

Functional characterization of the Venus Flytrap domain of the human TAS1R2 sweet taste receptor

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Supplementary

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      10      20      30      40      50      60
MGSSHHHHHH SGLVPRGSH MAENSDFYLP GDYLLGGLFS LHAMRGIHV INFLQVPMCK
      70      80      90     100     110     120
EYEVKVIQYN LMQAMRFAVE EINNDSLLP GVLLGYEIVD VCYISNNVQP VLYFLAHEDN
     130     140     150     160     170     180
LLPIQEDYSN YISRVVAVIG PDNSESVM TV ANFLSLFLLP QITYSAISDE LRDKVRFPAL
     190     200     210     220     230     240
LRTTPSADHH IEAMVQLMLH FRWNWIIIVLV SSDTYGRDNG QLLGERVARR DICIAFQETI
     250     260     270     280     290     300
PTLQPNQNT SEERQLVTI VDKLQOSTAR VVVVFSPLT LYHFFNEVLR QNFTGAVWIA
     310     320     330     340     350     360
SESWAIDPVL HNLTELRLHG TFLGITIQSV PIPGFSEFRE WGPQAGPPPL SRTSQSYTCN
     370     380     390     400     410     420
QECDNCLNAT LSFNTILRLS GERVVYSVYS AVYAVAHALH SLLGCDRSTC TRRVVYPWQL
     430     440     450     460     470     480
LEEIWKVNFT LLDHQIFDDP OGDVALHLEI VQWQWDRSQN PFQSVASYYP LQRLKNIQD
     490
ISWHTINNTI PMSHHHHHH
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Figure S1. Amino acid sequence of hTAS1R2-VFT. The hTAS1R2-VFT sequence (Ala22-Ser493) is shown with a green background. Numbers refer to amino acid residues of hTAS1R2 (signal peptide: 1–19). His-Tags and thrombin cleavage sites are shown with yellow and pink backgrounds, respectively.

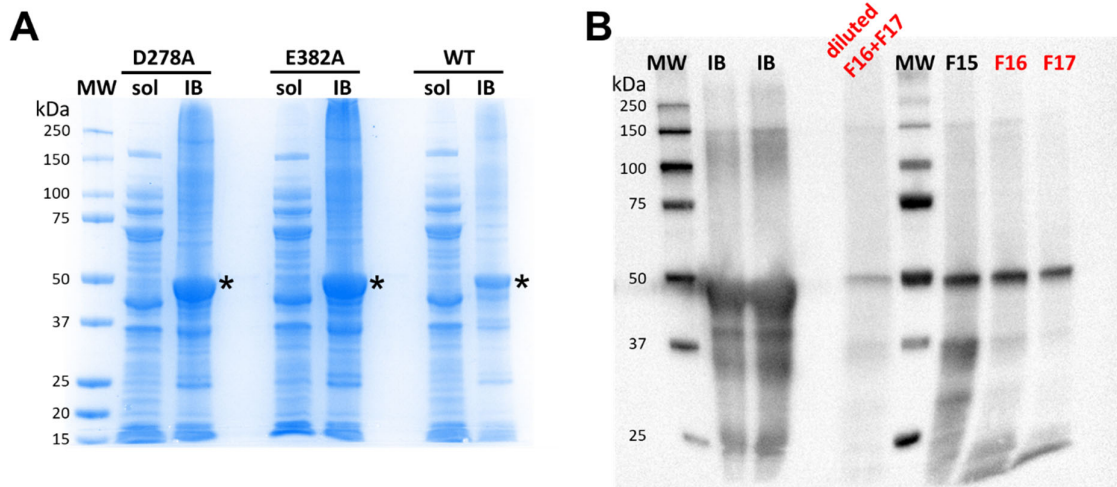


Figure S2. SDS-PAGE and western blot analysis of hTAS1R2-VFT wild-type (WT) and mutant hTAS1R2-VFT-D278A and hTAS1R2-VFT-D382A expressed in *E. coli* using the pET28 vector. (A) The proteins from the soluble fraction (sol) or insoluble fraction, known as inclusion bodies (IB), were separated by 10% acrylamide gels and stained with Coomassie blue. MW: molecular weight markers. Position of hTAS1R2-VFT is indicated by an asterisk. (B) Western blot analysis using mouse anti-His primary antibody and goat anti-mouse horseradish peroxidase conjugated secondary antibody revealed the presence of hTAS1R2-VFT wild-type in inclusion bodies (IB) (Lanes 1 and 2) and in the hTAS1R2-VFT wild-type purified fraction eluted by size exclusion chromatography from Figure 2.

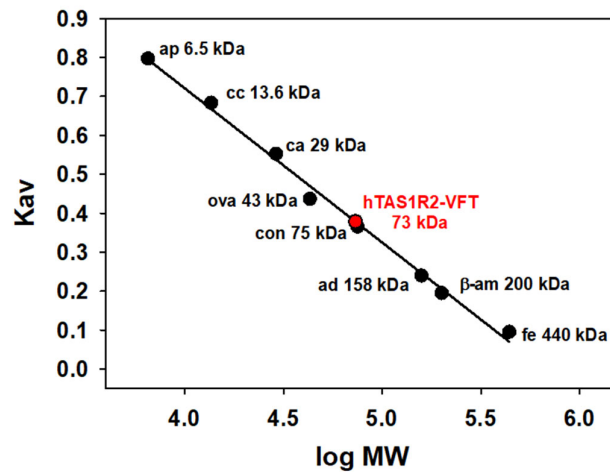


Figure S3. Gel filtration chromatography of purified hTAS1R2-VFT. The calibration curve for the HiLoad 16/600 Superdex 200 preparative grade column (GE Healthcare) was established with ferritin (fe, 440 kDa), β-amylase (β-am, 200 kDa), alcohol dehydrogenase (ad, 158 kDa), conalbumin (con, 75 kDa), ovalbumin (ova, 43 kDa), carbonic anhydrase (ca, 29 kDa), cytochrome c (cc, 13.6 kDa) and aprotinin (ap, 6.5 kDa). The estimated molecular mass of hTAS1R2-VFT is 73 kDa.

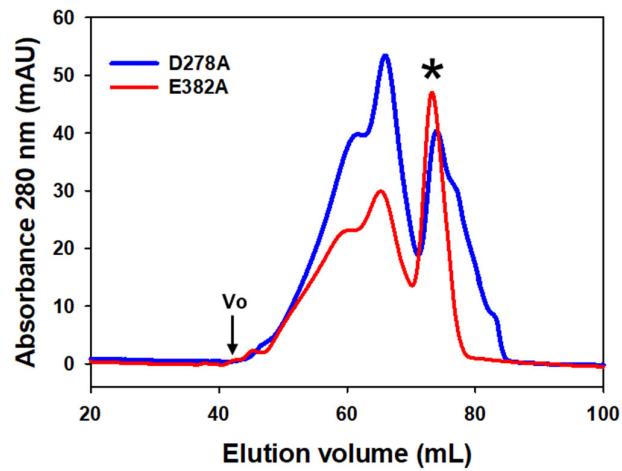


Figure S4. Preparative gel filtration chromatography of refolded hTAS1R2-VFT-D278A (solid blue line) and hTAS1R2-VFT-E382A (solid red line). The arrow indicates the position of the void volume (V_o), and the asterisk indicates the peak containing purified hTAS1R2-VFT. Gel filtration was performed using HiLoad 16/600 Superdex 200 preparative grade equilibrated with 50 mM Tris-HCl pH 8, 150 mM NaCl, 0.5 mM DDM, and 1 mM DTT at 1 mL/min.

Table S1. Secondary structure evaluation of the refolded hTAS1R2-VFT and mutants D278A and E382A.

Protein	α -helices (%)	B-sheets (%)
hTAS1R2-VFT	72	9
hTAS1R2-VFT-D278A	66	13
hTAS1R2-VFT-E382A	69	13