Figure S1

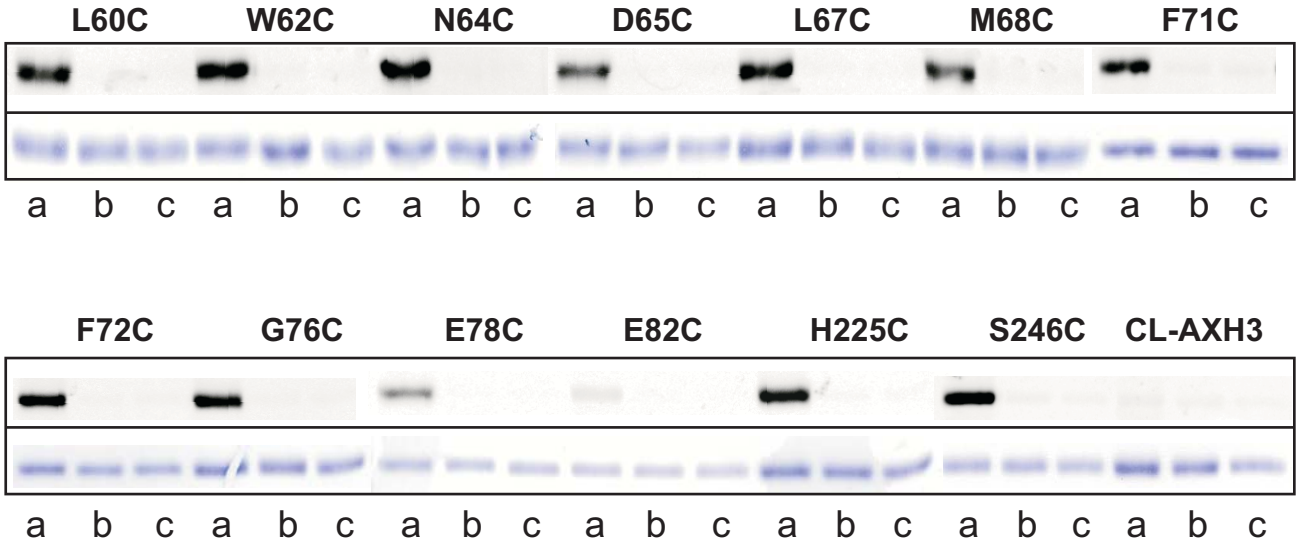
L60C
W62C
N64C
D65C
L67C
M68C
F71C
a b c a b c a b c a b c a b c a b c a b c
F72C
G76C
E78C
E82C
H225C
S246C
CL-AXH3
a b c a b c a b c a b c a b c a b c a b c

Figure S1. Reactivity of purified and denatured proteins of the NhaA Cys replacement variants with fluorescent maleimide, NEM, MTSES and MTSET.

Proteins of the Cys replacement variants of NhaA were affinity purified (Ni^{+2}-NTA) from membrane vesicles isolated from TA16 Cells expressing the Cys replacement variants, left bound on the beads, denatured and resuspended in 300 µl of SDS-urea buffer (6M urea, 2% SDS, 0.5 M NaCl, 20 mM Tris pH 7.5) as described under Experimental Procedures. Then each of the loaded beads was divided into three samples. One sample, the untreated control (lane a) was treated with 0.5 mM flourescein-5- maleimide (30 min at 23 °C) to obtain the level of 100 % reactivity with the maleimide. The other two samples were first exposed to either 0.5 mM NEM (lane b) or 10 mM MTSES (lane c) for 30 min at 23 °C and then washed and treated with fluorescein maleimide as above. The beads were washed, the protein eluted, resolved on SDS gel and processed as described under Experimental Procedures. The upper panel shows the fluorescence level and the lower panel the amount of the protein as described in the legend to Fig. 4. It is evident that both reagents fully react with the Cys replacements, totally eliminating the labeling by fluorescent maleimide. Similar results were obtained with MTSET instead of MTSES.