SUPPLEMETARY DATA

shRNA sequences.

sh-LPAR$_1$: 5'-GCAATCGAGAGGCACATTACG-3'

sh-LPAR$_2$: 5'-GCCTACCTCTTCCTCATGTTC-3'

sh-LPAR$_3$: 5'-GGAATTGCCTCTGCAACATCT-3'

sh-Tiam1-1: 5'-GCAGGAGGCAGCATTCCTATA-3'

sh-Tiam1-2: 5'-GCACCTGTCAGAGAGCCAATT-3'

sh-SOS1-1: 5'-GCTGATGCCCAATCAGCTATT-3'

sh-SOS1-2: 5'-GCTTGATGTAACAATGCTACA-3'

sh-EPS8-1: 5-GCTTGATGCCAAGGGCAAAGT-3'

sh-EPS8-2: 5'-GCCAACTTCTAATCGCCATAT-3'

sh-ABI1-1: 5'-GGTATATTCGGAAACCTATCG-3'

sh-ABI1-2: 5'-GCACACTGTCGAGAACAAATC-3'

Plasmids. Luciferase shRNA lentiviral vector (pSi-Luc), retroviral vector (pBabe) encoding H-RasV12G and H-RasT17N were purchased from Addgene. Lentiviral vector encoding Rac1G12V, Rac1T17N, SOS1, EPS8 and ABI1 were prepared by subcloning the coding sequence into pCDH-puro vector (SBI).

Immunoblotting assay. Primary antibodies and titer used were as follows,

Rac mAb (Cat #：05-389; Millipore Corp.); titer: 1:1,000

Cdc42 mAb (Cat#: 610929; BD Biosciences); titer: 1:250

Rho mAb (Cat#: 610991; BD Biosciences); titer 1:250

SOS1 mAb (Cat#: sc-55528, Santa Cruz Biotechnology Inc); titer: 1:2,000

EPS8: mAb (Cat#: 610144; BD Biosciences); titer: 1:1,000

ABI1 mAb (Cat#: D147-3; MBL international Corporation); titer: 1:1,000

Tiam1 Polyclonal antibody (Cat#: A300-099A; Bethyl Laboratories Inc.); titer: 1:8,000

βactin polyclonal antibody (Cat#: JM-3662-100; MBL international Corp.); titer: 1:1,000

βPIX polyclonal antibody (Cat#: 4515; Cell Signaling Technology); titer: 1,000.

Other reagents. LPA (18:1) was purchased from Avantis Lipid (Alabaster, AL); cell culture medium, fetal calf serum and cell culture supplements were purchased from Hyclone (Waltham, MA). βPIX siRNA pool was purchased from Thermal-Fisher (Dharmacon).

Ras Activity Assays. Ras activation was measured with the Ras activity assay kit (Cellbio Labs). To determine the effect of LPA on Ras, cells (2×10^6 cells/10-cm dish) were serum-starved for two days and LPA (10μM) was then added to cells for various times. Cells were lysed and cell lysates analyzed for Ras activity.

Effect of βPIX on LPA-induced Rac activation. HEY and SK-OV3 cells were treated with 2 μM βPIX siRNA pool for 2 days and then starved for another 2 days. Cells were treated with 10μM LPA for 5 min, lysed and analyzed for Rac activity using the Rac/Cdc42 Activity Assay kit.

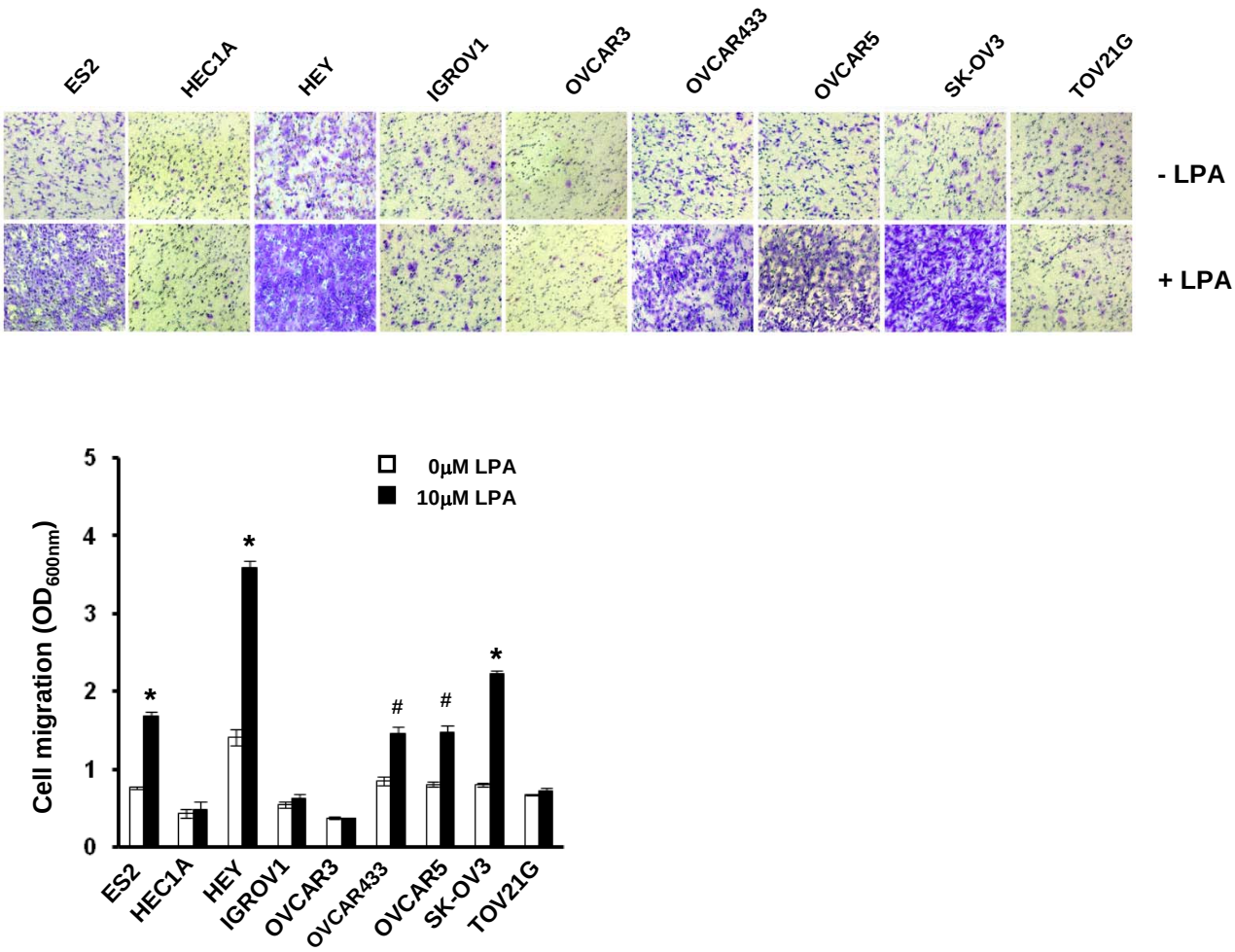

Fig.S1. LPA-stimulated ovarian cancer cell migration on laminin-coated surface. Cell migration was analyzed using Transwells with or without 10μM LPA contained in the lower chambers as described in Materials and Methods. The lower phase of transwells was coated with 10μg/ml laminin. Images are stained cells on the lower phase of transwells. Cell migration is presented as the OD_{600nm}. Data are means ± SE. n=3. *, $P < 0.001$ *vs* 0μM LPA; #, $P < 0.05$ *vs* 0μM LPA.

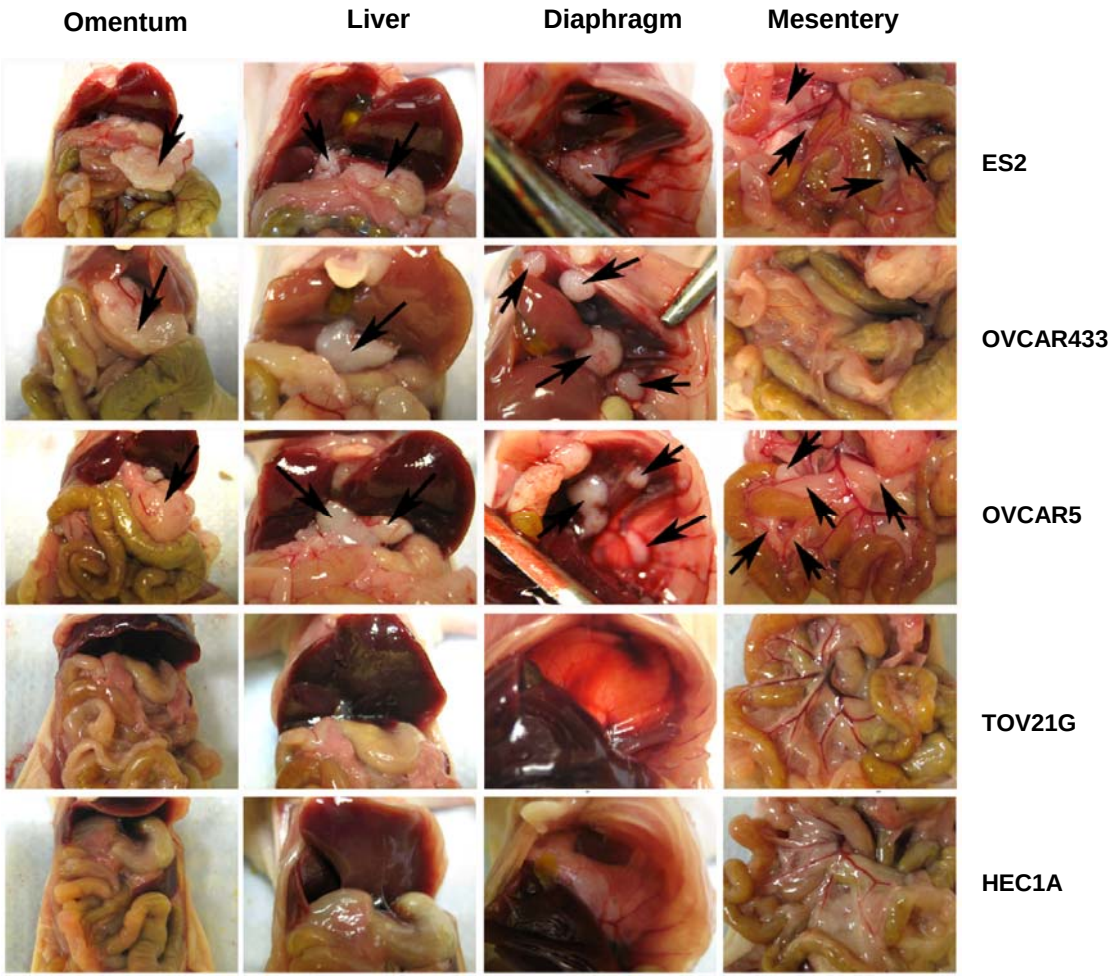

Fig.S2. Ovarian cancer cell lines with LPA migratory response can undergo peritoneal metastatic colonization. Peritoneal metastatic colonization assay. Cells were intraperitoneally injected into nude mice for 5 weeks to allow metastatic colonization. Images are the views of various areas in peritoneal cavity. Arrows point to metastatic implants.

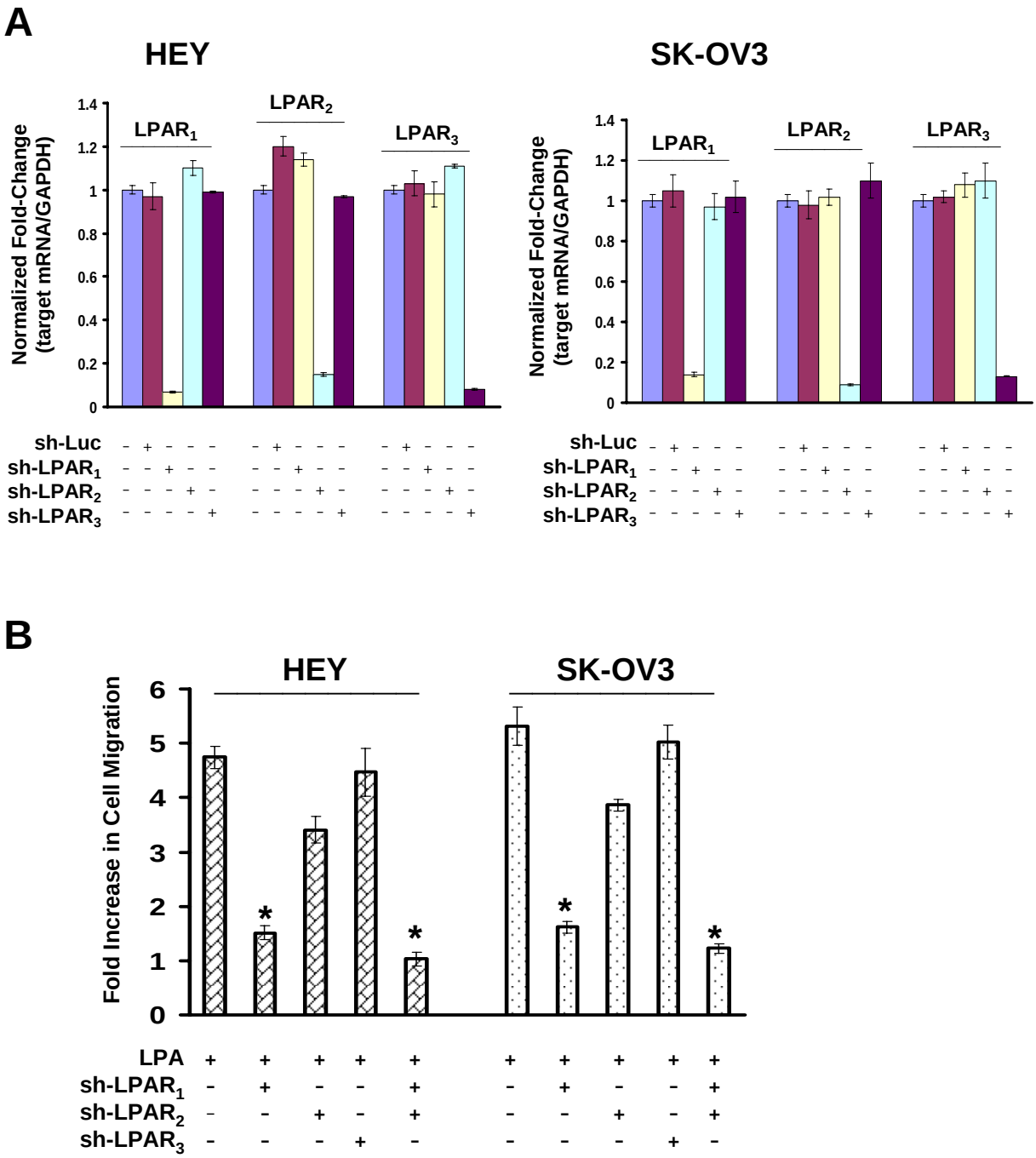

Fig.S3. Effect of LPA receptor knockdown on LPA-stimulated cell migration. ***A.*** $LPAR_1$, $LPAR_2$ and $LPAR_3$ shRNAs were lentivirally introduced into HEY or SK-OV3 cells. Total RNA was isolated and subjected to qRT-PCR to measure $LPAR_1$, $LPAR_2$ and $LPAR_3$ mRNA. GAPDH mRNA was used as an internal control for normalization. ***B.*** LPAR subtype knockdown HEY and SK-OV3 cells were analyzed for LPA-stimulated cell migration. Results are presented as Fold increase of cell migration [(LPA-stimulated cell migration)/(basal cell migration)]. Data are means ± SE. *, $P < 0.001$ *vs* luciferase shRNA.

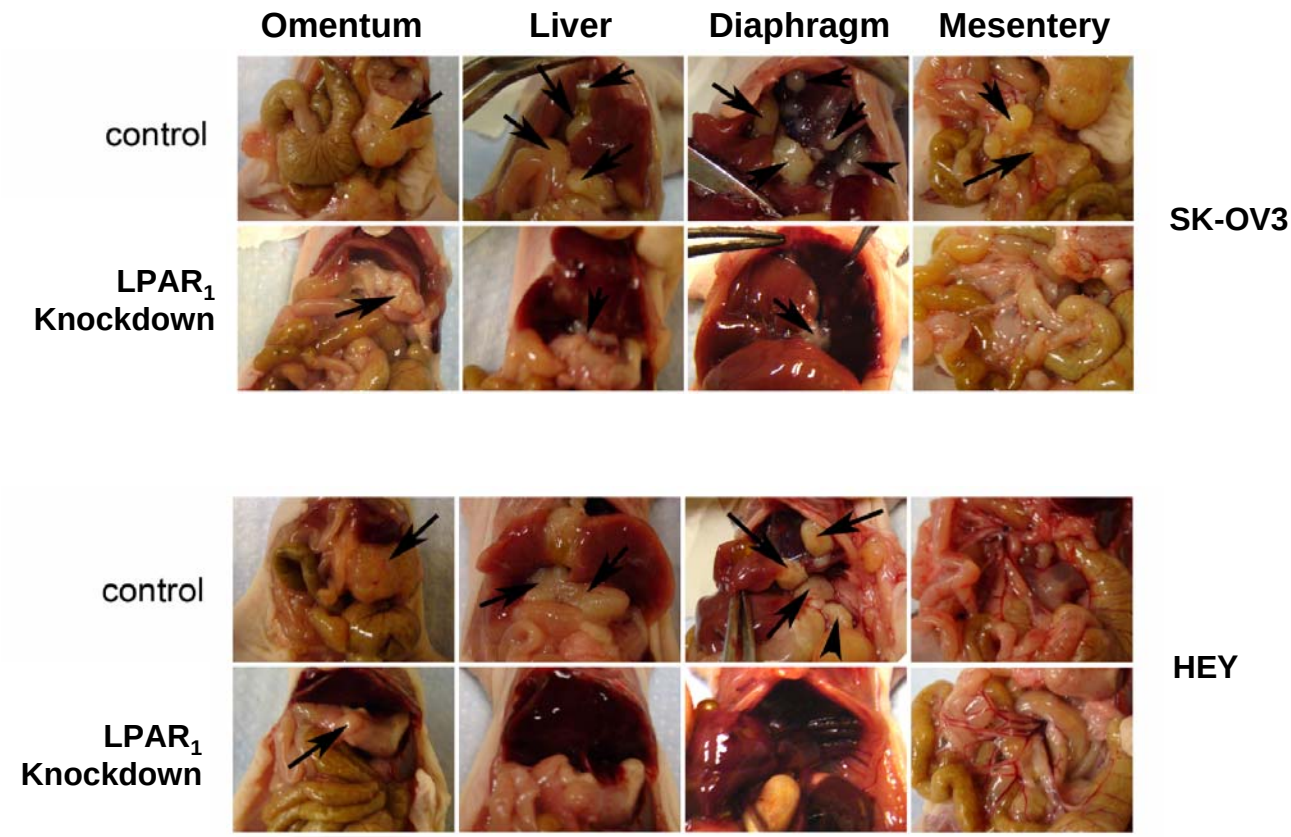

Fig.S4. Silencing $LPAR_1$ diminishes peritoneal metastatic colonization of SK-OV3 and HEY cells. Control (luciferase shRNA) or $LPAR_1$-knockdown cells were intraperitoneally injected to athymic female nude mice. Five weeks after injection, mice were sacrificed and peritoneal metastatic colonization was assessed. Images are the views of various areas in peritoneal cavity. Arrows point to metastatic implants.

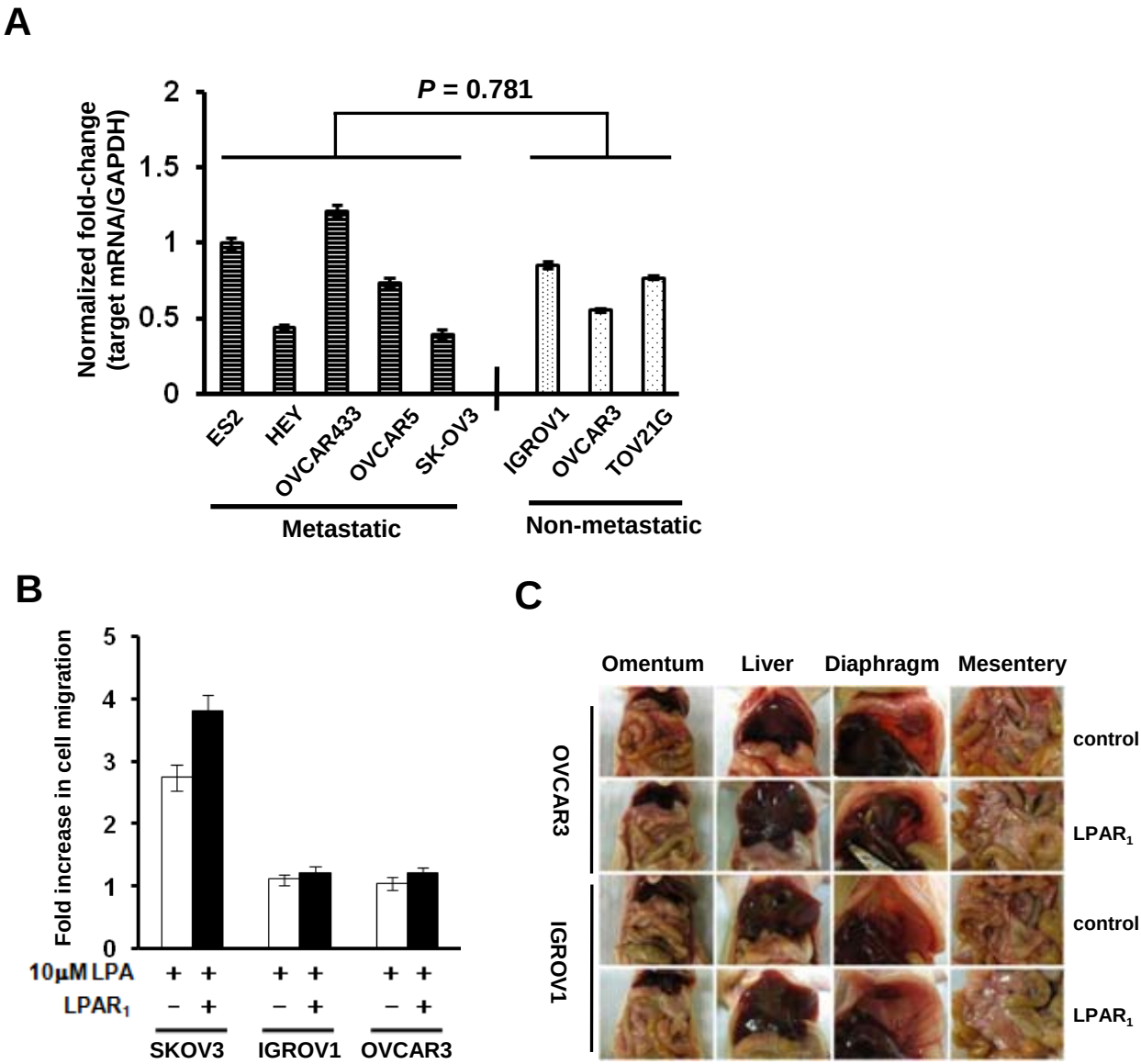

Fig.S5. Difference in LPA responsiveness between metastatic and non-metastatic ovarian cancer cell lines lays at downstream of $LPAR_1$. ***A.*** qRT-PCR of $LPAR_1$ mRNA in ovarian cancer cell lines. GAPDH mRNA was used as internal control for standardization. Data are the mean ± SE. n=3. Differences between metastatic and non-metastatic groups were assessed using Student *t* test. ***B.*** SK-OV3, IGROV1 and OVCAR3 cells were transduced with lentiviral vector containing $LPAR_1$. Transduced cells were analyzed for cell migration using Transwells with 10μg/ml Collagen I-coated lower phase. 10μM LPA was added into lower chambers as stimulant. Data are means ± SE. n=3. ***C.*** Control (empty vector) or $LPAR_1$-overexpressing cells were intraperitoneally injected to athymic female nude mice. Five weeks after injection, mice were sacrificed and peritoneal metastatic colonization was assessed. Images are the views of various areas in peritoneal cavity. There was no detectable intraperitoneal tumor implants.

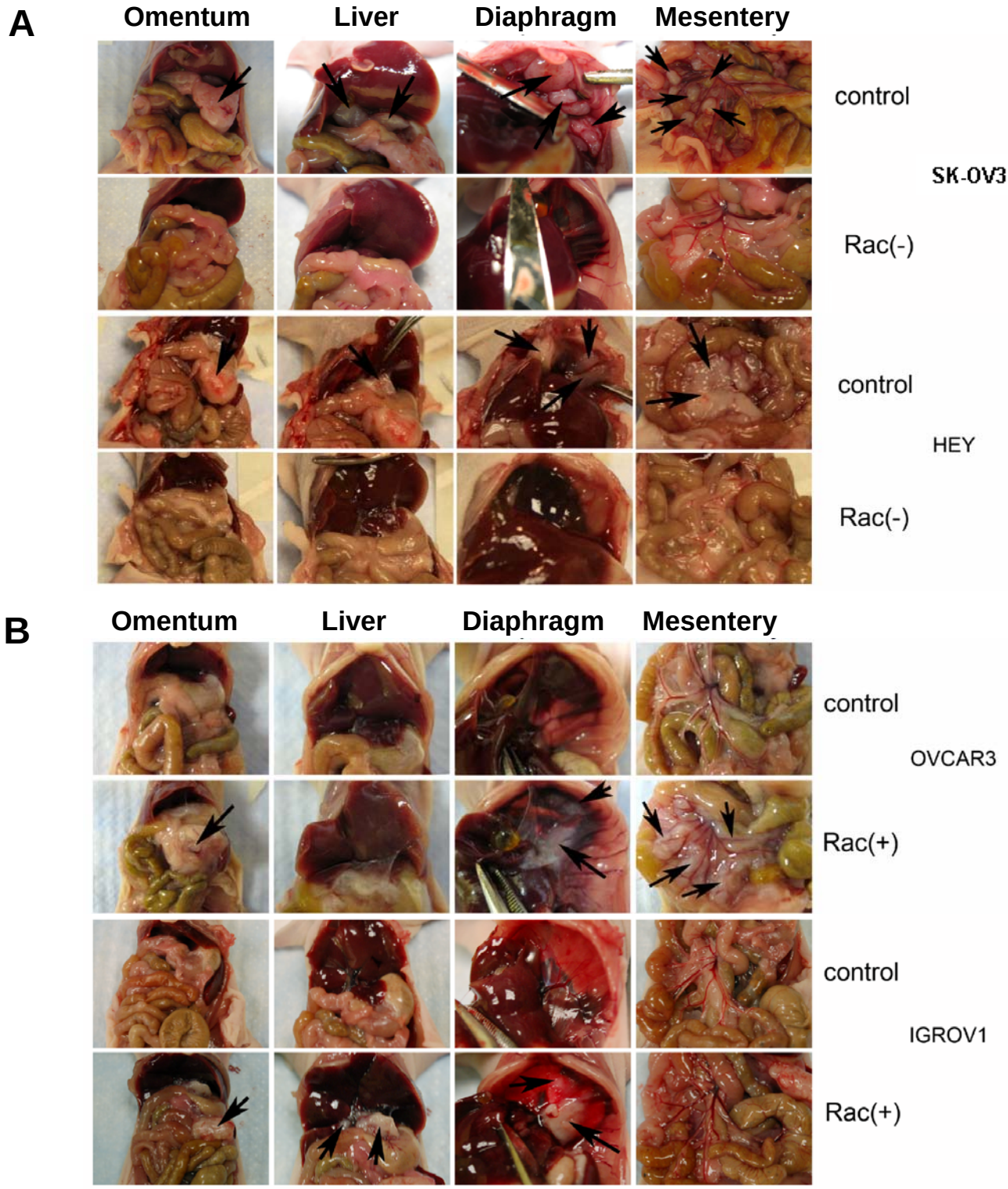

Fig.S6. Rac activity is essential for peritoneal metastatic colonization of ovarian cancer cells. (A) Control (empty vector) or dominant negative Rac1 (Rac1T17N)-expressing SK-OV3 or HEY cells were intraperitoneally injected to nude mice for 5 weeks to allow metastatic colonization. (B) Control (empty vector) or constitutively active Rac1 (Rac1G12V)-expressing OVCAR3 or IGROV1 cells were intraperitoneally injected to nude mice for 5 weeks to allow metastatic colonization. Images are the views of various areas in peritoneal cavity. Arrows point to metastatic implants.

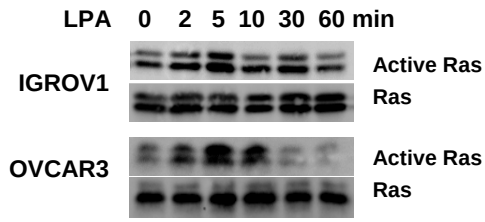

Fig.S7. LPA can effectively activate Ras in non-metastatic ovarian cancer cells. IGROV1 and OVCAR3 cells were stimulated with 10μM LPA for various times, then lysed and cell lysates analyzed for Ras activity using the Ras activity assay kit.

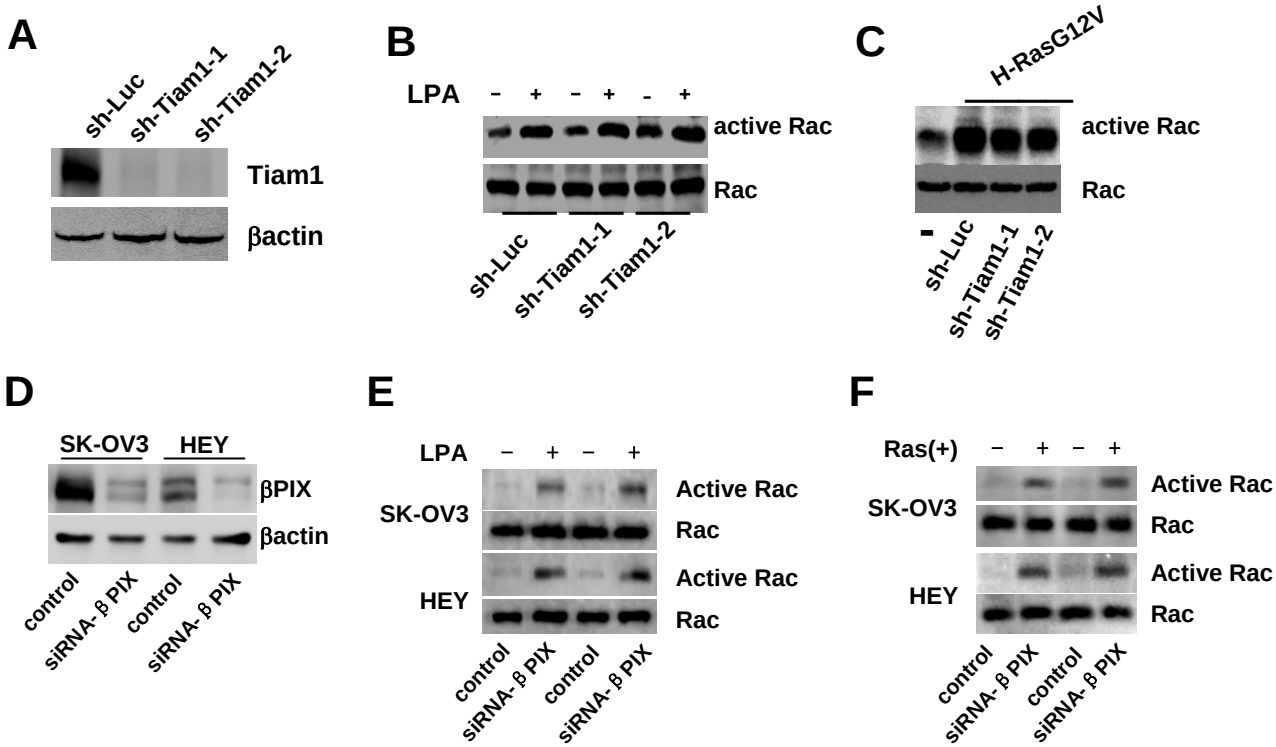

Fig.S8. Tiam1 and βPIX are not involved in LPA or Ras-induced Rac activation. ***A.*** SK-OV3 cells were lentivirally transduced with Tiam1 shRNAs for 4 days. Cells were lysed and cell lysates subjected to immunoblotting to detect Tiam1 and βactin expression with the respective antibodies. ***B.*** Control (luciferase shRNA) or Tiam1 shRNA-expressing SK-OV3 cells were stimulated with 10μM LPA for 5 min. Cells were lysed and cell lysates analyzed for Rac activity. An aliquot of cell lysate was also analyzed for Rac expression with anti-Rac mAb. ***C.*** H-RasG12V-expressing SK-OV3 cells were lentivirally transduced with luciferase or Tiam1 shRNAs for 4 days. Cells were lysed and cell lysates analyzed for Rac activity. "-", no transfection/transduction control. ***D.*** SK-OV3 and HEY cells were treated with 2μM βPIX siRNA pool for 4 days. Cells were lysed and cell lysates subjected immunoblotting to detect βPIX and βactin with the respective antibodies. ***E.*** Control or βPIX siRNA-treated SK-OV3 or HEY cells were stimulated with 10μM LPA for 5 min. Cells were lysed and cell lysate analyzed for Rac activity. ***F.*** H-RasG12V-expressing SK-OV3 or HEY cells were treated with 2μM βPIX siRNA pool for 4 days. Cells were lysed and cell lysate analyzed for Rac activity.

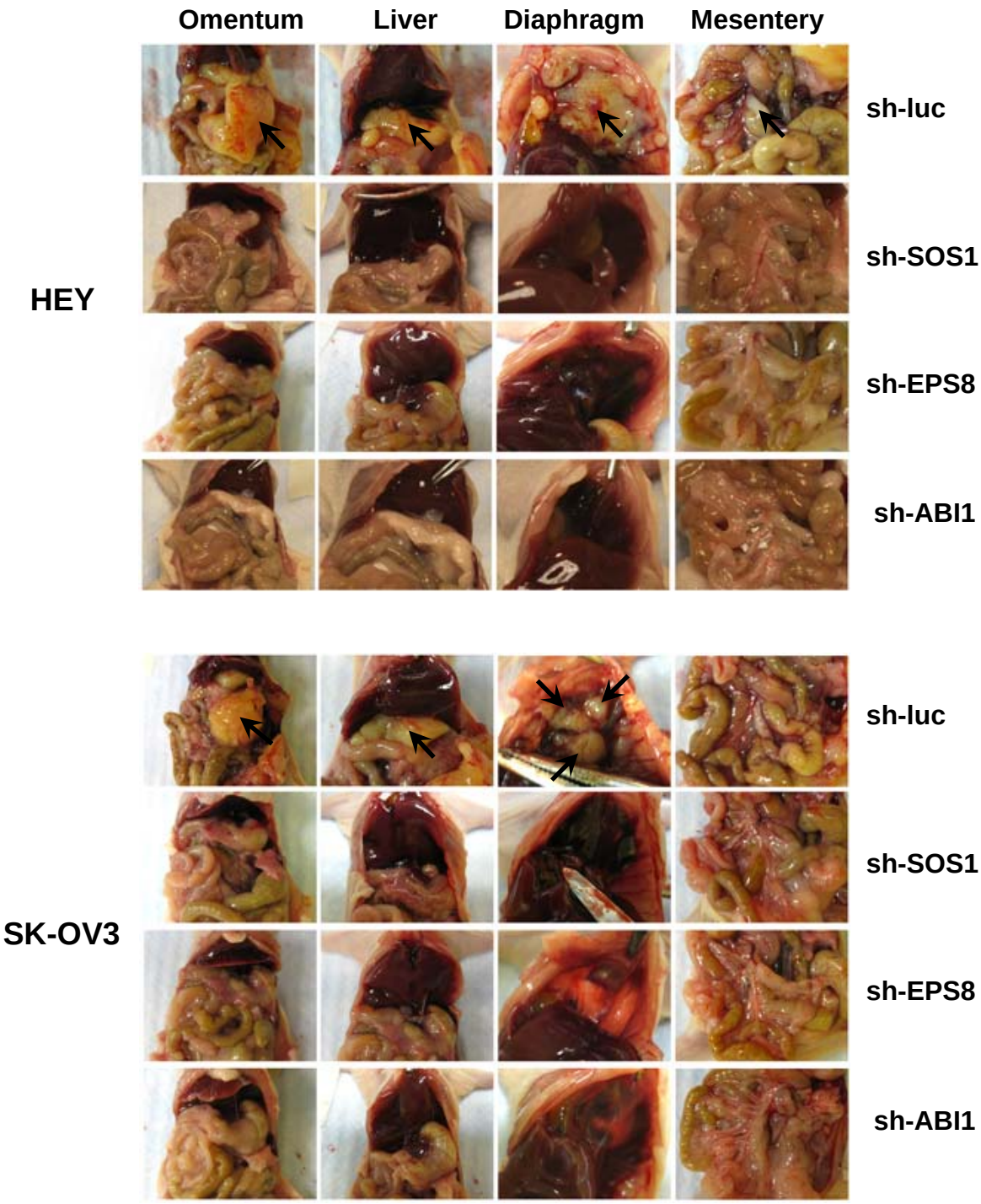

Fig.S9. Integrity of SOS1/EPS8/ABI1 tri-complex is essential for peritoneal metastatic colonization of ovarian cancer cells. Control (luciferase shRNA), SOS1-, EPS8- or ABI1-knockdown HEY and SK-OV3 cells were intraperitoneally injected to nude mice for 5 weeks to allow metastatic colonization. Images are the views of various areas in peritoneal cavity. Arrows point to metastatic implants.

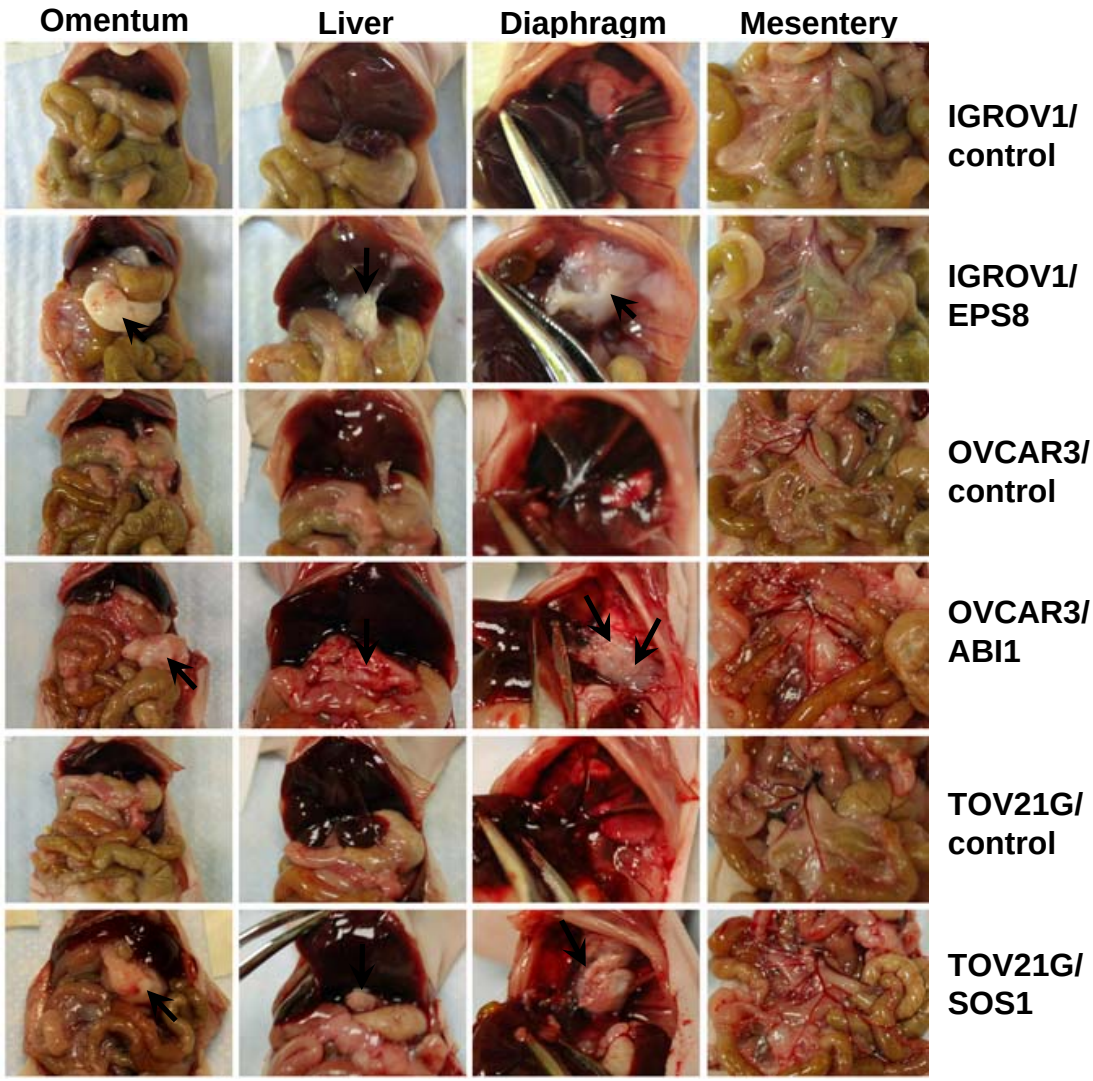

Fig.S10. Re-expressing the missing member of SOS1/EPS8/ABI1 tri-complex converts non-metastatic ovarian cancer cells to the metastatic ones. IGROV1/EPS8, OVCAR3/ABI1 and TOV21G/SOS1 as well as their empty vector-transduced control cells were intraperitoneally injected to nude mice for 5 weeks to allow metastatic colonization. Images are the views of various areas in peritoneal cavity. Arrows point to metastatic implants.

Table S1. Clinicopathological parameters of Ovarian Cancer Patients

Age	Tpye	Stage	Grade	survival status	survival time (month)	ABI1	EPS8	SOS1	Co-expression
62	Serous	I	well	live	84	+	-	-	No
36	Serous	III	poor	deceased	63	+	+++	++	Yes
32	Serous	I	well	deceased	10	-	+	-	No
22	Endometroid	I	well	deceased	5	++	+	-	No
22	Mucinous	II	moderate	live	44	+	+	+	Yes
50	Serous	III	pooor	deceased	9	+	++	+	Yes
46	Serous	III	poor	live	36	+	++	+	Yes
40	Serous	III	moderate	deceased	4	+++	+	++	Yes
46	Mucinous	I	well	live	36	++	+	-	No
66	Serous	III	poor	deceased	14	-	-	-	No
28	Serous	I	moderate	live	84	++	+	-	No
43	Endometroid	III	moderate	lost	lost	++	++	-	No
69	Serous	III	poor	deceased	48	-	-	-	No
43	Serous	III	poor	deceased	21	+++	+	+	Yes
55	Serous	III	poor	deceased	3	+++	+	+	Yes
50	Serous	I	moderate	live	84	-	+	-	No
53	Serous	II	well	live	84	++	-	++	No
33	Serous	I	well	live	77	-	-	-	No
55	Endometroid	III	poor	deceased	60	++	-	-	No
46	Serous	III	moderate	lost	lost	+++	+	-	No
51	Serous	III	moderate	deceased	31	+++	++	++	Yes
63	Serous	III	moderate	live	84	+++	+++	-	No
33	Serous	I	moderate	deceased	24	++	-	+	No
50	Serous	II	well	deceased	15	+++	+	-	No
65	Undifferentiated	I	well	deceased	7	++	-	-	No
56	Mucinous	I	moderate	live	74	++	-	-	No
50	Undifferentiated	IV	poor	live	13	+++	+	+	Yes
49	Serous	III	well	live	69	+++	+++	++	Yes
27	Serous	I	well	live	68	-	-	-	No
39	Endometroid	I	moderate	live	68	+++	+	+	Yes
53	Serous	III	moderate	live	66	+++	-	-	No
48	Serous	IV	well	deceased	35	-	-	-	No
58	Serous	III	moderate	deceased	6	+++	++	+	Yes
49	Serous	IV	poor	deceased	21	+++	+	+	Yes
68	Serous	III	poor	deceased	18	-	-	-	No

57	Serous	II	poor	live	66	-	-	-	No
55	Serous	II	poor	deceased	7	+++	-	-	No
71	Serous	IV	well	live	61	+++	++	-	No
46	Endometroid	IV	well	lost	lost	+++	+	+	Yes
37	Clear cell	III	moderate	deceased	7	-	-	-	No
43	Serous	I	moderate	live	49	++	-	+	No
52	Serous	III	poor	live	53	+++	++	-	No
70	Serous	III	poor	deceased	39	+++	++	+++	Yes
43	Serous	IV	well	deceased	44	+++	++	+++	Yes
39	Endometroid	III	poor	live	48	+++	-	-	No
42	Serous	IV	poor	deceased	43	+++	+	+	Yes
49	Clear cell	II	well	lost	lost	+++	-	-	No
84	Mucinous	I	moderate	live	44	-	+	+	No
54	Serous	II	poor	live	48	+++	+	+	Yes
57	Serous	IV	moderate	deceased	22	+	+	+	Yes
23	Serous	I	well	live	43	++	-	-	No
60	Endometroid	II	moderate	deceased	15	+++	+	+	Yes
52	Serous	IV	well	live	41	+++	+	+++	Yes
55	Endometroid	III	moderate	live	42	+++	-	-	No
56	Serous	III	well	live	39	-	-	-	No
52	Serous	II	moderate	live	44	-	+	-	No
60	Serous	III	well	deceased	14	++	++	+++	Yes
50	Serous	III	moderate	deceased	24	+	++	+	Yes
54	Serous	II	poor	live	36	-	-	-	No
60	Serous	III	moderate	deceased	10	-	-	-	No
50	Serous	III	well	deceased	4	+++	+	++	Yes
59	Undifferentiated	IV	moderate	live	36	+++	+	+	Yes
45	Serous	IV	well	live	36	+++	+	+	Yes
52	Endometroid	I	moderate	live	36	-	++	+	No

Lost: patient that was lost on track.

“-”, negative staining; “+”, “++” and “+++” are positive staining (10-25%, 25-50% and >50% cells with positive staining)

Yes: positive for co-expression of SOS1, EPS8 and ABI1.
No: devoid of at least one member of SOS1/EPS8/ABI1 tri-complex.

Table S2. Correlation between the Status of SOS1, ABI1 or EPS8 Staining and Clinicopathological Parameters of Ovarian Cancer Patients

	No.	SOS1 (%)	*P* value	ABI1 (%)	*P* value	EPS8 (%)	*P* value
Age *			0.313		0.194		0.081
≤ 50	33	18 (54.5%)		27 (81.8%)		24 (72.7%)	
> 50	31	13 (41.9%)		21 (67.7%)		16 (51.6%)	
Histological types			0.817		0.411		0.999
Serous	38	20 (52.6%)		27 (71.1%)		24 (63.2%)	
Mucinous	13	5 (38.5%)		9 (69.2%)		8 (61.5%)	
Endometroid	8	4 (50.0%)		7 (87.5%)		5 (62.5%)	
Clear cell and undifferentiated	5	2 (40.0%)		5 (100.0%)		3 (60.0%)	
Pathologic Grade			0.510		0.983		0.565
Well differentiated	21	8 (38.1%)		16 (76.2%)		12 (57.1%)	
Moderately differentiated	24	6 (54.2%)		18 (75.0%)		17 (70.8%)	
Poorly differentiated	19	10 (52.6%)		14 (73.7%)		11 (57.9%)	
Stage			0.069		0.382		0.137
I	16	5 (31.3%)		10 (62.5%)		8 (50.0%)	
II	10	4 (40.0%)		7 (70.0%)		5 (50.0)	
III	27	13 (48.1%)		21 (77.8%)		17 (63.0%)	
IV	11	9 (81.8%)		10 (90.9%)		10 (90.9%)	

* medium age, 51 (27 ~ 69)