Supporting Information

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SI Methods

Detection thresholds of off-flavor substances were examined by healthy panelists who were selected from employees of Daiwa Can Company, after informed consent was obtained. They trained for and passed the panel selection test that has been conducted yearly. Among possible panelists who do not hesitate to smell wines, nonexperts for evaluating wines were randomly selected; wine specialists can detect contamination of 2,4,6-trichroloanisole (TCA) easily based on their own criteria. The panelists included 15 men and 5 women ranging from 25 to 60 y in age. This test complied with ethics guidelines regarding human epidemiological studies at Osaka University, Daiwa Can Company, and the Ministry of Education, Culture, Sports, Science and Technology of Japan.

The triangle test, which is approved by the Japanese Industrial Standards (JIS) Committee or British Standards Institution/International Organization for Standardization (ISO), was used for the determination of threshold. The detail protocols are summarized in JIS form Z 9080, which includes the concept of international standard (from ISO). In short, the entire processes were conducted as follows. In the evaluation, a complete double-blind procedure was established; neither technician who presented samples, nor the panelists, were involved in electrophysiology or data analysis. For presentation of wines, three possible order combinations were randomized, TCC, CTC, and CCT (C, original wine; T, wine with off-flavor substance). Wines used were California Franzia (red and white). The temperatures of wines were 15° C to 17 °C (at presentation). Concentrations of tested off-flavor compounds were as follows: TCA, 3 ppt, 10 ppt, and 15 ppt; 2,4,6-tribromoanisole, 10 ppt, 30 ppt, and 100 ppt; and 2,4,6-trichlorophenol, 30 ppb, 100 ppb, and 300 ppb. Off-flavors were added in wines with an ascending order in concentration until the number of correct judgments became large enough to be statistically significant in the binomial distribution (i.e., 0.05 level): in the case of 20 panelists, 11 or more correct judgments are estimated to be significant. Evaluations were performed in ventilated booths at room temperature (23 ± 2 °C, 50 ± 5% relative humidity).

After panelists received instructions regarding the evaluation procedure only, they entered into isolated booths designed for sensory evaluation. They sniffed three brown glasses (20 mL/50 mL vessel) of wine, one by one, several times each ($n = 1$–5), at intervals (5–30 s) that erase olfactory adaptation and/or fatigue. To avoid the order effect, the sequence of glass selection was counterbalanced across trials more than 1 min apart. Trials were examined in each concentration. The panelists indicated on a report paper which glass included wine with less odor, and left the room; they did not see anyone else from the beginning until the end of the evaluation. Later, the technician collected the report sheets for analyses. For individual panelists, evaluation was conducted twice per day (in the morning and afternoon) with a different concentration or different substance.

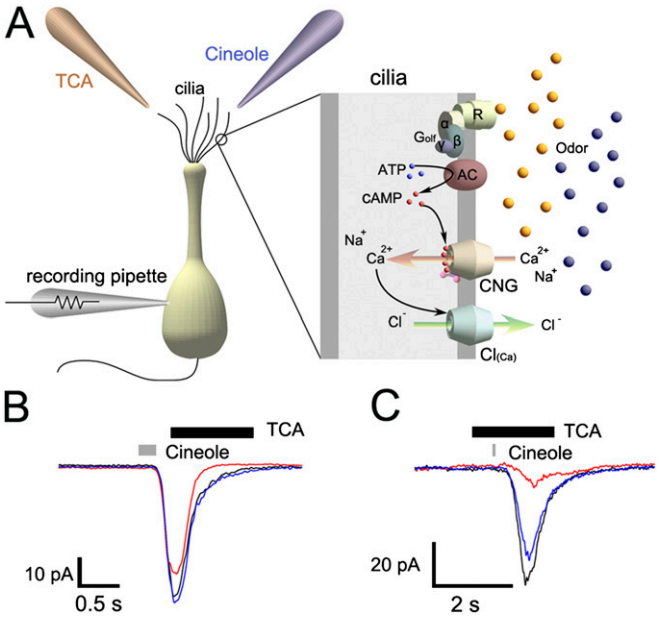

Fig. S1. Effect of TCA on the odorant-induced current. (*A*) Schema of cineole response and its suppression. (*B*) Current suppression by TCA stimulation at the peak. Black, control; red, 1 μM TCA; blue, recovery. (*C*) Current responses induced by cineole stimulation during TCA stimulation. Black, cineole response; red, 1 mM cineole response during 1 μM TCA application; blue, recovery.

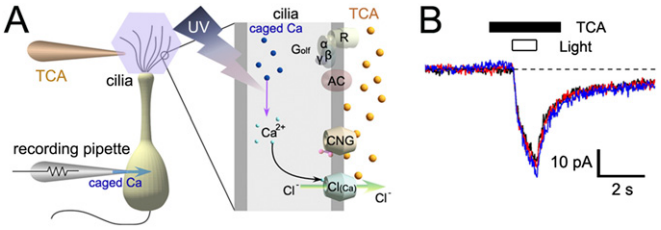

Fig. S2. Effect of TCA on the Ca^{2+}-activated Cl [$Cl_{(Ca)}$] channel. (*A*) Schema of Ca^{2+} response generation in the olfactory cilia. Cilia were loaded with 10 mM caged Ca (DM-nitrophen). When the UV light stimulation is applied to the ciliary region, uncaged Ca molecules open the $Cl_{(Ca)}$ channel. Note: In this experiment, only $Cl_{(Ca)}$ channels are open, without affecting cyclic nucleotide-gated (CNG) channels. (*B*) $Cl_{(Ca)}$ current responses induced by the light stimulation during 10 μM TCA stimulation. Traces: black, control; red, 10 μM TCA; blue, recovery.

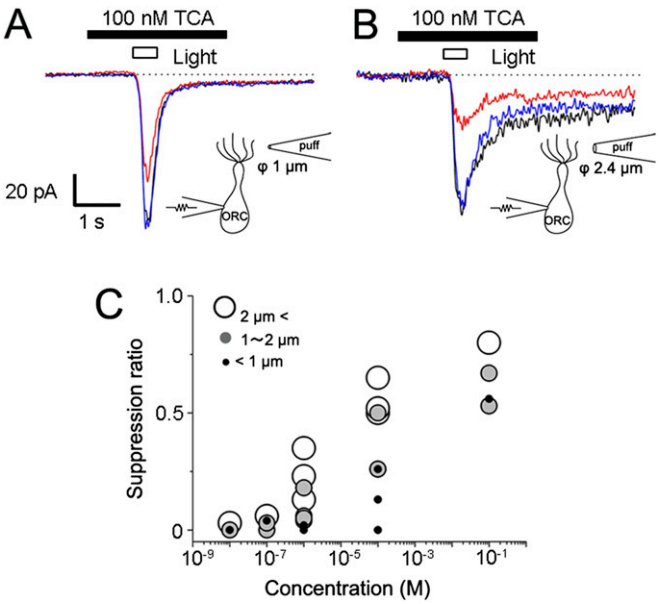

Fig. S3. Dependence on pipette diameter. (*A*) Current suppression induced with a pipette 1 μm in diameter. Traces: black, control; red, 100 nM TCA; blue, recovery. (*B*) Current suppression induced with a pipette 2.4 μm in diameter in the same cell as *A*. The time course of the light response is dependent on the time after the establishment of recording configurations. (*C*) Dose-suppression relations with pipettes with different tip diameters. Plots: black filled small circles, <1 μm; gray circles, 1–2 μm; open circles, >2 μm.