Supplemental figure legends

Fig. S1. Treatment with Nam, but not TSA, increases acetylation levels of endogenous SREBP-1c in mouse liver extracts. TSA or Nam was included during preparing nuclear extracts and immunoprecipitation and acetylated SREBP-1c in mouse liver was detected by western analysis using acetyl-lys antibody. SREBP-1c levels were also detected using western analysis (bottom).

Fig. S2.Expression levels of flag-SREBP-1c wild type and mutants in Cos-1 cells used for in vitro acetylation assays. In vitro acetylation assays were done using flag-SREBP-1c wild type or mutants according to the Experimental Procedures. Briefly, Cos-1 cells were transfected with plasmids expressing flag-SREBP-1c wild type or mutants and flag-SREBP-1c proteins were isolated and utilized for in vitro acetylation assays. Protein levels of flag-SREBP-1c wild type, K309, or K289/309R double mutants in the input samples were detected by western analysis using M2 antibody. SREBP-1c and nonspecific (NS) band are indicated by an arrow and asterisk, respectively.

Fig. S3. SIRT1 decreases association of SREBP-1c at the *FAS* gene promoter. HepG2 cells were transfected with plasmids as indicated and association of SREBP-1c and RNA polymerase II with the *FAS* gene promoter was examined by ChIP assays. After semi-quantitative PCR, band intensities were quantified from 3 experiments using the Image J program and occupancy of SREBP-1c from the control group (cells expressing flag-SREBP-1c only) were set to 1. Statistical significance was determined by the Student's t test, * indicates $p < 0.05$ (n=3).

Fig. S4. P300 increases SREBP-1c transactivation activity on *FAS* promoter. (A) Mouse Hepa1c1c7 cells were cotransfected with 200 ng of *FAS*-luc, 50 ng of pcDNA3.1 SREBP-1c, and 100 ng of CMV-p300. Twenty-four hr after transfection, cells were harvested for reporter assays. The values for firefly luciferase activity were normalized by dividing by values for ß-galactosidase activity. The SEM was calculated from triplicate experiments.

Fig. S5. SIRT1 inhibits SREBP-1c transactivation activity on *FAS* promoter. (A) Mouse Hepa1c1c7 cells were infected with increasing amounts of either Ad-siSIRT1 or Ad-empty virus, and 48 hr later, cells were cotransfected with 200 ng of *FAS*-luc, 50 ng of pcDNA3.1 SREBP-1c, and 100 ng of CMV-p300. Twenty-four hr after transfection, cells were harvested for reporter assays. The values for firefly luciferase activity were normalized by dividing by values for ß-galactosidase activity. The SEM was calculated from triplicate experiments. (B) Cells were infected with increasing amounts of Ad-siSIRT1 or control Ad-empty and 2 days after infection, SIRT1 protein levels were detected by western analysis. Nonspecific (NS) bands are shown (lower panel).

Fig. S6. Occupancy of SREBP-1c at its own promoter (*SREBP-1c*) is severely impaired with K289/309R double mutant. HepG2 cells were transfected with SREBP-1c wild type and mutant plasmids as indicated and 24 hr later, cells were treated with insulin (100 nM) for 3 hr in the presence of 0.1 % BSA. Cells were collected for ChIP assays to detect association of RNA polymerase II at the *SREBP-1c* promoter. Consistent results were observed from two independent assays.

Fig. S7. Overepxression of acetylation-defective SREBP-1c mutants decreases expression of the *FAS* gene. Mouse Hepa1c1c7 cells were transfected with expression plasmids and 36 h later, cells were treated with insulin (100 nM) and glucose (25 mM) for 2 hr and cells were collected for q-RTPCR. The mRNA levels of *FAS* gene and control *36B4* gene were detected by q-RTPCR in parallel. The *FAS* mRNA levels from three samples were normalized to those of 36B4 and average values are plotted.

Supplemental Figures

Fig. S1

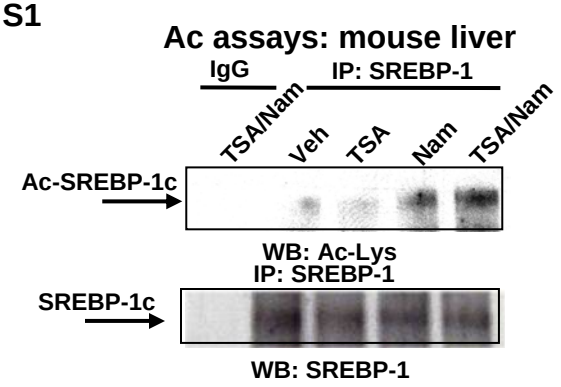


Fig. S2

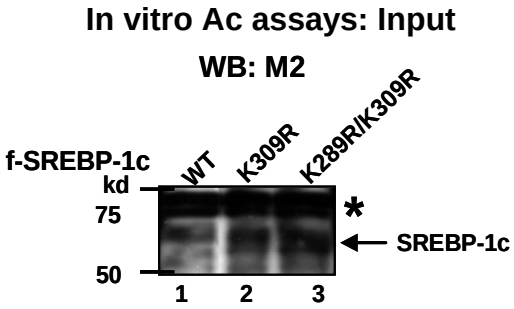


Fig. S3

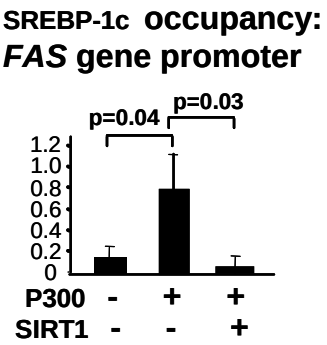


Fig. S4

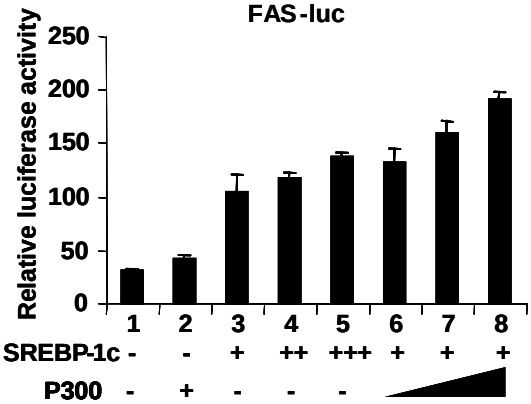
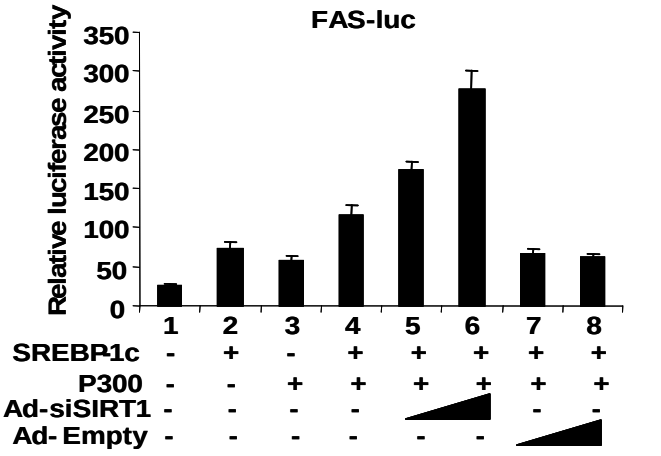


Fig. S5



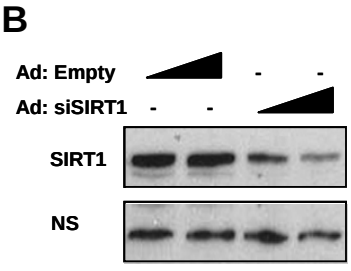


Fig. S6

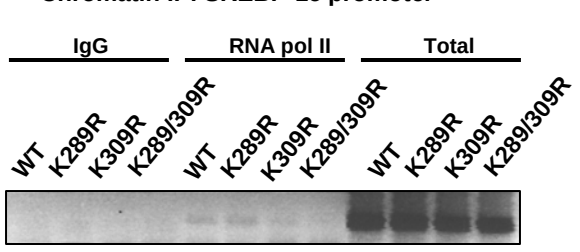


Fig. S7