

Supplementary Material

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Pharmacological analysis of negative allosteric modulation of the $\alpha 7$ nicotinic receptor by polyunsaturated free fatty acids

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Martini lipid name	Mol %
CHOL	20%
POPC	13%
PIPC	10.5%
DPSM	10.5%
PAPI	5.5%
IAPC	5.5%
OUPE	5.5%
IUPE	4%
IGPE	4%
DLSM	4%
DXSM	4%
POPE	3.5%
PAPS	2.5%
DUSM	2%
DOPS	1.5%
POPG	1%
DPCE	1%
DBCE	0.5%
DXCE	0.5%
XNCE	0.5%

Supplementary Table 1: Membrane composition (mol %) in absence of fatty acid, for use in CG-MD simulations with the MARTINI 2.2 force field. Adapted from Hill et al., Am J Physiol: Renal Physiol 2005. To calculate mol % in presence of 5% fatty acid, multiply all values by 0.95

A

	% of inhibition (Mean $\pm$ s.d.)	n
SA	-9.52 $\pm$ 4.22	4
OA	-3.47 $\pm$ 6.62	5
LA	46.62 $\pm$ 14.62	6
GLA	15.15 $\pm$ 5.16	5
ALA	34.66 $\pm$ 11.16	6
SDA	47.78 $\pm$ 10.85	8
ESA	-3.45 $\pm$ 3.73	6
DGLA	0.23 $\pm$ 6.7	7
AA	94.04 $\pm$ 2.59	4
EPA	65.38 $\pm$ 15.1	5
DSA	-3.34 $\pm$ 5.53	6
DTA	-3.68 $\pm$ 3.99	6
DPA	12.67 $\pm$ 6.42	7
DHA	62.17 $\pm$ 24.7	12
NA	-4.78 $\pm$ 12.68	6

B

	% of inhibition (Mean $\pm$ s.d.)	n
AA-(C)	93.34 $\pm$ 3.64	11
AA-(E)	75.5 $\pm$ 11.46	6
AA-(ME)	53.09 $\pm$ 14.55	6
AA-(EE)	20.29 $\pm$ 13.84	6
DHA-(C)	63.55 $\pm$ 19.26	9
DHA-(G)	81.22 $\pm$ 12.45	5
DHA-(E)	83.72 $\pm$ 18.67	6
DHA-(ME)	54.01 $\pm$ 14.5	6
DHA-(EE)	40.23 $\pm$ 12.07	6

Supplementary Table 2 : Pharmacological characterization of different fatty acids and AA derivatives on $\alpha 7$ nAChR wild-type. **A.** Table summarizing the mean percentage of inhibition (% of inhibition) and number of replicates (n) for different types of fatty acids. The values correspond to Fig.2B. Data are presented as mean $\pm$ s.d. These values were determined from n independent experiments per condition as indicated. **B.** Table summarizing the mean percentage of inhibition (% of inhibition) and number of replicates (n) for different types of AA head groups corresponding to the values of Fig.3B, and DHA head groups corresponding to the values of Fig.3C. Data are presented as mean $\pm$ s.d. These values were determined from n independent experiments per condition as indicated.

	% of contact frequency		% of contact frequency		% of contact frequency
L213	6.91	L228	13.97	T461	11.61
I217	9.92	L450	12.82	N214	1.14
P218	1.58	M451	15.55	L215	0.99
C219	0.96	A452	8.47	M254	0.06
V220	15.75	F453	7.63	V268	2.71
L221	19.76	S454	20.79	I271	0.3
I222	6.48	V455	13.86	A272	0.93
S223	14.11	F456	13.88	Q273	5.48
A224	19.58	T457	16.65	F275	2.25
L225	8.3	I458	19.99	A276	6.15
A226	0.75	I459	10.21	M279	2.42
L227	6.54	C460	10.61		

Supplementary Table 3: Contact frequency of AA with residues surrounding site 1 and site 2. The table shows the percentage of contact events for each residue with AA represented in Fig.4.

	% of inhibitor (Mean $\pm$ s.d)	n
L213A	92.07 $\pm$ 15.96	5
L213S	103.9 $\pm$ 16.86	5
I217A	107.3 $\pm$ 23.53	5
I217S	114.3 $\pm$ 12.84	5
V220A	99.86 $\pm$ 35.22	5
V220S	129.6 $\pm$ 19.27	6
L221A	105.3 $\pm$ 4.67	5
L221F	108.6 $\pm$ 12.09	5
L221S	107.4 $\pm$ 9.42	5
A224L	75.46 $\pm$ 25.81	12

	% of inhibitor (Mean $\pm$ s.d)	n
A224S	90.62 $\pm$ 23.01	8
L228A	162.8 $\pm$ 26.34	5
M451A	98.04 $\pm$ 14.75	4
M451S	85.73 $\pm$ 18.86	11
S454A	96.76 $\pm$ 12.43	5
T457A	123.8 $\pm$ 16.58	11
T457V	119.3 $\pm$ 13.22	7
I458A	110.5 $\pm$ 10.77	8
I458S	109.7 $\pm$ 5.84	5

	% of inhibitor (Mean $\pm$ s.d)	n
N214F	37.83 $\pm$ 30.96	7
M254L	58.76 $\pm$ 33.1	6
V268S	95.56 $\pm$ 21.13	5
A276M	41.63 $\pm$ 31.61	6
M279A	48 $\pm$ 14.22	3

Supplementary Table 4: Pharmacological characterization of mutants screening with 1mM ACh. The table summarizes the percentage of inhibition (mean $\pm$ s.d.) relative to the α 7nAChR WT and number of replicates (n) for various point mutants represented in Fig.6B.

	EC ₅₀ (μ M)	nH	n
α7 WT	129.3 $\pm$ 40.8	1.489 $\pm$ 0.6	5
N214F	41.29 $\pm$ 3.3	2.435 $\pm$ 0.44	5
V220S	208.1 $\pm$ 39	1.074 $\pm$ 0.17	5
A224L	275.1 $\pm$ 20.8	1.802 $\pm$ 0.20	5
L228A	444.7 $\pm$ 45.5	1.903 $\pm$ 0.3	5
M254L	56.6 $\pm$ 12	2.210 $\pm$ 1.03	5
V268S	310 $\pm$ 32.9	2.046 $\pm$ 0.45	5
A276M	32.86 $\pm$ 3.6	1.989 $\pm$ 0.4	5
M279A	24.68 $\pm$ 12	1.261 $\pm$ 0.62	5
T457A	162 $\pm$ 26.9	1.128 $\pm$ 0.16	5

	IC ₅₀ (μ M)	nH	n
α7 WT	2.13 $\pm$ 0.19	2.432	8
N214F	5.96 $\pm$ 3.6	2.236 $\pm$ 1.43	6
V220S	1.95 $\pm$ 0.28	1.679	6
A224L	3.92 $\pm$ 0.47	3.205 $\pm$ 1.47	6
L228A	1.172 $\pm$ 0.23	1.650	6
M254L	3.284 $\pm$ 0.47	2.915 $\pm$ 1.71	6
V268S	2.294 $\pm$ 0.41	2.323 $\pm$ 0.7	8
A276M	3.372 $\pm$ 0.23	4.726 $\pm$ 2	6
M279A	2.580 $\pm$ 0.42	4.043 $\pm$ 3.68	6
T457A	1.693 $\pm$ 0.42	2.622	5

Supplementary Table 5: Pharmacological characterization of α 7nAChR wild-type and mutants. The table summarizes EC₅₀ and IC₅₀ values (in μ M), Hill coefficients (nH) and number of replicates (n) for the WT receptor and various point mutants. EC₅₀ values represent the concentration of agonist ACh required to elicit the half-maximal response summarizing Fig.6C, while IC₅₀ values represent the concentration of AA required to inhibit 50% of the maximal response summarizing Fig.7. Mutations include N214F, V220S, A224L, L228A, M254L, V268S, A276M, M279A and T457A. Data are presented as mean $\pm$ s.d. The nH indicates the Hill slope of the dose-response or inhibition curve. These values were determined from n independent experiments per condition as indicated.

	% of inhibition (Mean $\pm$ s.d.)	n
N214F	25.5 $\pm$ 17.19	14
V220S	97.2 $\pm$ 16.71	6
A224L	53.69 $\pm$ 18.57	6
L228A	120 $\pm$ 6.21	6
M254L	65.84 $\pm$ 32.32	12
V268S	91.98 $\pm$ 17.26	13
A276M	73.97 $\pm$ 8.99	12
M279A	76.25 $\pm$ 13.97	13
T457A	121.1 $\pm$ 11.03	5

Supplementary Table 6 : Pharmacological characterization of mutants screening with EC80 ACh. The table summarizes the percentage of inhibition (mean $\pm$ s.d.) relative to the WT and number of replicates (n) for various point mutants represented in Fig.6D.

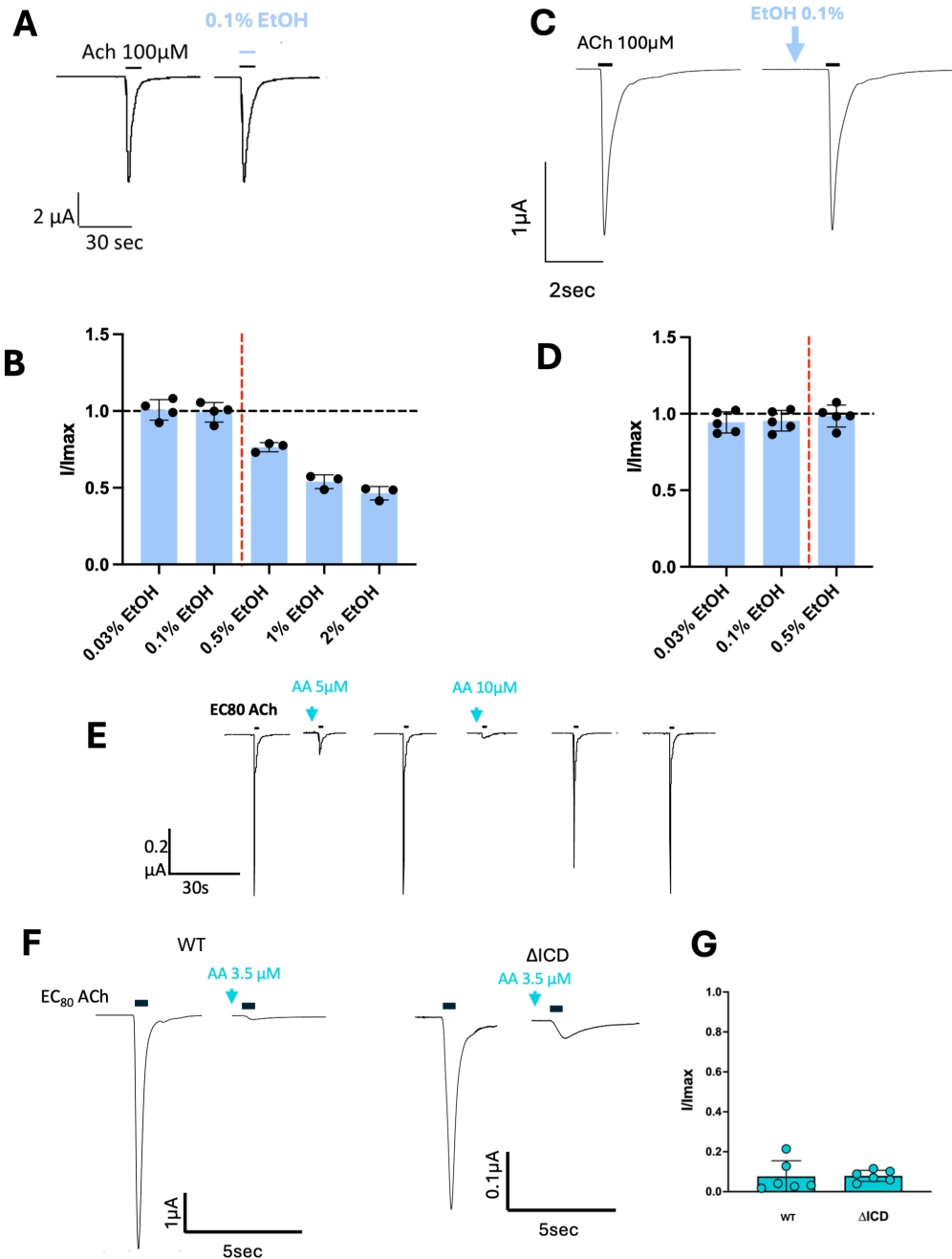
Data from Fig7B

Dunnett's multiple comparisons test	Mean diff.	Summary	Adjusted P Value
WT vs. L213A	7.933	ns	>0.9999
WT vs. L213S	-3.929	ns	>0.9999
WT vs. I217A	-7.286	ns	>0.9999
WT vs. I217S	-14.30	ns	0.9689
WT vs. V220A	0.1427	ns	>0.9999
WT vs. L221A	-5.340	ns	>0.9999
WT vs. V220S	-29.58	ns	0.0858
WT vs. A224L	24.54	ns	0.0802
WT vs. A224S	9.385	ns	0.9987
WT vs. L228A	-62.76	****	<0.0001
WT vs. M451A	1.964	ns	>0.9999
WT vs. M451S	14.27	ns	0.8116
WT vs. S454A	3.236	ns	>0.9999
WT vs. I458A	-10.46	ns	0.9993
WT vs. T457A	-23.77	ns	0.1181
WT vs. L221F	-8.625	ns	>0.9999
WT vs. I458S	-9.744	ns	0.9998
WT vs. L221S	-7.354	ns	>0.9999
WT vs. T457V	-19.35	ns	0.5603
WT vs. N214F	62.17	****	<0.0001
WT vs. M254L	41.24	**	0.0023
WT vs. A276M	58.37	****	<0.0001
WT vs. V268S	4.439	ns	>0.9999
WT vs. M279A	52.00	**	0.0030

Data from Fig 7D

Dunnett's multiple comparisons test	Mean diff.	Summary	Adjusted P Value
WT vs. N214F	74.50	****	<0.0001
WT vs. M254L	34.16	***	0.0002
WT vs. A276M	26.03	**	0.0079
WT vs. V268S	8.017	ns	0.8743
WT vs. M279A	23.75	*	0.0164
WT vs. T457A	-21.10	ns	0.1839
WT vs. A224L	46.31	****	<0.0001
WT vs. V220S	2.804	ns	>0.9999
WT vs. L228A	-19.97	ns	0.1827

Supplementary Table 7 : One-Way ANOVA multi-comparison analysis for data presented in Fig 7B and 7D. Significant differences are highlighted in gradient shades of blue



Supplementary Figure 1 : Solvent characterization for lipid solubilization and recovery from arachidonic acid inhibition. A, C. Representative traces of ACh dose-response curves at EC₅₀ of the $\alpha 7$ nAChR WT, recorded with (blue) or without (black) co-application (A) or pre-application (C) of different concentrations of 0.1 % ethanol. Recordings were performed by TEVC on *Xenopus* oocytes, with a 3-mins washout period between each application. B, D. Inhibition rates of 100 μ M ACh induced currents with co-application (B) or pre-application (D) of increasing concentrations of EtOH, normalized to responses elicited by 100 μ M ACh alone. Data are represented as mean $\pm$ s.d.. E: Recovery from arachidonic acid inhibition is slow as high concentration. Each ACh pulse with or without AA pre-application is separated by 3 minutes buffer wash. F,G: Effect of 3.5 μ M AA 30 sec pre-application on 206 μ M ACh currents on the WT and on a construct truncated from the disordered ICD loop and MA (Δ ICD) of $\alpha 7$ nAChR. I/I_{max} =current after/before AA pre-application.

Signal peptide

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WT          MRCSPPGGVWLALAASLLHVSLQGEFQRKLYKELVKNYNPLERPVANDSQPLTVYF
ΔICD_TEVC   MRCSPPGGVWLALAASLLHVSLQGEFQRKLYKELVKNYNPLERPVANDSQPLTVYF
ΔICD_GCMD   -----EFQRKLYKELVKNYNPLERPVANDSQPLTVYF

WT          SLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDGQIWKP
ΔICD_TEVC   SLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDGQIWKP
ΔICD_GCMD   SLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDGQIWKP

WT          DILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDVQHCK
ΔICD_TEVC   DILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDVQHCK
ΔICD_GCMD   DILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDVQHCK

WT          LKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPYPDV
ΔICD_TEVC   LKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPYPDV
ΔICD_GCMD   LKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPYPDV

WT          TFTVTMRRTLYYGLNLLIPCVLISALALLVFLLPADSGEKISLGITVLLSLTVF
ΔICD_TEVC   TFTVTMRRTLYYGLNLLIPCVLISALALLVFLLPADSGEKISLGITVLLSLTVF
ΔICD_GCMD   TFTVTMRRTLYYGLNLLIPCVLISALALLVFLLPADSGEKISLGITVLLSLTVF

WT          MLLVAEIMPATSDSVPLIAQYFASTMIIVGLSVVTVIVLQYHHHDPDGGKMPKW
ΔICD_TEVC   MLLVAEIMPATSDSVPLIAQYFASTMIIVGLSVVTVIVLQYHHHDPDGGKMPKW
ΔICD_GCMD   MLLVAEIMPATSDSVPLIAQYFASTMIIVGLSVVTVIVLQYHH-----

                               MX
WT          TRVILLNWCAWFLRMKRPGEDKVRPACQHKQRRCSLASVEMSAVAPPPASNGNLL
ΔICD_TEVC   TRVILLNWCAWFLRMKR-----
ΔICD_GCMD   -----

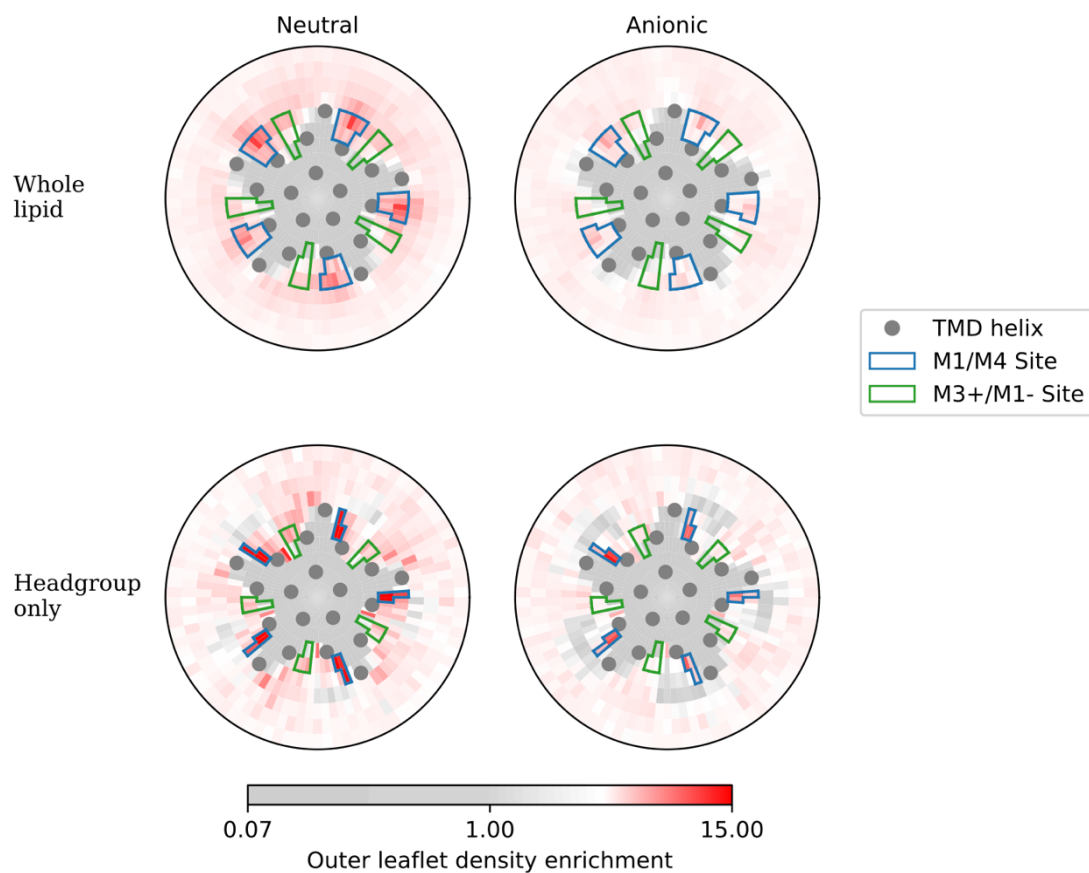
WT          YIGFRGLDGVHCVPTPDSGVVCGRMACSPTHDEHLLHGGQPPEGDPDLAKILEEV
ΔICD_TEVC   -----DGV-----
ΔICD_GCMD   -----

                               MA
WT          RYIANRFRQDESEAVCSEWKFAACVVDRCLCLMAFSVFTIICTIGILMSAPNFVE
ΔICD_TEVC   -----AVCSEWKFAACVVDRCLCLMAFSVFTIICTIGILMSAPNFVE
ΔICD_GCMD   -----WKFAACVVDRCLCLMAFSVFTIICTIGILMSAPNFVE

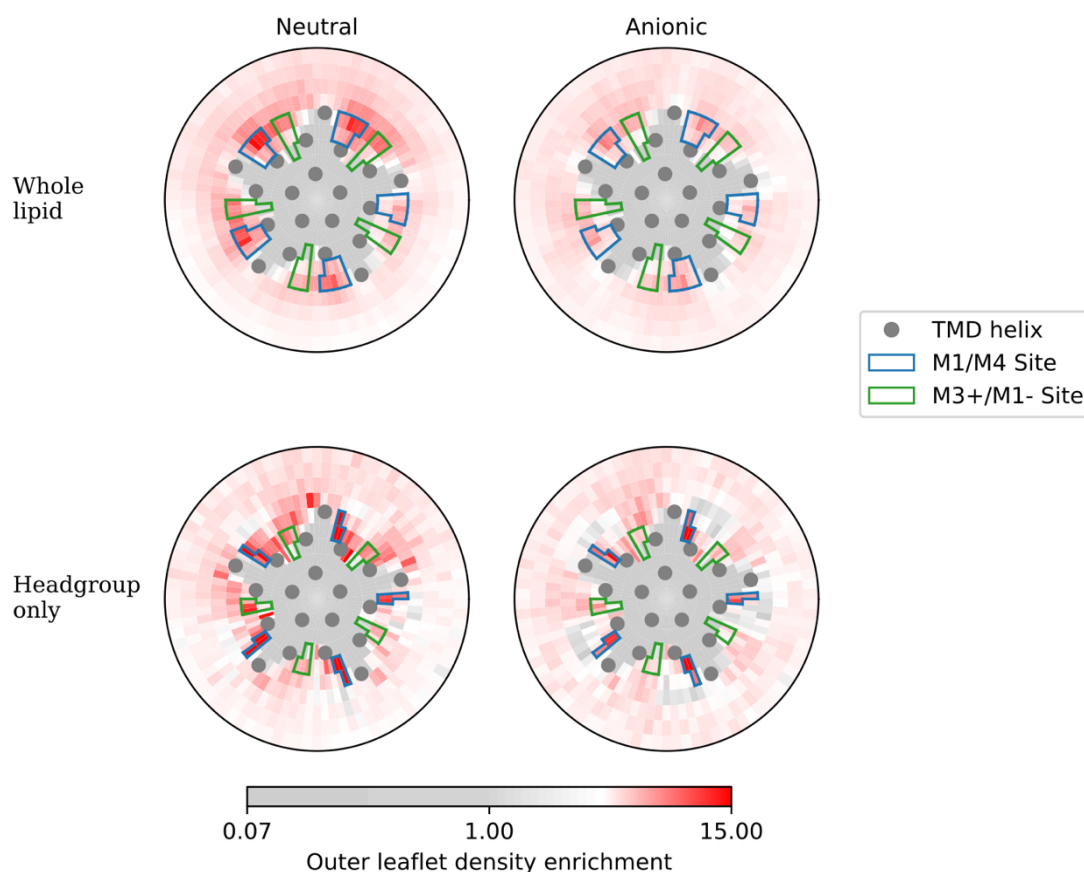
                               Rho1D4 tag
WT          AVSKDFA-----
ΔICD_TEVC   AVSKDFASAGLTETSQVAPA
ΔICD_GCMD   AVSKDF-----

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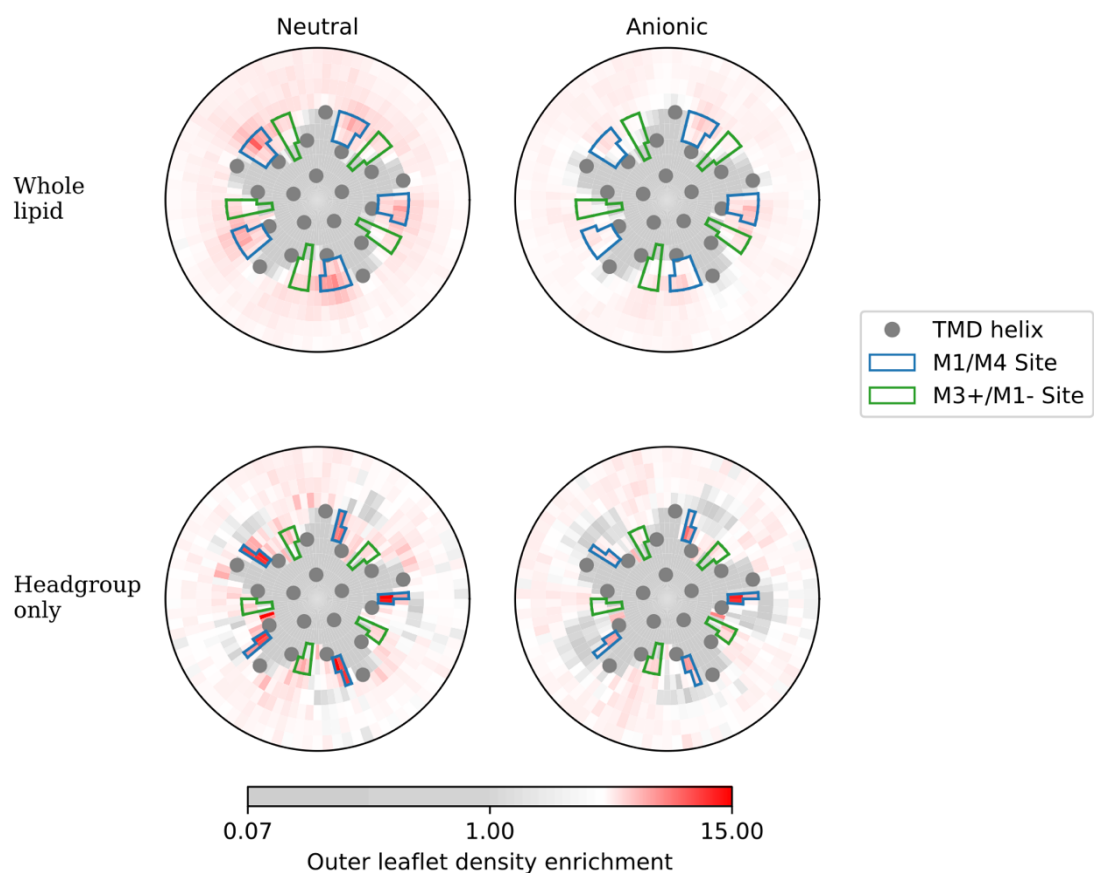
Supplementary Figure 2: Alignment of the sequences used in the study. WT: wild type $\alpha 7$ nAChR, Δ ICD_TEVC: construct used in TEVC, Δ ICD_GCMD: sequence of the model used in the simulations. MX and MA delimitations are shown in pink.



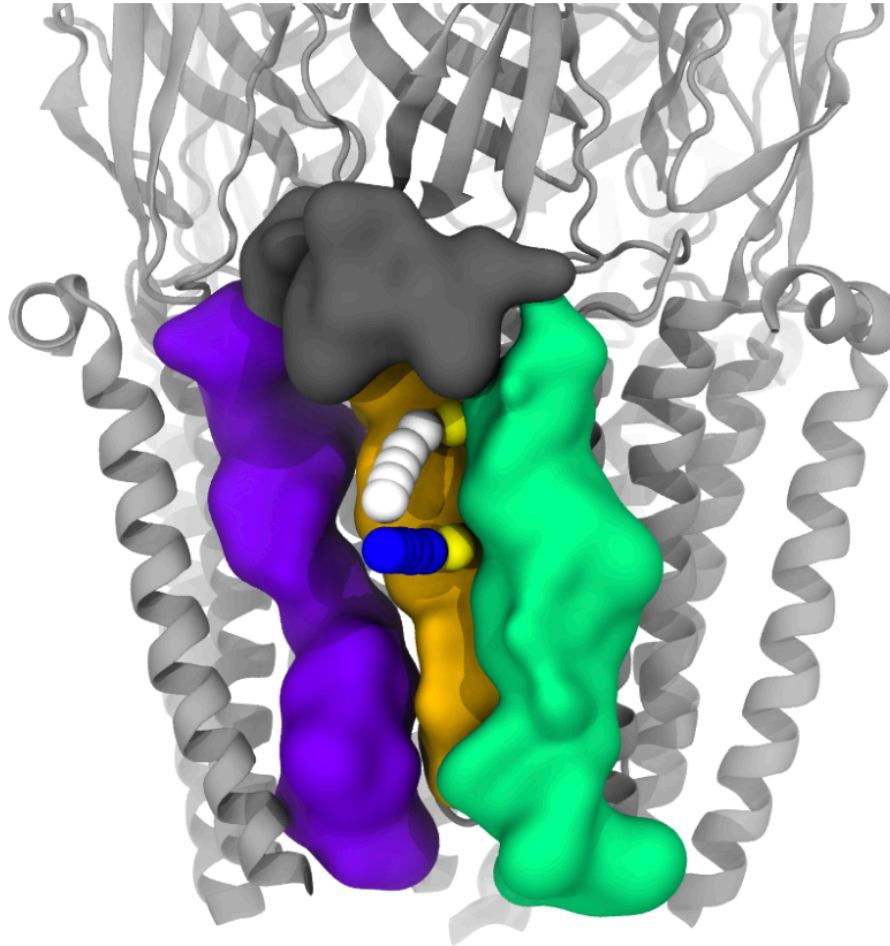
Supplementary Figure 3: Average density enrichment of AA in outer leaflet from CG-MD simulations. Average density enrichment (see eq. 1) of all beads (top row) or only the headgroup bead (bottom row) of neutral AA (left column) or anionic AA (right column). Time averages are taken over the second half of a 10 μ s trajectory and then averaged over four independent replicas. The M1/M4 intrasubunit site (blue) and M3+/M1- intersubunit site (green) are outlined in each heatmap. The center of mass of each TMD helix in the outer leaflet is shown with grey circles.



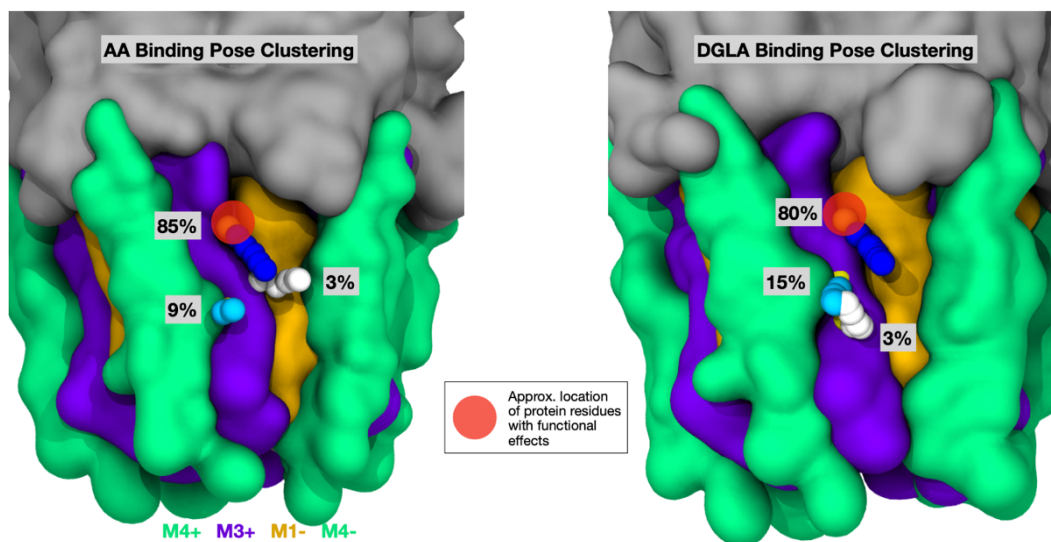
Supplementary Figure 4: Average density enrichment of DHA in outer leaflet from CG-MD simulations. Average density enrichment (see eq. 1) of all beads (top row) or only the headgroup bead (bottom row) of neutral DHA (left column) or anionic DHA (right column). Time averages are taken over the second half of a 10 μ s trajectory and then averaged over four independent replicas. The M1/M4 intrasubunit site (blue) and M3+/M1- intersubunit site (green) are outlined in each heatmap. The center of mass of each TMD helix in the outer leaflet is shown with grey circles.



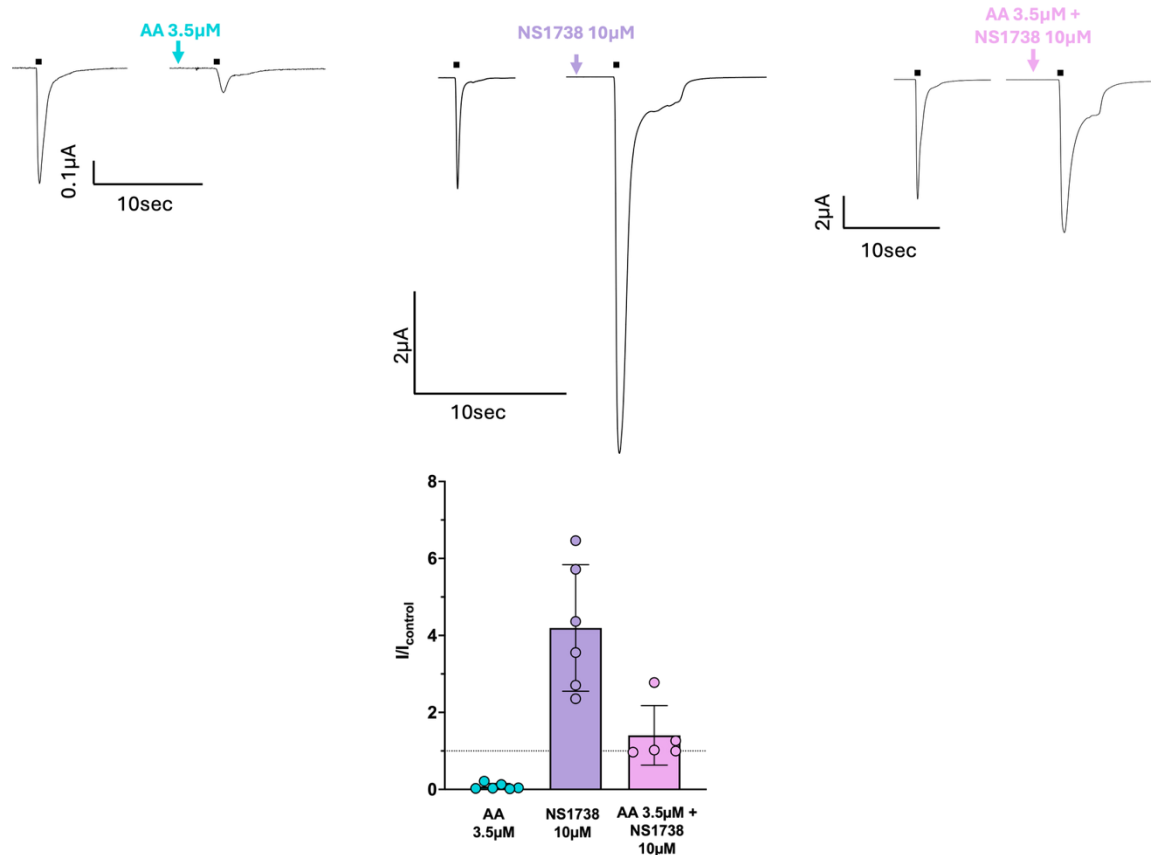
Supplementary Figure 5: Average density enrichment of DGLA in outer leaflet from CG-MD simulations. Average density enrichment (see eq. 1) of all beads (top row) or only the headgroup bead (bottom row) of neutral DGLA (left column) or anionic DGLA (right column). Time averages are taken over the second half of a 10 μ s trajectory and then averaged over four independent replicas. The M1/M4 intrasubunit site (blue) and M3+/M1- intersubunit site (green) are outlined in each heatmap. The center of mass of each TMD helix in the outer leaflet is shown with grey circles.



Supplementary Figure 6 : Most occupied binding modes of polar AA headgroups in M1/M4 site in CG-MD simulations, determined by cluster analysis. Initial binding modes are calculated using the measure cluster tool in VMD, clustering based off of the conformations of fatty acid beads except the most distal tail bead; clusters with headgroups within 4Å of each other are subsequently combined. Polar head groups are shown in yellow. Binding mode 1 (dark blue) represents 79% of all poses. Binding mode 2 (white) represents 17% of all poses. The remaining 5% of poses were not able to be clustered or generated clusters with less than 5% occupancy. M3 (purple), M1 (orange), M4 (green), and the loop over M1 (grey) are shown in surface representation to more accurately depict the binding cavity. The rest of the protein is displayed in new cartoon.



Supplementary Figure 7: Most occupied binding modes of polar AA and DGLA headgroups in M3+/M1- site in CG-MD simulations, determined by cluster analysis. Initial binding modes are calculated using the measure cluster tool in VMD, clustering based off of the conformations of the headgroup and first tail bead of the molecules; clusters with headgroups within 4Å of each other are subsequently combined. Polar head groups are shown in yellow. M4 (green), M3 (purple), M1 (orange), and the rest of the protein (grey) are shown in surface representation to more accurately depict the binding cavity. The approximate location of residues identified in TEVC as being functionally significant are denoted by the red circle.



Supplementary Figure 8: Effect of AA and NS1738 on $\alpha 7$ nAChR currents. AA, NS1738 were pre-applied alone or together for 30sec before 206μM ACh pulse. I/I_{control} = current after application / current before application (n=6.) Each condition was tested on a separate cell