



# Guide to image data on FlyLight websites

For: [splitgal4.janelia.org](http://splitgal4.janelia.org) & [gen1mcfo.janelia.org](http://gen1mcfo.janelia.org)

Home  FlyLight Split-GAL4 Driver Collection 

Aso Lab Branson Lab Card Lab Descending Interneuron Dickson Lab Rubin Lab Cart (0)

## SS00043

Upcoming top cell-type-specific line

Primary publication (DOI) [10.1002/cne.24512](https://doi.org/10.1002/cne.24512) Projections and movies are opened in a new browser window. For browsers that block pop-up windows by default, please allow them for this site.

Robot ID 2502208

AD [R65B12-p65ADZp in attP40; MKRS/TM6B](#) This page allows download of image stacks in LSM and/or h5j formats. To open and view these stacks, use Fiji (<http://fiji.sc>). Fiji has built-in plugins for stacks in LSM format (Zeiss microscope) and h5j format (a "visually lossless" compression format).

DBD [R30E10-ZpGdbd in attP2](#)

Fly Core Project Split\_GAL4

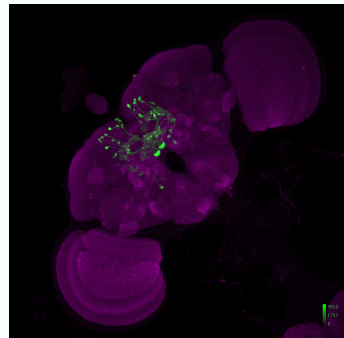
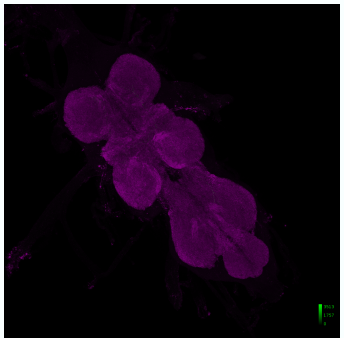
Genotype w; R65B12-p65ADZp in attP40; R30E10-ZpGdbd in attP2

Grade A


[View on NeuronBridge](#) [View on Virtual Fly Brain](#) [Order from Bloomington Stock Center](#)

### Adult 20x Objective Images [2 images]

Reporter: 20XUAS-CsChrimson-mVenus trafficked in attP18

Sex	Female		
Age	Day 3-10	<input type="text" value="Select an image to view"/>	<input type="text" value="Select an image to view"/>
Slide code	20180411_33_C1	Brain	Ventral nerve cord
Release	Wolff 2018		

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## Contents:

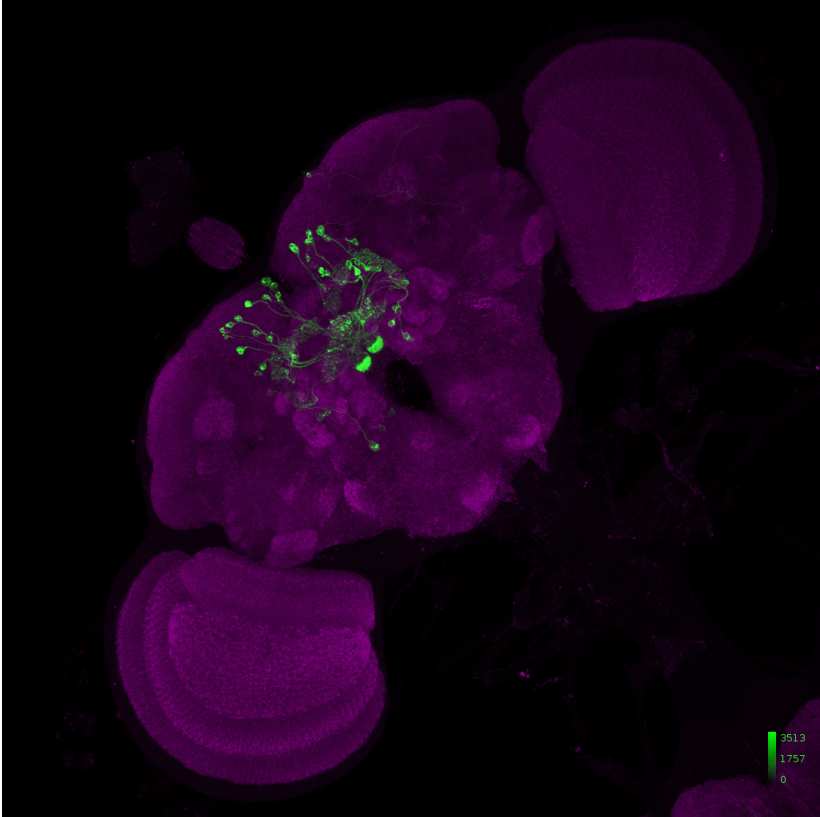
1. Image formats
2. FlyLight sample labeling methods
3. 63x and 40x image tile names

# Organization

Adult 20x Objective Images [2 images]

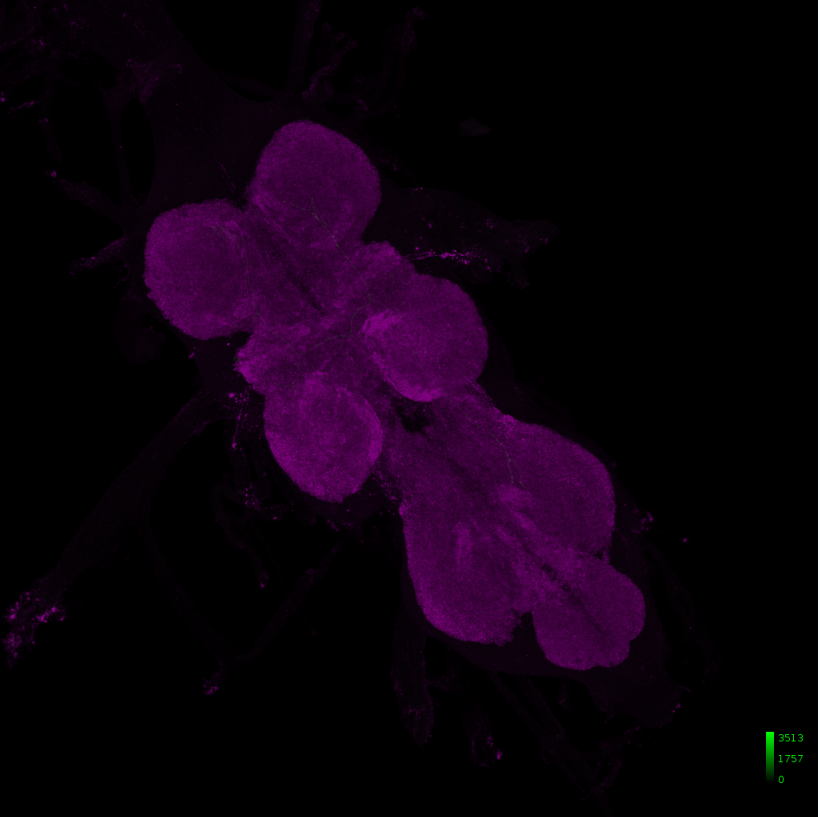
Reporter: 20XUAS-CsChrimson-mVenus trafficked in attP18

Sex	Female
Age	Day 3-10
Slide code	20180411_33_C1
Release	Wolff 2018



Select an image to view

Brain

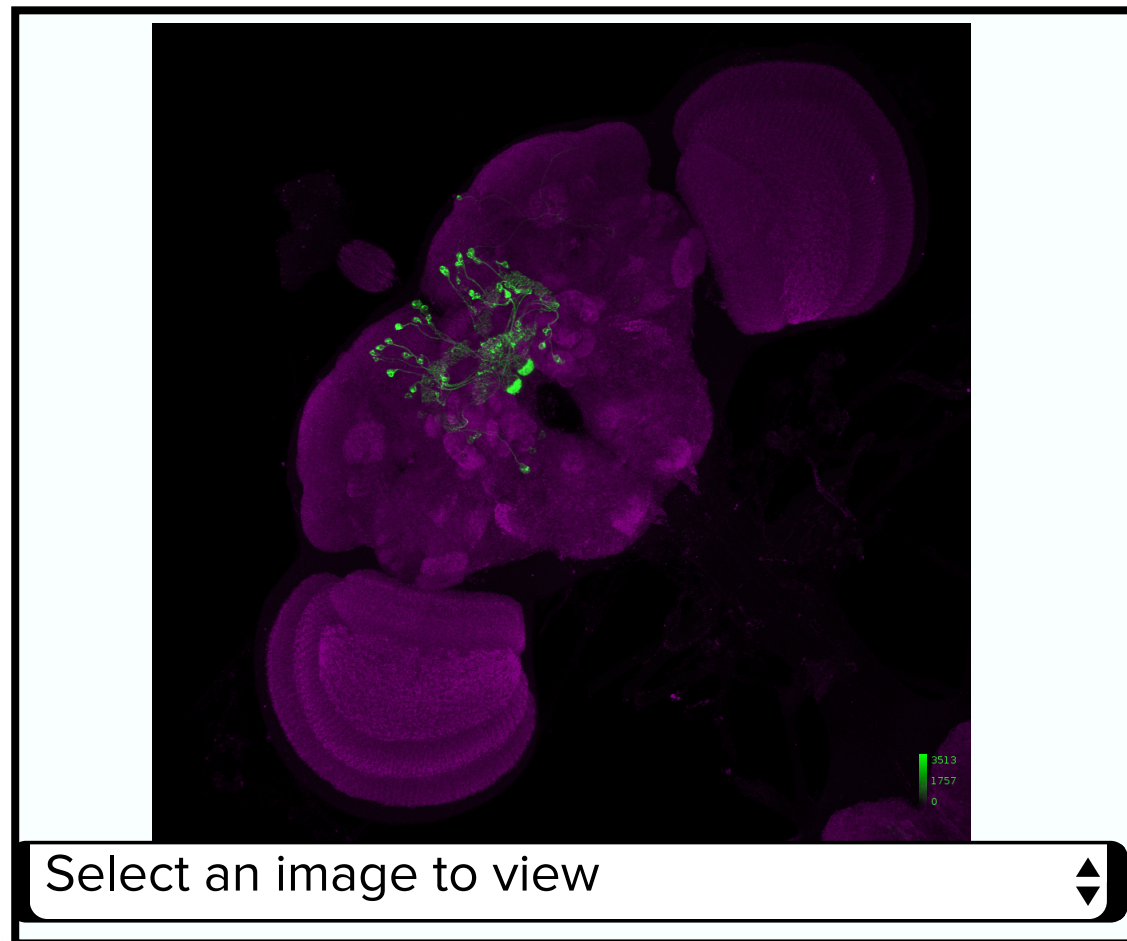


Select an image to view

Ventral nerve cord

- Images for each line are grouped by objective, reporter, and sample (fly), with image tiles shown as thumbnails
- Select the menu beneath a thumbnail to view or download a variety of 2D image, movie, or microscope format 3D stacks, detailed on next page

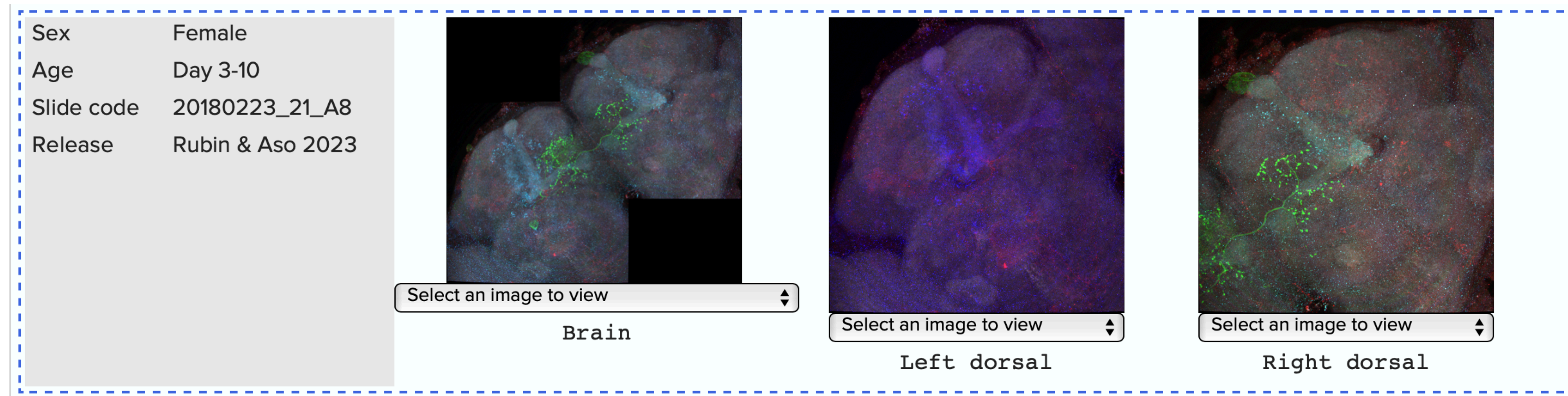
# Image formats



- ✓ Select an image to view
- MIP: Aligned gendered (all channels)
- MIP: Aligned unisex (all channels)
- MIP: Unaligned (all channels)
- MIP: Unaligned (signal channels)
- Color depth MIP: Aligned (Channel 1)
- Color depth MIP: Aligned (Channel 2)
- Movie: Unaligned (all channels)
- Movie: Unaligned (signal channels)
- Download H5J stack: Aligned gendered
- Download H5J stack: Aligned unisex
- Download H5J stack: Unaligned
- Download LSM: Raw data

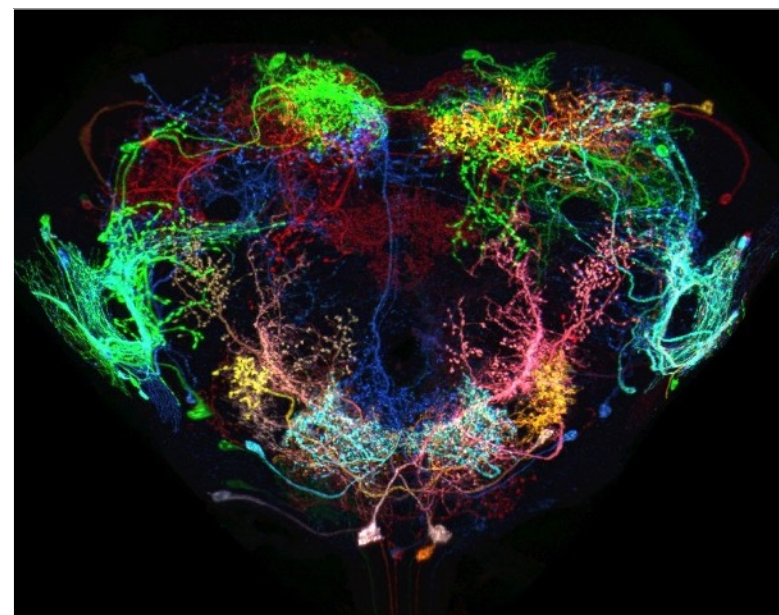
- Thumbnails show unaligned neuropil reference and neuron signal channels
- Maximum Intensity Projections (MIPs) give 2D views of data with original colors
- Images can be unaligned (as captured) or aligned to JRC2018 template (<https://doi.org/10.1371/journal.pone.0236495>)
- Color Depth MIPs (CDM) use color to indicate depth in z dimension; blue is close and red is far (<http://dx.doi.org/10.1101/318006>)
- Most stacks are H5J compressed (<http://data.janelia.org/h5j>)
- Original data capture format is a minimally-compressed LSM stack

# Image processing details



- The number of samples per line varies
- Slide code indicates date of slide creation, pipeline code, and location of individual sample on slide
- Images are individually optimized for brightness
- Some types of image outputs may show saturation; raw data (LSM stacks) have minimal saturation
- Brain and VNC images may be stitched from multiple tiles. Tiles for each sample/region are captured with consistent imaging parameters but are shown scaled independently.

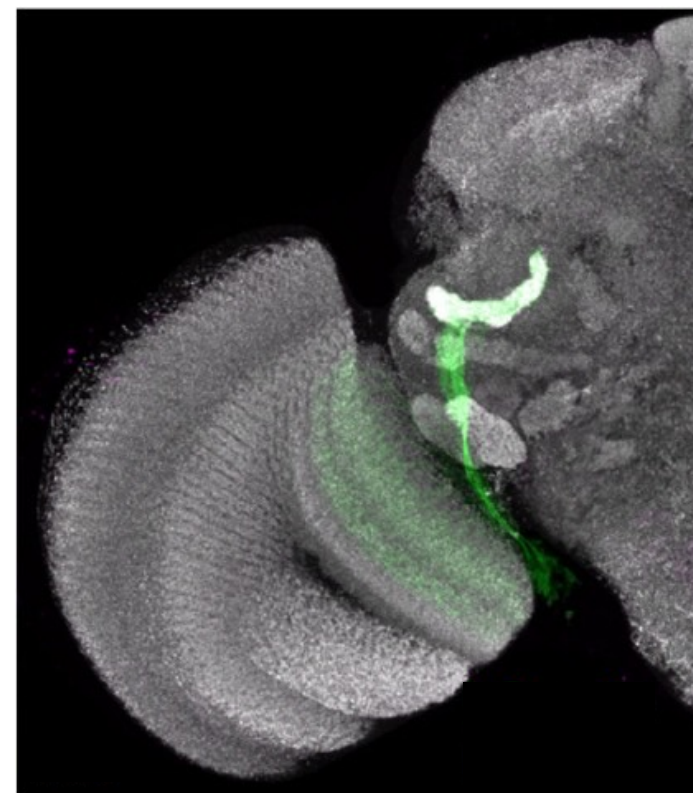
# FlyLight pipelines characterizing genetic lines



**Generation 1**

**MCFO:**

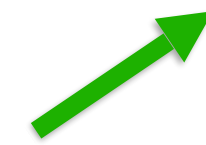
Identifying potential  
Gen1 GAL4 lines



Wu, Nern, et al., 2016

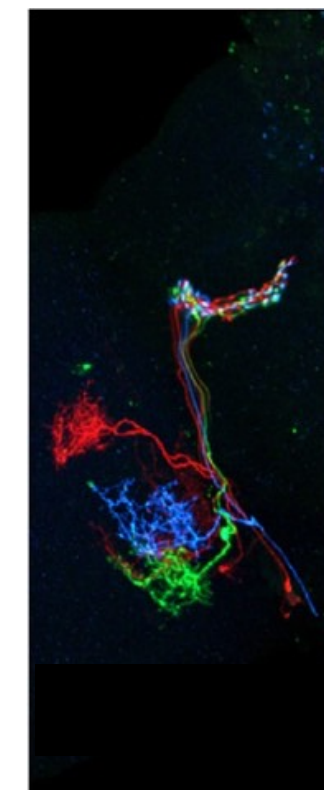
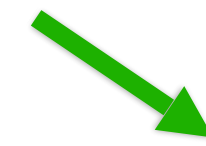
**Screen:**

Identifying  
the best split-  
GAL4  
combinations



**Polarity:**

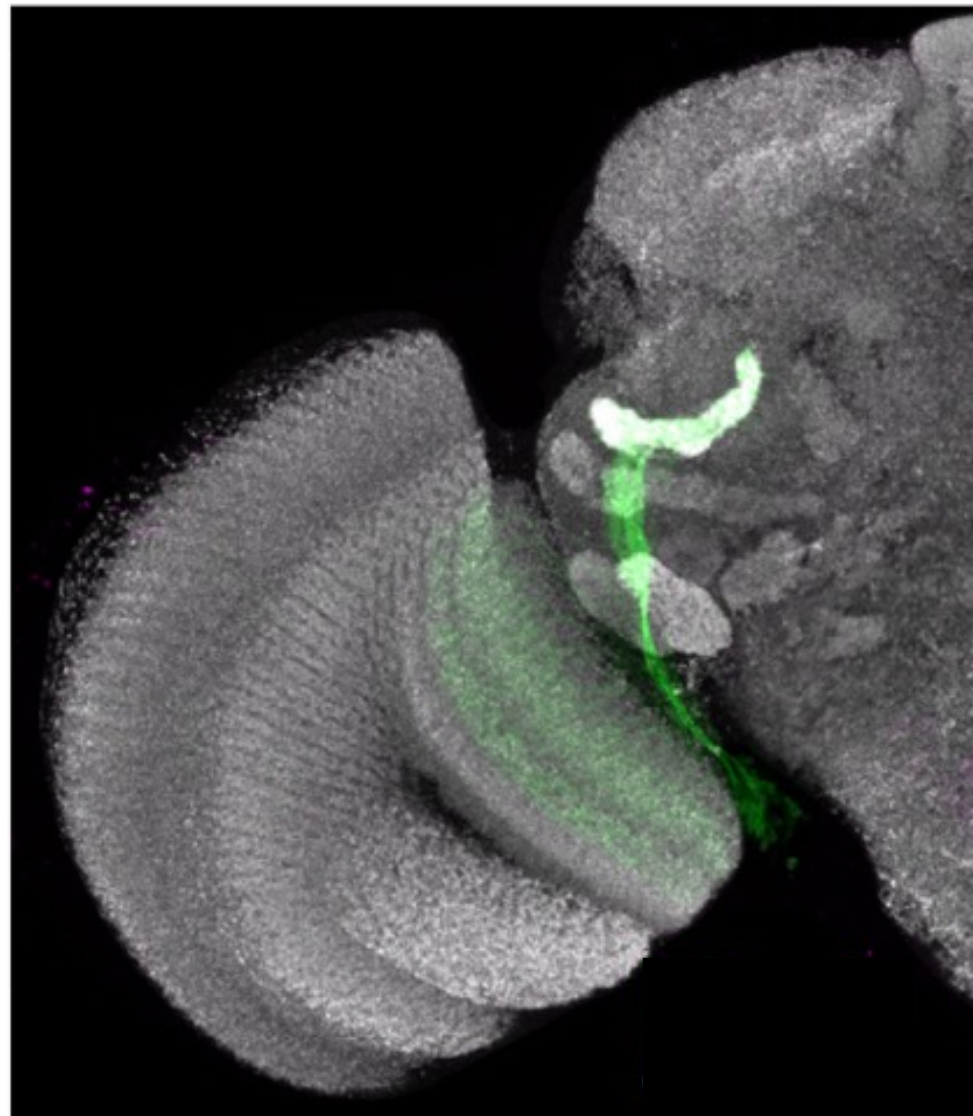
Determining  
neuronal  
input and output



**Multicolor Flp-  
out (MCFO):**

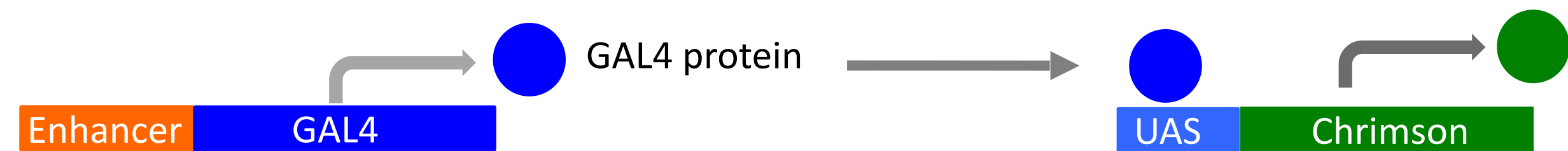
Understanding  
cells within a  
line

# Screen pipeline



UAS reporter: 20XUAS-Cs-Chrimson-mVenus trafficked in attP18

	Screen	
Target	Reference neuropil	neuron
Genetic marker	endogenous	UAS-GFP
Primary	mouse nc82	rabbit anti-GFP
Secondary	568 anti-mouse	488 anti-rabbit



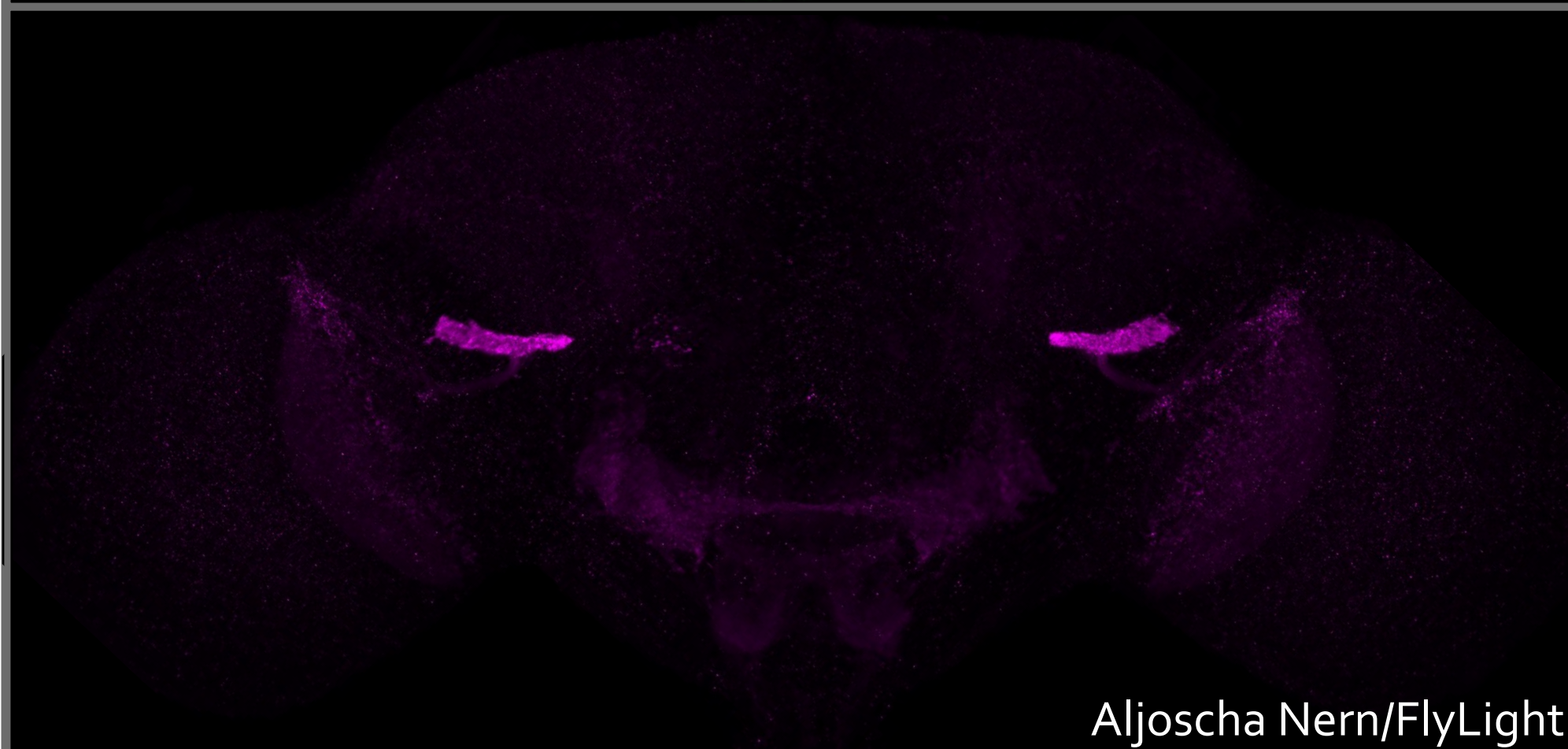
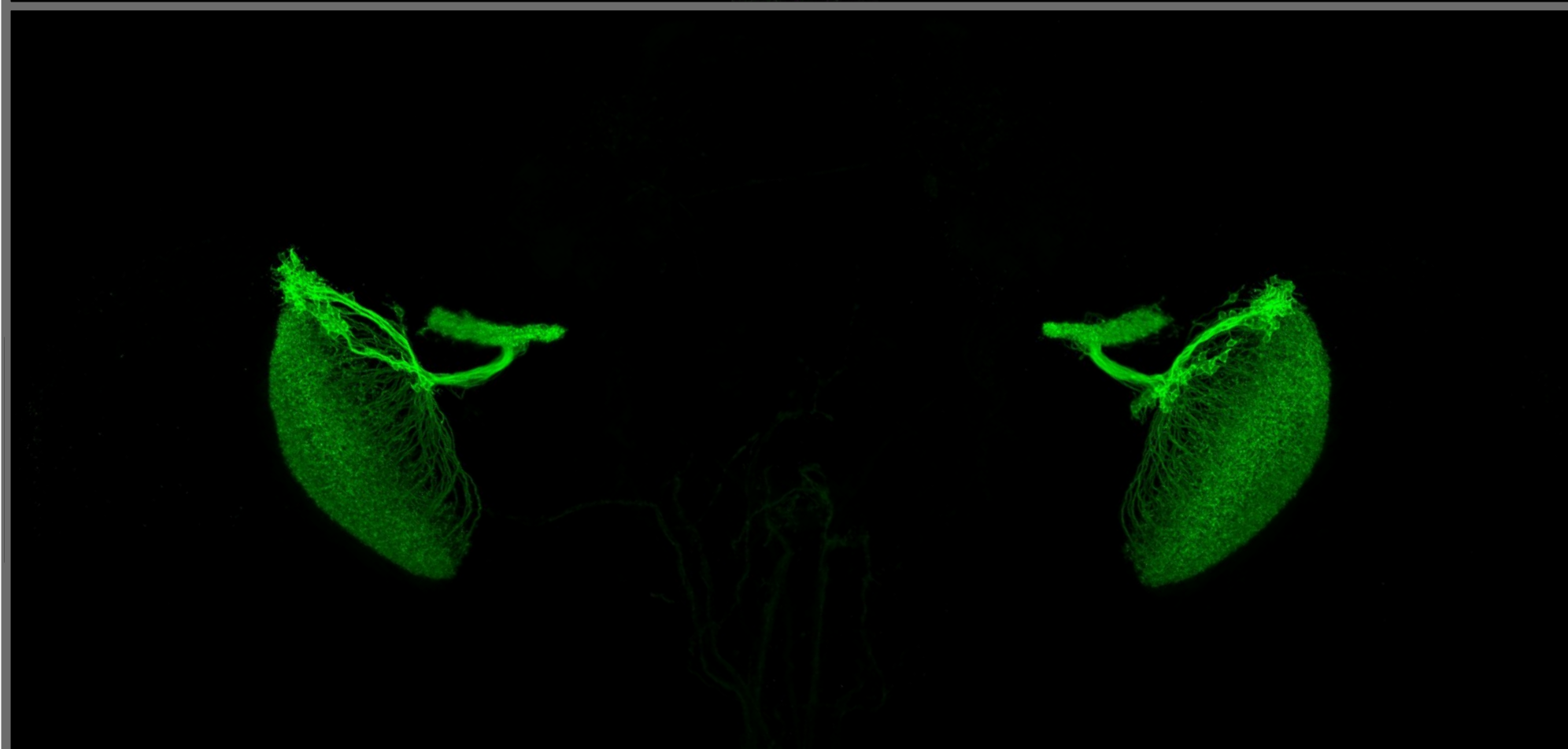
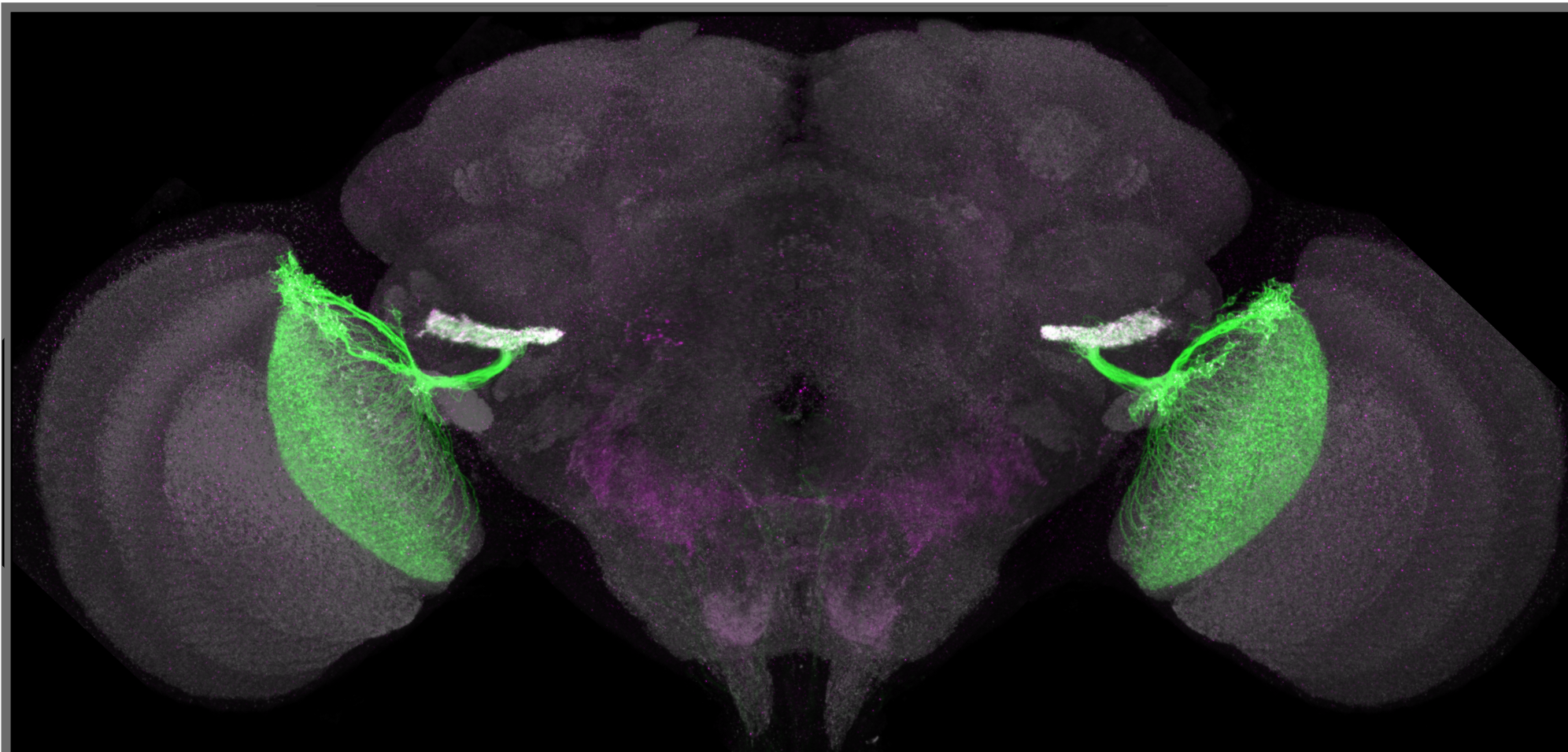
# Polarity pipeline

Standard approach is to label:

- GAL4-specific neuronal membrane
- GAL4-specific presynaptic terminal
- nc82 neuropil reference

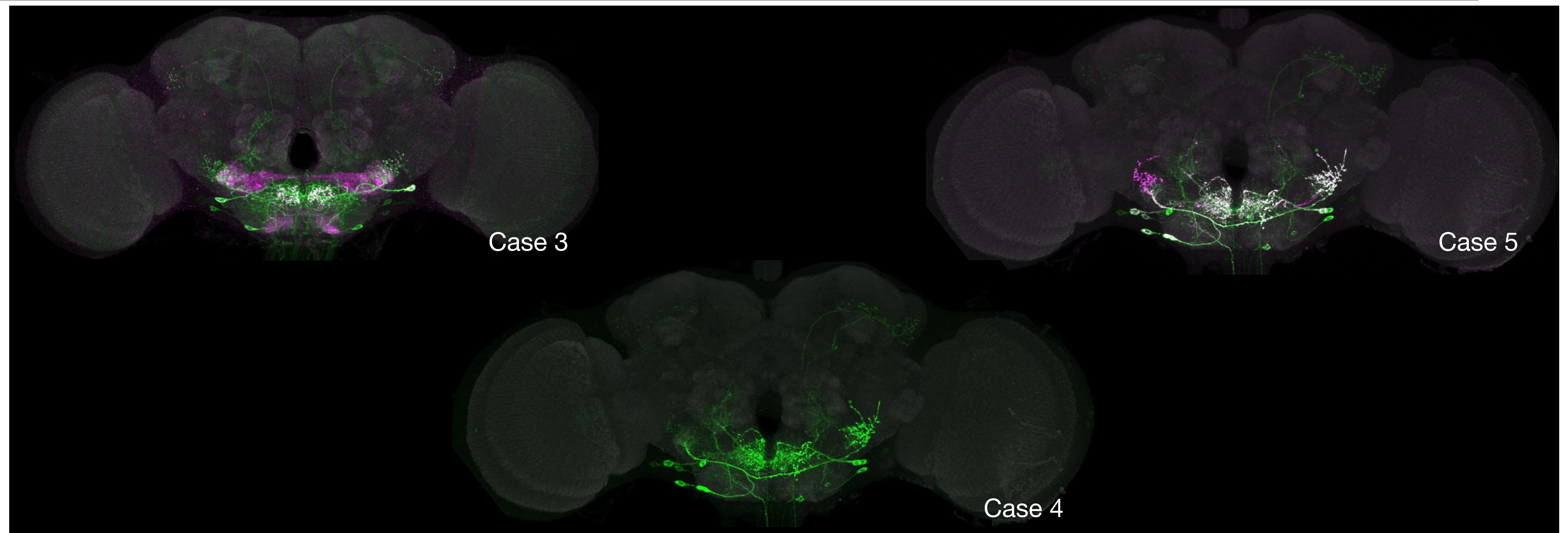
Two main purposes:

- Determine polarity (inputs and outputs) of neurons
- High-quality GAL4 membrane labeling



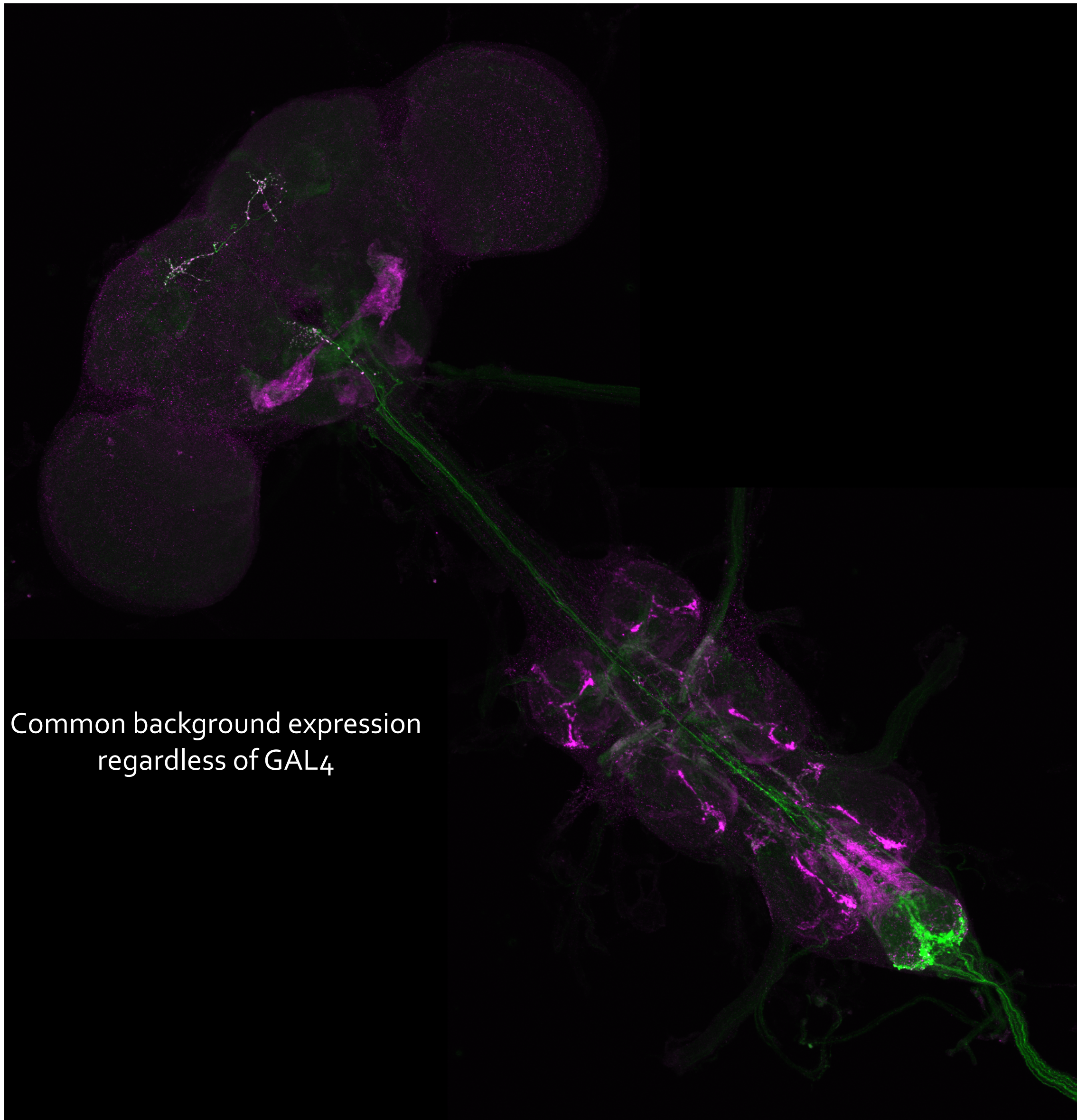
# Polarity Cases (variations on labeling methods)

	Case 3		Case 4		Case 5	
	Primary Ab	Secondary Ab	Primary Ab	Secondary Ab	Primary Ab	Secondary Ab
<b>nc82 (neuropil)</b>	mouse nc82	Cy2 anti-mouse	mouse nc82	AF568 anti-mouse	mouse nc82	AF568 anti-mouse
<b>UAS-Syt-HA (synapse)</b>	Rabbit anti-HA	Cy3 anti-rabbit	-	-	Rat anti-HA	ATTO647N anti-rat
<b>UAS-myr-FLAG (membrane)</b>	Rat anti-FLAG	ATTO647N anti-rat	-	-	-	-
<b>UAS-Chrimson (membrane)</b>	-	-	Rabbit anti-GFP	AF488 anti-rabbit	Rabbit anti-GFP	AF488 anti-rabbit



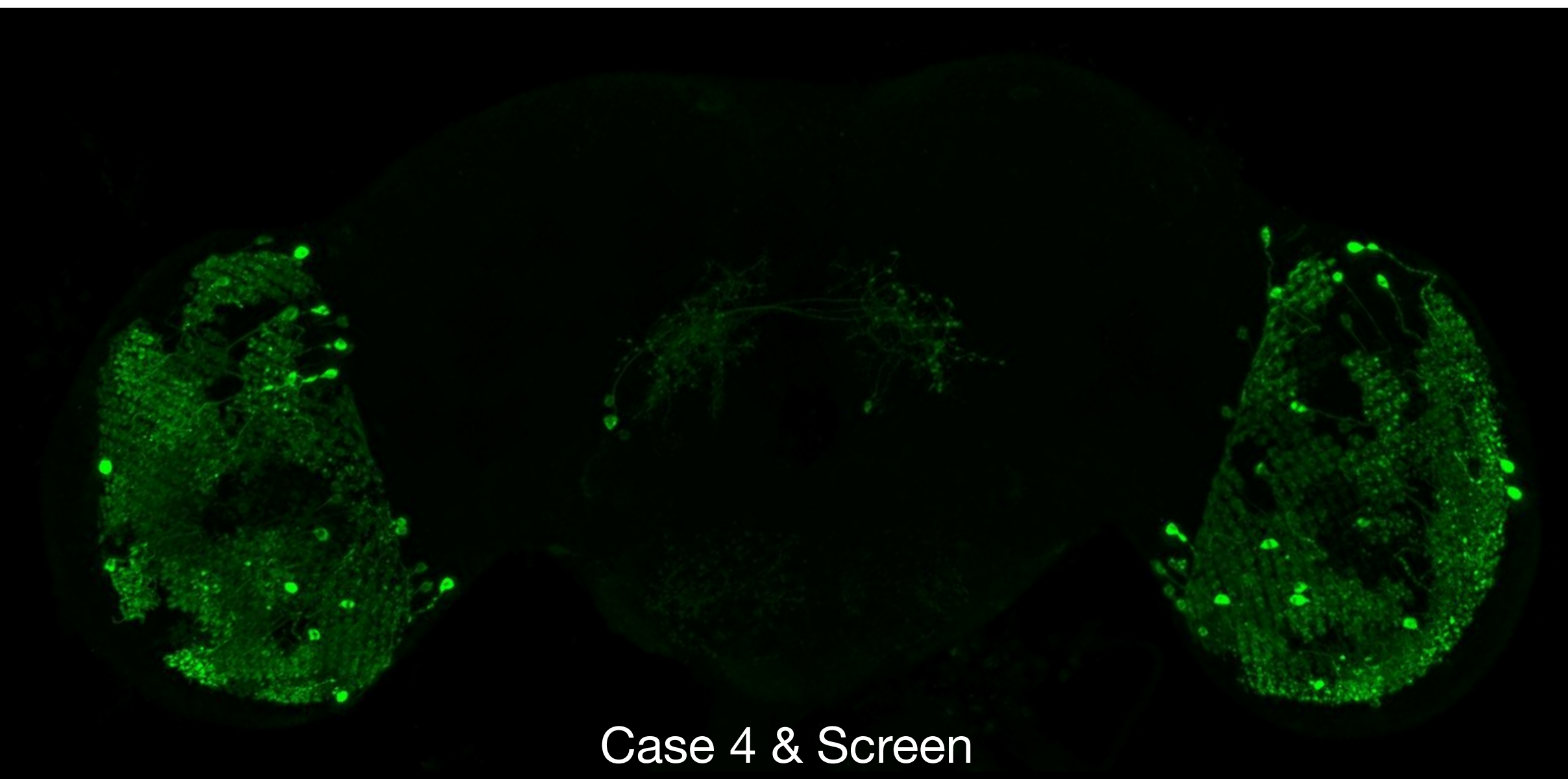


# Polarity Case 3

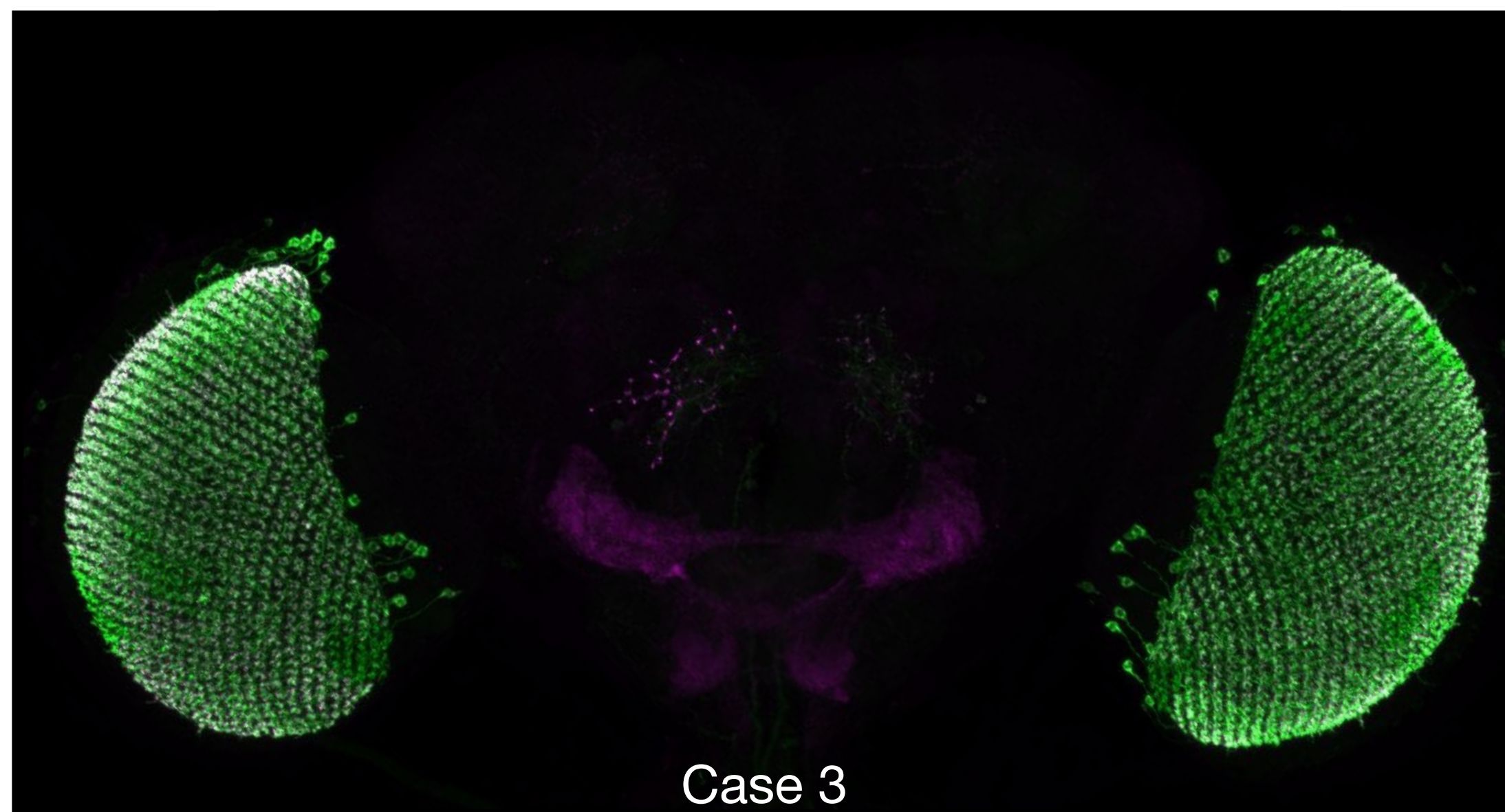


- UAS Reporters:
- Membrane: pJFRC225-5XUAS-IVS-myr::smGFP-FLAG in VK00005
- Synapse: pJFRC51-3XUAS-IVS-Syt::smGFP-HA in su(Hw)attP1
  
- Excellent intracellular synaptic labeling specificity
- Membrane label reliably fills GAL4 patterns
- But high background expression of membrane and synaptic reporters
  
- UAS-myr-FLAG reporter instead of Chrimson-mVenus
  - Different reporter from Screen & behavioral studies
    - Different attP insertion site
  - Harder to correlate Polarity pattern with other studies

# Polarity Case 4



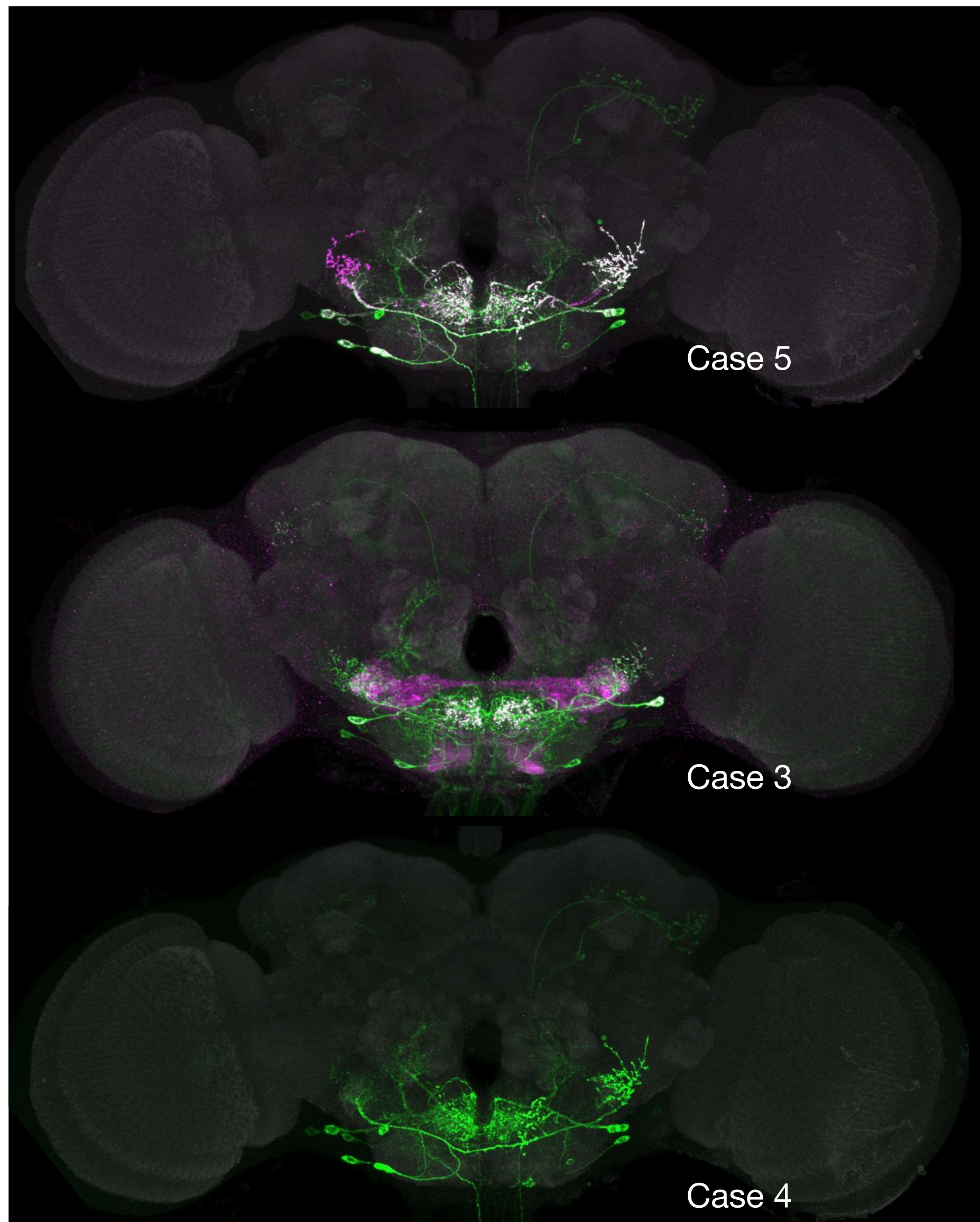
- UAS reporter: 20XUAS-Cs-Chrimson-mVenus trafficked in attP18
- Polarity Case 4 and Screen pipelines use the same UAS reporter and antibodies
- UAS-Chrimson-mVenus reporter allows direct comparison to physiology
- No synaptic label, despite Polarity name
- Very low background
- Less uniform labeling of GAL4 pattern than UAS-myr-FLAG
  - Greater variation between cell body and projection intensities
  - Doesn't always label all neurons in GAL4 pattern



# Polarity Case 5

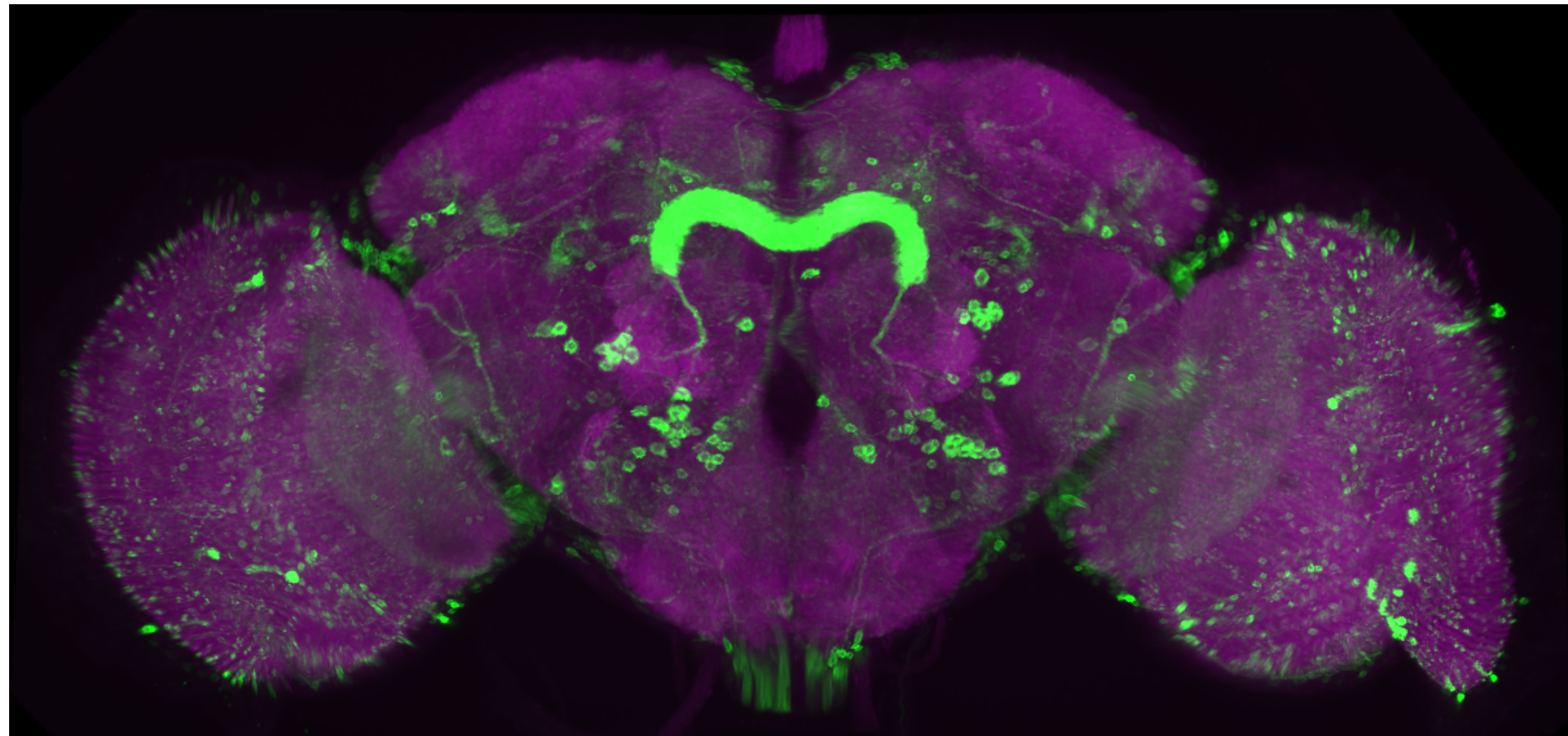
- UAS Reporters:
- Membrane: 20XUAS-Cs-Chrimson-mVenus trafficked in attP18
- Synapse: UAS-Syt-HA (different from Case 3)

- Chrimson-mVenus instead of myr-FLAG
  - Maximizes consistency with screening & physiology results
- Labels presynaptic terminals, but less accurate intracellular localization than Case 3
- Very low background in most cases
- Common contaminants can grow during IHC and be labeled by anti-GFP antibody, requiring sodium azide suppression

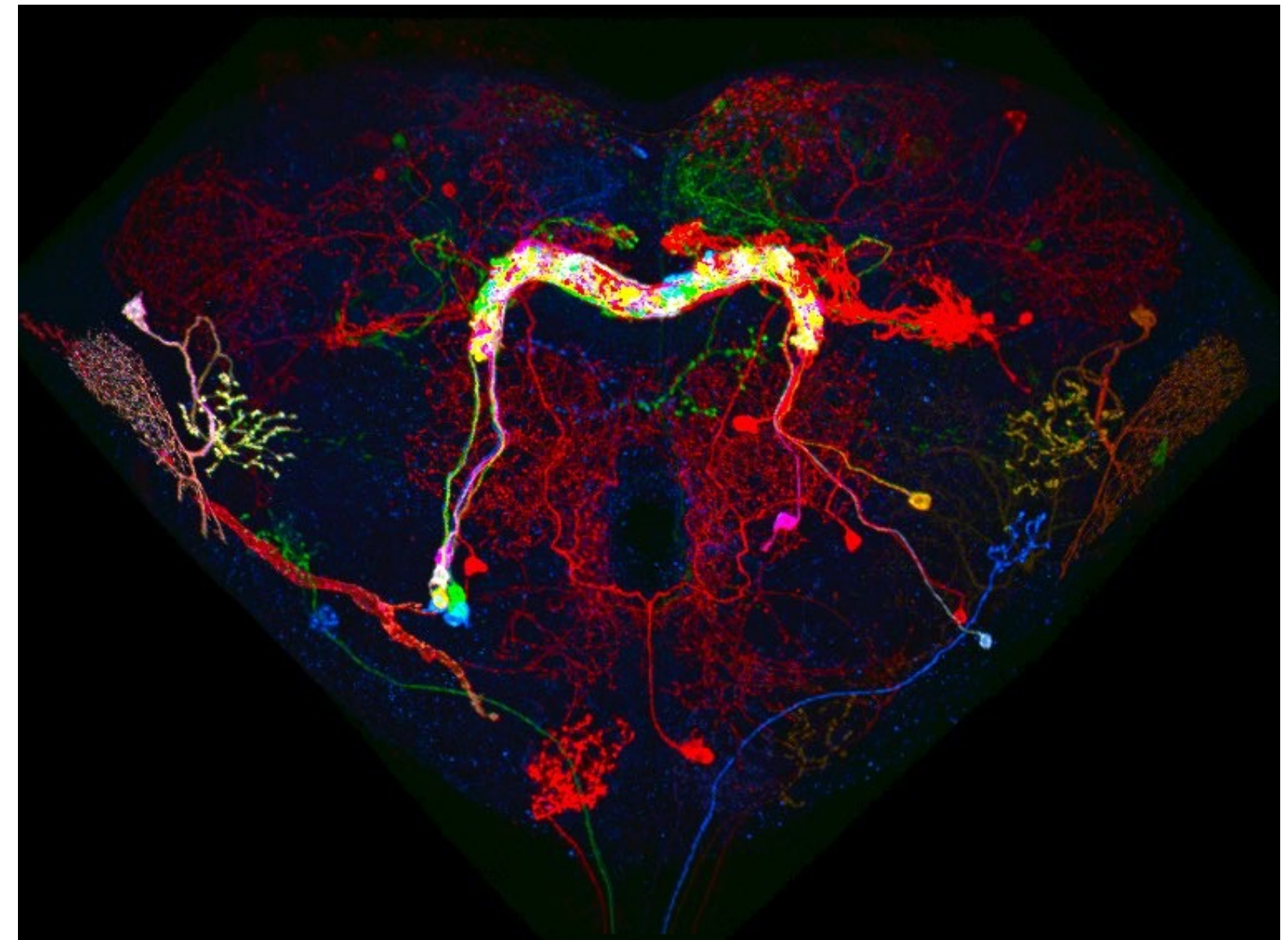


# MultiColor FlpOut (MCFO) subdivides GAL4 & split-GAL4 lines

Full GAL4 pattern with UAS-Chrimson-  
mVenus reporter



Subsets of neurons labeled in different  
colors by MultiColor FlpOut (MCFO)



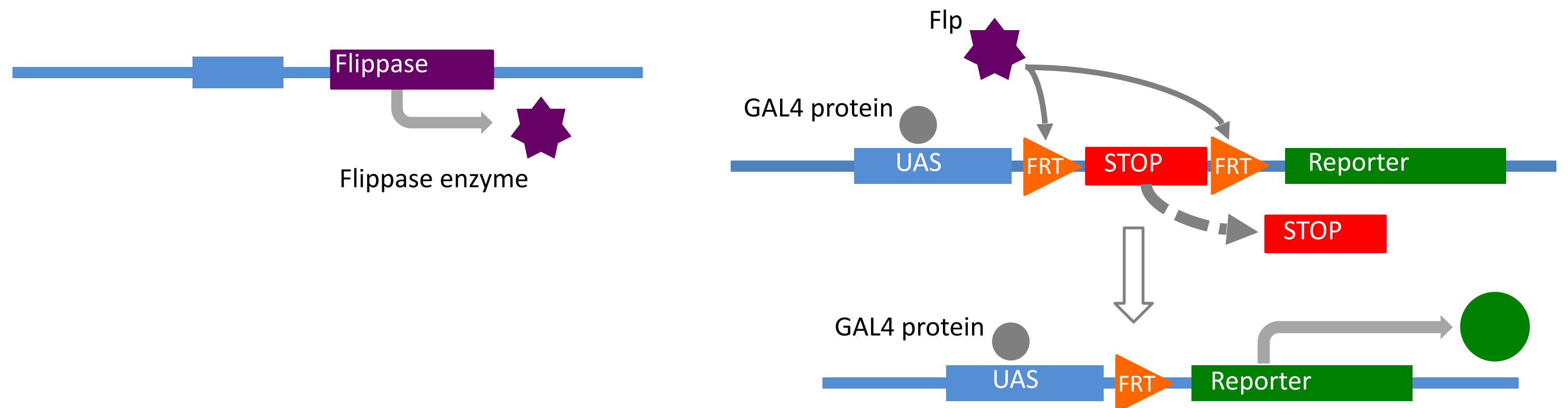
# Multicolor FlpOut (MCFO)

MCFO-1 UAS Reporter:  
 pBPhsFlp2::PEST in attP3;  
 pJFRC201-10XUAS>STOP>myr::smGFP-HA in  
 VK0005,  
 pJFRC240-10XUAS>STOP>myr::smGFP-V5-  
 THS-10XUAS>STOP>myr::smGFP-FLAG in  
 su(Hw)attP1

Target	Multicolor Flp-out			
	Reference neuropil	neuronal membrane 1	neuronal membrane 2	neuronal membrane 3
Genetic marker	endogenous	UAS>>HA	UAS>>V5	UAS>>FLAG
Primary/Direct Ab	mouse nc82	rabbit anti-HA	DL550 anti-V5	rat anti-FLAG
Secondary Ab	AF488 anti-mouse	AF594 anti-rabbit		ATTO647N anti-rat

See Nern et al., 2015 for more details on MCFO variations

<https://doi.org/10.1073/pnas.1506763112>



# 63X Adult Tiles - Brain

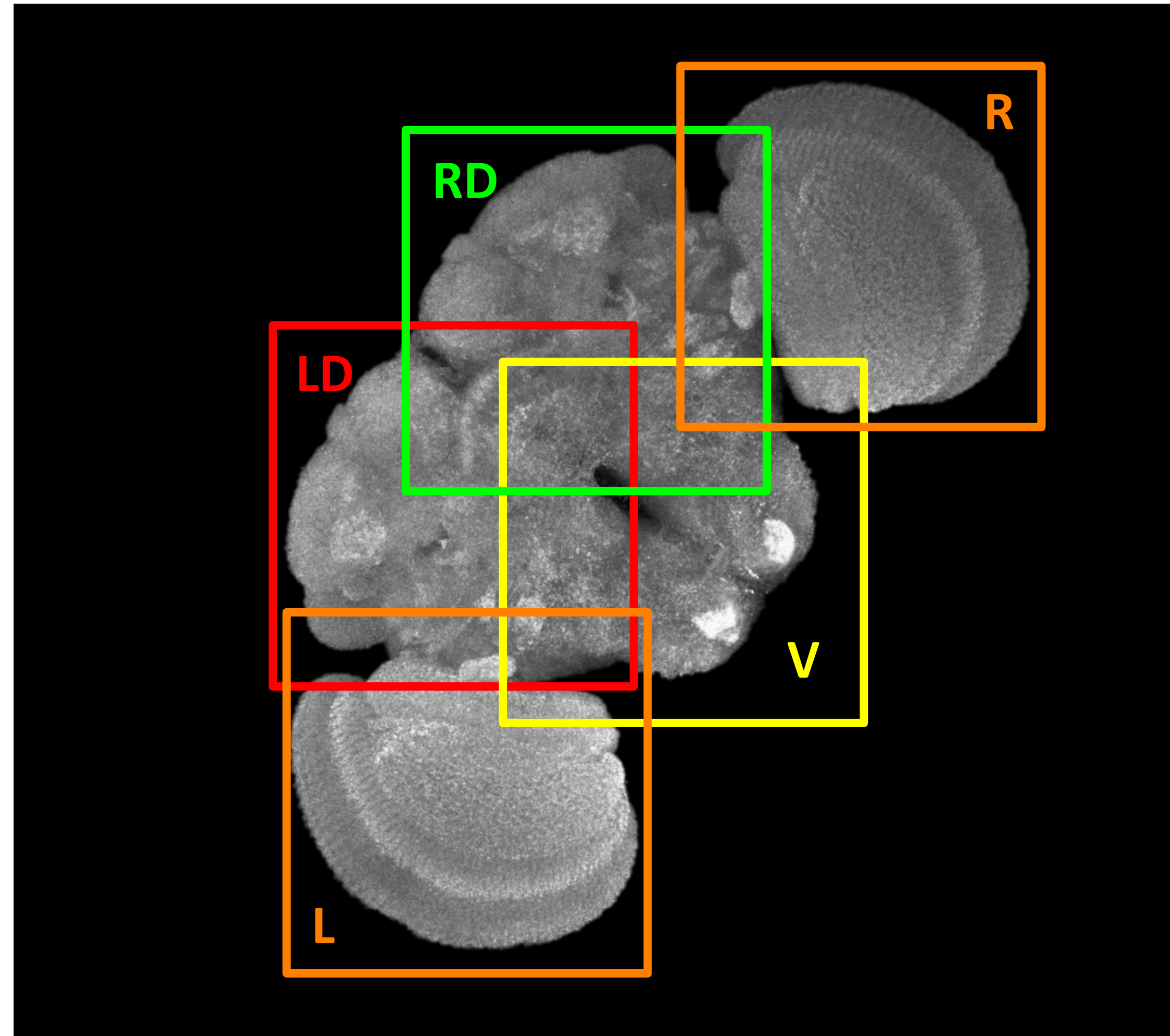
**L** – Left Optic Lobe

**V** – Ventral

**LD** – Left Dorsal

**RD** – Right Dorsal

**R** – Right Optic Lobe



# 63X Adult Tiles – Additional Brain Tiles

**DM** – Dorsal Medial

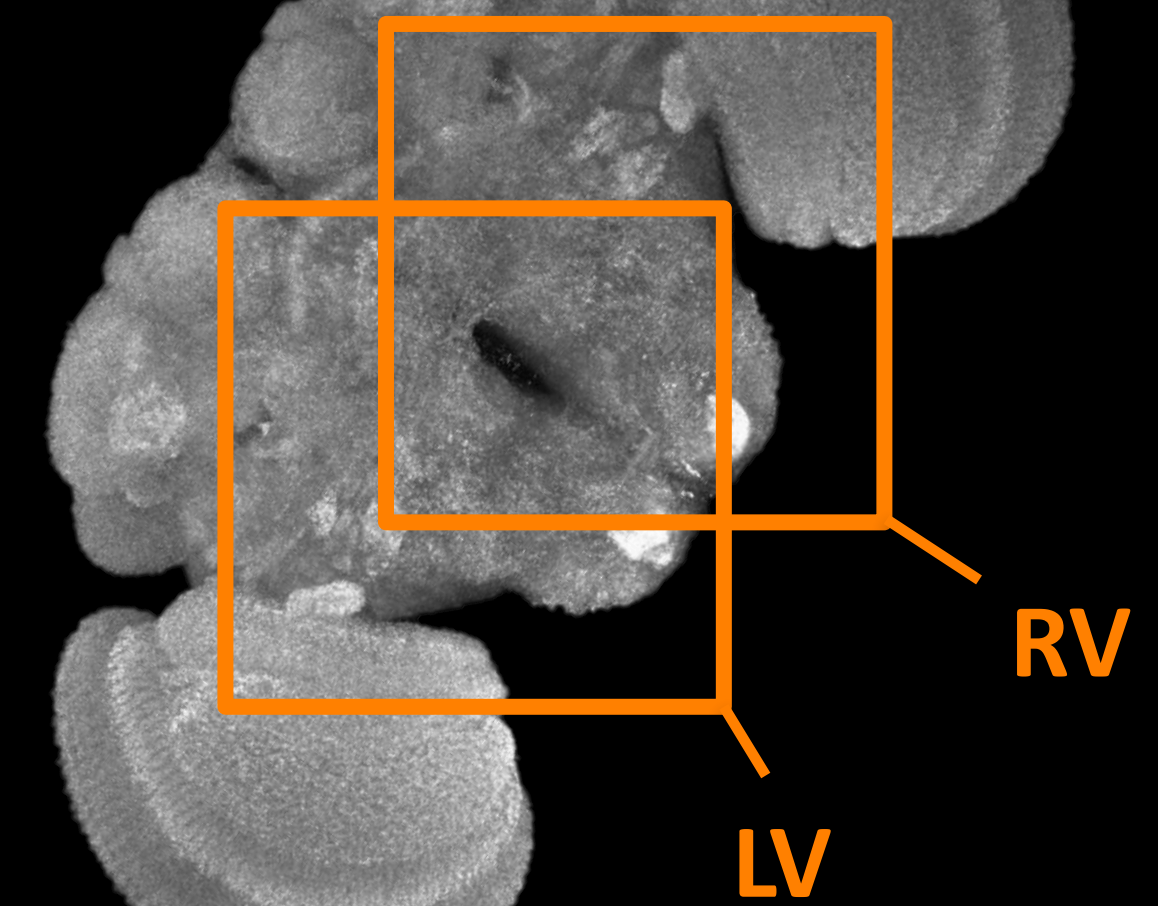
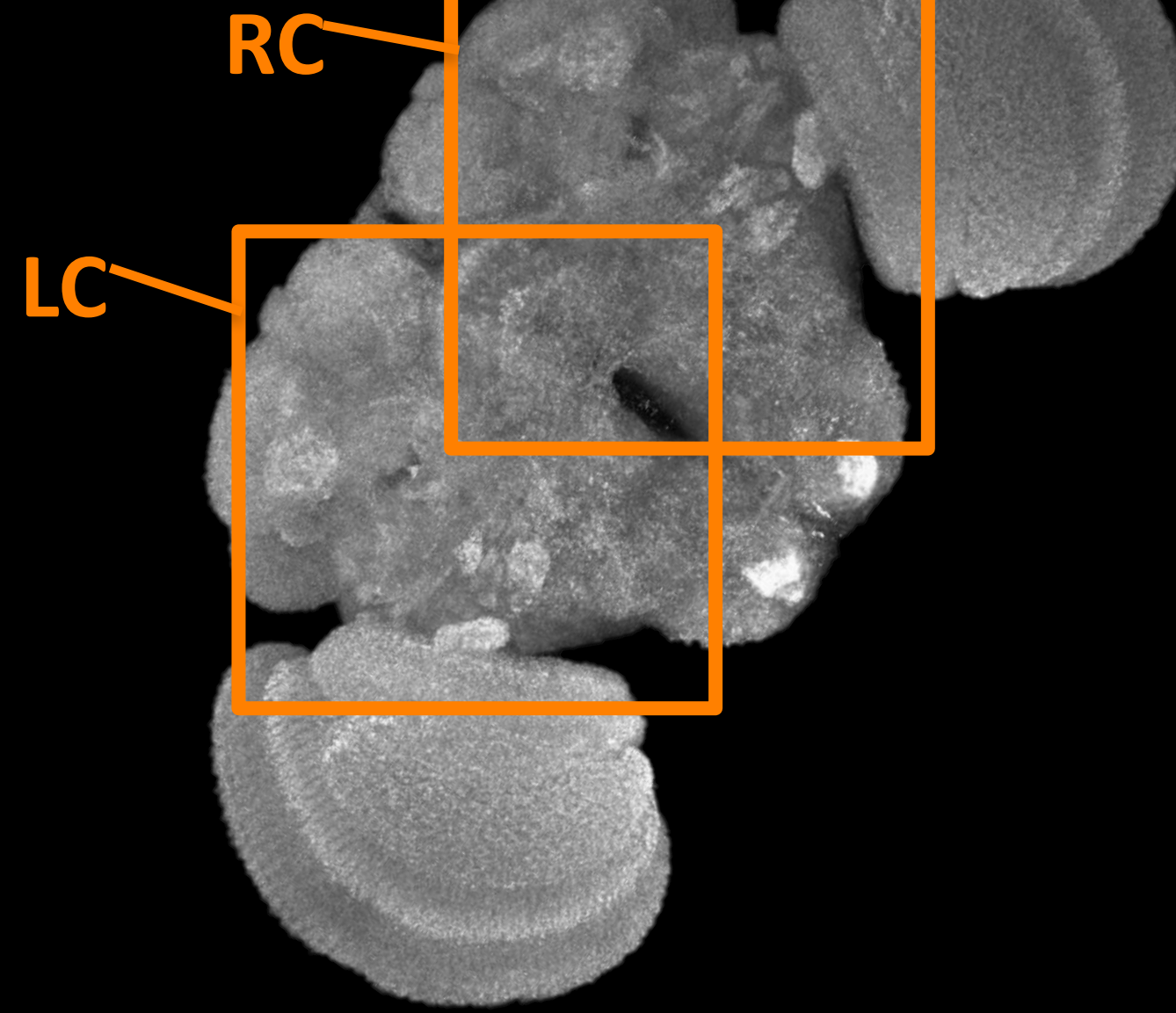
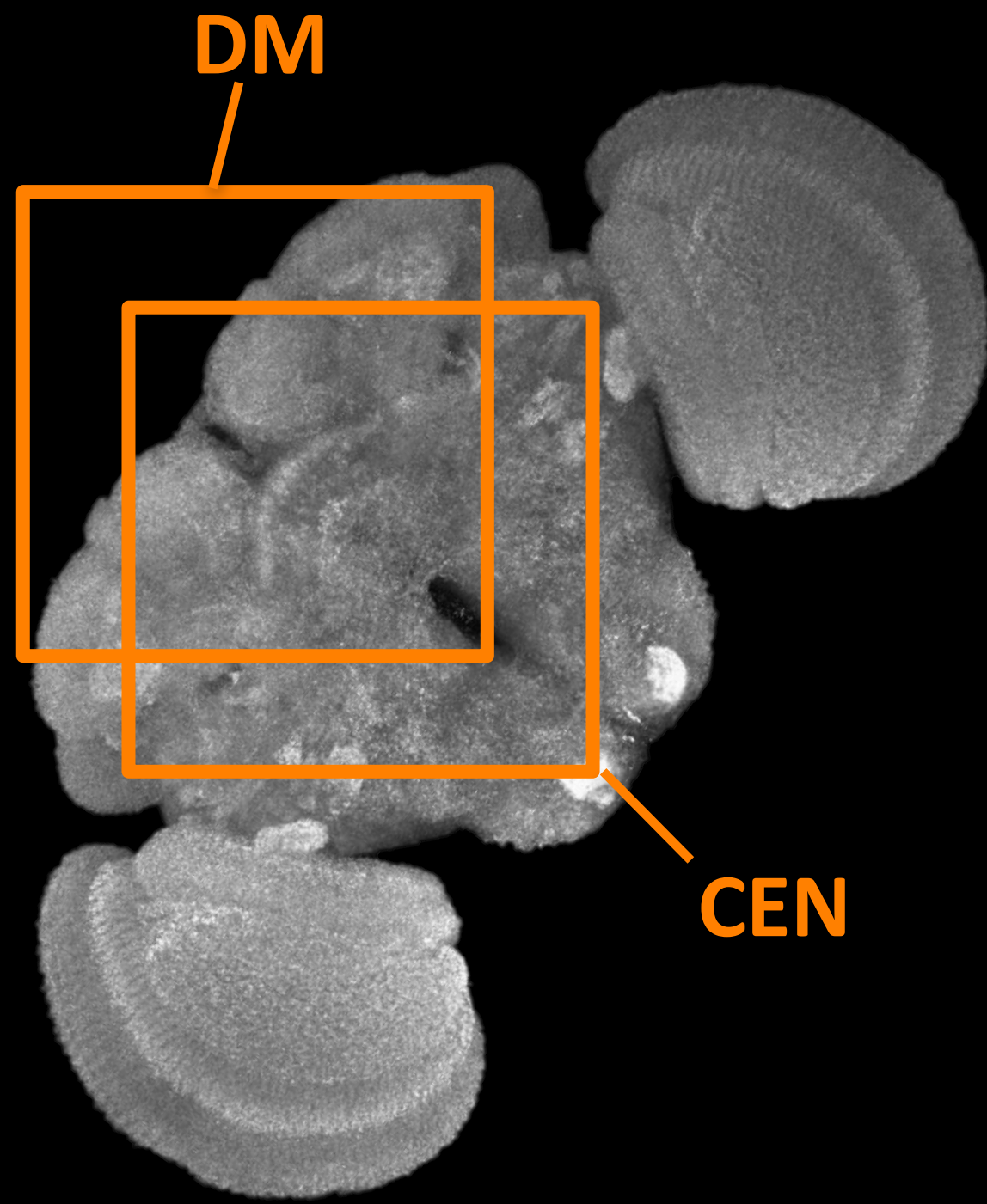
**CEN** – Central

**LC** – Left Central

**RC** – Right Central

**LV** – Left Ventral

**RV** – Right Ventral



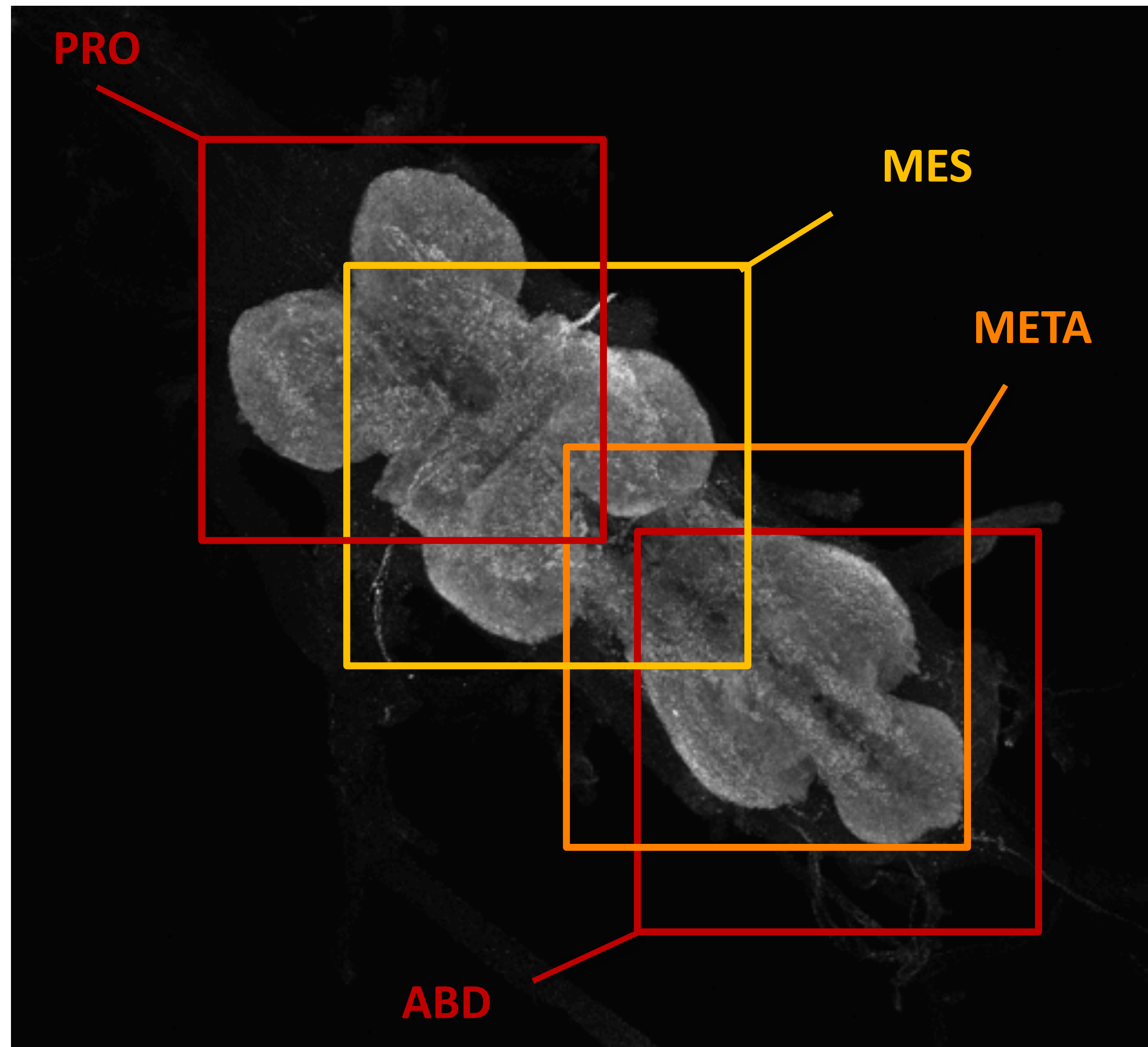
# 63X Adult Tiles - VNC

**PRO** – Prothoracic

**MES** – Mesothoracic

**META** – Metathoracic  
(sometimes covers Abd)

**ABD** – Abdominal

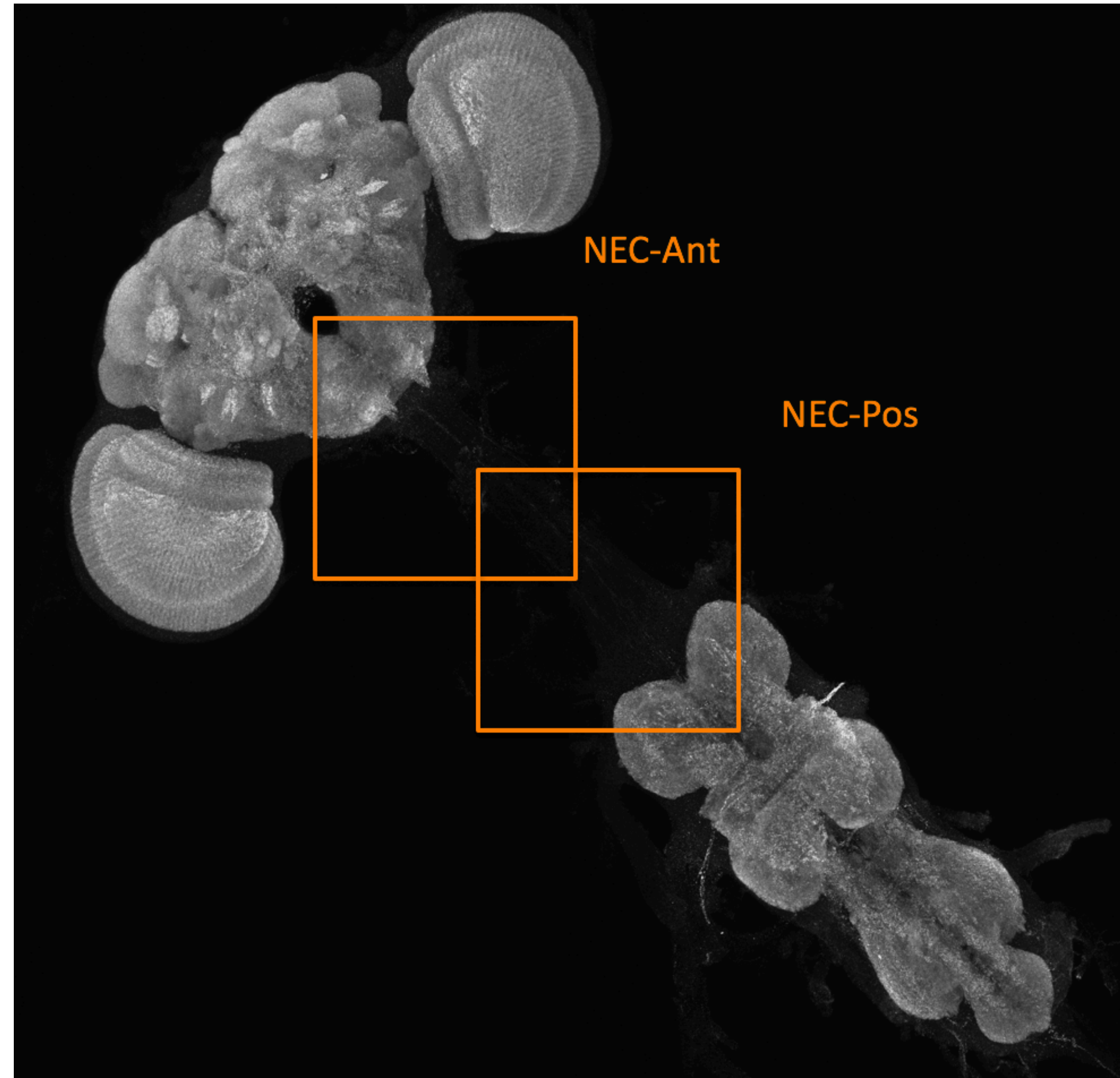




# 63X Adult Tiles - Neck

**NEC-Ant** – Anterior Neck

**NEC-Pos** – Posterior Neck



# 40X Adult Tiles - Brain & VNC

**BRAIN – 1 tile:**

**CEN** - Central brain

**VNC – 2 tiles:**

**PRO** - Prothoracic (anterior VNC)

**META** - Metathoracic (posterior VNC)

