# Supplementary Information

## DETERMINANTS INVOLVED IN SUBTYPE-SPECIFIC FUNCTIONS OF RAT TRACE AMINE-ASSOCIATED RECEPTORS 1 AND 4

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Running title: Determinants defining Taar-specific function

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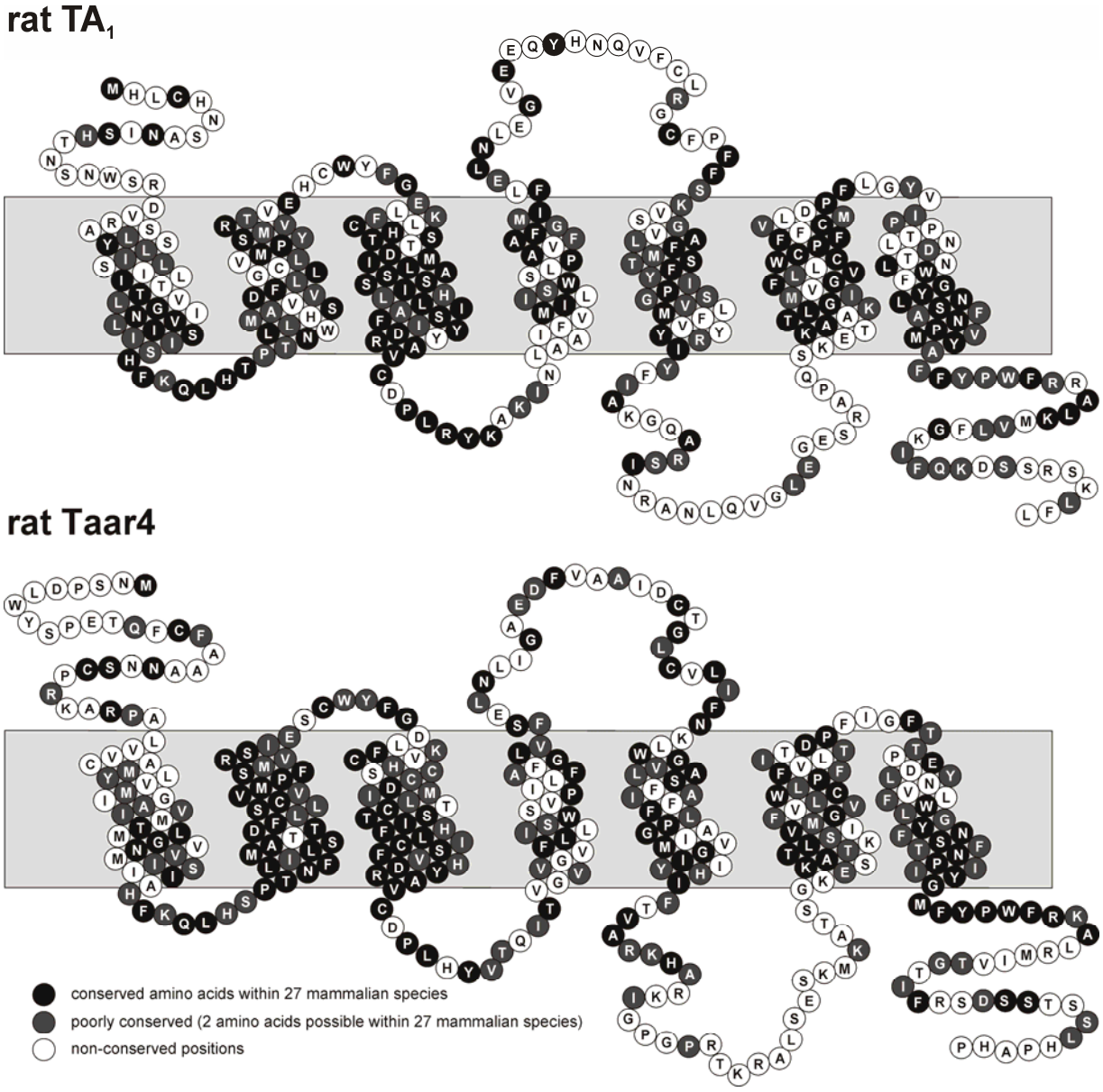


**Figure S1. Structural conservation of rat $TA_1$ and rat Taar4.** Amino acid sequences of rat TA1 and rat Taar4 are shown. Positions conserved in 27 mammalian species (*Bos taurus, Choloepus hoffmanni, Cavia porcellus, Dasypus novemcinctus, Dipodomys ordii, Equus caballus, Erinaceus europaeus, Echinops telfairi, Felis catus, Loxodonta africana, Monodelphis domestica, Macropus eugenii, Myotis lucifugus, Macaca mulatta, Microcebus murinus, Mus musculus, Ornithorhynchus anatinus, Oryctolagus cuniculus, Papio hamadryas, Pongo pygmaeus, Pteropus vampyrus, Rattus norvegicus, Sorex araneus, Saguinus oedipus, Saimiri sciureus, Sus scrofa, Tupaia belangeri*) are depicted in black. Positions that vary only by two amino acids are shown in gray. Positions given in white are not preserved during evolution. With 83.0% identity Taar4 is slightly higher conserved than $TA_1$ (80.7%).

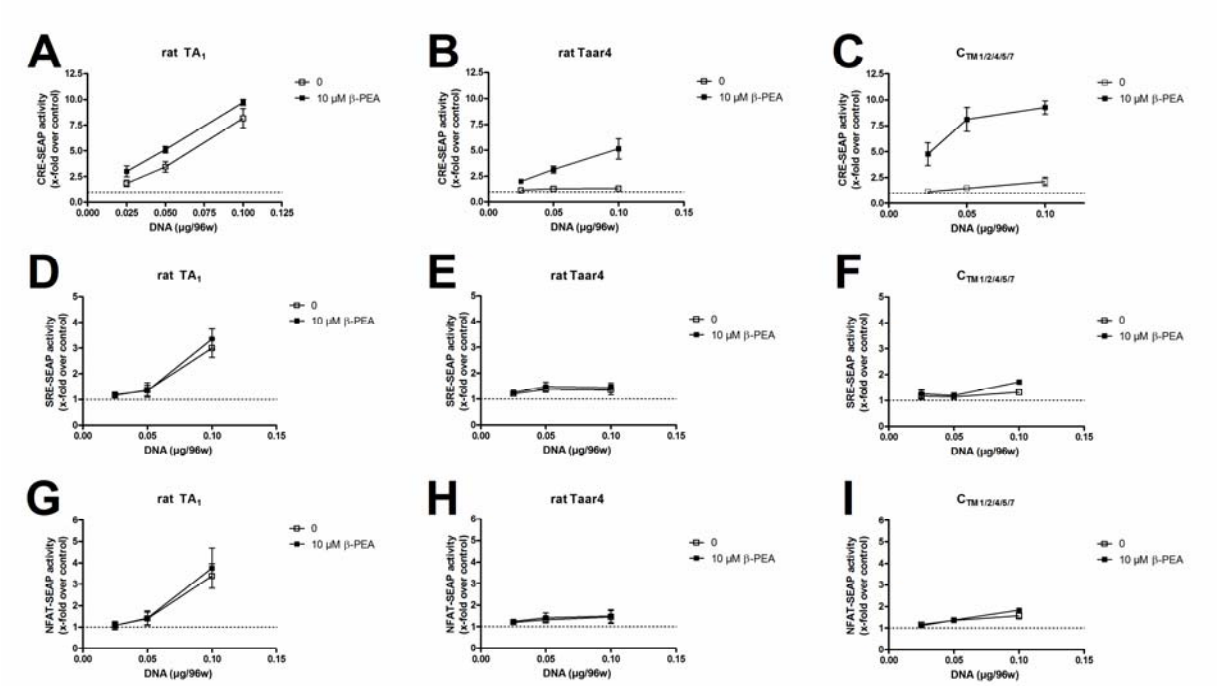


**Figure S2. Functional characterization of rat $TA_1$, rat Taar4 and $C_{TM1/2/4/5/7}$ in SEAP reporter gene assays.** Correlation of basal or stimulated receptor activity and the amount of transfected plasmid DNA. HEK-293 cells were transfected with 25 ng, 50 ng or 100 ng of the indicated plasmid DNA per well in 96-well plate. The total amount of transfected plasmid DNA was constant since differences were compensated by addition of respective amount empty vector. A-C: CRE-SEAP reporter gene assay in presence or absence of β-PEA D-F: SRE-SEAP reporter gene assay. G-I: NFAT-SEAP reporter gene assay. SEAP activity is expressed as fold over basal levels of HEK293 cells transfected with empty vector pcDps. Data are presented as mean ± SEM of two to four independent experiments, each carried out in triplicate.

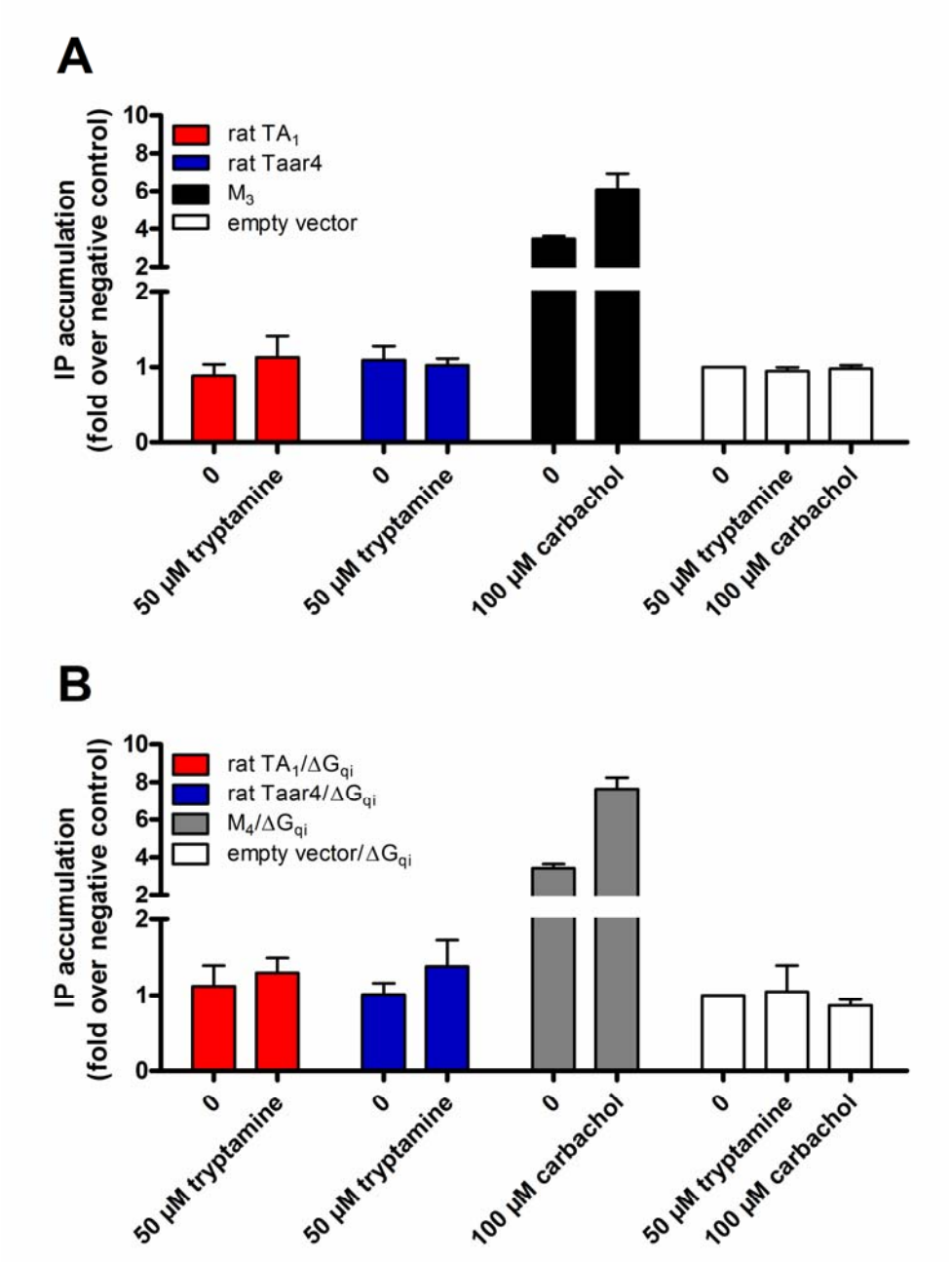


**Figure S3. Functional characterization of rat $TA_1$ and rat Taar4 in inositol phosphate (IP) assays.** A: IP accumulation in presence and absence of agonist. $M_3$ muscarinic acetylcholine receptor (NCBI Reference Sequence: NM_000740.2) served as positive control. B: $G\alpha_{\Delta 6qi4myr}$ (abbreviated $\Delta G_{qi}$) turns the $G\alpha_i$-coupled signal into the $G\alpha_q$ pathway (phospholipase C activation measured as PI-turnover). $M_4$ muscarinic acetylcholine receptor (NCBI Reference Sequence: NM_000741.2) served as positive control.

**Table S1. Primers used for rat Taar1 and rat Taar4 amplification and generation of chimeras.**

| ID | Sequence 5' -> 3' | Purpose |
|---|---|---|
| OLIGO.A | GTGCAAATCAAAGAACTGCTCCTC | pcDps forward (amplification/sequencing) |
| OLIGO.B | CCTGGTTCTTTCCGCCTCAGAAG | pcDps reverse (amplification/sequencing) |
| OLIGO.12 | ATTTTAGCACAATGTCCAGCTCCAA | rat Taar1 amplification S |
| OLIGO.13 | TAAAAGCCCATGAAATCAGTGCCTA | rat Taar1 amplification AS |
| OLIGO.9 | CATCGTCCTTATAGTCCAAAAATAGC TTAGACC | rat Taar1 FLAG adaptor AS |
| OLIGO.10 | CCCGACTACGCCCATCTTTGCCACAAT AG | rat Taar1 HA adaptor S |
| OLIGO.c | TTTGGGGAAAACAAGTTCCTGGT | rat Taar4 amplification S |
| OLIGO.d | TCTGTGAGGTTCAGCACTCATGC | rat Taar4 amplification AS |
| OLIGO.e | CATCGTCCTTATAGTCAGGATGTGCA GGATGC | rat Taar4 FLAG-adaptor AS |
| OLIGO.f | CCCGACTACGCCAATTCACCTGACCT CT | rat Taar4 HA-adaptor S |
| OLIGO.0 | CGCGAATTCCCCACCATGTACCCCTA CGACGTCCC | HA uni EcoRI+Coseq S |
| OLIGO.1 | CGCCGCACTAGTTCACTTATCGTCATC GTCCTTAT | FLAG uni SpeI AS |
| OLIGO.114 | CCCAAACTTCTACGTGCCTTTCTCCAA CAAGACGGGCGTGGTGCGCCATCTTT GCCACAATAGCGCGAAT | rat Taar1 HA-Rhodopsin adaptor S |
| OLIGO.115 | CCCAAACTTCTACGTGCCTTTCTCCAA CAAGACGGGCGTGGTGCGCAATTCAC CTGACCTCTGGTACTCC | rat Taar4 HA-Rhodopsin adaptor S |
| OLIGO.113 | CTCGAATTCCCCACCATGTACCCCTAC GACGTCCCCGACTACGCCAACGGGAC CGAGGGCCCAAACTTCTACGTGCCTT TC | HA-Rhod-uni S |
| OLIGO.70 | CGTGTGAAGTTGCTTGAAGTGGGCAA TG | TMD1-rat Taar4 AS |
| OLIGO.71 | CATTGCCCACTTCAAGCAACTTCACA C | TMD2-rat Taar1 S |
| OLIGO.72 | GGAAGACATTATACAAATCTTCAGGG ATAACATAGCCCAGGAAA | TMD6-rat Taar1 AS |
| OLIGO.73 | CCTTTCCTGGGCTATGTTATCCCTGAA GATTTGTATAATGTCTTCCTC | TMD7-rat Taar4 S |
| OLIGO.74 | CCGCCACTATGCTGTCTGTGACCCTTT AAGATACAAAGCCAAGA | TMD4-rat Taar1 S |
| OLIGO.75 | TCTTGGCTTTGTATCTTAAAGGGTCAC AGACAGCATAGTGGCGG | TMD3-rat Taar4 AS |
| OLIGO.76 | AGTGGGTCACAGACAGCATAGTAGCG GTCAATGG | TMD3-rat Taar1 AS |
| OLIGO.77 | CCATTGACCGCTACTATGCTGTCTGTG ACCCACT | TMD4-rat Taar4 S |
| OLIGO.78 | GGAGCTGCTTGAAGTGGGATATCGAA AT | TMD1-rat Taar1 AS |
| OLIGO.79 | AGTTCCCCAAAGTACCAGCACGACTC GATAGACCGGACCATACTGAAGGGCA TGACCACGCAGCTCAACAGGAAGTCT GTGGTAGCCAT | TMD2-rat Taar4 AS |

| | | |
|---|---|---|
| OLIGO.80 | TCGATATCCCACTTCAAGCAGCTCCACTCCCCGACCAACTTCCTTATTCTCTCCATGGCTACCACAGACTTCCTGTT | TMD2-rat Taar4 S |
| OLIGO.81 | CTATCGAGTCGTGCTGGTACTTTGGGGAACT | TMD3-rat Taar1 S |
| OLIGO.82 | AGGTCTCCGAAGTACCAGCAGTGCTCAACTG | TMD2-rat Taar1 AS |
| OLIGO.83 | AGGGTCGCACACAGCATAGTGGCGGTCCACTGAGATGAAACAGAGGTGGAAAATGGAGGTGGTACAGAGCATGATGTCACAGCA | TMD3-rat Taar4 AS |
| OLIGO.84 | CAGTTGAGCACTGCTGGTACTTCGGAGACCTCTTTTGCAAAGTCCACAGCTGCTGTGACATCATGCTCTG | TMD3-rat Taar4 S |
| OLIGO.85 | CGACGCCGACGGTGATCTTGGCTTTGTATCTTAAAGG | TMD3-rat Taar1 AS |
| OLIGO.86 | CCTGATTGTGATACTGCTCCTCAGCACCAATCAAATTTAATTCTGAGAACACC | TMD4-rat Taar4 AS |
| OLIGO.87 | AGGCCAAAGGCAAACAGGATGGGCCTTTAAGATACAAAGCCAAGATCACCGTCGGCGTCGTGGGGGTCTTTCTACTCATCAGTTGGTCTGTCCCCATCCTGTTTGCCTTTG | TMD4-rat Taar4 S |
| OLIGO.88 | ATTAAATTTGATTGGTGCTGAGGAGCAGTATCACAATCAGG | TMD5-rat Taar1 S |
| OLIGO.89 | ATATCAACACACACAAACCTGTACAGACCTGATTGTGATACTGCTCCT | TMD4-rat Taar1 AS |
| OLIGO.90 | GACCTCGCTTGTCCTTTAGCAACTGTGAAAATGTGTATATAAATCCCCACCATGATTGCCCCAGGCAGAAAGAAAGCTATAAAGGAAGCCAGC | TMD5-rat Taar4 AS |
| OLIGO.91 | AGGAGCAGTATCACAATCAGGTCTGTACAGGTTTGTGTGTGTTGATATTTAACAAGCTCTGGGGAGTGCTGGCTTCCTTTATAGCTTTCT | TMD5-rat Taar4 S |
| OLIGO.92 | GGATTTATATACACATTTTCACAGTTGCTAAAGGACAAGCGAGGTC | TMD6-rat Taar1 S |
| OLIGO.93 | CGGATGTGGCCTTCATTTCCCCTTCCAATCCAACT | TMD5-rat Taar1 AS |
| OLIGO.94 | AGTTGGATTGGAAGGGGAAATGAAGGCCACATCCGGAAAGGAAAGCAAGGCCACCAAGACTTTGAGCATAGTCATGGGAGT | TMD6-rat Taar4 AS |
| OLIGO.95 | GGCCCTCTCAGAAAGCAAAAGCAGAGCGCCACAAAGCAAGGAAACAAAAGC | TMD6-rat Taar4 S |
| OLIGO.96 | CGCGAAAACCTTAGGGATCATGGTGCATGGTCCTGGACCCTTTCATTGGTTTTACAACCC | TMD7-rat Taar1 S |
| OLIGO.109 | CACAGCACAAATACTCCCATGAC | TMD6-intra-ratTaar4/extra-rat Taar1 AS |
| OLIGO.110 | GTCATGGGAGTATTTGTGCTGTGCTGGTGCCCGTTCTTTTTCTGCA | TMD6-intra-rat Taar4/extra-rat Taar1 S |
| OLIGO.111 | AGAAGGGCAGCCAACACAGGAGGAAAACGCCCACCA | TMD6-intra-rat Taar1/extra-rat Taar4 AS |
| OLIGO.112 | CTGTGTTGGCTGCCCTTCT | TMD6-intra-rat Taar1/extra-rat Taar4 S |
| OLIGO.116 | TGGAGCTGCTTGAAGTGGGATATCGA | TMD1 rat Taar1 AS |

| | | |
|---|---|---|
| | AATGA | |
| OLIGO.117 | TTCGATATCCCACTTCAAGCAGCTCCA | TMD2 rat Taar4 S |
| OLIGO.118 | ATTCAGTGTGTCATTCAGAGTGGGTG GGGTTGTAAAACCAAT | TMD6 rat Taar4 AS |
| OLIGO.119 | TTGGTTTTACAACCCCACCCACTCTGA ATGACACACTGAAT | TMD7 rat Taar1 S |

**Table S2. NCBI database accession numbers and sequence description**

| | | $TA_1$ | | Taar4 | |
|---|---|---|---|---|---|
| | | **length** | **source** | **length** | **source** |
| ***Mammalia*** | | | | | |
| ***Protheria*** | | | | | |
| *Ornithorhynchus anatinus* | platypus | full length | NCBI *Ornithorhynchus anatinus* trace archive: gnl\|ti\|649529709 and others | full length | NCBI *Ornithorhynchus anatinus* trace archive: gnl\|ti\|1246961726 and others |
| ***Metatheria*** | | | | | |
| *Monodelphis domestica* | gray short-tailed opossum | full length | NCBI *Monodelphis domestica* trace archive: gnl\|ti\|337177360 and others | full length 3x | NCBI *Monodelphis domestica* trace archive: gnl\|ti\|473024684 and others |
| *Macropus eugenii* | tammar wallaby | full length | NCBI *Macropus eugenii* trace archive: gnl\|ti\| 1649014544 and others | full length | NCBI *Macropus eugenii* trace archive: gnl\|ti\|1275406430 and others |
| ***Eutheria*** | | | | | |
| ***Afrotheria*** | | | | | |
| *Procavia capensis* | cape rock hyrax | full length (P) | NCBI *Procavia capensis* trace archive: gnl\|ti\| 1303042942 and others | full length 3x | NCBI *Procavia capensis* trace archive: gnl\|ti\|1203477241 and others |
| *Loxodonta africana* | African savanna elephant | full length | NCBI *Loxodonta africana* trace archive: gnl\|ti\|537131216 and others | full length 2x | NCBI *Loxodonta africana* trace archive: gnl\|ti\|1695377756 and others |
| *Echinops telfairi* | lesser hedgehog tenrec | full length 2x (1P) | NCBI *Echinops telfairi* trace archive: gnl\|ti\| 691100554 and others | full length 2x (1P) | NCBI *Echinops telfairi* trace archive: gnl\|ti\|688316620 and others |
| ***Xenarthra*** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| *Dasypus novemcinctus* | nine-banded Armadillo | full length | NCBI *Dasypus novemcinctus* trace archive: gnl\|ti\|566803806 and others | full length | NCBI *Dasypus novemcinctus* trace archive: gnl\|ti\|567276505 and others |
| *Choloepus hoffmanni* | Hoffmann's two-fingered sloth | full length | NCBI *Choleopus hoffmanni* trace archive: gnl\|ti\|1302995278 and others | full length | NCBI *Choleopus hoffmanni* trace archive: gnl\|ti\|1357779584 and others |
| ***Laurasiatheria*** | | | | | |
| ***Insectivora*** | | | | | |
| *Erinaceus europaeus* | western European hedgehog | full length | NCBI *Erinaceus europaeus* trace archive: gnl\|ti\|909479654 and others | full length 2x (1P) | NCBI *Erinaceus europaeus* trace archive: gnl\|ti\|932971697 and others |
| *Sorex araneus* | European shrew | full length | NCBI *Sorex araneus* trace archive: gnl\|ti\|877297697 and others | full length 2x (1P) | NCBI *Sorex araneus* trace archive: gnl\|ti\|893813953 and others |
| ***Chiroptera*** | | | | | |
| *Pteropus vampyrus* | large flying fox | full length | NCBI *Pteropus vampyrus* trace archive: gnl\|ti\|1320431729 and others | full length | NCBI *Pteropus vampyrus* trace archive: gnl\|ti\|1329134482 and others |
| *Myotis lucifugus* | little brown bat | full length (2x) | NCBI *Myotis lucifugus* trace archive: gnl\|ti\|1874379801 and others | full length | NCBI *Myotis lucifugus* trace archive: gnl\|ti\|1899487221and others |
| ***Cetartiodactyla*** | | | | | |
| *Bos taurus* | cattle | full length | NCBI *Bos taurus* trace archive: gnl\|ti\|393291934 and others | full length | NCBI *Bos taurus* trace archive: gnl\|ti\|620739238 and others |
| *Sus scrofa* | boar | full length (2x) | NCBI *Sus scrofa* trace archive: gnl\|ti\|2042809629 and others | full length | NCBI *Sus scrofa* trace archive: gnl\|ti\|1579919251 and others |
| ***Perissodactyla*** | | | | | |
| *Equus caballus* | horse | full length | NCBI *Equus caballus* trace archive: gnl\|ti\|1400434870 and others | full length | NCBI *Equus caballus* trace archive: gnl\|ti\|1234728610 and others |
| ***Carnivora*** | | | | | |
| *Feliformia* | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| *Felis catus* | cat | full length | NCBI *Felis catus* trace archive: gnl\|ti\|711855444 and others | full length | NCBI *Felis catus* trace archive: gnl\|ti\|965531899 and others |
| *Canidae* | | | | | |
| *Canis familiaris* | dog | full length (P) | NCBI *Canis familiaris* trace archive: gnl\|ti\|313292271 and others | full length | NCBI *Canis familiaris* trace archive: gnl\|ti\|302723376 and others |
| ***Euarchontoglires*** | | | | | |
| ***Scandentia*** | | | | | |
| *Tupaia belangeri* | northern tree shrew | full length | NCBI *Tupaia belangeri* trace archive: gnl\|ti\|1044829742 and others | full length 2x | NCBI *Tupaia belangeri* trace archive: gnl\|ti\|1046628937 and others |
| ***Glires*** | | | | | |
| *Lagomorpha* | | | | | |
| *Leporidae* | | | | | |
| *Oryctolagus cuniculus* | european rabbit | full length | NCBI *Oryctolagus cuniculus* trace archive: gnl\|ti\|1940715560 and others | full length | NCBI *Oryctolagus cuniculus* trace archive: gnl\|ti\|638702844 and others |
| *Rodentia* | | | | | |
| *Cavia porcellus* | domestic guinea pig | full length | NCBI *Cavia porcellus* trace archive: gnl\|ti\|1617005707 and others | full length 2x | NCBI *Cavia porcellus* trace archive: gnl\|ti\|1596694171 and others |
| *Dipodomys ordii* | Ord's kangaroo rat | full length | NCBI *Dipodomys ordii* trace archive: gnl\|ti\|1535262597 and others | full length | NCBI *Dipodomys ordii* trace archive: gnl\|ti\|1586901258 and others |
| *Mus musculus* | house mouse | full length | NCBI accession: NM_053205 | full length | NCBI accession: FJ372497, NM_001008499 |
| *Rattus norvegicus* | Norway rat | full length | NCBI accession: NM_134328 | full length | NCBI accession: FJ372521, NM_175583 |
| ***Primates*** | | | | | |
| *Strepsirrhini* | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| *Lemuriformes*<br>*Microcebus murinus* | gray mouse lemur | full length | NCBI *Microcebus murinus* trace archive: gnl\|ti\|1576501568 and others | full length | NCBI *Microcebus murinus* trace archive: gnl\|ti\|1563038889 and others |
| *Haplorrhini* | | | | | |
| *Simiiformes*<br>*Platyrrhini* | | | | | |
| *Cebidae*<br>*Callithrix jacchus* | common marmoset | full length | NCBI *Callithrix jacchus* trace archive: gnl\|ti\|974234240 and others | full length (P) | NCBI *Callithrix jacchus* trace archive: gnl\|ti\|1133228298 and others |
| *Saguinus oedipus* | cotton-top tamarin | full length | NCBI accession: GQ892034 | full length | NCBI accession: GQ892037 |
| *Saimiri sciureus* | common squirrel monkey | full length | NCBI accession: EF549698 | full length | NCBI accession: EF549700 |
| *Catarrhini*<br>*Cercopithecoidea* | | | | | |
| *Macaca mulatta* | rhesus monkey | full length | *Macaca mulatta* trace archive: gnl\|ti\|402223230 and others | full length | NCBI *Macaca mulatta* trace archive: gnl\|ti\|486761772 and others |
| *Papio hamadryas* | hamadryas baboon | full length | NCBI *Papio hamadryas* trace archive: gnl\|ti\|1943570175 and others | full length | NCBI *Papio hamadryas* trace archive: gnl\|ti\|1959076673 and others |
| *Hominoidea*<br>*Hylobatidae* | | | | | |
| *Nomascus leucogenys* | white-cheeked Gibbon | full length | NCBI *Nomascus leucogenys* trace archive: gnl\|ti\|1891571032 and others | full length (P) | NCBI *Nomascus leucogenys* trace archive: gnl\|ti\|2088070237 and others |

| | | | | | |
|---|---|---|---|---|---|
| *Hominidae* | | | | | |
| *Homo sapiens* | human | full length | NCBI accession: NM_138327 | full length (P) | NCBI accession: NG_004855 |
| *Pan troglodytes* | chimpanzee | full length | NCBI accession: NM_001009145 | full length (P) | NCBI accession: FJ372513, FJ372514, AY702310 |
| *Pongo pygmaeus* | orangutan | full length | NCBI *Pongo pygmaeus abelii* trace archive: gnl\|ti\|820557702 and others | full length (P) | NCBI *Pongo pygmaeus abelii* trace archive: gnl\|ti\|886956191 and others |

**Table S3. Detailed description of rat $TA_1$-Taar4 chimeras generated in this study.**

| chimera | rat $TA_1$ | rat Taar4 | HA-tag | FLAG-tag | Rhod-tag |
|---|---|---|---|---|---|
| $\mathbf{C_{TM1-3}}$ | 129-332 | 2-139 | + | + | - |
| $\mathbf{C_{TM1-3}R}$ | 129-332 | 2-139 | + | + | + |
| $\mathbf{C_{TM4-7}}$ | 2-128 | 140-347 | + | + | - |
| $\mathbf{C_{TM4-7}R}$ | 2-128 | 140-347 | + | + | + |
| $\mathbf{C_{TM1}}$ | 55-332 | 2-65 | + | + | - |
| $\mathbf{C_{TM1}R}$ | 55-332 | 2-65 | + | + | + |
| $\mathbf{C_{TM2}}$ | 2-54; 92-332 | 66-102 | + | + | - |
| $\mathbf{C_{TM2}R}$ | 2-54; 92-332 | 66-102 | + | + | + |
| $\mathbf{C_{TM3}}$ | 2-91; 129-332 | 103-139 | + | + | - |
| $\mathbf{C_{TM3}R}$ | 2-91; 129-332 | 103-139 | + | + | + |
| $\mathbf{C_{TM4}}$ | 2-134; 169-332 | 146-179 | + | + | - |
| $\mathbf{C_{TM4}R}$ | 2-134; 169-332 | 146-179 | + | + | + |
| $\mathbf{C_{TM5}}$ | 2-175; 217-332 | 187-226 | + | + | - |
| $\mathbf{C_{TM5}R}$ | 2-175; 217-332 | 187-226 | + | + | + |
| $\mathbf{C_{TM6}}$ | 2-235; 274-332 | 248-285 | + | + | - |
| $\mathbf{C_{TM6}R}$ | 2-235; 274-332 | 248-285 | + | + | + |
| $\mathbf{C_{TM6i}}$ | 2-235; 262-332 | 248-273 | + | + | - |
| $\mathbf{C_{TM6i}R}$ | 2-235; 262-332 | 248-273 | + | + | + |
| $\mathbf{C_{TM6o}}$ | 2-261; 274-332 | 274-285 | + | + | - |
| $\mathbf{C_{TM6o}R}$ | 2-261; 274-332 | 274-285 | + | + | + |
| $\mathbf{C_{TM7}}$ | 2-279 | 292-347 | + | + | - |
| $\mathbf{CR_{TM7}R}$ | 2-279 | 292-347 | + | + | + |
| $\mathbf{C_{TM1/7}}$ | 55-279 | 2-65; 292-34 | + | + | - |
| $\mathbf{C_{TM1/7}R}$ | 55-279 | 2-65; 292-34 | + | + | + |
| $\mathbf{C_{TM2/3}}$ | 2-54; 129-332 | 66-139 | + | + | - |
| $\mathbf{C_{TM2/4}}$ | 2-54; 92-134; 169-332 | 66-102; 146-179 | + | + | - |
| $\mathbf{C_{TM2/4}R}$ | 2-54; 92-134; 169-332 | 66-102; 146-179 | + | + | + |
| $\mathbf{C_{TM2/5}}$ | 2-54; 92-175; 217-332 | 66-102; 187-226 | + | + | - |
| $\mathbf{C_{TM2/5}R}$ | 2-54; 92-175; 217-332 | 66-102; 187-226 | + | + | + |
| $\mathbf{C_{TM3/6}}$ | 2-91; 129-235; 274-332 | 103-139; 248-285 | + | + | - |
| $\mathbf{C_{TM4/5}}$ | 2-134; 169-175; 217-332 | 146-179; 187-226 | + | + | - |
| $\mathbf{C_{TM4/5}R}$ | 2-134; 169-175; 217-332 | 146-179; 187-226 | + | + | + |
| $\mathbf{C_{TM2/4/5}}$ | 2-54; 92-134; 169-175; 217-332 | 66-102; 146-179; 187-226 | + | + | - |
| $\mathbf{C_{TM2/4/5}R}$ | 2-54; 92-134; 169-175; 217-332 | 66-102; 146-179; 187-226 | + | + | + |
| $\mathbf{C_{TM2/3/4/5}}$ | 2-54; 217-332 | 66-226 | + | + | - |
| $\mathbf{C_{TM2/4/5/6}}$ | 2-54; 92-134; 169-175; 274-332 | 66-102; 146-179; 187-285 | + | + | - |
| $\mathbf{C_{TM1/2/4/5/7}}$ | 92-134; 169-175; 217-279 | 2-102; 146-179; 187-226, 292-347 | + | + | - |
| $\mathbf{C_{TM2-7}}$ | 2-54 | 66-347 | + | + | - |
| $\mathbf{C_{TM1-6}}$ | 280-332 | 2-291 | + | + | - |
| $\mathbf{C_{TM2-6}}$ | 2-54; 280-332 | 66-291 | + | + | - |

Various rat $TA_1$-Taar4 chimeras were generated as described in *experimental procedures*. The construct designation and the exact constitution are outlined. Numbers given refer to the exact rat $TA_1$ and rat Taar4 amino acid position (without the epitope tags), respectively. + epitope tag present; -, no epitope tag; HA-tag: YPYDVPDYA; FLAG-tag: DYKDDDDK; RHOD-tag: NGTEGPNFYVPFSNKTGVVR

**Table S4. Structural comparison of $TA_1$ and Taar4 orthologs**

| | % identity between rat $TA_1$ and rat Taar4 | % identity among 27 mammalian $TA_1$ | % identity among 27 mammalian Taar4 |
|---|---|---|---|
| full length | 46.8 | 80.7 | 83.0 |
| N-term | 20.0 | 66.2 | 66.1 |
| TM1 | 40.0 | 81.0 | 76.0 |
| IL1 | 83.3 | 98.8 | 96.3 |
| TM2 | 66.7 | 90.9 | 95.7 |
| EL1 | 83.3 | 78.6 | 94.0 |
| TM3 | 63.6 | 93.6 | 93.4 |
| IL2 | 54.5 | 83.6 | 87.9 |
| TM4 | 47.8 | 77.4 | 79.5 |
| EL2 | 35.7 | 74.6 | 83.9 |
| TM5 | 42.3 | 82.1 | 90.3 |
| IL3 | 23.3 | 64.1 | 66.1 |
| TM6 | 59.4 | 84.7 | 86.3 |
| EL3 | 50.0 | 73.7 | 82.8 |
| TM7 | 42.3 | 84.3 | 90.1 |
| C-term | 51.5 | 78.0 | 79.8 |

Protein sequence of rat $TA_1$ und rat Taar4 were compared for all domains. The sequence information of 27 full-length mammalian $TA_1$ and Taar4 orthologs (see suppl. Figure S1) was used to determine the structural conservation of the respective receptor (given as % identity determined by pairwise comparison using MegAlign).

**Table S5. Functional characterization of rat $TA_1$, rat Taar4 and chimeras in HEK-293 cells using a cAMP accumulation assay.**

| | Basal cAMP | β-PEA $E_{max}$ | Tyr $E_{max}$ | Trp $E_{max}$ | (+) Pseph $E_{max}$ | (+) Eph $E_{max}$ | (+) MAmp $E_{max}$ | MDMA $E_{max}$ | LSD $E_{max}$ | Naph $E_{max}$ | Xylo $E_{max}$ | Oxy $E_{max}$ | Tram $E_{max}$ | Neo-Syn $E_{max}$ | Amio $E_{max}$ | Betahis $E_{max}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R1 | 2.3 ± 0.5 (5) | 22.2 ± 7.3 (5) | 24.6 ± 6.5 (3) | 17.9 ± 5.8 (3) | 3.2 ± 0.3 (3) | 3.0 ± 0.5 (5) | 10.4± 1.7 (5) | 9.0 ± 1.5 (5) | 3.7 ± 0.9 (5) | 11.0 ± 2.5 (5) | 8.2 ± 1.7 (5) | 4.9 ± 1.2 (5) | 11.7 ± 4.3 (5) | 5.4 ± 1.1 (5) | 2.8 ± 0.5 (3) | 12.8 ± 1.6 (3) |
| R1-R | 7.1 ± 1.4 (5) | 55.9 ± 15.3 (5) | 70.8 ± 29.4 (3) | 57.7 ± 23.2 (3) | 10.5 ± 3.6 (3) | 13.4 ± 3.8 (5) | 40.8± 12.3 (5) | 35.1 ± 8.5 (5) | 19.8 ± 4.9 (5) | 44.1 ± 14.4 (5) | 28.4 ± 6.1 (5) | 23.2 ± 7.9 (5) | 34.4 ± 12.7 (5) | 18.6 ± 4.5 (5) | 11.6 ± 2.2 (3) | 55.8 ± 12.6 (3) |
| R4 | 0.9 ± 0.4 (4) | 1.2 ± 0.2 (4) | 1.2 ± 0.3 (3) | 1.2 ± 0.3 (3) | 0.7 ± 0.2 (3) | 1.0 ± 0.3 (4) | 0.8 ± 0.4 (4) | 0.7 ± 0.3 (4) | 1.1 ± 0.3 (4) | 1.1 ± 0.3 (4) | 0.8 ± 0.3 (4) | 0.9 ± 0.4 (4) | 0.6 ± 0.3 (4) | 0.8 ± 0.3 (4) | 1.5 ± 0.3 (3) | 0.9 ± 0.4 (3) |
| R4R | 1.2 ± 0.3 (4) | 1.5 ± 0.4 (4) | 1.6 ± 0.4 (3) | 1.9 ± 0.6 (3) | 0.9 ± 0.3 (3) | 1.0 ± 0.3 (4) | 1.1 ± 0.4 (4) | 1.2 ± 0.5 (4) | 1.4 ± 0.6 (4) | 1.4 ± 0.5 (4) | 1.3 ± 0.5 (4) | 1.0 ± 0.4 (4) | 1.2 ± 0.3 (4) | 1.0 ± 0.3 (4) | 2.5 ± 1.1 (3) | 1.8 ± 0.1 (3) |
| $C_{TM1-3}$ | 1.2 ± 0.2 (3) | 1.5 ± 0.3 (3) | 1.7 ± 0.3 (3) | 1.5 ± 0.3 (3) | 1.6 ± 0.5 (3) | 2.0 ± 0.7 (3) | 1.5 ± 0.3 (3) | 1.5 ± 0.4 (3) | 1.5 ± 0.3 (3) | 1.9 ± 0.5 (3) | 1.4 ± 0.2 (3) | 1.9 ± 0.3 (3) | 1.7 ± 0.5 (3) | 1.5 ± 0.2 (3) | 2.0 ± 0.1 (3) | 1.6 ± 0.2 (3) |
| $C_{TM4-7}$ | 1.0 ± 0.1 (3) | 0.7 ± 0.3 (3) | 0.8 ± 0.3 (3) | 0.7 ± 0.1 (3) | 1.2 ± 0.6 (3) | 1.1 ± 0.3 (3) | 1.3 ± 0.6 (3) | 0.7 ± 0.2 (3) | 1.0 ± 0.4 (3) | 1.1 ± 0.5 (3) | 0.9 ± 0.3 (3) | 0.9 ± 0.2 (3) | 0.9 ± 0.3 (3) | 0.6 ± 0.4 (3) | 1.0 ± 0.3 (3) | 0.8 ± 0.3 (3) |
| $C_{TM1}$ | 0.9 ± 0.1 (3) | 0.8 ± 0.1 (3) | 0.9 ± 0.3 (3) | 0.9 ± 0.1 (3) | 0.8 ± 0.1 (3) | 1.0 ± 0.2 (3) | 0.8 ± 0.1 (3) | 0.6 ± 0.1 (3) | 0.7 ± 0.1 (3) | 0.8 ± 0.1 (3) | 0.8 ± 0.1 (3) | 0.9 ± 0.2 (3) | 0.7 ± 0.1 (3) | 0.6 ± 0.1 (3) | 2.6 ± 0.2 (3) | 1.8 ± 0.1 (3) |
| $C_{TM2}$ | 3.3 ± 0.5 (4) | 31.2 ± 6.1 (4) | 37.5 ± 7.4 (3) | 33.4± 5.4 (3) | 6.3 ± 1.0 (3) | 7.5 ± 0.9 (4) | 28.1 ± 4.9 (4) | 19.9 ± 0.8 (4) | 8.7 ± 0.7 (4) | 22.7 ± 5.6 (4) | 14.8 ± 3.7 (4) | 11.1 ± 4.6 (4) | 18.4 ± 4.5 (4) | 8.1 ± 2.0 (4) | 4.7 ± 0.3 (3) | 24.4 ± 5.5 (3) |
| $C_{TM3}$ | 0.8 ± 0.3 (3) | 0.9 ± 0.4 (3) | 0.6 ± 0.2 (3) | 0.9 ± 0.4 (3) | 0.8 ± 0.3 (3) | 0.7 ± 0.3 (3) | 0.8 ± 0.4 (3) | 0.7 ± 0.3 (3) | 0.9 ± 0.4 (3) | 1.0 ± 0.3 (3) | 0.8 ± 0.3 (3) | 0.5 ± 0.2 (3) | 0.6 ± 0.2 (3) | 1.0 ± 0.5 (3) | 1.1 ± 0.4 (3) | 1.0 ± 0.3 (3) |
| $C_{TM4}$ | 2.0 ± 0.7 (3) | 42.9 ± 3.3 (3) | 57.6 ± 16.4 (3) | 38.9 ± 6.9 (3) | 7.4 ± 0.5 (3) | 6.8 ± 0.6 (3) | 27.5 ± 1.3 (3) | 20.6 ± 1.9 (3) | 6.0 ± 0.6 (3) | 23.2 ± 4.5 (3) | 15.2 ± 2.1 (3) | 12.9 ± 3.4 (3) | 26.3 ± 5.8 (3) | 10.9 ± 2.3 (3) | 4.2 ± 0.8 (3) | 23.7 ± 4.6 (3) |
| $C_{TM5}$ | 2.3 ± 0.5 (4) | 28.1 ± 6.4 (4) | 29.4 ± 4.8 (3) | 25.4 ± 5.2 (3) | 7.1 ± 1.6 (3) | 10.6 ± 1.4 (4) | 28.3 ± 6.1 (4) | 16.9 ± 1.8 (4) | 5.5 ± 1.2 (4) | 22.1 ± 5.9 (4) | 15.9 ± 3.8 (4) | 5.8 ± 1.9 (4) | 8.6 ± 1.7 (4) | 5.2 ± 1.1 (4) | 3.2 ± 1.6 (3) | 28.4 ± 6.1 (3) |
| $C_{TM6}$ | 1.0 ± 0.6 (3) | 0.7 ± 0.3 (3) | 0.7 ± 0.4 (3) | 0.7 ± 0.3 (3) | 1.5 ± 0.3 (3) | 1.1 ± 0.2 (3) | 0.9 ± 0.4 (3) | 0.6 ± 0.3 (3) | 0.5 ± 0.3 (3) | 0.7 ± 0.3 (3) | 0.7 ± 0.1 (3) | 1.1 ± 0.2 (3) | 0.9 ± 0.1 (3) | 0.6 ± 0.3 (3) | 1.0 ± 0.4 (3) | 0.8 ± 0.3 (3) |
| $C_{TM6i}$ | 1.1 ± 0.6 (3) | 3.1 ± 0.8 (3) | 3.1 ± 0.5 (3) | 2.7 ± 0.6 (3) | 1.4 ± 0.4 (3) | 1.2 ± 0.3 (3) | 2.5 ± 0.9 (3) | 2.5 ± 1.0 (3) | 1.1 ± 0.3 (3) | 4.4 ± 0.6 (3) | 2.7 ± 0.5 (3) | 2.0 ± 0.5 (3) | 1.9 ± 0.1 (3) | 0.8 ± 0.1 (3) | 2.0 ± 0.9 (3) | 1.7 ± 0.6 (3) |
| $C_{TM6o}$ | 0.8 ± 0.2 (3) | 1.6 ± 0.5 (3) | 2.7 ± 0.7 (3) | 2.0 ± 0.9 (3) | 0.8 ± 0.2 (3) | 1.3 ± 0.1 (3) | 0.8 ± 0.3 (3) | 0.9 ± 0.3 (3) | 0.8 ± 0.3 (3) | 2.3 ± 0.6 (3) | 1.6 ± 0.3 (3) | 1.2 ± 0.5 (3) | 1.0 ± 0.2 (3) | 1.1 ± 0.2 (3) | 1.1 ± 0.4 (3) | 0.7 ± 0.3 (3) |
| $C_{TM7}$ | 1.3 ± 0.1 (3) | 5.2 ± 0.2 (3) | 5.1 ± 0.4 (3) | 5.2 ± 0.4 (3) | 1.3 ± 0.4 (3) | 2.1 ± 0.9 (3) | 1.5 ± 0.3 (3) | 2.1 ± 0.6 (3) | 3.2 ± 0.5 (3) | 4.5 ± 0.5 (3) | 4.3 ± 0.8 (3) | 2.5 ± 0.8 (3) | 6.0 ± 0.8 (3) | 1.2 ± 0.4 (3) | 1.2 ± 0.1 (3) | 0.8 ± 0.1 (3) |
| $C_{TM1/7}$ | 1.1 ± 0.1 (3) | 2.5 ± 0.8 (3) | 3.1 ± 0.9 (3) | 3.1 ± 0.6 (3) | 1.4 ± 0.3 (4) | 1.1 ± 0.2 (3) | 1.4 ± 0.2 (3) | 0.8 ± 0.1 (3) | 1.6 ± 0.4 (3) | 2.8 ± 0.6 (3) | 2.3 ± 0.6 (3) | 1.5 ± 0.5 (3) | 3.0 ± 0.7 (3) | 1.5 ± 0.4 (3) | 1.1 ± 0.1 (3) | 0.9 ± 0.1 (3) |
| $C_{TM2/3}$ | 0.9 ± 0.4 (3) | 0.9 ± 0.3 (3) | 0.9 ± 0.4 (3) | 1.2 ± 0.4 (3) | 1.0 ± 0.6 (3) | 1.1 ± 0.3 (3) | 1.1 ± 0.3 (3) | 1.0 ± 0.5 (3) | 1.0 ± 0.3 (3) | 1.4 ± 0.7 (3) | 1.0 ± 0.1 (3) | 1.1 ± 0.3 (3) | 1.2 ± 0.5 (3) | 1.1 ± 0.5 (3) | 0.9 ± 0.2 (3) | 1.0 ± 0.4 (3) |
| $C_{TM2/4}$ | 2.5 ± 0.5 (5) | 38.8 ± 4.2 (5) | 43.2 ± 15.6 (3) | 34.9 ± 9.5 (3) | 12.6 ± 5.8 (3) | 8.2 ± 1.7 (5) | 30.9 ± 4.9 (5) | 24.7 ± 2.9 (5) | 6.0 ± 1.4 (5) | 26.6 ± 5.8 (5) | 24.2 ± 4.2 (5) | 18.4 ± 5.0 (5) | 21.6 ± 3.2 (5) | 9.5 ± 2.4 (5) | 2.2 ± 0.3 (3) | 22.0 ± 4.6 (3) |
| $C_{TM2/5}$ | 2.7 ± 0.5 (5) | 45.2 ± 8.1 (5) | 33.3 ± 8.6 (3) | 37.2 ± 7.8 (3) | 11.7 ± 2.0 (3) | 15.7 ± 3.5 (5) | 42.8 ± 5.2 (5) | 35.5 ± 4.1 (5) | 7.9 ± 1.5 (5) | 31.8 ± 3.6 (5) | 32.9 ± 5.4 (5) | 12.2 ± 2.9 (5) | 11.2 ± 1.3 (5) | 11.3 ± 1.7 (5) | 3.1 ± 0.4 (3) | 26.4 ± 2.3 (3) |
| $C_{TM3/6}$ | 1.2 ± 0.4 (3) | 0.8 ± 0.3 (3) | 0.7 ± 0.2 (3) | 1.3 ± 0.4 (3) | 0.9 ± 0.1 (3) | 1.1 ± 0.4 (3) | 1.2 ± 0.2 (3) | 0.8 ± 0.2 (3) | 1.0 ± 0.3 (3) | 1.0 ± 0.2 (3) | 0.8 ± 0.1 (3) | 0.9 ± 0.3 (3) | 0.9 ± 0.1 (3) | 1.0 ± 0.2 (3) | 1.4 ± 0.3 (3) | 1.0 ± 0.3 (3) |
| $C_{TM4/5}$ | 2.0 ± 0.5 (5) | 41.2 ± 9.1 (5) | 18.4 ± 2.7 (3) | 26.7 ± 5.1 (3) | 6.2 ± 2.2 (3) | 8.9 ± 2.7 (5) | 47.4 ± 11.4 (5) | 26.2 ± 7.4 (5) | 7.4 ± 2.1 (5) | 30.0 ± 7.3 (5) | 30.1 ± 9.0 (5) | 16.1 ± 5.1 (5) | 13.8 ± 2.7 (5) | 7.3 ± 0.9 (5) | 2.1 ± 0.5 (3) | 23.5 ± 6.8 (3) |
| $C_{TM2/4/5}$ | 1.6 ± 0.4 (5) | 50.0 ± 12.0 (5) | 12.8 ± 2.4 (3) | 43.3 ± 3.7 (3) | 2.4 ± 0.8 (3) | 2.8 ± 0.5 (5) | 50.9 ± 12.1 (5) | 29.1 ± 7.3 (5) | 5.2 ± 1.7 (5) | 47.6 ± 13.1 (5) | 40.7 ± 10.2 (5) | 32.2 ± 11.4 (5) | 10.3 ± 1.5 (5) | 5.0 ± 1.0 (5) | 1.9 ± 0.2 (3) | 4.4 ± 1.2 (3) |
| $C_{TM2/3/4/5}$ | 0.4 ± 0.1 (3) | 0.3 ± 0.1 (3) | 0.4 ± 0.2 (3) | 0.3 ± 0.1 (3) | 0.5 ± 0.1 (3) | 0.6 ± 0.3 (3) | 0.4 ± 0.1 (3) | 0.3 ± 0.1 (3) | 0.9 ± 0.6 (3) | 0.6 ± 0.2 (3) | 0.4 ± 0.2 (3) | 0.3 ± 0.1 (3) | 0.2 ± 0.1 (3) | 0.8 ± 0.5 (3) | 0.5 ± 0.1 (3) | 0.3 ± 0.2 (3) |
| $C_{TM2/4/5/6}$ | 0.7 ± 0.2 (3) | 0.6 ± 0.1 (3) | 0.6 ± 0.2 (3) | 0.8 ± 0.1 (3) | 0.6 ± 0.2 (3) | 0.8 ± 0.1 (3) | 0.7 ± 0.2 (3) | 0.5 ± 0.1 (3) | 0.7 ± 0.4 (3) | 0.7 ± 0.3 (3) | 0.5 ± 0.1 (3) | 0.7 ± 0.2 (3) | 0.9 ± 0.3 (3) | 0.7 ± 0.3 (3) | 0.9 ± 0.2 (3) | 0.9 ± 0.1 (3) |
| $C_{TM1/2/4/5/7}$ | 2.4 ± 0.1 (3) | 3.4 ± 0.5 (3) | 1.9 ± 0.6 (3) | 4.2 ± 0.7 (3) | 2.9 ± 1.3 (3) | 4.4 ± 1.5 (3) | 2.4 ± 0.5 (3) | 1.9 ± 0.6 (3) | 3.3 ± 1.1 (3) | 9.6 ± 2.7 (3) | 25.3 ± 6.4 (3) | 11.6 ± 2.3 (3) | 13.9 ± 2.1 (3) | 2.3 ± 0.8 (3) | 4.2 ± 1.5 (3) | 3.0 ± 1.3 (3) |
| $C_{TM2-7}$ | 0.6 ± 0.1 (3) | 1.2 ± 0.1 (3) | 1.2 ± 0.2 (3) | 1.0 ± 0.2 (3) | 0.9 ± 0.2 (3) | 1.2 ± 0.3 (3) | 1.2 ± 0.2 (3) | 0.9 ± 0.1 (3) | 1.3 ± 0.2 (3) | 1.4 ± 0.2 (3) | 1.6 ± 0.1 (3) | 1.3 ± 0.2 (3) | 0.9 ± 0.1 (3) | 1.1 ± 0.1 (3) | 1.3 ± 0.1 (3) | 1.3 ± 0.2 (3) |
| $C_{TM1-6}$ | 1.3 ± 0.5 (3) | 1.1 ± 0.5 (3) | 1.2 ± 0.5 (3) | 0.7 ± 0.3 (3) | 1.2 ± 0.2 (3) | 1.3 ± 0.6 (3) | 1.1 ± 0.4 (3) | 1.0 ± 0.4 (3) | 1.3 ± 0.4 (3) | 1.3 ± 0.4 (3) | 1.2 ± 0.4 (3) | 1.1 ± 0.4 (3) | 1.1 ± 0.5 (3) | 0.8 ± 0.1 (3) | 1.3 ± 0.3 (3) | 0.8 ± 0.2 (3) |
| $C_{TM2-6}$ | 1.4 ± 0.3 (3) | 1.3 ± 0.3 (3) | 1.0 ± 0.4 (3) | 1.4 ± 0.5 (3) | 1.0 ± 0.3 (3) | 1.4 ± 0.4 (3) | 1.7 ± 0.5 (3) | 1.2 ± 0.3 (3) | 1.1 ± 0.4 (3) | 1.6 ± 0.7 (3) | 1.3 ± 0.6 (3) | 1.7 ± 0.6 (3) | 1.1 ± 0.4 (3) | 1.1 ± 0.2 (3) | 1.3 ± 0.4 (3) | 1.3 ± 0.7 (3) |