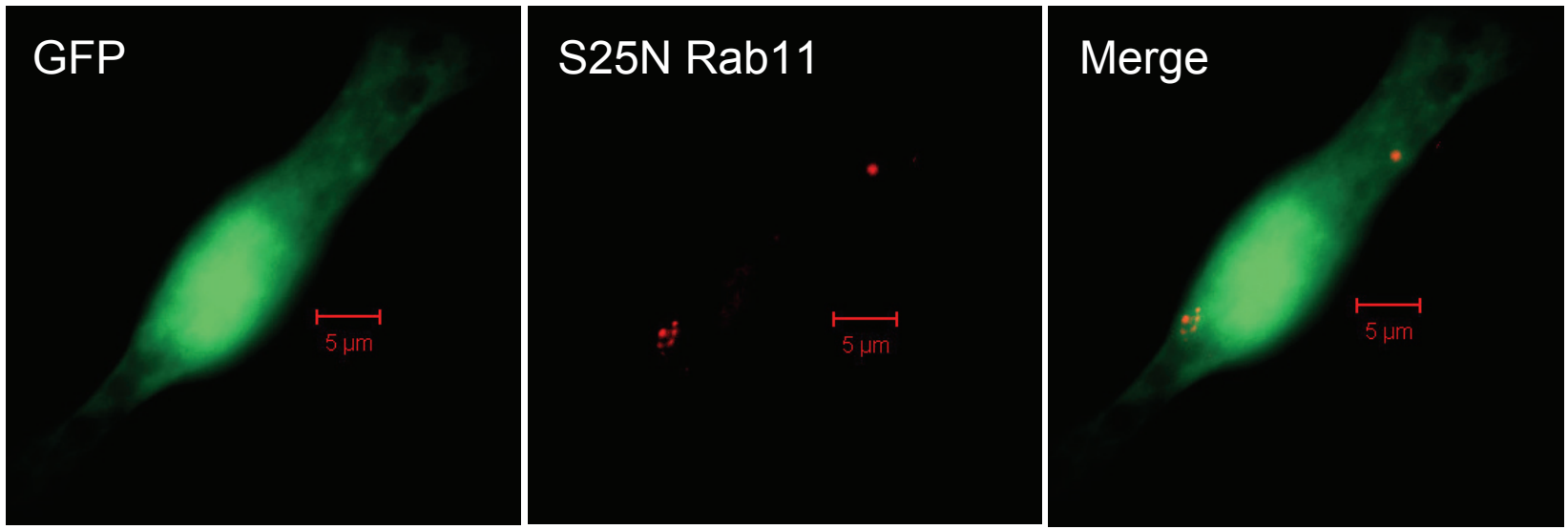
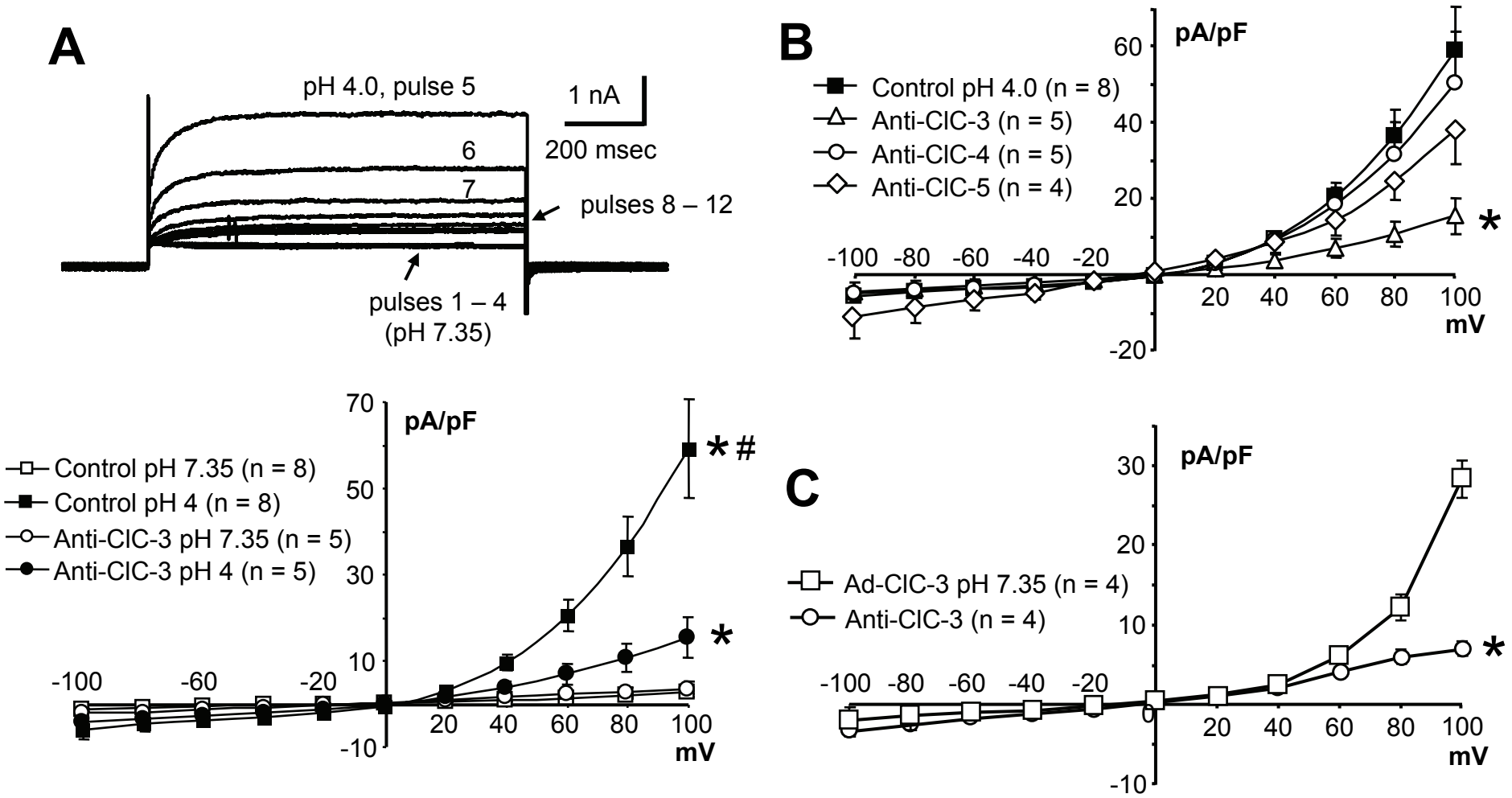


GFP = green
S25N Rab11-myc = red

Supplemental Figure 1.



Supplemental Figure 2.

Supplemental Figure 1. GFP and myc-tagged S25N Rab11 are both expressed in VSM cells co-transfected with plasmids encoding the two proteins. Although transfection efficiency was very low (roughly 0.5 - 1%), cells expressing GFP consistently also expressed the myc tag in a pattern consistent with the distribution of Rab 11 to intracellar vesicles. See the Methods section for transfection protocol with GFP and S25N Rab11-myc plasmids. For microscopy cells were grown on 2 well chamber slides. 48 hours after transfection they were fixed with 4% paraformaldehyde for 20 min, washed and permeabilized in PBS with 1% bovine serum albumin, 10% NGS and 0.1% Triton at room temperature for 45 min. Immunolabelling of the myc epitope was performed by incubation with a rabbit anti-myc antibody (GenScript, A00172-1) at a titer of 1:300 for one hour at room temperature. Immunostaining was performed by a one hour incubation in a 1:200 dilution of a goat anti-rabbit antibody conjugated to a Texas Red label (Invitrogen, T-6391). Imaging was performed by confocal microscopy (Zeiss LSM).

Supplemental Figure 2. Anti-ClC-3 antibody inhibits both the endogenous acid-induced anion current and the current induced by ClC-3 overexpression at neutral pH. Low pH activates a current that likely represents endogenous ClC-3 (2). If anti-ClC-3 antibody (30 μg/ml) is present in the pipette solution this current is inhibited in what appears to be a use-dependent manner. (A) The top panel shows superimposed currents induced by sequential depolarizing pulses (holding potential -40 mV, test potential +100 mV, pulses delivered every X seconds) before (pulses 1- 4) and after lowering extracellular pH to 4.0 (pulses 5 – 12). Pulse number 5 elicited a robust current which subsequently diminished with each successive depolarization until the response stabilized at a very low level. The I/V plot in the bottom panel summarizes this effect from multiple cells. The anti-ClC-3 pH 4.0 data (black circles) represents the size of the current after it stabilized at a reduced size. (B) Comparison of the effects of antibodies specific for ClC-3 -4, and -5 at identical concentrations (30 μg/ml). Only anti-ClC-3 significantly impaired the current activated by pH 4.0. (C) ClC-3 current can be induced in HEK293 cells at pH 7.35 by ClC-3 expression using adenovirus. These currents are also inhibited by anti-ClC-3 antibody. METHODS: HEK293T cells (adenoviral propagation-resistant) were obtained from the American Tissue Culture Collection and maintained in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Atlanta Biological). Pipette solution for dialyzed whole cells recording and bath solutions were the same as previously described (1,2). Anti-ClC antibodies were all rabbit polyclonals directed at epitopes in the intracellular C-terminus of the respective proteins. Anti-ClC-3 was obtained from Alomone Labs (ACL-001), anti-ClC-4 (CLC41-A) and anti-ClC-5 (CLC51-A) were obtained from Alpha Diagnostic.