



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

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Supplementary

10	20	30	40	50	60
MGSSHHHHHH	SSGLVPRGSH	MAENSDFYLP	GDYLLGGLFS	LHANMRGIVH	INFLQVPMCK
70	80	90	100	110	120
EYEVKIVIGYN	LMQAMRFAVE	EINNDSSLLP	GVLLGYEIVD	VCYISNNVQP	VLYFLAHEDN
130	140	150	160	170	180
LLPIQEDYSN	YISRVVAVIG	PDNSESVMTV	ANFLSLFLP	QITYSAISDE	LRDKVRFPAL
190	200	210	220	230	240
LRTTPSADHH	TEAMVQIMLH	FRWNWIIIVL	SSDTYGRDNG	QLLGERVARR	DICIAFOETI
250	260	270	280	290	300
PTLQPNQNM	SEERQRLVTI	VDRLOQSTAR	VVVVFSPDLT	LYHFFNEVLR	QNFTGAVWIA
310	320	330	340	350	360
SESWAIDPVL	HNLTELRLH	TFLGITIQSV	PIPGFSEFRE	WGPQAGPPPL	SRTSQSYTCN
370	380	390	400	410	420
QECDNCLNAT	LSFNTILRLS	GERVVYSVYS	AVYAVAHALH	SLLGCDRSTC	TRRVVYPWQL
430	440	450	460	470	480
LEEIWKVNFT	LLDHQIFDP	OGDVALHLEI	VQWQWDRSQN	PFQSVASYYP	LQRQLKNIQD
490					
ISWHTINNTI	PMSHHHHHH				

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.

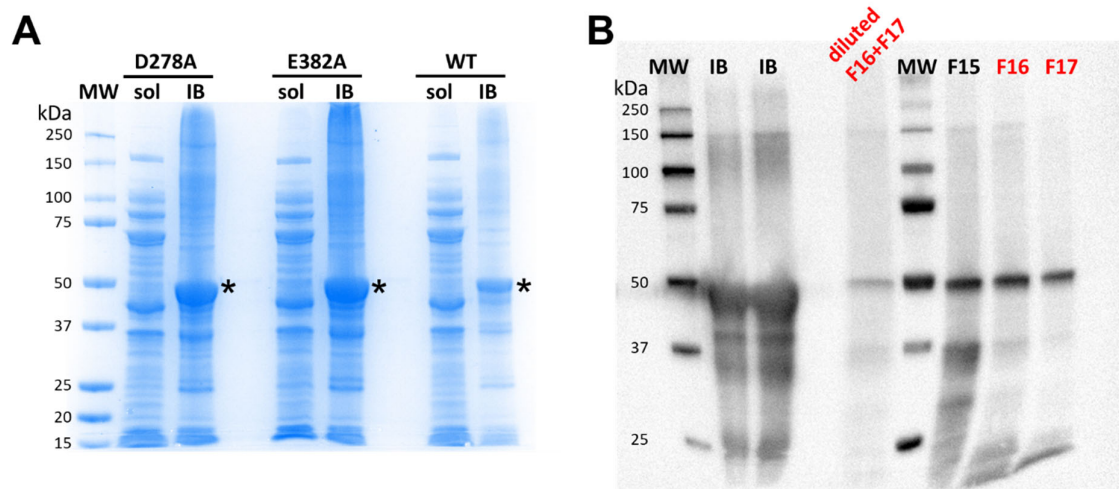


Figure S2. SDS-PAGE and western blot analysis of hTAS1R2-VFT wild-type (WT) and mutant hTAS1R2-VFT-D278A and hTAS1R2-VFT-D382A expressed in *E. coli* using the pET28 vector. (A) The proteins from the soluble fraction (sol) or insoluble fraction, known as inclusion bodies (IB), were separated by 10% acrylamide 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.

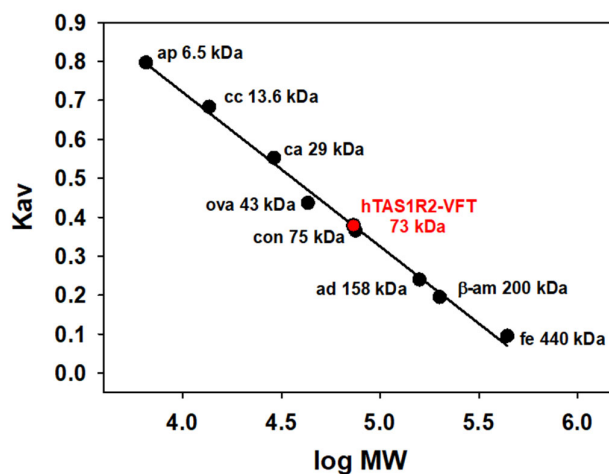


Figure S3. Gel filtration chromatography of purified hTAS1R2-VFT. The calibration curve for the HiLoad 16/600 Superdex 200 preparative grade column (GE Healthcare) was established with ferritin (fe, 440 kDa), β -amylase (β -am, 200 kDa), alcohol dehydrogenase (ad, 158 kDa), conalbumin (con, 75 kDa), 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.

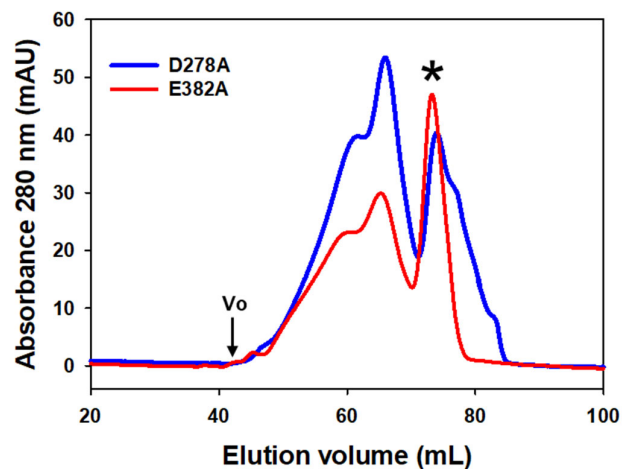


Figure S4. Preparative gel filtration chromatography of refolded hTAS1R2-VFT-D278A (solid blue line) and hTAS1R2-VFT-E382A (solid red line). The arrow indicates the position of the void volume (V_o), and the asterisk indicates the peak containing purified hTAS1R2-VFT. Gel filtration was performed using 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 (%)	β -sheets (%)
hTAS1R2-VFT	72	9
hTAS1R2-VFT-D278A	66	13
hTAS1R2-VFT-E382A	69	13