Supplementary Figures and Figure Legends

**Supplementary Figure 1.** PDK3 promotes glucose metabolism and chemoresistance in GC Cells.

A-E: Alternations in glucose consumption (A) production of pyruvic acid (B), lactic acid (C), mitochondrial membrane potential (D) and ROS generation (E) were determined in SGC7901 cells with or without stable PDK3 overexpression.

F-J: Alternations in glucose consumption (F) production of pyruvic acid (G), lactic acid (H), mitochondrial membrane potential (I) and ROS generation (J) were determined in SGC-R and BGC-R cells with or without PDK3 depletion.

K: DDP-induced apoptosis in SGC7901 cells with or without stable PDK3 overexpression were evaluated by Annexin V and PI double staining.

L: DDP-induced apoptosis in SGC-R and BGC-R cells with or without PDK3 depletion were evaluated by Annexin V and PI double staining.
Supplementary Figure 2. Chemical inhibition of PDK3 impairs glycolysis to reverse chemoresistance

A: Cell viability was evaluated by MTS assay after exposure to indicated concentrations of DDP for 24 h in MFC cells.

B: Cell viability was evaluated by MTS assay after exposure to indicated concentrations of DCA for 72 h in MFC cells.

C: DDP-induced apoptosis in SGC-R, BGC-R and MFC cells treated as indicated were evaluated by Annexin V and PI double staining.

D: Cell viability was evaluated by MTS assay after exposure to indicated concentrations of Radicicol for 48h in MFC cells.

E, Viability of various cells treated with various doses of DDP and Radicicol were evaluated by MTS assay.

F-H, Apoptosis of SGC-R, BGC-R and MFC cells after treatment with Radicicol and/or DDP were analyzed by immunoblot (F) and flowcytometry analysis (G and H).
Supplementary Figure 3. HSF1 upregulates PDK3 expression at transcription level.

A: Immunoblot analysis of endogenous PDK3 expression in SGC-R cells before and after HIF1α depletion.
B: ChIP assay assessing HSF1 binding at the putative HSE site in the PDK3 promoter in SGC7901 and SGC-R cells. The results were normalized with 1% of input.
C: RT-PCR analysis of HSP70 mRNA levels in gastric cancer cells transfected with (WT) or MT (mutated) HSF1.
D: RT-PCR analysis of a HSF1 mRNA levels in gastric cancer cells transfected with WT or MT HSF1.
E: Immunoblot analysis of ectopic PDK3 expression in SGC7901 cells before and after HSF1 depletion.
Supplementary Figure 4. PDK3 alternations changes the expression of HSF1 downstream targets

A: RT-PCR analysis of HSF1 downstream targets after transfected with PDK3 plasmid in GC chemosensitive cells
B: RT-PCR analysis of alternation of HSF1 downstream targets induced by PDK3 knocking down in GC chemoresistant cells.
Supplementary Figure 5. PDK3 upregulates HSF1 protein expression.

A: RT-PCR analysis of HSF1 expression in SGC7901 cells before and after PDK3 overexpression.
B: RT-PCR analysis of HSF1 expression in SGC-R cells before and after PDK3 depletion.
C: Immunoblot analysis of HSF1 protein levels in SGC7901 and SGC-R cells treated with cycloheximide (50 μg/mL).
D: Immunoblot analysis of HSF1 protein levels in cycloheximide-treated SGC7901 cells before and after PDK3 overexpression.
Supplementary Figure 6. FBXW7 inhibits the expression of HSF1.

A: Immunoblot analysis of HSF1 protein levels in gastric cancer cells treated with proteasome inhibitor MG132 (50 μg/mL) for 6 h, lysosomotropic agent chloroquine (CQ, 20 μM) for 24 h or calpain inhibitors PD150606 (400 μM) for 24 h, respectively.

B: Immunoblot analysis of HSF1 protein levels in gastric cancer cells treated with either MG132 (50 μg/mL) for 6 h or MLN4924 (100 nM) for 24 h.

C: Immunoblot analysis of HSF1 protein levels in gastric cancer cells before and after FBXW7 depletion.

D: Immunoblot analysis of HSF1 protein levels in SGC7901 cells before and after β-TrCP1 depletion.

E: Immunoblot analysis of the effect of FBXW7 on HSF1 protein levels.

F: The interaction of HSF1 with FBXW7 was explored with Co-immunoprecipitation (Co-IP).
Supplementary Figure 7. PDK3 interacts with HSF1 to disrupt its phosphorylation by GSK3β.

A: Cytoplasmic and nuclear fractionated from HEK293 cells expressing Myc-PDK3 and Flag-HSF1 were immunoprecipitated by anti-Myc antibody.

B: Effect of GSK-3β knock-down on DDP-induced cleavage of PARP1 and caspase3 were determined in HSF1 high expressing SGC7901 cells by immunoblot analysis.
Supplementary Figure 8. HSF1 regulates the glucose metabolism and chemoresistance.

A-C: Alternations in glucose consumption (A) production of pyruvic acid (B), lactic acid (C) were determined in SGC7901 cells with or without stable HSF1 overexpression.

D-F: Alternations in glucose consumption (D) production of pyruvic acid (E), lactic acid (F) were determined in SGC-R and BGC-R cells with or without HSF1 depletion.

G: DDP-induced apoptosis in SGC7901 cells with or without stable HSF1 overexpression were evaluated by Annexin V and PI double staining.

H: DDP-induced apoptosis in SGC-R and BGC-R cells with or without HSF1 depletion were evaluated by Annexin V and PI double staining.
Supplementary Figure 9. Chemical inhibition of HSF1 reverses chemoresistance.

A: Cell Viability was evaluated by MTS assay after exposure to indicated concentrations of KNK437 for 72 h.

B: DDP-induced apoptosis in gastric cancer cells treated with or without KNK437 were evaluated by Annexin V and PI staining.

C: Cell Viability was evaluated by MTS assay after exposure to indicated concentrations of KRIIBB11 for 48h in MFC cells.

D: Viability of various cells treated with various doses of DDP and KRIIBB11 were evaluated by MTS assay.

E-G, Apoptosis of SGC-R, BGC-R and MFC cells after treatment with KRIIBB11 and/or DDP were analyzed by immunoblot (E) and flow cytometry analysis (F and G).
### Supplementary Table 1: siRNAs used in this study

| siRNA       | Sequence                  |
|-------------|---------------------------|
| HSF1-1#     | S: CCUGAAGAGUGAAGACAUATT  |
|             | AS: UAUGUCUUCUCUCUUCAGGTT |
| HSF1-2#     | S: GGACAAGAAUGAGCUCAGUTT  |
|             | AS: ACUGAGCUCAUUCUUGUCCCTT|
| PDK3-1#     | S: GCCGCUCUCCAUAAACAATT   |
|             | AS: UUGUUUGAUGGAGAGCGGCTT|
| PDK3-2#     | S: GGUUCCUACAAUGGCACAATTT|
|             | AS: UUGUGCACUAUGGAACCTT   |
| FBXW7-1#    | S: GCACACUGCAAGAUGGUTT    |
|             | AS: ACCAUCCUUGCAGUGUGCTT  |
| FBXW7-2#    | S: GGAGUAUGGUCAUCAAAATT   |
|             | AS: UUUGUGAUGACCAUACUCTT  |
| Beta-TRCP1-1# | S: GUGGAAUUUGUGGAACAUCTT |
|             | AS: GAUGUUCACAAUUCCACTT   |
| Beta-TRCP12# | S: CACAUUACUCGUACUUATT    |
|             | AS: UAAGAUACGAGUUAUGUGTT  |
| HIF-1α 1#   | S: GCCGAGGAAGACAUUGAATT   |
|             | AS: UUCAUAGUUCUCCUCGGCTT  |
| HIF-1α 2#   | S: GCUGAUUUGUGAACCAUUATT  |
|             | AS: AAUGGCUUCAAAUCAAGCTT  |
Supplementary Table 2 Primers used in this study

| Primers | Sequence |
|---------|----------|
| HSF1    | F: AC... |
|         | R: C...  |
| PDK1    | F: CT... |
|         | R: CC... |
| PDK2    | F: G...  |
|         | R: CAT... |
| PDK3    | F: CTG... |
|         | R: GTT... |
| PDK4    | F: CTT... |
|         | R: CAA... |
| FBXW7   | F: GGT... |
|         | R: TGA... |
| HSP70   | F: ACC... |
|         | R: CGC... |
| HSP27   | F: GGC... |
|         | R: AGC... |
| HSP90   | F: TGC... |
|         | R: GAA... |
| HSPA6   | F: ACA... |
|         | R: TTC... |
| DNAJB4  | F: GGA... |
|         | R: ATG... |
| β-actin | F: CAC... |
|         | R: ACA... |
| HSFK80Q | F: CTG... |
| MT      | R: ATG... |
| Target         | F               | R               |
|---------------|-----------------|-----------------|
| PDK3 (CHIP)   | GCCGAGGTCTTTTACTGTGTATG | CAGCTTACTTTAGCCATTTCCAC |
| PDK3 (Luciferase) + 28 | GCAGGTACCGCTACCCACCAATAATCTCACATTCCAG | GCAAAGCTTTGAGTCGTTGCTGCACGTAC |
| GST-HSF11-529 | GGAGAATTCTCGATCTGCCCGTGCCGCCCAGG | GCGCTCGAGGAGAGACGTGAGGTGTCTTTG |
| GST-HSF11-385 | GGAGAATTCTCGATCTGCCCGTGCCGCCCAGG | GCGCTCGAGACTGAGCTCATTCTTTCA |
| GST-HSF11-123 | GGAGAATTCTCGATCTGCCCGTGCCGCCCAGG | GCGCTCGAGCTTTATGTCTTCA |
| GST-HSF1123-387 | GGAGAATTCTCCACCCTGAAGAGTGAAGAC | GCACTCGAGGTGTCTGAGGTACTCATTCTTTG |
| GST-HSF1123-315 | GGAGAATTCTCCACCCTGAAGAGTGAAGAC | GCACTCGAGGACTCCTCCTCTACC |
| GST-HSF1129-226 | GGAGAATTCTCACCCTGAAGAGTGAAGAC | GCACTCGAGGCTATACCTTGGGCATGGAATG |
| GST-HSF1220-315 | GGAGAATTCCATTCCATGCCCCAAAGTGATAAGC | GCACTCGAGGGAGACTCGCCTCCTTACC |
### Supplementary Table 3: Antibodies used in this study

| Antibody                  | Identifier, Source  | Dilution for WB |
|---------------------------|---------------------|-----------------|
| anti-GSK3                 | 9832S, CST          | 1:1000          |
| anti-ERK1/2               | 4695S, CST          | 1:1000          |
| anti-Cleaved-PARP         | 9541S, CST          | 1:1000          |
| anti-Cleaved-Caspase3     | 9661S, CST          | 1:1000          |
| anti-β-actin              | 4970L, CST          | 1:1000          |
| anti-beta-TRCP1           | 4394S, CST          | 1:1000          |
| anti-Myc                  | 2776s, CST          | 1:1000          |
| anti-HSF1                 | ab2757, Abcam       | 1:1000          |
| anti-HSF1 (p-S303/S307)   | ab81281, Abcam      | 1:5000          |
| anti-Histone3             | Ab1791, Abcam       | 1:2000          |
| anti-PDK3                 | sc-365378, SCBT     | 1:500           |
| anti-UB                   | sc-8017, SCBT       | 1:200           |
| HIF-1α                    | sc-53546, SCBT      | 1:500           |
| anti-flag (M2)            | F1804-1, Sigma-Aldrich | 1:2000        |
| anti-α-tubulin            | F5168, Sigma-Aldrich | 1:5000         |
**Supplementary Table 4  The VIP and FC values metabolites involved in glycolysis.**

| Name                        | SGCR-SGC7901 FC | VIP | MYCPDK3-SGC7901 FC | VIP | Flag-HSF1-SGC7901 FC | VIP |
|-----------------------------|-----------------|-----|-------------------|-----|---------------------|-----|
| 1,5-Anhydroinositol         | 1.1             | 0.4 | 1                 | 0.7 | 0.7                 | 1.2 |
| 2-Hydroxy-3-methylbutyric   | 0.6             | 1.7 | 0.8               | 1.5 | 0.8                 | 1.8 |
| Acid                        |                 |     |                   |     |                     |     |
| 3-Methyl-2-oxovaleric acid  | 1.8             | 1.7 | 2.5               | 1.7 | 1.4                 | 1.8 |
| 4-Hydroxybenzoic acid       | 0.8             | 1.3 | 0.9               | 0.7 | 0.9                 | 1.1 |
| Allose                      | 1.1             | 0.4 | 2                 | 1.3 | 1.4                 | 0.7 |
| Alpha-Lactose               | 1.1             | 0.5 | 0.9               | 0.1 | 1.1                 | 0.2 |
| Benzoic acid                | 1.3             | 1.6 | 1.2               | 0.8 | 0.9                 | 0.6 |
| Citric acid                 | 1               | 0.1 | 1                 | 0.6 | 0.8                 | 0.5 |
| D-Fructose                  | 1               | 0.8 | 1                 | 0.1 | 1.2                 | 0.1 |
| D-Galactose                 | 1.1             | 0.2 | 0.4               | 1.7 | 1.1                 | 1.2 |
| D-Glucose                   | 0.8             | 0.5 | 0.2               | 1.5 | 0.6                 | 1.3 |
| D-Mannose                   | 1.5             | 0.7 | 1.8               | 1.4 | 1.1                 | 0.3 |
| D-Ribose                    | 2.5             | 1.2 | 2.6               | 1.2 | 1.6                 | 1.6 |
| D-Tagatose                  | 1               | 0   | 1.1               | 0.3 | 0.9                 | 1.1 |
| D-Threitol                  | 0.8             | 1   | 1                 | 0.5 | 0.7                 | 1.1 |
| Fructose 6-phosphate        | 0.6             | 1.2 | 0.6               | 1.3 | 0.8                 | 0.7 |
| Fumaric acid                | 0.5             | 1.5 | 0.8               | 0.6 | 0.6                 | 1.1 |
| Galactonic acid             | 4.9             | 1.7 | 4.7               | 1.7 | 1.6                 | 0.8 |
| Gluconic acid               | 0.7             | 0.2 | 1                 | 0.1 | 0.9                 | 0.4 |
| Gluconolactone              | 0.1             | 1.2 | 0.1               | 1.2 | 0.1                 | 1.2 |
| Glyceric acid               | 1.2             | 0.3 | 1.3               | 0.1 | 1.1                 | 0.7 |
| Glycolic acid               | 1               | 0.2 | 1                 | 0.1 | 0.9                 | 0.8 |
| L-3-Cyanoalanine            | 1               | 0.1 | 1.1               | 0   | 1.1                 | 0.2 |
| L-Arabinose                 | 0.6             | 1   | 0.4               | 1.7 | 0.8                 | 1.9 |
| L-Arabinol                  | 0.9             | 0.3 | 0.6               | 1.6 | 0.8                 | 1.5 |
| L-Lactic acid               | 1.6             | 1.1 | 1.8               | 0.9 | 1.3                 | 0.7 |
| L-Pipecolic acid            | 1.1             | 0.5 | 1.6               | 0.1 | 1.5                 | 0.5 |
| L-Sorbose                   | 0.4             | 1.7 | 0.5               | 1.7 | 0.6                 | 1.8 |
| Maleic acid                 | 0.4             | 0.9 | 1.3               | 1   | 1.5                 | 1   |
| Malic acid                  | 0.6             | 1.5 | 1.1               | 0.8 | 0.6                 | 1.3 |
| Mannitol                    | 0.6             | 1.6 | 0.5               | 1.6 | 0.7                 | 1.5 |
| Oxalic acid                 | 0.4             | 1   | 0.5               | 1.3 | 0.7                 | 0.9 |
| Oxoglutaric acid            | 1.9             | 1.1 | 0.8               | 0.4 | 1.6                 | 1   |
| p-Aminobenzoic acid         | 1.1             | 0.5 | 0.9               | 0.4 | 1.1                 | 0.5 |
| Petroselinic acid           | 0.8             | 0.5 | 0.9               | 0.1 | 1.1                 | 0.3 |
| Pyruvic acid                | 9.1             | 1.7 | 4.9               | 1.5 | 3.5                 | 1.9 |
| Ribonolactone               | 2.4             | 1.2 | 2.4               | 1.2 | 1.6                 | 1.8 |
| Sorbitol                    | 0.2             | 1.6 | 0.5               | 1.6 | 0.7                 | 1.5 |
| Succinic acid               | 0.4             | 0.4 | 1.1               | 0.8 | 0.7                 | 1   |
|              | 0.9 | 1.4 | 0.6 | 1.7 | 0.8 | 1.7 |
|--------------|-----|-----|-----|-----|-----|-----|
| Sucrose      |     |     |     |     |     |     |
| Threonic acid| 1.1 | 0.4 | 1.2 | 1.2 | 1.1 | 0.5 |
| Uric acid    | 1.1 | 1.1 | 1.1 | 0.6 | 1   | 0.6 |