Glomerular Maps without Cellular Redundancy at Successive Levels of the *Drosophila* Larval Olfactory Circuit

Ariane Ramaekers, Edwige Magnenat, Elizabeth C. Marin, Nanaë Gendre, Gregory S.X.E. Jefferis, Liqun Luo, and Reinhard F. Stocker

Supplemental Experimental Procedures

Frequency Comparison between MARCM and FLP-out Clones

When studying MARCM clones, we observed 16% of the labeled PNs to establish dendrites in two glomeruli of the LAL [S1], whereas when we used the FLP-out method, we found that larval PNs were always uniglomerular. Also, the MARCM study revealed that 29% of PNs targeted two calycal glomeruli, whereas 4% did so in the FLP-outs. However, these frequencies cannot be directly compared for the two techniques. MARCM labeling is linked to cell division [S2] and therefore is likely to result in a sampling bias if heat shock is applied nonrandomly during development to induce recombination. If rare multiglomerular PNs are born at specific times relative to heat-shock induction of recombinase, they may be overrepresented in MARCM studies. FLP-out labeling, on the other hand, occurs postmitotically [S3]; it is therefore expected to reflect more faithfully the actual frequencies of given cell types. As an alternative explanation, MARCM may create a cell that is homozygous for a mutation or background modifier that changes the morphology of some PNs compared to wild-type PNs. Finally, because the MARCM clones were analyzed slightly later in development, i.e., during the wandering third instar, recruitment of additional glomeruli by some of the PNs is also a possibility.

Supplemental References

S1. Marin, E.C., Watts, R.J., Tanaka, N.J., Ito, K., and Luo, L.L. (2005). Developmentally programmed remodeling of the *Drosophila* olfactory circuit. Development 132, 725–737.

S2. Lee, T., and Luo, L. (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22, 451–461.

S3. Wong, A.M., Wang, J.W., and Axel, R. (2002). Spatial representation of the glomerular map in the *Drosophila* protocerebrum. Cell 109, 229–241.

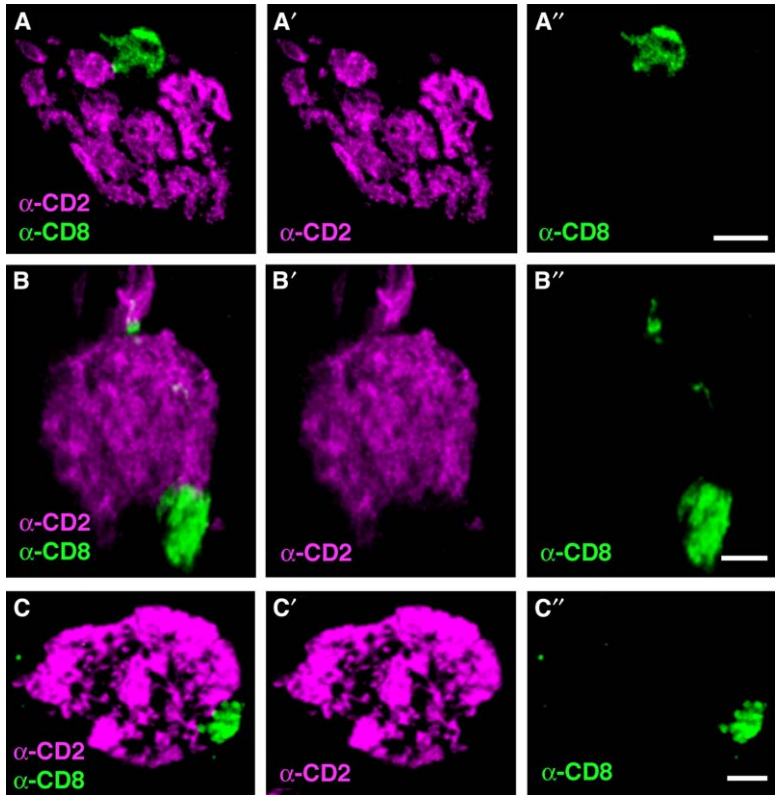

Figure S1. Expression Domains of CD2 and CD8 in Single-Cell FLP-out Clones Overlap Neither in Odorant-Receptor Neuron Terminals nor in Input and Output Glomeruli of Projection Neurons

(A–A″) LAL: Mutually exclusive expression domains of CD2 and CD8 in FLP-outs performed in the ORN-specific OR83b-GAL4 line demonstrate that each LAL glomerulus is the target of a single ORN.

(B–B″) LAL: Mutually exclusive expression of CD2 and CD8 in FLP-outs of the GH146-GAL4 line (which labels PNs) show that each LAL glomerulus is innervated by dendrites from a single GH146-positive PN.

(C–C″) MB calyx: Mutually exclusive expression of CD2 and CD8 in FLP-outs of GH146-GAL4 show that calycal glomeruli are essentially targets of single GH146-positive PNs.

All panels represent stacks of multiple confocal sections, with dorsal on top and lateral to the right. Scale bars represent 5 μm (for each row).

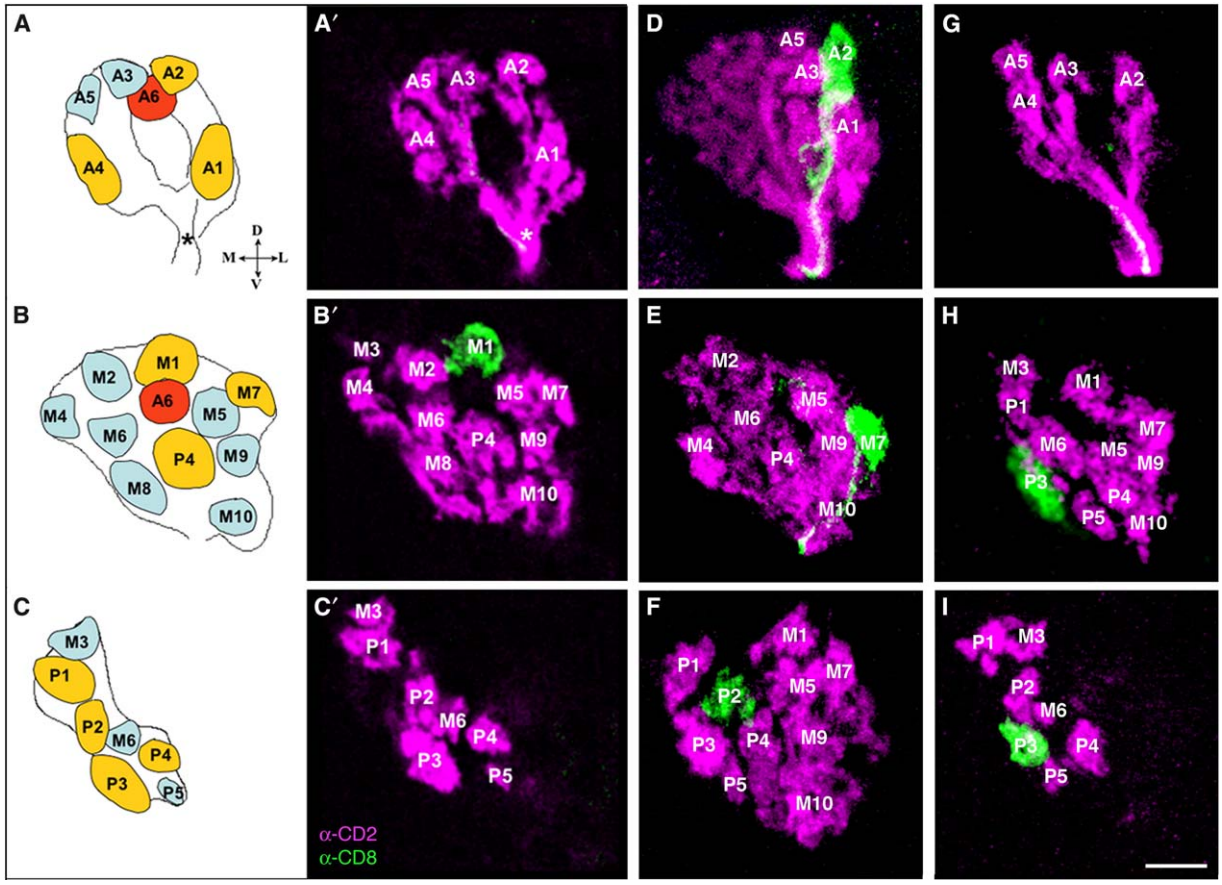

Figure S2. Conservation of the Glomerular Map in the Larval Antennal Lobe

The glomerular map of the LAL based on ORN projections is conserved among individuals. Top, middle, and bottom rows represent anterior, middle, and posterior levels of the LAL, respectively. In addition to panels (A)–(C′) and (D)–(F), which were taken from Figure 1, an additional individual is shown for each level (G, H, and I). The spatial orientation for all panels is given in panel (A). For glomerular terminology and other details, see Figure 1 in the main text. The scale bar in (I) represents 5 μm (valid for all panels).