Supplementary Figure Legends

Figure S1. PKM2, but not PKM1, associates with PAR *in vivo* (This figure is related to **Figure 1**)**.** (**A**) Binding of PAR with GST-PKM2 was shown by surface Plasmon resonance. (**B**) PKM2 is not PARylated by PARP1 ***in vitro***. Recombinant PKM2, Histone H3 and PARP1 were subjected to *in vitro* ribosylation assays in the absence or presence of NAD^+. The recombinant proteins were detected using indicated antibodies and the ribosylated proteins were determined with anti-PAR antibody. (**C**) Validating the anti-PAR antibody for immunofluorescence staining. U87/EGFR wt cells were mock treated or treated with PARP inhibitor olaparib (0.5 μM) for 8 hrs. Cells were fixed and immunostained using anti-PAR antibodies. (**D**) Schematic diagram of the putative PAR-binding motif (C2H2 motif) in PKM1. (**E**) PKM1 does not associate with PAR *in vivo*. Nuclear and cytoplasmic fractions were prepared from U87/EGFR wt cells without or with EGF treatment. IP/Western was conducted using indicated antibodies. (**F**) Localization of PKM1 and PAR before and after EGF treatment. U87/EGFR wt cells were serum starved for 12 hrs and then treated without or with EGF (100 ng/ml) for 6 hrs. Cells were immunostained with antibodies as indicated. (**G**) Localization of PKM1 and PAR after laser micro-irradiation. U87/EGFR wt cells were serum starved for 12 hrs and then treated without or with EGF (100 ng/ml) for 6 hrs. Cells were micro-irradiated and immunostained using indicated antibodies.

Figure S2. Abundance of PARP1 and PARP2 protein levels in whole organism and cell lines (This figure is related to **Figure 2**)**.**

Summary of PARP1 and PARP2 protein abundance in whole organism obtained from pax-db.org (http://pax-db.org/#!home). (**B**) Summary of PARP1 and PARP2 protein abundance in more than 50 different cell lines obtained from pax-db.org (http://pax-db.org/#!home).

Figure S3. The PAR-binding activity of PKM2-S37A mutant and the pyruvate kinase activity of wild-type and various PKM2 mutants were determined by *in vitro* assays (This figure is related to **Figure 3**).

(**A**) PKM2-S37A binds PAR *in vitro*. The recombinant GST, GST-PKM2 and GST-PKM2-S37A were incubated with Biotin-PAR, and protein-associated PAR was examined by dot blot using anti-PAR antibody. (**B**) The pyruvate kinase activities of GST, GST-PKM2 and mutants were determined by *in vitro* assays. The kinetics of pyruvate kinase activity was measured using *in vitro* pyruvate kinase assays. Briefly, we first used the pyruvate standard (provided in the PK activity kit from BioVision) and the PK activity samples to measure the amount of pyruvate for each sample using the pyruvate standard curve (**y=0.1139B+0.016**; **y** refers the OD 570nm value, while **B** is the amount of pyruvate). We next measured the OD 570nm value for each sample to obtain the kinetics of pyruvate kinase activity over time in order to calculate the PK activity. PK activity calculation: **PK activity= B*Sample Dilution Factor/(T2-T1)*V** (**B** is the amount of pyruvate previously measured; **T1** is the time of first reading in min; **T2** is the time of second reading in min; **V** is the sample volume added to the reaction).

Figure S4. Growth inhibition of lung cancer cells by PARP and EGFR inhibitors (This figure is related to **Figure 5**).

(**A**) Cells in Table S1 were treated with olaparib (0.5 μM for 8 hrs) and cell lysates were prepared for immunoblotting using the indicated antibodies. (**B**) H358 and H460 cells were treated without or with olaparib (0.5 μM), and cell proliferation was determined. Data presented are means ±SD (n=4 independent experiments). Immunostaining analyses were carried out using PKM2 and PAR

antibodies. (**C**) HCC2935 and HCC4006 cells were treated with gefitinib or olaparib and cell proliferation were measured at day 6 (n=4). Immunostaining analyses were carried out using indicated antibodies.

Figure S5. The effect of gefitinib and/or olaparib treatment on EGFR phosphorylation and PAR expression was determined in multiple cell lines (This figure is related to **Figure 5**)**.**

(**A**) The levels of PKM2 in PC9, H1650 and H1975 PKM2 knockdown cells were determined by immunoblotting as indicated. (**B**) The effect of gefitinib and olaparib treatment on p-EGFR and PAR levels in A549, PC9, H1650 and H1975 cells was confirmed by immunoblotting as indicated.

Figure S6. Validate p-EGFR, PAR, and PKM2 specific antibodies for IHC assay (This figure is related to **Figure 6**)**.**

(**A**) The p-EGFR antibody was validated using mock treated (with low p-EGFR level) and EGF-treated (with high p-EGFR level) U87/EGFR wt cells. The PKM2 antibody was validated using U87/EGFR wt PKM2 stable knockdown and control cells.The PAR antibody was validated using mocked treated (with low level of PAR) and H_2O_2 treated (with high level of PAR) U87/EGFR wt cells together with U87/EGFR cells with shRNA vector or PTEN downregulation (shPTEN). Scale bars, 50 μm. (**B**) Correlations between expression status of p-EGFR and PAR in human Glioblastoma and Lung Carcinoma samples are presented. Similarly, correlations between expression status of PAR and nuclear PKM2 in human glioblastoma and lung carcinoma samples with negative p-EGFR expression are also presented.

Table S1. Summary of 13 lung cancer cell lines with EGFR status, PKM2 localization and

sensitivity to gefitinib and olaparib (This table is related to **Figure 5**).

Figure S1

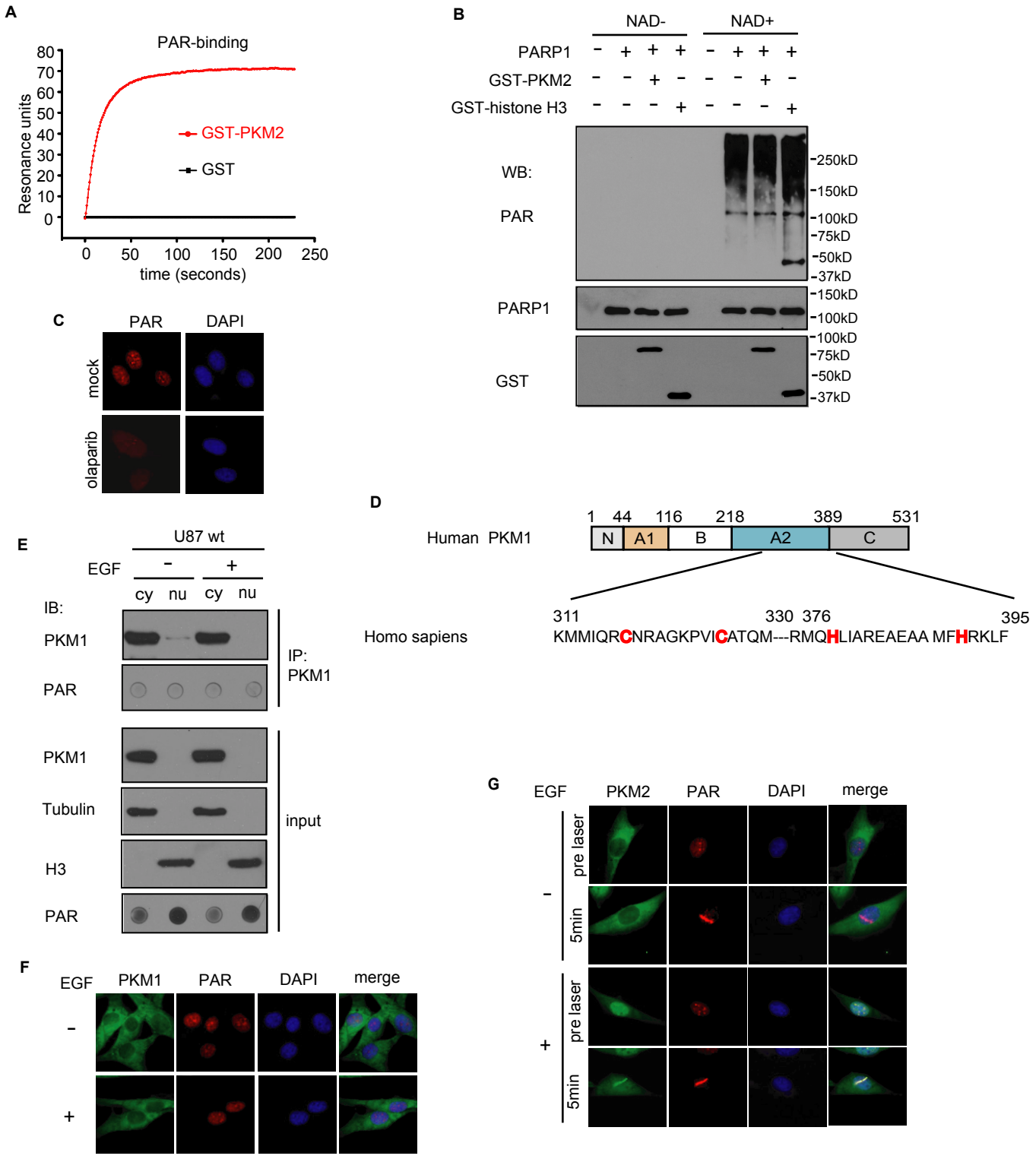
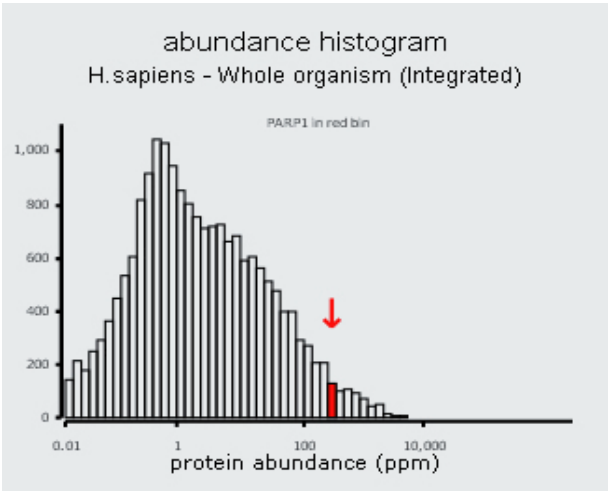
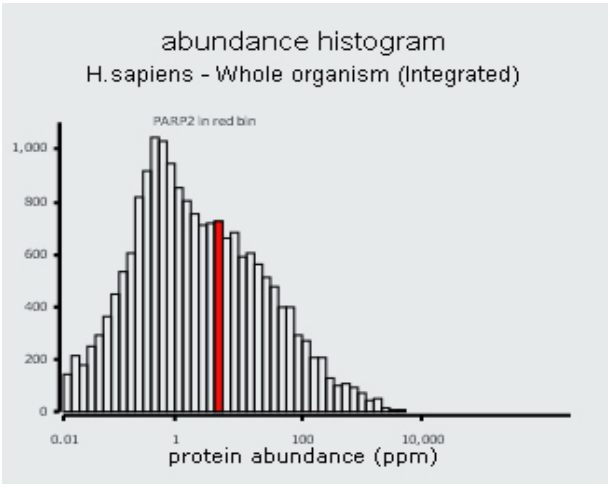

A
PAR-binding
Resonance units
time (seconds)
GST-PKM2
GST
B
NAD-
NAD+
PARP1
GST-PKM2
GST-histone H3
WB:
PAR
PARP1
GST
250kD
150kD
100kD
75kD
50kD
37kD
C
PAR
DAPI
mock
olaparib
D
Human PKM1
N
A1
B
A2
C
Homo sapiens
KMMIQRCNRAGKPVICATQM---RMQHLIAREAEAA MFHRKLF
E
U87 wt
EGF
cy
nu
IB:
PKM1
PAR
IP: PKM1
Tubulin
H3
input
F
EGF
PKM1
PAR
DAPI
merge
G
PKM2
pre laser
5min

Figure S2

A

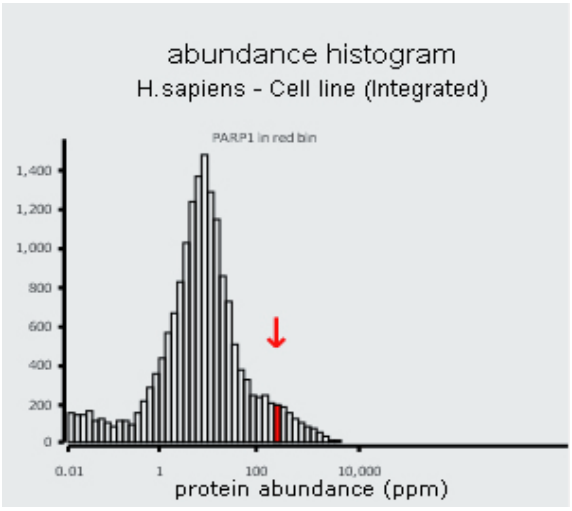


PARP1 Abundance (291ppm) Rank (586/19949 top 5%)

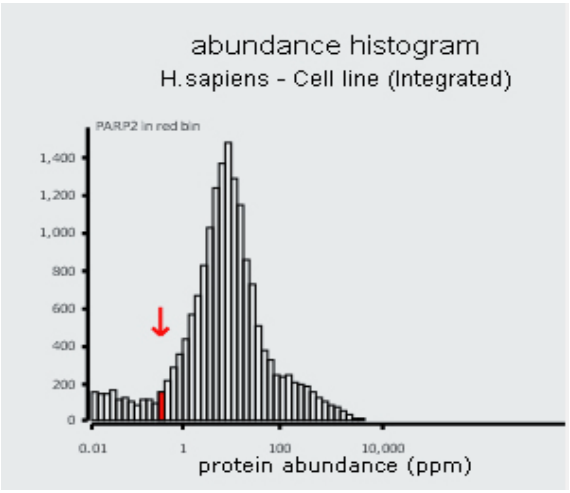


PARP2 Abundance (3.48ppm) Rank (7260/19949)

B



PARP1 Abundance (217ppm) Rank (941/18473 top 10%)



PARP2 Abundance (0.32ppm) Rank (15710/18473 bottom 25%)

Figure S3

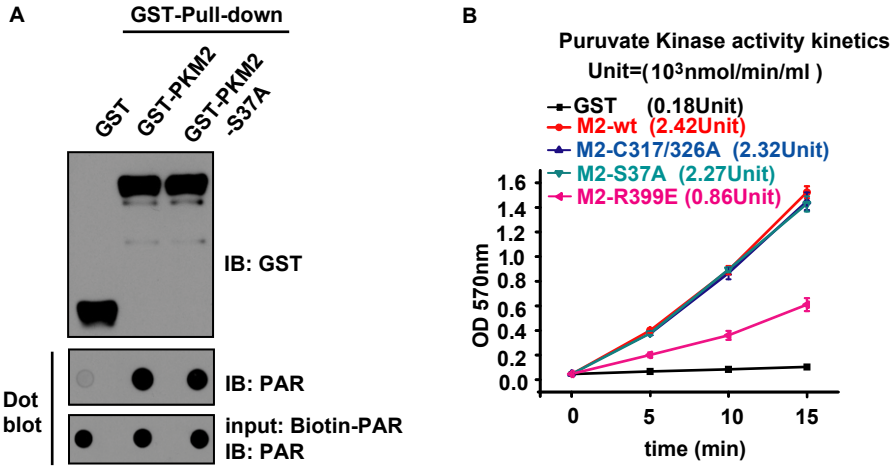

A
GST-Pull-down
GST
GST-PKM2
GST-PKM2 -S37A
IB: GST
IB: PAR
Dot blot
input: Biotin-PAR
IB: PAR
B
Puruvate Kinase activity kinetics
Unit=(10³nmol/min/ml)
GST (0.18Unit)
M2-wt (2.42Unit)
M2-C317/326A (2.32Unit)
M2-S37A (2.27Unit)
M2-R399E (0.86Unit)
OD 570nm
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
time (min)

Figure S4

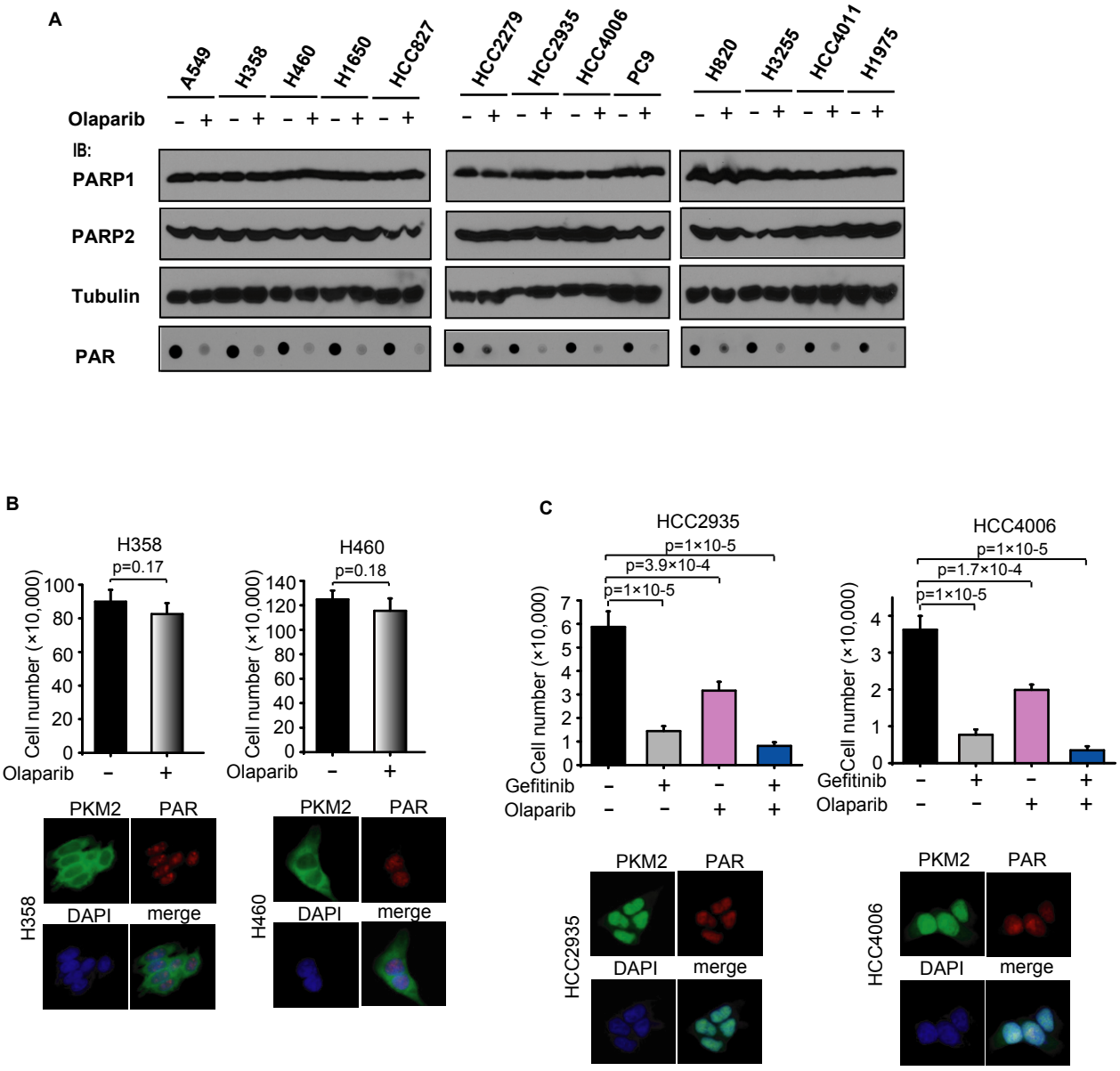

A
A549
H358
H460
H1650
HCC827
HCC2279
HCC2935
HCC4006
PC9
H820
H3255
HCC4011
H1975
Olaparib
IB:
PARP1
PARP2
Tubulin
PAR
B
H358
p=0.17
Cell number (×10,000)
Olaparib
H460
p=0.18
PKM2
PAR
DAPI
merge
C
HCC2935
HCC4006
p=1×10-5
p=3.9×10-4
p=1.7×10-4
Gefitinib
Olaparib

Figure S5

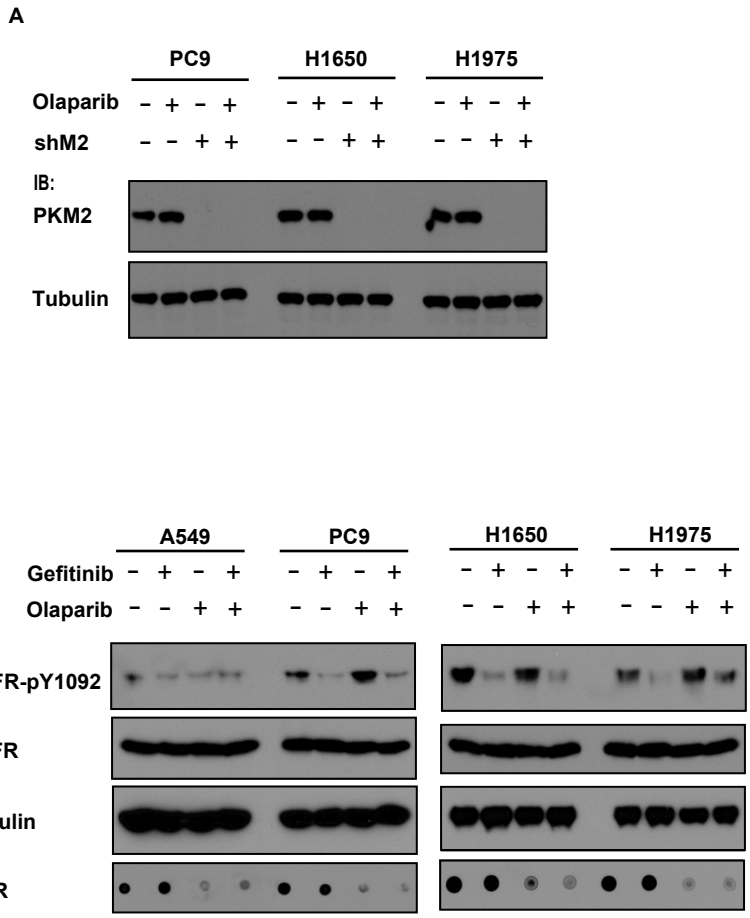
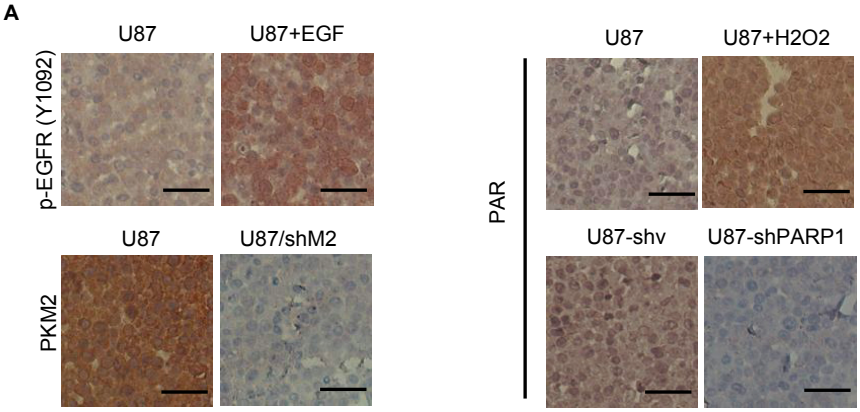

A
PC9
H1650
H1975
Olaparib - + - + - + - + - + - +
shM2 - - + + - - + + - - + +
IB:
PKM2
Tubulin
B
A549
PC9
H1650
H1975
Gefitinib - + - + - + - + - + - + - + - +
Olaparib - - + + - - + + - - + + - - + +
IB:
EGFR-pY1092
EGFR
Tubulin
PAR

Figure S6

A



B

Glioblastoma

		p-EGFR(Y1092)		total
		negative	positive	
PAR	low	15	14	29
	high	20	31	51
total		35	45	80
P= 0.3502				

p-EGFR negative		PAR		total
		negative	positive	
nuclear PKM2	low	9	18	27
	high	4	4	8
total		13	22	35
P= 0.4327				

Lung Carcinoma

		p-EGFR(Y1092)		total
		negative	positive	
PAR	low	38	20	58
	high	34	28	62
total		72	48	120
P= 0.2662				

p-EGFR negative		PAR		total
		negative	positive	
nuclear PKM2	low	27	29	56
	high	9	7	16
total		36	36	72
P= 0.7775				

Supplemental Experimental Procedures (These are related to **Experimental Procedures**)

Antibodies and Reagents

The following antibodies were used: antibodies against PKM1 (7067S), PKM2 (4053S), Histone H3 (9715S), PARP1 (9532S) were purchased from Cell Signaling Technology. Anti-PARP2 (ab176330) was obtained from Abcam. Anti-PKM2 (sc-365684) and anti-GST (sc-138) antibodies were from Santa Cruz Biotechnology. Anti-PAR antibody (551813) was from BD Bioscience. Anti-BrdU antibody (MA3-071) was from Thermo Scientific and anti-PAR (4335-MC-100) from Trevigen. Anti-EGFR-pY1172 (SAB400177), anti-α-tubulin (T6199) and anti-Flag (F1804) were purchased from Sigma-Aldrich.

EGF, Puromycin, G418, Doxycycline, LMB, and BrdU were purchased from Sigma-Aldrich, PARP inhibitors olaparib, PJ34, ABT-888, EGFR inhibitor Gefitinib, and MEK/ERK inhibitor U0126 were form Selleckchem.

Constructs and shRNAs

The PKM2 and RNF146 constructs were obtained from the Human ORFeome version 5.1 collection, and PKM1 and Histone H3 constructs were kindly provided by Dr. Zhimin Lu (MD Anderson Cancer Center). All constructs were subcloned into pDONOR201 vector and then into S-protein/Flag/SBP triple-tagged, and GST-tagged destination vectors using Gateway Technology (Invitrogen). The PKM2△301-400 mutant was generated by deleting the 301-400 amino acids of PKM2. The PKM2-C317/326A, PKM2-H379/391A and PKM2-S37A mutants were generated by site-directed mutagenesis and verified by sequencing. The pGIPZ lentiviral shRNA for PKM2 was generated with CATCTACCACTTGCAATTA oligonucleotide targeting exon 10 of the PKM2 transcript. The pLKO.1 PARP1 and PARP2 shRNA was purchased from Sigma

(PARP1:TRCN0000007929, PARP2:TRCN0000235598). The shRNA-resistant expression constructs of PKM2 and PKM2-C/A were generated by mutating the nucleotide sequence but not changing the amino acid sequence. The original shRNA targeting sequence for PKM2 (5'-CATCTACCACTTGCAATTA -3') was changed to (5'-AATATATCATCTCCAGCTG -3') and verified by sequencing.

Cell culture and Cell transfection

The A549, H358, H460, H1650, HCC827, HCC2279, HCC2935, HCC4006, PC9, H820, H3255, HCC4001, H1975, and 293T cells were purchased from American Type Culture Collection (ATCC) and cultured under conditions specified by the manufacturer. The U87/EGFR wt and U87/EGFRvIII cells were kindly provided by Dr. Zhimin Lu (MD Anderson Cancer Center) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin. Plasmid transfection was performed using Lipofectamine 2000 or polyethyleneimine reagent according to the manufacturer's protocol.

Immunoprecipitation and Immunofluorescence staining and Subcellular Fractionation

Cells were lysed with NETN buffer (20 mM Tris-HCl, pH 8.0; 100 mM NaCl; 1 mM EDTA; and 0.5% Nonidet P-40) containing protease inhibitors, the whole cell lysates obtained by centrifugation were incubated with protein A agarose beads coupled with indicated antibodies overnight at 4°C degree. The immunocomplexes were washed and boiled in 5×SDS loading buffer, and immunoblotting was carried out with indicated antibodies.

For immunofluorescence staining assays, cells cultured on cover slips were fixed by 4% paraformaldehyde for 10 min at room temperature, followed by permeabilization with 0.5% Triton

X-100 in PBS for 5 min. After for blocking with 5% goat serum in PBST for 30 min, cells were incubated with indicated primary antibodies for 1 h at room temperature, then cells were washed with PBS and incubated with rhodamine or FITC-conjugated secendary antibodies for 1 h. The cover slips were mounted on slides using anti-fade mounting medium with DAPI. Immunoflurescence images were acquired on a Nikon ECLIPSE E800 fluorescence microscope with a Nikon Plan Fluor ×40 objective lens.

Fractionation of cytoplasmic and nuclear proteins was performed as described previously(Andersen et al., 2002).

GST fusion protein expression, pull-down and mass spectrometry analysis

GST fusion proteins were expressed in E. coli and purified. The GST fusion proteins (5 pmol) were incubated with Biotin-PAR (10 pmol, Trevigen) conjugated streptavidin beads for 2 hrs at 4℃. The beads were washed by NETN buffer and then subjected to western blot using anti-GST antibody.

For Biotin-PAR pull-down followed by mass spectrometry analysis, Biotin-PAR (50 pmol, Trevigen) were conjugated to streptavidin sepharose beads for 2 hrs at 4℃, while Biotin conjugated streptavidin sepharose beads were included as negative control. HEK293T cells were lysed with NETN buffer on ice for 20 min. After removal of cell debris by centrifugation, cell lysates were incubated with streptavidin sepharose beads conjugating with Biontin-PAR or Biotin at 4℃ overnight. The beads were washed three times with NETN buffer. The proteins bound to streptavidin sepharose beads were separated by SDS-PAGE and visualized by Coomassie Blue staining. The proteins on gel were excised and identified by mass spectrometry analysis, which was performed by the Taplin Biological Mass Spectrometry Facility (Harvard Medical School).

Pyruvate kinase assay

The activity of GST, GST-PKM2, GST-PKM2-C/A, GST-PKM2-S37A and GST-PKM2-R399E (0.2 μg) towards PEP was measured with a pyruvate kinase assay (BioVision) according to the manufacturer's instruction. The pyruvate kinase activity was expressed as relative pyruvate kinase activity by defining the activity of PKM2 as 100.

Glucose uptake assay

Cells were plated in 6-cm dishes, after 24 hrs, cells were refreshed with glucose-free DMEM with 10% FBS and incubated for 16 hrs. Then cells were treated with 2-NBDG (50 uM, Invitrogen) for 1 hr and washed with PBS. Glucose uptake was quantified for the percentage of PI-negative cells using fluorescence-activated cell sorting analysis.

Lactate production assay

Cells were seeded in 6-well plates and cultured overnight, cell medium was changed with no-serum RPMI-1640 for 12 hrs, then cell medium was removed and lactate concentration was determined by using lactate Plus test trips and a Lactate Plus meter (Nova Biomedical). The remaining cells were harvested and counted using microscope. The lactate production was calculated the determined as lactate concentration per 10^4 cells.

BrdU incorporation

Cells growing on cover slips were pulse-labelled with 10 uM BrdU for 1 hour. Then cells were washed with PBS and fixed by 4% paraformaldehyde for 15 min. Afterwards, cells were permeabilized with 0.5% Triton for 5 min and 4M HCl for 30 min followed by sequential staining

with anti-BrdU antibody and FITC- conjugated secondary antibody. The nuclei were stained with 4′ and 6-diamidino-2-phenylindole (DAPI) and BrdU-positive cells were counted.

Laser Microirradiation

U87-EGFR wt cells with or without EGF treatment were plated on glass-bottomed culture dishes (Mat Tek). Laser microirradiation was performed using an IX 71 microscope (Olympus) coupled with the MicroPoint Laser Illumination and Ablation System (Photonic Instruments). A 337.1-nm laser diode transmits thought a specific cell and then yield a 365-nm wavelength laser beam that is focused through a ×60 oil objective to yield a spot size of 3.5 ns. The pulse energy is 170 μJ at 10 Hz. Immunoflurescence images were acquired on a Nikon ECLIPSE E800 fluorescence microscope with a Nikon Plan Fluor ×40 objective lens.

Surface Plasmon Resonance

Interactions between PKM2 and PAR were analyzed by surface plasmon resonance (SPR) using BIAcore 3000. Anti-GST antibody (2mg/ml) was dialyzed in 10 mM sodium acetate pH 5 overnight and covalently immobilized via amine coupling on channels 1 and 2 of a CM5 sensor chip (BD Bioscience) as per manufacturers protocol (GE Healthcare, Uppsala, Sweden) at a response of approximately 10000 resonance units. Both GST-PKM2 and GST were captured by anti-GST antibody onto channel-1 and as a control channel-2 respectively. Binding measurements were performed at 25℃ by injecting 5 μM PAR (Trevigen) in binding buffer (10 mM phosphate buffer pH7.5 containing 150 mM NaCl) over the surface of channel 1 and 2 at a flow rate of 10 uL/min for 220 seconds. Sensograms were corrected by subtraction of GST control signal from channel 1. After analysis in BiaEvalution (Biacore), the normalized resonance units were plotted over time with

the assumption of one-to-one binding.

In vitro PARsylation assay

Samples containing 0.2 µg recombinant PARP1 (purchased from Trevigen) and 2 µg recombinant GST-PKM2 or GST-Histone H3 were incubated in 50 µl PARP reaction buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 7.5 mM $MgCl_2$, 0.2 mM TCEP) with 1µM double-strand DNA (5‘-GAGTGTTGCATTCCTCTCTGGGCGCC GGG*CAGGTACCT*GCTG-3‘) and 5 mM NAD^+ for 30 min at room temperature, the reactions were stopped by the addition of SDS-loading buffer containing 0.1 M EDTA and samples were analyzed by immunoblotting using an anti-PAR antibody.