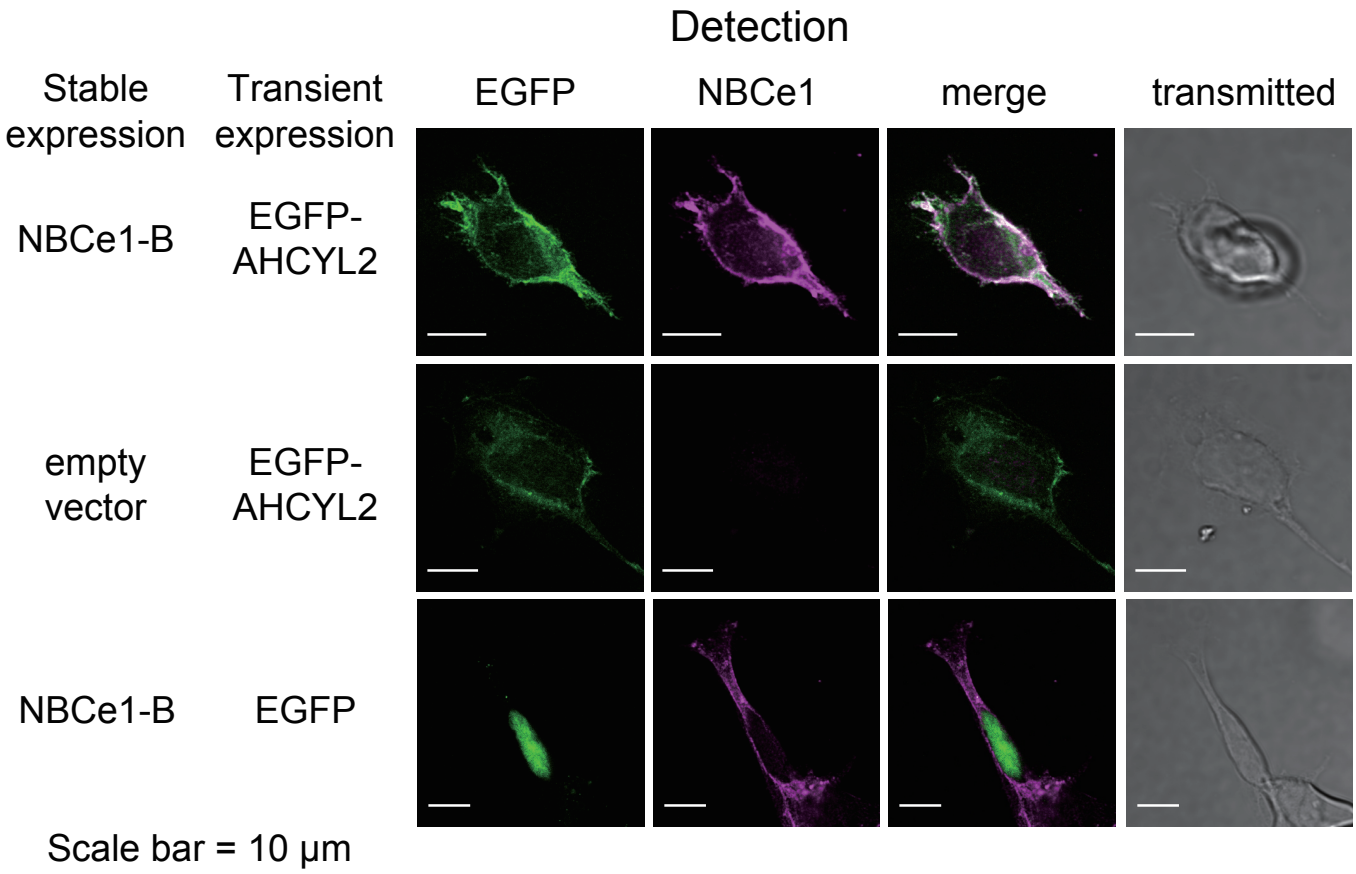
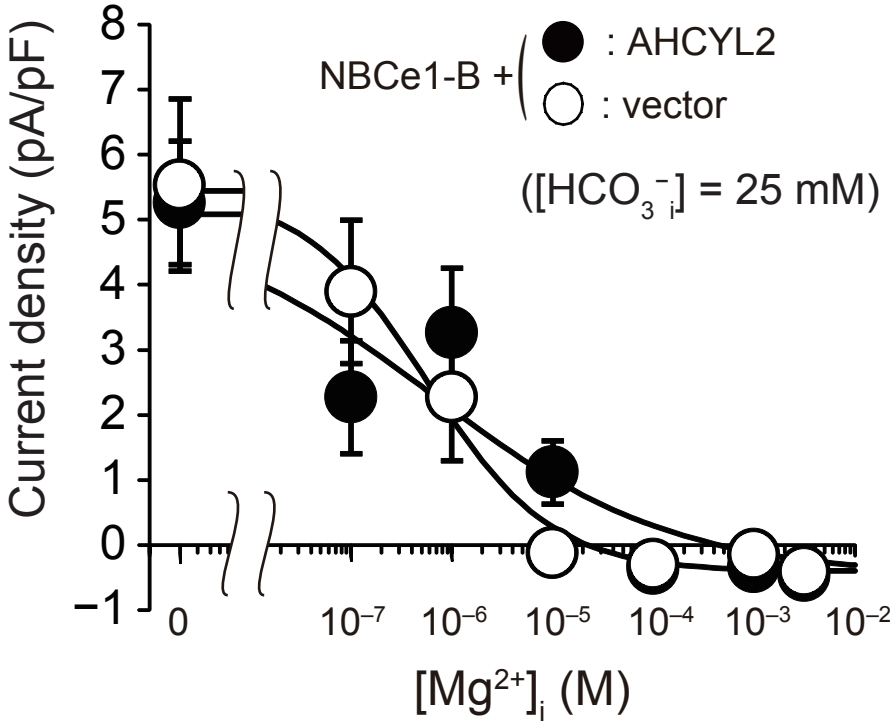
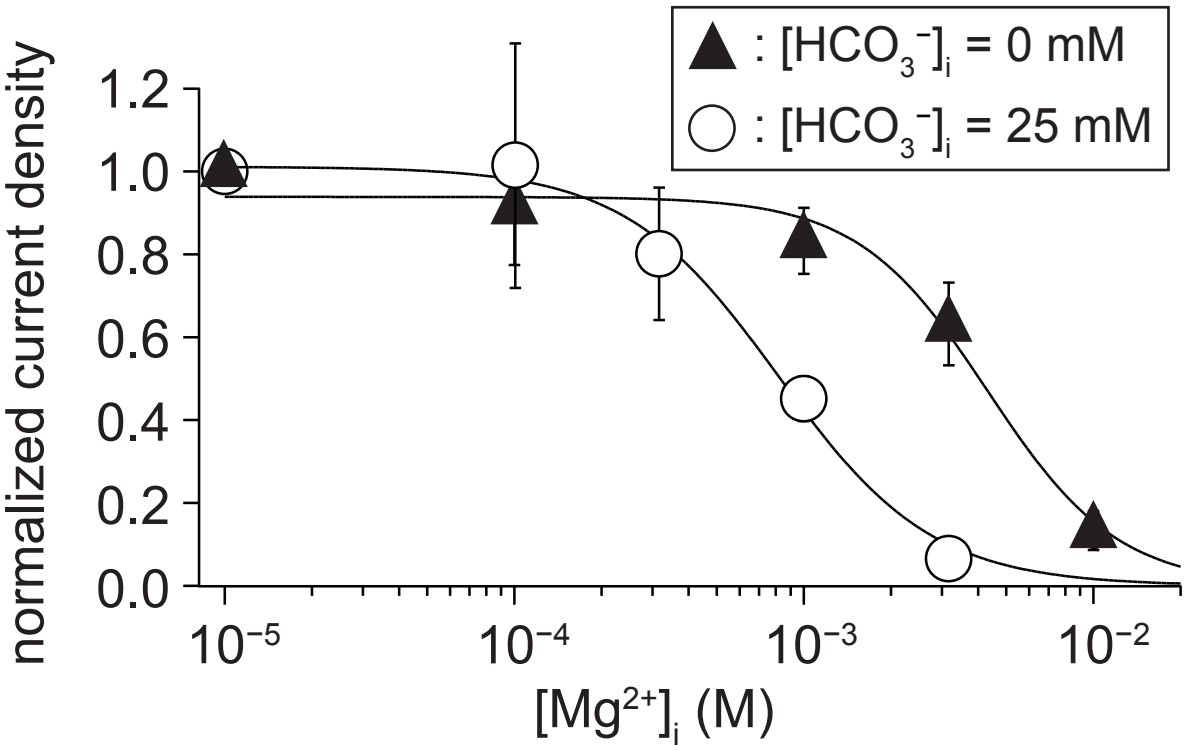


Supplemental Figure S1 Yamaguchi S. and Ishikawa T.



Supplemental Figure S2 Yamaguchi S. and Ishikawa T.



Supplemental Figure S3 Yamaguchi S. and Ishikawa T.

SUPPLEMENTAL FIGURE LEGEND

Supplemental Figure S1

Co-localization of AHCYL2 and NBCe1-B exogenously expressed in HEK293 cells

EGFP-tagged AHCYL2 or EGFP were transiently expressed in HEK293 cells stably transfected with NBCe1-B or mock transfected cells (empty vector). Cells were stained with mouse anti-NBCe1 antibody and Alexa 594 goat anti-mouse IgG antibody. AHCYL2 was detected by the fluorescence of EGFP (green), and NBCe1-B by fluorescence of Alexa 594 (magenta) by confocal microscopy. Cells were permeabilized with saponin to wash out cytosolic unbound proteins prior to fixation. Co-localization of AHCYL2 and NBCe1-B is indicated as merge and with white color. Scale bars, 10 μm.

Supplemental Figure S2

AHCYL2 does not change Mg^{2+}_i-sensitivity of NBCe1-B currents stably transfected in HEK293 cells when recorded using pipette solutions with added 25 mM HCO_3^-

Dose-response curves for the inhibition of NBCe1-B currents by increasing free Mg^{2+}_i concentrations in the stably NBCe1-B-expressing cells, which were transiently transfected with AHCYL2 (solid circles). Shown are mean ± S.E. (n = 8-11). The lines were fits to the Hill equation. The data for the stably NBCe1-B-expressing cells transiently transfected with empty vector alone (open circles) were taken from a recently published work (Yamaguchi & Ishikawa 2012) and are shown for comparison.

Supplemental Figure S3

Mg^{2+}_i sensitivity of native NBCe1-B-like current in BPA cells under a nominally

$HCO_3^-{}_i$-free condition

Dose-response curve for $Mg^{2+}{}_i$-inhibition of native NBCe1-B-like currents recorded from BPA cells using a nominally-HCO_3^--free pipette solution (filled triangles). NBCe1-B-like current densities at 0 mV were normalized to that at 10^{-5} M free Mg^{2+} obtained from a BPA cell derived from the same bovine parotid gland. Shown are mean ± S.E. (n = 3-11). The line is a fit to the Hill equation (K_i value: 4.3 $\times 10^{-3}$ M; Hill coefficient: 1.93). The data obtained under a HCO_3^--loaded condition (open circles) were taken and adapted from Yamaguchi & Ishikawa (2008) and plotted for comparison. The current density values before normalization at 10^{-5} M free Mg^{2+} with/without HCO_3^- were 5.4 ± 0.7 and 8.1 ± 0.7 pA/pF (n = 23 and 14), respectively.

MATERIALS AND METHODS FOR SUPPLEMENTAL FIGURES

Immunofluorescence confocal microscopy

N-terminally EGFP-tagged AHCYL2 (pEGFPC1 vector) or EGFP alone was expressed in HEK293 cells stably transfected with bovine NBCe1-B or empty vector (pCIneo). The transfected cells were permeabilized in permeabilization buffer (80 mM PIPES, 1 mM $MgCl_2$, 1 mM EGTA, and 4% polyethylene glycol, pH 7.2 with KOH) containing 0.1% saponin for 10 min on ice, and washed twice with ice-cold permeabilization buffer prior to fixation. The cells were fixed in PLP fixative (2% paraformaldehyde, 75 mM lysine, 37 mM Na phosphate, 10 mM Na periodate, pH 7.4) overnight at 4 °C, permeabilized in 1% SDS in PBS for 5 min, and blocked in image-iT signal enhancer (Life Technology) for 30 min and in PBS containing 5% normal goat serum and 0.2% BSA for 60 min. Cells were then stained with rabbit anti-NBCe1 antibody (1:1,000,

Chemicon). Following four times 5-min PBS washes, Alexa 594-conjugated goat anti-rabbit IgG (Life Technology) was applied for 1 hr at room temperature. Following four times 5-min PBS washes, the coverslips were mounted with ProLong Gold Antifade Reagent (Life Technology) and observed under IX-70 confocal fluorescence microscopy (Olympus) with a 60× oil-immersion objective.

Whole-cell patch clamp experiment

Pipette solutions (pH = 7.4 with NMDG (N - methyl - D - glucamine)) contained (in mM): 10 BAPTA (1,2-Bis (2-aminophenoxy) ethane- N,N,N',N'- tetraacetic acid), 100 HEPES, 4 EDTA-2Na (ethylenediaminetetraacetic acid disodium salt), 2 $NaHCO_3$, 23 $cholineHCO_3$, 0-8 $MgCl_2$ (no $MgCl_2$ or appropriate amounts of $MgCl_2$ were added to yield Mg^{2+}-free or 10^{-7} to $10^{-2.5}$ M free Mg^{2+}, respectively), 0.044 - 0.202 $CaCl_2$ (10^{-9} M free Ca^{2+}), 13-25 NMDG-glutamate, and 0-15 NMDG-Cl. Free Mg^{2+} and Ca^{2+} concentrations were calculated using the program Maxchelator (http://www.stanford.edu/~cpatton/maxc.html). The concentrations of NMDG-glutamate and NMDG-Cl were also varied to maintain the chloride concentration (14-16 mM Cl^-). Pipette solutions with no added HCO_3^- were the same as those described in the manuscript. The solutions containing HCO_3^- were bubbled with 5% CO_2/95% O_2. The average series resistance of HEK293 cells or BPA cells , which was not electrically compensated, was 23.1 ± 0.6 MΩ (n = 60) or 22.3 ± 0.7 MΩ (n = 45), respectively. The cell capacitance was 18.3 ± 1.0 pF (n = 60) or 32.6 ± 1.3 pF (n = 45), respectively. Other experimental conditions were the same as those described in the manuscript.

SUPPLEMENTAL REFERENCES

Yamaguchi S & Ishikawa T (2008). The electrogenic Na^+-HCO_3^- cotransporter NBCe1-B is regulated by intracellular Mg^{2+}. *Biochem Biophys Res Commun* **376,** 100-104.

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