

Fibrinogen in Neurological Diseases: Mechanisms, Imaging, Therapeutics

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Gladstone Institutes**

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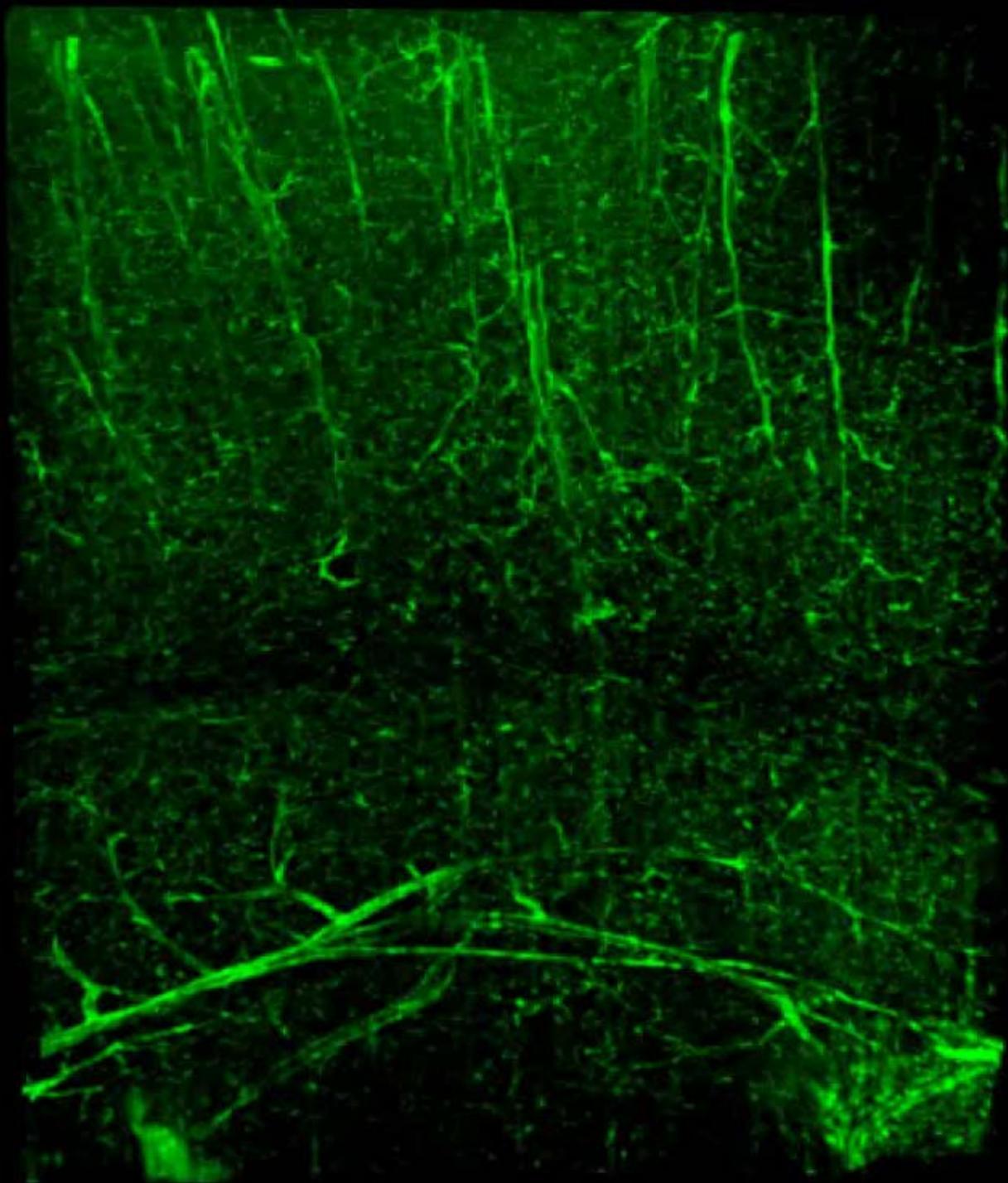
Disclosures:

Named Inventor: US 8980836, 8877195, 7807644, 8569242

Brain Vasculature

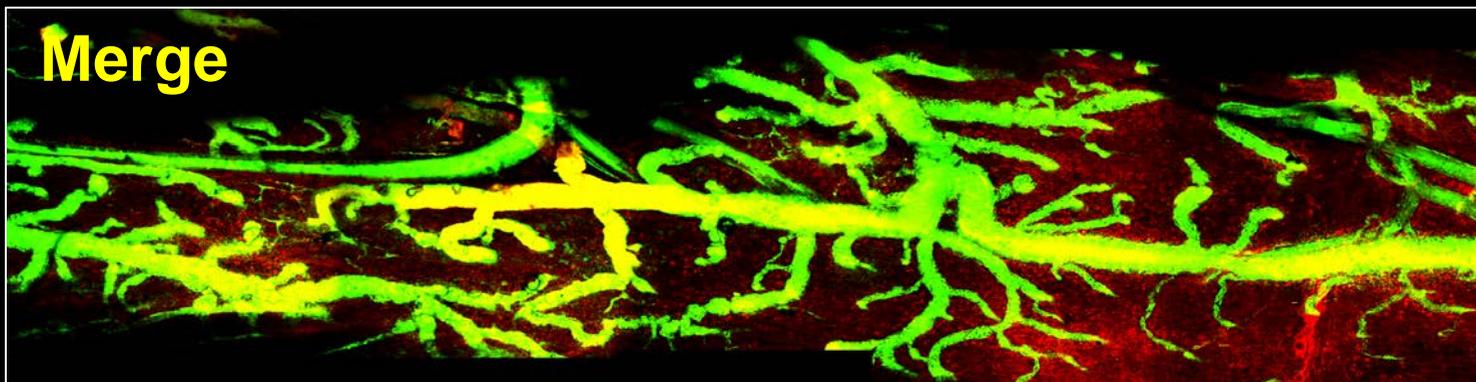
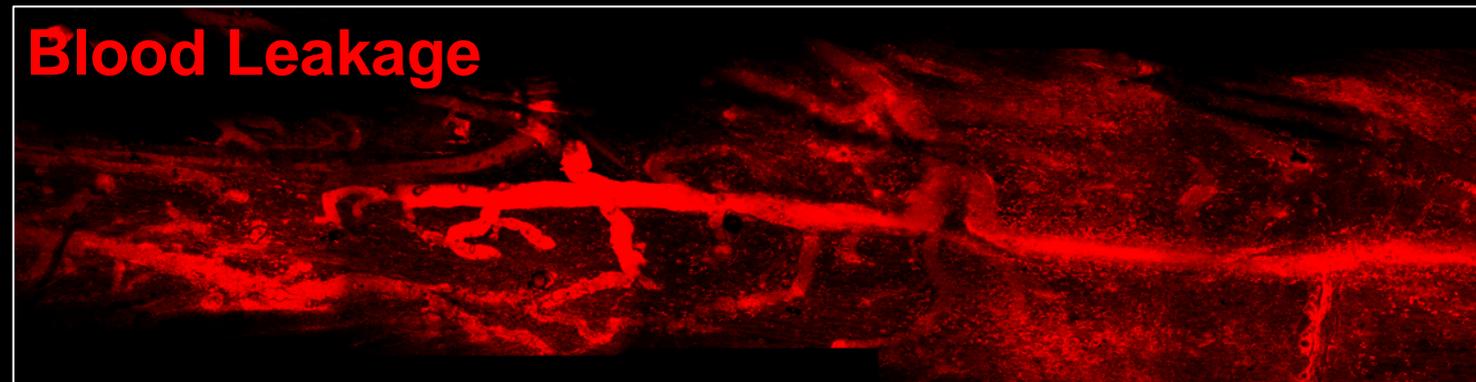
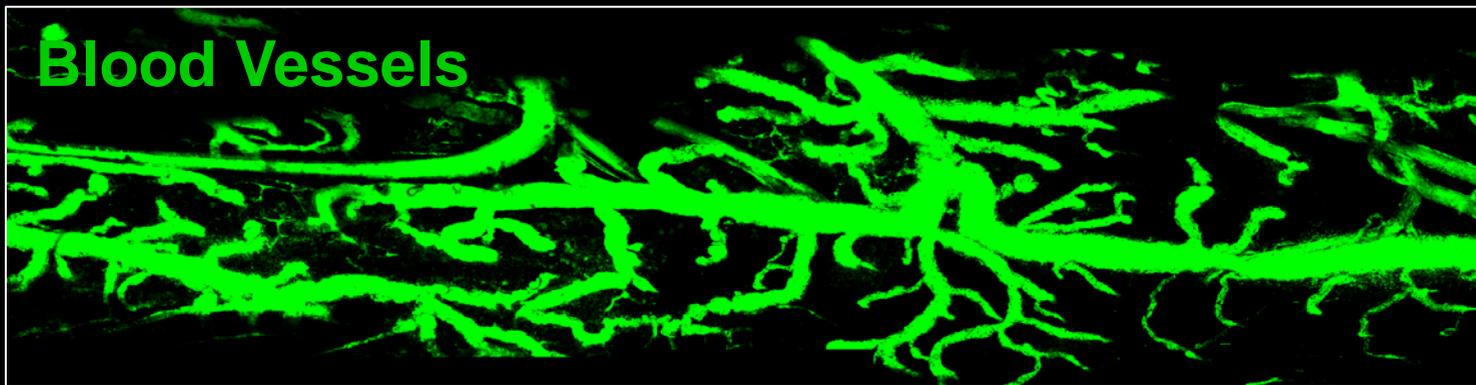
3D- Immuno- labeling in Cleared Brain

Pamela Rios
Victoria Rafalski



Blood-Brain Barrier Disruption

Multiple
Sclerosis
Animal
Model



Pamela Rios,
Maya Ellisman

Glia, Neurons

Microglial Activation
Neurite Outgrowth
Oxidative Stress
Axonal Damage
Scar Formation
Remyelination

Vasculature

BBB Disruption

Immune Cells

Innate Immunity
Autoimmunity

Multiple Sclerosis, Stroke,
Brain Trauma, Neurodegeneration

Imaging

Therapeutics

In-Vivo Imaging
Molecular Probe
Development

CNS Targeting
New Animal Model
Biomarkers

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**Peripheral Triggers?
Molecular Determinants?**

Imaging

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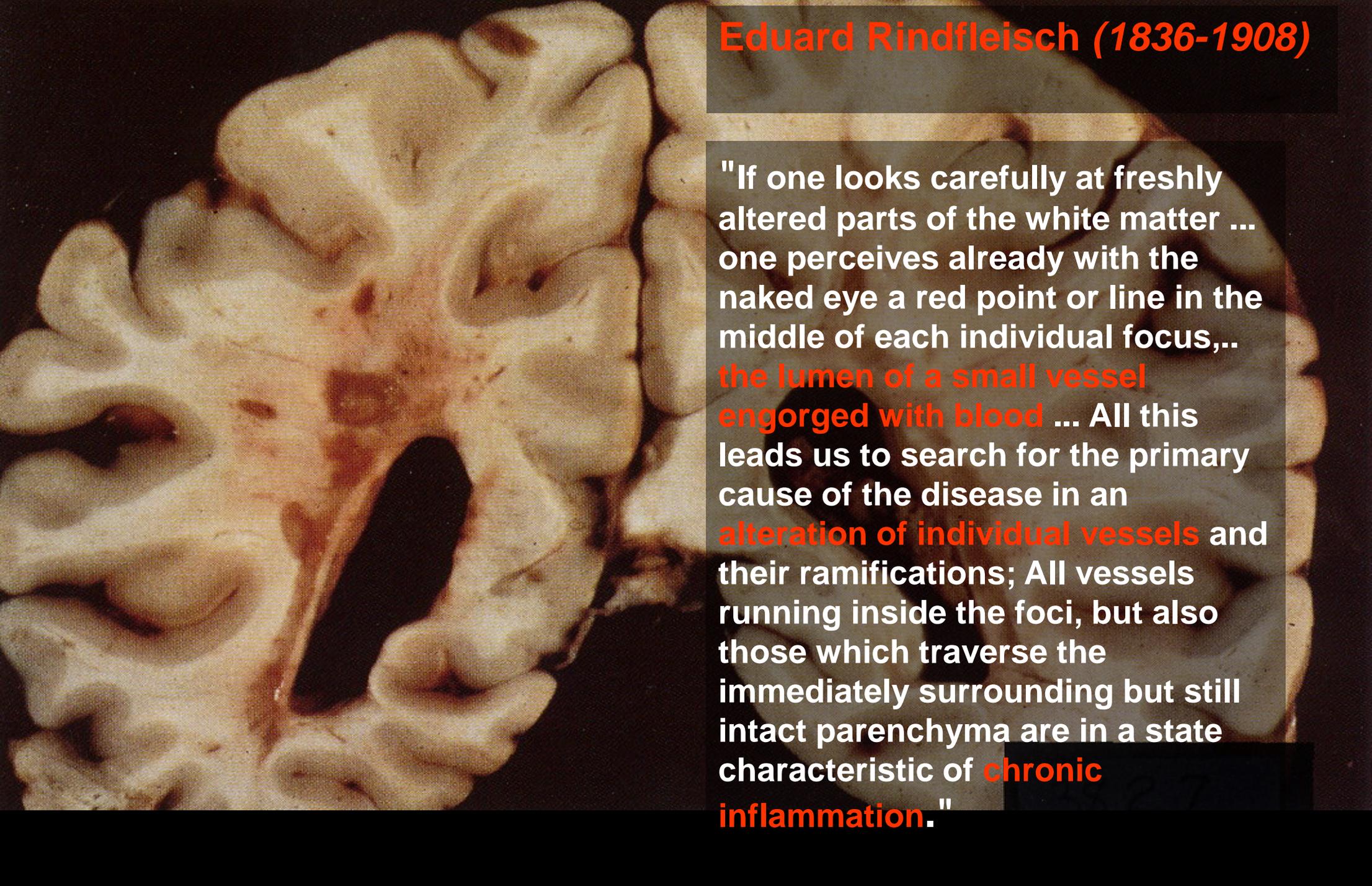
**Blood coagulation factors
Fibrinogen**

Imaging

Therapeutics

In-Vivo Imaging
Molecular Probe
Development

CNS Targeting
New Animal Model
Biomarkers



Eduard Rindfleisch (1836-1908)

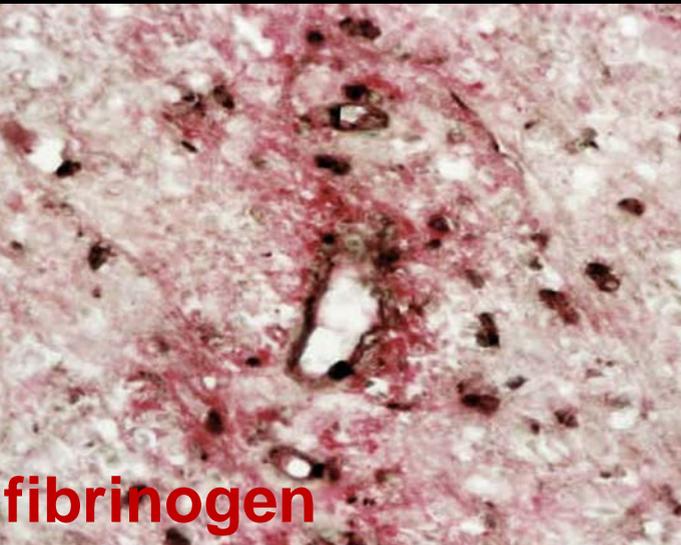
"If one looks carefully at freshly altered parts of the white matter ... one perceives already with the naked eye a red point or line in the middle of each individual focus, ... **the lumen of a small vessel engorged with blood** ... All this leads us to search for the primary cause of the disease in an **alteration of individual vessels** and their ramifications; All vessels running inside the foci, but also those which traverse the immediately surrounding but still intact parenchyma are in a state characteristic of **chronic inflammation.**"

Fibrinogen is abundantly deposited in neurological diseases

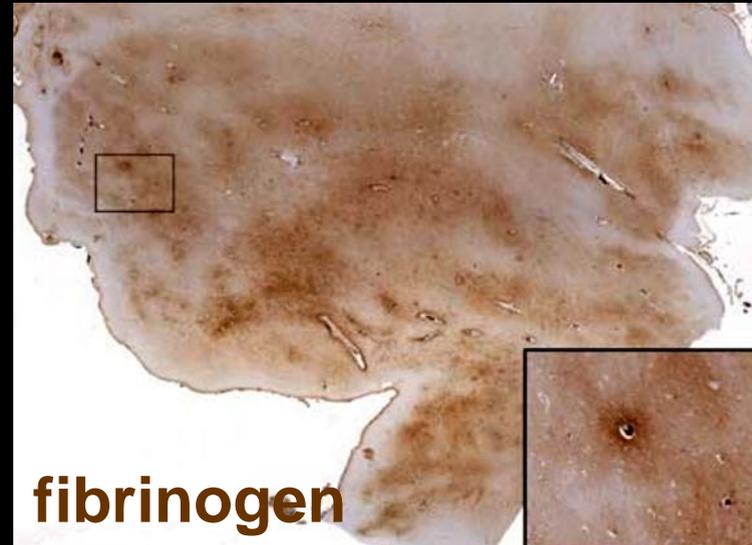
Human Brain

Multiple sclerosis (MS), Traumatic brain injury (TBI)
Alzheimers disease (AD)

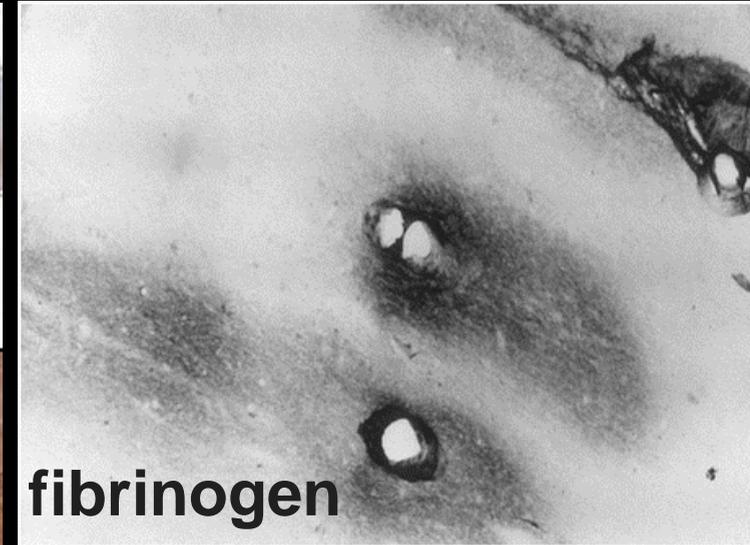
MS



TBI



AD



Fibrinogen is abundantly deposited in neurological diseases



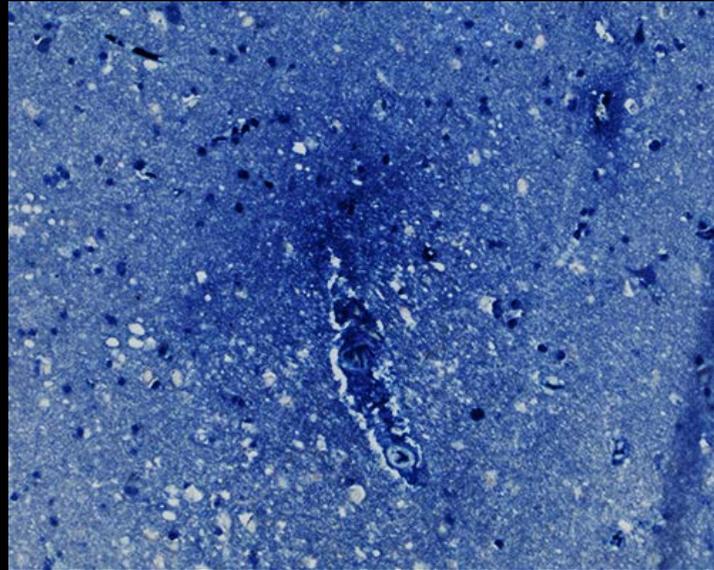
Fibrinogen

Fibrinogen is abundantly deposited in neurological diseases

Control



Active MS lesion



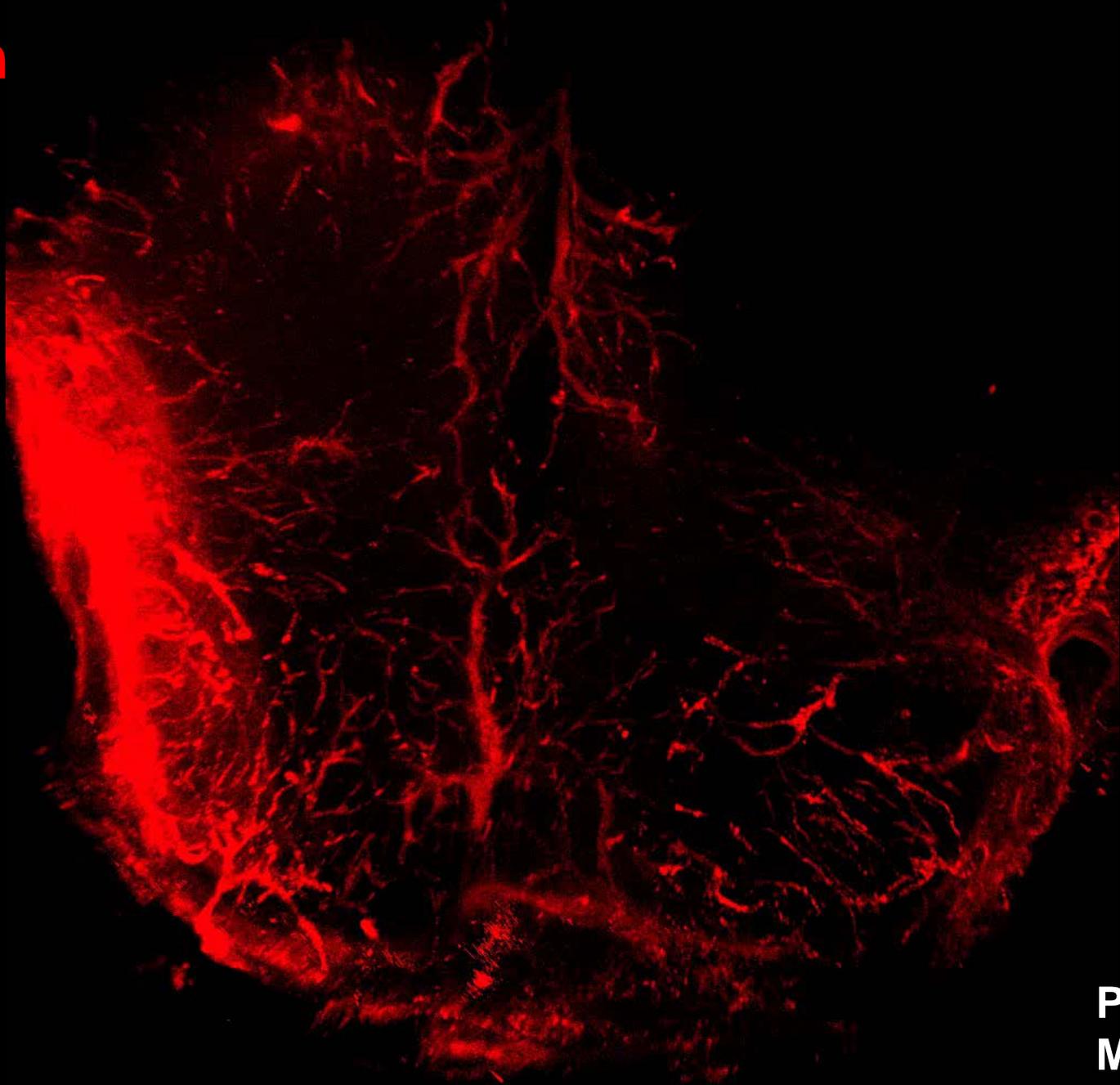
Chronic Inactive MS



Fibrinogen

Fibrinogen

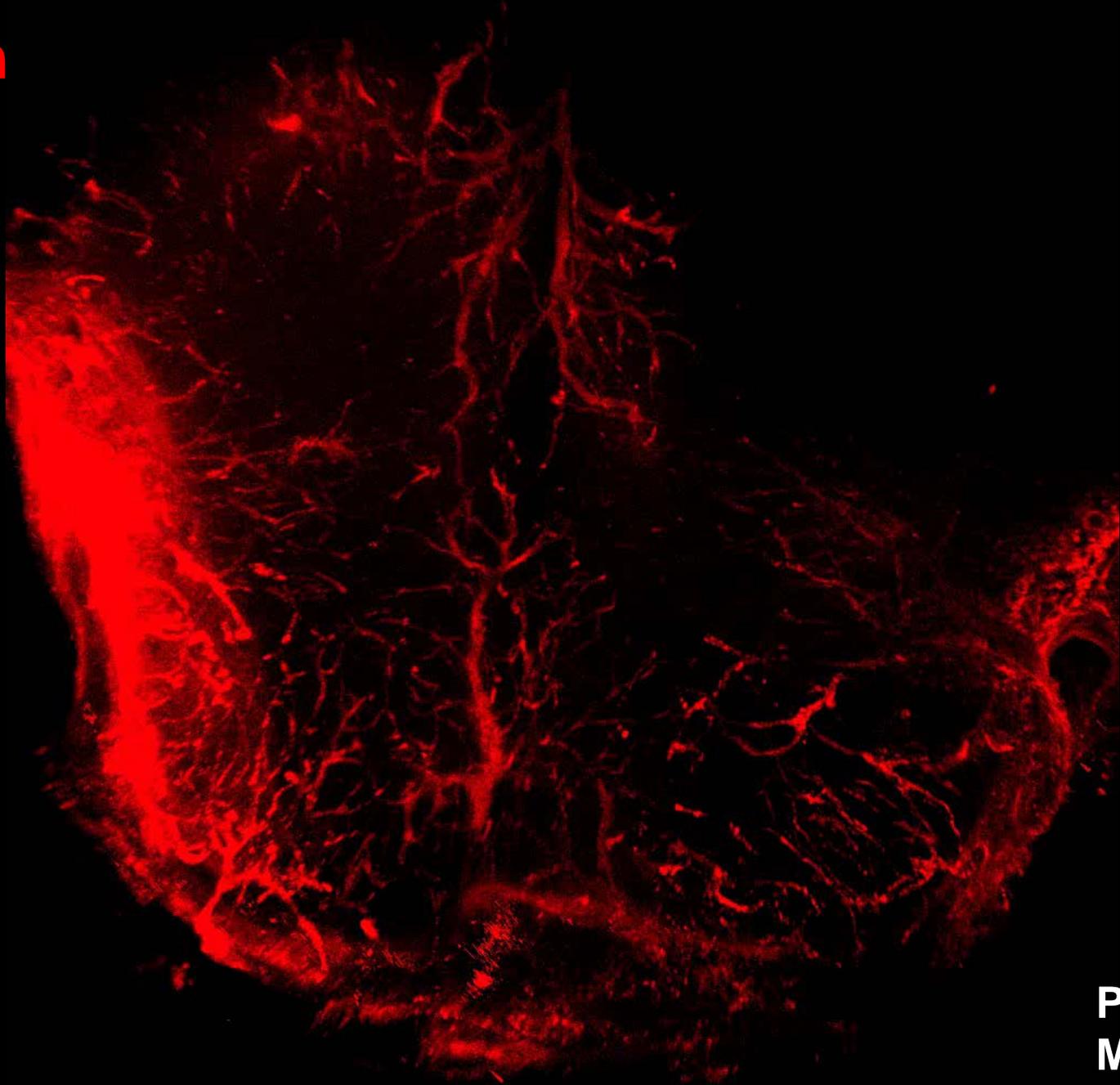
Multiple
Sclerosis
EAE
Animal
Model



Pamela Rios
Maya Ellisman

Fibrinogen

Multiple
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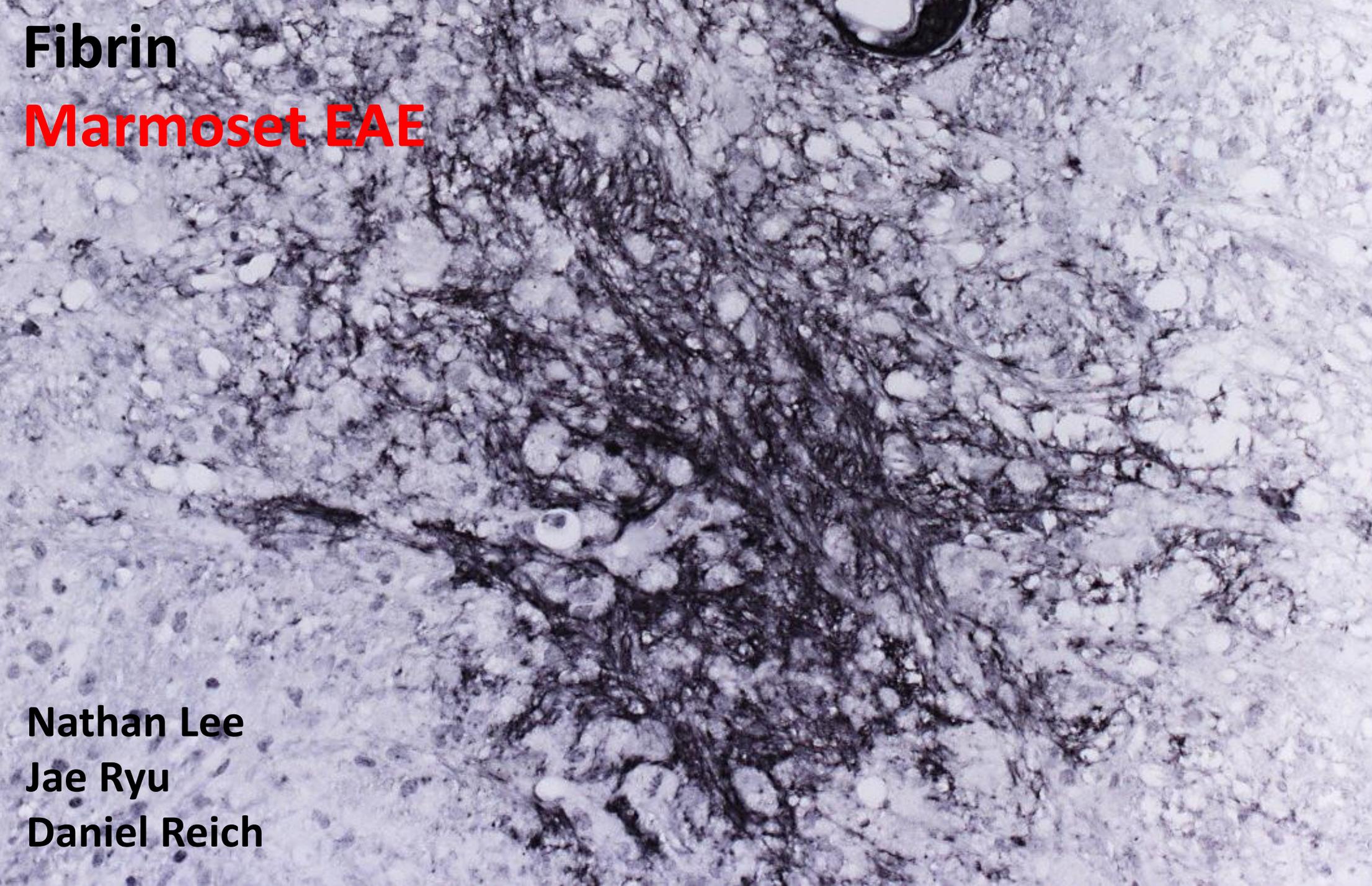
Fibrin

Marmoset EAE

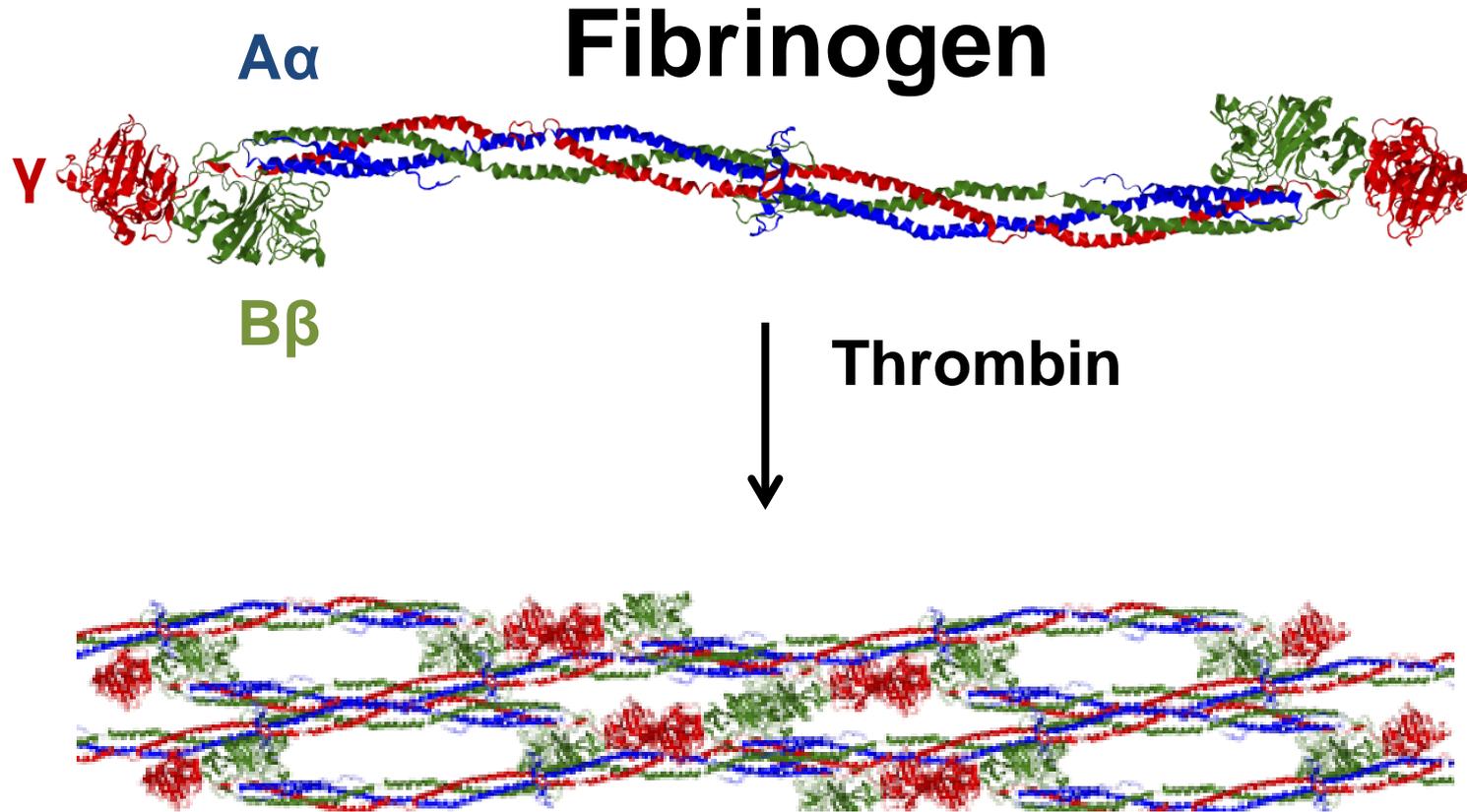
Nathan Lee

Jae Ryu

Daniel Reich



Fibrinogen is converted to inflammatory fibrin



Coagulation

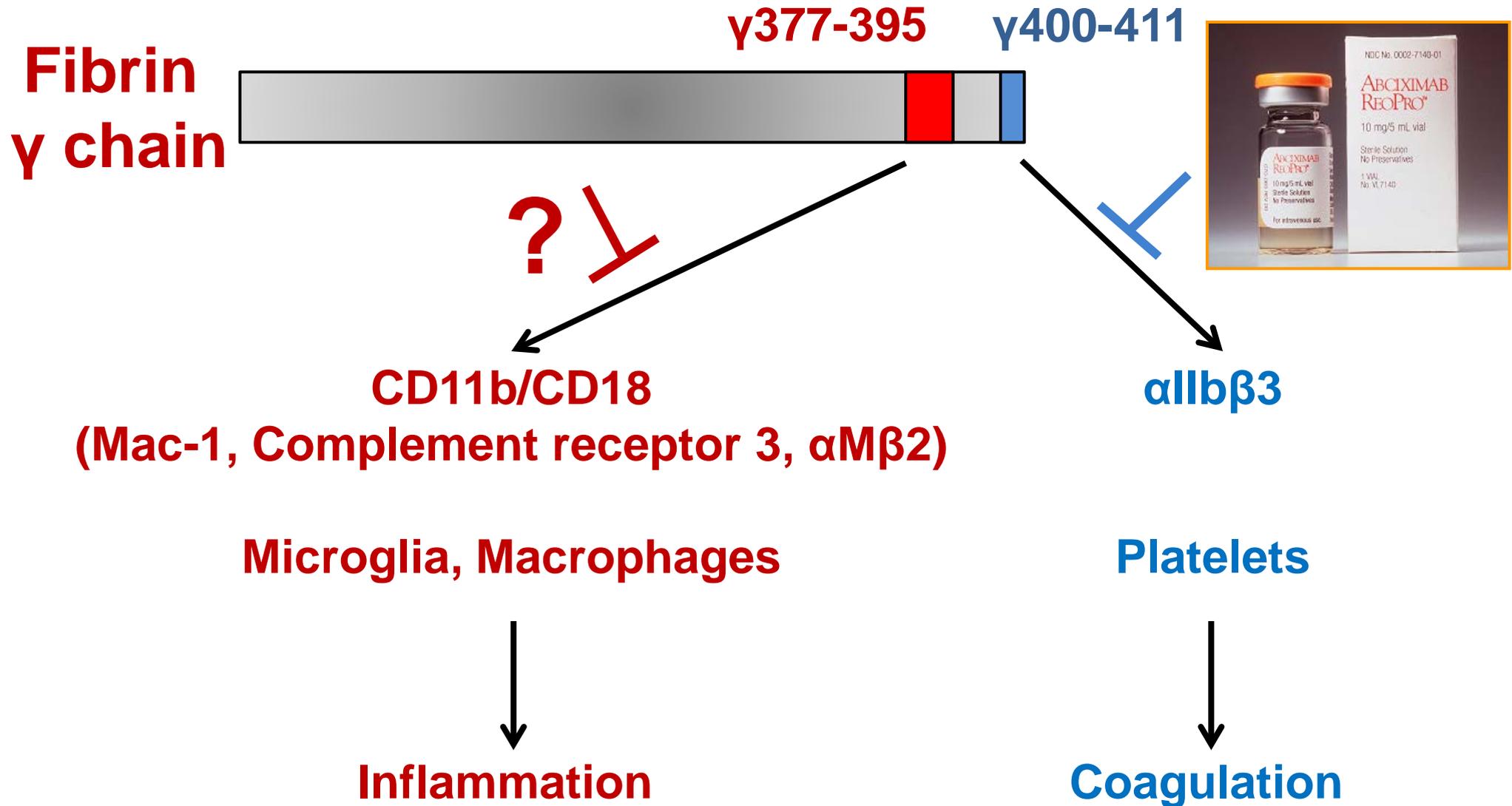


Fibrin



Inflammation

Fibrin is a “Druggable” Target



Fibrin is required for nervous system disease

PNS

- Nerve regeneration

Fibrin depletion enhances nerve remyelination

*Akassoglou et al., 2002, **Neuron**, 33:861-875*

CNS

- Multiple Sclerosis

Fibrin depletion decreases inflammation and delays the onset of demyelination

*Akassoglou et al., 2004, **Proc Natl Acad Sci USA**, 101:6698-6703*

Fibrin depletion suppresses relapsing paralysis

*Adams et al. 2007, **J Exp Med**, 35:2428-34*

Fibrin depletion protects from axonal damage

*Davalos et al. 2012, **Nature Commun**, 35:2428-34*

- Brain Trauma

Fibrin depletion reduces astrocyte scar formation

*Schachtrup et al., 2010, **J Neurosci**, 30:5843-54*

- Alzheimer Disease

Fibrin depletion reduces neuroinflammation and cognitive impairment

*Cortes-Canteli et al., 2010, **Neuron**. 66:695-709.*

Blood vessel

Fibrinogen

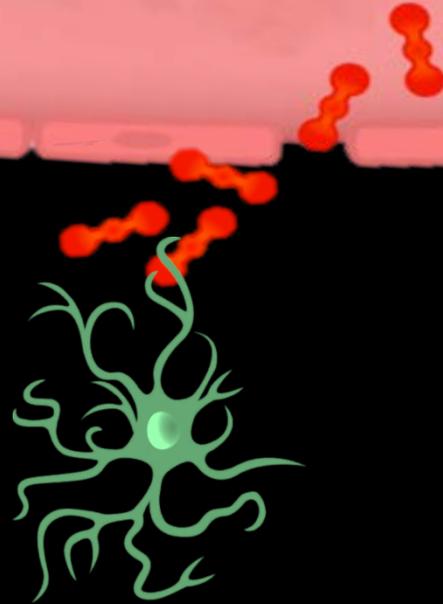
Nervous system



Multiple sclerosis, Traumatic brain injury, Alzheimers disease

Blood vessel

Fibrinogen



Microglia

**CD11b/CD18
(CR3, Mac-1)**

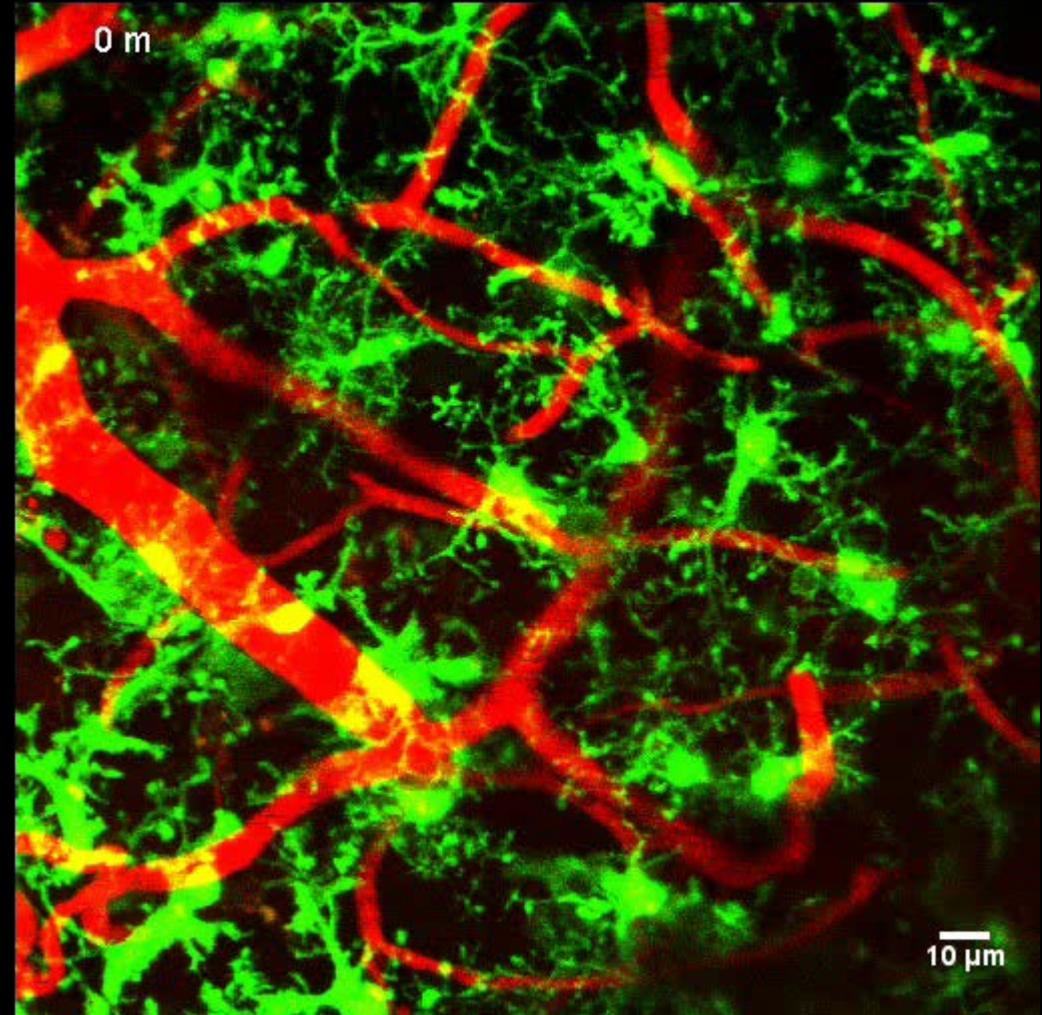
Adams et al. 2007, J Exp Med, 35:2428-34

Multiple sclerosis, Traumatic brain injury, Alzheimers disease

Dynamic Interaction between Microglia-Vasculature



Del Río-Hortega, 1921. Perivascular microglia.

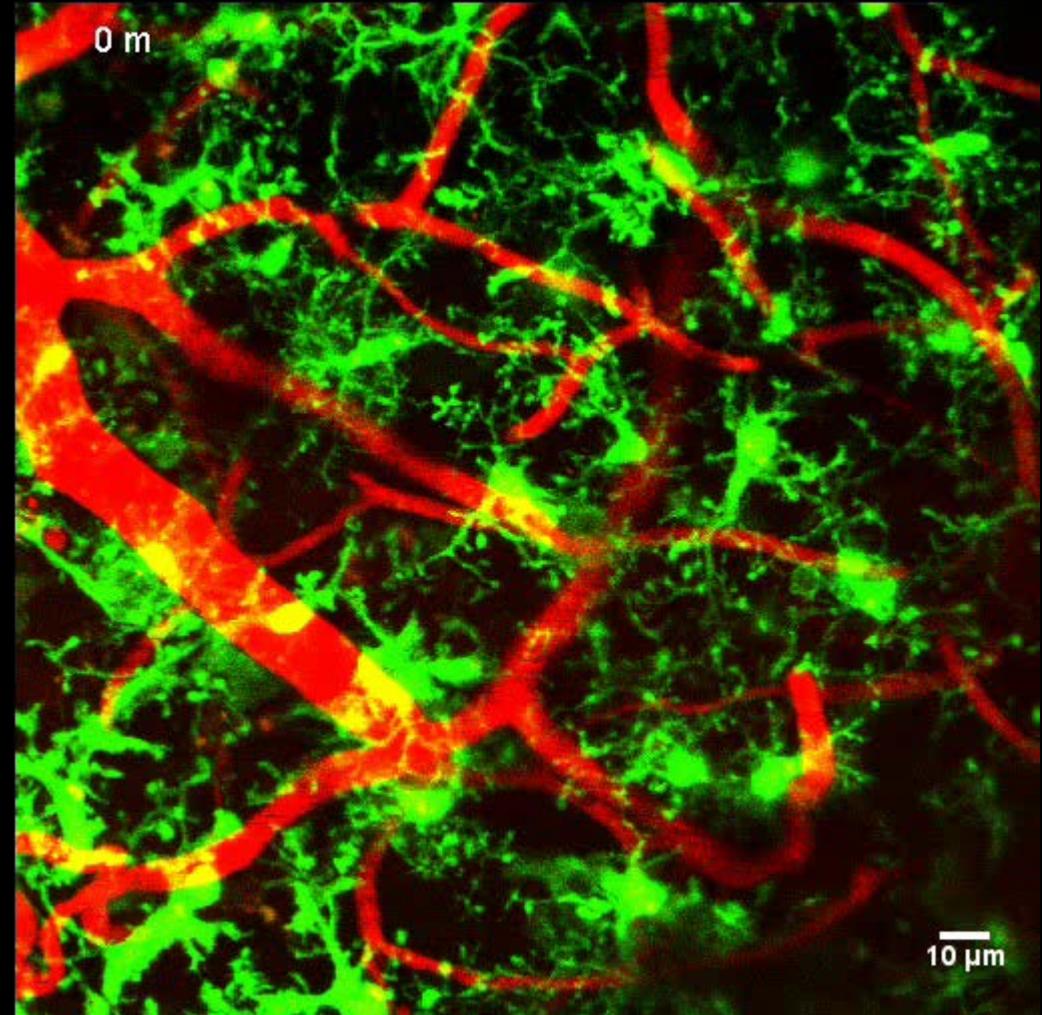


Davalos et al., 2012, *Nat Commun*, 3:1227

Dynamic Interaction between Microglia-Vasculature



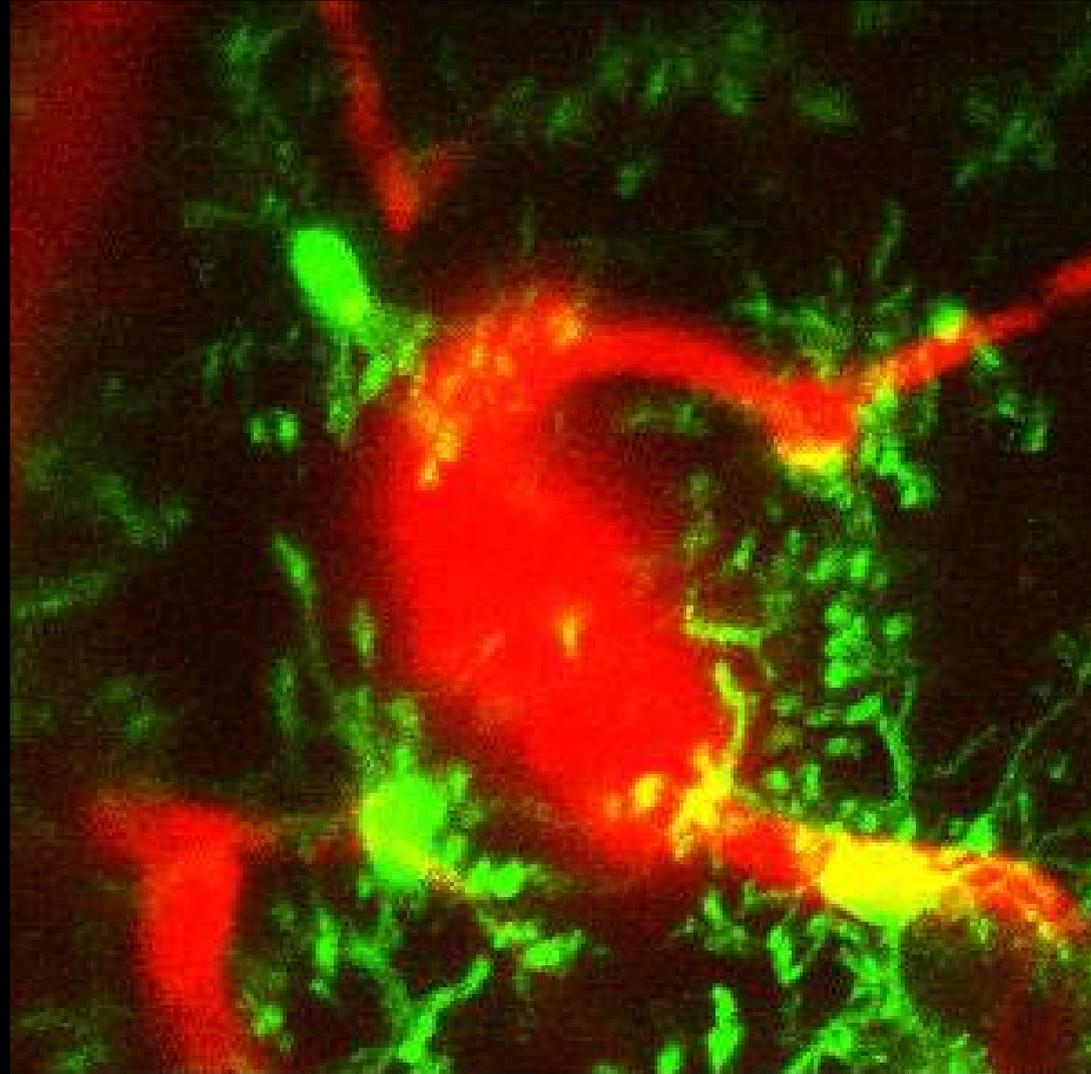
Del Río-Hortega, 1921. Perivascular microglia.



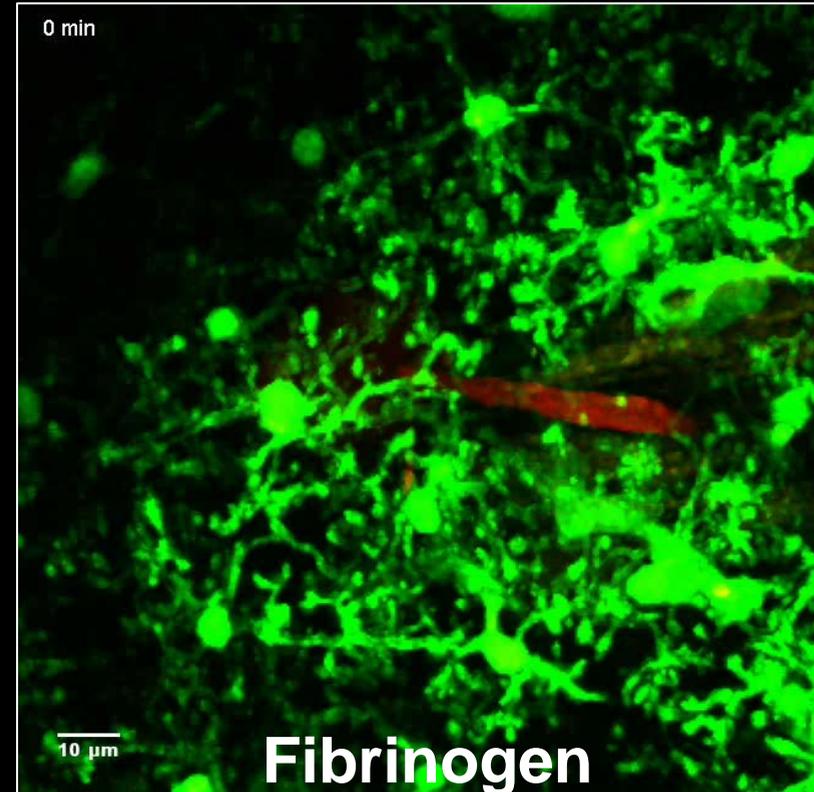
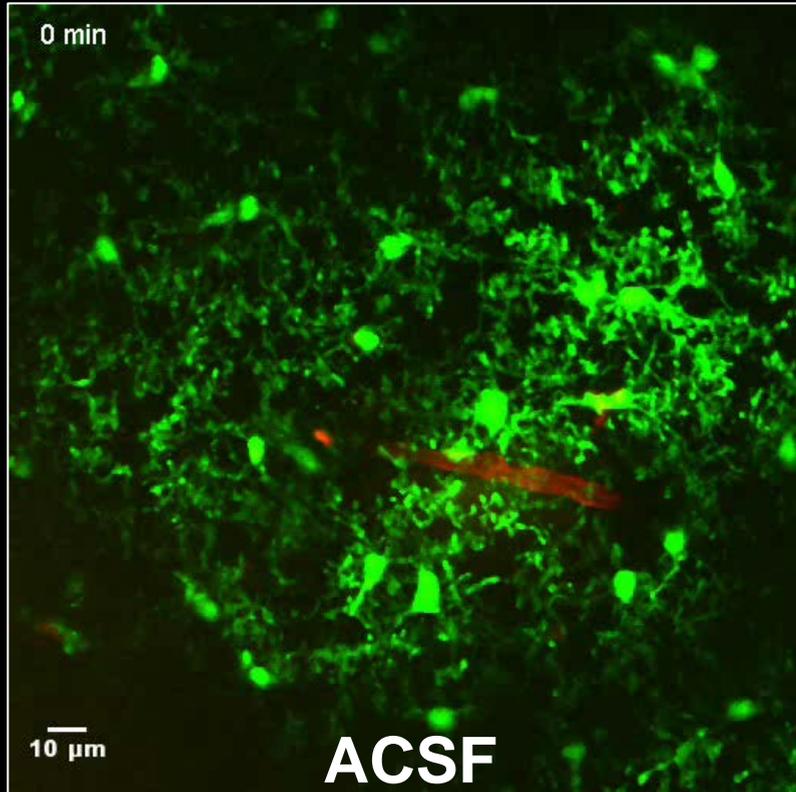
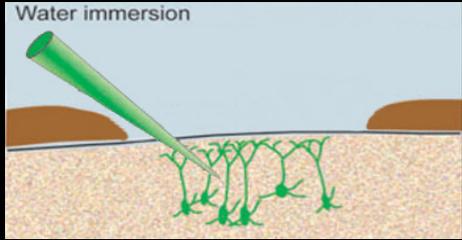
Davalos et al., 2012, *Nat Commun*, 3:1227

Vascular damage is a robust inducer of microglia responses

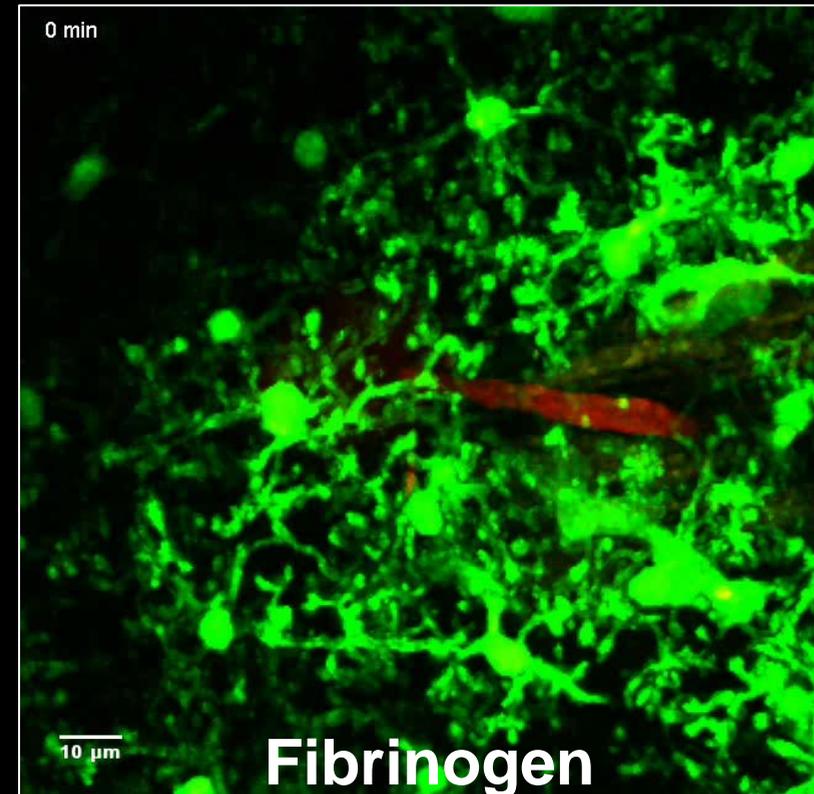
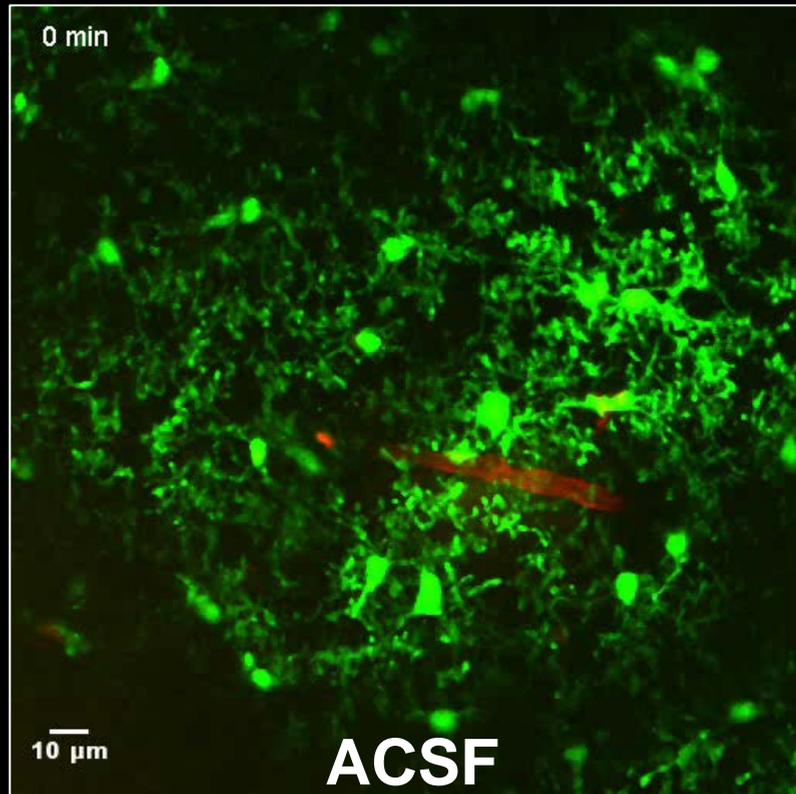
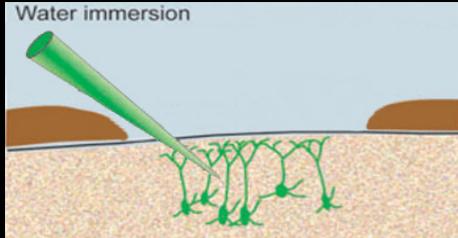
- Microglia
- Blood vessel
- Laser ablation



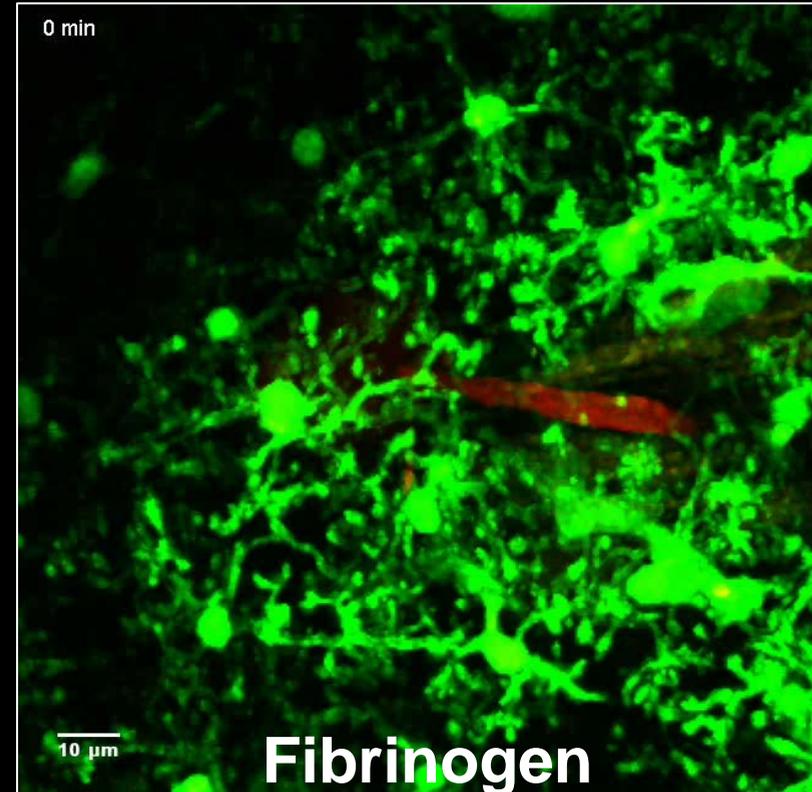
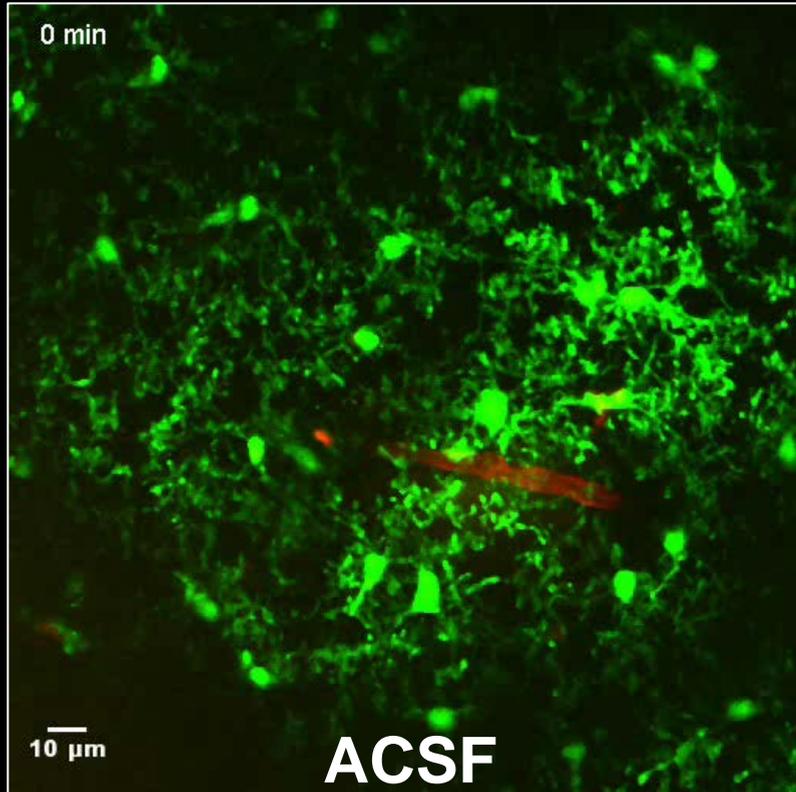
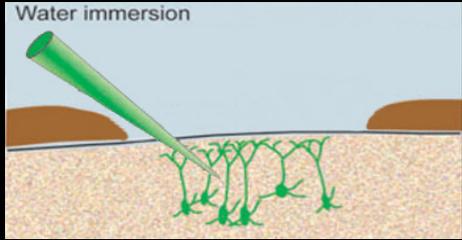
Fibrinogen is sufficient to induce rapid microglial responses in vivo



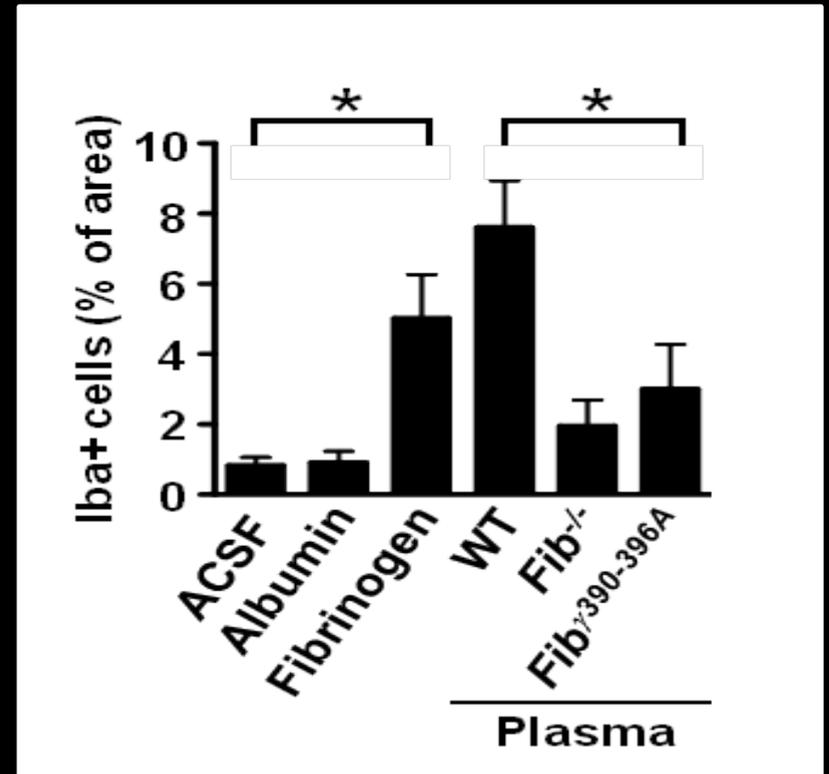
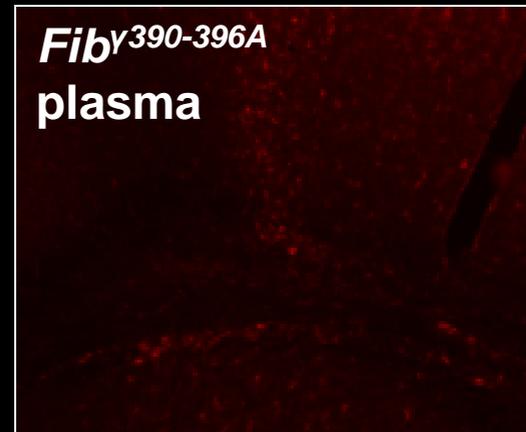
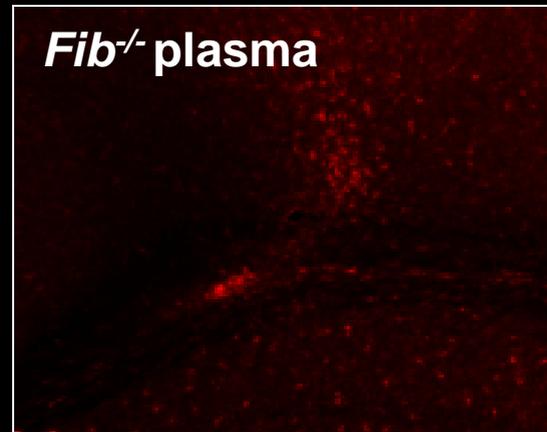
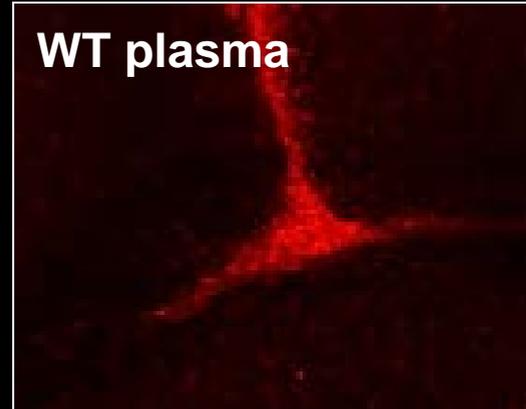
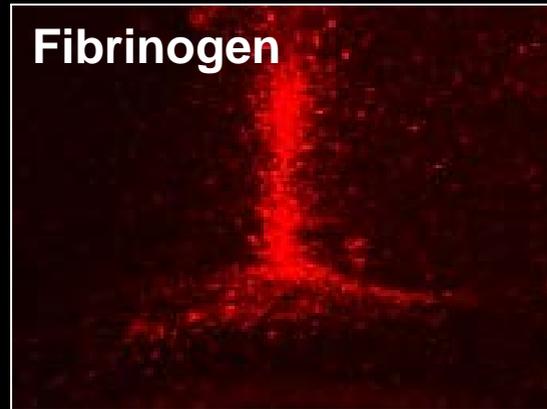
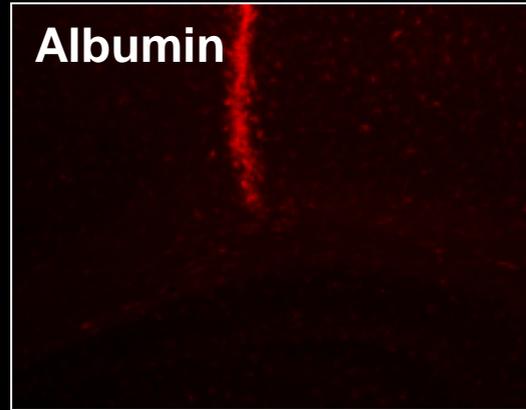
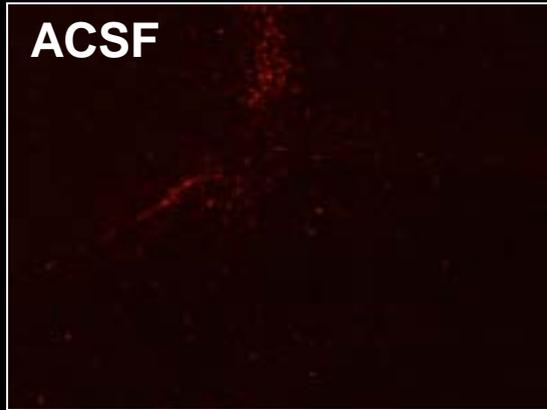
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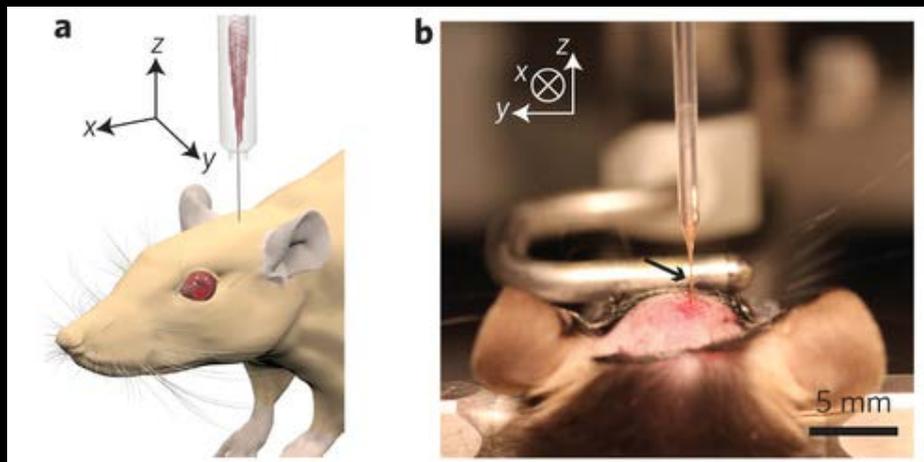
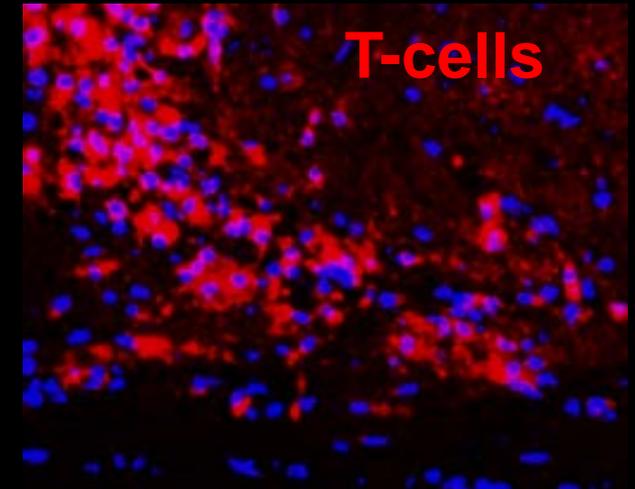
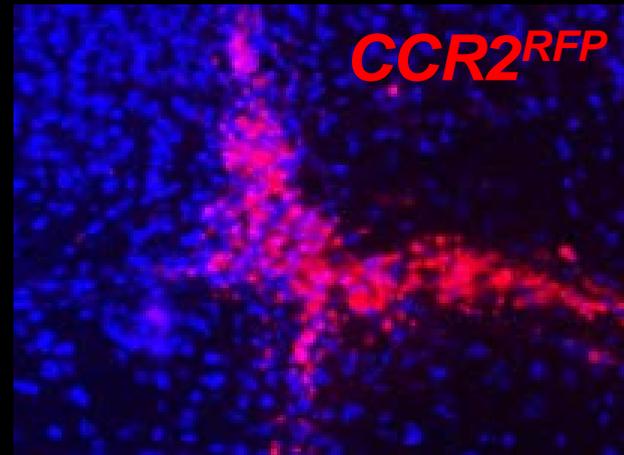
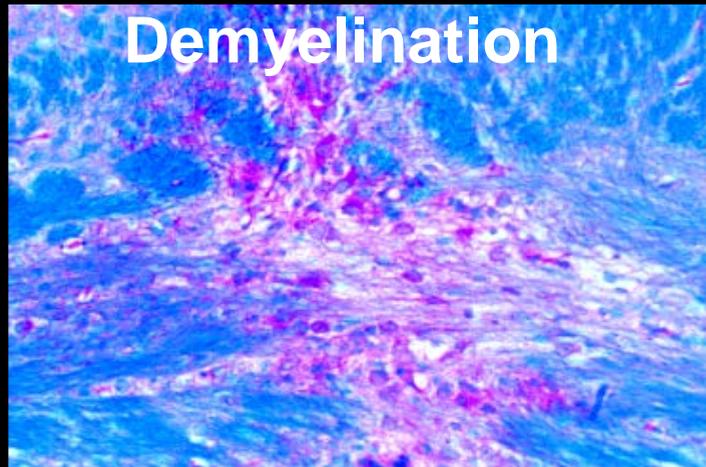


Fibrinogen is specific among plasma proteins to induce microglial activation



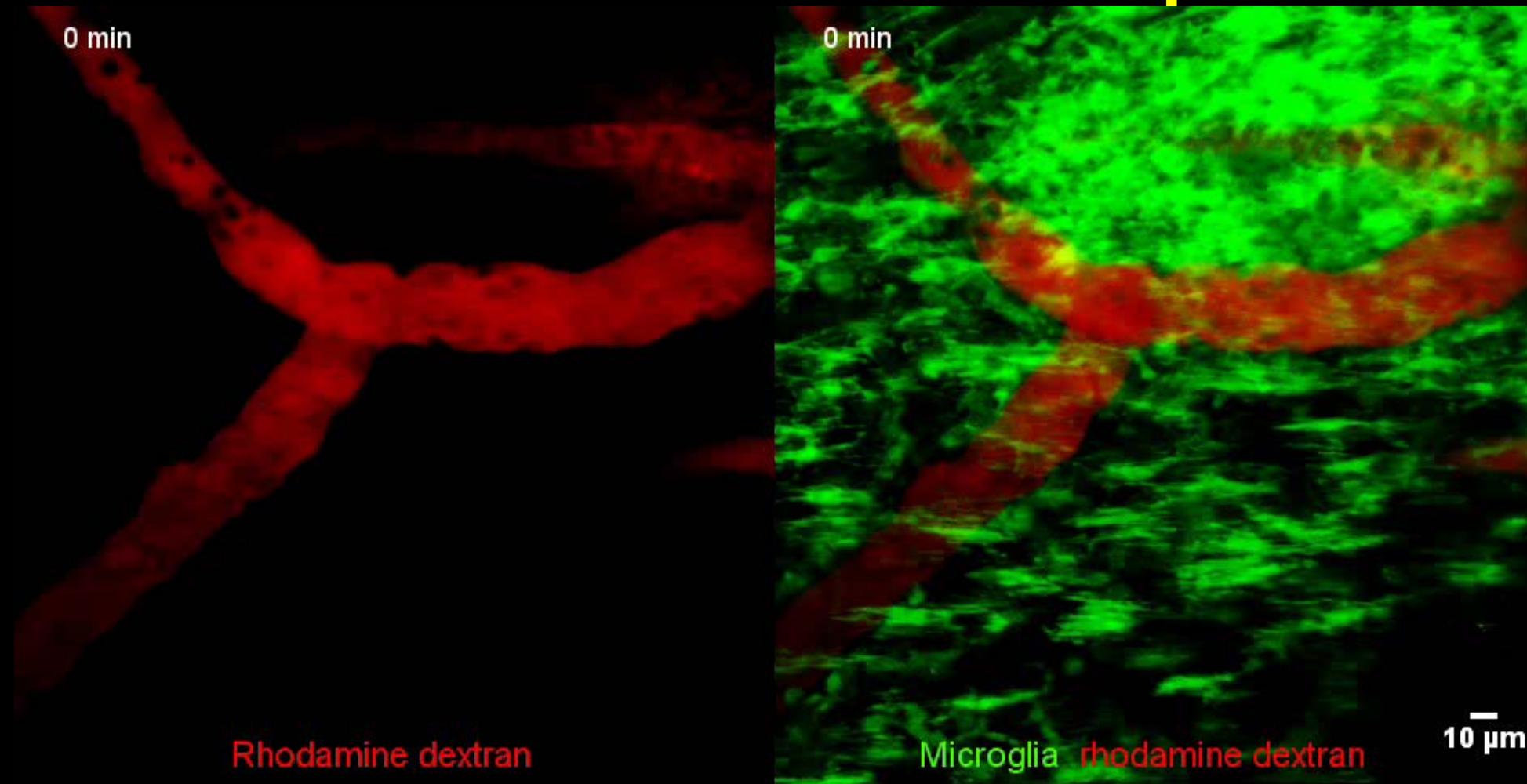
Fibrinogen-Induced Encephalomyelitis (FIE)

Ryu et al., 2015, *Nat Commun*:6:8164

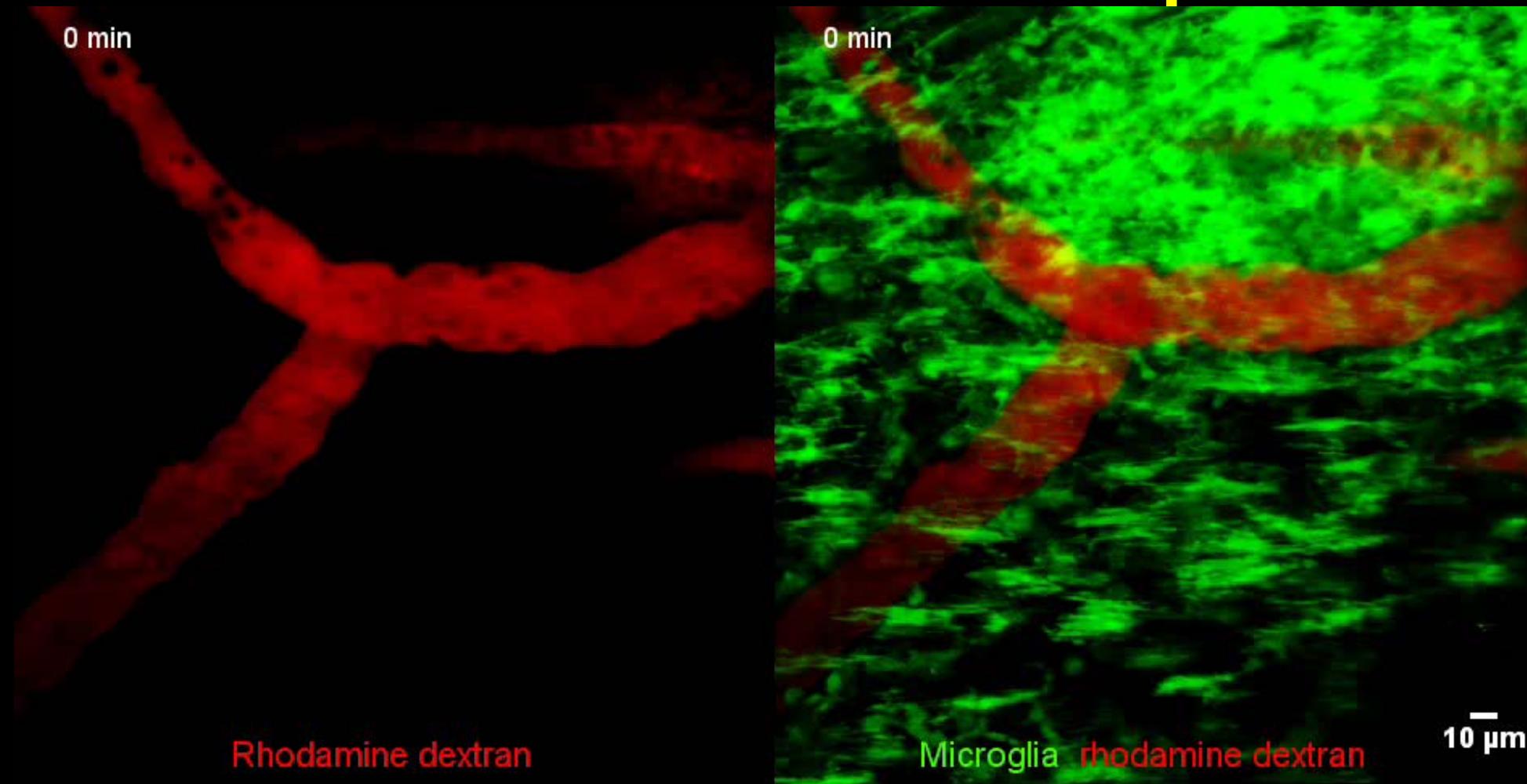


- Novel experimental setting to study vascular-driven neuroinflammation
- Fibrin-induced, microglia-driven

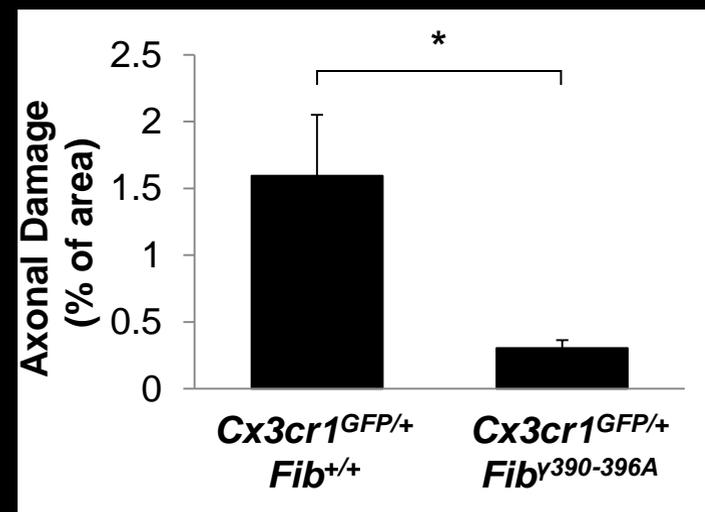
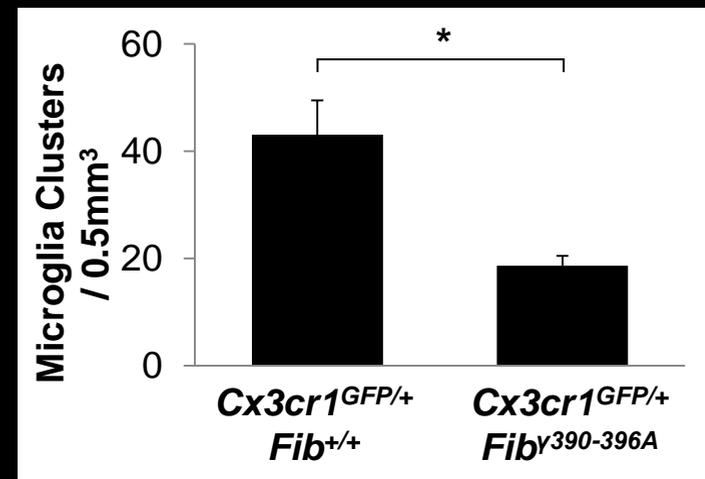
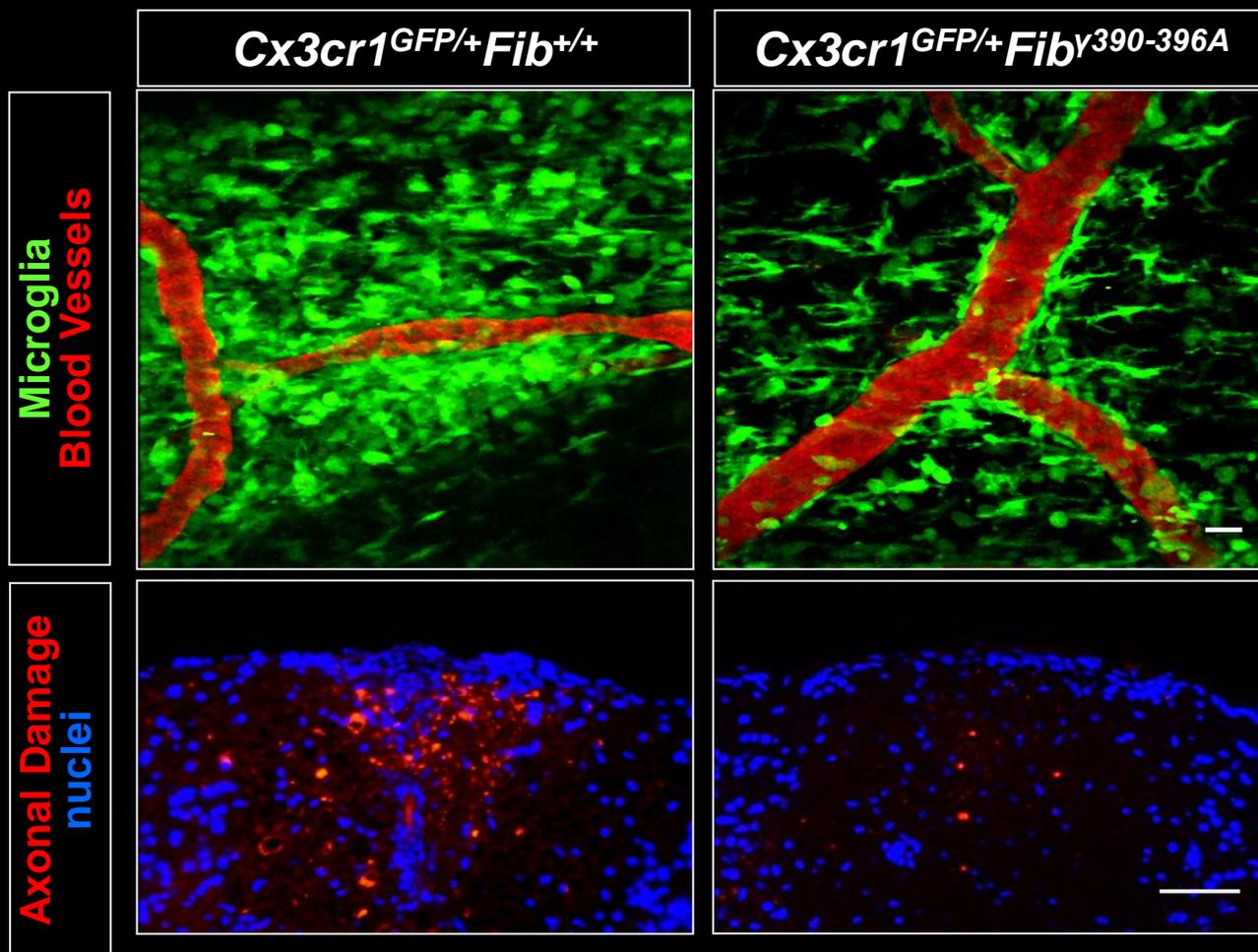
Microglia cluster around blood vessels at sites of active BBB disruption



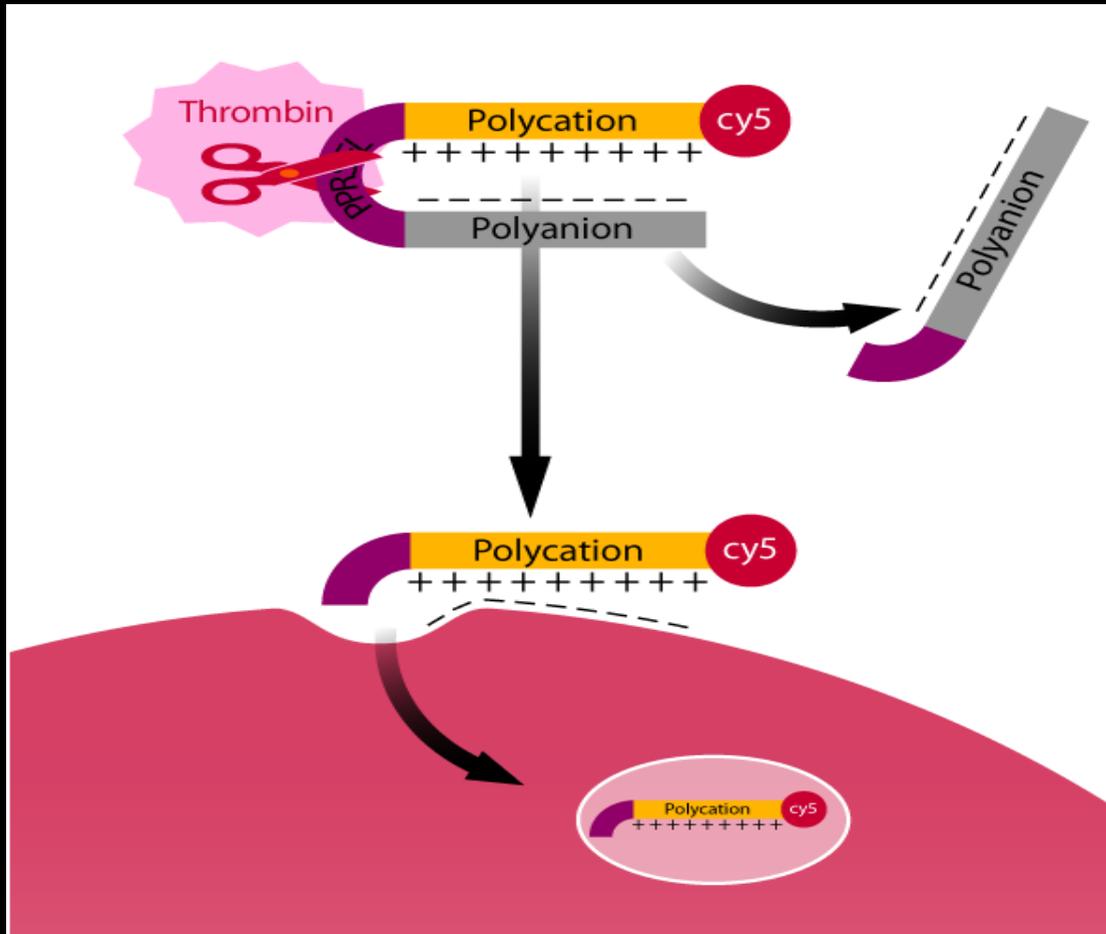
Microglia cluster around blood vessels at sites of active BBB disruption



Fibrin / CD11b signaling is required for microglial perivascular clustering and axonal damage



Coagulation activity in the CNS as a sensitive imaging biomarker



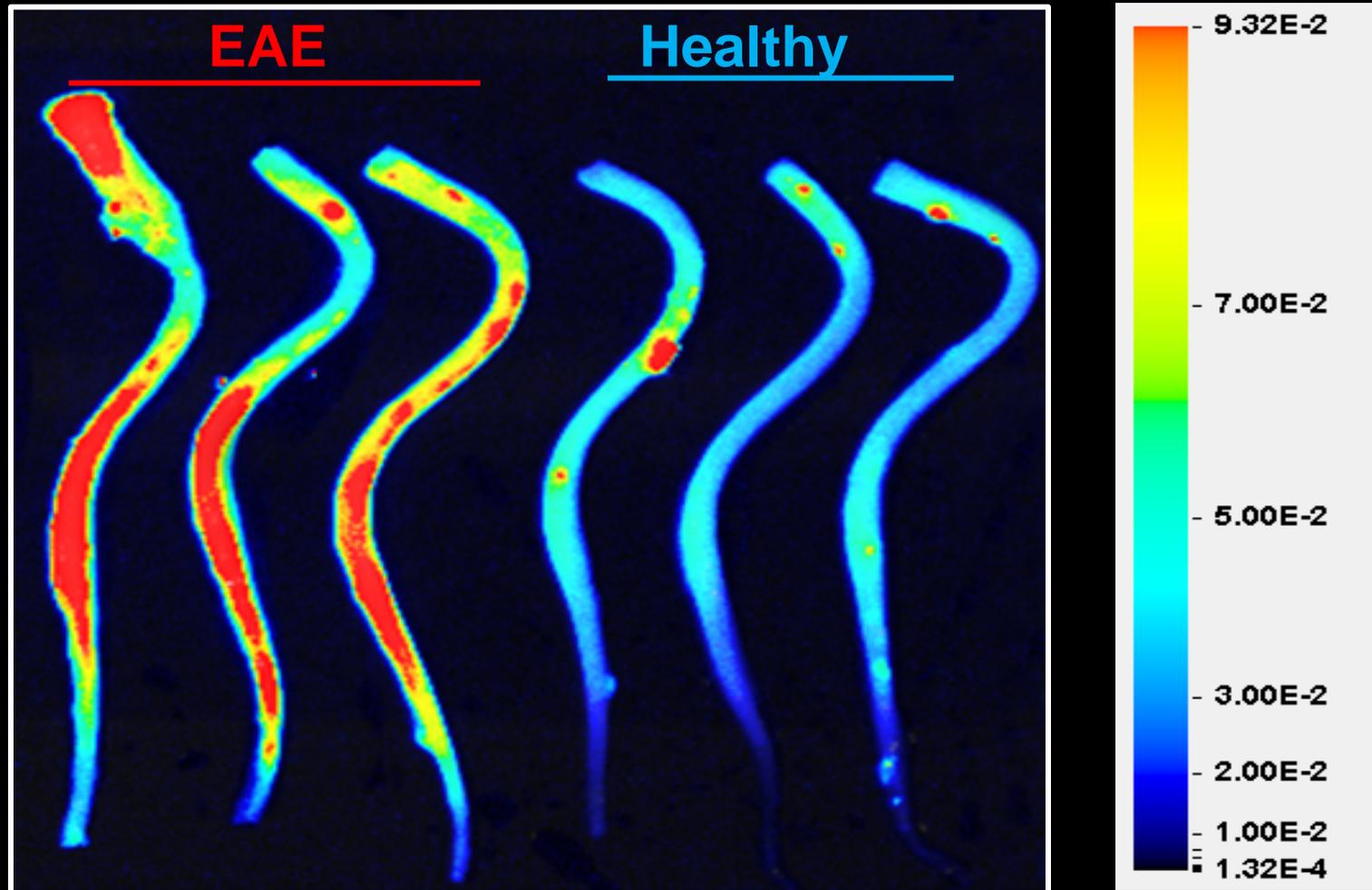
- **Thrombin ACPP** (Activatable Cell Penetrating Peptide): thrombin specific sequence links two oppositely charged peptides
- *Thrombin* activity releases polycation with cy-5 cargo that is uptaken by cells
- Clinical applications for ACPPs due to dual labeling with fluorescence and Gd.
- MMP ACPPs used in cancer detection and removal

*In collaboration with
Michael Whitney, Roger Tsien, UCSD*

Davalos et al., 2014, *Ann Neurol*, 75:303-8

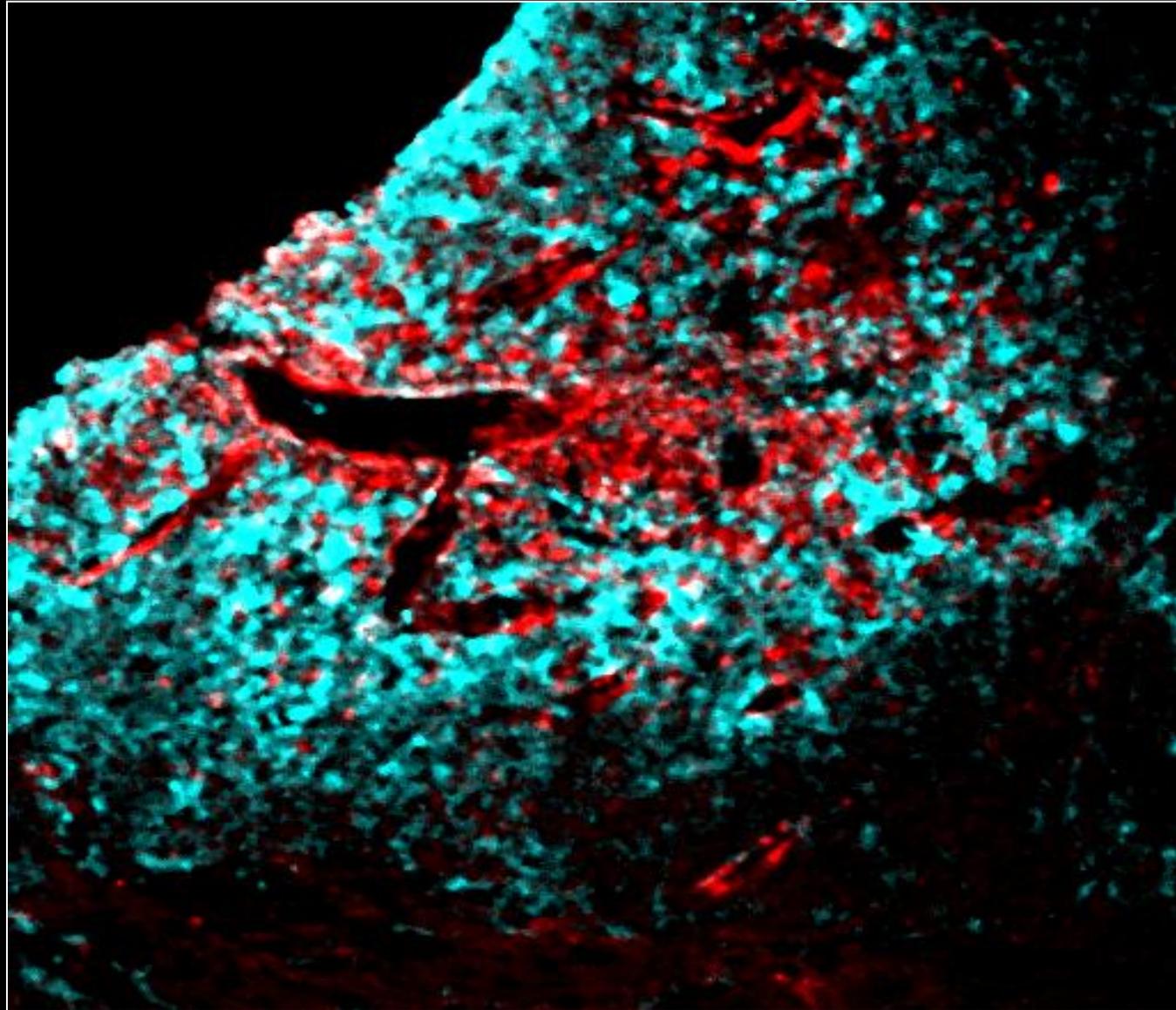
Coagulation activity in the CNS as a sensitive imaging biomarker

Whole Spinal Cord Imaging



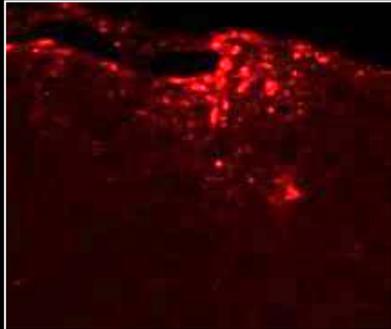
Coagulation activity correlates with demyelinated lesions

Thrombin Probe / Myelin

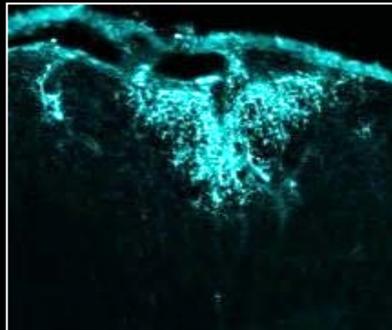


Coagulation activity detected early in EAE

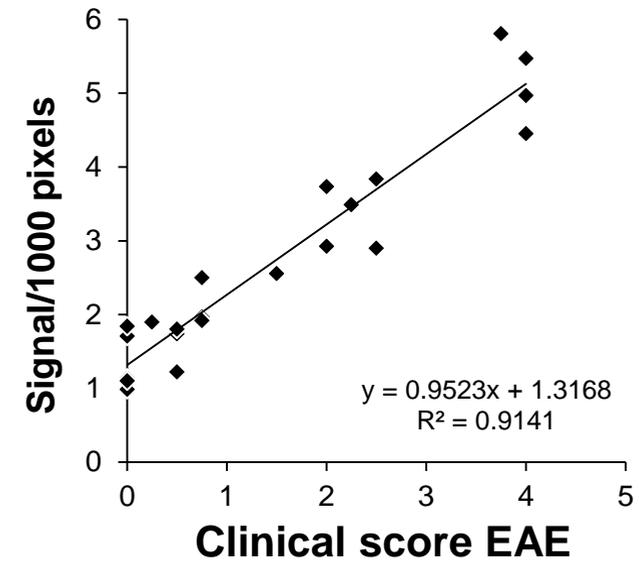
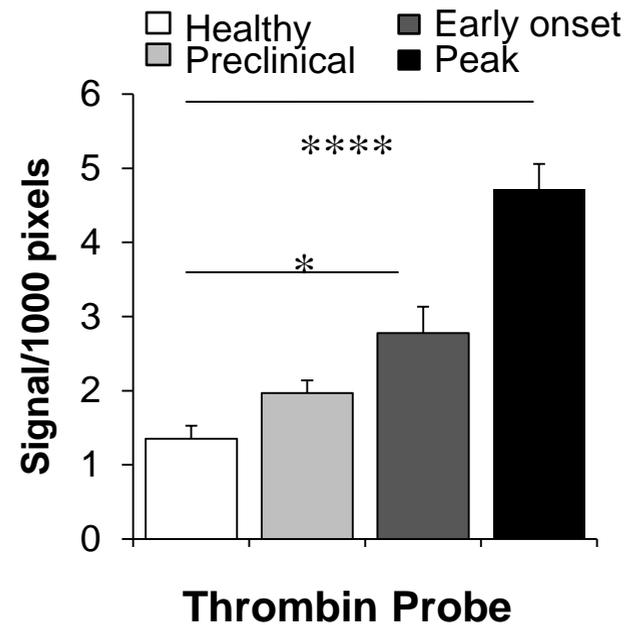
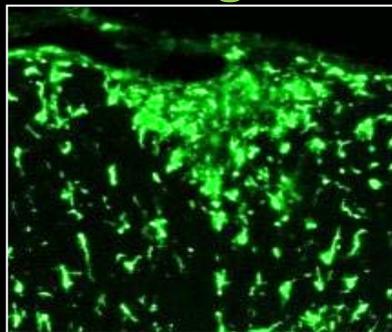
Thrombin Probe



Fibrin

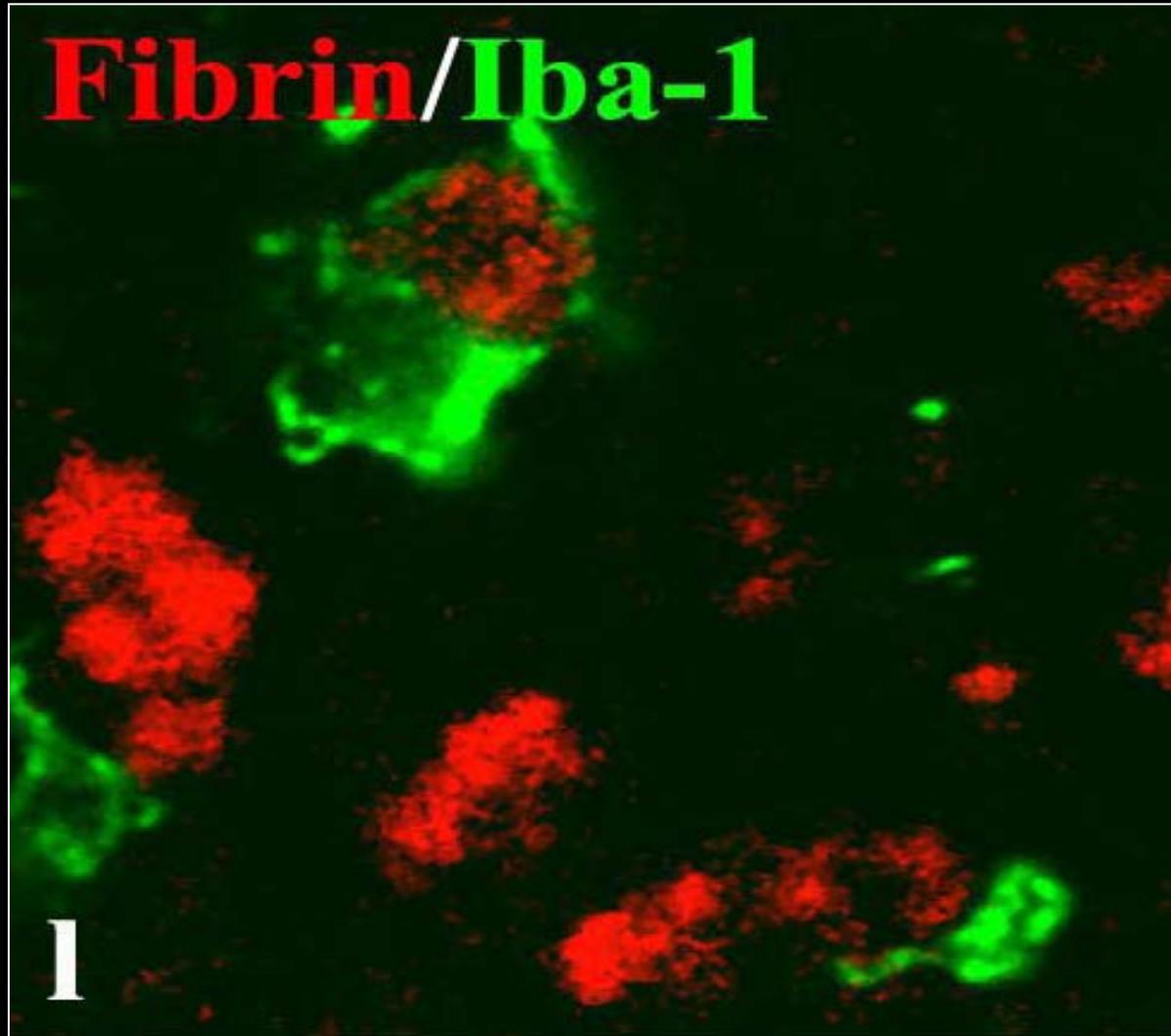


Microglia



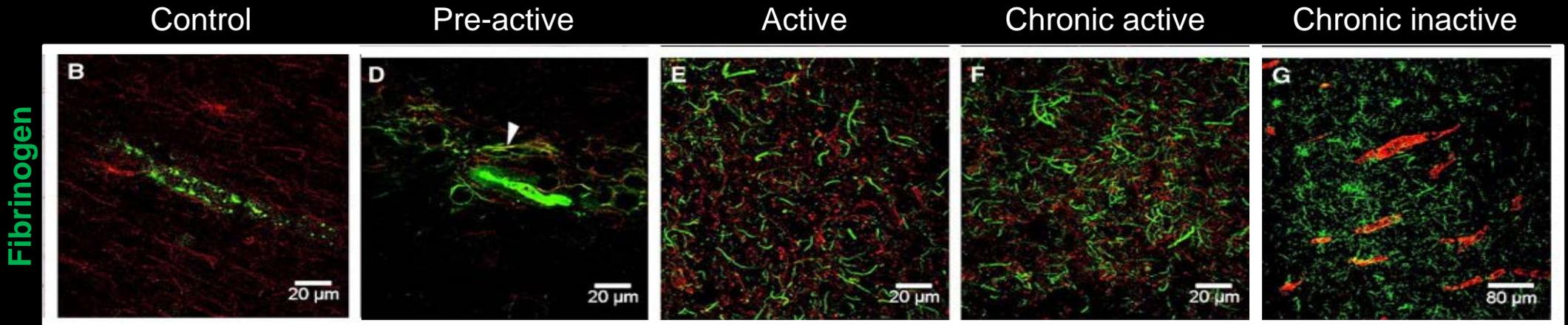
Fibrin in early MS lesions

Human MS



Fibrin deposition during the course of MS

Human MS

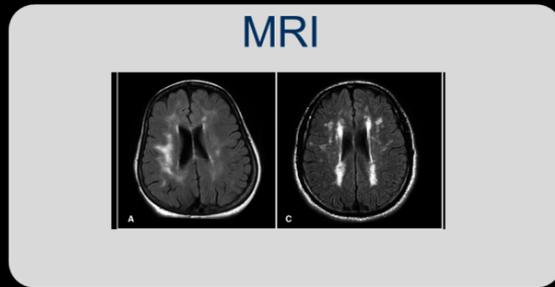


Vos et al., 2005, *Neurobiol Dis.* 20(3):953-60.

- Proteomic analysis of chronic active plaques reveals activation of the coagulation cascade
 - Han et al., 2008, *Nature*, 451:1076-81
- BBB opens 4 weeks prior to demyelination in marmoset EAE
 - Maggi et al., 2014, *Ann Neurol*, 76:594-608
- Impaired fibrinolysis in MS lesions
 - Gveric et al., 2003, *Brain*, 126:1590-98

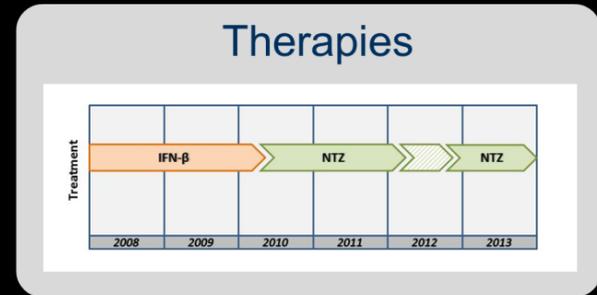
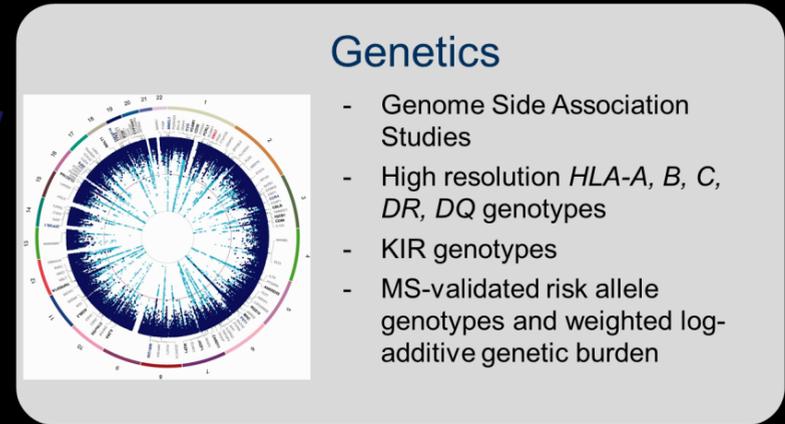
Excessive/persistent fibrin deposition in MS lesions

Coagulation Cascade Biomarkers for MS?



**EPIC MS
COHORT
N=582**

BIOMARKERS



Blood vessel

Fibrinogen

Macrophages

T Cells

Autoimmunity

Demyelination

Microglia

CD11b/CD18
(CR3, Mac-1)

Neurons

Oxidative Stress

Neurodegeneration

Adams et al. 2007, *J Exp Med*, 35:2428-34 ; Ryu et al., 2015, *Nat Commun*;6:8164 ; Davalos et al., 2012, *Nat Commun*, 3:1227

Multiple sclerosis, Traumatic brain injury, Alzheimers disease

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Mark Petersen
Victoria Rafalski
Pamela Rios
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Hans Lassmann

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Jay Degen

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Mario Merlini



Mark Petersen

