NMR resolved multiple anesthetic binding sites in the TM domains of the α4β2 nAChR

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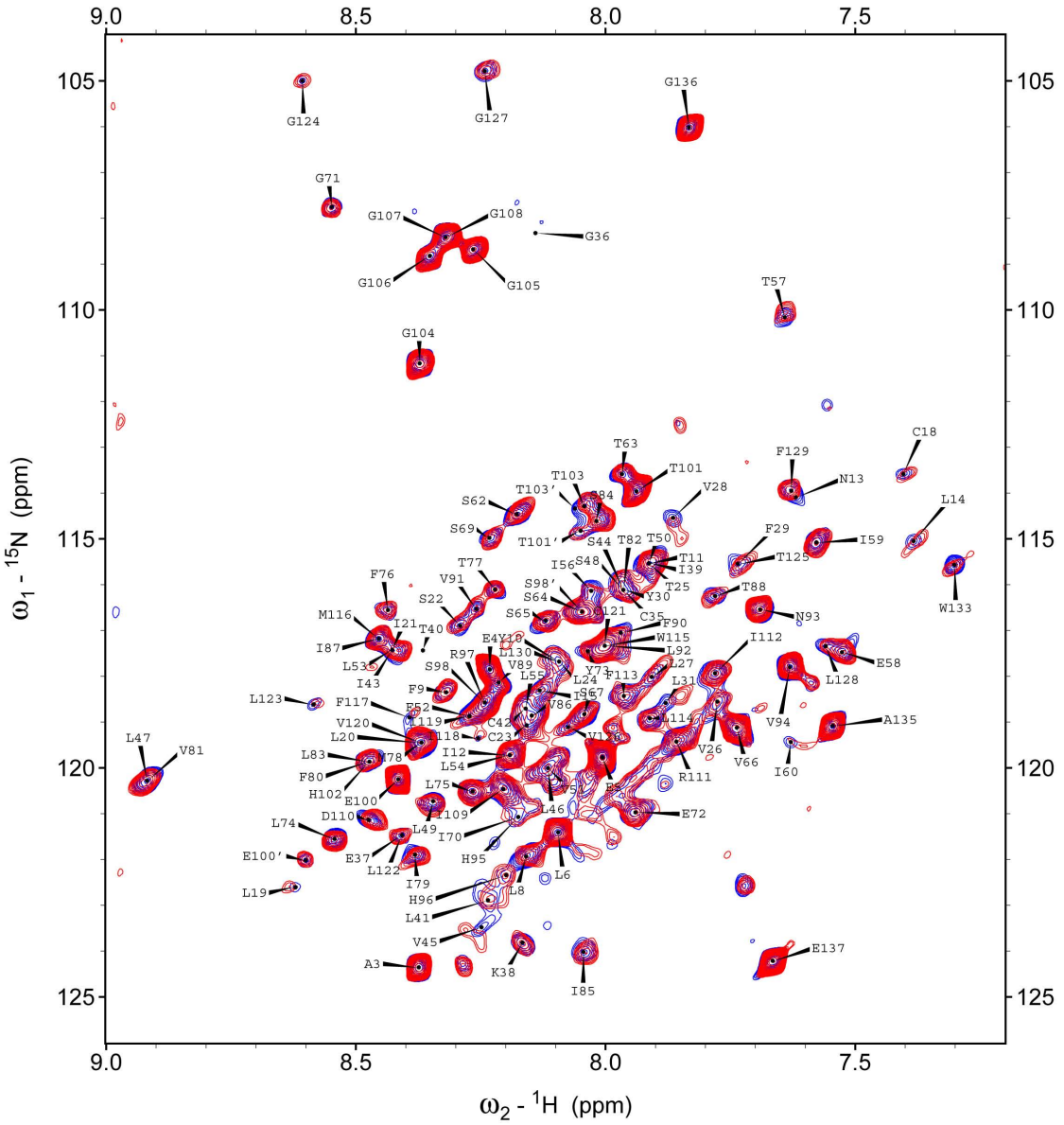

Fig. S1. Overlay of ^{1}H-^{15}N TROSY-HSQC spectra of α4(β2) (only α4 is ^{15}N-labeled and visible) in the absence (blue) and the presence (red) of 2 mM halothane. One-letter amino acid code followed by the sequence number was used for the chemical shift assignment. To convert the numbering in the spectra to the numbering for the full-length α4 nAChR, one needs to add 208 for residues labeled 1 to 105 and 461 for residues labeled 106 to 137.

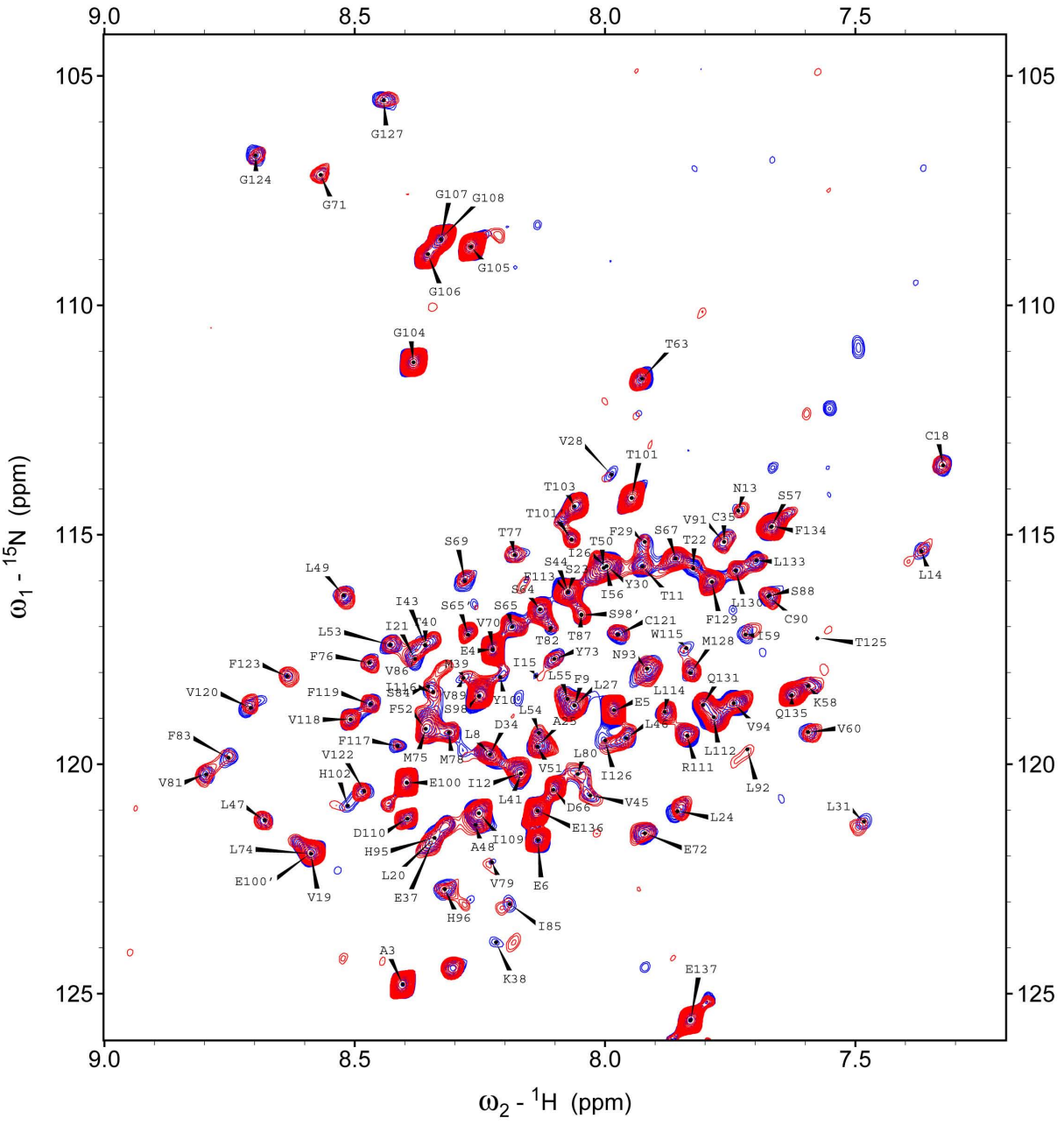

Fig. S2. Overlay of ^{1}H-^{15}N TROSY-HSQC spectra of β2(α4) (only β2 is ^{15}N-labeled and visible) in the absence (blue) and the presence (red) of 2 mM halothane. One-letter amino acid code followed by the sequence number was used for the chemical shift assignment. To convert the numbering in the spectra to the numbering for the full-length β2 nAChR, one needs to add 202 for residues labeled 1 to 105 and 324 for residues labeled 106 to 137.

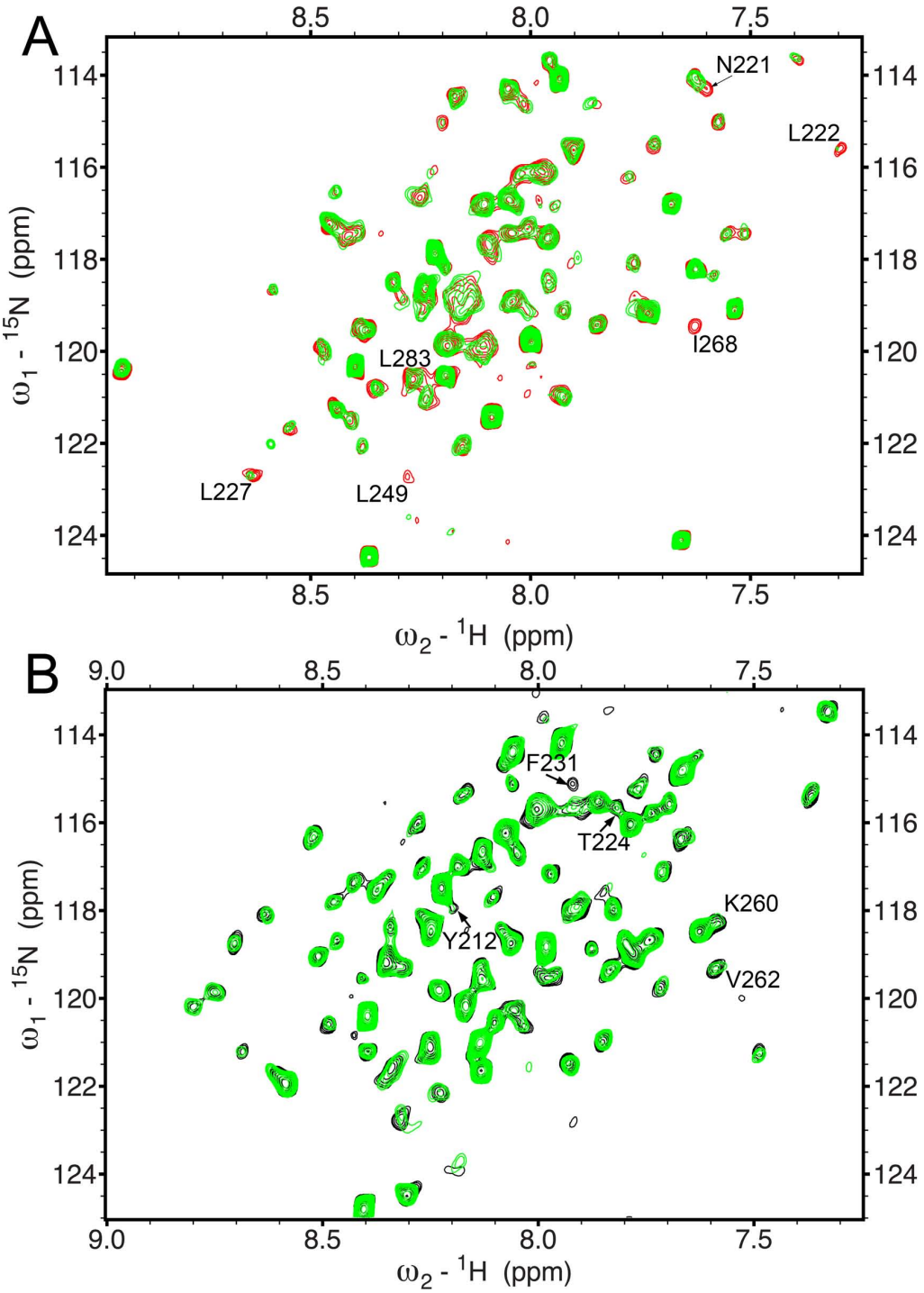

Fig. S3. The 2D saturation transfer experiment showed specific interactions between halothane and residues of α4β2. (**A**) Overlay of the α4(β2) spectra with (green) and without (red) saturation of the proton resonance of halothane (2 mM). (**B**) Overlay of the β2(α4) spectra with (green) and without (black) saturation of the proton resonance of halothane (2 mM). The labeled resonance peaks showed significant intensity decrease when the proton resonance of halothane was saturated.

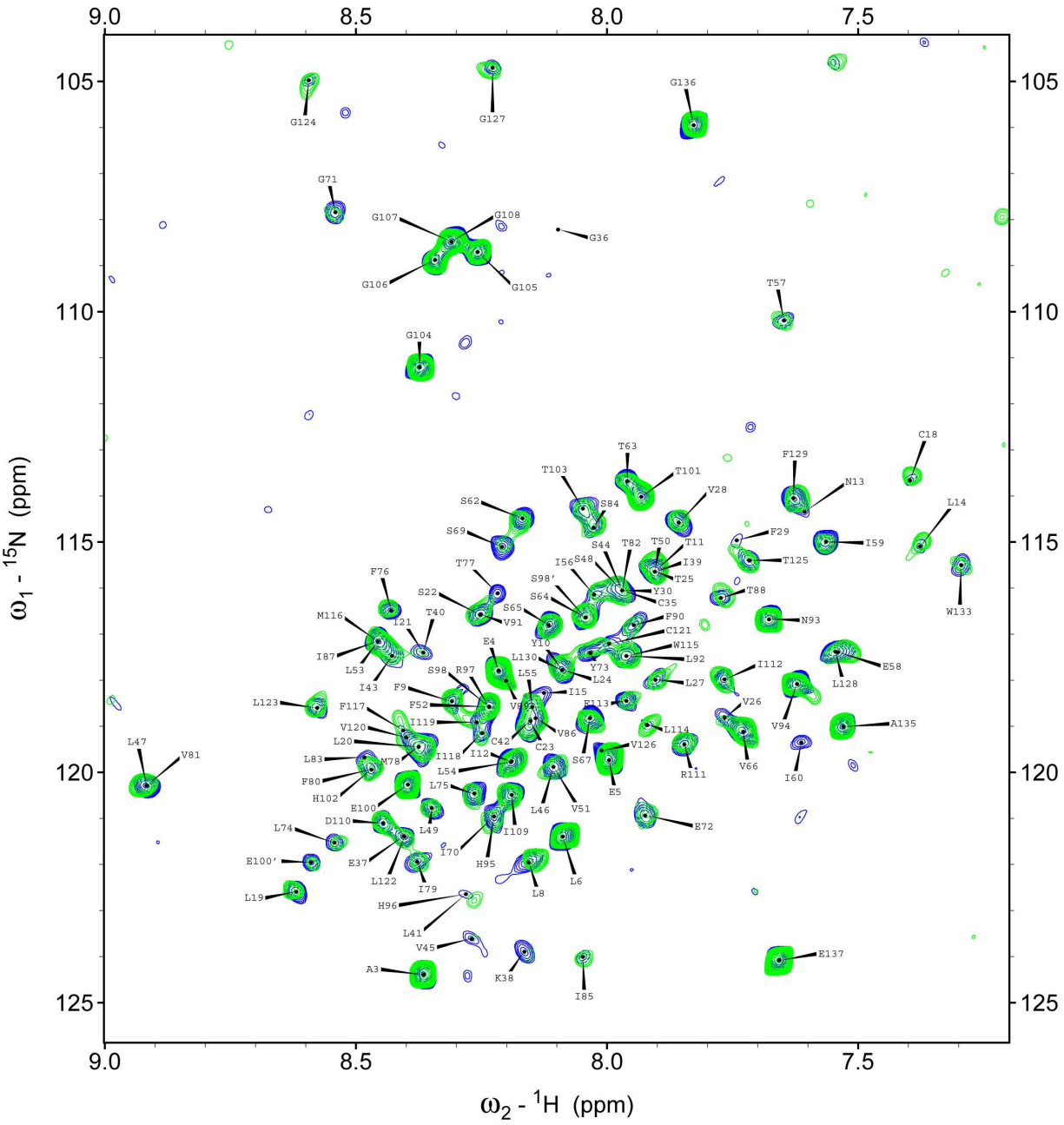

Fig. S4. Overlay of ^{1}H-^{15}N TROSY-HSQC spectra of α4(β2) (only α4 is ^{15}N-labeled and visible) in the absence (blue) and the presence (green) of 80 μM ketamine. One-letter amino acid code followed by the sequence number was used for the chemical shift assignment. To convert the numbering in the spectra to the numbering for the full-length α4 nAChR, one needs to add 208 for residues labeled 1 to 105 and 461 for residues labeled 106 to 137.

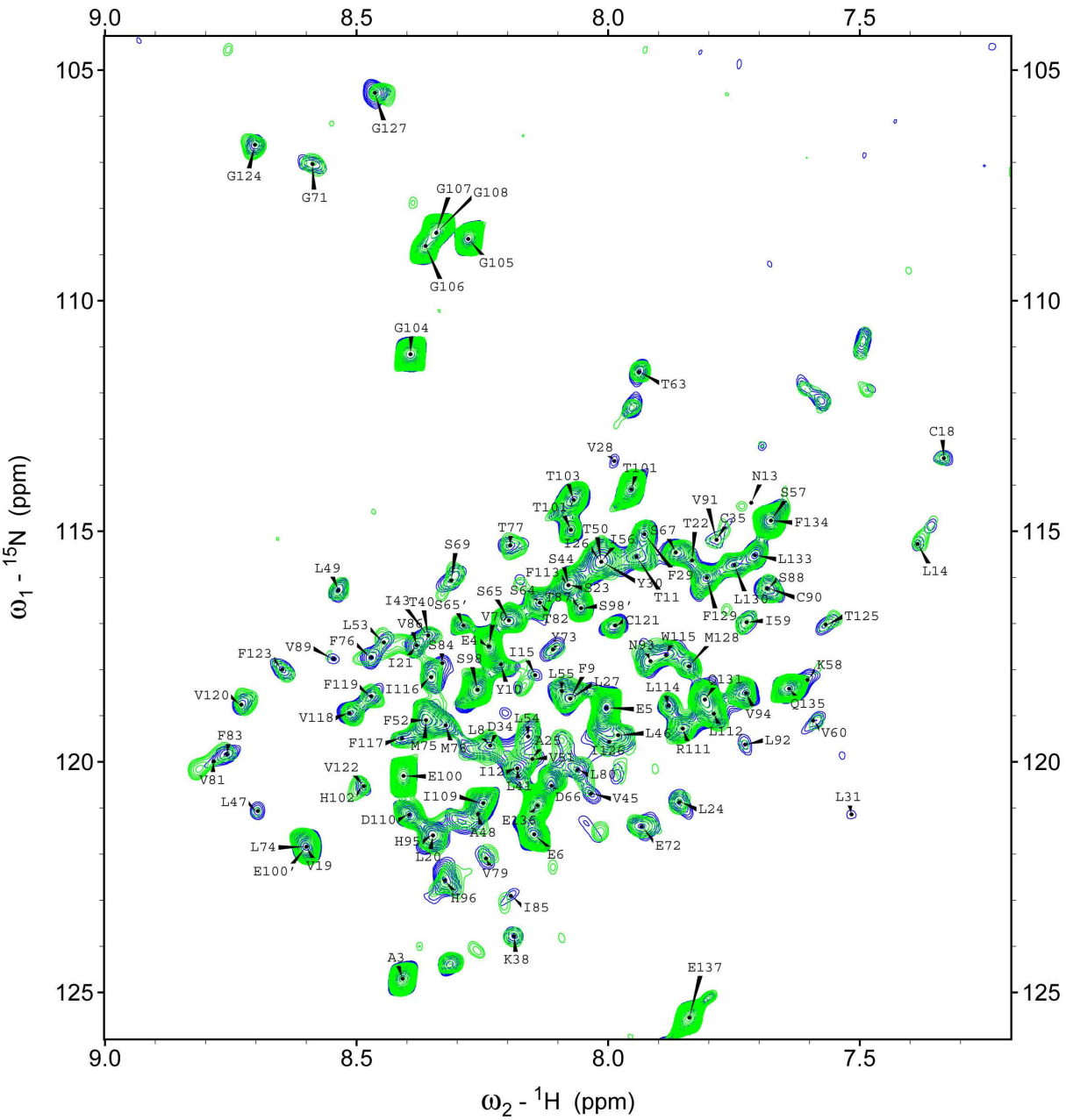

Fig. S5. Overlay of ^{1}H-^{15}N TROSY-HSQC spectra of β2(α4) (only β2 is ^{15}N-labeled and visible) in the absence (blue) and the presence (green) of 80 μM ketamine. One-letter amino acid code followed by the sequence number was used for the chemical shift assignment. To convert the numbering in the spectra to the numbering for the full-length β2 nAChR, one needs to add 202 for residues labeled 1 to 105 and 324 for residues labeled 106 to 137.

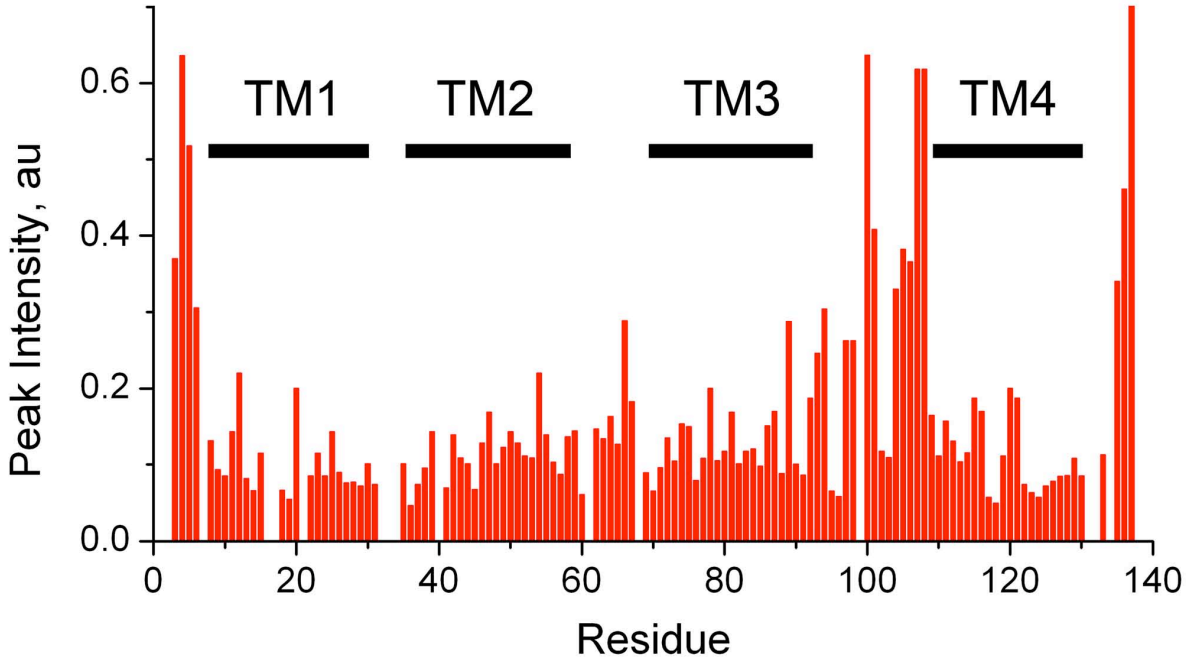

Fig. S6. Distribution of ^{1}H-^{15}N HSQC cross-peak signal intensities of residues in α4(β2). The locations of secondary structures are indicated on top. Note that high intensities in N- and C-termini and exposed loops indicate fast motions (ps-ns), whereas low intensities or missing signals in the helical segments indicate possible slow motions (μs-ms).

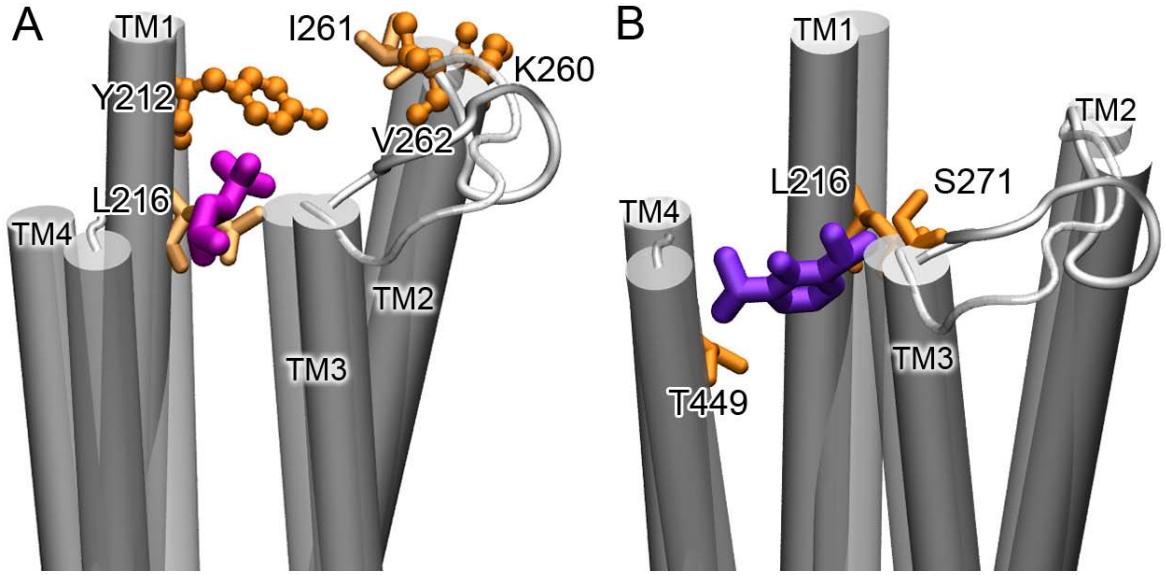

Fig. S7. Comparisons of the intra-subunit anesthetic binding sites within the β2 subunit (silver) with the crystal structures anesthetic-bound GLIC (white, transparent). (**A**) The NMR determined residues showing halothane cross-saturation (orange ball and stick representation) as well as changes in chemical shift (orange sticks) are in remarkable agreement with the X-ray determined binding position for desflurane (magenta). (**B**) Likewise, residues showing changes in chemical shift in response to ketamine binding agree well with the binding position of propofol (purple) in GLIC.