Falin et al., http://www.jgp.org/cgi/content/full/jgp.200810080/DC1

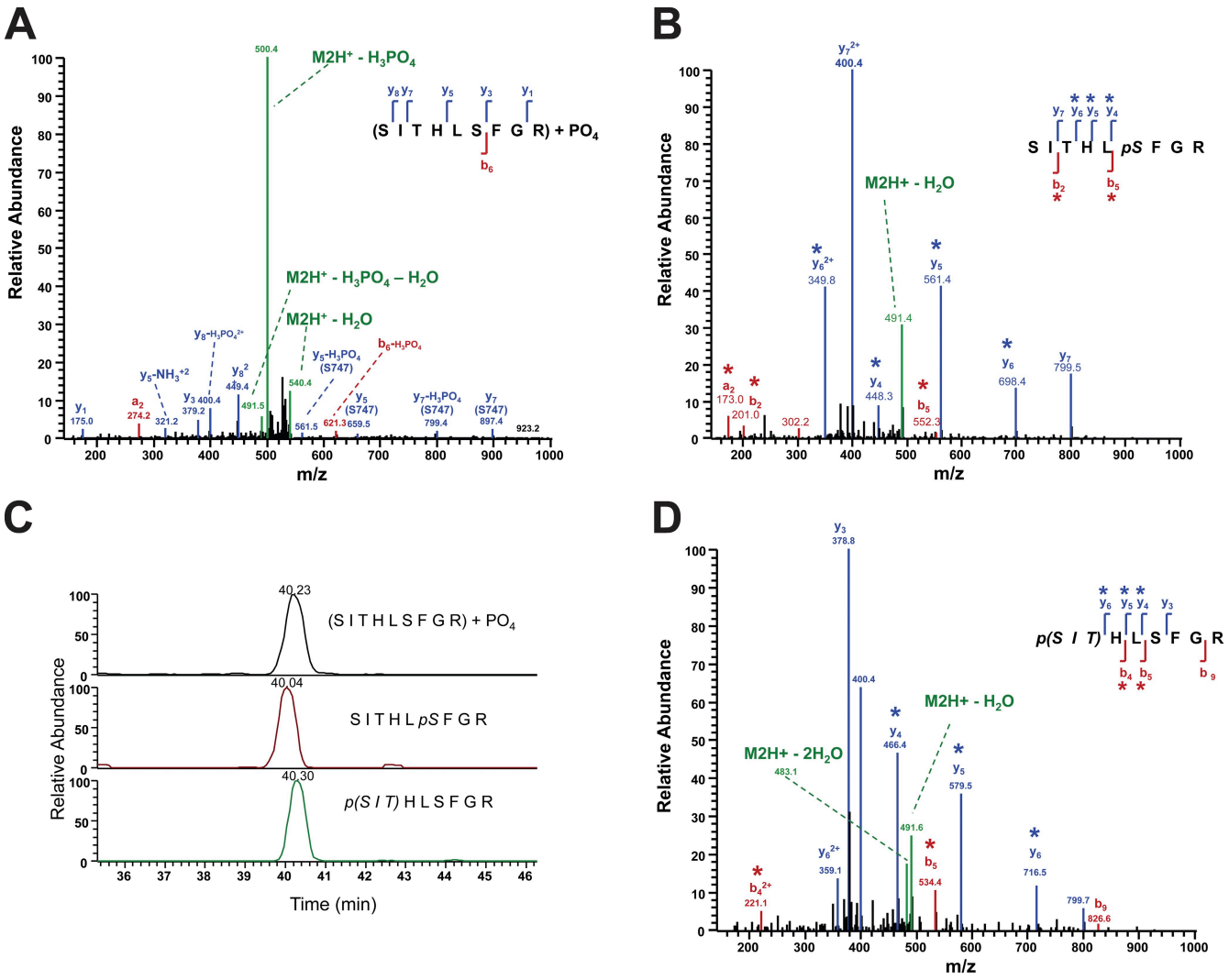

Figure S1. Chromatograms and spectra from targeted mass spectrometry (MS) analysis of the phosphorylated SITHLSFGR peptide. (A) MS/MS spectrum of 549.26 m/z averaged from 40.30–40.62 min (B) MS/MS/MS spectrum of 549.26 to 500.27 m/z averaged from 40.24–40.63 min. Asterisks indicate fragment ions that are specific for the phosphorylation of S747. (C) Extracted ion trace for phosphorylated peptides. Top trace is the MS/MS ion trace of the precursor 549.26 m/z and the extracted fragmentation ion 500.27 m/z that results from the neutral loss of phosphoric acid. The neutral loss of phosphoric acid is indicative of a phosphorylation on the peptide. The middle trace is the MS/MS/MS ion trace of 549.26 to 500.27 m/z transition with the extracted fragment ions 448.2 and 698.4 m/z, which are specific for the phosphorylation on S747. The bottom trace is the MS/MS/MS ion trace of 549.26 to 500.27 m/z transition with the extracted fragment ions 466.2 and 716.4 m/z, which are specific for the phosphorylation of either S742 or T744. (D) Average MS/MS/MS spectrum of 549.26 to 500.27 m/z averaged from 39.45–39.97 min. Asterisks indicate fragment ions that are specific for the phosphorylation of S742 or T744.

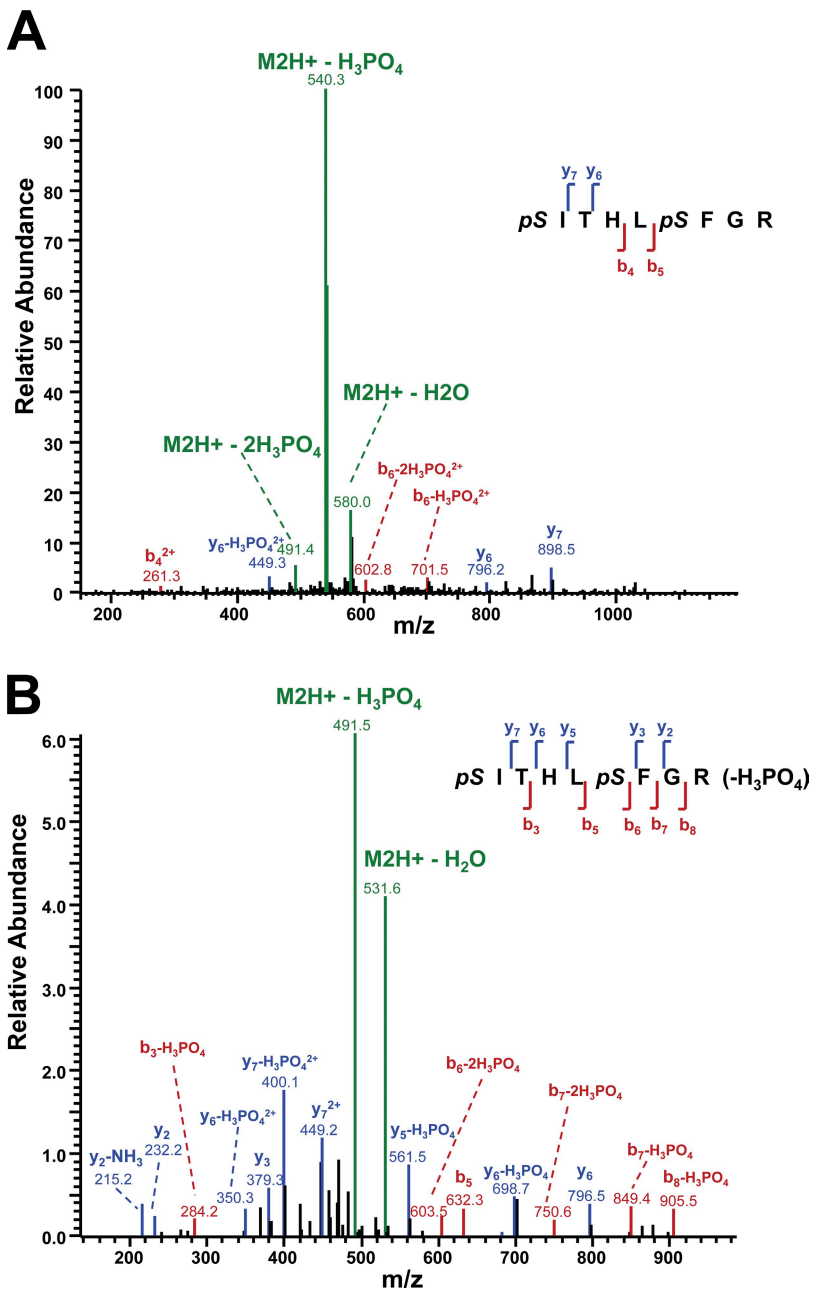

Figure S2. Data-dependent LC-MS analysis of the SITHLSFGR peptide phosphorylated at both S742 and S747. (A) MS/MS spectrum of the precursor 589.4 m/z. The ions at 540.3 and 491.4 m/z result from the neutral loss of one and two phosphoric acid moieties, respectively. The two neutral losses of phosphoric acid are indicative of two phosphorylated amino acids in the peptide. The y6 and y7 ions indicate the presence of phosphorylation at S742 and S747. (B) MS/MS/MS spectrum of 540.3 m/z from the spectrum in A. The ion at 491.5 m/z results from the neutral loss of phosphoric acid from S747. This spectrum further supports the conclusion that both S742 and S747 are phosphorylated.

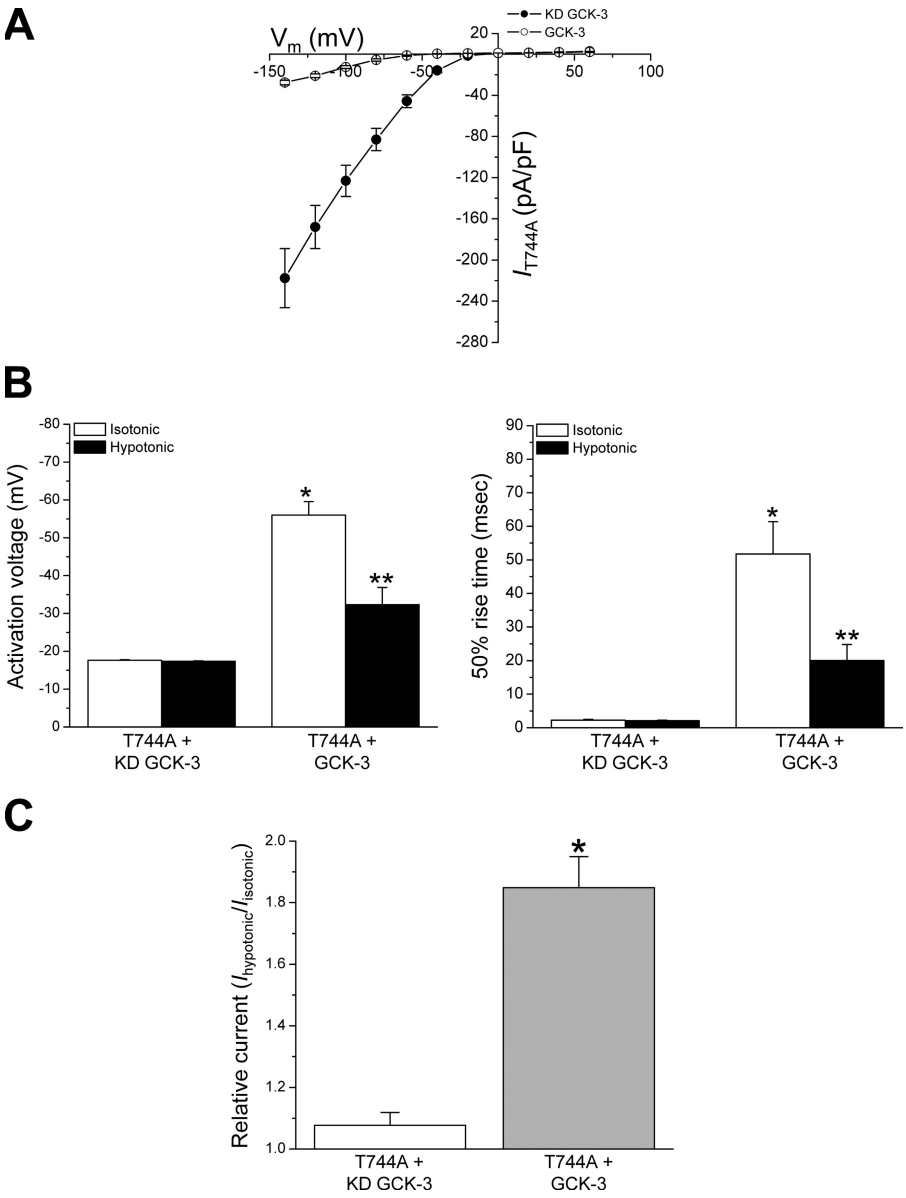

Figure S3. The CLH-3b T744A mutant exhibits wild type channel properties. (A) Current-to-voltage relationships of the T744A mutant coexpressed with functional or KD GCK-3. Coexpression of T744A with GCK-3 significantly ($P < 0.001$) reduced current density over the entire range of potentials where the channels were active. Values are means ± SE ($n = 7–8$). (B) Activation voltages and 50% rise times of whole cell currents in cells coexpressing the T744A mutant and KD GCK-3 or GCK-3. Values are means ± SE ($n = 7–8$). *, $P < 0.001$ compared with KD GCK-3 isotonic; **, $P < 0.002$ compared with GCK-3 isotonic. (C) Effect of cell swelling on current amplitude in cells coexpressing the T744A mutant and KD GCK-3 or GCK-3. Values are means ± SE ($n = 7–8$). *, $P < 0.005$. Data were obtained as described in the legend to Fig. 1.

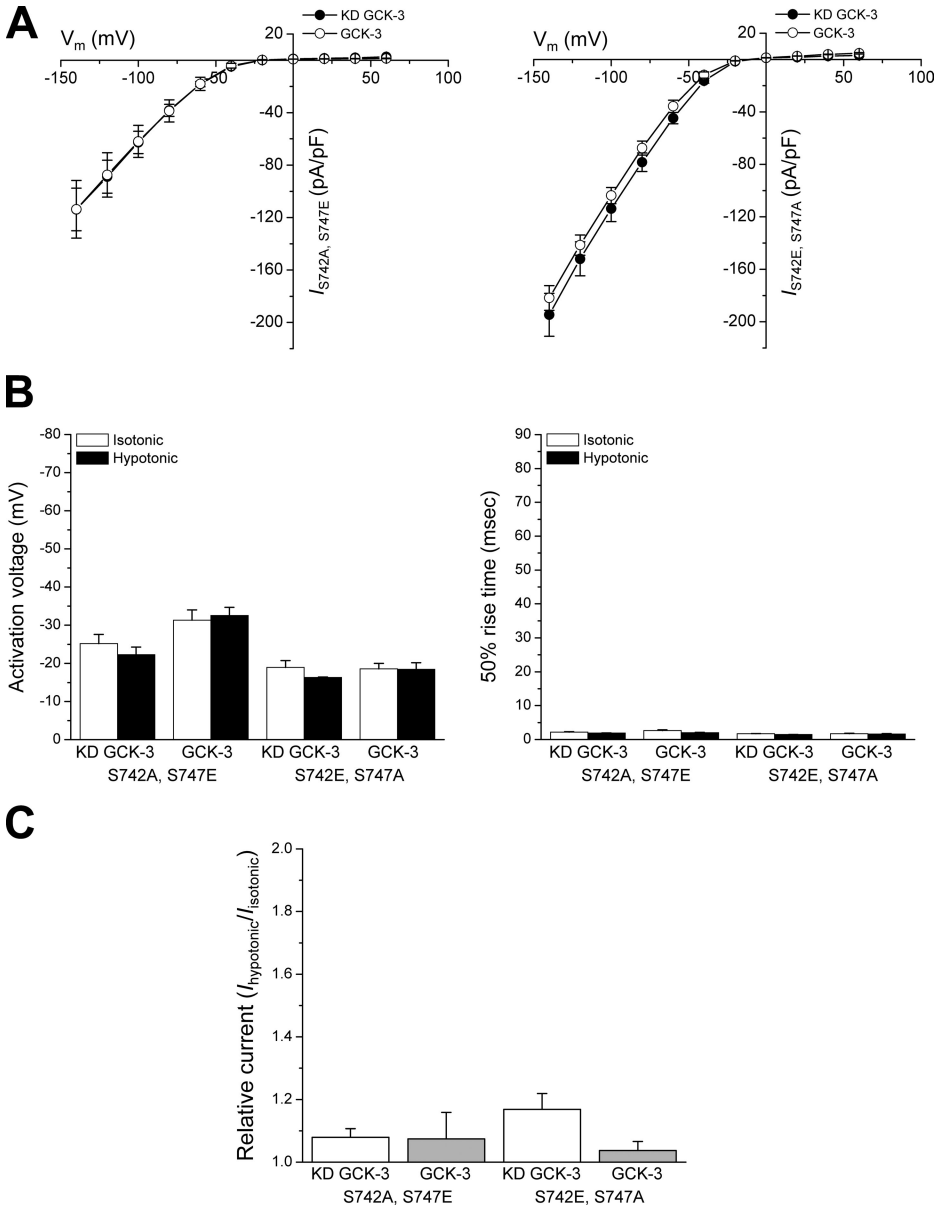

Figure S4. CLH-3b S742A, S747E and S742E, S747A mutants are unaffected by cell swelling and GCK-3. Current-to-voltage relationships of the S742A, S747E and S742E, S747A mutants coexpressed with functional or KD GCK-3. Values are means ± SE (n = 8–10). (B) Activation voltages and 50% rise times of whole cell currents in cells coexpressing the S742A, S747E or S742E, S747A mutants with KD GCK-3 or GCK-3. Values are means ± SE (n = 8–10). (C) Effect of cell swelling on current amplitude in cells coexpressing the S742A, S747E or S742E, S747A mutants with KD GCK-3 or GCK-3. Values are means ± SE (n = 8–10). Data were obtained as described in the legend to Fig. 1.