**Supplementary materials**

## EXPERIMENTAL PROCEDURES

## METHODS

**Materials.** Rabbit polyclonal antibodies recognizing KHK-A, KHK-C, and phospho-PRPS1 T225 were obtained from Signalway Antibody (College Park, Maryland). For the antibodies recognizing KHK-A and KHK-C, a peptide containing Exon 3A-coded regions (72-VLDDLRRYSVDLRYTVFQTTGSVPIATVIINEASGSRTILYYDR-115) or Exon 3C-coded regions (72-LVADFRRRGVDVSQVAWQSKGDTPSSCCIINNSNGNRTIVLHDT-115), was injected into rabbits. The rabbit serum was collected and purified by an affinity column conjugated with Exon 3A- or Exon 3C-peptide, followed by elution and concentration. For the antibody recognizing phospho-PRPS1 T225, a peptide containing PRPS1 pT225 was injected into rabbits. The rabbit serum was collected and purified using an affinity column conjugated with non-phosphorylated PRPS1 T225 peptide to exclude the antibodies recognizing non-phosphorylated PRPS1, followed by an affinity column conjugated with phosphorylated PRPS1 T225 peptide to bind to and purify the phospho-PRPS1 T225 antibody. The phospho-PRPS1 T225 antibody was then eluted and concentrated. Working concentration of 1 μg/ml and 5 μg/ml was used for immunoblotting and immunohistochemical staining, respectively.

Rabbit polyclonal antibodies recognizing hnRNPH1/2 and c-Myc, and mouse monoclonal antibody recongnizing V5 tag were obtained from Abcam (Cambridge, MA). Monoclonal antibodies against GST, tubulin, PRPS1/2, and pan-KHK-A/C were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Active recombinant human IMPDH2 (inosine monophosphate

dehydrogenase 2) protein, a mouse monoclonal antibody for Flag, anti-Flag M2 agarose beads, streptavidin-conjugated agarose beads, fructose, EGTA, ADP, NADH, IMP, AMP, GMP, N-Acetyl-L-cysteine (NAC), phosphorenolpyruvate, pyruvate kinase, myokinase, and lactic dehydrogenase were purchased from Sigma (St. Louis, MO). HyFect transfection reagents were from Denville Scientific (Metuchen, NJ). Normal human hepatocytes and Williams' Medium E were purchased from Invitrogen (Carlsbad, CA). Synthesized biotinylated RNA containing the exons 3A and exon 3C and their adjacent intron sequences were purchased from Selleck Chemicals (Houston, TX). [$\gamma$-$^{32}$P] ATP was purchased from MP Biomedicals (Santa Ana, CA). D-[5-$^{3}$H]-fructose and, D-[6-$^{14}$C]-glucose were purchased from American Radiolabeled Chemicals (St. Louis, MO). [8-$^{14}$C] ADP was purchased from Perkin Elmer (Waltham, Ma). Scintillation vials were purchased from Thermo Fisher Scientific (Pittsburgh, PA). Streptavidin beads were purchased from Pierce (Rockford, IL).

Supplementary Table 2 contains the detailed information of the used antibodies.

**Cell lines and cell culture conditions.** Hep3B and Huh-7 cells were from ATCC and were maintained in Dulbecco's modified Eagle's medium supplemented with 10% dialyzed bovine calf serum (HyClone). Normal human hepatocytes were maintained in Williams' Medium E (Invitrogen). No cell lines used in this study were found in the database of commonly misidentified cell lines that is maintained by ICLAC and NCBI Biosample. Cell lines were authenticated by Short Tandem Repeat (STR) profiling and were routinely tested for mycoplasma contamination.

***In vitro* kinase assay.** Kinase reactions were performed as described previously [37]. In brief, bacterially purified recombinant KHK-A or KHK-C (10 ng) was incubated with PRPS1 (100 ng) in 25 μl of kinase buffer (50 mM Tris-HCl, pH 7.5, 100 mM KCl, 50 mM $MgCl_2$, 1 mM $Na_3VO_4$, 1 mM DTT, 5% glycerol, 0.2 mM ATP, 10 μCi of [$\gamma$-$^{32}$P] ATP) at 25 °C for 1 h. The reactions were terminated with the addition of SDS-PAGE loading buffer and heated to 100 °C. The reaction mixtures were then subjected to SDS-PAGE analysis.

**Purification of recombinant proteins.** His-KHK-A, His-KHK-C, GST-KHK-A, GST-KHK-C, His-PRPS1 and His-PRPS2 were expressed in bacteria and purified as described previously [38]. Briefly, BL21(DE3)pLysS cells expressing $(His)_6$- or GST-tagged KHK-A, KHK-C, PRPS1 and PRPS2 proteins were cultured in 250 ml LB medium and were treated with IPTG for 16 h at 30 °C before lysis by sonication. For $(His)_6$-tagged proteins, cell lysates were loaded onto a Ni-NTA column (GE Healthcare Life Sciences, PA) followed by washing with 5 column volumes of 20 mM imidazole and subsequent elution with 250 mM imidazole. For GST-tagged proteins, cell lysates were loaded onto a GSTrap HP column (GE Healthcare Life Sciences, PA) followed by washing with 5 column volumes of PBS and subsequent elution with 10 mM reduced glutathione. The proteins in 10 kDa spin columns were desalted by washing twice with ice-cold PBS. We then loaded the proteins onto a HiPrep 16/60 Sephacryl S-200 HR gel filtration column (GE Healthcare Life Sciences, PA) to remove the contaminated proteins. Purification efficiency was examined by SDS-PAGE and colloidal coomassie blue (G-250) staining with superior sensitivity that detects as low as 1 ng protein/band [39].

**Immunoprecipitation and immunoblot analysis.** Extraction of proteins from cultured cells using a modified buffer was followed by immunoprecipitation and immunoblot analyses with corresponding antibodies as described previously [40]. The band intensity was quantified using the Image Lab software program (Bio-Rad).

**Transfection.** Cells were plated at a density of $4 \times 10^5$ per 60-mm dish 18 h before transfection. Transfection of cells was performed as described previously [38].

**Fructose metabolic rate assay.** The fructose metabolic rate in HCC cells was measured by monitoring the conversion of D-[5-$^3$H]-fructose to $^3H_2O$. Briefly, $10^6$ cells were washed once in phosphate-buffered saline (PBS) prior to incubation in 2 ml of Krebs buffer (126 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 1.2 mM $NaH_2PO_4$, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$) without fructose for 30 min at 37 °C. The Krebs buffer was then replaced with 2 ml of Krebs buffer containing 10 mM fructose spiked with 10 μCi of D-[5-$^3$H]-fructose (1 μM). Following incubation for 1 h at 37 °C, triplicate 50 μl aliquots of the Krebs buffer were transferred to uncapped PCR tubes containing 50 μl of 0.2 N HCl, and an uncapped PCR tube was then transferred into an Eppendorf tube containing 0.5 ml of water for diffusion of $^3H_2O$ into unlabeled water. The tubes were sealed, and diffusion was allowed to proceed for a minimum of 24 h at 37 °C. The amounts of diffused $^3H_2O$ were measured using scintillation counting. The fructose glycolytic rate (nmole/$10^6$ cells/h) was calculated using the formula ($^3H_2O$ formed [CPM] × concentration of fructose [nmole/ml] × $10^{-3}$)/(radioactivity of D-[5-$^3$H]-fructose [CPM/pmole] × concentration of D-[5-$^3$H]-fructose [nmole/ml] × cell number [$10^6$] × time [h]). CPM stands for counts per minute.

**Human liver tumor samples.** Liquid nitrogen-frozen HCC and adjacent matched non-tumor tissue samples were obtained from the Tumor Bank of Sun Yat-sen University Cancer Center in Guangzhou, People's Republic of China. About 25-200 mg of each sample was obtained and homogenized for TRIzol-based RNA extraction. The use of human HCC specimens and the database was approved by the Institutional Review Board at Sun Yat-sen University Cancer Center. All tissue samples were collected in compliance with informed consent policy.

Tissue microarray (diameter 1.5 mm) was obtained from Shanghai Outdo Biotech Co., Ltd (Shanghai, China). This microarray was constructed with primary HCC tumors and their matched, non-tumor adjacent tissues from 90 patients diagnosed between January of 2007 and November of 2009. Consecutive sections (five-micrometer in thickness) from the microarray tissue block were made with a microtome.

**RT-PCR.** Total RNA was extracted from cultured HCC cells and human HCC samples using TRIzol (Invitrogen) according to the manufacturer's instructions. Total RNA (1 μg) was used for each sample in a 20 μl reaction using an iScript cDNA synthesis kit (Bio-Rad). One microliter of the cDNA library was used in a 50 μl PCR. One microgram of the PCR products was digested with *Bsm*AI, and the products of digestion were resolved on a 2% agarose gel. The intensity of the bands in agarose gel was quantified using the Image Lab software program (Bio-Rad). Human KHK forward and reverse primers were used in the PCRs. For distinguishing the *PRPS1* and *PRPS2* cDNA, one microgram of the PCR products was digested by *Hind*III, and the products of digestion were resolved on a 2% agarose gel. Human *PRPS* forward and reverse primers were used in the PCRs. For *in vivo* splicing assays, KHKmini forward and reverse

primers were used in the PCRs. For *in vitro* splicing assays, AdML forward and reverse primers were used in the PCRs.

Supplementary Table 3 contains the detailed information of the primer sequences.

**RNA affinity binding assay.** RNA affinity binding assay was performed as described previously [41] with some modifications. 5'-Biotinylated EI3A (-30 to +30) and EI3C (-30 to +30) RNA oligonucleotides were purchased from Midland Certified Reagent Company. One nanomole of both biotinylated RNA oligonucleotides was bound with 100 μl of streptavidin-conjugated agarose beads in 500 μl of binding buffer (10 mM Tris-HCl, pH 7.5, 1 mM ethylenediaminetetraacetic acid, 2 M NaCl) overnight at 4 °C with rotation. The beads were washed twice with binding buffer and twice with buffer D (920 mM HEPES, pH 7.9, 20% glycerol, 100 mM KCl, 0.2 mM ethylenediaminetetraacetic acid, 0.5 mM dithiothreitol [DTT]).

A nuclear extract mixture was prepared in a 500 μl volume (10 mM HEPES, pH 7.9, 10% glycerol, 50 mM KCl, 2 mM $MgCl_2$, 0.75 mM ATP, 25 mM creatine phosphate, 0.1 mM ethylenediaminetetraacetic acid, 0.25 mM DTT, 30 μg of *Escherichia coli* transfer RNA) containing 125 μl of a Hep3B nuclear extract and preincubated at 30 °C for 10 min. RNA-immobilized beads were mixed with a preincubated nuclear extract mixture and incubated at 30 °C for 30 min with constant rotation. After RNA and protein binding, beads were washed with buffer D twice and buffer D without glycerol twice and eluted by adding 40 μl of Laemmli sample buffer and boiled for 5 min. Twenty-microliter aliquots of the eluted bound proteins were resolved using 10% SDS-PAGE followed by Coomassie Brilliant Blue staining, and the protein bands were excised for mass spectrometric analysis.

**Cross-linking and RNA immunoprecipitation.** RNA immunoprecipitation was performed as described previously [42] with some modifications. Briefly, following ultraviolet cross-linking, Hep3B cells were harvested, and nuclear extracts were prepared and sonicated. Protein A-Sepharose beads (Sigma) were incubated with a control rabbit IgG antibody or an anti-hnRNPH1/2 antibody for 4 h at 4 °C. Antibody-conjugated beads were washed twice with lysis buffer followed by incubation with precleared lysates at 4 °C overnight. After washing the beads with lysis buffer three times, one fourth volume of total beads was eluted in SDS sample buffer for immunoblot analysis to monitor hnRNPH1/2 immunoprecipitation efficiency. The remaining beads were incubated with lysis buffer in the presence of RNase-free DNase (QIAGEN) for 15 min at 37 °C followed by incubation with 50 μg of proteinase K (Sigma) for 15 min at 37 °C. Coimmunoprecipitated RNA was then extracted using TRIzol and used for RT-PCR analysis. The primers (hnRNPH1/2-binding forward and hnRNPH1/2-binding reverse) were used in the PCRs.

Supplementary Table 3 contains the detailed information of the primer sequences.

**Mass spectrometric analysis.** An *in vitro* KHK-phosphorylated sample of purified PRPS1 was digested in-gel in 50 mM ammonium bicarbonate buffer containing RapiGest (Waters Corporation) overnight at 37 °C with 200 ng of modified sequencing-grade trypsin (Promega). The digested samples were analyzed using high-sensitivity liquid chromatography tandem mass spectrometry with an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific). Proteins were identified by searching the fragment spectra against the Swiss-Prot protein database (EBI) using Mascot search engine (version 2.3; Matrix Science) via the Proteome Discoverer software

program (version 1.4; Thermo Fisher Scientific). Phosphopeptide matches were analyzed using the phosphoRS algorithm implemented in Proteome Discoverer and manually curated [43].

**GST pull-down assay.** Glutathione agarose beads were incubated with purified protein for 12 h. The beads were then washed with a lysate buffer three times.

**Cell proliferation assay.** A total of $1 \times 10^5$ cells were plated and counted 3 days after seeding in Dulbecco's modified Eagle's medium with 10% bovine calf serum. The data were presented as the mean ± standard deviation (s.d.) from three independent experiments.

**KHK minigene construction.** A KHK minigene containing exons 2, 3A, 3C, and 4 and introns adjacent to exons 3A and 3C was amplified from genomic DNA from Hep3B cells using a *Pfu* PCR kit (Promega) for 35 cycles at an annealing temperature of 58 °C and a 150 s extension time. The KHK-specific primers were E2F and E4R with *Nhe*I and *Xba*I overhangs. The resulting PCR products were gel-purified, digested, and subcloned into pcDNA3.1 (Invitrogen). The ligation reaction was transformed into TOP10 competent cells. Plasmid DNA was prepared, and the constructed vectors were confirmed using restriction digests and sequencing.
Supplementary Table 3 contains the detailed information of the primer sequences.

**DNA constructs and mutagenesis.** PCR-amplified human KHK-A, KHK-C, and PRPS1 were cloned into a pCold I, pGEX-4T-1, pcDNA 3.1, or pET22b+ vector. PcDNA3.1 Flag KHK-A L73A, pcDNA3.1 Flag KHK-A V81A, pcDNA3.1 Flag KHK-A L83A, pcDNA3.1 Flag KHK-A V87A, pcDNA3.1 Flag KHK-A I96A, pcDNA3.1 Flag PRPS1 T225A, pcDNA3.1 V5 PRPS1

T225A, pcDNA3.1 V5 PRPS1 A190T, pcDNA3.1 V5 rKHK-A L83A, pcDNA3.1 KHK minigene G1C, pGEX-4T-1 KHK-A L83A, and pCold I PRPS1 T225A were constructed using a QuikChange site-directed mutagenesis kit (Stratagene). PcDNA3.1 V5 rKHK contains nonsense mutations of G1084A, T1087C, and C1090A. PcDNA3.1 V5 rPRPS1 contains nonsense mutations of A568C, T571C, T574C, and T577C.

The pGIPZ vector was used for construction of the control shRNA and shRNAs for KHK, PRPS1, c-Myc, and hnRNPH1/2.

Supplementary Table 3 contains the detailed information of the shRNA-targeted nucleotide sequences of the indicated genes.

***In vivo* and *in vitro* splicing assays.** WT and mutated minigene vectors were transfected into Hep3B cells. Twenty-four hours after transfection, cells were collected, and the *KHK-A*:*KHK-C* mRNA ratio was analyzed using RT-PCR followed by *Bsm*AI digestion of PCR products. *In vitro* splicing substrates were constructed by replacing the first exon and downstream intronic sequence of AdML pre-mRNA with KHK exon 3C and the downstream intron 3 sequence with or without a mutated hnRNPH1/2 binding site. Pre-mRNA substrates were synthesized via *in vitro* transcription using T7 RNA polymerase (Promega) following the product's protocol. *In vitro* splicing of the WT and mutated pre-mRNA was carried out using Hep3B nuclear extracts.

**ChIP assay.** ChIP was performed using SimpleChIP Enzymatic Chromatin IP kit (Cell Signaling Technology). Chromatin prepared from cells in a 10-cm dish was used to determine total DNA input and for overnight incubation with specific antibodies or normal mouse IgG. The human hnRNPH1 and hnRNPH2 promoter-specific primers (forward and reverse) were used in PCR.

Supplementary Table 3 contains the detailed information of the primer sequences.

**ADP binding assay.** After a KHK-A kinase assay, GST-KHK-A protein was removed from the reaction using glutathione agarose beads. His-PRPS1 variants (2 μg each) with or without phosphorylation were pretreated with 1 mM ADP for 5 min at 25 °C and washed twice with kinase buffer (50 mM Tris-HCl, pH 7.5, 100 mM KCl, 50 mM $MgCl_2$, 1 mM $Na_3VO_4$, 1 mM DTT, 5% glycerol) using 10 kDa spin columns (Millipore) followed by incubation with 4 μCi of [8-$^{14}$C] ADP for 12 h at 4 °C in 100 μl of kinase buffer with shaking. The protein was then washed twice with 0.5 ml of ice-cold kinase buffer using 10 kDa spin columns, and His-PRPS1–associated radioactivity was detected via liquid scintillation counting.

**ATP binding assay.** After a KHK-A kinase assay, GST-KHK-A protein was removed from the reaction using glutathione agarose beads. His-PRPS1 variants (2 μg each) with or without phosphorylation were washed twice with kinase buffer (50 mM Tris-HCl, pH 7.5, 100 mM KCl, 50 mM $MgCl_2$, 1 mM $Na_3VO_4$, 1 mM DTT, 5% glycerol) using 10 kDa spin columns (Millipore) followed by incubation with 2 mM ATP spiked with 20 μCi of [γ-$^{32}$P] ATP for 5 min at 30 °C in 100 μl of kinase buffer. To determine the binding of ATP to WT GST-KHK-A and GST-KHK-A G257R, we incubated each protein (2 μg) with 2 mM ATP spiked with 20 μCi of [γ-$^{32}$P] ATP for 5 min at 30 °C in 100 μl kinase buffer. The proteins were then washed twice with 0.5 ml ice-cold kinase buffer using 10 kDa spin columns, and protein–associated radioactivity was detected via liquid scintillation counting.

**Measurement of PRPS1 activity.** Purified recombinant WT or mutant PRPS1 (10 ng) was incubated in 100 μl of reaction buffer (110 mM $NaH_2PO_4$, pH 7.0, 4.7 mM ribose 5-phosphate, 0.4 mM NADH, 3.2 mM ATP, 1.8 mM phosphoenolpyruvate, 6 mM $MgCl_2$, 31 mM $NaHCO_3$, 7 U of pyruvate kinase, 10 U of lactic dehydrogenase, 10 U of myokinase) at 25 °C in a 96-well plate. The absorbance was read at 340 nm in kinetic mode for 5 min using multi-detection microplate readers (BMG LABTECH, Cary, NC).

**Intracellular inosine monophosphate level measurement.** Cells cultured in a 10-cm dish at 70-80% confluence were harvested and homogenized in 1 ml of hypotonic lysis buffer (20 mM HEPES, pH 7.0, 5 mM KCl, 1 mM $MgCl_2$, 5 mM DTT). The homogenates were centrifuged at 4 °C for 10 min at maximum speed, and the supernatants were placed in Amicon Ultra tubes with a 10 kDa cut-off filter (EMD Millipore). Fifty microliters of the flow-through was mixed with 50 μl of 2× reaction buffer (100 mM Tris-HCl, pH 8.0, 2 mM NAD, 20 mM KCl, 2 mM ethylenediaminetetraacetic acid, 2 mM DTT) in a 96-well plate. Active recombinant human inosine monophosphate dehydrogenase 2 protein (30 nM) was added to each well and incubated at 25 °C for 30 min. The absorbance was read at 340 nm using multi-detection microplate readers.

**Influx of glucose into RNA assay.** Subconfluent cells seeded in a six-well plate were incubated in 2 ml of fresh medium supplemented with 10% fetal bovine serum and 5.5 mM glucose spiked with 1 μCi of D-[6-$^{14}$C]-glucose (0.01 mM; American Radiolabeled Chemicals) for 6 h followed by PBS washing. RNA was then extracted using TRIzol, and $^{14}$C-RNA was assayed using a scintillation counter. Influx of glucose (nmole/$10^6$ cells/h) into RNA was calculated using the

formula (formed $^{14}$C-RNA [CPM] × glucose concentration [nmole/ml] × $10^{-3}$)/(D-[6-$^{14}$C]-glucose radioactivity [CPM/pmole] × D-[6-$^{14}$C]-glucose concentration [nmole/ml] × cell number [$10^6$] × time [h]).

**Measurement of ROS.** We assessed intracellular ROS with the fluorescence probe DCFDA (Abcam) following the manufacturer's instructions. Briefly, 2.5 × $10^4$ cells were seeded in a clear bottom of 96-well plate. After fructose treatment for 2 h, cells were washed with HBSS buffer followed by incubation in HBSS buffer containing DCFDA (25 μM) for 45 min at 37 °C in dark. The cells were then washed with HBSS buffer, and green fluorescence was measured using a Synergy HT microplate reader at 485/535 nm (BioTek, Winooski, VT).

**Animal studies.** One million Huh-7 cells with depletion of endogenous KHK or PRPS1 and reconstituted expression of their WT or mutant proteins were collected in 20 μl DMEM with 33% Matrigel and intrahepatically injected into livers of 4-week-old male BALB/c athymic nude mice. The injections were performed as described in a previous publication [44]. Five mice per group in each experiment were used. Animals were sacrificed 28 days after injection. The liver of each mouse was dissected, which is followed by fixation in 4% formaldehyde and embedment in paraffin. Tumor formation and phenotype were determined by histologic analysis of H&E-stained sections. The tumor volume was calculated using the formula: $V = ½ a^2b$ (V: volume, a: shortest diameter, b: longest diameter). The animals were treated in accordance with relevant institutional and national guidelines and regulations. The use of the animals was approved by the Institutional Review Board at The University of Texas MD Anderson Cancer Center. Animals arriving in our facility were randomly put into cages with five mice each. No statistical method

was used to predetermine sample size. The investigators were not blinded to allocation during experiments and outcome assessment.

**Histologic evaluation and immunohistochemical staining.** Mouse tumor samples were fixed and prepared for staining. The samples were stained with Mayer's hematoxylin and eosin (BioGenex). The slides were then mounted using Universal mount (Research Genetics).

Sections of paraffin-embedded human HCC tissue were stained with antibodies against phospho-PRPS1 T225, KHK-A, hnRNPH1/2, c-Myc, or nonspecific IgG as a negative control. The tissue sections were quantitatively scored according to the percentage of positive cells and staining intensity as described previously [45]. The following proportion scores were assigned to the sections: 0 if 0% of the tumor cells exhibited positive staining, 1 for 0-1%, 2 for 2-10%, 3 for 11-30%, 4 for 31-70%, and 5 for 71-100%. In addition, the staining intensity was rated on a scale of 0-3: 0, negative; 1, weak; 2, moderate; and 3, strong. The proportion and intensity scores were then combined to obtain a total score (range, 0-8) as described previously[37]. Scores were compared with overall survival duration, defined as the time from date of diagnosis to death or last known follow-up examination. All patients had received standard therapies after surgery. The use of human HCC specimens and the database was approved by the Institutional Review Board at Shanghai JiaoTong University School of Medicine. All tissue samples were collected in compliance with informed consent policy.

**Determining Km of fructose to KHK-A.** Purified recombinant WT KHK-A (10 ng) was incubated in 100 μl of reaction buffer (50 mM Tris-HCl [pH 7.0], 0.2 mM NADH, 5 mM ATP, 1.8 mM phosphoenolpyruvate, 6 mM $MgCl_2$, 100 mM KCl, 7 U of pyruvate kinase, 10 U of

lactic dehydrogenase) with indicated D-fructose concentration at 25°C in 96-well plate and were read by Multi-detection microplate readers (BMG LABTECH, Cary, NC) at 340 nm in kinetic mode for 5 minutes. The reaction velocity (V) was obtained by measuring the product concentration as a function of time. Km was calculated from a plot of 1/V vs. 1/ [Fructose] according to the Lineweaver-Burke plot model. Data represent the means ± s.d. of three independent experiments.

**Determining Km of PRPS1 to KHK-A.** Purified recombinant WT KHK-A (10 ng) was incubated in 100 μl of reaction buffer (50 mM Tris-HCl [pH 7.0], 100 mM KCl, 50 mM $MgCl_2$, 1 mM $Na_3VO_4$, 1 mM DTT, 5% glycerol, 0.2 mM ATP, 10 μCi of [$\gamma$-$^{32}$P] ATP) with indicated PRPS1 recombinant protein concentration at 25°C in 1.5 ml Eppendorf tubes for 5 minutes. The reactions were stopped with 10% ice-cold trichloroacetic acid and collected the precipitated protein by centrifugation at 14: 13,400g. Proteins were washed three times with ice-cold acetone. The amount of bound radioactivity was measured with a scintillation counter and used the reactions without adding substrate (PRPS1) as a blank. The reaction velocity (V) was obtained by measuring the CPM as a function of time. Km was calculated from a plot of 1/CPM vs. 1/ [PRPS1] according to the Lineweaver-Burke plot model. Data represent the means ± s.d. of three independent experiments.

**Apoptosis assay.** A total of $1 \times 10^6$ cells suspended in 3 ml of medium were seeded in 60-mm dishes 18 h before the assays. Apoptosis was measured by staining with Annexin V-Cy5 Apoptosis Detection Kit (Abcam, MA) followed by flow cytometry on a FACS flow cytometer (BD Biosciences, CA). Data represent the means ± s.d. of three independent experiments.

**Measurement of intracellular ATP concentrations.** A total of $5 \times 10^6$ cells was washed 3 times with PBS and then lysed in 300 μl assay buffer. Samples were deproteinized using 10 kDa spin columns (Millipore), and ATP levels were determined using a BioVision ATP assay kit (BioVision).

**Measurement of intracellular phosphate concentrations.** A total of $5 \times 10^6$ cells was washed 3 times with 20 mM HEPES [pH 7.0] and then homogenized in 0.3 ml of hypotonic lysis buffer (20 mM HEPES [pH 7.0], 5 mM KCl, 1 mM $MgCl_2$, 5 mM DTT, and protease inhibitor cocktail). Samples were deproteinized using 10 kDa spin columns (Millipore), and phosphate levels were determined using a BioVision phosphate assay kit (BioVision).

**Lentiviral transduction studies.** Hepatocytes expressing either vector or a *MYC* cDNA were established using a pCDH Lentivector Expression System (System Biosciences, Mountain View, CA) according to the manufacturer's instructions. Briefly, c-Myc open reading frame were cloned into pCDH lentiviral vector using restriction enzyme *XbaI* and *BamHI*. Lentiviruses were produced by cotransfecting 293FT cells with one of the expression plasmids and three packaging plasmids (pLP1, pLP2, pLP/VSVG). Infectious lentiviruses were harvested 72 h after transfection, centrifuged to remove cell debris, and filtered through 0.45 μm filter (Millipore, Bedford, MA). Hepatocytes were lysed for total RNA extraction or immunoblot 24 h after transduction with lentiviruses.

**Determining the intracellular PRPS1 concentration.** Cell pellets (100 μl) and same volume of His-PRPS1 recombinant protein standard were mixed with cell lysate buffer (900 μl), respectively. Equal volume (10 μl from each sample) of cell lysate and His-PRPS1 recombinant protein standard was mixed and used for immunoblot.

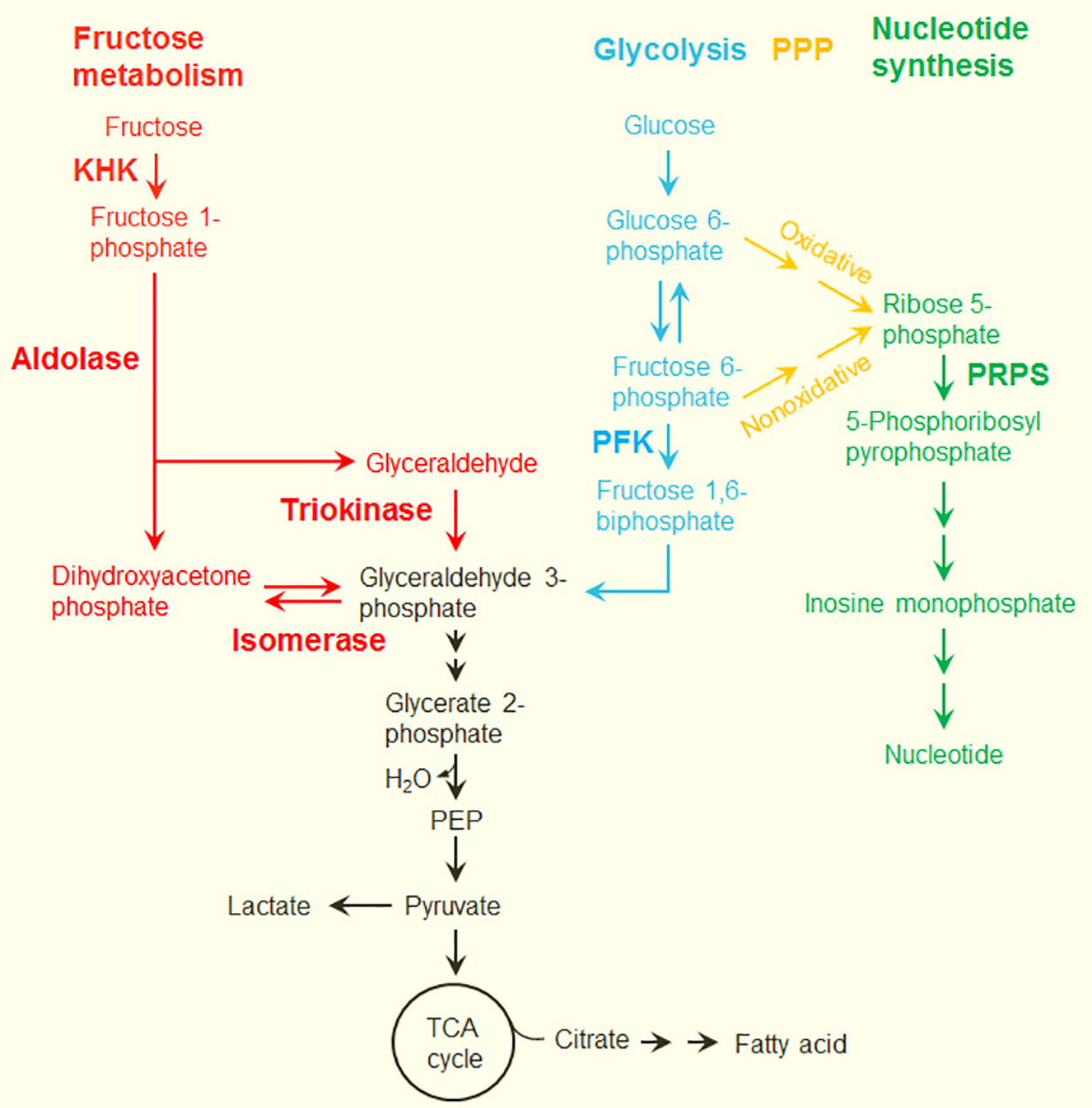
**Statistics and reproducibility.** All immunoblotting experiments were independently repeated at least three times. The results for significance tests are included in the legend of each figure. The fructose or glucose metabolism assays in Fig. 1a and Fig. 5a-d were independently repeated three times. In Fig. 1d and 6f, pair-wise comparisons were performed using a two-tailed Student's t-test. The ATP or ADP binding assays in Fig. 4b and 4e were independently repeated three times. The PRPS1 activity assays in Fig. 4c, d, f, and g were independently repeated three times. The ROS measurement in Fig. 5e was independently repeated three times. The cell proliferation assays in Fig. 6a-c were independently repeated three times. Survival rate comparison in the HCC patients was calculated using a log-rank test. Multivariate analyses of HCC patient survival and samples were performed using Cox regression model. P values $< 0.05$ were considered significant.

## SUPPLEMENTARY REFERENCES

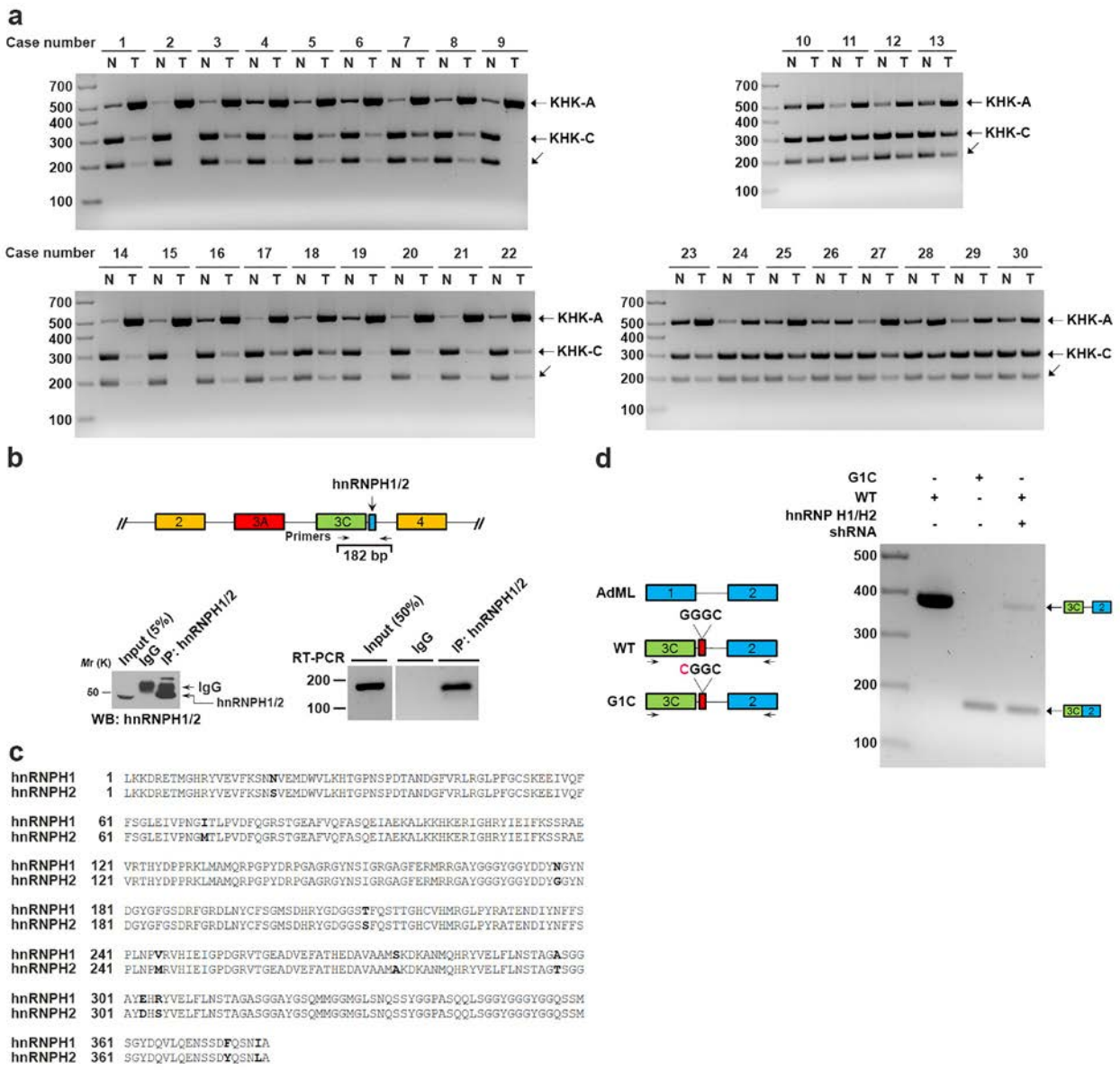

37. Ji, H. *et al.* EGF-induced ERK activation promotes CK2-mediated disassociation of alpha-Catenin from beta-Catenin and transactivation of beta-Catenin. *Molecular cell* **36**, 547-559 (2009).
38. Xia, Y. *et al.* c-Jun downregulation by HDAC3-dependent transcriptional repression promotes osmotic stress-induced cell apoptosis. *Molecular cell* **25**, 219-232 (2007).
39. Dyballa, N. & Metzger, S. Fast and sensitive colloidal coomassie G-250 staining for proteins in polyacrylamide gels. *J Vis Exp* 30, 1431 (2009).
40. Lu, Z. *et al.* Activation of protein kinase C triggers its ubiquitination and degradation. *Molecular and cellular biology* **18**, 839-845 (1998).

41. Kashima, T., Rao, N., David, C.J. & Manley, J.L. hnRNP A1 functions with specificity in repression of SMN2 exon 7 splicing. *Human molecular genetics* **16**, 3149-3159 (2007).
42. Xu, Y. *et al.* Cell type-restricted activity of hnRNPM promotes breast cancer metastasis via regulating alternative splicing. *Genes & development* **28**, 1191-1203 (2014).
43. Taus, T. *et al.* Universal and confident phosphorylation site localization using phosphoRS. *Journal of proteome research* **10**, 5354-5362 (2011).
44. Chen, Z.Y. *et al.* Dovitinib preferentially targets endothelial cells rather than cancer cells for the inhibition of hepatocellular carcinoma growth and metastasis. *Journal of translational medicine* **10**, 245 (2012).
45. Yang, W. *et al.* Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. *Nature* **480**, 118-122 (2011).

**Supplementary Information**




**Supplementary Figure 1** Pathways of fructose metabolism, glycolysis, tricarboxylic acid (TCA) cycle-derived fatty acids, and *de novo* synthesis of nucleotides and nucleic acids from the pentose phosphate pathway (PPP).



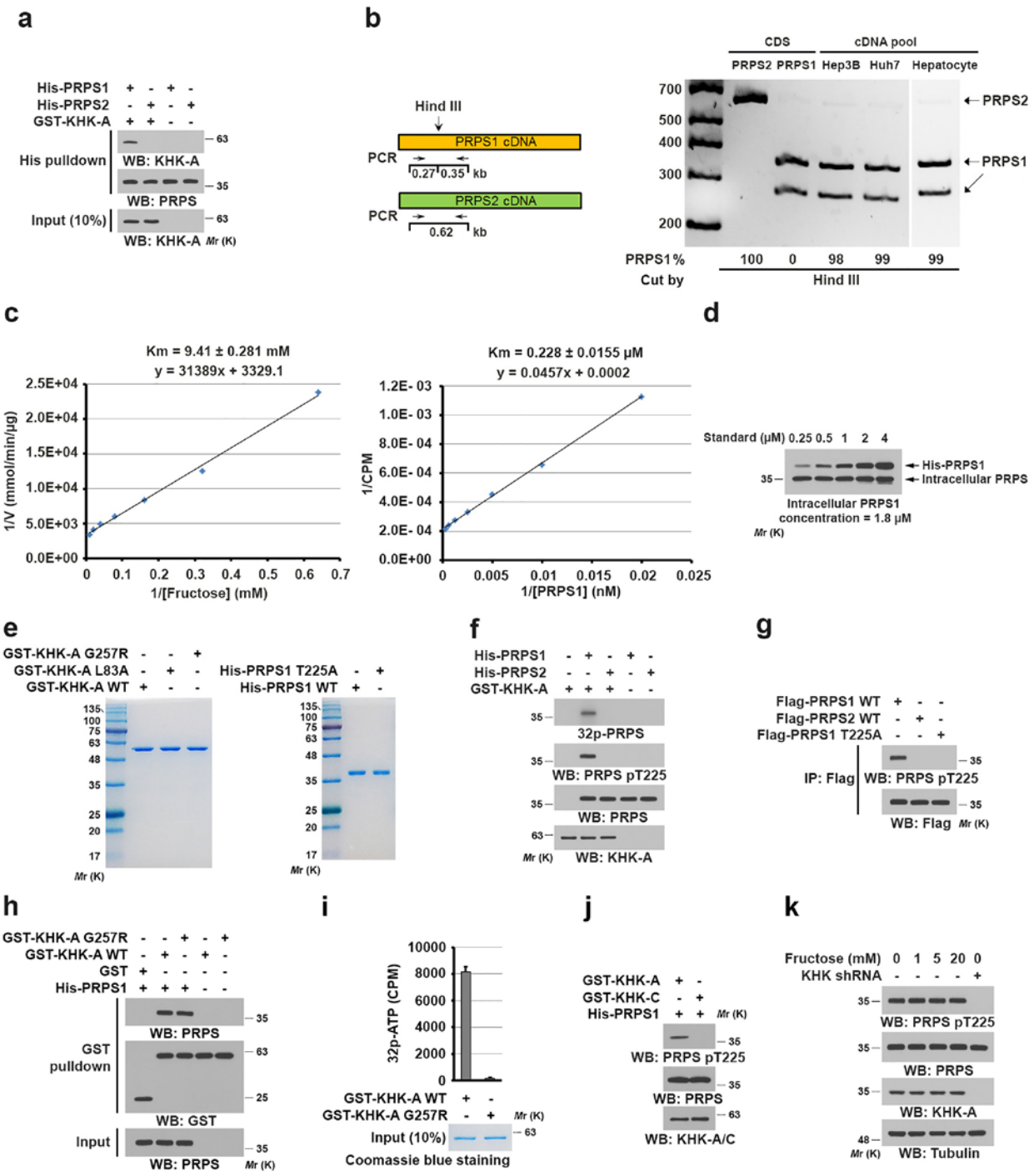

**Supplementary Figure 2** hnRNPH1/2 expression switches KHK-C expression to KHK-A expression in HCC cells.

(**a**) Total RNA was extracted from 30 human HCC and paired non-tumor tissue samples. The RT-PCR products were digested by *Bsm*AI and resolved on agarose gels. N, non-tumor tissue; T, tumor tissue.

(**b**) Immunoprecipitation of hnRNPH1/2 (bottom left panel) from Hep3B cells was followed by RT-PCR analysis of hnRNPH1/2-associated RNAs (bottom right panel) with the indicated primers (top panel).

(**c**) Amino acid sequences of hnRNPH1 and hnRNPH2 were aligned. Different amino acids in two proteins are highlighted.

(**d**) Schematic diagram of synthesized pre-mRNAs of AdML and the indicated AdML-*KHK* exon 3C fused products (left panel). Incubation of the synthesized pre-mRNAs with a Hep3B nuclear extract with or without hnRNPH1/2 depletion was followed by PCR analysis (right panel).




**Supplementary Figure 3** KHK-A but not KHK-C phosphorylates PRPS1 at Thr225.

(**a**) Purified recombinant GST-KHK-A was incubated with immobilized purified recombinant His-PRPS1 or His-PRPS2. An *in vitro* pulldown assay was performed. Immunoblot analysis was conducted with the indicated antibodies.

(**b**) Schematic diagram of the restriction site of *Hind*III in *PRPS1* cDNA (left panel). Total RNA was extracted from normal hepatocytes and the indicated HCC cells (right panel).

(**c**) The Km of purified GST-KHK-A for fructose with representative plotting of 1/V vs. 1/[fructose] (left panel) or for purified His-PRPS1 with representative plotting of 1/CPM vs. 1/[PRPS1] (right panel) was determined.

(**d**) Hep3B cells were centrifuged and washed with PBS. 100 μl of the cell pellets with removal of PBS were mixed with 900 μl lysate buffer. 10 μl cell lysate was mixed with 10 μl of purified recombinant his-PRPS1 with the indicated concentrations were analyzed by immunoblot assay with an anti-PRPS antibody.

(**e**) Bacterially purified indicated KHK-A and PRPS1 proteins were stained with colloidal coomassie blue.

(**f**) *In vitro* phosphorylation analysis**,** SDS-PAGE and autoradiography were performed by mixing purified GST-KHK-A with purified His-PRPS1 or His-PRPS2 in the presence of [$\gamma^{32}$P]-ATP. Immunoblot analysis was conducted with the indicated antibodies.

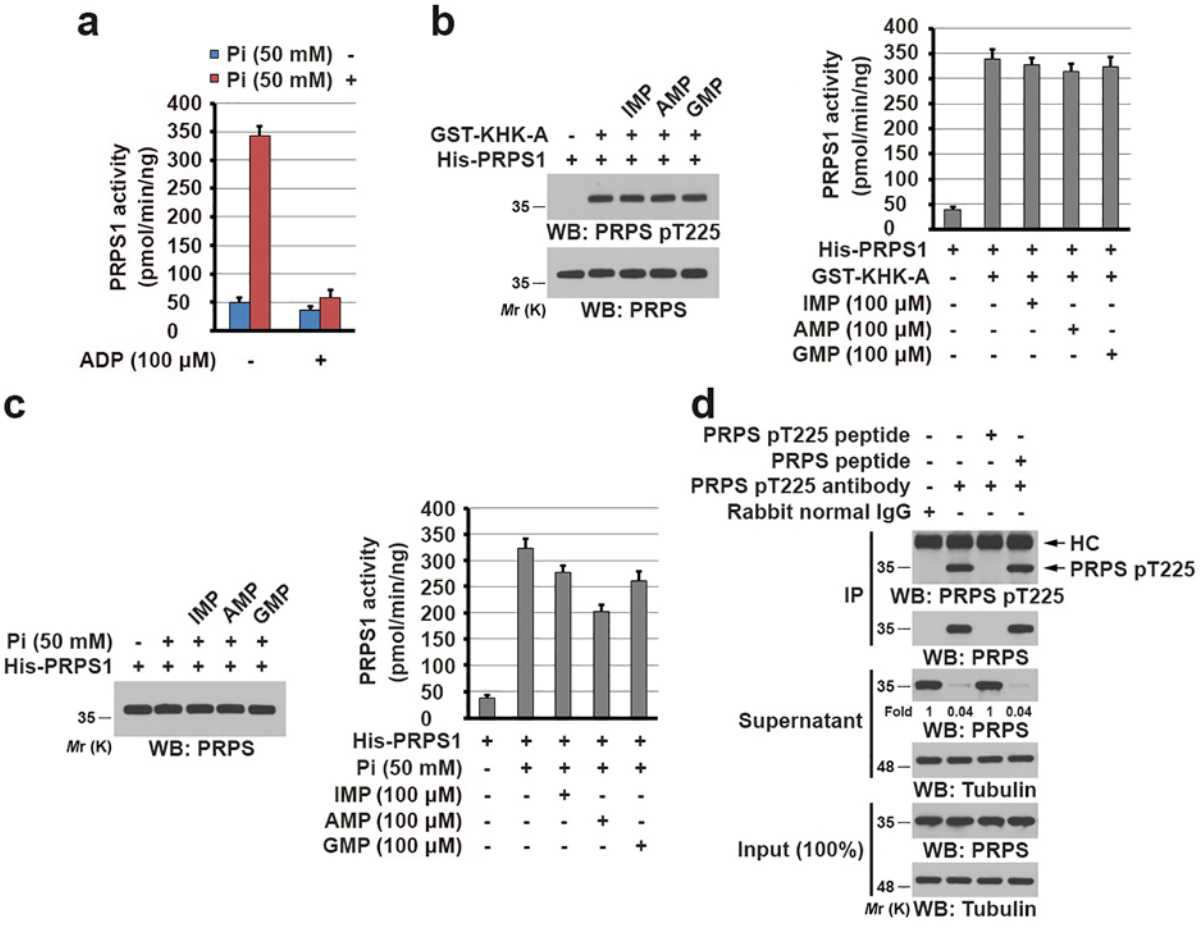
(**g**) Hep3B cells were transiently transfected with a vector expressing the indicated Flag-PRPS proteins. Immunoblot analysis was conducted with the indicated antibodies.

(**h**) GST pull-down analysis was performed by mixing purified immobilized WT GST-KHK-A, GST-KHK-A G257R on glutathione agarose beads with purified His-PRPS1.

(**i**) [$\gamma^{32}$P]-ATP was mixed with WT KHK-A or the KHK-A G257R mutant. KHK-A–bound [$\gamma^{32}$P]-ATP was measured. The data represent the mean ± s.d. from $n$ = 3 independent experiments.

(**j**) *In vitro* phosphorylation assays were performed by mixing purified WT KHK-A or KHK-C with purified PRPS1 in the presence of ATP. Immunoblot analysis was conducted with the indicated antibodies.

(**k**) Hep3B cells were cultured in the medium with added fructose with the indicated concentrations. Immunoblot analysis was conducted with the indicated antibodies.



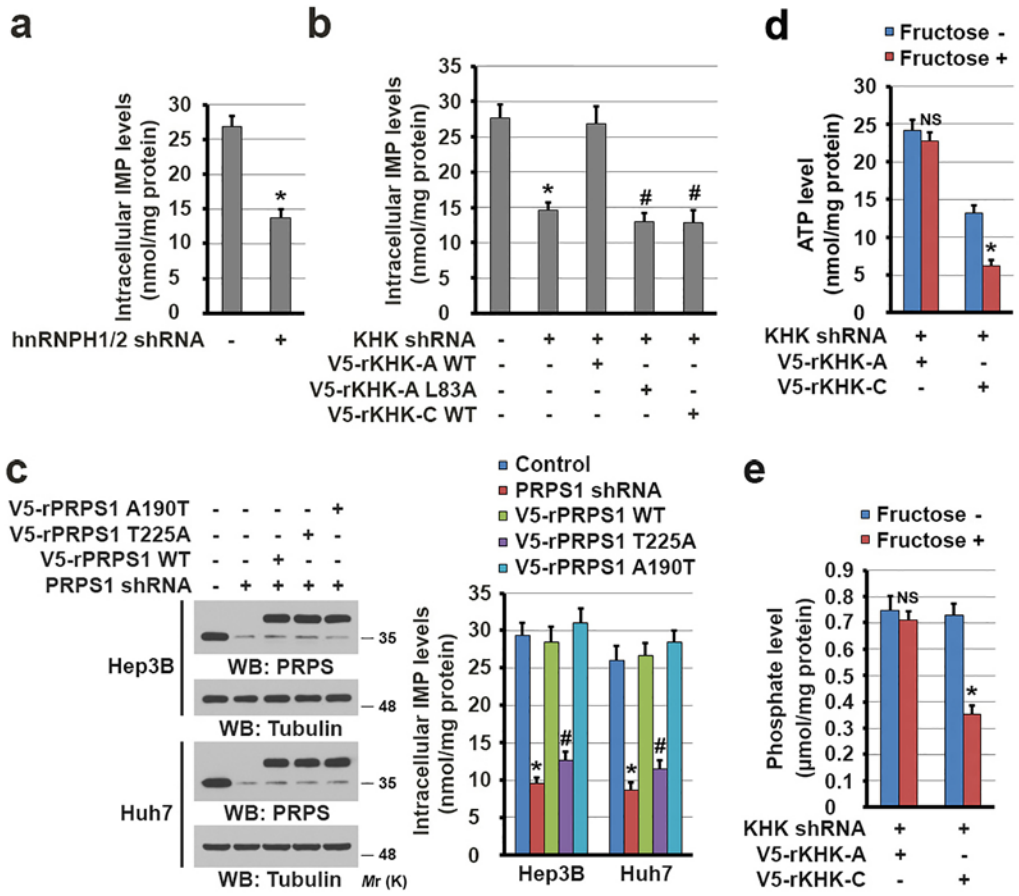

**Supplementary Figure 4** Phosphorylated PRPS1 was not regulated by the allosteric regulators.

(**a**) Activity of bacterially purified WT His-PRPS1 was measured in the presence or absence of phosphate (50 mM), ADP (100 μM). The data represent the mean ± s.d. from $n$ = 3 independent experiments.

(**b**) The activity of bacterially purified WT His-PRPS1 with or without phosphorylation by GST-KHK-A was measured in the presence or absence of IMP (100 μM), AMP (100 μM), or GMP (100 μM). The data represent the mean ± s.d. from $n = 3$ independent experiments. Immunoblot analysis was conducted with the indicated antibodies.

(**c**) The activity of bacterially purified WT His-PRPS1 was measured in the presence or absence of phosphate (50 mM), IMP (100 μM), AMP (100 μM), or GMP (100 μM). The data represent the mean ± s.d. from $n = 3$ independent experiments. Immunoblot analysis was conducted with the indicated antibody.

(**d**) Immunoprecipitation analyses with PRPS1 pT225 antibody was performed using Hep3B cell lysates. Immunoblot analysis was conducted with the indicated antibodies. The amount of phosphorylated PRPS1 was quantified. HC represents heavy chain.




**Supplementary Figure 5** KHK-A–mediated PRPS1 phosphorylation promotes glucose-derived *de novo* nucleotide synthesis.

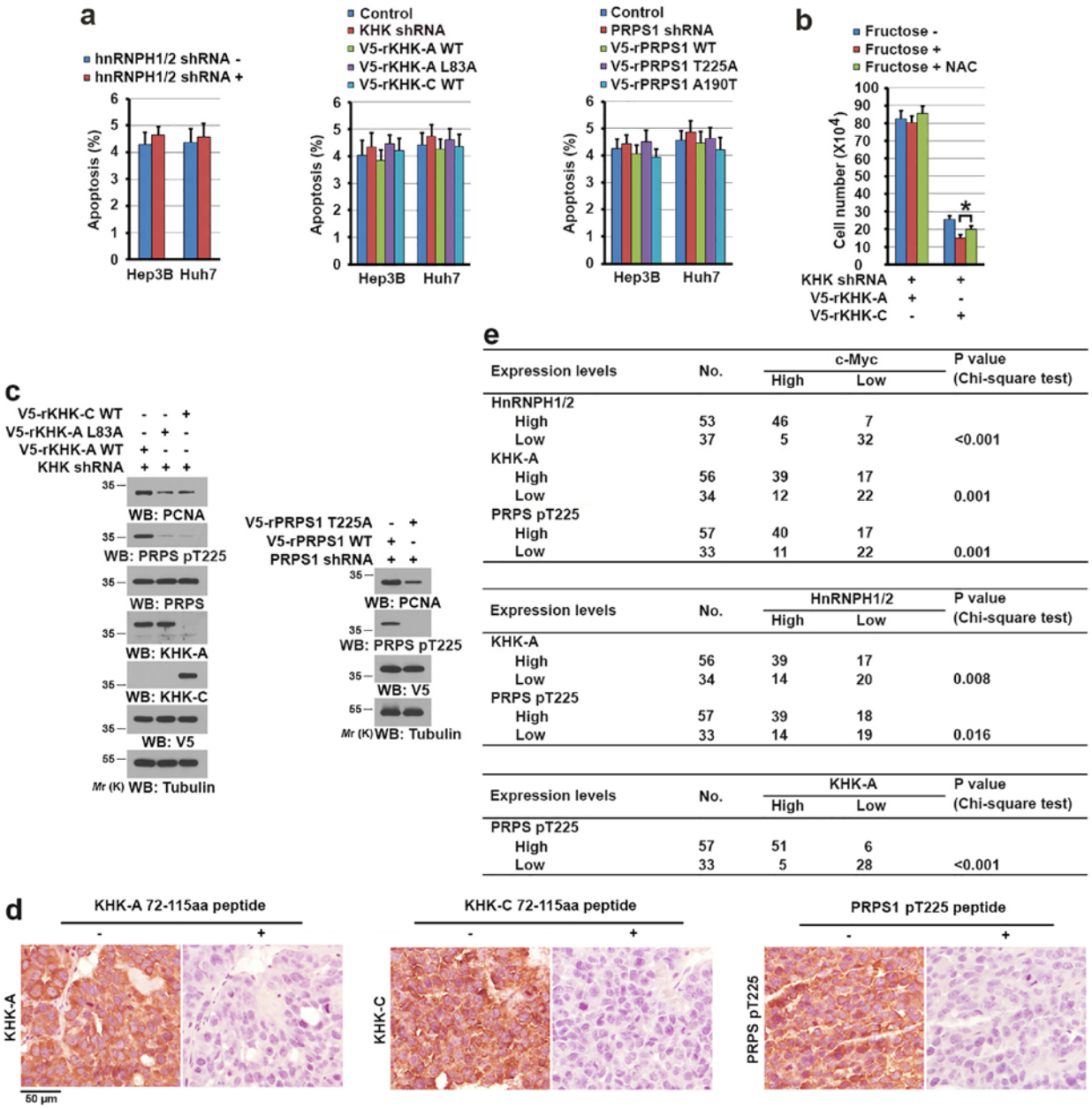
(**a**) Intracellular IMP levels in lysates of Hep3B cells expressing hnRNPH1/2 shRNA were measured. The data represent the mean ± s.d. from $n = 3$ independent experiments. A two-tailed Student's t test was used. ∗ represents $P < 0.01$ between the cells with or without hnRNPH1/2 depletion.

(**b**) Intracellular IMP levels in lysates of Hep3B cells expressing KHK shRNA with or without reconstituted expression of WT rKHK-A, rKHK-A L83A, or WT rKHK-C were measured. The data represent the mean ± s.d. from $n = 3$ independent experiments. A two-tailed Student's t test was used. ∗ represents $P < 0.01$ between the cells with or without KHK depletion. # represents P

< 0.01 between the KHK-depleted cells reconstituted expression of WT rKHK-A and the KHK-depleted cells reconstituted expression of rKHK-A L83A and WT rKHK-C.

(**c**) Intracellular IMP levels in lysates of Hep3B cells expressing PRPS1 shRNA with or without reconstituted expression of WT rPRPS1, rPRPS1 T225A, rPRPS1 A190T were measured. The data represent the mean ± s.d. from $n = 3$ independent experiments. A two-tailed Student's t test was used. ∗ represents $P < 0.01$ between the cells with or without PRPS1 depletion. # represents $P < 0.01$ between the PRPS1-depleted cells reconstituted expression of WT rPRPS1 and the rPRPS1 T225A.

(**d, e**) Hep3B cells with reconstituted expression of KHK-C or KHK-A were cultured in the presence or absence of fructose (10 mM). The intracellular levels of ATP (d) and phosphate (e) were measured. The data represent the mean ± s.d. from $n = 3$ independent experiments. A two-tailed Student's t test was used. ∗ represents $P < 0.01$; NS represents not significant difference between the indicated cells with or without fructose treatment.




e

| Expression levels | No. | c-Myc High | c-Myc Low | P value (Chi-square test) |
|---|---|---|---|---|
| HnRNPH1/2 | | | | |
| High | 53 | 46 | 7 | |
| Low | 37 | 5 | 32 | <0.001 |
| KHK-A | | | | |
| High | 56 | 39 | 17 | |
| Low | 34 | 12 | 22 | 0.001 |
| PRPS pT225 | | | | |
| High | 57 | 40 | 17 | |
| Low | 33 | 11 | 22 | 0.001 |

| Expression levels | No. | HnRNPH1/2 High | HnRNPH1/2 Low | P value (Chi-square test) |
|---|---|---|---|---|
| KHK-A | | | | |
| High | 56 | 39 | 17 | |
| Low | 34 | 14 | 20 | 0.008 |
| PRPS pT225 | | | | |
| High | 57 | 39 | 18 | |
| Low | 33 | 14 | 19 | 0.016 |

| Expression levels | No. | KHK-A High | KHK-A Low | P value (Chi-square test) |
|---|---|---|---|---|
| PRPS pT225 | | | | |
| High | 57 | 51 | 6 | |
| Low | 33 | 5 | 28 | <0.001 |

**Supplementary Figure 6** KHK-A–mediated PRPS1 phosphorylation promotes hepatocellular tumorigenesis and is associated with the pathogenesis of HCC.

(**a**) The apoptosis of Hep3B and Huh-7 cells with depletion of hnRNP1/2 (left panel), KHK-A (middle panel), and PRPS1 (right panel) and with or without reconstituted expression of the indicated proteins were examined. The data represent the mean ± s.d. from $n$ = 3 independent experiments.

(**b**) The proliferation rates of Hep3B cells with the depletion of KHK-A and reconstituted expression of the indicated proteins were examined in the presence or absence of added fructose (10 mM) and NAC (2 mM). The data represent the mean ± s.d. from $n$ = 3 independent experiments. * $P < 0.05$.

(**c**) Lysates of tumors derived from Huh-7 cells with KHK or PRPS1 depletion and with or without reconstituted WT rKHK-A, rKHK-A L83A, WT rKHK-C, WT rPRPS1, or rPRPS1 T225A expression were prepared. Immunoblot analyses with the indicated antibodies were conducted.

(**d**) The antibody specificities were validated. Immunohistochemical analysis of the tissues from the tumors derived from Huh-7 cells (left and right panel) or Huh-7 cells with KHK depletion and reconstituted expression of WT rKHK-C (middle panel) with the indicated antibodies was performed in the presence or absence of blocking peptides specific for KHK Exon 3A- or Exon 3C-coded regions or phosphorylated PRPS1 T225.

(**e**) IHC staining of $n$ = 90 human HCC samples with anti-phospho-PRPS1 T225, anti-KHK-A, anti-hnRNPH1/2, and anti-c-Myc antibodies was performed. Chi-square analysis was performed depending on the staining score for each antibody (high staining score, 4.1-8.0; low staining score, 0-4.0).

Figure 1c

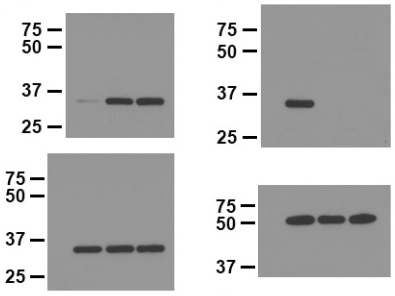



Figure 1f

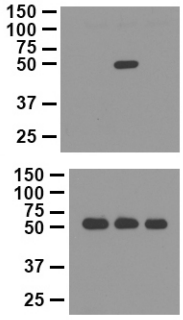



Figure 1g

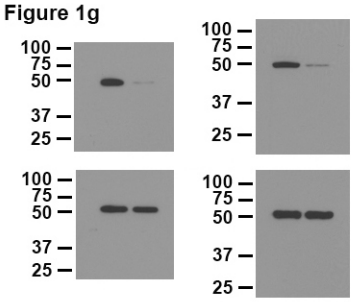



Figure 1h

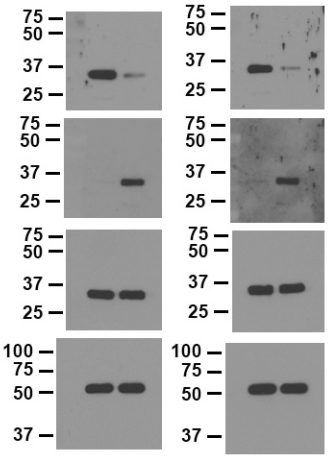



Figure 2b

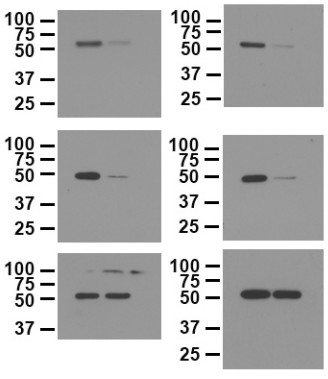



Figure 2c

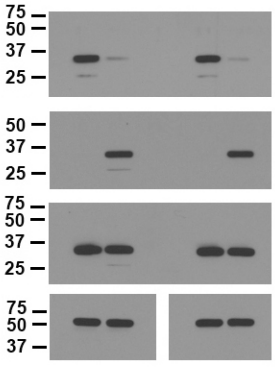
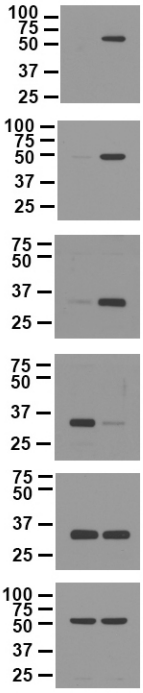
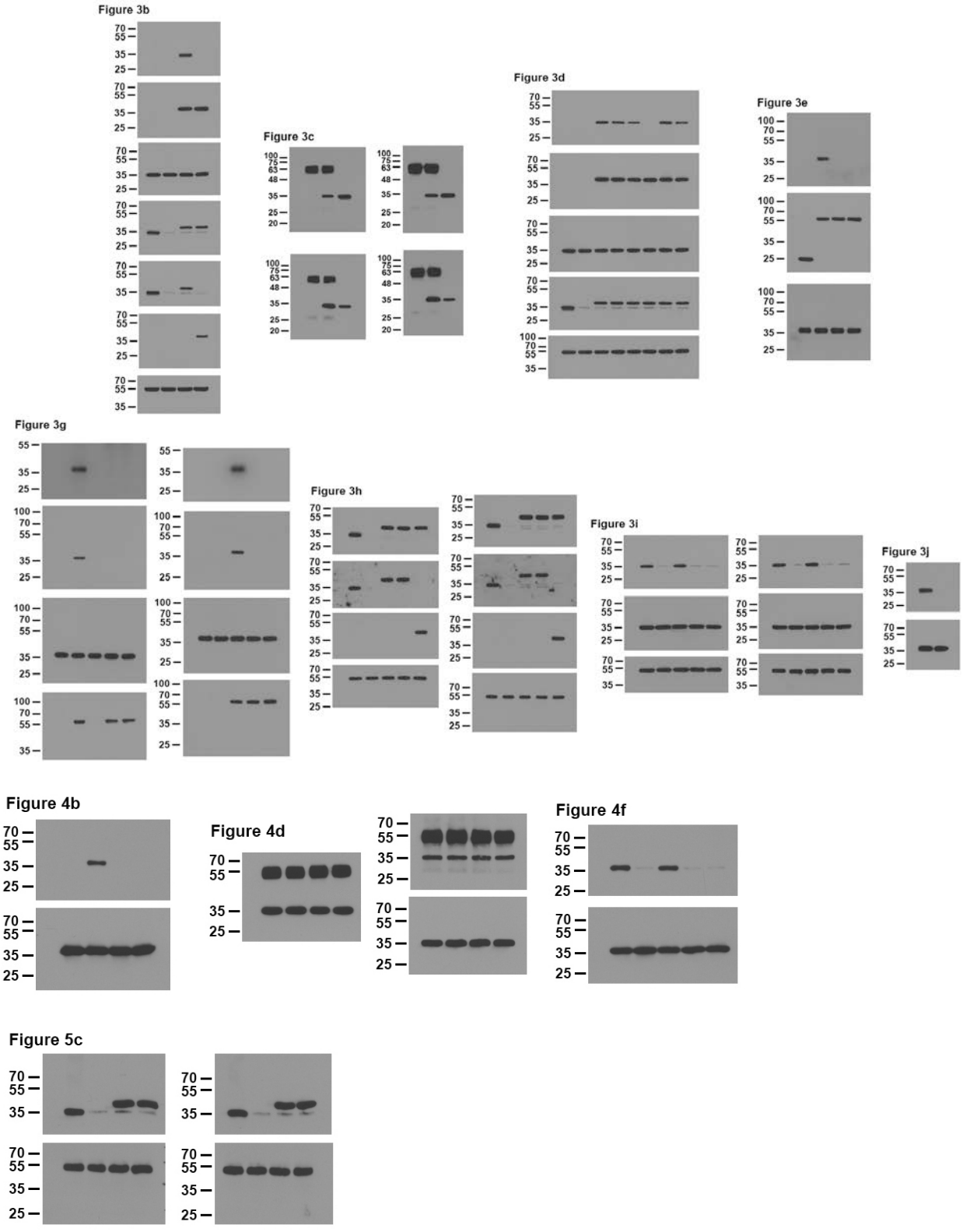



Figure 2d






**Supplementary Figure 7.** The unprocessed blots.

**Supplementary Table 1** Multivariate analysis of c-Myc, hnRNPH1/2, KHK-A, and PRPS1 pT225 expression in HCC samples and patient information (Cox regression model).

| Proteins | Characteristics | HR | 95% CI | *P* |
|---|---|---|---|---|
| c-Myc | Age, y (>49 vs ≤49) | 0.774 | 0.442-1.355 | 0.369 |
| | Sex (female vs male) | 0.898 | 0.355-2.273 | 0.821 |
| | Clinical stage (III/IV vs I/II) | 2.227 | 1.256-3.950 | **0.006** |
| | c-Myc expression (high vs low) | 1.879 | 1.052-3.359 | **0.033** |
| hnRNPH1/2 | Age, y (>49 vs ≤49) | 0.8 | 0.457-1.400 | 0.434 |
| | Sex (female vs male) | 0.901 | 0.355-2.289 | 0.827 |
| | Clinical stage (III/IV vs I/II) | 2.49 | 1.423-4.356 | **0.001** |
| | hnRNPH1/2 expression (high vs low) | 1.862 | 1.061-3.268 | **0.03** |
| KHK-A | Age, y (>49 vs ≤49) | 0.773 | 0.441-1.353 | 0.367 |
| | Sex (female vs male) | 0.934 | 0.370-2.361 | 0.886 |
| | Clinical stage (III/IV vs I/II) | 2.456 | 1.401-4.305 | **0.002** |
| | KHK-A expression (high vs low) | 2.07 | 1.151-3.724 | **0.015** |
| PRPS1 pT225 | Age, y (>49 vs ≤49) | 0.723 | 0.410-1.274 | 0.261 |
| | Sex (female vs male) | 0.888 | 0.351-2.245 | 0.802 |
| | Clinical stage (III/IV vs I/II) | 2.509 | 1.429-4.406 | **0.001** |
| | PRPS1 pT225 expression (high vs low) | 2.011 | 1.116-3.622 | **0.02** |

HR, hazard ratio; CI, confidence interval.

**Supplementary Table 2** The antibody information.

| Antibodies | Company | Catalogue | Clone | Dilution |
|---|---|---|---|---|
| hnRNPH1/2 | Abcam | ab10374 | | 1:1,000 for WB, 1:200 for IHC, 1:100 for IP |
| c-Myc | Abcam | ab32072 | | 1:1,000 for WB, 1:200 for IHC, 1:50 for ChIP |
| V5 | Abcam | ab27671 | clone SV5-Pk1 | 1:5,000 for WB |
| GST | Santa Cruz | sc-138 | clone B-14 | 1:1,000 for WB |
| tubulin | Santa Cruz | sc-5286 | clone B-7 | 1:2,000 for WB |
| PRPS1/2 | Santa Cruz | sc-100822 | clone EE-17 | 1:1,000 for WB, 1:200 for IP |
| pan-KHK-A/C | Santa Cruz | sc-377411 | clone B-6 | 1:1,000 for WB |
| Flag | Sigma | F3165 | clone M2 | 1:5,000 for WB |
| KHK-A | Signalway | SAB513 | | 1 μg/ml for WB, 5 μg/ml for IHC or IP |
| KHK-C | Signalway | SAB514 | | 1 mg/ml for WB, 5 mg/ml for IHC or IP |
| PRPS1 pT225 | Signalway | SAB517p | | 1 μg/ml for WB, 5 μg/ml for IHC or IP |

**Supplementary Table 3** The PCR primers and shRNA information.

| | |
|---|---|
| KHK forward | 5'-GTGGACAAGTACCCTAAGGAG-3' |
| KHK reverse | 5'-GTAGCCAAACAGCTGGAAGAG-3' |
| PRPS forward | 5'-GACCGCCTGGGCCTGGAGC-3' |
| PRPS reverse | 5'-GTGTCAGCCATGTCATCCAC-3' |
| KHKmini forward | 5'-CAGAGATGGCAGCGCGGAG-3' |
| KHKmini reverse | 5'-TAGAAGGCACAGTCGAGG-3' |
| AdML forward | 5'-CTTCCTGGTGGCCGACTTCAG-3' |
| AdML reverse | 5'-GAGTACTGGAAAGACCGCGAAG-3' |
| hnRNPH1/2-binding forward | 5'-GCTCCTGCTGCATCATCAACAAC-3' |
| hnRNPH1/2-binding reverse | 5'-GAGCAACTTCTGGTACACAGAAGC-3' |
| E2F | 5'-CTGCAGCTAGCCTGCTGTCAGCTTGAATTTAGCCTG-3' |
| E4R | 5'-CTAGATCTAGACTCAATGTGGATCCACTTGAACTG-3' |
| pGIPZ control shRNA | GCTTCTAACACCGGAGGTCTT |
| pGIPZ KHK shRNA | TTGCTGACAAACACCACGT |
| pGIPZ PRPS1 shRNA | GATAATATGATCTGCACCT, |
| pGIPZ c-Myc shRNA | AGTCGAGGTCATAGTTCCT |
| pGIPZ hnRNPH1/2 shRNA | TCAACACCCAATCCATTTC |
| hnRNPH1 promoter-specific forward | 5'-AACCCAAGCGTGTAATAATCCGCC-3' |
| hnRNPH1 promoter-specific reverse | 5'-CCTGCGCAACCTAAATAAGGTCCCTT-3' |
| hnRNPH2 promoter-specific forward | 5'-AGGAAACGAGGGCCAGGAAGCGAA-3' |
| hnRNPH2 promoter-specific reverse | 5'-TCATCGGTGATTGGTCCGCCCCTGA-3' |