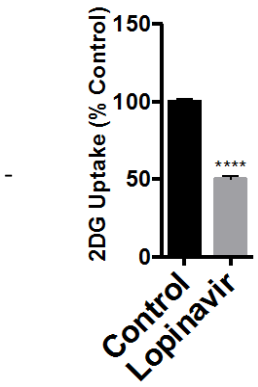
A



B

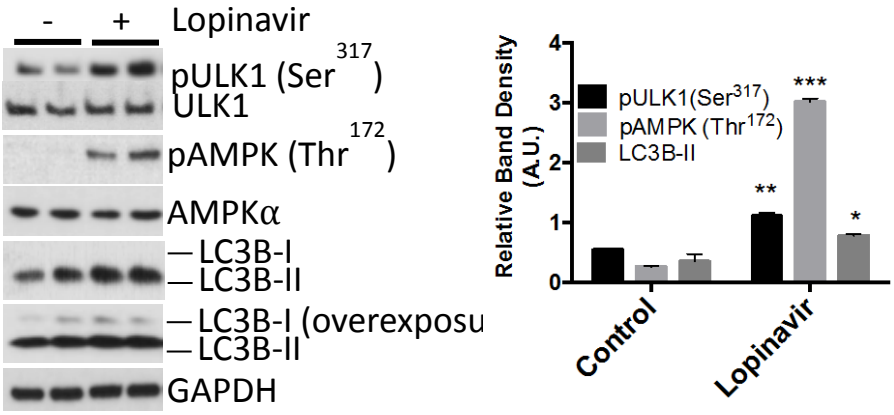


Fig. S1. The effect of the HIV protease inhibitor Lopinavir on 2-DG uptake, AMPK activation, and autophagic flux. (A) Uptake of 2-DG in primary hepatocytes exposed to DMSO (control) and Lopinavir (40 μM) for 5 minutes. 2-DG uptake was measured after 6 minutes incubation in radiolabel. Data are mean % control group uptake ± SEM of , N = 3 independent experiments, each with n = 3 replicates per experiment. p values calculated by 2-tailed T-test versus control. (B) Western blot analysis and quantification of the indicated proteins. Membrane with LC3B-I and LC3B-II is shown at two exposures to visualize LC3B-I. Primary hepatocytes were exposed to growth medium with DMSO (-) or growth medium with Lopanivir (40 μM) for 15 minutes. The amount of pULK1, pAMPK, and LC3B-II were normalized to total ULK1, AMPKα, and GAPDH, respectively. Data were quantified from 3 independent experiments and expressed as mean band density:loading control density ± SEM in arbitrary units (A. U.). p values were calculated by 2-tailed T-test versus control. (*, P< 0.05; **, P<0.01, ***, P<0.001 Lopinavir versus control samples of the same protein.

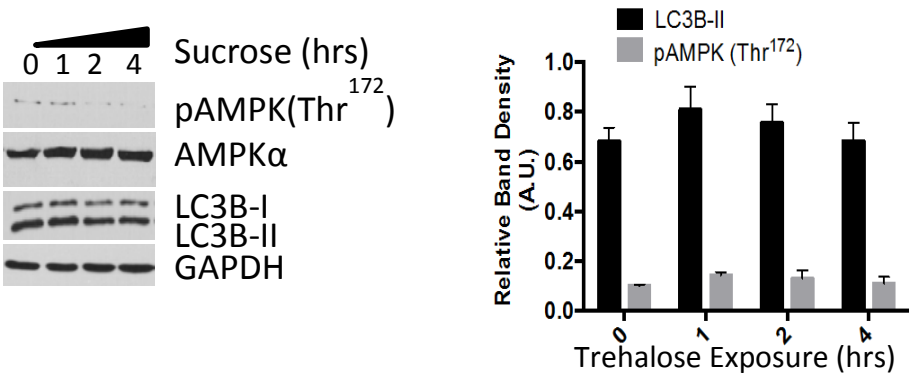


Fig. S2. The effect of sucrose on primary hepatocyte autophagy and AMPK signaling. Timecourse of AMPK phosphorylation in primary hepatocytes exposed to sucrose (100 mM). (B) The abundance of LC3B-II in primary hepatocytes exposed to sucrose (100 mM) for 1 - 4 hours. Cultures denoted as "0 hours" received regular growth medium and were analyzed four hours thereafter. The amount of pAMPK, and LC3B-II were normalized to AMPKa, and GAPDH, respectively. Data were quantified from 2 independent experiments, n = 2 replicates per group and expressed as mean band density:loading control density ± SEM in arbitrary units (A. U.). No significant differences between among LC3B-II:GAPDH density or among pAMPK(Thr^{172}):AMPKα densities were detected by one-way ANOVA.

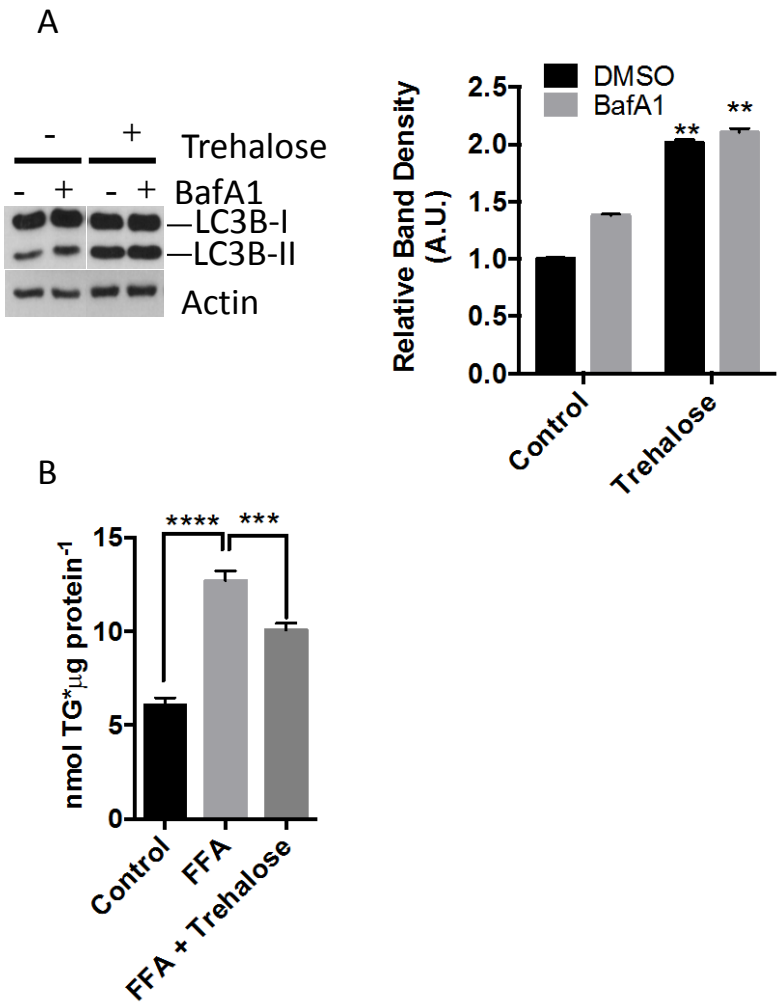


Fig. S3. The effect of trehalose on LC3B-II accumulation and triglyceride accumulation in HepG2 cells. (A) Western blot analysis and quantification of LC3B-II in HepG2 cells exposed to growth medium with or without trehalose (100 mM) in the presence of vehicle (DMSO) or Bafilomycin A1 (BafA1, 400 nM) for 1 hour. LC3B-II abundance was normalized to Actin. Data were quantified from 3 independent experiments and expressed as mean relative band density:actin density in arbitrary units (A.U.) ± SEM; P values were calculated by 2-tailed T-test versus growth media control. (**, P<0.01 versus growth media control). (B) Quantification of triglycerides (TG) in HepG2 cells exposed to growth medium with or without trehalose (100 mM) in the presence or absence of mixed bovine serum albumin-conjugated fatty acids (oleic, palmitic, stearic, linoleic acid in a 1:1:1:1 ratio; 500 μM). Data are quantified from 2 independent experiments, each run with n = 4-8 replicates per experiment and expressed as mean ± SEM in nmol triglyceride per μg protein; P values were calculated by one-way ANOVA with Sidak's post hoc test for multiple comparisons (***, P<0.001 and ****, P<0.0001 between the indicated samples).

Transcript Quantified	Forward Primer Reverse Primer	GLUT1	GLUT2	GLUT3	GLUT4	GLUT8
hGLUT1	AACTCTTCAGCCAGGGTCCAC CACAGTGAAGATGATGAAGAC	462327 ± 4566****	3725 ± 326	4145 ± 501	4527 ± 768	2288 ± 553
hGLUT2	TTTCAGGCCTGGTTCCTATG GATGGCCAGCTGATGAAAAG	5 ± 0	84758 ± 13107****	ND	7 ± 4	13 ± 10
hGLUT3	ACTTTGACGGACAAGGGAAATG ACCAGTGACAGCCAACAGG	288 ± 124	403 ± 54	1358605 ± 33835****	1223 ± 366	1269 ± 437
hGLUT4	CATGCTGGTCAACAATGTCC CCAATGAGGAATCGTCCAAG	155 ± 76	170 ± 49	268 ± 83	288864 ± 84501****	271 ± 57
hGLUT8	CCATCTTTGAAGAGGCCAAG ATGACCACACCTGACAAGACC	4096 ± 727	1208 ± 147	1967 ± 608	2761 ± 929	2083812 ± 86****

Table S1. Absolute mRNA copy numbers in the 293 GLUT expression system. Absolute quantification of GLUT1, GLUT2, GLUT3, GLUT4 and GLUT8 were measured by quantitative real-time RT-PCR in 293 cells stably expressing overexpresing GLUT1 (column labeled "GLUT1") or in 293 cells stably co-expressing both GLUT1 shRNA (to knock down endogenous GLUT1) and an overexpression vector encoding human GLUT2 (column labeled "GLUT2"), GLUT3 (column labeled "GLUT3"), GLUT4 (column labeled "GLUT4') or GLUT8 (column labeled "GLUT8"). Each row represents the transcript quantified by real-time RT-PCR. Values are expressed in mean copies*ng $cDNA^{-1}$ ± SEM for N = 3 independent cultures each read in triplicate. ****P < 0.0001 versus all other rows within a column by one-way ANOVA and Sidak's post-hoc testing for multiple comparisons. All primers are listed 5' to 3'.