

1 . 21 . 41 . 1.30 1.29 1.34

4AMJ MG[]A**ELLSQQWEAGMSLIM**ALV**VLLIVAGNV**LVI**AAIG**sTQR
β1AR MGDGWLPPDCGPHNRSGGGGATAAPTGSRQVSA**ELLSQQWEAGMSLIM**ALV**VLLIVAGNV**LVI**AAIG**RTQR
humanTAAR1 -----MMPFCHNIINISCVKNNWSNDRASLYSLMVLII**LTTLVGNLIVIVS**ISHFKQ
mouseTAAR3 -----MDLIYIPEDLSSCPKFGNKSPPTNRSFRVRMIMYLFMTGAMVITIFGNLVI**II**SISHFKQ
elephantTAAR7a -----MSTELSPAPVQLCYENLNGSCVKTSYSPGPRVMILYLVFGSGAVLAVFGNLLVMISMLHFKQ
mouseTAAR8a -----MTSNFSQPALQLCYENTNGSCIKTPYSPGPRVILY**VMYVGF**GAVLAVCGNLLVVISVLHFKQ
<-----TM1-----><-----

54 61 . 81 . 101 . 121 2.67 3.21

4AMJ LQT**LTNLFITSLACADLV**v**GLLVVPFGATLV**VRGT**TWLWGSFLCEI**W**TSIDVLCV**TAS**IETLCVIAIDRYLA**
β1AR LQT**LTNLFITSLACA**L**VLMGLLVVPFGATLV**VRGT**TWLWGSFLCEI**W**TSIDVLCV**TAS**IETLCVIAIDRYLA**
humanTAAR1 LHTPTN**WLIHSMATVDFLIGCL**MPY**SMVRSABHCWYFGEV**FCKI**HTST**IMLS**ASIFHLSFISIDRYYA**
mouseTAAR3 LHSP**TN**FL**ILSMAT**TDFLL**GFVIM**PY**SMVRSV**ESCWYFGDS**FCKFHAS**FD**MMLSLTSIFHLCSIAIDRFYA**
elephantTAAR7a LHSPAN**FLIASLACAD**FLV**GV**TV**MPFSTVRSV**ESCWYFG**EIYCTFHSCFNGS**F**CYASIFHLCFISVDK**FI**A**
mouseTAAR8a LHSPAN**FLIASL**AS**ADFLV**GIS**VMPF**SMVRS**IESCWYF**GD**AFCSLHSCCDVAF**CY**SSV**LHL**CFISVDR**YIA
IC1--<-----TM2-----><-----EC1-----><-----TM3-----><-----

125 . 151 . 181 3.65 4.61 5.36

4AMJ **ITSPFRYQSLMTRARAKV**IICT**VWAI**SALV**SFLPIMM**HWRDED**PQ**-**ALKCYQDPGCDFV**T**NRAYAI**ASS
β1AR **ITSPFRYQSLMTRARAKV**IICT**VWAI**SALV**SFLPIMM**HWRDED**PQ**-**ALKCYQDPGCCDFV**T**NRAYAI**ASS
humanTAAR1 VCDPLRY**KAKMNILVICVM**IFIS**WSPAVAF**FG**MI**FL**ENFKG**AEEI**YKHVHCRGGCSV**ES**SKISGVL**IF
mouseTAAR3 VCDPLHY**TTMTVSMIKRLLAF**CWA**APALF**S**FGLV**LSEANVSG**MQS**-YE**ILVAC**FN**F**CAL**T**FN**KFWG**TIL**F**
elephantTAAR7a VTDPLI**YPT**R**FTASV**SGIC**IA**FS**WLLSILY**S**FL**CSGAN**ETGLEE**-LV**SG**LS**CVGGCQ**IAV**NSN**W**V**FV**NE**
mouseTAAR8a VTDPLV**YPT**K**FTV**SVSGIC**IS**IS**WIL**PLV**YSSAV**F**Y**T**GISAK**GIES-LV**SAL**NC**CVGGCQ**IVIN**QDF**VL**ISE**
-----IC2-----><-----TM4-----><-----EC2-----><-----

196 201 . 231 . 241 5.65 6.26

4AMJ **IISFYI**PL**LIMIFV**a**LRVYREAKEQ**IRKIDR[]ASKRK**TSRVMIM**
β1AR **IISFYI**PL**LIMIFV**Y**LRVYREAKEQ**IRKIDR**CEGRFYGSQEQPP**PL**PQHQP**IL**GN**RASKRK**TSRVMIM**
humanTAAR1 **MTSFYI**PG**SIM**LCV**YRIY**LI**AKEQ**AR**LIS**DAN**QKLQIGL**-----EMK**NGISQS**
mouseTAAR3 **TTCFF**T**PGS**IM**VGIY**GK**IFIV**RR**HARALS**D**MPANT**KG-----AVG**KNLSK**
elephantTAAR7a **V**-**LF**FI**PTL**VM**IIV**YSK**IFL**VAK**QQA**RK**IESLS**N**KTET**SS-----DSY**KDR**VAK
mouseTAAR8a **L**-**LF**FI**PTL**VM**IIV**YSK**IFL**VAK**QQA**VKI**ETSV**SG**NR**G**ESS**-----ESH**KAR**VAK
-----TM5-----><-----IC3-----><-----

245 . 261 . 301 . 6.71 6.78 7.54

4AMJ **REHKALK**TLGI**IMGV**FT**LCWLP**FF**LVNIVN**VFN**RDLV**P**DWLE**V**aFNW**LG**YANS**a**mNP**I**IYCR**-**SPDFR**KAF
β1AR **REHKALK**TLGI**IMGV**FT**LCWLP**FF**LVNIVN**VFN**RDLV**P**DWLE**V**FFN**W**LG**YANS**aFN**I**IYCR**-**SPDFR**KAF
humanTAAR1 **KERKAV**KT**LGIV**MG**VFLIC**CP**FC**IC**IVM**P**FLHYI**IP**PTIND**V**LIFG**Y**LN**ST**FN**PM**YAF**F**Y**P**WFR**KAL
mouseTAAR3 **KDRKA**AK**TLGI**VM**GV**FL**ACWLP**CF**LAVL**ID**PYLD**YST**PIIV**LD**LLV**LG**YFN**ST**CN**PL**HGF**F**Y**P**WFR**KAL
elephantTAAR7a **RERKA**AK**TLGI**AV**IAFL**IS**WLP**Y**FDI**VID**AF**LG**FIT**PT**YI**E**ILV**WF**AYNS**AM**NPLI**Y**AF**F**Y**P**WFR**KAI
mouseTAAR8a **RERKA**AK**TLG**V**TVV**AF**MSW**LP**Y**T**ID**AL**VDA**FM**GFIT**PA**YV**Y**EIC**WG**TYNS**AM**NPLI**Y**AF**F**Y**P**WFR**KAI
-----><-----TM6-----><-----EC3-----><-----TM7----->

316 321 .

4AMJ **KRL**L**a**F**PRKAD**RR**Lh**hhhhhh[]
β1AR **KRL**LC**FPRKAD**RR**LHAGGQ**PAP**LP**GG**FIST**LG**SP**EH**SPGGT**WSD**CNGG**TR**GG**SE**SSLE**ER**HSK**TS**RS**ES**KM**
humanTAAR1 **KM**ML**FGK**IF**QK**D**SSR**CK**LF**LE**SS**-----
mouseTAAR3 **QFIV**SG**KIF**RS**NSD**TAN**LF**PE**AH**-----
elephantTAAR7a **KLI**IT**GK**VL**RE**NS**ST**N**LF**SD-----
mouseTAAR8a **KLI**LS**GE**IL**KG**HS**ST**N**LF**SE-----

4AMJ]
β1AR **EREK**NIL**AT**TR**FY**CT**FL**GN**G**DK**AV**FC**TV**LR**IV**KL**FED**AT**CT**CP**HT**KL**KM**K**WR**FK**QHQA**
humanTAAR1 -----
mouseTAAR3 -----
elephantTAAR7a -----
mouseTAAR8a -----

S5 Fig. Multiple alignment of the four TAAR and the turkey β_1 -adrenergic receptor proteins.

Protein sequences of two primary amine detecting TAARs (human TAAR1: NP_612200 and mouse TAAR3: NP_001008429) and two tertiary amine detecting TAARs (elephant TAAR7a: XP_003404143 and TAAR8a: NP_001010830) are aligned with the sequence of the turkey β_1 -adrenergic receptor (β_1 AR: P07700). The position number at the top of the alignment starts at the beginning of the human TAAR1 sequence. Position numbers based on the scheme proposed by Ballesteros and Weinstein (1995) are also shown diagonally for the start and end of each transmembrane region of β_1 AR. Approximate regions for transmembranes (TM1-TM7), intracellular loops (IC1-IC3), and extracellular loops (EC1-EC3) are indicated below each alignment block. The first lines of the alignment show the sequence the protein structure (4AMJ) is based on. In order to improve expression and to obtain crystals, eight thermostabilizing point mutations, a His-tag at the C-terminus, and truncations (at N-terminus, third intracellular loop, and C-terminus) were introduced (Warne *et al.*, 2012). These changes are indicated by lower cases and square brackets in the 4AMJ sequence. Residues assigned for alpha helices in 4AMJ are shown with white letters on black background. 26 residues suggested to involve with agonist binding to the β_1 AR are shown with blue background (Warne *et al.*, 2011, 2012). For the β_1 AR and TAAR protein sequences, residues predicted to be in transmembrane regions by Phobius (Kall *et al.*, 2007) are shown with gray background. The residues surrounding the main and minor ligand-binding pockets in the β_1 AR are shown with cyan and magenta background (Nygaard *et al.*, 2009; Rosenkilde *et al.*, 2010). 29 ligand-binding sites identified by Kleinau *et al.*, (2011) are shown with green background in the human TAAR1. Among them, the residues conserved among human TAARs (including both primary amine detectors and tertiary amine detectors), adrenergic receptors, as well as other biogenic amine receptors are shown with red fonts. Those in the human TAAR1 identical or similar to the residues in the corresponding position of biogenic amine receptors are shown with yellow fonts. Positively selected sites identified by our PAML analysis are shown with triangles below the alignment: red and green are sites identified by the site and branch-site models, respectively, in TAAR7, and purple and brown are sites identified by the site and branch-site models, respectively, in TAAR8. Closed triangles indicate sites identified with posterior probabilities higher than 0.95. See S3 and S4 Tables for details.

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