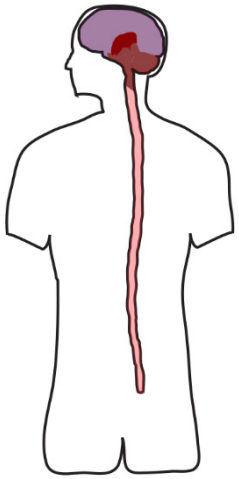
Optical control of neural ablation in zebrafish as a model for secondary injury mechanisms

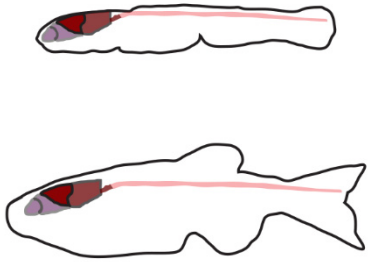
Karen Mruk, Patrick A. Piza, and James K. Chen
Departments of Chemical and Systems Biology,
Developmental Biology, and Chemical Engineering

Stanford, University, Stanford, CA

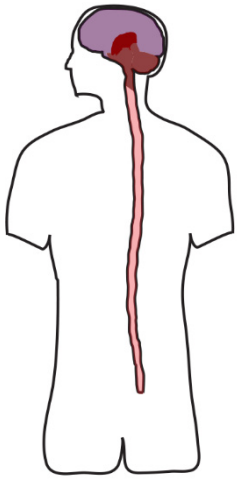
Behavioral consequences of neuronal loss



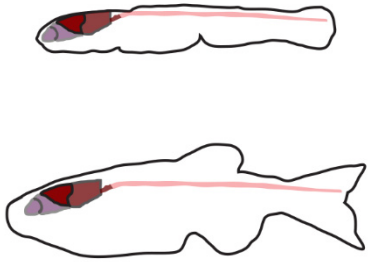
Complex Vertebrates
Myelinated Axons
Neurotransmitters



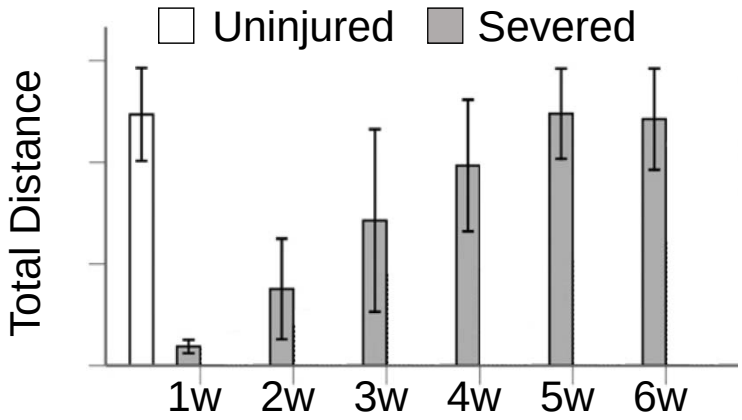
Behavioral consequences of neuronal loss



Complex Vertebrates
Myelinated Axons
Neurotransmitters



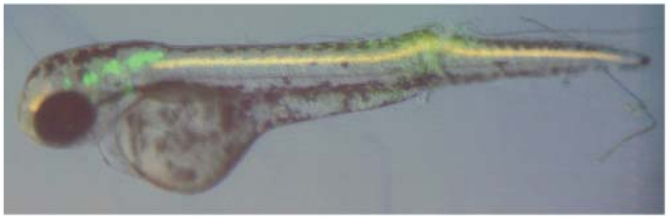
Permanent
Dysfunction



Adapted from: Yu, Y and Schachner, M. *Eur J Neurosci*. 2013

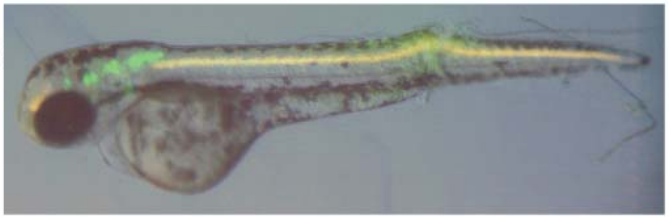
Current models of spinal injury in zebrafish are limited

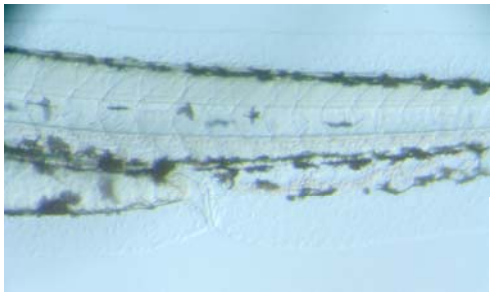
Manual
Stabbing

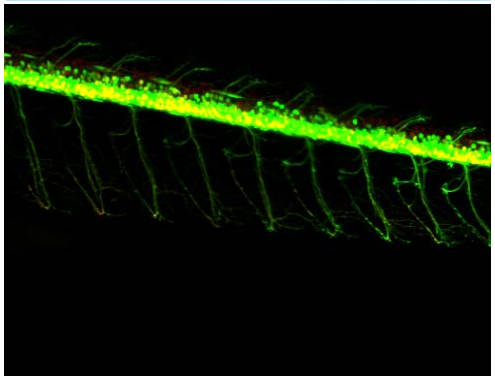


Current models of spinal injury in zebrafish are limited

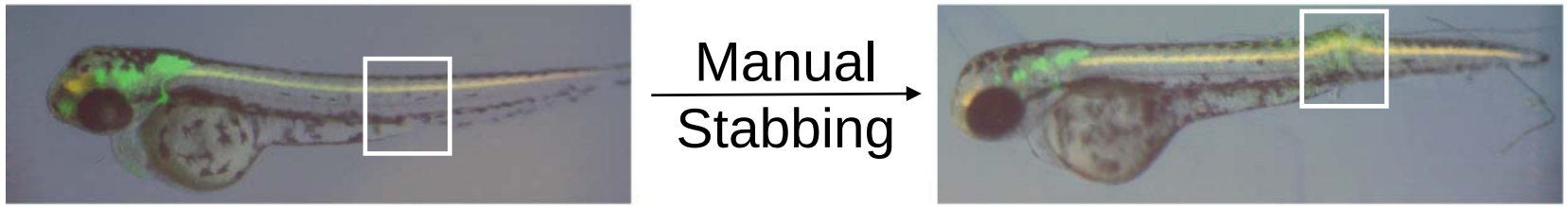
Manual
Stabbing

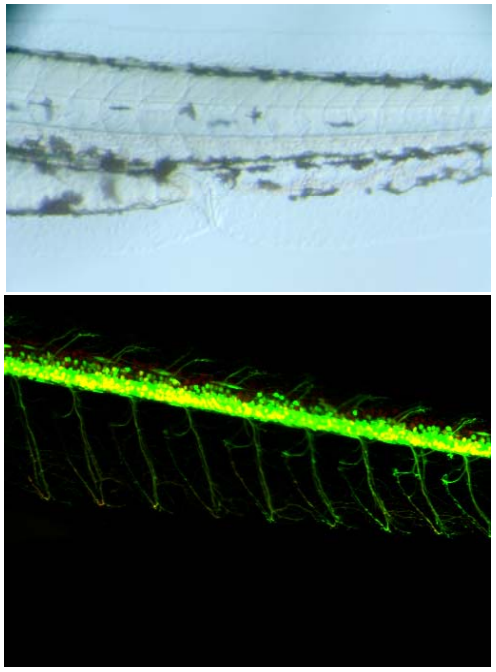


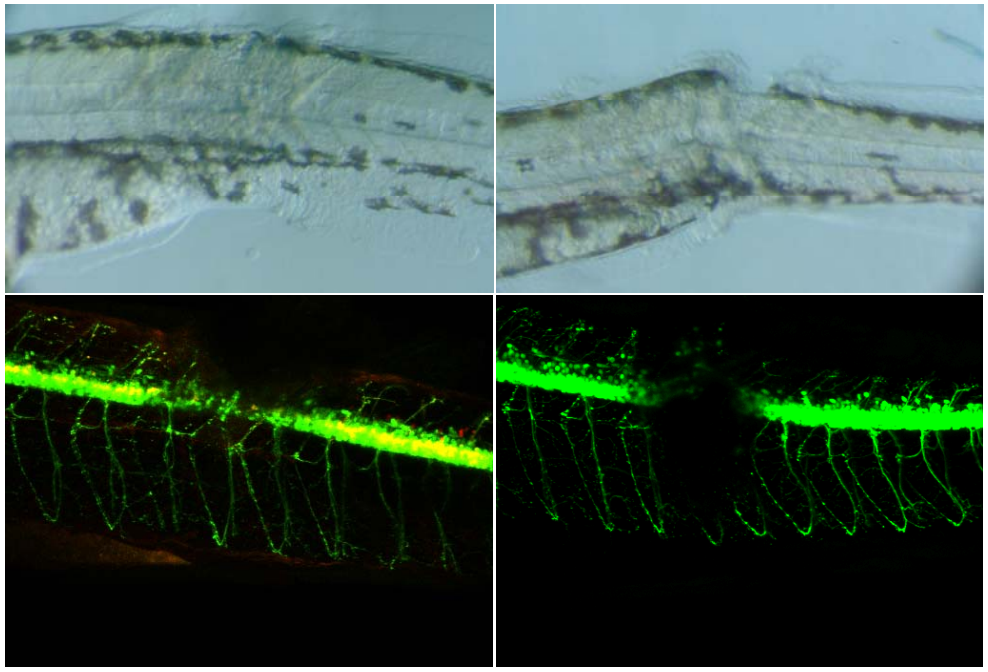




Current models of spinal injury in zebrafish are limited

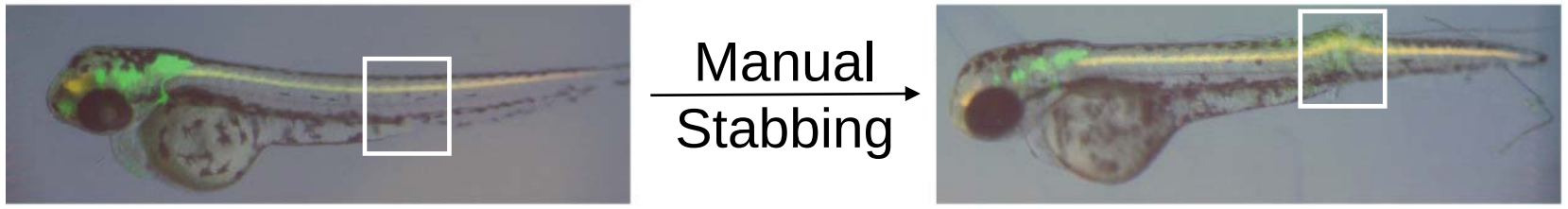






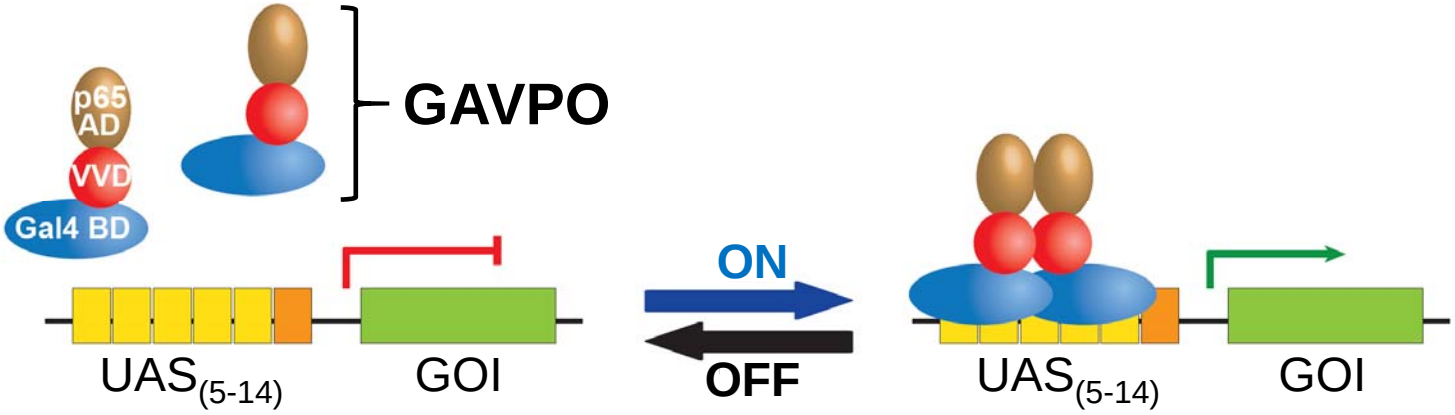
Less reproducible
Extraneous damage
Higher mortality

Current models of spinal injury in zebrafish are limited



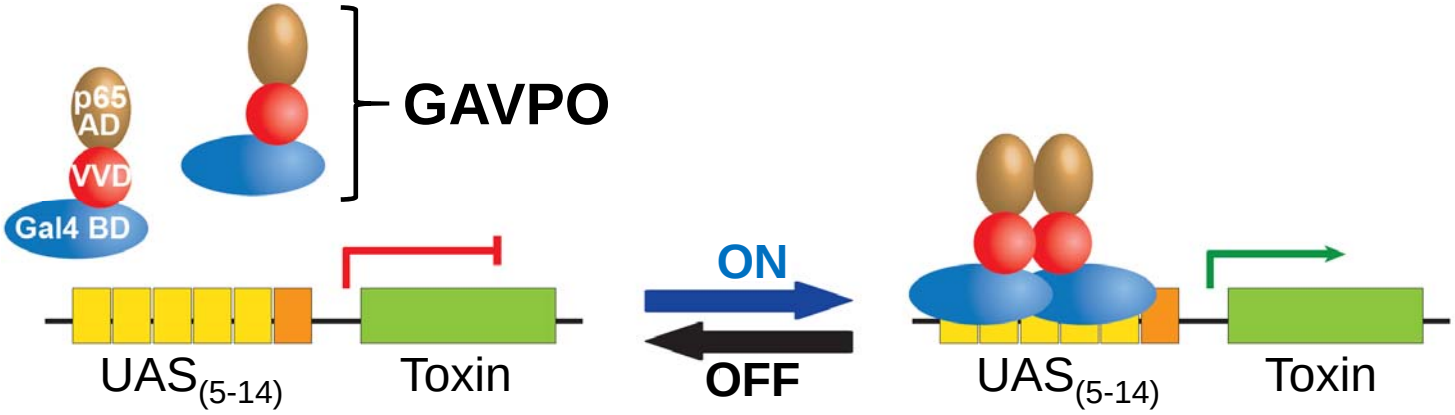
Can we use an optogenetic approach to reproducibly kill neurons as a model for spinal cord injury and degeneration?

Using light to spatiotemporally ablate CNS cells

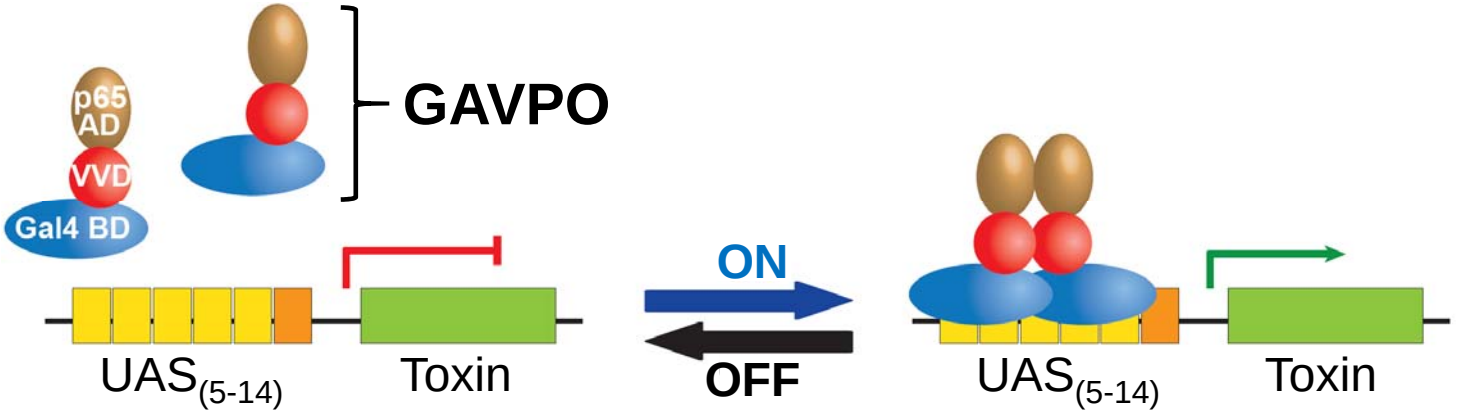


Wang et al. *Nature Methods* 2012

Using light to spatiotemporally ablate CNS cells

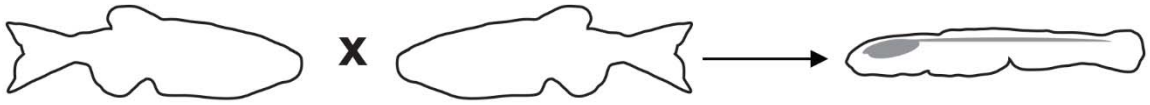


Using light to spatiotemporally ablate CNS cells

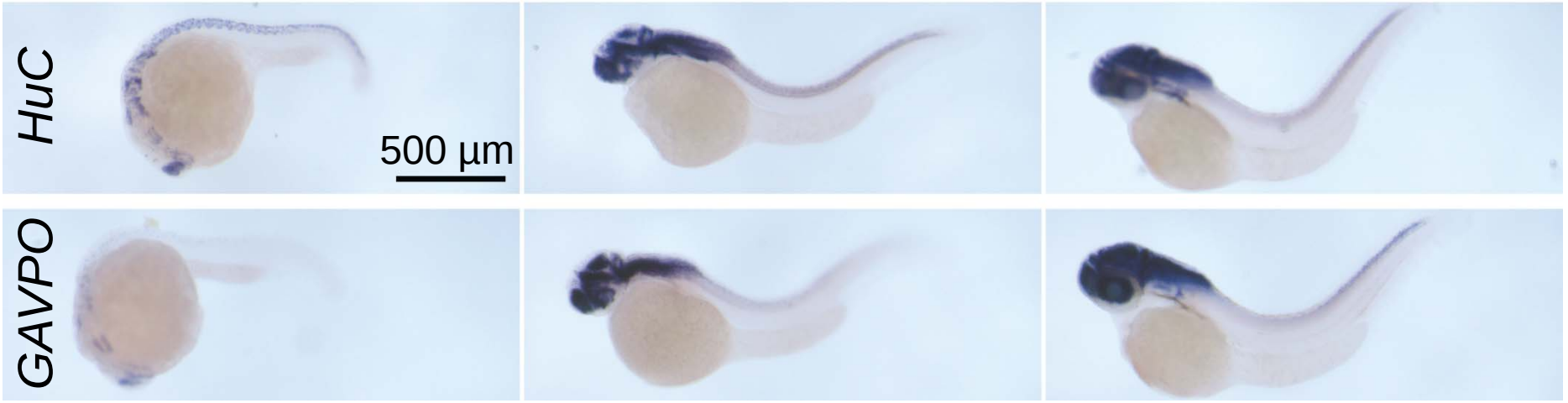


- Does GAVPO drive transcription in zebrafish?
- Can we model neural death with toxins?
- Can we induce neural death using the GAVPO system?

Generating and characterizing transgenic GAVPO lines



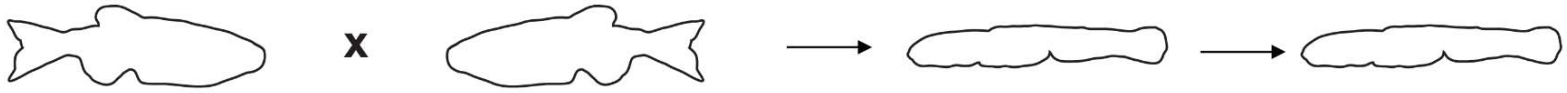
Tg(*HuC:GAVPO*) WT Fix and stain for mRNA expression



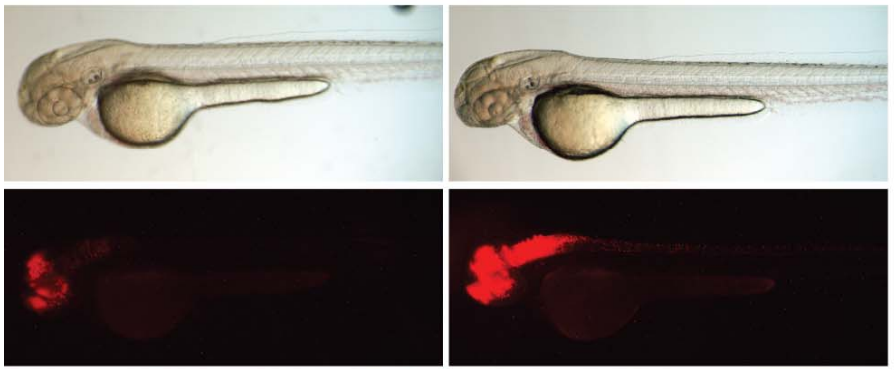
24 hpf 48 hpf 72 hpf

n = 20-25 larvae for each condition

GAVPO-induced expression increases with irradiation duration and intensity



Tg(*HuC:GAVPO*) Tg(*UAS:mCherry*) Irradiate at 60 hpf Image at 72 hpf



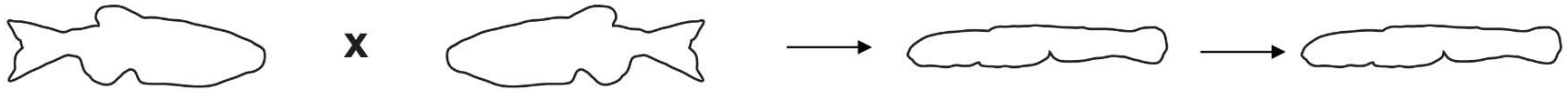
20 min 120 min

Global Irradiation

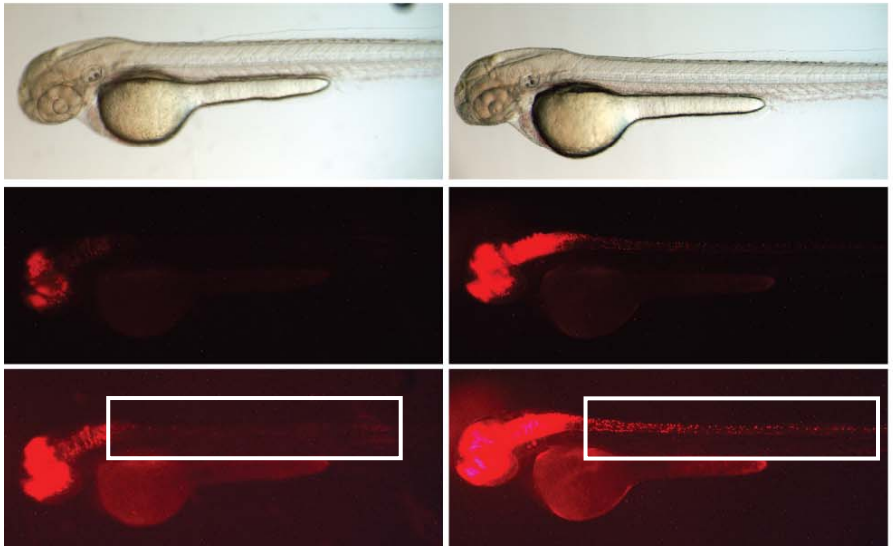
3 mW/cm ^{2}

n = 25-30 larvae for each condition

GAVPO-induced expression increases with irradiation duration and intensity



Tg(*HuC:GAVPO*) Tg(*UAS:mCherry*) Irradiate at 60 hpf Image at 72 hpf

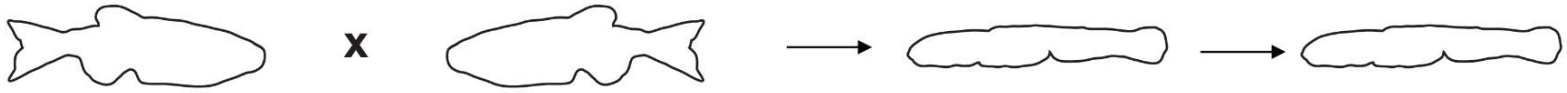


20 min 120 min

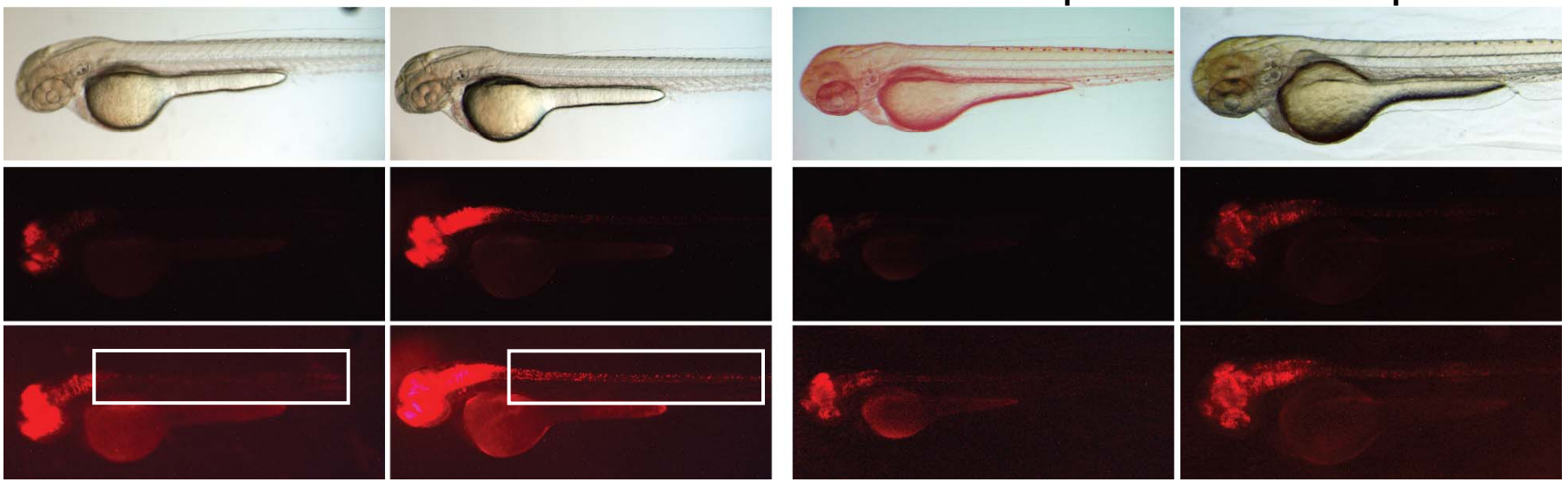
Global Irradiation
3 mW/cm ^{2}

n = 25-30 larvae for each condition

GAVPO-induced expression increases with irradiation duration and intensity



Tg(*HuC:GAVPO*) Tg(*UAS:mCherry*) Irradiate at 60 hpf Image at 72 hpf



20 min 120 min 5 min 1 min

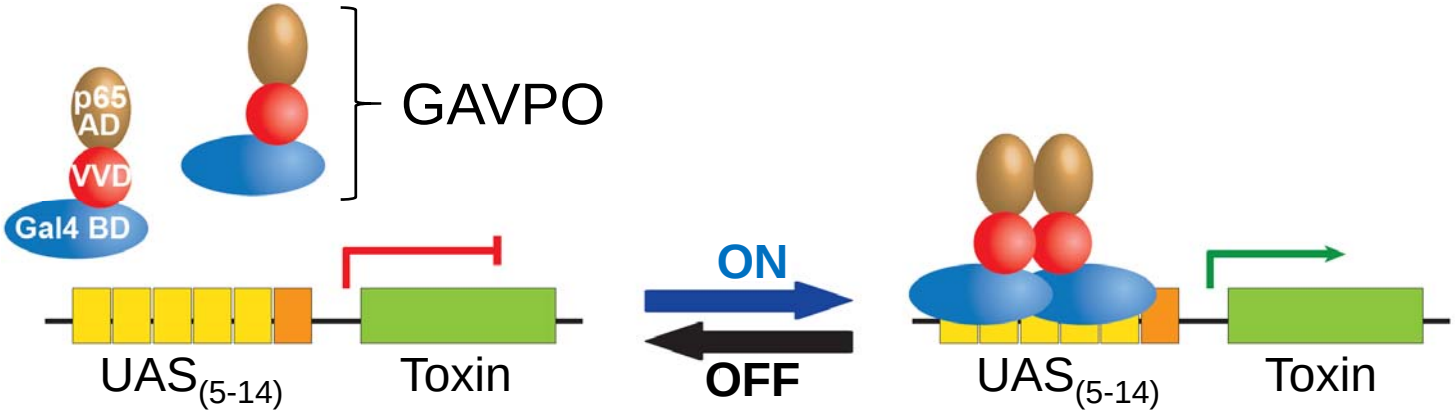
Global Irradiation
3 mW/cm ^{2}

Head Irradiation
900 mW/cm ^{2}

Head Irradiation
2000 mW/cm ^{2}

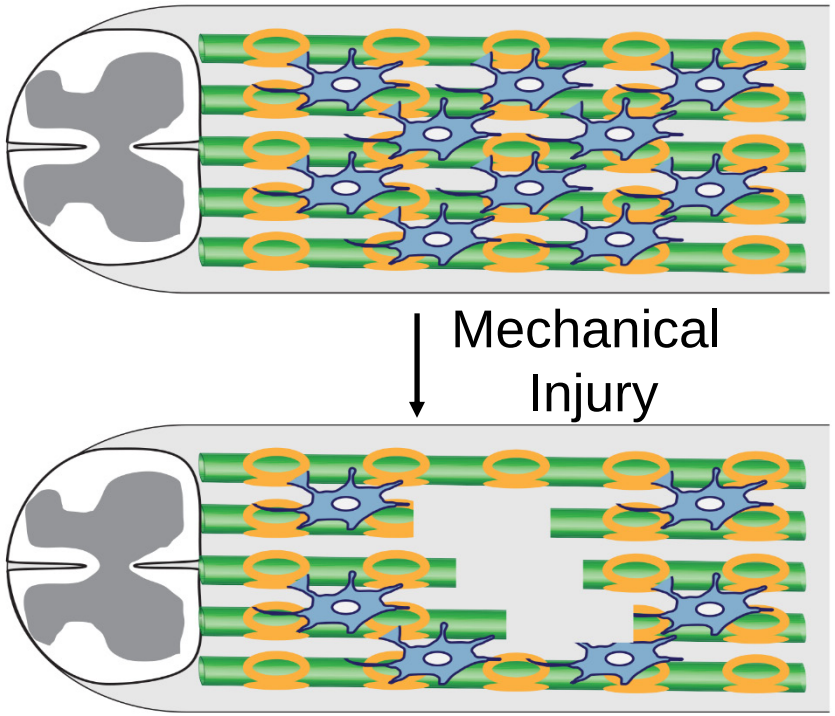
n = 25-30 larvae for each condition

Using light to spatiotemporally ablate CNS cells

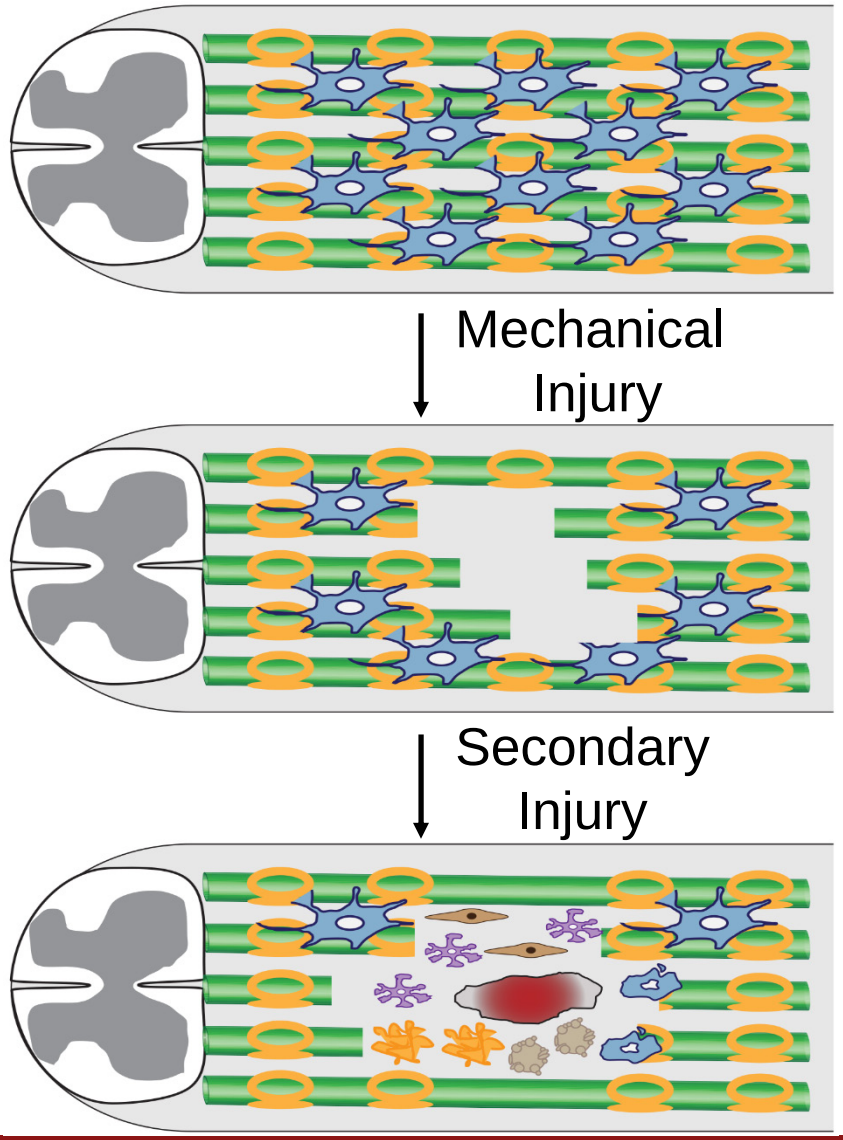


- ✓ Does GAVPO drive transcription in zebrafish?
- ❑ Can we model neural death with toxins?
- ❑ Can we induce neural death using the GAVPO system?

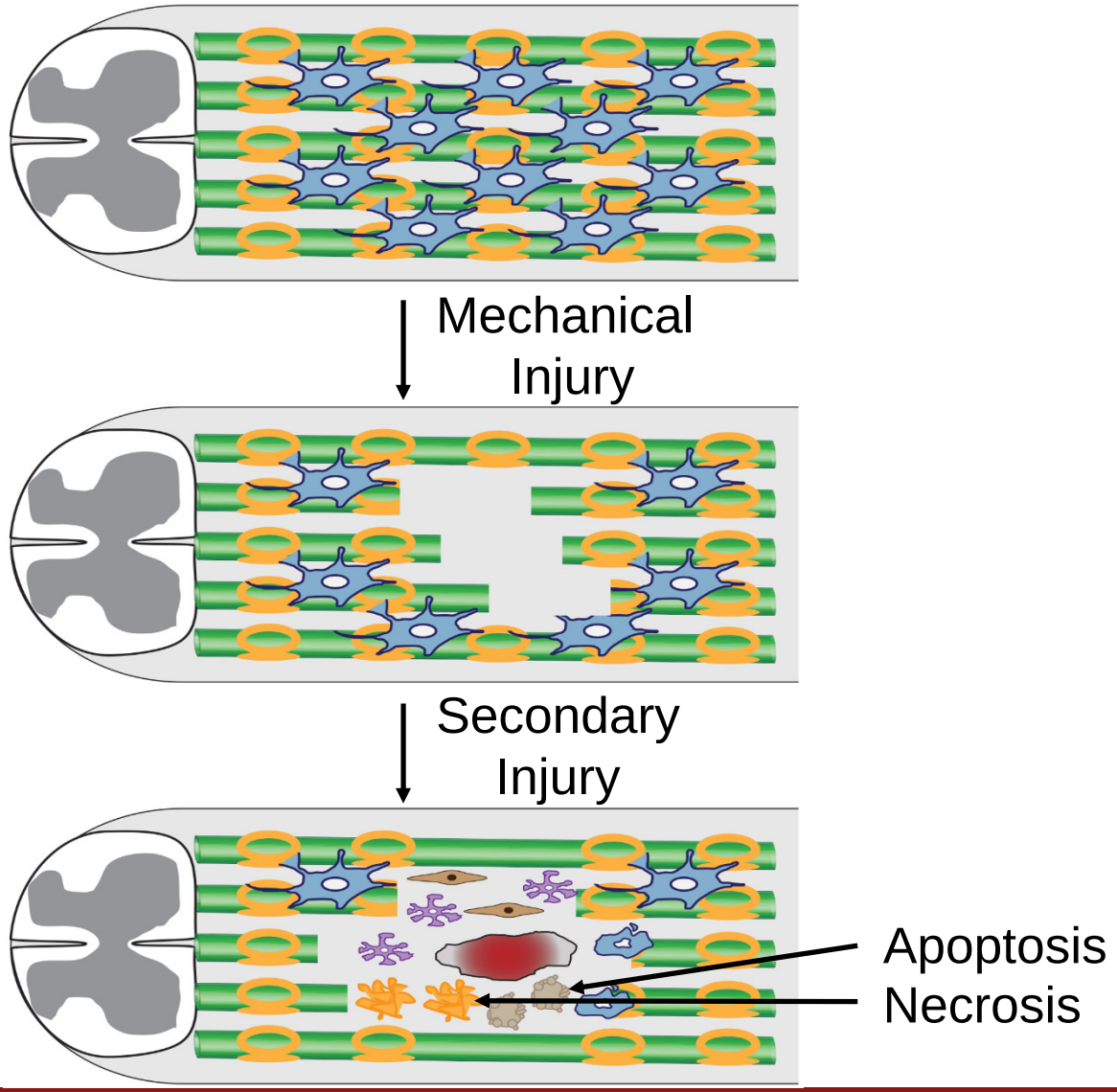
Modeling secondary injury via toxin-mediated ablation



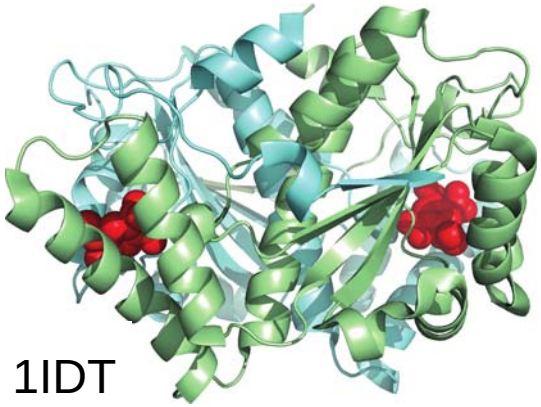
Modeling secondary injury via toxin-mediated ablation



Modeling secondary injury via toxin-mediated ablation



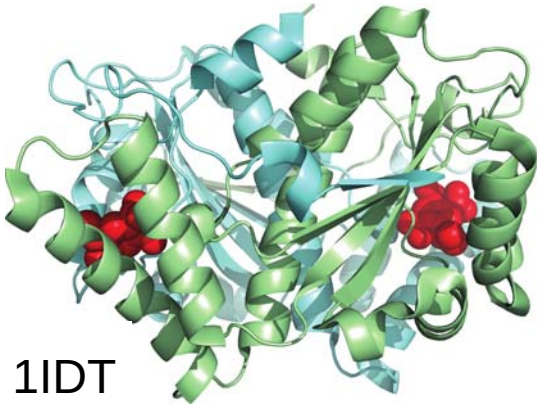
Modeling neuronal loss via development of toxin-mediated ablation



PDT: 1IDT

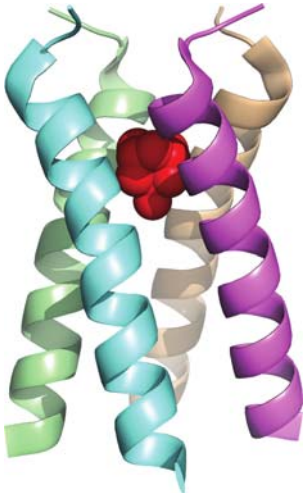
- Nitroreductase
  - Enzyme converts a prodrug (metronidazole) into a cytotoxic compound
  - Gold standard for zebrafish
  - Disadvantage – slow acting

Modeling neuronal loss via development of toxin-mediated ablation



PDT: 1IDT

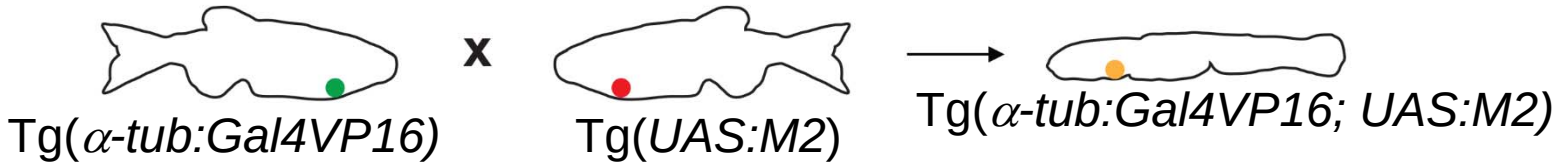
- Nitroreductase
  - Enzyme converts a prodrug (metronidazole) into a cytotoxic compound
  - Gold standard for zebrafish
  - Disadvantage – slow acting

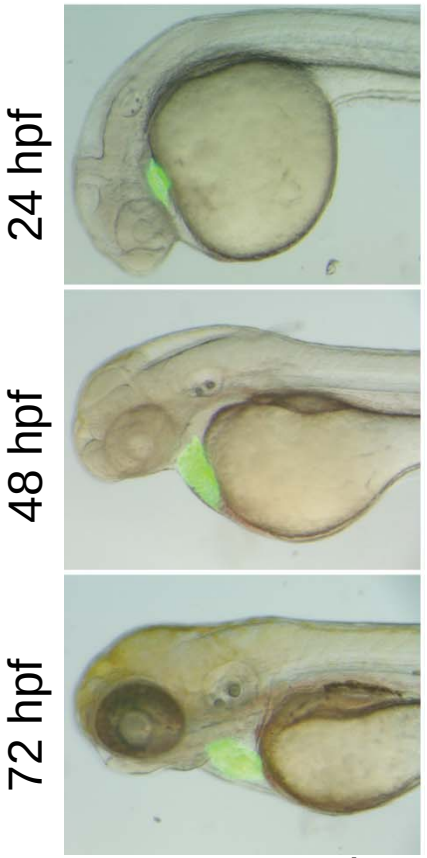


PDT: 2KQT

- M2
  - Ion channel from flu
  - Can be blocked with FDA approved drug (rimantadine)
  - Never used in zebrafish
  - Advantage – fast acting

Generating and characterizing Tg(*UAS:M2)* lines

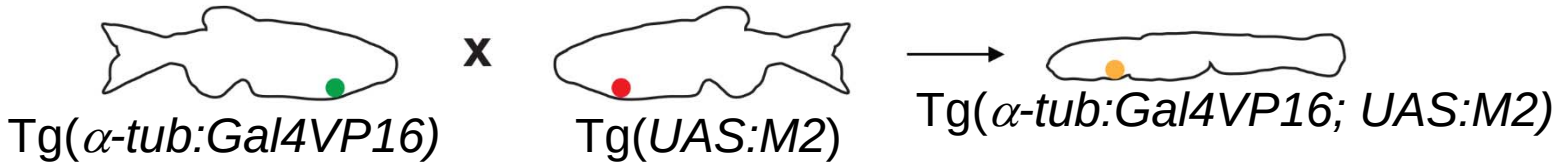


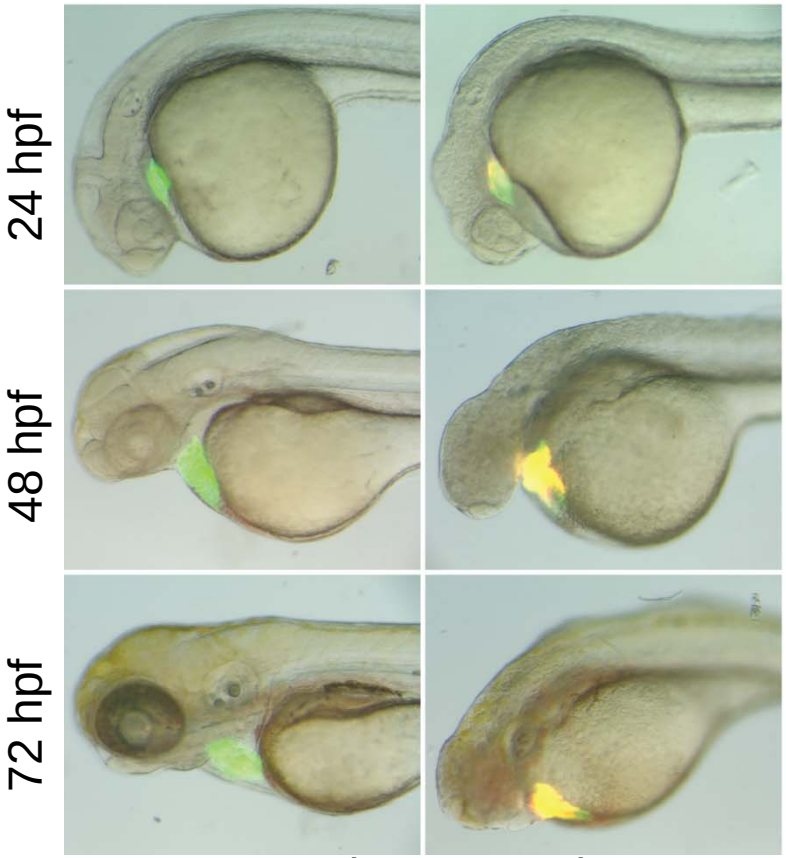


No drug control

n = 30-50 larvae for each condition

Generating and characterizing Tg(*UAS:M2)* lines

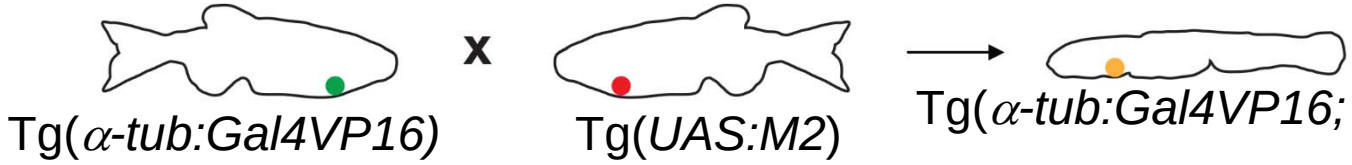




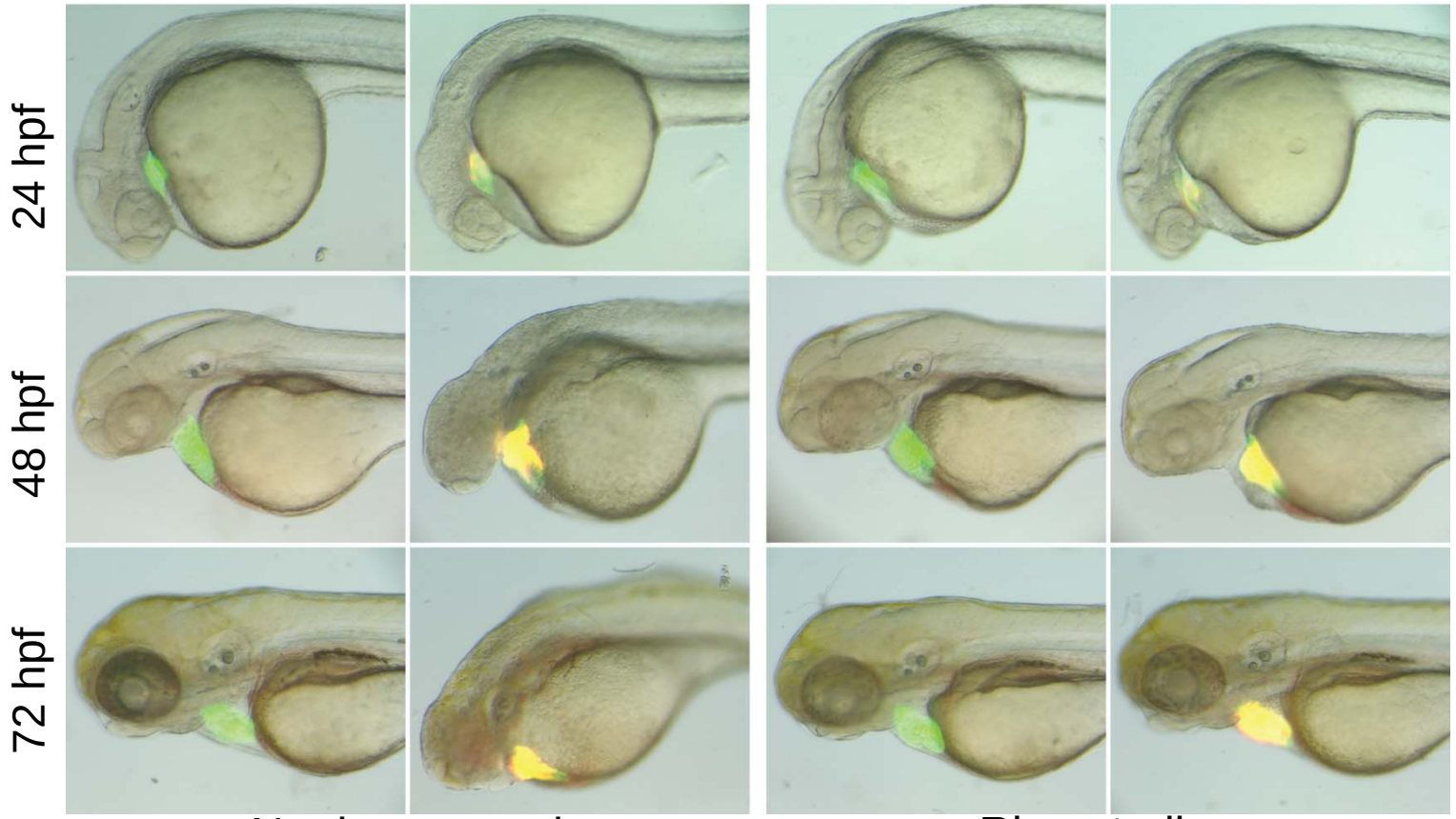
No drug control

n = 30-50 larvae for each condition

Generating and characterizing Tg(*UAS:M2)* lines



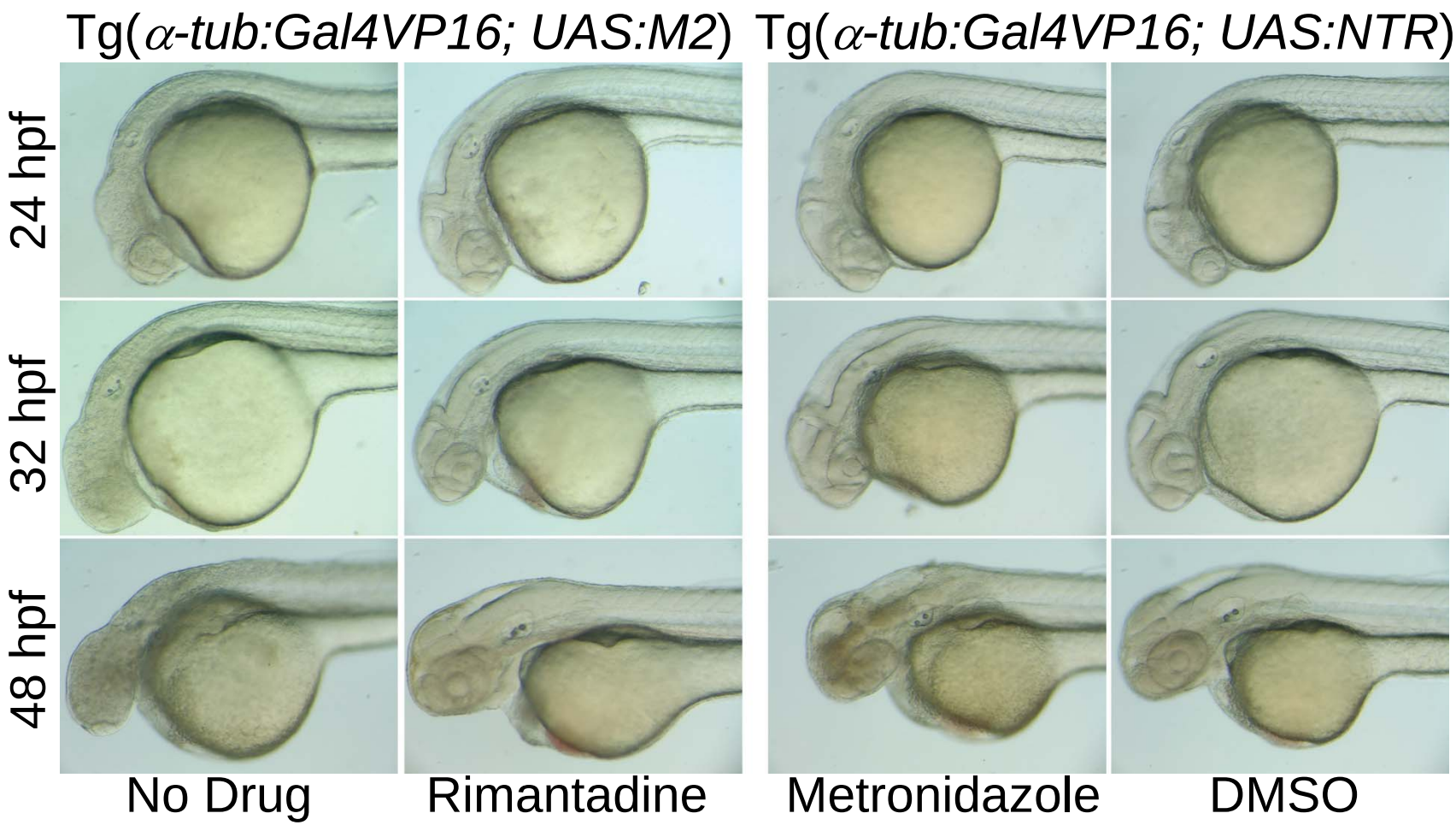
Tg(*α-tub:Gal4VP16)* Tg(*UAS:M2*) Tg(*α-tub:Gal4VP16; UAS:M2)*



No drug control Rimantadine

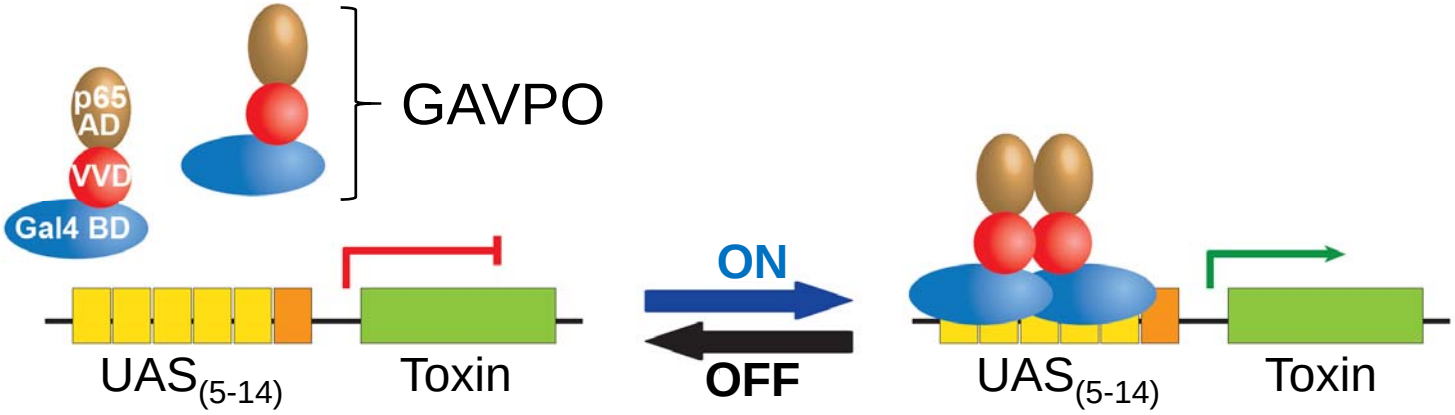
n = 30-50 larvae for each condition

M2-induced defects are more pronounced than NTR-mediated ablation



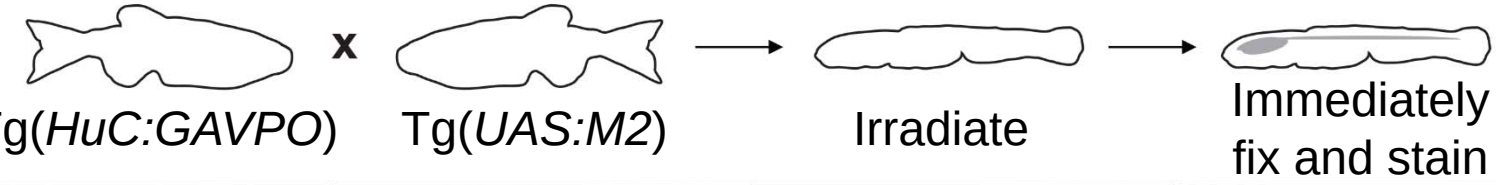
n = 30-50 larvae for each condition

Using light to spatiotemporally ablate CNS cells

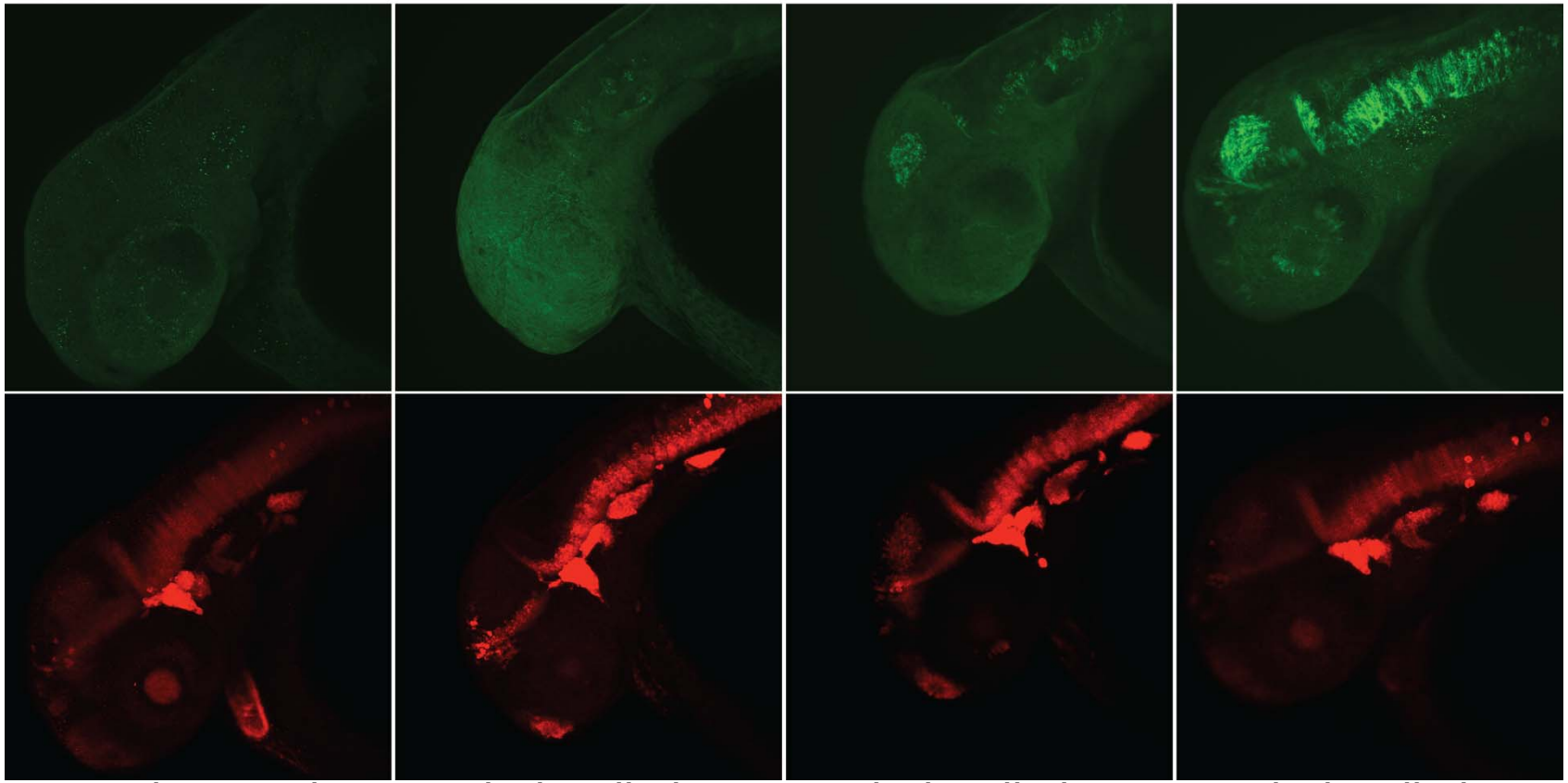


- ✓ Does GAVPO drive transcription in zebrafish?
- ✓ Can we model neural death with toxins?
- ❑ Can we induce neural death using the GAVPO system?

Irradiation induces M2 expression

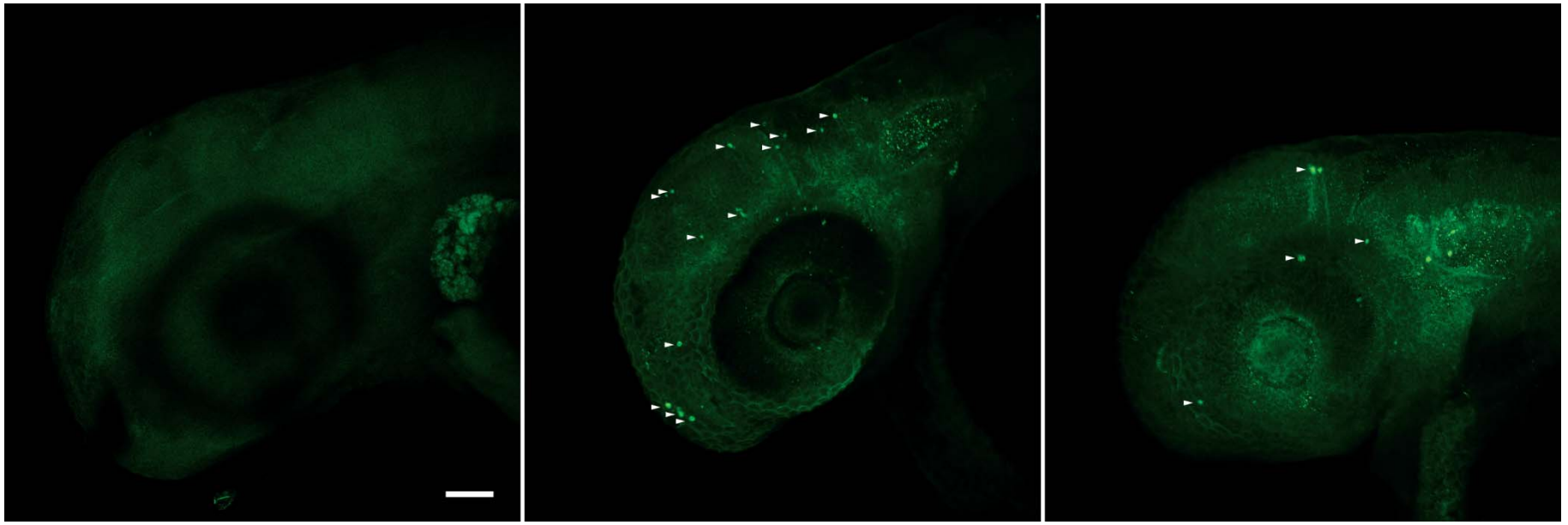


Tg(*HuC:GAVPO*) x Tg(*UAS:M2*) → Irradiate → Immediately fix and stain



n = 10-20 larvae for each condition

Irradiation induces activation of Caspase-3 which can be attenuated with rimantadine

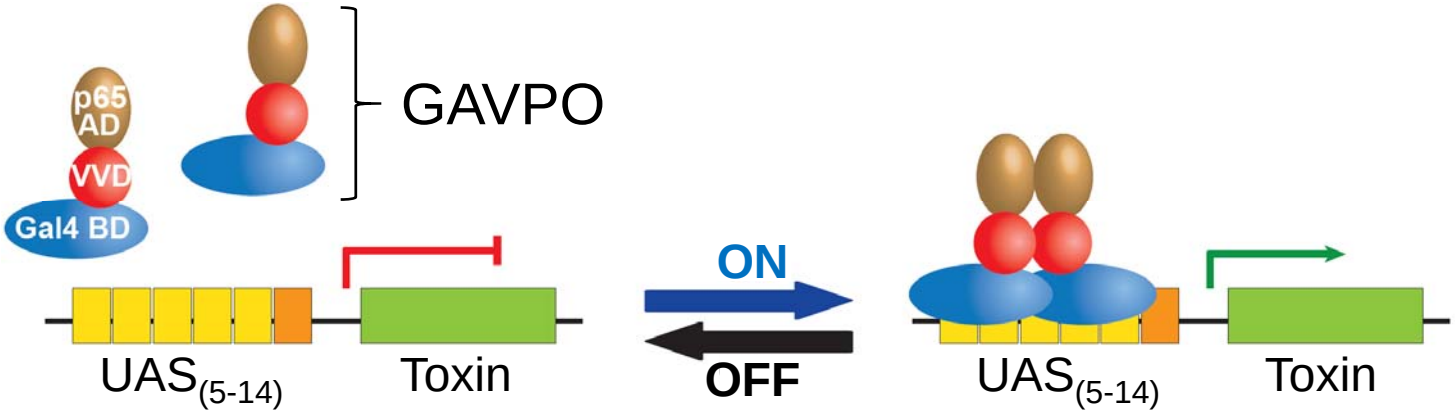


Dark control | 12 hr irradiation | 12 hr irradiation + rimantadine

Global irradiation (470-nm light 3 mW/cm^2)

n = 10-20 larvae for each condition

Using light to spatiotemporally ablate CNS cells

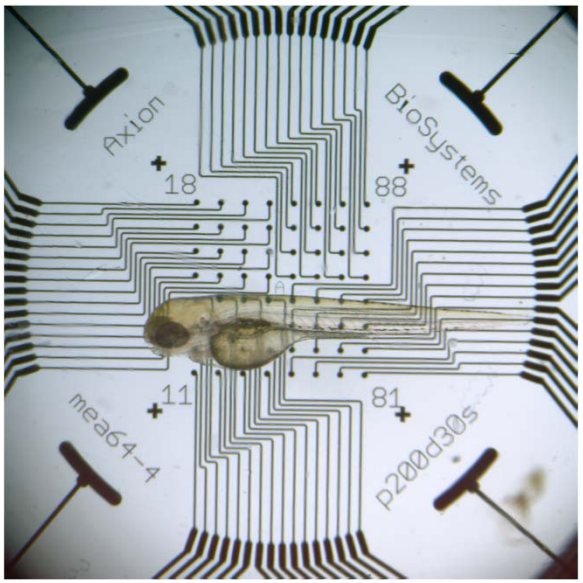


- ✓ Does GAVPO drive transcription in zebrafish?
- ✓ Can we model neural death with toxins?
- ✓ Can we induce neural death using the GAVPO system?

How does the nervous system response to optogenetic ablation compare to manual injury?

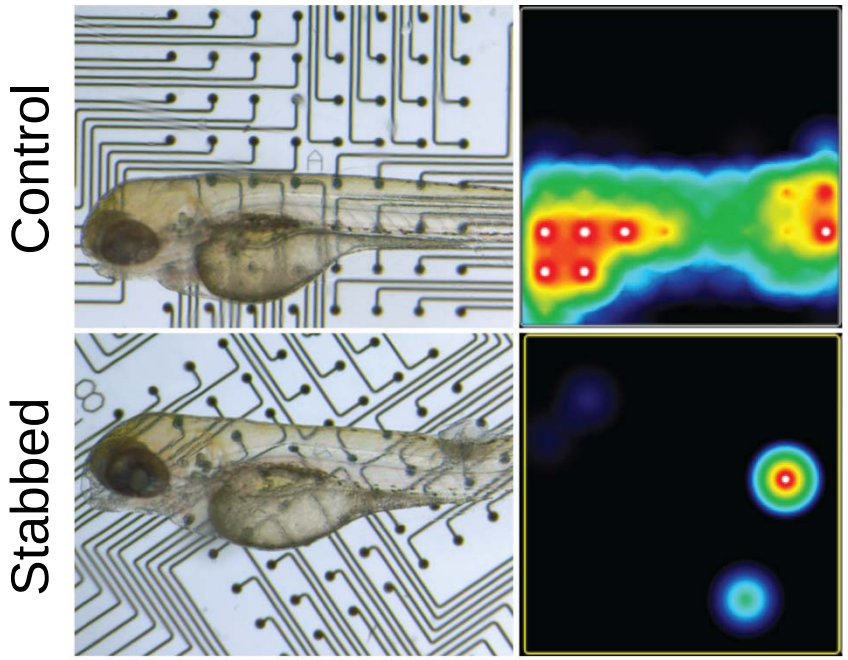
Comparing neural ablation to traditional spinal cord injury in zebrafish

Maestro MEA system
(Axion BioSystems)

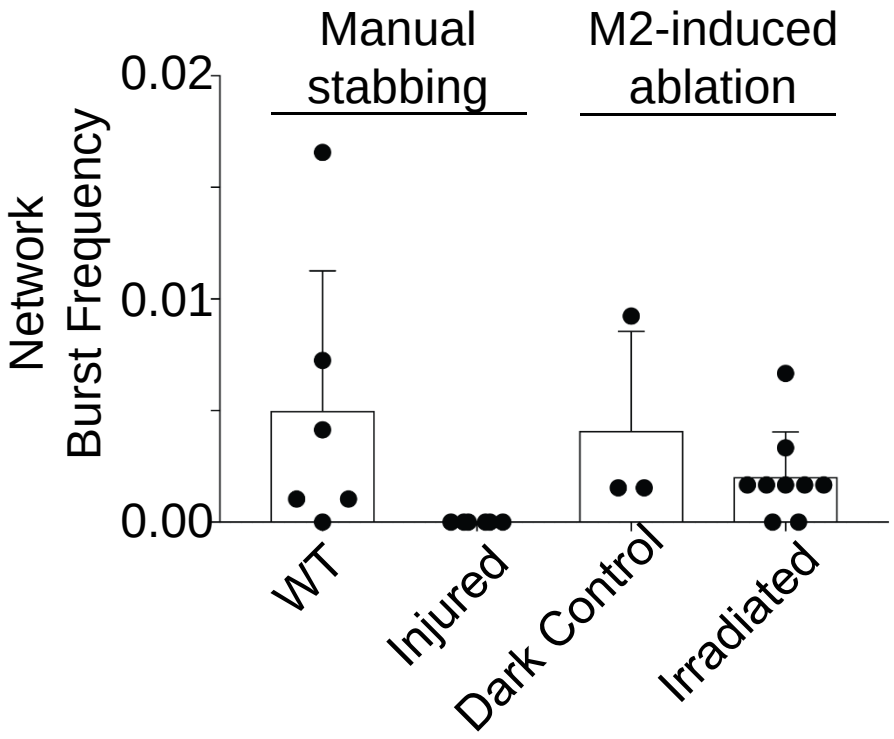


Comparing neural ablation to traditional spinal cord injury in zebrafish

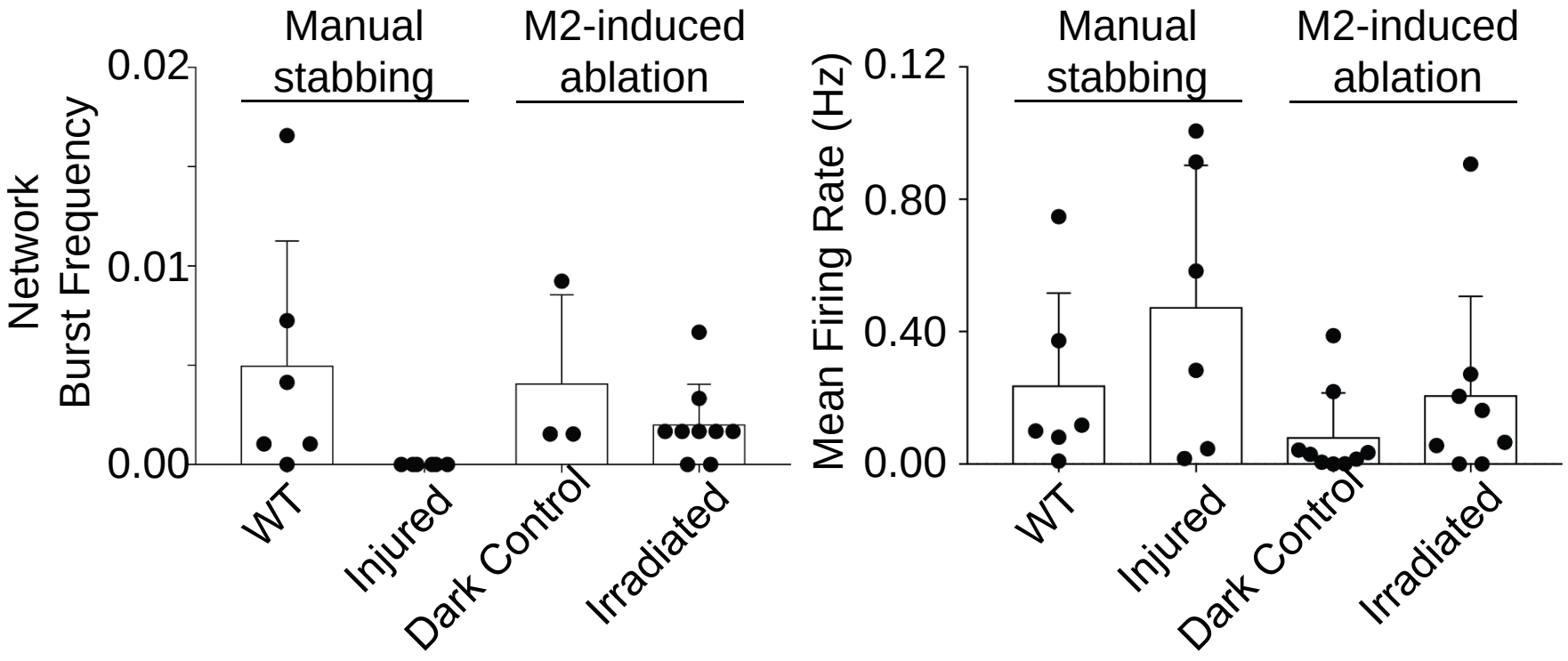
Maestro MEA system
(Axion BioSystems)



Injury reduces network bursting and increases firing frequency



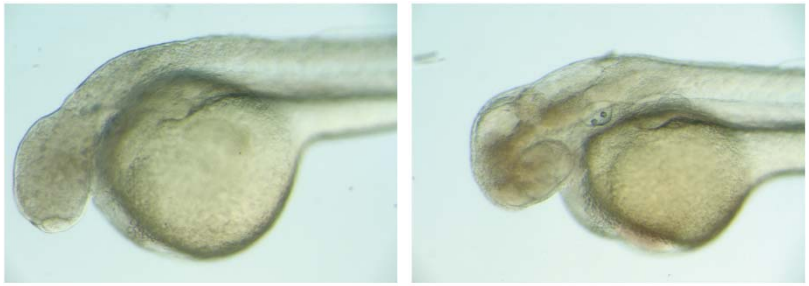
Injury reduces network bursting and increases firing frequency

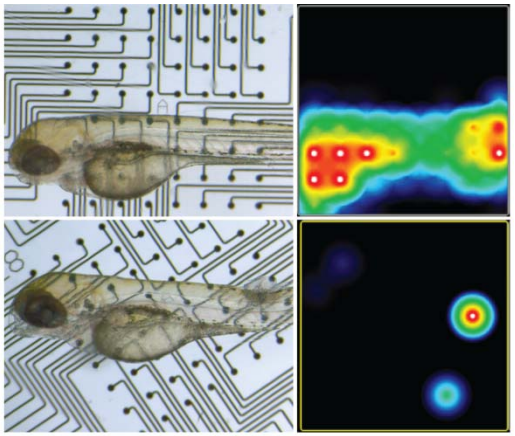


n = 3-10 larvae for each condition

Summary and future directions

- Developed transgenic zebrafish for light-inducible gene expression
- Used multiple toxins to model secondary injury
- Studying neuronal circuit-connectivity and physiology and SCI pathology





Acknowledgements

- Chen Laboratory
  - **James Chen**
  - Moe Alnaqid
  - Patrick Piza
- Plant Laboratory
  - **Giles Plant**
- Axion Biosystems
  - Stacie Chvatal
  - Emily Matheu

CRAIG·H NEILSEN FOUNDATION

STANFORD BIO-X

AXION BIOSYSTEMS