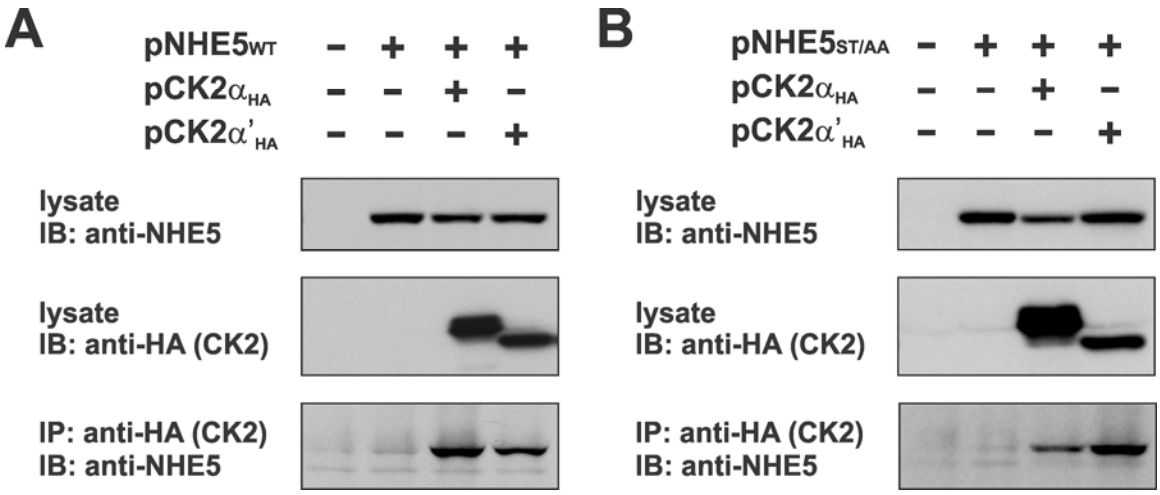
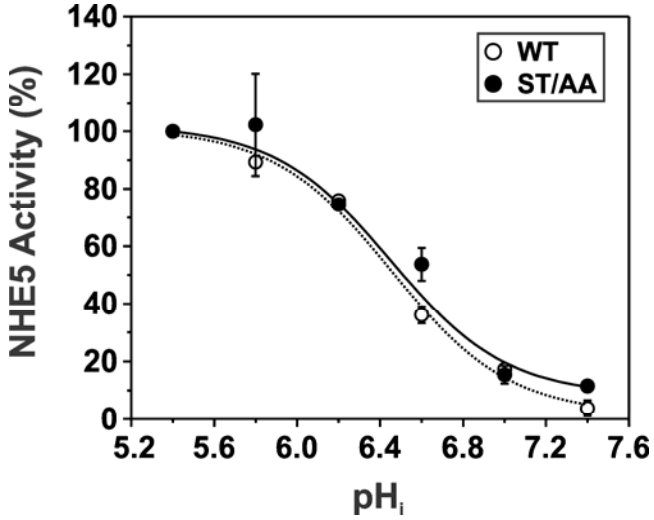
Supplemental Figures

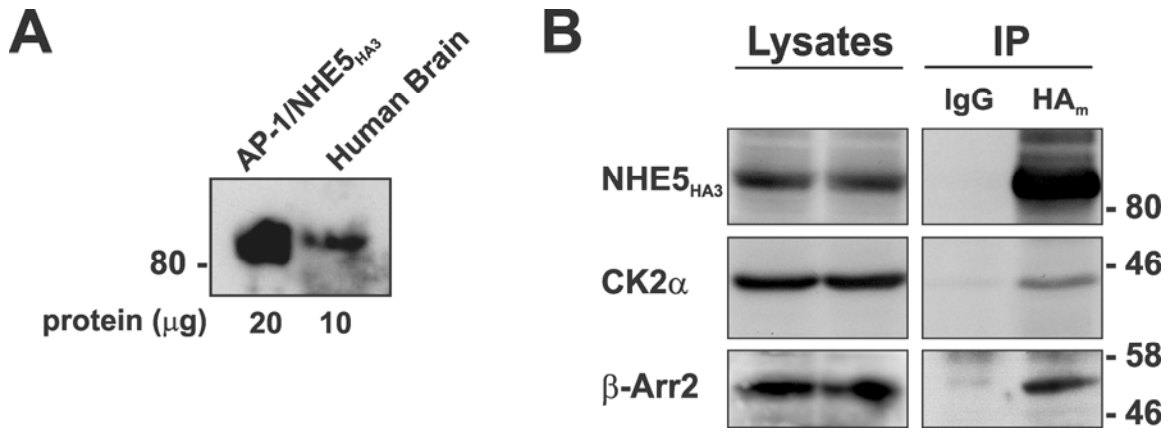


Supplemental Fig. 1. Catalytic α/α' subunits of protein kinase CK2 form a complex with NHE5 and regulate its activity. *A*, full-length NHE5 wild type (WT) and *B,* mutant (^{702}ST/AA^{714}) constructs were transiently transfected alone or together with CK2α_{HA} or CK2α'_{HA} in Chinese hamster ovary AP-1 cells. Cells were lysed after 24 h transfection. Aliquots of the lysates were removed for Western blot analysis of total cellular expression of NHE5 and CK2α_{HA} or CK2α'_{HA}, whereas the remainder was incubated with a monoclonal anti-HA antibody to precipitate CK2α_{HA}- or CK2α'_{HA}-containing protein complexes. The immunoprecipitates were fractionated by SDS-PAGE, followed by immunoblotting with a rabbit polyclonal antibody to detect NHE5.



Supplemental Fig. 2. Comparison of the affinity of NHE5 wild-type and ST/AA mutant for intracellular protons. AP-1 cells stably expressing NHE5 wild-type (WT) and ^{702}ST/AA^{714} mutant were cultured to confluence in 24-well plates. Initial rates of amiloride-inhibitable H^+-activated $^{22}Na^+$ influx were measured at various intracellular H^+ concentrations over the range of pH_i 5.4 to 7.4. The pH_i was adjusted by the K^+-nigericin method, as described in *Zaun et al (2008) J. Biol. Chem. 283(18):12456-12467*. To facilitate the comparison, the data were normalized to their respective maximal rates of uptake. Values represent the mean ± S.E. of three experiments, each performed in triplicate. Error bars smaller than the symbol are absent.

Supplemental Figures



Supplemental Fig. 3. Ectopically expressed NHE5 forms a complex with endogenous CK2α and β-arrestin in Chinese hamster ovary AP-1 cells.

A, *Comparison of the expression of human NHE5 in a stably transfected AP-1 cell line compared to whole normal human brain.* Aliquots of lysates of AP-1 cells stably expressing $NHE5_{HA3}$ (20 μg/lane) and commercially-prepared, whole human brain (10 μg/lane; Abcam) were analyzed by SDS-PAGE and immunoblotting using a rabbit polyclonal anti-NHE5 primary antibody (0.5 μg/mL) (generated in our laboratory) and a HRP-conjugated goat anti-rabbit secondary antibody (0.8 μg/mL; Jackson ImmunoResearch). Immunoreactive bands were detected with ECL detection reagents (Western Lighting Ultra Perkin Elmer kit) and Kodak X-ray film. The relative signal intensities (determined by densitometry) of exogenous $NHE5_{HA3}$ in AP-1 cells compared to endogenous NHE5 in human brain, when corrected for protein loading of the gel, were approximately 2 to 1, respectively. Given that NHE5 is expressed in only a subset of brain cells (primarily neurons), these data would suggest that the stable expression of NHE5 in this particular AP-1 subclone is roughly comparable to that found in neurons, at least within the limits of this type of analysis.

B, *Association of exogenous $NHE5_{HA3}$ with endogenous CK2α and β-arrestin in Chinese hamster ovary AP-1 cells*. Cell lysates of AP-1 cells stably expressing $NHE5_{HA3}$ were separated into two equal fractions (1 mL each). After removing an aliquot to represent the total cell lysates, the remainders were incubated with 1 μL of either mouse IgG (isotype control; 5 mg/ml) or anti-HA monoclonal antibody (HA.11 clone 16B12, 5-7 mg/ml; Covance, Richmond, CA) at 4 °C overnight on a tilt rocker, followed by addition of 25 μL of a 50% slurry of protein G sepharose beads (Amersham Pharmacia Biotech) for a further 2 h incubation. The beads were washed 4 times for 5 min each in lysis buffer, and the various protein fractions were resolved by SDS-PAGE and immunoblotting. Membranes were blocked for 1 h with 5% non-fat skim milk and PBS-T, and then probed with either 0.5 μg/mL of rabbit polyclonal anti-NHE5 antibody, 0.5 μg/mL of rabbit polyclonal anti-CK2α or 1 μg/mL of rabbit polyclonal anti-β-arrestin2/arrestin3 (Abcam) antibodies for 1 h at room temperature. The signals were visualized with 0.8 μg/mL of HRP-conjugated goat anti-rabbit secondary antibody (Jackson ImmunoResearch) after 1 h incubation. Immunoreactive bands were detected with ECL detection reagents (Western Lighting Ultra Perkin Elmer kit) and Kodak X-ray film.