Supplementary information

# Novel phorbol ester-binding motif mediates hormonal activation of $Na^+/H^+$ exchanger

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Movie captions

## Supplementary figures

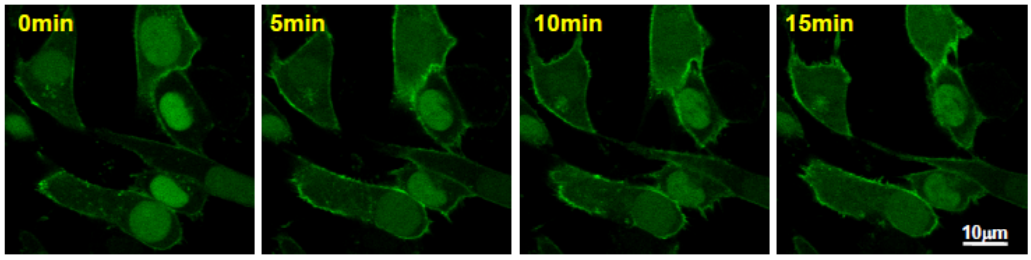


**Figure S1:** Thrombin-induced translocation of GFP-labeled LID to the plasma membrane. Thrombin (1 U/ml) was added at time zero. NHE1 is known to be activated in response to thrombin in this cell line.

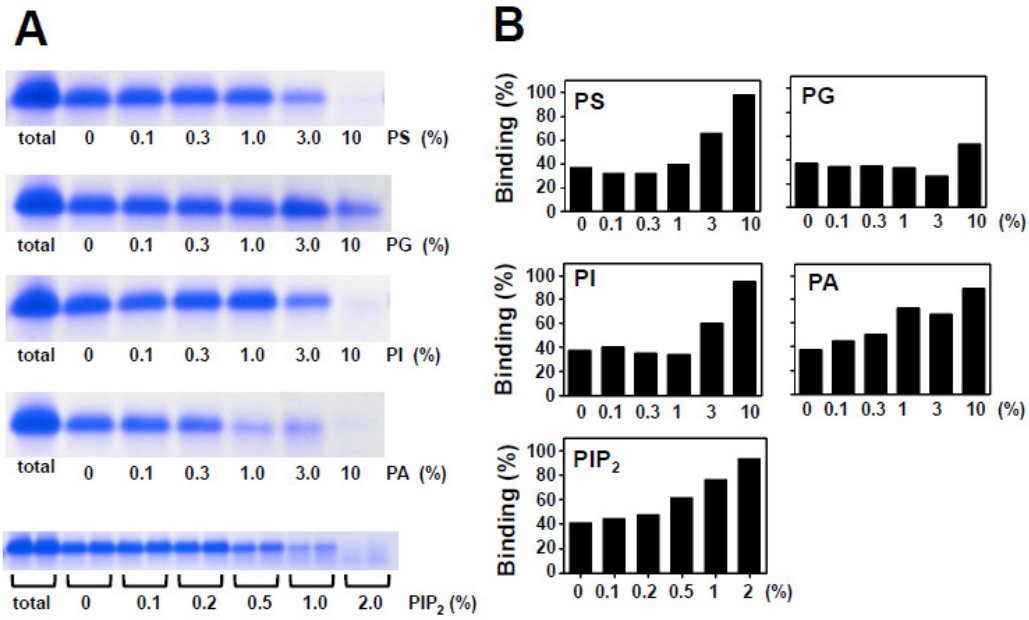


**Figure S2.** Binding of GP57 to liposomes. Liposomes containing various lipids (weight %, adjusted to 100% with PC) were prepared as described under "Materials and Methods", mixed with GP57, and centrifuged for 1h at 100,000 × *g*. (**A**) Peptides before centrifugation (total) and an aliquot of each supernatant were subjected to PAGE and stained with CBB. (**B**) Relative amounts of GP57 bound to liposomes were calculated. GP57 exhibited the following order of binding strength: $PIP_2$ > PS, PI, PA > PG > PC.

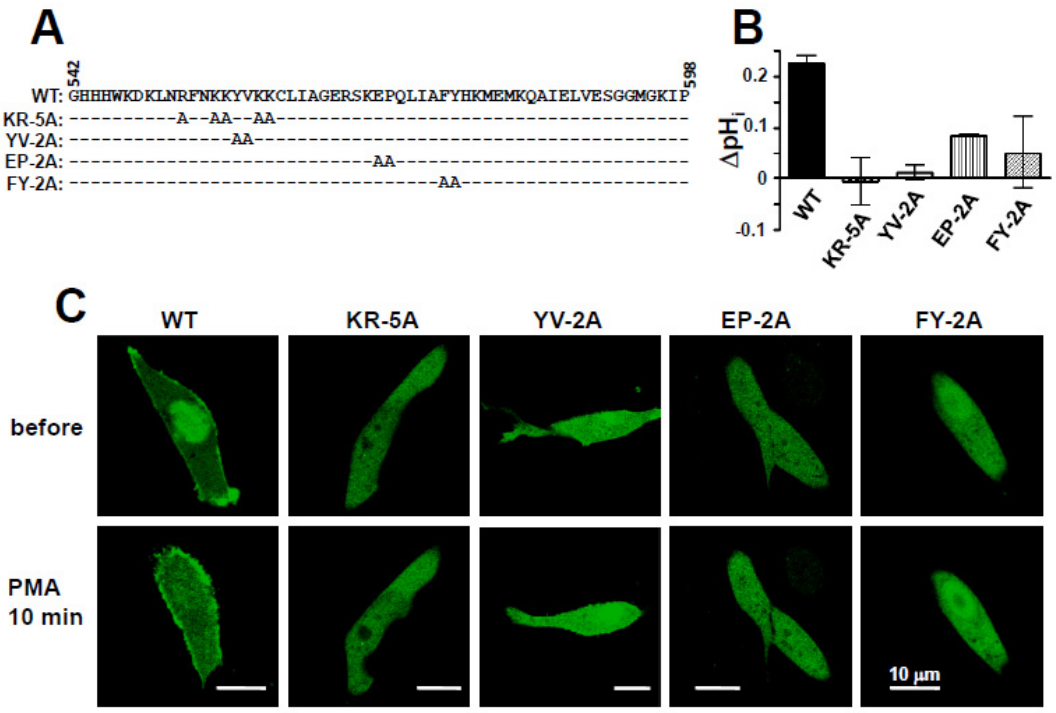


**Figure S3:** Effect of mutations in LID on PMA-induced changes in $pH_i$ and translocation to plasma membrane. (**A**) Locations of mutations. (**B**) PMA (1 μM)-induced change in $pH_i$ was measured in cells expressing the whole NHE1 molecule with the indicated mutations. These mutations markedly inhibited PMA-induced cytoplasmic alkalinization. Means ± S.D. (n = 3). (**C**) GFP-tagged LID (aa 542-598) with the indicated mutations was transiently expressed in cells. GFP fluorescence was observed before and 10 min after PMA addition. PMA failed to promote the translocation of mutant LID to plasma membrane. Scale, 10 μm.

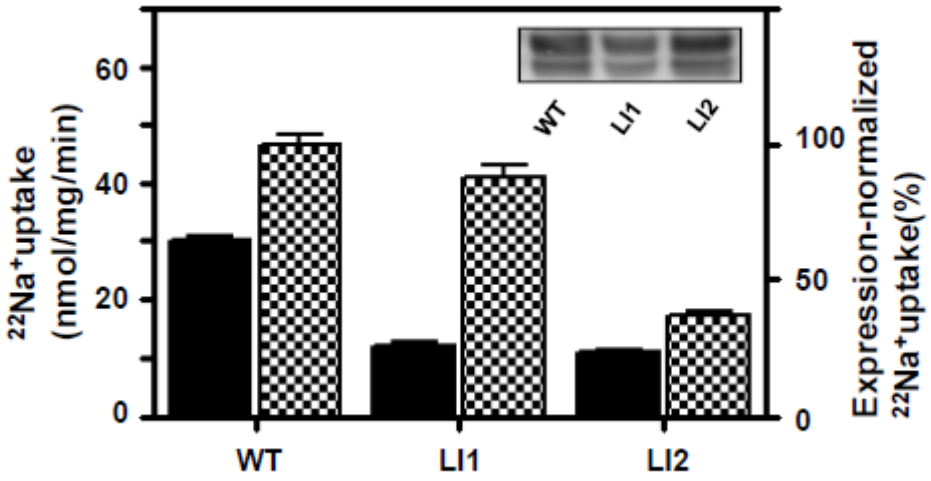


**Figure S4:** The maximal $^{22}Na^+$ uptake activity ($V_{max}$) of NHE1 variants. $^{22}Na^+$ uptake activity was measured at $pH_i$ 5.4 in cells expressing the wild-type (WT) NHE1 and two NHE1 mutants (LI1 and LI2). Inset represents the western blot stained with NHE1-specific antibody to estimate the expression levels of the protein. The upper and lower bands correspond to N-glycosylated mature NHE1 and immature NHE1, respectively. The $^{22}Na^+$ uptake was normalized by total protein expression (hatched bar). The normalized $V_{max}$ of mutant LI2 was significantly reduced. Means ± S.D (n = 3)

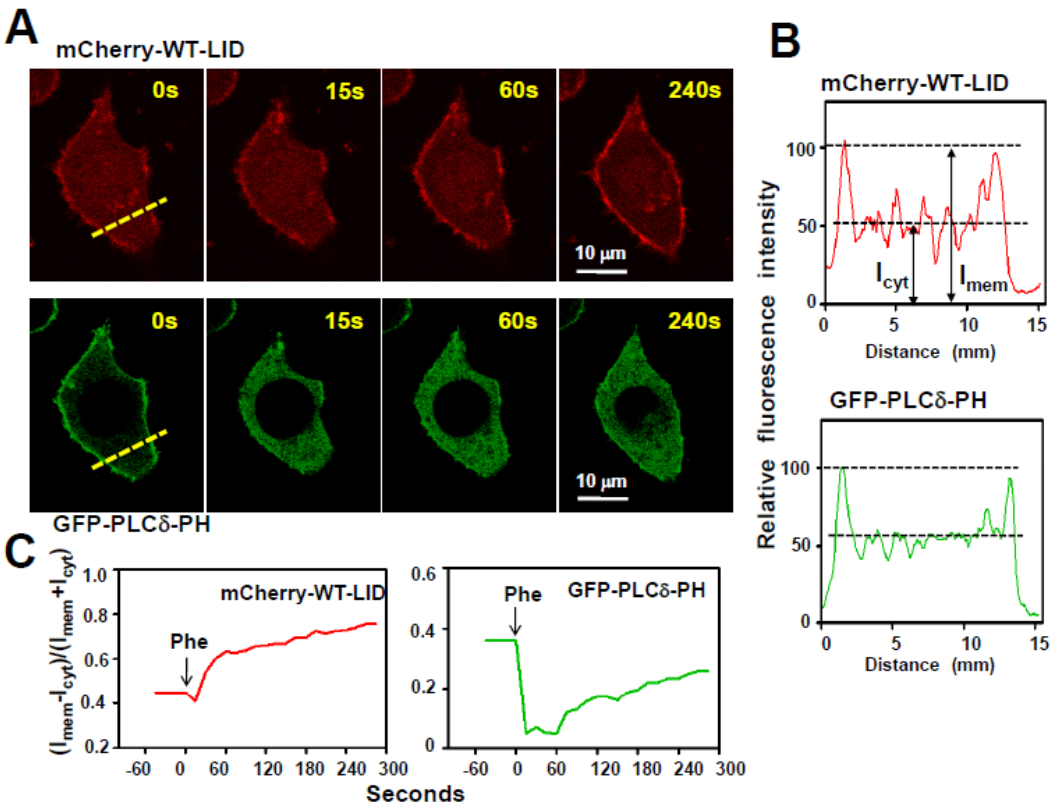


**Figure S5:** Phenylephrine (Phe)-induced translocation of mCherry-labeled NHE1-LID compared to that of $PIP_2$-monitoring probe GFP-PLCδ-PH in cells expressing mouse α1 adrenergic receptor. (**A**) Confocal microscopic observation of 2 fluorescent probes. Before phenylephrine (1 μM) addition (0 s), mCherry-LID was localized to plasma membrane but not extensively localized to the nucleus, unlike GFP-tagged LID (cf. Fig.1B). This difference may be due to the properties of the fluorescent probes (GFP vs. mCherry). **(B)** Line scanning of fluorescence intensity was performed along the dotted lines in (**a**)**,** fluorescence intensities in membrane ($I_{mem}$) and cytosol ($I_{cyt}$) were measured using Image-Pro Plus software (Media Cybernetics, Inc.), and relative membrane-localization index $(I_{mem}-I_{cyt})/(I_{mem}+I_{cyt})$ was calculated. **(C)** Time courses of relative membrane-localization index. Concomitant with rapid breakdown and slow re-synthesis of $PIP_2$, PLCδ-PH was translocated from the membrane to the cytosol immediately (<15s) after phenylephrine addition, and then partially relocated to plasma membrane. In contrast, only a small fraction of membrane LID was translocated to the cytoplasm, followed by continuous increase in membrane localization, consisting with the findings that in addition to $PIP_2$, LID can interact with multiple membrane lipids.

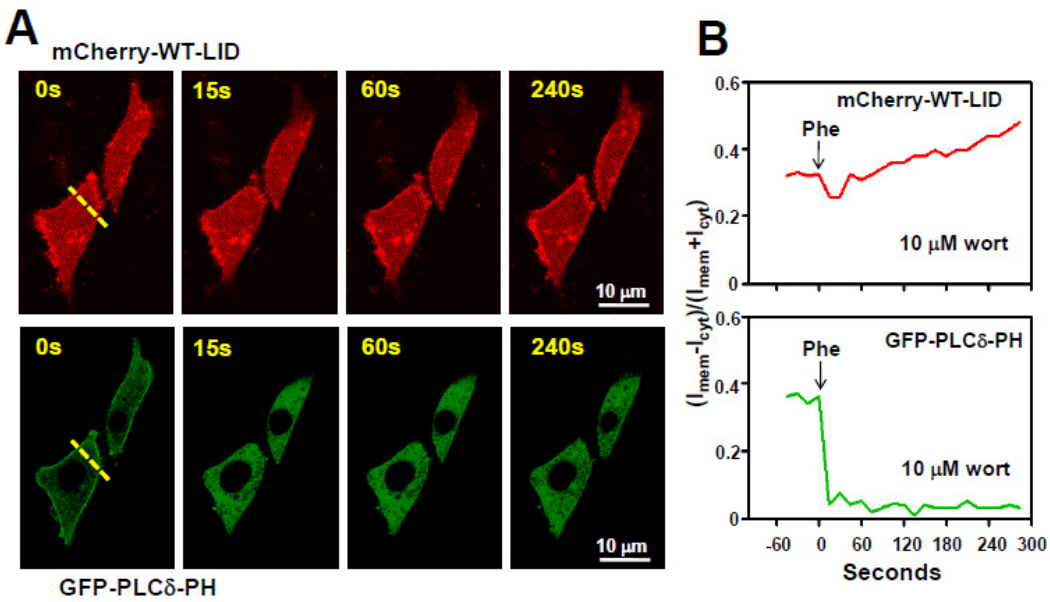


**Figure S6:** Effect of wortmannin (wort) on phenylephrine (Phe)-induced translocation of mCherry-labeled NHE1-LID compared to that of GFP-PLCδ-PH in cells expressing mouse α1-adrenergic receptor. **(A)** Confocal microscope observation of two fluorescent probes. Cells were preincubated for 15 min with wortmannin (10 µM) to prevent synthesis of $PIP_2$, and then phenylephrine (1 µM) + wort were added at time zero. **(B)** Time courses of relative membrane index of these probes. Although PLCδ-PH was translocated from the membrane to the cytosol immediately (<15s) after phenylephrine addition due to rapid breakdown of $PIP_2$, re-translocation to plasma membrane was strongly inhibited. In contrast, only a small fraction of membrane LID was translocated to the cytosol, followed by continuous accumulation to the plasma membrane even under conditions of reduced $PIP_2$.

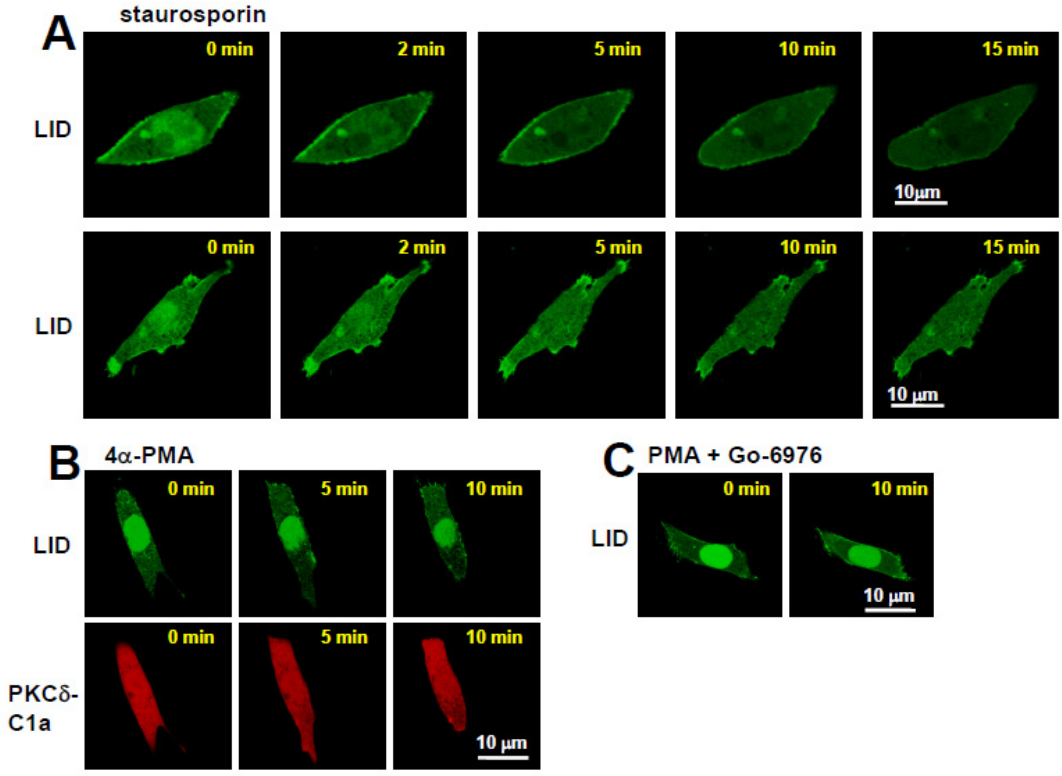


**Figure S7:** Effect of staurosporin, 4α-PMA and Go-6976 on the subcellular localization of GFP-LID. (**A**) Staurosporin (1 μM) itself did not promote the plasma membrane-translocation of LID, but at least partly reduced the amount of LID initially localized in plasma membrane, suggesting that St may reduce the affinity of LID for membrane lipids. Results of two experiments are represented. (**B**) 4α-PMA (100 μM) itself did not promote the plasma membrane translocation of LID (upper panel) or PKCδ-C1a (lower panel). (**C**) Go-6976 (1 μM) partially inhibited PMA-induced plasma membrane translocation of LID.

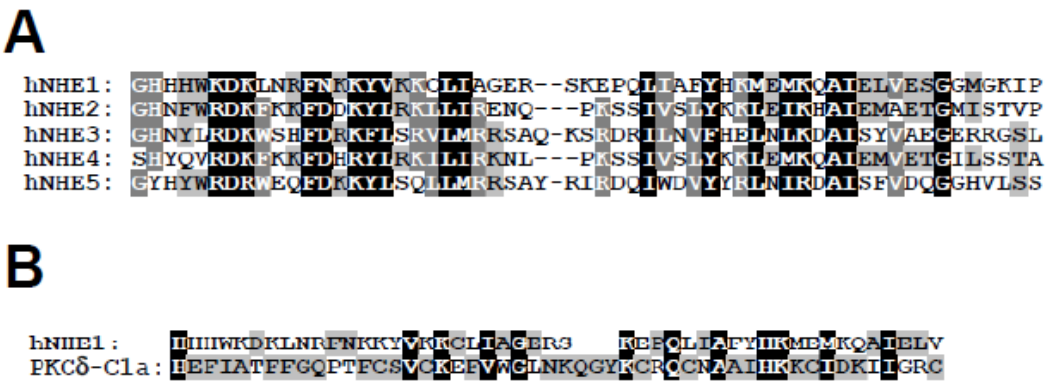


**Figure S8:** Amino acid sequence alignments of LID. (**A**) Sequence alignment among homologous regions of human $Na^+/H^+$ exchanger isoforms. Amino acids were shaded according to conservation. (**B**) Sequence alignment between LID of human NHE1 and PKCδ-C1a domain. There is a partial homology between these sequences, although NHE1 has no homologous cystein residues to the PKCδ-C1a domain, which are required for forming the zinc finger structure, the binding pocket for PEs.

## Movie captions.

**Movie 1**: PMA-induced translocation of GFP-LID (left) and mCherry-PKCδ-C1a (right) to the plasma membrane. In this movie, we selected cells showing the unremarkable initial plasma membrane localization of LID in order to clearly see its plasma membrane-translocation.

**Movie 2**: PMA does not facilitate the plasma membrane-translocation of GFP-tagged N-terminal 27 residues of LID (left), while it does facilitate that of mCherry-PKCδ-C1a (right).

**Movie 3**: OAG can facilitate the plasma membrane-translocation of GFP-LID (left), but not mCherry-PKCδ-C1a (right). Failure for PKCδ-C1a would be due to decreased effectiveness of DAG compared to PEs for the plasma membrane-translocation of this domain alone.

**Movie 4**: In cells expressing α1-adrenergic receptor, phenylephrin can facilitate the plasma membrane-translocation of GFP-LID (left), but not mCherry-PKCδ-C1a (right).

**Movie 5**: PMA does not facilitate the plasma membrane-translocation of GFP-tagged mutant LID (LI2) to plasma membrane (left), while it does facilitate that of mCherry-PKCδ-C1a (right).

**Movie 6**: In cells expressing α1-adrenergic receptor, GFP-PLCδ-PH (left) is translocated from the plasma membrane to the cytosol immediately (<15s) after phenylephrine addition, and then partially relocated to the plasma membrane. In contrast, only a small fraction of mCherry-LID (right) was translocated to the cytoplasm, followed by continuous accumulation in the plasma membrane.

**Movie 7**: In presence of wortemannin, re-translocation of PLCδ-PH (left) to the plasma membrane was strongly inhibited after rapid translocation from the plasma membrane to the cytosol in response to phenylephrin in cells expressing α1-adrenergic receptor. Even under this condition, only a small fraction of mCherry-LID (right) was translocated to the cytosol, followed by continuous accumulation to the plasma membrane.

**Movie 8**: Staurosporin completely blocks PMA-induced plasma membrane-translocation of GFP-LID (left), but not that of mCherry-PKCδ-C1a (right).

**Movie 9**: Calphostin C does not inhibit PMA-induced plasma membrane-translocation of GFP-LID (left), while it does inhibit that of mCherry-PKCδ-C1a (right).

**Movie 10**: 4α-PMA at least partially blocks PMA-induced translocation of GFP-LID (left), but not that of mCherry-PKCδ-C1a (right).