

Figure S1. Characterization of adenoviral vector for constitutively active PI3K (Adeno/Myr-p110-Myc). a) Immunoblot detection of the expression of chimeric protein Myr-p110-Myc with Myc monoclonal antibody. b) Evidence that adenoviral vector Adeno/Myr-p110-Myc delivers constitutively active PI3K in HK-2 cells. Expression of Myr-p110-Myc can substitute serum in activation of protein kinase Akt by phosphorylation. Serum is known to stimulate protein kinase Akt via activation of PI3K.

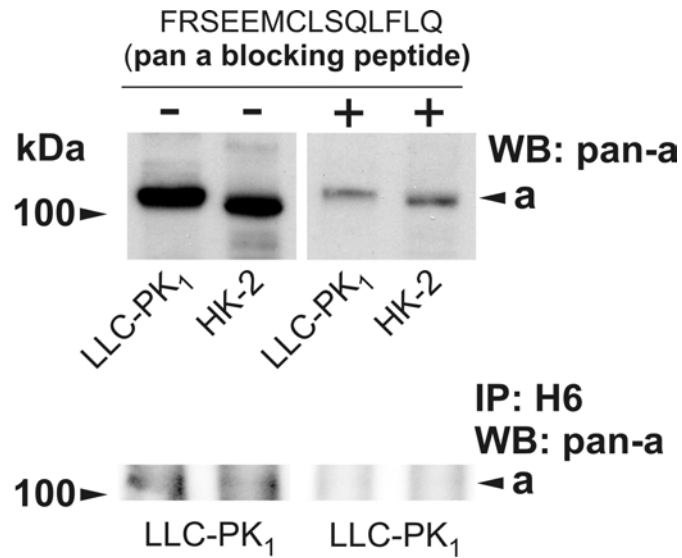


Figure S2. Analysis of immunospecificity of pan-a antibody by peptide competition analysis. 20 μ g protein samples of cell lysates obtained from LLC-PK₁ and HK-2 cells (upper panel) or aliquots of V-ATPase immunoprecipitated from LLC-PK₁ cells with H6 mAb (lower panel) were resolved by SDS-PAGE followed by electroblotting onto PVDF membrane. Portions of the membrane were incubated with primary antibody 'pan-a' against 'a' subunit with or without 5 mg/ml FRSEEMCLSQLFLQ followed by incubation with secondary HRP-linked antibody and simultaneous visualization by chemiluminescent detection. Pan a blocking peptide decreased significantly immunodetection of the protein with $M_r \approx 105-115$ corresponding to deduced molecular weight of the subunit 'a'.

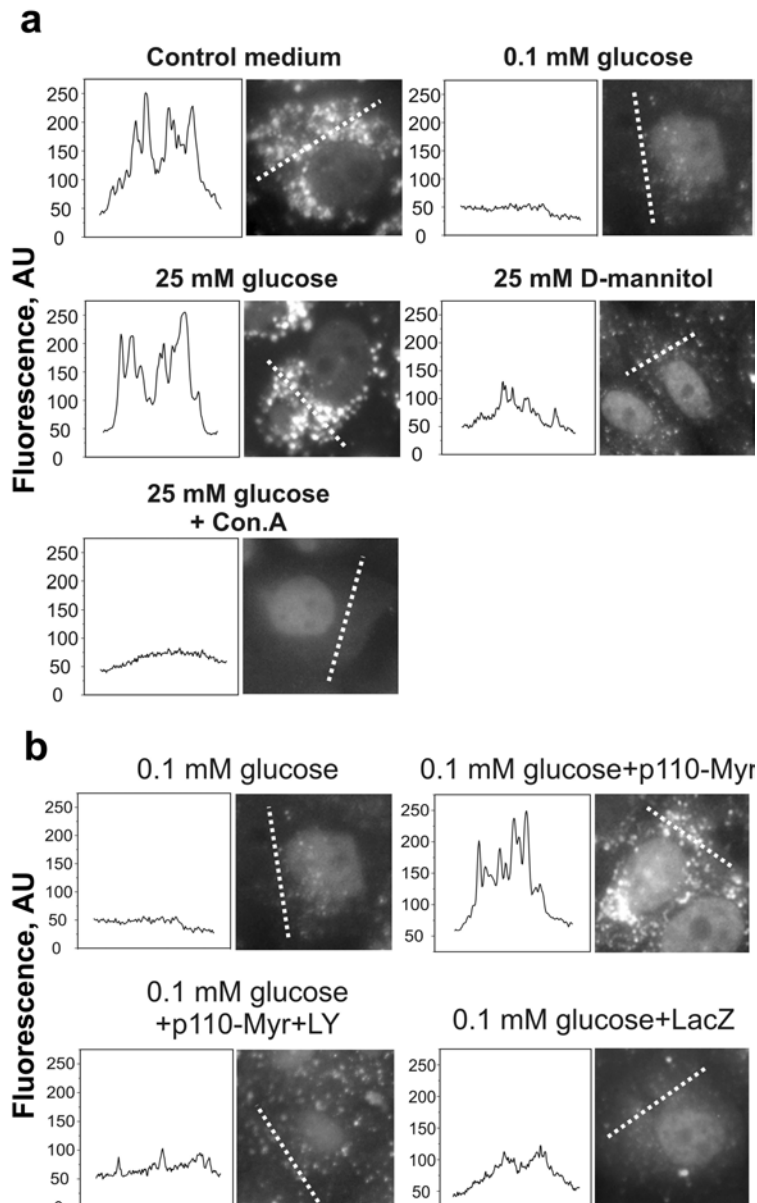


Figure S3. V-ATPase-dependent acidification of intracellular compartments of renal epithelial cells in response to glucose deprivation/stimulation and overexpression of constitutively active PI3K: fluorescence intensity profiles. LLC-PK₁ cells were incubated in standard medium containing glucose and serum on coverslips. **(a)** 16-18 h prior to DAMP labeling cells were transferred to the glucose-free medium containing dialysed serum and 0.1 mM glucose. Concanamycin A (100 nM), LY294002 (25 μ M) and vehicle were added 60 min before stimulation with glucose or D-mannitol. **(b)** Cells were infected with Adeno/Myr-p110-Myc or Adeno/LacZ at MOI 10 for 48 h. 16-18 h prior to DAMP labeling cells were transferred to the glucose-free medium containing dialysed serum and 0.1 mM glucose. LY294002 (25 μ M) and vehicle were added 60 min before DAMP labeling. 60 min incubation with DAMP was followed by fixation and staining with fluorescein-conjugated anti-dinitrophenol antibody. Fluorescence intensity profiles of acidic vesicles scattered in the cytoplasm were analyzed for several randomly chosen cross sections (dotted line) or rectangular area (not shown) using image analysis software NIH Scion Image. Representative fluorescence profiles and corresponding cross-sections are shown. Peaks on histograms represent high-intensity fluorescence of acidic vesicles whereas baseline shows nonspecific background fluorescence of the cytoplasm.