

Supplementary Table 2: Fly genotypes for anatomical analyses.

| Figure | Driver line(s) | Reporter transgene(s) | Description of displayed image | Details of original image (objective, mounting medium, voxel size) | Sample size |
|--|--|--|---|--|---|
| 1a; Ext. 1d; Ext. 9a,d | OL0047B [<i>R19G02-p65ADZp (attP40)</i> ; <i>R12E04-ZpGdbd (attP2)</i>] | MCFO-1 [<i>pBPhsFlp2::PEST (attP3)</i>]; <i>pJFRC201-10XUAS-FRT>STOP>FRT-myr::smGFP-HA (VK0005)</i> , <i>pJFRC240-10XUAS-FRT>STOP>FRT-myr::smGFP-V5-THS-10XUAS-FRT>STOP>FRT-myr::smGFP-FLAG (su(Hw)attP1)</i>] | Reconstructed view generated from 3D image stack (Vaa3D/NeuronAnnotator screenshot). | 63x/ NA 1.4, DPX, 0.19 μm x 0.19 μm x 0.38 μm | MCFO-labeled cells from 14 fly brains imaged at high resolution (63x) (~ 120 labeled LPLC2 cells). |
| 1d,e; 5j,k; Ext. 1c,e,f; Supplementary Video 1 | OL0047B or OL0048B [<i>R19G02-p65ADZp (attP40)</i> ; <i>R75G12-ZpGdbd (attP2)</i>] | MCFO-1 | Reconstructed view generated from 3D image stack (Vaa3D/NeuronAnnotator screenshot). Cells were manually segmented and colored based on approximate layer positions (using Fluorender and Fiji). Illustration in Fig. 5j,k is based on the image from Ext. Fig. 1f (cell2). | 63x/ NA 1.4, DPX, 0.19 μm x 0.19 μm x 0.38 μm | Segmentation and layer-specific coloring was done for the six cells shown. The general pattern of LPLC2 arbors in the LP illustrated by these examples was similar for ~ 110 MCFO-labeled cells from 20 brains [OL0047B (14 brains, see above) or OL0048B (6 brains, images generated in a previous study ^{ref.10}). (also see Methods). |
| Ext. 1a; Ext. 9c,f | SS00810 [<i>R28D05-p65ADZp (attP40)</i> ; <i>R55H05-ZpGdbd (attP2)</i>] | MCFO-1 | Reconstructed view generated from 3D image stack (Vaa3D/NeuronAnnotator screenshot). | 63x/ NA 1.4, DPX, 0.19 μm x 0.19 μm x 0.38 μm | 3 optic lobes with from 3 flies imaged at high resolution (63x). |
| 5k; Ext. 9b,e | SS03752 [<i>R38G02-p65ADZp (attP40)</i> ; <i>R24A07-ZpGdbd (attP2)</i>] | MCFO-1 | Reconstructed views generated from 3D image stack (Vaa3D/NeuronAnnotator screenshots) (Ext. 9b,e). Illustration in Fig. 5k is based on manually segmented cells (Fluorender). | 63x/ NA 1.4, DPX, 0.19 μm x 0.19 μm x 0.38 μm | 3 optic lobes from 3 flies imaged at high resolution (63x) (~ 20 LPi43 cells total). |
| 1b | VT014708 (GF), OL0047B (LPLC2) | MCFO-7 [<i>R57C10-Flp2::PEST (attP18)</i>]; <i>pJFRC210-10XUAS-FRT>STOP>FRT-myr::smGFP-OLLAS (attP2)</i> , <i>pJFRC201-10XUAS-FRT>STOP>FRT-myr::smGFP-HA (VK0005)</i> , <i>pJFRC240-10XUASFRT>STOP>FRT-myr::smGFP-V5-THS-10X-UAS-FRT>STOP>FRT-myr::smGFP-FLAG (su(Hw)attP1)</i> (GF) <i>pJFRC51-3XUAS-IVS-syt::smHA (su(Hw)attP1)</i> , <i>pJFRC225-5XUAS-IVS-myr::smFLAG (VK00005)</i> (LPLC2; only myr::smFLAG pattern shown) | Illustration based on maximum intensity projection of an overlay of computationally aligned image stacks. Images were manually edited to only show GF and LPLC2 cells. | 20x/ NA 0.8, DPX, 0.52 μm x 0.52 μm x 1 μm | Overlap of GF dendrites with LPLC2 target region (identified from anti-Brp reference pattern ^{ref.10}) was confirmed for 5 additional MCFO-labeled GF cells from 5 fly brains. |
| 2b; 3a | OL0048B | <i>pJFRC51-3XUAS-IVS-syt::smHA (su(Hw)attP1)</i> , <i>pJFRC225-5XUAS-IVS-myr::smFLAG (VK00005)</i> (only myr::smFLAG pattern shown) | Reconstructed view generated from 3D image stack (Vaa3D/NeuronAnnotator screenshot). | 63x/ NA 1.4, DPX, 0.19 μm x 0.19 μm x 0.38 μm | Image (same in 2b, 3a) illustrates approximate ROI for calcium imaging. OL0048B pattern has been described ^{ref.10} . |
| 5a; Ext. 6a | SS03752 | <i>pJFRC51-3XUAS-IVS-syt::smHA (su(Hw)attP1)</i> , <i>pJFRC225-5XUAS-IVS-myr::smFLAG (VK00005)</i> (only myr::smFLAG pattern shown in Ext. Fig. 6a) | Reconstructed view generated from 3D image stack (Vaa3D/NeuronAnnotator screenshot). | 63x/ NA 1.4, DPX, 0.19 μm x 0.19 μm x 0.38 μm | 5 optic lobes from 4 flies imaged at high resolution (63x). |
| Ext. 1b | OL0047B | <i>pJFRC51-3XUAS-IVS-syt::smHA (su(Hw)attP1)</i> , <i>pJFRC225-5XUAS-IVS-myr::smFLAG (VK00005)</i> | Reconstructed view generated from 3D image stack (Vaa3D/NeuronAnnotator screenshot). | 63x/ NA 1.4, DPX, 0.19 μm x 0.19 μm x 0.38 μm | 3 fly brains imaged at high resolution (63x). |
| Ext. 2a | SS00324 [<i>R59E08-p65ADZp (attP40)</i> ; <i>R42F06-ZpGdbd (attP2)</i>] (T4/T5), VT049479- <i>LexAp65 (attP40)</i> (LPLC2) | <i>LexAop2-Syn21-opGCaMP6s (su(Hw)attP8)</i> , <i>10XUAS-Syn21-Chrimson-tdT-3.1(attP18)</i> | Maximum intensity projection from substack. | 40x/ NA 1.3, Slow Fade Gold, 0.21 μm x 0.21 μm x 1 μm | Pattern confirmed for 2 fly brains. SS00324 pattern has been described ^{ref.20} . |
| 5e; Ext. 7a | SS03752 (Lpi4-3), VT049479- <i>LexAp65 (attP40)</i> (LPLC2) | <i>LexAop2-Syn21-opGCaMP6s (su(Hw)attP8)</i> , <i>10XUAS-Syn21-Chrimson-tdT-3.1(attP18)</i> | Maximum intensity projection from substack. | 40x/ NA 1.3, Slow Fade Gold, 0.21 μm x 0.21 μm x 1 μm | Pattern confirmed for 2 fly brains. |