

**Fig. S1.** Alignment of α7-AChR and β-GluCl sequences. The top sequence corresponds to the α7-AChR from chicken, and the bottom one corresponds to β-GluCl from *C. elegans*. The sequence of the α7-AChR–β-GluCl ECD–TMD chimera is indicated with a green background, and that of the reverse construct is indicated with a dark-yellow background; identical residues are indicated with a superimposed gray background. Residues that, on the basis of atomic models of other pLGlCs, are expected to be at the ECD–TMD interface are indicated in bold. The alignment was generated in ClustalW using 15 sequences that (in addition to those of the chicken α7-AChR and *C. elegans* β-GluCl) also included *C. elegans* α1-GluCl; mouse α1-, β1-, δ-, and ε-AChR; mouse 5-HT<sub>3A</sub>R; rat α1-, β1-, and γ2-γ-aminobutyric-acid type-A receptor; rat α1- and β-GlyR; and the bacterial GLIC and ELIC. Disregarding the highly variable M3–M4 linker, the sequences of chicken α7-AChR and *C. elegans* β-GluCl are 17.7 % identical (28.6 % similar). For comparison, the mouse-muscle AChR (the average of the four adult-type subunits) and the bacterial pLGIC GLIC are 19 % identical (33 % similar); the mouse-muscle AChR and the—also bacterial—ELIC are 16.9 % identical (30.4 % similar); and GLIC and ELIC are 20.5 % identical (35.8 % similar). For all pairwise sequence comparisons, the identity and similarity are higher when calculated only for the structural elements at the ECD–TMD interface.



**Fig. S2.** Mutations in M2 speed up desensitization of the  $\alpha$ 7-AChR- $\beta$ -GluCl CS chimera. Much as they do in the background of the PPSV -> AAAA mutant, the M2 mutations G4'C and T12'S accelerated the time course of entry into desensitization of the CS construct. Normalized inward currents recorded in the whole-cell configuration under asymmetrical KCl-concentration conditions in response to the application of 1-min pulses of 100- $\mu$ M ACh. The membrane potential was ~-60 mV. Black dashed lines denote the zero-current baseline. Two representative responses from each mutant are shown. Each displayed response was recorded from a different cell. For comparison, the averaged response (mean ± 1SD) of the CS chimera (without any additional mutation) to 1-min pulses of 100- $\mu$ M ACh is also shown, as in Fig. 2D (mean: black solid line; SD: gray error bars).



**Fig. S3.** The gating kinetics of the  $\alpha$ 7-AChR- $\beta$ -GluCl CS chimera are similar to those of the rat  $\alpha$ 1-GlyR. Normalized inward currents recorded from the rat  $\alpha$ 1-GlyR in the whole-cell configuration under asymmetrical KCl-concentration conditions in response to the application of 20-ms or 1-min pulses of 1-mM glycine. The membrane potential was  $^{\sim}$ -60 mV. Black dashed lines denote the zero-current baseline. (*A*, *B*) Three representative responses to 20-ms pulses, and four to 1-min pulses, are shown. Each displayed response was recorded from a different cell. For comparison, the averaged responses (mean ± 1SD) of the CS chimera (without any additional mutation) to 20-ms or 1-min pulses of 100- $\mu$ M ACh are also shown , as in Fig. 2 *C* and *D* (mean: black solid line; SD: gray error bars).



**Fig. S4.** Probing the effect of mutations to the C-terminal tail on gating. Normalized inward currents recorded from the indicated CS-chimera mutants in the whole-cell configuration under asymmetrical KCl-concentration conditions in response to the application of 20-ms or 1-min pulses of 100- $\mu$ M ACh. The membrane potential was ~-60 mV. Black dashed lines denote the zero-current baseline. (*A*, *B*) One representative response from each mutant is shown. For comparison, the averaged responses (mean ± 1SD) of the CS chimera (without any additional mutation) to 20-ms or 1-min pulses of 100- $\mu$ M ACh are also shown , as in Fig. 2 *C* and *D* (mean: black solid line; SD: gray error bars).

Loop 2 D <sup>ME</sup> KNQV         D66A + E67A + N69A + Q70A <u>A<sup>MSA</sup>KAAV</u> Yes         0.011 + 0.0068 (4)           D <sup>ME</sup> KNQV         β-GluC1 loop 2         D <sup>MSA</sup> VMME         Yes         1.3 ± 0.078 (4)           W156A         C <sup>LSM</sup> /IDVRMPFDVQXC         Yes         0.63 ± 0.11 (2)           P158A         C <sup>LSM</sup> /IDVRMP_GPVQXC         No         0.035 ± 0.11 (2)           P158A         C <sup>LSM</sup> /IDVRMP_GPVQXC         No         0.00052 ± 0.00075 (4)           P158G         C <sup>LSM</sup> /IDVRMPF_GPVQXC         No         0.0012 ± 0.0038 (4)           D160A + Q162A         C <sup>LSM</sup> /IDVRMPF_GPVQXC         Yes         0.047 ± 0.012 (4)           D160A + Q162A         C <sup>LSM</sup> /IDVRMPF_GPVQXC         Yes         0.047 ± 0.012 (4)           D160A + Q162A         C <sup>LSM</sup> /IDVRMPF_GPVQXC         Yes         0.047 ± 0.012 (4)           D160A + Q162A         C <sup>LSM</sup> /IDVRMFFGPVQXC         Yes         0.047 ± 0.012 (4)           G1694         S <sup>LSM</sup> /IDVRMFFGPVQXC         Yes         0.054 ± 0.002 (4)           JEOGP 9         E195A         S <sup>LSM</sup> /LDVRMFGPVQXC         Yes         0.15 ± 0.010 (4)           S <sup>LSM</sup> /LDVRMFFGPVQXC         Yes         0.024 ± 0.0025 (4)         Yes         0.026 ± 0.0021 (4)           JEOGP 9         E195A         S <sup>LSM</sup> /LDVRMFFGPVQXC         Yes </th <th>Structural element/ wild-type sequence</th> <th>Mutation(s)</th> <th>Mutated sequence<sup>*</sup></th> <th>Function<sup>†</sup></th> <th>Cell-surface expression mutant-to-CS ratio (mean ± SE) (replicates)</th>	Structural element/ wild-type sequence	Mutation(s)	Mutated sequence <sup>*</sup>	Function <sup>†</sup>	Cell-surface expression mutant-to-CS ratio (mean ± SE) (replicates)
D <sup>65</sup> EKNQV         β-GluCl loop 2         D <sup>66</sup> UVMME         Yes         1.3 ± 0.078 (4)           V156A         C <sup>150</sup> YIDVR.4PPTDVQKC         Yes         0.69 ± 0.057 (4)           F157A         C <sup>150</sup> YIDVRW.4PPTDVQKC         No         0.35 ± 0.11 (12)           P158Q         C <sup>150</sup> YIDVRW.4PPTDVQKC         No         0.005 ± 0.0075 (4)           P158Q         C <sup>150</sup> YIDVRW.4PPTDVQKC         No         0.005 ± 0.00075 (4)           Q162A         C <sup>150</sup> YIDVRW.4PFDVQKC         No         0.001 ± 0.0038 (4)           Q162A         C <sup>150</sup> YIDVRW.4PFDVQKC         Yes         0.047 ± 0.022 (4)           Q162A         C <sup>150</sup> YIDVRW.4PFDVQKC         Yes         0.047 ± 0.022 (4)           Q162A         C <sup>150</sup> YIDVRW.4PFDVQKC         Yes         0.047 ± 0.022 (4)           Q162A         C <sup>150</sup> YIDVRW.4PFDVQKC         Yes         0.15 ± 0.010 (4)           β-GluCI (75-loop         C <sup>150</sup> MIQULQIYPIDYQSC         Yes         0.15 ± 0.010 (4)           β-GluCI (75-loop         S <sup>150</sup> NG&MDL         Yes         0.32 ± 0.0081 (4)           Q154A         S <sup>150</sup> NG&MDL         Yes         0.32 ± 0.0021 (4)           G194A         S <sup>150</sup> NG&MDL         Yes         0.017 ± 0.012 (4)           G194P ± 1515N ± W196F         S <sup>150</sup> NG&MDL         Yes	Loop 2 D <sup>66</sup> EKNQV	D66A + E67A + N69A + Q70A	<u>A<sup>66</sup>AKAA</u> V	Yes	0.011 ± 0.0068 (4)
Loop 7		β-GluCl loop 2	D <sup>66</sup> VVNME	Yes	1.3 ± 0.078 (4)
Loop 7         F157A         C <sup>150</sup> /1D/RWAPFDVQKC         No         0.35 ± 0.11 (12)           P158A         C <sup>150</sup> /1D/RWFQFDVQKC         No         Undetectable (4)           P158Q         C <sup>150</sup> /1D/RWFQFDVQKC         No         0.0052 ± 0.00075 (4)           P158Q         C <sup>150</sup> /1D/RWFQFDVQKC         No         0.0012 ± 0.00075 (4)           Q150A         C <sup>150</sup> /1D/RWFPGDVQKC         No         0.010 ± 0.0038 (4)           Q162A         C <sup>150</sup> /1D/RWFPGDVQKC         Yes         0.047 ± 0.012 (4)           Q162A         C <sup>150</sup> /1D/RWFPGDVQKC         No         0.0096 ± 0.0041 (4)           β-GluCl Cys-loop         C <sup>159</sup> /1D/RWFPFAVGKC         No         0.0096 ± 0.0041 (4)           β-GluCl Cys-loop         C <sup>159</sup> /1D/RWFPFAVGKC         No         0.0095 ± 0.001 (4)           β-GluCl Cys-loop         C <sup>159</sup> /1D/RWFPFAVGKC         No         0.0025 ± 0.001 (4)           G194A         S <sup>159</sup> /NGEWDL         Yes         0.33 ± 0.0088 (4)           S <sup>152</sup> NGEWDL         G194P + E195N + W196F         S <sup>152</sup> /NGEWDA         Yes         0.017 ± 0.010 (4)           G194P + E195N + W196F         S <sup>152</sup> /NGEWDA         Yes         0.018 ± 0.0022 (4)         1198A         S <sup>152</sup> /NGEWDA         Yes         0.018 ± 0.0026 (4)           Pre-M1         Iffer	<b>Loop 7</b> C <sup>150</sup> YIDVRWFPFDVQKC	W156A	C <sup>150</sup> YIDVR <u>A</u> FPFDVQKC	Yes	0.69 ± 0.057 (4)
Loop 7 C <sup>150</sup> YIDVRWFPFDVQKC         No         Undetectable (4)           P158Q         C <sup>150</sup> YIDVRWFQFDVQKC         No         0.0052 ± 0.00075 (4)           C <sup>150</sup> YIDVRWFPFDVQKC         P158G         C <sup>150</sup> YIDVRWFPFDVQKC         Yes         0.010 ± 0.0038 (4)           D160A         C <sup>150</sup> YIDVRWFPFDVQKC         Yes         0.047 ± 0.012 (4)         0.0062 ± 0.00075 (4)           D160A         C <sup>150</sup> YIDVRWFPFDVQKC         Yes         0.047 ± 0.012 (4)         0.0066 ± 0.0041 (4)           D160A + 0.162A         C <sup>150</sup> YIDVRWFPFDVQKC         Yes         0.15 ± 0.010 (4)         0.0096 ± 0.0041 (4)           B1000 9         S <sup>151</sup> DCGVS-loop         C <sup>150</sup> YIDVRWFPDVQKC         Yes         0.54 ± 0.019 (4)           G194A         S <sup>151</sup> NQEWDL         Yes         0.33 ± 0.0088 (4)         10.0025 ± 0.0017 (4)           G194P + E195N + W196F         S <sup>151</sup> NQEWDA         Yes         0.033 ± 0.0046 (4)           R <sup>217</sup> RG         R227A + R229A         A <sup>223</sup> RG         Yes         0.013 ± 0.0029 (4)           Pre-M1 linker         R <sup>227</sup> RG         No         0.0023 ± 0.00019 (4)         Arg insertion         R <sup>223</sup> RG         Yes         0.013 ± 0.0029 (4)         Yes         0.12 ± 0.033 (4)         Yes         0.12 ± 0.033 (4)         Yes         0.12 ± 0.033 (4)         Yes         0.0023 ±		F157A	C <sup>150</sup> YIDVRW <u>A</u> PFDVQKC	No	0.35 ± 0.11 (12)
Loop 7 C <sup>150</sup> YIDVRWFPFDVQKC         P158Q         C <sup>150</sup> YIDVRWFQFDVQKC         No         0.0052 ± 0.00075 (4)           C <sup>150</sup> YIDVRWFPFDVQKC         P158G         C <sup>150</sup> YIDVRWFQFDVQKC         No         0.010 ± 0.0038 (4)           D160A         C <sup>150</sup> YIDVRWFPFAVQKC         Yes         0.047 ± 0.012 (4)           Q162A         C <sup>150</sup> YIDVRWFPFAVQKC         Yes         0.047 ± 0.012 (4)           D160A + Q162A         C <sup>150</sup> YIDVRWFPFAVQKC         Yes         0.15 ± 0.010 (4)           β-GIUCI (ys-loop         C <sup>150</sup> YIDVRWFPFAVQKC         No         0.0096 ± 0.0041 (4)           β-GIUCI (ys-loop         C <sup>150</sup> YIDVRWFPFAVQKC         Yes         0.54 ± 0.019 (4)           G194A         S <sup>152</sup> JQGEWDL         Yes         0.54 ± 0.019 (4)           G194A         S <sup>152</sup> NGEWDL         Yes         0.33 ± 0.0088 (4)           V1956A         S <sup>152</sup> NGEWDL         No         0.0025 ± 0.0017 (4)           G194P + E195N + W196F         S <sup>152</sup> NGEWDA         Yes         0.033 ± 0.0064 (4)           R227A + R229A         A <sup>223</sup> NA         No         0.0023 ± 0.0013 (4)           R227A + R229A         A <sup>223</sup> NA         No         0.0023 ± 0.0013 (4)           Pre-M1 linker         β-GIUCI pre-M1         K <sup>223</sup> NA         No         0.054 ± 0.0024 (4)		P158A	C <sup>150</sup> YIDVRWF <u>A</u> FDVQKC	No	Undetectable (4)
Loop J         P158G         C130YIDVRWFGFDVQKC         No         0.010 ± 0.0038 (4)           C150YIDVRWFFPDVQKC         D160A         C120YIDVRWFFFQVQKC         Yes         0.071 ± 0.012 (4)           Q162A         C120YIDVRWFFFQVQKC         Yes         0.71 ± 0.062 (4)         D160A + 0162A         C150YIDVRWFFQVQKC         Yes         0.71 ± 0.062 (4)           D160A + 0162A         C150YIDVRWFFPQVQKC         No         0.0096 ± 0.00041 (4)         G194A         S192NGEWDL         Yes         0.54 ± 0.019 (4)           Loop 9         E195A         S192NGEWDL         Yes         0.33 ± 0.0088 (4)         W196A           S192NGEWDL         W196A         S192NGEADL         Yes         0.33 ± 0.0025 ± 0.0017 (4)           G194P + E195N + W196F         S192NGEWDA         No         0.0025 ± 0.0017 (4)           G194P + E195N + W196F         S192NGEWDA         Yes         0.033 ± 0.0026 (4)           L198A         S192NGEWDA         Yes         0.017 ± 0.010 (4)           R227A R R229A         A227RA         No         0.0023 ± 0.00019 (4)           M2-M3 linker         β-GluCl pre-M1         K227RQ         Yes         0.17 ± 0.010 (4)           R2270 R         R227A + R229A         R227AR         No         0.0023 ± 0.00031 (4)		P158Q	C <sup>150</sup> YIDVRWF <u>Q</u> FDVQKC	No	0.0052 ± 0.00075 (4)
C HOHMH PEQUAL         D160A         C <sup>150</sup> YIDVRWFPF2AVQKC         Yes         0.047 ± 0.012 (4)           Q162A         C <sup>150</sup> YIDVRWFPF2AVQKC         Yes         0.71 ± 0.052 (4)           D160A + Q162A         C <sup>150</sup> YIDVRWFPF2AVQKC         No         0.0096 ± 0.0041 (4)           β-GILC (2y-loop         C <sup>150</sup> YIDVRWFPF2AVQKC         No         0.0096 ± 0.0041 (4)           β-GILC (2y-loop         C <sup>150</sup> PMCUPLDYQSC         Yes         0.15 ± 0.010 (4)           G194A         S <sup>192</sup> NGEWDL         Yes         0.54 ± 0.019 (4)           G194A         S <sup>192</sup> NGAWDL         Yes         0.33 ± 0.0088 (4)           S <sup>192</sup> NGEWDL         W196A         S <sup>192</sup> NGEMDL         No         0.0025 ± 0.0017 (4)           G194P + E195N + W196F         S <sup>192</sup> NGEWDL         No         0.0025 ± 0.0017 (4)         10.0026 (4)           L198A         S <sup>192</sup> NGEWDA         Yes         0.018 ± 0.0029 (4)         11.98         12.92 NGEWDA         Yes         0.018 ± 0.0029 (4)           R <sup>227</sup> RR         R227A + R229A         A <sup>227</sup> RQ         Yes         0.018 ± 0.0029 (4)         14.92 NGEWDA         Yes         0.023 ± 0.00019 (4)           R <sup>237</sup> RR         S <sup>192</sup> SYY         β-GILC IPr-M1         K <sup>227</sup> RQ         Yes         0.78 ± 0.055 (4)         14.92 NGEWDA         Y232A + Y233		P158G	C <sup>150</sup> YIDVRWF <u>G</u> FDVQKC	No	0.010 ± 0.0038 (4)
Q162A         C150YIDVRWFPFDVAKC         Yes         0.71 ± 0.062 (4)           D160A + Q162A         C150YIDVRWFPFAVAKC         No         0.0096 ± 0.0041 (4)           β-GluCl Cys-loop         C150PMRIQLYPLOYQSC         Yes         0.15 ± 0.010 (4)           A         S1972AGEWDL         Yes         0.54 ± 0.019 (4)           G194A         S197AGEWDL         Yes         0.54 ± 0.019 (4)           B         E195A         S197NGEWDL         Yes         0.33 ± 0.0088 (4)           B         E195A         S197NGEADL         No         0.0025 ± 0.0017 (4)           G194P + E195N + W196F         S192NGEWDA         Yes         0.038 ± 0.0026 (4)           L198A         S192NGEWDA         Yes         0.018 ± 0.0029 (4)           R227A + R229A         A227RA         Yes         0.018 ± 0.0029 (4)           R277R R         β-GluCl pre-M1         K227RQ         Yes         0.017 ± 0.010 (4)           R2273R R         Y232A + Y233A         P230SAA         Yes         0.78 ± 0.055 (4)           P285A + P286A + S288A + V290A         L284AVAYAK         Yes         0.78 ± 0.055 (4)           P285A + P286A + S288A + V290A         L284AVAYAK         Yes         0.53 ± 0.056 (3)           P285A + P286A + S288A + V290A         L284AV		D160A	C <sup>150</sup> YIDVRWFPF <u>A</u> VQKC	Yes	0.047 ± 0.012 (4)
$ \frac{10160A + Q162A}{P_{1}} = \frac{C_{150}^{150} VIDVRWFPF_{A}VAC}{P_{150}^{150} VID_{10}^{10} VID_{10}$		Q162A	C <sup>150</sup> YIDVRWFPFDV <u>A</u> KC	Yes	0.71 ± 0.062 (4)
$ \beta \cdot GluCl Cys \cdot loop \\ I = 0 + GluCl Cys \cdot $		D160A + Q162A	C <sup>150</sup> YIDVRWFPF <u>A</u> V <u>A</u> KC	No	0.0096 ± 0.0041 (4)
Loop 9         N193A         S <sup>132</sup> ΔGEWDL         Yes         0.54 ± 0.019 (4)           G194A         S <sup>192</sup> NΔEWDL         Yes         1.2 ± 0.15 (4)           S <sup>132</sup> NGEWDL         W195A         S <sup>192</sup> NGAWDL         Yes         0.33 ± 0.0088 (4)           W196A         S <sup>192</sup> NGAWDL         Yes         0.33 ± 0.0088 (4)           G194P ± E195N + W196F         S <sup>192</sup> NGEDL         No         0.0025 ± 0.0017 (4)           G194P ± E195N + W196F         S <sup>192</sup> NGEWDA         Yes         0.053 ± 0.0046 (4)           R270A + R229A         A <sup>227</sup> NG         Yes         0.018 ± 0.0029 (4)           R277A + R229A         A <sup>227</sup> NG         Yes         0.018 ± 0.0029 (4)           Arg insertion         R <sup>227</sup> RQ         Yes         0.018 ± 0.0029 (4)           Arg insertion         R <sup>227</sup> AR         No         0.066 ± 0.0084 (4)           N-terminus of M1 F <sup>230</sup> SYY         Y232A + Y233A         F <sup>230</sup> SAA         Yes         0.78 ± 0.055 (4)           A <sup>230</sup> SYY         Y232A + Y233A         F <sup>230</sup> SAA         Yes         0.78 ± 0.058 (4)           M <sup>240</sup> SYVK         Yes         0.78 ± 0.058 (4)         280 + V290A + 6266 (2)         284 APVAYAK and M2 G4'C         Yes         0.99 ± 0.075 (4)           P285A + P286A + S288A + V290A + 6266 C         L <sup>284</sup> AAVAY		β-GluCl Cys-loop	C <sup>150</sup> PMRLQLYPLDYQSC	Yes	0.15 ± 0.010 (4)
	<b>Loop 9</b> S <sup>192</sup> NGEWDL	N193A	S <sup>192</sup> AGEWDL	Yes	0.54 ± 0.019 (4)
		G194A	S <sup>192</sup> N <u>A</u> EWDL	Yes	1.2 ± 0.15 (4)
$\begin{split} S^{192}\text{NGEWDL} & \frac{W196A}{G194P + E195N + W196F} & S^{192}\text{NGEADL} & No & 0.0025 \pm 0.0017 (4) \\ \hline G194P + E195N + W196F & S^{192}\text{NgENEDL} & No & 0.084 \pm 0.0026 (4) \\ \hline & L198A & S^{192}\text{NGEWDA} & Yes & 0.053 \pm 0.0046 (4) \\ \hline & L198A & S^{192}\text{NGEWDA} & Yes & 0.053 \pm 0.0046 (4) \\ \hline & R227A + R229A & A^{222}RA & Yes & 0.018 \pm 0.0029 (4) \\ \hline & & & & & & & & & & & & & & & & & &$		E195A	S <sup>192</sup> NG <u>A</u> WDL	Yes	0.33 ± 0.0088 (4)
$ \frac{G194P + E195N + W196F}{G194P + E195N + W196F} \frac{S^{192}NPFDL}{S^{192}NGEWDA} No 0.084 \pm 0.0026 (4) \\ \hline H198A S^{192}NGEWDA Yes 0.053 \pm 0.0046 (4) \\ \hline R227A + R229A A^{222}RA Yes 0.018 \pm 0.0029 (4) \\ \hline \beta - GluCl pre-M1 K^{222}RQ Yes 0.17 \pm 0.010 (4) \\ \hline R228A R^{227}AR No 0.0023 \pm 0.00019 (4) \\ \hline Arg insertion R^{227}RR No 0.0023 \pm 0.00019 (4) \\ \hline Arg insertion R^{227}RR No 0.66 \pm 0.0084 (4) \\ \hline N-terminus of M1 Y232A + Y233A F^{230}SAA Yes 0.78 \pm 0.055 (4) \\ \hline P285A + P286A + S288A + V290A L^{284}AAVAYAK Yes 0.53 \pm 0.056 (4) \\ \hline \alpha 7 - AChR M2 - M3 linker M^{284}PATSDSV No 1.5 \pm 0.10 (4) \\ \hline \alpha 7 - AChR M2 - M3 linker + D289K M^{284}PATSDSV No 2.0 \pm 0.083 (4) \\ \hline P285A + P286A + S288A + V290A L^{284}AVAYAK and M2 G4'C Yes 0.99 \pm 0.075 (4) \\ \hline P285A + P286A + S288A + V290A L^{284}AVAYAK and M2 G4'C Yes 0.99 \pm 0.075 (4) \\ \hline P285A + P286A + S288A + V290A L^{284}AAVAYAK and M2 G4'C Yes 0.14 \pm 0.014 (4) \\ + T274S L^{284}AVAYAK And M2 G4'C Yes 1.2 \pm 0.056 (3) \\ \hline P415A M^{408}SANASTPESLV Yes 1.0 \pm 0.10 (3) \\ \hline N411A P415A + E416A M^{408}SANASTAESLV Yes 1.0 \pm 0.10 (3) \\ \hline N411A + P415A + E416A M^{408}SANASTAESLV Yes 1.1 \pm 0.076 (3) \\ \hline N4113top M^{408}SA Yes 0.74 \pm 0.036 (3) \\ \hline \alpha 7 - AChR C-terminal tail P^{408}NFVEAVSDFA Yes 0.27 \pm 0.033 (3) \\ \hline \$		W196A	S <sup>192</sup> NGE <u>A</u> DL	No	0.0025 ± 0.0017 (4)
$\frac{1198A}{M^{408}SANASTPESLV} \left\{ \begin{array}{c c c c c } \hline L198A} & S^{192}NGEWD\underline{A}} & Yes & 0.053 \pm 0.0046 (4) \\ \hline R227A + R229A & \underline{A^{227}RA} & Yes & 0.018 \pm 0.0029 (4) \\ \hline \beta - GluCl pre-M1 & \underline{K^{227}RQ} & Yes & 0.17 \pm 0.010 (4) \\ \hline R228A & R^{227}\underline{AR} & No & 0.0023 \pm 0.00019 (4) \\ \hline Arg insertion & R^{227}RRB & No & 0.066 \pm 0.0084 (4) \\ \hline \textbf{N-terminus of M1} & Y232A + Y233A & F^{230}\underline{SAA} & Yes & 0.78 \pm 0.055 (4) \\ \hline \mu 285A + P286A + S288A + V290A & L^{284}\underline{AAV}\underline{AY}\underline{AK} & Yes & 0.53 \pm 0.056 (4) \\ \hline \alpha 7 - AChR M2 - M3 linker & \underline{M^{284}PATSDSV} & No & 1.5 \pm 0.10 (4) \\ \hline \mu 27 - AChR M2 - M3 linker + D289K & \underline{M^{284}PATSDSV} & No & 2.0 \pm 0.083 (4) \\ \hline \mu 285A + P286A + S288A + V290A & L^{284}\underline{AAV}\underline{AY}\underline{AK} and M2 G4'C & Yes & 0.99 \pm 0.075 (4) \\ \hline \mu 285A + P286A + S288A + V290A & L^{284}\underline{AAV}\underline{AY}\underline{AK} and M2 G4'C & Yes & 0.14 \pm 0.014 (4) \\ \hline \mu 1274S & M^{408}\underline{SANASTPESLV} & Yes & 1.0 \pm 0.010 (3) \\ \hline N411A + P415A & M^{408}\underline{SANASTP}\underline{SLV} & Yes & 1.0 \pm 0.010 (3) \\ \hline N4113top & M^{408}\underline{SA}A \\ \hline \mu 408\underline{SANASTPESLV} & Yes & 0.74 \pm 0.036 (3) \\ \hline \mu 7 - AChR C-terminal tail & \underline{P^{408}NFVEAVSDFA} & Yes & 0.74 \pm 0.036 (3) \\ \hline \mu 7 - AChR C-terminal tail & \underline{P^{408}NFVEAVSDFA} & Yes & 0.74 \pm 0.033 (3) \\ \hline \mu 408\underline{SANASTPESLV} & Ne1 \\ \hline \mu 408\underline{SANASTPESLV} & Yes & 0.74 \pm 0.036 (3) \\ \hline \mu 408\underline{SANASTPESLV} & Ne1 \\ \hline \mu 408\underline{SANASTPESLV} & Yes & 0.74 \pm 0.036 (3) \\ \hline \mu 408\underline{SANASTPESLV} & Ne1 \\ \hline \mu 408\underline{SANASTPESLV} & Yes & 0.74 \pm 0.036 (3) \\ \hline \mu 408\underline{SANASTPESLV} & Yes & 0.74 \pm 0.036 (3) \\ \hline \mu 408\underline{SANASTPESLV} & Yes & 0.74 \pm 0.036 (3) \\ \hline \mu 408\underline{SANASTPESLV} & Yes & 0.74 \pm 0.036 (3) \\ \hline \mu 408\underline{SANASTPESLV} & Yes & 0.74 \pm 0.036 (3) \\ \hline \mu 408\underline{SANASTPESLV} & Yes & 0.74 \pm 0.036 (3) \\ \hline \mu 408\underline{SANASTPESLV} & Yes & 0.74 \pm 0.036 (3) \\ \hline \mu 408\underline{SANASTPESLV} & Yes$		G194P + E195N + W196F	S <sup>192</sup> NPNFDL	No	0.084 ± 0.0026 (4)
$ \begin{array}{l l l l l l l l l l l l l l l l l l l $		L198A	S <sup>192</sup> NGEWD <u>A</u>	Yes	0.053 ± 0.0046 (4)
$ \begin{array}{l c c c c c c c c c c c c c c c c c c c$	<b>Pre-M1 linker</b> R <sup>227</sup> RR	R227A + R229A	<u>A<sup>227</sup>RA</u>	Yes	0.018 ± 0.0029 (4)
$ \frac{R^{227}RR}{R} = \frac{R228A}{R^{227}\underline{A}R} \\ No \\ 0.0023 \pm 0.00019 (4) \\ Arg insertion \\ R^{227}RR\underline{R} \\ No \\ 0.66 \pm 0.0084 (4) \\ P285A + P286A + S288A + V290A \\ \frac{\alpha^{7}-AChR M2-M3 linker}{M^{284}PATSDSV} \\ R^{7}-AChR M2-M3 linker + D289K \\ R^{7}-AChR M2-M3 linker \\ R^{7}-AChR + R^{7}-AChR \\ R^{7}$		β-GluCl pre-M1	<u>K<sup>227</sup>RQ</u>	Yes	0.17 ± 0.010 (4)
$\frac{Arg insertion}{R^{227}RR} \qquad No \qquad 0.66 \pm 0.0084 (4)$ $\frac{Arg insertion}{F^{230}SYY} \qquad Y232A + Y233A \qquad F^{230}SAA \qquad Yes \qquad 0.78 \pm 0.055 (4)$ $\frac{P285A + P286A + S288A + V290A}{\alpha^{7}-AChR M2-M3 linker} \qquad M^{284}PATSDSV \qquad No \qquad 1.5 \pm 0.10 (4)$ $\frac{\alpha^{7}-AChR M2-M3 linker + D289K}{P285A + P286A + S288A + V290A} \qquad L^{284}AAVAYAK \qquad Yes \qquad 0.53 \pm 0.056 (4)$ $\frac{\alpha^{7}-AChR M2-M3 linker + D289K}{P285A + P286A + S288A + V290A} \qquad L^{284}PATSDSV \qquad No \qquad 2.0 \pm 0.083 (4)$ $\frac{P285A + P286A + S288A + V290A}{+ G266C} \qquad L^{284}AAVAYAK and M2 G4'C \qquad Yes \qquad 0.99 \pm 0.075 (4)$ $\frac{P285A + P286A + S288A + V290A}{+ T274S} \qquad L^{284}AAVAYAK and M2 T12'S \qquad Yes \qquad 0.14 \pm 0.014 (4)$ $\frac{P415A}{P415A} \qquad M^{408}SAAASTPESLV \qquad Yes \qquad 1.0 \pm 0.10 (3)$ $\frac{N411A + P415A + E416A}{P415A + E416A} \qquad M^{408}SAAASTPASLV \qquad Yes \qquad 1.1 \pm 0.076 (3)$ $\frac{Arg insertion}{A^{7}-AChR C-terminal tail} \qquad P^{408}NFVEAVSKDFA}{P408} \qquad Yes \qquad 0.27 \pm 0.033 (3)$		R228A	R <sup>227</sup> <u>A</u> R	No	0.0023 ± 0.00019 (4)
$ \frac{\textbf{N-terminus of M1}}{F^{230}SYY}  Y232A + Y233A  F^{230}S\underline{AA}  Yes  0.78 \pm 0.055 (4) \\ \hline P285A + P286A + S288A + V290A  L^{284}\underline{AAV}\underline{AY}\underline{AK}  Yes  0.53 \pm 0.056 (4) \\ \hline \alpha7 - AChR M2 - M3 linker  M^{284}\underline{PATSDSV}  No  1.5 \pm 0.10 (4) \\ \hline \alpha7 - AChR M2 - M3 linker + D289K  M^{284}\underline{PATSKSV}  No  2.0 \pm 0.083 (4) \\ \hline P285A + P286A + S288A + V290A  L^{284}\underline{AAV}\underline{AY}\underline{AK} and M2 G4'C  Yes  0.99 \pm 0.075 (4) \\ \hline P285A + P286A + S288A + V290A  L^{284}\underline{AAV}\underline{AY}\underline{AK} and M2 T12'S  Yes  0.14 \pm 0.014 (4) \\ \hline P285A + P286A + S288A + V290A  L^{284}\underline{AAV}\underline{AY}\underline{AK} and M2 T12'S  Yes  0.14 \pm 0.014 (4) \\ \hline P285A + P286A + S288A + V290A  L^{284}\underline{AAV}\underline{AY}\underline{AK} and M2 T12'S  Yes  1.2 \pm 0.056 (3) \\ \hline P415A  M^{408}SANAST\underline{PESLV}  Yes  1.0 \pm 0.10 (3) \\ \hline M411A + P415A + E416A  M^{408}SANAST\underline{ASTP}\underline{SLV}  Yes  1.0 \pm 0.10 (3) \\ \hline M411A + P415A + E416A  M^{408}SA  Yes  0.74 \pm 0.036 (3) \\ \hline \alpha7 - AChR C-terminal tail  \underline{P^{408}NFVEAVSKDFA}  Yes  0.27 \pm 0.033 (3) \\ \hline \end{array}$		Arg insertion	R <sup>227</sup> RR <u>R</u>	No	0.66 ± 0.0084 (4)
$ \frac{P285A + P286A + S288A + V290A}{\alpha^{7}-AChR M2-M3 linker} \frac{M^{284}PATSDSV}{M^{284}PATSDSV} No 1.5 \pm 0.10 (4) \\ \frac{\alpha^{7}-AChR M2-M3 linker + D289K}{\alpha^{7}-AChR M2-M3 linker + D289K} \frac{M^{284}PATSKSV}{M^{284}PATSKSV} No 2.0 \pm 0.083 (4) \\ \frac{\alpha^{7}-AChR M2-M3 linker + D289K}{P285A + P286A + S288A + V290A} \frac{L^{284}AAVAYAK and M2 G4'C}{P285A + P286A + S288A + V290A} \frac{L^{284}AAVAYAK and M2 G4'C}{P285A + P286A + S288A + V290A} \frac{L^{284}AAVAYAK and M2 G4'C}{P285A + P286A + S288A + V290A} \frac{L^{284}AAVAYAK and M2 T12'S}{P285A + P286A + S288A + V290A} \frac{L^{284}AAVAYAK and M2 T12'S}{P285A + P286A + S288A + V290A} \frac{L^{284}AAVAYAK and M2 T12'S}{P285A + P286A + S288A + V290A} \frac{L^{284}AAVAYAK and M2 T12'S}{P415A} \frac{Ves}{P415A} \frac{1.2 \pm 0.056 (3)}{P415A} \frac{P408SANASTPESLV}{P408} \frac{Ves}{P415} \frac{1.0 \pm 0.10 (3)}{P415A} \frac{P408SANASTPASLV}{P408SANASTPASLV} \frac{Ves}{P415} \frac{1.0 \pm 0.10 (3)}{P415A} \frac{N408SAAASTAASTAASLV}{P408SAAASTAASLV} \frac{Ves}{P415} \frac{1.0 \pm 0.076 (3)}{P415A} \frac{N4118 + P415A + E416A}{P408SAAASTAASLV} \frac{Ves}{P415} \frac{0.74 \pm 0.036 (3)}{P415A} \frac{Ves}{P403} \frac{0.74 \pm 0.036 (3)}{P403SAA} \frac{Ves}{P415} \frac{0.74 \pm 0.036 (3)}{P415A} \frac{P408NFVEAVSKDFA}{P408SAA} \frac{Ves}{P415} \frac{0.74 \pm 0.033 (3)}{P403SA} \frac{Ves}{P415} \frac{0.72 \pm 0.033 (3)}{P403SA} \frac{Ves}{P415} \frac{Ves}{P415} \frac{0.72 \pm 0.033 (3)}{P403SA} \frac{Ves}{P415} \frac{0.72 \pm 0.033 (3)}{P403SA} \frac{Ves}{P415} \frac{Ves}{P415} \frac{Ves}{P415} \frac{Ves}{P415} \frac{Ves}{P415} \frac{Ves}{P415} \frac{Ves}{P415} \frac{Ves}{P403S} \frac{Ves}{P415} \frac{Ves}{P403S} \frac{Ves}{P415} \frac{Ves}{P403S} \frac{Ves}{P415} \frac{Ves}{P403S} \frac{Ves}{P415} \frac{Ves}{P415} \frac{Ves}{P403S} \frac{Ves}{P403S} \frac{Ves}{P403S} \frac{Ves}{P415} \frac{Ves}{P403S} \frac{Ves}{P415} \frac{Ves}{P403S} \frac{Ves}{P403S} \frac{Ves}{P403S} \frac{Ves}{$	N-terminus of M1 F <sup>230</sup> SYY	Y232A + Y233A	F <sup>230</sup> S <u>AA</u>	Yes	0.78 ± 0.055 (4)
	<b>M2–M3 linker</b> L <sup>284</sup> PPVSYVK	P285A + P286A + S288A + V290A	L <sup>284</sup> <u>AA</u> V <u>A</u> Y <u>A</u> K	Yes	0.53 ± 0.056 (4)
$ \begin{array}{c} \textbf{M2-M3 linker} \\ L^{284} PPVSYVK \\ \hline \end{tabular}{llet} \\ L^{284} PPVSYVK \\ \hline \end{tabular}{llet} \\ P285A + P286A + S288A + V290A \\ + G266C \\ \hline \end{tabular}{llet} \\ P285A + P286A + S288A + V290A \\ + G266C \\ \hline \end{tabular}{llet} \\ P285A + P286A + S288A + V290A \\ + T274S \\ \hline \end{tabular}{llet} \\ P285A + P286A + S288A + V290A \\ + T274S \\ \hline \end{tabular}{llet} \\ P285A + P286A + S288A + V290A \\ + T274S \\ \hline \end{tabular}{llet} \\ P285A + P286A + S288A + V290A \\ + T274S \\ \hline \end{tabular}{llet} \\ P285A + P286A + S288A + V290A \\ + T274S \\ \hline \end{tabular}{llet} \\ P285A + P286A + S288A + V290A \\ + T274S \\ \hline \end{tabular}{llet} \\ P285A + P286A + S288A + V290A \\ + T274S \\ \hline \end{tabular}{llet} \\ P408SANASTPESLV \\ \hline \end{tabular}{llet} \\ P4015A \\ \hline \e$		$\alpha$ 7-AChR M2–M3 linker	M <sup>284</sup> PATSDSV	No	1.5 ± 0.10 (4)
$ \begin{array}{c} L^{284} PPVSYVK & \begin{array}{c} P285A + P286A + S288A + V290A \\ + G266C & \begin{array}{c} L^{284}\underline{AAV}\underline{AY}\underline{AK} \text{ and } M2 \ G4'C & Yes & 0.99 \pm 0.075 \ (4) \\ \end{array} \\ \hline P285A + P286A + S288A + V290A \\ + T274S & \begin{array}{c} L^{284}\underline{AAV}\underline{AY}\underline{AK} \text{ and } M2 \ T12'S & Yes & 0.14 \pm 0.014 \ (4) \\ \end{array} \\ \hline P285A + P286A + S288A + V290A \\ + T274S & \begin{array}{c} M^{408}SA\underline{A}STPESLV & Yes & 1.2 \pm 0.056 \ (3) \\ \hline P415A & M^{408}SANAST\underline{A}ESLV & Yes & 1.4 \pm 0.13 \ (3) \\ \hline P415A & M^{408}SANAST\underline{A}ESLV & Yes & 1.0 \pm 0.10 \ (3) \\ \hline N411A + P415A + E416A & M^{408}SA\underline{A}AST\underline{A}SLV & Yes & 1.1 \pm 0.076 \ (3) \\ \hline N411stop & M^{408}SA & Yes & 0.74 \pm 0.036 \ (3) \\ \hline \alpha7 - AChR C - terminal tail \\ \hline P408NFVEAVSKDFA & Yes & 0.27 \pm 0.033 \ (3) \end{array} $		α7-AChR M2–M3 linker + D289K	<u>M<sup>284</sup>PATSKSV</u>	No	2.0 ± 0.083 (4)
$\frac{P285A + P286A + S288A + V290A}{+ T274S} L^{284}\underline{AAVAYAK} and M2 T12'S Yes 0.14 \pm 0.014 (4)$ + T274S Yes 0.14 \pm 0.014 (4) $\frac{N411A}{P415A} = \frac{M^{408}SAAASTPESLV}{M^{408}SAAASTPESLV} Yes 1.2 \pm 0.056 (3)$ + 1.4 \pm 0.13 (3) $\frac{P415A}{P415A} = \frac{M^{408}SANASTAESLV}{M^{408}SAAASTPASLV} Yes 1.0 \pm 0.10 (3)$ + 1.1 \pm 0.076 (3) + 1.1 \pm 0.036 (3) + 1.1 \pm 0.03		P285A + P286A + S288A + V290A + G266C	L <sup>284</sup> AAVAYAK and M2 G4'C	Yes	0.99 ± 0.075 (4)
$ \begin{array}{c c} \mbox{N411A} & \mbox{M408SAAASTPESLV} & \mbox{Yes} & 1.2 \pm 0.056 (3) \\ \hline \mbox{P415A} & \mbox{M408SANASTAESLV} & \mbox{Yes} & 1.4 \pm 0.13 (3) \\ \hline \mbox{P415A} & \mbox{M408SANASTPASLV} & \mbox{Yes} & 1.0 \pm 0.10 (3) \\ \hline \mbox{M408SANASTPESLV} & \mbox{N411A} + \mbox{P415A} + \mbox{E416A} & \mbox{M408SAAASTAASLV} & \mbox{Yes} & 1.1 \pm 0.076 (3) \\ \hline \mbox{N411A} + \mbox{P415A} + \mbox{E416A} & \mbox{M408SAAASTAASLV} & \mbox{Yes} & \mbox{1.1} \pm 0.076 (3) \\ \hline \mbox{N411A} + \mbox{P415A} + \mbox{E416A} & \mbox{M408SAA} & \mbox{Yes} & \mbox{0.74} \pm 0.036 (3) \\ \hline \mbox{N411stop} & \mbox{M408SA} & \mbox{Yes} & \mbox{0.74} \pm 0.036 (3) \\ \hline \mbox{\alpha7-AChR C-terminal tail} & \mbox{P408NFVEAVSKDFA} & \mbox{Yes} & \mbox{0.27} \pm 0.033 (3) \\ \hline \end{array} $		P285A + P286A + S288A + V290A + T274S	L <sup>284</sup> <u>AA</u> V <u>A</u> Y <u>A</u> K and M2 T12'S	Yes	0.14 ± 0.014 (4)
P415A         M <sup>408</sup> SANAST <u>A</u> ESLV         Yes         1.4 ± 0.13 (3)           C-terminal tail         E416A         M <sup>408</sup> SANASTP <u>A</u> SLV         Yes         1.0 ± 0.10 (3)           M <sup>408</sup> SANASTPESLV         N411A + P415A + E416A         M <sup>408</sup> SA <u>A</u> AST <u>A</u> SLV         Yes         1.1 ± 0.076 (3)           N411stop         M <sup>408</sup> SA         Yes         0.74 ± 0.036 (3) $\alpha$ 7-AChR C-terminal tail         P <sup>408</sup> NFVEAVSKDFA         Yes         0.27 ± 0.033 (3)	<b>C-terminal tail</b> M <sup>408</sup> SANASTPESLV	N411A	M <sup>408</sup> SA <u>A</u> ASTPESLV	Yes	1.2 ± 0.056 (3)
$ \begin{array}{c c} \textbf{C-terminal tail} \\ M^{408} \text{SANASTPESLV} & Fes \\ M^{408} \text{SANASTPESLV} & 1.0 \pm 0.10  (3) \\ \hline M^{408} \text{SANASTPESLV} & 1.1 \pm 0.076  (3) \\ \hline M^{408} \text{SANASTPESLV} & Yes \\ \hline M^{411} \text{stop} & M^{408} \text{SA} & Yes \\ \hline M^{408} \text{SA} & Yes \\ \hline M^{7-A} \text{ChR C-terminal tail} & \underline{P^{408} \text{NFVEAVSKDFA}} & Yes \\ \hline 0.27 \pm 0.033  (3) \\ \hline \end{array} $		P415A	M <sup>408</sup> SANAST <u>A</u> ESLV	Yes	1.4 ± 0.13 (3)
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		E416A	M <sup>408</sup> SANASTP <u>A</u> SLV	Yes	1.0 ± 0.10 (3)
N411stop         M <sup>408</sup> SA         Yes         0.74 ± 0.036 (3)           α7-AChR C-terminal tail         P <sup>408</sup> NFVEAVSKDFA         Yes         0.27 ± 0.033 (3)		N411A + P415A + E416A	M <sup>408</sup> SA <u>A</u> AST <u>AA</u> SLV	Yes	1.1 ± 0.076 (3)
$\alpha$ 7-AChR C-terminal tail P <sup>408</sup> NFVEAVSKDFA Yes 0.27 ± 0.033 (3)		N411stop	M <sup>408</sup> SA	Yes	0.74 ± 0.036 (3)
		$\alpha$ 7-AChR C-terminal tail	P <sup>408</sup> NFVEAVSKDFA	Yes	0.27 ± 0.033 (3)

## Table S1. Characterization of $\alpha$ 7-AChR- $\beta$ -GluCl CS-chimera mutations

\*Mutated residues are underlined.

<sup>+</sup>For some mutants, poor expression on the plasma membrane may underlie the failure to observe currents in response to ACh applications.