Supplementary Materials and Methods

Cell line and Treatments. The HLE human HCC cell line was grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum and used for the experiments. Transfection experiments of HLE cells with *AKT1* cDNA in pCMV6-XL5 vector (OriGene Technologies) was performed following the manufacturer's protocol using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) for transfection procedures. For stable transfection of *AKT1*, stable transfectants were selected with cloning cylinders after 3–4 weeks in medium containing Geneticin (600 μg/ml). Transient transfection experiments with a dominant negative form of Spry2 (Spry2Y55F; Supplementary Reference 1) were performed using Lipofectamine 2000 (Invitrogen). *In vitro* growing HLE cells were treated with small interfering RNA (siRNA) against *PKM2* (Santa Cruz Biotechnology, Santa Cruz, CA) gene, according to the manufacturer's recommendations, and incubated for 24 and 48 hours. For the treatment with chemical inhibitors, HLE cells were plated at 2.0 X 10^3/well in 96-well plate and grown for 12 hours. After 24-hour serum deprivation, NVP/BEZ235 (PI3K/mTOR dual inhibitor; Selleck Chemicals, Houston, TX) or UO126 (MEK inhibitor; Cell Signaling Technology, Danvers, MA), were added to the medium at 1 μM and 20 mM final concentration, respectively, and cells incubated for 24 and 48 hours. To assess cell proliferation, HLE cells were plated at the concentration of 2.0 x 10^3/well in 96-well plates, allowed to attach and adjust for the next 12 hours (which corresponds to the 0 hour time point in our graphs), and grown for the additional 24 and 48 hours. The proliferation was assessed at these three time points - 0, 24, and 48 hours with the BrdU Cell Proliferation Assay Kit (Cell Signaling Technology) by measuring the absorbance at 450 nm following the manufacturer's protocol. To measure apoptosis, HCC cell lines were plated at the concentration of 2.0 x 10^3/well in 96-well plates, incubated for 12 hours, and then subjected to 24-hour serum deprivation, which corresponded to the 0 hour time point in the apoptosis graphs. HCC cells continue to growth in serum-free medium for additional 24 and 48 hours. Apoptosis was assessed at these three time

points - 0, 24, and 48 hours with the Cell Death Detection Elisa Plus Kit (Roche Molecular Biochemicals) by measuring the absorbance at 405 nm, following the manufacturer's instructions.

Protein Extraction and Western blotting. Mouse liver specimens and HLE cells were homogenized in lysis buffer [30 mM Tris (pH 7.5), 150 mM NaCl, 1% NP-40, 0.5% Na deoxycholate, 0.1% SDS, 10% glycerol, and 2 mM EDTA] containing the Complete Protease Inhibitor Cocktail (Roche Molecular Biochemicals, Indianapolis, IN) and sonicated. Membranous extracts were prepared with the MEM-PER membrane extraction kit (Thermo Fisher Scientific, Rockford, IL.) following the manufacturer's recommendations. Protein concentrations were determined with the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA) using bovine serum albumin as standard. Aliquots of 100 μg were denatured by boiling in Tris-Glycine SDS Sample Buffer (Invitrogen), separated by SDS-PAGE, and transferred onto nitrocellulose membranes (Invitrogen) by electroblotting. Membranes were blocked in 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween 20 for 1 h and probed with specific antibodies listed in Supplementary Table 1. Each primary antibody was followed by incubation with horseradish peroxidase-secondary antibody diluted 1:5000 for 1 h and then revealed with the Super Signal West Pico (Pierce Chemical Co., New York, NY). For each protein, densities were calculated by ImageQuaNT 5.1 software (GE Healthcare, Piscataway, NJ), normalized to β-Actin (Santa Cruz Biotechnology; dilution 1:2000) levels and mean values evaluated for statistical significance.

Histopathologic Analysis. Liver histopathologic analysis was performed by two experienced pathologists (FD and ME) on tissue slides stained with H&E and the PAS reaction in accordance with the criteria by Frith et al. (Supplementary Reference 2). Naphthol AS-D Chloroacetate (CLAE) enzyme histochemistry was performed using a standard protocol. In contrast to hepatocellular tumors that were usually already visible macroscopically as white nodules, preneoplastic lesions showed no expansive growth. Liver lesions were diagnosed as hepatocellular adenomas (HCAs) if: (1.) the acinar morphology was lost; (2.) the lesion showed a normal trabecular pattern; (3.) the lesions displayed no or only mild atypia; (4.) the lesions compressed the adjacent parenchyma

(expansive growth). Hepatocellular carcinomas (HCCs) were instead diagnosed if the lesion showed in addition a pseudoglandular or a macrotrabecular pattern with more than three cell layers of trabecular width in at least two different areas or frank signs of malignancy such as large areas of necrosis in combination with severe nuclear atypia, a high mitotic/apoptic rate, and/or vascular invasion.

Immunohistochemistry. Immunohistochemical staining on mouse liver tissue specimens was performed on 4% paraformaldehyde-fixed, paraffin-embedded sections. Deparaffinized sections were incubated in 3% H_2O_2 dissolved in phosphate-buffered saline (PBS) 1X for 30 minutes to quench the endogenous peroxidase. For antigen retrieval, slides were microwaved in 10 mmol/L citrate buffer (pH 6.0) for 12 minutes. The mouse monoclonal anti-HA-Tag (Cell Signaling Technology, Danvers, MA; 1:2000) and anti-V5-Tag (Invitrogen; 1:200), the rabbit polyclonal anti-phosphorylated AKT, anti-phosphorylated ERK1/2, and anti-PKM2 (Cell Signaling Technology; 1:100), and the anti-Ki-67 (Bethyl Laboratories, Montgomery, TX; 1:2000) antibody were used. The immunoreactivity was visualized with the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) and 3,3'-diaminobenzidine as the chromogen. Slides were counterstained with hematoxylin.

Proliferation and Apoptotic Indices, Microvessel Density. Proliferation and apoptotic indices were determined in wild-type livers and preneoplastic and neoplastic lesions from AKT and AKT/Spry2Y55F mice by counting Ki67-positive cells and apoptotic figures stained with the ApoTag peroxidase in situ apoptosis kit (Millipore, Billerica, MA), respectively, on 3000 hepatocytes. To assess the microvessel density (MVD), the mouse liver collection was stained with a rabbit polyclonal antibody anti-PODXL1 (Applied Genomics, Huntsville, AL; dilution 1:800). Any brown-stained endothelial cell or endothelial cell cluster was counted as one microvessel, irrespective of the presence of a vessel lumen. In particular, tumors were first screened at low power (x 40) to identify the areas of highest MVD. The four highest MVD areas for each tumor were photographed at high power (x 200) and the size of each area standardized using the ImageJ 1.41

software. MVD indicates the percentage (mean ± SD) of the total PODXL1-stained spots per section area (0.94 mm^2).

Supplementary References

1. Hanafusa H, Torii S, Yasunaga T, Nishida E. Sprouty1 and Sprouty2 provide a control mechanism for the Ras/MAPK signalling pathway. Nat Cell Biol 2002;4:850-858.
2. Frith CH, Ward JM, Turusov VS. Tumours of the liver. IARC Sci Publ 1994;111:223-269.