Supplementary Materials and Methods

Plasmids and antibodies. pBabe-Flag-*LKB1* was constructed as previously described (1). pLKO-sh*NEDD9#1* and pLKO-sh*NEDD9*#2, pLKO-sh*Nedd*9 were provided by Dr. Lynda Chin. The target sequence of sh*LKB1#1*, sh*LKB1*#2, sh*CRTC1* and sh*SIK2* were listed in Supplementary Table S1. The plasmids pCDH1-*CREB*-*Neo* and pCDH1-*CRTC1*-*Neo* were constructed by cloning either *CREB* or *CRTC1* cDNA into pCDH1-CMV-EF1-*Neo* using *EcoR* I and *Not* I sites or *EcoR* I and *BamH* I sites, respectively. The *NEDD9* cDNA was cloned into a modified plasmid pCDH1-CMV-BXN-EF1-*Neo* (BXN) containing additional one restriction enzyme site (*Xho* I) between *EcoR* I and *Xho* I sites as pCDH1-CMV-*NEDD9*-BXN-EF1-*Neo* (BXN-*NEDD9*). The cDNA of *Cre* was then cloned into either BXN or BXN-*NEDD9* for Lenti-*Cre* or *NEDD9*-*Cre*, respectively. The pLKO-*Cre* (Ctrl-*Cre*) and pLKO-sh*Nedd9*-*Cre* (sh*Nedd9*-*Cre*) were constructed by cloning the cDNA of *Cre* (Kpn I and Bgl II) into pLKO-*puromycin* (pLKO-*Puro*) or pLKO-sh*Nedd9*-*puromycin* (sh*Nedd9*-*Puro*) using Kpn I site and BamH I. The sequence for all the primers were listed in Supplementary Table S1.

A series of truncated human *NEDD9* promoter region sequences (0~-2957bp) were PCR amplified and cloned into PGL3-basic using *Mlu* I and *Xho* I sites. Mutated *NEDD9* promoter (7bp deletion from -146 to -139) was generated by site-directed mutagenesis and confirmed by direct sequencing. The CRE, SRE, NF-κB, GRE, HSE, TAL and AP1 reporters were purchased from Clontech.

The following antibodies were used: mouse monoclonal anti-LKB1 (Upstate, 05-832), rabbit monoclonal anti-CREB (Cell Signaling, #9197), rabbit polyclonal anti-CRTC1 (Santa Cruz , SC-67146), rabbit anti-SIK2 antiserum (generously provided by Dr. Hiroshi Takemori), mouse monoclonal anti-Flag (Sigma, F1804), rabbit polyclonal anti-HSP90 (Santa Cruz, SC-7947), rabbit polyclonal anti-Fibrillarin (Santa Cruz, SC-25397), mouse monoclonal anti-Actin (Sigma, A2228), Normal rabbit IgG (Santa Cruz, SC-2027) , rabbit monoclonal anti-Ki-67 (Zymed, ZA-0502), rabbit polyclonal anti-cleaved caspase 3 (Cell Signaling, #9661), polyclonal anti-NEDD9 raised in rabbit using peptide from residues 422-623 of human NEDD9. Peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz, SC-2004) or goat anti-mouse IgG (Santa Cruz, SC-2005) were used as the secondary antibodies.

Immunofluorescence. Cells were grown on cover slips for 24 hrs, fixed with 4% paraformaldehyde at room temperature for 10 min, blocked with 1% bovine serum albumin in phosphate-buffered saline (PBS) for 1 hr, and stained with corresponding primary antibodies (Flag, CRTC1, SIK2) for 1 hr. The cells were then incubated with Alexa Fluor 555 or 488 conjugated secondary antibodies (Invitrogen) in PBS for another hour. Nuclei were counterstained with DAPI (Sigma). Cells were mounted with anti-fade (Thermo Electron Corporation). Photos were taken using a laser scanning confocal microscope (Leica TCS ST5-MT).

Soft agar assay. Cells were suspended on a top layer of RPMI 1640 containing 10% FBS and 0.4% agar (Gibco/Invitrogen) in 6-well plates in triplicate and plated on a

bottom layer of RPMI 1640 containing 10% FBS and 1% base agar. Colonies were photographed and stained with 0.005% crystal violet for 1 hr after 2-week culture and then colonies were counted and analyzed by Image-J 2008.

Boyden chamber assay. Cells were plated into the upper chamber of a polycarbonate transwell filter chambers (Corning) coated with matrigel (1mg/ml, BD biosciences) in serum-free medium, followed by incubation in the 24-well plates with the complete medium containing 10% FBS for 24 hours. Cells inside the chamber were lightly removed with cotton swabs while the migrated cells on the outside membrane were fixed in 1% paraformaldehyde and stained with hematoxylin. The migrated cells were counted at high-power field for at least 10 views randomly under microscope.

Supplementary References

1. Gao Y, Xiao Q, Ma H, Li L, Liu J, Feng Y*, et al.* LKB1 inhibits lung cancer progression through lysyl oxidase and extracellular matrix remodeling. Proc Natl Acad Sci U S A 2010;107:18892-7.