

**Additional File 4. F(DFn, DFd) and p values from one- and two population fittings to ACh concentration-response curves, calculated using the Extra sum-of-squares F test.**

| Species                   | cRNA ratio | Receptor               | Type of curve   | F(DFn, DFd)                | P value |
|---------------------------|------------|------------------------|-----------------|----------------------------|---------|
| <i>Xenopus tropicalis</i> | 1:3        | $\alpha 4\beta 2$ (FS) | One population  | 11.51 (2, 3)               | 0.04    |
|                           |            | $\alpha 4\beta 2$ (FC) | One population  | 8.062 (2, 3)               | 0.06    |
|                           |            | $\alpha 4\beta 2$ (LC) | One population  | The other fit is ambiguous |         |
|                           | 7:1        | $\alpha 4\beta 2$ (FS) | One population  | 5.998 (2, 3)               | 0.09    |
|                           |            | $\alpha 4\beta 2$ (FC) | One population  | The other fit is ambiguous |         |
|                           |            | $\alpha 4\beta 2$ (LC) | -               | -                          | -       |
| <i>Nanorana parkeri</i>   | 1:3        | $\alpha 4\beta 2$ (FS) | Two populations | The other fit is ambiguous |         |
|                           |            | $\alpha 4\beta 2$ (FC) | Two populations | The other fit is ambiguous |         |
|                           |            | $\alpha 4\beta 2$ (LC) | Two populations | The other fit is ambiguous |         |
|                           | 7:1        | $\alpha 4\beta 2$ (FS) | Two populations | The other fit is ambiguous |         |
|                           |            | $\alpha 4\beta 2$ (FC) | -               | -                          | -       |
|                           |            | $\alpha 4\beta 2$ (LC) | Two populations | The other fit is ambiguous |         |

A value of  $P < 0.10$  means the preferred model is the “Two populations” (biphasic curve);  $P > 0.10$  means the preferred model is the “One population” (monophasic curve). The amino acids between parentheses stand for the residues at locations 106 and 108, respectively; if there is a substitution, the letter is in bold font.

DFn: degree of freedom for the numerator of the F ratio

DFd: degree of freedom for the denominator of the F ratio