Supporting Material

Alkaline pH block of CLC-K kidney chloride channels mediated by a pore lysine residue.

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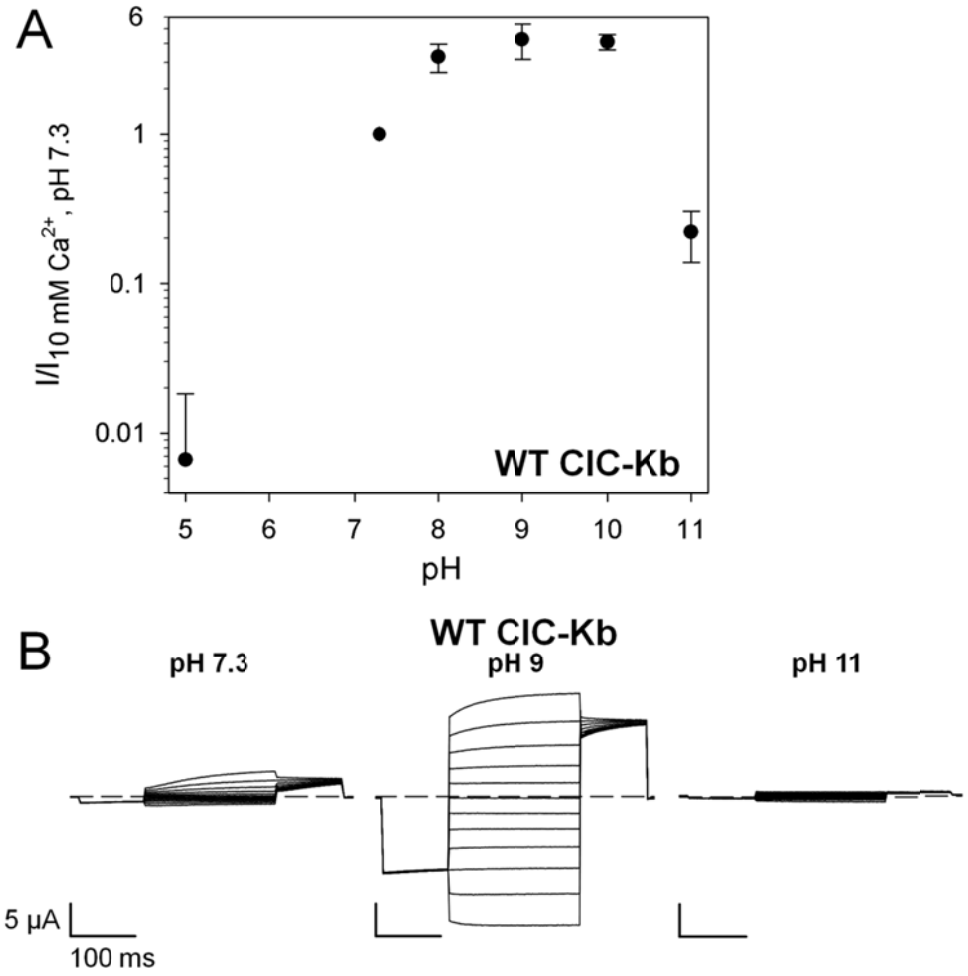

FIGURE S1. Effect of alkaline pH on WT ClC-Kb. ClC-Kb is inhibited by alkaline pH with currents at pH 11 that are ~ 22% of the currents at pH 7.3, but only 5% of the maximum level of current recorded at pH 9. **(**A) Mean currents of ClC-Kb at 60 mV as a function of pH, normalized to the current at pH 7.3 (n = 4, except pH 5 for which n = 3). Error bars indicate SD. (B) Typical current traces of WT ClC-Kb in response to the "IV pulse" protocol at pH 7.3, 9, and 11.

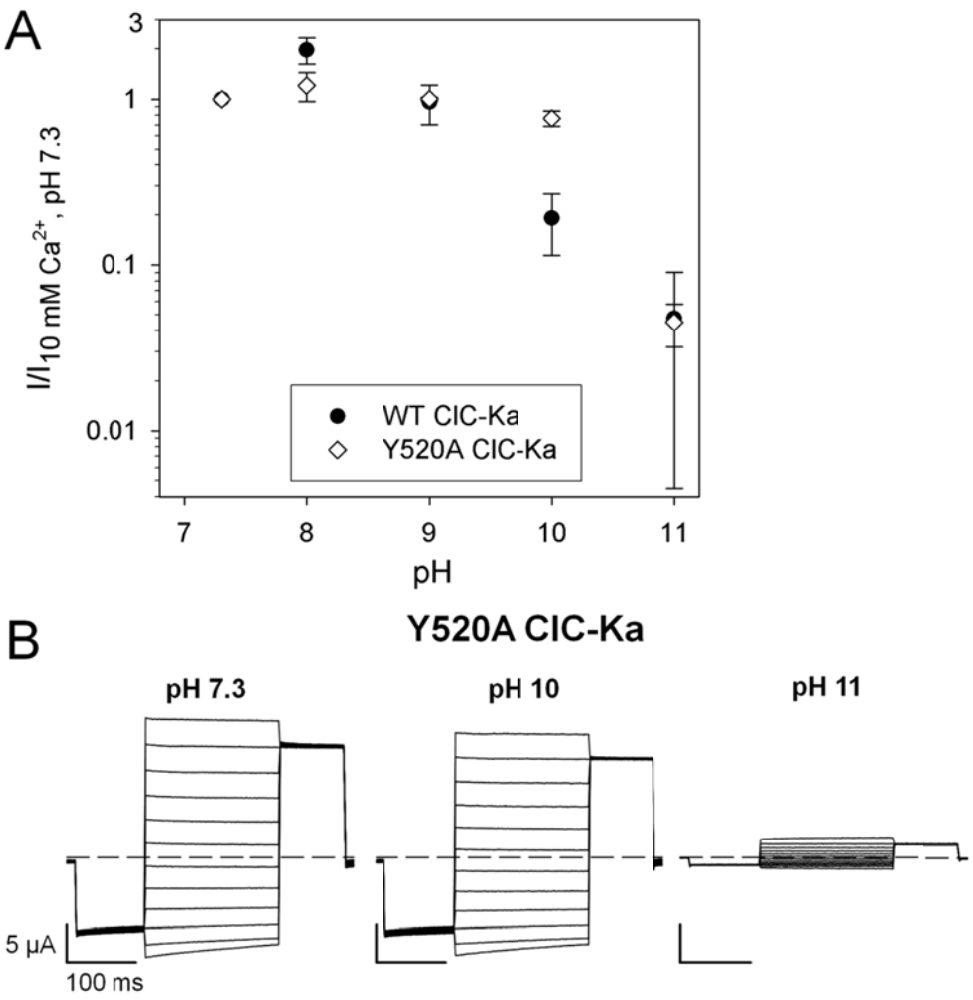

FIGURE S2. Y520A ClC-Ka currents are affected only by extreme alkaline pH. (A) Mean currents of WT ClC-Ka ($n \geq 11$; filled circles) and Y520A ClC-Ka ($n \geq 4$; empty rhombi) recorded at 60 mV were normalized to current at pH 7.3 and plotted versus pH values. Data for WT ClC-Ka are the same as in Fig. 1A. Error bars indicate SD. (B) Representative current traces for Y520A ClC-Ka in response to the "IV pulse" protocol at different pH values.

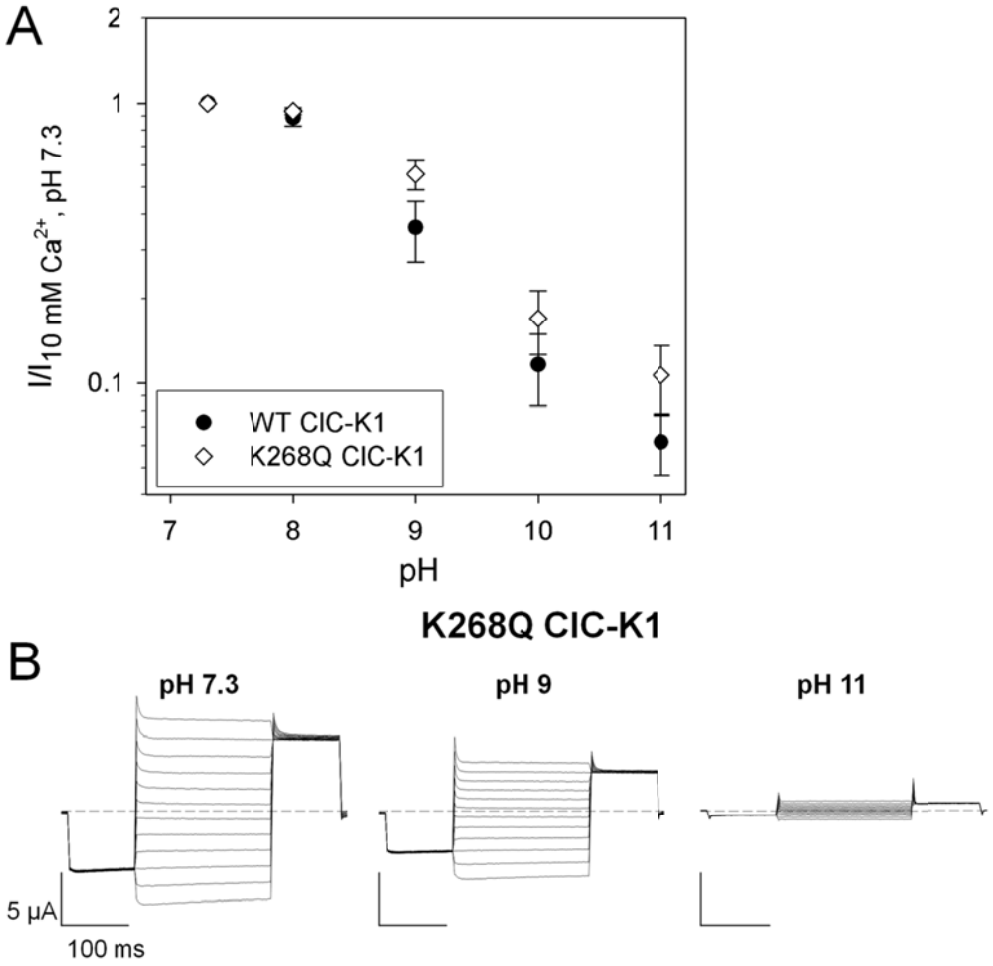

FIGURE S3. K268Q ClC-K1currents are affected by alkaline pH similarly to WT. (A) Mean currents of WT ClC-K1 (n = 10; filled circles) and K268Q ClC-K1 (n = 3; empty rhombi) recorded at 60 mV were normalized to current at pH 7.3 and plotted versus pH values. Error bars indicate SD. Data for WT ClC-K1 are the same as in Fig. 1A, Fig. 5B, Fig. 6C, Fig. 7C, and Fig. 8B. (B) Current traces of K268Q ClC-K1 in response to the "IV pulse" protocol at different pH values.

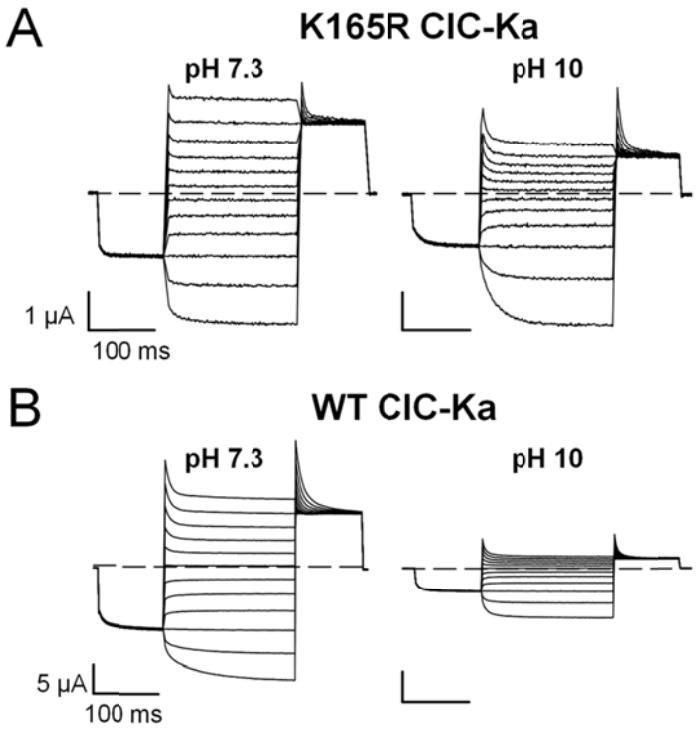

FIGURE S4. The mutant K165R ClC-Ka is less affected by alkaline pH than WT. At all pH tested this mutant shows a minor response to alkaline pH, for example at pH 10 the K165R currents are ~ 50% of the currents in control conditions, while the currents of WT ClC-Ka are only ~ 19% of the current at 7.3. Voltage clamp traces of oocytes expressing K165R ClC-Ka (A) and WT ClC-Ka (B) in response to the "IV pulse" protocol (see Materials and Methods) at pH 7.3 and 10.

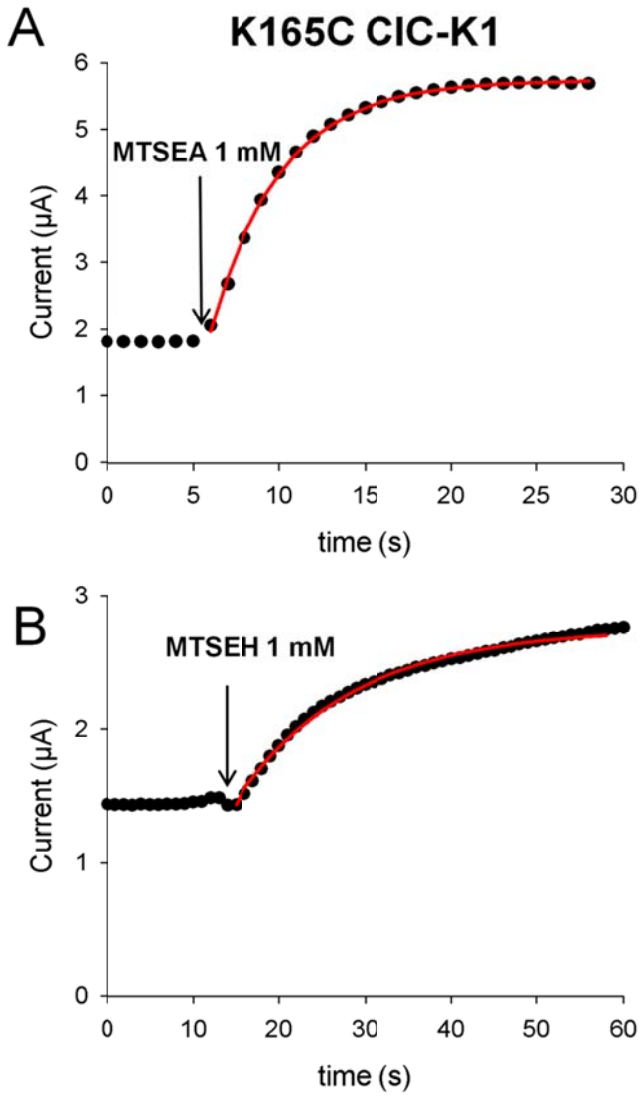

FIGURE S5. Modification by MTS reagents of K165C ClC-K1. The channels were stimulated with repetitive pulse to 60 mV and 1 mM MTS reagent was continuously applied. The current response of the mutant was plotted as function of time. Red lines represent exponential fits. (A) For MTSEA time constant τ = (4.9 +/- 1.0) s and reaction rate k = (206 +/- 40) s^{-1} M^{-1} came from n = 5 experiments. (B) For MTSEH τ = (8.9 s +/- 1.8) s, k = (112 +/- 23) s^{-1} M^{-1} were estimated by n = 3 experiments. All errors are SD.