SUPPLEMENTAL MATERIAL

Methods

Isolation of mouse Lung Endothelial Cells (MLEC)

MLECs were isolated from 8-week-old *LKB1*$^{endo-/-}$ and control mice as described previously.[1] Briefly, lungs were excised, minced, and digested with 0.1% collagenase in PBS for 45 min. The digest was homogenized by multiple passages through a 14-gauge needle, and then filtered through a 100-µm tissue sieve. The endothelial cells were isolated by immunoselection with CD31-conjugated (BD Pharmingen) magnetic beads (Invitrogen). When plated cells reached confluency, a second immuno-isolation was performed by ICAM-2-conjugated (BD Pharmingen) magnetic beads.

Biotin pull-down analysis

For in vitro synthesis of biotinylated transcripts, cDNA from mouse endothelial cells was used as a template for PCRs, whereby the T7 RNA polymerase promoter sequence (CCAAGCTTCTAATACGACTCACTATAGGGAGA [T7]) was added to the 5' end of all fragments. To prepare partial caveolin-1 3' UTR transcripts, the primers used were (T7)GGGACATTTCAAGGATGAAAG and GGGTTTAAACATTCCAGACTGTCA for ARE1, (T7)ATTCCTGCTCTCTCTTTTATA and TCTGTATTTCAGAGTCAGAAACAA for ARE2, (T7)ACCTGTTGATGTTGATATCTG and AAACTTGAATTTATTACAGCAAGA for ARE3, and (T7)TTCTGAACCCAAACTGAGGAA and TAACTGTTAAACAATTTTATTGTG for ARE4. (T7)GAAACCCTGGACCACCCACCCCAG and GGGTGCAGCGAACTTTATTGATG for GAPDH as negative control. (T7)ACTGACCTAACTCGAGGAGGAG and GTATTTTTTCCAATTATTTTAT for c-Myc as positive control. Biotinylated RNAs were synthesized using a MaxiScript T7 kit (Ambion) according to the manufacturer's instruction by using 1:10 ratios of CTP and Biotin-11-CTP (New England Biolabs). Whole-cell lysates (120 µg

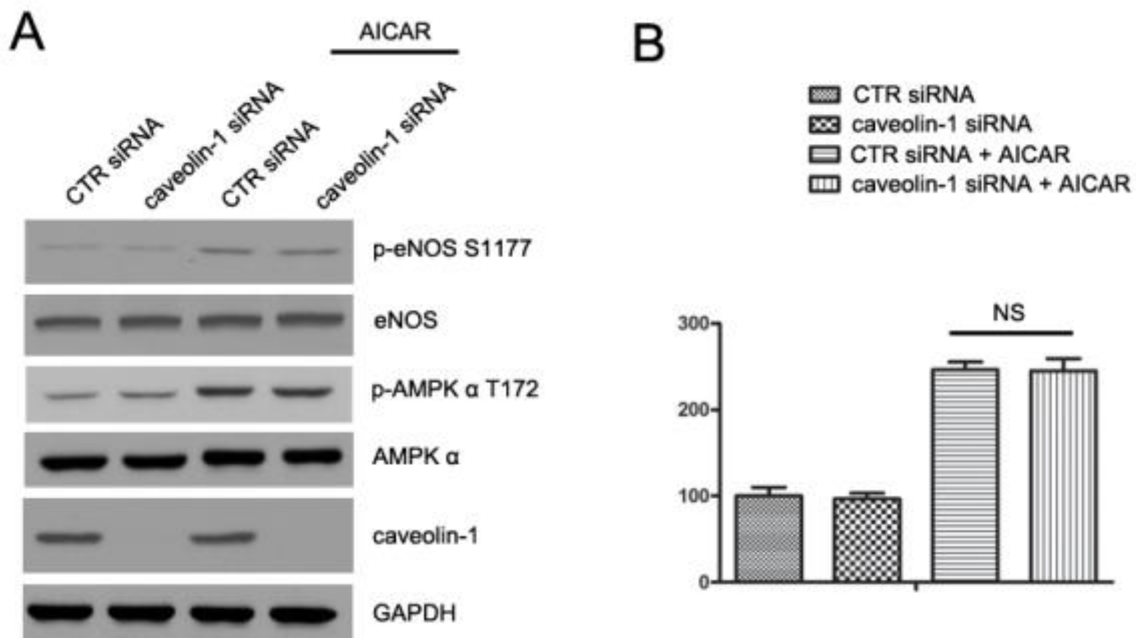
for each sample) were incubated with 2 μg of purified biotinylated transcripts for 1 h at room temperature. Complexes were isolated with paramagnetic streptavidin-conjugated Dynabeads (Dynal), and bound proteins in the pulldown material were assayed by Western blot.

Immunohistochemistry and immunofluorescence

Eight- to 10-week-old mice were perfused with PBS and then with 4% paraformaldehyde for 5 min. Blood vessels were harvested, embedded in paraffin or OCT (Sakura) and then 5 μm sections were cut and blocked with 3% normal goat serum. For immunofluorescence studies, the frozen slides were incubated with mouse anti-LKB1 antibody (Santa Cruz) and rabbit anti-CD31 (Abcam) at 4 °C for overnight, Alexa Fluor 488 goat anti-rabbit /555 goat anti-mouse IgG (Invitrogen) were used as secondary antibody (1:400 dilution; at room temperature for 1 h). For immunohistochemistry, paraffin embedded slides were probed with rabbit anti- caveolin-1 antibody or anti-phospho-AMPKα T172 (Cell Signaling Technology) at 4 °C for overnight and then secondary antibody at room temperature for one hour. Then the signal was visualized by using the Liquid DAB+ Substrate Chromogen System (Dako).

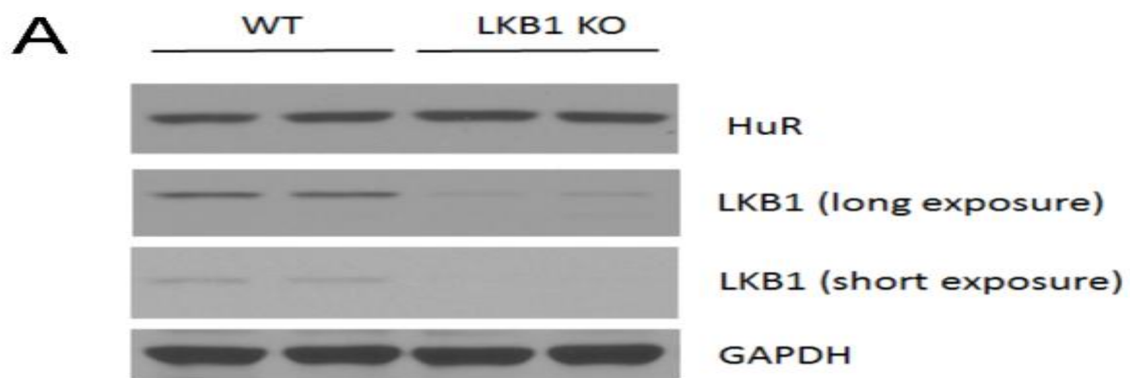
Reference

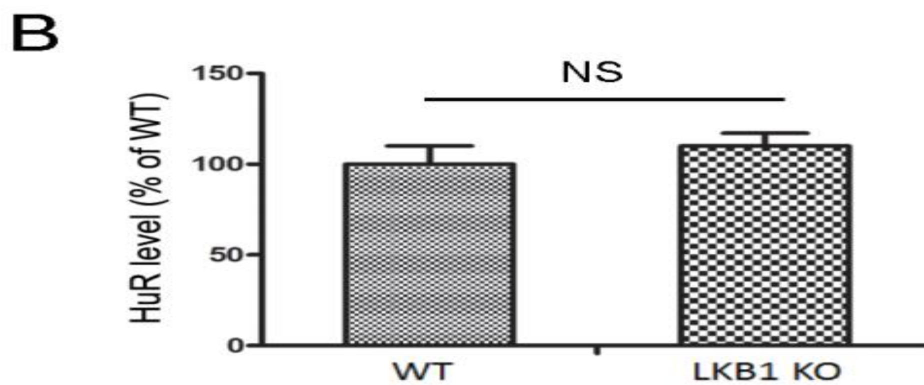
1. Ohashi K, Ouchi N, Higuchi A, Shaw RJ, Walsh K. LKB1 deficiency in Tie2-cre-expressing cells impairs ischemia-induced angiogenesis. *J Biol Chem.* 2010;285:22291-22298.



Supplemental Figure 1

Caveolin-1 association with eNOS does not affect the ability of AMPK to phosphorylate eNOS. **A** and **B**, HUVEC were transfected with CTR siRNA or caveolin-1 siRNA for 48 hours followed by AICAR (2 mM) treatment for 30 min. Western blot analysis was performed to detect phospho-eNOS levels. The blot is a representative of 3 blots from 3 independent experiments. NS, not significantly.





Supplemental Figure 2

LKB1 deletion does not affect total HuR level.

A and **B**, Western blot analysis to detect total HuR level in WT and LKB1-deficient MLECs. The blot is a representative of 3 blots from 3 independent experiments. NS, not significantly.