Ingleby et al., http://www.jgp.org/cgi/content/full/jgp.200810133/DC1

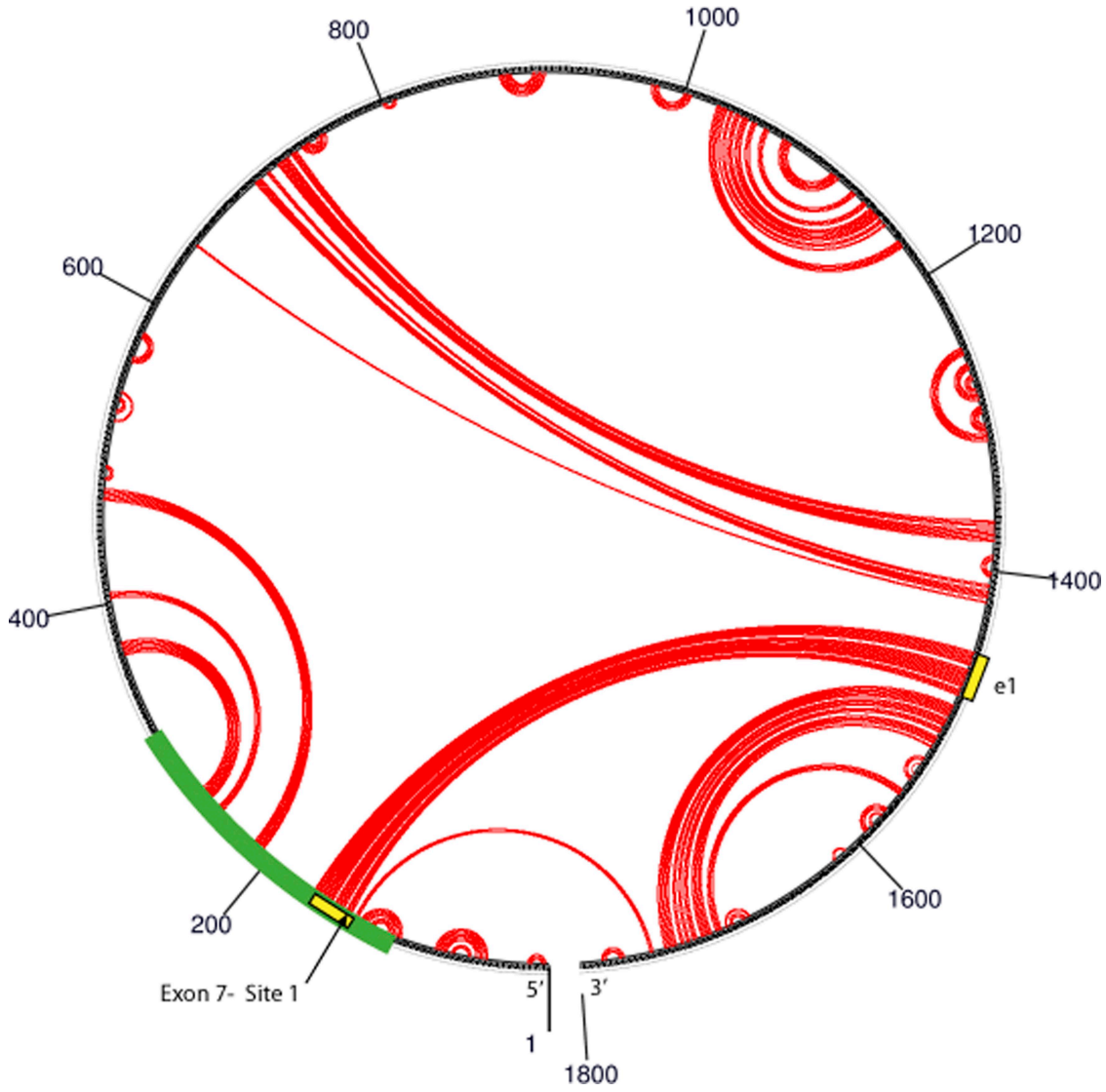


**Figure S1.** SFOLD analysis of *Shaker* editing site 1 (exon 7). Sequences were submitted to the SFOLD server (http://sfold.wadsworth.org/) spanning (1,800 nt) the exon 7 editing sites. Intron 6, 100 nt; exon 7, 172 nt; intron 7, 1,528 nt. Results are presented in the sir_graph format, depicting base-pairing interactions as connecting lines (red) (Ding, Y., C.Y. Chan, and C.E. Lawrence. 2005. *RNA*. 11:1157–1166). Sequence coordinates are indicated around the circle. Exonic regions are highlighted in green, and conserved pairing regions are indicated in yellow. For *Shaker* site 1, two centroids were predicted, both of which paired e1 with the editing site (see Fig. S3 A). Thus, 100% of the probability mass was contained in the ensemble-predicted pairing.

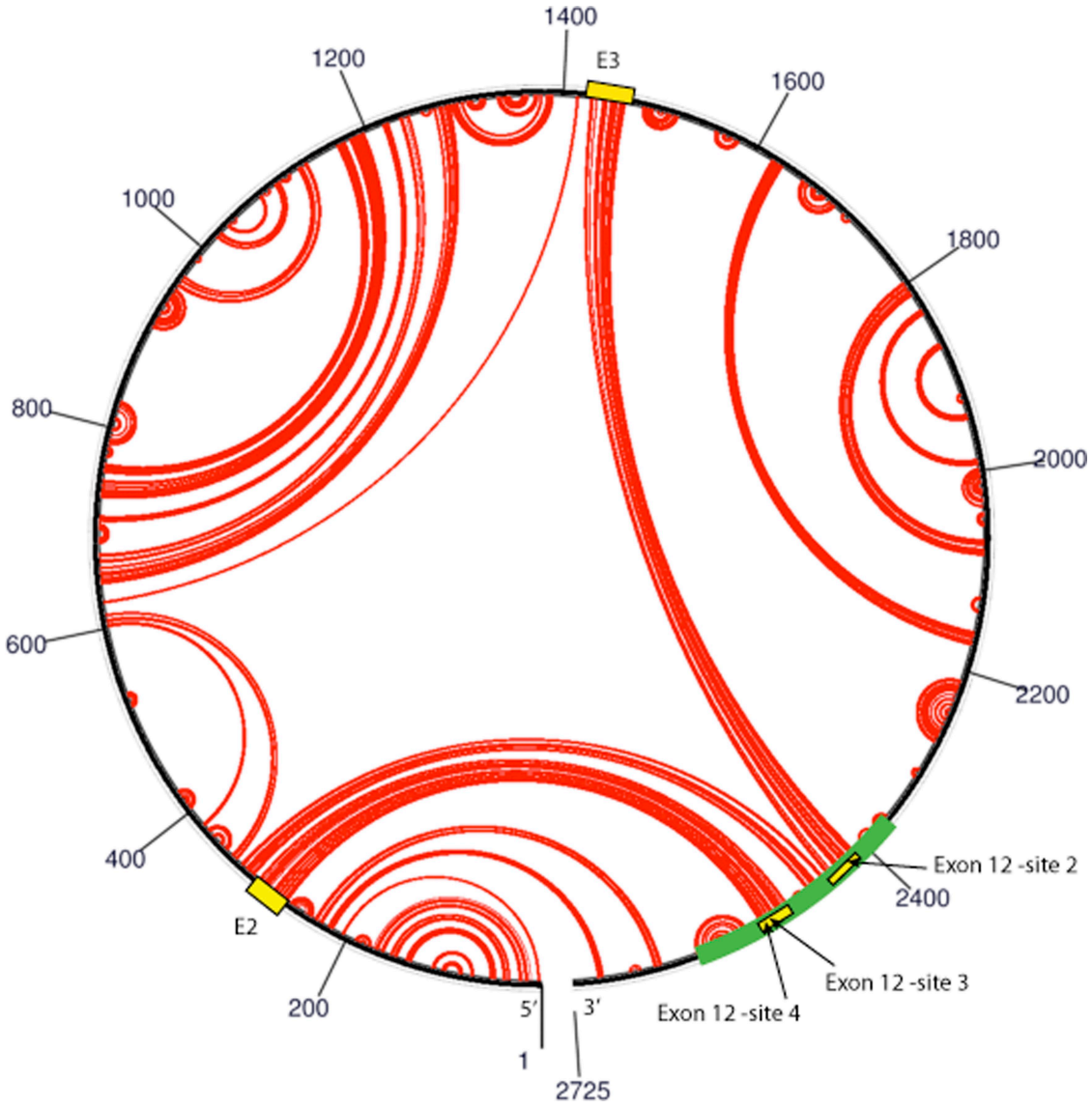


**Figure S2.** SFOLD analysis of *Shaker* editing sites 2–4 (exon 12). Sequences were submitted to the SFOLD server (http://sfold.wadsworth.org/) spanning (2,725 nt) the exon 12 editing sites. Intron 11, 2,386 nt; exon 12, 194 nt; intron 12, 145 nt. Results are presented in the sir_graph format, depicting base-pairing interactions as connecting lines (red) (Ding, Y., C.Y. Chan, and C.E. Lawrence. 2005. *RNA.* 11:1157–1166). Sequence coordinates are indicated around the circle. Exonic regions are highlighted in green, and conserved pairing regions are indicated in yellow. For Shaker sites 2–4, two centroids were predicted. Only centroid 1 is shown here. Centroid 1 (51.9%) and centroid 2 (48.1%) both pair e2 with sites 3–4 (see Fig. S3 C). Only centroid 1 pairs e3 with site 2 (see Fig. S3 B). Thus, the structure predicted for site 2 encompasses only 51.9% of the probability mass of the predicted structures.

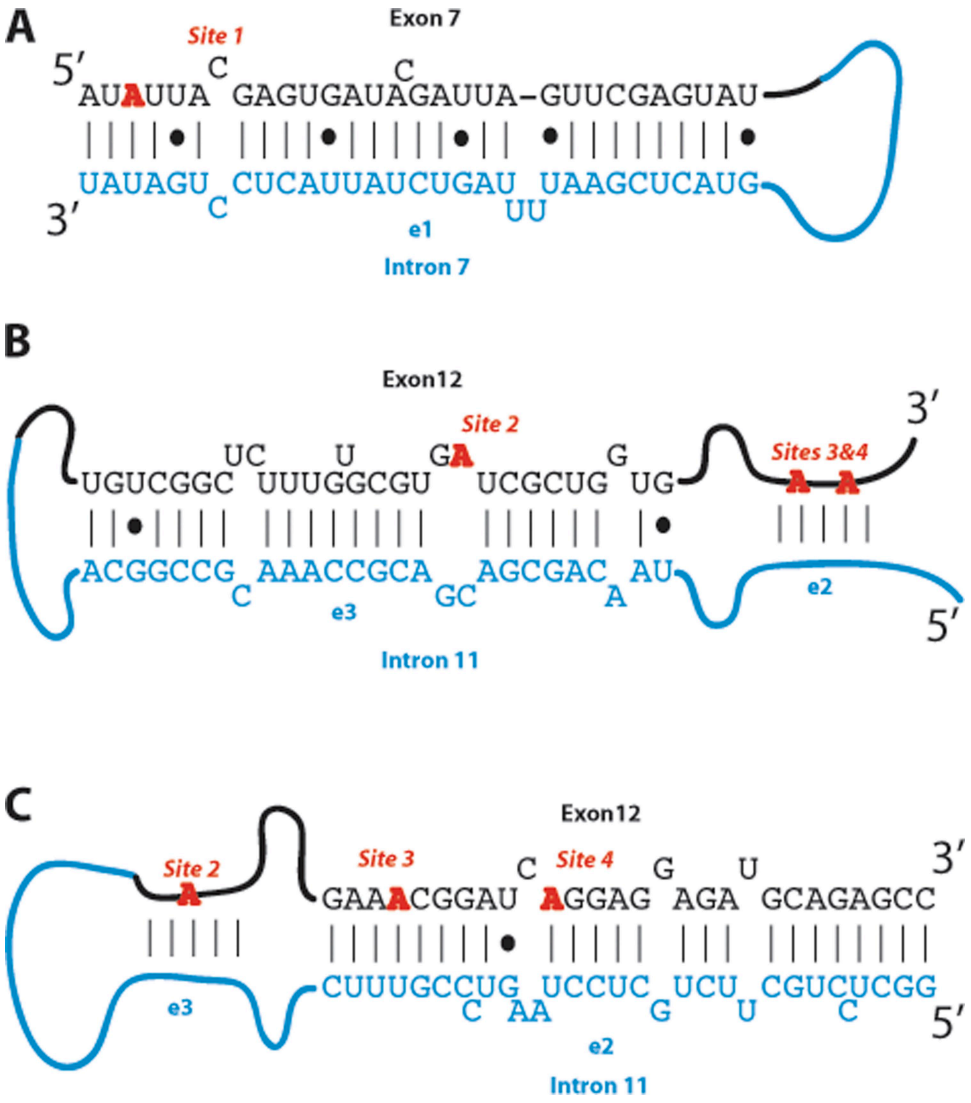


**Figure S3.** Predicted local RNA secondary structures of *Shaker* editing sites 1–4. All predicted structures show *Shaker* exonic sequences (black) containing the edited adenosines (red) pairing with absolutely conserved intronic pairing partners (e1–e3; blue). (A) *Shaker* editing site 1 structure. (B) *Shaker* editing site 2 structure with sites 3–4 shown in less detail. (C) *Shaker* editing sites 3–4 with site 2 shown in less detail.

TABLE S1

*Detailed Editing Isoform Profiles*

| | AAAA | AAAG | AAGA | AGAA | GAAA | AAGG | AGGA | GGAA | AGAG | GAGA | GAAG | AGGG | GGGA | GGAG | GAGG | GGGG | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Male Head | 11 | 0 | 7 | 2 | 1 | 27 | 3 | 0 | 0 | 2 | 0 | 14 | 5 | 0 | 19 | 9 | 100 |
| Female Head | 8 | 2 | 3 | 8 | 0 | 28 | 4 | 1 | 0 | 2 | 0 | 14 | 3 | 1 | 13 | 13 | 100 |
| Male Thorax | 16 | 0 | 8 | 10 | 5 | 13 | 4 | 2 | 0 | 9 | 0 | 8 | 2 | 0 | 15 | 8 | 100 |
| Female Thorax | 19 | 1 | 5 | 8 | 9 | 9 | 3 | 2 | 1 | 18 | 0 | 8 | 6 | 0 | 8 | 4 | 101 |
| Male Antennae | 1 | 0 | 11 | 1 | 52 | 11 | 8 | 0 | 0 | 21 | 0 | 1 | 3 | 0 | 1 | 0 | 110 |
| Male eye | 3 | 0 | 4 | 4 | 32 | 4 | 4 | 1 | 0 | 11 | 0 | 22 | 1 | 0 | 5 | 5 | 96 |
| Male Wing | 3 | 0 | 3 | 0 | 75 | 0 | 1 | 0 | 0 | 18 | 0 | 7 | 0 | 0 | 2 | 2 | 111 |
| Larvae (L1) | 25 | 0 | 7 | 2 | 12 | 9 | 2 | 0 | 0 | 22 | 0 | 3 | 8 | 0 | 9 | 4 | 103 |
| Totals | 86 | 3 | 48 | 35 | 186 | 101 | 29 | 6 | 1 | 103 | 0 | 77 | 28 | 1 | 72 | 45 | 821 |

Sequence analyses were performed on 821 cDNAs from the tissue samples as indicated. The cDNAs were characterized by the editing status of Shaker editing sites 1–4 into one of 16 possible isoforms. Only the GAAG isoform was not observed in these studies.

TABLE S2

*Inactivation Parameters*

| | $V_{mid}$ (mV) | $q$ | $\tau_{rec}$ (ms) |
|---|---|---|---|
| AAAG | −59.4 ± 2.5 | 7.18 ± 0.26 | 85.6 ± 10.7 |
| AGAA | −58.5 ± 1.8 | 7.90 ± 0.16 | 89.3 ± 5.4 |
| AGGA | −55.7 ± 0.7 | 7.41 ± 0.30 | 101.5 ± 8.4 |
| AAGG | −54.7 ± 0.8 | 7.19 ± 0.15 | 65.3 ± 4.4 |
| AAAA | −53.4 ± 0.7 | 7.39 ± 0.17 | 75.2 ± 4.5 |
| GGGG | −56.1 ± 1.8 | 8.02 ± 0.14 | 134.3 ± 22.2 |
| AAGA | −51.0 ± 1.2 | 7.16 ± 0.22 | 87.0 ± 8.9 |
| GGGA | −52.3 ± 1.5 | 6.64 ± 0.18 | 214.5 ± 23.8 |
| GAAA | −47.6 ± 1.9 | 7.21 ± 0.22 | 144.1 ± 10.9 |

Steady-state inactivation and time constant of recovery from inactivation at −120 mV. Steady-state inactivation relationships (Fig. 5 B) were fit by a standard Boltzmann function with estimates of the midpoint ($V_{mid}$) and slope ($q$ with units of elementary electric charge) shown here. Single exponential relaxations were fit to recovery from inactivation (Fig. 5 C) with estimates of the time constants ($\tau_{rec}$) shown here.

TABLE S3

*Parameters for* G-V *Fits Using Single or Double (When Necessary) Boltzmann Functions*

| | $V_{mid1}$ (mV) | $q_1$ | $w_1$ | $V_{mid2}$ (mV) | $q_2$ |
|---|---|---|---|---|---|
| AAAG | −51.73 ± 0.80 | 6.11 ± 0.16 | 0.80 ± 0.02 | −9.32 ± 20.34 | 0.59 ± 0.22 |
| AGAA | −51.72 ± 0.92 | 5.77 ± 0.33 | 0.82 ± 0.03 | 1.66 ± 28.48 | 0.48 ± 0.14 |
| AGGA | −48.24 ± 1.56 | 5.61 ± 0.20 | 0.83 ± 0.01 | −13.49 ± 5.01 | 0.88 ± 0.10 |
| AAGG | −47.06 ± 1.89 | 4.89 ± 0.14 | 0.76 ± 0.01 | -3.26 ± 7.91 | 0.95 ± 0.14 |
| AAAA | −46.64 ± 1.95 | 5.31 ± 0.27 | 0.75 ± 0.03 | −8.81 ± 5.87 | 0.80 ± 0.18 |
| GGGG | −46.22 ± 1.21 | 5.18 ± 0.08 | 1.00 | | |
| AAGA | −43.53 ± 1.29 | 5.44 ± 0.27 | 0.74 ± 0.03 | −17.22 ± 5.98 | 0.87 ± 0.04 |
| GGGA | −41.33 ± 1.16 | 5.04 ± 0.26 | 1.00 | | |
| GAAA | −40.61 ± 1.04 | 5.20 ± 0.16 | 0.82 ± 0.01 | 0.56 ± 3.45 | 0.86 ± 0.14 |

The normalized double Boltzmann function is

$$G(V) = \frac{W_1}{1+\exp\left\{q_1\left(V_{mid1}-V\right)\right\}} + \frac{1-W_1}{1+\exp\left\{q_2\left(V_{mid2}-V\right)\right\}},$$

where $W_1$ is the fractional weight of the first component, the midpoints are in units of mV, and the slopes are in units of elementary charge.

TABLE S4

*Deactivation Time Constants (in ms) at Indicated Membrane Potentials*

| V (mV) | AAGG | AAAA | GAAA | GGGG | AGGA | GGGA | AGAA | AAGA | AAAG |
|---|---|---|---|---|---|---|---|---|---|
| −140 | 0.94 ± 0.06 | 1.07 ± 0.06 | 1.52 ± 0.10 | 1.60 ± 0.09 | 1.49 ± 0.15 | 1.51 ± 0.07 | 1.84 ± 0.15 | 0.83 ± 0.04 | 1.02 ± 0.03 |
| −135 | 1.10 ± 0.07 | 1.38 ± 0.14 | 1.77 ± 0.11 | 1.88 ± 0.10 | 1.78 ± 0.18 | 1.79 ± 0.07 | 2.14 ± 0.10 | 0.97 ± 0.05 | 1.19 ± 0.03 |
| −130 | 1.26 ± 0.07 | 1.49 ± 0.09 | 2.03 ± 0.11 | 2.25 ± 0.14 | 2.08 ± 0.21 | 2.11 ± 0.09 | 2.62 ± 0.12 | 1.08 ± 0.06 | 1.40 ± 0.04 |
| −125 | 1.36 ± 0.09 | 1.78 ± 0.16 | 2.35 ± 0.16 | 2.71 ± 0.18 | 2.43 ± 0.21 | 2.55 ± 0.09 | 3.21 ± 0.16 | 1.24 ± 0.06 | 1.63 ± 0.06 |
| −120 | 1.57 ± 0.13 | 2.07 ± 0.14 | 2.79 ± 0.18 | 3.24 ± 0.21 | 3.08 ± 0.29 | 3.09 ± 0.11 | 3.92 ± 0.21 | 1.42 ± 0.07 | 1.92 ± 0.07 |
| −115 | 1.78 ± 0.14 | 2.54 ± 0.30 | 3.26 ± 0.18 | 3.89 ± 0.28 | 3.55 ± 0.43 | 3.78 ± 0.11 | 4.68 ± 0.38 | 1.67 ± 0.07 | 2.28 ± 0.09 |
| −110 | 2.04 ± 0.17 | 2.81 ± 0.23 | 3.83 ± 0.24 | 4.84 ± 0.37 | 4.31 ± 0.48 | 4.73 ± 0.16 | 6.33 ± 0.30 | 1.93 ± 0.08 | 2.71 ± 0.11 |
| −105 | 2.39 ± 0.16 | 3.23 ± 0.25 | 4.50 ± 0.22 | 5.90 ± 0.46 | 5.15 ± 0.57 | 5.56 ± 0.12 | 8.07 ± 0.46 | 2.23 ± 0.08 | 3.21 ± 0.15 |
| −100 | 2.60 ± 0.26 | 3.66 ± 0.23 | 5.18 ± 0.26 | 7.18 ± 0.61 | 6.29 ± 0.68 | 6.85 ± 0.15 | 10.19 ± 0.77 | 2.51 ± 0.10 | 3.75 ± 0.17 |
| −95 | 2.96 ± 0.29 | 4.27 ± 0.28 | 6.03 ± 0.28 | 8.68 ± 0.75 | 7.53 ± 0.81 | 8.11 ± 0.20 | 12.23 ± 0.59 | 2.86 ± 0.11 | 4.49 ± 0.24 |
| −90 | 3.41 ± 0.29 | 5.15 ± 0.50 | 6.93 ± 0.33 | 10.58 ± 0.93 | 8.82 ± 1.04 | 9.76 ± 0.32 | 14.71 ± 0.71 | 3.29 ± 0.12 | 5.24 ± 0.28 |
| −85 | 3.82 ± 0.36 | 5.83 ± 0.53 | 8.00 ± 0.39 | 12.69 ± 1.18 | 10.33 ± 1.17 | 11.60 ± 0.49 | 17.82 ± 0.91 | 3.67 ± 0.15 | 6.12 ± 0.32 |
| −80 | 4.29 ± 0.44 | 6.91 ± 0.83 | 9.07 ± 0.50 | 15.22 ± 1.36 | 12.36 ± 1.47 | 13.60 ± 0.68 | 21.78 ± 1.19 | 4.25 ± 0.22 | 7.30 ± 0.43 |
| −75 | 4.98 ± 0.55 | 7.79 ± 0.80 | 10.47 ± 0.59 | 18.27 ± 1.81 | 14.25 ± 1.78 | 16.15 ± 0.93 | 26.14 ± 1.50 | 4.89 ± 0.25 | 8.71 ± 0.51 |
| −70 | 5.91 ± 0.67 | 9.18 ± 0.99 | 12.29 ± 0.79 | 22.10 ± 2.21 | 17.04 ± 2.25 | 19.21 ± 1.36 | 29.25 ± 2.60 | 5.63 ± 0.25 | 10.70 ± 0.66 |
| −65 | 7.16 ± 0.86 | 11.10 ± 1.21 | 14.55 ± 1.12 | 26.80 ± 2.55 | 20.28 ± 2.48 | 23.65 ± 2.03 | 34.19 ± 2.31 | 6.81 ± 0.39 | 13.51 ± 0.84 |
| −60 | 8.60 ± 0.86 | 13.67 ± 1.46 | 18.13 ± 1.38 | 32.02 ± 2.42 | 23.27 ± 2.16 | 30.31 ± 3.03 | 32.24 ± 1.54 | 8.48 ± 0.49 | 16.38 ± 0.93 |
| −55 | 11.12 ± 1.21 | 15.37 ± 0.96 | 22.13 ± 1.68 | 34.74 ± 0.73 | 24.80 ± 1.09 | 37.89 ± 3.80 | 34.62 ± 5.93 | 10.72 ± 0.72 | 17.26 ± 0.82 |
| −50 | 14.01 ± 1.91 | 17.77 ± 0.71 | 24.92 ± 1.77 | 32.57 ± 1.21 | 25.35 ± 1.23 | 39.52 ± 2.91 | 28.45 ± 1.56 | 12.29 ± 0.62 | 18.93 ± 1.41 |

From single exponential fits to deactivation (Fig. 8).