SUPPORTING INFORMATION

Table S1. Assays used for Real Rime RT-PCR.

Gene Symbol (Human)	Context Sequence	Assay ID	Vendor
TNFSF10	TGTCTTCTCCAAACTCCAAGAATGA	Hs00234356_m1	Life Technologies
CXCL10	CCACGTGTTGAGATCATTGCTACAA	Hs01124251_g1	Life Technologies
CHAC1	TGAAGATCATGAGGGCTGCACTTGG	Hs00225520_m1	Life Technologies
TGFB2	GCACAGCAGGGTCCTGAGCTTATAT	Hs00234244_m1	Life Technologies
AKR1D1	GTACCTACTCAGAACCTAAATCGAC	Hs00818881_m1	Life Technologies
ASNS	TACTGCTGCCCATGGTCTTGAACTG	Hs00370265_m1	Life Technologies
SLC22A9	GAAAAATGAGAGAAAAGACCCCAGA	Hs00971067_m1	Life Technologies
ULBP1	AGGATGGGTCGACACACACTGTCTT	Hs00360941_m1	Life Technologies
SLC17A1	TATTGCTCCCAGATATTTTGGATTT	Hs00192656_m1	Life Technologies
STC2	GATGCCCAGGGCAAGTCATTCATCA	Hs00175027_m1	Life Technologies
NRG4	GTGCCCACCACAGTCATGAACAACA	Hs00945535_m1	Life Technologies
SLC7A11	ATGCAGTGGCAGTGACCTTTTCTGA	Hs00921938_m1	Life Technologies
HIST1H2BM	AAAGGCTCCAAGAAGGCCATTAACA	Hs00605772_s1	Life Technologies
PCK2	CAAGTACAATAACTGCTGGCTGGCC	Hs00388934_m1	Life Technologies
FOXP2	ATCTCCCAAACCTCTAAATCTGGTG	Hs00475034_m1	Life Technologies
DDIT4	CGGAGGAAGACACGGCTTACCTGGA	Hs01111686_g1	Life Technologies
DGKK	TCCAATCTTTGTTCCAGAGGAAAAA	Hs01385661_m1	Life Technologies
CTH	GCTGAGCTTCCGGCAATCATGACTC	Hs00542284_m1	Life Technologies

Table S2. Antibodies used in Signaling Studies.

Target	Vendor	Catalog number
DDIT4/REDD1	proteintech	10638-1-AP
Phospho-mTOR (Ser396)	Cell Signaling	5536
mTOR total	Cell Signaling	2983
Phospho-4E-BP1 (Thr37/46)	Cell Signaling	2855
4E-BP1 total	Cell Signaling	9644
Phospho-p70 S6 Kinase (Thr389)	Cell Signaling	9234
p70 S6 Kinase	Cell Signaling	2708
Phosphor-FoxO1 (Ser256)	Cell Signaling	9461
FoxO1 total	Cell Signaling	2880
Phospho-FoxO3a (Ser318/321)	Cell Signaling	9465
FoxO3a total	Cell Signaling	2497
Phospho-AMPKa (Thr172)	Cell Signaling	2535
AMPKa total	Cell Signaling	2532
Phospho-PERK (Thr980)	Cell Signaling	3179
PERK total	Cell Signaling	3192
Actin	Santa Cruz	sc-1616
Stat1 p84/p91	Santa Cruz	sc-346
Phospho-eIF2a (Ser51)	Cell Signaling	3398
eIF2a total	Cell Signaling	5324
ATF-4	Cell Signaling	11815
Phospho-Acetyl-CoA Carboxylase (Ser79)	Cell Signaling	11818
Acetyl-CoA Carboxylase total	Acetyl-CoA Carboxylase	3662

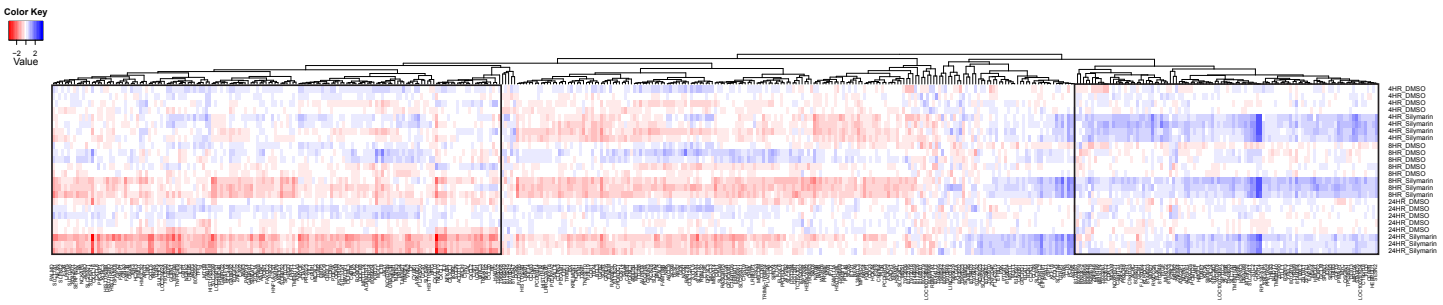

Figure S1. Global Transcriptional Profile of the Hepatocellular Response to Silymarin Treatment. Huh7.5.1 cells were treated with 80μM of silymarin or DMSO (vehicle control). Total cellular RNA was isolated at four, eight, and 24 h post-silymarin treatment. RNA was analyzed and processed in Affymetric arrays. Data represent genes whose expression profiles varied by more than $\log_2$ 1.5-fold change relative to the DMSO control. The results reflect four separate technical repeats.

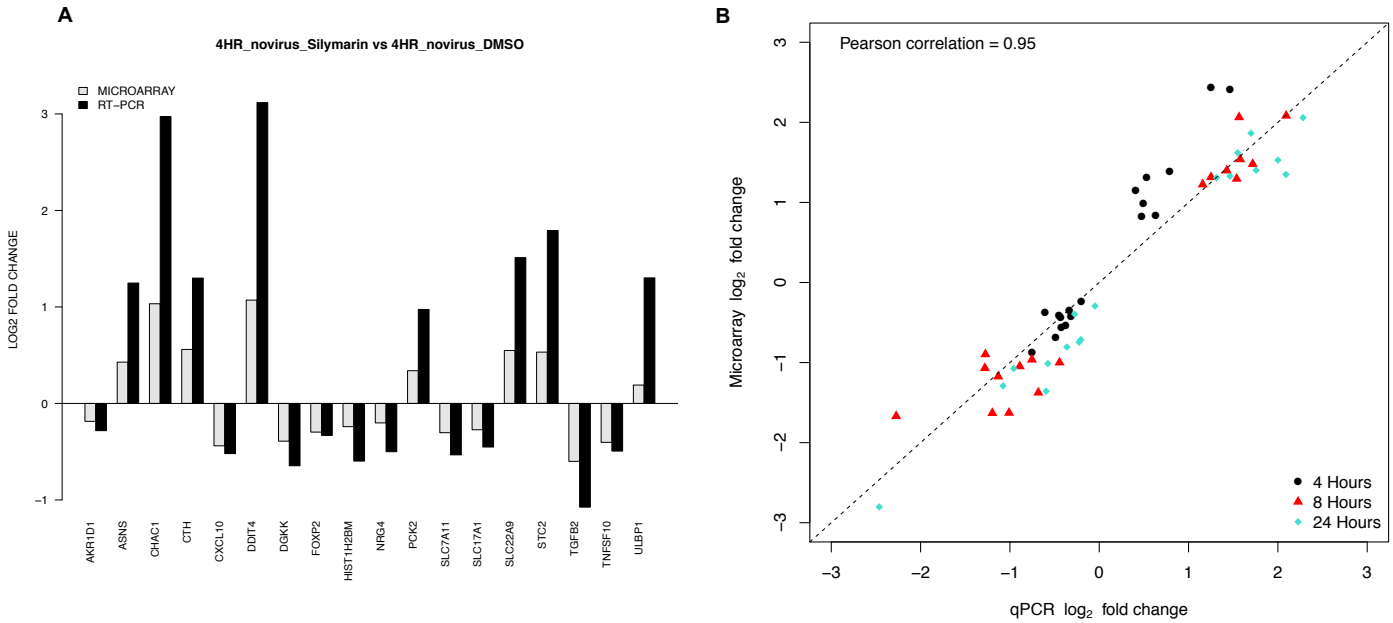

Figure S2. Microarray Gene Expression Validation by Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR). A, The log-fold change comparison of several of the most significantly up- and down-regulated genes with silymarin treatment at four h are shown between the microarray data (grey bars) and qRT-PCR validation experiments (black bars). B, The log-fold change of the microarray data and the qRT-PCR data for the three time points (i.e., four [black], eight [red], and 24 [light blue] h) were calculated to have a Pearson correlation of 0.95. Data from all 18 genes across all three time points are represented.

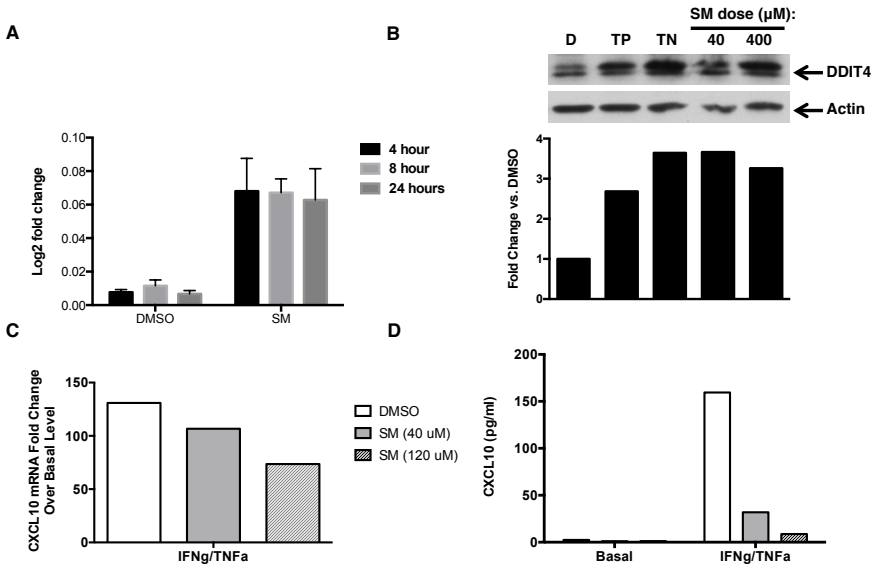

Figure S3. Validation of Silymarin-Induced Changes in DDIT4 and CXCL10 mRNA and Protein Expression. A, qRT-PCR for *DDIT4* mRNA on Huh7.5.1 cellular RNA and normalized to housekeeping gene expression as described in the Methods. B, *Above:* protein expression of DDIT4 versus Actin (loading control) in Huh7.5.1 cells at four h post-silymarin (SM) treatment (40 and 400 μM) in comparison to DMSO (D; vehicle control) and ER stress control treatments (Thapsigargin [TP; 100 nM] and Tunicamycin [TN; 10 mg/mL]). Blot is a representative of two independent experiments. *Below:* fold-change in protein band pixel intensity was compared between D versus ER stress control treatments or silymarin treatments. C, Silymarin inhibition of *CXCL10* represented as fold-change over basal *CXCL10* mRNA expression in Jurkat cells. D, CXCL10 protein expression (*p*g/mL) in both untreated and IFN-γ and TNF-α stimulated Jurkat cells that were incubated with DMSO (D) or silymarin (SM) treatment. The expression of *CXCL10* mRNA and protein was measured 24 h post-silymarin treatment by qRT-PCR or Luminex ELISA, respectively. *CXCL10* mRNA and protein expression data were performed in technical triplicates and the graph shown are representative of two independent experiments.

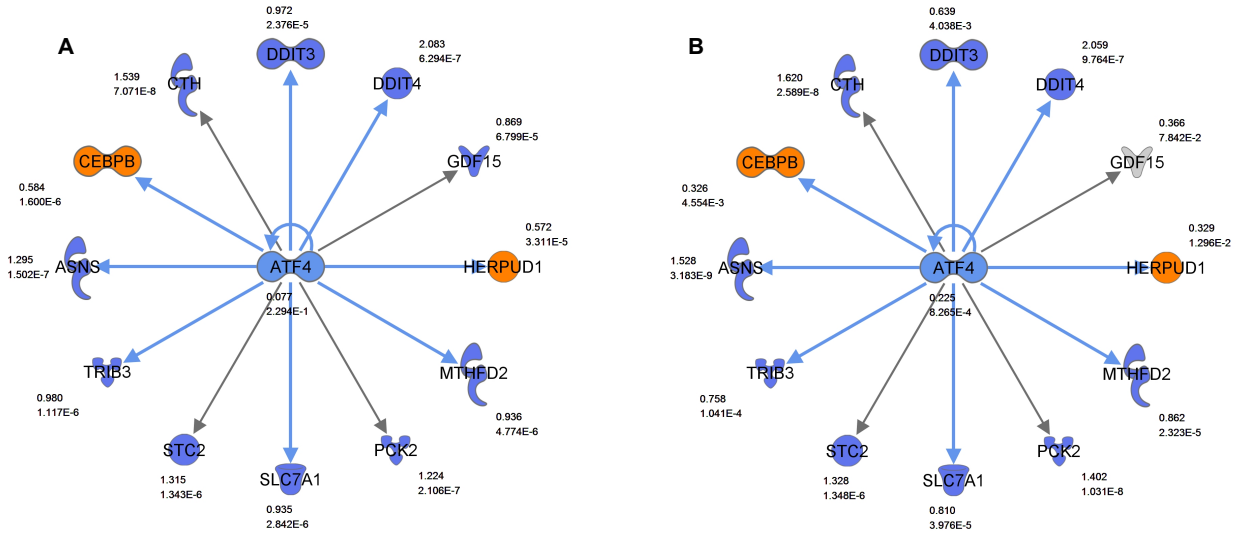

Figure S4. Silymarin Induces Endoplasmic Reticulum (ER) Stress via Activation of ATF4 Signaling. IPA results from eight (A) and 24-h (B) silymarin treatments of Huh7.5.1 cells. For legend describing shapes, colors, and lines of pinwheels, please see Figure S9, below.

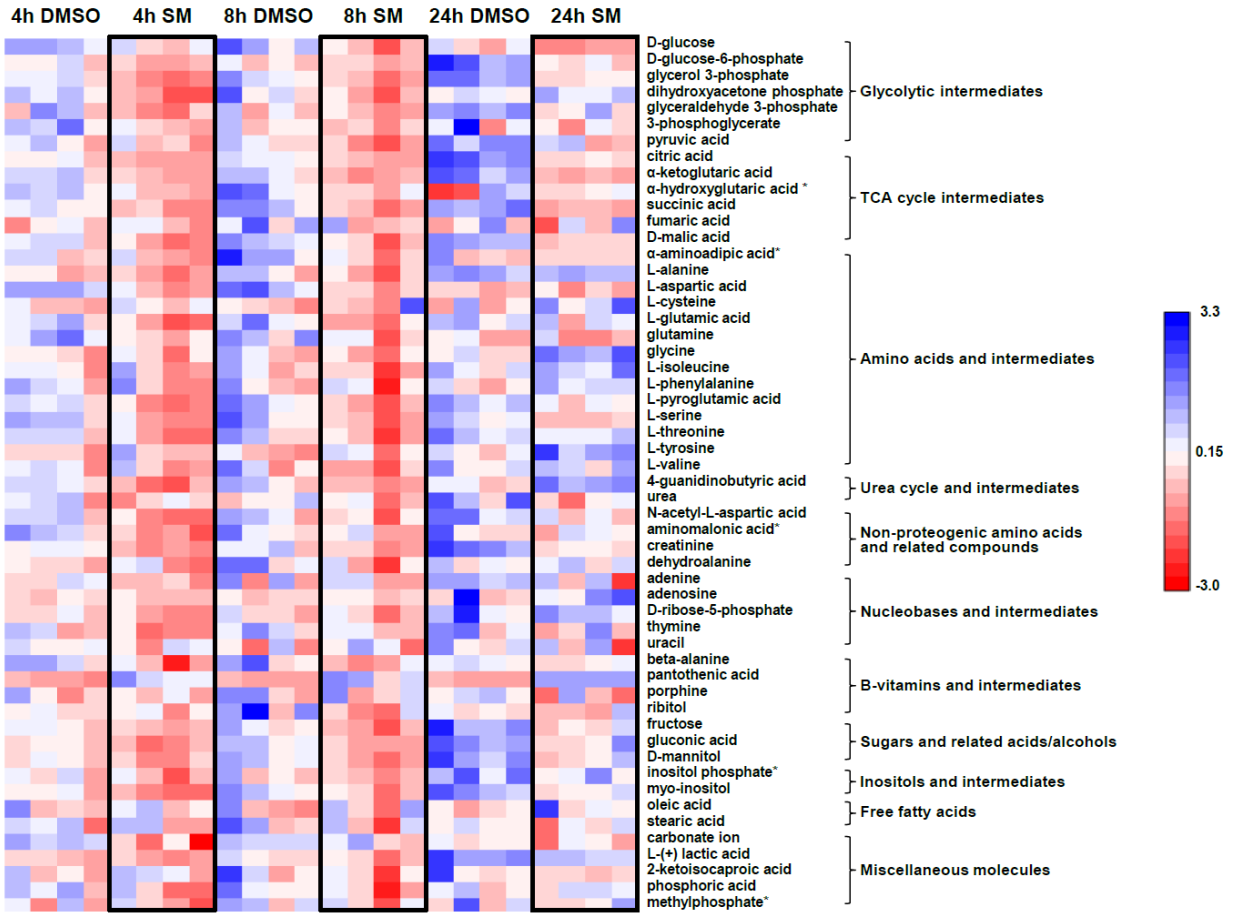

Figure S5. Silymarin Suppresses Cellular Metabolism. The abundances of 54 identified metabolites were z-score transformed to facilitate data visualization. Each row corresponds to a metabolite, and each column corresponds to a biological replicate. Two different treatment conditions (DMSO and silymarin) and three time points (four, eight and 24 h) were studied in quadruplicate. The scale bar indicates the z-score transformed abundance values of the metabolites. Asterisk (*) indicates metabolites identified using mass spectral information alone.

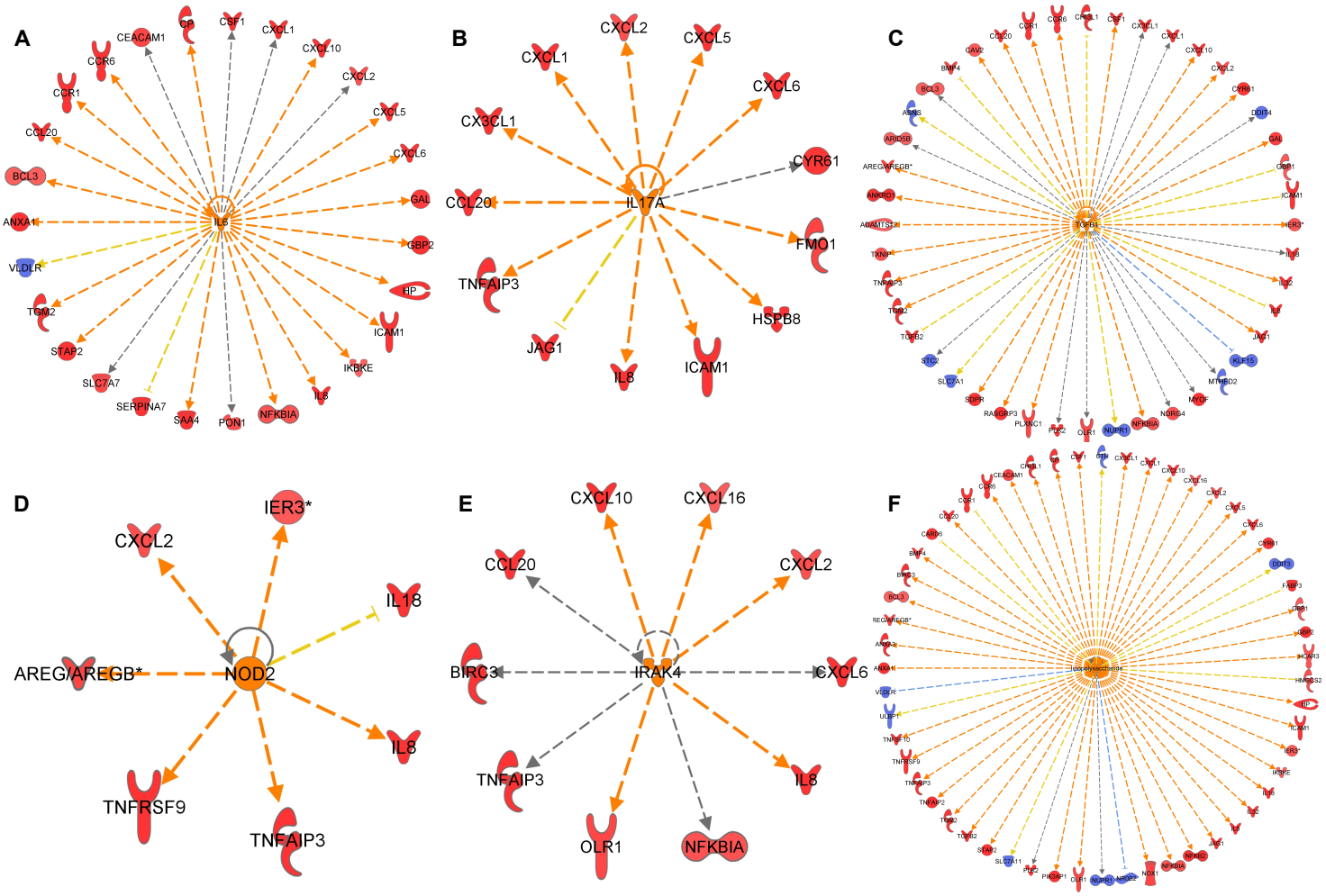

Figure S6. Silymarin Inhibits Multiple Upstream Regulators of Immune Responses. Silymarin treatment for 24 h in Huh7.5.1 cells resulted in the down-regulation of many inflammatory mRNAs. IPA predicted silymarin-induced changes in upstream regulators such as interleukin 6 (IL-6 (A)), interleukin 17-A (IL-17A (B)), transforming growth factor beta (TGFβ (C)), nucleotide-binding oligomerization domain containing 2 (NOD2 (D)), interleukin-1 receptor-associated kinase 4 (IRAK4 (E)), and the response to lipopolysaccharide (LPS (F)).

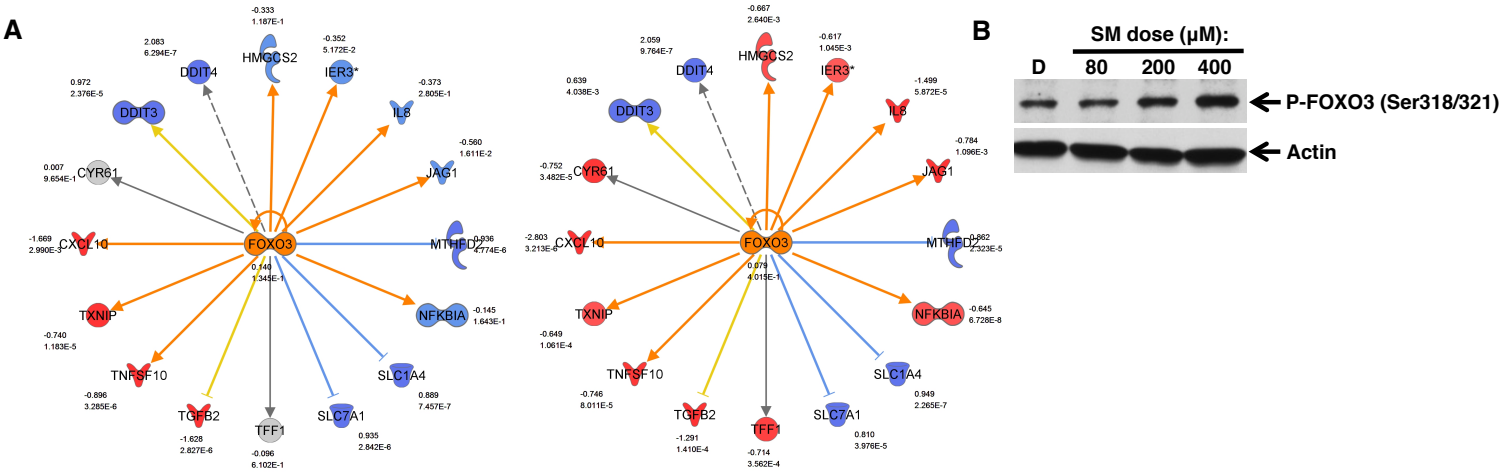

Figure S7. Silymarin Inhibits FOXO3 Activity. A, IPA software predicted FOXO3 as a key upstream regulator involved in silymarin-induced signaling as indicated by the significant modulation of FOXO3-regulated genes at eight (left) and 24 (right) h post silymarin-treatment. B, Jurkat T cells were serum-starved overnight before treatment with DMSO (D) or the indicated doses of silymarin (μM) for one h, followed by addition of FBS for 10 minutes. Whole cell extracts were probed for phosphorylated FOXO3 and Actin by Western blot.

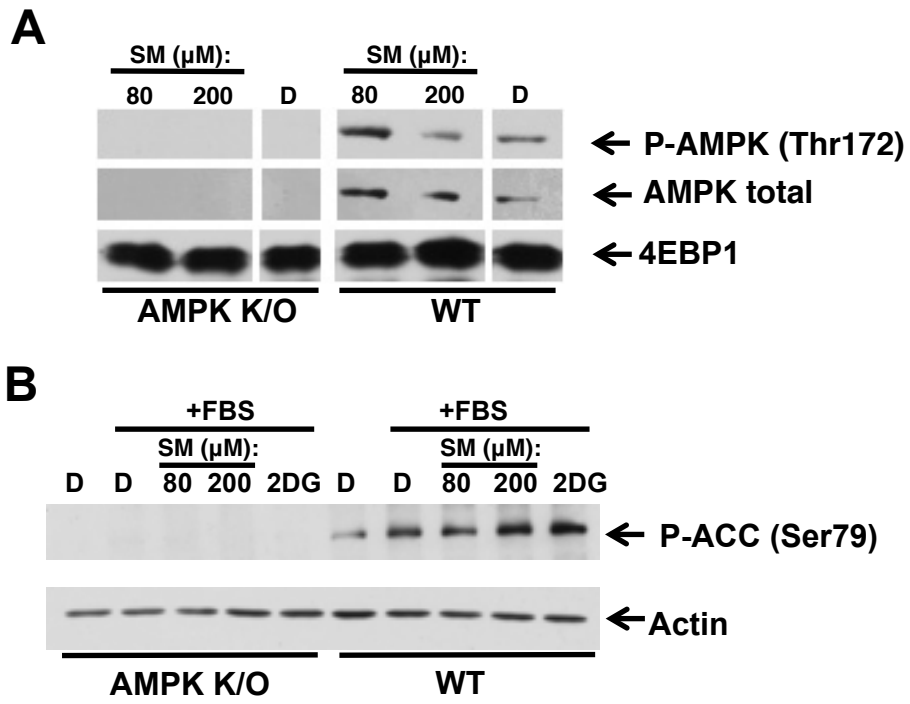

Figure S8. Silymarin Activates AMPK in Wild Type (WT) but not AMPK Knock Out (KO) MEFs. A, WT or AMPK KO MEFs were treated with DMSO (D) or silymarin (SM) at 80 or 200μM. Four h later, whole cell lysate was extracted and probed by Western blot. B, WT and AMPK KO MEFs were serum starved overnight followed by treatment with D, silymarin (at 80 or 200μM), or 50mM 2-deoxyglucose (2DG) in the presence of stimulation with 10% fetal bovine serum (FBS) for 10 minutes. Whole cell extracts were probed by Western blot for phosphorylated ACC, an AMPK target.

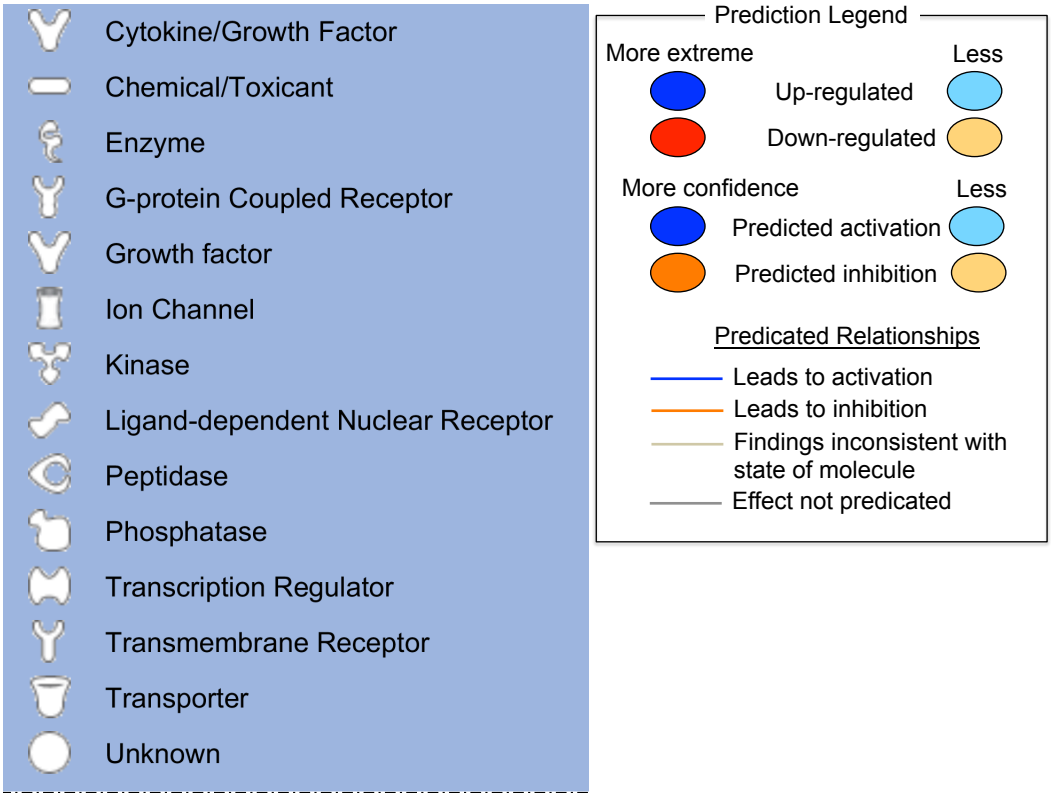

Figure S9. Legend for IPA Path Designer Upstream Regulator Figures. *Left:* the shapes of the molecules involved in the upstream regulator relationships are described. *Right*: the prediction legend for the relationships shows the color details between genes and regulators. Relationships between genes and regulators are designated by: arrowheads (i.e., pointed, activating; blunt, inhibitory relationships), lines (i.e., solid, direct; dotted, indirect interaction), and color (i.e., blue, activation; orange, inhibition; yellow, mixed activity; and gray, unknown association); all based on previously published data in the IPA Knowledge database. Colors of the upstream regulators, located in the middle of each pinwheel, are designated by color (i.e., darker, stronger association; blue, activating; orange, inhibitory).