**Supplementary File 1**

**Supplementary File 1–Table 1:** Parameters from Boltzmann function fits of normalized activation curves of KCNQ1-KCNE1 WT and cysteine mutants which were used in crosslinking experiments.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **Control** | | **DTT** | | **Cu-phenanthroline** | |
| **KCNQ1** | **KCNE1** | **Na** | **V1/2app**  **(mV)b** | **Slope kb** | **V1/2app**  **(mV)** | **Slope k** | **V1/2app**  **(mV)** | **Slope k** |
| WT | - | 9 / 5 / 5 | -9.9 ± 0.9 | 13.1 ± 0.7 | -14.4 ± 0.8 | 12.7 ± 0.7 | -12.3 ± 1.3 | 11.8 ± 1.0 |
| V141C | - | 13 / 7 / 5 | -10.5 ± 0.6 | 14.6 ± 0.5 | -13.7 ± 0.7 | 13.5 ± 0.6 | -5.1 ± 0.5 | 13.9 ± 0.4 |
| I274C | - | 12 / 7 / 7 | -21.2 ± 1.9 | 17.7 ± 1.4 | -26.6 ± 1.8 | 11.2 ± 1.5 | -31.3 ± 1.9 | 12.5 ± 1.5 |
| WT | WT | 10 / 4 / 6 | 27.7 ± 1.0 | 13.8 ± 0.9 | 31.3 ± 0.9 | 12.3 ± 0.8 | 24.9 ± 0.9 | 14.2 ± 0.8 |
| V141C | WT | 18 / 12 / 9 | 28.4 ± 1.0 | 13.6 ± 0.9 | 30.1 ± 0.9 | 12.6 ± 0.8 | 30.0 ± 1.0 | 13.1 ± 0.9 |
| I274C | WT | 20 / 9 / 11 | 24.8 ± 1.1 | 15.4 ± 1.0 | 21.6 ± 1.0 | 15.4 ± 0.9 | 24.9 ± 1.2 | 15.3 ± 1.1 |
| I274C | L45C | 6 / 6 / 5 | 28.9 ± 1.2 | 14.5 ± 1.1 | 24.9 ± 1.0 | 14.9 ± 0.9 | 25.2 ± 1.1 | 14.7 ± 1.0 |
| V141C | V47C | 8 / 6 / 6 | 12.7 ± 1.2 | 18.7 ± 1.1 | 17.0 ± 1.0 | 16.9 ± 0.9 | 16.2 ± 1.2 | 17.9 ± 1.0 |
| I274C | V47C | 13 / 10 / 7 | 25.3 ± 1.1 | 15.1 ± 1.0 | 20.5 ± 1.0 | 15.7 ± 0.9 | 23.8 ± 1.1 | 15.5 ± 1.0 |
| V141C | L48C | 7 / 5 / 5 | 10.4 ± 0.9 | 18.0 ± 0.8 | 10.9 ± 0.9 | 17.8 ± 0.8 | 6.9 ± 0.9 | 18.7 ± 0.8 |
| I274C | L48C | 12 / 7 / 7 | -11.1 ± 1.2 | 22.6 ± 0.8 | -12.0 ± 1.0 | 22.6 ± 0.7 | -13.0 ± 1.1 | 22.0 ± 0.7 |

a N refers to the number of cells that were treated with control solution, DTT, or Cu-phenanthroline, respectively.

b The V1/2app of activation and the slope factor k were obtained by least-squares fitting with a Boltzmann function: *I*tail/ *I*tailmax = (1 - *I*Bottom) / (1+exp[(*V*1/2app - *V*)/*k*]) + *I*Bottom

**Supplementary File 1–Table 2:** Distance restraints used in generation of the closed state KCNQ1-KCNE1 docking model.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **KCNQ1 Residue** | **KCNE1 Residue** | **Analysis Method** | **Reference** | **Restrained Cα-Cα Distance (Å)** | **Distance (Å)**  **Rosetta Model** | **Mean (Min – Max) Distance (Å) MD Sim.** |
| ***Restraints from disulfide crosslinking experiments*** | | | | | | |
| V141\* | E43\* | Disulfide crosslinking | (Chan et al. 2012) | 12.0 | 10.6 | 14.3 ( 10.7 - 17.4)  12.2 ( 8.9 - 16.4)  11.1 ( 7.6 - 15.0)  9.8 ( 5.6 - 16.5) |
| V141 | A44 | Disulfide crosslinking | (Chan et al. 2012) | 12.0 | 11.6 | 14.0 ( 10.2 - 16.1)  12.4 ( 9.0 - 15.8)  10.2 ( 5.5 - 15.3)  10.5 ( 5.8 - 14.2) |
| S143\* | G40\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 11.6 | 20.4 ( 11.0 - 26.5)  17.1 ( 12.1 - 20.9)  17.9 ( 14.2 - 22.3)  14.5 ( 6.2 - 23.7) |
| T144\* | G40\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 14.5 | 18.9 ( 9.2 - 24.8)  15.4 ( 8.5 - 19.6)  16.5 ( 12.4 - 20.2)  12.7 ( 3.4 - 22.0) |
| T144 | K41 | Disulfide crosslinking | (Wang et al. 2011) | 12.0 | 13.3 | 17.3 ( 10.2 - 21.6)  12.9 ( 8.9 - 17.1)  14.2 ( 9.5 - 18.7)  12.3 ( 4.3 - 20.3) |
| T144\* | E43\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 11.7 | 13.9 ( 9.6 - 18.4)  12.8 ( 7.4 - 18.9)  12.9 ( 8.2 - 18.3)  10.9 ( 5.6 - 17.5) |
| I145\* | G40\* | Disulfide crosslinking | (Chung et al. 2009)  (Wang et al. 2011) | 12.0 | 11.3 | 15.3 ( 5.9 - 21.2)  12.4 ( 7.2 - 17.2)  13.8 ( 10.0 - 18.1)  11.6 ( 4.1 - 18.6) |
| I145 | K41 | Disulfide crosslinking | (Chung et al. 2009)  (Wang et al. 2011) | 12.0 | 10.6 | 13.9 ( 7.6 - 17.9)  10.0 ( 6.1 - 14.2)  11.9 ( 7.0 - 16.3)  10.5 ( 4.0 - 17.2) |
| I145 | L42 | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 7.0 | 12.0 ( 6.5 - 18.4)  8.0 ( 5.1 - 13.0)  9.4 ( 5.5 - 13.6)  8.1 ( 4.0 - 14.2) |
| I145\* | E43\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 8.6 | 10.6 ( 6.4 - 15.4)  9.9 ( 4.7 - 16.8)  11.0 ( 6.0 - 16.8)  8.7 ( 4.1 - 16.3) |
| E146\* | G40\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 12.8 | 15.0 ( 4.1 - 21.6)  13.3 ( 6.2 - 20.8)  15.8 ( 11.2 - 21.0)  13.1 ( 4.2 - 20.6) |
| E146\* | K41\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 13.1 | 14.0 ( 5.7 - 19.4)  11.4 ( 7.7 - 17.1)  14.0 ( 8.5 - 19.3)  12.2 ( 3.9 - 19.8) |
| Q147 | S37 | Disulfide crosslinking | (Wang et al. 2011) | 12.0 | 9.4 | 16.5 ( 3.6 - 28.8)  14.0 ( 4.5 - 23.5)  16.3 ( 5.1 - 26.7)  13.2 ( 5.3 - 25.6) |
|  |  |  |  |  |  |  |
| W323\* | E43\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 10.2 | 5.5 ( 4.3 - 8.8)  9.1 ( 6.3 - 13.1)  8.9 ( 5.3 - 14.2)  9.2 ( 5.2 - 14.5) |
| V324 | K41 | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 7.6 | 8.7 ( 6.4 - 16.1)  10.6 ( 6.4 - 17.3)  9.4 ( 7.0 - 16.2)  14.0 ( 7.5 - 22.3) |
| ***Restraints from additional functional experiments*** | | | | | | |
| I274 | L45 | Higher IKs of double Cys mutant under reducing cond (+DTT) | This study | 12.0 | 15.5 | 16.5 ( 14.6 - 19.2)  15.9 ( 13.8 - 20.2)  15.9 ( 13.7 - 18.1)  15.8 ( 13.5 - 18.7) |
| I328 | L51 | Lower IKs of double Cys mutant when Cd(II) present  Cd(II)-cysteine crosslinking | (Tapper and George 2001) | 15.0 | 15.3 | 15.2 ( 12.6 - 17.6)  15.2 ( 11.9 - 18.1)  15.3 ( 12.9 - 17.7)  14.7 ( 11.6 - 18.1) |
| C331 | F54 | Lower IKs of Q1 with E1 F54C mutant when Cd(II) present  Cd(II)-cysteine crosslinking | (Tapper and George 2001) | 15.0 | 13.4 | 12.5 ( 8.9 - 14.7)  12.3 ( 8.9 - 14.9)  12.3 ( 8.7 - 16.3)  11.2 ( 7.8 - 16.0) |
| C331 | G55 | Lower IKs of Q1 with E1 G55C mutant when Cd2+ present  Cd(II)-cysteine crosslinking | (Tapper and George 2001) | 15.0 | 15.5 | 15.3 ( 11.4 - 17.7)  14.9 ( 12.1 - 17.6)  14.9 ( 10.6 - 17.5)  14.2 ( 10.4 - 19.0) |
| F340 | T58 | Double mutant cycle analysis | (Strutz-Seebohm et al. 2011)  (Li et al. 2014) | 12.0 | 17.4 | 17.3 ( 13.6 - 21.7)  17.5 ( 14.7 - 21.1)  17.5 ( 15.1 - 20.3)  17.8 ( 14.4 - 21.3) |
| A344 | Y65 | Double mutant cycle analysis | (Li et al. 2014) | 12.0 | 22.7 | 24.8 ( 20.1 - 30.8)  24.1 ( 20.3 - 28.7)  22.9 ( 19.6 - 27.8)  22.7 ( 19.9 - 26.6) |
| \*For those restraints, the functional channel state under the experimental conditions was unclear. They were included in the restraint list of both the open and closed state model. | | | | | | |

**Supplementary File 1–Table 3:** Distance restraints used in generation of the open state KCNQ1-KCNE1 docking model.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **KCNQ1 Residue** | **KCNE1 Residue** | **Analysis Method** | **Reference** | **Restrained Cα-Cα Distance (Å)** | **Distance (Å)**  **Rosetta Model** | **Mean (Min – Max) Distance (Å) MD Sim.** |
| ***Restraints from disulfide crosslinking experiments*** | | | | | | |
| V141\* | E43\* | Disulfide crosslinking | (Chan et al. 2012) | 12.0 | 8.5 | 10.4 ( 7.2 - 14.0)  11.7 ( 8.7 - 14.9)  11.7 ( 8.3 - 16.6)  12.1 ( 7.3 - 16.6) |
| V141 | A44 | Disulfide crosslinking | (Wang et al. 2011)  (Chan et al. 2012) | 12.0 | 6.3 | 8.7 ( 5.9 - 11.7)  9.1 ( 6.5 - 11.7)  8.4 ( 6.0 - 13.5)  9.7 ( 6.2 - 13.5) |
| S143\* | G40\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 11.4 | 11.6 ( 8.4 - 17.7)  13.4 ( 9.8 - 20.3)  13.6 ( 10.0 - 19.8)  13.5 ( 8.8 - 19.9) |
| T144 | G40 | Disulfide crosslinking | (Chung et al. 2009)  (Wang et al. 2011) | 12.0 | 10.5 | 10.6 ( 7.1 - 16.9)  12.4 ( 7.4 - 19.6)  11.3 ( 7.4 - 19.3)  12.4 ( 8.2 - 19.3) |
| T144\* | K41\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 11.8 | 12.8 ( 9.0 - 16.2)  13.5 ( 9.4 - 17.9)  10.7 ( 7.0 - 19.1)  13.8 ( 9.9 - 19.1) |
| T144\* | E43\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 9.6 | 11.1 ( 8.4 - 15.2)  12.2 ( 8.6 - 16.2)  12.3 ( 8.9 - 18.9)  13.2 ( 8.7 - 18.9) |
| I145 | G40 | Disulfide crosslinking | (Chung et al. 2009)  (Wang et al. 2011) | 12.0 | 6.9 | 7.1 ( 4.9 - 13.1)  8.8 ( 5.1 - 15.9)  8.8 ( 5.2 - 15.6)  8.7 ( 5.0 - 15.6) |
| I145 | K41 | Disulfide crosslinking | (Chung et al. 2009)  (Wang et al. 2011) | 12.0 | 8.6 | 9.5 ( 6.2 - 13.2)  10.1 ( 6.5 - 14.8)  7.8 ( 4.9 - 15.4)  10.4 ( 6.1 - 15.4) |
| I145 | L42 | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 10.2 | 11.2 ( 9.3 - 14.9)  12.0 ( 9.3 - 15.5)  10.5 ( 6.6 - 18.0)  12.5 ( 8.6 - 18.0) |
| I145\* | E43\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 7.4 | 8.6 ( 6.5 - 13.1)  9.4 ( 6.0 - 14.0)  10.5 ( 6.5 - 16.3)  10.3 ( 6.3 - 16.3) |
| E146\* | G40\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 8.1 | 8.5 ( 5.2 - 14.8)  9.6 ( 6.2 - 17.0)  10.7 ( 4.8 - 17.3)  9.7 ( 4.8 - 17.3) |
| E146\* | K41\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 10.5 | 11.1 ( 7.5 - 15.4)  11.5 ( 7.8 - 15.9)  9.9 ( 6.7 - 17.2)  11.7 ( 7.1 - 17.2) |
| Q147 | G38 | Disulfide crosslinking | (Wang et al. 2011) | 12.0 | 10.9 | 10.0 ( 3.6 - 16.9)  9.8 ( 4.6 - 18.8)  13.7 ( 5.2 - 21.3)  11.7 ( 4.0 - 21.3) |
| Q147 | G40 | Disulfide crosslinking | (Wang et al. 2011) | 12.0 | 6.7 | 6.9 ( 4.4 - 13.0)  7.7 ( 4.6 - 15.4)  10.8 ( 4.6 - 15.8)  7.9 ( 4.6 - 15.6) |
| Q147 | K41 | Disulfide crosslinking | (Wang et al. 2011) | 12.0 | 8.4 | 8.7 ( 5.0 - 13.9)  9.0 ( 5.6 - 13.5)  9.2 ( 5.6 - 15.4)  9.1 ( 5.5 - 15.4) |
| W323\* | E43\* | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 10.5 | 10.8 ( 7.4 - 14.1)  9.9 ( 6.0 - 15.9)  8.6 ( 5.1 - 17.1)  10.9 ( 5.0 - 17.1) |
| V324 | L42 | Disulfide crosslinking | (Chung et al. 2009) | 12.0 | 12.8 | 13.5 ( 9.6 - 17.6)  12.8 ( 9.8 - 19.9)  14.0 ( 10.5 - 20.7)  14.8 ( 9.9 - 20.7) |
| ***Restraints from additional functional experiments*** | | | | | | |
| V141 | L48 | Lower IKs of double Cys mutant under reducing cond. (+DTT) | This study | 12.0 | 8.6 | 9.9 ( 8.2 - 12.4)  9.0 ( 6.1 - 12.9)  8.8 ( 5.7 - 15.0)  10.6 ( 7.9 - 15.0) |
| C331 | F54 | Positive shift in V1/2 of Q1 with E1 F54C mutant under reducing cond. (+DTT).  Constitutive current under oxidizing cond. (+H2O2) that is reversed by H2O2 washout and DTT. | (Wang et al. 2012) | 12.0 | 13.0 | 10.8 ( 8.1 - 13.6)  13.6 ( 9.5 - 16.7)  12.8 ( 9.5 - 17.9)  13.3 ( 9.5 - 17.9) |
| \*For those restraints, the functional channel state under the experimental conditions was unclear. They were included in the restraint list of both the open and closed state model. | | | | | | |

**Supplementary File 1–Table 4:** MolProbity statistics for the KCNQ1-KCNE1 Rosetta models.

|  |  |  |
| --- | --- | --- |
|  | **Open state model** | **Closed state model** |
| Molprobity score | 1.34 (98th percentile) | 1.35 (98th percentile) |
| Clash score | 2.36 (99th percentile) | 2.39 (99th percentile) |
| Ramachandran statistic |  |  |
| Favored regions (%) | 95.3 | 95.2 |
| Allowed regions (%) | 4.5 | 3.9 |
| Disallowed regions (%) | 0.2 | 0.9 |
| Rotamer statistic |  |  |
| Favored rotamers (%) | 99.5 | 99.5 |
| Poor rotamers (%) | 0.0 | 0.0 |
| Cβ deviations (%) | 0.0 | 0.1 |
| Bad bonds (%) | 0.0 | 0.0 |
| Bad angles (%) | 0.06 | 0.04 |
| Rosetta ΔGBinding (REU)a | -63.3 ± 1.3 | -64.7 ± 1.8 |
| Amber ΔGBinding (kcal/mol)a | -59.6 ± 1.5 | -59.2 ± 1.0 |

a mean ± SEM

**Supplementary File 1–Table 5:** KCNQ1-KCNE1 residue contacts observed in MD simulations of the KCNQ1-KCNE1 RC and AO channel models. For every KCNE1 residue, the KCNQ1 residues that made more than one heteroatom contact on average (≤ 4 Å distance) are listed. Residue numbers are colored differently to indicate different KCNQ1 subunits. The average contact number is given in parentheses.

|  |  |  |
| --- | --- | --- |
| **KCNE1 Residue** | **Interacting KCNQ1 Residue – RC Model** | **Interacting KCNQ1 Residue – AO Model** |
| S37 | **E290** (1.3) | **Q147** (1.0), **G297** (1.9), **D301** (2.0) |
| G38 | **Q321** (1.1), **W323** (1.2) | **Q147** (1.3) |
| D39 | **Q321** (2.2), **T322** (2.0), **W323** (2.4) | **Q147** (2.4) |
| G40 | **W323** (3.4) | **I145** (1.7), **Q147** (2.4), **Y148** (2.3) |
| K41 | **S298** (1.2), **D301** (1.8), **Q321** (1.8), **W323** (6.1) | **Q147** (2.3), **Y148** (5.3) |
| L42 | **I145** (2.4), **Q147** (2.1), **Y148** (1.8), **W323** (1.2) | **W323** (2.9) |
| E43 | **I145** (1.1), **Q147** (1.1), **Y148** (2.0), **W323** (2.5) | **I145** (1.8), **S298** (6.4), **Y299** (1.9), **A300** (2.7), **W323** (5.9) |
| A44 | **W323** (5.0) | **L142** (1.3), **I145** (1.2), **Y148** (1.4) |
| L45 | **W323** (4.1) | - |
| Y46 | **A300** (3.8), **L303** (4.2), **W304** (11.9), **V307** (2.0), **W323** (5.5), **K326** (2.2), **T327** (6.8), **S330** (6.1) | **A300** (3.1), **L303** (1.2), **W304** (3.3), **W323** (14.7), **V324** (2.0), **T327** (2.5) |
| V47 | **I138** (1.1), **V141** (2.4), **Y299** (2.4), **A300** (3.3), **L303** (2.3) | **V141** (1.6), **Y299** (3.7), **A300** (1.6), **L303** (1.1) |
| L48 | **I138** (3.8), **V141** (1.1) | **I138** (2.7) |
| M49 | **T327** (2.3) | - |
| V50 | **F270** (1.4), **L303** (2.6) | **F270** (1.6), **L303** (1.6) |
| L51 | **L134** (4.5), **L137** (2.0), **I138** (2.5) | **L134** (3.2), **L137** (2.5), **I138** (3.4), **I274** (1.2) |
| G52 | - | - |
| F53 | **F270** (3.0), **C331** (1.7) | **F270** (2.9) |
| F54 | **F130** (3.7), **F232** (5.8), **Y267** (6.3), **F270** (11.7), **L271** (1.6), **I274** (2.5) | **F130** (1.5), **M238** (2.1), **Y267** (6.3), **F270** (10.5), **L271** (3.0), **I274** (3.3) |
| G55 | **F127** (1.4), **F130** (2.6), **L134** (1.1) | **F130** (3.4), **L134** (1.3) |
| F56 | - | - |
| F57 | **I263** (2.6), **L266** (4.5), **Y267** (9.3), **F270** (4.4) | **I263** (2.8), **L266** (4.9), **Y267** (8.1), **F270** (4.7) |
| T58 | **F127** (2.4), **F130** (7.5), **Y267** (6.1) | **F130** (8.2), **V241** (1.2), **Y267** (8.1) |
| L59 | **F127** (7.8) | **F123** (2.0), **F127** (6.5) |
| G60 | - | - |
| I61 | **L239** (1.5), **I263** (4.5), **Y267** (4.3) | **G245** (2.7), **T247** (1.8), **Q260** (1.8), **I263** (4.3), **T264** (1.9), **Y267** (7.3) |
| M62 | **F123** (7.5), **F127** (4.7), **L239** (1.5) | **F123** (6.6), **H126** (1.6), **F127** (3.4), **F130** (1.6), **V241** (2.1), **Q244** (10.0), **G245** (1.5) |
| L63 | - | - |
| S64 | **R259** (3.4), **Q260** (2.3) | **R259** (1.7), **Q260** (7.0), **I263** (1.4) |
| Y65 | **F123** (6.5), **L239** (6.8), **H240** (2.0), **D242** (3.7), **R243** (15.1), **Q244** (5.5), **R259** (1.0), **Q260** (5.4) | **R116** (4.1), **P117** (2.8), **R243** (1.8), **Q244** (10.4), **G245** (13.4), **G246** (7.0), **T247** (1.4), **Q260** (4.2) |
| I66 | **W120** (3.1), **F123** (3.9) | **T118** (3.6), **F123** (2.9), **Q244** (3.0) |
| R67 | **F256** (1.4), **R259** (1.4) | **R259** (3.6), **Q260** (1.3) |
| S68 | **R243** (3.4), **R259** (2.6) | **R259** (1.5), **Q260** (3.1), **K362** (1.2) |
| K69 | **D242** (1.8), **R243** (2.6) | **R116** (2.9), **T118** (3.8), **R366** (1.0) |
| K70 | **W120** (1.6), **R243** (1.1) | **T118** (1.0), **R366** (1.4) |
| L71 | **R243** (1.2) | **Q359** (1.3), **Q361** (1.0), **K362** (1.8), **N365** (1.1), **R366** (1.1) |

**Supplementary File 1–Table 6:** Biophysical properties of IKs channels formed with KCNQ1 or KCNE1 mutants.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Q1/E1**  **Variant** | **Current**  **(%WT)** | **V1/2app**  **(mV)c,e** | **Slope kc,e** | **Nd** | **Activation τ (sec)** | | | | | | | **Deactivation τ (sec)** | | | | | | | | |
|  |  |  |  |  | 0 mV | 10 mV | 20 mV | 30 mV | 40 mV | 50 mV | 60 mV | -100 mV | -90 mV | -80 mV | -70 mV | -60 mV | -50 mV | -40 mV | -30 mV | -20 mV |
| **KCNQ1a** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| WT | 100.0 | 28.6 ± 0.7 | 11.4 ± 0.2 | 77 / 45 | - | - | 9.94 | 6.92 | 5.53 | 3.84 | 3.52 | 0.11 | 0.12 | 0.12 | - | 0.27 | 0.24 | 0.25 | 0.26 | 0.28 |
| Y267F | 20.0 | **42.1 ± 0.7** | **8.5 ± 0.3** | 61 / 22 | - | - | 5.43 | 4.81 | 2.90 | 2.79 | 3.48 | - | - | - | - | - | - | - | **0.10** | **0.11** |
| W323A | 25.4 | **22.2 ± 0.7** | **15.2 ± 0.8** | 56 / 22 | 6.49 | 4.02 | 3.21 | 2.65 | **2.30** | **1.80** | **1.59** | 0.10 | - | - | - | - | - | 0.24 | 0.24 | 0.26 |
| W323L | 17.8 | **17.3 ± 1.5** | **19.0 ± 1.0** | 61 / 25 | 1.98 | 1.79 | **1.62** | **1.52** | **1.60** | **1.49** | **1.44** | **0.09** | 0.10 | - | - | - | - | 0.30 | 0.38 | 0.33 |
| W323F | 83.3 | 29.1 ± 0.5 | **12.8 ± 0.2** | 72 / 58 | 5.28 | 4.69 | 4.57 | 3.15 | **2.58** | **2.17** | **1.81** | **0.08** | **0.08** | 0.09 | - | **0.17** | **0.19** | **0.22** | 0.27 | 0.28 |
| K362A | 23.2 | **43.3 ± 0.6** | **7.8 ± 0.4** | 65 / 31 | - | - | - | - | - | 8.59 | 7.57 | **0.07** | - | - | - | - | - | **0.10** | **0.11** | **0.12** |
| N365A | 25.4 | **43.3 ± 0.6** | **7.8 ± 0.4** | 67 / 24 | - | - | 4.13 | 3.63 | 2.99 | 2.66 | 2.54 | **0.09** | **0.09** | - | - | - | - | **0.11** | **0.12** | **0.14** |
| **KCNE1b** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| WT | 100.0 | 24.4 ± 0.5 | 13.6 ± 0.1 | 320 / 270 | 6.86 | 6.89 | 7.30 | 5.81 | 4.40 | 3.24 | 2.54 | 0.14 | 0.14 | 0.13 | 0.29 | 0.28 | 0.27 | 0.27 | 0.26 | 0.26 |
| Y46A | 26.2 | **-11.9 ± 5.1** | 15.1 ± 1.6 | 41 / 14 | **1.23** | **1.20** | **1.12** | **1.07** | **1.15** | **1.05** | **0.99** | 0.13 | 0.15 | 0.12 | - | - | 0.42 | 0.41 | 0.42 | 0.29 |
| Y46L | 8.5 | **-3.3 ± 3.1** | 15.8 ± 1.8 | 52 / 14 | 4.70 | **2.71** | **1.71** | **1.45** | **1.33** | **1.18** | **1.61** | 0.13 | 0.15 | 0.13 | - | - | - | - | - | - |
| Y46F | 70.6 | 23.2 ± 1.3 | 13.5 ± 0.4 | 39 / 28 | - | - | 6.87 | 5.80 | 3.47 | 2.94 | 2.19 | 0.12 | 0.12 | - | - | - | 0.24 | 0.24 | 0.24 | **0.22** |
| V50A | 41.4 | 22.0 ± 0.9 | 14.4 ± 0.4 | 74 / 43 | 6.94 | 8.20 | 7.51 | 3.72 | 4.81 | **2.16** | 1.96 | 0.14 | 0.14 | 0.20 | - | **0.36** | **0.37** | **0.38** | **0.38** | **0.38** |
| F56A | 50.3 | 24.9 ± 1.2 | 13.5 ± 0.3 | 42 / 30 | - | - | 12.40 | 7.82 | 4.84 | 3.80 | 2.38 | **0.11** | **0.12** | - | - | - | - | 0.28 | 0.23 | **0.22** |
| F57A | 11.4 | 18.6 ± 3.0 | **17.1 ± 1.0** | 47 / 14 | 6.94 | 4.12 | 4.39 | **2.79** | **2.38** | **1.54** | 2.21 | 0.19 | 0.17 | - | - | - | - | - | - | - |
| F57L | 169.7 | **6.7 ± 1.5** | **15.5 ± 0.3** | 73 / 58 | **0.90** | **0.74** | **0.64** | **0.57** | **0.70** | **0.51** | **0.46** | 0.13 | 0.13 | 0.11 | 0.31 | 0.24 | 0.24 | 0.24 | 0.25 | 0.27 |
| L59A | 48.9 | **29.6 ± 1.2** | **11.9 ± 0.5** | 46 / 29 | - | 13.30 | 8.64 | 6.39 | 4.24 | 3.36 | 2.03 | **0.10** | 0.12 | - | - | 0.22 | 0.19 | **0.19** | **0.21** | 0.22 |
| L63A | 115.8 | 25.3 ± 1.3 | 12.8 ± 0.4 | 30 / 20 | 7.36 | 6.40 | 5.69 | 5.36 | 4.75 | 2.98 | 2.57 | 0.12 | 0.12 | 0.13 | 0.23 | 0.29 | 0.26 | 0.31 | 0.34 | 0.35 |
| Y65A | 55.7 | 28.7 ± 2.7 | 12.1 ± 0.9 | 27 / 18 | 5.62 | 6.00 | **3.30** | **2.66** | **2.48** | **2.10** | 2.03 | **0.08** | **0.10** | 0.11 | - | **0.12** | 0.18 | **0.18** | **0.17** | **0.17** |
| Y65L | 12.3 | **36.7 ± 1.6** | 13.2 ± 1.5 | 66 / 23 | - | - | - | **3.02** | **1.79** | 2.50 | 2.27 | **0.09** | 0.10 | 0.11 | **0.09** | - | - | - | - | - |
| Y65F | 66.0 | **33.8 ± 0.7** | **11.2 ± 0.3** | 34 / 23 | - | - | - | 13.71 | 6.85 | 5.54 | 5.43 | 0.12 | **0.11** | - | - | 0.23 | **0.23** | **0.23** | 0.27 | 0.26 |
| I66A | 97.7 | **32.6 ± 0.6** | **11.3 ± 0.3** | 41 / 32 | 5.91 | 7.10 | 5.52 | 4.11 | 3.41 | **1.98** | **1.44** | **0.08** | **0.10** | 0.11 | - | **0.15** | **0.16** | **0.18** | **0.19** | **0.21** |

a KCNQ1 variants were measured in CHO-K1 cells stably expressing human KCNE1 and transiently transfected with WT or mutant KCNQ1 cDNA (see Methods & Materials).

b KCNE1 variants were measured in CHO-K1 cells co-transfected with human KCNQ1 cDNA and WT or mutant KCNE1 cDNA (see Methods & Materials).

c The V1/2app of activation and slope factor k were obtained by least-squares fitting with a Boltzmann function (mean ± SEM).

d N refers to the number of cells used for determination of peak current / voltage-dependence of activation.

e Bold text indicates variants of KCNQ1 or KCNE1 that were significantly different from WT KCNQ1 or WT KCNE1 in terms of the specified parameter (Student’s t-test, P < 0.001).

**Supplementary References**

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