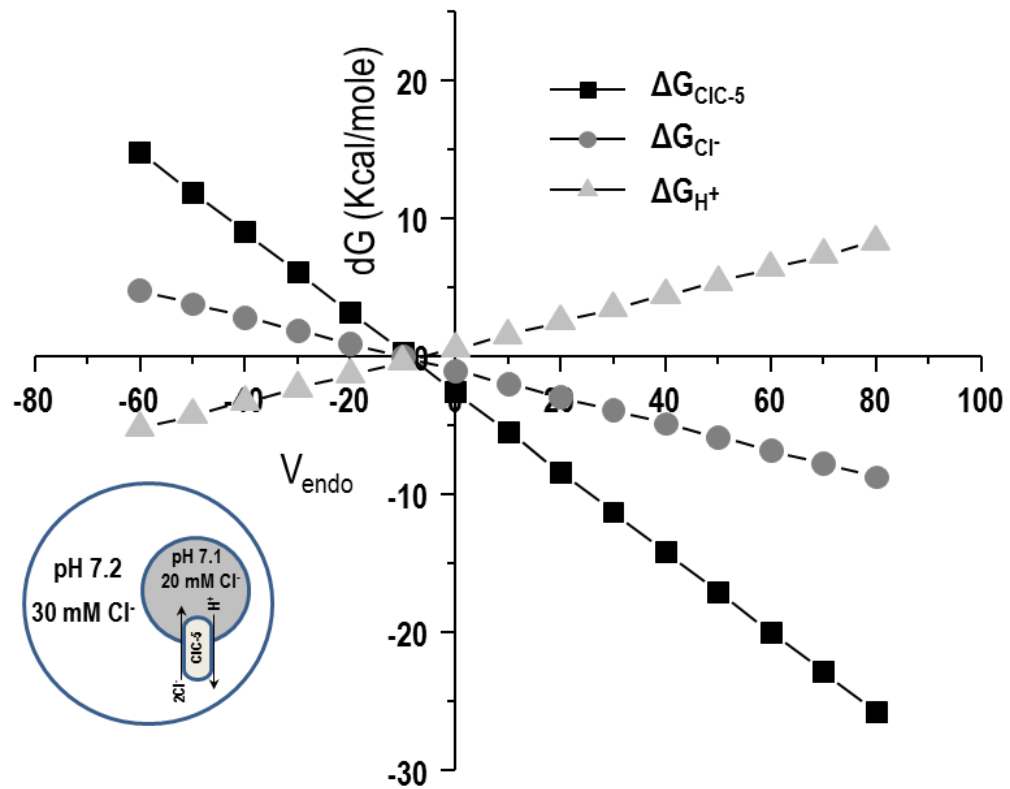


**Supplementary Figure 1. Quantification of CIC-5 (EGFP) colocalization with early endosomes marker (EEA1-Red) in renal epithelial cells transfected with CIC-5 WT or mutants.** CIC-5<sup>+</sup>/EEA1<sup>+</sup> double positive pixels were measured using ImageJ and normalized to total EEA1<sup>+</sup> pixels. CIC-5 WT, S244L, and R345W strongly colocalized with EEA1, while expression of Q629\* in early endosomes was significantly blunted. \*\*\*, p<0.001.



**Supplementary Figure 2.** Hypothetical driving force of CIC-5 transport at early endosome estimated using Gibbs free energy equation in relation to various endosomal membrane potential ( $V_{\text{endo}}$ ). The total free energy for CIC-5 transport  $\Delta G_{\text{CIC-5}} = 2x(\Delta G_{\text{Cl}^-}) - 1x(\Delta G_{\text{H}^+})$ ;  $[\text{Cl}^-_{\text{out}}] = 30 \text{ mM}$ ,  $[\text{Cl}^-_{\text{in}}] = 20 \text{ mM}$  and  $\text{pH}_{\text{out}} = 7.2$ ,  $\text{pH}_{\text{in}} = 7.1$ .