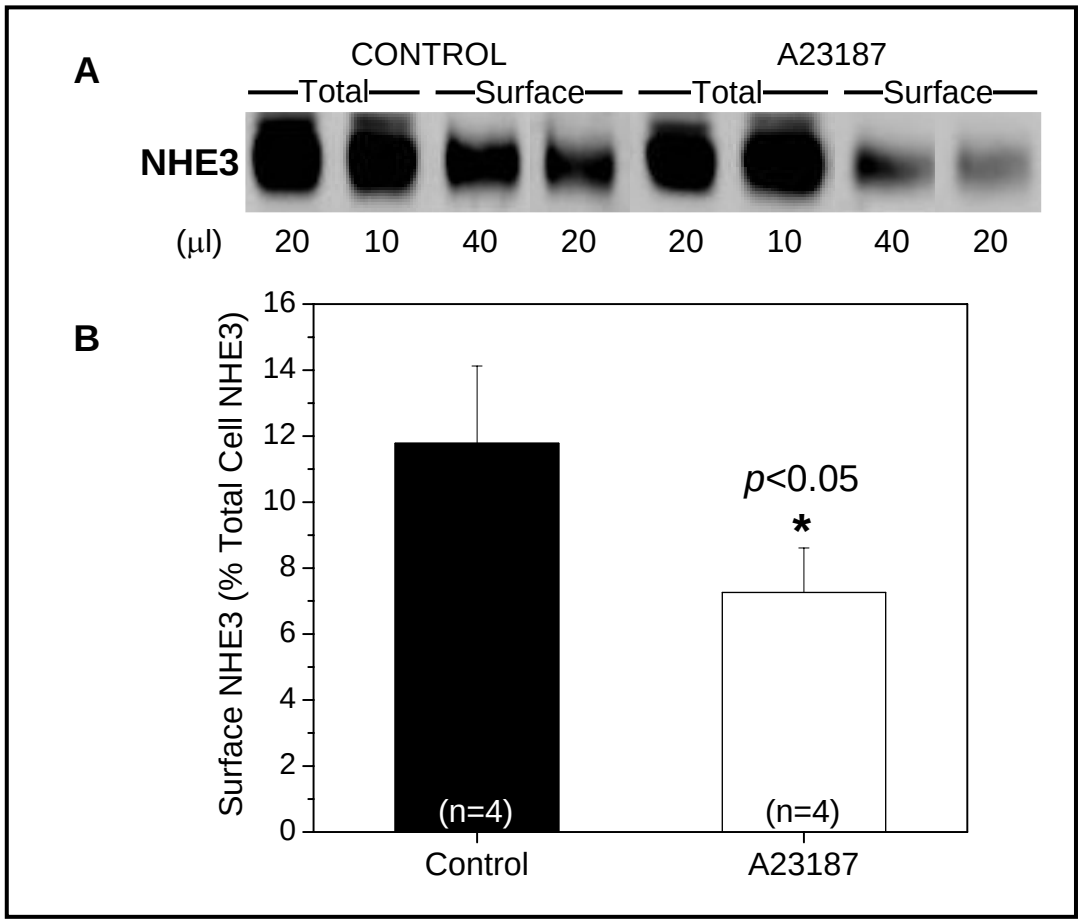
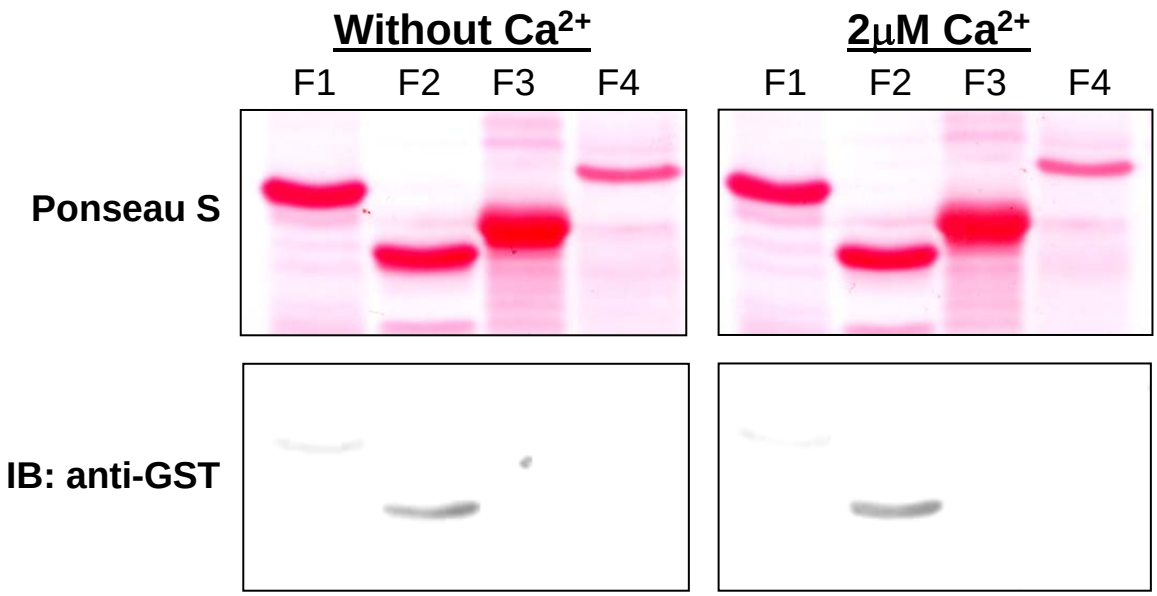
Supplemental Figure 1.



Supplemental Figure 2.



Supplemental Fig. 1: Inhibition of NHE3 activity is due to decreased surface expression of NHE3. PS120 cells expressing NHE3 and NHERF3 were grown to 70-80% confluency in 10cm Petri dishes and treated with vehicle or 4-Br-A23187 for 15 minutes. Cells were washed and incubated twice with Sulfo-NHS-SS biotin (0.5mg/mL) for 20 minutes. Cell lysates were collected and subjected to avidin precipitation and analyzed by western blot analysis. A. Western blot demonstrating decreased surface expression after elevated intracellular calcium of biotinylated NHE3 compared to total NHE3 expression (numbers below indicate amount in μl of sample loaded). B. Surface biotinylation of PS120/NHE3V/NHERF3 cells showed that basal levels of surface NHE3 (11.9 ± 2.3%) decreased by 39.0 ± 16.4% ($p<0.05$) in response to elevated calcium (7.6 ± 1.4%). Mean and SE of four experiments similar to results demonstrated in A.

Supplemental Fig. 2: Direct binding of NHERF3 to F2 fragment of NHE3 C-terminus is not altered by 2mM free Ca^{2+}. Purified His6-tagged NHE3 fusion proteins were separated by SDS-PAGE and proteins transferred to nitrocellulose membranes. Membranes were blocked and incubated overnight at 4ºC with GST-tagged NHERF3 purified proteins in buffer containing 1mM EDTA with or without 2mM free Ca^{2+}. Direct binding of NHERF3 with NHE3 C-terminal fragments was visualized by anti-GST antibody (to detect NHERF3) and goat anti-mouse IRdye 800 secondary antibody (1:20,000) on the Odyssey Infrared Imaging System (Lower Panel). Upper panel demonstrates loading of NHE3 C-terminal fragments onto SDS-PAGE as visualized by Ponseau S staining. Similar results were obtained from three independent experiments.