

Supplementary data

Supplementary data 1.

BLASTP search of ORs with MAYDRYVAIC, KAFSTCASH, and PMLNPFYIY motifs in rat, mouse and human

Supplementary data 2.

BLASTP search of ORs with VTPMLNPFYSLRNRDMK motif in rat, mouse and human

Table S1. Sequence of specific primers for ORs

Olr1493	Forward	5'-TCACGCGTATGAACAACAAAAGTGCATCACC-3'
	Reverse	5'-AAGCGGCCGCTACCATGGCAGAGAGATT-3'
Ofr394	Forward	5'-TCACGCGTATGAACAACAAAAGTGCAT-3'
	Reverse	5'-AAGCGGCCGCTAAGTAGAGACCAATAATTG-3'
OR1E1	Forward	5'-GCACGCGTATGATGGGACAAAATCAAACCA-3'
	Reverse	5'-GCGGCCGCTCAGAGAGAGAAGAAAGTTTT-3'
OR7A5	Forward	5'-GCACGCGTATGGAACCAAGGAAATGATAC-3'
	Reverse	5'-GCGGCCGCTCTCATGGGCACTTCTGAAA-3'
OR7D4	Forward	5'-TCGAGAATTCACGCGTATGGAAGCAGAAAACCTTAC-3'
	Reverse	5'-CTGCTCGAAGCGGCCGCTCATGGACAAGAATCGGC-3'
OR7C1	Forward	5'-GCACGCGTATGGAACAGGAAATCAAAC-3'
	Reverse	5'-GCGGCCGCTCTTATGAAAGTCTGCAGTGA-3'
OR1D2	Forward	5'-GCACGCGTATGGATGGAGGCAACCAGAG-3'
	Reverse	5'-GCG GCC GCT CTCATGTCAGCCTCTTAAAGT-3'
OR7E24	Forward	5'-CGACGCGTATGTCCTATTTTCCAATTC-3'
	Reverse	5'-ATAAGAATGCGGCCGCTATCCCATATAACAAAAGG-3'
OR4D1	Forward	5'-CGACGCGTATGGAACCAAGAACACC-3'
	Reverse	5'-ATAAGAATGCGGCCGCTTACTCCCTGCAAACTACTAG-3'
OR10J5	Forward	5'-TCACGCGTATGAAGAGAAAGAACTTAC-3'
	Reverse	5'-AAGCGGCCGCTAAGAAATATTTCTGCCACAAC-3'
OR2AG1	Forward	5'-GCACGCGTATGGAGCTCTGGAACCTCACCTTG-3'
	Reverse	5'-GCGCGGCCGCTAGAGCGTGGAGTGTGCTGGC-3'
OR1G1	Forward	5'-GCACGCGTATGGAGGGGAAAATCTGACCAGC-3'
	Reverse	5'-CCGCGGCCCTAAGGGGAATGAATTTCCG-3'

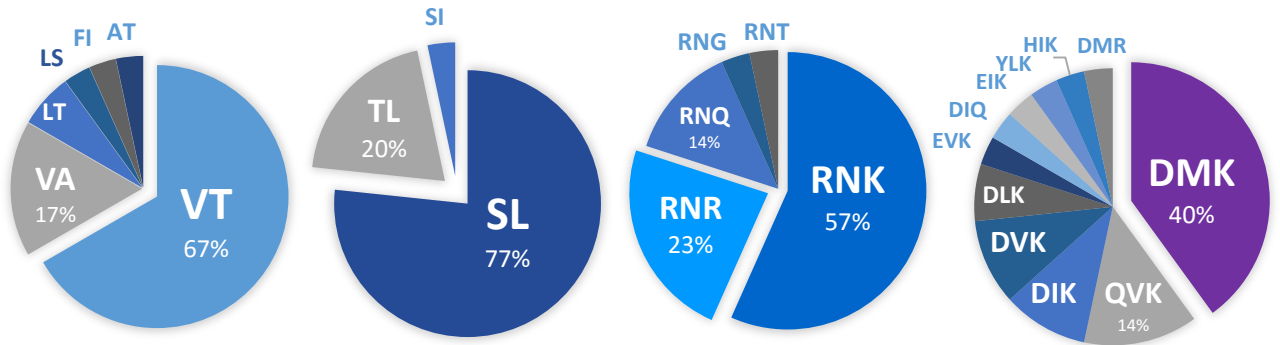
Table S1.

Following primers were used to obtain full length of genomic DNA of ORs from the tissues.

Supplementary data
Figure S1.

CONSERVED 18 AMINO ACID SEQUENCES: PMLNPFYI

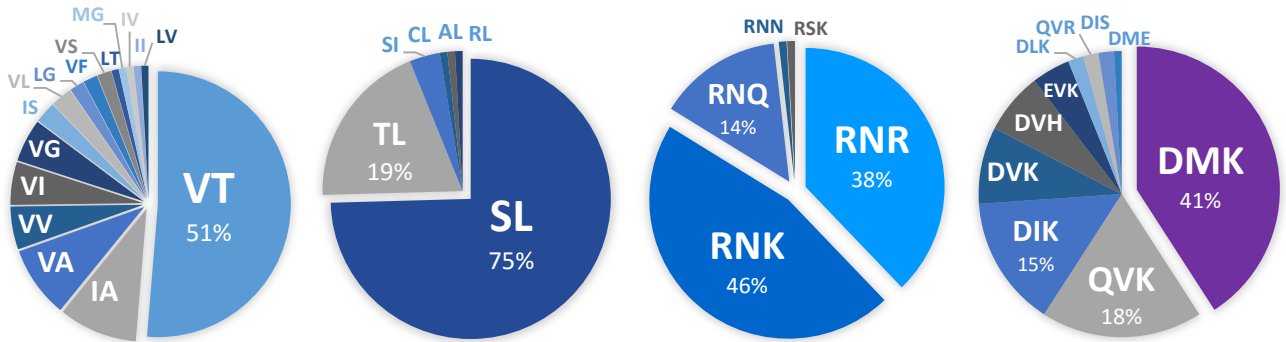
HUMAN



1) VTPMLNPFYISLRNKDMK

2) VTPMLNPFYISLRNRDMK

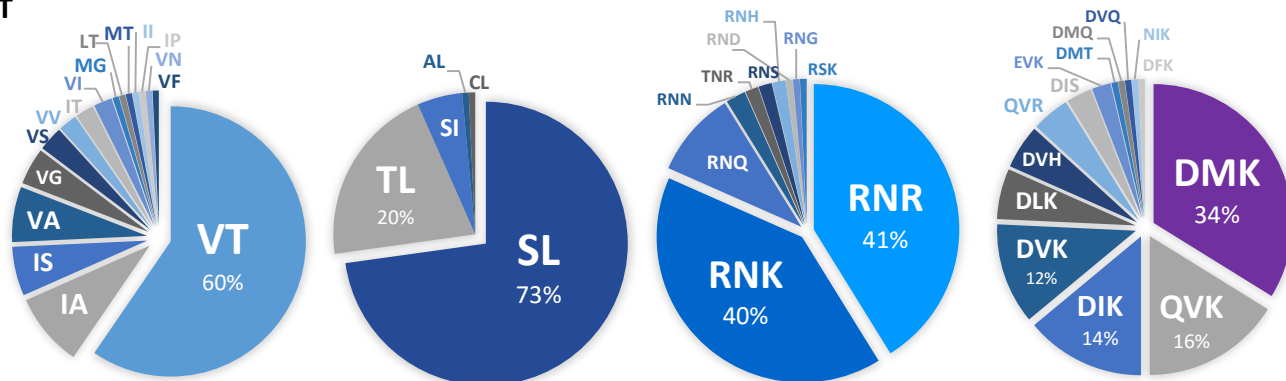
MOUSE



1) VTPMLNPFYISLRNKDMK

2) VTPMLNPFYISLRNRDMK

RAT



1) VTPMLNPFYISLRNRDMK

2) VTPMLNPFYISLRNKDMK

Figure S1. Analysis of conserved sequences among species

To identify the most identical amino acids in the TM7 among species; human, mouse, and rat, we analyzed amino acid sequences of ORs that have 100% identical PMLNPFYI motif from PLASTP searches. 30 ORs in human, 115 ORs in mice, and 136 ORs in rats have PMLNPFYI sequence in their TM7, and the rest of 10 amino acids were determined according to the ratio of occupation at each region. The human TM7 was consist of relatively various amino acids. In this reason, full conserved 18 amino acids were determined by sequence analysis of mouse and rat.

Supplementary data

Figure S2. Characterization of OR antibody

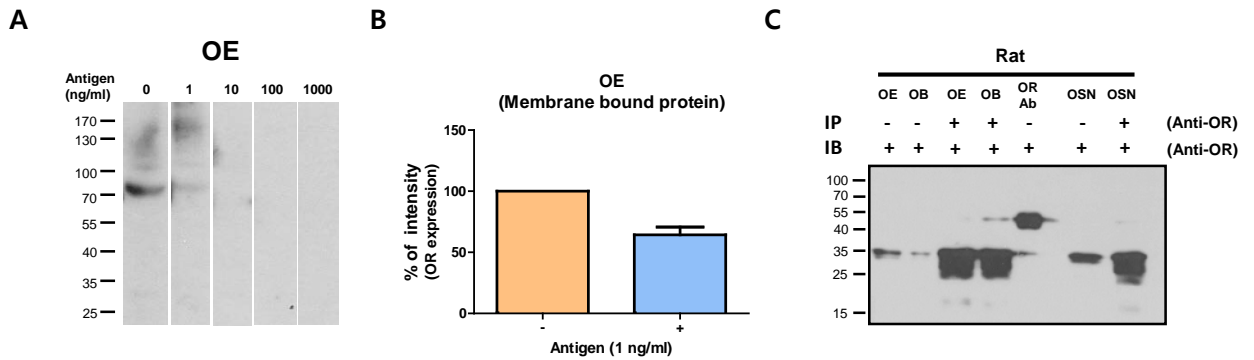
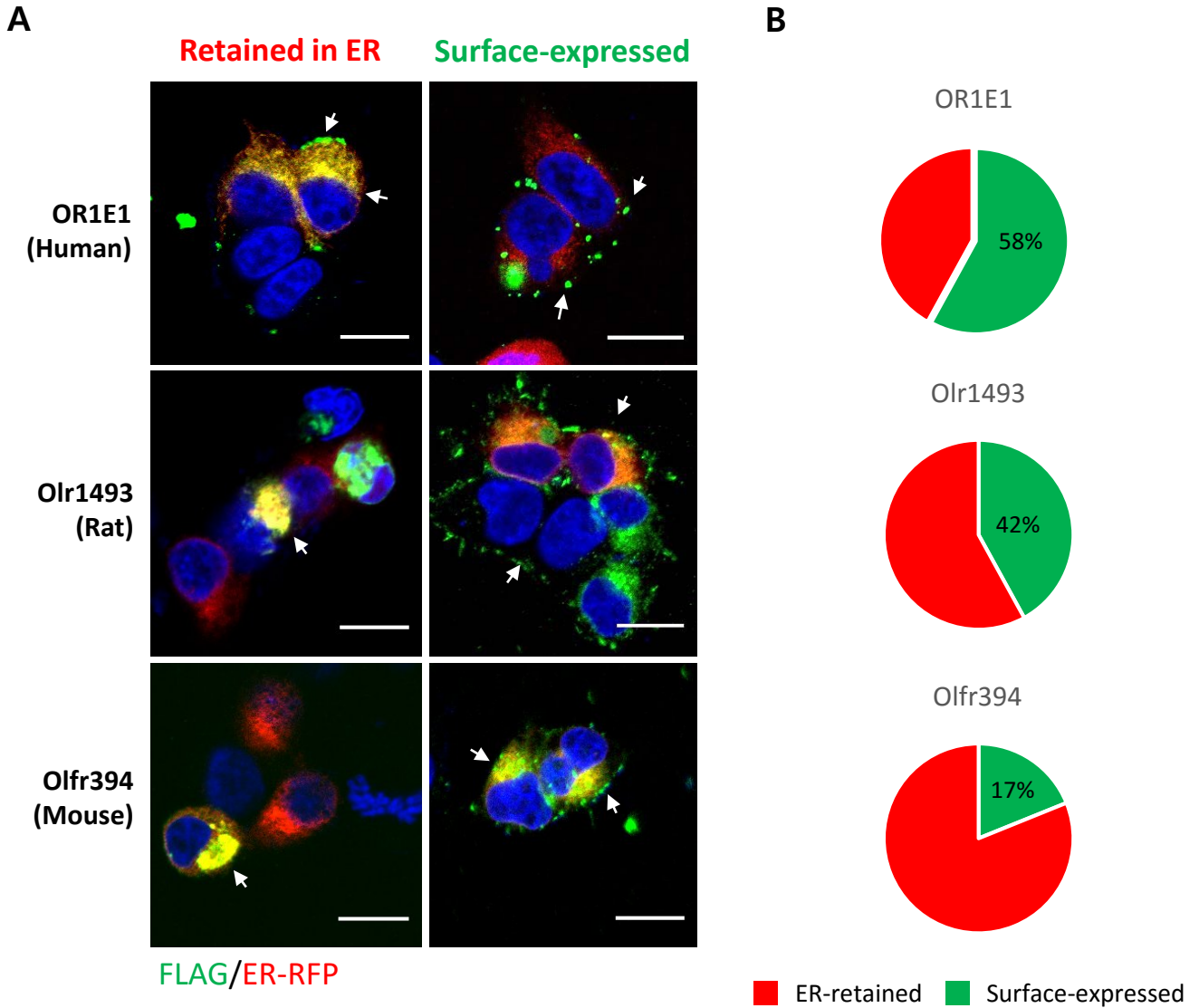


Figure S2. Characterization of OR antibody

Identify the specificity for ORs of sequence specific antibody **A**. Antigen pre blocking test in whole lysate of rat OE. Preincubation with blocking peptide at concentration of 1, 10 100, and 1000 ng/ml. The immunoreaction of OR antibody was completely blocked at the concentration of 10, 100 and 1000 ng/ml. **B**. Statistical analysis of peptide blocking at 1 ng/ml of concentration with the membrane fractionated protein of rat OE (n=3). The immunoreaction was consistently decreased after preincubation of peptide. **C**. Immunoprecipitation of OR antibody (against PMLNPFIY motif at TM7) with rat OE, OB and primary cultured olfactory sensory neurons. The immunoprecipitated OR proteins immunoblotted with OR antibody for confirmation. **The specific bands were observed at the 35 kDa from all samples. The IgG used as a negative control for IP assay with CAS-TM7 antibody.**

Supplementary data
Figure S3.



FigS3. Localization of ORs from three species (human, mouse and rat) in Hana3A cells.

A. Immunocytochemical images of ORs present in the ER in Hana3A cells. N-terminally FLAG tagged ORs were shown in every figure through green fluorescence labeling. Transiently expressed ER-RFP shown in red color and the localization of ORs in the ER and plasma membrane is discriminated with yellow and green color (The arrows indicate ORs in the ER and plasma membrane). **B.** Approximately, 58% of OR1E1 was in the plasma membrane and 42% ORs were retained ER. In case of Olr1493, 42% of ORs were plasma membrane and 58% of ORs were retained in the ER. The Olf394 is the OR that mostly retained in the ER (73%). (Scale bar; 15 μ M).