NMR structures of the human α7 nAChR transmembrane domain and associated anesthetic binding sites

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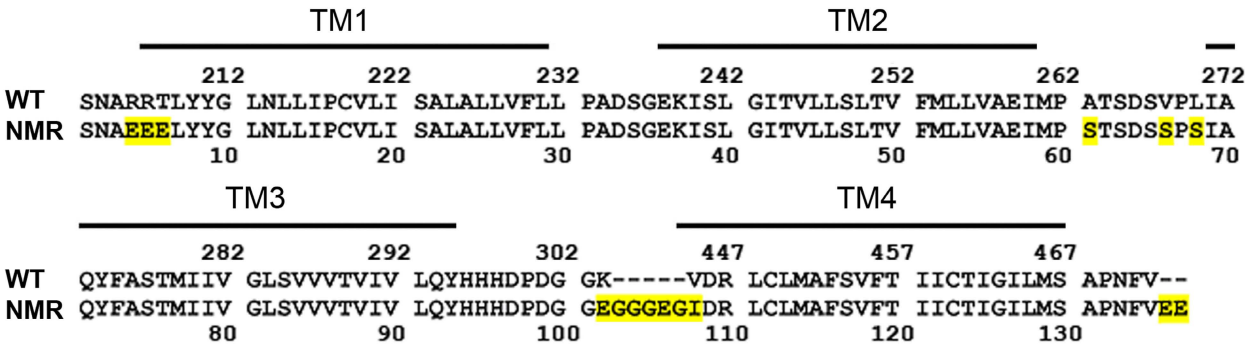

Fig. S1. The amino acid sequences of the wild-type TM domain **(WT)** of the human α7 nAChR and the α7 TM domain used in our NMR study **(NMR)** with the mutated sites highlighted. The residue numbering for each sequence is shown above and below the sequences, respectively. To convert the residue numbering used in the NMR study to the numbering for the full-length a7 AChR, add 202 for residues labeled 1 to 102 and add 337 for residues labeled 103 to 137.

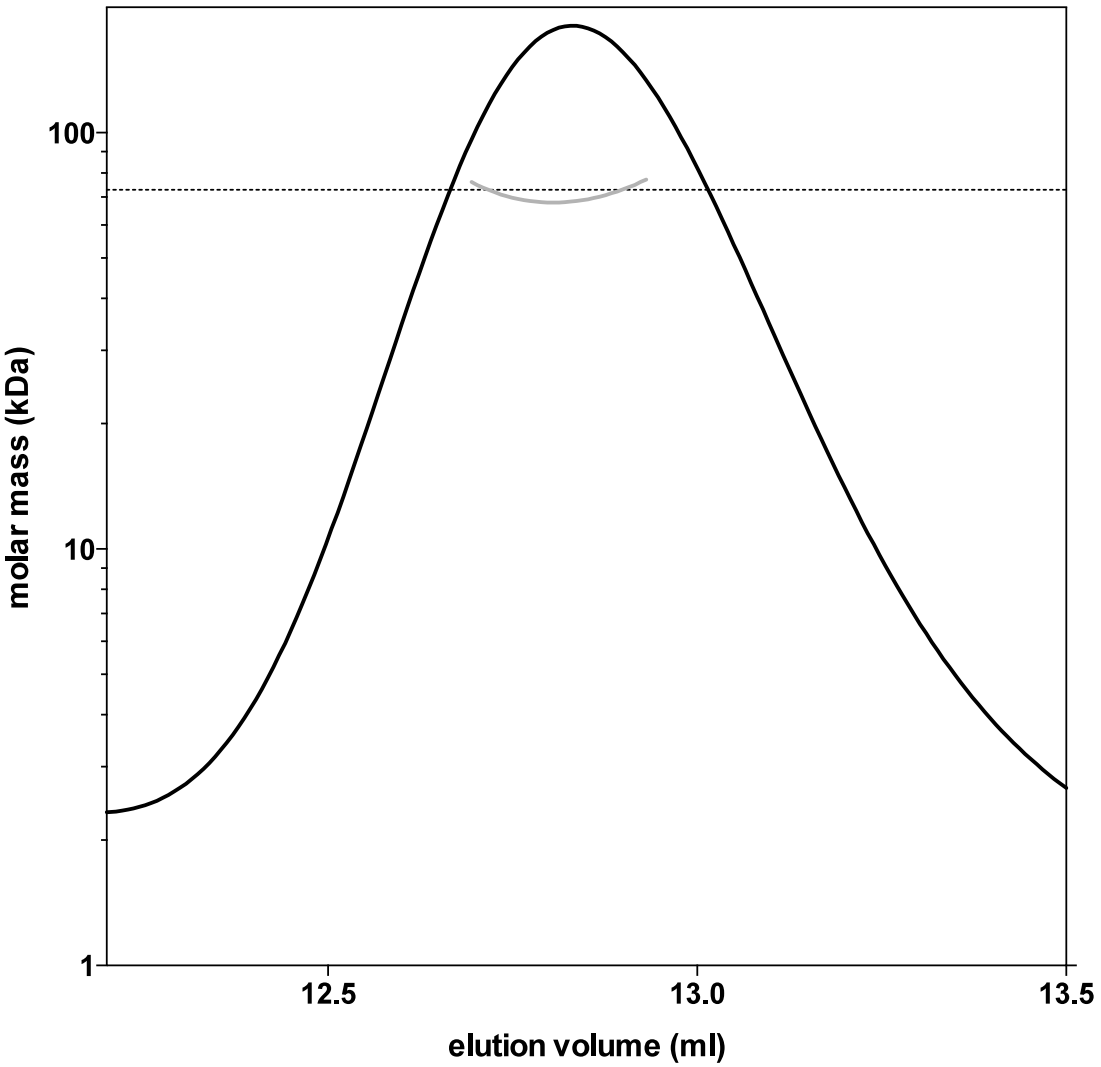

Fig. S2. Size exclusion chromatography-multi-angle light scattering analysis indicated the formation of a α7 TM domain pentamer. The molar mass (gray) of the α7 TM domain was calculated from conjugate analysis of the protein-detergent complex and is shown across the UV elution peak (black) after size exclusion chromatography. The average molar mass of the α7 TM domain assembly was 71.2 kDa. The dotted line indicates the expected molar mass of 72.9 kDa.

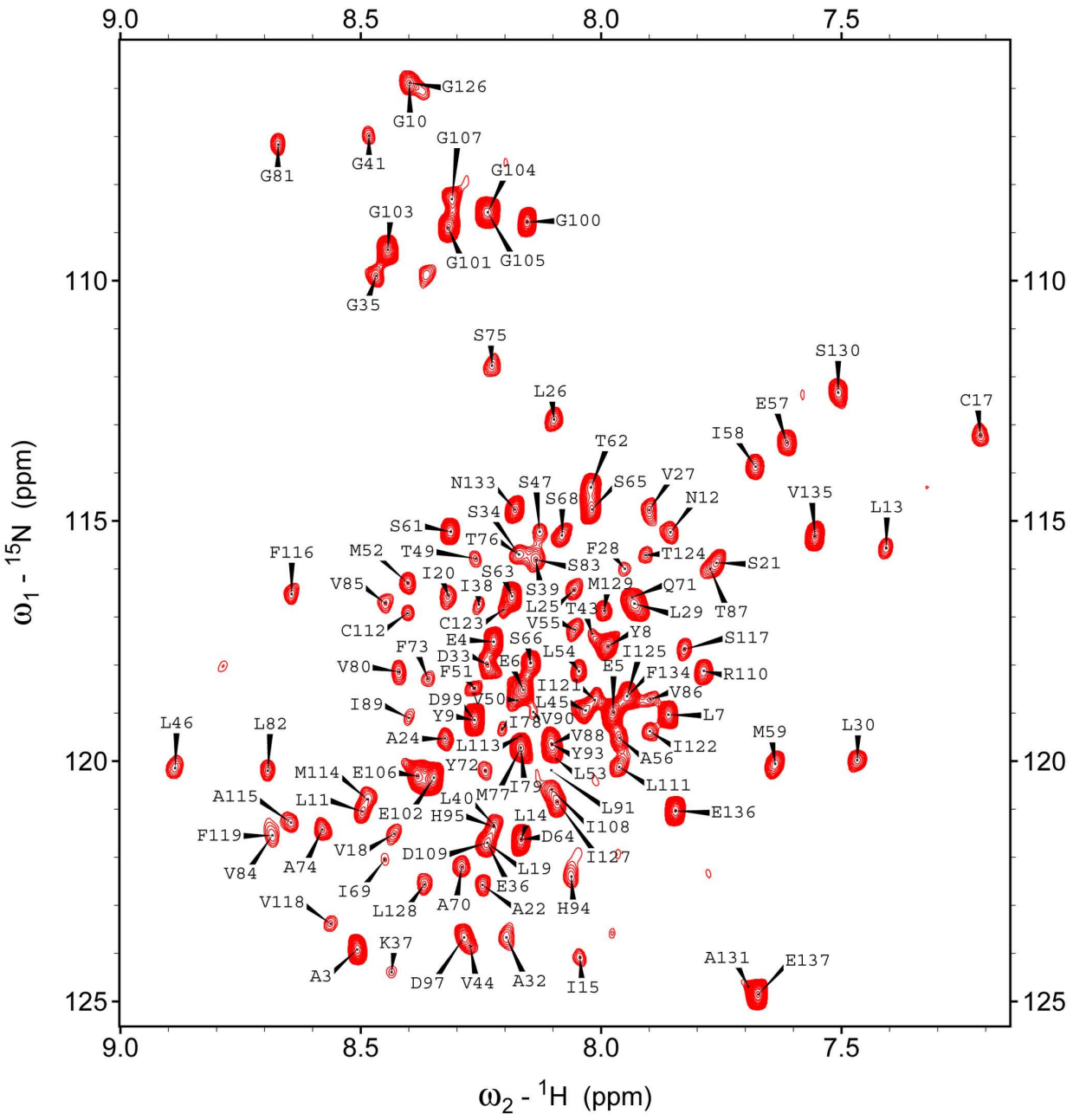

Fig. S3. ^{1}H-^{15}N TROSY-HSQC spectrum of the TM domain of the human α7 nAChR. The NMR sample contained 0.25 mM α7, 60 mM LDAO detergent, 5 mM sodium acetate pH 4.7, 10 mM NaCl, and 20 mM 2-mercaptoethanol to prevent disulfide bond formation. The NMR spectrum was acquired at 900 MHz and 45 ºC. The peak assignment is shown with the one-letter amino acid code and the sequence number. To convert the numbering in the spectra to the numbering for the full-length α7 nAChR, add 202 for residues labeled 1 to 102 and add 337 for residues labeled 103 to 137.

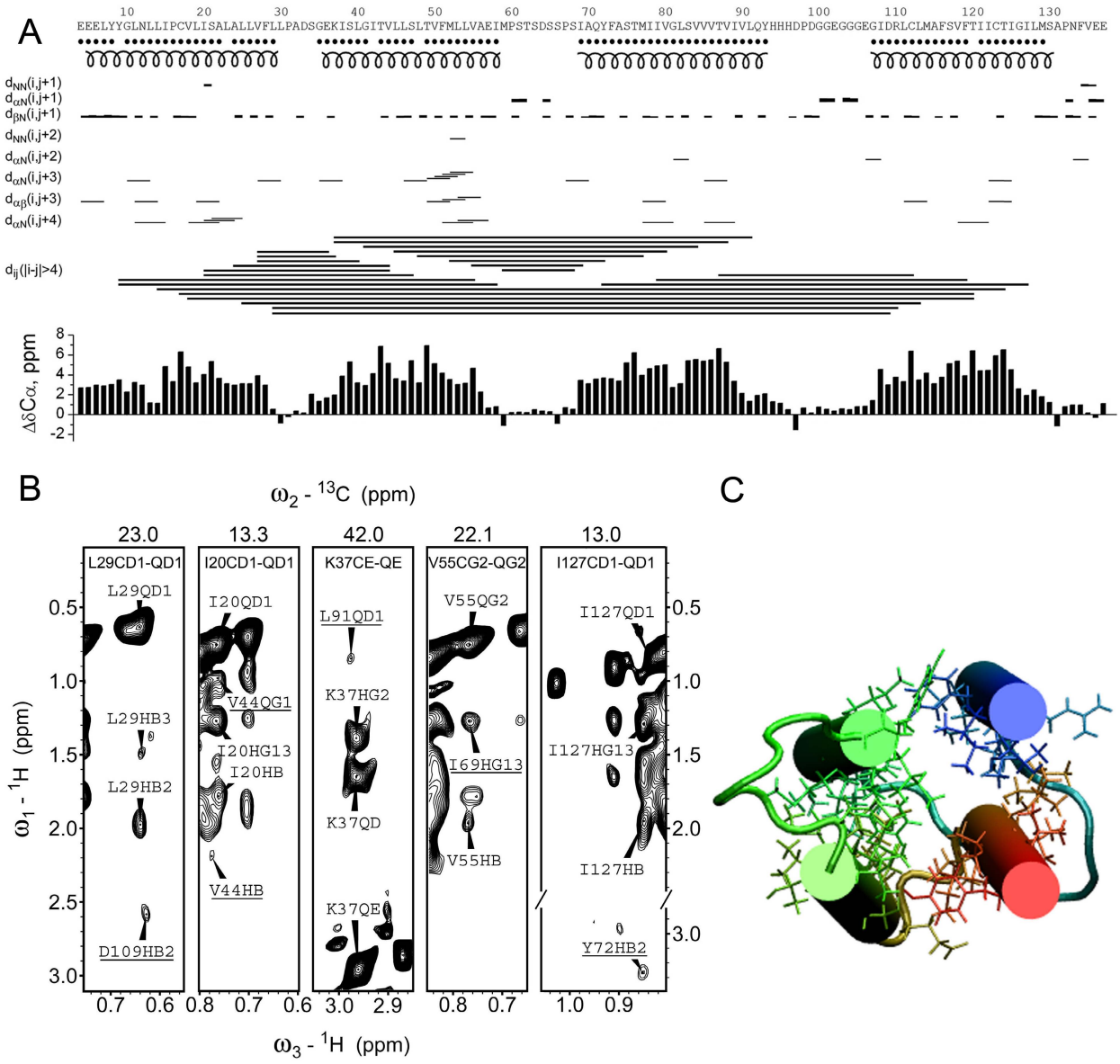

Fig. S4. Summary of NOE connectivity and hydrogen bonding restraints used for structural calculations of the TM domain of the human α7 nAChR. (**A**) Residues with temperature coefficients for amide proton chemical shifts smaller than 4.5 ppb/K were considered to be involved in hydrogen bonding and are marked with (●) below the protein sequence. Sequential, midrange, and long-range NOE connectivities are demonstrated by lines. The Cα chemical shift index is shown below the long-range NOE connectivities. The helical regions of the calculated α7 structure are indicated below the sequence. (**B**) Representative strip plots from the 3D ^{13}C-edited NOESY spectrum of the α7 TM domain acquired at 800 MHz and 45 ºC demonstrating long-range NOEs (underlined). (**C**) The α7 structure highlighting all α7 residues involved in long-range NOEs. The α7 structure is shown in a cartoon presentation and is color-coded from red for TM1 through green for TM2 and TM3 to blue for TM4. The residues are shown in a line presentation and colored in the same scheme as that for the four helices. To convert the residue

numbering used in the NMR study to the numbering for the full-length a7 AChR, add 202 for residues labeled 1 to 102 and add 337 for residues labeled 103 to 137.

Table S1. Statistics for the bundle of 20 calculated structures of the TM domain of the human α7 nAChR.

NMR structure	***Statistics***
Number of distance restraints	614
Intraresidue ($\|i - j\| = 0$)	239
Short range ($\|i - j\| = 1$)	223
Medium range ($1 < \|i - j\| \leq 4$)	109
Long-range, inter-helical ($\|i - j\| \geq 5$)	43
Number of dihedral angle restraints	196
(Residues 4-15, 17-29, 34, 36-58, 69-93, 107-130)	
Number of hydrogen bond restraints	152×2
(Residues 4-8,10-22, 24-25, 35-41, 43-47, 49-54, 69-89, 107-119, 121-125)	
Number of upper limit restraints violations > 0.5 Å	0
Number of dihedral angle restraints violations > 5°	0
Backbone RMSD (Residues 5-29, 36-58, 69-93, 107-130)	1.24 ± 0.32 Å
Heavy atom RMSD (Residues 5-29, 36-58, 69-93, 107-130)	1.64 ± 0.30 Å
Ramachandran plot	
Residues in most favored regions	86.2 %
Residues in additionally allowed regions	13.6 %
Residues in generously allowed regions	0.1 %
Residues in disallowed regions	0.1 %

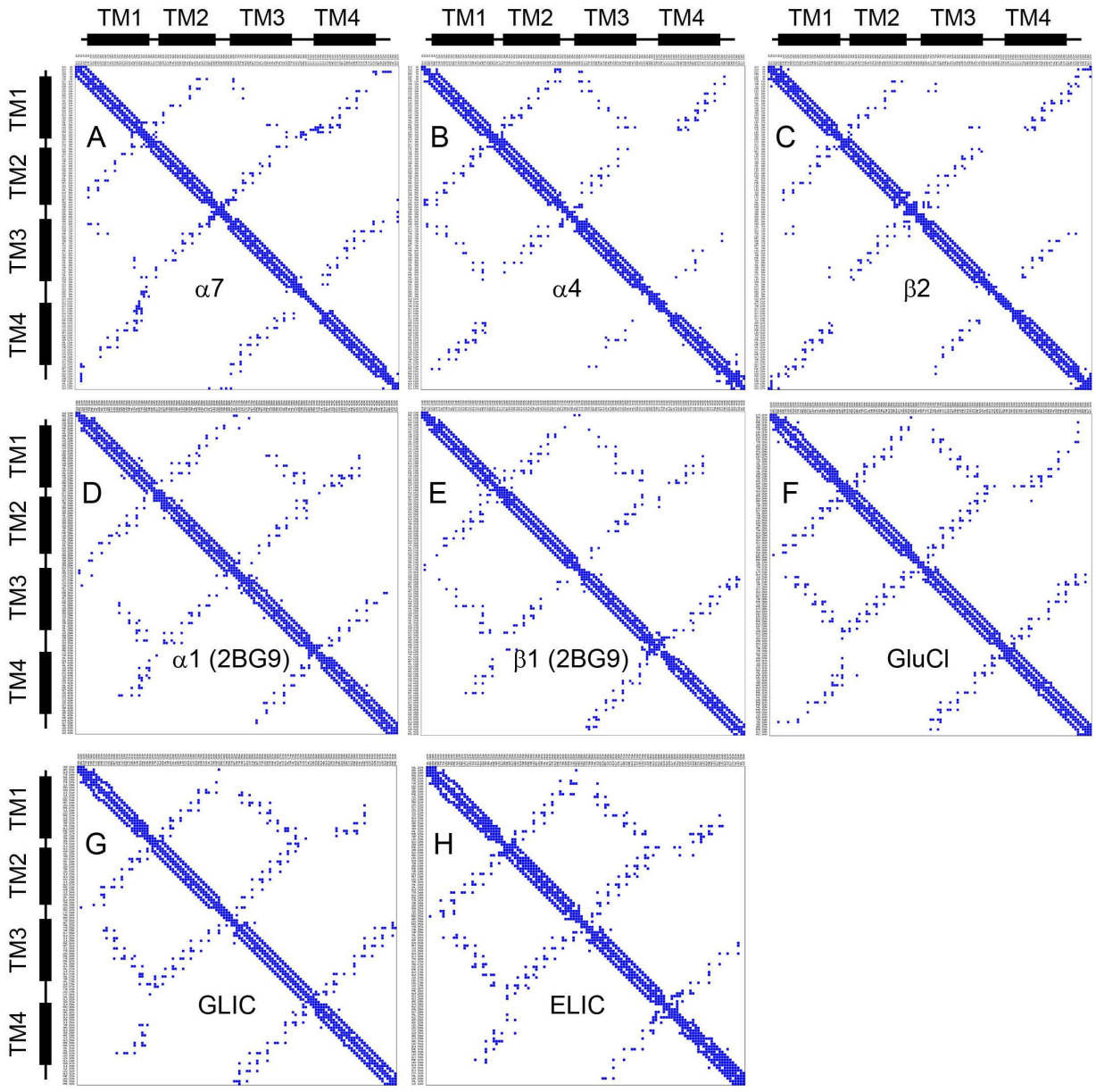

Fig. S5. Comparisons of contact maps for the TM domain structures of (**A**) α7 (PDB ID: 2MAW)**,** (**B**) α4 (PDB ID: 2LLY), and (**C**) β2 (PDB ID: 2LM2) in LDAO micelles, (**D**) α1 and (**E**) β1 of the *Torpedo* nAChR (PDB ID: 2BG9), (**F**) GluCl (PDB ID: 3RHW), (**G**) GLIC (PDB ID: 3EAM), and (**H**) ELIC (PDB ID: 2VL0). All protein structures show contact maps typical for four-helix bundle structures, but some differences are observed. For example, α7 shows more contacts between TM1 and TM3 than β2 but less than the other proteins. Also, there are some contacts between the TM1 – TM2 loop and the TM3 – TM4 loop in α7 that are not present in α4 and β2.

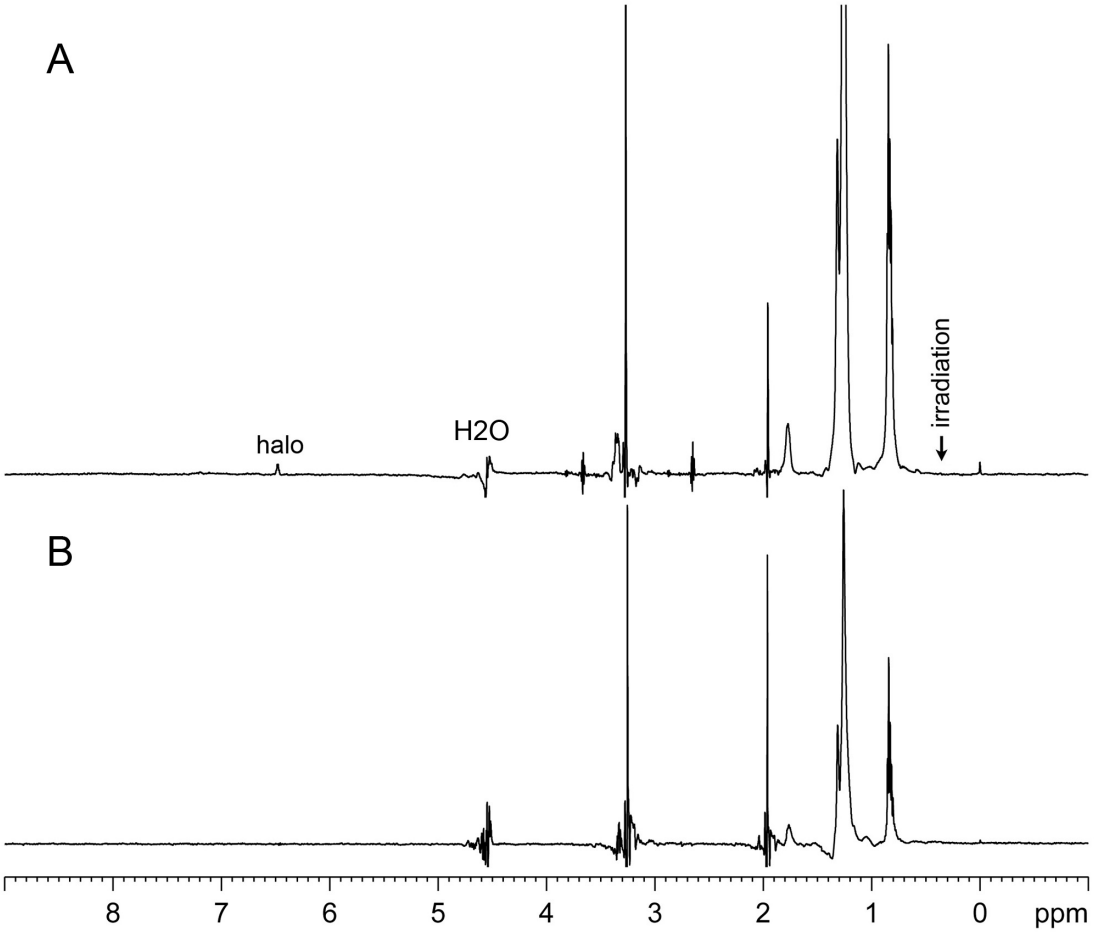

Fig. S6. 1D saturation transfer difference (STD) NMR confirms direct interaction between halothane and the TM domain of the human α7 nAChR. The STD spectra of 3.2 mM halothane in 60 mM LDAO detergent in (**A**) the presence and (**B**) the absence of the 0.25 mM α7 TM domain. The spectra were acquired at 600 MHz and 45 ºC with the same NMR parameters. A 10 s saturation time and a 12 s relaxation delay were used. The on- and off-resonance frequencies for saturation were set at 0.4 ppm and 25 ppm, respectively. The anesthetic signal (6.48 ppm) was observed only in the α7 STD spectrum confirming that the halothane signal result from direct anesthetic interactions with the protein. The peaks around 0.83, 1.26, 1.32, 1.77, 3.27, and 3.34 ppm were residual signals of LDAO.

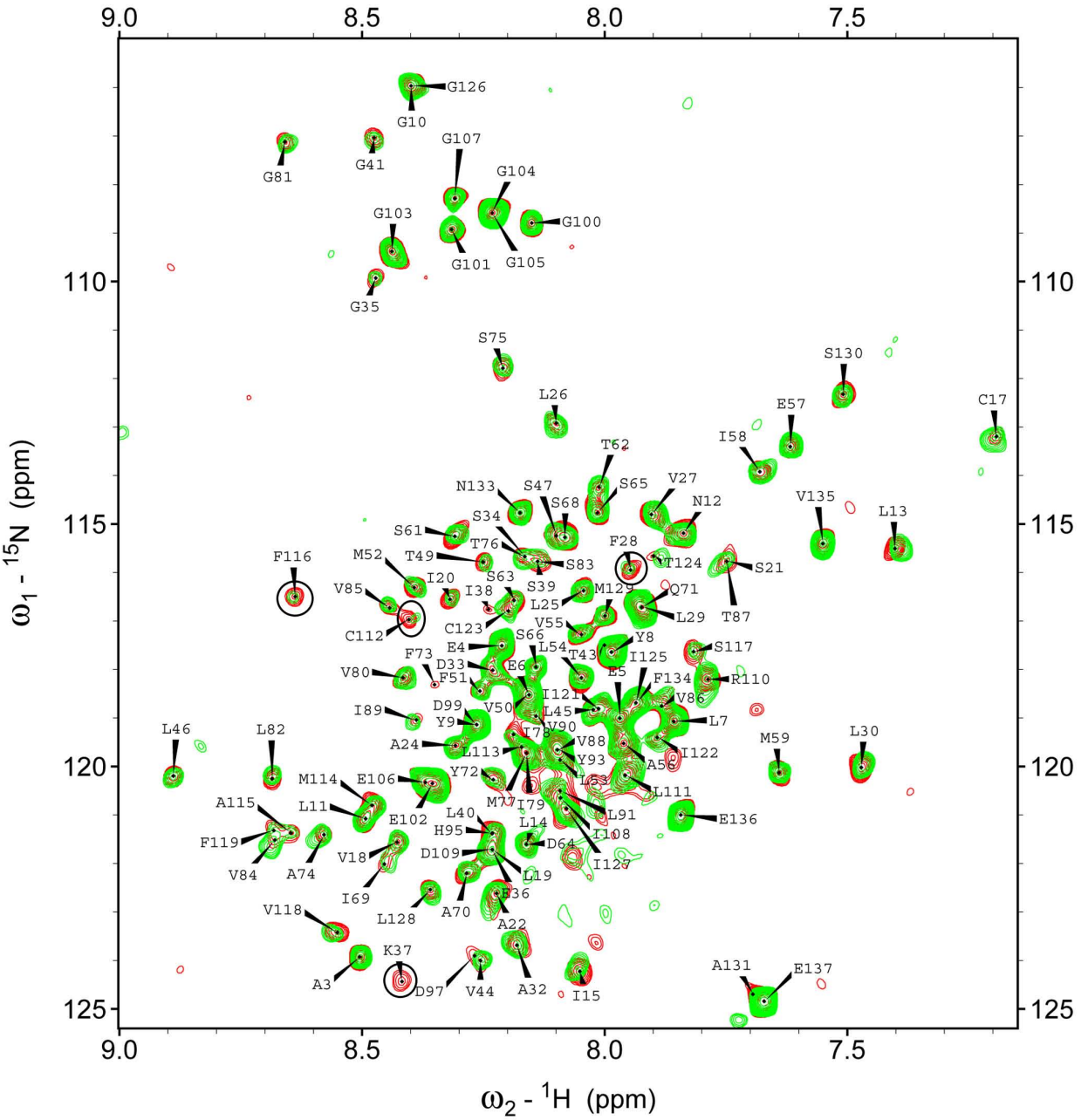

Fig. S7. 2D saturation transfer NMR demonstrates direct interaction between halothane and the specific residues of the TM domain of the human α7 nAChR. Overlay of 2D saturation transfer spectra of the α7 TM domain with (green) and without (red) saturation of the proton resonance of halothane (3.2 mM). The spectra were acquired at 600 MHz and 45 ºC. The peak assignment is shown with the one-letter amino acid code and the sequence number. The residues directly interacting with the anesthetic revealed significant decreasing in peak intensity upon the halothane peak saturation and are labeled with ovals. To convert the numbering in the spectra to the numbering for the full-length α7 nAChR, add 202 for residues labeled 1 to 102 and add 337 for residues labeled 103 to 137.

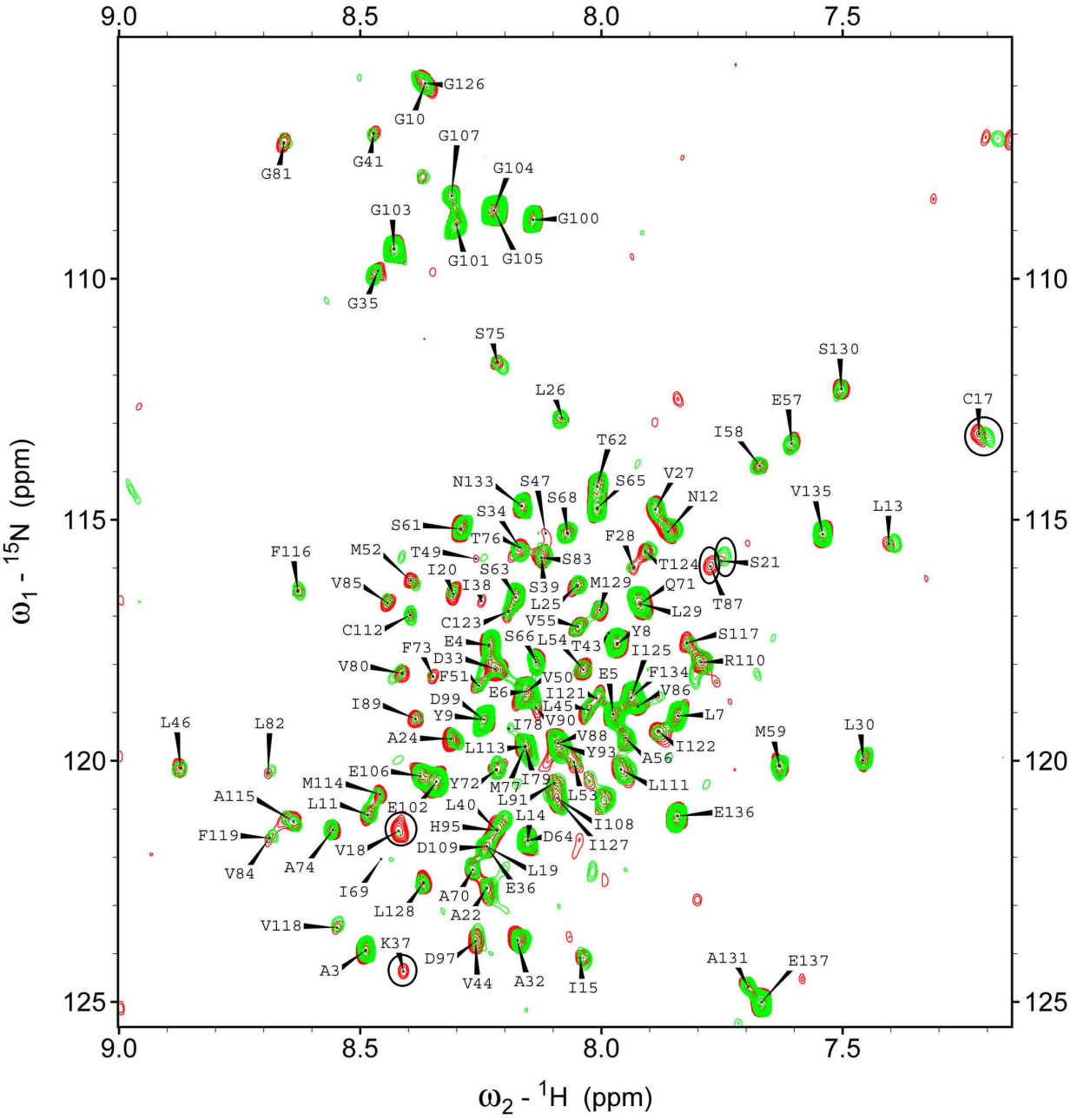

Fig. S8. Halothane effects on the TM domain of the human α7 nAChR. Overlay of ^{1}H-^{15}N TROSY-HSQC spectra of the α7 TM domain in the absence (red) and the presence (green) of 1.7 mM halothane. The spectra were acquired at 600 MHz and 45 ºC. The peak assignment is shown with the one-letter amino acid code and the sequence number. The residues significantly affected by the anesthetic are marked with ovals. To convert the numbering in the spectra to the numbering for the full-length α7 nAChR, add 202 for residues labeled 1 to 102 and add 337 for residues labeled 103 to 137.

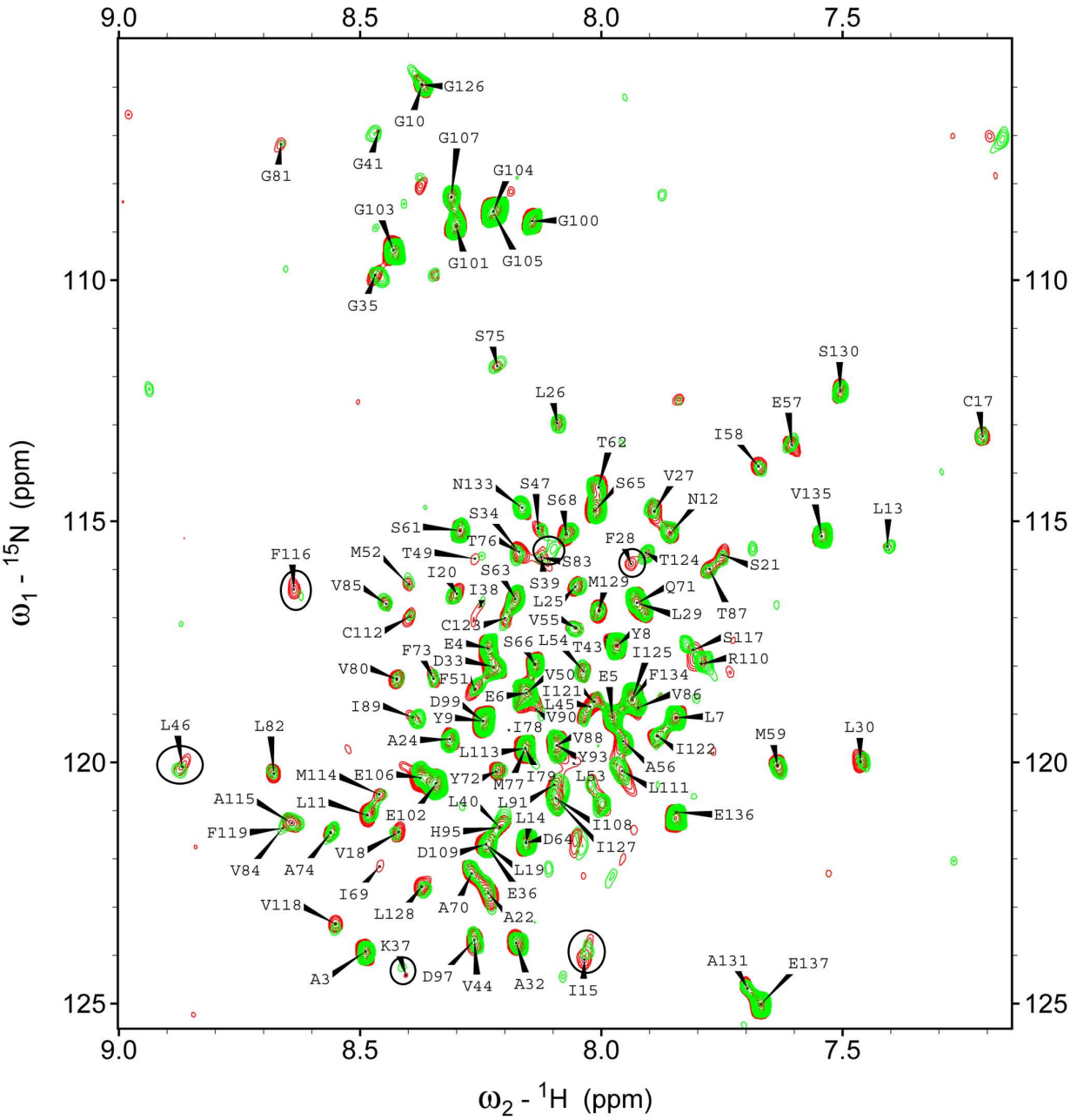

Fig. S9. Ketamine effects on the TM domain of the human α7 nAChR. Overlay of ^{1}H-^{15}N TROSY-HSQC spectra of the α7 TM domain in the absence (red) and the presence (green) of 80 μM ketamine. The spectra were acquired at 600 MHz and 45 ºC. The peak assignment is shown with the one-letter amino acid code and the sequence number. The residues significantly affected by the anesthetic are marked with ovals. To convert the numbering in the spectra to the numbering for the full-length α7 nAChR, add 202 for residues labeled 1 to 102 and add 337 for residues labeled 103 to 137.

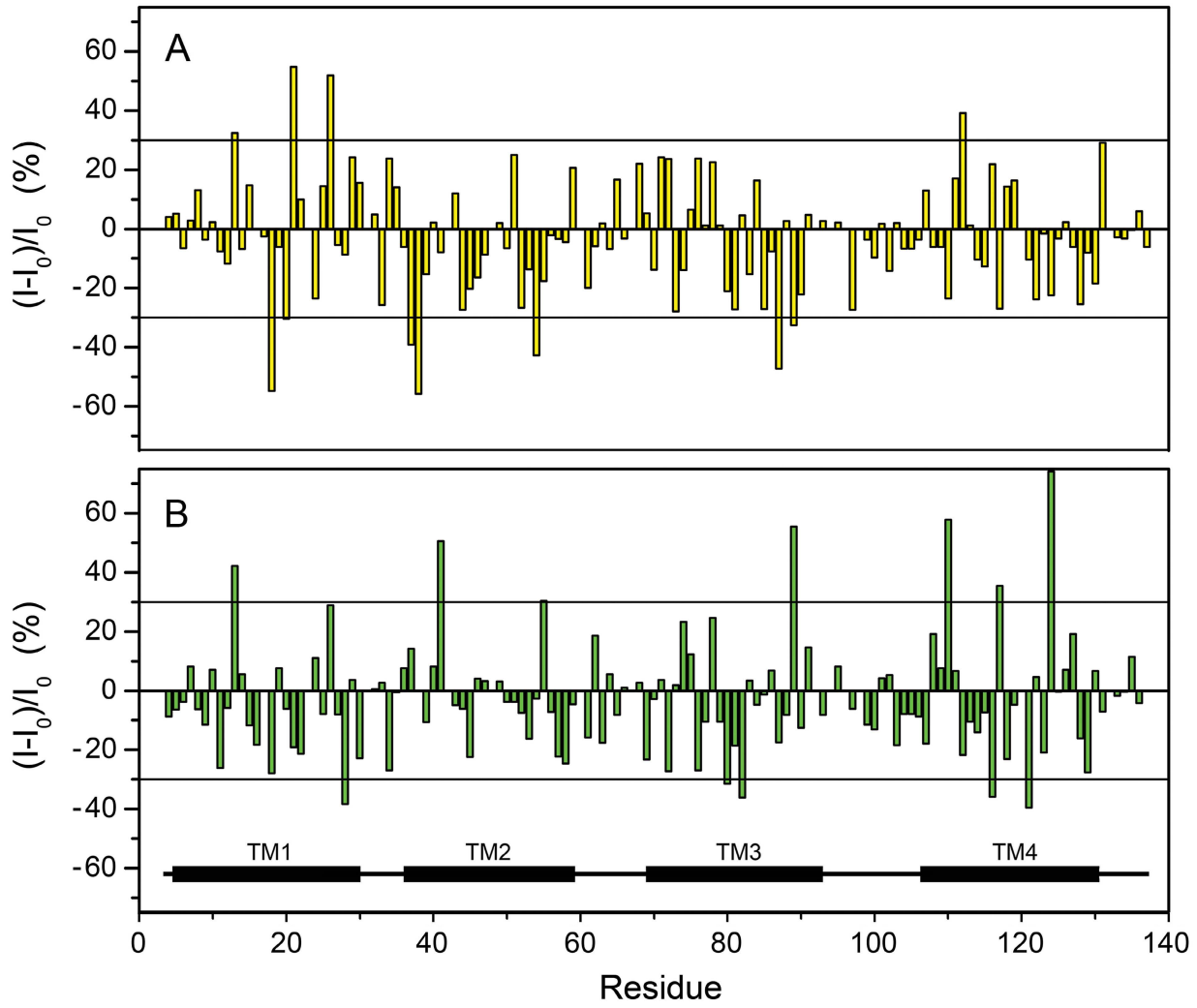

Fig. S10. Anesthetics modulate backbone dynamics of the TM domain of the human α7 nAChR. Relative changes in peak intensity of the α7 TM domain induced by (**A**) halothane (1.7 mM) and (**B**) ketamine (80 μM) versus the residue number. Residues significantly affected by halothane included L13, S21, L26, and C112 (peak intensity increased) as well as V18, I20, K37, I38, L54, T87, and I89 (peak intensity decreased). The ketamine binding affected the following residues: L13, G41, I89, R110, S117, and T124 (peak intensity increased) as well as F28, V80, L82, F116, and I121 (peak intensity decreased). To convert the numbering in this figure to the numbering for the full-length α7 nAChR, add 202 for residues labeled 1 to 102 and add 337 for residues labeled 103 to 137.