Steinberg et al., http://www.jcb.org/cgi/content/full/jcb.200911083/DC1

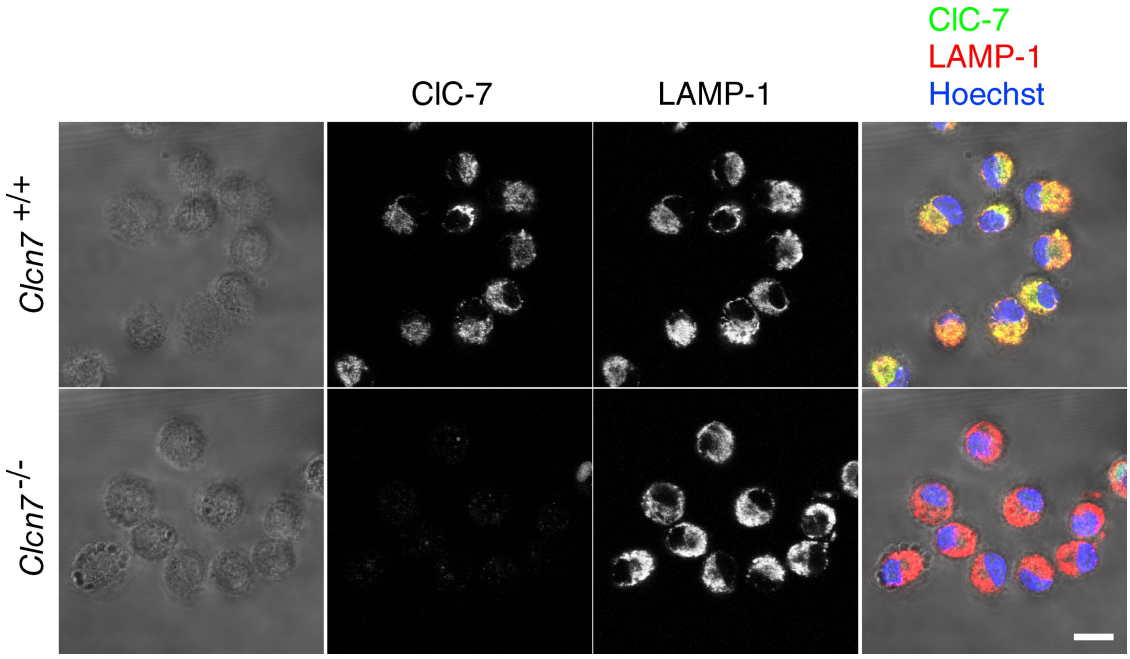

Figure S1. **ClC-7 colocalizes with LAMP-1 in alveolar macrophages.** Alveolar macrophages obtained from wild-type (*Clcn7*$^{+/+}$) and ClC-7 knockout mice (*Clcn7*$^{-/-}$) were immunostained for ClC-7 (second column; green in right column) and for LAMP-1 (third column; red in rightmost column), and their nuclei stained with Hoechst 33342 (blue). Corresponding bright-field images are shown in leftmost column. Bar, 10 µm.

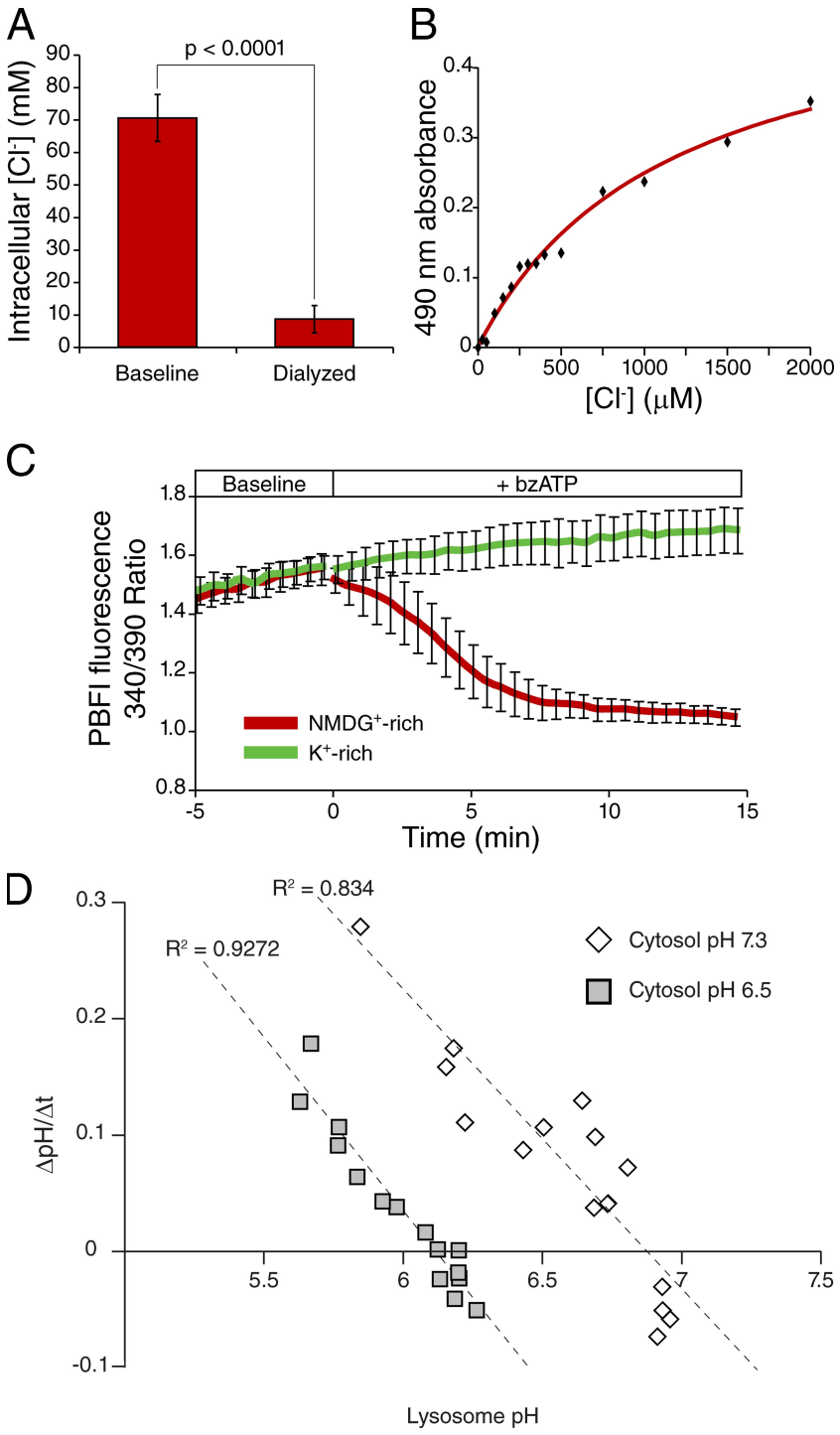

Figure S2. **Validation of the cellular dialysis procedure.** (A–C) Effectiveness of the dialysis accomplished by $P2X_7$ activation. (A) RAW264.7 cells were either left untreated or dialyzed of their intracellular Cl^- by incubation in a Cl^--free medium during stimulation of $P2X_7$ receptors for 15 min. The intracellular Cl^- content of cells before and after dialysis was assayed colorimetrically as described in Materials and methods. Data are means ± SE of three and six experiments for the control and dialysis treatments, respectively. A sample calibration curve for the assay is shown in B. (C) Intracellular K^+ was measured using the ratiometric K^+-sensitive fluorescent dye PBFI. In brief, RAW264.7 cells were incubated for 30 min with the acetoxymethyl ester of PBFI before imaging. After 5 min of baseline measurements, the plasmalemma was selectively permeabilized using bzATP in the presence of either a cytosol-like (K^+-rich) or $NMDG^+$-rich solution. Data (shown as means ± SD of six cells in a single experiment) are representative of at least three similar experiments performed for each condition. (D) Comparison of two independent null-point titrations of lysosomal K^+ content. To confirm our initial lysosomal K^+ determination (Fig. 4 B, reproduced here using squares), we conducted a second, independent null-point titration of lysosomal K^+. In the second titration, illustrated by the open diamonds, the cytosolic pH was increased from 6.5 to 7.3. Elevating the cytosolic pH is expected to change the value of the null-point, but should nevertheless yield an estimate of the lysosomal K^+ content comparable to that calculated at pH 6.5. Using the dataset obtained at cytosolic pH 7.3 we calculate a null-point of pH 6.81 ± 0.04 corresponding to a luminal K^+ content of 61.7 ± 6.2 mM, in reasonable agreement with the value of 50.1 ± 2.0 mM estimated at cytosolic pH 6.5.

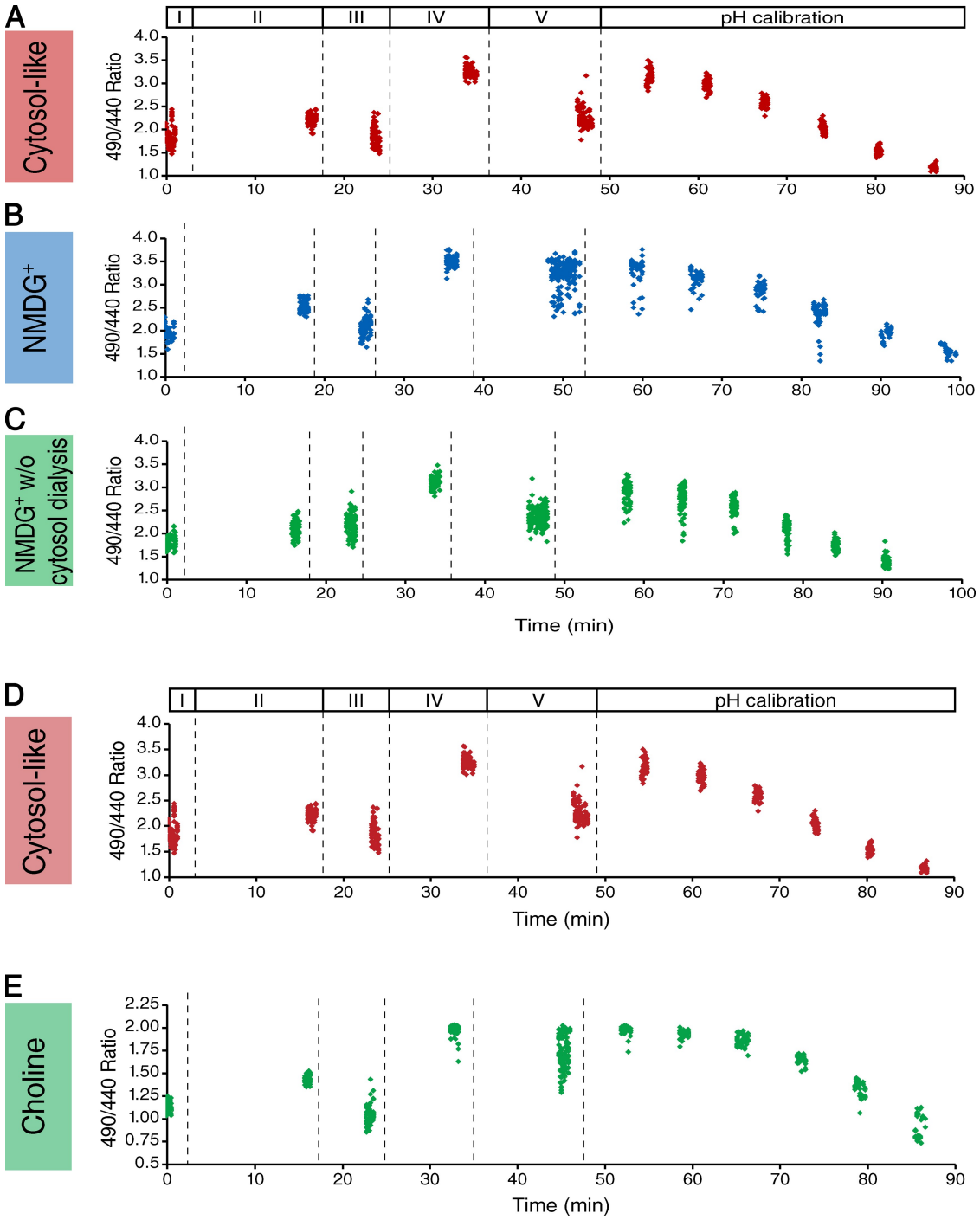

Figure S3. **Lysosome re-acidification after luminal cation dialysis.** Raw data of representative lysosome cation-substitution experiments as described for Fig. 7. Lysosomes were either dialyzed with a cytosol-like (A) or $NMDG^+$-rich (B) solution. As a control, the same protocol as that used in B was performed but without $P2X_7$ receptor activation, and thus without cytosolic dialysis (C). (D and E) Lysosome re-acidification after luminal cation dialysis with choline. Lysosomes were either dialyzed with a K^+- (D) or choline-based (E) solution. The different stages of the experimental protocol are indicated by Roman numerals as described for Fig. 7. Each data point represents a single region of interest.

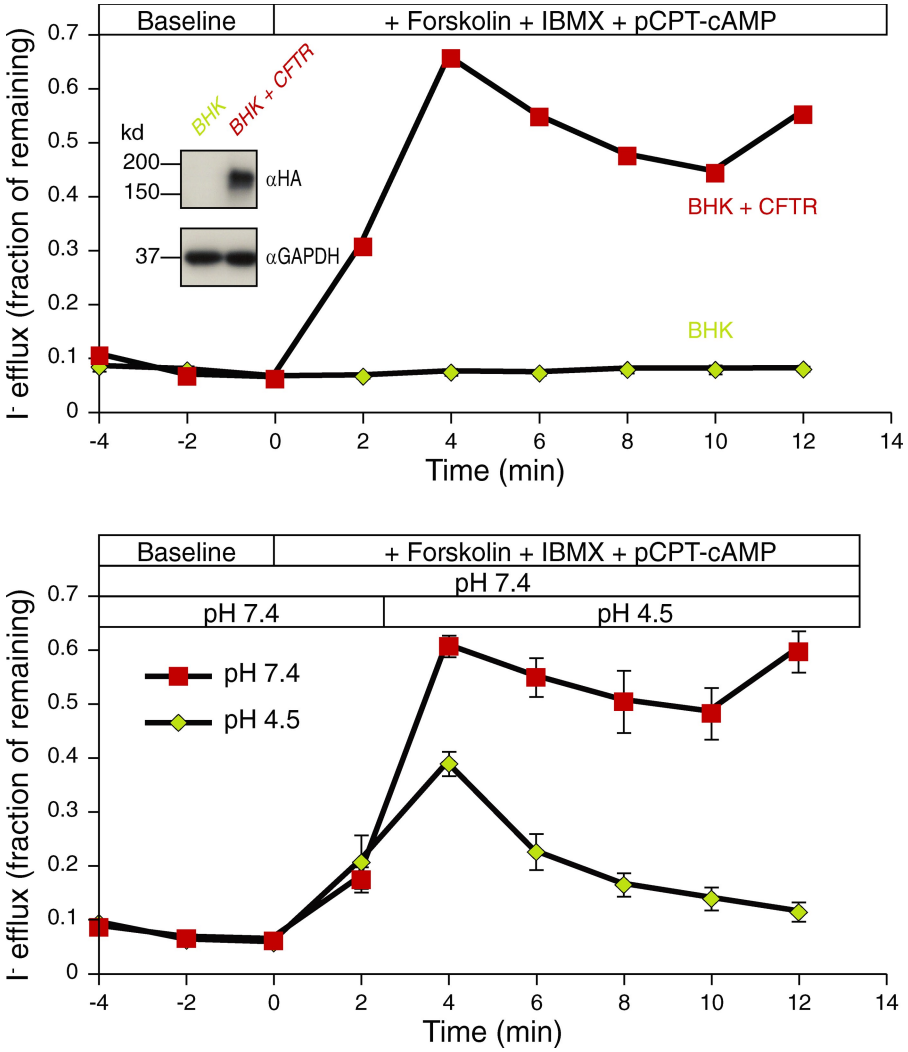

Figure S4. **Effect of pH on CFTR-mediated iodide flux.** Top: cAMP-dependent iodide efflux was readily detectable in CFTR-expressing BHK cells but not in their untransfected counterparts. BHK cells were stably transfected with human CFTR tagged with the HA epitope. Inset: immunoblot showing expression of the tagged protein in the transfectants, but not in wild-type BHK cells. GAPDH was blotted to verify equal loading. Main panel: representative traces of I^- efflux from wild-type (green diamonds) and CFTR-transfected BHK cells (red squares) before and immediately after stimulation with a cocktail of forskolin, IBMX, and pCPT-cAMP. Bottom: effect of extracellular acidification on CFTR function. Iodide flux was measured in CFTR-transfected BHK cells as above. After stimulation, the pH of the extracellular medium was lowered to 4.5 while in the continued presence of CFTR agonists. The exposure of the extracellular surface (topologically equivalent to the lysosome luminal side) to acid induced a marked decrease in iodide efflux. Representative experiments are shown for the control (red squares) and acid-treated (green diamonds) cases.

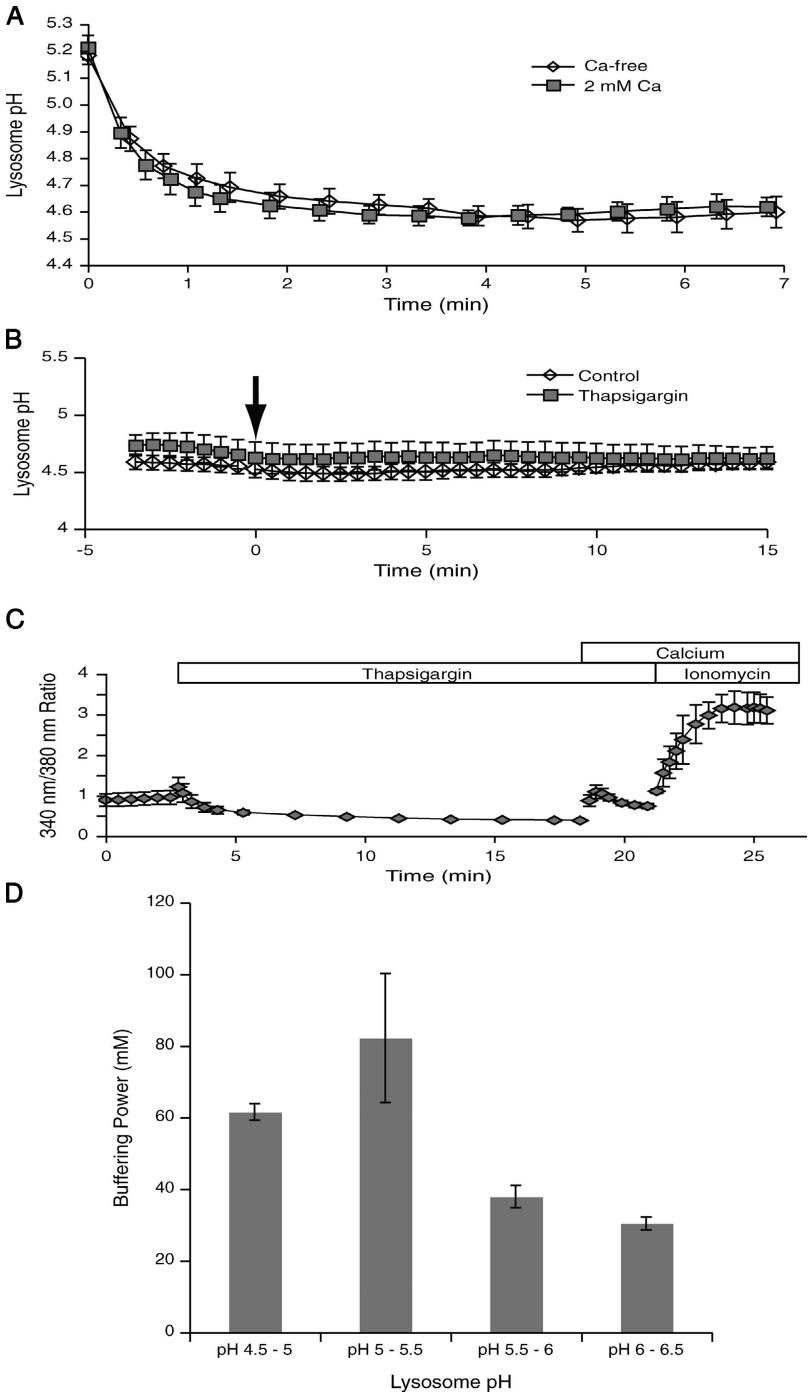

Figure S5. **Analysis of the effect of calcium on lysosomal pH.** (A) RAW264.7 cells were permeabilized as described in Fig. 3 and resealed in the absence (diamonds) or presence of 2 mM Ca^{2+}. Lysosomal re-acidification was measured as described in Materials and methods. (B) RAW cells were suspended in medium devoid of Ca^{2+} and either left untreated (Control) or treated with 2 µM thapsigargin. Lysosomal pH was measured as above. Where indicated by the arrow, 2 mM Ca^{2+} was added to the medium. (C) Cytosolic-free Ca^{2+} was measured in RAW cells using Fura-2. Cells were suspended in Ca^{2+}-free medium and thapsigargin (2 µM), Ca^{2+} (2 mM), and ionomycin (10 µM) were added where indicated. Data in A–C are means ± SE of at least three experiments of each type. (D) pH dependence of the lysosomal buffering power. Lysosomal pH was measured as described in Materials and methods. Buffering power was estimated by measuring the ΔpH induced by addition of NH_4Cl. To measure the buffering power at pH values above the steady-state lysosomal pH, 500 nM CcA was added to the bathing medium to slowly dissipate the pH gradient. At various times (i.e., when various, defined pH levels had been attained) NH_4Cl was added, the change in pH recorded, and the buffering power calculated as described by Roos and Boron (1981). Results were binned in 0.5 pH unit categories between 4.5 and 6.5.

Reference

Roos, A., and W.F. Boron. 1981. Intracellular pH. *Physiol. Rev.* 61:296–434.