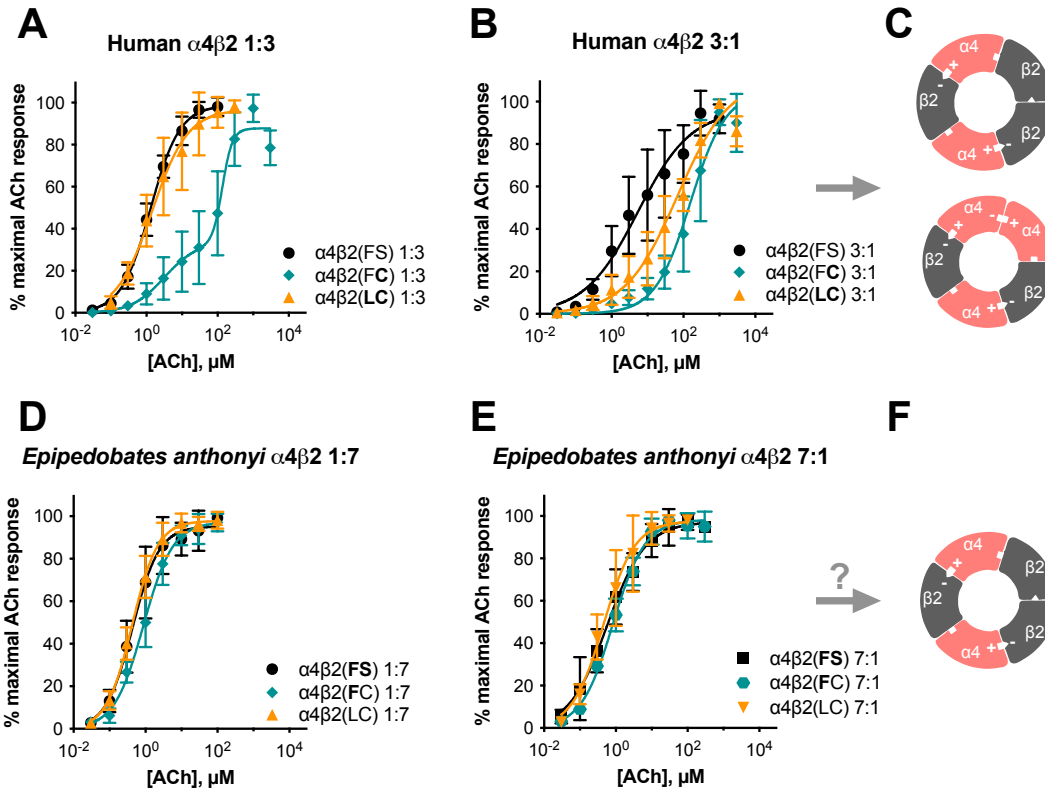




Additional file 2. Effects of reciprocal substitutions on ACh concentration-response curves (CRC) in the $\beta 2$ subunit of human and dendrobatid frog, *Epipedobates anthonyi*.

Data redrawn from Tarvin et al.¹, presented as mean $\pm$ SD. (A) A high ratio of $\beta 2$ to $\alpha 4$ ($1\alpha:3\beta$) of cRNA of the wild type human receptor subunits produces a monophasic CRC with a single EC_{50} indicating only high sensitivity (HS) binding sites (black curve: $\beta 2$ (FS) represents F106 and S108 in $\beta 2$ subunit; $n=7$). Introduction of the S108C substitution adds a low sensitivity (LS) binding site so that the CRC is now best fit with a biphasic curve reflecting both HS and LS sites (green curve: $\beta 2$ (FC), amino acid in bold indicates a substitution; $n=6$). Further addition of F106L to S108C eliminates the LS sites, thus compensating for the effect of S108C [orange curve: $\beta 2$ (LC); $n=6$]. (B) A low ratio of $\beta 2$ to $\alpha 4$ ($3\alpha:1\beta$) of the wild type human receptor subunits produces an ACh CRC shifted rightward and best fit with a monophasic curve with a shallow slope [black curve: $\beta 2$ (FS); $n=13$]. Introduction of the S108C substitution shifted the curve further right (green curve: $\beta 2$ (FC); $n=6$). Addition of F106L to S108C partially compensates for the effect of S108C alone [orange curve: $\beta 2$ (LC); $n=5$]. (C) When the ratio of injected human β subunit cRNA/ α subunit cRNA is high, the nAChR stoichiometry is $2\alpha:3\beta$. However, with paucity of β subunits the stoichiometry shifts to $3\alpha:2\beta$. (D,E) Even with more extreme ratios of α and β subunits (1:7 and 7:1) and the introduction of the ancestral amino acids (FS, FC), there was no change in the CRC of *Epipedobates anthonyi* ($n=5-13$). (F) The stoichiometry of frog nAChR receptors is unknown but we conjectured it is $2\alpha:3\beta$ because they show a single kind of binding site (HS). This conjecture is noted by the question mark over the grey arrow.

¹ Tarvin RD, Borghese CM, Sachs W, Santos JC, Lu Y, O'Connell LA, et al. Interacting amino acid replacements allow poison frogs to evolve epibatidine resistance. Science. 2017;357(6357):1261-6.