Malignant pleural effusion and ascites induce epithelial–mesenchymal transition and cancer stem-like cell properties via VEGF/PI3K/Akt/mTOR pathway

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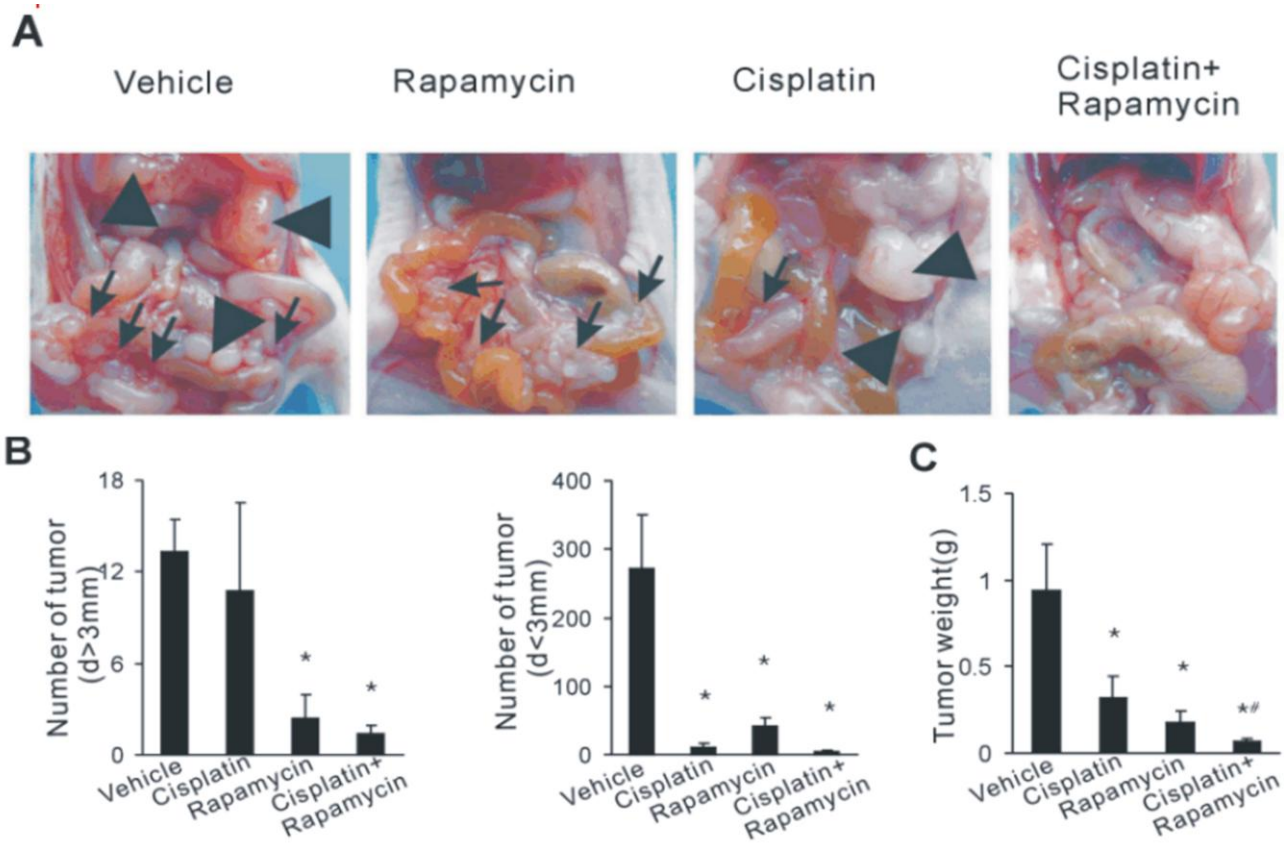
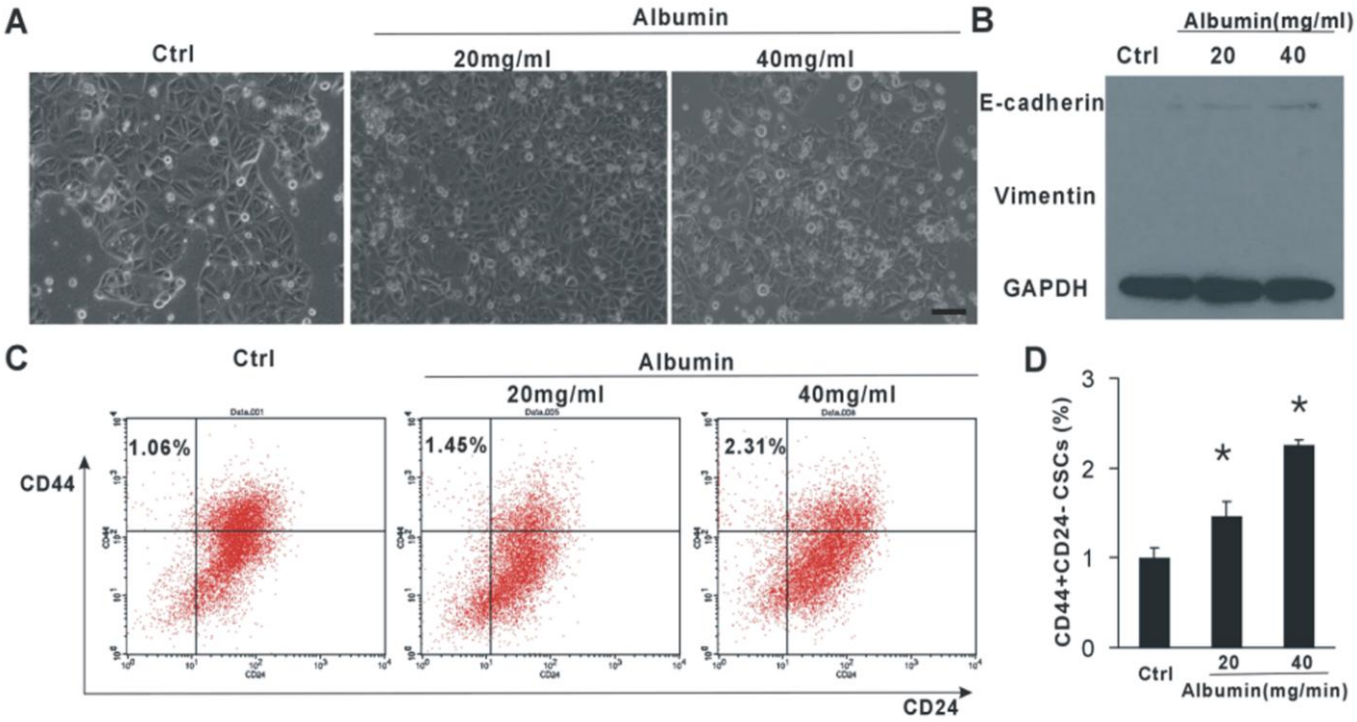
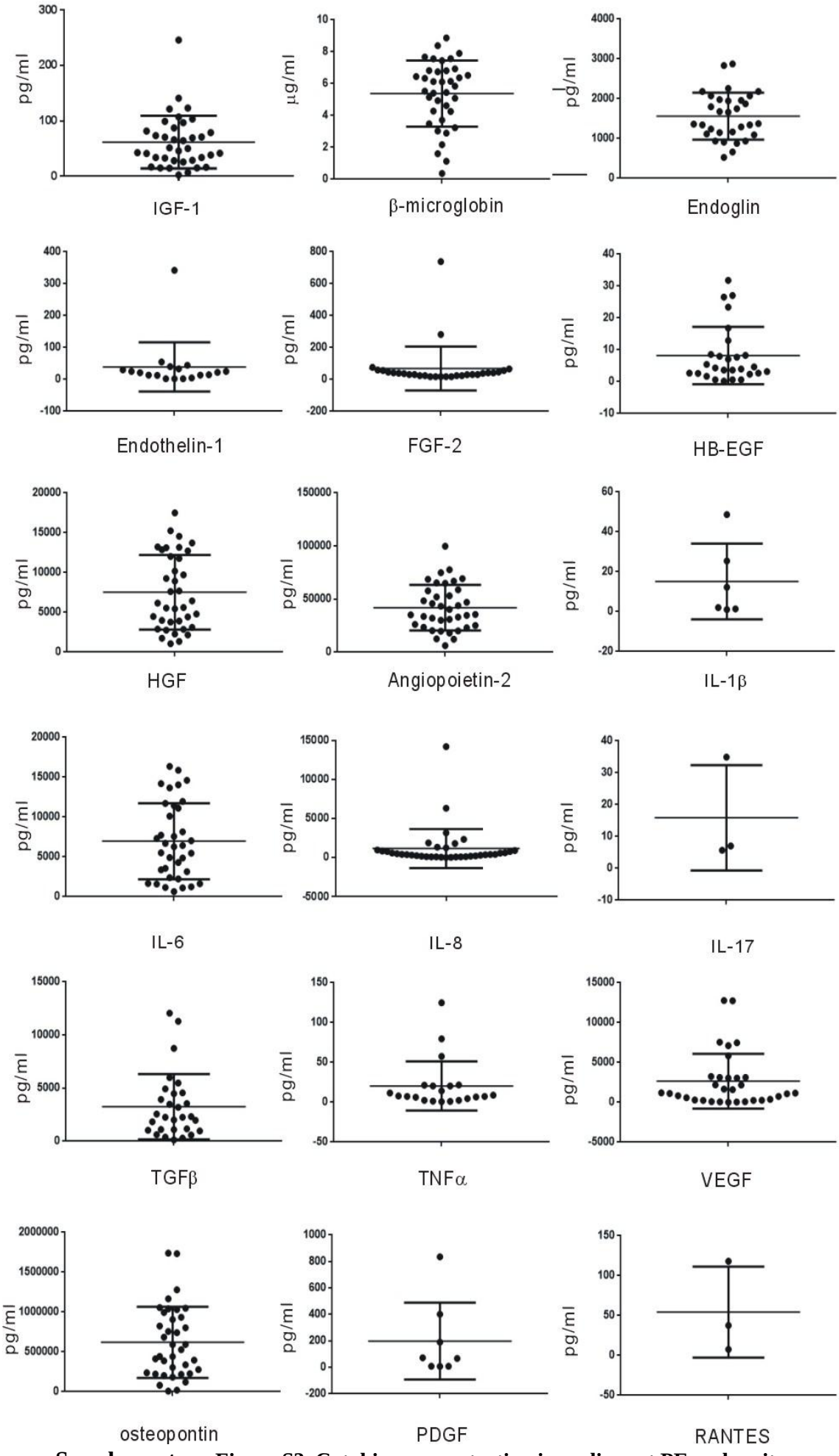
Supplementary Information



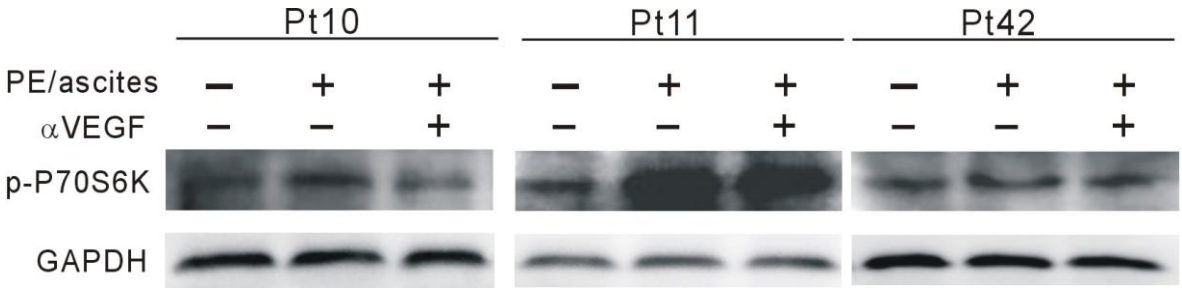
Supplementary Figure S1. Targeting PI3K/AKT/mTOR pathway is effective for ascetic ovarian cancers. A, BALB/c-nude mice bearing SKOV3 ovarian cancer were treated with vehicle, cisplatin, rapamycin, and cisplatin plus rapamycin, respectively. Representative pictures of tumors in peritoneal cavity with large (arrowhead) and small (arrow) diameters. **B**, the numbers of large (left panel) and small (right panel) tumors was depicted for the respective treatment groups (n=3-5). *, $P < 0.05$. **C**, tumor weight was depicted for the respective treatment groups (n=3-5). *, $P<0.05$ versus vehicle, #, $P<0.05$ versus rapamycin and cisplatin.



Supplementary Figure S2. Albumin does not induce epithelial-mesenchymal transition in tumor cells. A, representative phase contrast images of human breast cancer cells MCF-7 cultured with various concentrations of human albumin for twenty days. Scale bar, 50 μm. **B**, expression of E-cadherin and vimentin in MCF-7 cells treated with albumin. **C-D**, flow cytometry analysis of MCF-7 cells treated with albumin and their parental cells for CD44 and CD24. CD44+/CD24- stem cells were quantified. *, $P < 0.05$.



Supplementary Figure S3. Cytokine concentration in malignant PE and ascites.



Supplementary Figure S4. VEGF neutralization reversed the mTOR pathway activation induced by malignant PE and ascites. MCF-7 cells were treated with malignant PE and ascites for 30 min in the absence or presence of 10μg/ml VEGF antibody. Cell lysates were analyzed by western blot with antibodies against p-P70S6K. GAPDH served as the loading control.

Supplementary Table S1. Characteristics of human malignant and ascites specimens.

Patients	Sex	Age (years)	Cancer type	Effusion type
1	F	42	Breast Cancer	Pleural effusion
2	F	72	Lung Cancer	Pleural effusion
3	M	59	Gastric Cancer	Ascites
4	M	17	Lymphoma	Pleural effusion
5	M	59	Colorectal Cancer	Pleural effusion
6	M	76	Lung cancer	Pleural effusion
7	F	65	Lung Cancer	Pleural effusion
8	F	73	Breast Cancer	Pleural effusion
9	F	50	Lung Cancer	Pleural effusion
10	F	48	Colorectal Cancer	Pleural effusion
11	F	53	Lung Cancer	Ascites
12	M	68	Lung Cancer	Pleural effusion
13	M	76	Lung Cancer	Pleural effusion
14	F	36	Lung Cancer	Pleural effusion
15	M	51	Peritoneal mesothelioma	Ascites
16	M	47	Lung Cancer	Pleural effusion
17	F	62	Lung Cancer	Pleural effusion
18	M	43	Colorectal Cancer	Ascites

19	M	65	Lung Cancer	Pleural effusion
20	F	72	Ovarian Cancer	Ascites
21	F	25	Lymphoma	Pleural effusion
22	M	90	Lymphoma	Pleural effusion
23	F	44	Lung Cancer	Pleural effusion
24	F	45	Gastric Cancer	Pleural effusion
25	M	48	Gastric Cancer	Ascites
26	M	47	Peritoneal mesothelioma	Ascites
27	M	69	Lung Cancer	Pleural effusion
28	M	55	Hepatocarcinoma	Ascitis
29	M	43	Lung Cancer	Pleural effusion
30	F	67	Liver metastatic adenocarcinoma	Ascites
31	M	51	Left axillary lymph nodes metastatic adenocarcinoma	Pleural effusion
32	M	55	Lung Cancer	Pleural effusion
33	F	73	Lung Cancer	Pleural effusion
34	M	35	Lung Cancer	Pleural effusion
35	M	38	Lung Cancer	Pleural effusion
36	F	60	Ovarian Cancer	Ascites
37	F	64	Lung Cancer	Pleural effusion
38	F	48	Colorectal Cancer	Pleural effusion
39	F	53	Lung Cancer	Pleural effusion
40	M	71	Lung Cancer	Pleural effusion
41	F	55	Lung Cancer	Pleural effusion
42	M	38	Lung Cancer	Pleural effusion
43	M	55	Gastric Cancer	Ascites
44	M	66	Colorectal Cancer	Ascites
45	M	59	Gastric Cancer	Ascites
46	M	46	Lung Cancer	Pleural effusion
47	F	51	Lung Cancer	Pleural effusion

Supplementary Table S2. Primer sequences in the study are listed below.

gene	Forward	Reverse
Sox2	AGAACCCCAAGATGCACAAC	CGGGGCCGGTATTTATAATC
ABCB1	AGGTTCCAGGATTGGCGTCTT	CCAGTCATTGCTGCGGTTTCA
ABCG2	AATACATCAGCGGATACTACAGAG	AGCCACCATCATAAGGGTAAACAT
Patch	AAACCTCCTTTGCGGTGGACAAAC	TGTAACCATGACCAACCTCAGCCT
Smo	ACCTATGCCTGGCACACTTC	GTGAGGACAAAGGGGAGTGA
Gli-1	CCCAATCACAAGTCAGGTTCCT	CCTATGTGAAGCCCTATTTGCC
Nodal	AGCATGGTTTTGGAGGTGAC	CCTGCGAGAGGTTGGAGTAG
Cripto-1	TCCTTCTACGGACGGAACTG	ATCACAGCCGGGTAGAAATG
Activin	AAAGCTTCATGTGGGCAAAG	AATCTCGAAGTGCAGCGTCT
GAPDH	CAGGAGCGAGATCCCT	GGTGCTAAGCAGTTGGT