

Supplementary Materials for **Tyrosine Phosphorylation Inhibits PKM2 to Promote the Warburg Effect and Tumor Growth**

Taro Hitosugi, Sumin Kang, Matthew G. Vander Heiden, Tae-Wook Chung, Shannon Elf, Katherine Lythgoe, Shaozhong Dong, Sagar Lonial, Xu Wang, Georgia Z. Chen, Jianxin Xie, Ting-Lei Gu, Roberto D. Polakiewicz, Johannes L. Roesel, Titus J. Boggon, Fadlo R. Khuri, D. Gary Gilliland, Lewis C. Cantley, Jonathan Kaufman, Jing Chen*

*To whom correspondence should be addressed. E-mail: jchen@emory.edu

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Fig. S6. FGFR1 binds PKM2 in a tyrosine phosphorylation–dependent manner but this binding is dispensable for FGFR1-dependent inhibition of PKM2.

A

Protein Name	NCBI Accession	NCBI Site	Peptides
PKM2	NP_035229	83	LNFSHGTHEyHAETIK
PKM2	NP_035229	105	EATESFASDPILyRPVAVALDTK
PKM2	NP_035229	148	ITLDNAyMEKCDENILWLDYK
PKM2	NP_035229	175	lyVDDGLISLQVK
PKM2	NP_035229	370	AEGSDVANAVLDGADCIMLSGETAKGDyPLEAVR
PKM2	NP_035229	390	EAEAAlyHLQLFEELR
LDH-A	NP_034829	172	FRyLMGER
LDH-A	NP_034829	239	EVHKQVVDSAyEVIK
G6PD	NP_032088	112	NSYVAGQyDDAASYK
G6PD	NP_032088	503	VGfQyEGTYK
MDH2	NP_032643	253	AGAGSATLSMAyAGAR

B

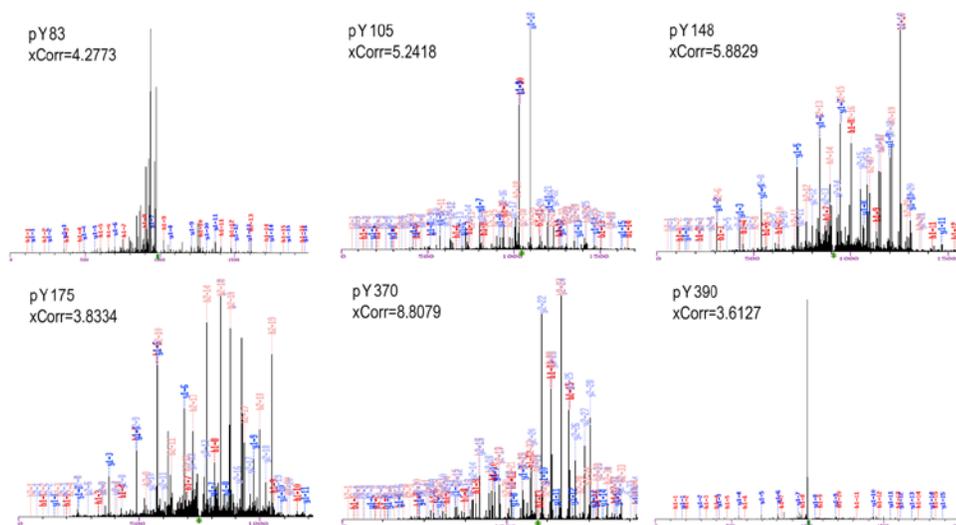


Fig. S1. Six tyrosine residues of PKM2 are phosphorylated in cells harboring active FGFR1 mutants. (A) Metabolic enzymes identified as tyrosine phosphorylated by phospho-proteomic analysis in cells expressing active FGFR1 mutants. (B) MS spectra of peptide fragments of PKM2 with identities of the phospho-Tyr residues and the SEQUEST cross-correlation (xCorr) scores.

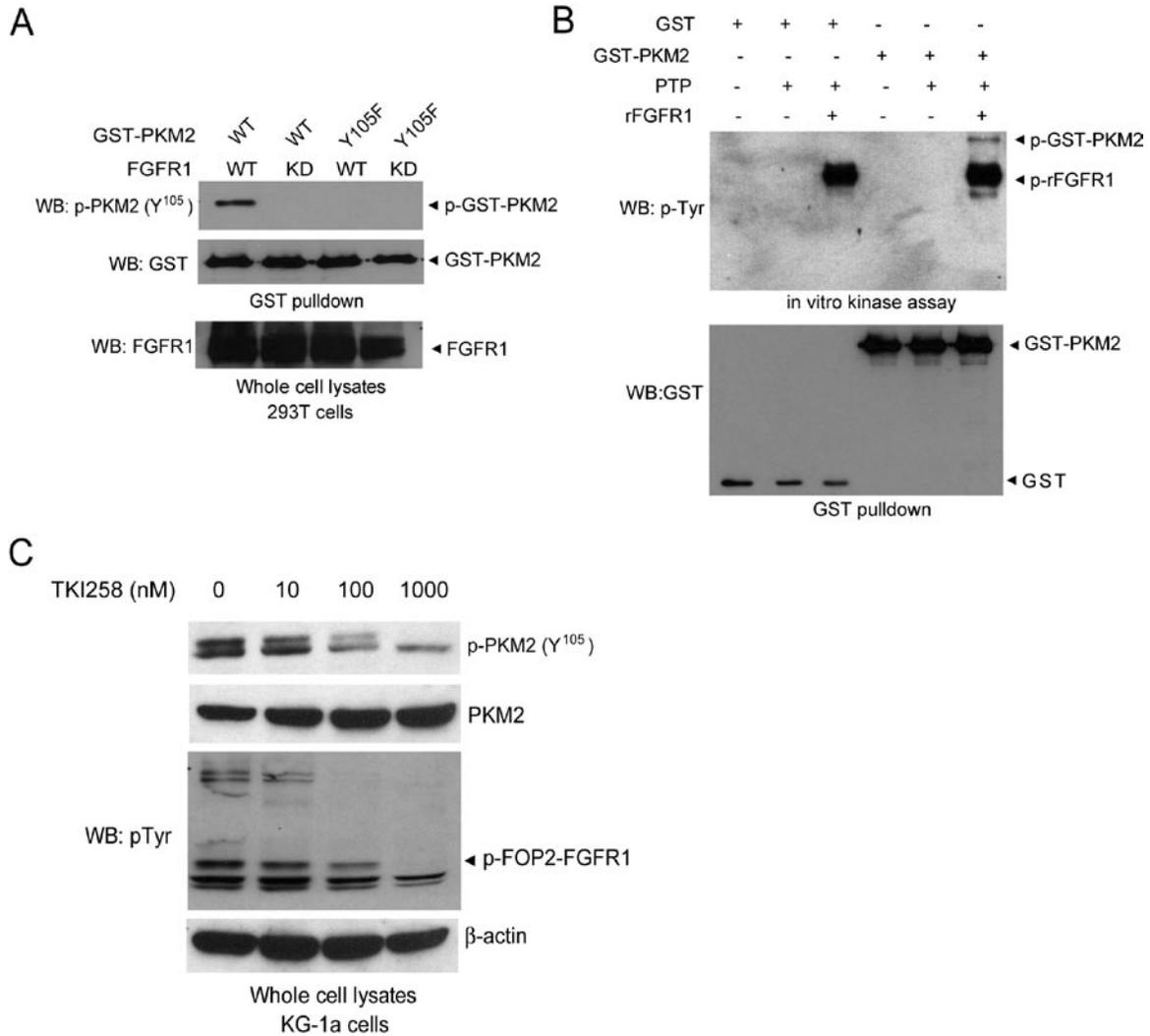


Fig. S2. FGFR1 directly phosphorylates PKM2. **(A)** Detection of Y¹⁰⁵ phosphorylation of GST-PKM2 with an antibody that specifically recognizes phospho-PKM2 (Y¹⁰⁵) antibody in lysates of 293T cells coexpressing FGFR1 WT but not kinase dead (KD) mutant. **(B)** Active recombinant FGFR1 (rFGFR1) directly phosphorylates purified GST-PKM2 at tyrosine residues in an in vitro kinase assay. GST-PKM2 was pulled down by beads from transfected 293T cell lysates and treated with protein tyrosine phosphatase (PTP), followed by treatment with active rFGFR1. Phosphorylation at tyrosine residues in GST-PKM2 was detected with an antibody that specifically recognizes phosphotyrosines (pY99). **(C)** FGFR1 fusion tyrosine kinase phosphorylates PKM2 at Y¹⁰⁵. Immunoblotting reveals that inhibition of FGFR1 by TKI258 in KG-1a cells expressing FOP2-FGFR1 fusion tyrosine kinase leads to a decrease in Y¹⁰⁵ phosphorylation of endogenous PKM2.

fig. S3

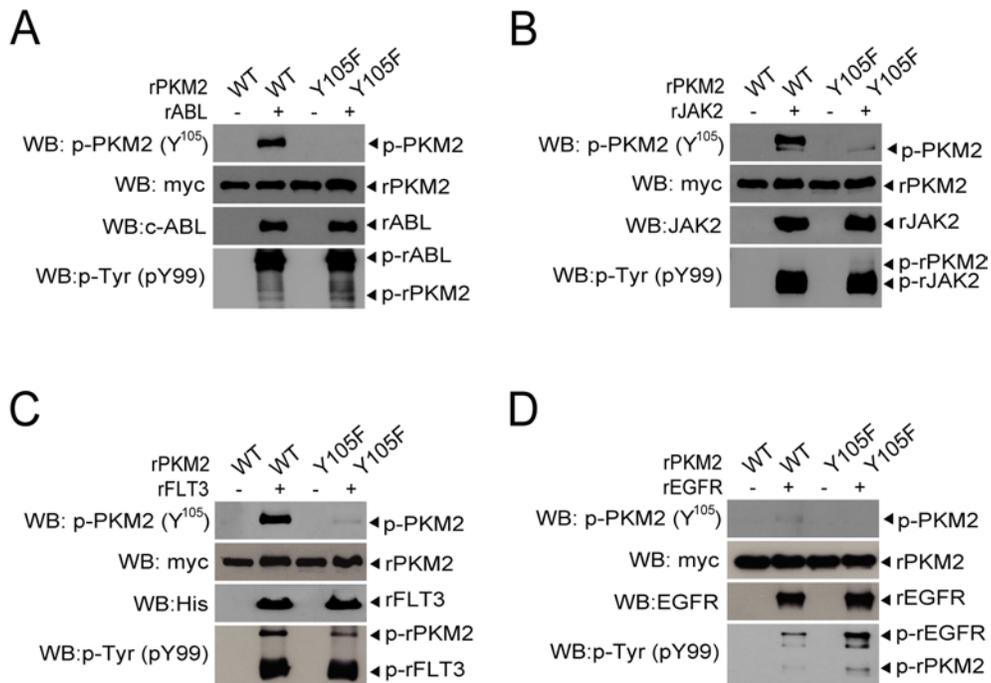


Fig. S3. ABL, JAK2 and FLT3 effectively and directly phosphorylate PKM2 at Y¹⁰⁵ in vitro, whereas epidermal growth factor receptor (EGFR) is less effective. Active, recombinant ABL (A), JAK2 (B), FLT3 (C), and EGFR (D) were incubated with recombinant PKM2 in in vitro kinase assays, followed by immunoblotting using the specific anti-phospho-PKM2 (pY¹⁰⁵) antibody.

fig. S4

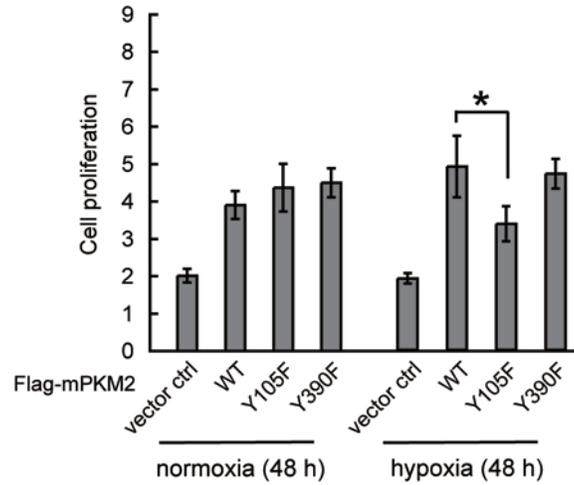


Fig. S4. Presence of the PKM2 Y105F mutant in lung cancer H1299 cells leads to decreased proliferation under hypoxic conditions. Rescue with mPKM2 Y105F in H1299 cells results in a reduced rate of cell proliferation under hypoxic conditions (1% oxygen) but not under normal oxygen conditions (normoxia; 17% oxygen) compared with cells expressing PKM2 WT and the Y390F mutant. Cell proliferation is presented as cell number 48 hours after seeding divided by that at seeding (T=0) for each cell line.

fig. S5

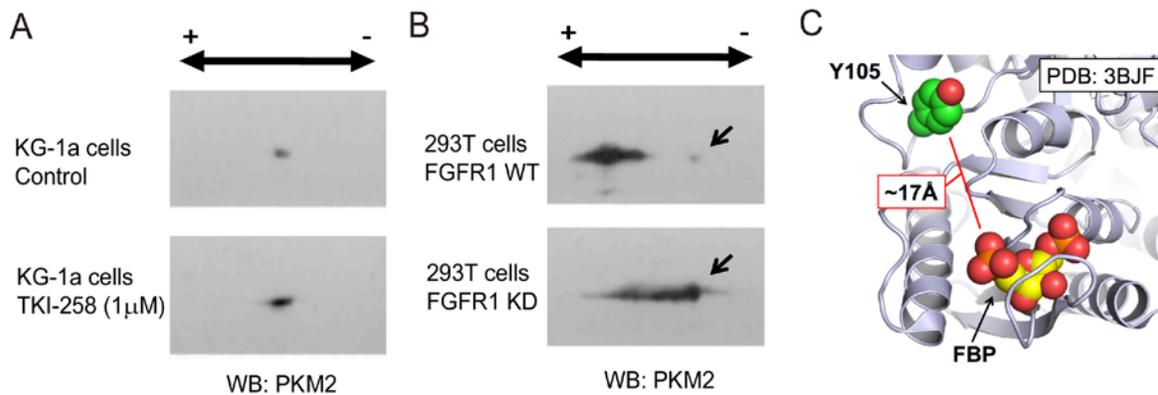


Fig. S5. Y¹⁰⁵ phosphorylation may affect FBP-binding to PKM2 in an “intra-molecular manner” in cellular contexts with high tyrosine phosphorylation stoichiometry of PKM2. (A) Inhibiting FGFR1 with TKI258 did not result in a stoichiometric shift of PKM2 to a less phosphorylated form in KG-1a cells. Cell lysates were applied to an isoelectric focusing (IEF) gel and Western blot was performed using PKM2 antibody. (B) FGFR1 WT causes a stoichiometric shift of PKM2 to a more phosphorylated form in 293T cells, compared PKM2 in cells carrying the FGFR1 KD mutant. Cell lysates were applied to IEF gel and Western blot was performed using PKM2 antibody. The arrows indicate the origin of the IEF. (C) Cartoon representation of PKM2 structure [PDB: 3BJF (8)] using Pymol (www.pymol.org). Y¹⁰⁵ is located at the interface between the A and C domains of PKM2, ~17Å from the FBP binding site.

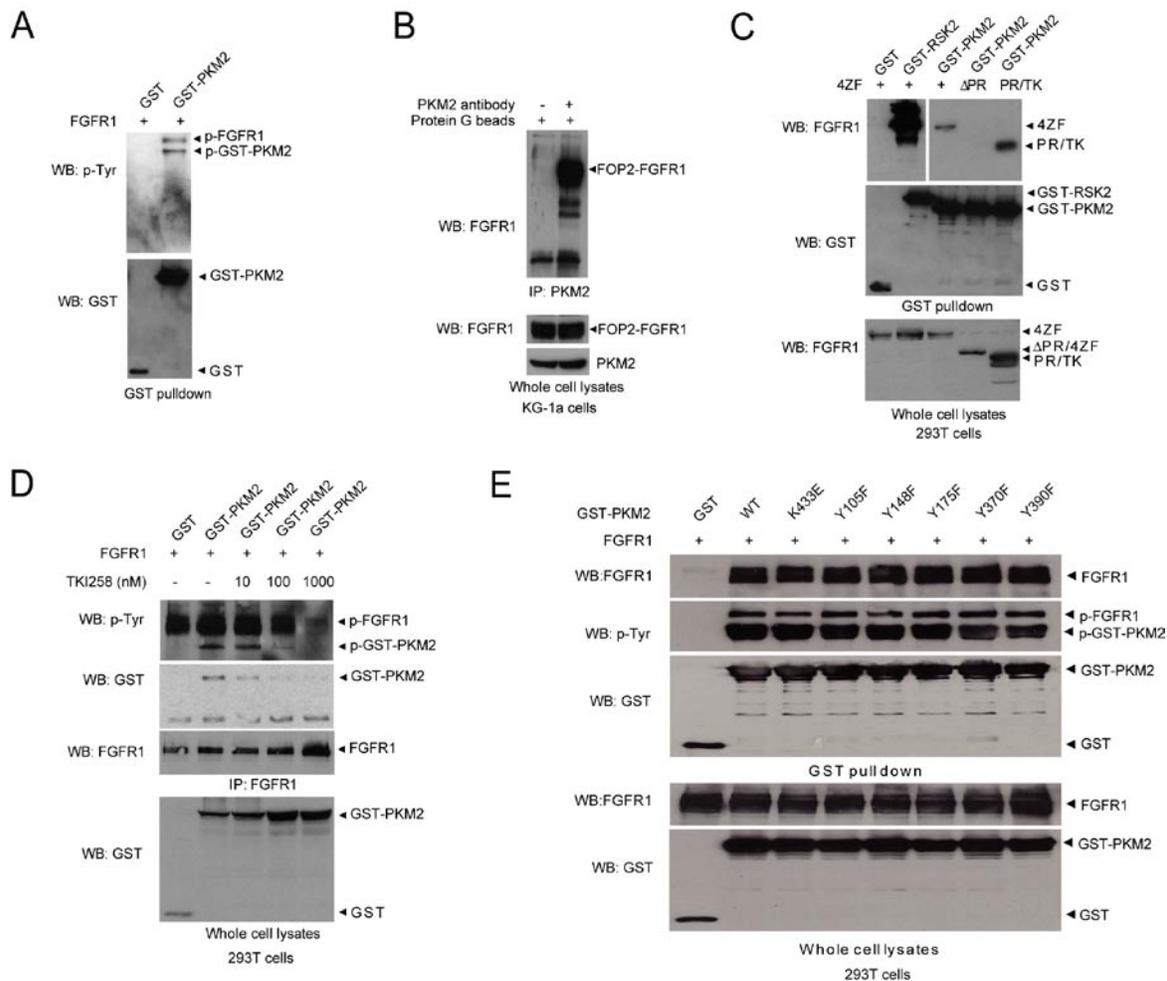


Fig. S6. FGFR1 binds PKM2 in a tyrosine phosphorylation-dependent manner but this binding is dispensable for FGFR1-dependent inhibition of PKM2. **(A)** FGFR1 binds to and phosphorylates GST-PKM2 in 293T cells. **(B)** Co-IP experiment detected the interaction between endogenous FOP2-FGFR1 and PKM2 in KG-1a cells under physiological conditions. **(C)** Constitutively activated FGFR1 fusion tyrosine kinases 4ZF and PR/TK binds to PKM2, whereas a kinase dead mutant 4ZF/ΔPR fails to bind to PKM2. GST and GST-tagged p90RSK2 were included as negative and positive controls, respectively. **(D)** FGFR1-PKM2 interaction depends on FGFR1 kinase activity. Cells coexpressing FGFR1 and GST-PKM2 were treated with TKI258 at increasing concentrations as indicated for two hours, followed by immunoprecipitation using a FGFR1 antibody. **(E)** GST pull down assay shows that single mutations at the identified tyrosine phosphorylation sites of PKM2 do not affect FGFR1-dependent tyrosine phosphorylation of PKM2, or binding of FGFR1 to PKM2. 293T cells coexpressing FGFR1 WT and diverse GST-tagged PKM2 variants were used for the GST pull down experiment.