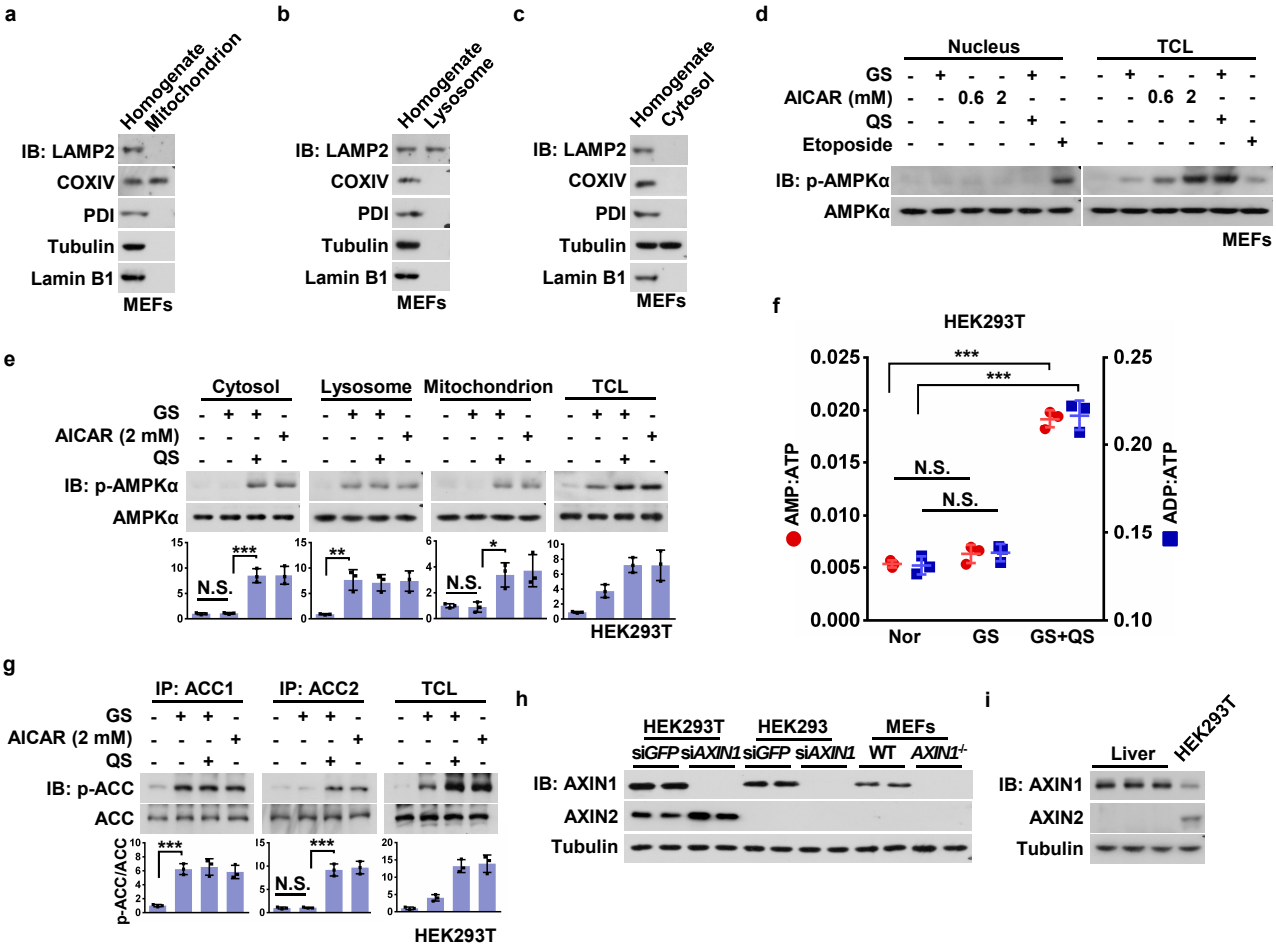

Supplementary information, Fig. S1. Basal AMP Is Sufficient for Activation of the Lysosomal Pool of AMPK

(**a-c**) Validation of methods for subcellular fractionation. MEFs were homogenized and subjected to fractionation of mitochondrion (**a**), lysosome (**b**) and cytosol (**c**) following individual methods (described in "Materials and Methods" section). Fractions were then analyzed by immunoblotting using the antibodies against LAMP2 (a lysosome marker), COXIV (a mitochondrion marker), PDI (an ER marker), tubulin (a cytosol marker) and Lamin B1 (a nuclear marker), respectively.

(**d**) No nuclear AMPK activation can be detected under glucose starvation or severe nutrient stress. MEFs were starved for glucose, glucose and glutamine, treated with 0.6 or 2 mM AICAR, or 20 μM etoposide (all for 2 hr). The nucleus fraction was then prepared, followed by analysis of p-AMPKα levels by immunoblotting.

(**e**) Low glucose exclusively activates lysosomal pool of AMPK in HEK293T cells. Cells were starved for glucose, or for both glucose and glutamine, or treated with 2 mM AICAR, all for 2 hr, followed by fractionation and immunoblotting.

(**f**) Adenine nucleotide ratios are unaltered in HEK293T cells after glucose starvation. Cells were starved as in (**e**), followed by determination of adenylate nucleotide ratios by CE-MS. Results are mean ± SD; *** $p < 0.001$, N.S., not significant by ANOVA, $n = 3$.

(**g**) ACC1, but not ACC2, is phosphorylated in in HEK293T cells starved for glucose. Cells were starved or treated with AICAR as in (**e**). Endogenous ACC1 and ACC2 were individually immunoprecipitated, followed by immunoblotting.

(**h, i**) AXIN2 can be readily detected in HEK293T cells but not in MEFs, HEK293 cells and mouse liver. MEFs, HEK293 cells, HEK293T cells (with AXIN1 knocked down/knocked out as controls) and mouse liver were lyzed and analyzed by immunoblotting.

Experiments in (**a**), (**b**), (**c**), (**d**), (**f**), (**h**), and (**i**) were performed twice and those in (**e**) and (**g**) three times.