Supplementary Materials: The Allosteric Activation of α7 nAChR by α-conotoxin MrIC is Modified by Mutations at the Vestibular Site

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Table S1. The average scores and standard deviations (S.D.) calculated for the ten lowest-scoring poses from the WT, G83V, A102V, and T106A peptide docking calculations. All units are in Rosetta Energy Units (REU).

Protein	Average Score (REU)	S.D. (REU)
WT	-25.9	0.8
G83V	-28.7	0.8
A102V	-28.0	0.6
T106A	-26.8	1.0

Table S2. The Shapiro-Wilk test statistic values and the p-values calculated for the different MrIC, GAT107, and PNU-120596 applications at different mutations to test whether the measurements fall within normal distribution. A p-value > 0.05 indicates a normal distribution for the set of measurements and a p-value < 0.05 indicates a non-normal distribution for the set of measurements.

Application	Shapiro Test Statistic	Shapiro p-value
WT MrIC + PNU	0.599909306	0.0004576
WT MrIC + PNU + TMP-TQS	0.859702408	0.227181956
α7C190A GAT107	0.963213861	0.845787644
α7C190A GAT107 + TMP-TQS	0.95107913	0.739512026
α7C190A MrIC + PNU	0.945044458	0.700046778
α7C190A MrIC + PNU + TMP-TQS	0.805926859	0.046861239
α7G83V MrIC + PNU	0.871676564	0.304304093
α7G83V PNU	0.94775176	0.709198117
α7A102V MrIC + PNU	0.669861794	0.002928978
α7A102V PNU	0.802413106	0.043275245
α7T106A MrIC + PNU	0.625163436	0.000912655
α7T106A PNU	0.633596778	0.000668759

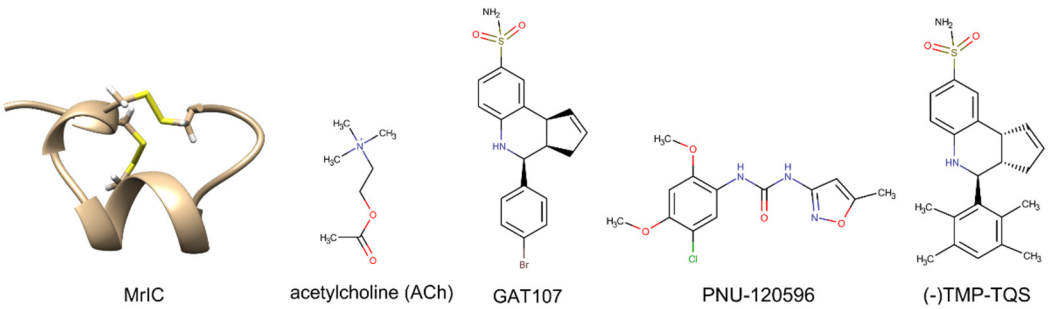

Figure S1. The structures of an MrIC model, ACh, GAT107, PNU-120596, and (-)TMP-TQS that were used in the experimental and computational studies.

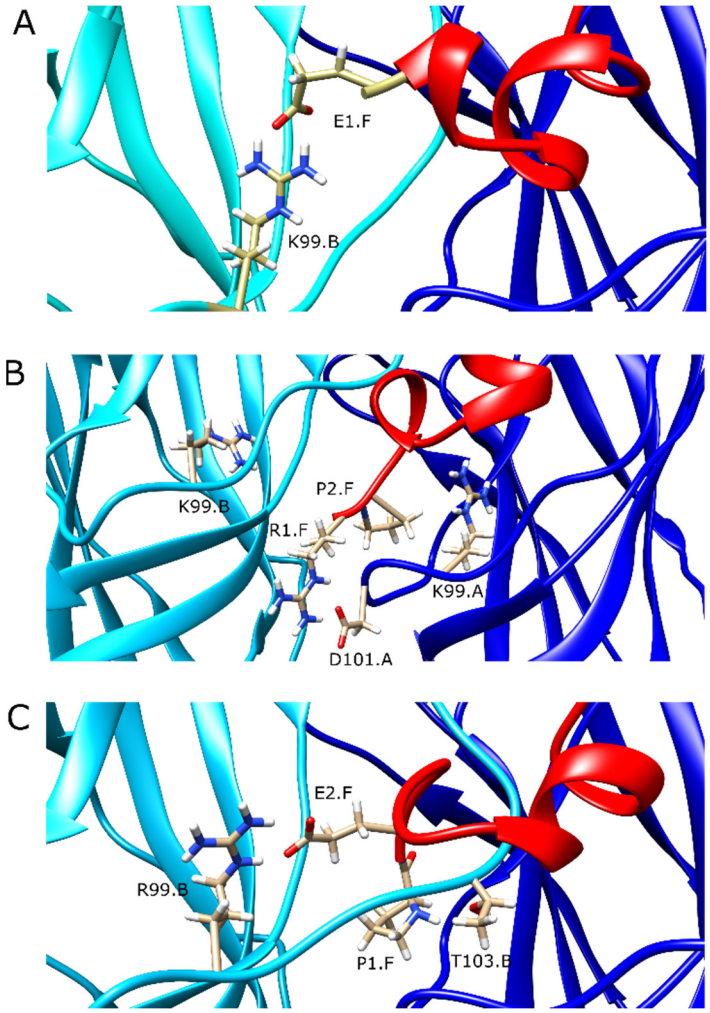

Figure S2. The interactions between MrIA (A), MrIB (B), and MrIC (C) and the vestibular residues. Chain A (blue) indicates subunit A, chain B (cyan) indicates subunit B, and chain F (red) indicates MrIC. The residues important for α7 nAChR – MrIC interactions are shown explicitly.

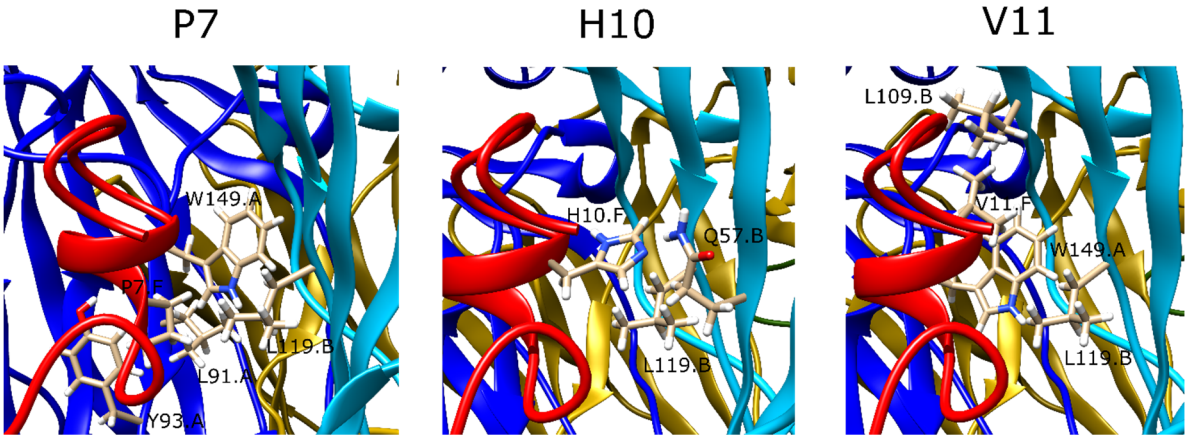

Figure S3. The lowest-scoring docking pose of MrIC at the WT α7 nAChR orthosteric site viewed with the important interactions that interact with P7MrIC (left), H10MrIC (middle), and V11MrIC (right) labeled. Chain A (blue) stands for the positive subunit of the interface bearing the C-loop, chain B (cyan) stands for the negative subunit of the interface, and chain F (red) stands for the peptide.

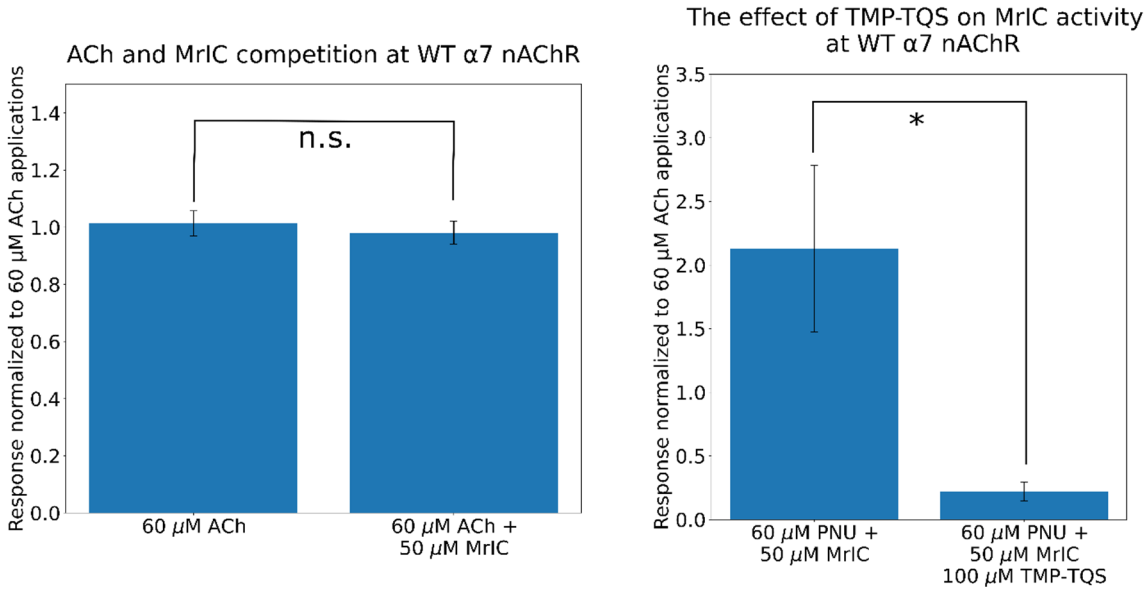

Figure S4. The effect of 50 μM MrIC application on the activity of 60 μM ACh at WT α7 nAChR (n=7) (A), and the effect of 100 μM (-)TMP-TQS (n=6) on the potentiated response evoked by 60 μM PNU-120596 + 50 μM MrIC (n=7) at WT α7 nAChR. All responses were normalized to the responses evoked by 60 μM ACh applications. n.s. stands for not significant, * stands for $p < 0.05$, ** stands for $p < 0.01$, *** stands for $p < 0.001$, and **** stands for $p < 0.0001$.

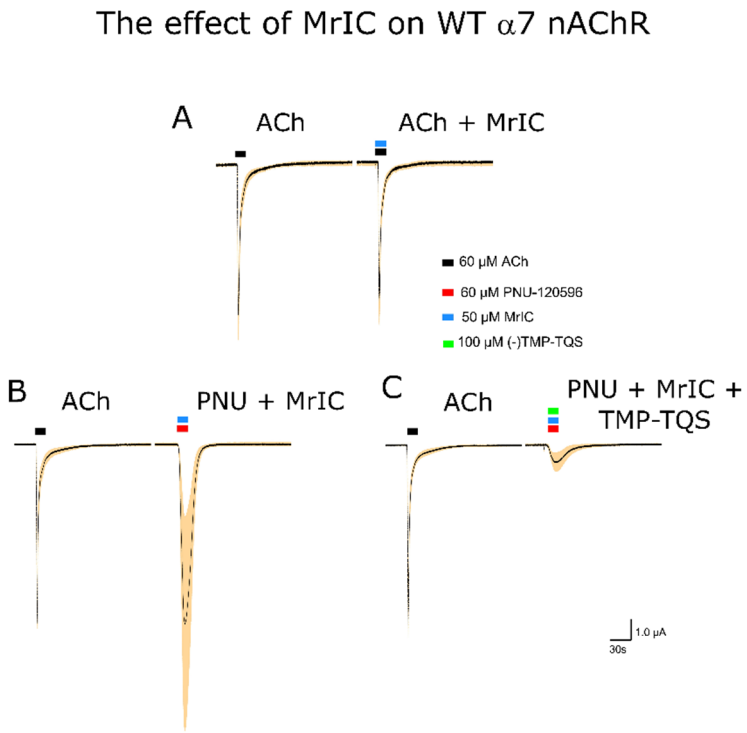

Figure S5. The current traces measured for (A) 60 μM ACh and 60 μM ACh + 50 μM MrIC applications, (B) 60 μM ACh and 60 μM PNU-120596 + 50 μM MrIC, and (C) 60 μM ACh and 60 μM PNU-120596 + 50 μM MrIC + 100 μM (-)TMP-TQS applications. The x-axis stands for the time scale of the measurement and the y-axis stands for the current induced by each application. Black bars indicate the application time of 60 μM ACh, red bars indicate the application time of 60 μM PNU-

120596, blue bars indicate the application time of 50 μM MrIC, and green bars indicate the application time of 100 μM (-)TMP-TQS. See the Materials and Methods section for a more detailed explanation on the measurements and how the error bands are calculated.

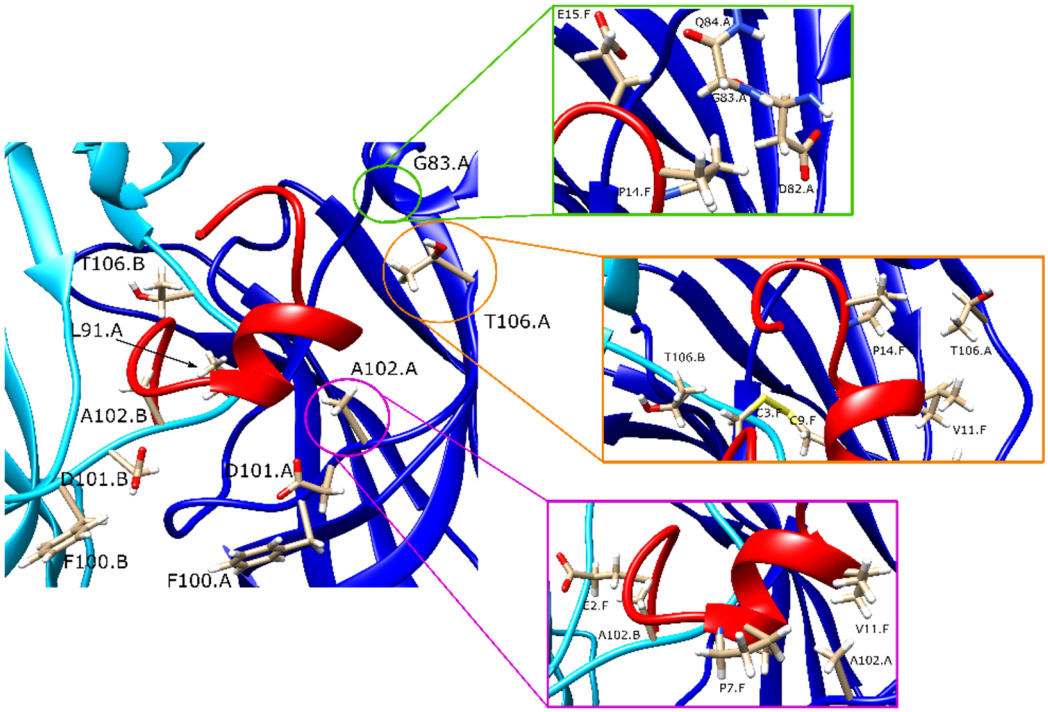

Figure S6. The lowest-scoring MrIC pose at the WT α7 nAChR AA site and the peptide-protein interactions at the vicinity of the residues G83 (green box), T106 (orange box), and A102 (purple box). The chain A (blue) indicates subunit A, the chain B (cyan) indicates subunit B, and the chain F (red) indicates MrIC. The residues important for α7 nAChR – MrIC interactions are shown explicitly.

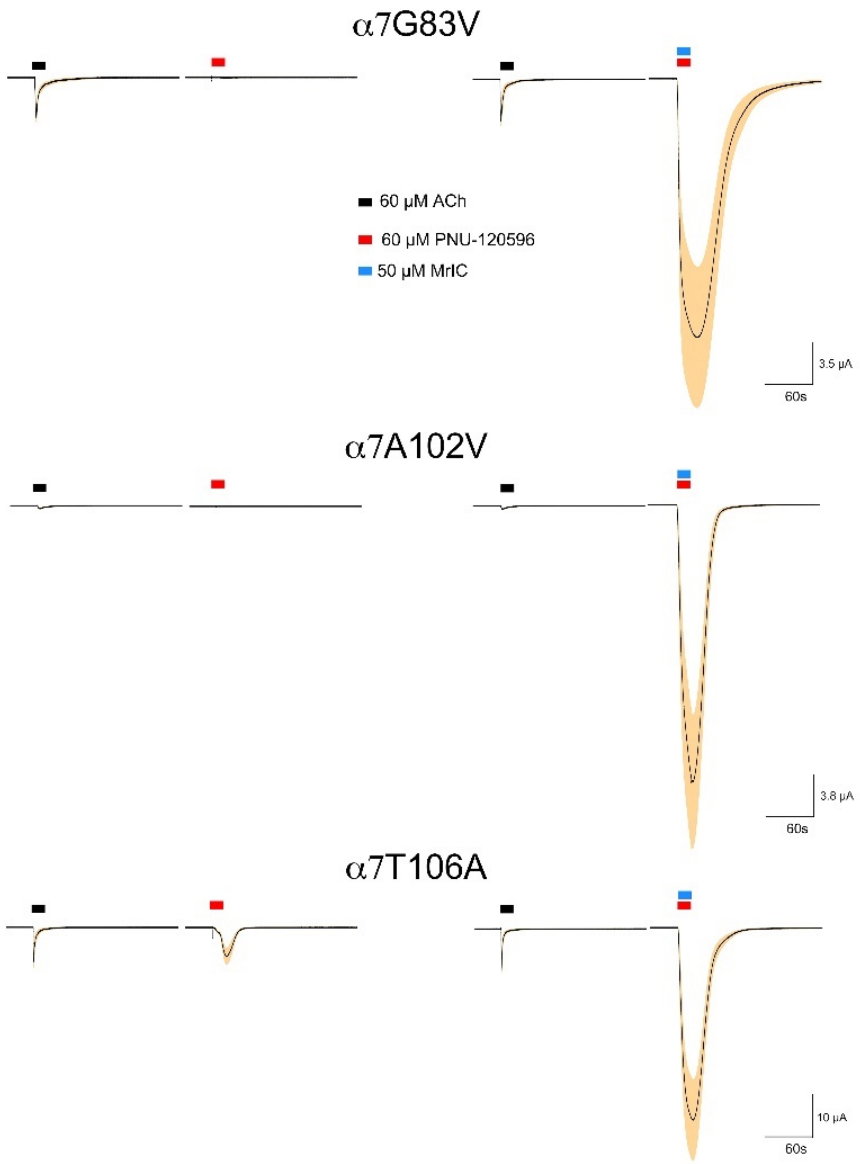

Figure S7. The current traces measured for *α*7G83V (top), *α*7A102V (middle), and *α*7T106A mutations. The left panels show the current induced by standalone application of 60 μM ACh (black) and 60 μM PNU-120596 (red). The right panels show the current induced by the 60 μM ACh control application (black) and the 60 μM PNU-120596 + 50 μM MrIC application. The x-axis stands for the time scale of the measurement and the y-axis stands for the current induced by each application. See the Materials and Methods section for a more detailed explanation on the measurements and how the error bands are calculated.