Receptor-transporting Protein 1 Short (RTP1S) Mediates Translocation and Activation of Odorant Receptors by Acting through Multiple Steps*1

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Background: RTP1S facilitates the translocation of odorant receptors (ORs).

Results: Different domains in RTP1S are important for different stages of OR trafficking, odorant-mediated responses, and interaction with ORs.

Conclusion: RTP1S mediates the trafficking and ligand-induced response of ORs by acting through multiple steps.

Significance: Probing the structure-function of RTP1S is crucial for understanding the mechanism of OR trafficking and activation.

Odorant receptor (OR) proteins are retained in the endoplasmic reticulum when heterologously expressed in cultured cells of non-olfactory origins. RTP1S is an accessory protein to mammalian ORs and facilitates their trafficking to the cell-surface membrane and ligand-induced responses in heterologous cells. The mechanism by which RTP1S promotes the functional expression of ORs remains poorly understood. To obtain a better understanding of the role(s) of RTP1S, we performed a series of structure-function analyses of RTP1S in HEK293T cells. By constructing RTP1S deletion and chimera series and subsequently introducing single-site mutations into the protein, we found the N terminus of RTP1S is important for the endoplasmic reticulum exit of ORs and that a middle region of RTP1S is important for OR trafficking from the Golgi to the membrane. Using sucrose gradient centrifugation, we found that the localization of RTP1S to the lipid raft microdomain is critical to the activation of ORs. Finally, in a protein-protein interaction analysis, we determined that the C terminus of RTP1S may be interacting with ORs. These findings provide new insights into the distinct roles of RTP1S in OR translocation and activation.

The mammalian olfactory system is capable of detecting and discriminating a large number of odors. The binding of odorant molecules to odorant receptors (ORs)2 located on the cell surface of olfactory sensory neurons (1) leads to the production of second messengers and the subsequent neuronal depolarization (2). The OR family contains as many as 1200 intact genes in mice and 400 in human, which are G-protein-coupled receptors with seven transmembrane (TM) domains (3–5). Understanding the fundamental properties of the olfactory system requires investigation of diverse OR proteins and a large number of odorant molecules (6), making heterologous OR expression systems, where ORs are robustly expressed to mimic their native states in olfactory sensory neurons, a compelling model to study OR ligand specificity and selectivity.

It is known that OR proteins are usually retained in the endoplasmic reticulum (ER) and subsequently degraded in cultured cell lines of non-olfactory origins (7, 8). Extensive efforts have been made to enhance the cell-surface expression of ORs in heterologous cells. It was first discovered that appending the first 20 amino acids of rhodopsin to the N terminus of ORs facilitates the surface expression of some ORs (9). Coexpression with other G-protein-coupled receptors is known to enhance the expression and function of certain ORs: the β2-adrenergic receptor dramatically increases the expression of a mouse OR (M71) in HEK293 cells (10), whereas the M₁ muscarinic receptor modulates the signaling transduction of ORs (11). The use of accessory factors was also shown to be effective for the expression of a subset of ORs. For example, ODR-4, a protein that promotes the trafficking of a chemosensory receptor in nematodes, has a small effect on the cell-surface expression of rat olfactory receptor U131 (12). On the other hand, Ric-8B, a putative guanine nucleotide exchange factor, can amplify sig-

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2 The abbreviations used are: OR, odorant receptor; TM, transmembrane; ER, endoplasmic reticulum; RTP, receptor-transporting protein; REEP, receptor expression-enhancing protein.
naling in the G_{olf} signaling cascade (13, 14). Given the limited effect of these accessory factors, it is likely that there are other conserved mechanisms for OR trafficking that are absent in heterologous cells. Saito et al. (15) first cloned receptor-trans- 
porting protein (RTP) and receptor expression-enhancing pro- 
tein (REEP) family members, of which RTP1, RTP2, and, to a 
lesser degree, REEP1 promote the functional expression of a 
large number of ORs in HEK293T cells. Subsequently, a shorter 
form of RTP1 (RTP1S) was discovered to promote the cell- 
surface expression of ORs even more efficiently than the origi- 
nal RTP1 (16). These findings provided the basis for a high-
throughput screening platform of the chemical selectivity of the 
mammalian OR repertoire (16–18).

As members of the putative chaperone protein families, 
RTPs and REEPs induce the functional expression of ORs; 
selected members also play important roles in other chemosen- 
sory organs. It has been reported that coexpression of RTP3 and 
RTP4 enhances the function of the human bitter taste receptor 
TAS2R (19), whereas REEP2 promotes the function of the 
sweet taste receptors TAS1R2 and TAS1R3 by recruiting them 
to the lipid raft microdomains on the plasma membrane (20). In 
addition, RTP4 increases the cell-surface expression of a het- 
erodimer of two non-chemosensory G-protein-coupled recep- 
tors, the μ and δ opioid receptors (21). Finally, RTP1 forms a 
complex with Homer to increase the surface expression and to 
promote the signal transduction of TRPC2 (transient receptor 
potential channel type 2) through interaction with TRPC2 (22).

It has been hypothesized that the trafficking of ORs from the 
ER to the plasma membrane involves at least two steps (12); 
however, the exact mechanism underlying the promotion of OR 
functional expression by RTP1S remains unknown, and the 
functional domains of RTP1S are unidentified. Here, we 
employed a structure-function analysis of RTP1S to examine its 
role as an OR chaperone. We show a multifaceted mode of 
function for RTP1S, which regulates the functional expression 
of ORs in multiple steps. We identified specific domains that 
are crucial for these steps and for interacting with ORs. These 
findings may provide clues to the function of RTP family 
members.

EXPERIMENTAL PROCEDURES

Chemicals—The odorant compounds octanoic acid and 
2-coumaranone were purchased from Sigma. Odorant solu-
tions were diluted to 1 M stock solutions and kept at −20 °C 
until used.

Plasmid Construction—Rho (MNGTEGPNFYVPSNAT-
GVVR), FLAG (DYKDDDDK), and HA (MYPYDVPDYA) tags 
were subcloned into the pCI mammalian expression vector as 
described previously (16). Olfr62 (mOR258-5) and Olfr599 
(mOR23-1) open reading frames were amplified from mouse 
genomic DNA and subcloned into pCI expression vectors con-
taining the primary antibody at room temperature for 45 min. 
The cells were then washed with phosphate-buffered saline, 
followed by incubation with secondary antibodies at room tem-
perature for 30 min. Anti-calnexin antibody (Abcam) was used 
for ER staining. For Golgi staining, cells were incubated with 
Alexa Fluor 488-conjugated wheat germ agglutinin (Invitro-
gen) for 20 min following incubation with a secondary anti-
body. Slides were mounted with Mowiol and visualized by con-
focal microscopy (Leica TCS SP5).

To quantify the percentages of OR or RTP1S colocalization 
with markers for ER or the Golgi apparatus, cells were double-
staing with the respective epitope tags for the OR or RTP 
(Cy3) and for ER or Golgi markers (Alexa Fluor 488). As 
assessed by a cotransfected blue fluorescent protein plasmid, 
we estimated the transfection efficiency of the system to be 
~40%; thus, for each experiment, we first counted 100 cells 
with Cy3 signals and subsequently recorded the number of cells 
in which Cy3 and Alexa Fluor 488 overlapped. To quantify the 
percentages of cells that were expressed on the cell surface, we 
cotransfected the cells with GFP, counted 100 cells with GFP, 
and recorded the numbers of cells that had punctate cell-surface 
signals.

In addition, we used the JACoP plug-in in ImageJ to calculate 
the Manders’ coefficients (which range from 0 to 1 and in which 
M1 and M2 of 0.8 and 0.2 for a red-green pair imply that 80% of 
red pixels colocalize with green, but only 20% of green pixels 
colocalize with red) for three selected images from each exper-
mint. M1 is the more meaningful coefficient in the context of 
this experiment, as the Cy3 signal depends on transfection 
efficiency.

FACS Analysis—FACS analysis was performed as described 
previously (16). Briefly, HEK293T cells were seeded in 35-mm 
dishes and then transfected with the same amount of plasmid 
DNA as used for immunocytochemistry. 2 ng of GFP expres-
sion vector was transfected per dish as a control for transfection 
efficiency. 24 h post-transfection, the cells were dissociated in 
Cellstripper™ (Corning cellgro) and transferred to a tube for 
incubation with the anti-rhodopsin antibody as described for 
immunocytochemistry and then with phycoerythrin-conju-
gated donkey anti-mouse IgG (Jackson ImmunoResearch La-
boratories, Inc.). Fluorescence was analyzed using a 
FACSCalibur (BD Biosciences).
Luciferase Assay—The Dual-Glo® luciferase assay system (Promega) was used for luciferase assay essentially as described previously (16). HEK293T cells were plated on poly-D-lysine-coated 96-well plates (Greiner). Plasmid DNAs of ORs and accessory factors were transfected using Lipofectamine 2000 (Invitrogen). In addition, two luciferase constructs were used, including a firefly luciferase gene driven by a 4× cAMP-response element (CRE-Luc) and a Renilla luciferase gene driven by a constitutively active SV40 promoter (pRL-SV40; Promega) that was used as an internal control for cell viability and transfection efficiency. When ORs are activated, the downstream second messenger cAMP is produced, and binding of cAMP to the cAMP-response element region leads to luciferase gene transcription and luminescence. For each 96-well plate, 1 μg of CRE-Luc, 1 μg of pRL-SV40, 5 μg of OR, and 2 μg total of all accessory proteins (RTP1S variants) or the pC1 empty vector were transfected. 24 h post-transfection, the medium was replaced with CD293 chemically defined medium (Invitrogen) and then incubated for 30 min at 37 °C. The medium was replaced with 25 μl of odorant solution diluted in CD293 and incubated for 4 h at 37 °C. We followed the manufacturer's protocols for measuring firefly luciferase (Luc) and Renilla luciferase (RL) activities. Luminescence was measured using a SpectraMax M5 plate reader (Molecular Devices). Normalized luciferase activity for RTP1S variants was calculated as (Luc/RLNL2)/Luc/RL(RTP1S) – Luc/RLmin, where Luc/RLNL2 represents the mean value from the replicate wells of a certain sample, and Luc/RLmin represents the mean value from the minimal response in the experiment. Student's t test was used to compare the responses of RTP1S variants with that of RTP1S. Bonferroni corrections were applied to correct for problems associated with multiple comparisons.

Lipid Raft Fractionation—HEK293T cells grown to near confluence in four 100-mm dishes were transfected with N-terminally FLAG-tagged Olfr599 and C-terminally HA-tagged RTP1S or TM modifications. Lipid raft fractionation was performed as described in a published protocol (23). Anti-caveolin-1 (Cell Signaling) and anti-transferrin receptor (CD71; Abnova) antibodies were used for probing lipid rafts and nonrafts in Western blotting, respectively.

Immunoprecipitation—HEK293T cells in 60-mm dishes were transfected with N-terminally FLAG-tagged OR, C-terminally HA-tagged RTP1S variants, and/or the negative control, C-terminally HA-tagged ANP32B. 16 h after transfection, cells were lysed with lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, 0.5 mM PMSF, and 1% protease inhibitor mixture). The lysates were incubated with anti-FLAG M2 affinity gel (Sigma) or anti-HA affinity matrix (Roche Applied Science) for 4 h at 4 °C and washed with lysis buffer. The bound proteins were eluted by incubation with 1× SDS sample buffer at room temperature for 2 h and at −80 °C overnight and then subjected to Western blotting. The intensity of the bands in each of the Western blots was quantified using Quantity One 1-D analysis software (Bio-Rad). The relative levels of interactions between FLAG-tagged OR and HA-tagged RTP1S deletion mutants and chimeras were calculated as the normalized ratios of immunoprecipitated proteins over protein lysis, blotted with both antibodies.

Multiple Steps for OR Translocation and Activation

Western Blotting—Proteins from immunoprecipitation or lipid raft fractionation were resolved by SDS-PAGE using a mini-gel apparatus (Bio-Rad) and subsequently electrophoretically transferred onto nitrocellulose membranes. The membranes were blocked with blocking solution (Tris-buffered saline with 5% nonfat milk and 0.1% Tween 20) for 2 h at room temperature, incubated with the primary antibodies (anti-DYKDAPAP (FLAG; Cell Signaling) or anti-HA), dissolved in blocking solution overnight at 4 °C, and finally incubated with horseradish peroxidase-conjugated secondary antibodies (Cell Signaling) dissolved in blocking solution for 1.5 h at room temperature. The signals were detected using Immobilon Western chemiluminescent HRP substrate (Millipore) according to the manufacturer's instructions.

RESULTS

RTP1S N-terminal Domain and Middle Segments Are Crucial, whereas TM Domain Is Not Required for Functional Expression of ORs—Scanning the amino acid sequence and the predicted secondary structure of RTP1S revealed very few known motifs or domains. To pinpoint the regions in RTP1S that are important for OR expression and function, we constructed a series of sequence modifications of RTP1S, including N- and C-terminal deletions, in which progressively longer fragments of up to 70 amino acids were truncated from both ends of the protein, and RTP1/RTP4 chimera, in which 20-amino acid-long fragments in the middle of RTP1S were replaced with the corresponding fragments in RTP4 (Fig. 1A and supplemental Fig. S1), which is not expressed in the olfactory epithelium and does not promote OR functional expression (15). We intercalated the deletions and the chimeras so that all regions in the middle of the protein that were not covered by deletions were accounted for by the chimeras. The deletion mutants or chimeras, along with the mouse OR Olfr599, were transiently transfected into HEK293T cells. We then assayed for the cell-surface expression of Olfr599 by flow cytometry (Fig. 1B). We found that the OR could still be expressed on the cell surface when coexpressed with N1, C2, C3, C4, Ch1, and Ch7, but had little (Ch6) or no surface expression with the other variants, indicating that certain parts of the N terminus may be more important for RTP1S function. The activation of Olfr599 upon exposure to the ligand octanoic acid, as measured by cAMP-mediated luciferase reporter gene assays, was detected with the same constructs as those showing OR cell-surface expression (Fig. 1C). Notably, the C3 deletion is a shorter form of RTP1S missing only the TM domain. Olfr599 cotransfected with C3 retained at least 50% of the cell-surface expression and activation compared with RTP1S, suggesting that the TM domain is not essential for RTP1S function as an OR accessory protein. We also repeated the experiments using another mouse OR, Olfr62, and obtained similar results, corroborating that reduced OR response is always paralleled by loss of OR surface expression (supplemental Fig. S2).

Trp-60, Trp-62, and CXXC Motifs of RTP1S Are Essential for OR Functional Expression—To further identify the residues that are key to RTP1S function, we carried out site-directed mutagenesis on 65 amino acids in the regions that abolished RTP1S function, namely N2, C1, and Ch2–Ch6 (blue in
supplemental Fig. S1 and supplemental Table S1). This included all amino acid residues that are conserved in RTP1S and RTP2 but not in RTP3 and RTP4, and these were mutated to the corresponding residues in RTP4. We then measured the cell-surface expression and activation of Olfr599 when cotransfected with each of the RTP1S mutants (supplemental Fig. S3). The results showed that the W60R (M14) and W62C (M15) mutations led to the complete loss of OR surface expression and function (p < 0.0065) (Fig. 2, A and B, and supplemental Fig. S3), whereas mutations of some of the other residues (A52L (M12), K93G (M27), S107P (M36), S108K (M37), and M109F (M38)) caused partial disruption of RTP1S function (supplemental Fig. S3).

We next investigated the significance of a known CXXC motif that is seen three times throughout RTP1S and is conserved among all four members of the RTP family. We carried out site-directed double-residue mutagenesis of the three conserved pairs of cysteine residues (C58S and C61S, C96S and C99S, and C155S and C158S). As expected, we found that these mutations totally abolished both the cell-surface expression and function of the OR (Fig. 2, C and D).

RTP1S N Terminus Is Important for ER Exit of OR, and RTP1S Middle Segment Is Important for OR Trafficking from Golgi to Membrane—It has long been known that heterologously expressed ORs are retained in the ER (7), a problem that can be readily resolved when the OR and RTP1/RTP2 are cotransfected (15). In a previous study, we found that the majority of the signals for the OR and RTP1S colocalized (16), suggesting a possible association between the two proteins in intracellular compartments. Here, we used both live-cell staining and permeabilized staining to detect the subcellular localization of both Olfr599 and RTP1S in combinations with the markers for the ER and Golgi apparatus. We found that both Olfr599 and RTP1S colocalized with the ER marker calnexin when transfected on their own; both are found on the cell surface (15) and also colocalized with the Golgi marker when the two were cotransfected (Fig. 3A).

We next investigated the specific regions in RTP1S that are important for the trafficking of the OR using the deletion and chimera series. First, for all RTP1S deletion mutants and chimeras, RTP1S and Olfr599 immunofluorescence was almost always colocalized, reinforcing the reciprocal trafficking of the two molecules (Fig. 3, B and C, and supplemental Tables S2 and S3). Second, we found that, consistent with the cell-surface expression of the OR shown in Fig. 1B, chimeras Ch1 and Ch7, as well as most of the C-terminal deletion mutants, were presented at the cell-surface as punctate signals. In contrast, when Ch2–Ch5 were transfected with the OR, Olfr599 reached the
Golgi but not the cell surface, whereas when cotransfected with deletion mutants N2 and C1, Olfr599 was found exclusively in the ER (Fig. 3, B and C). Interestingly, when cotransfected with Olfr599, the immunofluorescence of C2 and C3, which are C-terminal deletions lacking the TM domain, accumulated near the intracellular surface of the plasma membrane (supplemental Fig. S4), indicating that the C-terminal domain, including the TM domain, is not required for the Golgi exit of RTP1S or the OR.

**Lipid Raft Fractions of RTP1S and Activation of ORs**—The results from the deletion series showed that the RTP1S TM domain is not required for the function of RTP1S. To explore the specific function of the TM domain, whether it is the mere anchoring of RTP1S to the membrane or other functions, we appended a series of lipid modifications to the C3 deletion, namely the signals for geranylgeranylation (GCLLL), farnesylation (GCVVM), and palmitoylation (KQFRNCMLTTICCG-KNPLG, the last 19 amino acids from human rhodopsin Rho19), all of which would aid in the insertion of the protein into the plasma membrane (Fig. 4A). We found that lipid-modified RTP1S retained the ability to promote the cell-surface expression of OR (Fig. 4B). However, in contrast to other experiments in which OR cell-surface expression and odor-mediated responses were always in agreement with each other, Olfr599 cotransfected with lipid-modified RTP1S exhibited severely impaired activation by its cognate ligand (Fig. 4C).

To address the apparent discrepancy in OR expression and function, we performed sucrose gradient centrifugation to probe the microdomain localization of RTP1S and Olfr599 on the plasma membrane. It is known that the addition of lipid modifications may not be sufficient for localization to the lipid rafts, and certain modifications, such as prenylation, are less likely to be found in the rafts due to the bulky branched nature of their lipid moiety (24, 25). If RTP1S plays an integral role in

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**FIGURE 3.** Altering segments in RTP1S impacts cell-surface expression and subcellular localization of OR and RTP1S. Live-cell and permeabilized staining was performed to examine the cell-surface expression and subcellular localization of the OR and RTP1S deletion mutants and chimeras. A, subcellular localization of the OR and RTP1S when they were transfected alone or cotransfected. B, subcellular localization of Olfr599 when cotransfected with representative RTP1S variants, including N2 and Ch2. Calnexin is a marker for the ER, whereas wheat germ agglutinin marks the trans-Golgi apparatus. Blue signals in the lower panel are DAPI nuclear staining. C, quantification of OR and RTP1S subcellular localization when Olfr599 and RTP1S were transfected alone and when Olfr599 was cotransfected with various RTP1S deletion mutants and chimeras. Large dots represent >30% colocalization with markers for the ER or Golgi, and small dots represent 10–30% with these markers. The actual numerical values (mean ± S.D., n = 3) that were converted to dots are shown in supplemental Table S2. N/A, not applicable.
OR activation, mislocalization of RTP1S could affect the odorant-mediated response of the OR. Indeed, although wild-type RTP1S was clearly detected in both the lipid raft and non-raft microdomain fractions, all lipid-modified RTP1S variants were found exclusively in the non-raft fractions. Interestingly, ORs were consistently localized to the lipid raft fractions regardless of the RTP1S variants transfected (Fig. 4D).

**OR Interacts with C-terminal Half of RTP1S**—Finally, we investigated which regions of RTP1S mediate stable protein-protein interaction with ORs. We cotransfected RTP1S deletion mutants and chimeras with Olfr599 and used co-immunoprecipitation to assess protein-protein interaction. Despite the fact that the RTP1S N-terminal deletion mutants were, in general, expressed to a lesser degree than the C-terminal deletion mutants (Fig. 5, A (left panel) and B, and supplemental Fig. S5), Olfr599 always co-immunoprecipitated with the RTP1S N-terminal deletion mutants and vice versa, but not in the case of C-terminal deletion mutants (Fig. 5A, left panel). The same trend was seen for the RTP1S chimera series (Fig. 5, A (right panel) and B, and supplemental Fig. S5). Therefore, our results demonstrated that the OR physically interacted primarily with the C-terminal half of RTP1S.

**DISCUSSION**

Past successes in the heterologous expression of ORs highlighted the importance of a group of specific accessory proteins that regulate the trafficking of ORs to the plasma membrane. Of these, RTP family members appear to most dramatically improve the cell-surface expression and function of many ORs. In this study, we identified key regions conferring OR functional expression. Specifically, the 17 amino acids in the RTP1S N-terminal domain are responsible for the ER exit of the OR, whereas a region of ~80 amino acids in the middle of RTP1S is required for the OR to pass through the Golgi apparatus. We also showed that the identity of the TM domain is critical for the localization of RTP1S to the lipid rafts and consequently for the OR to signal at the cell surface.

What is the exact role of RTP1S in OR maturation, trafficking, and function? It is plausible to hypothesize that multiple steps are required for OR trafficking. In other words, the OR has to overcome several barriers along the secretory pathway to the membrane, and different domains of RTP1S are responsible for the OR to pass these steps. Our data are highly supportive of a reciprocal trafficking mechanism in which RTP1S and the OR rely on each other for trafficking to the membrane. As proposed in a previous study (15), RTPs may contribute to the folding and trafficking of the OR or function as co-receptors to the OR. Our data showed a possible sequential functionality of RTP1S in all of these steps. First, the RTP1S N-terminal domain stabilizes the OR by ensuring its correct folding in the ER, which is in turn required for successful ER exit. Next, RTP1S aids the OR in its vesicular transportation to the membrane. Our results showed some RTP1S/RTP4 chimeric proteins that are retained in the Golgi, and this is consistent with the notion that Golgi exit is another critical checkpoint in OR trafficking.

Once the OR is at the membrane, RTPs could function as co-receptors to the OR to carry out the proper signal transduction of ORs by localizing to the lipid raft microdomains where ORs are located. Our data showed that, when cotransfected with the lipid-modified versions of RTP1S, the association between the OR and RTP1S was lost at the cell surface, contributing to the loss of OR function. This is in contrast to a previous report in which the REEP2 accessory protein promoted the localization of sweet taste receptors to lipid rafts (20). It was proposed that lipid rafts act as a platform on which molecules in the G-protein-coupled receptor signal transduction pathway are brought to close proximity to selectively associate with each other, activating the downstream signal transduction cascade (26). We thus hypothesize that, in addition to its chaperone activities, the presence of RTP1S in the lipid rafts side by side with the OR is required to form a functional receptor complex with the OR. An analogous example would be the receptor activity-modifying proteins, which complex with the calcitonin receptor and calcitonin-like receptor to give receptor complexes with divergent ligand specificities (27). Due to its short extracellular C-terminal domain, it is unlikely that RTP1S contributes to OR ligand binding, but it may still modulate OR pharmacology through orthosteric or allosteric mechanisms that may eventually contribute to downstream signaling effi-
ciency. Notably, wild-type RTP1S is also found in non-raft/cyttoplasmic fractions, whereas the OR is found exclusively in lipid raft fractions. The exact role of non-raft RTP1S remains to be investigated.

Finally, the three pairs of cysteine residues conserved among RTP family members point to a possible common secondary structure. The loss-of-function mutations at these sites could be a result of structural perturbation, and this is consistent with the notion that the CXXC motif may contribute to protein conformation through the formation of disulfide bonds. For example, the CXXC motif in the protein-disulfide isomerase, a member of the thioredoxin family of chaperone proteins, can catalyze the formation of disulfide bonds in vitro (28). Interestingly, the two key tryptophan residues, Trp-60 and Trp-62, may be important for the structural integrity of RTP1S deletion mutants and chimeras with anti-FLAG antibody. Fourth panels, co-immunoprecipitation of Olfr599 with anti-HA antibody when transfected with certain RTP1S deletion mutants and chimeras. Refer to supplemental Fig. S5 for the original blots.

**FIGURE 5.** **RTP1S C-terminal half is important for protein-protein interaction with ORs.** A, left panel, interactions between Olfr599 and RTP1S deletion mutants. Right panel, interactions between Olfr599 and RTP1S chimeras. First and second panels, protein lysates of HEK293T cells transfected with FLAG-tagged Olfr599 and/or HA-tagged RTP1S deletion mutants and chimeras and blotted with anti-HA or anti-FLAG antibody. Third panels, co-immunoprecipitation of certain RTP1S deletion mutants and chimeras with anti-FLAG antibody.

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