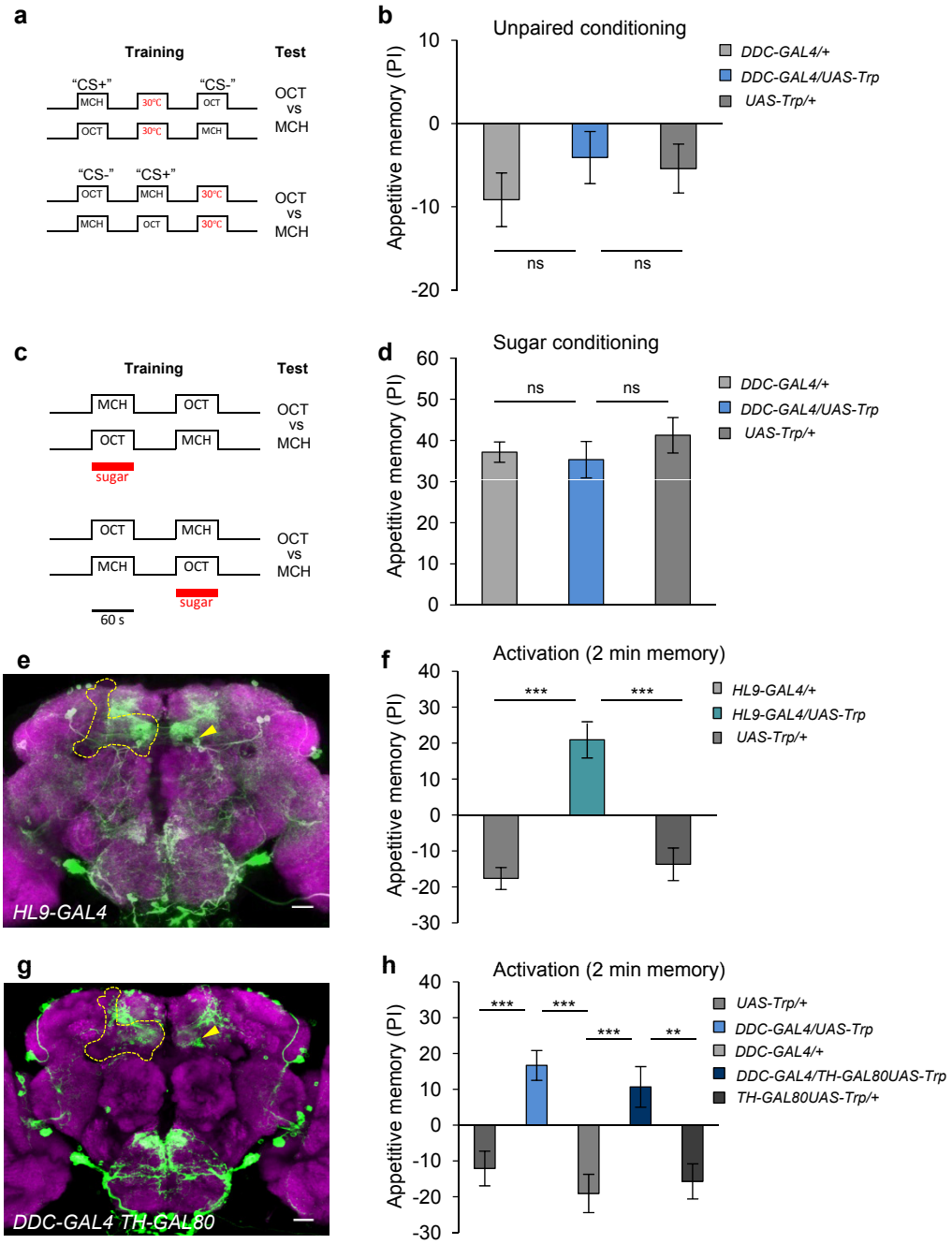
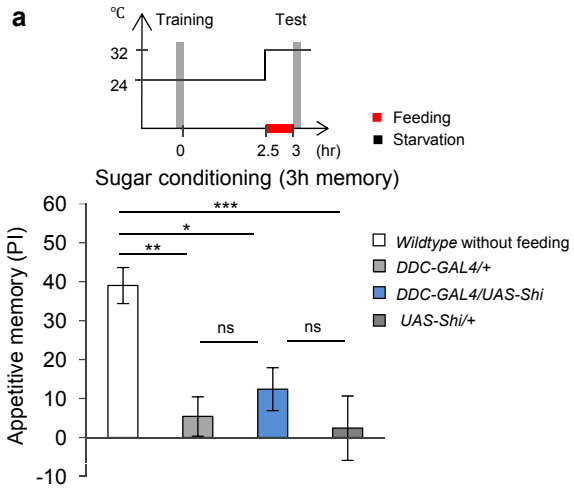
doi:10.1038/nature11304



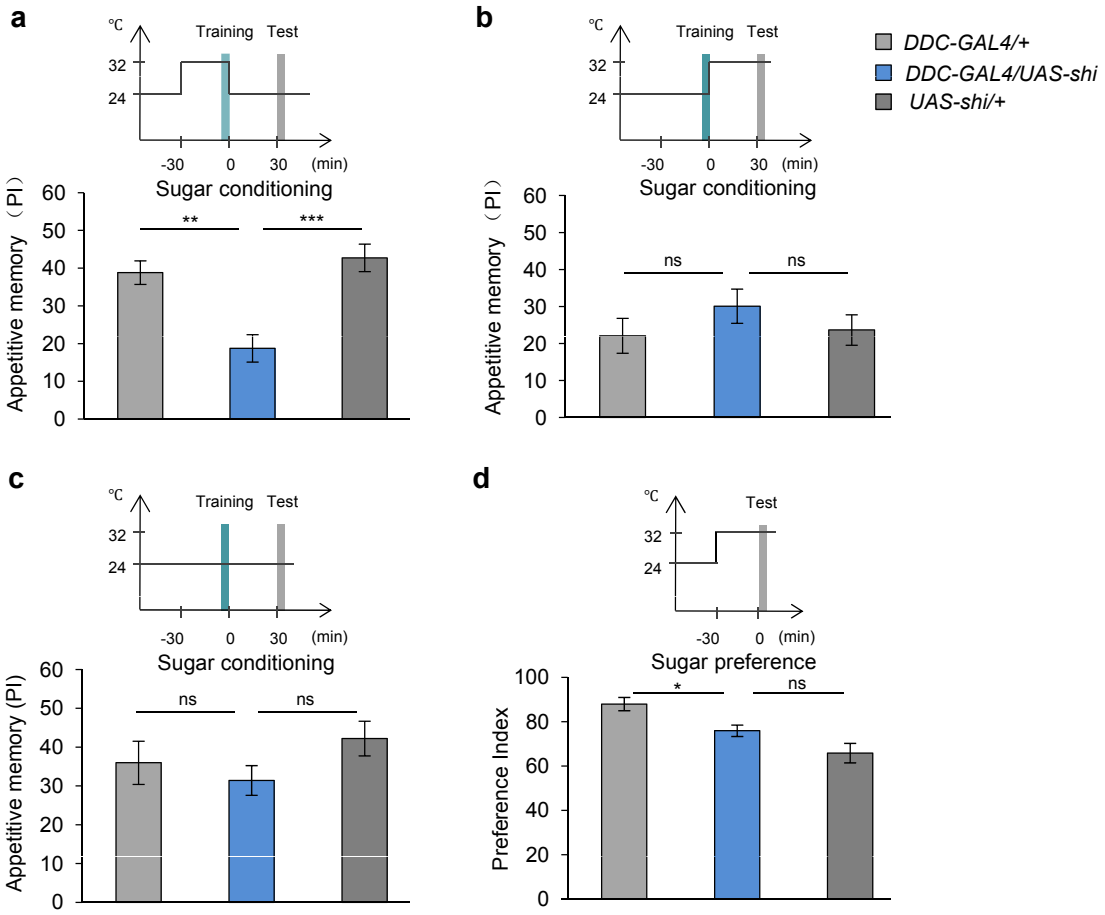
Supplementary Figure 1 | Control behavioural experiments for thermo-activation with *DDC-GAL4*.

a, The protocol for unpaired thermo-activation (see also Methods). **b**, Unpaired thermo-activation with *DDC-GAL4* did not induce appetitive odour memory. **c-d**, The lack of appetitive memory in genetic control flies (i.e., *DDC-GAL4/+* and *UAS-Trp/+*) is not due to a general learning defect, as their appetitive memory with sugar reward is not impaired. **c**, The protocol for sugar-induced memory (see Methods for detail). **d**, Sugar-induced memory of *DDC-GAL4/UAS-dTrpA1* is comparable to the controls. **e**, Expression pattern of *HL9-GAL4* in the brain (Frontal view, dorsal up) is visualized by

UAS-mCD8::GFP (green) with counterstaining of neuropile (magenta). The MB is outlined, and the cell bodies of PAM neurons are indicated with arrowheads. Scale bar 20 µm. **f**, Thermo-activation with *HL9-GAL4* induces significant appetitive memory. *n*=15-16. **g**, Expression pattern of *DDC-GAL4 TH-GAL80*. **h**, *TH-GAL80* did not block appetitive memory by thermo-activation with *DDC-GAL4*. Mean and s.e.m. are shown (**b**, **d**, **f** and **h**).

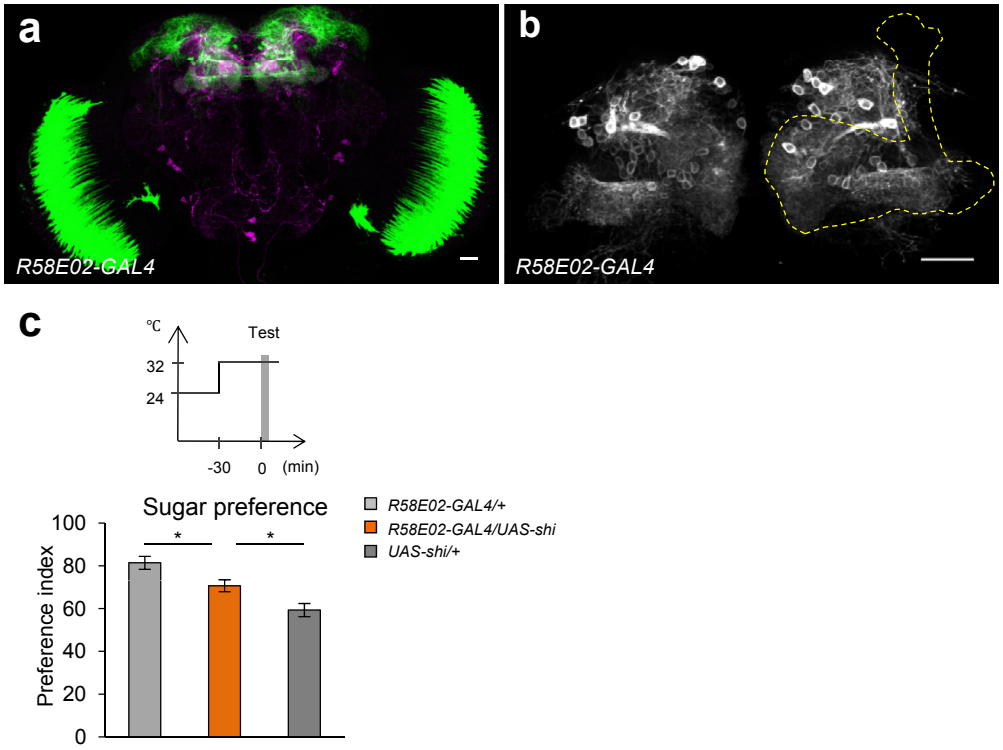


Supplementary Figure 2 | Satiation leads to the suppression of appetitive memory. Brief feeding before the test suppresses the behavioural expression of sugar-induced memory. Blockade in *DDC-GAL4/UAS-shi*^{ts1} flies during feeding does not reverse feeding-mediated suppression of conditioned odour approach. *n*=15-16.

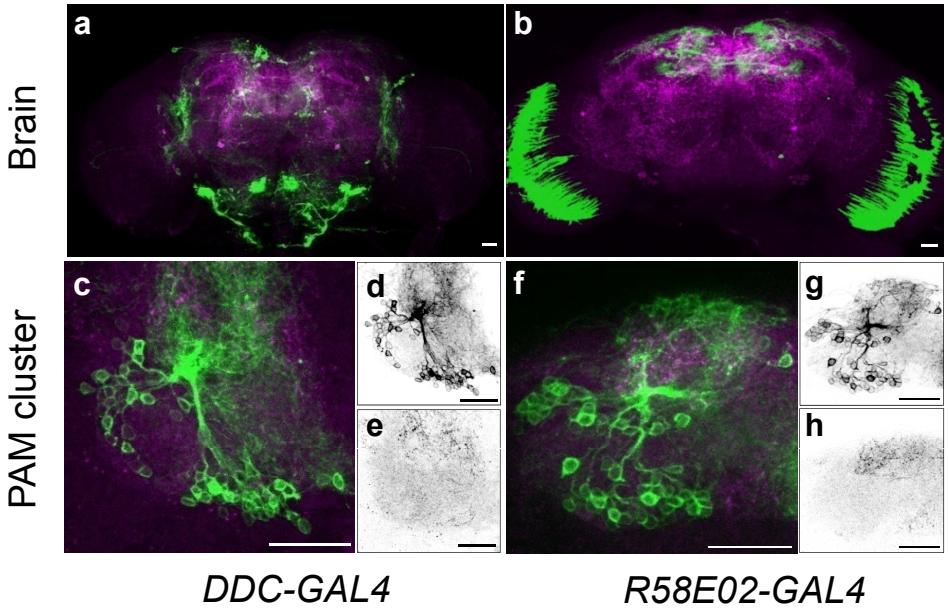


Supplementary Figure 3 | Acquisition of sugar-rewarded memory requires the output of neurons in *DDC-GAL4*.

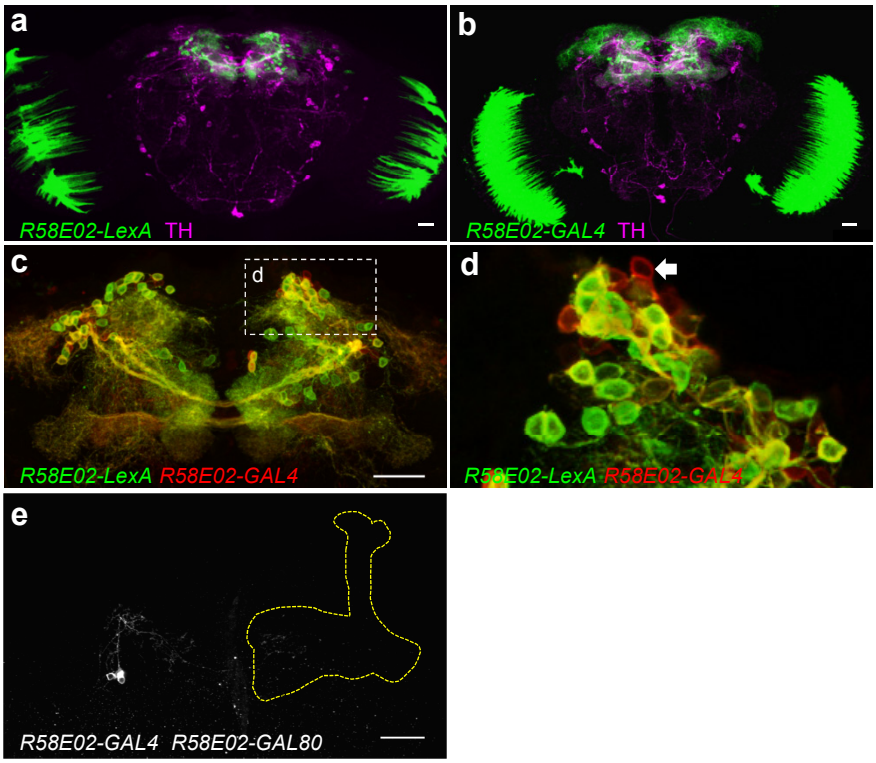
a, A transient block during the training (blue shading) induced in *DDC-GAL4/UAS-shi*^{ts1} by a 30-min temperature elevation significantly impairs sugar-induced olfactory memory. No significant defect is found under the same block applied after training (**b**) or at permissive temperature (**c**). *n*=15-16 (**a-c**). **d**, There is no consistent defect in *DDC-GAL4/UAS-shi*^{ts1} sugar preference at restrictive temperature. *n*=12.



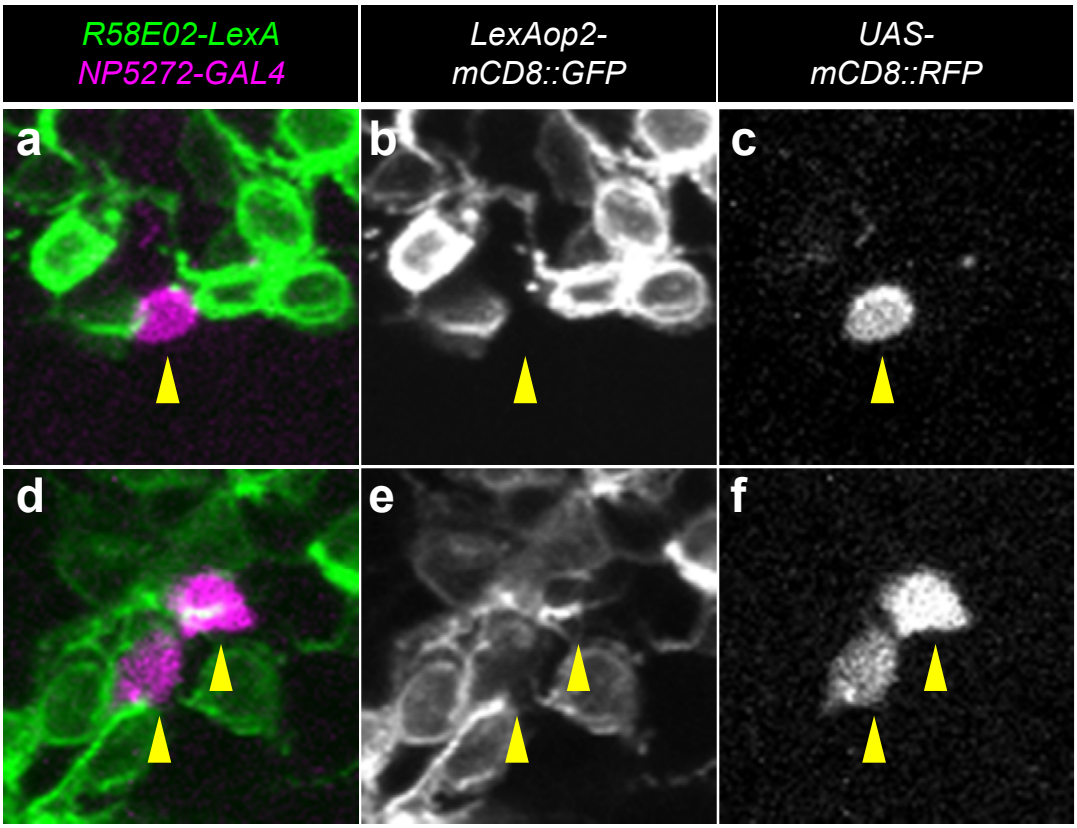
Supplementary Figure 4 | Expression pattern of *R58E02-GAL4* and sugar preference under blockade of these neurons. a, The whole-mount brain expressing *UAS-mCD8::GFP* (green) under the control of *R58E02-GAL4* with counterstaining with the anti-TH (magenta). *R58E02-GAL4* prominently labels the PAM cluster neurons and glia cells in the optic lobes with little additional expression. **b**, Magnification of the region including the MB lobes (outlined). The PAM cluster neurons supply dense arbours in the medial lobes of the MB. **c**, Blockade of the output of the PAM neurons in *R58E02-GAL4/UAS-shi*^{ts1} does not impair the reflexive choice of sugar. n=12.



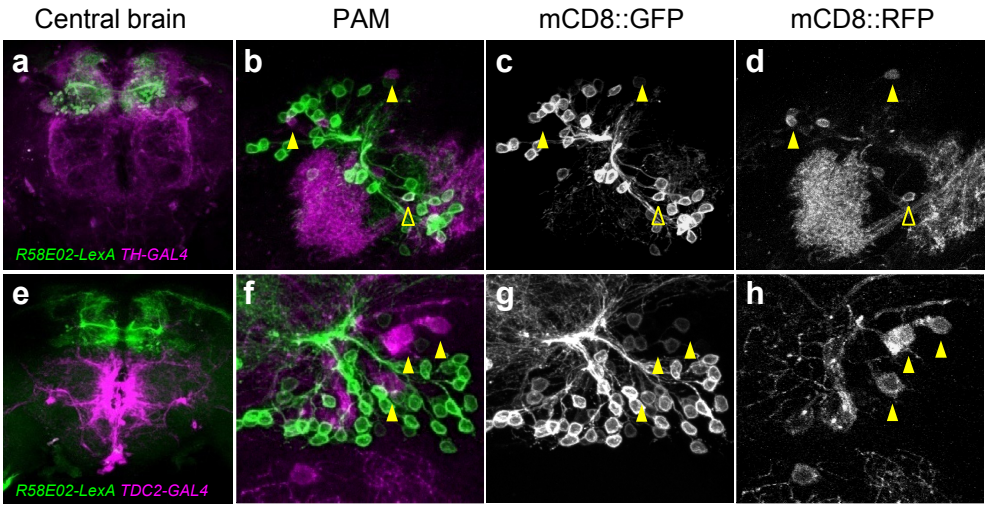
Supplementary Figure 5 | Serotonin-immunoreactivity is undetectable in the PAM cluster neurons in *DDC-GAL4* and *R58E02-GAL4*.
Double labelling of serotonin (magenta) and *DDC-GAL4* (**a**, **c-e**) or *R58E02-GAL4* (**b**, **f-h**) visualized with *UAS-mCD8::GFP* (green). **c-f,** There is no detectable colocalization in the cell bodies of the PAM cluster neurons (**d**, **e** and **g**, **h** are split channels of **c** and **f**, respectively).



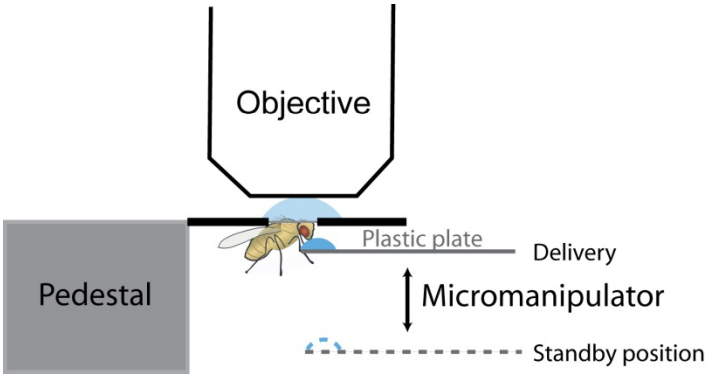
Supplementary Figure 6 | Consistency of the regulatory element *R58E02* when inserted at different genomic locations.
a-d, *R58E02-LexA* (inserted in *attP40*) (**a**) and *R58E02-GAL4* (inserted in *attP2*) (**b**) have similar expression patterns (Frontal view, dorsal up; counterstaining with TH [magenta]). The PAM cluster neurons and glial cells in the optic lobes are strongly labelled (green). Scale bars 20 µm. **c, d**, Double labelling of *R58E02-LexA* and *R58E02-GAL4* in a single brain visualized using *pJFRC21-10XUAS-IVS-mCD8::RFP* (*attp18*; red), *pJFRC15-13XLexAop2-mCD8::GFP* (*su(Hw)attP8*; green). The expression pattern in the PAM cluster is largely overlapping (**c**), although *R58E02-GAL4* labels a few more cells (arrow in **d**). **e**, *R58E02-GAL80* suppresses expression of *UAS-mCD8::GFP* in most, if not all, PAM neurons in *R58E02-GAL4* .



Supplementary Figure 7 | Heterogeneity of the PAM cluster neurons.
a-f, Projection of several confocal slices (~3 µm) for magnified cell body regions of MB-M3. The cell bodies of MB-M3 neurons (arrowheads) visualized with mCD8::RFP (magenta) under the control of *NP5272* do not overlap with *R58E02-LexA*-labelled neurons visualized with mCD8::GFP (green).



Supplementary Figure 8 | Neurons of the PAM cluster and the OA-ASM cluster in *TH-GAL4* and *TDC2-GAL4*.
a-d, The PAM neurons labelled in *R58E02-LexA* (green) and *TH-GAL4* (magenta) are visualized by mCD8::GFP and mCD8::RFP, respectively. Several, but not all, neurons are labelled by both drivers (see also Fig. 4). Solid and hollow arrowheads indicate non-overlapping and overlapping neurons, respectively. **e-h**, The PAM neurons and the OA-ASM neurons labelled with *R58E02-LexA* (green) and *TDC2-GAL4* (magenta), respectively, do not overlap. Arrowheads indicate the cell bodies of *TDC2-GAL4* labelled neurons.



Supplementary Figure 9 | Experimental setup of combined *in vivo* calcium imaging and gustatory stimulation.
A fly is prepared for *in vivo* imaging on a custom-made accessible surgery cell. The cell is placed under the objective of a confocal microscope[33]. A droplet of water, sucrose or caffeine solution is deposited on a plastic plate driven by a micromanipulator. To deliver the stimulus, the plate was lifted up to reachable distance for the fly.

Supplementary Movie 1 | The expression pattern of *R58E02-GAL4* in the brain

R58E02-GAL4 visualized with *UAS-mCD8::GFP* (green) with neuropile counterstaining using the anti-Synapsin antibody (magenta).

Supplementary Movie 2 | The expression pattern of *DDC-GAL4* in the central brain

mCD8::GFP (green) is expressed under the control of *DDC-GAL4* with counterstaining with the anti-TH antibody (magenta) in the central brain.

Supplementary Movie 3 | The expression pattern of *DDC-GAL4* with *R58E02-GAL80* in the central brain

R58E02-GAL80 silences transgene expression of most PAM cluster neurons in *DDC-GAL4* visualized with *UAS-mCD8::GFP* (green). The brain is counterstained with the anti-TH antibody (magenta).

Supplementary Movie 4 | Differential labelling of *NP5272-GAL4* and *R58E02-LexA* in the cell body region of the PAM cluster

mCD8::GFP (green) and mCD8::RFP (magenta) are expressed under the control of *R58E02-LexA* and *NP5272-GAL4*, respectively.

Supplementary Movie 5 | Differential labelling of *TH-GAL4* and *R58E02-LexA* in the MB

mCD8::GFP (green) and mCD8::RFP (magenta) are expressed under the control of *R58E02-LexA* and *TH-GAL4*, respectively.