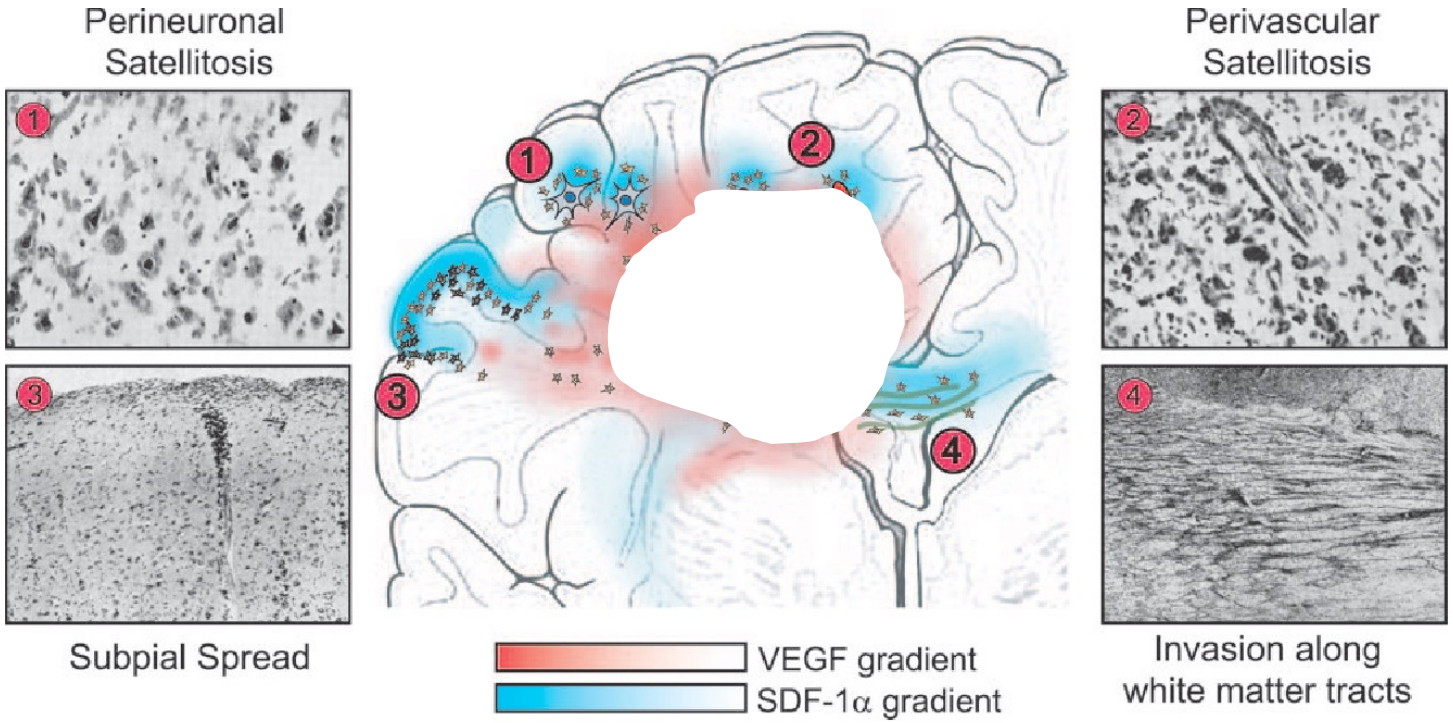
METABOLISM IN CANCER – NICE 2023

How glioblastoma cells adapt their metabolism when colonizing the brain ?

Thomas Daubon, PhD - CNRS Research Director
Group leader « GBmetabo group »
CNRS UMR5095, University of Bordeaux – France

Glioblastoma (GB) : a highly heterogeneous and invasive tumour

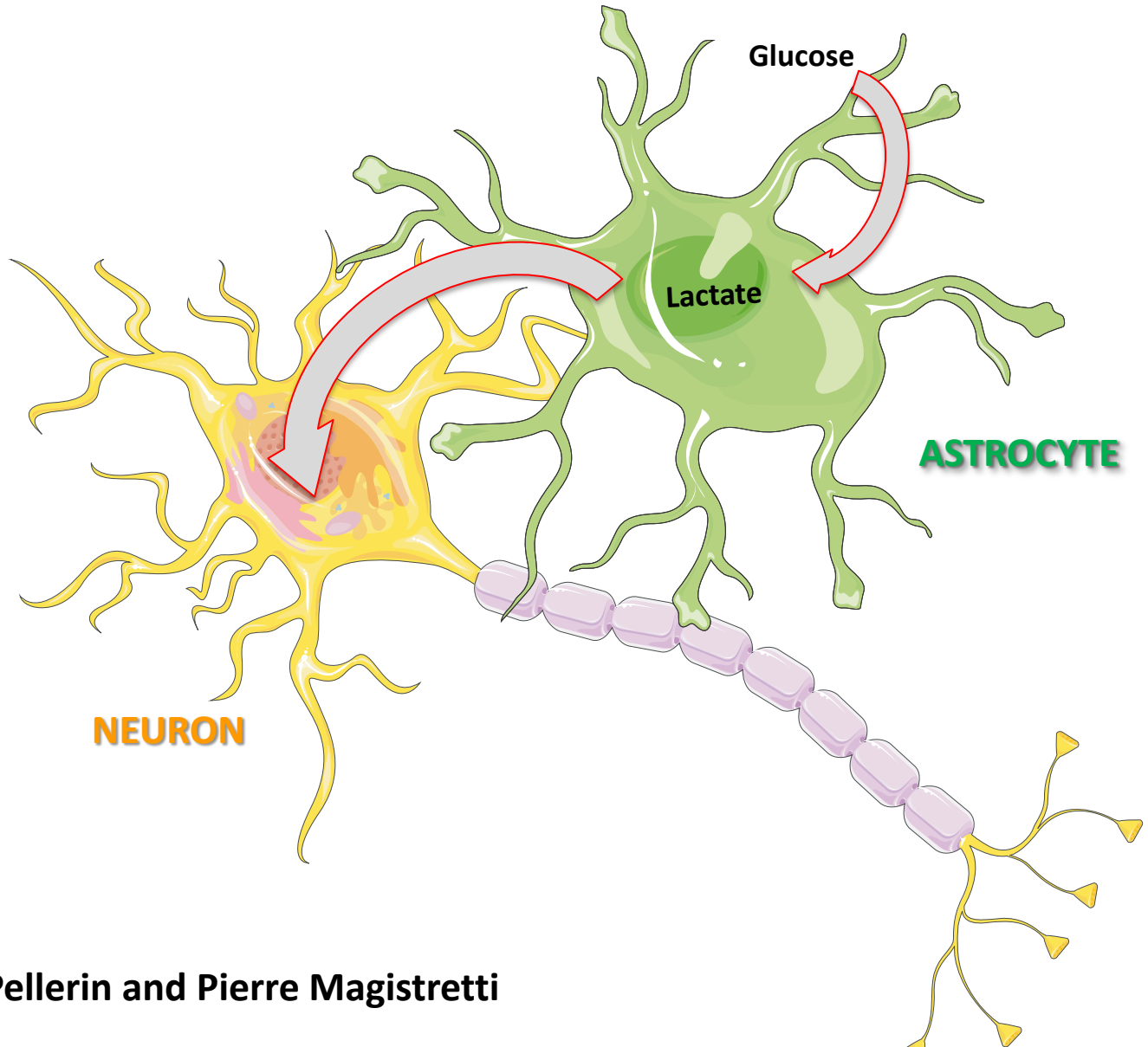


Role of TGFβ1/thrombospondin

- Daubon Nat Com 2019, NeuroOnco Adv 2019
- Joseph, Magaut et al (Daubon/Miletic groups) NeuroOnco 2022

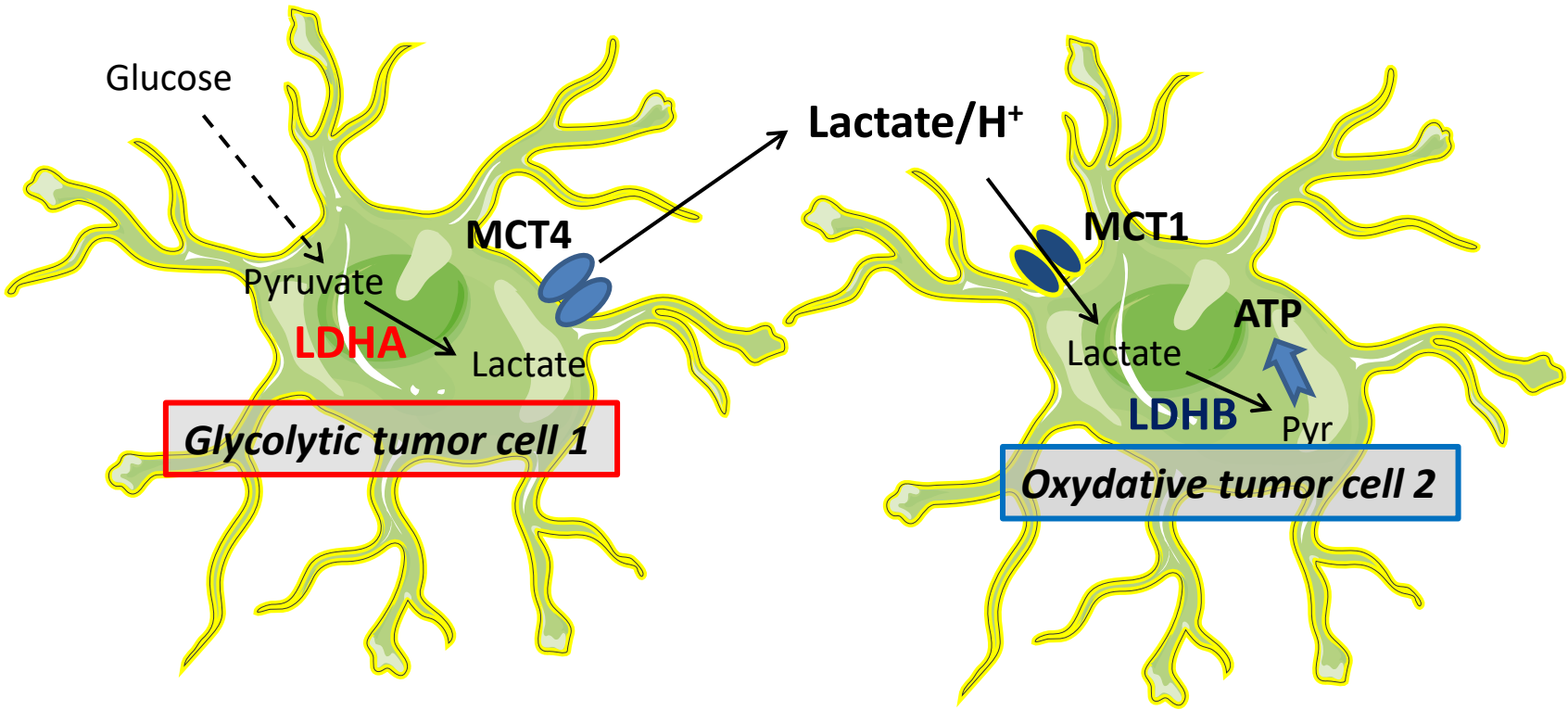
→ Invasive cells are the seeds of recurrent GB.

From a neuron-astrocyte lactate shuttle concept …

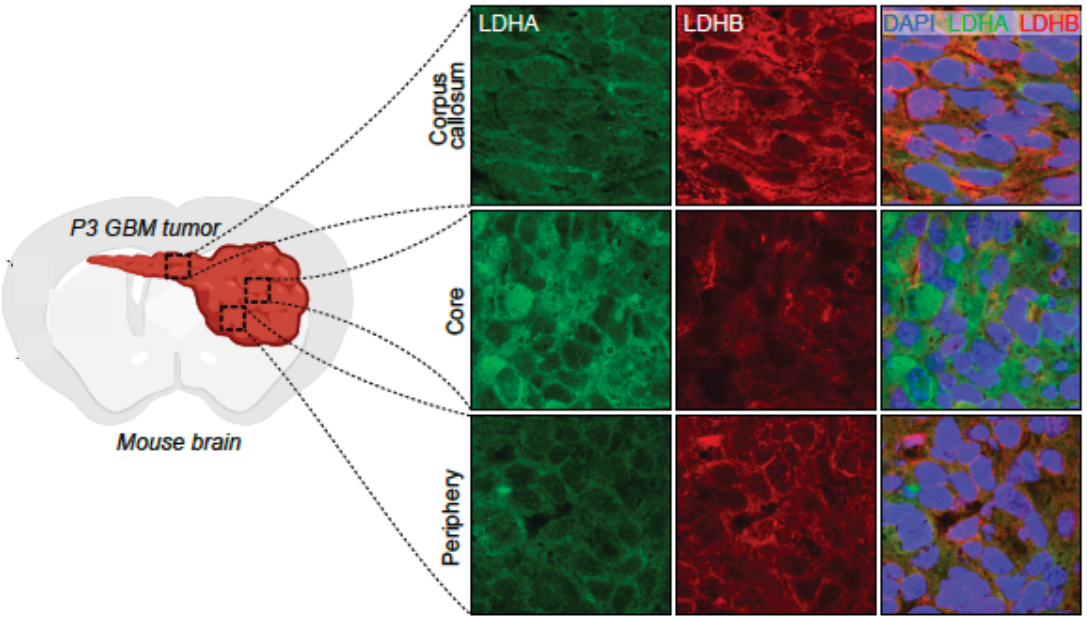


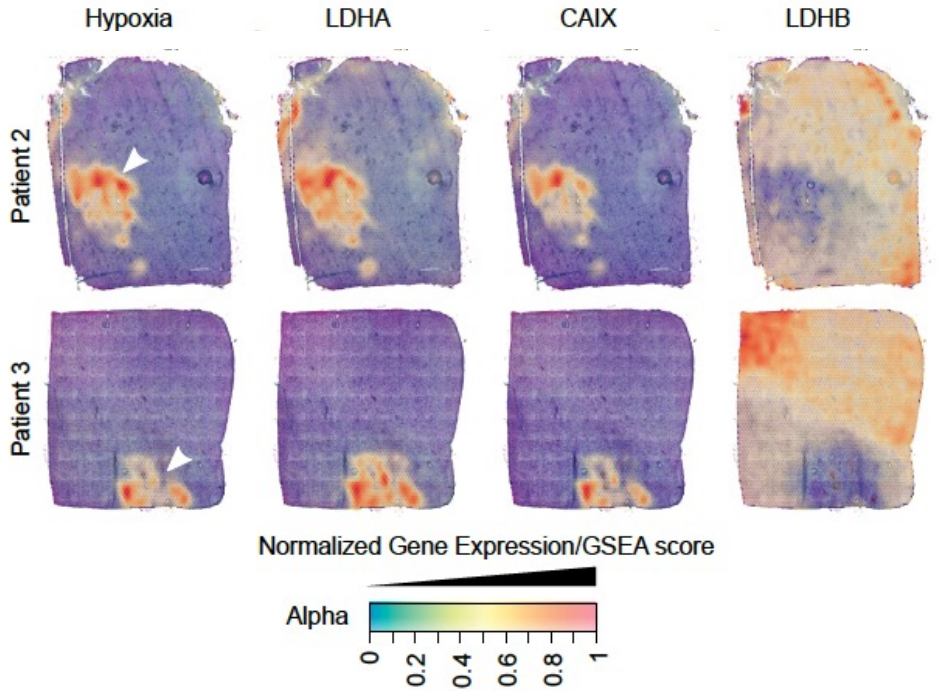
Work of Luc Pellerin and Pierre Magistretti

… to neuro-oncology with glioblastoma metabolic symbiosis ?



Determining LDHA and LDHB regional expression by IHC and spatial transcriptomics in xenograft derived from patient stem-like cells



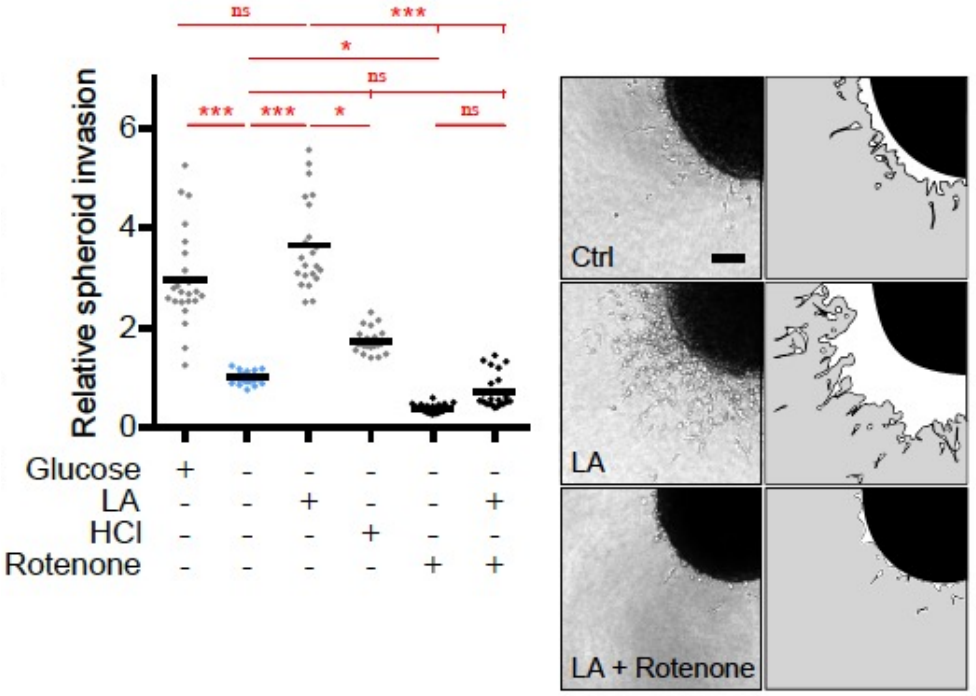


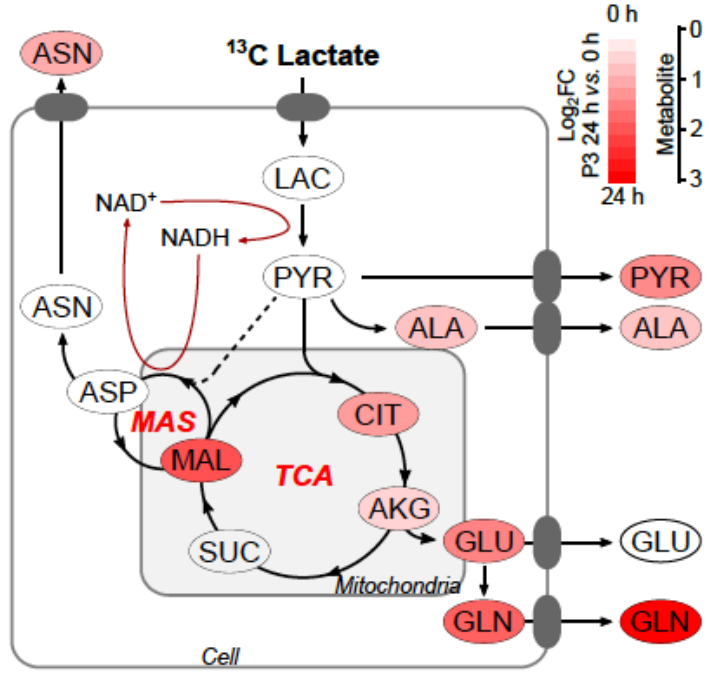
Collaboration with DH Heiland/V Ravi/K Joseph ; data extracted from Ravi et al Cancer Cell 2022

→ **LDHA is expressed in hypoxic area and some invasive cells while LDHB is highly expressed in invasive areas.**

Lactate itself fuels TCA cycle for a full cell respiration, triggering invasion

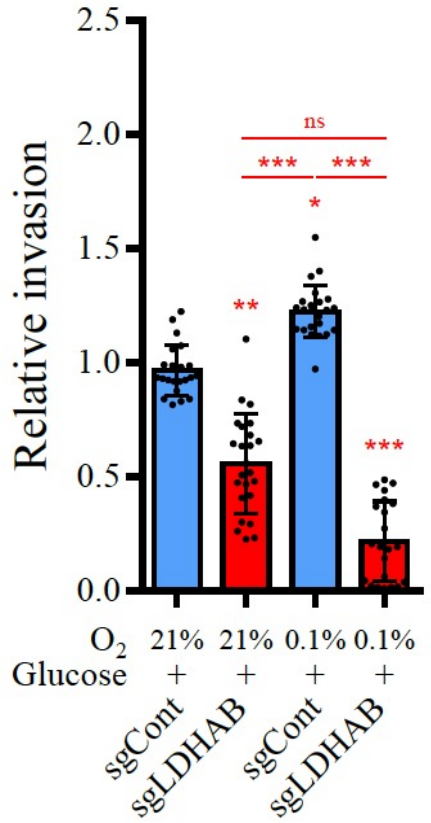
Glucose/Glutamine-free medium and complemented with Lactate

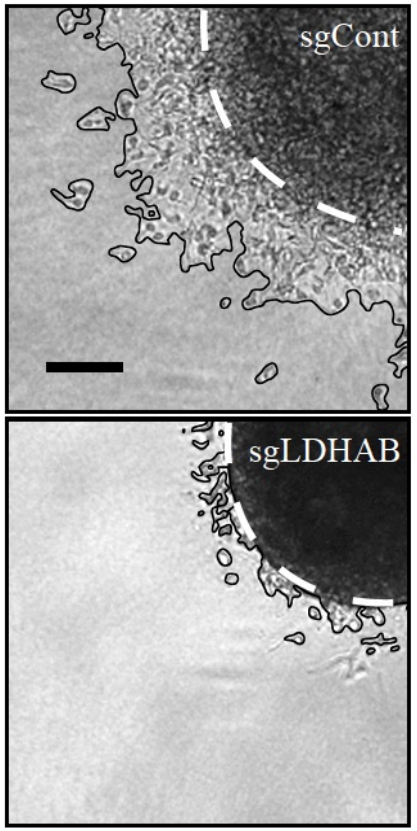


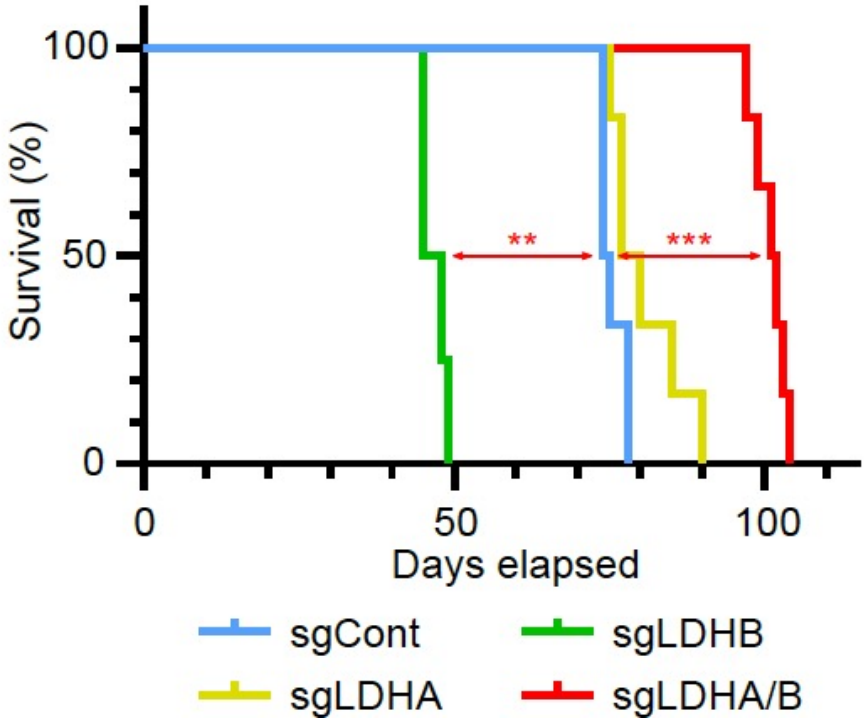


→ **Lactate is retroconverted into pyruvate to full TCA cycle in absence of glucose : importance of LDHB ?**

LDHA/B KO induces an increase in mouse survival





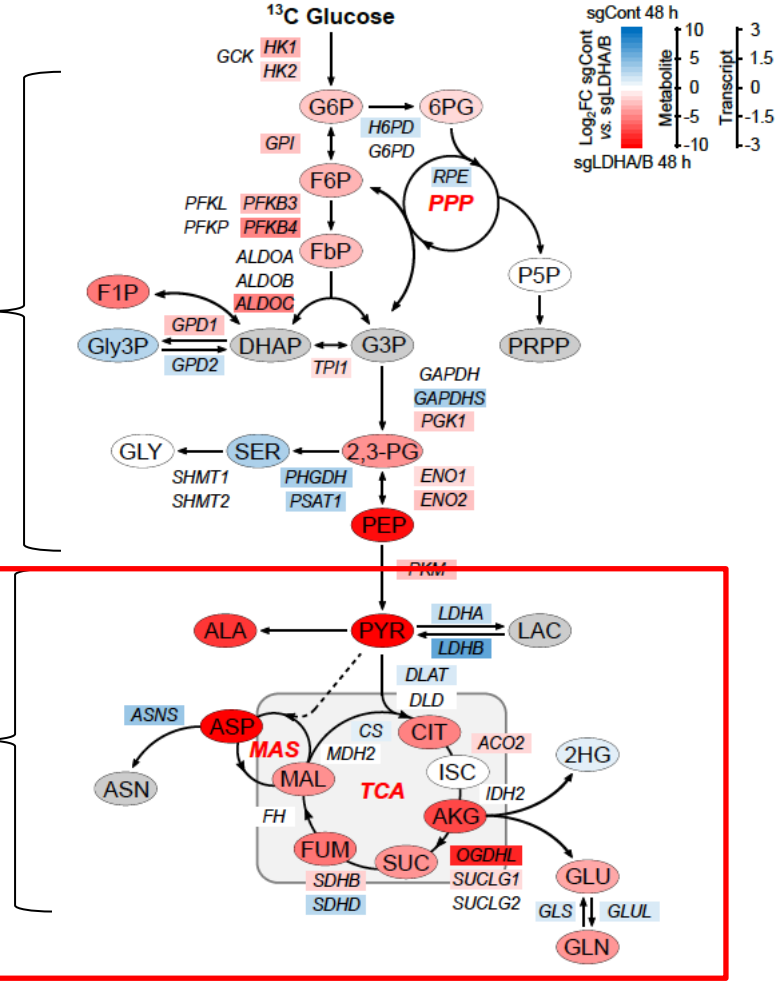


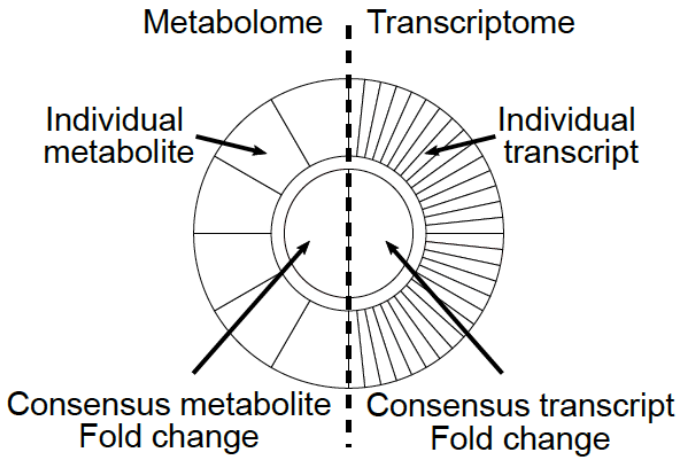
RNAsequencing and metabolomics define metabolic profiles of LDHA/B KO cells

Comparison of sgCont cells vs sgLDHA/B after 48 hours in 0.1% O2

Glycolysis

TCA cycle and associated amino acids

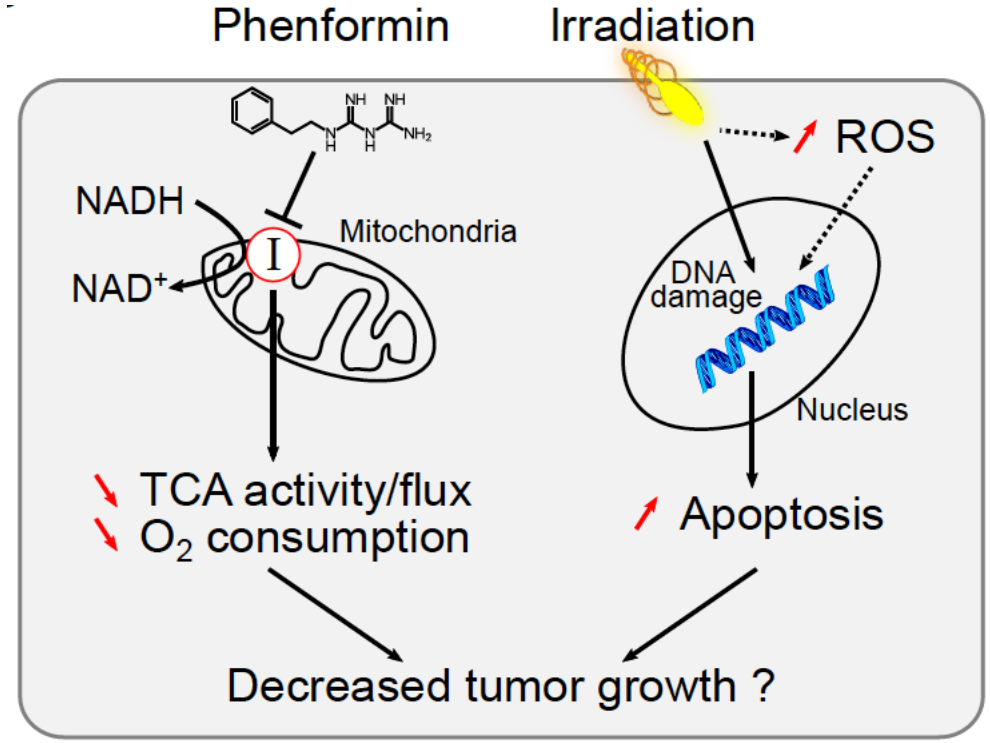


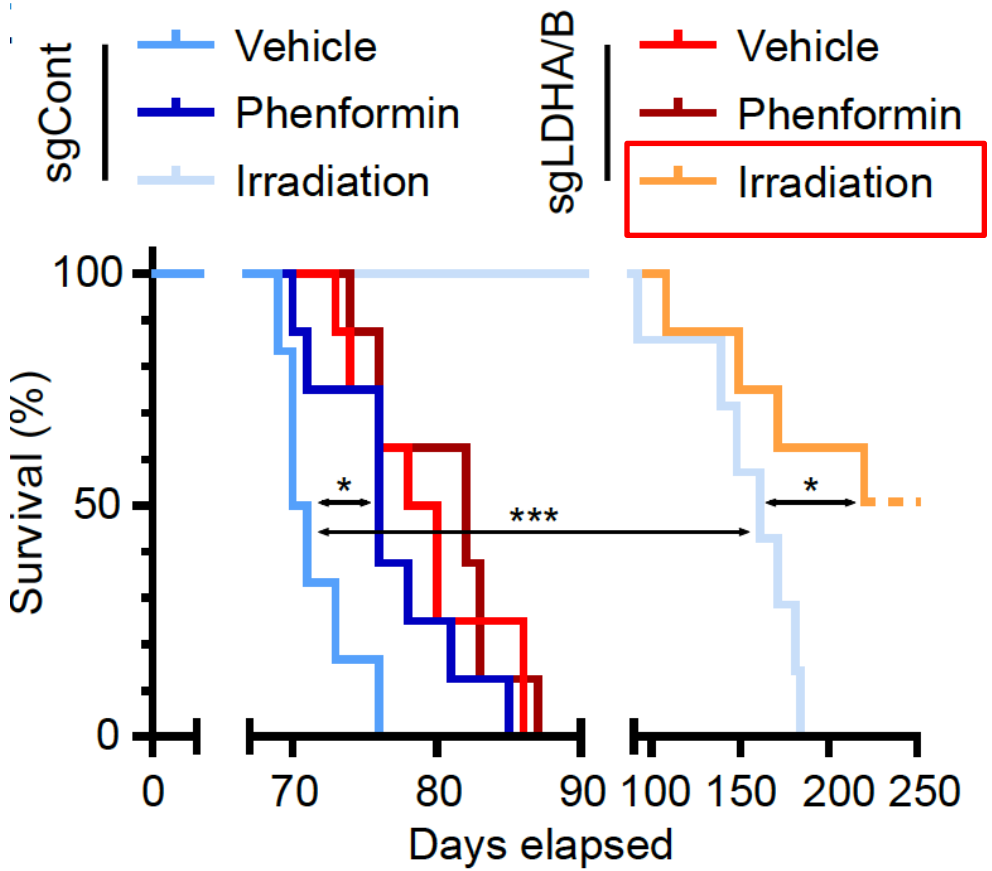


→ ***Publication DIMet in revision (Galvis et al)***
https://github.com/cbib/DIMet

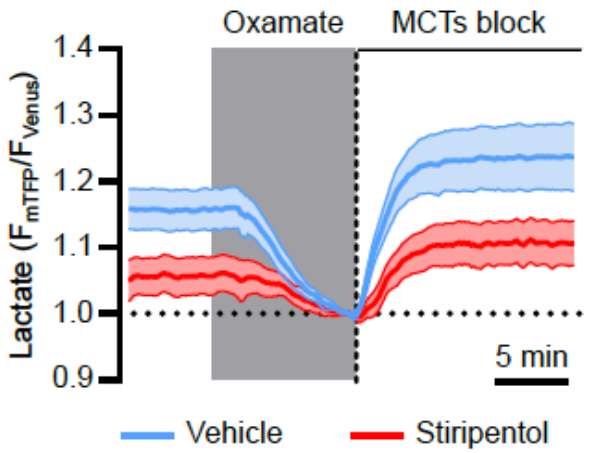
→ **Even under 0.1% O_2, LDHA/B KO cells rewire their metabolism through oxydative phosphorylation**

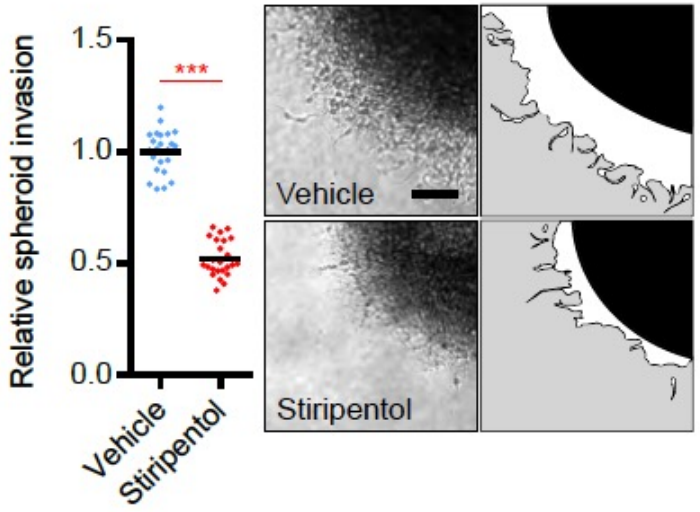
Irradiation of LDHA/B KO tumors leads to increase of mouse survival

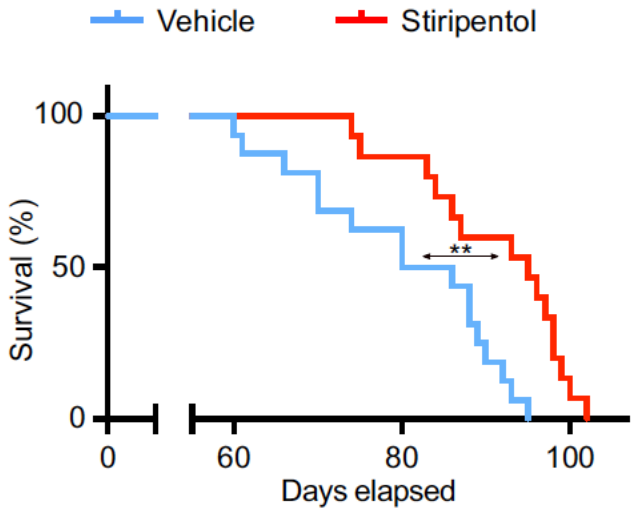


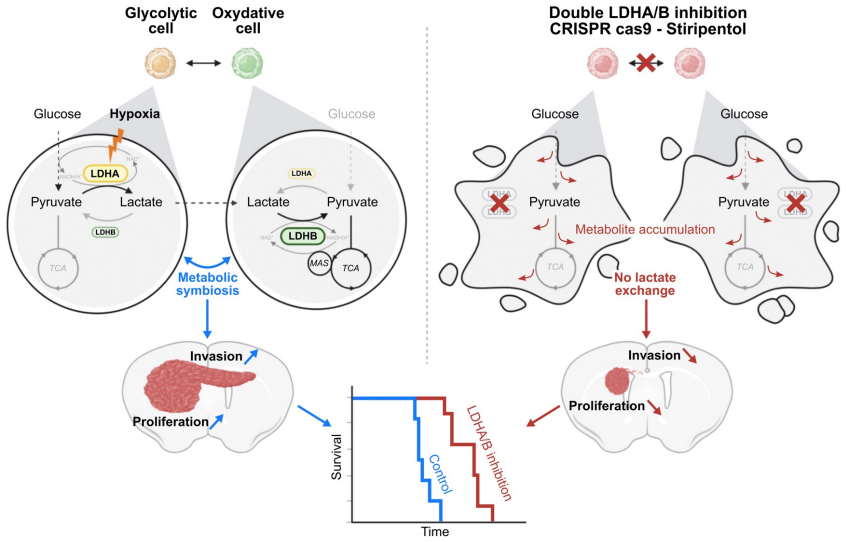


Antiepileptic drug inhibiting LDH activity and cell respiration led to increased mouse survival







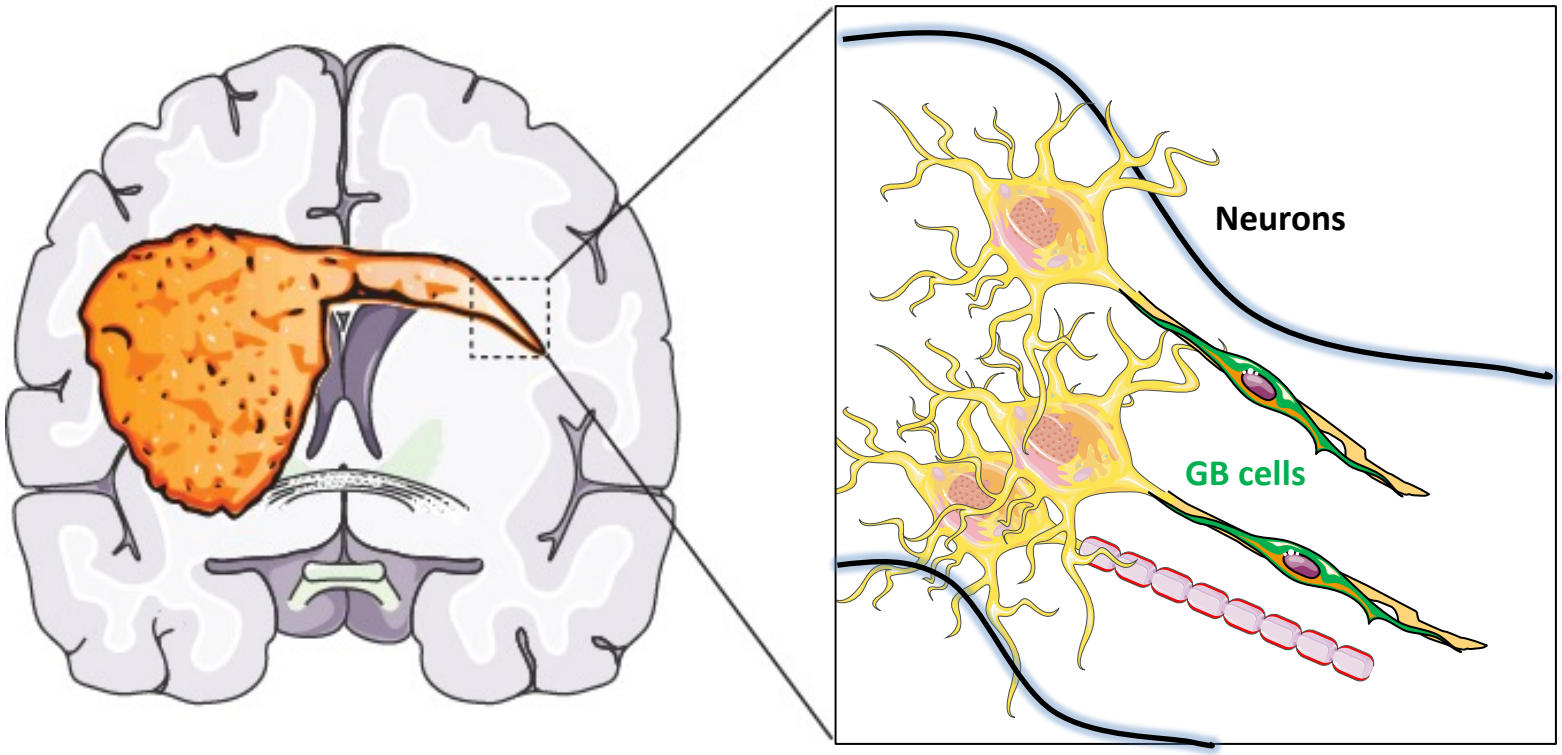


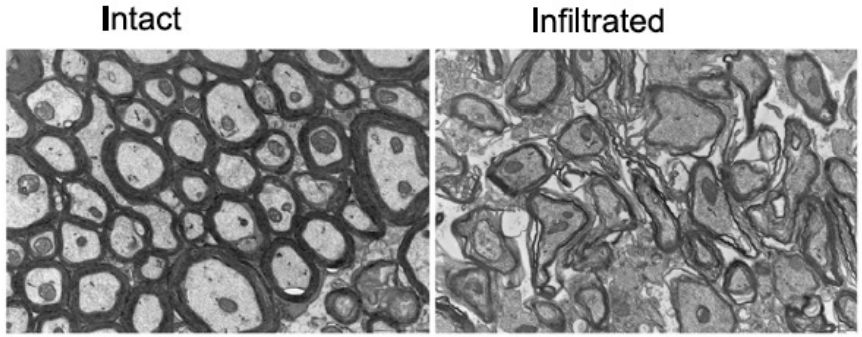
Guyon et al., Nov22- EMBO Mol Med

+ role of MPST in GB invasion

Saurty-Seerunghen MS, Daubon T et al Cell Death Dis. 2022

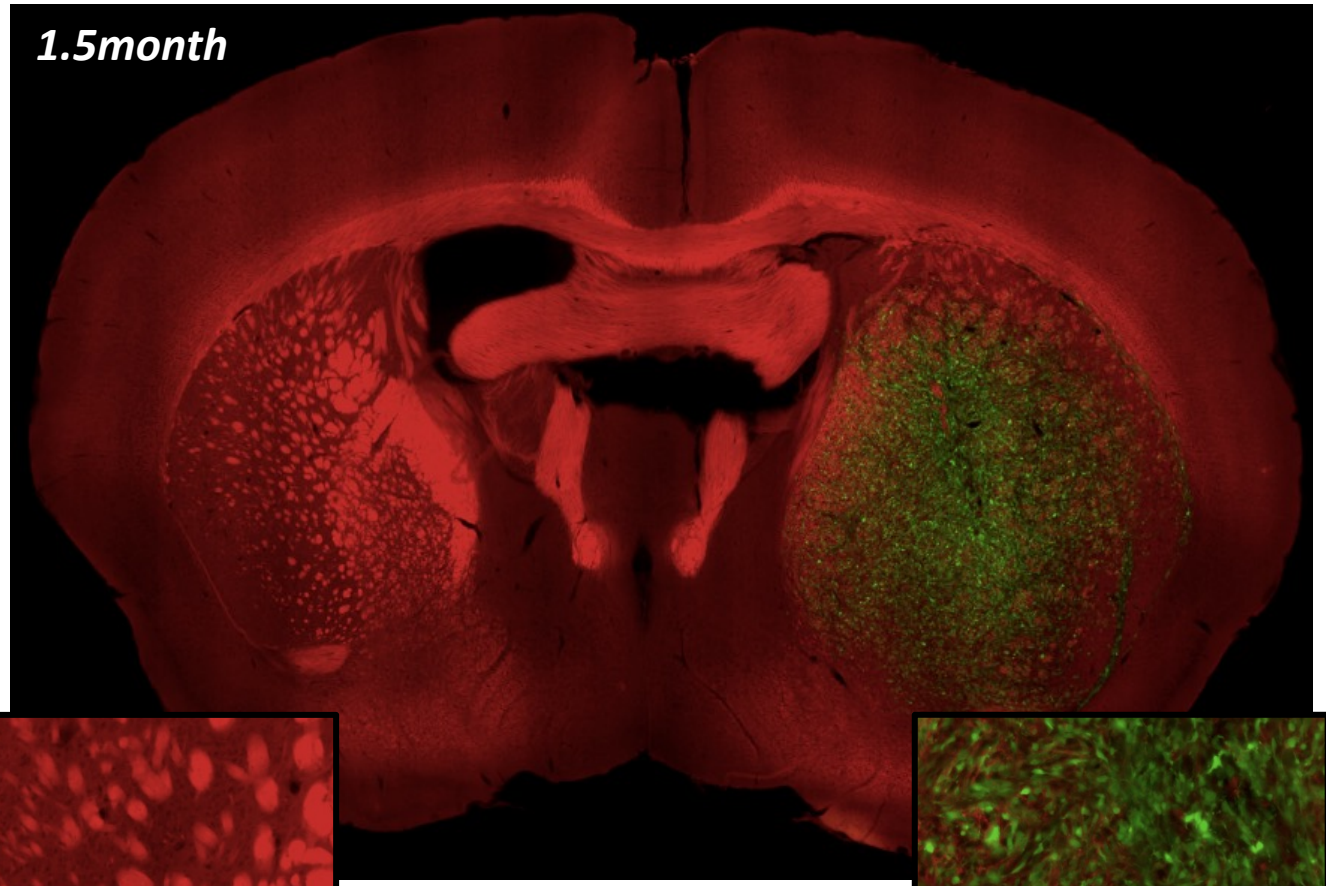
How tumor cells modulate their metabolism during perineural invasion ?

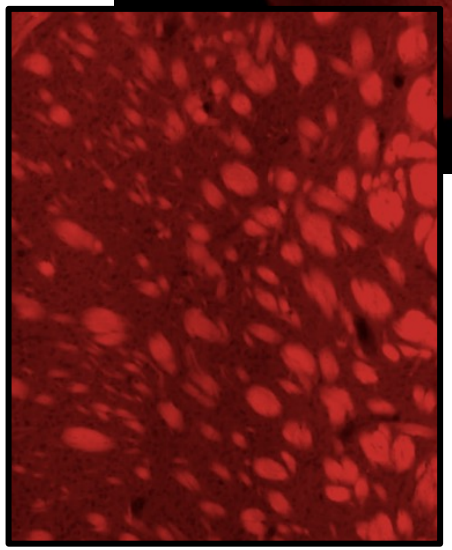


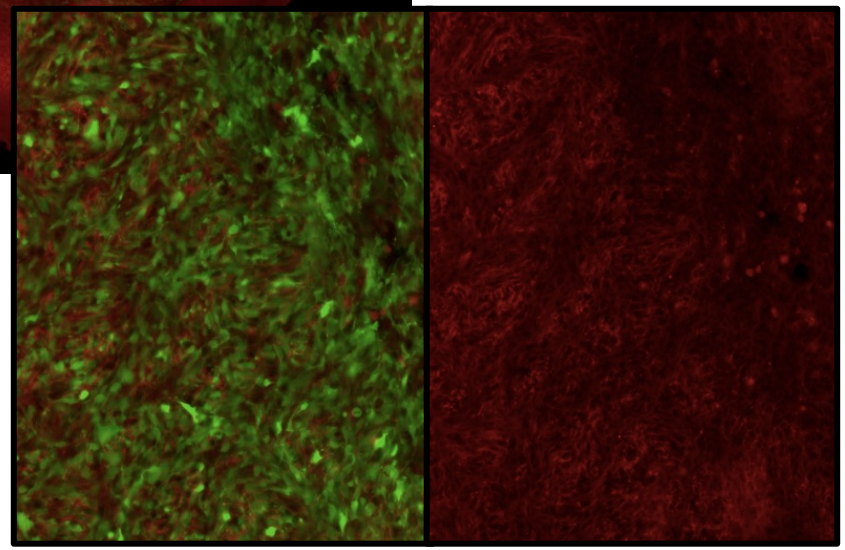


Brooks et al Nat Com 2021

Destruction of myelin sheath in striasomes





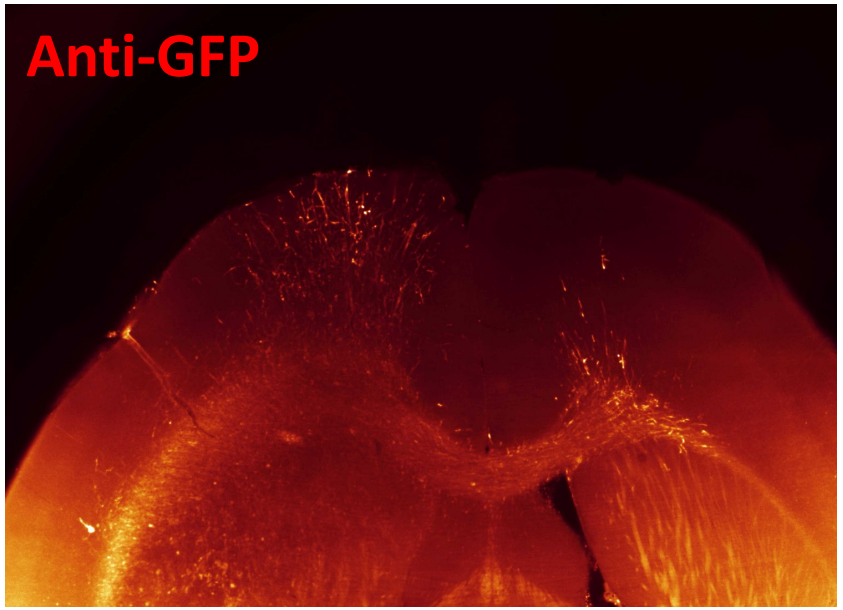


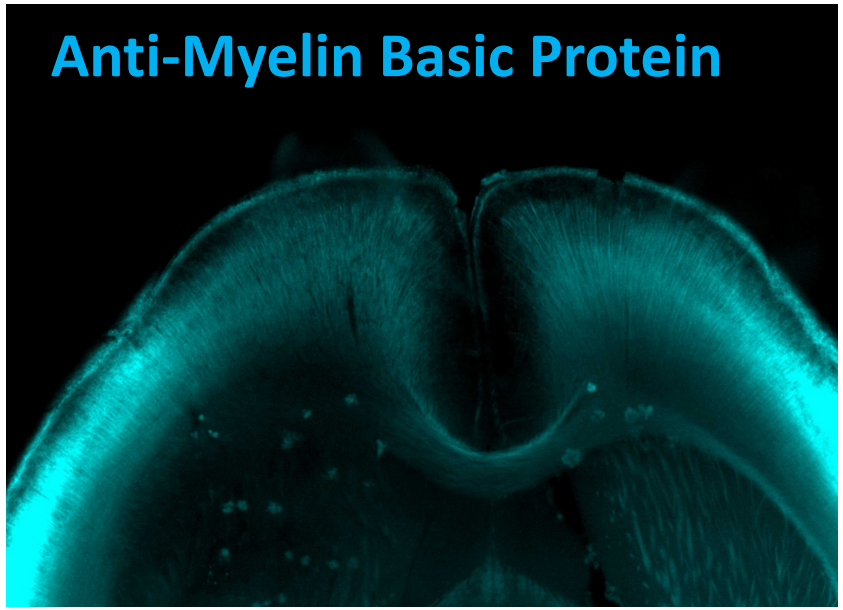
Fluoromyelin

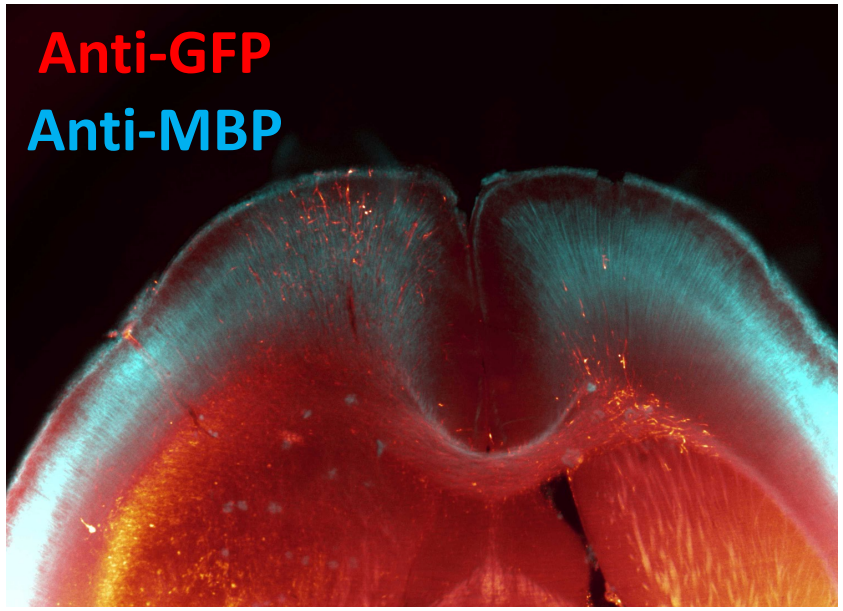
GFP-GB cells

DO NOT POST

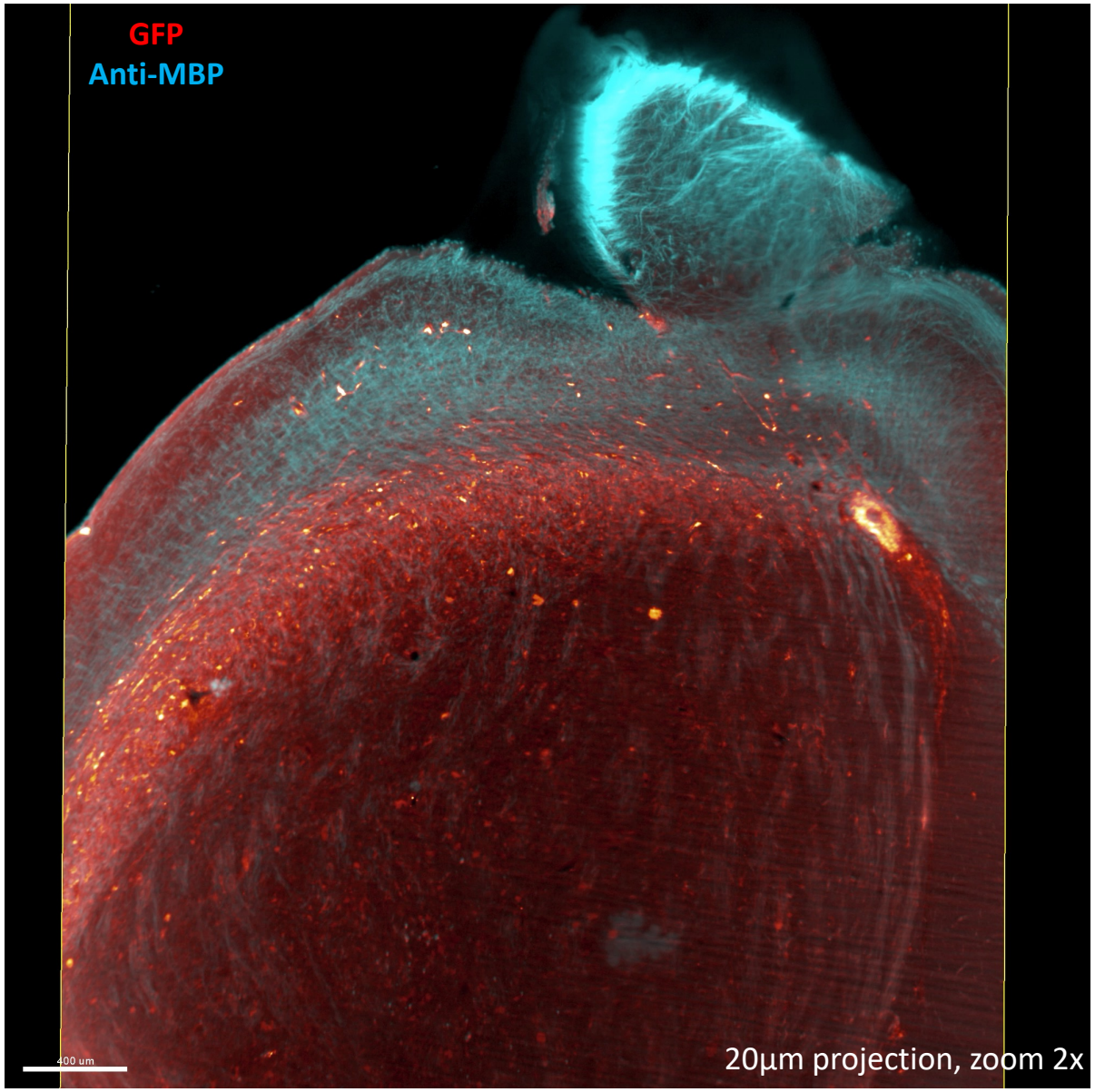
Destruction of myelin sheath in corpus callosum







Destruction of myelin sheath in corpus callosum



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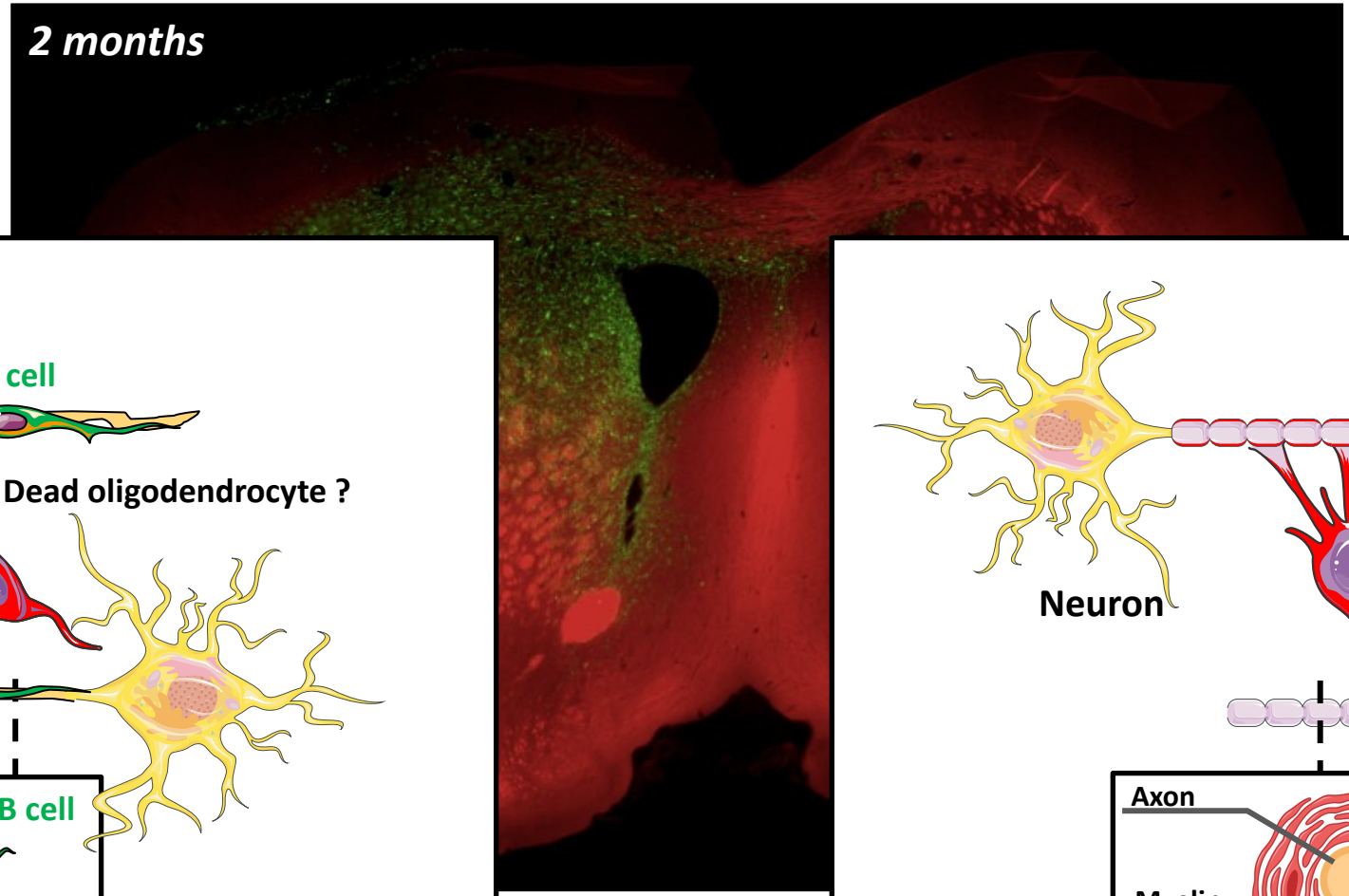
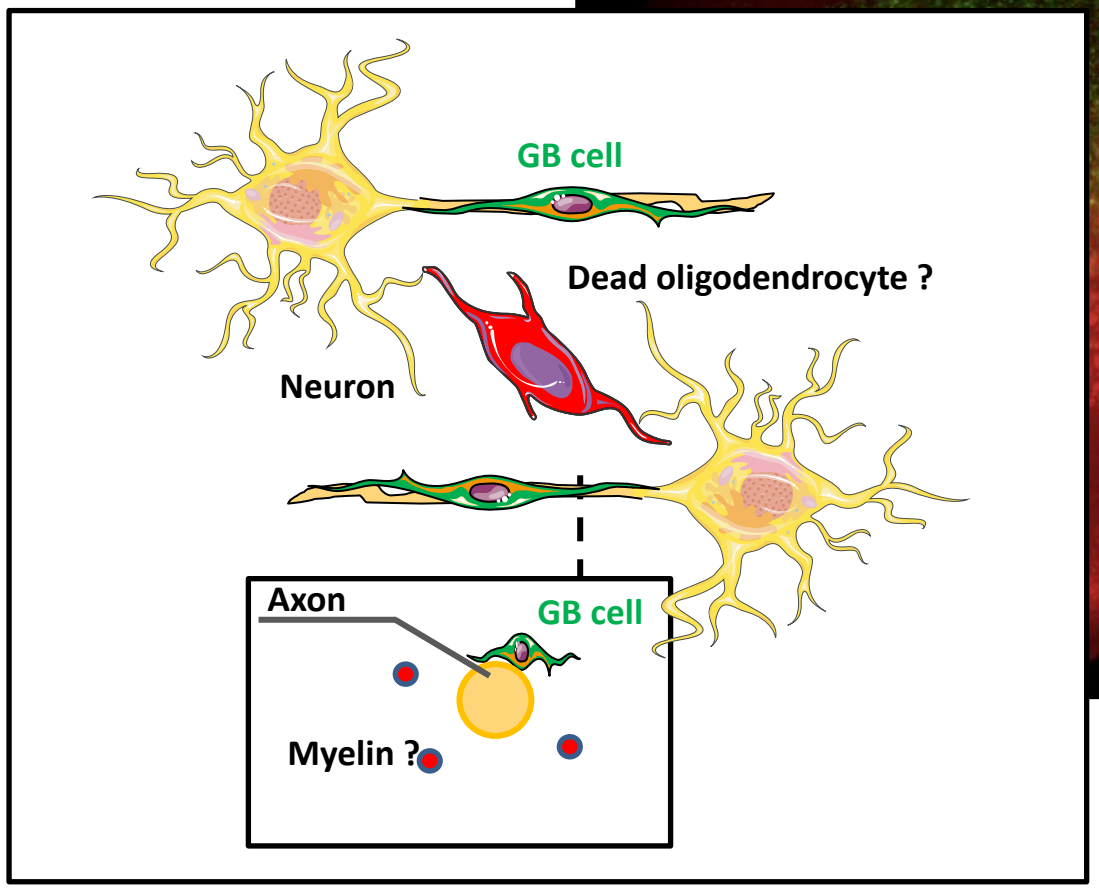
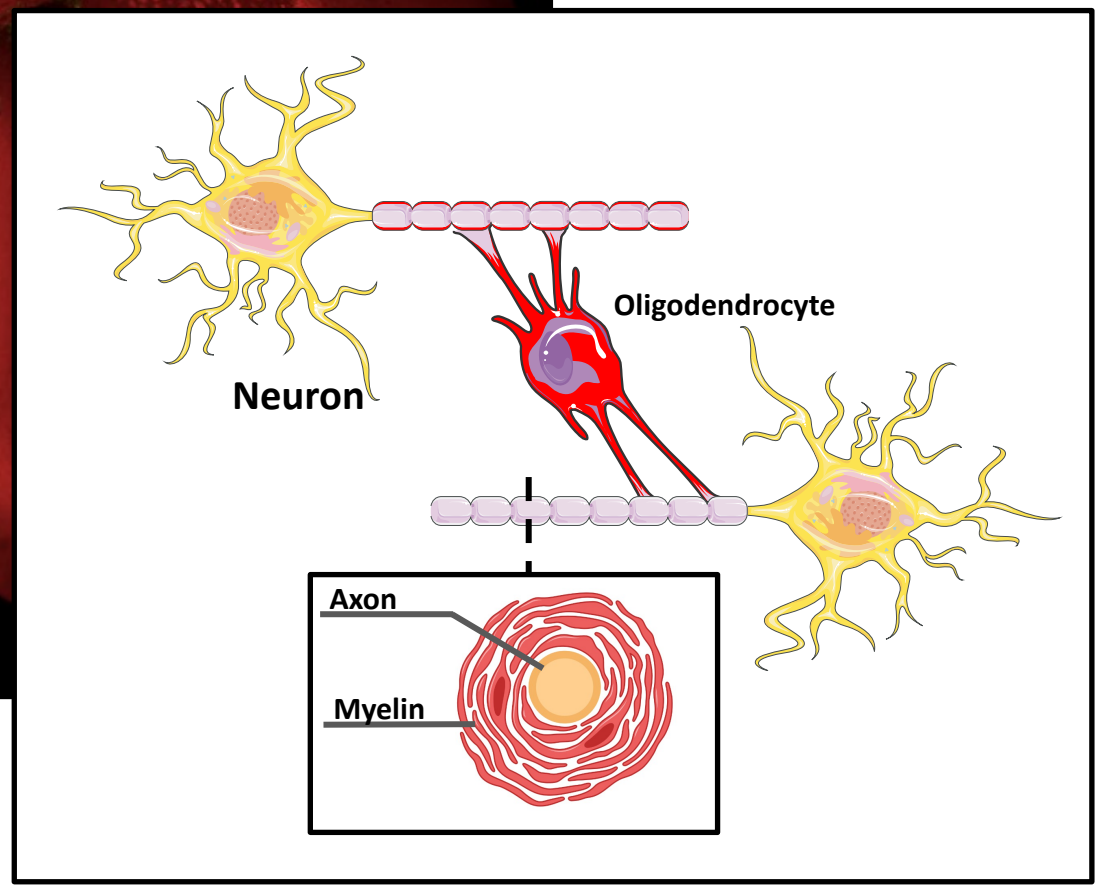
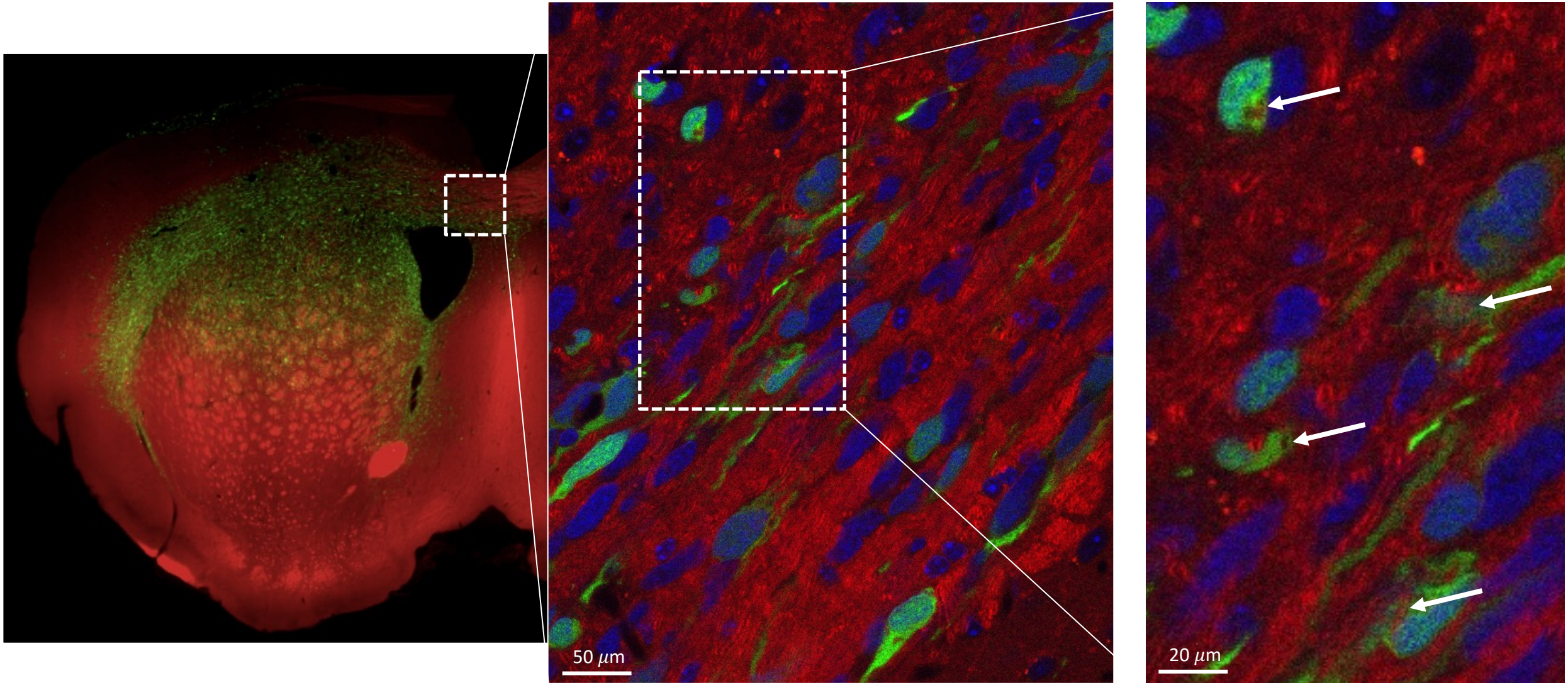
Destruction of myelin sheath in corpus callosum







In vivo engulfment of myelin particles by glioblastoma cells



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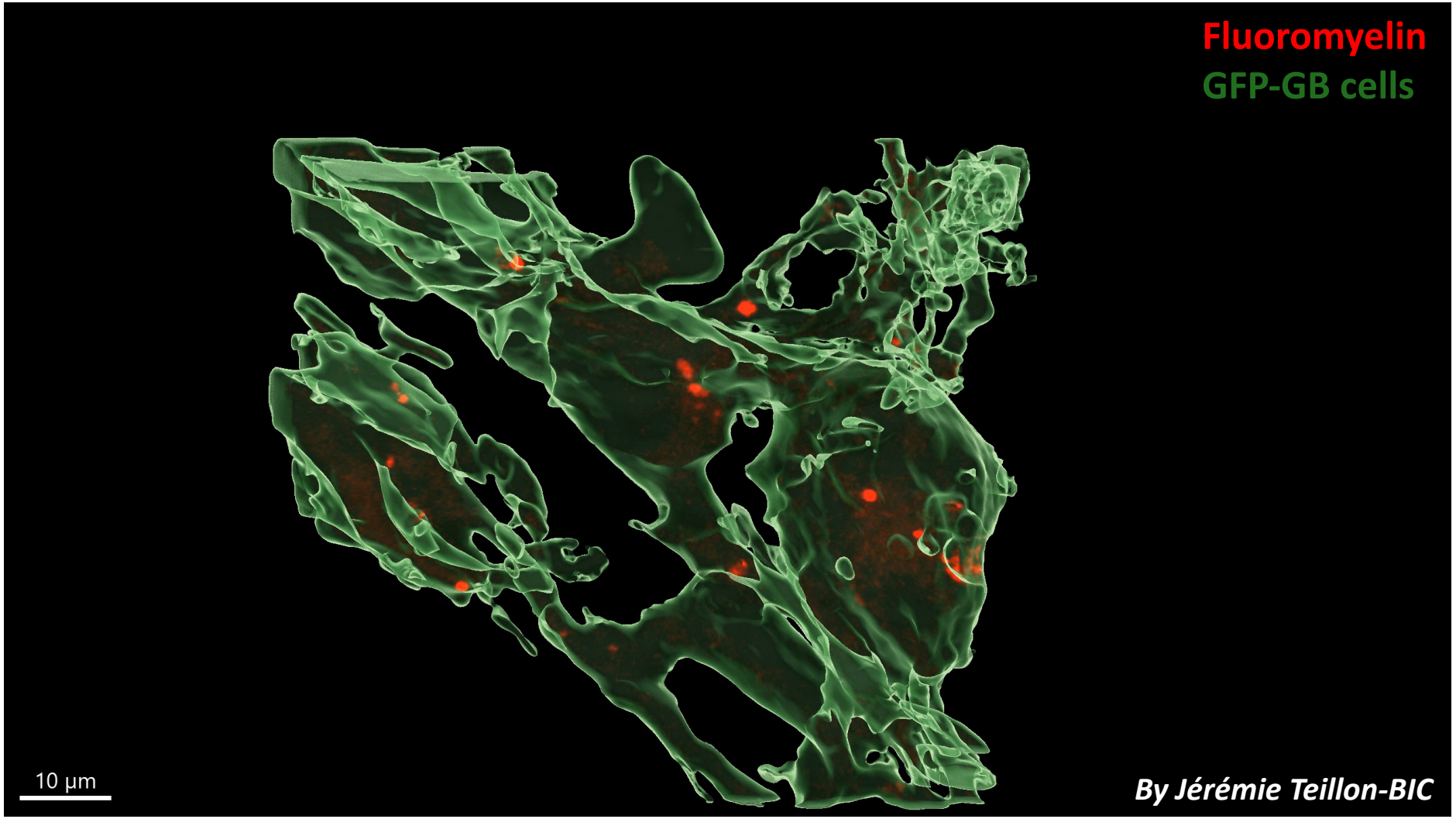
In vivo engulfment of myelin particles by glioblastoma cells



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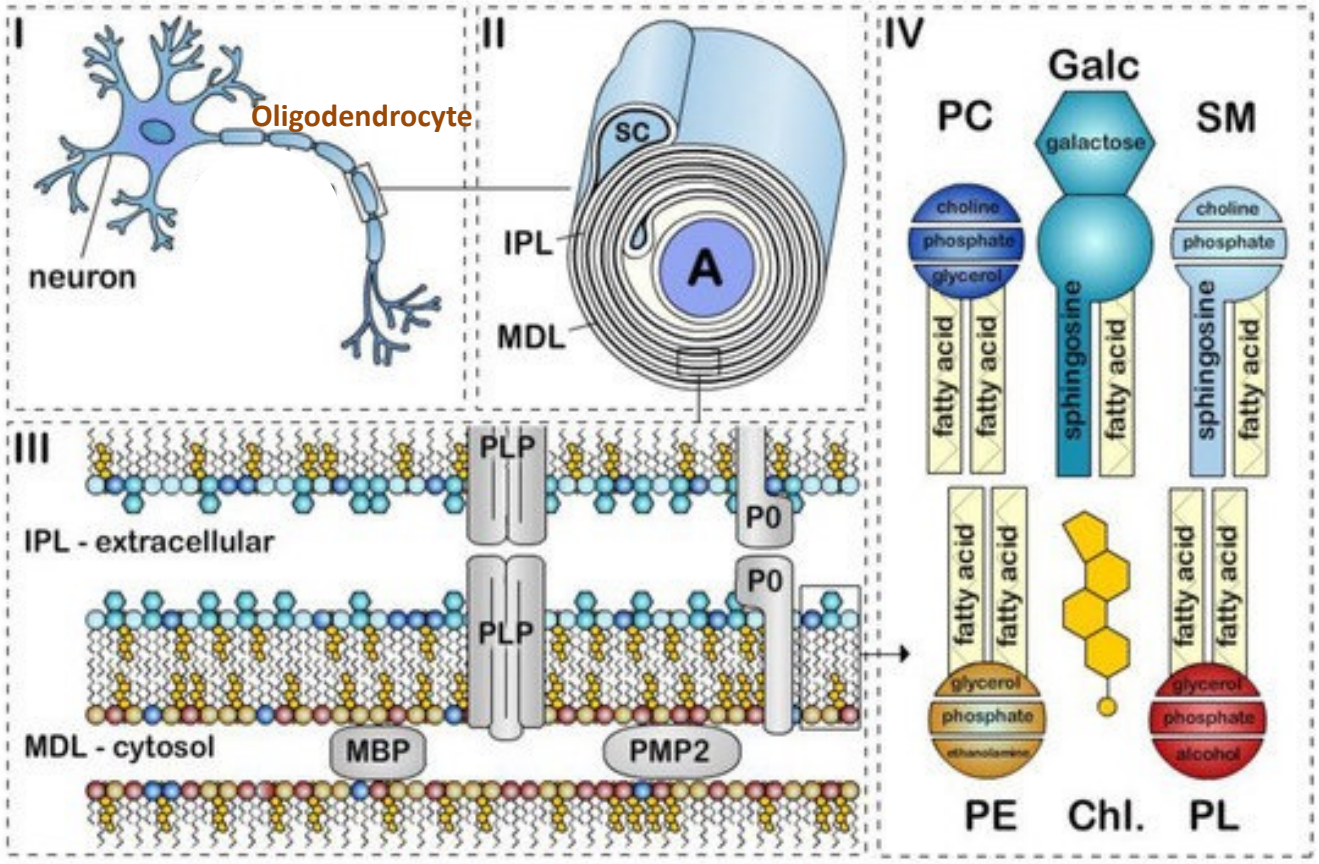
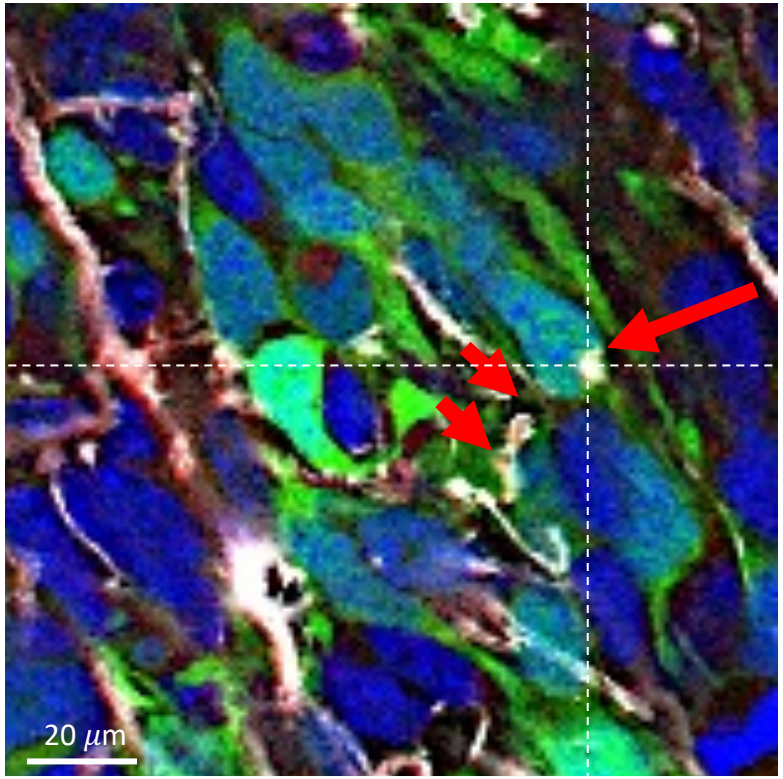
Myelin composition : 70-85% lipids, 15-30% proteins

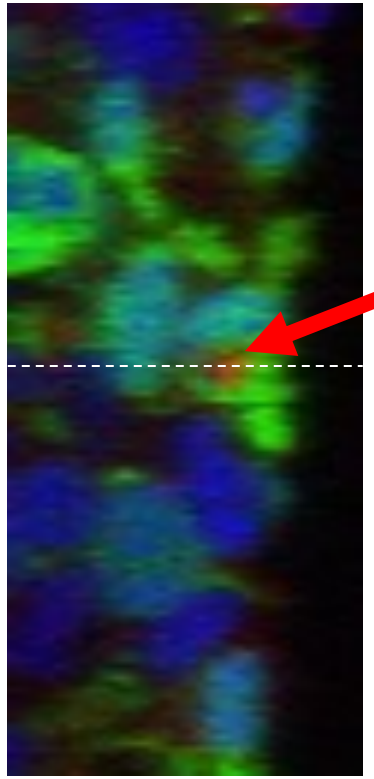


Reviewed in Poitelon et al, 2020

In vivo engulfed-vesicles contain Myelin-Basic Protein

GFP-GB cells
Fluoromyelin
Anti-MBP
Hoescht



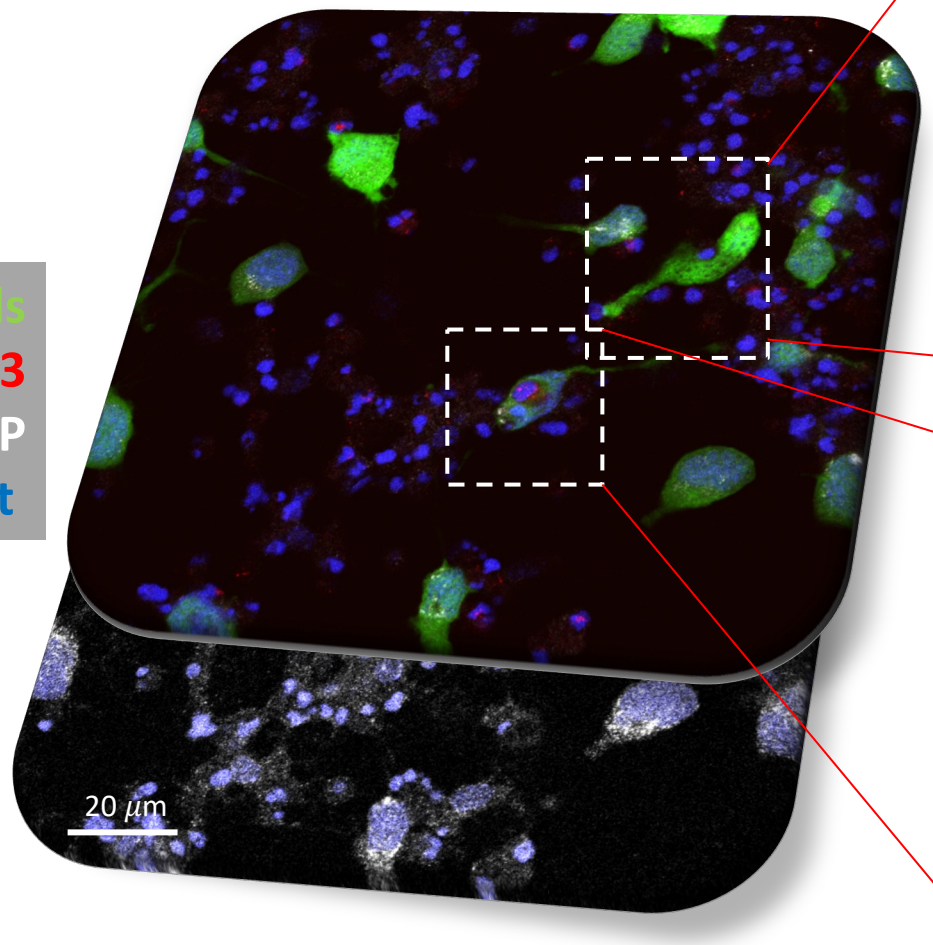


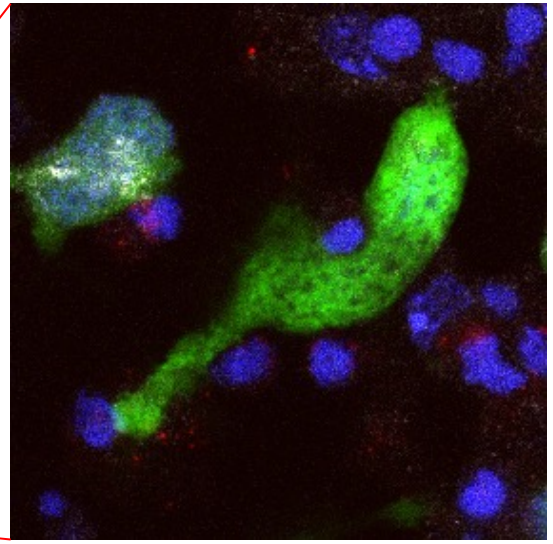
GFP-GB cells
Fluoromyelin
Hoescht

GB cells phagocyte oligodendrocytes and expressed MBP-positive vesicles

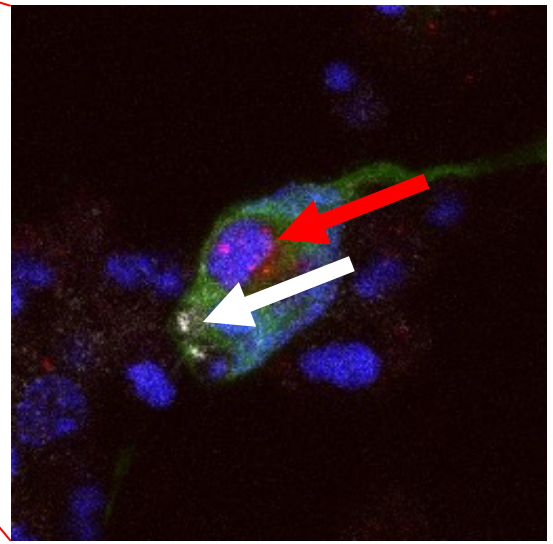
In vitro

GFP-GB cells
Cleaved-Casp3
Anti-MBP
Hoescht





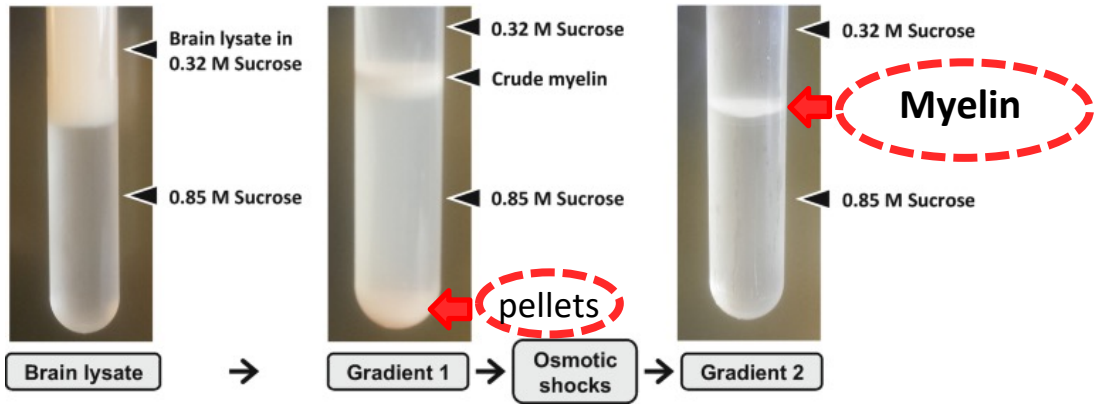
Tumor cell engulfing oligodendrocyte



Dead oligodendrocyte in a tumor cell which expresses MBP

GB cells phagocyte purified myelin extracts

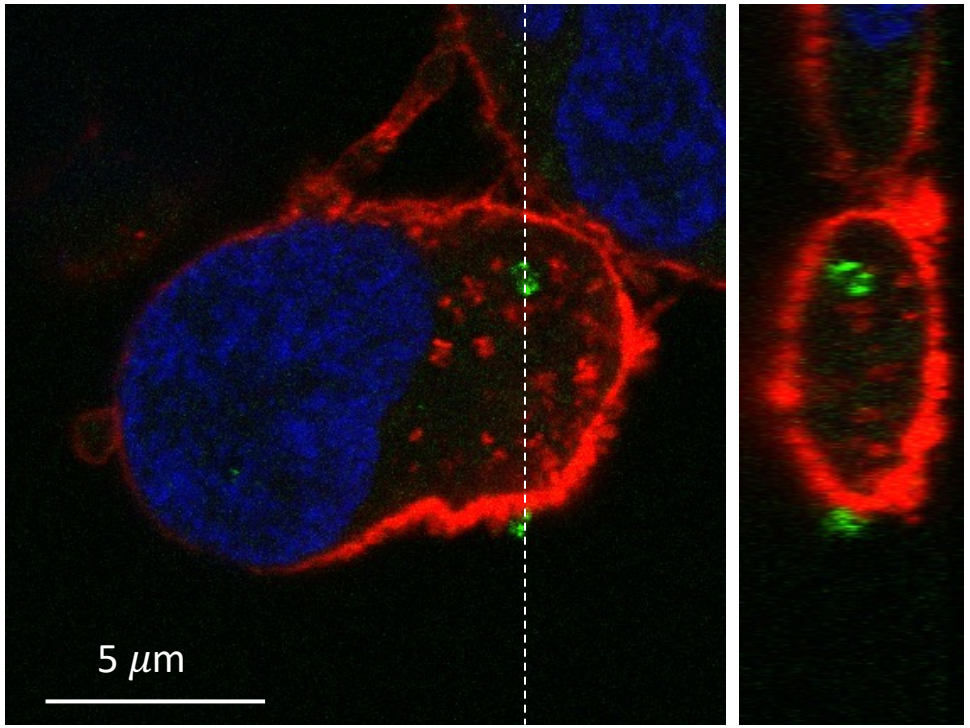
Home-made purification of myelin extracts



Erwing et al, 2019

→ ***Collaboration with Dr Arne Battefeld – IINS-Bordeaux***

MBP
Phalloidin
DAPI



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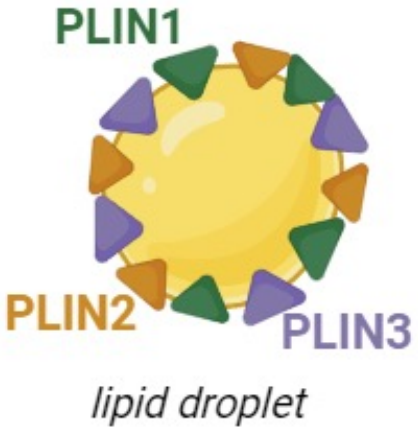
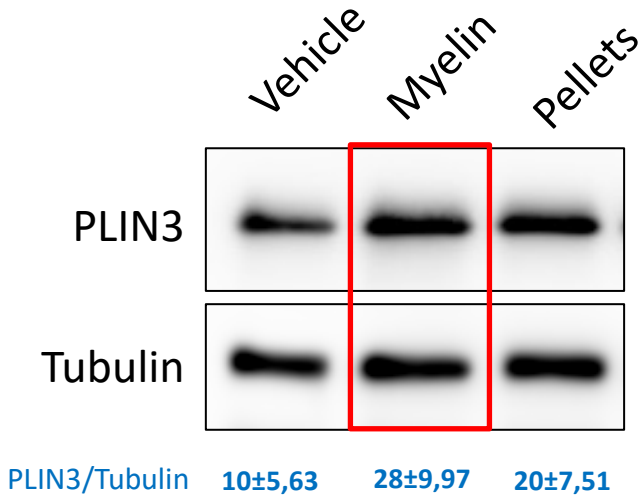
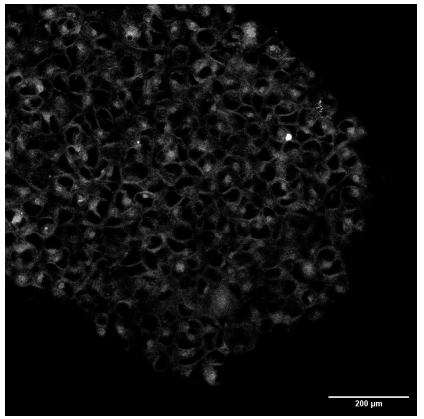
Myelin engulfment triggers lipid droplet formation in GB cells



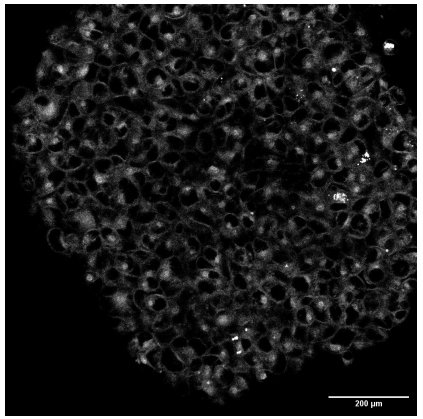


Lipiblue staining in spheroids

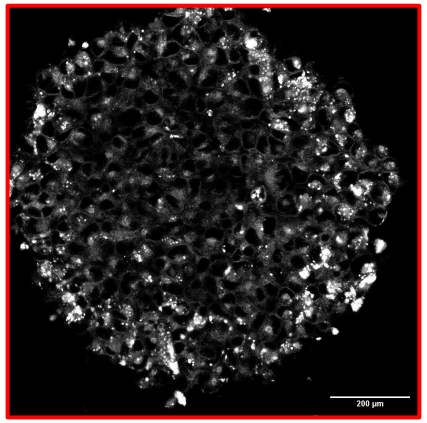
Vehicle

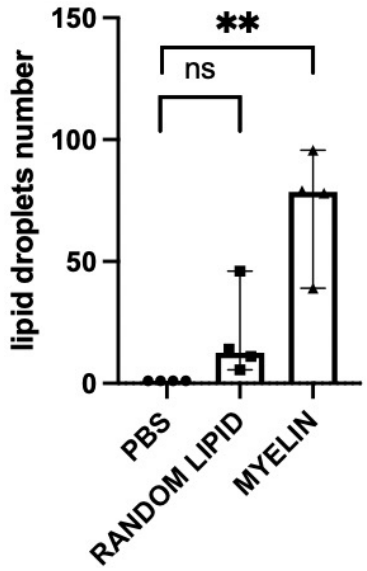


Pellets



Myelin





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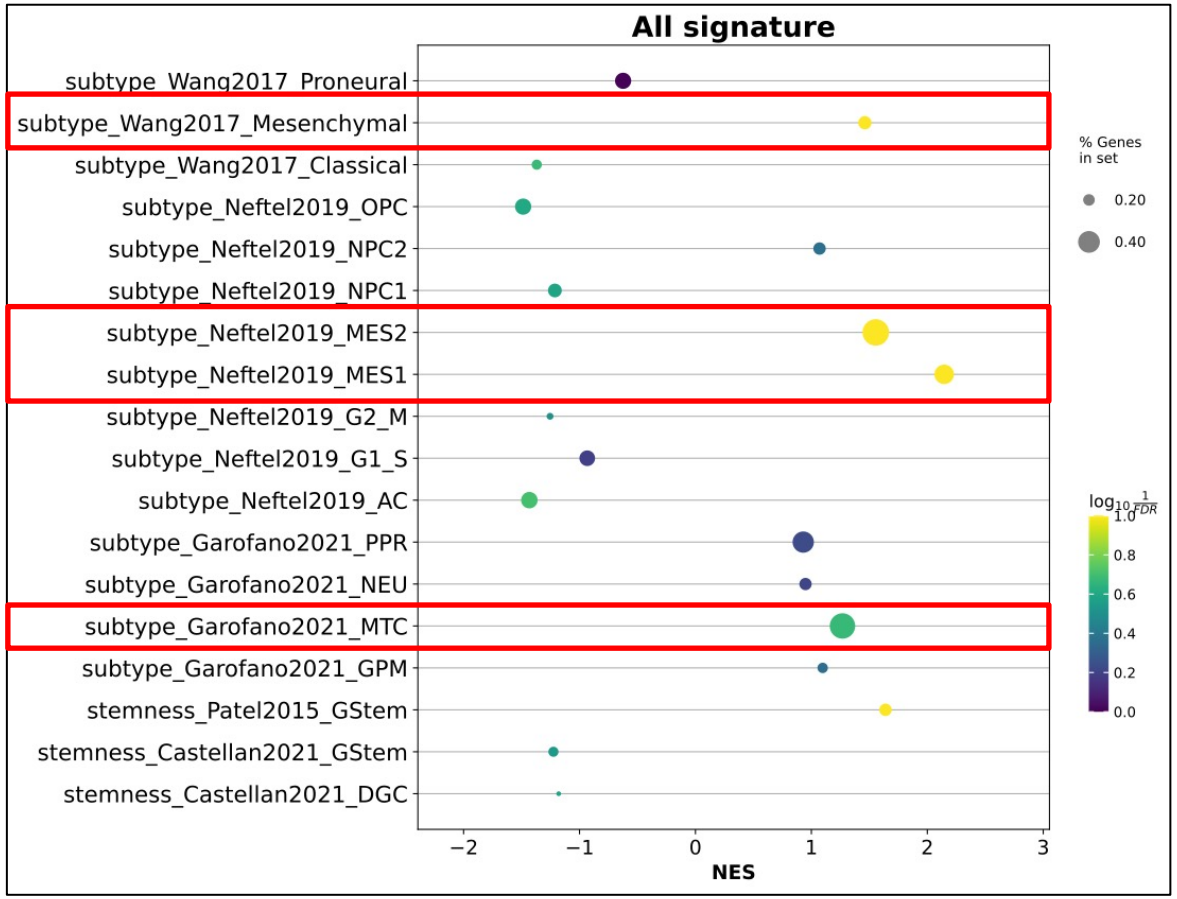
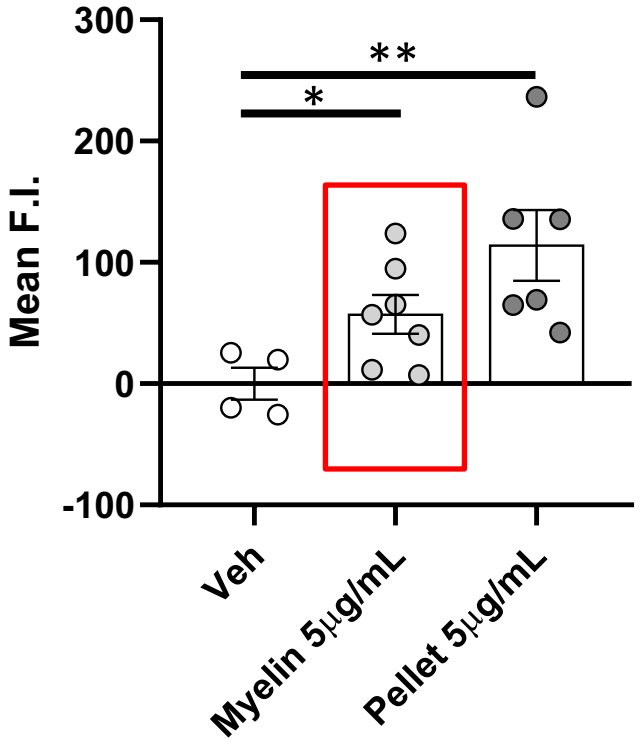
Comparison between Myelin-engulfed and control cells by RNAseq



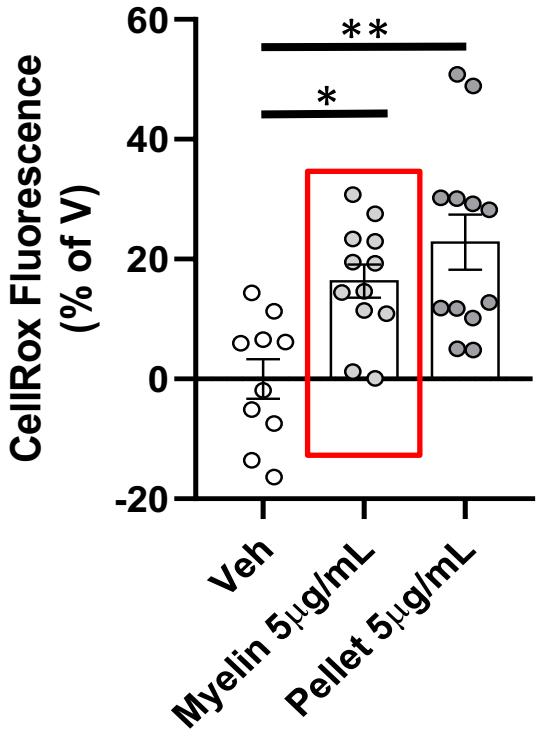
DO NOT POST

Myelin engulfment induces an increase in cell respiration and ROS production

TMRM detection for mitochondria membrane potential



CellROX detection for ROS production



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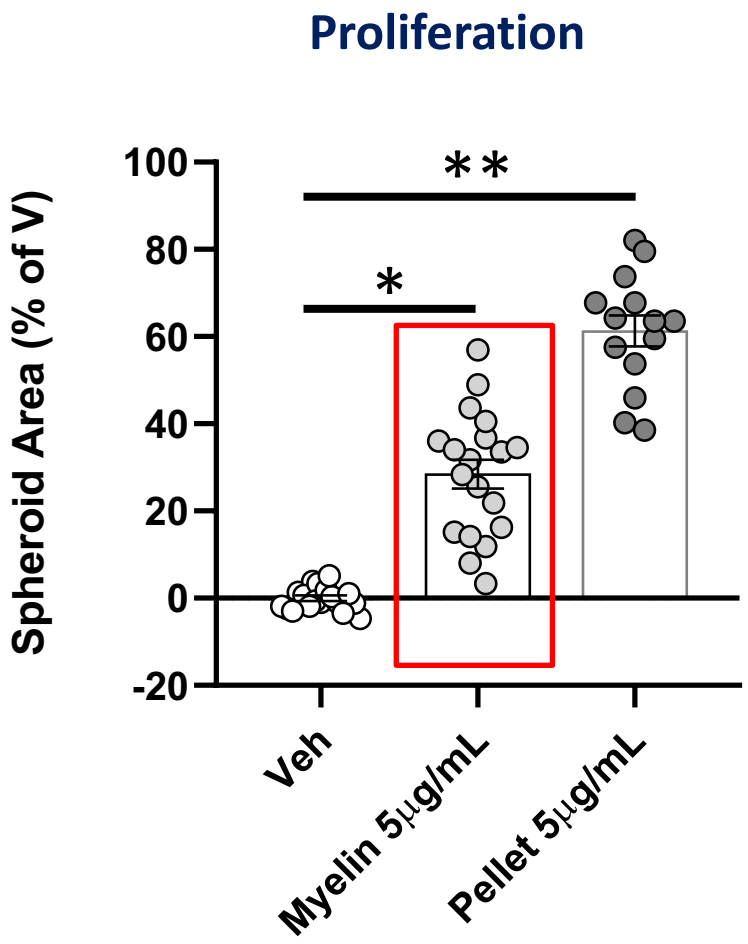
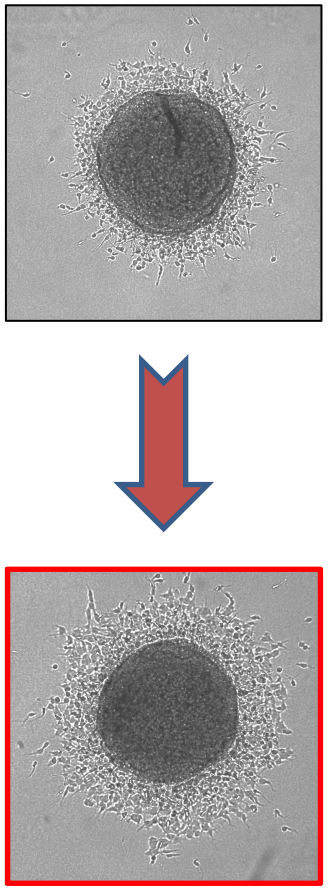
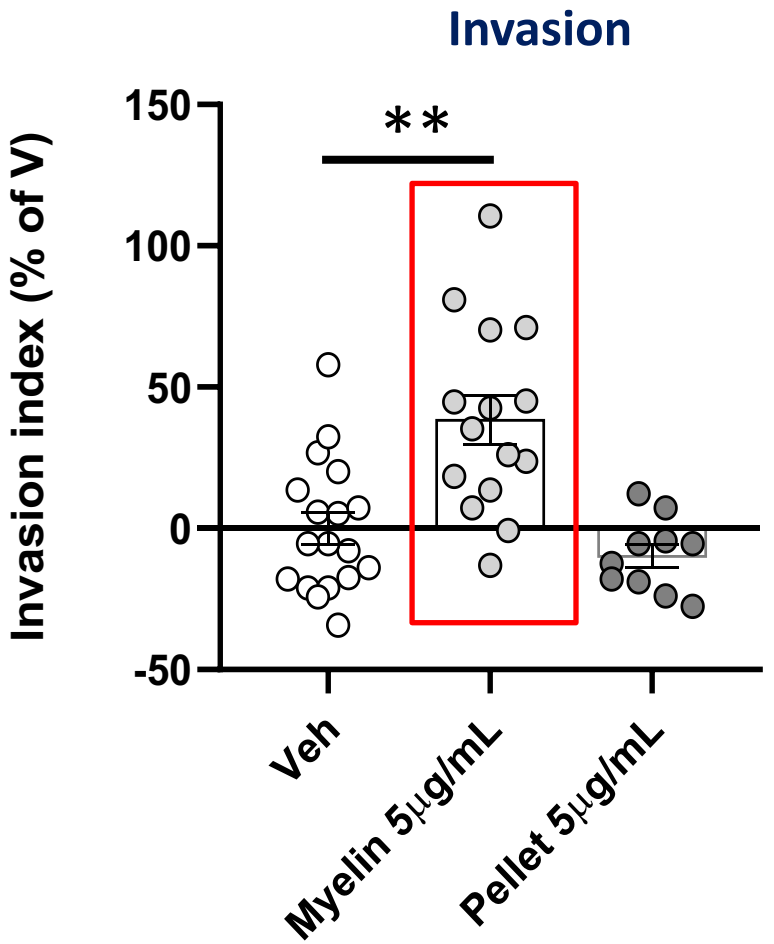
Myelin specifically increases GB cell invasion, but also cell proliferation







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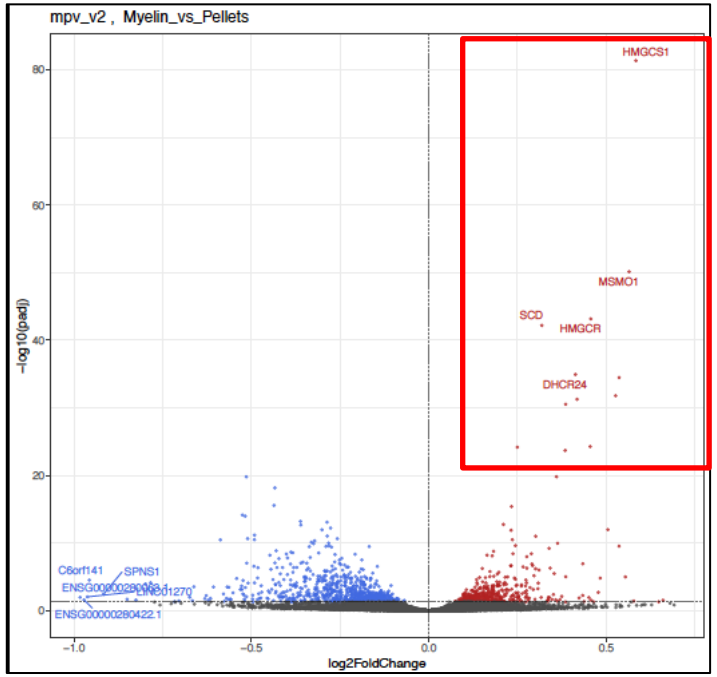
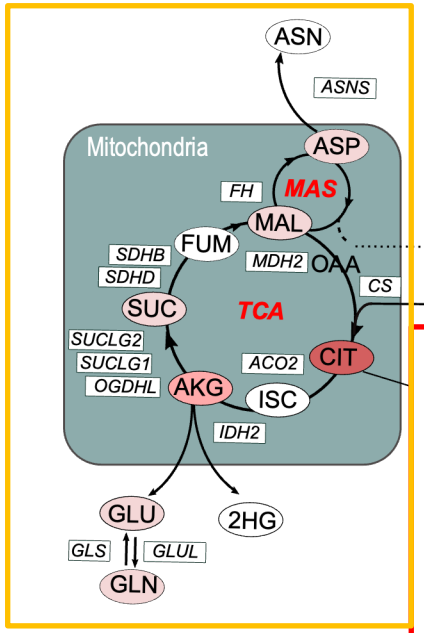
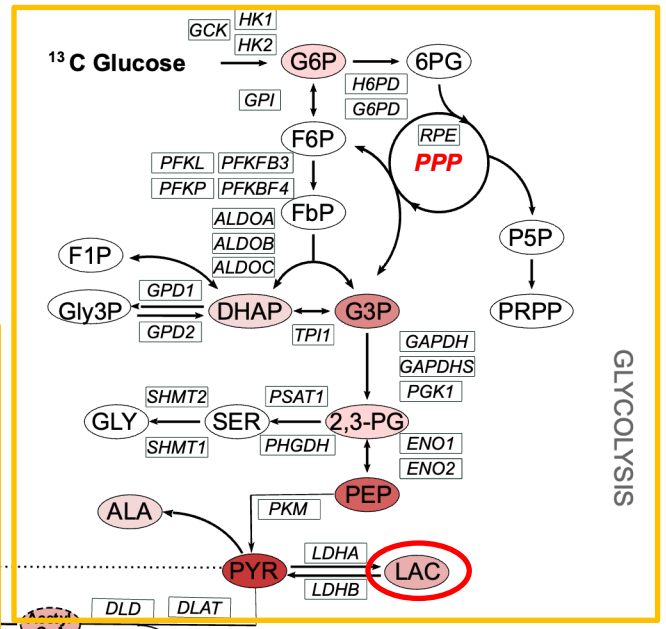
Myelin increases production of glycolysis, TCA intermediates and mevanolate pathways

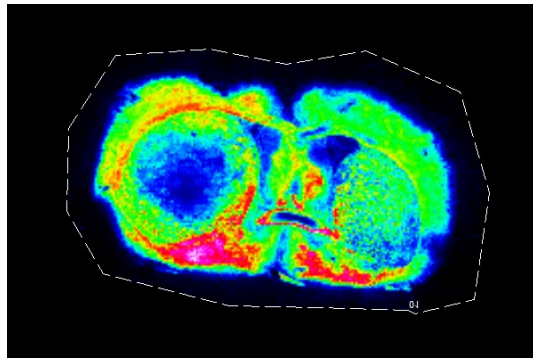
Comparison between myelin- and pellets-treated cells







Spatial metabolomics on lipids - on going -



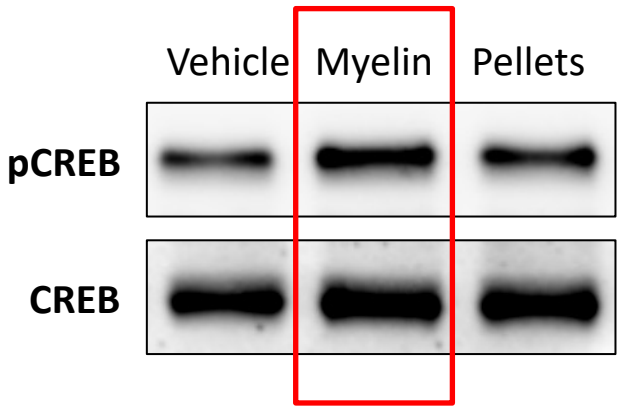
Yanis Zirem

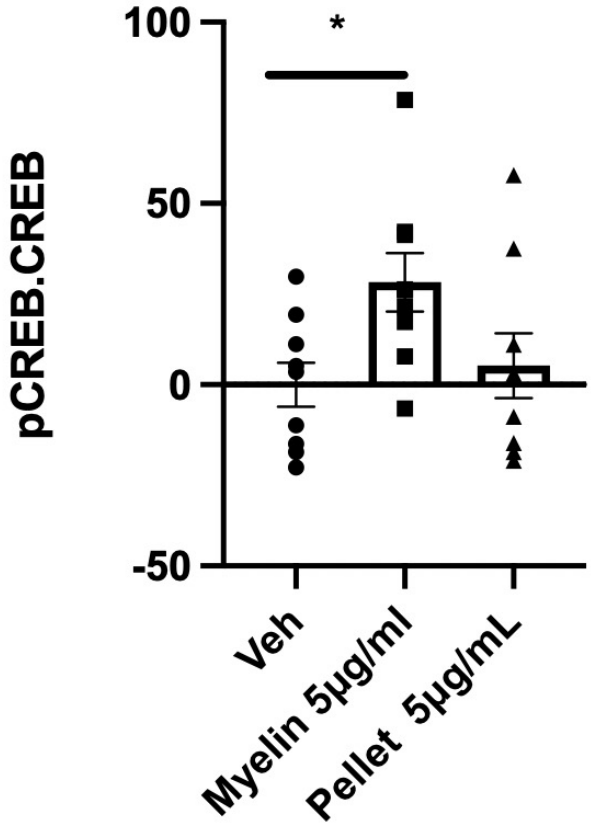
+ Lipodomics experiment with Leuven VIB facility (Jonas Dehairs)
- on going -

DO NOT POST

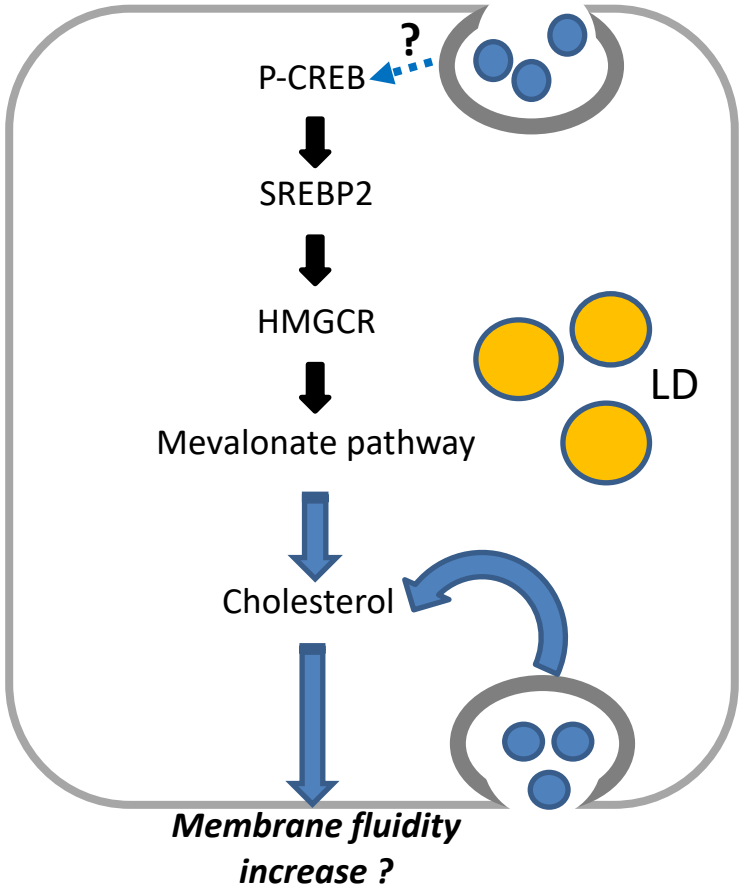
Increase of Phospho-CREB and SREBP2 expression in myelin-treated samples

Rationale *= in RNAseq, increase of CREBRF and SREBF2 expressions*





Myelin engulfment



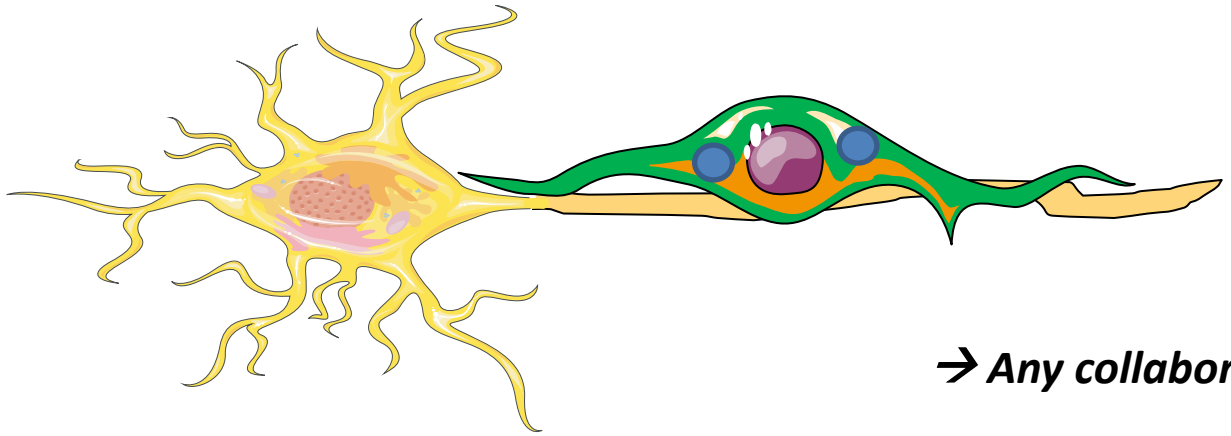
→ ***In vivo*** **experiment with late simvastatin treatment to evaluate myelin engulfment**

DO NOT POST

Take-home messages

<u>Part 1</u> : Inhibiting lactate metabolic symbosis reduces glioblastoma development and invasion.

<u>Part 2</u> : GB cells induces oligodendrocyte death and engulf myelin, triggering metabolic adaptations and higher invasion capacities.



→ Any collaboration on this topic is welcome !

Work from Claire Larrieu – PhD Student – on GB tumor resection

Role of lactate metabolism in glioblastoma development and relapse

Claire M. Larrieu[1], Antonio C. Pagano Zottola[1,2], Manon Lemaître[1], Marie-Alix Derieppe[3], Olivier Mollier[4], Thomas Daubon[1]

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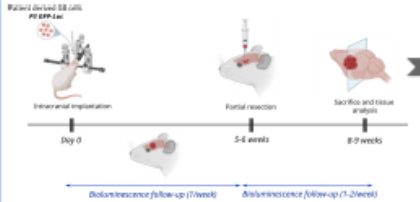
SURGICAL RESECTION & METABOLIC REGPROGRAMMING

In vivo model of surgical tumor resection

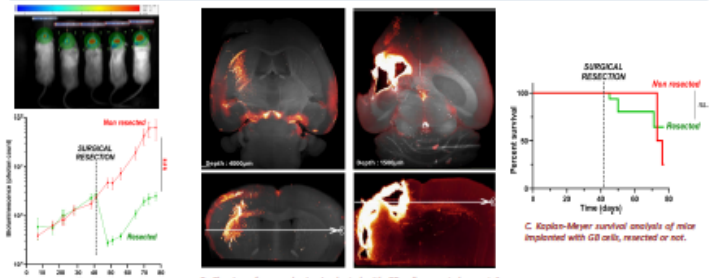


Histological analysis :
- *Tumor size & invasiveness*
- *Tumor architecture before/after resection*

Laser Capture Microdissection for RNA Sequencing

Immunostainings for metabolic targets

Efficient surgical resection, controlled by bioluminescence & histology, does not affect mice survival

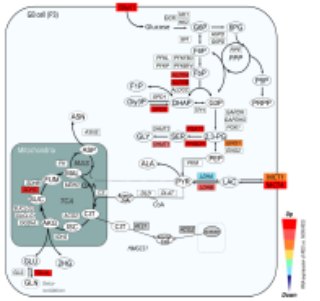


A. Bioluminescence follow-up of resected (n=14) or non-resected mice (n=6).

B. Clearing of mouse brains implanted with GB cells, resected or not 1 month post-implantation. Light Sheet imaging in transversal (upper panel) or coronal (lower panel) plane.

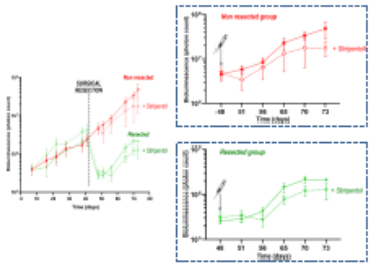
C. Kaplan-Meyer survival analysis of mice implanted with GB cells, resected or not.

Surgical resection reshapes GB cell metabolism



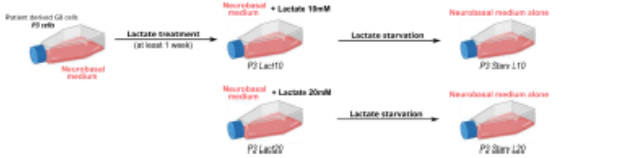
RNA Sequencing data from laser capture microdissection samples of hypoxic, angiogenic and invasive areas of GB tumors in mice brains. RNA expression of metabolic enzymes in resected (RES) brains were compared to non-resected (NON RES) brains.

Blockade of lactate metabolism by repurposing an anti-epileptic drug tends to decrease tumor growth

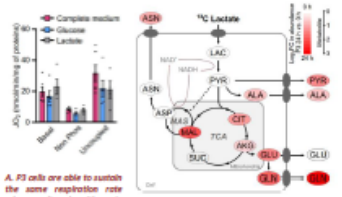


Mice resected or not were administered Stiripentol, LDH inhibitor, 150mg/kg (intra-peritoneal, 3 times per week) or vehicle, starting from 6 days post-resection. Mild decrease of bioluminescence was observed with Stiripentol treatment.

Mimicking resection-induced lactate fluctuations in vitro



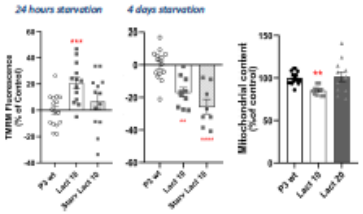
GB cells use lactate to fuel TCA cycle



A. P3 cells are able to sustain the same respiration rate when cultured either in complete medium or in medium supplemented only with glucose or only lactate (data obtained by oxygraphy analysis).

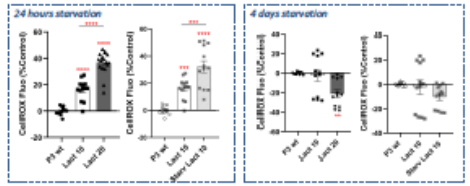
B. Lactate can directly fuel TCA cycle in P3 cells. Data obtained from P3 cell infused with [13C3] lactate for 24 hours followed by labeled-metabolite detection.

Lactate starvation impacts mitochondrial activity



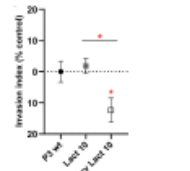
Mitochondrial membrane potential, but not mitochondrial content, is enhanced with lactate treatment. This effect is reversed when cells are then starved from lactate for 24h. When starved 4 days, mitochondrial activity is strongly decreased.

Lactate starvation promotes transient ROS production, even more than lactate treatment



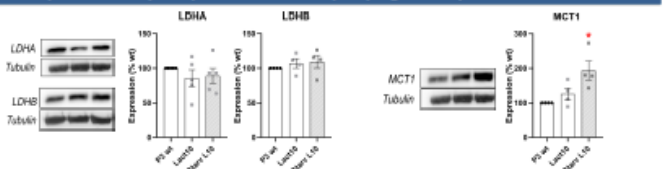
P3 cells were cultured without or with lactate 10mM/20mM and then starved from lactate for 24h or 4 days. ROS production was measured using CellROX Green probe (n=4 independent exp ; 4-5 technical replicates each).

GB cell invasion is impaired with lactate starvation



P3 cell invasion in a collagen I matrix (n=4 independent exp ; 8-10 technical replicates each).

MCT1, lactate transporter, but not LDH A or B, is upregulated upon lactate starvation



Detection of lactate metabolism actors by Western Blot shows modulations of protein expression of lactate transporter MCT1 (n=4-5 independent experiments, 2 technical replicates each).

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- ***Manon Lemaitre*** *as Engineer*
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Thank you for your attention, any questions ?