Supplementary Material

Figure Legends

Supplementary Figure 1: **ACC1 protein levels and gene expression in quiescent fibroblasts.** Early passage human fibroblasts were incubated either in complete media (with 10 % FBS) (Ctl), made quiescent by incubating in media with 0.1% FBS for three days (Quies) or incubated in complete media with doxorubicin (Doxo) as described in figure 1. (A) ACC1 levels, ATM phosphorylation (p-ATM), p53 phosphorylation (p-p53), p53, p21 and pRb phosphorylation (p-pRB) were assessed by Western blot; tubulin was used as loading control. (B) Cell proliferation was measured by immunofluorescence as BrdU incorporation to nuclear DNA, original magnification X200. The percentage of cells with positive nuclear staining is shown below the picture. (C) The graph shows the data obtained after quantifying independent Western blots and normalizing ACC1 levels by tubulin. (D) ACC1 mRNA levels were determined. Results are expressed as the mean ± SEM ($n \geq 4$, *P<0.05, **P<0.005).

Supplementary Figure 2: **Inhibition of lipid synthesis induces senescence in human fibroblasts.** (A and B) Early passage fibroblasts were transduced with lentiviral particles harboring either a control plasmid or shRNA constructs directed against either ACC1 or FAS and selected with puromycin. Enzyme levels were assessed by Western blot four days after transduction. (C) SA-β-Gal staining in cells transduced with lentiviral particles carrying either a control plasmid, a shRNA sequence that does not target any known human gene (NT) or different shRNA constructs directed against either ACC1 or FAS. (D) Treatment with the FAS inhibitor

C75 demonstrating a concentration dependent inhibition of lipid synthesis. (E) Similar analysis using the ACC1 inhibitor TOFA. (F) Corresponding SA-β-Gal levels in cells treated with a vehicle control, C75 (50 μM) or TOFA (4.5 μM) for 5 days. (Results are expressed as the mean ± SE ($n \geq 3$, *P<0.05, **P<0.005, ***P<0.0005).

Supplementary Figure 3: N-Acetyl cysteine inhibits p38 MAPK and H2AX phosphorylation. Early passage human fibroblasts were transduced with lentiviral particles containing either a control or an ACC1 specific shRNA. Twenty-four hours after the infection the cells were switched to media with NAC (0.75 mM) or with the p38 inhibitor SB203580 (8 μM). (A) Five days after infection, p-p38 and p38 and ACC1 levels were assessed by Western blot. The bands corresponding to p-p38 MAPK and p38 MAPK were quantified, the ratio between them calculated and expressed relative to shACC1 (B) Five days after infection γ-H2AX were assessed by Western blot, the bands were quantified and expressed relative to γ-H2AX levels in shACC1 treated cells. Results are expressed as the mean ± SEM (n≥2, ***P<0.0005 Ctl vs. shACC1; #P≤0.05 shACC1+NAC vs. ACC1 or shACC1+SB vs. shACC1).

Supplementary Figure 4: N-Acetyl cysteine does not restore proliferation of cells lacking ACC1. Human fibroblasts were transduced with lentiviral particles carrying either a control plasmid or shRNA directed at ACC1 and selected with puromycin. Cells were incubated in absence and in presence of 0.75 mM N-acetylcysteine (NAC) as described in Figure 5C. (A) Cell proliferation was measured as [methyl ^{3}H]-thymidine incorporation into DNA per mg of protein and expressed relative to control values. (B) pRb phosphorylation (p-pRB) was assessed by Western blot, and GAPDH was used as loading control. Results are expressed as the mean ± SE (n=3, *P<0.05).

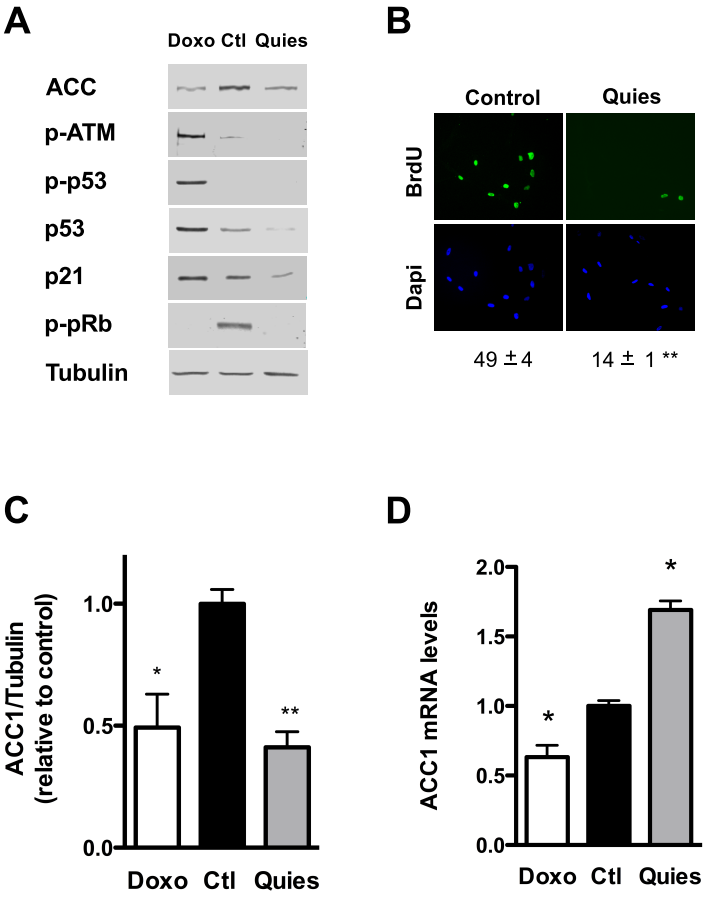

A
Doxo Ctl Quies
ACC
p-ATM
p-p53
p53
p21
p-pRb
Tubulin
B
Control
Quies
BrdU
Dapi
49 ± 4
14 ± 1 **
C
ACC1/Tubulin
(relative to control)
1.0
0.5
0.0
*
**
Doxo Ctl Quies
D
ACC1 mRNA levels
2.0
1.5
1.0
0.5
0.0
*
*
Doxo Ctl Quies

Figure S1

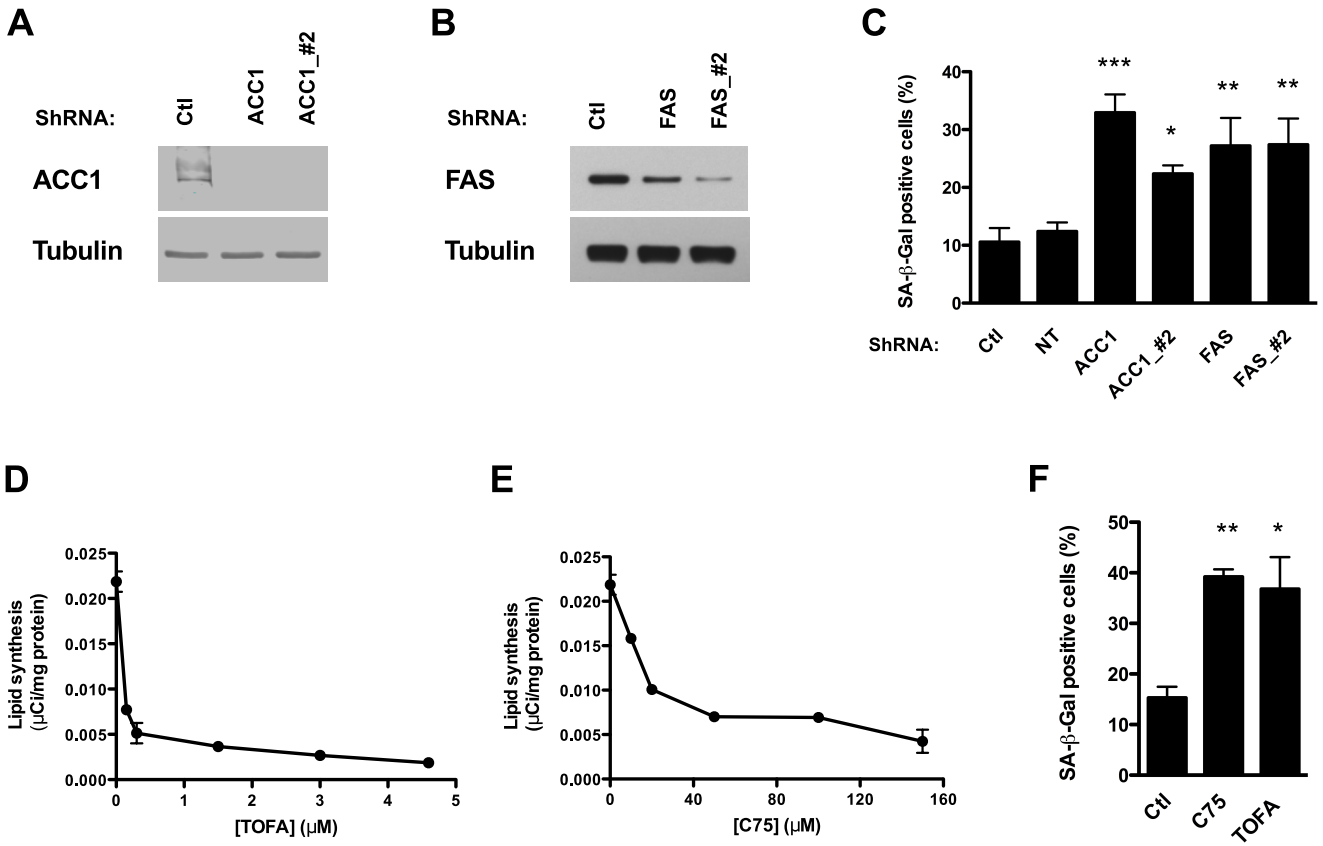

A
ShRNA:
Ctl
ACC1
ACC1_#2
ACC1
Tubulin
B
ShRNA:
Ctl
FAS
FAS_#2
FAS
Tubulin
C
SA-β-Gal positive cells (%)
ShRNA:
Ctl
NT
ACC1
ACC1_#2
FAS
FAS_#2
D
Lipid synthesis (µCi/mg protein)
[TOFA] (µM)
E
Lipid synthesis (µCi/mg protein)
[C75] (µM)
F
SA-β-Gal positive cells (%)
Ctl
C75
TOFA

Figure S2

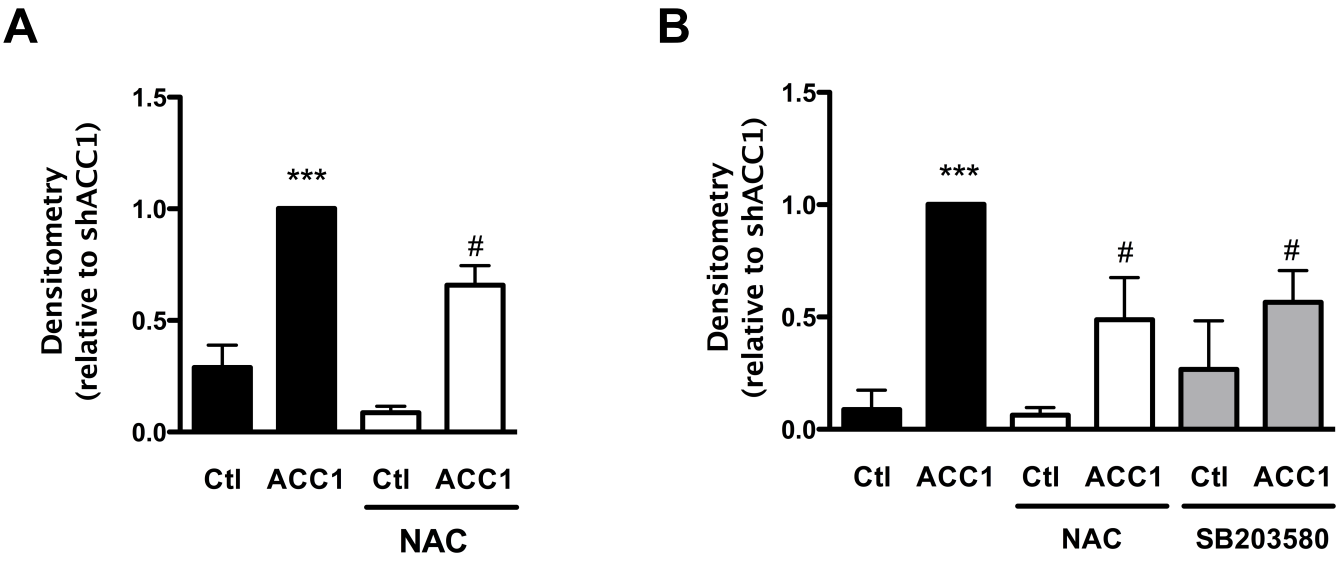
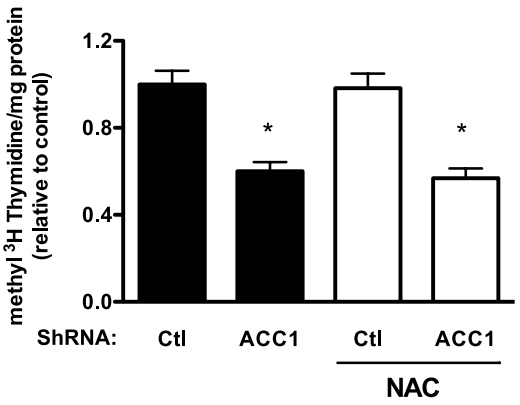

A
B
Densitometry
(relative to shACC1)
1.5
1.0
0.5
0.0
***
#
Ctl
ACC1
NAC
SB203580

Figure S3

A


methyl ^{3}H Thymidine/mg protein
(relative to control)
1.2
0.8
0.4
0.0
*
*
ShRNA:
Ctl
ACC1
Ctl
ACC1
NAC

B

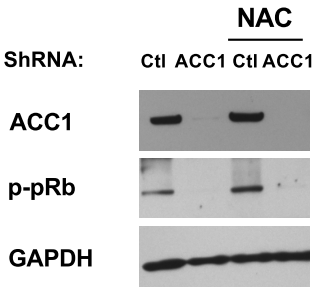

NAC
ShRNA:
Ctl ACC1 Ctl ACC1
ACC1
p-pRb
GAPDH

Figure S4