SUPPLEMENTAL MATERIAL

Pinelli et al., http://www.jgp.org/cgi/content/full/jgp.201611623/DC1

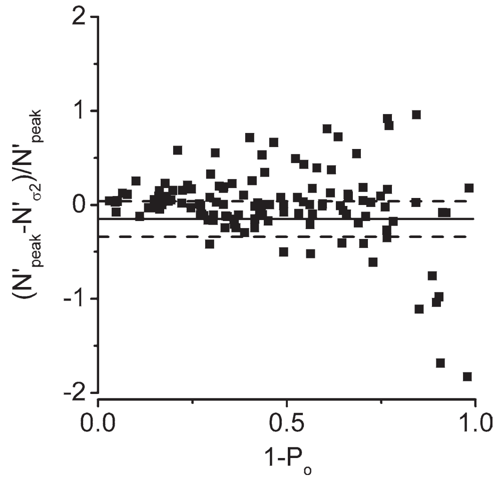

Figure S1. **Comparison of two methods for estimating the number of active channels on patches.** The number of active channels (N′) was determined in each stretch of data from (1) the peak current amplitude ($N'_{peak} = I_{peak}/i$) and (2) the variance of the patch current ($N'_{\sigma 2}$) as described in Materials and methods. The graph is a modified Bland Altman analysis where $(N'_{peak} - N'_{\sigma 2})/N'_{peak}$ is plotted against $1 - P_o$ ($=\sigma^2/\langle I \rangle * i$). Each point is a measurement from a stretch of recording at either V_m −80 mV or V_m 80 mV. The continuous line is the mean of 130 determinations, and 95% agreement interval limits (mean ± 1.96*SEM) are represented as dashed lines. Only recordings yielding values within the 95% agreement interval where taken as valid for the estimation of N′ and P_o.

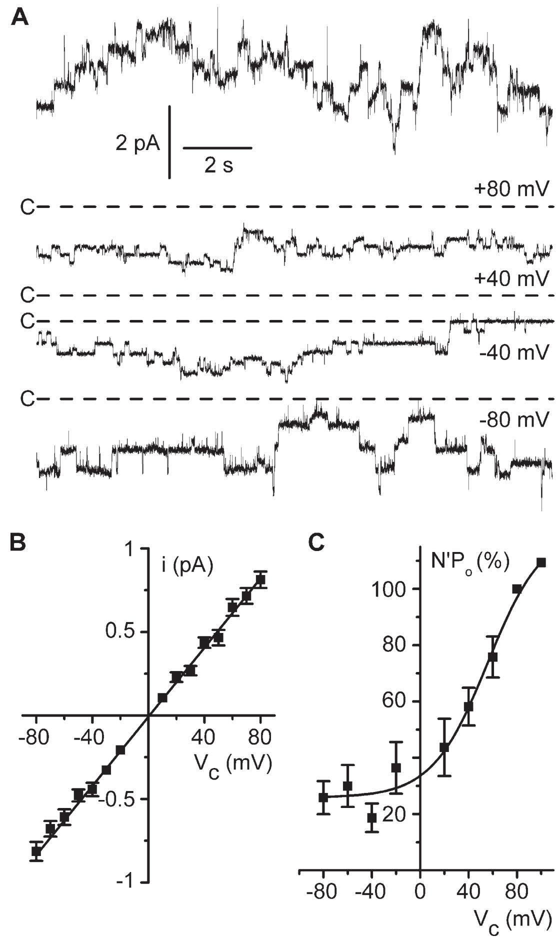

Figure S2. **Channel voltage dependence in the cell-attached configuration.** (A) Representative current recordings at the clamp membrane potentials (V_c) values indicated on the right side of each trace. CNTs were bathed in physiological saline solution, and pipettes were filled with a 5 mM Ca^{2+}–containing NMDG-Cl solution set at pH 7.4. The dashed lines (C-) indicate the closed channel current level for each clamp potential, measured after acidification of the intracellular compartment as described in Materials and methods. (B) Mean i/V_c relationship obtained in the condition given in A. Each point is the mean of 12 determinations, and SEM is shown as error bars when larger than symbols. (C) N′P_o/V_c relationship obtained in the conditions given in A. N′P_o values were normalized to values at V_c 80 mV on the same patch. Each point is the mean of nine measurements, except at V_c 100 mV where *n* = 2, and SEM is shown as error bars. The line is a nonlinear least squares fit of mean data points using the Boltzmann equation.

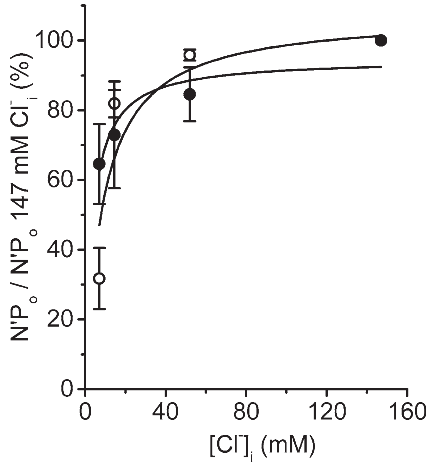

Figure S3. **Channel sensitivity to intracellular chloride concentration.** Cell-excised inside-out membrane patches were exposed to 7–147 mM internal Cl⁻ solutions, and currents were recorded at V_m −80 mV (●) or V_m 80 mV (○). Each N′P_o value at a given internal Cl⁻ concentration was normalized to the paired N′P_o at 147 mM Cl⁻ on the same patch. Data are means of measurements from three to seven patches, and SEM is shown as error bars. Lines are fits of mean data points using a rectangular hyperbola equation yielding $K_{1/2}$ values of 3.6 ± 1.3 mM internal chloride at V_m −80 mV (R^2 = 0.879) and 9 ± 5.5 mM internal chloride at V_m 80 mV (R^2 = 0.832).

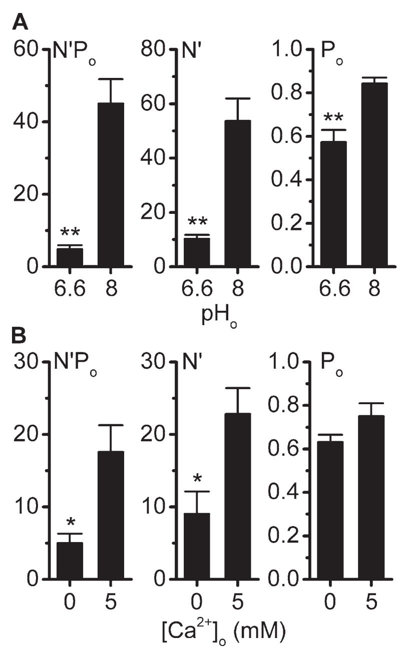

Figure S4. **pH_o and $[Ca^{2+}]_o$ also modulate the number of active channels.** Experiments were performed under symmetrical NMDG-Cl solutions, at V_m 80 mV. (A and B) N′P_o was measured within 2 min after patch excision into a pH_i 7.4 and calcium-free solution under either pH_o 6.6 or 8 ($[Ca^{2+}]_o$ 5 mM; A) or external calcium-free or 5 mM Ca^{2+} conditions (pH_o 7.4; B). The number of active channels (N′) was determined by peak current measurements and validated by stationary noise analysis (see Materials and methods), and only recordings yielding ΔN′/N′ values within the 95% agreement interval were taken as valid (pH_o 6.6: *n* = 5 out of 7; pH_o 8: *n* = 6 out of 7; $[Ca^{2+}]_o$ 0: *n* = 3 out of 5; $[Ca^{2+}]_o$ 5 mM: *n* = 6 out of 6). Results are given as means, and SEM is shown as error bars. *, P < 0.01; **, P < 0.005, unpaired Student's *t* test.

Table S1. **Effects of pH_i, pH_o, and $[Ca^{2+}]_o$ on single-channel conductive properties**

Condition	**g**	$\mathbf{E_{rev}}$
	pS	*mV*
pH_i 7.0 (7)	11.4 ± 0.3	0.1 ± 0.3
pH_i 7.4 (12)	13.0 ± 0.5	1.0 ± 0.4
pH_i 7.8 (6)	11.6 ± 0.3	0.5 ± 0.4
pH_o 6.6 (6)	12.4 ± 0.5	1.1 ± 0.4
pH_o 8.0 (9)	13.4 ± 0.1	0.4 ± 0.2
$[Ca^{2+}]_o$ 0 mM (5)	12.5 ± 0.2	0.3 ± 0.6
$[Ca^{2+}]_o$ 5 mM (5)	12.3 ± 0.5	0.5 ± 0.7

Single-channel conductances (g) and reversal potentials (E_{rev}) were determined in the conditions given in the left column. Data are given as means ± SEM for the number of observations in parentheses. Neither g nor E_{rev} were significantly affected by pH_i, pH_o, or $[Ca^{2+}]_o$ ($P > 0.2$ for each condition, unpaired *t* test).