Chan et al., http://www.jcb.org/cgi/content/full/jcb.201404067/DC1

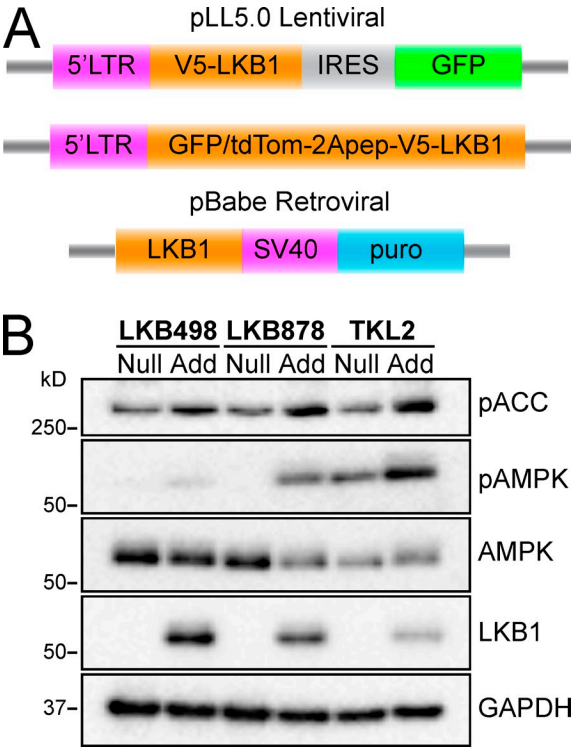


Figure S1. **Reconstitution of LKB1-deficient melanoma cell lines.** (A) Schematic of lentiviral and retroviral constructs for LKB1 reconstitution. Lentiviral constructs utilized the 5′ long terminal repeat (LTR) of the murine stem cell virus to drive expression. (B) Western blots of LKB1 reconstitution in three *Kras*^{G12D}/*Lkb1*-null melanoma cell lines (LKB498, LKB878, and TKL2) were probed with specific antibodies. GAPDH was used as a loading control. Add, addback; pACC, phosphorylated acetyl-CoA carboxylase; IRES, internal ribosome entry site; tdTom, tdTomato.

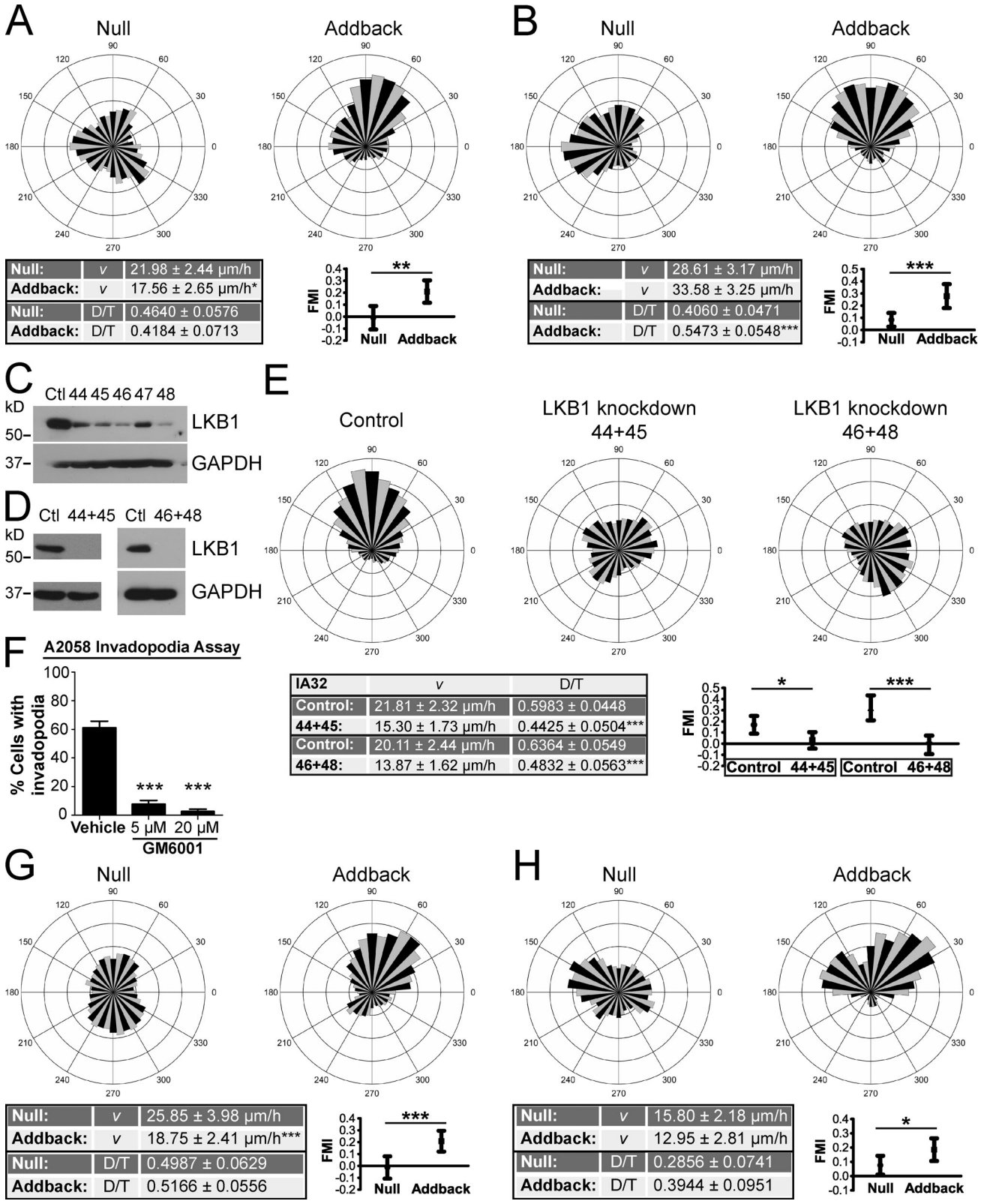
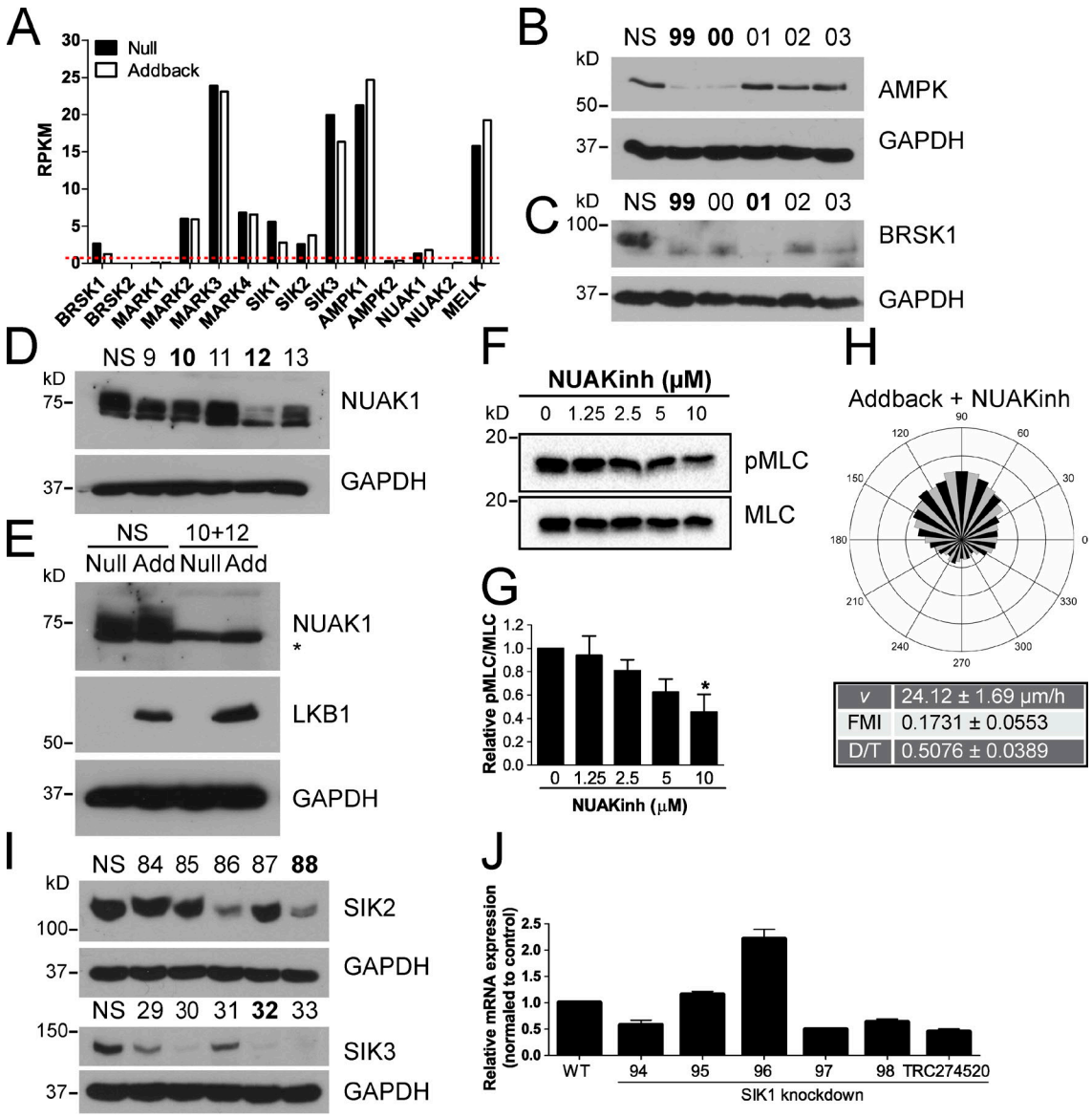


Figure S2. **LKB1 is required for haptotaxis.** (A and B) LKB1 is required for haptotaxis in LKB878 (A) and TKL2 (B) melanoma. (LKB878: null, *n* = 58; addback, *n* = 45. TKL2: null, *n* = 131; addback, *n* = 59.) (C and D) Western blot showing LKB1 knockdown in IA32 mouse fibroblasts using a single shRNA (C) or two shRNA (D) hairpins. The last two digits of the GE Healthcare TRC1 shRNA library identifier for LKB1 are shown to denote each individual hairpin. Ctl, control. (E) LKB1 is required for haptotaxis in IA32 mouse fibroblasts. (control, *n* = 54; LKB1 knockdown, *n* = 82.) (F) Invadopodia assay of human A2058 cells treated with the MMP inhibitor GM6001. Data are shown as means ± SEM. ***, P < 0.001 by one-way ANOVA with Dunnett's post-test, as compared with vehicle control. (vehicle, *n* = 108; 5 μM, *n* = 114; 20 μM, *n* = 112.) (G and H) GM6001 has no effect on haptotaxis in LKB498 cells in 2D (20 μM: null, *n* = 72; addback, *n* = 76; G) or 3D (5 μM: null, *n* = 41; addback, *n* = 36; H). All haptotaxis data are shown as mean ± 95% confidence intervals. *, P < 0.05; **, P < 0.01; ***, P < 0.0001 by two-tailed unpaired *t* test. *v*, velocity; D/T, displacement (D) over the total path length (T).



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LKB498	*v*	D/T	FMI	N	Haptotaxis?
Addback:	23.81 ± 3.35 μm/h	0.4453 ± 0.0799	0.2120 ± 0.0930	40	Yes
AMPK-KD#99:	27.71 ± 3.69 μm/h	0.4376 ± 0.0573	0.2764 ± 0.0755	42	Yes
Addback:	24.26 ± 3.61 μm/h	0.4897 ± 0.0643	0.1937 ± 0.0643	49	Yes
AMPK-KD#00	23.74 ± 3.07 μm/h	0.4274 ± 0.0565	0.2459 ± 0.0725	46	Yes
Addback:	25.65 ± 3.43 μm/h	0.5005 ± 0.0675	0.2567 ± 0.0938	49	Yes
BRSK1-KD#99:	29.52 ± 4.57 μm/h	0.5165 ± 0.0585	0.1949 ± 0.0897	68	Yes
Addback:	19.42 ± 3.12 μm/h	0.5278 ± 0.0665	0.2329 ± 0.1098	52	Yes
BRSK1-KD#01	21.91 ± 4.23 μm/h	0.4569 ± 0.0688	0.1919 ± 0.1135	43	Yes
Addback:	18.55 ± 2.20 μm/h	0.3706 ± 0.0581	0.1487 ± 0.0606	68	Yes
NUAK1-KD#10+12:	21.87 ± 3.58 μm/h	0.4064 ± 0.0722	0.2035 ± 0.0865	55	Yes
Addback:	22.76 ± 3.08 μm/h	0.5125 ± 0.0751	0.1296 ± 0.1180	50	Yes
SIK1-KD#98 + SIK2-KD#88 + SIK3-KD#32	25.04 ± 4.04 μm/h	0.4974 ± 0.0587	0.1716 ± 0.0870	70	Yes

Figure S3. **Testing LKB1 substrates for their role in haptotaxis.** (A) Transcript levels of known LKB1 substrates are shown from RNA-Seq analysis (Table S1) of LKB498 LKB1-null and addback cells, which was completed once. Dashed red line shows threshold (reads per kilobase per million reads [RPKM] = 1) of expression. (B–E) Western blots showing lentiviral shRNA depletion in LKB498 cells of AMPK (B), BRSK1 (C), and NUAK1 (D) with a single hairpin and (E) NUAK1 with two hairpins. Asterisk indicates a nonspecific band. The last two digits of the GE Healthcare TRC1 shRNA library identifier for each target are shown to denote each individual hairpin. Hairpins in bold were used in cells for haptotaxis experiments. (F and G) Western blot (F) of myosin light chain phosphorylation at serine 19 and quantification (G) in LKB498 addback cells treated with increasing concentrations of the NUAK inhibitor (NUAKinh) WZ4003. Data are relative phosphorylated myosin light chain (pMLC)/total phosphorylated myosin light chain and are shown as means ± SEM. *, $P < 0.05$ by one-way ANOVA with Dunnett's post-test, as compared with vehicle control (0 μM; $n = 3$). (H) NUAK1 activity is not necessary for haptotaxis in LKB498 addback cells treated with 5 μM WZ4003 ($n = 183$). (I) Western blots of SIK2 and SIK3 by lentiviral shRNA depletion. (J and K) Expression of *Sik1* by qRT-PCR (relative mRNA expression shown as mean ± SD, $n = 3$; J) and SIK2 and SIK3 by Western blot in LKB498 addback cells with triple knockdown of SIK family kinases (K). WT, wild type. (L) Table of several LKB1 substrates that are not required for haptotaxis. All haptotaxis data are shown as mean ± 95% confidence intervals. KD, knockdown; NS, nontargeting sequence; *v*, velocity; D/T, displacement (D) over the total path length (T).

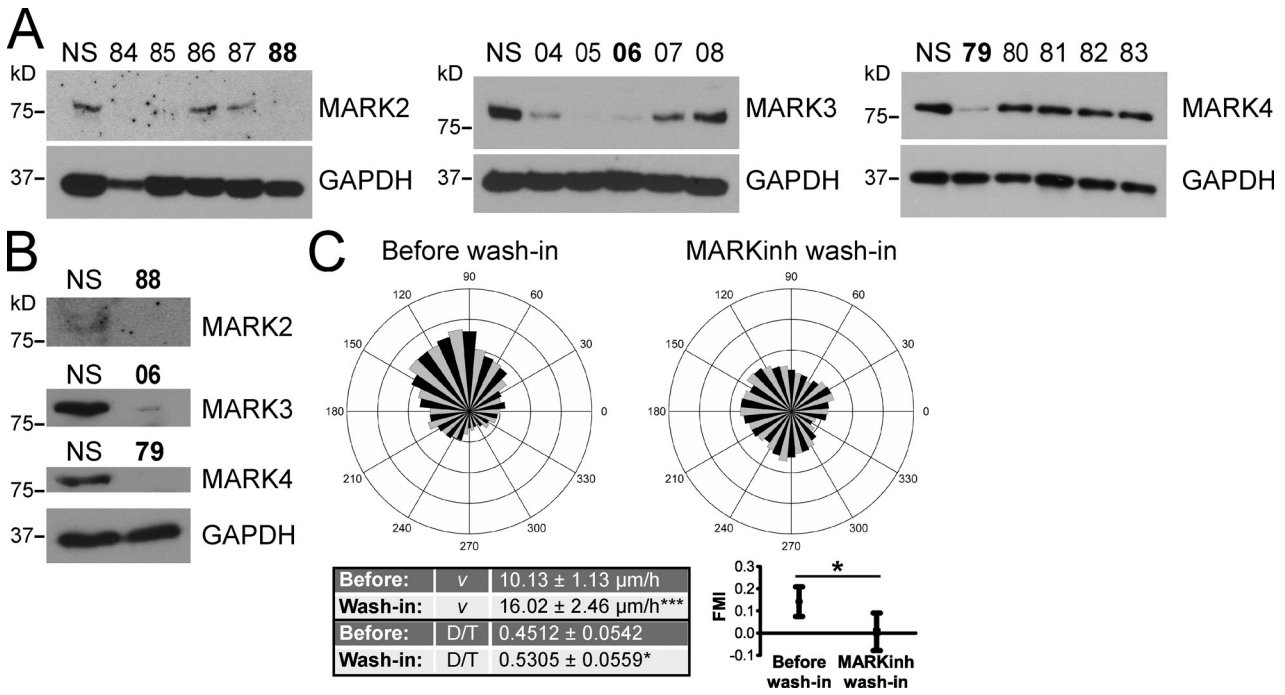


Figure S4. **MARK family kinases regulate haptotaxis.** (A) Western blots showing lentiviral depletion with a single shRNA hairpin against MARK2, MARK3, and MARK4 in LKB498 addback cells. The last two digits of the GE Healthcare TRC1 shRNA library identifier for MARK are shown to denote each individual hairpin. Hairpins in bold were used in cells for haptotaxis experiments in B and C. (B) Western blots showing triple knockdown of MARK family kinases. (C) Wash in of MARK inhibitor blocks haptotaxis in IA32 fibroblasts. (before wash in, *n* = 103; MARK inhibitor [MARKinh] wash in, *n* = 82.) Data are shown as mean ± 95% confidence intervals. *, P < 0.05; ***, P < 0.001 by two-tailed unpaired *t* test. NS, nontargeting sequence; *v*, velocity; D/T, displacement (D) over the total path length (T).

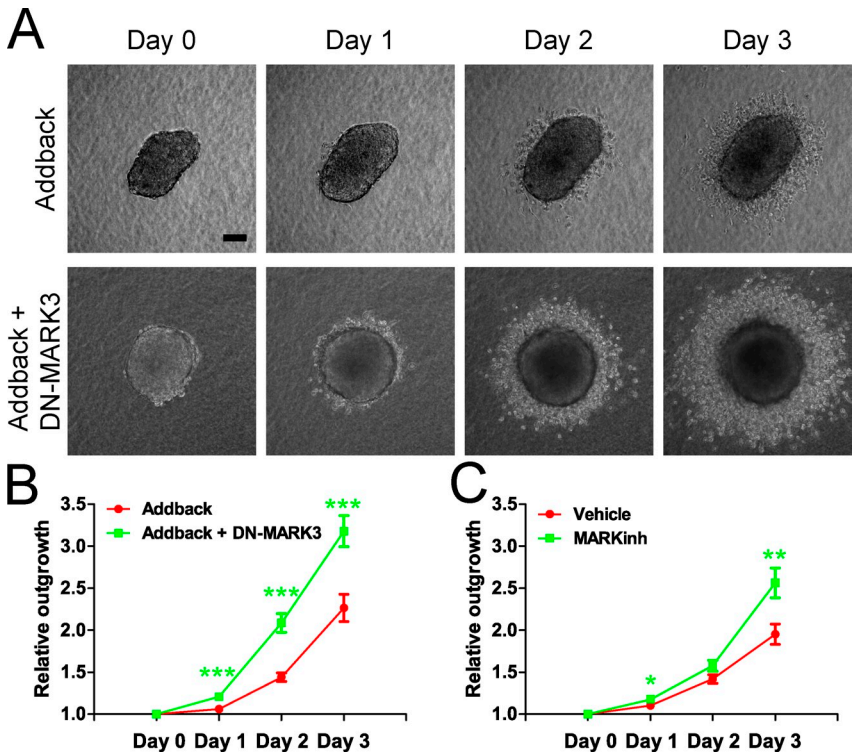
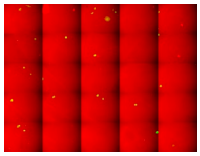
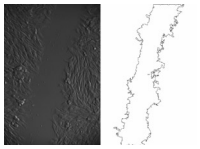


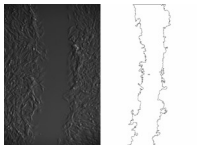
Figure S5. **DN-MARK enhances collagen invasion.** (A and B) Overexpression of DN-MARK3 in LKB498 LKB1 addback cells increases spheroid invasion into 6 mg/ml 3D collagen. Bar, 100 µm. (day 0–2: null, *n* = 24; addback, *n* = 23. day 3: null, *n* = 16; addback, *n* = 22.) (C) LKB498 addback cell spheroid invasion is increased with 20 µM MARK inhibitor (MARKinh) 39621. (day 0–3: null, *n* = 28; addback, *n* = 30.) Data are shown as means ± SEM. *, P < 0.05; **, P < 0.01; ***, P < 0.001 by two-tailed unpaired *t* test.



Video 1. **Live-cell invadopodia assay.** WM-266-4 cells stably expressing Lifeact-GFP (green) were plated on Alexa Fluor 568–gelatin (red). Images were analyzed by time-lapse epifluorescence microscopy using an incubator microscope (VivaView FL; Olympus). Frames were taken every 30 min for 27 h. Video is played at seven frames per second.



Video 2. **LKB1-null cells in wound-healing assay.** LKB498 LKB1-null cell monolayers were scratch wounded. Images were analyzed by differential interference contrast microscopy using an incubator microscope (VivaView FL; Olympus), and ImageJ software was used to highlight the wound boundary. Frames were taken every 10 min for 12 h. Video is played at seven frames per second.



Video 3. **LKB1 addback cells in wound-healing assay.** LKB498 LKB1 addback cell monolayers were scratch wounded. Images were analyzed by time-lapse differential interference contrast microscopy using an incubator microscope (VivaView FL; Olympus), and ImageJ software was used to highlight the wound boundary. Frames were taken every 10 min for 12 h. Video is played at seven frames per second.

Table S1 is provided online as an Excel file and shows RNA-Seq results of LKB498 LKB1-null and addback cells.