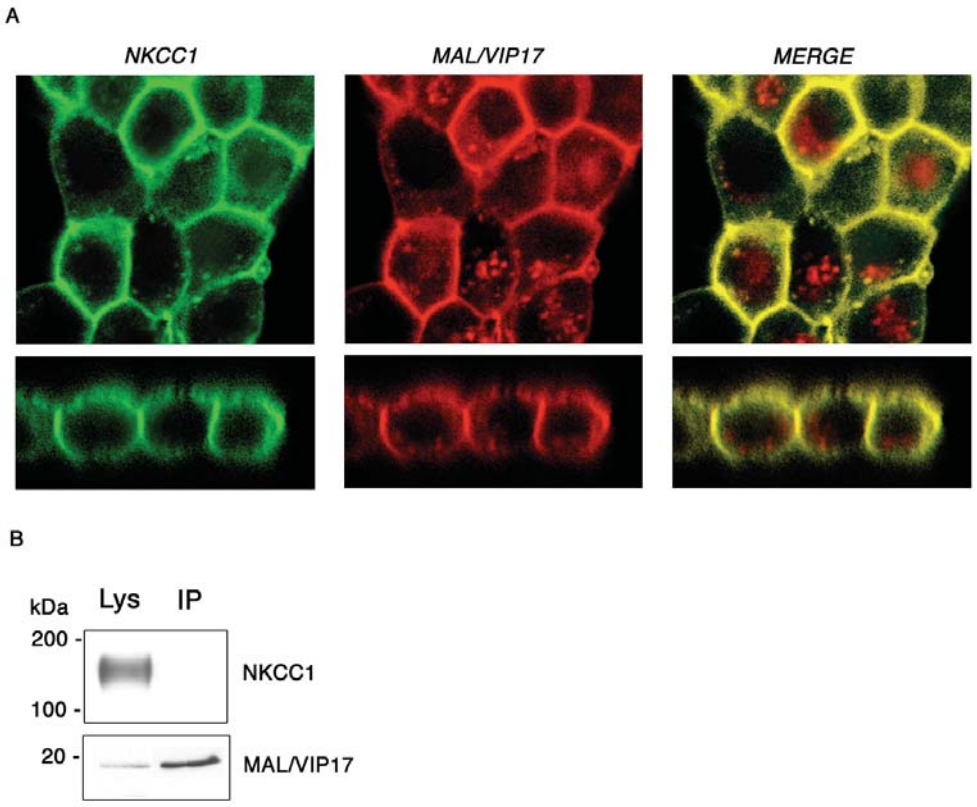
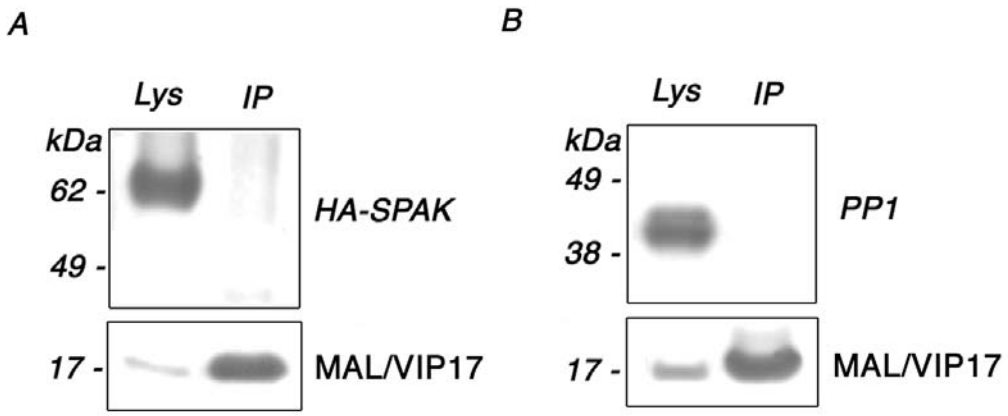
Supplemental Figure 1



Supplemental Figure 1: Colocalization and coimmunoprecipitation experiments in MAL/VIP17 and NKCC1 stably transfected HEK cells.
A) Stable HEK cell expressing NKCC1 were transfected with MAL/VIP17. Co-localization experiments were then performed using a monoclonal anti-HA antibody to stain c-NKCC2 (green) and a polyclonal anti Flag antibody to stain MAL/VIP17 (red). Images were collected with a confocal laser-scanning microscope. For each protein x-y and x-z sections are shown. The merge showed that NKCC1 and MAL/VIP17 colocalize at plasma membrane. B) The same cells were lysed in 2 % CHAPS and subjected to Immunoprecipitation with the polyclonal anti-Flag antibody. Western blotting analysis using HA antibody, showed that NKCC1 did not coimmunoprecipitate with MAL/VIP17.

Supplemental Figure 2



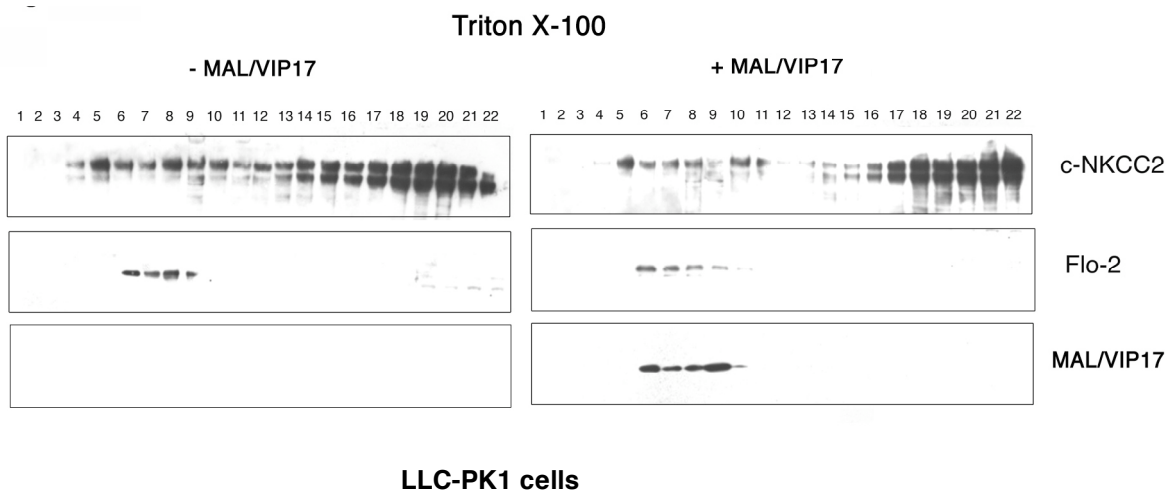
Supplemental Figure 2. Coimmunoprecipitation of MAL/VIP17 and HA-SPAK or the protein phosphatase-1 (PP1) in MAL/VIP17-expressing LLC-PK1 cells

A) LLC-PK1 cells, stably expressing Flag-tagged MAL/VIP17, were transiently transfected with the human HA-tagged SPAK construct. Cells were lysed in 2% CHAPS and subjected to Immunoprecipitation using anti Flag antibody. Western blotting analysis using anti HA antibodies showed that VIP17 did not co-immunoprecipitate with SPAK.

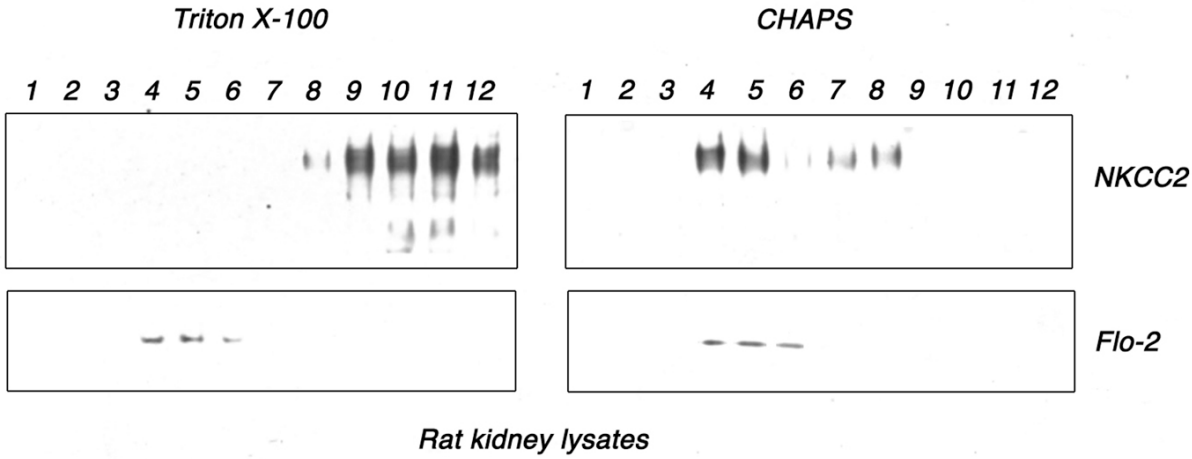
B) LLC-PK1 cells, stably expressing Flag-tagged MAL/VIP17, were lysed in 2% CHAPS and subjected to Immunoprecipitation using anti Flag antibody. Western blotting analysis antibodies against the anti-Protein Phosphatase-1 catalic subunit (PP1) showed that MAL/VIP17 did not co-immunoprecipitate with PP1.

Supplemental Figure 3

A



B



Supplemental Figure 3: Floatation assay of LLC-PK1 cell **A)** and rat kidney medulla **B)**. Cells or rat kidney medullae were lysed in the specific detergent at 4°C. Lysates were then subjected to floatation assay as described in methods. Twelve fractions, taken from the top to the bottom of the gradient, were subjected to immunoblotting with antibodies specific for the membrane proteins indicated on the right.