UNIVERSITY OF CAMBRIDGE

BTS British Transplantation Society

The Voice of Transplantation in the UK

Accurate viability assessment and cryopreservation of pancreatic islets

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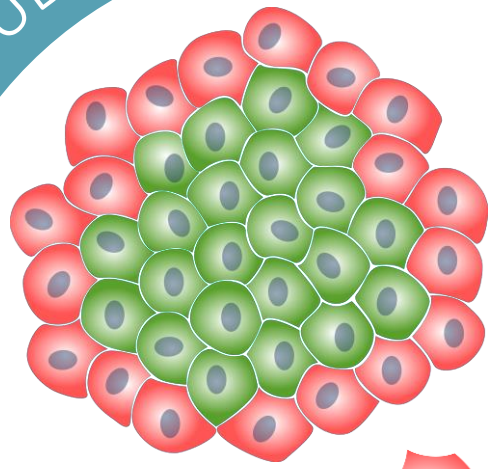
LONG INCUBATION

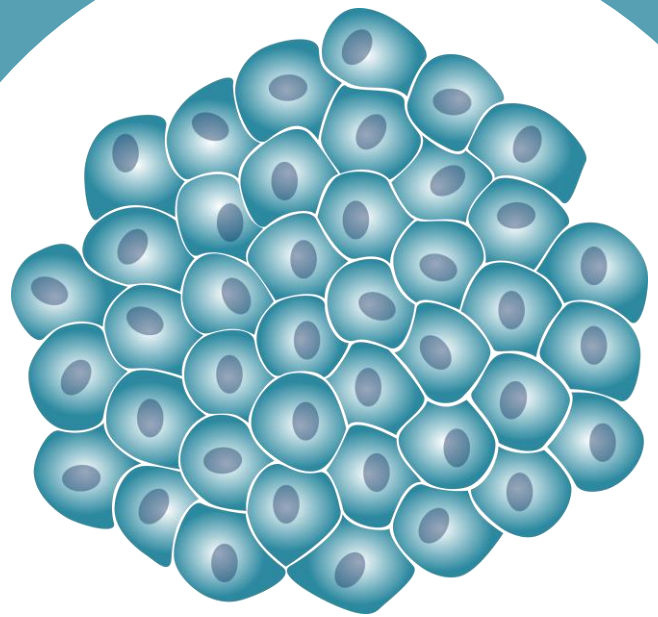


dead cell

live cell

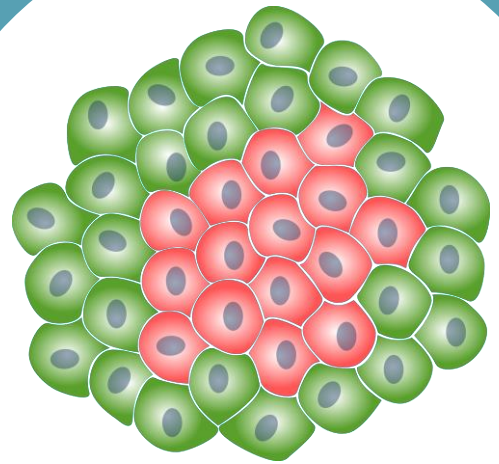
Unde
develo

s is key to
on protocols

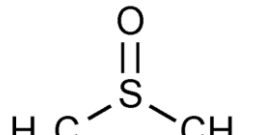


pancreatic islet

SHORT INCUBATION



DMSO



PANCREATIC ISLET CRYOPRESERVATION

AIMS

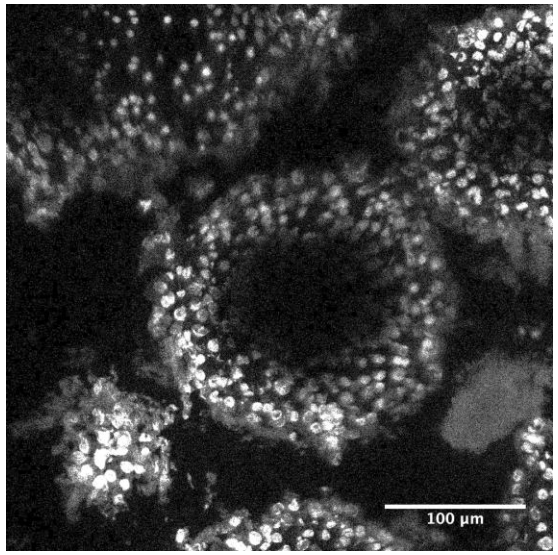
1. Determine kinetics of molecule diffusion into pancreatic islets
2. Optimise methods for viability assessment of pancreatic islets
3. Improve cryopreservation of pancreatic islets

DIFFUSION KINETICS IN PANCREATIC ISLETS

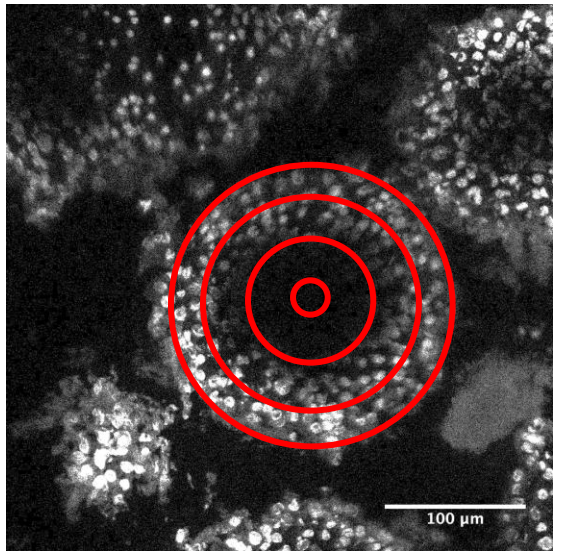
Experiments performed on mouse islets isolated in-house

Pre-staining with nuclear dye → Embedding and cryosectioning → Confocal imaging → Image analysis

Original image

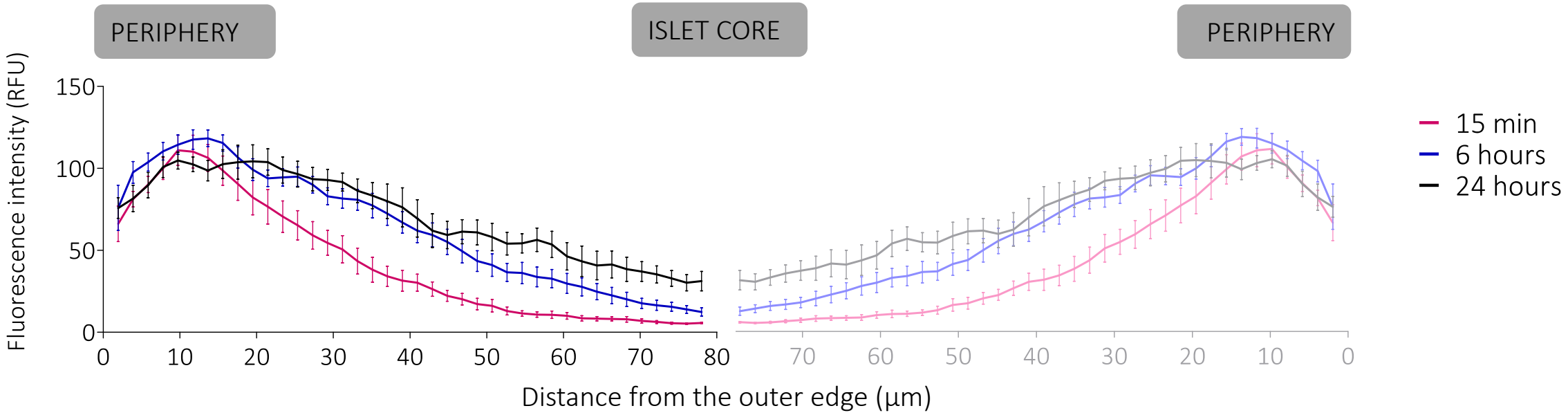


Concentric Circles Plugin in ImageJ

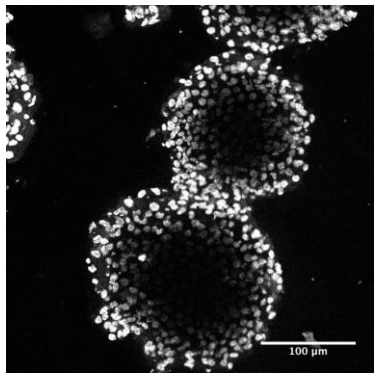


DIFFUSION KINETICS IN PANCREATIC ISLETS

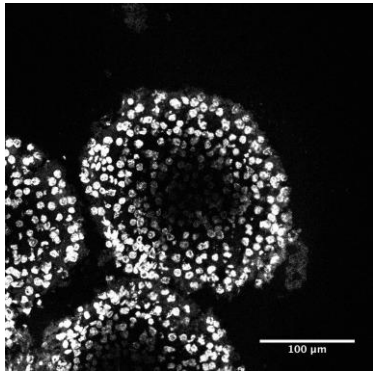
20°C



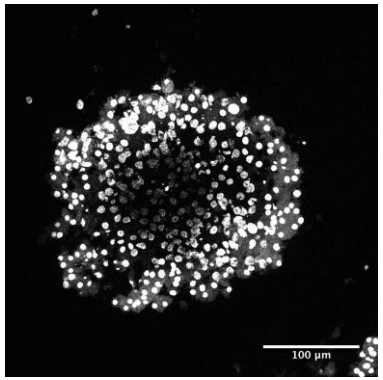
15 min



6 hours

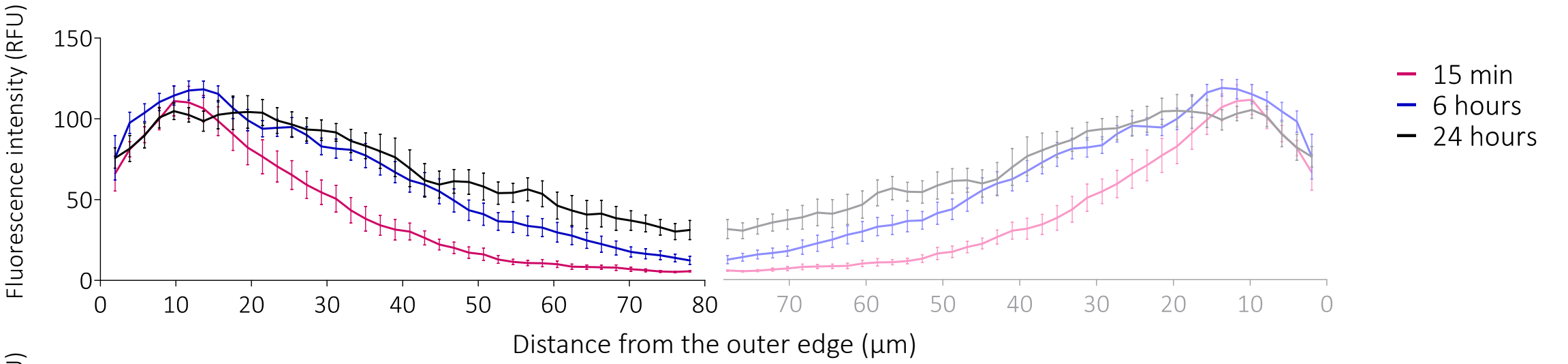


24 hours

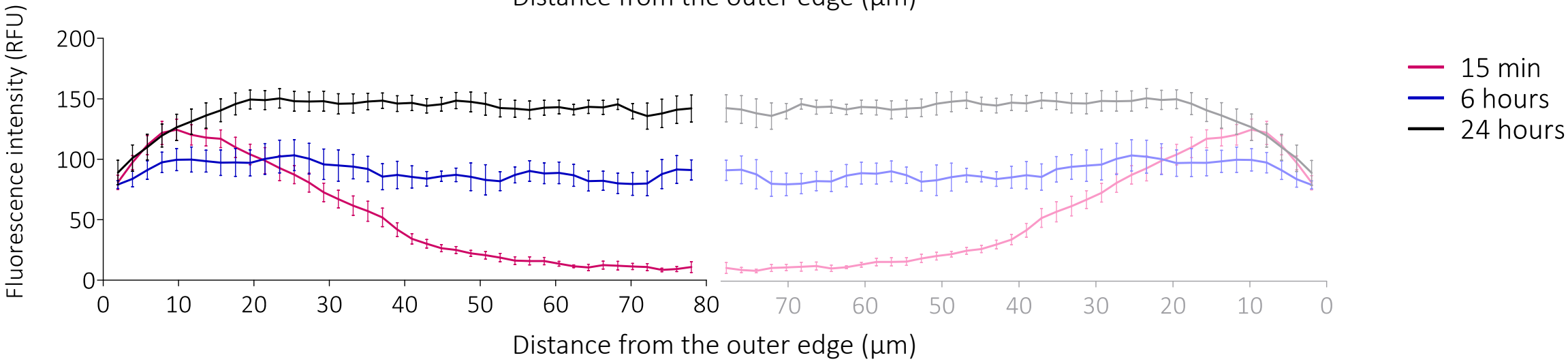


DIFFUSION KINETICS IN PANCREATIC ISLETS – TEMPERATURE EFFECT

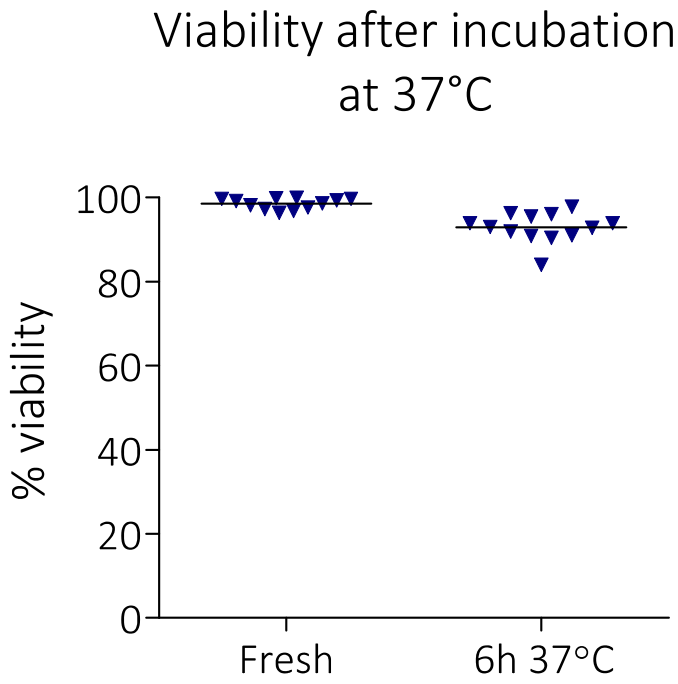
20°C



37°C

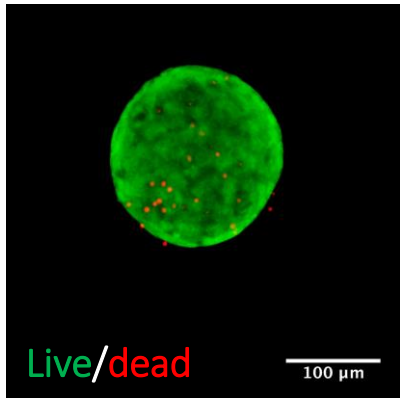


EFFECT OF PROLONGED INCUBATION AT 37 °C ON ISLET VIABILITY

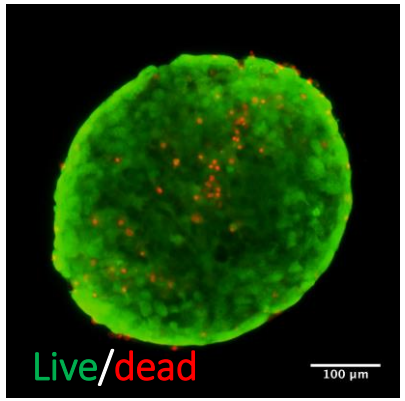


Fluorescein diacetate / Propidium iodide

Fresh

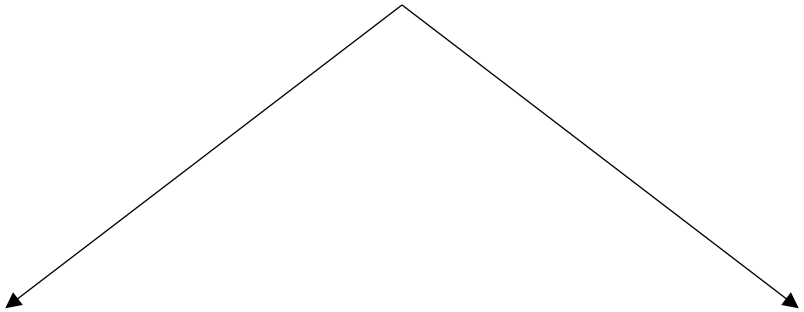


6h at 37 °C



IMPORTANCE OF DIFFUSION KINETICS

Diffusion of solutes takes up to 6 hours to reach the islet core at 37 °C

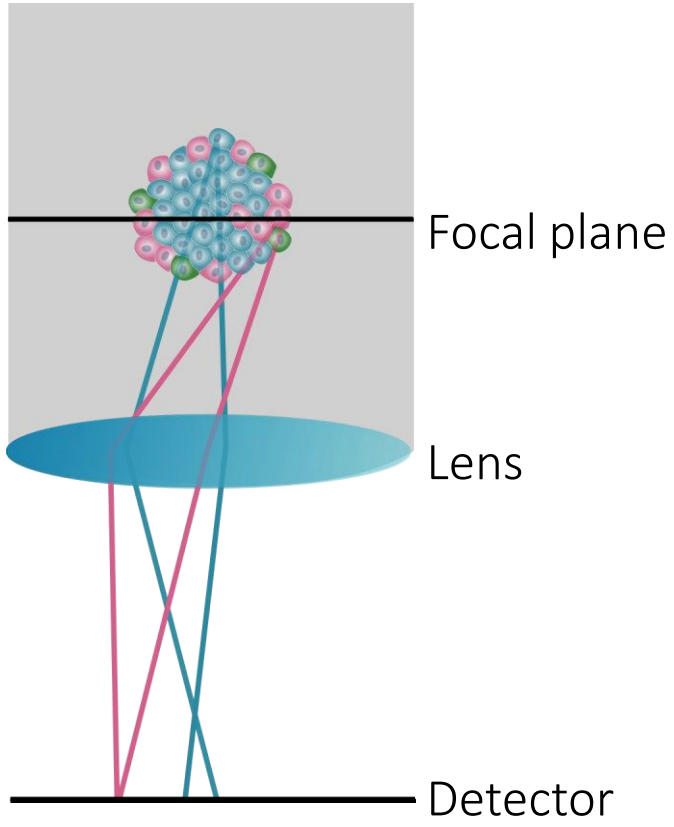


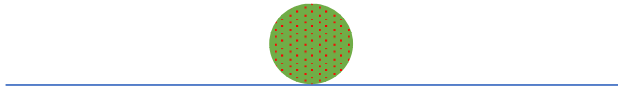
Current viability staining and imaging

Development of new cryopreservation protocols

CURRENT VIABILITY STAINING AND IMAGING

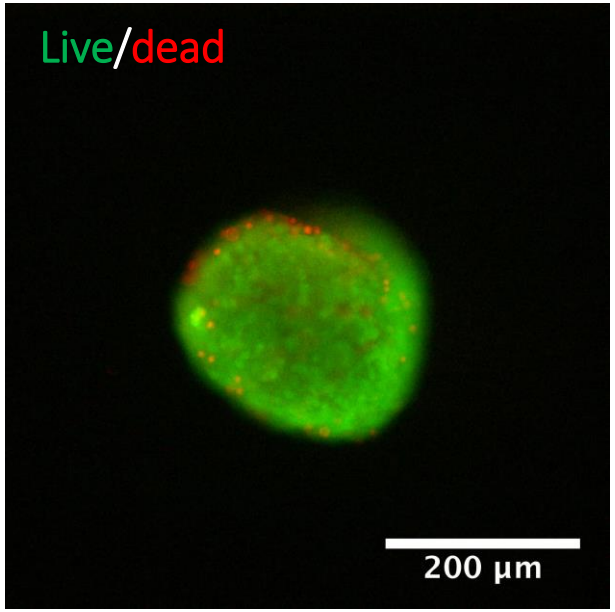
WIDEFIELD

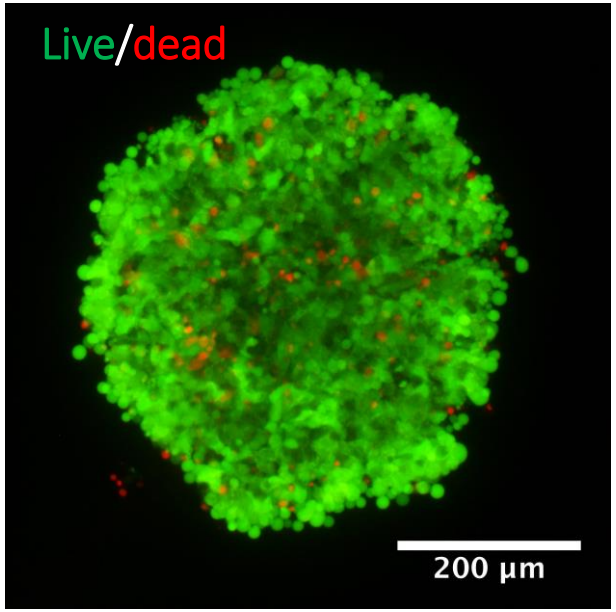




On top of a coverslip

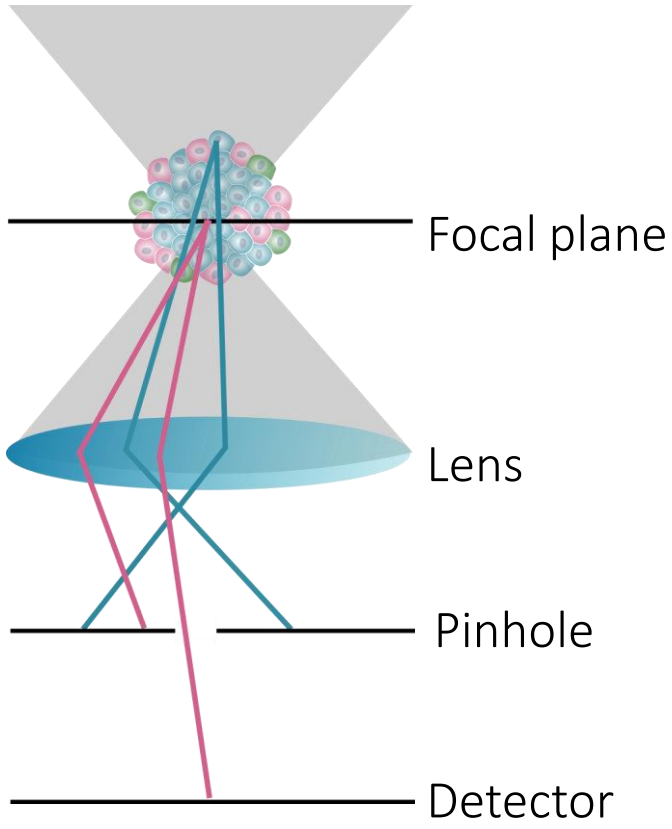
Squashed in between two coverslips

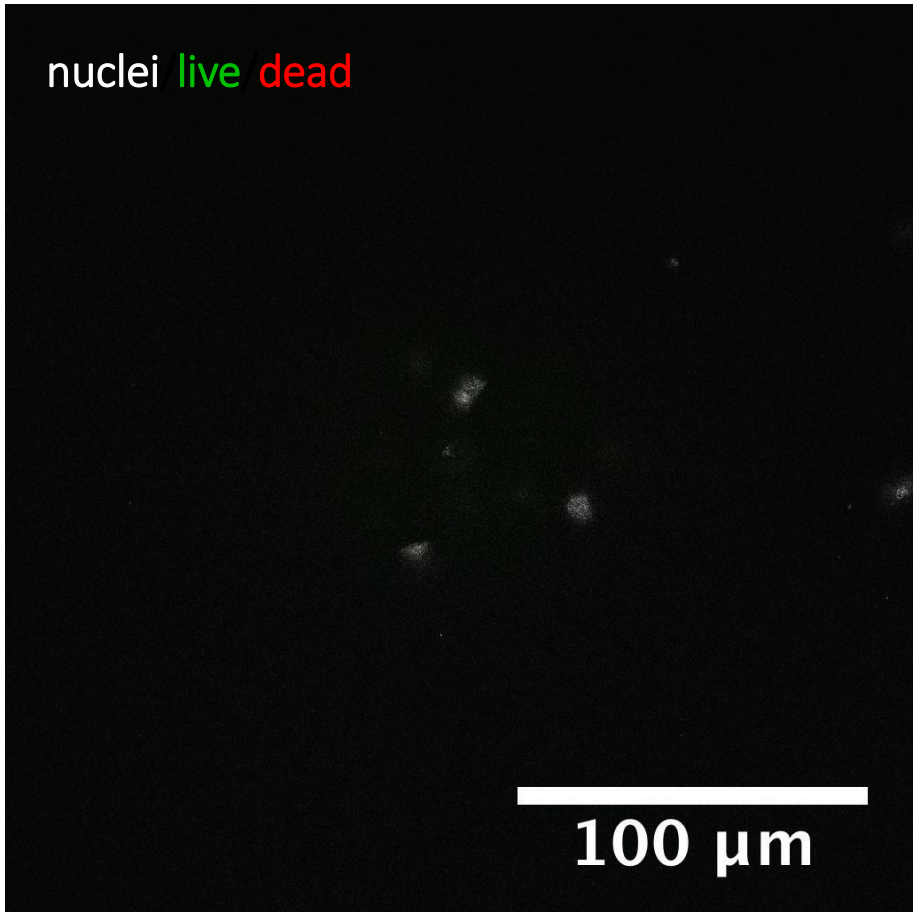




VIABILITY STAINING AND IMAGING

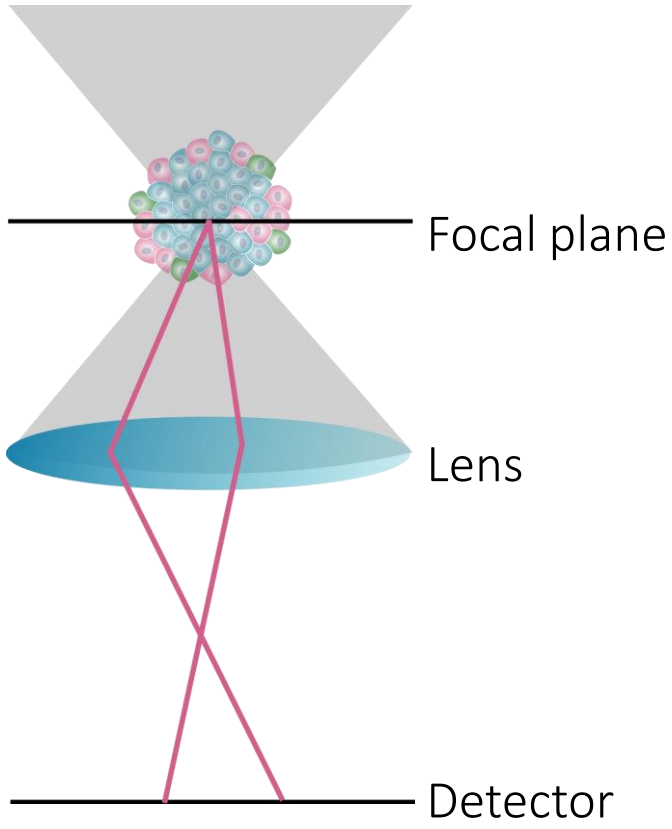
CONFOCAL

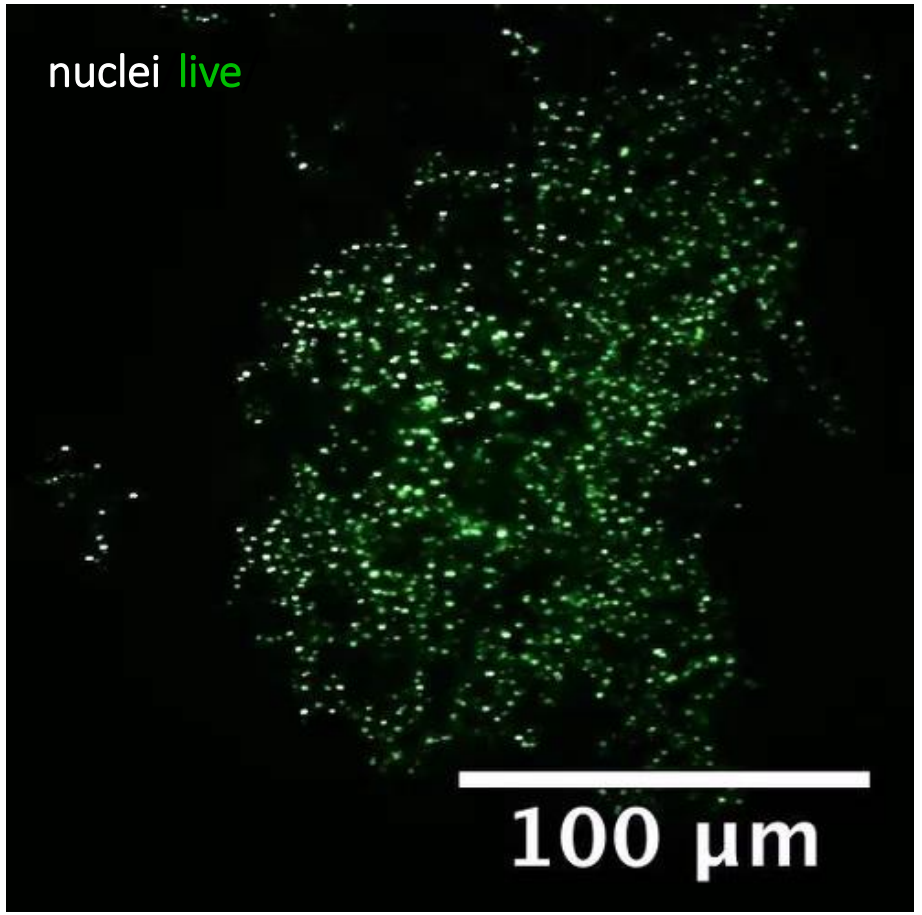




VIABILITY STAINING AND IMAGING

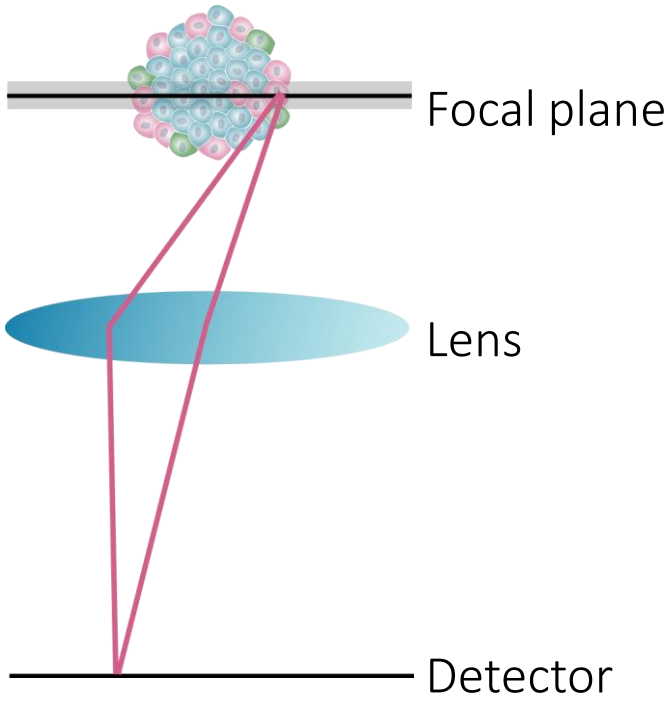
MULTIPHOTON

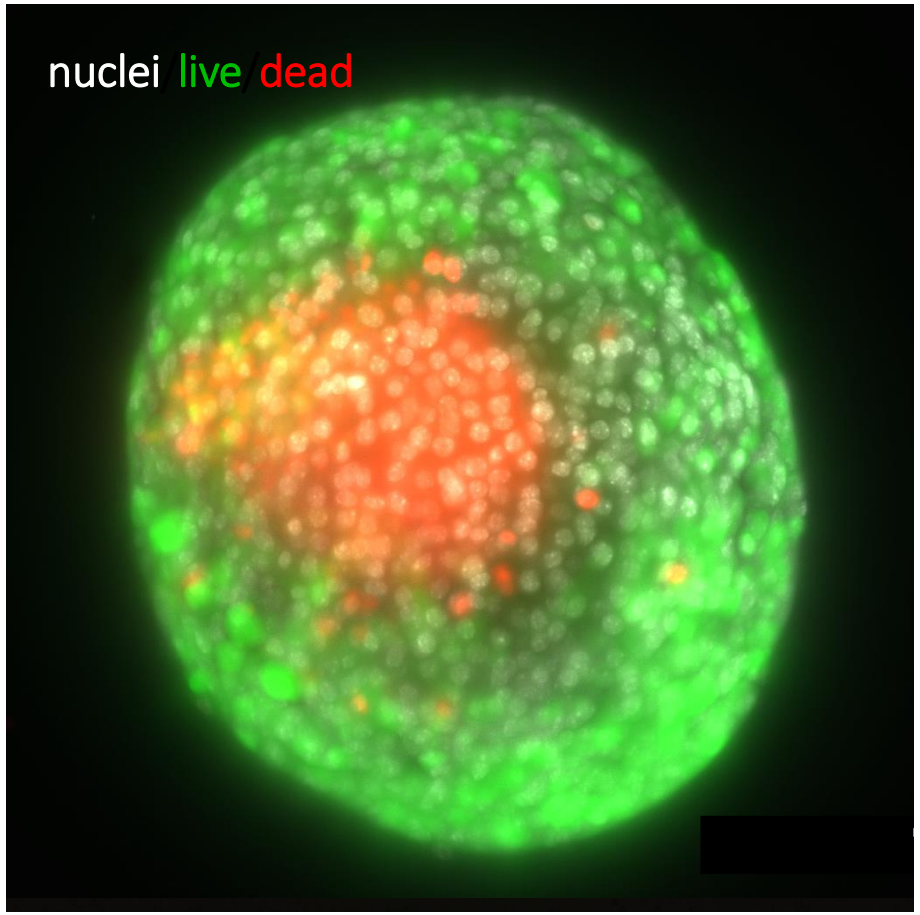




VIABILITY STAINING AND IMAGING

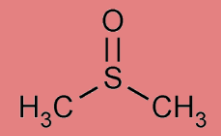
LIGHT-SHEET



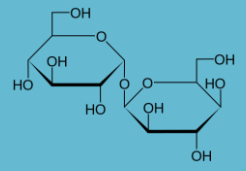


DEVELOPMENT OF NEW CRYOPRESERVATION PROTOCOL

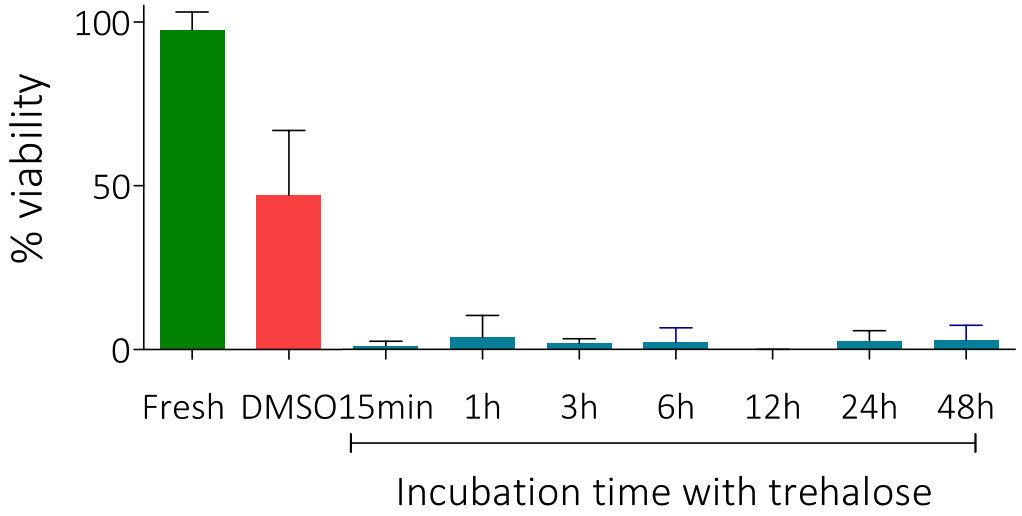
DMSO



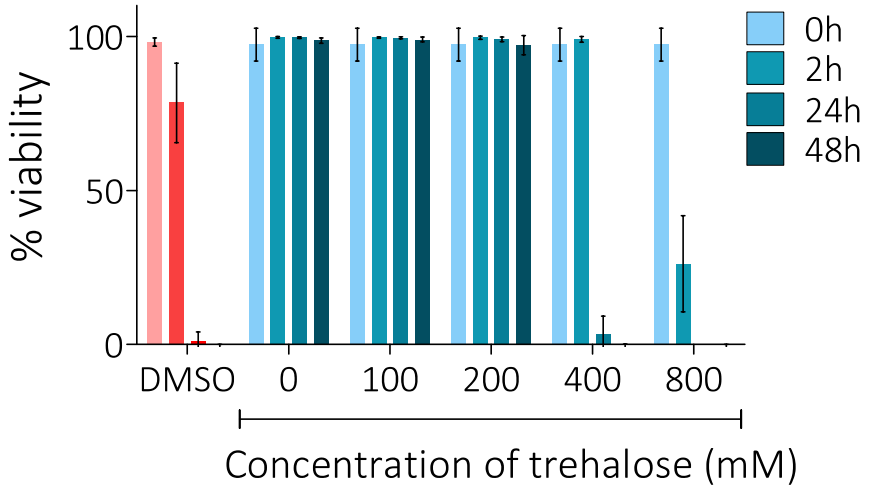
Trehalose



Viability immediately post-thaw

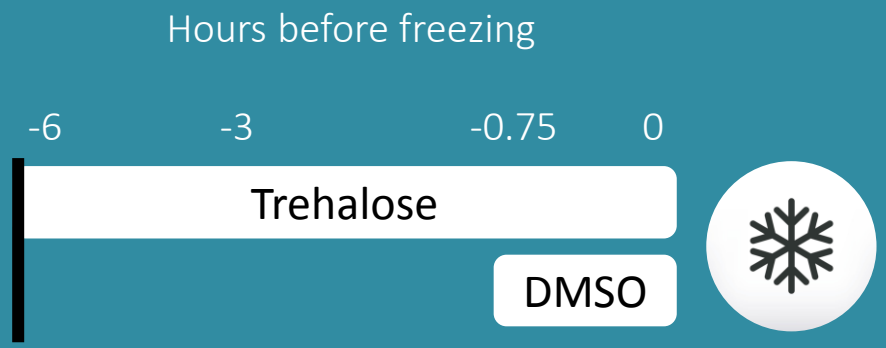


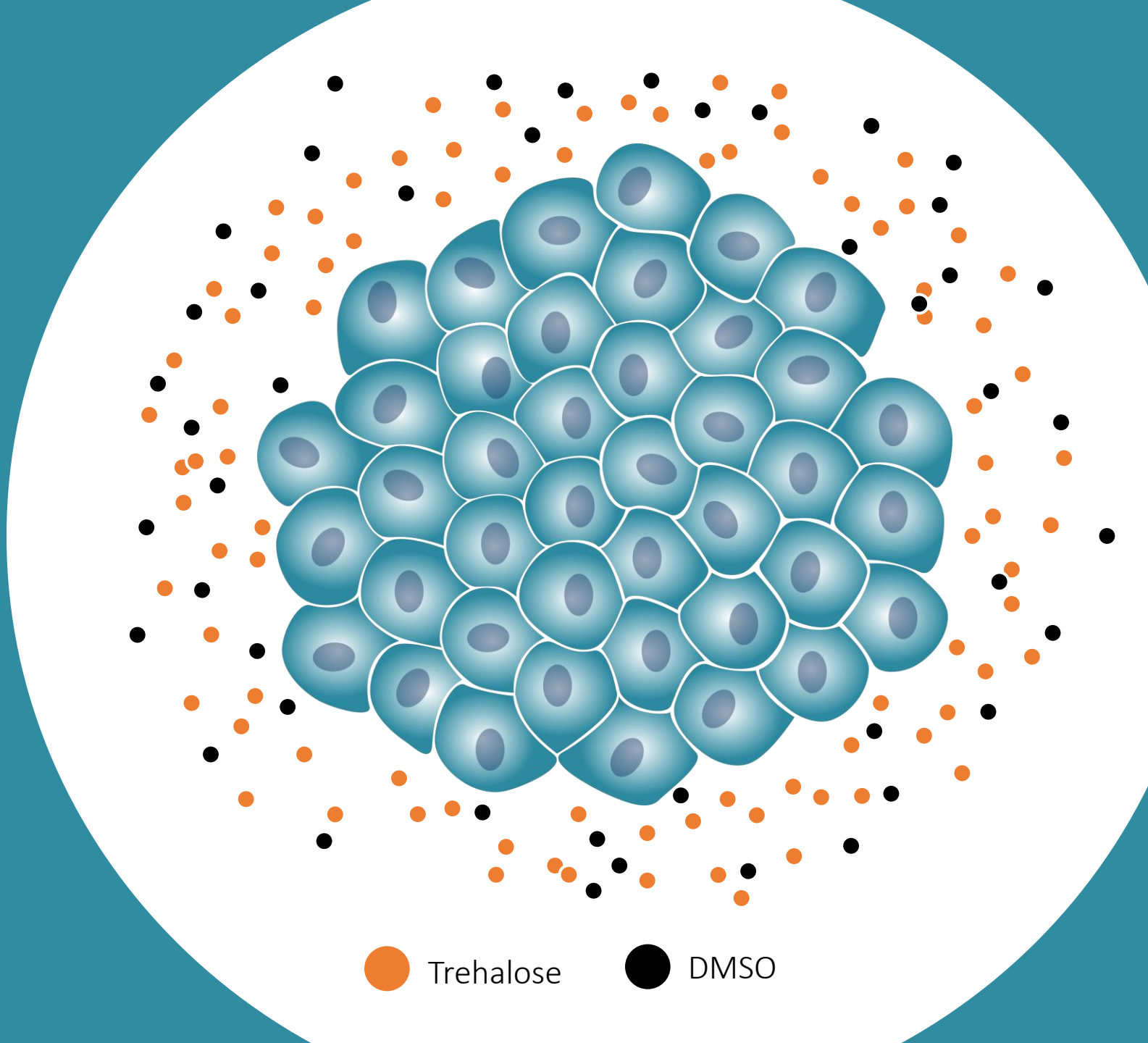
Toxicity of DMSO and trehalose



OUR PROTOCOL

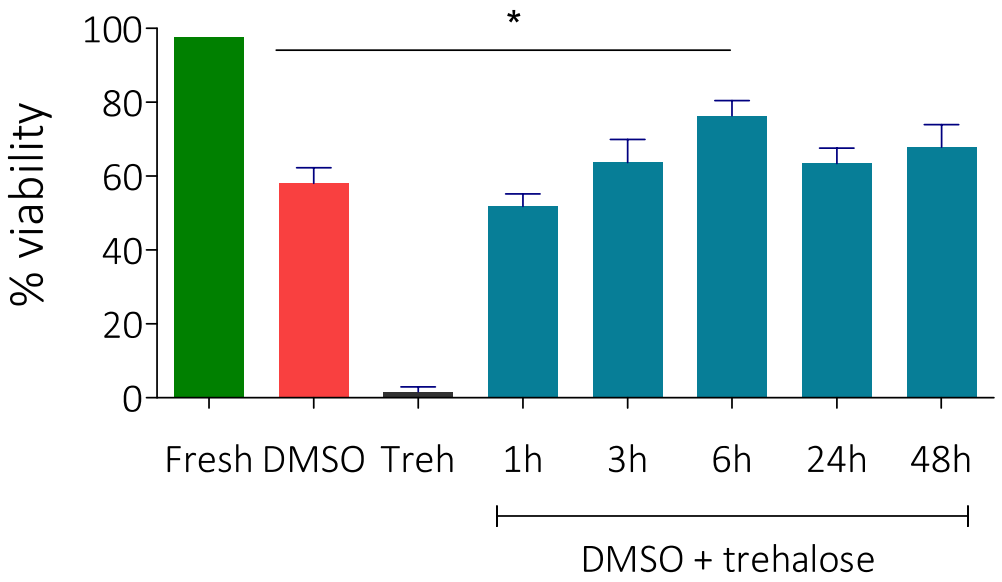
1. Pre-incubation with 200mM trehalose for 1-48 hours
2. DMSO added step-wise in the last 45 minutes
3. Slow-freezing in programmable freezer



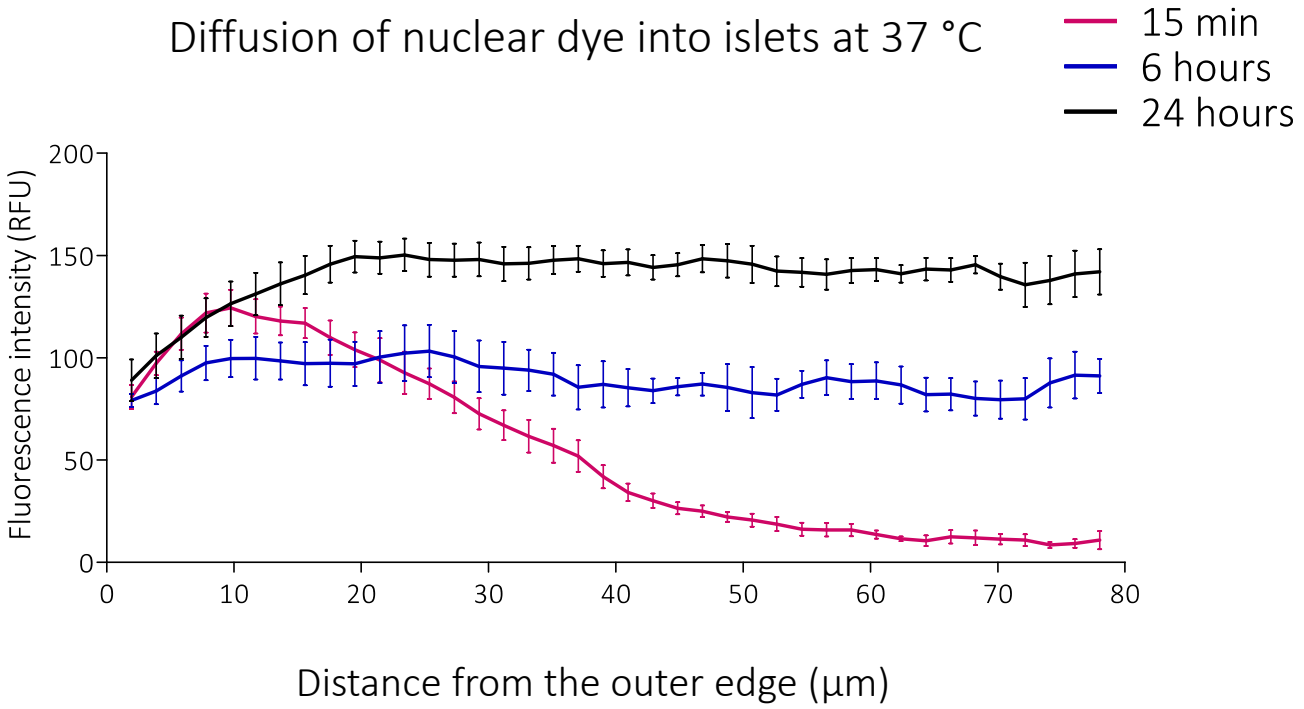


DEVELOPMENT OF NEW CRYOPRESERVATION PROTOCOL

Viability immediately post-thaw



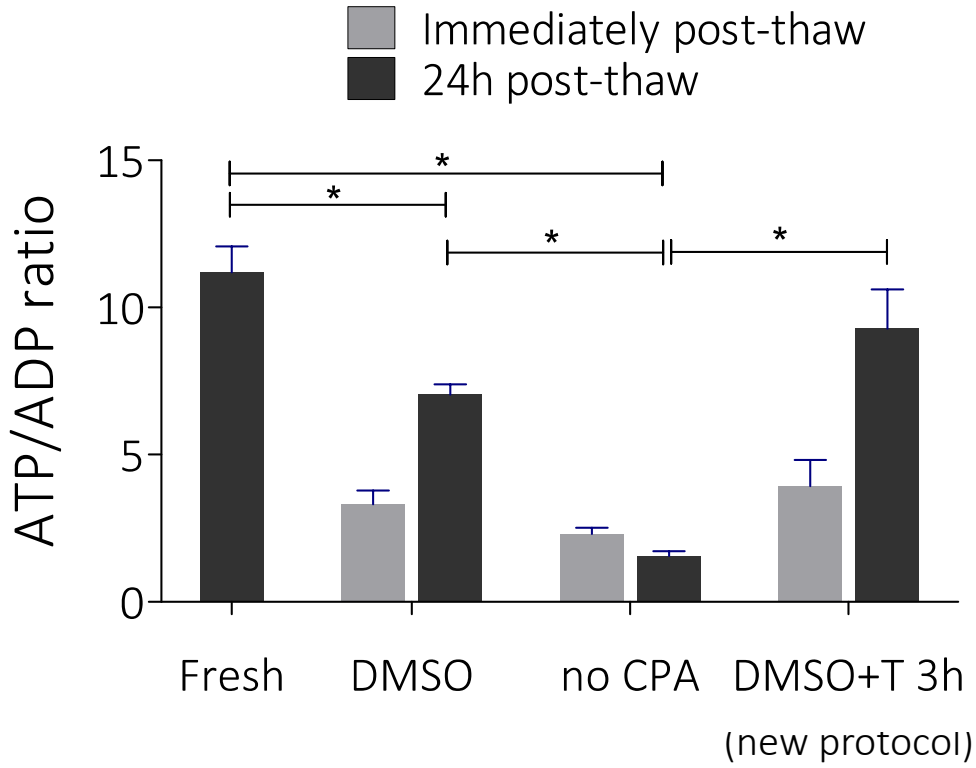
Diffusion of nuclear dye into islets at 37 °C



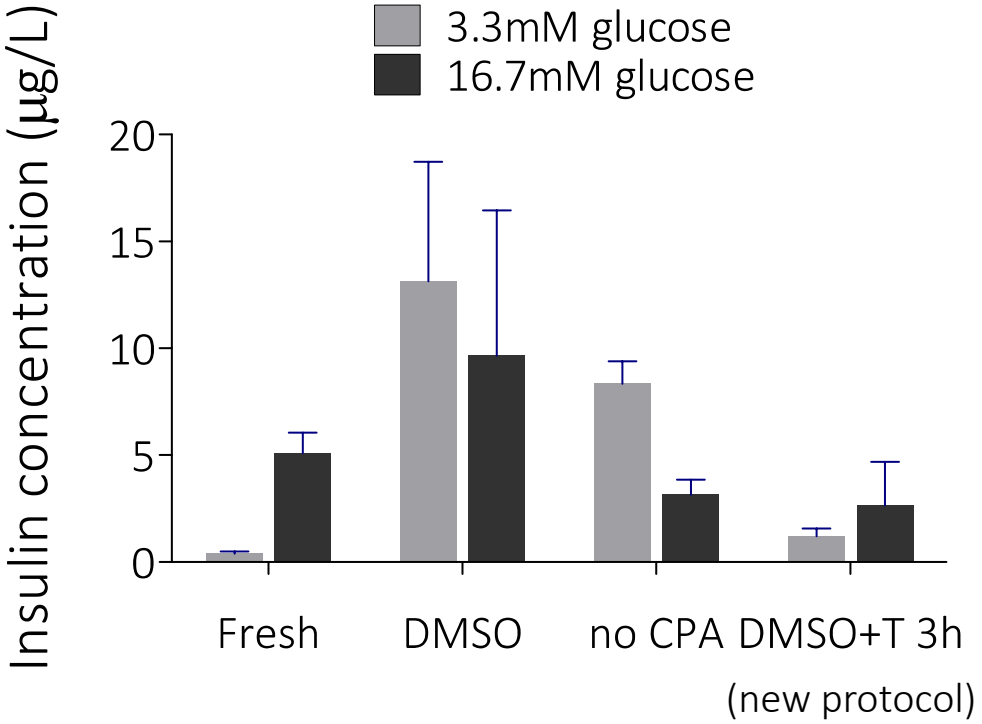
* $p \leq 0.05$

FUNCTIONAL ASSAYS

1 ATP/ADP ratio



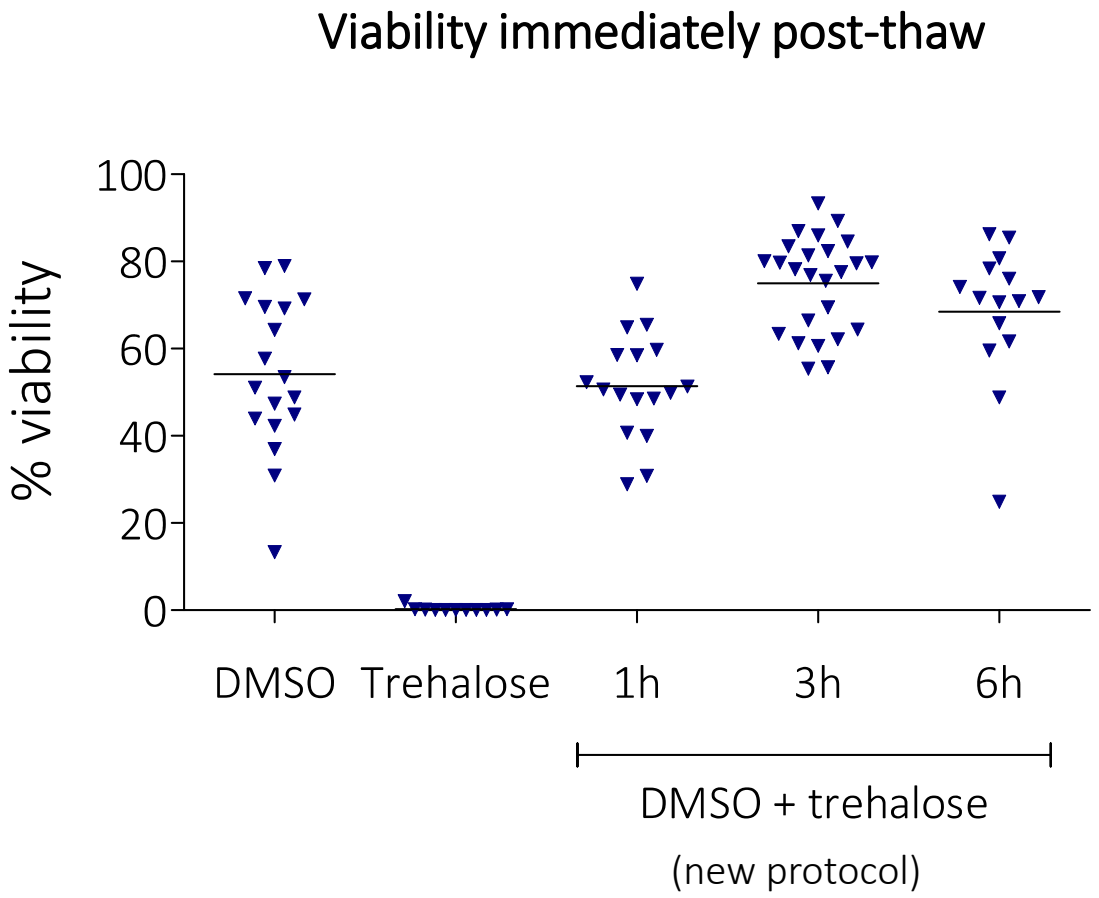
2 Glucose-stimulated insulin secretion

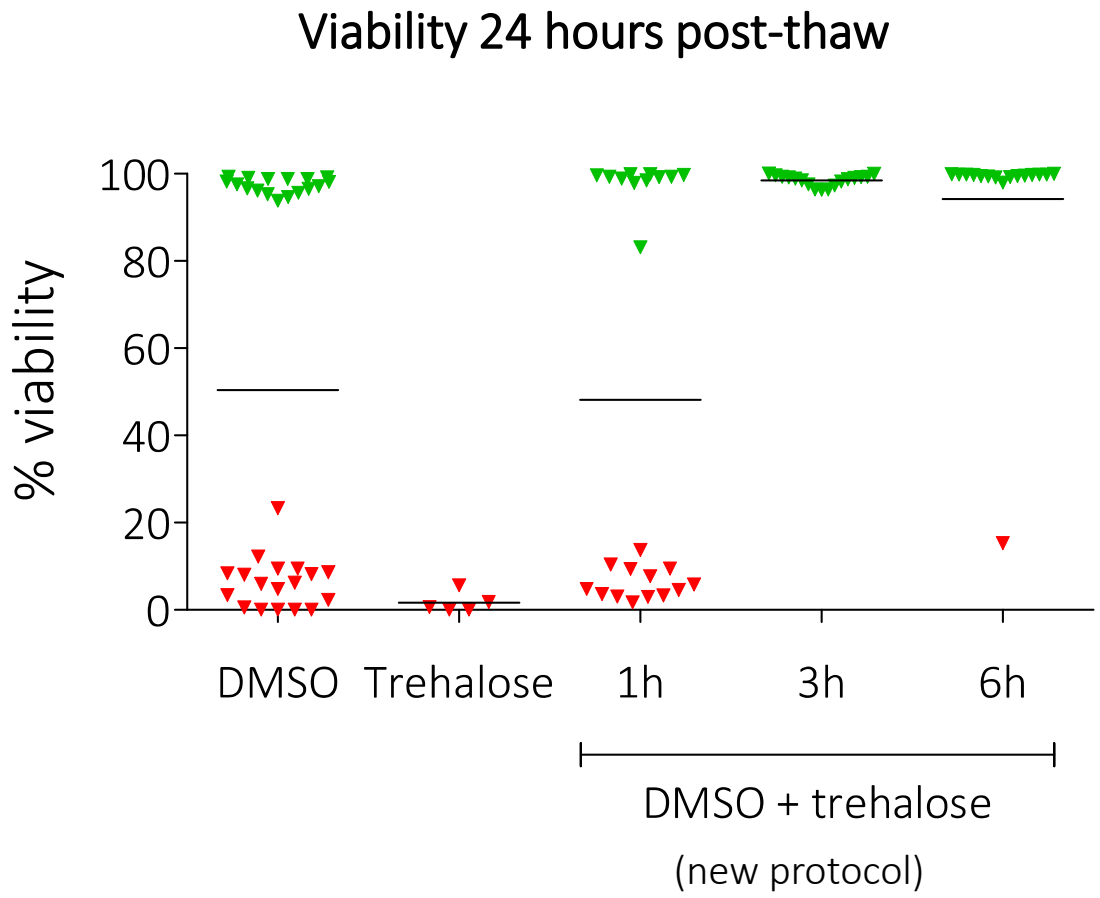


3 Transplantation under mouse kidney capsule

* $p \leq 0.05$

BENEFITS OF NEW CRYOPRESERVATION PROTOCOL





CONCLUSIONS

1. Diffusion into islet core increases at 37 °C but still takes up to 6 hours

2. Current viability assessment methods are sub-optimal and disregard islet core

3. Pre-incubation of islets with non-toxic cryoprotectants can improve cryosurvival

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ACKNOWLEDGEMENTS

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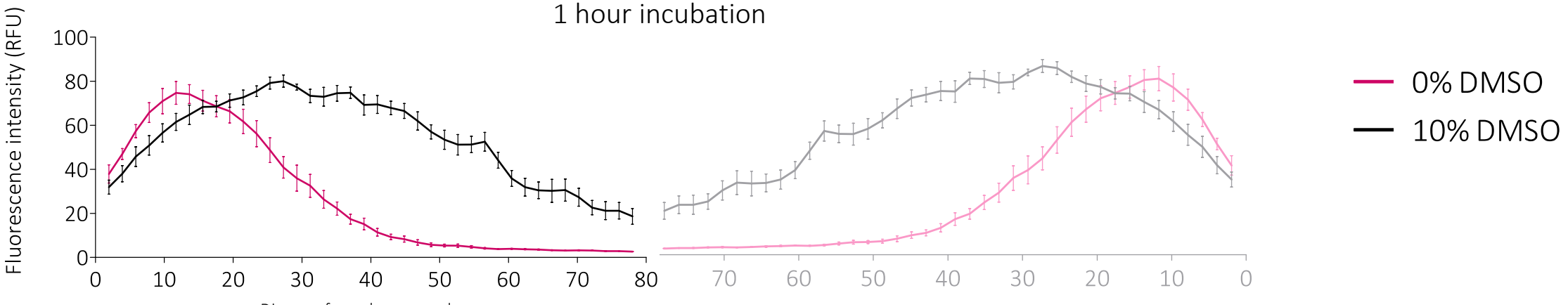
SUPPLEMENTARY INFORMATION: DIFFUSION STUDIES

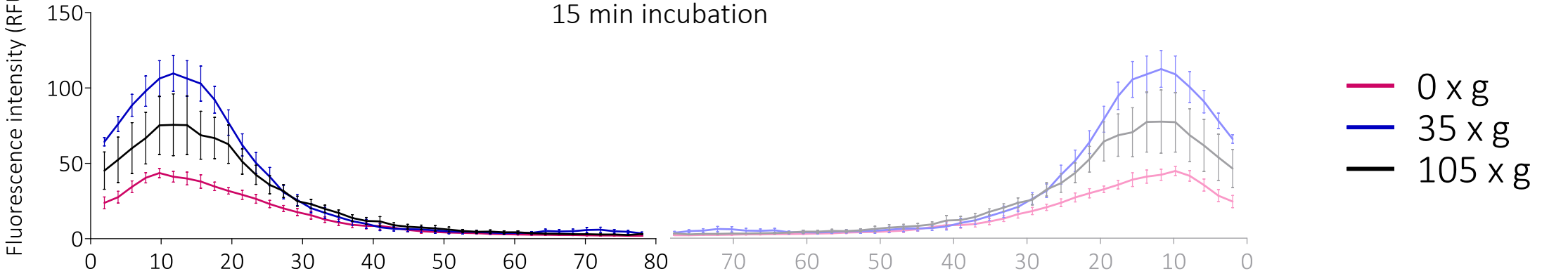
Adding DMSO

1 hour incubation

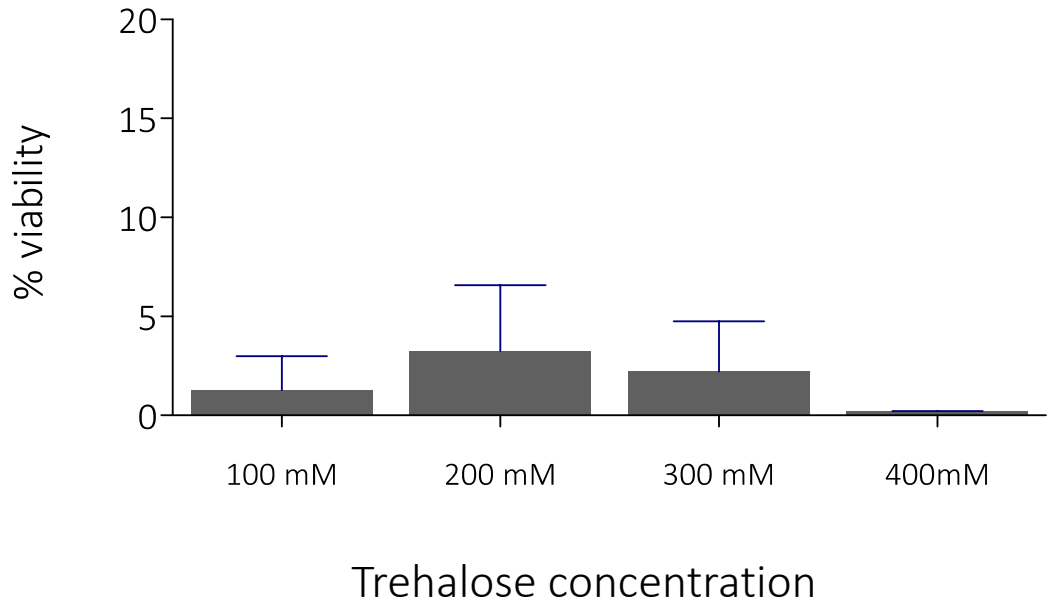


Increasing hydrostatic pressure

15 min incubation



SUPPLEMENTARY INFORMATION: TREHALOSE CONCENTRATION



SUPPLEMENTARY INFORMATION: HUMAN ISLET CRYOPRESERVATION