

Supplemental Data

Regulation of M₃ Muscarinic Receptor Expression and Function by Transmembrane
Protein 147

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Supplemental Table 1

M3R-interacting proteins identified in a membrane-based yeast two-hybrid screen

Protein	Accession No.
Tetraspanin family	
CD82	AAB23825
CD9	P21926
CD63	AAV38940
Ubiquitin-associated proteins	
Small ubiquitin-related modifier 2 precursor (SUMO-1)	AAC50996
Small ubiquitin-related modifier 2 precursor (SUMO-2)	P61956
UBC protein	AAH08955
Ubiquitin C	NP_066289
Receptor proteins/Transmembrane proteins	
Transmembrane protein 147	AAH01118 / BC001118
G protein-coupled receptor 37	NP_005293
Homo sapiens G protein-coupled receptor, family C, group 5, member B (GPRC5B)	NM_016235 / AAF05331
Rhodopsin	NP_000530
Aquaporin-4 (AQP-4)	P55087
Glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1	NP_001009184
Sodium channel, voltage gated, type VIII, alpha	NP_055006
Transmembrane 9 superfamily member 2	CAH71381
Transmembrane protein 14A	AAH19328
Signaling molecules	
Phosphatidic acid phosphatase type 2C	AAP35667
Calmodulin 2	AAH08437
Protein kinase Njmu-R1	AAH54035
2',3'-Cyclic nucleotide 3' phosphodiesterase (CNP)	AAH06392
Solute carrier proteins	
Solute carrier family 39 (zinc transporter), member 3 isoform a	NP_653165
Solute carrier family 22 (organic cation transporter), member 17 isoform b	NP_057693
Solute carrier family 31 (copper transporters), member 2	NP_001851
Solute carrier family 35, member B1	NP_005818
FXFD domain-containing ion transport regulator 6	NP_071286

PLP domain-containing proteins

Glycoprotein M6B isoform 1 variant	BAD92762
Proteolipid protein 1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomplicated)	CAA98191

Signal sequence proteins

Homo sapiens signal sequence receptor, gamma (translocon-associated protein gamma) [synthetic construct]	AAP36250
Signal sequence receptor gamma subunit	AAH17203
Signal peptidase complex subunit 1 homolog	NP_054760
Signal peptidase 12 kDa subunit	AAL31361

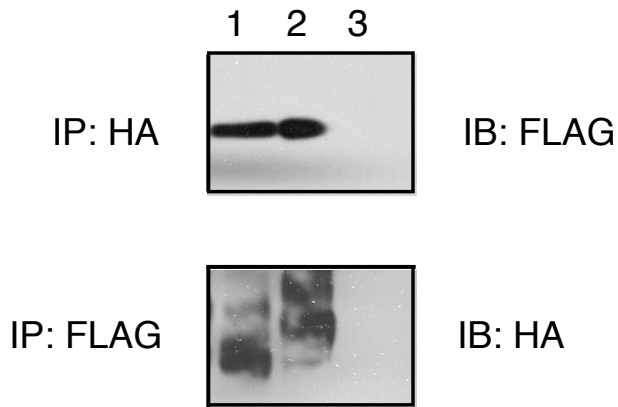
Other proteins

Nogo-A protein short form	AAG40878
LAG1 longevity assurance homolog 2, isoform 1	AAH10032
FLJ20489 protein	AAH65033
Yip1 domain family, member 6	NP_776195
HCV F-transactivated protein 1	AAT35812
BCL2/adenovirus E1B 19kDa interacting protein 3-like	AAV38308
Growth-inhibiting gene 5 protein	AAS00486
B-cell receptor-associated protein 31 variant (BAP31)	BAD96214
Delta-notch-like EGF repeat-containing transmembrane	NP_620711
Claudin 5	AAP35918
Immediate early response 3 interacting protein 1	NP_057181
BAX inhibitor 1	AAU29521
Tumor differentially expressed protein 1 variant	BAD96643
HSPC288	AAF28966
Leptin receptor overlapping transcript-like 1	AAH00642
Interferon induced transmembrane protein 1	AAH00897
Homolog of yeast long chain polyunsaturated fatty acid elongation	AAH67123
BM88 antigen	AAH34732
Thymopoietin isoform gamma	NP_001027455
Myelin and lymphocyte protein (T-lymphocyte maturation-associated protein)	P21145
Ovarian carcinoma immunoreactive antigen	AAG45220
Syntaxin 8	NP_004844
ZMYM6 protein	AAH29439
β -amyloid precursor-like protein 2 (APLP-2)	AAA35526
Prostaglandin D2 synthase 21kDa	CAI12758
Intercellular adhesion molecule 2 precursor variant	BAD93115
Thymic dendritic cell-derived factor 1	AAH16374
NADH dehydrogenase subunit 4	ABB91083
NADH dehydrogenase subunit 4L	AAX53836
Lysosomal-associated protein transmembrane 4 alpha	AAH00421
Sterol-C4-methyl oxidase-like isoform 1	NP_006736

Dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit, isoform 1	AAH15233
Histone deacetylase-like protein	CAA09893
ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit	AAI06882
ATPase, H ⁺ transporting, lysosomal 21kDa, V0 subunit c"	CAI16801
ATP synthase F0 subunit 6	AAU02441
Selenoprotein K	Q9Y6D0

D.melanogaster	MTLYHFGNCVALL-TPYYFTYKYSGLSEYGAFWKCVQAGGIYIFTQLVKMLVLATFFYS	59
Anopheles	MTLYHFGNCAALVYVPYYFTYKYSGLSEYGAFWKCVQAGGIYVFTQLCKMLVLATFFPDT	60
Xenopus	MTLFHFGNCFALAYFPYFITYKCSGLSEYNAFWRCVQAGATYLCVQLCKMLFLATFFPTW	60
Zebrafish	MTLFHFGNCFALAYFPYFITYKCSGLSEYNAFWRCVQAGATYLFVRLCKMLFLATFFPTW	60
Human	MTLFHFGNCFALAYFPYFITYKCSGLSEYNAFWRCVQAGVTYLFVQLCKMLFLATFFPTW	60
Bovine	MTLFHFGNCFALAYFPYFITYKCSGLSEYNAFWRCVQAGVTYLFVQLCKMLFLATFFPTW	60
Mouse	MTLFHFGNCFALAYFPYFITYKCSGLSEYNAFWRCVQAGVTYLFVQLCKMLFLATFFPTW	60
C.elegans	MSFFHFINCFALAFAPYFIVYKYSGLINEYSSIWKCATASGGYLLTQLAKLLIIATFFPAL	60
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D.melanogaster	APSSSGE-FNFFAEILRCSMDIADLLGFALILS-RIPGKGHSLKITAGLGWATAEVIISR	117
Anopheles	DTFTEGAPFNFAEFLRCSVDLDFLGLAFVLS-RIPGKGHSLKITAGLGWATAEVIISR	119
Xenopus	EGA--AGAYDFIGEFMKATVDLADLLGLHLVMS-RNAGKGEYKIMVAAMGWATAELVMSR	117
Zebrafish	EGG--AGVYDFVGEFMKATVDMADLLGLHLVMS-RNAGKGEYKIMVAAMGWATAELIMSR	117
Human	EG---GIYDFIGEFMKASVDVADLIGLNLVMS-RNAGKGEYKIMVAALGWATAELIMSR	115
Bovine	EG---GIYDFIGEFMKASVDVADLIGLNLVMS-RNAGKGEYKIMVAALGWATAELIMSR	115
Mouse	EG---GIYDFIGEFMKASVDVADLIGLNLVMS-RNAGKGEYKIMVAALGWATAELIMSR	115
C.elegans	DS---EGFSIVPEFLKSSADIIDVIGLHLLMTNFLAGKGEVRFVVGGLGWGFAHVAHR	116
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D.melanogaster	GIMLWVGARGTEFSWIYILKCLESNVLLVQHITATLIWLFTRHDLNALKPLVSLLLAV	177
Anopheles	GLMLWVGARGAEFSWLYIQKCLESNVLLVQHLSTATLLWLFSTRHDLDRKLVPLVLLVA	179
Xenopus	CLPLWVGARGIEFDWKYIQMSIDSNISLVHYMAVAALVMMWTRYDLPHTYRLPVTVLLGL	177
Zebrafish	CIPLWVGARGIEFDWKYIQMSFDSNISLVHYIAMAAVVMFTRYDLPKSFRLPVAILLGL	177
human	CIPLWVGARGIEFDWKYIQMSIDSNISLVHYIVASAQVMMITRYDLYHTFRPAVLLLMFL	175
Bovine	CIPLWVGARGIEFDWKYIQMSIDSNISLVHYIVASAQVMMITRYDLYHTYRPAVLLLMFL	175
Mouse	CIPLWVGARGIEFDWKYIQMSIDSNISLVHYIVASAQVMMITRYDLYHTFRPAVLLLMFL	175
C.elegans	LVLLWVGARGTAFTWRVQTSLDSSADLLVIVSLACLWMTIR----TPNKFVSPILAM	172
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D.melanogaster	TVFKGVWLEGMLHILTIGPWLTVAVKALVAAVIGFCTLHIYSGLAQQIGI---	227
Anopheles	TSYRGVWLEGLTHAMSAGPWLSLALKALITSCMGVGLTHIYSGLAQQIGL---	229
Xenopus	SMYKAFLMDCFVHMFIMGSWTALLKAVITGVLSLSCLTLFVSLVHGN-----	225
Zebrafish	CVYKGFLELFLVHVFLGSGWTALLVKAVLTGALSLSLFLFVTLVHSN-----	225
Human	SVYKAFVMETFVHLCSLGSWAALLARAVVTGLLALSTLALYVAVVNVHS----	224
Bovine	SVYKAFVMETFVHLCSLGSWTALLARALVTGLLALSTLALYVAVVNVHS----	224
Mouse	SVYKAFVMETFVHLCSLGSWTALLARAVVTGLLALSTLALYVAVVNVHS----	224
C.elegans	CVFSTFVYQTVQHTFSLYGWLLAFRFAYSIATAILTVVVYSANRTASTRKNE	225
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Supplemental Figure 1. Tmem147 is highly conserved among species. Human Tmem147 has 99% amino acid homology to bovine and mouse Tmem147, 78% homology to the corresponding zebrafish and *X. laevis* proteins, and ~50% homology to *Anopheles*, *D. melanogaster*, and *C. elegans* Tmem147. "*", amino acids are identical in all sequences in the alignment; ":", conserved substitutions occur at the indicated positions; ".", semi-conserved substitutions are observed at the indicated positions.



Supplemental Figure 2. Co-immunoprecipitation of Tmem147 with M₁ muscarinic or V₂ vasopressin receptors in co-transfected COS-7 cells. Co-immunoprecipitation studies were carried out as described in the legend to Fig. 4 and under Materials and Methods. Lysates were prepared from COS-7 cells that had been co-transfected with Tmem147-FLAG and HA-tagged versions of the M₁ muscarinic or the V₂ vasopressin receptors. Immunoprecipitates were analyzed via Western blotting using an anti-FLAG (upper panel) or an anti-HA antibody (lower panel), respectively. COS-7 cells were co-transfected with either Tmem147-FLAG and the M₁ receptor construct (lane 1), Tmem147-FLAG and the V₂ receptor construct (lane 2), or Tmem147-FLAG and vector DNA (lane 3). Note that the anti-HA antibody detected multiple immunoreactive receptor bands ranging in size from ~60-80 kDa, most likely to heterogeneous glycosylation of the receptors.