Article:
Sato, K, Mogi, C, Mighell, AJ orcid.org/0000-0002-9624-6923 et al. (1 more author) (2020) A missense mutation of Leu74Pro of OGR1 found in familial amelogenesis imperfecta actually causes the loss of the pH-sensing mechanism. Biochemical and Biophysical Research Communications, 526 (4). pp. 920-926. ISSN 0006-291X

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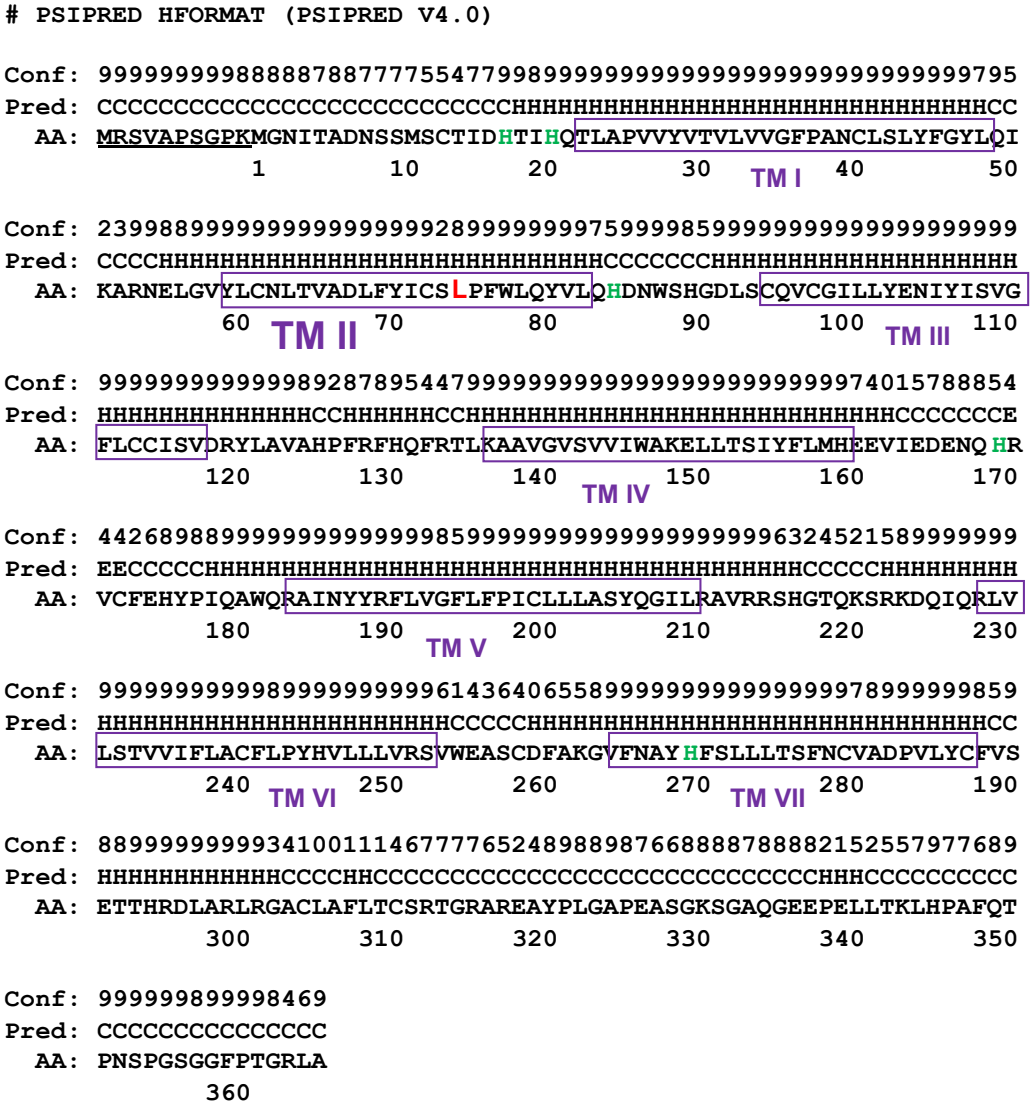
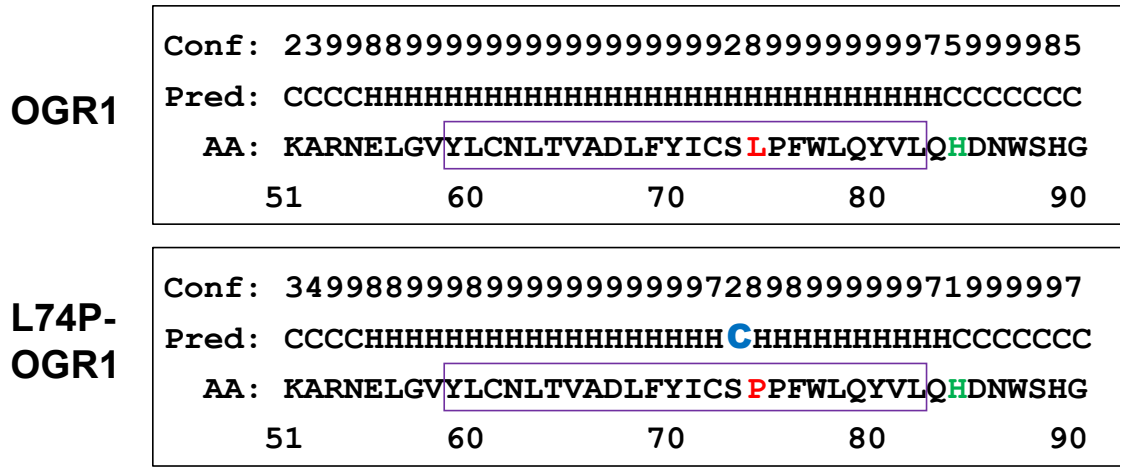
Supplementary Material

Supplementary Table 1. Primers for PCR

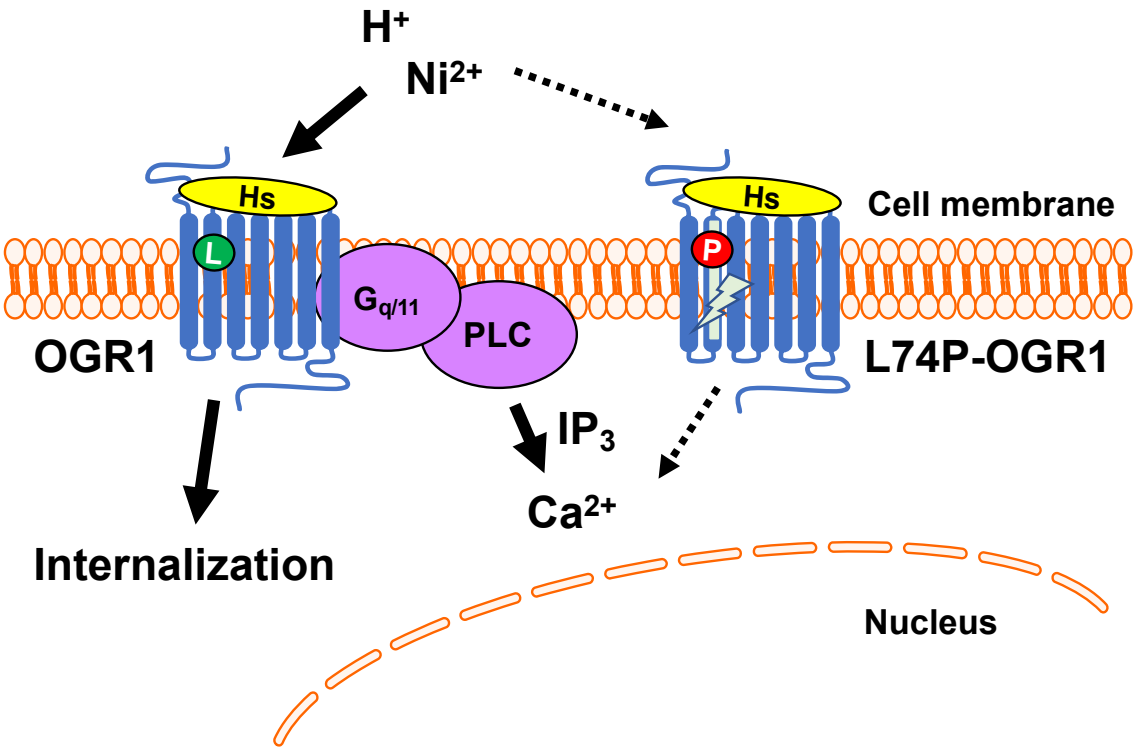
Name	Sequence
GPR68-1	aagcttccaccATGAGGAGTGTGGCCCCT
GPR68-2	gaattcCTAGGCCAACCTGCCCGTGGGGA
GPR68-2HA	gaattcCTAAGCGTAATCTGGAACATCGTATGGGTAGGCCAACCTG CCCGTGGGGA
pcDNA3-T7	TAATACGACTCACTATAGGG
pcDNA3-BGH	TAGAAGGCACAGTCGAGGCT
GAPDH-1	TGATGACATCAAGAAGGTGGTGAAG
GAPDH-2	TCCTTGGAGGCCATGTAGGCCAT



Supplementary Fig. 1. Prediction of secondary structure for human OGR1 by PSIPRED V4.0 (The PSIPRED Protein Sequence Analysis Workbench at http://bioinf.cs.ucl.ac.uk/psipred/). Abbreviations used were: Conf, configuration values (0-9); Pred, prediction of secondary structure (H, helix; E, strand; C, coil) as described in The PSIPRED Protein Sequence Analysis Workbench (http://bioinf.cs.ucl.ac.uk/psipred/). The transmembrane domains (TMI-TMVII) are boxed in purple, and the histidine residues that have been shown to be involved in proton-sensing in OGR1 (H17, H20, H84, H169, and H269) are shown in green letter as reported previously [2, 8]. A notable leucine (L74) in TMII is shown in red letter. A putative amino acid sequence (10 residues) are underlined in the N-terminal of the OGR1 (GenBank: NM_003485).



Supplementary Fig. 2. Prediction of secondary structure around second transmembrane domain in human OGR1 by PSIPRED V4.0. The positions of L74P and H84 are shown in red and green letters, respectively. The predicted secondary structure around L74 and TMII (boxed in purple) for WT and L74P are affected by the substitutions as shown in blue letters. Thus, the mutations in OGR1 may become damaged in its receptor functions.



Supplementary Fig. 3. The postulated Ca^{2+} signaling by OGR1 and different modes of WT and L74P receptors. Extracellular protons or Ni^{2+} induce OGR1 activation through histidine residues and subsequent activation of G protein/Ca^{2+} signaling pathways. Similar to other GPCRs, OGR1 is internalized in response to the extracellular stimuli. The L74P receptor is distributed in the plasma membranes without sensing extracellular stimuli, and dose not influence anything on the proton/intrinsic OGR1-receptor signaling. See text more detail.