

Supplemental Materials

Molecular Biology of the Cell

Boyd-Shiwarski et al.

Potassium-Regulated Distal Tubule WNK Bodies are Kidney-Specific WNK1 Dependent

Supplemental Materials

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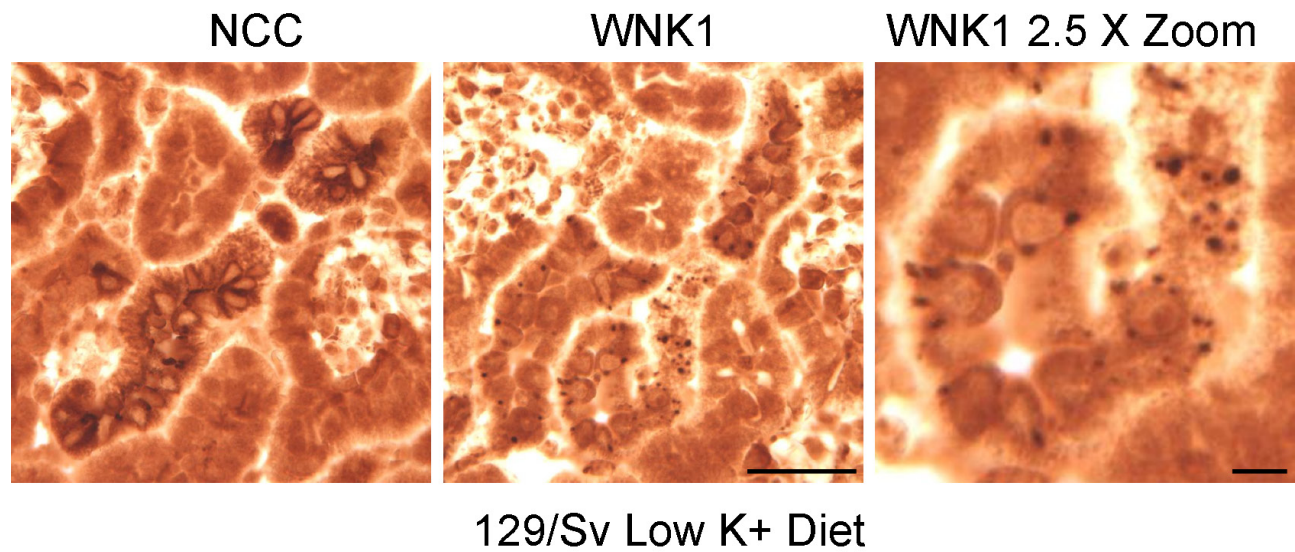


Figure S1. 129/Sv mice form WNK1 puncta on a low potassium diet.

Representative immunohistochemical staining of 129/Sv mice subjected to dietary potassium depletion for 10 days. Distal convoluted tubules were identified by NCC staining in contiguous sections. Note the presence of pan-WNK1 positive perinuclear puncta in NCC positive tubules. Whole blood [K⁺] was 3.0 mEq/L at the time of kidney harvesting and fixation. Scale bars = 50 μ m in 1x images, 10 μ m in the 2.5X image.

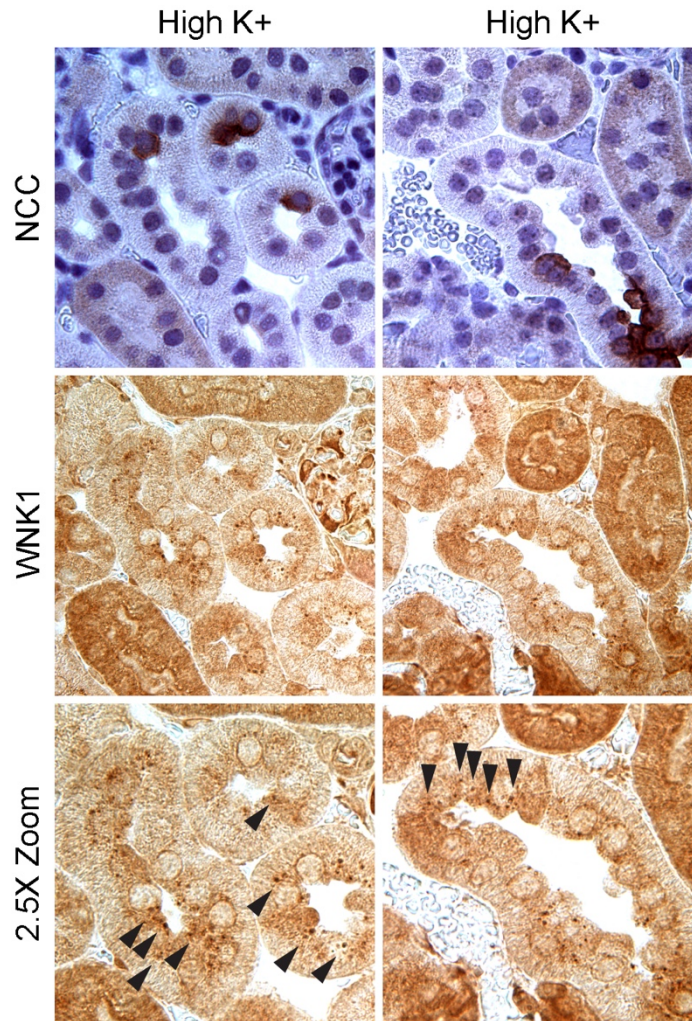


Figure S2. WT mice on high potassium diets form WNK1 puncta in both the DCT and CNT.

Representative immunohistochemical staining of kidney tissue from C57Bl/6 mice placed on a high potassium diet for 10d. DCTs were identified by NCC and nuclear costaining in contiguous sections. Pan-WNK1 antibody detected subapical WNK1 puncta in both NCC positive (representing DCT) and NCC negative cells (representing connecting tubule, CNT). CNT puncta are indicated by arrowheads. Whole blood [K⁺] at the time of sacrifice and kidney harvest was 4.8 mEq/L.

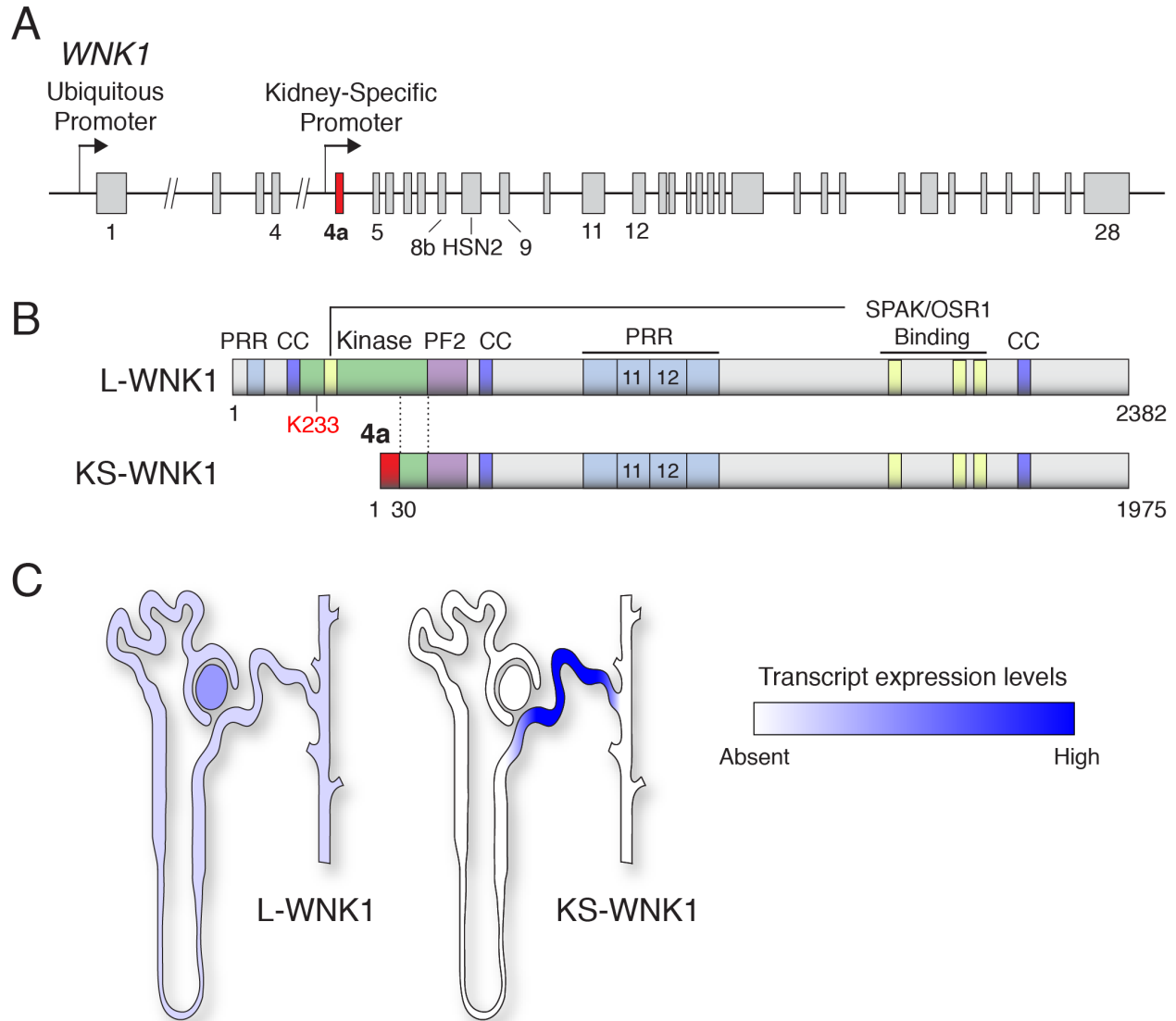


Figure S3. KS-WNK1 is highly expressed in renal distal tubules.

- A) Schematic representation of the *WNK1* gene. A ubiquitously expressed promoter drives the transcription of L-WNK1, which contains an intact kinase domain. A kidney-specific promoter drives the expression of KS-WNK1, a truncated *WNK1* isoform.
- B) Domain architecture of L-WNK1 and KS-WNK1. Note that KS-WNK1 lacks the first 437 amino acids of L-WNK1 including the catalytic site (K233); these exons are replaced by exon 4a (red), which is 30 amino acids long and unique to KS-WNK1 isoforms. The location of three coiled coil domains (CC), PF2-like domain (Moon *et al.*, 2013) [PF2, formerly known as the “auto-inhibitory domain” (Xu *et al.*, 2002)], proline-rich regions [PRR(Roy *et al.*, 2015)], and SPAK/OSR1 binding sites are shown.
- C) Schematic representation of a nephron highlighting relative L-WNK1 and KS-WNK1 mRNA expression. KS-WNK1 mRNA is predominantly expressed in the DCT and CNT, whereas L-WNK1 is diffusely expressed at low levels throughout the renal tubule. Graphical representation of empirical data derived from Vidal-Petiot *et al* (Vidal-Petiot *et al.*, 2012).

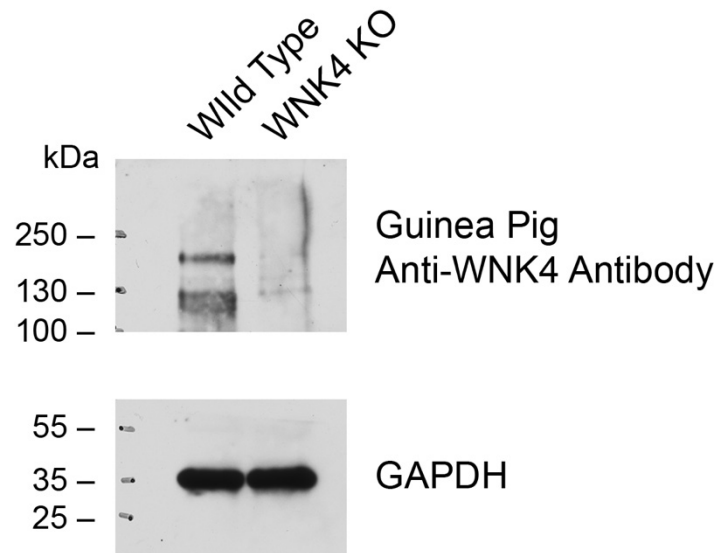


Figure S4. Guinea pig anti-WNK4 antibody.

A purified GST fusion protein containing the first 167 amino acids of mouse WNK4 was injected into guinea pigs, and the antiserum was validated in immunoblots, comparing mouse kidney lysates from WNK4 KO mice (Castaneda-Bueno *et al.*, 2012) and control C57Bl/6 mice. A corresponding GAPDH blot is shown as a loading control.

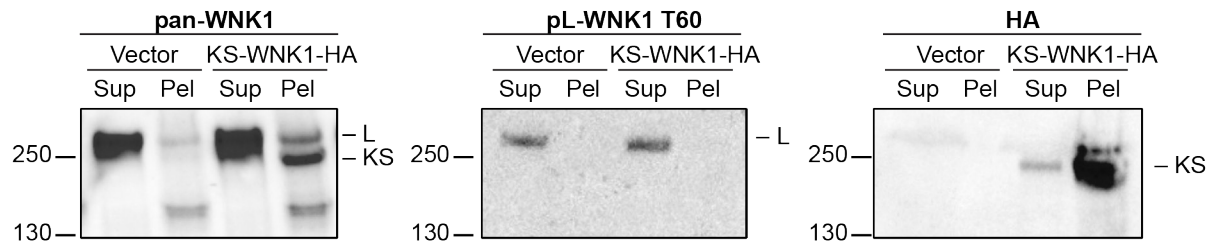


Figure S5. The fraction of L-WNK1 protein that is sequestered by KS-WNK1 is dephosphorylated. Immunoblots of HEK-293 cell lysates transiently transfected with either empty vector or KS-WNK1-HA, subjected to SP assay. *Left*, pan-WNK1 antibody immunoblot. Endogenous L-WNK1 (“L”) migrates as a >250kDa band that is enriched in the Triton-soluble supernatant; coexpression of KS-WNK1 (“KS”) causes the endogenous L-WNK1 protein to partially redistribute into the SDS-soluble pellet. *Center*, phosphospecific L-WNK1 immunoblot. The pThr60 antibody exclusively detected endogenous phosphorylated L-WNK1 species that were localized to the Triton-soluble Sup. *Right*, HA antibody immunoblot, confirming expression of transfected KS-WNK1 and enrichment in the SDS-soluble pellet.

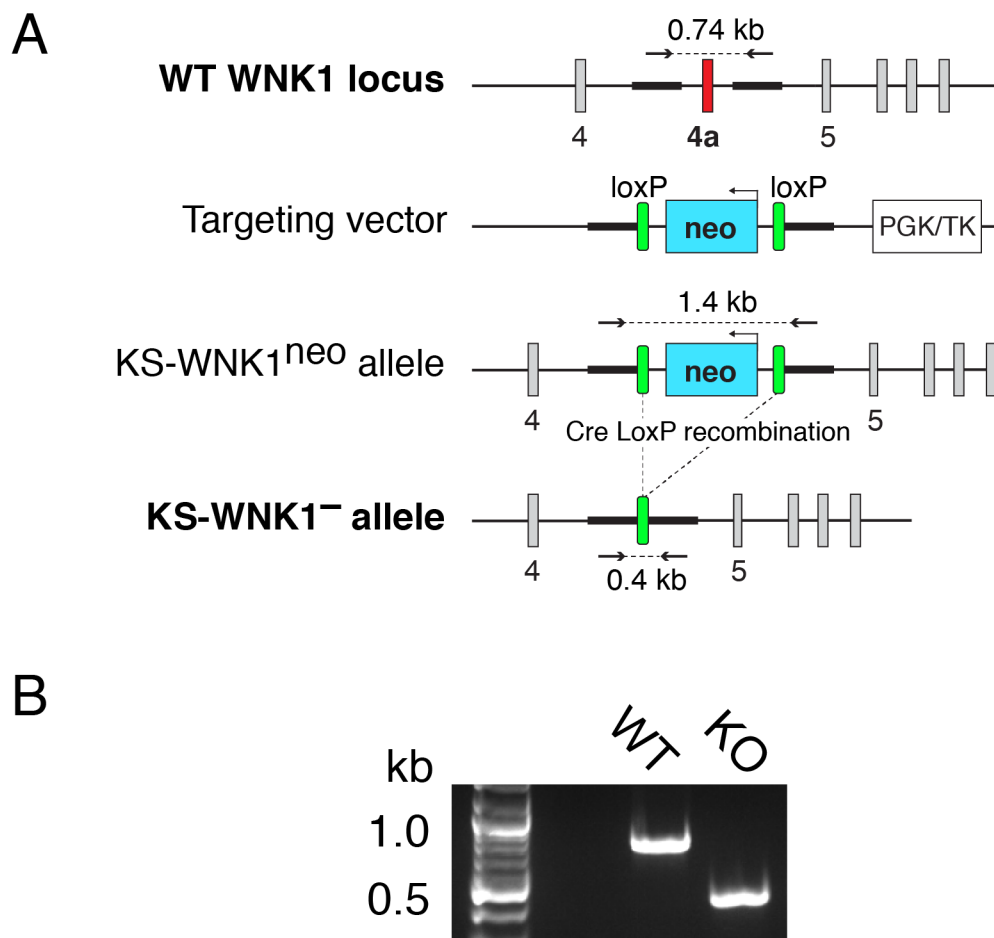


Figure S6. KS-WNK1 knockout mice.

- A) The KS-WNK1 knockout mice used for this study were derived from a mouse line originally reported by Liu *et al.* (Liu *et al.*, 2011). These mice were generated with a targeting construct containing a loxP-flanked reverse-oriented neomycin (neo) resistance cassette and a herpes virus thymidine kinase negative selection gene; the neo cassette was flanked by homology arms upstream and downstream of exon4a. Homologous recombination replaced exon 4a with the loxP-flanked neo cassette, resulting in the KS-WNK1^{neo} allele. Cre-LoxP mediated recombination in KS-WNK1^{neo} ES cells resulted in the KS-WNK1⁻ allele. Mice homozygous for the KS-WNK1⁻ allele were used for this study. Arrows denote previously reported binding sites for genotyping primers (F1 and R1 (Liu *et al.*, 2011)). Note the expected sizes of the WT and KS-WNK1⁻ genotyping PCR products (0.74kb and 0.4kb, respectively). The KO PCR product was TA cloned and sequenced to confirm presence of a loxP site, and the deletion of exon 4a (not shown).
- B) Genotyping of WT and KS-WNK1^{-/-} (KO) mice.

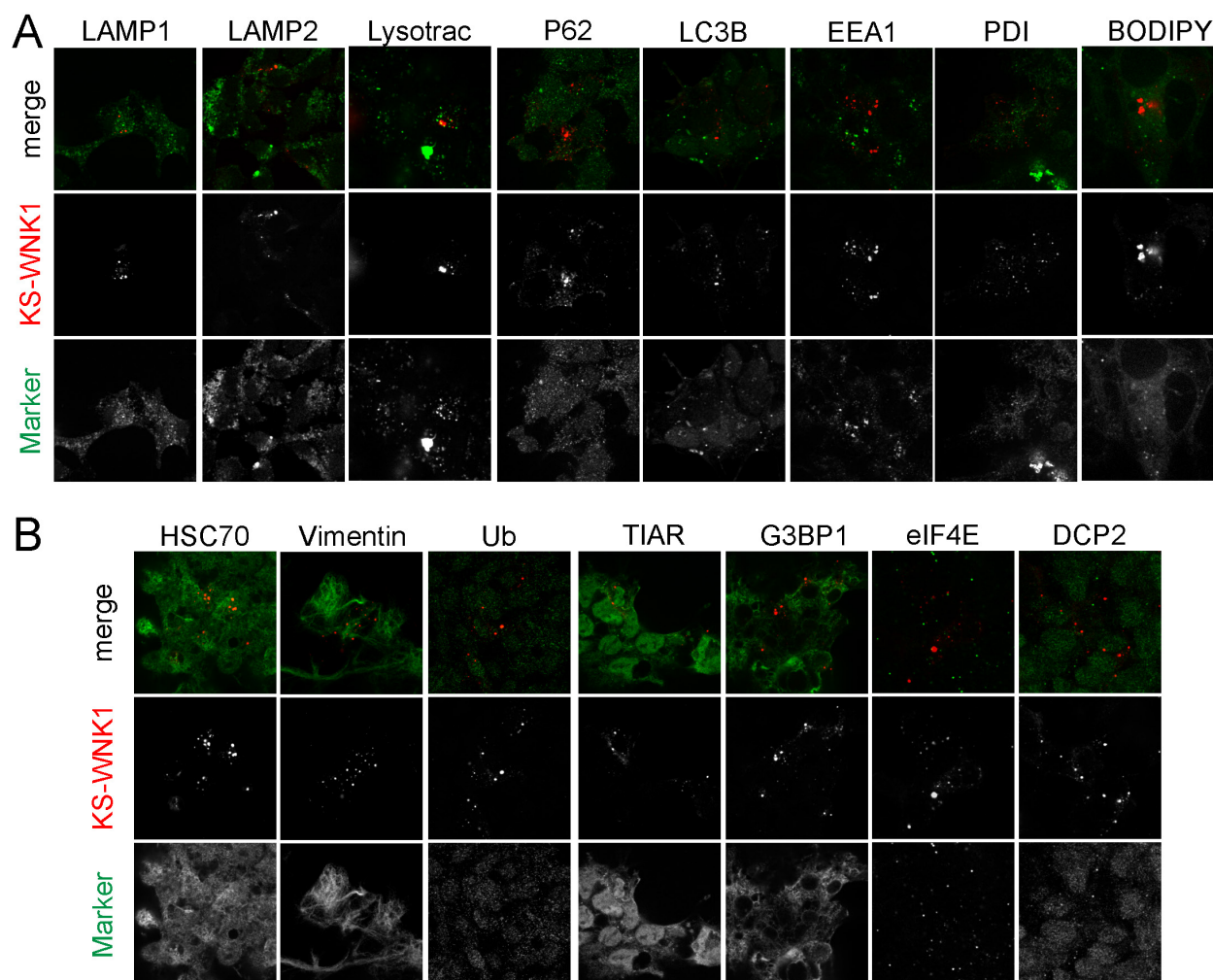


Figure S7. KS-WNK1 puncta do not colocalize with markers of membrane bound organelles, membraneless foci, or proteotoxic aggregates under unstressed conditions.

- A) HEK293 cells were transiently transfected with C-terminal tagged KS-WNK1 (either HA tag, GFP tag, or mRuby tag) and endogenous proteins associated with membrane-bound organelles were detected using antibodies listed in [Table S1](#). Antibodies tested were directed against early endosomes (EEA1), Lysosomes (LAMP1, LAMP2, Lysotracker), Autophagosomes (P62, LC3B), Endoplasmic Reticulum (PDI), and lipid droplets (BODIPY). See also [Movies S2](#) and [S3](#).
- B) No co-localization was detected between tagged KS-WNK1 and endogenous proteins that associate with membraneless organelles or proteotoxic aggregates using double immunofluorescence of fixed cells. Antibodies tested were directed against chaperone-mediated autophagic substrates (Hsc70), Aggresomes (vimentin, ubiquitin), stress granules (TIAR, G3BP1, eIF4E), and P bodies (eIF4E, DCP2).

Movie Legends:

Movie S1. KS-WNK1 puncta are dynamic and cluster L-WNK1.

Movie of a HEK-293 cell transfected with C-terminally tagged L-WNK1-eGFP (green) and KS-WNK1-mRuby (red). Live cell images of L-WNK1 and KS-WNK1 trafficking were performed using a Nikon Ti-E-2000 confocal microscope, as described in the Methods. The arrow denotes a mobile KS-WNK1 punctate structure. Note how L-WNK1 is concentrated within this structure and tracks with KS-WNK1.

Movie S2. KS-WNK1 puncta do not colocalize with lysosomes.

Movie of a HEK-293 cell transfected with C-terminally tagged KSWNK1-mRuby (red), and incubated with LysoTracker Green (green) to stain lysosomes. Live cell images of KS-WNK1 and LysoTracker trafficking were performed using a Zeiss 700 LSM confocal microscope, as described in the Methods. No apparent colocalization, or co-trafficking is observed with KS-WNK1 and LysoTracker.

Movie S3. KS-WNK1 puncta do not colocalize or traffic with lipid droplets.

Movie of a HEK-293 cell transfected with C-terminally tagged KSWNK1-mRuby (red), and incubated with BODIPY FL (green) to stain lipid droplets. Live cell images of KS-WNK1 and BODIPY trafficking were performed using a Zeiss 700 LSM confocal microscope, as described in the Methods. No apparent colocalization or co-trafficking is observed with KS-WNK1 and BODIPY.

Movie S4. 3-dimensional colocalization of KS-WNK1 and RPL22.

3-dimensional Z-stack movie of a HEK-293 cell transfected with C-terminal HA tagged KS-WNK1, fixed and stained using antibodies against HA epitopes (red) and endogenous RPL22 (green). Fifty four Z stack confocal slices were taken at Nyquist resolution, then rendered in 3D in Imaris. Movie created in Imaris.

| | Marker | Target | Host | Source |
|-----------------|---|--|----------------------------------|---|
| ANTIBODY | WNK1 | With No Lysine Kinase 1 C-terminus (AA 2031-2117) ["Pan-WNK1"] | Rb | Atlas Antibodies (HPA059157) |
| | WNK1 | With No Lysine Kinase 1 Exon 28 | Rb | Sigma Aldrich (SAB1300464) |
| | pL-WNK1 | Phosphorylated "Long" WNK1 (Thr 60) | Rb | Cell Signaling (4946) |
| | SPAK | SPS1-related proline/alanine-rich kinase | Rb | Cell Signaling (2281) |
| | WNK4 | With No Lysine Kinase 4 | Gp | (this study) |
| | NCC | Na ⁺ /Cl ⁻ cotransporter | Gp | Jim Wade/David Ellison(Wyse <i>et al.</i> , 2002) |
| | NCC | Na ⁺ /Cl ⁻ cotransporter | Rb | StressMarq (SPC-402) |
| | GAPDH | Housekeeping protein | Rb | Cell Signaling (2118) |
| | HA.11 | HA-tag | Mo | Covance (MMS-101P) |
| | HA | HA-tag | Rb | Sigma Aldrich (H6908) |
| | Myc | c-Myc-tag | Rb | Thermo Fisher (PA1-981) |
| | RPL22 | Ribosomal Protein L22 | Gt | Acris/OriGene (AP23832PU-N) |
| | Vimentin | Intermediate filaments/Aggresomes | Rb | Cell Signaling (5741) |
| | DCP2 | Processing bodies | Rb | Bethyl (A302-597A) |
| | eIF4E | Stress Granule, Processing bodies | Rb | Bethyl (A301-153A) |
| | G3BP1 | Stress Granule | Rb | Udai Pandey(Daigle <i>et al.</i> , 2016) |
| | TIAR | Stress Granule | Mo | Udai Pandey(Daigle <i>et al.</i> , 2016) |
| | LAMP1 (CD107A) | Lysosome | Mo | BD Biosciences (555798) |
| | LAMP2 | Lysosome | Mo | DS Hybridoma Bank (H4B4) |
| | EEA1 | Endosomes | Rb | Gerard Apodaca(Oztan <i>et al.</i> , 2007) |
| Ub (P4D1) | Ubiquitin/Aggresomes | Mo | Santa Cruz (sc-8017) | |
| PDI | Endoplasmic Reticulum | Mo | Abcam (ab2792) | |
| P62/SQSTM1 | Autophagosome | Rb | Cell Signaling (5114) | |
| LC3A | Autophagosome | Rb | Novus (2331) | |
| Hsc70 | Chaperone-mediated autophagy substrates | Rb | Assay Designs/Enzo (ADI-SPA-819) | |
| DYE | LysoTracker Green | Lysosomes in live cells | N/A | Thermo Fisher/Invitrogen (L7526) |
| | BODIPY 493/503 | Lipid droplet in live cells | N/A | Thermo Fisher/Life Technologies (D3922) |

Table S1. Antibodies and Dyes.

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