

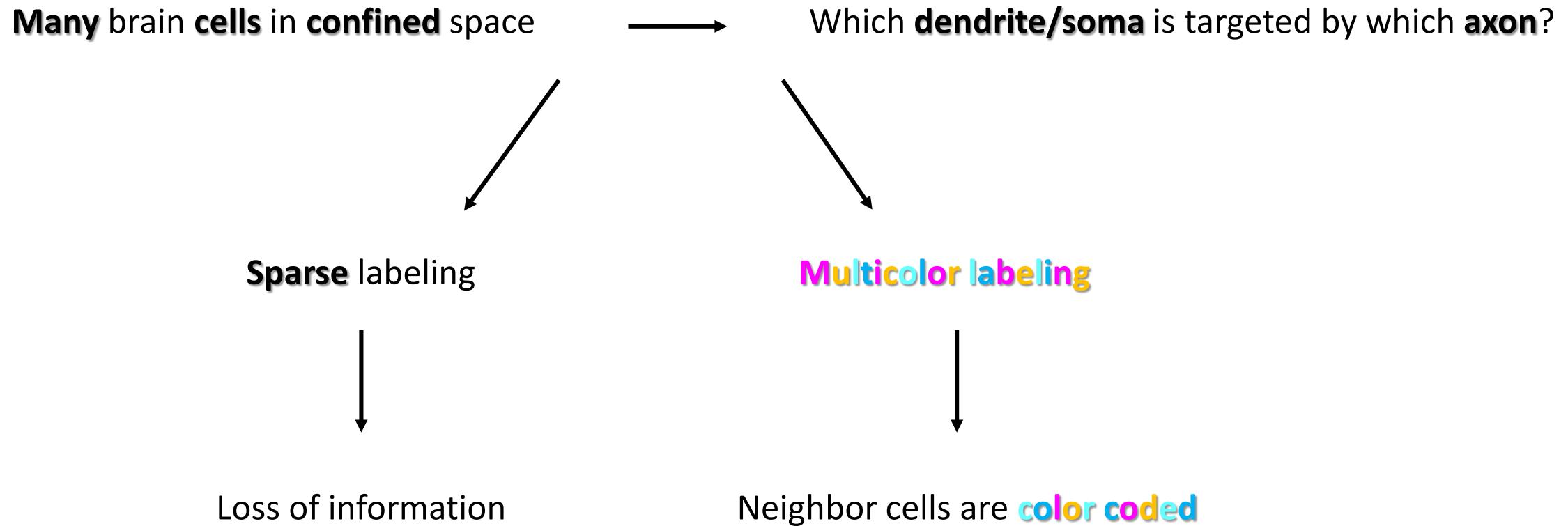
# From Brainbow to Tetbow – multicolor labeling for connectomics

Anna Maria Reuss

Technical Journal Club

12/05/2020

# The problem of connectivity mapping

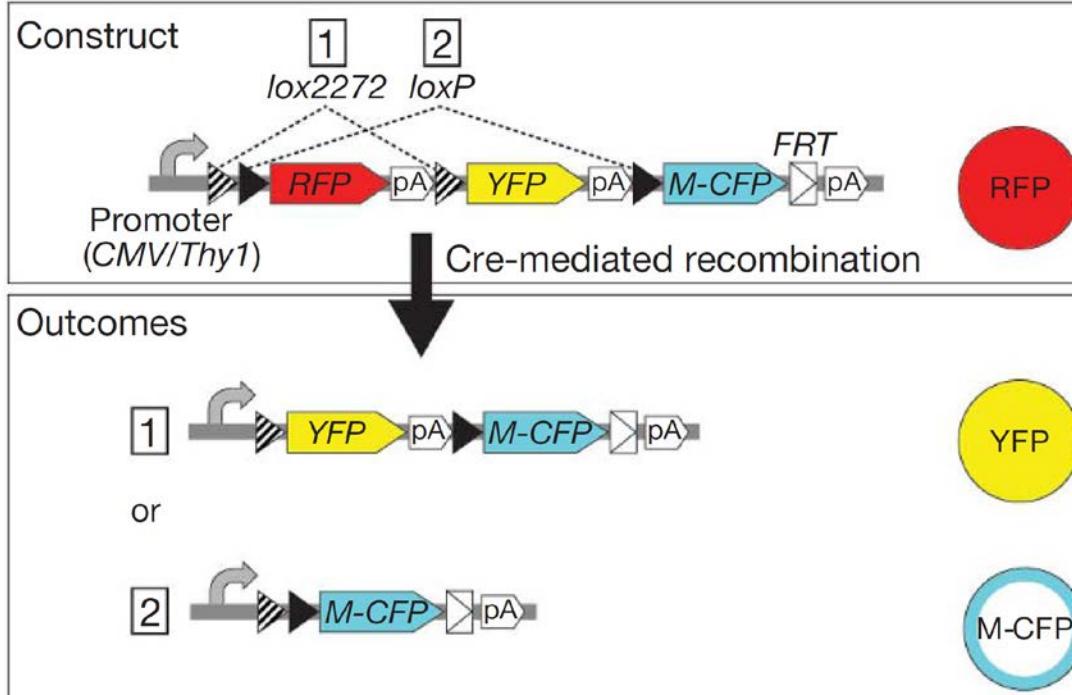
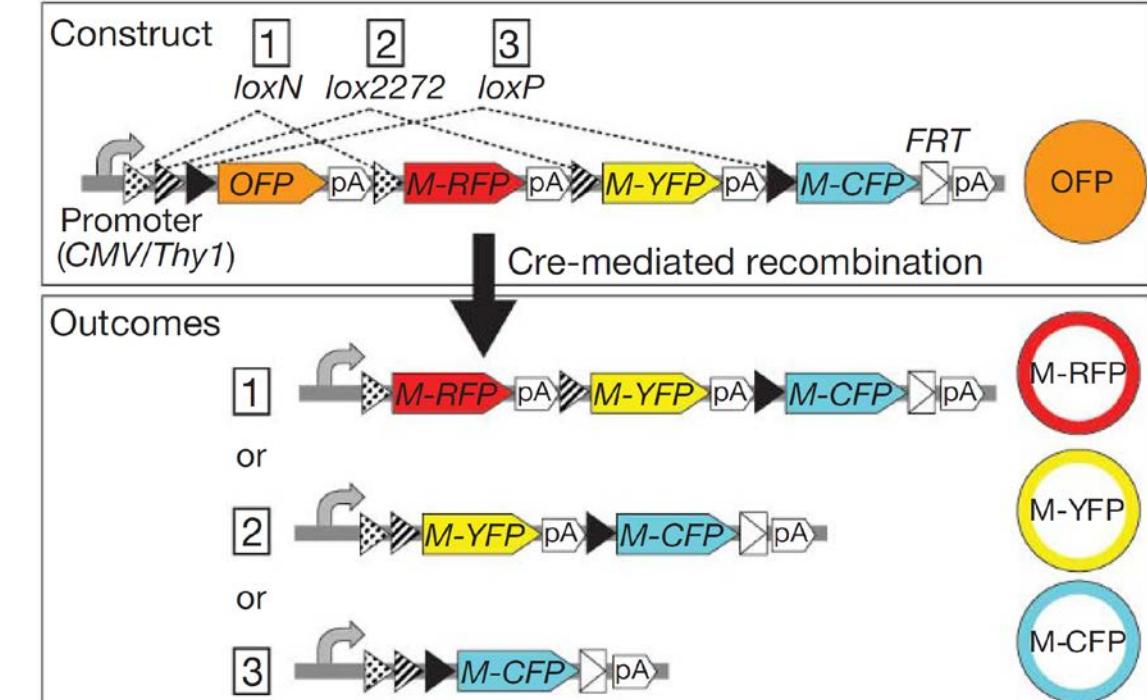
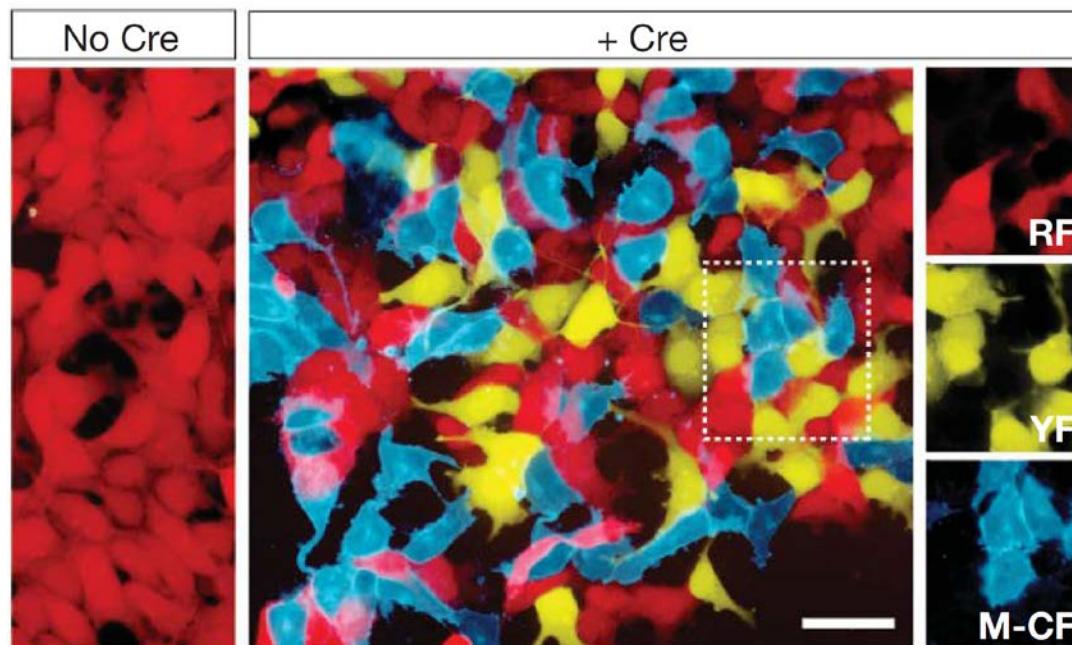
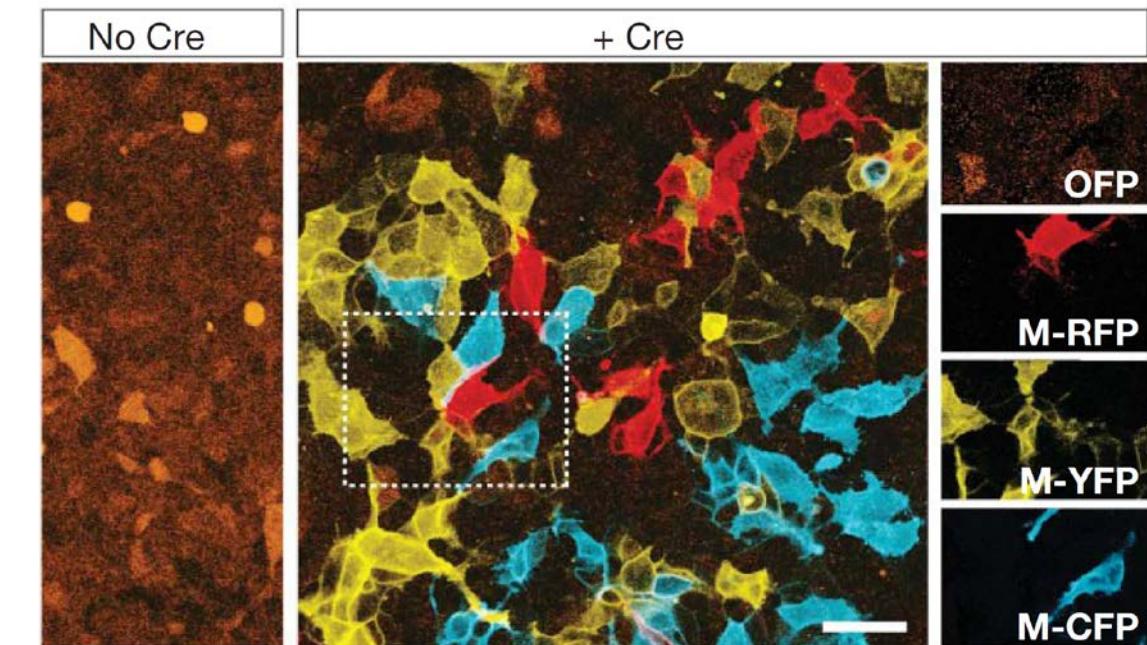


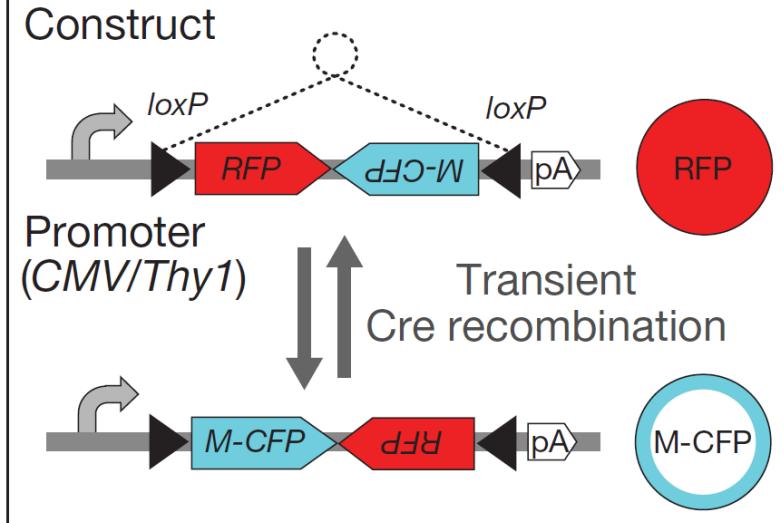
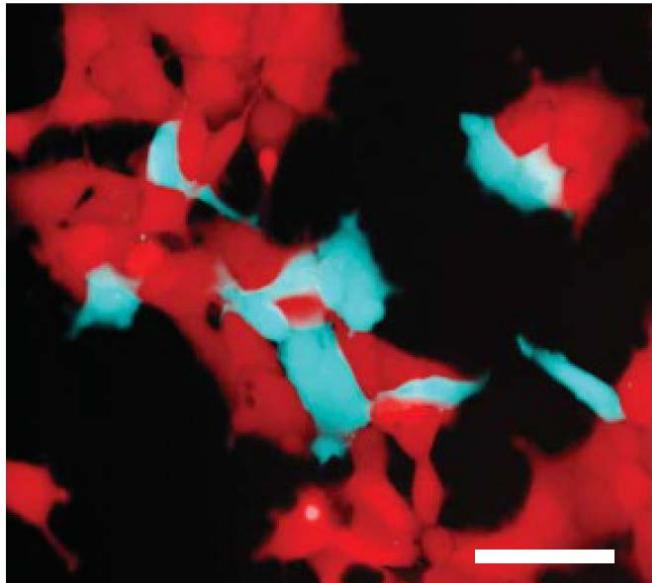
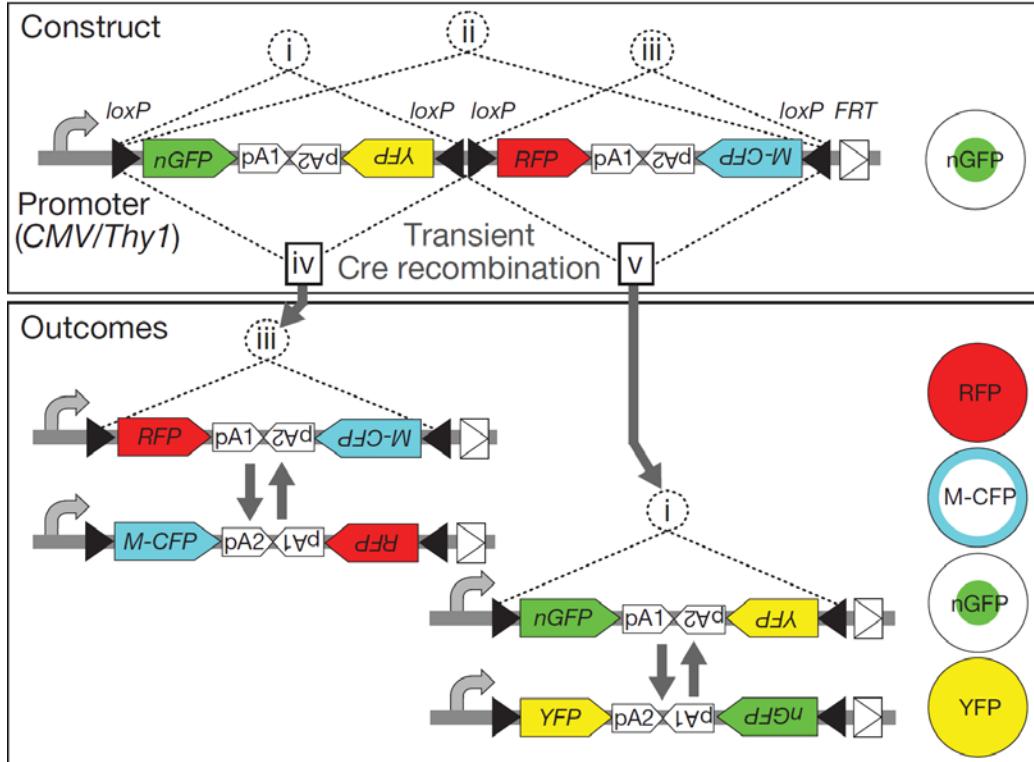
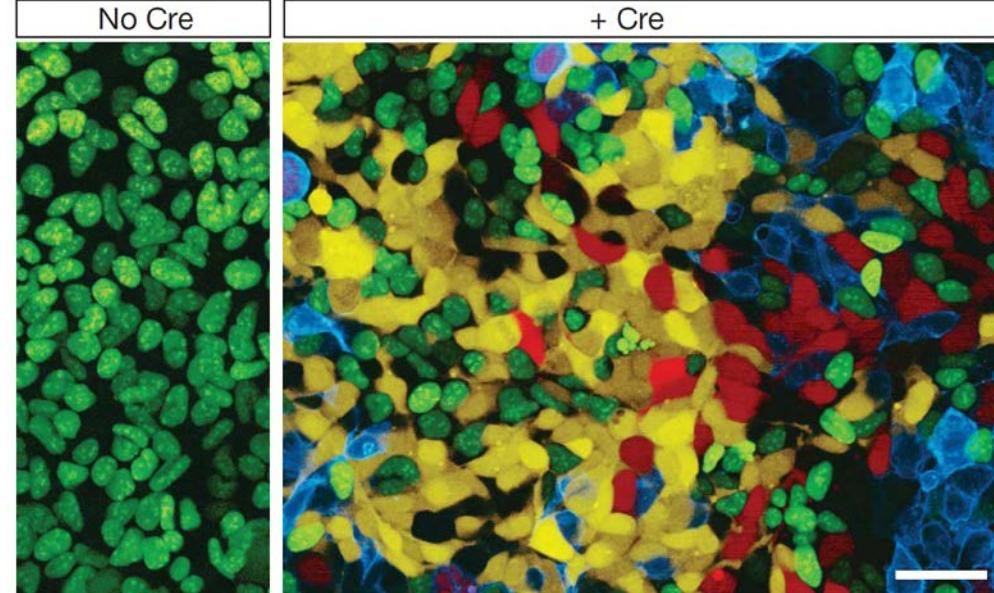
## ARTICLES

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# Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system

Jean Livet<sup>1</sup>, Tammy A. Weissman<sup>1</sup>, Hyuno Kang<sup>1</sup>, Ryan W. Draft<sup>1</sup>, Ju Lu<sup>1</sup>, Robyn A. Bennis<sup>1</sup>, Joshua R. Sanes<sup>1</sup>  
& Jeff W. Lichtman<sup>1</sup>

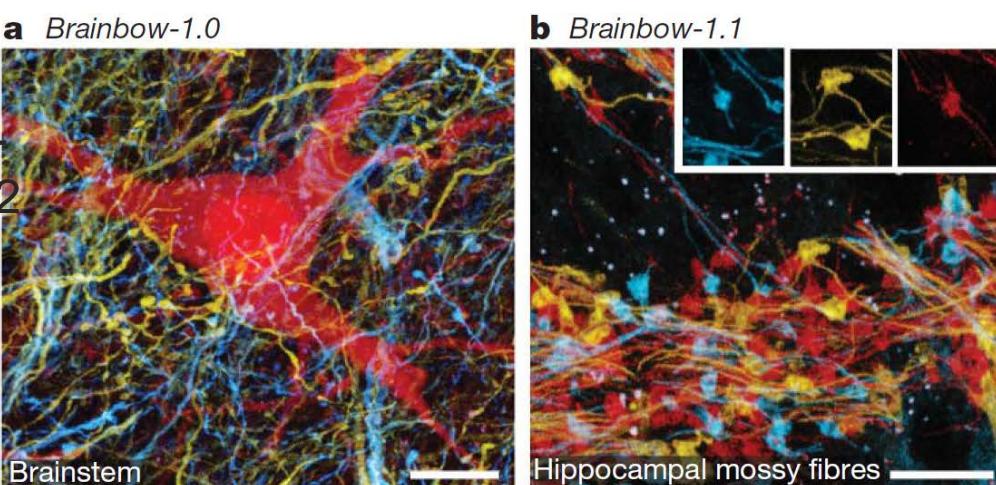
**a Brainbow-1.0****c Brainbow-1.1****b Test *in vitro*****d Test *in vitro***

**a Brainbow-2.0****b Test *in vitro*****c Brainbow-2.1****d Test *in vitro***

Thy1-Brainbow<sup>+-</sup>  
CAGGS-CreERT2  
 $\frac{+/-}{+/-}$

↓

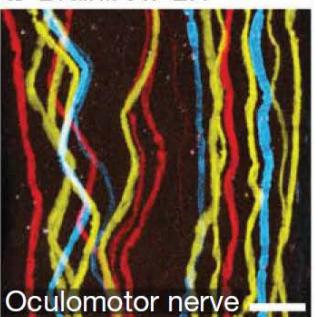
# Tamoxifen induction



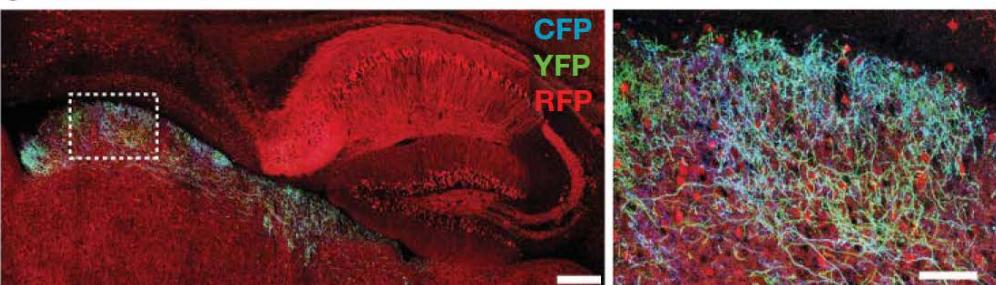
## **c** Brainbow-2.0



• Brainbow-2.1



### e Restriction of recombination

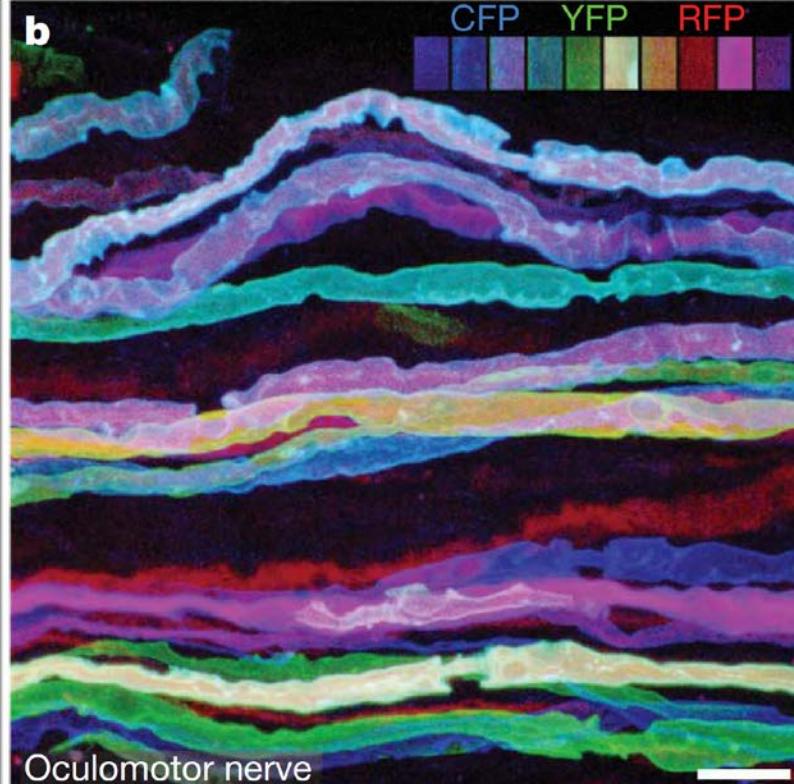


## Combinatorial XFP expression

## a XFP combinations

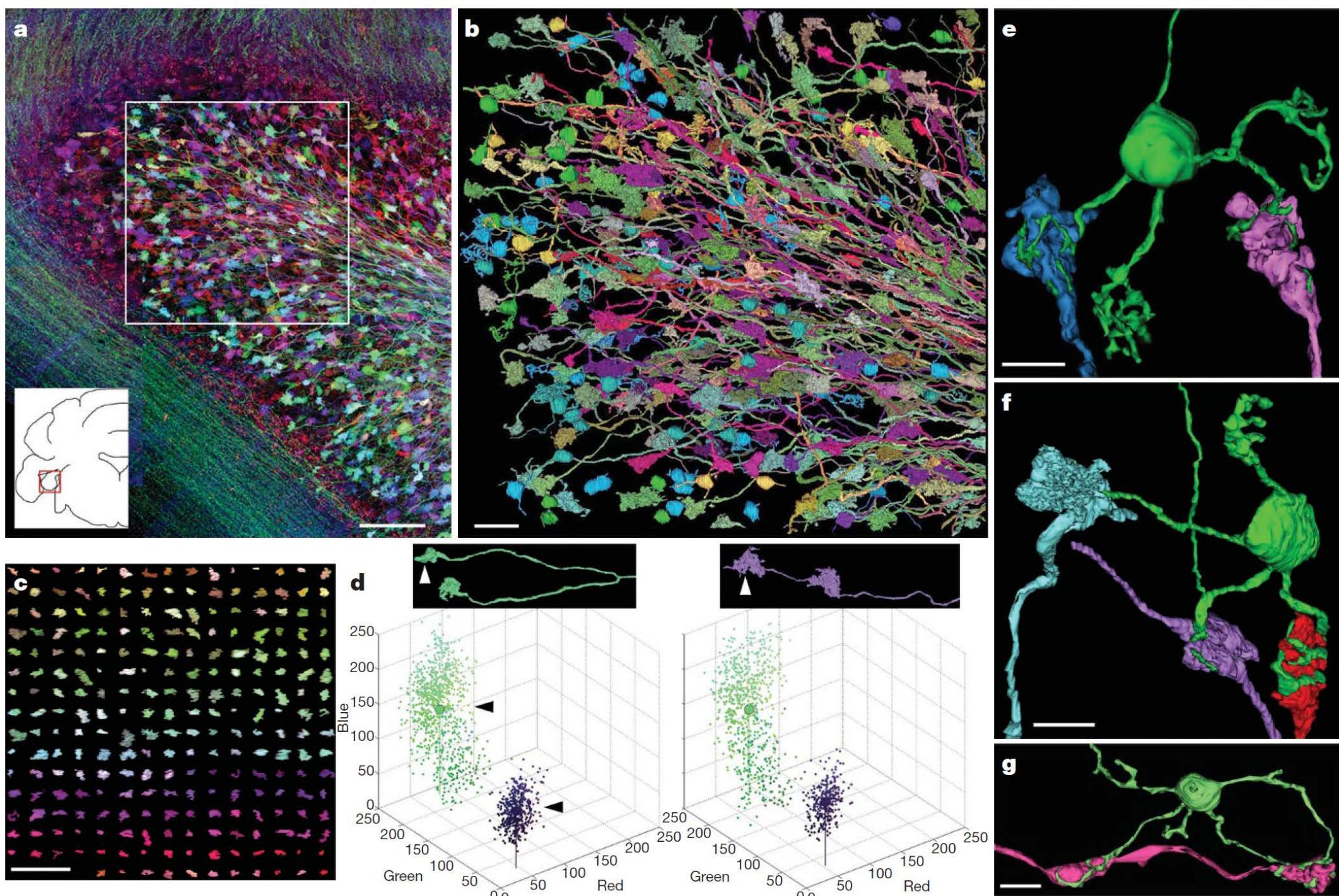
Outcome for each copy			Resulting colour
1	2	3	
C	C	C	Blue
C	C	Y	Light blue
C	Y	Y	Blue-green
Y	Y	Y	Green
Y	Y	R	Light green
Y	R	R	Orange
R	R	R	Red
R	R	C	Magenta
R	C	C	Purple
R	C	Y	Grey

b



# Cerebellar Circuit Tracing

Reconstruction of  
341 axons,  
236 mossy fibre  
presynaptic rosettes,  
and 93 granule cells

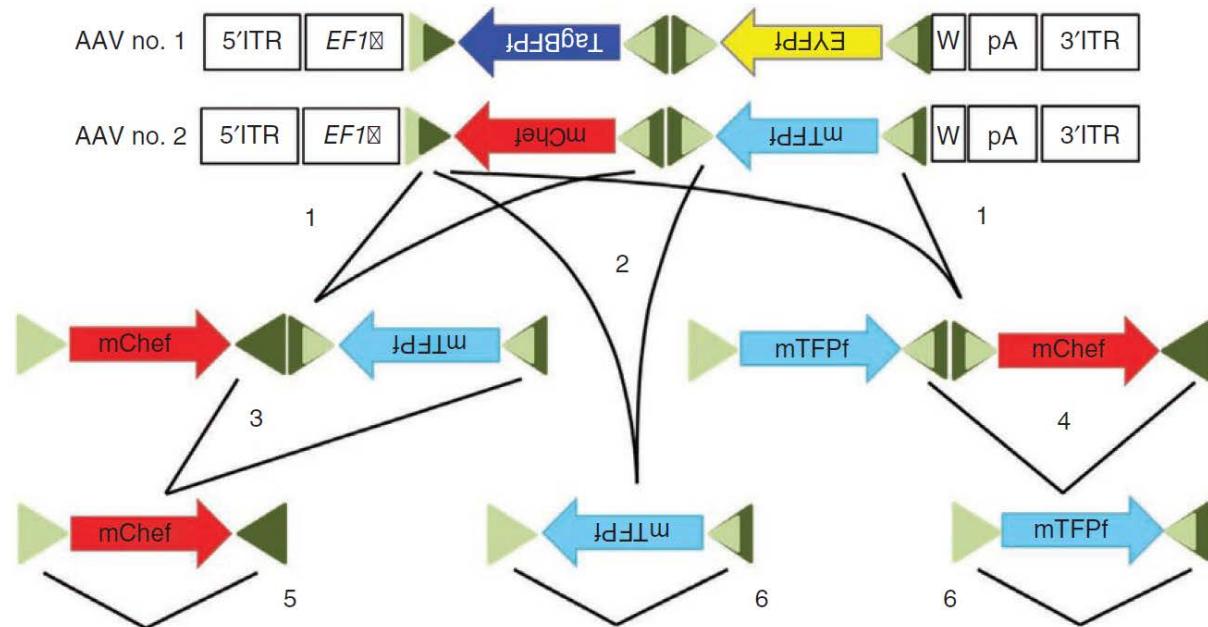
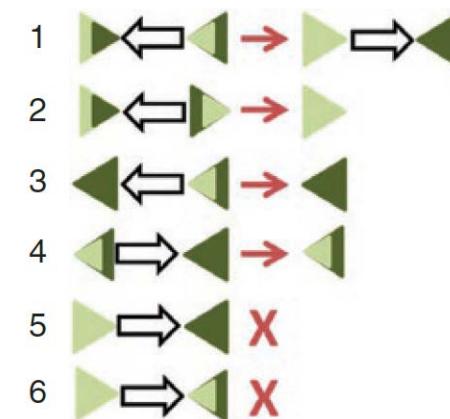


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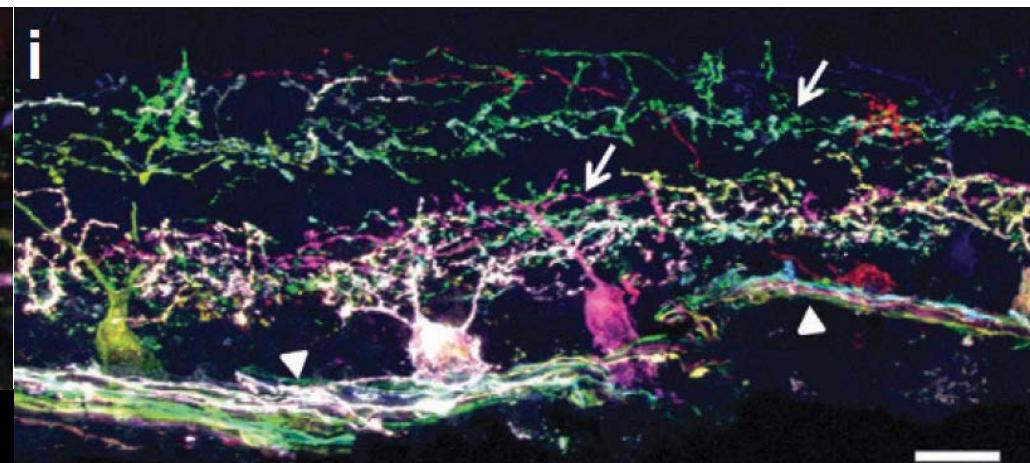
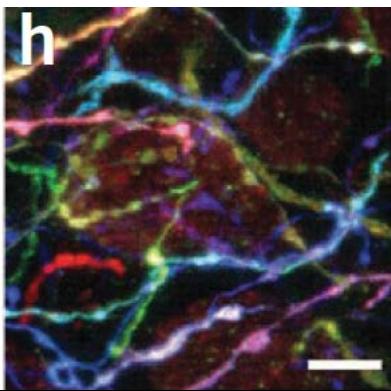
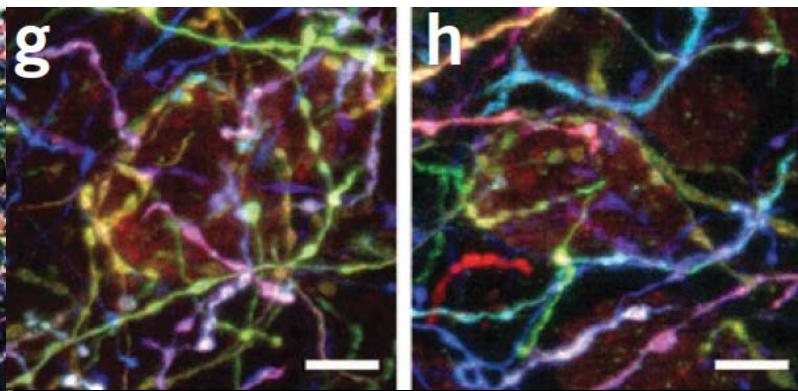
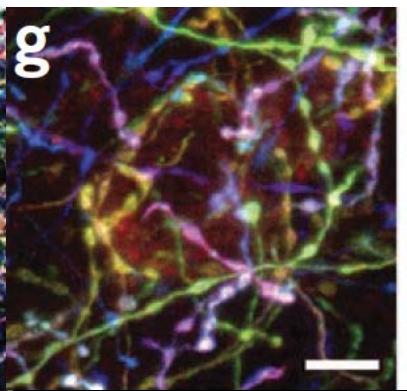
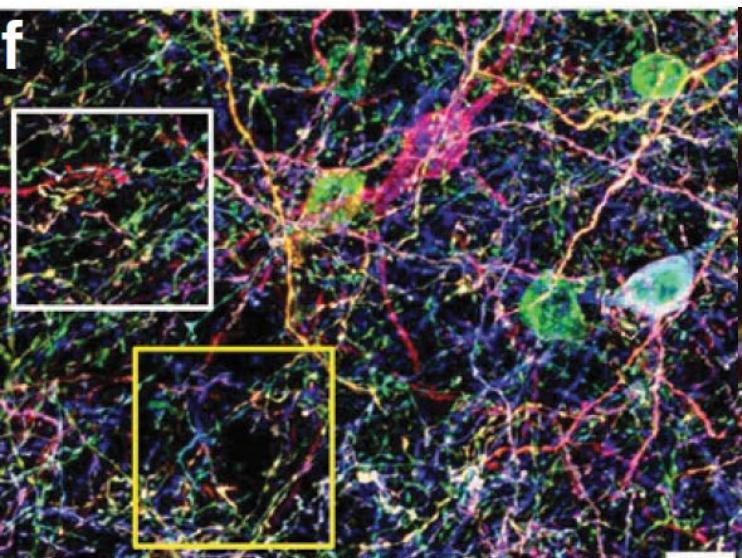
**RESOURCE**

# Improved tools for the Brainbow toolbox

Dawen Cai<sup>1,2</sup>, Kimberly B Cohen<sup>1,2</sup>, Tuanlian Luo<sup>1,2</sup>, Jeff W Lichtman<sup>1,2</sup> & Joshua R Sanes<sup>1,2</sup>

**a****b****c**

AAV no. 1	AAV no. 2	Output
Blue arrow	Red arrow	Magenta
Blue arrow	Blue arrow	Blue
Black	Black	Black
Yellow arrow	Red arrow	Orange
Yellow arrow	Blue arrow	Green
Red arrow	Red arrow	Red
Black	Blue arrow	Blue

**f**



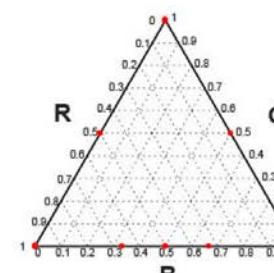
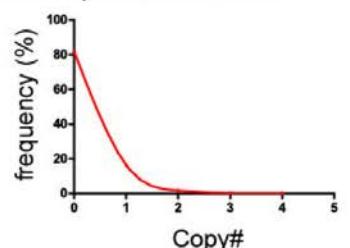
# Bright multicolor labeling of neuronal circuits with fluorescent proteins and chemical tags

**Richi Sakaguchi<sup>1,2,3</sup>, Marcus N Leiwe<sup>1,3</sup>, Takeshi Imai<sup>1,2,3\*</sup>**

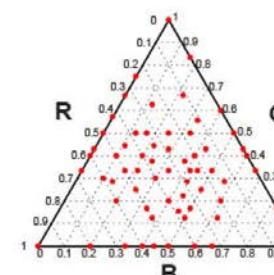
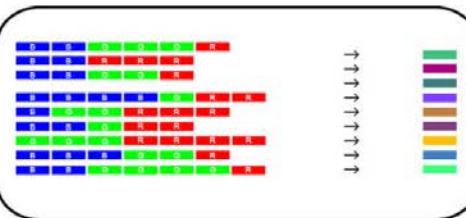
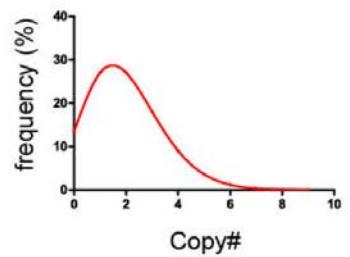
<sup>1</sup>Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan;

<sup>2</sup>Graduate School of Biostudies, Kyoto University, Kyoto, Japan; <sup>3</sup>Laboratory for Sensory Circuit Formation, RIKEN Center for Developmental Biology, Kobe, Japan

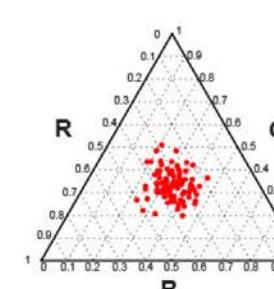
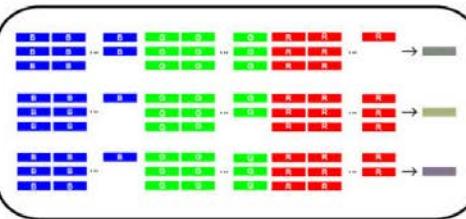
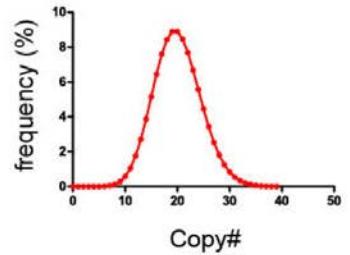
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**A**  
0.2 copies / cell / color

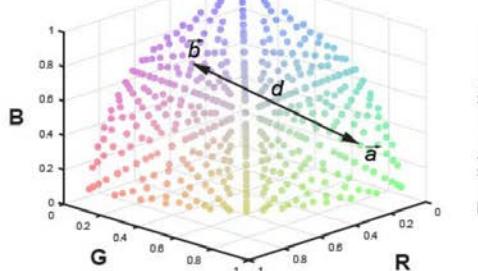
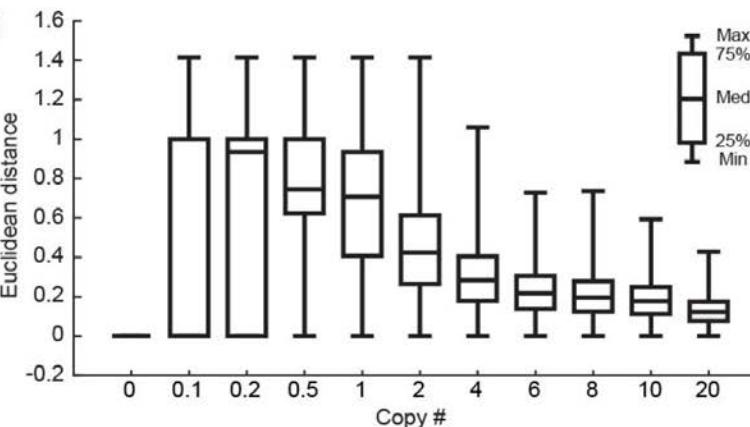
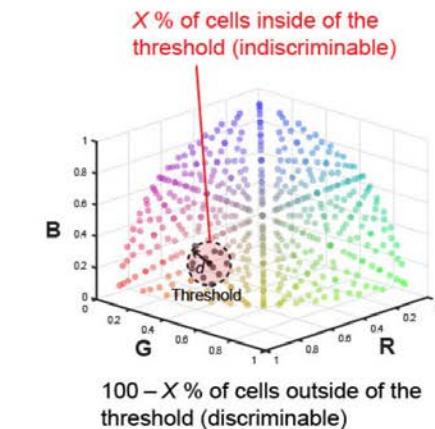
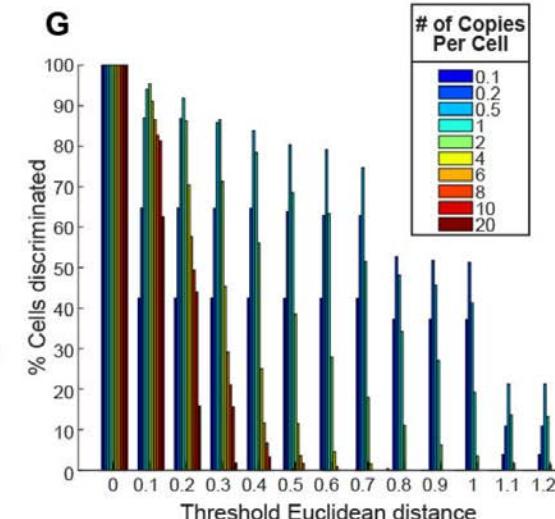
Copy number too small → only 1 of the 3 genes expressed in most cells

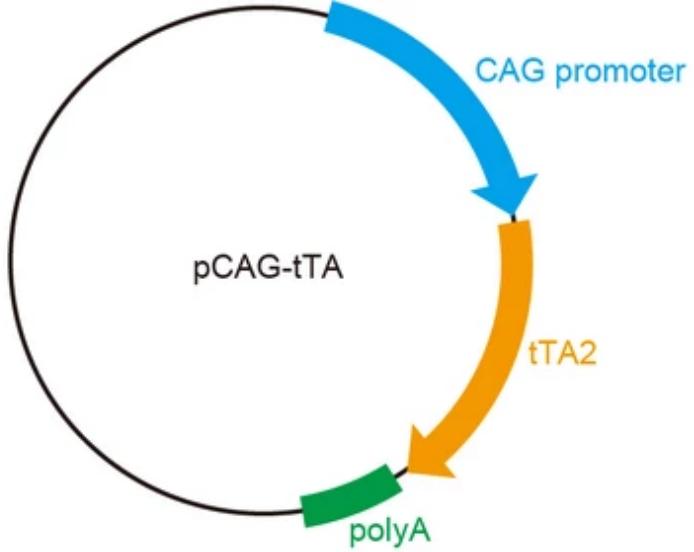
**B**  
2 copies / cell / color

2 copies → stochastic expression of 3 different genes

**C**  
20 copies / cell / color

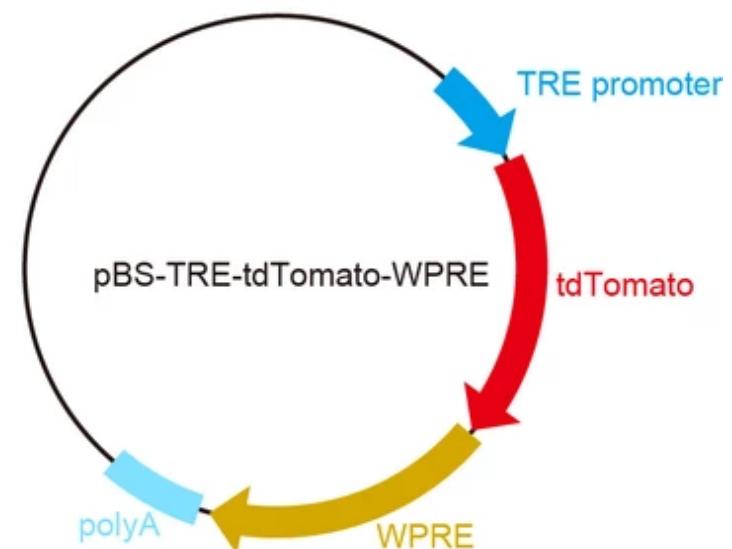
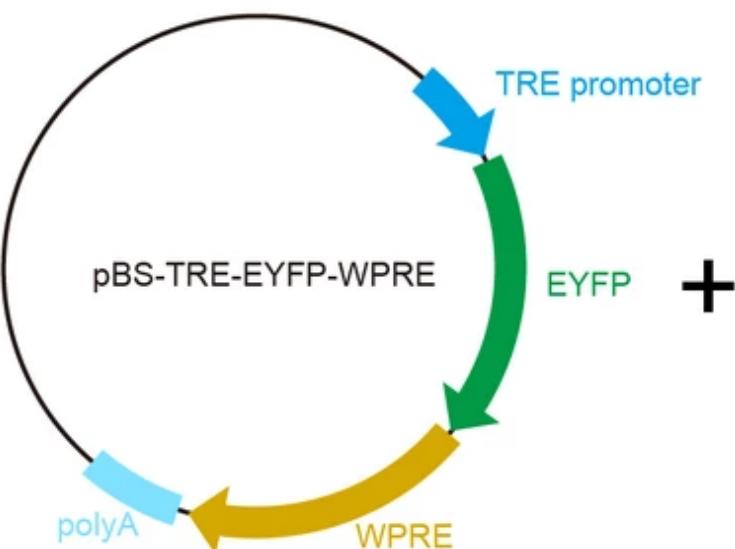
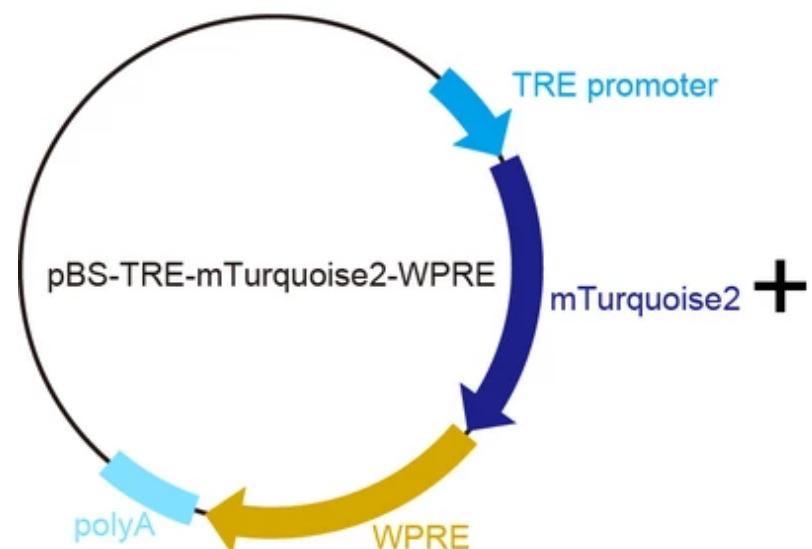
Copy number too high → reduced color variation

**D**  
Euclidean distance ( $d$ ) =  $\sqrt{\sum(\vec{a} - \vec{b})^2}$ **E**Max 75%  
Med 25%  
Min**F****G**



tTA-TRE (Tet-Off) system

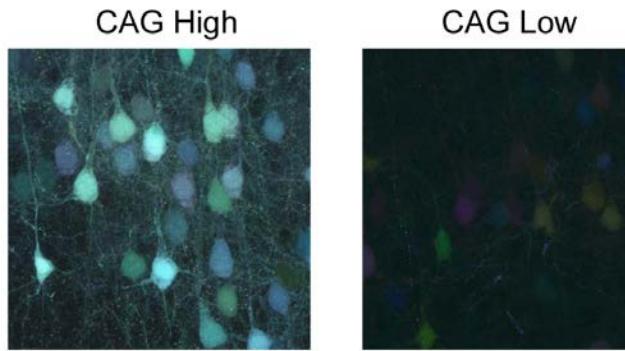
+



**A**

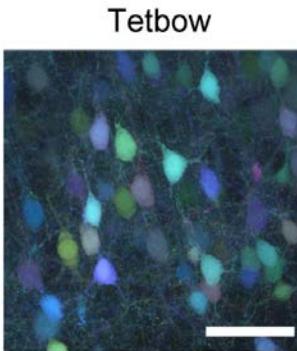
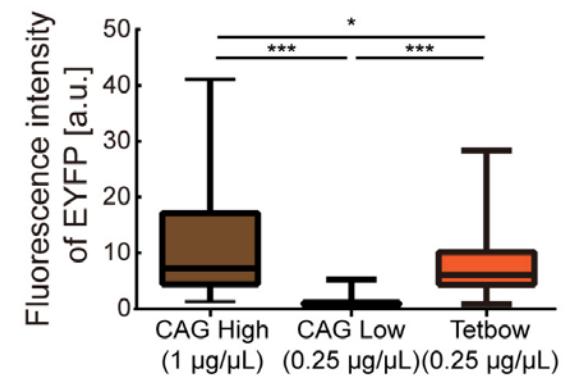
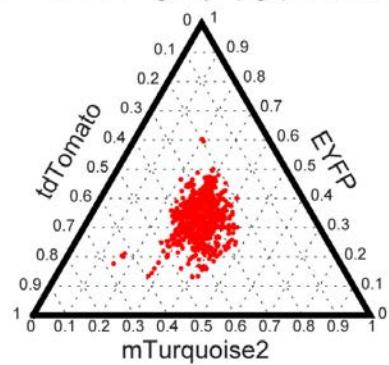
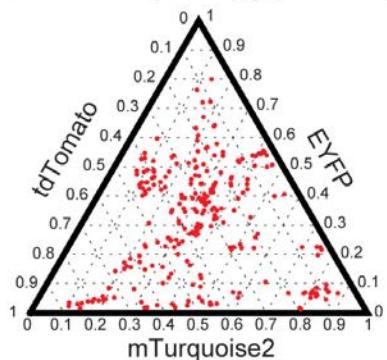
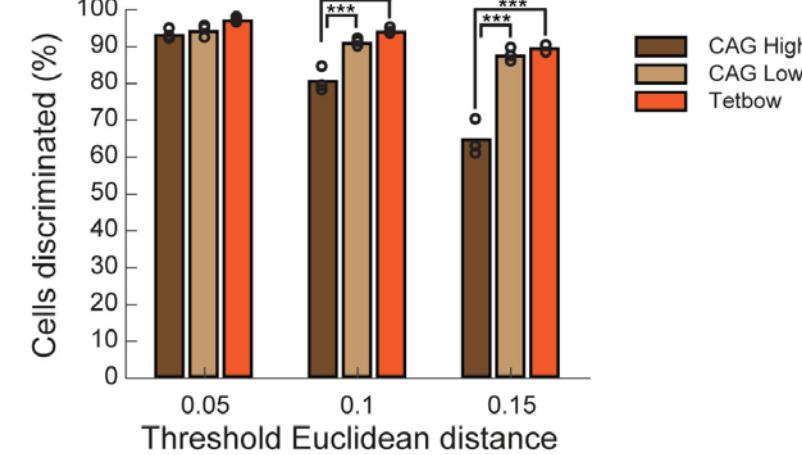
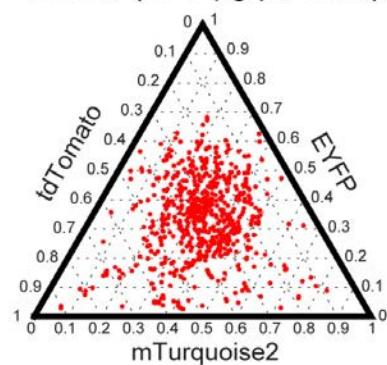
CAG-mTurquoise2  
CAG-EYFP  
CAG-tdTomato

1 µg/µL each      0.25 µg/µL each

**B**

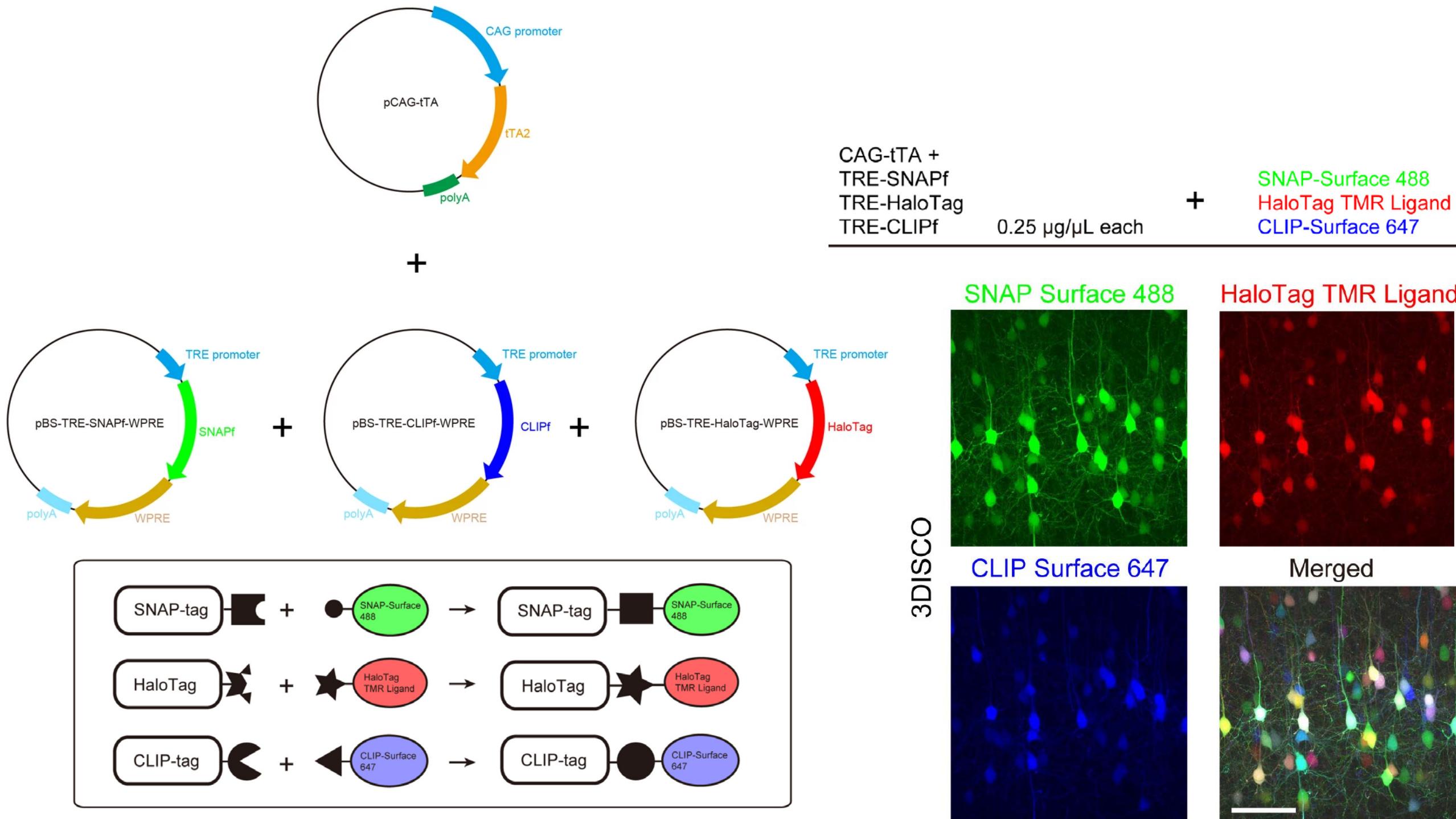
CAG-tTA +  
TRE-mTurquoise2  
TRE-EYFP  
TRE-tdTomato

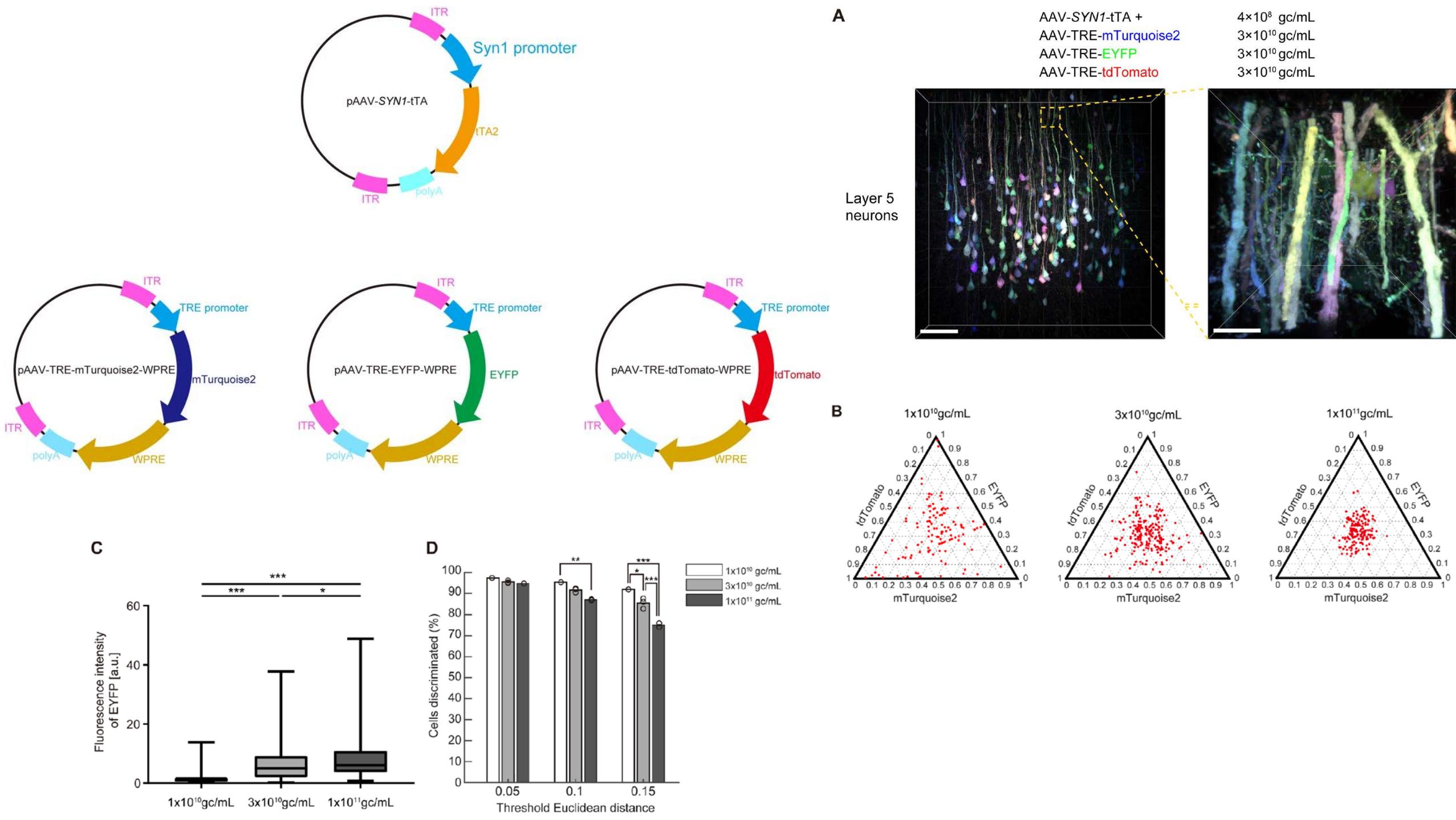
0.25 µg/µL each

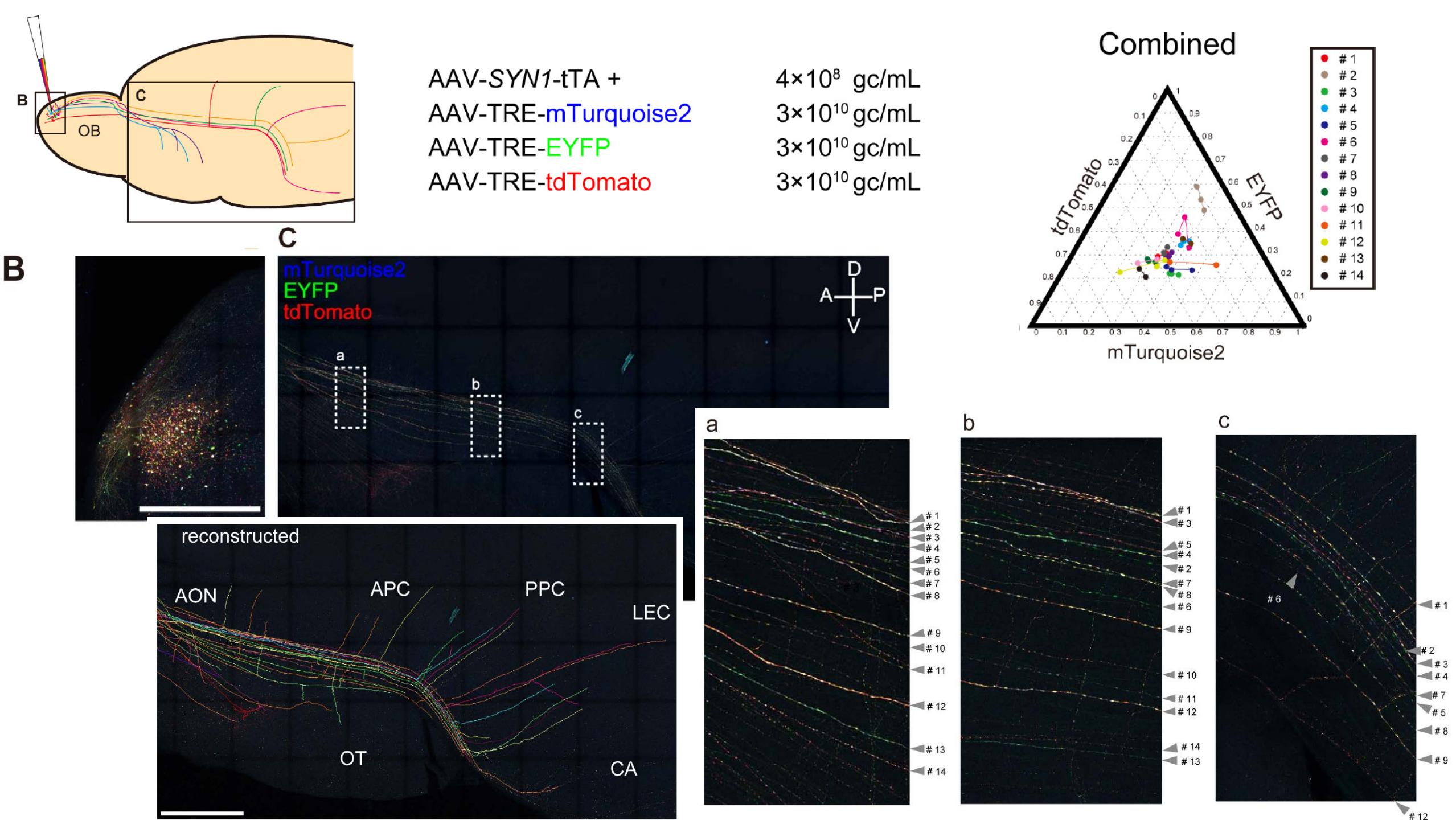
**E****C** CAG High (1 µg/µL each)**C** CAG Low (0.25 µg/µL each)**D** Tetbow (0.25 µg/µL each)

CAG High  
CAG Low  
Tetbow









# From Brainbow to Tetbow – Summary

## Development

- Enhanced **expression** by **color variation** maintenance → improved **neuronal tracing**
- **AAVs** for adult and less genetically tractable animals
- Combination with **tissue clearing** by using **chemical tags** → **large scale** tracing

## Limitations and future challenges

- Too **sparse** labeling → improve multicolor labeling and color hue consistency
- **Multicolor 3D STORM** imaging of cleared tissues by **autonomously blinking chemical tags**