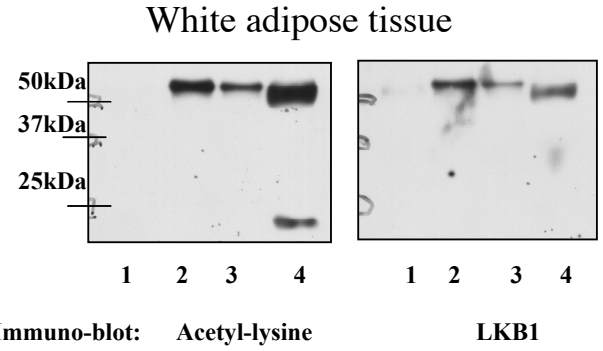
Fig.S1.

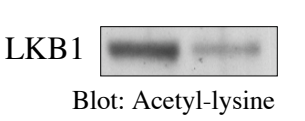


Lysine acetylation of endogenous LKB1. In white adipose tissue from the mouse, LKB1 was immunoprecipitated with covalently linked anti-LKB1 antibody-protein G beads. Lane 1: beads only, 2: 200 µg lysate, 3: 100 µg lysate, 4: IgG.

Fig.S2A. **Acetylated lysines (K*) identified by MALDI-TOF and LC-MS/MS**

Acetylated lysine	MALDI-TOF		LC-MS/MS
	M/z	Mass matched sequence	Acetylated fragment
K44, K48	2197.1579	(R)AK*LIGK*YLMGDLLGEGSYGK(V)	RAK*LKGK*
K96, 97	983.5162 1267.7123	(R)IPNGEANVK*(K) (R)RIPNGEANVK*K(E)	KLRRIPNGEANVK* LRRIPNGEANVK*K*
K296	1235.6094	(K)GMLEYEPAK*R(F)	
K310	1427.766	(R)QIRQHSWFRK*(K)	
K416,423	1542.7698	(R)APNPARK*ACSASSK*(I)	APNPARK*ACSASSK*
K431	1045.5941	(K)IRRLSACK*(Q)	RLSACK*

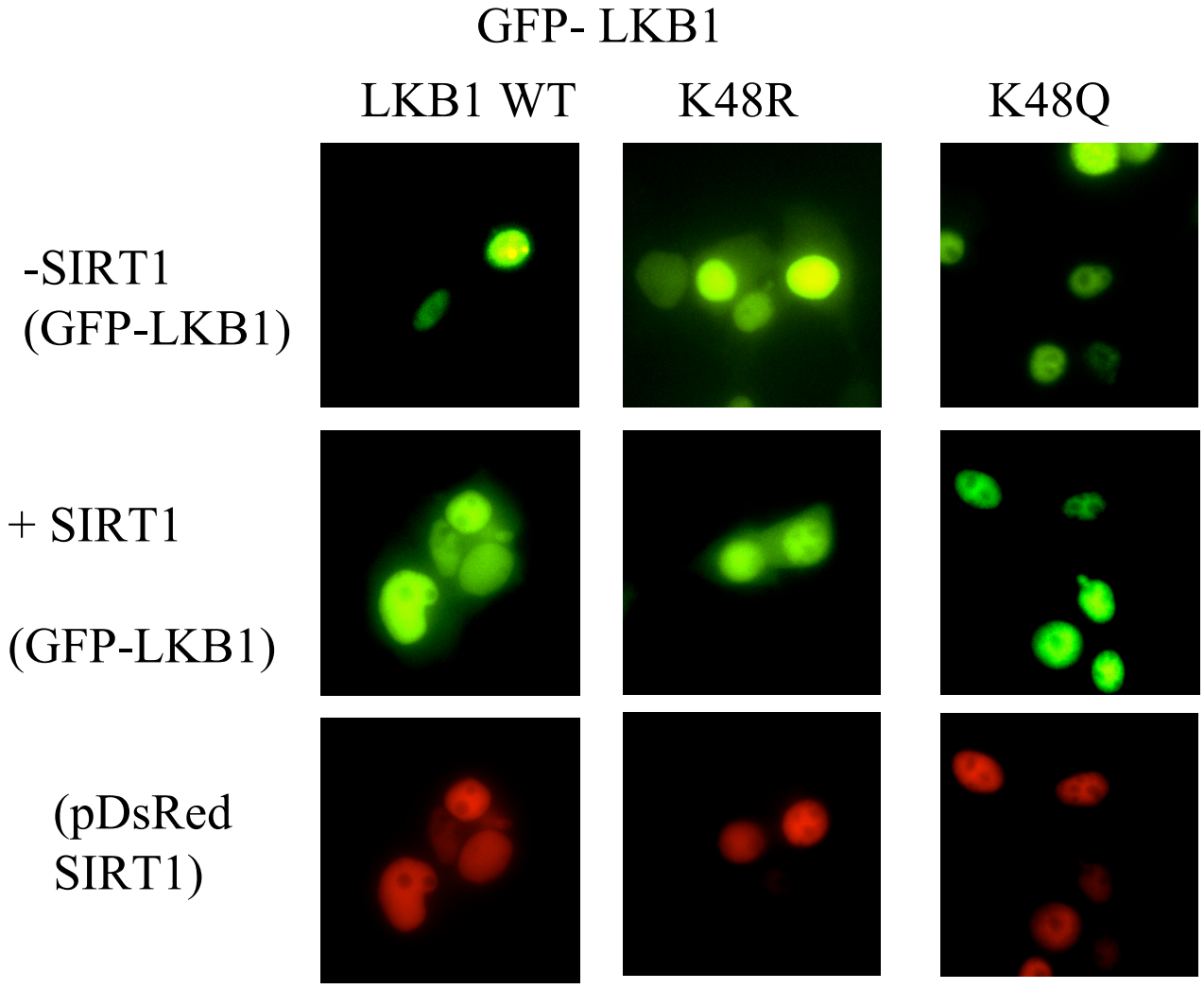
Fig.S2B.



Treatment	Mass observed	Sequence
DMSO	2197.1579	43-62: (R)AK^{Ac}LIGK^{Ac}YLMGDLLGEGSYGK(V) (K44 and K48 acetylation)
Resveratrol	1502.7201	49-62: (K)YLMGDLLGEGSYGK(V)

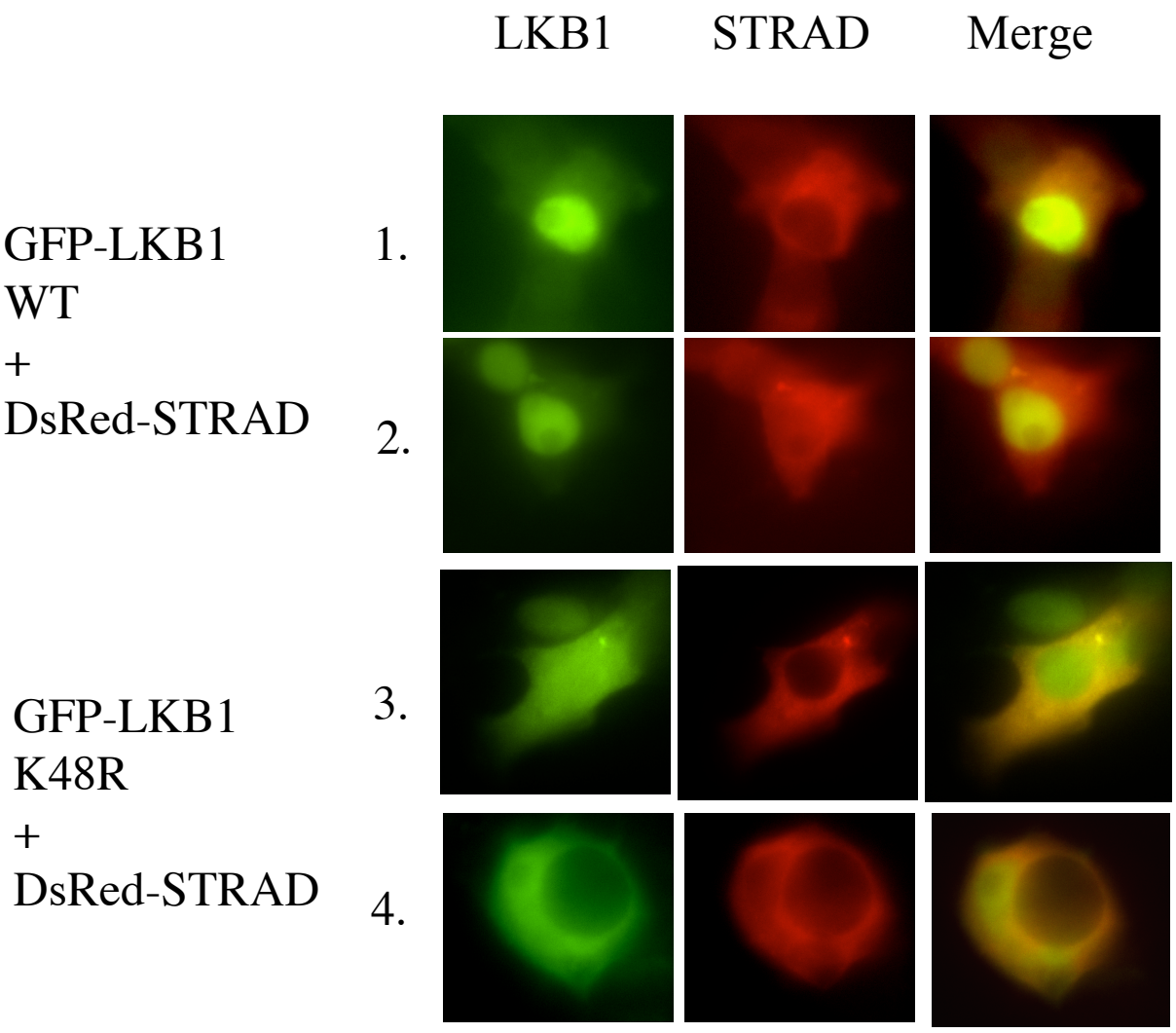
Deacetylation of LKB1-K48 by resveratrol. (Left Panel) Resveratrol (50 µM for 60 minutes), a putative SirT1 activator, decreased total LKB1 acetylation. (Right Panel). A mass spec analysis (MALDI-TOF) was carried out in which trypsin was used to cleave LKB1 enzymatically. Unacetylated lysines are susceptible to cleavage by trypsin; however acetylated lysines are resistant. In keeping with this, the mass signature corresponding to amino acids (aa) 43-62, containing AcK44 and AcK48, was only observed under baseline (DMSO) condition. Treatment with resveratrol caused the disappearance of the aforementioned fragment and the mass appearance of aa 49-62 without any acetylation, suggesting that stimulation with resveratrol leads to deacetylation of AcK48.

Fig.S3.



GFP-tagged (green) LKB1 WT, K48R and K48Q mutant was transfected with/ without pDsRed-tagged SIRT1 (red). SIRT1 expression increases LKB1 cytoplasmic localization and mutation of K48 to R mimicked the effect. The differences are most recognizable on the screen in the Windows computer.

Fig.S4.



Plasmids (0.5 μg) expressing GFP-tagged (green) LKB1 WT or the K48Rmutant were co-transfected with 0.2 μg of pDsRed-tagged STRAD (red) into HEK293T cells grown in 6-well plates. The cells were fixed 12 hrs later. STRAD was predominantly localized in cytoplasm in all cells. STRAD co-expression enhanced the cytoplasmic localization of both WT and K48R (see Fig. S3) LKB1, with its effect greater in the latter. Thus, after transfection with STRAD, WT-LKB1 was still predominantly localized in the nucleus (1, 2), whereas K48R-LKB1 was either equally distributed between the cytoplasm and nucleus (3) or predominantly in the cytoplasm (4).