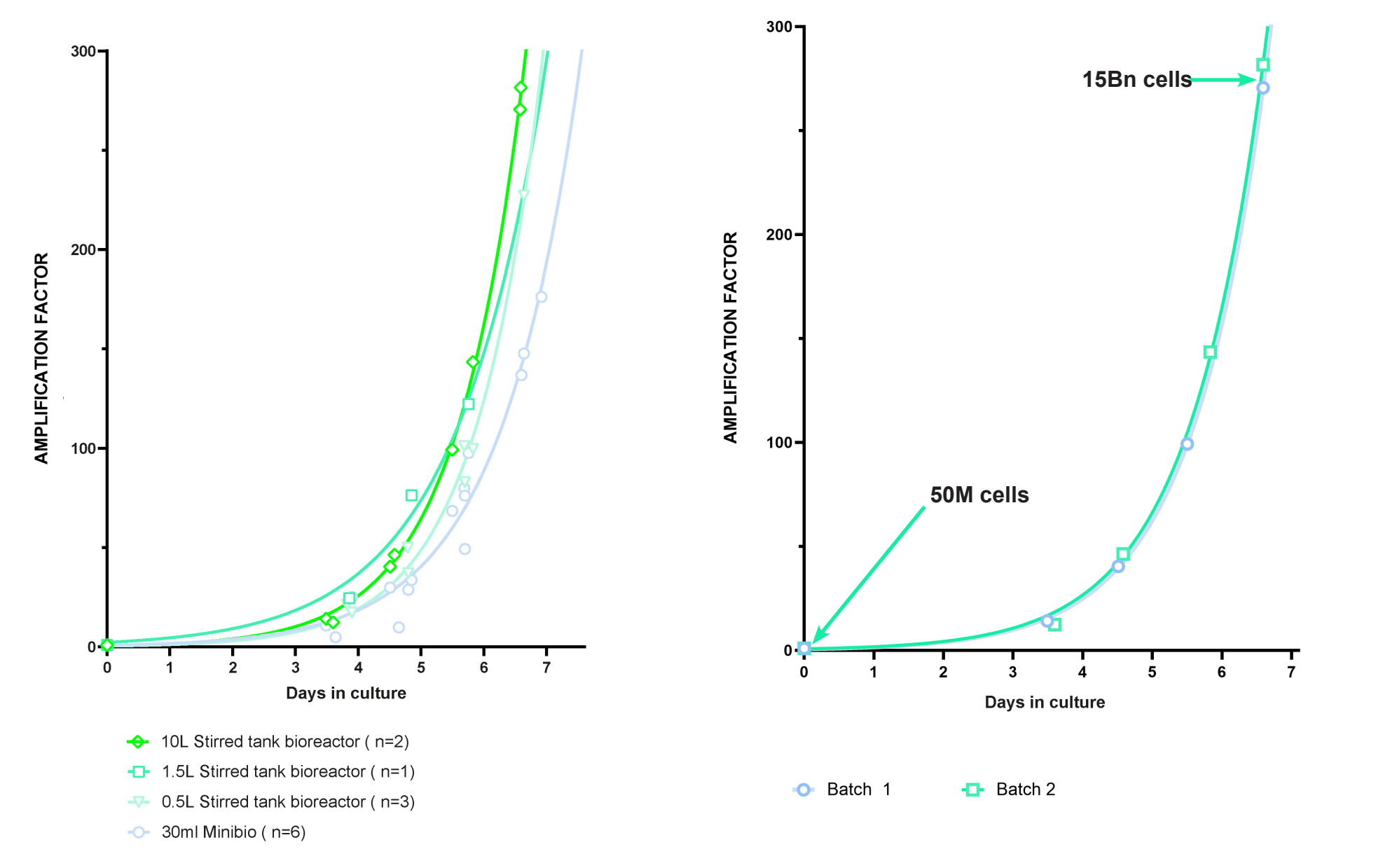


Manufacturing process in place at commercial scale

hiPSC expansion & differentiation in bioreactors from 200mL to 10L

By shielding hiPSCs within alginate capsules, the C-Stem™ technology removes hydrodynamic stress constraints usually found in stirred-tank bioreactors, thus permitting easy scale-up to larger volumes while conserving core cell culture parameters.

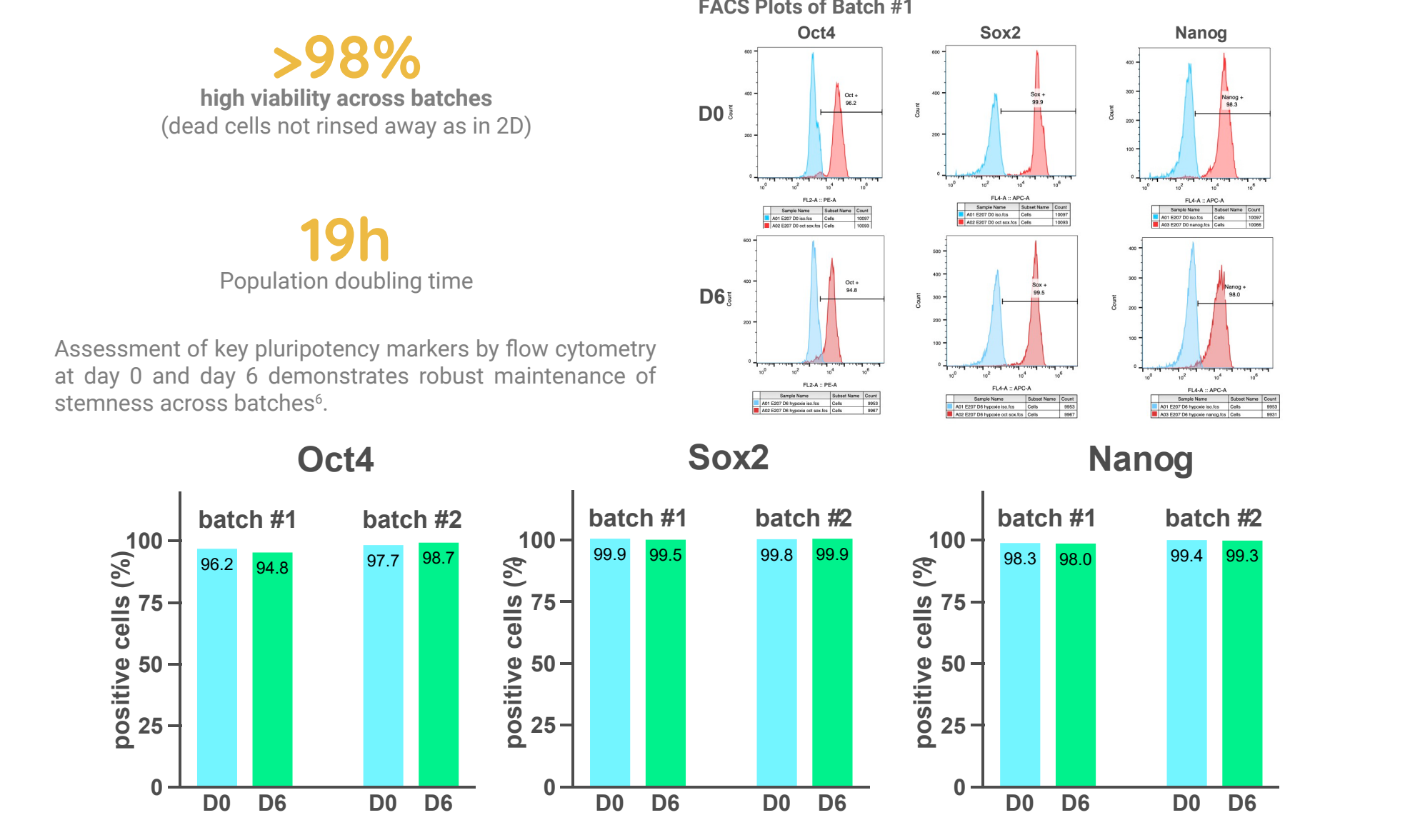
Highly reproducible hiPSC amplification: >277-fold per week in 10L bioreactors



C-Stem™ enables robust exponential hiPSC growth across scales, while conserving key cell culture parameters.

hiPSC amplification data in two independent 10L bioreactors. For each run, 50 million C-Stem™-encapsulated hiPSCs were cultivated under hypoxic conditions for 6 days. Each run resulted in a 15Bn hiPSC batch⁶.

Preservation of hiPSC viability in 10L bioreactors: viability & pluripotency

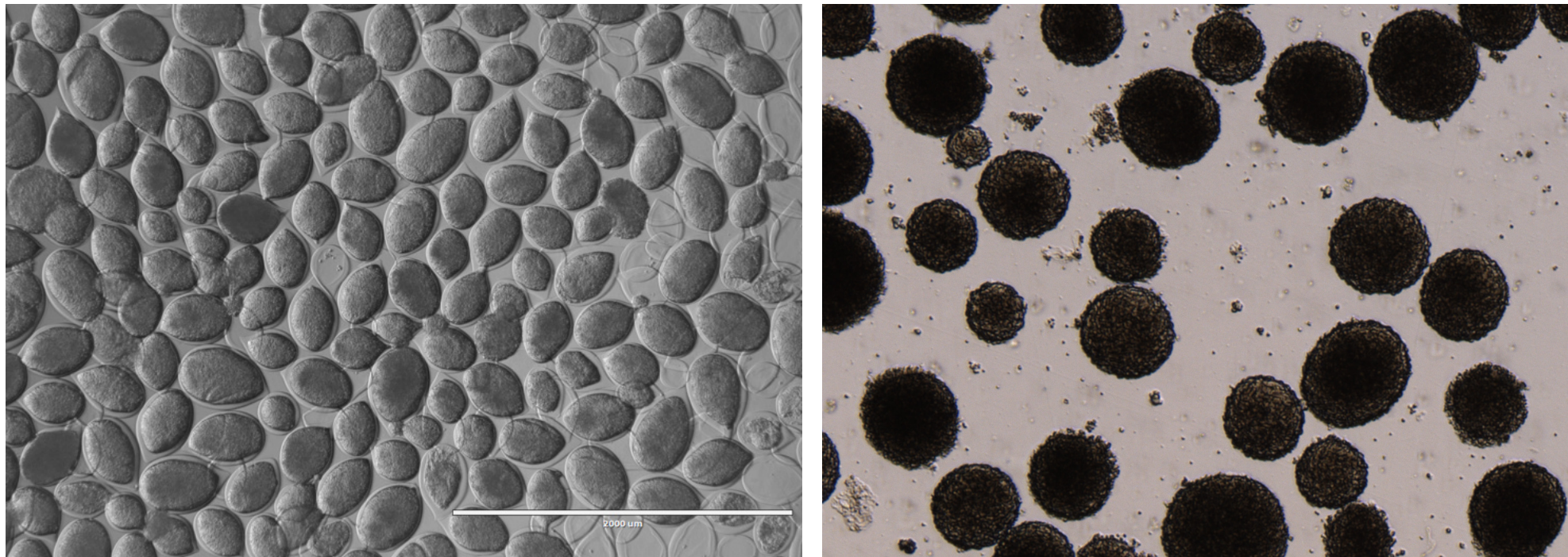


Genomic integrity

Preliminary results assessing the genomic integrity of hiPSCs amplified with C-Stem™ indicated an additional benefit of lumenized hiPSC culture in capsule over 2D or aggregate culture. In particular, monitoring the variation in 20q11.21 copy number variation over an extended 28-day culture in 2D vs aggregates vs C-Stem™ suggests that the limited cell death seen in C-Stem™ appears to reduce the selective advantage of mutations that affects cell survival. This observation is very relevant in the context of the production of clinical batches. TreeFrog continues to explore this aspect of capsule culture internally and through external collaborations.

Scalable differentiation in 3D

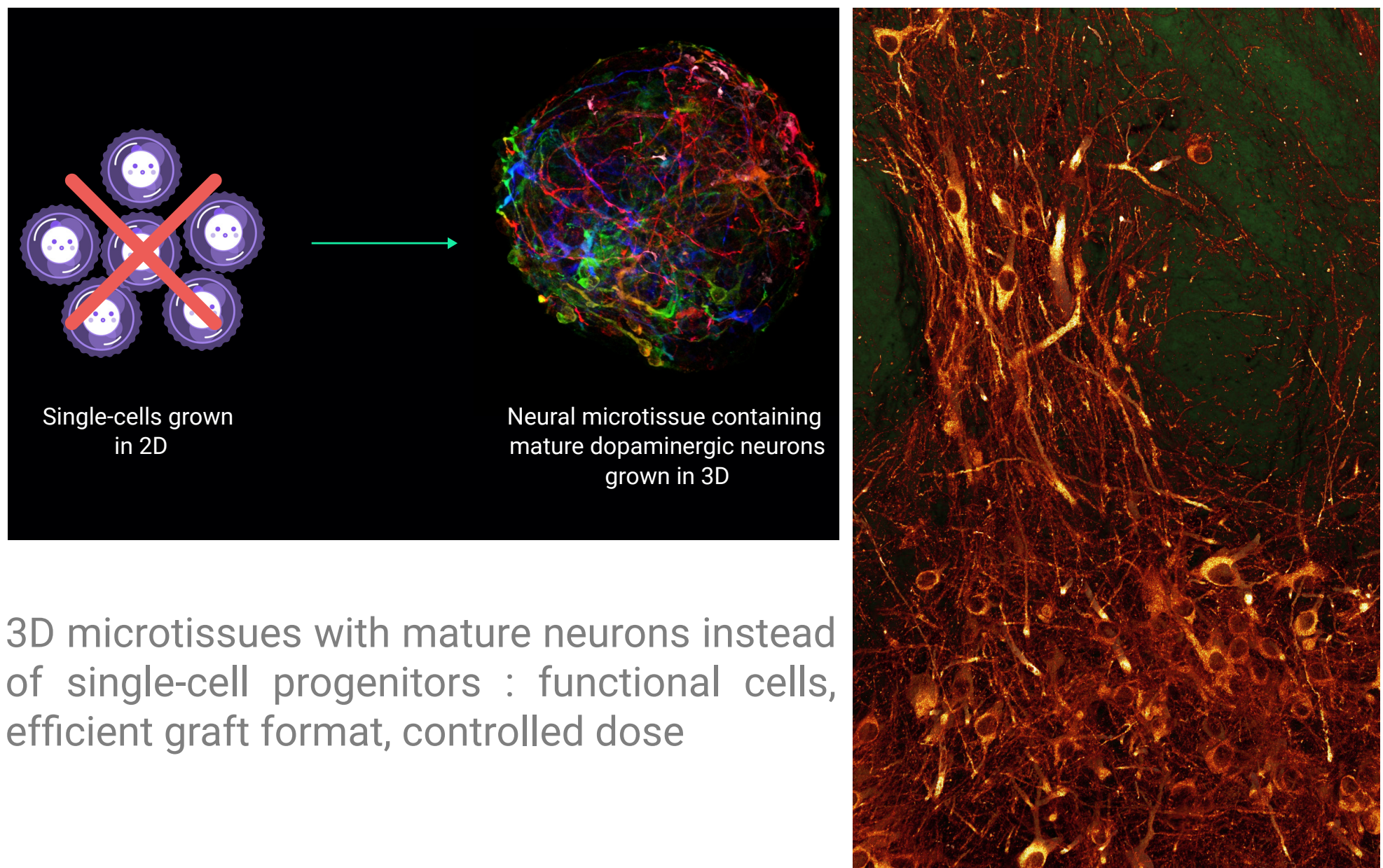
Single-step differentiation in 500mL bioreactors



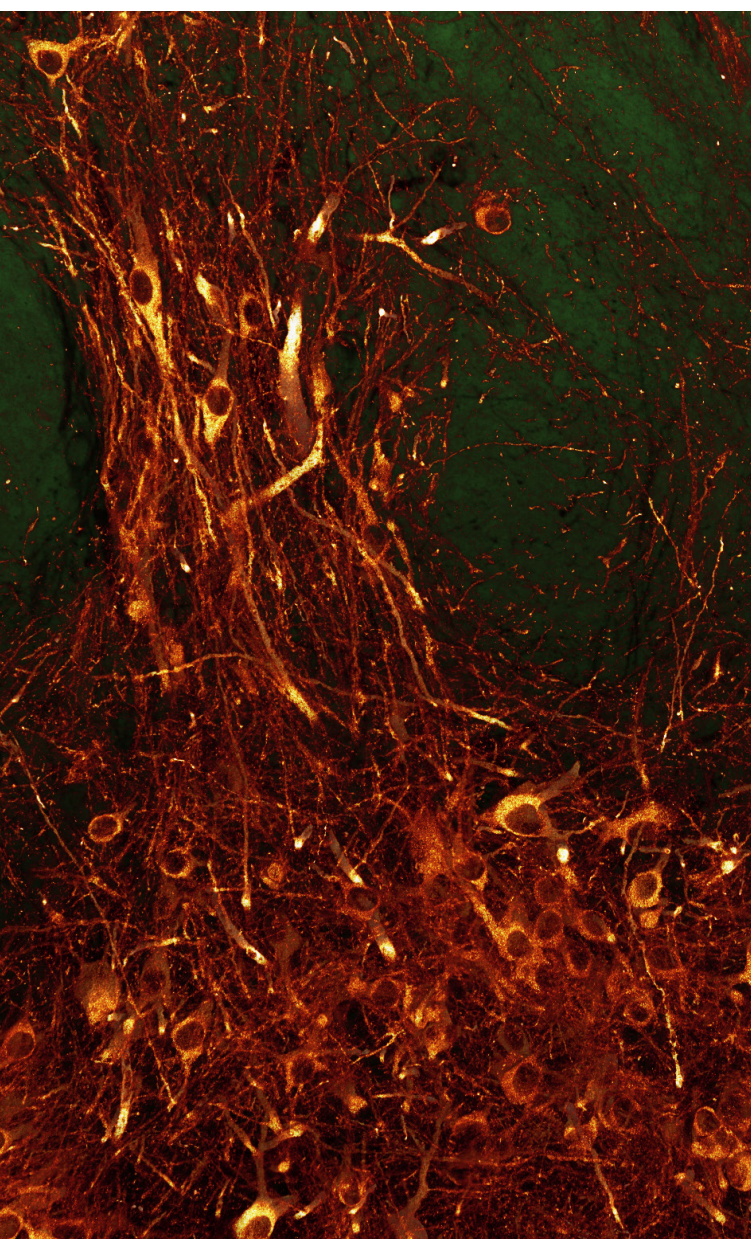
C-Stem™ generated micro-tissues after differentiation from hiPSCs in 500mL bioreactor.

24 hours post-thawing microtissues.

C-Stem™-generated neural microtissues contain mature dopaminergic neurons

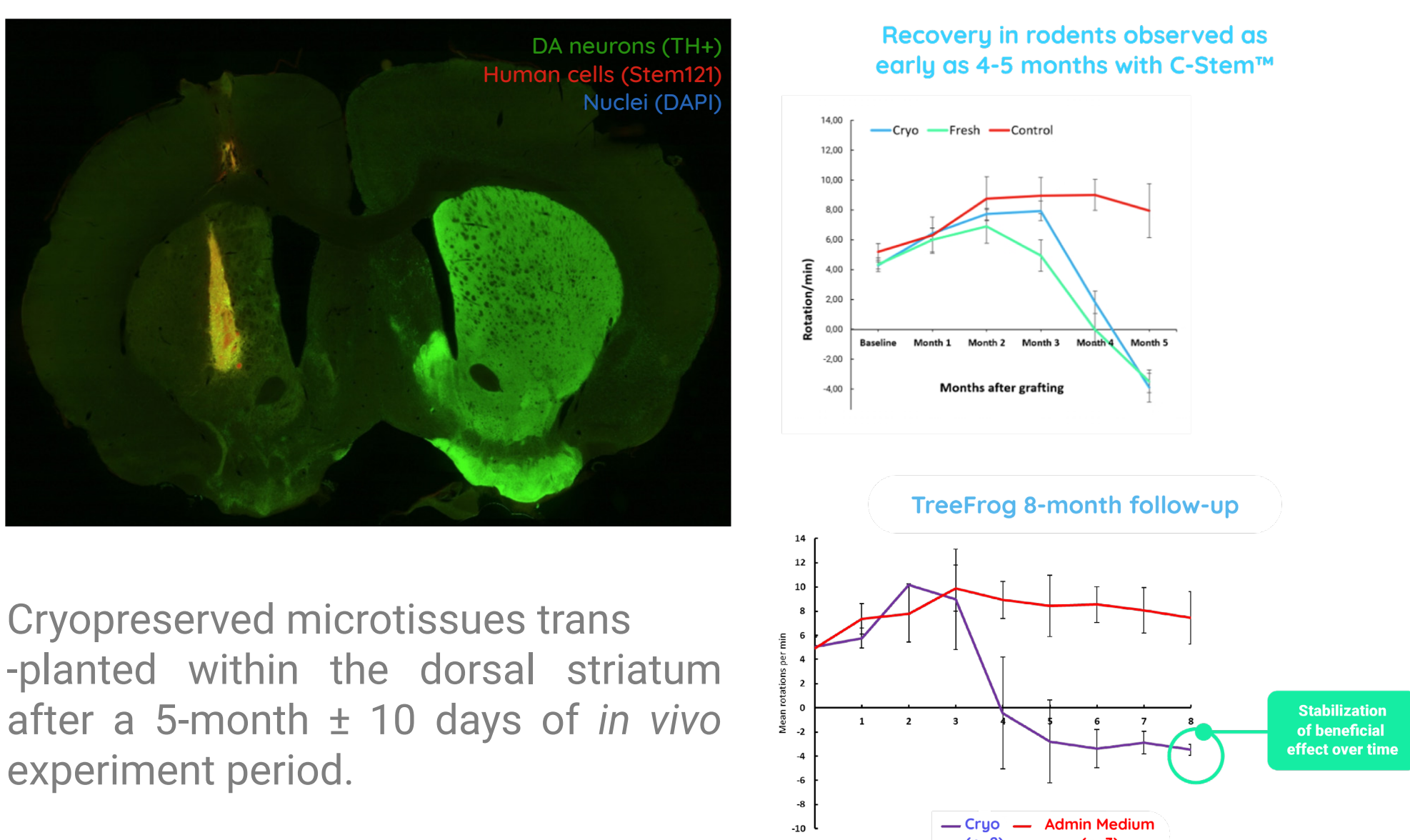


3D microtissues with mature neurons instead of single-cell progenitors : functional cells, efficient graft format, controlled dose

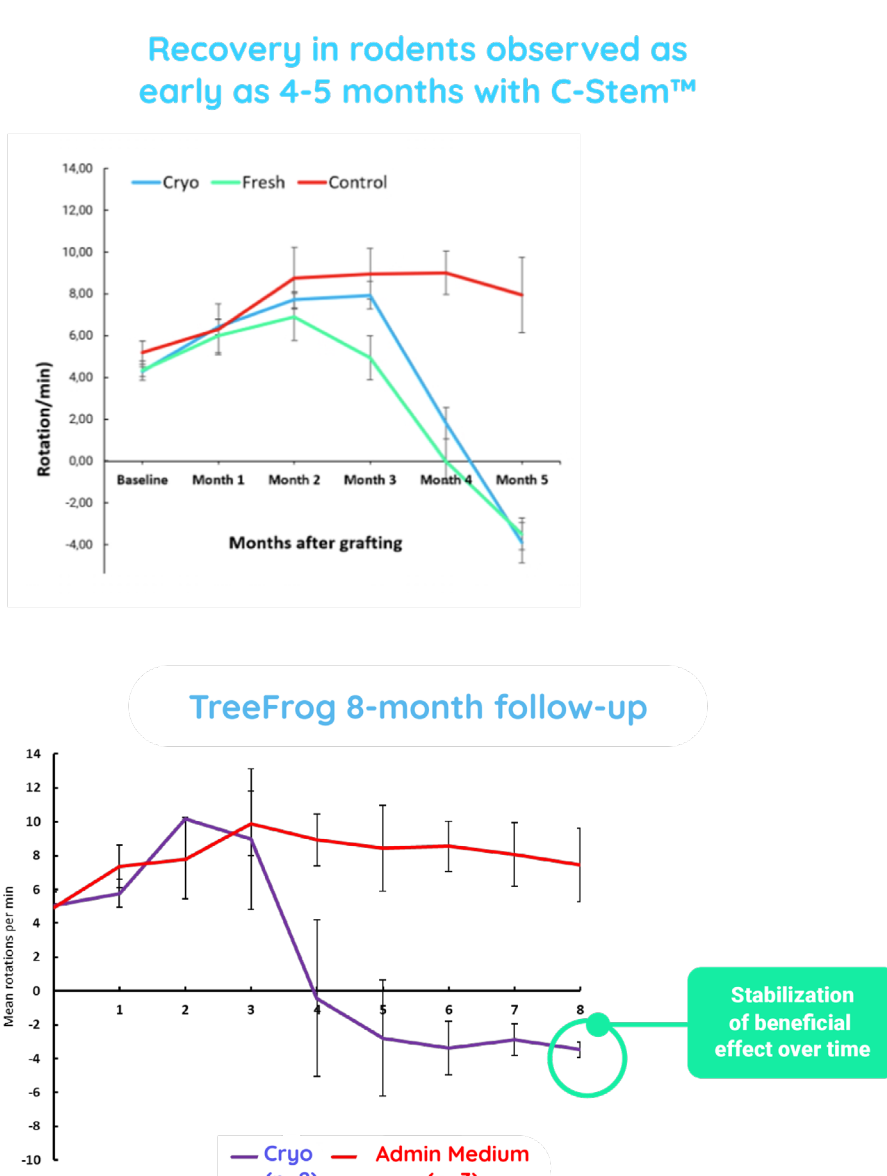


Human dopaminergic neurons network integrated into rodents brain (confocal microscopy)

Full motor function recovery in preclinical models with cryopreserved product



Cryopreserved microtissues trans-planted within the dorsal striatum after a 5-month ± 10 days of *in vivo* experiment period.



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References

(1) Knouse, K. A.; Lopez, K. E.; Bachofner, M.; Amon, A. Chromosome Segregation Fidelity in Epithelia Requires Tissue Architecture. *Cell* 2018, 175 (1), 200-211.e13. <https://doi.org/10.1016/j.cell.2018.07.042>.

(2) Singla, S.; Iwamoto-Stohl, L. K.; Zhu, M.; Zernicka-Goetz, M. Autophagy-Mediated Apoptosis Eliminates Aneuploid Cells in a Mouse Model of Chromosome Mosaicism. *Nat. Commun.* 2020, 11 (1), 2958. <https://doi.org/10.1038/s41467-020-16796-3>.

(3) Lima, A.; Lubatti, G.; Burgstaller, J.; Hu, D.; Green, A. P.; Di Gregorio, A.; Zawadzki, T.; Pemaute, B.; Mahammadov, E.; Perez-Montero, S.; Dore, M.; Sanchez, J. M.; Bowling, S.; Sancho, M.; Kolbe, T.; Karimi, M. M.; Carling, D.; Jones, N.; Srinivas, S.; Scialdone, A.; Rodriguez, T. A. Cell Competition Acts as a Purifying Selection to Eliminate Cells with Mitochondrial Defects during Early Mouse Development. *Nat. Metab.* 2021, 3 (8), 1091-1108. <https://doi.org/10.1038/s42255-021-00422-7>.

(4) Alessandri, K.; Sarangi, B. R.; Gurchenkov, V. V.; Sinha, B.; Kießling, T. R.; Fetter, L.; Rico, F.; Scheuring, S.; Lamaze, C.; Simon, A.; Geraldo, S.; Vignjević, D.; Doméjean, H.; Rolland, L.; Furfak, A.; Bibette, J.; Bremond, N.; Nassoy, P. Cellular Capsules as a Tool for Multicellular Spheroid Production and for Investigating the Mechanics of Tumor Progression in Vitro. *Proc. Natl. Acad. Sci.* 2013, 110 (37), 14843-14848. <https://doi.org/10.1073/pnas.1309482110>.

(5) Alessandri, K.; Feyeux, M.; Gurchenkov, B.; Delgado, C.; Trushko, A.; Krause, K.-H.; Vignjević, D.; Nassoy, P.; Roux, A. A 3D Printed Microfluidic Device for Production of Functionalized Hydrogel Microcapsules for Culture and Differentiation of Human Neuronal Stem Cells (HNSC). *Lab. Chip* 2016, 16 (9), 1593-1604. <https://doi.org/10.1039/C6LC00153E>.

(6) Philippe, J.R.; Cohen, Elisa; Luquet, Justine; Pletenka, Andrea; Leonard, Elise; Warter, Basile; Gurchenkov, Jessica; Carrere, Clément; Rieu, Jérôme; Hardouin, Fabien; Monseubeig, Michael; Lanero, Eddy; Quelenec, Helene; Wurtz, Emilie; Jamet, Masile; Demarco, Celine; Banal, Paul; Van Liedekerke, Pierre; Nassoy, Maxime; Feyeux, Nathalie; Lefort, Kevin; Alessandri, Engineering 3D micro-compartments for highly efficient and scale-independent expansion of human pluripotent stem cells in bioreactors. *Biomaterials* 2023, 295, 122033. <https://doi.org/10.1016/j.biomaterials.2023.122033>

Ask for our white paper : The Lumenized Rosette - Mimicking the *in vivo* micro-environment of human pluripotent stem cells
<https://treefrog.fr/stemcelljungle/release-of-the-white-paper-on-the-hiPSC-lumenized-rosette/>