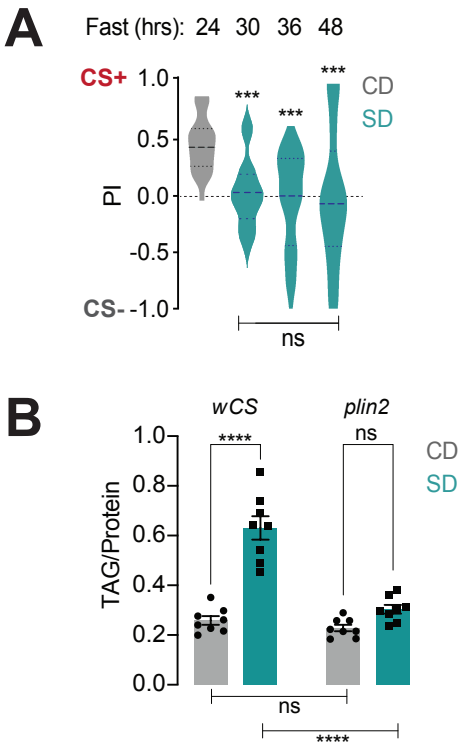
A
Fast (hrs): 24 30 36 48
CS+
CS-
1.0
0.5
0.0
-0.5
-1.0
PI
***
***
***
CD
SD
ns
B
wCS
plin2
****
ns
ns
****
CD
SD
TAG/Protein
1.0
0.8
0.6
0.4
0.2
0.0

Figure S1. Changes in food memories on a high-sugar diet occur independently of fasting time. Related to Figure 1.

A) The preference index (PI) of *wCS* flies on a CD (Gray) or SD (Teal) diet under different fasting times. n=22 (30 hr SD), n=38 (36 hrs SD), n=26 (48 hrs SD), and n=20 (24 hrs CD). Kruskal-Wallis with Dunn's multiple comparison test, ****p<0.001.*

B) Triglyceride levels normalized to protein in age-matched male *wCS* and *plin2* mutant flies on CD (gray) or SD (teal). n=8, one-way ANOVA with Tukey's test, comparisons as shown, ****p<0.0001.

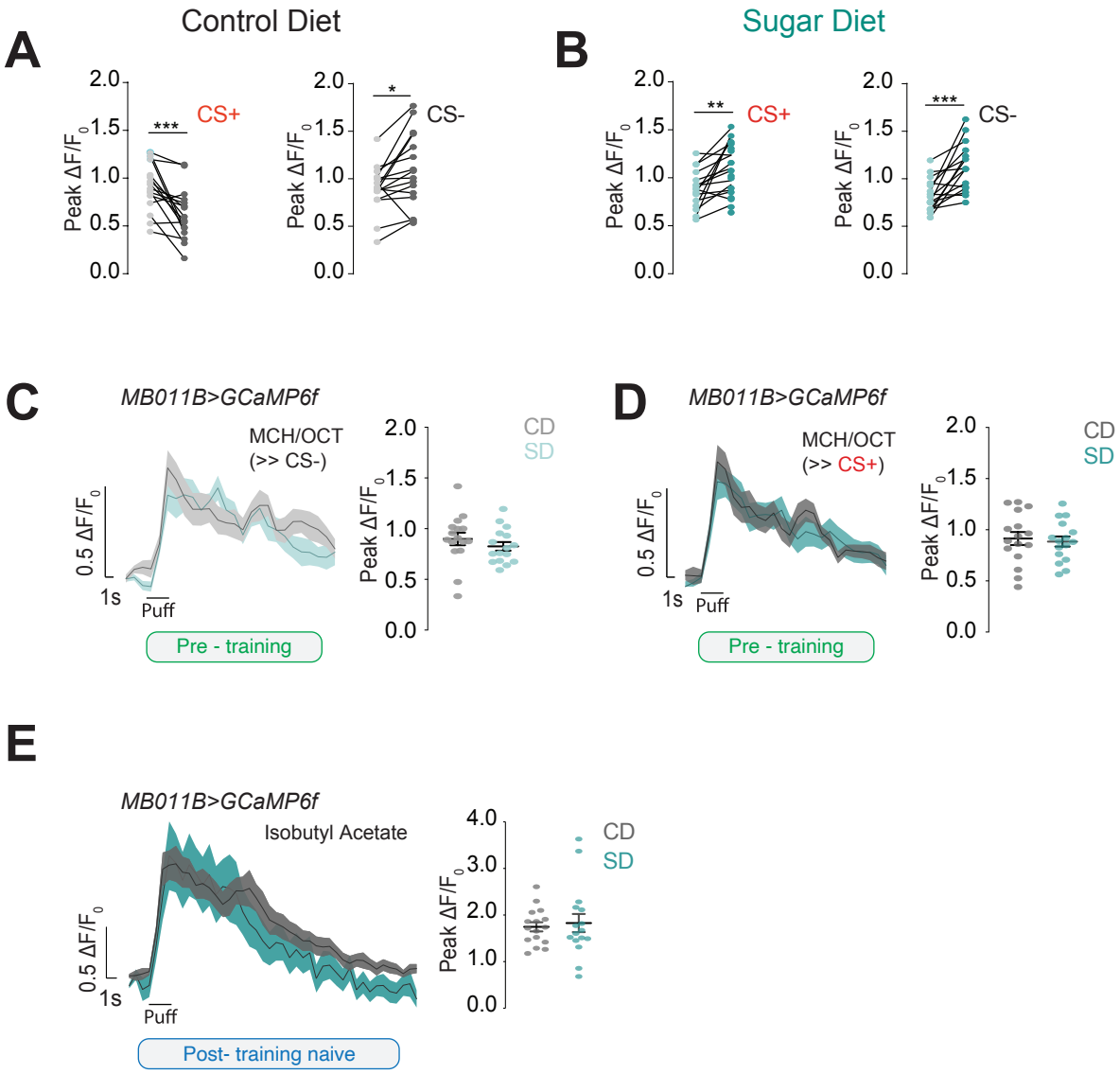
A
Control Diet
Peak ΔF/F0
2.0
1.5
1.0
0.5
0.0
***
CS+
*
CS-
B
Sugar Diet
**
CS+
***
CS-
C
MB011B>GCaMP6f
MCH/OCT
(>> CS-)
0.5 ΔF/F0
1s
Puff
Pre - training
CD
SD
D
MB011B>GCaMP6f
MCH/OCT
(>> CS+)
Pre - training
E
MB011B>GCaMP6f
Isobutyl Acetate
4.0
3.0
Post- training naive

Figure S2. A high-sugar diet alters the neural signatures of food learning. Related to Figure 2.

A and B) Comparison between the peak mean $\Delta F/F_0$ calcium responses at the β′2 dendrites of *MB011B>GcAMP6f* neurons to an odor (MCH or OCT) before (left, lighter color) and after training (right, darker color) (indicated with CS+, CS-) of flies either on a CD (A) or an SD (B). Data are shown as mean +/- SEM. n=16; student's t-test; **p<0.05, **p<0.01, ***p<0.001.*

C-D) The naive calcium responses to MCH and OCT (puff) in the β′2mp dendrites of *MB011B>GcAMP6f* neurons before training in CD (gray shades) and SD (teal shades) flies; data are shown $\Delta F/F_0$ traces and quantified as maximum peak $\Delta F/F_0$ response. Data correspond to that of Figures 2C and D.

E) The calcium responses to a novel odor (Isobutyl acetate) post-training are shown as mean $\Delta F/F_0$ traces and quantified as maximum peak $\Delta F/F_0$ in CD (gray) and SD flies (teal). n=16; Student's t-test.

Data are shown as +/- SEM.

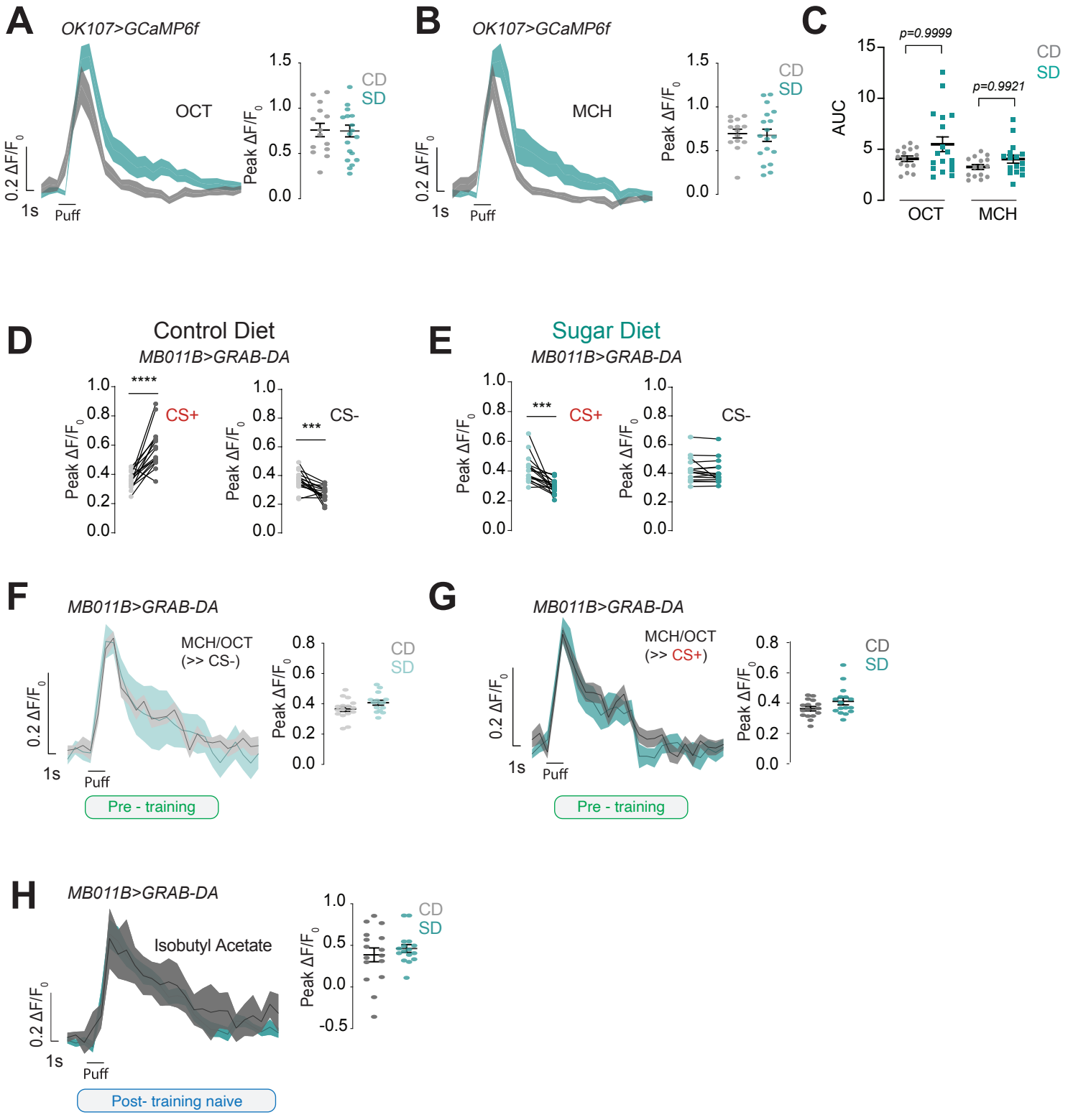
A
OK107>GCaMP6f
OCT
0.2 ΔF/F0
1s
Puff
Peak ΔF/F0
CD
SD
B
OK107>GCaMP6f
MCH
C
p=0.9999
p=0.9921
AUC
OCT
MCH
D
Control Diet
MB011B>GRAB-DA
****
CS+
***
CS-
E
Sugar Diet
MB011B>GRAB-DA
F
MB011B>GRAB-DA
MCH/OCT
(>> CS-)
Pre - training
G
MB011B>GRAB-DA
MCH/OCT
(>> CS+)
Pre - training
H
MB011B>GRAB-DA
Isobutyl Acetate
Post- training naive

Figure S3. Diet-dependent changes in DA-induced plasticity during learning. Related to Figure 3.

A-C) The calcium responses to OCT (A) and MCH (B) (puff) in the β′2 axons of Kenyon Cells of naive *OK107>GCaMP6f* flies fed a CD (gray) or SD (teal) shown as mean $\Delta F/F_0$ traces and quantified as maximum peak $\Delta F/F_0$ response (A and B) or area under the curve (C). n=14-18, A) Student's t-test, B) Mann-Whitney Test, C) One-way ANOVA Kruskal Wallis test.

D-E) Comparison between the peak mean $\Delta F/F_0$ calcium responses at the β′2 dendrites of *MB011B>GRAB-DA* neurons to a naïve odor (MCH or OCT) before (left, lighter color) and after training (right, darker color) (indicated with CS+, CS-) of flies either on CD (D) or SD (E). Data are shown as mean +/- SEM. n=16; student's t-test and Wilcoxon test (SD CS-). ****p<0.001, ****p<0.0001.*

F-G) The mean $\Delta F/F_0$ traces and quantification of maximum peak $\Delta F/F_0$ response to CS- (F) and CS+ (G) pretraining in the β'2 dendrites of CD (gray) and SD (teal) *MB011B>GRAB-DA* flies before training. Data correspond to Figure 3C and Figure 3D. n=16; Student's t-test.

H) The calcium responses to a novel odor (Isobutyl acetate) post-training shown as mean $\Delta F/F_0$ traces and quantified as maximum peak $\Delta F/F_0$ in CD (gray) and SD flies (teal). n=16; Student's t-test.

Data are shown as mean +/- SEM.

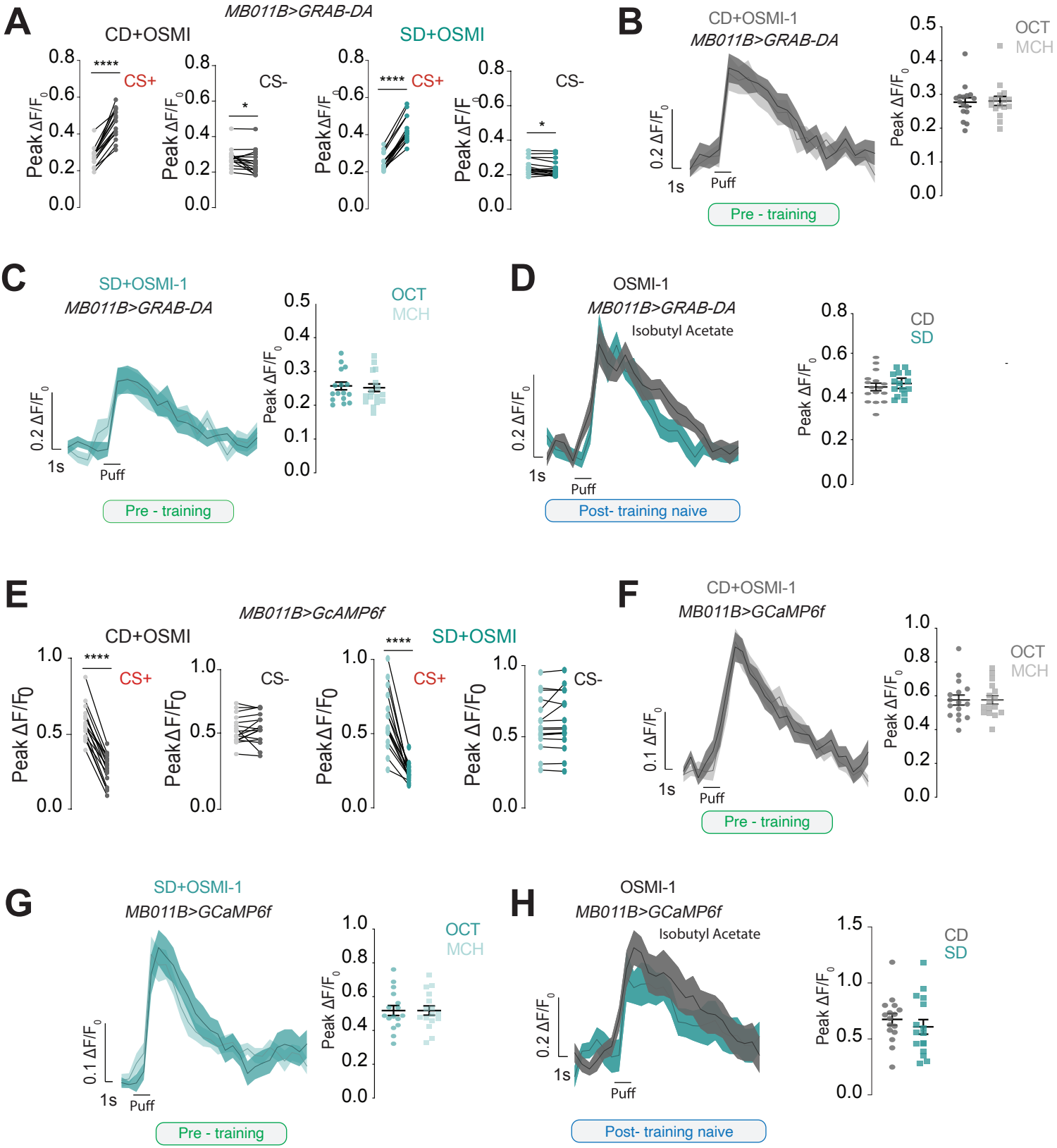
A
MB011B>GRAB-DA
CD+OSMI
SD+OSMI
CS+
CS-
Peak ΔF/F0
****
*
B
CD+OSMI-1
MB011B>GRAB-DA
0.2 ΔF/F0
1s
Puff
Pre - training
OCT
MCH
C
SD+OSMI-1
MB011B>GRAB-DA
D
OSMI-1
MB011B>GRAB-DA
Isobutyl Acetate
Post- training naive
CD
SD
E
MB011B>GcAMP6f
F
CD+OSMI-1
MB011B>GCaMP6f
0.1 ΔF/F0
G
SD+OSMI-1
MB011B>GCaMP6f
H
OSMI-1
MB011B>GCaMP6f
Isobutyl Acetate

Figure S4. Effects of OGT inhibition on DA-plasticity and MBON activity. Related to Figure 4.

A) Comparison between the peak mean $\Delta F/F_0$ calcium responses at the β′2 dendrites of *MB011B>GRAB-DA* neurons to a naïve odor (MCH or OCT) before (left, lighter color) and after training (right, darker color) (indicated with CS+, CS-) of flies either on a CD (gray) or an SD (teal) + OSMI. n = 16; student's t-test (CD CS+) and Wilcoxon test (CD CS-, SD CS+ and CS-); **p<0.05, ****p<0.0001.*

B-D) The mean $\Delta F/F_0$ traces and quantification of maximum peak $\Delta F/F_0$ response to MCH and OCT pretraining (B-C) or to a novel odor post-training (D) in the β'2 dendrites of CD+OSMI-1 (B, gray, Mann-Whitney), SD+OSMI-1 (C, teal, Mann-Whitney), and CD vs. SD both with OSMI-1 (D, Student's t-test) in *MB011B>GRAB-DA* flies. n=16 flies,

E) Comparison between the peak mean $\Delta F/F_0$ calcium responses at the β′2 dendrites of *MB011B>GcAMP6f* neurons to a naïve odor (MCH or OCT) before (left, lighter color) and after training (right, darker color) (indicated with CS+, CS-) of flies either on a CD (gray) or an SD (teal) + OSMI. n = 16; student's t-test; ****p<0.0001.

F-H) The mean $\Delta F/F_0$ traces and quantification of maximum peak $\Delta F/F_0$ response to MCH and OCT pretraining (F, G) or to a novel odor post-training (H) in the β'2 dendrites of CD+OSMI-1 (F, gray, Student's t-test), SD+OSMI-1 (G, teal, Student's t-test), and CD vs. SD both with OSMI-1 (H, Student's t-test) in *MB011B>GCaMP6f* flies. n=16 flies.

Data are shown as mean +/- SEM.

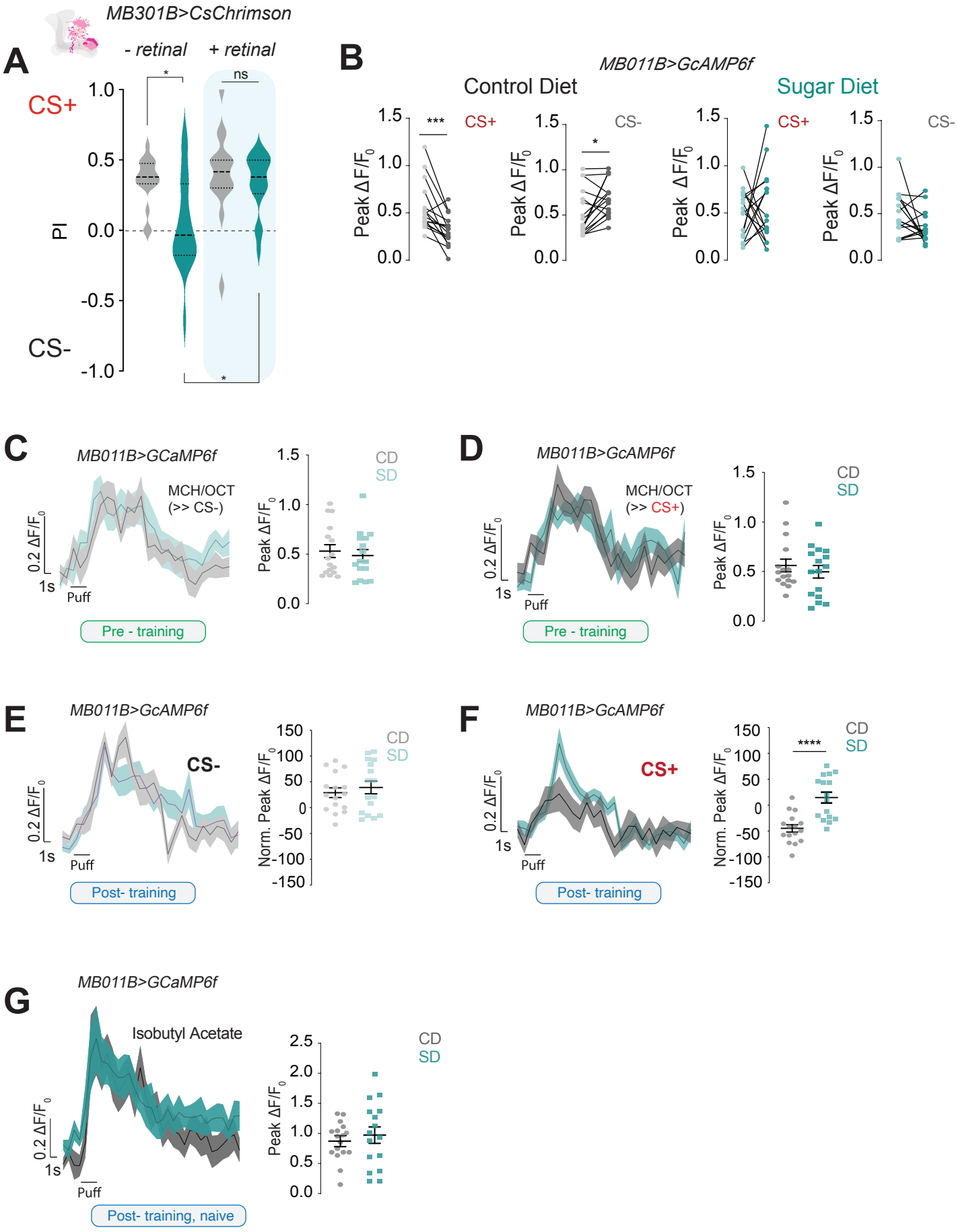
A
MB301B>CsChrimson
- retinal
+ retinal
CS+
CS-
PI
1.0
0.5
0.0
-0.5
-1.0
*
ns
*
B
MB011B>GcAMP6f
Control Diet
Sugar Diet
Peak ΔF/F0
CS+
CS-
***
*
C
MB011B>GCaMP6f
MCH/OCT
(>> CS-)
0.2 ΔF/F0
1s
Puff
Pre - training
CD
SD
D
MB011B>GcAMP6f
MCH/OCT
(>> CS+)
E
MB011B>GcAMP6f
CS-
Post- training
Norm. Peak ΔF/F0
F
MB011B>GcAMP6f
CS+
****
G
MB011B>GCaMP6f
Isobutyl Acetate
Post- training, naive

Figure S5. The effect of diet on the presynaptic responses of MBONs to cues. Related to Figure 5.

A) The preference index (PI) to the CS- (water) or CS+ (sucrose + light) of *MB301B>CsChrimson* flies on a CD (Gray) or SD (Teal) with or without retinal. n=16-18. Kruskal-Wallis test, **p<0.05.*

B) Comparison between the peak mean $\Delta F/F_0$ presynaptic calcium responses of *MB011B>GcAMP6f* neurons to a naïve odor (MCH or OCT) before (left, lighter color) and after training (right, darker color) (indicated with CS+, CS-) of flies either on a CD (gray) or an SD (teal). n=16; Wilcoxon test (CD CS- and CS+, SD CS-) and student's t-test (SD CS+); ** p<0.05, ***p<0.001.*

C-D) The pre-training mean calcium $\Delta F/F_0$ traces and quantification of maximum peak $\Delta F/F_0$ response to CS- and CS+ in the axons of CD (gray) and SD (teal) in *MB011B>GCaMP6f* flies. n=16 flies, Mann-Whitney test.

E-G) The post-training mean calcium $\Delta F/F_0$ traces and quantification of maximum peak $\Delta F/F_0$ response to CS- (E, Mann-Whitney) and CS+ (F, Student's t-test), or to a novel odor post-training (G, Student's t-test) in the axons of CD (gray), and SD (teal) *MB011B>GCaMP6f* flies. Data corresponds to that in Figure 5C' and Figure 5D'. n=16.

Data are shown as +/- SEM.

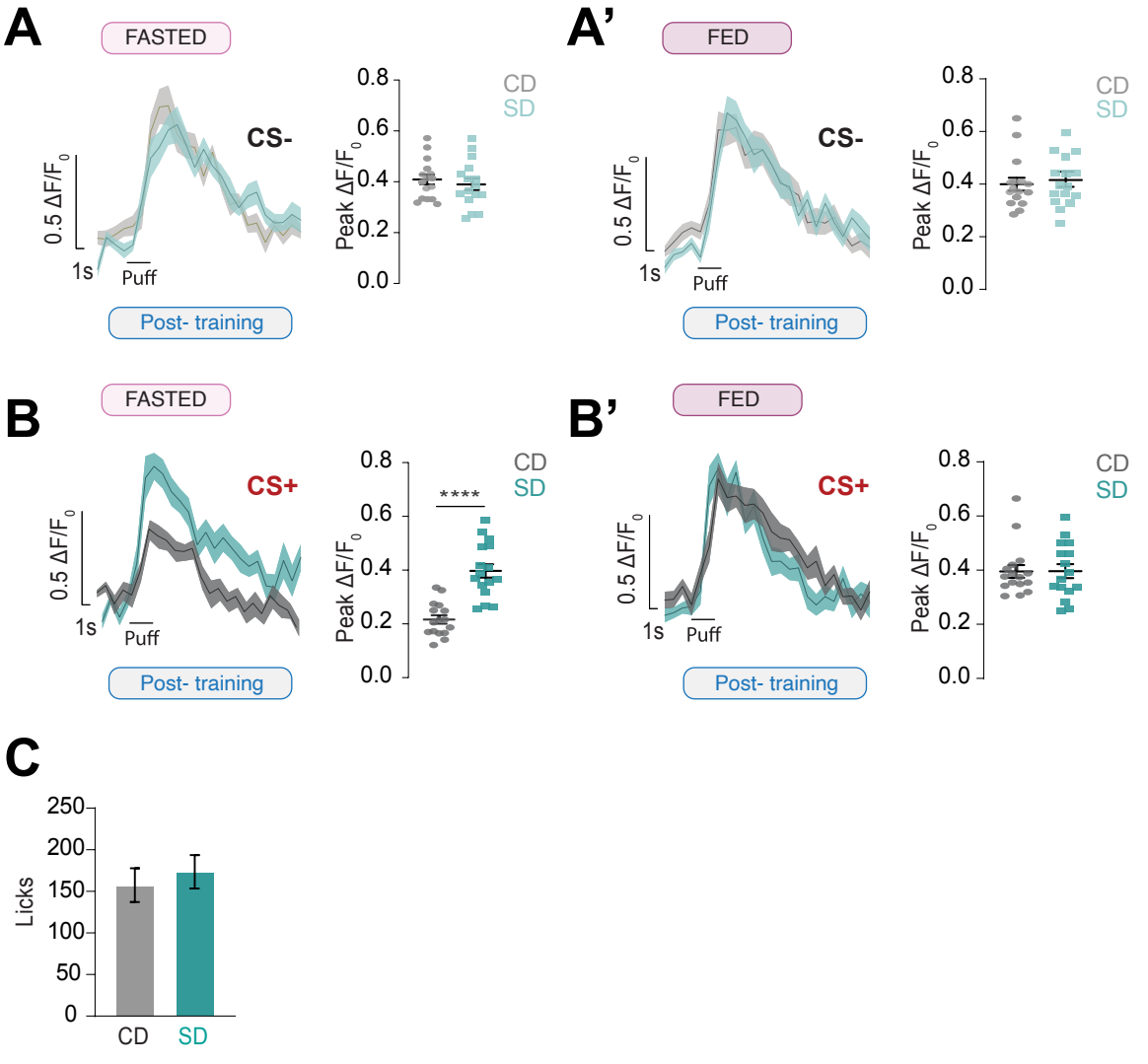
A
FASTED
CS-
0.5 ΔF/F0
1s
Puff
Post- training
Peak ΔF/F0
0.8
0.6
0.4
0.2
0.0
CD
SD
A'
FED
CS-
0.5 ΔF/F0
1s
Puff
Post- training
Peak ΔF/F0
0.8
0.6
0.4
0.2
0.0
CD
SD
B
FASTED
CS+
0.5 ΔF/F0
1s
Puff
Post- training
Peak ΔF/F0
****
0.8
0.6
0.4
0.2
0.0
CD
SD
B'
FED
CS+
0.5 ΔF/F0
1s
Puff
Post- training
Peak ΔF/F0
0.8
0.6
0.4
0.2
0.0
CD
SD
C
Licks
250
200
150
100
50
0
CD
SD

Figure S6. The presynaptic responses of MBONs to cues change during eating. Related to Figure 6.

A-B') The presynaptic calcium responses to the CS- (A, A') and CS+ (B, B') in *MB011B>GCaMP6f* CD (gray) and SD (teal) flies after appetitive conditioning in fasted (A and B, fasted) or refed flies (A' and B'). Data from Figure 6B and Figure 6C. n=16. A) Student's t-test, A') Mann-Whitney test, B) Student's t-test, ****$p<0.0001$, B') Mann-Whitney test.

C) The number of food interactions (licks) for flies feeding on 2M sucrose during a 30 min period; CD (gray) and SD (teal). n=16-24; Student's t-test.

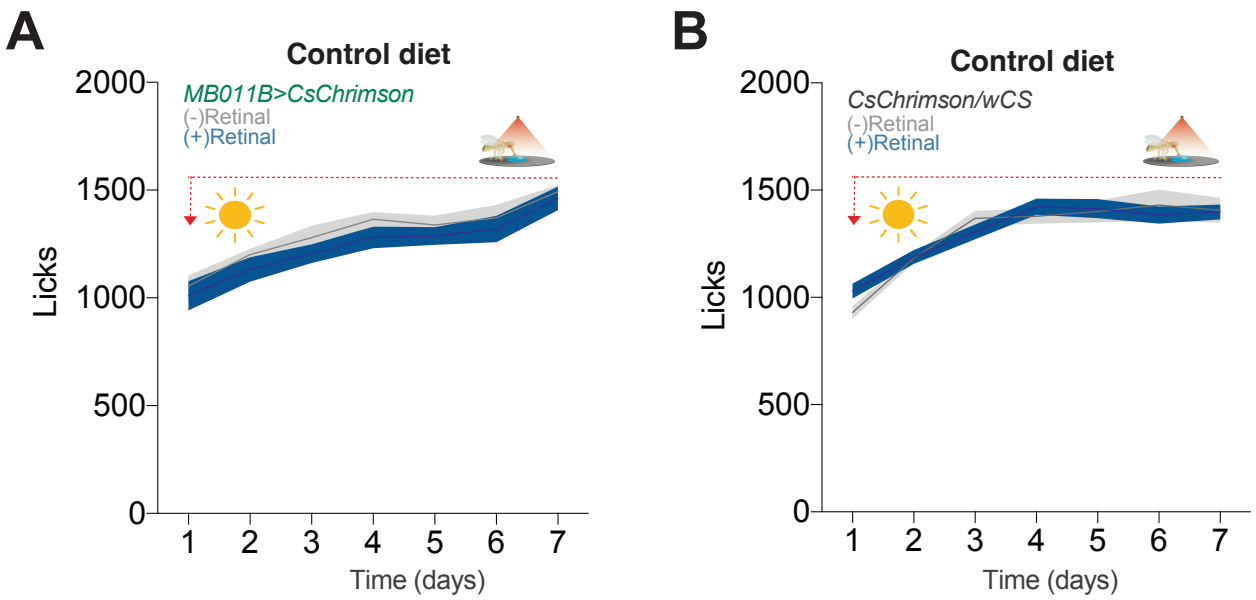
A
Control diet
MB011B>CsChrimson
(-)Retinal
(+)Retinal
Licks
2000
1500
1000
500
0
1
2
3
4
5
6
7
Time (days)
B
Control diet
CsChrimson/wCS
(-)Retinal
(+)Retinal
Licks
2000
1500
1000
500
0
1
2
3
4
5
6
7
Time (days)

Figure S7. The activity of MB011B+ MBONs affects eating and energy balance. Related to Figure 7.

A) The number of food interactions (licks) per day on a control diet in experimental *MB011B>CSChrimson* +retinal (blue) flies or control *MB011B>CSChrimson* - retinal (gray) flies. n=26 flies, Two-way Repeated Measure ANOVA with Sidak's test, *p>0.05.* **B)** The number of food interactions (licks) per day on a high sugar diet in control *CSChrimson>wCS* + retinal (blue) or - retinal (gray) flies. n=26 flies, Two-way Repeated Measure ANOVA with Sidak's test, *p>0.05.*

Data are shown as +/- SEM.