Supplementary Table 2:

|     | ΔF mean | SEM | N | p-value (vs. natives) | p-value (mutant vs. WT) | p-value (-G4 vs. +G4) |
|-----|---------|-----|---|----------------------|------------------------|-----------------------|
| native | 0.01    | 0.00 | 109 | >0.999               | >0.999                 | >0.999                |
| WT    | 0.28    | 0.03 | 136 | <0.001               | >0.999                 | <0.001                |
| WT+G4 | 1.00    | 0.04 | 142 | <0.001               | >0.999                 | <0.001                |
| S132Y | 0.21    | 0.03 | 25  | <0.001               | 0.468                  | <0.001                |
| S132Y+G4 | 0.66    | 0.08 | 24  | <0.001               | 0.007                  | <0.001                |
| L135I | 0.20    | 0.03 | 25  | <0.001               | 0.135                  | <0.001                |
| L135I+G4 | 0.77    | 0.12 | 23  | <0.001               | 0.729                  | <0.001                |
| F136L | 0.28    | 0.06 | 19  | 0.060                | >0.999                 | <0.001                |
| F136L+G4 | 0.65    | 0.11 | 20  | 0.015                | 0.007                  | <0.001                |
| E139K | 0.26    | 0.03 | 19  | <0.001               | >0.999                 | <0.001                |
| E139K+G4 | 1.00    | 0.13 | 19  | <0.001               | >0.999                 | <0.001                |
| E140M | 0.11    | 0.02 | 14  | 0.045                | <0.001                 | <0.001                |
| E140M+G4 | 1.00    | 0.12 | 14  | <0.001               | 0.830                  | <0.001                |
| A142T | 0.43    | 0.08 | 23  | 0.060                | 0.748                  | <0.001                |
| A142T+G4 | 2.48    | 0.35 | 19  | <0.001               | 0.055                  | <0.001                |
| G145A | 0.32    | 0.08 | 23  | 0.072                | >0.999                 | 0.015                 |
| G145A+G4 | 0.66    | 0.13 | 24  | 0.045                | 0.570                  | <0.001                |
| R149Q | 0.23    | 0.02 | 24  | <0.001               | 0.748                  | <0.001                |
| R149Q+G4 | 1.36    | 0.33 | 24  | 0.101                | >0.999                 | <0.001                |
| R149P | 0.36    | 0.08 | 24  | 0.052                | >0.999                 | <0.001                |
| R149P+G4 | 1.12    | 0.24 | 23  | 0.060                | >0.999                 | <0.001                |
| I151N | 0.13    | 0.02 | 28  | 0.008                | <0.001                 | 0.503                 |
| I151N+G4 | 0.29    | 0.09 | 29  | 0.015                | <0.001                 | <0.001                |
| G158S | 0.11    | 0.02 | 27  | <0.001               | <0.001                 | 0.393                 |
| G158S+G4 | 0.15    | 0.06 | 29  | 0.262                | <0.001                 | <0.001                |
| G178D | 0.14    | 0.02 | 24  | 0.015                | <0.001                 | <0.001                |
| G178D+G4 | 1.28    | 0.18 | 24  | <0.001               | 0.830                  | <0.001                |
| M184I | 0.23    | 0.03 | 20  | 0.024                | 0.748                  | 0.004                 |
| M184I+G4 | 0.99    | 0.10 | 17  | 0.101                | >0.999                 | <0.001                |
| S185Y | 0.17    | 0.03 | 23  | 0.024                | 0.072                  | <0.001                |
| S185Y+G4 | 1.23    | 0.13 | 24  | <0.001               | 0.830                  | <0.001                |
| Q186R | 0.29    | 0.04 | 22  | <0.001               | >0.999                 | <0.001                |
| Q186R+G4 | 1.67    | 0.24 | 19  | <0.001               | 0.729                  | <0.001                |
Supplementary Table 3:

|                  | $l_{\text{total}}$ mean | SEM | N   | p-value (vs. natives) | p-value (mutant vs. WT) | p-value (-G4 vs. +G4) |
|------------------|--------------------------|-----|-----|-----------------------|-------------------------|----------------------|
| native           | 0.02                     | 0.00| 109 |                       |                         |                      |
| G4 (homo)        | 0.20                     | 0.02| 130 | <0.001                |                         | <0.001               |
| WT               | 0.07                     | 0.00| 133 | <0.001                | <0.001                  | <0.001               |
| WT+G4            | 1.00                     | 0.03| 137 | <0.001                |                         |                      |
| S132Y            | 0.03                     | 0.01| 25  | 0.635                 | <0.001                  | <0.001               |
| S132Y+G4         | 0.57                     | 0.07| 24  | 0.004                 | <0.001                  | <0.001               |
| L135I            | 0.04                     | 0.00| 25  | 0.012                 | <0.001                  | <0.001               |
| L135I+G4         | 0.95                     | 0.10| 23  | <0.001                | 0.649                   | <0.001               |
| F136L            | 0.02                     | 0.00| 24  | >0.999                | <0.001                  | <0.001               |
| F136L+G4         | 0.72                     | 0.07| 25  | <0.001                | <0.001                  | <0.001               |
| E139K            | 0.02                     | 0.01| 25  | >0.999                | <0.001                  | <0.001               |
| E139K+G4         | 0.21                     | 0.04| 24  | >0.999                | <0.001                  | <0.001               |
| E140M            | 0.03                     | 0.00| 24  | >0.999                | <0.001                  | <0.001               |
| E140M+G4         | 1.22                     | 0.13| 23  | <0.001                | 0.252                   | <0.001               |
| A142T            | 0.10                     | 0.01| 20  | <0.001                | 0.034                   | <0.001               |
| A142T+G4         | 1.31                     | 0.14| 20  | <0.001                | 0.166                   | <0.001               |
| G145A            | 0.03                     | 0.00| 24  | >0.999                | <0.001                  | <0.001               |
| G145A+G4         | 0.32                     | 0.09| 23  | 0.678                 | <0.001                  | <0.001               |
| R149Q            | 0.02                     | 0.00| 24  | >0.999                | <0.001                  | <0.001               |
| R149Q+G4         | 0.71                     | 0.09| 25  | <0.001                | 0.039                   | <0.001               |
| R149P            | 0.02                     | 0.00| 24  | >0.999                | <0.001                  | <0.001               |
| R149P+G4         | 0.72                     | 0.12| 23  | 0.018                 | 0.166                   | <0.001               |
| I151N            | 0.02                     | 0.00| 23  | >0.999                | <0.001                  | <0.001               |
| I151N+G4         | 0.42                     | 0.09| 24  | 0.480                 | <0.001                  | <0.001               |
| G158S            | 0.02                     | 0.00| 27  | >0.999                | <0.001                  | <0.001               |
| G158S+G4         | 0.21                     | 0.03| 29  | >0.999                | <0.001                  | <0.001               |
| G178D            | 0.04                     | 0.01| 25  | 0.535                 | <0.001                  | <0.001               |
| G178D+G4         | 0.68                     | 0.06| 25  | <0.001                | <0.001                  | <0.001               |
| M184I            | 0.13                     | 0.01| 16  | <0.001                | <0.001                  | <0.001               |
| M184I+G4         | 1.63                     | 0.22| 15  | <0.001                | 0.093                   | <0.001               |
| S185Y            | 0.03                     | 0.00| 23  | 0.479                 | <0.001                  | <0.001               |
| S185Y+G4         | 0.27                     | 0.03| 24  | 0.480                 | <0.001                  | <0.001               |
| Q186R            | 0.05                     | 0.00| 24  | <0.001                | <0.001                  | <0.001               |
| Q186R+G4         | 0.43                     | 0.07| 23  | 0.039                 | <0.001                  | <0.001               |
### Supplementary Table 4:

|          | $I_ACh/I_{total}$ mean | SEM  | N  | $p$-value (mutant vs. WT) |
|----------|------------------------|------|----|----------------------------|
| WT       | 0.42                   | 0.01 | 137| >0.999                     |
| S132Y    | 0.42                   | 0.03 | 24 | >0.999                     |
| L135I    | 0.37                   | 0.03 | 23 | 0.980                      |
| F136L    | 0.42                   | 0.03 | 25 | >0.999                     |
| E139K    | 0.43                   | 0.03 | 24 | >0.999                     |
| E140M    | 0.31                   | 0.04 | 23 | 0.143                      |
| A142T    | 0.42                   | 0.03 | 20 | >0.999                     |
| G145A    | 0.34                   | 0.03 | 23 | 0.234                      |
| R149Q    | 0.43                   | 0.04 | 25 | >0.999                     |
| R149P    | 0.38                   | 0.04 | 23 | >0.999                     |
| I151N    | 0.51                   | 0.02 | 24 | 0.049                      |
| G158S    | 0.49                   | 0.03 | 29 | 0.237                      |
| G178D    | 0.37                   | 0.02 | 25 | >0.999                     |
| M184I    | 0.46                   | 0.03 | 15 | >0.999                     |
| S185Y    | 0.28                   | 0.02 | 24 | <0.001                     |
| Q186R    | 0.44                   | 0.03 | 23 | >0.999                     |