Supporting information

Mankouri et al. 10.1073/pnas.0912426107

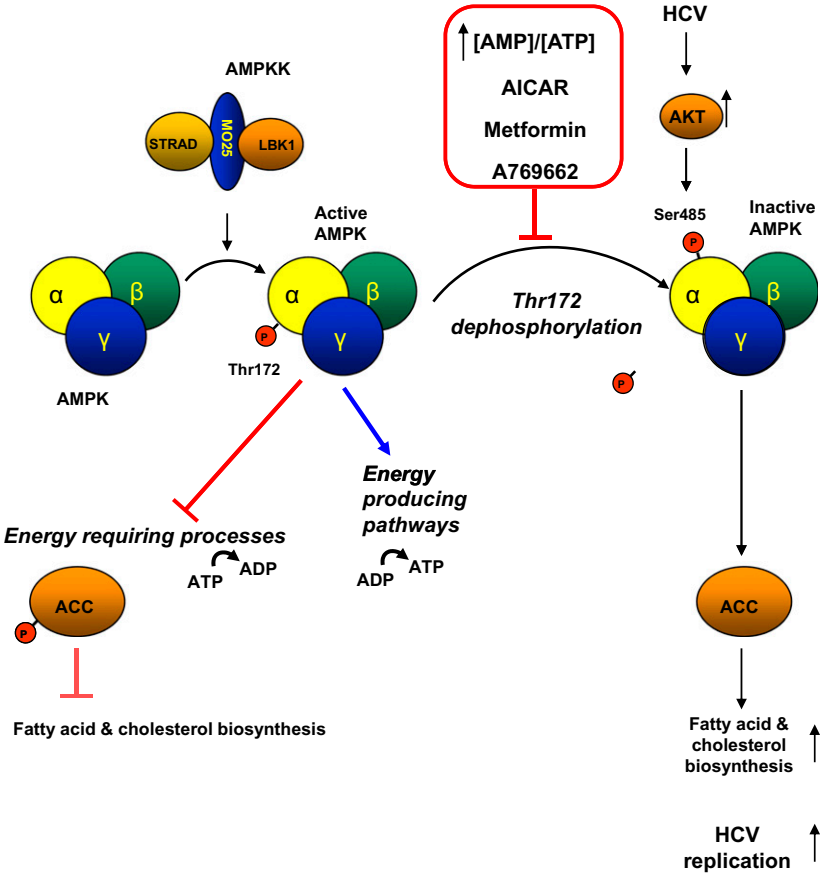

Fig. S1. Schematic of the regulation of AMP-activated protein kinase (AMPK) activity. When activated by ATP consumption or pharmacological activation, AMPK is phosphorylated at threonine 172 on the α subunit by an upstream complex, AMPK kinase. Active AMPK then phosphorylates a number of substrates. Key targets include the two isoforms of acetyl-CoA carboxylase (ACC1/2). Importantly, phosphorylation of ACC1/2 on serine 79/220 by AMPK inhibits enzymatic activity, concomitantly decreasing cellular fatty acid synthesis which could be deleterious to hepatitis C virus (HCV) infection. HCV (most likely via proteins NS4B and NS5A) activates the serine/threonine kinase, AKT, which phosphorylates AMPK on an inhibitory residue (serine 485), leading to activation of ACC1/2 and increased cellular lipid synthesis.

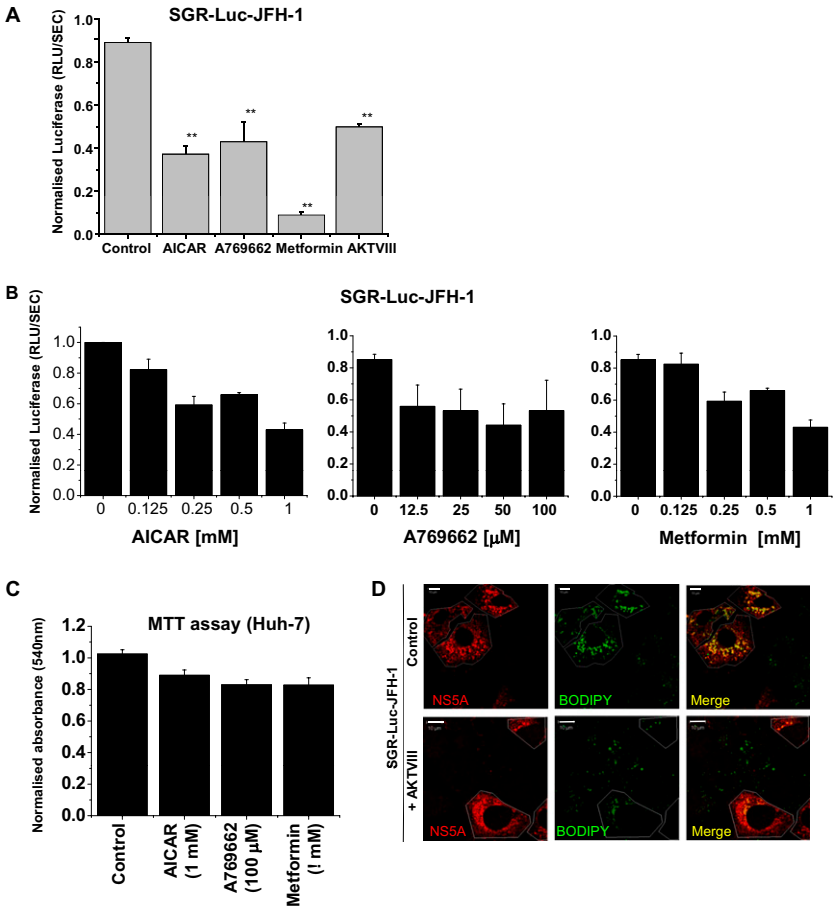

Fig. S2. Restoration of AMPK activity using either AMPK agonists or AKTVIII is detrimental to the replication of a genotype 2a (JFH-1) subgenomic replicon (SGR). (*A*) Huh-7 cells were transfected with in vitro transcripts of a transient luciferase subgenomic replicon (JFH-1: genotype 2a) (1) and left untreated (Control) or treated with the indicated compounds overnight. Luciferase activity measured 24 h posttransfection shows the relative level of genome replication. Error bars indicate mean ± SEM. (*B*) Huh-7 cells were transfected with in vitro transcripts of a transient luciferase subgenomic replicon (JFH-1: genotype 2a) and treated with the AMPK agonists AICAR, A769662, or metformin at the indicated concentrations overnight. Luciferase activity was measured 24 h posttransfection and shows the relative level of genome replication. Error bars show SEM. (*C*) Huh-7 cells were left untreated (Control) or treated with AICAR, A769662, or metformin at the indicated concentrations for 24 h before analysis of cell viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Error bars indicate mean ± SEM. (*D*) Distribution and abundance of NS5A or cellular lipids (BODIPY) was evaluated in Huh-7 cells transfected with in vitro transcripts of a transient luciferase subgenomic replicon (JFH-1: genotype 2a) after fixation and permeabilization. Cells were stained for lipid content with BODIPY dye for 1 h after NS5A labeling. Identical settings were maintained for image capturing. Representative confocal images are shown. Cells were left untreated (Control) or treated with AKTVIII for 4 h before processing. Replicon transfected cells are outlined in white. (Scale bars, 10 μm.)

1. Targett-Adams P, McLauchlan J (2005) Development and characterization of a transient-replication assay for the genotype 2a hepatitis C virus subgenomic replicon. *J Gen Virol* 86: 3075–3080.

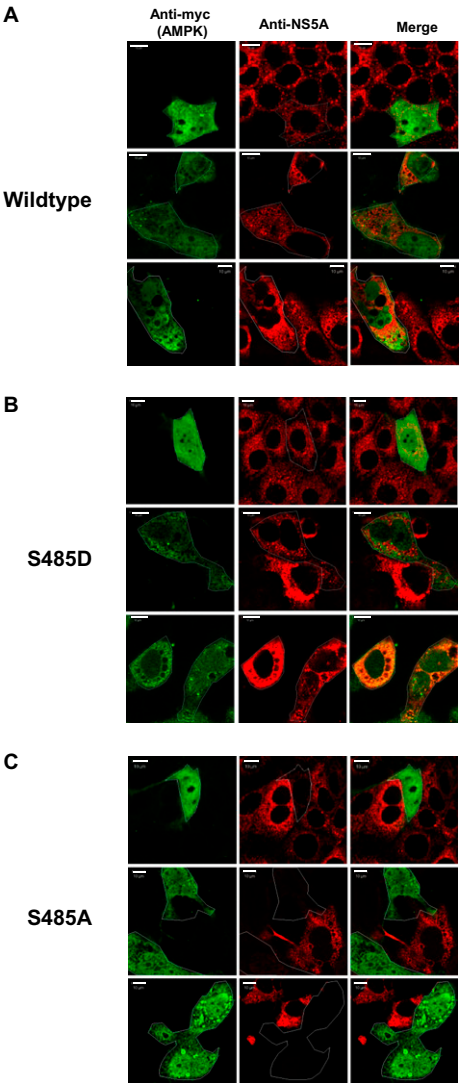

Fig. S3. Expression of an exogenous AMPK Ser485Ala mutant blocks HCV RNA replication. Huh-7 cells stably harboring an HCV genotype 1b culture-adapted subgenomic replicon (1) were transfected with the indicated Myc-tagged AMPKα constructs and analyzed by immunofluorescence 48 h posttransfection. Representative confocal images are shown. The upper row of images in *A–C* shows a wide-field image. (Scale bars, 10 μm.)

1. Krieger N, Lohmann V, Bartenschlager R (2001) Enhancement of hepatitis C virus RNA replication by cell culture-adaptive mutations. *J Virol* 75:4614–4624.

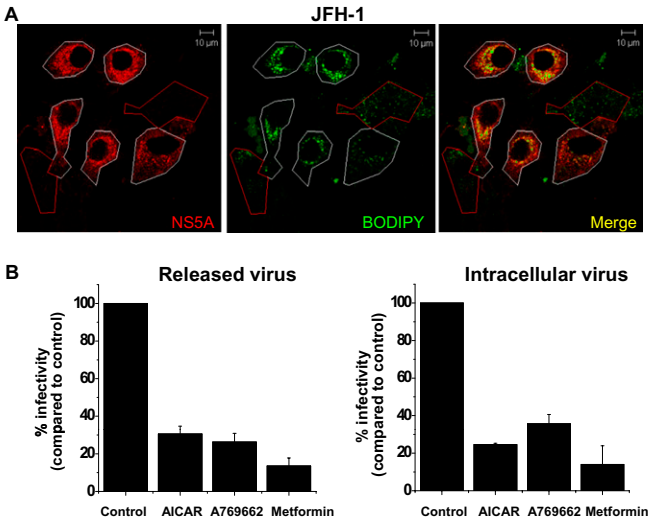

Fig. S4. Restoration of AMPK activity with AMPK agonists inhibits assembly and release of infectious genotype 2a (JFH-1) virus. (*A*) Additional images of Huh-7 cells transfected with full-length in vitro transcripts of JFH-1. Cells were stained for lipid content with BODIPY dye for 1 h after NS5A labeling. Identical settings were maintained for image capture. Representative confocal images are shown. JFH-1 transfected cells are outlined in white; untransfected cells in the same field are outlined in red. (Scale bars, 10 μm.) (*B*) Huh-7 cells were infected with JFH-1 virus at a multiplicity of infection of 0.5 focus-forming units per cell. At 24 h posttransfection, the indicated AMPK agonists were added. Virus in both supernatant and clarified cell lysates was titered after a further 24-h incubation (n = 3). All error bars indicate mean ± SEM.