Supplementary Information

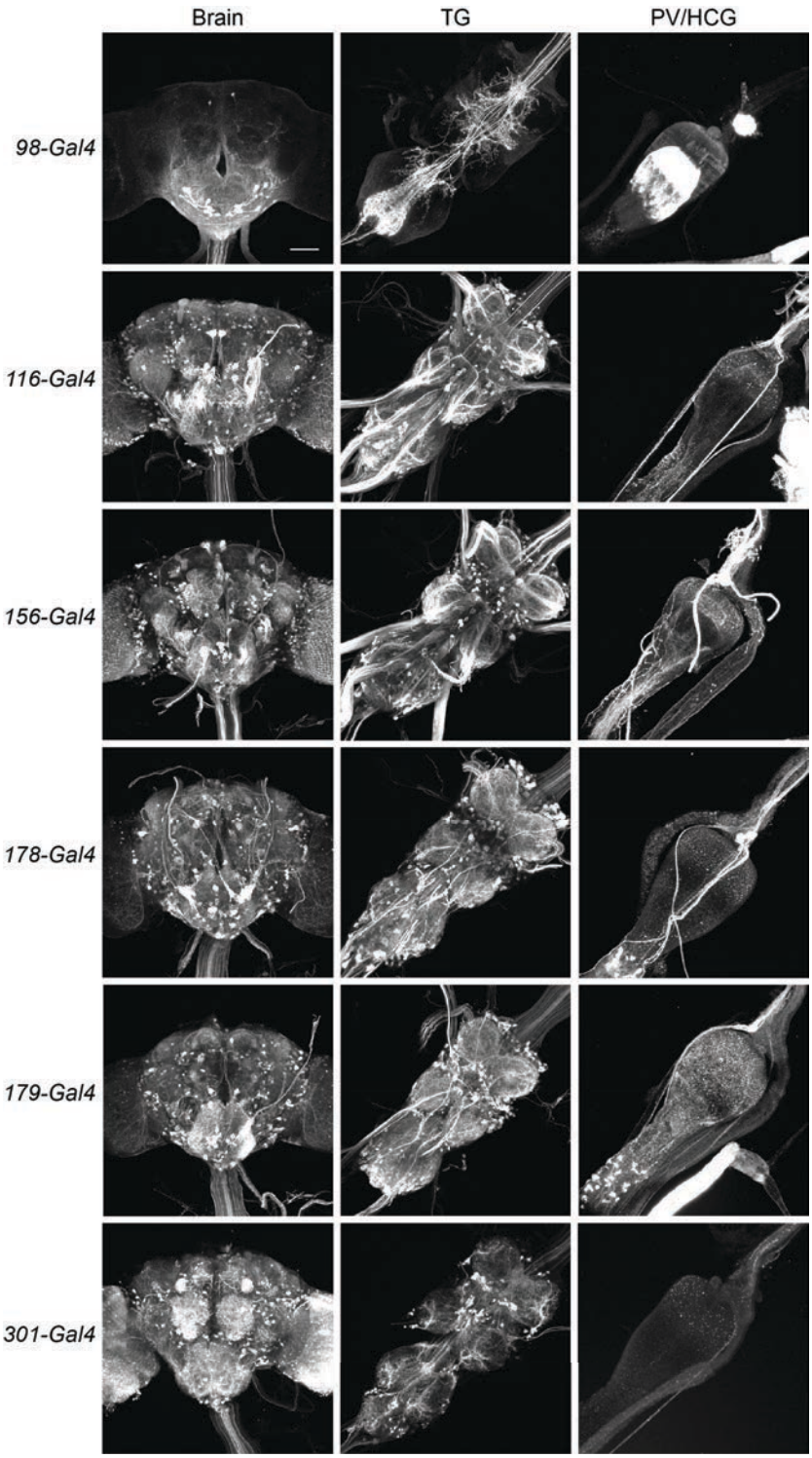

Figure S1: Expression pattern of Gal4 lines with an overconsumption phenotype, related to Figure 1. Expression in brain, ventral nerve cord (VNC) and proventriculus (PV, *98-Gal4*) or hypocerebral ganglion (HG, other lines) is shown for Gal4 lines expressing *UAS-CD8:GFP*. Scale is 50 μm.

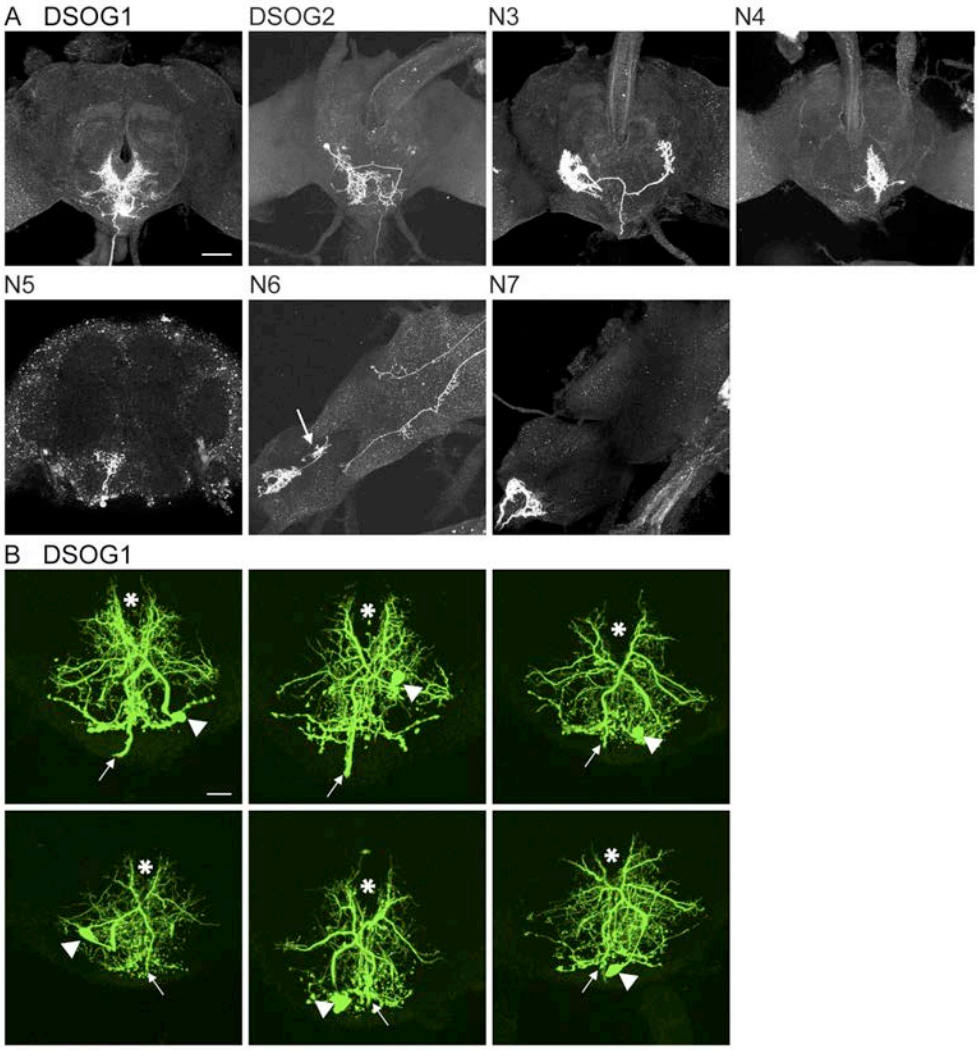

Figure S2: Different cell-types in the *98-Gal4* line and similarity of DSOG1 cells, related to Figure 2. A. Single cell clones for the seven cell-types identified in mosaic animals (flies contained *hs-FLP*, *tub>Gal80>, 98-Gal4, UAS-GFP, UAS-Kir2.1*). N6 and N7 are located in the ventral nerve cord, other neurons are localized in the brain. Scale is 50 μm. B. The DSOG1 cell cluster is comprised of morphologically indistinguishable neurons. Anatomical diversity among DSOG1 neurons was explored by photoconverting photo-activatable GFP (pa-GFP) by 2-photon microscopy to visualize single DSOG1 cells. Flies were *tub>Gal80>*; *98-Gal4/276B-FLP*; *UAS-C3PA-GFP*) 6 individual photoactivated DSOG1 cells. All 6 cells were characterized by a large cell body (arrow head) that sends widely arborizing projections throughout the SOG, with characteristic innervation around the oesophageal passage (asterisk), and a descending projection on the contralateral side of the cell body (arrow). Scale: 20 μm.

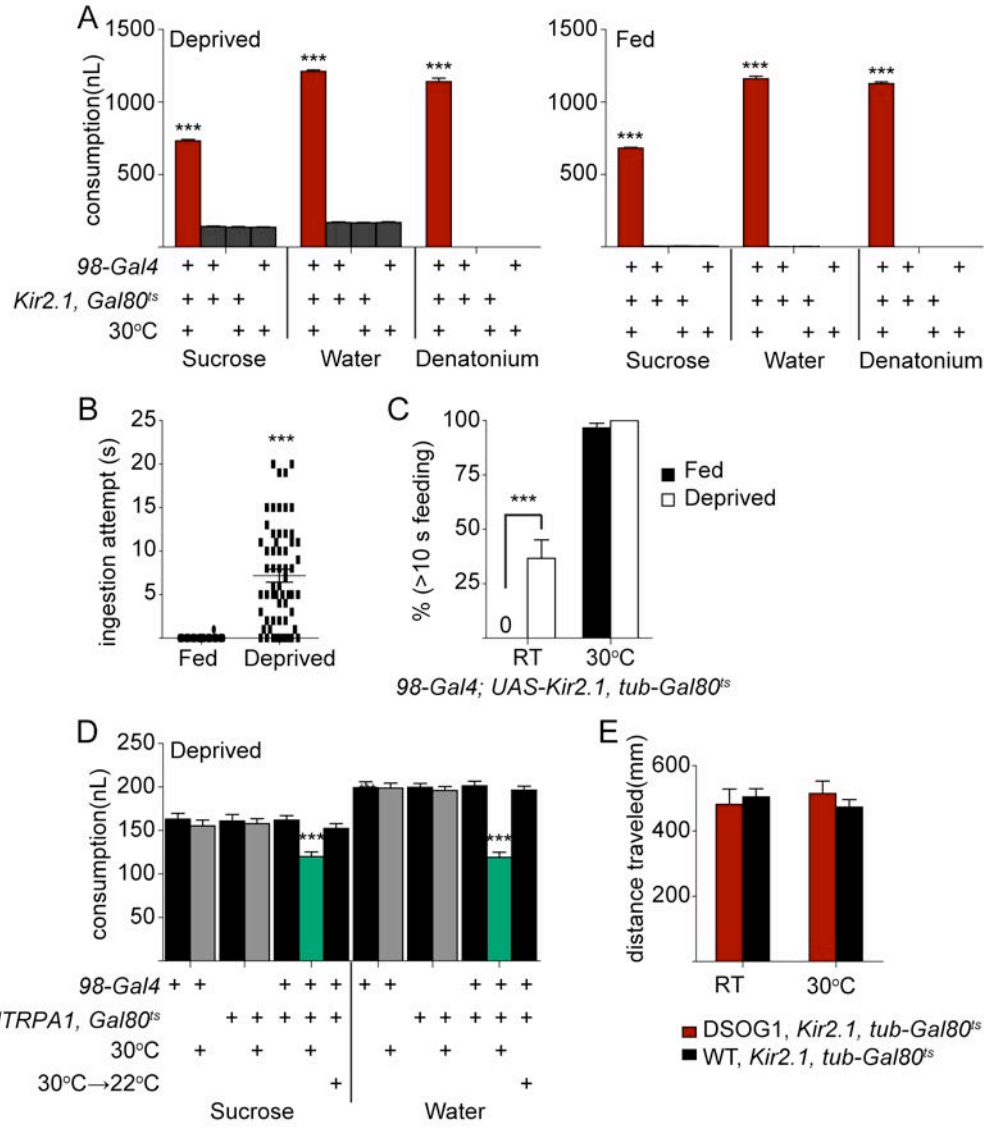

Figure S3: Feeding intake and locomotion phenotypes for flies with altered DSOG1 activity, related to Figure 3. A. Conditional inactivation of *98-Gal4* neurons with Kir2.1 increased volume consumed in deprived (24 hr food-deprived) and fed (non-deprived) conditions. *98-Gal4, 276-FLP, tub>Gal80>, tub-Gal80^{ts}, UAS-Kir2*.1 flies were shifted to 30ºC for 2 days for Kir2.1 induction or remained at 22ºC for non-induced controls. n=20; mean +/- SEM; one-way ANOVA, Tukey post-hoc, ***p<0.001. B. Dry food feeding attempts on crystallized sucrose measured in pumping time. n=60 isoD1 flies/condition. Student's t test, *** p < 0.001. C. Inactivation of DSOG1 neurons leads to persistent feeding attempts on dry food regardless of feeding state. Genotype *98-Gal4; UAS-Kir2.1, tub-Gal80^{ts}*, n= 60/condition. Student's t test, *** p < 0.001. D. Heat-induced dTRPA1 activation of DSOG1 neurons (*98-Gal4, 276-FLP, tub>Gal80>, UAS-dTRPA1*) reduced consumption volume for sucrose and water. n=30; mean +/- SEM; one-way ANOVA, Tukey post-hoc, ***p<0.001. E. DSOG1 inactivation in *tub>Gal80>; 98-Gal4/276B-FLP; UAS-Kir2.1, Gal80^{ts}* does not change baseline locomotion as compared to genetic (*tub>Ga80>; +/276B-FLP; UAS-Kir2.1, Gal80^{ts})* and temperature controls. n = 10 flies/condition. Two-way ANOVA NS.

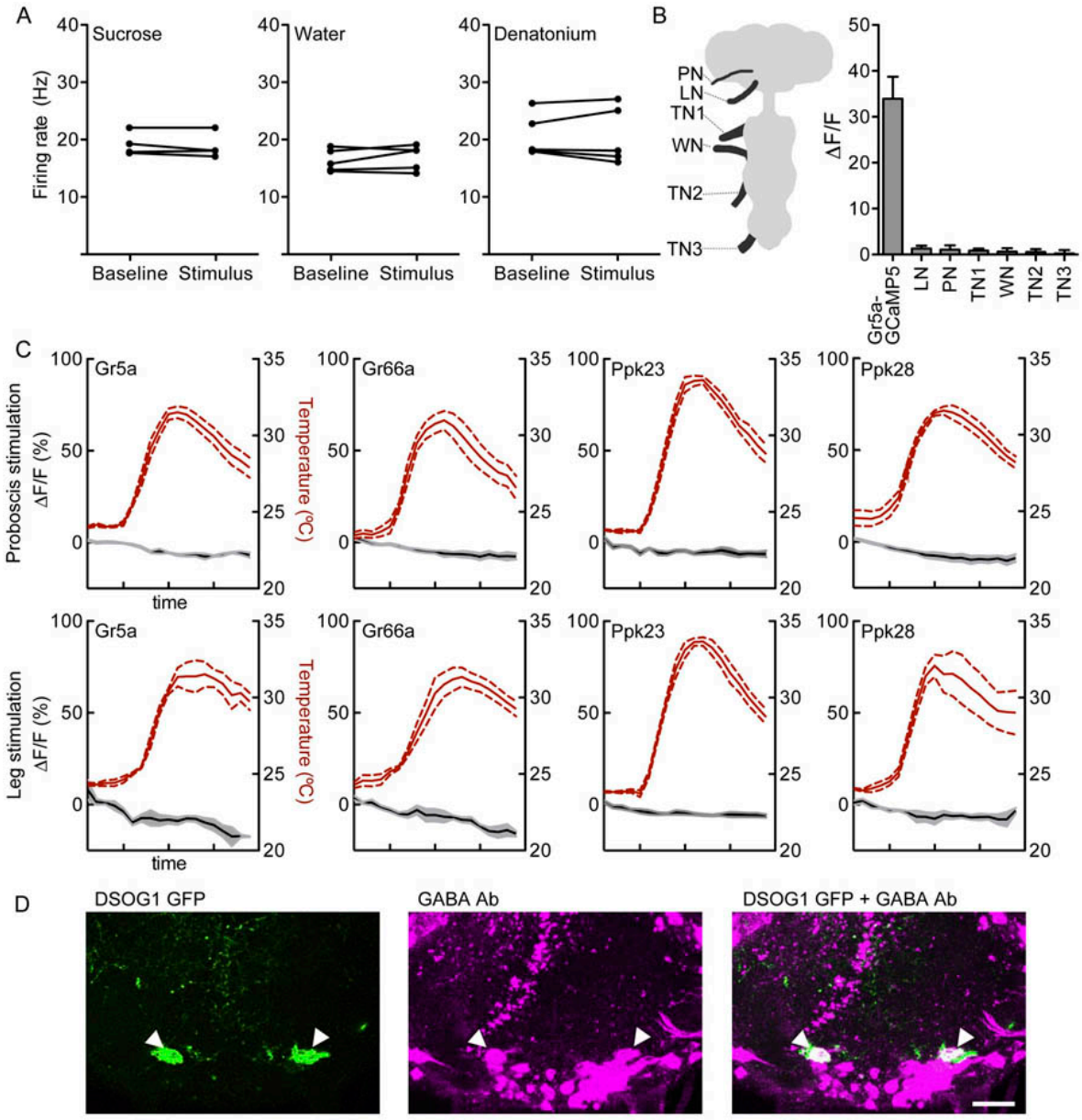

Figure S4: DSOG1 neurons do not respond to taste stimuli, related to Figure 5. A. DSOG1 firing rate before (Baseline) and during 1s stimulation (Stimulus) with 1M sucrose, water or 1mM denatonium in deprived conditions (24 hrs food deprivation). Baseline rate was averaged 10s pre-stimulus. n=4-5 flies, Wilcoxon matched pairs test. B. Brain plus nerve cord schematic showing nerves stimulated: Pharyngeal nerve (PN), Labellar Nerve (LN), Thoracic Nerve segment 1-3 (TN1, TN2, TN3), Wing Nerve (WN). Nerve stimulation (10V) activated Gr5a sugar-sensing senosry projections but did not activate DSOG1 by GCaMP fluorescence changes. n=6-8 flies/condition, mean±SEM, ANOVA with Tukey post-hoc test. C. Flies expressing the heat-activated dTRPA1 ion channel in different sensory classes were stimulated with a heat probe (temperature marked in red) and GCamp fluorescence of DSOG1 was monitored (greay lines). LexA/lexAop system was used for dTRPA1 expression and Gal4/UAS system was used for GCaMPexpression in DSOG1. D. Anti-GABA immunostaining shows co-expression of DSOG1 (green) and GABA (magenta). Scale is 20 μm.

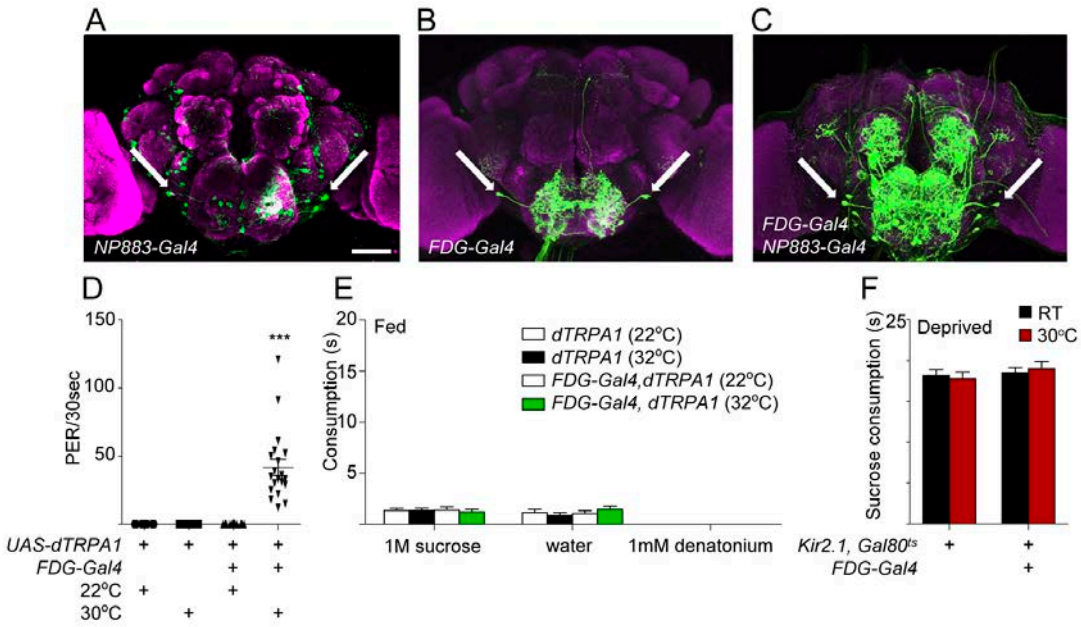

Figure S5: FDG, a putative feeding command neuron, controls meal initiation but not consumption, related to Figure 6. A. *NP883-Gal4* line that was previously reported to label a putative feeding command neuron FDG (arrows). Scalebar 50 μm. B. *FDG-Gal4* - FDG neuron selective line identified in the FlyLight collection (Gmr81e10). C. *NP883-Gal4, FDG-Gal4* co-immunostain showing a single FDG neuron labeled, suggesting that both lines label the FDG neuron. D. Thermogenetic activation of neurons labeled by *FDG-Gal4* leads to spontaneous proboscis extensions (similar to *NP833-Gal*4, see Figure 5B), suggesting that it is an interneuron for proboscis extension. E. Thermogenetic activation of neurons labeled by *FDG-Gal4* does not induce consumption of appetitive nor aversive substances suggesting that the FDG neuron does not function as a feeding command neuron. F. Inactivation of the *FDG-Gal4* does not reduce post-starvation consumption of 1M sucrose (Two-way ANOVA, NS).

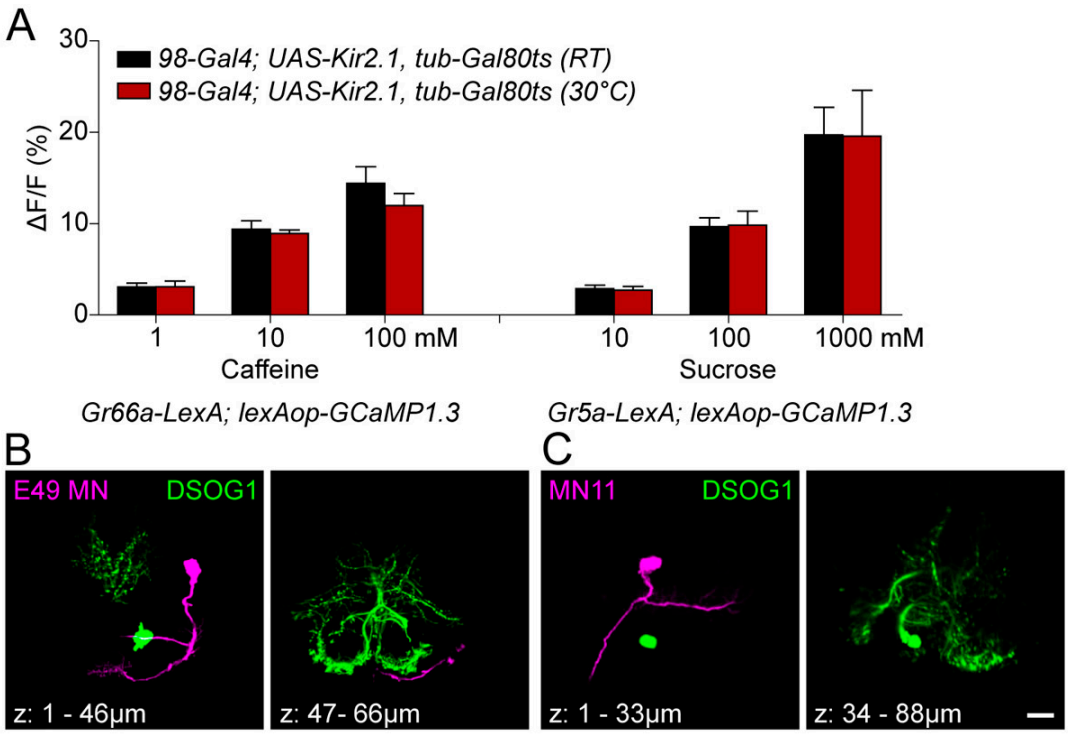

Figure S6: Sensory responses are not affected upon DSOG1 silencing, related to Figure 8. A. The response of Gr66a, bitter-sensing cells to 1,10, 100 mM caffeine was monitored in flies with (RT) or without *98-Gal4* neural activity (30ºC) (left) using GCaMP calcium imaging. *lexAop-GCaMP1.3* was used to monitor calcium changes and *UAS-Kir2.1, tub-Gal80^{ts}* was used for conditional inactivation of *98-Gal4* cells. The response of Gr5a, sugar-sensing cells to 10, 100 and 1000 mM sucrose was also monitored (right). Responses with or without *98-Gal4* neural silencing were not statistically different. n=3-5; mean+/- SEM; t-test, NS. B. DSOG1 (green, pa-GFP)and E49 motor neuron (magenta, pa-GFP) project to non-overlapping compartments in the SEZ neuropil. Genotype: *98-Gal4/E49-Gal4; UAS-C3PA:GFP*. C. DSOG1 (green, pa-GFP) and MN11 motorneuron (magenta, pa-GFP) project to non-overlapping compartments in the SEZ. Genotype: *98-Gal4/UAS-C3PA:GFP; NP534-Gal4/UAS-C3PA:GFP*. Scalebar: 20 µm.

Supplemental Videos

Supplemental Video 1: This movie shows the water consumption of a wild-type, water-deprived fly. Blue dye tracer is used to monitor water uptake (Quicktime; 6.7 MB).

Supplemental Video 2: This movie shows the water consumption of a *98-Gal4* fly conditionally expressing Kir2.1 under water-deprived conditions. Blue dye is included for visualization purposes (Quicktime; 28.9 MB).

Supplemental Video 3: This movie shows a three dimensional visualization of DSOG1 neurons. DSOG1 is labeled with CD8-GFP (green), nervous system is labeled with nc82 (purple). Genotype is *98-Gal4*, *276B-FLP*, *tub>Gal80>*, *UAS-CD8:GFP* (Quicktime; 23.9 MB).