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Supporting Information

Probing the Effects of Residues Located Outside the Agonist Binding Site on Drug-Receptor Selectivity in the Nicotinic Receptor

Nyssa L. Puskar[†], Henry A. Lester[‡], Dennis A. Dougherty[†*]

[†]Division of Chemistry and Chemical Engineering, California Institute of Technology, 164-30, Pasadena, CA USA
[‡]Division of Biology, California Institute of Technology, 156-29, Pasadena, CA USA

Contents

Figure S1

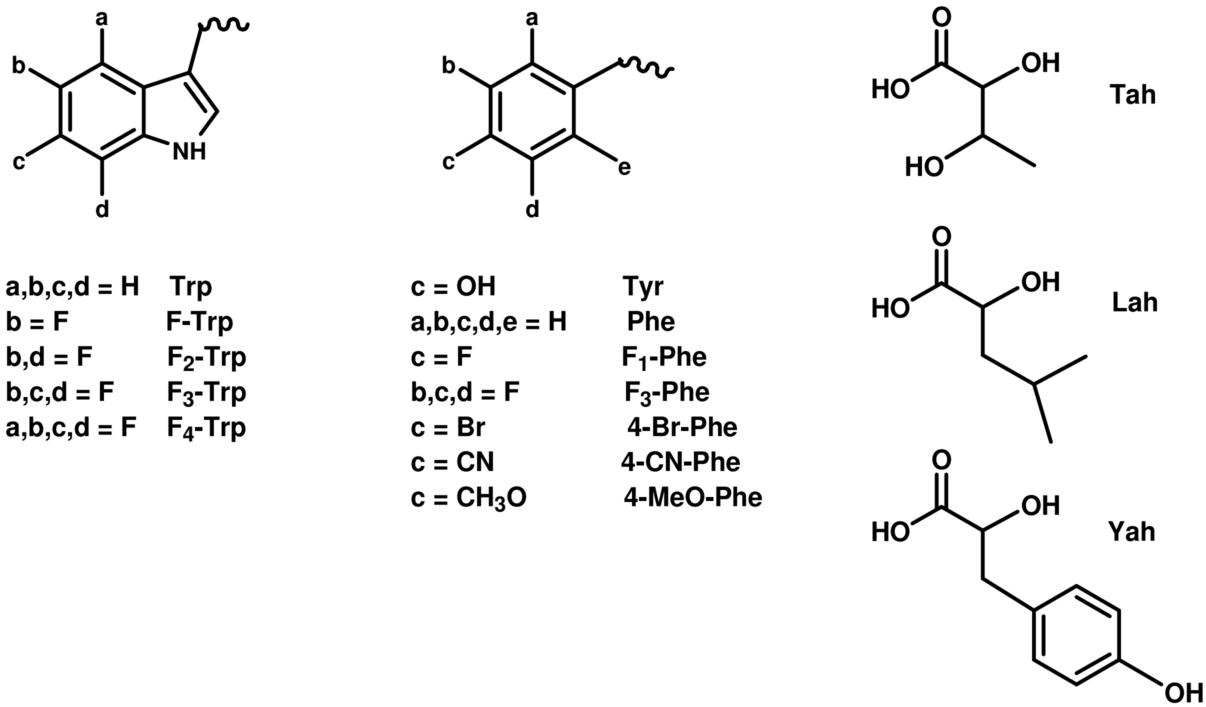
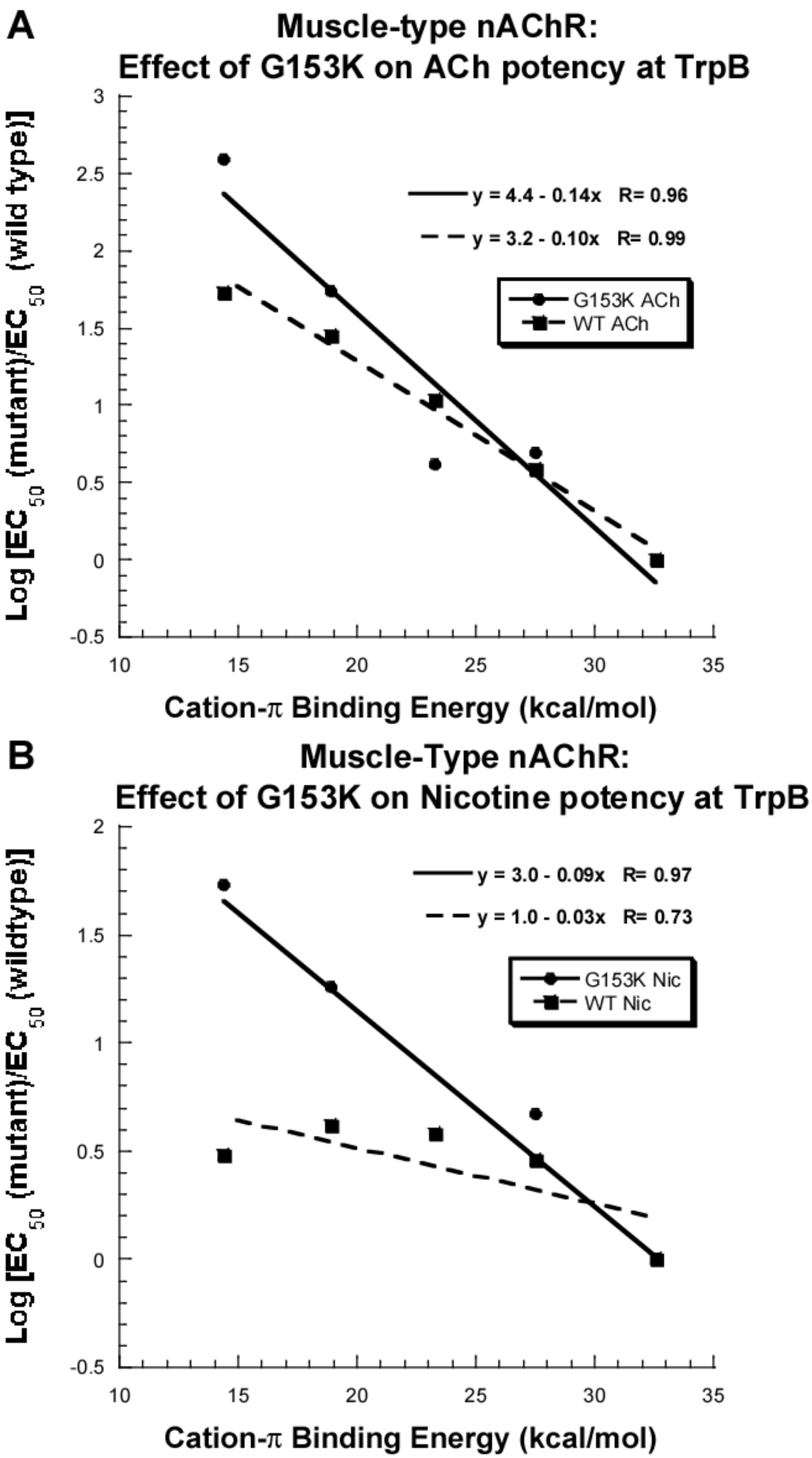


Figure S1: Unnatural amino acids and α-hydroxy acids used in the present study. If not indicated, a, b, c, or d group is H. *F-Trp*, 5-fluoro-tryptophan; *F_2-Trp*, 5,7-difluoro-tryptophan; *F_3-Trp*, 5,6,7-trifluoro-tryptophan; *F_4-Trp*, 4,5,6,7-tetrafluoro-tryptophan; *F_1-Phe*, 4-flouro-phenylalanine; *F_3-Phe*, 3,4,5-triflouro-phenylalanine; *4-Br-Phe*, 4-bromo-phenylalanine; *4-CN-Phe*, 4-cyano-phenylalanine; *4-MeO-Phe*, 4-methoxy-phenylalanine; *Tah*, threonine, α-hydroxy; *Lah*, leucine, α-hydroxy; *Yah*, tyrosine, α-hydroxy.

Figure S2


A
Muscle-type nAChR:
Effect of G153K on ACh potency at TrpB
y = 4.4 - 0.14x R= 0.96
y = 3.2 - 0.10x R= 0.99
G153K ACh
WT ACh
Log [EC50 (mutant)/EC50 (wild type)]
Cation-π Binding Energy (kcal/mol)
B
Muscle-Type nAChR:
Effect of G153K on Nicotine potency at TrpB
y = 3.0 - 0.09x R= 0.97
y = 1.0 - 0.03x R= 0.73
G153K Nic
WT Nic
Log [EC50 (mutant)/EC50 (wildtype)]
Cation-π Binding Energy (kcal/mol)

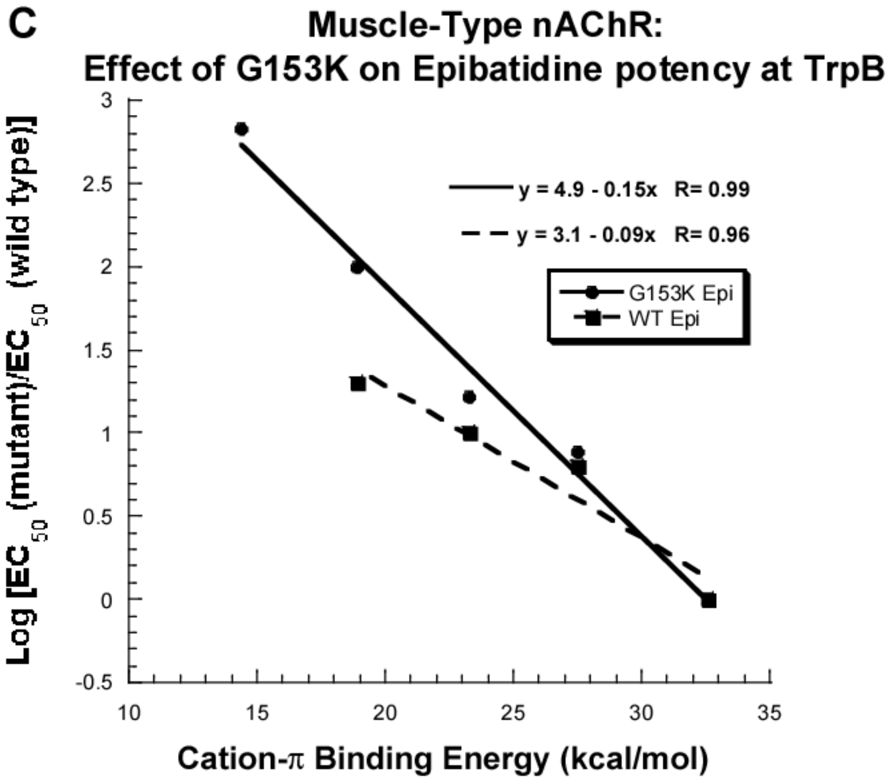
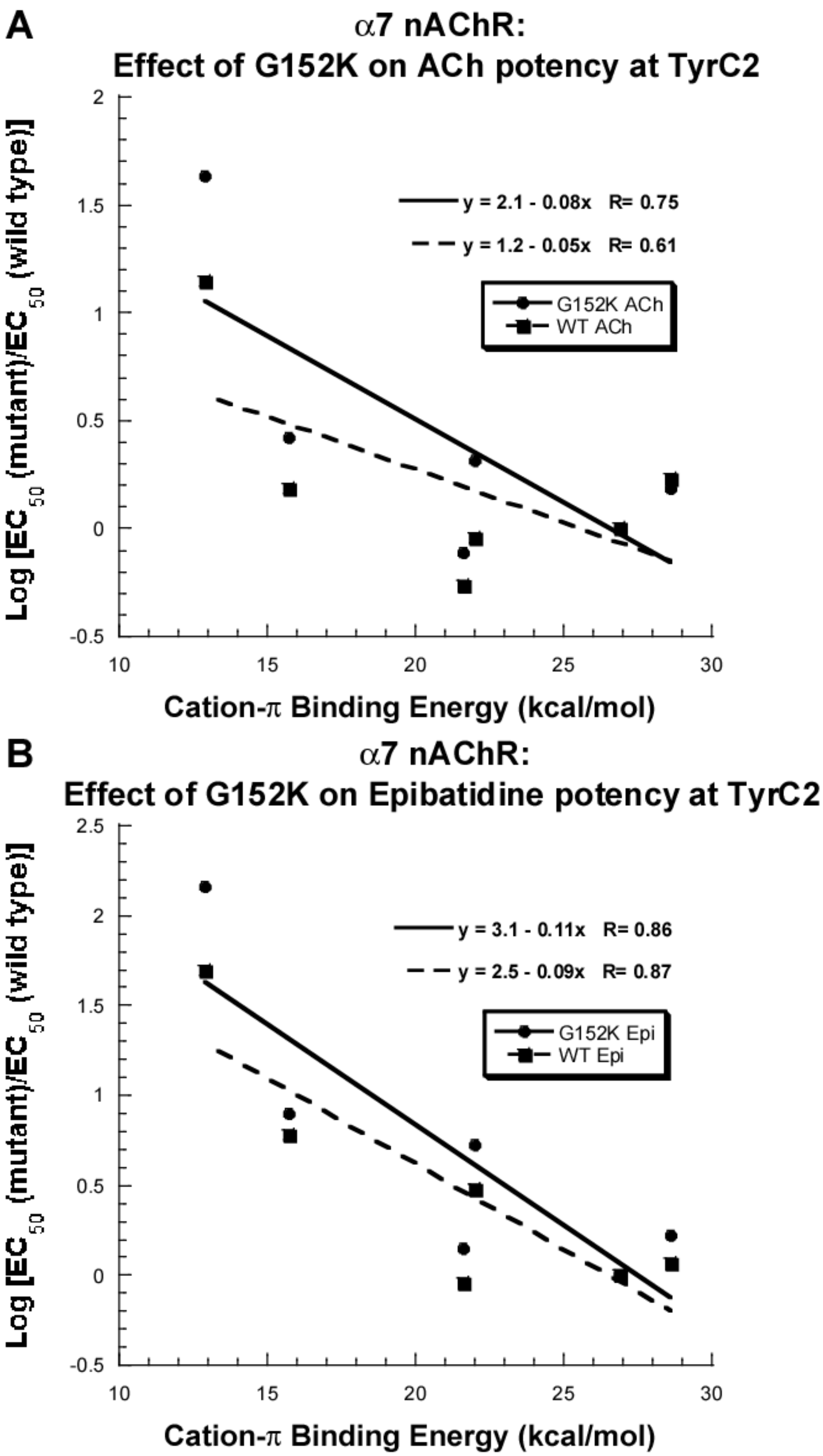


Figure S2: Fluorination plots probing the effect of the α1 G153K mutation on the muscle-type nAChR. Log[EC_{50} (mutant)/EC_{50} (wild type)] is plotted versus quantitative cation-π binding energies (REF). The data are from Supporting Table S1. Fluorination plots are shown for (A) ACh, (B) nicotine, and (C) epibatidine at the TrpB position. Moving to the left corresponds to Trp, F_1-Trp, F_2-Trp, F_3-Trp, and F_4-Trp.

Figure S3



A
α7 nAChR:
Effect of G152K on ACh potency at TyrC2
y = 2.1 - 0.08x R= 0.75
y = 1.2 - 0.05x R= 0.61
G152K ACh
WT ACh
Log [EC50 (mutant)/EC50 (wild type)]
Cation-π Binding Energy (kcal/mol)
B
α7 nAChR:
Effect of G152K on Epibatidine potency at TyrC2
y = 3.1 - 0.11x R= 0.86
y = 2.5 - 0.09x R= 0.87
G152K Epi
WT Epi
Log [EC50 (mutant)/EC50 (wild type)]
Cation-π Binding Energy (kcal/mol)

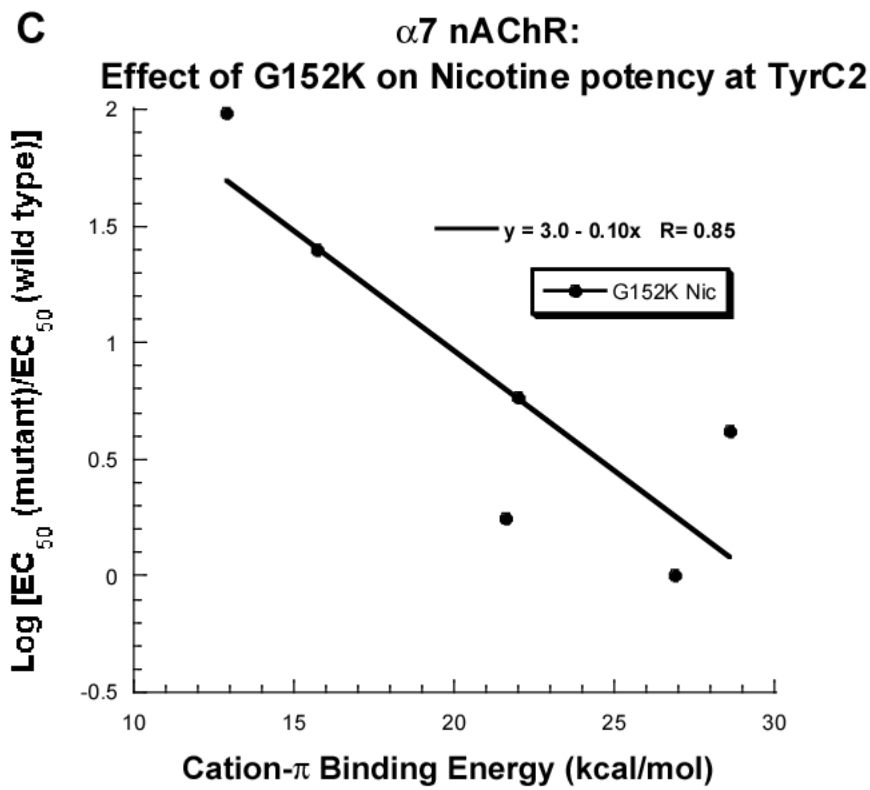
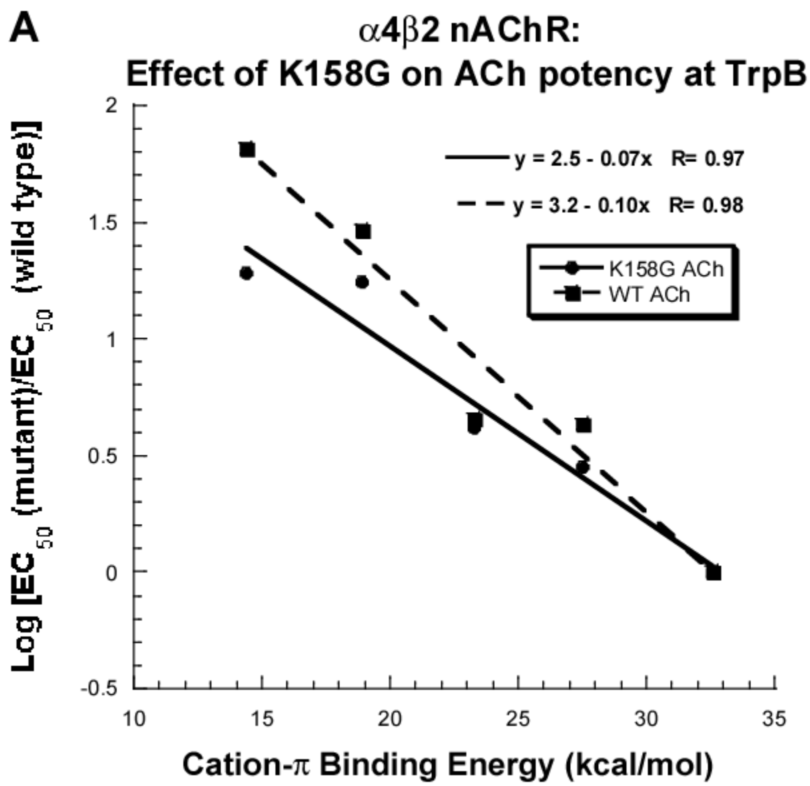


Figure S3: Fluorination plots probing the effect of the α7 G152K mutation on the $(\alpha7)_5$ nAChR. Log[EC_{50} (mutant)/EC_{50} (wild type)] is plotted versus quantitative cation-π binding energies (REF). The data are from Supporting Table S2. Fluorination plots are shown for (A) ACh, (B) epibatidine, and (C) nicotine at the TyrC2 position. Moving to the left corresponds to 4-MeO-Phe, Tyr, F_1-Phe, 4-Br-Phe, 4-CN-Phe, F_3-Phe.

Figure S4


A
α4β2 nAChR:
Effect of K158G on ACh potency at TrpB
y = 2.5 - 0.07x R= 0.97
y = 3.2 - 0.10x R= 0.98
K158G ACh
WT ACh
Log [EC50 (mutant)/EC50 (wild type)]
Cation-π Binding Energy (kcal/mol)
2
1.5
1
0.5
0
-0.5
10
15
20
25
30
35

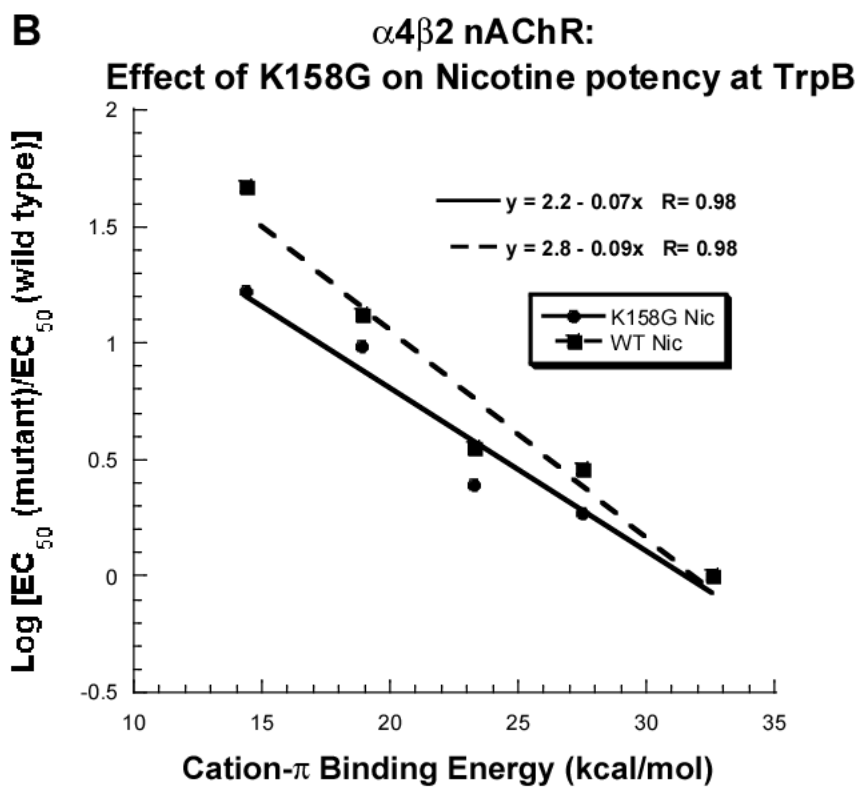

B
α4β2 nAChR:
Effect of K158G on Nicotine potency at TrpB
y = 2.2 - 0.07x R= 0.98
y = 2.8 - 0.09x R= 0.98
K158G Nic
WT Nic
Log [EC50 (mutant)/EC50 (wild type)]
Cation-π Binding Energy (kcal/mol)
2
1.5
1
0.5
0
-0.5
10
15
20
25
30
35

Figure S4: Fluorination plots probing the effect of the α4 K158G mutation on the $(\alpha4)_2(\beta2)_3$ nAChR. Log[EC_{50} (mutant)/EC_{50} (wild type)] is plotted versus quantitative cation-π binding energies (REF). The data are from Supporting Table S3. Fluorination plots are shown for (A) ACh and (B) nicotine at the TrpB position. Moving to the left corresponds to Trp, F_1-Trp, F_2-Trp, F_3-Trp, and F_4-Trp.

Figure S5

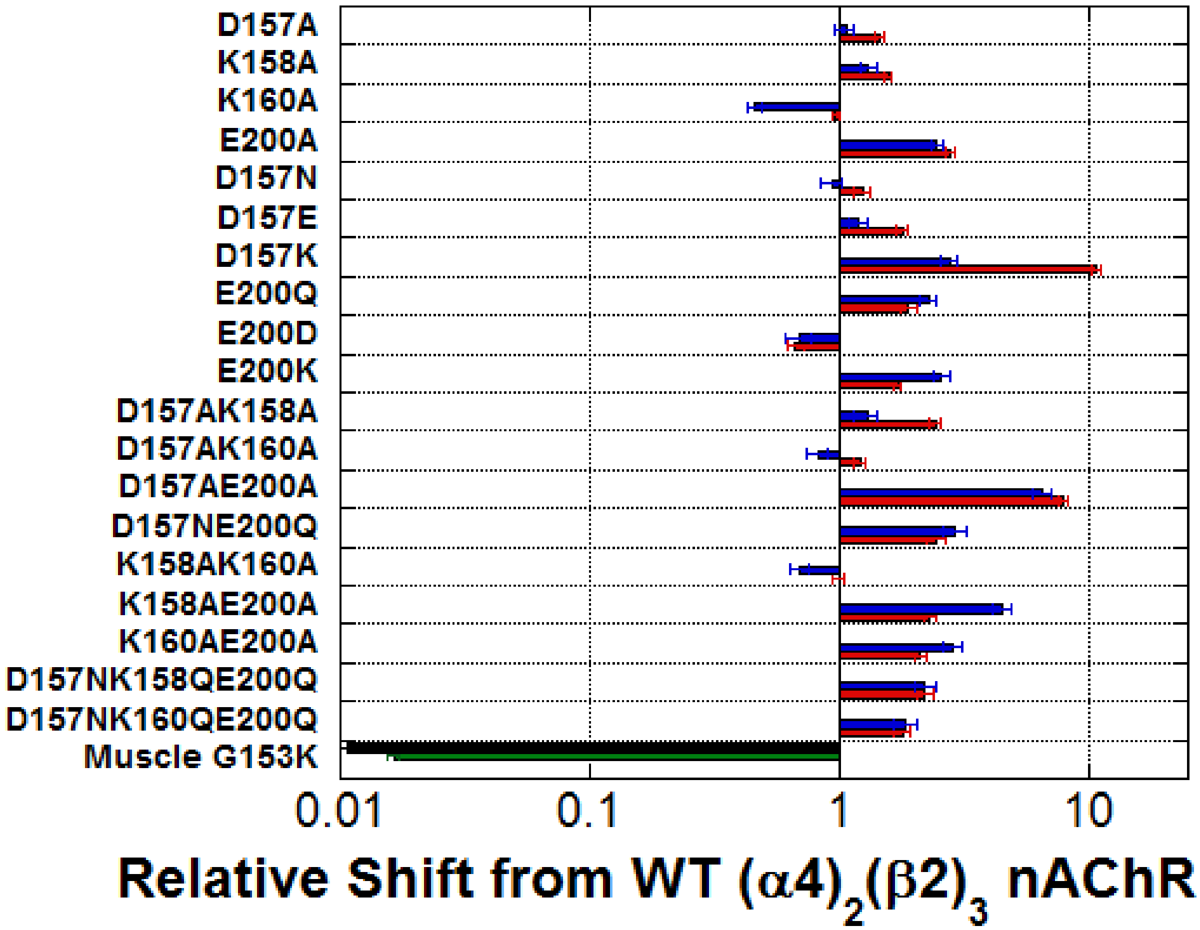


Figure S5: Bar graph comparing the effect on agonist potency of mutating select residues located outside of the $(\alpha4)_2(\beta2)_3$ agonist binding site. The data are from Supporting Table S3. For each mutation, the relative shift in agonist potency from the wild type $(\alpha4)_2(\beta2)_3$ receptor is shown for ACh (red) and nicotine (blue). The effect of α1 G153K on ACh (green) and nicotine (black) potency for the muscle receptor are shown for reference.

Supporting Tables

Table S1: EC_{50} values (μM) and Hill coefficients for mutant $(\alpha 1)_2\beta 1\gamma\delta$ nAChRs. N = number of cells. The EC_{50} values are ± S.E. ND, not determined; N/A, not available. [‡]Previously reported in Cashin 2005; [†]previously reported in Xiu 2009. All other values in this table were determined in the present work.

$(\alpha 1)_2\beta 1\gamma\delta$ nAChR									
Mutation	**ACh**	**n_H**	**N**	**Nicotine**	**n_H**	**N**	**Epibatidine**	**n_H**	**N**
Wild Type	1.2 ± 0.1	1.6 ± 0.1	9	56 ± 4	2.2 ± 0.3	14	0.83 ± 0.08[‡]	N/A	N/A
G153K	0.027 ± 0.001	1.5 ± 0.1	12	0.76 ± 0.05	1.6 ± 0.2	13	0.011 ± 0.001	1.5 ± 0.1	10
G153A	0.029 ± 0.001	1.7 ± 0.1	9	1.2 ± 0.1	1.5 ± 0.1	6	ND	ND	ND
G153T	0.030 ± 0.001	1.5 ± 0.1	15	1.2 ± 0.1	1.8 ± 0.1	14	ND	ND	ND
$(\alpha 1\ G153K)_2\beta 1\gamma\delta$ – TrpB (W149)									
Trp	0.019 ± 0.001[†]	1.5 ± 0.1[†]	6	0.59 ± 0.04[†]	1.8 ± 0.2[†]	11	0.010 ± 0.001	1.4 ± 0.1	9
F_1-Trp	0.094 ± 0.004[†]	1.6 ± 0.1[†]	7	2.8 ± 0.1[†]	1.3 ± 0.1[†]	16	0.078 ± 0.001	1.2 ± 0.1	9
F_2-Trp	0.079 ± 0.004[†]	1.3 ± 0.1[†]	5	2.3 ± 0.1[†]	1.3 ± 0.1[†]	7	0.17 ± 0.01	1.2 ± 0.1	9
F_3-Trp	1.05 ± 0.03[†]	1.3 ± 0.1[†]	9	11 ± 1[†]	1.5 ± 0.1[†]	10	1.0 ± 0.1	1.3 ± 0.1	13
F_4-Trp	7.5 ± 0.5[†]	1.2 ± 0.1[†]	8	32 ± 4[†]	1.5 ± 0.2[†]	6	6.8 ± 0.9	1.2 ± 0.1	8
$(\alpha 1\ G153K)_2\beta 1\gamma\delta$ – Thr(B+1) (T150)									
Thr	0.024 ± 0.001	1.3 ± 0.1	8	0.62 ± 0.03	1.6 ± 0.1	8	0.012 ± 0.001	1.2 ± 0.1	7
Tah	0.028 ± 0.002	1.1 ± 0.1	11	9.0 ± 0.6	1.5 ± 0.1	11	0.13 ± 0.01	1.3 ± 0.1	8

Table S2: EC_{50} values (μM) and Hill coefficients for mutant $(\alpha7)_5$ nAChRs. N = number of cells. The EC_{50} values are ± S.E.

$(\alpha7)_5$ nAChR										
Residue	**Mutation**	**ACh**	**n_H**	**N**	**Nicotine**	**n_H**	**N**	**Epibatidine**	**n_H**	**N**
Wild type		66 ± 1	2.9 ± 0.1	15	23 ± 1	3.1 ± 0.1	9	0.26 ± 0.01	3.3 ± 0.2	11
G152K		3.7 ± 0.1	1.8 ± 0.1	12	0.76 ± 0.03	2.4 ± 0.2	10	0.016 ± 0.001	2.9 ± 0.4	10
$(\alpha7\ G152K)_5$										
TyrA (Y92)	**Tyr**	5.1 ± 0.3	2.1 ± 0.3	6	0.55 ± 0.01	3.3 ± 0.3	12	0.017 ± 0.001	2.8 ± 0.3	9
	F_3-Phe	240 ± 11	2.9 ± 0.4	10	10 ± 1	2.8 ± 0.5	13	0.47 ± 0.01	3.4 ± 0.2	6
TrpB (W148)	**Trp**	4.1 ± 0.2	2.7 ± 0.3	14	0.77 ± 0.03	2.9 ± 0.3	16	0.016 ± 0.001	3.6 ± 0.5	10
	F_3-Trp	9.0 ± 0.3	1.9 ± 0.1	11	1.2 ± 0.1	2.4 ± 0.2	13	0.23 ± 0.02	2.1 ± 0.2	12
TyrC2 (Y194)	**Tyr**	3.9 ± 0.1	3.2 ± 0.2	12	0.61 ± 0.01	3.5 ± 0.3	13	0.015 ± 0.001	3.8 ± 0.2	9
	F_1-Phe	8.0 ± 0.5	1.9 ± 0.2	12	3.5 ± 0.1	2.9 ± 0.1	13	0.079 ± 0.001	3.4 ± 0.2	9
	F_3-Phe	170 ± 8	2.2 ± 0.2	14	60 ± 2	2.1 ± 0.1	14	2.2 ± 0.1	2.6 ± 0.3	12
	4-Br-Phe	3.0 ± 0.2	1.9 ± 0.2	10	1.1 ± 0.1	3.4 ± 0.3	10	0.021 ± 0.001	2.6 ± 0.2	11
	4-CN-Phe	10 ± 1	2.0 ± 0.2	8	15 ± 1	2.6 ± 0.2	9	0.12 ± 0.01	3.4 ± 0.3	16
	4-MeO-Phe	6.0 ± 0.4	2.3 ± 0.3	11	2.5 ± 0.1	3.2 ± 0.1	11	0.025 ± 0.001	3.0 ± 0.2	11
Ser(B+1) (S149)	**S149T**	1.8 ± 0.1	2.1 ± 0.1	9	0.29 ± 0.01	4.1 ± 0.4	14	0.009 ± 0.001	3.1 ± 0.4	11
	Thr	1.7 ± 0.1	2.0 ± 0.1	14	0.29 ± 0.01	4.6 ± 0.4	20	0.012 ± 0.001	3.5 ± 0.4	20
	Tah	0.6 ± 0.1	1.7 ± 0.2	9	2.3 ± 0.1	2.0 ± 0.1	6	0.031 ± 0.002	2.7 ± 0.5	9

Table S3: EC_{50} values (ACh and Nicotine, μM; Epibatidine, nM) and Hill coefficients for mutant $(\alpha4)_2(\beta2)_3$ nAChRs. N = number of cells. The EC_{50} values are ± S.E. †Previously reported in Xiu 2009. All other values in this table were determined in the present work.

α4β2 nAChR								
Mutation	**ACh**	**n_H**	**N**	**Nicotine**	**n_H**	**N**	**Norm. I (+70mV)**	**N**
$(\alpha4)_3(\beta2)_2$	0.023 ± 0.001†	1.3 ± 0.1†	6	0.01 ± 0.001†	1.7 ± 0.2†	3	0.297 ± 0.041†	24
$(\alpha4)_2(\beta2)_3$	0.42 ± 0.01†	1.2 ± 0.1†	12	0.08 ± 0.01†	1.2 ± 0.1†	15	0.041 ± 0.005†	9
$(\alpha4)_3(\beta2)_2$ K158G	0.11 ± 0.01	0.99 ± 0.05	11	0.045 ± 0.001	1.5 ± 0.1	13	0.268 ± 0.015	21
$(\alpha4)_2(\beta2)_3$ K158G	1.3 ± 0.1	1.1 ± 0.1	14	0.30 ± 0.02	1.6 ± 0.1	10	0.015 ± 0.006	20
$(\alpha4\ K158G)_2(\beta2)_3$ – TrpB (W154)								
Trp	1.3 ± 0.1	1.2 ± 0.1	10	0.27 ± 0.02	1.6 ± 0.2	13	0.014 ± 0.006	17
F_1-Trp	3.7 ± 0.1	1.2 ± 0.1	14	0.50 ± 0.04	1.4 ± 0.1	12	0.034 ± 0.005	23
F_2-Trp	5.4 ± 0.2	1.2 ± 0.1	10	0.67 ± 0.06	1.3 ± 0.1	13	0.024 ± 0.008	19
F_3-Trp	23 ± 1	1.3 ± 0.1	9	2.6 ± 0.2	1.2 ± 0.1	13	0.017 ± 0.009	17
F_4-Trp	25 ± 3	0.99 ± 0.08	8	4.5 ± 0.5	1.2 ± 0.1	6	0.021 ± 0.010	12
$(\alpha4\ K158G)_2(\beta2)_3$ – Thr (B+1) (T155)								
Thr	0.99 ± 0.03	1.1 ± 0.1	8	0.25 ± 0.01	1.5 ± 0.1	9	0.023 ± 0.004	13
Tah	0.53 ± 0.02	1.2 ± 0.1	8	3.4 ± 0.2	1.2 ± 0.1	10	0.024 ± 0.006	16
$(\alpha4)_2(\beta2)_3$ – Side Chain Mutations in the α4 Subunit								
D157A	0.58 ± 0.02	1.3 ± 0.1	9	0.18 ± 0.01	1.4 ± 0.1	8	0.013 ± 0.009	9
D157N	0.61 ± 0.03	1.2 ± 0.1	7	0.14 ± 0.01	1.5 ± 0.1	7	0.032 ± 0.004	7
D157E	0.86 ± 0.02	1.2 ± 0.1	12	0.19 ± 0.01	1.5 ± 0.1	13	0.017 ± 0.005	15
D157K	6.0 ± 0.2	1.3 ± 0.1	9	0.39 ± 0.01	1.7 ± 0.1	11	-0.023 ± 0.015	7
K158A	0.57 ± 0.01	1.2 ± 0.1	9	0.21 ± 0.01	1.4 ± 0.1	7	0.032 ± 0.008	10
K160A	0.37 ± 0.01	1.1 ± 0.1	9	0.081 ± 0.005	1.5 ± 0.1	10	0.039 ± 0.006	9
E200A	1.1 ± 0.1	1.1 ± 0.1	15	0.44 ± 0.02	1.4 ± 0.1	12	0.037 ± 0.006	12
E200Q	0.93 ± 0.05	1.3 ± 0.1	6	0.34 ± 0.01	1.5 ± 0.1	9	0.019 ± 0.004	6
E200D	0.32 ± 0.02	1.2 ± 0.1	11	0.11 ± 0.01	1.5 ± 0.1	12	0.025 ± 0.003	15
E200K	0.96 ± 0.03	1.2 ± 0.1	11	0.36 ± 0.01	1.5 ± 0.1	11	0.025 ± 0.008	11
D157AK158A	1.3 ± 0.1	1.2 ± 0.1	12	0.22 ± 0.02	1.4 ± 0.1	7	0.032 ± 0.008	11
D157AK160A	0.63 ± 0.03	1.3 ± 0.1	12	0.14 ± 0.01	1.4 ± 0.1	10	0.031 ± 0.007	13
D157AE200A	4.1 ± 0.1	1.3 ± 0.1	10	1.1 ± 0.1	1.4 ± 0.1	10	0.024 ± 0.006	9
D157NE200Q	1.2 ± 0.1	1.2 ± 0.1	7	0.41 ± 0.03	1.5 ± 0.1	13	0.029 ± 0.010	11
K158AK160A	0.58 ± 0.02	1.2 ± 0.1	9	0.096 ± 0.004	1.6 ± 0.1	7	0.021 ± 0.004	8

K158AE200A	1.3 ± 0.1	1.2 ± 0.1	6	0.63 ± 0.03	1.5 ± 0.1	7	0.031 ± 0.004	8
K160AE200A	1.2 ± 0.1	1.2 ± 0.1	12	0.40 ± 0.02	1.4 ± 0.1	11	0.026 ± 0.003	12
D157NK158QE200Q	1.1 ± 0.1	1.2 ± 0.1	10	0.31 ± 0.02	1.5 ± 0.1	12	0.049 ± 0.007	13
D157NK160QE200Q	0.93 ± 0.05	1.3 ± 0.1	9	0.24 ± 0.02	1.5 ± 0.1	6	0.035 ± 0.005	10
$(\alpha 4)_2(\beta 2)_3$ – TrpB (W154)								
Mutation	**±Epibatidine**	**n_H**	**N**	**Norm. I (+70mV)**	**N**			
Trp	0.58 ± 0.03	1.6 ± 0.1	13	0.036 ± 0.008	18			
F_1-Trp	6.8 ± 1.1	1.1 ± 0.2	12	0.039 ± 0.005	22			
F_2-Trp	12.0 ± 1.5	1.1 ± 0.1	11	0.062 ± 0.006	22			
F_3-Trp	35.4 ± 2.0	1.1 ± 0.1	14	0.032 ± 0.006	24			
F_4-Trp	23.1 ± 1.3	1.0 ± 0.1	8	0.021 ± 0.007	17			
$(\alpha 4)_2(\beta 2)_3$ – Thr (B+1) (T155)								
Thr	0.67 ± 0.04	1.4 ± 0.1	12	0.022 ± 0.004	24			
Tah	3.7 ± 0.1	1.5 ± 0.1	11	0.026 ± 0.004	13			

Table S4: EC_{50} values (µM) and Hill coefficients for mutant $(\alpha4)_2(\beta2)_3$ nAChRs probing the Loop B-Loop C hydrogen bond. N = number of cells. The EC_{50} values are ± S.E.

$(\alpha4)_2(\beta2)_3$ – K158								
Mutation	**ACh**	**n_H**	**N**	**Nicotine**	**n_H**	**N**	**Norm. I (+70mV)**	**N**
K158L	0.13 ± 0.01	1.2 ± 0.1	17	0.035 ± 0.003	1.5 ± 0.1	10	-0.005 ± 0.023	13
Leu	0.15 ± 0.01	1.3 ± 0.1	8	0.031 ± 0.001	1.3 ± 0.1	11	0.038 ± 0.010	14
Lah	0.060 ± 0.001	1.2 ± 0.1	11	0.011 ± 0.001	1.3 ± 0.1	10	0.026 ± 0.004	15
$(\alpha4)_2(\beta2)_3$ – TyrC2 (Y202)								
Tyr	0.44 ± 0.01	1.2 ± 0.1	10	0.096 ± 0.006	1.5 ± 0.1	8	0.035 ± 0.007	11
Yah	0.73 ± 0.03	1.2 ± 0.1	13	0.42 ± 0.03	1.4 ± 0.1	8	-0.008 ± 0.026	5

Table S5: Injection ratios of α4 K158G:β2 mRNA used to control α4β2 receptor stoichiometry in *Xenopus* oocytes. N = number of cells. EC_{50} values (μM) and Hill coefficients are shown. The EC_{50} values are ± S.E. ND, not determined.

α4 K153G:β2 mRNA Ratios								
Ratio	**ACh**	**n_H**	**N**	**Nicotine**	**n_H**	**N**	**Norm. I (+70mV)**	**N**
100:1	0.11 ± 0.01	1.0 ± 0.1	11	0.045 ± 0.001	1.5 ± 0.1	13	0.268 ± 0.015	21
30:1	0.08 ± 0.01	1.0 ± 0.1	5	ND	ND	ND	0.248 ± 0.027	9
10:1	0.35 ± 0.04	0.71 ± 0.05	11	ND	ND	ND	0.242 ± 0.021	13
6:1	0.49 ± 0.02	0.80 ± 0.02	8	ND	ND	ND	0.215 ± 0.016	17
3:1	0.68 ± 0.02	1.1 ± 0.1	13	ND	ND	ND	0.045 ± 0.008	11
1:1	1.3 ± 0.1	1.1 ± 0.1	14	0.30 ± 0.02	1.7 ± 0.2	10	0.015 ± 0.006	20
1:3	1.1 ± 0.1	1.3 ± 0.1	9	0.26 ± 0.02	2.1 ± 0.3	8	0.059 ± 0.006	17
1:10	1.0 ± 0.1	1.2 ± 0.1	12	0.26 ± 0.03	1.7 ± 0.3	7	0.043 ± 0.032	6