Supporting Information

Molecular determinants of α-conotoxin potency for inhibition of human and rat α6β4 nicotinic acetylcholine receptors

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Running title: Determinants of α-Ctx potency for human α6β4 nAChRs

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Table S1

Crystallography statistics

Data collection	
Resolution range (Å)	48.82 - 2.34 (2.42 - 2.34)
Space group	C 2 2 2_1
Unit cell	145.12 147.89 146.31 90 90 90
Completeness (%)	98.9
Unique reflections	66,040 (6,263)
Wilson B-factor (Å^2)	34.8
Refinement	
Number of residues	
AChBP	1032
PeIA	68
Water	332
R(work) (%)	0.1959
R(free) (%)	0.228
RMS bonds (Å)	0.008
RMS angles	1.29
Ramachandran favored (%)	97.1
Ramachandran allowed (%)	2.9
Ramachandran outliers (%)	0.0
Average *B* (Å^2)	39.89
AChBP	39.92
PeIA	63.31
Water	38.66

Table S2
Statistical analysis of [P13A]PeIA structures

Experimental restraints	
total no. distance restraints	90
intraresidue	36
sequential	36
medium range, i-j<5	14
long range, i-j≥5	4
hydrogen bond restraints	4
dihedral angle restraints	
phi	8
psi	6
Violations	
NOE violations exceeding 0.2 Å	0
Dihedral violations exceeding 2.0 Å	0
Rms deviation from mean structure, Å	
backbone atoms	0.60 ± 0.16
all heavy atoms	1.13 ± 0.22
Stereochemical quality[b]	
Residues in most favored Ramachandran region, %	93.2 ± 5.8
Ramachandran outliers, %	0.0 ± 0.0
Unfavorable sidechain rotamers, %	17.7 ± 3.5
Clash score, all atoms	0.5 ± 1.5
Overall MolProbity score	1.8 ± 0.2

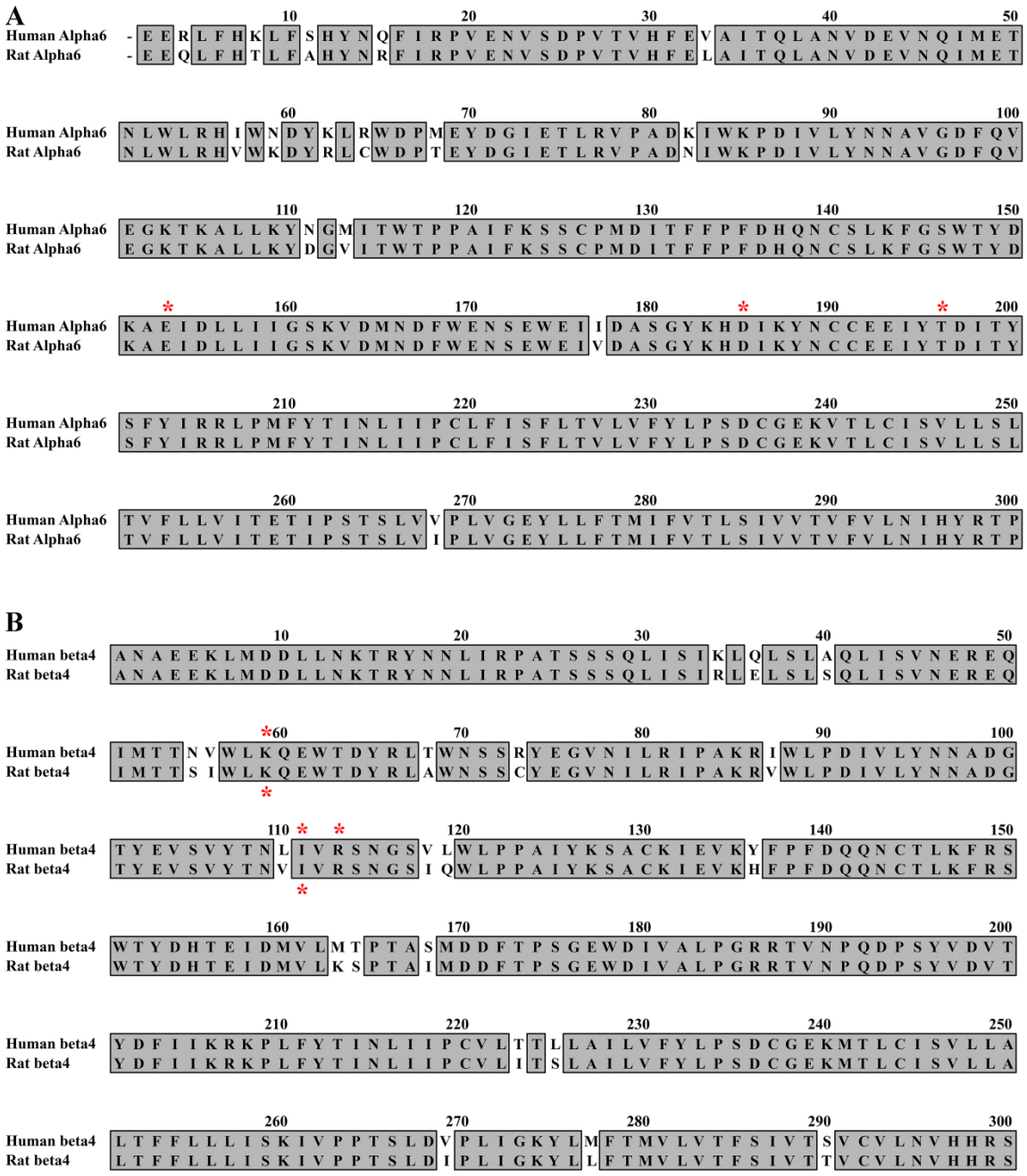

FIGURE S1. **Sequence homology of human and rat α6 and β4 subunits.** (A) Sequence alignment of human and rat α6 subunits. A pairwise analysis of the extracellular ligand-binding domains (residues 1-206) indicated 192 (93%) conserved identities. Red asterisks indicate residues that have previously been shown in functional assays to be important for α-Ctx binding to rat α6/α3β2β3 nAChRs (1). (B) Sequence alignment of human and rat β4 subunits. A pairwise analysis of the extracellular ligand-binding domains (residues 1-208) indicated 193 (93%) conserved identities. Red asterisks indicate residues that have previously been shown in functional assays to be important for α-Ctx binding to α3β4 nAChRs (2). Asterisks above the sequence line indicate residues identified in human receptors and those below indicate rat receptors. Alignments and sequence comparisons were performed using MacVector.

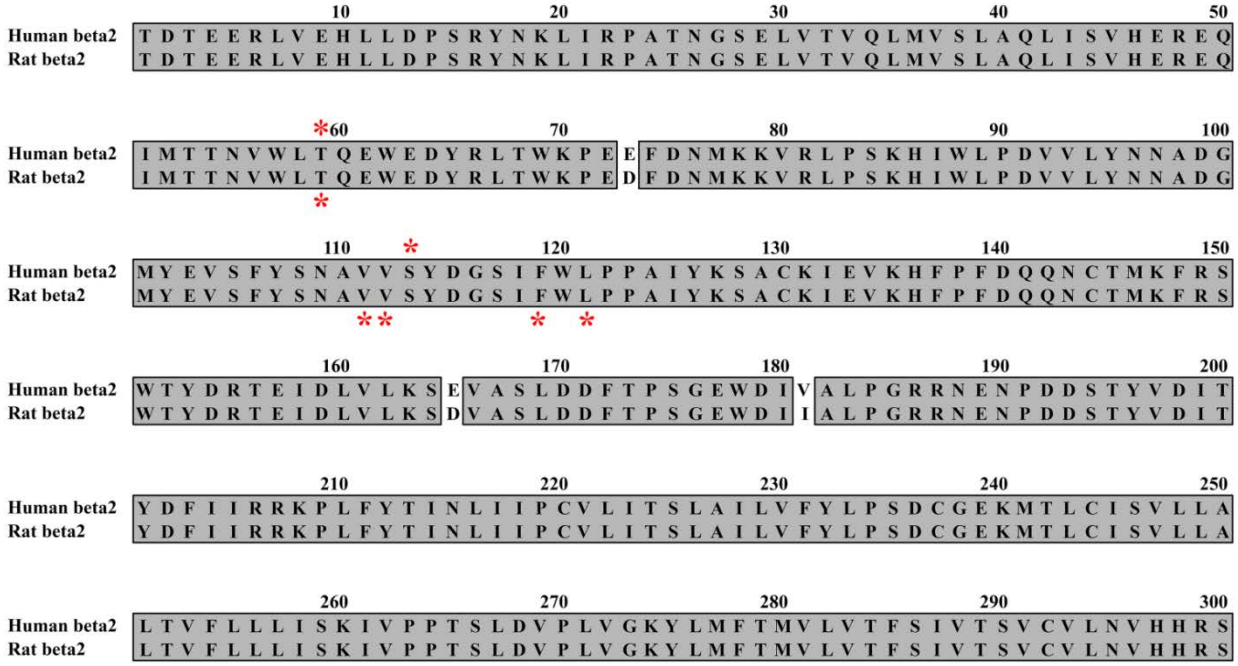

FIGURE S2. **Sequence homology of human and rat β2 subunits.** (A) Sequence alignment of human and rat β2 subunits. A pairwise analysis of the extracellular ligand-binding domains (residues 1-208) indicated 205 (99%) conserved identities. Red asterisks indicate residues that have previously been shown in functional assays to be important for α-Ctx binding to human (2) and rat α3β2 nAChRs (3-5). Asterisks above the sequence line indicate residues identified in human receptors and those below indicate rat receptors. Alignments and sequence comparisons were performed using MacVector.

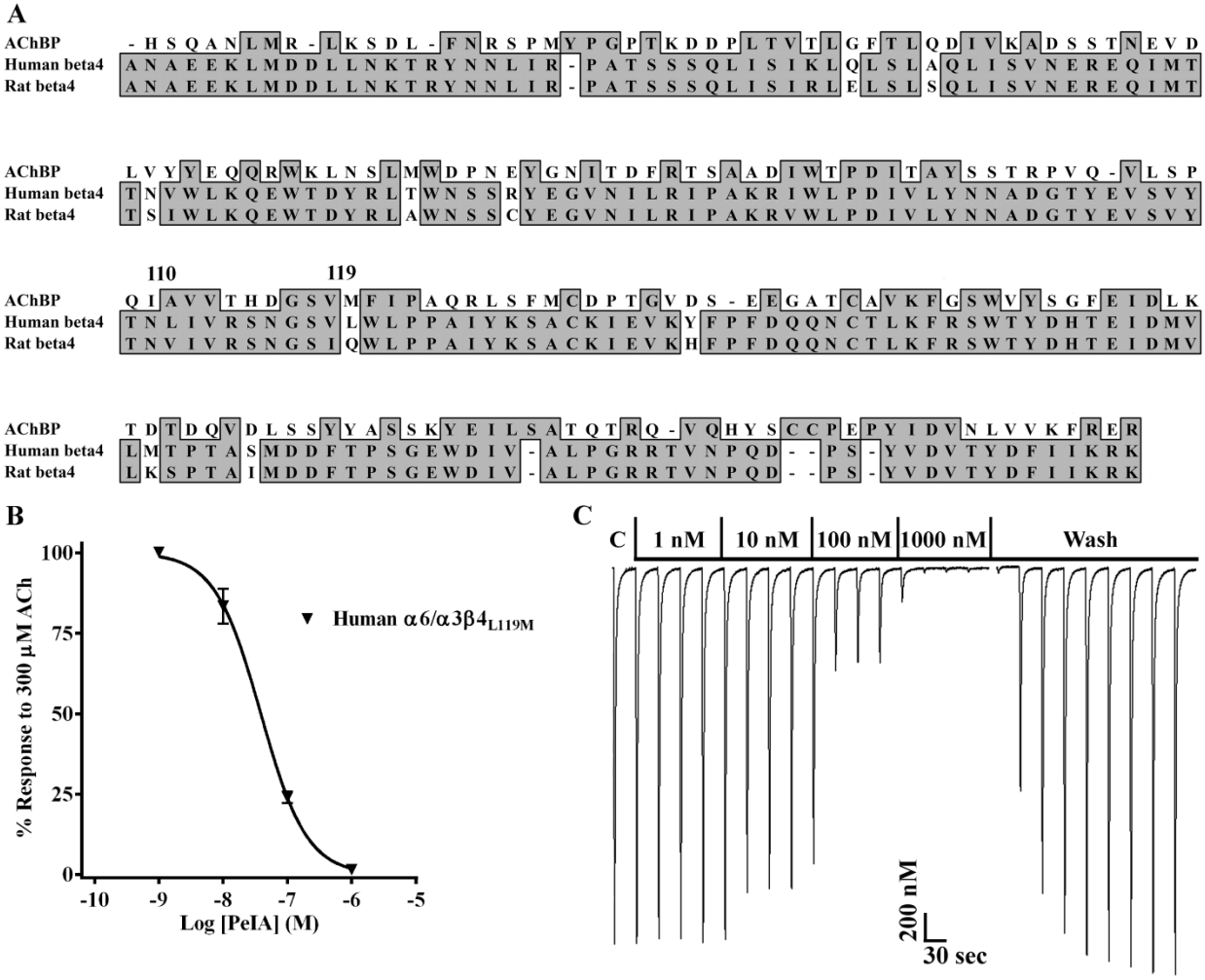

FIGURE S3. **Sequence comparison of the *Aplysia californica* AChBP with nAChR β4 subunits, and the effect of Met substitution of human $\beta4_{Leu119}$ on the potency of PeIA.** (A) Sequence alignment of the AChBP with human and rat β4 subunits. Note that for key residues 110, 118, and 119 of the ligand-binding pocket, only position 119 varies significantly among the three sequences. (B) Concentration-response analysis of the potency of PeIA on mutant human $\alpha6/\alpha3\beta4_{L119M}$ nAChRs. PeIA inhibited $\alpha6/\alpha3\beta4_{L119M}$ nAChRs with and IC_{50} of 38.5 (34.4-43.1) nM. Values in parentheses indicate the 95% confidence interval and the error bars represent ± SDM of the data obtained from 4 individual oocytes. (C) Current traces showing the inhibition of the ACh-evoked responses by the indicated concentrations of PeIA. The traces have been concatenated for brevity. C indicates a control response in the absence of PeIA. Alignments and sequence comparisons were performed using MacVector; numbering follows that of the AChBP sequence.

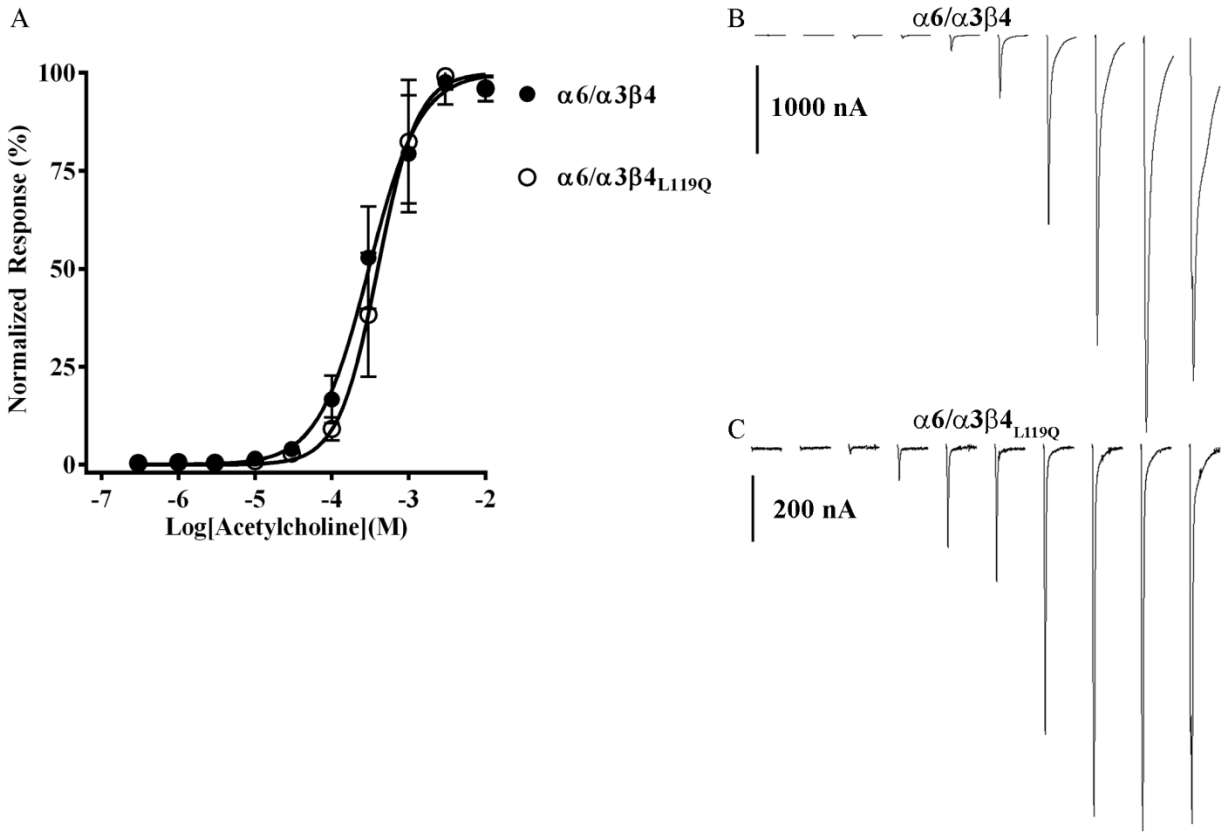

FIGURE S4. **Concentration-response relationship for activation of human α6/α3β4 and α6/α3β4$_{L119Q}$ nAChRs by acetylcholine.** *Xenopus laevis* oocytes expressing α6/α3β4 or α6/α3β4$_{L119Q}$ nAChRs were subjected to TEVC electrophysiology and the EC_{50} values determined for activation by acetylcholine as described in Experimental Procedures. (A) Concentration-response analysis of ACh-gated currents obtained by applying ascending concentrations of agonist. Acetylcholine activated α6/α3β4 nAChRs with an EC_{50} value of 303 (267-344) μM and a Hill slope of 1.3 (1.1-1.5) (n=6) and α6/α3β4$_{L119Q}$ mutant nAChRs with an EC_{50} value of 396 (355-441) μM and a Hill slope of 1.7 (1.4-2.0) (n=7). The values in parentheses denote the 95% confidence interval. Error bars denote ± SDM. (B) Representative current traces for activation α6/α3β4 nAChRs by 300 nM through 10 mM ACh. (C) Representative current traces for activation of α6/α3β4$_{L119Q}$ nAChRs by 300 nM through 10 mM acetylcholine. ACh was applied every 65 sec from the lowest to highest concentration and each current trace is 30 sec in duration.

Reference

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