SUPPLEMENTARY ONLINE DATA

SPAK/OSR1 regulate NKCC1 and WNK activity: analysis of WNK isoform interactions and activation by T-loop trans-autophosphorylation

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Figure S1 Specificity of human WNK isoform antibodies

(A) HEK-293 cells were untransfected or transfected with FLAG-tagged human WNK2, WNK3 and WNK4. Lysates (20 μg) were subjected to immunoblot analysis with the indicated antibodies. Moesin levels were monitored as a loading control. (B) Using the human FLAG–WNK-transfected cell lysates, immunoprecipitations with the individual antibodies were performed on each lysate as indicated. The immunoprecipitations were next subjected to immunoblot analysis with the anti-FLAG antibody to check the specificity of the individual isoform immunoprecipitations. (C) HEK-293 lysates (20 μg) were subjected to immunoblot analysis with the indicated isoform-specific antibodies. Lysates expressing the individual FLAG-tagged WNK isoforms were employed as a positive control. Molecular masses are indicated in kDa on the left-hand side of the Western blots. IP, immunoprecipitation.

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**Figure S2  Characterization of the mouse WNK2 and WNK3 antibodies**

(A) Lysates (40 μg) from a panel of mouse tissues and mouse cell lines were subjected to immunoblot analysis with the anti-WNK2 mouse antibody. (B) WNK2 was immunoprecipitated from mouse brain lysates and probed with the anti-WNK2 mouse antibody. Immunoprecipitation with the preimmune IgG was used as a negative control. (C) Same as (A), except that the lysate panel of mouse tissues and cell lines was immunoblotted with the anti-WNK3 mouse antibody. (D) WNK3 was immunoprecipitated using the mouse-specific WNK3 antibody from the indicated mouse tissues and cell lines. The immunoprecipitates were subjected to immunoblot analysis with the WNK3 mouse-specific antibody. Molecular masses are indicated in kDa on the left-hand side of the Western blots. IB, immunoblot; IP, immunoprecipitation; KO, knockout ES cells derived from the Gene Trap Consortium; mpkDCT, mouse-derived kidney distal-convoluted-tubule-derived cells; WT, wild-type.

**Figure S3  ES cell genotyping**

(A) Genomic DNA from the WNK3 wild-type and knockout ES cells was PCR amplified with the WNK3 primers described in the Materials and methods section of the main text. For the WNK3 knock-out, a 500 bp insert product used to disrupt the WNK3 gene is detected. (B) Genomic DNA from each SPAK/OSR1 ES wild-type, single and double cell line was PCR amplified with previously reported SPAK and OSR1 primers [17]. For SPAK, the wild-type allele generates a 679 bp product, whereas the knockin allele generates a 587 bp product. For OSR1, the wild-type allele generates a 290 bp product, whereas the knockin allele generates a 337 bp product. The larger knockin allele product is due to the presence of the 92 bp FRT site and flanking region, which remains in an intronic region following Flp-mediated excision of the neomycin selection cassette. Molecular size (MW) markers are indicated in kb on the left-hand side of the gels.
HEK-293 cells were transfected with the indicated FLAG-tagged or GST-tagged WNK isoform constructs encoding the wild-type (WT) and T-loop mutant in which the residue equivalent to Ser382 in WNK1 is changed to an alanine residue to prevent activation (Ser356 in WNK2, Ser308 in WNK3, and Ser335 in WNK4). At 36 h post-transfection, the cells were lysed and whole cell lysates (20 μg) were subjected to immunoblot analysis with the indicated antibodies. Molecular masses are indicated in kDa on the left-hand side of the Western blots.