Supporting Information

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SI Materials and Methods

Cell Culture. Unless otherwise stated, cell culture reagents were purchased from Invitrogen. Paired isogenic clones of HCT116 p53+/+ and p53−/− cells have been described in refs. 1 and 2. The human cancer cell line HepG2 was obtained from ATCC. HCT116 and HepG2 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM glutamine, 10% heat-inactivated FCS (Gemini), 100 unit/mL penicillin, 100 μg/mL streptomycin, and 10 mM Hepes at 37 °C and 5% CO_2. IL-3-dependent cell lines derived from Bax−/−Bak−/− mice were maintained in the presence or absence of IL-3 as described in ref. 3.

RNA and Protein Analysis. RNA was extracted from samples in triplicate according to the TRIzol (Invitrogen) protocol. cDNA was synthesized from RNA using SuperScript II reverse transcriptase (Invitrogen). Quantitative PCR was performed with Taqman Gene Expression Assay products (Applied Biosystems) on a 7900HT Sequence Detection System (Applied Biosystems). Quantitative PCR data were normalized to actin and reported as the mean ± SD of triplicate samples.

Cells grown in tissue culture plates were rinsed with phosphate buffer saline (PBS) and lysed in RIPA buffer (20 mM Tris·HCl pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% Na-deoxycholate, 1 mM EDTA, 0.1% SDS, 1× Roche complete mini protease inhibitor mixture, and 1× Sigma phosphatase inhibitor mixture). Tumor samples were cut into small pieces and homogenized in ice-cold RIPA on ice for 1 min. The cell lysate and tumor homogenate were spun (14,000 rpm, 5 min, 4 °C) and the supernatant was saved for protein quantification. Protein concentration was determined by the bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific). Equivalent amounts of total protein were loaded onto 3–8% Tris-Acetate or 4–12% Bis-Tris gels (Invitrogen) and Western blot analysis was performed as described in ref. 4. The following primary antibodies were used: anti-ChREBP antibody (Novus Biologicals), anti-Stat-3 antibody (Cell Signaling), anti-α-tubulin antibody (Sigma), anti-phospho-p53 (Ser-15) antibody (Cell Signaling), anti-p53 antibody (Calbiochem), anti-p21 antibody (Santa Cruz Biotechnology), and anti-TIGAR antibody (Abcam).

Analysis of Cellular ROS and GSH and NAC Treatment. 48 h after transfection, cellular ROS levels were measured by incubating HCT116 cells transfected with either the control or ChREBP2 siRNA with 2 μM CM-DCFDA (Molecular Probes) for 30 min, followed by flow cytometry using FACSCalibur flow cytometer (BD Biosciences). The GSH-Glo Glutathione Assay kit (Promega) was used to measure cellular glutathione level according to the manufacturer's protocol. Four hours after transfection with either the control or ChREBP2 siRNA, HCT116 cells were treated with either 0 or 10 mM NAC (Sigma) for 72 h before FACS and Western blot analysis.

Statistical Analysis. Error bars represent standard deviations obtained from experiments performed in triplicate. *P* values were calculated by paired, two-tailed Student's *t* test.

1. Bunz F, et al. (1999) Disruption of p53 in human cancer cells alters the responses to therapeutic agents. *J Clin Invest* 104:263–269.
2. Zhang L, Yu J, Park BH, Kinzler KW, Vogelstein B (2000) Role of BAX in the apoptotic response to anticancer agents. *Science* 290:989–992.
3. Lum JJ, et al. (2005) Growth factor regulation of autophagy and cell survival in the absence of apoptosis. *Cell* 120:237–248.
4. Hatzivassiliou G, et al. (2005) ATP citrate lyase inhibition can suppress tumor cell growth. *Cancer Cell* 8:311–321.

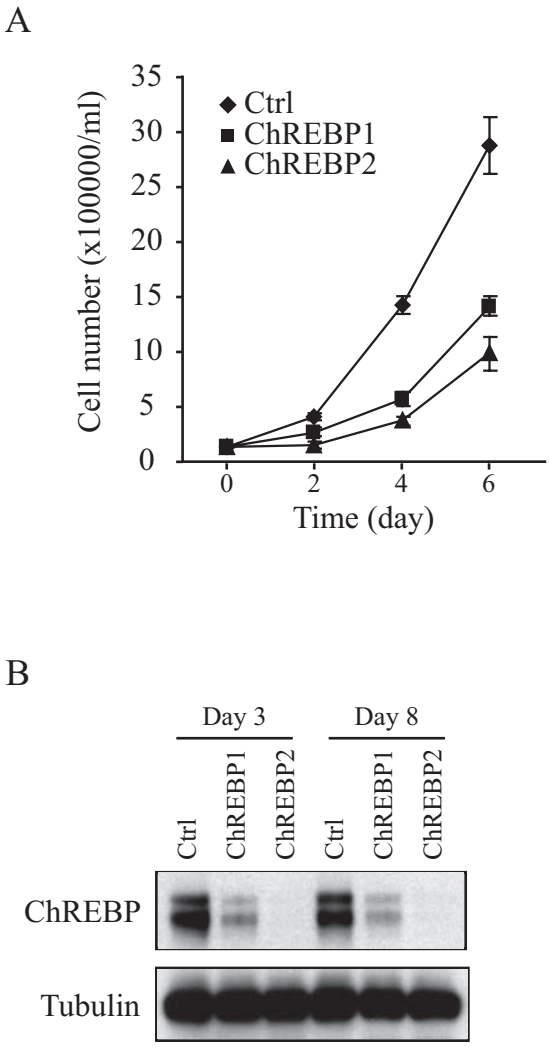

Fig. S1. Suppression of ChREBP by siRNA inhibits cell proliferation in HepG2 cells. (*A*) Cell proliferation of HepG2 cells transfected with control (Ctrl) and ChREBP (ChREBP1 and 2) siRNA. Data are presented as the mean ± SD of triplicate samples. (*B*) Western blot analysis of protein extracts of HepG2 cells transiently transfected with siRNA for control and ChREBP using antibodies to ChREBP and tubulin at days 3 and 8 posttransfection. Data are representative of at least three experiments.

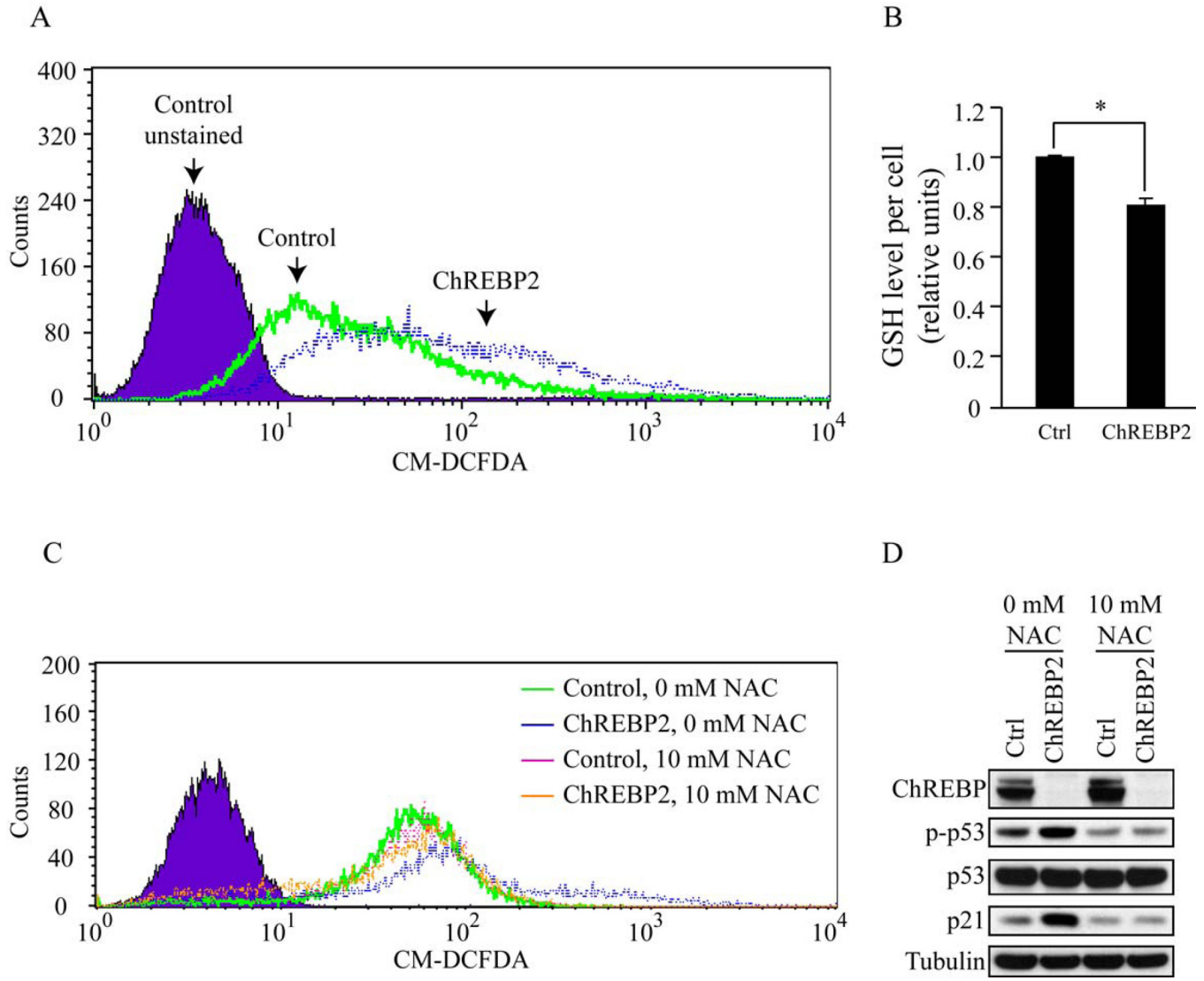

Fig. S2. Increased ROS generation and p53 activation in ChREBP-deficient cells is reduced by the antioxidant NAC in HCT116 cells. (*A*) Increased ROS level in ChREBP2 siRNA-transfected cells compared with control cells detected by flow cytometry using CM-DCFDA at day 2 post-transfection. The histogram is representative of three experiments. (*B*) Reduced cellular GSH level in ChREBP2 siRNA-transfected cells compared with control cells at day 3 post-transfection. Data are normalized by cell number and presented as the mean ± SD of triplicate samples. *, $P < 0.01$. (*C*) FACS analysis of CM-DCFCA-stained cells treated with 0 or 10 mM NAC for 72 h from 4 h after transfection with control or ChREBP2 siRNA. The histogram is representative of three experiments. (*D*) Western blot analysis of cells treated with 0 or 10 mM NAC for 72 h from 4 h after transfection with control or ChREBP2 siRNA. Data are representative of at least three experiments.