



**Figure S1. Potentiation of the current of E166A<sub>CLC0</sub> by intracellular Mg<sup>2+</sup>.** (A) Nearly complete current block of E166A<sub>CLC0</sub> by 10 mM CPA at -160 mV to ensure that the majority of the current in the recording patch was from the CLC-0 mutant. Horizontal dashed line indicates zero-current level. Inset shows the voltage protocol (from 0 mV to -160 mV) used to induced the current. (B) Representative traces showing the effect of 5 mM (upper panel) and 20 mM Mg<sup>2+</sup> (lower panel) on the current of E166A<sub>CLC0</sub> at +20 mV. Inset shows the voltage protocol (0 mV to +20 mV) used for the experiments. Dashed lines are the zero-current level. (C) Dose-dependent Mg<sup>2+</sup> potentiation of the E166A<sub>CLC0</sub> current (n = 3). The solid green line represents the Mg<sup>2+</sup>-dependent potentiation of WT<sub>16A</sub>.