

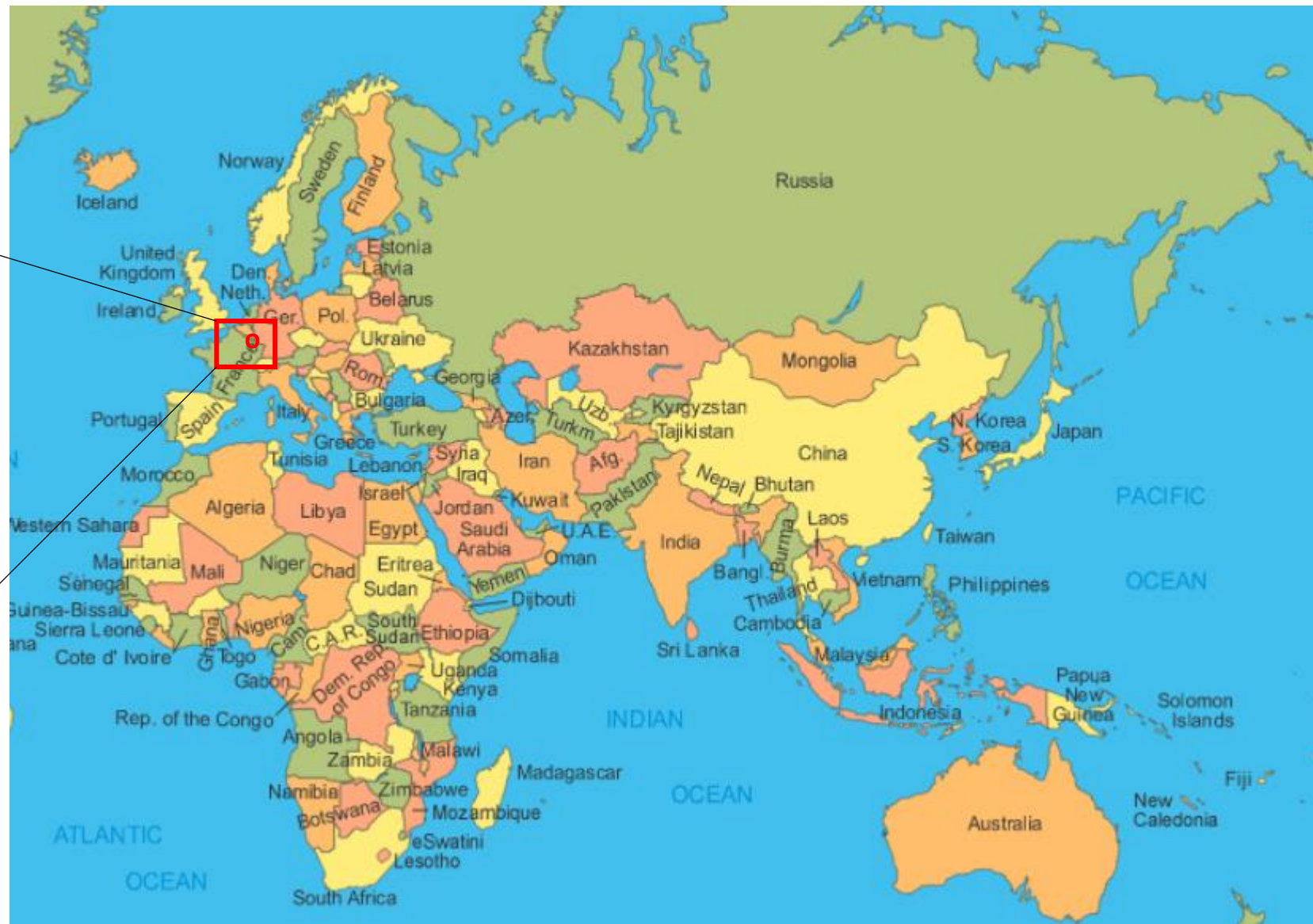
# Studying cancer cell invasion in Glioblastoma

**Simone P. Niclou, PhD**

Director, Department of Oncology  
Group leader, NORLUX Neuro-Oncology Laboratory

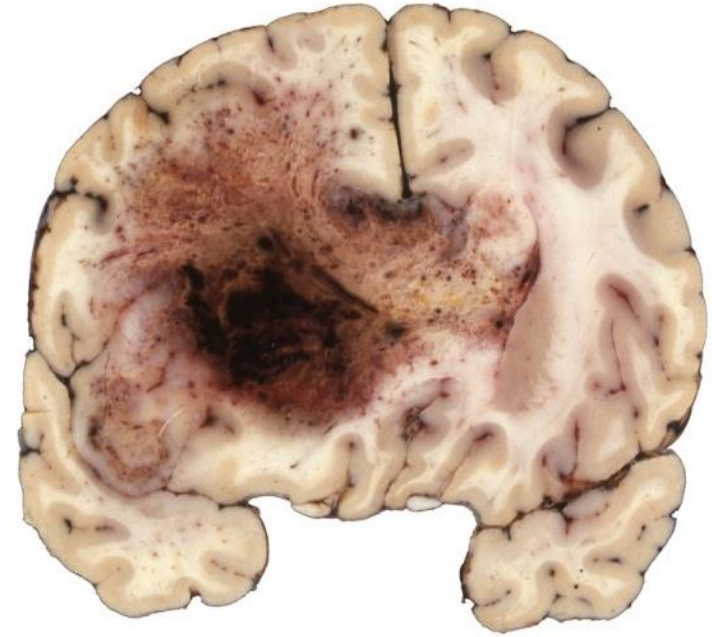
**Luxembourg Institute of Health**





**Luxembourg**  
2,586.4 km<sup>2</sup>  
Population: 615,000  
GDP/capita: 125,000 \$

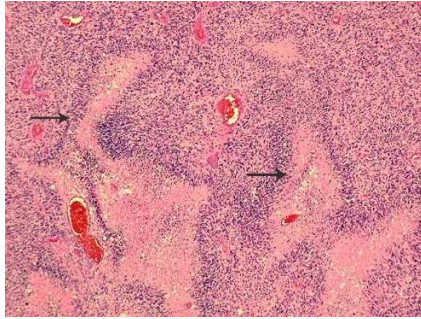
# Overview



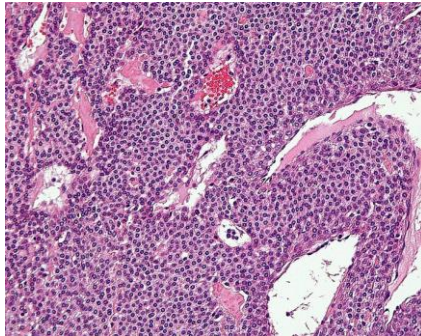
- Introduction to *Diffuse Glioma*
- Modes and routes of glioma cell invasion
- Experimental models to study glioma invasion: *in vitro*, *ex vivo*, *in vivo*
- Identification of novel invasion essential genes
- Conclusion & Perspectives

# Biological and Clinical Challenges of Glioblastoma

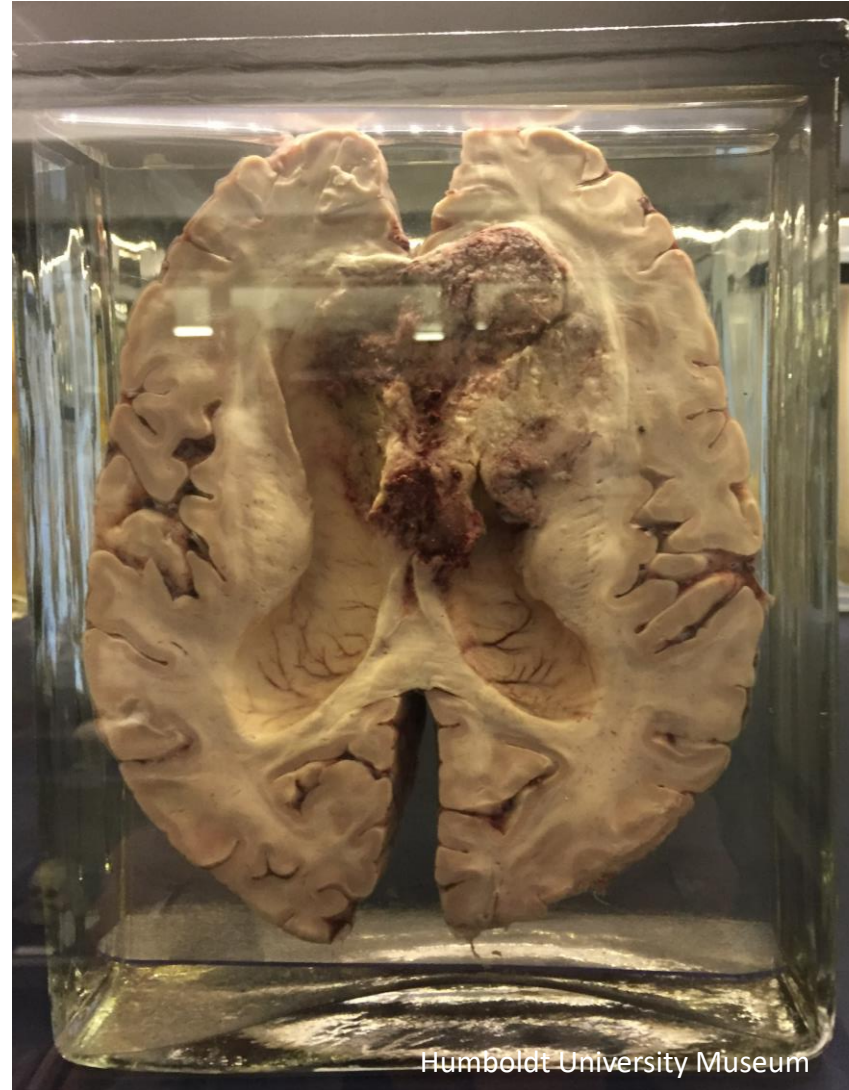
- Aggressive, fast growing tumor
- Hypoxia and necrosis



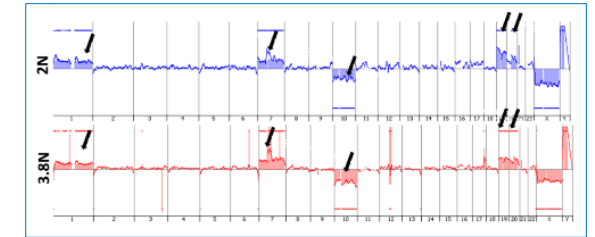
- Abnormal vessels and neo-angiogenesis



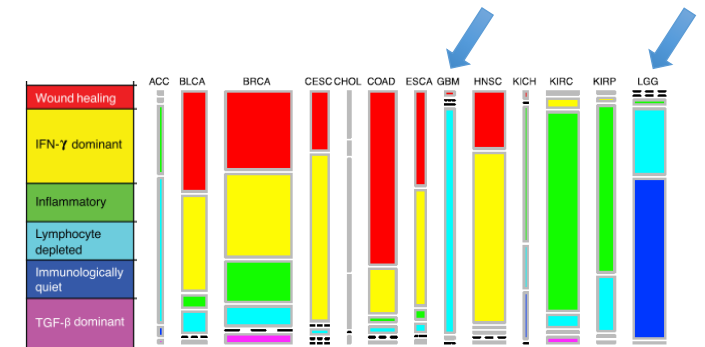
- No clear boundary
- Invasion of brain parenchyme



- Genetic instability: mutations and CNAs



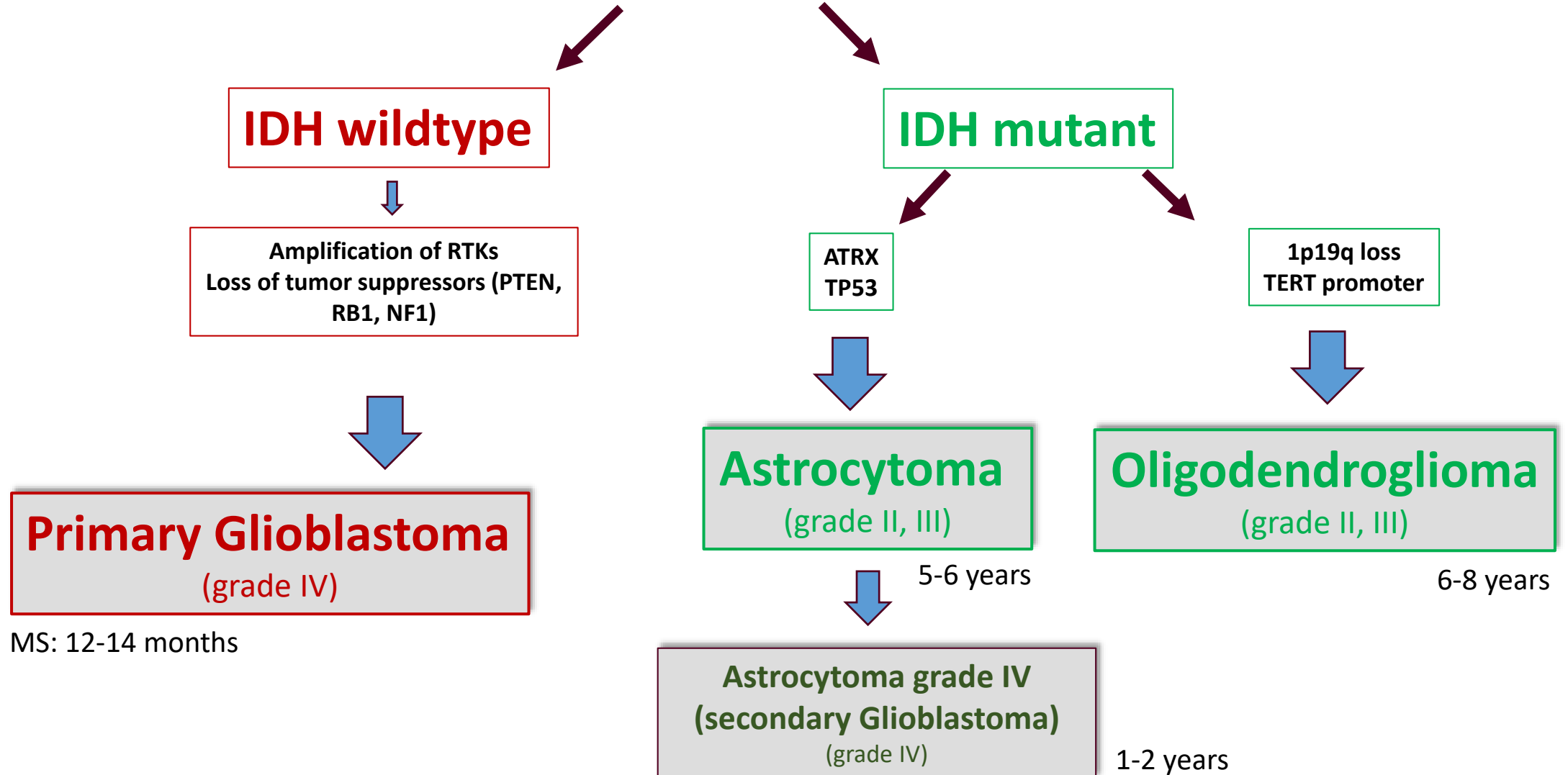
- Immunologically cold tumor



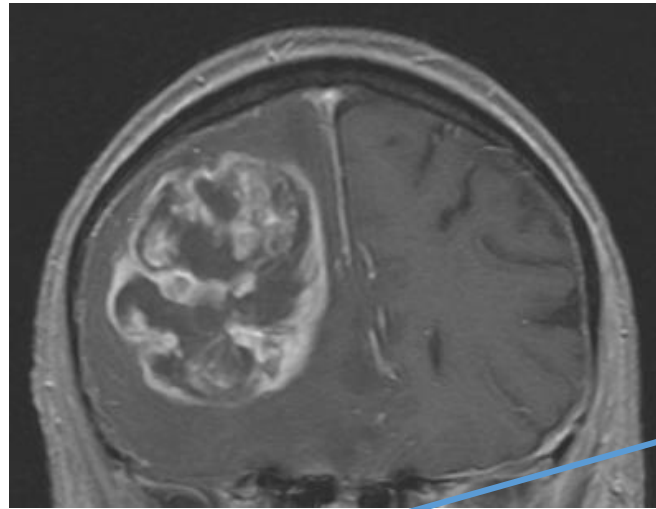
# Classification of Malignant Gliomas (= Diffuse Gliomas)

## Diffuse Gliomas

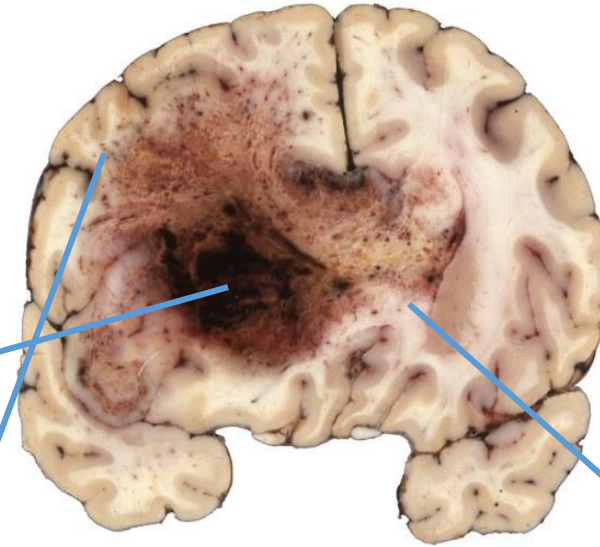
IDH: Isocitrate Dehydrogenase



# Glioma: 'a disease of the whole brain'



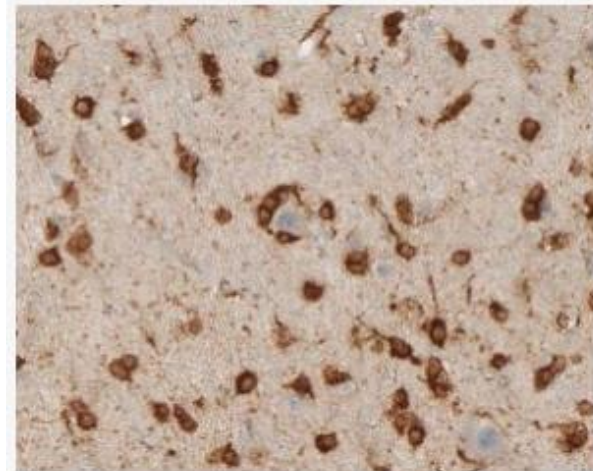
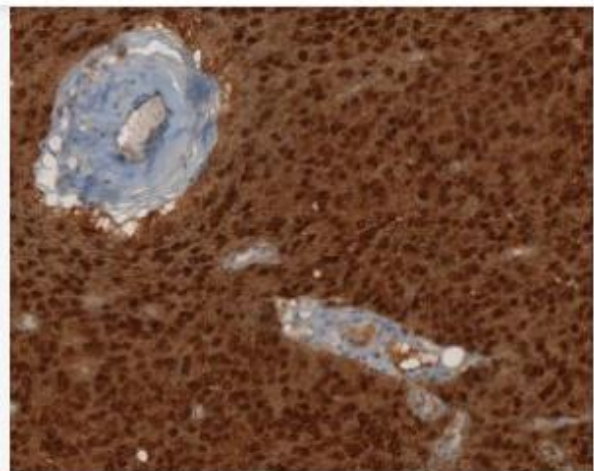
**Tumor core**



**Cortical area**

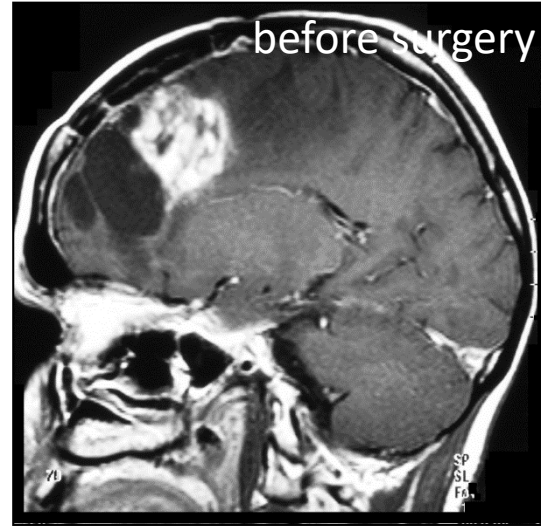
**Infiltration zone**

IHC for mIDH

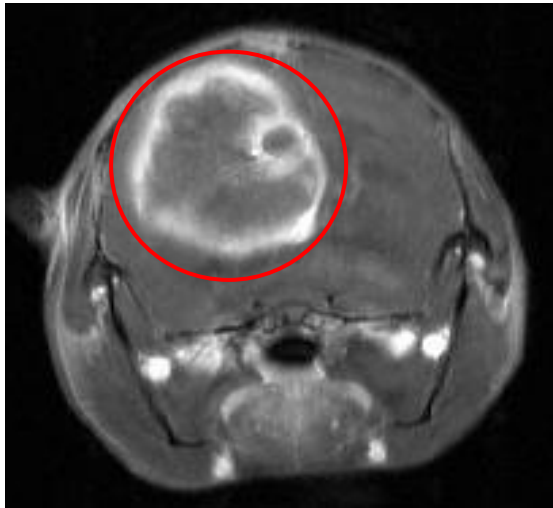


# Invading tumor cells are not removed by surgery

GBM in patient



Patient GBM in mouse brain  
(PDOX)



Visualization of tumor cells  
on brain section

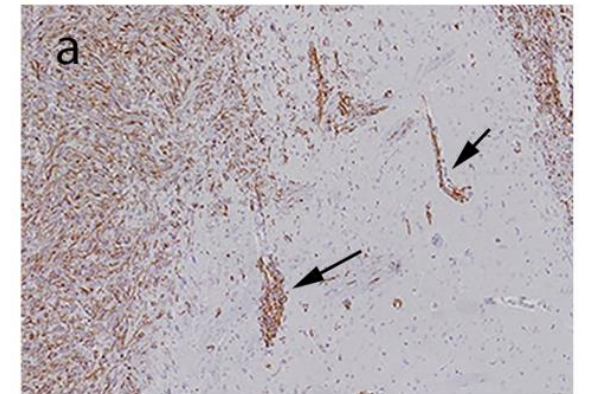
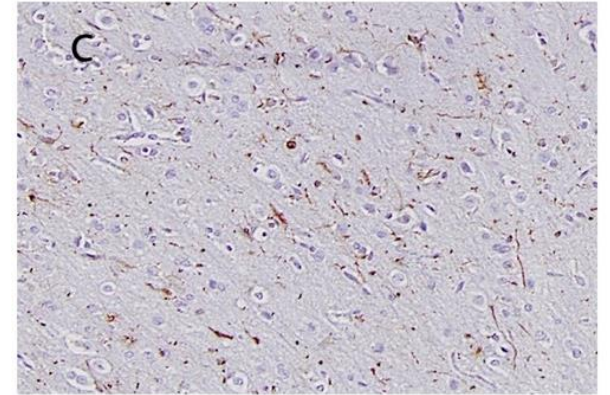
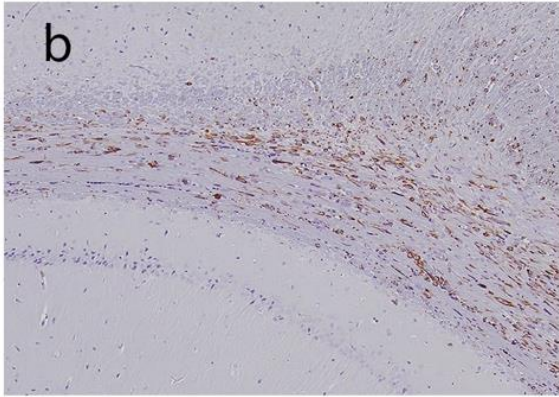
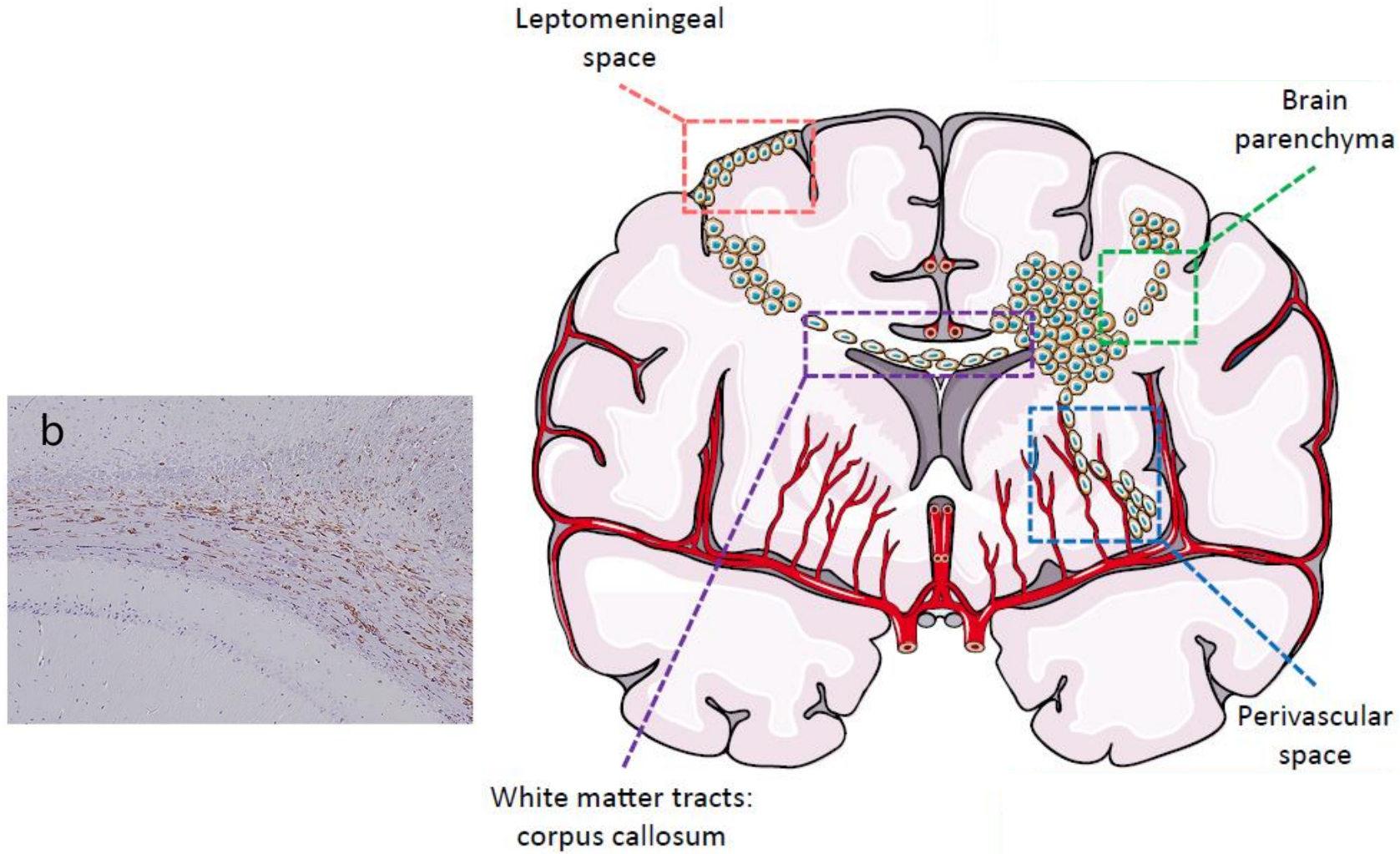
Invading tumor cells

# Factsheet about glioma invasion

- ✓ Glioma cells **do not metastasize** to other organs (although CTCs can sometimes be detected in blood)
- ✓ However they 'metastasize' **within the brain** – concept of Diffuse Glioma
- ✓ Invading glioma cells are largely **shielded from current therapeutic interventions**:
  - **Surgical resection** removes the tumor core, but not the invasive front
  - **Radiotherapy** focuses on the tumor core & limited margin
  - **Systemic therapy** often does not reach invading glioma cells, which are hidden in normal brain tissue and protected by an intact blood brain barrier
- ✓ Hence **recurrence is inevitable**
- ✓ Targeting invading glioma cells is like **guerilla warfare**

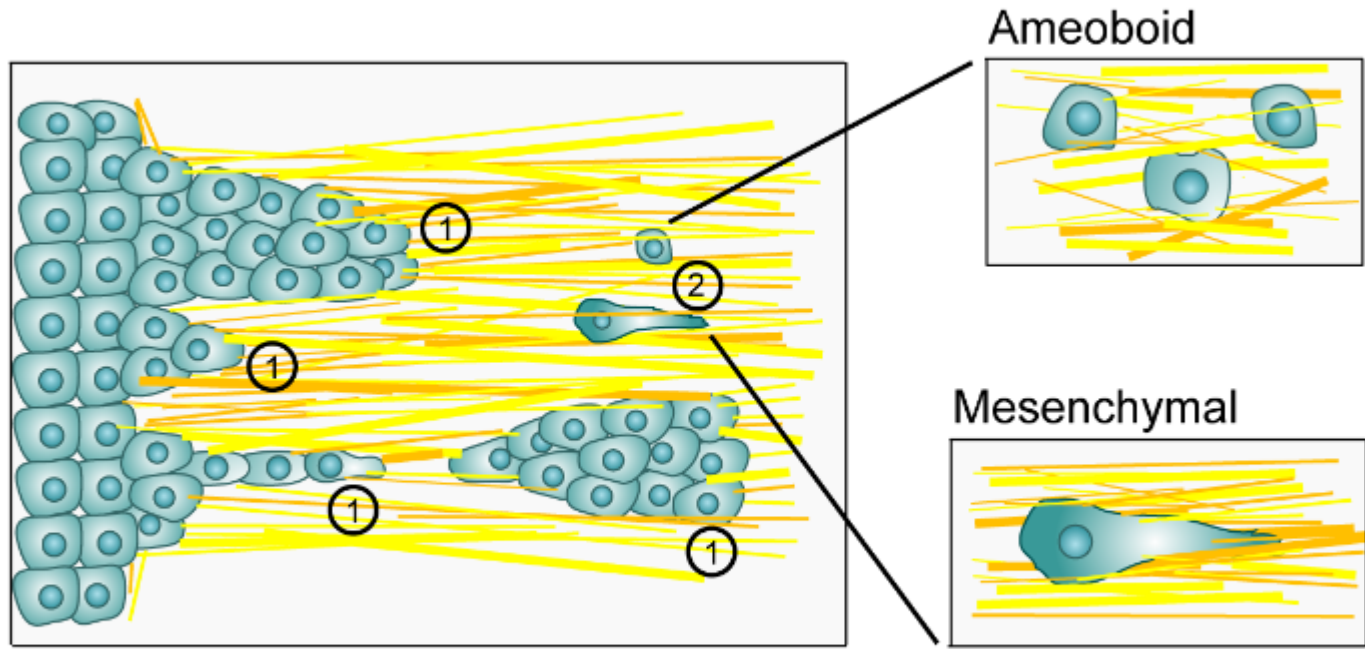


# Preferred routes of glioma cell invasion

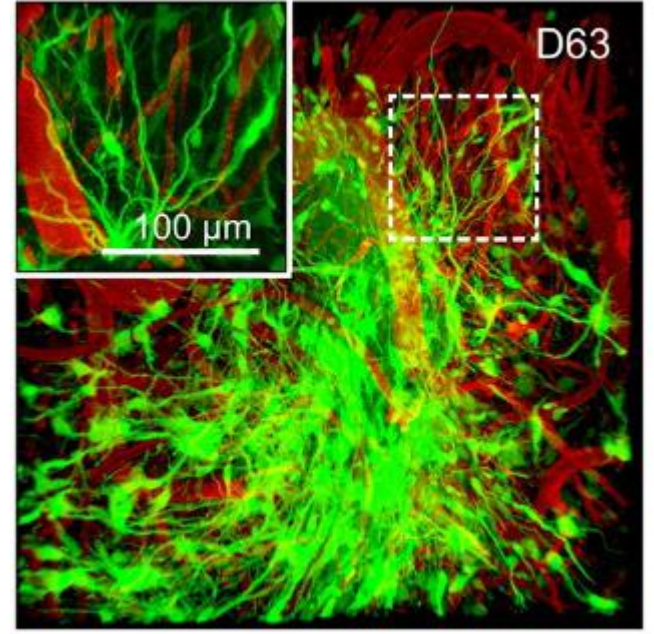


# Single Cell Invasion vs Collective Invasion

## Collective and Single Cell Invasion Through the Collagenous Extracellular Matrix



- ① **Collective Invasion: Sheets, Strands, and Clusters**
- ② **Single Cell Motility: Ameoboid and Mesenchymal**

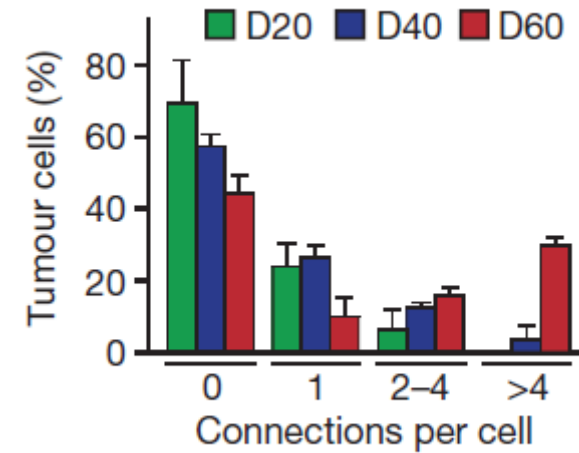
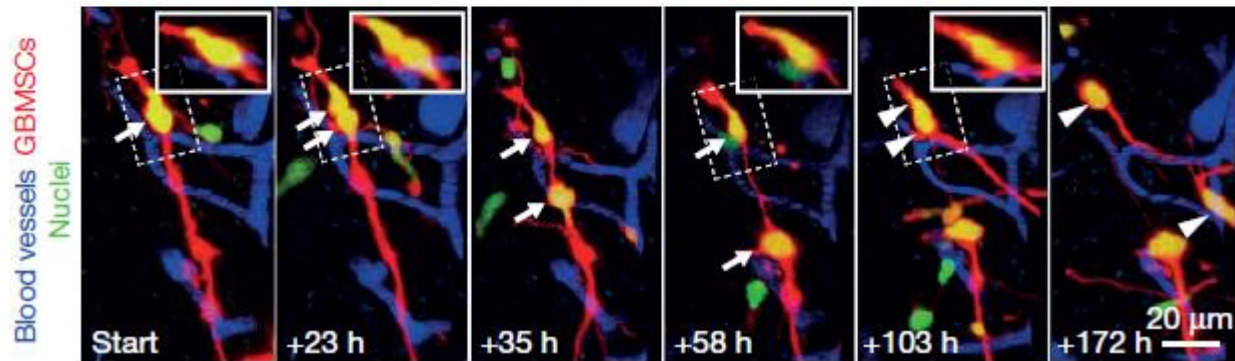
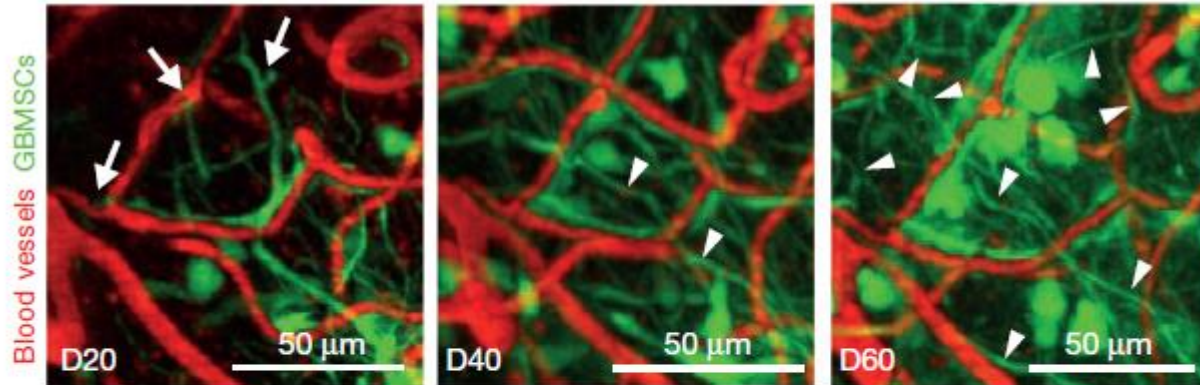


Collective invasion through cellular networks in GBM  
(Tumor microtubes)

# Tumor microtubes: thin cellular protrusions between glioma cells

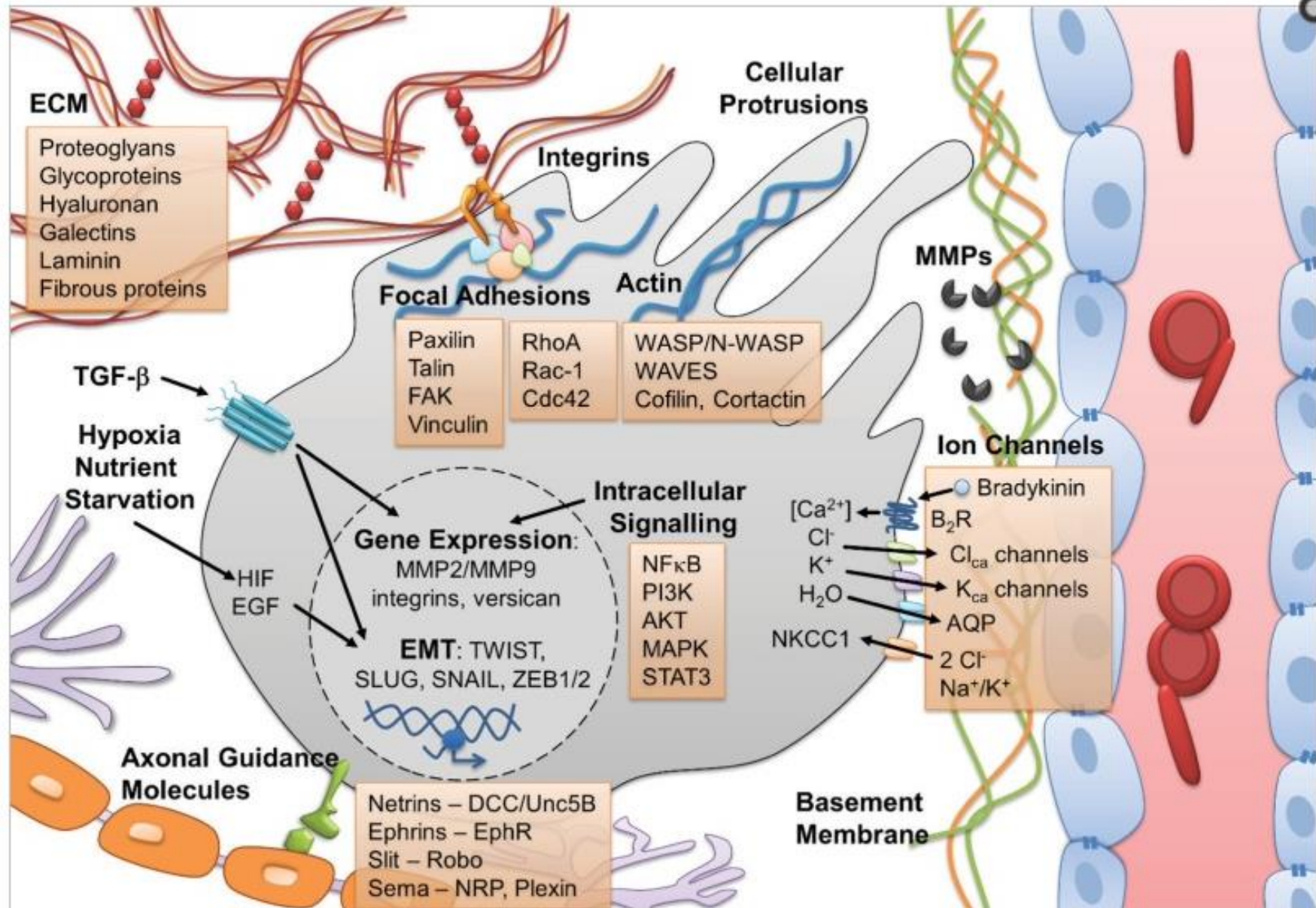
- Facilitate invasion
- Increase tumor cell resistance

Protrusions extending into the brain parenchyma

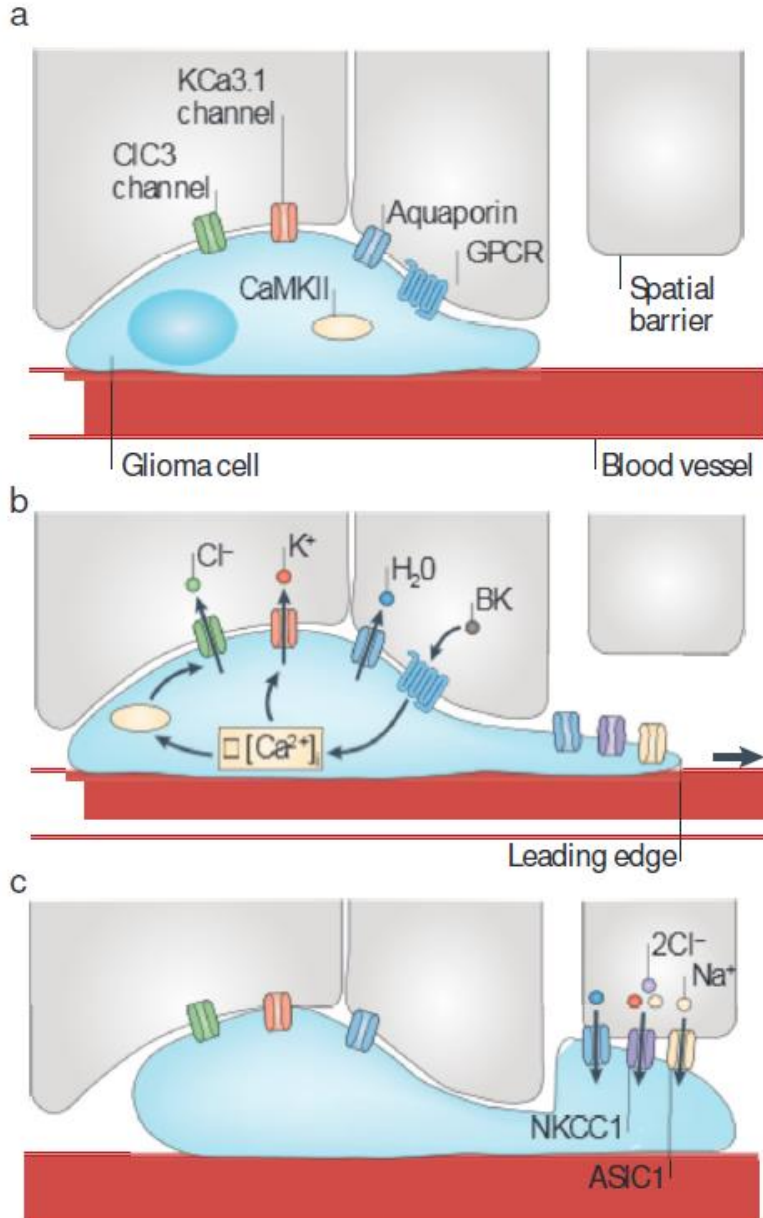


Travel of nuclei after nuclear division in cellular protrusions of migrating cells

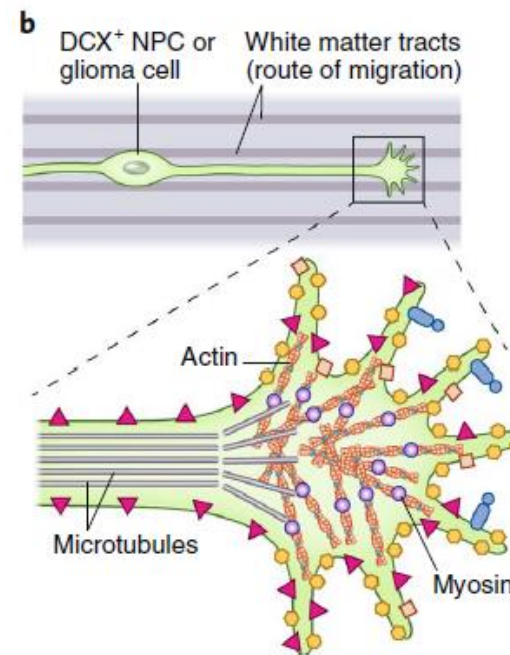
# Molecular mechanisms of glioma cell invasion



# Ca<sup>2+</sup> signaling and role of cytoskeleton

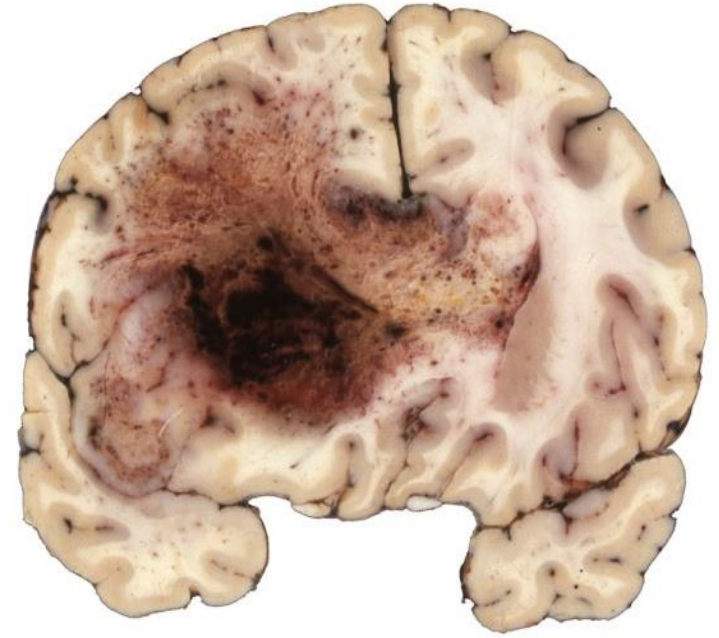


- Intracellular Ca<sup>2+</sup> increase leads to opening of channels (Cl<sup>-</sup>, K<sup>+</sup> channels, aquaporins) and water efflux, volume loss allowing to squeeze into small spaces
- Followed by regain of volume through ion influx



- Tumor microtubes at the invasive front (similar to growth cones during axon elongation)

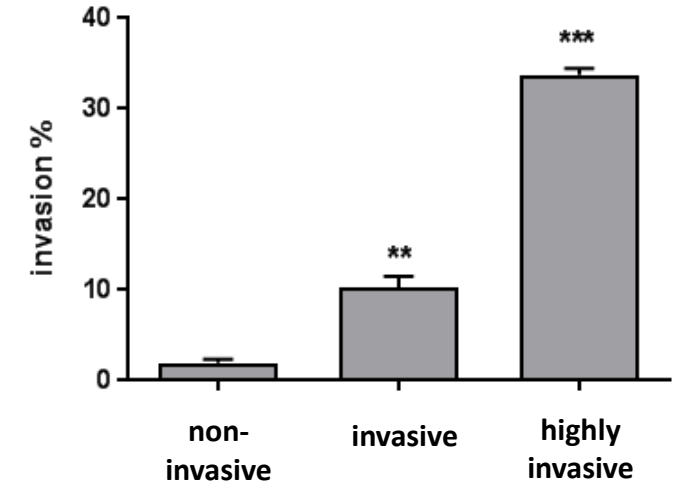
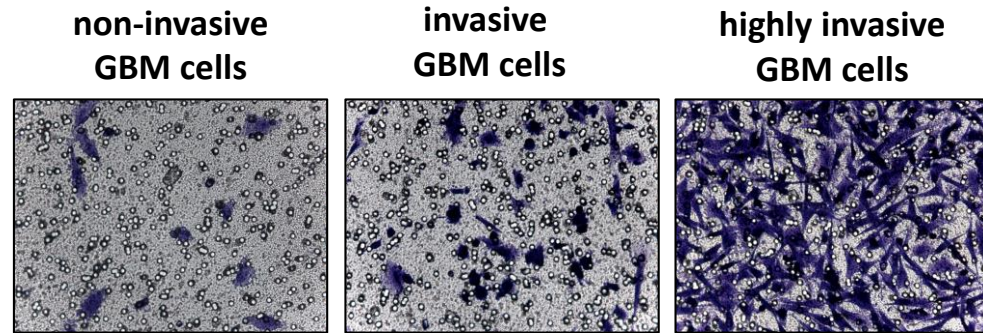
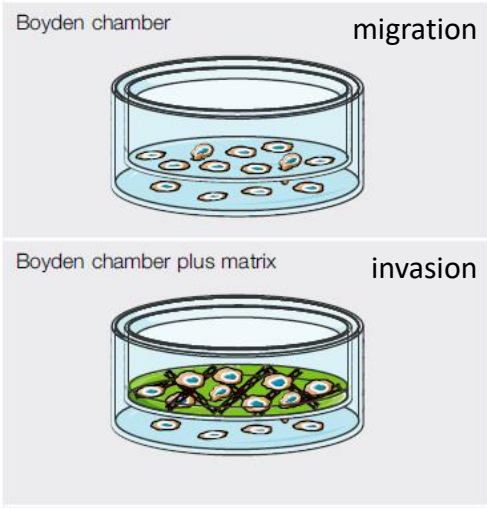
# Overview



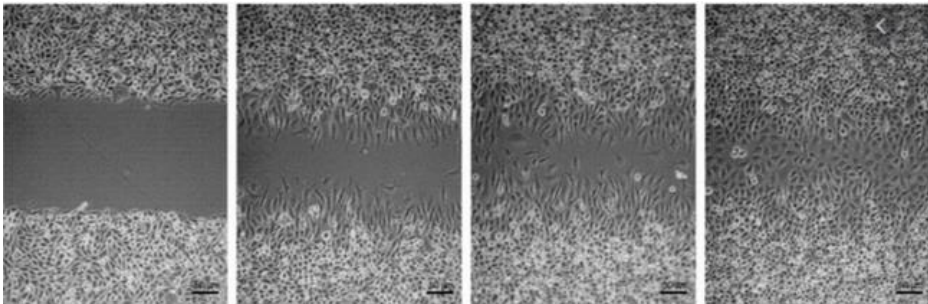
- Introduction to *Diffuse Glioma*
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# In vitro invasion assays in 2D format

## Transwell assay



## Scratch assay

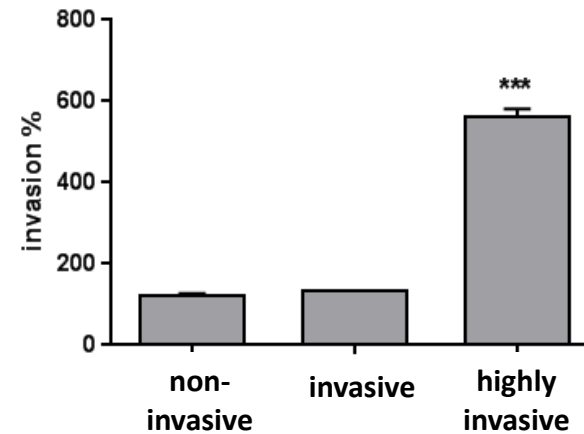
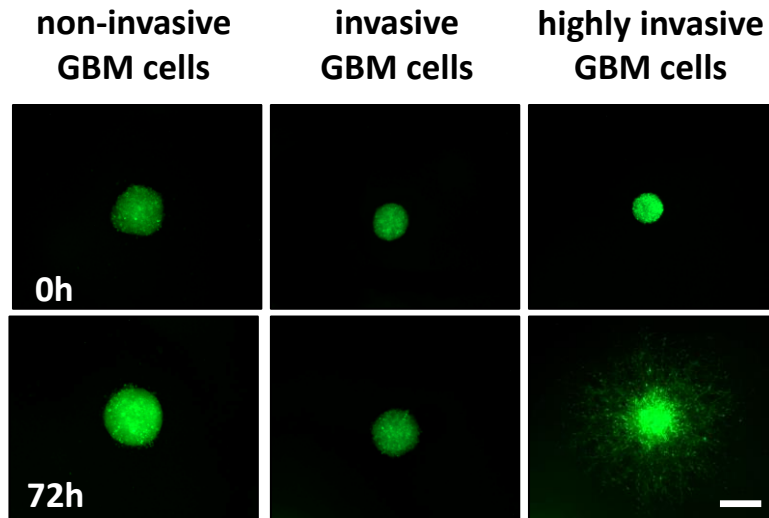
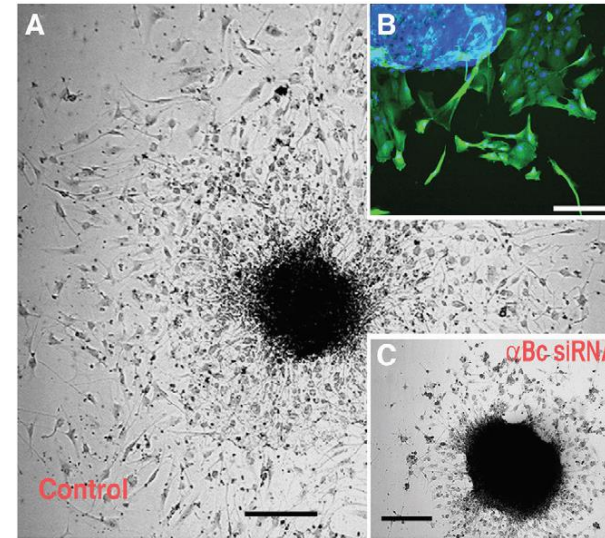


- Easy and straightforward
- Monolayer cultures
- Control for proliferation required!

# Invasion assay in 3D format: tumor spheroids



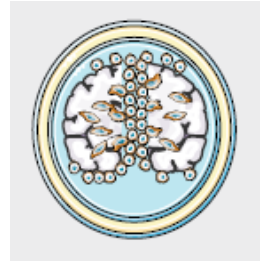
- on plastic (migration)
- in matrigel/ECM (invasion)





# Glioma invasion assays within *ex vivo* brain tissue

## Organotypic brain slice cultures



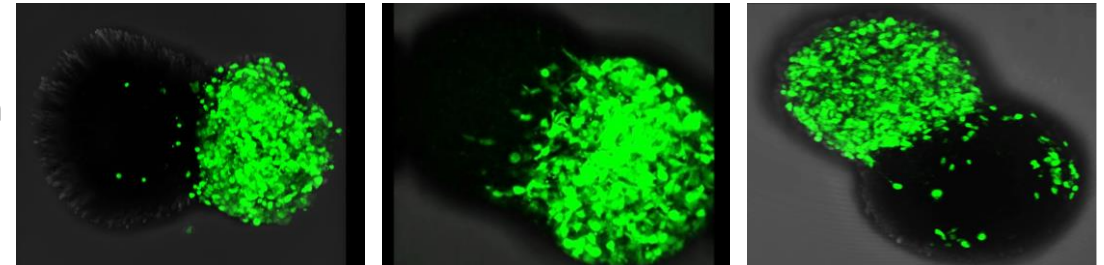
## Brain organoids

non-invasive  
GBM cells

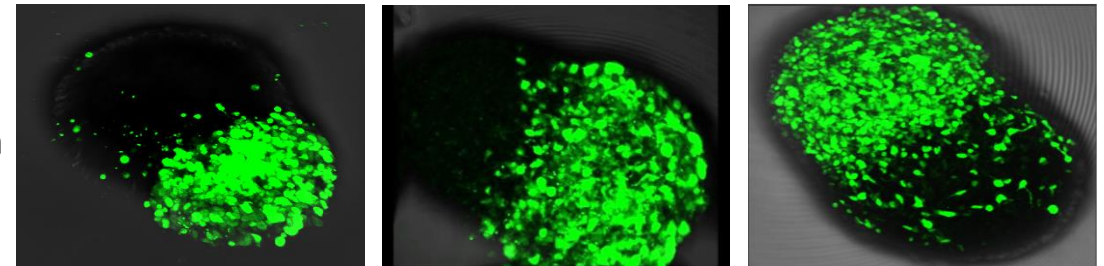
invasive  
GBM cells

highly-invasive  
GBM cells

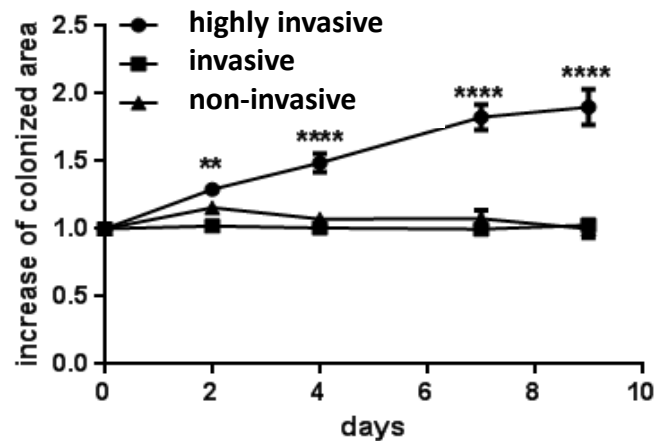
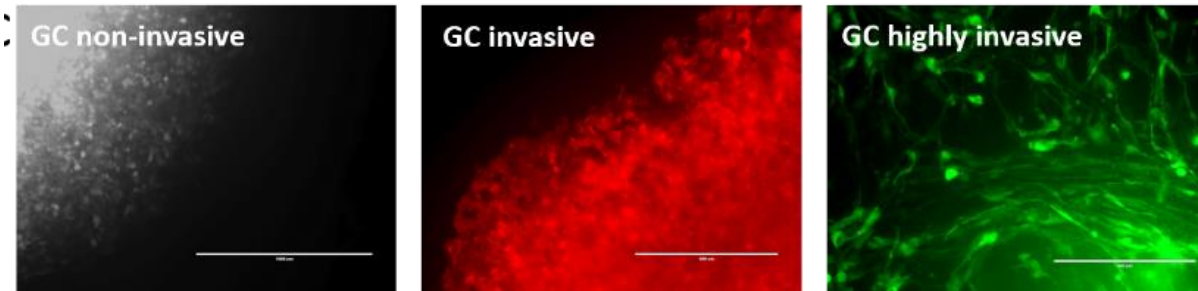
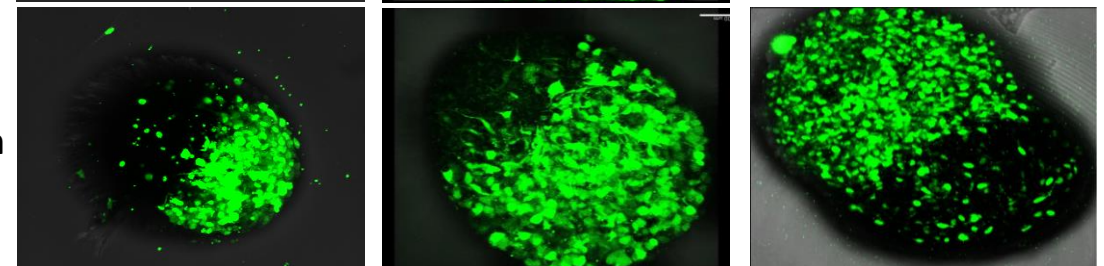
24h



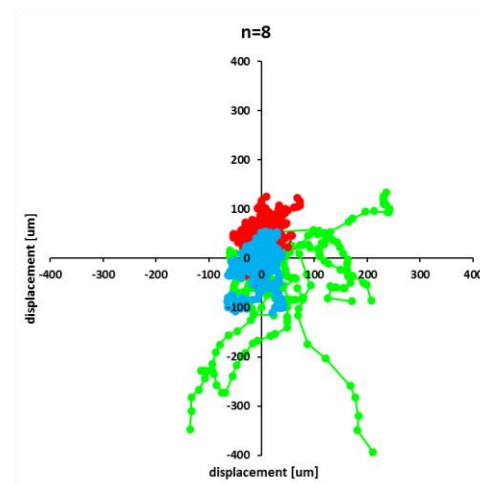
48h



72h



Colonized area

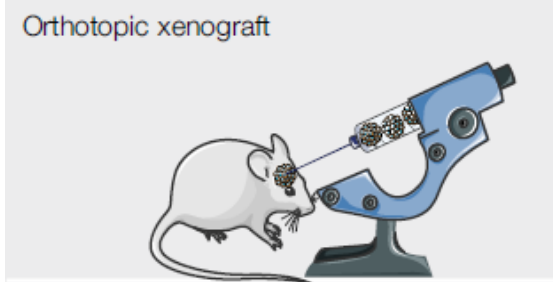


Single cell velocity

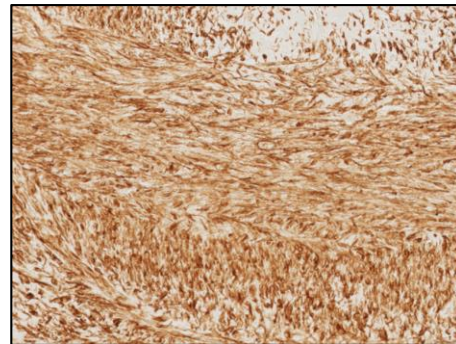
# Glioma invasion *in vivo*

## Immunohistochemistry

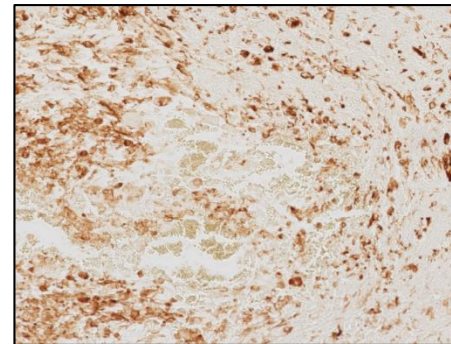
Orthotopic xenograft



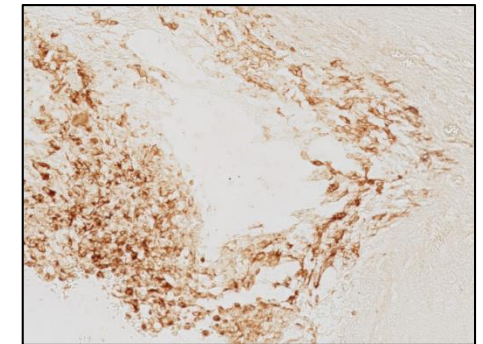
**Highly invasive GBM**



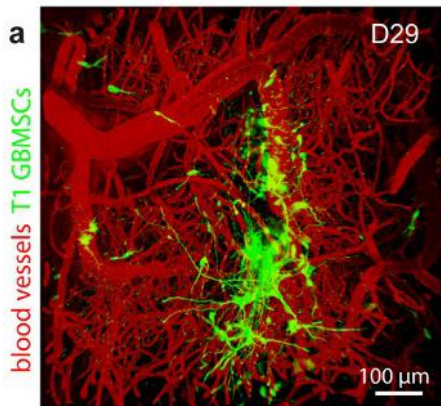
**Invasive GBM**



**Non-invasive GBM**



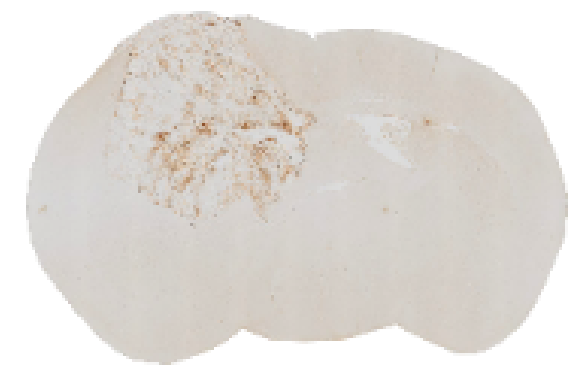
## Intravital microscopy



Endpoint: 20 weeks

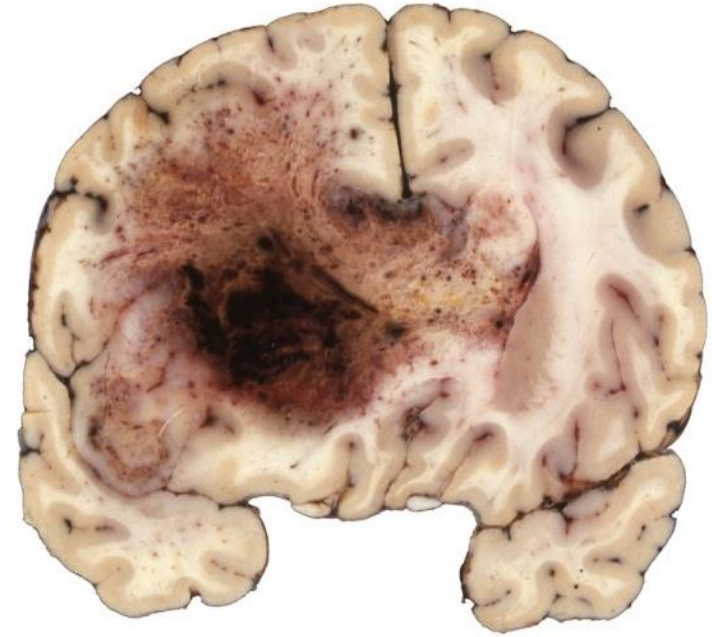


Endpoint: 8 weeks



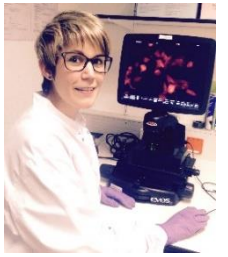
Endpoint: 5 weeks

# Overview

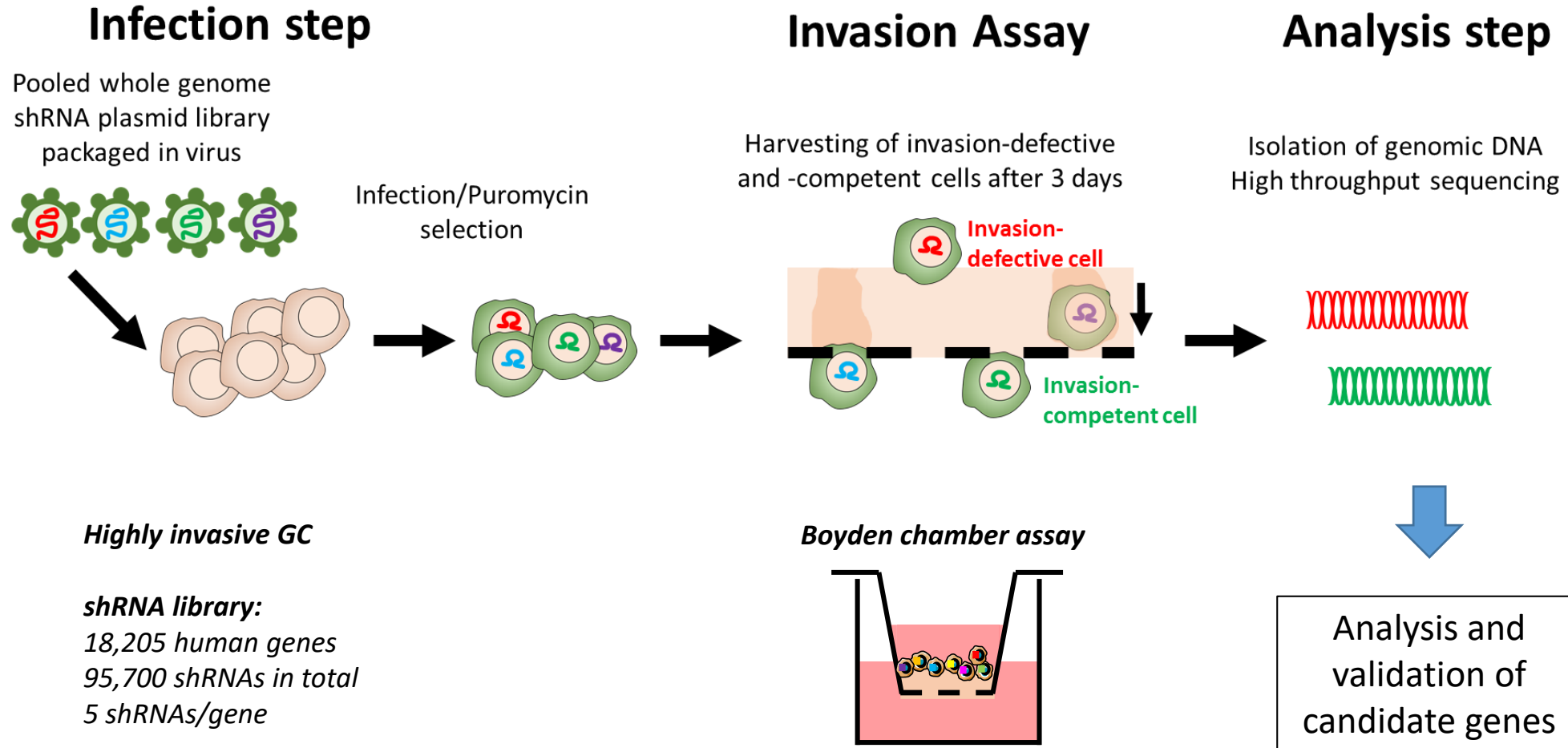


- Introduction to *Diffuse Glioma*
- Modes and routes of glioma cell invasion
- Experimental models to study glioma invasion: *in vitro*, *ex vivo*, *in vivo*
- **Identification of novel invasion essential genes**
- **Conclusion & Perspectives**

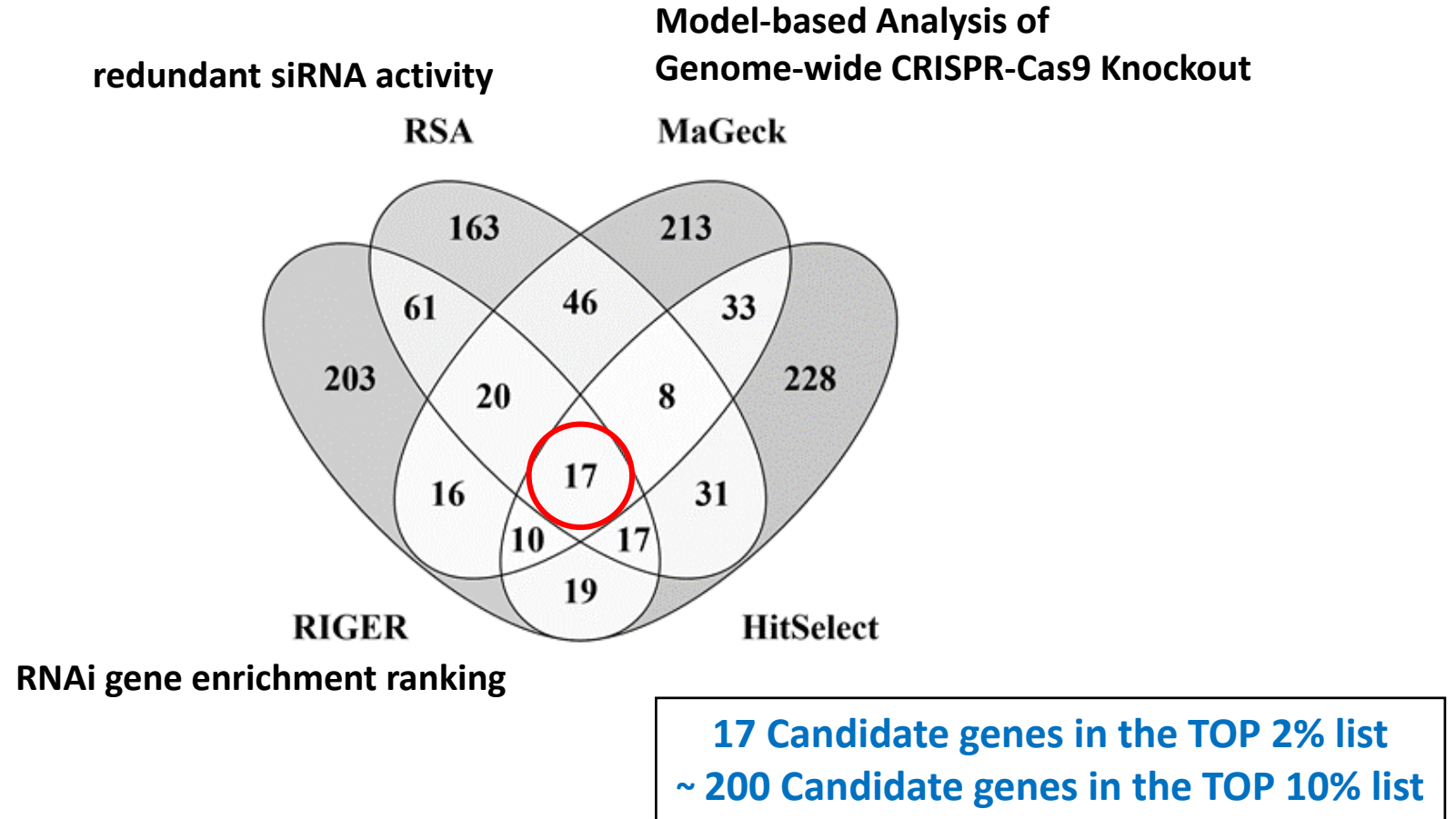
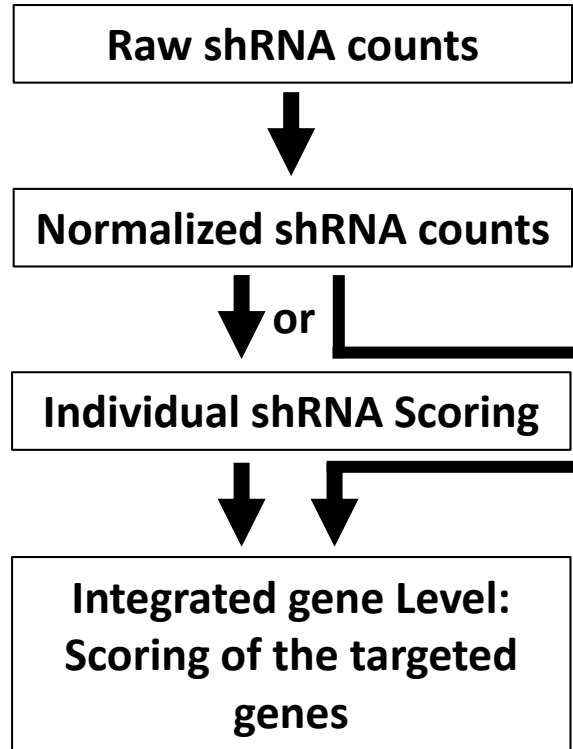
# RNA interference screen to identify invasion essential genes



Anne Schuster

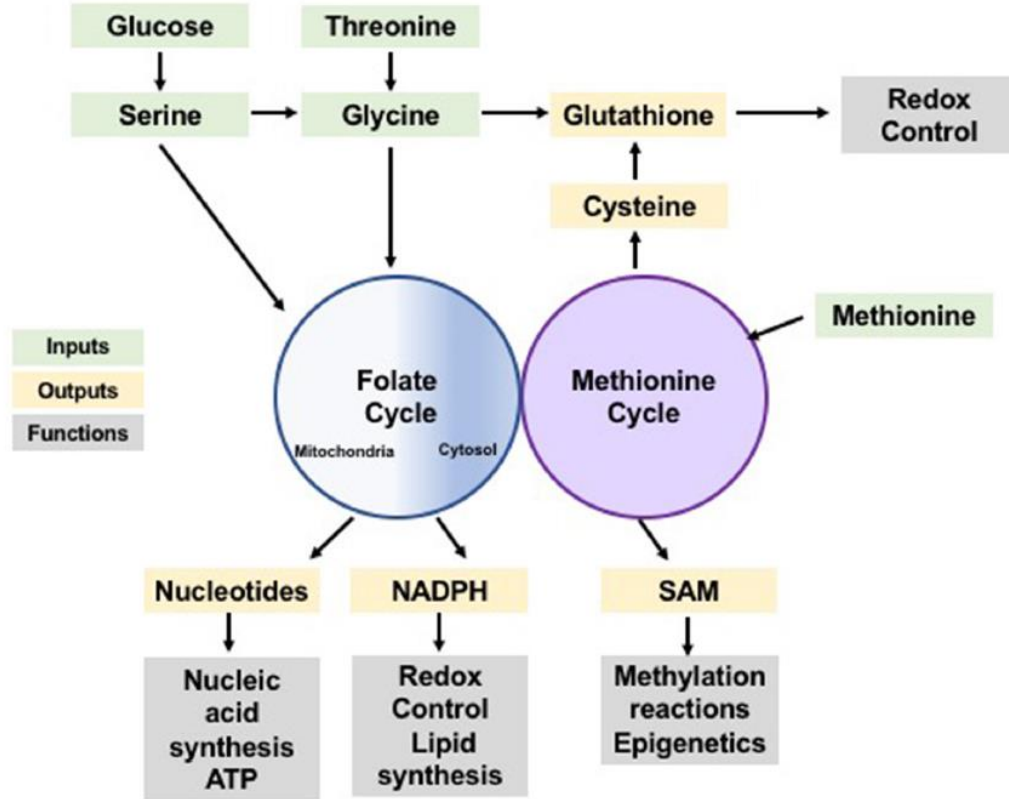


# Data analysis of shRNA screen



- **Metabolic enzyme: Methylene Tetrahydrofolate Dehydrogenase 1like (MTHFD1L)**
- **Novel transcriptional regulator: Zinc finger AN-type protein 3 (ZFAND3)**

# Serine One-Carbon Metabolism



**FIGURE 1** | One-carbon metabolism as a cellular process integrating nutrient status and availability. Glucose and amino acids input to the folate and methionine cycles (green) contributing with one-carbon units which can be used in anabolic synthesis of many building blocks, reducing species and co-factors (yellow). These synthesis products support a variety of cellular functions (gray) including synthesis of biomolecules, redox control and post-translational modification, sustaining cellular homeostasis.

Rosenzweig et al. 2018

SCIENCE ADVANCES | RESEARCH ARTICLE 2016

HEALTH AND MEDICINE

## Serine one-carbon catabolism with formate overflow

Johannes Meiser,<sup>1</sup> Sergey Tumanov,<sup>1,2</sup> Oliver Maddocks,<sup>2</sup> Christiaan Fred Labuschagne,<sup>1</sup>  
 Dimitris Athineos,<sup>1</sup> Niels Van Den Broek,<sup>1</sup> Gillian M. Mackay,<sup>1</sup> Eyal Gottlieb,<sup>1</sup> Karen Blyth,<sup>1</sup>  
 Karen Vousden,<sup>1</sup> Jurre J. Kamphorst,<sup>1,2</sup> Alexei Vazquez<sup>1\*</sup>

## ARTICLE

2017 doi:10.1038/nature23481

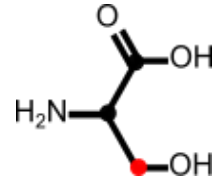
## Mammals divert endogenous genotoxic formaldehyde into one-carbon metabolism

Guillermo Burgos-Barragan<sup>1</sup>, Niek Wit<sup>1\*</sup>, Johannes Meiser<sup>2\*</sup>, Felix A. Dingler<sup>1</sup>, Matthias Pietzke<sup>2</sup>, Lee Mulderrig<sup>1</sup>,  
 Lucas B. Pontel<sup>1</sup>, Ivan V. Rosado<sup>3</sup>, Thomas F. Brewer<sup>4</sup>, Rebecca L. Cordell<sup>5</sup>, Paul S. Monks<sup>5</sup>, Christopher J. Chang<sup>4</sup>,  
 Alexei Vazquez<sup>2</sup> & Ketan J. Patel<sup>1,6</sup>

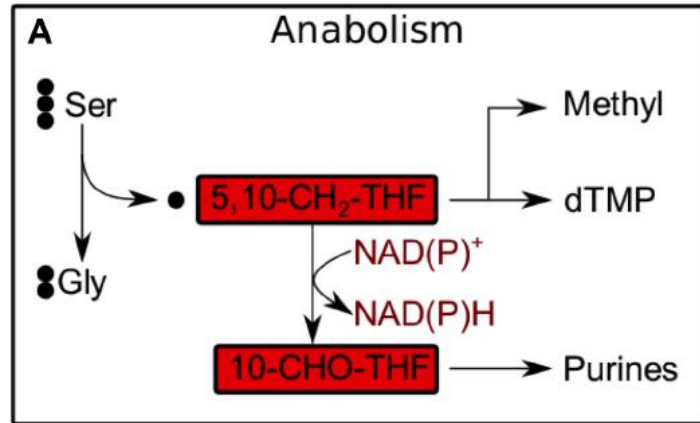


# A novel route for serine catabolism coupled to ATP synthesis and formate release

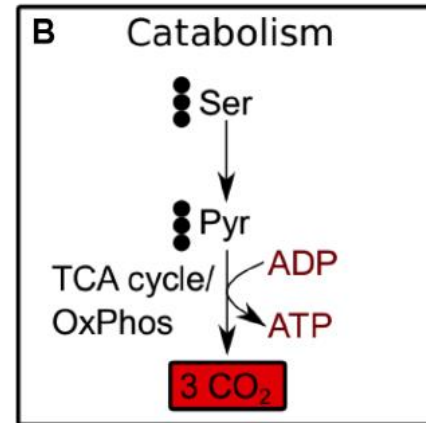
amino acid serine



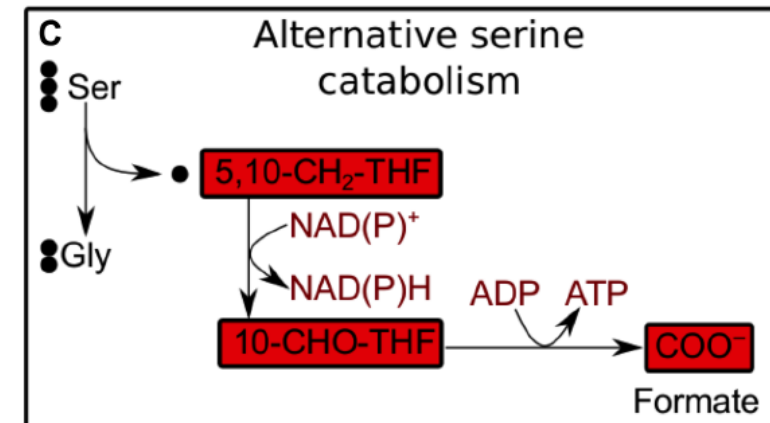
**Novel catabolic pathway**



**Nucleotide synthesis**



**ATP production**

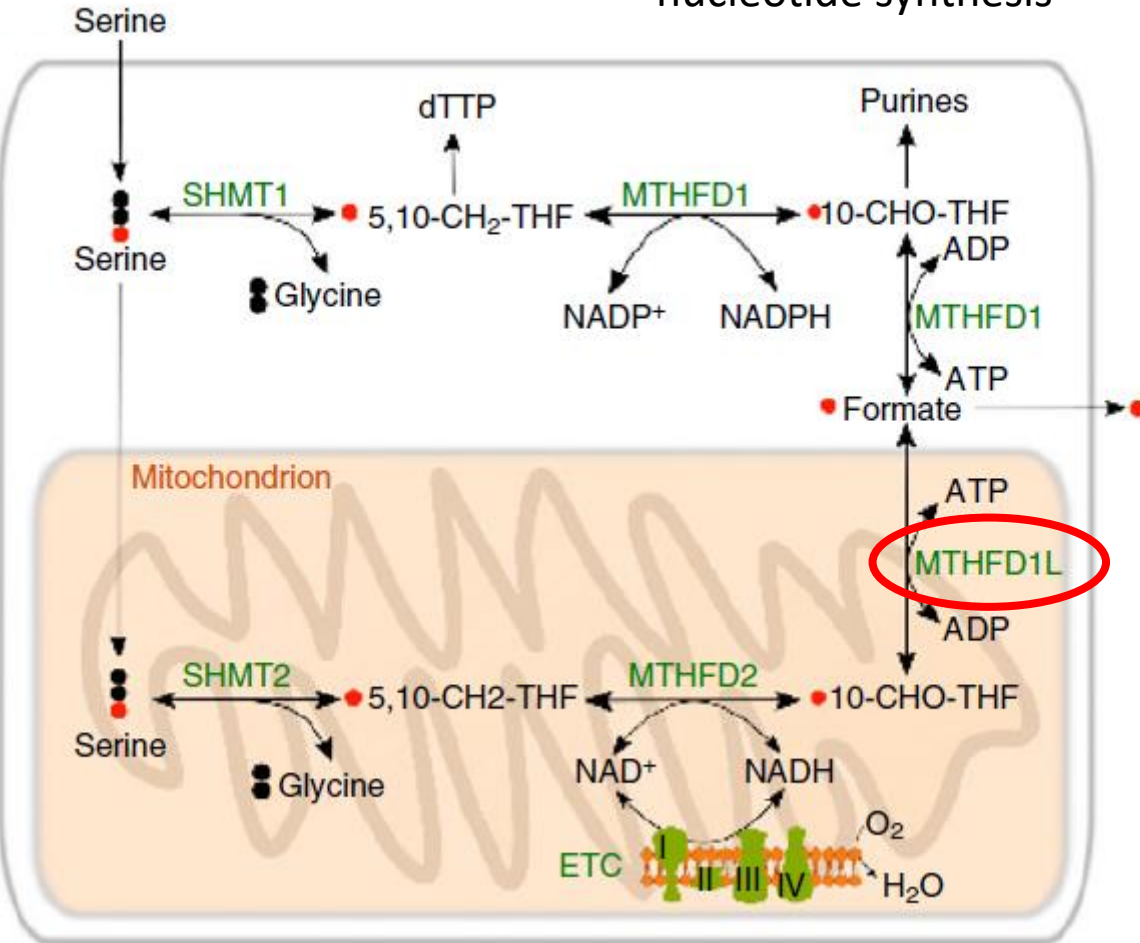


**ATP production and  
formate release**

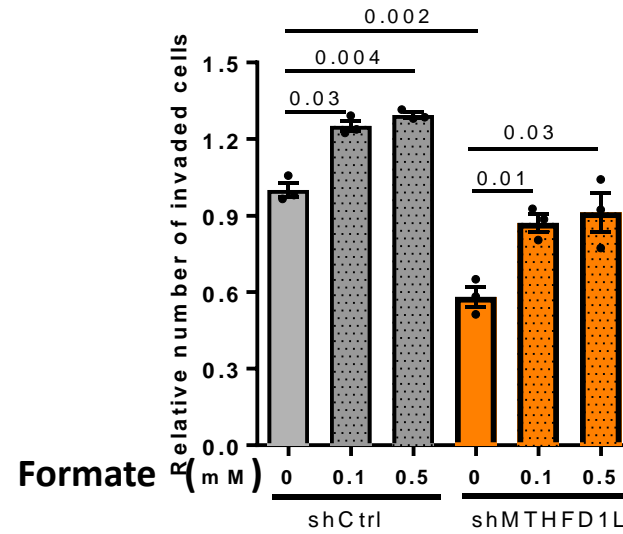
# MTHFD1L knockdown leads to reduced invasion, which is rescued by formate

## Folate cycle

Anabolic pathway:  
nucleotide synthesis

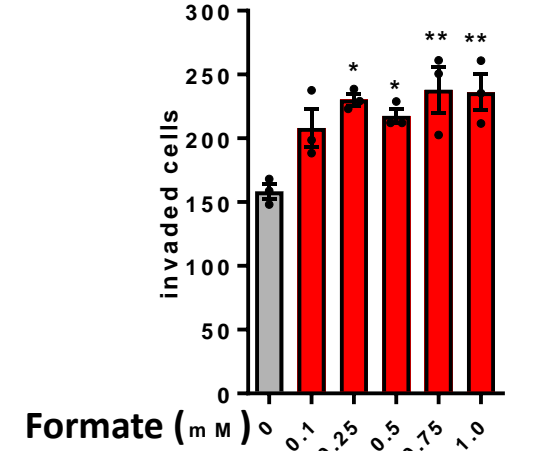


## MTHFD1L-KD & rescue



highly invasive GSC

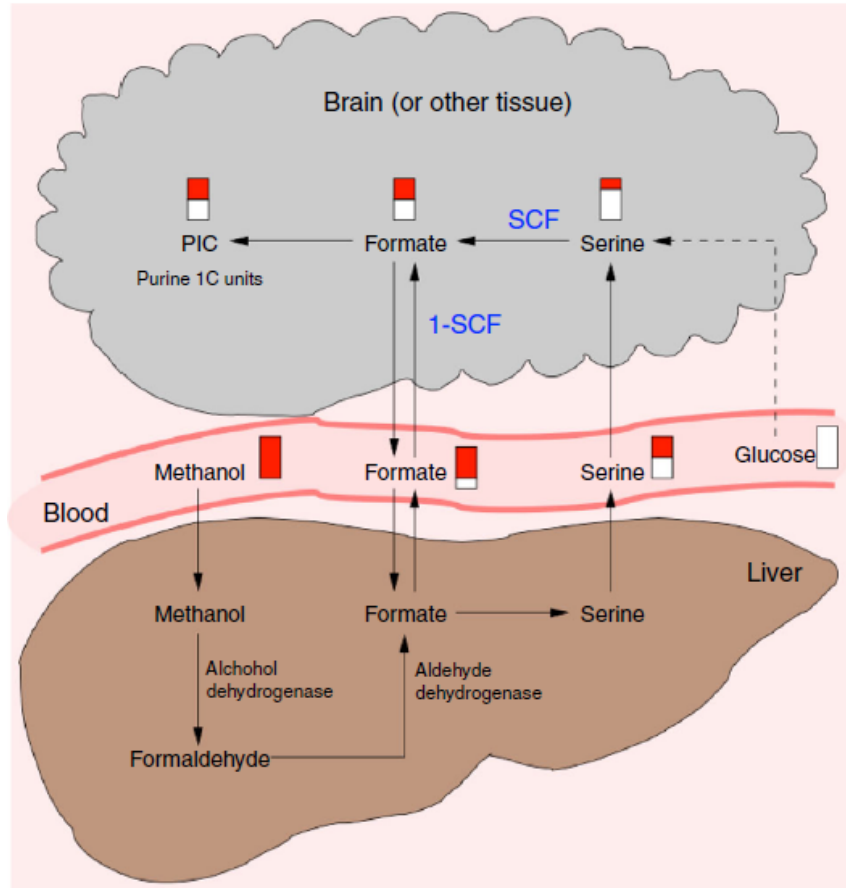
## Formate supply



Non-invasive GSC

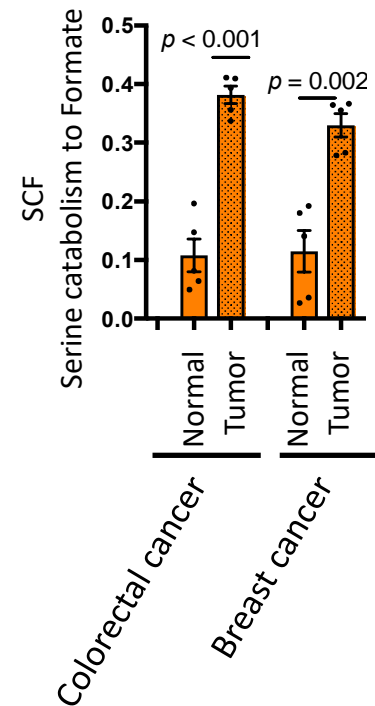


# Serine catabolism is increased in tumors *in vivo*

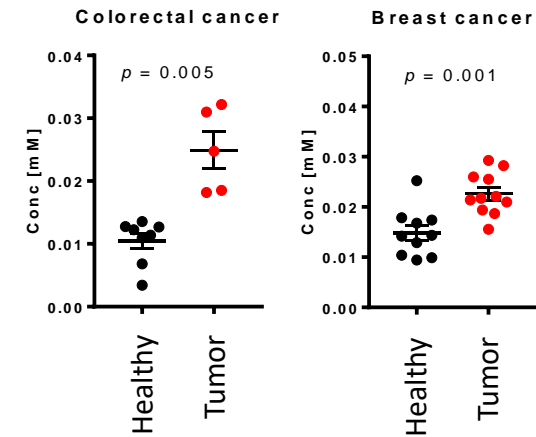


*in vivo* Metabolic Flux Analysis  
in GEMMs

## Increased formate in tumors

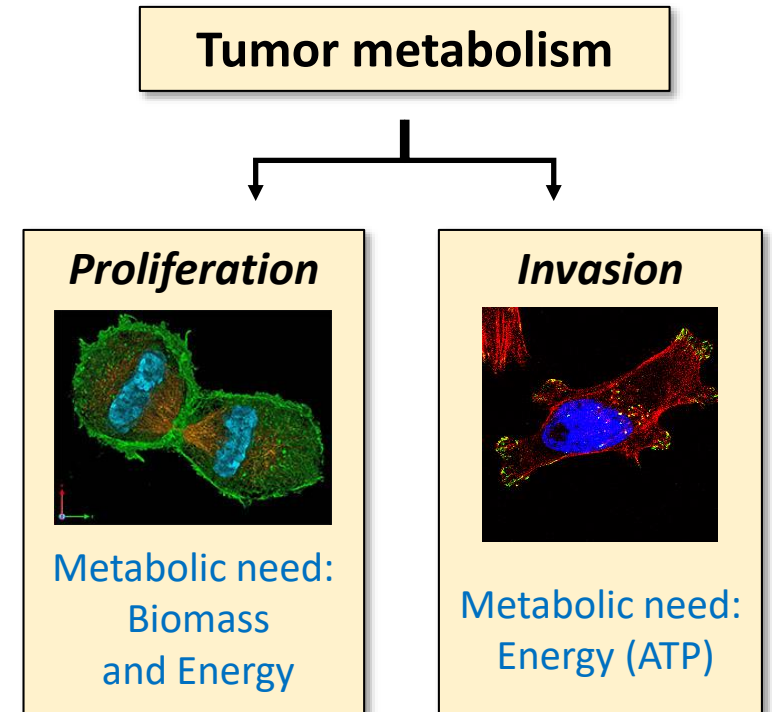


## Increased formate in blood

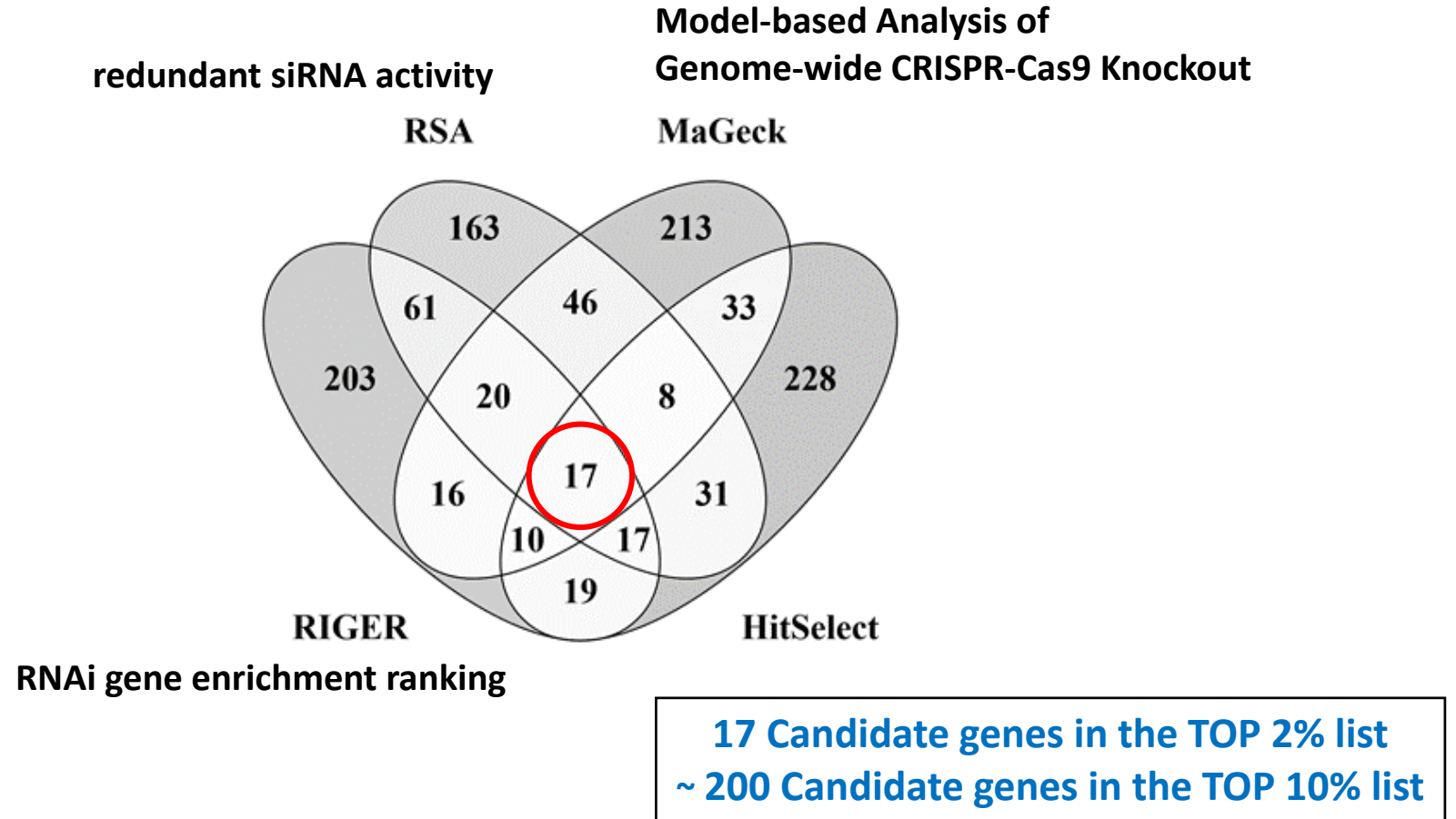
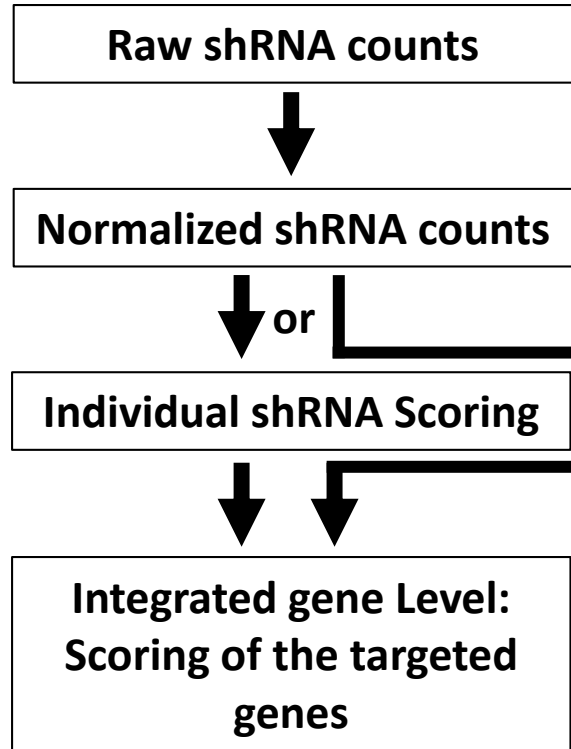


# Novel role of metabolism in tumor cell invasion

- ✓ **MTHFD1L** activity promotes glioma invasion through formate release
- ✓ **Formate** accumulates in tumors and in plasma of tumor-bearing mice – role in metastatic process remains to be elucidated
- ✓ Tumor metabolism not only regulates proliferation, but also invasion (energy driven processes)
- ✓ MTHFD1L is localized to **mitochondria**, which may provide **local ATP production** at the migratory tip of tumor cells (cf tumor microtubules)



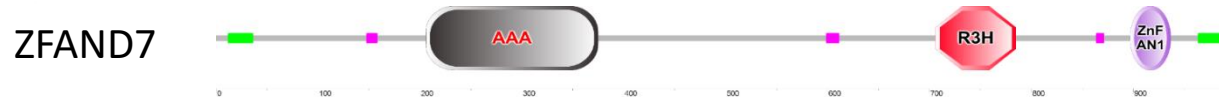
# Data analysis of shRNA screen



- Metabolic enzyme: Methylene Tetrahydrofolate Dehydrogenase 1like (**MTHFD1L**)
- Putative transcription factor: Zinc finger AN-type protein 3 (**ZFAND3**)

# ZFAND3: Zinc Finger AN1-Type containing 3 domain

## 8 family members



### ZFAND3 (or testis expressed sequence 27, *TEX27*)

- AN1 and A20 domain
- essential for spermatogenesis in mice
- associated with development of Diabetes Type II
- exact function remains unknown

### ZFAND1 Recruits p97 and the 26S Proteasome to Promote the Clearance of Arsenite-Induced Stress Granules

### Integrated bioinformatics analysis reveals role of the LINC01093/miR-96-5p/ZFAND5/NF-κB signaling axis in hepatocellular carcinoma

YAHUI ZHENG<sup>a</sup>, KANGKANG YU<sup>a</sup>, CHONG HUANG, LU LIU, HAO ZHAO, MEISI HUO and JUBO ZHANG

Department of Infectious Diseases, Huashan Hospital, Fudan University, Shanghai 200040, P.R. China

Received February 24, 2019; Accepted August 19, 2019

### ZFAND5/ZNF216 is an activator of the 26S proteasome that stimulates overall protein degradation

Donghoon Lee<sup>a</sup>, Shinichi Takayama<sup>b</sup>, and Alfred L. Goldberg<sup>a,1</sup>

<sup>a</sup>Department of Cell Biology, Harvard Medical School, Boston, MA 02115; and <sup>b</sup>Research Division, Chugai Pharma USA, Berkeley Heights, NJ 07922

SCIENTIFIC REPORTS

OPEN Characterizing the *ZFAND3* gene mapped in the sex-determining locus in hybrid tilapia (*Oreochromis spp.*)

Received: 14 January 2016  
Accepted: 18 April 2016  
Published: 03 May 2016

Keyi Ma<sup>1</sup>, Minghui Liao<sup>1</sup>, Feng Liu<sup>2</sup>, Baoqing Ye<sup>1</sup>, Fei Sun<sup>1</sup> & Gen Hua Yue<sup>1,2,3</sup>



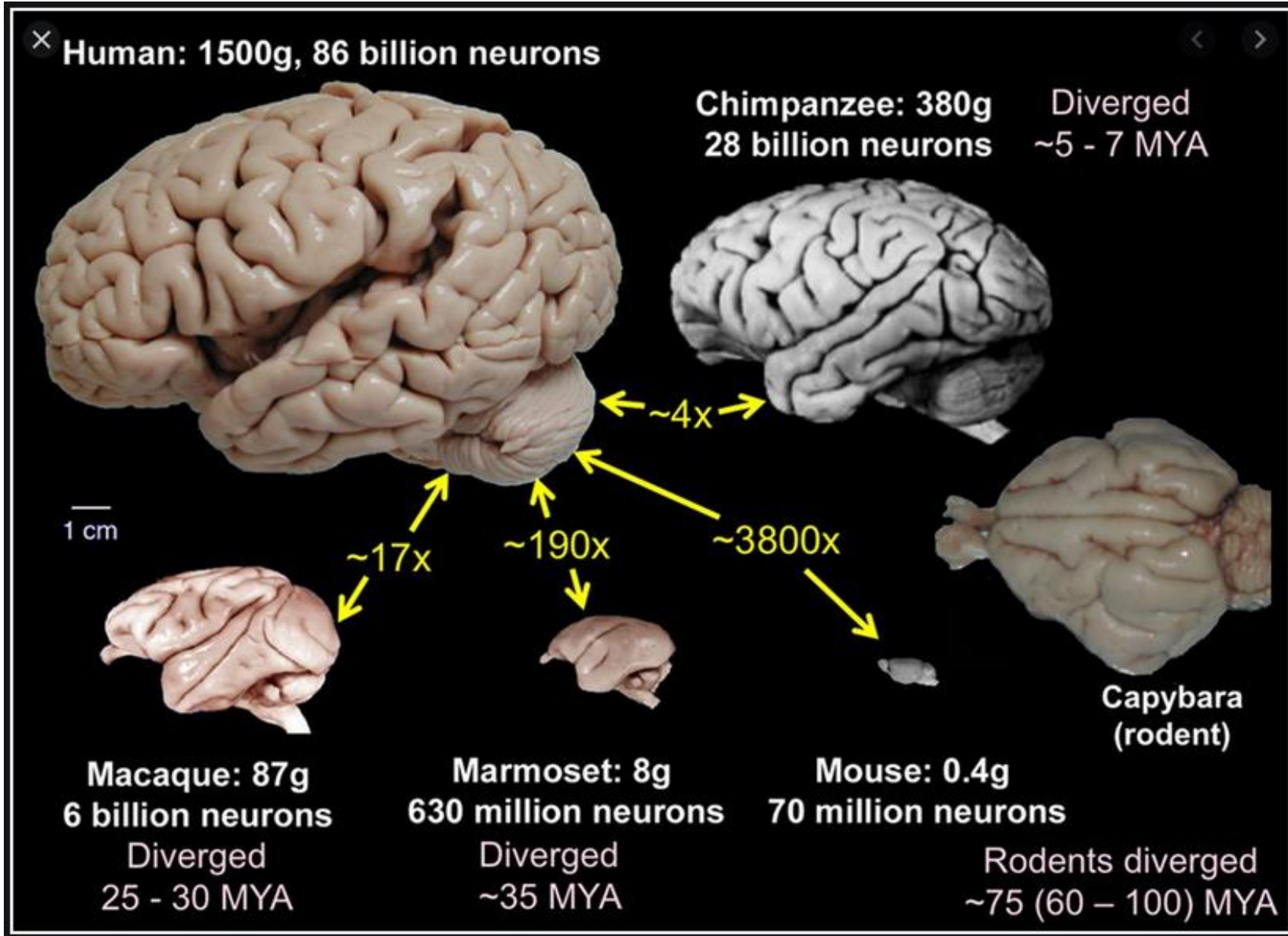
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## Summary and Perspectives

- ✓ Glioma invasion is a major **clinical challenge**
- ✓ Specific routes and modes of glioma invasion in the **brain microenvironment**
- ✓ RNA interference screen reveals multiple genes regulating glioma cell invasion
- ✓ **MTHFD1L** activity promotes GBM invasion through formate release
- ✓ **ZFAND3**: a novel transcriptional regulator of GBM invasion
- ✓ Therapeutic targeting remains a challenge, but may become feasible with **tumor-specific targets**

Is there a role for **mathematical modeling**?

- Modeling invasion in the brain taking into account different parameters
- Predict potential of recurrence at distant sites?
- Is invasion in mouse and human brain comparable?
- ...



# Acknowledgements

## NORLUX Neuro-Oncology Laboratory, LIH

**Anna Golebiewska**

Anne Dirkse

Ann-Christin Hau

**Anais Oudin**

Virginie Baus

Vanessa Barthelemy

Monika Dieterle

Amandine Bernard

**Virginie Neirinckx**

**Anne Schuster**

Sabrina Fritah

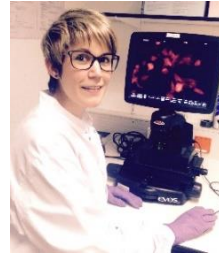
Andrés Cano

**Carina Fabian**

Yahaya Yabo

Hugo Dosquet

**Eliane Klein**



**Rolf Bjerkvig**

Luxembourg Institute of Health  
& University of Bergen, Norway



**Johannes Meiser**

Tumor Metabolism Group, Luxembourg  
Institute of Health

**Petr V. Nazarov, Arnaud Muller, Tony Kaoni**

Biostatistics/Bioinformatics, LIH, Luxembourg

**Alexei Vazquez**

Beatson Institute, Glasgow, UK

**Bjarne Winther Kristensen**

Department of Pathology, Odense Universitetshospital,  
Odense, Denmark

**Barbara Klink, Michel Mittelbronn**

Laboratoire national de santé, LNS, Luxembourg

**Frank Hertel**

Neurosurgery Department, Centre Hospitalier de  
Luxembourg, Luxembourg

**Christel Herold-Mende**

Department of Neurosurgery, University Hospital  
Heidelberg, Germany

**Hakan Hedman**

Radiation biology, University of Umeo, Sweden

