

# *Passaggio d'anno, II year*

## PhD course in Nanoscience

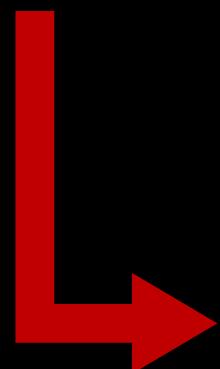
Pisa, October 20<sup>th</sup> 2020

Student: Gabriele Nardi

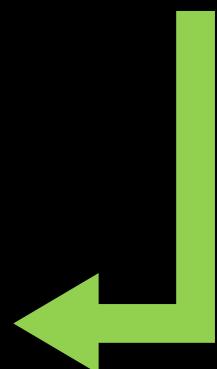
Supervisor: Gian Michele Ratto

Genetic  
manipulation

2-Photon  
imaging

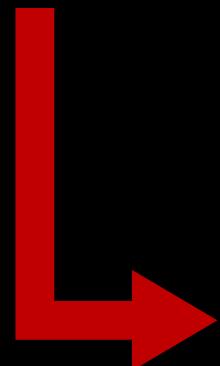


*In vivo*  
neurophysiology



Genetic  
manipulation

Beatrix



2-Photon  
imaging

GCaMP



*In vivo*  
neurophysiology

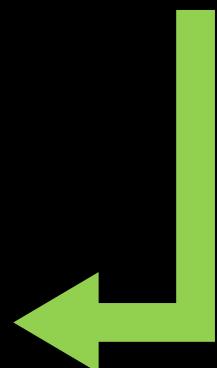
Genetic  
manipulation

Beatrix 5.0

2-Photon  
imaging

ddGCaMP

Study of  
mosaicism-based  
autism models

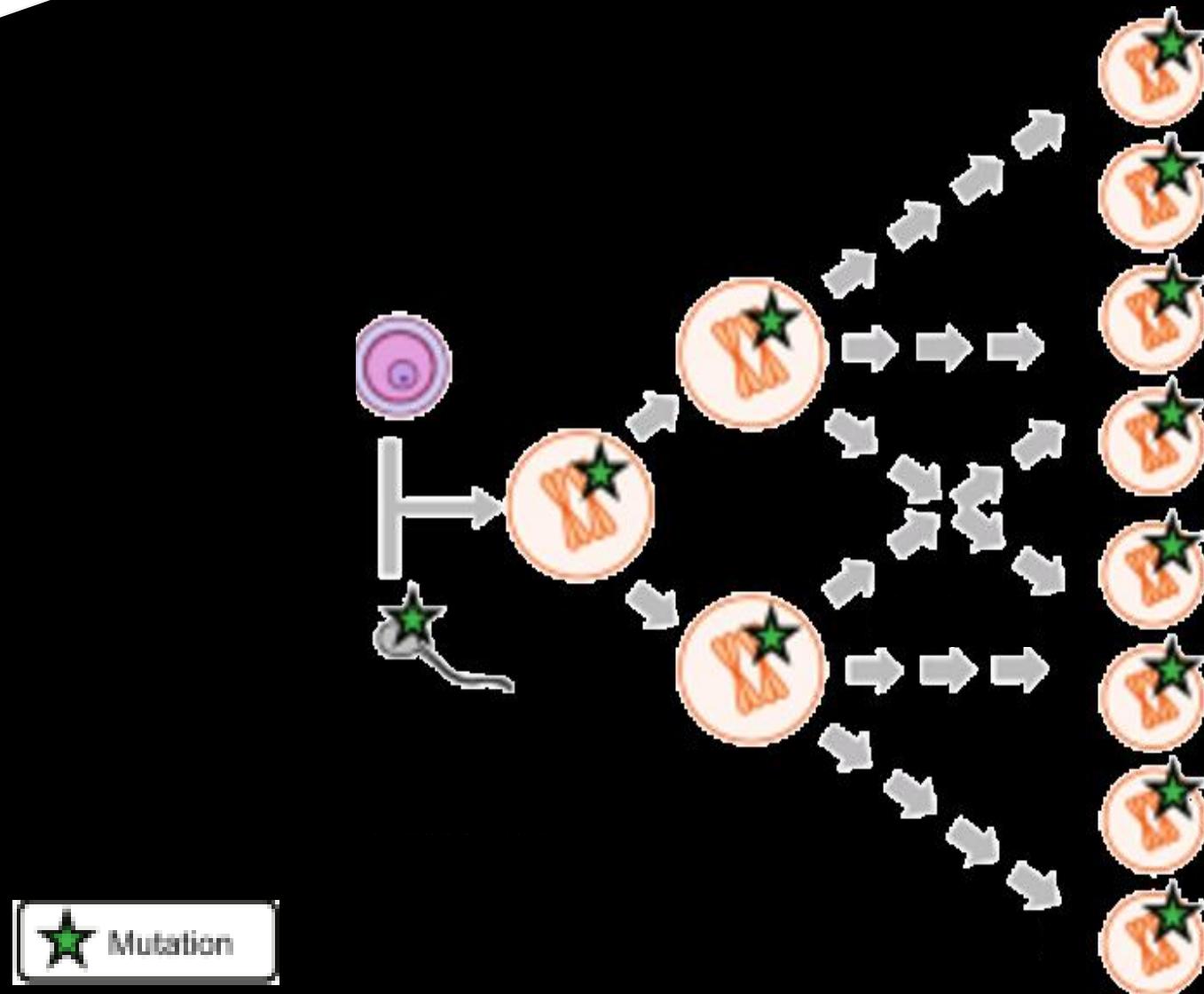


## Somatic mutations and mosaicism

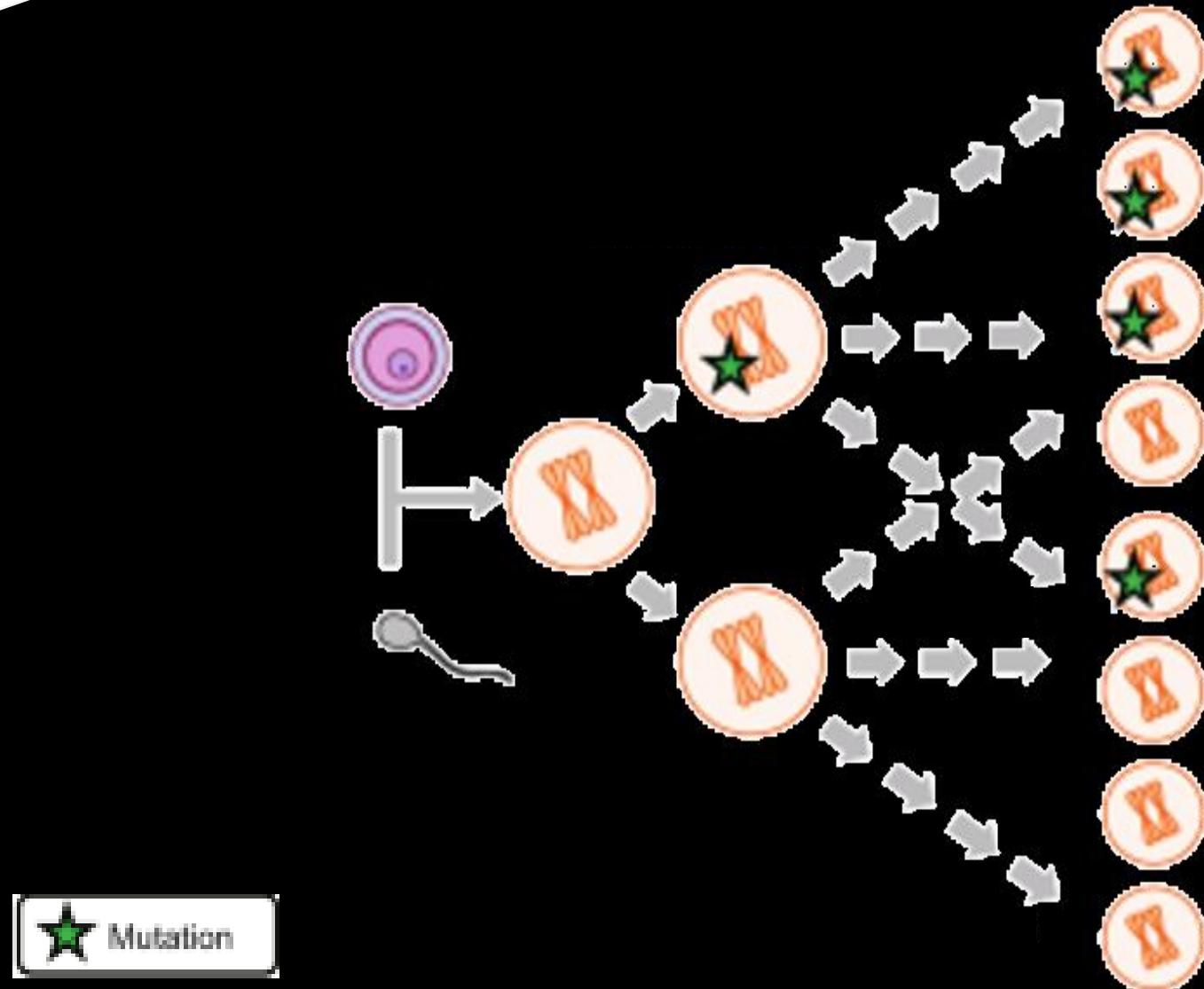


 Mutation

## Somatic mutations and mosaicism



## Somatic mutations and mosaicism

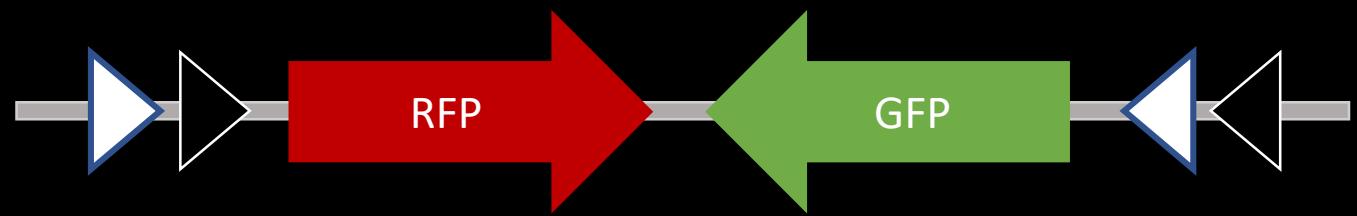


# KO reporting

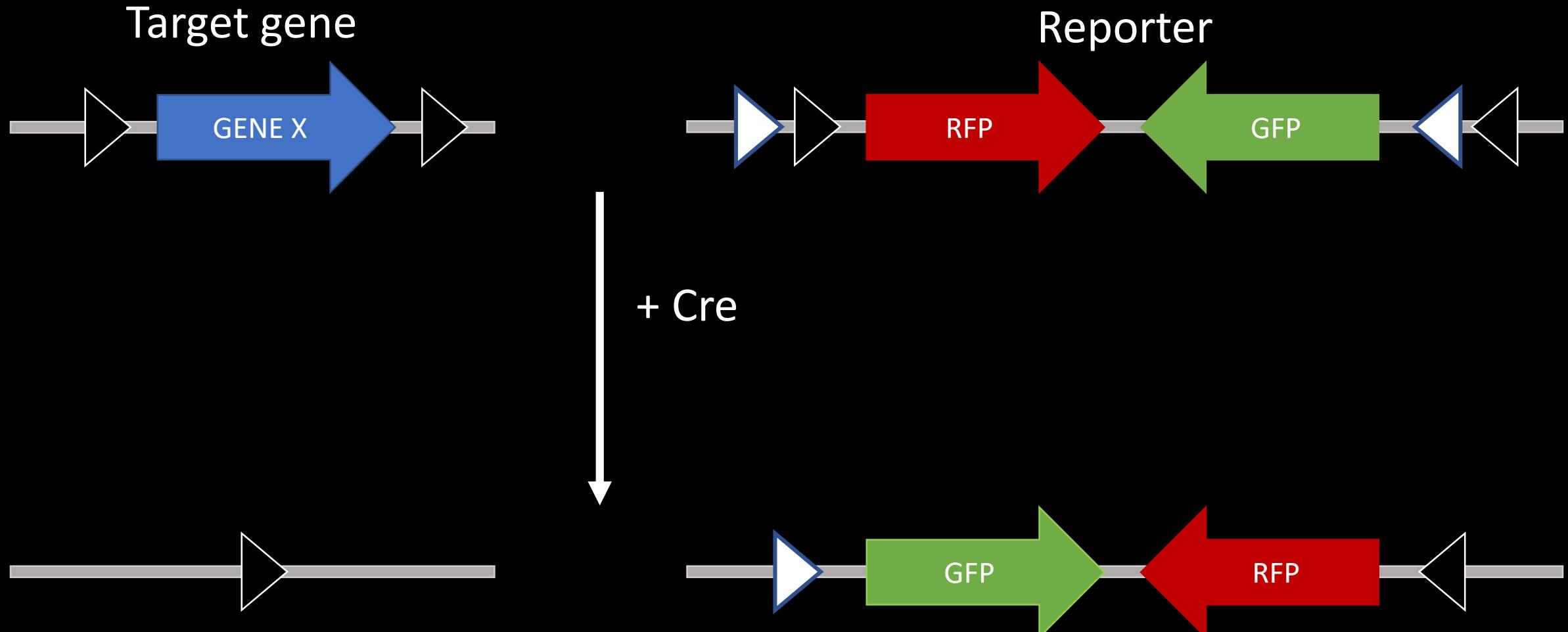
Target gene



Reporter



# KO reporting

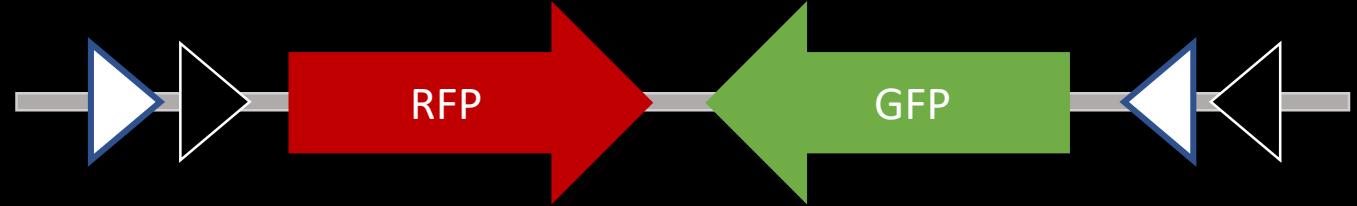


# KO reporting

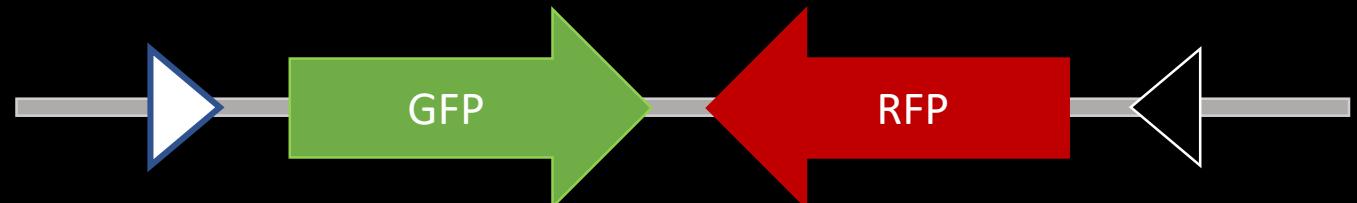
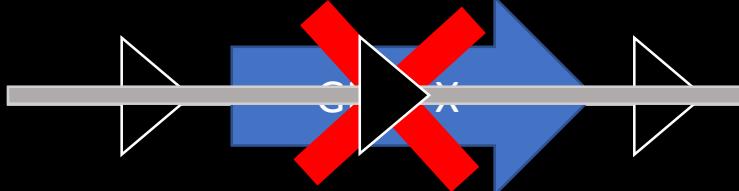
Target gene



Reporter

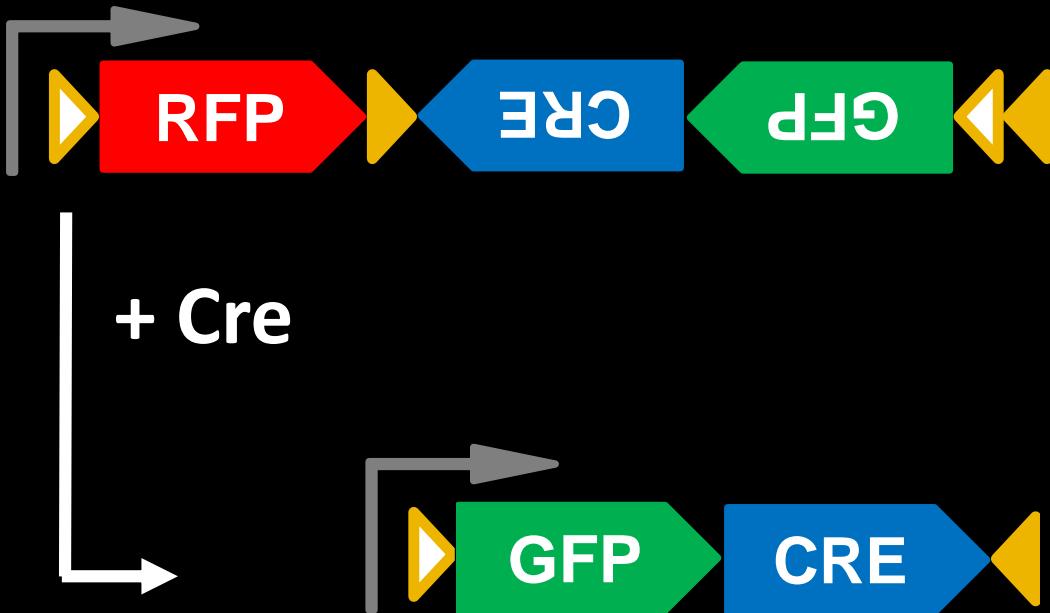


+ Cre

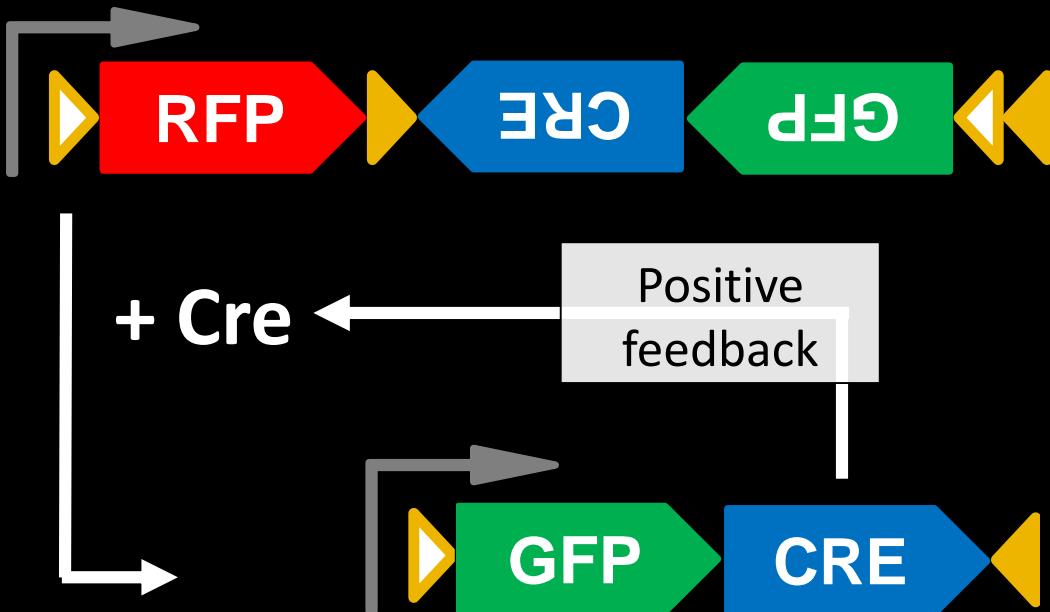




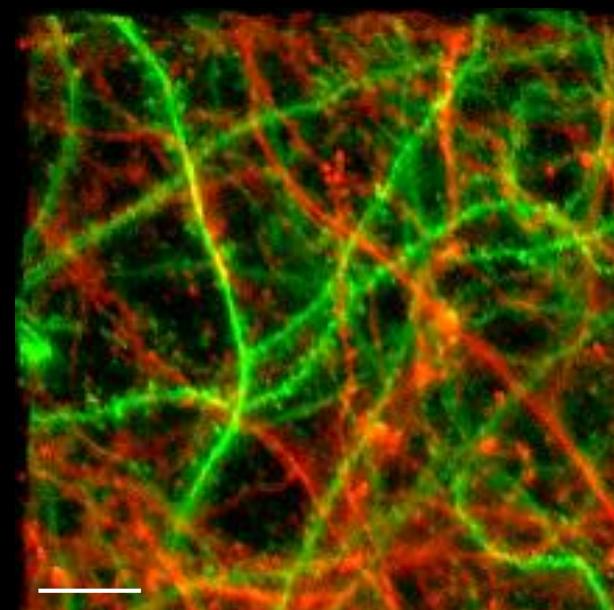
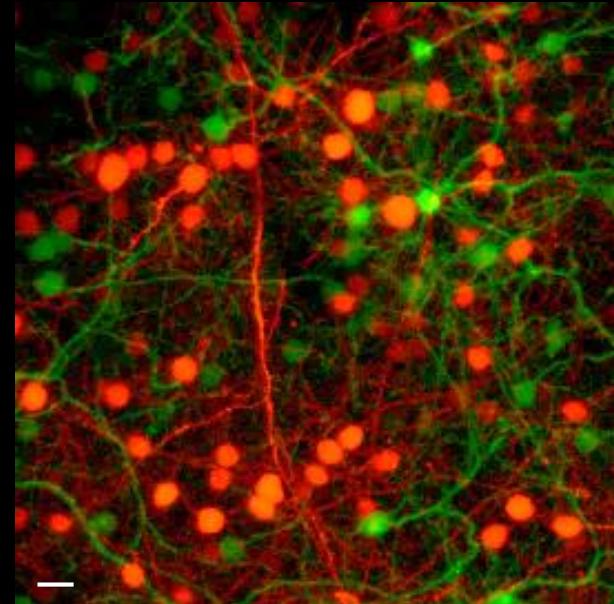
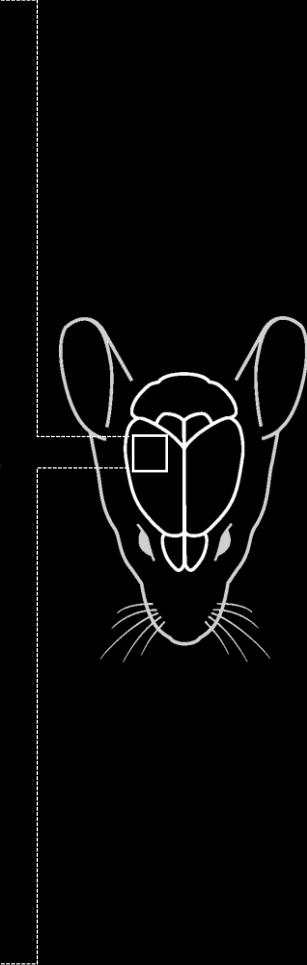
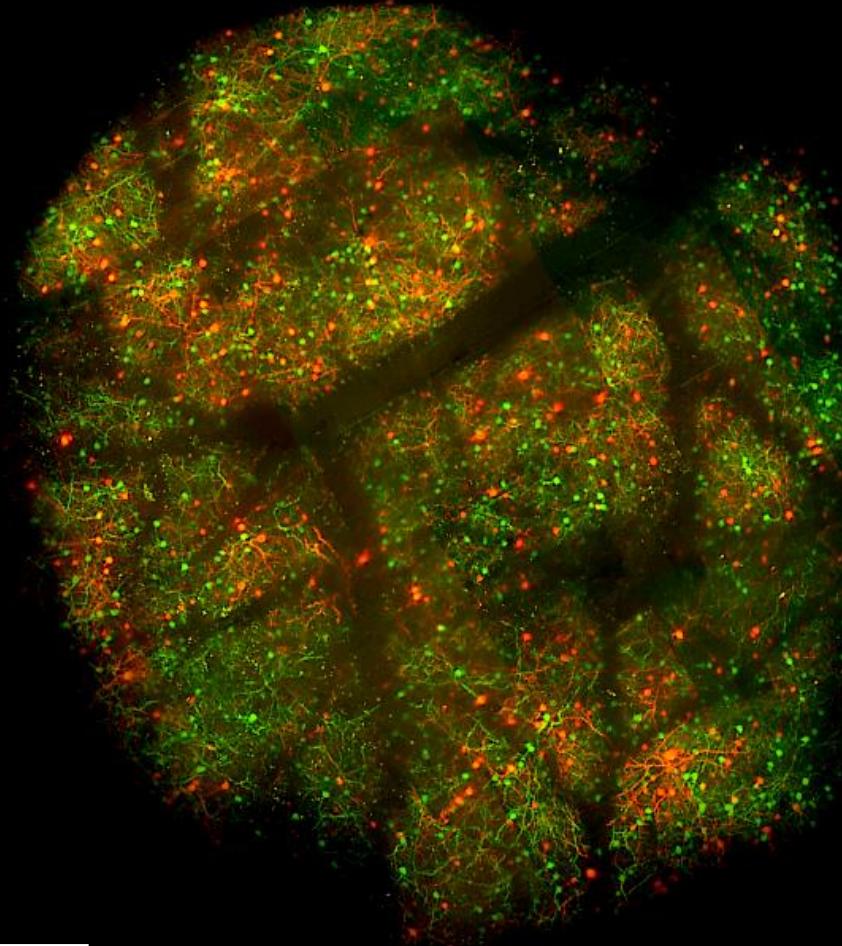
# Beatrix how it works

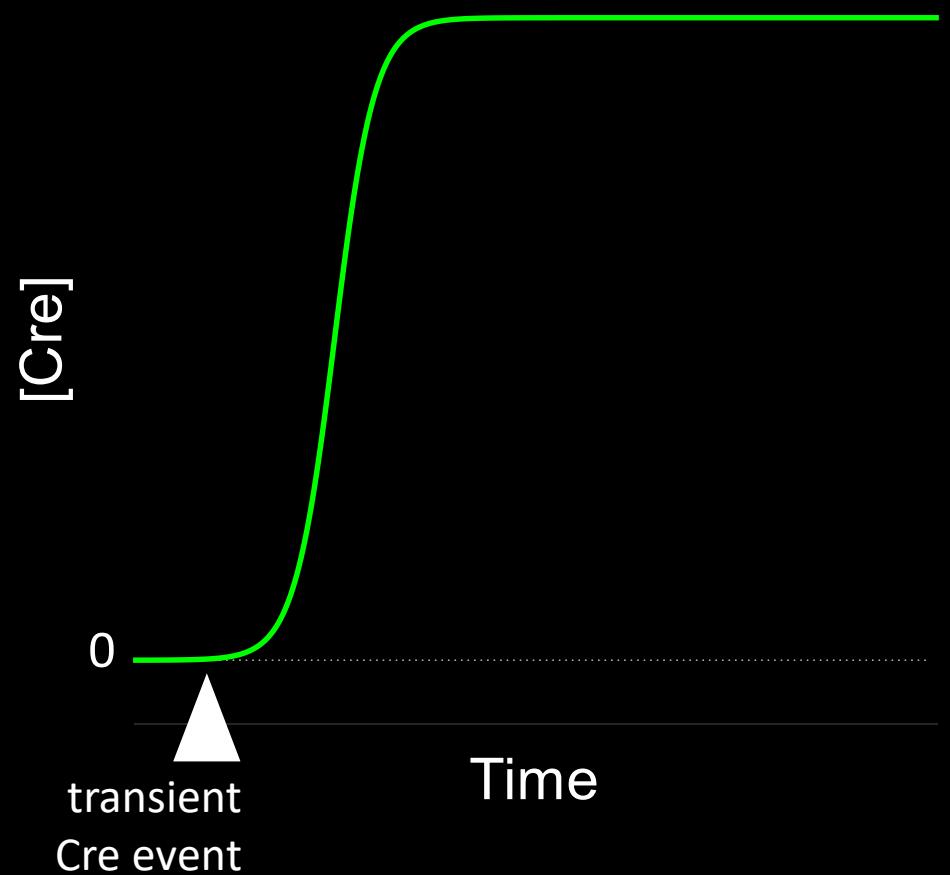
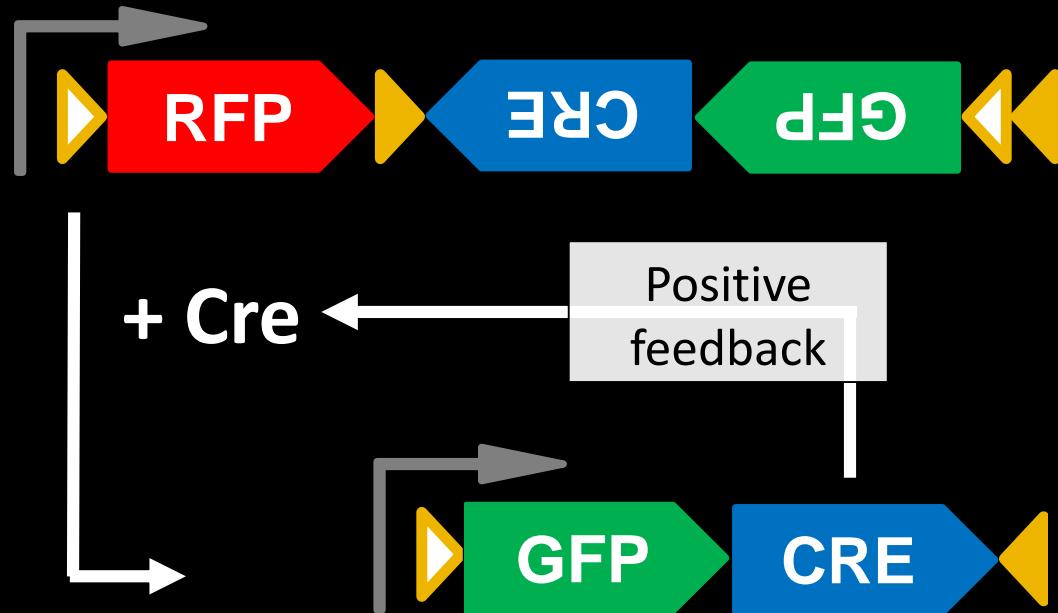


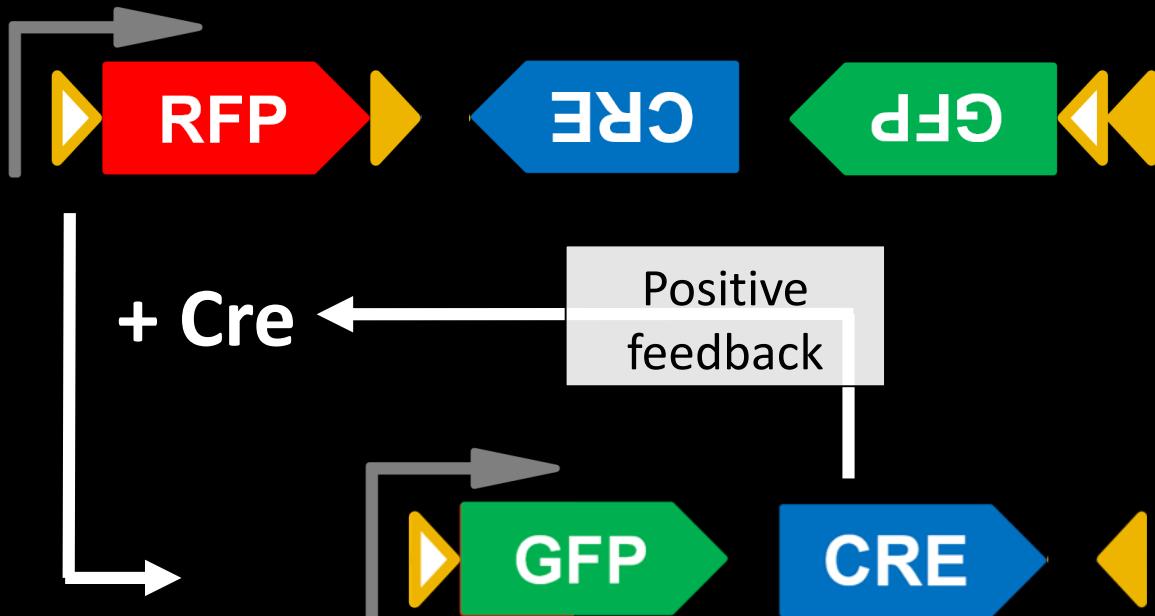
# Beatrix how it works

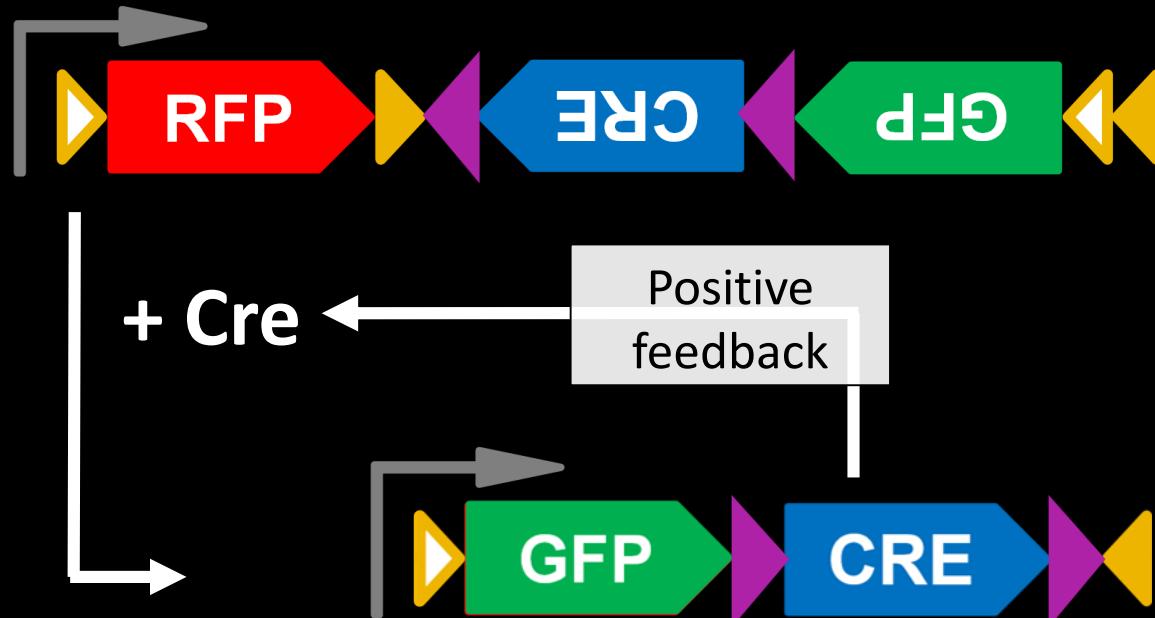


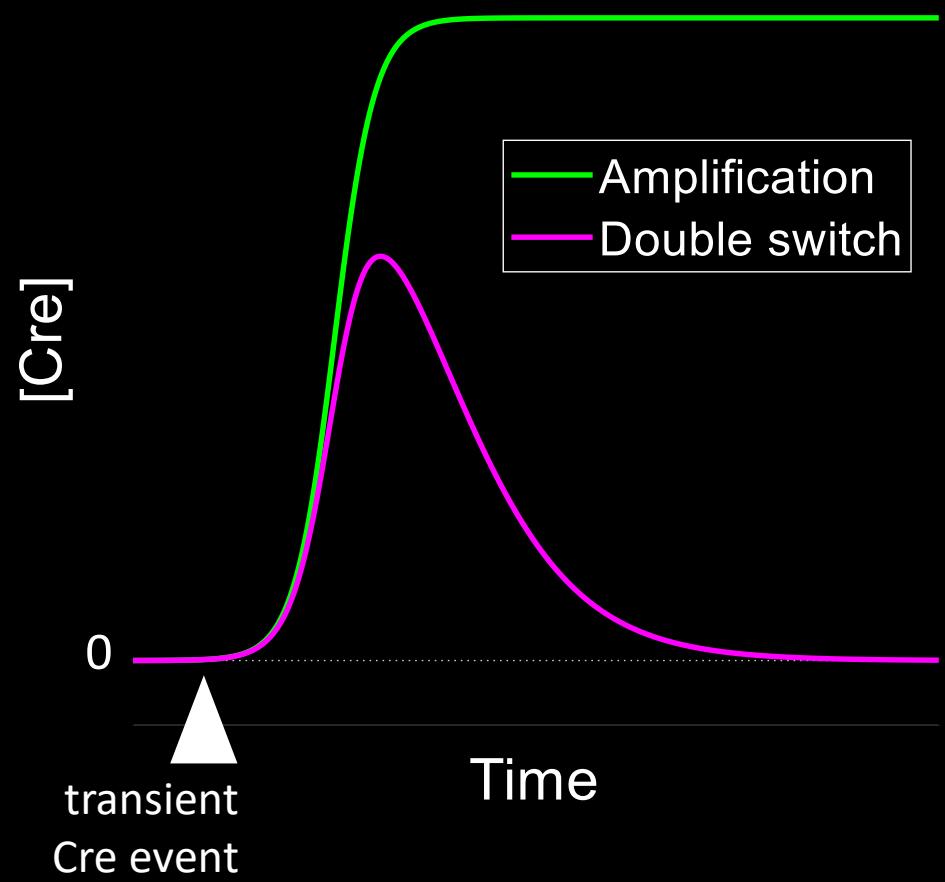
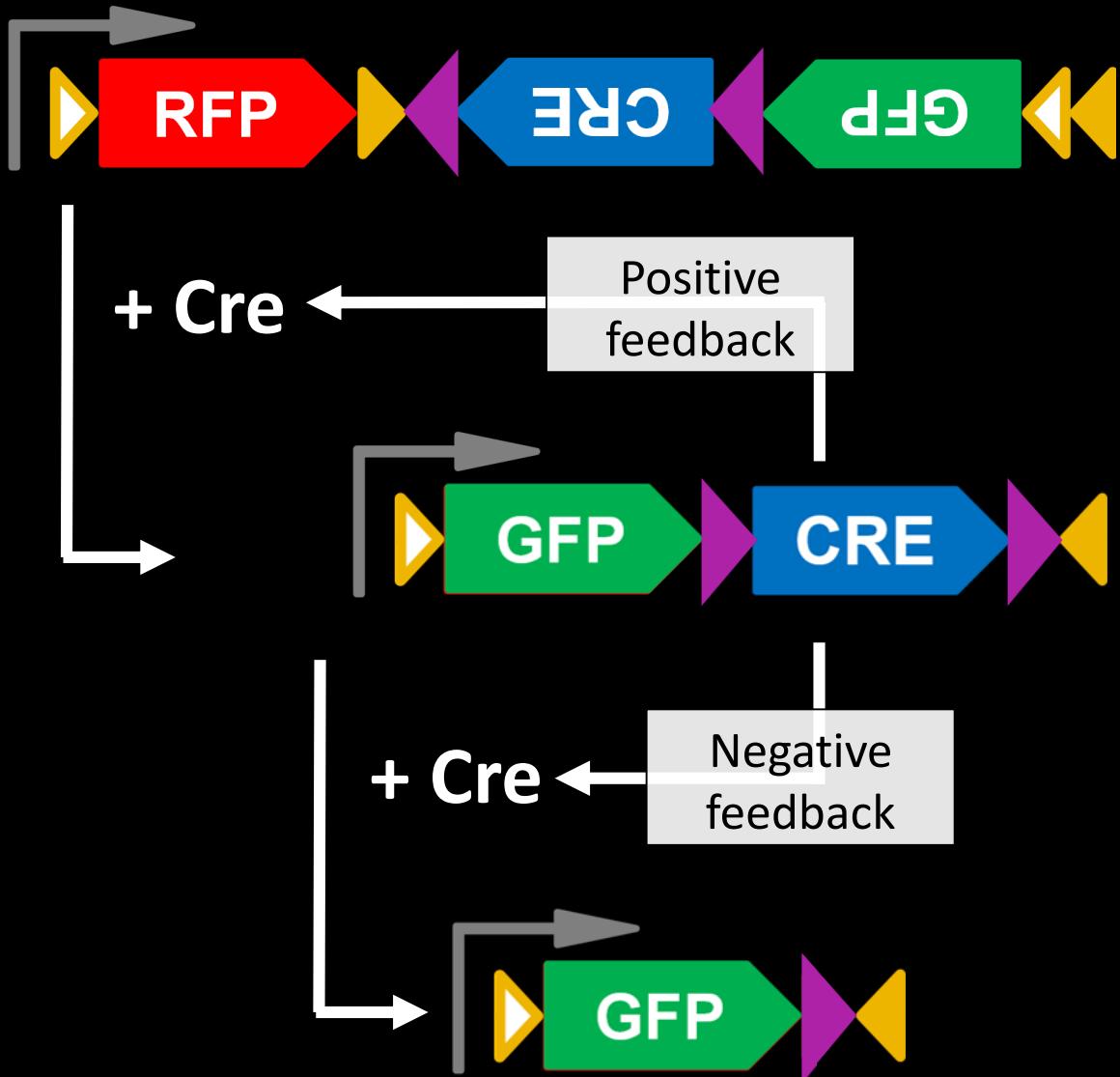
## *In vivo* imaging





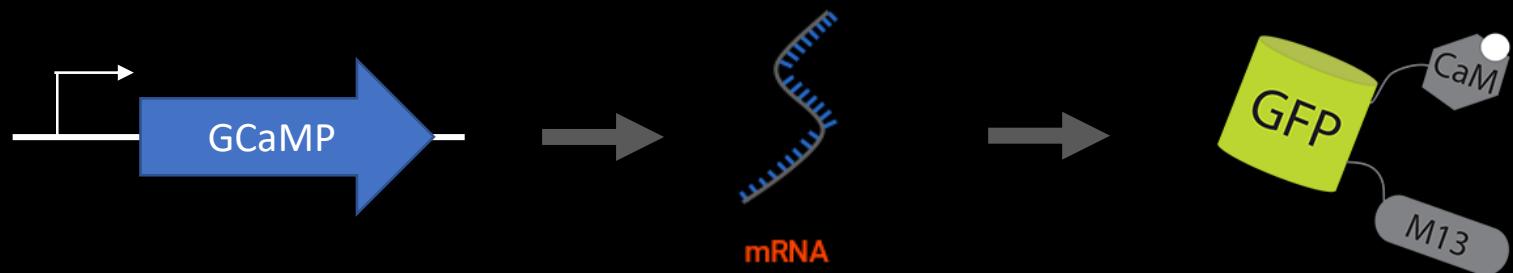


 = Low affinity lox sites



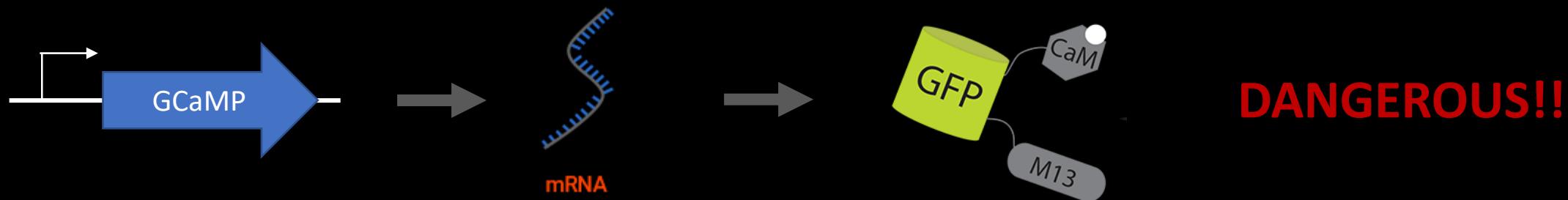
# ddGCaMP: how it works

## How it works



# ddGCaMP

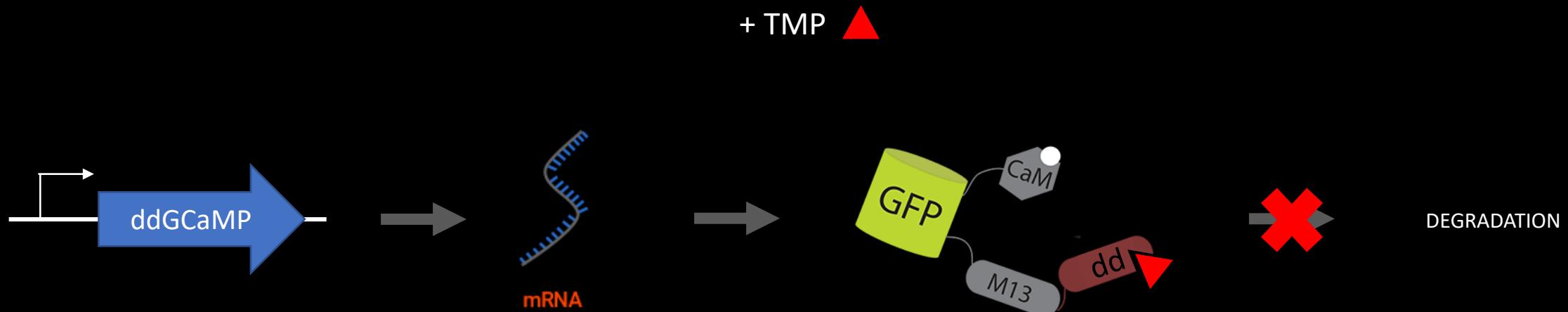
## How it works



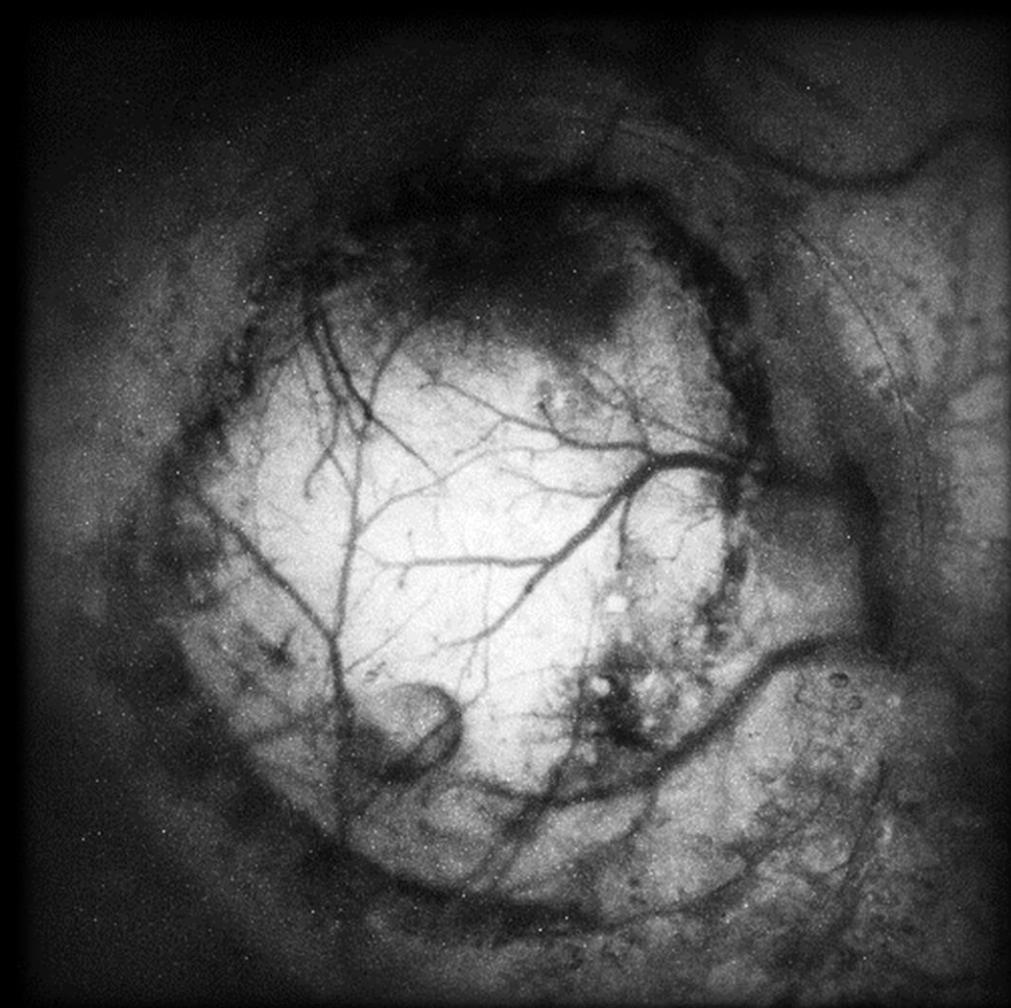
# ddGCaMP is constitutively expressed and degraded



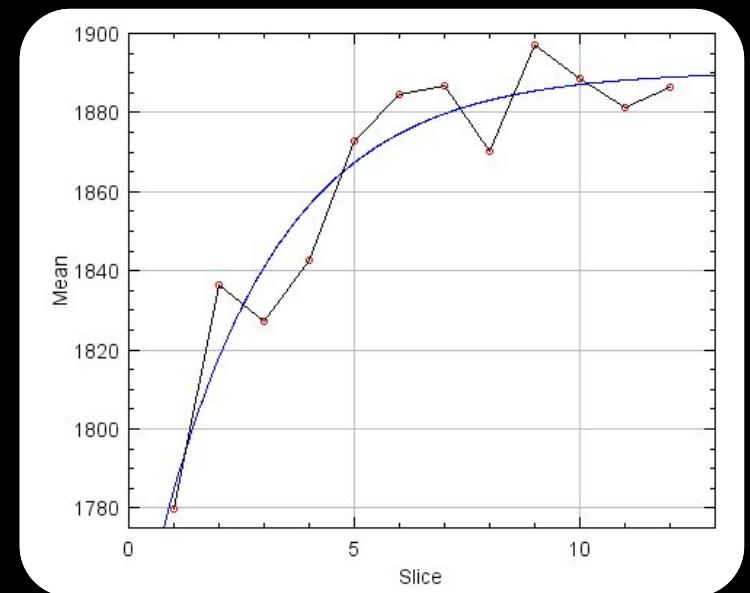
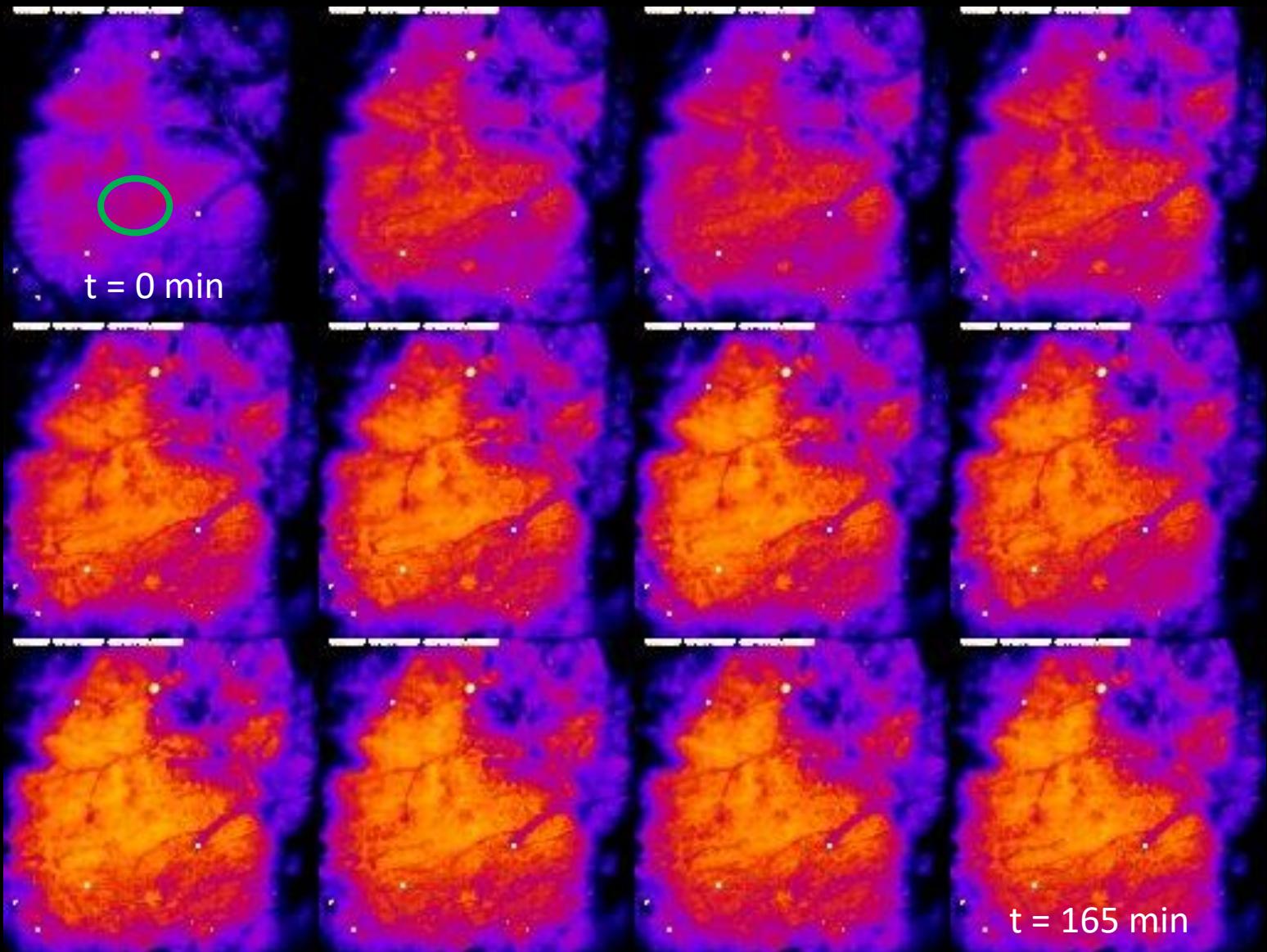
# Trimetoprim (TMP) administration prevents ddGCaMP degradation



# Widefield imaging



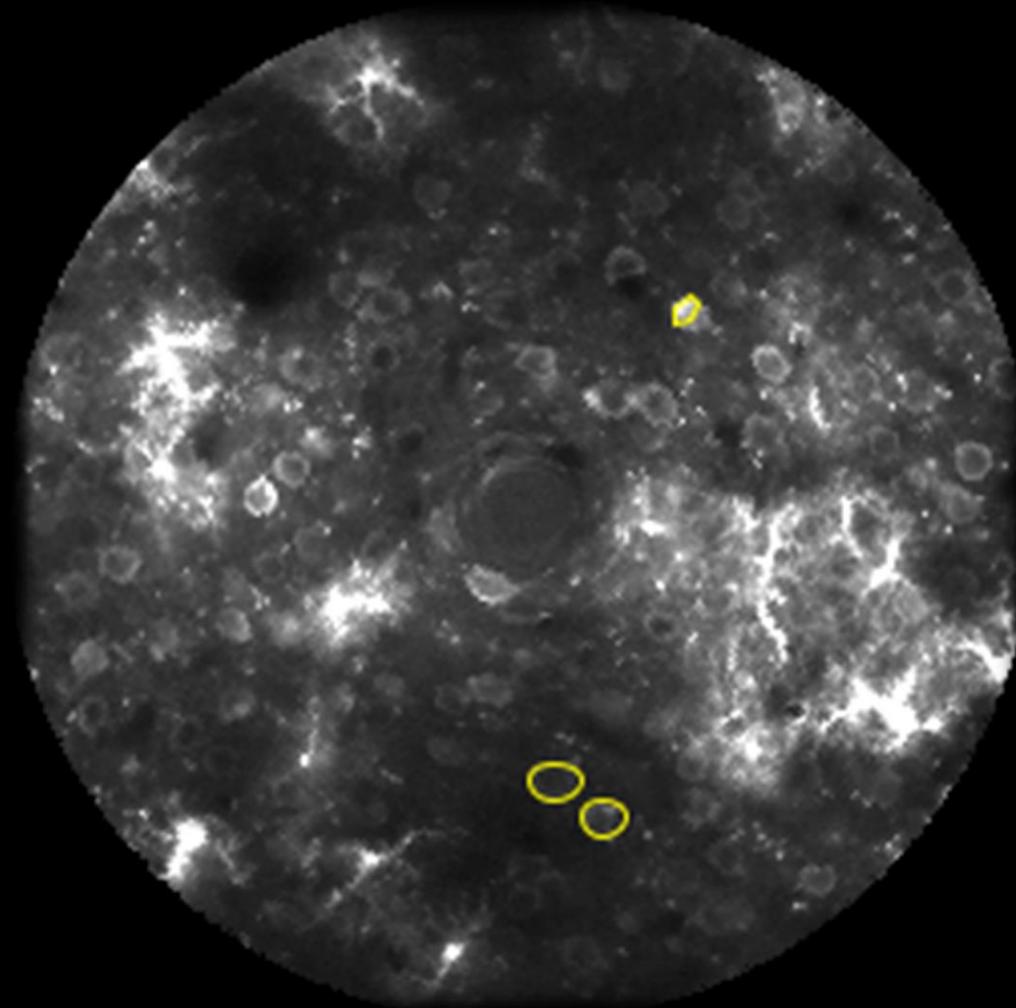
## Activation



$\Delta t = 15 \text{ min}$

25

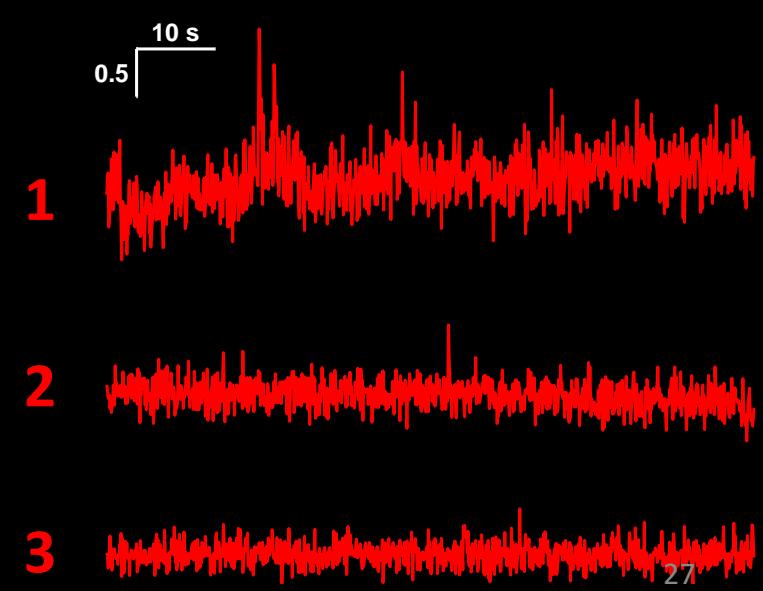
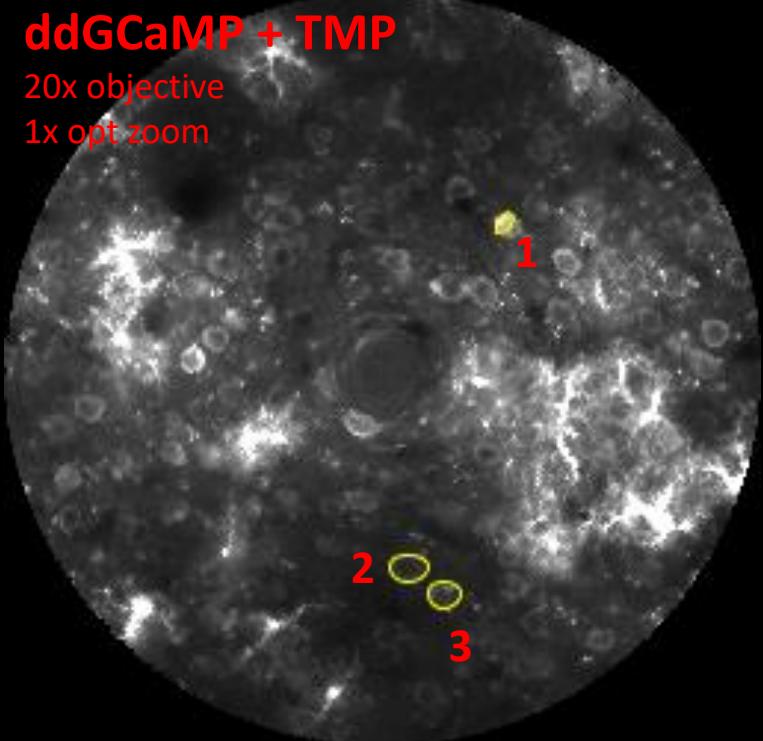
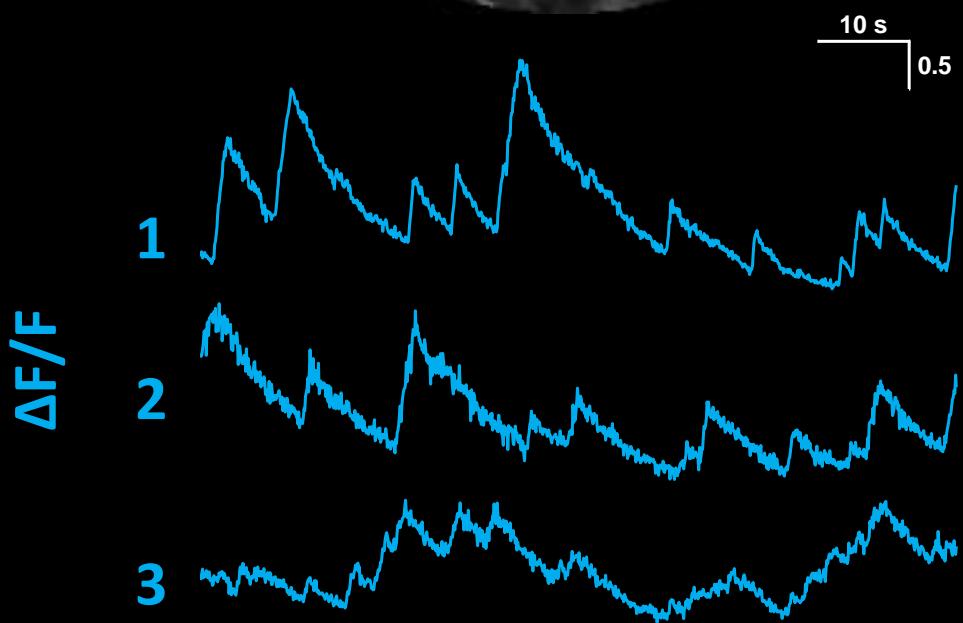
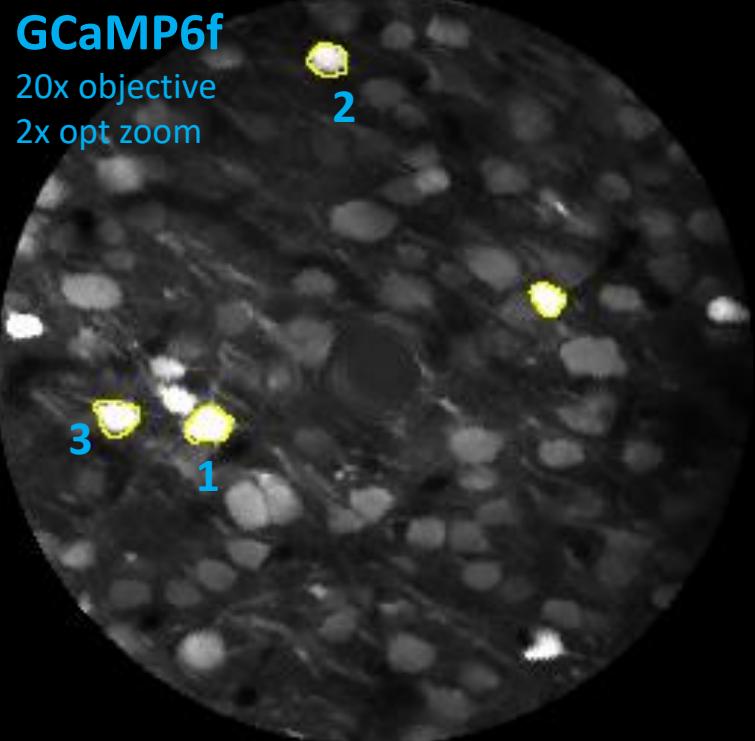
# 2Photon imaging



## IntraVentricular Injections

### Two Photon imaging:

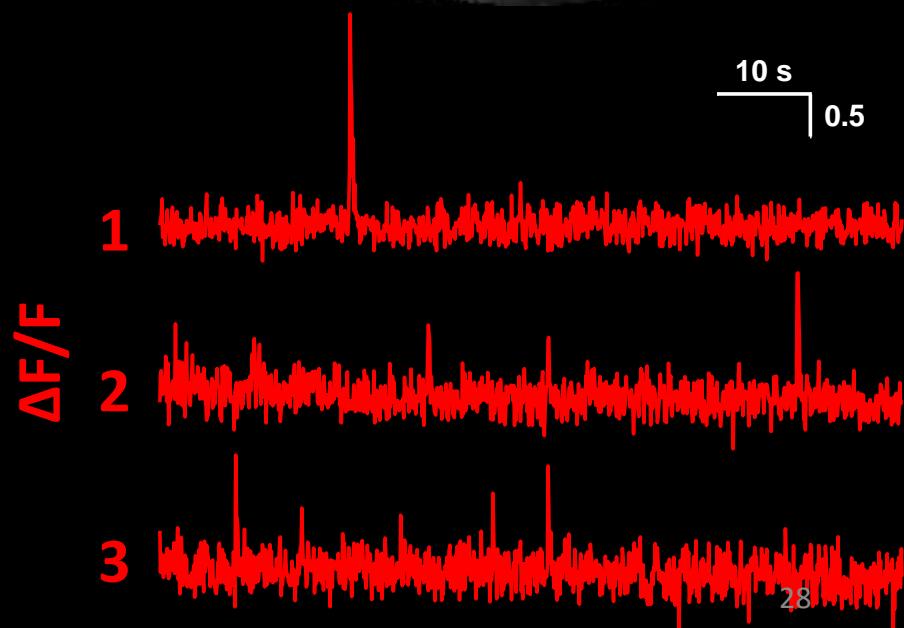
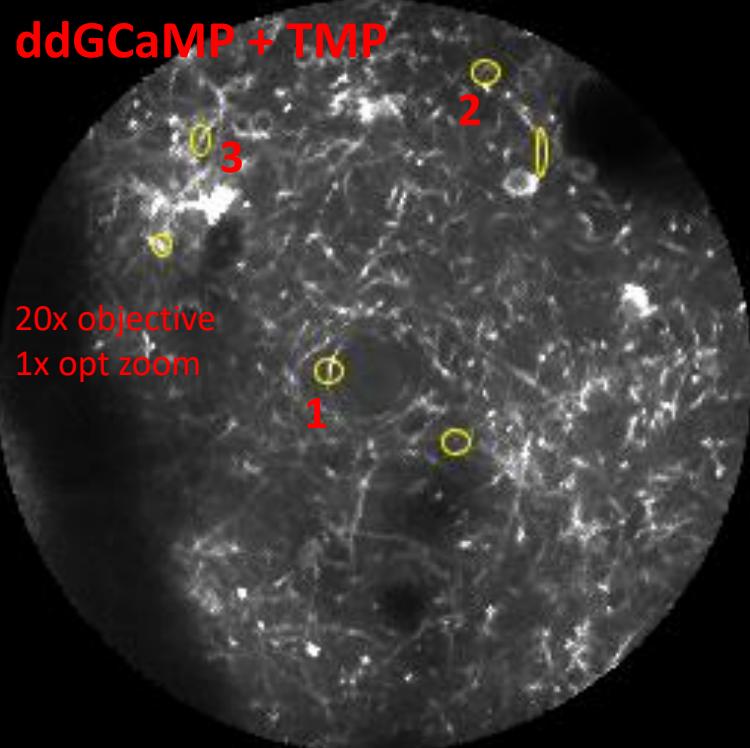
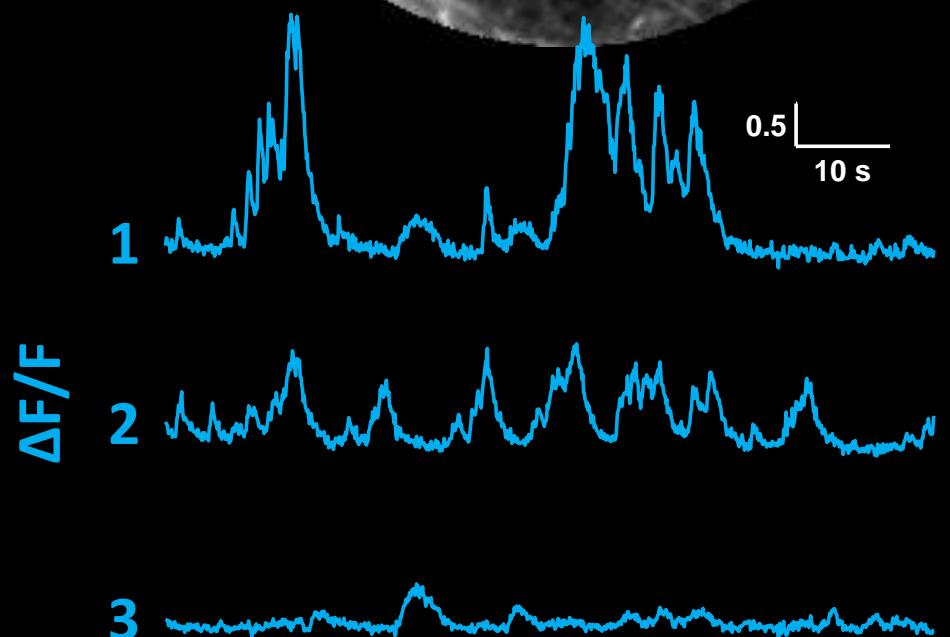
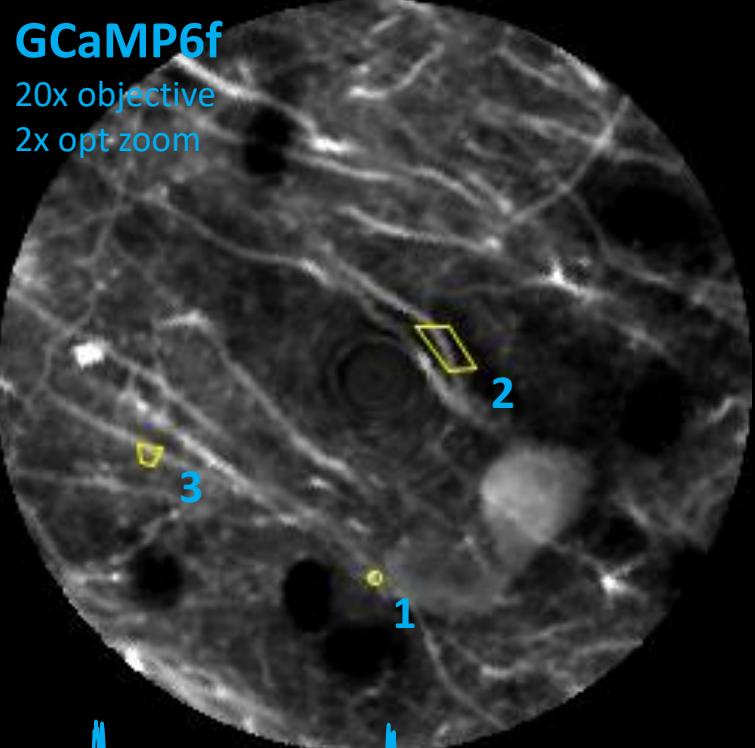
- Cell bodies
- Dendrites



## IntraVentricular Injections

### Two Photon imaging:

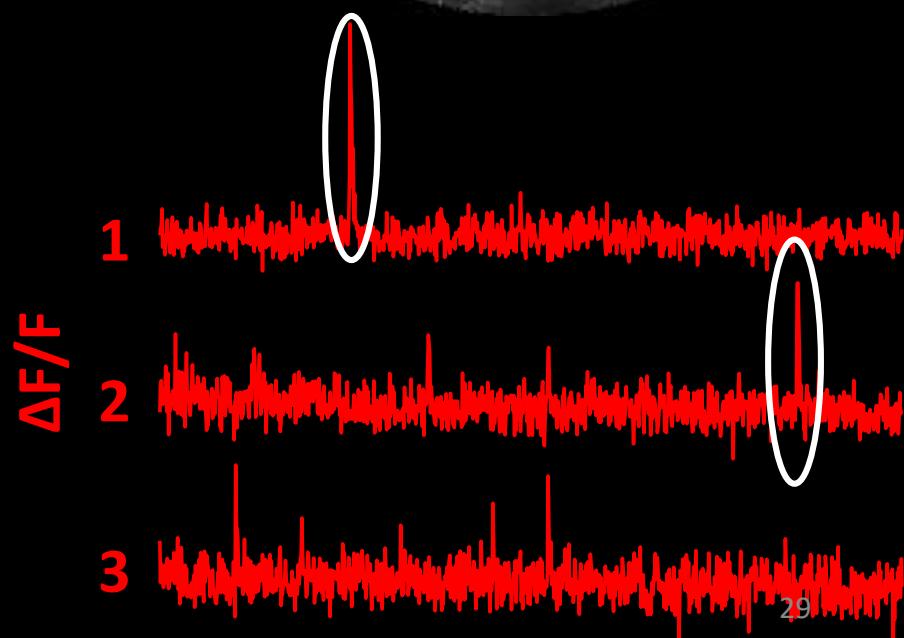
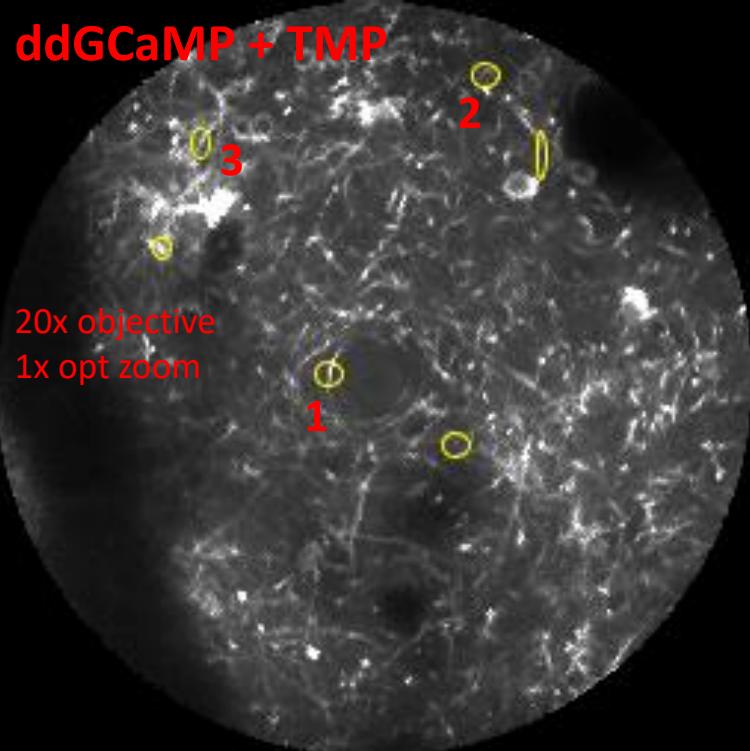
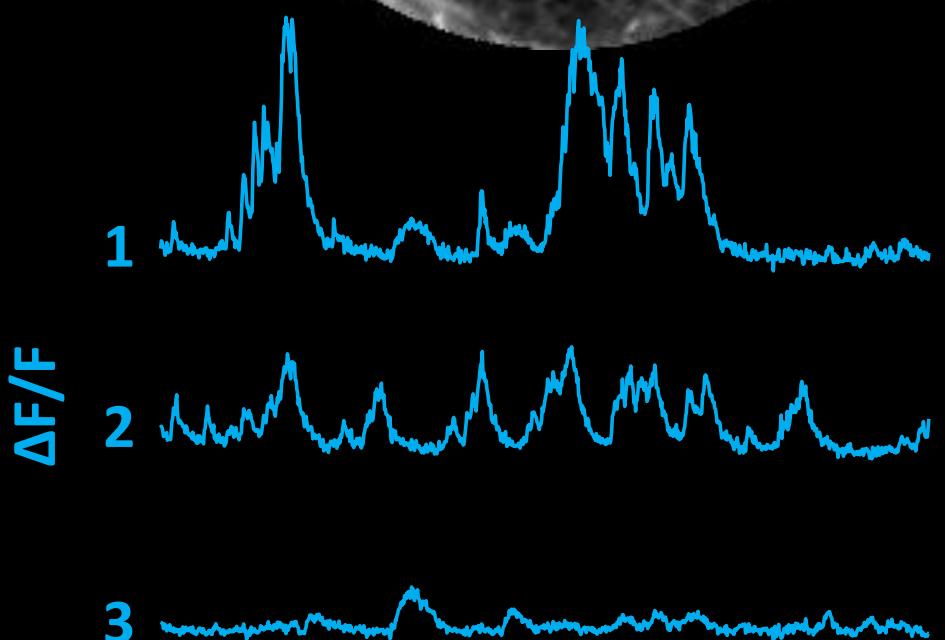
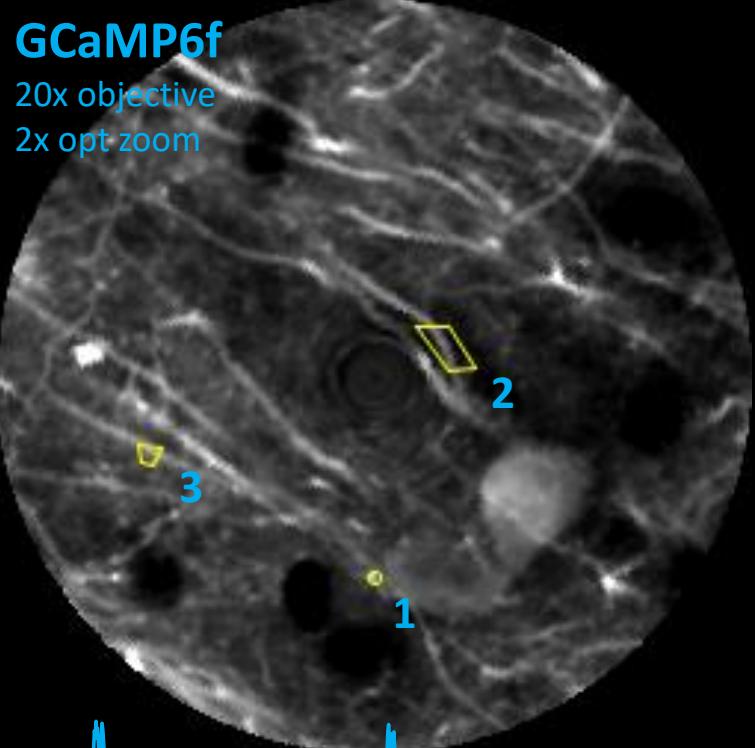
- Cell bodies
- Dendrites



## IntraVentricular Injections

### Two Photon imaging:

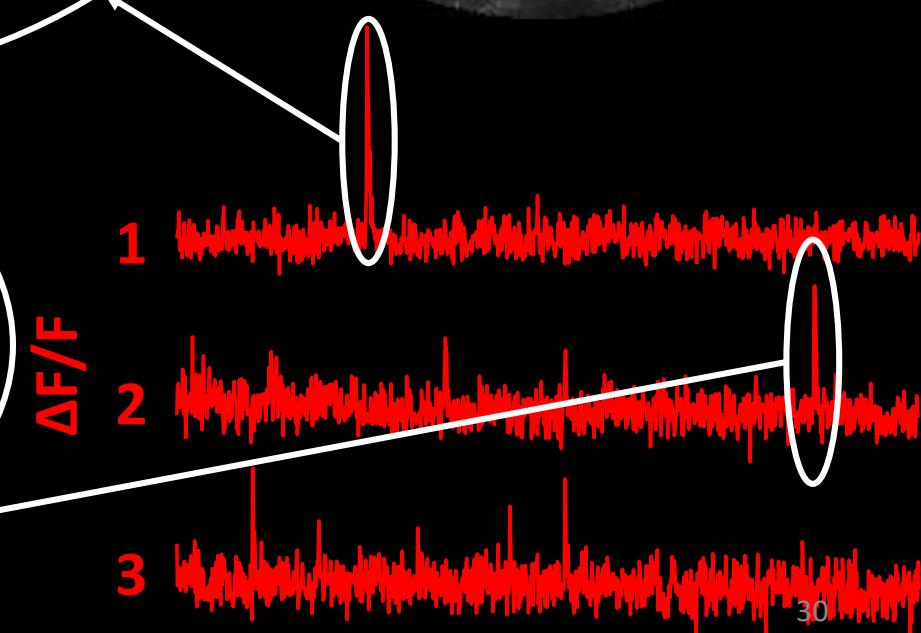
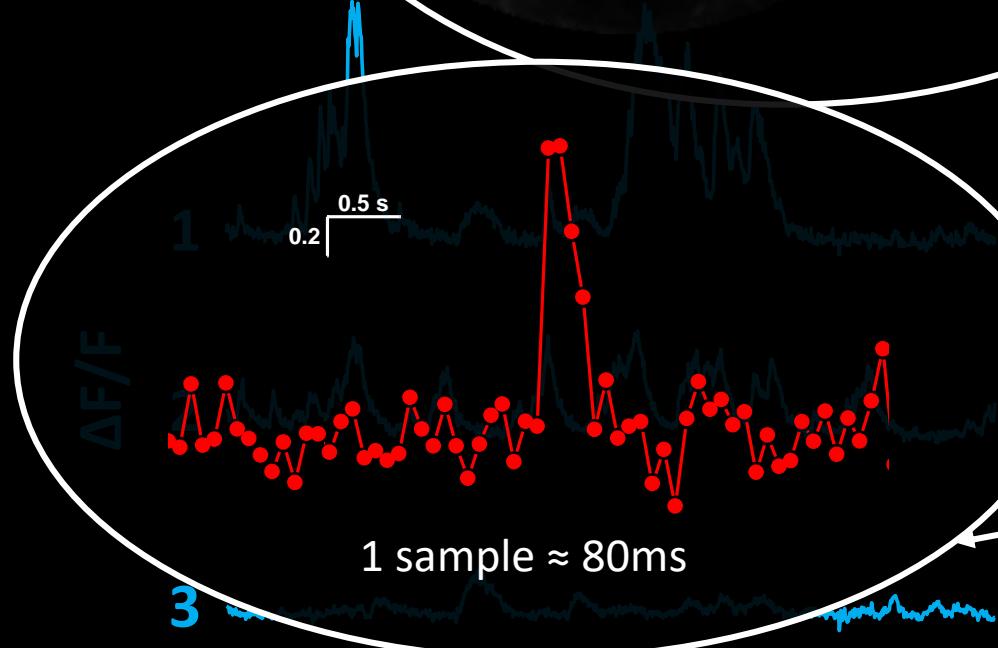
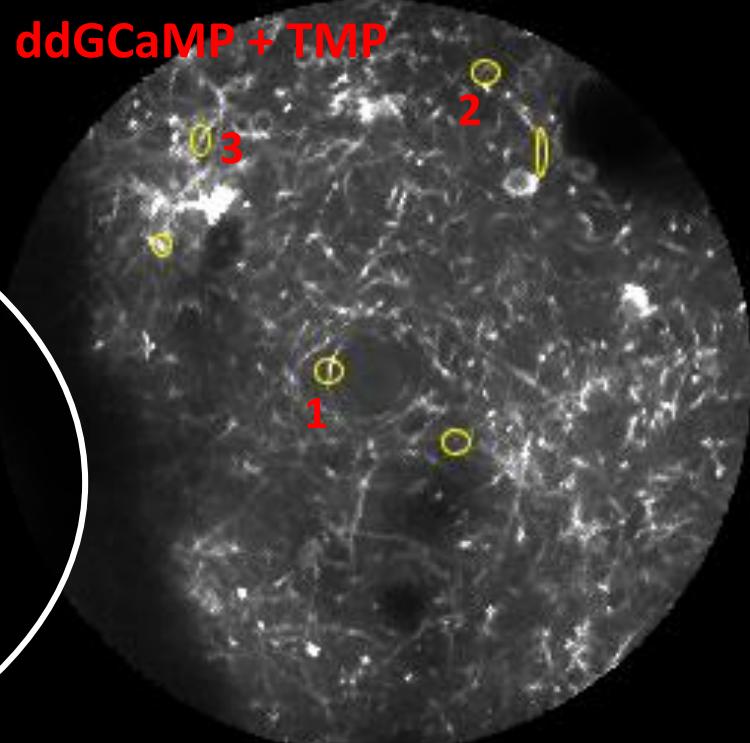
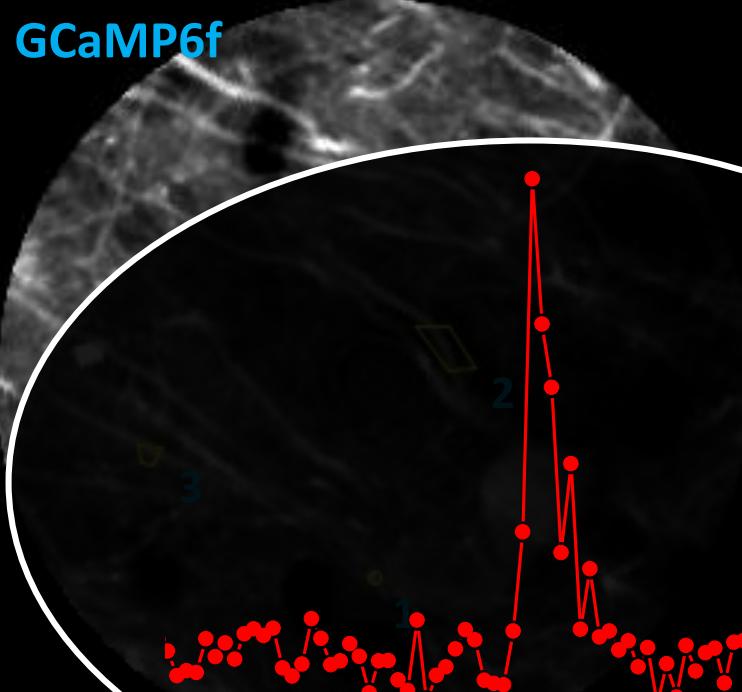
- Cell bodies
- Dendrites



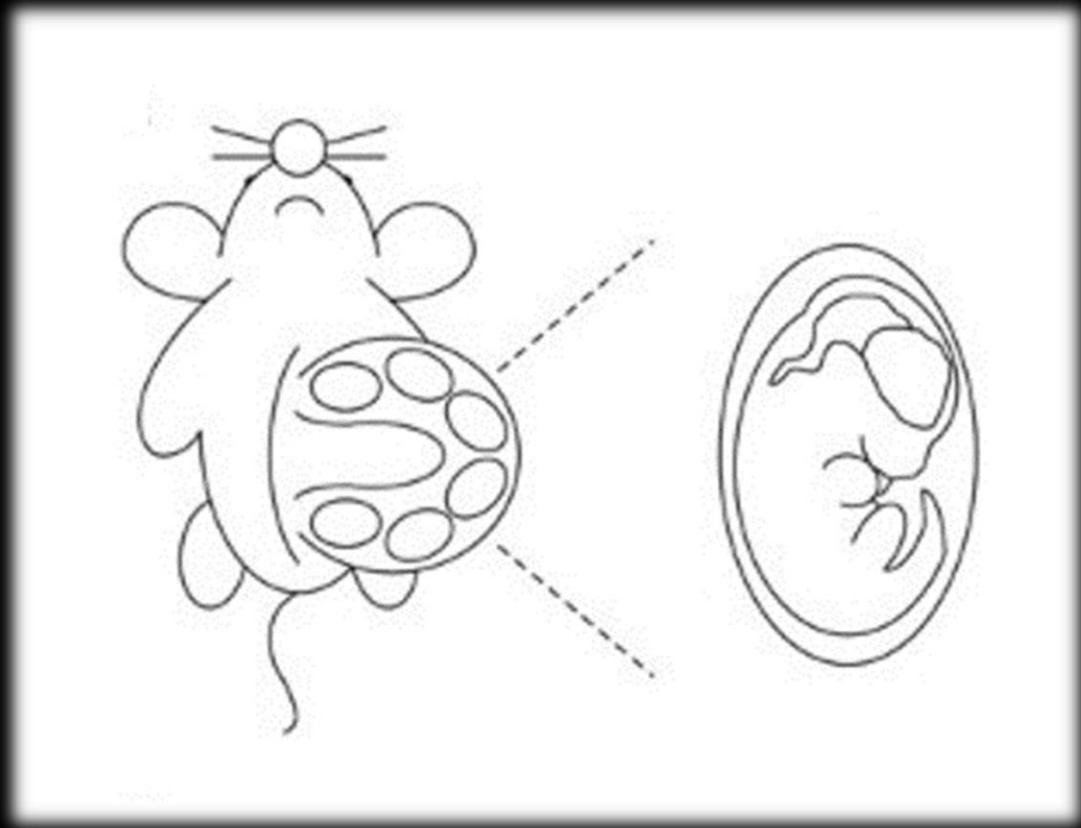
IntraVentricular  
Injections

Two Photon  
imaging:

- Cell bodies
- Dendrites



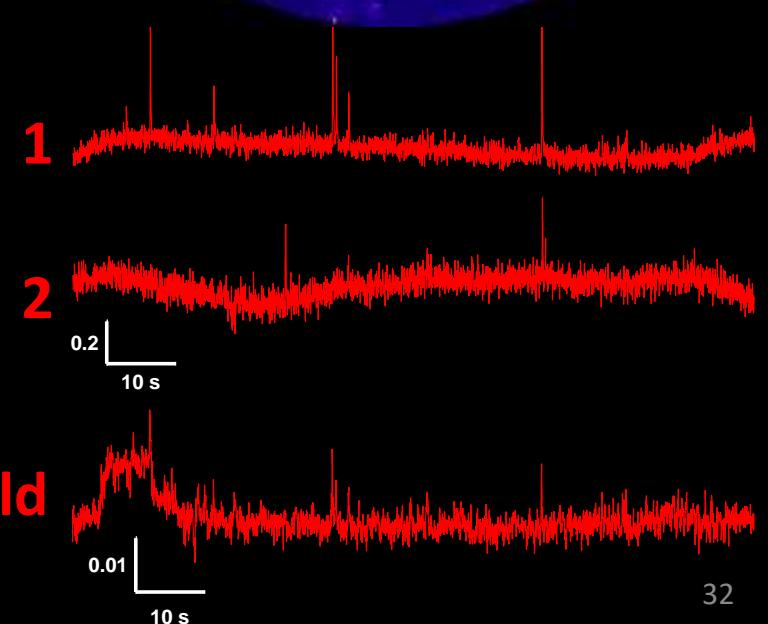
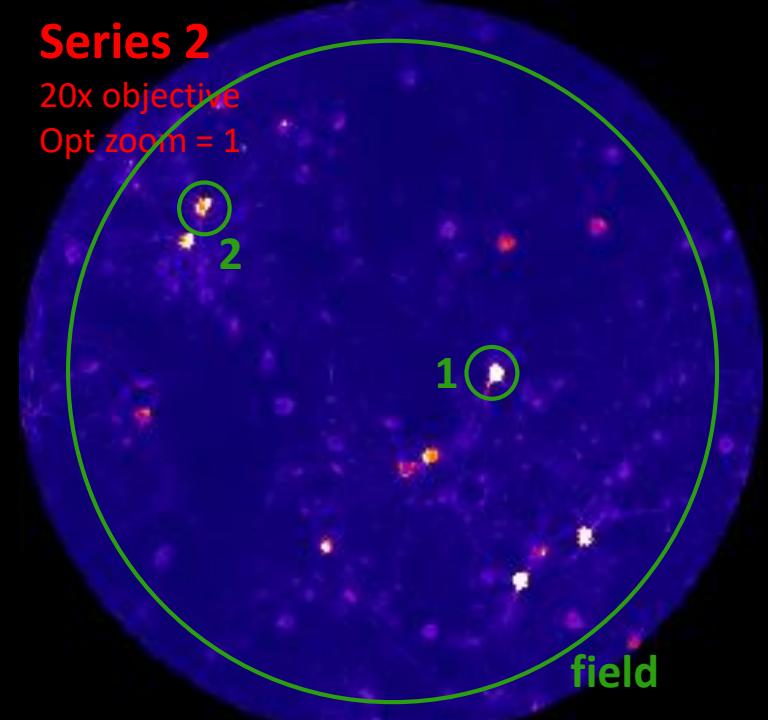
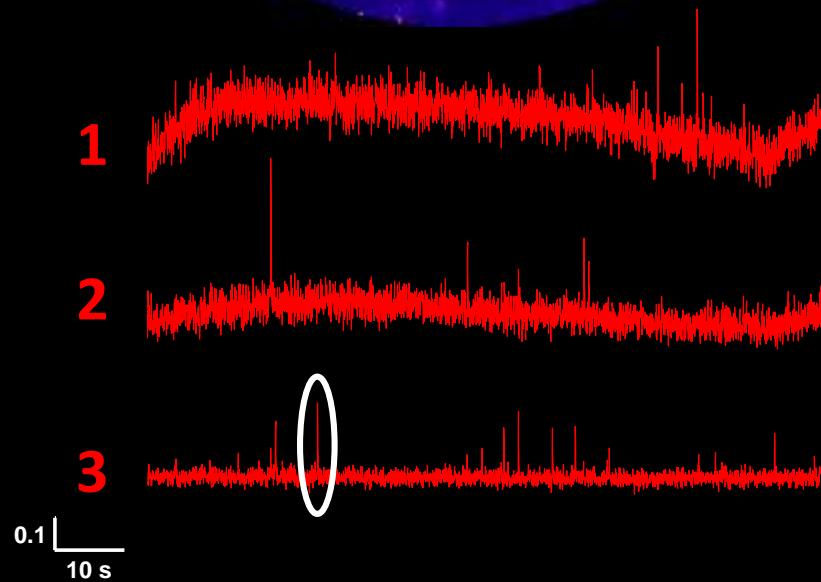
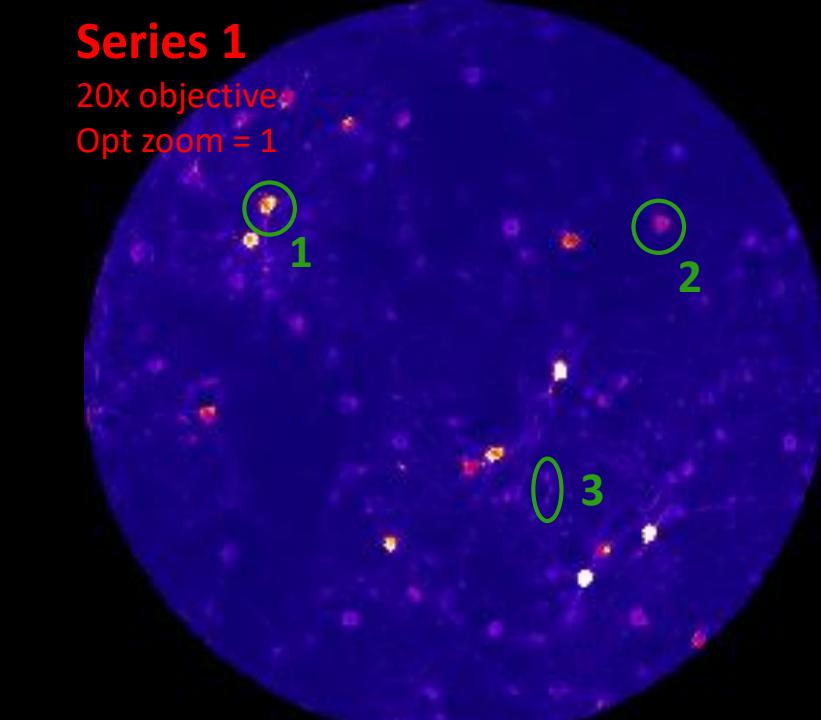
In utero  
electroporation

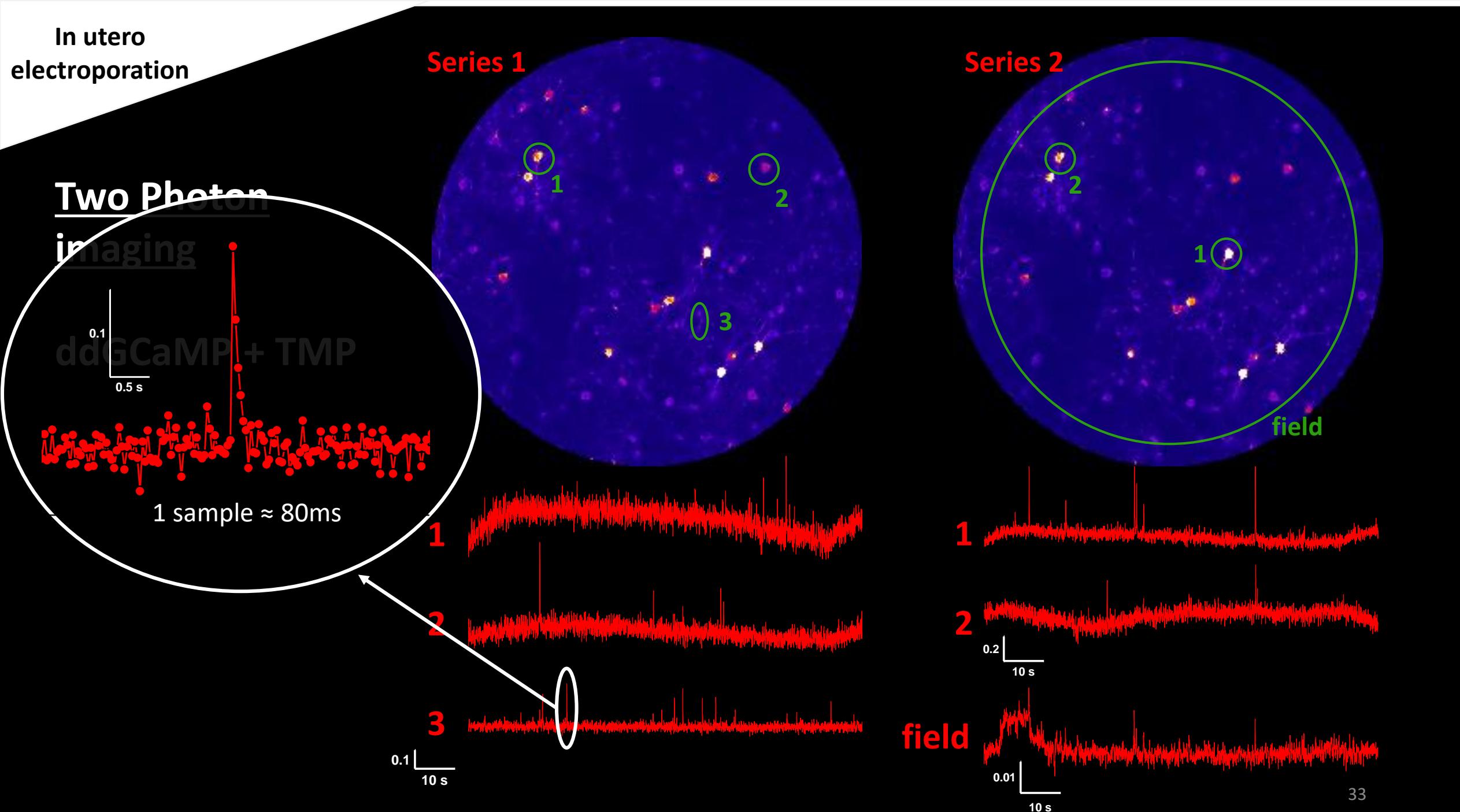


In utero  
electroporation

## Two Photon imaging

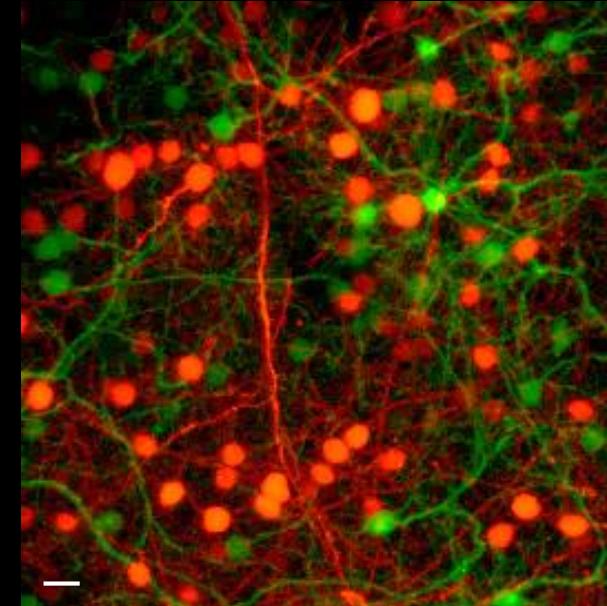
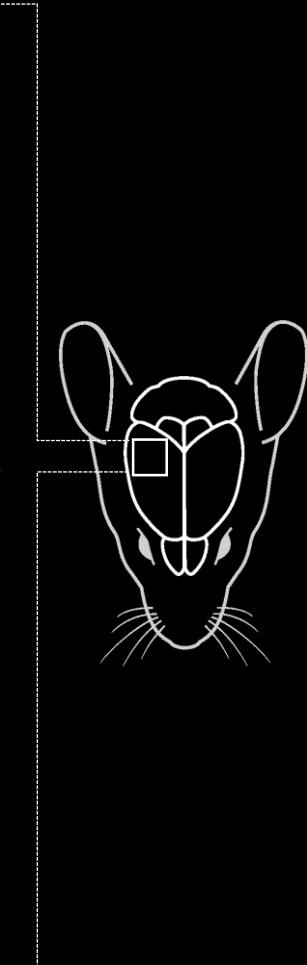
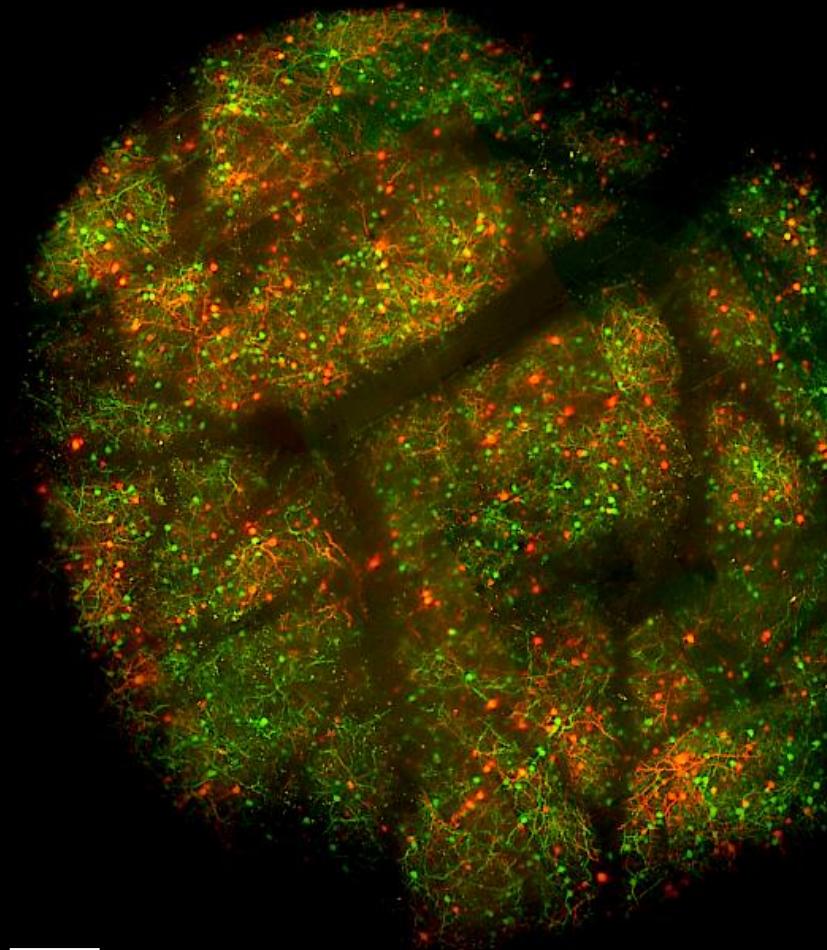
ddGCaMP + TMP



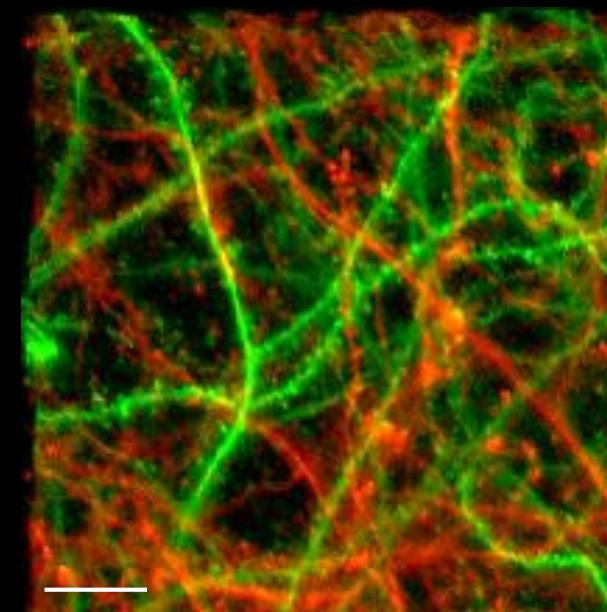


## *In vivo* imaging

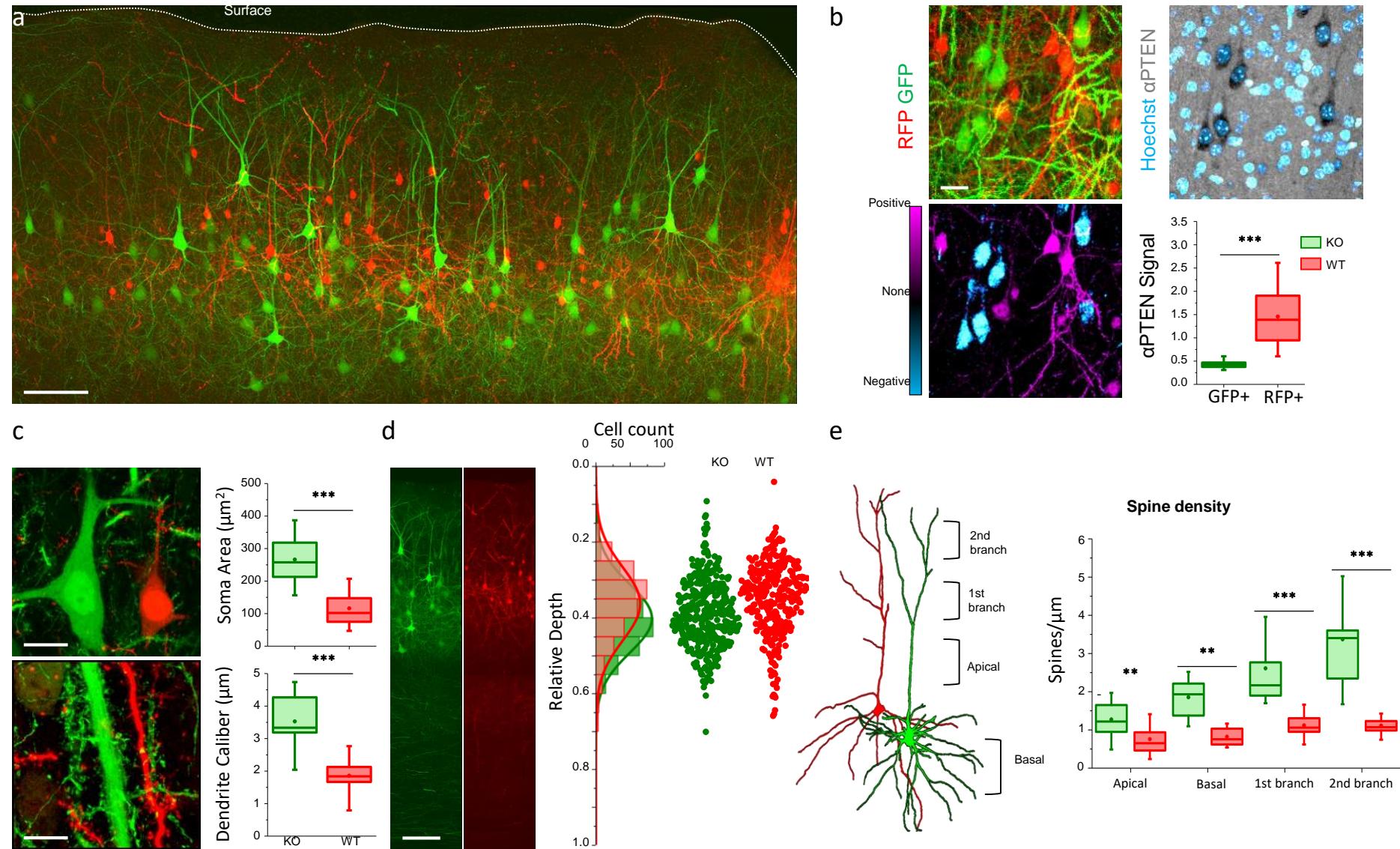
RFP = WT



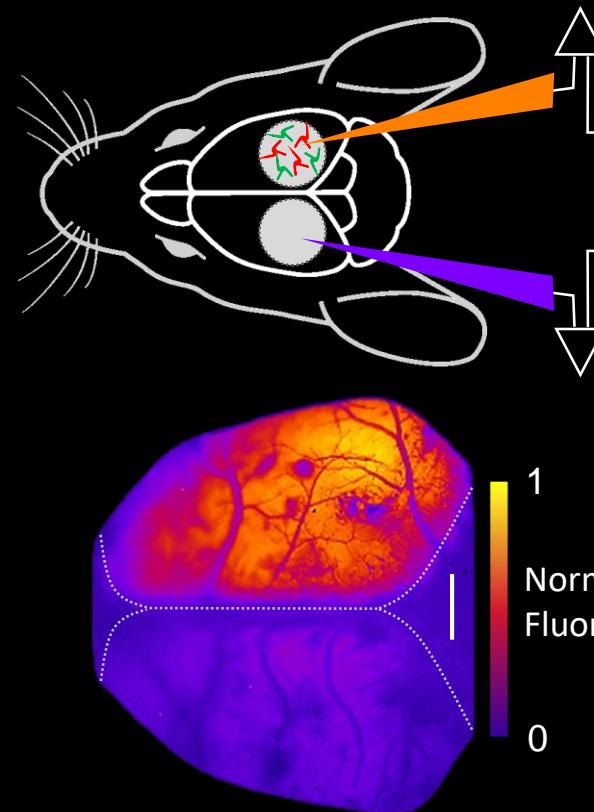
GFP = KO



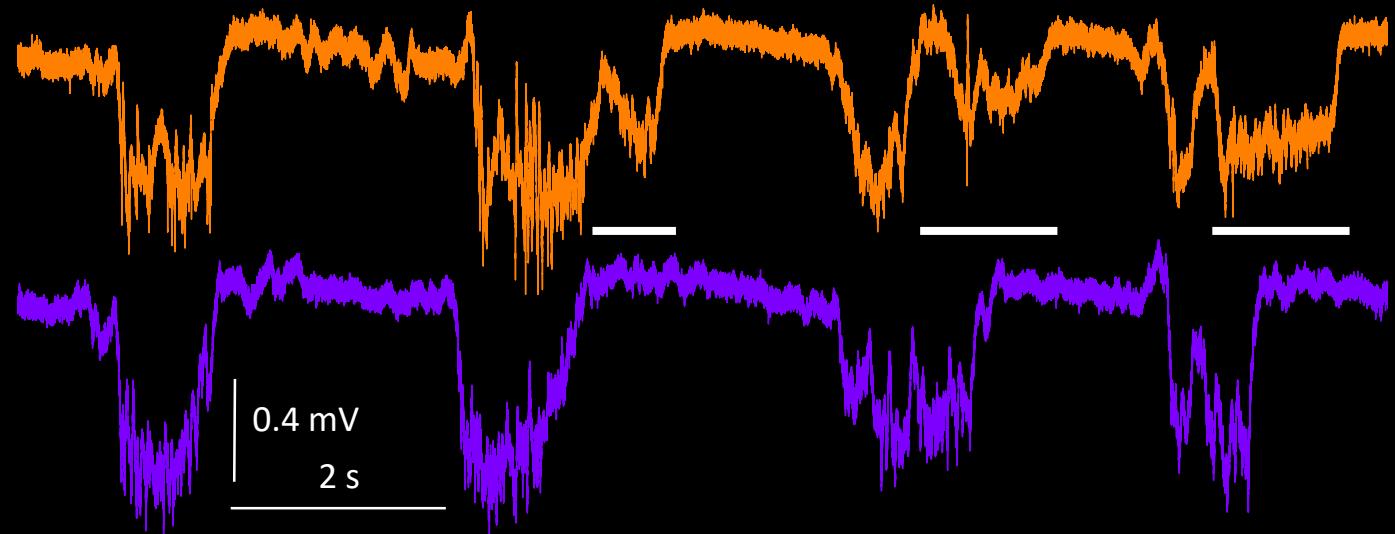
**In vivo physiology:  
PTEN knock out  
mosaic**



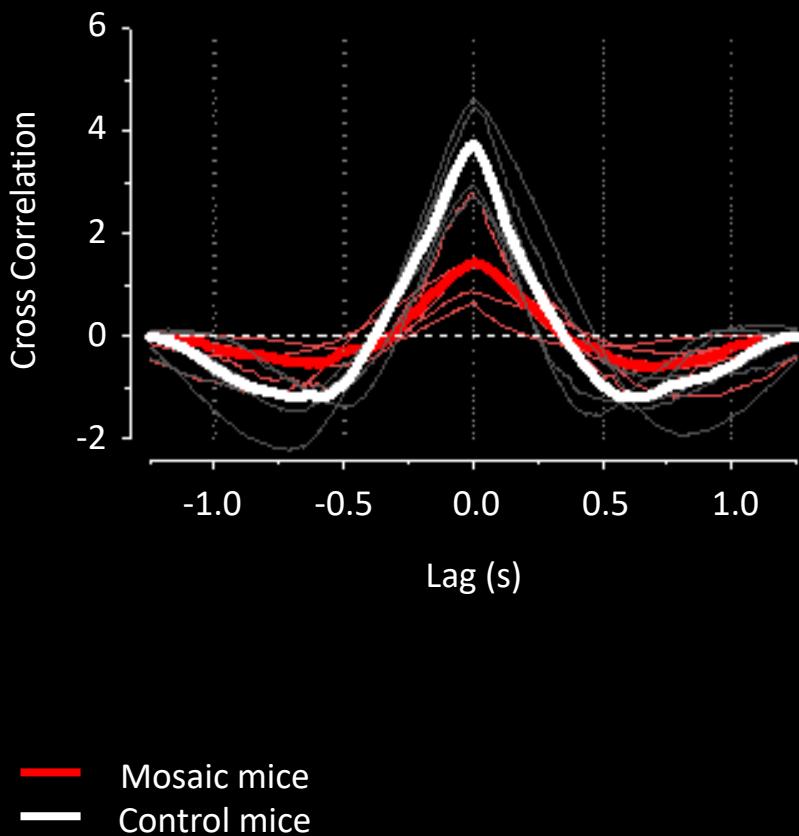
*In vivo* physiology:  
PTEN knock out  
mosaic



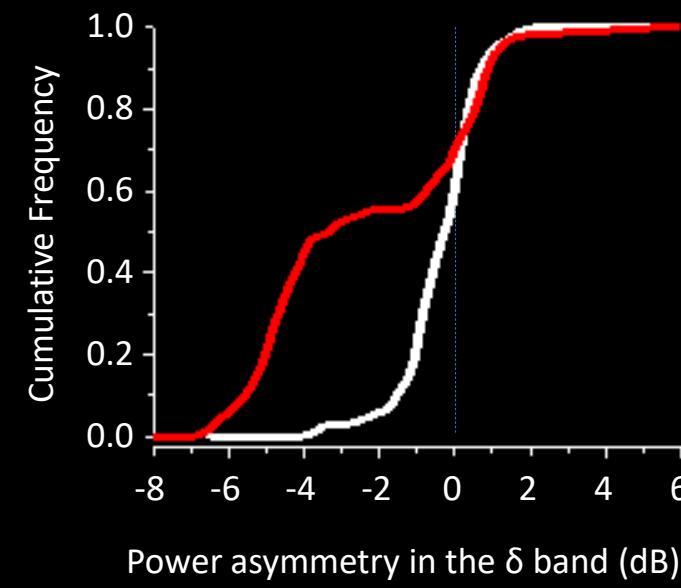
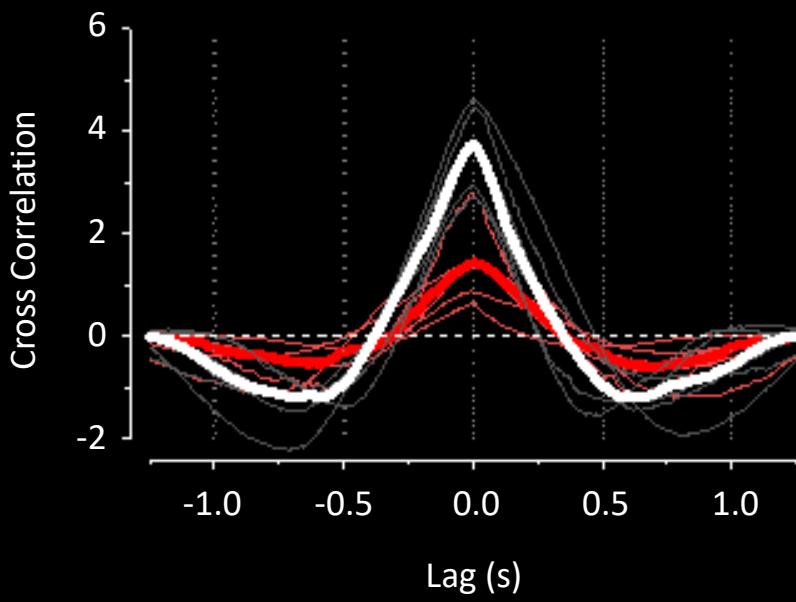
— Mosaic hemisphere  
— Control hemisphere



*In vivo* physiology:  
PTEN knock out  
mosaic

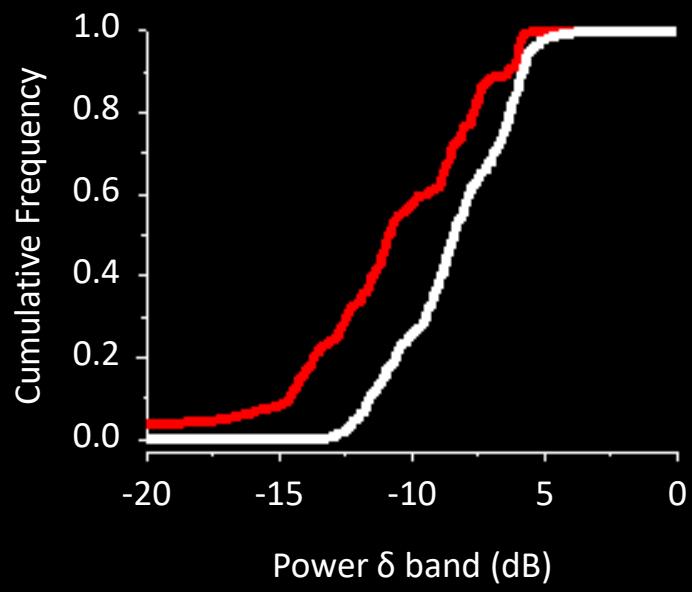
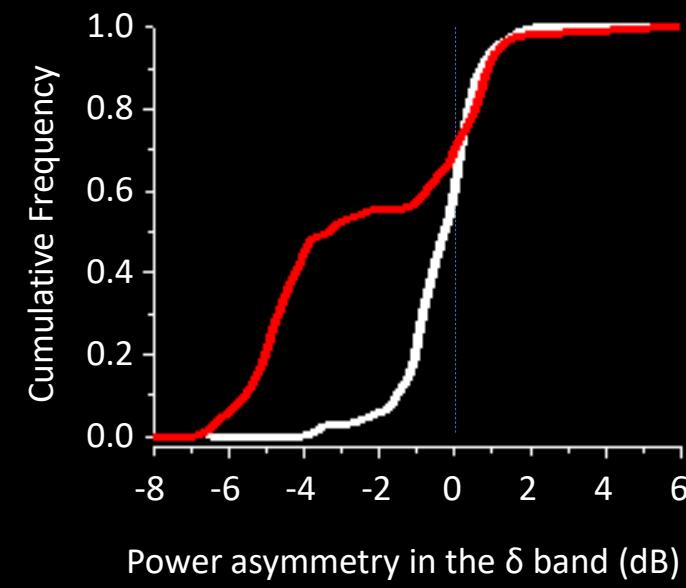
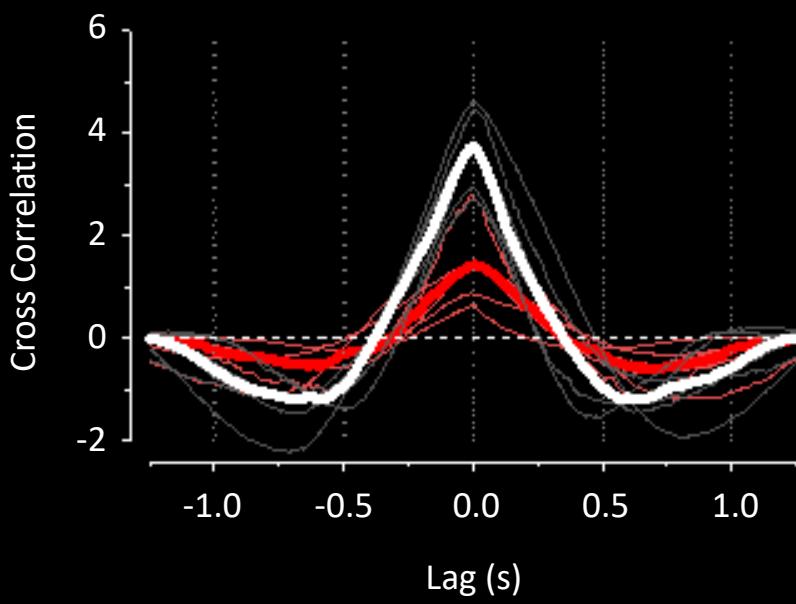


*In vivo* physiology:  
PTEN knock out  
mosaic



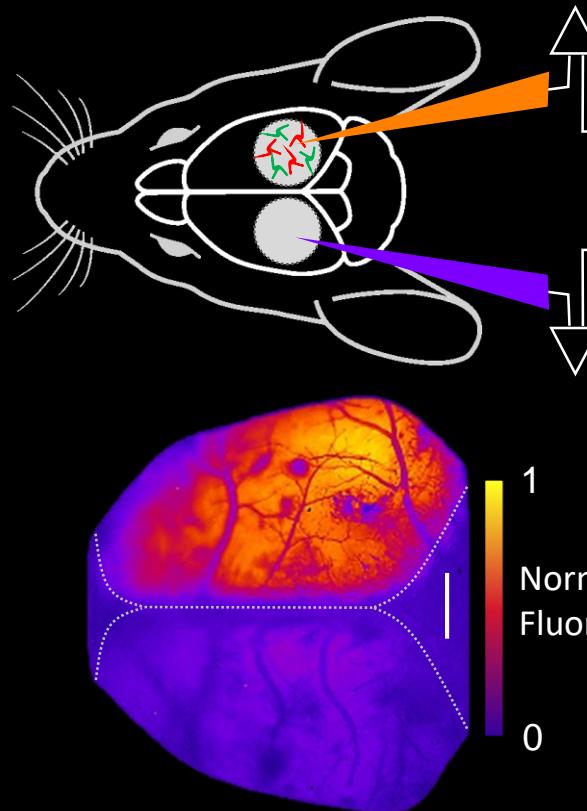
— Mosaic mice  
— Control mice

*In vivo* physiology:  
PTEN knock out  
mosaic

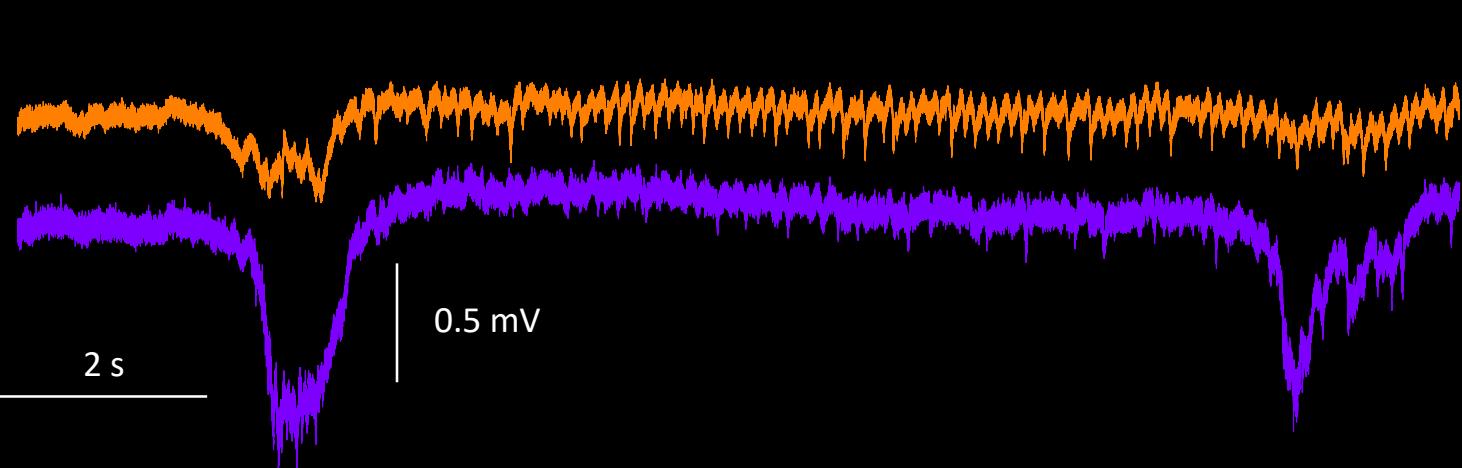


— Mosaic mice  
— Control mice

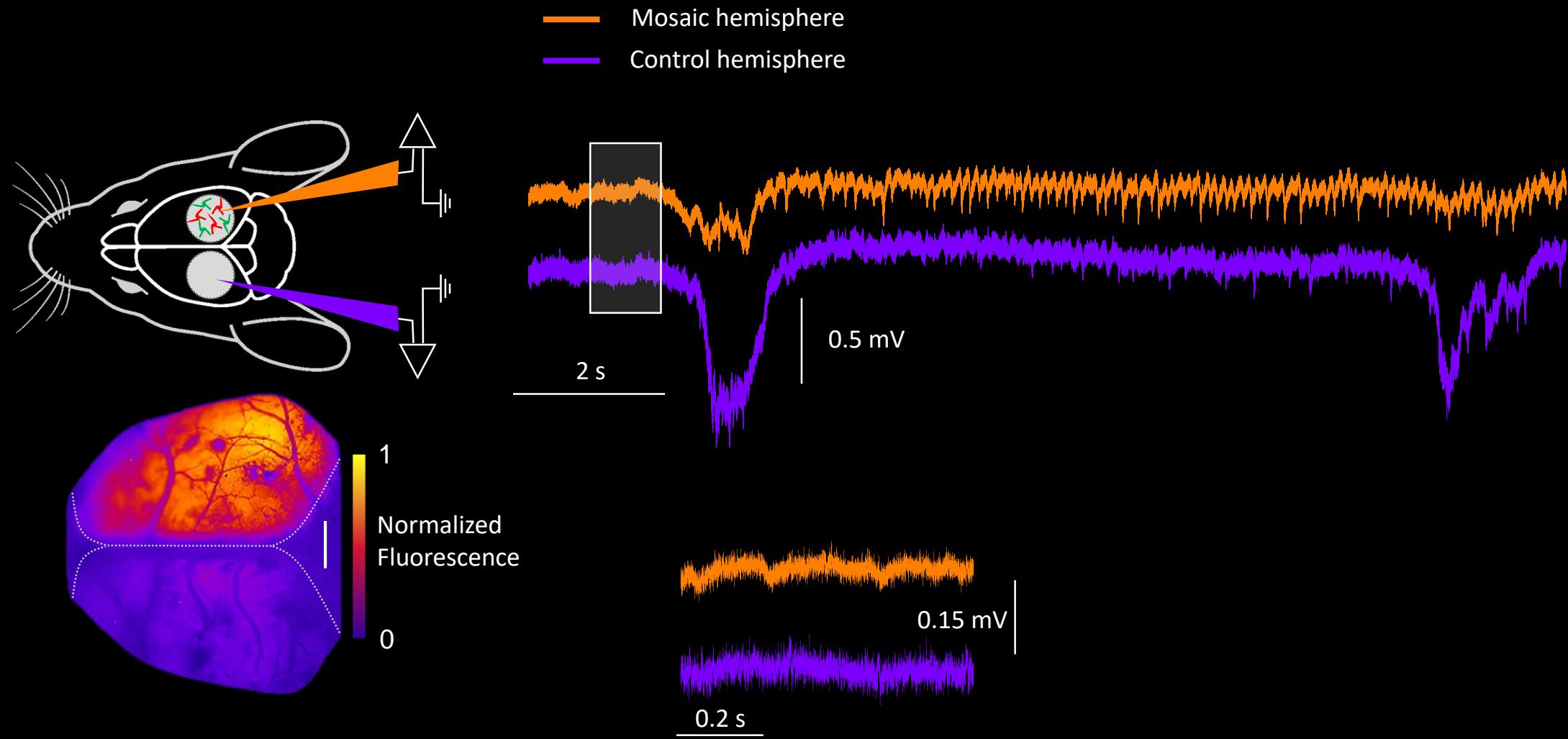
*In vivo* physiology:  
PTEN knock out  
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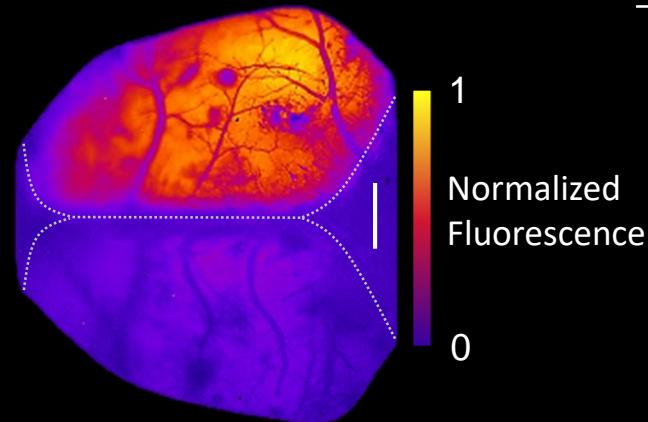
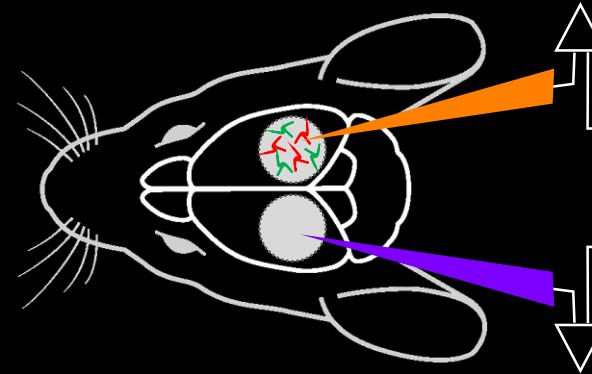
— Mosaic hemisphere  
— Control hemisphere



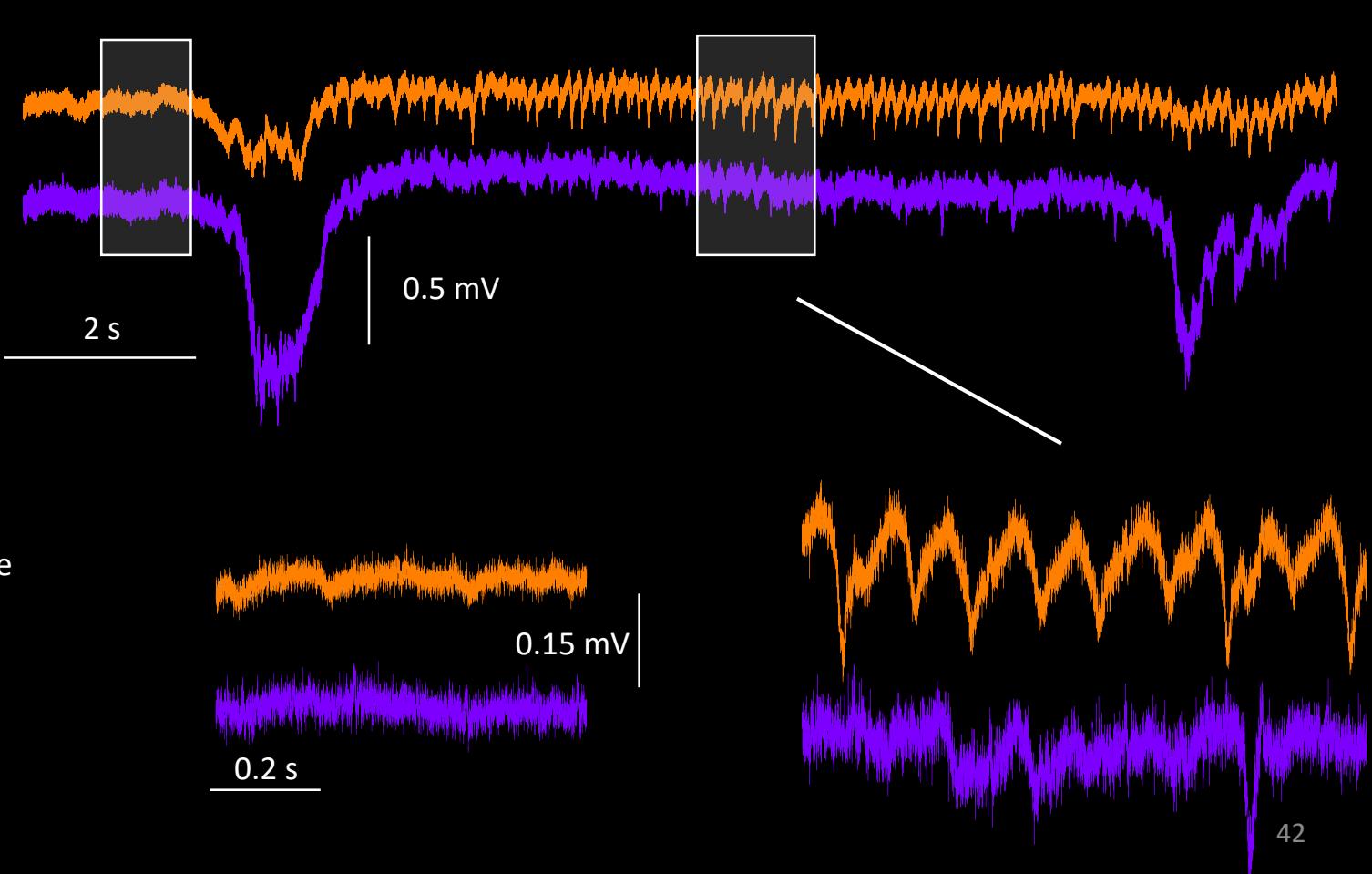
*In vivo* physiology:  
PTEN knock out  
mosaic



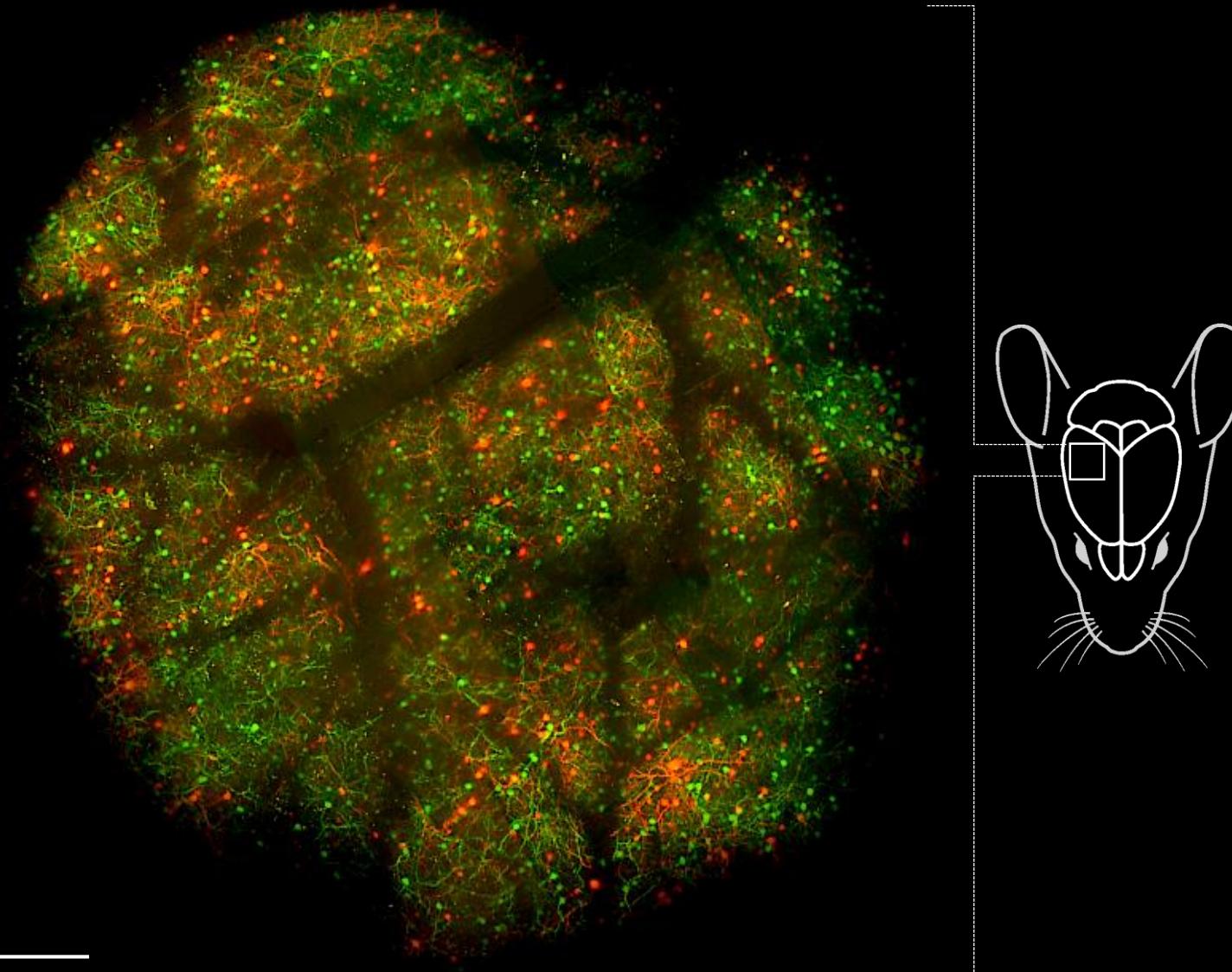
*In vivo* physiology:  
PTEN knock out  
mosaic



— Mosaic hemisphere  
— Control hemisphere



*In vivo* physiology:  
PTEN knock out  
mosaic



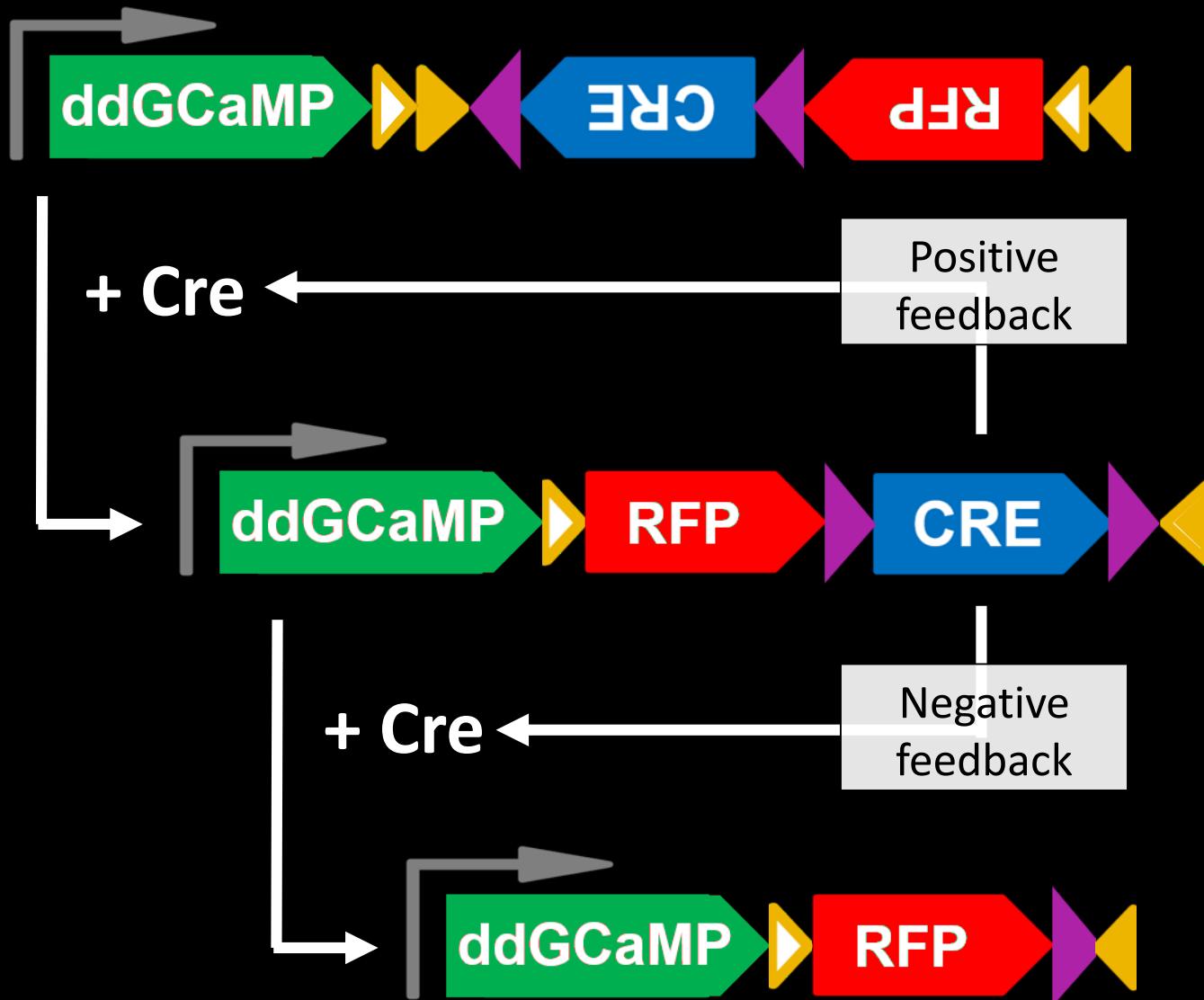
**RFP: KO cells**

**ddGCaMP: all the  
transfected cells**

“Ultimate” tool



“Ultimate” tool



ddGCaMP = WT cell

RFP + ddGCaMP = KO cell

Thanks to:

