SUPPLEMENTAL DATA

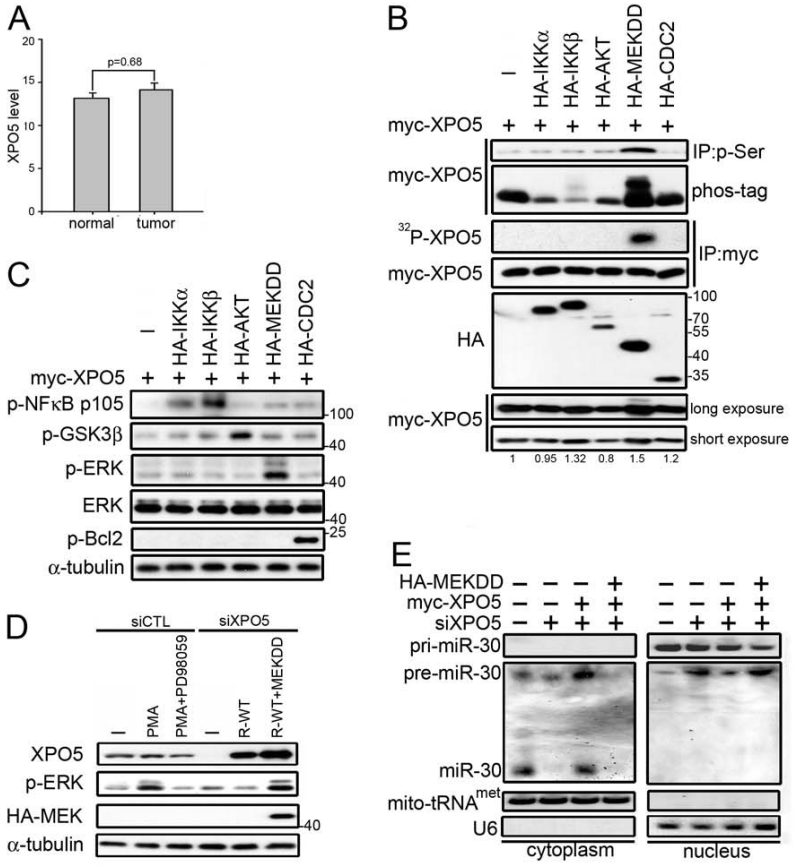

Figure S1, related to Figure 1. ERK activation suppresses XPO5-mediated nuclear export.

(A) Comparison of XPO5 protein expression in paired human normal and liver tumor tissues at Human HistoArray IMH-342 and IMH-318 by IHC staining (n=35 per group, data represents mean ± SEM, p values were calculated by Student's t test).

(B) Lysates of 293T cells transfected as indicated were subjected to IP and analyzed by SDS-PAGE or phos-tag SDS-PAGE. For quantification of the XPO5 expression, the density of each band was quantified by Image J software and indicated below the blots. For detection of the XPO5 phosphorylation, cells were labeled with [^{32}P] orthophosphate.

(C) Substrate phosphorylation in 293T cells co-transfected with oncogenic kinase was analyzed by IB. The activities of IKKα and IKKβ were detected by p-NFκB p105 (Ser932), AKT by p-GSK3β (Ser9), MEK by p-ERK, and CDC2 by p-Bcl2 (Thr56).

(D) IB analysis of siCTL and siXPO5 293T cells. siCTL cells were pre-treated with or without 30 μM PD98059 for 1 hr then treated with 100 nM PMA for 8 hr, and siXPO5 cells were transfected with siRNA-resistant XPO5 (R-WT) with or without MEKDD for 48 hr.

(E) siXPO5 293T cells were transfected with siRNA-resistant XPO5 (R-WT) with or without MEKDD for 48 hr. All cells were co-transfected with pCMV-miR-30. Cytoplasmic and nuclear RNA was extracted using PARIS kit before Northern Blot.

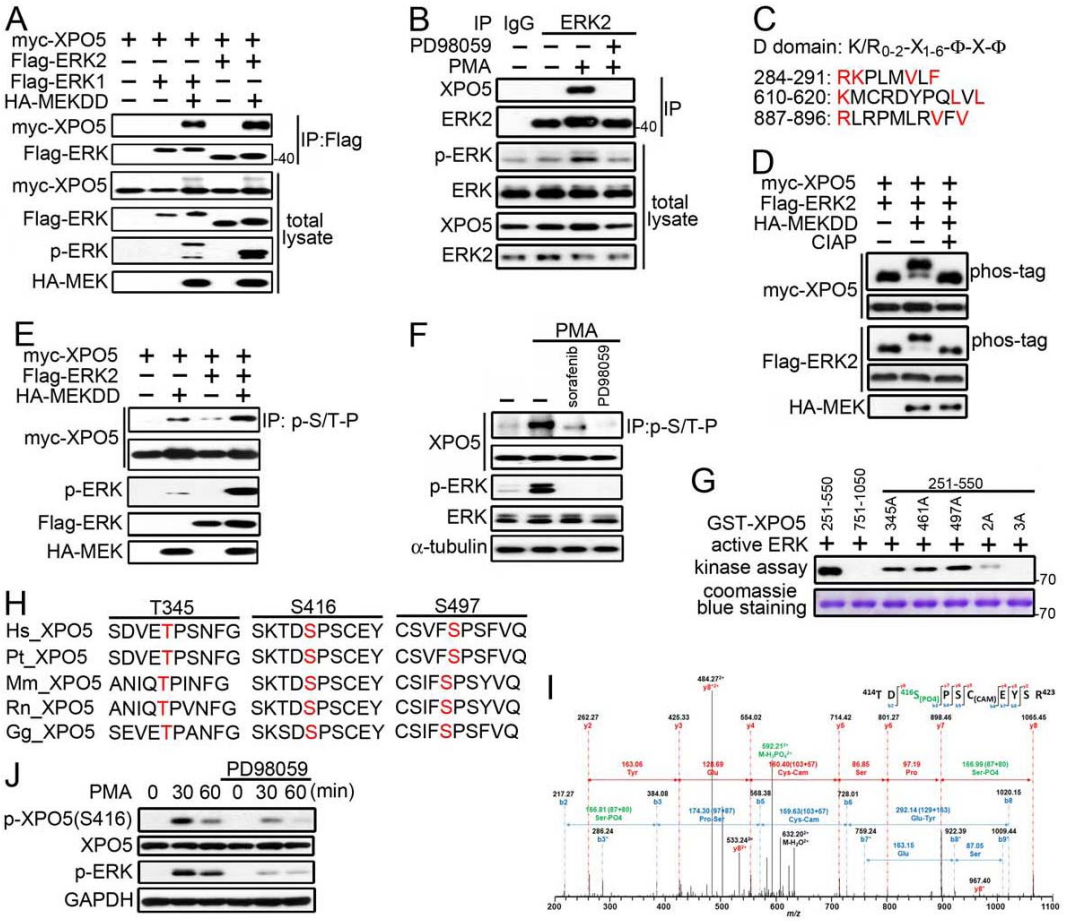

Figure S2, related to Figure 2. ERK interacts with and phosphorylates XPO5.

(A) Lysates of 293T cells transfected as indicated were subjected to IP with anti-Flag antibody followed by IB.

(B) Huh-7 cells were pre-treated with or without 30 μM PD98059 for 1 hr then treated with 100 nM PMA for 30 min. Cell lysates were analyzed by IP and IB.

(C) Putative D-domain on XPO5 predicted by the Eukaryotic linear motif website. D domain possess a consensus binding sequence of $(Lys/Arg)_{0-2}$-$(X)_{1-6}$-Φ-X-Φ; where Φ is a hydrophobic residue such as Leu, Ile, Val, Phe, and X is any amino acid.

(D) Lysates from 293T cells transfected as indicated were treated with calf intestine alkaline phosphatase (CIAP) then subjected to SDS-PAGE or phos-tag SDS-PAGE.

(E-F) Lysates of 293T cell transfected as indicated (E) or Huh-7 cells treated with 100 nM PMA, 5 μM sorafenib, and 30 μM PD98059 (F) were subjected to IP and IB.

(G) In vitro kinase assays were conducted by incubating recombinant activated ERK2 with GST-fusion XPO5 fragment constructs 251-550, 751-1050 that contained 6 potential ERK phosphorylation sites (S/T-/P), that are T345, S416, S497, S826, S913, and T1004.

(H) Sequence alignment of the conserved serine/threonine residues on XPO5 that are phosphorylated by ERK. Hs, *Homo sapiens*; Pt, *Pan troglodytes;* Mm, *Mus musculus;* Rn, *Rattus norvegicus;* Gg, *Gallus gallus.*

(I) Mass spectrometry detected ERK phosphorylated XPO5 at S416 in Huh-7 cells after treatment with 100 nM PMA for 30 min.

(J) Huh-7 cells were pre-treated with or without 30 μM PD98059 for 1 hr then treated with 100 nM PMA for 30 min. Cell lysates were analyzed by IB.

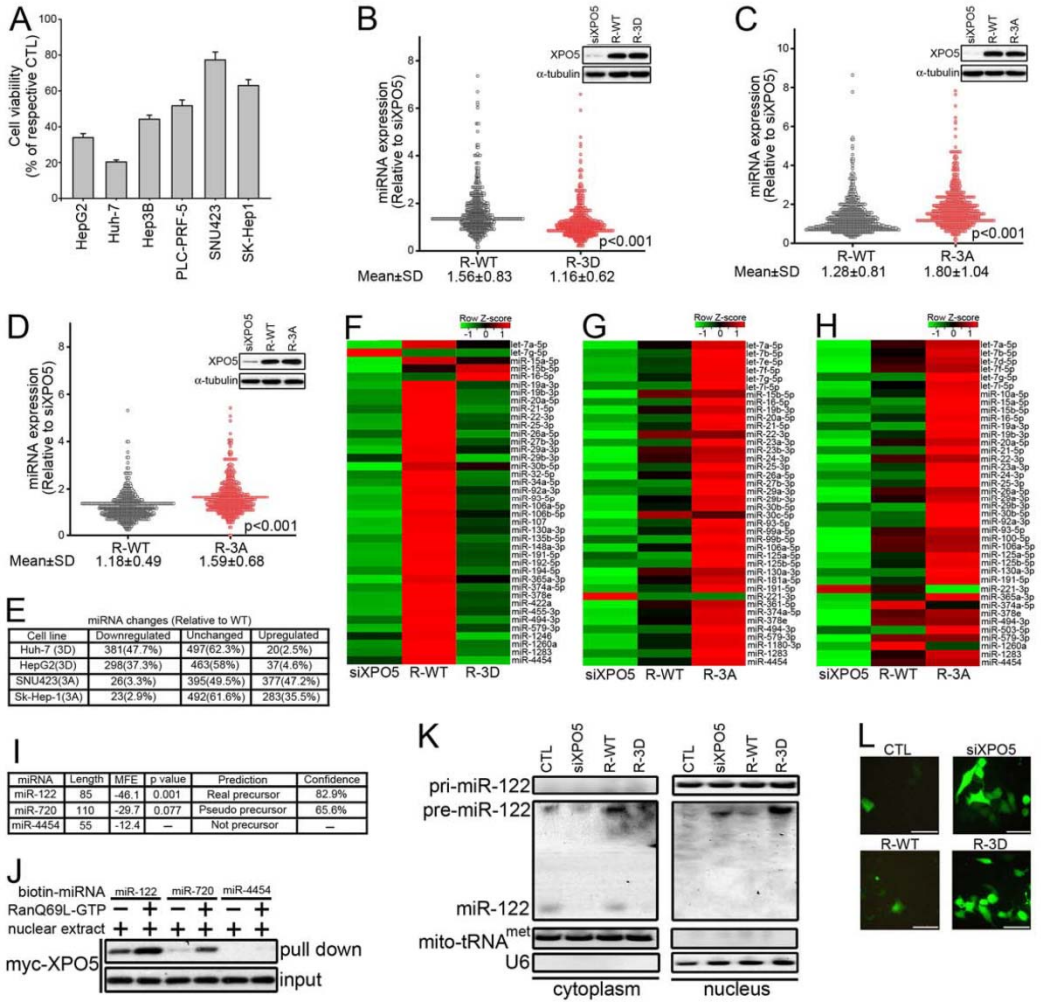

Figure S3, related to Figure 3. XPO5 phosphorylation globally downregulates miRNA.

(A) Liver cancer cells were treated with 5 μM sorafenib for 48 hr before MTT assay. Data were presented as relative to the cells without sorafenib treatment (n=3 per group, data represents mean ± SEM, p values were calculated by Student's t test).

(B-D) Dot density plots show relative miRNA expression in the HepG2 (B), SNU-423 (C), and SK-Hep-1(D) stable transfectants profiled by nanostring assay. Data were presented as relative to the miRNA expression in siXPO5 cells. Inset: IB of XPO5.

(E) miRNA changes in XPO5 mutant compared to R-WT stable transfectants. Based on relative expression levels, miRNAs were classified into three sets: downregulated (<0.67-fold), unchanged (0.67- to 1.5-fold), and upregulated (> 1.5-fold).

(F -H) Heat maps generated using R platform shows relative miRNA expression in HepG2 (F), SNU-423 (G), and SK-Hep-1 (H) stable transfectants as indicated by the green to red key bar at the top of the map. Nanostring counts >350 are shown.

(I) Classification of pre-miR122, pre-miR-720, and pre-miR-4454 by MiPred.

(J) Biotinylated pre-miRNA was bound to the Strepavidin magnetic beads and incubated with 293T cell lysates with or without RanQ69LGTP. The precipitated proteins were analyzed by IB.

(K) Cytoplasmic and nuclear RNA was extracted from Huh-7 stable transfectants using PARIS kit before Northern blot.

(L) EGFP reporter containing miR-122-binding site in the 3'UTR was transfected into Huh-7 stable transfectants for 48 hr before fluorescence microcopy. Scale bars: 50 μm.

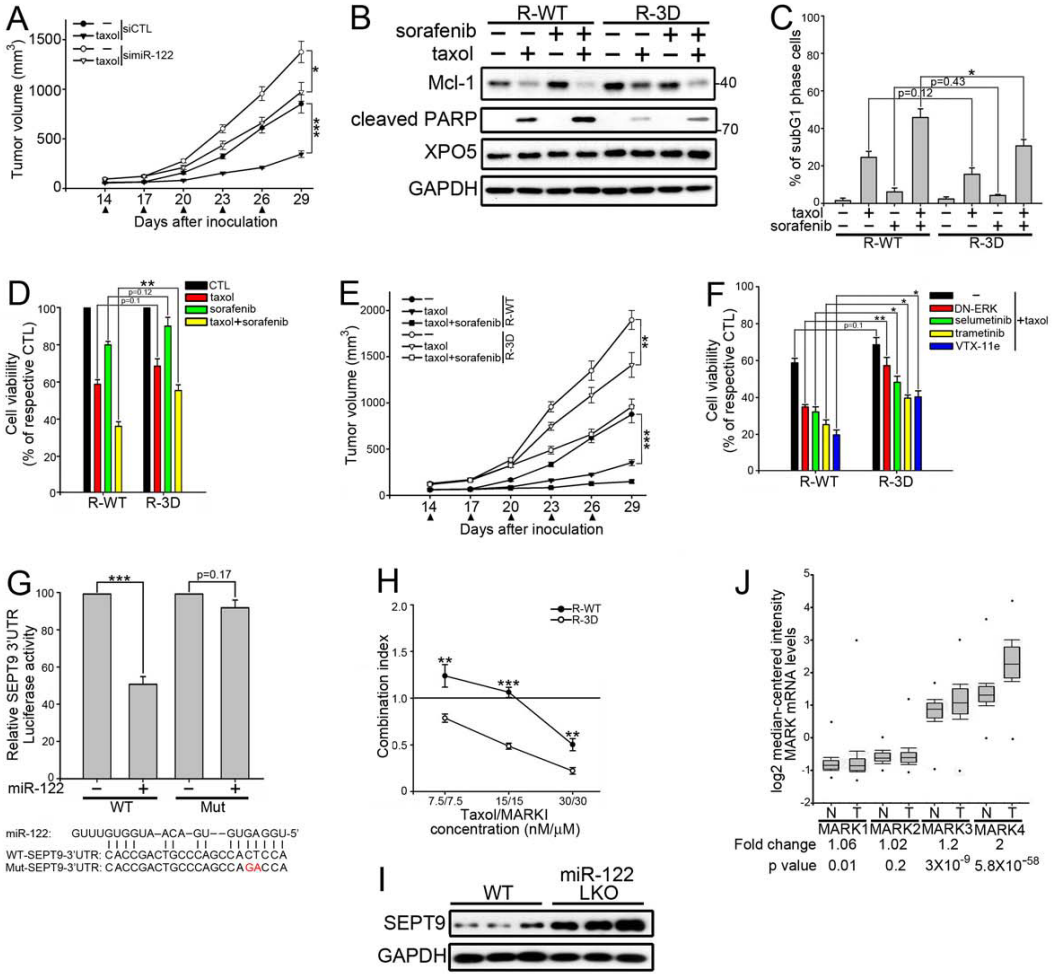

Figure S4, related to Figure 4. XPO5 phosphorylation decreases sorafenib but increases MARK inhibitor synergism with taxol.

(A) Huh-7 stable transfectants were inoculated s.c. in SCID mice for 14 days then treated with taxol (20 mg/kg/q3d, i.v.) for 5 cycles (n=5 per group, data represents mean ± SEM, p values were calculated by Student's t test). Tumor volumes were determined by caliper. Arrowheads indicate dates of taxol injection.

(B-D) Huh-7 stable transfectants were treated with 30 nM taxol and 3 μM sorafenib for 48 hr before IB (B) , flow cytometry (C) , and MTT assay (D) (n=3 per group, data represents mean ± SEM, p values were calculated by Student's t test).

(E) Huh-7 stable transfectants were inoculated s.c. into SCID mice for 14 days, then treated with taxol

(20 mg/kg/q3d, i.v.) with or without sorafenib (20 mg/kg/d, p.o.) for 5 cycles (n=5 per group, data represents mean ± SEM, p values were calculated by Student's t test). Tumor volumes were determined by caliper. Arrowheads indicate dates of taxol injection.

(F) Huh-7 stable transfectants were treated with 30 nM taxol with or without MEK inhibitors (1 μM selumetinib, 0.3 μM trametinib), ERK inhibitor (0.3 μM VTX-11e) for 48 hr before measuring viability by MTT assay. Data were presented as relative to the cells without drug treatment (n=3 per group, data represents mean ± SEM, p values were calculated by Student's t test).

(G) 293T cells were transfected with WT or mutated pLightSwtich-SEPT9-3′UTR, with or without miR-122 before measuring Renilla luciferase expression at 48 hr. Data were presented as relative to the cells transfected with the 3'UTR reporter without miR-122 overexpression (n=3 per group, data represents mean ± SEM, p values were calculated by Student's t test). The lower panel shows the sequence of WT and miR-122 binding site mutant of SEPT9-3′UTR.

(H) The combination index plot for the Huh-7 stable transfectants exposed to fixed molar ratios, 1:1000, of taxol to MARKI for 48 hr (n=3 per group, data represents mean ± SEM, p values were calculated by Student's t test).

(I) IB of SEPT9 expression in isolated WT and liver-specific miR-122 knockout (LKO) hepatocytes.

(J) Oncomine analysis of deposited Roessler dataset (Roessler et al., 2010) shows upregulation of MARK4 mRNA in tumor (T) (n=225) compared to normal (N) (n=220) tissues. The box extends from the 25th to 75th percentiles with the central line being the median, and whiskers stretching from 10th to 90th percentiles. The dots outside the whiskers represent minimum and maximum values.

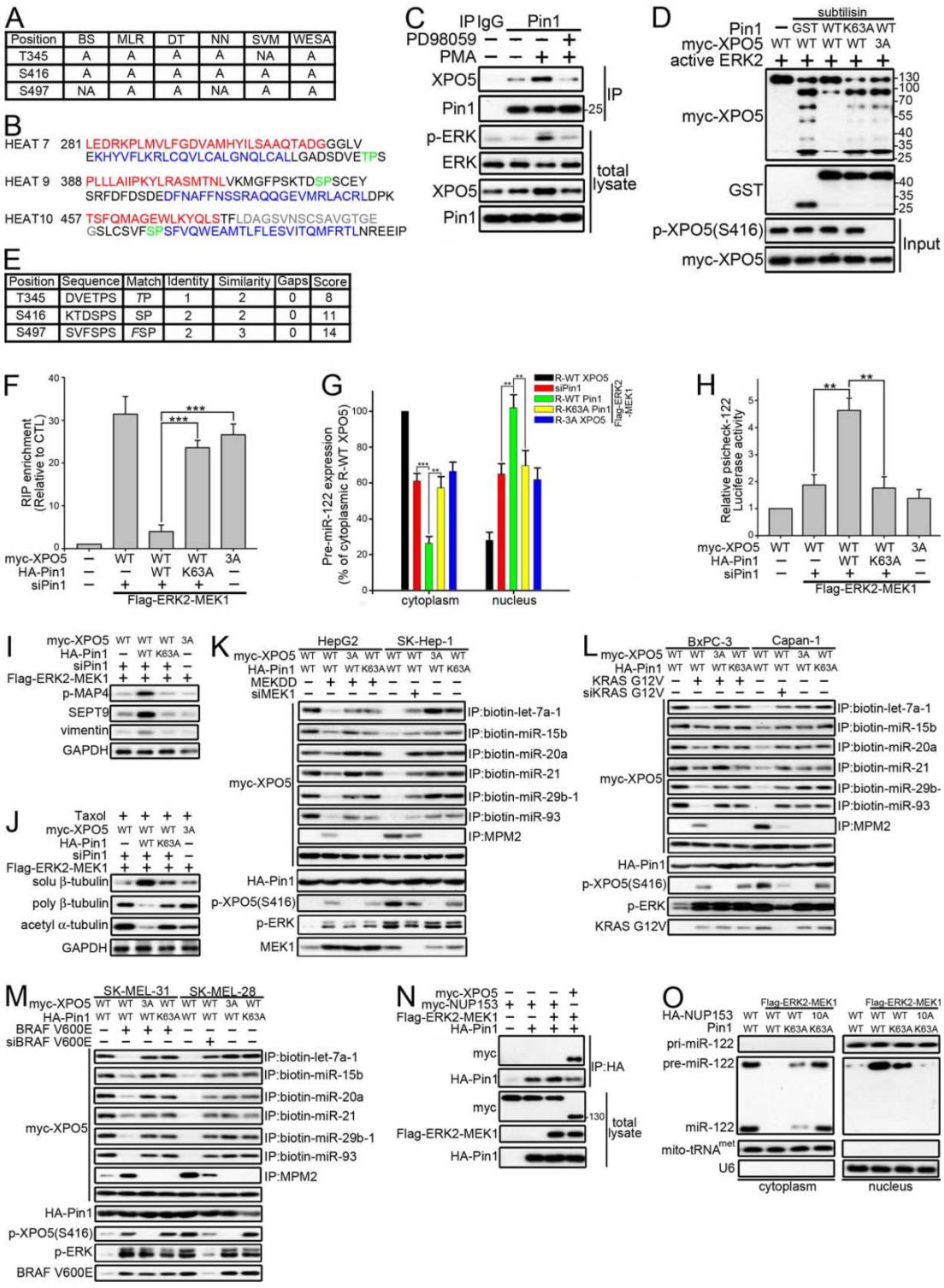

Figure S5, related to Figure 5. Pin1-induced conformational changes inhibit XPO5 function.

(A) Prediction of surface accessibility of potential Pin1-interacting sites on XPO5 using the WESA algorithm. Possibility of accessible (A) or non-accessible (NA) was calculated by Bayesian statistics (BS), multiple linear regression (MLR), decision tree (DT), neutral network (NN), and support vector machine (SVM). WESA score is based on a weighted sum of these five predictions.

(B) Corresponding secondary structures around Pin1-interacting sites on XPO5 are represented.

Residues in helical conformations are shown in blue (inner helix) and red (outer helix). Potential Pin1 interacting sites were marked in green. Residues with unknown atomic coordinates are shown in grey.
(C) Huh-7 cells were pre-treated with or without 30 μM PD98059 for 1 hr then treated with 100 nM PMA for 30 min. Cell lysates were analyzed by IP and IB.
(D) Ectopically expressed myc-XPO5 was purified from Pin1 knocked down 293T cells followed by phosphorylation with active ERK2. Purified proteins bound to beads then incubated with GST, GST-Pin1, or GST-Pin1 (K63A) for 30 min before subtilisin treatment for 5 min.
(E) Pairwise sequence alignment with Pin1 binding model sequence WFYS*PR using EMBOSS with local sequence alignment algorithm WATER. Perfectly matched amino acids are referred as the identity, and similar types (acid/basic) were designed as similarity. A gap penalty of 10 as well as extended penalty of 0.5 was set to obtain the score for alignment.
(F) Lysates of Huh-7 stable transfectants were subjected to RIP analysis. Extracts of Huh-7 stable transfectants were subjected to IP with anti-myc tag antibody. Pull-down RNA was analyzed by qRT-PCR using specific primers for pre-miR-122. Data were presented as relative to the CTL Huh-7 cells (n=3 per group, data represents mean ± SEM, p values were calculated by Student's t test).
(G) Cytoplasmic and nuclear RNA were isolated from Huh-7 stable transfectants using PARIS kit then subjected to pre-miR-122 qPCR analysis. Data were presented as relative to the cytoplasmic pre-miR-122 expression in R-WT XPO5 Huh-7 cells (n=3 per group, data represents mean ± SEM, p values were calculated by Student's t test).
(H) Huh-7 stable transfectants were transfected with a reporter with miR-122-binding sites for 48 hr before luciferase assay. Data were presented as relative to R-WT XPO5 Huh-7 cells (n=3 per group, data represents mean ± SEM, p values were calculated by Student's t test).
(I) IB of miR-122 targeted proteins in Huh-7 stable transfectants.
(J) Total cell lysates or tubulin fractions of Huh-7 stable transfectants treated with 100 nM taxol for 24 hr were analyzed by IB.
(K-M) Lysates of liver cancer (K), pancreatic cancer (L), or melanoma (M) stable transfectants were incubated with biotinylated pre-miRNA or MPM2 antibody. Pull-down proteins were analyzed by IB.
(N) Lysates of 293T cells transfected as indicated were subjected to IP with anti-HA antibody followed by IB.
(O) Cytoplasmic and nuclear RNA was extracted from Huh-7 stable transfectants using PARIS kit before Northern blot.

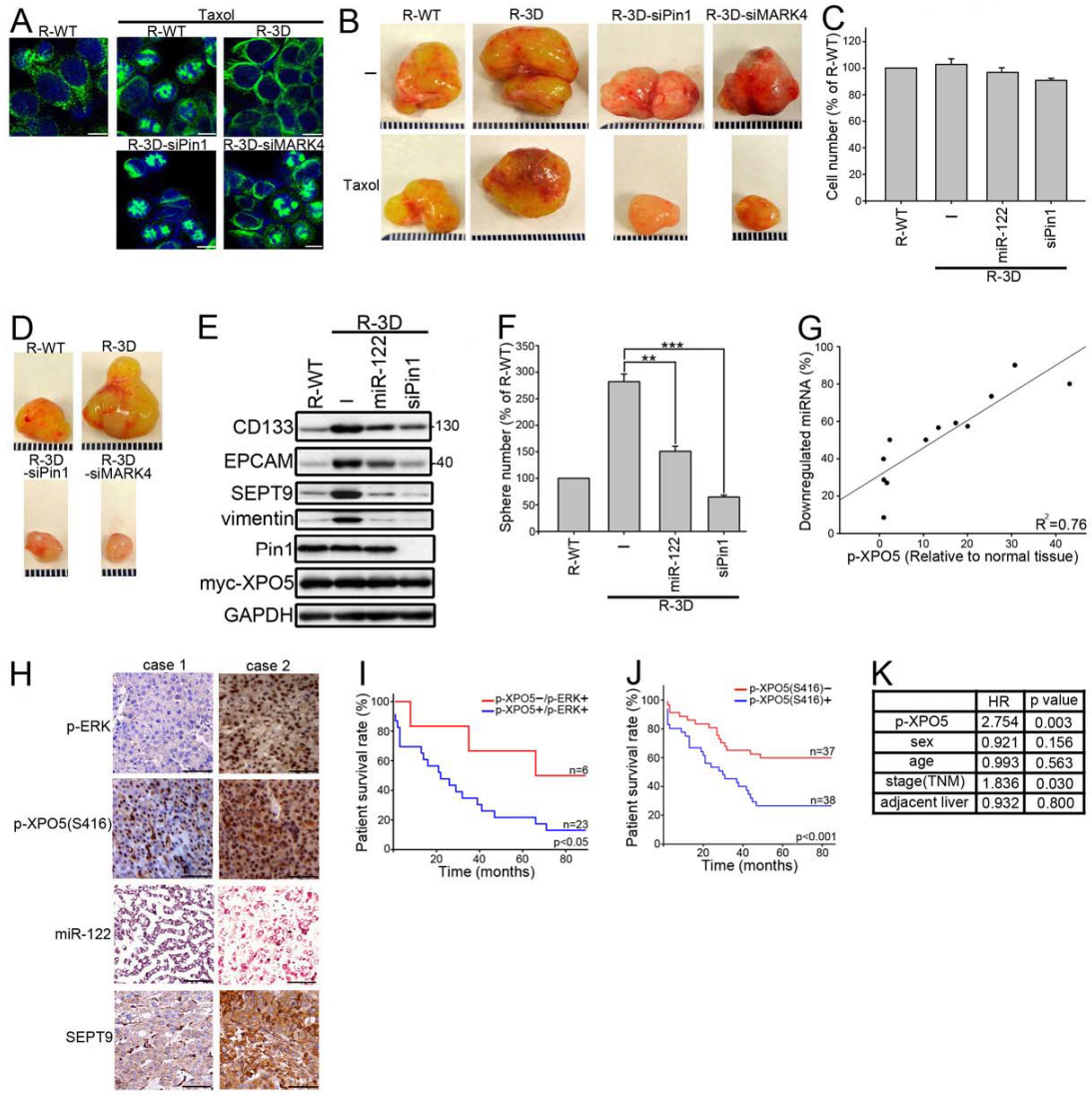

Figure S6, related to Figure 6. Clinical association between ERK phosphorylation and miRNA downregulation.

(A) Immunofluorescence analysis of β–tubulin (green) in Huh-7 stable transfectants treated with 100 nM taxol for 24 hr. DAPI (blue) was used to mark the nucleus. Scale bars: 10 μm.

(B) Representative photograph of engrafted Huh-7 tumors from mice that received taxol treatments for 10 days.

(C) Growth of Huh-7 stable trasfectants after 2 days was measured by MTT assay. Data were presented as relative to the R-WT Huh-7 cells (n=3 per group, data represents mean ± SEM).

(D) Representative photograph of engrafted Huh-7 tumors from mice that received transplants after 24 days.

(E) IB of cancer stem cell surface marker CD133 and EPCAM in Huh-7 stable transfectants.

(F) The amount of tumor spheres on day 7 formed from 20000 cells was counted under microscopy. Data were presented as relative to the R-WT Huh-7 cells (n=3 per group, data represents mean ± SEM, p values were calculated by Student's t test).

(G) The correlation between miRNA downregulation (Figure 6F) and p-XPO5 (Figure 6G) were

analyzed by linear regression (n=12). Compared to normal tissue, expression levels lower than 0.67 were classified as downregulated miRNA. The density of p-XPO5 was quantitiated using Image J software.

(H) Representative liver cancer specimens for p-ERK, p-XPO5, miR-122 or SEPT9 staining on Human Histoarray IMH-318. DAB chromogen (brown) was used for monitoring protein expression while NBT/BCIP (blue) was used for miRNA expression. Scale bars: 50 μm.

(I) Kalplan-Meier survival curves of liver cancer patients on Human HistoArray IMH-318 grouped by p-XPO5/p-ERK double positive and p-XPO5 negative/p-ERK positive expression.

(J) Kalplan-Meier survival curves of liver cancer patients on validation cohort liver tissue microarray LVC1504 grouped by p-XPO5 expression.

(K) Multivariate analyses of parameters associated with survival of HCC patients (n=75) on validation cohort liver tissue microarray LVC1504. HR: hazard ratio.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmids, antibodies, and chemicals

pKmyc-XPO5 (#12552), pCMV-Luc-miR-30 (#20875), pCMV-miR-30 (#20670), pCMV-VSVG (#8454), and pCMV-dR8.2 dvpr (#8455) were ordered from Addgene. Flag-IKKα, Flag-IKKβ, HA-AKT, HA-MEKDD, HA-CDC2, Flag-DN-ERK, and GST-Pin1 were kindly provided by M.C. Hung (M.D. Anderson Cancer Center, Houston, TX). siCTL (SHC002), siXPO5 (TRCN0000158915), siMARK1 (TRCN000 0006330), siMARK2 (TRCN0000001584), siMARK3 (TRCN0000001567), siMARK4 (TRCN0000007158), siPin1 (TRCN0000010577), siMEK1 (TRCN0000002329), or siNUP153 (TRCN0000296605) were purchased from Sigma. The annealed KRAS G12V (Sunaga et al., 2011) or BRAF V600E (Kumar et al., 2007) specific targeted sequence was inserted into pLK0.1 (Addgene # 10879). GFP-NLS-eEF1A was a gift from J. Dahlberg (University of Wisconsin, Madison, WI). The full length ERK1 (Addgene #23509), ERK2 (Addgene # 23498) or ERK2-L4A-MEK1 fusion (Addgene 39197, a constitutively active and nuclear form of the ERK2 (Robinson et al., 1998) cDNA was subcloned into the pCMV5-Flag. IKKα, IKKβ, Pin1 or NUP153 (Addgene #64268) cDNA was subcloned into the pCMV5-HA. NUP153 cDNA was also subcloned into pkmyc. All mutants were generated using the Quikchange site-directed mutagenesis kit (Strategene). XPO5 and various mutants were subcloned into pCDH-CMV-MCS-EF1-Neo vector, Flag-ERK2-L4A-MEK1 fusion, KRAS G12V (Origene # RC400109), BRAF V600E (Addgene 17544) was cloned into pCDH-CMV-MCS-EF1-RFP vector, and HA-NUP153 was cloned into pCDH-CMV-MCS-EF1-Hygro vector (System Bioscience). GST-XPO5 fusion constructs were generated by subcloning the optimized XPO5 fragment sequence (synthesized by Genscript) into the pGEX4T3 (GE healthcare). MiRZip-122 , lenti-miR-122, and pBLIV-EF1-GFP-T2A-Luciferase were obtained from System Bioscience. EGFP-122 was kindly provided by P. Sarnow (Stanford University, Stanford, CA) and psiCHECK-122 was provided by A. Deiters (University of Pittsburgh, Pittsburgh, PA). pLightSwtich empty and SEPT9 3'UTR were ordered from Switchgear genomics. GST-RanQ69L was a generous gift from Y. Zheng

(Carnegie Institution for science, Baltimore, MD).

Antibodies used in this work were myc-tag, α-tubulin, Histone H3, p-ERK, ERK, ERK2, GAPDH, Mcl-1, cleaved PARP, vimentin, acetyl-α-tubulin, Pin1, EPCAM, p-NFκB p105(Ser932), p-GSK3β(Ser9), p-Bcl2(Thr56), MEK1, KRAS G12V (Cell Signaling), Flag (Sigma), HA (Roche), p-serine, p-tyrosine, p-threonine-proline, SEPT2, MPM2, CD133 (Millipore), XPO5 (Abnova), SEPT9 (Thermo Scientific), p-MAP4 (Ser941), MAP4, β-tubulin (Abcam) and BRAF V600E (RevMab). Antibodies to the Thr345, Ser416, and Ser497 phosphorylation sites of XPO5 were generated in collaboration with Lifetein LLC. Synthetic phosphorylated peptides as following were used as antigens for producing antibodies, phospho-345T: GADSDVE-pT-PSNFGKYL, phospho-416S: GFPSKVD-pS-PSCEYSR, phospho-497S: GSLCSVF-pS-PSFVQWE. ELISA and dot blot analysis were performed for the verification of antibody specificity (Lifetein LLC). Phorbol 12-myristate 13-acetate (PMA), PD98059, vincristine, and taxol were purchased from Cayman chemicals. Cisplatin, 5-fluorouracil, and VTX-11e were purchased from Tocris Bioscience. Active ERK2, TGF-α, PP2A, and MARK/Par-1 activity inhibitor (MARKI) were purchased from Millipore. Trametinib and Selumetinib were purchased from Selleckchem. Sorafenib was ordered from Biorbyt. Juglone, PiB, and subtilisin were purchased from Sigma.

Cell culture and transfection

293T cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS). HepG2, Huh-7, Hep3B, PLC-PRF-5, SNU-423, SK-Hep-1, BXPC-3 cells were cultured in RPMI medium with 10% FBS. Capan-1 cells were culture in IMDM medium with 20% FBS. SK-MEL-28 and SK-MEL-31 cells were culture in MEM medium with 10% FBS and nonessential amino acids. Primary hepatocytes were isolated from WT or miR-122 liver-specific knockout (LKO) mice and cultured as previously described (Hsu et al., 2012). For transient transfection, cells were transfected with DNA by Lipofectamine 2000 or with MiRIDIAN microRNA mimic (Dharmacon) by Lipofectamine RNAiMAX as previously described (Lee et al., 2007). For lentivirus production, lentiviral construct (pLKO.1-derived plasmids for knockdown XPO5, MARKs, Pin1, MEK1, KRAS G12V, or BRAF V600E expression, pCDH-CMV-MCS-EF1 plasmids for overexpressing XPO5, ERK2-L4A-MEK1 fusion, Pin1, KRAS G12V, BRAF V600E or NUP153, or pBLIV-EF1-GFP-T2A-Luciferase for luciferase expression used to monitor in vivo tumor growth) together with pCMV-dR8.2 dvpr and pCMV-VSVG were co-transfected into 293TN cells (System Bioscience). Viruses were concentrated by the PEG-*it* Virus precipitation solution and used for infecting cells in the presence of Transdux (System Bioscience). Pools of stable transfectants were selected by antibiotics or sorted by flow cytometry. Aldefluor assay were performed according to the commercial protocol (Stemcell Technologies) to detect the enzyme activity of Aldehyde Dehydrogenase. For sphere culture, 20000 cells were plated in ultralow attachment 6 well plates (Corning) with MammoCult medium (Stemcell Technologies) for 7 days. Tumor spheres that are larger than 60μm were counted under microscopy. Luciferase assays were performed using the Dual-Luciferase Assay System according to the commercial protocol (Promega).

Immunoprecipitation, immunoblotting, and in vitro kinase assay

Immunoprecipitation (IP), immunoblotting (IB), and in vitro ERK2 kinase assay were performed essentially as previously described (Lee et al., 2007). In IB, sizes of proteins were labeled with molecular weight marker when they appeared first in the manuscript. Recombinant GST-XPO5 proteins were isolated from the inclusion body pellet using inclusion body solubilization reagent (Pierce), then renatured according to the commercial protocol. Phosphate-affinity gel electrophoresis was performed in gels containing 60 μM $MnCl_2$, and 30 μM acrylamide-pendant Phos-tag ligand (AAL-107, Wako Chemicals). Nuclear-cytoplasmic fractionation was performed with the NE-PER kit (Pierce). In vivo labeling with [^{32}P] orthophosphate was performed as previously described (Huang et al., 2007). To quantitate the degree of in vivo tubulin polymerization, soluble and polymerized forms of tubulin were extracted and analyzed by IB (Wang et al., 2012). For the subtilisin-mediated partial proteolysis and PP2A-mediated dephosphorylation assay, ectopically expressed myc-XPO5 was purified from Pin1 knocked down 293T cells followed by phosphorylation with active ERK2. Purified proteins bound to beads then incubated with GST, GST-Pin1, or GST-Pin1 (K63A) for 30 min at 20°C. Subtilisin was added at 4°C while PP2A was added at 20°C for 5 min (Park et al., 2012). The reaction was stopped by the addition of the sample buffer then analyzed by IB.

For the GST pull-down assay, glutathione Sepharose 4B (GE healthcare) slurry was saturated with GST-Pin1, or GST-RanQ69L at 4°C overnight. For GTP loading, 2 mM GTP was incubated with RanQ69L in the loading buffer (50 mM Hepes (pH 7.3), 200 mM NaCl, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol) for 2 hr at room temperature. After loading, 50 mM $MgCl_2$ was added and incubated at 4°C for 20 min to stop the loading. GST protein then incubated with Huh-7 cell lysates at 4°C for 2 hr. The bound proteins were eluted with sample buffer and then analyzed by IB. RNA pull-down assay was performed using the Magnetic RNA-protein pull-down kit (Pierce) according to the manufacturer's instructions. Biotinylated pre-miRNA was bound to the Strepavidin magnetic beads and incubated with cell lysates and RanQ69LGTP for 1 hr at 4 ^{o}C. After the beads were washed three times, the bound protein was eluted by biotin elution buffer and analyzed by IB.

Mass spectrometry

To identify phosphorylation sites of XPO5, GST-XPO5 251-500 incubated with activated ERK2 or XPO5 precipitated from Huh-7 cells treated with PMA 30 min was analyzed by SDS-PAGE. The protein band corresponding to XPO5 was excised and subjected to in-gel digestion with tryspin and chemotrypsin. Samples were analyzed by ultimate capillary LC system (Dionex) directly coupled to LTQ Orbitrap mass analyzer (Thermo Scientific) using TopTenTM method. The data were searched on MASCOT (MassMatrix) against human Swiss-Prot database. All the identified phospho peptides were further confirmed by manually check.

Quantitation of miRNA

Total RNA was extracted by TRIzol (Invitrogen). Nuclear and cytoplasmic RNAs were extracted using the PARIS kit (Ambion) according to the manufacturer's instructions. RNA-binding protein immunoprecipitation (RIP) assay was performed using the Magna RIP kit (Millipore) according the

manufacture's instruction. Myc antibody was bound to the magnetic beads and added to each RIP reaction for incubation overnight at 4°C. 10% beads were used to show comparable amount of myc-XPO5 was precipitated, while the residual immunoprecipitate was digested with proteinase K, extracted by phenol-chloroform and precipitated by ethanol to isolate RNA for qRT-PCR. Pri- and mature miRNA were measured using the Taqman assays provided by Applied Biosystems. To measure the pre-miRNA, hairpin primers were designed to amplify both the pri-miRNA and pre-miRNA: miR-30 sense (GTAAACATCCTCGACTGGAAGCT) and antisense (GCTGCAAACATCCGACTGAA); miR-122 sense (GGAGTGTGACAATGGTGTTTG) and antisense (TTTAGTGTGATAATGGCGTTTG) (Jiang et al., 2005). The amount of pre-miRNA was calculated using the equation: pre-miRNA= $2^{-CT(\text{hairpin primer})} - 2^{-CT(\text{Taqman pri-miRNA primer})}$. Northern blot analysis was performed as described (Ramkissoon et al., 2006). 10% TBE-Urea acrylamide gel was used for pri-miRNA analysis while 15% TBE-Urea acrylamide gel was used for pre-miRNA and mature miRNA. DNA oligonucleotide probes (miR-30a: CTTCCAGTCGAGGATGTTTACA, miR-122: AAACACCATTGTCACACTCCA, U6: CACGAATTTGCGTGTCATCCTT, mitochondria tRNA met: GTATAACCAACATTTTCGGGGTATGGG) were end-labeled with digoxigenin-ddUTP using DIG oligonucleotide 3'end labeling kit (Roche). Probe detection was performed using the DIG luminescent detection kit (Roche) following the manufacturer's protocol. For the miRNA profiling, total RNA was submitted to the Ohio State University Comprehensive Cancer Center Genomics Shared Resource for further processing by the NanoString nCounter system. Dot density plots were created using sigmaplot and used to compare the miRNA expression distribution within study groups. Each dot indicates the individual level of miRNA expression.

Fluorescent microscopy and confocal microscopy

For imaging the GFP, 293T cells were transfected with GFP-NLS-eEF1A and Huh-7 cells were transfected with EGFP-122 then detected by fluorescence microscope (Nikon). For confocal microscopy, cells after treatments were fixed in 4% paraformaldehyde, permeabilized with 0.5% Triton X-100, blocked with 5% bovine serum albumin, incubated with primary antibodies overnight at 4°C, followed by incubation with the appropriate secondary antibody tagged with Alexa 488 or Alexa 568 (Molecular Probes). Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) before mounting. Confocal fluorescence images were captured using Olympus FV1000 confocal spectral microscope.

Evaluation of cell viability

The percentage of surviving cells after treatment was assessed by the MTT assay (Wang et al., 2012). For the application of Chou and Talay method to the drug combination study of taxol, fixed dose ratios based on the IC50 were applied (1:100 for taxol and sorafenib, 1:1000 for taxol and MARKI). The results were calculated by the Calcusyn software to obtain the combination index (CI). CI values of 0.9-1.1 are considered additive, 0.8 and below show moderate-to-strong synergism, while above 1.2 have moderate-to-strong antagonism for the drug pair tested. Cell cycle distribution was assessed by flow cytometry as described (Wang et al., 2012).

Mouse model for tumorigenesis

Each mouse was inoculated s.c. in the dorsal flank with 5×10^6 Huh-7 stable transfectants suspended in 0.1 ml serum-free medium containing 50% Matrigel (BD Biosciences). The tumor volume was measured by caliper and calculated using the following formula: volume (mm^3) = $(width)^2 \times length \times 0.52$. When the tumor volume reached about150 mm^3, mice were randomized into groups (n=5). Taxol and sorafenib were dissolved in Cremophor EL/ethanol (1:1) then diluted with 5% glucose or water before treatment. Taxol was given 20 mg/kg, i.v., once every three days or with sorafenib 20 mg/kg/d, p.o. For testing the efficacy of taxol and sorafenib, wild type Huh-7 cells were implanted at the left flank while simiR-122 or 3D XPO5 stable transfectants were at the right flank of mice. Treatments were started at day 14. For testing sensitivity to taxol, R-WT, R-3D, R-3D-siPin1, and R-3D-siMARK4 expressing cells were injected into mouse flanks on both sides. Because we noticed different growth rates of these stable transfectants, taxol 20 mg/kg q3d was started at different dates when tumor reaches 150 mm3. R-WT group starts on day 20, R-3D on day 16, R-3D-siPin1 on day 24, and R-3D-siMARK4 on day 28. Study was stopped when the tumor volume reached 1500 mm^3. Tumors were removed from sacrificed mice and photographed.

Human liver tumor samples and IHC staining

Primary human liver cancer and adjacent normal tissues for IP and IB were obtained from the Cooperative Human Tissue Network at the Ohio State University James Cancer Hospital. Tissue specimens were procured and studied in accordance with the Ohio state University Institutional Review Board approved protocol. Human HistoArray IMH-318 (liver cancer) and IMH-342 (matched normal tissue) were purchased from Imgenex as discovery cohort, while liver tissue microarray LVC1504 from Pantomics was used as validation cohort. In situ detection of miR-122 with the 5' DIG-labeled LNA-modified probe (Exiqon) and immunohistochemical analysis with p-ERK, p-XPO5, and SEPT9 were performed as previously described with minor modifications (Nuovo et al., 2009). The immunoreactivities were semi-quantitatively scored using a well-established score system in which immunoreactivity score was generated by incorporating both the percentage of positive tumor cells and the intensity of staining (Lee et al., 2007). Kaplan-Meier survival curves were generated using scores of negative versus positive p-XPO5(416) as strata.

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