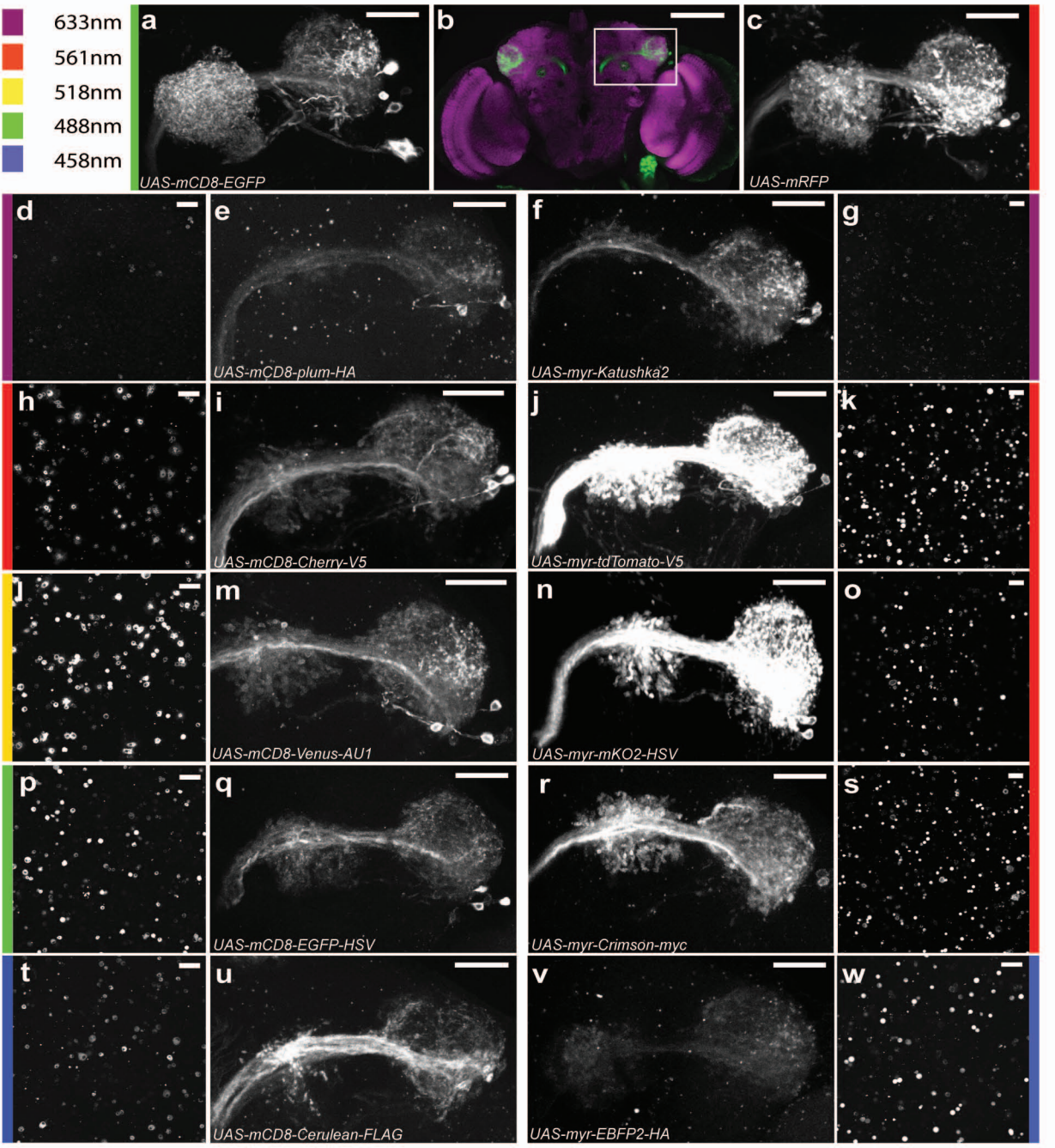
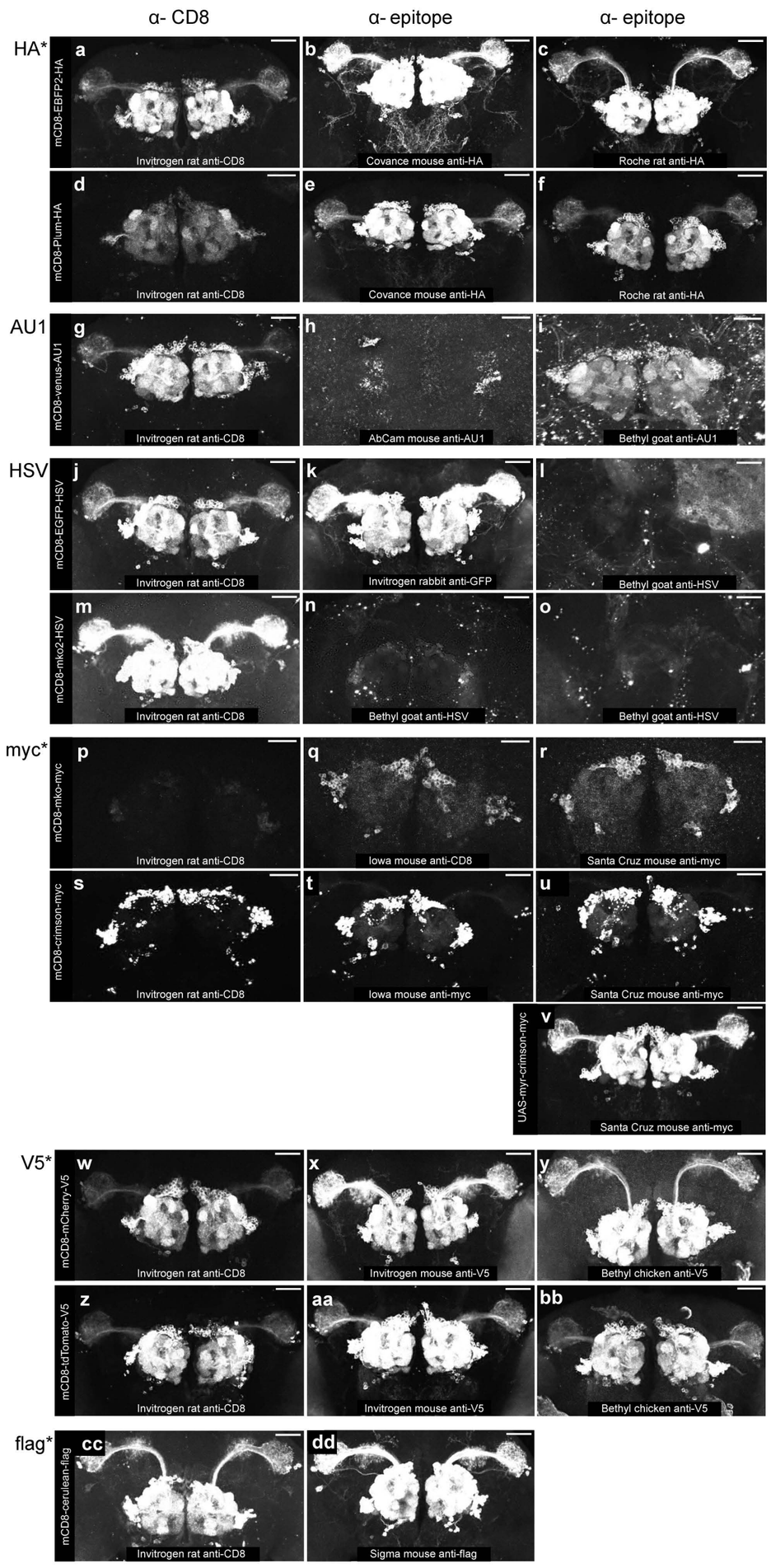
Supplementary Figure 1:



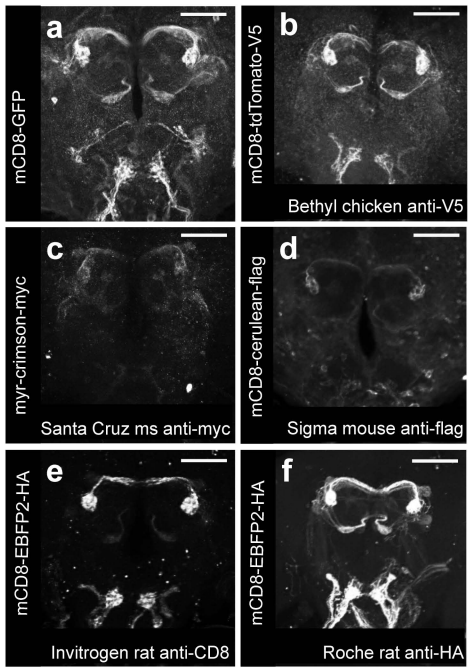
Supplementary Figure 1: Comparison of endogenous fluorescence of different fluorescent proteins in cell culture and adult Drosophila brains.
(**b**) shows a partial projection of a 20x confocal stack of *GH146-GAL4; UAS-mCD8-GFP.* The box shows the part of the pattern used in later panels. (**a**, **c**) show 40x images of *UAS-mCD8-GFP* (Bloomington stock number #5137) and *UAS-myr-mRFP* (#3127), the fluorescent reporter constructs most commonly used in fly. We compare constructs with the same sub-cellular localization: the images on the left (**e, i, m, q, u**) use the vector JFRC2 which contains mCD8 for membrane targeting, while those on the right (**f, j, n, r, v**) use JFRC12 that targets the membrane with a myristoylation sequence54. Outer panels (**d, g, h, k, l, o, p, s, t, w**) show the same constructs expressed in Drosophila S2 cells. The excitation lasers used are indicated by the color bars and the excitation and emission maxima are listed in Supplementary Table 1. Note that EBFP2 is reported to be maximally excited at 383nm but we used 458nm. We also tried a 405nm laser line but this generated so much auto-fluorescence that the weak EBFP2 signal was not detectable. The scale bar in (**b**) is 100 μm; all others are 50 μm.

Supplementary Figure 2:



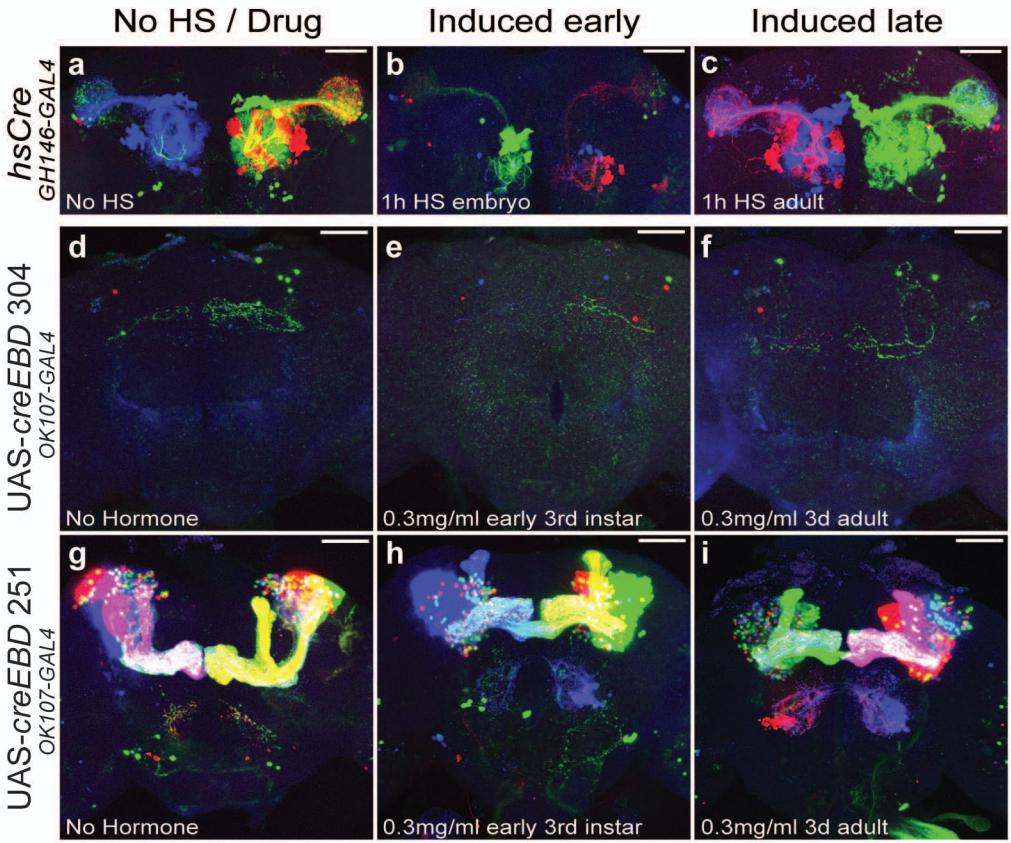
Supplementary Figure 2: Comparison of antibody-epitope performance.
The left column shows the expression of *GH146-GAL4* crossed to constructs (in vertical text at the left) each containing mCD8, plus a fluorescent protein, plus an epitope tag. By staining for mCD8 along with each experimental antibody for a tag (or fluorescent protein), we can control for differences in expression level between constructs and evaluate the performance of the experimental antibodies. At least two antibodies for each epitope were tested; the best performers are shown. When possible, two different constructs bearing the same epitope are presented. The antibodies used are listed under each image. The epitopes that performed best, and that are included in the *dBrainbow* construct, are marked with asterisks. More details about epitope sequence and antibody order numbers are given in **Supplementary Table 2.**

Supplementary Figure 3:



Supplementary Figure 3: Comparison of the best antibodies when a weaker GAL4 line drives the reporter constructs, *a81-GAL4*. The vertical text shows the description of the construct and the antibodies used are shown below each image.

Supplementary Figure 4:



Supplementary Figure 4: Comparison of available Cre recombinase sources and induction regimes.

(**a**) *hs-Cre; GH146-GAL4; UAS-dBrainbow* flies were raised at 18ºC and tested under several induction regimes: no heat-shock (**a**) and late (adult) heat shock (**c**) resulted in similar lineage-based labeling. Heat shock at 24hrs after egg-laying (b) resulted in fewer labeled cells, suggesting that very high levels of Cre are toxic in PNs. (**d**) *OK107-GAL4; UAS-dBrainbow; UAS-EBD-Cre 304* flies were tested in the absence of hormone induction (**d**) and with drug induction early (**e**) and late (**f**). Very few labeled cells were observed. Two different concentrations of hormone failed to induce fluorescent protein selection, suggesting that it is difficult to induce high enough Cre expression levels, rather than that the cells are dying due to Cre toxicity. *GH146-GAL4; UAS-dBrainbow; UAS-EBD-Cre 304* failed to show expression under any of the induction conditions tested. (**g**) *OK107-GAL4; UAS-dBrainbow; UAS-EBD-Cre 251* flies were tested under the same conditions as in (**d**). There was significant fluorescent protein expression in the absence of hormone (**g**) and early or adult exposure to hormone did not increase expression or toxicity (**h** and **i**), suggesting that this construct has significant basal expression. Interestingly, *GH146-GAL4; UAS-dBrainbow; UAS-EBD-Cre 251* flies did not show expression in any condition with this construct and we were unable to determine if this was due to poor inducibility or toxicity (data not shown).

Supplementary Tables

Supplementary Table 1: Fluorescent Proteins Tested

Fluorescent Protein	Published Excitation wavelength (nm)	Published Emission wavelength (nm)	Zeiss 710 confocal excitation laser used (nm)	Zeiss 710 Spectral detector emission range used (nm)	Our Rating	Reference
EBFP2	383	448	405 and 458	470-500 BP	*	Ai 2007
Cerulean	433	475	458	470-510 BP	****	Rizzo 2004
EGFP	488	507	488	500-540 BP	***	Zhang 1996
Venus	515	528	514	520-550 BP	***	Nagai 2002
mKO2	551	565	561	565-645 BP	*****	Sakaue-Sawana 2008
Crimson	551	565	561	575-695 BP	****	Lin 2009
tdTomato	554	581	561	550-695 BP	*****	Shaner 2004
mCherry	587	615	561	565-695 BP	***	Shaner 2004
Katushka2	588	635	633	590-695 BP	***	Shcherbo 2009
mPlum	590	649	633	610-675 BP	*	Wang 2004

Supplementary Table 1: Published emission and excitation wavelengths of tested fluorescent proteins. Since the different colors were imaged with different lasers and filters, one cannot directly compare brightness, but we optimized settings for each fluorescent protein. Our subjective performance rating, based on brightness, ability to detect small neurites, and appropriate cellular localization is listed in the right column, where the best fluorescent proteins received five stars. References for the fluorescent proteins are listed in the table: EBFP2[21], Cerulean[55], EGFP[56], Venus[57], mKO2[20], crimson[58], mCherry and tdTomato[59], Katushka2[60], mPlum[61]. We include the laser lines and spectral detector filter settings used to assess these proteins.

Supplementary Table 2: Epitopes and Antibodies Tested

Epitope	protein sequence	antibody options	our performance rating
HA	YPYDVPDYAG	mouse (Covance MMS-101P) 1:100 mouse (Santa Cruz sc-7392) 1:50 mouse (Abcam ab16918) 1:100 rat (Roche 11867423001) 1:100	***** * ** *****
V5	GKPIPNPLLGLDST	mouse (Invitrogen R960-25) 1:100 chicken (Bethyl Lab A190-118A) 1:500	***** ***
HSV	QPELAPEDPED	goat (Bethyl Lab A190-136A) 1:10 goat (Abcam ab19354) 1:10	** *
myc	MEQKLISEEDLN	mouse (DSHB 9E10) 1:10 mouse (Santa Cruz sc-40) 1:50	**** **
AU1	DTYRYI	mouse (Abcam ab24620) 1:100 goat (Bethyl A190-124A) 1:100	* **
FLAG	DYKDHDG	mouse (Sigma F1804) 1:100 mouse (Sigma F3165) 1:200	**** **
mCD8	Q60965	rat (Invitrogen/CalTag MCD0800) 1:500	****
GFP	P42212	rabbit (Invitrogen A11122) 1:500 goat (Abcam 6673) 1:2,000 chicken (Abcam 13970) 1:1000	***** ND ND

Supplementary Table 2: Detailed epitope and antibody information. The protein sequences of the epitopes are listed; the subjective rating criteria are the same as that used for endogenous fluorescence.

Supplementary Table 3: Color distribution in PN lineages

hs-Cre; GH146-GAL4; UAS-dBrainbow

Sample #	Left adPN	Left lPN	Left vPN	Right adPN	Right lPN	Right vPN
1	G	R	B	B	R	G
2	G	G	R	R	G	G
3	B	R	R	G	G	R
4	B	B	R	B	B	B
5	B	G	B	B	B	R
6	G	R	B	B	R	B
7	R	G	B	R	G	B
8	G	G	B	G	B	B
9	B	G	G	G	B	R
10	B	G	R	G	R	R
11	B	G	G	G	B	G
12	R	B	B	G	B	G
13	B	B	B	R	B	R
14	G	R	B	R	G	G
15	R	R	G	B	G	B
16	B	R	B	R	G	B
17	R	G	G	R	G	G
18	B	G	G	B	R	G
19	B	R	B	G	G	G
20	R	R	B	B	B	R
21	B	B	G	G	R	G
22	R	R	G	B	R	B
23	B	G	B	B	G	G
24	B	G	G	B	G	G
25	B	G	R	G	B	G

hs-Cre; GH146-GAL4, UAS-dBrainbow; UAS-dBrainbow

Sample #	Left adPN	Left lPN	Left vPN	Right adPN	Right lPN	Right vPN
1	M	M	G	B	C	G
2	R	C	R	C	B	B
3	M	Y	Y	C	Y	G
4	M	C	M	G	M	M
5	B	G	B	C	Y	C
6	C	M	B	M	Y	Y
7	C	M	Y	Y	C	C
8	M	C	C	Y	M	C
9	Y	C	B	Y	M	Y
10	M	B	M	M	G	R
11	G	M	C	R	C	C
12	C	Y	G	M	C	M
13	C	Y	B	B	M	M
14	M	Y	C	M	M	C
15	M	M	R	G	R	B

Supplementary Table 3: Color distribution within projection neuron lineages. Whole confocal stacks of 25 single-copy *UAS-dBrainbow* and 15 double-copy *UAS-dBrainbow* preparations of adult antennal lobes were taken and the color of each lineage scored. Grey boxes indicate hemispheres in which each of the three lineages was labeled in a different color. The genotypes of the flies were *hs-Cre; GH146-GAL4; UAS-dBrainbow* and *hs-Cre; GH146-GAL4, UAS-dBrainbow, UAS-dBrainbow* as indicated. Selection of each fluorescent protein was approximately equal: the first cassette – green – was observed 37.3% of the time, the second cassette – blue –

37.3%, and the third cassette – red – 25.3% (p=0.12; n=150; Chi-squared test), resulting in a useable mixture of colors in many of our samples (11 out of 50 brain hemispheres had each lineage in a different color; equal probabilities predicts 16/50). Color choice in adjacent lineages is independent (p=0.67; Bartel's test of randomness). Analysis of color selection frequencies when two copies of *UAS-dBrainbow* are present suggests that recombination of each copy also occurs independently (p=0.65 n=90 chi-squared test; p=0.67 n=90 Fisher's exact test, and p=0.54 Bartel's test for randomness.) The Cre recombinase is efficient: failure to remove the stop cassette was rare, as evidenced by the fact that none of our 25 samples had an unlabeled lineage.

Supplementary Table 4: Quantification of cell types labeled in R12D05-GAL4

		right SOG hemisphere										left SOG hemisphere										NMJs	
brain	color	**dorsal MN**	dim dorsal neurons	isolated frontal cells	large frontal neurons near labial nerve	ventral ascending neurons	isolated ventral cells	V-L cluster	**V-L MN**	total right		**dorsal MN**	dim dorsal neurons	isolated frontal cells	large frontal neurons near labial nerve	ventral ascending neurons	isolated ventral cells	V-L cluster	**V-L MN**	total left		rostrum NMJs	haustellum NMJs
1	G				3	4		6	1									3					x
	R											1				6		4				x	
	B	1	1			5		5		25					2	4		4	1	24		x	x
2	G	1	1		3		2	9				1		1				7				xx	
	R					7		1					2			5		2					
	B		1				1	1		27					3			2		23			
3	G	1	1					3	1				1	1				5				x	x
	R		3		3	4		4								6		4	1				x
	B						1	2		22		1			3			3		24		x	
4	G			1		11		3	1			1					2	9	1			x	xx
	R		1		3			1							3		3	2					
	B	1						4		25						7		1		28		x	
5	G	1		1		9		9	1				2		3		2	9	1			x	xx
	R				3		1							1				1					
	B						1	3		28		1				9		1		29		x	
6	G		1		3		2	4				1			3	10	1	6				x	
	R	1	1	1				1									1	6	1			x	x
	B					9		6	1	29										28			x
7	G	1			3	7	1	2							3	8	1	7				x	
	R					3	1	3	1				1					2					x
	B		1					4		26		1					1		1	24		x	x
8	G		3		3	14		5				1	3		3	10		5				x	
	R	1	1					4					2				1	5	1			x	x
	B						2	4	1	37								3		33			x
9	G	1	1					3				1		2		9		4	1			xx	x
	R					6		7					1					2					
	B				3			3	1	24			1		3			3		26			x
10	G	1			5			6	1			1				9		5					
	R						1	2				1		1			1						
	B	1				4		7		27					3		2	4		27			
11	G		1	4				5				1			1		1	3	1				
	R				1	5		4	1			1	1			6		3					
	B	1					4	3		28							3	2		22			

Supplementary Table 4: Quantification of cell types labeled in *hs-Cre; R12D05-GAL4; UAS-dBrainbow.* Cells were counted in the SOG of 11 brains using confocal stacks and cell count marking software (a custom plug-in for ImageJ, a gift of Dr. A. Jenett, JFRC). The large, bright single neuron in the dorsal SOG was clearly a motor neuron ("dorsal MN") because its color corresponded to the color of the rostrum NMJs in all 9 samples examined. (Samples 10 and 11 were omitted from the proboscis NMJ color analysis because more than one of these bright dorsal neurons was visible.) Additional dim dorsal neurons were sometimes found nearby. Although the ventrolateral (V-L) cluster of cells always contains multiple colors, the V-L motor neuron can be singled out because of its distinctive C-shaped arbor, corresponding to the color of the haustellum NMJs in all 9 samples examined. (In two other brains the arbors could not be seen or were present in one hemisphere but not the other.) We did not count a population of cells on the back of the SOG, which were difficult to image through the thickness of the brain, and sometimes damaged by the dissection. dBrainbow makes it clear that some cell clusters likely derive from a single lineage (for example, a small group of large frontal neurons near the labial nerve were always labeled in a single color), while other clusters often showed multiple colors.