Supplemental Material and Methods

Establish gene silenced HCC cell lines. NRF2-silenced HCC cell lines were established by transduction of NRF2 lentiviral particles produced by employing co-transfection of pLKO.1 shRNA and packaging vectors in HEK 293FT cells. The sh*NRF2* constructs targeted 5'-guggcugcucagaauugcaga-3' (sh*NRF2*-1) and 5'-guaagaagccagauguuaaga-3' (sh*NRF2*-2). The shRNA control (shCtl) construct targets 5'-ccuaagguuaagucgcccucg-3'. After shRNA transduction, HCC cell lines were selected with 1.5 μg/ml puromycin until analysis.

Mouse chemical compound treatment. Mice were induced with DEN at 14 days of age and treated with chemical compounds Brusatol (2 mg/kg), ML385 (30 mg/kg) or vehicle DMSO via intraperitoneal injection three times a week (Monday, Wednesday, Friday) for 4 weeks starting at 32-week post-DEN treatment of age. At the end of treatment at 36-week of age, mouse sera were collected for ALT and AST assay and liver tumors were collected for RNA and protein assay, acetyl CoA level determination.

Luciferase reporter cell lines. A pGreenFire1 plasmid that expressing destabilized copGFP reporter and firefly luciferase was purchased from System Biosciences and kindly provided by Dr. Hasan Korkaya (Georgia Cancer Center, Augusta University). Lentiviral production was performed in 293FT cells by co-transfection of the pGreenfire1 plasmid with a three-plasmid packaging system (Gag-Pol, Rev and VSV-G) using Lipofectamine 2000 (Thermo Fisher). shCtl and sh*NRF2* HCC cells were transduced with viral particles, and single colonies with GFP expression were screened for luciferase activity by measurement of bioluminescence intensity using D-Luciferin (Gold Biotechnology, St Louis MO) as substrate.

Long-term clonogenic assays. 2 x 10^4 HepG2, HuH7, Hep3B, and SNU449 cells were cultured in a 6-cm plate with medium changed twice a week for 12 (HuH7, Hep3B and SNU449) or 21 (HepG2) days. Cells were then fixed with 4% formaldehyde in PBS and stained with 0.5% crystal violet (Sigma). Images were captured using an ImageQuant800 biomolecular imager (Amersham, Marlborough, MA).

Ectopic protein expression and immunoprecipitation. Plasmids FLAG-Nrf2 (#159129) and HA-CBP (#16701) were obtained from Addgene (Watertown, MA). Ectopic expression of the tagged NRF2 and CBP protein was achieved after transfection of Hepa1-6 (DT81 subline) with lipofectamine 2000. Protein-protein interactions were determined by immunoprecipitation with antibody against FLAG (F3165, Sigma) followed by immunoblot analysis with antibody against HA (H9658, Sigma), and vice versa.

Quantitative RT-PCR analysis. Total RNA was extracted from mouse liver tumors and HCC cells with Trizol (Invitrogen). cDNA was synthesized from 500 ng RNA using MMLV reverse transcriptase (Promega), and quantitative real-time PCR (qPCR) was performed using SYBR Green Supermix (Biorad) with gene-specific primers (Supplementary Table 1). Mouse or human 18s rRNA was used as an internal control.

Histology, immunohistochemistry and image analysis. Tissues were either fixed in 10% neutral buffered formalin for at least a week, dehydrated, and embedded in paraffin or embedded in Tissue Tek OCT. compound and snap-frozen in liquid nitrogen, then kept at -80°C until examination. Tissue sections of 5 μm thickness were subjected to hematoxylin and eosin (H&E) staining and subsequent assays. Pathological characterization was assessed by a clinic pathologist. Frozen liver sections (5 μm) were stained with an Oil Red O staining kit (IHC World) to detect lipid accumulation. Fibrosis was assessed using FFPE sections with a Picro-Sirius red staining kit (IHC World). Oil Red O and Sirius Red positive areas were quantified in more than 6 random fields on each slide using Image J software (NIH).

For immunohistochemistry, FFPE sections were de-paraffinized, rehydrated, and antigen retrieval by boiling. After blocking in 5% BSA at room temperature for 1 hour, tissues were incubated with primary antibody overnight at 4°C with anti-Nrf2 (ab62352, Abcam, with a 1:300 dilution), anti-AcH3K27 (ab4729, Abcam, with a 1:500 dilution) or Ki67 (RM-9106-S0, Epredia, with a 1:250 dilution) followed by incubation with either Biotin-conjugated secondary antibody (Thermo Fisher Scientific) for 1 h, Extravidin-Alkaline Phosphatase (E2636, Sigma) for 30 min at room temperature and developed with FAST Red (Vector Laboratories), or with ImmPRESS® Excel Amplified Polymer Staining Kit (Vector Laboratories). Tissue sections were counterstained with Hematoxylin QS (Vector Laboratories). Expression levels were scored based on IHC staining intensity and area of tumor cells. All slides were examined employing an all-in-one fluorescence microscope (Keyence) and the staining intensity of immunoreactivity was evaluated by two independent observers. Positive immunostaining of NRF2 was detected both in the nucleus and in the cytoplasm, while positive immunostaining of AcH3K27 was detected in the nucleus. Ten fields were randomly selected at a magnification of x400 from each slide. In terms of the final IHC staining score, each section was calculated according to the intensity and percentage of cells with positive staining.

Immunofluorescence and microscopy. Cells cultured on glass coverslips were fixed with 4% PFA and permeabilized with 0.2% Triton-X-100. Primary Nrf2 antibody (ab62352, Abcam) incubation was performed overnight in blocking buffer (5% BSA in PBS) at 4 °C. The following day, cells were washed twice in PBS, then incubated 1 h in secondary antibody in 5% BSA in PBS at room temperature. After air dried, slides were mounted with fluorescence mounting medium (Vector Laboratories) containing DAPI.

ATP and ADP assay. Liver tumors were homogenized in ice-cold PBS and the supernatant was collected after centrifugation. The levels of ATP and ADP in the supernatant were measured using a firefly luciferase-based ATP Assay System Bioluminescence Detection Kit (Promega) and ADP-Glo™ Kinase Assay (Promega). The ATP/ADP concentration was normalized with total protein.

Liver enzymes. Mouse plasma ALT and AST activities were measured using Alanine Transaminase Colorimetric Activity Assay Kit (700260, Cayman Chemical) and Aspartate Aminotransferase Colorimetric Activity Assay Kit (701640, Cayman Chemical).

Hepatic triglycerides. Triglycerides in mouse liver tumors were determined using a Triglyceride Assay kit (Cayman Chemical) following the manufacturer's instructions with the levels of triglycerides calculated after normalized to sample weight.

Glucose measurement. Glucose in blood. Tail blood was collected for mice in the random-fed condition at 09:00 -10:00 am and glucose levels were determined using a Onetouch Ultra2 glucose monitor (LifeScan Inc., Milpitas CA).

Glucose in the liver tumor. Mouse normal liver tissues or liver tumors were homogenized in deionized water, heated at 70 ^{o}C for 20 min, and centrifuged at 10,000 rpm for 10 min at room temperature. Glucose content in the supernatant was measured using a Glucose (GO) Assay Kit (Sigma) and the level of glucose was calculated after normalized to the sample weight.

Liver glycogen content. Mouse liver tumor was homogenized in 0.4 ml of 30% (m/v) KOH and boiled for 15 min. After centrifugation at 6,000 rpm, 200 µl of supernatant was spotted onto a Whatman filter paper disc, washed twice with 70% (v/v) ethanol and once with acetone. Glycogen was hydrolyzed to glucose by adding 1 ml of amyloglucosidase (A9913, Sigma) (0.2 mg/ml in 50 mM sodium acetate, pH 4.8) to the dried disc followed by incubation at 55°C for 1 h. Glucose concentration was measured using Glucose (GO) Assay Kit (GAGO20, Sigma) and glycogen content was calculated to standards (G1765, Sigma) after normalized to the sample weight.

NAD+/NADH levels. Mouse liver tumor tissue was washed with ice-cold PBS and homogenized in NAD or NADH extraction buffer before assayed with an NAD+/NADH assay kit (BioAssay Systems) according to the manufacturer's instructions.