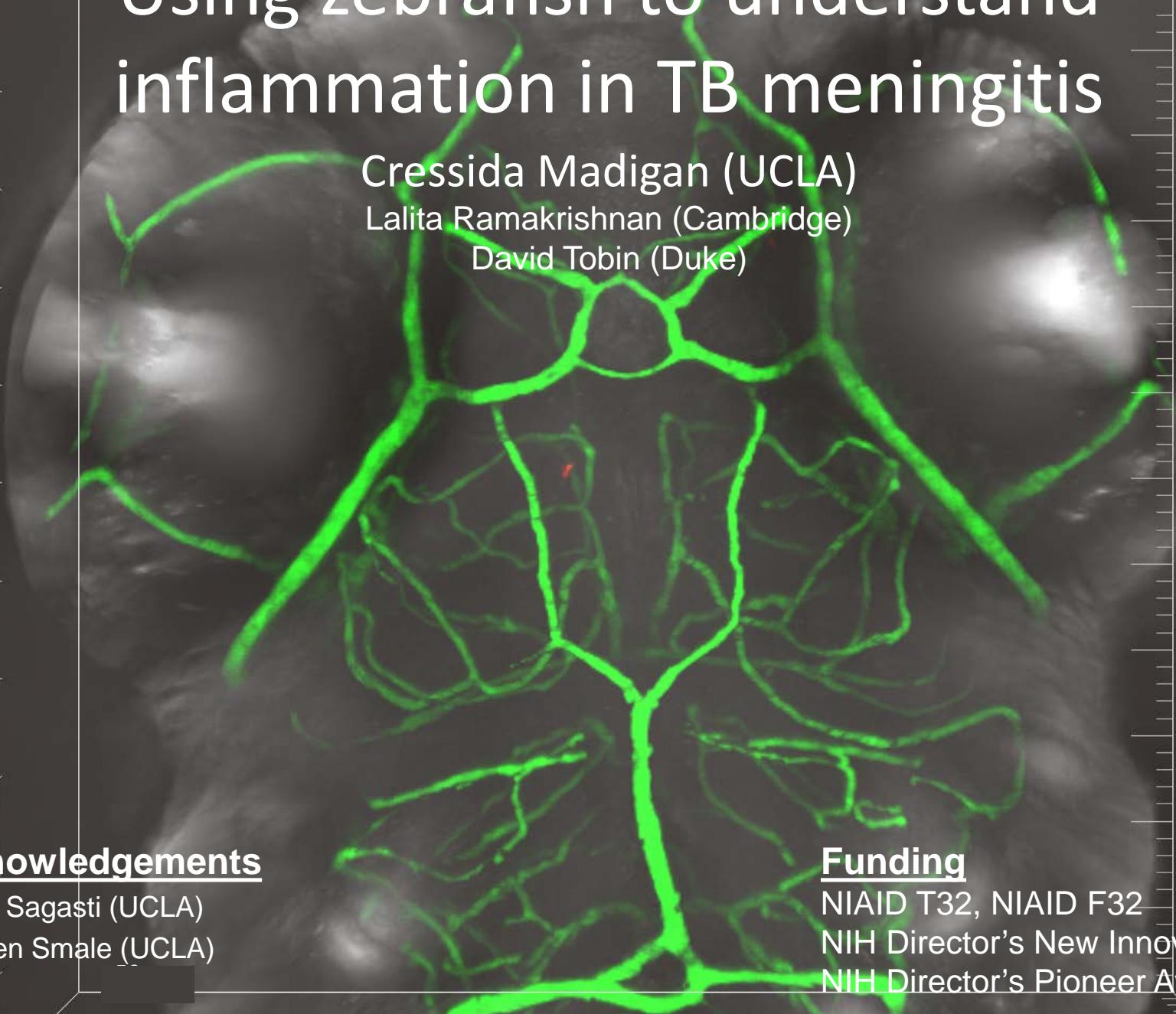


# Using zebrafish to understand inflammation in TB meningitis



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## Acknowledgements

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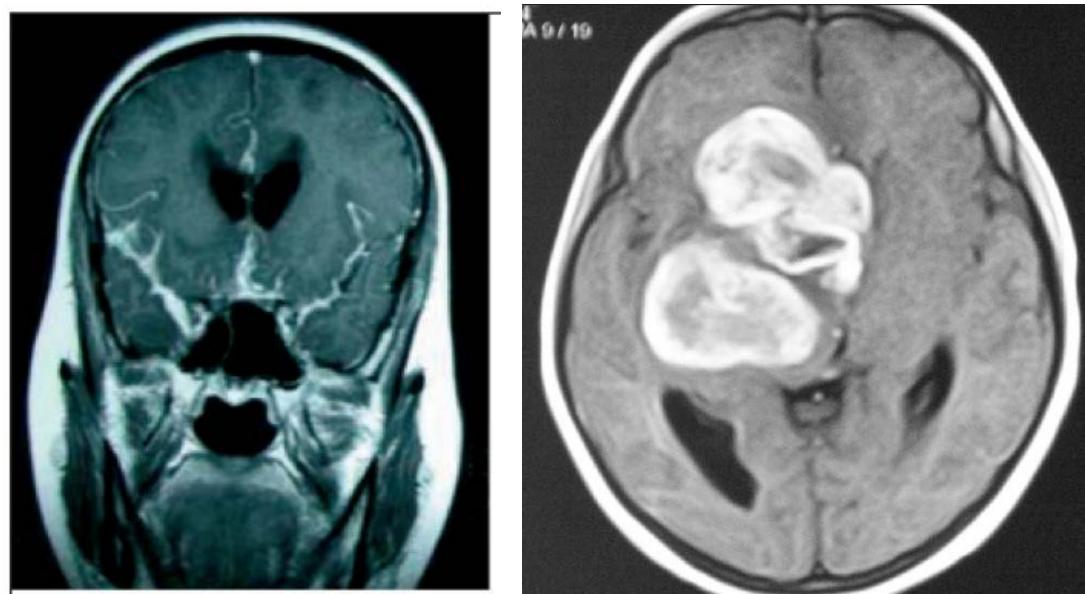
NIAID T32, NIAID F32

NIH Director's New Innovator Award

NIH Director's Pioneer Award

# What are the initial events in TBM pathogenesis?

- Host factors: TNF, laminin A, TIRAP, TLR2, LTA4H
- Bacterial factors: pknD
- Route of CNS entry? Cells involved?
- What initially triggers the neuroinflammation?
- *In vivo* models: rabbit, mouse, guinea pig



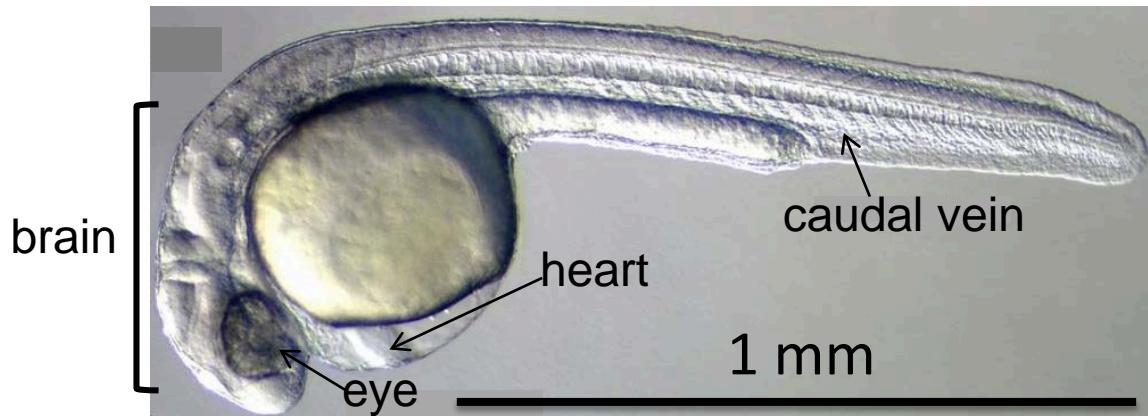
Zucchi 2012; Tsanova 1999; Jain 2006; Be 2008, 2012; Rock 2008;  
Thwaites 2000; Chatterjee 2011; Tobin 2010; Yang 2007; Klein 2017

1. Why zebrafish?
  2. LTA4H modulates inflammation in TBM
- 
1. Modeling CNS invasion

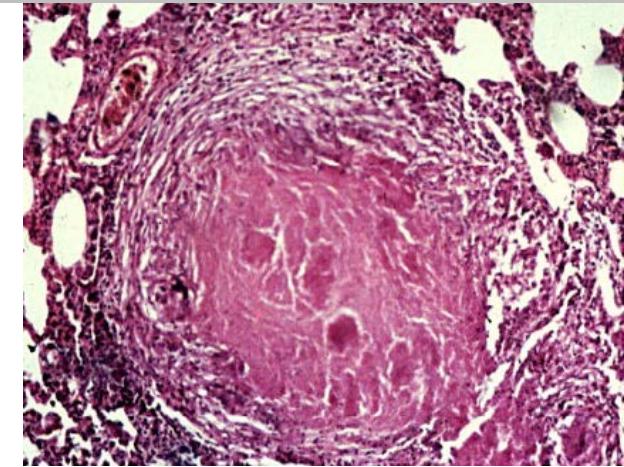
1. Why zebrafish?
  2. LTA4H modulates inflammation in TBM
1. Modeling CNS invasion

# Advantages of the zebrafish model to study mycobacterial pathogenesis

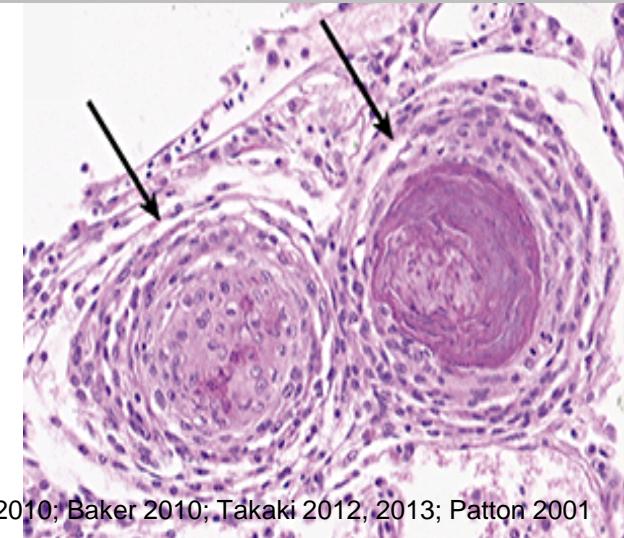
2 days post fertilization



human + *M. tuberculosis*



zebrafish + *M. marinum*

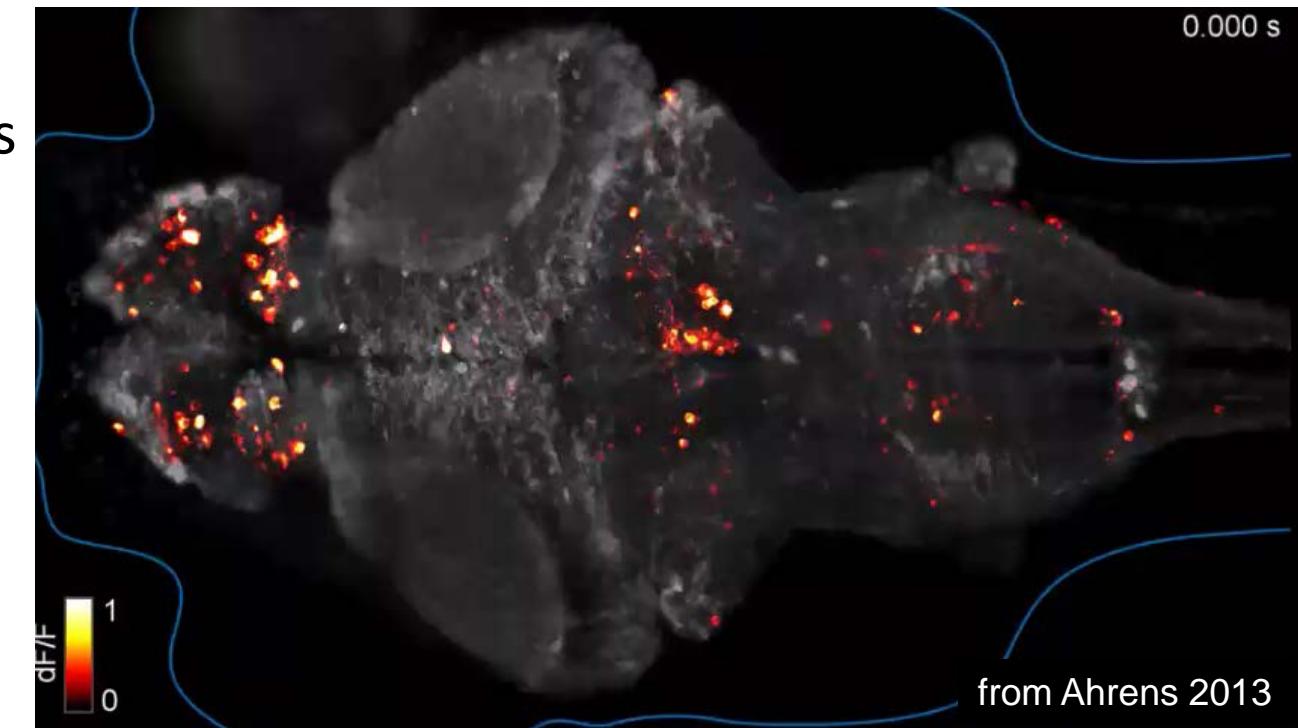


1. Orthologs for 70% of human genes
2. Genetics: CRISPR, morpholinos, transgenics
3. Established TB model (*M. marinum*)
4. Optically transparent → live imaging

# Live imaging neuroinflammation in an intact animal

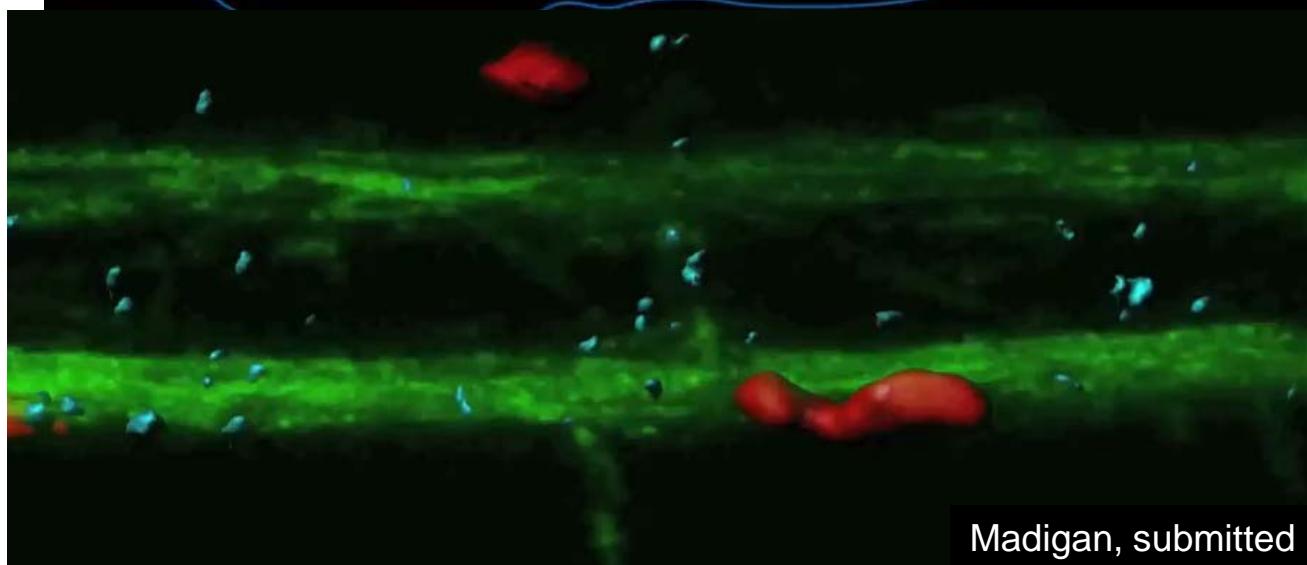
brain - action potentials

intracellular calcium



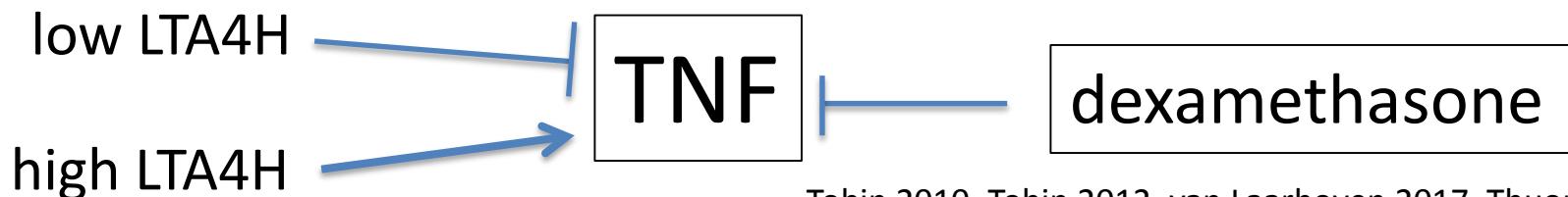
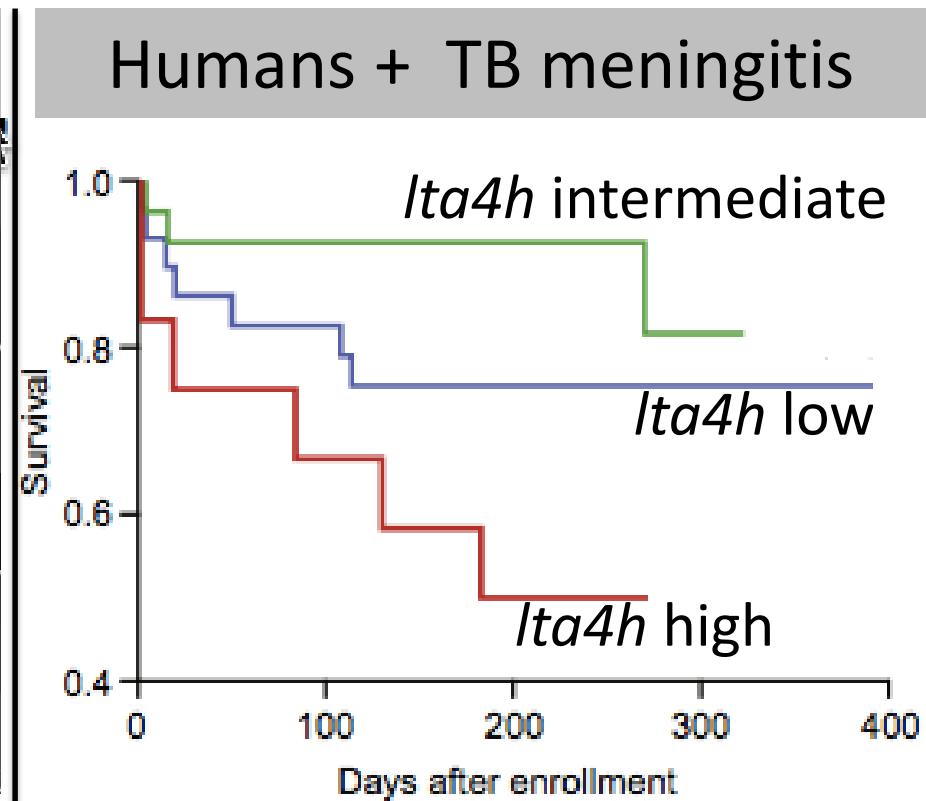
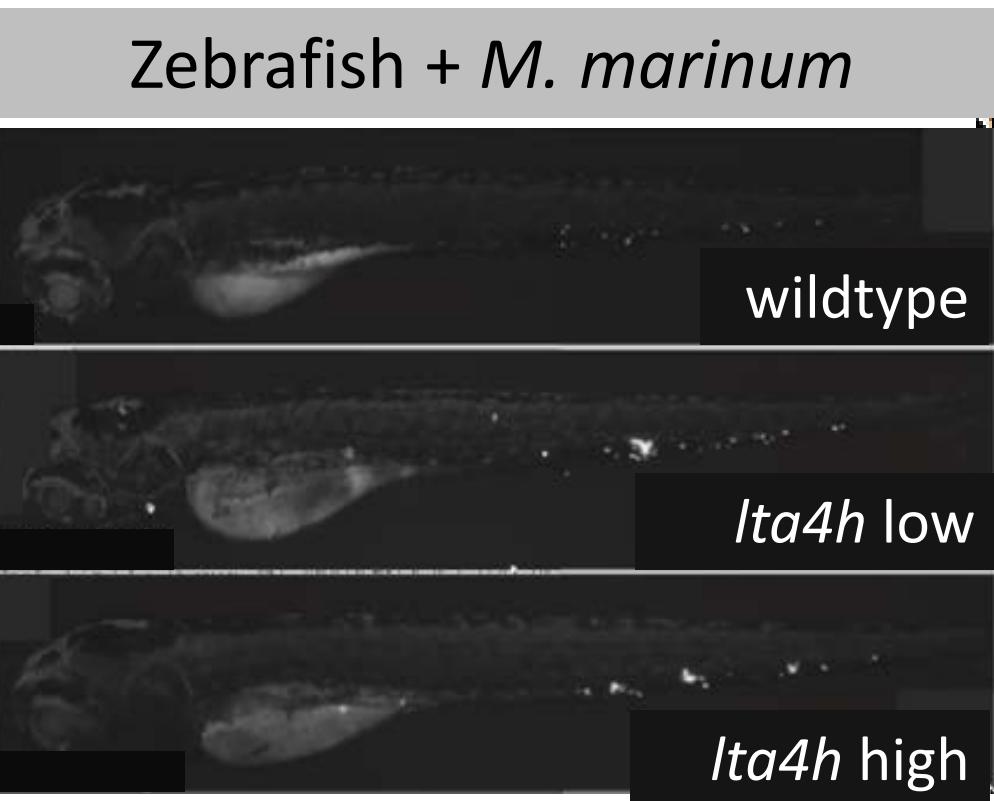
spinal cord - infection

*M. marinum*  
myelin  
macrophages



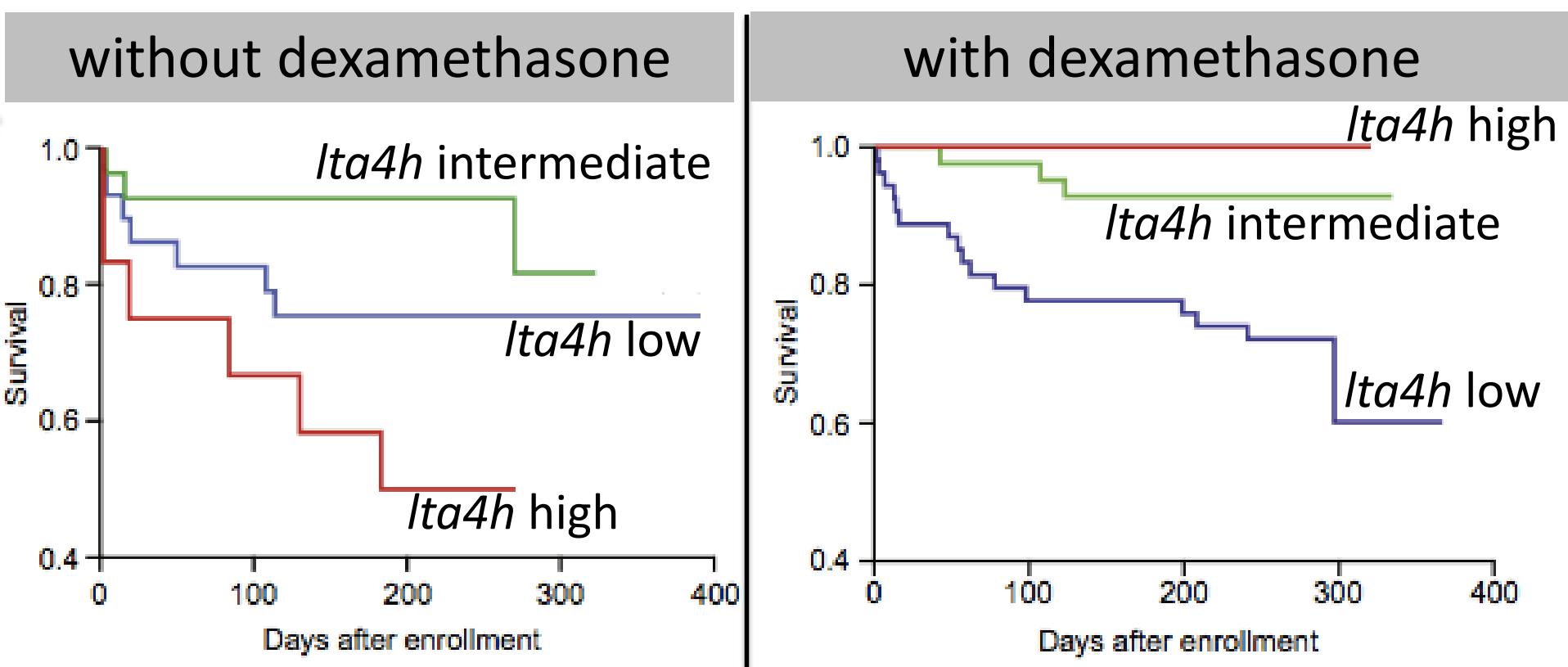
1. Why zebrafish?
  2. LTA4H modulates inflammation in TBM
- 
1. Modeling CNS invasion

# Humans and fish: low or high *Ita4h* = increased mycobacterial disease severity



Tobin 2010, Tobin 2012, van Laarhoven 2017, Thuong 2017

# *LTA4H* genotype of TB meningitis patients predicts treatment response to dexamethasone



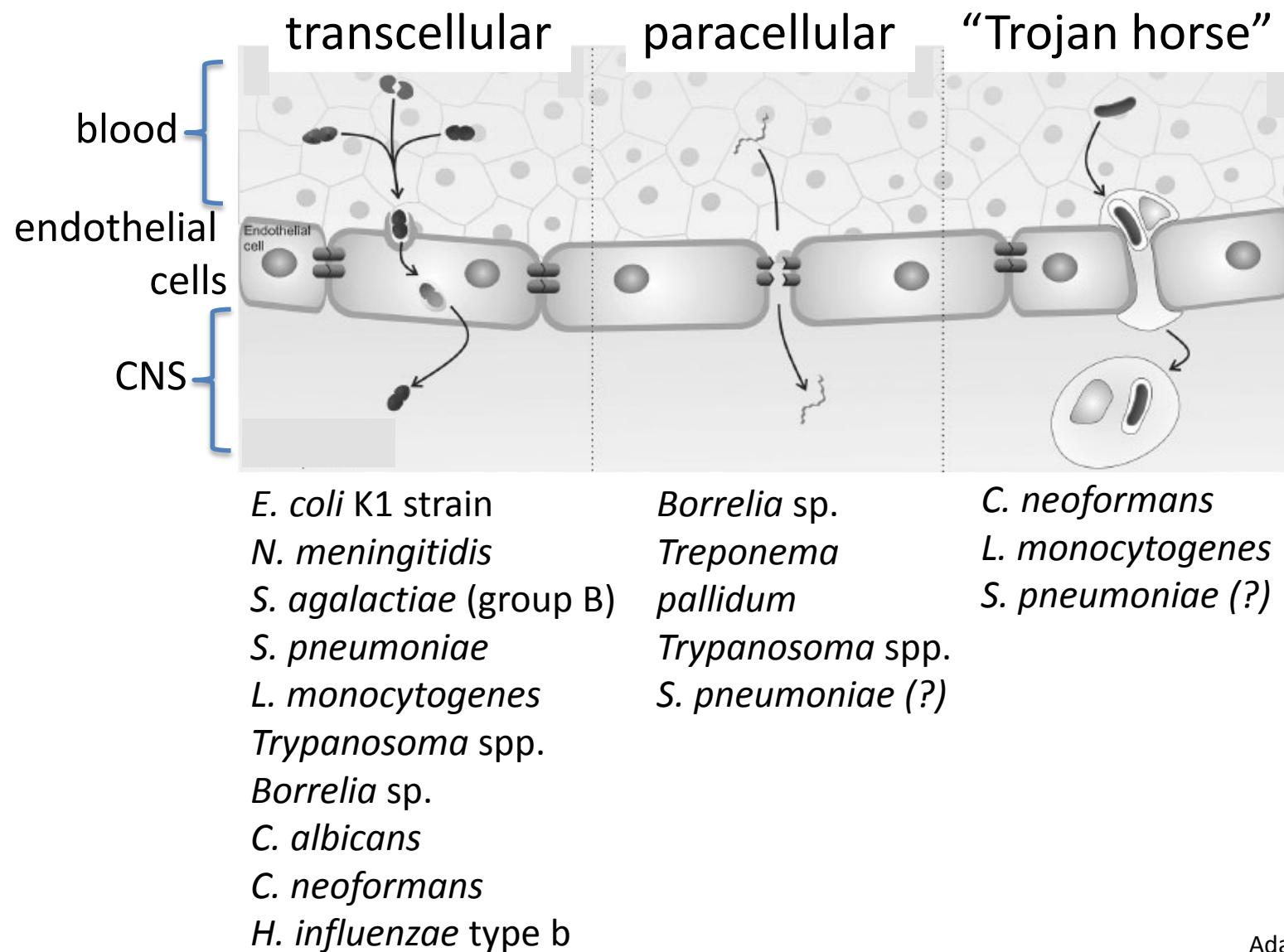
*Ita4h low* → low TNF → low inflammation → dexamethasone hurts

*Ita4h high* → high TNF → high inflammation → dexamethasone helps

1. Why zebrafish?
2. LTA4H modulates inflammation in TBM
  1. Modeling CNS invasion

1. Why zebrafish?
  2. LTA4H modulates inflammation in TBM
1. Modeling CNS invasion
    - entry?
    - first mediators of inflammation?

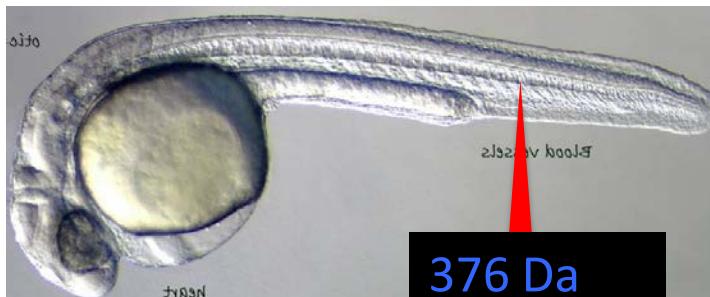
# How do mycobacteria enter the CNS?



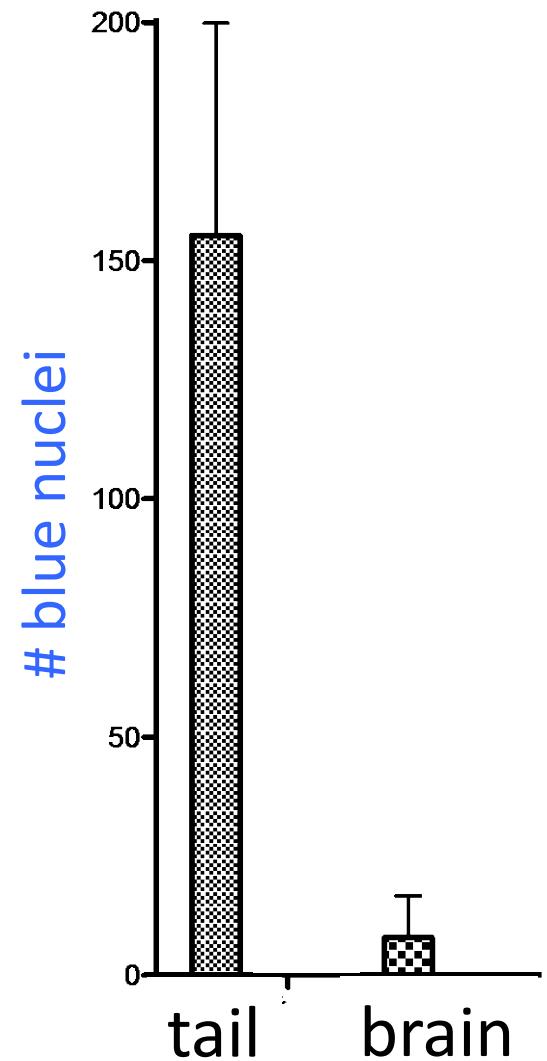
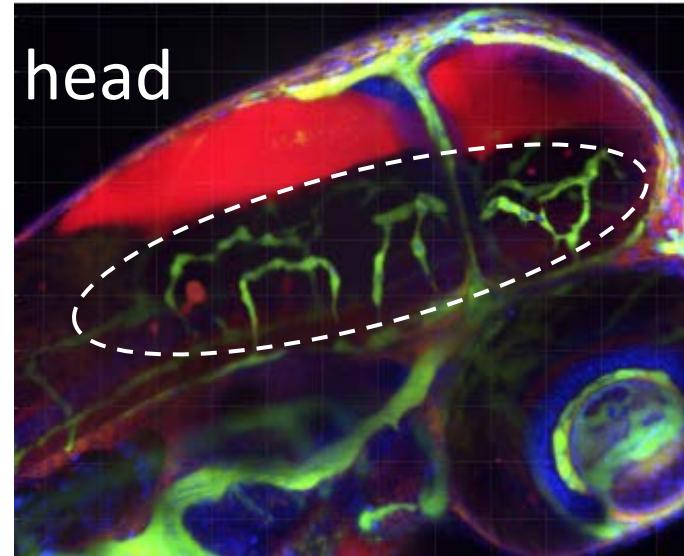
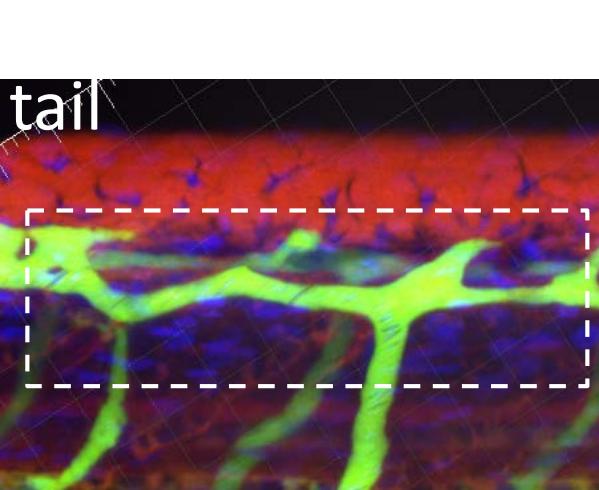
# Zebrafish larvae have a functional blood-brain barrier

## Hallmarks of mammalian blood-brain barrier:

1. Endothelial tight-junction proteins
2. Size-selective

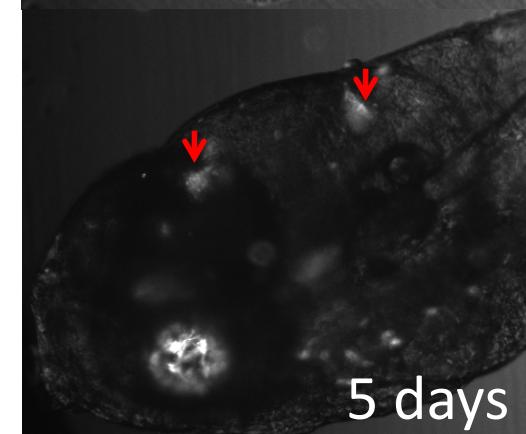
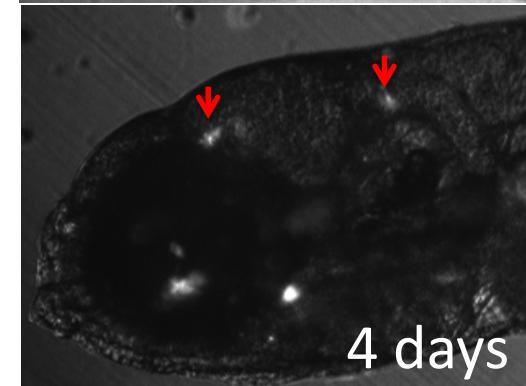
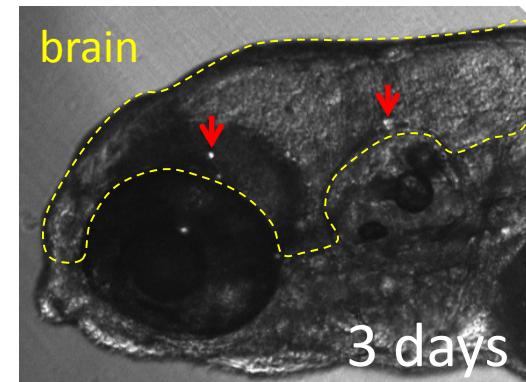
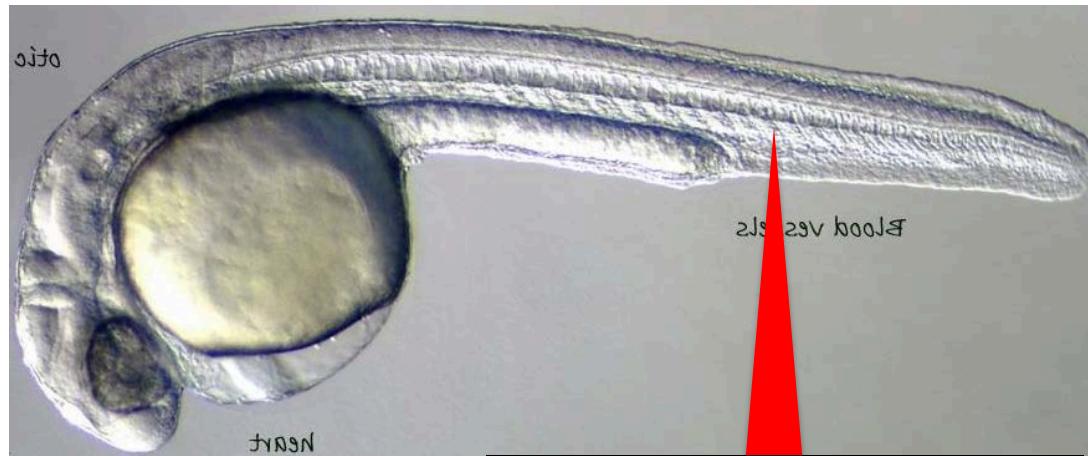


376 Da  
10 kDa  
2,000 kDa

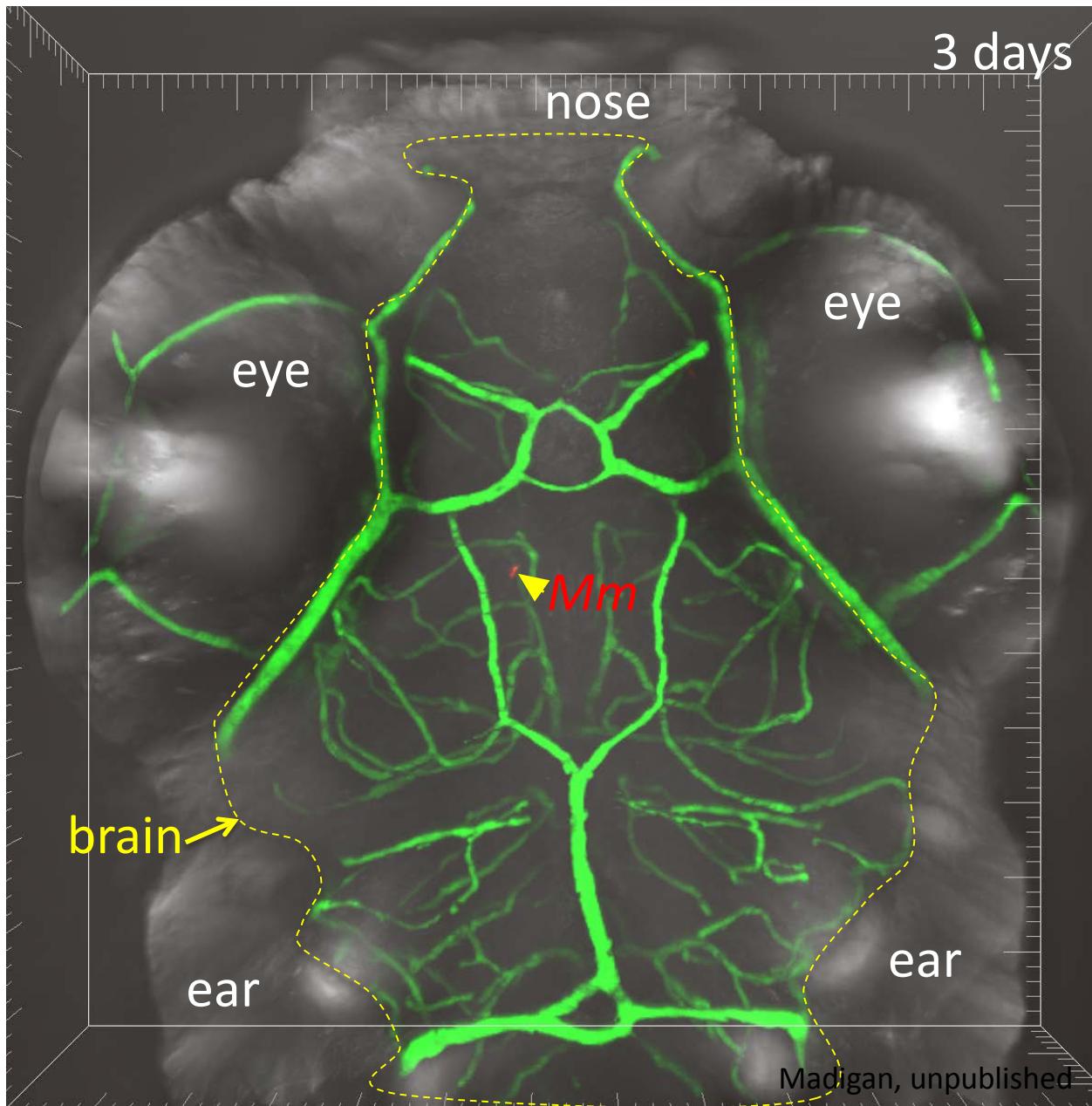
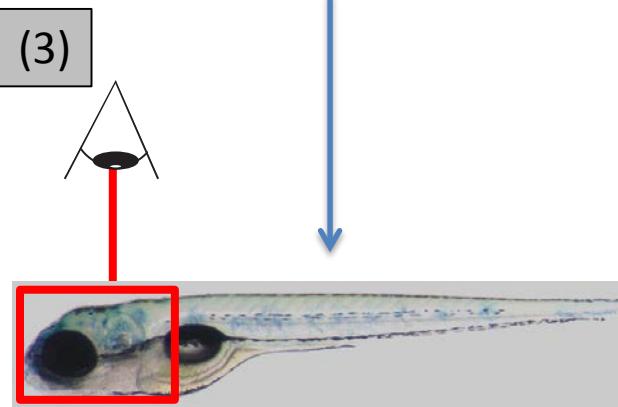
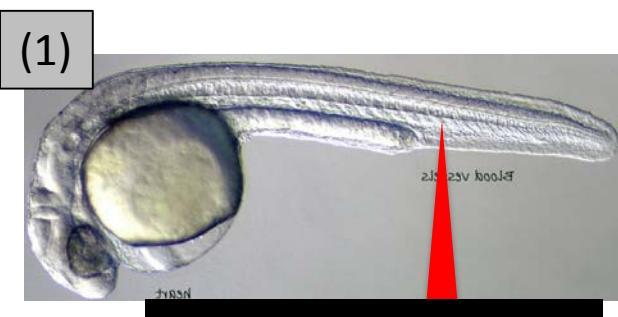


Madigan, unpublished

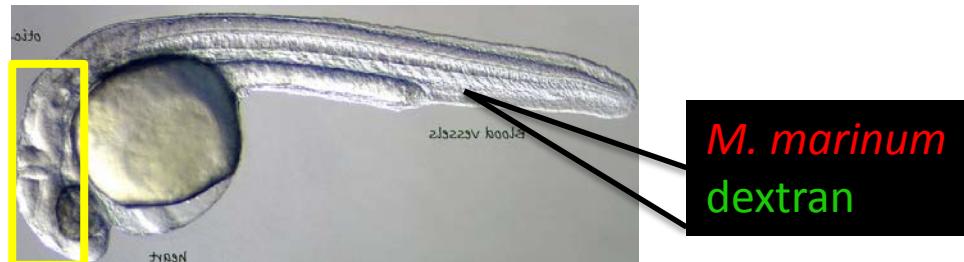
# *M. marinum* attach to CNS blood vessels and replicate



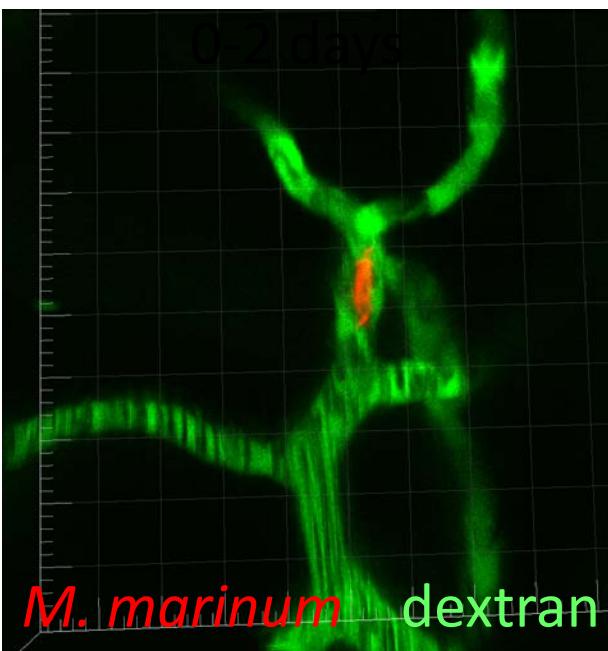
# Live imaging infection in an intact animal



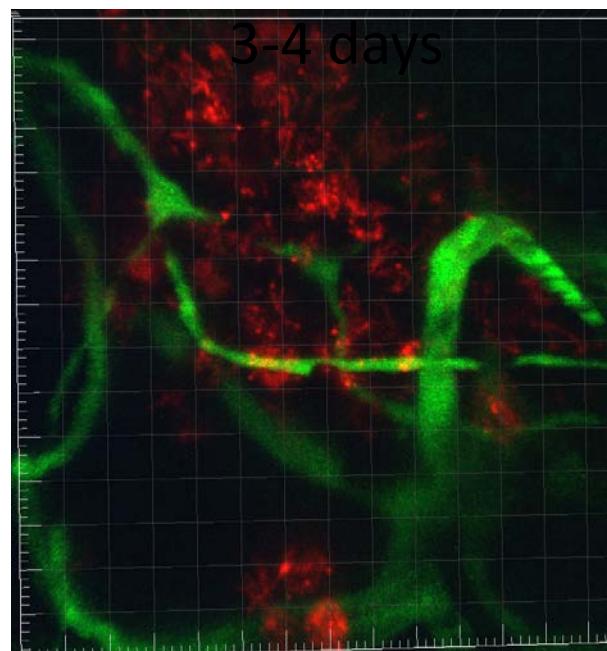
# Mycobacterial entry into CNS: working model



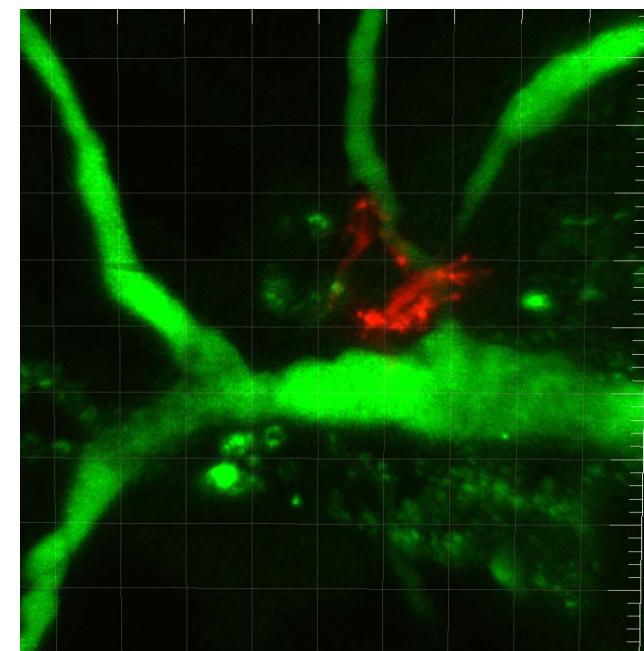
1. vessel attachment



2. vessel exit



3. vessel leakage

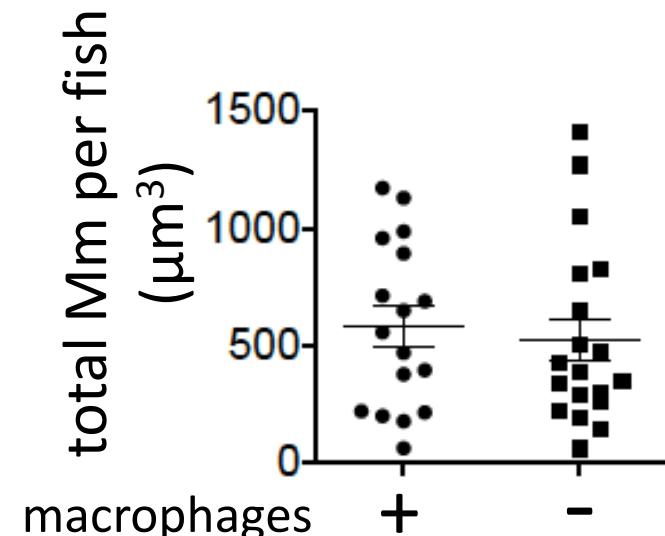
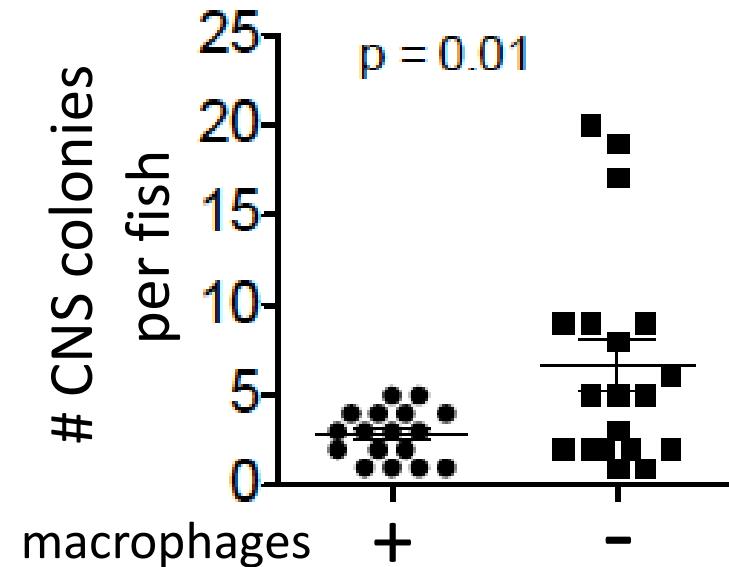
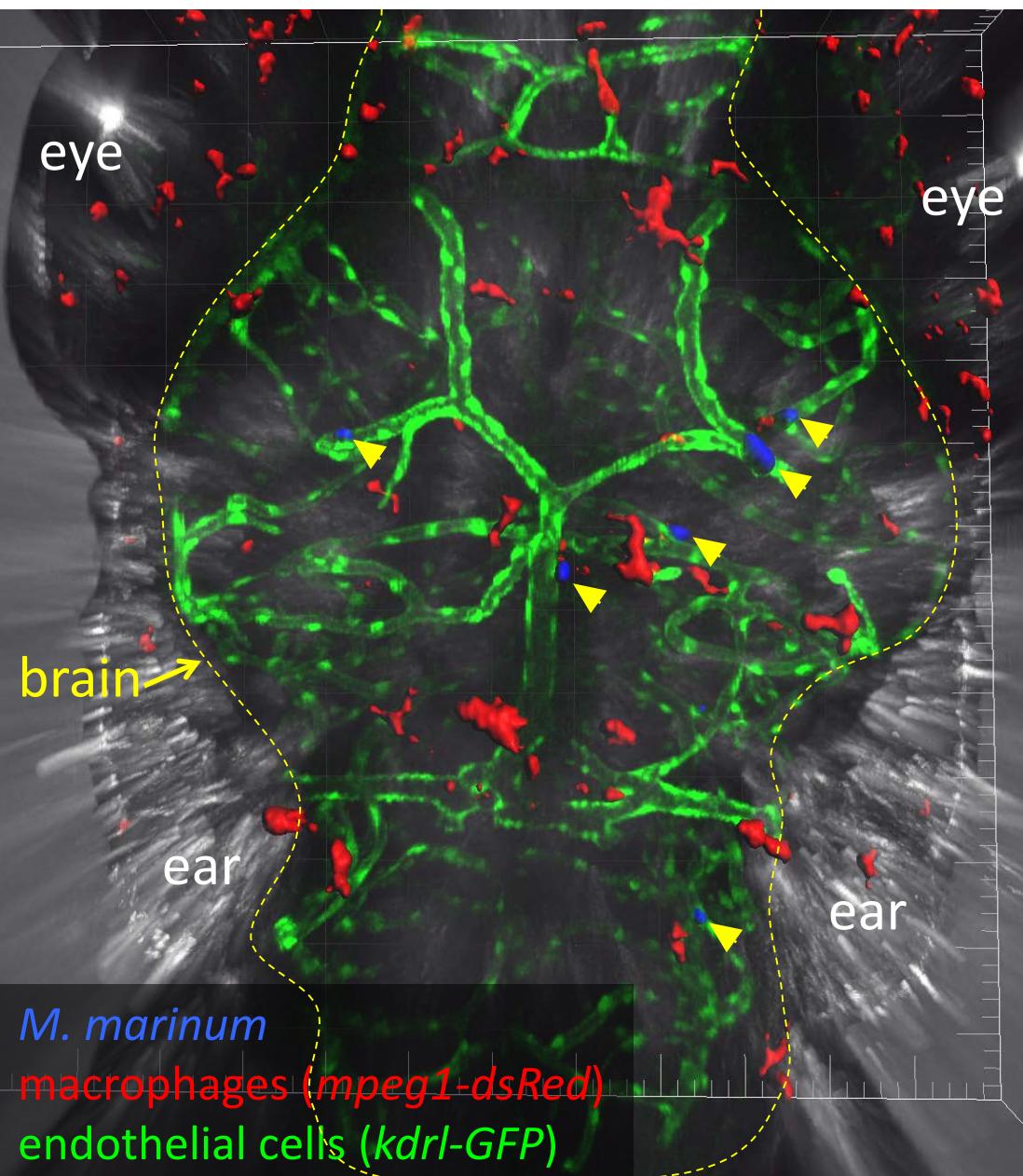


0-2 days

3-4 days

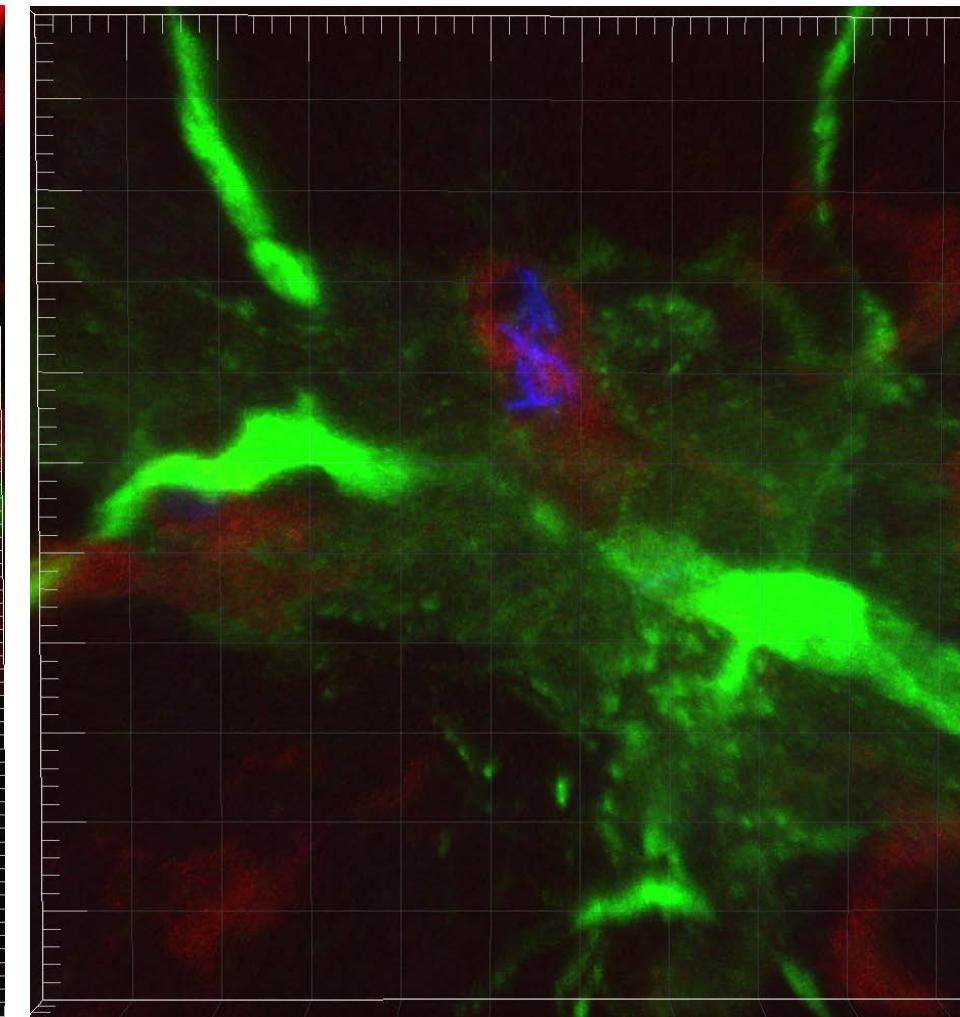
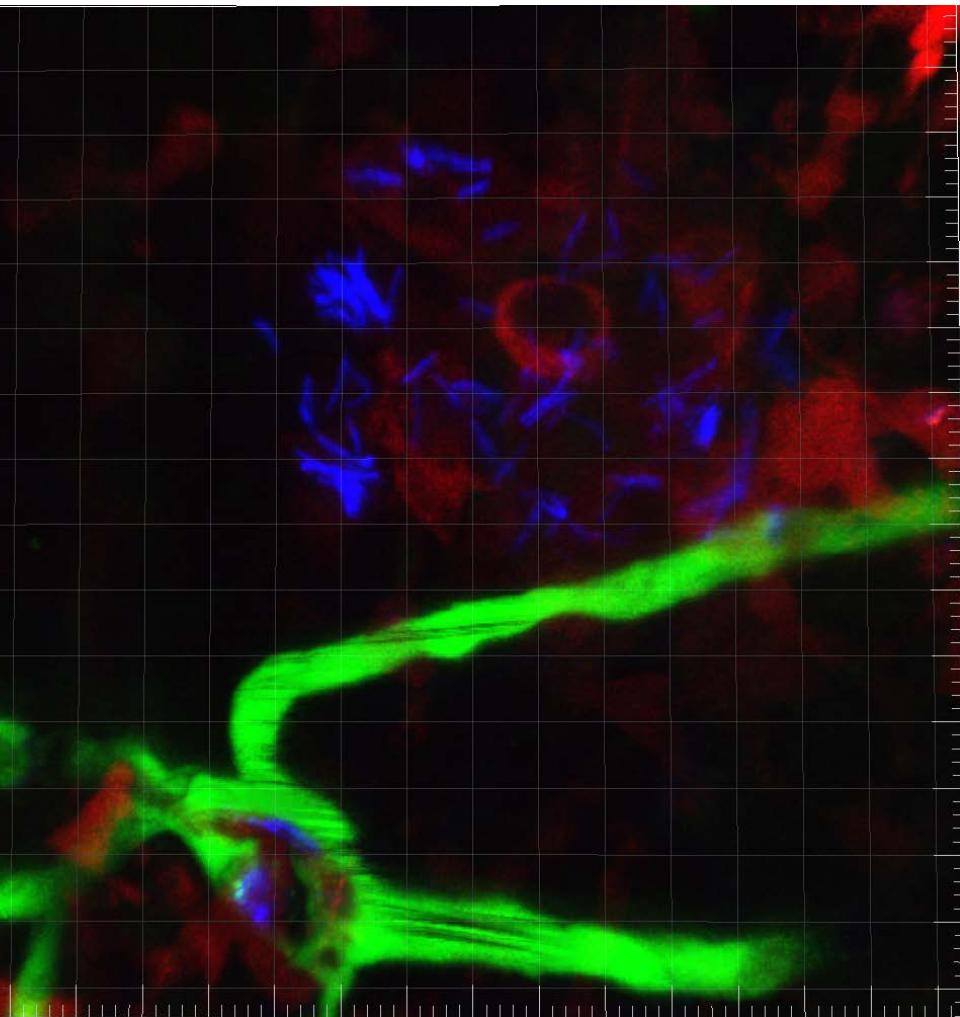
5 days

# *M. marinum* attach to vessels without macrophages...



Madigan, unpublished

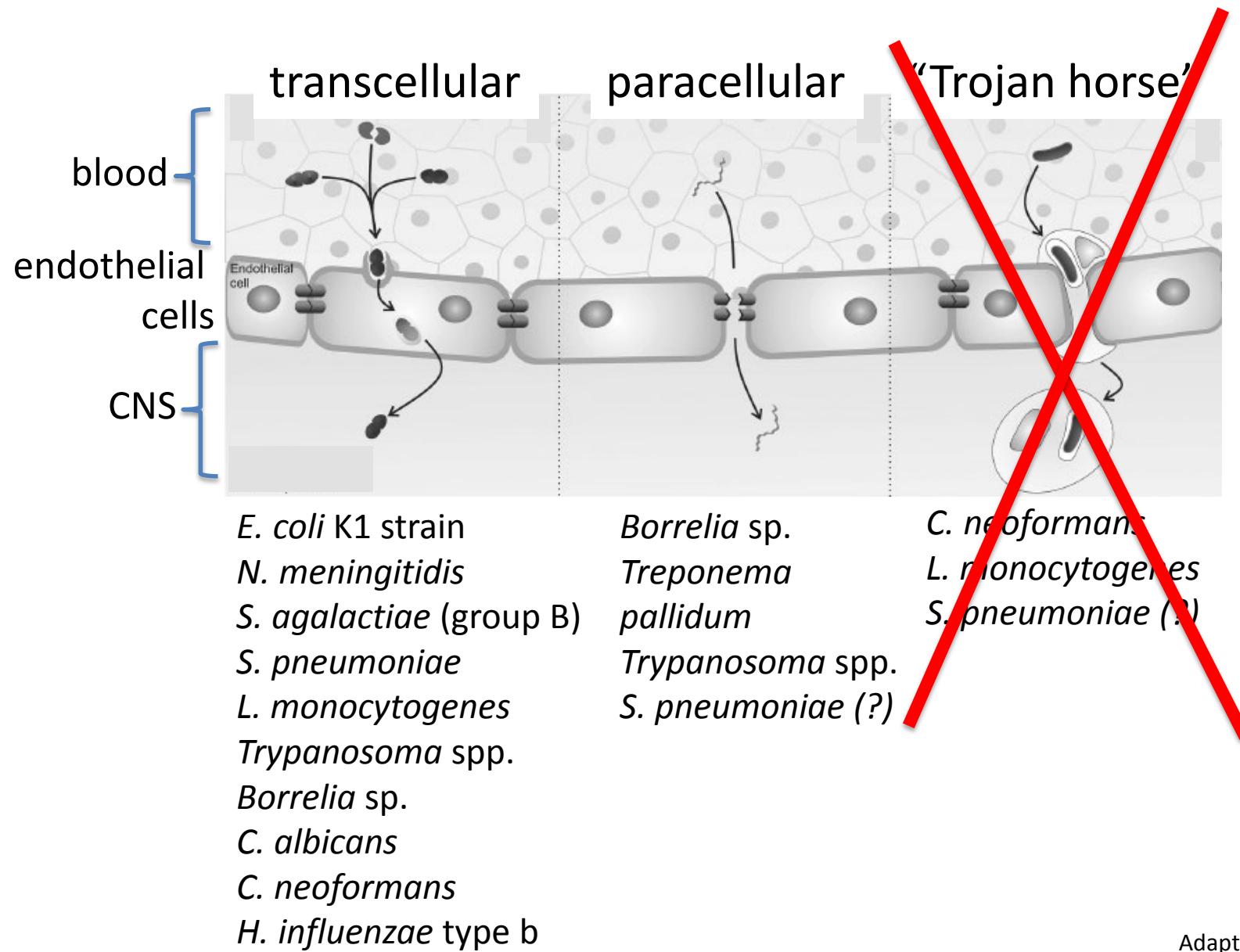
...but after vessel exit, macrophages/microglia arrive



*M. marinum*  
macrophages/microglia (*mpeg1-dsRed*)  
dextran

Madigan, unpublished

# How do mycobacteria enter the CNS?



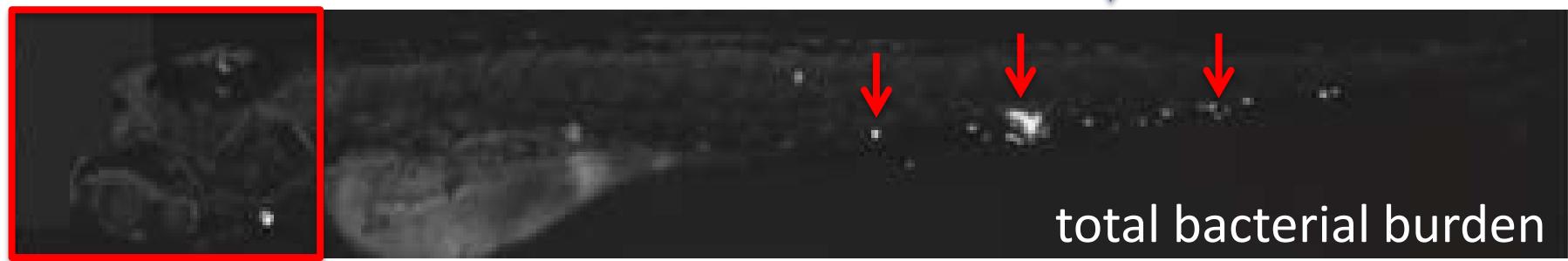
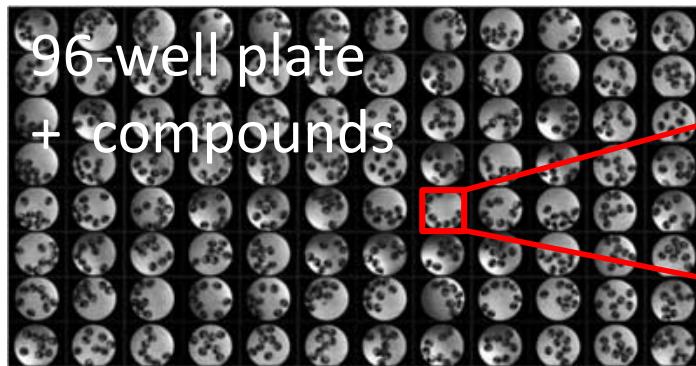
## Summary

1. LTA4H modulates inflammation in TBM
  - high *lta4h* = high TNF = give dexamethasone  
→ *New therapies?*

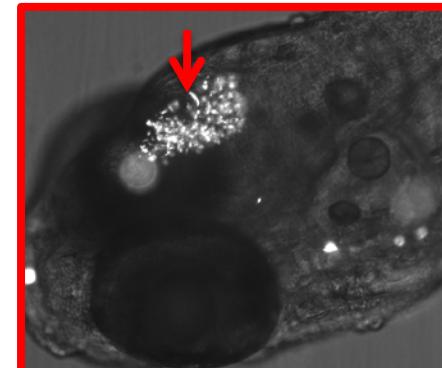
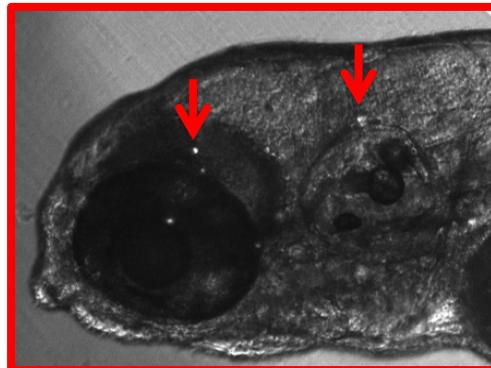
# Summary

1. LTA4H modulates inflammation in TBM
  - high *lta4h* = high TNF = give dexamethasone  
→ *New therapies?*
2. Modeling CNS invasion in zebrafish
  - Mycobacterial entry does not require macrophages
  - Blood-brain barrier breakdown  
→ *Mechanism of entry & vessel leakage?*

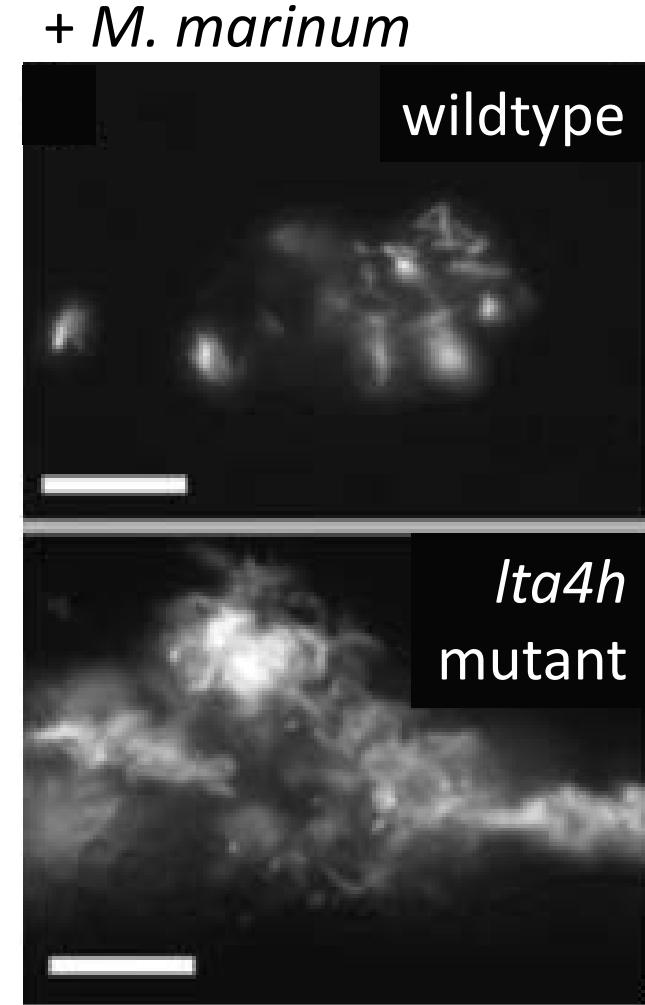
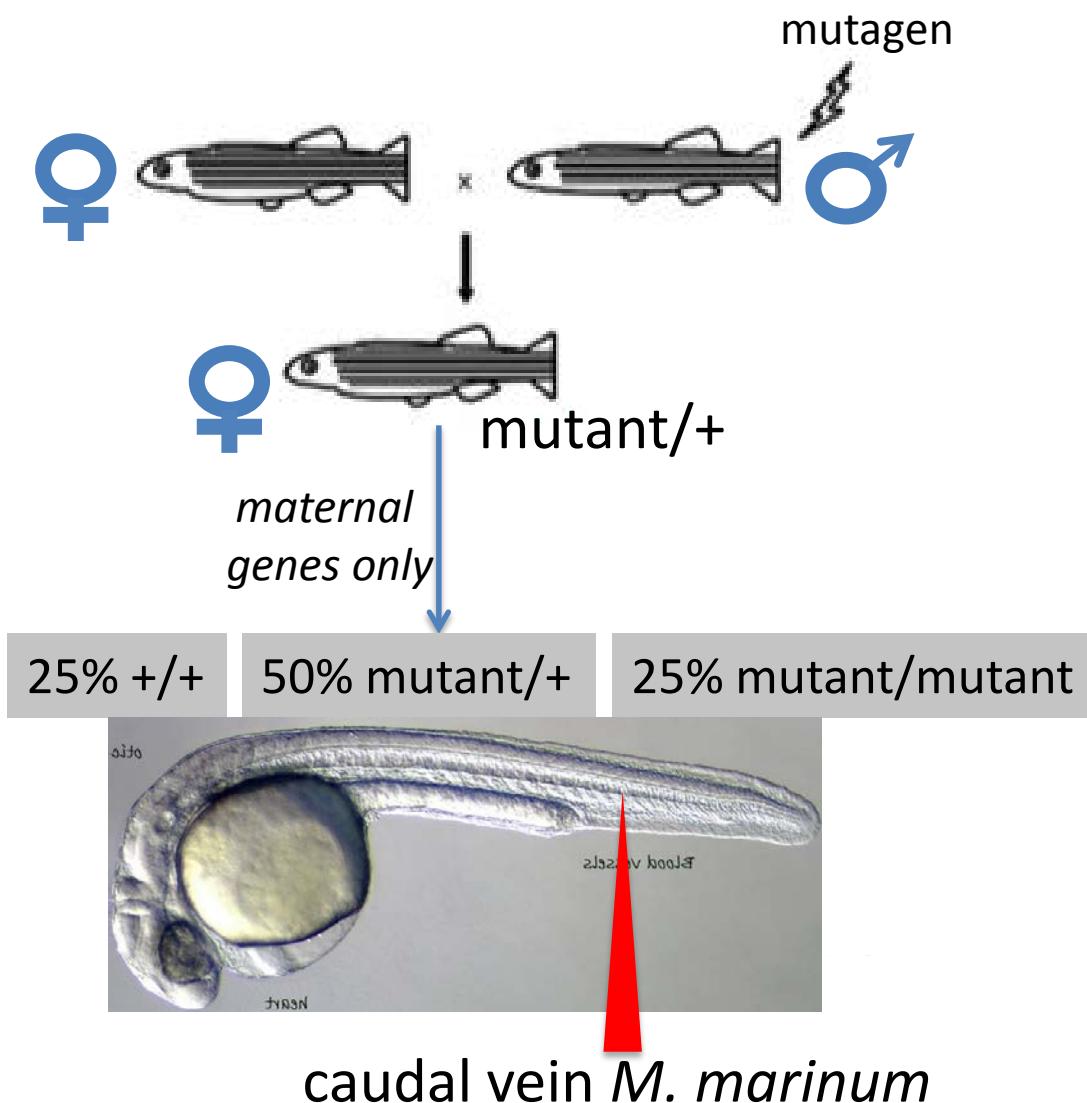
# Future Directions: whole animal screening for new TBM therapies



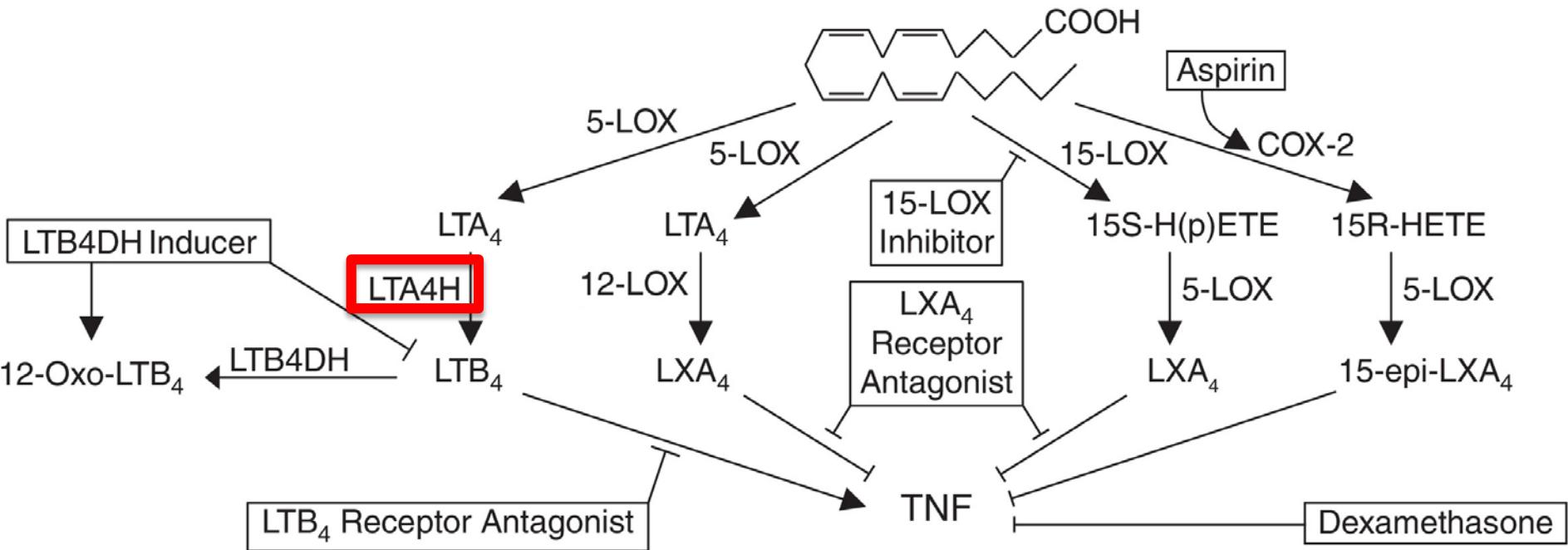
vessel  
attachment



# Screen identifies zebrafish mutants with increased *M. marinum* susceptibility



# Other genotype-specific therapies?

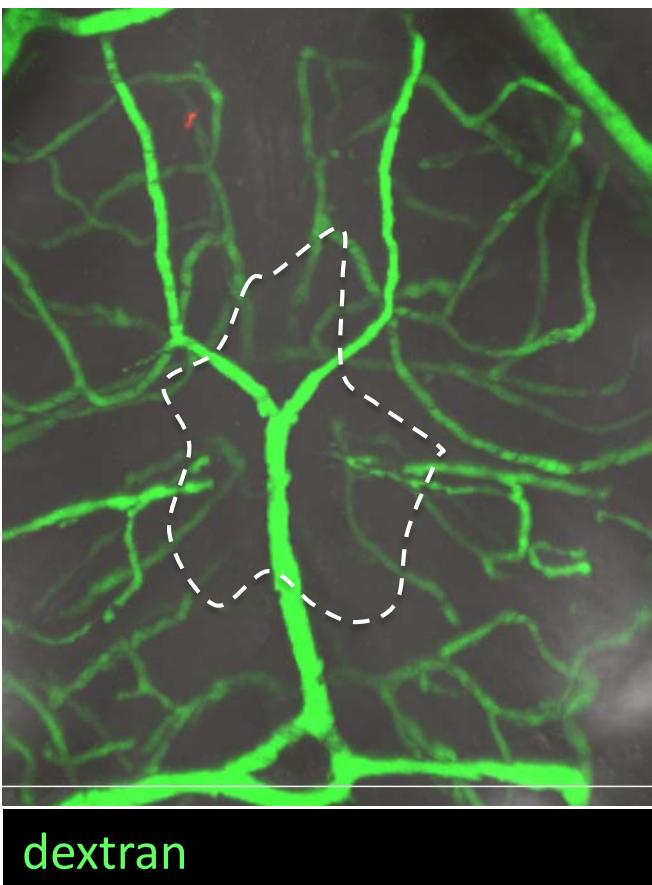


inadequate inflammation: 15-LOX inhibitor, LXA<sub>4</sub>R antagonist

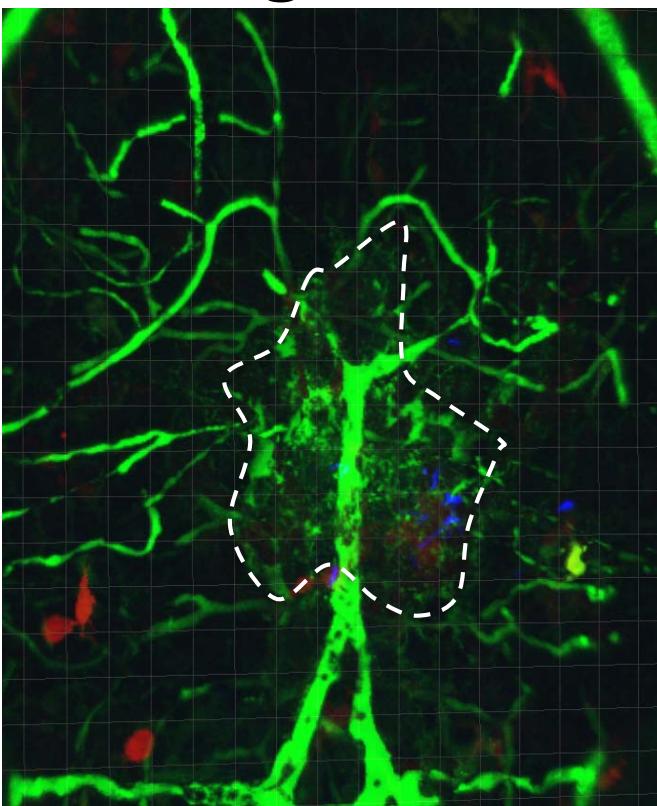
excess inflammation: aspirin, LTB4DH inducer, LTB<sub>4</sub>R antagonist

# Vessels near *M. marinum* become permeable

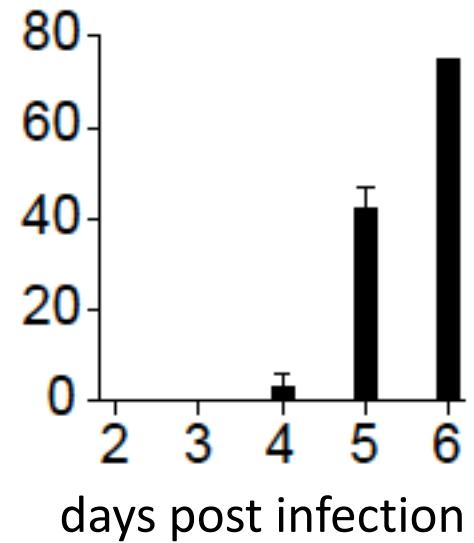
Intact



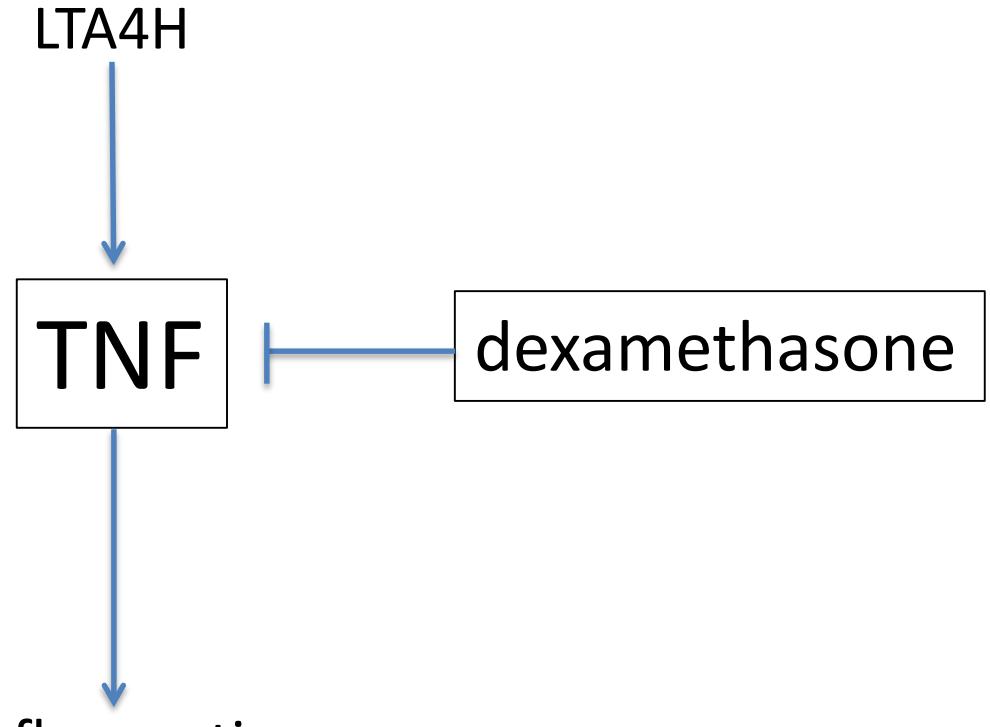
Leaking



% animals with dextran leakage



# Zebrafish-*M. marinum* model shows *LTA4H* activity increases TNF and inflammation



Prediction:

*lta4h* low → low TNF → low inflammation → dexamethasone hurts

*lta4h* high → high TNF → high inflammation → dexamethasone helps