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Supplementary Information for

A novel allosteric PGAM1 inhibitor effectively suppresses pancreatic ductal adenocarcinoma

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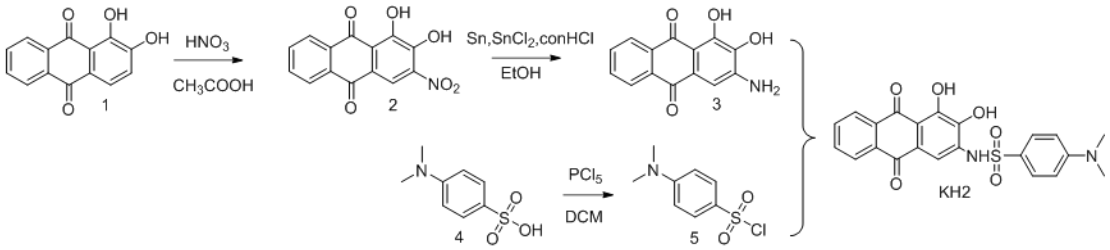
Supplementary Information Text

Chemistry：

Synthesis of KH2



Synthesis of 1,2-dihydroxy-3-nitroanthracene-9,10-dione(2)

Nitric acid (1.5mL, 33.33mmol) was added dropwise to the suspension of 1,2-dihydroxyanthracene-9,10-dione (5g, 20.8mmol) in acetic acid (350mL) at 50℃. Then the mixture was cooled to room temperature and filtered to obtain crude product 2 as yellow solid (4.15g,70 %) (1).

Synthesis of 3-amino-1,2-dihydroxyanthracene-9,10-dione (3)

Sn (10.5g, 341mmol), $SnCl_2 \cdot 2H_2O$ (12.5g, 55.4mmol) and concentrated HCl (50.4mL, 604.8mmol) were added to the suspension of 1, 2-dihydroxy-3-nitroanthracene-9, 10-dione (1.75g, 6.14mmol) in ethanol (350mL). The mixture was stirred at room temperature overnight, then concentrated under reduced pressure to get rid of part of ethanol and the residue was poured into water (1L). A red solid precipitated and was filtered, dried under vacuum to obtain crude product 3 as black solid (1.41g, 90 %)(1).

Synthesis of 4-(dimethylamino) benzenesulfonyl chloride (5)

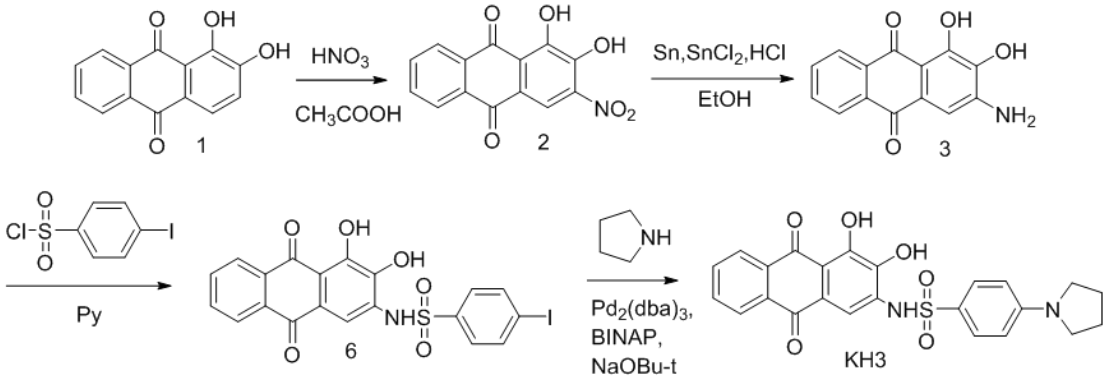
Phosphorus pentachloride (458mg, 2.2mmol) was added to the solution of 4-(dimethylamino) benzenesulfonic acid (402mg, 2mmol) in dry dichloromethane(5.5mL).The mixture was stirred for 1.5hours and to react with 3-amino-1, 2-dihydroxyanthracene-9, 10-dione (3) without further treatment or purification.

Synthesis of N-(3,4-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl) 4-(dimethylamino) benzenesulfonamide (**KH2**)

The reaction mixture of 4-(dimethylamino) benzenesulfonyl chloride (5) was added to the solution of 3-amino-1, 2-dihydroxyanthracene-9, 10-dione (255mg, 1mmol) in dry pyridine (5mL). The mixture was stirred at room temperature for 4h then added to 10% aqueous HCl (50mL).The suspension was extracted withethyl acetate and the organic layer washed with brine, dried overanhydrous Na_2SO_4, filtered. After removal of ethyl acetate, the residue was purified by silica chromatography to give **KH2** as an ared solid (117mg, 20%) (1). H NMR (400 MHz, DMSO-d_6) δ 12.59 (brs, 1H), 10.79 (brs, 1H), 9.79 (brs, 1H), 8.23 – 8.11 (m, 2H), 7.95 – 7.79 (m, 3H), 7.67 (d, J = 8.8 Hz, 2H), 6.74 (d, J = 8.8 Hz, 2H), 2.95 (s, 6H).^{13}C NMR (151 MHz, Pyr) δ 188.83, 181.86, 153.65, 151.79, 143.29, 135.01, 134.82, 134.57, 134.14, 134.07, 129.88, 127.65, 127.11, 126.53, 125.23, 113.56, 112.37, 111.70, 39.81.

MS (ESI) (*m/z*): 437.0 $(M-H)^-$.HRMS (ESI) calcd for $C_{22}H_{18}N_2O_6S$ $[M-H]^-$: 437.0813; found: 437.0814.

Synthesis of KH3



Synthesis of N-(3,4-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-4-iodobenzenesulfonamide (6)

Compound **6** was synthesized according to the route of **KH2**. The 4- 4-iodobenzenesulfonyl chloride was used to give compound 6 as a yellow solid (313mg, 60%).^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (brs, 1H), 10.87 (brs, 1H), 10.44 (brs, 1H), 8.23 – 8.10 (m, 2H), 7.99 (d, J = 8.0 Hz, 2H), 7.96 – 7.85 (m, 2H), 7.73 (s, 1H), 7.64 (d, J = 8.0 Hz, 2H).

Synthesis of N-(3,4-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl) -4-(pyrrolidin-1-yl) benzenesulfonamide (**KH3**)

N-(3,4-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)4-iodobenzene-sulfonamide(52.1mg, 0.1mmol), tris (dibenzylideneacetone) dipalladium (9.2mg,0.01mmol), (+/-)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (9.3mg,0.015mmol), sodium tert-butoxide (48.1mg, 0.5mmol) were added to piperidine (1mL) underargon atmosphere. The mixture was stirred at85℃overnight, then cooled to room temperature and added to 10% aqueous HCl (20mL).The suspension was extracted with ethyl acetate and the organic layer washed with brine, dried over anhydrous Na_2SO_4, filtered. After removal of ethyl acetate, the residue was purified by silica chromatography to give KH3 as a yellow solid (27.8mg, 60%).^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (brs, 1H), 10.83 (brs, 1H), 9.80 (brs, 1H), 8.20 – 8.08 (m, 2H), 7.93 – 7.84 (m, 2H), 7.82 (s, 1H), 7.66 (d, J = 8.8 Hz, 2H), 6.56 (d, J = 8.8Hz, 2H), 3.24 (t, J=6.4Hz, 4H), 1.96 – 1.84 (m, 4H).^{13}C NMR (151 MHz, DMSO) δ 187.53, 180.68, 150.17, 150.09, 140.96, 134.86, 134.15, 133.26, 132.82, 132.22, 128.58, 126.72, 126.30, 123.86, 123.85, 112.25, 110.89, 110.83, 47.23, 24.89.

MS (ESI) (*m/z*): 463.0(M-H)$^{-}$.HRMS (ESI) calcd for $C_{24}H_{20}N_2O_6S$ [M-H]$^{-}$: 463.0969; found: 463.0973.

SI Materials and Methods.

PGAM1 activity analysis of tissues from patients

10 mg cancerous tissue or adjacent normal tissue was homogenized in 100 μL buffer containing 150 mM NaCl and 50 mM Tris-HCl. After centrifugation, 50 μL supernatant was diluted into 450 μL buffer. Then 1 μL diluted supernatant incubated with 49 μL enzyme mix containing 3 units/ml enolase (Sigma–Aldrich), 3 units/ml recombinant pyruvate kinase M2 (Sigma–Aldrich), 0.6 units/ml recombinant LDH (Sigma–Aldrich), 100 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$, 1 mM ADP, 0.2 mM NADH. Finally, 50μL 3PG with concentration of 4 mM was added. The decrease in OD at 340 nm was measured as PGAM1 activity.

Immunohistochemistry (IHC)

IHC staining was performed as previously described (2).The primary antibodies [anti-PGAM1 (ab2220 Abcam, UK), anti-Ki67, anti-Caspase3, anti-CyclinD1 (27309, 19677, 60186, Proteintech, USA),] used in IHC was diluted in 0.1 M phosphate buffered saline (PBS). The IHC score is calculated by combining the quantity score (percentage of positive stained cells) with the staining intensity score (0： negative, 1: weak, 2: moderate and 3: strong). The quantity score ranges from 0 to 4, i.e. 0, no immunostaining; 1, 1–10% of cells are stained; 2, 11–50% are positive; 3, 51–80% are positive; and 4, ⩾ 81% of cells are positive. Raw data were converted to IHC score by multiplying the quantity score (0 – 4) by the staining intensity score (0 – 3). Theoretically, the scores range from 0 to 12. Samples whose IHC score were more than 4 were considered to be high, and less than 4 were considered to be low.

Co-crystallization of PGAM1 with small molecules and data collection and refinement

For small molecules soaking, crystals were soaked in reservoir solution containing 1% DMSO with compounds for 2 hours. The crystals were then quickly frozen in liquid nitrogen with cryo-buffer containing 25% glycerol. All diffraction data were collected at 100K at Shanghai Synchrotron Radiation Facility (SSRF) beam line BL17U1, BL18U1 and BL19U1, respectively. The data were processed with HKL2000 and the scaled data were used for molecular replacement. Crystallographic statistics are summarized in Table S1.

For phasing, model building and refinement, the structures were determined by molecular replacement using Phaser in the CCP4 suite, with the template protein as the search model (pdb code: 1YFK). The structures were then refined by using Phenix. The ligand restraints were generated with the electronic ligand builder and optimization workbench (eLBOW) in Phenix. Manual rebuilding of the model was carried out using the molecular graphics program COOT based on electron density interpretation. Water molecules were incorporated into the model if they gave rise to peaks exceeding 3σ in Fo-Fc density maps. The final refined models had good stereochemistry (Table S1).

Isothermal titration calorimetry (ITC)

Binding constants and thermodynamic parameters of the interaction between PGAM1 and **KH3** were determined with an iTC200 instrument (GE Healthcare) at 25 °C. Samples were equilibrated with a buffer composed of 20mM TRIS (pH 8.0), 100mM NaCl, and

0.5% (v/v) DMSO. **KH3** at 15μM was titrated with PGAM1 0.25mM. Data were analyzed with ORIGIN7 using a one-site binding model.

Cell culture

The cell lines (HEK293T, AsPC-1, SW1990, PANC-1 and MIA PaCa-2) were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China) and maintained according to the supplier's culture protocol. The primary cell lines were obtained from PDAC patient tumor samples, were maintained and confirmed by the WuXi Apptec Co. (Shanghai, China).

HEK293T, PANC-1, SW1990 and MIA PaCa-2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (GIBCO), 100 units/ml of Penicillin and 100μg/ml Streptomycin, Liquid (GIBCO). AsPC-1 cells were maintained in RPMI-1640 medium containing 10% FBS, 100 units/ml of Penicillin and 100μg/ml Streptomycin, Liquid (GIBCO).

Construction of PGAM1 knockdown cells

The sequences of the shRNA reagents used in this study were as follows:

shPGAM1#1 (5′-GAGAGTCTGAAGGATACTATT-3′)

shPGAM1#2 (5′-TGTCAAGCATCTGGAGGGTCT-3′)

The shRNA sequences were ligated into GV248 vector (Genechem, China) to generate shPGAM1 RNA. The construct was confirmed by sequencing. Empty vectors were used as negative controls. Then the viral vector was co-transfected with pMD2-VSVG and pDelta8.9 into HEK293T cells, and the viral supernatant was collected.PANC-1 cells stably expressing shRNA #1 or #2 targeting PGAM1 (shPGAM1#1 or shPGAM1#2) were generated after the puromycin selection and further confirmed by western blot.

Cell viability assays

Cell proliferation assay was performed with cell counting kit 8 (CCK8, Dojindo, Japan). Briefly, 1000 cells (AsPC-1, SW1990, PANC-1, MIA PaCa-2, sh-control and PGAM1 knockdown cells) or 5000 primary cells (0001,0015,0034,0037,0045, 0049) per well were seeded in 96-well plate and treated with **KH3** of gradient concentration. The amount of viable cells was quantified at each 24 h interval by measuring OD_{450} with micro-plate reader (Epoch; BioTek, Winooski, VT).

Seahorse

Inhibition of glycolysis, mitochondrial respiration and Cell Energy Phenotype were performed using Agilent Seahorse XF Cell Mito Stress Test Kit, Agilent Seahorse XF Glycolysis Stress Kit and Agilent Seahorse XF Cell Energy Phenotye Test Kit (Agilent Technologies, Santa Clara, CA, USA) according to manufacturer's instructions. The assay was conducted in a Seahorse XF System (Agilent Technologies, Wilmigton, USA). The data were analyzed using the software "Wave" (Agilent Technologies, Wilmigton, USA) and Prism 6 (GraphPad Software).

PLGA entrapment assay.

815mg PLGA was dissolved in the solution of 220mg **KH3** in 110mL 20:1 (v/v) dichloromethane : methanol. The organic phase was added to 550mL aqueous solution of

1%poly(vinyl alcohol), stirred by shear mixer for 5 minutes and the suspension continued being stirred at 30℃,500 rpm for 8h. Then 1% poly (vinyl alcohol)aqueous solution was removed and the left suspension was washed by 1L double distilled water in total for three times. A yellow solid of 950mg was obtained by freeze-drying under vacuum in which **KH3** was 180mg detected by the method of high performance liquid chromatography (HPLC).

Pharmacokinetic study *in vivo.*

Male ICR mice were administered intraperitoneally at a single dose of 90 mg/kg of **KH3** loaded with PLGA. Blood samples about 0.1mL were collected at 0.25, 0.5, 1, 2, 4, 6, 10, 24h in microtubes containing heparin as anticoagulant. After centrifugation at 3500 rpm for 3 minutes, the plasma was obtained and extracted with methanol. After centrifugation at 12,000 rpm for 10 minutes, the supernatant was transferred for HPLC detection.

The method of HPLC for quantifying KH3.

KH3 was quantified by HPLC using a standard curve. Ten μL of every sample were injected by Agilent 1100 HPLC system and column compartment was 25 ℃. Mobile phase A was water and mobile phase B was methanol containing 0.1% trifluoroacetic acid. Flow rate was 1ml/min using linear gradients as follow:0-2min was 40%A/60%B, 2-10 min was from 40%A/60%B to 5%A/95%B, 10-13min was5%A/95%B,13-14min was from 5%A/95%B to 40%A/60%B,14-18 min was 40%A/60%B. Peak areas of **KH3** in every sample were detected and analyzed according to the standard curve.

Xenograft Studies

Dosage and treatment schedule The dosage and administration schedule of **KH3** in mice was investigated based on PK results. We firstly applied 50mg/kg **KH3** twice per day in the animal study, however the blood concentration of **KH3** in mice dropped quickly due to its short half-life. Also, twice administrations of **KH3** per day caused more adverse events in mice. Thus, we loaded **KH3** with PLGA to slowly release the compound into blood. By monitoring that PLGA-KH3, administered by 90mg/kg in mice, showed half-life of 5.2 hours and maximum concentration of 55μg/ml, we gradually altered the dosage and treatment schedule to 125mg/kg i.p. every four days, to ensure **KH3** preserved excellent efficacy with limited adverse events in animal model.

PDAC orthotopic xenograft. For experimental orthotopic assays, each mouse was then injected with 100μL of MIA PaCa-2 cell suspension (1×10^6 cells) into the pancreas. Animal health conditions and metastatic progression were monitored. At day 6 post-implantation, the mice were randomly assigned to treat with control (PLGA) (n=8), 30mg/kg Gemzar (Eli Lilly, USA) intravenously at day 9, 12, 16 (n=8) and **KH3** 100mg/kg at day 6,125mg/kg intraperitoneally at day 9, 12, 15 (n=8). The body weights were evaluated during the experiment. At day 20 post-implantation the mice were sacrificed, all tumors were harvested, weighed and measured using the formula ($L \times W \times W) \times (\pi/6)$, where L is the major tumor axis and W is the minor tumor axis.

PDAC PDX models Mice were maintained and handled in accordance with the WuXi AppTec Co. (Shanghai, China). All the studies with mice were approved by the Ruijin

Hospital Ethics Committee. Patient tumor materials were collected in culture medium and kept on wet ice for engraftment within 24 h after resection. Approximately 20-30 mg tissue fragments were implanted subcutaneously into the flank region of athymic nude (nu/nu) male mice using a trocar. Successfully engrafted tumor models were then passaged and banked after three passages in mice. The identity of the established PDXs was confirmed by gene sequencing. Tumors were subcutaneously implanted into nude mice at passages p4. Tumors (~100 mm^3) were enrolled on treatment with **KH3**. About 2 weeks post-implantation, the mice were evenly distributed to treat with control (PLGA), Gemzar (Eli Lilly, USA) intravenously and **KH3** intraperitoneally. The tumor size and body weight were evaluated and the approximate volume of the mass was calculated using the formula $(L \times W \times W) \times (\pi/6)$, where L is the major tumor axis and W is the minor tumor axis. At day 14, all tumors were harvested, weighed and the blood was collected for hematological analysis, kidneys and livers for histopathological analysis. All samples were fixed overnight in 40% formaldehyde (pH 7.4) and then dehydrated in graded concentrations of ethanol. Immunochemical staining and analysis were performed using previously methods.

Statistical analysis

Survival curve was estimated by Kaplan-Meier methods. Two-way analysis of variance (ANOVA) or one-way ANOVA together was used for multiple groups analysis. Unpaired t-test was used to determine statistical significance in the rest experiments, and p value less than 0.05was considered significant: * for $p<0.05$, ** for $p<0.01$, ***for $p<0.001$. Values are expressed as mean±SEM or mean±SD (Prism 6; GraphPad Software).

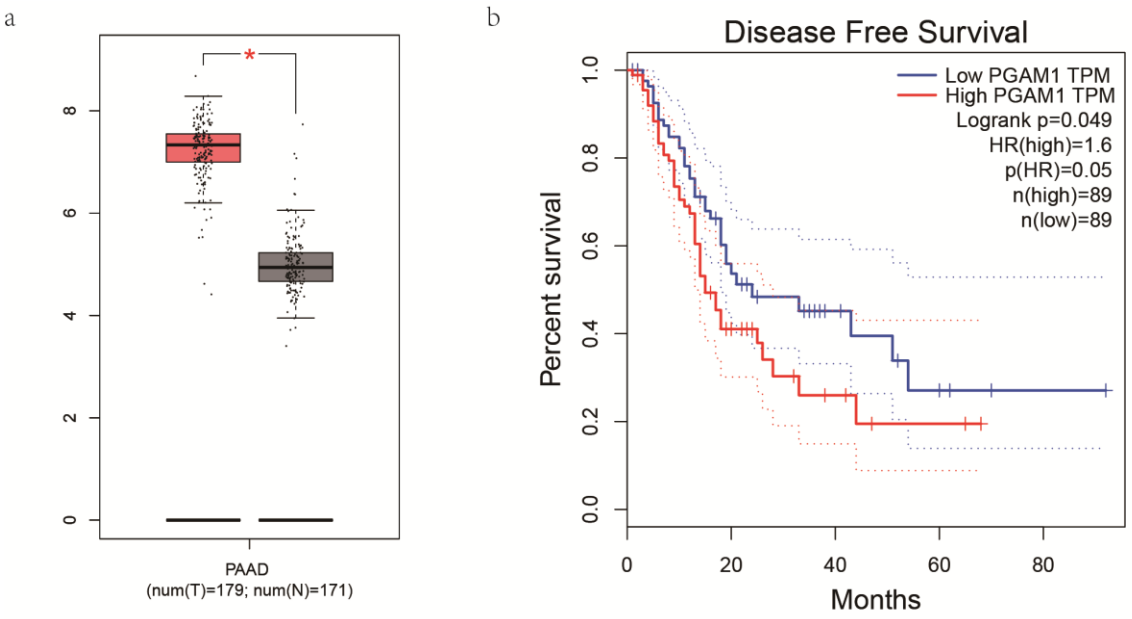


Fig. S1. PGAM1 expression level in PDAC patients and their correlated prognosis analyzed by the data of TCGA.

a. PGAM1 expression level in cancerous tissue was significantly higher than adjacent normal tissue in PDAC patients.

b. Patients with higher PGAM1 expression level showed worse prognosis (Disease Free Survival) than those with low PGAM1 level.

(*: $0.01<p<0.05$)

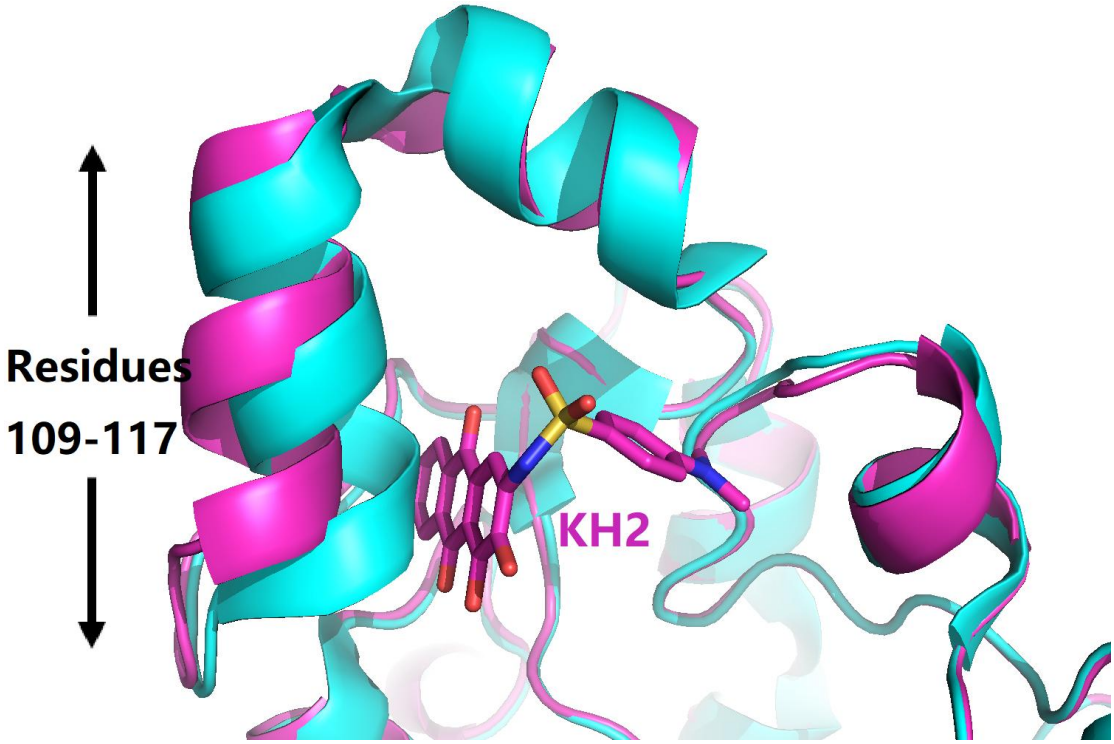


Fig. S2. Structure comparison of PGAM1-KH2 complex (purple) and apo-PGAM (cyan). Protein scaffolds are shown in cartoon.

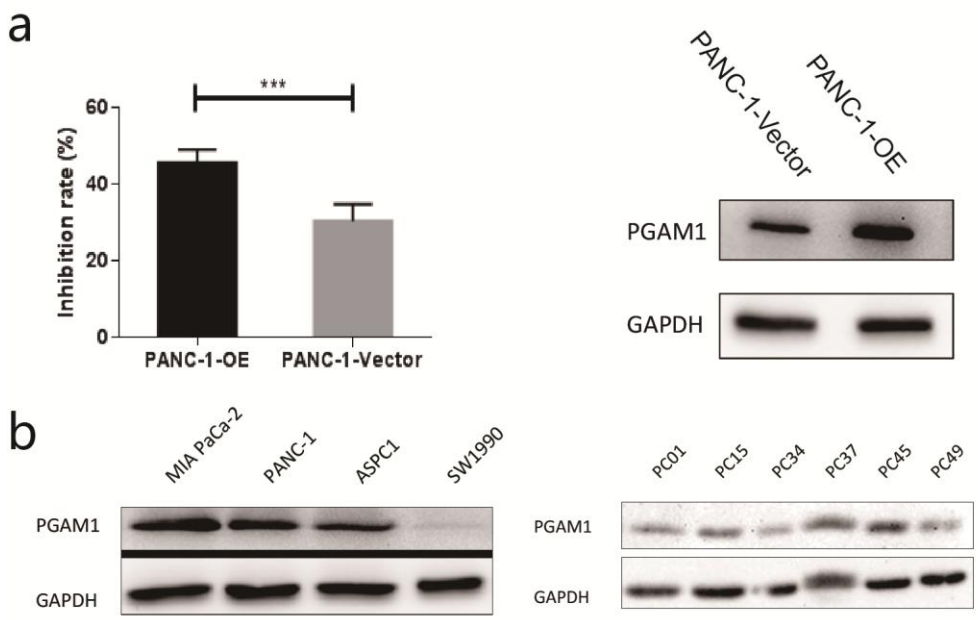


Fig. S3. PGAM1 overexpression impaired the inhibition of **KH3** on cell proliferation and the expression levels of PGAM1 in PDAC cell lines and tissue of patient.

a. Overexpression of PGAM1 impaired the inhibition of **KH3** (1 μM) on proliferation of PANC-1 cells when compared to control. Overexpression of PGAM1 in PANC-1 cells was verified by western blot. The data was presented as mean ±s.d. (n=5) and *p*-values was obtained from unpaired *t*-test (***: $0.0001<p<0.001$).

b. The expression levels of PGAM1 in PDAC commercial and primary cells.

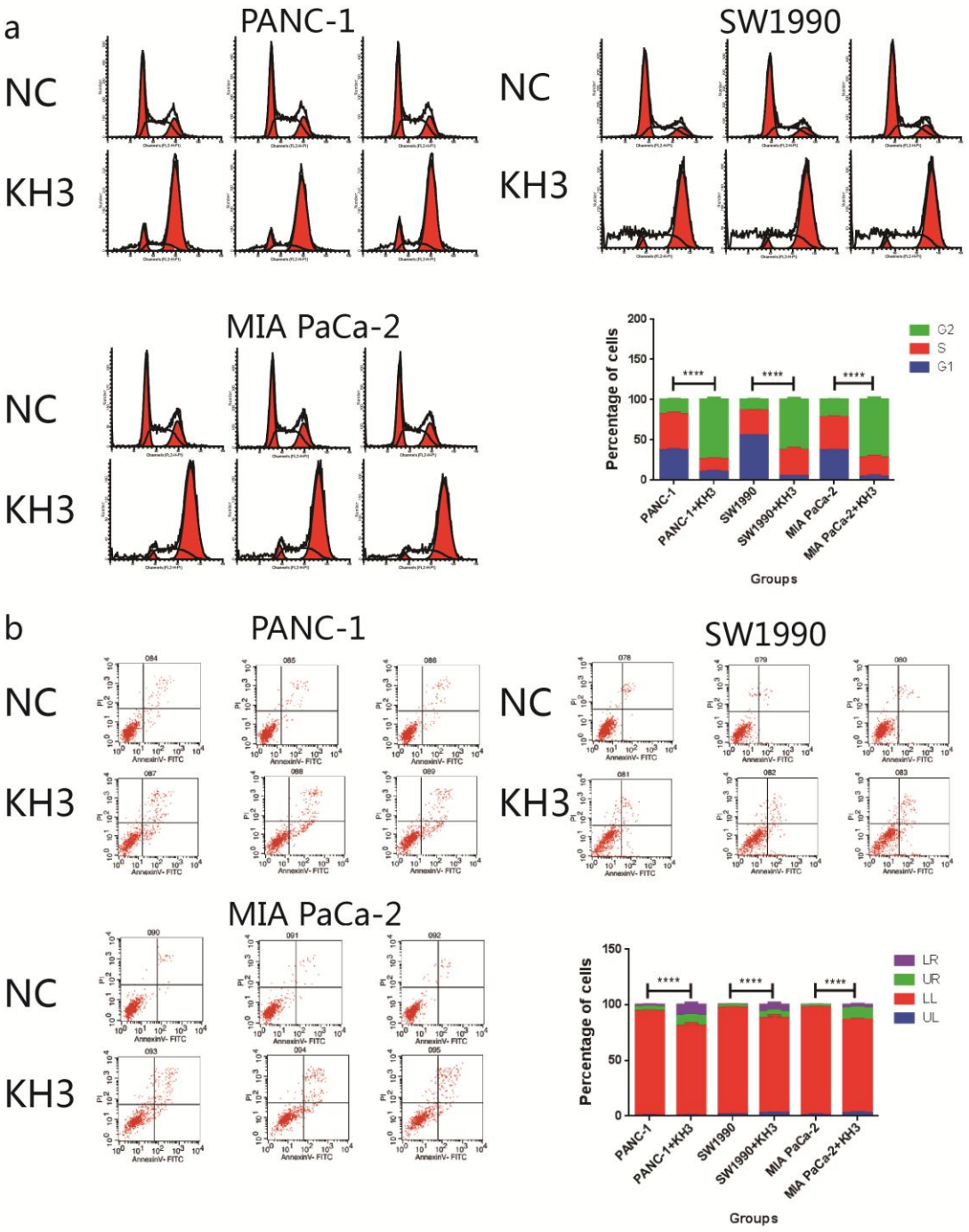


Fig. S4. The impact of **KH3** on PDAC cell apoptosis and cycle arrest *in vitro*.
a. The effect of **KH3** on cycle arrest was analyzed by FACS and the percentage of cells positive for FL2-H-PI staining was quantified. PDAC cells were treated with 2μM **KH3** for 48 h.
b. The effect of KH3 on cell apoptosis was analyzed by FACS and the percentage of cells positive for Annexin V and PI staining was quantified. PDAC cells were treated with 2μM **KH3** for 48 h.

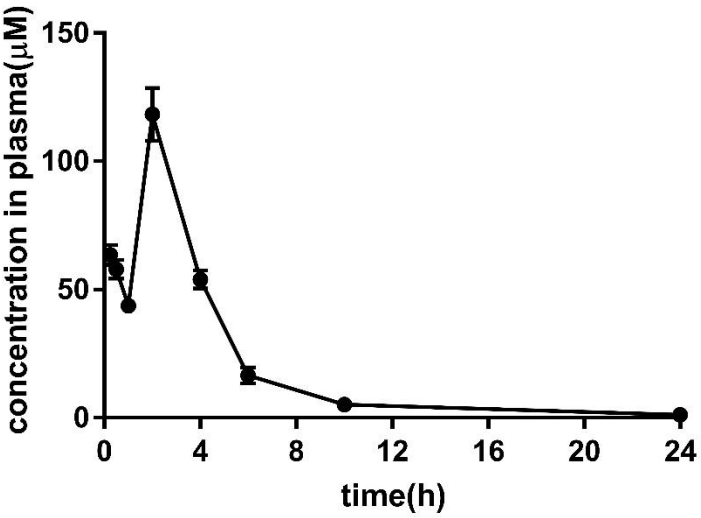


Fig. S5. Plasma drug concentration-time profile of **KH3** in ICR mice (n=4) after intraperitoneal administration at 90 mg/kg loaded with PLGA (KH3: PLGA=1:3.7). The data are presented as mean ± s.e.m.

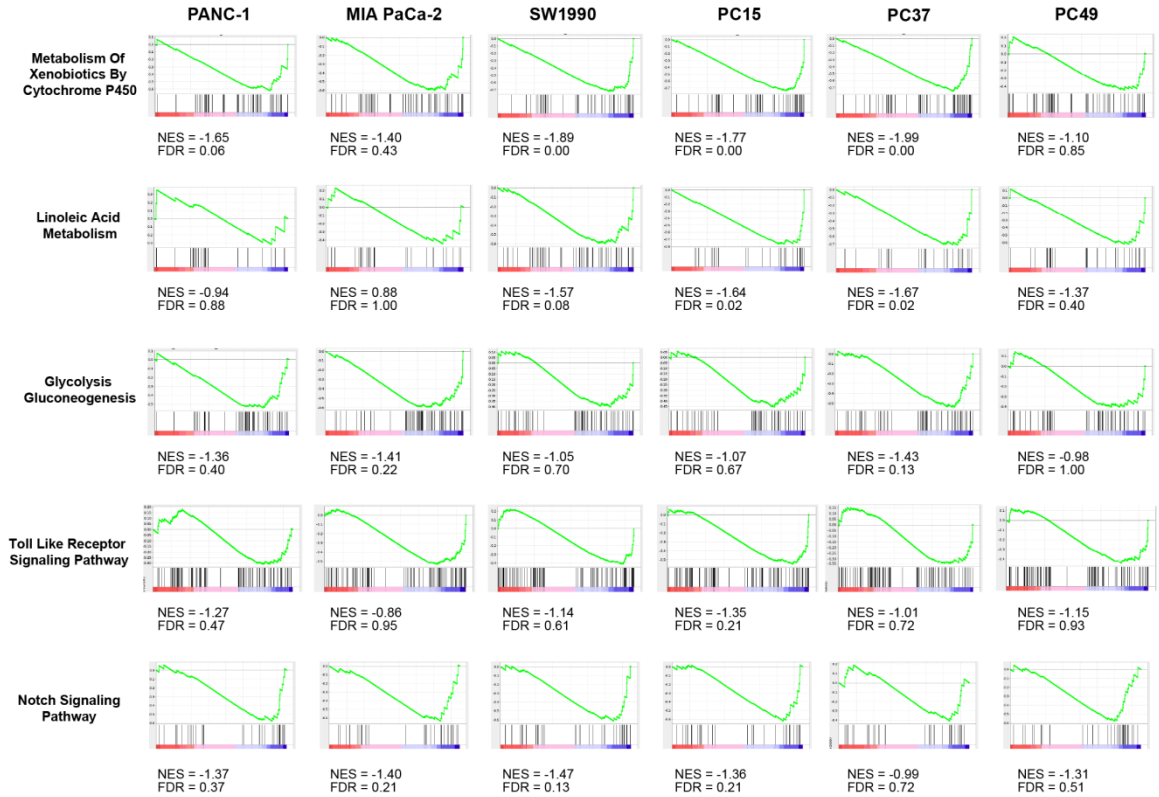


Fig. S6. The representative and core-enriched signaling pathways decreased by **KH3** treatment in six PDAC cells were shown by gene set enrichment analysis (GSEA).

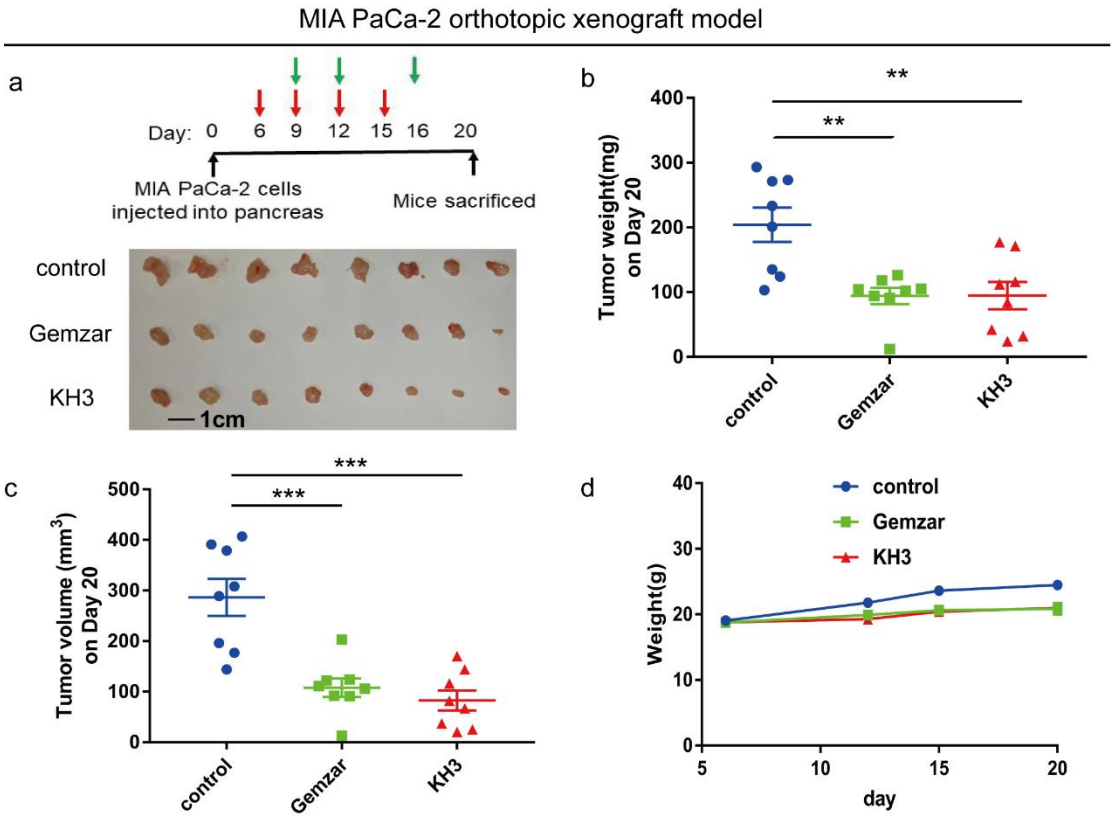


Fig. S7. KH3 attenuates pancreatic tumor growth in orthotopic PDAC mouse models.

a. Flow chart of MIA PaCa-2 orthotopic xenografts treated with control (n=8), Gemzar 30mg/kg at day 9, 12, 16 (n=8) as the green arrows indicated and **KH3** 100mg/kg at day 6, 125mg/kg at day 9, 12, 15 (n=8) as the red arrows indicated.

b. Gemzar and **KH3** decrease the tumor weights compared with the control group after they were harvested on day 20.

c. Gemzar and **KH3** decrease the tumor volumes compared with the control group after they were harvested on day 20.

d. The weight of mice in this model.

The data was presented as mean±s.e.m. and *p*-values were obtained from unpaired t-test, (n.s., not significant. *: $0.01<p<0.05$, **: $0.001<p<0.01$, ***: $0.0001<p<0.001$, **** $p<0.0001$)

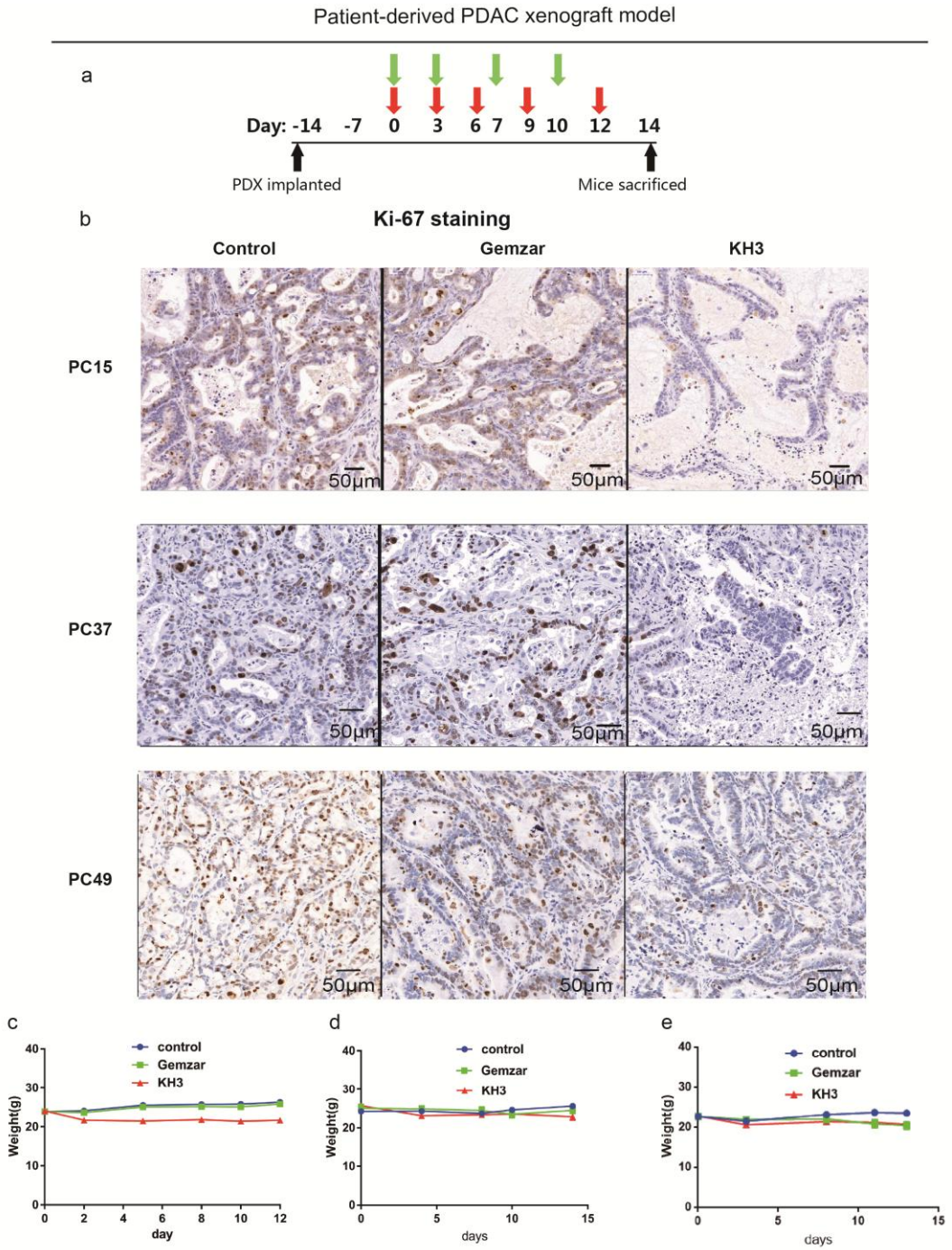


Fig. S8. Flow chart, Ki67 staining and body weights in PDX models.

a. Flow chart of PDX xenografts treated with control (n=8), Gemzar 30mg/kg(n=8) twice a week as the green arrows indicated and **KH3** 125mg/kg (n=8) once every four days as the red arrows indicated.
b. Ki67 staining of patient-derived tumor tissues in three PDAC PDX models (PC15, PC37 and PC49)
c. Body weights of mice in the model of PDX (PC15).
d. Body weights of mice in the model of PDX (PC37).
e. Body weights of mice in the model of PDX (PC49).

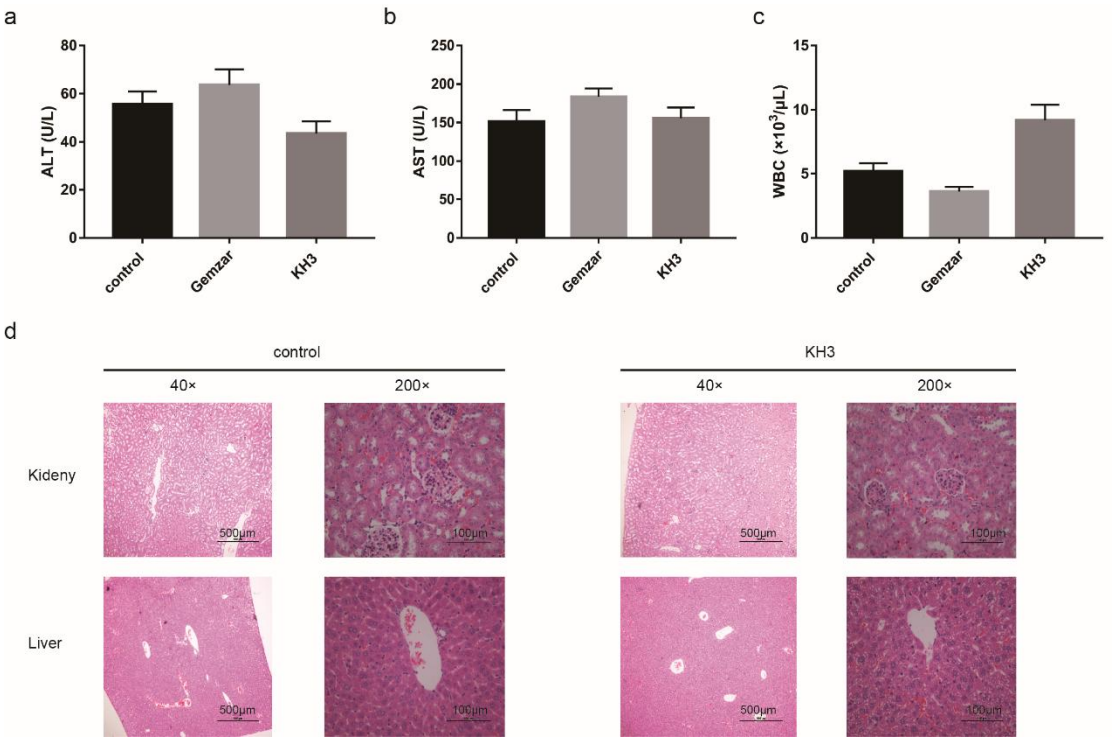


Fig. S9. Toxicity study of **KH3** in mice

a. Plasma levels of the ALT in mice in the model of PDX (PC 15).
b. Plasma levels of the AST in mice in the model of PDX (PC 15).
c. Plasma levels of the WBC in mice in the model of PDX (PC 15).
d. Haematoxylin and eosin histology of kidneys and livers from mice in the model of PDX (PC 15).

The data was presented as mean±s.e.m.

Table. S1. Data collection and refinement statistics

	PGAM1-RedS	PGAM1-KH2
Data collection		
Space group	P 2_1 2_1 2_1	P 2_1 2_1 2_1
Cell constants a, b, c(Å)	82.64 85.27 102.47	79.19 82.93 99.80
α, β, γ(°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution *(Å)	50-1.98 (2.05-1.98)	50-2.55 (2.64-2.55)
Data completeness (%)	92.2 (90.8)	99.4 (99.9)
No. Observations	321781	133070
No. Unique Reflections	51433	21972
Redundancy	6.8 (6.8)	6.1 (6.1)
R_{merge}	0.08 (0.78)	0.11 (0.74)
$< I/\sigma(I) >^1$	2.47(1.98Å)	1.95(2.54Å)
Refinement		
Resolution (Å)	43.54-1.97 (2.02-1.97)	49.90-2.55 (2.67-2.55)
Data completeness (%)	92.0 (87)	99.4 (100)
No. reflections	47382	21790
R_{work}/R_{free}	0.200/0.226	0.198/0.248
R_{free} test set	2402 (5.07%)	1115 (5.12%)
Wilson B-factor ($Å^2$)	35.1	51.5
F_o,F_c correlation	0.95	0.94
Total number of atoms	4055	3912
Average B, all atoms ($Å^2$)	38.0	34.0
Ligands	MES: 1;	MES: 1;

	RedS: 2; CL: 1;	KH2: 1; CL: 1;
Ramachandran favored (%)	98.1	97.9
Ramachandran allowed (%)	1.9	2.1
Ramachandran outliers (%)	0	0
R.m.s deviations		
Bond lengths (Å)	0.008	0.009
Bond angles(°)	1.157	1.237
PDB accession code	5Y2U	5Y65

Values in parentheses are for the highest resolution shell. All datasets were collected from a single crystal each.

Table. S2. *In vivo* pharmacokinetic studies of **KH3** via intraperitoneal injection

$AUC_{0\text{-}24h}$	$AUC_{0\text{-}inf}$	CL/F	C_{max}	MRT	T_{max}	V/F	$t_{1/2}$
ug*h/mL	ug*h/mL	mL/h	ug/mL	h	h	mL	h
214	218	12.1	55	4.4	2	90	5.2

AUC: area under curve
CL: clearance
F: bioavailability
MRT: mean residence time
V: volume of distribution

Table. S3. Athymic nude mouse hematology
Nude mice (n=5) were treated with either vehicle control or Gemcitabine or KH3 for 12 days. CBC analysis shows no significant difference in the hematopoietic properties between the two groups of mice.

Test Name (units)	Reference range (low-high)	Control day 12 (mean)	30 mg/kg Gemcitabine twice a week day 12 (mean)	125 mg/kg KH3 once four days day 12 (mean)
WBC	2.6-10.1	5.18 ±1.47	3.62 ±0.84	9.2 ±3.0
LYM%	0-99.9	21.6 ±2.2	33.8 ±9.7	29.8 ±9.6
MONO%	0-99.9	17.74 ±6.6	13.7 ±3.4	18.3 ±5.9
NEUT%	0-99.9	59.22 ±7.7	52.7 ±9.2	72.7 ±11.7
LYM($\times 10^3$μL)	1.3-8.4	0.98 ±0.19	1.18 ±0.19	2.85 ±1.95
MONO($\times 10^3$μL)	0-0.3	0.78 ±0.26	0.5 ±0.19	1.7 ±0.9
NEUT($\times 10^3$μL)	0.4-2.0	2.64 ±0.70	1.93 ±0.74	6.72 ±2.51
RBC($\times 10^6$μL)	6.5-10.1	8.722 ±0.64	8.15 ±0.54	7.78 ±1.47
HGB(g/L)	110-150	133 ±11	128 ±6	117 ±21
HCT%	32.6-48	38.9 ±3.1	38.6 ±1.5	34.5 ±6.4
MCV (fL)	38.5-44.1	44.6 ±0.5	47.4 ±1.6	44.4 ±0.7
MCH (pg)	13.7-18.1	15.3 ±0.4	15.7 ±0.6	15.1 ±0.2
MCHC (pg)	320-362	343 ±8	332 ±7	341 ±5
RDW-CV	10.9-15.4	12.8 ±0.7	14.0 ±0.3	14.6 ±0.9
PLT($\times 10^3$μL)	250-1540	673 ±117	539 ±60	802 ±161
MPV(fL)	7.6-13.6	4.14 ±0.58	4.73 ±0.68	6.22 ±0.98
PCT	0.108-0.282	0.28 ±0.07	0.26 ±0.06	0.51 ±0.17
PDW	9.0-17	17.62 ±0.6	17.9 ±0.7	18.4 ±0.8

Table. S4. PGAM1 expression and clinical characteristics of 50 PDAC patients

Patient	Gender	Age	PGAM1 IHC Expression H/L（1/0）	Months elapsed	Destination D/A（1/0）	Perineural invasion	T	M	N	Stage
1	F	80	1	10	1	1	T3	M0	N0	IIa
2	M	49	0	37	0	0	T2	M0	N0	Ib
3	F	66	1	27	1	1	T3	M0	N0	IIa
4	F	62	1	6	1	1	T4	M0	N0	IIa
5	M	62	1	10	1	1	T3	M0	N0	IIa
6	M	56	1	10	1	1	T3	M0	N0	IIa
7	M	53	1	23	1	1	T3	M0	N0	IIa
8	M	60	0	32	1	1	T4	M0	N2	III
9	F	61	0	26	1	0	T3	M0	N0	IIa
10	F	59	0	13	1	0	T3	M0	N0	IIa
11	F	71	1	5	1	1	T3	M0	N0	IIa
12	M	54	0	32	0	1	T3	M0	N0	IIa
13	F	71	1	8	1	1	T3	M0	N0	IIa
14	M	60	1	31	0	1	T3	M0	N0	IIa
15	M	72	0	14	1	1	T3	M0	N0	IIa
16	M	58	1	10	1	0	T2	M0	N0	Ib
17	M	51	1	22	1	1	T4	M0	N1	III
18	F	63	1	26	0	0	T2	M0	N0	Ib
19	F	65	0	28	0	1	T3	M0	N0	IIa
20	F	55	0	8	1	0	T3	M0	N0	IIa
21	M	59	1	11	1	1	T2	M0	N1	IIb
22	M	42	0	15	1	1	T3	M0	N0	IIa
23	M	72	1	12	1	1	T4	M0	N2	III
24	M	73	1	24	0	1	T4	M0	N0	III
25	M	56	0	24	0	1	T3	M0	N2	III
26	M	66	1	24	0	1	T3	M0	N0	IIa
27	M	43	0	25	0	1	T3	M0	N0	IIa
28	F	68	0	22	1	1	T3	M0	N0	IIa
29	M	73	0	24	0	0	T2	M0	N1	IIb
30	M	45	0	18	0	1	T3	M0	N0	IIa
31	M	52	1	6	1	1	T3	M0	N2	III
32	F	59	1	5	1	1	T2	M0	N1	IIb
33	M	61	0	12	1	1	T3	M1	N0	IV
34	F	61	0	10	1	1	T3	M1	N2	IV
35	M	71	1	1	1	1	T3	M0	N0	IIa
36	F	67	1	1	1	1	T3	M1	N0	IV
37	M	59	1	8	1	1	T3	M0	N0	IIa

38	M	69	1	5	1	1	T3	M0	N0	IIa
39	F	56	0	12	1	1	T3	M0	N0	IIa
40	M	66	1	6	1	1	T3	M0	N0	IIa
41	M	65	0	10	1	1	T3	M0	N0	IIa
42	F	60	1	13	1	1	T3	M0	N0	IIa
43	M	44	1	6	1	1	T4	M0	N1	III
44	M	52	1	7	1	1	T3	M0	N0	IIa
45	M	51	0	18	0	0	T2	M0	N1	IIb
46	M	71	1	9	1	1	T3	M1	N0	IV
47	M	51	1	9	1	1	T3	M1	N1	IV
48	M	69	1	6	1	1	T3	M0	N1	IIb
49	M	67	1	2	1	1	T3	M0	N0	IIa
50	M	62	1	6	1	1	T3	M0	N0	IIa

Table. S5. Correlation between PGAM1 expression and clinical characteristics of 50 PDAC patients

Clinical characteristics	Low expression (n = 19)	High expression (n = 31)	P value
Gender			
Male	12	22	0.566
Female	7	9	
Age			
< 60	10	11	0.233
≥ 60	9	20	
pT stage			
T2	3	3	
T3	15	23	0.234
T4	1	5	
pN stage			
N0	14	23	
N1	2	6	0.606
N2	3	2	
pTNM stage			
Ib	1	2	
IIa	12	18	0.838
IIb	2	3	
III	2	5	
IV	2	3	
Perineural invasion			
No	6	2	0.041*
Yes	13	29	

*P<0.05

Supplemental reference

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2. Chen J*, et al.* (2014) Snail recruits Ring1B to mediate transcriptional repression and cell migration in pancreatic cancer cells. *Cancer research* 74(16):4353-4363.