Supporting Information

Wang et al. 10.1073/pnas.1103746108

SI Materials and Methods

Cell Culture. NIH 3T3 and HeLa cells were purchased from American Type Culture Collection. Primary WT and $SIN1^{-/-}$ MEFs were provided by B. Su (Yale University, New Haven, CT). HCT15 and SW620 colorectal carcinoma cells were provided by R. S. Warren (University of California, San Francisco, San Francisco, CA). HCC cells were provided by A. Goga (University of California, San Francisco, San Francisco, CA). NIH 3T3 cells were cultured in DMEM supplemented with 10% BCS and penicillin/streptomycin. WT and $SIN1^{-/-}$ MEFs and HCT15 and SW620 cells were cultured in DMEM containing 10% FBS, glutamine, and penicillin/streptomycin. HCC cells were cultured in Eagle minimal essential medium supplemented with 10% FBS and penicillin/streptomycin. HeLa cells were cultured in DMEM supplemented with 10% FBS and penicillin/streptomycin. For culture under low-sterol conditions, HeLa cells were grown in media containing 10% LPDS (Intracel) for 24 h, then treated with inhibitor for an additional 18 h and harvested.

Immunoblotting. Cells were treated with DMSO or inhibitor for the indicated lengths of time and then lysed in ice-cold lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, Roche protease inhibitor mixture, Roche phosphatase inhibitor mixture). For SREBP-2 immunoblot analysis, cells were treated with 25 μg/mL ALLN for 1 h before lysis. Lysates were cleared by centrifugation, protein concentrations were normalized by using Bradford reagent, and samples were resolved by SDS/PAGE, transferred to nitrocellulose, and immunoblotted. Immunoblots were quantified by using ImageJ software.

qRT-PCR. After DMSO or inhibitor treatment, total RNA was isolated from cells by using QIAshredder and RNeasy Mini kits (Qiagen). RNA (2–5 μg) was reverse-transcribed into cDNA by using SuperScript III Reverse Transcriptase (Invitrogen). Real-time PCR was performed on the StepOnePlus Real-Time PCR System (Applied Biosystems) using SYBR Green Master Mix (Applied Biosystems) and the following thermal cycles: 10 min at 95 °C, 42 cycles of 20 s at 95 °C, 30 s at 57 °C, and 30 s at 72 °C. The data were analyzed by the ΔΔCt method, and ribosomal RPL17 was used as an internal control for normalization of transfected NIH 3T3 cells whereas RPL3 was used as a control in all other instances.

Microarray Analysis. Sample preparation, labeling, and array hybridizations were performed according to standard protocols from the University of California, San Francisco, Shared Microarray Core Facilities and Agilent Technologies. Total RNA was isolated by using QIAshredder and RNeasy Mini kits (Qiagen). RNA was quantified by Nanodrop and integrity was assessed on an Agilent 2100 Bioanalyzer. RNA was amplified by using whole-transcriptome amplification kits (Sigma) following the manufacturer's protocol, and subsequent Cy3-CTP labeling was performed by using one-color labeling kits (NimbleGen). The size distribution and quantity of the amplified product was assessed by Nanodrop and Bioanalyzer, and equal amounts of Cy3-labeled target were hybridized to Agilent mouse whole-genome 4x44K Ink-jet arrays. Hybridizations were performed for 14 h, according to the manufacturer's protocol. Arrays were scanned by using the Agilent microarray scanner, and raw signal intensities were extracted with Feature Extraction software (version 10.1).

The dataset was normalized by using the quantile normalization method (1). No background subtraction was performed, and the median feature pixel intensity was used as the raw signal before normalization. A one-way ANOVA linear model was fit to the comparison to estimate the mean $\log_2$-based fold changes and calculate moderated t-statistic, B statistic, FDR, and *P* value for each gene for the comparison of interest. All procedures were carried out by using functions in the R package limma in Bioconductor (2, 3). Duplicate probes sets for the same gene were eliminated from the final gene list. Data were deposited in the National Center for Biotechnology Information Gene Expression Omnibus database under accession number GSE27784.

1. Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* 19:185–193.
2. Smyth GK (2004) Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 3:article3.
3. Gentleman RC, et al. (2004) Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biol* 5:R80.

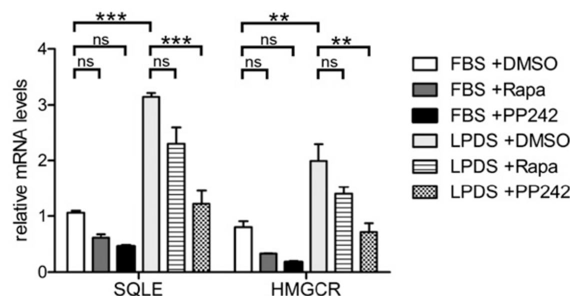

Fig. S1. mTOR inhibition down-regulates cholesterol biosynthetic gene expression similarly in an unstimulated and an LPDS-stimulated cell system. HeLa cells were cultured in media containing FBS or LPDS for 24 h, then treated with DMSO, 50 nM rapamycin, or 2 μM PP242 for 18 h. Cells were harvested, and mRNA levels were assessed by qRT-PCR (**$P < 0.01$; ***$P < 0.001$; ns, not significant).

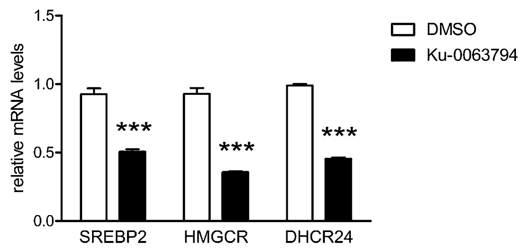

Fig. S2. ATP-site mTOR inhibitor Ku-0063794 inhibits cholesterol biosynthetic gene expression. Relative amounts of mRNA in NIH 3T3 cells after 18 h treatment with DMSO or 10 μM Ku-0063794 (***$P < 0.001$).

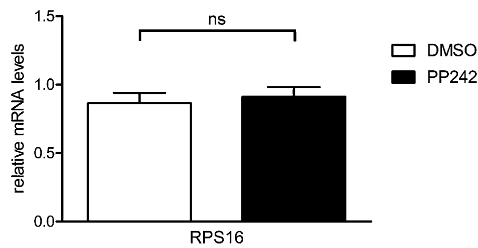

Fig. S3. PP242 does not affect *RPS16* gene expression. Relative amounts of mRNA in NIH 3T3 cells after treatment with DMSO or 2 μM PP242 for 18 h (ns, not significant).

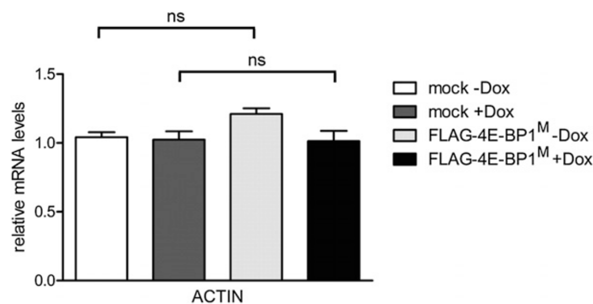

Fig. S4. Expression of FLAG-4E-BP1^{M} does not affect ACTIN mRNA levels. Relative mRNA levels in NIH 3T3 cells transfected with FLAG-4E-BP1^{M} as described in Fig. 2*C* (ns, not significant).

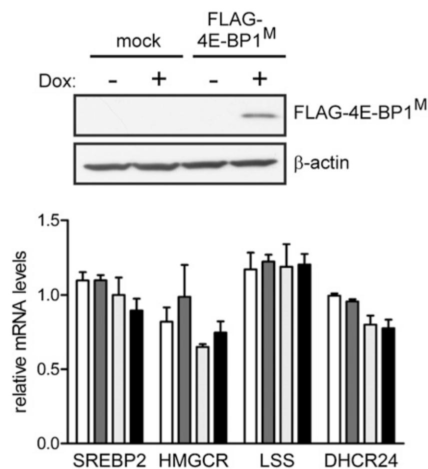

Fig. S5. Expression of FLAG-4E-BP1^{M} does not affect cholesterol biosynthetic gene expression in SW620 cells. SW620 cells were cotransfected with FLAG-4E-BP1^{M} and Tet activator expression constructs, and induced with 1 μg/mL doxycycline, and cell lysates were prepared for immunoblotting and RNA was harvested for cDNA synthesis and subsequent qRT-PCR analysis. Legend is the same as in Fig. S4. No differences observed by qRT-PCR were statistically significant.

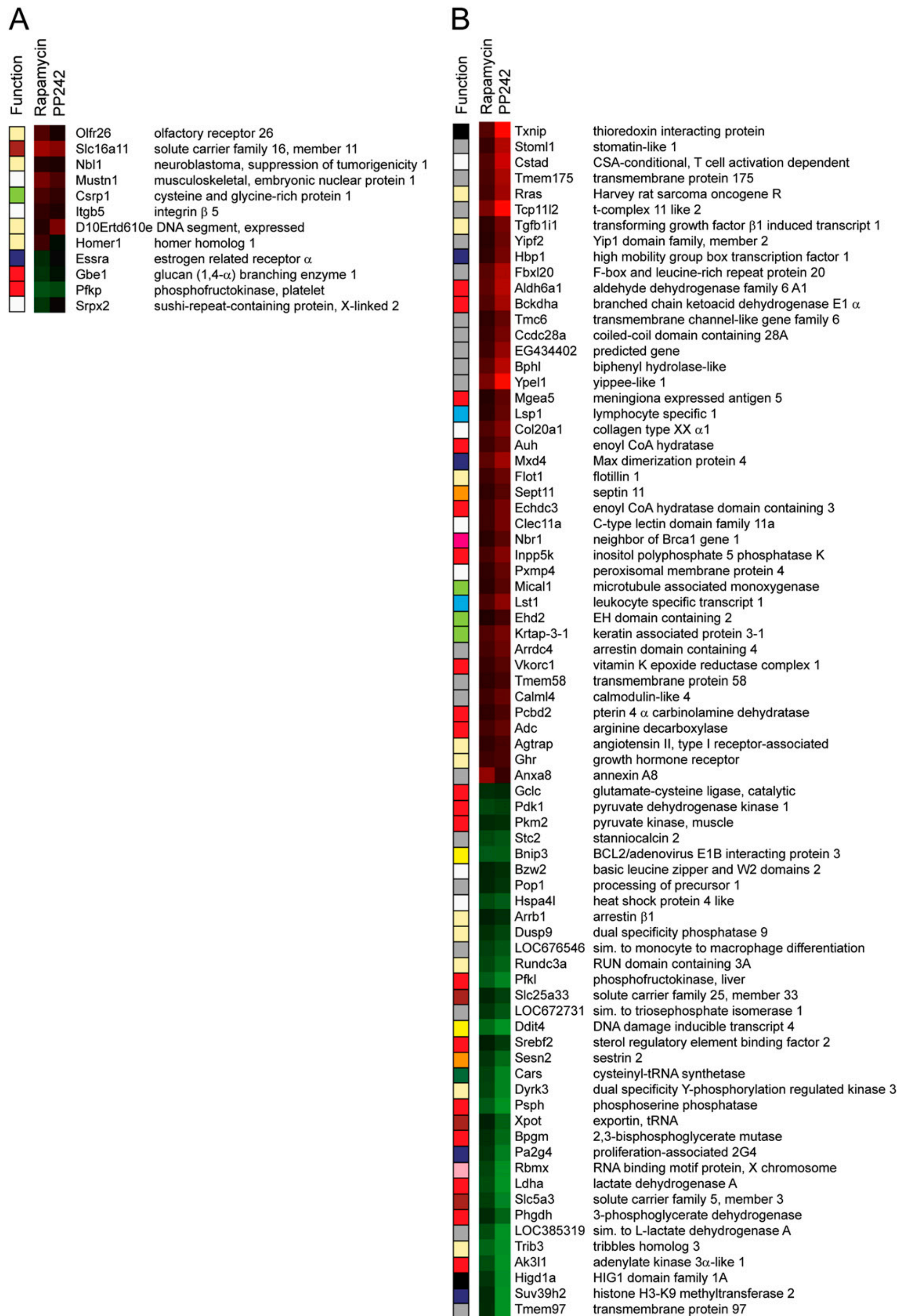

Fig. S6. Functional annotation of the 88 rapamycin-sensitive genes. (*A*) List and functional annotation of the 12 genes that are uniquely rapamycin-sensitive ($B > 0$). (*B*) List and functional annotation of the 76 genes that are differentially affected by rapamycin and PP242 ($B > 0$). Legend is the same as in Fig. 4*C*.

Table S1. Differential gene expression induced by PP242 in NIH 3T3 cells

Table S1

Table S2. Primers used for qRT-PCR analysis

Table S2