PKM2 methylation by CARM1 activates aerobic glycolysis to promote tumorigenesis

Fabao Liu1, Fengfei Ma3, Yuyuan Wang3, Ling Hao2, Hao Zeng1,9, Chenxi Jia2,9, Yidan Wang3, Peng Liu4,5, Irene M. Ong4,5, Baobin Li2, Guojun Chen3, Jiaoyang Jiang2, Shaoqin Gong3,6,10, Lingjun Li12,7,8,10 and Wei Xu1,10

Metabolic reprogramming is a hallmark of cancer. Herein we discover that the key glycolytic enzyme pyruvate kinase M2 isoform (PKM2), but not the related isoform PKM1, is methylated by co-activator-associated arginine methyltransferase 1 (CARM1). PKM2 methylation reversibly shifts the balance of metabolism from oxidative phosphorylation to aerobic glycolysis in breast cancer cells. Oxidative phosphorylation depends on mitochondrial calcium concentration, which becomes critical for cancer cell survival when PKM2 methylation is blocked. By interacting with and suppressing the expression of inositol-1,4,5-trisphosphate receptors (InsP₃,Rs), methylated PKM2 inhibits the influx of calcium from the endoplasmic reticulum to mitochondria. Inhibiting PKM2 methylation with a competitive peptide delivered by nanoparticles perturbs the metabolic energy balance in cancer cells, leading to a decrease in cell proliferation, migration and metastasis. Collectively, the CARM1–PKM2 axis serves as a metabolic reprogramming mechanism in tumorigenesis, and inhibiting PKM2 methylation generates metabolic vulnerability to InsP₃, R-dependent mitochondrial functions.

One hallmark of cancer is the Warburg effect, whereby tumour cells rely mainly on aerobic glycolysis for adenosine-5’-triphosphate (ATP) production, even with sufficient oxygen. However, metabolic adaptation in tumours extends beyond the Warburg effect, including balancing energy needs with equally important needs for macromolecular synthesis and redox homeostasis. Emerging evidence suggests that mitochondrial respiration is crucial for tumorigenesis and presents a target for cancer therapy.

Pyruvate kinase (PK) catalyses the final step in glycolysis, converting phosphoenolpyruvate to pyruvate while phosphorylating ADP to produce ATP. The M1 and M2 isoforms of PK are produced by mutually exclusive alternative splicing of PKM pre-messenger RNA. Although PKM1 and PKM2 differ by only 22 amino acids, PKM2 is not allosterically regulated and exists in a tetrameric form with high PK activity. PKM2 shifts between inactive dimeric and active tetrameric forms, modulated by phosphotyrosine signalling, metabolic intermediates (for example, FBP, serine and SAICAR) and post-translational modifications. Switching PKM2 to PKM1 reverses aerobic glycolysis to oxidative phosphorylation and reduces tumour formation in nude mice, identifying PKM2 as a potential cancer therapy target. However, a recent report challenged the PKM2-catalysed reaction as a rate-limiting step in cancer cell glycolysis and a possible protein kinase activity of PKM2 remains controversial.

Co-activator-associated arginine methyltransferase 1 (CARM1), also known as PRMT4, is a type I protein arginine methyltransferase (PRMT) that asymmetrically dimethylates protein substrates including histones, transcriptional factors and co-regulators, splicing factors and RNA polymerase II. CARM1 is overexpressed in breast cancer to promote cancer growth, and elevated CARM1 expression correlates with poor prognosis. Recently, we discovered that chromatin remodelling factor BAF155 methylation by CARM1 promotes breast cancer progression and metastasis. However, whether CARM1 regulates energy metabolism in cancer cells remains unknown.

Here, we discovered CARM1–PKM2 interaction as a major contributor to metabolic reprogramming in cancer. CARM1 methylates the dimeric form of PKM2 at Arg445/447/455. Methylated PKM2...
promotes tumour cell proliferation, migration and lung metastasis by reprogramming oxidative phosphorylation to aerobic glycolysis, and this effect was reversed by a competitive PKM2 peptide delivered using nanoparticles. We showed that methylated PKM2 localized in mitochondria-associated endoplasmic reticulum membrane, through interaction with inositol 1,4,5-trisphosphate receptors (InsP$_3$Rs), decreasing mitochondrial membrane potential ($\Delta$\psi$_{m}$) and Ca$^{2+}$ uptake, which is essential for activating pyruvate dehydrogenase (PDH) to support oxidative phosphorylation$^{32}$. Blocking PKM2 methylation elevates InsP$_3$R expression, increasing mitochondrial Ca$^{2+}$ uptake, PDH activation and oxidative phosphorylation. Thus, PKM2 methylation represents an important regulator of switching between oxidative phosphorylation to aerobic glycolysis in cancer cells.

RESULTS

CARM1 interacts with and methylates PKM2

Consistent with CARM1 promotion of tumour development and progression$^{21,23}$, knocking out CARM1 decreased DNA synthesis in MCF7 cells (Supplementary Fig. 1a). CARM1 knockout (KO) also increased mitochondrial oxygen consumption rate (OCR) but decreased lactate production without affecting glucose uptake (Supplementary Fig. 1b–f). These results prompted us to test whether CARM1 modulates energy metabolism in breast cancer cells. We identified PKM2 as a putative CARM1-interacting protein by mass spectrometry when Halo-tagged CARM1 was overexpressed in HEK293T cells (Supplementary Table 1). Endogenous CARM1–PKM2 interaction was confirmed by reciprocal co-immunoprecipitation in MCF7 cells (Fig. 1a). To determine whether CARM1 directly interacts with PKM2, we performed glutathione S-transferase (GST) pulldown using recombinant GST-tagged CARM1, Flag-tagged PKM2 and Flag-PABP1 (positive control)$^{25}$ and observed that GST-CARM1, but not GST alone, bound PKM2, indicating direct CARM1–PKM2 interaction (Fig. 1b). Interestingly, recombinant PKM1 also associated with CARM1 in vitro (Fig. 1b). To map the PKM2 region that binds CARM1, we expressed truncated Flag-PKM2 using in vitro transcription and translation and performed in vitro interaction assays with GST-CARM1. Deleting PKM2 domain C (A1) or N/A1 (A4) significantly decreased the interaction of PKM2 with CARM1, and truncation of both domains (A8) completely abolished the interaction, suggesting that the C and N/A1 domains are responsible for the interaction (Fig. 1c,d). Although these domains are identical between PKM1 and PKM2, intriguingly only PKM2, but not PKM1, can be methylated by CARM1 in vitro (Fig. 1e).

Since non-methylatable PKM1 forms only tetramers while methylatable PKM2 forms tetramers or dimers, we hypothesized that tetramer formation may prevent CARM1-mediated methylation. TEPP-46 (ref. 26), a PKM2 activator, or FBP stimulated PKM2 tetramer formation (Supplementary Fig. 2a,b), inhibiting PKM2 methylation by CARM1 (Fig. 1f). Neither TEPP-46 nor FBP affected histone H3 methylation by CARM1 (Fig. 1f), excluding the possibility that TEPP-46 and FBP interfere with CARM1 methyltransferase activity. Thus, only the dimeric form of PKM2 is methylated by CARM1.

Mammalian genomes encode nine PRMTs that sometimes share the same substrates$^{27}$. Using purified Halo-tagged$^{28}$ PRMTs (1–8), we assayed methylation of recombinant PKM2 by PRMTs and showed that PKM2 is uniquely methylated by CARM1 (Fig. 1g).

CARM1 methylates PKM2 at Arg445/447/455

To narrow down the PKM2 methylation site(s), we assayed methylation in vitro using purified GST-tagged full-length (FL) or truncated PKM2 (Fig. 2a). Among the three truncated fragments, only the C domain was significantly methylated (Fig. 2b), suggesting that it might contain the methylation site(s) of PKM2. Three arginine residues (Arg445, Arg447 and Arg455) were identified in the in vitro methylated GST-PKM2 (390–531 amino acid) fragment using liquid chromatography coupled tandem mass spectrometry (LC–MS/MS) (Fig. 2c). To further discern the major methylation site(s) of PKM2, we substituted lysine for the three methylated arginines to preserve their positive charge, individually or in combination, in GST-PKM2 (Fig. 2d). While individually mutating each arginine site slightly affected PKM2 methylation (Fig. 2e), mutating any two sites dramatically decreased methylation, and mutating all three sites abolished methylation (Fig. 2e). While all three arginine residues reside in the C domain that fosters tetramer formation, none is at the tetrameric interface (Supplementary Fig. 2a). Size-exclusion chromatography using purified recombinant His-tagged proteins showed that neither PKM2 (Supplementary Fig. 2b) nor PKM1 (Supplementary Fig. 2c) tetramer formation was affected by mutating Arg445, Arg447 and Arg455 to lysine. Thus, while CARM1 predominantly methylates dimeric PKM2, PKM2 tetramer formation is not affected by PKM2 methylation. PKM1 is not methylated by CARM1 possibly because the corresponding arginines in tetrameric PKM1 are inaccessible to CARM1.

Inhibiting PKM2 methylation decreases breast cancer cell proliferation and migration and tumour growth

To investigate the function of PKM2 methylation in breast cancer cell lines, we employed CRISPR/Cas9 technology to knock out endogenous PKM2. Genomic DNA sequencing of two representative PKM2-KO clones of MCF7 or MDA-MB-231 revealed non-homologous end-joining-induced random insertions or deletions at the targeted site (Supplementary Fig. 3a), causing mRNA degradation and PKM2 protein loss (Fig. 3a,b). Specific PKM2 KO led to compensatory PKM1 expression and arrested proliferation, with reduced DNA synthesis due to PKM1 expression, rather than PKM2 loss$^{27}$. Similarly, EdU incorporation and S-phase accumulation revealed dramatically reduced DNA synthesis when PKM2 was knocked out in MCF7 cells (Fig. 3d,e). However, the mechanism of reduced DNA synthesis in MCF7 cells appears to differ from that of MEF cells. PKM2 KO
induced massive reduction of nucleotides in MEF cells, but not in MCF7 and MDA-MB-231 cells (Supplementary Table 3). Thus, reduced DNA synthesis in MCF7 cells was not due to lack of nucleotides, as in MEFs. Also in contrast to MEFs, PKM1 overexpression in MCF7 cells did not inhibit cell proliferation (Supplementary Fig. 3d) in the presence of PKM2 expression. To delineate the functions of PKM2 methylation on energy metabolism without interference from PKM1, we restored PKM2WT or PKM2mut (where mut = mut1,2,3 from Fig. 2d) in PKM2-KO cell lines, followed by knocking down PKM1 (Fig. 3f). While knocking down PKM1 60–80% in PKM2-KO cells did not affect cell proliferation and oxidative phosphorylation (Supplementary Fig. 3e–g), cell viability was dramatically reduced when PKM1 knockdown (KD) reached nearly 100%, possibly because these cells have insufficient PK to support survival. To ensure that PKM2 methylation was defective in PKM2 methylation, we generated an antibody against an asymmetrically dimethylated Arg445 and Arg447 peptide expressed by in vitro transcripational and translational systems. (e) In vitro methylation assays using recombinant CARM1, GST-PKM1 or PKM2 protein in the presence of 3H-SAM. (f) In vitro methylation assays of PKM2 by CARM1 in the presence of FBP (100 μM) or TEPP-46 (1 μM). Histone proteins were used as negative controls. (g) In vitro methylation assays of PKM2 by PRMTs. Data represent one of three independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Fig. 9.

**Figure 1** Only the dimeric form of PKM2 is methylated by CARM1. (a) Reciprocal co-immunoprecipitation of PKM2 and CARM1 in MCF7 cells followed by western blot analysis. (b) Direct interaction of recombinant PKM1, PKM2 and PABP1 (positive control) proteins with CARM1 in GST pulldown assays. GST and GST-CARM1 were detected by anti-GST antibody, and Flag-tagged PKM1, PKM2 and PABP1 were detected by anti-Flag antibody, respectively. (c,d) Schematic showing truncations of PKM2 and in vitro interaction assays with CARM1 using full-length PKM and truncated proteins.
of PKM2, referred to as the methyl-PKM2 antibody. While PKM2\textsuperscript{WT} and PKM2\textsuperscript{mut} were restored to similar levels, methylated PKM2 was detected only in parental and PKM2\textsuperscript{WT}-expressing cells, but not in PKM2-KO or PKM2\textsuperscript{mut}-expressing cells (Fig. 3f) or in CARM1-KO cells (Supplementary Fig. 3h), demonstrating the antibody specificity. We used these cell lines to investigate the effects of methylated PKM2 on cell proliferation and colony formation in MCF7 cells and cell migration in MDA-MB-231. PKM2 methylation-defective cells (for example, PKM2 KO and PKM2\textsuperscript{mut}) elicited reduced cell proliferation and colony formation (Fig. 3g and Supplementary Fig. 3i) compared with parental and PKM2\textsuperscript{WT} MCF7 cells; however, these effects appeared not to be caused by apoptosis (Supplementary Fig. 3j). PKM2 methylation status also affected cell migration (Fig. 3h) and tumour growth of MDA-MB-231 xenografts (Fig. 3i). Therefore, PKM2 methylation is required for promoting cell proliferation, migration and tumour growth in various breast cancer cell models.

Figure 2 CARM1 methylates PKM2 at Arg445, Arg447 and Arg455. (a) Schematic diagram of full-length PKM2 and its truncated derivatives. (b) Mapping of the methylation domain of PKM2 by CARM1 in in vitro methylation assays. (c) Identification of dimethylated Arg445, Arg447 and Arg455 of the in vitro methylated GST-PKM2 protein. (d) Schematic showing arginine to lysine mutations on the GST-PKM2 protein. (e) In vitro methylation assays of PKM2 mutants relative to the wild-type PKM2. In b,e, data represent one of three independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Fig. 9.
Blocking PKM2 methylation results in elevated mitochondrial respiration in breast cancer cells

We next examined whether PKM2 methylation regulates metabolic fluxes. The switch from PKM2 to PKM1 in MCF7 and MDA-MB-231 PKM2-KO cells significantly increased OCR and reduced lactate production (Fig. 4a–c). Remarkably, the balance of OCR and lactate production could be reversed by restoring PKM2WT, but not PKM2mut (Fig. 4b,c). TEPP-46 treatment, which triggers PKM2 tetramerization, thus blocking its methylation (Figs 1f and 4d), also significantly increased OCR while decreasing lactate production.
Figure 4 Inhibiting PKM2 methylation increased mitochondrial oxidative phosphorylation. (a) The OCR curves in parental MCF7, PKM2-KO, PKM2WT/shPKM1 and PKM2mut/shPKM1 cells treated with oligomycin, FCCP and rotenone/antimycin A (n = 6 independent experiments). (b) Basal OCR and lactate production normalized to the cell numbers in parental MCF7, PKM2-KO, PKM2WT/shPKM1 and PKM2mut/shPKM1 cells (n = 6 independent experiments). (c) Basal OCR and lactate production normalized to cell numbers in parental MDA-MB-231, PKM2-KO, PKM2WT/shPKM1 and PKM2mut/shPKM1 cells (n = 6 independent experiments). (d) Western blot analysis of methyl-PKM2 in MCF7 cells treated with DMSO or TEPP-46. (e) The OCR curves in parental MCF7 cells treated with DMSO or TEPP-46 (n = 6 independent experiments). (f,g) Basal OCR and lactate production normalized to cell numbers in MCF7 cells (f) or MDA-MB-231 (g) treated with DMSO or TEPP-46 (n = 6 independent experiments). In a–e,g, data are shown as mean ± s.d. and statistics source data are available in Supplementary Table 7. Statistical significance was assessed using two-tailed t-test (f,g) and ANOVA (b,c). **P < 0.01, ***P < 0.001. In d, data represent one of three independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Fig. 9.

(Fig. 4e–g). Thus, PKM2 methylation modulates energy metabolism in cancer cells.

Reactive oxygen species (ROS), an inevitable by-product of mitochondrial oxidative phosphorylation, are often scavenged by nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (GSH). As blocking PKM2 methylation increases oxidative phosphorylation, it may elevate ROS and deplete NADPH and GSH. Indeed, ROS levels were elevated by knocking out PKM2 (Supplementary Fig. 4a), mutating PKM2 methylation sites (Supplementary Fig. 4b) or knocking out CARM1 (Supplementary Fig. 4c) in MCF7 cells relative to corresponding controls, and increased ROS was accompanied by a decreased NADPH/NADP+ ratio (Supplementary Fig. 4d,e) and GSH concentration (Supplementary Fig. 4f,g). Similarly, the NADPH/NADP+ ratio (Supplementary Fig. 4h) and GSH
concentration (Supplementary Fig. 4i) were higher in parental and PKM2WT-expressing cells than PKM2-KO and PKM2mut-expressing MDA-MB-231 cells. However, mitoTEMPO, a specific scavenger of mitochondrial superoxide, and glutathione did not alter cell proliferation and migration in cells producing high levels of ROS (that is, PKM2-KO expressing PKM2mut and CARM1-KO cells) (Supplementary Fig. 4j–o). Thus, glycolytic metabolism and growth effects regulated by PKM2 methylation are largely independent of ROS.

**Inhibiting PKM2 methylation increases Ca\(^{2+}\) uptake and mitochondrial membrane potential**

To investigate the mechanism by which mitochondrial respiration was elevated in PKM2 methylation-defective cells relative to PKM2 methylation-competent cells, we evaluated the effects of PKM2 methylation on mitochondrial membrane potential (ΔΨm), an indicator of oxidative energy metabolism. Incorporation of mitochondrial-specific JC-1 dye followed by flow cytometry showed that ΔΨm increased after knocking out PKM2 in MCF7 cells, and that restoring PKM2WT, but not PKM2mut, in PKM2-KO cells abrogated increased ΔΨm (Fig. 5a). To validate this, we used tetramethylrhodamine ethyl ester (TMRE) to measure ΔΨm. Similar results were obtained in both MCF7 (Fig. 5b) and MDA-MB-231 cells (Supplementary Fig. 5a). In accordance with the PKM2 methylation-dependent ΔΨm change, CARM1 KO also increased ΔΨm in MCF7 cells (Fig. 5c). Increased mitochondrial DNA copy number is another indicator of increased mitochondrial activity. Ablating PKM2 expression or PKM2 methylation resulted in increased mitochondrial DNA content in MCF7 cells (Supplementary Fig. 5b). Therefore, PKM2 methylation suppresses mitochondrial function and loss of PKM2 methylation releases this suppression and elevates ΔΨm.

Mitochondrial Ca\(^{2+}\), primarily driven by ΔΨm, stimulates oxidative phosphorylation to maintain cellular energy homeostasis\(^{24,29}\). ΔΨm sensitivity to PKM2 methylation prompted us to assess basal mitochondrial Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{\text{mito}}\)) using a Ca\(^{2+}\)-sensitive Rhod-2 AM dye and confocal imaging (Fig. 5d). PKM2 KO profoundly increased basal [Ca\(^{2+}\)]\(_{\text{mito}}\) in MCF7 (Fig. 5e,f) and MDA-MB-231 (Fig. 5g) cells. The effect is not cancer-cell-specific, as [Ca\(^{2+}\)]\(_{\text{mito}}\) also increased in PKM2-KO MEF cells (PKM2Wt/fl, Cre-oestrogen receptor)\(^{27}\) (Fig. 5h). Restoring PKM2WT, but not PKM2mut, abrogated the elevated basal [Ca\(^{2+}\)]\(_{\text{mito}}\) caused by PKM2 KO in MCF7 and MDA-MB-231 cells (Fig. 5f,g). Basal [Ca\(^{2+}\)]\(_{\text{mito}}\) was also elevated in CARM1-KO MCF7 cells (Fig. 5i). Thus, methylated PKM2 suppresses mitochondrial Ca\(^{2+}\) uptake. Mitochondrial matrix calcium regulates oxidative phosphorylation through activating several dehydrogenases, including PDH, which couples glycolysis to the tricarboxylic acid (TCA) cycle by pyruvate decarboxylation\(^{28}\). PDH activity is suppressed by phosphorylation by PDH kinase and enhanced by dephosphorylation by Ca\(^{2+}\)-dependent pyruvate phosphatases\(^{24,30}\). To investigate whether altered [Ca\(^{2+}\)]\(_{\text{mito}}\) levels change PDH activity, we measured phosphorylated PDH levels in PKM2-KO, CARM1-KO and PKM2WT- or PKM2mut-restored cell lines by western blot. PKM2 KO dramatically decreased PDH phosphorylation in MCF7 (Fig. 5j), MDA-MB-231 (Fig. 5k) and MEF (Fig. 5l) cells, implying increased PDH activity by [Ca\(^{2+}\)]\(_{\text{mito}}\) influx. Restoring PKM2WT, but not PKM2mut, increased phosphorylated PDH in MCF7 and MDA-MB-231 cells (Fig. 5j,k), implying attenuated PDH activity. CARM1 KO similarly decreased PDH phosphorylation in MCF7 (Fig. 5m). As expected, treating cells with dichloroacetate (DCA), a PDH kinase inhibitor, also decreased PDH phosphorylation and lactate production while increasing oxidative phosphorylation (Fig. 5n–q). Thus, PKM2 methylation is critical for restraining mitochondrial oxidative phosphorylation via decreasing mitochondrial ΔΨm and Ca\(^{2+}\) uptake, and increasing PDH phosphorylation.

**Methylated PKM2 decreases InsP3R expression**

A previous study reported PKM2 in mitochondria\(^{31}\). We confirmed PKM2 in the mitochondrial fraction by subcellular fractionation (Supplementary Fig. 6a) and observed a portion of PKM2 co-localizing with HSPA9 in mitochondrial outer membrane (Supplementary Fig. 6b). However, the mitochondrial localization of PKM2 appeared not to be affected by its methylation (Supplementary Fig. 6c). To elucidate how methylated PKM2 modulates mitochondrial oxidative phosphorylation, we overexpressed Flag-tagged PKM2WT or PKM2mut in HEK293T cells, and performed MS analyses on Flag-PKM2 co-immunoprecipitated proteins to identify differentially interacting proteins. Many interacting proteins were identical between PKM2WT and PKM2mut (Supplementary Tables 4 and 5); however, the endoplasmic reticulum (ER) calcium-releasing proteins InsP3R1, 2 and 3 showed the most notable difference between PKM2WT and PKM2mut (Supplementary Fig. 6d). Interestingly, InsP3R1 and InsP3R3 are not only putative PKM2-interacting proteins but their levels also increased in PKM2-KO MCF7 cells (Fig. 3c and Supplementary Fig. 6e). To confirm the proteomics results, we examined InsP3R1 and InsP3R3 expression levels by western blotting in different PKM2-KO clones from MCF7 and MDA-MB-231 cells. Both InsP3R1 and InsP3R3 were significantly elevated in three different PKM2-KO clones of two cell lines (Fig. 6a). As a negative control, levels of HSPA9, another PKM2-interacting protein, were insensitive to PKM2 KO. To test whether increased InsP3Rs resulted from increased PKM1 in PKM2-KO cells, we measured the kinetics of protein changes in immortalized, tamoxifen-inducible PKM2-KO (PKM2Wt/fl, Cre-oestrogen receptor) MEFs. As reported previously\(^{27}\), PKM1 expression was elevated after a two-day 4-hydroxytamoxifen (4-OHT) treatment and plateaued after four-day treatment (Fig. 6b). However, increased InsP3R1 and InsP3R3 levels were detected only later when PKM2 was substantially lower (Fig. 6b). Moreover, overexpressing PKM1 failed to increase InsP3R expression (Supplementary Fig. 6f), reinforcing that InsP3R1 and InsP3R3 are regulated by PKM2 but not PKM1. Co-immunoprecipitation showed that PKM2, but not PKM1, interacted with the endogenous InsP3R1 and InsP3R3 in breast cancer cells (Fig. 6c and Supplementary Fig. 6g). Thus, elevated InsP3R levels in PKM2-KO cells probably result from PKM2 loss rather than PKM1 gain. Inverse expression changes of InsP3R1 and InsP3R2 were also observed in The Cancer Genome Atlas (TCGA) breast tumour specimens\(^{32}\). Across 1,093 primary breast tumours in TCGA, CARM1 and PKM2 mRNA levels were positively correlated but negatively correlated with InsP3R1 and InsP3R2 expression (Fig. 6d). Similarly, in the CPTAC 77 breast tumour proteogenomics database\(^{33}\), InsP3R2 protein level was negatively correlated with CARM1 or PKM (Fig. 6e).

To determine whether PKM2–InsP3R interaction is affected by PKM2 methylation, we precipitated PKM2 from MCF7 cells...
Figure 5 Inhibiting PKM2 methylation increases mitochondrial membrane potential and [Ca\textsuperscript{2+}]\textsubscript{mito}. (a-c) Measurement of mitochondrial membrane potential (ΔΨ) by incorporation of JC-1 dye (a) or TMRE dye (b,c) followed by flow cytometry. The ΔΨ was measured by incorporation of JC-1 (a) or TMRE dyes (b) in parental MCF7, PKM2-KO, PKM2\textsuperscript{WT}/shPKM1 and PKM2\textsuperscript{mut}/shPKM1 cells (n=3 independent experiments). Alternatively, the ΔΨ was measured by TMRE dye incorporation in parental MCF7, PKM2-KO and CARM1-KO cells (c) (n=3 independent experiments). (d) Representative images of co-localized mitochondrial tracker and Rhod-2 in MCF7 PKM2-KO cells. Scale bars, 5 μm. (e) Representative images of Rhod-2-labelled mitochondria in parental MCF7, PKM2-KO, PKM2\textsuperscript{WT}/shPKM1 and PKM2\textsuperscript{mut}/shPKM1 cells. Scale bars, 10 μm. (f-i) Relative basal [Ca\textsuperscript{2+}]\textsubscript{mito} in Rhod-2-labelled parental, PKM2-KO, PKM2\textsuperscript{WT}/shPKM1 and PKM2\textsuperscript{mut}/shPKM1 MCF7 (f) (n=3 independent experiments) or corresponding MDA-MB-231 cells (g) (n=3 independent experiments); or parental MEF and PKM2-KO cells (h) (n=3 independent experiments); or parental MCF7, CARM1-KO and PKM2-KO cells (i) (n=3 independent experiments). (j-m) Western blotting of phosphorylated PDH and total PDH in the indicated MCF7 (j) or corresponding MDA-MB-231 cells (k); or parental MEF and PKM2-KO (l); or parental MCF7, CARM1-KO and PKM2-KO cells (m). (n,o) Western blot analysis of phosphorylated PDH and total PDH in MCF7 (n) or MDA-MB-231 (o) cells treated with DCA. (p,q) Basal OCR (p) and lactate production (q) normalized to the cell numbers in MCF7 or MDA-MB-231 cells treated with DCA (n=6 independent experiments). In b,c,f-h,p–q, data are shown as mean ± s.d. and statistics source data are available in Supplementary Table 7. Statistical significance was assessed using two-tailed t-test (h,p,q) and ANOVA (b,c,f,g,l), *P<0.05, **P<0.01, ***P<0.001. NS, not significant. In d,e, data represent one of two independent experiments with similar results. In a,j–o, data represent one of three independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Fig. 9.

Stably expressing Flag-tagged PKM2\textsuperscript{WT} or PKM2\textsuperscript{mut} using anti-Flag M2 resin. PKM2–InsP\textsubscript{3R} interaction was detected in PKM2\textsuperscript{WT} but not PKM2\textsuperscript{mut}–expressing cells (Fig. 6f, upper panel). Even when Flag-PKM1 was overexpressed in HEK293T PKM2-KO cells, no PKM1–InsP\textsubscript{3R} interaction was detected (Fig. 6f, lower panel). Interestingly, InsP\textsubscript{3R} expression appears sensitive to PKM2 methylation since restoring PKM2\textsuperscript{WT}, but not PKM2\textsuperscript{mut}, abrogated elevated InsP\textsubscript{3R} expression in PKM2-KO cells (Fig. 6g). Furthermore,
Methylated PKM2 decreases InsP₃R expression. (a) Western blotting of InsP₃R₁, InsP₃R₂, p53, and HSPA9 in parental and PKM2-KO MCF7 or MDA-MB-231 cells. (b) Western blotting of InsP₃R₁, InsP₃R₃, p53, PKM1 and PKM2 in MEF (PKM2KO, Cre-ER) cells treated with 4-OHT for the indicated time. (c) Co-immunoprecipitation of InsP₃Rs and HSPA9 with PKM2 but not PKM1. Flag-tagged PKM1 or PKM2 is immunoprecipitated from cell lysates derived from parental MCF7 or PKM2-KO cells using anti-Flag antibody followed by detection of PKM1, PKM2, InsP₃R₁, InsP₃R₃, and HSPA9 by western blot. (d) mRNA correlation of CARM1, InsP₃R₁, InsP₃R₂, InsP₃R₃, and PKM2 in 1,093 primary breast tumours of TCGA (n = 1,093 biologically independent patient samples). (e) Protein correlation of CARM1, InsP₃R₁, InsP₃R₂, InsP₃R₃, and PKM in 77 breast tumours of the CPTAC collection (n = 77 biologically independent patient samples). (f) Co-immunoprecipitation of InsP₃R₁ and InsP₃R₃ with PKM2WT but not PKM2mut in MCF7 (top panel) and overexpressed PKM1 in HEK293T PKM2-KO cells (bottom panel). InsP₃R₁, InsP₃R₃, PKM1, PKM2, and methyl-PKM2 were detected by western blot in Flag-PKM1/2 immunoprecipitates using corresponding antibodies. (g) Western blotting of p53, InsP₃R₁, InsP₃R₃, and PKM1 in MCF7 PKM2-KO and MDA-MB-231 PKM2-KO cells expressing control shRNA and p53 shRNA. (h) Western blotting of p53 in parental MCF7 and CARM1-KO cells. In a-f, data represent one of three independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Fig. 9.

Methylated PKM2 restrains mitochondrial addiction to Ca²⁺ through InsP₃Rs

To test whether methylated PKM2 regulates mitochondrial functions through modulating InsP₃Rs, which sustain mitochondrial functions, we stably knocked down InsP₃R₃ in MCF7 PKM2-KO and MDA-MB-231 cell lines, which highly express InsP₃R₃ (Supplementary Fig. 7a,b). InsP₃R₃ KD reduced the basal [Ca²⁺]ᵢ_{mito} level (Fig. 7a,b) and Δψₘ (Fig. 7c and Supplementary Fig. 7c). Similarly, OCR was decreased by knocking down InsP₃R₃ in MCF7 PKM2-KO (Fig. 7d) and MDA-MB-231 cells (Fig. 7e). To delineate the roles of InsP₃Rs in PKM2-modulated mitochondrial activity, we knocked down InsP₃R₃ in PKM2WT- or PKM2mut-expressing cells. InsP₃R₃ KD significantly reduced OCR in PKM2WT or PKM2mut cells. Basal OCR in PKM2mut/shInsP₃R₃ cells was higher than in

Figure 6

Figure 6: Methylated PKM2 decreases InsP₃R expression. (a) Western blotting of InsP₃R₁, InsP₃R₃, p53 and HSPA9 in parental and PKM2-KO MCF7 or MDA-MB-231 cells. (b) Western blotting of InsP₃R₁, InsP₃R₃, p53, PKM1 and PKM2 in MEF (PKM2KO, Cre-ER) cells treated with 4-OHT for the indicated time. (c) Co-immunoprecipitation of InsP₃Rs and HSPA9 with PKM2 but not PKM1. Flag-tagged PKM1 or PKM2 is immunoprecipitated from cell lysates derived from parental MCF7 or PKM2-KO cells using anti-Flag antibody followed by detection of PKM1, PKM2, InsP₃R₁, InsP₃R₃, and HSPA9 by western blot. (d) mRNA correlation of CARM1, InsP₃R₁, InsP₃R₂, InsP₃R₃, and PKM2 in 1,093 primary breast tumours of TCGA (n = 1,093 biologically independent patient samples). (f) Co-immunoprecipitation of InsP₃R₁ and InsP₃R₃ with PKM2WT but not PKM2mut in MCF7 (top panel) and overexpressed PKM1 in HEK293T PKM2-KO cells (bottom panel). InsP₃R₁, InsP₃R₃, PKM1, PKM2, and methyl-PKM2 were detected by western blot in Flag-PKM1/2 immunoprecipitates using corresponding antibodies. (g) Western blotting of p53, InsP₃R₁, InsP₃R₃, and PKM1 in MCF7 PKM2-KO and MDA-MB-231 PKM2-KO cells expressing control shRNA and p53 shRNA. (h) Western blotting of p53 in parental MCF7 and CARM1-KO cells. In a−f, data represent one of three independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Fig. 9.

Methylated PKM2 restrains mitochondrial addiction to Ca²⁺ through InsP₃Rs

To test whether methylated PKM2 regulates mitochondrial functions through modulating InsP₃Rs, which sustain mitochondrial functions, we stably knocked down InsP₃R₃ in MCF7 PKM2-KO and MDA-MB-231 cell lines, which highly express InsP₃R₃ (Supplementary Fig. 7a,b). InsP₃R₃ KD reduced the basal [Ca²⁺]ᵢ_{mito} level (Fig. 7a,b) and Δψₘ (Fig. 7c and Supplementary Fig. 7c). Similarly, OCR was decreased by knocking down InsP₃R₃ in MCF7 PKM2-KO (Fig. 7d) and MDA-MB-231 cells (Fig. 7e). To delineate the roles of InsP₃Rs in PKM2-modulated mitochondrial activity, we knocked down InsP₃R₃ in PKM2WT- or PKM2mut-expressing cells. InsP₃R₃ KD significantly reduced OCR in PKM2WT or PKM2mut cells. Basal OCR in PKM2mut/shInsP₃R₃ cells was higher than in
PKM2WT/shInsP3R3 cells, possibly due to other InsP3Rs (InsP3R1 and 2) that remain abundant in PKM2null cells (Fig. 7f,g). Calcium transport between the ER and mitochondria is essential for Ca2+ homeostasis and cell survival. To assess the importance of calcium haemostasis, we treated cells with xestospongin B (XeB), a specific InsP3R inhibitor, to inhibit IP3R-mediated ER Ca2+ release. PKM2-KO or PKM2null cells were more vulnerable to XeB than parental and PKM2WT cells (Fig. 7h–j and Supplementary Fig. 7d), indicating that addiction to oxidative phosphorylation following loss of PKM2 methylation plays essential roles in cell survival. Thus, methylated PKM2 repressed mitochondrial addiction to Ca2+ via interacting with and suppressing the expression of InsP3Rs.

**Inhibiting PKM2 methylation with a nanoparticle-delivered competitive peptide blocks cancer cell metastasis in vivo**

Having established that PKM2 methylation controls ER-mitochondrial Ca2+ signalling and promotes breast cancer cell proliferation and migration, we investigated whether PKM2 methylation can be therapeutically targeted. To assess PKM2 methylation dynamics, we estimated the extent of endogenous PKM2 methylation in cancer cells. We used excess methyl-PKM2 antibody for immunoprecipitation and measured the proportion of PKM2 in the supernatant and pellets (that is, in the methylated form) in MCF7 cells. Approximately 40% of endogenous PKM2 was methylated (Supplementary Fig. 8a). This partial PKM2 methylation in cancer cells implies that PKM2 methylation is dynamic and...
regulatable. Peptide drugs have made huge impacts on cancer treatment\textsuperscript{35}. We evaluated whether a competitive, non-methylated PKM2 peptide encompassing the methylation sites could inhibit endogenous PKM2 methylation and reverse aerobic glycolysis to oxidative phosphorylation. As a negative control, we also synthesized a corresponding peptide with Arg445 and Arg447 asymmetrically dimethylated. In vitro, the non-methyl-peptide, but not the control methyl-peptide, abrogated CARM1-mediated methylation of PKM2 (Fig. 8a). In contrast, the peptides only partially inhibited methylation of histone H3, a control CARM1 substrate, suggesting that PKM2 is the primary target of inhibition by this peptide.

We employed unimolecular nanoparticles (UMNPs) to deliver a peptide to inhibit methylation of PKM2 in vivo (Supplementary Fig. 8b–f). To ensure that UMNPs encapsulation did not alter the PKM2 peptide's inhibitory activity, we performed PKM2 in vitro methylation assay in the presence of non-methyl-peptide-loaded UMNPs or methyl-peptide-loaded UMNPs. The UMNPs–non-methyl-peptide, but not UMNPs–methyl-peptide, inhibited CARM1-mediated PKM2 methylation (Fig. 8h). Under this condition, histone H3 methylation was not inhibited. The results imply that UMNPs–non-methyl-peptide selectively inhibited PKM2 methylation, an effect similar to expressing PKM2\textsuperscript{mut} and TEPP-46 treatment. MDA-MB-231 cells efficiently took up the 6-carboxyfluorescein-conjugated peptide (FAM-peptide)-loaded UMNPs in a dose-dependent manner (Fig. 8c). In addition, only UMNPs–non-methyl-peptide inhibited endogenous PKM2 methylation, increasing InsP\textsubscript{3} protein levels (Fig. 8d). Treatment with UMNPs–non-methyl-peptide significantly increased OCR in MCF7 and MDA-MB-231 cells relative to the UMNPs–methyl-peptide control (Fig. 8e,f). Just as CARM1 KO did not affect PK activity (Supplementary Fig. 3c), inhibiting PKM2 methylation by UMNPs–non-methyl-peptide did not alter PKM2 PK activity (Supplementary Fig. 8g). Moreover, the non-methyl-peptide, but not methyl-peptide, inhibited MCF7 cell proliferation (Fig. 8g) and MDA-MB-231 cell migration (Fig. 8h). To test whether the competitive PKM2 peptide inhibits breast cancer lung metastasis in vivo, LM2 cells\textsuperscript{36}, a metastatic MDA-MB-231 derivative clone, were tail-vein-injected into nude mice. While the majority of cells did not survive one day after injection, the remaining cells colonized in the lungs and reached 30–40% of the initial cell numbers by day 7. The mice were treated with UMNPs loaded with methyl-peptide or non-methyl-peptide on day 8 and treatment continued for three weeks. LM2 colonization and outgrowth in the lungs of the two cohorts were monitored by bioluminescence imaging\textsuperscript{23}. Bioluminescence intensities in the UMNPs–non-methyl-peptide-treated group were significantly decreased compared with those in the UMNPs–methyl-peptide-treated group, indicating that non-methyl-peptide inhibited LM2 lung colonization (Fig. 8i,j). Thus, targeting PKM2 methylation is a feasible therapeutic strategy to reverse oncogenic processes.

**DISCUSSION**

We show here that reversible PKM2 methylation reprogrammes cancer metabolism from oxidative phosphorylation to aerobic glycolysis. PKM2 methylation by CARM1 inhibits Ca\textsuperscript{2+} influx from ER to mitochondria. In breast cancer cells, mitochondrial oxidative phosphorylation dramatically increased following the loss of CARM1, PKM2 or PKM2 methylation, increasing basal mitochondrial [Ca\textsuperscript{2+}] and ΔΨ\textsubscript{m} (Supplementary Fig. 8h). These findings provide mechanistic insights into the metabolic reprogramming controlled by the CARM1–PKM2 axis in breast cancer cells and show that inhibiting PKM2 methylation has therapeutic applications.

PKM2 plays an important role in aerobic glycolysis by distributing glycolytic intermediates for anabolic and catabolic purposes in cancer cells\textsuperscript{37}. Several post-translational modifications of PKM2 (refs 37,38) have been reported to modulate PKM2 function by inhibiting PK activity. However, a recent study challenged the PKM2-catalysed reaction as a rate-limiting step in cancer cell glycolysis\textsuperscript{35}. Consistent with this, PKM2 PK activity was not affected by knocking out CARM1 (Supplementary Fig. 3c), or by inhibiting PKM2 methylation using non-methyl-PKM2 peptide (Supplementary Fig. 8g), suggesting that PKM2 methylation has little effect on its PK activity. Mitochondrial oxidative phosphorylation dramatically increased in CARM1-KO, PKM2-KO or PKM2\textsuperscript{mut}-expressing breast cancer cells, suggesting that non-glycolytic function of PKM2 regulates aerobic glycolysis rather than PK activity. Indeed, we found that PKM2 methylation elicits profound effects on energy production by altering mitochondrial oxidative phosphorylation. Notably, mitochondria have well-recognized roles in producing ATP and intermediates for macromolecule biosynthesis in normal and cancerous cells, and are promising chemotherapeutic targets\textsuperscript{8}. In breast cancer cells, knock down of mitochondrial p32, a critical regulator of tumour metabolism via maintenance of oxidative phosphorylation, shifted metabolism from oxidative phosphorylation to glycolysis, yet tumorigenesis was impaired\textsuperscript{2}, suggesting that high levels of glycolysis without adequate oxidative phosphorylation do not always benefit tumour growth. Thus, our results support the notion that the balance between aerobic glycolysis and mitochondrial respiration is essential for tumour progression.

Cancer cells rely on mitochondria for TCA cycle intermediates to fuel lipid, nucleic acid and protein biosynthesis essential for growth\textsuperscript{8}. The TCA cycle is regulated by mitochondrial Ca\textsuperscript{2+}, which activates matrix dehydrogenases, including pyruvate-, α-ketoglutarate- and isocitrate-dehydrogenases\textsuperscript{29}, to promote oxidative phosphorylation and ATP production\textsuperscript{30}. Mitochondrial Ca\textsuperscript{2+} is primarily taken from ER at mitochondria-associated ER membrane contacts. A minor fraction is from cytosol through low-affinity mitochondrial calcium uniporters. Both processes are tightly controlled by InsP\textsubscript{3}R, the ubiquitous family of ER Ca\textsuperscript{2+}-release channels\textsuperscript{40}. Interestingly, we found that InsP\textsubscript{3}R expression levels are inversely associated with PKM2 expression, and are sensitive to PKM2 methylation, that is, high InsP\textsubscript{3}R expression and mitochondrial Ca\textsuperscript{2+} uptake increases in PKM2 methylation-defective cells. Accordingly, mitochondrial Ca\textsuperscript{2+} uptake increases in PKM2 methylation-defective cells, activating PDH and increasing oxidative phosphorylation. PKM2 methylation, on the contrary, decreases InsP\textsubscript{3}R expression and [Ca\textsuperscript{2+}]\textsubscript{mito}, increasing PDH phosphorylation and inactivation, decreasing ΔΨ\textsubscript{m}, and switching energy homeostasis from mitochondrial respiration to aerobic glycolysis. We found that methylated PKM2 suppresses the expression of InsP\textsubscript{3}R via negatively regulating p53, the transcription factor regulating InsP\textsubscript{3}R expression. In addition to controlling InsP\textsubscript{3}R expression, methylated PKM2 co-precipitates with InsP\textsubscript{3}R1 and InsP\textsubscript{3}R3 (Fig. 6f). Thus, methylated PKM2, through regulating InsP\textsubscript{3}R expression and interaction, delicately controls Ca\textsuperscript{2+} uptake by mitochondria.

Mitochondrial Ca\textsuperscript{2+} addiction was recently identified as a feature of cancer cells\textsuperscript{41}. While inhibiting ER-to-mitochondria Ca\textsuperscript{2+} transfer
creates a bioenergetic crisis in normal and tumour cells, normal cells trigger autophagy to sustain survival, whereas the same autophagic response in tumour cells is insufficient for survival. Tumour cell survival uniquely depends on InsP$_3$R-regulated, constitutive ER-to-mitochondria Ca$^{2+}$ transfer, since inhibiting InsP$_3$R activity reduces cancer cell line proliferative potential in vitro and impairs tumour cell methylation using a competitive PKM2 peptide reduces proliferation, migration and lung metastasis of cancer cells due to increased oxidative phosphorylation. (a) In vitro methylation assays showing the inhibitory effects of the methyl- or non-methyl-PKM2 peptides on the methylation of PKM2 or a control histone H3 protein. (b) In vitro methylation assays showing the inhibitory effects of the methyl- or non-methyl-PKM2 peptides encapsulated in the UMNPs on the methylation of PKM2 or a control histone H3 protein. (c) Flow cytometric measurement of FAM-labelled peptide uptake delivered by UMNPs in MDA-MB-231 cells. (d) Western blotting of endogenous PKM2 methylation and the InsP$_3$R3 protein levels following cellular uptake of UMNPs–methyl-peptide or UMNPs–non-methyl-peptide. (e) Normalized basal OCR values in MCF7 cells under the indicated treatment conditions (n=10 independent experiments). (h) The relative migratory cell numbers of MDA-MB-231 cells treated with UMNPs–methyl-peptide or UMNPs–non-methyl-peptide. Scale bars, 50 $\mu$m. (n=3 independent experiments.) (i) Bioluminescence in lungs of mice treated with UMNPs–methyl-peptide or UMNPs–non-methyl-PKM2 peptide (n=6 biologically independent mice per group). (j) Representative bioluminescence images of mice after 28 days of treatment. The colour scale depicts the photon flux (photons per second) emitted from the lung. In e–i, data are shown as mean ± s.d. and statistics source data are available in Supplementary Table 7. Significance was assessed using two-tailed t-test (e,f,h) and ANOVA (g,i). ***P < 0.001, **P < 0.01, *P < 0.05. In a,b,d,g data represent one of three independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Fig. 9.
growth in vivo. Accordingly, increased InsP3R expression and/or activity are associated with cancer cell proliferation, survival and invasiveness. All three InsP3R subtypes are expressed in breast cancer cells at various levels to regulate intracellular Ca2+ release, which is essential for growth control of these cells. Although inhibiting PKM2 methylation appears to reduce tumour cell growth, migration and metastasis in various breast cancer cell lines, it is insufficient to alter cell survival (Supplementary Fig. 3j), whereas inhibiting both PKM2 methylation and InsP3R activity is lethal to cancer cells (Fig. 7h–j). The results indicate a gain of dependence on mitochondrial Ca2+ by cancer cells in order to maintain cell viability. The acquired mitochondrial addiction to Ca2+ renders cancer cells susceptible to therapies based on inhibiting InsP3R (for example, XeB). Thus, combinatory inhibition of InsP3R activity and PKM2 methylation may elicit synergistic therapeutic effects. Targeting cancer-specific metabolism pathways (that is, aerobic glycolysis and ER-to-mitochondria Ca2+ transfer) should provide new therapeutic avenues for cancer treatment, as exemplified by the UMN peptide delivery system here.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of this paper.

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Supplementary Information is available in the online version of this paper.

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MCF7, MDA-MB-231 and HEK293T cell lines were purchased from ATCC, LM2 was provided by J. Massagué (Howard Hughes Medical Institute), and immortalized MEFs (PKM2\textsuperscript{fl}, Cre-ER) were kindly provided by M. G. Vander Heiden (Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology) and were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) purchased from Gibco. None of the cell lines used in this study was found in the database of commonly misidentified cell lines that are maintained by ICLAC and NCBI Biosample. Cell lines were authenticated by short tandem repeat profiling and were routinely tested for mycoplasma contamination. PKM2-KO cell lines were generated using a PKM2-specific CRISPR/Cas9/EGFP plasmid. For PKM2 KO in MEF cells, MEF cells were treated with 1\textmu M 4-hydroxytamoxifen (4-OHT) at least for 8–10 days to allow complete knockout of PKM2.

For lentivirus packaging, three plasmids (PAX2, VSVG- and pLKO-shRNA-expressing plasmid) were transfected into HEK293T cells. For retrovirus packaging, the three plasmids pHIT600, VSVG- and pLNCX-PKM2-expressing plasmid were employed. Supernatant containing the virus was harvested for cell line infection after 48 h. To generate stable cell lines expressing PKM2 wild type or methylation-defective mutant in accompanying with PKM1 KO, 2 × 10\textsuperscript{6} PKM2-KO cells were seeded into six-well plates. For infection the next day, 1 ml of retrovirus was mixed with 1 ml fresh cell culture medium; Polybrene was added to a final concentration of 5 \textmu g/ml\textsuperscript{23} to increase the infection efficiency. On the second day, the cells were cultured with fresh medium containing 400 \textmu g/ml\textsuperscript{23} G418 (or 200 \textmu g/ml\textsuperscript{23} G418 for MDA-MB-231) for at least four weeks to obtain cell lines stably expressing the PKM2 wild type or the methylation-defective mutant. To knock down PKM1, the above cell lines were infected with pLKO-PKM1 shRNA lentivirus and selected with 2 \textmu g/ml\textsuperscript{23} puromycin for at least 2 weeks. The expression of PKM2 in stable cell lines was individually examined by western blotting.

Co-immunoprecipitation. Co-immunoprecipitation was performed as previously described\textsuperscript{23}.

In vitro methylation assay. In vitro methylation assay was performed as previously described\textsuperscript{23}.

Quantitative real-time PCR. Quantitative real-time PCR was performed as described previously\textsuperscript{23}.

Gel filtration. Reconstituted His-tagged PKM2 protein was incubated with TEPP-46 (10\mu M) for 1 h on ice, and then separated in the Superdex 200 Increase 10/300 GL column (GE Healthcare) in 0.01 M phosphate buer and 0.14 M NaCl at pH 7.4. The flow speed rate is 0.5 ml min\textsuperscript{-1}. Fractions (0.3 ml) were collected and analysed by UV absorbance or SDS–PAGE and western blot.

Pyruvate kinase and lactate dehydrogenase assays. Pyruvate activity was measured as described previously\textsuperscript{23}. Briefly, 2 \mu g whole cell lysate was incubated in 1 ml buffer (Tris pH 7.5 (50 mM), KC1 (100 mM), MgCl\textsubscript{2} (5 mM), ADP (0.6 mM), phosphoenolpyruvate (0.5 mM), NADH (180 \mu M) and LDH (8 units)). The change in absorbance at 340 nm owing to the oxidation of NADH was measured using a Nanodrop ND-2000 1-position spectrophotometer (Thermo). LDH activity was determined by measuring the decreased fluorescence intensity at 340 nm from the NADH oxidation in buffer (Tris pH 7.5 (30 mM), KC1 (100 mM), MgCl\textsubscript{2} (5 mM), pyruvate (20 mM), NADH (180 \mu M)).

Subcellular fractionation. The isolation of cytosol and mitochondria was conducted as described previously\textsuperscript{23}. Briefly, the cell pellets were resuspended in 11 ml ice-cold RSB hypo buffer (10 mM NaCl 1.5 mM MgCl\textsubscript{2} 10 mM Tris-HCl (pH 7.5)) and the cells were allowed to swell for 5–10 min; then the swollen cells were broken open with several strokes in the presence of 8 ml of 2.5× MS homogenization buffer (525 mM mannitol, 175 mM sucrose, 12.5 mM Tris-HCl, 2.5 mM EDTA, pH 7.5) to give a final concentration of 1× MS homogenization buffer. The homogenate was centrifuged at 1,300g for 5 min, repeated several times. After centrifugation at 7,000g for 15 min, the supernatant (cytosolic fraction) and the crude mitochondria fraction were separated. The pellet was resuspended with 1× MS homogenization buffer followed by 7,000g sedimentation several times.

Confocal imaging. MAD-MB-231 cells were fixed with 4% paraformaldehyde in culture media for 15 min at 37 °C and permeabilized with 0.2% Triton X-100 for 10 min at room temperature. The nonspecific binding was blocked by incubation with 4% BSA in PBS for 60 min, and cells were subsequently stained with primary PKM2 and HSP90 antibodies overnight at 4 °C. The slides were washed in PBS three times (5 min each time) and were incubated for 1 h with the following secondary antibodies: FITC-conjugated goat anti-mouse IgG and Dylight 594-conjugated goat anti-rabbit IgG. After being washed three times in PBS and air-dried, the coverslips were mounted in ProLong Gold anti-fade reagent with DAPI (Invitrogen). Fluorescence was examined using a Leica SP8 3X STED Super-resolution microscope equipped with a 63× objective lens with laser excitation at 405 nm, 488 nm or 592 nm. For z-stack analysis, optical sections were obtained along the z axis at 0.5- \mu m intervals. Images were analysed with ImageJ software.

Expression and purification of recombinant proteins. Human PRMT1-8 cDNAs were cloned into pFN21K HaloTag CMV Flexi Vector (Promega) and the corresponding proteins were purified as described previously\textsuperscript{23}.

GST pulldown assays. GST and GST-CARM1 proteins were expressed in E. coli BL-21 competent cells and purified by glutatione Sepharose 4B resin (GE Healthcare Life Sciences). Reconstituent, Flag-tagged PKM2 proteins were purified from HEK 293T cells. The GST pulldown experiment was conducted as described previously\textsuperscript{23}.

In vitro protein–protein interaction assay. Flag-tagged full-length PKM2 and its truncation constructs were translated by the T7 Quick Coupled Translation/Transcription system (Promega). Interaction with GST-CARM1 fusion protein (1 \mu g ml\textsuperscript{-1}) was conducted as described previously\textsuperscript{23}. For 5-ethyl-2'-deoxyuridine (EdU) incorporation assay, 3 × 10\textsuperscript{3} cells were seeded into a six-well plate and incubated with 10 \mu M EdU for 1 h, followed by procedures described in the Click-IT EdU cytometry assay kit on a BD LSRII. For the clonogenicity assay, 1,000 viable transfected cells were cultured in six-well plates for two weeks. Colonies were washed with PBS and fixed with 3.7% formaldehyde at room temperature for 15 min, and then stained for 20 min with 0.05% crystal violet.

Transwell cell migration assays. The Transwell cell migration assay was performed as described previously\textsuperscript{23}. Briefly, 1 × 10\textsuperscript{3} cells in 100 \mu l serum-free media were added into the upper chamber; 500 \mu l culture media with 20% FBS was in the lower well. After 12-h culture, cells on the upper surface of the membrane were removed and migrating cells on the lower surface were fixed with 3.7% formaldehyde in culture media at 37 °C for 15 min, and then stained with 0.05% crystal violet for 20 min.

Generation of methylated PKM2 (methyl-PKM2) specific antibody. Methyl-PKM2 specific anti-peptide antibody was generated by Genemed Synthesis. The KLH-conjugated PKM2 peptide RVR(aPr)xAPIAVT, with Arg445 and Arg447 asymmetrically dimethylated, was synthesized. This peptide corresponding to human PKM2 (amino acids 443–454) was used to immunize rabbits. Purification of antibody was conducted as described previously\textsuperscript{23}.

Measurement of oxygen consumption rate. The OCR was measured in an XF96 extracellular analyser (Seahorse Bioscience). A total of 2 × 10\textsuperscript{4} cells per well were seeded into a 96-well plate and incubated in DMEM media with 10% FBS at 37 °C. The next day, the medium was changed to analysis media containing 10 mM glucose. The cells were incubated in a CO\textsubscript{2} free incubator at 37 °C for 1 h. Cells were sequentially exposed to oligomycin (1 \mu M), FCCP (1 \mu M) and rotenone (0.5 \mu M). Each point in the traces represents the average measurement from six different wells.

Measurement of glucose uptake. Cells were washed and subsequently studied in a modified balanced salt solution (MBSS) containing (in mM): 140 NaCl, 5.4 KCl, 0.5 MgCl\textsubscript{2}, 0.4 MgSO\textsubscript{4}, 3.3 NaHCO\textsubscript{3}, 2.0 CaCl\textsubscript{2}, 10 HEPES, 5.5 glucose, pH 7.4; 2-NBDG (0.1–0.3 mM) was added to the bathing media, and after 20 min incubation and several washes, uptake of 2-NBDG was measured by fluorescence spectrometry.

Mass spectrometry analysis of arginine methylation. In-gel digestions. Experiments were performed with a previously described protocol with modifications\textsuperscript{1}. Briefly, the gel was destained twice with 100 mM (NH\textsubscript{4})HCO\textsubscript{3}/50% methanol, followed by dehydration with 25 mM (NH\textsubscript{4})HCO\textsubscript{3}/50% acetonitrile and 100% acetonitrile. The gel pieces were rehydrated with freshly prepared 25 mM dithiothreitol at 56 °C. The proteins were then alkylated with 55 mM iodoacetamide in the dark. The gel pieces were incubated with 50 ng of trypsin in 25 mM (NH\textsubscript{4})HCO\textsubscript{3}/0.01% ProteaseMAX (Promega) at 42 °C. The tryptic peptides were extracted by adding 2.5% TFA solution.

Samples were analysed on a Waters nanoAcquity UPLC system coupled to a Q-Exactive quadrupole Orbitrap mass spectrometer (Thermo). Tryptic peptides were identified and quantified using a previously described workflow\textsuperscript{1}.
Unimolecular nanoparticle (UMNP) synthesis. β-benzyl t- aspartate N-carboxyanhydride (BLA-NAc) monomer, poly (β-benzyl t-aspartate)-poly(ethylene glycol) (PBLA-PEG) block copolymers (that is, PBLA-mPEG and PBLA-Maleimide (Mal)), and poly (amidoamine)-poly(β-benzyl t-aspartate) (polyethylene glycol)-lysozyme (PAMAM-PBLA-PEG) were synthesized following the methods previously reported44. 1H NMR (400 MHz, CDCl3), BCLA-NAc: 7.42-7.28 (5H, m, Ar-H), 6.30 (1H, s, NH), 5.20 (2H, s, CH2-Ar), 4.6 (1H, t, CH), and 2.9 (2H, t, COCH2) ppm. 13C NMR (100 MHz, DMSO-D6), BCLA-mPEG: 7.26-7.38 (10H2, m, Ar-H), 5.0-5.10 (4OH, s, CH2-Ar), 4.53-4.68 (2OH, m, COCH2), 3.35-3.53 (45OH, m, CH2CH2O from PEG), and 2.48-2.90 (41H, m, COCH2) ppm. PAMAM-PBLA-PEG-Mal: 7.30-7.10 (105H, m, Ar-H), 6.90 (0.5H, s, Mal), 4.90-5.00 (4OH, s, CH2-Ar), 4.60-4.50 (20H, m, COCH2), 3.22-3.50 (45OH, m, CH2CH2O from PEG), and 2.50-2.80 (41H, m, COCH2) ppm.

To synthesize poly(amidoamine)-poly(aspartyl diethylenetriamine)-poly(ethylene glycol)-OCH3/Mal (PAMAM-PAsp(DET-Im)-PEG-Mal), PAMAM-PBLA-PEG-Mal (20 mg) was dissolved in 5 ml DME. Diethylthiuram (224 µl) was added to the solution dropwise at 4 ºC and the resulting mixture was stirred at room temperature for 4 h. Thereafter, it was added to 10 ml deionized water, neutralized by 1 M HCl and dialyzed against deionized water (MWC = 15 kDa) for 48 h. 1H NMR (400 MHz, D2O): 6.90 (0.5H, s, Mal), 4.80-4.30 (20H, s, COCH2), 4.00-3.60 (45OH, m, CH2CH2O from PEG), 3.30 (40H, s, CONHCH2), 3.10-2.98 (80H, m, CH2NHCH2), 2.75 (41H, CH2NH), and 2.70-2.50 (41H, CH2COOCH2) ppm.

To synthesize poly(amidoamine)-poly(aspartyl diethylenetriamine)-4-imidazole-carboxylate-poly(ethylene glycol)-OCH3/Mal (PAMAM-PAsp(DET-Im)-PEG-Mal) was synthesized using PAMAM-PAsp(DET-Im)-PEG-Mal following a previously reported method44. 1H NMR (400 MHz, D2O): 8.24−8.22 (5H, s, Im), 7.53−7.52 (5H, d, Im), 6.90 (0.5H, H, Mal), 4.80−4.50 (20H, s, COCH2), 4.00−3.50 (45OH, m, CH2CH2O from PEG), 3.30 (40H, s, CONHCH2), 3.10−2.98 (80H, m, CH2NHCH2), 2.75 (41H, CH2NH), and 2.70−2.50 (41H, CH2COOCH2) ppm.

To synthesize PAMAM-PAsp(DET-Im)-PEG-TAT, PAMAM-PAsp(DET-Im)-PEG-Mal was dissolved in 10 ml DME and dialyzed against deionized water (MWC = 15 kDa) for 48 h. 1H NMR (400 MHz, D2O): 8.24−8.22 (5H, s, Im), 7.35−7.32 (5H, d, Im), 6.10 (0.5H, H, Mal), 4.80−4.50 (20H, s, COCH2), 4.00−3.50 (45OH, m, CH2CH2O from PEG), 3.30 (40H, s, CONHCH2), 3.10−2.98 (80H, m, CH2NHCH2), 2.75 (41H, CH2NH), and 2.70−2.50 (41H, CH2COOCH2) ppm.

To synthesize PAMAM-PAsp(DET-Im)-PEG-TAT (PAMAM-PAsp(DET-Im)-PEG-Mal) was dissolved in 10 ml DME and dialyzed against deionized water (MWC = 15 kDa) for 48 h. 1H NMR (400 MHz, D2O): 8.24−8.22 (5H, s, Im), 7.53−7.52 (5H, d, Im), 6.90 (0.5H, H, Mal), 4.80−4.50 (20H, s, COCH2), 4.00−3.50 (45OH, m, CH2CH2O from PEG), 3.30 (40H, s, CONHCH2), 3.10−2.98 (80H, m, CH2NHCH2), 2.75 (41H, CH2NH), and 2.70−2.50 (41H, CH2COOCH2) ppm.

To prepare PKM2 peptide-loaded UMNP s, 6-carboxyfluorescein (FAM)-poly(ethylene glycol)-OCH3/Mal (PAMAM-PAsp(DET-Im)-PEG-Mal) was synthesized using pH 7.4. After 24 h stirring, the solution was purified by dialysis against deionized water (MWC = 15 kDa). 1H NMR (400 MHz, D2O): 8.24−8.22 (5H, s, Im), 7.53−7.50 (15H, s, Im and Tat), 5.82−5.72 (15H, s, COOHCH2COOH), 4.80−4.50 (20H, s, COCH2), 4.00−3.50 (45OH, m, CH2CH2O from PEG), 3.30 (40H, s, CONHCH2), 3.10−2.98 (80H, m, CH2NHCH2), 2.75 (41H, CH2NH), and 2.70−2.50 (41H, CH2COOCH2) ppm.

To prepare PKM2 peptide-loaded UMNP s, 6-carboxyfluorescein (FAM)-conjugated PKM2 peptide (FAM-PKM2 peptide, 0.5 mg) was dissolved in 0.5 ml deionized water under stirring, while the PAMAM-PAsp(DET-Asc-Im)-PEG-TAT polymer (UMNP, 2 mg) was dissolved in 1 ml deionized water at pH 7. The UMNP solution was slowly added to the peptide solution under stirring at room temperature for 4 h and dialyzed against deionized water (MWC = 100 kDa). The peptide loading level was determined by a UV-Vis spectrometer (Cary 5000 UV-Vis-NIR, Agilent Technologies) with the absorbance of FAM at 495 nm. UMNP-n-methyl-aryl-UMNP-methyl-peptide without FAM conjugation were prepared using the same method.

Characterization. The 1H NMR spectra were collected on a Bruker Advance 400 NMR spectrometer. The hydrodynamic size distribution and zeta-potential of the UMNP s were characterized using a dynamic light scattering (DLS) spectrometer (Malvern Zetasizer Nano ZS) at a polymer concentration of 0.1 mg mL−1.

Principle of PKM2 peptide delivery using UMNP. Positively charged PKM2 peptides were loaded onto the charge conversional polyauranic (PAsp(DET-Asc-Im)-PEG-Mal) segments through electrostatic interaction. PAMAM-PAsp(DET-Asc-Im) were also (for example, through the blood stream), the PAsp (DE-T-Asc-Im) segments carried negative charges, allowing for the complexation of the PKM2 peptides. Once the UMNP s were endocytosed by target cancer cells, the polyauranic PAsp(DET-Asc-Im) segments
were converted to polycationic PAsp(DET-r-IIm) segments in the acidic endocytic compartments due to the acid-induced cleavage of the aconitc acid side groups, thereby facilitating the release of PKM2 peptides. The imidazole functional groups were incorporated into the PAsp(DET-Aco-r-IIm) segments to enhance the endosomal escape capability, thereby preventing potential damage of the peptides in the acidic endosomes/lysosomes. A cell-penetrating peptide TAT was conjugated onto the surfaces of the nanocarriers to enhance their cellular uptake.

Animal experiments. All animal work was performed in accordance with protocols approved by the Research Animal Resource Center of UW-Madison and the study was compliant with ethical regulations regarding animal research. BALB/c nude female mice at 4–6 weeks old were used for all xenograft experiments (Harlan). For xenograft tumour assays, 1 × 10^6 cells (MDA-MB-231 PKM2wt/shPKM1 or PKM2mut/shPKM1 cells) were injected into the inguinal mammary fat pads of nude mice (n=6 per group). Tumour size was determined using calliper measurement and the tumour volume was calculated using the formula \( V = \frac{1}{2} \times L \times W^2 \). For lung metastasis assays, 1 × 10^6 LM2, a metastatic MDA-MB-231 derivative clone, was resuspended in 0.1 ml PBS and tail-vein-injected into mice. Mice were imaged for luciferase activity immediately after injection (day 0) to exclude any mice that were not successfully xenografted. Luciferase-based non-invasive bioluminescent imaging and analysis were performed as described previously using an IVIS Imaging System (Caliper Life Sciences). Briefly, mice were anaesthetized and injected intraperitoneally with 2 mg \( \delta \)-luciferin (20 mg mL^{-1} in PBS) (Gold Biotechnology). Imaging was completed between 5 to 15 min after injection. For bioluminescence plots, total photon flux was calculated for each mouse in the gated areas. Then, the mice were retro-orbitally injected with UMNP-methyl-peptide or UMNP-non-methyl-peptide (100 µl, 1 g l^{-1}) at the indicated time interval. Imaging was performed every week and endpoint assays were conducted four weeks after injection.

Statistics and reproducibility. Statistical testing was performed using the unpaired two-tailed Student's t-test and/or ANOVA analysis. All experiments were repeated at least three times unless otherwise indicated. N numbers are indicated in the figure legends. P value < 0.05 (\^{}*) was considered as statistically significant.

Data availability. Mass spectrometry data have been deposited in ProteomeXchange with the primary accession code PXD007671 (http://www.ebi.ac.uk/pride/archive\#). Global metabolic nucleotide data have been deposited in MetaboLights with the primary accession code MTBLS533 (http://www.ebi.ac.uk/metabolights/index\#). The RNA-seq data of 1,093 human primary solid breast tumour samples were derived from the TCGA Research Network: http://cancergenome.nih.gov and they are available in FireBrowse (http://fgdac.broadinstitute.org/rums/sidda_2016_01_26/data/BRCA/20160126/gdac.broadinstitute.org_BRCA.Merge_rnaseqv2_illuminahiseg_rnaseqv2_unc_edu_Level_3_RSEM_genes_normalized_data.Level_3.2016012800.0.0.tar.gz).

The proteomics data of 77 human breast tumour samples were derived from the CPTAC Research Network (https://proteomics.cancer.gov/programs/cptac) and they are available in (http://prot-shiny-vm.broadinstitute.org:3838/BC2016). Data related to CARM1- or PKM2-interacting proteins are provided in Supplementary Tables 1, 4 and 5. Source data for Figs 3a,d,g,i, 4a–c,e–g, 5b,c,f–h,p,q, 7a,b,d–g,i and 8e–i and Supplementary Figs 1a–f, 2b–d,g, 4a–l, 5a,b, 6i and 8g have been provided in Supplementary Table 7. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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METHODS
Erratum: PKM2 methylation by CARM1 activates aerobic glycolysis to promote tumorigenesis

Fabao Liu, Fengfei Ma, Yuyuan Wang, Ling Hao, Hao Zeng, Chenxi Jia, Yidan Wang, Peng Liu, Irene M. Ong, Baobin Li, Guojun Chen, Jiaoyang Jiang, Shaoqin Gong, Lingjun Li and Wei Xu

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In the version of this Article originally published, an amino acid (aa) range in Fig. 2a incorrectly read 390–53 aa. The correct range is 390–531 aa. In addition, two labels from Fig. 8j were displaced during production and instead appeared over Fig. 8g. These errors have now been corrected in the online version of the Article.
**Supplementary Figure 1** CARM1 KO decreases EdU incorporation and increases OCR in MCF7 cells. (a) EdU incorporation assays in parental MCF7 and CARM1 KO cells (n=3 independent experiments). Cells were incubated with 10 μM EdU for 1 hour prior to flow cytometric analysis. (b, c) Basal OCR values normalized to cell numbers in parental MCF7 and CARM1 KO cells (b) or in parental MDA-MB-231 and CARM1 KO cells (c) (n=6 independent experiments). (d, e) Relative lactate production in parental MCF7 and CARM1 KO cells (d) or in parental MDA-MB-231 and CARM1 KO cells (e) (n=3 independent experiments). (f) Relative glucose uptake in parental MCF7 and CARM1 KO cells (n=3 independent experiments). In a-f, data are shown as Mean ± SD and statistics source data are available in Supplementary Table 7. Statistical significance was assessed using two-tailed t-test. **p<0.01, ns: not significant.
Supplementary Figure 2 TEPP-46 promotes PKM2 tetramer formation whereas R445/447/455K mutations on neither PKM1 nor PKM2 alter their di-/tetra-merization status. (a) PDB structure of PKM2 tetramer (PDB ID: 3SRH) showing the positions of R445, 447 and 455 residues. Neither of the three R methylation sites is localized to the tetrameric interface. (b) Size exclusion chromatography and western blot analyses of His-tagged PKM2 in the presence and absence of TEPP-46 treatment. Wild type PKM2 and PKM2 R445/447/455K mutant peaks are completely overlapped. (c) Size exclusion chromatography and western blot analyses of His-tagged PKM1 and the corresponding R445/447/455K mutant. Mutations at R methylation sites do not alter PKM1 tetramer status. In b and c, data represent one of the two independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Figure 9.
Supplementary Figure 3 Characterization of PKM2 KO clones. (a) Genomic DNA sequencing results of selected PKM2 KO clones shows frame-shifts in PKM2 specific exon, resulting in knockout of PKM2 in MCF7 and MDA-MB-231 cells. (b) The relative pyruvate kinase activity in parental and MCF7 PKM2 KO clones (n=3 independent experiments). (c) The relative pyruvate kinase activity in parental MCF7, MCF7 CARM1 KO, parental MDA-MB-231 or MAD-MB-231 CARM1 KO cells (n=3 independent experiments). (d) Cell growth measured by MTT assays in parental MCF7 and MCF7 cells overexpressing PKM1 (n=10 independent experiments). (e) Western blot analysis of PKM1 in MCF7 PKM2 KO or MDA-MB-231 PKM2 KO cells expressing ctrl shRNA or PKM1 shRNA (#1). (f) Cell growth measured by MTT assays in MCF7 PKM2 KO cells with ctrl shRNA or PKM1 shRNA (#1) knockdown (n=6 independent experiments). (g) Basal OCR values normalized to cell numbers in MCF7 PKM2 KO cells with ctrl shRNA or PKM1 shRNA (#1) knockdown (n=3 independent experiments). (h) Western blot analysis of methylated PKM2 in immunoprecipitated PKM2 from parental and CARM1 KO cells. (i) Colony formation assays in parental MCF7, PKM2 KO, PKM2^wt/shPKM1 and PKM2^mut/shPKM1 cells. (j) Cell apoptosis measured by Annexin V and propidium iodide (PI) staining in parental MCF7, PKM2 KO, PKM2^wt/shPKM1 and PKM2^mut/shPKM1 cells. In b-d, f and h, data are shown as Mean ±SD and statistics source data are available in Supplementary Table 7. Statistical significance was assessed using two-tailed t-test (b, c and g) and ANOVA (d and f). ns: not significant. In e, h-j, data represent one of the three independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Figure 9.
Supplementary Figure 4  Inhibition of PKM2 methylation leads to increase of mitochondrial ROS levels. (a-c) the ROS levels in parental MCF7 and MCF7 PKM2 KO cells (a); MCF7 expressing PKM2^wt^/shPKM1 and PKM2^mut^/shPKM1 (b); parental MCF7 and CARM1 KO cells (c) (n=3 independent experiments). (d, f) Relative NADPH/NADP⁺ ratio (d) and GSH concentration (f) in parental MCF7, PKM2 KO, PKM2^wt^/shPKM1 and PKM2^mut^/shPKM1 cells (n=3 independent experiments). (e, g) Relative NADPH/NADP⁺ ratios (e) and GSH concentrations (g) in parental MCF7 and CARM1 KO cells (n=3). (h, i) Relative NADPH/NADP⁺ ratios (h) and GSH concentration (i) in parental MDA-MB-231, PKM2 KO, PKM2^wt^/shPKM1 and PKM2^mut^/shPKM1 cells (n=3 independent experiments) (j-l) Cell growth measured by MTT assays in MCF7 PKM2^mut^/shPKM1 (j) or MDA-MB-231 PKM2^mut^/shPKM1 (k) or MCF7 CARM1 KO (l) cells treated with mitoTEMPO (n=10 independent experiments). (m) Images of migrated MDA-MB-231 PKM2^mut^/shPKM1 cells treated with mitoTEMPO. Scale bars, 50µm. (n) Cell growth in MCF7 PKM2^mut^/shPKM1 or MDA-MB-231 PKM2^mut^/shPKM1 or MCF7 CARM1 KO cells treated with glutathione (1mM) (n=3 independent experiments). (o) Images of migrated MDA-MB-231 PKM2^mut^/shPKM1 cells treated with glutathione. Scale bars, 50µm. In a-l and n, data are shown as Mean ±SD and statistics source data are available in Supplementary Table 7. Statistical significance was assessed using two-tailed t-test (a-c, e and g) and ANOVA (d, f, h-l and n). In m and o, data represent one of the two independent experiments with similar results. *p<0.05, **p<0.01***p<0.001, ns: not significant.
Supplementary Figure 5 PKM2 methylation suppresses mitochondrial membrane potential and mitochondrial DNA content. (a) Mitochondrial membrane potential ($\Delta\Psi$) measured by the incorporation of TMRE dye in MDA-MB-231 cells (n=3 independent experiments). (b) Mitochondrial DNA (mtDNA) content in parental MCF7, PKM2 KO, PKM2$^{wt/shPKM1}$ and PKM2$^{mut/shPKM1}$ cells (n=3 independent experiments). In a and b, data are shown as Mean ±SD and statistics source data are available in Supplementary Table 7. Statistical significance was assessed using ANOVA (a and b). *$p<0.05$, **$p<0.01$***$p<0.001$, ns: not significant.
Supplementary Figure 6  MAM localized PKM2 interacts with and suppresses IP3Rs expression in methylation-dependent manner. (a) Western blot analyses of PKM1 and PKM2 in cytosolic and mitochondria fractions derived from parental MCF7 or PKM2 KO cells. VDAC and tubulin serve as mitochondria and cytoplasm markers, respectively. (b) Confocal images of PKM2 localization in mitochondria. HSPA9 serves as a positive control which largely overlap with PKM2 staining. (c) Western blot analyses of wild type or mutant PKM2 in cytosolic and mitochondria fractions from MCF7 PKM2\textsuperscript{wt/shPKM1 and PKM2\textsuperscript{mut/shPKM1 cells. (d) List of selected ER and mitochondrial proteins that interact with wild type PKM2 or methylation-defective PKM2. Flag-tagged wild type or mutant PKM2 were transiently transfected into HEK293T cells. Flag-tagged PKM2 was pulled down from cell lysates and the interacting proteins were analyzed by mass spectrometry. The numbers of the detected peptides for each protein are indicated. (e) Venn diagram of PKM2 interacting proteins identified in Fig. S 6d overlapped with the altered proteins in response to PKM2 KO in MCF7 cells (Fig. 3c). 22 PKM2 interacting proteins were upregulated and 13 PKM2 interacting proteins were downregulated. ITPRs are also known as IP3Rs. (f) Western blot analysis of IP3R1 and IP3R3 in MCF7 cells overexpressing Flag-PKM1. (g) Co-immunoprecipitation of IP3R3 with PKM2 from MCF7 and MDA-MB-231 cell lysates. (h) Western blot analyses of IP3R3 protein levels in parental MCF7, CARM1 KO, or parental MCF7 treated with DMSO or TEPP-46. (i) Q-PCR analyses of mRNA levels of IP3R1, IP3R2 and IP3R3 in parental MCF7 and MDA-MB-231 cells and their respective PKM2 KO clones (n=3 independent experiments). Data are shown as Mean ±SD and statistics source data are available in Supplementary Table 7. In a-c and f-h, data represent one of the three independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Figure 9.
Supplementary Figure 7 Methylated PKM2 restrains mitochondrial addiction to Ca^{2+} through IP3Rs. (a) Western blot analysis of relative IP3R3 in MCF7 and MDA-MB-231 cells. (b) Western blot analysis of IP3R3 knockdown efficiency in MCF7 PKM2 KO and MDA-MB-231 cells. (c) The gating strategy of flow cytometry. (d) Representative images of parental MDA-MB-231, PKM2 KO, PKM2\textsuperscript{wt/shPKM1} and PKM2\textsuperscript{mut/shPKM1} cells after treatment with 5 µM XeB for 24 hours. Scale bars, 50µm. In a and b, data represent one of the three independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Figure 9.
Supplementary Figure 8  Cellular PKM2 methylation can be inhibited by unimolecular nanoparticle (UMNP) loaded with non-methyl-PKM2 peptide. (a) Assessing the proportion of the endogenous methylated PKM2 by immunoprecipitation using the excess amount of methyl-PKM2 antibody. The amount of precipitated methyl-PKM2 is estimated by subtracting the PKM2 left in the flow-through (FT) fraction from the input following detection with PKM2 antibody. The western blot bands were quantified using ImageJ software. (b) The chemical structure of the unique unimolecular nanoparticles (UMNP) for PKM2 peptide delivery. (c) Illustration of the UMNP used for PKM2 peptide delivery. (d) Synthesis scheme of the multi-arm star block copolymer poly(amineamine)-poly(aspartate diethyltriamine-acetic acid-Imidazole)-polyethylene glycol (PAMAM-PAsp(DET-Aco-Im)-PEG-TAT). (e) 1H NMR spectrum of the multi-arm star block copolymer PAMAM-PAsp(DET-Aco-Im)-PEG-TAT. The * represents the solvent residual peak. (f) Dynamic light scattering (DLS) histogram of the UMNPs. (g) The relative pyruvate kinase activity of PKM2 in MDA-MB-231 cells treated with UMNP-methyl-peptide or UMNP-non-methyl-peptide (n=3 independent experiments). Data are shown as Mean ±SD and statistics source data are available in Supplementary Table 7. Statistical significance was assessed using two-tailed t-test. ns: not significant. (h) The schematic diagram of energy homeostasis regulated by PKM2 methylation in cancer cells. CARM1 methylates dimeric PKM2 which associates with IP3Rs to inhibit Ca\(^{2+}\) influx from ER to mitochondria, resulting in increased PDH phosphorylation and decreased oxidative phosphorylation. Inhibiting PKM2 methylation by knocking out CARM1 or PKM2 or with a competitive PKM2 peptide increases IP3Rs expression, consequently increased \([\text{Ca}^{2+}]_{\text{mito}}\), de-phosphorylated PDH, and increased oxidative phosphorylation. The cell survival depends on \([\text{Ca}^{2+}]_{\text{mito}}\) and is sensitive to IP3R inhibition. In a, data represent one of the three independent experiments with similar results. Unprocessed original scans of blots are shown in Supplementary Figure 9.
Supplementary Figure 9  Unprocessed original scans of blots.
Supplementary Figure 9 Continued
Supplementary Figure 9 Continued
Supplementary Figure 9 Continued
Supplementary Figure 9 Continued
Supplementary Figure 9 Continued
Supplementary Table Legends

Supplementary Table 1 List of CARM1 interacting proteins in HEK293T cells.

Supplementary Table 2 List of global protein changes between parental MCF7 and PKM2 KO cells.

Supplementary Table 3 Heatmap of components of nucleotide super pathways in parental cells and PKM2 KO cells.

Supplementary Table 4 List of wild type of PKM2 interacting proteins in HEK293T cells.

Supplementary Table 5 List of PKM2 mutant interacting proteins in HEK293T cells.

Supplementary Table 6 List of antibodies, reagents and primers used in this study.

Supplementary Table 7 Statistics Source Data.
## Experimental design

1. **Sample size**
   - **Describe how sample size was determined.**
     - No statistical method was used to predetermine sample size.

2. **Data exclusions**
   - **Describe any data exclusions.**
     - No samples or animals were excluded from the analyses.

3. **Replication**
   - **Describe whether the experimental findings were reliably reproduced.**
     - All attempts at replication were successful.

4. **Randomization**
   - **Describe how samples/organisms/participants were allocated into experimental groups.**
     - Animals arriving in our facility were randomly put into cages with five mice each. The mice were randomly assigned to experimental groups.

5. **Blinding**
   - **Describe whether the investigators were blinded to group allocation during data collection and/or analysis.**
     - The investigators were not blinded to allocation during data collection and/or analysis.

*Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.*

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   | n/a | Confirmed |
   |-----|-----------|
   | ☒ | The exact sample size \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
   | ☒ | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
   | ☒ | A statement indicating how many times each experiment was replicated |
   | ☒ | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
   | ☒ | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
   | ☒ | The test results (e.g. \(P\) values) given as exact values whenever possible and with confidence intervals noted |
   | ☒ | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
   | ☒ | Clearly defined error bars |

*See the web collection on statistics for biologists for further resources and guidance.*
Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Nikon Elements Workstation and ImageJ were used for confocal images analysis; Origin 2016 and graphpad were used for graphs output and statistical analysis; FlowJo 9.8.5 was used for flow data analysis.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique material was used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The commercial rabbit polyclonal anti-PKM1 (Clone [D30G6], Cat. No. 70675, 1:1000 dilution), rabbit polyclonal anti-PKM2 (Clone [D78A4], Cat. No. 40535, 1:3000 dilution), rabbit polyclonal anti-VDAC (Clone [D73D12], Cat. No. 46615, 1:1000 dilution), rabbit monoclonal anti-PDH (Clone [C54G1], Cat. No. 3205T, 1:1000 dilution), rabbit anti-Tubulin/β (Cat. No. 2148S, 1:1000 dilution) antibodies were purchased from Cell Signaling Technology. Rabbit anti-IP3R1 (Cat. No. A302-158A, 1:1000 dilution, Bethyl laboratories, Montgomery, TX), rabbit anti-IP3R3 (Cat. No. A302-159A, 1:1000 dilution, Bethyl laboratories), Mouse monoclonal anti-HSPA9 (Cat. No. MA1-094, 1:1000 dilution, Thermo Fisher scientific, Waltham, MA), rabbit polyclonal anti-p(Ser293)-PDH (Cat. No. NB110-93479SS, 1:1000 dilution, Novus Biologicals, Littleton CO) Mouse monoclonal anti-TP53 (Clone [PAb122], Cat. No. MAS-12453, Thermo Fisher scientific) and Flag M2 Affinity Gel (Sigma-Aldrich, St. Louis, MO) were purchased from individual vendors. Secondary goat anti-rabbit IgG (H+L), anti-mouse IgG (H +L) and mouse Anti-Rabbit IgG (Light Chain Specific) were from Jackson ImmunoResearch (West Grove, PA). FITC-conjugated goat anti-mouse IgG (Cat. No. A90-116F), Dylight®594 conjugated goat anti-rabbit IgG (Cat. No. A120-101D4) were obtained from Bethyl laboratories. Positive and/or negative controls such as protein knockdown, knockout, overexpression or purified proteins were used to validate the relevant antibodies used in this work.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

b. Describe the method of cell line authentication used.

ATCC

STR profiling

yes, cell lines were routinely tested for mycoplasma

None of the cell lines used in this study was found in the database of commonly misidentified cell lines that are maintained by ICLAC.

c. Report whether the cell lines were tested for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Balb/c nude female mice at 4-6 week old were used for all xenograft experiments.
Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.
Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☑️ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☑️ 3. All plots are contour plots with outliers or pseudocolor plots.

☑️ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.

1x10^6 cells were centrifuged and washed twice with PBS. The cell pellet was resuspended in 200 μl of ice-cold PBS, and then 800 μl of ice cold ethanol was added. Cells were fixed at 4°C overnight. Fixed cells were centrifuged and washed twice in PBS. Cells were treated with RNase and resuspended in the wash buffer with propidium iodide (PI, 50 μg/ml). Cells were analyzed for PI content on a BD FACSCalibur flow cytometer.

6. Identify the instrument used for data collection.

BD Biosciences, FACSCalibur and LSR II

7. Describe the software used to collect and analyze the flow cytometry data.

FlowJo 9.8.5

8. Describe the abundance of the relevant cell populations within post-sort fractions.

We collect 10,000 single cells for each sample.

9. Describe the gating strategy used.

We gate the cells using SSC-A/FSC-A, and the gate single cells using SSC-A/SSC-H. We use negative control to define the boundary of positive and negative population. Gating strategy is shown in the Supplementary fig. 7c.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☑️