Chapter 2

Prediction of the 3D Structure for FMRF-amide Peptides Bound to Mouse MrgC11 Receptor with Subsequent Experimental Verification¹

2.1 Introduction

G protein-coupled receptors (GPCRs) play an essential role in cell communications and sensory functions as mentioned in chapter 1. Consequently they are involved in wide variety of diseases and are targets for many drug therapies. Particularly important is the large number of orphan GPCRs (for which the native ligands remain unknown), which may play important, albeit unknown, functions in various cells. To understand their respective physiological roles, it is important to identify their endogenous ligands, and to find small molecule ligands that would serve as selective agonists or antagonists. One example here is the family of GPCRs called the Mas-related gene (Mrg) receptor for mouse or the sensory neuron specific receptor (SNSR) in mouse and human[1, 2]. A subset of these receptors including mMrgC11 and mMrgA1 is localized mainly to isolectin B4⁺, the small diameter nociceptors in the dorsal root ganglia (DRG). Dong et al. showed that some of these receptors were activated by RFamide neuropeptides such as NPFF and NPAF and suggested them to be involved in pain sensation or modulation[1]. These Mrg receptors have been paired with structurally diverse transmitter peptides[3].

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Clearly deorphanization would be greatly aided by having three-dimensional (3D) structures of the orphan receptors to help select the most promising new ligands for experimental assays, but it is not yet possible to obtain experimental 3D structures for human GPCRs. Consequently our group developed the MembStruk computational method[4, 5] to predict such structures and we demonstrate in this study that the predicted structures are sufficiently accurate to predict binding sites and relative binding energies. Previously MembStruk was applied to several GPCRs, obtaining ligand binding sites in excellent agreement with experiments. However in these studies the structural data were known prior to our calculations. Although any experimental data were not utilized in making our predictions, such validations are not completely convincing. We undertook this study on mMrgC11 and mMrgA1 receptors for the specific purpose of validating the MembStruk method. Thus prior to our calculations there were no data on how mutations affect binding. In addition the experiments had shown that the F-M-R- $F-NH_2$ (D) $F-M-R-F-NH_2$ and $F-(D)M-R-F-NH_2$ tetrapeptides activate mMrgC11 receptor at ~100 nM concentration, while F-M-(D)R-F-NH2 and F-M-R-(D)F-NH2 are inactive (>10 µM). We assumed that explaining such an effect of chirality on binding should provide a strong test of the predicted structures.

2.2 Computational methods

All energy and force calculations were done using DREIDING force field (FF)[6] with the charges from CHARMM22[7] FF and were executed in the molecular dynamics program, MPSIM[8]. The cell multipole method[9] was used for the calculation of nonbond interaction. Unless otherwise specified all simulations were performed in gas phase with the dielectric constant of 2.5.

2.2.1 Structure predictions of the Mrg receptor

The 3D structure of the mMrgC11 and mMrgA1 receptors were predicted independently using MembStruk (version 4.05)[10]. The details of the MembStruk (version 3.5) were described

	TM1	
mMrgC11	MDPTISSHDTESTPLN-ETGHPNCTPILTLSFLVLITTLVGLAGNTIVLWLLGFRMRRKA	59
mMrgA1	MDNTIPGGINITILIPNLMIIIFGLVGLTGNGIVFWLLGFCLHRNA 	46
mMrgC11	ISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNAAIIPYISGLSILSA	119
mMrgAl	FSVYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTIMMVLYIAGLSMLSA	103
	[†] TM4 [†]	
mMrgC11	ISTERCLCVLWPIWYHCHRPRNMSAIICALIWVLSFLMGILDWF-SGFLGETHHH-LWKN	177
mMrgA1	ISTERCLSVLCPIWYHCHRPEHTSTVMCAVIWVLSLLICILNSYFCGFLNTQYKNENGCL	163
	TM5 TM6	
mMrgC11	-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIALTVMVYLICGLPLGL	236
mMrgA1		
-	ALNFFTAAYLMFLFYVLCLSSLALVARLFCGTGQIKLTRLYVTIILSILVFLLÇGLPFGI	223
-	ALNFFTAAYLMFLFYVLCLSSLALVARLFCGTGQIKLTRLYVTIILSILVFLLCGLPFGI TM7	223
mMrgC11	ALNFFTAAYLMFLFYVLCLSSLALVARLFCGTGQIKLTRLYVTIILSILVFLLCGLPFGI TM7 YLFLLYWFGVHLHYPFCHIYQVTAVLSCVNSSANPIIYFLVGSFRQHRKHRSLKRVLKRA	223 296
mMrgC11 mMrgA1	ALNFFTAAYLMFLFYVLCLSSLALVARLFCGTGQIKLTRLYVTIILSILVFLLCGLPFGI TM7 YLFLLYWFGVHLHYPFCHIYQVTAVLSCVNSSANPIIYFLVGSFRQHRKHRSLKRVLKRA HWFLLFKIKDDFHVFDLGFYLASVVLTAINSCANPIIYFFVGSFRHRLKHQTLKMVLQNA	223 296 283
mMrgC11 mMrgA1 mMrgC11	ALNFFTAAYLMFLFYVLCLSSLALVARLFCGTGQIKLTRLYVTIILSILVFLLCGLPFGI TM7 YLFLLYWFGVHLHYPFCHIYQVTAVLSCVNSSANPIIYFLVGSFRQHRKHRSLKRVLKRA HWFLLFKIKDDFHVFDLGFYLASVVLTAINSCANPIIYFFVGSFRHRLKHQTLKMVLQNA LEDTPEEDEYTDSHLHKTTEISESRY 322	223 296 283

Figure 2.1 Predicted transmembrane (TM) regions. The sequence alignment of mMrgC11 and mMrgA1 is based on the alignment with the entire set of sequences obtained by BLAST search with the mMrgC11 sequence (see Fig. S2.1). The hydrophobic center of each TM is indicated with an arrow. The residues involved in the mutagenesis experiment are highlighted.

in reference 10. Here we outline the procedure, highlighting aspects also relevant to Mrg receptors or that were improved in version 4.05.

Prediction of transmembrane regions

The transmembrane (TM) regions and the hydrophobic maximum for each TM helix were predicted using the TM2ndS method[5]. We used NCBI BLAST[11] to search the non redundant protein database to find sequences homologous to the mMrgC11 receptor with bit scores greater than 200. These 27 sequence hits had sequence identities to mMrgC11 ranging from 41% to 88%. This set of sequences included the mMrgA1 receptor whose sequence identity to mMrgC11 is 44%. Twenty-two of these 27 sequences belong to Mrg receptor family, with remaining 5 corresponding to unnamed GPCRs. We then carried out a multiple sequence alignment with these 27 sequences using ClustalW[12]. These results (Fig. S2.1) were used as input to TM2ndS. The hydrophobicity profile (Fig. S2.2) resulting from TM2ndS had no clear separation between TM2 and TM3, leading to uncertainty in the boundaries between TM2 and TM3. A similar ambiguity

was observed between TM6 and TM7. To eliminate such problems, the MembStruk 4.05 procedure calculates the hydrophobicity profile from a second round of seven TM predictions in which each sequence of the core of the seven TM (15 amino acids around the hydrophobic center) was used as a template. This second set of independent BLAST searches was executed under high gap penalty with each TM core. Here we selected GPCR sequences with sequence identities of >50% (the identity with the entire sequence of mMrgC11 was as low as 23%), see table S1. Then a second round of TM predictions was performed using the multiple sequence alignment of these 7 sets of sequences, see Table S1. The final refined TM region and its hydrophobic center for each of the 7 TM domains were determined from this second round of prediction. For mMrgA1 we used the same TM regions as assigned from alignment with mMrgC11.

Assembly of TM helical bundle

For each TM domain we built canonical α -helices with fully extended conformation of side chains. These were assembled such that the 7 predicted hydrophobic centers are all in the xy plane with the x and y coordinates adapted from the 7.5 Å electron density map of frog rhodopsin[13]. Each helix oriented about its axis so that its hydrophobic moment pointed away from the center of the seven helices (toward the membrane). The tilt of each helix with respect to the z axis and its azimuthal angle were adapted from the 7.5 Å electron density map of frog rhodopsin[13].

Then we carried out 200 ps of molecular dynamics (MD) at 300 K without solvent or lipid, but with charged side chains neutralized by adding Na^+ or Cl^- ions. This allows the conformation of each individual helix to bend or kink as appropriate. We then selected the snapshot with the lowest potential energy from the last 100 ps of the MD trajectory and the net hydrophobic moment was calculated for the middle 15 residues around the hydrophobic center for each helix using this conformation. Each helix was rotated again so that its hydrophobic moment faces toward the membrane. This hydrophobicity-based rotation works well for the six TM helices with extensive contacts to lipid bilayers. Moreover, since the optimal orientation of a helix depends on the relative orientations of the neighboring helices, we often carry out a combinatorial rotation of the 7 helices. However we found that the structure predicted by the above process placed the highly conserved Asn44-Asp71 pair between TM1 and TM2 and the Asn66-Trp151 pair between TM2 and TM4 close enough to form hydrogen bonds (based only on the coarse hydrophobicitybased rotation step). Therefore we carried out extensive 360° rotational orientation optimization only for TM3, TM5 and TM6. Here the rotational angle of TM3 was scanned for 360° (in 30° increments) because TM3 has the least surface area exposed to lipid, but TM5 and 6 were rotated only over the range of -60° to 60° since the orientation had already been optimized roughly using the hydrophobic moment. For every rotation we reassigned the side chain conformation using SCWRL3.0[14] before energy-minimization. The orientation with the best energy was then selected. The results of these scans are shown in Table S2. The rotational orientation of TM7 was scanned over 360° in 5° increments, where for each angle all atoms were optimized. In fact the initial orientation showed the best energy.

Rigid body dynamics in lipid bilayers and addition of loops

Next we added two layers of explicit lipid molecules (52 molecules of dilauroylphosphatidyl choline (DPC) lipid) surrounding the TM bundle. The initial structures for the lipid DPC these were based on the crystal structure in Cambridge Structural Database (ID: LAPETM10). To achieve proper packing of the TM helices, the 7-helix-lipid complex was optimized using rigid body MD for 50 ps where each helix and lipid molecule was treated as a rigid body, with just 6 degrees of freedom (translation and rotation).

The conformation of each TM helix was further optimized in the lipid environment with full atom Cartesian MD simulation for 50 ps while the coordinates of lipid molecules were kept fixed. Then we carried out an additional equilibration of the whole system for 40 ps and selected the structure with the lowest potential energy. For this structure each side chain conformation was re-assigned using SCWRL and the bundle (helices plus lipid) was minimized to an RMS force of 0.5 (kcal/mol)/Å using conjugate gradients.

The loops were added to the helices using MODELLER6v2[15]. The side chains were reassigned using SCWRL and subsequently a full atom conjugate gradient minimization of the receptor was performed.

In many GPCRs (including bovine rhodopsin and the catechol amine receptors, such as dopamine and adrenergic receptors) there are conserved cysteines near the top of TM3 and in the second extracellular loop (EC2) that are expected to form a disulfide bond leading to a closed loop. However the mMrgC11 and mMrgA1 receptors do *not* contain such cysteines so the loops were allowed to remain in an open conformation. From five loop structures generated with MODELLER6v2 we selected the one with the lowest internal strain and then optimized the coordinates using annealing MD while keeping the coordinates of TM helices fixed. In this process the system was heated from 50 K to 600 K and cooled down back to 50 K in 50 K steps, with 1 ps of equilibration between the temperature jumps. At the end of the annealing cycle the structure was fully optimized using the conjugate gradients. This final structure shown in Figure 2.3 (top and side views) was used for all docking studies.

2.2.2 Docking predictions with peptide ligands

Using the 3D structure of the mMrgC11 structure we used a refined version (MSCDock) of the docking procedure described in Cho et al.[16]. Since peptide ligands are highly flexible we modified the step in HierDock2.0 (described in Vaidehi et al.[4]), involving scan of the entire receptor with RFa to locate the binding site. This hierarchical docking protocol to predict the binding sites for various ligands as used in this study is described below.

Scanning of the binding sites



Figure 2.2 The scanning regions used to determine the binding sites for the mMrgC11 receptor. (a) The 9186 Spheres generated with SPHGEN to fill the void spaces of the receptor. The 40 cubic boxes used for docking are shown. (b) The four regions pre-selected for the docking studies. The region enclosed by the dotted circle was identified as the best site.

The entire receptor structure was scanned with the Arg-Phe-NH₂ (RFa) dipeptide known to agonize the receptor (EC50 = 460 nM) to locate the putative binding site. First, the molecular surface was created using the autoMS utility in DOCK4.0[17] with the default values for surface density (3.0dots/Å²) and probe radius (1.4Å). Then we generated spheres from each that filled the void space in the receptor. To do this we used SPHGEN in DOCK4.0. We then constructed a total of 40 cubic boxes (sides of 10Å) and spaced by 8Å that covered this set of spheres. Assuming that the ligand binds inside the TM bundle from the extracellular region, we analyzed these

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spheres using a buried surface criterion to pre-select four non-peripheral regions, located on the upper half of the receptor as shown in Figure 2.2. The spheres inside each box were used to define the docking region as input to DOCK4.0.

The RFa ligand was docked independently into each of the four regions as follows. Since the peptide ligands have a significant number of independent dihedral angles (the smallest dipeptide, RFa, contains 10 torsions), we wanted to ensure that this extensive conformation space is sampled in the docking. Thus for each peptide ligand we used the Metropolis Monte Carlo (MC) Method in Cerius2[18] (with a MC temperature of 5000 K in vacuum) to generate a set of 1,000 low energy conformations having a diversity of CRMS = 1.0 Å.

Level0: Then for each of the 1,000 conformers we used DOCK4.0 to generate a set of 3,000 configurations within each of the four binding regions of the receptor. From these we selected the 100 best configurations for each of the 1,000 conformers based on the DOCK score. This led to a total of 100*1000 = 100,000 configurations which were combined together and saved for the next scoring step. In these configurational searches, the rigid ligand and torsion drive options in DOCK4.0 were used. The bump filter option was turned on (maximum bump = 10) and the reduced (to 75%) van der Waals radius was used.

Level1: The configurations from level0 with a ligand-buried surface area below 65% were discarded and the remaining configurations were ranked by the number of hydrogen bonds between receptor and ligand, then by the percentage of buried surface area, and then by DOCK4.0 energy score. This ordered list was trimmed using a diversity criterion of CRMS = 0.6 Å and the top 100 configurations selected. Each of these was minimized in MPSIM using 100 steps of conjugate gradient method, while the receptor coordinates were fixed.

Level2: The 10 best configurations by energy were selected from level1 and the full ligandprotein complex was minimized in MPSIM with 100 steps. The side chain rotamers for all the residues within 5 Å of the ligand were reassigned using the SCREAM side chain replacement program [Kam, Vaidehi and Goddard unpublished], which uses a side chain rotamer library of 1,478 rotamers with a diversity of 1.0 Å in coordinates.

Level3: The binding energies were then calculated for these 10 optimized ligand-receptor complex configurations. The calculated binding energy (BE) is defined by

BE = E (ligand in fixed protein) – E (ligand in water)

where the E (ligand in fixed protein is the potential energy of the ligand calculated in the ligandreceptor complex with the coordinates of the receptor fixed. This potential energy includes the internal energy of the ligand and the interaction energy of the ligand with the receptor. E (ligand in water) is the potential energy of the free ligand in its docked conformation (snap bind energy) and its solvation energy calculated using the analytical volume generalized born (AVGB) continuum solvation method[19]. In these calculations the dielectric constant was set to 78.2 for the exterior region and to 1.3 for the interior region. The final best ligand-receptor structure was selected as the one with the most negative binding energy.

Among four regions we found that the RFa ligand had the best binding energy in the region involving TM3, 4, 5 and 6 (blue in Fig. 2.2(b)), which we call the putative binding site. The best structure of the RFa-receptor complex was further refined using one cycle of annealing MD heating from 50 K to 600 K and cooling down back to 50 K in 50 K steps, with 1 ps of equilibration between the temperature jumps. Here only the ligand and the residues within 10 Å of the binding pocket (including backbone atoms) were allowed to move during the annealing cycle. At the end of the annealing cycle, the system was minimized to an RMS force of 0.3 (kcal/mol)/Å and the side chains of the residues in the receptor within 4 Å from the ligand was reassigned again with SCREAM. The spheres for docking other peptide ligands were defined with this final optimized RFa-receptor complex.

Docking of other peptide ligands

For the five F-M-R-F tetra-peptide stereoisomers, we first docked the R-F amide part of the C-terminal. This motif is common to most peptide agonists of mMrgC11[20]. Indeed the efficacy results for the five chirally modified F-M-R-F-amides (see Fig. 2.11) show that the chirality of the R-F part dramatically affects activation. Therefore we first docked the three dipeptides: acetylated R-F-NH₂, (D)R-F-NH₂ and R-(D)F-NH₂. Then we used these as an anchor in building the remaining F-M amino acids to construct the docked tetrapeptide.

For docking the three dipeptides, we used the Dock-Diversity Completeness protocol (DDCP) described in Cho et al.[16] to generate a set of diverse configurations and improve completeness in searching the configurations in DOCK (Level0). Briefly, DDCP attempts to generate a complete set of ligand configurations families with a fixed coordinate diversity (1.0 Å). Completeness is defined as the point where the fraction of new configuration that belong to previously generated families to the fraction that leads to a new family is 2.2 (but restricted the list to 5000 families). Then we selected the 50 families with the best energies (by DOCK4.0 energy score) and continued generating configurations while keeping only those that belonged to one of these 50 families until there was an average of six members in each family. Then 50 family heads (best energy in each family) were conjugate gradient minimized (100 steps or 0.1 kcal/mol/Å of RMS force) with the ligand atoms movable and the receptor atoms fixed. Then the 10 best scoring ligands (one from each family by binding energy) were selected for further side chain optimization. Here the binding energy was calculated as the difference between the energy of the ligand in the fixed receptor and the energy of the ligand in solution. The energy of the free ligand was calculated for the docked conformation and its solvation energy was calculated using surface generalized Born model (SGB)[21]. The side chain rotamers of the residues in the receptor within 5 Å of the bound ligand were reassigned by using the SCREAM side chain replacement program. After side chain optimization, the final 10 complex structures were minimized (100 steps or 0.1 kcal/mol/Å of RMS force) with all atoms movable.

The above docking procedure was applied to each of the 1,000 conformers of each peptide ligand generated using the MC Method in Cerius2 (with a MC temperature of 5,000 K in vacuum) using diversity of CRMS = 2.0 Å. Prior to docking the structures of the 1,000 conformers were minimized in gas phase and ordered by energy. Then they were re-clustered with the diversity of 2.0 Å and the conformer of each family head (the best energy among the family) was chosen for docking. This led at least 10 family heads for the R-F dipeptides and over 20 family heads for acetylated R-F dipeptides.

For each such structure the docking process ends up with 10 structures for the ligand/protein complex. Thus we obtained ~100 structures for the dipeptide and ~200 for acetylated peptides. The number of hydrogen bonds (intermolecular between receptor and ligand and intramolecular for a ligand) was calculated for each structure of each ligand/protein complex. This was combined with the binding energy and the number of hydrogen bonds to select the final best structure.

The final structure of ligand-receptor complex obtained from the hierarchical docking procedure was further refined by annealing MD as described in section 2.1.3. Here only the ligand and the side chains of residues within 3.5 Å of the binding pocket were allowed to move. At the end of the annealing cycle, the system was minimized to an RMS force of 0.1 (kcal/mol)/Å.

Building the terminal F-M residues from the bound acetylated R-F-NH₂

The conformations of the terminal F-M residues were sampled using moleculeGL, a recursive, Metropolis Monte Carlo-based rotamer design technique [Kekenes-Huskey, Vaidehi and Goddard in preparation] from the R-F-NH₂ dipeptide docked in mMrgC11 receptor where the extracellular loops were removed. Either the psi angle of Met or the phi angle of Arg is defined as an anchor. We used moleculeGL to generate 1000 structures for the terminal FM, using a diversity of 1.0. Then we selected the lowest energy conformation and minimized the ligand structure (0.3 kcal/mol/Å of RMS force) with the coordinates of receptor fixed. Then the side

chain rotamers of residues within 5 Å of the ligand were assigned using SCREAM and the structure of the whole complex was minimized. The final best structure was refined by annealing as described in previous section.

2.3 Experimental procedures

2.3.1 In vitro mutagenesis

The point mutation was incorporated into mMrgC11-GFP coding sequence in pcDNA3.1/Zeo (+) plasmid (Invitrogen) using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The mutagenic oligonucleotide primers were synthesized and purified in the oligonucleotide synthesis center of Caltech. All mutant constructs were verified by DNA sequencing. Later the wild type and mutant gene in pcDNA3.1/Zeo (+) were sub-cloned into pcDNA5/FRT expression vector (Invitrogen) for stably expressing cell lines.

2.3.2 Cell culture and transfection

Flp-InTM-293 cells (Invitrogen) were co-transfected with mMrgC11-GFP gene in pcDNA5/FRT vector and pOG44 plasmid (Invitrogen) using FuGENE-6 reagent (Roche Applied Science) according to the manufacturer's instructions. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin and L-glutamine. The cells were split into fresh medium 48 h after transfection and then selected with 400 µg/ml of hygromycin. After two weeks of selection period the hygromycin-resistant clones were picked and then maintained in the selective medium with 200 µg/ml of hygromycin.

2.3.3 Biotinylation and immunoprecipitation

Flp-InTM-293 cells stably expressing wild type and mutant receptors were placed into 10 cm culture dish coated with poly-L-lysine and cultured for 24h. The cells were washed twice with ice-cold PBS and incubated with 3 mL of 0.5 mg/mL Sulfo-NHS-LC-Biotin (Pierce

Biotechnology) in PBS supplemented with 0.1 mM HEPES, pH 7.5 at room temperature for 30 min. The biotinylation reaction was quenched by washing cells three times with Tris-buffered saline (10 mM Tris pH 7.5, 154 mM NaCl). The washed cells were incubated with 5 mL of cold lysis buffer (10 mM HEPES, pH 7.4, 1 mM EGTA) supplemented with 100 µM 4-(2-aminoethyl)-benzene sulfonyl fluoride hydrochloride at 4 °C for 15 min. Cells were scraped from the dish and homogenized with a Dounce homogenizer (20-25 strokes with a tight pestle). The cell lysate was centrifuged at 750x g for 10min at 4 °C to remove the nuclei and cell debris. The resulting supernatant was centrifuged at 75,000x g for 30 min at 4 °C. The membrane pellet was solubilized in 500 µL of ice-cold TX/G buffer (300 mM NaCl, 1% TX-100, 10% Glycerol, 1.5 mM MgCl₂, 1 mM CaCl₂, 50 nM Tris pH 7.4, 0.5 mM PMSF, protease inhibitor cocktail) and incubated with gentle mixing at 4 °C for 1h. Insoluble material was removed by centrifugation at 10,000x g for 15 min at 4 °C. The protein concentration was estimated using the *DC* Protein Assay Kit (Bio-Rad).

The solubilized protein was incubated with 50 µL of streptavidin-agarose (Pierce Biotechnology) overnight at 4 °C on an inversion wheel. The streptavidin-agarose was washed four times with ice-cold TX/G buffer in absence of protease inhibitor and then twice with ice-cold PBS. The precipitates were resuspended with protein sample buffer and then boiled for 15 min. The protein sample was analyzed by SDS-PAGE and transferred to nitrocellulose membrane. The membrane was blocked in Tris-buffered saline with 0.1% Tween 20 containing 5% non-fat milk for 1h. GFP-tagged mMrgC11 receptors were detected by blotting with anti-GFP polyclonal primary antibody (Molecular Probes) in blocking solution followed by anti-rabbit horseradish peroxidase-conjugated secondary antibody and an ECL detection kit (Amersham Biosciences).

2.3.4 Intracellular calcium assay

The cells were placed into 96-well cell culture plate coated with MATRIGEL matrix (BD Biosciences). After 16-24 h, the cells were washed twice with Hank's balanced salt solution

supplemented with 10 mM D-glucose, 20 mM HEPES and 1.6 mM NaOH (assay buffer) and loaded with 2 μ M fura-2/AM (Molecular Probes) in assay buffer at room temperature for 20min. Then the cells were washed four times with assay buffer to get rid of the residual fura-2/AM present outsides cell membranes. The fluorometric imaging plate reader (FLIPR) assay was carried out at various concentrations of peptide ligands (1 nM to 10 μ M) with the FlexStation II system (Molecular Devices). The fluorescence emitted from the excitation at 340 nm and 380 nm was measured respectively along the time and the ratio of emission at two excitation wavelengths was evaluated together. The difference between maximum and minimum value of the ratio was plotted along with the logarithm of the ligand concentration. The curve was fitted with ORIGIN6.0 software to compute EC₅₀ value.

2.4 Results and discussion

2.4.1 Characteristics of the predicted mMrgC11 receptor structure

The predicted TM regions for mMrgC11 are given in Figure 2.1 and the predicted 3D structure of the mMrgC11 receptor is shown in Figure 2.3. TM6 is bent by 28° at Pro233 and TM7 is bent by 15° at Pro271. These two prolines are highly conserved over all family A GPCRs including rhosopsin (in rhodopsin TM 6 and TM7 are bent by 24° and 33°, respectively). Moreover, Pro109 in the middle of TM3 leads to bending of 23° (in rhodopsin TM3 is bent by 13°). We find that these distortions lead to a cavity lined by TM3, TM5, and TM6 that provides the space required for binding our tetrapeptides. The remaining four TMs have relatively straight α -helical conformations.

The predicted 3-D structure of mMrgC11 receptor is superimposed with the 2.2 Å X-ray crystal structure of bovine rhodopsin[22] in Figure 2.3. Here each TM between mMrgC11 and rhodopsin was aligned separately with Clustal-W, imposing a high gap penalty and only the TM



Figure 2.3 Comparison of the predicted 3D structure for the RFa/mMrgC11 complex (green) with the X-ray crystal structure of retinal/rhodopsin (PDB code: 1U19, 2.2 Å resolution). The RFa dipeptide is colored red while the retinal is blue. (a) top view (from extracellular region); (b) side view (with EC at the top). As expected by the low sequence identity (22% for the TM regions) there are significant differences. The CRMS difference in the C α atoms is 3.75 Å.

regions were fitted with each other for superposition. The sequence identity between the TM regions is ~22%, averaged over the seven TM region sequences. The RMSD in coordinates (CRMSD) of the C α atoms in the TM regions between bovine rhodopsin and mMrgC11 is 3.75 Å. As expected from the low sequence identity the structures are rather different, but they share such structural features as the kink in the TM6 and TM7 helices. Indeed TM3 of rhodopsin has a slight kink at the two consecutive glycines present at the same position as the proline in mMrgC11.

Several conserved residues participate in the inter-helical hydrogen bonds that maintain the stability of the mMrgC11 receptor structure just as in the rhodopsin crystal structure. Thus Asn44 (TM1) (highly conserved in the family A GPCRs) forms a hydrogen bond with the Ser268 carbonyl group of the backbone in TM7 as shown in Figure 2.4. Asp71 (TM2) forms an interhelical hydrogen bond with this Asn in rhodopsin is in the proximity, but is not in hydrogen bond contact in the mMrgC11 receptor. Such differences are plausible since Miura and Karnik reported TM2 movement from activation in angiotensin II type 1 receptor (using substituted

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Figure 2.4 Interhelical hydrogen bond networks in the mMrgC11 receptor. The interhelical hydrogen bonds (dashed lines) are specified with residues participating in hydrogen bonds. The highly conserved residues in the family A of GPCRs that form interhelical hydrogen bonds in rhodopsin are colored by yellow. (a) Viewed from the intracellular region. (b) Viewed from the extracellular region. The HBPLUS[23] program was used to calculate hydrogen bonds (maximum D-A distance = 3.9 Å, minimum D-H-A angle = 90.0°).

cystein accessibility mapping)[24]. Thus Asp in TM2 might interact differently, compared to one in the inactive rhodopsin structure. The Asn66 (TM2)–Trp151 (TM4) pair does form a hydrogen bond just like the analogous pair in rhodopsin.

Important points to note in the structure are:

Tyr63 (TM2) (one of residues conserved in the Mrg receptor family (with 39 sequences available on Swiss-Prot and TrEMBL)) participates in hydrogen bonding with Ser112 (TM3) as shown in Figure 2.4.

Another conserved residue, Ser143 (TM4) forms a hydrogen bond with the hydroxyl group in Thr122 (TM3) as shown in Figure 2.4.

Arg215 (TM6) contacts with the backbone carbonyl group of Val277 in TM7, as shown in Figure 2.4.

Asp179 (TM5), which is identified as a key residue for the ligand binding in this study is in contact with Lys99 (TM3) in the apo protein. Asp161 (TM4) also interacts with Thr183 (TM5) in the absence of a ligand.

Several other inter-helical hydrogen bonds are formed with non-conserved hydrophilic residues. Most of these are found in the regions of the TM regions near the intracellular loop. These regions pack more compactly than the near-extracellular regions as appropriate for ligand binding.

No direct contact between TM3 and TM6 or between TM3 and TM7 is found in the TM regions. However, these TM helices interact with each other through well-stacked aromatic rings as shown in Figure 2.5. Tyr110 (TM3), one of the aromatic residues participating in these interactions is conserved through the Mrg receptors (5 of 39 have Phe at this position instead of Tyr). Also Trp265 in TM6 known to be responsible in activating rhodopsin is replaced with Gly



Figure 2.5 Aromatic interactions in TM regions of mMrgC11 receptor. Aromatic residues involved in the π -stacking through TM3, 5, 6 and 7 are shown with the closest C–C distance between two benzyl rings (Å).

in the mMrgC11 receptor. Thus activation in mMrgC11 might involve a different mechanism. As discussed in section 4.2 we find that the agonists to MrgC11 bind in the pocket located between TM3, 4, 5 and 6, which might affect the aromatic–aromatic interactions to help induce activation.

2.4.2 Description of the peptide binding sites

The predicted RFa binding site is located between TM3, TM4, TM5 and TM6 as shown in Figure 2.3. In contrast to 11-cis retinal in rhodopsin, we find that RFa orients vertically in the binding pocket. As seen in Figure 2.3, the aromatic rings stacked between TM3 and TM6 confines the ligand to the region between TM3, TM4, TM5 and TM6. A similar binding orientation has been suggested for the formylated peptide, *f*MLF[25], which binds parallel to the helix in the formyl peptide receptor (FPR). Since RFa is a small peptide ligand (like *f*MLF) it can be placed parallel in the pocket but for longer peptides, the additional amino acids might be kinked towards TM2 and TM7, having contact with these TMs mainly in the loop regions.

Predicted binding site of the dipeptides



Figure 2.6 Predicted 5 Å binding pocket of the RFa and RF dipeptide agonists. The intermolecular hydrogen bonds calculated with explicit hydrogens using the same criteria as in Figure 2.4 are indicated by the dotted lines. A residue whose side chain participates in the hydrogen bond is specified in red, while one whose backbone is involved is in blue. The residues showing good hydrophobic interactions are specified in black. The top of the picture corresponds to the extracellular regions.

The detailed interactions of bound dipeptides with mMrgC11 receptors are described in Figure 2.6. The binding mode of R-F-OH (RF) is similar to R-F-NH₂ (RFa) although the side chain rotamers of certain residues are different. The common features are that the positively charged moieties are stabilized through the salt bridges and other hydrophilic interactions. Thus the Arg has a good electrostatic interaction with Asp179 (TM5) and the N-terminus has good electrostatic interaction with Asp161 (TM4). The N-terminus of RFa also forms a hydrogen bond with the hydroxyl group of Thr183 (TM5). In addition the C-terminus of RFa makes a hydrogen bond with the hydroxyl group of Tyr110 (TM3).



Figure 2.7 Predicted 3D structure for the FMRFa/mMrgC11 complex. The C α atoms in the TM regions are traced in cartoon while the three key residues (Y110, D161, and D179) are shown in stick. The top view is from extracellular (EC) region and in the side view the EC region is at the top.

The phenyl group of the Phe is stabilized by several aromatic residues present in the binding pocket. Tyr110 interacts most closely with Phe of both dipeptides. For RFa the phenyl ring is in a sandwiched geometry with Tyr110 while for RF these two rings have the displaced T-shape. Phe190 (TM5) also has a good π - π interaction with Phe of the ligand, while Leu186 (TM5) also contributes a good hydrophobic environment for Phe.

Predicted binding sites of the tetrapeptide agonists, F-M-R-F-NH₂, (D)F-M-R-F-NH₂ and F-(D)M-R-F-NH₂

Three tetra-peptides known to be good agonists for mMrgC11[20] were docked into the binding region identified for RFa. The common C-terminal dipeptide part, which is parallel to the average helical axis with the C-terminus of the peptide toward the intracellular region, is bound similarly to RFa (or RF). The extra F-M peptide stretches out horizontally toward TM6 as shown

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in Figure 2.7 for the F-(D)M-R-F-NH₂ (FdMRFa) case, where the chirality of Met is modified to be left-handed. In FdMRFa, the amide group of the C-terminus forms hydrogen bonds with the side chain of Asp161 (TM4) and the backbone carbonyl group of Gly158 (TM4). Phe at the C-terminus resides in good aromatic and hydrophobic environment formed by Tyr110 (TM3), Phe190 (TM5) and Leu186 (TM5). Arg is stabilized through the electrostatic interactions with Asp161 (TM4) and Asp179 (TM5). Thr183 (TM5) also interacts with the side chain of Arg. Asp161 (TM4) forms a hydrogen bond with a nitrogen atom of the backbone. Met located in the peripheral region between TM5 and TM6 is nearby such hydrophobic residues as Leu238 (TM6), Phe239 (TM6) and Ile187 (TM5), but has no specific interaction. The N-terminal Phe is sandwiched between Trp162 (TM4) and Tyr237 (TM6), leading to good aromatic interactions. The N-terminus is exposed to the extracellular region. Thus for longer peptide agonists the extra residues might be added starting from this N-terminal position. This might account for the binding of Met-Enk-RF-amide. This is all shown in Figure 2.8.

In F-M-R-F-NH₂ (FMRFa), the overall binding mode is similar to FdMRFa. Some differences are that Thr183 (TM5) no longer participates in the hydrogen bonding with the peptide and the side chain of the right-handed Met is closer to TM5 and interacts at the edge of aromatic ring of Phe180 (S–C distance = 4.0 Å). The preference of S atoms at the edge of aromatic ring has been observed in the study of the non-bond interaction involving sulfur atom of Met by analyzing the protein crystal structures[26].

(D)F-M-R-F-NH₂ (dFMRFa) shows similar interactions. Although the N-terminal Phe has a different chirality from the previous two ligands, it has a similar conformation of the side chain and fits in between Trp162 (TM4) and Tyr237 (TM6). In this case the Met leads to an intraresidue S...O interaction and an inter-residue interaction with Leu240, where the sulfur atom behaves as an electrophile [26].



Figure 2.8 Predicted 5 Å binding site to mMrgC11 of the agonist tetra-peptides, F-(D)M-R-F-NH₂, F-M-R-F-NH₂ and (D)F-M-R-F-NH₂.



Figure 2.9 Predicted 5 Å binding pocket of the non-agonist tetra-peptides, F-M-(D)R-F-NH₂ and F-M-R-(D)F-NH₂ (neither case was observed experimental to bind even at 30 μ M).

We calculate FdMRFa to bind strongest, with FMRFa and dFMRFa having binding energies just 7% and 11% weaker.

Predicted binding sites of the non-agonists, F-M-(D)R-F-NH₂ and F-M-R-(D)F-NH₂

The two other chirally modified FMRFa peptides, FMdRFa and FMRdFa, do not agonize mMrgC11. Our predicted 5 Å binding sites for them are shown in Figure 2.9. In both cases, the C-terminal Phe interacts with Tyr110 (TM3) and Phe190 (TM5) as seen for other agonists.

In F-M-(D)R-F-NH₂ (FMdRFa) the side chain of Arg is located near Asp161 (TM4) and Asp179 (TM5), with good electrostatic interactions. However the contact is less tight and the non-bond interaction energies with Asp161 and Asp179 decrease by 41% and 12% respectively,

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compared with FdMRFa. We see the intra-residue S...O interaction for Met in this case. The N-terminal Phe loses π - π interaction with Trp162 (TM4).

In the other non-agonist, F-M-R-(D)F-NH₂ (FMRdFa), the side chain of Arg is between Asp161 (TM4) and Asp179 (TM5) and the interaction is weaker than in FdMRFa. The sulfur of Met shows the interaction with the backbone carbonyl group of Asp179. The N-terminal Phe is sandwiched with Trp162 (TM4) and Tyr237 (TM6). Overall these two non-agonist peptides show the similar binding characteristics to the agonist peptides, but the interaction energy is much weaker by 34% for FMdRFa and by 32% for FMRdFa, compared with FdMRFa.

Summary of binding sites

This study identified several residues critical for peptide binding in mMrgC11. The two aspartic acids, Asp161 (TM4) and Asp179 (TM5) contribute to good electrostatic interactions for the electropositive groups of the ligands; Arg for tetrapeptides and Arg and N-terminus for dipeptides. Several aromatic residues contribute to good π - π interactions. Tyr110 (TM3) and Phe190 (TM5) contact with the common C-terminal Phe of all five agonists. Tyr110 is highly conserved across MRG family of receptors. In the tetrapeptide agonists, the additional phenyl group interacts with Trp162 (TM4) and Tyr237 (TM6). As mentioned previously, these aromatic residues are well stacked in the receptor in the absence of a ligand and provide the interhelical interactions among TM3, TM5, TM6 and TM7. This coupling with two phenyl groups of the tetrapeptide ligand along with the strong electrostatic interaction of Arg with Asp161 (TM4) and Asp179 (TM5) is likely to induce the conformational change responsible for the activation.

2.4.3 Mutagenesis experimental results

Based on the predictions described above, we expect that Tyr110 (TM3) (highly conserved aromatic residue among Mrg family), Asp161 (TM4), and Asp179 (TM5) are all critical to binding. Thus we embarked on a series of mutation experiments to validate theses predictions.



Figure 2.10 The expression of mMrgC11 wild type and mutant receptors in the Flp-In293 cells. (a) GFP images of wild type and mutant cells. The GFP was fused into the C-terminus of the receptor. (b) Biotinylation of the cell surface where receptors are localized and folded. The biotinylated cell extract is blotted with anti-GFP after immunoprecipitation (IP) with streptavidin. Lanes 1-5 are before IP and Lanes 6-10 are after IP. The molecular weight markers are shown on the left in kDa.

Peptide	Sequence	EC50, nM	Han et. al[20]
RF	RF	1255 ± 239	632 ± 124
RFa	RF-NH ₂	682 ± 186	460 ± 35
FMRF	FMRF	666 ± 228	544 ± 117
FMRFa	FMRF-NH ₂	168 ± 26	114 ± 32
dFMRFa	(D)F-M-R-F-NH ₂	276 ± 56	108 ± 1
FdMRFa	F-(D)M-R-F-NH ₂	113 ± 18	11 ± 4
FMdRFa	F-M-(D)R-F-NH ₂	inactive	inactive
FMRdFa	F-M-R-(D)F-NH ₂	inactive	inactive
Bam15	VGRPEWWMDYQKRYG	292 ± 19	53 ± 2
γ1-MSH	YVMGHFRWDRF-NH ₂	398 ± 189	17 ± 3
γ2-MSH	YVMGHFRWDRFG	340 ± 66	11 ± 5
NPFF	FLFQPQRF-NH ₂	358 ± 25	54 ± 5

 Table 2.1 The EC50 values of various peptide ligands determined by the intracellular calcium release

 assay with Flp-In293 cells expressing mMrgC11 receptor

Inactive means that no activation was detected up to the highest concentration tested, 10μ M. Data represent the mean (\pm SEM) of four independent experiments.

Expression and localization of mMrgC11 wild type and mutant receptors

Based on the predictions, we carried out three sets of experiments in which key residues were mutated to alanine – Tyr110Ala, Asp161Ala and Asp179Ala. Figure 2.10(a) shows the GFP images for mMrgC11 wild type and for the three mutant receptors. All mutant cells show fluorescence signals as intense as the wild type and the cell boundaries are clearly identified. These images indicate that the mutant receptors are expressed at level similar to the wild type and are well localized at the cell membranes.

To determine whether the mutants properly fold across the cell membrane, we combined immunoprecipitation (IP) experiments with biotinylation. Lanes 1-5 in Figure 2.10(b) show total mMrgC11 receptor proteins including ones that are not biotinylated but present in cytosol and those that have not crossed properly through the membranes. These blots indicate again that all

three mutants are well expressed in the cells, although the expression levels of D161A and D179A mutants seem slightly lower. The results of blots after IP with streptavidin (lanes 6-10) show that the mutant receptors localized on the cell membranes take apical positions at similar amounts to the wild type. Since the band corresponding to the non-specific binding of streptavidin (lane 6) is much weaker, we conclude that the major portions of blots in lane 7-10 come from the biotin-specific binding. This suggests the mutant proteins folds properly on the membranes as well as the wild type protein.

Dose-dependent intracellular calcium release assay with stably expressed MrgC11 receptors

Table 2.1 shows the EC50 values of various peptide ligands determined by intracellular calcium assay experiment with Flp-In293 cells expressing the mMrgC11 receptor. The di- and tetra-peptides and some longer peptide agonists were selected from the ligands previously identified by Han *et al.*[20]. We obtained slightly higher EC50 values in our cellular system, compared to the previous measurements. This difference might result from a variety of sources such as different coupling efficiencies, different expression levels of receptor, and different cellular environment[27]. Nonetheless, the selectivity observed in this study is consistent with the previous results– for example; FMdRFa and FMRdFa still show no activity.

Out of the twelve ligands tested for the wild type receptor, we selected the six most potent ligands to measure the potencies for Y110A, D161A and D179A mutant receptors, as shown in Table 2.2.

We find that the Y110A mutant is not activated by any of the six tested ligands up to a concentration of 33 μ M, indicating that Y110 is critical for binding and activation.

The D179A mutants show no potency for the three tetrapeptide ligands, while the other three are activated only under 10 times higher concentration of the ligand.

	Binding ^a	Wild Type	Y110A ^b	D161A ^b	D179A ^b	Y110F ^c	Y110W ^c
FdMRFa	100%	113 ± 18	>33000	>33000	>33000	714 (6.3)	334 (3.0)
FMRFa	93%	168 ± 26	>33000	+ (18000)	>33000	1795 (10.7)	1531 (9.1)
dFMRFa	88%	276 ± 56	>33000	>33000	>33000	1500 (5.4)	1513 (5.5)
Bam15		292 ± 19	>33000	>33000	+ (3000)	749 (2.6)	1713 (5.9)
. γ1-MSH		398 ± 189	>33000	>33000	+ (3000)	331 (0.8)	302 (0.8)
. γ2-MSH		340 ± 66	>33000	+ (33000)	+ (2000)	340 (1.0)	349 (1.0)
FMdRFa	67%	>10000					
FMRdFa	68%	>10000					

 Table 2.2 Binding constants (EC50 values in nM) of mutant mMrgC11 receptors from intracellular calcium assays

^a Calculated binding energy relative to FdMRFa (absolute value = 117kcal/mol). ^b + means that activation starts at a given concentration. ^c Numbers in parentheses are the ratio with respect to the EC50 values of WT.

For mutants D161A we find that 4 of the 6 ligands no longer activate while that other two only activate for 100 times the concentration.

These results that mutation of Tyr110, Asp161 and Asp179 very strongly reduce or eliminate the activity of mMrgC11 receptor validate the predictions that these residues are involved in the ligand binding.

For a positive control experiment, the mutant of Asp81 in TM2 to Ala was transiently expressed in HEK293 cells along with the Y110A, D161A, and D179A mutant receptors also transiently expressed under the same condition. Then the intracellular calcium assay experiment was carried out with 0.33 μ M of FMRFa. Except for the D81A mutant, the other three showed no activity.

We investigated the implication of the hydroxyl group on the Tyr110 in ligand recognition by replacing this tyrosine with phenylalanine or tryptophan. The potencies of γ 1-MSH and γ 2-MSH ligands are not affected by the absence of the hydroxyl group, indicating that the hydroxyl group does not contribute to ligand activation for these ligands. For the three tetrapeptide agonists the Y110F or Y110W mutations leads to a factor of 5 to 10 reduction in the potency. This is consistent with our predicted structure (Figures 2.7 and 2.8) which does not have the hydroxyl group of Tyr110 interacting with the ligand, but instead forms a hydrogen bond with the carbonyl group of the backbone. The missing hydroxyl group should results in a dangling hydrogen bond donor which might induce an overall conformational change in the binding pocket to explain the loss in activity. Since mutation of Tyr110 to Ala totally extinguishes the activity for all six ligands, we conclude that the aromatic ring must be significant for all six cases.

To investigate whether the two non-agonist tetrapeptides, FMdRFa and FMRdFa are antagonists or weak binders (or non-binders), we saturated the receptors either with FMdRFa or with FMRdFa in three concentrations, 3.3, 16 and 33 μ M and then measured the EC50 value for FdMRFa. The intensity of calcium signal remained on the same level as in the absence of FMdRFa or FMRdFa and the EC50 values did not change much (within standard deviation). This result shows that FMdRFa and FMRdFa do not block the efficacy of FdMRFa and at best bind only weakly to the receptor.

Summarizing, the experimental results show that Tyr110 (TM3), Asp161 (TM4) and Asp179 (TM5) are possibly in the binding site in agreement with the predictions. These predicted mutations focused on the dipeptide binding region. Using the binding region for the tetrapeptide, we now suggest that mutations of Trp162 (TM4), Phe190 (TM5), and Tyr237 (TM6) to Ala would also dramatically decrease binding. Additional validations could be to mutate either the receptor or the peptide ligand and to carry out other cell assay experiments such as radiolabelled ligand binding assays. Such studies should further improve our understanding of the structure and ligand binding site.

2.4.4 Prediction of the structure of the mMrgA1 receptor and the binding site for ligands

The 3D structure of mMrgA1 was predicted using MembStruk procedure described in this chapter. The CRMSD of Cα atoms between mMrgC11 and mMrgA1 is 2.49Å in the TM



Figure 2.11 Comparison between mMrgC11 and mMrgA binding sites. (a) The electrostatic potential map of the binding pocket in mMrgC11 and mMrgA1. The residues within 5 Å from RFa ligand were selected for visualization. Asp161, Asp179 and Lys99 of mMrgC11 are specified in stick and Asn145 of mMrgA1 in stick. The electrostatic potential was computed using APBS and visualized on PyMOL. The van der Waals radii of DREIDING forcefield were used for APBS calculation. (b) The predicted 5 Å binding pocket of RFa in mMrgC11 and mMrgA1 receptor.

regions and 4.94 Å if the loops are included. The sequence identity between them is 53% for the TM regions and 46% for the entire sequence. It was observed experimentally that mMrgA1 receptor is activated much less potently by tetra-peptide ligands containing the RF-amide motif as compared to the mMrgC11 receptor, and that neither the amide nor acidic form of RF di-peptide activates mMrgA1[20].

We docked the RFa ligand into the mMrgA1 receptor by superimposing it with RFa-bound mMrgC11 receptor. The side chains of residues within 5 Å were reassigned using SCREAM and then the potential energy of the ligand-receptor complex structure was minimized. We found that Tyr94 (TM3), F177 (TM5) and L173 (TM5) (homologous residues of Tyr110, Phe190 and Leu186 in mMrgC11) form a hydrophobic pocket for Phe as in mMrgC11, but they are located slightly farther (the closet C–C distance between aromatic rings is 4 to 5 Å). Asn145 (TM4), the homologous residue of Asp161 in mMrgC11, is involved in the hydrogen bonding with the N-terminus. The Arg side chain of the peptide is surrounded with hydrophobic residues and does not have any favorable interaction with receptor. The calculated binding energy (positive value) predicts that it does not bind to mMrgA1 receptor.

Figure 2.11 shows the electrostatic potential maps of the binding pocket in mMrgC11 and of the corresponding region in mMrgA1. The electrostatic potential was calculated for the entire receptor using adaptive Poisson-Boltzmann solver (APBS)[28]. The binding pocket within 5 Å from RFa docked in mMrgC11 receptor is selectively presented here. We can see that the pocket of mMrgA1 is more hydrophobic than that of mMrgC11. In mMrgC11, two aspartic acids (Asp161 and Asp179) are located in the spot showing the fairly negative potential. We observed that in mMrgC11 the positively charged side chain of Arg and the N-terminus are favored in this region. In the absence of the ligand, Lys in TM3 (Lys99) compensates for this highly negative potential. For mMrgA1 these Asp residues are replaced by Asn. We expect that highly polar ligands such as a peptide containing an Arg residue might be unfavorable for the hydrophobic

character of the pocket in mMrgA1. This might explain why this ligand fails to bind strongly to the mMrgA1 receptor, explaining the low potency for RFa ligand to mMrgA1. This provides additional confirmation of our predicted binding site and protein structure for mMrgC11. We expect that the potency for these ligands to mMrgA1 might increase if these Asn residues are mutated to Asp, an experiment we intend to do soon.

2.4.5 Comparison of Mrg sequences

The 39 verified Mrg sequences were aligned using Clustal-W (v. 1.83) with the default parameters (protein gap open penalty = 10.0, protein gap extension penalty = 0.2, protein matrix = Gonnet) as shown in Figure S2.4. It includes 19 mouse, 13 rat, 1 monkey and 6 human Mrg receptors. The sequence identities range from 21% to 97 %. The mouse MrgF and rat MrgF have the highest sequence identity. The human MrgF also shows the relatively high sequence identity with rat and mouse orthologs (85% and 86% respectively). Across the 39 sequences we examined the sequence variations in the six key residues (Tyr110, Phe190, Asp161, Asp179, Trp162 and Tyr256) that we identified in this study. As mentioned before, Tyr110 is conserved throughout the Mrg sequences except for the 5 Mrgs that have the homologous Phe at the same position. Other five residues show various range of alteration;

- D161: 14 D or E, 7 N, 5 L, 2 A, 3 H, 2 P, 2 T, 1 Q, 1 K, 1 V and 1 Y.
- W162: 6 W, 14 G, 8 S, 4 N, 3 R, 2 A, 1 M and 1 E.
- D179: 14 D, 10 N, 5 I, 3 M, 2 A, 2 H, 2 W and 1 S.
- F190: 20 F, 8 C, 3 T, 2 M, 2 S, 1 A, 1 I, 1 L and 1 V.
- Y256: 20 Y or F, 3 C, 3 D, 3 Q, 2 H, 2 L, 2 N, 2 I, 1 S, and 1 T.

We observed that only rat MrgC has all six residues conserved and mouse MrgB1, mouse MrgB2 and rat MrgB2 have Tyr110, Asp161, Asp179, Phe190 and Y256 at their homologous positions. Trp162 are replaced with Gly for these MrgB receptors. It has been shown that γ2-MSH is the most potent at activating rat MrgC and the active moiety recognized by rat MrgC is the Cterminal of γ 2-MSH, F-R-W-D-R-F-G[29]. The rat MrgC also binds Met-Enkephalin RF-amide with F-M-R-F-NH₂ at the C-terminus. These experimental results suggest the similar characteristics in the binding site of rat MrgC receptor to that of mMrgC11, further supporting our predictions.

2.5 Summary and conclusions

We predicted the 3D structure of the mMrgC11 receptor and used it to predict the binding sites for a number of di- and tetra-peptide ligands. We find that in each case the peptide ligand binds in a pocket among TM3, 4, 5 and 6 oriented parallel to the helical axis. These predictions suggested that three residues (Tyr110 (TM3), Asp161 (TM4) and Asp179 (TM5) in the binding pocket) play a key role in the binding.

To test these predictions, we carried out several mutagenesis experiments. For 6 ligands exhibiting EC50 of 100 to 400 nM in wild type, we find that the EC50 for the Y110A, D161A and D179A mutant receptors are higher than 33 μ M for 14 of 18 combinations and 50 to 100 times higher for the other 4 combinations. This validates the implication of these residues for the activation or binding of the ligand.

Since the peptide forms a zwitterion at pH 7 giving it relatively polar character and since the ligands that bind to MrgC11 contain an Arg whose side chain is positively charged at pH 7, it is plausible that the two aspartic acids in the binding pocket participate. On the other hand, mMrgA1 has increased hydrophobic character in the corresponding region (these Asp are replaced by Asn). This might be responsible for the low efficacy of the ligand.

Our predicted binding site also suggests additional mutation candidates to be tested, especially residues involving hydrophobic interaction such as Trp162, Leu186, Phe190, Tyr237, and Leu238.

This study indicates how collaboration between theory and experiment can provide insight into the structural characterization of these Mrg receptors to determine how they are related with function. This could lead to the design of small molecule antagonists to selectively inhibit these receptors as candidate drugs for treating pain. Such studies would be equally valuable for many other GPCR receptors, indicating that a systematic combination of computational tools along with biochemical experiments can provide an increased understanding membrane protein receptors and their activation.

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Supporting figures and tables

mMı	rgC11	MDPTISSHDTESTPLN-ETGHPNCTPILTLSFLVLITTLVGLAGNT	45
tr	Q91YB7	-MGESFTGTGFINLNTSASTIAVTTTNPMDKTIPGSFNGRTLIPNLLIIISGLVGLIGNA	59
tr	Q7TN49	MDKTIPGSFNSRTLIPNLLIIISGLVGLTGNA	32
tr	Q91WW5	MDNTIPGGINITILIPNLMIIIFGLVGLTGNG	32
tr	Q91ZC6	IRILITNLMIVILGLVGLTGNA	28
tr	Q91WW3	MNETIPGSIDIETLIPDLMIIIFGLVGLTGNA	32
tr	Q8R4G1	MVCVLRDTTGRFVSMDPTISSLSTESTTLN-KTGHPSCRPILTLSFLVPIITLLGLAGNT	59
tr	Q7TN42	MDPTISSLSTESTTLN-KTGHPSCRPILTLSFLVPIITLLGLAGNT	45
tr	Q96LB0	MDSTIPVLGTELTPINGREETPCYKQTLSFTGLTCIVSLVALTGNA	46
дb	AX923125 40216229	MDSTIPVLGTELTPINGREETPCYKQTLSFTGLTCIVSLVALTGDA	46
gp	AX647081 28800069	MDSTIPVLGTELTPINGREETPCYKQTLSFTGLTCIVSLVALTGNA	46
tr	Q8TDE1	MDPTIPVLGTELTPINGREETPCYKQTLSFTGLTCIVSLVALTGNA	46
tr		MDPTVPVLGTELTPINGREETPCYKQTLSFTGLTCIVSLVALTGNA	46
gp	AX657514 29160254		24
tr		CARTER AND AND A AN	46
tr		CICCYKQTLSLTVLTCIVSLVGLTGNA	46
tr		MDPTVSTLDTELTPINGTEETLCYKQTLSLTVLTCIVSLVGLTGNA	46
τr		MDPTVPVFGTKLTPINGREETPCINQTLSFTVLTCIISLVGLTGNA	46
gp	AX646849 28799318	MDPTVPVFGTKLTPINGREETPCYNQTLSFTVLTCIISLVGLTGNA	46
tr		MDPTVPVFGTKLTPINGREETPCYNQTLSFTVLTCIISLVGLTGNA	40
Cr.			40
gp	AX657510 29160250		24 40
tr tr	001700	MSPITQAWSINNIVVKENYYTELLSUITTFNILNFLIVIISVVGMAGNA	49
ι. + ~			40
			49
ι <u>ι</u> + γ			56
tr			56
ιı	Q0CD14		50
mMi	raCl 1	TVI.WI.I.GERMRRKATSVYTI.NI.ALADSFFI.CCHFTDSLI.RTIDFYGLYAHKI.SKDTI.GNA	105
mMu tr	rgC11	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLKFSYPNIIFLPCFNTV	105 117
mMu tr tr	cgCl1 Q91YB7 07TN49	IVIWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV	105 117 90
mMu tr tr tr	cgCll Q91YB7 Q7TN49 O91WW5	IVLWLLG FRMRRK AISVYILNLALADSFFLCCHFIDSL LRIIDFYGLYAHKLS KDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCLHRNAFSVYILNLALADFFFLLGHIIDSILLLNVFYP-ITFLLCFYTI	105 117 90 89
mMi tr tr tr	rgCll Q91YB7 Q7TN49 Q91WW5 Q91ZC6	IVLWLLG FRMRRK AISVYILNLALADSFFLCCHFIDSL LRIIDFYGLYAHKLS KDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCLHRNAFSVYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLLFRLRRNAFSIYILNLALADFLFLLCHIIASTEHILTFSSPNSIFINCLYTF	105 117 90 89 86
mMu tr tr tr tr	rgCll Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCHRNAFSVYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMHRTAFLYYILNLALADFLFLLCHIIASTEHILTFSSPNSIFINCLYFF IVFWLLGFRMHRTAFLYYILNLALADFLFLLCHIINSTVDLLKFTLPKGFFAFCFHTI	105 117 90 89 86 90
mMJ tr tr tr tr tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 (08R4G1	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRLRNAFSVYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMRTAFLYYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVFWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA	105 117 90 89 86 90 119
mMn tr tr tr tr tr tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q8R4G1 Q7TN42	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRCHRNAFSVYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMRTAFLYYILNLALADFLFLLCHIIASTEHILTFSSPNSIFINCLYTF IVFWLLGFRMRRKAISVYULNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYULNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYULNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA	105 117 90 89 86 90 119 105
mMi tr tr tr tr tr tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCLHRNAFSVYILNLALADFLFLLCHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMHRTAFLYYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA VVLWLLGCRMRRNAVSIYILNLALADFLFLSGHIICSPLRLINIRHPISK-ILSPV	105 117 90 89 86 90 119 105 101
mMi tr tr tr tr tr tr gp	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRLHRNAFSVYILNLALADFLFLLCHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMHRTAFLVYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA VVLWLLGCRMRRNAVSIYILNLALADFLFLSGHIICSPLRLINIRHPISK-ILSPV	105 117 90 89 86 90 119 105 101
mMn tr tr tr tr tr tr gp	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRLHRNAFSVYILNLALADFLFLLCHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMHRTAFLVYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV	105 117 90 89 86 90 119 105 101 101
mMn tr tr tr tr tr tr gp tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRLHRNAFSVYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMHRTAFLVYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMHRTAFLVYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGGRMRRNAVSIYILNLALADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV	105 117 90 89 86 90 119 105 101 101 101
mMn tr tr tr tr tr tr gp tr tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCLHRNAFSVYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMHRTAFLVYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGCRMRRNAVSIYILNLALADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV	105 117 90 89 86 90 119 105 101 101 101 101
mMn tr tr tr tr tr tr gp tr gp	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRLRRNAFSYYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMHRTAFLVYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGGRMRRNAVSIYILNLALADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV	105 117 90 89 86 90 119 105 101 101 101 101 79
mMn trrtrtrtr trtgp gprrt gp tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCLHRNAFSYYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMHRTAFLYYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGCRMRRNAVSIYILNLALADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV	105 117 90 89 86 90 119 105 101 101 101 101 79 101
mMn tr tr tr tr tr tr gp gr tr gp tr tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MYFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRLRNAFSVYILNLALADFLFLLCHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMRRAFSIYILNLALADFLFLLCHIINSTVDLLKFTLPKGFFAFCFHTI IVLULGFRMRRKAISVYVLNLSLADFFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV	105 117 90 89 86 90 119 105 101 101 101 101 79 101 101
mMn trrtrtrtr gpptr gtrtr tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8 Q8TDD8 Q8TDD8	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRLARNAFSYYILNLALADFLFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMRRAFSIYILNLALADFLFLLCHIINSTVDLLKFTLPKGFFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADFFFLLCHIINSTVDLLKFTLPKGFFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAAADFLFLSGRLIYSLLSFISIPHTISK-ILYPV	105 117 90 89 109 105 101 101 101 101 79 101 101
mMn trrtrtrtr ggprrrgp trrtr tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8 Q8TDD9 Q96LA9	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCHRNAFSVYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMRTAFLYYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVFWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNN VVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAAADFLFLSGRLIYSLSFISIPHTISK-ILYPV	105 117 90 89 86 90 119 105 101 101 101 101 101 101 101 101
mMn tr tr tr tr tr gpptr r gtr tr tr tr gp	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD9 Q96LA9 AX646849 28799318	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCHRNAFSVYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMRTAFLYYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNN VVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINISHTISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINISHTISK-ILSPV VVLWLLGCRMRRNAYSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV	105 117 90 89 109 105 101 101 101 101 101 101 101 101 101
mM tr tr tr tr tr gpptr gptr tr tr tr gptr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCHRNAFSVYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMRTAFLYYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNN VVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINISHTISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINISHTISK-ILSPV VVLWLLGCRMRRNAYSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAYSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLSFISIPHTISK-ILYPV	105 117 90 89 109 105 101 101 101 101 101 101 101 101 101
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mMn trrtrtrtrtrgp gprtrgp trrtrtrgp grrtrgp	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8 Q8TDD9 Q96LA9 AX64849 28799318 Q8TDD6 Q8TDD7 AX657510 29160250	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV VVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCLHRNAFSVYILNLALADFFFLLGHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMRRAFSYYILNLALADFLFLLCHIIASTEHILTFS-SPNSIFINCLYTF IVFWLLGFRMRRKAISVYULNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYULNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYULNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINISHTISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILSPV VVLWLLGCRMRRNAYSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINISHTISK-ILYPV VVLWLLGCRMRRNAYSIYILNLAADFLFLSFQIIRSPLRLINISHTISK-ILYVV VVLWLLGCRMRRNAYSIYILNLAADFLFLSFQIIRSPLRLINISHTISK-ILVSV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGYRMRNAVSIYILNLAAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV	105 117 90 89 109 105 101 101 101 101 101 101 101 101 101
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mMr trrttrrtr trr g g r tr g r trr g trr g trr g trr g trr tr g trr	rgCl1 Q91YB7 Q7TN49 Q91WV5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6 Q8TDD6 Q8TDD7 AX657510 29160250 Q7TN45 Q91ZC0	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV WVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCLHRNAFSVYILNLALADFLFLLCHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMRRAFSVYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYULNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYULNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINISHTISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINISHTISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGHIICSPLRLINISHTISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGLIYSLLSFISIPHTISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGLIYSLLSFISIPHTISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINISHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINISHTISK-ILYPV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIKK-ILVSV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIKK-ILVSV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIKK-ILVSV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIKK-ILVSV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIKK-ILVSV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIKK-ILVSV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIKK-ILVSV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIKK-ILVSV	105 117 90 89 86 90 119 105 101 101 101 101 101 101 101 101 101
mMrttrtttrtrgggtrtggtrtrgttrgttrgttr	rgCl1 Q91YB7 Q7TN49 Q91WV5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6 Q8TDD7 AX657510 29160250 Q7TN45 Q91ZC0 Q91ZC3	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCLHRNAFSVYILNLALADFLFLLCHIIDSILLLLNVFYP-ITFLLCFYTI IVFWLLGFRMRRAFSYYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYULNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYULNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINISHISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGHIICSPLRLINISHISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGHIICSPLRLINISHISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGPIRLINISHISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGCRMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGCRMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGCRMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGCRMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGCRMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGCRMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGCRMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV	105 117 90 89 86 90 119 105 101 101 101 101 101 101 101 101 101
mMrttrtttrtrgggtrtggtrtrgftrggtrtrggtrtrg	rgCl1 Q91YB7 Q7TN49 Q91WV5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD7 AX657510 29160250 Q7TN45 Q91ZC3 Q7TN48	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCHRNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRLRNAFSVYILNLALADFLFLLCHIIDSTLLLKFSYPNIFLPCFYTI IVFWLLGFRMRRTAFLVYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGYMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGYRMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGYRMRNAVSIYILNLAADFLFLSFQIIR	105 117 90 89 105 101 101 101 101 101 101 101 101 101
mt trtrtrtr gg gg trt gg trtrt gg trt gg trtrtrt gg trtrtrt	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q95LB2 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6 Q8TDD7 AX657510 29160250 Q7TN45 Q91ZC3 Q7TN48 Q91ZC2	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFFFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCHRNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFRMRAFSVYILNLALADFLFLLCHIIDSTLLLKFSYPNIFLPCFNTV IVFWLLGFRMRRTAFLVYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSGRLIYSLLSFISIPHTISK-ILYPV VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINISHLIKK-ILVSV VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINISHLIKK-ILVSV VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINS VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINISHLIKK-ILVSV VVLWLLGCRMRRNAFSIYILNLAADFLFLSFQIIRSPLRLINS VVLWLLGCRMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINS <	105 117 90 89 105 101 101 101 101 101 101 101 101 101
mttrtrtrtrtgggttrgtrtrtgtrgtttgttttt	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q95LB2 Q8TDD8 Q8TDD8 Q8TDD8 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6 Q8TDD7 AX657510 29160250 Q7TN45 Q91ZC3 Q7TN48 Q91ZC2 Q8CDY4	IVLWLLGFRMRRKAISVYILNLALADSFFLCCHFIDSLLRIIDFYGLYAHKLSKDILGNA MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV MVFWLLGFRLARNAFSVYILNLALADFLFLLCHIIDSTLLLLKFSYPNIIFLPCFNTV IVFWLLGFCLHRNAFSVYILNLALADFFFLLGHIIDSILLLNVFYP-ITFLLCFYTI IVFWLLGFCHRNAFSYYILNLALADFLFLLCHIINSTVDLLKFTLPKGIFAFCFHTI IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA IVLWLLGFRMRRKAISVYVLNLSLADSFFLCCHFIDSLMRIMNFYGIYAHKLSKEILGNA VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINIRHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINISHPISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINISHISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGHIICSPLRLINISHISK-ILSPV VVLWLLGCRMRRNAVSIYILNLVAADFLFLSGRLIYSLLSFISIPHTISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGRLIYSLLSFISIPHISK-ILSPV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGRLIYSLLSFISIPHISK-ILVSV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGRLIYSLLSFISIPHISK-ILVSV VVLWLLGCRMRRNAVSIYILNLAADFLFLSGRLIYSLLSFISIPHISK-ILVSV VVLWLLGCRMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINISHLIRK-ILVSV VVLWLLGYMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINSHLIRK-ILVSV VVLWLLGYMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINSHLIRK-ILVSV VVLWLLGYMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINSHLIRK-ILVSV VVLWLLGYMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINSHLIRK-ILVSV VVLWLLGYMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINSHLIRK-ILVSV VVLWLLGYMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINSHLIRK-ILVSV VVLWLLGFMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINSHLIRK-ILVSV VVLWLLGFMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINSHLIRK-ILVSV VVLWLLGFMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINSHLIRK-ILVSV VVLWLLGFMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINSHLIRK-ILVSV VVLWLLGFMRRNAVSIYILNLAADFLFLSFQIIRSPLRLINSHLIRK-ILVSV VVLWLLGFMRRNAVSIYILNLAADFLFLSFQIVSSPVCVQHVSLEVVLVFV	105 117 90 89 105 101 101 101 101 101 101 101 101 101

Figure S2.1 Multiple sequence alignment for mMrgC11 with 27 homologous sequences.

mMa	rgC11	AIIPYISGLSILSAISTERCLCVLWPIWYHCHRPRNMSAIICALIWVLSFLMGILDWF-S	164
tr	Q91YB7	${\tt MMVPYIAGLSMLSAISTERCLSVVCPIWYRCRRPKHTSTVMCSAIWVLSLLICILNRYFC}$	177
tr	Q7TN49	MMVPYIAGLSMLSAISTERCLSVVCPIWYRCRRPKHTSTVMCSAIWVLSLLICILNRYFC	150
tr	Q91WW5	MMVLYIAGLSMLSAISTERCLSVLCPIWYHCHRPEHTSTVMCAVIWVLSLLICILNSYFC	149
tr	Q91ZC6	RVLLYIAGLSMLSAISIERCLSVMCPIWYRCHSPEHTSTVMCAMIWVLSLLLCILYRYFC	146
tr	Q91WW3	KRVLYITGLSMLSAISTERCLSVLCPIWYHCRRPEHTSTVMCAVIWVLSLLICILDGYFC	150
tr	08R4G1	AIIPYISGLSILSAISTERCLSVLWPIWYHCHRPRNMSAIICVLIWVLSFLMGILDWFFS	179
tr	07TN42	AFIPYISGLSILSAISTERCLSVLWPIWYHCHRPRNMSAIICVLIWVLSFLMGILDWFFS	165
tr	096LB0	MTFPYFIGLSMLSAISTERCLSILWPIWYHCRRPRYLSSVMCVLLWALSLLRSILEWMFC	161
ap	AX923125 40216229	MTFPYFIGLSMLSAISTERCLSILWPIWYHCRRPRYLSSVMCVLLWALSLLRSILEWMFC	161
ap	AX647081 28800069	MTFPYFIGLSMLSAISTERCLSILWPIWYHCRRPRYLSSVMCVLLWALSLLRSILEWMFC	161
tr		MTFPYFTGI.SMI.SATSTERCI.STI.WPTWYHCRPPRYI.SSVMCVI.LWALSLID.STI.FWMFC	161
tr		MTFPYFTGLSMLNATSTERCLSTLWPTWYHCRRPRYLSSVMCVLLWAPSLLRSTLEWMFC	161
an	AX657514 29160254	MTEDVETCI.SMI.SATSTERCI.STI.WDTWYHCPPDPYI.SSVMCVI.LWAI.SLIPSTI.FWMEC	139
9P tr	096LB2	MITTIFICLORIDATOTERCLOTIANTWINCKRERTLOOVALUALOLLROTLEWIC MMESYFACLSELSAVSTERCLSVLWDIWYRCHRDTHLSAVVCVLUWALSLLRSTLEWIC	161
τ. τ.			161
LI + 20		MMERCERAL CELONICEEDOL CUL WDIWINCHNPIHLSAVVCVLLWALSLLKSILEWMLC	161
			161
LI			101
gp	AX646849 28799318	MTFPYFTGLSMLSAISTERCLSVLWPIWYRCRRPTHLSAVVCVLLWGLSLLFSMLEWRFC	101
τr		MTFPYFTGLSMLSAISTERCLSVLWPIWYRCRRPTHLSAVVCVLLWGLSLLFSMLEWRFC	101
tr		MTFPYFTGLSMLSAISTERCLSVLWPIWYRCRRPTHLSAVVCVLLWGLSLLFSMLEWRFC	101
дb	AX657510 29160250	MTFPYFTGLSMLSAISTERCLSVLWPIWYRCRRPTHLSAVVCVLLWGLSLLFSMLEWRFC	139
tr	Q7TN45	LMFNYLAGFCMIAAISTERCLSVTWPIWYHCQRPRHTSATVCALFWAFSLLLSLLLGQGC	164
tr	Q91ZC0	TMFAYLAGLCMIAAISAERCLSVMWPIWYHCQRPRHTSAIMCALVWVSSLLLSLVVGLGC	163
tr	Q91ZC3	LNIAYLSGLSILTVISTERFLSVMWPIWYRCQRPRHTSAVICTVLWVLSLVLSLLEGKEC	167
tr	Q7TN48	LNIGYLCGMSILSAISIERCLSVMWPIWYRCQRPRHTSAVICTLLWVLALVWSLIEGKEC	145
tr	Q91ZC2	PIFAYLSGLSILSTISIERCLSVIWPIWYRCKRPRHTSAITCFVLWVMSLLLGLLEGKAC	175
tr	Q8CDY4	PIFAYLSGLSILSTISIERCLSVIWPIWYRCKRPRHTSAITCFVLWVMSLLLGLLEGKAC	175
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mMa		COLORADUU IWWN-UNDTITANDI TOI DWI I CCCCI AI I I DII CCNDDUNI CDI VUMTAI	222
mMi	rgCl1	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL	222
mMı tr	rgC11 Q91YB7 C7my40	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRSFCGAGRMKLTRLYATIML	222 237
mMu tr tr	cgC11 Q91YB7 Q7TN49	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRSFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRLFCGAGRMKLTRLYATIML	222 237 210
mMı tr tr tr	cgCl1 Q91YB7 Q7TN49 Q91WW5	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRSFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRLFCGAGRMKLTRLYATIML GFLNTQYKNENGCLALNFFTAAYLMFLFVVLCLSSLALVARLFCGTGQIKLTRLYVTIIL	222 237 210 209
mMi tr tr tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRSFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRLFCGAGRMKLTRLYATIML GFLNTQYKNENGCLALNFFTAAYLMFLFVVLCLSSLALVARLFCGTGQIKLTRLYVTIIL GFLDTKYEDDYGCLAMNFLTTAYLMFLFVVLCVSSLALLARLFCGAGRMKLTRLYVTIIL	222 237 210 209 206
mMi tr tr tr tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRSFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRLFCGAGRMKLTRLYATIML GFLNTQYKNENGCLALNFFTAAYLMFLFVVLCLSSLALVARLFCGTGQIKLTRLYVTIIL GFLDTKYEDDYGCLAMNFLTTAYLMFLFVVLCLSSLALLARLFCGAGRMKLTRLYVTITL GYLDNHYFNYSVCQAWDIFIGAYLMFLFVVLCLSSTLALLARLFCGARNMKFTRLFVTIML	222 237 210 209 206 210
mMı tr tr tr tr tr	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRSFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRLFCGAGRMKLTRLYATIML GFLNTQYKNENGCLALNFFTAAYLMFLFVVLCLSSLALVARLFCGTGQIKLTRLYVTIIL GFLDTKYEDDYGCLAMNFLTTAYLMFLFVVLCVSSLALLARLFCGAGRMKLTRLYVTITL GYLDNHYFNYSVCQAWDIFIGAYLMFLFVVLCLSTLALLARLFCGARNMKFTRLFVTIML GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL	222 237 210 209 206 210 237
mMi tr tr tr tr tr tr	rgCll Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRSFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRLFCGAGRMKLTRLYATIML GFLDTKYEDDYGCLAMNFITTAYLMFLFVVLCLSSLALLVRLFCGAGRMKLTRLYVTITL GYLDNHYFNYSVCQAWDIFIGAYLMFLFVVLCVSSLALLARLFCGAGRMKLTRLYVTITL GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL	222 237 210 209 206 210 237 223
mMi tr tr tr tr tr tr	CgC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRIFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRIFCGAGRMKLTRLYATIML GFLDTKYEDDYGCLANNFFTAAYLMFLFVVLCLSSLALLVRIFCGAGRMKLTRLYVTITI GYLDNHYFNYSVCQAWDIFIGAYLMFLFVVLCUSSLALLARLFCGAGRMKLTRLYVTITL GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL DFLFSGADS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRRKMPLTRLYVTILL	222 237 210 209 206 210 237 223 220
mMi tr tr tr tr tr tr gp	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRSFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRLFCGAGRMKLTRLYATIML GFLDTKYEDDYGCLANNFLTTAYLMFLFVVLCLSSLALLVRLFCGAGRMKLTRLYVTITL GYLDNHYFNYSVCQAWDIFIGAYLMFLFVVLCLSSLALLARLFCGARMKLTRLYVTITL GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL DFLFSGADS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL	222 237 210 209 206 210 237 223 220 220
mMi tr tr tr tr tr tr gp	rgCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRSFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRLFCGAGRMKLTRLYATIML GFLDTKYEDDYGCLANNFLTTAYLMFLFVVLCLSSLALLVRLFCGAGRMKLTRLYVTII GYLDNHYFNYSVCQAWDIFIGAYLMFLFVVLCLSSLALLARLFCGARMKLTRLYVTITL GFLGETHHH-LWKN-VDFIVTAFLIFLFWLLFGSSLALLVRILCGSRRKPLSRLYVTISL GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL DFLFSGADS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL	222 237 210 209 206 210 237 223 220 220 220
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mMn tr tr tr tr tr tr gp tr tr	rgC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRSFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRLFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAAYLMFLFVVLCLSSLALLVRLFCGAGRMKLTRLYVTIIL GFLDTKYEDDYGCLAMNFLTTAYLMFLFVVLCLSSLALLVRLFCGAGRMKLTRLYVTIIL GFLGETHHH-LWKN-VDFIVTAFLIFLFWLLFGSSLALLVRILCGSRRKPLSRLYVTISL GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL DFLFSGADS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL	222 237 210 209 206 210 237 223 220 220 220 220 220
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mMn trrtrtrtr gpprr gtr	rgCll Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRIFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRIFCGAGRMKLTRLYATIML GFLNTQYKNENGCLALNFFTAAYLMFLFVVLCLSSLALLVRIFCGAGRMKLTRLYVTIIL GFLDTKYEDDYGCLAMNFLTTAYLMFLFVVLCVSSLALLARLFCGAGRMKLTRLYVTITL GYLDNHYFNYSVCQAWDIFIGAYLMFLFVVLCVSSLALLARLFCGAGRMKLTRLYVTITL GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL DFLFSGADS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-WCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-WCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-WCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL	222 237 210 209 206 210 237 223 220 220 220 220 220 220 198 220
mMr tr tr tr tr tr tr g p tr g tr tr	rgC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8	GFLGETHHH-LWKN-VDFIITAFLIFLFMLLSGSSLALLLRILCGPRRKPLSRLYVTIAL GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRIFCGAGRMKLTRLYATIML GFLDTKYEKDNRCLASNFFTAACLIFLFVVLCLSSLALLVRIFCGAGRMKLTRLYATIML GFLDTKYEDDYGCLANNFFTAAYLMFLFVVLCLSSLALVARLFCGAGRMKLTRLYVTITL GYLDNHYFNYSVCQAWDIFIGAYLMFLFVVLCVSSLALLARLFCGAGRMKLTRLYVTITL GYLDTHYPNYSVCQAWDIFIGAYLMFLFVVLCUSSLALLARLFCGAGRMKLTRLYVTITL GFLGETHHH-LWKN-VDFIVTAFLIFLFMLLFGSSLALLVRILCGSRRKPLSRLYVTISL DFLFSGADS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGADS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL GFLFSGANS-VWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-WCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-WWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-WWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-WWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-WWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-WWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-WWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL DFLFSGANS-WWCETSDFITIAWLVFLCVVLCGSSLVLLVRILCGSRKMPLTRLYVTILL	222 237 210 209 206 210 237 223 220 220 220 220 220 220 198 220 220
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Figure S2.1 (continued)

mM	raC11	TVMVYLICGLPLGLYLFLLYWFGVHLHYPFCHIYOVTAVLSCVNSSANPIIYFLVC	SFRO 2	282
tr	091YB7	TVLVFLLCGLPFGIHWFLLIWIKIDYGKFAYGLYLAALVLTAVNSCANPIIYFFV	SFRH 2	297
tr	07TN49	TVLVFLLCGLPFGIHWFLLIWIKIDYGKFAYGLYLAALVLTAVNSCANPIIYFFVC	SFRH 2	270
tr	091WW5	SILVFLLCGLPFGIHWFLLFKIKDDFHVFDLGFYLASVVLTAINSCANPIIYFFVC	SFRH 2	269
tr	091ZC6	TLLVFLLCGLPCGFYWFLLSKIKNVFTVFEFSLYLASVVLTAINSCANPIIYFFVC	SFRH 2	266
tr	091WW3	TVLVFLLCGLPWGITWFLLFWIAPGVFVLDYSPLLVLTAINSCANPIIYFFV(SFRO 2	267
tr	08R4G1	TVMVYLICGLPLGLYLFLLYWFGIHLHYPFCHIYOVTVLLSCVNSSANPIIYFLVC	SFRH 2	297
tr	Q7TN42	TVMVYLICGLPLGLYLFLLYWFGIHLHYPFCHIYQVTVLLSCVNSSANPIIYFLV	SFRH 2	283
tr	096LB0	TVLVFLLCGLPFGIOWALFSRIHLDWKVLFCHVHLVSIFLSALNSSANPIIYFFV(SFRO 2	280
qp	AX923125 40216229	TVLVFLLCGLPFGIQWALFSRIHLDWKVLFCHVHLVSIFLSALNSSANPIIYFFV	SFRQ 2	280
gp	AX647081 28800069	TVLVFLLCGLPFGIQWALFSRIHLDWKVLFCHVHLVSIFLSALNSSANPIIYFFV	SFRQ 2	280
tr	Q8TDE1	TVLVFLLCGLPFGIQWALFSRIHLDWKVLFCHVHLVSIFLSALNSSANPIIYFFV	SFRQ 2	280
tr	Q8TDE0	TVLVFLLCGLPFGIQWALFSRIHLDWKVLFCHVHLVSIFLSALNSSANPIIYFFM	SFRQ 2	280
qp	AX657514 29160254	TVLVFLLCGLPFGIQWALFSRIHLDWKVLFCHVHLVSIFLSALNSSANPIIYFFV	SFRQ 2	258
tr	Q96LB2	TVLVFLLCGLPFGIQFFLFLWIHVDREVLFCHVHLVSIFLSALNSSANPIIYFFV	SFRQ 2	280
tr	Q8TDD8	TVLVFLLCGLPFGIQFFLFLWIHVDREVLFCHVHLVSIFLSALNSSANPIIYFFV	SFRQ 2	280
tr	O8TDD9	TVLVFLLCGLPFGIOFFLFLWIHVDREVLFCHVHLVSIFLSALNSSANPIIYFFV	SLRO 2	280
tr	Q96LA9	TVLVFLLCGLPFGILGALIYRMHLNLEVLYCHVYLVCMSLSSLNSSANPIIYFFV	SFRQ 2	280
qp	AX646849 28799318	TVLVFLLCGLPFGILGALIYRMHLNLEVLYCHVYLVCMSLSSLNSSANPIIYFFV	SFRQ 2	280
tr	Q8TDD6	TVLVFLLCGLPFGILGALIYRMHLNLEVLYCHVYLVCMSLSSLNSSANPIIYFFV	SFRQ 2	280
tr	Q8TDD7	TVLVFLLCGLPFGILGALIYRMHLNLEVLYCHVYLVCMSLSSLNSSANPIIYFFV	SFRQ 2	280
qp	AX657510 29160250	TVLVFLLCGLPFGILGALIYRMHLNLEVLYCHVYLVCMSLSSLNSSANPIIYFFV	SFRQ 2	258
tr	Q7TN45	TVLVFIFFGLPIGICVFLLPWIHMMLSSFFYEMVTLLSCVNSCANPIIYFFV(SIRH 2	280
tr	Q91ZC0	TVVVFIYFGMPFGICWFLLSRIMEFDSIFFNNVYEIIEFLSCVNSCANPIIYFLVC	SIRQ 2	282
tr	Q91ZC3	TVLVFLIFGLPYGIYWFLLEWIREFHDNKPCGFRNVTIFLSCINSCANPIIYFLVC	SIRH 2	286
tr	Q7TN48	TVLVFLLCGLPYGIYWFLLEWTEKFNYNLPCGFHPVTVLLSCVNSCANPIIYFLVC	SIRH 2	264
tr	Q91ZC2	TVLVFLIFGLPFGIYWILYQWISNFYYVEICNFYLEILFLSCVNSCMNPIIYFLVC	SIRH 2	294
tr	Q8CDY4	TVLVFLIFGLPFGIYWILYQWISNFYYVEICNFYLEILFLSCVNSCMNPIIYFLVC	SIRH 2	294
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mMı	rgCll	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY	322	
mMu tr	cgC11 (091YB7	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY	322 331	
mMn tr tr	cgC11 (Q91YB7 (07TN49	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP OKHOTLKMVLORALODTPETAENTVEMSSSKVEP	322 331 304	
mMu tr tr tr	cgC11 Q91YB7 Q7TN49 Q91WW5	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHOTLKMVLONALODTPETAKIMVEMSRSKSEP	322 331 304 304	
mMn tr tr tr	29C11 Q91YB7 Q7TN49 Q91WW5 091ZC6	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQNALQDTPETAKIMVEMSRSKSEP R-LKHOTLKMVLOSALODTPETPENMVEMSRNKAEL	322 331 304 304 301	
mMn tr tr tr tr	cgC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 091WW3	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQNALQDTPETAKIMVEMSRNKAEL R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LNKOTLKMVLOKALODTPETPENMVEMSRNKAEP	322 331 304 304 301 302	
mMn tr tr tr tr tr	2911 291YB7 27TN49 291WW5 291ZC6 291WW3 08R4G1	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENMVEMSSKVEP R-LKHQTLKMVLQSALQDTPETAKIMVEMSRNKAEL R-LNKQTLKMVLQKALQDTPETPENMVEMSRNKAEP R-LNKQTLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLKRALEETPEEDEYTDSHV-0KPTEISERRC	322 331 304 304 301 302 337	
mMn tr tr tr tr tr tr	rgC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENMVEMSRSKSEP R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LNKQTLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLKRALEETPEEDEYTDSHV-0KPTEISERRC	322 331 304 301 302 337 323	
mMn tr tr tr tr tr tr	rgC11 Q91YB7 Q7TN49 Q91W95 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q97E0	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENMVEMSRSKSEP R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LNKQTLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLGRALQDTPEVDEGGGWLP-OETLELSGSRLEO	322 331 304 301 302 337 323 322	
mMi tr tr tr tr tr tr tr	rgC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQNALQDTPETAENMVEMSRSKSEP R-LKHQTLKMVLQALQDTPETPENMVEMSRNKAEL R-LNKQTLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLKALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ	322 331 304 304 301 302 337 323 322 322	
mMn tr tr tr tr tr tr gp	rgC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQNALQDTPETPAKIMVEMSRSKSEP R-LKHQTLKMVLQXALQDTPETPENMVEMSRNKAEL R-LNKQTLKMVLQKALQDTPETPENMVEMSRNKAEL R-KKHRSLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ	322 331 304 301 302 337 323 322 322 322	
mMn trrtrtrtr trttr gp tr	rgC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 Ax923125 40216229 Ax647081 28800069 Q8TDE1	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQSALQDTPETPENMVEMSRSKSEP R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LKKQTLKMVLQKALQDTPETPENMVEMSRNKAEL R-KKHRSLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ	322 331 304 301 302 337 323 322 322 322 322	
mMn tr tr tr tr tr tr gp gr tr	rgC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 Ax923125 40216229 Ax647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQSALQDTPETPENMVEMSRSKSEP R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LKKQTLKMVLQKALQDTPETPENMVEMSRNKAEL R-KKHRSLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ L-QNRKTLKLVLQRALQDTPEVDEGGWLP-QETLELSGSRLEQ	322 331 304 301 302 337 323 322 322 322 322 322 322	
mMn tr tr tr tr tr tr gp gr tr gp	gC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENMVEMSRNKAEP R-LKHQTLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGWLP-QETLELSGSRLEQ	322 331 304 301 302 337 323 322 322 322 322 322 304	
mMn trrtrtrtr trtrgpptrgp trgp	gC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAKIMVEMSRNKAEL R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALYTIRSRESYSLSREQQREDPTHDSILS	322 331 304 301 302 337 323 322 322 322 322 322 322 322 304 322	
mMn trrtrtrtr trgp gtr gp tr gp tr	gC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDB8	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQALQDTPETAKIMVEMSRNKAEL R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LKHSLKMVLQKALQDTPETPENMVEMSRNKAEL R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ	322 331 304 301 302 337 323 322 322 322 322 322 322 304 322	
mMn trrtrtrtr trgpprrgprrtr trtr	gCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDB8 Q8TDD8 Q8TDD8	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQNALQDTPETAKIMVEMSRNKAEL R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LKHSLKMVLQKALQDTPETPENMVEMSRNKAEL R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGWLP-QETLELSGSRLEQ	322 331 304 301 302 337 323 322 322 322 322 322 322 304 322 322 322 322 322	
mMn trrtrrtrrgp grrtg trrtr tr	gC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8 Q8TDD9 Q96LA9	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQNALQDTPETAKIMVEMSRSKSEP R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LKKTSLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGWLP-QETLELSGSKLEQ R-QNRQNLKLVLQRALQDTPEVDEGGWLP-QETLELSGSKLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ	322 331 304 301 302 337 323 322 322 322 322 322 322 322 32	
mMn trrtrtrtrgp gptrtgptrtrtr	rgCl1 Q91YB7 Q7TN49 Q91W5 Q91ZC6 Q91W3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQNALQDTPETAENMVEMSRSKSEP R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LKKQTLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGWLP-QETLELSGSRLEQ	322 331 304 301 302 337 323 322 322 322 322 322 324 322 322 322	
mM tr tr tr tr tr gp gtr tr gp tr tr tr tr gp gtr tr gp tr tr tr tr gp tr	gC11 Q91YB7 Q7TN49 Q91W5 Q91ZC6 Q91W3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX647081 29160254 Q96LB2 Q8TDD8 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQNALQDTPETAENMVEMSRSKSEP R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LKHQTLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ	322 331 304 301 302 337 323 322 322 322 322 322 322 322 32	
mMrtrrrrrrggprrgprrrgtrrr	rgCl1 Q91YB7 Q7TN49 Q91W5 Q91ZC6 Q91W3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDB8 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6 Q8TDD7	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENMVEMSRNKAEL R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEP R-LKHRSLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ	322 331 304 301 302 337 323 322 322 322 322 322 322 322 32	
M trrtrtrtrtgggtrggtrtrgtrtrgtrg	rgCl1 Q91YB7 Q7TN49 Q91W5 Q91ZC6 Q91W3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6 Q8TDD7 AX657510 29160250	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENMVEMSRNKAEL R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEP R-LKHRSLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ	322 331 304 301 302 337 323 322 322 322 322 322 322 322 32	
mM trttrtttrggptrgptrtgttrg trgprtrgpr	gC11 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDD8 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6 Q8TDD7 AX657510 29160250 Q7TN45	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAKIMVEMSRSKSEP R-LKHQTLKMVLQKALQDTPETPENMVEMSRNKAEL R-KKHRSLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKHRSLKMVLQRALQDTPEVDEGDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGUP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP	322 331 304 301 302 337 323 322 322 322 322 322 322 322 32	
mMrttrtttrtf999ttr99ttrtgttrgttrgttr	gC11 Q91YB7 Q7TN49 Q91W95 Q91ZC6 Q91ZC6 Q91W3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDB8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6 Q8TDD6 Q8TDD7 AX657510 29160250 Q7TN45 Q91ZC0	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY -QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENMVEMSRSKSEP R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-KKRSLKMVLQKALQDTPETPENMVEMSRNKAEP R-KKRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDKPEVDKGEQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP	322 331 304 301 302 337 323 322 322 322 322 322 322 322 32	
mm trrtrrtrrgg grrtg trrtrg trg trrg trr	gCl1 Q91YB7 Q7TN49 Q91W5 Q91W5 Q91ZC6 Q91ZC6 Q91W3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDB8 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6 Q8TDD7 AX657510 29160250 Q7TN45 Q91ZC0 Q91ZC3	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENMVEMSRNKAEL R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-KKRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGUP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLEQ R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP	322 331 304 301 302 327 323 322 322 322 322 322 322 322 32	
mMr trrtrtrrggtrrgptrrtgrtrgptrrgrtrr	gCl1 Q91YB7 Q7TN49 Q91W5 Q91ZC6 Q91ZC6 Q91W3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDB8 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6 Q8TDD7 AX657510 29160250 Q7TN45 Q91ZC3 Q7TN48	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENMVEMSRNKAEL R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LKHQTLKMVLQRALQDTPETPENMVEMSRNKAEP R-KKRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGULP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGULP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLEQ R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKL	322 331 304 301 302 327 322 322 322 322 322 322 322 322 32	
m trtrtrtrtrgggtrtgtrtrtrgtrtgtrtrtrtr	gCl1 Q91YB7 Q7TN49 Q91W5 Q91ZC6 Q91ZC6 Q91W3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDB8 Q8TDB8 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD7 AX657510 29160250 Q7TN45 Q91ZC0 Q91ZC3 Q7TN48 Q91ZC2	H-RKHRSLKRVLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKMVLQNALQDTPETAENMVEMSRSKSEP R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-LKHQTLKMVLQSALQDTPETPENMVEMSRNKAEL R-KKRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-KKHRSLKMVLKRALEETPEEDEYTDSHV-QKPTEISERRC R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDTPEVDEGGGWLP-QETLELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGQLP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGULP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDASEVDEGGGULP-EEILELSGSRLEQ R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP R-QNRQNLKLVLQRALQDKPEVDKGEGQLP-EESLELSGSRLGP	322 331 304 301 302 323 322 322 322 322 322 322 322 322	
mtrttrtrtrtgggtrtgrtrtrtgttggtrtrgttt	gCl1 Q91YB7 Q7TN49 Q91WW5 Q91ZC6 Q91WW3 Q8R4G1 Q7TN42 Q96LB0 AX923125 40216229 AX647081 28800069 Q8TDE1 Q8TDE0 AX657514 29160254 Q96LB2 Q8TDB8 Q8TDB8 Q8TDD8 Q8TDD9 Q96LA9 AX646849 28799318 Q8TDD6 Q8TDD7 AX657510 29160250 Q7TN45 Q91ZC3 Q7TN48 Q91ZC2 Q8CDY4	H-RKHRSLKR VLKRALEDTPEEDEYTDSHL-HKTTEISESRY QKHQTLKM VLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKM VLQRALQDTPETAENTVEMSSSKVEP R-LKHQTLKM	322 331 304 301 302 323 322 322 322 322 322 322 322 322	

Figure S2.1 (continued)



Figure S2.2 Hydrophobicity profile for mMrgC11 sequence set (window size = 12).

mrgC11	MDPTISSHDTESTPLNETGHPNC	: 23
sp Q91WW5 MGA1_MOUSE	GGINGGIN	10
sp Q91WW4 MGA2_MOUSE	GSIN	- 10
sp Q91WW3 MGA3 MOUSE	GSIDGSID	- 10
sp Q91WW2 MGA4 MOUSE	GSIDMAPTTTNPMNETIPGSID	- 18
sn1091ZC71MGA5_MOUSE	KYGHKYGH	- 10
an LOGIZCELMGNE MOUSE		. 6
splQ912C5 mGA/_mOUSE	R51DR51D	· 10
sp Q91ZC4 MGA8_MOUSE	GSID	10
tr Q91ZC3 MrgB1	MDLVIQDWTINITALKESNDNGISFCE	27
tr Q91ZC2 MrgB2	MSGDFLIKNLSTSAUKTNITVLNGSYYIDTSVCV	134
tri091ZC1 MrgB3	MALRTSLITTTAPDKTSLPISICI	24
triogizen MrgB4	MGTTTL & MNITNT & FNGS-VIEWFSCI	26
CELOSIZES MIGDS	RGLIIPAWNINNIVVNGSNNIEHFSCV	21
tr Q7TN51 MigB8	MDSSFPDWNIEFREQNESYFMESSSCD	27
sp Q91ZB8 MRGD_MOUSE	MNSTLDSSPAPGLTISPTMD-LVTW	1 24
sp Q91ZB7 MRGE MOUSE	MTSLSVHTDSPSTQGEN	[17
sp Q8VCJ6 MRGF MOUSE	MAGNCSWEAHSTNQNKMCPGMSEARELYSRGFLTIEQIATL	41
an1 091 ZB51 MRGG MOUSE	MFSTFNING	. g
aplo7TN49 MDC) DiT	MDVTTPCSEN	. 10
spiermus MrgB1		
CE Q7IN48 MigDi		
tr Q7TN47 MIGB2	MSSCG	; 5
tr Q7TN45 MrgB4	MSPTTQAWSINNTVVKENYYTEILSCI	27
tr Q7TN44 MrgB5	MPDSPTESYGPDREYHVFISLFLCRNTSGKFLSVGPATPGWSINNTVVKENYYTEKLSCI	60
tr107TN43 MrgB6	MDINISTLDIDIIELNGSNYTNTEICF	27
triozTN50 MrgB8	MDSSIPDPFADLTOLNGSVHTFTSPCV	27
triozmuas MrgC	NDDTIGI GTEGTTI NUTCUDG	
		. 23
sp Q7TN41 MRGD_RAT	MNYTPYSSPAPGLTISPTMD-PVTW	1 24
sp Q7TN40 MRGE_RAT	MSLRVHTHSPSTQGDM	1 16
sp P23749 MRGF RAT	MAGNCSWEAHSTNONKMCPGMSEALELYSRGFLTIEQIATL	41
sp Q7TN39 MRGG RAT	MLSIFNIWG	9
triozTN38 MraH	MEPL&TTLCPOECTOTTRNETPNETTWSSEHVTKY	35
am LOGI 7961 MDCD MACEA		25
SpigeLieeinker A		. 23
sbloaerbslwkgi_Howaw	MDPTISTLDTELTPINGTEETLCY	24
sp Q96LB1 MRG2_HUMAN	MDPTTPAWGTESTTVNGNDQALLLLCG	; 27
sp Q96LB0 MRG3_HUMAN	MDSTIPVLGTELTPINGREETPCY	24
sp Q96LA9 MRG4 HUMAN	MDPTVPVFGTKLTPINGREETPCY	24
SDIOSTDS7 MRGD HUMAN	MNOTLNSSGTVESALNYSRGSTVHT	25
SDIO964M11MRGF HUMAN	MAGNCSHEAHPGNRNKMCPGLSEAPELVSRGFLTIEOIAMI	. 41
sp Q96AM1 MRGF_HUMAN	MAGNCSWEAHPGNRNKMCPGLSEAPELYSRGFLTIEQIAML	41
sp Q96AM1 MRGF_HUMAN	MAGNCSWEAHPGNRNKMCPGLSEAPELYSRGFLTIEQIAM	. 41
sp Q96AM1 MRGF_HUMAN mrgC11	TAGNCSWEAHPGNRNKMCPGLSEAPELYSRGFLTIEQIAML	, 41 82
sp Q96AM1 MRGF_HUMAN mrgC11 sp Q91WW5 MGA1_MOUSE	TPILTLSFLVLITTLVGLAGNTIVLWLLGFR-MRRKAISVYILNLALADSFFLCCHFIDS ITILIPNLMIIIFGLVGLTGNGIVFWLLGFC-LHRNAFSVYILNLALADFFFLLGHIDS	, 41 82 69
sp Q96AM1 MRGF_HUMAN mrgC11 sp Q91WW5 MGA1_MOUSE sp Q91WW4 MGA2_MOUSE	TPILTLSFLVLITTLVGLAGNTIVLULLGFR-MRRKAISVYILNLALADSFFLCCHFIDS ITILIPNLMIIIFGLVGLTGNGIVFWLLGFC-LHRNAFSVYILNLALADFFFLLGHIIDS IRILIPRLMIIIFGLVGLMGNAIVFWLLGFH-LRRNAFSVYILNLALADFLFLSSIIAS	, 41 82 69 69
sp Q96AM1 MRGF_HUMAN mrgC11 sp Q91WW5 MGA1_MOUSE sp Q91WW4 MGA2_MOUSE sp Q91WW3 MGA3_MOUSE	MAGNCSWEAHPGNRNKMCPGLSEAPELYSRGFLTIEQIAMI TPILTLSFLVLITTLVGLAGNTIVLWLLGFR-MRRKAISVYILNLALADSFFLCCHFIDS ITILIPNLMIIIFGLVGLTGNGIVFWLLGFC-LHRNAFSVYILNLALADFFFLLGHIDS IRILIPKLMIIIFGLVGLMGNAIVFWLLGFH-LRRNAFSVYILNLALADFLFLLSSIIAS IETLIPDLMIIIFGLVGLTGNAIVFWLLGFR-MHRTAFLVYILNLALADFLFLLCHIDS	, 41 82 69 69 69
sp Q96AM1 MRGF_HUMAN mrgCl1 sp Q91WW5 MGA1_MOUSE sp Q91WW4 MGA2_MOUSE sp Q91WW3 MGA3_MOUSE sp Q91WW3 MGA4_MOUSE	TPILTLSFLVLITTLVGLAGNTIVLWLLGFR-MRRKAISVYILNLALADSFFLCCHFIDS ITILIPNLMIIIFGLVGLTGNGIVFWLLGFC-LHRNAFSVYILNLALADFFFLLGHIIDS IRILIPKLMIIIFGLVGLTGNAIVFWLLGFC-LHRNAFSVYILNLALADFFFLLGHIIDS IETLIPDLMIIIFGLVGLTGNAIVFWLLGFH-LRNAFLVYILNLALADFLFLLSSIIAS IETLIPNLMIIIFGLVGLTGNVIFWLLGFH-LHRNAFLVYILNLALADFLFLLCHIINS	, 41 82 69 69 69 77
sp Q96AM1 MRGF_HUMAN mrgC11 sp Q91WW5 MGA1_MOUSE sp Q91WW4 MGA2_MOUSE sp Q91WW3 MGA3_MOUSE sp Q91WW2 MGA4_MOUSE sp Q91WZ2 MGA4_MOUSE	TPILTLSFLVLITTLVGLAGNTIVLULGFR-MRRKAISVYILNLALADSFFLCCHFIDS ITILIPNLMIIIFGLVGLTGNGIVFWLLGFC-LHRNAFSVYILNLALADFFFLLGHIIDS IRILIPKLMIIIFGLVGLTGNAIVFWLLGFC-LHRNAFSVYILNLALADFFFLLGHIIDS IETLIPDLMIIIFGLVGLTGNAIVFWLLGFR-MHRTAFLVYILNLALADFLFLLCHIINS IETLIPNLMIIIFGLVGUTGNVIFWLLGFS-LHRNAFSVYILNLALADFLFLLCHIINS	, 41 82 69 69 69 77 68
sp Q96AM1 MRGF_HUMAN mrgC11 sp Q91WW5 MGA1_MOUSE sp Q91WW4 MGA2_MOUSE sp Q91WW4 MGA3_MOUSE sp Q91WW2 MGA4_MOUSE sp Q91ZC7 MGA5_MOUSE sp Q91ZC7 MGA5_MOUSE	MAGNCSWEAHPGNRNKMCPGLSEAPELYSRGFLTIEQIAMI TPILTLSFLVLITTLVGLAGNTIVLWLLGFR-MRRKAISVYILNLALADSFFLCCHFIDS ITILIPNLMIIIFGLVGLTGNGIVFWLLGFR-LRNAFSVYILNLALADFFFLLGHIDS IRILIPKLMIIIFGLVGLTGNAIVFWLLGFH-LRNAFSVYILNLALADFLFLLSSIIAS IETLIPDLMIIIFGLVGLTGNVILFWLLGFH-LHRNAFLVYILNLALADFLFLLCHIINS IETLIPNLMIIIFGLVGLTGNVILFWLLGFS-LHRNAFSVYILNLALADFVFLLCHIINS LDB-DFKLMIIIFRLVOMTGNAIVFWLLGFS-LHRNAFSVYILNLALADFVFLLCHIIDS	, 41 82 69 69 69 77 68
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sp Q96AN1 MRGF_HUMAN mrgC11 sp Q91WW5 MGA1_MOUSE sp Q91WW3 MGA2_MOUSE sp Q91WW3 MGA3_MOUSE sp Q91WW3 MGA3_MOUSE sp Q91WW3 MGA3_MOUSE sp Q91ZC7 MGA5_MOUSE sp Q91ZC6 MGA6_MOUSE sp Q91ZC6 MGB4 tr Q91ZC0 MrgB3 tr Q91ZC0 MrgB4 tr Q91ZB6 MRG6_MOUSE sp Q91ZB6 MRG6_MOUSE sp Q91ZB6 MRG6_MOUSE sp Q91ZB5 MRG6_MOUSE sp Q91ZB5 MRG6_MOUSE sp Q91ZB6 MRG6_MOUSE sp Q91ZB6 MRG6_MOUSE sp Q91ZB6 MRG6_MOUSE sp Q91ZB6 MRG6_MOUSE sp Q7TN49 MRG6_RAT tr Q7TN45 MrgB4 tr Q7TN45 MrgB4 tr Q7TN46 MrgB6 tr Q7TN47 MrgB2 tr Q7TN40 MRG6_RAT sp Q7TN40 MRG6_RAT sp Q7TN40 MRG6_RAT sp Q7TN40 MRG6_RAT sp Q7TN30 MRG6_RAT tr Q7TN36 MRG6_MAT sp Q6LB2 MRG1_HUMAN <td><pre></pre></td> <td>41 82 69 69 69 69 69 88 88 88 70 69 64 64 88 88 88 69 64 88 88 88 88 88 88 88 88 88 88 88 88 88</td>	<pre></pre>	41 82 69 69 69 69 69 88 88 88 70 69 64 64 88 88 88 69 64 88 88 88 88 88 88 88 88 88 88 88 88 88
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Figure S2.3 Multiple alignment of 39 verified Mrg sequences, including 19 mouse (orange), 13 rat (navy), 1 monkey and 6 human (violet) receptors. The positions of six key residues are specified in red boxes.

mrgC11 sp|Q91WW5|MGA1 MOUSE sp|091WW4|MGA2 MOUSE sp|Q91WW3|MGA3_MOUSE sp|Q91WW2|MGA4 MOUSE sp|Q91ZC7|MGA5_MOUSE sp|Q91ZC6|MGA6_MOUSE sp|Q91ZC5|MGA7 MOUSE sp|Q91ZC4|MGA8 MOUSE tr/Q91ZC3 MrgB1 tr | Q91ZC2 MrgB2 tr|Q91ZC1 MrgB3 tr|Q91ZC0 MrgB4 tr|Q91ZB9 **MrgB5** tr | Q7TN51 MrgB8 sp|Q91ZB8|MRGD MOUSE sp|Q91ZB7|MRGE MOUSE sp|Q8VCJ6|MRGF MOUSE sp|Q91ZB5|MRGG_MOUSE sp|Q7TN49|MRGA_RAT tr|Q7TN48 MrgB1 tr|Q7TN47 MrgB2 tri07TN45 MraB4 tr | Q7TN44 MrgB5 tr|Q7TN43 MrgB6 tr/Q7TN50 MrgB8 tr|Q7TN42 MrgC sp|Q7TN41|MRGD_RAT sp|Q7TN40|MRGE RAT sp|P23749|MRGF RAT sp|Q7TN39|MRGG_RAT tr|Q7TN38 MrgH sp|Q6L786|MRGD_MACFA sp|Q96LB2|MRG1_HUMAN sp|Q96LB1|MRG2_HUMAN sp|Q96LB0|MRG3 HUMAN sp|Q96LA9|MRG4 HUMAN sp|Q8TDS7|MRGD_HUMAN sp|Q96AM1|MRGF_HUMAN mrgC11 sp|Q91WW5|MGA1 MOUSE sp|Q91WW4|MGA2 MOUSE sp|Q91WW3|MGA3 MOUSE sp|Q91WW2|MGA4 MOUSE sp|Q91ZC7|MGA5_MOUSE sp|Q91ZC6|MGA6_MOUSE sp|Q91ZC5|MGA7 MOUSE sp|Q91ZC4|MGA8 MOUSE tr | Q91ZC3 MrgB1 tr | Q91ZC2 MrgB2 tr|Q91ZC1 MrgB3 tr|Q91ZC0 MrgB4 tr | Q91ZB9 MrgB5 tr|Q7TN51 MrgB8 sp|Q91ZB8|MRGD MOUSE sp|Q91ZB7|MRGE MOUSE sp|Q8VCJ6|MRGF MOUSE sp|Q91ZB5|MRGG MOUSE sp|Q7TN49|MRGA_RAT tr|Q7TN48 MrgB1 tr|Q7TN47 MrgB2 tr|Q7TN45 MrgB4 tr|Q7TN44 MrgB5 tr|Q7TN43 MrgB6 tr/Q7TN50 MrgB8 tr | Q7TN42 MrgC sp|Q7TN41|MRGD_RAT sp|Q7TN40|MRGE RAT sp|P23749|MRGF RAT sp|Q7TN39|MRGG_RAT tr|Q7TN38 MrgH sp|Q6L786|MRGD MACFA sp|Q96LB2|MRG1 HUMAN SD|096LB1|MRG2 HUMAN sp|Q96LB0|MRG3 HUMAN sp|Q96LA9|MRG4 HUMAN sp|Q8TDS7|MRGD_HUMAN .sp|Q96AM1|MRGF_HUMAN

 FYIIFDIYT-IPIKLPLF----FIVMLNFATLCGLSILSAVSIERCLCVMRPFWY-RCQL
 118

 LECVLQFDN----SYFYF---LLTILMFMYLAGFCMIAAISTERCLSVTWPIWY-HCQR
 137

 LECVLQFDN----SYFYF---LLTILMFATLAALCMIPAISTERCLSVTWPIWY-HCQR
 140

 LAIISYKYSISFRIPFL----FEVLAKVANLSGLSILSISISERCLCIMWPIWY-HCQR
 141

 LEKIIVLFHSVHIHIPLL---FYTLSTLAYLAGVSMVTAISAEYWLSGIWPYWY-QGQR
 141

 LMRIMNFYGIYAHKLSK---FILGNVAFIPYISGLSILSAISTERCLSVLWPIWY-HCRR
 138

 SLETGPLLTASTSARVYE---GMKRIKYFAYTAGLSLLTAISTORCLSVLFPIWY-KCRR
 139

 IPELLQOLNFPEFVHIS----LIMLRFFCYIVGLSLLVAISTEQCLATLFPSGY-LCRR
 130

 LLMMGTF-LGSFPDYVRR---VSRIVCLCTF AGVSLLPAISIERCVSVIFPMWY-WRRR
 155

 IAKIASGYEDTLYFP------VTFLWFAVGLWLLAAFIVDCCLSYMFPSFCGPNCR
 118

 LVNT----FHIYDSTLVS---YAVLFMIFGYNTGLHLLTAISVERCLSVLYFIWF-HCRR
 142

 SLETQPL--VSTDKVHE---LMKKLKYFAYTVGLSLLTAISTQRCLSVLFPIWF-KCRR
 134

 LLSFISIP----HTISK----ILYPVMMFSYFAGLSFLSAVSTERCLSVLWPIWY-RCHR 134 LVYLSNFF----RISK---INFYMMISIE AGLSNISAGSIEGAUSTERCLSUUPIWY-RCRR 134 PLRLINIR----HPISK----ILSPVMTFPYFIGLSMLSAISTERCLSILWPIWY-RCRR 134 PLRLINIS----HLIRK----ILVSVMTFPYFIGLSMLSAISTERCLSULWPIWY-RCRR 134 SLETQPL--VNTTDKVHE---LMKRLMYFATTVGLSLLTAISTQRCLSVLFPIWF-KCHR 137 ILNTGGF-LGTFADYIRS---VCRVLGLCMFLTGVSLLPAVSAERCASVIFPAWY-WRRR 155 . : PRNMSAIICALIUVLSFLMGILDUF-SGFLGETH-HHLUKN-VDFIITAFLIFLFMLLSG 195 PEHTSTVMCAVIUVLSLLICILNSYFCGFLNTQYKNENGCLALNFFTAAYLMFLFVVLCL 182 PVHTSTVMCAVIWVLSLLICIL<mark>NS</mark>YFCAVLHTRYDNDNECLATNIFTASYMIFLLVVLCL 183 PEHTSTVMCAVIWVLSLLICIL<mark>D</mark>GYFCGYLDNHYFNYSVCQAU<mark>D</mark>IFIGAYLMFL<mark>FV</mark>VLCL 183 PEHTSTVLCAVIWFLPLLICIL<mark>NG</mark>YFCHFFGPKYVIDSVCLAT<mark>N</mark>FFIRTYPMFL<mark>F</mark>IVLCL 191 PEHTSTVMCAAIUWLPLLUCILMQYFCHFFGPKVVIDSVCLAIMFFIRTPHLFIVLCL 191 PEHTSTVMCAAIUWLPLLVCILMRYFCSFLDINYNNDKQCLASNFFTRAYLMFLFVVLCL 182 PEHTSTVMCAMIUWLSLLCILVRYFCSFLDTKYEDDYGCLAMNFLTATVLMFLFVVLCL 183 PEHTSTVMCAVIWIFSVLICILMRYFCDFFGTKLGNYTVCQASNFFMGAYLMFLFVVLCL 183 PEHTSTAMCAVIWVMSLLISILMGYFCNFSSPKYVNNSVCQASDIFIRTYPIFLFVLLCL 183 PRHTSAVICTVLWVLSLVLSLLPGKECGFLYYTS-GPGLCKTFDLITTAWLIVLFVVLLG 199 PRHTSATICFVLWVMSLLGLLPGKACGLFNSF-DSYNCETTDVITNIWSVVFFGVLCG 207 PRHTSAVICVLLWALSLLFPAL<mark>C</mark>MEKCSVLFNTF-EYSWCGIINIISGAWLVVL<mark>FV</mark>VLCG 196 PRHTSAIMCALVWVSSLLLSLV<mark>VG</mark>LGCGFLFSYY-DYYFCITL<mark>N</mark>FITAAFLIVL<mark>S</mark>VVLSV 195 PRHTSSIMCALLWAFCLLLNFLLGEGGGLFSDP-KYYFCITGALITALIILLTVVBSV 196 PRHTSSIMCALLWAFCLLLNFLLGEGGGLFSDP-KYYFCITGALITALIILLTVVBSV 196 PRHTSSVVSGALWALAFLMNFILGSFFCVQFWHPNK-HQCFKVDIVFNSLILGIFMPVMIL 198 PRYLTTCVCALIWVLCLLLDLLSGACTQFFGAP-SYHLCDMLWLVVAVLLAALCCTMCV 190 PKRLSAGVCALLUMLSFLVTSIHNYFCMFLGHEAPGTVCRNMDIALGILLFFLFCPLMVL 215 PRFTSVVLCLVIUALTMPAVLLPANACGLLKNGM-SLLVCLKYHUTSVTULAVLSGMACG 177 PKHTSTVMCSAIWVLSLLICIL<mark>NR</mark>YFCGFLDTKYEKDNRCLAS<mark>M</mark>FFTAACLIFL<mark>F</mark>VVLCL 183 PRHTSAVICTLLWVLALVWSLI<mark>E</mark>GKECGFLFDTN-GPGWCETF<mark>D</mark>LIATAWLIVLIVVLLG 177 PRHTSAVICTMLWVLSLVLSLS<mark>E</mark>GKECGFLPGTN-ISDWCKTI<mark>D</mark>LIITSWLIVL<mark>FV</mark>VLLV 177 PRHTSATVCALFWAFSLLLSLL<mark>LG</mark>QGCGFLFSKF-DYSFCRYC<mark>N</mark>FIATAFLIVI<mark>FM</mark>VLFV 196 PRHTSATVCALFWAFSLLLRLLLGQGCGFLFGKY-DYYFCRYCSFITTAFLIVLFWVPFV 229 PRYLTTCVCAFIWVLCLLLDLL<mark>L</mark>SGACTQFFGAP-SYHLCGML<mark>W</mark>LVVAVLLAALCCTMCV 189 PKRLSAGVCALLULLSFLVTSI<mark>HN</mark>YFCMFLGHEASGTACLNMD<mark>I</mark>SLGILLFFLF<mark>C</mark>PLMVL 215 PRYTSFVLCLVIWALTMLAVLLPANACGLLYNRM-SLLVCLKYHWVSVVWLGVLASTACG 177 PRYISFULCIUMALIHLAVICHANACGLINNA-SLUVCHATHWSVVWLGVLSIACG PRHOSTVACTLIWALSVUSGLDYFFCILEVKPOF-DECRYVYIFSCILTFLVFVPLWVF PRHLSAVVCALLWALSLLSSILDWHLCGFJFSGA-DSAWCOTSDFITVAWLIFLCVVLCG PRHLSAVVCVLLWALSLLSSILDWHLCGFJFSGA-DSAWCOTSDFITVAWLIFLCVVLCG PRYLSSVMCVLLWALSLLSSILDWHFCDFJFSGA-DSWCETSDFITVAWLIFLCVVLCG PRYLSSVMCVLLWALSLLSSILDWHFCDFJFSGA-DSWCETSDFITVAWLIFLCVVLCG PRYLSSVMCVLLWALSLLSSILDWHFCDFJFSGA-DSSWCETSDFITVAWLIFLCVVLCG PRYLSSVMCVLLWGLSLLSSILDWHFCDFJFSGA-DSSWCETSDFITVAWLIFLCVVLCG PRYLSSVMCVLLWGLSLLSSILDWHFCDFJFSGA-DSSWCETSDFITVAWLIFLCVVLCG PRYLSSVMCVLLWGLSLLSSILDWHFCDFJFSGA-DSSWCETSDFITVAWLIFLCVVLCG PRYLSSVMCVLLWGLSLLSSILDWHFCDFJFSGA-DSSWCETSDFITVAWLIFLCVVLCG PRYLSSVMCVLLWGLSLLSSILDWHFCDFJFSGA-DSSWCETSDFITVAWLIFLCVVLCG PRYLSSVMCGLLWJCZLJNG PRYLSSVMCGLLWJCZLJNG PRYLSSVMCGALLWJCZLVCZ PRYLSSVMCGALLWJCZUVCZ PRYLSSVMCGALWJCZ PRYLSSVMCGALWJCZ PRYLSSVMCZZ PRYLSSVMCZ PKRLSAVVCALLWVLSLLVTCLHNYFCVFLGRGAPGAACRHMD<mark>I</mark>FLGILLFLLC<mark>C</mark>PLMVL 215 . .* :

Figure S2.3 (continued)

68

LLRIIDFYGLYAHKLSK---DILGNAAIIP<mark>Y</mark>ISGLSILSAISTERCLCVLWPIWY-HCHR 138

ILLLLNVFYP-ITFLLC----FYTINMVL<mark>Y</mark>IAGLSMLSAISTERCLSVLCPIWY-HCHR 122 TLFLLKVSYLSIIFHLC----FNTINMVVYITGISMLSAISTECCLSVLCPTWY-RCHR 123 TVDLLKFTLPKGIFAFC----FHTIKRVL<mark>Y</mark>ITGLSMLSAISTERCLSVLCPIWY-HCRR 123

TMLLLKVHLPNNILNHC----FDIIMTVL<mark>Y</mark>ITGLSMLSAISTERCLSVLCPIWY-RCRR 131

MLLLLTVFYPNNIFSGY----FYTIMTVP<mark>Y</mark>IAGLSMLSAISTELCLSVLCPIWY-RCHH 122

MLLLITVFYPNNIFSGY----FYTINTVPNIAGLSMLSAISTELCLSVLCPIWY-RCHH 122 TEHILTFSSPNSIFINC----LYTFRVLLTIAGLSMLSAISIERCLSVNCPIWY-RCHS 123 ANFLLKVPIPNGIFVYC----FYTIKNVLTITGLSMLSAISTERCLSVLCPIWY-RCHR 123 INFLLKVPSPNIILDHC----FYTINIVLTITGLSMLSAISTERCLSVLCPIWY-RCHR 123 FYIILDIYF-IPTNFFSS---YTINIVLNIATLSGLSILTVISTERFLSVNWPIWY-RCQR 140 LLIAFYIFYSIDINIPLV---LYVVPIFATLSGLSILSTISIERCLSVIWPIWY-RCRR 148 FLIVCKLHY-FLFYIRQL---LDTVTNFATVFGLSITTIISIERCLSNWPIWY-HQR 137

LECVLQLDN----NSFYI----LLIVTMFAYLAGLCMIAAISAERCLSVMWPIWY-HCQR 136

LECVLQLDN----NSFYI---LIVINFARLAGLCMIAAISAERCLSVMWPIWY-HCQR 136 LECFLQLNR----RHTFF---LIVVFMFARLAGLCMIAAISVERSLSVMWPIWY-HCQR 137 LENLIRQFHYIDIHMALF---SVNVTILAYLAGVSMITAISVEFWLSVLWPTWY-HAQR 140 SLETGPLIVMISAKIYE---GMRRIKYFAYTAGLSLLTAISTQRCLSVLFPIWY-KCHR 139 IPELLQDQLNFPEFVHIS---LTMLRFFCYIVGLSLLAAISTEQCLATLFPAWY-LCRR 131 LLNMGTF-LGSFPDYIRR---VSRIVGLCTFFTGVSLLPAISIERCVSVIFPTWY-WRRR 155 IATIVSGHEDTLYFP-----VTFLWFAVGLWLLAAFSVDCCLAYMFPSFCSPNRR 118

TLLLLKFSYPNIIFLPC----FNTVMMVFYIAGLSMLSAISTERCLSVVCPIWY-RCRR 123 SHINLDMYY-IPIKFPLF---SIVVLNIGYLCGMSILSAISIERCLSVMWPIWY-RCQR 118 FYIIFDIYT-IPIKLPLF----FIVMLNFAYLCGLSILSAVSIERCLCVMRPFWY-RCQL 118

Figure S2.3 (continued)

mraC11 sp|091WW5|MGA1 MOUSE sp|Q91WW4|MGA2_MOUSE SDI 091WW3 | MGA3 MOUSE sp|Q91WW2|MGA4 MOUSE sp|Q91ZC7|MGA5_MOUSE sp|Q91ZC6|MGA6 MOUSE sp|Q91ZC5|MGA7 MOUSE sp|Q91ZC4|MGA8_MOUSE tr | Q91ZC3 MrgB1 tr|Q91ZC2 MrgB2 tr|Q91ZC1 MrgB3 tr|Q91ZC0 MrgB4 tr | Q91ZB9 MrgB5 tr|Q7TN51 MrgB8 sp|Q91ZB8|MRGD MOUSE sp|Q91ZB7|MRGE_MOUSE sp|Q8VCJ6|MRGF_MOUSE sp|Q91ZB5|MRGG_MOUSE sp|Q7TN49|MRGA_RAT tr|Q7TN48 MrgB1 trlQ7TN47 MrgB2 tr107TN45 MraB4 tr|Q7TN44 MrgB5 tr|Q7TN43 MrgB6 tr|Q7TN50 MrgB8 tr|Q7TN42 MrgC spl07TN41|MRGD RAT SDIO7TN401MRGE RAT sp|P23749|MRGF RAT sp|Q7TN39|MRGG_RAT tr|Q7TN38 MrgH sp|Q6L786|MRGD_MACFA [sp|Q96LB2|MRG1_HUMAN sp1096LB11MRG2 HUMAN sp|Q96LB0|MRG3 HUMAN sp|Q96LA9|MRG4 HUMAN sp|Q8TDS7|MRGD_HUMAN .sp|Q96AM1|MRGF_HUMAN mrgC11 sp|Q91WW5|MGA1 MOUSE sp|Q91WW4|MGA2_MOUSE sp|Q91WW3|MGA3 MOUSE sp|Q91WW2|MGA4 MOUSE sp|Q91ZC7|MGA5 MOUSE sp|Q91ZC6|MGA6 MOUSE sp|Q91ZC5|MGA7 MOUSE sp|Q91ZC4|MGA8_MOUSE tr|Q91ZC3 MrgB1 tr|Q91ZC2 MrgB2 tr|Q91ZC1 MrgB3 tr|Q91ZC0 MrgB4 tr|Q91ZB9 MrgB5 tr|Q7TN51 MrgB8 sp|Q91ZB8|MRGD_MOUSE sp|Q91ZB7|MRGE_MOUSE sp|Q8VCJ6|MRGF MOUSE sp|Q91ZB5|MRGG_MOUSE sp|Q7TN49|MRGA_RAT tr|Q7TN48 MrgB1 tr107TN47 MrgB2 tr|Q7TN45 MrgB4 tr|Q7TN44 MrgB5 tr|Q7TN43 MrgB6 tr | Q7TN50 MraB8 tr|Q7TN42 MrgC sp|Q7TN41|MRGD_RAT sp|Q7TN40|MRGE RAT spiP237491MRGF RAT sp|Q7TN39|MRGG RAT tr | Q7TN38 MrgH sp|Q6L786|MRGD_MACFA sp|Q96LB2|MRG1 HUMAN sp|Q96LB1|MRG2_HUMAN sp|Q96LB0|MRG3_HUMAN sp|Q96LA9|MRG4_HUMAN sp|Q8TDS7|MRGD_HUMAN sp|Q96AM1|MRGF_HUMAN

CHIYQVTAVLSCVNSSANPIIYFLVGSFRQHRKHRS--LKRVLKRALEDTPEEDEYTDSH 310 LGFYLASVVLTAINSCANPIIYFFVGSFRHRLKHQT--LKMVLQNALQDTPETAKI---- 293 VNFYLALEVLTAINSCANPIIYFFVGSFRHQLKHQT--LKMVLQSALQDTPETAEN---- 294 YS---PLLVLTAINSCANPIIYFFVGSFRQRLNKQT--LKMVLQKALQDTPETPEN---- 291 YILFOTSLVLTSVNSCANPIIYFFVGSFRHRLKHKT--LKMVLOSALODTPETPEN---- 302 FRFYMSTHVLTAINNCANPIIYFFEGSFRHOLKHOT--LKMVLOSVLODTPEIAEN---- 293 FSLYLASVVLTAINSCANPIIYFFVGSFRHRLKHQT--LKMVLQSALQDTPETPEN---- 290 YRLYLASIVLTVVNSCANPIIYFFVGSFRHRLKHQT--LKMVLQSALQDTPETHEN---- 294 YRLFFASVVLTVVNSCANPIIYFFVGSFRHRLKQQT--LKMFLQRALQDTPETPEN---- 294 G-FRNVTIFLSCINSCANPIIYFLVGSIRHHRFQRKT-LKLLLQRAMQDSPEEEECGEMG 315 N-FYLEILFLSCVNSCMNPIIYFLVGSIRHRRFRRKT-LKLLLQRAMQDTPEEEQSGNKS 323 G-FSHHILYVYCINICVNATIYFLVGSIRHGKFQKMT-LKLILQRAIQGTPEEEGGERGP 312 N-VYEIIEFLSCVNSCANPIIYFLVGSIROHRLRWOS-LKLLLORAMODTPEEESGERGP 311 H-VLEVTIFLSCVNSCANPIIYFLVGSIROHRLOWOS-LKLLLORAMODTPEEDSGERVP 312 GYFYETILLLSGVNSCANPIICLFVGSIKHCQFQCGT-LRLILQRAIQESPEEEDEEVEE 314 -LYSCVSRFSSSLSSSANPVIYFLVGSQKSHRLQE--SLGAVLGRALRDEPEPEGRETPS 314 -YFYHFSFFMASVHSAAKPAIYFFLGSTPGQRFREP--LRLVLQRALGDEAELGAGREAS 301 -FPEYVTDLCICINSSAKPIVYFLAGRDKSQRLWE--PLRVVFQRALRDGAEPGDAASST 327 -FFFPLATLLACIDSSAKPLLYYMKG---RQLRKDP--LQVALNRALGEESQSGLGGLSL 284 YGLYLAALVLTAVNSCANPIIYFFVGSFRHQ-KHQT--LKMVLQRALQDTPETAEN----293 G-FHPVTVLLSCVNSCANPIIYFLVGSIRHHRFORKT-LKLLLOKAMODTPEEEECGEMG 293 G-FLPVTIFLSCINTCANPIIYFLVGSVRHHRFORKS-LKLLLOOARODTPETEEYVEMG 293 ---YEMVTLLSCVNSCANPIIYFFVGSIRHHRLQRQT-LKLLLQRAMQDTPEEEGGERGP 309 ---YKIVTFLYSVNCCANPIIYFLIGSIRHHRLQRQS-LKLLLQRAMQDTPEEEGGVKGP 343 NSFHETILLLSYINSCANPIICLLVGSIRHCRFQCGT-LRLLLHRAMQDIPEEEGEELEE 317 CHIYQVTVLLSCVNSSANPIIYFLVGSFRHRKKHRS--LKMVLKRALEETPEEDEYTDSH 311 -LYVCSSRFSSSLSSSANPVIYFLVGSQKSHRLQE--SLGAVLGRALQDEP--EGRETPS 312 -YEYHESEEMASVHSAAKPATYFELGSTPGORFOEP--LRLVLORALGDEAELGAVREAS 300 -FPEYVTDLCICINSSAKPIVYFLAGRDKSORLWE--PLRVVFORALRDGAEPGDAASST 327 -FFFPLATLLACIDSSAKPLLYYLKG---RQLRKEP--LQVALNRALGEESQSSSGGISL 284 -KSLPYLNMLSTINCSINPIVYFVVGSLRRKRSRK--SLKEALQKVFEEKP----VVASR 311 -LYFNLSRLSSSMSSSANPLIYFLVGSRRSRRLQG--SLGTVLQRALREEPELEGGETPT 312 CHVHLVSIFLSALNSSANPIIYFFVGSFRQRQNRQN--LKLVLQRALQDASEVDEGGGQL 308 CHIHPVSVVLSSLNSSANPIIYFFVGSFRKQWRLQQPILKLALQRALQDIAEVDHSEGCF 317 CHVHLVSIFLSALNSSANPIIYFFVGSFRQRQNRQN--LKLVLQRALQDTPEVDEGGGWL 308 CHVYLVCMSLSSLNSSANPIIYFFVGSFRQRQNRQN--LKLVLQRALQDKPEVDKGEGQL 308 -LCFSLSRLSSSVSSSANPVIYFLVGSRRSHRLPTR-SLGTVLOOALREEPELEGGETPT 313 -FPEYVTDLCICINSSAKPIVYFLAGRDKSQRLWE--PLRVVFQRALRDGAELGEAGGST 327

SSLALLLRILCG--PRRKPLSRLYVTIALTVMVYLICGLPLGLYLFLLYWFGVH-LHYPF 252 SSLALVARLFCG--TGOIKLTRLYVTIILSILVFLLCGLPFGIHWFLLFKIKDD-FHVFD 239 SSLALLARLFCG--AGQMKLTRFHVTILLTLLVFLLCGLPFVIYCILLFKIKDD-FHVLD 240 STLALLARLFCG--ARNMKFTRLFVTIMLTVLVFLLCGLPWGITWFLLFWIAPG-VFVLD 240 STLALLARLFCG--GGKTKFTRLFVTIMLTVLVFLLCGLPLGFFWFLVPWINRD-FSVLD 248 SSMALLARLFCG--TGQMKLTRLYVTIMLTVLGFLLCGLPFVIYYFLLFNIKDG-FCLFD 239 SSLALLARLFCG--AGRMKLTRLYVTITLTLLVFLLCGLPCGFWWFLLSKIKNV-FTVFE 236 STLALLARLFCG--AEKMKFTRLFVTIMLTILVFLLCGLPWGFFWFLLIWIKGG-FSVLD 240 STLALLARLFSG--AGKRKFTRLFVTIMLAILVFLLCGLPLGFFWFLSPWIEDR-FIVLD 240 SSLALVLTIFCG--LHKVPVTRLYVTIVFTVLVFLIFGLPYGI<mark>YW</mark>FLLEWIREFHDNKPC 257 SSLTLLVRIFCG--SQRIPMTRLYVTITLTVLVFLIFGLPFGI<mark>YW</mark>ILYQWISNFYYVEIC 265 FSLILLRISCG--SQQIPVTRLNVTIALRVLLLLIFGIPFGIFWIVDKWNEENFFVRAC 254 SSLALLVKIVWG--SHRIPVTRFFVTIALTVVVFIYFGMPFGICWFLLSRIMEFDSIFFN 253 SSLALLVKMICG--SHRIPVTRFYVTIALTLVVFIFLGLPFGI<mark>YS</mark>SFLIMFKEFQSIFSY 254 SNOALLFRVFCG--SOOTPVTRLLVTIMLTALVVLICGFGIGICFF--YWKKEENSIMPC 255 TSTILFIRVRKNSLMQRRRPRRLYVVILTSILVFLTCSLPLGINWFLLYWVDVKRDVRL- 257 TSLLLLLRVERG--PERHQPRGFPTLVLLAVLLFLFCGLPFGIFWLSKNLSWHIPL----244 PCLALILHVECR-ARRRORSAKLNHVVLAIVSVFLVSSIYLGIDWFLFWVFQIPAP---- 270 ASKFLLIFGNCC--SSQPPPK-FCKLAQCSGILLFFCRLPLVVYWCLRPVLKFLLP----230 SSLALLVRLFCG--AGRMKLTRLYATIMLTVLVFLLCGLPFGIHWFLLIWIKID-YGKFA 240 SSLALVINIFCG--LYRIPVTRLYVTIVFTVLVFLLCGLPYG SSLALVITIFCG--LYRIPVTRLYVVIVFTVLFFLLFGLPYGTYLFLLVWAETFYYVFPC 235 SSLALLAKIICG--SHRIPVTRFYVTIALTVLVFIFFGLPIGICVFLLPWIHMMLSSFF- 253 SSLAMLTKIICG--SHRIPVTRFYVTIAVTVLVFTFFGLPVGI<mark>I</mark>SLLLPRIVVFRGVFYI 287 SSLILLIRIFCG--SQRIPVTRLYVTIVLTVLFFLICCLPFGI<mark>S</mark>WLI---245 SIQVLLVRIFCG--SQRTPVTKLHVTIVLTALVLVICGFPFGI SSLALLVRILCG--SRRKPLSRLYVTISLTVMVYLICGLPLGL<mark>YL</mark>FLLYWFGIH-LHYPF 253 TSAIIFIRMRKNSLLORROPRRLYVVILTSVLVFLTCSLPLGINWFLLYWVELPOAVRL- 257 TSLLLLLRVERG--PERHOPRGFPTLVLLVILLFLFCGLPFGIFWLSKNLSWHTPL--- 243 PCLALILHVECR-ARRRQRSAKLNHVVLAIVSVFLVSSIYLGIDWFLFWVFQIPAP---- 270 ASMFLLVFGNCC--SSOPPSK-FCKLAOCSGILLFFCRLPLVFWUCLRPVIKFLLP---- 230 SNLILFIQVCCN-LKPRQ-PAKLYVIIMATVILFLVFAMPMKV<mark>L</mark>LIIGYYSNSTDASVW- 258 SSLTLFVRVRRSSQQWRRQPTRLFVVVLASVLVFLICSLPLGF<mark>Y</mark>WFVLYWLNLPPDTKV- 255 SSLVLLIRILCG--SRKIPLTRLYVTILLTVLVFLLCGLPFGICFFLFLWIHVD-REVLF 250 SSLALLVRILCG--SRGLPLTRLYLTILLTVLVFLLCGLPFGICWFLILWIWKD-SDVLF 257 SSLVLLVRILCG--SRKMPLTRLYVTILLTVLVFLLCGLPFGIQWALFSRIHLD-WKVLF 250 SSLVLLVRILCG--SRKMPLTRLYVTILLTVLVFLLCGLPFGILGALIYRMHLN-LEVLY 250 SSLTLFVWVRRSSQQWRRQPTRLFVVVLASVLVFLICSLPLSI<mark>Y</mark>WFVLYWLSLPPEMQV- 255 PCLALILHVECR-ARRRQRSAKLNHVILAMVSVFLVSSIYLGIDWFLFWVFQIPAP---- 270

mrgC11	LHKTTEISESRY	322
sp Q91WW5 MGA1 MOUSE	MVEMSRSKSEP	304
sp Q91WW4 MGA2 MOUSE	MVEMSSNKAEP	305
sp Q91WW3 MGA3 MOUSE	MVEMSRNKAEP	302
sp Q91WW2 MGA4 MOUSE	MVEMSRSKAEP	313
sp Q91ZC7 MGA5 MOUSE	MVEMSRNIPKP	304
sp Q91ZC6 MGA6 MOUSE	MVEMSRNKAEL	301
sp Q91ZC5 MGA7 MOUSE	MVEMSRIKAEQ	305
sp Q91ZC4 MGA8 MOUSE	MVEMSRSKAEP	305
tr Q91ZC3 MrgB1	SSRRPREIKTVWKGLRAALIRHK	338
tr Q91ZC2 MrgB2	SSEHPEELETVQSCS	338
tr Q91ZC1 MrgB3		
tr Q91ZC0 MrgB4	SQRSGELETV	321
tr Q91ZB9 MrgB5	SQRSGELESV	322
tr Q7TN51 MrgB8	VVEQEGGEEDEESTTL	330
sp Q91ZB8 MRGD_MOUSE	TCTNDGV	321
sp Q91ZB7 MRGE_MOUSE	QGGLVDMTV	310
sp Q8VCJ6 MRGF_MOUSE	PNTVTMEMQCPSGNAS	343
sp Q91ZB5 MRGG_MOUSE	PMHQV	289
sp Q7TN49 MRGA_RAT	TVEMSSSKVEP	304
tr Q7TN48 MrgB1	S	294
tr Q7TN47 MrgB2	SLGRSREVN-SLQGTESCFDQA-	314
tr Q7TN45 MrgB4	SQKSEDLEVVRCSS	323
tr Q7TN44 MrgB5	SQKSNELEIV	353
tr Q7TN43 MrgB6		
tr Q7TN50 MrgB8	VVGERVQNSIP	328
tr Q7TN42 MrgC	VQKPTEISERRC	323
sp Q7TN41 MRGD_RAT	TCTNDGV	319
sp Q7TN40 MRGE_RAT	QGGLVDMTV	309
sp P23749 MRGF_RAT	PNTVTMEMQCPSGNAS	343
sp Q7TN39 MRGG_RAT	PMSRV	289
tr Q7TN38 MrgH	ENEVQFSLPL	321
sp Q6L786 MRGD_MACFA	TGTNEMGA	320
sp Q96LB2 MRG1_HUMAN	PEEILELSGSRLEQ	322
sp Q96LB1 MRG2_HUMAN	RQGTPEMSRSSLV	330
sp Q96LB0 MRG3_HUMAN	PQETLELSGSRLEQ	322
sp Q96LA9 MRG4_HUMAN	PEESLELSGSRLGP	322
sp Q8TDS7 MRGD_HUMAN	VGTNEMGA	321
sp Q96AM1 MRGF_HUMAN	PNTVTMEMQCPPGNAS	343
_		

Figure S2.3 (continued)

Sequence	Identity (%) ^a	TM1	TM2	TM3	TM4	TM5	TM6	TM7
tr Q91YB7	46	х	х	х	х		х	х
tr Q7TN49	49	x	x	x	x		x	
tr Q91WW5	46	х	x	x	x		x	х
tr Q91ZC6	47	х	x		x	x	x	х
tr Q91WW3	47	х	x	x			x	
tr Q8R4G1	88	х	x	x	x	x	x	х
tr Q7TN42	88	х	x	x	x	x	x	х
tr Q96LB0	51	x	x	x			x	х
gp AX923125 40216229	51			x	x		x	х
gp AX647081 28800069	51	х	x	x	x		x	
tr Q8TDE1	51	х	x	x	x		x	х
tr Q8TDE0	49	х	x		x		x	х
gp AX657514 29160254	46	х	x	x	x		x	х
tr Q96LB2	53	х			x		x	х
tr Q8TDD8	53	x			x		x	x
tr Q8TDD9	53	х			x		x	х
tr Q96LA9	51	х		x	x		x	х
gp AX646849 28799318	50	х		x	x		x	х
tr Q8TDD6	50	х		x	x		x	x
tr Q8TDD7	50	x		x	x		x	x
gp AX657510 29160250	50	х		x	x		x	х
tr Q7TN45	43	х	x			x	x	x
tr Q91ZC0	42	х						х
tr Q91ZC3	44	х	x	x	x	x	x	x
tr Q7TN48	48	х	x				x	x
tr Q91ZC2	42		x		x		x	x
tr Q8CDY4	42		x		x		x	x
tr Q91ZB9	41	x						x
tr Q96LB1	49	x	x		x	x	x	х

Table S2.1 Hit sequences from independent BLAST search of each TM

^a w.r.t. the sequence of mMrgC11

Table S2.1 (continued)

Sequence	Identity (%) ^a	TM1	TM2	TM3	TM4	TM5	TM6	TM7
tr Q91ZC5	45	х	х	х			х	х
tr Q7TN39	23	x						
tr Q91ZC4	42	x	x	x	x		x	x
tr Q91WW4	42	x	x	x	x		x	
tr AAH64040	40	x	x	x	x		x	
tr Q8NGK7	37	x					x	
tr Q8TDS7	37	x						
tr Q91ZB8	35		x					
tr Q91ZC7	45		x	x			x	
tr Q91WW2	42	x	x	x			x	x
tr Q7TN47	42	x					x	x
tr Q7TN50	39	x	x					
tr Q7TN41	34		x					
tr Q91ZB5	23	x						
tr Q91ZB7	32		x		x		x	
tr Q7TN40	30		x					
tr Q7TN43	44		x	x				
tr Q91ZC1	36		x					
tr Q7TN51	34		x		x			
sp MRG_HUMAN	29		x					
tr Q7TN44	40		x					
tr Q8IXE2	31				x			x
tr Q7TN46	42				x		x	x
tr Q8N7J6	33				x			x
sp MRGF_HUMAN	31				x			x
sp MRGF_RAT	31							x
sp MRGF_MOUSE	31							x

^a w.r.t. the sequence of mMrgC11

TM5	TM6	-150	-120	-90	-60	-30	0	30	60	90	120	150	
0	0	613.5	603.1	627.3	644.4	631.5	575.9	615.7	604.7	656.5	702.2	674.7	
0	-30	632.9	585.6	626.5	633.4	652.2	579.3	593.9	593.6	635.3	706.1	683.0	
0	30	610.7	597.1	633.8	618.1	642.8	589.1	608.6	593.7	634.9	679.9	670.2	
0	-60	627.0	606.7	669.5	641.1	686.5	598.7	638.6	641.9	657.2	742.7	678.5	
0	60	609.9	588.9	653.4	626.7	631.6	586.4	694.7	593.8	636.3	676.6	729.2	
-30	0	514.4	566.8	599.9	568.0	595.5	555.5	594.9	571.3	570.8	623.7	624.1	
-30	-30	552.1	569.9	580.8	557.2	612.1	566.0	567.9	569.3	571.6	604.1	606.5	
-30	30	545.4	549.1	605.0	542.8	597.2	569.4	593.8	574.5	581.1	624.9	629.9	
-30	-60	555.4	560.5	643.8	552.6	610.8	596.2	593.6	593.3	643.2	686.4	678.4	
-30	60	534.2	558.9	610.2	553.3	600.0	547.8	599.5	569.3	560.0	627.8	631.9	
30	0	593.3	554.0	616.0	581.1	571.7	551.1	585.6	594.1	607.7	645.8	649.7	
30	-30	609.5	538.9	609.6	531.6	551.6	509.1	573.2	600.4	576.0	606.7	607.8	
30	30	595.0	539.8	609.3	553.5	555.3	546.1	640.1	597.0	581.9	624.6	618.1	
30	-60	841.2	569.8	646.4	596.6	583.8	566.5	618.5	604.0	594.8	650.5	745.2	
30	60	624.0	575.4	627.3	555.8	572.1	571.7	583.4	658.1	555.4	635.2	667.7	
-60	0	550.2	526.2	546.3	547.7	536.7	478.3	584.3	561.9	583.6	634.3	587.1	
-60	-30	565.5	479.0	513.7	544.1	537.9	511.8	540.8	552.3	596.1	580.7	564.4	
-60	30	558.0	551.4	532.3	519.8	555.2	488.3	560.7	555.2	557.7	612.9	569.3	
-60	-60	557.4	524.0	562.2	553.9	587.8	574.4	615.6	558.8	606.9	609.3	573.2	
-60	60	520.5	556.1	545.6	533.6	545.3	536.8	561.1	571.6	588.4	621.0	606.1	
60	0	605.5	564.7	632.0	609.2	588.3	551.0	600.1	575.0	604.0	673.8	655.2	
60	-30	611.1	542.6	601.8	549.0	569.0	563.0	1082.5	557.3	580.7	747.9	641.6	
60	30	614.4	617.6	626.6	545.2	592.0	556.3	698.1	574.1	578.9	693.8	631.5	
60	-60	613.3	588.0	664.1	554.4	619.8	587.1	616.1	583.0	591.5	694.0	658.7	
60	60	616.3	638.9	681.2	569.1	599.2	607.3	606.9	591.2	590.9	704.2	658.1	

angle of TM3 was scanned for 360 degrees (in 30 degree increments) Table S2.2 Calculated energies (in kcal/mol) of configurations generated in combinatorial rotations of TM3, 5 and 6; the rotational

Ligand	B.E.	Coulomb	VDW	Hbonds	Desolvation	EC50, nM
FMRFa	-109	-83	-34	-81	90	168 ± 26
dFMRFa	-103	-75	-35	-79	86	276 ± 113
FdMRFa	-117	-90	-33	-82	88	113 ± 37
FMdRFa	-78	-46	-51	-56	75	inactive
FMRdFa	-80	-60	-43	-61	84	inactive
acetylated RFa	-97	-67	-19	-45	34	
acetylated dRFa	-82	-48	-25	-40	31	
acetylated RdFa	-75	-49	-27	-29	30	
RF	-71	-80	-19	-47	75	1255 ± 478
RFa	-74	-86	-19	-62	94	682 ± 371
RFa/mMrgA1	58	-0.03	-15	-20	93	inactive

Table S2.3 Calculated binding energy (in kcal/mol) and its component contribution for ligands in mMrgC11;the binding energy of RFa in mMrgA1 is also included on the last row for comparison