Rapid Deorphanization of Human Olfactory Receptors in Yeast

Emily A. Yasi[1], Sara L. Eisen[1], Hanfei Wang[1], Widianti Sugianto[1], Anita R. Minniefield[1], Kaitlyn A. Hoover[1], Paul J. Branham[1], Pamela Peralta-Yahya[1,2*]

Table of Contents

Materials. Tert-butylbenzene (B90602**)** and 3-phenylbutyraldehyde (289027) were purchased from Sigma-Aldrich. Nonanoic acid (N0288) and nerol (N0077) were purchased from TCI Chemicals. Supplier information for the chemical panels can be found in **Table S5**.

Chemical toxicity to yeast-based OR sensor. Overnight cultures of PPY1802, PPY1804, PPY1806, and PPY1807 where diluted to an OD_{600}= 1 in 5 mL of SD(HL^-). Cultures were incubated for 4 hours at 30°C, 250 rpm with varying chemical concentrations with a final DMSO concentration of 1%. OD_{600} was measured after incubation. The experiments were run in triplicate.

mRNA Quantification. Following the OR biosensing protocol, between 2 x 10^7 and 5 x 10^7 cells were pelleted and total RNA was extracted using Qiagen RNeasy Mini Kit. RNA concentration was measured using a Nanodrop Lite spectrophotometer and stored at -80°C. Reverse transcription was executed using 700 µg of total RNA using the QuantiTect Reverse Transcription kit (Qiagen). Quantitative PCR reactions were set up using the QuantiTect SYBR Green PCR kit (Qiagen) using cDNA from 42 ng of mRNA and read using a StepOnePlus Real-Time PCR system (Applied Biosciences). Reactions were set up as technical triplicates using primers EY270/EY271 for OR10S1, EY272/EY273 for OR2A7, EY274/EY275 for OR2T4, and ACT-F/ACT-R for actin. OR expression were normalized to the housekeeping gene, ACT1, that encodes actin and relative expression of chemical versus no chemical (DMSO) was compared using the comparative C_T method (Livek and Schmittgen, 2001) using the equations below.

$$\Delta C_{T\_Sample} = avgC_{T\_Sample} - avgC_{T\_Actin}$$
$$\Delta\Delta C_T = \Delta C_T - \Delta C_{T\_DMSO}$$
$$2^{-\Delta\Delta C_T} = Mean\ relative\ expression\ of\ gene\ of\ interest\ compared\ to\ DMSO\ control$$
$$2^{-(\Delta\Delta C_T \pm StDev)} = Range\ of\ fold\ change\ in\ expression\ relative\ to\ DMSO\ control$$
$$StDev = standard\ deviation\ of\ \Delta C_{T\_Sample}$$

Table S1. Sequence identity of ectopically expressed olfactory receptors in the colon

	OR2A7	**OR2T4**	**OR2A42**	**OR2W3**	**OR51B5**	**OR2L13**	**OR10S1**
OR2A7							
OR2T4	39.90%						
OR2A42	71.30%	44.00%					
OR2W3	42.70%	41.60%	46.90%				
OR51B5	31.80%	28.80%	32.20%	27.60%			
OR2L13	39.50%	49.20%	42.20%	39.30%	26.20%		
OR10S1	39.60%	36.40%	41.70%	41.60%	28.20%	40.30%	

Table S2. Table of primers

Name	**Sequence**
EY46	ACTTCTTGCTCATTAGAAAG
HW12	CTTTTCGGTTAGAGCGGATC
EY270	CTGTCAGCCGTTACATTATCC

EY271	CCACAATACAGCAGACGAAAG
EY272	CCGTGAAGTTCAGCGTAAG
EY273	TTCAGCATCGGGTTAAACAG
EY274	TTCAGACAGAGCAAGCACC
EY275	ACGAAAATGACGACACACAG
ACT-F	TTCTGAGGTTGCTGCTTTGG
ACT-R	ACCGACGATAGATGGGAAGAC
HW1	GCAATCTAATCTAAGTTTTAATTACAAAGGATCC
HW4	AGAATGAGATTCCTTGCCC
HW7	TTCCTTGCCTAGTTCTCTCTTACC
HW8	TTCCATTGCCTTTTGGAAGGC
HW9	GGTACCAATTCTATGGGTGGTG
PB89	TTCCTTCAGGAAGCTGAAGATACC
PB116	CGGCGGCGGGCTA
PB118	CAGTGCACGTTCAACACCC
HW15	AGGAGGGCTTTGGGCAAGGAATCTCATTCTGTGAGCAAGGGCGAGG
HW18	TTGTTGGGTAAGAGAGAACTAGGCAAGGAAGTGAGCAAGGGCGAGG
HW19	GTTGAACCAGCCTTCCAAAAGGCAATGGAAGTGAGCAAGGGCGAGG
HW20	CATTTGTTCACCACCCATAGAATTGGTACCGTGAGCAAGGGCGAGG
PB88	GTGTTCGGTATCTTCAGCTTCCTGAAGGAAGTGAGCAAGGGCGAG
PB115	AGCACCGCAGGTAGCCCGCCGCCGGTGAGCAAGGGCGAGG
PB117	GTTCTGGGTGTTGAACGTGCACTGGTGAGCAAGGGCGAGG

Table S3. Table of Plasmids.

Number	**Name**	**Description**	**Source**
PPY111	pKM111	pESC-His3-P_{TEF1}-P_{ADH1}	Mukherjee et al. (2015)
PPY586	pKM586	pRS415- P_{FIG1}-eGFP-Leu2	Mukherjee et al. (2015)
PPY1658	pHW3	pESC-HIS3-P_{TEF1}-OR2A42- P_{ADH1}	This study
PPY1661	pHW6	pESC-HIS3-P_{TEF1}-OR2W3- P_{ADH1}	This study
PPY1663	pHW7	pESC-HIS3-P_{TEF1}-OR2T4- P_{ADH1}	This study
PPY1715	pHW18	pESC-HIS3-P_{TEF1}-OR51B5- P_{ADH1}	This study
PPY1795	pPB8	pESC-HIS3-P_{TEF1}-OR2L13- P_{ADH1}	This study
PPY1717	pHW20	pESC-HIS3-P_{TEF1}-OR10S1- P_{ADH1}	This study
PPY1718	pHW21	pESC-HIS3-P_{TEF1}-OR2A7- P_{ADH1}	This study
PPY1719	pHW22	pESC-HIS3-P_{TEF1}-OR2A42-GFP	This study
PPY1720	pHW23	pESC-HIS3-P_{TEF1}-OR51B5-GFP	This study

PPY1734	pHW30	pESC-HIS3-P_{TEF1}-OR2T4-GFP	This study
PPY1737	pHW33	pESC-HIS3-P_{TEF1}-OR2W3-GFP	This study
PPY1947	pPB59	pESC-HIS3-P_{TEF1}- OR2A7-GFP	This study
PPY1948	pPB60	pESC-HIS3-P_{TEF1}-OR10S1-GFP	This study
PPY1930	pPB54	pESC-HIS3-P_{TEF1}-OR2L13-GFP	This study

Table S4. Table of Strains.

Strain Number	**Description**	**Source**
PPY140	*S. cerevisiae* MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3 leu2-112 can1-100 Δ*far1,* Δ*sst2,* Δ*ste2*	Mukherjee et al. (2015)
PPY1800	PPY140 carrying pKM111 and pKM586	This study
PPY1801	PPY140 carrying pHW3 and pKM586	This study
PPY1802	PPY140 carrying pHW6 and pKM586	This study
PPY1803	PPY140 carrying pHW7 and pKM586	This study
PPY1804	PPY140 carrying pHW18 and pKM586	This study
PPY1805	PPY140 carrying pPB8 and pKM586	This study
PPY1806	PPY140 carrying pHW20 and pKM586	This study
PPY1807	PPY140 carrying pHW21 and pKM586	This study
PPY1949	PPY140 carrying pHW22	This study
PPY1950	PPY140 carrying pHW23	This study
PPY1951	PPY140 carrying pHW30	This study
PPY1952	PPY140 carrying pHW33	This study
PPY1953	PPY140 carrying pPB59	This study
PPY1954	PPY140 carrying pPB60	This study
PPY1955	PPY140 carrying pPB54	This study

Table S5*.* Table of chemicals in the chemical panel

Chemical	**CAS #**	**Supplier**	**Catalog #**
(-)-limonene	5989-54-8	TCI Chemicals	L0132
(+)-carvone	6485-40-1	TCI Chemicals	C0703
(+)-menthol	2216-51-5	TCI Chemicals	M0826
1,2,3-trimethylbenzene	526-73-8	TCI Chemicals	T0468
1-butanol	71-36-3	TCI Chemicals	B0944
1-butoxy-2-propanol	5131-66-8	TCI Chemicals	B0864
1-dodecanol	112-53-8	Alfa Aesar	A12228
1-octanol	111-87-5	TCI Chemicals	O0036
1-propanol	71-23-8	TCI Chemicals	P0491

2,3-heptadione	96-04-8	TCI Chemicals	H0422
2,5-dimethylpyrazine	123-32-0	TCI Chemicals	D1526
2-methyl-1-butanol	137-32-6	TCI Chemicals	M0175
3-hexanone	589-38-8	TCI Chemicals	H0115
3-pentanol	584-02-1	TCI Chemicals	P0057
acetic acid	64-19-7	VWR	BDH3092
alpha-pinene	80-56-8	Sigma-Aldrich	305715
anisaldehyde	123-11-5	TCI Chemicals	A1674
beta-pinene	127-91-3	Sigma-Aldrich	CRM40433
butyric acid	107-92-6	TCI Chemicals	B0754
coumarin	91-64-5	TCI Chemicals	C0395
ethyl decanoate	110-38-3	TCI Chemicals	D0022
ethyl laurate	106-33-2	TCI Chemicals	L0013
ethyl myristate	124-06-1	TCI Chemicals	M0479
ethyl octanoate	106-32-1	TCI Chemicals	O0030
ethyl palmitate	628-97-7	Alfa Aesar	A15694
ethyl stearate	111-61-5	TCI Chemicals	S0079
eugenol	97-53-0	Alfa Aesar	A14332
farnesol	4602-84-0	TCI Chemicals	T0608
geraniol	106-24-1	TCI Chemicals	G0027
heptanoic acid	111-14-8	Alfa Aesar	A17704
hexanoic acid	142-62-1	Alfa Aesar	A13789
isovaleric acid	503-74-2	TCI Chemicals	M0182
lactic acid	50-21-5	Alfa Aesar	36415
lauric acid	143-07-7	TCI Chemicals	L0011
lilial	80-54-6	TCI Chemicals	B1145
linalool	78-70-6	TCI Chemicals	L0048
lyral	31906-04-4	Sigma-Aldrich	95594
melatonin	73-31-4	Sigma-Aldrich	M5250
methyl decanoate	110-42-9	Alfa Aesar	A15658
methyl laurate	111-82-0	Alfa Aesar	A12492
methyl myristate	124-10-7	Alfa Aesar	A10257
methyl octanoate	111-11-5	Alfa Aesar	A10991
methyl palmitate	112-39-0	TCI Chemicals	S0311
methyl stearate	112-61-8	TCI Chemicals	S0312
m-xylene	108-38-3	Sigma-Aldrich	95670
myristic acid	544-63-8	TCI Chemicals	M0476
nonanal	124-19-6	TCI Chemicals	N0296
octanal	124-13-0	Alfa Aesar	A10901

octanoic acid	124-07-2	Alfa Aesar	A11149
palmitic acid	57-10-3	TCI Chemicals	P0002
p-cymene	99-87-6	TCI Chemicals	C0513
pentadecanoic acid	1002-84-2	Alfa Aesar	A14664
propionic acid	79-09-4	TCI Chemicals	P05000
sotolon	28664-35-9	TCI Chemicals	D1851
stearic acid	57-11-4	TCI Chemicals	S0163
undecanal	112-44-7	TCI Chemicals	U0009
vanillin	121-33-5	Alfa Aesar	A11169

Table S6. Abbreviations and Chemical Descriptors for PCA Analysis

Descriptors	Abbreviations
Molecular Weight	MW
Carbon atom count	CAC
Hydrogen atom count	HAC
Oxygen atom count	OAC
Nitrogen atom count	NAC
Hydrogen-bond donors	HBD
Hydrogen-bond acceptor	HBA
Rotatable bonds	RB
Stereocenter count	SC
Topological polar surface area	TPSA
Number of rings	NOR
Aromatic ring count	ARC
Ring system count	RSC
Size of largest ring	SLR
Van der Waals surface area	VSA
Amine	-
Alcohol	-
Ether	-
Aldehyde	-
Ketone	-
Carboxylic Acid	-
Ester	-
Amide	-

Table S7. Chemical space coordinates of the screened chemicals

Chemical Space	PC1 (28% variance)	PC2 (19% variance)	PC3 (16% variance)	Members	Population (%)
1	-	-	-	9	15.8
2	+	-	-	8	14.0
3	-	-	+	6	10.5
4	+	-	+	8	14.0
5	+	+	-	2	3.5
6	-	+	-	13	22.8
7	+	+	+	6	10.5
8	-	+	+	5	8.8
			Total:	57	100

Table S8. R^2 values for dose response fits

Figure	OR	Chemical	R^2
3A, 4A	OR2A7	α-pinene	0.98946
3A, 4B	OR2T4	α-pinene	0.99269
3B, 4C	OR2A7	lilial	0.97004
3B, 4D	OR2T4	lilial	0.99854
3B	OR10S1	lilial	0.99623
3C	OR51B5	farnesol	0.99585
3D	OR2T4	undecanal	0.90762
3D	OR10S1	nonanal	0.99651
4A	OR2A7	β-pinene	0.99659
4C	OR2A7	tert-butylbenzene	0.99853

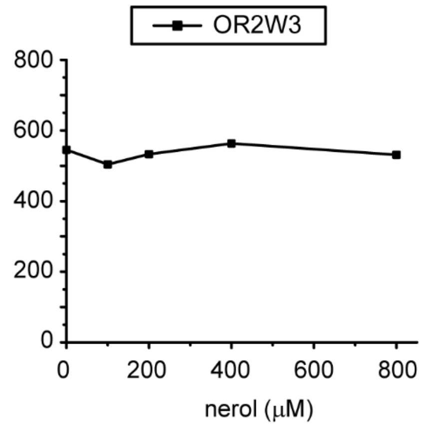

FIGURE S1. Dose response curve of OR2W3 with nerol.

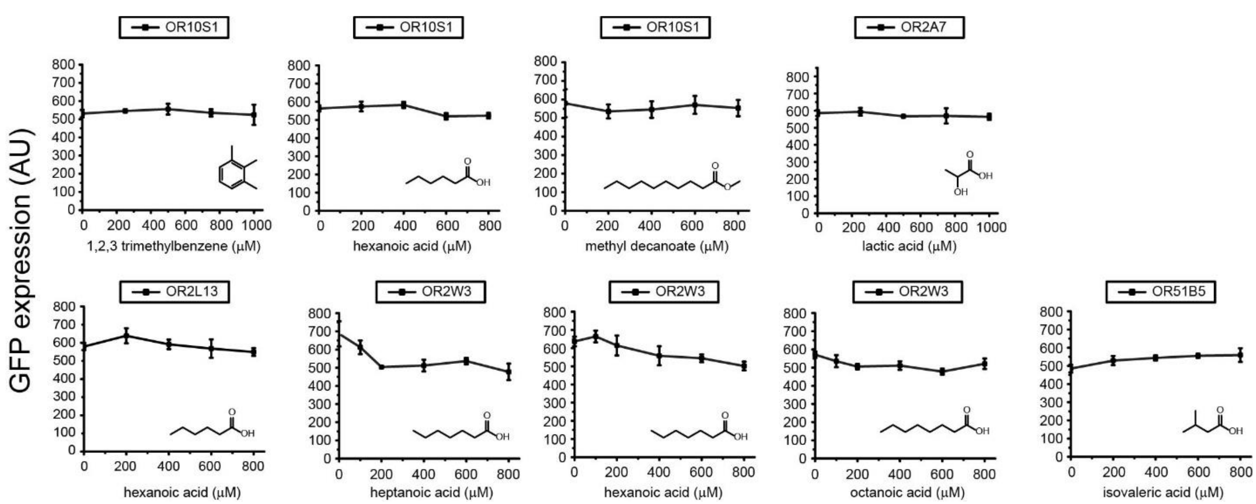

Figure S2. Dose response curves of colon ORs with chemical hits (false positives). Chemicals shown above did not elicit a statistically significant increase in GFP expression.

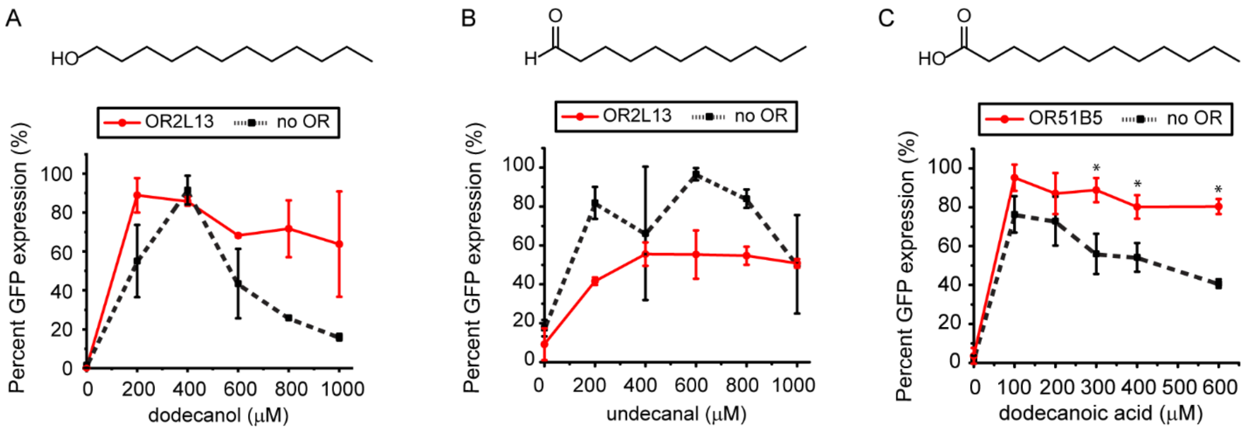

Figure S3. Dose response curves of OR2L13 and OR51B5 with validated chemicals. A. OR2L13 with dodecanol. B. OR2L13 with undecanal. C. OR51B5 with dodecanoic acid.

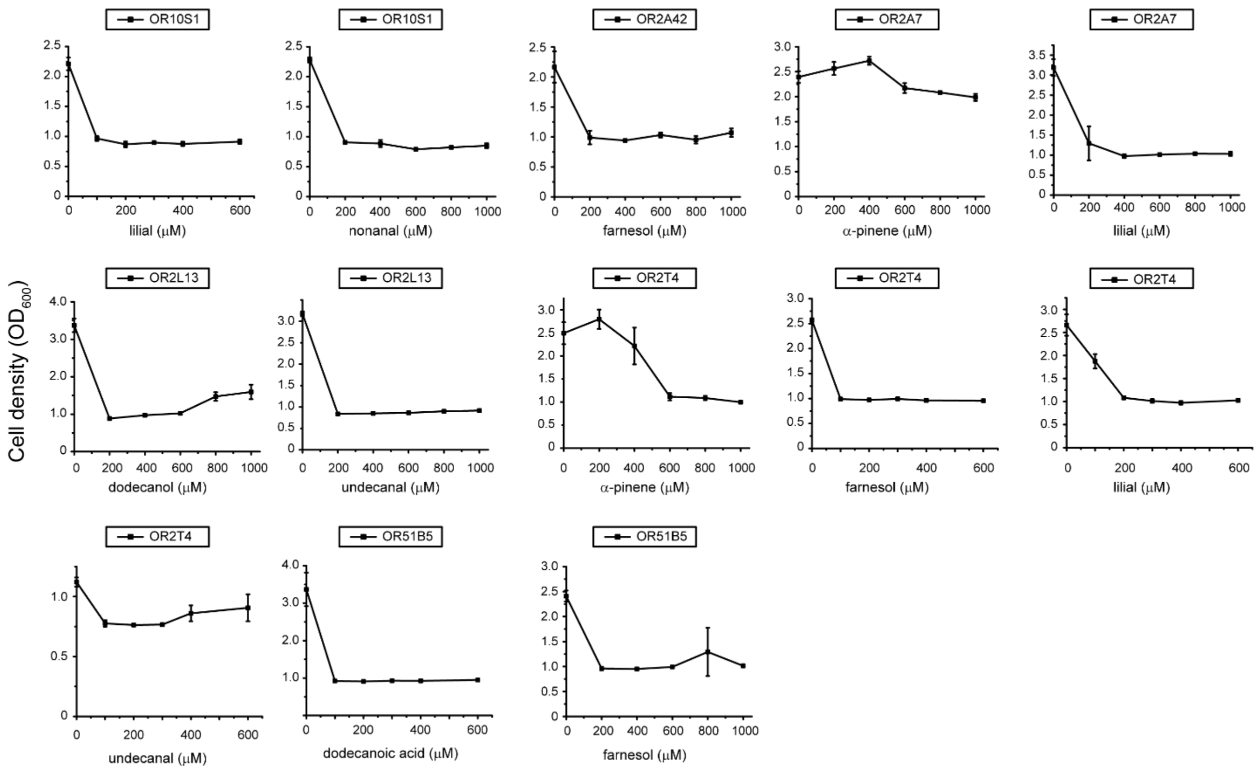

FIGURE S4. Chemical toxicity to yeast-based OR sensor. Yeast cell density was measured after the 4 hour incubation step in the OR chemical sensing protocol. The no chemical data point has 1% DMSO. While the yeast cells double in the absence of chemical, their cell density is static in the presence of chemical.

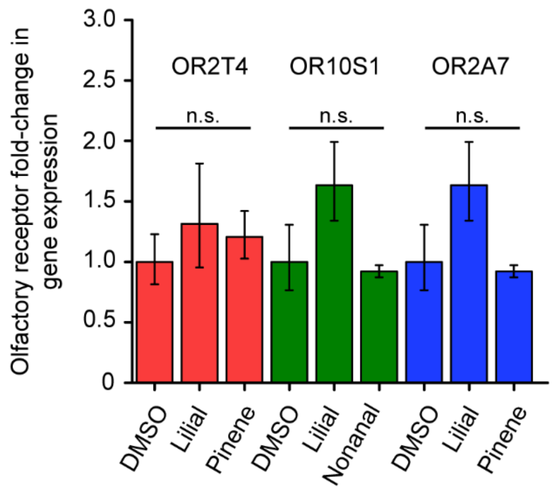

FIGURE S5. Changes in olfactory receptor gene expression levels in the presence of chemicals. Red bars: OR2T4. Green bars: OR10S1.Blue bars: OR2A7. The gene expression experiments were run 600 µM lilial and nonanal, and 1000µM pinene. DMSO controls were compared to OR/chemical pairs for OR2T4, OR10S1, and OR2A7 and in all cases P>0.05.

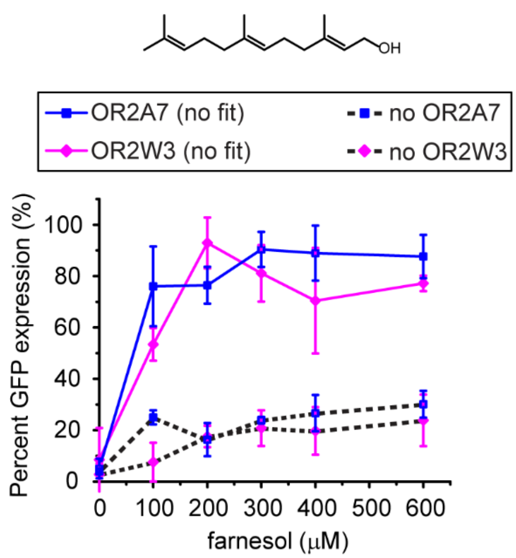

FIGURE S6. Dose response curves of OR2A7 and OR2W3 with farnesol.

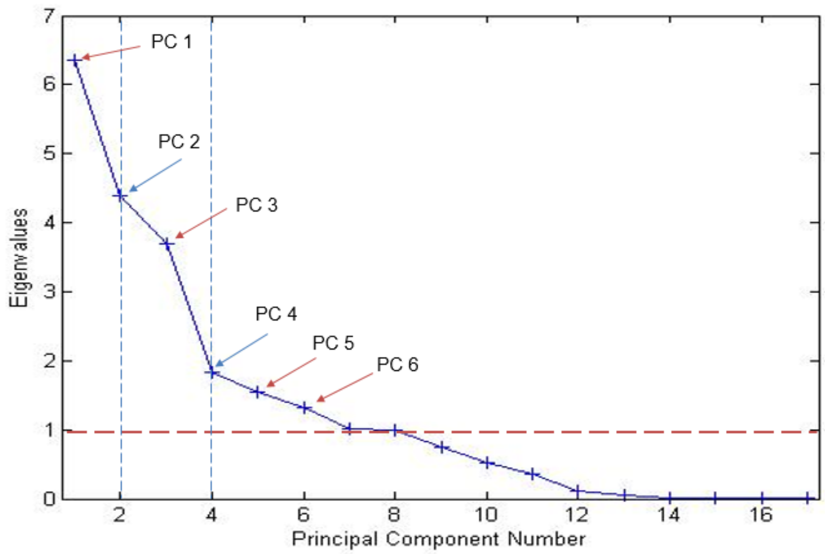

Figure S7. Eigenvalues for principle component determination. Two criteria to be considered in determining number of principle components are: 1) number of PCs with eigenvalues greater than one (red dashes and red arrows), 2) PCs located after bends or knees (blue dashes and blue arrow). Solo analysis resulted in six eigenvalues greater than one, thus six PCs (PCs 1 to 6) could be considered for the analysis. Two bends were also observed on the graph at PC2 and PC4, meaning that the PCs after the bends (PC3 and PC5) need to be included in the analysis. These criteria led us to include three PCs (PCs 1-3) in our analysis.

Sequences

Homo sapiens OR2A42 (UniprotKB - Q8NGT9)
ATGGGTGAAAATCAAACTATGGTTACCGAATTTCTACTACTAGGTTTCCTACTAGGTCCAAG
AATCCAAATGCTACTATTCGGTTTGTTCTCCTTGTTCTACATTTTCACCTTGTTGGGTAACG
GTGCTATTTTGGGTTTGATTTCCTTGGATTCCAGATTGCATACCCCAATGTATTTCTTCCTAA
GTCATTTGGCTGTTGTTGATATTGCTTACACCAGAAACACCGTTCCACAAATGTTGGCTAAC
TTGTTGCACCCTGCCAAGCCTATTAGCTTCGCCGGATGCATGACCCAAACCTTCTTATGTTT
ATCCTTCGGTCATTCCGAATGTTTGTTGTTGGTTTTGATGTCTTACGATAGATACGTTGCTA
TTTGTCATCCATTGAGATACTCCGTTATTATGACTTGGAGAGTTTGTATTACTTTGGCTGTTA
CTTCCTGGACTTGTGGTTCCTTGTTGGCTTTGGCTCATGTTGTTTTGATTTTGAGATTGCCA
TTCTCCGGCCCACATGAAATTAACCATTTCTTCTGTGAAATTTTGTCCGTTTTGAGATTGGC
TTGTGCTGATACTTGGTTGAACCAAGTTGTTATTTTCGCTGCTTGTGTCTTCTTCCTAGTTG
GTCCACCATCTTTGGTTTTGGTTTCTTACTCTCATATTTTGGCTGCTATTTTGAGAATACAAT
CTGGCGAAGGCAGAAGAAAGGCTTTCTCTACTTGTTCTTCTCATTTGTGTGTTGTTGGTTTG
TTCTTCGGTTCTGCTATTATTATGTACATGGCTCCAAAGTCTAGACATCCAGAAGAACAACA
AAAGGTTTTCTTCTTGTTCTACTCTTTCTTCAACCCAACTTTGAACCCATTGATTTACTCTTT
GAGAAACGGCGAAGTTAAGGGTGCTTTGAGGAGGGCTTTGGGCAAGGAATCTCATTCTTA
A

Homo sapiens OR2W3 (UniprotKB – Q7Z3T1)
ATGGATGGTACTAACGGTTCCACCCAAACCCACTTTATTCTATTGGGTTTCTCCGATAGACC
ACATTTGGAAAGAATTTTGTTCGTTGTTATTTTGATTGCTTACTTGTTGACCTTGGTTGGTAA
CACCACCATTATTTTGGTTTCCAGACTAGACCCACATTTGCATACCCCAATGTACTTCTTCT
TGGCTCATTTGTCCTTCTTGGATTTGTCTTTCACCACCTCCTCCATTCCACAATTGTTGTACA

ACTTGAACGGTTGTGATAAGACCATTTCCTACATGGGTTGTGCTATTCAATTGTTCTTGTTC
TTGGGTTTGGGTGGTGTTGAATGTTTGTTGTTGGCTGTTATGGCTTACGATAGATGTGTTG
CTATTTGTAAGCCATTGCATTACATGGTTATTATGAACCCAAGATTGTGTAGAGGTTTGGTT
TCTGTTACCTGGGGTTGTGGTGTCGCTAACTCTTTGGCTATGTCTCCAGTCACTCTACGTC
TACCAAGATGTGGCCATCATGAAGTTGATCATTTCTTGAGAGAAATGCCAGCTTTGATTAGA
ATGGCTTGTGTTTCTACTGTTGCTATTGAAGGTACTGTTTTCGTTTTGGCTGTTGGTGTTGT
TCTAAGTCCATTGGTTTTCATTTTGTTGTCTTACTCTTACATTGTTAGAGCTGTTCTACAAAT
TAGATCTGCTTCTGGTAGACAAAAGGCTTTCGGTACTTGTGGTTCTCATTTGACTGTTGTTT
CTTTGTTCTACGGTAACATTATTTACATGTACATGCAACCAGGTGCTTCTTCTTCTCAAGATC
AAGGTATGTTTCTAATGCTATTCTACAACATTGTTACTCCATTGTTGAACCCCCTAATCTACA
CTCTACGTAACCGTGAAGTCAAGGGTGCTTTGGGTAGATTGTTGTTGGGTAAGAGAGAACT
AGGCAAGGAATAA

Homo sapiens OR2T4 (UniprotKB - Q8NH00)
ATGGGTCTGTTCAGACAGAGCAAGCACCCAATGGCTAACATTACCTGGATGGCTAACCATA
CCGGTTGGAGCGATTTCATTCTGCTGGGTCTGTTCAGACAATCCAAGCATCCAGCTCTGCT
GTGTGTCGTCATTTTCGTTGTTTTCTTGATGGCTTTGTCCGGTAACGCTGTCCTAATCTTGT
TGATTCATTGTGATGCTCATTTGCATACCCCAATGTACTTCTTCATTTCCCAATTGTCCTTGA
TGGATATGGCTTACATTTCCGTTACCGTTCCAAAGATGTTGTTGGATCAAGTTATGGGTGTT
AACAAGATTTCCGCTCCAGAATGTGGTATGCAAATGTTCTTCTACGTTACCTTGGCTGGTTC
CGAGTTCTTTCTGTTGGCTACTATGGCTTACGATAGATACGTTGCTATTTGTCATCCATTGA
GATACCCAGTTTTGATGAACCATAGAGTTTGTTTGTTCTTGTCCTCCGGTTGTTGGTTCTTG
GGTTCCGTCGATGGTTTTACCTTCACCCCAATTACCATGACCTTCCCATTCAGAGGTTCCA
GAGAAATTCACCACTTCTTCTGTGAAGTTCCAGCTGTTTTGAACTTGTCCTGTTCCGATACT
TCCTTGTACGAAATTTTCATGTACTTGTGTTGTGTTTTGATGTTGTTGATTCCAGTTGTTATT
ATTTCTTCTTCTTACTTGTTGATTTTGTTGACTATTCATGGTATGAACTCTGCTGAAGGTAGA
AAGAAGGCTTTCGCTACTTGTTCTTCTCATTTGACTGTTGTTATTTTGTTCTACGGTGCTGCT
ATTTACACTTACATGTTGCCATCTTCTTACCATACTCCAGAGAAGGATATGATGGTTTCTGTT
TTCTACACTATTTTGACTCCAGTTGTTAACCCATTGATTTACTCTTTGCGTAACAAGGATGTT
ATGGGTGCTCTAAAGAAGATGTTGACTGTTGAACCAGCCTTCCAAAAGGCAATGGAATAA

Homo sapiens OR51B5 (UniprotKB - Q9H339)
ATGTCCAGCTCCGGCTCCTCCCATCCATTCTTGTTGACCGGCTTCCCAGGTTTGGAAGAAG
CTCATCATTGGATTTCCGTTTTCTTCTTGTTCATGTACATTTCCATTTTGTTCGGTAACGGTA
CCCTACTACTACTAATTAAGGAAGATCATAACTTGCATGAGCCTATGTATTTCTTCTTGGCT
ATGTTGGCTGCTACCGATTTGGGTTTGGCTTTGACCACCATGCCAACCGTTTTGGGTGTTT
TGTGGTTGGATCATAGAGAGATTGGTTCTGCCGCCTGTTTCTCCCAAGCCTACTTCATTCAT
TCCTTGTCCTTCTTGGAATCCGGCATTTTGTTGGCTATGGCTTACGATCGTTTCATTGCTAT
TTGTAACCCATTGAGATACACCTCCGTTCTAACTAATACTCGTGTTGTTAAGATTGGCTTGG
GTGTTTTGATGAGAGGTTTCGTTTCCGTTGTTCCACCAATTAGACCATTGTACTTCTTCTTG
TACTGTCATTCCCATGTTTTGTCCCATGCTTTCTGTTTGCATCAAGATGTTATTAAGTTGGCT
TGTGCTGATACTACTTTCAACAGATTGTACCCAGCTGTTTTGGTTGTTTTCATTTTCGTTTTG
GATTACTTGATCATCTTCATTAGTTACGTCCTAATTTTGAAGACTGTTTTGTCCATTGCTTCC
AGAGAAGAAAGAGCTAAGGCTTTGATTACTTGTGTTAGTCATATTTGTTGTGTTTTGGTTTT
CTACGTTACTGTTATTGGTCTAAGTTTGATTCATCGTTTCGGAAAACAGGTCCCACATATTG
TTCATTTGATTATGTCCTACGCTTACTTCCTATTCCCTCCACTAATGAACCCAATTACTTACT
CCGTTAAGACCAAGCAAATTCAAAACGCTATTTTGCATTTGTTCACCACCCATAGAATTGGT
ACCTAA

Homo sapiens OR2L13 (UniprotKB - Q8N349)
ATGGAGAAGTGGAATCATACATCAAACGACTTCATCCTTCTGGGTCTGTTACCTCCTAATCA
GACCGGTATCTTCCTGCTCTGTCTGATCATTCTGATCTTCTTCCTGGCAAGCGTTGGTAATA

GCGCAATGATTCATCTGATTCACGTTGATCCGCGTCTGCATACCCCGATGTATTTCCTGCT
GAGCCAGCTGAGCCTGATGGATCTGATGTATATTAGTACCACCGTTCCGAAGATGGCATAT
AATTTCTTAAGTGGTCAGAAGGGCATCAGTTTCTTAGGTTGTGGTGTTCAAAGTTTCTTCTT
CTTAACAATGGCCTGTAGTGAAGGTCTGTTACTCACCTCAATGGCCTACGATCGTTATTTAG
CAATTTGTCATTCATTATATTATCCTATTAGAATGTCAAAGATGATGTGCGTTAAGATGATTG
GTGGTTCTTGGACATTAGGTTCAATTAACAGTTTAGCACATACAGTGTTCGCATTACATATT
CCGTATTGTAGAAGTAGAGCAATTGATCATTTCTTCTGTGACGTTCCTGCTATGTTATTATTA
GCTTGTACCGATACCTGGGTTTACGAATATATGGTGTTCGTTAGTACCAGTCTGTTCTTGCT
GTTCCCATTCATTGGTATTACCAGTAGTTGTGGTCGTGTTCTGTTCGCAGTTTATCACATGC
ATAGCAAGGAAGGCAGAAAGAAGGCATTCACTACTATTAGCACCCATCTGACCGTTGTTAT
CTTCTATTACGCACCTTTCGTTTACACCTATCTGCGTCCTCGTAACCTGCGTAGTCCGGCA
GAAGATAAGATTCTGGCAGTGTTCTACACCATTCTGACCCCGATGCTGAACCCGATTATTTA
TAGCCTGCGTAATAAAGAAGTTCTGGGTGCAATGCGTCGTGTGTTCGGTATCTTCAGCTTC
CTGAAGGAATAA

Homo sapiens OR10S1 (UniprotKB - Q8NGN2)
ATGACCAGTCGTAGTGTTTGTGAAAAGATGACCATGACCACCGAAAATCCGAATCAGACAG
TTGTTAGCCATTTCTTCTTAGAAGGTTTACGTTATACAGCAAAGCATAGTAGTTTATTCTTCT
TATTATTCTTATTAATTTACAGTATTACAGTTGCAGGTAACTTATTAATTTTATTAACCGTTGG
TAGTGATAGCCATTTAAGTTTACCGATGTATCATTTCTTAGGTCATTTAAGTTTCTTAGACGC
CTGTTTAAGTACCGTTACAGTTCCGAAGGTTATGGCAGGTTTATTAACCTTAGACGGTAAAG
TTATTAGTTTCGAAGGTTGTGCAGTTCAGTTATATTGTTTCCACTTCTTAGCAAGTACAGAGT
GTTTCTTATATACAGTTATGGCATACGATCGTTATTTAGCAATCTGTCAGCCGTTACATTATC
CGGTTGCCATGAATCGTCGTATGTGTGCCGAAATGGCAGGTATTACCTGGGCAATTGGTG
CAACCCACGCAGCAATTCATACCAGCCTGACCTTTCGTCTGCTGTATTGTGGTCCGTGTCA
TATTGCATATTTCTTCTGTGATATTCCGCCGGTTCTGAAACTGGCCTGTACCGATACCACCA
TTAACGAACTGGTTATGTTAGCAAGTATTGGTATTGTTGCAGCAGGTTGTCTGATTCTGATT
GTTATTAGTTATATTTTCATTGTTGCAGCAGTTTTACGTATTCGTACAGCACAGGGTCGTCA
GCGTGCATTCAGTCCGTGTACAGCACAATTAACAGGTGTTTTATTATATTACGTTCCGCCG
GTTTGCATTTATTTACAACCGCGTAGCAGTGAAGCAGGTGCCGGTGCACCGGCAGTTTTCT
ATACCATTGTTACACCGATGCTGAATCCGTTTATTTATACATTACGTAATAAGGAGGTTAAA
CACGCATTACAACGTTTACTGTGTAGTAGTTTTCGTGAAAGCACCGCAGGTAGCCCGCCGC
CGTAA

Homo sapiens OR2A7 (UniprotKB - Q96R45)
ATGGGTGACAACATTACCAGCATTACCGAATTTCTGCTGCTGGGTTTTCCTGTTGGTCCAC
GCATCCAGATGCTGCTGTTTGGTCTGTTCAGCCTGTTCTACGTATTTACACTGCTGGGTAA
CGGTACCATTTTAGGTTTAATTAGCTTAGATAGCCGTTTACACGCACCTATGTACTTCTTCTT
AAGCCATTTAGCAGTTGTTGATATTGCATACGCTTGCAACACAGTTCCTCGTATGTTGGTTA
ACTTATTACATCCGGCTAAACCTATTAGCTTCGCAGGTCGTATGATGCAAACCTTCTTATTT
AGCACCTTCGCAGTTACCGAGTGTTTATTATTAGTTGTTATGAGCTACGATTTATACGTTGC
AATTTGCCATCCATTACGTTACTTAGCAATTATGACCTGGCGTGTTTGTATTACCTTAGCAG
TTACAAGCTGGACAACAGGTGTTCTGTTAAGTCTGATTCACTTAGTTTTATTATTACCTTTAC
CTTTCTGTCGTCCTCAAAAGATTTATCATTTCTTCTGTGAAATTTTAGCAGTTTTAAAGTTAG
CCTGTGCAGACACACACATTAACGAAAACATGGTTTTAGCAGGTGCAATTAGCGGTTTAGT
TGGTCCTTTAAGTACCATTGTTGTTAGCTACATGTGTATTCTGTGTGCAATTTTACAAATTCA
AAGCCGTGAAGTTCAGCGTAAGGCATTCTGCACCTGTTTCAGCCACCTGTGTGTTATTGGT
TTATTCTACGGTACAGCAATTATTATGTACGTTGGTCCGCGTTACGGTAACCCTAAGGAACA
AAAGAAGTACTTATTATTATTCCATAGCCTGTTTAACCCGATGCTGAACCCGCTGATTTGTA
GCCTGCGTAACAGCGAAGTTAAGAACACCCTGAAGCGCGTTCTGGGTGTTGAACGTGCAC
TGTAA

REFERENCES

Mukherjee, K., Bhattacharyya, S. & Peralta-Yahya, P. (2015) GPCR-Based Chemical Biosensors for Medium-Chain Fatty Acids. *ACS Synthetic Biology 4*, 1261-1269.

Livak, K. J. & Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods 25*, 402-408.