Supplementary figures

Supplementary figure 1. Expression of PDZK1 protein was totally abolished in renal tissue of the *Pdzk1 -/- mouse*. Both brush border membrane vesicles (BBMV) and total homogenates were tested with a polyclonal rabbit anti-PDZK1, kindly provided by David Silver, by western blot analysis.

Supplementary figure 2. Specificity of NaPi-2c polyclonal rabbit antibody was tested by peptide blocking assay. A) Western blot analysis of renal BBMV of wild type mice fed high Pi (H Pi) or low Pi (L Pi) diets with anti-NaPi2c (left; Ab) and with anti-NaPi-2c incubate with its antigenic peptide (right; Ab + peptide). B) Immunofluorescence of *Pdzk1* -/- kidney slices stained with anti-NaPi-2c (upper row), anti-NaPi-2c + peptide (middle row) and the same slice with digital increase of the NaPi-2c signal (red) intensity (lower row). Simultaneously actin was stained with phalloidin Alexa-488 (Green). A zoomed image of the merge staining is showed at the side for comparison. Both the apical and the intracellular staining obtained with the NaPi-2c antibody were prevented by using the antigenic peptide.

Supplementary figure 3. BBMV protein expression in male wild type (WT) and *Pdzk1* -/- mice in response to chronic adaptation to high (1.5%) and low (0.1%) Pi diets. A similar adaptive response was observed in male *Pdzk1* -/- animals with normal adaptation to low Pi diets of NaPi-2a expression in BBM and impaired upregulation of NaPi-2c in *Pdzk1* -/- model under the same conditions.

Supplementary figure 4. NaPi-2c shows diminished apical expression in *Pdzk1* -/- mice. Renal section derived from wild type and *Pdzk1* -/- mice were stained for actin (with phalloidin, green), NaPi-2c (red) and nuclei (blue). NaPi-2c staining in the superficial cortex (SC) tubules (first row) is clearly weaker than in juxtamedullary tubules (third row) in the wild type. NaPi-2c apical membrane expression is totally blunted in SC tubules of *Pdzk1* -/- resulting in an increased intracellular perinuclear staining (second row). JM tubules of *Pdzk1* -/- showed a diminished apical expression with increased subapical staining but still conserved partial NaPi-2c expression in the apical membrane (fourth row).

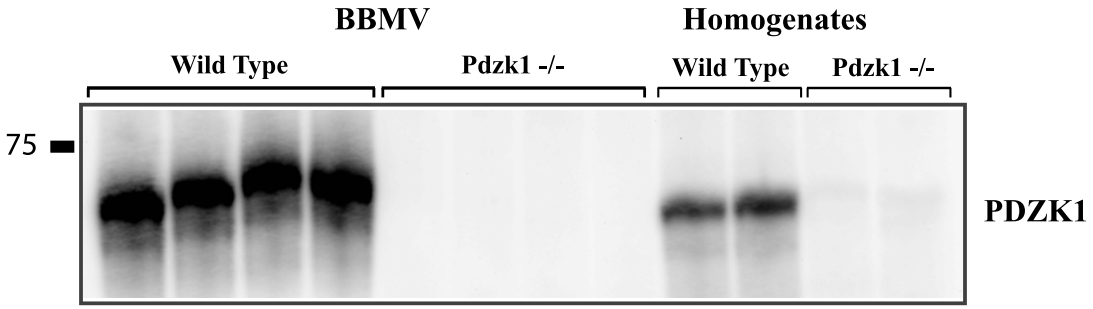
Supplementary figure 5. Analysis of FRET data in the phasor plot. Given the phasors of the unquenched donor (D_{unq}) and of the autofluorescence (af), the phasor of the quenched donor (D_q) is expected to lie along a calculated trajectory of variable efficiency (black solid line). The phasor of a mixture of quenched and unquenched donors is a linear combination of the phasors of D_q and D_{unq} and will lie along the segment connecting them (dashed line), at a distance from D_{unq} proportional to the fraction of interacting donors (f_q).

Supplementary figure 6. Procedure to calculate the FRET efficiency and the fraction of interacting donors. The phasor coordinates associated with the Donor only (D only) and Donor plus Acceptor samples (D+A) are fitted to a linear trend. The intersection between the trend line and the calculated trajectory of variable efficiency yields the extrapolated phasor of the Donor quenched with efficiency E. The fraction of quenched and unquenched donors in the Donor plus Acceptor samples is then calculated. Experiment sample: Cerulean-NaPi-2a vs EYFP-NHERF-1.

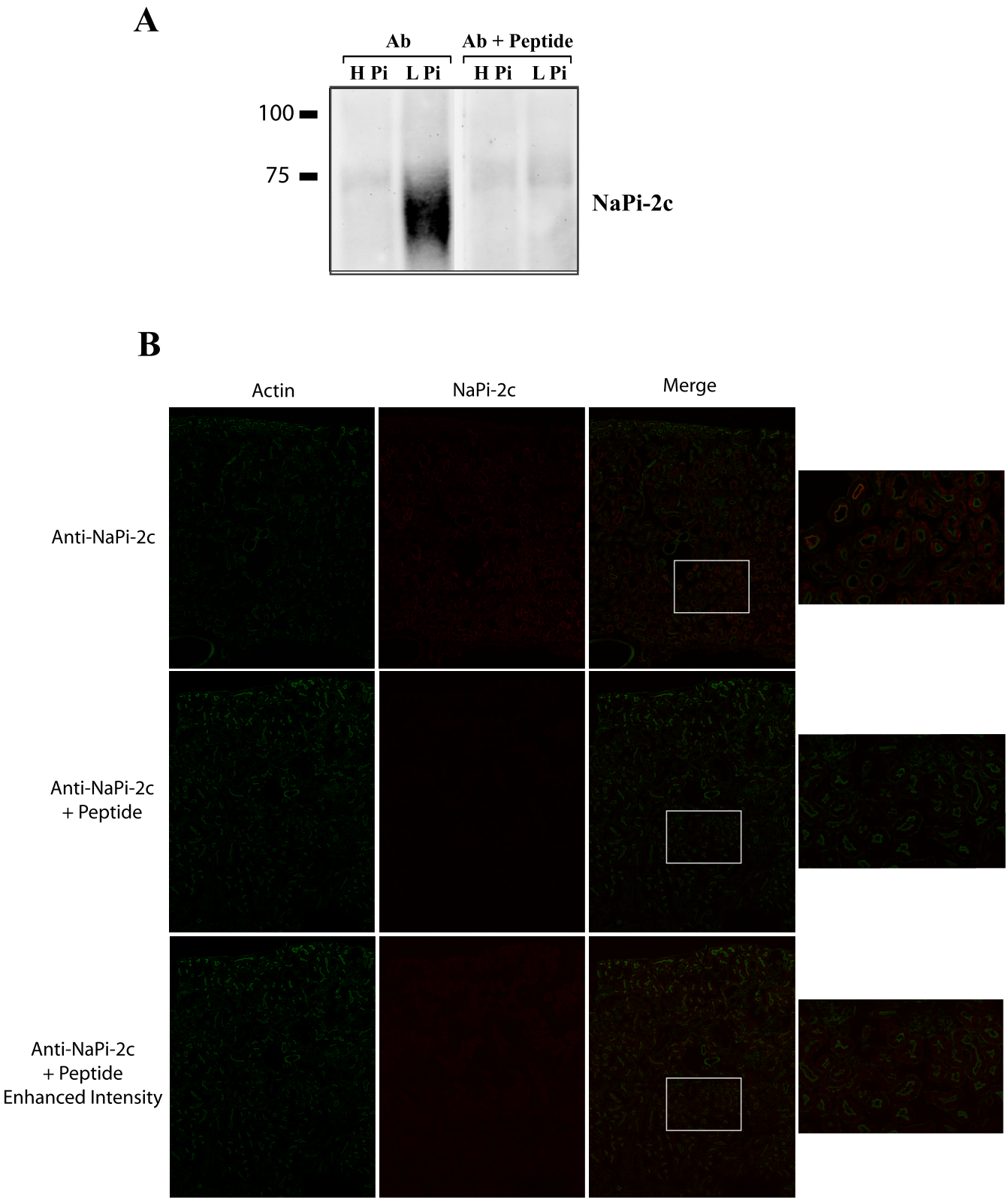
Supplementary figure 7. Phosphate excretion measurements. Urine levels of phosphate and creatinine were measured in wild type and *Pdzk1* -/- animals fed high and low Pi diets during one week. Data was plotted as Urine Pi/Urine Creatinine ratio and Fractional Excretion Index (FEI) of Pi defined previously as Urine Pi/(Urine Creatinine*Plasma Pi). Both measured parameters did not show any significant differences between wild type (WT) and *Pdzk1* -/- animals. An extensive increase in phosphaturia was observed in the animals fed a high Pi diet in both groups.

Supplementary figure 8. Schematic representation of the molecular interactions between the Na/Pi transporters and the PDZ proteins PDZK1 and NHERF-1. The Na/Pi transporters are represented as transmembrane proteins (NaPi-2c-green; NaPi-2a-yellow) fused to the Cerulean fluorescent protein (Blue) and PDZK1 (with 4 different PDZ domains) and NHERF-1 (with 2 PDZ domains and the ERM-binding motif in the C-terminus) fused to EYFP (Green). NaPi-2c interacts with the second while NaPi-2a interacts with the third domain of PDZK1 suggesting that the distance between the fluorescent proteins (FPs) will be larger for the interaction NaPi-2a/PDZK1. In the other hand, both NaPi-2c and NaPi-2a have been proved to interact with the first PDZ domain of NHERF-1 and presumably the distance between the FPs should be in the same range. The relative distances showed below have been calculated considering: FP length - 4 nm, PDZ domain length- 3.5 nm and interdomain aminoacid chain being in alpha-helix structure- 0.15nm/residue. It is important to notice that these distances are referring to the totally extended protein conformation and that under physiological conditions the multiprotein complex could be folded in a more compact structure.

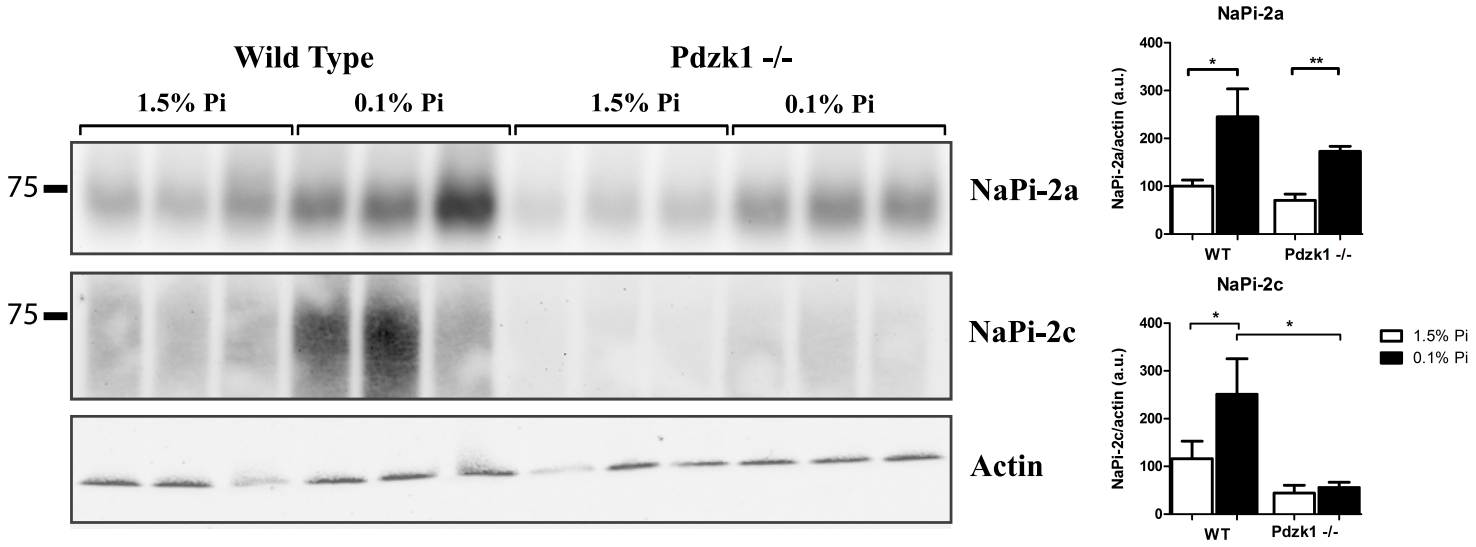
Supplementary Figure 1



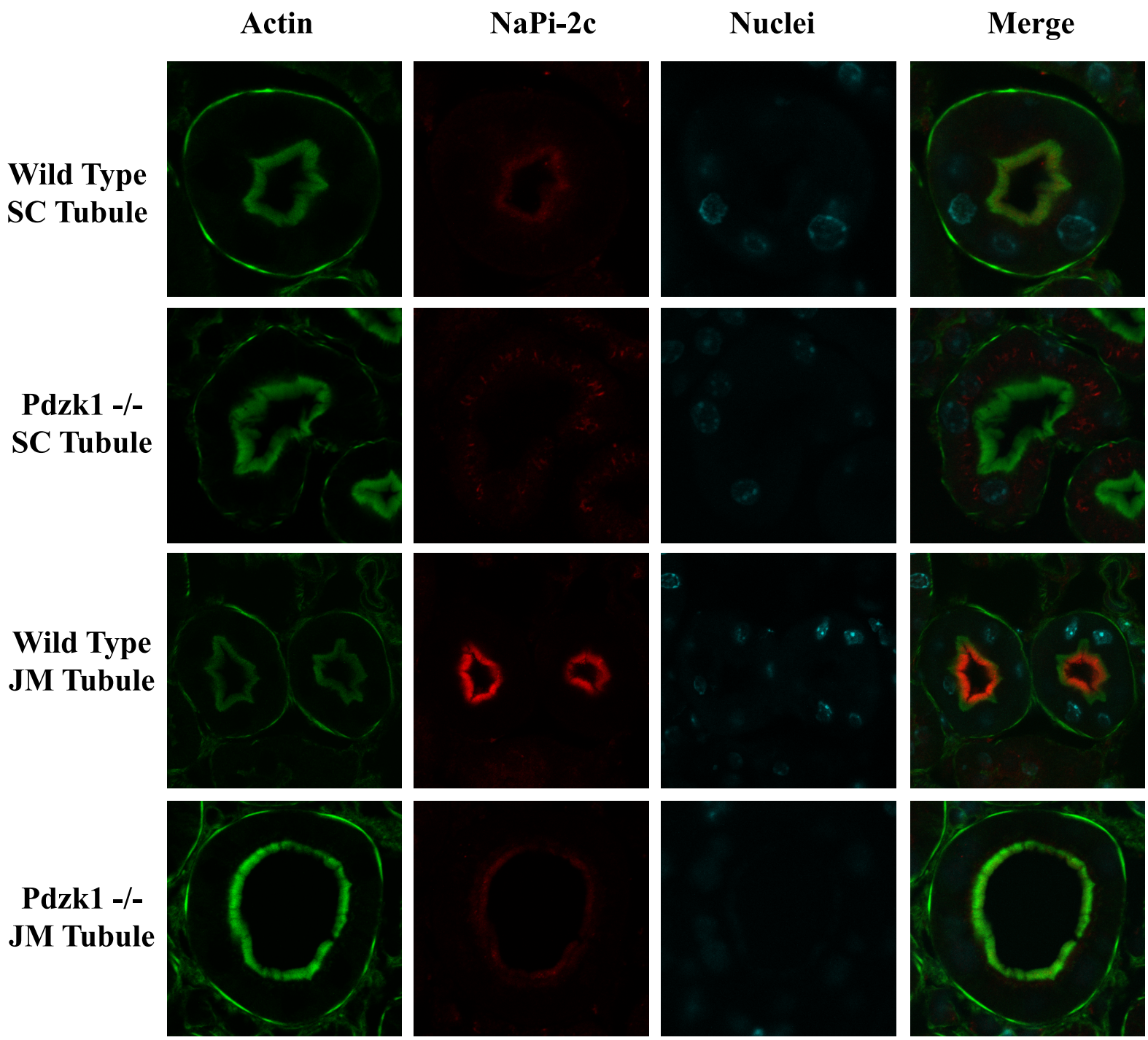
Supplementary Figure 2



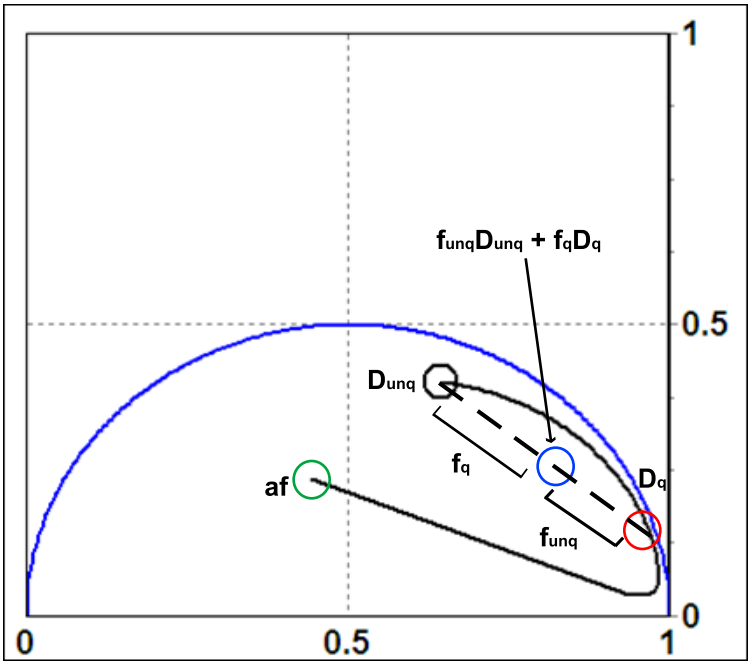
Supplementary Figure 3



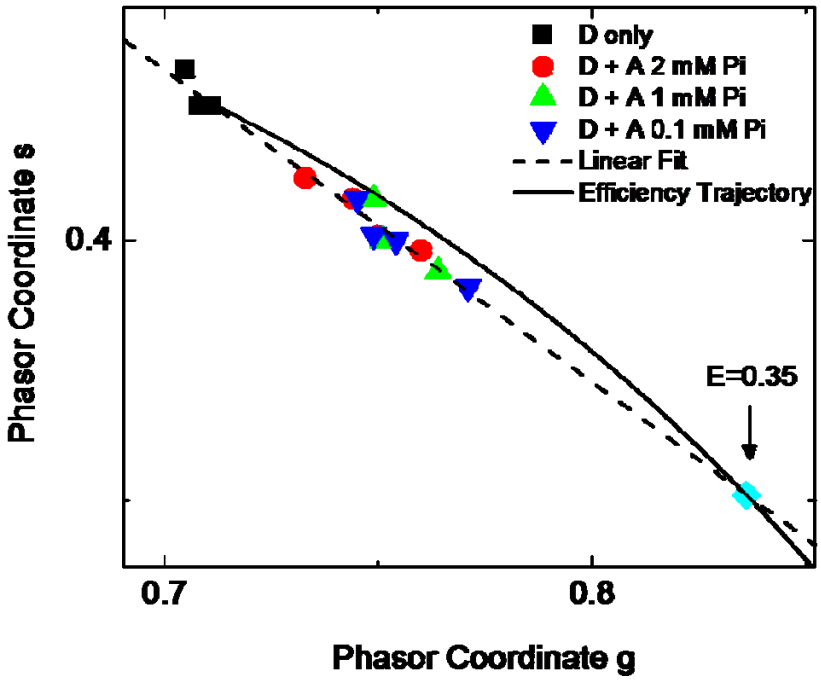
Supplementary Figure 4



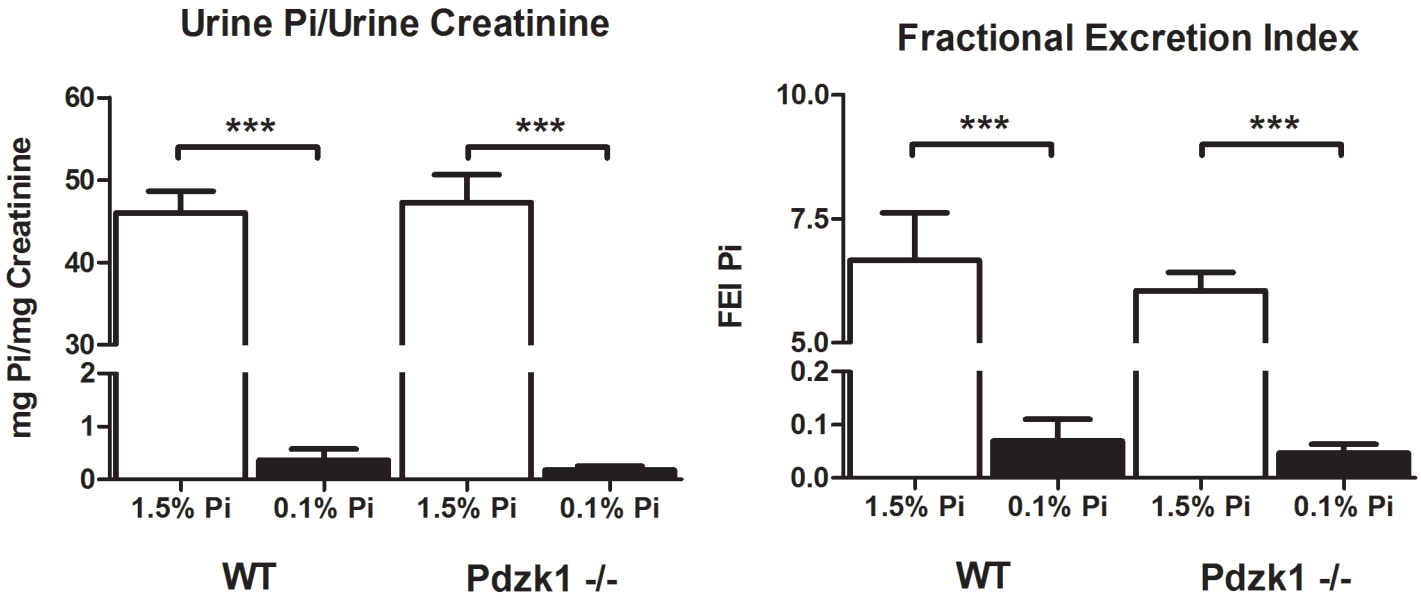
Supplementary Figure 5



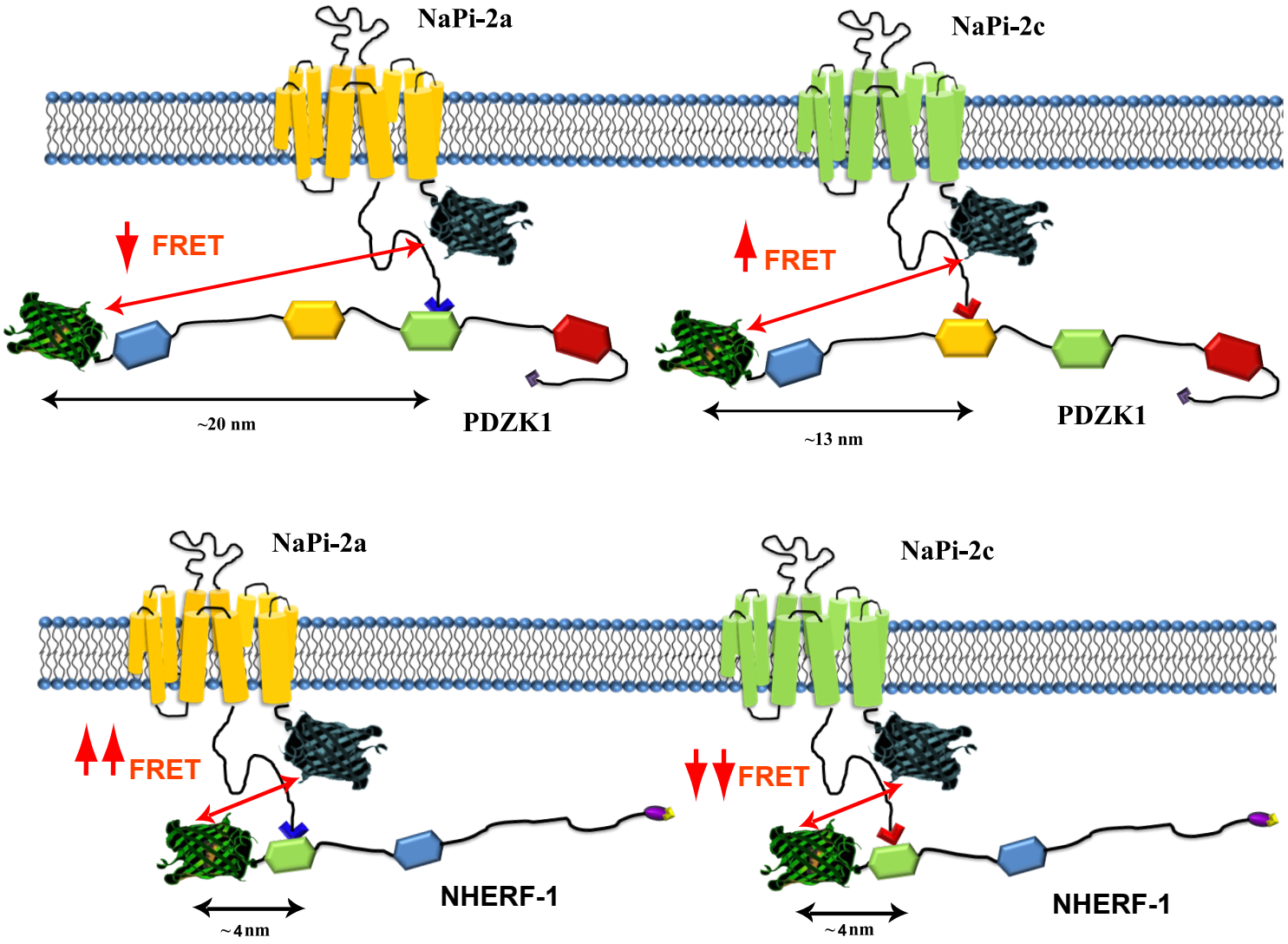
Supplementary figure 6.



Supplementary Figure 7



Supplementary Figure 8



Legend

- Green Fluorescent protein
- Cerulean Fluorescent protein
- PDZ domain
- PDZ binding motif
- ERM binding domain