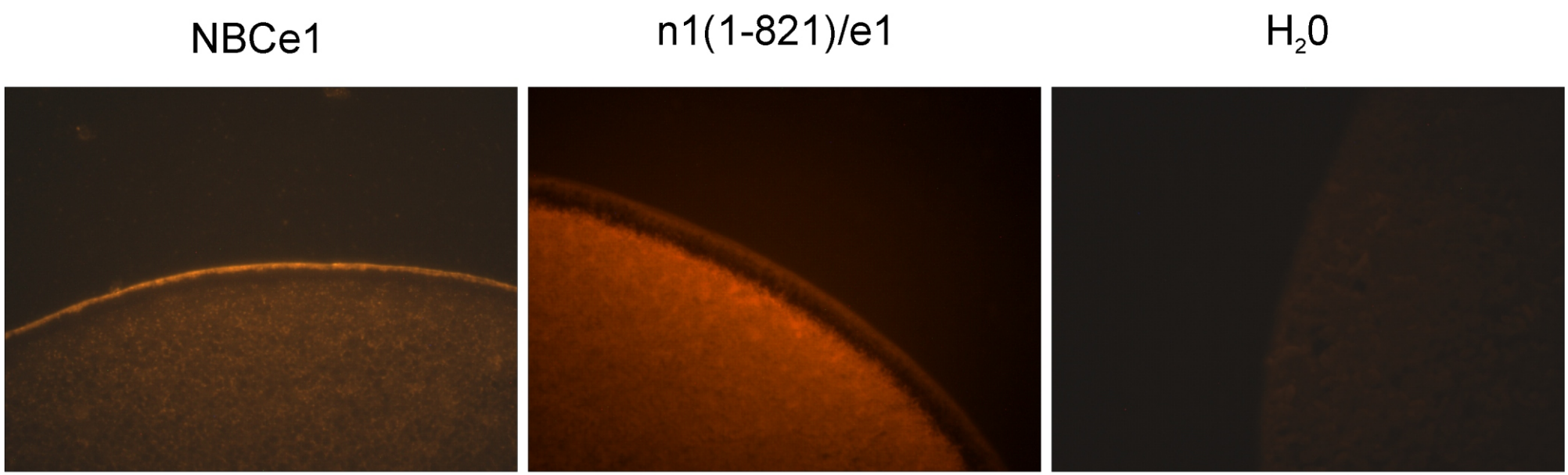
Supplemental Data 1: Immunocytochemistry



Legend for immunocytochemistry

Immunocytochemistry. Oocytes were fixed overnight in 4% paraformaldehyde, embedded in paraffin, and sectioned at a thickness of 5 μm. The sections were deparaffinized in a series of xylene, and then rehydrated with a series of ethanol washes. For antigen retrieval, the sections were heated in a citrate butter (pH 6.0) for 10 min in a microwave and incubated at room temperature for 20 min. After treating with 1.2% hydrogen peroxide for 30 min to inhibit the endogenous peroxidase, sections were washed with PBS for 20 min. Sections were then pre-incubated (30 min) with 10% normal goat serum in PBS and then incubated for 24 h with the primary antibody (1:500 for NBCe1). After washes with PBS containing 0.1% Triton X 100 (PBT), sections were incubated with horse radish peroxidase-conjugated secondary antibody (Chemicon; 1:600). After rinsing with PBS, sections were incubated with TSA-Plus-Tetramethylrhodamine (Perkin Elmer) for 1 min. Sections were placed on cover-slips using DAKO Glycergel (Dakopatts) plus 2.5% 1,4-diazabicyclo[2.2.2.]octane (DABCO, Sigma) as a fading retardant. Immunofluorescence were revealed by fluorescence light microscopy (Polyvar, Reichert-Jung, Austria), using narrow-band filter systems for rhodamine.

Supplemental Data 2:

Constructions of chimeric transporters:

e1(Nt)/n1: The pGH19/NBCn1 (Choi *et al.*, 1999) was digested with Afl II and Nhe I to obtain a 2.5 kb DNA fragment, which includes the portion of the N-terminal domain, the entire TMs, and the C-terminal domain of NBCn1. This fragment was then used to replace the Afl II/Nhe I fragment of the pTLN2/NBCe1 (Romero *et al.*, 1998). To complete the boundary between the electrogenic N-terminal domain and the electroneutral TM1, two DNA fragments were then prepared: i) the portion of the N-terminal domain of pTLN2/NBCe1 was prepared by Afl II/Ssp I digestion, and ii) nucleotides 2,013-2,499 of NBCn1 (GenBank accession number NM_058211) was obtained by PCR. Primers were GATGCATTAAATATTCAGTGCCTGGC CTCGATTCTTTTC (forward) and ACTGGTCAATGCATCCAAGTTGTTGTGCATGTTAAA GGCATA (reverse). Primers contained sites for either Ssp I or Nsi I. The two DNA fragments were subsequently ligated with the above plasmid at the Afl II/Nsi I sites.

e1(L)/n1: The electrogenic loop domain was obtained by PCR of pGH19/NBCn1 with hybrid primers, which contain both NBCn1 and NBCe1 sequences. These primers are TATGAAGCCTTGGAGAAACTCTTTCACCTGGCAGATTACTATCCCATCAACTCTGAC (forward) and GTAGTCAATTGCAACCATTATTACTATTGTGAGAAATACGGCAAAAT CGCTGATCAGTTTTCTTGC (reverse). The PCR product was digested with Sty I and Mfe I, and then ligated with the pGH19/NBCn1 at the corresponding sites. The ligation product contained the electrogenic loop domain and a portion of the electrogenic TMs. To replace the electrogenic TMs, SnaB I was introduced by PCR at the junction between the loop and TM6 of the ligation product. The plasmid was then digested with SnaB I and Xho I, and the region of the TM6 and the C-terminal end was replaced with the homologous region of NBCn1.

e1(Ct)/n1: The C-terminal domain of NBCe1 was obtained by PCR. Primers were GCCCTGGTAGCTGTGCGCAAAGGTATGGAC (forward) and CTTGGACTGGGTAAAAC TAGTGACTCAAGG (reverse). The PCR product was digested with Hha I and Spe I, and ligated with pGH19/NBCn1 at the corresponding sites.

e1(NtLCt)/n1: The e1(NtLCt)/n1 construct was made with enzymatic digestion and ligation of the above three constructs: e1(Nt)/n1, e1(L)/n1, and e1(Ct)/n1. The electrogenic loop domain was obtained by digesting the e1(L)/n1 with Sty I and Mfe I. The electrogenic C-terminal domain was obtained by digesting the e1(Ct)/n1 with Mfe I and Spe I. These two DNA fragments were then simultaneously ligated with the e1(Nt)/n1 at Sty I and Spe I sites.

n1(Nt)/e1: The pGH19/NBCn1 was digested with Nsi I and Hind III to remove the entire TMs and the C-terminal domain of NBCn1. The Hind III site was blunted with T4 DNA ligase. To insert the homologous regions of NBCe1 into this deletion plasmid, two DNA fragments were prepared. First, nucleotides 1,431-1,805 of NBCe1 (GenBank accession number NM_053424) were obtained by PCR with primers GACTTTTATGATGCATTAAATATTCAG (forward) and CGTGAAACGGGTGAAGTACTG AACCAG (reverse). The PCR product was digested with Nsi I and Sca I. Second, nucleotides 1,805-3,270 of NBCe1 were isolated from pTLN2/NBCe1 by Sca I/Hpa I digestion. These two NBCe1 DNA fragments were subsequently ligated with pGH19/NBCn1 at the Nsi I/Hind III sites.

n1(L)/e1: PCR was performed on the n1(1-821)/e1 (see below) to obtain nucleotides corresponding to the electroneutral loop and a portion of the 3' flanking electrogenic TMs. Primers were AAACTCTTTAAGCTTGGAGAAATATATGC (forward) and GGACCTGTTTTGGGTCGCCATCCTCATGGTGG (reverse). The PCR product was digested with Hind III and PflM I. To produce the boundary between the electrogenic TM5 and electroneutral loop, another PCR was performed on the pTLN2/NBCe1 to obtain the electrogenic upstream flanking region of the loop was obtained by PCR with primers amplifying nucleotides 1234-5678 of NBCe1. Primers were GGAAGAGCCATCGCCACCTTGATGTCTGAC (forward) and GGATAGTAATCTGCAAGCTTGATCATCTTC (reverse). This PCR product was digested with Ava I and Hind III. The two PCR products were subsequently ligated with the pTLN2/NBCe1 at the Ava I/PflM I sites.

n1(Ct)/e1: The C-terminal domain of NBCn1 was obtained by PCR on pGH19/NBCn1 with primers ATCTATGAAGCCTTGGAGAAACTCATTCACTTAGGAGAA (forward) and the commercial SP6 promoter primer (reverse). The PCR product was digested with FspI and Spe I. Another PCR was performed on the pTLN2/NBCe1 to produce the boundary between the electrogenic TM14 and the electroneutral C-terminal domain. Primers were GGAAGAGCCATCGCCACCTTGATGTCTGAC (forward) and GTCCATACCTTTGCGCACAGCTACCAGGGC (reverse). This PCR product was digested with Ava I and Fsp I. Both PCR products were subsequently ligated with the pTLN2/NBCe1 at the Ava I/Avr II sites.

n1(NtLCt)/e1: The n1(NtLCt)/e1 construct was made with enzymatic digestion and ligation of the above three constructs: n1(Nt)/e1, n1(L)/e1, and n1(Ct)/e1. The electroneutral C-terminal domain and the flanking electrogenic region were obtained by digesting the n1(Ct)/e1 with Ava I and Mlu I. The fragment was ligated with the n1(Nt)/e1 at the corresponding sites. To replace the loop domain, the n1(L)/e1 was digested with Ava I and PflM I, and the 0.4 kb fragment was ligated with the above plasmid at the Ava/PflM I sites

n1(1-821)/e1: The modified pGH19/NBCn1, which has a SnaB I site just before TM6, was digested with SnaB I and Spe I. The region of TM6 to the C-terminal end was thus removed. The homologous region of NBCe1 was obtained by PCR on pTLN2/NBCe1 with primers ACTGTGACTACGTACCTGACATCA (forward) and CTTGGACTGGGTAAAACTAGTGAC TCAAGG (reverse). The PCR product was digested with SnaB I and Spe I and ligated with the above pGH19/NBCn1.

n1(822-1218)/e1: The region of the N-terminal domain to the loop of NBCe1 was obtained by PCR on pBluescript/NBCe1. Primers were the T7 promoter (forward) and TGATGTCAGGTACGTAGTCACAGT (reverse). The PCR product was subcloned into pGH19, and then digested with SnaB I and Nhe I. The region of TM6 to the C-terminal domain of NBCn1 was obtained by digesting the modified pGH19/NBCn1 with SnaB I and Nhe I. This fragment was subsequently ligated with the SnaB I/Nhe I-digested plasmid.