SUPPLEMENTARY FIGURES ONLINE

Consequences of SPAK inactivation on Hyperkalemic Hypertension caused by *WNK1* mutations: evidence for differential roles of WNK1 and WNK4.

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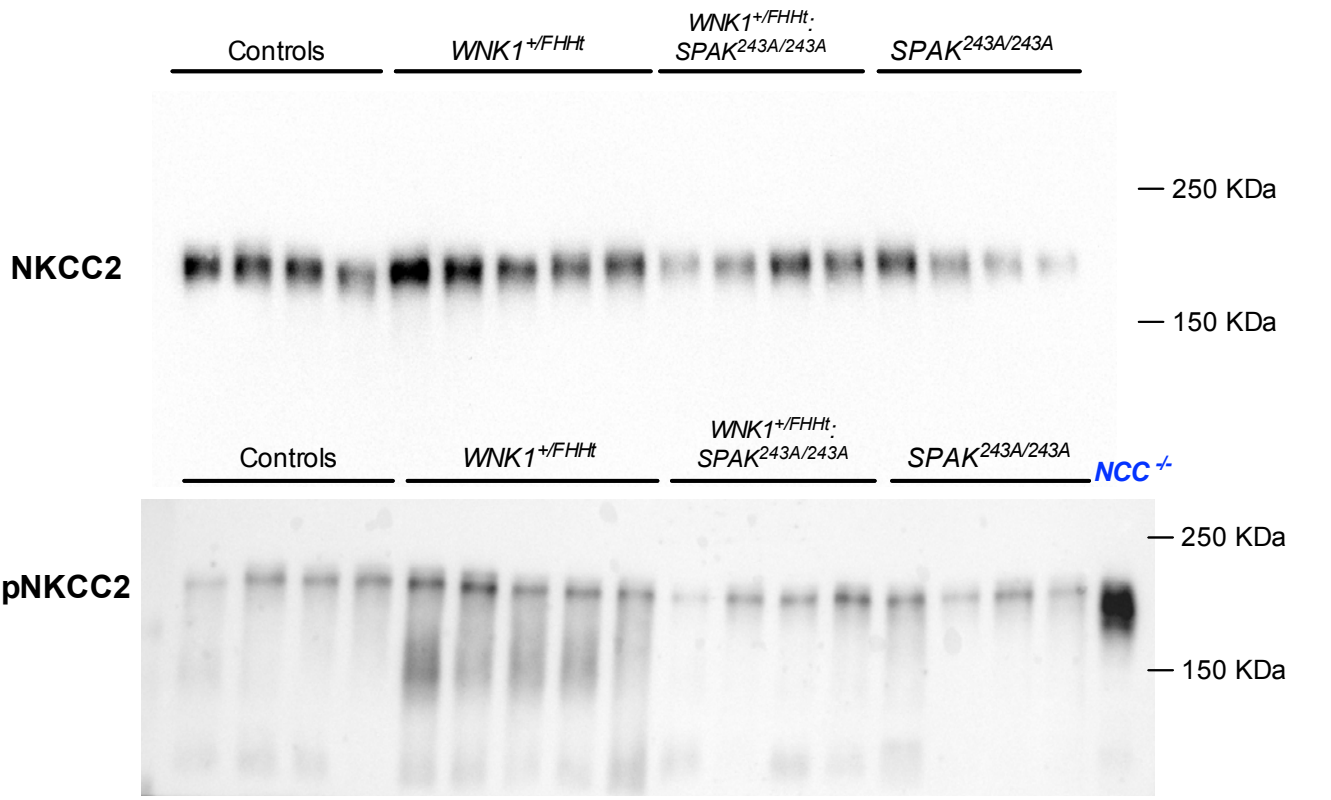
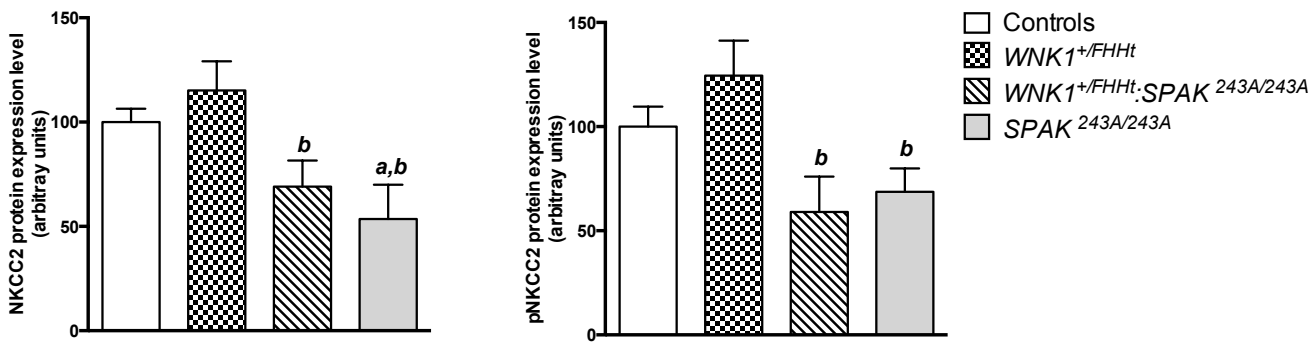
Supplementary Figure S1



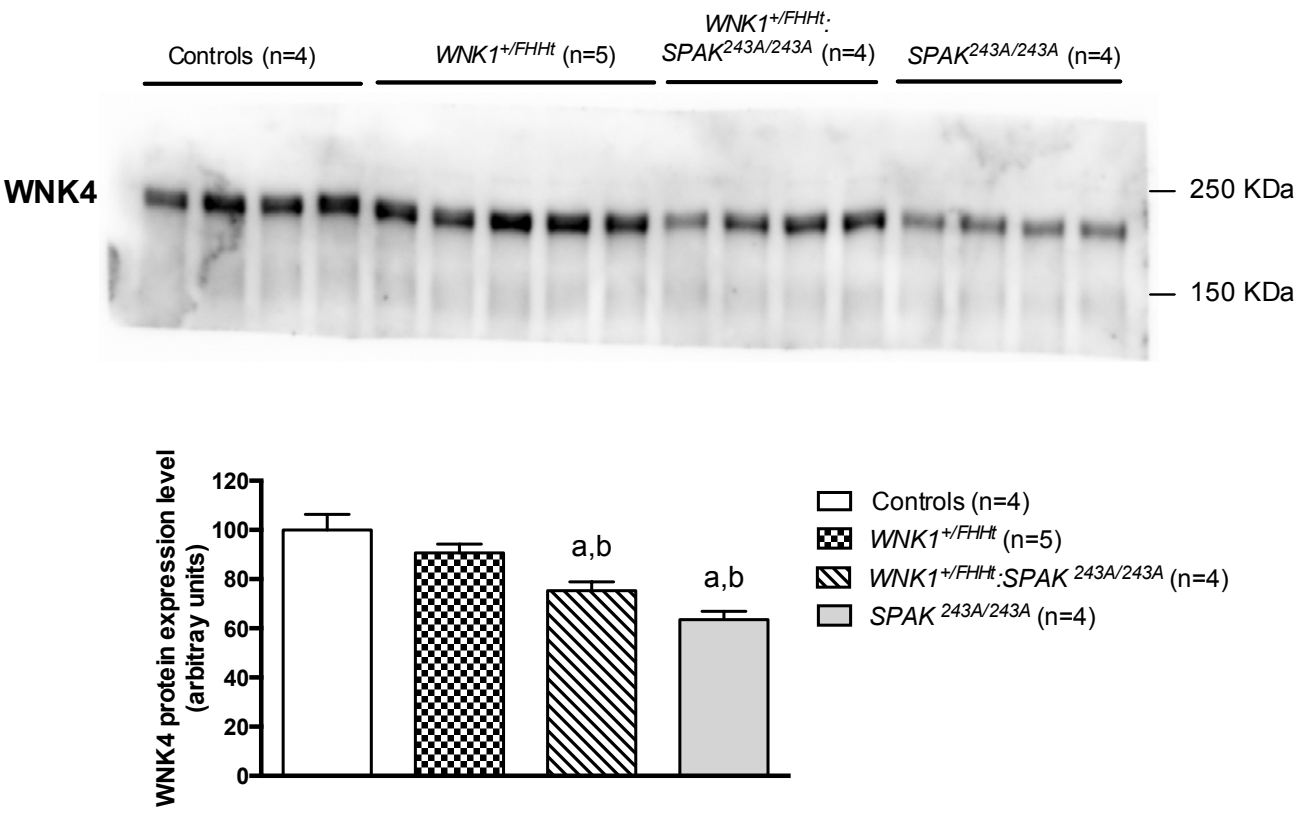


Supplementary Figure S1. The expression and phosphorylation of NKCC2 are decreased in *WNK1$^{+/FHHt}$:SPAK$^{243A/243A}$* mice.

Upper panel: Representative immunoblots of NKCC2 and phosphorylated NKCC2 performed on the membrane-enriched fractions of the renal cortex of mice of each genotype. As pNKCC2 antibody is also able to recognize pNCC, we loaded a protein sample extracted from a *NCC$^{-/-}$* mouse kidney (in blue, last lane of the pNKCC2 blot) to identify the band corresponding specifically to pNKCC2.

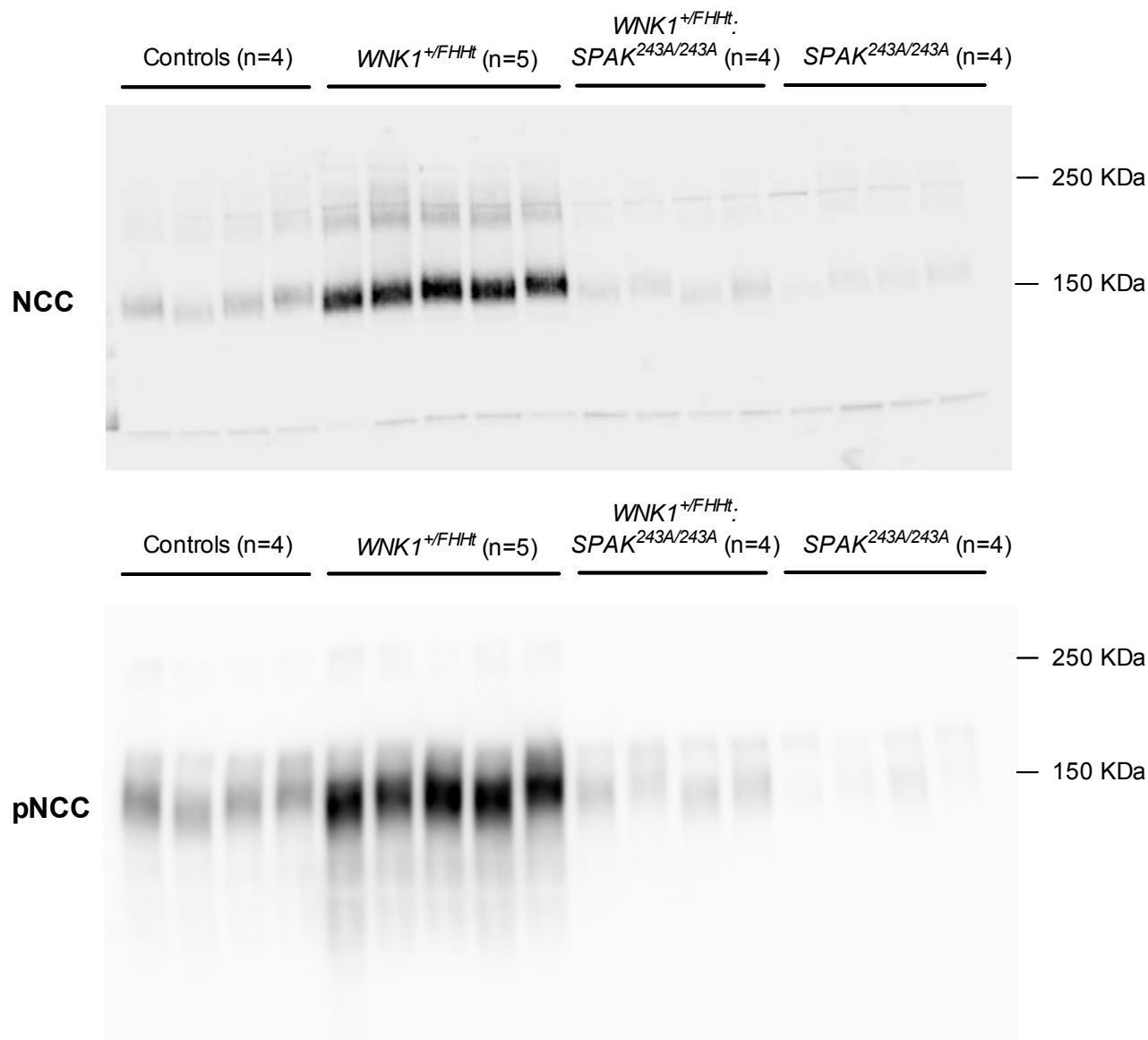
Lower panel: Densitometric analysis. NKCC2 abundance and phosphorylation are decreased in *WNK1$^{+/FHHt}$:SPAK$^{243A/243A}$* and *SPAK$^{243A/243A}$* mice. In contrast to NCC, there is no difference between these two groups. Number of animals: 4 control, 5 *WNK1$^{+/FHHt}$*, 4 *WNK1$^{+/FHHt}$:SPAK$^{243A:243A}$* and 4 *SPAK$^{243A:243A}$* male mice. Values are means ± s.e.m. [a] $p<0.05$ vs. controls. [b] $p<0.05$ vs. *WNK1$^{+/FHHt}$* mices (Kruskal-Wallis one-way analysis of variance, followed by Mann-Whitney test). Immunoblot images are full-lenght images.

Supplementary Figure S2



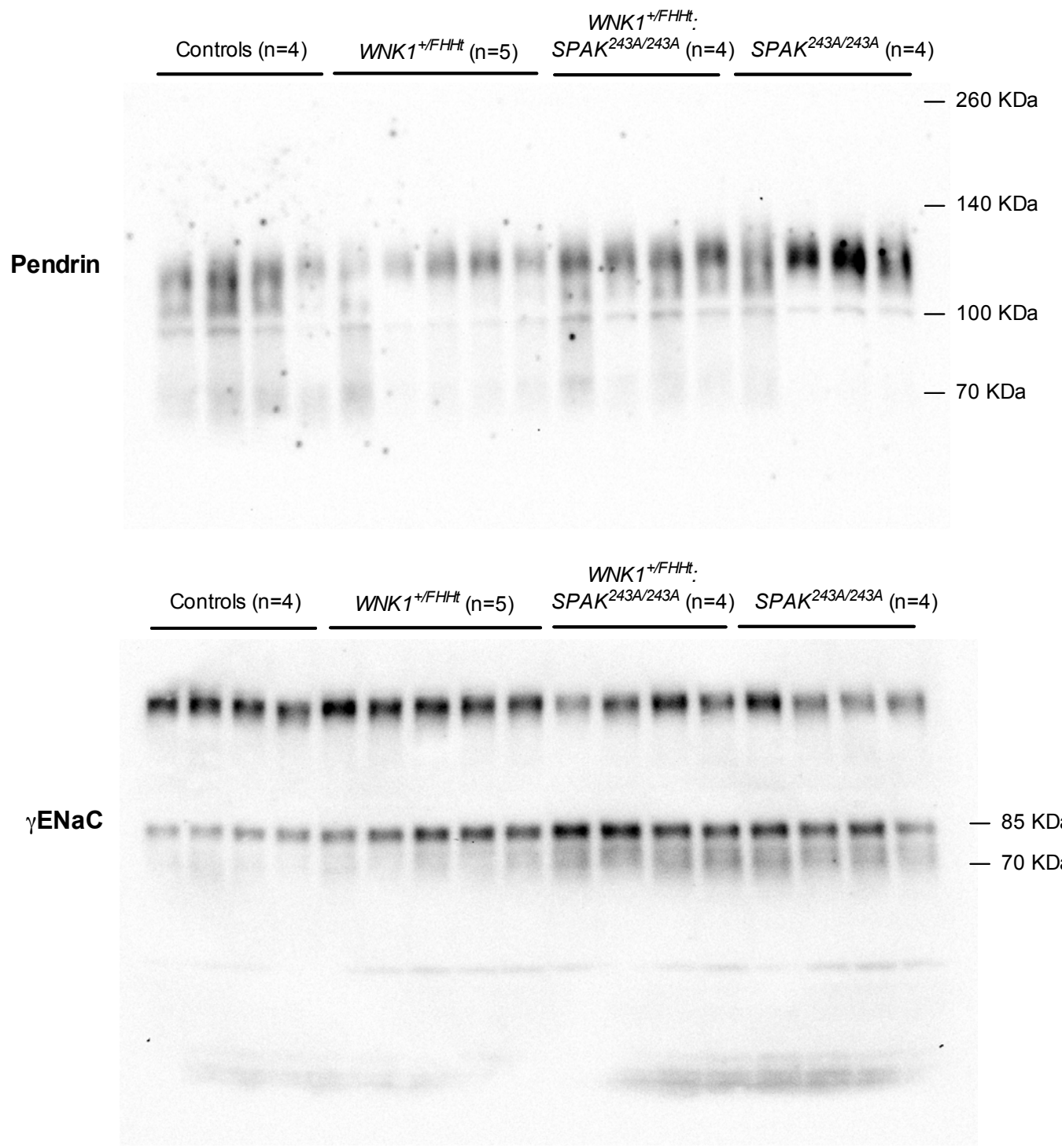
Supplementary Figure S2. WNK4 expression is decreased in *WNK1$^{+/FHHt}$:SPAK$^{243A:243A}$* mice. **Upper panel:** Representative immunoblot of WNK4 performed on protein extracted from the renal cortex of mice of each genotype. **Lower panel:** Densitometric analysis. WNK4 abundance is decreased in *WNK1$^{+/FHHt}$:SPAK$^{243A/243A}$* and *SPAK$^{243A/243A}$* mice compared to control mice. Number of animals: 4 control, 5 *WNK1$^{+/FHHt}$*, 4 *WNK1$^{+/FHHt}$:SPAK$^{243A:243A}$* and 4 *SPAK$^{243A:243A}$* male mice. Values are means ± s.e.m. [a] $p<0.05$ vs. controls. [b] $p<0.05$ vs. *WNK1$^{+/FHHt}$* mice. The immunoblot image is a full-lenght image.

Supplementary Figure S3



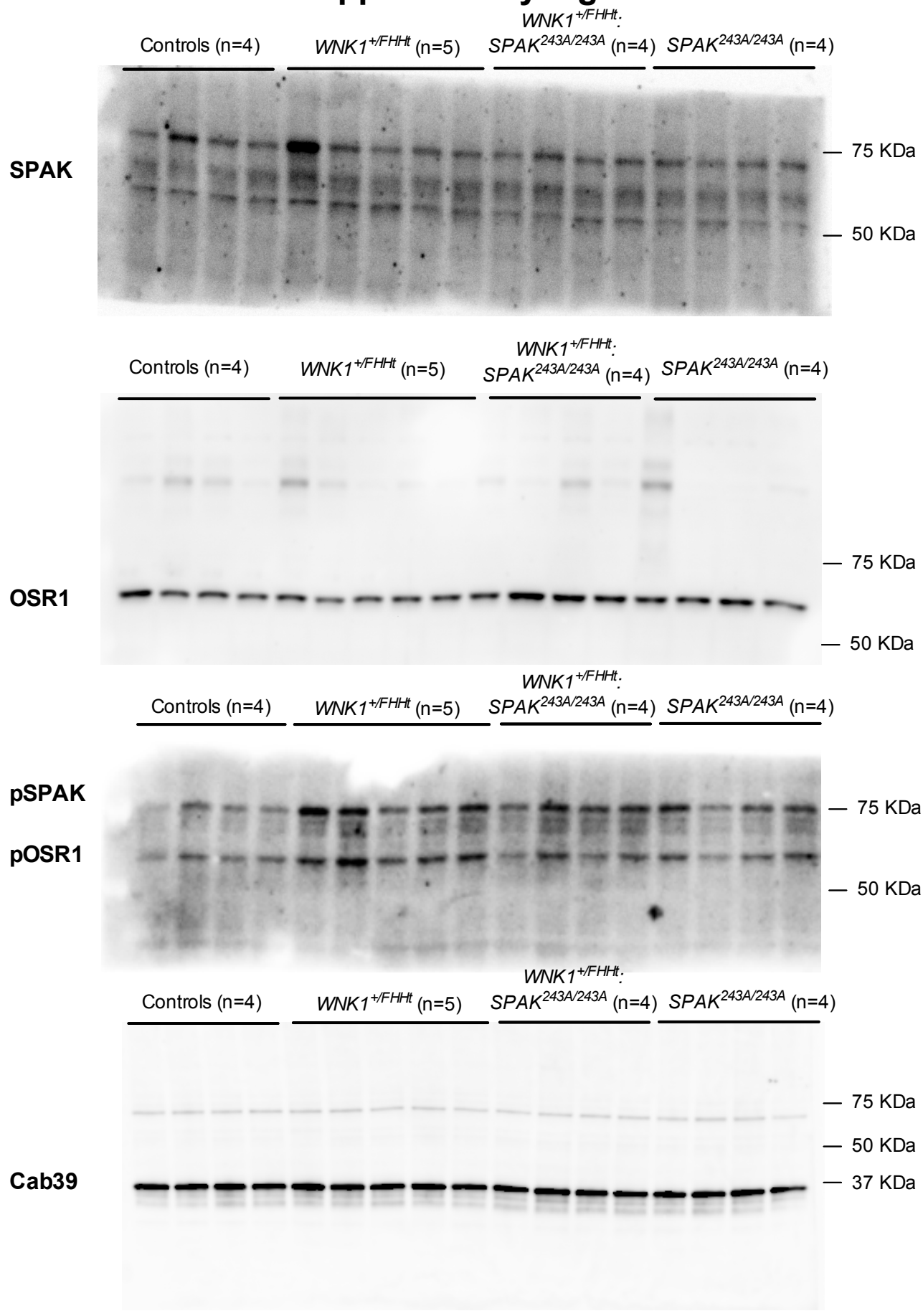
Supplementary Figure S3. Full-lenght iamges of the NCC and pNCC immunoblots, corresponding to Figure 2.

Supplementary Figure S4



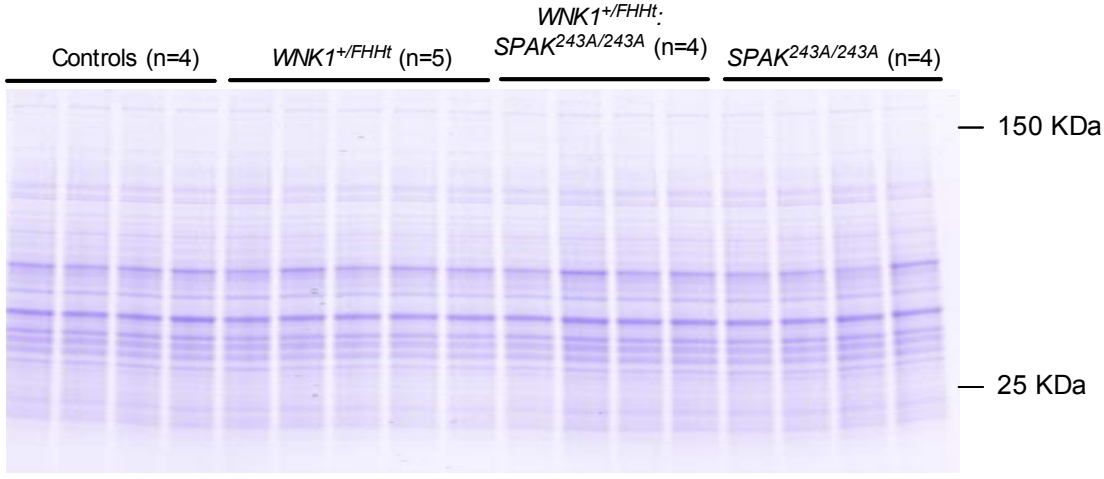
Supplementary Figure S4. Full-lenght iamges of the pendrin and γENAC immunoblot, corresponding to Figure 3.

Supplementary Figure S5

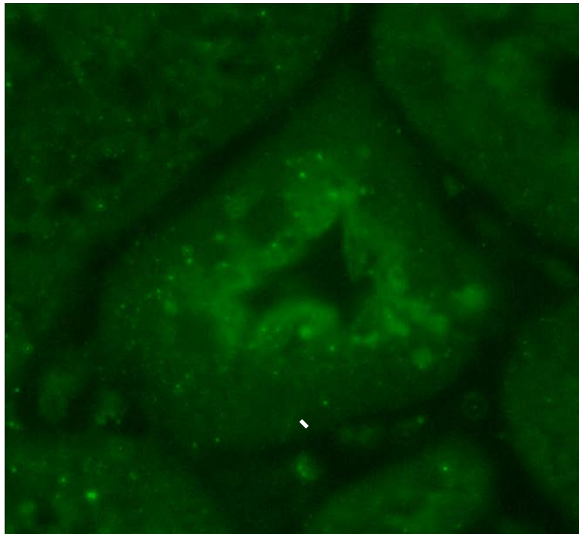


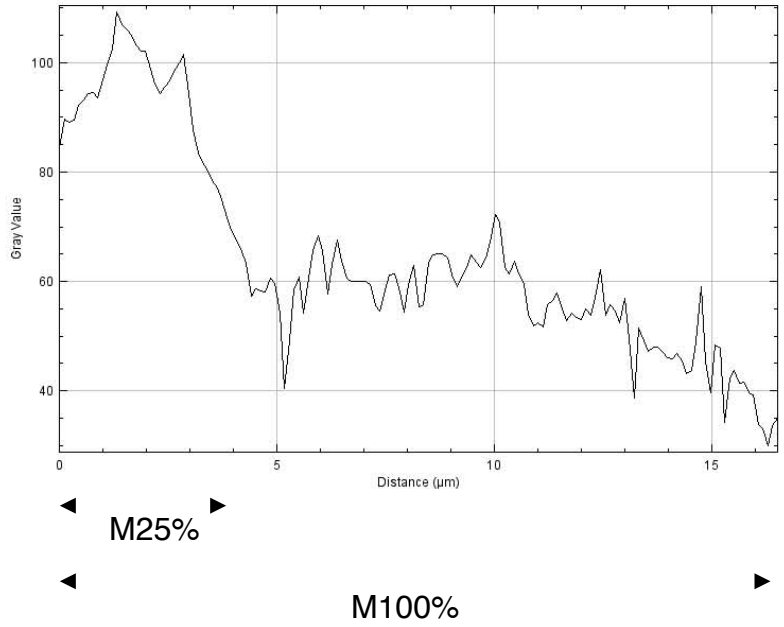
Supplementary Figure S5. Total SPAK, total OSR1, pSPAK/pOSR1 (S-motif) and Cab39 full-lenght immunoblot images.

Supplementary Figure S6



Supplementary Figure S6. Representative Coomassie gel staining. Preliminary 10% SDS-PAGE gels were run and stained with Coomassie blue to confirm equality of loading in each lane.





L = length of the ROI

M25% = Mean intensity of pixels from 0 (apical membrane) to L/4

M100% = Mean intensity of all pixels

Apical OSR1 = M25% / M100%

Supplementary Figure S7. Schematic description of the method used to quantify OSR1 apical staining. A detailed description is provided in the Material and Methods section.