Figure S1
(Related to Figure 1)

A.

| Protein | Nb unique peptides | FDR  |
|---------|--------------------|------|
| WNK1    | 11                 | 0.001|

B.

Peptides

| Probability | Weight | Charge / Sequence |
|-------------|--------|-------------------|
| 0.9900      | 0.09   | ESPVQQDVR         |
| 0.9900      | 0.09   | FVPSIPER          |
| 0.9900      | 0.09   | IGDIATLK          |
| 0.9955      | 1.00   | SIVPPSPNLQ        |
| 0.9900      | 1.00   | SGGDCDIEETK       |
| 0.9905      | 1.00   | EGPVLTSSCAGDFVK   |
| 0.9903      | 1.00   | VTSIKPAFDK        |
| 0.9808      | 0.99   | ILNMSKNDR         |
| 0.9909      | 1.00   | VAPEVK            |
| 0.9982      | 1.00   | QQGEQSSGADQGK     |
| 0.9943      | 1.00   | EGDVLQR           |

C.

D.

(A-C) Mass Spectrometric evidence for WNK1-PCF11 interaction. PCF11 IP was performed in nuclear extract and co-immunoprecipitated interacting proteins analyzed by MS. The only kinase found in the experiment was WNK1. A. Summary of detected peptides. FDR is derived from comparison with control mock IP experiment (CPFP SIRQ). B. Identified WNK1 peptides. C. Coverage of the identified WNK1 peptides both at N- and C-terminus of the sequence indicates likely presence of full-length protein in the IP. D. Western blot for the detection of WNK1 in cytoplasmic and nuclear HeLa cell extracts. Tubulin and the export factor NXF1 were used as controls for fractionation.
Splicing isoforms of *WNK1*. 6 of the 21 total annotated isoforms contain the catalytic kinase domain. Red box represents the recognition epitope of the antibody used in previous studies (Tu et al. 2011), a blue box represents the epitope of the antibody used in this study (Bethyl, A301-514). NLS stands for Nuclear Localization Signal (see also Material and Methods) and is shown in green.
**Figure S3**

(Related to Figures 1 and 2)

A. Detection of WNK1 depletion levels upon siRNA treatment. Two different siRNAs were used: siWNK1A (single siRNA targeting) and siWNK1 Pool (OnTARGET Dharmakon siRNA Smart pool). Western blot with a-WNK1 antibody (Bethyl, A301-515A). siLUC and siPCF11 siRNA were used as control. B. Effect of depletion of WNK1 with different siRNAs on mRNA export. Measurement of nuclear and cytoplasmic mRNA levels of TBP and MYC genes upon WNK1 depletion (Error bars represent standard error). C. GO-analysis of the genes with affected mRNA export upon WNK1 depletion. Details for GO analysis in Supplemental Table 1. No GO categories showed significant enrichment.
Figure S4
(Related to Figure 3)

A

ChIP experiment for Aly recruitment. siRNA mediated depletion of WNK1 does not have a significant effect on Aly recruitment on the Actin and MYC genes (Summary data of three technical repeats, error bars represent standard error).

B

Levels of TBP and SGK1 mRNA associated with immunoprecipitated PCF11 for DNase treated extracts of control and WNK1 depleted cells (Summary data of three technical repeats of, error bars represent standard error).
Mass spectrometry evidence of in vivo PCF11 CID phosphorylation. PCF11 was immunoprecipitated from nuclear extract, resolved on SDS-PAGE, the coomassie band corresponding to the PCF11 protein cut out, digested with trypsin and subject to Mass Spectrometry. Shown are the peptide spectra and fragment ions of the CID phosphorylated peptide from MASCOT. Three independent biological repeats showed evidence of phosphorylated S120/T121 peptides (Supplemental Table 2). Note that we were unable to unambiguously determine which of the neighbouring S120/T121 residues is phosphorylated in vivo.
Mass spectrometry analysis of recombinant WNK1 kinase domain activity on recombinant GST-PCF11-CID in vitro. GST-PCF11-CID was incubated with WNK1 as described, resolved on SDS-PAGE, the coomassie band corresponding to GST-PCF11-CID cut out, digested with trypsin and subject to Mass Spectrometry. Shown are the peptide spectra and fragment ions of the CID phosphorylated peptide from MASCOT. Note that we were unable to unambiguously determine which of the neighbouring S120/T121 residues is phosphorylated. All identified peptides for GST-PCF11-CID are shown in Supplemental Table 3.
Figure S7

(Related to Figure 5)

A. Western blot for the detection of OSR1 S325 phosphorylation by WNK1 upon inhibition of WNK kinase domain. Cells were incubated with the WNK463 inhibitor for 6 hrs, followed by 30 min treatment with 0.5M Sorbitol to induce OSR1 S325 phosphorylation by WNK1. A range of concentrations from 1mM to 100nM was tested.

B. Detection of WNK1 levels upon its inhibition with WNK463. Cells were incubated with WNK463 for 6 hrs, followed by 30min treatment with 0.5M Sorbitol, same concentrations as panel A were tested.

C. Fluorescent oligo-dT FISH for the detection of mRNA levels in cells expressing WT or S120A/T121A PCF11. Alexa Fluor 488-dT23 was used as a probe and DAPI for DNA staining. α-HA antibody was used for staining of ectopically expressed PCF11.

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C. Fluorescent oligo-dT FISH for the detection of mRNA levels in cells expressing WT or S120A/T121A PCF11. Alexa Fluor 488-dT23 was used as a probe and DAPI for DNA staining. α-HA antibody was used for staining of ectopically expressed PCF11.
Figure S8
(Related to Figure 5)

A. Co-IP experiments of ectopically expressed PCF11. WT PCF11 co-immunoprecipitates with the nuclear pore complex (NPC) and the export factor ALY. Expression of the phosphomimetic PCF11 does not show a significant change in this interaction.

B. Peptide coupling efficiency of S120/T121 unmodified, as well as phosphorylated S120ph and T121ph peptides (sequences available in Supplemental table 5). Peptide concentration was monitored by ponceau red staining on a dot blot before (input) and after coupling of the peptides to sulfolink beads (flow-through). All peptides coupled very efficiently to the resin as evidenced by only trace quantities detectable in the flow-through.
**Supplemental tables list**

**Supplemental table 1**
GO analysis of genes whose export is affected by WNK1 depletion.

**Supplemental table 2**
*In vivo* PCF11 phosphorylation sites detected in Mass Spectrometry in HeLa cells (3 experiments).

**Supplemental table 3**
Phosphorylation sites on GST-PCF11CID detected by Mass Spectrometry after in vitro kinase assay with WNK1 kinase domain.

**Supplemental table 4**
Mass Spectrometry analysis of proteins bound in peptide affinity purification using PCF11 CID peptides. Shown are Spectral index (MIC SIn) and ratios of protein abundance between phosphorylated and unphosphorylated peptide used as bait obtained through CPFP.

**Supplemental table 5**
Sequences of oligonucleotides and peptides used in this study.