Supplementary Information

Diverse populations of local interneurons integrate into the
*Drosophila* adult olfactory circuit

Liou et al.
Supplementary Note 1. The soma position of larval LNs.

Larval LNs form two clusters in the larval AL, one in the lateral region and the other in the ventral region (Fig. 2a, Supplementary Fig. 1a). Our data show that all 189Y-positive larval LNs (in the ventral cluster) and NP3056-positive larval LNs (in both lateral and ventral clusters) reintegrate into the adult circuit. Furthermore, the reintegrated cells all have somas that are located in the lateral LN cluster of adult brains (Fig. 4c, e), suggesting that the positions of LN clusters in the larval AL and the adult AL are not directly related. Therefore, it is not clear whether the two larval LN clusters arise from the same or different lineages. In addition, 670-positive larval LNs, which eventually degenerate, are found in the ventral cluster among 189Y-positive and NP3056-positive larval LNs. Thus, the position of larval LN somas did not dictate whether the cells are pruned or degenerate.

Supplementary Note 2. The gap between the birth time of LNs and their emergence and integration to the olfactory circuit.

Previous studies have used clonal analyses to demonstrate that LNs are born from embryonic to late pupal stages1-3. Therefore the previous studies provided only the birth time of distinct types of LNs and no information about how and when those LNs develop and integrate to the circuit. In this work, we found a certain time delay between the birth time of a given LN and the emergence and integration of the LN to the circuit. In addition, not counting those LNs born at the embryonic stage, at least 48 lateral LNs are born during the larval stage and remain in the adult brains2. However we found that approximately 22 larval LNs survive metamorphosis and remain in the AL of 24 h APF pupae. In other words, it is likely that more than 26 adult-specific LNs (after deducting 22 LNs from 48 LNs born at the larval stage plus embryonic born LNs) in the lateral cluster emerge and integrate to the circuit after 24 h APF.

Supplementary Note 3. The types of larval LNs.

Four types of 189Y-positive larval LNs in late 3rd instar larval brains were identified in this work (Fig. 4b1-b4). They are either found with dense processes restricted to and covering the whole AL (Fig. 4b1, n = 22/68 single cell clones (SCCs)), covering the whole AL and innervating SEZ (Fig. 4b2, n = 30/68 SCCs), sparse processes covering the whole AL (Fig. 4b3, n = 9/68 SCCs), or dense processes covering a part of the AL (Fig. 4b4, n = 7/68 SCCs). The first and second types of 189Y-positive larval LNs were reported previously4. Four types of NP3056-larval LNs in late 3rd instar larval brains were also identified in this work (Fig. 4d1-d4). They either exhibit processes restricted to and covering the entire AL (Fig. 4d1,
dashed circle, \( n = 21/86 \) SCCs), covering a part of the AL and innervating the SEZ (Fig. 4d\(_2\), contoured by white dashed line, \( n = 24/86 \) SCCs), covering the entire AL and innervating the SEZ (Fig. 4d\(_3\), \( n = 25/86 \) SCCs), or with most processes in the AL and a few processes to dorsal brain regions (arrows) and the SEZ (Fig. 4d\(_4\), \( n = 16/86 \) SCCs). Two types of NP3056-positive larval LNs shown in Fig. 4d\(_2\) and Fig. 4d\(_3\) were reported previously\(^4\).

**Supplementary Note 4. Glomerular index.**

The glomerular index shown in figures are as follows:

(Fig. 6h, 6i, Supplementary Fig. 12a, 12b, 12c)

DA1, DA2, DA3, DA4m, DA4l, DM1, DM2, DM3, DM4, DM5, DM6, VA1d, VA1v, VA2, VA3, VA4, VA5, VA6, VA7m, VA7l, VM1, VM2, VM3, VM4, VM5v, VM5d, VM6, VM7, 1, D, DC1, DC2, DC3, DC4, DL1, DL2d, DL2v, DL3, DL4, DL5, V, VL1, VL2a, VL2p, VC1, VC2, VC3, VC4, DP1m, DP1l, VP1, VP2, VP3, Arm, Column

(Fig. 6j, Supplementary Fig. 8c right, 8e, 12d)

V, VM6, VA3, VM4, VM1, VL1, VA4, VA2, VA5, VM3, VA7m, VA7l, VP1, VM2, VP2, VC2, VC3, VA1v, VC4, VP3, VC1, VL2p, VM5v, VL2a, VA6, 1, DM5, VM7, VM5d, VA1d, Column, DC3, DM4, Arm, DA4l, DA2, DC2, DM2, DM6, DC4, DA4m, DL2v, DP1l, DC1, DA1, DL2d, DM1, DA3, DP1m, DM3, DL4, D, DL1, DL5, DL3

(Fig. 6k, Supplementary Fig. 8d right)

V, VA3, VL1, VA5, VA4, VM6, VM4, VA7l, VA2, VA7m, VA1v, VM1, VM3, VC2, VP3, VC4, VL2p, VC3, VA6, VM2, VL2a, VP1, VM5v, VC1, VA1d, VP2, DC3, 1, Column, VM5d, DA4l, DM5, VM7, DA2, DA4m, DA1, DM4, DL2d, Arm, DL2v, DC4, DC1, DC2, DA3, DM6, DP1l, DM2, D, DL4, DM1, DP1m, DL1, DL3, DM3, DL5

(Supplementary Fig. 8c left and middle, 8d left and middle, 8g left, 9d left and middle)

V, VM6, VA3, VL1, VM4, VA5, VA4, VA2, VM1, VM3, VA7m, VA7l, VA1v, VP1, VM2, VC2, VC3, VP3, VP2, VC4, VL2p, VL2a, VC1, VM5v, VA1d, 1, VA6, DM5, Column, VM7, DC3, DM4, VM5d, Arm, DA4l, DM6, DA2, DP1l, DC2, DA1, DL2d, DC1, DC4, DM2, DA4m, DL2v, DA3, DL4, DP1m, DM1, DM3, D, DL1, DL5, DL3

(Supplementary Fig. 9d right)

V, VM6, VL1, VM4, VA3, VM1, VA4, VA5, VA2, VM3, VP1, VP3, VA1v, VP2, VA7m, VA7l, VL2p, VC2, VC3, VM2, VC4, VC1, VL2a, VM5v, VA6, Column, VA1d, 1, DM5, VM7, Arm, DC3, VM5d, DM4, DL2d, DL2v, DA2, DA4l, DA1, DP1l, DC4, DC2, DM2, DM2, DC1, DA4m, DP1m, DM1, DA3, DL4, DM3, D, DL1, DL3, DL5
Supplementary Fig. 1 The development of distinct subsets of LNs. Projected confocal images show larval, pupal and adult brains stained with neuropil markers DNcad (magenta, larval and pupal brains) or Bruchpilot (magenta, adult brains). LNs were visualized by GAL4-driven mCD8GFP. Developmental stages are indicated by 3rd L: third larval instar, hours after puparium formation (APF): pupal stages, and adult. a Group 1: GAL4 lines that label LNs as early as late 3rd instar larvae. Identical images of 189Y, NP3056, 449 and 670 are also shown in Fig. 2a. b Group 2: GAL4 lines labeling LNs that emerge from early to mid-pupal stages (48 h APF). Identical images of 351 and 501 are also shown in Fig. 2b. c Group 3: GAL4 lines labeling LNs that emerge after 48 h APF. Identical images of 95 and 988 are also shown in Fig. 2c. Asterisks denote glia or neurons that are not LNs. Scale bars, 20 μm. All ALs in this and following figures are oriented such that the midline is in the left. D, dorsal; L, lateral.
Supplementary Fig. 2 Estimated number of LNs labeled in individual and combined GAL4 lines. **a** Projected confocal images show GAL4-labeled LNs (GFP, green) and neuropil (DNcad, magenta). Scale bars, 20 μm. **b** Estimated number of labeled LNs per AL are shown as mean ± s.d., based on the scoring of 7-26 ALs. Asterisks denote strong genetic interaction among GAL4 insertions in combined GAL4 lines. From the results of two combined GAL4 drivers, 449 and NP3056 label non-overlapping subsets of larval LNs. 189Y-labeled larval LNs are either non-overlapping, or overlap by one LN with those labeled by 449. Since 189Y- and 449-positive larval LNs innervate distinct regions of the AL at 24 h APF (Fig. 2a), these two GAL4s probably label non-overlapping subsets of larval LNs. Therefore, the estimated lower bound number of total larval LNs is either 26 or 27 (22 derived from NP3056+449+670-positive, plus five derived from 189Y-positive, with or without one LN labeled by both 189Y and one of the other GAL4 drivers). Note that c739-GAL4 labels a subset of larval LNs⁵ that has strong genetic interaction with 189Y and the combination of NP3056, 449 and 670, thus precludes further estimation of larval LN number.
Supplementary Fig. 3 189Y-positive, NP3056-positive and 449-positive larval LNs express EcRB1 and undergo pruning through the ecdysone signaling pathway. a (Left) Projected confocal image showing 0 h APF pupal brain carrying 189Y-positive, NP3056-positive or 449-positive larval LNs, co-stained with EcRB1 (blue) and Dncad (magenta). (Right) Single confocal section shows larval LNs (green) express EcRB1 (arrows, blue in the top panel and white in the bottom panel). A subset of 449-positive larval LNs does not express EcRB1 (arrowheads). n = 11 brains for each GAL4. Scale bars, 20 μm (left panels) and 10 μm (right panels). b Blocking ecdysone signal in 189Y-positive or 449-positive larval LNs by overexpressing truncated Ecdysone receptor (EcR) leads to failure of pruning. Compared to 189Y-LN processes, which occupy the medial region, 449-LN processes occupy the central region of the AL in 24 h APF control pupal brains (n = 19 and 22 brains for 189Y and 449, respectively), LNs expressing truncated EcR have processes in the ventral region of the AL and SEZ, which is reminiscent of their innervation patterns in 0 h APF pupal brains (n = 23 and 9.
brains for 189Y and 449, respectively). Failed pruning of 189Y-processes is more prevalent in the central AL, which is likely due to weak GAL4 expression in these LNs, indicating partial penetrance of the phenotype. Scale bars, 20 μm. c 0 h APF and 36 h APF pupal brains from control flies (n = 12 and 14 ALs for 0 h and 36 h, respectively) and 670-GAL4 driven UAS-GAL4 flies (n = 12 and 14 ALs for 0 h and 36 h, respectively). Sustained GAL4 expression in larval LNs (arrowheads) did not result in labeling of these neurons in 36 h APF pupal brains. Images for 670-GAL4 control flies were reproduced from Fig. 2a. Scale bars, 20 μm.
Supplementary Fig. 4 Morphogenesis of NP3056-positive larval LNs during pupal development. a-d The same labeling strategy as in Fig. 4e was used to examine the morphologies of NP3056-positive larval LNs in 24 h APF (n = 26 SCs/128 ALs) (a), 36 h APF (n = 33 SCs/114 ALs) (b), 48 h APF (n = 68 SCs/380 ALs) (c) and 72 h APF (n = 53 SCs/245 ALs) (d) pupal brains. Brains were stained with V5 and DNcad (not shown) to visualize neurons and neuropil, respectively. These larval LNs may have processes that innervate both ALs (a1-d1, bilateral LN), the central portion of the AL (a2-c2) or the posterior of the AL (a3-c3, d2). Middle panels show a presumably regional LN, and right panels show a presumably oligo-glomeruli LN in the right AL, with 1-2 bilateral LNs (magenta arrowheads) in the ipsi- and/or contralateral AL. ALs are indicated by yellow dashed contours. Yellow arrowheads indicate LN soma. Red arrowheads indicate the processes of presumably regional LNs or oligo-glomerular LNs. Red arrow indicates glomerulus V that is likely innervated by the regional LN. Right panels are partial projections of the posterior ALs. Soma that are not covered in the projections are denoted with dashed ovals. Asterisk denotes non-LN cells. Scale bars, 20 μm.
Supplementary Fig. 5 Visualizing 449-positive larval LNs in adult brains. Similar to Fig. 4a, c but examining 449-positive larval LNs in adult brains. 

a) No GFP-labeled LNs were found in the adult brain of flies raised at a constant 18°C throughout development (n = 35 brains).

b) When the flies were raised at a constant 29°C during development, both larval and adult-specific LNs were labeled (n = 39 brains).

c) When flies were raised at 29°C from embryonic stages to larval stage and shifted to 18°C at puparium formation, 3-24 LNs per AL were observed in adult brains (n = 51 brains).

e1, e2 A single confocal section of the area outlined in the projected brain shown in e reveals that most of labeled 449-positive larval LNs are GABA-positive. Arrowheads indicate the soma of GdP-positive LNs. Scale bars, 20 μm.
Supplementary Fig. 6 Relative distribution of LN processes and PN dendrites in developing ALs. **a** Partially projected confocal images show pupal brains carrying NP3056-GAL4 and GH146-QF, co-stained with GFP for LNs (white in left, and green in the middle and right panels), mtdT for PNs (magenta in the middle and right panels) and DNcad for neuropils (blue in the right panel) (n = 28 and 15 brains at 24 h APF and 36 h APF, respectively). GH146-QF is expressed in approximately two-thirds of PNs. **b** Similar to (a) but with 449-positive LNs and GH146-positive PNs (n = 17 and 20 brains at 24 h APF and 36 h APF, respectively). **c** Similar to (a) but with 670-positive LNs and GH146-positive PNs (n = 12 and 11 brains at 12 h APF and 24 h APF, respectively). Scale bars, 20 μm.
**Supplementary Fig. 7** Ectopically expressed DTI effectively ablated larval LNs. a Schematic of temperature shift. Larvae were transiently raised at 29°C from late 3rd instar larvae to 6.5 h APF. b, d, f Control or LN>DTI pupal brains were stained with Dncad (magenta) and GFP (green). b Compared to majority control brains with 2-3 larval LNs (1.86 ± 0.15, n = 50 ALs), 75% of NP3056>DTI brains have only 0-1 larval LNs (0.76 ± 0.12, n = 68 ALs). d Compared to control brains with ~20 larval LNs (20.24 ± 0.44, n = 34 ALs), 449>DTI brains have ~14 larval LNs (14.45 ± 0.57, n = 29 ALs). f Compared to control brains with 3-5 larval LNs (3.09 ± 0.10, n = 34 ALs), 70% of 670>DTI brains have only 0-2 larval LNs (1.67 ± 0.22, n = 33 ALs). The majority of larval LNs in the 670>DTI brains exhibited extremely dim fluorescence and relatively few processes, implying that they were dying. Arrowheads indicate single LNs, arrows indicate a group of LNs, and open arrowhead indicates area that was previously occupied by ablated LNs. Scale bars, 20 μm. c, e, g Quantification of NP3056-positive larval LNs (c), 449-positive larval LNs (e), and 670-positive LNs (g) in control and DTI-expressing brains. The graph shows mean ± s.e.m. Student’s t-test was used to compare the groups. **p < 0.005, ***p < 0.001.
Supplementary Fig. 8 Genetic ablation of larval LNs causes aberrant glomeruli organization. a, b Bar graphs showing the distance (a) and y-position (b) of UAS-DTI control, LN-GAL4 control or LN>DTI ALs to the reference origin. c The y-position of individual glomeruli in UAS-DTI, NP3056 control and NP3056>DTI ALs was determined. Glomeruli were aligned based on their y-position value in UAS-DTI (left and middle panels) or UAS-GAL4 (right panel) control ALs. Note that NP3056>DTI glomeruli have larger y-position values. The right panel is reproduced from Fig. 6j. d Same as (c) but for 670>DTI. 670>DTI glomeruli do not show obvious differences with UAS-DTI or 670 control glomeruli. The right panel is reproduced from Fig. 6k. e The relative shift of individual glomeruli in NP3056>DTI ALs was quantified as the deviation. Glomeruli were aligned as in the left panel of Fig. 6j. The mean deviation of all glomeruli is 14.0318 (black line). Grey lines indicate one standard deviation (SD) from the mean, which contains 68.3% of the glomeruli. f 3D-reconstructed ALs show glomeruli with deviation > 1 SD (pink) and < -1 SD (orange). Identical results in (e) and the anterior view of NP3056 control AL (f) were also shown in Fig. 6j. g, h Same as (e) and (f), respectively, but UAS-DTI was used as control for comparison. The directions of relative X, Y, Z axes are indicated. The axis origin was the tip of the MB peduncle. The information of glomerular index is provided in Supplementary Note 4. a-d, mean ± s.e.m.
Supplementary Fig. 9 The effect of genetic ablation of 449-positive larval LNs at a late larval stage on glomeruli organization. a Schematic of temperature shift experiments. Larvae were transiently raised at 29°C from late 3rd instar larvae to 6.5 h APF. b PN dendrites targeting to glomeruli DA1, VA1d and DC3 of GAL4 and UAS-DTI controls and 449>DTI brains. Adult brains were stained for Bruchpilot (magenta) to visualize individual glomeruli and GFP to visualize PN dendrites (green). Scale bars, 20 μm. UAS-DTI result was reproduced from Fig. 6b. c 3D reconstruction of ALs from 449-GAL4 and UAS-DTI control (top) and 449>DTI brains were shown in anterior view (left) and lateral view (right), respectively. D, dorsal; L, lateral; P, posterior. d The y-position of individual glomeruli in 449-GAL4 and UAS-DTI control (left), UAS-DTI control and 449>DTI (middle) and 449-GAL4 control and 449>DTI (right) ALs. Glomeruli were aligned based on their y-position value in the UAS-DTI (left and middle panels) or UAS-GAL4 (right panel) control ALs. Note that 449>DTI glomeruli have similar y-position values to UAS-DTI control glomeruli. These results suggest 449-GAL4 alone has some effect on glomerular organization; in other words, it is not clear whether 449>DTI causing glomerular organization defect or not. The bar graphs show mean ± s.e.m. The information of glomerular index is provided in Supplementary Note 4.
Supplementary Fig. 10 3D reconstruction of ALs from control and LN>DTI brains. All 6 reconstructed ALs from control and LN>DTI brains were shown in anterior view (top) and lateral view (bottom). The outlined AL of each group was reproduced from Fig. 6c, e and Supplementary Fig. 9c, respectively.
Supplementary Fig. 11 Schematic diagram of a brain 3D reconstruction by Imaris. The surface of individual glomeruli, AL and MB medial lobe were manually outlined (grey) based on neuropil marker (red). The origin for the axes was set at the tip of MB peduncle (orange ball). The Y-Z plane was set such that the two brain hemispheres were evenly divided, with Y-axis parallel to the MB dorsal lobe. Accordingly, Z-axis was perpendicular to the MB dorsal lobe. The X-axis was set perpendicular to the Y-Z plane. Scale bar, 30 μm.
Supplementary Fig. 12 The relative distance between any two glomeruli in the ALs of flies with genetically ablated larval LNs. 

**a** The relative distance between any two glomeruli in GAL4 and UAS-DTI controls and LN>DTI ALs was estimated as in Fig. 6h. Results for NP3056-GAL4 and NP3056>DTI were reproduced from Fig. 6h.  

**b** The differences between a given pair of glomeruli in GAL4 control and LN>DTI ALs were shown. Results of NP3056 and 670 were reproduced from Fig. 6h, i.  

**c** The differences between a given pair of glomeruli in UAS-DTI control and LN>DTI ALs were shown.  

**d** The relative distance between any two glomeruli in NP3056-GAL4 controls and NP3056>DTI ALs was estimated and realigned by the glomerulus index used in the y-position shift analysis (Fig. 6j). The result demonstrates no clear correlation between changes of glomerulus-glomerulus distance and y-position shifts. The information of glomerular index is provided in Supplementary Note 4.
Supplementary Table 1  Number of LNs labeled by individual GAL4 lines and their neurotransmitters.

| GAL4 | LNs  | AL  | GABA+ (mean ± s.e.m.) | GABA- (mean ± s.e.m.) |
|------|------|-----|----------------------|----------------------|
| 68   | 2.75 ± 0.16 | 8   | 0 ± 0                | 2.75 ± 0.13          | 12 |
| 95   | 2.69 ± 0.15 | 16  | 0 ± 0                | 1.8 ± 0.2            | 5  |
| 154  | 9.43 ± 0.43 | 7   | N.D.*                | N.D.                 | N.D. |
| 191  | 58.25 ± 1.18 | 8   | N.D.                 | N.D.                 | N.D. |
| 330  | 8.71 ± 0.29 | 7   | N.D.                 | N.D.                 | N.D. |
| 351  | 3.0 ± 0     | 6   | 0 ± 0                | 2.89 ± 0.11          | 9  |
| 361  | 44.83 ± 5.45 | 6   | N.D.                 | N.D.                 | N.D. |
| 382  | 36.6 ± 2.01 | 5   | 18.0 ± 3.03          | 19.5 ± 2.78          | 4  |
| 388  | 4.83 ± 0.40 | 6   | N.D.                 | N.D.                 | N.D. |
| 415  | 9.0 ± 0.38  | 7   | N.D.                 | N.D.                 | N.D. |
| 421  | 21.375 ± 0.73 | 8   | 9.38 ± 0.91          | 12.0 ± 0.73          | 8  |
| 449  | 60.5 ± 1.45 | 8   | 53.25 ± 3.89         | 9.38 ± 4.35          | 8  |
| 501  | 20.63 ± 0.84 | 8   | 18.83 ± 1.85         | 4.0 ± 0.63           | 6  |
| 551  | 4.17 ± 0.31 | 6   | N.D.                 | N.D.                 | N.D. |
| 584  | 1.0 ± 0     | 5   | 0 ± 0                | 1.0 ± 0              | 11 |
| 658  | 30.6 ± 0.95 | 10  | 24.63 ± 1.49         | 2.75 ± 0.88          | 8  |
| 666  | 19.83 ± 1.30 | 6   | N.D.                 | N.D.                 | N.D. |
| 670  | 3.90 ± 0.18 | 10  | 3.0 ± 0.37           | 1.33 ± 0.42          | 6  |
| 674  | 27.17 ± 1.17 | 6   | 20.11 ± 1.55         | 6.67 ± 1.04          | 9  |
| 829  | 23.17 ± 1.35 | 6   | 23 ± 1.73            | 2.67 ± 1.76          | 3  |
| 930  | 2.73 ± 0.14 | 11  | 0 ± 0                | 2.92 ± 0.08          | 12 |
| 988  | 23.75 ± 1.25 | 4   | 19.67 ± 1.56         | 3.83 ± 0.40          | 6  |
| 1008 | 5.33 ± 0.21 | 6   | 1.7 ± 0.3            | 3.4 ± 0.22           | 10 |
| 1078 | 25.67 ± 0.92 | 6   | 12.4 ± 2.87          | 12.0 ± 1.92          | 5  |
| 1081 | 94.17 ± 0.21 | 6   | 41.5 ± 5.31          | 29.5 ± 5.30          | 4  |

* N.D., not determined.
**Supplementary Table 2** Summary of PN dendrite targeting in brains with genetically ablated larval LNs.

| Genotype       | Reporter of PNs | Glomerular target(s) | Frequency of phenotype (mistargeted glomerulus) | No. of analyzed ALs |
|----------------|-----------------|----------------------|-----------------------------------------------|---------------------|
| UAS-DTI        | MZ19-mCD8GFP    | DA1, VA1d, DC3       | 10.6 % (1) 2.1 % (VA1v)                        | 47                  |
| NP3056         | MZ19-mCD8GFP    | DA1, VA1d, DC3       | 0 %                                            | 38                  |
| NP3056>DTI     | MZ19-mCD8GFP    | DA1, VA1d, DC3       | 10 % (VA2) 6.7 % (1) 3.3 % (VM5v)              | 30                  |
| 449            | MZ19-mCD8GFP    | DA1, VA1d, DC3       | 0%                                             | 60                  |
| 449>DTI        | MZ19-mCD8GFP    | DA1, VA1d, DC3       | 0%                                             | 46                  |
| 670            | MZ19-mCD8GFP    | DA1, VA1d, DC3       | 0 %                                            | 26                  |
| 670>DTI        | MZ19-mCD8GFP    | DA1, VA1d, DC3       | 0%                                             | 24                  |
**Supplementary Table 3** Statistical test results for Fig. 3h and Supplementary Fig. 7c, 7e and 7g.

| Figure                  | Pair      | LN No. (mean ± s.e.m.) | No. of AL | p-value          |
|-------------------------|-----------|------------------------|-----------|------------------|
| Fig. 3h                 | 670       | 0.03 ± 0.03            | 36        | 1.08E-07***      |
|                         | 670>p35   | 1.95 ± 0.25            | 20        |                  |
| Supplementary Fig. 7c   | NP3056    | 1.86 ± 0.15            | 50        | 0.00287***       |
|                         | NP3056>DTI| 0.76 ± 0.12            | 68        |                  |
| Supplementary Fig. 7e   | 449       | 20.24 ± 0.44           | 34        | 4.33E-11***      |
|                         | 449>DTI   | 14.45 ± 0.57           | 29        |                  |
| Supplementary Fig. 7g   | 670       | 3.09 ± 0.10            | 34        | 2.39E-07***      |
|                         | 670>DTI   | 1.67 ± 0.022           | 33        |                  |

Student *t*-test was used. *p < 0.05, **p < 0.01, ***p < 0.001.
**Supplementary Table 4** Statistical test results for Fig. 6f and Supplementary Fig. 8a, 8b.

| Figure     | Pair (No. of AL) | Feature                          | p-value  |
|------------|------------------|----------------------------------|----------|
| **GAL4 control vs GAL4>DTI** |                  | **NP3056 vs NP3056>DTI**         |          |
| Supplementary Fig. 8a | (6 vs 6)          | Area                             | 0.65     |
|              |                   | Distance from Origin Reference Frame | 0.05578  |
|              |                   | Ellipticity (oblate)              | 0.4088   |
|              |                   | Ellipticity (prolate)             | 0.3885   |
|              |                   | Intensity Mean (nc82)             | 0.812    |
|              |                   | Position X Reference Frame        | 0.7491   |
| Supplementary Fig. 8b |                  | Position Y Reference Frame        | 0.04123* |
|              |                   | Position Z Reference Frame        | 0.1207   |
|              |                   | Sphericity                       | 0.266    |
| Fig. 6f     |                  | Volume                           | 0.4479   |
| **449 vs 449>DTI** |                  | **Area**                         | 0.7817   |
| Supplementary Fig. 8a | (6 vs 6)          | Distance from Origin Reference Frame | 0.0008963*** |
|              |                   | Ellipticity (oblate)              | 0.1473   |
|              |                   | Ellipticity (prolate)             | 0.4135   |
|              |                   | Intensity Mean (nc82)             | 0.7231   |
|              |                   | Position X Reference Frame        | 0.7565   |
| Supplementary Fig. 8b |                  | Position Y Reference Frame        | 0.004953** |
|              |                   | Position Z Reference Frame        | 0.9847   |
|              |                   | Sphericity                       | 0.03125* |
| Fig. 6f     |                  | Volume                           | 0.7988   |
| **670 vs 670>DTI** |                  | **Area**                         | 0.8683   |
| Supplementary Fig. 8a | (6 vs 6)          | Distance from Origin Reference Frame | 0.8068   |
|              |                   | Ellipticity (oblate)              | 0.2814   |
|              |                   | Ellipticity (prolate)             | 0.6089   |
|              |                   | Intensity Mean (nc82)             | 0.799    |
|              |                   | Position X Reference Frame        | 0.3887   |
|                         | Position Y Reference Frame | Position Z Reference Frame | Sphericity | Volume |
|-------------------------|----------------------------|----------------------------|------------|--------|
| **Supplementary Fig. 6f** |                            |                            |            | 0.5079 |
| **Supplementary Fig. 8b** |                            |                            |            | 0.5781 |
| **Supplementary Fig. 8a** |                            |                            |            | 0.3289 |
|                         |                            |                            | 0.4506     |        |

**UAS-DTI control vs GAL4>DTI**

|                         | Position Y Reference Frame | Position Z Reference Frame | Sphericity | Distance from Origin Reference Frame | Ellipticity (oblate) | Ellipticity (prolate) | Intensity Mean (nc82) | Position X Reference Frame | Area |
|-------------------------|----------------------------|----------------------------|------------|---------------------------------------|----------------------|-----------------------|---------------------------|--------------------------------|------|
| **Supplementary Fig. 8a** |                            |                            |            | 0.2865                               | 0.5536               | 0.7616                | 0.3239                    | 0.08424                          | 0.06171 |
|                         |                            |                            |            |                                       |                      |                       |                           |                                  |      |
| **Supplementary Fig. 8b** |                            |                            | 0.8318     |                                       |                      |                       |                           |                                  |      |
| **Fig. 6f**              |                            |                            |            | 0.3395                               | 0.02188*             |                       |                           |                                  |      |

**UAS-DTI vs NP3056>DTI**

|                         | Position Y Reference Frame | Position Z Reference Frame | Sphericity | Distance from Origin Reference Frame | Ellipticity (oblate) | Ellipticity (prolate) | Intensity Mean (nc82) | Position X Reference Frame | Area |
|-------------------------|----------------------------|----------------------------|------------|---------------------------------------|----------------------|-----------------------|---------------------------|--------------------------------|------|
| **Supplementary Fig. 8a** |                            |                            |            | 0.1398                               | 0.7339               | 0.3812                | 0.3269                    | 0.8169                          | 0.3395 |
|                         |                            |                            |            |                                       |                      |                       |                           |                                  |      |
| **Supplementary Fig. 8b** |                            |                            | 0.8098     |                                       |                      |                       |                           |                                  |      |
|                         |                            |                            |            | 0.2795                               |                      |                       |                           |                                  |      |

**UAS-DTI vs 449>DTI**

|                         | Position Y Reference Frame | Position Z Reference Frame | Sphericity | Distance from Origin Reference Frame | Ellipticity (oblate) | Ellipticity (prolate) | Intensity Mean (nc82) | Position X Reference Frame | Area |
|-------------------------|----------------------------|----------------------------|------------|---------------------------------------|----------------------|-----------------------|---------------------------|--------------------------------|------|
| **Supplementary Fig. 8a** |                            |                            |            | 0.6474                               | 0.7125               | 0.884                 |                           |                                  |      |
| **Supplementary Fig. 8b** |                            |                            | 0.9211     |                                       |                      |                       |                           |                                  |      |
| **Fig. 6f**              |                            |                            |            | 0.3781                               | 0.3781               |                       |                           |                                  |      |

**UAS-DTI vs 670>DTI**
| Supplementary Fig. 8b | Position X Reference Frame | 0.9664 |
|-----------------------|----------------------------|--------|
|                       | Position Y Reference Frame | 0.966  |
|                       | Position Z Reference Frame | 0.5201 |
|                       | Sphericity                  | 0.1854 |
| Fig. 6f               | Volume                      | 0.02084* |

**UAS-DTI control vs GAL4 control**

|                              | Area            | 0.04082* |
|-----------------------------|-----------------|----------|
| Supplementary Fig. 8a       | UAS-DTI vs NP3056 | Distance from Origin Reference Frame | 0.1208 |
|                             |                 | Ellipticity (oblate) | 0.2735 |
|                             |                 | Ellipticity (prolate) | 0.541  |
|                             |                 | Intensity Mean (nc82) | 0.2964 |
|                             |                 | Position X Reference Frame | 0.08935 |
| Supplementary Fig. 8b       | Position Y Reference Frame | 0.7893 |
|                             | Position Z Reference Frame | 0.3107 |
|                             | Sphericity       | 0.1504  |
| Fig. 6f                     | Volume           | 0.1043  |

|                              | Area            | 0.8559 |
|-----------------------------|-----------------|--------|
| Supplementary Fig. 8a       | UAS-DTI vs 449  | Distance from Origin Reference Frame | 0.004897** |
|                             |                 | Ellipticity (oblate) | 0.297 |
|                             |                 | Ellipticity (prolate) | 0.9678 |
|                             |                 | Intensity Mean (nc82) | 0.3858 |
|                             |                 | Position X Reference Frame | 0.4899 |
| Supplementary Fig. 8b       | Position Y Reference Frame | 0.004188** |
|                             | Position Z Reference Frame | 0.9282 |
|                             | Sphericity       | 0.02187* |
| Fig. 6f                     | Volume           | 0.4226 |

|                              | Area            | 0.5616 |
|-----------------------------|-----------------|--------|
| Supplementary Fig. 8a       | UAS-DTI vs 670  | Distance from Origin Reference Frame | 0.3505 |
Student *t*-test was used. *p* < 0.05, **p** < 0.01, ***p*** < 0.001.

|                          | Value     |
|--------------------------|-----------|
| Ellipticity (oblate)     | 0.7273    |
| Ellipticity (prolate)    | 0.543     |
| Intensity Mean (nc82)    | 0.1719    |
| Position X Reference     | 0.367     |
| **Frame**                |           |
| Supplementary Fig. 8b    |           |
| Position Y Reference     | 0.2779    |
| **Frame**                |           |
| Position Z Reference     | 0.1733    |
| **Frame**                |           |
| Sphericity               | 0.1232    |
| Figure 6f                |           |
| Volume                   | 0.7897    |
### Supplementary Table 5 Genotypes of flies used in experiments described in Fig. 1-6 and Supplementary Fig. 1-8.

| Figure | Genotype |
|--------|----------|
| **Fig. 1b** | (330-GAL4, 388-GAL4) w, IS-GAL4/y w; UAS-nuclacZ UAS-mCD8GFP/+; |
| | (95-GAL4, 154-GAL4, 351-GAL4, 1078-GAL4, 1081-GAL4) y w/w; IS-GAL4/UAS-nuclacZ UAS-mCD8GFP; |
| | (68-GAL4, 191-GAL4, 361-GAL4, 382-GAL4, 415-GAL4, 421-GAL4, 449-GAL4, 501-GAL4, 551-GAL4, 584-GAL4, 658-GAL4, 666-GAL4, 670-GAL4, 674-GAL4, 829-GAL4, 930-GAL4, 988-GAL4, 1008-GAL4) y w/w; UAS-nuclacZ UAS-mCD8GFP/+; IS-GAL4/+; |
| **Fig. 2a** | (y) w; 189Y-GAL4/UAS-nuclacZ UAS-mCD8GFP; |
| | (y) w; UAS-nuclacZ UAS-mCD8GFP/+; NP3056/+; |
| | y w/w; UAS-nuclacZ UAS-mCD8GFP/+; 449-GAL4/+; |
| | y w/w; UAS-nuclacZ UAS-mCD8GFP/+; 670-GAL4/+; |
| **Fig. 2b** | y w/w; UAS-nuclacZ UAS-mCD8GFP/+; 501-GAL4/+; |
| | y w/w; 351-GAL4/UAS-nuclacZ UAS-mCD8GFP; |
| **Fig. 2c** | y w/w; UAS-nuclacZ UAS-mCD8GFP/+; 988-GAL4/+; |
| | y w/w; 95-GAL4/UAS-nuclacZ UAS-mCD8GFP; |
| **Fig. 3a** | w; UAS-GAL4 (12B)/+; UAS-Kaeda.A (3)/NP3056; |
| **Fig. 3c** | (y) w; UAS-FLP/13xLexAop2(FRT. stop)myr::smGdP-V5 (attP40); NP3056/tubP-GAL80[ts] (2), nSyb-LexA.DBD::QF.AD (attP2) |
| **Fig. 3d** | (control) (y) w; tubP-GAL80[ts] (20)/UAS-mCD8GFP.1; NP3056/+; |
| | (EcRDN) (y) w; UAS-EcR.B1-ΔC655,F645A (TP1), tubP-GAL80[ts] (20)/UAS-mCD8GFP.1; NP3056/+; |
| **Fig. 3e** | (y) w; UAS-FLP/QUAS(FRT.stop)mCD8GFP.P (10); 670-GAL4/tubP-GAL80[ts] (7), nSyb-QF2w.P (attP2); |
| **Fig. 3f** | y w/w; UAS-mCD8GFP/+; 670-GAL4/+; |
| **Fig. 3g** | (control) ey3.5-GAL80, w/w; tubP-GAL80[ts] (20)/UAS-mCD8GFP; 670-GAL4/+; |
| | (p35) ey3.5-GAL80, w/w; tubP-GAL80[ts] (20)/UAS-mCD8GFP.1/+; UAS-p35.H (BH2)/670-GAL4; |
**Fig. 4b**  
[w1118] hs-FLP5.PEST (attP3)/w; 189Y-GAL4/+; 10xUAS(FRT.stop)myr::smGdP-HA (VK00005), 10xUAS-(FRT.stop)myr::smGdP-V5-THS-10xUAS(FRT.stop)myr::smGdP-FLAG (su(Hw)attp1)/+;

**Fig. 4c**  
(y) w; UAS-Flip/189Y-GAL4; 13xLexAop2(FRT.stop)myr::smGdP-HA (VK00005)/tubP-GAL80[ts] (2), nSyb-LexA.DBD::QF.AD (attP2);

**Fig. 4d**  
[w1118] hs-FLP5.PEST (attP3)/w; NP3056-GAL4/10xUAS-(FRT.stop)myr::smGdP-HA (VK00005), 10xUAS-(FRT.stop)myr::smGdP-V5-THS-10xUAS(FRT.stop)myr::smGdP-FLAG (su(Hw)attp1);

**Fig. 4e**  
(y) w; UAS-FLP/13xLexAop2(FRT. stop)myr::smGdP-V5 (attP40); NP3056/tubP-GAL80[ts] (2), nSyb-LexA.DBD::QF.AD (attP2);

**Fig. 5a-d**  
(y) w; UAS-Flip/189Y-GAL4; 13xLexAop2(FRT.stop)myr::smGdP-HA (VK00005)/tubP-GAL80[ts], nSyb-LexA.DBD::QF.AD (attP2);

**Fig. 6b, f**  
(GAL4 control) ey3.5-GAL80, w/y w; tubP-GAL80[ts] (20)/MZ19-mCD8GFP[y+]; NP3056-GAL4/+;

(UAS-DTI control) ey3.5-GAL80, w/y w; UAS-DTI (18), tubP-GAL80[ts] (20)/MZ19-mCD8GFP[y+];

(DTI) ey3.5-GAL80, w/y w; UAS-DTI (18), tubP-GAL80[ts] (20)/MZ19-mCD8GFP[y+]; NP3056-GAL4/+

**Fig. 6d, f**  
(control) ey3.5-GAL80/ey3.5-GAL80; tubP-GAL80[ts] (20)/MZ19-mCD8GFP(y+); 670-GAL4/+  
(DTI) ey3.5-GAL80/+; UAS-DTI (18), tubP-GAL80[ts] (20)/MZ19-mCD8GFP(y+); 670-GAL4/+

**Fig. 6f**  
(control) ey3.5-GAL80, w/y w; tubP-GAL80[ts] (20)/MZ19-mCD8GFP[y+]; 449-GAL4/+  
(DTI) ey3.5-GAL80, w/y w; UAS-DTI (18), tubP-GAL80[ts] (20)/MZ19-mCD8GFP[y+]; 449-GAL4/+;

**Supplementary Fig. 1a**  
(y) w; UAS-nuclacZ UAS-mCD8GFP/+; krasavietz-GAL4/+

LCCH3-GAL4/y w; UAS-nuclacZ UAS-mCD8GFP;

(y) w; 189Y-GAL4/UAS-nuclacZ UAS-mCD8GFP;

(y) w; UAS-nuclacZ UAS-mCD8GFP/+; NP3056-GAL4/+

y w/w; 1081-GAL4/UAS-nuclacZ UAS-mCD8GFP;

(421-GAL4, 449-GAL4, 658-GAL4, 670-GAL4) y w/+; UAS-nuclacZ UAS-mCD8GFP/+; IS-GAL4/+
| Supplementary Fig. 1b | \((351\text{-}GAL4, 1078\text{-}GAL4)\) y w/w; IS\text;-\text{GAL4}/UAS\text{-}nuclacZ UAS\text{-}mCD8GFP; ; |
|-----------------------|--------------------------------------------------------------------------------------------------|
| Supplementary Fig. 1c | \((382\text{-}GAL4, 501\text{-}GAL4, 674\text{-}GAL4, 1008\text{-}GAL4)\) y w/w; UAS\text{-}nuclacZ UAS\text{-}mCD8GFP/+; IS\text{-}GAL4/+; |
| Supplementary Fig. 2a | \((68\text{-}GAL4, 930\text{-}GAL4, 988\text{-}GAL4)\) y w/w; UAS\text{-}nuclacZ UAS\text{-}mCD8GFP/+; IS\text{-}GAL4/+; |
| Supplementary Fig. 3b | (y) w; 189Y\text{-}GAL4/UAS\text{-}nuclacZ UAS\text{-}mCD8GFP; ; |
| Supplementary Fig. 3b | (y) w; 189Y\text{-}GAL4/UAS\text{-}nuclacZ UAS\text{-}mCD8GFP; NP3056\text{-}GAL4/449\text{-}GAL4/+; |
| Supplementary Fig. 3b | (y) w; 189Y\text{-}GAL4/UAS\text{-}nuclacZ UAS\text{-}mCD8GFP; 449\text{-}GAL4/+; |
| Supplementary Fig. 3b | (y) w; 189Y\text{-}GAL4/UAS\text{-}nuclacZ UAS\text{-}mCD8GFP; 670\text{-}GAL4/+; |
| Supplementary Fig. 3b | (y) w; 189Y\text{-}GAL4/c739\text{-}GAL4 UAS\text{-}nuclacZ UAS\text{-}mCD8GFP;+ |
| Supplementary Fig. 3b | (y) w; UAS\text{-}nuclacZ UAS\text{-}mCD8GFP/+; NP3056\text{-}GAL4 449\text{-}GAL4/+; |
| Supplementary Fig. 3b | (y) w; UAS\text{-}nuclacZ UAS\text{-}mCD8GFP/+; 449\text{-}GAL4/+; |
| Supplementary Fig. 3b | (y) w; UAS\text{-}nuclacZ UAS\text{-}mCD8GFP/+; 670\text{-}GAL4/+; |
| Supplementary Fig. 3b | (y) w; UAS\text{-}nuclacZ UAS\text{-}mCD8GFP/+; NP3056\text{-}GAL4 449\text{-}GAL4/670\text{-}GAL4; |
| Supplementary Fig. 3b | (y) w; c739\text{-}GAL4/UAS\text{-}nuclacZ UAS\text{-}mCD8GFP; NP3056\text{-}GAL4, 449\text{-}GAL4/670\text{-}GAL4; |
| Supplementary Fig. 3b | (y) w; 189Y\text{-}GAL4/UAS\text{-}nuclacZ UAS\text{-}mCD8GFP; NP3056\text{-}GAL4, 449\text{-}GAL4/670\text{-}GAL4; |
| Supplementary Fig. 3b | (y) w; 189Y\text{-}GAL4/UAS\text{-}nuclacZ UAS\text{-}mCD8GFP; NP3056\text{-}GAL4, 449\text{-}GAL4/670\text{-}GAL4; |
| Supplementary Fig. 3b | (y) w; UAS\text{-}nuclacZ UAS\text{-}mCD8GFP/+; NP3056\text{-}GAL4 449\text{-}GAL4/+; |
| Supplementary Fig. 3b | (y) w; UAS\text{-}nuclacZ UAS\text{-}mCD8GFP/+; 449\text{-}GAL4/+; |
| Supplementary Fig. 3b | (y) w; UAS\text{-}nuclacZ UAS\text{-}mCD8GFP/+; 670\text{-}GAL4/+; |
| Supplementary Fig. 3b | (y) w; UAS\text{-}nuclacZ UAS\text{-}mCD8GFP/+; NP3056\text{-}GAL4 449\text{-}GAL4/670\text{-}GAL4; |

**Footnotes:**
- (189Y, control)
- (189Y, EcRDN)
- (449, control)
| Supplementary Fig. 3c | (control) | (y) w; UAS-EcR.B1-ΔC655,F645A (TP1)/ UAS-mCD8GFP.1; 449-GAL4/+; UAS-mCD8GFP/++; 670-GAL4/+; UAS-GAL4.H (12B) UAS-mCD8GFP/+; 670-GAL4/+; |
| Supplementary Fig. 4a-d | (y) w; UAS-nuclacZ UAS-mCD8GFP/+; 670-GAL4/+; UAS-GAL4 |
| Supplementary Fig. 5a-e | (y) w; UAS-FLP/13xLexAop2(FRT. stop)myr::smGdP-V5 (attp40); NP3056/tubP-GAL80[ts] (2), nSyb-LexA,DBD::QF.AD (attp2); |
| Supplementary Fig. 6a | (y) w; UAS-mCD8GFP/+; NP3056-GAL4/GH146-QF (53), QUAS-mtdTomato-3xHA (24); |
| Supplementary Fig. 6b | (y) w; UAS-mCD8GFP/+; 449-GAL4/GH146-QF (53), QUAS-mtdTomato-3xHA (24); |
| Supplementary Fig. 6c | (y) w; UAS-mCD8GFP/+; 670-GAL4/GH146-QF (53), QUAS-mtdTomato-3xHA (24); |
| Supplementary Fig. 7b | (control) | ey3.5-GAL80, w/y w; tubP-GAL80[ts] (20)/UAS-mCD8GFP.1; NP3056/+; |
| Supplementary Fig. 7d | (DTI) | ey3.5-GAL80, w/y w; UAS-DTI (18), tubP-GAL80[ts] (20)/UAS-mCD8GFP.1; UAS-DTI control; ey3.5-GAL80, w/y w; tubP-GAL80[ts] (20)/UAS-mCD8GFP.1; 449-GAL4/+; |
| Supplementary Fig. 7f | control | ey3.5-GAL80/ey3.5-GAL80; tubP-GAL80[ts] (20)/UAS-mCD8GFP.1; 670-GAL4/+ |
| Supplementary Fig. 9b | (GAL4 control) | ey3.5-GAL80, w/y w; tubP-GAL80[ts] (20)/MZ19-mCD8GFP[y+]; 449-GAL4/+; |
| | (UAS-DTI control) | ey3.5-GAL80, w/y w; UAS-DTI (18), tubP-GAL80[ts] (20)/MZ19-mCD8GFP[y+]; |
| | (DTI) | ey3.5-GAL80, w/y w; UAS-DTI (18), tubP-GAL80[ts] (20)/MZ19-mCD8GFP[y+]; 449-GAL4/+; |
### Supplementary Table 6 Secondary antibodies used in this study.

| Antibody                                           | Catalogue number                        | Working dilution |
|----------------------------------------------------|-----------------------------------------|------------------|
| Goat anti-mouse IgG (H+L) Alexa Flour® 488         | Jackson ImmunoResearch Laboratories (115-545-166) | 1:500            |
| Goat anti-mouse IgG (H+L) DyLight™ 488             | Jackson ImmunoResearch Laboratories (115-485-166) | 1:500            |
| Goat anti-mouse IgG (H+L) Cy™3                     | Jackson ImmunoResearch Laboratories (115-165-166) | 1:500            |
| Goat anti-mouse IgG (H+L) DyLight™ 649             | Jackson ImmunoResearch Laboratories (115-495-166) | 1:500            |
| Goat anti-mouse IgG (H+L) Alexa Fluor® 674         | Jackson ImmunoResearch Laboratories (115-605-166) | 1:500            |
| Goat anti-rabbit IgG (H+L) Alexa Flour® 488        | Invitrogen (A-11034)                    | 1:500            |
| Goat anti-rabbit IgG (H+L) Alexa Flour® 488        | Jackson ImmunoResearch Laboratories (111-545-144) | 1:500            |
| Goat anti-rabbit IgG (H+L) DyLight™ 488            | Jackson ImmunoResearch Laboratories (111-485-144) | 1:500            |
| Goat anti-rabbit IgG (H+L) Cy™3                    | Jackson ImmunoResearch Laboratories (111-165-144) | 1:500            |
| Goat anti-rabbit IgG (H+L) Alexa Fluor® 647        | Jackson ImmunoResearch Laboratories (111-605-144) | 1:500            |
| Goat anti-rabbit IgG (H+L) DyLight™ 649            | Jackson ImmunoResearch Laboratories (111-495-144) | 1:500            |
| Goat anti-rat IgG (H+L) DyLight™ 488               | Jackson ImmunoResearch Laboratories (112-485-167) | 1:500            |
| Goat Anti-rat IgG (H+L) Alexa Fluor® 488           | Jackson ImmunoResearch Laboratories (112-545-167) | 1:500            |
| Goat anti-rat IgG (H+L) Cy™3                       | Jackson ImmunoResearch Laboratories (112-165-167) | 1:500            |
| Goat Anti-rat IgG (H+L) Alexa Fluor® 647           | Jackson ImmunoResearch Laboratories (112-605-167) | 1:500            |
| Goat anti-rat IgG (H+L) DyLight™ 649               | Jackson ImmunoResearch Laboratories (112-495-167) | 1:500            |
Supplementary References

1. Chou, Y. H. et al. Diversity and wiring variability of olfactory local interneurons in the Drosophila antennal lobe. *Nat Neurosci* **13**, 439-449, doi:nn.2489 [pii]10.1038/nn.2489 (2010).

2. Lin, S., Kao, C. F., Yu, H. H., Huang, Y. & Lee, T. Lineage analysis of Drosophila lateral antennal lobe neurons reveals notch-dependent binary temporal fate decisions. *PLoS Biol* **10**, e1001425, doi:10.1371/journal.pbio.1001425 (2012).

3. Lai, S. L., Awasaki, T., Ito, K. & Lee, T. Clonal analysis of Drosophila antennal lobe neurons: diverse neuronal architectures in the lateral neuroblast lineage. *Development* **135**, 2883-2893, doi:dev.024380 [pii]10.1242/dev.024380 (2008).

4. Thum, A. S., Leisibach, B., Gendre, N., Selcho, M. & Stocker, R. F. Diversity, variability, and suboesophageal connectivity of antennal lobe neurons in *D. melanogaster* larvae. *J Comp Neurol* **519**, 3415-3432, doi:10.1002/cne.22713 (2011).

5. Ramaekers, A. et al. Glomerular maps without cellular redundancy at successive levels of the Drosophila larval olfactory circuit. *Curr Biol* **15**, 982-992 (2005).

6. Potter, C. J., Tasic, B., Russler, E. V., Liang, L. & Luo, L. The Q system: a repressible binary system for transgene expression, lineage tracing, and mosaic analysis. *Cell* **141**, 536-548, doi:S0092-8674(10)00178-9 [pii]10.1016/j.cell.2010.02.025 (2010).