ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Sulforaphane selectively inhibits glucose metabolism in *PIK3CA*-mutated ovarian cancer cells

Supplementary Files

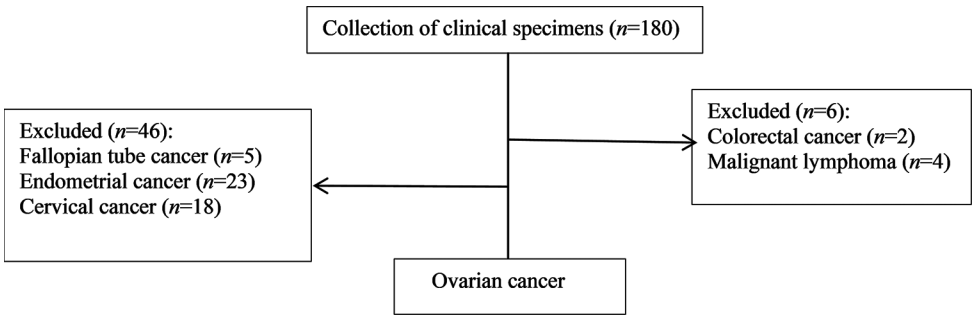

Figure S1. Flowchart of patient selection and exclusion process for ovarian cancer

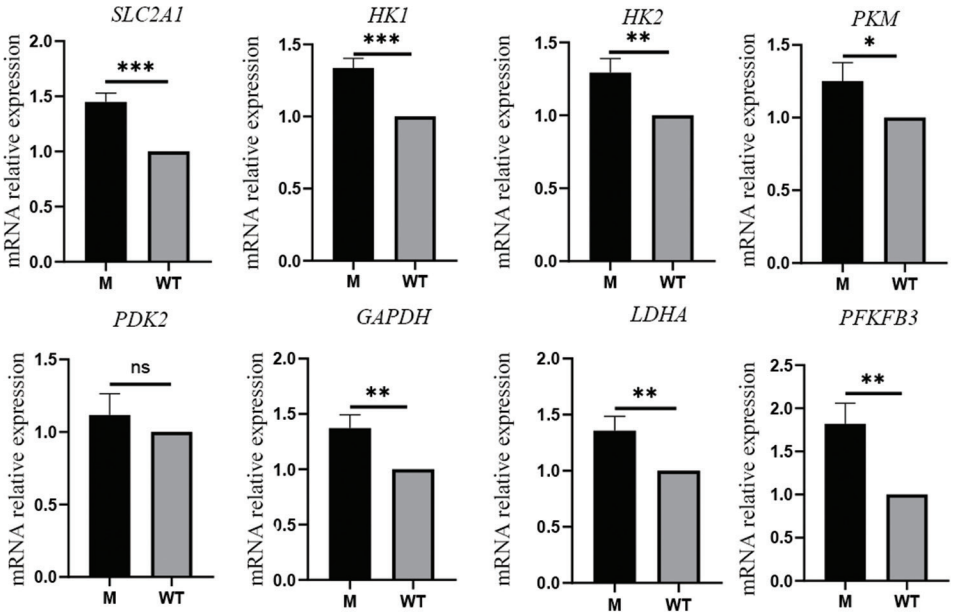

Figure S2. Expres=sion of glucose transporter and glycolysis-related enzymes detected by RT-qPCR. Statistical significance determined at *p<0.05; **p<0.01; ***p<0.001; and ****p<0.0001.
Abbreviations: M: Mutant; WT: Wild-type.

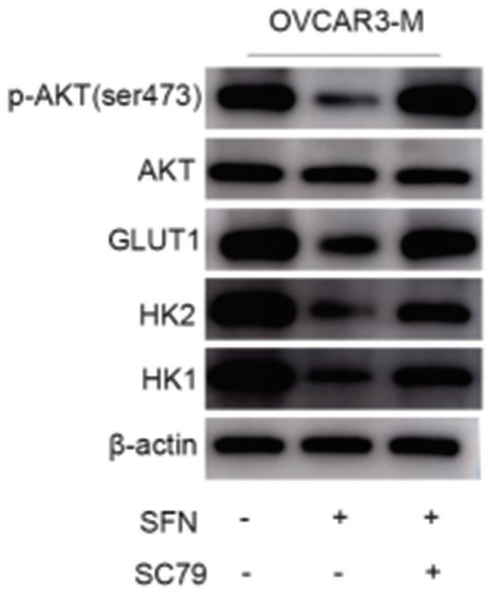

Figure S3. After SC79 treatment, the expression levels of glycolytic enzymes HK1, HK2de, and GLUT1, which are inhibited by SFN. "+" indicates with; "–" indicates without.
Abbreviations: AKT: Protein kinase B; GLUT1: Glucose transporter 1; HK: Hexokinase; SFN: Sulforaphane.

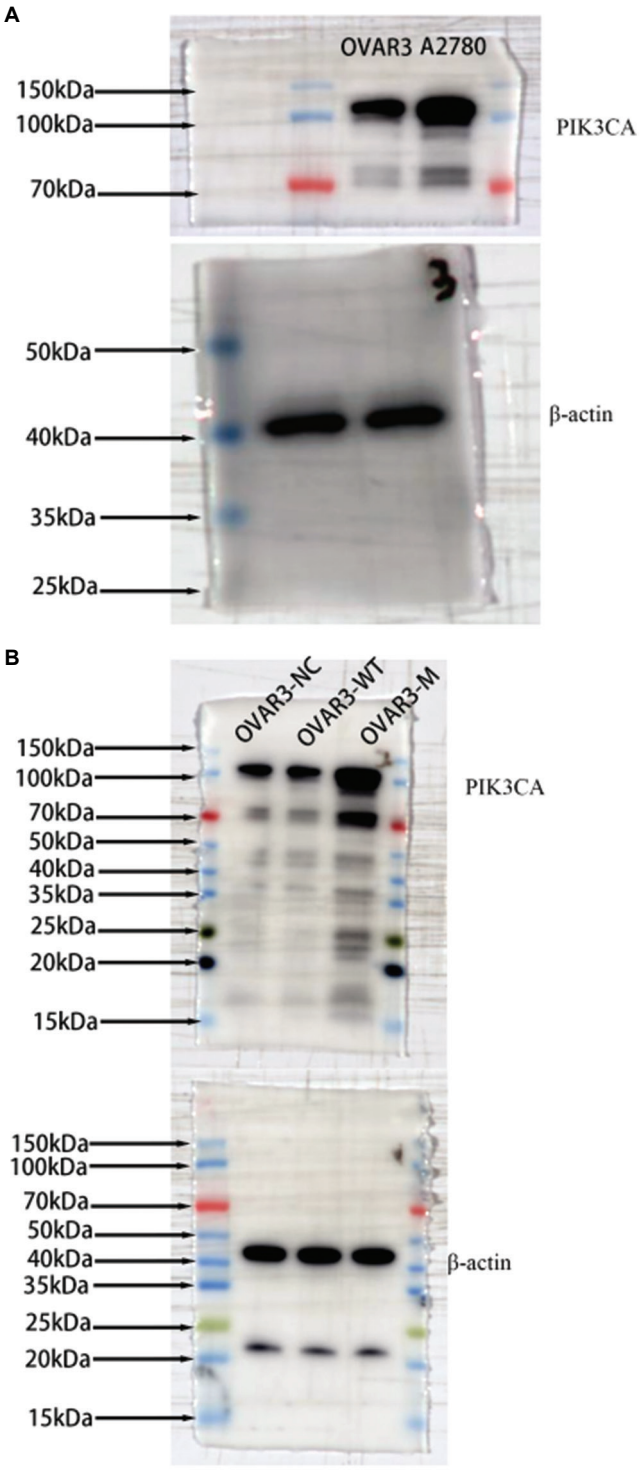

Figure S4. (A and B) Original images for Western blotting for A and C in Figure 2 (from the main manuscript)
Abbreviations: M: Mutant; PIK3CA: Phosphatidylinositol 3-kinase; WT: Wild-type.

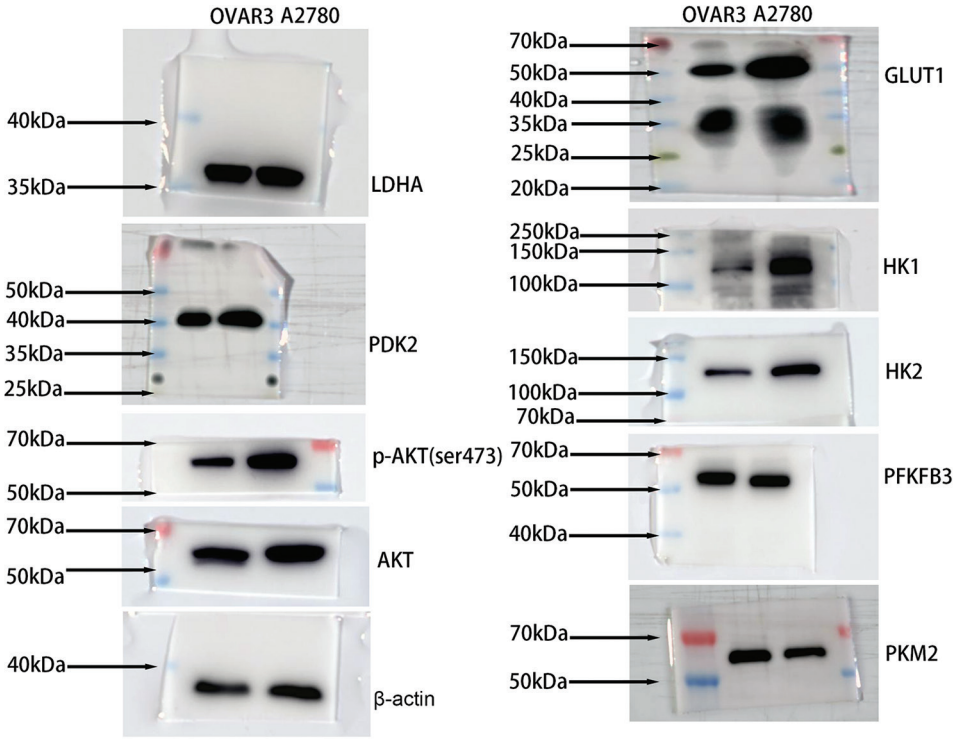

Figure S5. Original images for Western blotting for J in Figure 2 (from the main manuscript)
Abbreviations: AKT: Protein kinase B; GLUT1: Glucose transporter 1; HK: Hexokinase; LDHA: Lactate dehydrogenase A; PDK2: Pyruvate dehydrogenase kinase 2; PFKFB3: 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; PKM2: Pyruvate kinase M2.

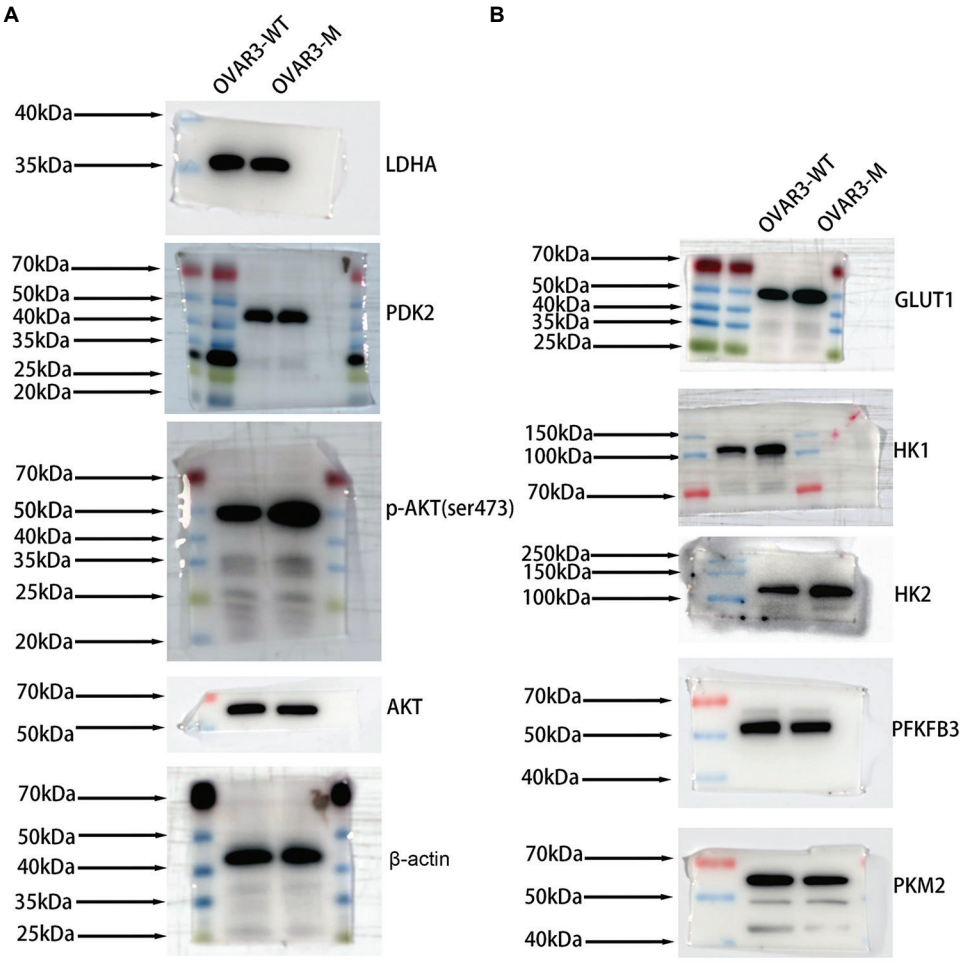

Figure S6. (A and B) Original images for Western blotting for K in Figure 2 (from the main manuscript)
Abbreviations: AKT: Protein kinase B; GLUT1: Glucose transporter 1; HK: Hexokinase; LDHA: Lactate dehydrogenase A; M: Mutant; PDK2: Pyruvate dehydrogenase kinase 2; PFKFB3: 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; PKM2: Pyruvate kinase M2; WT: Wild-type.

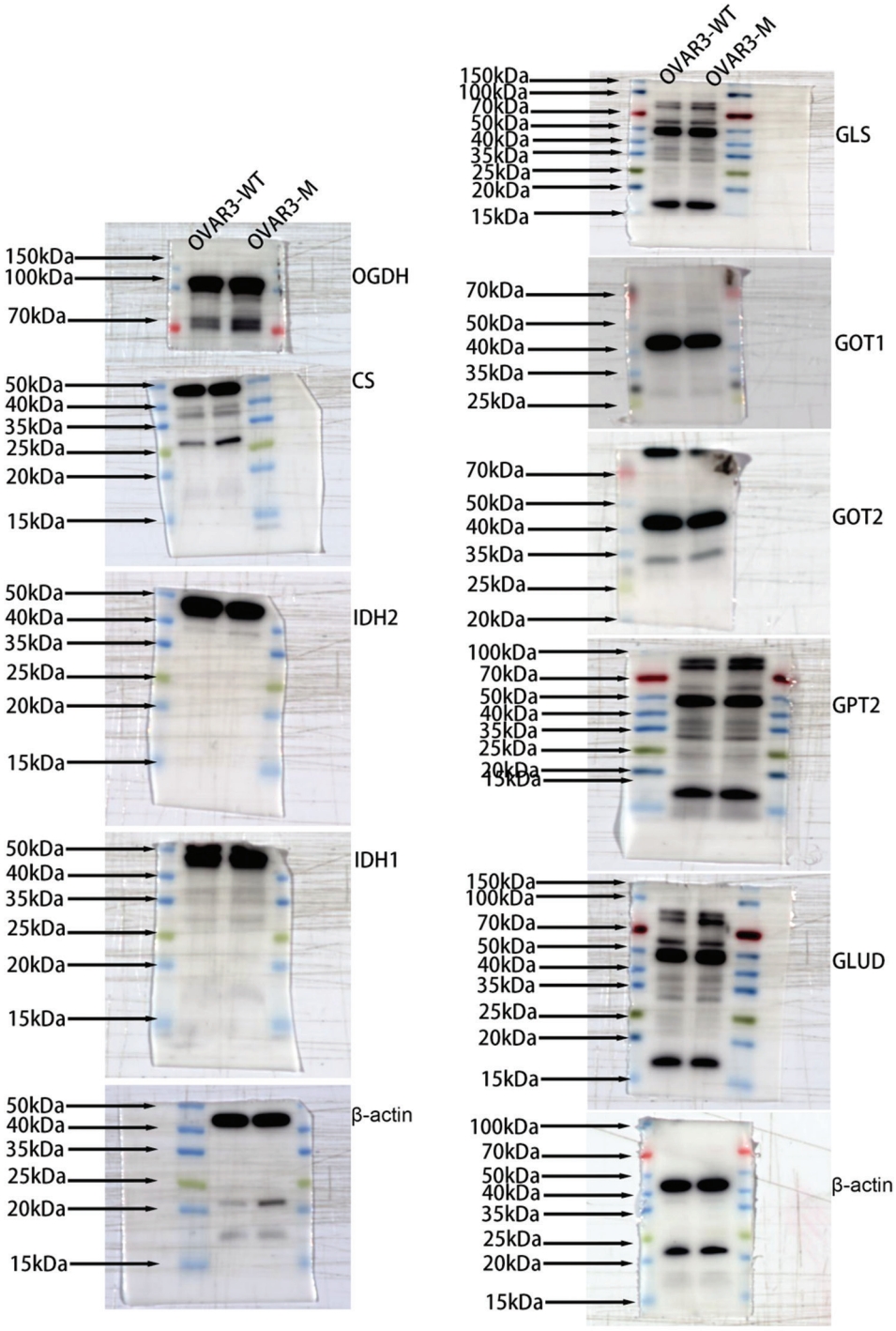

Figure S7. Original images for Western blotting for D and G in Figure 3 (from the main manuscript)
Abbreviations: CS: Citrate synthase; GLS: Glutaminase; GLUD: Glutamate dehydrogenase; GOT: Glutamic-oxaloacetic transaminase; GPT: Glutamic-pyruvate transaminase; IDH: Isocitrate dehydrogenase; M: Mutant; OGDH: Oxoglutarate dehydrogenase; WT: Wild-type.

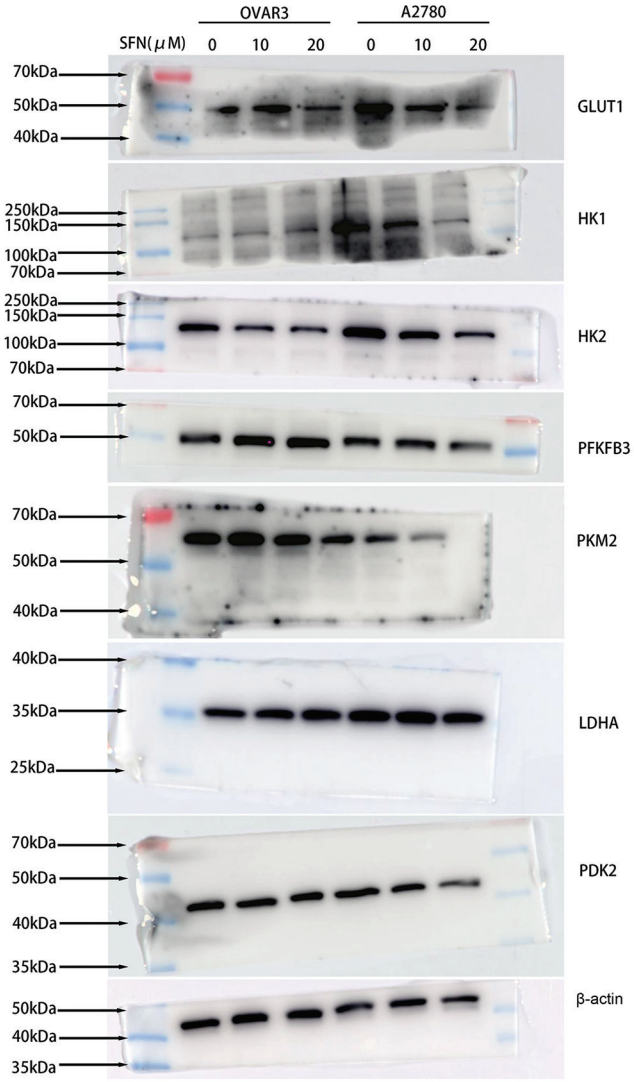

Figure S8. Original images for Western blotting for E in Figure 4 (from the main manuscript)
Abbreviations: AKT: Protein kinase B; GLUT1: Glucose transporter 1; HK: Hexokinase; LDHA: Lactate dehydrogenase A; PDK2: Pyruvate dehydrogenase kinase 2; PFKFB3: 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; PKM2: Pyruvate kinase M2; SFN: Sulforaphane.

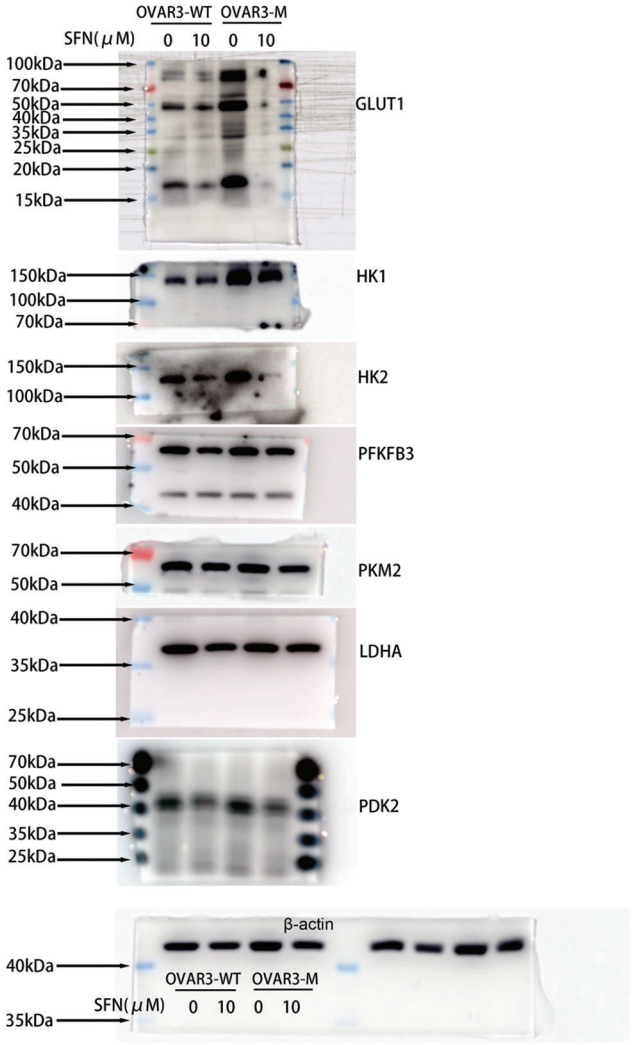

Figure S9. Original images for Western blotting for F in Figure 4 (from the main manuscript)
Abbreviations: AKT: Protein kinase B; GLUT1: Glucose transporter 1; HK: Hexokinase; LDHA: Lactate dehydrogenase A; M: Mutant; PDK2: Pyruvate dehydrogenase kinase 2; PFKFB3: 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; PKM2: Pyruvate kinase M2; SFN: Sulforaphane; WT: Wild type.

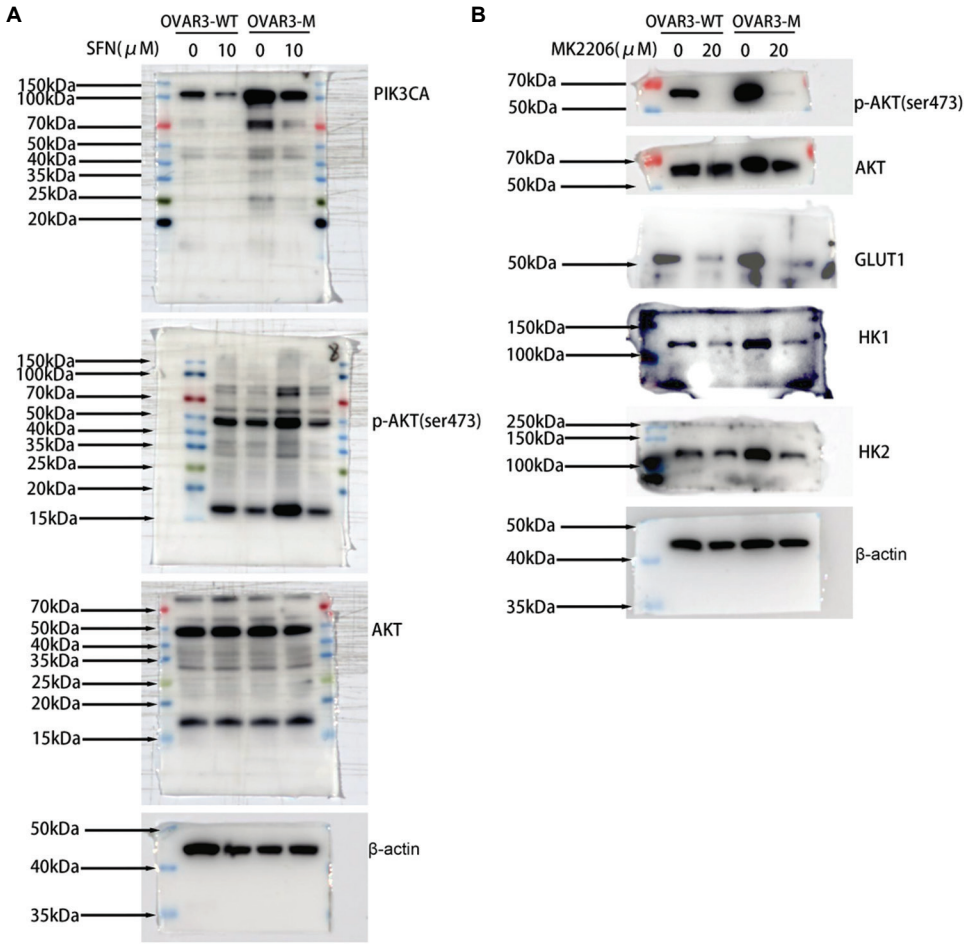

Figure S10. (A and B) Original images for Western blotting for A and C in Figure 5 (from the main manuscript)
Abbreviations: AKT: Protein kinase B; GLUT1: Glucose transporter 1; HK: Hexokinase; M: Mutant; PIK3CA: Phosphatidylinositol 3-kinase; SFN: Sulforaphane; WT: Wild-type.

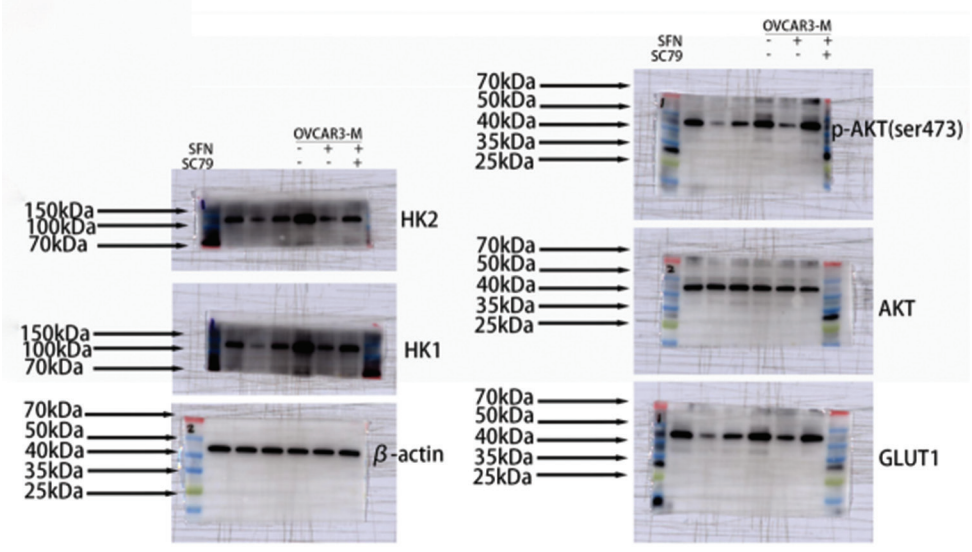

Figure S11. Original images for Western blotting in Figure S3
Abbreviations: AKT: Protein kinase B; GLUT1: Glucose transporter 1; HK: Hexokinase; M: Mutant; PIK3CA: Phosphatidylinositol 3-kinase; SFN: Sulforaphane.

Table S1. Baseline information of patients with high-grade ovarian cancer

ID number	[a]Age	Pathology type	Diagnosis date (Year.Month)	Presence of *PIK3CA* mutation
1	2	High-grade serous carcinoma	2007.12	No
2	1	Granular cell tumor	2009	No
3	2	Low-grade serous carcinoma	2013.02	Yes
4	1	High-grade serous carcinoma	2017.04	No
5	2	High-grade serous carcinoma	2022.02	No
6	1	High-grade serous carcinoma	2013.04	No
7	2	High-grade serous carcinoma	2014.03	Yes
8	1	High-grade serous carcinoma	2014.05	No
9	2	High-grade serous carcinoma	2015.04	No
10	2	High-grade serous carcinoma	2016.07	No
11	1	High-grade serous carcinoma	2016.12	No
12	2	High-grade serous carcinoma	2016.09	Yes
13	2	High-grade serous carcinoma	2016.08	No
14	2	High-grade serous carcinoma	2016.03	No
15	1	Low-grade serous carcinoma	2016.08	No
16	2	High-grade serous carcinoma	2016.11	No
17	2	Mucous carcinoma	2016.09	No
18	1	High-grade serous carcinoma	2016.09	No
19	1	High-grade serous carcinoma	2016.02	No
20	2	High-grade serous carcinoma	2016.07	No
21	1	High-grade serous carcinoma	2017.07	No
22	2	High-grade serous carcinoma	2017.07	No
23	1	High-grade serous carcinoma	2017.08	No
24	1	High-grade serous carcinoma	2017.12	Yes
25	1	High-grade serous carcinoma	2017.03	No
26	2	High-grade serous carcinoma	2017.03	No
27	2	High-grade serous carcinoma	2017.07	Yes
28	1	High-grade serous carcinoma	2017.05	No
29	2	High-grade serous carcinoma	2017.01	No
30	2	Clear-cell carcinoma	2017.04	Yes
31	2	High-grade serous carcinoma	2018.01	No
32	2	High-grade serous carcinoma	2018.07	No
33	2	High-grade serous carcinoma	2018	No
34	2	High-grade serous carcinoma	2018.06	No
35	1	Serous mucinous cystadenocarcinoma	2018.01	No
36	2	High-grade serous carcinoma	2018.10	No
37	2	High-grade serous carcinoma	2018.03	No
38	2	High-grade serous carcinoma	2018.07	No
39	2	High-grade serous carcinoma	2018.05	No
40	1	Sarcomatoid carcinoma	2018.07	Yes
41	2	Serous mucinous cystadenocarcinoma	2018.11	No

(*Cont'd...*)

Table S1. (*Continued*)

ID number	[a]Age	Pathology type	Diagnosis date (Year.Month)	Presence of *PIK3CA* mutation
42	2	Low-grade serous carcinoma	2018.05	No
43	2	Low-grade serous carcinoma	2018.04	No
44	1	High-grade serous carcinoma	2018.10	No
45	1	High-grade serous carcinoma	2018.11	Yes
46	1	Clear-cell carcinoma	2018.10	No
47	1	Mucous carcinoma	2018.10	No
48	1	High-grade serous carcinoma	2018.09	No
49	2	High-grade serous carcinoma	2018.11	Yes
50	1	High-grade serous carcinoma	2019.10	No
51	2	High-grade serous carcinoma	2019.02	No
52	2	High-grade serous carcinoma	2019.09	No
53	2	High-grade serous carcinoma	2019.06	No
54	1	Low-grade serous carcinoma	2019.04	No
55	2	Glioma peripheral	2019.11	No
56	2	Clear-cell carcinoma	2019.07	Yes
57	2	High-grade serous carcinoma	2019.04	No
58	2	High-grade serous carcinoma	2019.11	No
59	2	High-grade serous carcinoma	2019.06	No
60	2	High-grade serous carcinoma	2019.10	No
61	1	High-grade serous carcinoma	2019.04	No
62	2	High-grade serous carcinoma	2019.03	No
63	2	Carcinoma sareomatodes	2019.12	No
64	2	Endometrioid carcinoma	2019	Yes
65	2	High-grade serous carcinoma	2019.07	No
66	2	High-grade serous carcinoma	2019.09	No
67	1	High-grade serous carcinoma	2019.07	No
68	2	High-grade serous carcinoma	2020.07	No
69	2	High-grade serous carcinoma	2020.07	No
70	1	High-grade serous carcinoma	2020.04	No
71	1	High-grade serous carcinoma	2020.11	No
72	1	High-grade serous carcinoma	2020.09	No
73	2	Clear-cell carcinoma	2020.06	No
74	2	High-grade serous carcinoma	2020.08	No
75	1	High-grade serous carcinoma	2020.06	No
76	2	Granular cell tumor	2020.04	No
77	1	Borderline serous carcinoma	2020.07	No
78	2	High-grade serous carcinoma	2020.09	No
79	2	Sarcomatoid carcinoma	2020.08	Yes
80	2	Low-grade serous carcinoma	2020.01	No
81	2	High-grade serous carcinoma	2020.09	Yes
82	2	High-grade serous carcinoma	2020.01	No
83	2	Low-grade serous carcinoma	2020.11	No
84	1	High-grade serous carcinoma	2020.11	No

(*Cont'd*...)

Table S1. (*Continued*)

ID number	[a]Age	Pathology type	Diagnosis date (Year.Month)	Presence of *PIK3CA* mutation
85	2	High-grade serous carcinoma	2020.01	No
86	2	High-grade serous carcinoma	2020.01	No
87	2	High-grade serous carcinoma	2020.10	No
88	2	High-grade serous carcinoma	2020.02	No
89	2	High-grade serous carcinoma	2020.03	No
90	2	High-grade serous carcinoma	2020.09	No
91	1	High-grade serous carcinoma	2020.08	Yes
92	2	High-grade serous carcinoma	2020.07	No
93	2	High-grade serous carcinoma	2020.03	No
94	1	High-grade serous carcinoma	2021.06	No
95	1	High-grade serous carcinoma	2021.08	No
96	2	High-grade serous carcinoma	2021.03	No
97	2	High-grade serous carcinoma	2021.08	No
98	2	High-grade serous carcinoma	2021.04	No
99	1	High-grade serous carcinoma	2021.05	No
100	1	Endometrioid carcinoma	2021.03	No
101	1	High-grade serous carcinoma	2021.07	Yes
102	1	High-grade serous carcinoma	2021.03	No
103	1	Mucous carcinoma	2021.06	No
104	1	High-grade serous carcinoma	2021.02	No
105	2	High-grade serous carcinoma	2021.03	No
106	1	High-grade serous carcinoma	2021.07	No
107	1	High-grade serous carcinoma	2021.04	Yes
108	2	High-grade serous carcinoma	2021.04	No
109	1	High-grade serous carcinoma	2021.10	No
110	2	High-grade serous carcinoma	2021.09	No
111	1	High-grade serous carcinoma	2021.04	No
112	2	Granular cell tumor	2021.09	Yes
113	2	High-grade serous carcinoma	2021.05	No
114	2	High-grade serous carcinoma	2021.03	No
115	2	High-grade serous carcinoma	2021.02	No
116	1	High-grade serous carcinoma	2021.06	No
117	2	High-grade serous carcinoma	2021.05	No
118	1	Mucous carcinoma	2021.08	No
119	1	High-grade serous carcinoma	2021.09	No
120	2	High-grade serous carcinoma	2021.08	No
121	1	Granular cell tumor	2021.08	No
122	1	High-grade serous carcinoma	2021.07	No
123	2	High-grade serous carcinoma	2022.06	No
124	2	High-grade serous carcinoma	2022.10	No
125	2	Mucous carcinoma	2021.12	No
126	2	High-grade serous carcinoma	2022.01	No
127	2	High-grade serous carcinoma	2021.04	No
128	1	High-grade serous carcinoma	2021.09	No

Note: [a]Age was categorized as ≤50 years (denoted 1) and ≤50 years (denoted 2).

Table S2. Inclusion and exclusion criteria for study participants

Type	Criteria
Inclusion	Adult women aged 18 years or older
	Pathological diagnosis of ovarian cancer
	Obtained written informed consent from all participants or their legal guardians
	Acknowledged voluntary participation in the study and approved the utilization of their biospecimens and clinical data
Exclusion	History of other concurrent malignancies, unless disease-free for at least 5 years
	Severe comorbidities, such as significant cardiovascular disease or hepatic or renal dysfunction, which could affect survival or interpretation of study results
	Pregnant or lactating women
	Refused to sign the informed consent form
	Unwilling to cooperate with follow-up surveys
	Participants deemed ineligible by the investigators for reasons not specified

Table S3. The primer sequences used for real-time quantitative polymerase chain reaction

Gene	Sequence
SLC2A1	F: 5'-GGCTTCTTCTTCTACGGCAT-3'
	R: 5'-CAGATCGGCAATGAGGAAGT-3'
HK1	F: 5'-TGGAAGAGGTGGCTGAGAAG-3'
	R: 5'-GCCTTGTCAGAAGGAGCAAT-3'
HK2	F: 5'-CAAAGTGCGGCATCATCATC-3'
	R: 5'-TCCACATCTGGGGTCTCAAT-3'
PKM	F: 5'-GCCGCCTGGCTTATTCAG-3'
	R: 5'-TGGGTGTCAAAGCAATCAGA-3'
PDK2	F: 5'-TGCTGGCCAAGGTAAAGAGG-3'
	R: 5'-GCCTTGGCTTCTTCTTCACC-3'
GAPDH	F: 5'-GGAGCGAGATCCCTCCAAAAT-3'
	R: 5'-GGCTGTTGTCATACTTCTCATGG-3'
LDHA	F: 5'-ATGGCAGTGTCTCCAGAACG-3'
	R: 5'-TCCACCACCCTGTTGCTGTA-3'
PFKFB3	F: 5'-CAAGGAGCTGGAGGAGGAAG-3'
	R: 5'-GTCTGGGTGGGAGTTGAGAA-3'