Supplemental Materials

Mice. Mdr2−/− mice were a gift from D. Schuppan (Beth Israel Deaconess Medical Center, Boston, MA). 10 Mdr2−/− mice (age 51-59 weeks of age) were assigned to treatment with either Vehicle (DMSO, n=5) or 40mg/kg GDC-0449 (n=5) and treated for 9 days as previously described (1).

Immunohistochemistry. Liver specimens fixed in formalin and embedded in paraffin were cut into 4μm sections, dewaxed, hydrated, subsequently incubated in 3% hydrogen peroxide/methanol for 15 min to block endogenous peroxidase. To evaluate tissue architecture, slides were stained with hematoxylin and eosin (H&E) per standard protocol. Antigen retrieval was performed by heating in 10mM sodium citrate buffer or 0.25% pepsin (K19; Invitrogen, Carlsbad, CA) for 10 minutes. Sections were blocked (Dako Envision, Carpinteria, CA) and incubated with primary antibodies overnight at 4°C: Sonic Hedgehog (Epitomics 1843-1; 1:6000), Pyruvate kinase M2 (Cell Signaling; 1:1000), Glioblastoma-2 (Genway 18-732-292462; 1:2000); α smooth muscle actin (Dako; 1:800) (Dako, 1:1000); Mct4 (Santa Cruz; 1:1000), Indian hedgehog (Abcam; 1:750); Polymer-HRP anti-rabbit (K4003; Dako) or anti-mouse (K40011; Dako) were used as secondary antibodies. 3,3′-Diaminobenzidine (DAB) Substrate Chromogen System (K3466; Dako) and/or Vino Green (Biocare) was employed in the detection procedure. Omitting primary antibodies from the reactions eliminated staining which demonstrated staining specificity. Images were acquired on an Olympus IX71 (Tokyo, Japan) inverted microscope using the DP2-BSW (Olympus) image acquisition software system.

Quantitative Immunohistochemical Analysis. Formalin-fixed human and mouse tumor sections were stained for GLI2 and costained for GLI2 and PKM2. A minimum of 10 randomly
selected 200x field were evaluated for each mouse by counting the number of cells positive for GLI2 only and co-labeled for both GLI2 and PKM2.

**Quantitative Real-Time Reverse Transcription PCR.** RNA isolated from cells, whole liver, as well as from resected tumor specimens had standard TriZol extraction as previously described (2). 15 ng of cDNAs was used to perform QPCR assay in duplicate with SYBR Green Supermix. Primer sequences listed in Supplemental Table 1. Expression of the examined genes was normalized against internal control gene (rodent β-actin or human β-actin) based on the threshold cycle (C\(_T\)) and relative fold change calculated by the \(2^{-\Delta\Delta C_T}\) method (2).

**Morphometry.** Formalin-fixed human tumor sections (n=5) were stained for SHH, PKM2 and mouse tumor sections (n=5) as described in the previous section; staining was quantified by morphometric analysis with MetaView software (Universal Imaging, Downingtown, PA). A minimum of 10 randomly selected 200x fields/section were evaluated for each human and mouse section. Each stain quantified by morphometric analysis was normalized to the non-tumor liver group (n=5 patients/group) or Vehicle-treated group (n=5 mice/group).

**Cell culture.** Clonally derived rat myofibroblastic line (8B cells) were obtained from M. Rojkind (George Washington University, Washington D.C., USA) (3) were cultured alone (monoculture), in a Transwell co-culture system with HepG2 cells (ATCC, Manassas, VA, USA), Huh 7.5 cells (C. Rice, Rockefeller University), or Panc 10.05 cells (Duke Cell Culture Facility). MF monocultures were also treated with PBS-control or 1000 ng/ml recombinant Shh ligand (StemCell Technologies, Vancouver, Canada) +/- DMSO-vehicle or 3µM GDC-0449 (Selleck Chemicals, Houston, TX), or conditioned medium from the other cells +/- IgG-control (R&D) or 10µg/ml 5E1 Hh neutralizing antibody (Developmental Studies Hybridoma Bank, Iowa City, IA) for 24 hours. To characterize the effects of myofibroblast-tumor cell interactions *in vitro*, we
used a Transwell co-culture system in which liver MF and hepatoma lines were cultured for 24 hours alone or in the co-culture system. To assess the effects of lactate on HepG2 cells, cells were treated with control media and media containing 40μM of lactate (Sigma Aldrich) for 24 hours. Conversely, HepG2 cells were treated for 24 hours simultaneously with 40μM of lactate and either DMSO-vehicle or 10μM of FX11 (4), a LDH inhibitor and gift from C. Dang (Johns Hopkins University, Baltimore, MD), to assess the effects of blocking lactate utilization. All cell lines were plated in triplicate for each experiment and grown as previously described (2, 5). After normalization to respective controls, data from individual cultures were averaged to generate the result of each experiment. All experiments were repeated three times; replicate results were used to calculate final mean +/- SEM values.

**Measurement of cellular lipid droplets.** PFA fixed slide samples were stained with Oil Red O for 30 min at RT, followed by PBS washing. Oil Red O staining was quantified by morphometric analysis of at least 10 HPF per experiment using 200x magnification.

**Measurement of lactate and pyruvate.** Cell culture media or whole cell lysate was prepared with Lactate Assay Buffer (Biovision, Mountain View, CA). 250μL of media or lysate was filtered through 10 kd column (BioVision, Mountain View, CA) and stored at -80 ⁰C for lactate and pyruvate measurement and, when calculating intracellular amounts, for a BCA assay (BioRad, Hercules, CA) to determine protein concentration for normalization. Lactate was measured with the Lactate Assay Kit (BioVision) and pyruvate was measured using the Pyruvate Assay Kit (BioVision) according to the manufacturer’s manual and normalized to the protein concentration.

**Measurement of ATP.** Whole cell lysates were prepared with ATP Assay Mix (Sigma Aldrich, St. Louis, MO) and ATP was immediately measured using the ATP Bioluminescent Assay Kit.
(Sigma Aldrich) according to the manufacturer’s manual. Results were normalized to protein concentration measured by a BCA assay (BioRad, Hercules, CA).

**Luciferase Reporter Assay.** The Hh-responsive luciferase reporter assay was performed using Shh-LightII cells (ATCC, Manassas, VA, USA). Cells were treated with conditioned medium from HepG2 cells for two days. Briefly, Shh-LightII cells were stably co-transfected with a Gli-responsive firefly luciferase reporter and a pRL-TK constitutive Renilla luciferase reporter. Firefly and Renilla luciferase activities were determined via a dual luciferase kit (Promega, Madison, WI, USA). In a parallel experiments, Shh-lightII cells were co-cultured with HepG2 cells in a Transwell system. One day after plating, cells were harvested and Firefly and Renilla luminescence were measured.

**Supplemental References**

**Supplemental Figure Legends**

**Supplemental Figure 1. Panc 10.05 cells do not generate functional Hh ligands** (A) Gli-luciferase reporter activity in Shh–LightII cells incubated with control medium (white bar), grown in co-culture with Panc 10.05 cells (gray bar), or incubated with Panc 10.05-conditioned media (black bar). (B) Ptc mRNA levels in 8B MF grown in monoculture (white bar), grown in co-culture with Panc 10.05 cells (gray bar), or incubated with Panc 10.05-conditioned media (black bar). (C) Pkm2 and Mct4 mRNA levels in 8B MF grown in monoculture (white bar) or in co-culture with HepG2 (grey bar) or Panc 10.05 cells (black bar) (*p<0.05, **p<0.01 vs. control). (D) Pkm2 and Mct4 mRNA levels in monocultures of 8B MF treated with vehicle (white bar) or with conditioned media from HepG2 (grey bar) or Panc 10.05 cells (black bar) (*p<0.05, **p<0.01 vs. control).

**Supplemental Figure 2. Other malignant hepatoma lines can increase MF Hh and glycolytic activity.** (A) Gli-luciferase reporter activity in Shh–LightII cells incubated with control medium (white bar), grown in co-culture with Huh7.5 cells (gray bar), or incubated with Huh7.5-conditioned media (black bar) (*p<0.05 vs. control). (B) Pkm2, Mct4, and Ptc mRNA levels in 8B MFs grown in monoculture (white bar) or grown in co-culture with Huh7.5 cells (black bar) (*p<0.05, **p<0.01 vs. control).

**Supplemental Figure 3. Summary of glycolytic metabolism.** During glycolysis, glucose is converted to pyruvate, which can either be shunted to lactate via the enzyme lactate dehydrogenase (LDH) or taken into the tricarboxylic acid (TCA) cycle after conversion into acetyl Co-A. Entry into the TCA cycle is regulated by the enzyme pyruvate dehydrogenase kinase.
Supplemental Figure 4. MF-derived lactate provides energy source for lipogenesis in other malignant hepatoma lines. (A) Oil Red O staining quantified by morphometric analysis, (B) Intracellular ATP and (C) MCT1, PPARγ mRNA levels in Huh7.5 cells cultured alone (white bar), co-cultured with 8B MFs (black bar), or cultured alone and treated with 40mM of lactate (striped bar) (*p<0.05, **p<0.01 vs. control).

Supplemental Figure 5. Schematic of alterations in hepatoma cells due to release of metabolic end products by glycolytic MF. Hepatoma cells take up MF-derived lactate (denoted by blue circles) across the cellular membrane via the MCT1 lactate importer. Lactate is then converted to pyruvate via lactate dehydrogenase (LDH) and enters the TCA cycle, via a process that is gated by pyruvate dehydrogenase kinase (PDK1).