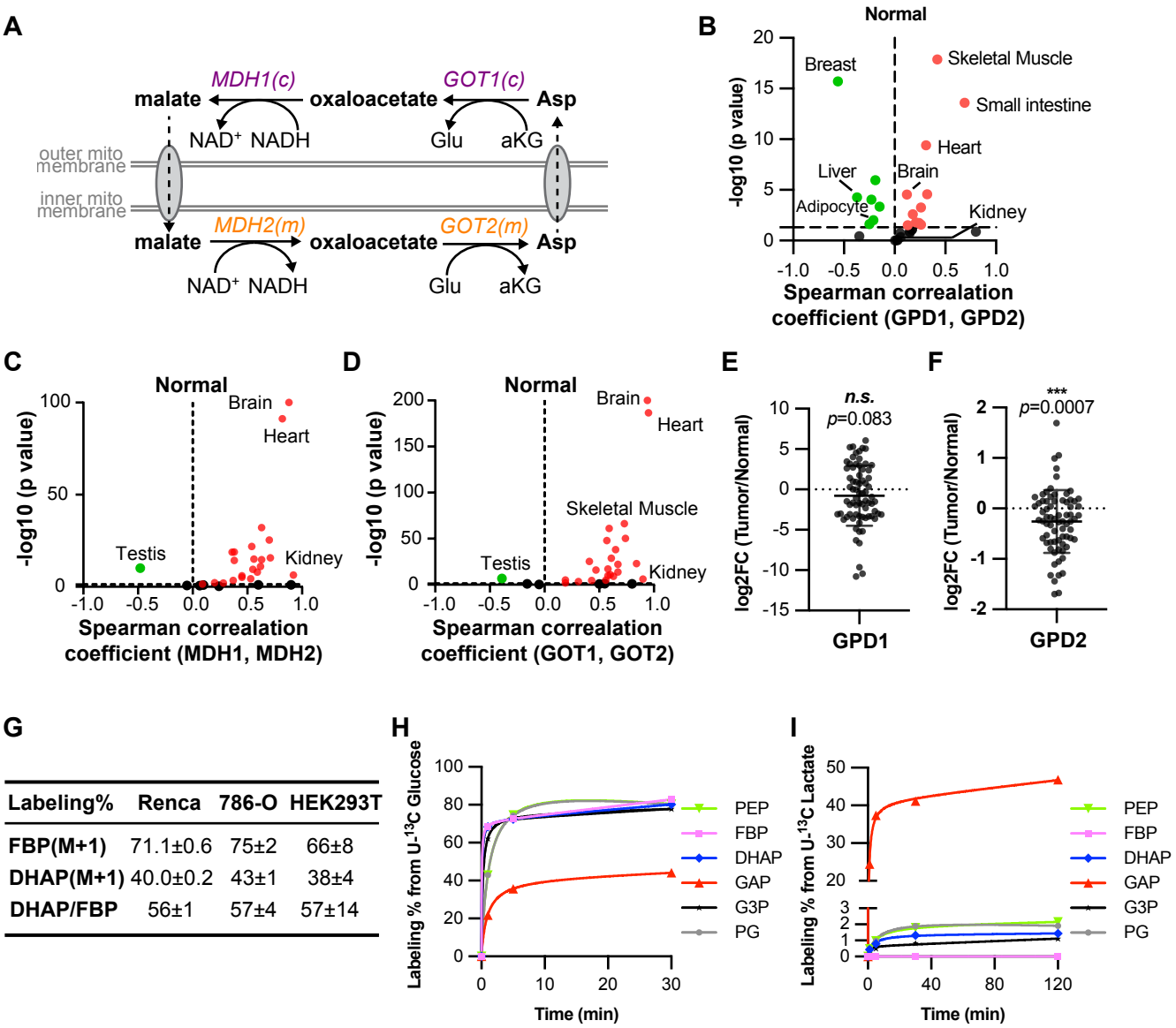
Figure S1



Labeling%	Renca	786-O	HEK293T
FBP(M+1)	71.1±0.6	75±2	66±8
DHAP(M+1)	40.0±0.2	43±1	38±4
DHAP/FBP	56±1	57±4	57±14

Figure S1 (Related to Figure 1). Correlation analysis and metabolic flux analysis of G3PS and MAS

(A) Diagram of malate-aspartate shuttle.

(B) Spearman correlation analysis of GPD1 and GPD2 expression in normal tissues. Red dot represents positive coefficients with p value < 0.05. Green dot represents negative coefficients with p value <0.05.

(C) Spearman correlation analysis of MDH1 and MDH2 expression in normal tissues. Red dot represents positive coefficients with p value < 0.05. Green dot represents negative coefficients with p value <0.05.

(D) Spearman correlation analysis of GOT1 and GOT2 expression in normal tissues. Red dot represents positive coefficients with p value < 0.05. Green dot represents negative coefficients with p value <0.05.

(E) $\log_2$ fold change of the expression of GPD1 in tumor compared to paired normal tissue in KIRC patients

(F) $\log_2$ fold change of the expression of GPD2 in tumor compared to paired normal tissue in KIRC patients

(G) Labeling percentage of M+1 isotopolgue of DHAP after cells were labeled with 1-^{13}C glucose for 4hr.

(H) Labeling kinetics of glycolytic intermediates with U-^{13}C glucose.

(I) Labeling kinetics of glycolytic intermediates with U-^{13}C lactate.

Figure S2

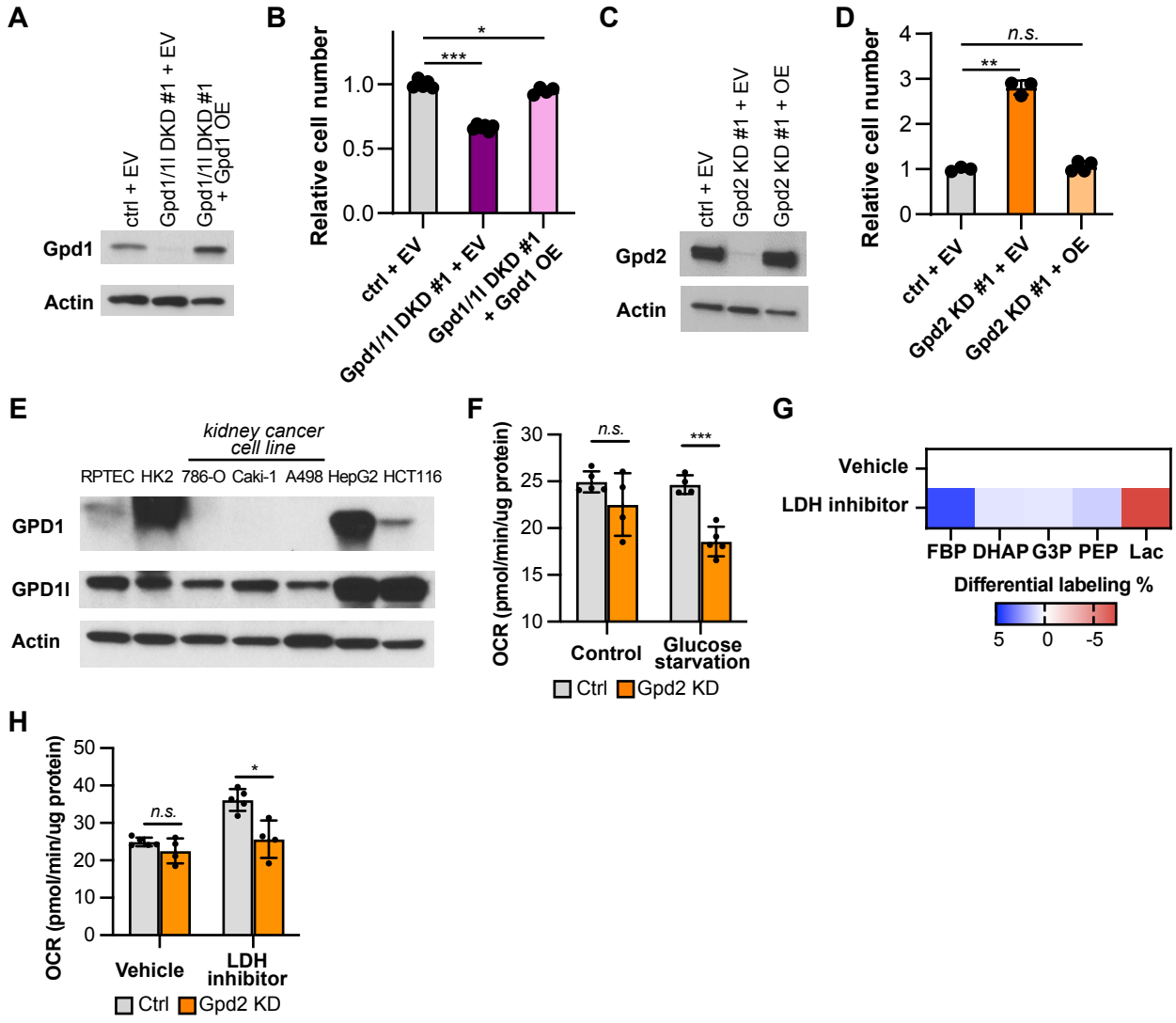


Figure S2 (Related to Figure 2). Protein expression and metabolic phenotypes of Gpd KD cells

(A) Western blot analysis of Gpd1 in control cells expressing empty vector (ctrl+EV), Gpd1/1l DKD cells expressing empty vector (Gpd1/1l DKD #1+EV), and Gpd1/1l DKD cells expressing shRNA-resistant Gpd1 (Gpd1/1l DKD #1+Gpd1 OE). β-actin was used as a loading control.

(B) Proliferation rate of ctrl+EV, Gpd1/1l DKD+EV, and Gpd1/1l DKD+Gpd1OE.

(C) Western blot analysis of Gpd2 in control cells expressing empty vector (ctrl+EV), Gpd2 KD cells expressing empty vector (Gpd2 KD#1+EV), and Gpd2 KD cells expressing shRNA-resistant Gpd2 (Gpd2 KD#1+Gpd2 OE). β-actin was used as a loading control.

(D) Proliferation rate of ctrl+EV, Gpd2 KD+EV, and Gpd2 KD+Gpd2OE.

(E) Western blot analysis of Gpd1 in non-transformed kidney cell lines (Primary Renal Proximal Tubule Epithelial CellsRPTEC and HK2), kidney cancer cell lines (786-O, Caki-1 and A498), liver cancer cell line HepG2 and colorectal cancer cell line HCT116.

(F) Oxygen consumption rate of control and Gpd2 KD cells cultured in the presence of 5mM glucose (Control) or 1mM glucose (glucose starvation) for 24hr.

(G) Differential labeling percentages in glycolytic metabolites after cells were labeled with U-^{13}C lactate in the presence of vehicle control or LDH inhibitor (GSK2837808A).

(H) Oxygen consumption rate of control and Gpd2 KD cells treated with vehicle control or 10μM LDH inhibitor (GSK2837808A) for 24hr.

Data are presented as mean ±SEM. *p<0.05, **p<0.01, ***p<0.001, n.s. not statistically significant.

Figure S3

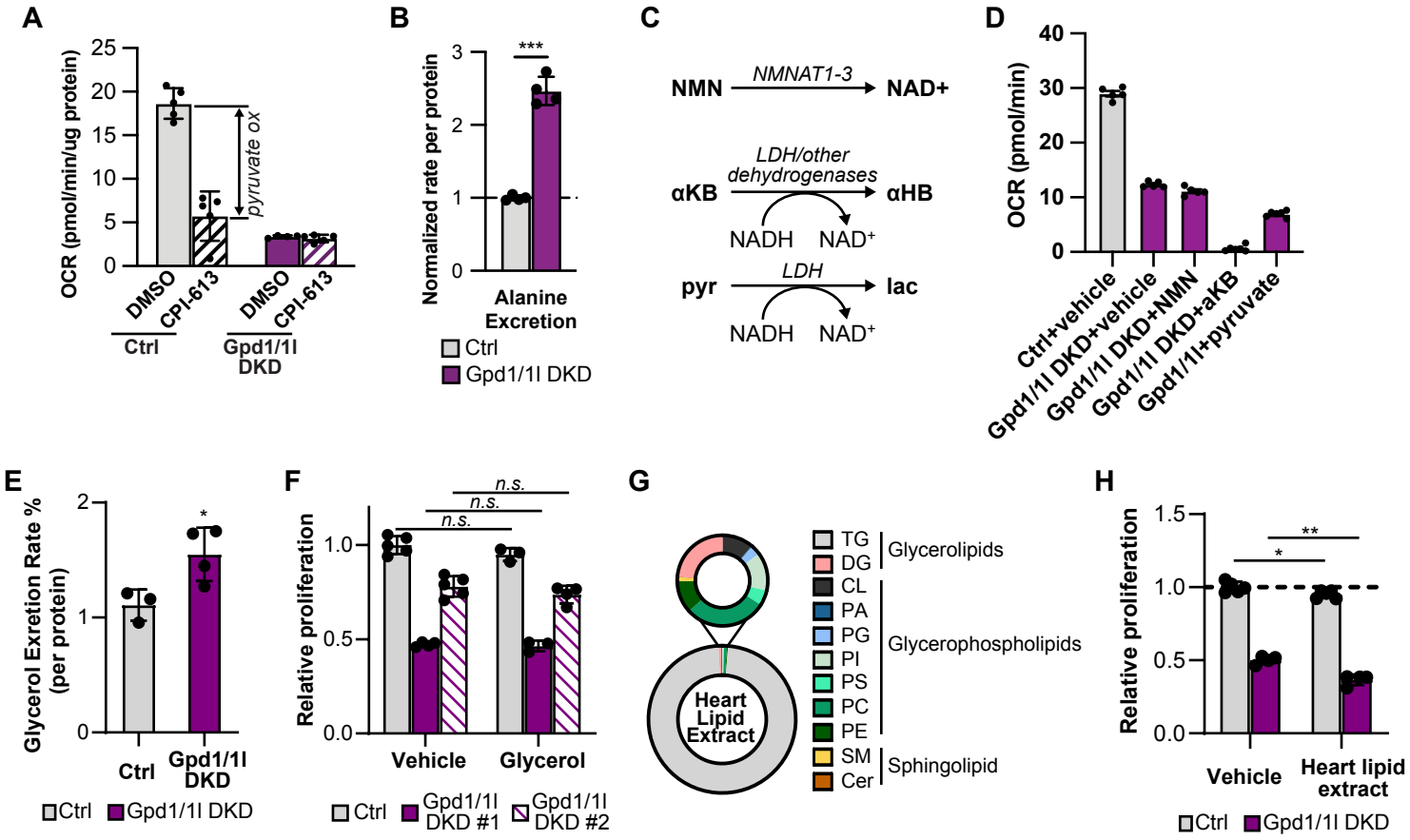


Figure S3 (Related to Figure 3). Rescue experiments in Gpd1/1l DKD renca cells

(A) Oxygen consumption rate of ctrl and Gpd1/1l DKD cells treated with vehicle (DMSO) or CPI-613.
(B) Relative alanine excretion rate in ctrl and Gpd1/1l DKD cells.
(C) Reactions that produce NAD+. NMN, nicotinamide mononucleotide. αKB, alpha-ketobutyrate. αHB, alpha-hydroxybutyrate. Pyr, pyruvate. Lac, lactate.
(D) Oxygen consumption rate of ctrl and Gpd1/1l DKD cells treated with vehicle, NMN, aKB or pyruvate.
(E) Glycerol secretion rate of ctrl and Gpd1/1l DKD cells.
(F) The relative proliferation of ctrl and Gpd1/1l DKD cells supplemented with water (vehicle) or 1mM glycerol.
(G) Lipid composition in mouse heart lipid extract.
(H) The relative proliferation of ctrl and Gpd1/1l DKD cells supplemented with ethanol (vehicle) or heart lipid extract.
Data are presented as mean ±SEM. *p<0.05, **p<0.01, ***p<0.001, n.s. not statistically significant.

Figure S4

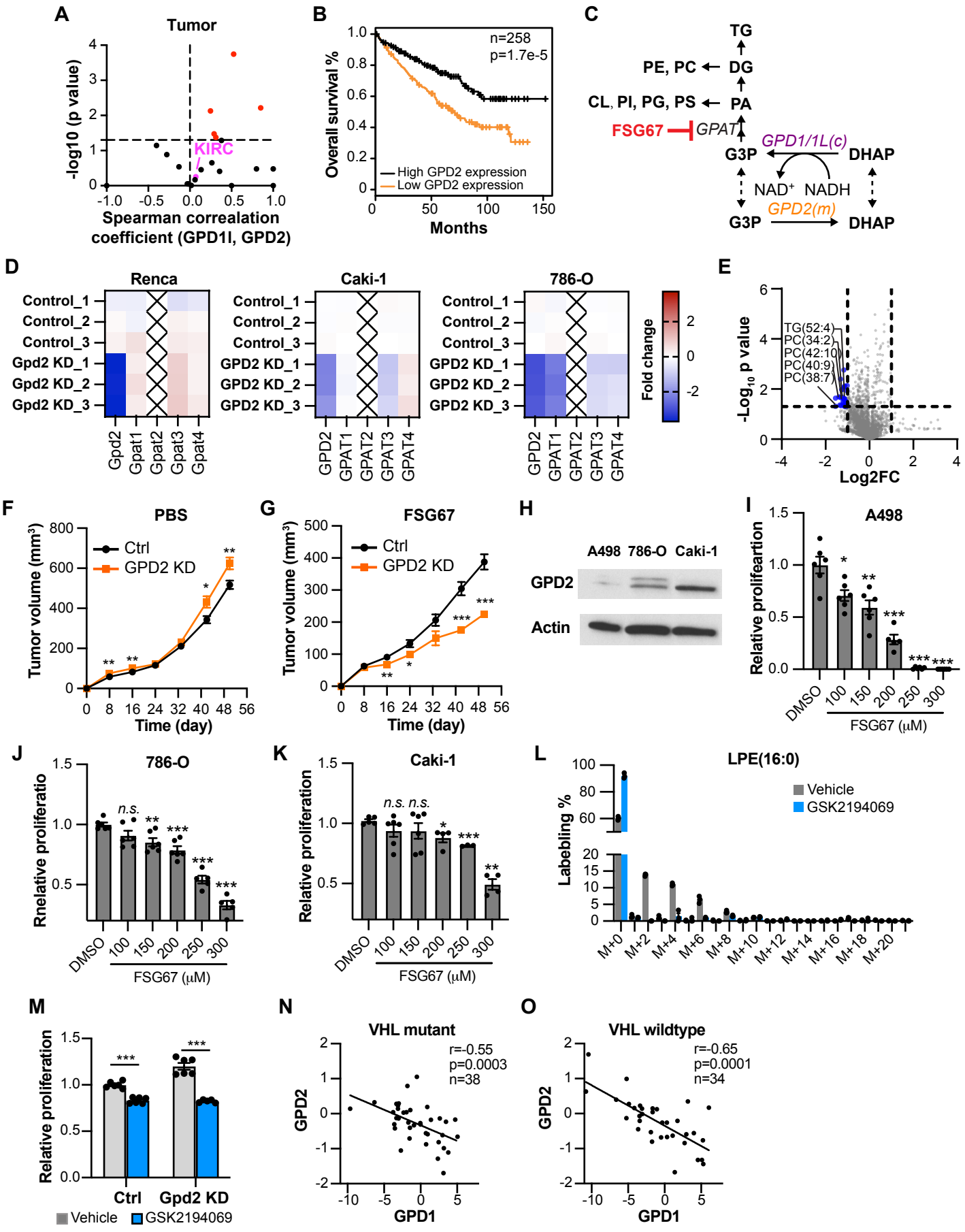

A
Tumor
-log10 (p value)
KIRC
Spearman correalation coefficient (GPD1l, GPD2)
B
n=258
p=1.7e-5
Overall survival %
High GPD2 expression
Low GPD2 expression
Months
C
TG
PE, PC
DG
CL, PI, PG, PS
PA
FSG67
GPAT
GPD1/1L(c)
G3P
DHAP
NAD+ NADH
GPD2(m)
D
Renca
Caki-1
786-O
Control_1
Control_2
Control_3
Gpd2 KD_1
Gpd2 KD_2
Gpd2 KD_3
GPD2 KD_1
GPD2 KD_2
GPD2 KD_3
Gpd2
Gpat1
Gpat2
Gpat3
Gpat4
GPD2
GPAT1
GPAT2
GPAT3
GPAT4
Fold change
E
-Log10 p value
TG(52:4)
PC(34:2)
PC(42:10)
PC(40:9)
PC(38:7)
Log2FC
F
PBS
Ctrl
GPD2 KD
Tumor volume (mm3)
Time (day)
G
FSG67
H
A498
786-O
Caki-1
GPD2
Actin
I
A498
Relative proliferation
DMSO
FSG67 (μM)
J
786-O
Rnelative proliferatio
n.s.
K
Caki-1
Relative proliferation
L
LPE(16:0)
Vehicle
GSK2194069
Labelling %
M+0
M+2
M+4
M+6
M+8
M+10
M+12
M+14
M+16
M+18
M+20
M
Relative proliferation
Ctrl
Gpd2 KD
N
VHL mutant
r=-0.55
p=0.0003
n=38
GPD2
GPD1
O
VHL wildtype
r=-0.65
p=0.0001
n=34

Figure S4 (Related to Figure 4). Gpd2 inhibition promotes lipid synthesis and correlates with worse overall survival in patients

(A) Spearman correlation analysis of GPD1l and GPD2 expression in normal tissue. Red dot represents positive coefficients with p value < 0.05.

(B) Overall survival of KIRC patients with high and low expression of GPD2.

(C) Diagram showing effect of FSG67 inhibition on Gpat which connects glycerol-3-phosphate shuttle and lipid synthesis.

(D) log2 fold change in the expression levels of GPATs in control and GPD2 KD cells.

(E) Volcano plot showing the log2 fold change and -log10 p value of the lipidome in plasma from animlas treated with FSG67 compared to PBS control. Blue dots represent metabolic features that had fold change <0.5 and p value < 0.05.

(F) Tumor growth curve of ctrl and GPD2 KD 786-O xenograft in SCID mice treated with PBS.

(G) Tumor growth curve of ctrl and GPD2 KD 786-O xenograft in SCID mice treated with FSG67.

(H) Western blot analysis of GPD2 in kidney cancer cell lines.

(I) Relative proliferation of A498 cells upon increasing dose of FSG67 treatments.

(J) Relative proliferation of 786-O cells upon increasing dose of FSG67 treatments.

(K) Relative proliferation of Caki-1 cells upon increasing dose of FSG67 treatments.

(L) Isotopologue distribution pattern of lysophosphatidylethanolamine (LPE(16:0)) in cells labeled with U-^{13}C acetate for 24hr in the presence of vehicle control or 75nM GSK2194069.

(M) Relative proliferation of ctrl and Gpd2 KD Renca cells treated wth vehicle or GSK2194069.

(N) Spearman correlation analysis showing negative correlation between the expression of GPD1 and GPD2 in KIRC patients with mutant VHL.

(O) Spearman correlation analysis showing negative correlation between the expression of GPD1 and GPD2 in KIRC patients with wildtype VHL.

Data are presented as mean ±SEM. *p<0.05, **p<0.01, ***p<0.001, n.s. not statistically significant.

Table S1 (Related to Figure 1). Labeling percentages of F6P and FBP when cells were labeled with U-^{13}C glutamine, U-^{13}C lactate or U-^{13}C glycerol until isotopic steady state.

Labeling %	**U-^{13}C Glutamine**		**U-^{13}C Lactate**		**U-^{13}C Glycerol**	
	F6P	**FBP**	**F6P**	**FBP**	**F6P**	**FBP**
Renca	0±0	0.47±0.06	0±0	0.6±0.1	0±0	0±0
786O	0±0	0.10±0.05	0±0	0.6±0.1	0±0	3±2
Caki-1	0±0	0.1±0.1	0.7±0.3	4.4±0.3	0±0	2±2
HEK293T	0±0	0±0	0±0	0±0	0±0	1±1

Table S3 (Related to Figure 3). Lipidomic quantitation of mouse heart and kidney extract

Lipid Specie	% in Heart Extract	% in Kidney Extract
CL(64:0) IS	-	-
CL(72:7)	1.05E-01	4.39E-01
CL(72:8)	1.64E-01	4.08E-01
PA(24:0) IS	-	-
PA(32:0)	0.00E+00	4.13E-03
PA(34:1)	5.81E-03	9.87E-03
PA(34:2)	2.13E-03	1.19E-02
PA(36:1)	0.00E+00	4.28E-03
PA(36:2)	0.00E+00	1.39E-02
PA(36:4)	0.00E+00	6.13E-03
PA(38:4)	0.00E+00	1.32E-02
PA(38:4)	0.00E+00	1.58E-02
PE(31:1) IS	-	-
PG(30:0) IS	-	-
PG(32:0)	1.30E-03	6.55E-02
PG(34:0)	6.23E-03	1.38E-02
PG(34:1)	3.19E-02	3.54E-01
PG(34:2)	8.58E-03	6.73E-02
PG(36:0)	5.66E-04	1.68E-03
PG(36:1)	7.37E-03	2.19E-02
PG(36:2)	8.70E-03	1.13E-01
PG(36:3)	4.50E-03	3.85E-02
PG(36:4)	6.35E-03	1.34E-02
PG(38:4)	5.36E-03	9.73E-03
PG(38:5)	2.45E-03	1.76E-02
PG(38:6)	1.51E-04	2.41E-02
PG(40:8)	0.00E+00	4.61E-02
PI(32:0)	1.77E-04	9.45E-03
PI(34:1)	9.86E-03	4.03E-02
PI(34:2)	6.02E-03	1.85E-02
PI(36:2)	1.19E-01	5.91E-02
PI(36:3)	1.67E-02	1.99E-02
PI(36:4)	3.99E-03	4.19E-02
PI(38:4)	1.54E-01	3.60E-01
PI(38:5)	1.97E-02	3.38E-02
PI(38:6)	1.41E-03	6.04E-02
PS(28:0) IS	-	-
PS(32:0)	5.85E-04	2.60E-03
PS(34:0)	1.96E-03	3.37E-03
PS(34:1)	3.17E-03	7.40E-03
PS(34:2)	4.85E-04	6.28E-03
PS(36:1)	4.62E-02	2.88E-02
PS(36:2)	5.11E-02	3.81E-02
PS(36:3)	2.83E-03	5.27E-03
PS(36:4)	6.40E-05	3.57E-02
PS(38:4)	1.39E-02	2.06E-01
PS(38:5)	1.14E-02	3.48E-02
PS(38:6)	0.00E+00	2.25E-02
PS(40:8)	0.00E+00	3.38E-03
Cer(d34:1)	1.99E-03	8.56E-02
Cer(d35:1) IS	-	-
Cer(d36:1)	2.68E-03	4.96E-03
Cer(d36:2)	0.00E+00	2.56E-03
DG(24:0) NH4 IS	-	-
DG(32:0) NH4	2.58E-02	1.80E-01
DG(34:0) NH4	6.95E-02	1.21E-01
DG(34:1) NH4	1.07E-01	5.73E-01
DG(36:0) NH4	9.25E-03	0.00E+00
DG(36:3) NH4	2.25E-01	1.70E+00
DG(36:4) NH4	2.20E-02	1.03E+00
DG(38:4) NH4	3.03E-02	6.91E-01
DG(38:5) NH4	6.62E-03	2.63E-01
DG(38:6) NH4	0.00E+00	9.67E-01
DG(40:8) NH4	0.00E+00	2.27E-01
PC(31:1) IS	-	-
PC(32:0)	1.77E-02	1.54E+00
PC(34:0)	7.59E-03	1.84E-01
PC(34:1)	7.53E-02	1.04E+00
PC(34:2)	3.58E-01	2.07E+00
PC(36:0)	4.90E-04	1.08E-02
PC(36:1)	2.04E-02	3.17E-01
PC(36:2)	5.25E-02	9.03E-01
PC(36:3)	2.77E-02	6.28E-01
PC(36:4)	2.58E-02	9.47E-01
PC(38:4)	7.26E-03	6.68E-01
PC(38:5)	7.59E-03	4.28E-01
PC(38:6)	1.36E-02	2.83E+00
PC(40:8)	1.41E-03	3.36E-01
PE(31:1) IS	-	-
PE(32:0)	1.51E-04	3.84E-03
PE(34:0)	4.31E-04	2.01E-03
PE(34:1)	1.34E-03	1.62E-02
PE(34:2)	4.46E-03	5.90E-02
PE(36:0)	3.95E-03	2.80E-02
PE(36:1)	4.22E-03	1.67E-02
PE(36:2)	5.08E-02	8.99E-02
PE(36:3)	7.22E-03	6.68E-02
PE(36:4)	2.91E-03	2.95E-01
PE(38:4)	1.46E-01	7.71E-01
PE(38:5)	1.29E-02	1.92E-01
PE(38:6)	4.85E-03	2.89E-01

PE(40:8)	4.84E-03	7.54E-02
SM(d34:1)	2.56E-02	2.28E+00
SM(d35:1) IS	-	-
SM(d36:1)	5.61E-03	1.60E-02
TG(48:0) NH4	8.43E-01	2.72E-01
TG(50:1) NH4	9.65E+00	2.23E+00
TG(50:2) NH4	8.00E+00	4.79E+00
TG(52:0) NH4	3.86E-01	5.66E-02
TG(52:1) NH4	1.23E+01	1.11E+00
TG(52:2) NH4	2.49E+01	6.44E+00
TG(52:3) NH4	9.33E+00	1.21E+01
TG(52:4) NH4	1.46E+00	1.03E+01
TG(54:0) NH4	1.09E-01	1.38E-02
TG(54:1) NH4	6.00E+00	1.81E-01
TG(54:2) NH4	1.56E+01	1.91E+00
TG(54:3) NH4	7.07E+00	6.56E+00
TG(54:4) NH4	1.43E+00	1.01E+01
TG(54:5) NH4	2.87E-01	8.96E+00
TG(54:6) NH4	6.64E-02	4.82E+00
TG(56:4) NH4	1.84E-01	1.51E+00
TG(56:5) NH4	1.25E-01	1.05E+00
TG(56:7) NH4	5.88E-02	7.53E-01
TG(56:7) NH4	1.56E-02	4.79E-01
TG(56:8) NH4	3.60E-03	7.36E-01
TG(57:0) NH4 IS	-	-
TG(58:10) NH4	0.00E+00	1.67E-01
TG(58:8) NH4	0.00E+00	3.78E-01
TG(58:9) NH4	1.71E-03	3.06E-01
TG(60:12) NH4	0.00E+00	8.49E-02

Table S4 (Related to Figure 1). Isotopologue distribution pattern of GAP and metabolites involved in metabolic flux analysis

GAP labeling %	M+0	M+1	M+2	M+3
786O_1	53.07	0.25	1.28	45.4
786O_2	51.95	0.33	1.66	46.05
786O_3	51.54	0.2	1.93	46.33
Gpd11LDKD_1	46.41	0.47	2.71	50.41
Gpd11LDKD_2	45.92	0.49	2.39	51.2
Gpd11LDKD_3	44.13	0.42	2.79	52.66
HEK293_1	49.67	0.51	1.09	48.74
HEK293_2	50.73	0.51	1.04	47.73
HEK293_3	51.67	0.47	1.37	46.49
Caki-1_1	68.11	0.52	0	31.37
Caki-1_2	67.34	0.33	2.59	29.73
Caki-1_3	66.42	0.21	2.23	31.14
Renca_1	62.17	0.49	2.79	34.55
Renca_2	56.77	1.24	2.88	39.12
Renca_3	54.61	1.31	2.67	41.41
Gpd2KD_1	53.17	0.98	6.78	39.07
Gpd2KD_2	50.03	0.85	6.83	42.29
Gpd2KD_3	51.76	0.82	8.27	39.15

G3P labeling %	M+0	M+1	M+2	M+3
786O_1	41.68	0.00	0.00	58.32
786O_2	40.87	0.00	0.00	59.13
786O_3	40.35	0.00	0.00	59.65
Gpd11LDKD_1	39.55	0.00	0.00	60.45
Gpd11LDKD_2	40.22	0.00	0.00	59.78
Gpd11LDKD_3	38.62	0.00	0.00	61.38
HEK293_1	51.95	0.85	0.00	47.21
HEK293_2	54.10	0.62	0.00	45.28
HEK293_3	53.03	0.74	0.00	46.25
Caki-1_1	48.13	0.00	0.00	51.87
Caki-1_2	48.79	1.62	0.00	49.59
Caki-1_3	48.08	1.76	0.00	50.16
Renca_1	32.92	1.58	1.44	64.06
Renca_2	41.82	1.82	1.53	54.84
Renca_3	42.20	2.77	1.14	53.89
Gpd2KD_1	22.64	0.88	0.64	75.83
Gpd2KD_2	23.08	0.88	0.69	75.34
Gpd2KD_3	23.85	0.96	0.82	74.37

DHAP labeling %	M+0	M+1	M+2	M+3
786O_1	29.81	0.32	0.00	69.87
786O_2	29.44	0.39	0.00	70.17
786O_3	28.29	0.26	0.00	71.45
Gpd11LDKD_1	22.53	0.79	0.00	76.68
Gpd11LDKD_2	19.86	0.69	9.28	70.17
Gpd11LDKD_3	18.94	0.67	10.09	70.30
HEK293_1	43.09	1.04	0.00	55.87
HEK293_2	52.60	2.72	0.00	44.68
HEK293_3	44.53	2.67	0.00	52.80
Caki-1_1	37.65	1.09	0.00	61.26
Caki-1_2	37.51	1.24	0.00	61.26
Caki-1_3	38.01	1.02	0.00	60.97
Renca_1	26.90	1.35	1.62	70.13
Renca_2	29.98	1.19	2.44	66.39
Renca_3	27.48	1.12	3.16	68.24
Gpd2KD_1	22.55	0.90	1.25	75.30
Gpd2KD_2	20.99	0.91	1.90	76.20
Gpd2KD_3	17.87	1.13	2.24	78.76

F6P labeling %	M+0	M+1	M+2	M+3	M+4	M+5	M+6
786O_1	26.74	0	0	8.1	0	0	65.16
786O_2	26.84	0	0	9.79	0	0	63.37
786O_3	24.25	0	0	8.4	0	0	67.35
Gpd11LDKD_1	0	2.32	0	4.61	1.69	2.39	88.99
Gpd11LDKD_2	0	0	0.93	4.41	1.16	0	93.5
Gpd11LDKD_3	0	1.95	0	4.71	0.53	0.56	92.25
HEK293_1	5.85	1.91	3.27	7.52	0	1.95	79.51
HEK293_2	0	1.72	1.4	3.13	0	4.89	88.87
HEK293_3	2.925	1.815	2.335	5.325	0	3.42	84.19
Caki-1_1	35.38	0	0	6.48	0	1.58	56.56
Caki-1_2	34	0	0.46	6.87	0	1.09	57.58
Caki-1_3	32.12	0.35	0	8.2	0	2	57.33
Renca_1	26.47	0.59	0.66	5.21	0.42	0.46	66.19
Renca_2	28.74	0.39	0.21	2.14	0.26	0.32	67.94
Renca_3	26.1	0.19	0	1.72	0.19	0.69	71.11
Gpd2KD_1	24.64	0	0	5.04	0	0	70.32
Gpd2KD_2	22.02	0	0.17	4.46	0.91	0.53	71.91
Gpd2KD_3	18.97	0	0.65	4.58	0.76	0.59	74.46