Supplementary materials

PSMC6 regulation of ovarian cancer cisplatin resistance unravels a new mode for proteasome targeting

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Table S1. Characteristics of patients involved in the pilot study

	N	%
Diagnosis		
Endometrioid carcinoma	25	18.66
High-grade serous carcinoma	80	59.70
Other[a]	29	21.64
Grade		
1	15	11.19
2	13	9.70
3	106	79.10
Stage		
I	26	19.40
II	21	15.67
III	71	52.99
IV	16	11.94

[a] Other, Mucinous adenocarcinoma, Clear cell carcinoma, Low-grade serous carcinoma

Table S2. Descriptive statistics of the relative expression of PSMC6 according to the diagnosis, tumor grade and stage.

	N	Min	Q1	Median	Q3	max	IQR
Diagnosis							
Endometrioid carcinoma	25	-10.55	-7.19	-6.25	-4.88	-3.02	2.31
High-grade serous carcinoma	79	-12.62	-7.57	-6.61	-5.95	-3.19	1.62
Other	29	-10.74	-7.85	-6.43	-5.71	-4.22	2.14
Grade							
1	15	-9.96	-8.11	-7.75	-5.31	-3.02	2.80
2	13	-10.55	-7.38	-6.76	-5.96	-4.41	1.43
3	105	-12.62	-7.46	-6.46	-5.72	-3.14	1.74
Stage							
I	26	-10.74	-6.76	-6.1	-4.92	-3.02	1.84
II	21	-10.55	-7.19	-6.43	-5.71	-4.22	1.48
III	71	-12.62	-7.63	-6.61	-5.81	-3.19	1.82
IV	15	-8.31	-7.85	-7.38	-6.42	-4.25	1.43

Table S3. Western blot quantification of Figure 4 as percentage of band intensity relative to negative control band at each time point[a].

Table S1						
PSMC6	**siRNA A 48h**	**siRNA B 48h**	**siRNA A 72h**	**siRNA B 72h**	**siRNA A 144h**	**siRNA B 144h**
IGROV-1/Pt1	8	7	9	12	33	36
IGROV-1	21	38	34	21	27	7

[a] The band intensity was quantified by using ImageQuant software, intensities were normalized to the control protein β-tubulin or actin; the siRNAs percentage of intensity was calculated by dividing their normalized band intensity to their negative control normalized band intensity of the same time point.

Table S4. Western blot quantification of Figure 6 as percentage of band intensity relative to negative control band at each time point[a].

Table S2						
AKT	**siRNA A 48h**	**siRNA B 48h**	**siRNA A 72h**	**siRNA B 72h**	**siRNA A 144h**	**siRNA B 144h**
IGROV-1/Pt1	165	211	210	211	210	128
IGROV-1	106	110	82	99	166	185
P-AKT	**siRNA A 48h**	**siRNA B 48h**	**siRNA A 72h**	**siRNA B 72h**	**siRNA A 144h**	**siRNA B 144h**
IGROV-1/Pt1	45	113	121	112	70	100
IGROV-1	119	86	71	98	120	173
ERK1/2	**siRNA A 48h**	**siRNA B 48h**	**siRNA A 72h**	**siRNA B 72h**	**siRNA A 144h**	**siRNA B 144h**
IGROV-1/Pt1	110	90	82	73	83	67
IGROV-1	106	114	97	100	155	164
P-ERK1/2	**siRNA A 48h**	**siRNA B 48h**	**siRNA A 72h**	**siRNA B 72h**	**siRNA A 144h**	**siRNA B 144h**
IGROV-1/Pt1	80	60	60	50	100	75
IGROV-1	42	19	22	29	36	154

[a] Quantification procedure as stated in Table S3.

Table S5. Western blot quantification of Figure 7 as percentage of band intensity relative to negative control band at each time point.

Table S3				
DUSP5	**siRNA A 48h**	**siRNA B 48h**	**siRNA A 72h**	**siRNA B 72h**
IGROV-1/Pt1	122	244	157	100
IGROV-1	47	33	71	121
DUSP6	**siRNA A 48h**	**siRNA B 48h**	**siRNA A 72h**	**siRNA B 72h**
IGROV-1/Pt1	94	112	220	300
IGROV-1	357	243	171	200
PSMC5	**siRNA A 48h**	**siRNA B 48h**	**siRNA A 72h**	**siRNA B 72h**
IGROV-1/Pt1	228	370	310	228
IGROV-1	502	413	304	259

[a] Quantification procedure as stated in Table S3.

Table S6. Western blot quantification of Figure S7c as percentage of band intensity relative to negative control band.

Table S4		
p21	**siRNA A**	**siRNA B**
IGROV-1/Pt1	250	133
IGROV-1	114	214
Cleaved caspase-3	**siRNA A**	**siRNA B**
IGROV-1/Pt1	143	221
IGROV-1	217	192

[a] Quantification procedure as stated in Table S3.

Figure S1: Complete data from the CRISPR/Cas9 dropout screening for both IGROV-1 and IGROV-1/Pt1 cell lines, analyzed independently. Notably, our findings reveal that among all tested proteasome subunits, PSMC6 resulted in the most significant lethality score in the IGROV-1/ Pt cells (average score of -1.20815) compared to IGROV-1 cells (average score of -0.694).

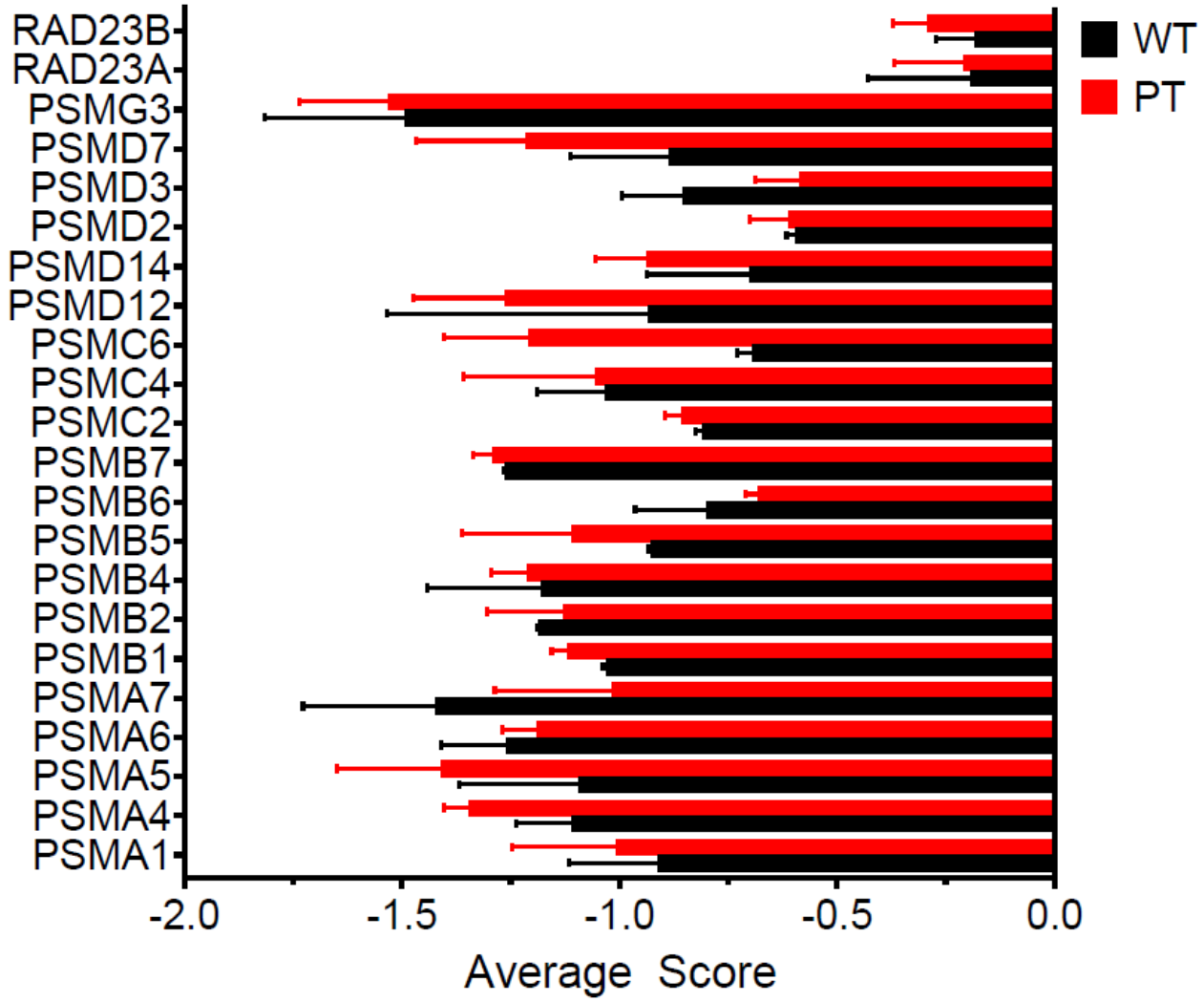


Figure S2: qRT-PCR of PSMC6 in silenced ovarian cancer cell lines. RNA of siRNA transfected cells was extracted and retrotranscribed into cDNA for RT-PCR for PSMC6 and GAPDH.

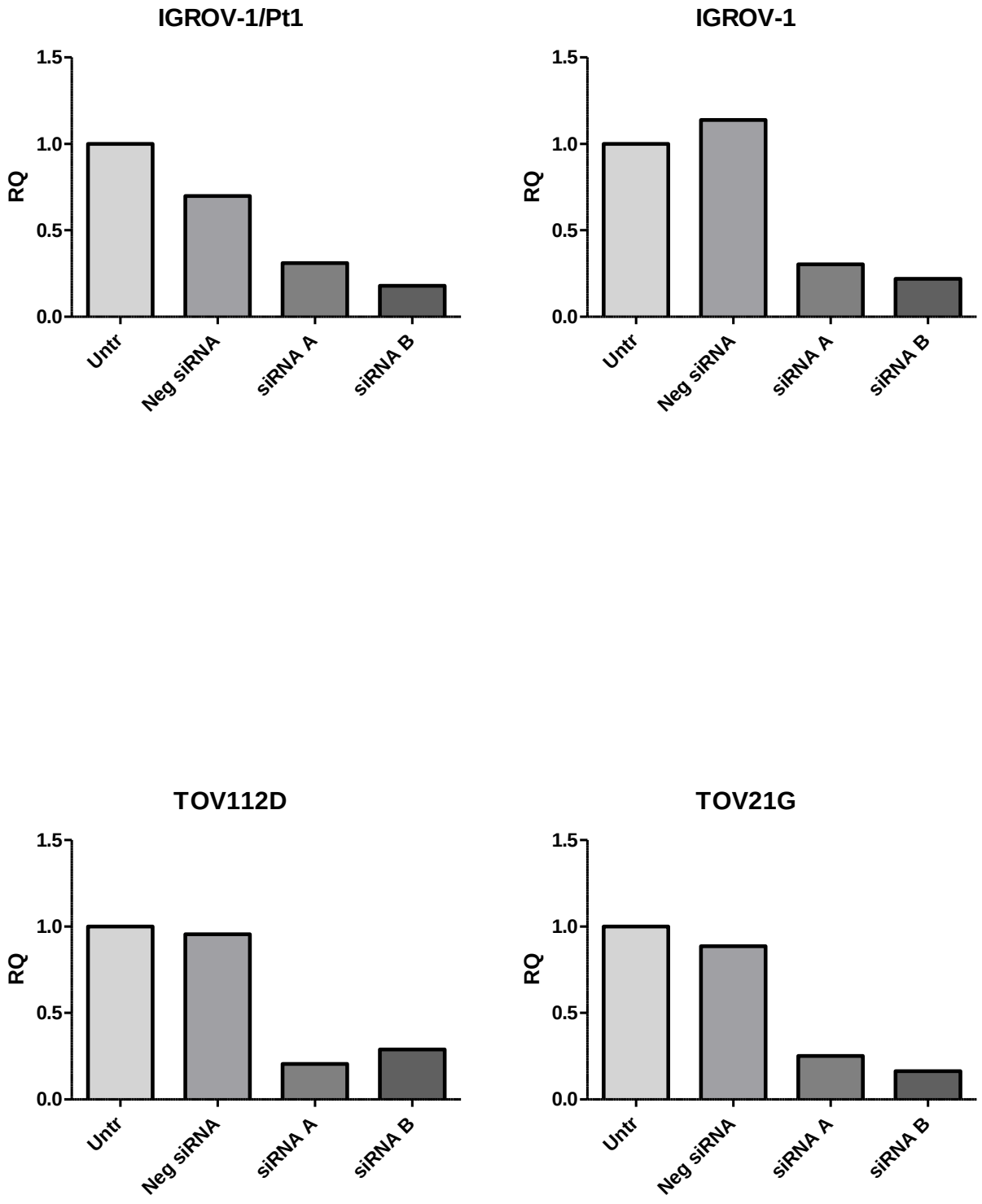


Figure S3: PSMC6 silencing in IGROV-1 cells and its effect on sensitivity to cisplatin by colony forming assay. a) Colony forming ability was evaluated in plastic dishes. Cells were harvested 48 h after siRNA transfection start and their colony forming ability was evaluated. **b)** Percentage of survival of IGROV-1 cells forming colonies in plastics after treatment with cisplatin at different concentrations. The IC_{50} values for each sperimental condition is reported.

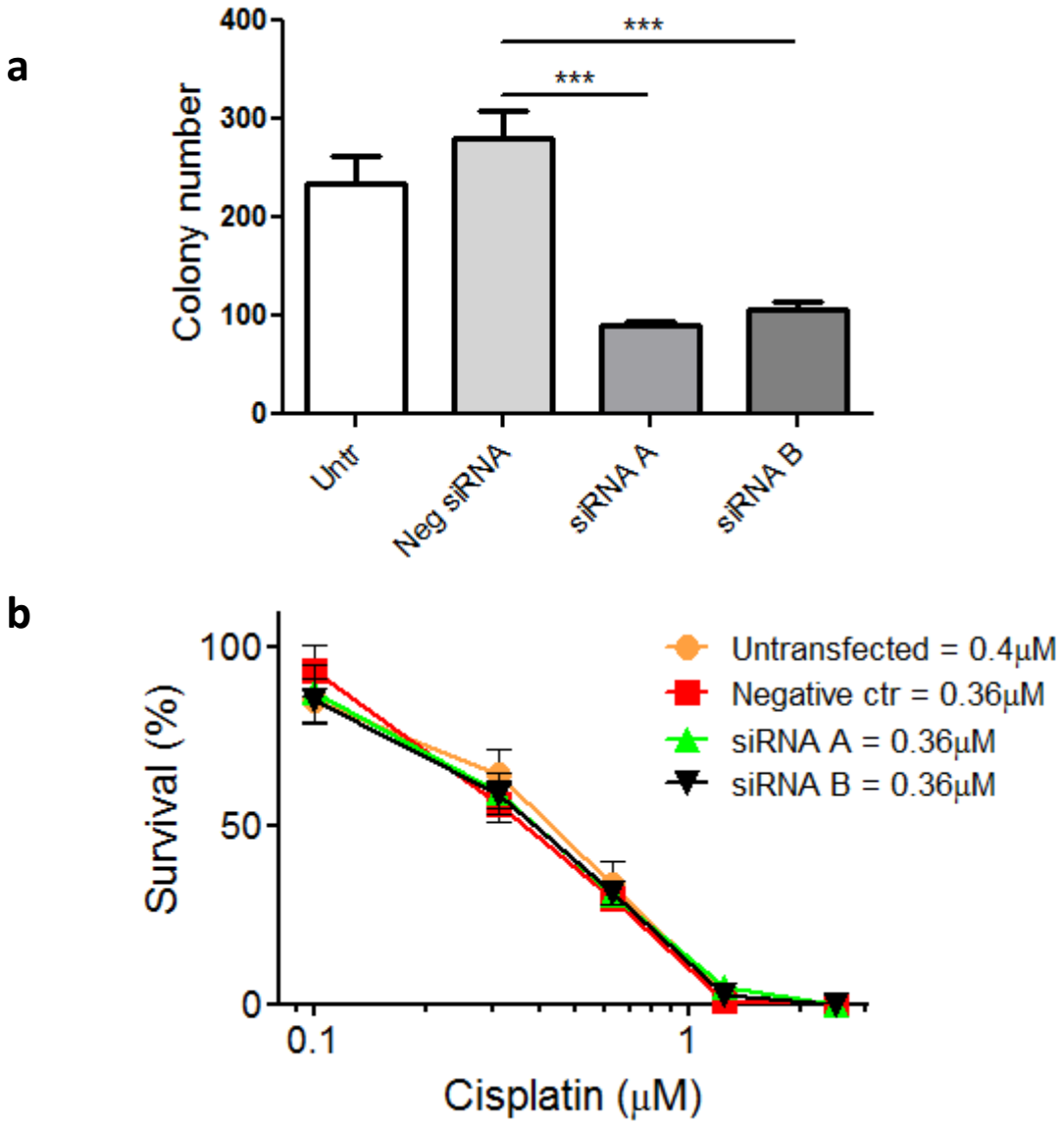


Figure S4. PSMC6 silencing in OSE-SV40 and FT240 cells and its effect on sensitivity to cisplatin by growth inhibition assay. Hystograms represent the qRT-PCR of PSMC6 in silenced OSE-SV40 and FT240 cell lines. RNA of siRNA transfected cells was extracted and retrotranscribed into cDNA for RT-PCR for PSMC6 and GAPDH. The graphs represent the percentage of cell growth in growth inhibition of OSE-SV40 and FT240 cells after treatment with cisplatin at different concentrations. The IC_{50} values for each experimental condition is reported.

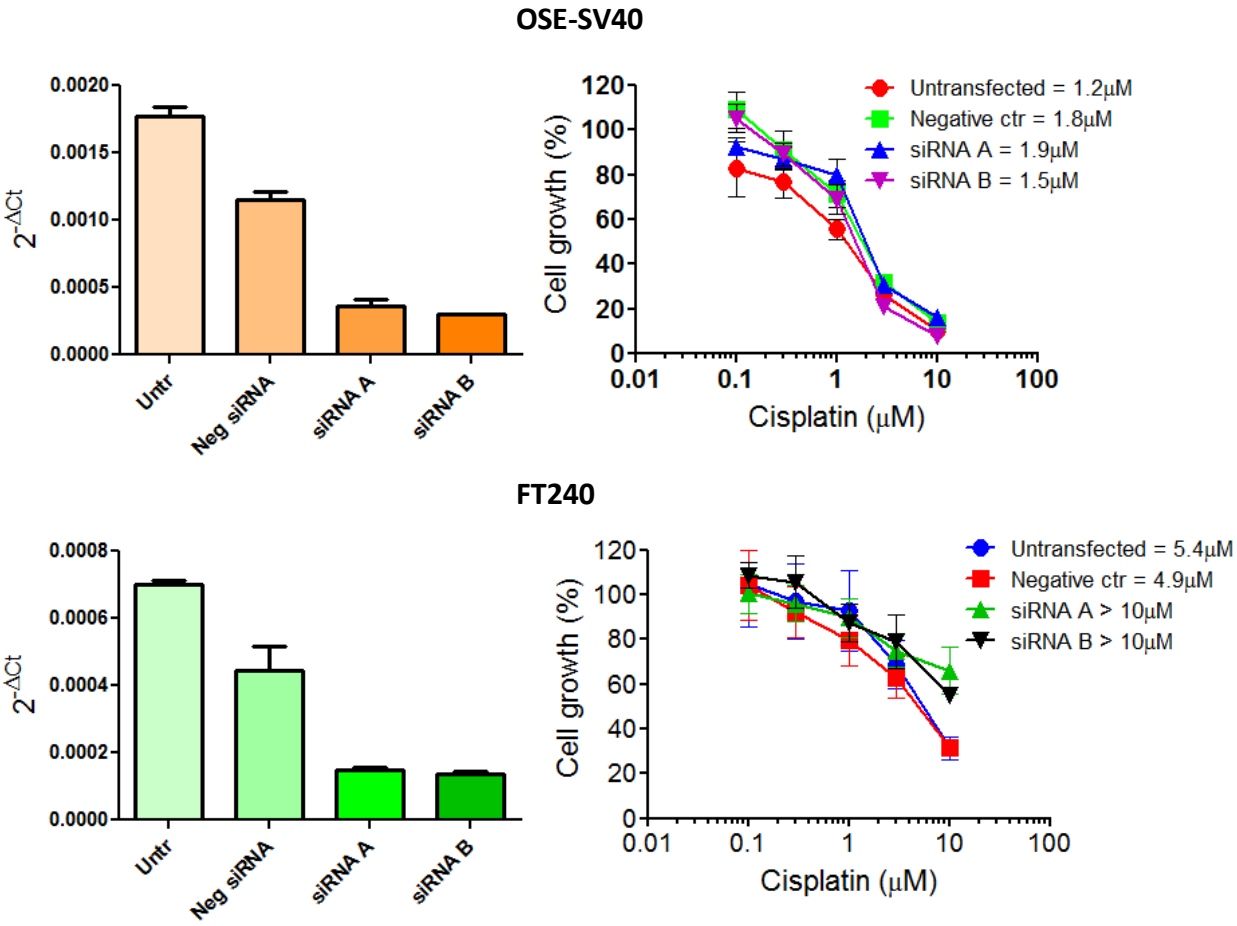


Figure S5. PSMC6 silencing in OVCAR-5, OVCAR-5/Pt and TOV21G/Pt and its effect on sensitivity to cisplatin by colony forming assay. Hystograms represent the qRT-PCR of PSMC6 in silenced OVCAR-5, OVCAR-5/Pt and TOV21G/Pt cell lines. RNA of siRNA transfected cells was extracted and retrotranscribed into cDNA for RT-PCR for PSMC6 and GAPDH. The graphs represent the percentage of survival in clonogenic assay of OVCAR-5, OVCAR-5/Pt and TOV21G/Pt cells after treatment with cisplatin at different concentrations. The IC_{50} values for each sperimental condition is reported.

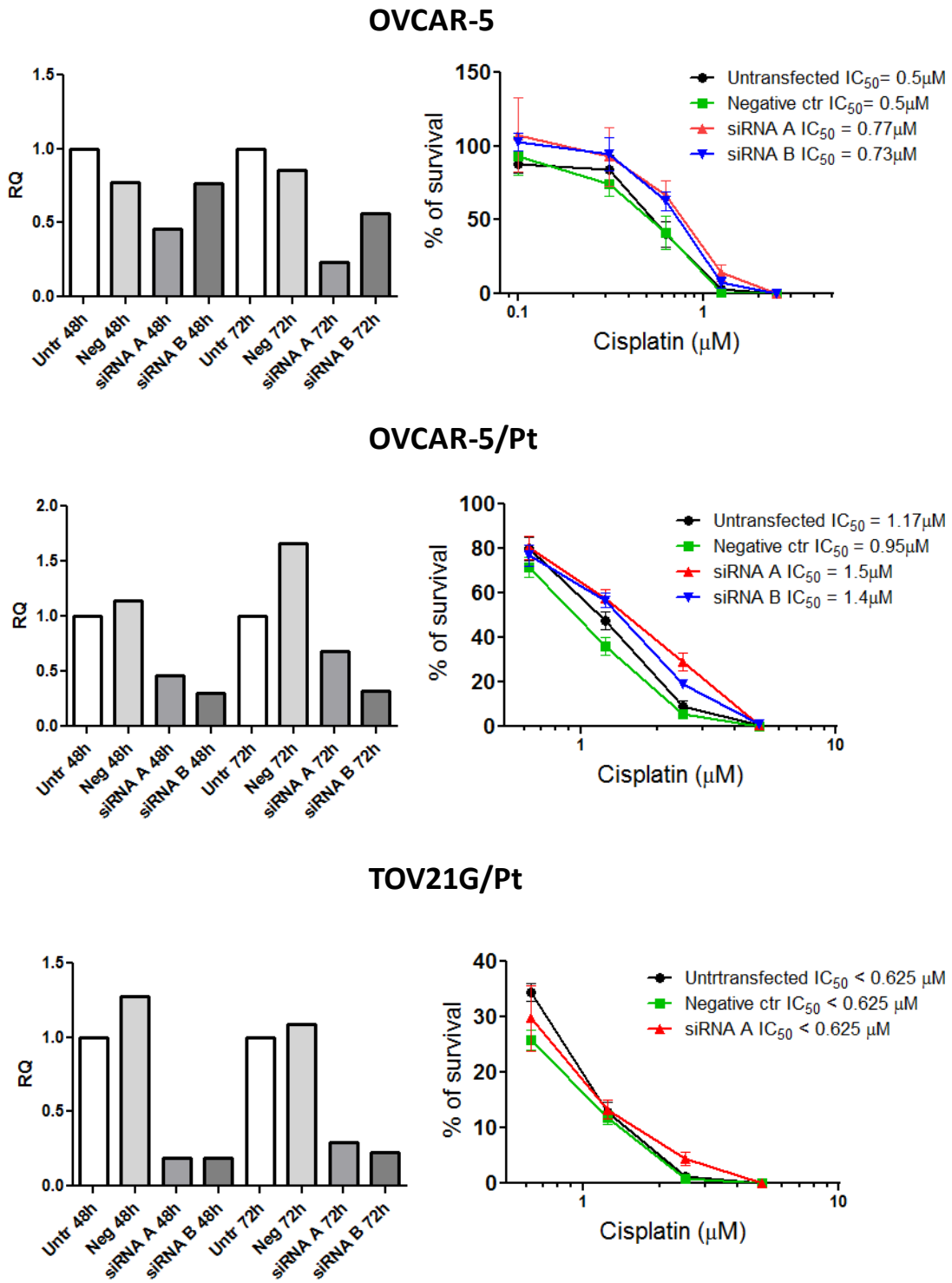


Figure S6. Analysis of ERK1/2 phosphorylation. Percentage of phospho-ERK1/2 over total ERK1/2 in IGROV-1 cell line after silencing with siRNAs. The extent of ERK1/2 phosphorylation has been measured by using an ELISA kit (Abcam)

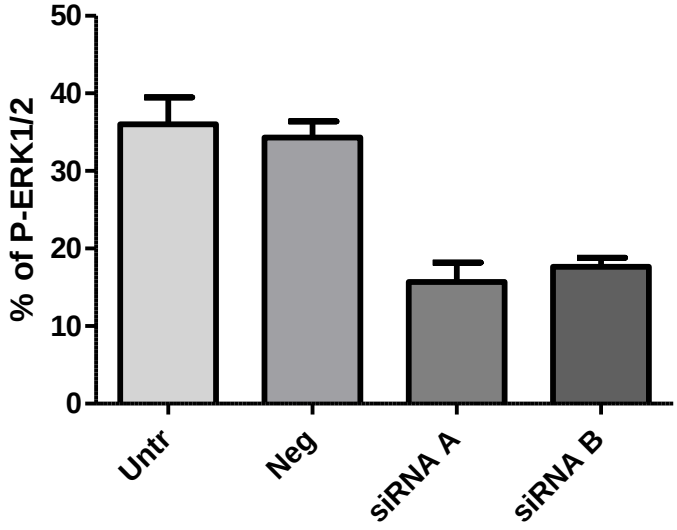


Figure S7. Human protein array and western blot analysis of selected proteins in PSMC6-silenced IGROV-1 and IGROV-1/Pt1 cells. Twenty for hours after seeding, cells were silenced with 100 nM control siRNA or siRNAs directed to PSMC6. Following 72 h, cells were harvested and lysed. Protein extracts were incubated overnight onto the array according to manufacturer's protocol. a), b) heatmap of the modulated proteins in IGROV-1/Pt1 (a) and IGROV-1cells (b). Western blot analysis of p21cip1/waf1 and cleaved Caspase-3 in IGROV-1/Pt1 and IGROV-1 cells (c). The band intensity was quantified by using the ImageQuant software. Western Blot quantification reported in **Table S6.**

a **IGROV-1/Pt1**

	UNTR	NEG	SiRNA A	SiRNA B
Bad				
Bax				
Bcl-2				
Bcl-x				
Pro-Caspase-3				
Cleaved Caspase-3				
Catalase				
cIAP-1				
cIAP-2				
Claspin				
Clusterin				
Cytochrome c				
TRAIL R1/DR4				
TRAIL R2/DR5				
FADD				
Fas/TNFRSF6/CD95				
HIF-1α				
H0-1/HM0X1/HSP32				
H0-2/HM0X2				
HSP27				
HSP60				
HSP70				
HTRA2/Omi				
Livin				
PON2				
p21/CIP1/CDKN1A				
p27/Kip1				
Phospho-p53 (S15)				
Phospho-p53 (S46)				
Phospho-p53 (S392)				
Phospho-Rad17 (S635)				
SMAC/Diablo				
Survivin				
TNF RI/TNFRSF1A				
XIAP				

b **IGROV-1**

	UNTR	NEG	siRNA A	siRNA B
Bad				
Bax				
Bcl-2				
Bcl-x				
Pro-Caspase-3				
Cleaved Caspase-3				
Catalase				
cIAP-1				
cIAP-2				
Claspin				
Clusterin				
Cytochrome c				
TRAIL R1/DR4				
TRAIL R2/DR5				
FADD				
Fas/TNFRSF6/CD95				
HIF-1α				
H0-1/HM0X1/HSP32				
H0-2/HM0X2				
HSP27				
HSP60				
HSP70				
HTRA2/Omi				
Livin				
PON2				
p21/CIP1/CDKN1A				
p27/Kip1				
Phospho-p53 (S15)				
Phospho-p53 (S46)				
Phospho-p53 (S392)				
Phospho-Rad17 (S635)				
SMAC/Diablo				
Survivin				
TNF RI/TNFRSF1A				
XIAP				

c

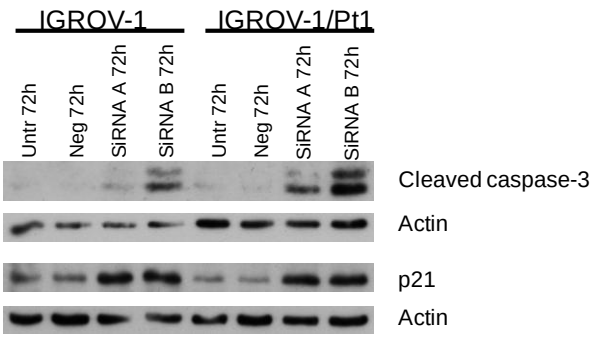


Figure S8. Human apoptosis antibody arrays of PSMC6-silenced IGROV-1 and IGROV-1/Pt1 cell lines. Untr, untransfected cells; Neg, negative control siRNA transfected cells; siRNA A, cells transfected with siRNA A; siRNA B, cells transfected with siRNA B

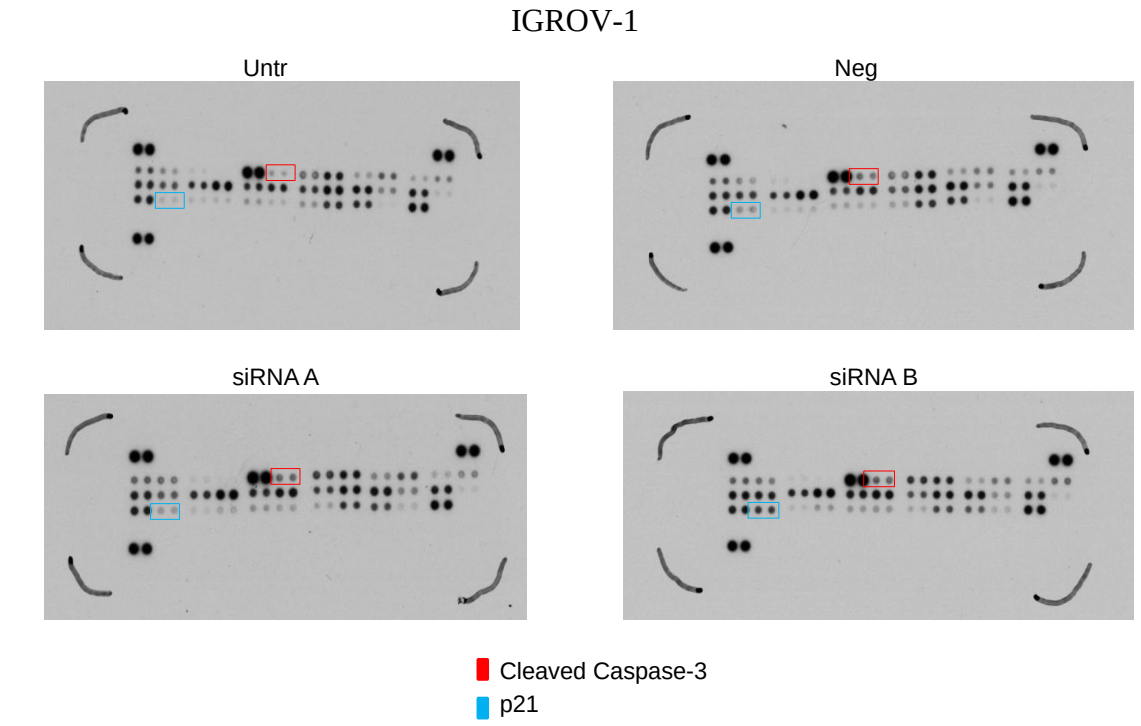


Figure S9. PSMC6 interaction network. The PSMC6 interaction network was built using STRING. The proteins functionally interacting with PSMC6 according to the results of the present study are reported. The thickness of the lines connecting the hubs represents the edge confidence.

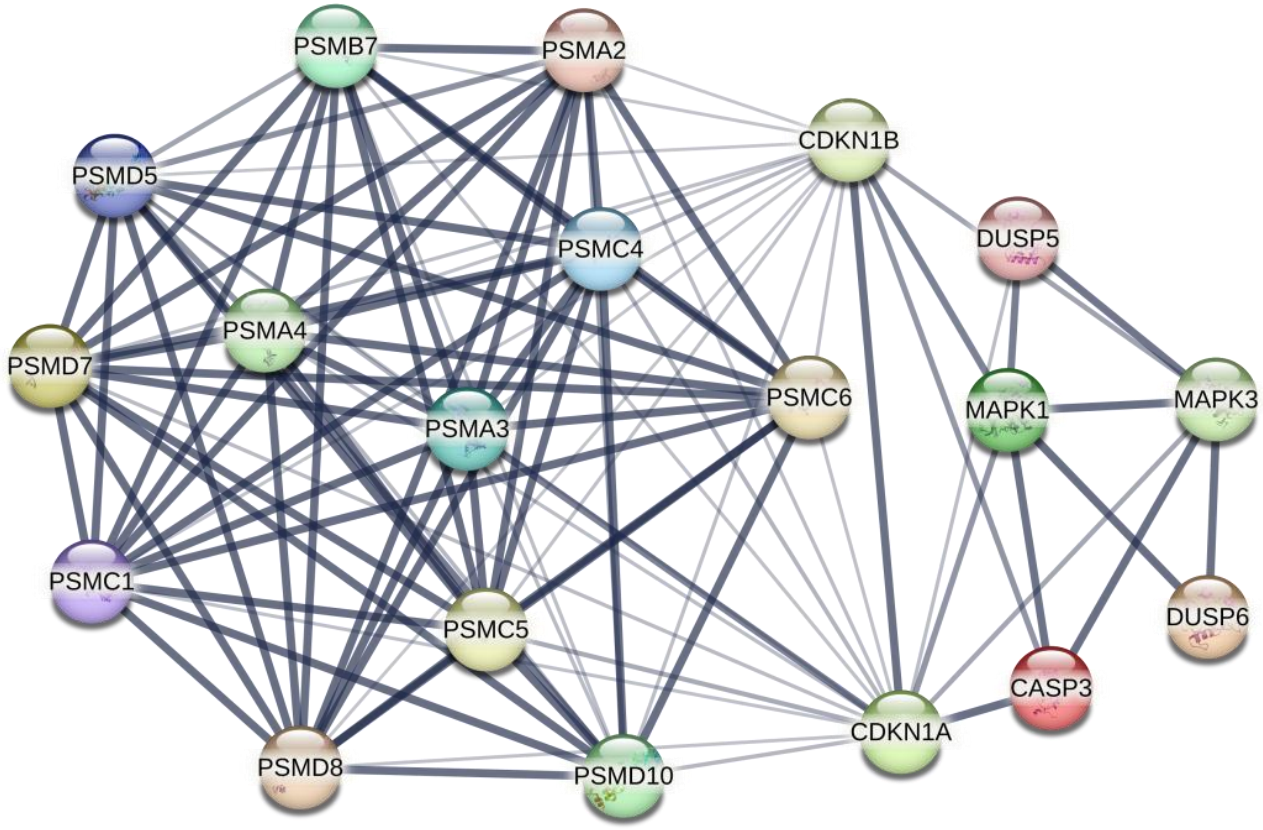


Figure S10. Sensitivity to proteasome inhibitors of IGROV-1/Pt1 (a) and IGROV-1 (b) cells following PSMC6 silencing. Cells were seeded and 24 h later silenced for 48 h with 100 nM siRNA A, siRNA B or Neg siRNA. Silenced cells were then harvested and seeded and 24 h later exposed to different concentrations of Bortezomib or bAP15 for 72h. Untr, untransfected cells; Neg siRNA, negative control siRNA transfected cells; siRNA A, cells transfected with siRNA A; siRNA B, cells transfected with siRNA B.

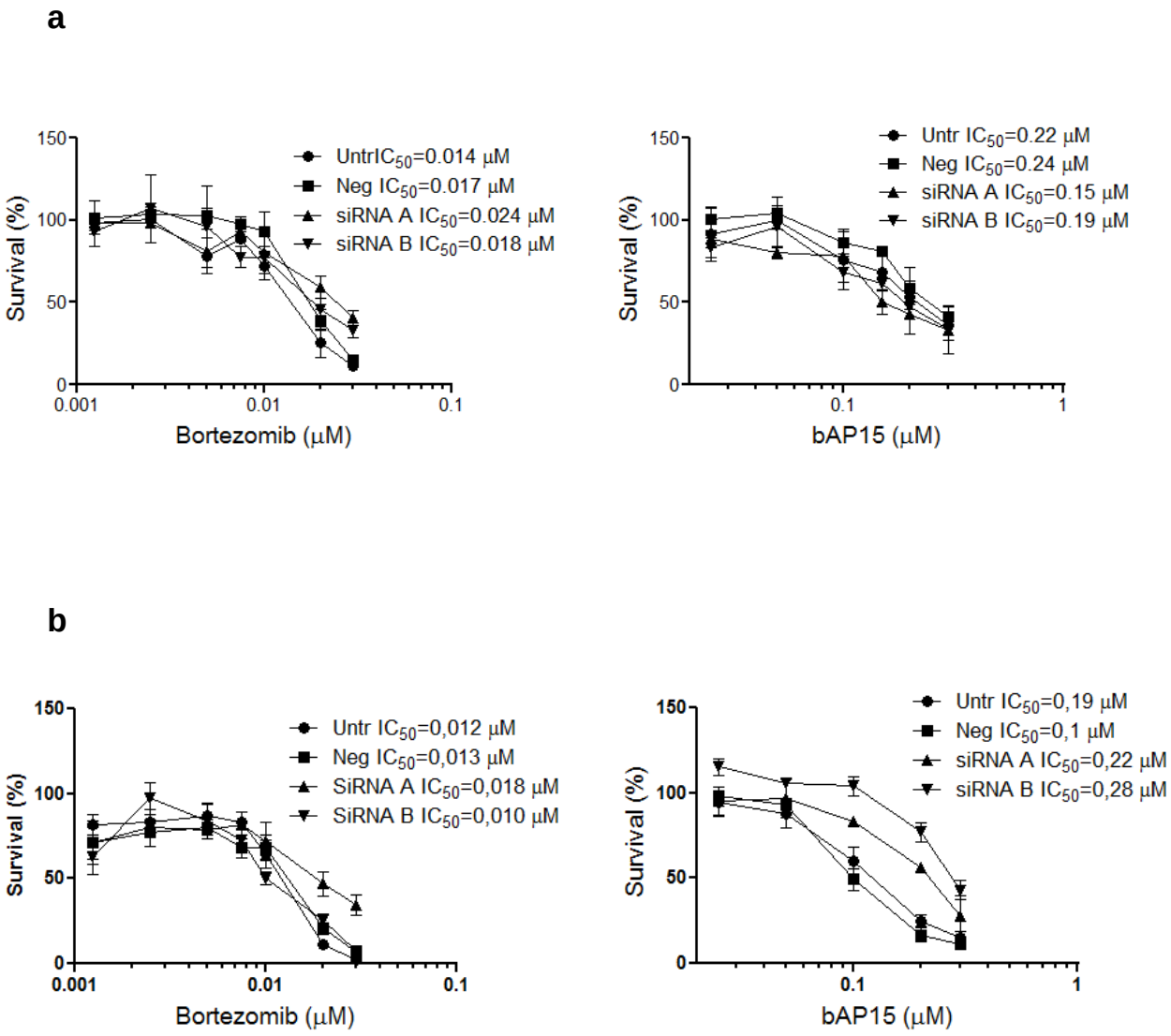
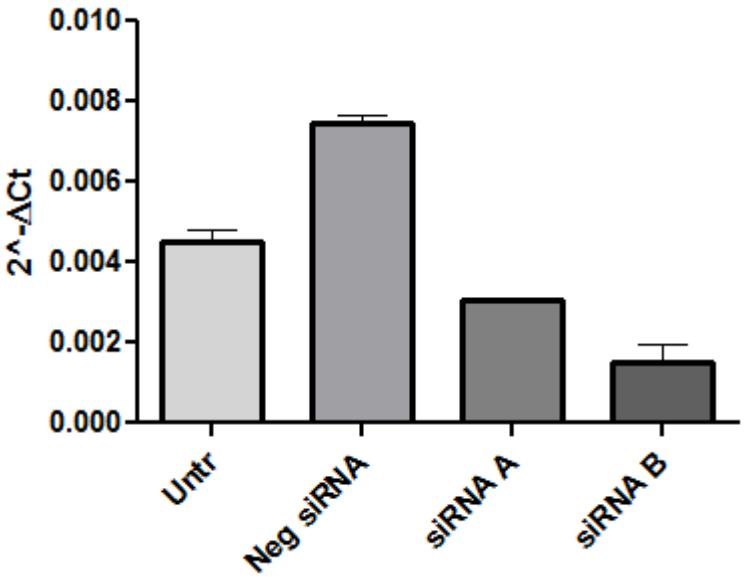


Figure S11. Phospho--ERK1/2 levels following PSMC6 silencing in serous OVCAR-5/Pt ovarian carcinoma cells. a) qRT-PCR of PSMC6 in silenced OVCAR-5/Pt cell lines. RNA of siRNA transfected cells was extracted and retrotranscribed into cDNA for RT-PCR for PSMC6 and GAPDH. **b)** Western blot assay of P-ERK1/2 and ERK in PSMC6 silenced OVCAR-5/Pt cells. Cells were harvested 72 h after transfection start for protein analysis. Cell lysates were analyzed by Western blotting. β-Tubulin represents control for loading.

a



b