

RESEARCH ARTICLE

USP7 facilitates brain tumor survival upon glucose deprivation by regulating phosphofructokinase muscle-type nuclear translocation in mice

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Abstract

Cancer cells reprogram the metabolic pathways to adapt to nutrient deficiency, while the underlying mechanism has not been fully understood. Phosphofructokinase 1 muscle type (PFKM) is the second rate-limiting step of glycolysis, catalyzing the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate. Here we show, using an orthotopic xenograft glioma mouse model, that PFKM is deubiquitinated and translocated into nucleus upon glucose deficiency, thereby activating fatty acid oxidation (FAO), which sustains tumor cell survival and ultimately promotes glioblastoma (GBM) development. Mechanistically, the levels of fructose-2,6-bisphosphate (F-2,6-BP) are decreased in tumor cells upon glucose deficiency, which enhances the interaction between ubiquitin carboxyl-terminal hydrolase 7 (USP7) and PFKM. USP7 removes the monoubiquitination of PFKM at lysine (K) 615, thereby promoting PFKM's translocation into the nucleus. Nuclear PFKM interacts with c-MYC, which upregulates the expression of carnitine o-palmitoyltransferase 1 muscle isoform (CPT1B) to activate FAO, thereby sustaining tumor cell survival upon glucose deficiency. Notably, USP7 inhibitor effectively dampens GBM development and extends the survival duration of the mice. The levels of nuclear PFKM correlate with the malignancy and prognosis of human GBM patients. Our findings reveal a novel mechanism through which USP7 senses fructose-2,6-bisphosphate levels to promote PFKM nuclear translocation, thereby sustaining tumor cell survival under

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Data availability statement: All relevant data are included in the manuscript and its [Supporting information](#) files. The numerical data underlying the main and supplementary figures are provided in S1 Data. All raw Western blot images for [Figs 1–5](#) and [S1–S5](#) are available in [S1 Raw Images](#).

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Abbreviations: AGC, automated gain control; ChIP, Chromatin Immunoprecipitation; co-IP, co-immunoprecipitation; CPT1B, carnitine o-palmitoyltransferase 1 muscle isoform; DMEM, Dulbecco's modified Eagle's medium; FAO, fatty acid oxidation; FBS, fetal bovine serum; FDR, false discovery rate; F1,6BP, fructose 1,6-bisphosphate; F-2,6-BP, fructose-2,6-bisphosphate; F6P, fructose-6-phosphate; GBM, glioblastoma multiforme; GD, glucose deprivation; HCC, hepatocellular carcinoma; IF, immunofluorescence; IHC, immunohistochemistry; NBS1, nijmegen breakage syndrome protein 1; NLS, nuclear localization sequence;

nutrient deficiency by activating FAO. This establishes the critical role of USP7 in brain tumor development and suggests the therapeutic potential of USP7 inhibitors for treating GBM.

Introduction

Cancer cells generally proliferate at a faster rate than normal cells, leading to an increased demand for energy and nutrients, particularly a greater reliance on glucose [1]. Glucose is crucial for energy production and anabolic metabolism in cancer [2,3]. Therefore, cancer cells greatly elevate the uptake and utilization of glucose from tumor microenvironment (TME). In addition, tumor-infiltrating immune cells also consume large amounts of glucose [4]. As a result, TME is characterized by limited nutrients, especially glucose deficiency [5]. Additionally, the inadequate and dysregulated vasculature in solid tumors may also contribute to insufficient nourishment, which trigger metabolic stress [6]. In response to metabolic stress, cancer cells often rewire their metabolic pathways and employ alternative nutrients, such as glutamine or fatty acids as the fuel source [7,8]. These adaptations help cancer cells survive and continue to grow, even in glucose-deficient environments. However, the underlying mechanisms of how cancer cells switch to alternative metabolic pathways under metabolic stress remain elusive.

Cancer cells typically consume glucose via the Warburg effect, relying mainly on glycolysis even in the presence of oxygen [1]. This effect is linked to heightened glycolysis due to the upregulation of key glycolytic enzymes. A primary control point in glycolysis is the conversion of fructose-6-phosphate (F6P) to fructose 1,6-bisphosphate (F1,6BP), which is catalyzed by phosphofructokinase 1 (PFK1) [9]. There are three isoforms of PFK1: platelet (PFKP), muscle (PFKM), and liver (PFKL) [10]. PFKL is most abundant in liver and kidneys, while PFKM and PFKP are found in adult muscles and platelets, respectively. Instead, all three isoforms are found in the brain [11]. PFK1 is often upregulated and hyperactivated in cancer cells [9]. Recent researches have shown that upregulation of PFKM is associated with progression and poor prognosis in various solid tumors. For instance, Zinc finger E-box-binding homeobox 1 (ZEB1), a transcription factor, could transcriptionally up-regulate the expression of PFKM and contribute to carcinogenesis and metastasis of hepatocellular carcinoma (HCC) [12]. Posttranscriptional modifications of PFKM also promote tumor progression. PFKM could be S-nitrosylated by nitric oxide synthase NOS1 at Cys351. S-nitrosylation stabilizes the tetramer of PFKM, promoting the growth and metastasis of ovarian cancer cells [13]. Intriguingly, PFKM was recently reported to reside in the nucleus. Nuclear PFKM could interact with nijmegen breakage syndrome protein 1 (NBS1) and safeguard genomic stability of glioblastoma cells [14]. However, how is PFKM nuclear translocation regulated and whether nuclear PFKM contributes to tumor cell survival upon nutrient stress remain unknown.

In this study, we investigated the role and regulatory mechanism of nucleus-translocated PFKM in cancer cells under glucose deficiency. We reveal a novel non-glycolysis-related function of PFKM in enhancing the growth and survival of

PFKM, phosphofructokinase 1 muscle type; PFK1, phosphofructokinase 1; PPP, pentose phosphate pathway; PTMs, post-translational modifications; TME, tumor microenvironment; USP7, ubiquitin carboxyl-terminal hydrolase 7; ZEB1, zinc finger E-box-binding homeobox 1.

cancer cells under glucose deficiency by switching glycolytic metabolic pathway to fatty acid oxidation (FAO), and demonstrate the regulatory mechanism underlying such role of PFKM.

Results

Nucleus-translocated PFKM sustains tumor cell survival after GD

To investigate the role of PFKM in the adaptation of tumor cells to nutrient deficiency, we subjected U87 and U251 human glioblastoma multiforme (GBM) cells to glucose deprivation (GD). Immunoblotting analysis of subcellular fractions showed that PFKM, but not PFKL or PFKP, was translocated into nucleus in the cells after GD (Figs 1A, 1B, and S1A). Similar results were obtained with immunofluorescence (IF) staining of PFKM in the cells (Fig 1C and 1D).

To determine the role of GD-induced PFKM's nuclear translocation, we mapped nuclear localization sequence (NLS) of PFKM by using sequence analysis programs (NLS-mapper) and identified the peptide from 746 to 777 as one potential NLS in PFKM (S1B Fig). To validate the prediction, we generated NLS-disrupted PFKM mutant (PFKM RKR) by mutating arginine (R) 772, lysine (K) 773, and arginine (R) 774 to alanine (A). PFKM was depleted in U87 cells by using CRISPR system and then rescued with guide RNA (gRNA)-resistant (r) PFKM WT or RKR (S1C Fig). Unlike PFKM WT, PFKM RKR was not translocated into the nucleus in response to GD (Fig 1E). Functionally, the cells rescued with rPFKM RKR exhibited much lower cell viability than the cells rescued with rPFKM WT after GD (Fig 1F). Collectively, these results demonstrate that PFKM is translocated into the nucleus upon GD, which supports tumor cell survival after GD.

Nuclear PFKM levels correlate with the malignancy and prognosis of GBM

To define clinical relevance of nucleus-translocated PFKM, we performed immunohistochemistry (IHC) analyses in tumor tissues dissected from human primary GBM patients using anti-PFKM antibody. The survival durations of 55 GBM patients, all of whom underwent standard adjuvant radiotherapy after surgery, and subsequently received treatment with an alkylating agent (primarily temozolomide), were compared based on their levels of nuclear PFKM staining, with one group exhibiting low staining (0–4) and the other high staining [5–8]. The representative image was shown in Fig 1G. Patients (21 cases) with tumors exhibiting low levels of nuclear PFKM had a median survival of 19 months, while patients (34 cases) with tumors showing high levels of nuclear PFKM had a notably lower median survival of 14 months (Fig 1H). Furthermore, we conducted an analysis of the association between nuclear PFKM levels and the malignancy of glioma. IHC analysis showed that patients (63 cases) with low-grade diffuse astrocytoma (WHO grade II; median survival time >5 years) had lower nuclear PFKM levels in tumors than those (60 cases) with high-grade GBM (Fig 1I and 1J). These results provide evidence for the involvement of nuclear PFKM in the clinical behavior of human GBM, highlighting the connection between nuclear PFKM and the prognosis of the tumor.

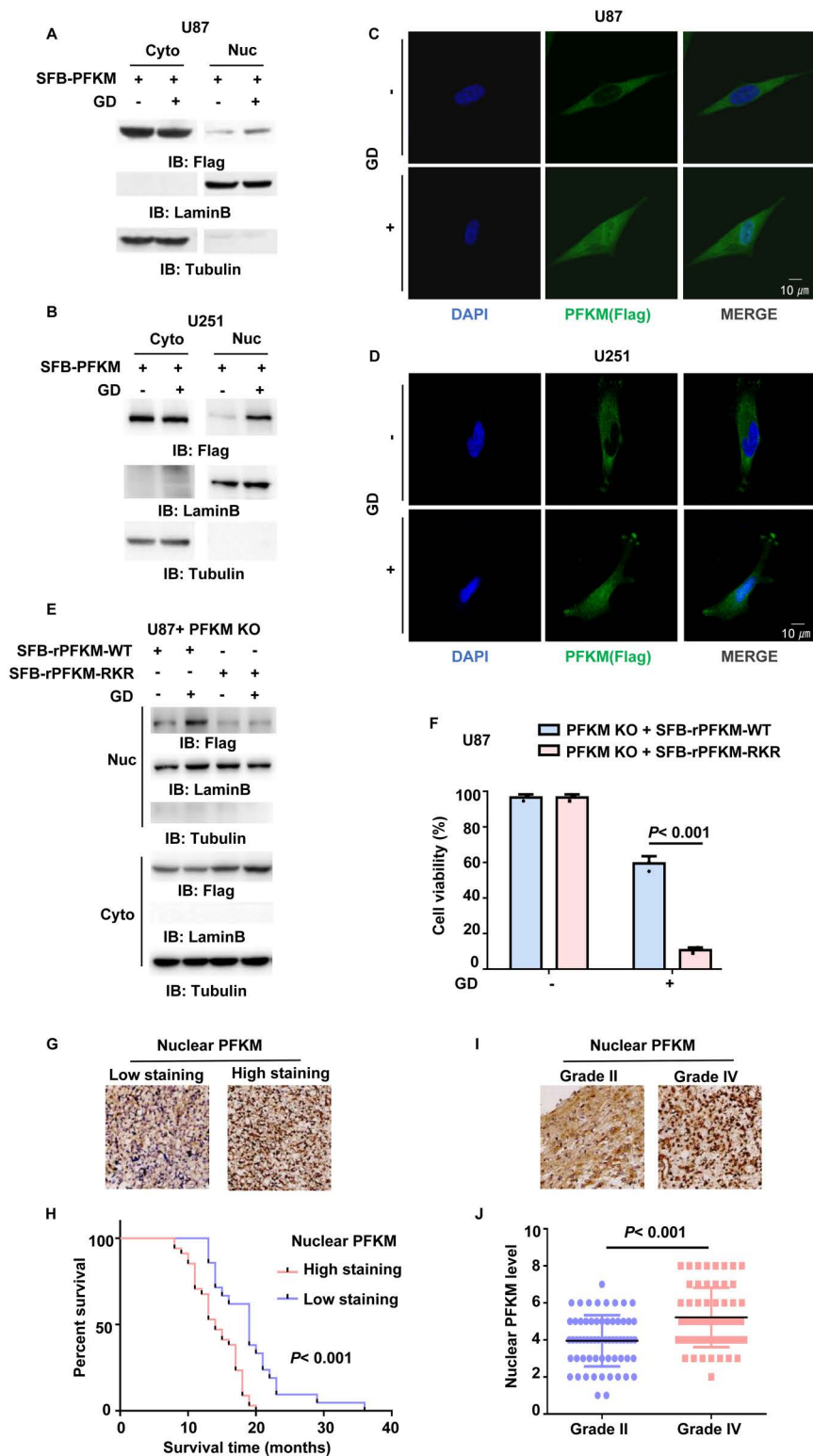


Fig 1. Nucleus-translocated PFKM sustains tumor cell survival after GD. Immunoblotting (IB) experiments were performed with the indicated antibodies. Data are representative of at least three independent experiments. **(A)** U87 cells stably expressing SFB-PFKM were treated with or without no glucose DMEM supplemented with 10% FBS (GD) for 6h. Cytosolic (Cyto) and nuclear (Nuc) fractions were prepared. **(B)** U251 cells stably expressing SFB-PFKM were treated with or without GD for 6h. Cytosolic (Cyto) and nuclear (Nuc) fractions were prepared. **(C)** U87 cells stably expressing

SFB-PFKM were treated with or without GD for 6h. IF staining was performed using anti-Flag antibody. Representative images were shown. (D) U251 cells stably expressing SFB-PFKM were treated with or without GD for 6h. IF staining was performed using anti-Flag antibody. Representative images were shown. (E) PFKM-depleted U87 cells were rescued with SFB-rPFKM WT or SFB-rPFKM RKR (NLS-disrupted PFKM mutant). Cells were treated with or without GD for 6h. Cytosolic (Cyto) and nuclear (Nuc) fractions were prepared. (F) PFKM-depleted U87 cells were rescued with SFB-rPFKM WT or SFB-rPFKM RKR. Cells were treated with or without GD for 48h. Cell viability was determined using trypan blue staining. Data represent the mean $\pm$ SD of the viability of the cells from three independent experiments (two-tailed Student *t* test). (G) Representative images for the nuclear staining of PFKM, low staining vs. high staining. (H) Survival of 55 patients with low (0–4 staining scores, blue curve) vs. high (5–8 staining scores, red curve) nuclear PFKM levels (low, 21 patients; high, 34 patients) was compared (Log-rank test). (I) Representative images for the nuclear staining of PFKM in Grade II vs. Grade IV glioma specimens. (J) 63 diffuse astrocytoma (Grade II) specimens were immunohistochemically stained using anti-PFKM antibody. Staining scores of the specimens were compared with 60 stained GBM (Grade IV) specimens (two-tailed Student *t* test). The data underlying this Figure can be found in [S1 Data](#) and [S1 Raw Images](#).

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PFKM K615 deubiquitination promotes its nuclear translocation and GBM development

Subcellular localization of proteins are frequently regulated by post-translational modifications (PTMs) [15–17]. To explore the regulatory mechanism of GD-induced PFKM nuclear translocation, we analyzed the PTMs of PFKM in response to GD. Mining the UniProt database confirmed that PFKM undergoes several PTMs, including ubiquitination, acetylation, and phosphorylation. Guided by this information, we began by evaluating GD-mediated alterations in these PFKM modifications. As shown in [Fig 2A](#), PFKM was detected to be mono-ubiquitinated. Intriguingly, this mono-ubiquitination was reduced following GD ([Fig 2B](#)). In contrast, the phosphorylation and acetylation levels of PFKM were unaffected by GD ([S2A Fig](#)). Thus, we focused our investigation on the functional role of mono-ubiquitination in regulating PFKM under GD conditions. To pinpoint the mono-ubiquitinated residue in PFKM, we generated a set of ubiquitination-deficient PFKM mutants, in which lysine (K) was mutated to arginine (R) and observed that K615R abrogated mono-ubiquitination of PFKM ([Fig 2C](#)), suggesting that PFKM was mono-ubiquitinated at K615.

We next examined whether K615 mono-ubiquitination (K615 mUb) regulated nuclear translocation of PFKM. PFKM-depleted U87 and U251 cells were rescued with rPFKM WT or K615R ([S2B Fig](#)). As shown in [Figs 2D](#) and [S2C](#), PFKM K615R had more nuclear localization in the cells after GD than PFKM WT. Moreover, the cells rescued with rPFKM K615R exhibited higher cell viability than the cells expressing rPFKM WT after GD ([Figs 2E](#) and [S2D](#)), indicating the removal of PFKM K615mUb is necessary for tumor cell survival after GD.

Furthermore, we evaluated the importance of K615 deubiquitination-promoted nuclear translocation of PFKM to GBM progression. We intracranially injected PFKM-depleted U87 cells stably expressing EGFRvIII (U87/EGFRvIII) rescued with rPFKM WT or K615R into randomized athymic nude mice ([S2E Fig](#)). Bioluminescence imaging of mice showed that the cells rescued with rPFKM K615R elicited more rapid tumor growth than the cells expressing rPFKM WT ([Fig 2F](#) and [2G](#)). Consistently, mice bearing tumors expressing rPFKM K615R exhibited significantly shorter survival durations compared to mice bearing tumors expressing rPFKM WT ([Fig 2H](#)). Of note, the expression of PFKM WT was comparable to that of PFKM K615R in glioblastoma tumors ([S2F Fig](#)).

Collectively, these results demonstrate that K615 deubiquitination promotes tumor cell survival after GD by enhancing nuclear translocation of PFKM, thereby accelerating brain tumor development.

Additionally, we further investigated how K615 mUb regulates nuclear translocation of PFKM. We performed mass spectrometry analysis of PFKM-associated proteins in U87 cells after GD, showing that IPO4 (Importin 4), a nuclear transport receptor, interacts with PFKM under GD conditions ([S2G Fig](#)). The interaction of the two proteins was validated by co-IP in HEK293T cells transfected with HA-IPO4 and SFB-PFKM after GD ([S2H Fig](#)). Importantly, GD-induced nuclear translocation of PFKM in U87 cells was dramatically inhibited by IPO4 knockdown ([S2I](#) and [S2J Fig](#)), suggesting that IPO4 mediates PFKM nuclear import upon GD. To determine whether K615 mono-ubiquitination regulated nuclear translocation of PFKM through IPO4, we examined the interaction between IPO4 and PFKM WT or the non-ubiquitinable mutant PFKM K615R. As shown in [S2K Fig](#), PFKM K615R exhibited stronger interaction with IPO4 under GD than PFKM

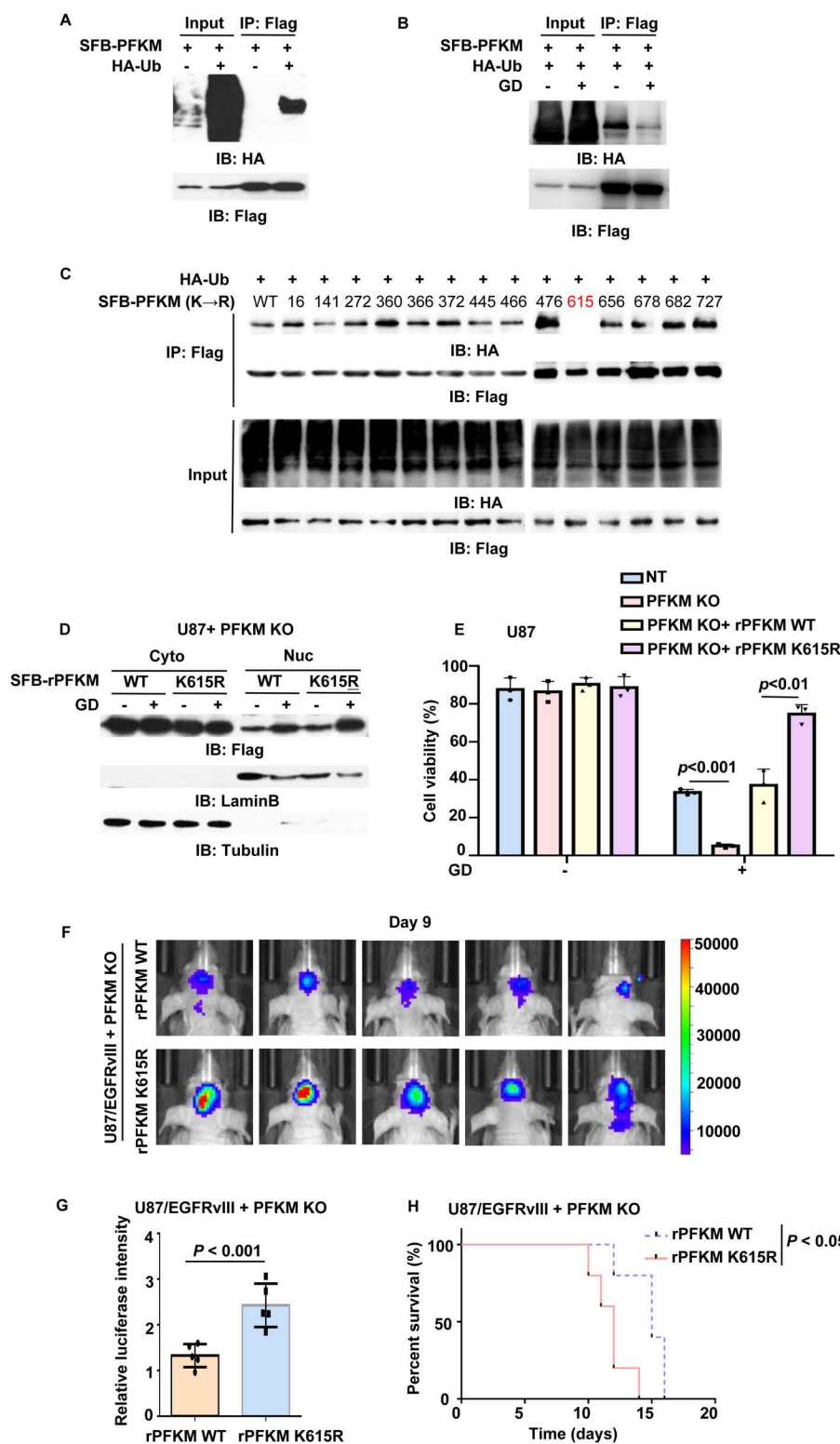


Fig 2. PFKM K615 deubiquitination promotes its nuclear translocation and GBM development. Immunoprecipitation (IP) and immunoblot (IB) analyses were performed with indicated antibodies. Data are representative of at least three independent experiments. **(A)** HEK293T cells were transfected with SFB-PFKM and HA-Ubiquitin (HA-Ub) or HA-pCDNA3. Co-IP experiment was performed with anti-Flag antibody. **(B)** HEK293T cells were

transfected with SFB-PFKM and HA-Ub. The cells were treated with or without GD for 6 h and then Co-IP experiment was performed with anti-Flag antibody. **(C)** HEK293T cells were transfected with HA-Ub and SFB-PFKM WT or K16R/K141R/K272R/K360R/K366R/K372R/K445R/K466R/K476R/K615R/K656R/K678R/K682R/K727R. Co-IP experiment was performed with anti-Flag antibody. **(D)** Endogenous PFKM in U87 cells was depleted by using CRISPR-Cas9 system and rescued these cells with gRNA-resistant (r) PFKM WT or K615R. These cells were then treated with GD treatment (6 h). Cytosolic (Cyto) and nuclear (Nuc) fractions were prepared. **(E)** PFKM-depleted U87 cells were rescued with rPFKM WT or K615R. These cells were then treated with or without GD (48 h). Cell viability was determined using trypan blue staining. Data represent the mean $\pm$ SD of the viability of the cells from three independent experiments (two-tailed Student *t* test). **(F–H)** U87/EGFRvIII cells stably expressing luciferase were depleted endogenous PFKM by using CRISPR-Cas9 system and the PFKM-depleted U87/EGFRvIII cells were reconstituted with the expression of rPFKM WT or K615R. These genetically modified cells (2×10^5 per mouse) were intracranially injected into randomized athymic nude mice (five mice per group). Bioluminescence imaging of tumor growth was carried out. Representative real-time images were presented (F) and the intensities of luciferase were quantified (G) using living image software (PerkinElmer). Data represent the mean $\pm$ SD of luciferase intensity of five mice per group (two-tailed Student *t* test). Survival durations of these implanted mice were compared (Log-rank test) **(H)**. The data underlying this Figure can be found in [S1 Data](#) and [S1 Raw Images](#).

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WT, suggesting that mono-ubiquitination at K615 prevents IPO4 binding, thereby inhibiting the nuclear translocation of PFKM. Together, these results demonstrate that IPO4 facilitates PFKM nuclear translocation upon GD, and that mono-ubiquitination at K615 inhibits this process by impairing the PFKM-IPO4 interaction.

USP7 deubiquitinates PFKM K615

Next, we explored how GD induced the deubiquitination of PFKM K615. Mass spectrometry analysis of PFKM-associated proteins in U87 cells after GD showed that ubiquitin carboxyl-terminal hydrolase 7 (USP7), a potent deubiquitinase, interacted with PFKM after GD ([S3A Fig](#)). The interaction of the two proteins was validated by co-immunoprecipitation (co-IP) in HEK293T cells transfected with HA-USP7 and SFB-PFKM after GD ([Fig 3A](#)). To confirm whether USP7 deubiquitinates PFKM upon GD, we reduced USP7 in the cells and observed that USP7 knockdown almost completely abrogated GD-induced PFKM deubiquitination ([Fig 3B](#)), suggesting that USP7 is responsible for PFKM deubiquitination after GD. Importantly, GD-induced nuclear translocation of PFKM in U87 cells was dramatically inhibited by USP7 knockdown, while that of PFKM K615R was not influenced by USP7 depletion ([Figs 3C](#) and [S3B](#)). Consistently, the viability of the cells rescued with rPFKM WT was dramatically decreased by USP7 depletion after GD treatment, while the viability of the cells rescued with rPFKM K615R was only slightly influenced ([Fig 3D](#)). Together, these results indicate that USP7 interacts with PFKM upon GD and catalyzes PFKM K615 deubiquitination, thereby promoting nuclear translocation of PFKM and tumor cell survival after GD.

Pharmacological inhibition of USP7 dampens brain tumor development

As demonstrated above, USP7 is essential for tumor cell survival upon nutrient deficiency by deubiquitinating PFKM to promote its nuclear translocation, suggesting that pharmacologically targeting USP7 could be a promising therapeutic approach to treat GBM. To test this hypothesis, we depleted endogenous PFKM in U87/EGFRvIII cells and rescued the cells with either rPFKM WT or the K615R mutant. These cells were implanted into randomized athymic nude mice, followed by treatment with the USP7 inhibitor P22077. Bioluminescence imaging and Kaplan–Meier survival analysis revealed that P22077 treatment strongly suppressed tumor growth and extended survival in mice bearing rPFKM WT tumors, whereas these anti-tumor effects were significantly attenuated in mice with rPFKM K615R tumors ([Fig 3E–3G](#)). These results implicate the therapeutic potential of USP7 inhibitor for GBM treatment and the efficacy of USP7 inhibition depends on PFKM, specifically on its deubiquitination at K615.

To further confirm the on-target effect of P22077, we combined pharmacological inhibition with genetic knockdown of USP7 using shRNA in vitro. Notably, P22077 treatment recapitulated the anti-proliferative effects observed upon USP7 knockdown. Moreover, the combined application of P22077 in USP7-depleted cells did not enhance the suppression of cell viability ([S3C Fig](#)), suggesting that P22077 exerts its function primarily through targeting USP7.

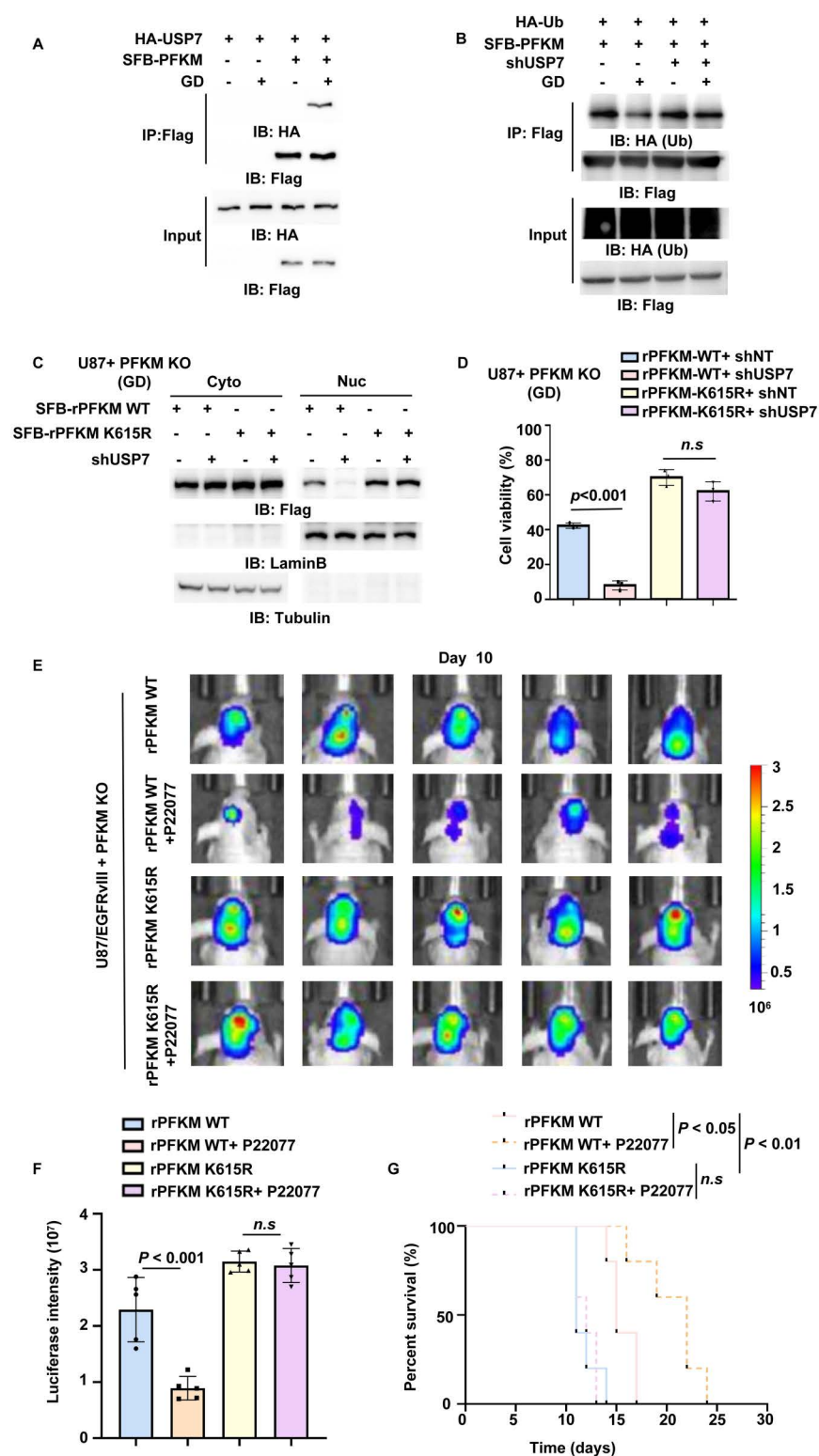


Fig 3. USP7 deubiquitinates PFKM K615. IP and IB analyses were performed with indicated antibodies. Data are representative of at least three independent experiments. **(A)** HEK293T cells were transfected with SFB-PFKM and HA-USP7. The cells were treated with or without GD for 6h and then Co-IP experiment was performed with anti-Flag antibody. **(B)** HEK293T cells were infected with the lentivirus expressing shNT or shUSP7 and then transfected with HA-Ub and SFB-PFKM. The cells were treated with or without GD for 6h and then Co-IP experiment was performed with anti-Flag

antibody. (C) PFKM-depleted U87 cells reconstituted with rPFKM WT or K615R were infected with the lentivirus expressing shNT or shUSP7. Cells were treated with GD for 6h. Cytosolic (Cyto) and nuclear (Nuc) fractions were prepared. (D) PFKM-depleted U87 cells reconstituted with rPFKM WT or K615R were infected with the lentivirus expressing shNT or shUSP7. Cells were treated with GD for 48h. Cell viability was determined using trypan blue staining. Data represent the mean $\pm$ SD of the viability of the cells from three independent experiments (two-tailed Student *t* test). (E–G) U87/EGFRvIII cells-depleted of endogenous PFKM and reconstituted with either rPFKM WT or the K615R mutant (2×10^5 per mouse) were intracranially injected into randomized athymic nude mice (five mice per group) and then treated with or without P22077 (15mg/kg/daily). Bioluminescence imaging of tumor growth were carried out. Representative real-time images were presented and the intensities of luciferase were quantified using living image software (PerkinElmer) (E, F). Data represent the mean $\pm$ SD of luciferase intensity of five mice per group (two-tailed Student *t* test). Survival durations of these implanted mice were compared (Log-rank test) (G). The data underlying this Figure can be found in [S1 Data](#) and [S1 Raw Images](#).

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F-2,6-BP prevents the interaction between PFKM and USP7

Several metabolites, including ATP, ADP, AMP, citrate, and fructose-2,6-bisphosphate (F-2,6-BP) can either activate or inhibit PFKM, thereby modulating the rate of glycolysis within cells [18,19]. It is very likely that the levels of these energy-producing metabolites will be altered in cells after GD. To explore how GD induces the interaction between USP7 and PFKM, we tested whether these metabolites can influence the interaction between these two proteins. SFB-PFKM was immunoprecipitated from glucose-starved HEK293T cells expressing SFB-PFKM and HA-USP7 and incubated with these metabolites. As shown in [Fig 4A](#), F-2,6-BP dramatically attenuated the interaction between USP7 and PFKM after GD compared to the other metabolites. F-2,6-BP, produced by PFK2 from F6P, is the most potent allosteric activator of PFKM [20]. Furthermore, we supplemented F-2,6-BP to GD-treated HEK293T cells expressing SFB-PFKM and HA-USP7 ([S4A Fig](#)). Immunoblotting analysis indicated that F-2,6-BP supplementation greatly abrogated the interaction between PFKM and USP7 after GD ([Fig 4B](#)), suggesting that F-2,6-BP inhibits the interaction of the two proteins. Consistently, F-2,6-BP supplementation abrogated GD-induced PFKM deubiquitination ([Fig 4C](#)). Notably, the levels of cellular F-2,6-BP were greatly decreased after GD ([Fig 4D](#)). In addition, we treated HEK293T cells with 3PO, an inhibitor of PFK2, to inhibit the production of F-2,6-BP. Indeed, 3PO treatment decreased F-2,6-BP levels in the cells ([S4B Fig](#)). Importantly, 3PO treatment enhanced the interaction between PFKM and USP7, thereby promoting PFKM deubiquitination ([Fig 4E](#) and [4F](#)), suggesting that the decrease in F-2,6-BP levels sufficiently enhances the interaction between PFKM and USP7 and PFKM deubiquitination. Together, these results suggest that GD promotes the interaction between USP7 and PFKM by decreasing cellular F-2,6-BP levels, thereby promoting PFKM deubiquitination.

Next, we investigated how F-2,6-BP inhibits the interaction between PFKM and USP7 under GD. PFKM undergoes dynamic conversion between its active tetrameric form and inactive dimeric form, and this process is strictly regulated. It is known that F-2,6-BP is the strongest allosteric activator of PFKM, which can directly bind to PFKM and stabilize it in the active tetrameric conformation [21]. To determine whether F-2,6-BP inhibits the PFKM-USP7 interaction by regulating PFKM oligomerization, we generated PFKM F639L mutant, which has been reported to mainly exist in a dimeric form [22], and examined its interaction with USP7, which showed that PFKM F639L exhibited stronger interaction with USP7 than PFKM WT ([Fig 4G](#)), suggesting that dimeric PFKM has a stronger interaction with USP7 than the tetrameric form. To further confirm that F-2,6-BP inhibits the PFKM-USP7 interaction by interacting with PFKM, we performed additional experiments. Firstly, Flag-PFKM and HA-USP7 were immunoprecipitated and purified from cells treated with GD. Next, we pre-incubated Flag-PFKM or HA-USP7 with F-2,6-BP, washed away the unbound F-2,6-BP, and then mixed pre-incubated Flag-PFKM or HA-USP7 with untreated HA-USP7 or Flag-PFKM for a Pull-down experiment. The results showed that Flag-PFKM pre-incubated with F-2,6-BP failed to interact with USP7 ([Fig 4H](#)), whereas HA-USP7 pre-incubated with F-2,6-BP could still interact with Flag-PFKM ([Fig 4I](#)). These results indicate that F-2,6-BP interacts with PFKM to disrupt the interaction between PFKM and USP7. Taken together, these results suggest that F-2,6-BP interacts with PFKM to promote its tetramerization, thereby attenuating its interaction with USP7.

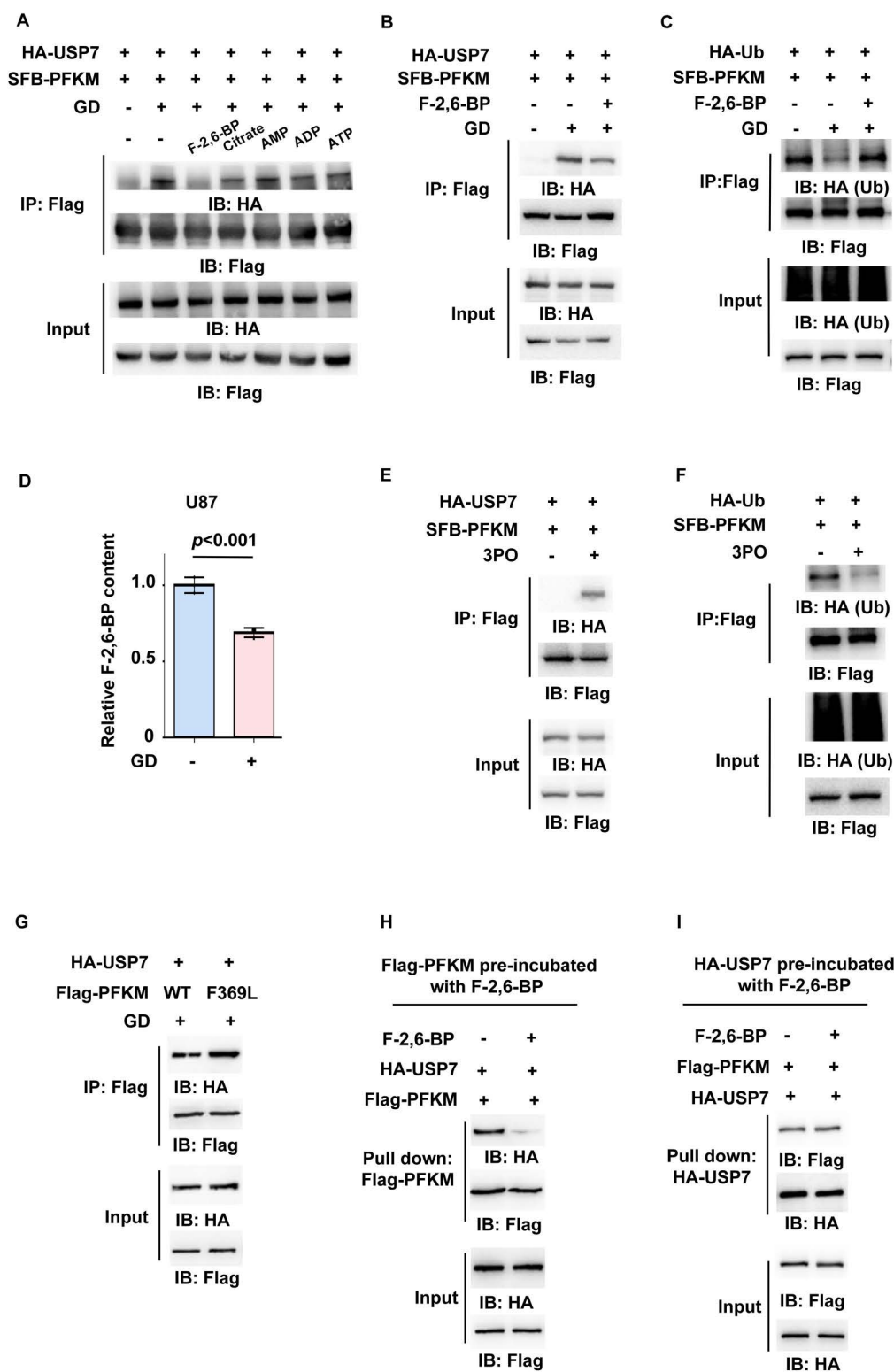


Fig 4. F-2,6-BP prevents the interaction between PFKM and USP7. (A) HEK293T cells were transfected with SFB-PFKM and HA-USP7 and then treated with GD for 6 h. SFB-PFKM is immunoprecipitated using Flag beads and the precipitate is incubated with or without F-2,6-BP/Citrate/AMP/ADP/ATP (100 μ M). (B) HEK293T cells were transfected with SFB-PFKM and HA-USP7. The cells were supplemented with or without F-2,6-BP (100 μ M) by

electroporation and then treated with or without GD for 6 h. Co-IP experiment was performed with anti-Flag antibody. **(C)** HEK293T cells were transfected with SFB-PFKM and HA-Ub. The cells were supplemented with or without F-2,6-BP (100 μ M) by electroporation and then treated with or without GD for 6 h. Co-IP experiment was performed with anti-Flag antibody. **(D)** U87 cells were treated with or without GD for 6 h. The cell lysis were collected for measurement of F-2,6-BP concentrations as determined by F-2,6-BP assay kit (Huabang Biotechnology, China). **(E)** HEK293T cells were transfected with SFB-PFKM and HA-USP7 and then treated with or without 3PO (20 μ M, 12 h). Co-IP experiment was performed with anti-Flag antibody. **(F)** HEK293T cells were transfected with SFB-PFKM and HA-Ub and then treated with or without 3PO (20 μ M, 12 h). Co-IP experiment was performed with anti-Flag antibody. **(G)** HEK293T cells were transfected with HA-USP7 and Flag-PFKM WT or F639L and then treated with GD for 6 h. Co-IP experiment was performed with anti-Flag antibody. **(H)** HEK293T cells were transfected with either HA-USP7 or Flag-PFKM and treated with GD for 6 h. Following this, Flag-PFKM and HA-USP7 were immunoprecipitated and purified from the respective cell lysates. For the pull-down assay, the purified Flag-PFKM was first pre-incubated with F-2,6-BP. After removing unbound F-2,6-BP through washing, it was then mixed with HA-USP7 to proceed with the pull-down assay. **(I)** HEK293T cells were transfected with either HA-USP7 or Flag-PFKM and treated with GD for 6 h. Following this, Flag-PFKM and HA-USP7 were immunoprecipitated and purified from the respective cell lysates. For the pull-down assay, the purified HA-USP7 was first pre-incubated with F-2,6-BP. After removing unbound F-2,6-BP through washing, it was then mixed with Flag-PFKM to proceed with the pull-down assay. The data underlying this Figure can be found in [S1 Data](#) and [S1 Raw Images](#).

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PFKM interacts with c-MYC to up-regulate CPT1B expression and fatty acid oxidation

Tumor cells exhibit remarkable metabolic plasticity in response to GD, enabling survival through two key adaptive strategies: the utilization of alternative nutrient sources to maintain energy production and the activation of mechanisms to mitigate oxidative stress. For example, under glucose-deprived conditions, many cancers enhance glutamine uptake and metabolism, which not only replenishes TCA cycle intermediates for energy generation but also contributes to redox homeostasis [7,8]. In melanoma cells, the nuclear receptor Nur77 promotes FAO to sustain NADPH and glutathione levels, thereby reducing ROS and ensuring survival during glucose starvation [23]. Additionally, GD also activates nuclear factor erythroid 2-related factor 2 (NRF2) via ROS, which upregulates Glucose-6-phosphate dehydrogenase (G6PD) to enhance the NADPH-producing pentose phosphate pathway (PPP), thereby providing metabolites for ROS scavenging and nucleic acid synthesis in gastric cancer cells [24]. The reliance on specific metabolic pathways varies across tumor types. To identify which pathway supports glioma cell survival under GD, we treated U87 cells with the glutaminase inhibitor BPTES, the FAO inhibitor Etomoxir, or the PPP inhibitor 6-AN, and examined the survival of these cells under GD. The treatment of Etomoxir, but not that of BPTES or 6-AN, dramatically reduced the viability of the cells after GD (Fig 5A), suggesting that FAO is required for the survival of glioma cells under GD.

As a key enzyme in FAO pathway, CPT1 is located on the outer mitochondrial membrane and is responsible for the transport of long-chain fatty acids into the mitochondria where they undergo beta-oxidation to produce energy [25]. And CPT1 has three isoforms, including CPT1A, CPT1B, and CPT1C. After GD, only CPT1B expression was upregulated (S5A and S5B Fig). And GD-induced CPT1B expression could be abrogated by PFKM depletion (S5B Fig). More importantly, rescued expression of rPFKM K615R could further up-regulate CPT1B expression compared to that of rPFKM WT (Fig 5B and 5C). Further, to confirm that nucleus-translocated PFKM promotes cell survival after GD through FAO upregulated by CPT1B, we measured whether FAO level was changed accordingly by tracing ^{13}C -labeled palmitate. We observed the proportions of ^{13}C incorporated citrate were increase in rPFKM K615R-U87 cells compared with rPFKM WT-U87 cells (Fig 5D). Moreover, we depleted CPT1B in PFKM-depleted U87 cells or U251 cells rescued with rPFKM WT or K615R (S5C Fig) and found that rescued expression of rPFKM K615R increased the viability of these cells after GD, while CPT1B depletion abrogated such viability increase (Figs 5E and 5SD). Similar results were obtained with the inhibition of FAO by Etomoxir in S5E and S5F Fig. Taken together, these results indicate that nucleus-translocated PFKM support tumor cell survival after GD by upregulating CPT1B expression and FAO.

Next, we explored how K615 deubiquitination-dependent nucleus-translocated PFKM upregulates CPT1B expression. Firstly, we performed Luciferase Reporter Assays using the CPT1B promoter. The results clearly show that PFKM WT enhances CPT1B promoter activity under GD conditions, with the PFKM K615R further increasing this activity, suggesting

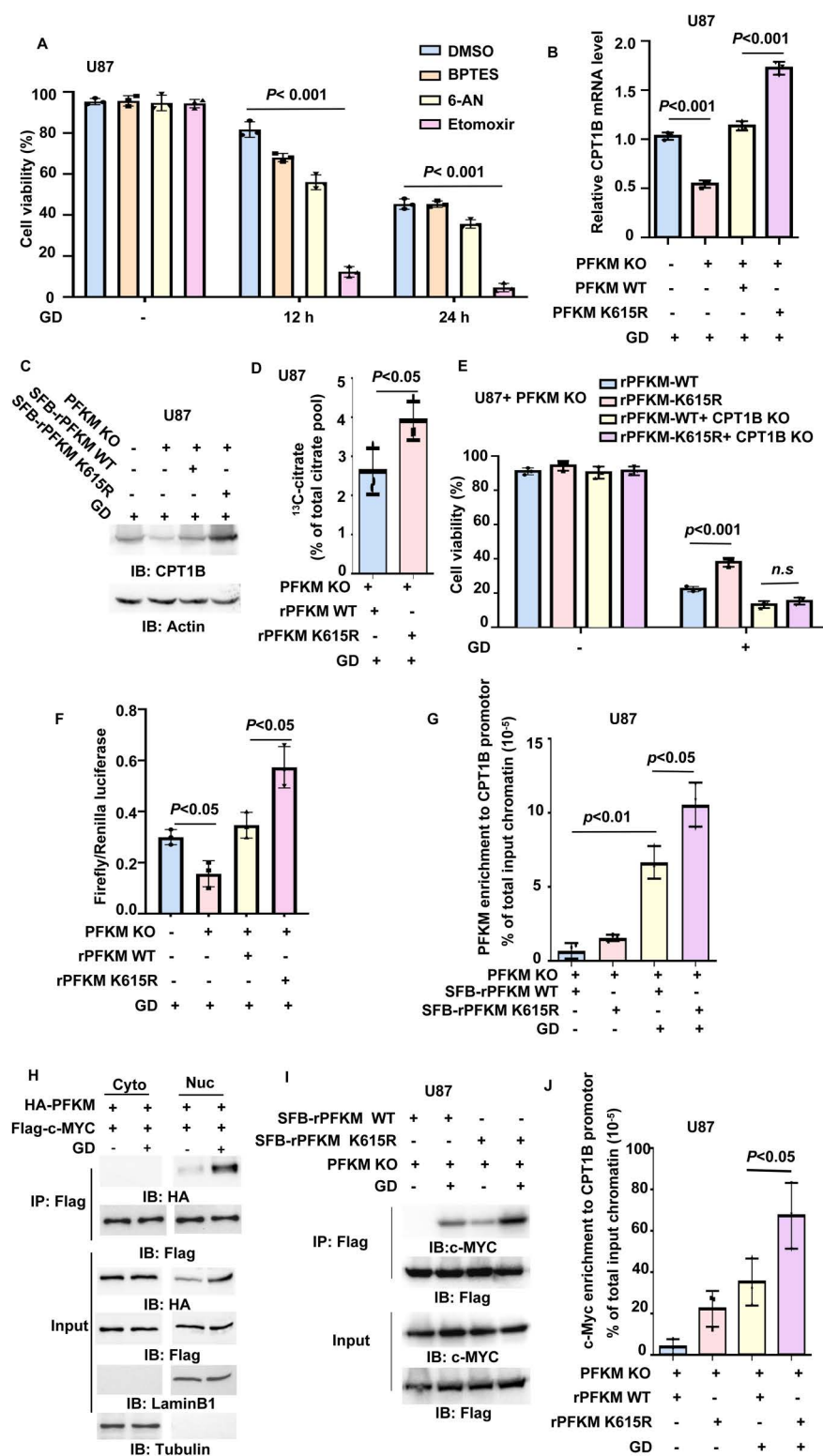


Fig 5. PFKM interacts with c-MYC to promote CPT1B upregulation and fatty acid oxidation. IP and IB analyses were performed with indicated antibodies. Data are representative of at least three independent experiments. (A) U87 cells were treated with or without CPT1 inhibitor (Etomoxir, 10 μM), glutaminase inhibitor (BPTES, 10 μM) or PPP inhibitor (6-AN, 100 nM) followed by GD treatment for indicated time. Cell viability was determined.

Data represent the mean $\pm$ SD of the viability of the cells from three independent experiments (two-tailed Student *t* test). **(B)** PFKM-depleted U87 cells rescued with or without rPFKM WT/K615R were treated with GD for 6 h. The mRNA level of CPT1B was examined by real-time PCR analyses. **(C)** PFKM-depleted U87 cells rescued with rPFKM WT or K615R were treated with GD for 6 h. **(D)** Labeling incorporation from ^{13}C -palmitate into citrate in PFKM-depleted U87 cells rescued with rPFKM WT or K615R after GD treatment. Data are shown as percentage of M+1 and M+2 labeling from $[\text{U-}^{13}\text{C}]$ palmitate in citrate compared to the total pool of citrate. **(E)** PFKM-depleted U87 cells rescued with rPFKM WT or K615R were infected with the lentivirus expressing lentiCRISPRv2-NT or lentiCRISPRv2-CPT1B. The cells were treated with or without GD for 72 h. Cell viability was determined. Data represent the mean $\pm$ SD of the viability of the cells from three independent experiments (two-tailed Student *t* test). **(F)** Luciferase activity was measured in PFKM-depleted HEK293T cells rescued with rPFKM WT or the K615R mutant. Cells were transfected with a CPT1B promoter-firefly luciferase reporter and a Renilla control plasmid, followed by GD treatment for 6 h. Data shown represent normalized firefly/Renilla luciferase ratios determined at 36 h post-transfection. **(G)** PFKM-depleted U87 cells rescued with SFB-rPFKM WT or K615R were treated with or without GD for 6 h. ChIP assays were performed with Flag antibody. **(H)** HEK293T cells were transfected with Flag-c-MYC and HA-PFKM and then treated with or without GD for 6 h. Cytosolic (Cyto) and nuclear (Nuc) fractions were prepared. Co-IP experiment was performed with anti-Flag antibody. **(I)** PFKM-depleted U87 cells rescued with rPFKM WT or K615R were treated with or without GD for 6 h. Co-IP experiment was performed with anti-Flag antibody. **(J)** PFKM-depleted U87 cells rescued with rPFKM WT or K615R were treated with or without GD for 6 h. ChIP assays were performed with c-Myc antibody. The data underlying this Figure can be found in [S1 Data](#) and [S1 Raw Images](#).

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that nuclear-localized PFKM transcriptionally activates CPT1B (Fig 5F). We also performed the ChIP assay to determine whether PFKM bound to the CPT1B promoter. As shown in Fig 5G, both PFKM WT and the K615R mutant were enriched at the CPT1B promoter following GD treatment, with the K615R mutant exhibiting stronger binding.

c-Myc is a multifunctional transcription factor and has been identified to regulate CPT1B expression [26]. We performed subcellular fractionation in cells treated with or without GD, which showed that GD treatment enhances the nuclear localization of PFKM. Moreover, the interaction between PFKM and c-MYC was observed in the nucleus after GD treatment (Fig 5H). More importantly, rescued expression of rPFKM K615R enhanced the interaction between PFKM and c-Myc compared to that of rPFKM WT (Fig 5I). Furthermore, ChIP assay also confirmed that c-Myc bound to CPT1B promoter in the cells rescued with rPFKM WT after GD. Moreover, rescued expression of rPFKM K615R further enhanced the binding of c-Myc to CPT1B promoter (Fig 5J). Taken together, these results suggest that nuclear PFKM interacts with c-Myc and directly enhances its binding to CPT1B promoter, thereby upregulating CPT1B expression and FAO.

Discussion

In order to achieve rapid proliferation, tumor cells must effectively adapt to and overcome the metabolic stress induced by glucose deficiency. In this study, we discover a novel function of PFKM, a rate-limiting enzyme in glycolysis, in promoting glioma cell survival after GD by switching metabolism from glycolysis to FAO. Upon GD, the levels of F-2,6-BP are decreased, leading to the enhanced interaction between USP7 and PFKM. USP7 then deubiquitinates PFKM K615 and promotes its nuclear translocation. Nuclear PFKM enhances c-Myc-dependent expression of CPT1B and the activity of FAO, and promotes tumor cell survival under nutrient stress (Fig 6).

As a rate-limiting glycolytic enzyme, PFK1 plays a central role in tumor progression. ZEB1 transcriptionally upregulates PFKM, thus promoting carcinogenesis and metastasis of HCC [12]. Oxidative stress-responsive microRNA-320a adjusts glycolysis via regulating PFKM expression and is involved in lung cancer progression [27]. In addition to the important role of PFK1 in cancer metabolism, several studies have reported other functions of PFK1 in tumors. Intriguingly, a study showed that PFKP could interact with AMPK after GD and promoted mitochondrial recruitment of AMPK, thereby alleviates GD-induced metabolic stress in lung cancer cells [28]. PFK1 can also bind the YAP/TAZ transcriptional cofactors TEADs and promotes the pro-tumorigenic function of YAP/TAZ [29]. More significantly, the interaction between PFK1 and TEADs occurs within the cell nucleus, implying the nuclear localization of PFK1. Recently, a research showed that PFKM interacts with NBS1 to maintain genomic stability [14]. These studies have hinted at the nuclear localization of PFK1, however, it remains unclear how the nuclear translocation of PFK1 is regulated and its potential contribution

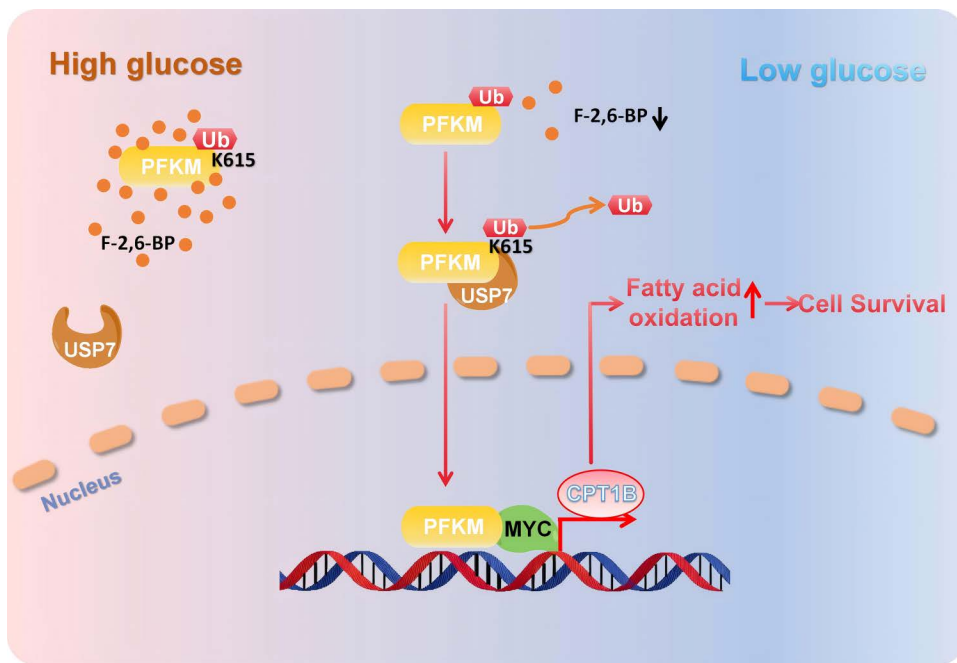


Fig 6. USP7-mediated deubiquitination and nuclear translocation of PFKM promotes brain tumor development by sensing fructose-2,6-bisphosphate. USP7 interacts with PFKM regulated by F-2,6-BP, resulting in the ubiquitination of PFKM K615 after GD treatment. Deubiquitinated PFKM translocates into nucleus and interacts with c-Myc. PFKM facilitates c-MYC binding to CPT1B promoter and promotes transcription of CPT1B, thereby enhancing FAO and cell survival.

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to tumor cell survival under nutrient stress are currently unknown. In our study, we found that the decrease in F-2,6-BP induced by GD promotes interaction between PFKM and USP7. The deubiquitination of PFKM at K615 mediates its nuclear translocation after GD.

Notably, PTMs of PFK1 are crucial for its role in cancer. For example, phosphorylation of S386 site of PFKP by AKT leads to increased expression of PFKP and promotes the growth of brain tumors [11]. S-nitrosylation at Cys351 of PFKM by NOS1 contributes to the metabolic reprogramming of ovarian cancer cells [13]. Glycosylation inhibits PFK1 activity and redirects glucose flux from glycolysis through the PPP, thereby providing cancer cells with a selective growth advantage [30]. Another research discovered that E3 ubiquitin ligase A20 interacts with PFKL and promotes its degradation, therefore inhibiting glycolysis in HCC [31]. Besides, USP14 interacts with PFKL and enhances its stability through deubiquitination in OSCC cells [32]. Moreover, PFKP deubiquitination and stabilization by USP5 activate aerobic glycolysis to promote triple-negative breast cancer progression [33]. In our study, unlike other PTMs affecting PFK1 activity and expression, the deubiquitination of PFKM at K615 promotes its nuclear translocation and non-glycolytic functions within the nucleus, thereby supporting tumor cell survival under nutrient stress.

USP7, a deubiquitinase, is overexpressed in various cancers including breast cancer, lung cancer, leukemia and prostate cancer, where it plays important roles in regulation of key oncogenic signaling pathways [34–39]. Jie Li and colleagues demonstrate that USP7 promotes GBM progression by deubiquitinating and stabilizing the nuclear receptor KPNB1. In our study, we discovered that USP7 is essential for glioma cell survival upon nutrient deficiency by deubiquitinating PFKM to promote its nuclear translocation. More importantly, we demonstrate that inhibiting USP7 by P22077 potently suppresses tumor growth of orthotopic GBM, suggesting that the pharmacological targeting of USP7 could represent a promising therapeutic strategy for the treatment of GBM.

Methods

Ethics statement

The use of human GBM and astrocytoma specimens and associated data was approved by Ethics Committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (XHEC-D-2025-019). Informed consent was obtained from all patients prior to their participation, with thorough review and documentation. The ethics committee evaluated the design, implementation process, and protection of the rights and interests of the subjects. The researchers strictly followed the “Declaration of Helsinki” and “International Ethical Guidelines for Research Involving Human Health,” and fully respected the subjects’ rights to know and privacy, and effectively protected the subjects’ rights and well-being. All animal experiments were conducted in compliance with relevant domestic and international ethical principles, and specifically adhered to the Guidelines for the Ethical Review of Laboratory Animal Welfare of the People’s Republic of China (National Standard GB/T 35892-2018). All procedures were approved by the Animal Ethics Committee of the Shanghai Institute of Biochemistry and Cell Biology (approval number: SIBCB-S355-2312-40), and every effort was made to minimize animal suffering.

Materials

Antibodies: Mouse monoclonal antibodies against Tubulin (1:5,000, T5201), Flag (1:5,000, F3165), and anti-Flag M2 affinity gel (A2220) were purchased from Sigma (St. Louis, MO, USA). Anti-HA Aagarose FF (AGM90054) was purchased from AGOMA. Rabbit monoclonal antibodies against HA (1:3,000, 3724S), β -actin (1:3,000, 3700S), LaminB1 (1:3,000, 12586s), and rabbit polyclonal antibody against Acetylated-Lysine (1:1,000, 9441S) were obtained from Cell Signaling Technology (Danvers, MA, USA). Rabbit polyclonal antibody against PFKM (1:2,000, 55028-1-AP) were purchased from Proteintech. Rabbit polyclonal antibody against IPO4 (1:2,000, A19902) were obtained from Abclonal Technology (Wuhan, China). Rabbit polyclonal antibody against Phosphoserine (1:1,000, ab9332) and rabbit monoclonal antibodies against c-Myc (1:2,000 for WB, 1:200 for Chip, ab32072) were purchased from Abcam (Cambridge, US). Mouse monoclonal antibody against p-Thr (1:1,000, sc-5267) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Reagents: Puromycin (540411-100MGCN) and hygromycin (400052-20MLCN) were bought from Merck/Millipore (Darmstadt, Germany). DNA transfection reagent Hieff TransTM Liposomal Transfection Reagent (H17520) was purchased from Yeasen Biotechnology (Shanghai, China). Trypan blue stain (0.4%, 15,250,061) and Dulbecco’s modified Eagle’s medium (DMEM) (no glucose, 11,966,025) were purchased from Gibco (USA). Fructose-2,6-bisphosphate Elisa kit (HBP37450R) was purchased from Huabang Biotechnology (Shanghai, China). Fructose-2,6-biphosphate (84364-89-6) was purchased from Tianshui Yiyao Chemical company (Gansu, China). The PFK2 inhibitor-3PO (HY-19824), USP7 inhibitor-P22077 (HY-13865), glutaminase inhibitor BPTES (HY-12683) and FAO inhibitor Etomoxir (HY-50202) were purchased from MCE (MedChemExpress, China). The inhibitor of PPP (6-AN, S9783) was purchased from Selleck.

Plasmids

PCR-amplified PFKM was cloned into pCDH-SFB. PCR-amplified USP7, UBB, and IPO4 were cloned into pCDNA3.0-HA. PCR-amplified c-MYC was cloned into pFlag-CMV. PFKM mutations were generated using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). For gRNA cloning, the lentiCRISPRv2 vector was digested with BsmBI and ligated with BsmBI-compatible pre-annealed oligo-nucleotides. The following sequence was used for CRISPR-knockout strategy: PFKM gRNA AATGAGGATCTTACCCACAG. CPT1B gRNA CGAGGCGCGCCGCCGTTTGT. The pGIPZ control was generated with the control oligonucleotide 5’-CTCGCTTGGGCGAGAGTAA-3’. pGIPZ USP7 shRNA was generated with 5’-AGAAGAGTCGAACGAGCTG-3’ oligonucleotide targeting the coding region of the USP7 transcript.

Cell culture and transfection

U87, U251 GBM cells, and HEK293T cells were obtained from the cell library of the Chinese Academy of Sciences and maintained in high glucose or no glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The protein expression and reconstitution experiments were conducted using the established stable cell lines. Cells were transfected with indicated plasmids using Liposomal Transfection Reagent according to the manufacturer's instructions.

Subcellular fractionation

Cells were treated with no glucose DMEM supplemented with 10% FBS (GD treatment) for indicated times and washed three times with cold PBS. Then the cells were lysed by buffer A (10 mM HEPES (contain 10mM KCl, pH 7.9), 1 mM DTT, 0.1 mM EDTA, 0.1 mM EGTA, 0.5mM PMSF) supplemented with 0.5% NP40, phosphatase inhibitors, and protease inhibitors. Following centrifugation for 5 min at 1,500 *g* and 4 °C, the supernatants were collected as the cytoplasmic fraction. Pellets were washed three times with cold PBS and then were lysed by vortexing in 50 μ l of buffer B (50mM Tris-HCl (pH 7.5), 150mM NaCl, 0.5mM EDTA, 1% Triton X-100, 0.1% SDS) supplemented with phosphatase inhibitors and protease inhibitors. After incubation on ice for 2 h, the lysates were centrifuged at 14,000 rpm for 20 min to remove the nuclear debris. The supernatants then were collected as the nuclear fraction.

Immunofluorescence analysis

Cells were fixed and incubated with primary antibodies, Alexa Fluor dye-conjugated secondary antibodies and DAPI according to standard protocols. Cell imaging was performed on a FLUOVIEW FV3000 laser scanning confocal microscope (OLYMPUS).

Cell viability assay

In total, 2×10^5 cells were seeded in six-well plates. After GD treatment for the indicated time, cells were harvested with trypsin. Cells was stained with trypan blue and cell viability was measured by Thermo Countess II FL.

Mass spectrometry analysis

Immunoprecipitated SFB-PFKM using Flag beads (Sigma) from U87 cells stably expressing SFB-PFKM which were treated with no glucose DMEM supplemented with 10% FBS for 6 h. The precipitated complexes were boiled at 95 °C for 10 min. PFKM-associated proteins were separated from the complexes using SDS-PAGE.

In-gel digestion

Briefly, gel band was de-stained with 50 mM NH_4HCO_3 in 50% acetonitrile. The protein was reduced with 10 mM TCEP (Thermo Scientific) for 30 min and alkylated with 55 mM iodoacetamide (Sigma) in the dark for 30 min, respectively. 12.5 ng/ μ L trypsin (Promega) in 50 mM NH_4HCO_3 was using for digestion overnight at 37 °C. The digestion was stopped by and extracted with 50% acetonitrile/5% formic acid and dried out by Speed Vacuum instrument. The sample was reconstituted with 0.1% formic acid, then desalted using a Mono-Spin C18 column (GL Science, Tokyo, Japan), and dried out by Speed Vacuum instrument.

HPLC-tandem MS (MS/MS) analysis of peptides

The peptide mixture was analyzed by a home-made 15 cm-long pulled-tip analytical column (75 μ m ID packed with Aqua C18-3 μ m resin, Phenomenex), the column was then placed in-line with an Easy-nLC 1200 nano HPLC (Thermo Scientific) for mass spectrometry analysis. The analytical column temperature was set at 55 °C during the experiments. The

mobile phase were 0.1% formic acid in water as buffer A and 0.1% formic acid in 80% acetonitrile as buffer B, 0–1 min, 1%–4% B; 1–91 min, 4%–35% B; 91–101 min, 35%–60% B, 101–111 min, 60%–100% B, 111–120 min, 100% B. The flow rate was set as 300 nl/min.

Data-dependent MS/MS analysis was performed with a Q Exactive Orbitrap mass spectrometer (Thermo Scientific). Peptides eluted from the LC column were directly electrosprayed into the mass spectrometer with the application of a distal 2.5-kV spray voltage. A cycle of one full-scan MS spectrum (m/z 300–1,800) was acquired followed by top 20 MS/MS events at a 30% normalized collision energy. Full scan resolution was set to 70,000 with automated gain control (AGC) target of 3×10^6 . MS/MS scan resolution was set to 17,500 with isolation window of 1.8 m/z and AGC target of 1×10^5 . MS scan functions and LC solvent gradients were controlled by the Xcalibur data system (Thermo Scientific).

Data analysis

The acquired MS/MS data were analyzed against a human UniProtKB database (released on August 12, 2019) using PEAKS Studio 8.5. Trypsin was set as the cleavage enzyme, with both-specific at peptide N/C term; the max missed cleavage was set as 3.

In order to accurately estimate peptide probabilities and false discovery rates (FDRs), a decoy database containing the reversed sequences of all the proteins was appended to the target database to accurately estimate peptide probabilities and FDR, the FDR was set at 0.01.

Fructose-2,6-bisphosphate assay

Fructose-2,6-bisphosphate concentration was measured using fructose-2,6-bisphosphate Elisa kit (Huabang Biotechnology, China) according to the manufacturer's instructions.

Metabolic flux analysis of FAO

FAO activity was measured by monitoring the conversion rate of [U- ^{13}C]-palmitate to ^{13}C -citrate by using LC/MS. In brief, the cells were treated with GD and incubated with 50 μM [U- ^{13}C]-palmitate conjugate for 12 h. After incubation, the metabolites were extracted and dried. Analysis was conducted using an LC–MS system comprising a Agilent 1290 Infinity II UHPLC system tandem with Agilent6545 Q-TOF/MS (Agilent). Extracted metabolites were dissolved in 100 μl of 80% methanol for analysis. Chromatographic separation was achieved on an ACQUITY UPLC BEH Amide column (100 mm $\times$ 2.1 mm, 1.7 μm). The mobile phase consisted of 15 mM ammonium acetate, 0.3% $\text{NH}_3 \cdot \text{H}_2\text{O}$ in water and 15 mM ammonium acetate, 0.3% $\text{NH}_3 \cdot \text{H}_2\text{O}$ in 9:1 acetonitrile/water (B) at a flow rate of 0.3 ml/min.

The column was eluted with 95% mobile phase B for 1 min, followed by a linear gradient to 50% mobile-phase B over 8 min, held at 50% for 3 min, a linear gradient to 95% mobile phase B over 0.5 min, then 1.5 min at 95% mobile-phase B. The sample volume injected was 5 μL .

MS data were acquired using electrospray ionization in negative ion mode over 50–1,250 m/z . Other MS settings include: sheath gas temperature 350°C, sheath gas flow 11 L/min, VCap 4,000 V, Nozzle voltage 1,000 V, gas temperature 350 °C, nebulizer gas 30 psi; drying gas flow rate 8 L/min; fragmentor 110 V, skimmer 65 V. Raw data were processed using Profinder 10.0 (Agilent) for peak detection, alignment, and integration.

Dual-Luciferase Reporter Assay

PFKM-depleted HEK293T cells reconstituted with either rPFKM-WT or rPFKM-K615R were plated in 24-well plates and co-transfected with the pGL3-CPT1B promoter-firefly luciferase reporter and a Renilla luciferase control plasmid. After 36 h, cells were harvested and lysed. Firefly and Renilla luciferase activities were sequentially measured using the

Dual-Luciferase Reporter Assay System (Promega) on a luminescence microplate reader, following the manufacturer's instructions. Firefly luciferase signals were normalized to the corresponding Renilla luciferase values to control for transfection efficiency.

Chromatin Immunoprecipitation (ChIP)

ChIP was performed with indicated antibodies using SimpleChIP Enzymatic Chromatin IP Kits (Cell Signaling Technology) according to manufacturer instructions. The enrichment of c-Myc or PFKM WT/K615R to CPT1B promoter was assessed by quantitative RT-PCR. Primer sequences used for the amplification of human CPT1B promoter associated with c-Myc and PFKM were 5'- AGATGGCTTCCCATAGATCTG -3' (forward) and 5'- AGCCCCTGGGAGAATAAGG -3' (reverse).

Intracranial injection and bioluminescence imaging

Approximately 2×10^5 (in 2 μ l of DMEM per mouse) luciferase-expressing PFKM-depleted U87/EGFRvIII cells with reconstituted expression of rPFKM WT/K615R or U87/EGFRvIII cells were intracranially injected into randomized 8-week-old female athymic nude mice. Briefly, a small hand-controlled twist drill that is 1 mm in diameter is used to make a hole in the animal's skull. The cell suspension is drawn up into the cuffed Hamilton syringe. The needle of the Hamilton syringe is slowly lowered into the central hole of the guide screw until the cuff rests on the screw surface. The cell suspension is slowly injected into the brain of mouse [40]. To examine the effect of USP7 inhibition with P22077 (HY-13865, MCE) on tumor growth, mice were treated with P22077 (15 mg/kg/daily) or vehicle control through tail vein injection. Five mice in each group were included. After inoculation, the mice were intraperitoneally injected with 100 μ l of 15 mg/ml D-luciferin (Xenogen) and subsequently anesthetized with isoflurane inhalation. Bioluminescence imaging with a CCD camera (IVIS Spectrum CT, PerkinElmer) was initiated 10 min after injection. All bioluminescent data were collected and analyzed using Living image software (PerkinElmer). Survival durations of the implanted mice were compared. The use of mice was in compliance with ethical regulations and was approved by the institutional review board at Institute of Biochemistry and Cell Biology.

Immunohistochemical analysis

The tissue sections from paraffin-embedded human GBM and astrocytoma specimens were stained with anti-PFKM antibody. We quantitatively scored the tissue sections according to the percentage of positive cells and staining intensity, as previously defined [41]. We rated the intensity of nuclear PFKM staining on a scale of 0–3: 0, negative; 1, weak; 2, moderate; and 3, strong. We assigned the following proportion scores: 0 if 0% of the tumor cells showed positive staining, 1 if 0%–1% of cells were stained, 2 if 2%–10% were stained, 3 if 11%–30% were stained, 4 if 31%–70% were stained, and 5 if 71%–100% were stained. We then combined the proportion and intensity scores to obtain a total score (range, 0–8). Scores were compared with overall survival, defined as the time from the date of diagnosis to death or last known date of follow-up. All patients received standard adjuvant radiotherapy after surgery, followed by treatment with an alkylating agent. The use of human brain tumor specimens and the database was approved by the Institutional Review Board at XinHua Hospital School of Medicine. Informed consent was obtained from all patients.

Statistical analysis

No statistical methods were used to predetermine sample size. A log-rank test was used to analyze the statistical significance of the survival correlations between groups (Figs 1H, 2H, and 3G). Other than the analyses mentioned above, an unpaired, two-tailed Student *t* test was used for two-group comparisons. $P < 0.05$ was considered to be significant. $P < 0.01$ was considered to be extremely significant.

Supporting information

S1 Fig. Related to Fig 1. IB analyses were performed with indicated antibodies. Data are representative of at least three independent experiments. **(A)** U87 cells stably expressing SFB-PFKL (left panel) or SFB-PFKP (right panel) were treated with or without GD for 6 h. Cytosolic (Cyto) and nuclear (Nuc) fractions were prepared. **(B)** The NLS of PFKM was predicted through sequence analysis programs (NLS-mapper). **(C)** U87 cells were depleted of endogenous PFKM and rescued with or without rPFKM WT or RKR. The data underlying this Figure can be found in [S1 Raw Images](#). (PDF)

S2 Fig. Related to Fig 2. IB analyses were performed with indicated antibodies. Data are representative of at least three independent experiments. **(A)** U87 cells stably expressing SFB-PFKM were treated with or without GD for 6 h. Co-IP was performed with anti-Flag antibody. **(B)** U87 cells (left panel) and U251 cells (right panel) were depleted of endogenous PFKM and rescued with or without rPFKM WT or K615R. **(C)** PFKM-depleted U251 cells were rescued with rPFKM WT or K615R and then treated with or without GD for 6 h. Cytosolic (Cyto) and nuclear (Nuc) fractions were prepared. **(D)** PFKM-depleted U251 cells were rescued with or without rPFKM WT/K615R and then treated with or without GD for 48 h. Cell viability was determined. Data represent the mean $\pm$ SD of the viability of the cells from three independent experiments (two-tailed Student *t* test). **(E)** U87/EGFRvIII cells stably expressing luciferase were depleted endogenous PFKM by using CRISPR-Cas9 system and the PFKM-depleted U87/EGFRvIII cells were reconstituted with or without the expression of SFB-rPFKM WT or K615R. **(F)** The expression of SFB-rPFKM WT or K615R was detected in the glioblastoma tumors. **(G)** Mass spectrometry analysis of PFKM-associated protein in U87 cells stably expressing SFB-PFKM with GD treatment (6 h). **(H)** HEK293T cells were transfected with SFB-PFKM and HA-IPO4, then treated with or without GD (6 h). Co-IP experiment was performed with anti-Flag antibody. **(I, J)** U87 cells were infected with the lentivirus expressing shNT or shIPO4-1/shIPO4-2, followed by infection with a lentivirus expressing SFB-PFKM. These cells were treated with or without GD for 6 h. Cytosolic (Cyto) and nuclear (Nuc) fractions were prepared. **(K)** HEK293T cells were transfected with SFB-PFKM WT or K615R and HA-IPO4, then treated with or without GD (6 h). Co-IP experiment was performed with anti-Flag antibody. The data underlying this Figure can be found in [S1 Data](#) and [S1 Raw Images](#). (PDF)

S3 Fig. Related to Fig 3. Data are representative of at least three independent experiments. **(A)** Mass spectrometry analysis of PFKM-associated protein in U87 cells stably expressing SFB-PFKM with GD treatment (6 h). **(B)** PFKM-depleted U87 cells were rescued with rPFKM WT or K615R and then infected with the lentivirus expressing shNT or shUSP7. The mRNA level of USP7 was examined by real-time PCR analyses. **(C)** PFKM-depleted U87 cells were rescued with rPFKM WT or K615R and then infected with the lentivirus expressing shNT or shUSP7. These cells were treated with P22077 (20 μ M) followed by GD treatment for 48 h. Cell viability was determined. Data represent the mean $\pm$ SD of the viability of the cells from three independent experiments (two-tailed Student *t* test). The data underlying this Figure can be found in [S1 Data](#). (PDF)

S4 Fig. Related to Fig 4. Data are representative of at least three independent experiments. **(A)** HEK293T cells were supplemented with or without F-2,6-BP (100 μ M) by electroporation. F-2,6-BP content were determined. Data represent the mean $\pm$ SD of three biologically independent experiments (two-tailed Student *t* test). **(B)** HEK293T cells were treated with or without 3PO (20 μ M, 12 h). F-2,6-BP content were determined. Data represent the mean $\pm$ SD of three biologically independent experiments (two-tailed Student *t* test). The data underlying this Figure can be found in [S1 Data](#). (PDF)

S5 Fig. Related to Fig 5. Data are representative of at least three independent experiments. **(A)** The mRNA level of CPT1A (left panel) or CPT1C (right panel) was examined by real-time PCR analyses in U87 cells treated with or without

GD for 6 h. **(B)** U87 cells were depleted endogenous PFKM by using CRISPR-Cas9 system and then treated with or without GD for 6 h. The mRNA level of CPT1B was examined by real-time PCR analyses. **(C)** PFKM-depleted U87 (left panel) or U251 (right panel) cells were rescued with rPFKM WT or K615R and then infected with the lentivirus expressing lentiCRISPRv2-NT or lentiCRISPRv2-CPT1B. **(D)** PFKM-depleted U251 cells rescued with rPFKM WT or K615R were infected with the lentivirus expressing lentiCRISPRv2-NT or lentiCRISPRv2-CPT1B. The cells were treated with or without GD for 72 h. Cell viability was determined. Data represent the mean $\pm$ SD of the viability of the cells from three independent experiments (two-tailed Student *t* test). **(E, F)** PFKM-depleted U87 cells (E) or U251 cells (F) rescued with rPFKM WT or K615R treated with or without CPT1 inhibitor (Etomoxir, 10 μ M) followed by GD treatment for 72 h. Cell viability was determined. Data represent the mean $\pm$ SD of the viability of the cells from three independent experiments (two-tailed Student *t* test). The data underlying this Figure can be found in [S1 Data](#) and [S1 Raw Images](#).
(PDF)

S1 Data. Source data for Figs 1–5 and S1–S5. Source data for Figs 1–5 and S1–S5 are organized in the accompanying Excel file. Each individual tab corresponds to the raw data for a specific figure panel.
(XLSX)

S1 Raw Images. Original, uncropped western blot images for Figs 1–5 and S1–S5.
(PDF)

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References

1. Vander Heiden MG, DeBerardinis RJ. Understanding the intersections between metabolism and cancer biology. *Cell*. 2017;168(4):657–69. <https://doi.org/10.1016/j.cell.2016.12.039> PMID: [28187287](#)
2. Hay N. Reprogramming glucose metabolism in cancer: can it be exploited for cancer therapy?. *Nat Rev Cancer*. 2016;16(10):635–49. <https://doi.org/10.1038/nrc.2016.77> PMID: [27634447](#)
3. DeBerardinis RJ, Chandel NS. We need to talk about the Warburg effect. *Nat Metab*. 2020;2(2):127–9. <https://doi.org/10.1038/s42255-020-0172-2> PMID: [32694689](#)

4. Reinfeld BI, Madden MZ, Wolf MM, Chytil A, Bader JE, Patterson AR, et al. Cell-programmed nutrient partitioning in the tumour microenvironment. *Nature*. 2021;593(7858):282–8. <https://doi.org/10.1038/s41586-021-03442-1> PMID: [33828302](#)
5. Martinez-Outschoorn UE, Peiris-Pagés M, Pestell RG, Sotgia F, Lisanti MP. Cancer metabolism: a therapeutic perspective. *Nat Rev Clin Oncol*. 2017;14(2):113. <https://doi.org/10.1038/nrclinonc.2017.1> PMID: [28094266](#)
6. Kouidhi S, Noman MZ, Kieda C, Elgaaid AB, Chouaib S. Intrinsic and tumor microenvironment-induced metabolism adaptations of T cells and impact on their differentiation and function. *Front Immunol*. 2016;7:114. <https://doi.org/10.3389/fimmu.2016.00114> PMID: [27066006](#)
7. Yang C, Ko B, Hensley CT, Jiang L, Wasti AT, Kim J, et al. Glutamine oxidation maintains the TCA cycle and cell survival during impaired mitochondrial pyruvate transport. *Mol Cell*. 2014;56(3):414–24. <https://doi.org/10.1016/j.molcel.2014.09.025> PMID: [25458842](#)
8. Carracedo A, Cantley LC, Pandolfi PP. Cancer metabolism: fatty acid oxidation in the limelight. *Nat Rev Cancer*. 2013;13(4):227–32. <https://doi.org/10.1038/nrc3483> PMID: [23446547](#)
9. Lu J, Tan M, Cai Q. The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism. *Cancer Lett*. 2015;356(2 Pt A):156–64. <https://doi.org/10.1016/j.canlet.2014.04.001> PMID: [24732809](#)
10. Mor I, Cheung EC, Voutsden KH. Control of glycolysis through regulation of PFK1: old friends and recent additions. *Cold Spring Harb Symp Quant Biol*. 2011;76:211–6. <https://doi.org/10.1101/sqb.2011.76.010868> PMID: [22096029](#)
11. Lee J-H, Liu R, Li J, Zhang C, Wang Y, Cai Q, et al. Stabilization of phosphofructokinase 1 platelet isoform by AKT promotes tumorigenesis. *Nat Commun*. 2017;8(1):949. <https://doi.org/10.1038/s41467-017-00906-9> PMID: [29038421](#)
12. Zhou Y, Lin F, Wan T, Chen A, Wang H, Jiang B, et al. ZEB1 enhances Warburg effect to facilitate tumorigenesis and metastasis of HCC by transcriptionally activating PFKM. *Theranostics*. 2021;11(12):5926–38. <https://doi.org/10.7150/thno.56490> PMID: [33897890](#)
13. Gao W, Huang M, Chen X, Chen J, Zou Z, Li L, et al. The role of S-nitrosylation of PFKM in regulation of glycolysis in ovarian cancer cells. *Cell Death Dis*. 2021;12(4):408. <https://doi.org/10.1038/s41419-021-03681-0> PMID: [33859186](#)
14. Lim YC, Jensen KE, Aguilar-Morante D, Vardouli L, Vitting-Seerup K, Gimble RC, et al. Non-metabolic functions of phosphofructokinase-1 orchestrate tumor cellular invasion and genome maintenance under bevacizumab therapy. *Neuro Oncol*. 2023;25(2):248–60. <https://doi.org/10.1093/neu-onc/noac135> PMID: [35608632](#)
15. Yang W, Zheng Y, Xia Y, Ji H, Chen X, Guo F, et al. ERK1/2-dependent phosphorylation and nuclear translocation of PKM2 promotes the Warburg effect. *Nat Cell Biol*. 2012;14(12):1295–304. <https://doi.org/10.1038/ncb2629> PMID: [23178880](#)
16. Spoden GA, Morandell D, Eehalt D, Fiedler M, Jansen-Dürr P, Hermann M, et al. The SUMO-E3 ligase PIAS3 targets pyruvate kinase M2. *J Cell Biochem*. 2009;107(2):293–302. <https://doi.org/10.1002/jcb.22125> PMID: [19308990](#)
17. Harreman MT, Kline TM, Milford HG, Harben MB, Hodel AE, Corbett AH. Regulation of nuclear import by phosphorylation adjacent to nuclear localization signals. *J Biol Chem*. 2004;279(20):20613–21. <https://doi.org/10.1074/jbc.M401720200> PMID: [14998990](#)
18. Icard P, Fournel L, Coquerel A, Gligorov J, Alifano M, Lincet H. Citrate targets FBPase and constitutes an emerging novel approach for cancer therapy. *Cancer Cell Int*. 2018;18:175. <https://doi.org/10.1186/s12935-018-0676-y> PMID: [30455595](#)
19. Jenkins CM, Yang J, Sims HF, Gross RW. Reversible high affinity inhibition of phosphofructokinase-1 by acyl-CoA: a mechanism integrating glycolytic flux with lipid metabolism. *J Biol Chem*. 2011;286(14):11937–50. <https://doi.org/10.1074/jbc.M110.203661> PMID: [21258134](#)
20. Yalcin A, Clem BF, Simmons A, Lane A, Nelson K, Clem AL, et al. Nuclear targeting of 6-phosphofructo-2-kinase (PFKFB3) increases proliferation via cyclin-dependent kinases. *J Biol Chem*. 2009;284(36):24223–32. <https://doi.org/10.1074/jbc.M109.016816> PMID: [19473963](#)
21. Fernandes PM, Kinkad J, McNaie I, Michels PAM, Walkinshaw MD. Biochemical and transcript level differences between the three human phosphofructokinases show optimisation of each isoform for specific metabolic niches. *Biochem J*. 2020;477(22):4425–41. <https://doi.org/10.1042/BCJ20200656> PMID: [33141153](#)
22. Lin P, Qi Y, Chu H, Wu H, Zhang Y, Huang X, et al. PFKM phosphorylates histone H3 and promotes mitotic progression by sensing the levels of citrate. *Nat Commun*. 2025;16(1):6736. <https://doi.org/10.1038/s41467-025-62111-3> PMID: [40695785](#)
23. Li X-X, Wang Z-J, Zheng Y, Guan Y-F, Yang P-B, Chen X, et al. Nuclear receptor Nur77 facilitates melanoma cell survival under metabolic stress by protecting fatty acid oxidation. *Mol Cell*. 2018;69(3):480–492.e7. <https://doi.org/10.1016/j.molcel.2018.01.001> PMID: [29395065](#)
24. Ping M, Li G, Li Q, Fang Y, Fan T, Wu J, et al. The NRF2-CARM1 axis links glucose sensing to transcriptional and epigenetic regulation of the pentose phosphate pathway in gastric cancer. *Cell Death Dis*. 2024;15(9):670. <https://doi.org/10.1038/s41419-024-07052-3> PMID: [39266534](#)
25. Guan L, Chen Y, Wang Y, Zhang H, Fan S, Gao Y, et al. Effects of carnitine palmitoyltransferases on cancer cellular senescence. *J Cell Physiol*. 2019;234(2):1707–19. <https://doi.org/10.1002/jcp.27042> PMID: [30070697](#)
26. Wang Y, Yin C, Chen Z, Li Y, Zou Y, Wang X, et al. Cardiac-specific LRP6 knockout induces lipid accumulation through Drp1/CPT1b pathway in adult mice. *Cell Tissue Res*. 2020;380(1):143–53. <https://doi.org/10.1007/s00441-019-03126-3> PMID: [31811407](#)
27. Tang H, Lee M, Sharpe O, Salamone L, Noonan EJ, Hoang CD, et al. Oxidative stress-responsive microRNA-320 regulates glycolysis in diverse biological systems. *FASEB J*. 2012;26(11):4710–21. <https://doi.org/10.1096/fj.11-197467> PMID: [22767230](#)
28. Chen J, Zou L, Lu G, Grinchuk O, Fang L, Ong DST, et al. PFKP alleviates glucose starvation-induced metabolic stress in lung cancer cells via AMPK-ACC2 dependent fatty acid oxidation. *Cell Discov*. 2022;8(1):52. <https://doi.org/10.1038/s41421-022-00406-1> PMID: [35641476](#)
29. Enzo E, Santinon G, Pocaterra A, Aragona M, Bresolin S, Forcato M, et al. Aerobic glycolysis tunes YAP/TAZ transcriptional activity. *EMBO J*. 2015;34(10):1349–70. <https://doi.org/10.15252/emj.201490379> PMID: [25796446](#)

30. Yi W, Clark PM, Mason DE, Keenan MC, Hill C, Goddard WA 3rd, et al. Phosphofructokinase 1 glycosylation regulates cell growth and metabolism. *Science*. 2012;337(6097):975–80. <https://doi.org/10.1126/science.1222278> PMID: [22923583](#)
31. Feng Y, Zhang Y, Cai Y, Liu R, Lu M, Li T, et al. A20 targets PFKL and glycolysis to inhibit the progression of hepatocellular carcinoma. *Cell Death Dis*. 2020;11(2):89. <https://doi.org/10.1038/s41419-020-2278-6> PMID: [32015333](#)
32. Zhang X, Geng L, Tang Y, Wang Y, Zhang Y, Zhu C, et al. Ubiquitin-specific protease 14 targets PFKL-mediated glycolysis to promote the proliferation and migration of oral squamous cell carcinoma. *J Transl Med*. 2024;22(1):193. <https://doi.org/10.1186/s12967-024-04943-z> PMID: [38388430](#)
33. Peng Z-M, Han X-J, Wang T, Li J-J, Yang C-X, Tou F-F, et al. PFKP deubiquitination and stabilization by USP5 activate aerobic glycolysis to promote triple-negative breast cancer progression. *Breast Cancer Res*. 2024;26(1):10. <https://doi.org/10.1186/s13058-024-01767-z> PMID: [38217030](#)
34. Cummins JM, Rago C, Kohli M, Kinzler KW, Lengauer C, Vogelstein B. Tumour suppression: disruption of HAUSP gene stabilizes p53. *Nature*. 2004;428(6982):1 p following 486. <https://doi.org/10.1038/nature02501> PMID: [15058298](#)
35. Li M, Brooks CL, Kon N, Gu W. A dynamic role of HAUSP in the p53-Mdm2 pathway. *Mol Cell*. 2004;13(6):879–86. [https://doi.org/10.1016/s1097-2765\(04\)00157-1](https://doi.org/10.1016/s1097-2765(04)00157-1) PMID: [15053880](#)
36. Cai J-B, Shi G-M, Dong Z-R, Ke A-W, Ma H-H, Gao Q, et al. Ubiquitin-specific protease 7 accelerates p14(ARF) degradation by deubiquitinating thyroid hormone receptor-interacting protein 12 and promotes hepatocellular carcinoma progression. *Hepatology*. 2015;61(5):1603–14. <https://doi.org/10.1002/hep.27682> PMID: [25557975](#)
37. Wang Q, Ma S, Song N, Li X, Liu L, Yang S, et al. Stabilization of histone demethylase PHF8 by USP7 promotes breast carcinogenesis. *J Clin Invest*. 2016;126(6):2205–20. <https://doi.org/10.1172/JCI85747> PMID: [27183383](#)
38. Zhao GY, et al. USP7 overexpression predicts a poor prognosis in lung squamous cell carcinoma and large cell carcinoma. *Tumour Biol*. 2015;36:1721–9. <https://doi.org/10.1007/s13277-014-2773-4> PMID: [25519684](#)
39. Carra G, et al. Therapeutic inhibition of USP7-PTEN network in chronic lymphocytic leukemia: a strategy to overcome TP53 mutated/deleted clones. *Oncotarget* 2017;8:35508–22. <https://doi.org/10.18632/oncotarget.16348> PMID: [28418900](#)
40. Lal S, et al. An implantable guide-screw system for brain tumor studies in small animals. *J Neurosurg* 2000;92:326–33. <https://doi.org/10.3171/jns.2000.92.2.0326> PMID: [10659021](#)
41. Wu S, et al. Pyruvate facilitates FACT-mediated gammaH2AX loading to chromatin and promotes the radiation resistance of glioblastoma. *Adv Sci (Weinh)*. 2022:e2104055. <https://doi.org/10.1002/adv.202104055> PMID: [35048565](#)