

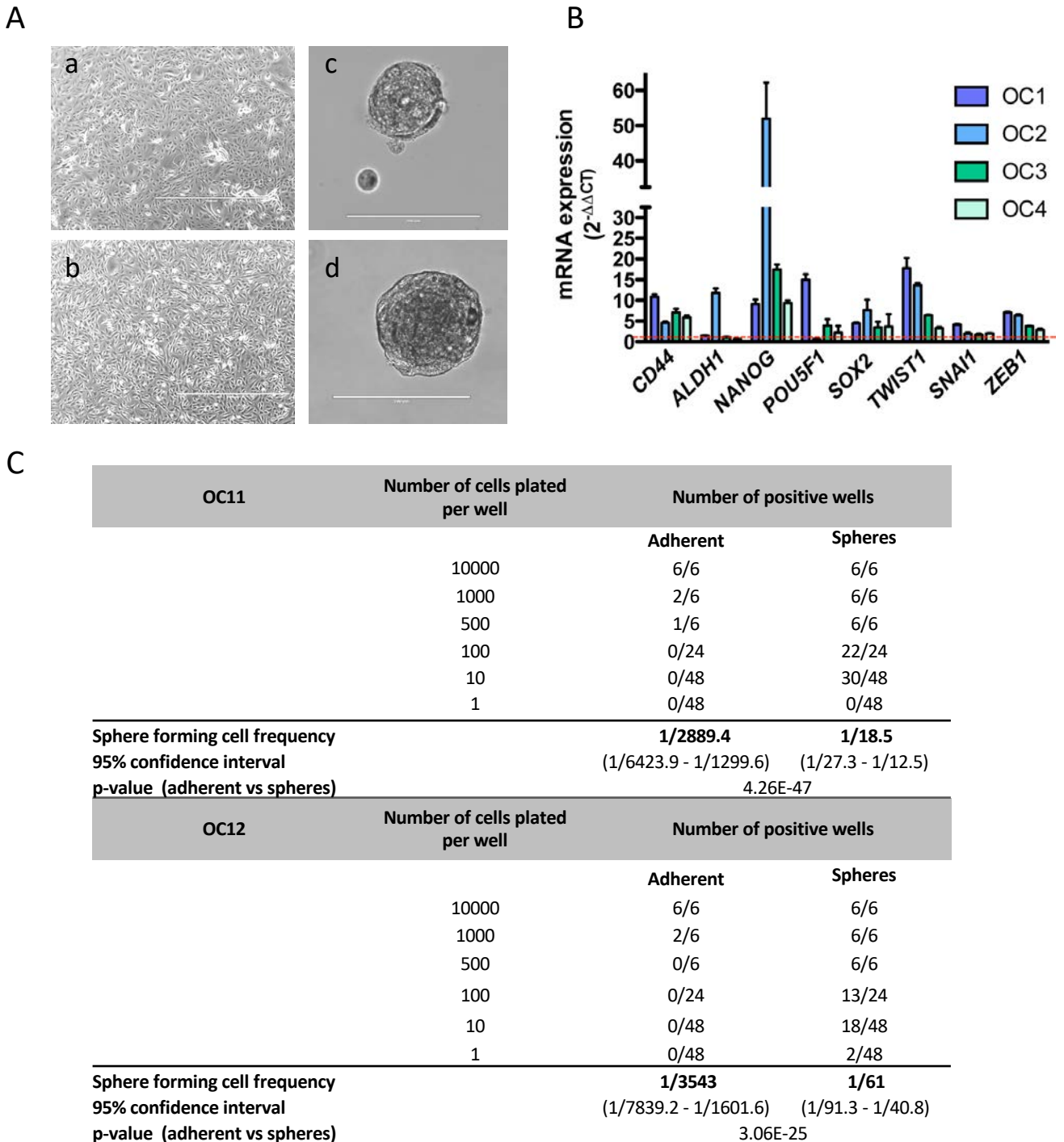
**Stem Cell Reports, Volume 10**

**Supplemental Information**

**CD73 Regulates Stemness and Epithelial-Mesenchymal Transition in  
Ovarian Cancer-Initiating Cells**

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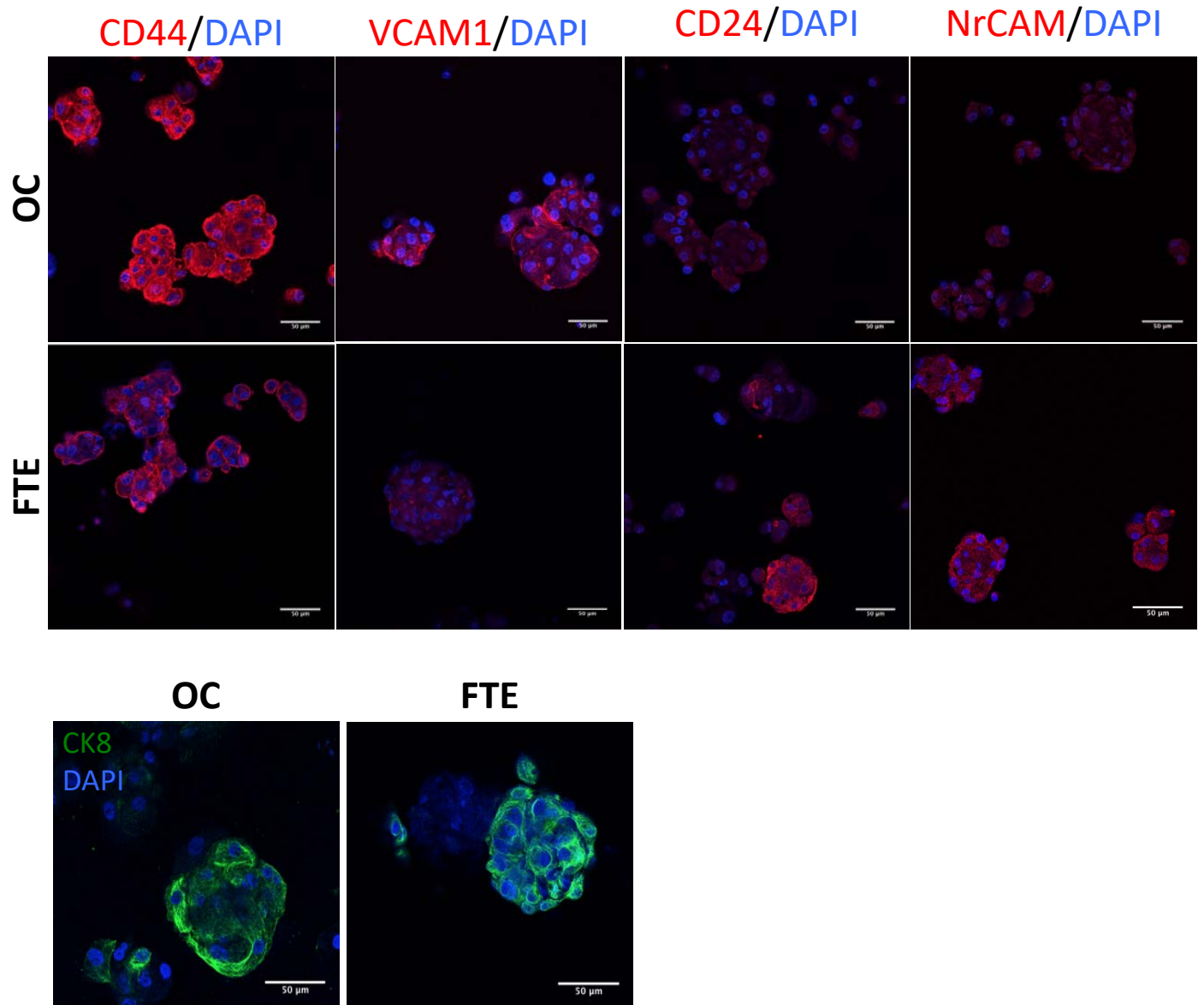
# Figure S1



**Figure S1. Primary cultures as adherent cells or spheres and enrichment for stem cells in OC-derived spheres.**

(A) Primary cultures were established and grown under adherent conditions from fallopian tube epithelium (FTE) (a) and from ovarian cancer (OC) (b). Scale bars, 400  $\mu$ m. Spheres were obtained from FTE (c) or OC primary cells (d), as described in Materials and Methods. Representative images are shown. Scale bar, 200  $\mu$ m. (B) Matched spheres and adherent cultures from four different OC samples were analyzed for the expression of genes related to stemness and EMT. Columns represent the relative mRNA expression ( $2^{-\Delta\Delta CT}$ ) of reported genes in second-generation spheres as compared to their adherent counterpart. Dashed red line indicates gene expression in control cells. The experiment was performed in triplicate and data are expressed as means  $\pm$  SD. (C) Extreme limiting dilution assay (ELDA) was carried out on two different OC samples. Briefly, adherent or sphere-derived OC cells (OC11, top panel, and OC12, bottom panel) were subjected to sphere formation assay under limiting dilution conditions, ranging from  $10^4$  to 1 cell/well. At day 7, the number of positive wells containing spheres was recorded and plotted using online ELDA analysis tool (<http://bioinf.wehi.edu.au/software/elda>). Related to Figure 1.

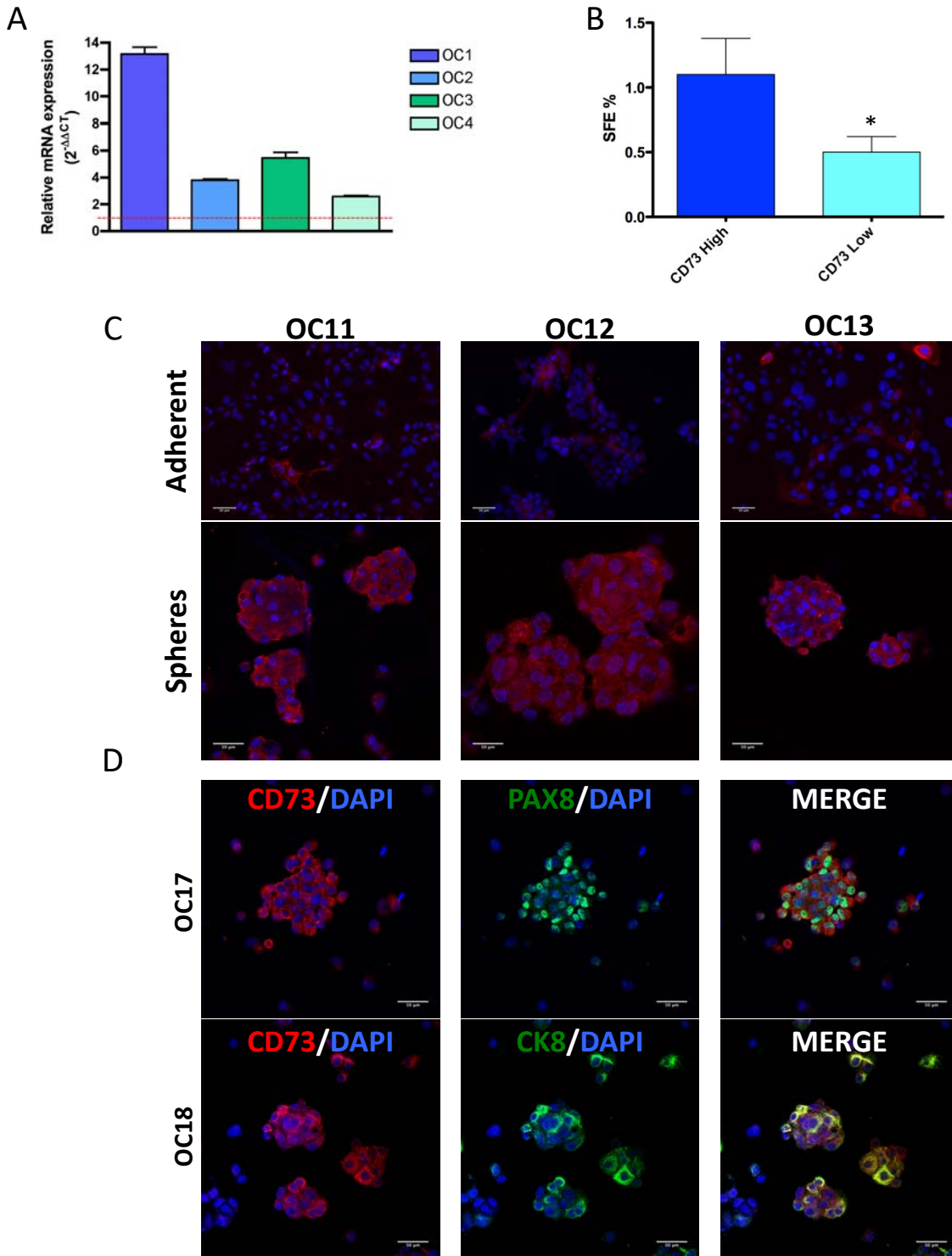
**Figure S2**



**Figure S2. Validation of selected genes from cDNA microarray.**

Representative images of second-generation spheres derived from primary samples (OC and FTE) stained for the indicated proteins. Nuclei were counterstained with DAPI. OC-derived spheres showed higher levels of CD44 and VCAM1 and lower levels of CD24 and NrCAM as compared to FTE-derived spheres, thus confirming the results of cDNA microarrays. Cytokeratin 8 (CK8), an established marker of epithelial cells, was abundant in all spheres, confirming the purity and the epithelial nature of primary cells. Scale bars, 50 μm. The experiment was performed on three independent samples with similar results. Related to Figure 1.

# Figure S3

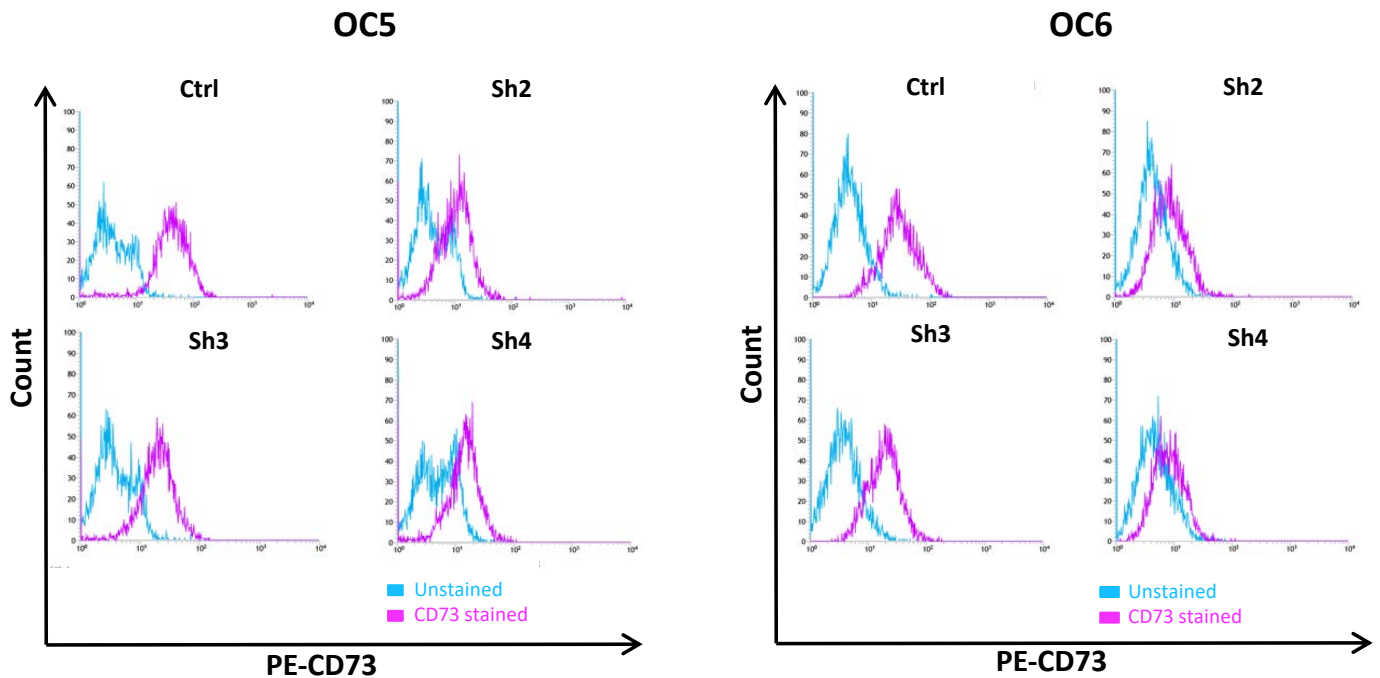


**Figure S3. CD73 is enriched in OC spheres.**

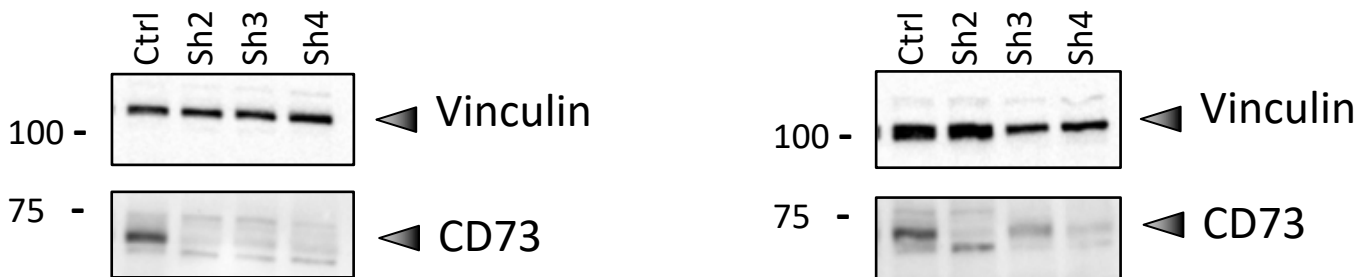
(A) Matched spheres and adherent cultures from four different OC samples were analyzed for the expression of CD73. Columns represent the relative mRNA expression ( $2^{-\Delta\Delta CT}$ ) of selected genes in second-generation spheres as compared to their adherent counterpart. The experiment was performed in triplicate and data are expressed as means  $\pm$  SD. (B) SFE data from the four independent samples shown in Figure 2E. Data are expressed as means  $\pm$  s.e.m.  $*p < 0.05$ . (C) Representative images of CD73 staining on matched bulk adherent and second-generation spheres derived from three primary OC samples. OC-derived spheres showed higher levels of CD73 as compared to adherent cells. Scale bars, 50  $\mu$ m. (D) Representative images of the co-staining of bulk spheres derived from primary OC samples for CD73 and for the OC markers PAX8 and cytokeratin-8 (CK8). Nuclei were counterstained with DAPI. OC-derived spheres showed the co-expression of CD73 with PAX8 and cytokeratin-8 (CK8). Scale bars, 50  $\mu$ m. The experiment was performed on three independent samples with similar results. Related to Figure 2.

**Figure S4**

**A**



**B**



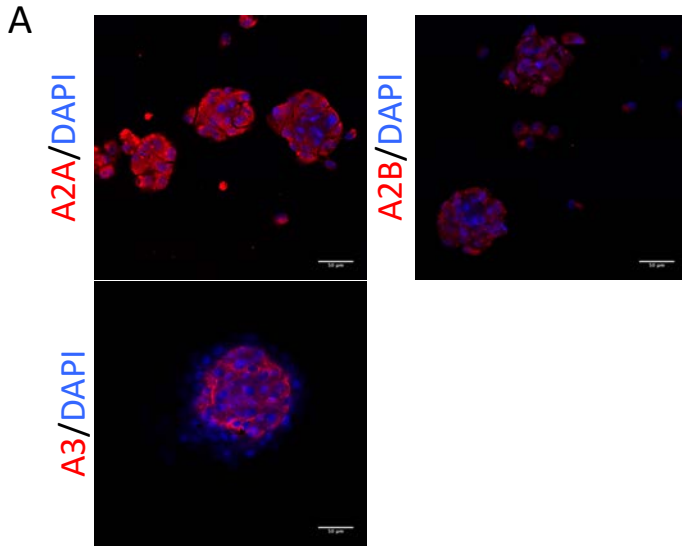
**Figure S4. CD73 knockdown on ovarian cancer cells.**

ShRNA-mediated knockdown of CD73 (with three independent shRNAs, Sh2-4) on two different primary ovarian cancer samples OC5 and OC6 was assessed by FACS analysis (**A**) and by western blotting (**B**). Stable CD73 knockdown resulted in dramatically reduced levels of CD73. Related to Figure 3A.

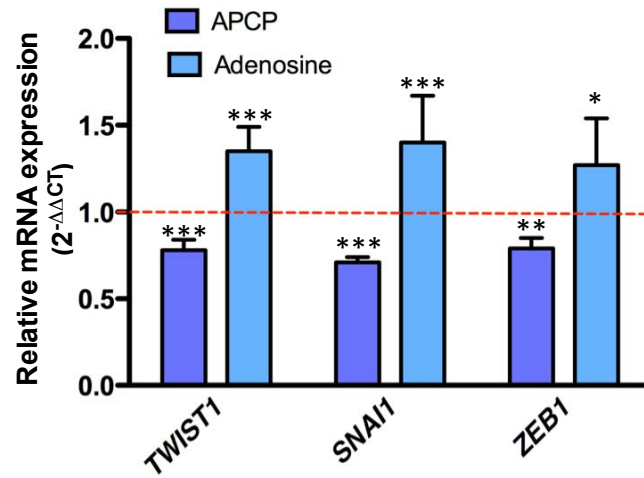


# Figure S5

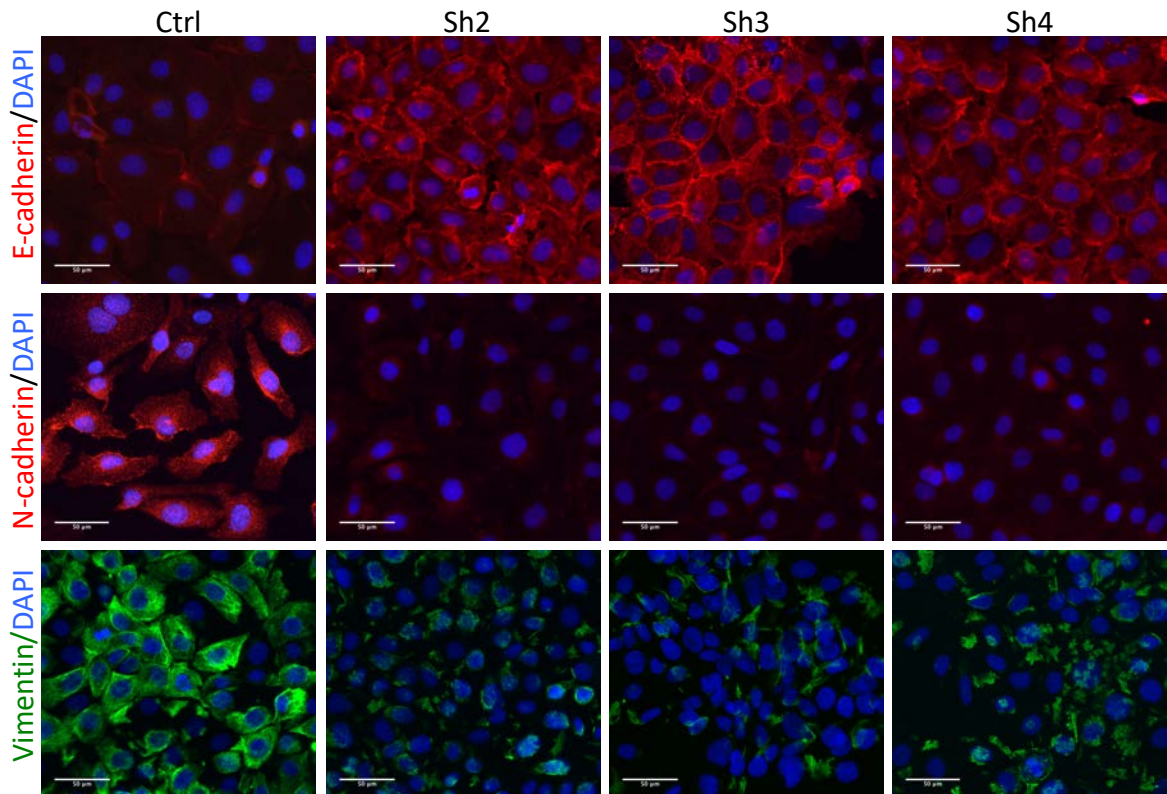
OC19



**B** OC20



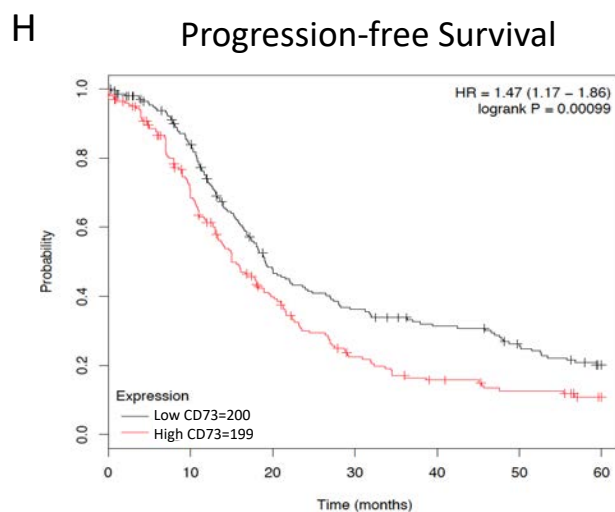
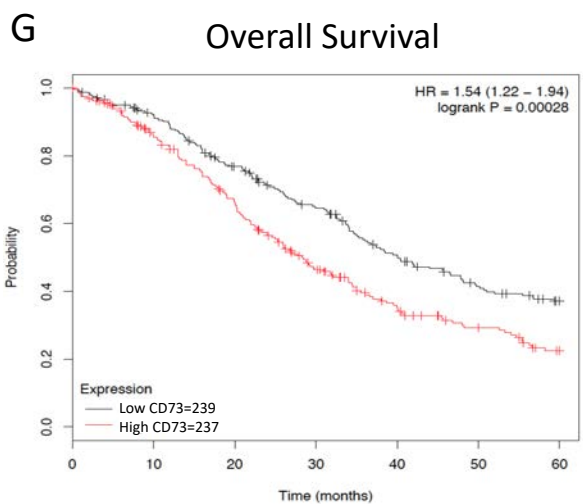
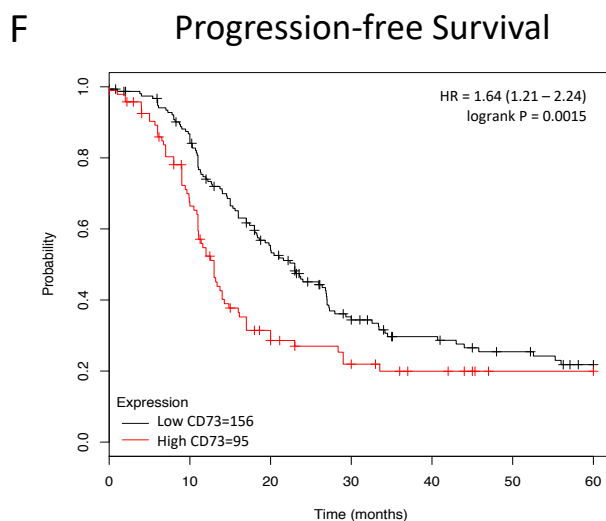
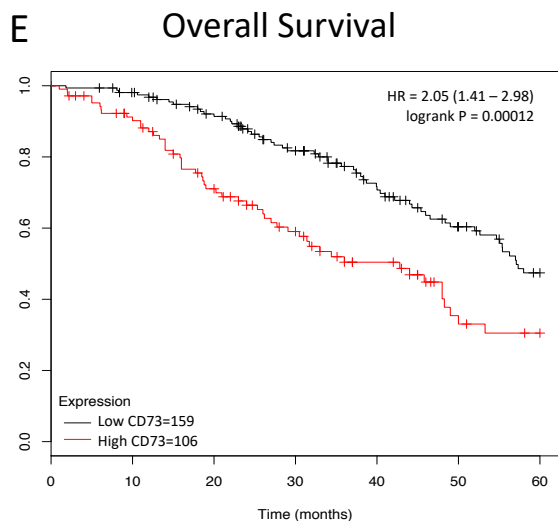
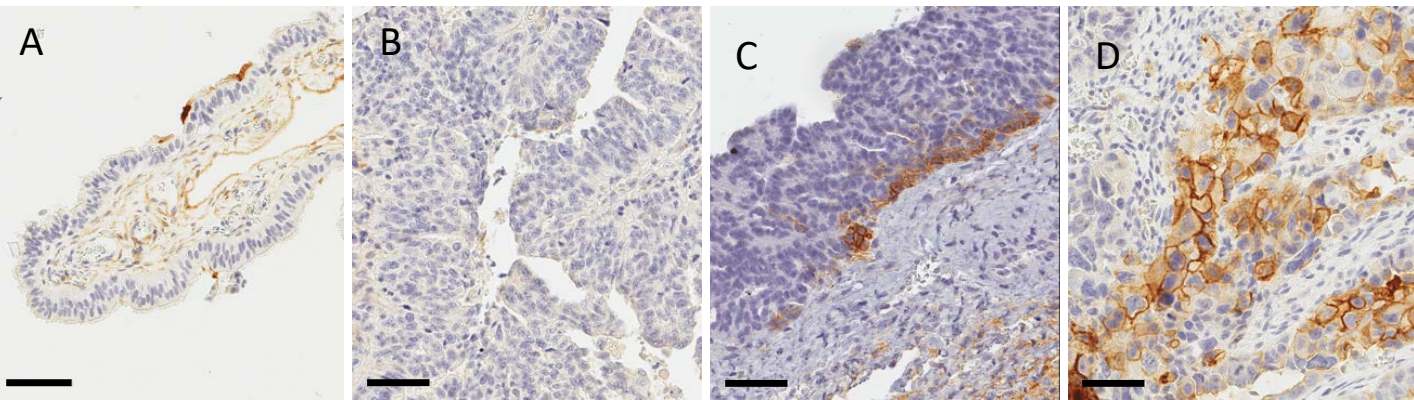
**C**



**Figure S5. CD73 regulates the expression EMT-related genes in OC cells.**

**(A)** Representative images of OC second-generation spheres analyzed for the expression of the adenosine receptors A2A, A2B and A3. OC spheres express predominantly the two adenosine receptors A2A and A3. Scale bars, 50  $\mu\text{m}$ . The experiment was performed on three independent samples with similar results. **(B)** Primary OC cells were treated with either 50 $\mu\text{M}$  APCP or 100 $\mu\text{M}$  adenosine for 24h and then analyzed by qRT-PCR for the expression of the EMT-associated transcription factors *TWIST1*, *SNAIL1* and *ZEB1*. Data are expressed as the relative mRNA expression ( $2^{-\Delta\Delta\text{CT}}$ ) of reported genes in treated cells as compared to control cells treated with vehicle only. Dashed red line indicates gene expression in control cells. APCP treatment resulted in down regulation of EMT-related genes while the adenosine treatment induced their up-regulation. Data refer to three independent experiments (each performed in triplicate) and are expressed as means  $\pm$  s.e.m. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . Figure S5A and B are related to Figure 3C. **(C)** OC5 primary CD73-silenced and control cells stained for E-cadherin, N-cadherin and vimentin. Nuclei were counterstained with DAPI. CD73-knockdown cells showed higher expression of the epithelial marker E-cadherin and low or absent expression of mesenchymal markers N-cadherin and vimentin as compared to control cells. Scale bars, 50  $\mu\text{m}$ . The experiment was performed on three independent samples with similar results. Figure S5C is related to Figure 6.

**Figure S6**



**Figure S6. Expression of CD73 in FTE and OC tissues and correlations with prognosis in OC patients.** Representative images of IHC staining for CD73 in FTE or in high-grade serous OC tissues. While no positive cells were detected in FTE (A), CD73 expression in high-grade serous OC was heterogeneous, ranging from negative (B) to positive only in a small subpopulation (C) or in a high fraction of tumor cells (D). Scale bars, 60 μm. (E, F) Kaplan-Meier curves with 5-year overall (E) and progression-free survival (F) in a cohort of grade-1/2 serous OC. Kaplan-Meier curves with 5-year overall (G) and progression-free survival (H) in a cohort of sub-optimally debulked OC. Data were obtained from [www.kmplot.com](http://www.kmplot.com).

**Table S1: Clinical-pathological parameters of patients.**

**Samples for primary cell pools. Related to Figure 1.**

Sample	Diagnosis	Grade	FIGO Stage	Age
<b>Pool 1</b>				
Patient 1	Serous cystadenocarcinoma	3	III C	72
Patient 2	Serous surface papillary carcinoma	3	III C	63
Patient 3	Serous surface papillary carcinoma	3	III C	72
Patient 4	Serous cystadenocarcinoma	3	III C	50
<b>Pool 2</b>				
Patient 5	Serous cystadenocarcinoma	3	III C	55
Patient 6	Papillary Cystadenocarcinoma	3	III C	42
Patient 7	Serous Surface Papillary Carcinoma	3	III C	47
<b>Pool 1</b>				
FTE 1	Endometrioid endometrium adenocarcinoma			40
FTE 2	Squamous cell carcinoma of uterine cervix			42
FTE 3	Endometrioid endometrium adenocarcinoma			67
FTE 4	Endometrioid endometrium adenocarcinoma			39
FTE 5	Ovarian cystadenoma			60
<b>Pool 2</b>				
FTE 6	Uterine inflammation			67
FTE 7	Squamous cell carcinoma of uterine cervix,			67
FTE 8	Endometrium atypical hyperplasia			48
FTE 9	Endometrium atypical hyperplasia			48



**Table S1, continued. OC samples for primary cell cultures**

Sample	Diagnosis	Grade	FIGO Stage	Age
OC1	Serous surface papillary carcinoma	3	III C	65
OC2	Serous cystadenocarcinoma	3	III C	53
OC3	Serous surface papillary carcinoma	3	III C	69
OC4	Serous surface papillary carcinoma	3	III C	58
OC5	Serous surface papillary carcinoma	3	III C	59
OC6	Serous surface papillary carcinoma	3	III C	46
OC7	Serous surface papillary carcinoma	3	III C	46
OC8	Serous surface papillary carcinoma	3	III C	44
OC9	Serous surface papillary carcinoma	3	III C	35
OC10	Serous surface papillary carcinoma	3	II B	47
OC11	Serous cystadenocarcinoma	3	III C	47
OC12	Serous surface papillary carcinoma	3	III B	46
OC13	Serous surface papillary carcinoma	3	III C	47
OC14	Serous surface papillary carcinoma	3	III C	44
OC15	Serous surface papillary carcinoma	3	III C	50
OC16	Serous cystadenocarcinoma	3	III C	47
OC17	Serous cystadenocarcinoma	3	III C	56
OC18	Serous surface papillary carcinoma	3	III C	70
OC19	Serous surface papillary carcinoma	3	III C	72
OC20	Serous surface papillary carcinoma	3	III C	51
OC21	Serous cystadenocarcinoma	3	III C	60
OC22	Serous surface papillary carcinoma	3	III B	55
OC23	Serous surface papillary carcinoma	3	III C	58
OC24	Serous surface papillary carcinoma	3	III C	49
OC25	Serous cystadenocarcinoma	3	III C	62

**Table S5. TaqMan® Gene Expression Assays for Quantitative RT-PCR Related to Experimental Procedures Section.**

<b>Gene</b>	<b>Assay ID</b>
<i>CD73</i>	Hs00159686_m1
<i>CD44</i>	Hs00153304_m1
<i>ALDH1</i>	Hs00946916_m1
<i>NANOG</i>	Hs02387400_g1
<i>POU5F1</i>	Hs04260367_gh
<i>SOX2</i>	Hs00602736_s1
<i>TWIST1</i>	Hs00361186_m1
<i>SNAI1</i>	Hs00195591_m1
<i>ZEB1</i>	Hs00232783_m1
<i>18s</i>	Hs99999901_s1
<i>GAPDH</i>	Hs99999905_m1
<i>GUSB</i>	Hs99999908_m1
<i>HPRT</i>	Hs99999909_m1