NEST
SCUOLA NORMALE SUPERIORE PISA

Passaggio d'anno, II year

PhD course in Nanoscience

Pisa, October 20th 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

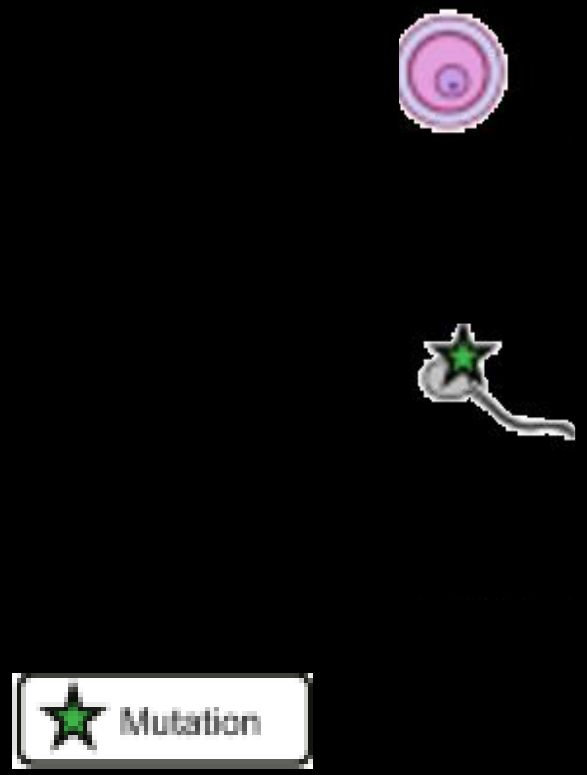
Beatrix **5.0**

2-Photon imaging

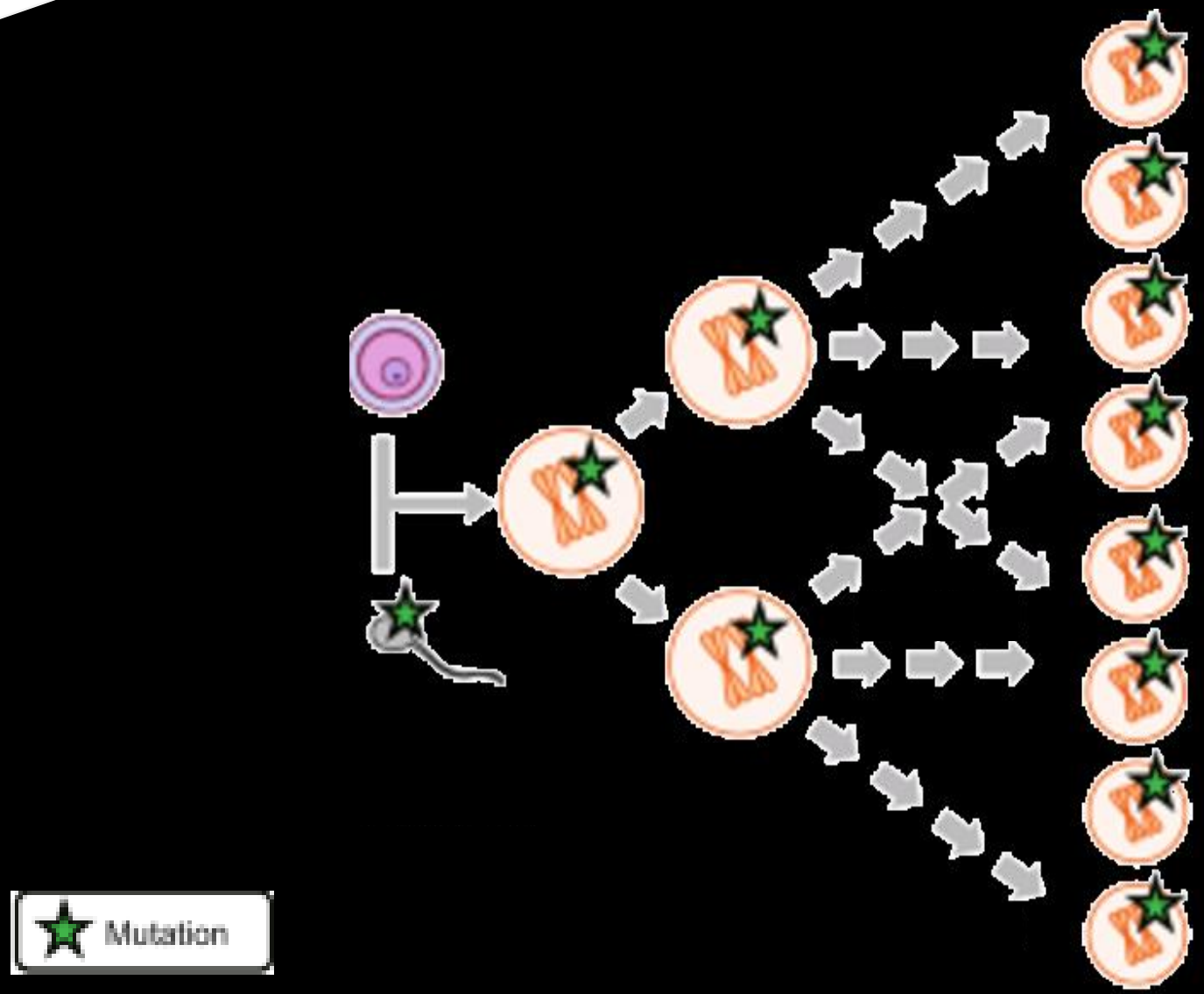
ddGCaMP

Study of mosaicism-based autism models

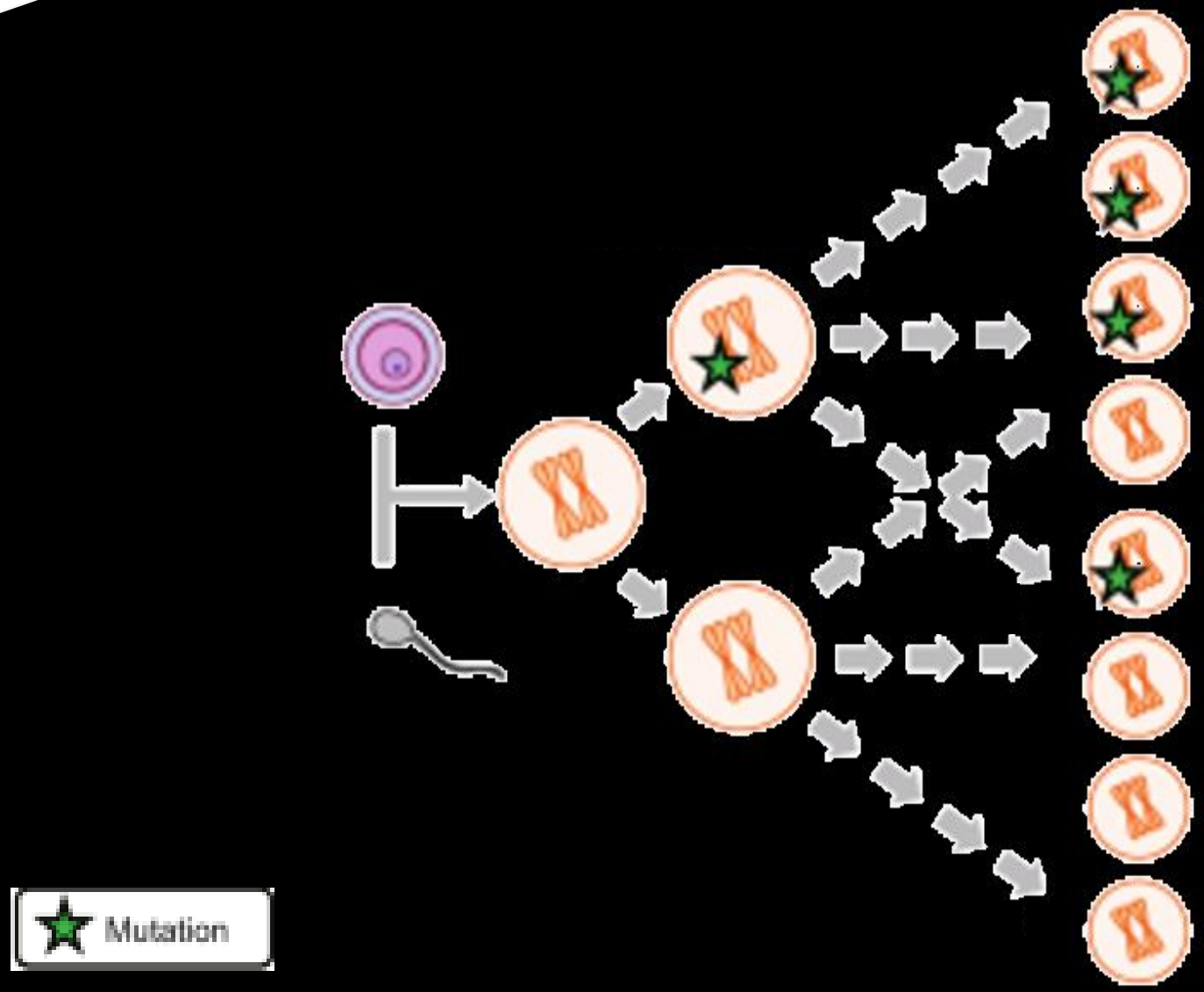
Somatic mutations and mosaicism



Somatic mutations and mosaicism



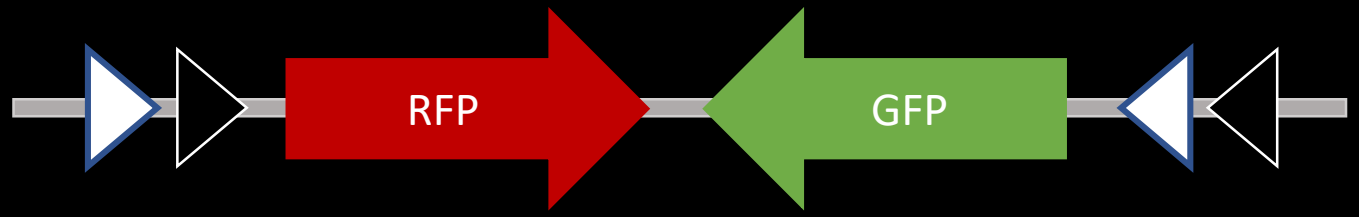
Somatic mutations and mosaicism



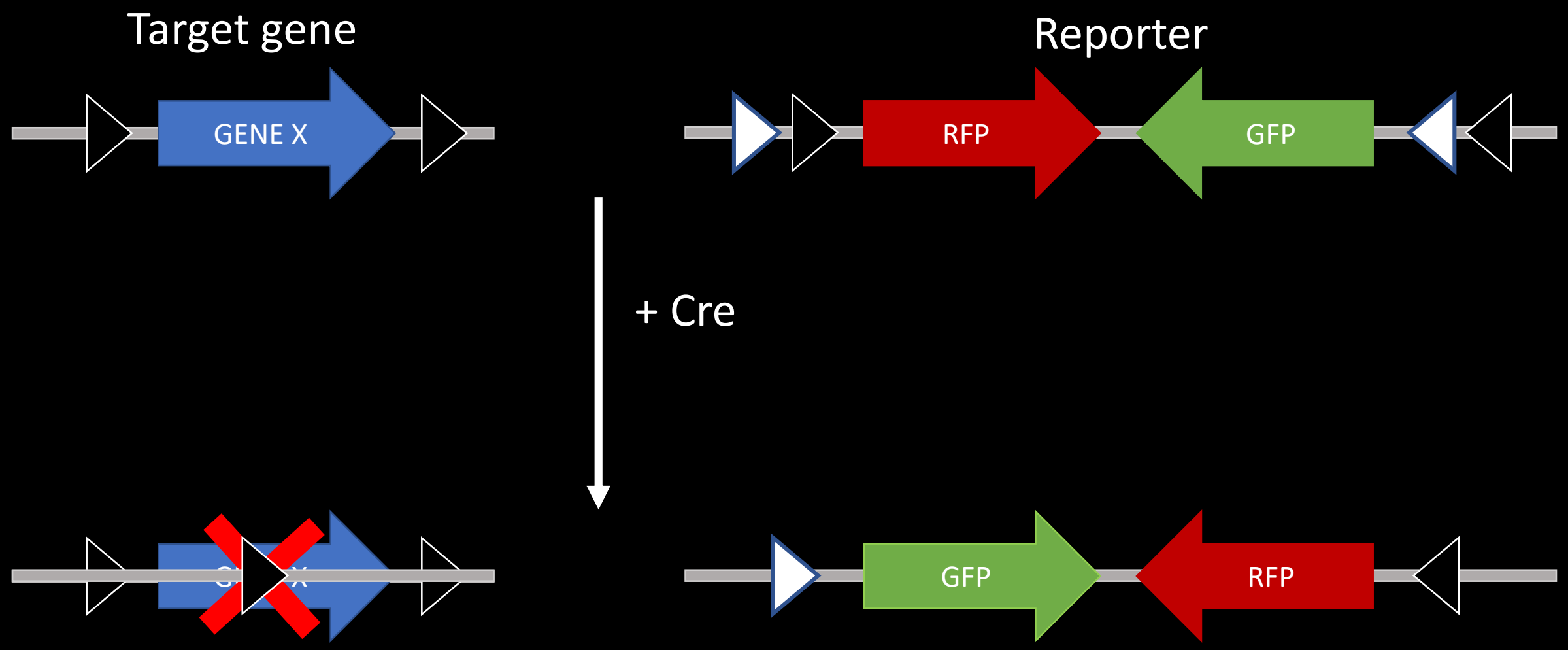
KO reporting

Target gene

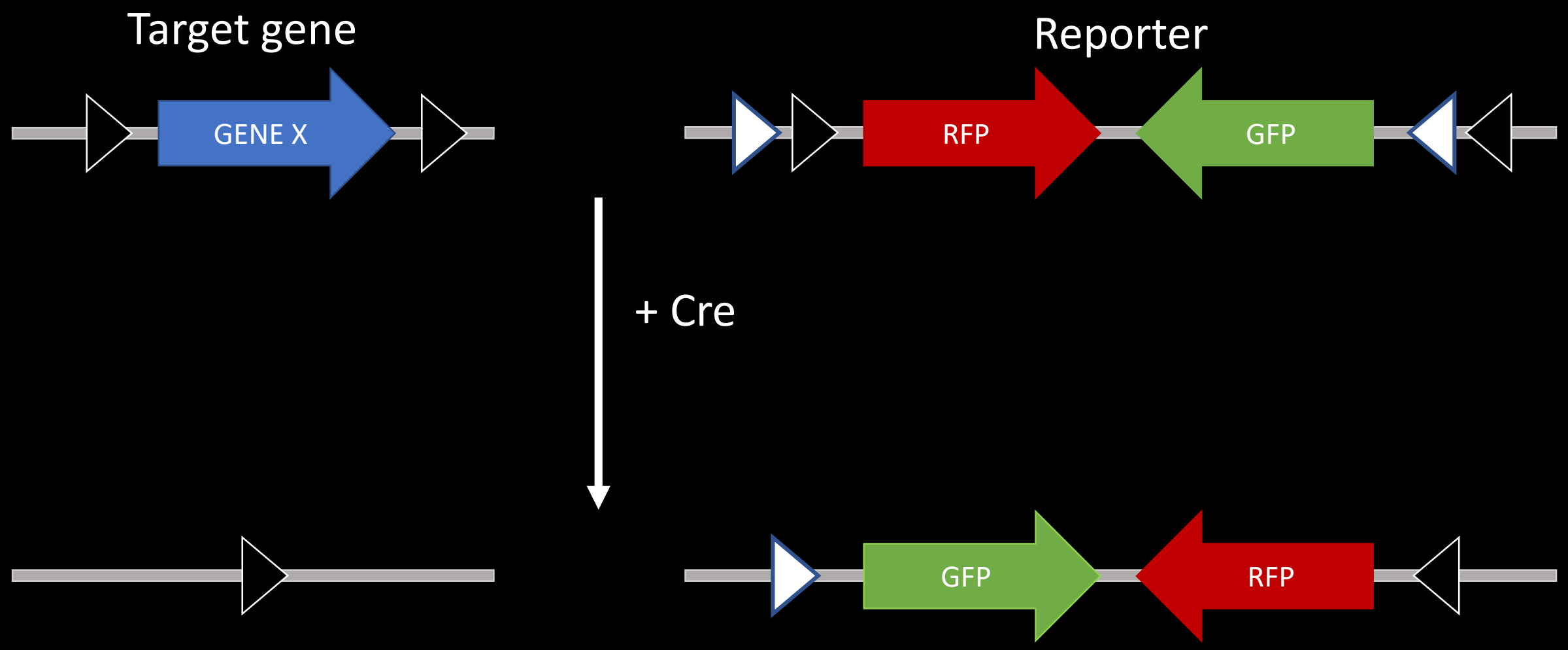
Reporter



KO reporting

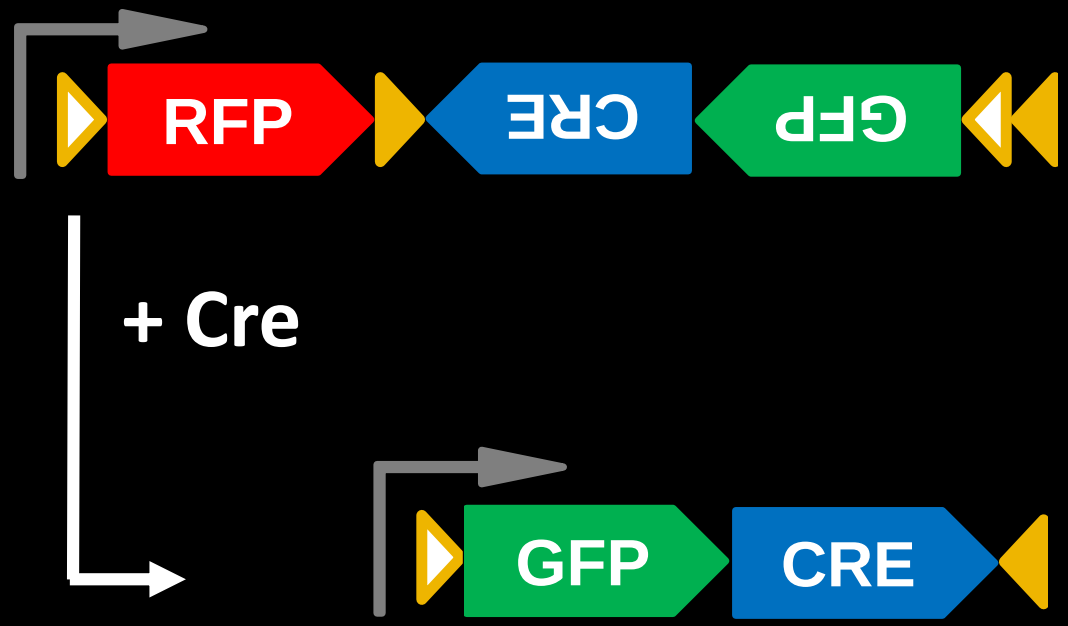


KO reporting

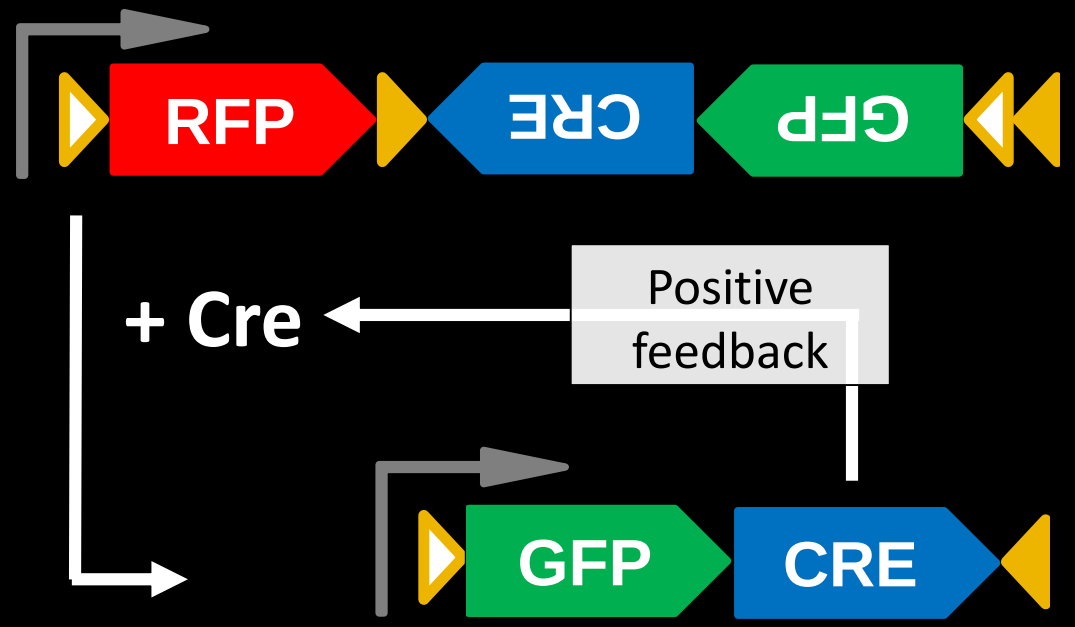


Beatrix
how it works

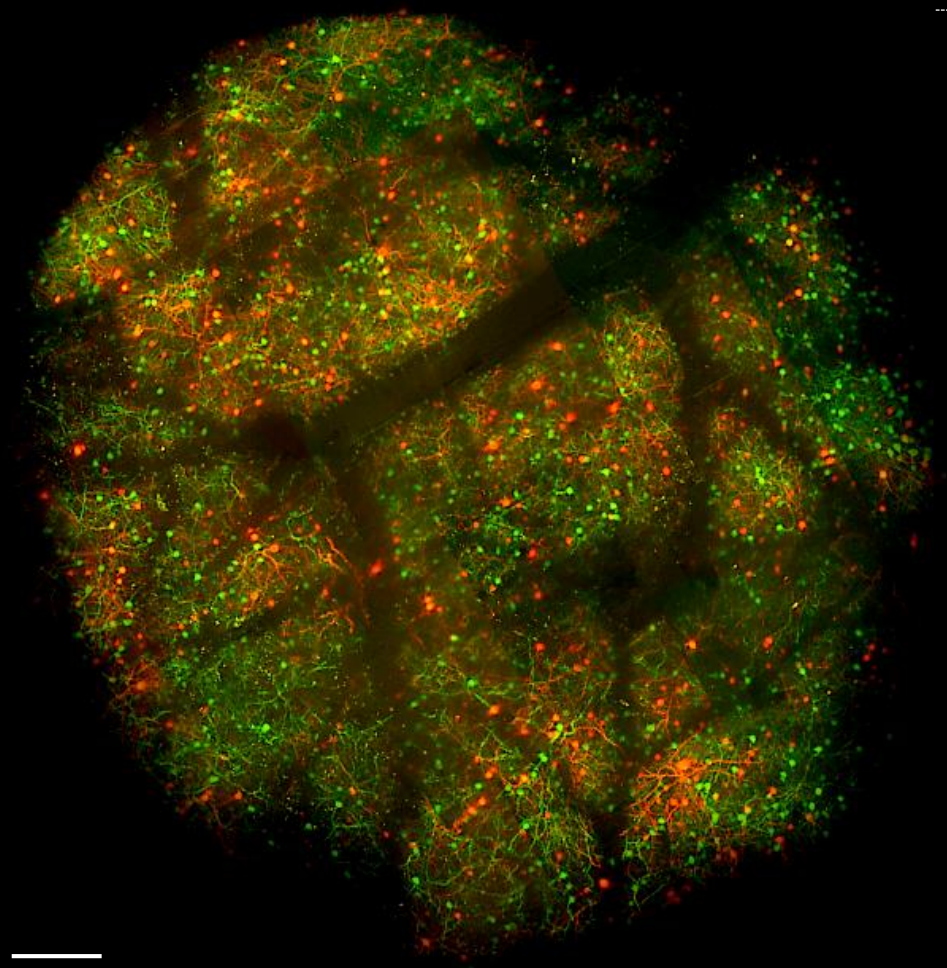
Beatrix
how it works

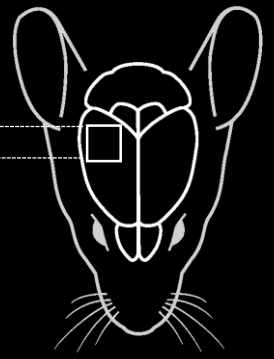


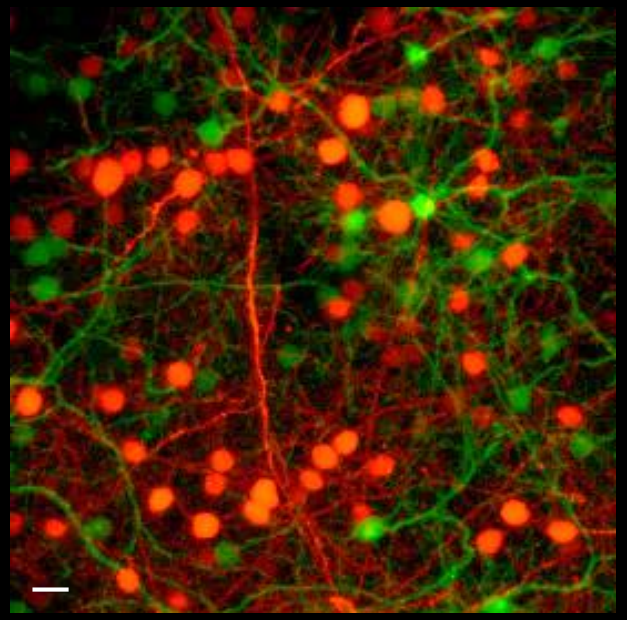
Beatrix
how it works

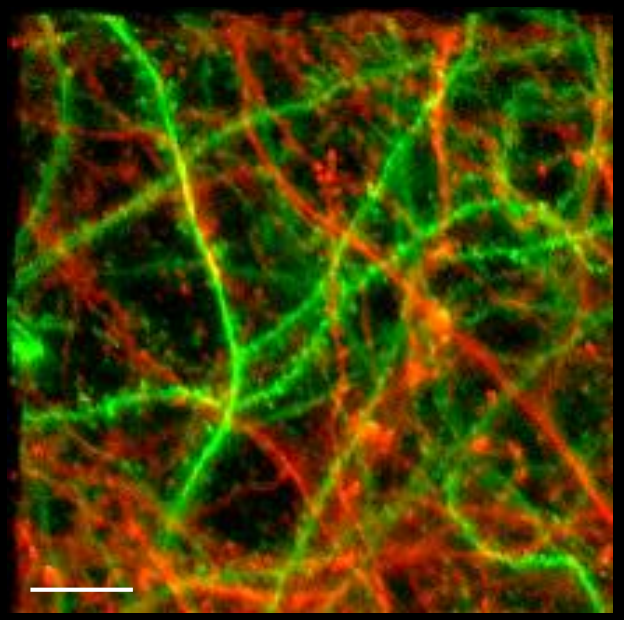


In vivo imaging

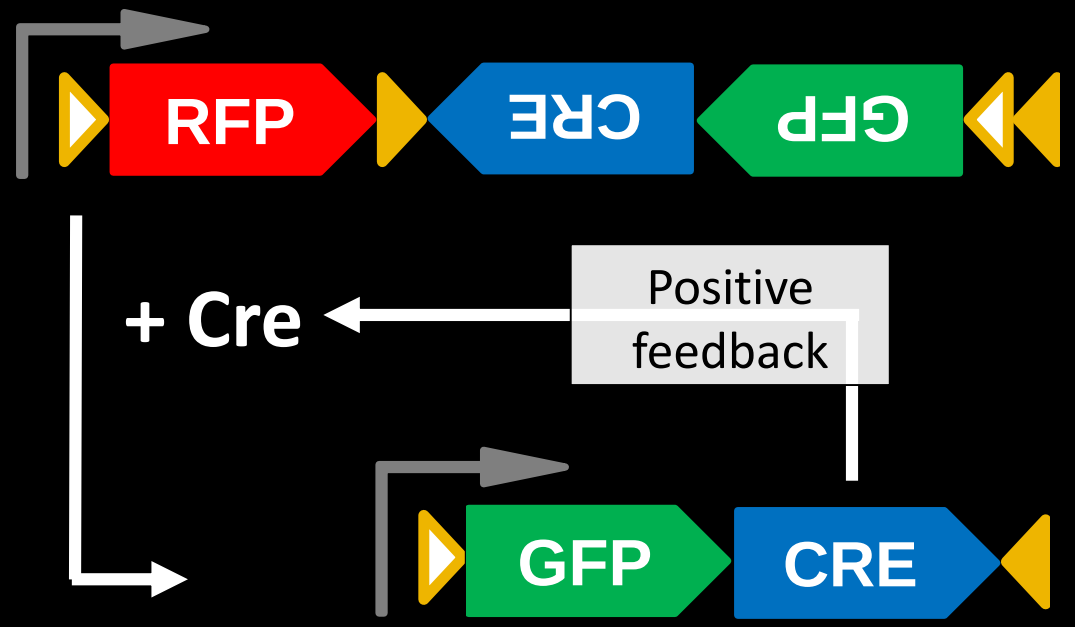


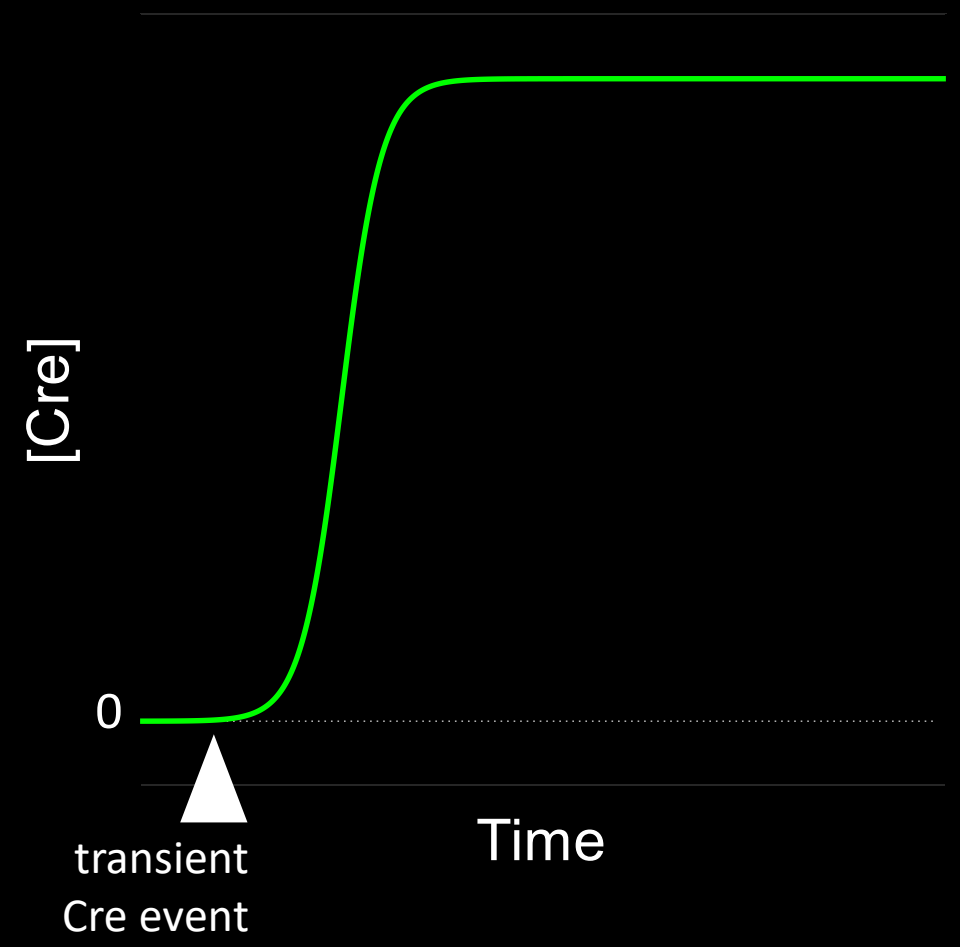




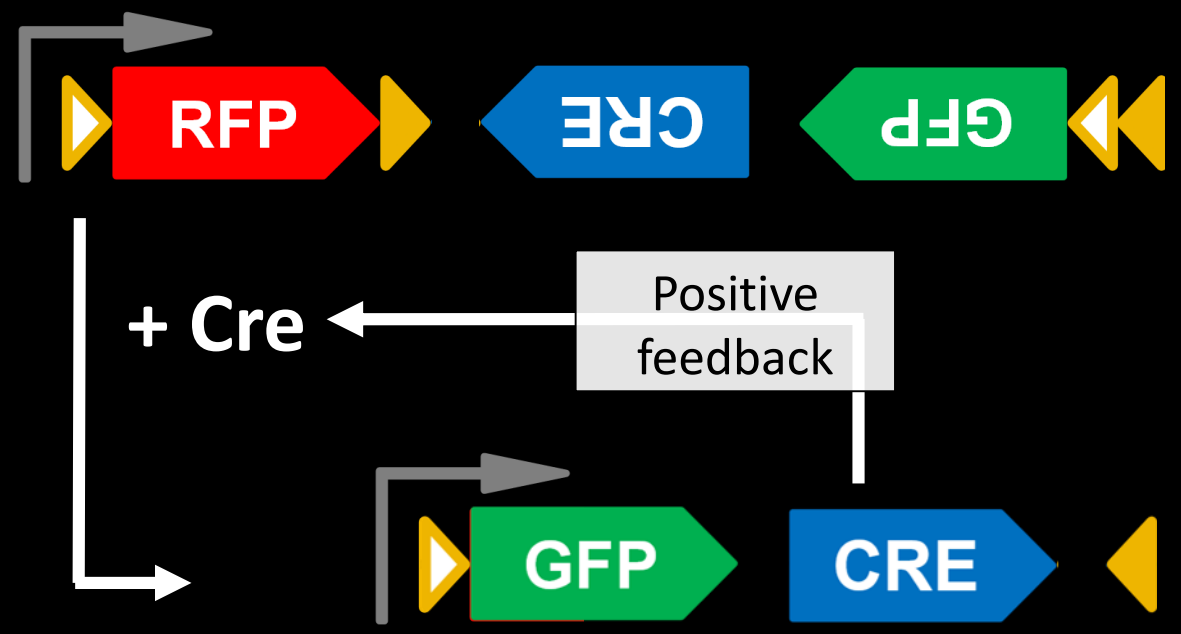


Beatrix


RFP
CRE
GFP
+ Cre
Positive feedback
GFP
CRE

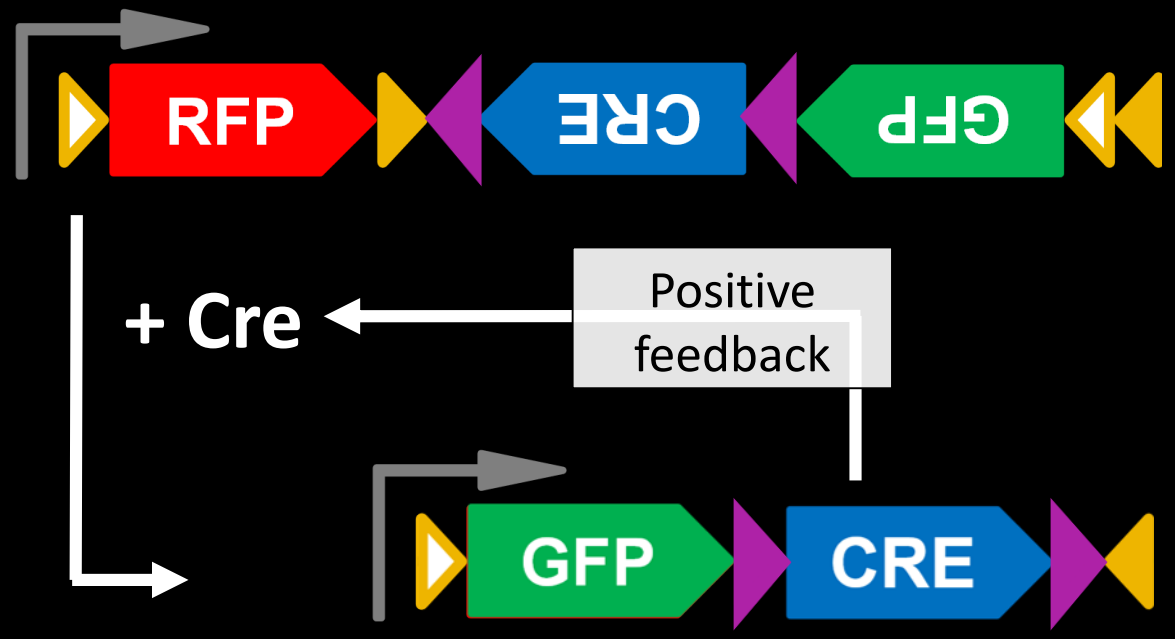

[Cre]
0
transient Cre event
Time

Beatrix

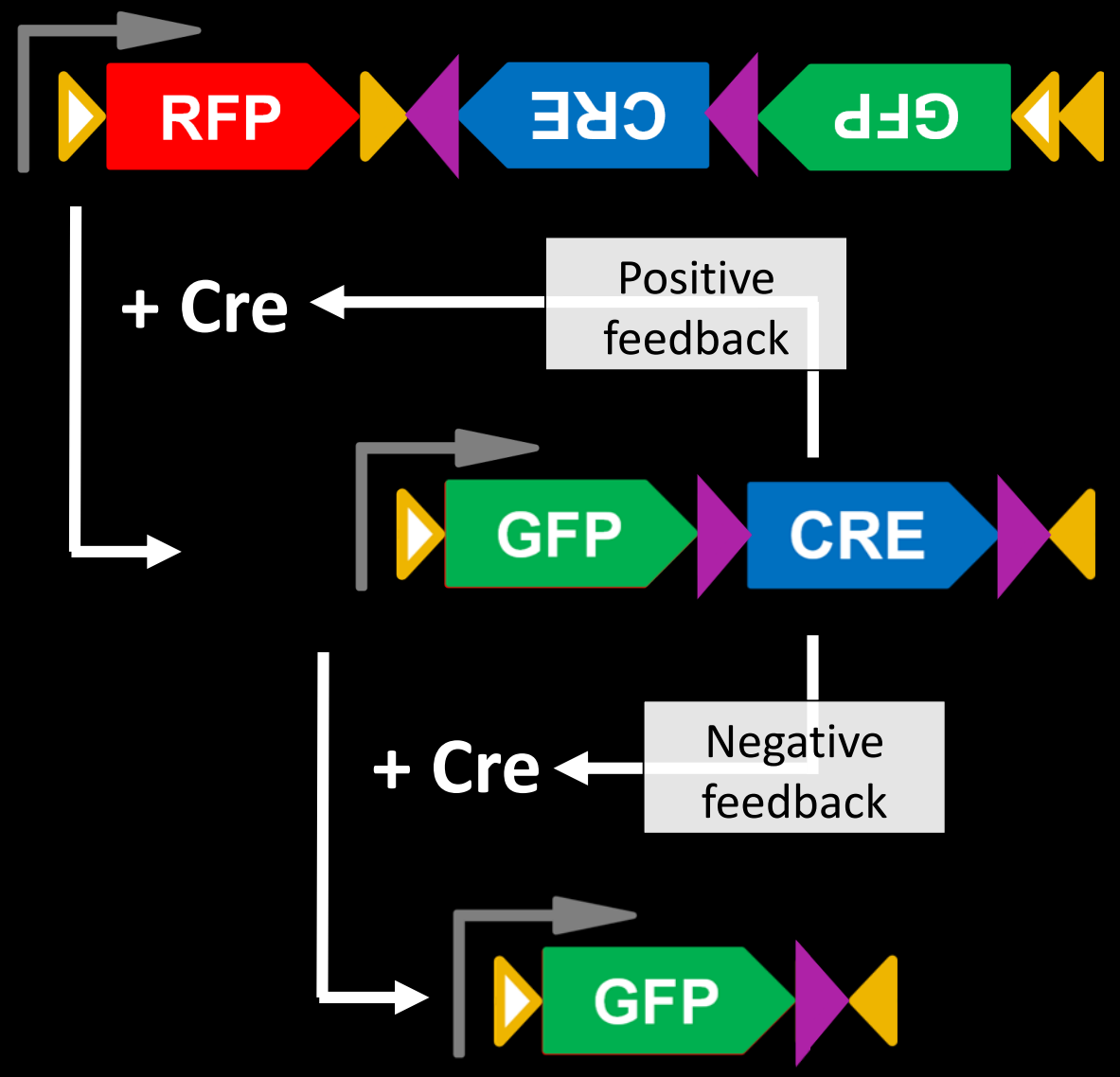


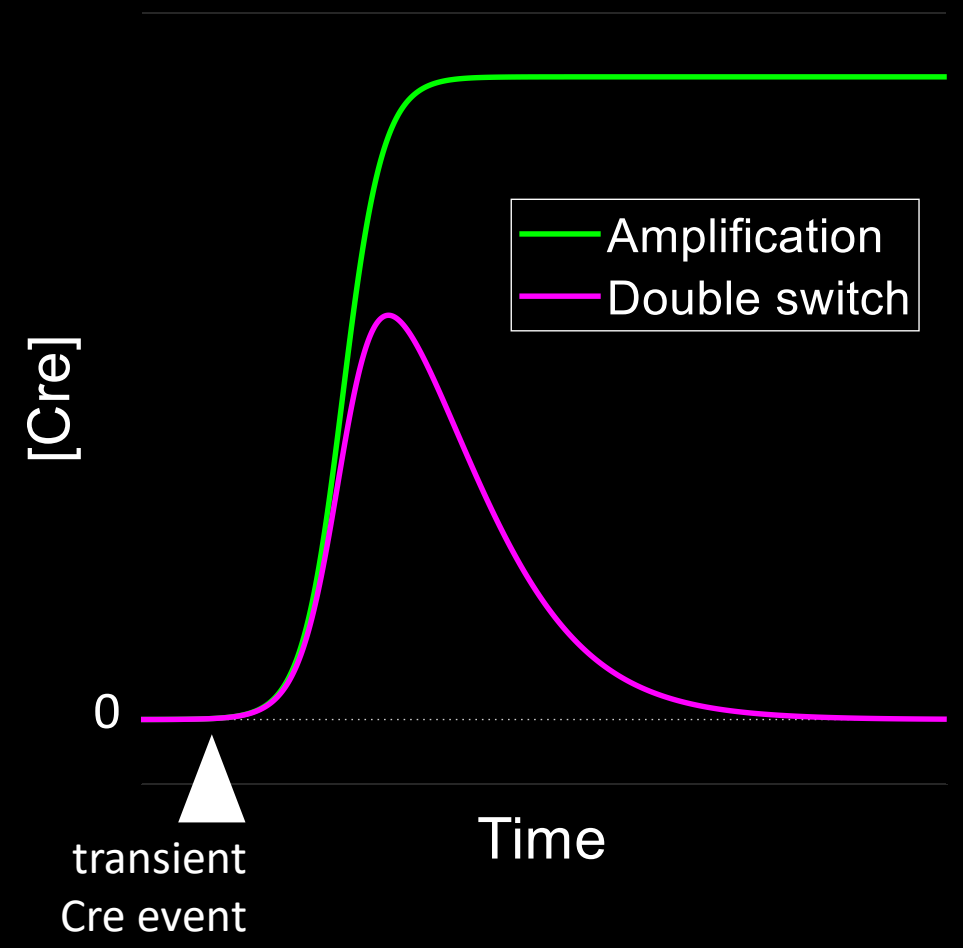
Beatrix

= **Low affinity** lox sites



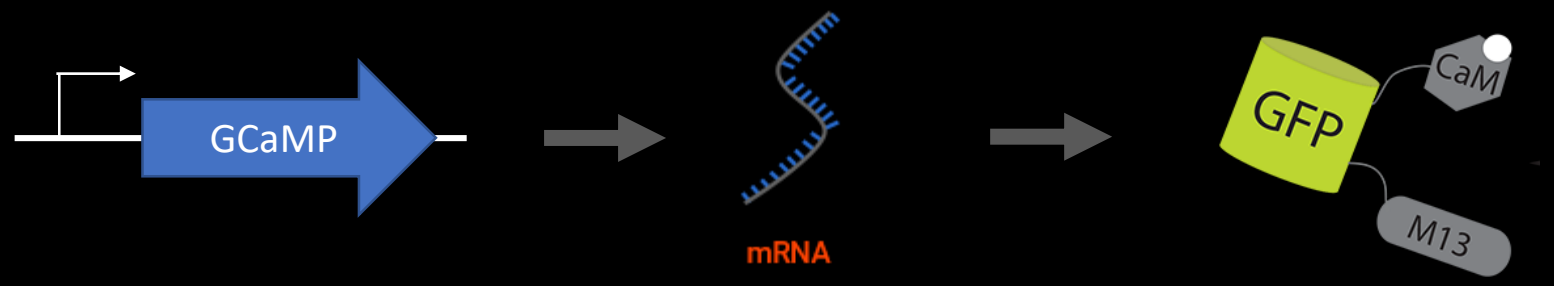
Beatrix 5.0


RFP
CRE
GFP
+ Cre
Positive feedback
GFP
CRE
+ Cre
Negative feedback
GFP

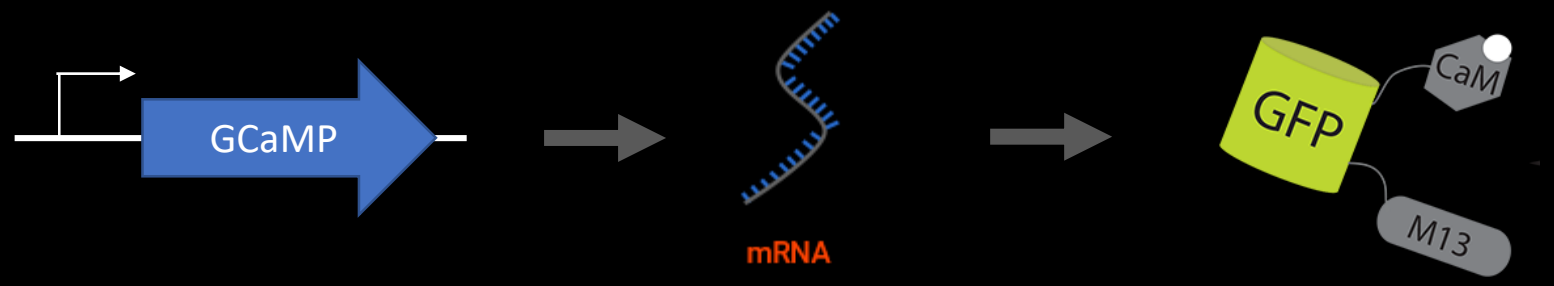

Amplification
Double switch
[Cre]
0
transient Cre event
Time

ddGCaMP:
how it works

How it works

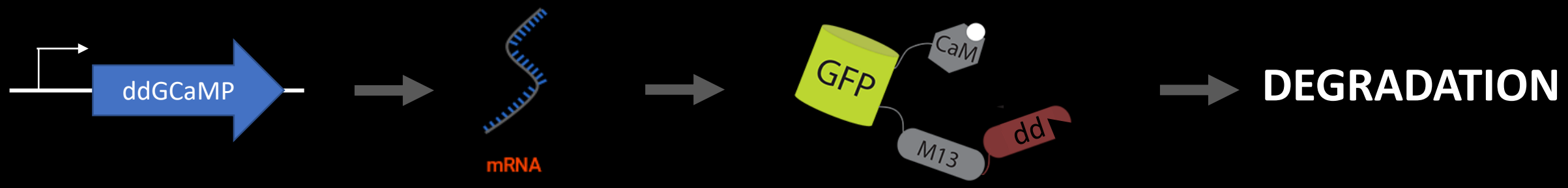


ddGCaMP
How it works

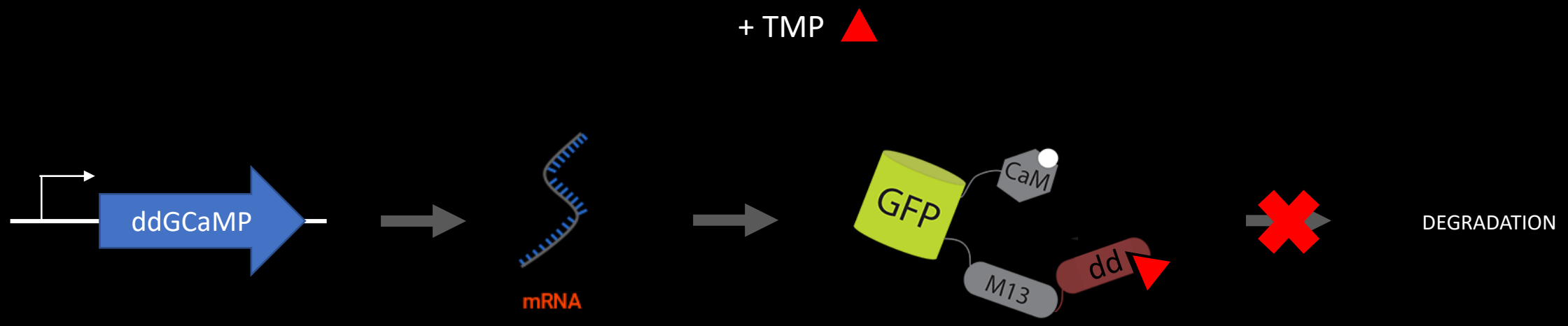


DANGEROUS!!

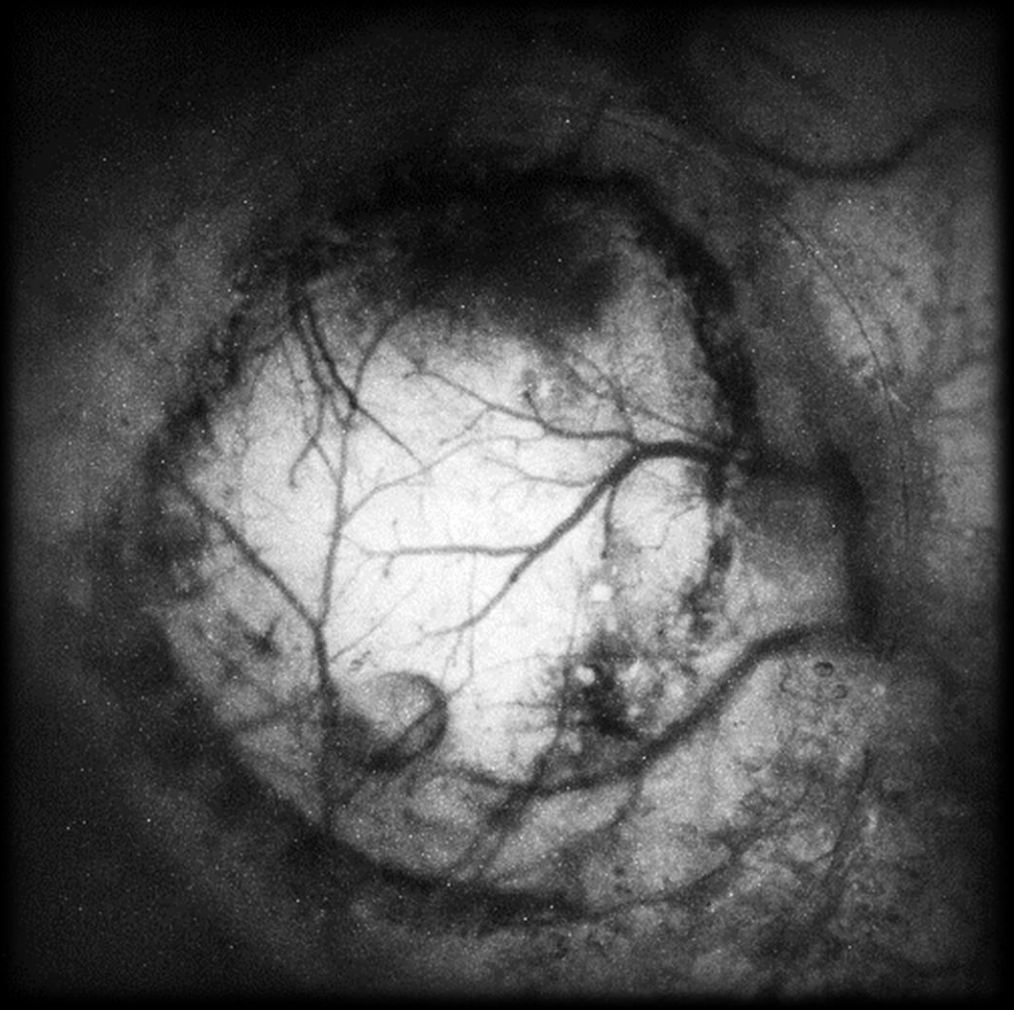
ddGCaMP is constitutively expressed and degraded



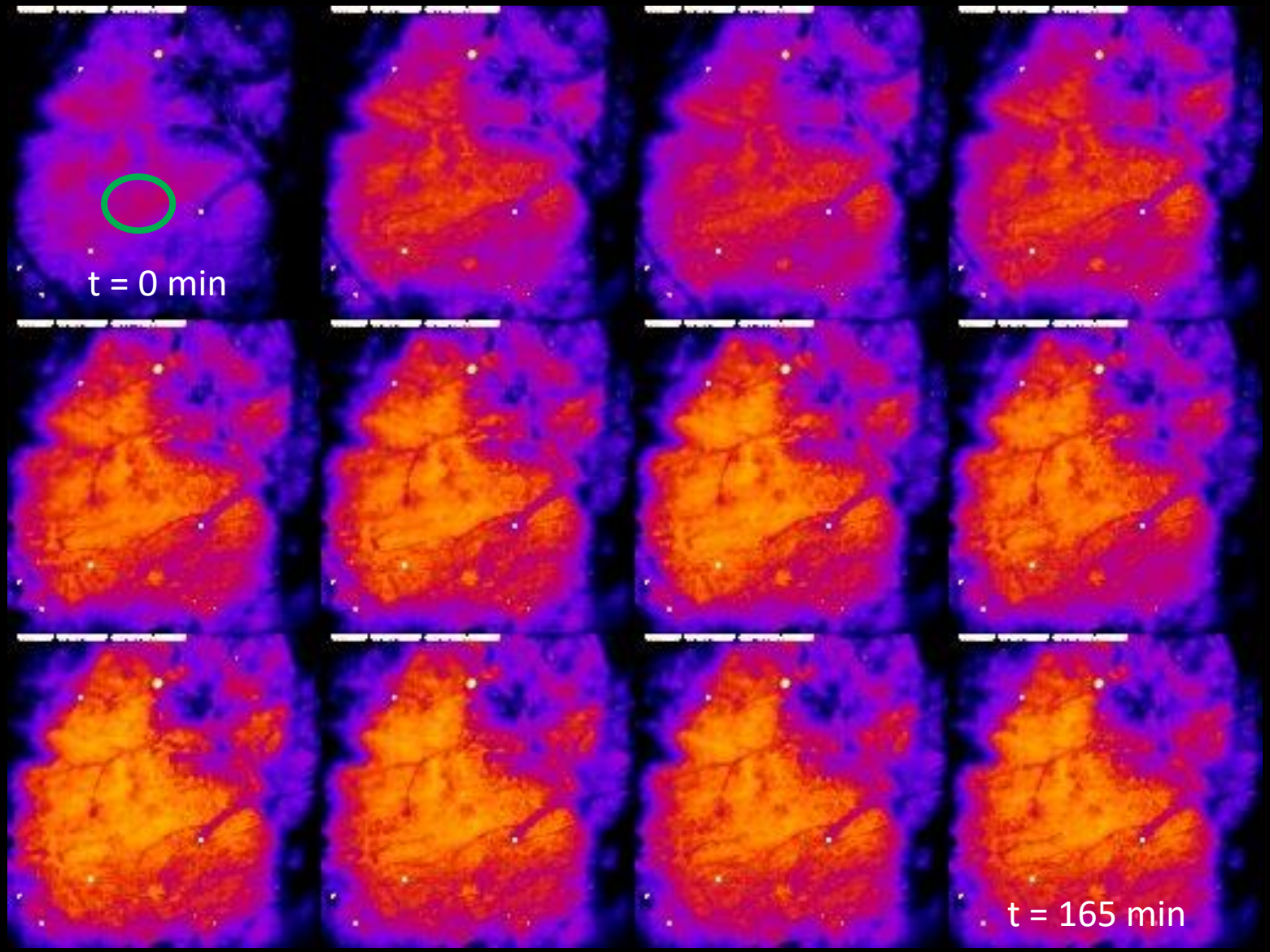
Trimetoprim (TMP) administration prevents ddGCaMP degradation

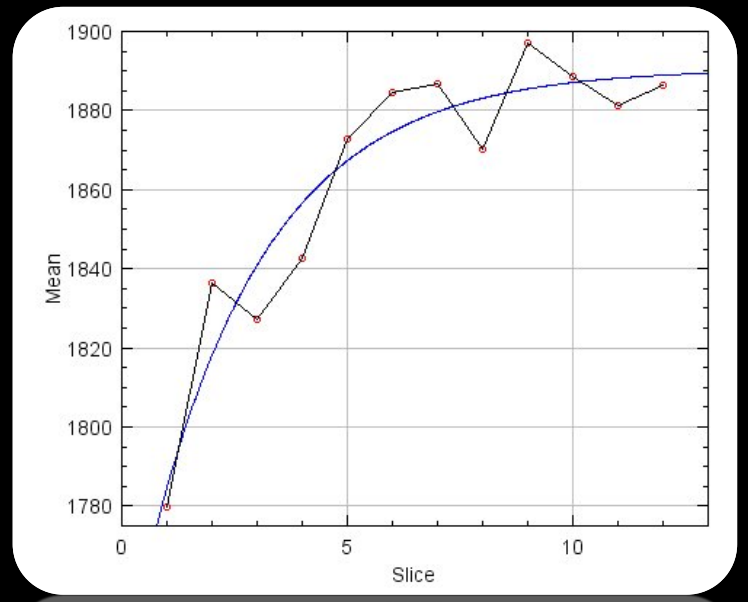


Widefield imaging



Activation

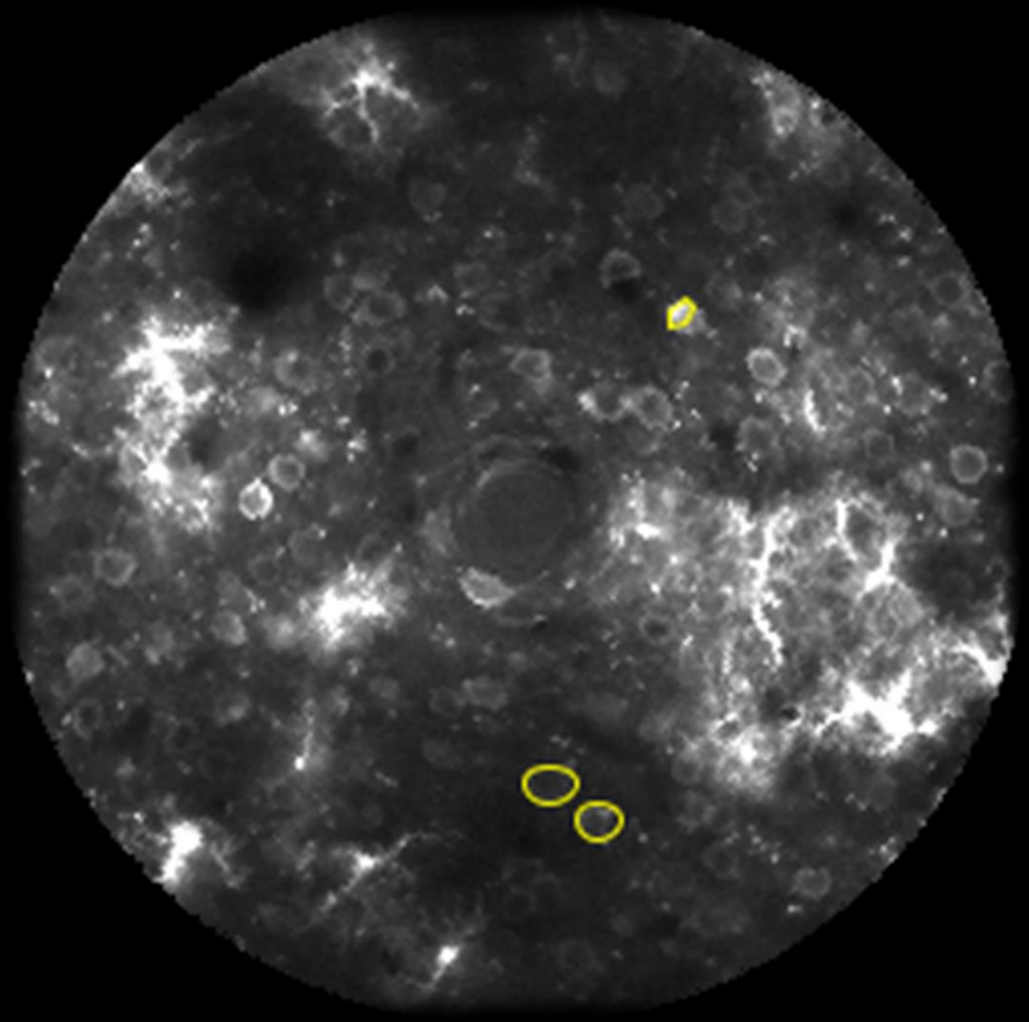




Δt = 15 min

IntraVentricular Injections

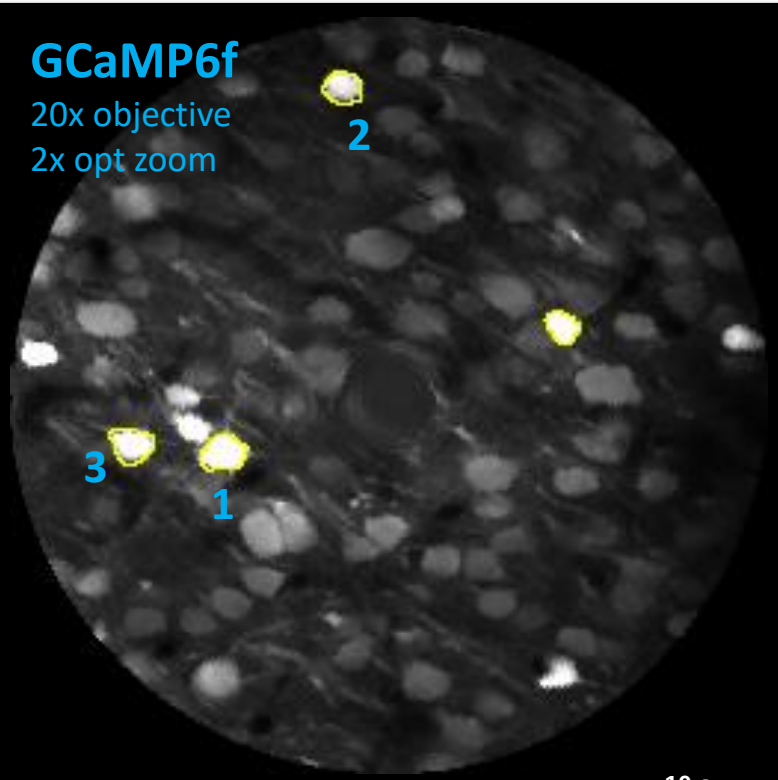
2Photon imaging

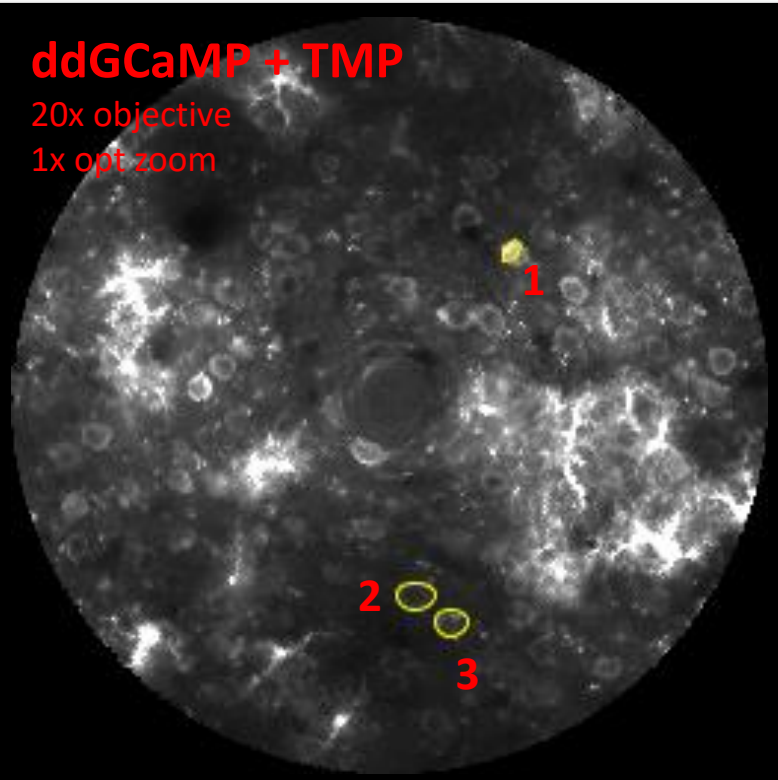


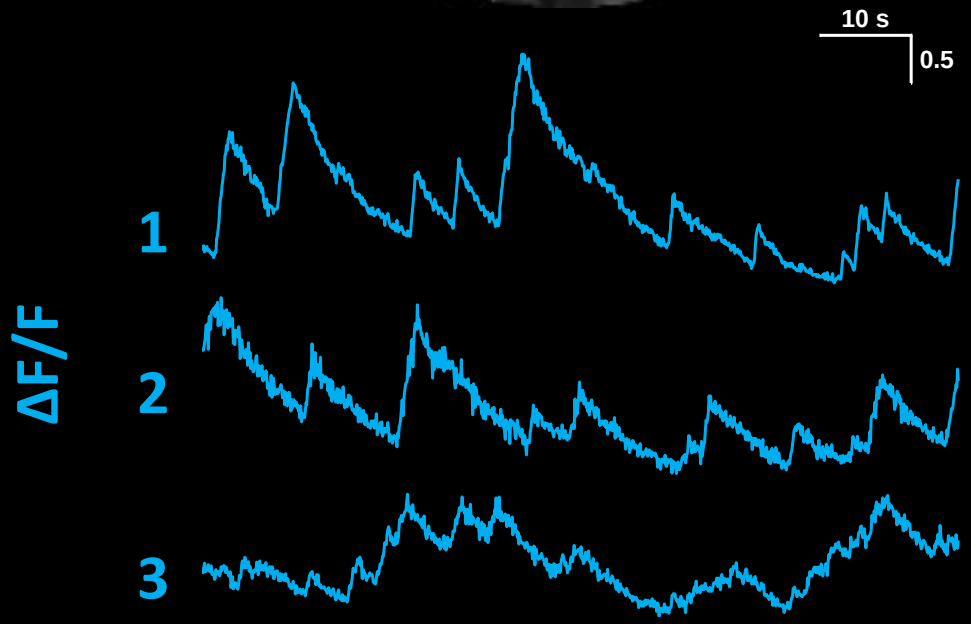
IntraVentricular Injections

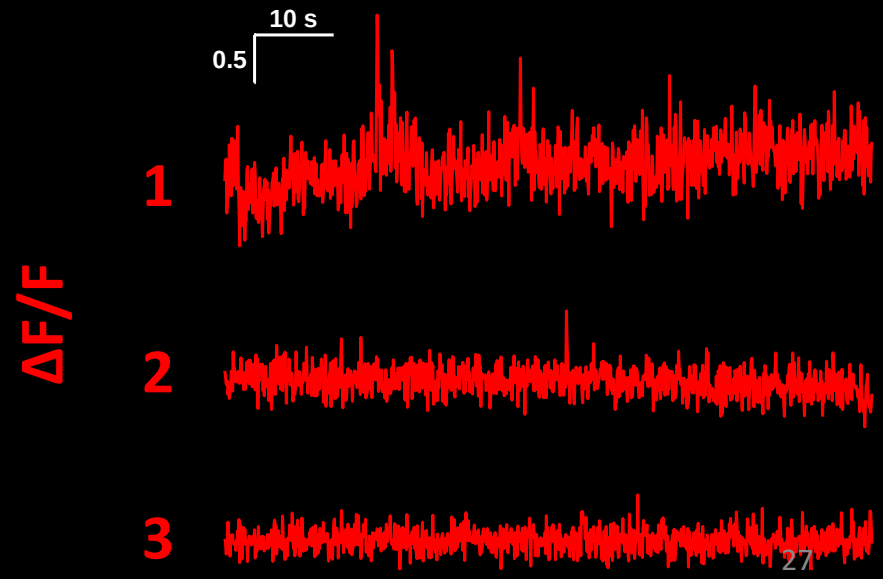
Two Photon imaging:

- Cell bodies
- Dendrites









IntraVentricular Injections

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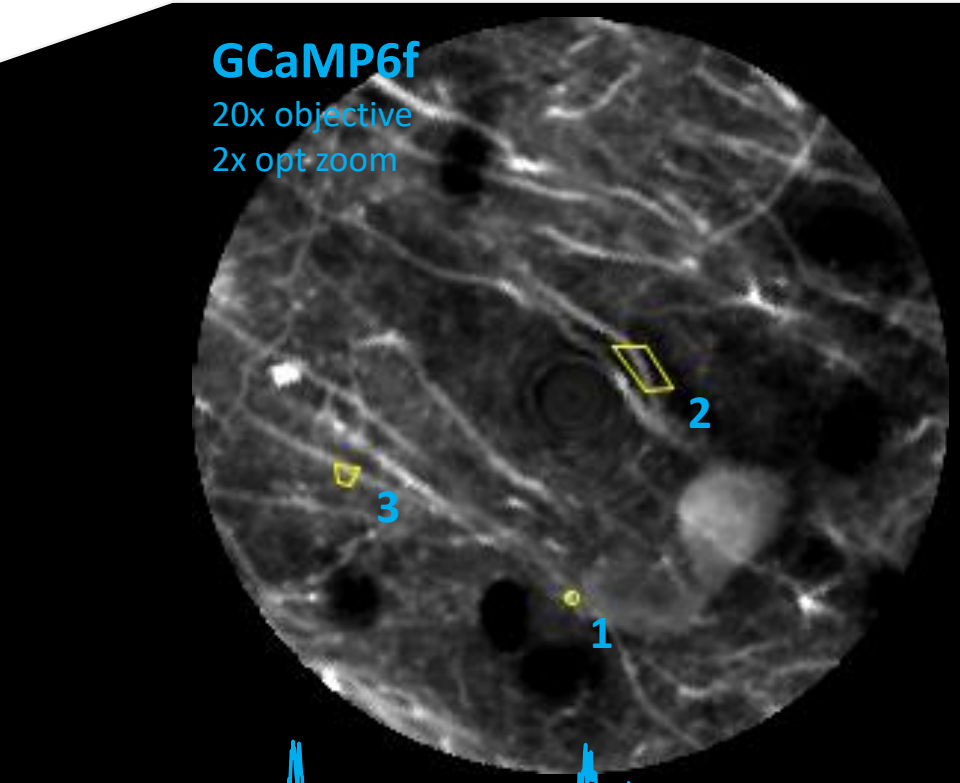

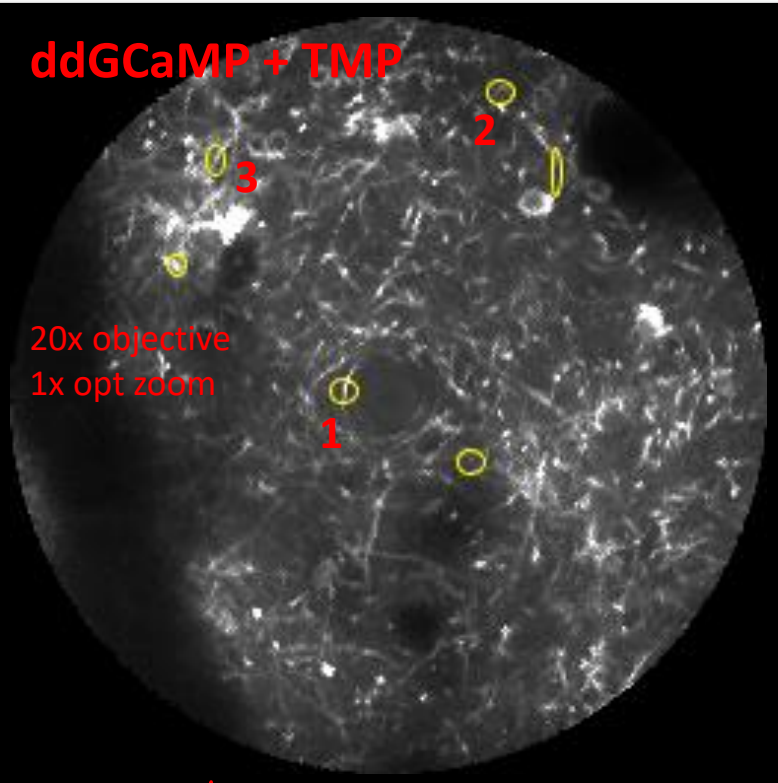

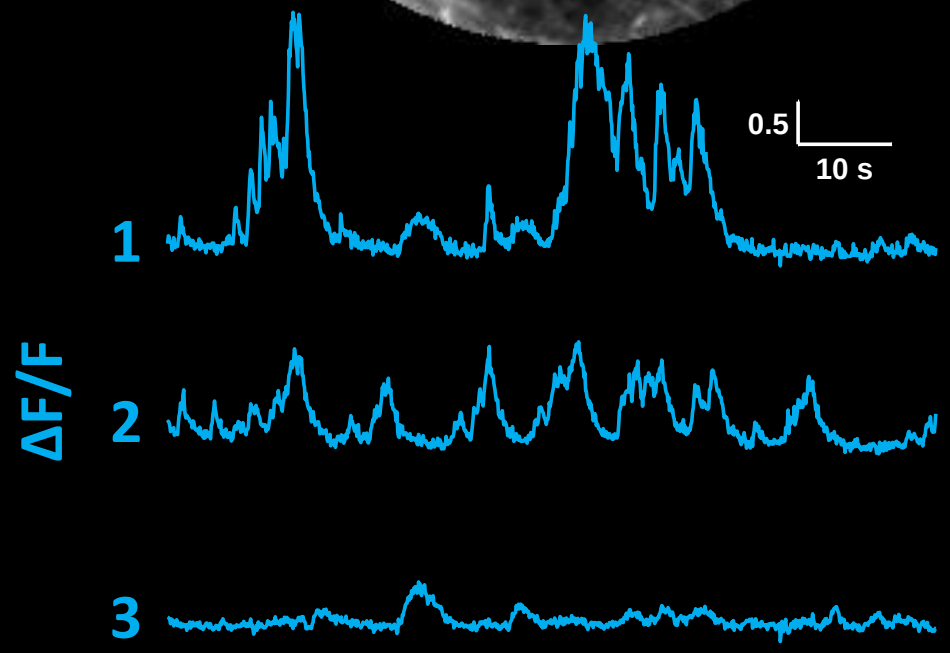

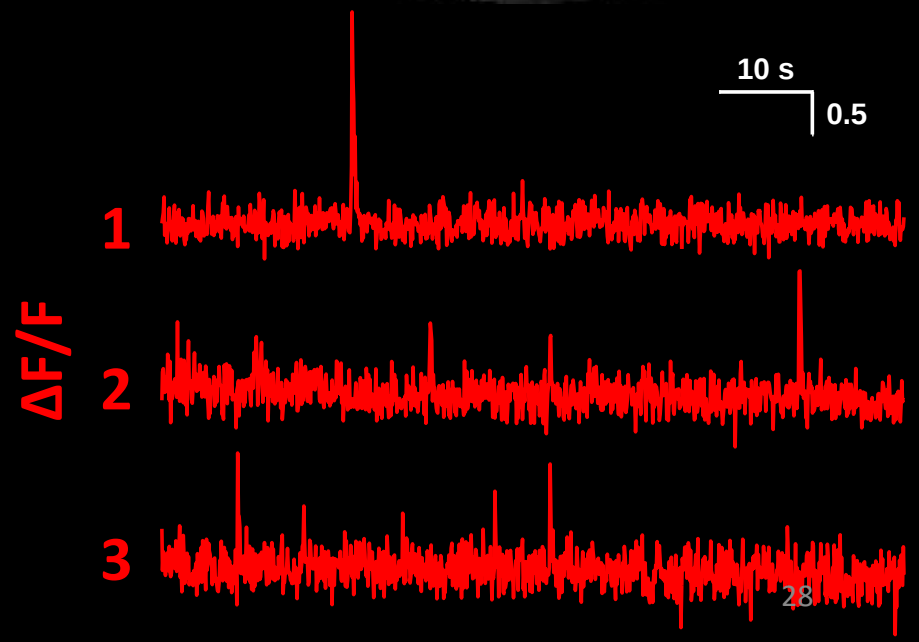

IntraVentricular Injections

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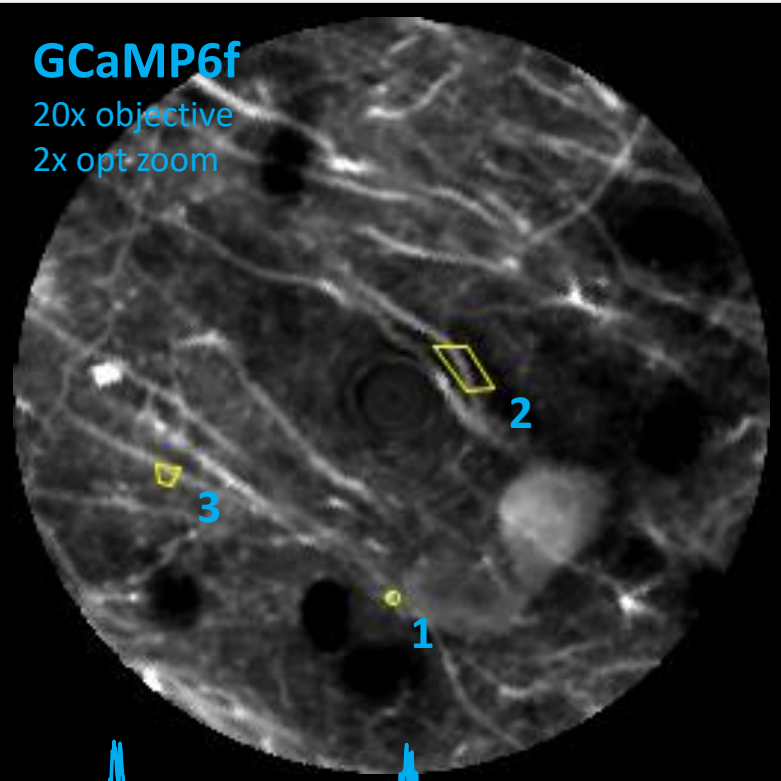
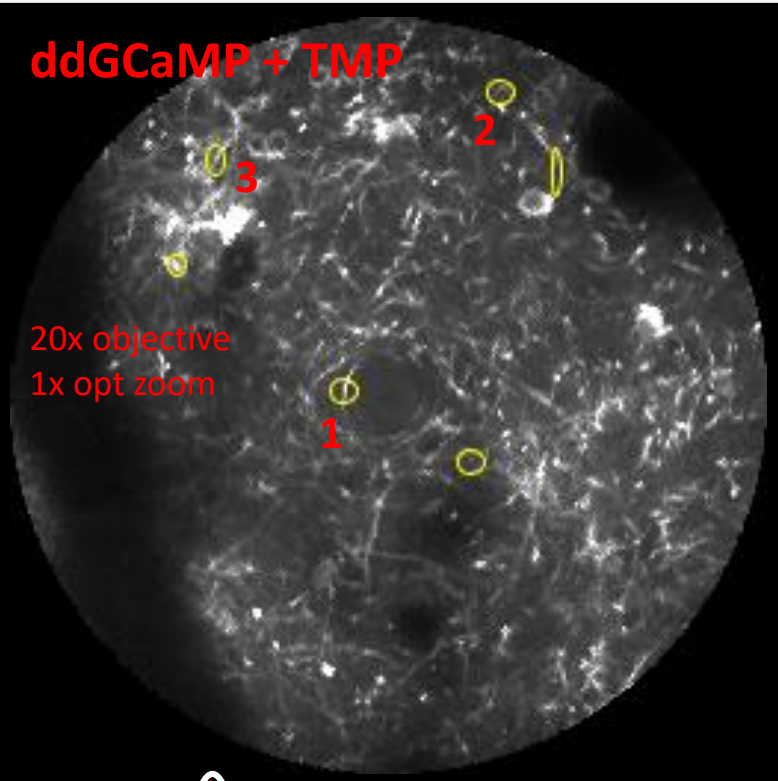
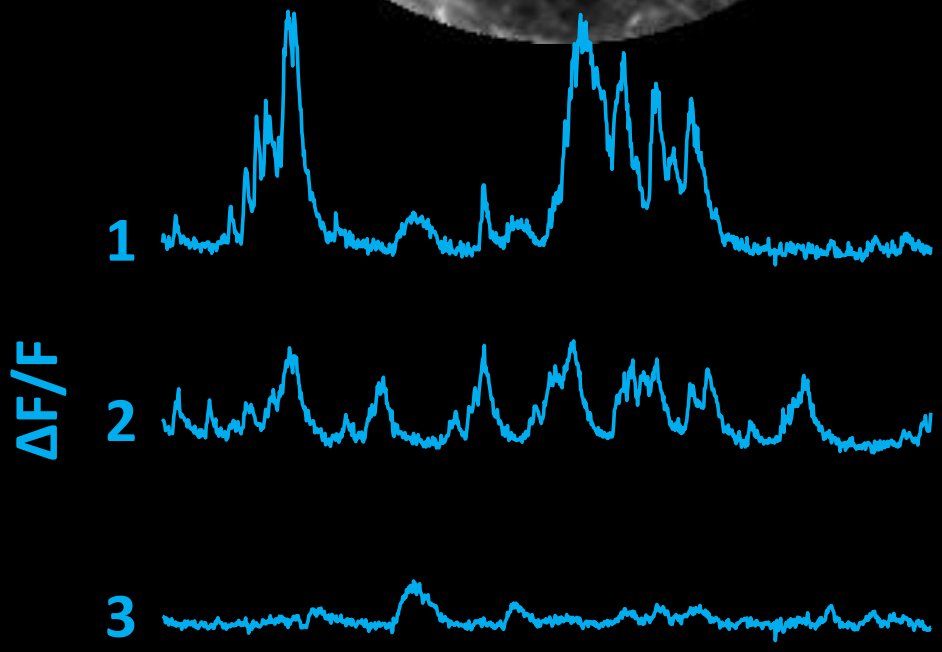
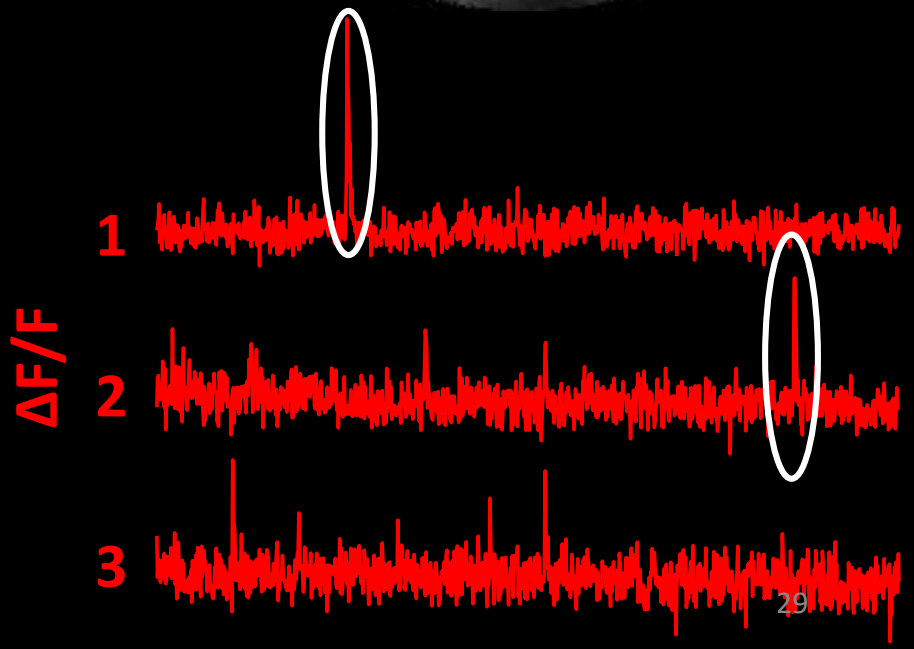




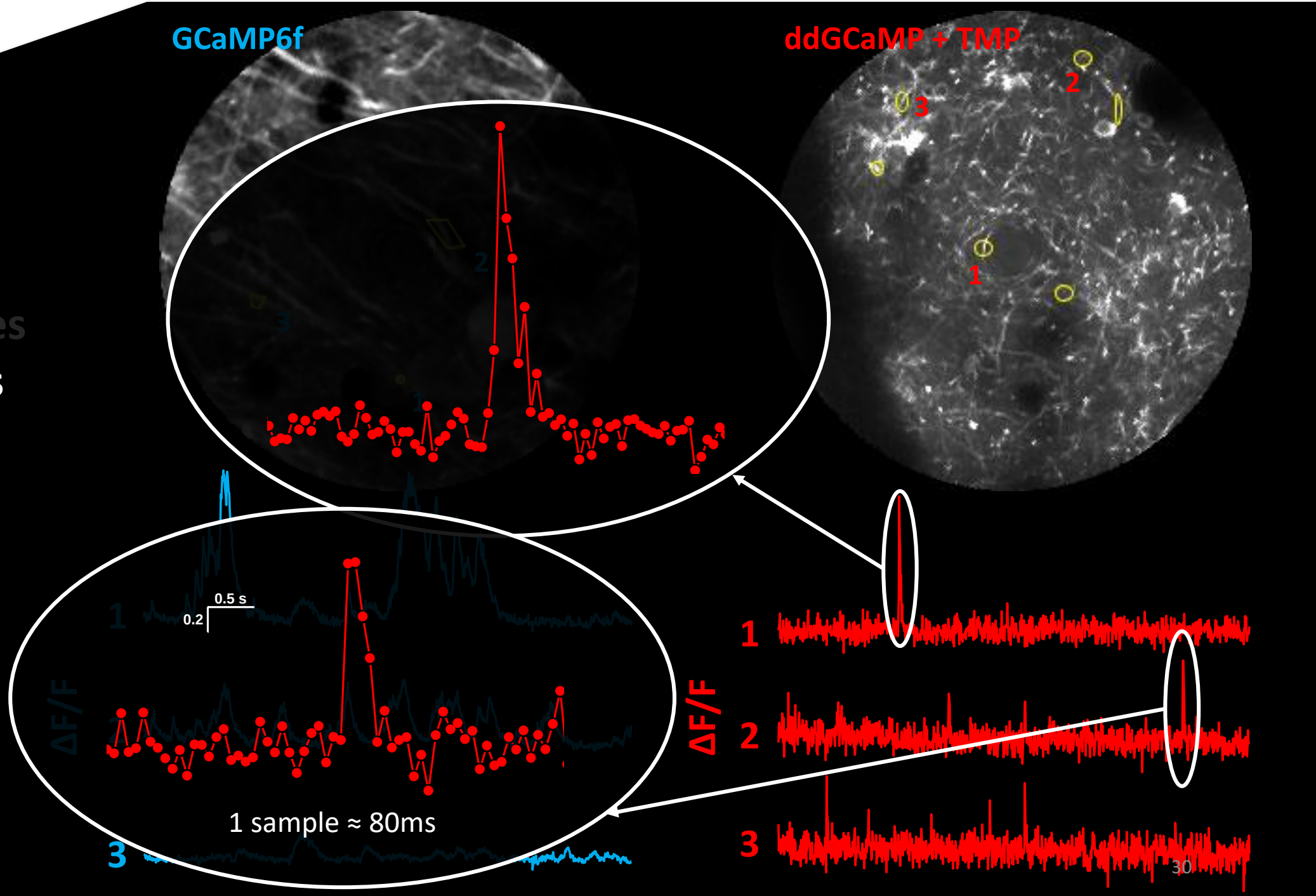




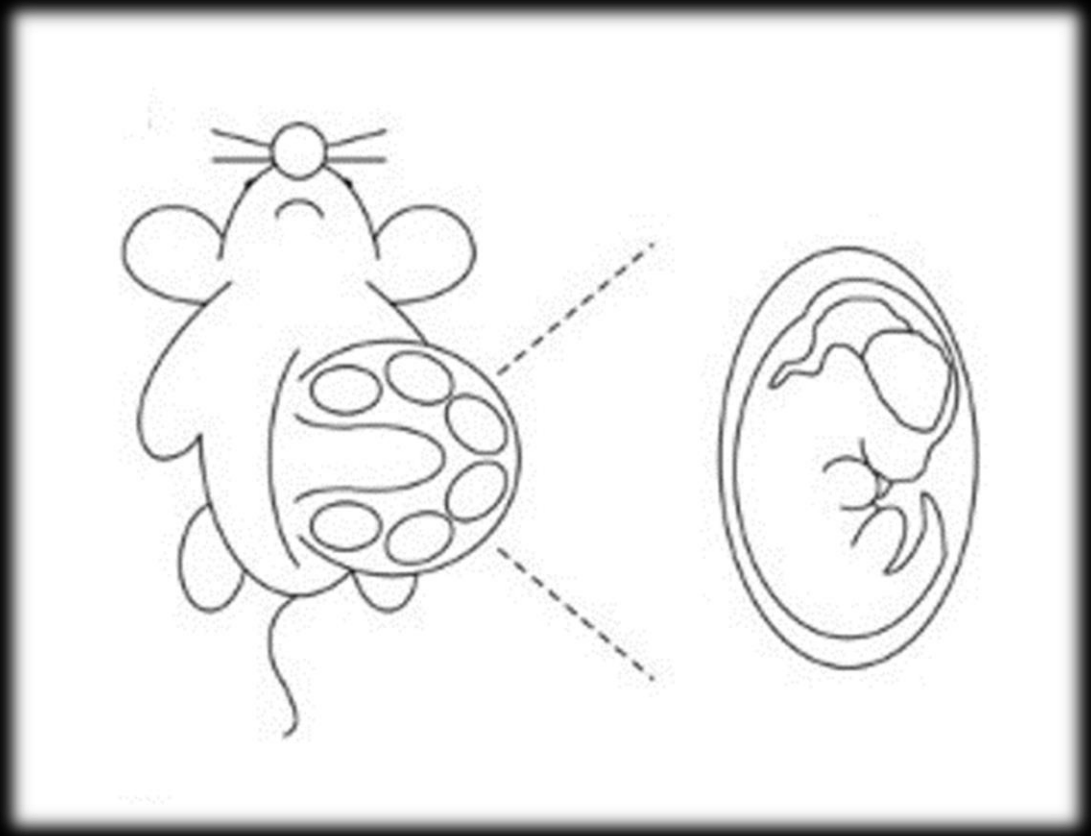
IntraVentricular Injections

Two Photon imaging:

- Cell bodies
- Dendrites



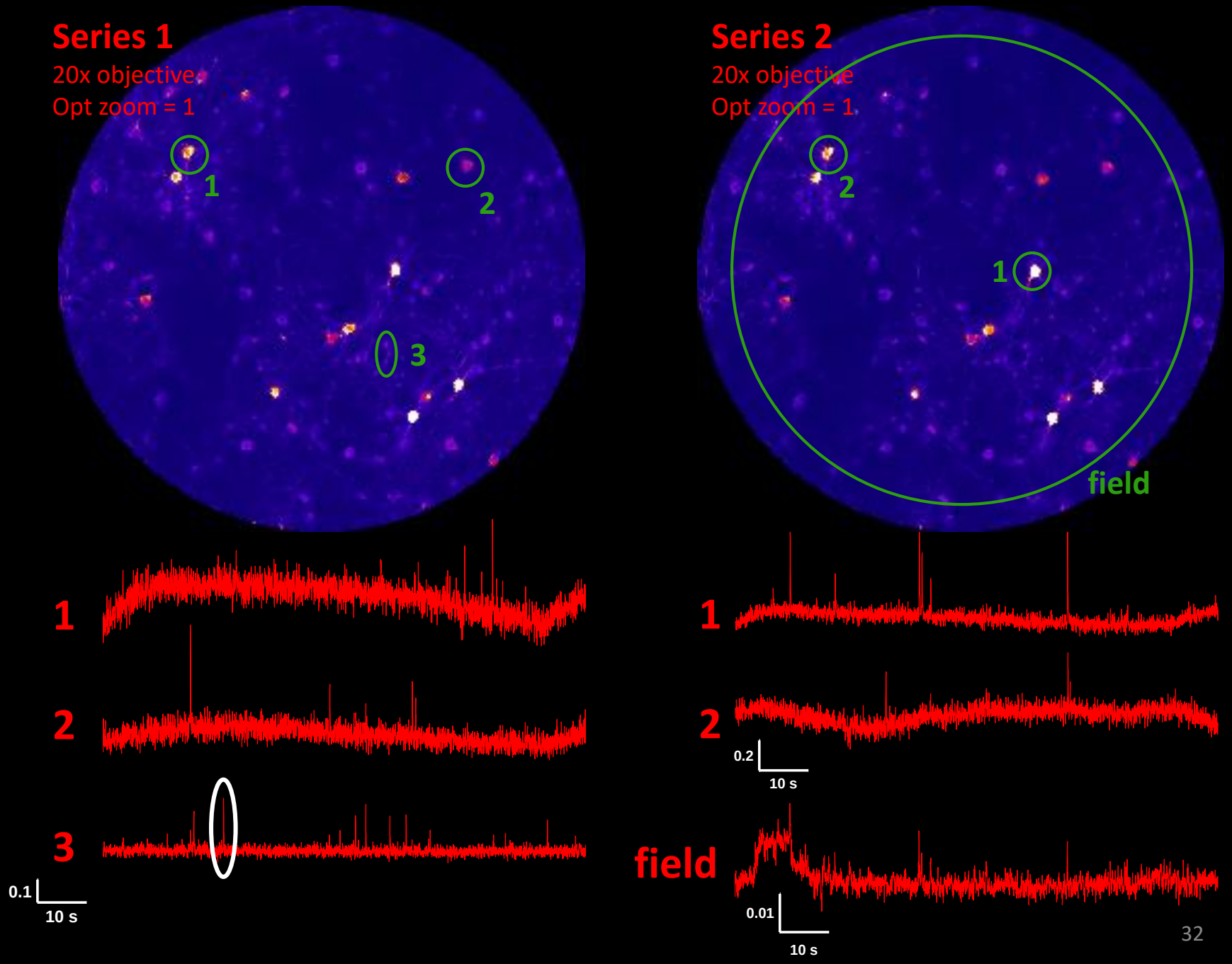
In utero electroporation



In utero electroporation

Two Photon imaging

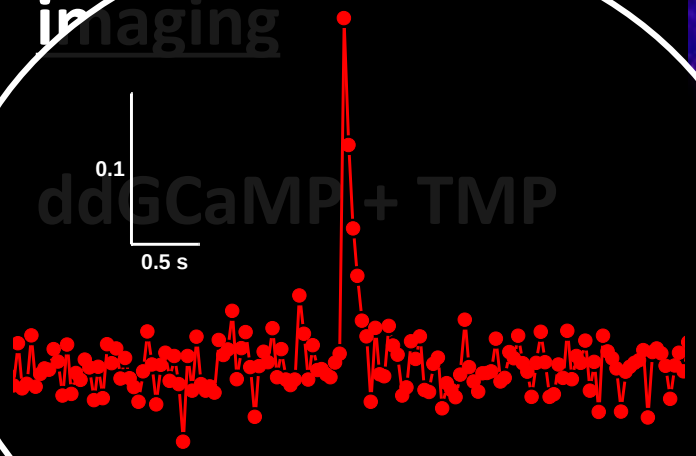
ddGCaMP + TMP



In utero electroporation

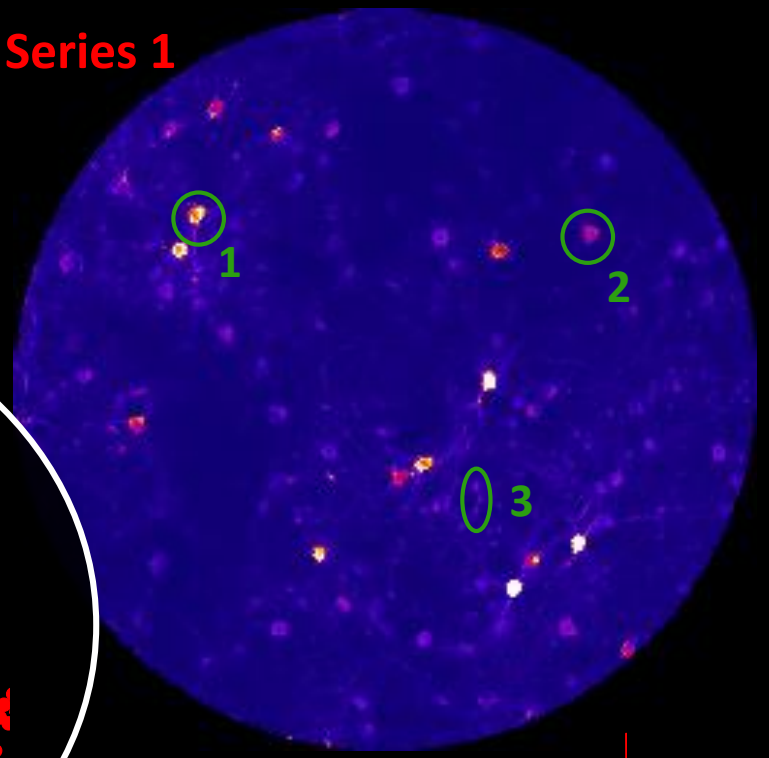
Two Photon imaging

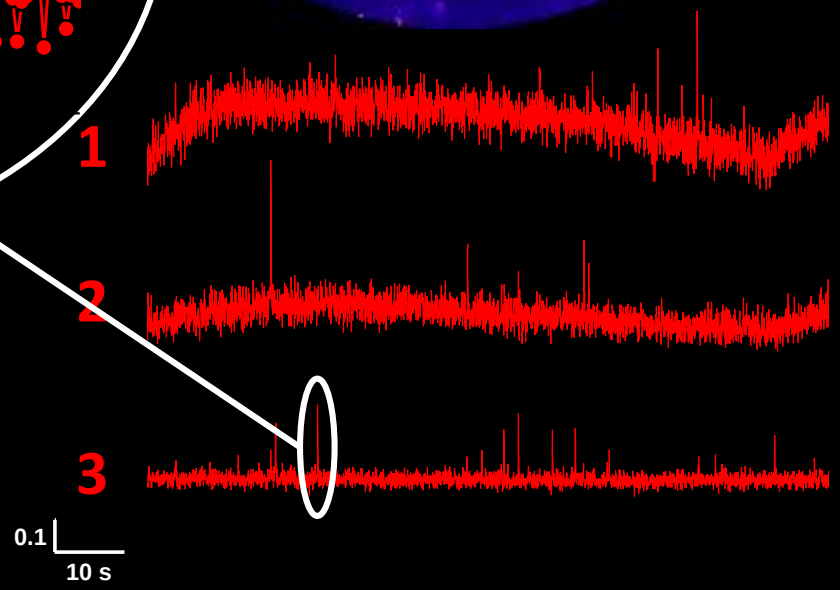
ddGCaMP + TMP



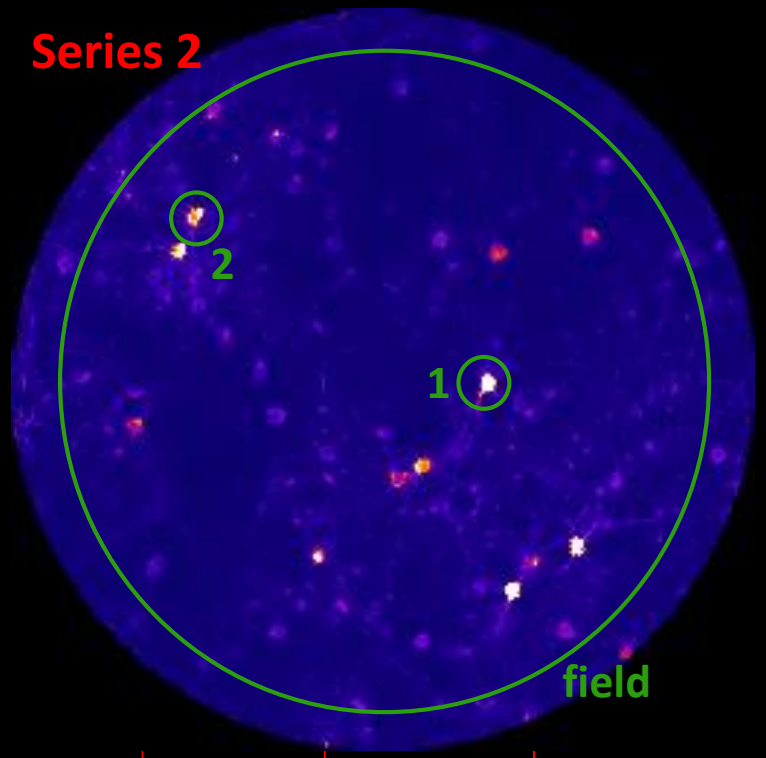
1 sample ≈ 80ms

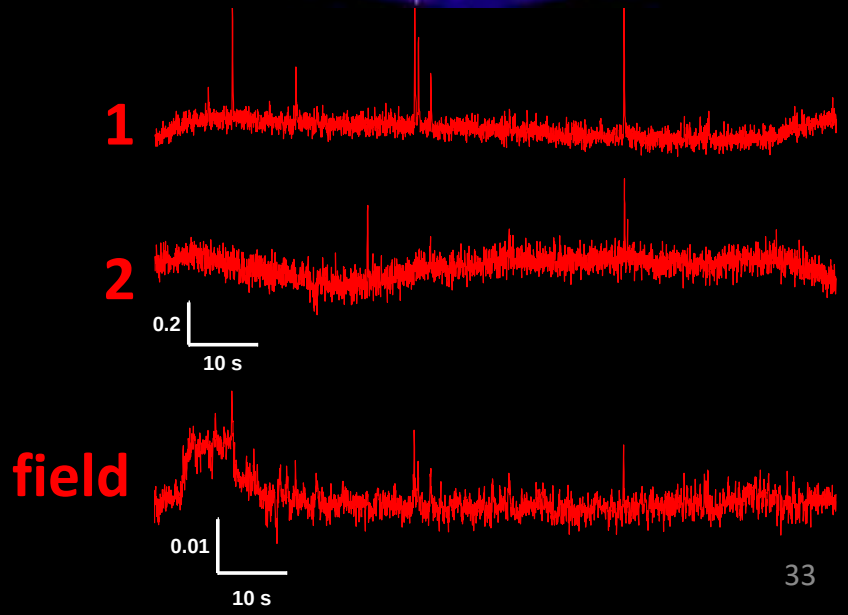
Series 1



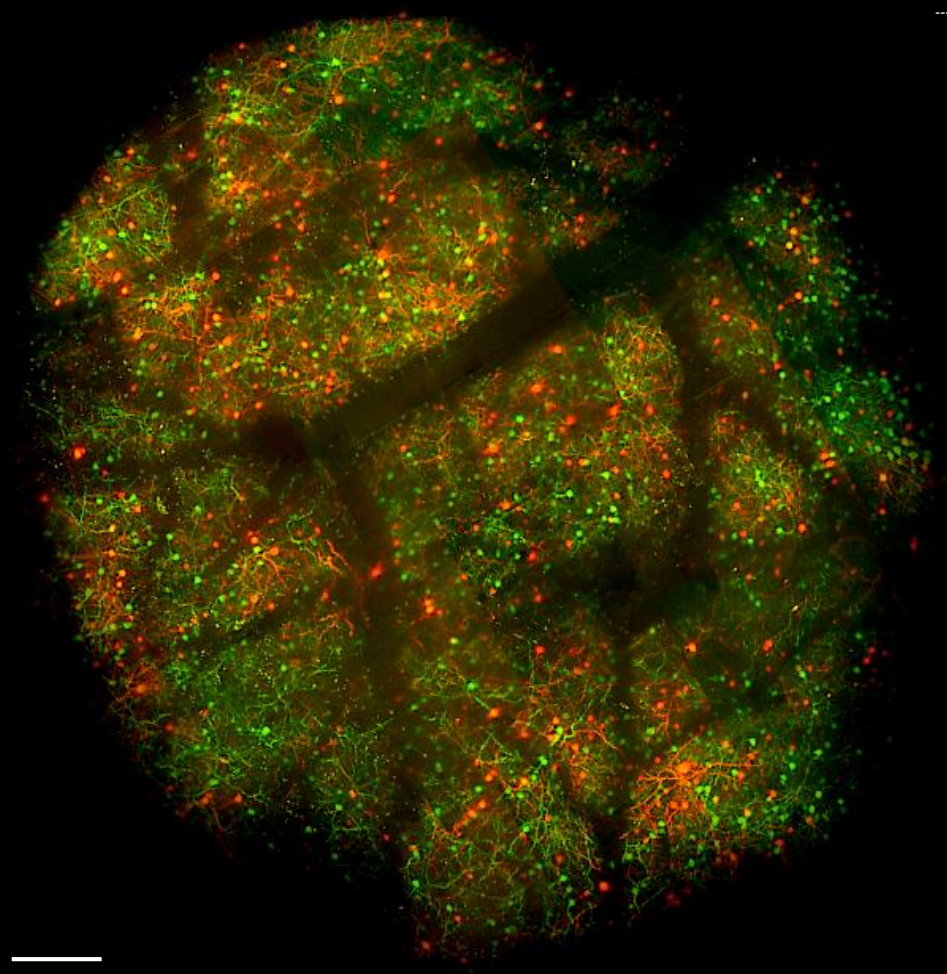


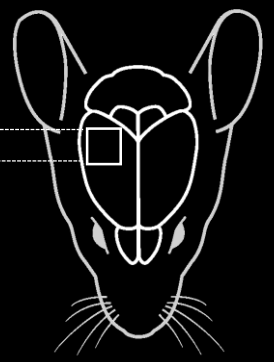
Series 2

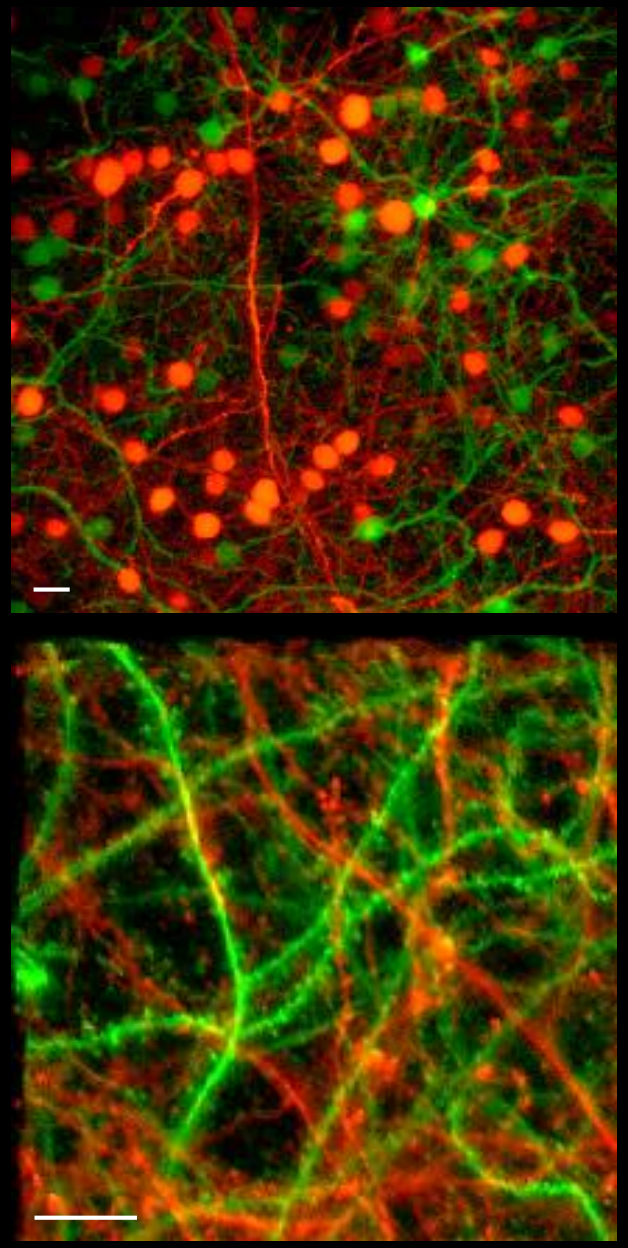




In vivo imaging



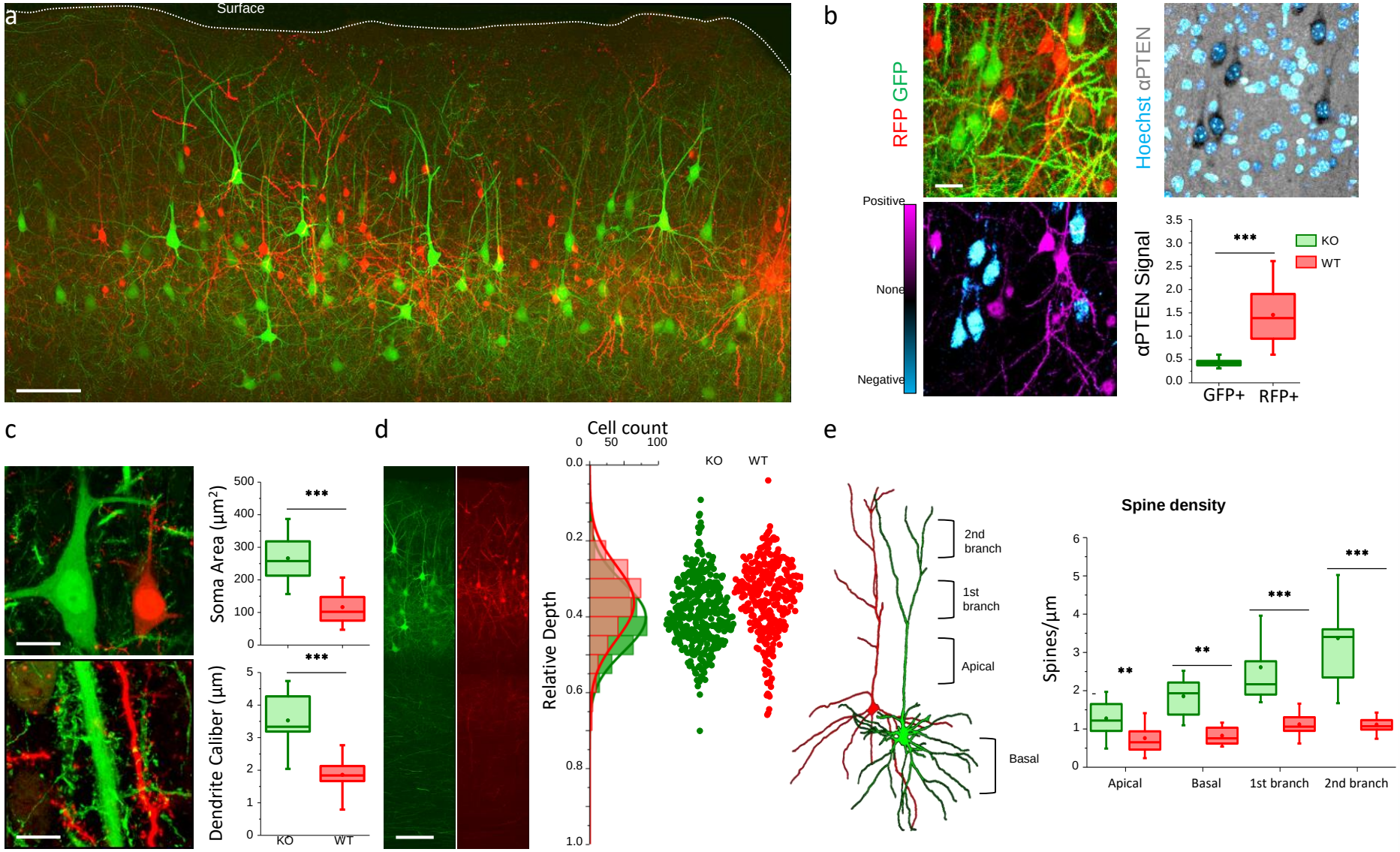




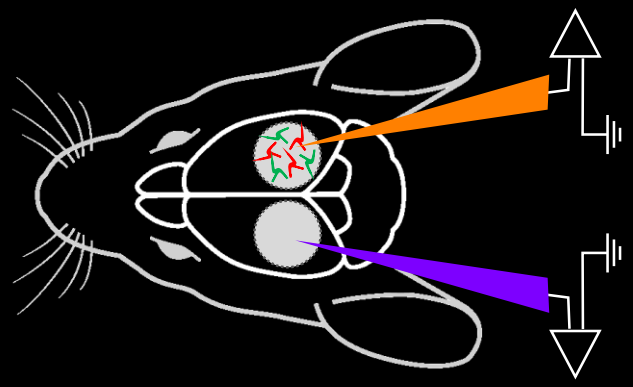
RFP = WT

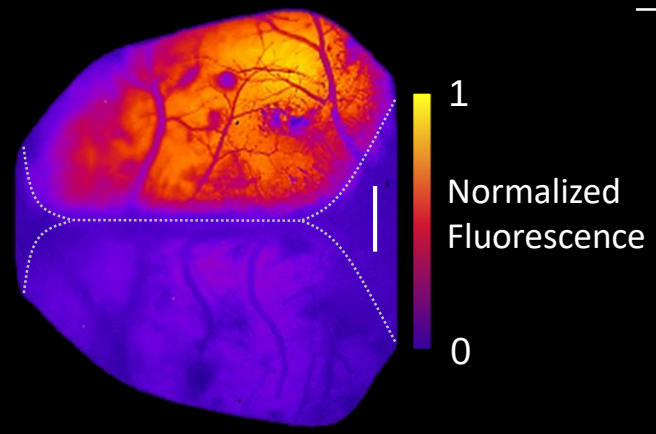
GFP = KO

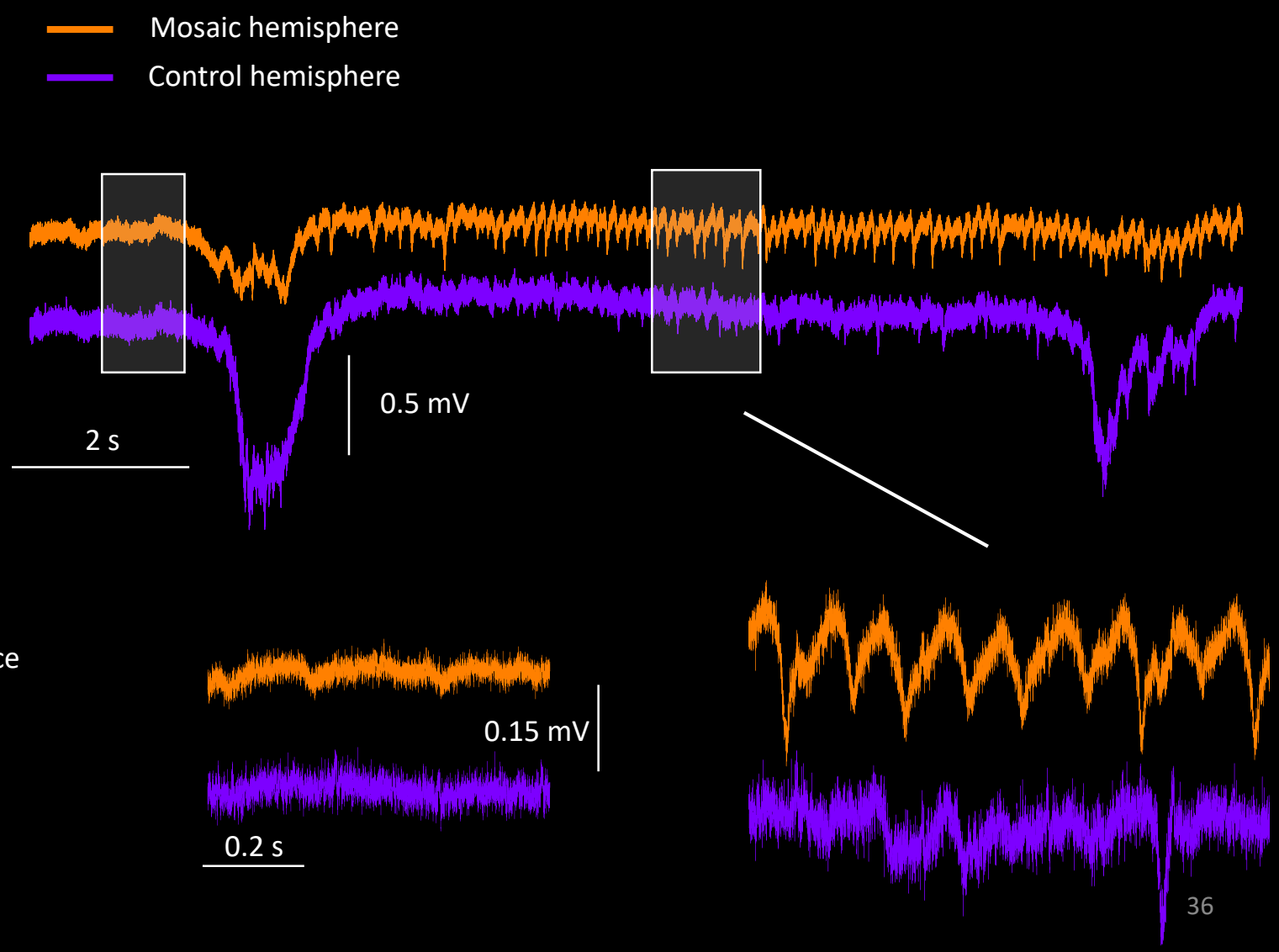
Ex vivo physiology: PTEN knock out mosaic



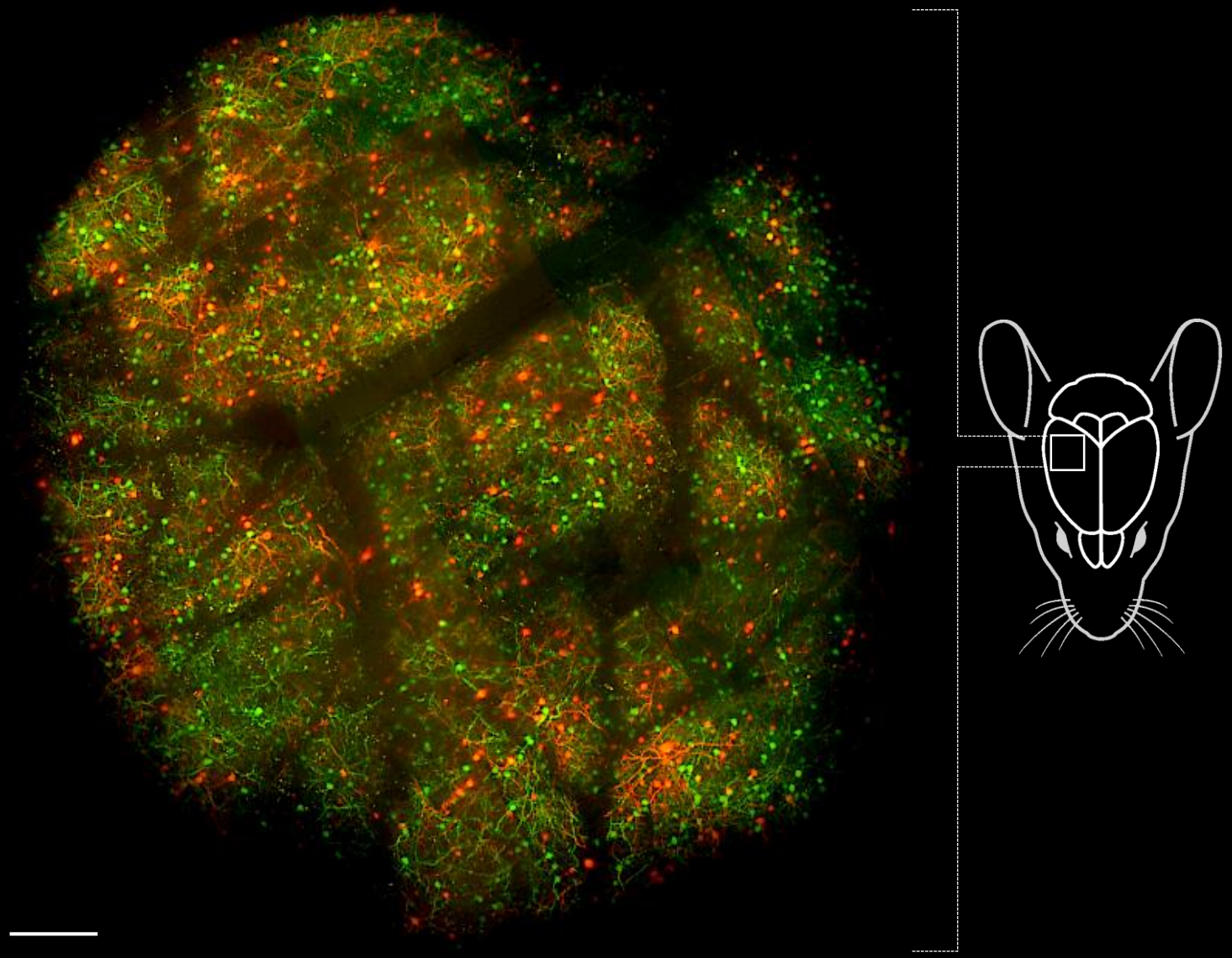
In vivo physiology: PTEN knock out mosaic







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

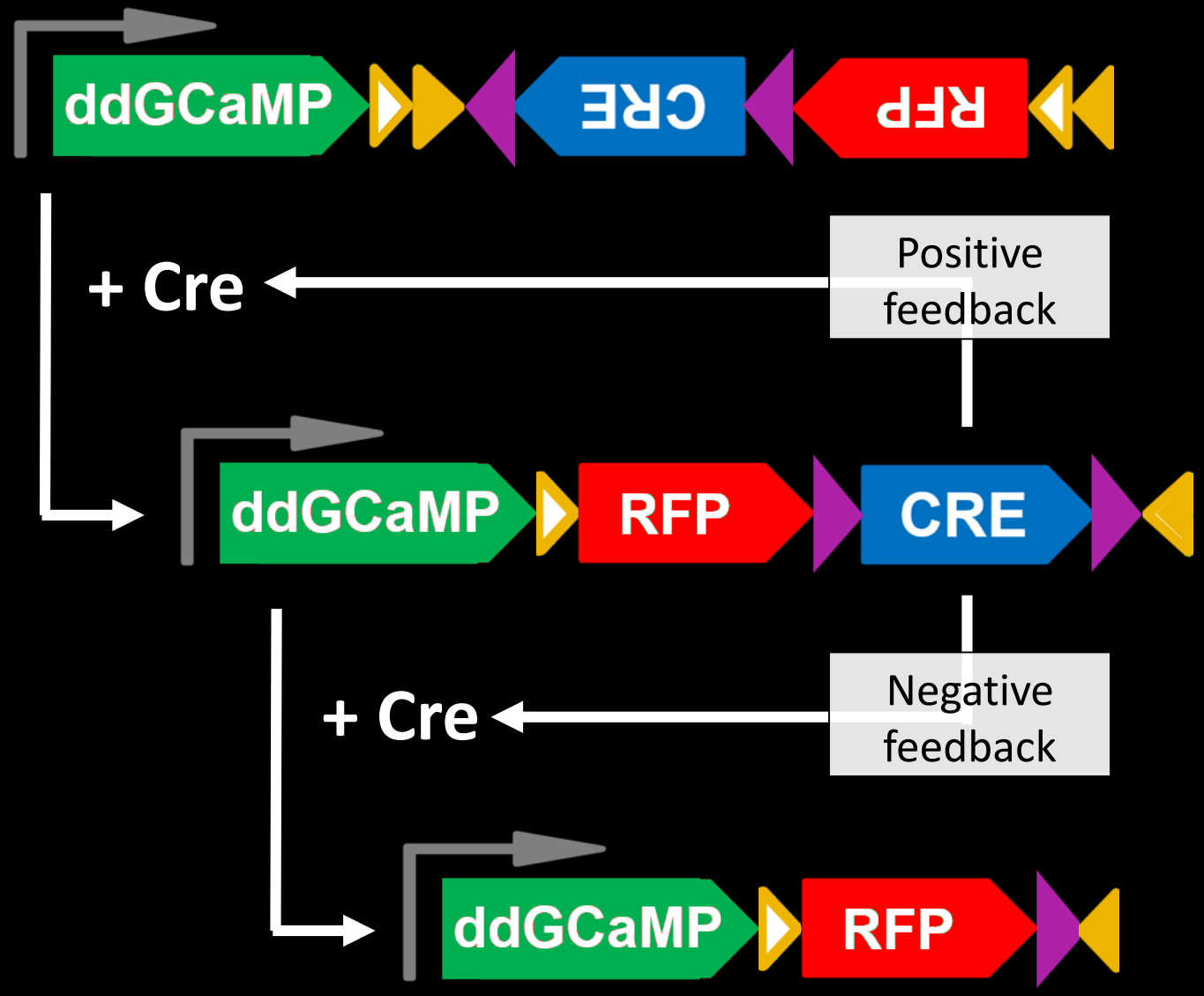


RFP: KO cells

ddGCaMP: all the transfected cells

“Ultimate” tool

“Ultimate” tool

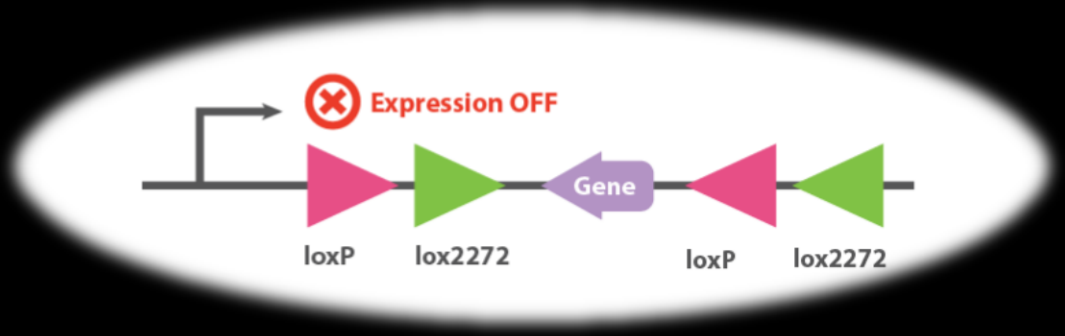


ddGCaMP = WT cell

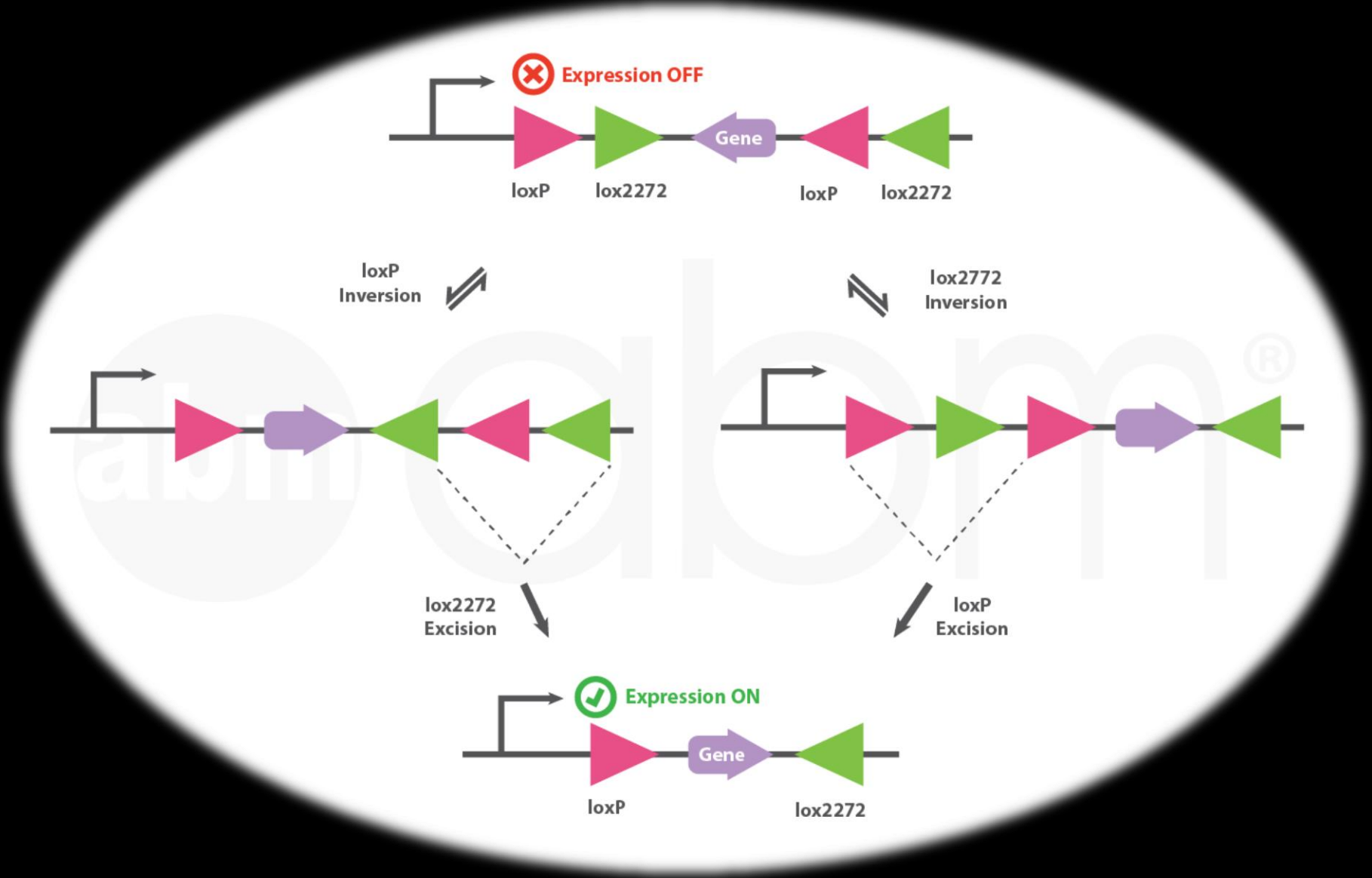
RFP + ddGCaMP = KO cell

Thanks to:

FLEx switch strategy



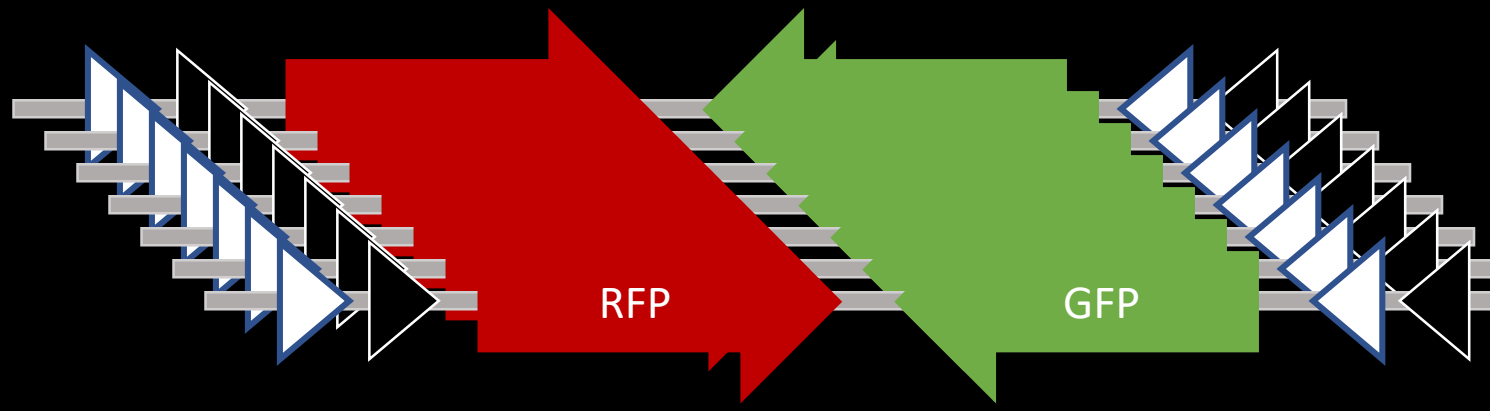
FLEx switch strategy



KO reporting

Target gene

Reporter



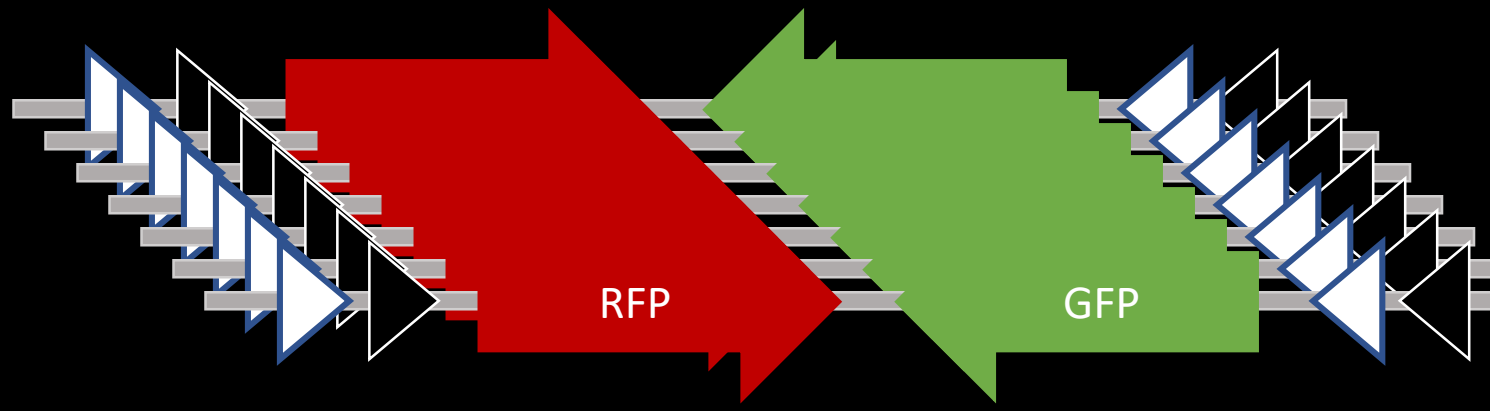
+

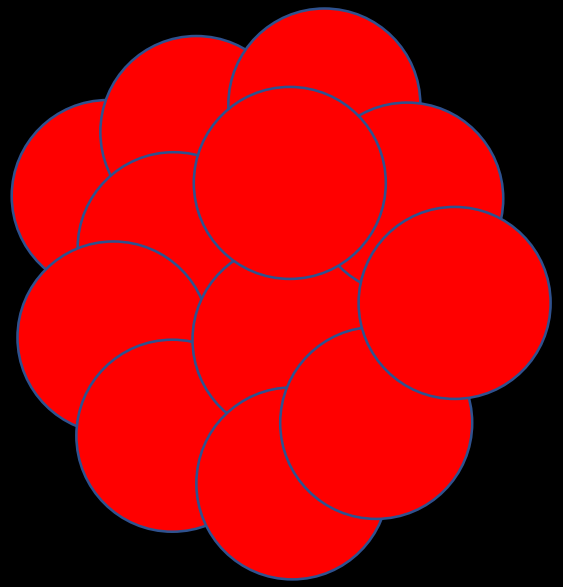
Inducer

KO reporting

Target gene

Reporter

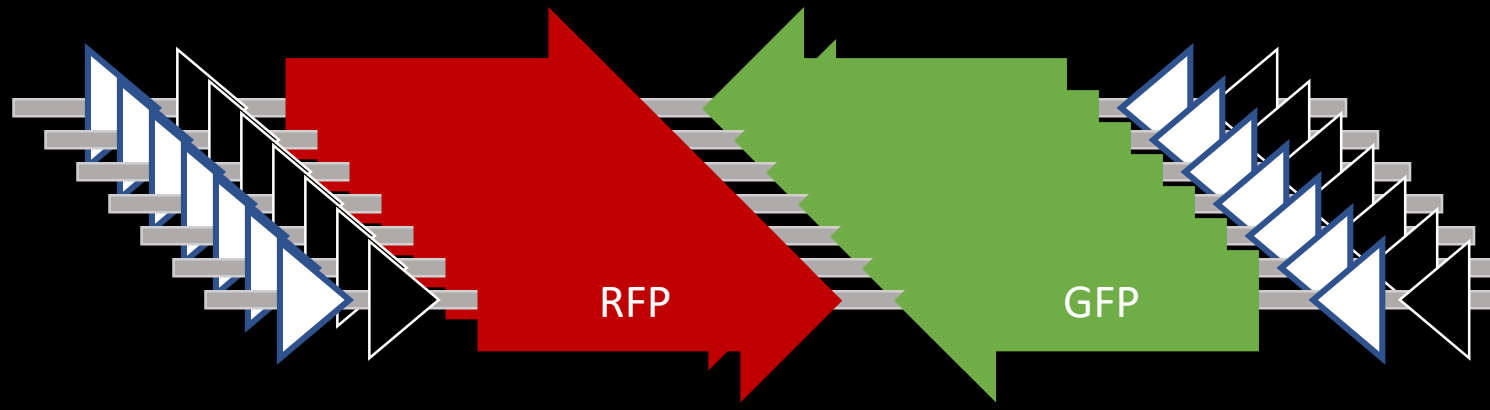


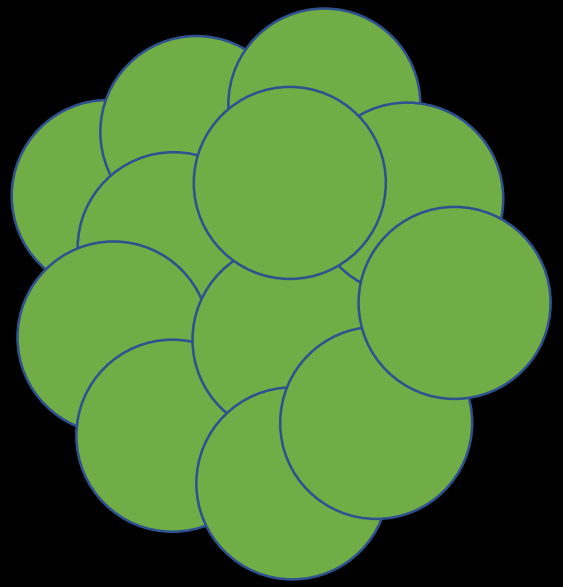


KO reporting

Target gene

Reporter





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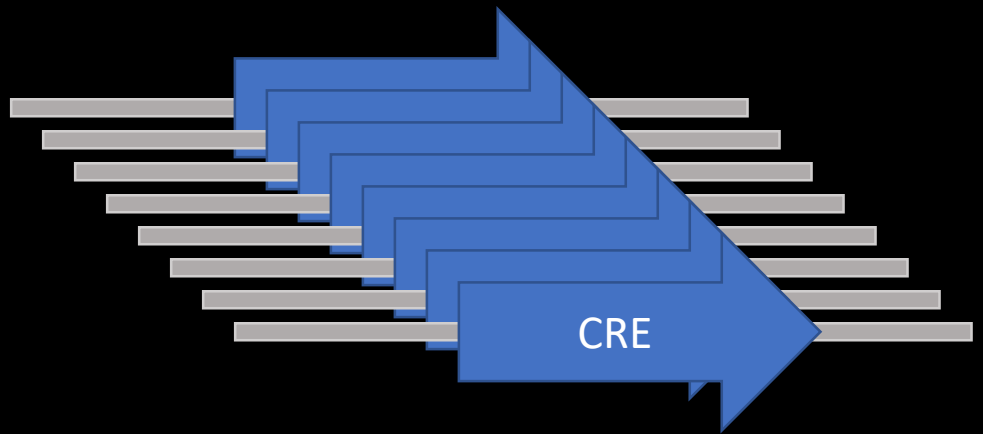
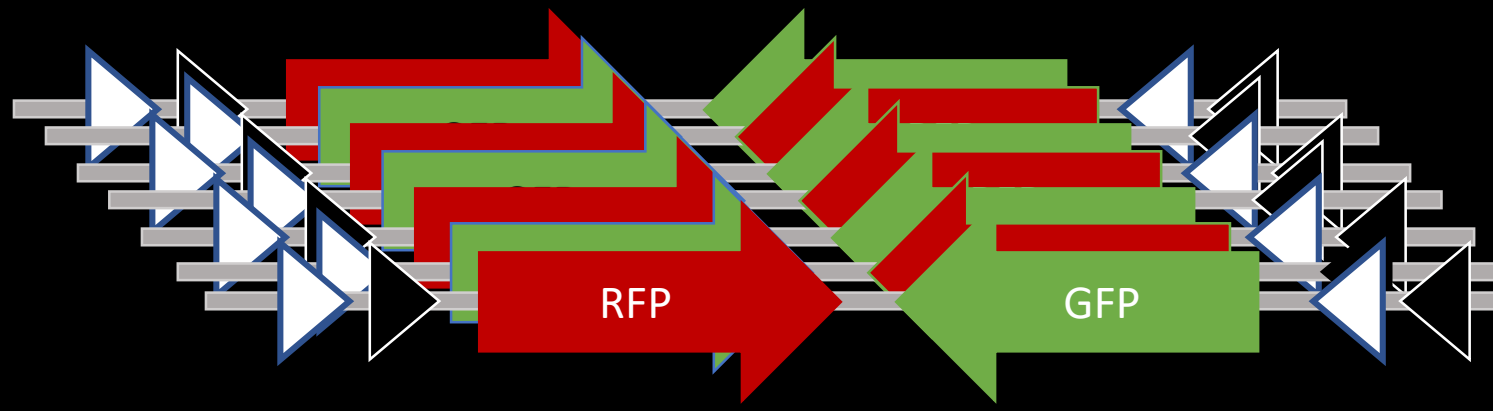


KO reporting

Target gene

Reporter



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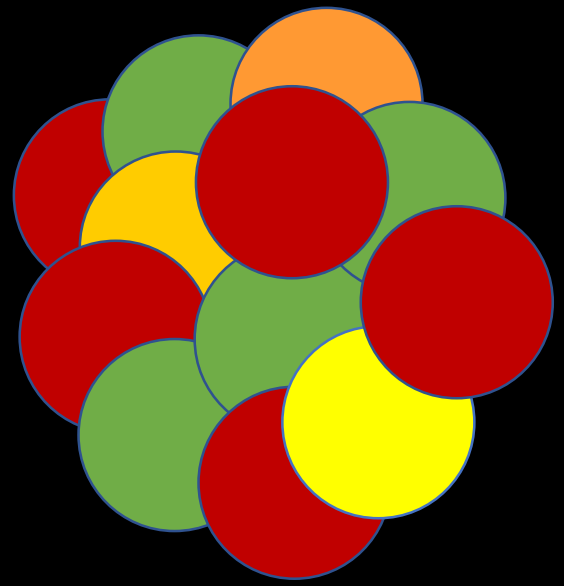
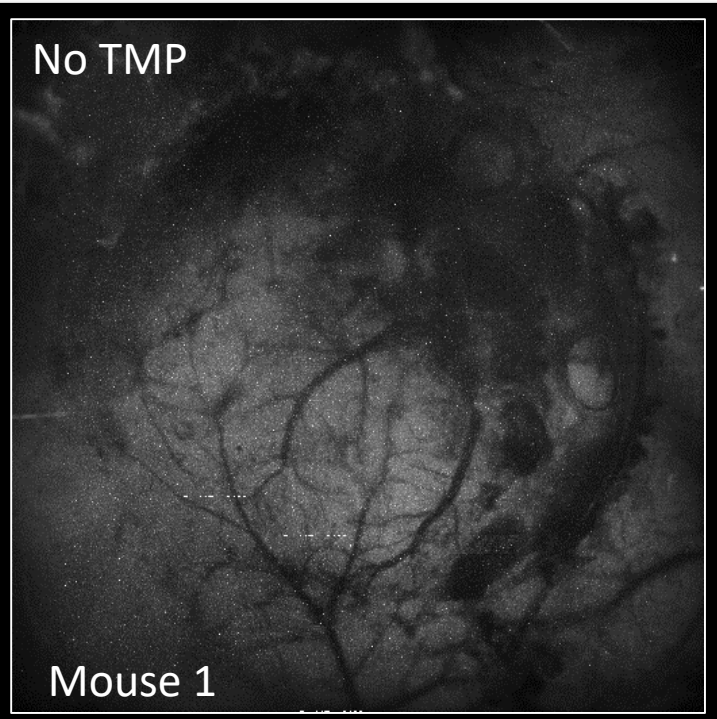
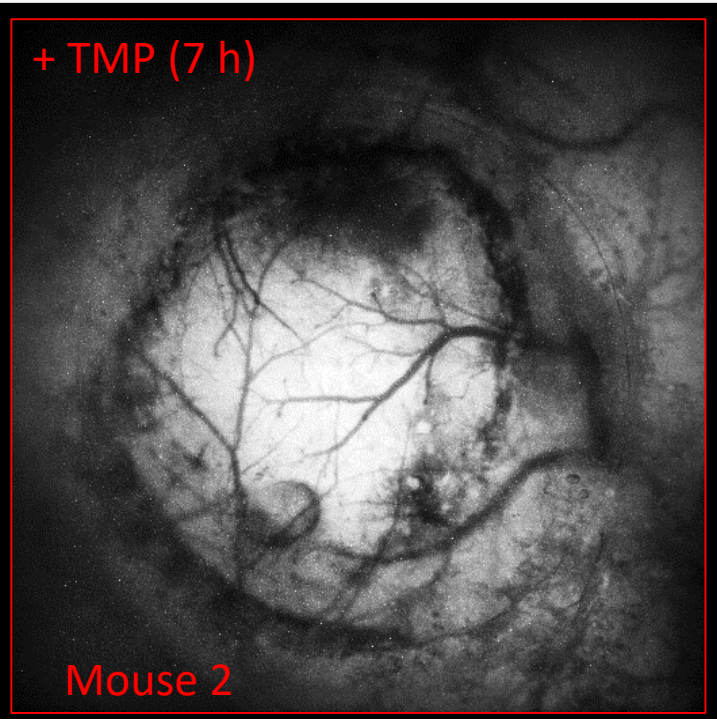
Inducer

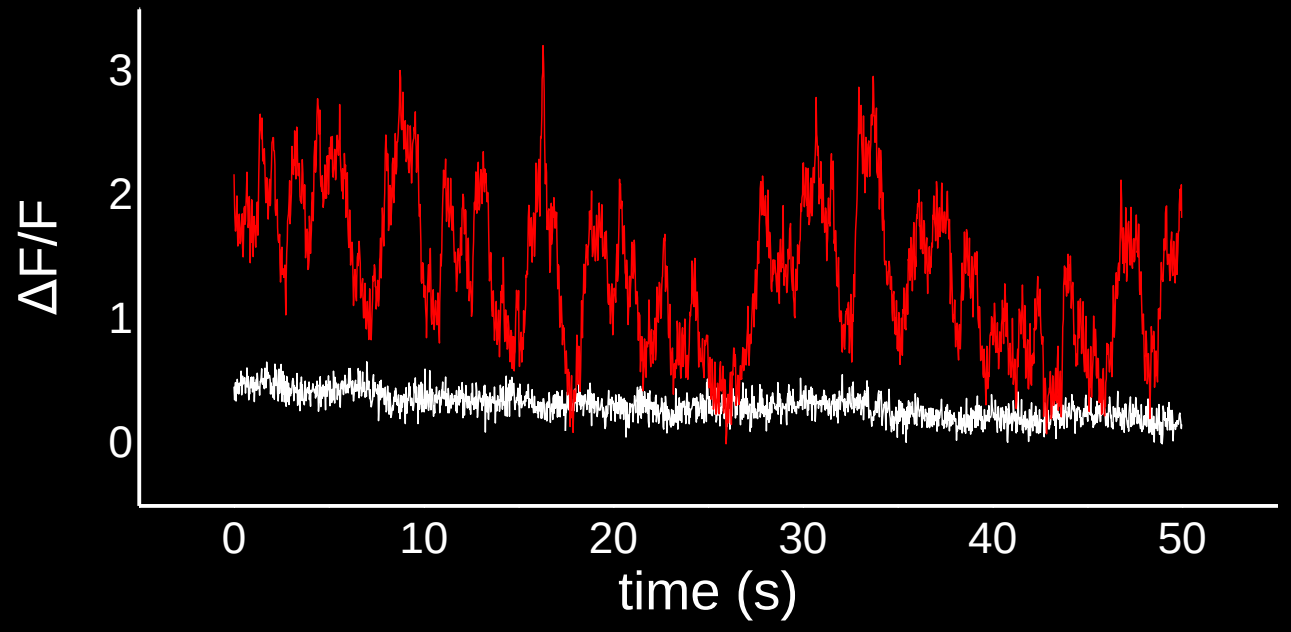


Wide-field imaging

- 2x objective
- 40 fps
- 20ms exp



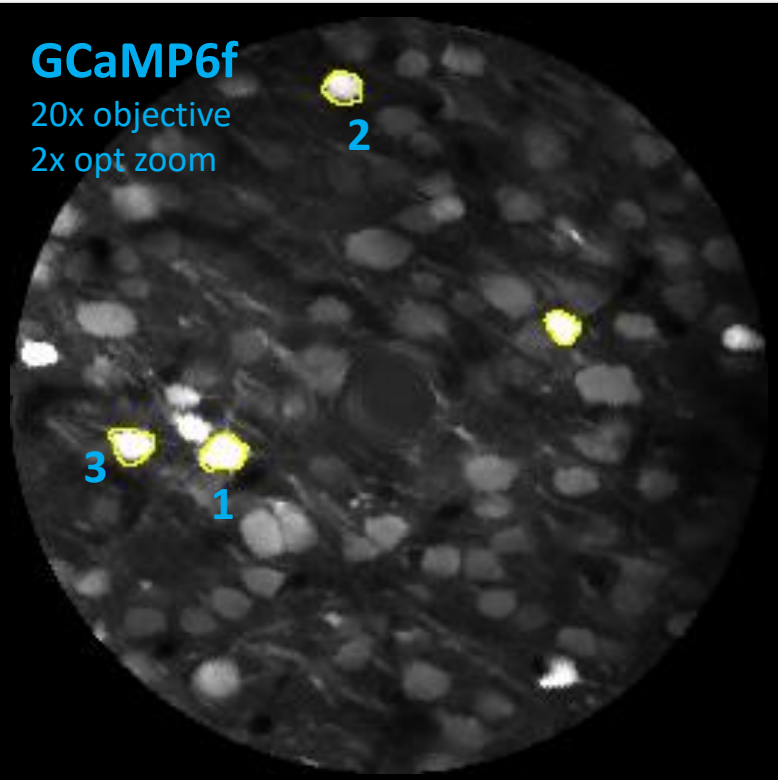


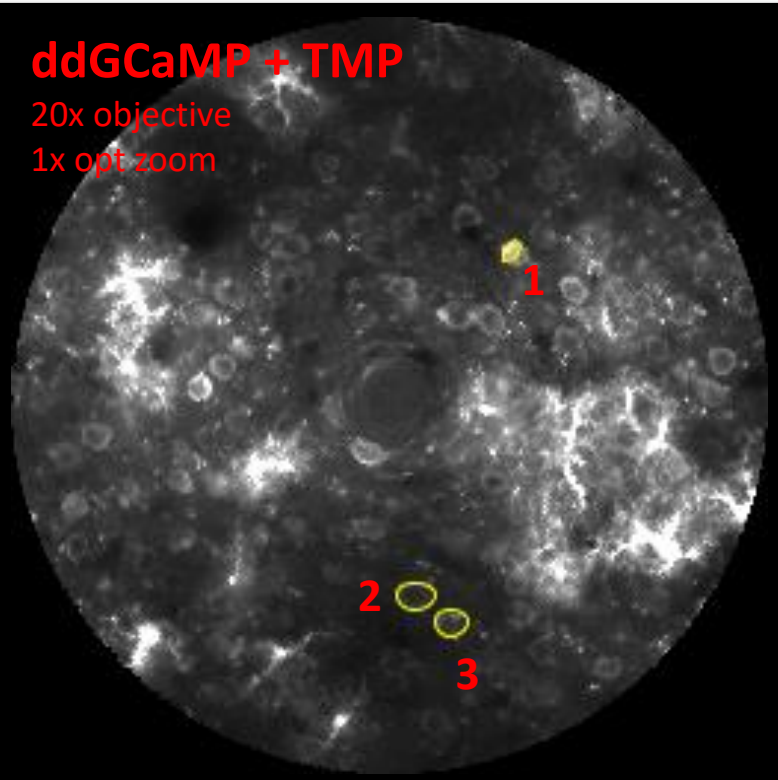


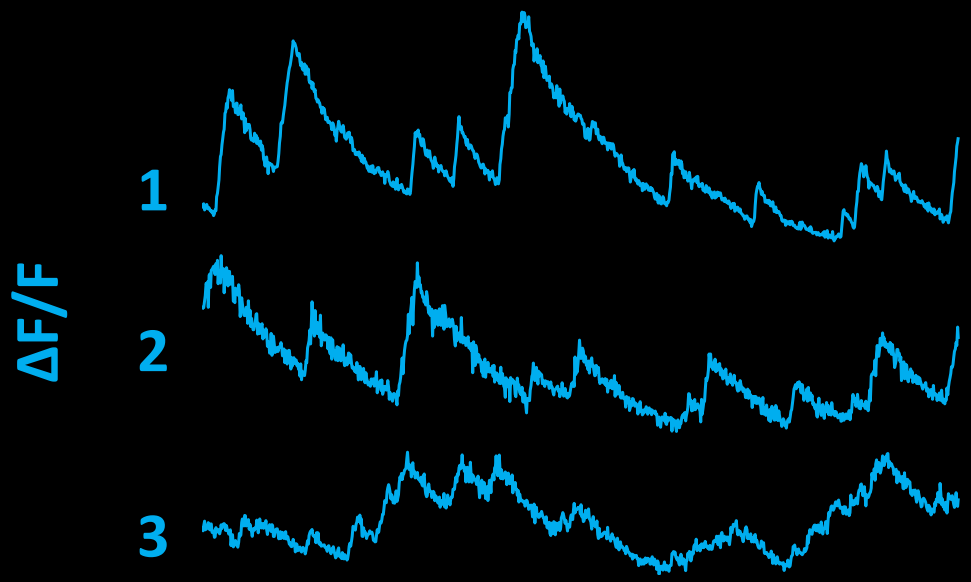
IntraVentricular Injections

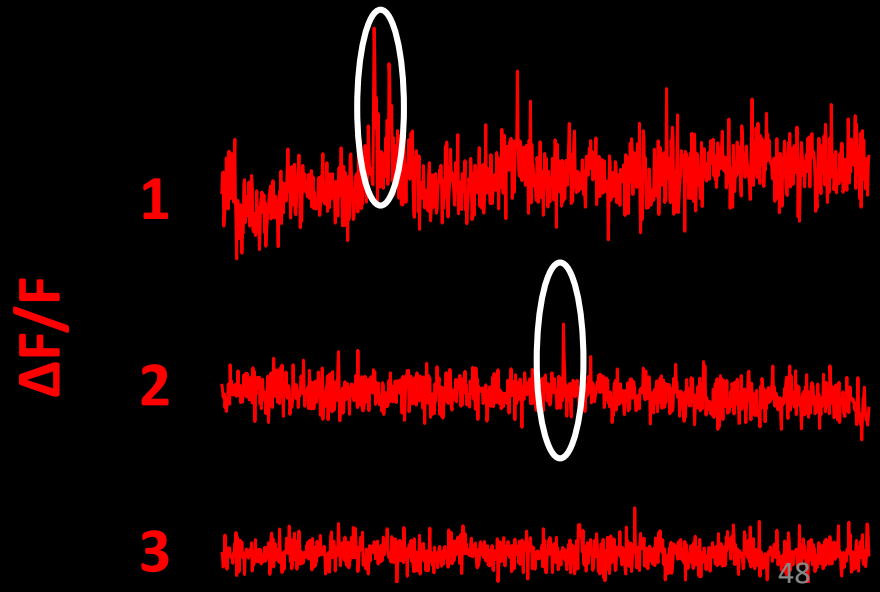
Two Photon imaging:

- Cell bodies
- Dendrites









IntraVentricular Injections

Two Photon imaging:

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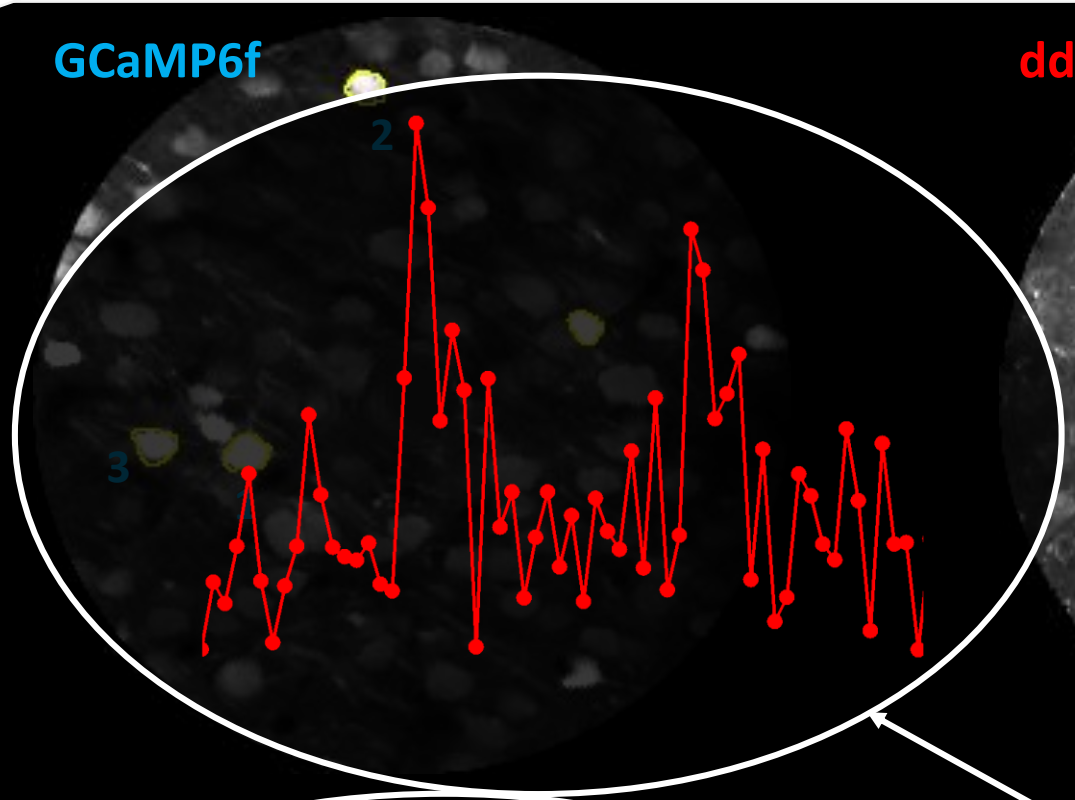
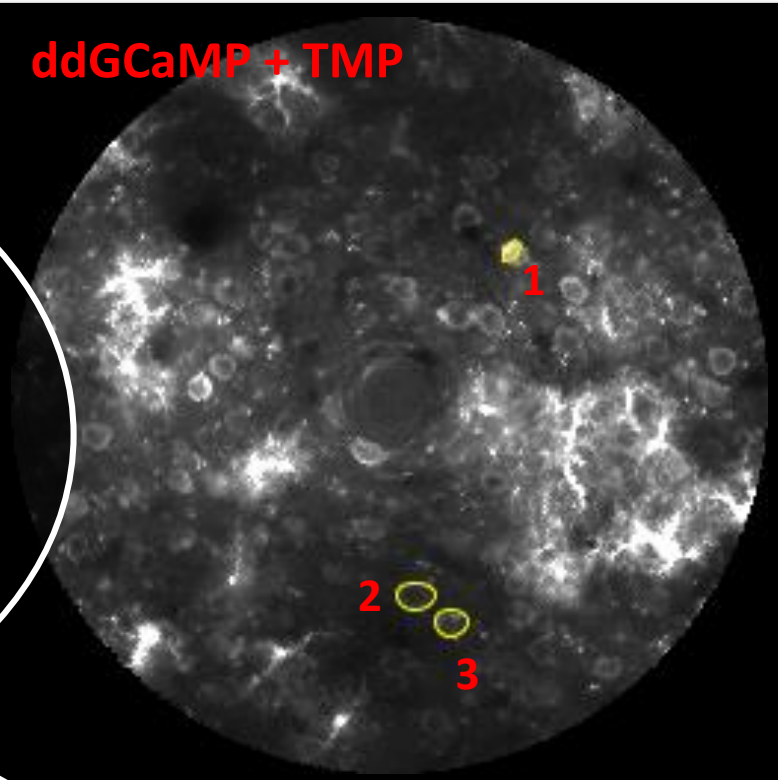
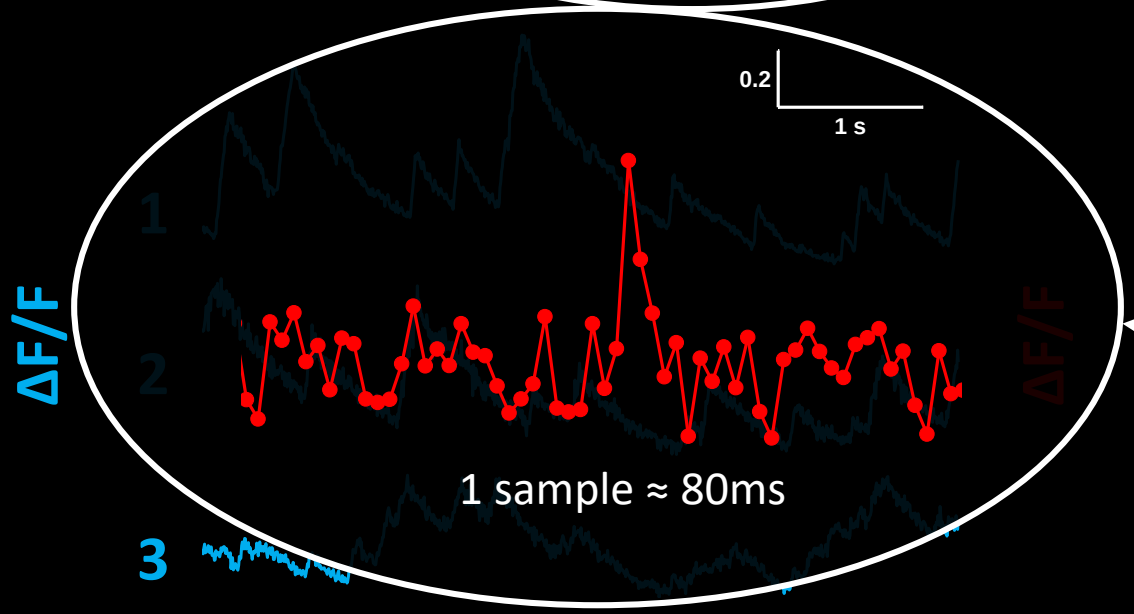
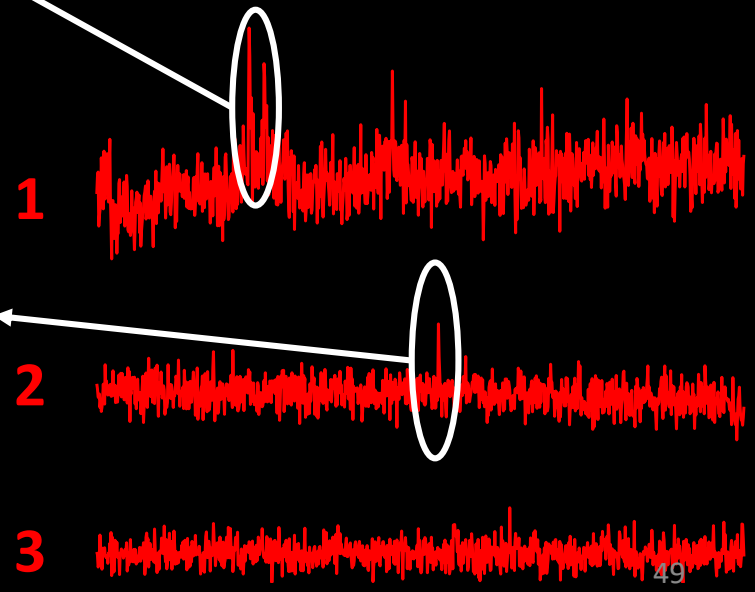
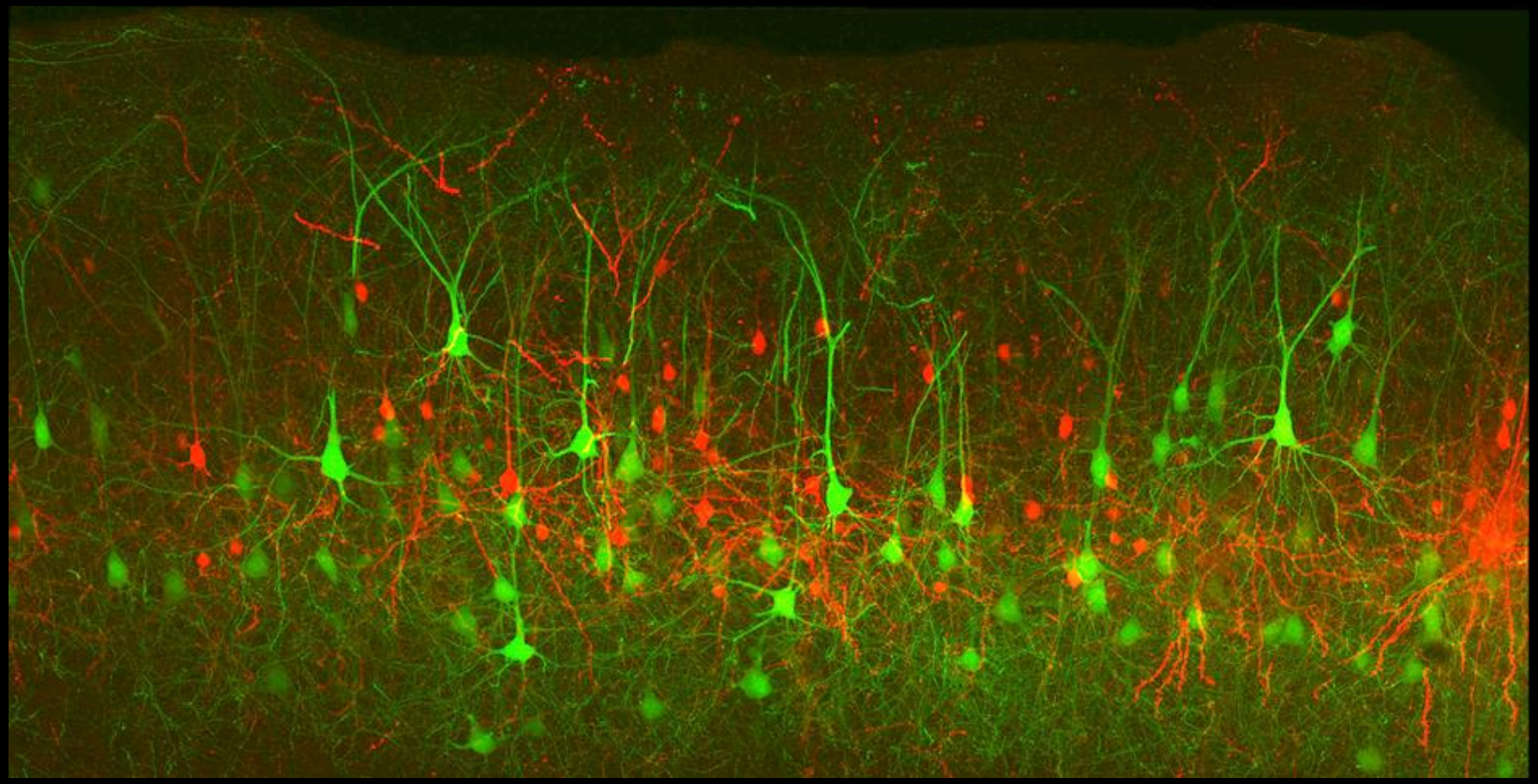






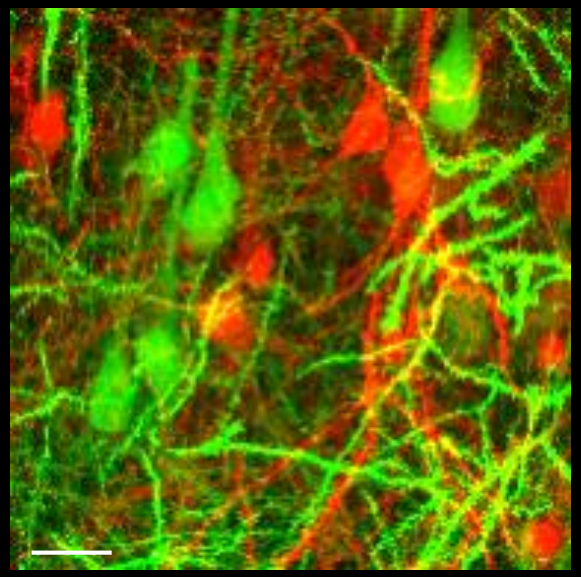


Ex vivo analysis: PTEN knock out mosaic

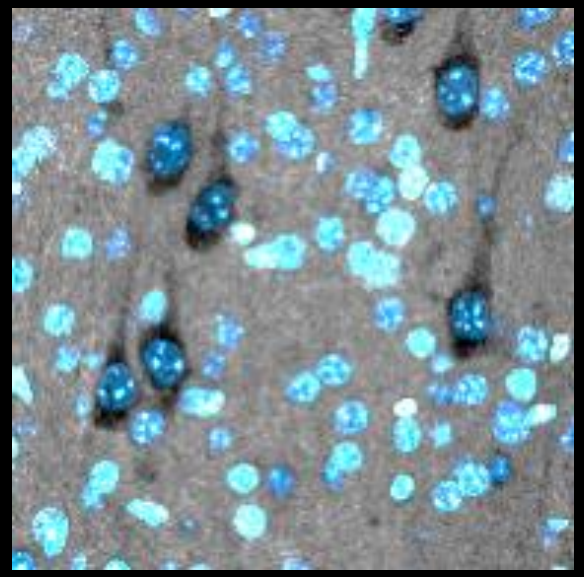


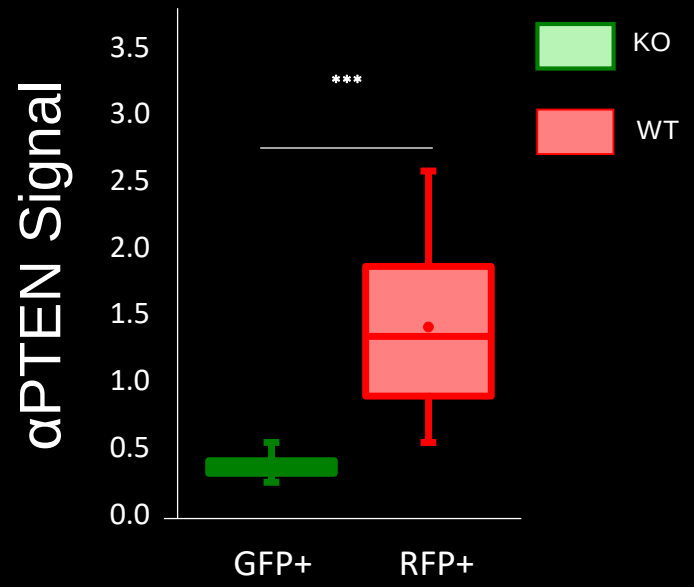
Ex vivo analysis: PTEN knock out mosaic

RFP GFP

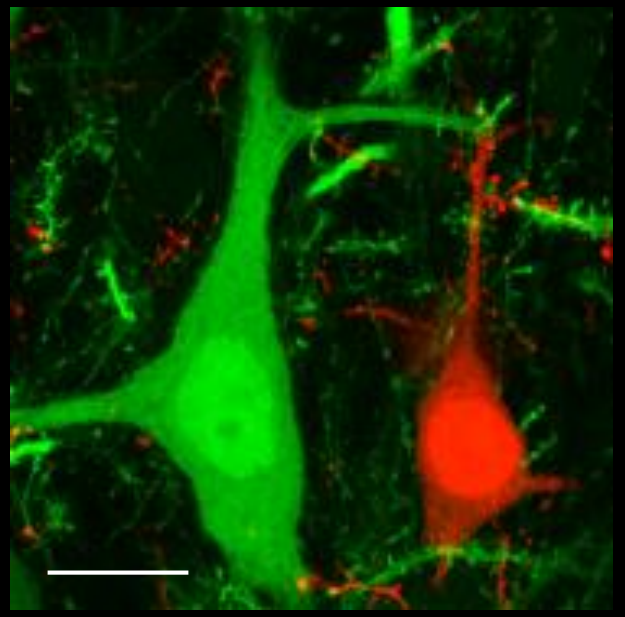


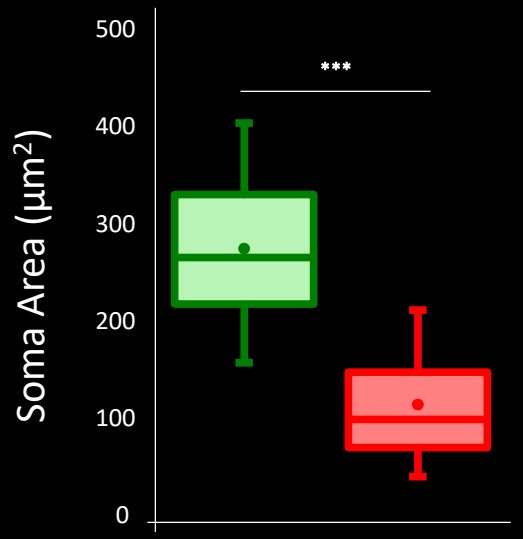
Hoechst αPTEN

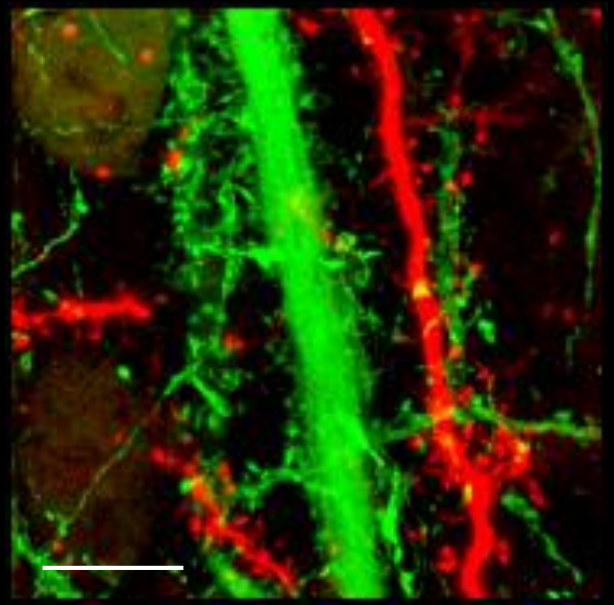


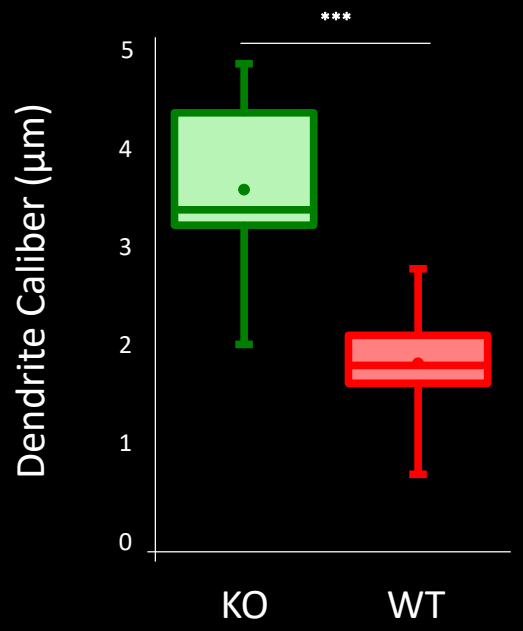


In vivo physiology: PTEN knock out mosaic



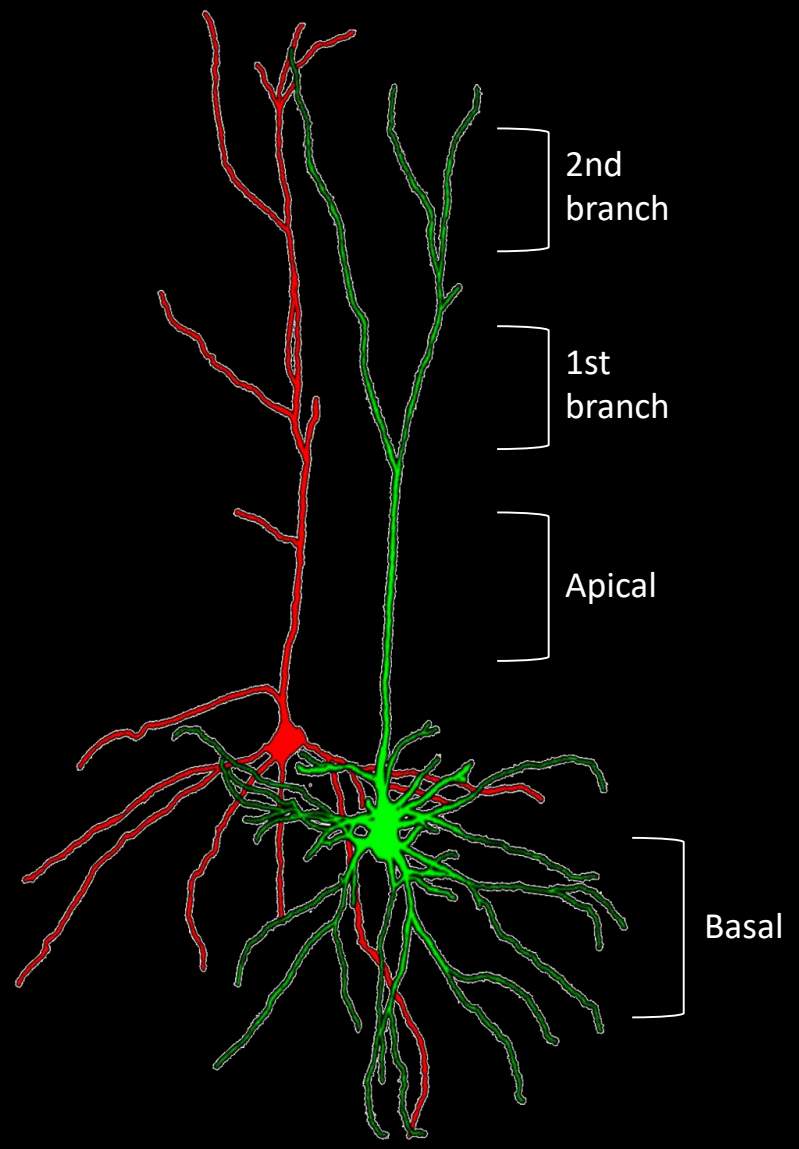


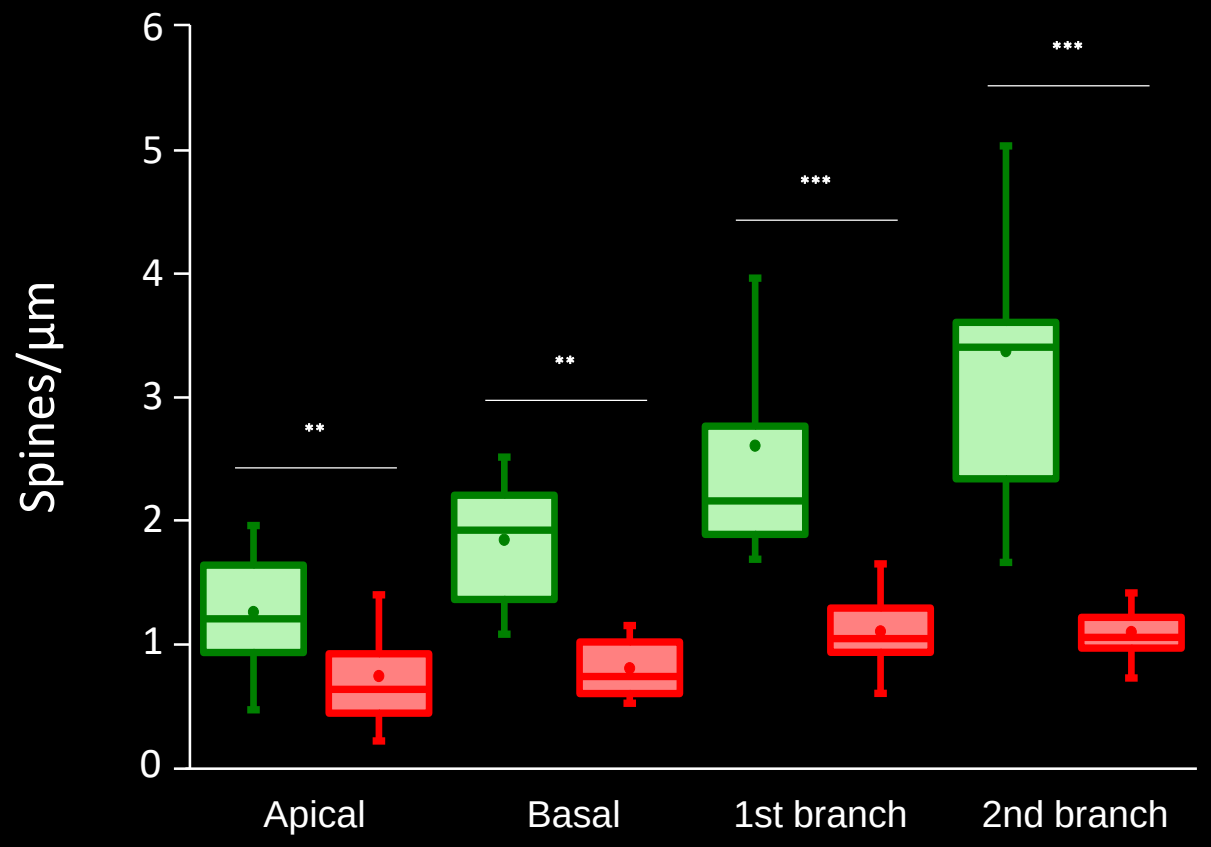




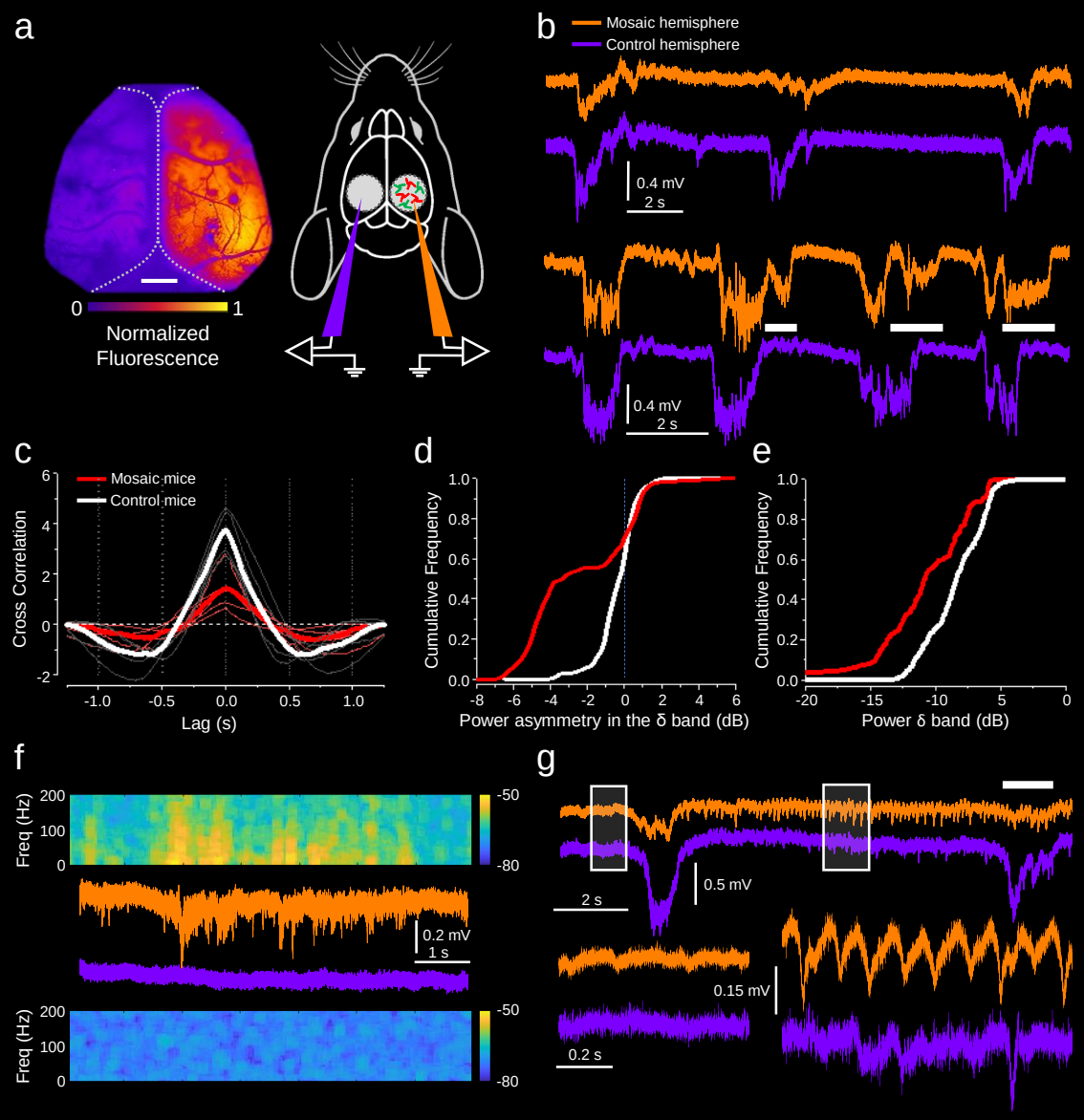
***Ex vivo* analysis: PTEN knock out mosaic**

Spine density

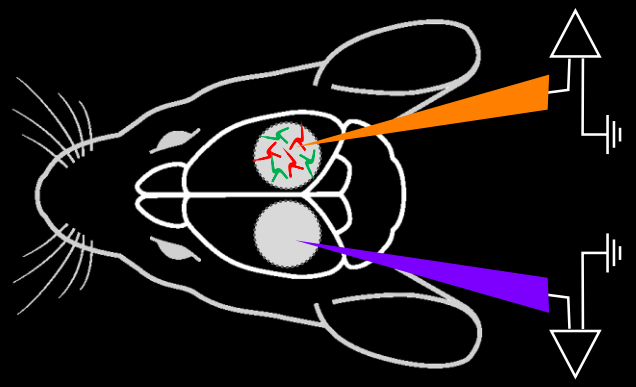


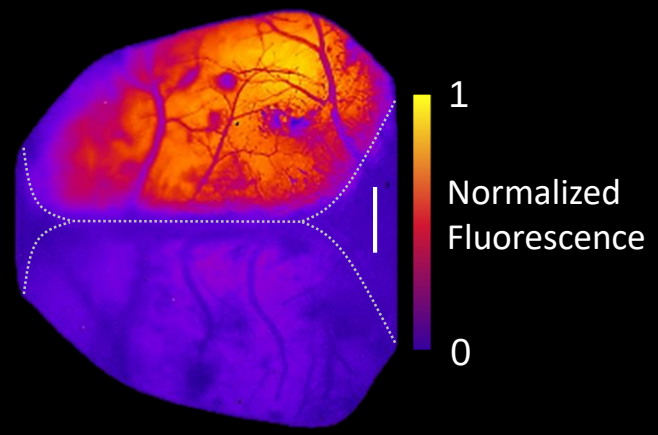


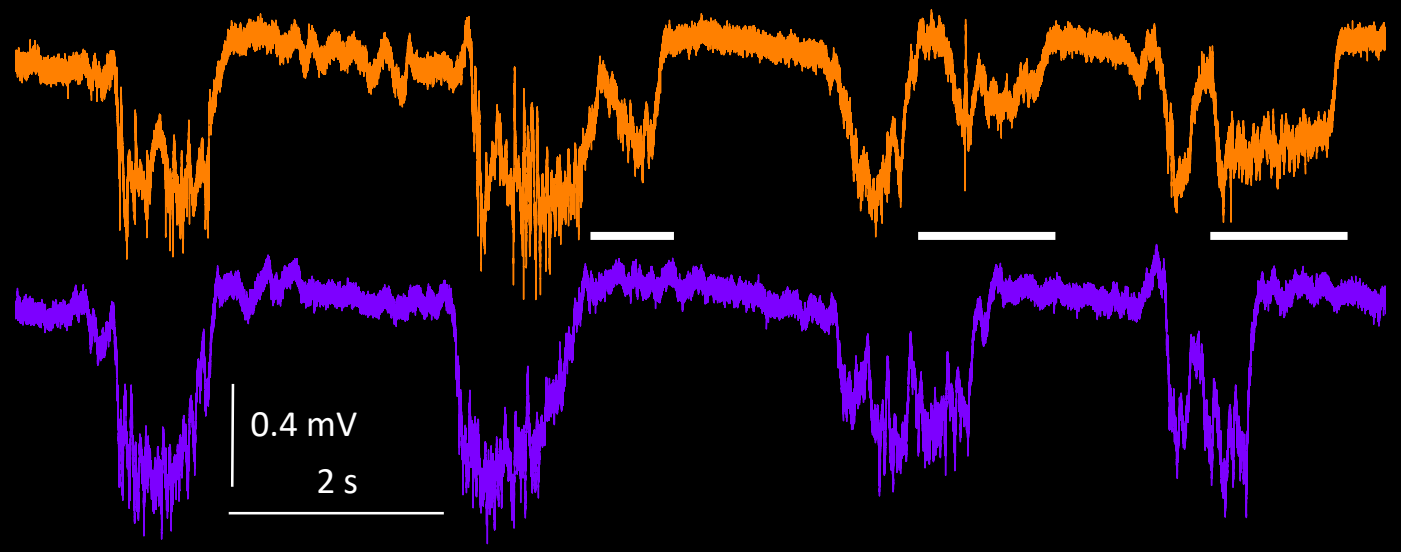
In vivo physiology: PTEN knock out mosaic



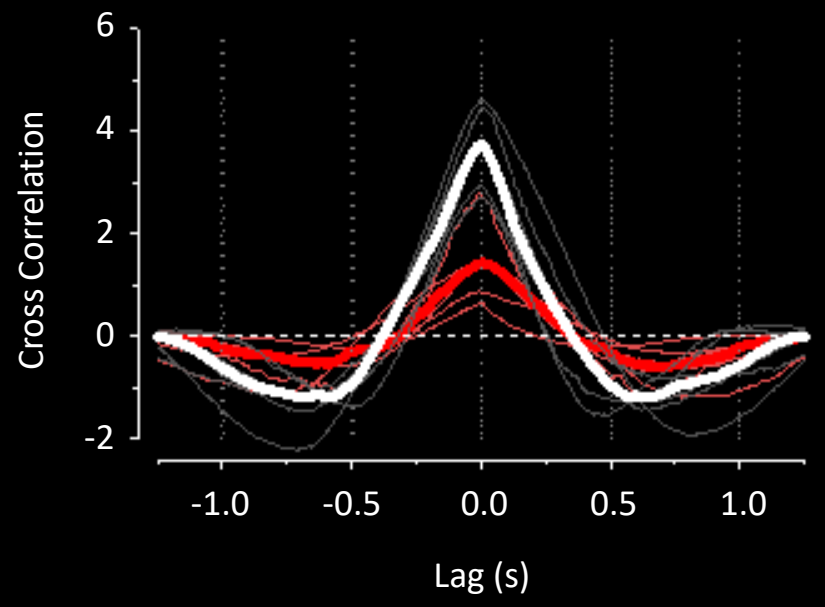
In vivo physiology: PTEN knock out mosaic





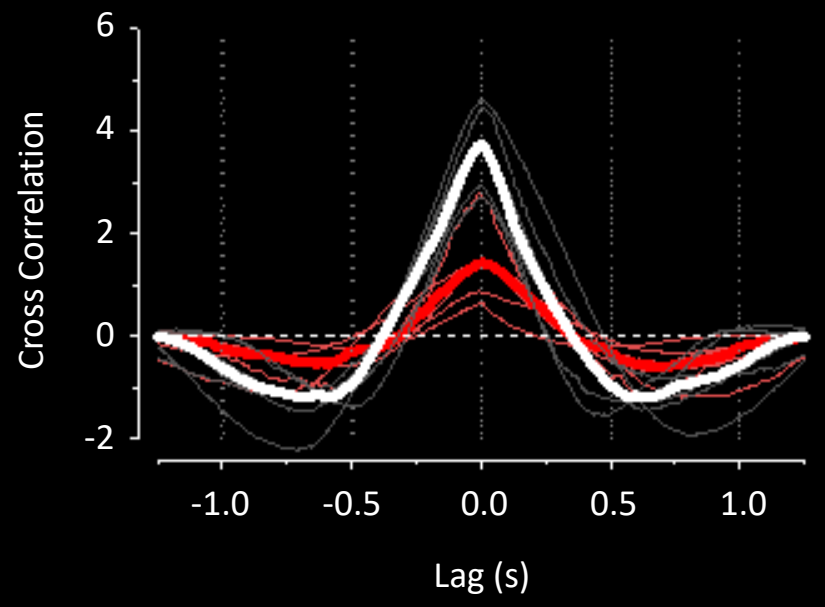


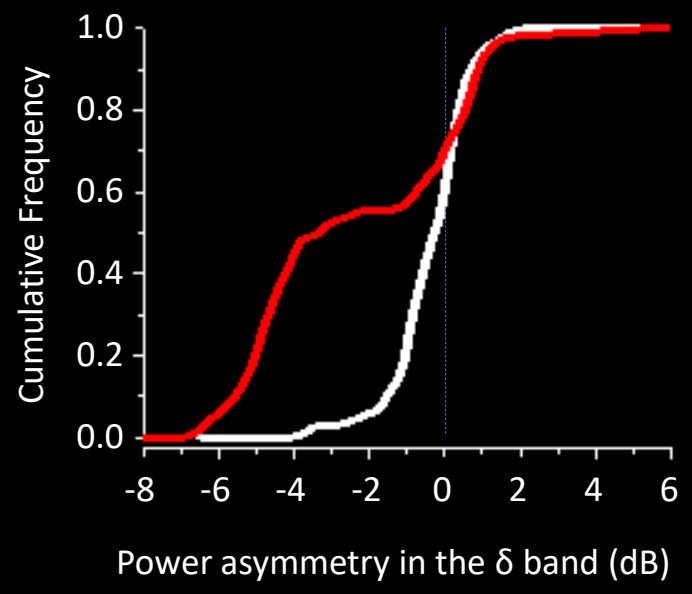
In vivo physiology: PTEN knock out mosaic



Mosaic mice
Control mice

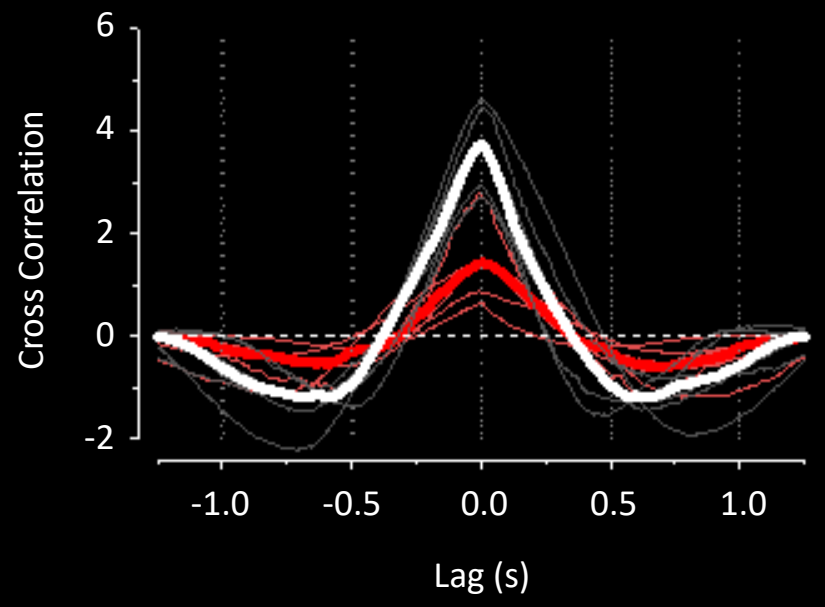
In vivo physiology: PTEN knock out mosaic

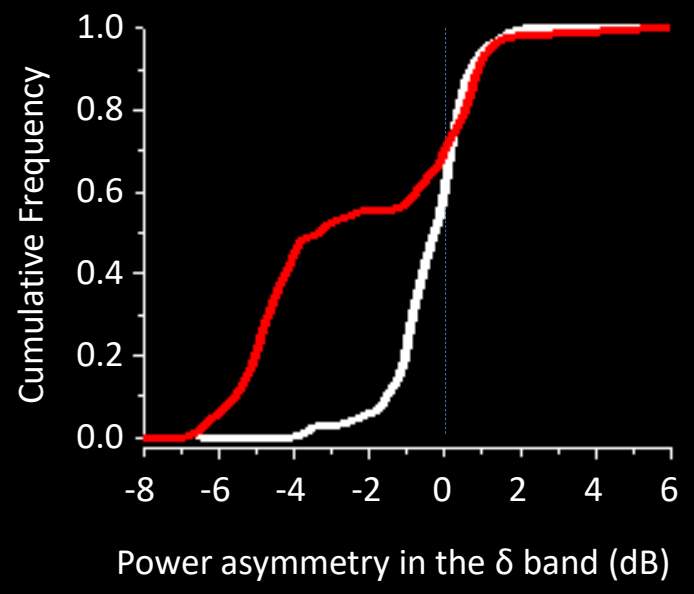


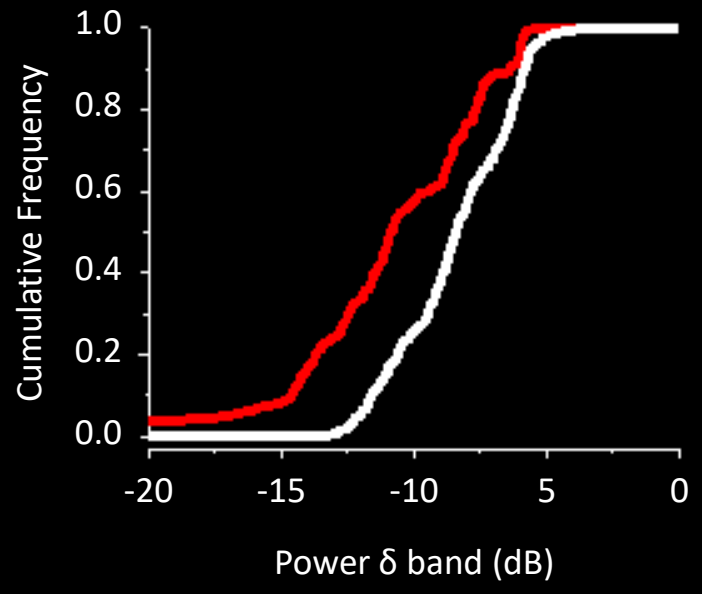


Mosaic mice
Control mice

In vivo physiology: PTEN knock out mosaic

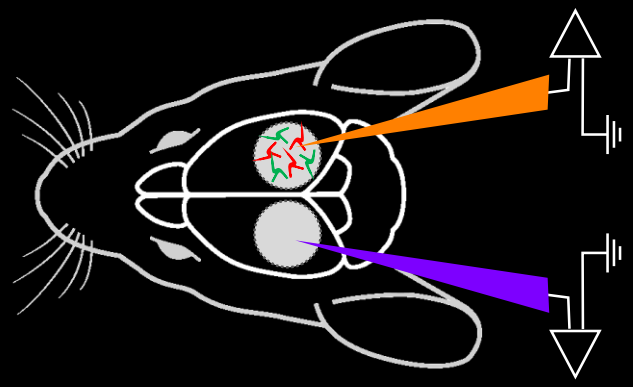


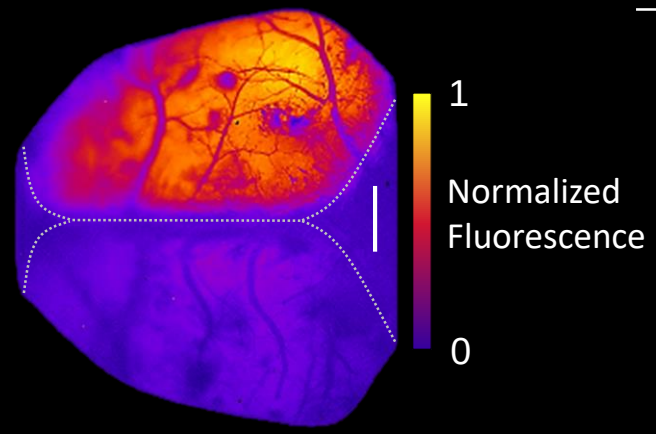


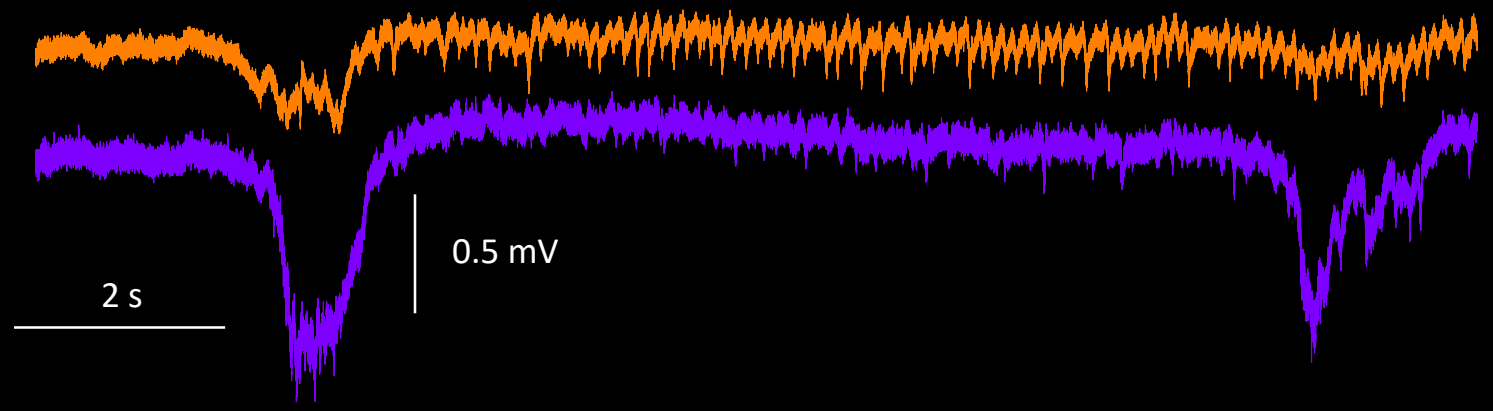


Mosaic mice
Control mice

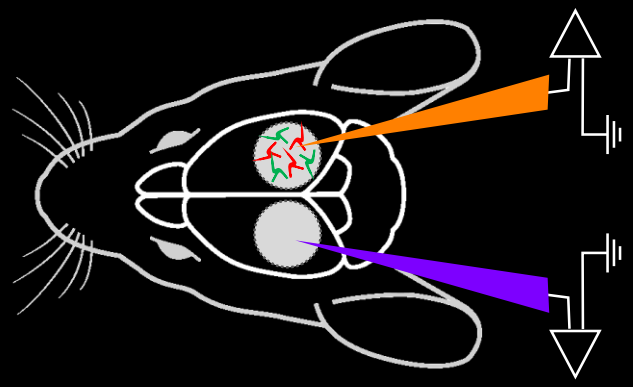
In vivo physiology: PTEN knock out mosaic

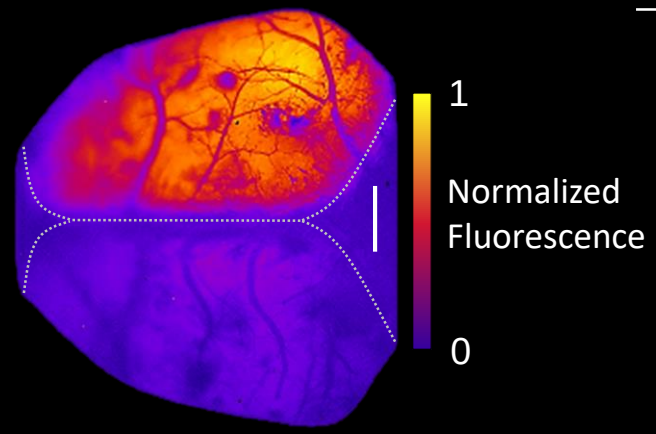


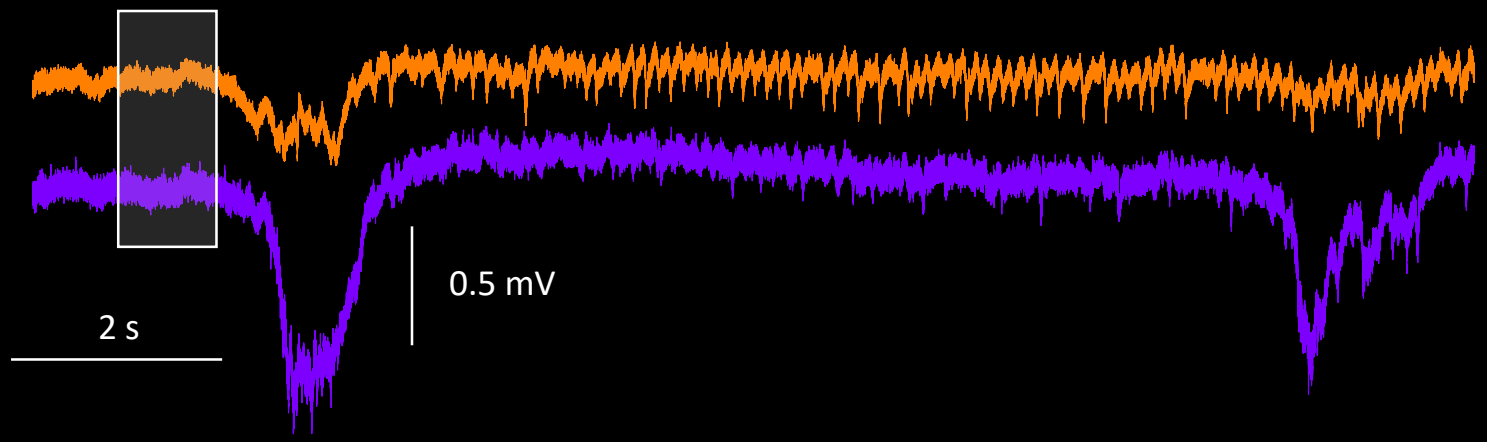


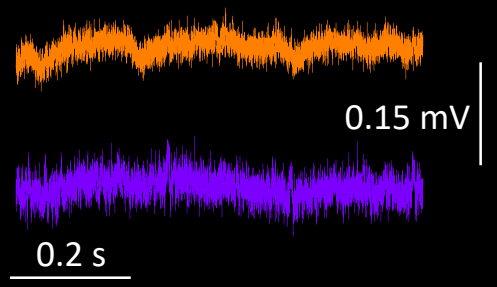


In vivo physiology: PTEN knock out mosaic









In vivo physiology: PTEN knock out mosaic