

Supplementary Information Zdebik *et al.*

Supplementary Figures

Figure S1

Currents and proton transport in oocytes expressing WT CIC-4 (A-C) and its 'proton glutamate' mutant E281A (D-F). Voltage was clamped in steps of 20 mV from -100 to +80 mV. Original voltage clamp traces are shown in (A) and (D) for WT CIC-4 and the E281A mutant, respectively. Averaged steady-state current-voltage (I/V) curves are given in (B) (CIC-4) (n=10) and (E) (CIC-4_{E281A}) (n=7). (C) (WT) and (F) (E281A) show extracellular pH changes observed before and during a depolarizing pulse trains to + 80 mV. Consistent with the data obtained with CIC-5 (Fig. 1 G, H, main text), E281A gave neither currents nor H⁺ transport. H⁺-transport was studied in 8 and 5 oocytes expressing WT and E281A mutant CIC-4, respectively.

Figure S2

Western blot analysis of total expression levels in *Xenopus* oocytes of CIC-5 and key mutants of the 'proton glutamate' E268, as indicated at the bottom of the lanes. All constructs were tagged with an extracellular HA-epitope as described by Schwake *et al.* (2001) (*J Biol Chem* **276**, 12049-12054). Expression times in hours are stated below, and apparent molecular weights are given in the free lane separating results from different expression times. CIC-5 proteins were detected with an antibody directed against the HA-epitope and detection of rab 4 (after stripping the membrane) served as loading control. CIC-5 appears as a doublet, probably due to differences in glycosylation. The gating glutamate mutations did not change expression levels within the experimental error.

Figure S3

Cl⁻, but not H⁺ transport is restored to the CIC-4 'proton glutamate' E281A mutant by additionally inserting the uncoupling mutation in the 'gating glutamate' (E224A). (A) I/V curves of the double mutant with different anions (n=12). SCN⁻ changed dramatically both the shape and the reversal potential of the

currents. Similar, but smaller currents were also observed in non-injected oocytes and mock-transfected cells (data not shown). We therefore assume that part of the current in the presence of SCN^- is endogenous. *Inset*, voltage clamp trace obtained with Cl^- . **(B)** Changing pH_o affects neither conductance nor reversal potentials, in accord with the lack of H^+ transport observed in Fluorocyte measurements **(C)**(n=14). Pulses from -60 mV (100ms) to +90 mV (400 ms) were applied repetitively as indicated by bars. Data are almost identical to the results obtained for CIC-5 (shown in Fig. 4 in the main text).

Figure S4

Average I/V curves in the presence of extracellular Cl^- , NO_3^- , or SCN^- for uninjected oocytes (n=10) (A), oocytes expressing the 'proton glutamate' mutant CIC-5_{E268A} (n=14) (B) and the mutant CIC-5_{E268D} (n=16) (C). Oocytes were from 2 batches in which at least 5 μA of WT CIC-5 currents were observed at +100 mV. Uninjected oocytes showed currents of comparable magnitude as oocytes injected with CIC-5_{E268A}. Although NO_3^- transport is largely uncoupled from H^+ -fluxes in WT CIC-5, it is not detectably transported by the E268A mutant that is thought to disrupt H^+ transport to the Cl^-/H^+ -exchange site. Significant, but smaller currents are observed with all anions in the conservative E268D mutant.

Figure S5

CIC-0 and CIC-1 are not converted into Cl^-/H^+ transporters by inserting a 'proton glutamate'. When the valine which they carry in the position of the "proton glutamates" in the transporter CLCs is mutated to glutamate. **(A)**, typical I/V trace for CIC-0_{V227E}, **(B)**, Fluorocyte recording of this mutant (n=5). No change in intracellular fluorescence was seen upon activation of the pulse protocol.

Table 1

Reversal potential changes measured upon changes of extracellular pH. Results were obtained from voltage-clamp experiments conducted on the constructs indicated in the first column. The last column indicates the expected change for a Cl⁻/H⁺ exchanger with a 2:1 stoichiometry (calculated as $\Delta E_H/3$, where ΔE_H is the change in the Nernst potential for protons). The measured values are clearly inconsistent with H⁺ transport activity.

Channel	pH change	ΔE_{rev} (mV) (mean \pm SD (n))	Expected ΔE_{rev} for a 2Cl ⁻ :1H ⁺ exchanger
ClC-0	7.4 to 5	0.3 \pm 0.2 (15)	46 mV
ClC-0 _{V227E}	7.3 to 6	0.1 \pm 0.1 (2)	25 mV
ClC-0 _{V227E}	7.5 to 5	1.8 \pm 0.6 (15)	48 mV
hClC-1 _{V292E}	7.3 to 6	0.5 \pm 0.3 (2)	25 mV
hClC-1 _{V292E}	7.3 to 5	0.7 \pm 0.5 (6)	44 mV

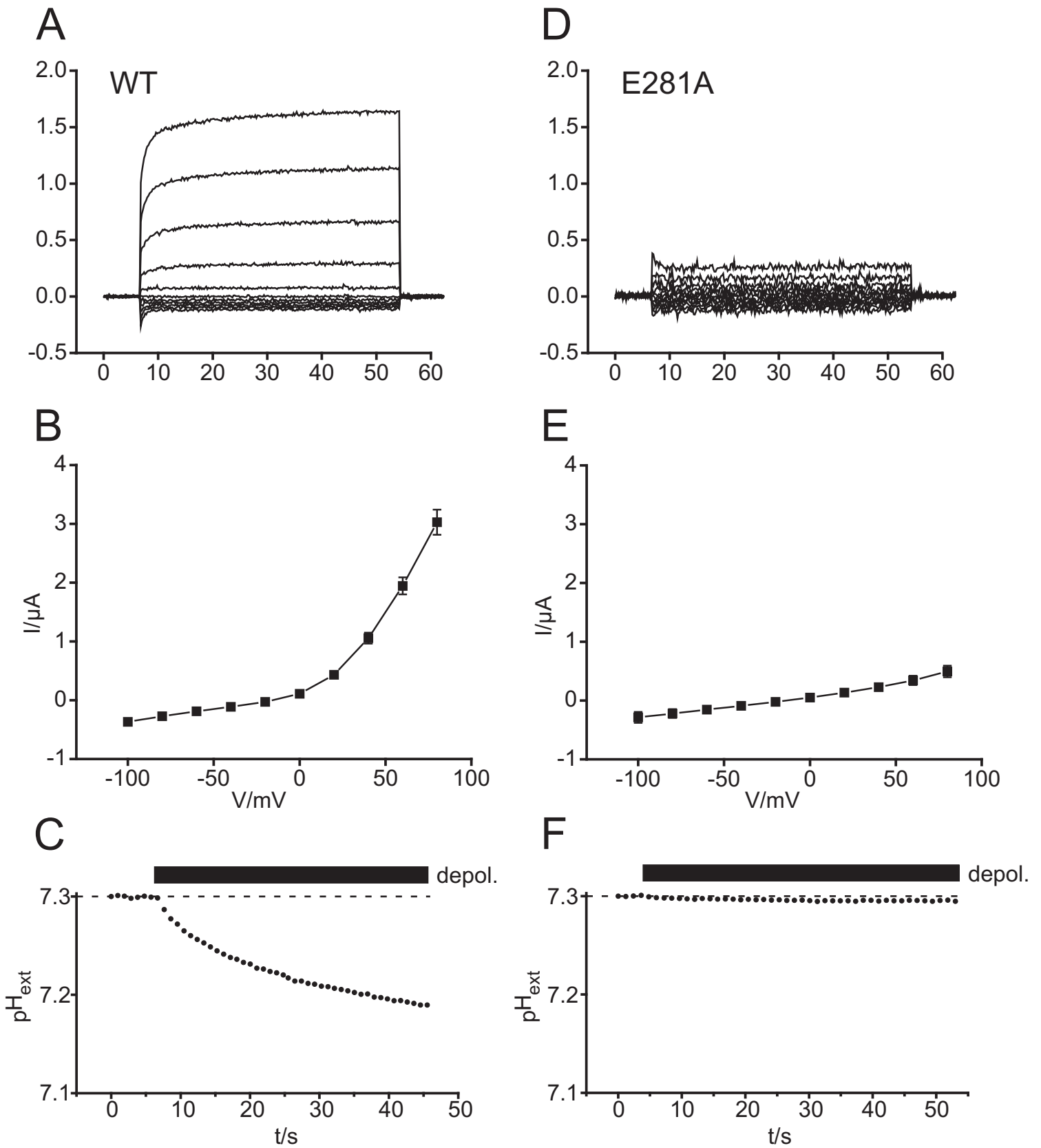


Fig. S1

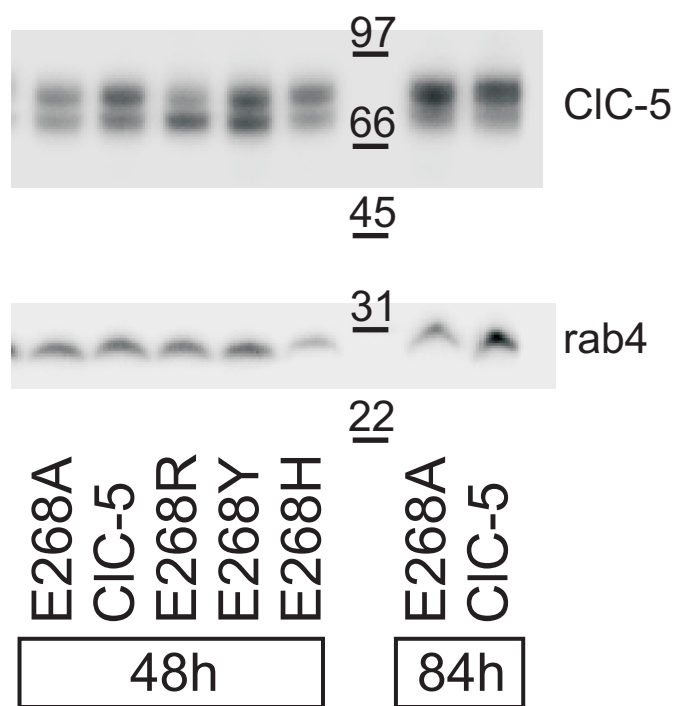


Fig. S2

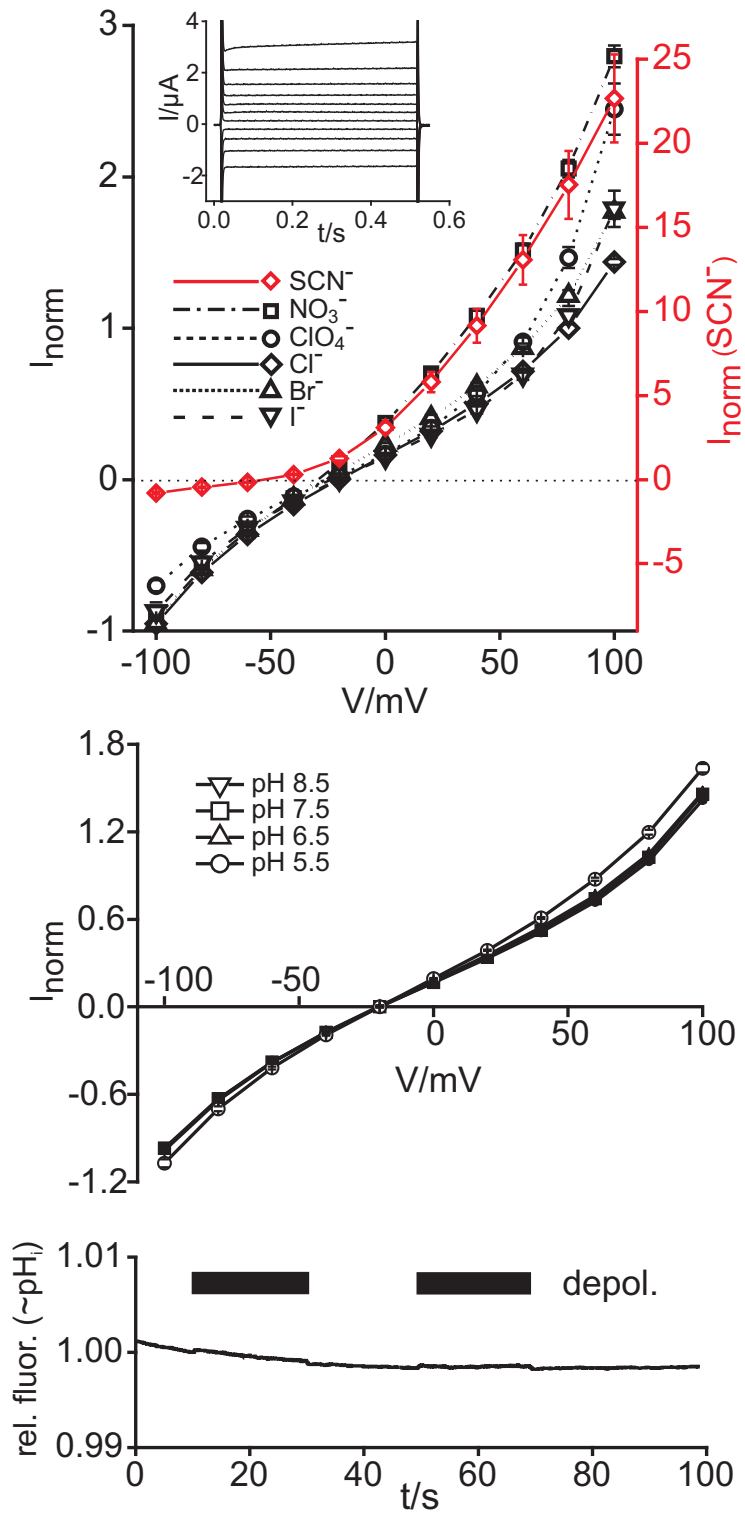


Fig. S3

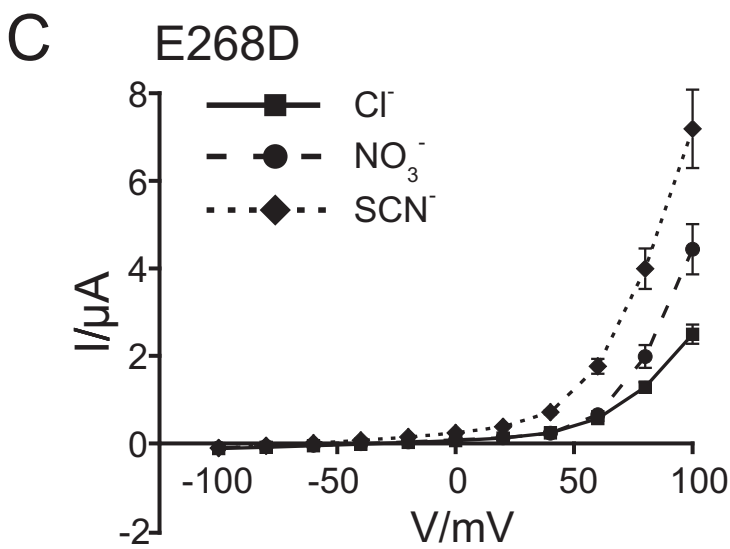
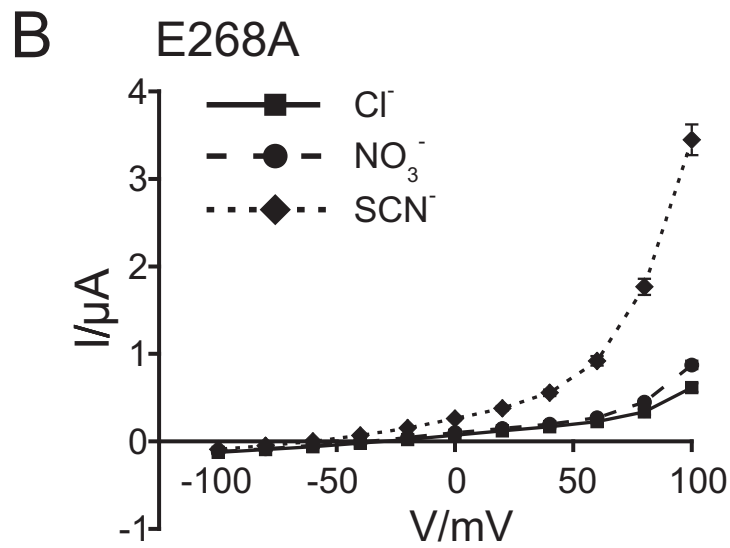
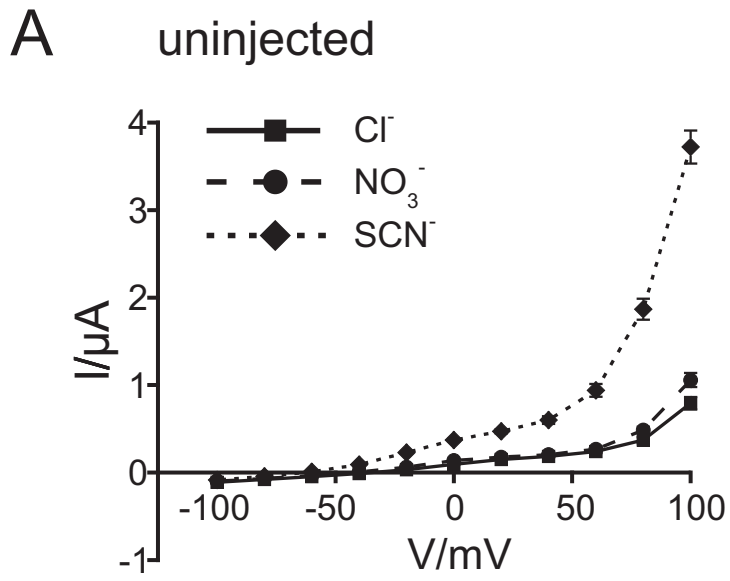
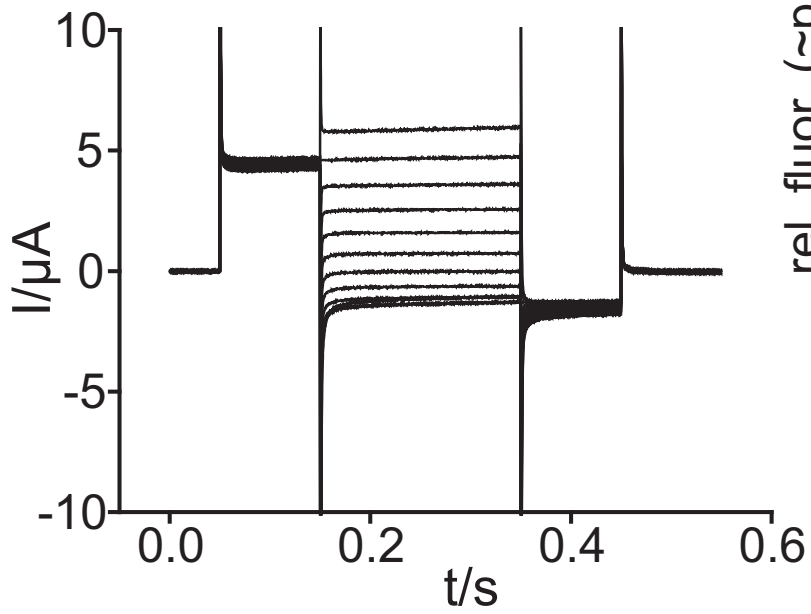


Fig. S4

A CIC-0_{V227E}



B

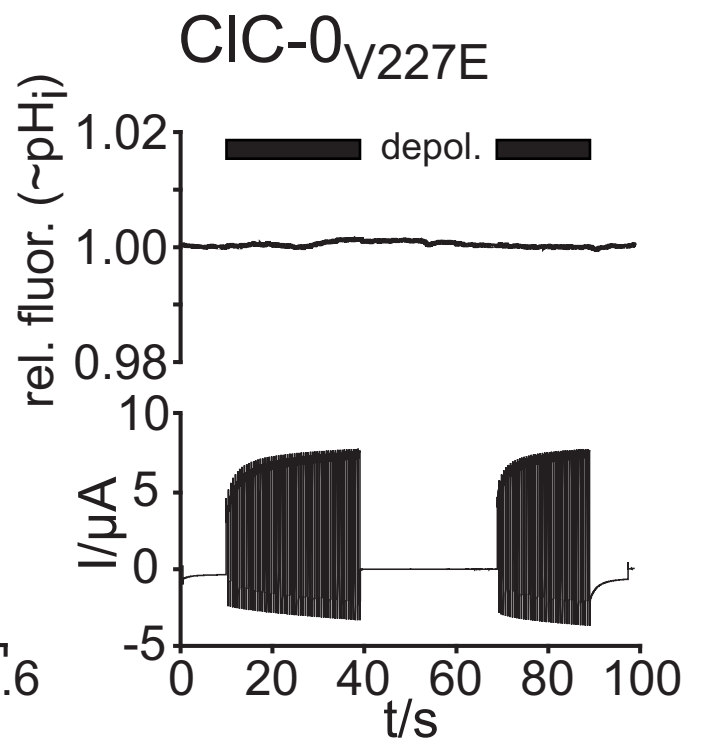


Fig. S5