Supplementary information

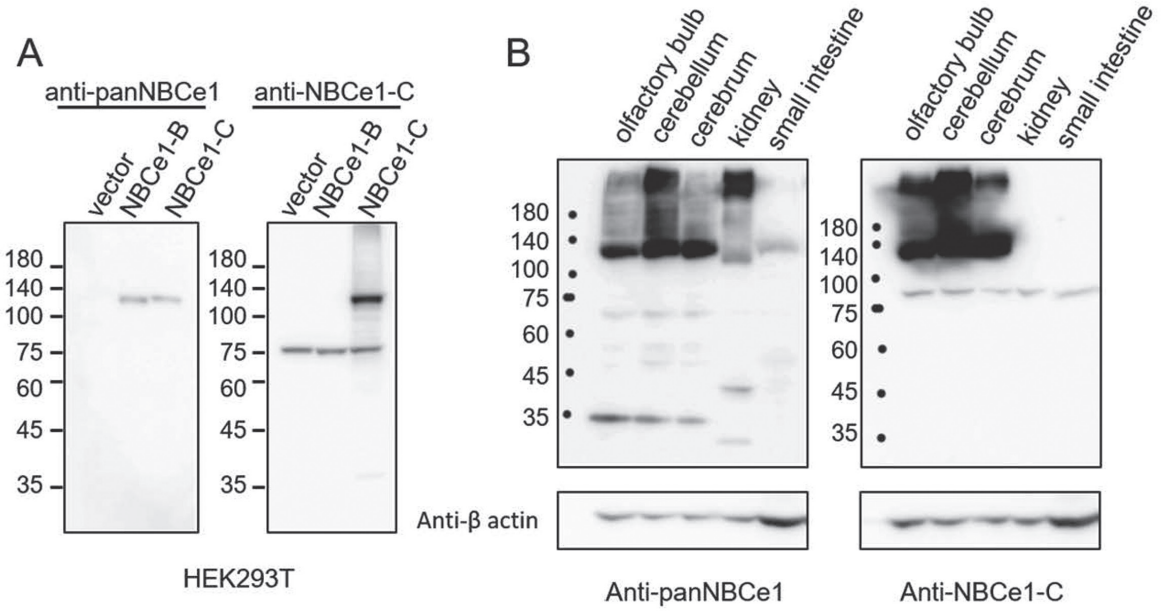

Figure S1. Production and Specificity of Anti-NBCe1-C Specific Antibody

(A) Anti-NBCe1-C specific antibody recognized NBCe1-C, but not NBCe1-B, heterologously expressed in HEK293T cells. (B) Tissue blot with anti-NBCe1 antibody showed that reactive signals around 130~140 kDa were observed only in tissues from central nervous system. Note that higher expression of NBCe1-C in cerebellum compared to other sub brain regions.

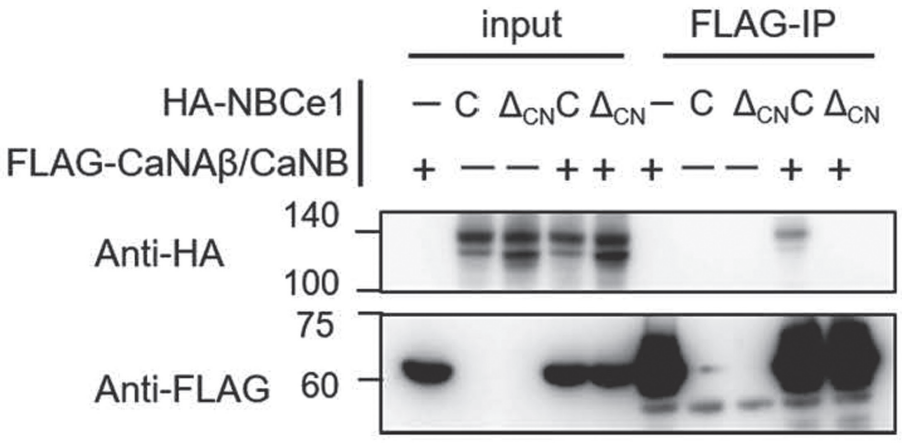

Figure S2. CaNAβ also Bound to NBCe1-C via PQIRIE Motif

Full-length CaNAβ binds to NBCe1-C in HEK293T cells revealed by co-immunoprecipitation assay. C; NBCe1-C, Δ_{CN}; Δ_{CN}NBCe1-C.

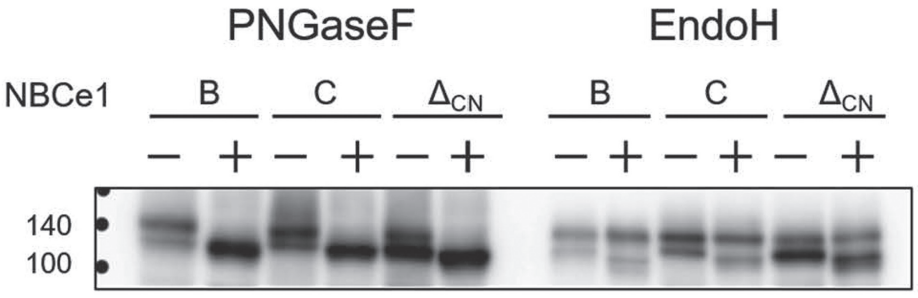

Figure S3. Faster-Migrating Moiety of each NBCe1 Construct Expressed Was Associated with Insufficient Glycosylation

Effects of glycosidase treatments of each NBCe1 protein were analyzed by migration shift on 7.5% SDS-PAGE. When NBCe1 construct was expressed, a doublet band appeared and Δ_{CN}NBCe1-C always showed faster-migrating-band dominant pattern. PNGaseF treatment showed a convergence of the doublet bands, while EndoH treatment affects only the lower bands resulting in their more faster migration. PNGase F; N-Glycosidase F, oligosaccharides. Endo H; Endoglycosidase H. B; NBCe1-B, C; NBCe1-C, Δ_{CN}; Δ_{CN}NBCe1-C.

Information about construction of expression vectors

Full-length NBCe1-C cDNA was cloned from human neuroblastoma cell line, IMR-32 (accession number AB470072) and was used in the series of experiments. Human full-length NBCe1-B expression plasmid was kindly gifted from Dr. Seki (Yaizu City Hospital, Yaizu, Japan). For extracellularly Hemagglutinin (HA)-tagged constructs, HA-tag epitope sequence was inserted between ^{613}P and ^{614}E of NBCe1-B/C according to the previous study reporting that the HA-tag insertion did not affect on either surface expression or transport activity of NBCe1,[1)] and resulting cDNA were subcloned into mammalian expression vector pcDNA3.1. We used extracellularly HA-tagged NBCe1 constructs in most experiments except in pHi measuring experiment. For recombinant protein expression, cDNA encoding the unique C-terminal region of NBCe1-C (1034-1094 amino acids in human NBCe1-C) was subcloned into *E. coli* expression vector pGEX6p (GE Healthcare) and pMal-c2 (New England BioLabs) for production of GST-fusion protein and MBP fusion protein, respectively. Deletion mutant of NBCe1-C lacking ^{1070}PQIRIE^{1075} (named Δ_{CN}NBCe1-C) was synthesized by PCR-based strategy. For pHi measuring experiments, non-tagged NBCe1-C and Δ_{CN}NBCe1-C were subcloned into pIRES2-AcGFP vector (Clontech), which enabled us to easily detect NBCe1 expressing cells. Mouse cDNAs of full-length calcineurin Aα (PPP3CA, CaNAα) and calcineurin Aβ (PPP3CB, CaNAβ) were amplified from mouse cerebellum (Reference sequence, NM_008913.5 and NM_001310426.1, respectively) and were subcloned into pcDNA3.1. N-terminally FLAG-tagged CaNAα was generated by inserting a FLAG-tag (DYKDDDDK) sequence into just after ATG of calcineurin. Mouse cDNAs in pME18s expression vectors of truncated constitutively active CaNAα (CA-CaNAα) and calcineurinB subunit (CaNB) were kindly gifted from Dr. Watanabe (Showa pharmaceutical university, Tokyo, Japan), and FLAG-tag was also N-terminally inserted into CA-CaNAα. When CaNA subunit, irrespective of CA- or PD-, was expressed, CaNB subunit was always co-expressed for activation and stabilization of CaNA subunit. Phosphatase activity deficient CaNAα (PD-CaNAα), which has a mutation in the catalytic domain (H151Q)[2)] was produced by PCR-based site directed mutagenesis using CA-CaNAα cDNA as a template. All cDNA constructs were produced by standard PCR-based strategies using KOD-plus DNA polymerase (TOYOBO), and were verified by DNA sequencing.

1) McAlear, SD, Liu X, Williams JB, McNicholas-Bevensee CM, Bevensee MO. Electrogenic Na/HCO3 cotransporter (NBCe1) variants expressed in Xenopus oocytes: functional comparison and roles of the amino and carboxy termini. *J. Gen. Physiol.,* **127**, 639-658 (2006).

2) Mertz P, Yu L, Sikkink R, Rusnak F. Kinetic and spectroscopic analyses of mutants of a conserved histidine in the metallophosphatases calcineurin and lambda protein phosphatase. *J. Biol. Chem.,* **272**, 21296-21302 (1997) .