Supporting Information

Tu et al. 10.1073/pnas.1018567108

SI Materials and Methods

Microinjection. Cell culture medium was replaced with CO_2-independent medium just before injection. A Transjector 5246 (Eppendorf) on an Eclipse TE300 microscope (Nikon) was used for microinjection. Cells were coinjected with 1 mg/mL dextran-Alexa 488 with 20 nM WNK1 siRNA oligonucleotides.

Time-Lapse Microscopy. Movie S3 was imaged as described in *Materials and Methods*, except using a 40× oil immersion lens.

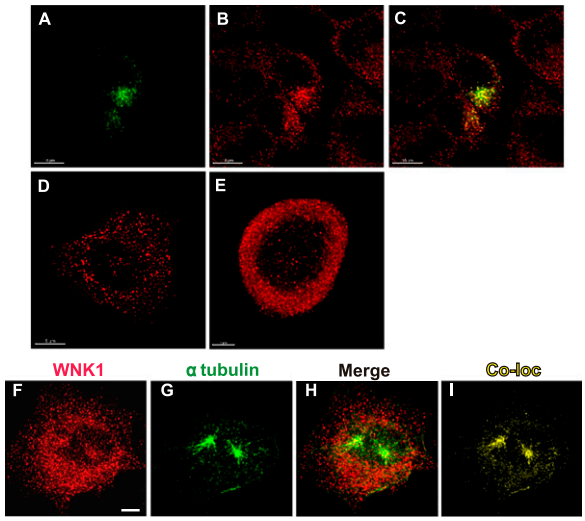


Fig. S1. Localization of WNK1 and GFP-WNK1 in multiple cell types. (*A*) HeLa cells expressing low levels of GFP-WNK1. (*B*) Immunostaining of endogenous HeLa WNK1 (red). (*C*) Color-merged image from *A* and *B*. WNK1 staining in MCF7 cells (*D*) or HT29 cells (*E*). (Scale bars: *A–C*, 10 μm; *D* and *E*, 5 μm. Immunostaining of HeLa cells: WNK1 (*F*, red), tubulin (*G*, green), merge of red and green channels (*H*), and computer-generated colocalization (*I*, yellow).

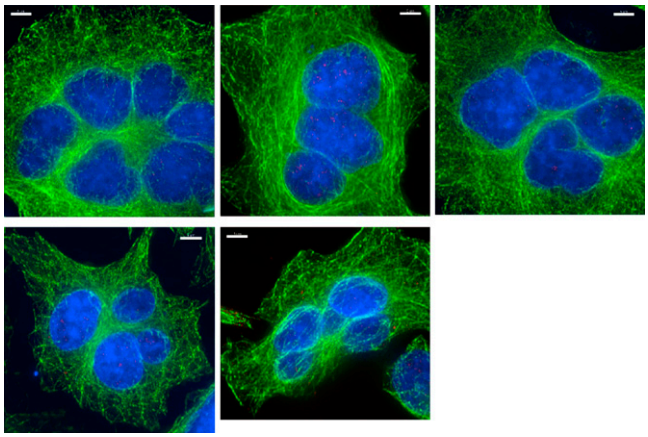


Fig. S2. Multinucleate WNK1 knockdown cells. Immunostaining of HeLa cells 72 h after transfection with WNK1 siRNA oligonucleotides. WNK1 (red), tubulin (green), and DAPI (blue channel). Because the protein has been knocked down, WNK1 staining is very faint. (Scale bar: 5 μm.)

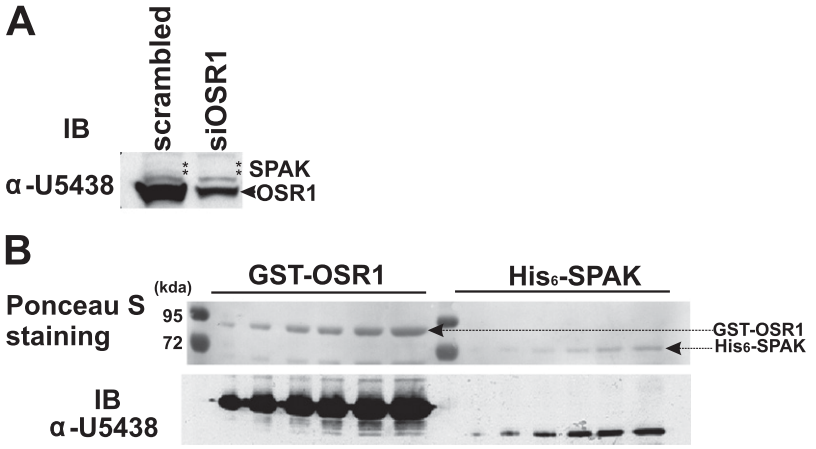
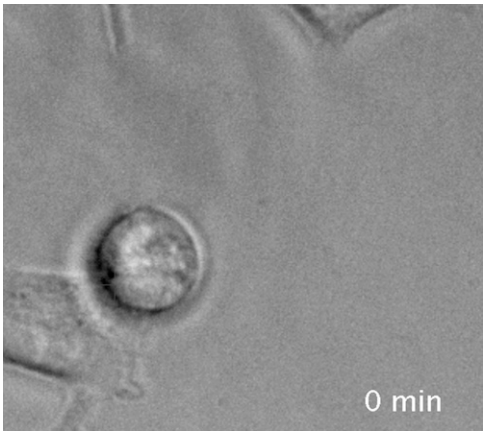


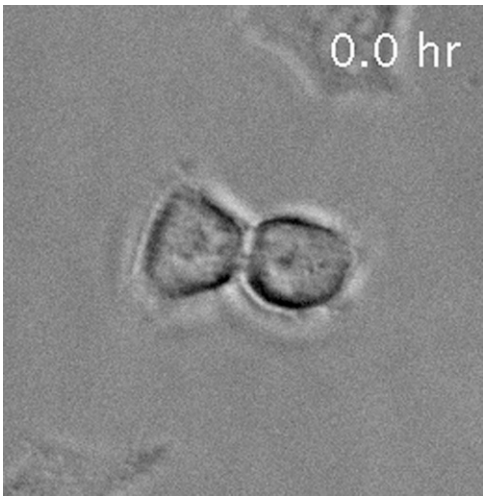
Fig. S3. Expression of OSR1 and SPAK in HeLa cells. (*A*) HeLa cells were transfected with OSR1 or scrambled control oligonucleotides. Lysates were immunoblotted with an antibody that recognizes both proteins (1). SPAK is ~5 kDa larger than OSR1. (*B*) Ponceau S staining of recombinant GST-OSR1 and His_6-SPAK (1) (*Upper*) and immunoblot of increasing amounts of GST-OSR1 and His_6-SPAK with the same antibody (*Lower*).

1. Anselmo AN, et al. (2006) WNK1 and OSR1 regulate the Na+, K+, 2Cl– cotransporter in HeLa cells. *Proc Natl Acad Sci USA* 103:10883–10888.



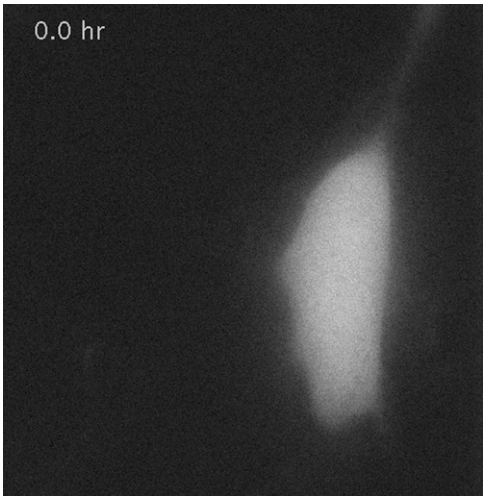
Movie S1. Live cell image movie file supporting Fig. 4*D*.

Movie S1



Movie S2. Live cell image movie file supporting Fig. 4*E*.

Movie S2



Movie S3. HeLa cells were microinjected with dextran-Alex 488 and WNK1 siRNA oligonucleotides. After 24 h, cells were imaged by time-lapse microscopy. This movie shows that defective abscission caused by WNK1 siRNA in two daughter cells results in a lengthened bridge as cells attempt to move away from each other during late telophase.

Movie S3