

Modification of the response of olfactory receptors to acetophenone by CYP1a2

Masashi Asakawa¹, Yosuke Fukutani¹, Aulaphan Savangsuksa¹, Keiich Noguchi², Hiroaki Matsunami^{3,4}, Masafumi Yohda^{1,4} *

¹ Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan.

² Instrumentation Analysis Center, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan.

³ Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, 27710, USA

⁴ Institute of Global Innovation Research, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan.

*Corresponding author:

Department of Biotechnology and Life Science, Graduate School of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan
yohda@cc.tuat.ac.jp

Supplementary Fig. S1 Screening of mouse ORs that are affected by co-expression of CYPs.

ORs that are known to respond to acetophenone were used for screening. Relative responses of cells transfected with odorant receptor and CYPs, CYP2a5 (Green), CYP1a2 (Yellow), CYP2f2 (Light blue) or an empty vector (White). The relative response of ORs was calculated as (Luc/RL)/(Luc/RL without odorant solution). The means \pm standard deviation of two separate experiments are shown.

Supplementary Fig. S2 Confirmation of functional expression of CYP1a2 in Hana3A cells.

CYP1a2 activity in the cell free extract of Hana3A cell was analyzed using the P450-GloTM assay with luciferin-ME as a substrate. Bioluminescence of Hana3A cells expressing CYP1a2 was compared with that of the mock transformants with pCI empty vector. (A) Dose response of CYP1a2 plasmid and control plasmid (pCI). Bioluminescence was measured after 3h incubation. (B) Time course of bioluminescence of Hana3A cells transfected with 20ng of CYP1a2 plasmid.

Supplementary Fig. S3 Control experiments for immunofluorescence and RNA in situ hybridization of mouse OE.

Mice were mock stimulated by a piece of filter paper spotted with 10 μ L water. pS6 and MOR161-2 were detected by Immunofluorescence and RNA in situ hybridization, respectively. (Nuclei) nuclei of olfactory sensory neurons, (MOR161-2) RNA in situ hybridization for MOR161-2, (pS6) Immunofluorescence for pS6, and (Merged) Merged image of Nuclei, MOR161-2 and pS6. And then the stained sections were observed by fluorescence microscope. Images were 20x magnification.

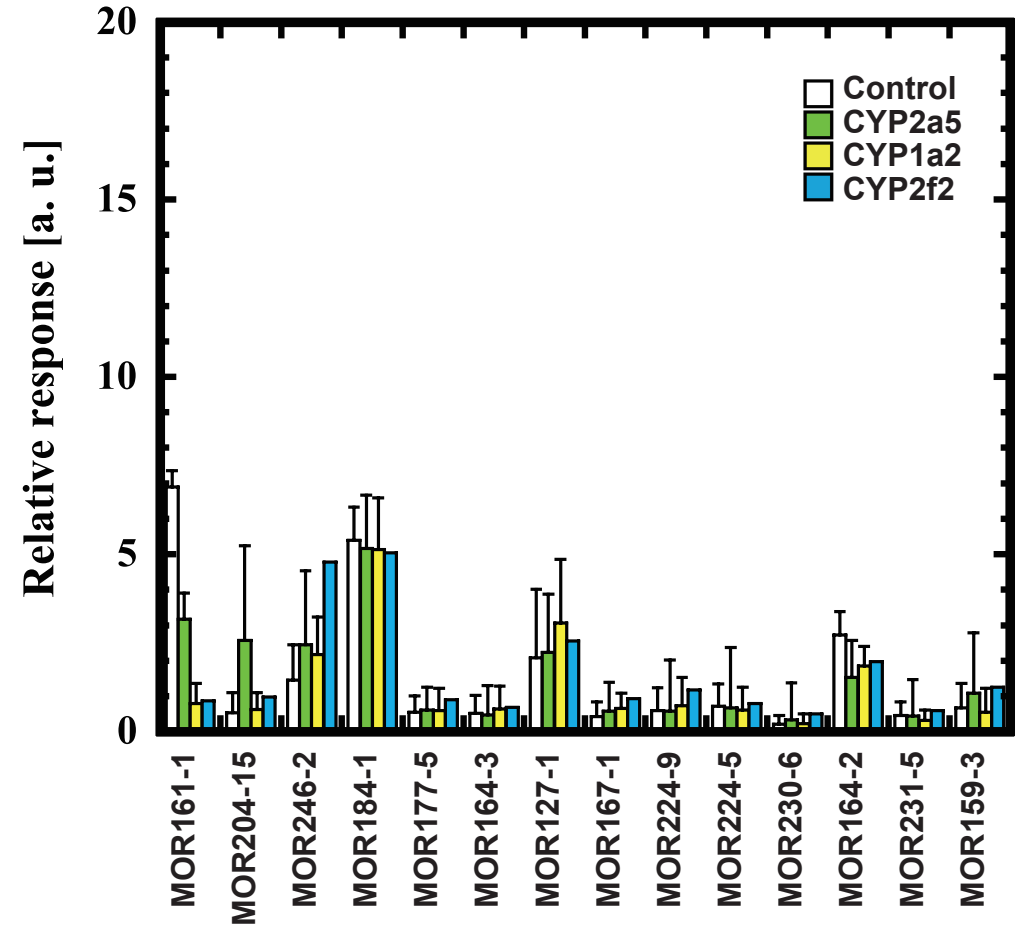
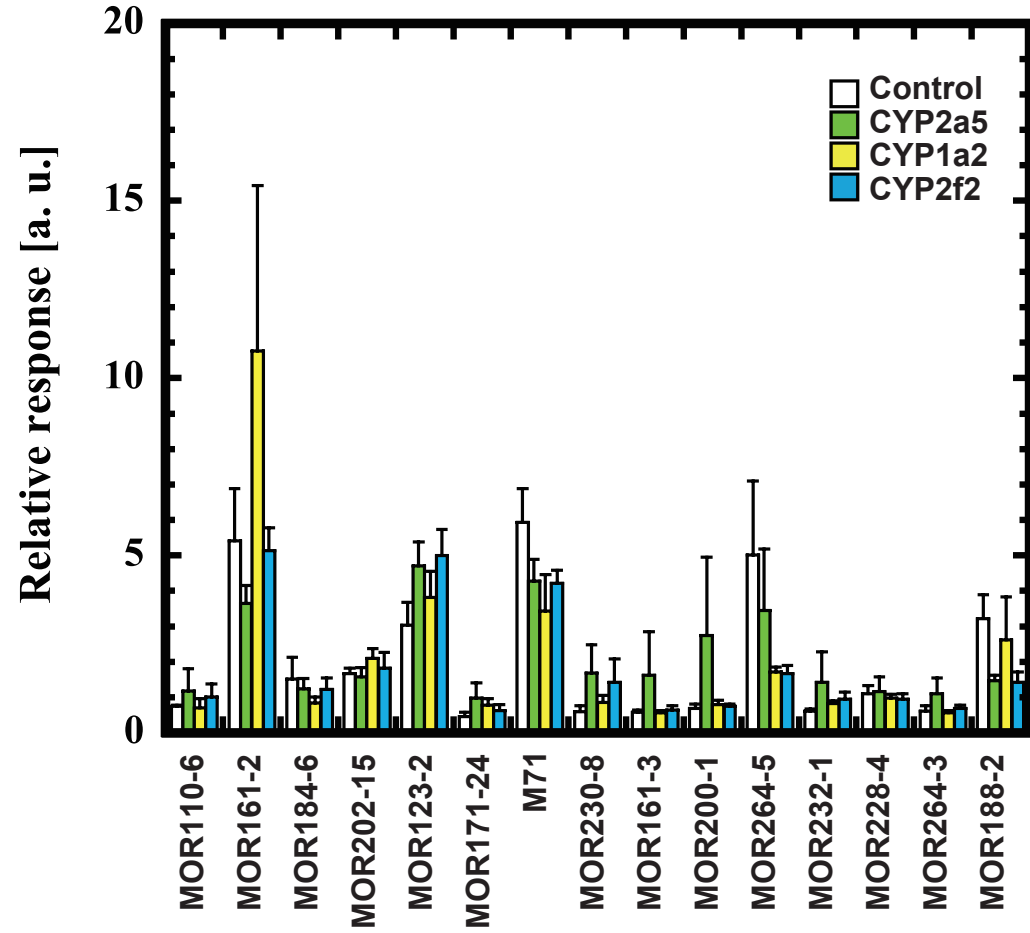
Supplementary Table S1 The list of ORs used in this study.

All odorant receptor open reading frames were amplified from mouse genomic DNA and subcloned into pCI expression vectors containing a Rho-tag.

Supplementary Table S2 The list of CYPs used in this study.

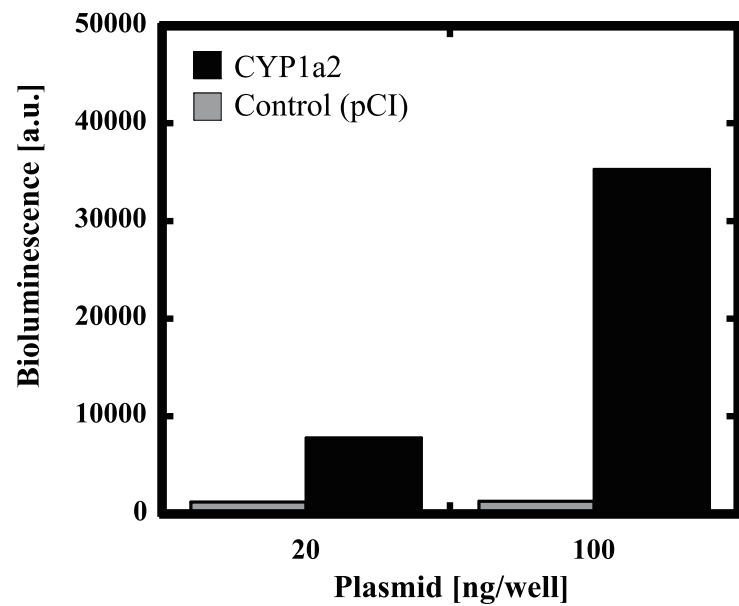
All CYPs were cloned from a cDNA library from mouse OE and inserted into pCI expression vectors.

Supplementary Fig. S1

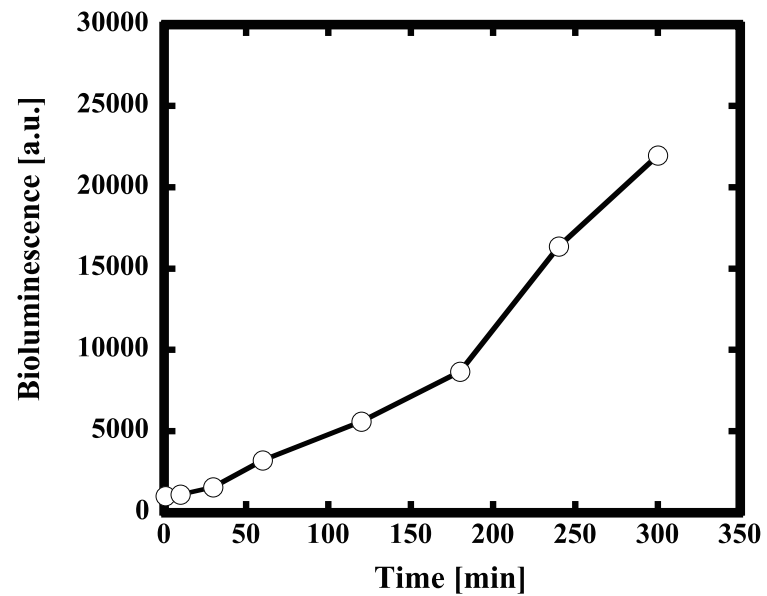


Supplementary Fig. S2

(A)

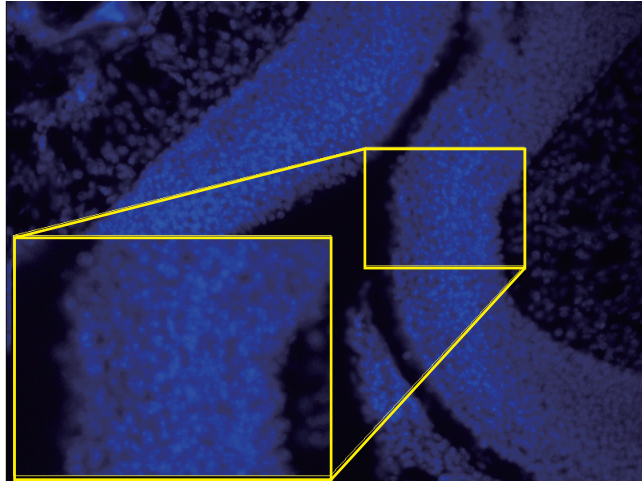


(B)

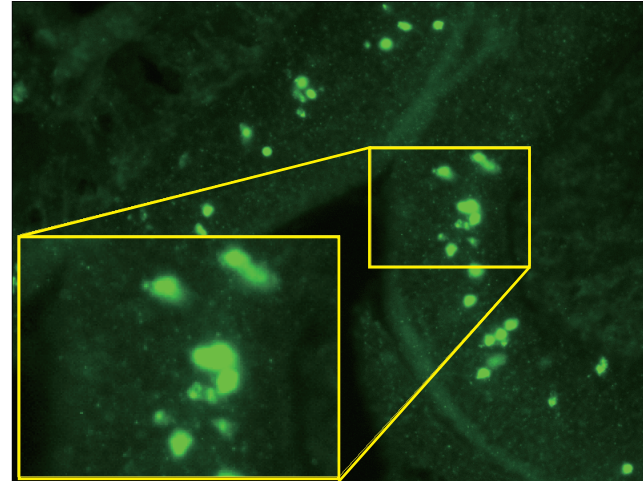


Supplementary Fig. S3

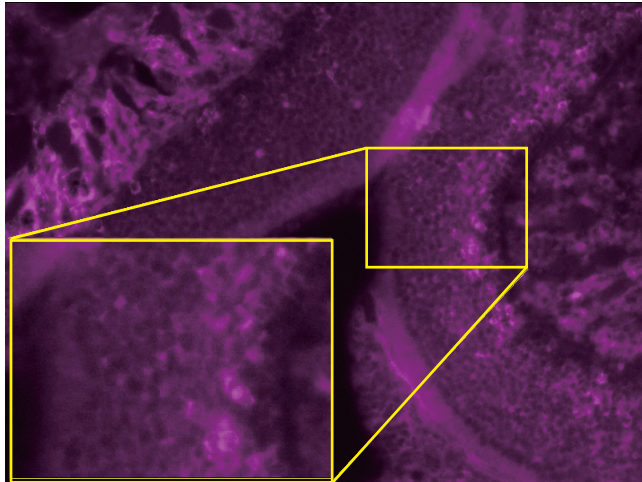
Nuclei



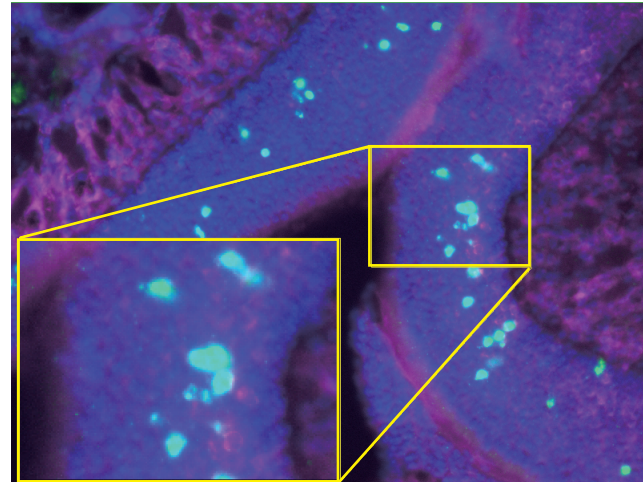
MOR161-2



pS6



Merged (Nuclei, pS6, MOR161-2)



Supplementary Table S1

The list of ORs used in this study

| NAME | ORDB | ACCESSION |
|-----------|----------|----------------|
| MOR110-6 | Oifr811 | NM_146552.1 |
| MOR161-2 | Oifr874 | NM_146882.2 |
| MOR184-6 | Oifr178 | NM_146997.2 |
| MOR202-15 | Oifr1467 | NM_146691.1 |
| MOR123-2 | Oifr432 | NM_146716.2 |
| MOR171-24 | Oifr937 | NM_146439.1 |
| M71 | Oifr151 | NM_207664.2 |
| MOR230-8 | Oifr1199 | NM_146458.1 |
| MOR161-3 | Oifr922 | NM_146781.2 |
| MOR200-1 | Oifr1031 | NM_001011759.2 |
| MOR264-5 | Oifr1126 | NM_146837.2 |
| MOR232-1 | Oifr1257 | NM_146982.1 |
| MOR228-4 | Oifr1274 | AY318479.1 |
| MOR264-3 | Oifr1124 | NM_147028.2 |
| MOR188-2 | Oifr1054 | AY318250.1 |
| MOR161-1 | Oifr876 | NM_146883.2 |
| MOR204-15 | Oifr488 | NM_146732.1 |
| MOR246-2 | Oifr727 | NM_146319.2 |
| MOR184-1 | Oifr174 | AY317259.1 |
| MOR177-5 | Oifr153 | NM_206823.1 |
| MOR164-3 | Oifr918 | NM_146375.2 |
| MOR127-1 | Oifr1496 | NM_146989.2 |
| MOR167-1 | Oifr905 | NM_146804.2 |
| MOR224-9 | Oifr958 | NM_146330.1 |
| MOR224-5 | Oifr961 | NM_146504.1 |
| MOR230-6 | Oifr1183 | NM_146529.2 |
| MOR164-2 | Oifr923 | NM_146816.2 |
| MOR231-5 | Oifr1242 | NM_146968.2 |
| MOR159-3 | Oifr361 | NM_146368.1 |
| MOR211-4P | Oifr1505 | NM_001011850.1 |

Supplementary Table S2

| NAME | ACCESSION |
|---------|-------------|
| CYP1a2 | NM_009993.3 |
| CYP2a5 | NM_007812.4 |
| CYP2f2 | NN_007817.2 |
| CYP2b10 | NM_009999.3 |
| CYP2b19 | NM_007814.1 |
| CYP2g1 | NM_013809.1 |
| CYP2j6 | NM_010008.4 |