Supplemental Experimental Procedures:

Lentiviral Preparation and Infection

Lentivirus was prepared using System Biosciences lentivirus technology. 293 TN cells (SBI # LV900A-1) were seeded in 150 mm plates at 7.5 x 10^6 cells per plate with 20 ml DMEM without antibiotics for 24h. Cells were then transfected with 22.5µg pPACKH1 HIV packaging mix (SBI # LV500A-1) and 4.5µg of pCDH lentiviral vector using BioT transfection reagent. Viral supernatant was collected 48h and 72h post-transfection. After centrifugation for 15 min at 3000 x g to remove debris, 1X PEG-it (SBI # LV810A-1) was added to precipitate virus. After incubation at 4°C for at least 16h, centrifugation for 30 min at 1500 x g was used to collect viral particles. Virus was resuspended in a small volume (300-500µl) 1X PBS (phosphate buffered saline). Virus was titered using the Global UltraRapid Lentiviral Titer Kit (SBI #LV961A-1). Transduction of target cells was performed according to SBI protocol. Briefly, cells were seeded at 1.0 x 10^5 cells per 12-well or 2.5 x 10^5 cells per 6-well plate. After 24h, cells were treated with fresh media, 1X TransDux (SBI # LV850A-1), and lentivirus at a Multiplicity of Infection (MOI) of 10. Infected cells were collected for subsequent assays after 72h.

Lentiviral shRNA Experiments

293T cells were seeded at 7.5 x 10^5 cells per well in 12-well plates for 24h. Cells were then transfected with 1.6µg pCMV packaging plasmid, 0.4µg pMP2G packaging plasmid and 2µg pLKO.1 scramble or shRNA plasmid (Thermo Scientific) using BioT transfection reagent. Media was changed 16h post infection, and viral supernatant was collected 48h later. Virus was sterile filtered to remove debris. Transduction of DLD-1 cells was performed according to SBI
protocol (see above), using 100µl of virus stock for infection per 6-well plate. 72h post transduction, infected cells were selected for with the addition of 5µg/ml puromycin to the culture media until a mock-infected culture indicated complete selection. Fully selected cultures were then used immediately for FLIM and western blot analysis.

**Cell Cycle Analysis**

SW480 cells (72h post transduction) and DLD-1 dnLEF-1 cells (24h after addition of doxycycline) were treated with 10µM EdU for 2h. Cells were harvested and treated according to the Click-iT EdU Alexa Fluor 488 Flow Cytometry Assay Kit (Molecular Probes #C-10420). After incubation with 0.2µg/µl RNaseA and 4µg/ml propidium iodide (PI) for 30 min, cells were analyzed on a BD FacsCalibur Flow Cytometer using a low flow rate. At least 75,000 events were counted for each condition. Cell cycle data analysis was performed using FlowJo software.

**Culturing Supplemental Cell Lines**

Colon cancer cell lines, LS 174T, SW620, HCT 116, and HT-29 were cultured in either Dulbecco's modified Eagle's medium (DMEM; Gibco 11960) or RPMI-1640 medium (Cellgro 15-040), supplemented with 10% Fetal Bovine Serum (FBS) and 2mM Glutamine (unless otherwise stated, experiments were performed in the same media).

**Luciferase, Lactate, and Glucose Consumption Assays for Supplemental Cell Lines**

SW620 cells, LS174T cells, HCT116 cells, HT-29 cells were lentivirally transduced (see above) with either dnLEF-1 or dnTCF-1Emut-carrying virus. 72h post transduction, stable transductants were selected for with addition of 400µg/ml (SW620 and LS 174T) or 500µg/ml (HCT116 and
HT-29) G418 to the culturing media. Cells were tested for Wnt luciferase activity, lactate production, and glucose consumption, and FLIM profile as described previously for the SW480 and DLD-1 cells, except the density for each cell line had to be optimized to obtain confluent cultures around day five for each experiment. For glucose consumption assays, low glucose media (DMEM or RPMI with 5.5mM glucose) was added 12h prior to media collection for each of these cell lines.

**Fluorescence Lifetime Imaging Microscopy (FLIM) of cells in vitro**

Fluorescence lifetime images were acquired with a two-photon microscope coupled with a Becker and Hickl 830 card (Becker and Hickl, Berlin). A Ti:Sapphire laser (Spectra-Physics Mai Tai) with 80 MHz repetition rate was used to excite the sample. The laser was coupled with a Zeiss Axiovert S100TV microscope. The scanning system used a Cambridge Technology Mirror (scanner 6350). A Zeiss 40 x 1.2 NA water immersion objective was used. For image acquisition the following settings were used: image size of 256 x 256 pixels, scan speed of 32µsec/pixel. A dichroic filter (700DCSPXR, Chroma Technologies) was used to separate the fluorescence signal from the laser light and the fluorescence was detected by a hybrid detector (HPM-100 of Hamamatsu). An additional barrier filter was used to block the near IR light. FLIM data were acquired and processed by the SimFCS software developed at the Laboratory of Fluorescence Dynamics. The excitation wavelengths used were 740nm. An average power of about 5mW was used to excite the live tissue. FLIM calibration of the system was performed by measuring the known fluorescence decay of fluorescein with a single exponential of 4.04ns. Every FLIM image was acquired over 10 frames of the same field of view.
Fluorescence lifetime images were acquired with a Zeiss 710 microscope coupled to a Ti:Sapphire laser system (Spectra-Physics Mai Tai) and a ISS A320 FastFLIM system. A 40×0.8 NA water immersion objective (LUMPlanFl Olympus) was used. For image acquisition the following settings were used: image size of 256×256 pixels or 1024x1024 pixels and scan speed of 25µsec/pixel. A dichroic filter (690nm) was used to separate the fluorescence signal from the laser light and the fluorescence. For the acquisition of FLIM images, fluorescence was detected by two photomultipliers (H7422P-40 of Hamamatsu). A 610nm short pass filter was placed in front of the detectors. A 495nm long pass filter separates the blue and the green fluorescence. NADH fluorescence was collected through a 460/80nm filter, while the fluorescence of the FITC Dextran was collected through a 540/50nm filter. FLIM data were acquired and processed by the SimFCS software developed at the Laboratory of Fluorescence Dynamics. The excitation wavelength used was 740nm. An average power of about 5mW was used to excite the live tissue. FLIM calibration of the system was performed by measuring the known fluorescence decay of fluorescein with a single exponential of 4.04nsec. FLIM data were collected until 100 counts in the brightest pixel of the image were acquired. Typically the acquisition time was on the order of a few seconds.

**Real-time PCR**

Primer pairs used for real-time PCR analysis include: human *GAPDH* (5’-TCGACAGTCAGCCGCATCTTCTT-3’) and reverse (5’-GCGCCCAATACGACCAAATCC-3’), human *PDK1* forward (5’-CGGATCAGAAACCGACACA-3’) and reverse (5’-ACTGAACATTCTGGCTGGTGA-3’), human *PDK2* forward (5’-
AAGGACACCTACGGCGATG-3’) and reverse (5’- ATGGAGATGCGGCTGAGG-3’), human

*PDK3* forward (5’- TTAATAAAGTCCGCATGCGC-3’) and reverse (5’-

TGAAGCATCCCTGGGTTCAC-3’), human *PDK4* forward (5’-

GGAGCATTTTCTCGCCTACA-3’) and reverse (5’- ACAGGCAATTCTTGTGCAAA-3’),

human *CMYC* forward (5’- CTACCCTCTCAACGACAGCA-3’) and reverse (5’-

AGAGCAGAGAATCCGAGGAC-3’), flag-dnLEF-1 transgene forward (5’-

GGATTACAAGGATGACGACGATAAG-3’) and reverse (5’-

TGCTTTCCGCTACGCGGGGTG-3’), human *Axin2* forward (5’-

GGACACTTCAAGGAACAATAC -3’) and reverse (5’- CCTCATACATTGGCAGAACTG -

3’), human *Uba52* forward (5’- AGACAAGGAGGGTATCC-3’) and reverse (5-

TGAAGGGCGAGCATGAC-3’), ChIP “Peak 1” forward (5’- CTCTGCCTCTCTGCCCCTTGTG-3’) and reverse (5’-

GCGGCCTGATGACCTGAG-3’), ChIP *PDK1* promoter forward (5’-

GCGCCTTAGGTGCTTCCTTC-3’) and reverse (5’- TAGCGCPCACCTGCACACGTG-3’),

ChIP “Peak 2” forward (5’- CTTGGCCCTCCAAAGTGCTG-3’) and reverse (5’-

TGCGCGGAATCTCTGGAAGCTG-3’), ChIP *PDK1* ORF forward (5’-

CGGATCAGAAACCGACACA-3’) and reverse (5’- ACTGAACATTCTGCTGTA-3’),

human *HIF1α* forward (5’ – CTCAAATACAAGAACCTACTGCTAA-3’) and reverse (5’-

GTGATGATGTGGCACTAGTA-3’), human *VEGFA* (5’- AGGGCAGAATCATCAGAAGT-3’) and reverse (5’- AGGTCTCGATGGATGGCA-3’), human *MCT-1* forward (5’ –

CACCGTACAGCAACTATAAGT-3’) and reverse (5’ – CAATGGTCCCTCTTGTGGA-3’),

human *GLUT1* forward (5’ - CTTGTGCTTCTGGAAAGT-3’) and reverse (5’-

CCACACAGTTGACTCAGC-3’).
Luciferase Reporter Plasmid Cloning

To create a luciferase reporter plasmid driven by the human \( PDK1 \) or herpes virus thymidine kinase promoter, the following PCR primers were used. 5’ flanking sequences of the primers (lower case) were designed for complementarity to the pGL2 or tkLUC vector backbone for use with a Cold Fusion cloning schema (Cold Fusion Cloning; System Biosciences). Human placental DNA was used as template for PCR amplification of the \( PDK1 \) core promoter using Pfu Turbo polymerase. The PCR fragment and SmaI digested pGL2 vector was purified following the manufacturer’s protocol (Fermentas GeneJET Gel Extraction Kit) and ligations were performed via an insert:plasmid ratio of 1:2 with Cold Fusion reagents. Clones were sequence-verified.

Forward primer (-257, upper case sequence is genomic, lower case sequence is complementary to pGL2):

5’- gag cta aca taa ccc cAC CCG ACG TGT ACG GGG AC - 3’

Reverse primer (+36, upper case sequence is genomic, lower case sequence is complementary to pGL2):

5’- age tct gta cct ccc tAC GTG AGG GAC GTG GCG GG - 3’

The following PCR primer sequences were used to amplify “Peak1” and “Peak2” regions of the \( PDK1 \) locus, regions identified as occupied by TCF-1, for cloning 5’ of the \( PDK1 \) endogenous promoter (-257, +36) or the heterologous herpes virus tk promoter (tkLUC):

Primers designed for amplification and insertion into a plasmid backbone containing a minimal Herpes Virus thymidine kinase reporter (at BamHI):

“Peak 1” (860nt; chr2: 173390266-173391125)

Forward primer (upper case sequence is genomic, lower case is complementary to tkLUC):

5’- atc tta tca tgt ctg  CTG GGC ACT CTG AAA AGG AA - 3’

Reverse primer (upper case sequence is genomic, lower case is complementary to tkLUC):

5’- ctc gga ccc cgg atc ATC AAG AGT GGG AAC GCA TG - 3’

“Peak 2” (299nt: chr2: 173469007-173469305)

Forward primer (upper case sequence is genomic, lower case is complementary to tkLUC):

5’- atc tta tca tgt ctg GCC ACC TCA AGG GAT CCA CC - 3’
**Reverse Primer** (upper case sequence is genomic, lower case is complementary to tkLUC):

5’-ctc gga ccc cgg atc GAG GCC ATG ATT TTT CTG AAC TGG AG - 3’

For insertion of PDK1 genomic fragments into the PDK1/pGL2 reporter vector, genomic regions were amplified using Taq polymerase and Betaine. PCR amplicons were purified following the manufacturer’s protocol (Fermentas GeneJET Gel Extraction Kit). The PDK1/PGL2 vector was digested with ApaI and purified. Each “peak region” was ligated and transformed into the vector with a ratio of insert:vector of 1:2 using the manufacturer’s protocol (Cold Fusion Cloning kit Systems Biosciences). Clones were sequenced verified prior to purification and transfection.

“Peak 1” (860nt; chr2: 173390266-173391125)

**Forward primer** (upper case sequence is genomic, lower case is complementary to pGL2):

5’ – gcg agg ggg cgg gcc CTG GGC ACT CTG AAA AGG AA - 3’

**Reverse primer** (upper case sequence is genomic, lower case is complementary to pGL2):

5’- ggc ggg cgc ttg cgg ATC AAG AGT GG G AAC GCA TG - 3’

“Peak 2” (299nt: chr2: 173469007-173469305)

**Forward primer** (upper case sequence is genomic, lower case is complementary to pGL2):

5’- gcg agg ggg cgg gcc GCC ACC TCA AGG GAT CCA CC - 3’

**Reverse primer** (upper case sequence is genomic, lower case is complementary to pGL2):

5’ – ggc ggg cgc ttg cgg GAG GCC ATG ATT TTT CTG AAC TGG AG - 3’

**FLIM Data Analysis**

Every pixel of the FLIM image was transformed in one pixel in the phasor plot as previously described (2,3). The coordinates g and s in the phasor plot were calculated from the fluorescence intensity decay of each pixel of the image by using the following transformations:

\[
g_{ij}(\omega) = \frac{\int_0^\infty I_{ij}(t) \cos(\omega t) dt}{\int_0^\infty I_{ij}(t) dt}
\]
where the indices i and j identify a pixel of the image and ω frequency \(\omega = 2\pi f\), where f is the laser repetition rate, i.e. 80 MHz in our experiment. All phasor plots were calculated at 80 MHz, i.e. the first harmonic of the laser repetition rate and for some cases for higher harmonics.

In the phasor plot if the decay is a single exponential \(I(t) = Ae^{-t/\tau}\) the coordinates are given by:

\[
\begin{align*}
    g(\omega) &= \frac{1}{1 + (\omega\tau)^2} \\
    s(\omega) &= \frac{\omega\tau}{1 + (\omega\tau)^2}
\end{align*}
\]  

(3) (4)

where \(\tau\) is the lifetime of the decay and \(\omega\) is the laser frequency. There is a direct relationship between a phasor location and lifetime. Every possible lifetime can be mapped into this universal representation of the decay (phasor plot). All possible single exponential lifetimes lie on the "universal circle" defined as the semicircle going from point \((0, 0)\) to point \((1, 0)\) with radius 1/2. Point \((1, 0)\) corresponds to \(\tau = 0\), while point \((0, 0)\) to \(\tau = \infty\). In the phasor coordinates the single lifetime components add directly because the phasor follows the vector algebra. A mixture of two distinct single lifetime components, each of which lie separately on the single lifetime semicircle, does not lie on the semicircle. All the combination of two single exponential components must be along the line joining the two lifetime points. In a system with many single lifetime components the phasor coordinate \(g\) and \(s\) are described as:

\[
\begin{align*}
    g(\omega) &= \sum_k \frac{h_k}{1 + (\omega \tau_k)^2} \\
    s(\omega) &= \sum_k \frac{h_k \omega \tau_k}{1 + (\omega \tau_k)^2}
\end{align*}
\]  

(5) (6)

where \(h_k\) is the intensity weighted fractional contribution of the single-exponential component with lifetime \(\tau_k\). The phasor location of the mixture of single-lifetimes is the intensity-weighted average of the contributions of each single-lifetime that lie separately on the semicircle.
In general in a system with multiple fluorescent components like a tissue the overall decay is a phasor that is the sum of the independent phasors of each fluorescence component:

\[ G(\omega) = \sum_n f_n g_n(\omega) \]  
\[ S(\omega) = \sum_n f_n s_n(\omega) \]

Where \( f_n \) is the fractional contribution of each component characterized by the phasor coordinates \( g_n \) and \( s_n \). Two molecular species with multi-exponential decay are identified by two specific points in the phasor plot inside the semicircle. All possible weighting of the two molecular species give phasors distributed along a straight line joining the phasors of the two species. In the case of three molecular species, all the possible combinations are contained in a triangle where the vertices correspond to the phasor of the pure species. The phasor plot of an N-component mixture will be contained in a polygon with N-vertices located in the position of the phasor of each contributing component.

The analysis of the phasor distribution was performed by cluster identification. Clusters of pixel values were detected in specific regions of the phasor plot. Fractional intensities of free and bound NADH in every pixel of the image were evaluated with a graphical analysis in the phasor plot as described (3). We performed image segmentation on the FLIM data by selecting the region of interest of cells in vitro or within the tissue. The region of interest of cells was selected by using a cursor with arbitrary shape. We calculated the phasor average values within these regions of interest and we represented them in the scatter plot. When measuring the cell phasor, all pixels of the cell (about 1000) were taken in account and the signal to noise ratio of the FLIM signature of cells was higher than in single pixels. A t-test was performed on the distributions of different types of the cell average phasor values. All phasor transformation and
the data analysis of FLIM data were performed using SimFCS software developed at the Laboratory for Fluorescent Dynamics.

Bioinformatic Patient Tumor Analysis

To analyze Wnt signaling and glycolysis pathway gene expression in human colon cancer tumors, the publicly available colon adenocarcinoma (COAD) mRNA expression data was downloaded from The Cancer Genome Atlas (TCGA) Data Portal (https://tcga-data.nci.nih.gov/tcga/). Two gene expression datasets were generated from next-generation sequencing of RNA (RNA-seq) using two different sequencing platforms; data for 192 COAD tumor samples was generated with the Illumina Genome Analyzer and data for 238 COAD tumor samples was generated with the Illumina HiSeq. A detailed description of the methods for generating the sequence data and the bioinformatics analysis can be found at the TCGA website or the TCGA colon cancer manuscript (4). Matched normal tissue RNA-seq data was not available. Given the potential for batch effects, the two gene expression datasets were analyzed separately with the larger sample of 238 individual tumor samples being the primary discovery set. The gene memberships of the Wnt signaling and glycolysis pathways were defined by the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The normalized gene expression measurements (FPKM) were extracted from the full genome dataset for the 142 Wnt signaling pathway genes and the 68 glycolysis pathway genes. Genes were excluded from the analysis if more than 25% of their normalized expression values were zero, with the filtering resulting in a discovery dataset of 238 independent tumor samples with gene expression data for 126 Wnt signaling and 51 glycolysis genes. Expression data from eight additional genes (PDK1, PDK2, PDK3, PDK4, HIF1A, EPAS1, ARNT and TFAP4) were included bringing the total number of
genes analyzed to 185. The normalized expression measurements were log2 transformed for all statistical analyses.

Hierarchical clustering of log2 transformed normalized expression was used to identify groups of tumor samples showing similar expression profiles for the 185 analyzed genes (Figure S10a). Correlations in gene expression was computed between genes using the full sample and within each of nine groups defined by the cluster analysis. A group of 21 independent tumors, indicated in Figure S10a (group4), showed a gene expression pattern that was characterized by strong correlations in gene expression both within the Wnt and glycolysis pathways as well as between the pathways. A symmetrical heatmap was produced to show the strength of correlations in Wnt and glycolysis gene expression signatures between all individuals and the clustering of individuals based on their similar correlations (Figure S10b), further identifying the group of 21 individuals with strongly correlated Wnt signaling and glycolysis gene expression signatures (dark blue square in the upper left corner of the graph).

In the full sample of 238 tumors, there were seven pairs of genes from the different pathways with correlations of less than -0.70 or greater than 0.70. Among the 21 tumor samples with strongly correlated Wnt and glycolysis gene signatures, there were 453 unique gene pairs from different pathways that showed a correlation that was greater than 0.70 or less than -0.70 (p-value < 0.01), including 129 of the 185 genes that had such a correlation with at least one other gene in the alternative pathway. Thirty-six genes had correlations of this magnitude with at least 10 other genes in the alternative pathway (with strong being defined as -0.70 > r > 0.70, p-value < 0.01) with at least 12 genes in the alternative pathway. \textit{PDK1} showed strong correlations with 25 genes, 9 from the Wnt signaling pathway and 16 from the glycolysis pathway. Of the 16 Wnt signaling genes strongly correlated with \textit{PDK1}, 15 were negative.
correlations (anti-correlations), with only MAPK8 showing a positive correlation with PDK1
[APC2 (-0.78), CSNK2A2 (-0.72), PRICKLE2 (-0.74), DVL1 (-0.71), DVL3 (-0.83), NFATC4 (-0.83),
PRKACA (-0.79), MAPK8 (0.72), TBL1X (-0.78), WNT9A (-0.75), CXXC4 (-0.70), CAMK2B (-0.74),
FZD7 (-0.73), FZD8 (-0.80), TCF7L1 (-0.79), NKD1 (-0.78), ADH1C (0.76), ENO2 (-0.72), ALDH3A2
(0.74), PFKM (-0.82), PGK1 (0.71), PGM1 (0.74), TPI1 (0.73), PDHX (0.72), ADPGK (0.72)]. Among
the nine glycolysis genes that were correlated with PDK1, seven showed positive correlations
while two genes, PFKM and ENO2, showed negative correlations. Among the identified 21
individuals, there are strong correlations between gene expression in the Wnt and glycolysis
pathways, and sets of genes within each pathway that showed similar signatures of expression.

The independent set of 192 samples that were sequenced on the Illumina Genome
Analyzer was analyzed by the same methods. Two of the tumor samples showed expression
patterns that were similar to the group of 21 individuals identified in the first set of 238 tumor
samples. However, with only two samples it was impossible to compute correlations in order to
determine if the Wnt signaling and glycolysis gene expression patterns were similar to the 21
tumor samples. The clinical data for each sequenced subject in TCGA was sparse and did not
allow for an analysis of clinical markers for the 21 subjects. Among the 21 subjects, the
prevalence of microsatellite instability, hypermutation, and somatic mutations among known
colon cancer genes was similar to the full sample.

The STRING (Search Tool for the Retrieval of Interacting Genes/Proteins; http://string-
db.org; (5)) database displays interaction networks based on known and predicted protein
interactions (genomic context, high-throughput experiments, co-expression, and literature). To
illustrate the possible interactions between the 25 Wnt and glycolysis genes that correlate
strongly with PDK1 in the 21 tumor subset, 25 gene IDs were submitted to STRING for analysis
and display. The functional protein network predicted by STRING is shown in (Fig. S10C) where each colored node is a gene from the submitted list.

References:


