

ORIGINAL RESEARCH ARTICLE

Secreted phosphoprotein 1 promotes ovarian cancer metastasis by regulating the function of cancer-associated fibroblasts

Supplementary Files
Table S1. Baseline information of 30 patients with HGSOc

ID number	Age (years)	FIGO stage	Lymph node metastasis	HE4 (pmol/L)	CA125 (U/mL)
1	73	IC	No	51.7	14.9
2	48	IIIC	No	304	344
3	46	IV	No	301	1,105
4	50	IVB	No	788	9,243
5	49	II	Yes	45.5	242
6	48	IB	No	205	10,275
7	52	IIIC	No	48.3	37.81
8	22	IA	No	73.6	43.9
9	75	IA	No	63.4	188
10	44	IC	No	93	347
11	57	IV	No	4412	1,261
12	54	II	No	80.3	76.8
13	55	IIIC	No	1,724	1,051
14	63	IV	Yes	86.3	681
15	64	I	No	96.3	13.64
16	50	IIIC	No	345	682
17	75	IIB	No	370	65.3
18	55	IIIC	No	701	253
19	47	IIIC	No	80.2	189
20	53	IA	No	96	251
21	56	IA	No	54.7	6.07
22	63	IIIC	No	1,098	11,884
23	59	IV	No	Unknown	336.8
24	49	III	No	1,500	4,119
25	61	IIA	No	116	22.8
26	37	IV	Yes	1,186	2,562
27	56	IIIC	No	401	790
28	18	IC	No	93.2	40.9
29	64	IIIC	No	1,198	1,640
30	62	IV	Yes	105	105

Abbreviations: CA125: Cancer antigen 125; FIGO: International Federation of Gynecology and Obstetrics; HE4: Human epididymis protein 4; HGSOc: High-grade serous ovarian carcinoma.

Table S2. Primer sequences of siRNAs used in this study

Primer/siRNA		Sequence (5'-3')
Spp1-Mus-620	Forward primer	GUCUAGGAGUCCAGGUUTT
	Reverse primer	AACUGGAAACUCCUAGACTT
Spp1-Mus-828	Forward primer	CUGUAGCAUCCAAAGAGATT
	Reverse primer	UCUCUUUGGAUGCUCUAAAGTT
Spp1-Mus-153	Forward primer	AUGAGAUAUUGGCAGUAUUUTT
	Reverse primer	AAAUCACUGCCAUCUCAUTT

Table S3. Primer sequences of shRNAs used in this study

Primer/shRNA	Sequence (5'-3')
mouse-sh1-SPP1	GAGGTCAAAGTCTAGGAGTTT
mouse-sh2-SPP1	AGGATGACTTTAAGCAAGAAA
mouse-sh3-SPP1	CCTAAGAGTAAGGAAGATGAT

Table S4. Primary antibodies used in this study

Antibody	Company (Cat no.)	Concentration
α-SMA	Proteintech (14395-1-AP)	WB: 1:6,000
FAP	Affinity (AF5344)	WB: 1:1,000
PI3K	Proteintech (20584-1-AP)	WB: 1:500
AKT	Proteintech (60203-2-Ig)	WB: 1:6,000
p-PI3K	Affinity (AF3242)	WB: 1:1,000
p-AKT	Proteintech (66444-1-Ig)	WB: 1:6,000
GAPDH	Proteintech (10494-1-AP)	WB: 1:5,000
SPP1	Zenbio (340690)	WB: 1:1,000; IHC: 1:100
p65	Proteintech (80979-1-RR)	WB: 1:6,000

Abbreviations: AKT: Protein kinase B; α-SMA: Alpha-smooth muscle actin; FAP: Fibroblast activation protein; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; IHC: Immunohistochemistry; p-AKT: Phosphorylated protein kinase B; p-P65: Phosphorylated nuclear factor kappa B p65; p-PI3K: Phosphorylated phosphoinositide 3-kinase; PI3K: Phosphoinositide 3-kinase; SPP1: Secreted phosphoprotein 1; WB: Western blot.

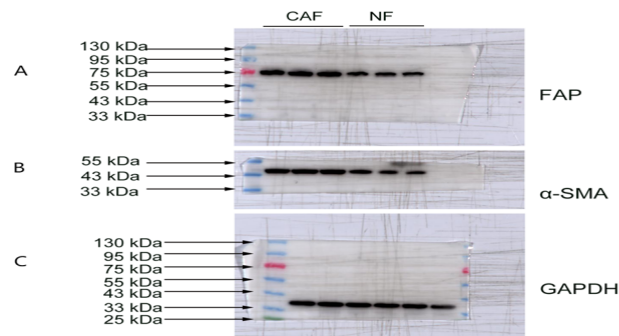


Figure S1. Original images for western blotting for FAP, α-SMA, and GAPDH in Figure 2B. (A) Western blotting for FAP (70–100 kDa). (B) Western blotting for α-SMA (40–50 kDa). (C) Western blotting for GAPDH (36 kDa).

Abbreviations: α-SMA: Alpha smooth muscle actin; CAF: Cancer-associated fibroblast; FAP: Fibroblast activation protein; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; NF: Normal fibroblast.

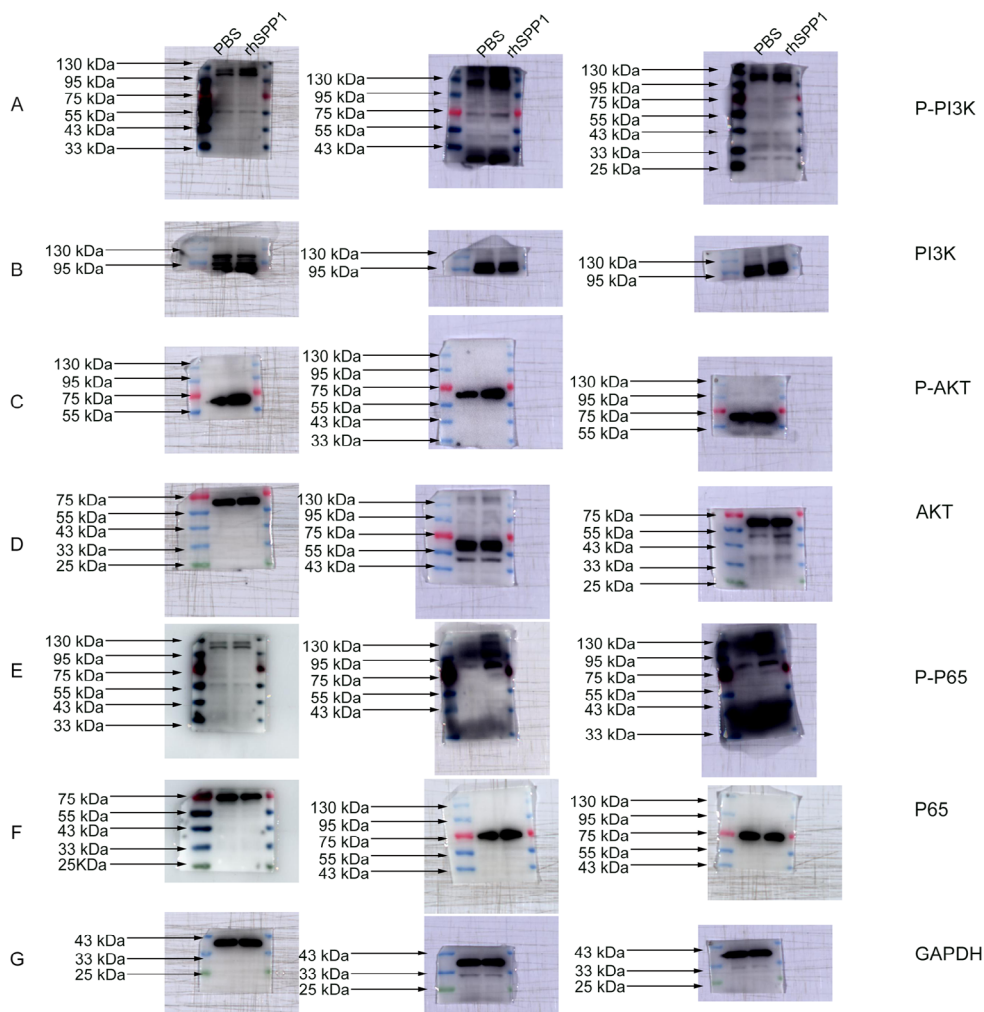


Figure S2. Original images for western blotting for P-PI3K, PI3K, P-AKT, AKT, P-P65, P65, and GAPDH in Figure 4B. (A) Western blotting for P-PI3K (84/54 kDa). (B) Western blotting for PI3K (110–130 kDa). (C) Western blotting for P-AKT (50–70 kDa). (D) Western blotting for AKT (56–62 kDa). (E) Western blotting for P-P65 (65–75 kDa). (F) Western blotting for P65 (65–75 kDa). (G). Western blotting for GAPDH (36 kDa).

Abbreviations: AKT: Protein kinase B; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; P: Phosphorylated; PBS: Phosphate-buffered saline; PI3K: Phosphoinositide 3-kinase; rhSPP1: Recombinant human SPP1

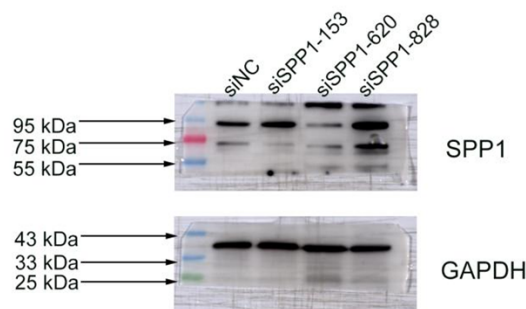


Figure S3. Original images for western blotting for SPP1 and GAPDH in Figure 4C. (A) Western blotting for SPP1 (35/60–70 kDa). (B) Western blotting for GAPDH (36 kDa).

Abbreviations: GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; NC: Control; si: Small interfering; SPP1: Secreted phosphoprotein 1.

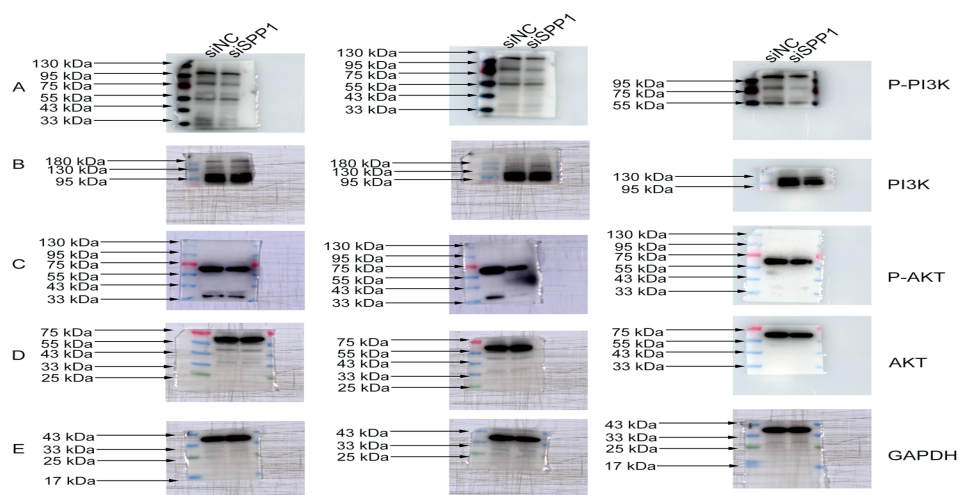


Figure S4. Original images for western blotting for P-PI3K, PI3K, P-AKT, AKT, and GAPDH in Figure 4E. (A) Western blotting for P-PI3K (84/54 kDa). (B) Western blotting for PI3K (110–130 kDa). (C) Western blotting for P-AKT (50–70 kDa). (D) Western blotting for AKT (56–62 kDa). (E) Western blotting for GAPDH (36 kDa).

Abbreviations: AKT: Protein kinase B; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; NC: Control; P: Phosphorylated; PI3K: Phosphoinositide 3-kinase; si: Small interfering; SPP1: Secreted phosphoprotein 1.