Supplemental Legends

Figure S1. Characterization and consequences of Stk25 expression in knockdown neurons, Reelin treated neurons and mouse brain; relates to Figure 1. A Expression of the Stk25 shRNA (GFP positive, green) resulted in an axonless phenotype in cells that also expressed RFP (Red). **B** Expression wild-type Stk25*-wt RFP rescued the production of axons. **C** Kinase inactive Stk25* K49-RFP fusion protein expression also rescued the phenotype. **D** The expression of the Stk25*-RFP fusion protein was approximately three fold higher than endogenous Stk25 in primary cortical neurons by immunoblotting. The β-actin Western blot served as a loading control. The graphic shows the sequence of Stk25 that is homologous to the stem portion of the shRNA and the silent alterations that were introduced in Stk25* (red). **E** In situ hybridization patterns for Stk25 in the E14.5 (Gene Paint [Internet]; Visel et al., 2004; Yaylaoglu et al., 2005). Goettingen, Germany: Genepaint.org and EuroExpress Project. Available from http://www.genepaint.org/ ID EH2512] **F** In situ hybridization patterns for Stk25 in adult mouse brain. Colors indicate provided intensity values. Allen Mouse Brain Atlas [Internet]. Seattle (WA): Allen Institute for Brain Science. ©2009. Available from: http://mouse.brain-map.org.

Figure S2. STRAD and GM130 coimmunoprecipitate with Stk25, implicating Stk25 in cell polarizing and Golgi functions respectively; relates to Figure 3. A Immunoprecipitation of myc-STRAD (IP; anti-myc) supported precipitation of HA-Stk25 wt (1st panel; anti-HA). In contrast, neither HA-Stk25 wt nor HA-Stk25 K49R were immunoprecipitated from total cell lysates (TCL) that expressed HA-Stk25 (3rd panel) but not myc-Strad (4th panel) indicating that the coimmunoprecipitations were specific (n=4).

B Myc-GM130 was immunopreciptated from cell lysates of transfected HEK293 cells (2^{nd} panel; anti-Myc 71D10). HA-Stk25 and HA-Stk25 K49R were co-immunoprecipitated (1^{st} panel; anti-HA 3F10) only when Myc-GM130 was present in the cell lysates (4^{th} panel), suggesting a complex is formed with GM130. Immunoblots of immunoprecipitates (IP; top 2 panels) and total cell lysates (TCL; bottom 3 panels) are shown (n=4). **C** Constitutive LKB1 expression does not induce W4 cell polarization in GFP- (green) expressing cells (a-c). However, adding doxycycline (dox) to induce STRAD expression led to filamentous actin-rich (red, d), polarized brush borders. Humanized (h) Stk25 shRNA expression blocks STRAD-induced cell polarization (e) but the control (con) shRNA does not (f). **D** Stk25 (red) was localized proximal to the Golgi apparatus (blue, anti-GM130) in GFP expressing cells (green) by indirect immunofluorescence. Stk25 shRNA expression reduced the immunodetection of Stk25, unlike control (con) Stk25 shRNA expression, which had little effect, suggesting that the antibody is specific for Stk25. **E** Stk25 reduction causes a statistically significant loss of polarization in LKB1- and STRAD-expressing cells (*$p<0.0001$, Student's t-test).
F W4 cells were infected with hStk25 shRNA virus and transfected with RFP or Stk25*-expressing vectors to assay for rescue. After 24 h of doxycyclin treatment, only 20% of RFP and hStk25 shRNA coexpressing cells had a polarized phenotype. In contrast, close to 70% of cells were polarized when they expressed the shRNA and either wild-type or kinase inactive Stk25*. The RFP- and myc-tagged fusions had similar activities demonstrating that rescue is not dependent upon a C-terminal RFP fusion (n indicated in bars). Bars in C, D, 5 μm.

Figure S3. LKB1 and GM130 shRNA vectors effectively diminish the expression of the respective proteins; relates to Figure 4. A Mouse B16 cells were infected with LKB1 shRNA target 1 and 2 viruses (Shelly et al., 2007). Target 1 was found to be the most effective and was used for this study. **B** Only GM130 shRNA target 2 (Puthenveedu et al., 2006) reduced GM130 expression in B16 cells and was therefore used for this study.

Figure S4. The extended Golgi morphology of layer V neocortical neurons is *reelin* and *dab1* gene dependent; relates to figure 5. A At P0 in wild-type animals, layers V and VI and the cortical plate is apparent in DAPI stained images (white). **B** Defects in the lamination of the *reelin*-/- neocortex are apparent. **C** The distribution of cells in *dab1*-/- brains is similarly disrupted. **D** The cis-Golgi apparatus (anti-GRASP65, green) is elongated in wild-type cells from layer VI up to the cortical plate particularly the layer V Ctip2 neurons (red nucleus, inset). **E** In contrast the Golgi apparati are convoluted in Reelin neocortices and are observed proximal to the nucleus in Ctip2-positive neurons. **F** The Golgi apparati in Dab1 mutant mice is also less often elongated than in the wild-type. **G** Golgi extension was quantified for Ctip2-positive neurons by measuring the distance from the nucleus to the furthest tip of the Golgi ribbon (* $p<0.0001$, Student's t-test). Bar: F, 50 μm; inset, 5 μm.

Movies

Movie S1. Axon initial segments of EV-control and Stk25 shRNA-expressing neurons in P7 brain were rendered as rotating 3D image; relates to Fig. 2C, 2D. The

first clip shows an EV-control neuron. The colocalization (yellow) of the anti-phospho-IκB (axon initial segment, red) and anti-GFP (green) immunoreactivity indicates that this neuron possesses an axon initial segment. The second clip shows an Stk25 shRNA-expressing neuron; the anti-phospho-IκB signal does not colocalize with the GFP-positive processes.

Movie S2. Golgi apparatus structure visualized by optical Z-sections through EV-control or Stk25 shRNA-expressing neurons in P7 brain; relates to Fig. 2G, 2H. In the first clip, the cis-Golgi apparatus (GRASP65 signal, red) is localized to the apical side of the EV-control neuron (green). The second clip shows that in an Stk25 shRNA expressing neuron (green), the Golgi apparatus is fragmented and dispersed throughout the neuron.

Movie S3. Three dimensional rotation of Golgi and nucleus in EV-control, Stk25 shRNA, LKB1 shRNA and GM130 shRNA-expressing neurons; relates to Fig. 6A. The first clip shows the appearance of the cis-Golgi (anti-GRASP65, yellow) and the nucleus (DAPI, blue) in a normal stage III neuron. The second through fourth clips show the appearance of the Golgi and nucleus in Stk25 shRNA, LKB shRNA and GM130 shRNA-expressing neurons, respectively.

Extended Experimental Procedures

Antibodies

For immunocytochemistry, we raised a rabbit anti-Stk25 antibody against a fusion of glutathione-S transferase (GST) and a region of Stk25 (NM_021537, residues 306-354) with minimal homology to Mst3 (NM_145465) or Mst4 (NM_133729). The immunoserum was depleted for anti-GST antibodies prior to use. Knockingdown Stk25 expression eliminated the majority of the anti-Stk25 immunoreactivity, suggesting that the antibody is specific (Fig. S2). For immunoprecipitation, western blotting and immunocytochemistry, the following antibodies were used: anti-phosphorylated Tau (pS202/pT205) AT8 (Thermo Scientific), anti-Tau 5E2 (Millipore), anti-Stk25/Ysk1 N-19 (Santa Cruz), anti-β-actin (AC-15, Sigma), anti-HA 3F10 (Roche), anti-myc 71D10 (Cell Signaling; Western blotting), anti-myc 9E10 (BD Transduction Laboratories; immunoprecipitation), anti-pan-axonal neurofilament marker SMI312 (Covance), anti-GFP (Invitrogen), anti-GM130 (BD, Transduction Laboratories), anti-GRASP65 (Abcam) anti-Ctip2 (Abcam), anti-RFP (rabbit, Rockland), anti-RFP (clone H8 mouse, Advanced Targeting Systems), and anti-phospho IκBα (clone 14D4 Cell signaling). Filamentous actin was visualized using phalloidin conjugated to Alexa Fluor 568 (Invitrogen). Images were collected with a DeltaVision deconvolution (Applied Precision; Fig. 2 low magnification) or LSM 510 meta confocal (Zeiss; all other figures) microscope.

Immunocytochemistry and Immunohistochemistry

Mouse hippocampal neurons were prepared from E17.5 mouse embryos and cultured at $1x10^4$ cells/cm^2 unless otherwise indicated. Cultures were fixed (4% PFA, 250mM Sucrose, 2.5mM KCl, 2.5mM Magnesium Acetate, 25mM HEPES [pH7.4]) for 10 min at

25° C followed by permeabilization (0.1% saponin in PBS). Antigen recovery was done for Stk25 immunodetection by pepsin digestion (0.2 mg/mL in 0.2N HCl) at 25° C for 10 min followed by three washes with PBS. Fixed cells were incubated in blocking solution [20 mM Tris-HCL (pH7.4), 3% BSA, 10% normal goat serum and 0.05% sodium azide, 0.1% Tween 20] for 30 min at 25° C.

For immunostaining of brain sections, animals were perfused with 4% paraformaldehyde in PBS and the isolated brains were imbedded in 10% gelatin and sectioned using a vibrating microtome (60 μm; Leica). Indicated antibodies were incubated with sections floating in blocking buffer (containing 0.5% Triton X-100 in place of Tween 20) overnight at 4° C followed washes with PBS and fluorescent secondary antibody incubations.

Cell polarization assay

W4 cell polarity was examined 48 h after infections with humanized (h) Stk25 shRNA-expressing or control lentiviruses and 24 h after doxycycline treatment (Baas et al., 2004). Actin filaments were visualized with fluorescently-tagged phalloidin in GFP-positive cells that were fixed (as above for neurons) and permeabilized (0.1% Triton X-100).

Immunoprecipitation and Western blotting

HEK293T cells (2X 10^5 cells per well) were plated in a six-well dish and transfected a day later by incubating 8 μl of polyethylenimine (PEI; 1mg/mL) and 2 μg of each

indicated pCAGGS expression vectors (Niwa et al., 1991); cloning details available upon request) for 15 min prior to addition to cells. Cells were lysed 48 h later in NP40-IPB (137 mM NaCl, 2.7 mM KCl, 1% NP-40, 25 mM Tris-HCl [pH7.4], 2 mM EDTA, 50 mM NaF, 0.1% 2-mercaptoethanol, protease inhibitor cocktail [complete mini, EDTA free; Roche], 1 mM sodium orthovanadate, 1 mM phenylarsine oxide) and clarified by centrifugation at 20,000 X g for 20 min. Clarified lysates were preincubated with protein A/G plus Sepharose beeds (Santa Cruz) for 1 h at 4° C to pre-adsorb non-specific binding molecules. Lysates were then incubated with anti-myc (0.5 μg, 9E10) for 2 h on ice followed by addition of protein A/G PLUS Sepharose beads for 1 h with rotation at 4° C. Immunocomplexes were collected by centrifugation and washed 4 times with NP40-IPB, eluted with 1X sample buffer (62.5 mM Tris-HCl [pH6.8], 25 mM dithiothreitol, 2% SDS, 10% sucrose and 0.004% Bromophenol blue) and resolved by SDS-polyacrylamide gel electrophoresis on a 4-12% gradient gel (Invitrogen). Resolved total cell lysates and immunoprecipitated proteins were transferred to a PVDF membrane (Immobilon-P, Millipore) and immunoblotted with the indicated antibodies.

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