

# Molecular regulation of the critical steps to initiate brain metastasis

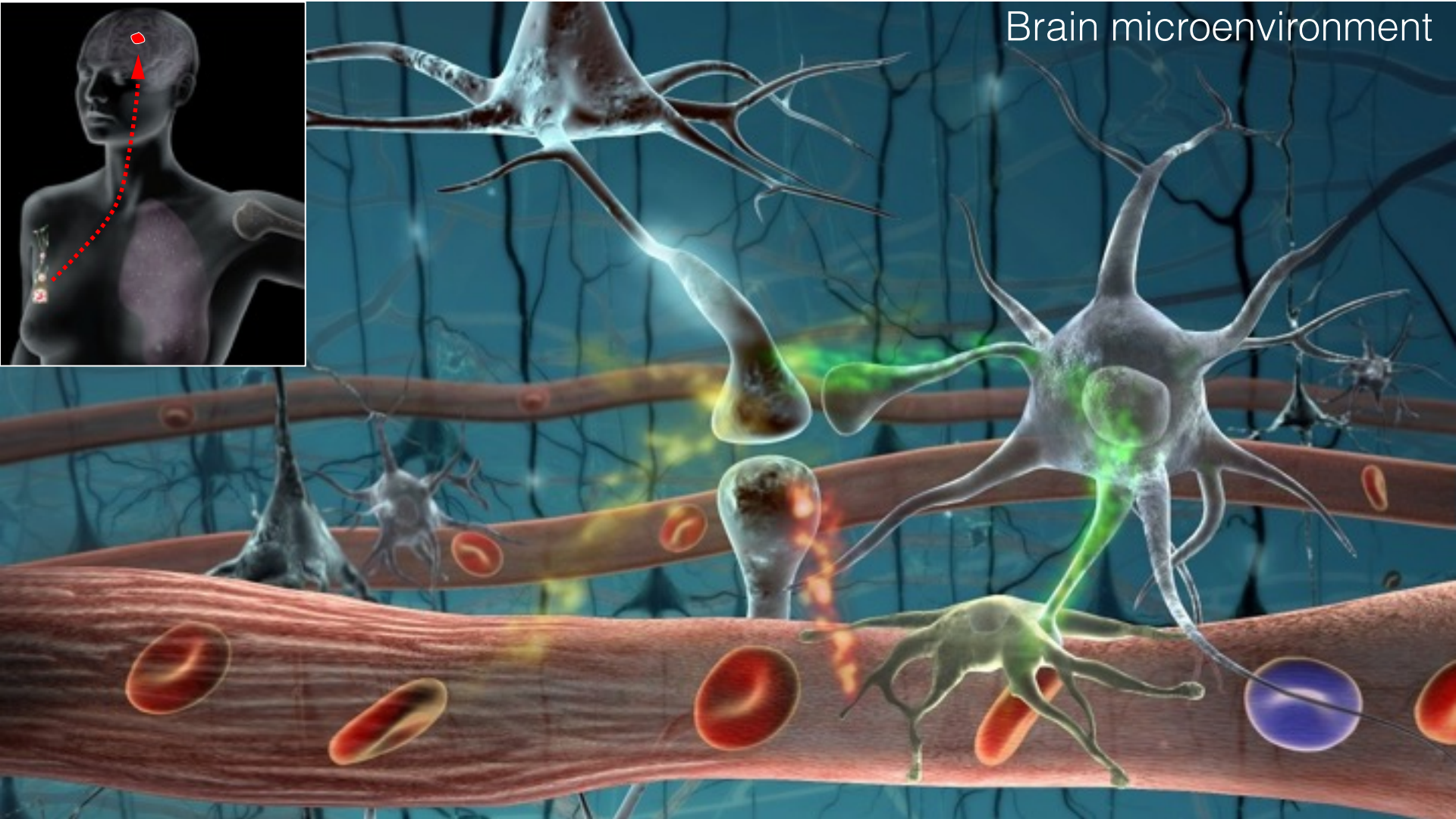
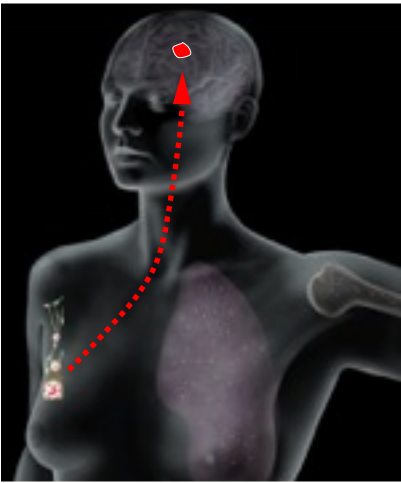
Manuel Valiente

***5<sup>th</sup> Brain Metastases  
Research and Emerging  
Therapies Conference***

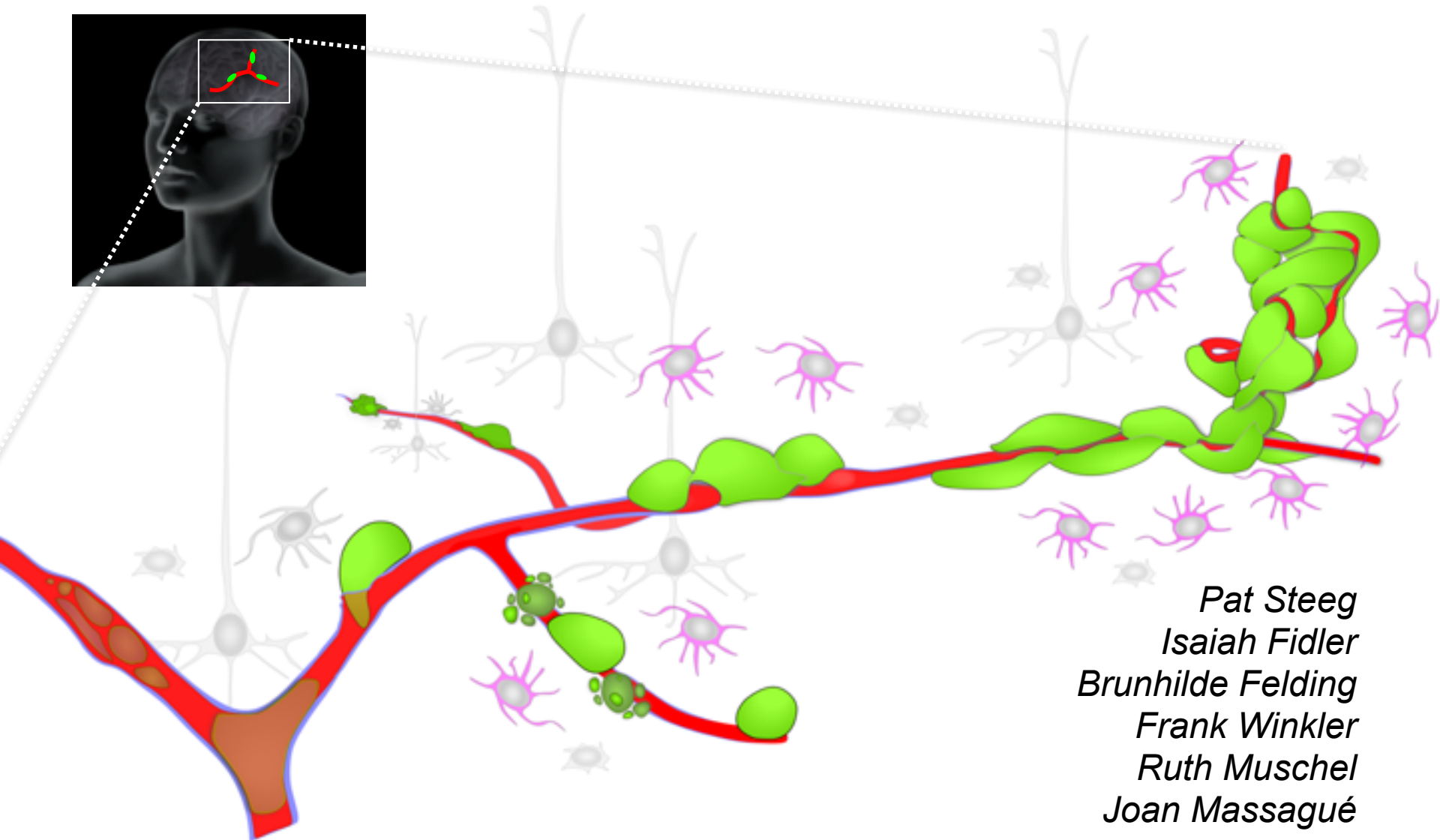
*Oct. 2nd-3th, 2015*

Facing an new microenvironment:

The need to find/ create a niche



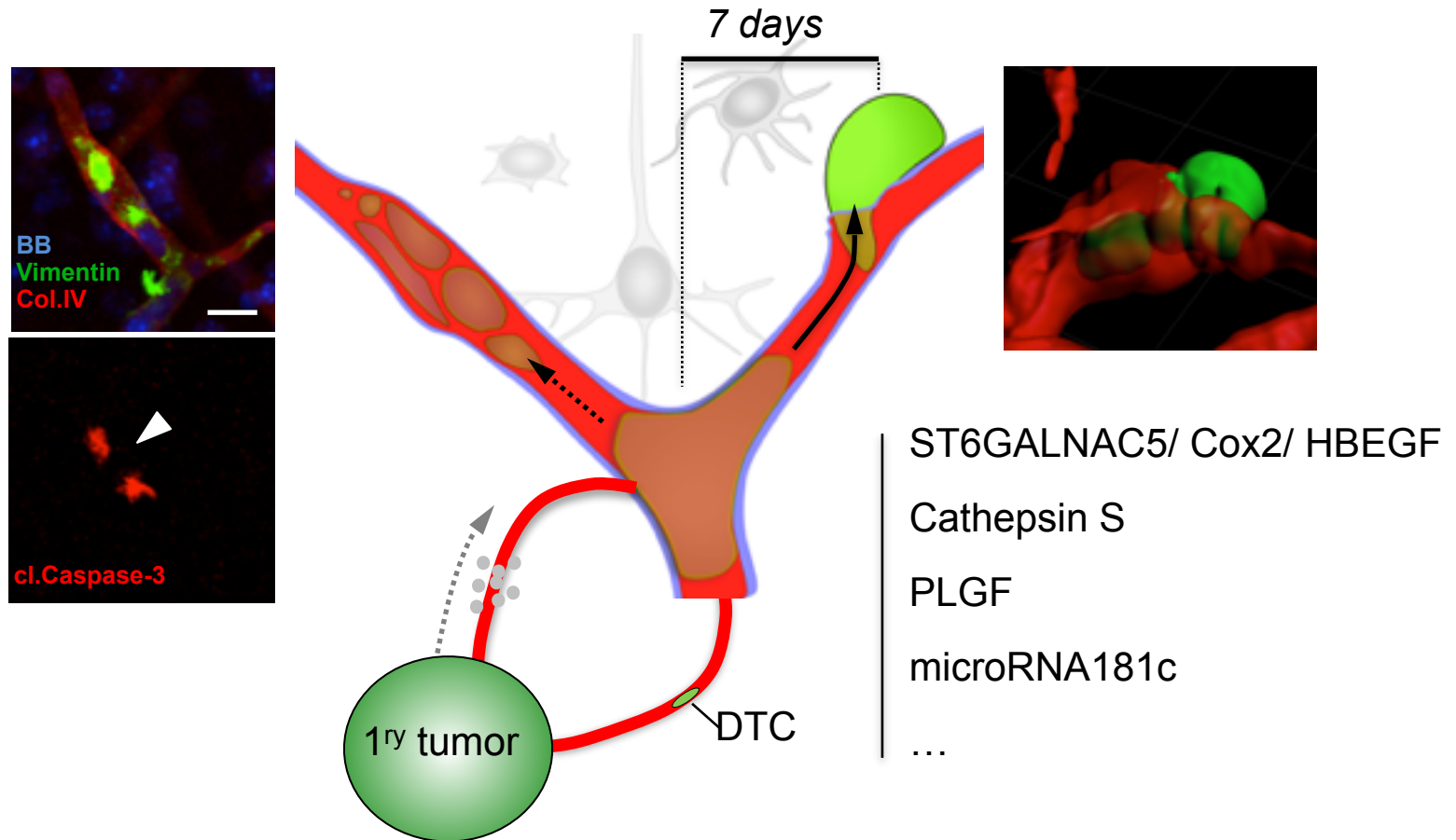
# First hours/ days of a brain metastatic cell



*Pat Steeg  
Isaiah Fidler  
Brunhilde Felding  
Frank Winkler  
Ruth Muschel  
Joan Massagué*

...

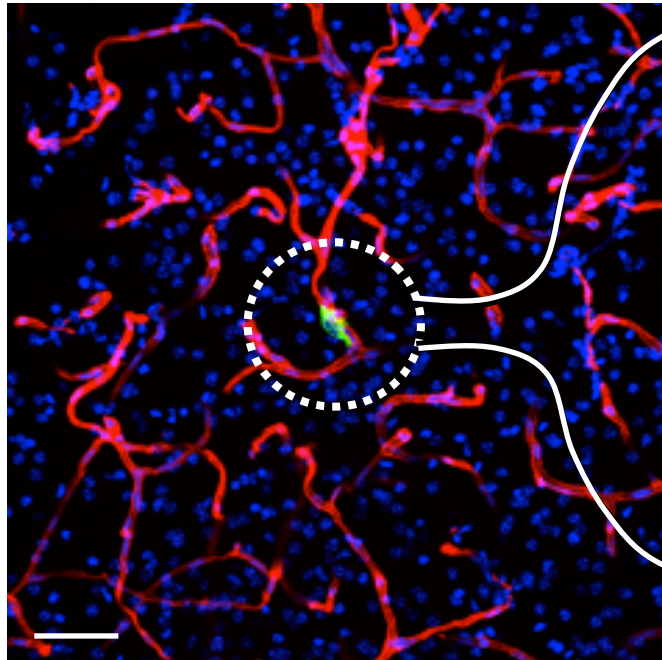
# Crossing the blood-brain barrier



**Knowledge of the mechanisms to cross the BBB  
is unlikely to be translated into clinical interventions**



# Metastasis initiating cell ?



↘ >90% perish



*Kienast et al. (2010) Nat. Med.*

*Heyn et al. (2006) Magn. Reson. Med*

*Valiente et al. (2014). Cell*

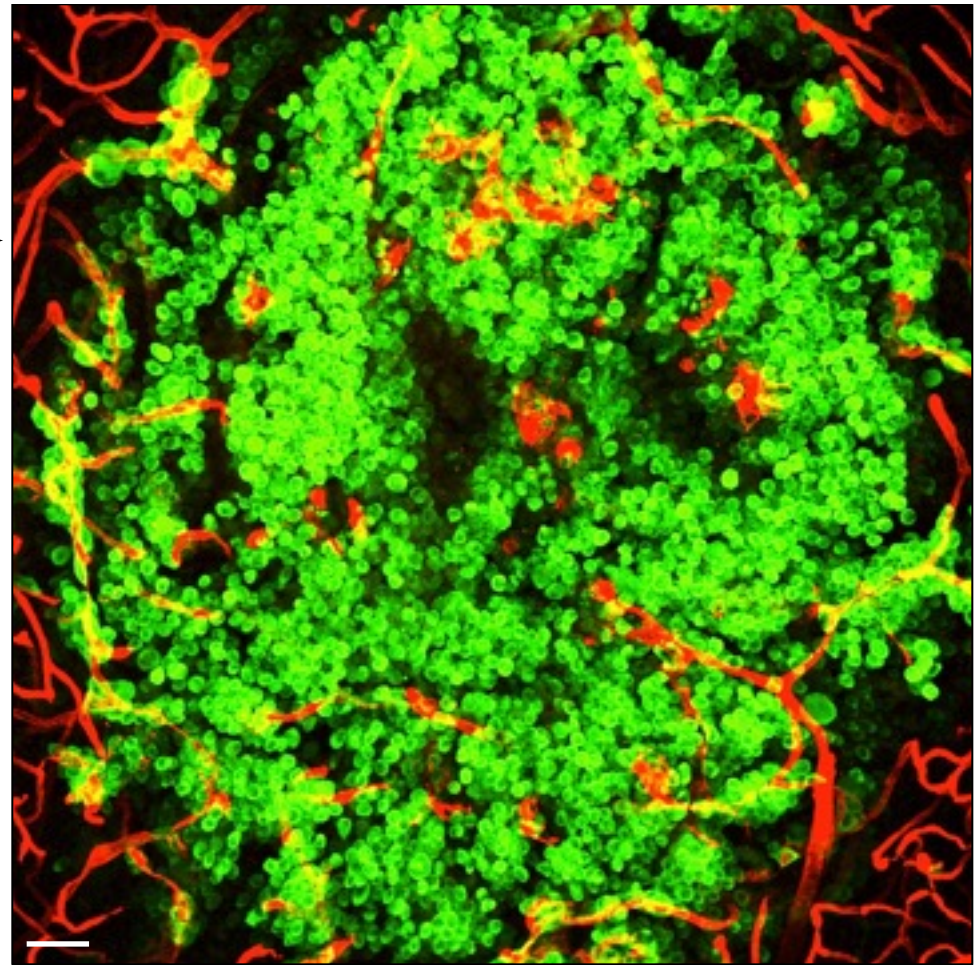
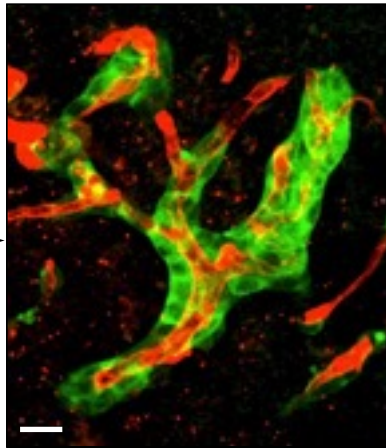
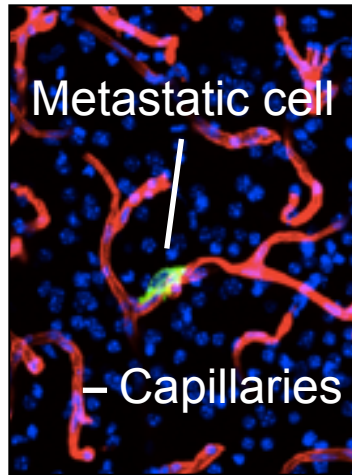
↘ <10% succeed

**Why do they dye and how they avoid it ?**

**How do they thrive?**

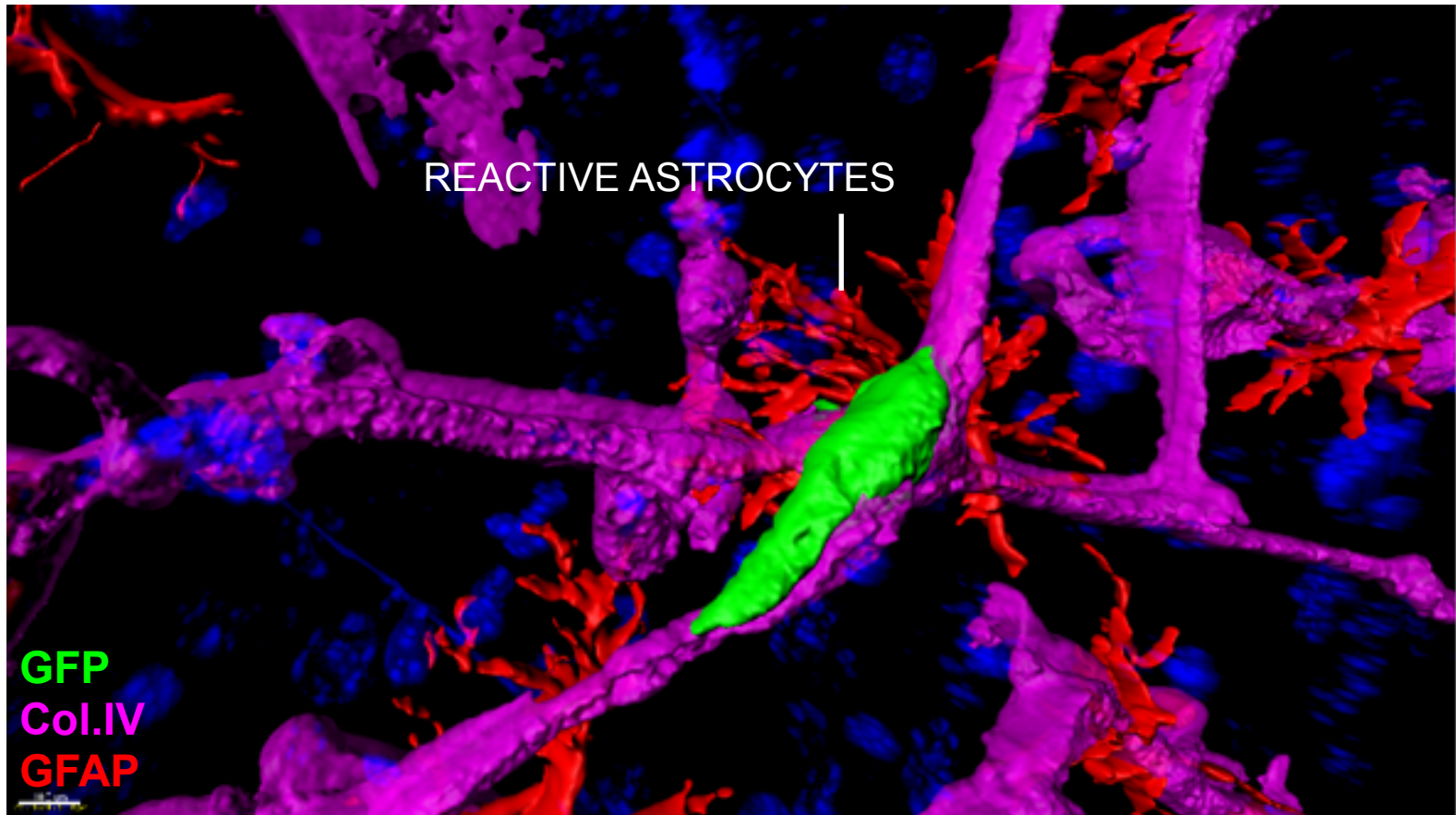
**Can they avoid dead without thriving?**

# When do they die?



MAXIMUM INEFFICIENCY

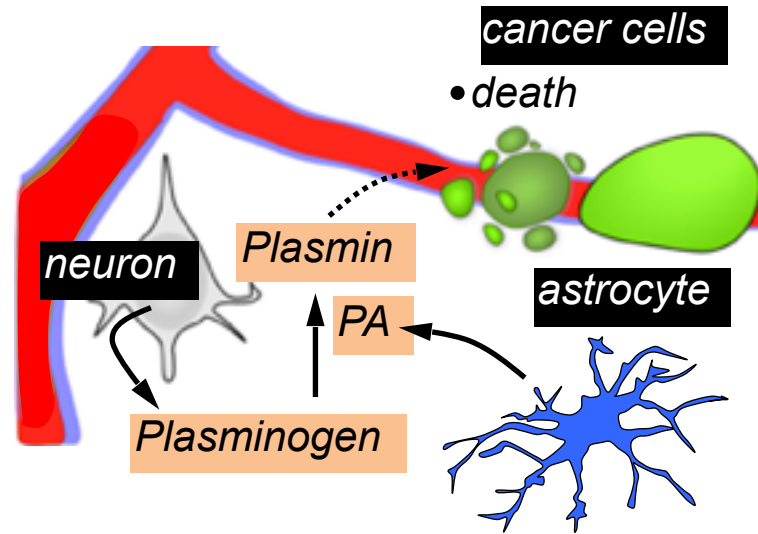
# Metastasis initiating cells: Not an easy soil ;



**Reactive microenvironment efficiently reduces  
the number of cancer cells**

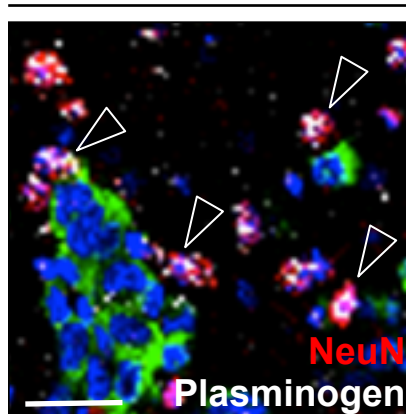


# A hostile microenvironment

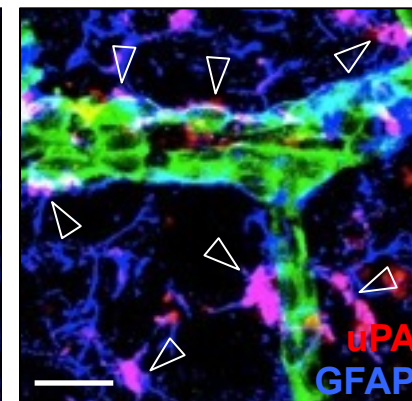
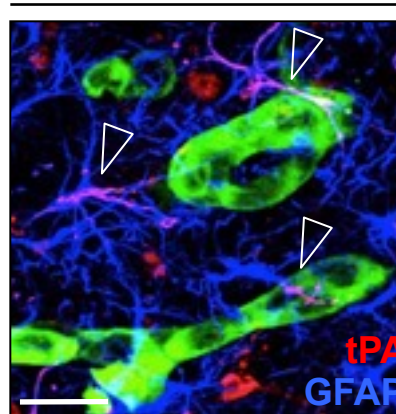


→ This defensive mechanism also in other brain injuries

Neurons

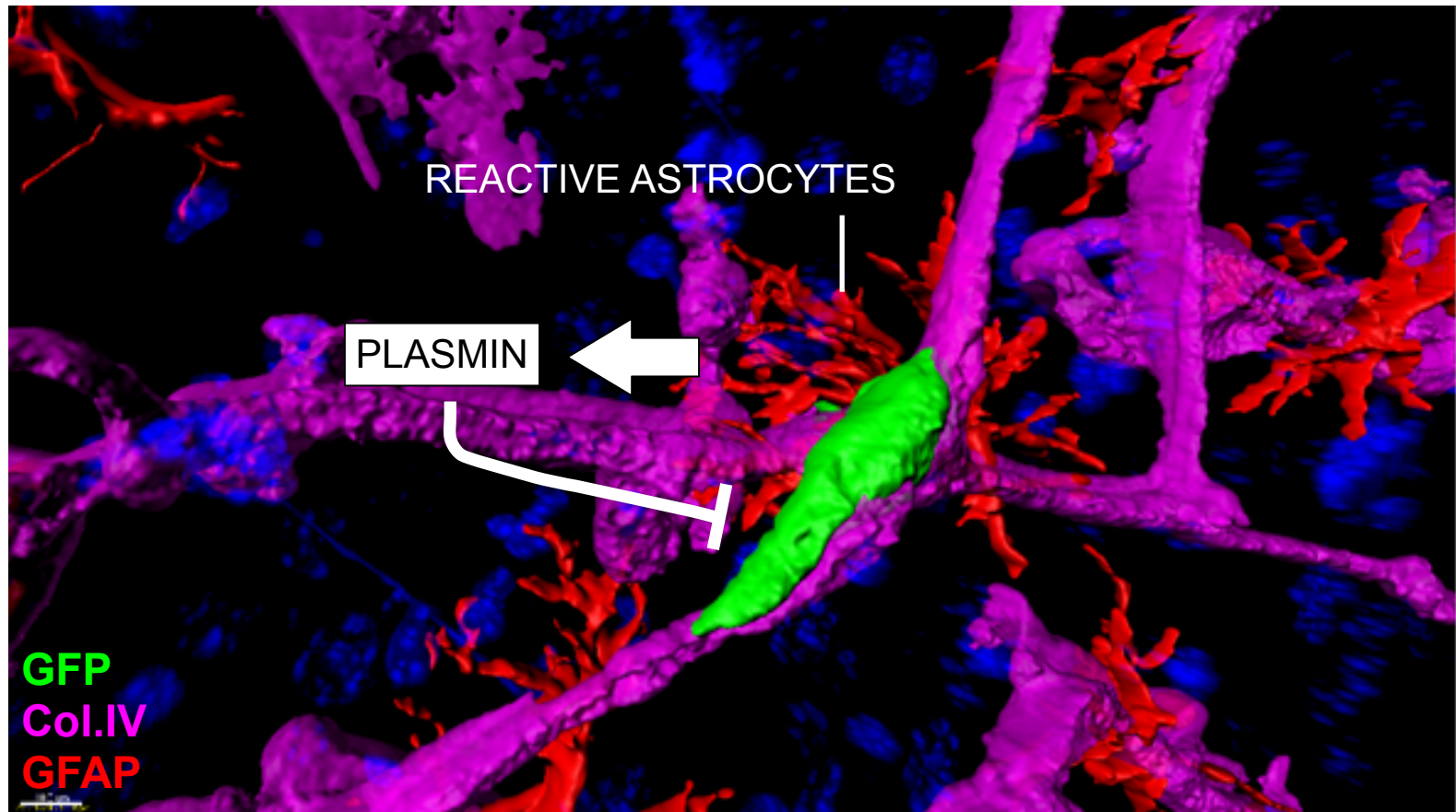


Reactive astrocytes



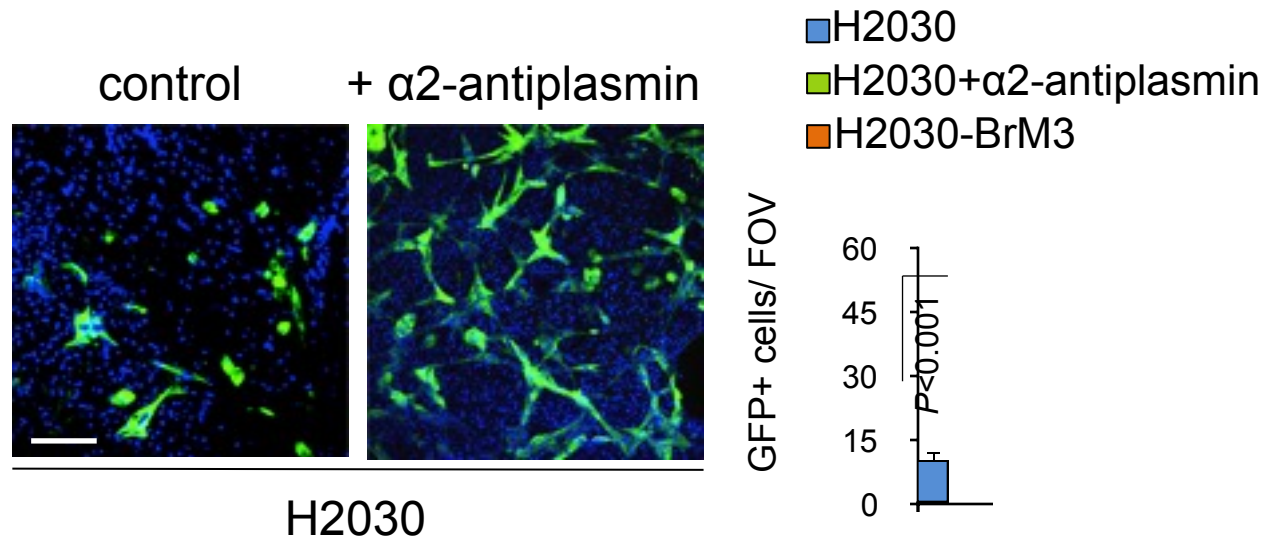
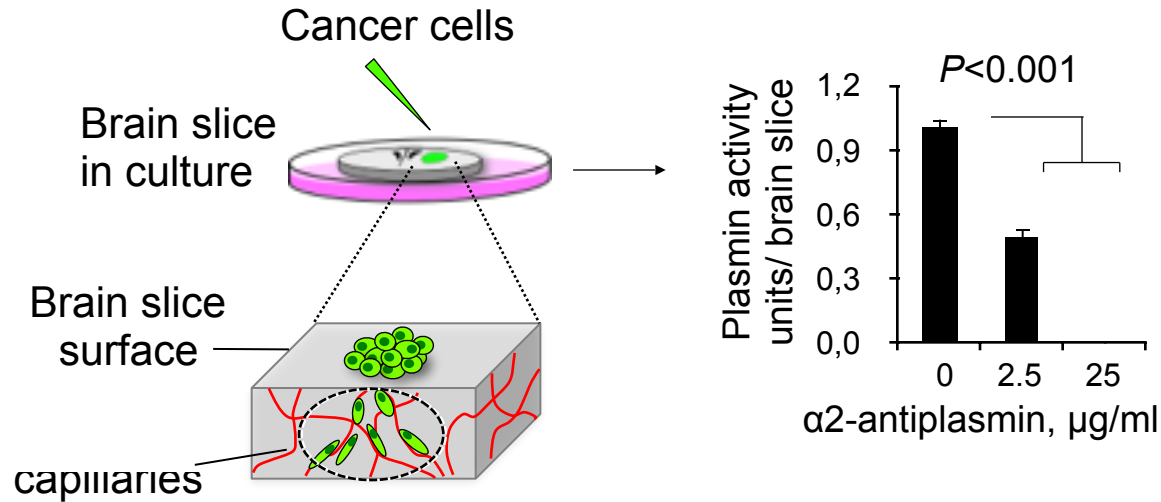


# Metastasis initiating cells: Not an easy soil ;

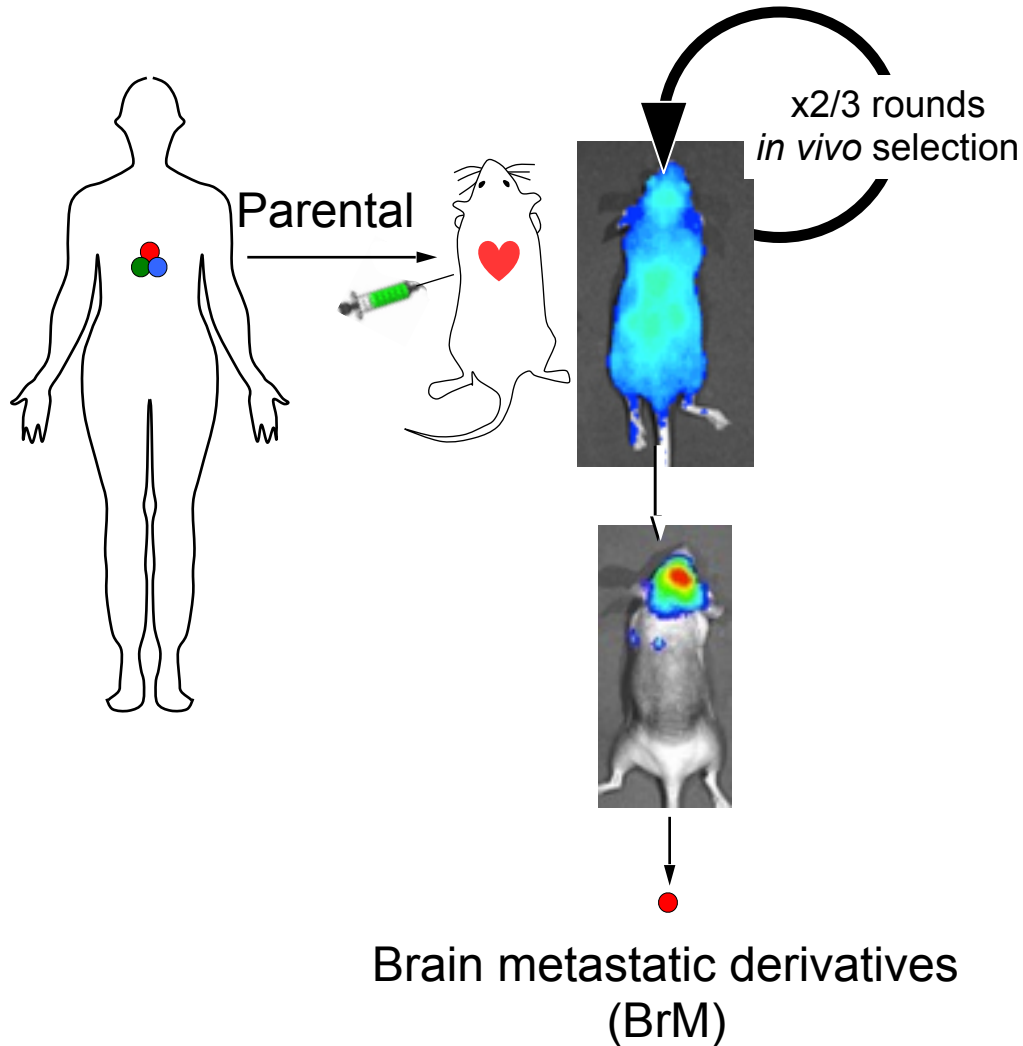


**Reactive microenvironment efficiently reduces the number of metastasis initiating cells by producing deleterious proteases**

# Endogenously generated Plasmin kills cancer cells



# Generation of BrM cell lines

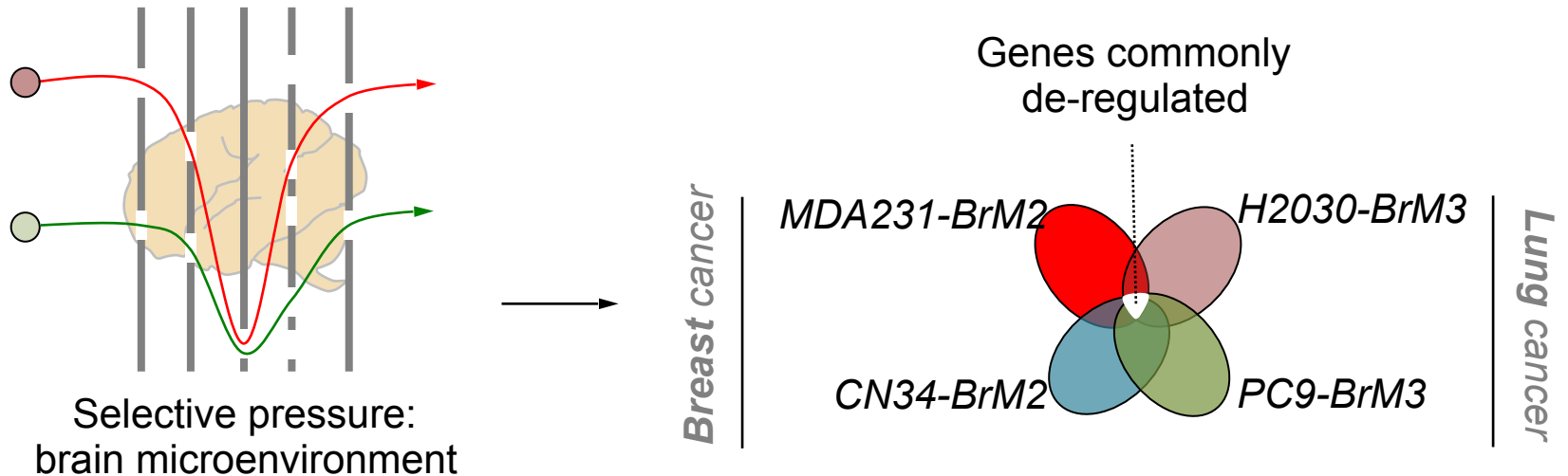


## BrM cell lines

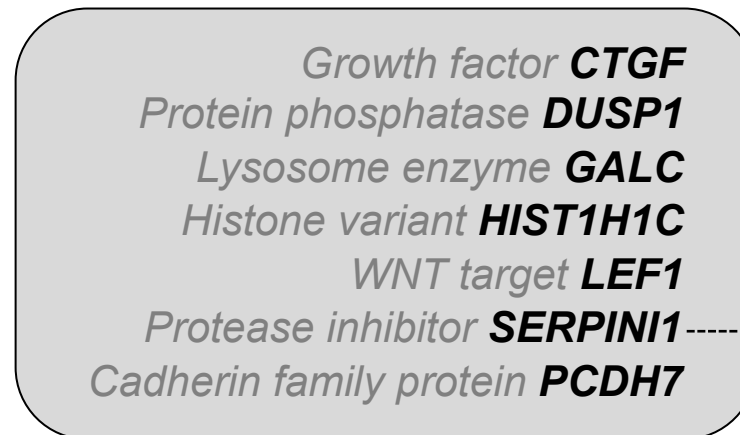
1. H2030-BrM3	H U M A N	Lu
2. PC9-BrM3		
3. MDA231-BrM2		Br
4. CN34-BrM2		
5. Hcc1954-BrM1a		
6. Hcc1954-BrM1b		
7. SCP6 <sup>MBrM</sup>		
8. LLC-BrM	m o u s e	Lu
9. 393N1		
10. 482N1		
11. ErbB2-BrM2		Br

Lu: lung cancer  
Br: breast cancer

# Anti-PA serpins are commonly among BrM cell lines



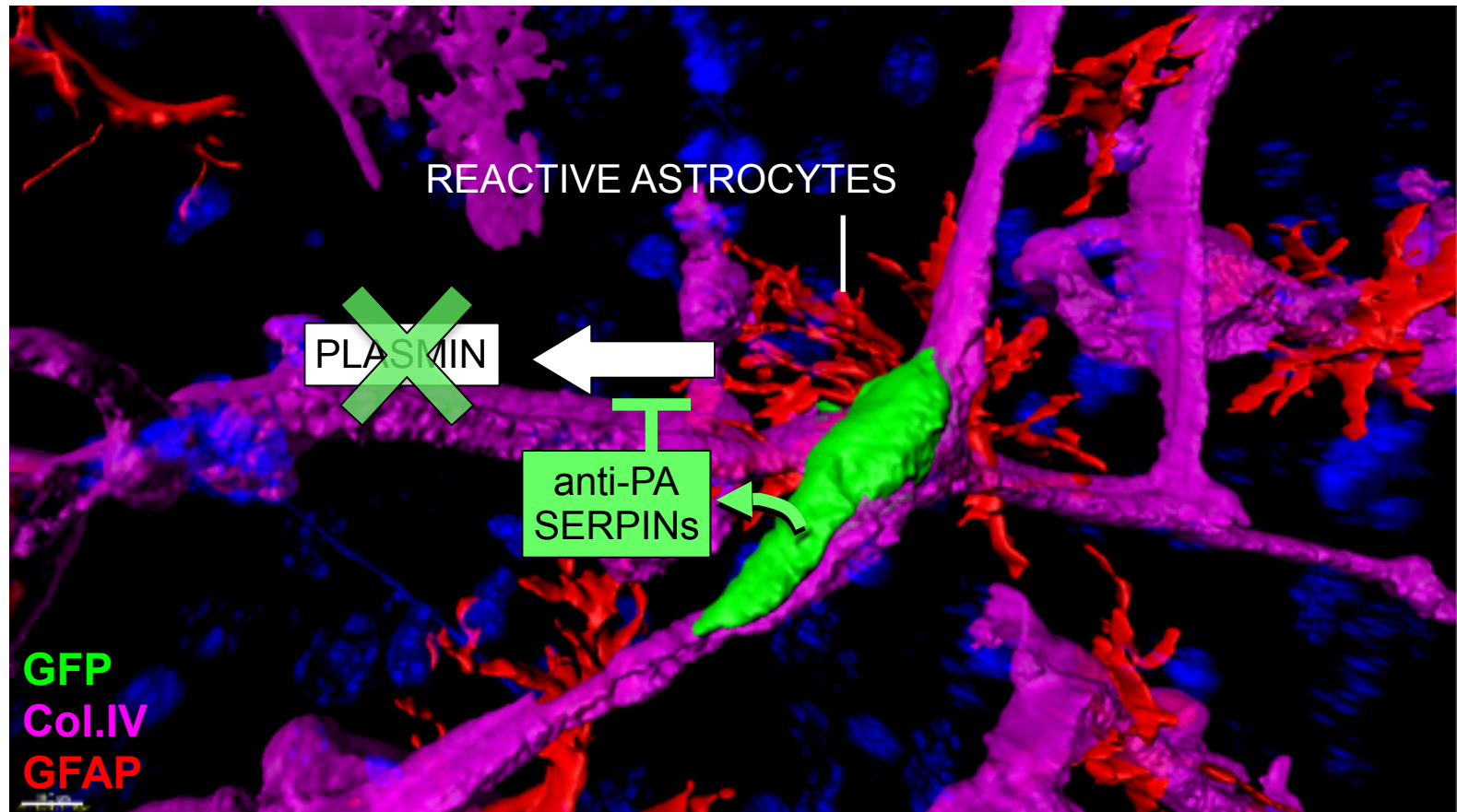
Brain metastatic >>> Parentals



anti-PA SERPIN

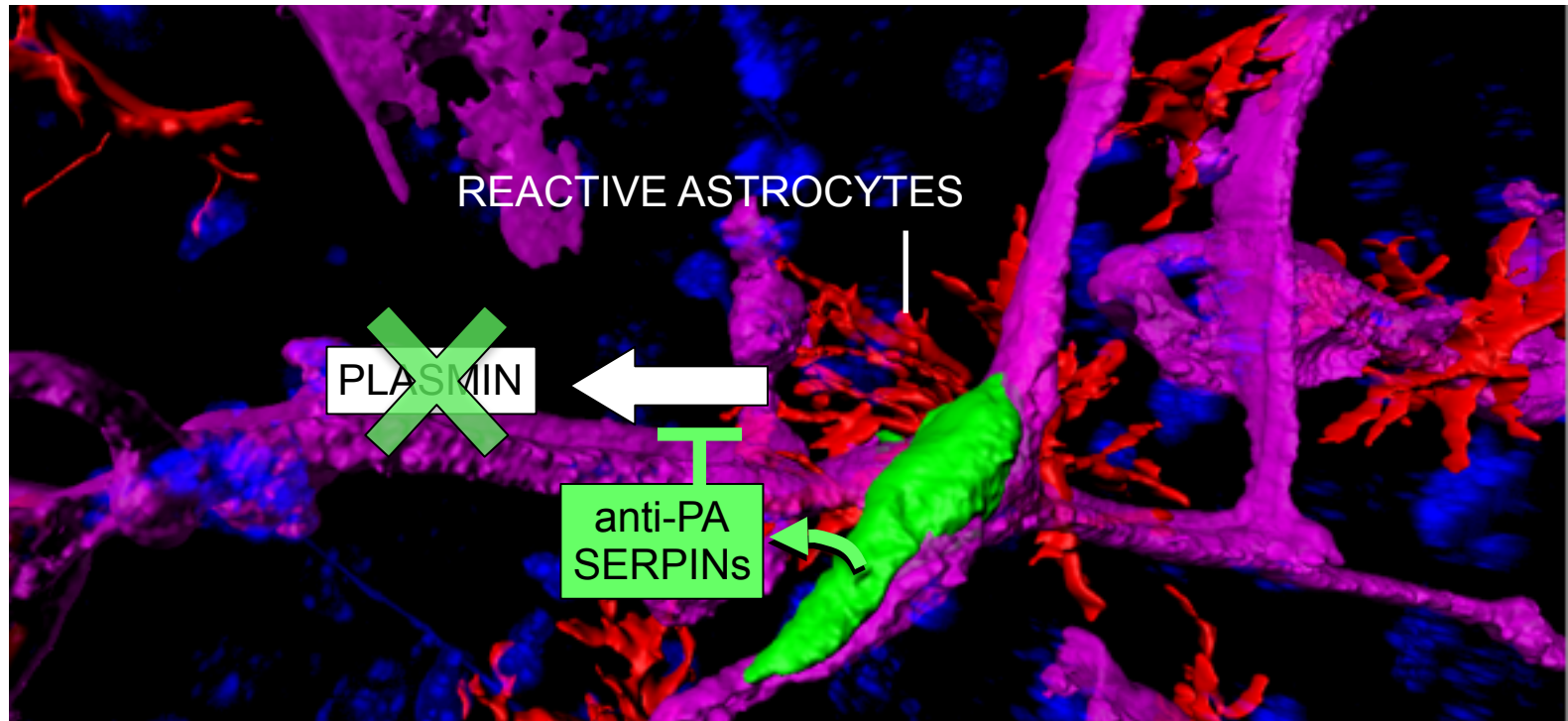


# Protection from a hostile environment: anti-PA Serpins

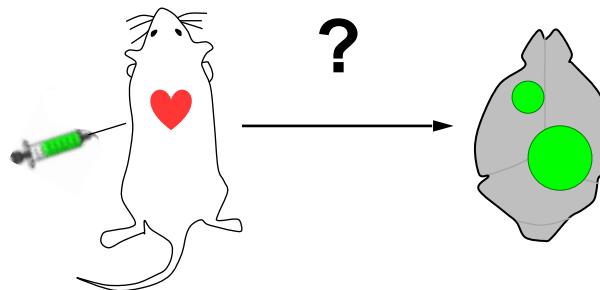


Could anti-PA serpins be required to colonize the brain?

# Protection from a hostile environment: anti-PA Serpins



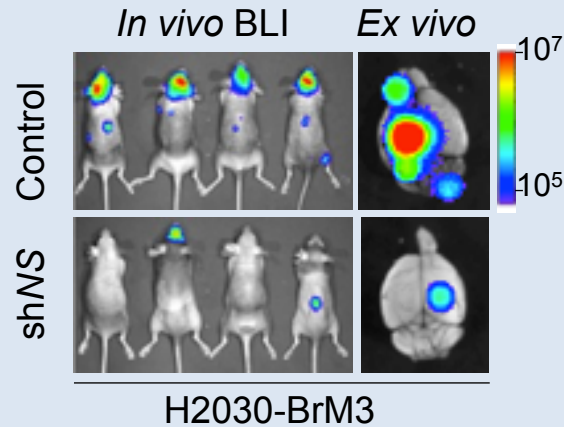
BrM cells  
- Control  
vs  
- Serpin KD



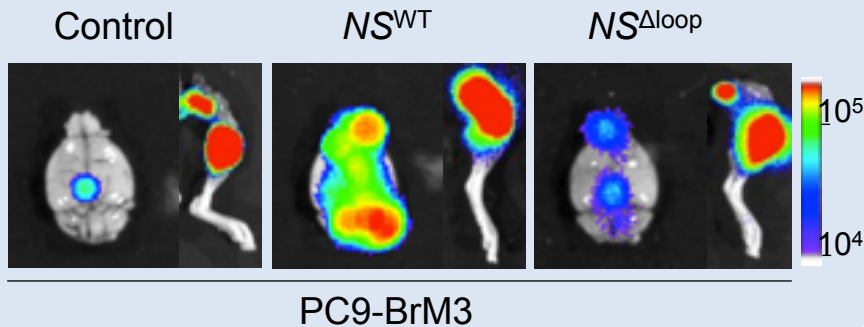
# anti-PA Serpins are critical for brain metastasis initiation

## ***NSCLC brain metastasis***

### **SINGLE SERPIN**

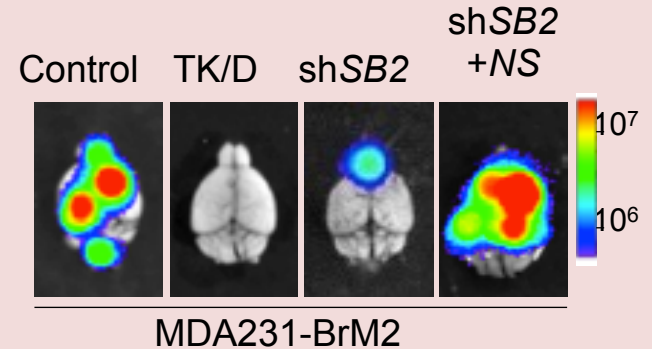


### **GOF IN LESS AGGRESSIVE MODELS**

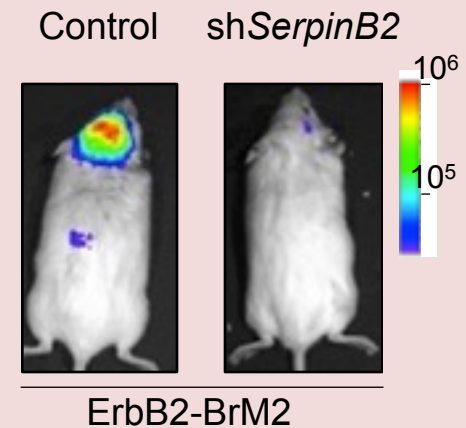


## ***Breast Cancer brain metastasis***

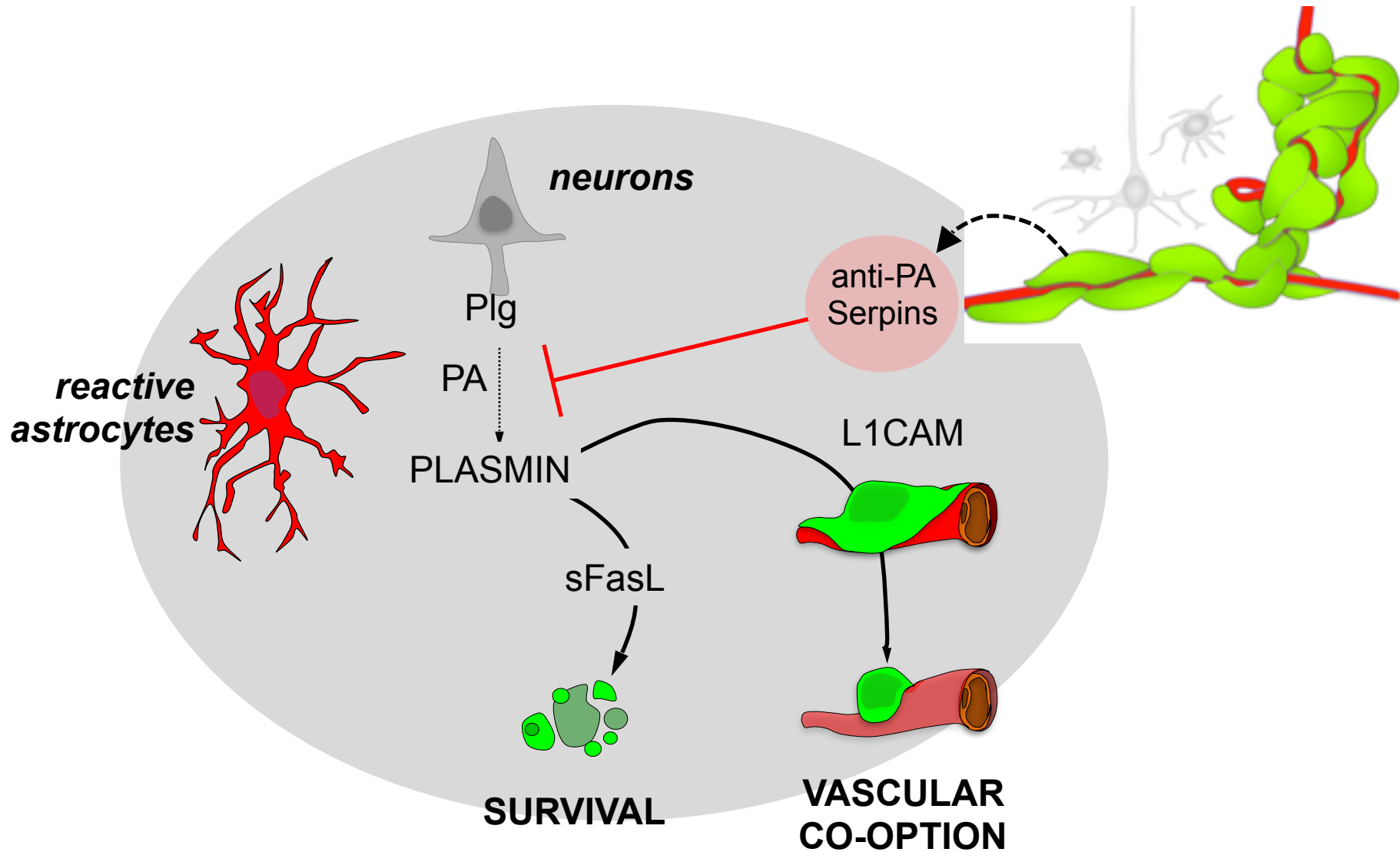
### **MULTIPLE SERPINS**



### **IMMUNE COMPETENT MODELS**

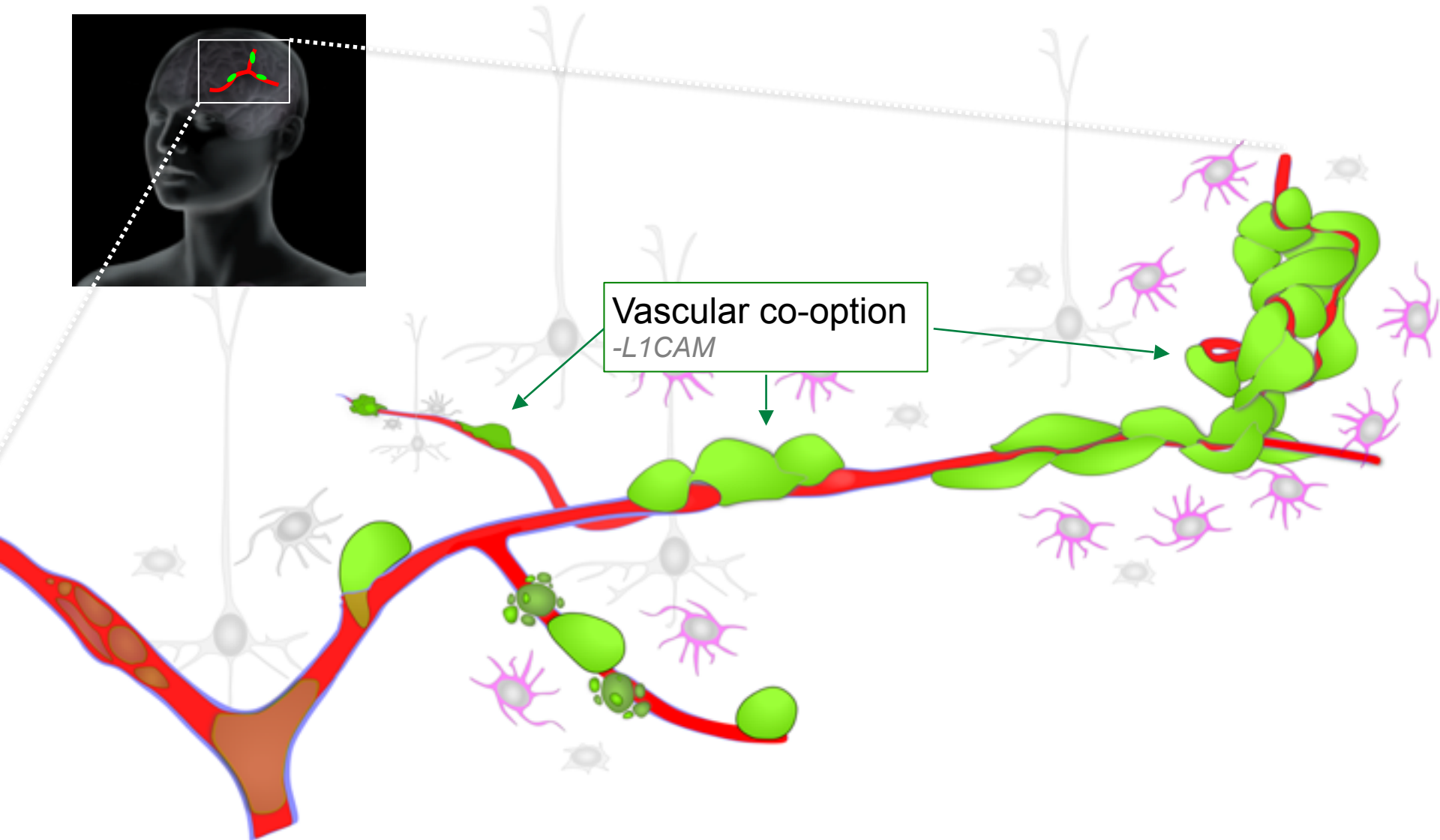


# Serpins are required to avoid Plasmin activity: survival and vascular co-option

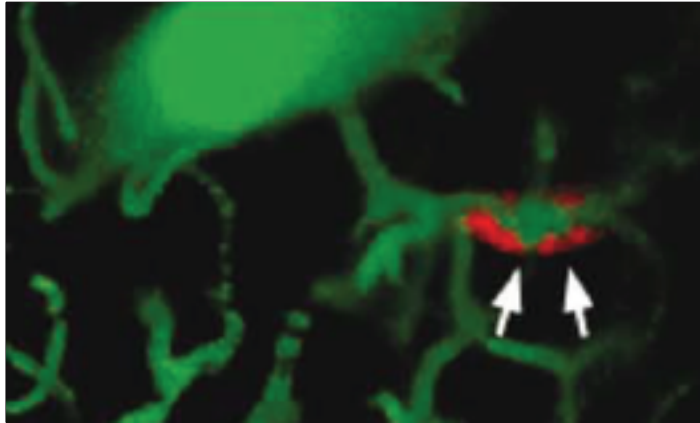




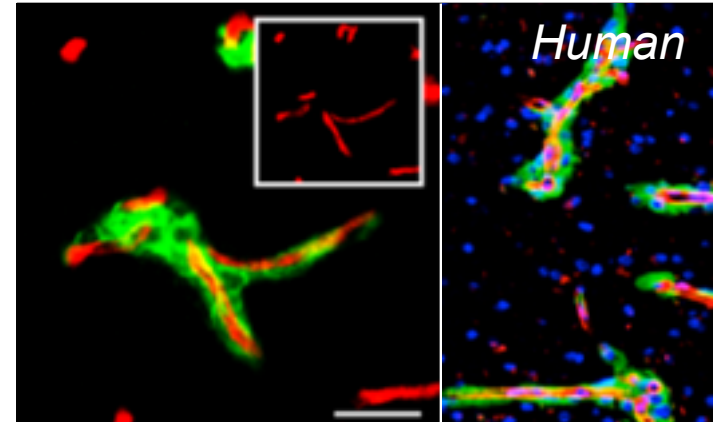
# First hours/ days of a brain metastatic cell



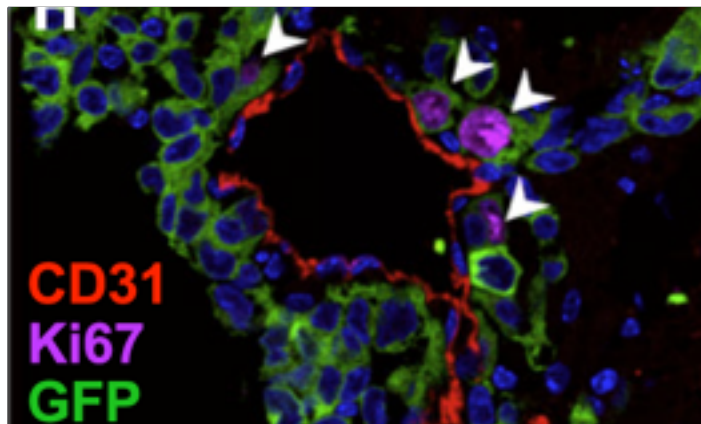
# Vascular co-option in brain metastasis



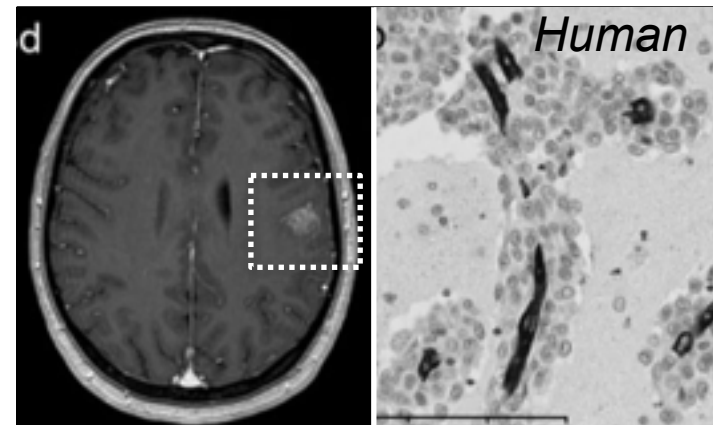
*Kienast et al. (2010) Nat. Med.*



*Carbonell et al. (2009) PLoS one.*

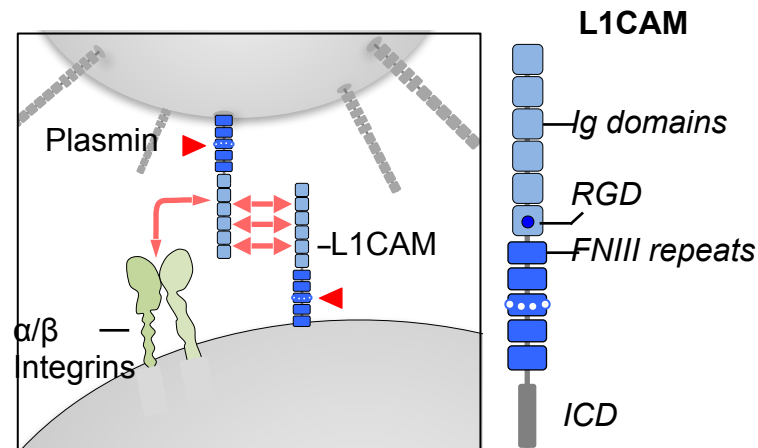
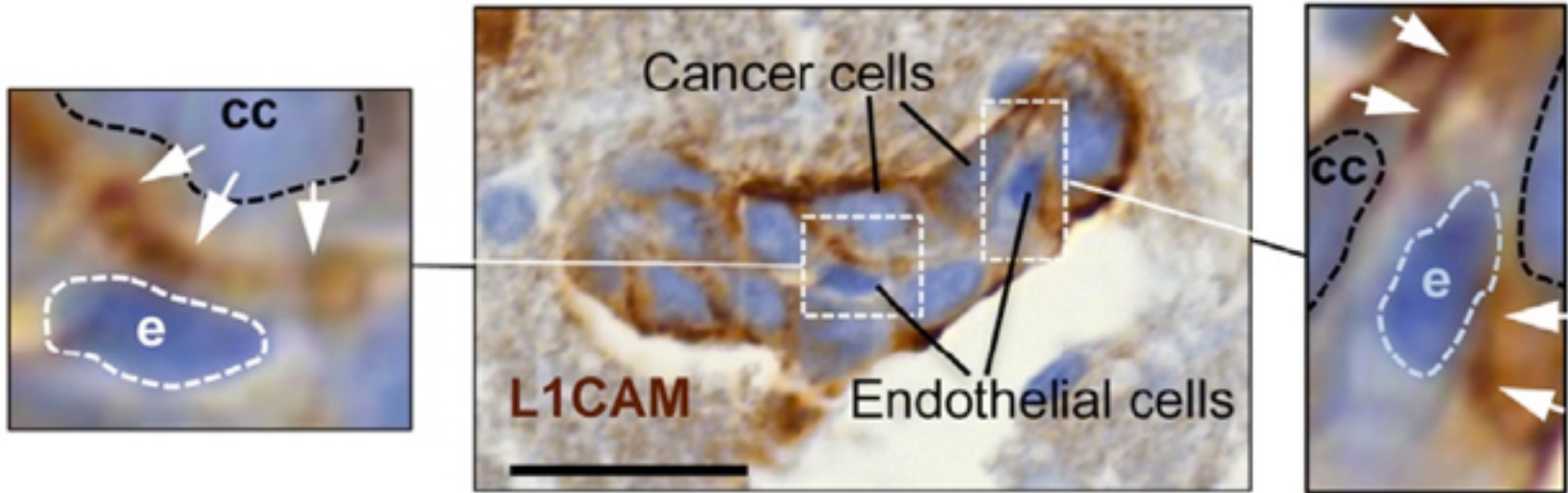


*Nguyen et al. (2009) Cell.*



*Berghoff et al. (2013) Neuro-Oncol.*

# Molecular regulation of vascular co-option: L1CAM



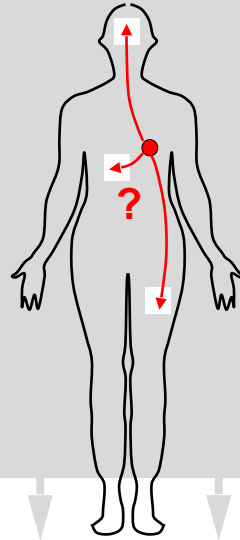




Vascular  
co-option

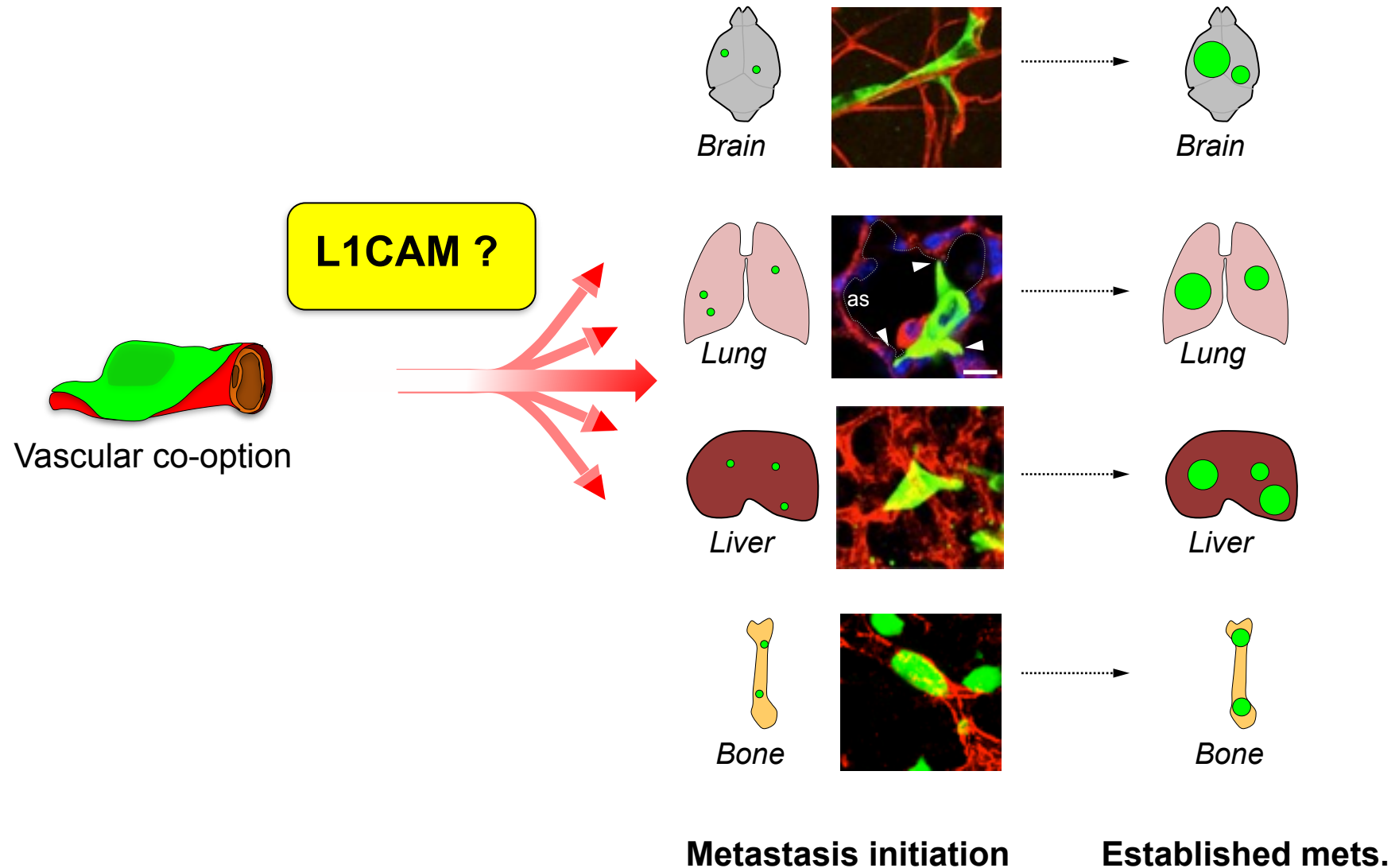
Clinical  
relevance

*L1CAM*



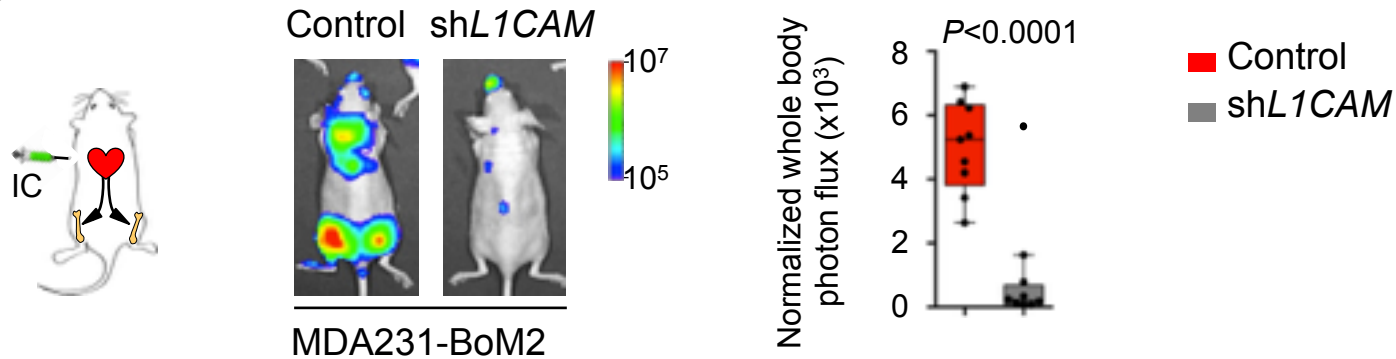
*Potential target  
for multi-organ  
metastases*

# Vascular co-option is a hallmark of metastasis initiation

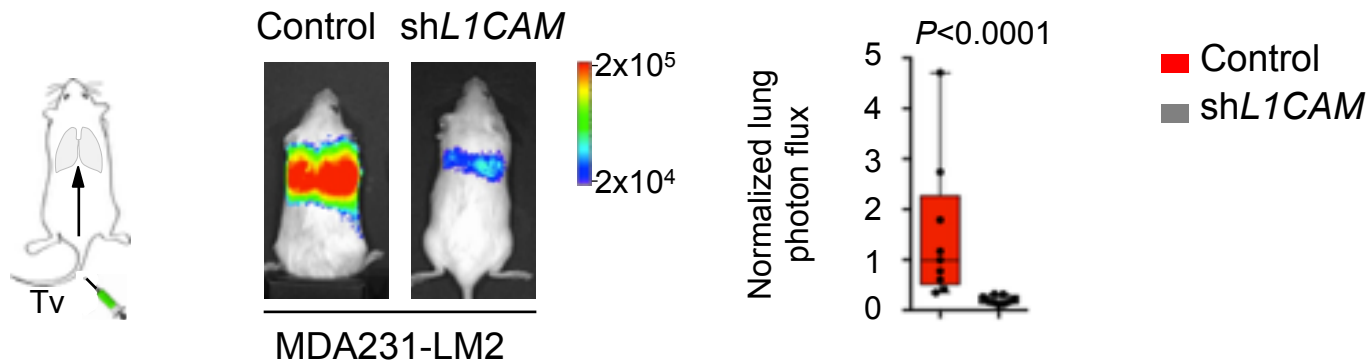


# L1CAM mediates multi-organ metastasis

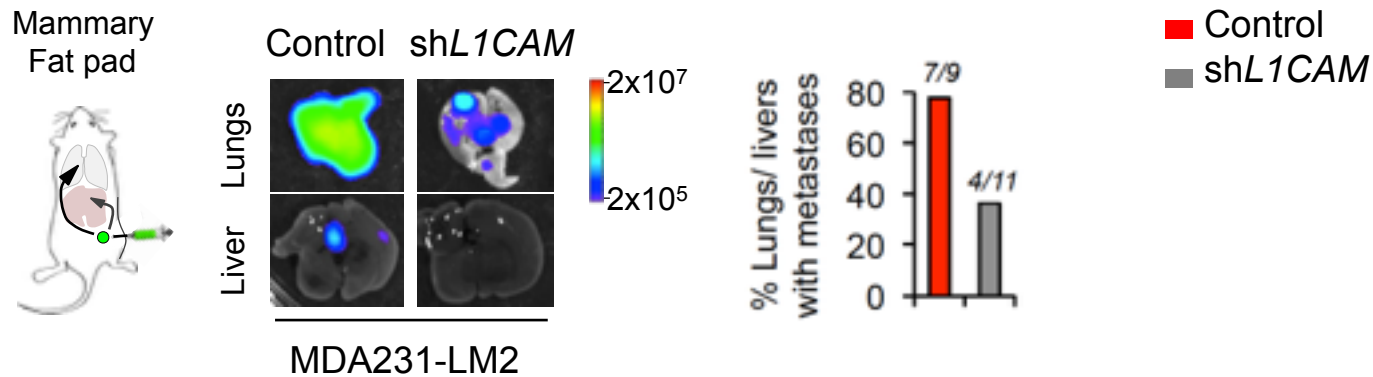
## BONE METS



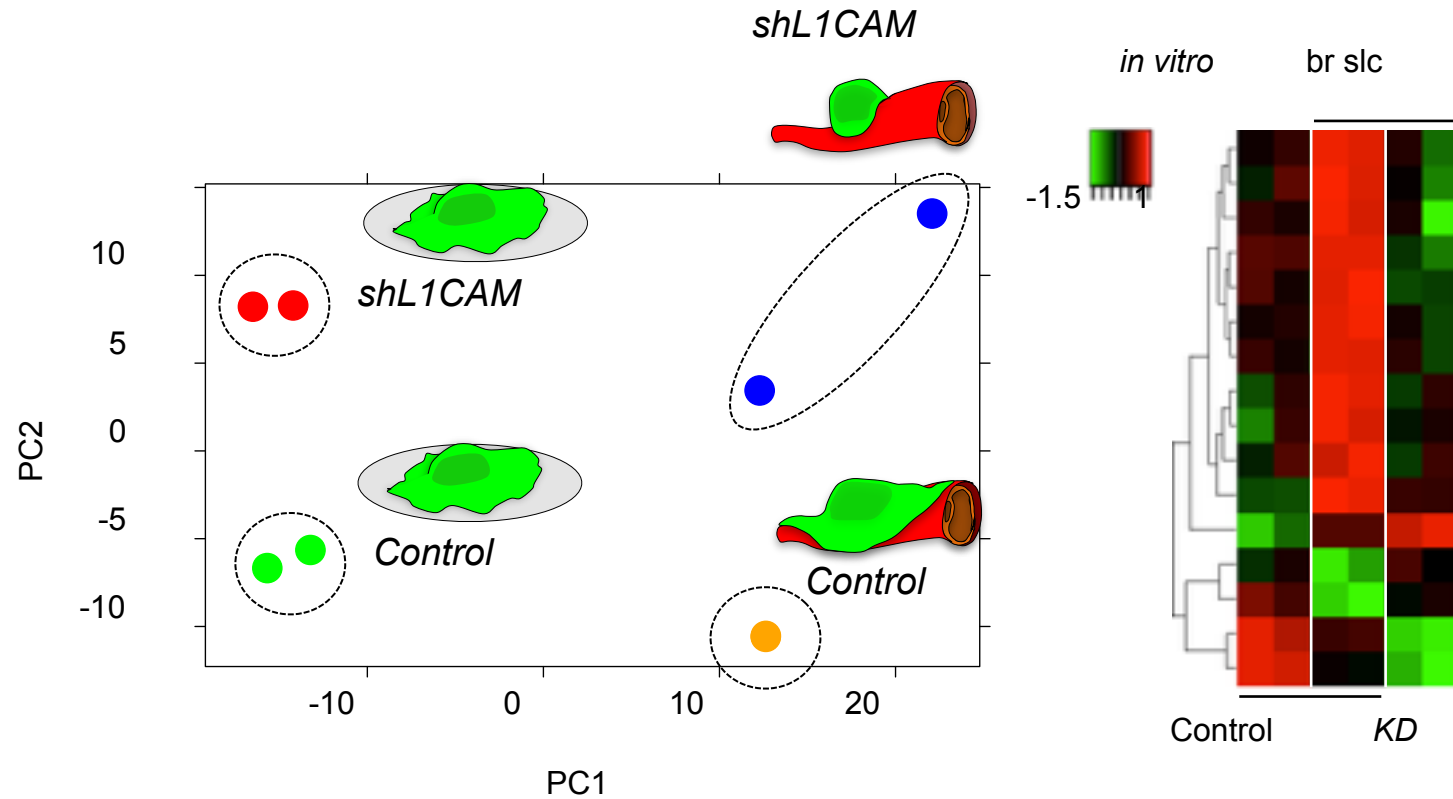
## LUNG METS



## LUNG & LIVER (spontaneous)

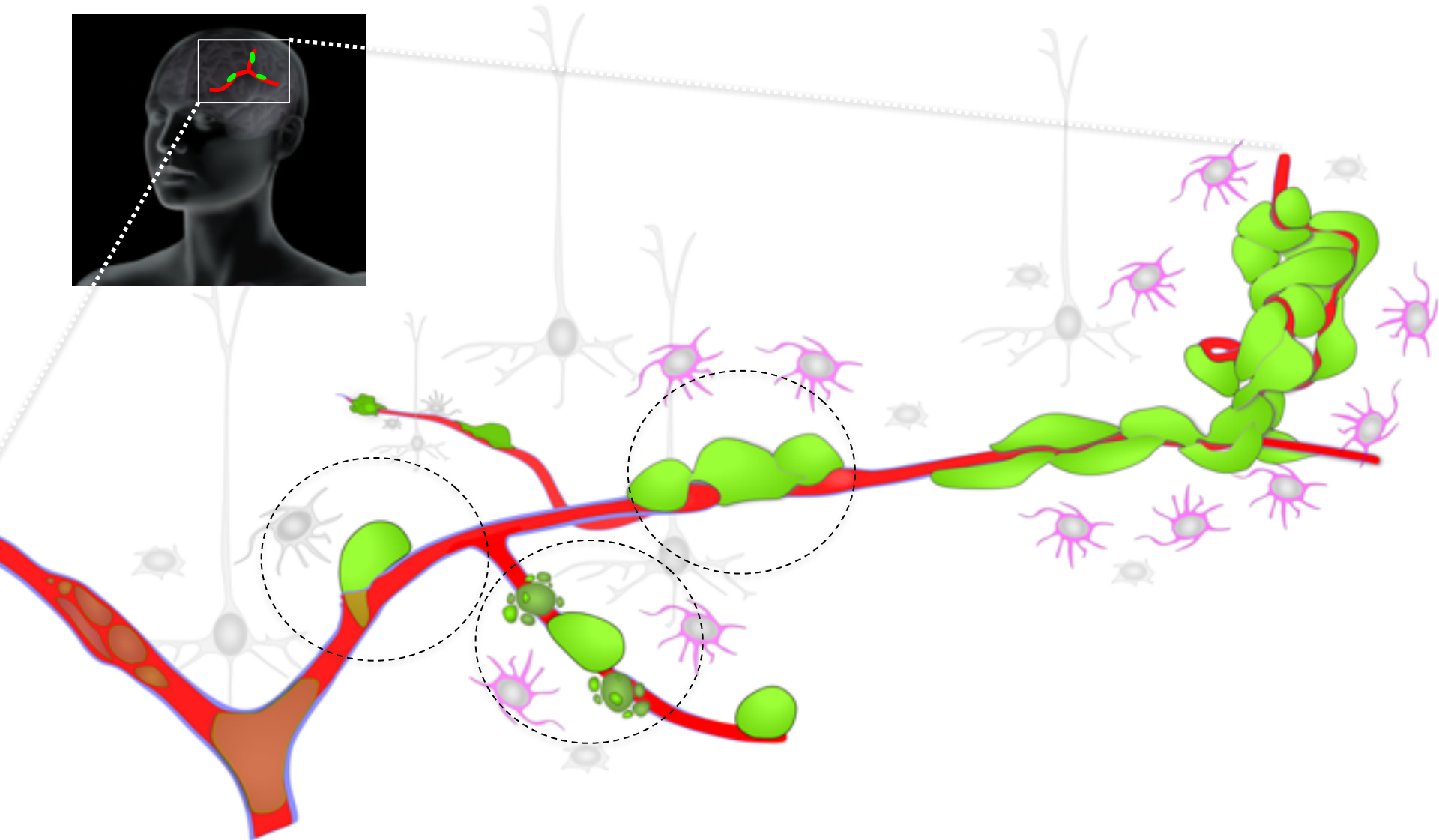


# L1CAM role in vascular co-option

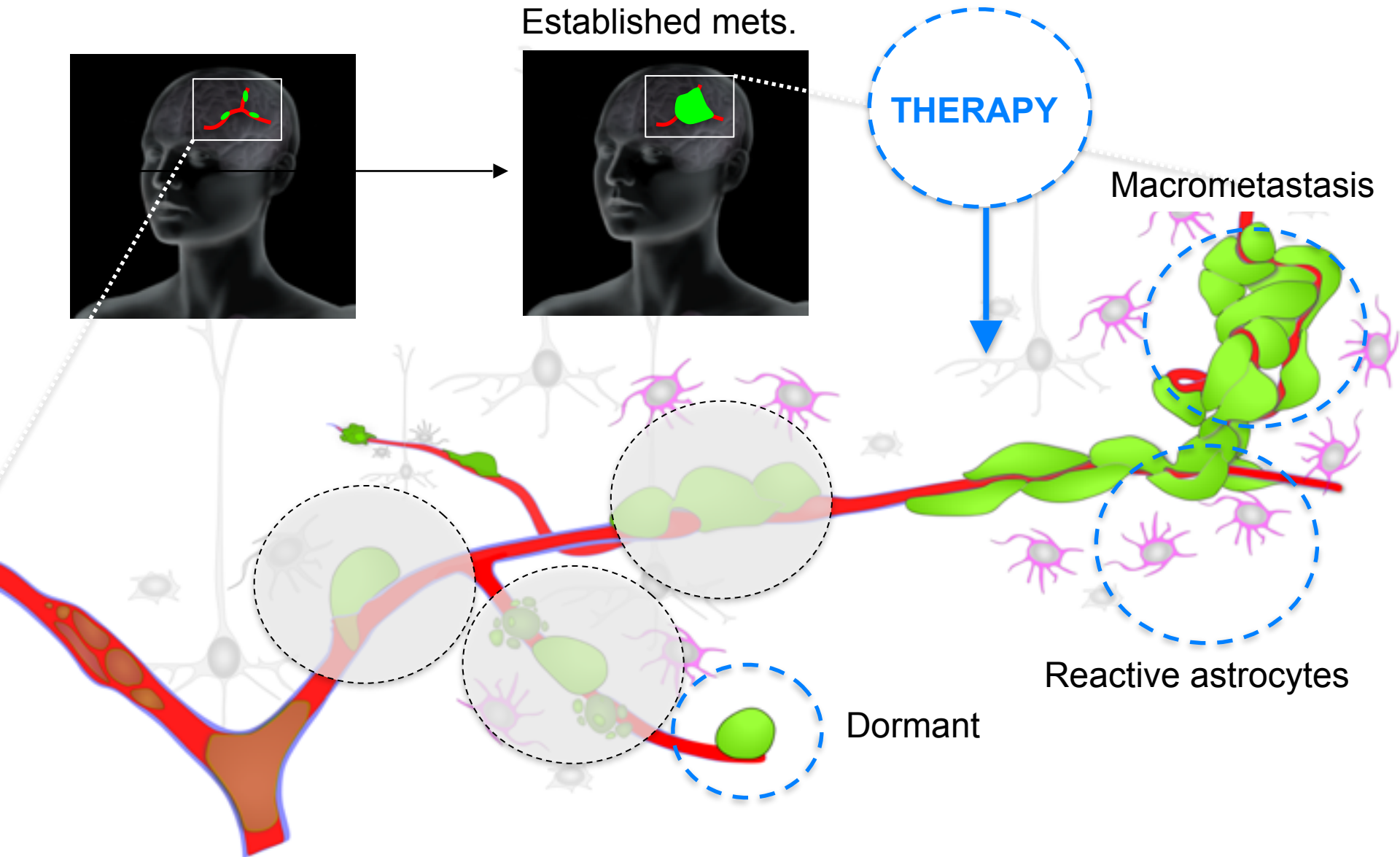




# First hours/ days of a brain metastatic cell

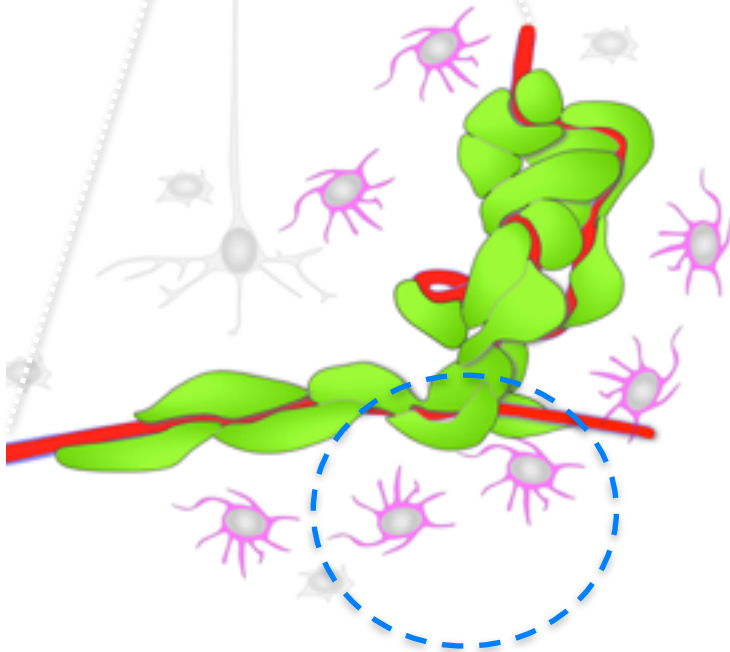
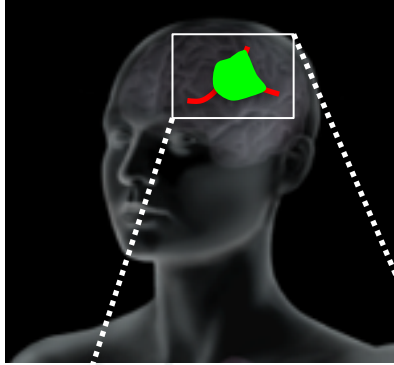


# Next steps in brain metastases



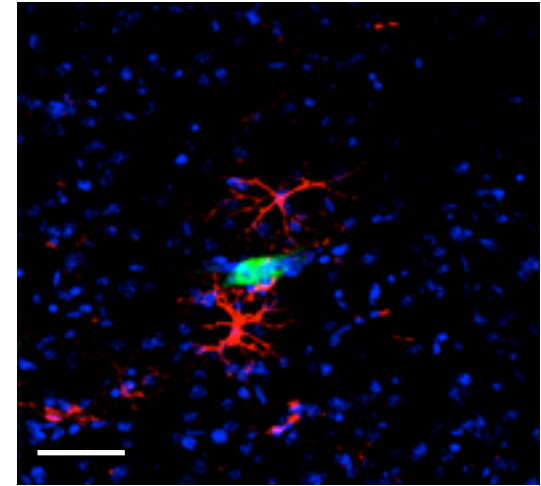
# Next steps in brain metastases: Reactive astrocytes

Established mets.

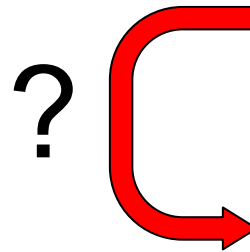
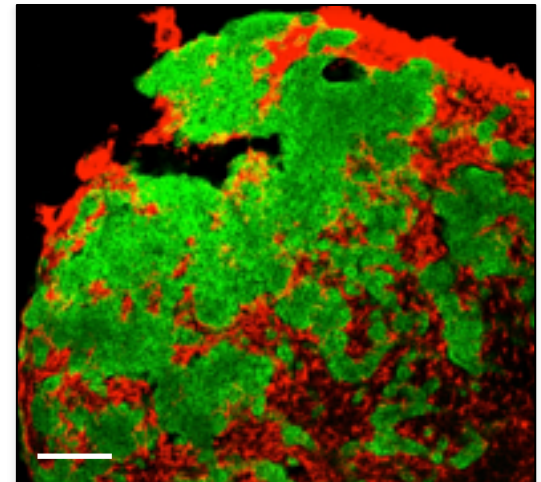


Reactive astrocytes

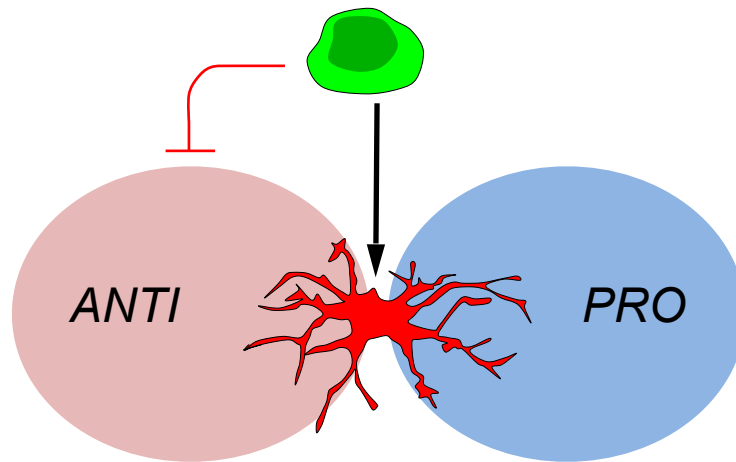
Early colonization



Late colonization

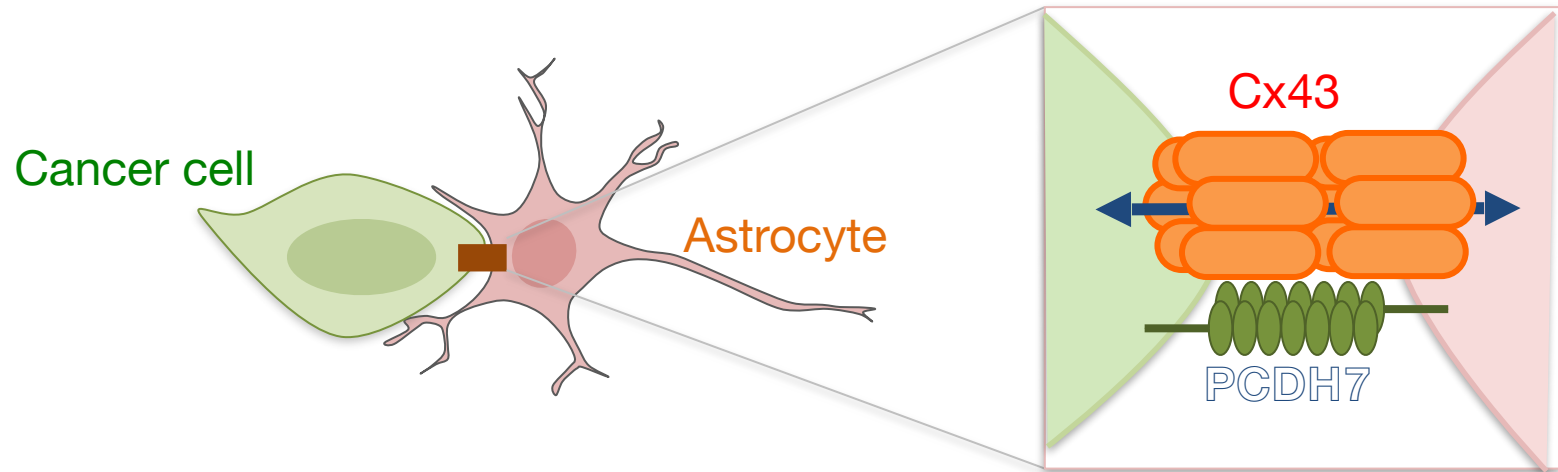


# Remodeling the microenvironment: Reactive astrocytes



- **Chen, Boire et al. Submitted.**  
**(Massagué lab)**
- *Heterogeneity in reactive astrocytes*

# Protocadherin 7 potentiates carcinoma-astrocyte connexin 43 gap junctions



Brain metastatic >>> Parentals

Growth factor **CTGF**

Protein phosphatase **DUSP1**

Lysosome enzyme **GALC**

Histone variant **HIST1H1C**

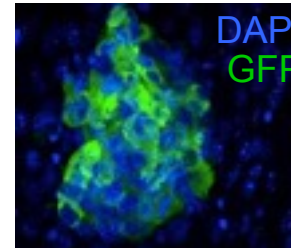
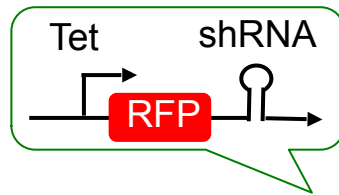
WNT target **LEF1**

Protease inhibitor **SERPINI1**

Cadherin family protein **PCDH7**

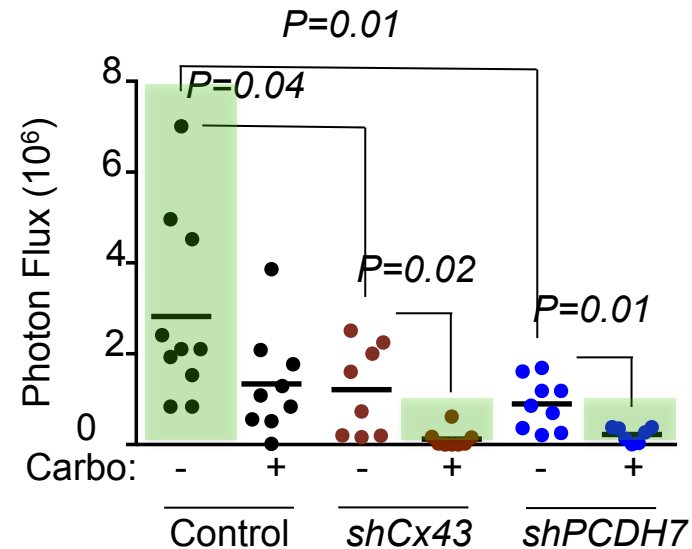
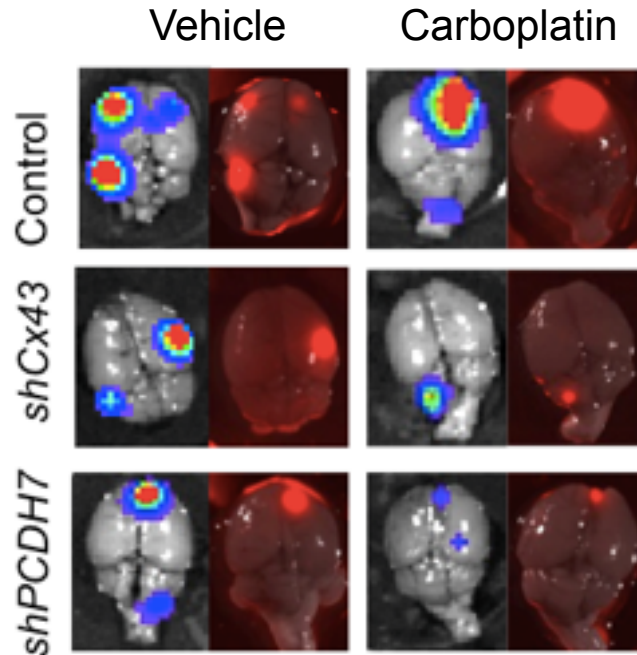
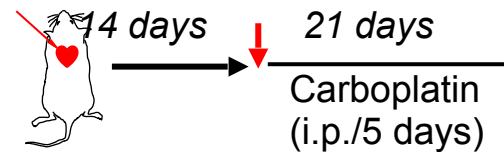


# Gap junction knockdown synergizes with chemotherapy



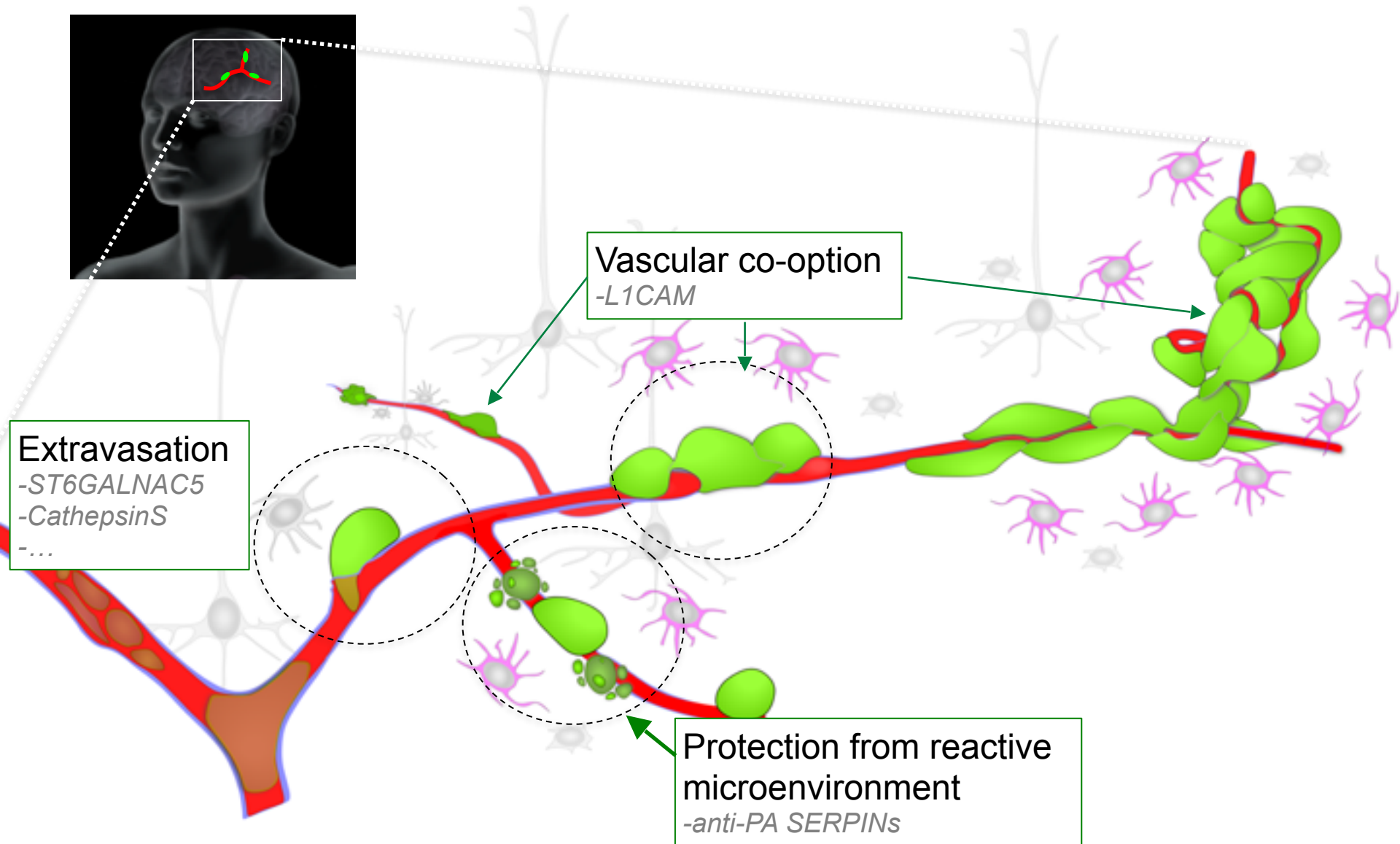
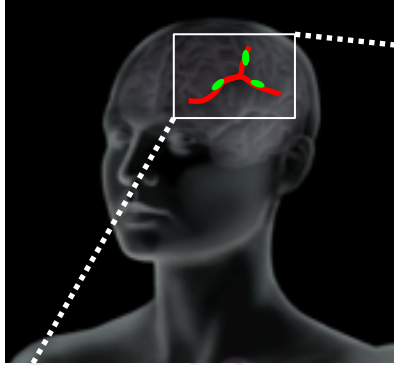
MDA231-BrM2

Dox



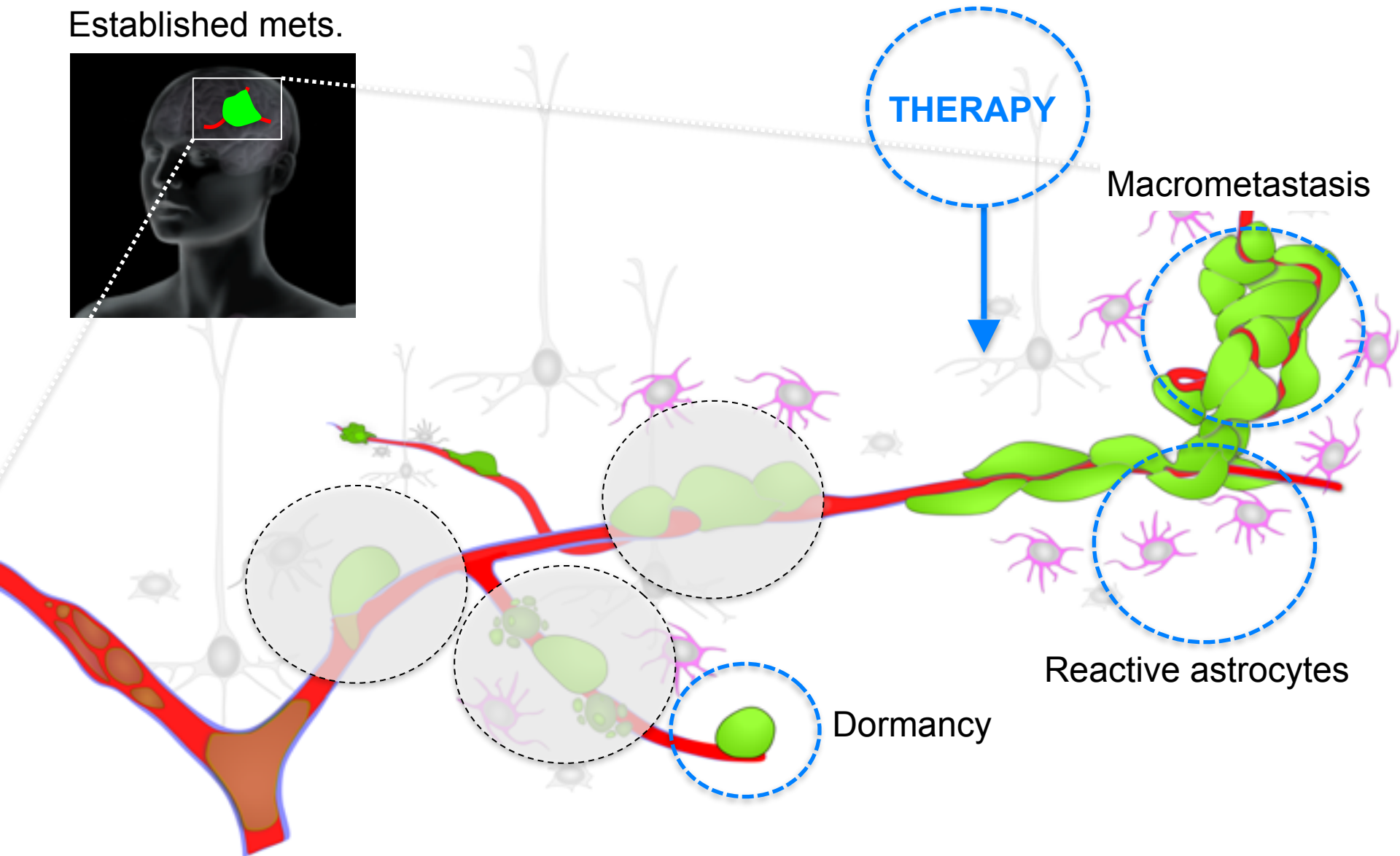
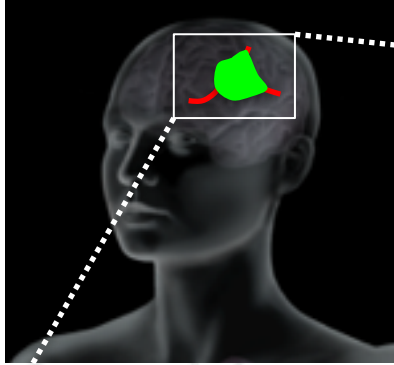
# First hours/ days of a brain metastatic cell

Met. initiating cells



# Next steps in brain metastases

Established mets.





## Valiente Lab

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[www.valientelab.com](http://www.valientelab.com)

## Collaborators

### *Basic research collaborators*

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Laura López-Mascaraque  
(Cajal Institute)

Massimo Squatrito (CNIO)

Fatima Al-Shahrour (CNIO)

### *Clinical research collaborators*

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Fernando Lopez-Rios (HM Hospital)

Santiago Ramón y Cajal (Vall d'Hebron)

## Funding:



# SAVE THE DATE



**CNIO FRONTIERS MEETINGS 2017**

**PRIMARY AND SECONDARY BRAIN TUMOR**

Madrid  
19-22 February 2017

**Organisers**

**Richard Gilbertson**  
CRUK Cambridge Institute  
Cambridge, UK

**Michael Weller**  
University Hospital Zurich,  
Zurich, CH

**Massimo Squatrito**  
CNIO, Madrid, Spain

**Manuel Valiente**  
CNIO, Madrid, Spain

**CNIO Frontier Meeting 2017:**

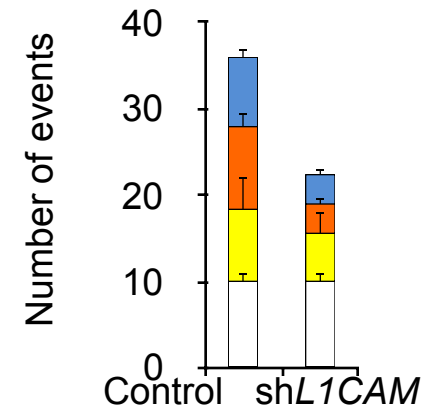
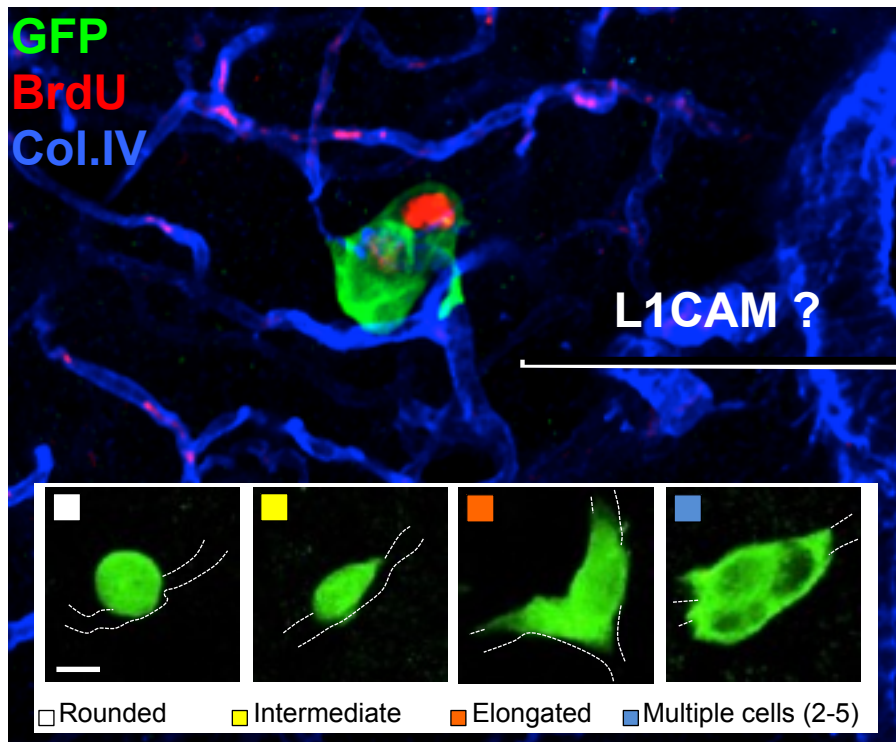
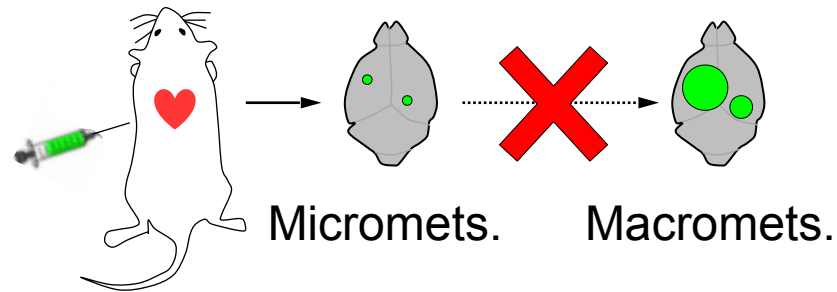
**Primary and Secondary  
Brain Tumors**

**19-22 February 2017**  
**Madrid, Spain**



# Indolent BrM cells

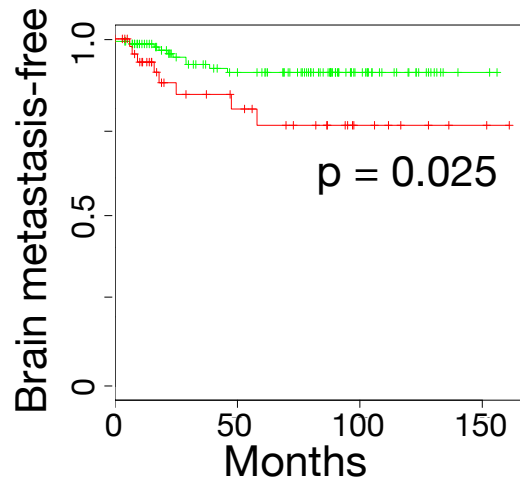
## *Indolent ErbB2 model*



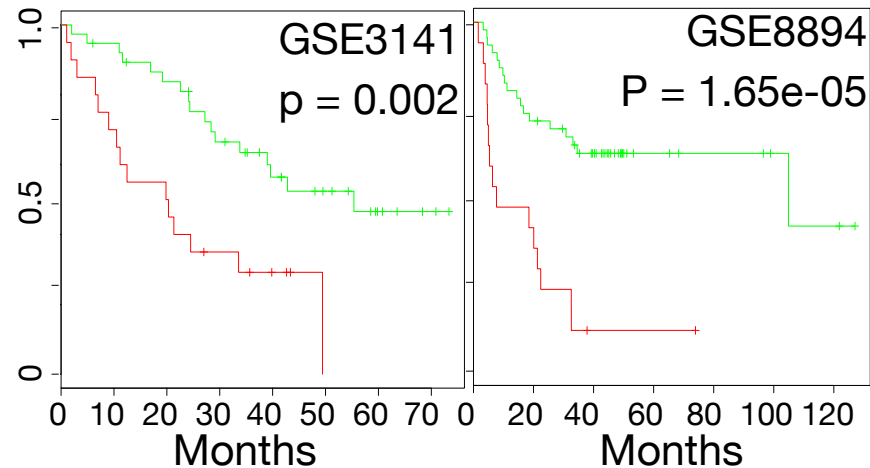
**L1CAM prevents growth of indolent metastasis initiating cells (without killing them)**

# Clinical association of Cx43/PCDH7 with brain metastasis

Triple negative breast cancer



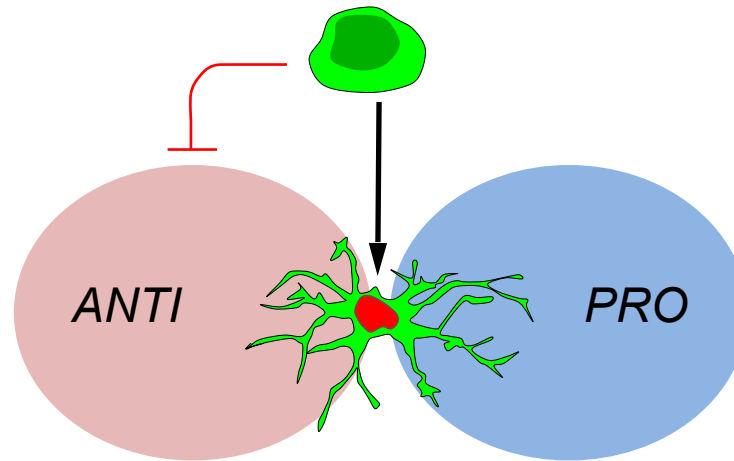
Lung adenocarcinoma



— PCDH7<sup>low</sup>, Cx43<sup>low</sup>

— PCDH7<sup>hi</sup>, Cx43<sup>hi</sup>

# Remodeling the microenvironment: Reactive astrocytes



*Fitzgerald et al. (2008) Clin. Exp. Metastasis.*

*Lin et al. (2010). Neoplasia.*

*Seike et al. (2011). Clin. Exp. Metastasis.*

*Kim et al. (2011). Neoplasia.*

*Chuang et al. (2013). Glia.*

*Gil et al. (2013). Am. J. Pathol.*

*Xing et al. (2013) EMBO Mol. Med.*

*Kim et al. (2014). Neuro-Oncol.*

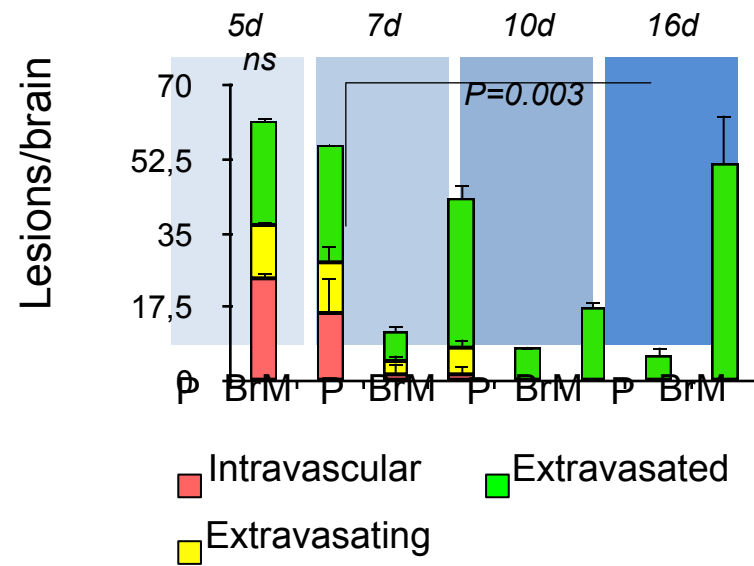
*Valiente et al. (2014). Cell.*

*Wu et al. (2015). J Biol. Chem.*

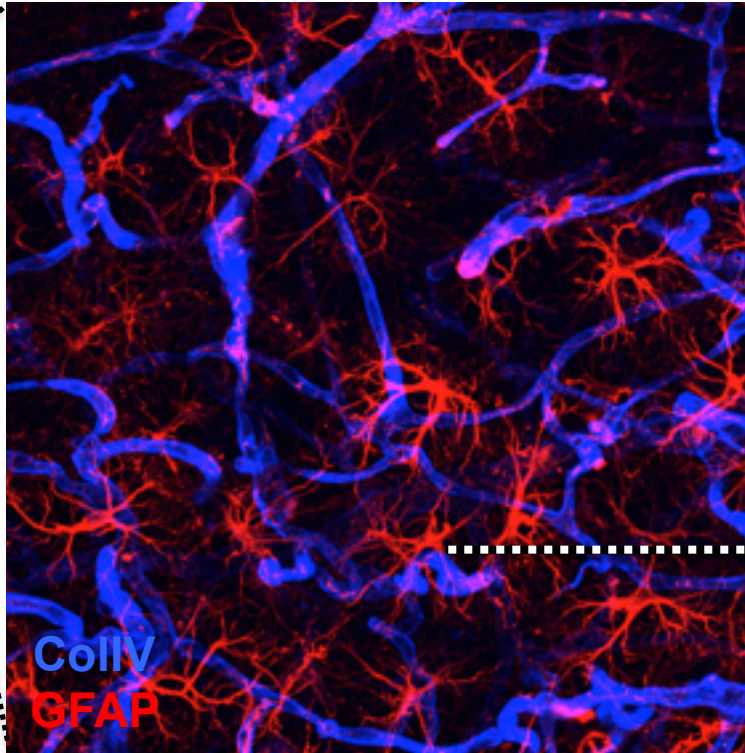
*Klein et al. (2015). J. Pathol.*

*Siam et al. (2015). Oncotarget.*

***Qing et al. Submitted (Massagué Lab)***



# Reactive astrocytes in BrM: who are they?



## Astrocyte Development and Heterogeneity

Omer Ali Bayraktar<sup>1,2,4</sup>, Luis C. Fuentealba<sup>3,4</sup>, Arturo Alvarez-Buylla<sup>1,3</sup>, and David H. Rowitch<sup>1,2</sup>

<sup>1</sup>Department of Pediatrics and Edythe Broad Institute for Stem Cell Research and Regeneration Medicine, University of California, San Francisco, San Francisco, California 94143

<sup>2</sup>Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, California 94143

<sup>3</sup>Department of Neurosurgery, Eli and Edythe Broad Institute for Stem Cell Research and Regeneration Medicine, University of California, San Francisco, San Francisco, California 94143

(2014). *Cold Spring Harb. Perspect. Biol.*

REACTIVE ASTROCYTES

Origin?

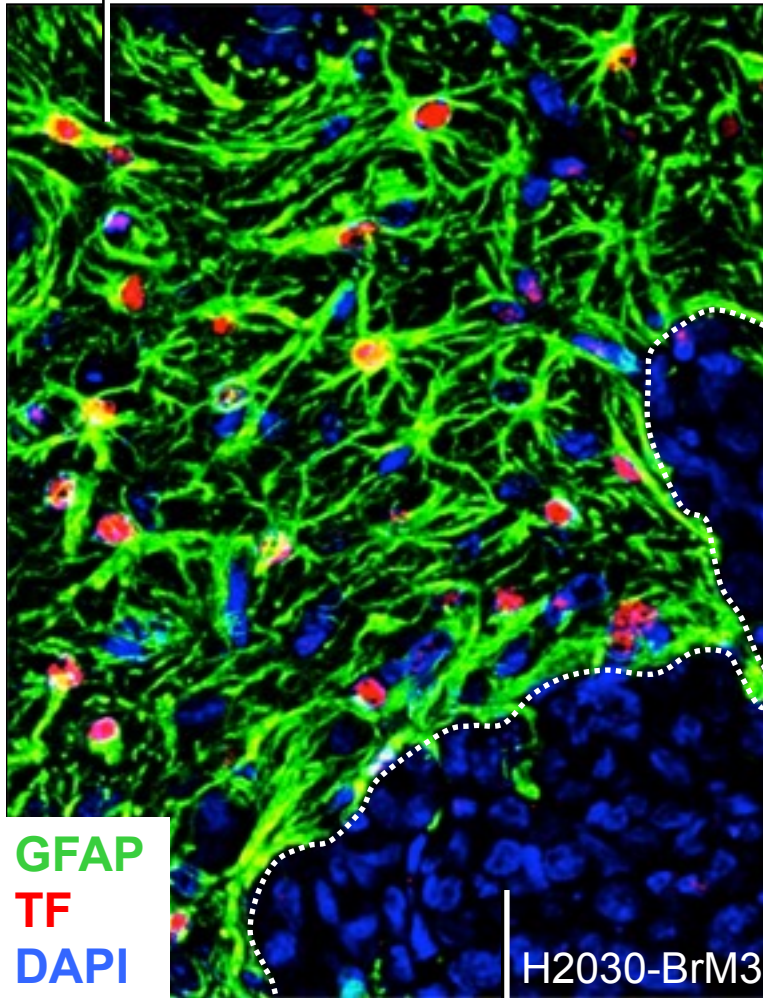
Heterogeneity?

Therapeutic value?

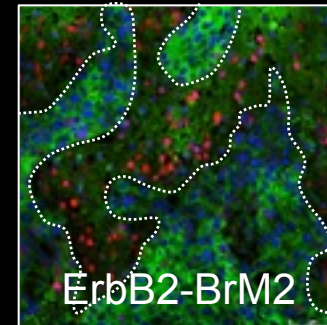
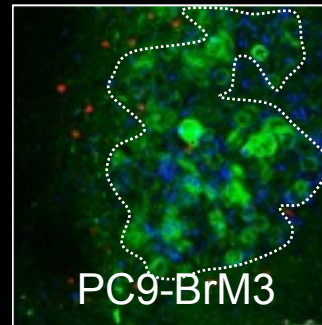
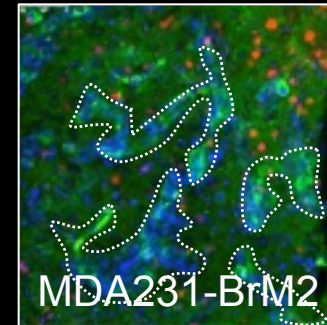
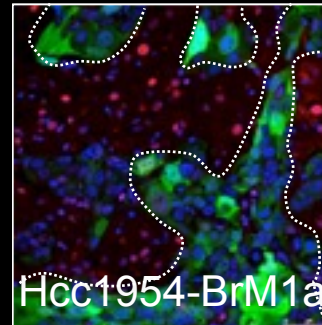


# Subpopulations of React. Astrocytes in Brain Metastases

REACTIVE  
ASTROCYTES



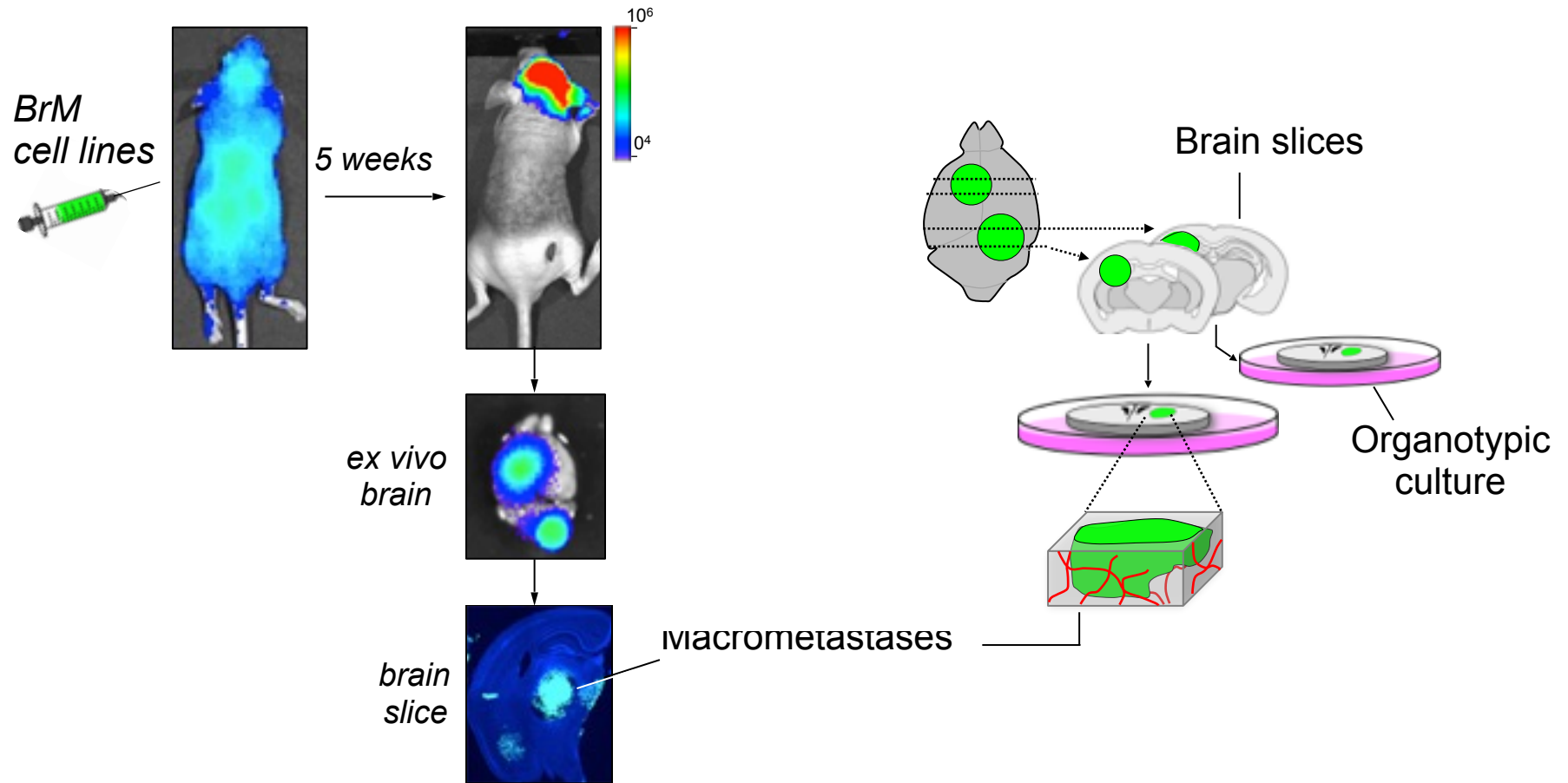
BrM CELLS



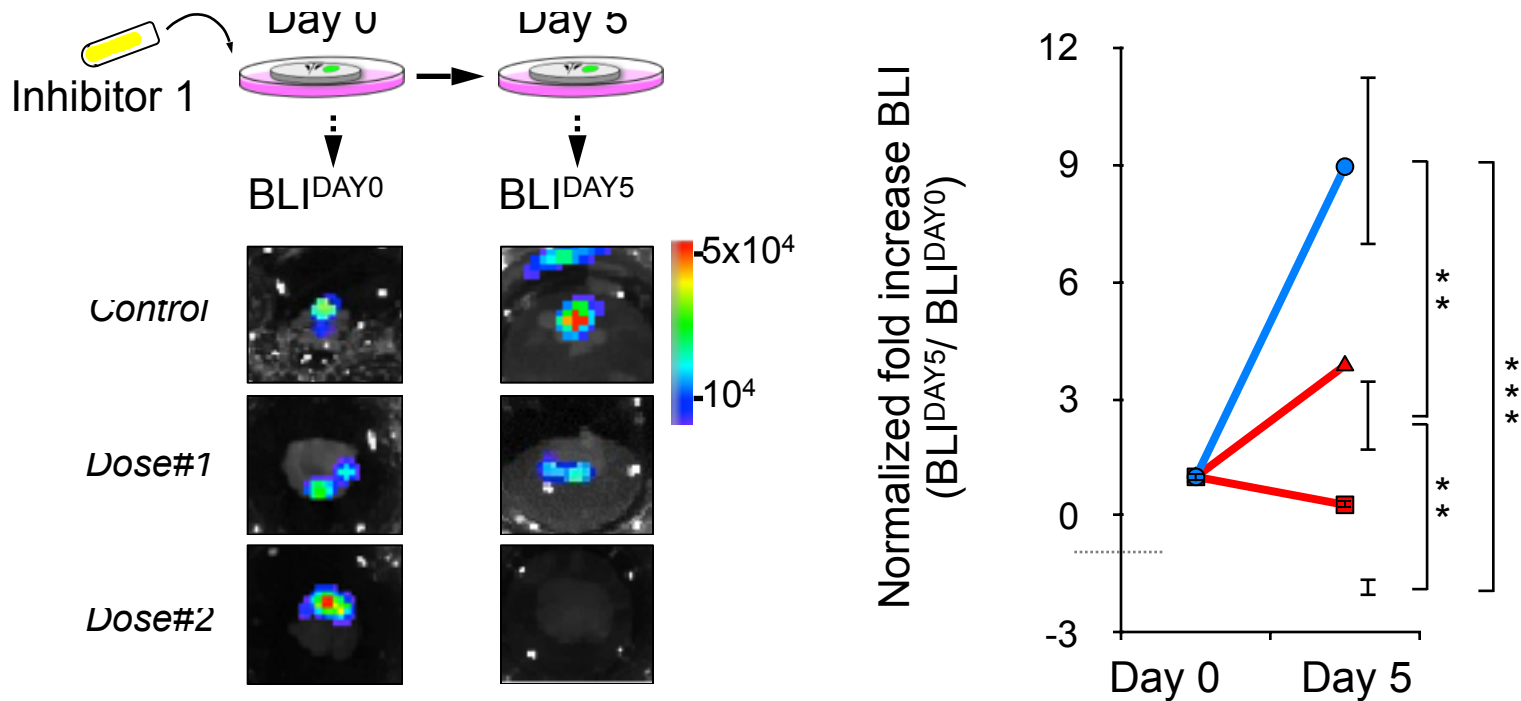
GFP TF DAPI

Is this (therapeutically) relevant?

# Functional relevance of microenvironment heterogeneity



# Targeting microenvironment heterogeneity



**Targeting (subtypes) reactive astrocytes could be a novel and effective strategy to treat brain metastasis**

# Organotypic cultures for drug discovery in BrM

