Supplemental methods and data:

Co-immunoprecipitation of untagged KCNE1 and the LQT5 mutants in CHO-K1 cells- For co-immunoprecipitation experiments CHO-K1 cells were grown to ~70 % confluence in 100 mm dishes. Cells were then transiently transfected with the α-subunit KCNQ1-GFP (10 µg) and or the β-subunit KCNE1 or LQT5 mutants (10 µg) using Lipofectamine (Invitrogen). 48 hours after transfection, the cells were washed once with ice-cold binding buffer (50 mM Tris-Base, pH 7.4) and scraped into 500 µl of ice-cold binding buffer. 50 µl of this cell suspension was then removed and combined with 25 µl of reducing 3 x SDS-PAGE loading buffer (R-STB) to provide a total cell lysate sample. A further 500 µl of ice-cold binding buffer was then added to the cell suspension. To the cell suspension, an equal volume of hypotonic buffer (10 mM Tris-base and 10 mM EDTA, pH 7.4) was added and the samples incubated on ice for ten minutes. After incubation, an equal volume of sucrose buffer (10 mM Tris-base and 500 mM Sucrose, pH 7.4) was then added to restore tonicity. The samples were then homogenised in a glass homogeniser (>60 strokes) and centrifuged at 600xg for 15 minutes at 4 °C to pellet the nuclear fraction. The supernatant was then decanted into ultracentrifuge tubes and centrifuged at 100,000xg for 1 hour at 4 °C to pellet the membranes. Membrane pellets were resuspended in 1 ml of IP-buffer (50 mM HEPES, 100 mM NaCl and 10 % Glycerol) containing 1 % NP-40 and protease inhibitors (Roche) on ice for 30 minutes with short bursts, 5 times for 5 seconds, of sonication if necessary. To pre-clear the membrane preparations 30 µl of Protein G sepharose (Zymed) was added and the samples incubated for 2 hours at 4 °C with end-over-end rotation. After rotation, the samples were centrifuged at 376xg for 3 minutes to pellet the sepharose beads and the supernatant removed and transferred to a fresh tube. To the supernatants 2 µg of α-GFP mouse monoclonal antibody (Roche (11814460001)) was then added and the samples incubated overnight at 4 °C with end-over-end rotation. To precipitate the α-GFP antibody, 20 µl of Protein G sepharose was added and incubated for 2 hours at 4 °C with end-over-end

rotation. The beads were then harvested by centrifugation at 376xg for 3 minutes and the supernatant discarded. After centrifugation, the beads were washed once with 500 µl ice-cold IP-buffer containing 0.1 % NP-40 and three times with 500 µl ice-cold IP buffer containing no detergent. Once washed, the precipitated proteins were eluted from the beads by incubating with 35 µl R-STB for 30 minutes at 37 °C.

Supplemental Figures:

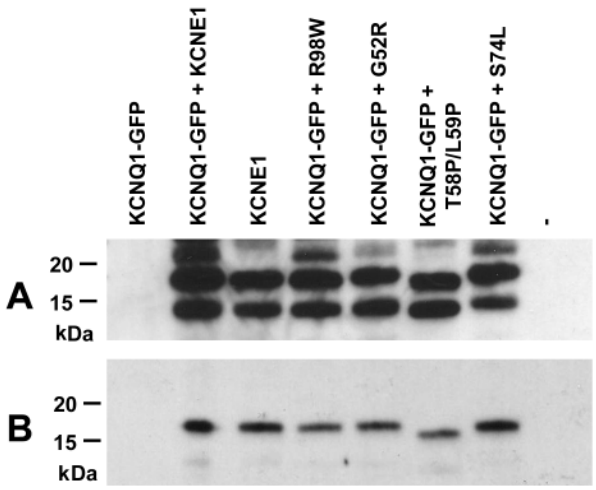

Fig. S1. 3XFlag tagging of KCNE1 and LQT5 mutants provides a more sensitive method for detection of KCNE1/LQT5 mutants than using a polyclonal antibody directed towards the C-terminus of KCNE1. In (*A*) and (*B*), CHO-K1 cells were transfected with 3XFlag-tagged KCNE1 or LQT5 mutants in the presence or absence of KCNQ1-GFP. 48 hours after transfection cells were lysed directly in 3 x R-STB. Equal amounts of each lysate was loaded onto two separate tricine gels. After separation, the proteins were transferred to stable membranes. The membranes were then probed with either; Panel (*A*): α-FLAG (M2) (mouse monoclonal (Sigma) (F3165)) (1:2000) or Panel (*B*): α-KCNE1 (rabbit polyclonal (Alomone) (APC-008) (1:500)) antibodies. The presence of bound primary antibody was then detected using either an anti-mouse-HRP conjugated antibody (Amersham) (1:3000) or an anti-rabbit-HRP conjugated antibody (Santa Cruz Biotechnology) (1:3000) and the blots were exposed for an equal length of time (2 minutes).

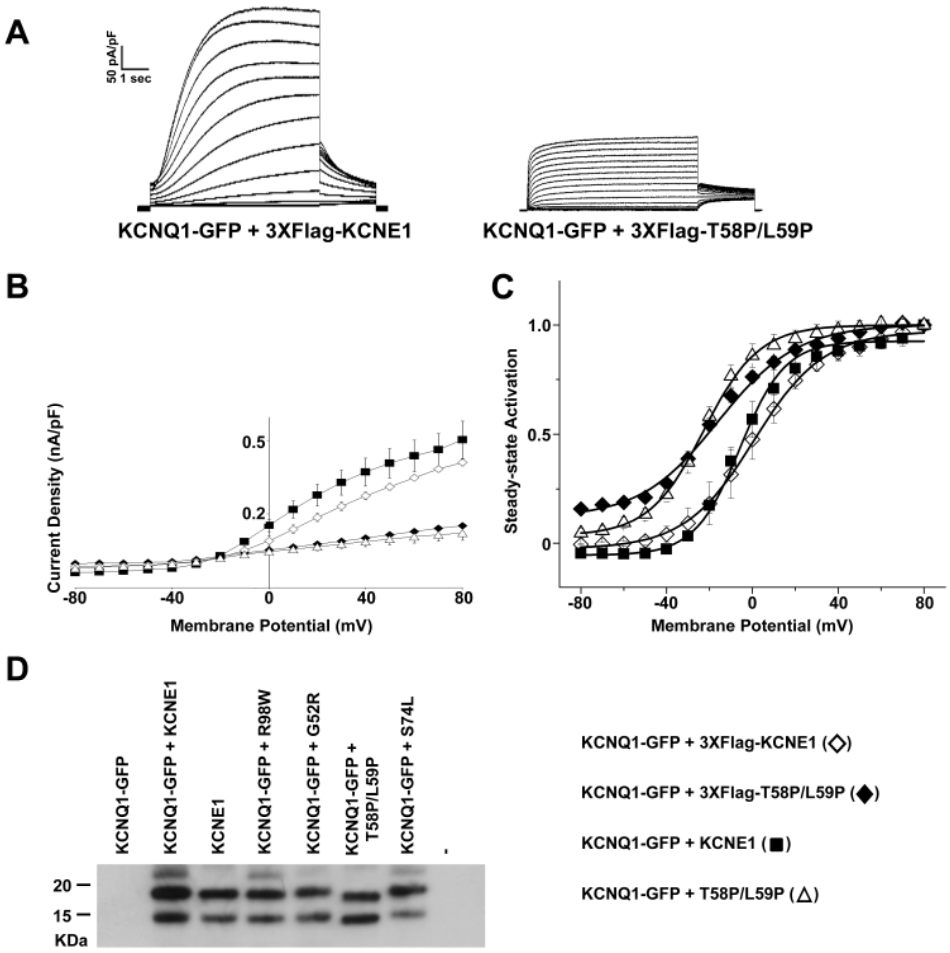

Fig. S2. Patch clamp and expression based characterisation of 3XFlag tagged KCNE1 and LQT5 mutants. (*A*) Representative traces of KCNQ1-GFP expressed in the presence of 3XFlag-KCNE1 or 3XFlag-T58P/L59P that are normalised to cell capacitance. (*B*) Analysis of the effects of 3XFlag tagging KCNE1 and T58P/L59P on maximal current density (normalised to cell capacitance (nA/pF)). (*C*) Normalised voltage-dependent activation curves of KCNQ1-GFP expressed with either 3XFlag-tagged or untagged KCNE1 and T58P/L59P. The activation curves are fitted with Boltzmann functions (solid lines) using non-linear regression. For KCNQ1-GFP + KCNE1, $V_{0.5}$ = -5.83±1.77 and slope factor = 10.5±1.06. For KCNQ1-GFP + 3XFlag-KCNE1, $V_{0.5}$ = 0.84±0.77 and slope factor = 16.2±0.74. For KCNQ1-GFP + T58P/L59P, $V_{0.5}$ = -22.67±1.58 and slope factor = 12.88±1.39. For KCNQ1-GFP + 3X-Flag-T58P/L59P, $V_{0.5}$ = -15.8±1.57 and slope factor = 18.3±1.18. Data are presented as mean ± S.E.M. (for untagged samples n= 5 cells, for Flag-tagged samples n= 3 cells). (*D*) Representative western blot analysis of the expression of Flag-tagged KCNE1 and LQT5 mutants in CHO-K1 cells. The presence of FLAG tagged KCNE1 and LQT5 mutants was detected using a mouse monoclonal antibody (Sigma (F3165)) that recognises the FLAG epitope. - = blank transfection control.