Lo et al., http://www.jcb.org/cgi/content/full/jcb.201208080/DC1

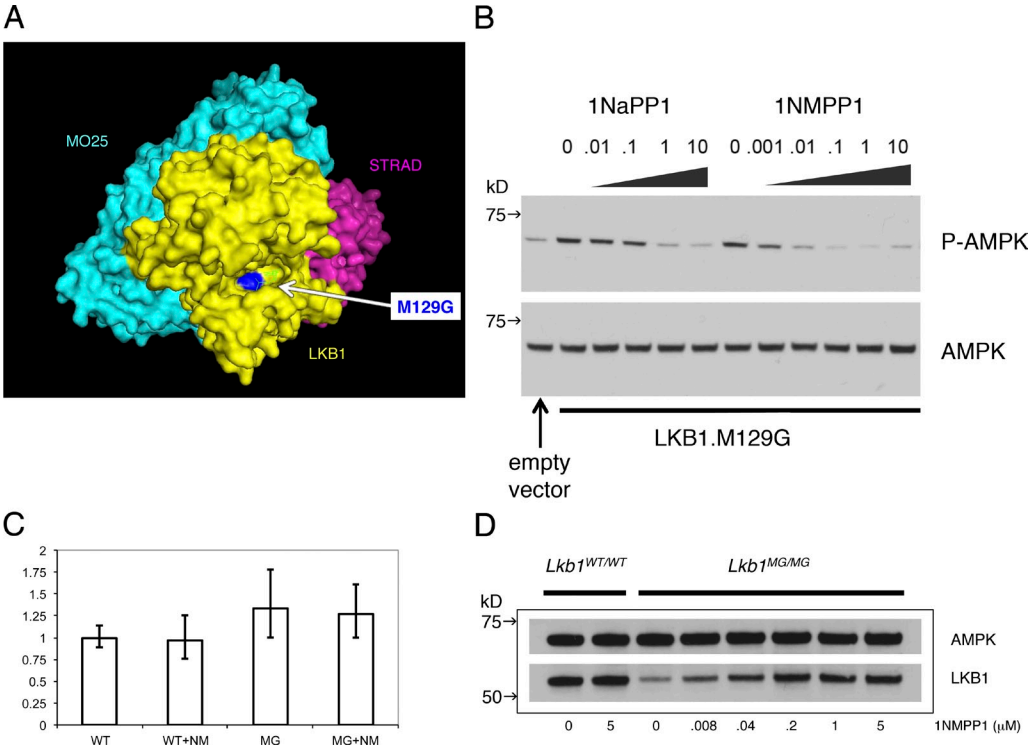


Figure S1. **Characterization of an analogue-sensitive kinase allele of Lkb1.** (A) Crystal structure of Lkb1 (yellow), Stradα (magenta), Mo25 (cyan; Zeqiraj et al., 2009) deposited with the PDB (accession number 2WTK) and visualized by Pymol. Met129 is shown in dark blue showing the gatekeeper location for the ATP binding pocket. (B) HeLa cells stably expressing Lkb1^{MG} were cultured with increasing concentrations of 1NaPP1 or 1NMPP1 in the presence of STO609 to inhibit CAMKK. Phospho-AMPK levels are reduced in a dose-dependent fashion by both PP1 analogues. Western blots were analyzed with antibodies to Phospo-AMPKα(Thr172) and AMPKα. (C) Total RNA was isolated from Lkb1$^{WT/WT}$ and Lkb1$^{MG/MG}$ MEFs cultured 16 h with or without 1 µM 1NMPP1. Real-time PCR was performed using the mouse Stk11 (Lkb1) TaqMan assay. Data were normalized to GAPDH and then expressed relative to the untreated Lkb1$^{WT/WT}$ expression levels. Error bars represent standard deviations (n = 3). (D) MEFs were cultured with increasing concentrations of 1NMPP1 for 16 h before collecting cell lysates. Lkb1^{WT} protein levels are unchanged with 1NMPP1, whereas Lkb1^{MG} protein levels increase with increasing concentrations of 1NMPP1. AMPKα serves as a loading control.

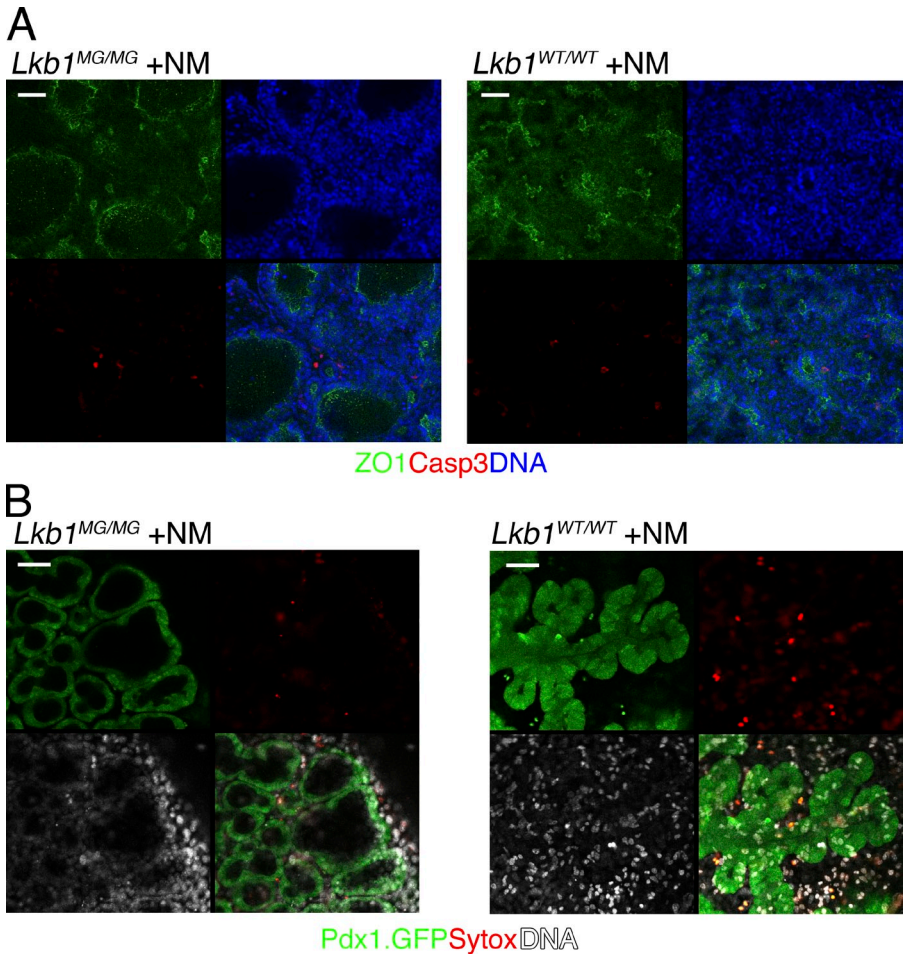


Figure S2. **Lkb1 inhibition did not result in increased cell death.** (A) Lkb1$^{MG/MG}$ (left) and Lkb1$^{WT/WT}$ (right) pancreatic explants treated with 1NMPP1 and then fixed and stained for activated caspase-3. (B) Live imaging shows that Lkb1$^{MG/MG}$ (left) pancreatic explant treated with 1NMPP1 did not exhibit increased numbers of Sytox-positive cells compared with Lkb1$^{WT/WT}$ (right) control. Bars, 50 µm.

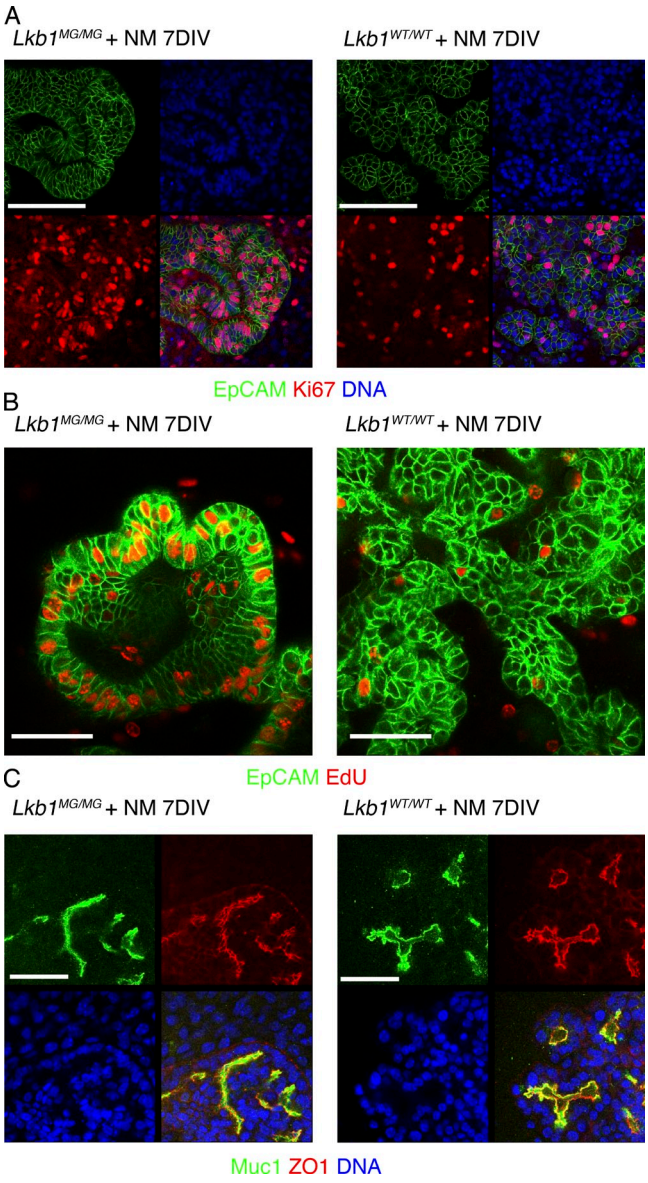


Figure S3. **Lkb1 inhibition results in increased proliferation in PanIN-like lesions without loss of apical-basal polarity.** Lkb1$^{MG/MG}$ (left) and Lkb1$^{WT/WT}$ (right) pancreatic explants were cultured 7 DIV with 1 μM 1NMPP1. Ki67 staining (A) and EdU incorporation (B) show that the Lkb1$^{MG/MG}$ epithelial structures demonstrate higher levels of proliferation. Muc-1 and ZO-1 patterns of staining (C) are similar between Lkb1$^{MG/MG}$ (left) and Lkb1$^{WT/WT}$ (right) explants. Bars: (A) 100 μm; (B and C) 50 μm.

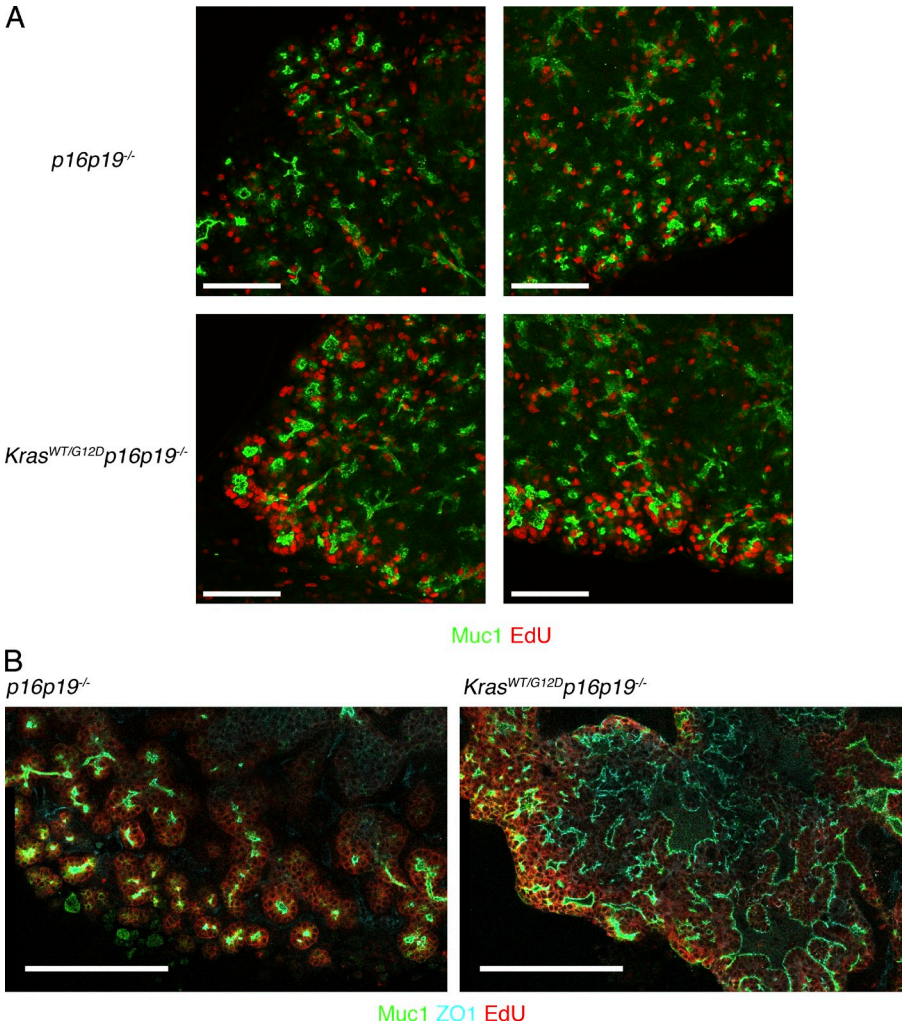


Figure S4. **Mutant K-Ras together with loss of p16/p19 increases cell proliferation and induces disruptions in tissue architecture.** (A) E14.5 p16p19$^{-/-}$ and Kras$^{G12D/WT}$p16p19$^{-/-}$ pancreatic explants were cultured for 5 d. EdU incorporation shows areas of increased proliferation for the Kras$^{G12D/WT}$p16p19$^{-/-}$ explants. (B) E14.5 p16p19$^{-/-}$ and Kras$^{G12D/WT}$p16p19$^{-/-}$(Pdx1-Cre) pancreatic explants were cultured for 8 d, fixed, and stained with Muc-1, ZO-1, and E-cadherin antibodies. Bars: (A) 100 µm; (B) 200 µm.

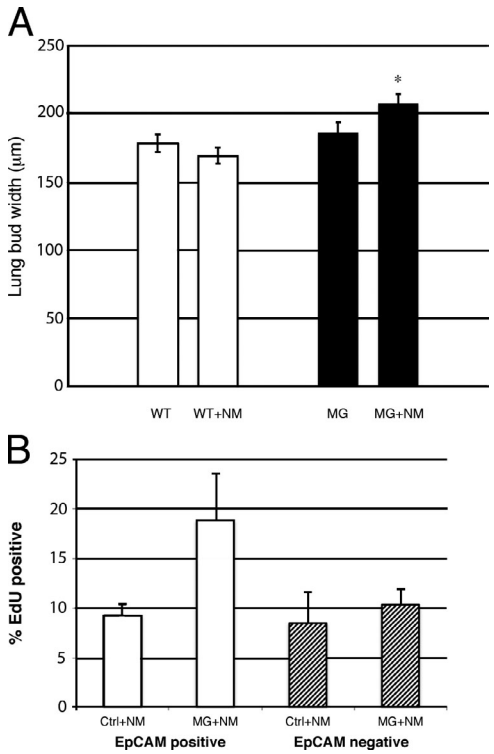
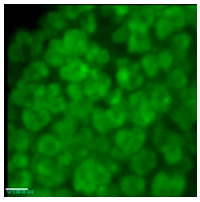


Figure S5. **Quantification of morphogenesis defect in lung explants and proliferation in pancreas explants.** (A) Quantification of bud widths in whole-mount lung explants from Lkb1$^{WT/WT}$ (WT) and Lkb1$^{MG/MG}$ (MG) embryos treated with or without 1NMPP1 (NM) for 24 h. Bud width was measured for each terminal bud at its widest point. Error bars represent standard errors ($n > 85$). Asterisk indicates a statistically significant difference in the mean ($P < 0.05$). (B) Percentage of EdU positivity in EpCAM-positive and -negative cells from dissociated pancreas explants from Lkb1$^{MG/MG}$ (MG) embryos and littermate controls treated with 1NMPP1 (NM). Error bars represent standard deviations ($n = 5$).



Video 1. **Fluorescence time-lapse imaging of cyst formation dynamics.** Lkb1$^{WT/MG}$ (left) and Lkb1$^{MG/MG}$ (right) Pdx1-GFP pancreatic explants cultured on transwell filters with 1NMPP1 and imaged at 37°C in 5% CO_2. Video begins ~24 h after addition of 1NMPP1 and continues for 52 h. Image stacks were acquired every 30 min using an Eclipse Ti-E (10× Plan Flour, 0.3 NA phase-contrast objective; Nikon) with an Revolution DSD white light spinning disk confocal (Andor Technology).

Table S1. **Percentage of EdU-positive cells in pancreatic explants**

	EpCAM positive		EpCAM negative	
Genotype	**Ctrl + NM**	**MG + NM**	**Ctrl + NM**	**MG + NM**
WT vs. MG	8.64	25.7	4.52	9.73
WT vs. MG	8.14	21.9	5.88	12.3
WT vs. MG	10.7	16	11	11.1
HET vs. MG	10.2	15.3	11.5	10.5
HET vs. MG	8.62	15.2	9.38	8.03
Mean	9.26	18.82	8.456	10.332
SD	1.118659913	4.750473661	3.111250552	1.59316352

Pairs of E16.5 pancreatic explants from $Lkb1^{MG/MG}$ (MG) embryos and littermate controls (either $Lkb1^{WT/WT}$ [WT] or $Lkb1^{WT/MG}$ [HET]) were cultured for 3 d with 1NMPP1 and then incubated with EdU for 2 h before dissociation. Dissociated cells were fixed and labeled for EdU and EpCAM and then subjected to FACS analysis.

Table S2. **Summary of mesenchyme-free lung epithelial culture experiments**

Genotype	No. of experiments	No. of explants	Branched	Unbranched	No growth
			%	%	%
WT	18	46	74	24	2
WT + NM	18	41	68	24	7
MG	18	41	49	49	2
MG + NM	18	49	18	80	2
WT + A7	12	26	81	12	8
WT + NM + A7	12	27	78	7	15
MG + A7	12	25	64	36	0
MG + NM + A7	12	31	61	39	0

Mesenchyme-free lung epithelial cultures were prepared from $Lkb1^{WT/WT}$ (WT) and $Lkb1^{MG/MG}$ (MG) embryos and then treated as indicated (1NMPP1 [NM] and/or A-769662 [A7]).

Table S3. **Quantification of cysts in pancreatic explants**

Genotype	No. of cysts	Mean diameter	Range of diameters	SD of diameters
		μm	μm	μm
WT + NM	1	32	NA	NA
MG + NM	16	221	76–688	181

E16.5 Pdx1-GFP–expressing $Lkb1^{WT/WT}$ (WT) and $Lkb1^{MG/MG}$ (MG) explants were cultured for 3 d in 1NMPP1 and then imaged live to avoid fixation artifacts and distortions from mounting between coverglass. Z-stacks were reconstructed and, in a volume of 1,200 × 1,200 × 200 µm, cystic structures were counted and maximal diameters in the XY plane were measured.

Reference

Zeqiraj, E., B.M. Filippi, M. Deak, D.R. Alessi, and D.M. van Aalten. 2009. Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation. *Science*. 326:1707–1711. http://dx.doi.org/10.1126/science.1178377