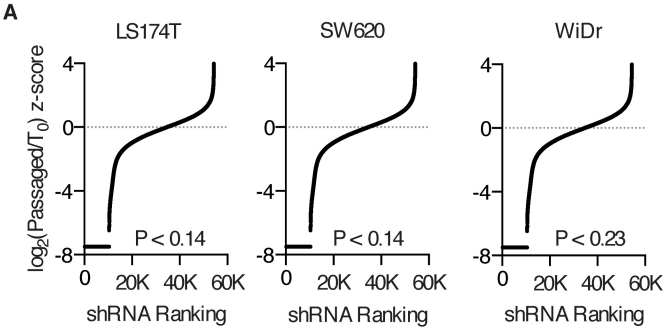
Nguyen et al., Supplemental Figures, Methods and References

Supplemental Figures

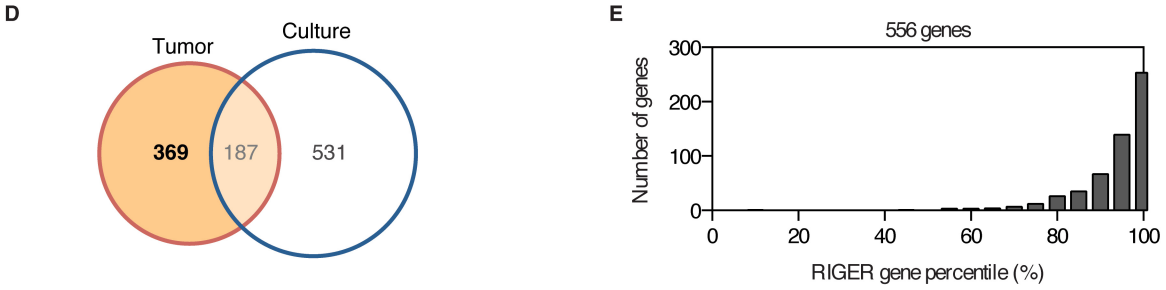


B

	shRNAs absent in tumors	Genes with 2+ shRNAs absent
LS174T	15,199 (27.8%)	3,682 (26.1%)
WiDr	18,432 (33.8%)	4,770 (33.8%)
SW620	8,111 (14.9%)	1,465 (10.4%)
All		556 (3.9%)

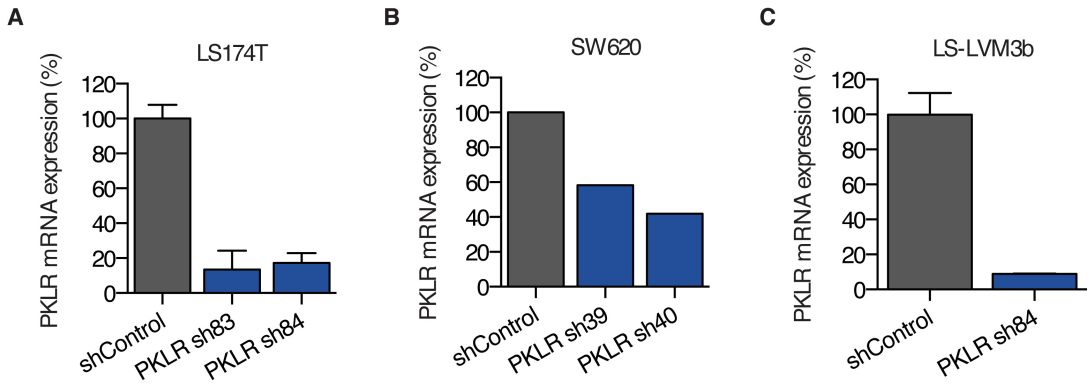
C

	shRNAs absent in culture	Genes with 2+ shRNAs absent
LS174T	10,548 (19.3%)	2,186 (15.5%)
WiDr	15,994 (29.3%)	3,973 (28.2%)
SW620	9,534 (17.5%)	1,891 (13.4%)
All		719 (5.1%)

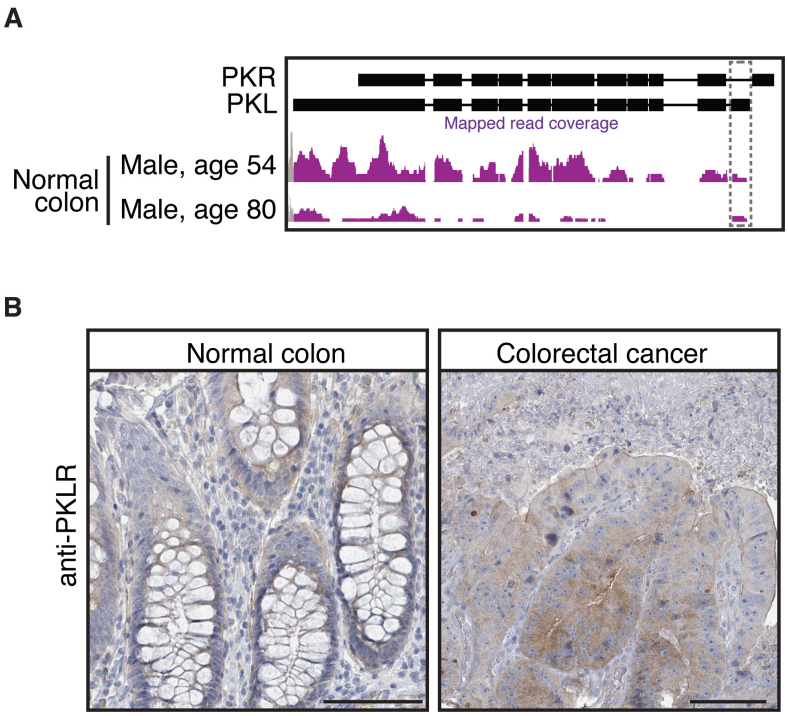


Supplemental Figure 1. Additional analyses of genome-wide shRNA screen for liver colonization and survival in culture

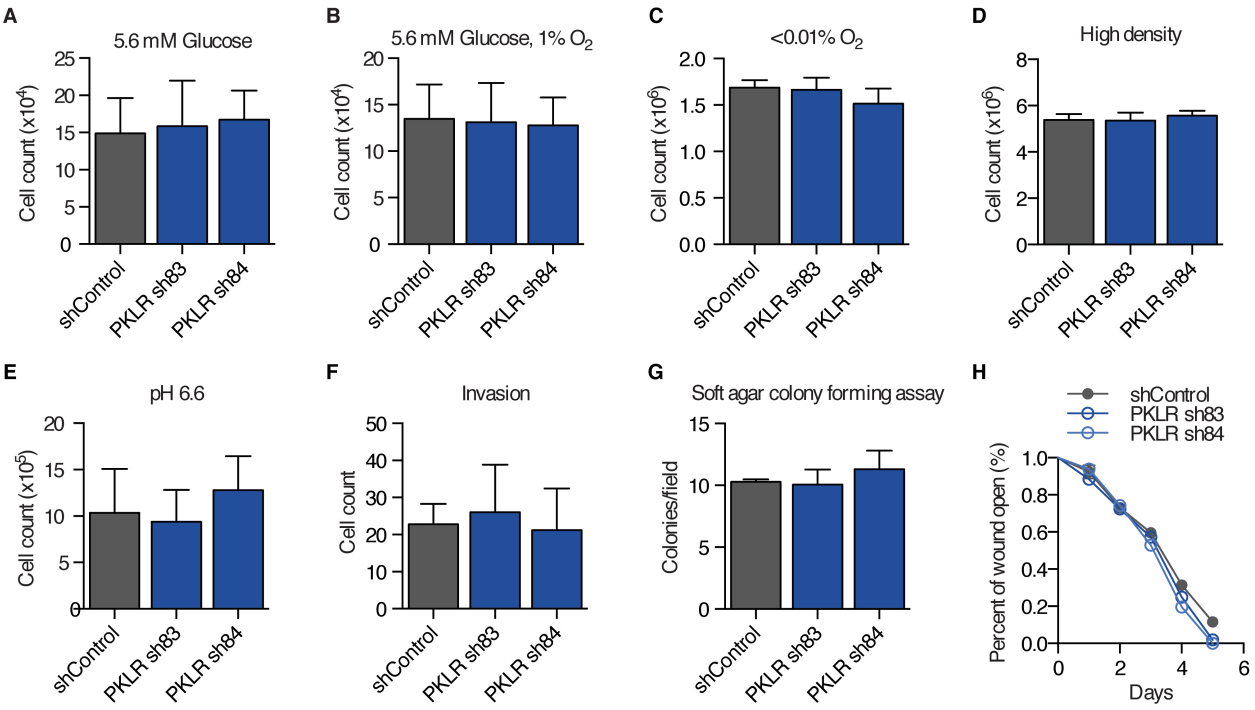
(**A**) Overview of shRNA depletion in cultured cells. shRNAs were ranked based on peak median absolute deviation normalized z-score of $\log_2(\text{Tumor}/T_0)$. shRNAs absent in tumors are black. P-values for absent shRNAs were derived by bootstrapping with 1000 random samplings. (**B**)(**C**) Summary of absent shRNAs and gene hits from tumor (B) and cultured cells (C). Percentage of total population is indicated in parentheses. (**D**) Overlap between gene hits scored in tumors and those scored in culture. (**E**) Distribution of 556 top genes as scored by RIGER and ranked by gene percentile.



Supplemental Figure 2. PKLR depletion in cell lines used.
(**A**)(**B**)(**C**) PKLR depletion as measured by qRT-PCR in LS174T cells (n=3) (A), SW620 cells (B), and LS-LVM3b cells (n=2) (C).



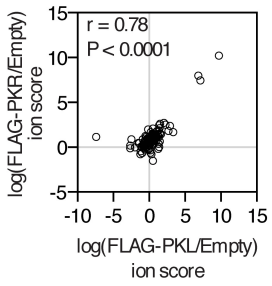
Supplemental Figure 3. PKLR expression in normal colon and colorectal cancer tissue.
(**A**) RNA-sequencing read density at PKLR locus from normal colon tissue. PKL-specific exon is indicated with dashed box. (**B**) PKLR protein expression in normal colon and colorectal cancer tissue as assessed by immunohistochemistry. Scale bar is 100um.



Supplemental Figure 4. PKLR is not required for survival under various *in vitro* conditions

Cells were seeded in triplicate under the following conditions: (**A**) 10^5 cells in 5.6mM glucose for 5 days, (**B**), 10^5 cells in 5.6mM glucose & hypoxia for 5 days, (**C**) 10^6 cells in anoxia for 3 days, (**D**) 10^6 cells seeded at a density of 1000 cells/mm^2 for 3 days, (**E**) 10^5 cells in media buffered at pH 6.6 for 5 days, (**F**) $25x10^3$ cells in quadruplicate in invasion chambers and were allowed to invade for 48 hours, (**G**) $5x10^3$ cells seeded in soft agar for 14 days. Data shown is from three independent experiments. (**H**) 10^6 cells seeded overnight and scratched using a pipette tip the next day. Cells were seeded in duplicate and imaged daily.

A



B

FLAG-PKL

Gene set	*NES*	*NOM p-val*	*FDR q-val*
REACTOME_METABOLISM_OF_CARBOHYDRATES	1.72	0.012	0.539
REACTOME_GLUCOSE_METABOLISM	1.72	0.003	0.274
REACTOME_GLYCOLYSIS	1.70	0.004	0.278
BANDRES_RESPONSE_TO_CARMUSTIN_MGMT_48HR_DN	1.59	0.012	1

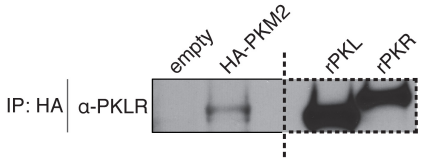
FLAG-PKR

Gene set	*NES*	*NOM p-val*	*FDR q-val*
REACTOME_GLUCOSE_METABOLISM	1.82	0.011	0.333
REACTOME_GLYCOLYSIS	1.81	0.007	0.214
KEGG_FRUCTOSE_AND_MANNOSE_METABOLISM	1.74	0	0.359
REACTOME_METABOLISM_OF_CARBOHYDRATES	1.74	0.011	0.276

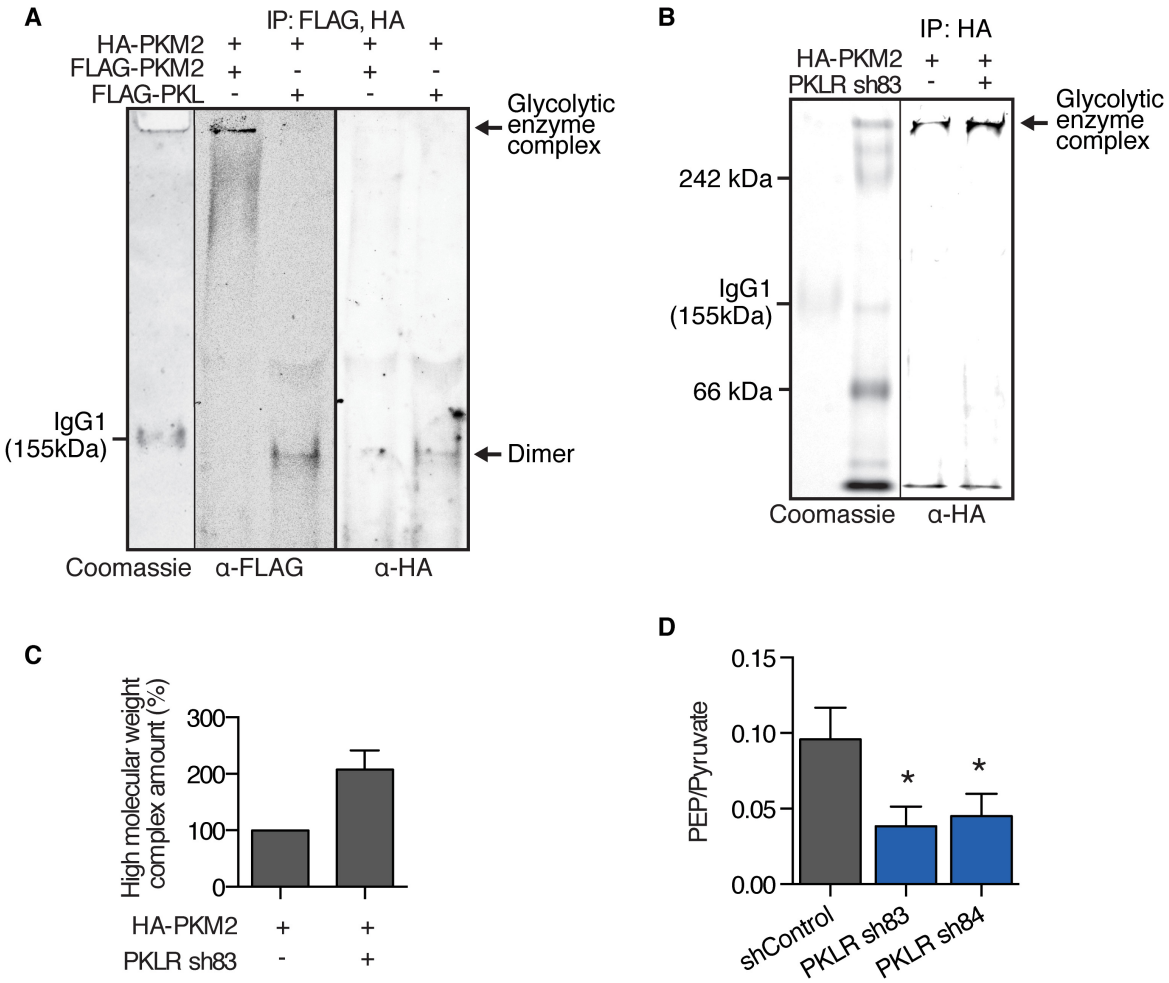
C

		peak area	
PKM2-specific peptides	Empty	FLAG-PKL	FLAG-PKR
LAPITSDPTEATAVGAVEASFK	0	3.688E7	1.846E7
RLAPITSDPTEATAVGAVEASFK	0	2.185E6	7.746E5
EAEAAIYHLQLFEELRR	0	9.152E6	3.930E6

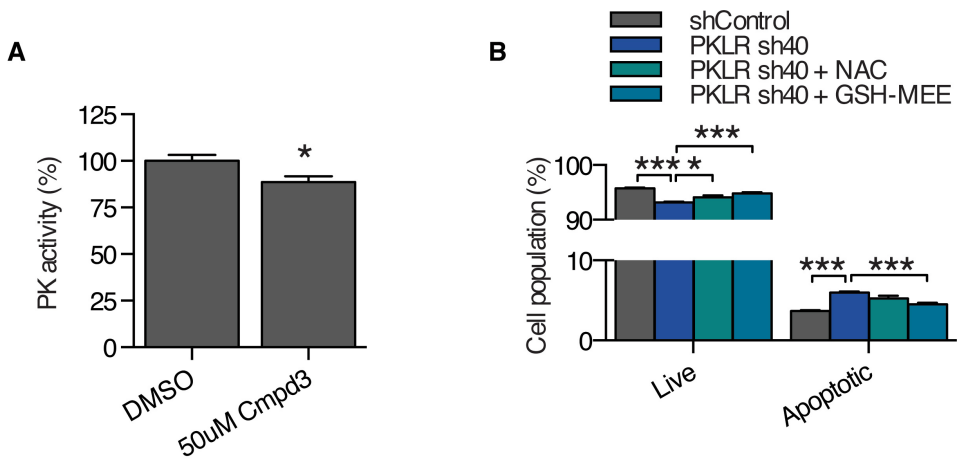
D



Supplemental Figure 5. PKL interacts with PKM2.
(**A**) LC-MS/MS ion scores of FLAG-PKL and FLAG-PKR co-immunoprecipitated proteins relative to empty vector in LS-LVM3b. Pearson's correlation coefficient and associated P-value are shown. (**B**) Gene set enrichment analysis was performed on co-immunoprecipitated proteins, and top scoring gene sets are shown. (**C**) PKM2-specific peptides as measured by LC-MS/MS are shown. (**D**) Western blot from Figure 6C with 20 ng of recombinant PKL (rPKL) and PKR (rPKR) analyzed as controls (dashed border) on same gel.

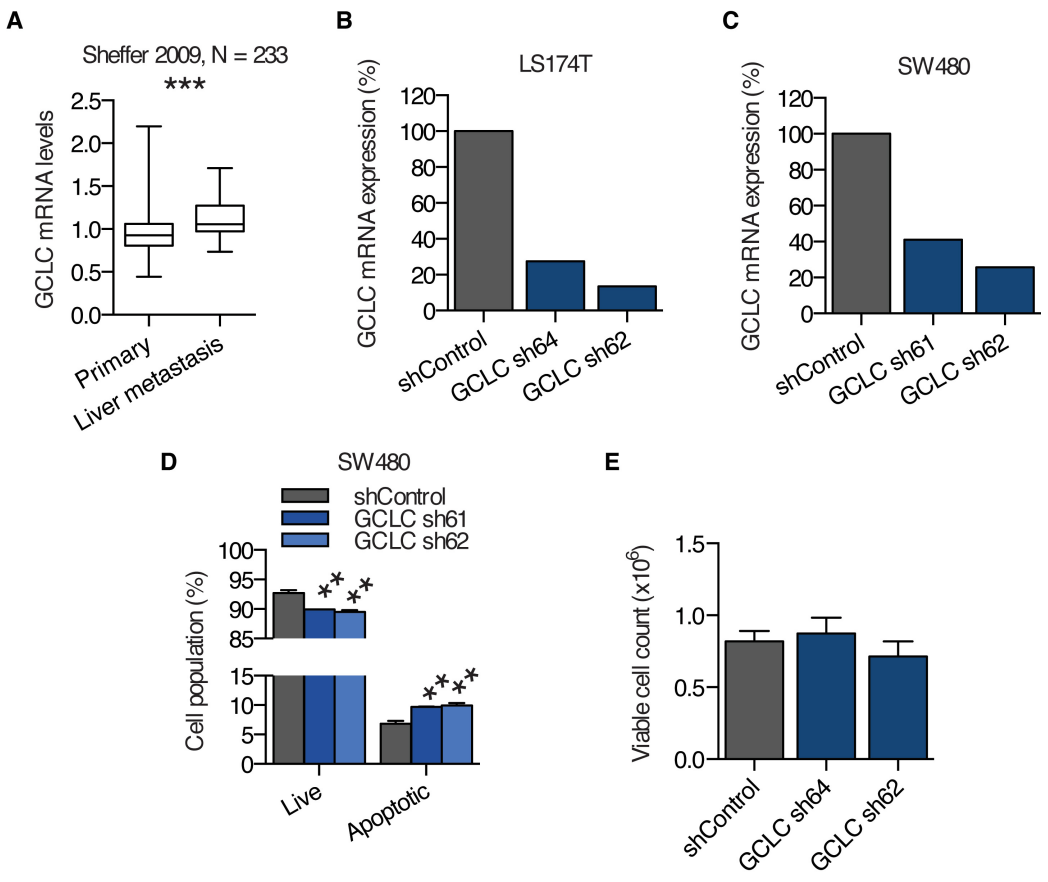


Supplemental Figure 6. PKLR inhibits active glycolytic complex formation
(**A**) Sequentially immunoprecipitated PKM2 complexes from transfected HEK293T cells were assayed for pyruvate kinase oligomerization by native PAGE followed by quantitative western blotting. Mouse IgG1 was loaded on the same gel as a molecular weight marker and visualized with Coomassie staining. Lanes were run on the same gel but were noncontiguous. (**B**) Immunoprecipitated PKM2 complexes from LS174T cells, were assayed for pyruvate kinase oligomerization by native PAGE followed by quantitative western blotting. Mouse IgG1 and a native protein standard was loaded on the same gel as molecular weight markers and visualized with Coomassie staining. Lanes were run on the same gel but were noncontiguous. (**C**) Quantitation of amount of high molecular weight complex was performed on LI-COR Odyssey software. Data shown is from two independent experiments. **(D**) Pyruvate levels and phosphoenolpyruvate (PEP) levels were measured from LS174T cell lysates after 24 hours under hypoxic, cell-dense conditions, and ratios were computed. Data shown is from three biological replicates and four independent experiments. P-values were derived using one-sided Student's t-test between indicated sample and shControl.



Supplemental Figure 7. Additional data supporting PKLR enhancement of cell survival through glutathione

(**A**) 10^6 LS174T cells were seeded in triplicate at a density of 1000 cells/mm^2, incubated with DMSO or 50uM Compound 3 for 3 hours in 1% O_2, and then assessed for pyruvate kinase ativity levels. Data is shown from 2 independent experiments. P-value was derived using two-sided Student's t-test. (**B**) 10^6 SW620 cells were seeded at a density of 1000 cells/mm^2, incubated with indicated compounds, and were assessed for apoptosis after 24 hours in 1% O_2. P-values were derived using one-sided Student's t-test between indicated samples.



Supplemental Figure 8. GCLC is up-regulated in human liver metastases and promotes survival under conditions of high cell density & hypoxia

A, GCLC expression in unmatched primary and liver metastasis tumors as shown by box plots. P-values were derived using one-sided Mann Whitney test. (**B**)(**C**) GCLC knockdown as measured by qRT-PCR in LS174T (B) and SW480 (C) cells. (**D**) 10^6 SW480 cells were seeded at a density of 1000 cells/mm^2 and were assessed for apoptosis after 24 hours in 1% O_2. P-values were derived using one-sided Student's t-test between indicated sample and shControl. (**E**) 10^5 LS174T cells were seeded in triplicate and viable cells were counted by trypan blue exclusion after 3 days under recommended cell culture conditions. Data shown is from four independent experiments.

Supplemental Methods

Recombinant protein production

PKL and PKR cDNAs were cloned into pGEX-6P-L vector, followed by transformation in BL21 (DE3) Gold (Agilent). 0.1mM IPTG was used to induce expression and after 3 hours, cells were lysed using 50mM Tris-HCl, pH 7.5, 5mM EDTA, 200mM NaCl, 0.1% NP-40, 1mM DTT, 5mg/ml lysozyme and sonicated (Misonix Sonicator S-4000). Purification was performed using glutathione-agarose beads (Sigma) and eluted using 5mM reduced glutathione (Sigma). Cleavage of GST-tag was performed using Prescission protease (GE).

Additional in vitro cell growth assays

Anoxia was generated using the AnaeroPack System (Mitsubishi Gas Chemical). Cell culture under acidic pH was obtained by using MES buffer and adjusting to pH 6.6. Anchorage-independent growth was performed as follows: cells were suspended in 0.4% agar in cell culture media and layered on top of 0.6% agar in 24-well plates. Colonies formed with feed media changed every 4 days and were counted and imaged by light microscopy at 14 days. Invasion assays were performed as previously described (1) except for the difference in number of cells seeding.

Pathway analysis

Gene Set Enrichment Analysis (2) was performed on a list ranked by enrichment of proteins in FLAG-PKL/FLAG-PKR immunoprecipitated samples using the most

abundant proteins relative to control based on the sum of peptide spectrum matches per protein. Curated C2 gene sets from version 4.0 of the MSigDB were used for analysis.

Native PAGE

HEK293T cells were transfected with plasmids encoding HA-PKM2, FLAG-PKM2, or FLAG-PKL using Lipofectamine 2000 (Invitrogen). 48 hours later, cells were lysed using 50mM Tris, pH 7.5, 150mM NaCl, 1mM EDTA, 1% NP40, 100uM fructose 1,6-bisphosphate, protease inhibitor (Roche), and phosphoSTOP (Roche). Immunoprecipitation was first performed using anti-FLAG M2 magnetic beads (Sigma) for 1 hour at 4 degrees Celsius. Beads were washed 2x with lysis buffer, and elution was performed using 3x FLAG peptide (Sigma). Eluate was then incubated with anti-HA magnetic beads (Pierce) for 1 hour at 4 degrees Celsius. Beads were washed 2x with lysis buffer, and elution was performed using HA peptide (Pierce). For stable cell lines, an equal number of cells were seeded the day before lysis, and BCA assay was used to confirm equivalent immunoprecipitated protein amounts. Samples were loaded into 3-8% Tris-acetate gel (ThermoFisher) using Novex Tris-Glycine Native Sample Buffer (ThermoFisher) and 0.5% digitonin (Sigma). Mouse IgG1 (Cell Signaling) and NativeMark Unstained Protein Standard (Life Technologies) were used as molecular weight markers. Samples were run in Tris-Glycine running buffer, and transferred to Immobilon-FL PVDF membranes (Millipore), blocked and probed with antibodies. Anti-FLAG (1:1,000; Sigma), anti-HA (1:1,000; Cell Signaling), and fluorescent secondary (1:10,000; Li-Cor) antibodies were used for detection on Odyssey SA Imaging System

(Li-Cor). Molecular weight markers run on same gels and were visualized by Coomassie staining.

Supplemental References

1. Pencheva N, Tran H, Buss C, Huh D, Drobnjak M, Busam K, and Tavazoie SF. Convergent multi-miRNA targeting of ApoE drives LRP1/LRP8-dependent melanoma metastasis and angiogenesis. *Cell.* 2012;151(5):1068-82.

2. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America.* 2005;102(43):15545-50.