**Supporting Information for**

Cholesterol taste avoidance in *Drosophila melanogaster*

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**This file includes:**

Figures S1 to S3

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**Figure 1—figure supplement 1.** Electrophysiological analysis of different doses of MβCD.(A) Dose-dependent neuronal responses of *w1118* adult flies to MβCD from S7, I8, and L6 sensilla (n=10).(B) Representative sample traces corresponding to the data in (A).Error bars represent standard errors of the means (SEMs). Statistical analysis was performed using single-factor ANOVA with Scheffe's post hoc analysis to compare multiple datasets.

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**Figure 2—figure supplement 1.** Electrophysiological analysis of different bitter GRs and TRP lines in the presence of 10-1% CHL, with subset expression of *Ir56d* in bitter GRNs.(A) Electrophysiology analysis of broadly tuned bitter GRs with 10-1% cholesterol (n=10). (B) Neuronal response analyses of TRP mutant lines with 10-1% cholesterol (n=10).(C, D, E) Tip recording analyses of control flies and candidate IRs mutant flies (*Ir7g1*, *Ir25a2*, *Ir51b1*, *Ir56d1*, and *Ir76b1*) with 10-3% stigmasterol (STG) from S6, S7, and S10 sensilla (n=10-12).(F) Relative spatial distributions of the *Gr66a* (green; anti-GFP) and *Ir56d* (red; anti-DsRed) reporters in the labella of *Gr66a-I-GFP*, *Ir56d-GAL4/UAS-DsRed* flies. Images were acquired by confocal microscopy. The scale bars represent 50 µm.All error bars represent standard errors of the means (SEMs). Statistical analysis was performed using single-factor ANOVA with Scheffe's post hoc analysis to compare multiple datasets. Asterisks indicate statistical significance compared to the control group (\*\**P* < 0.01).

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**Figure 3—figure supplement 1.** Binary food choice assay with CHL and MβCD.(A) Dose-dependent binary food choice assay using control flies with 10-3%, 10-2%, and 10-1% MβCD containing 2 mM sucrose vs 2 mM sucrose only (n=6).(B) Dose-dependent binary food choice assay comparing cholesterol (CHL) vs MβCD food. Sucrose (2 mM) was employed on both sides (n=6). (C) Behavioral analysis of control flies after switching the dye to 0.1% cholesterol (n=6).(D) Feeding assay of the RNAi lines for *Ir7g*, *Ir25a*, *Ir51b*, *Ir56d*, and *Ir76b* with *UAS*-*Dicer2* driven by *Gr33a-GAL4*. (E) Feeding assay of the RNAi lines for *Ir7g*, *Ir25a*, *Ir51b*, *Ir56d*, and *Ir76b* with *UAS*-*Dicer2* driven by *ppk23-GAL4* (n=6).(F) Binary food choice assay of control flies and *orco1* mutants with 0.1% cholesterol (n=6).(G) Evaluation of the role of different organs in 0.1% cholesterol perception via feeding assay (n = 6). All error bars represent standard errors of the means (SEMs). Statistical analysis was performed using single-factor ANOVA with Scheffe's post hoc analysis to compare multiple datasets. Asterisks indicate statistical significance compared to the control group (\*\**P* < 0.01).