Patient-derived ovarian cancer explants: preserved viability and histopathological features in long-term agitation-based cultures

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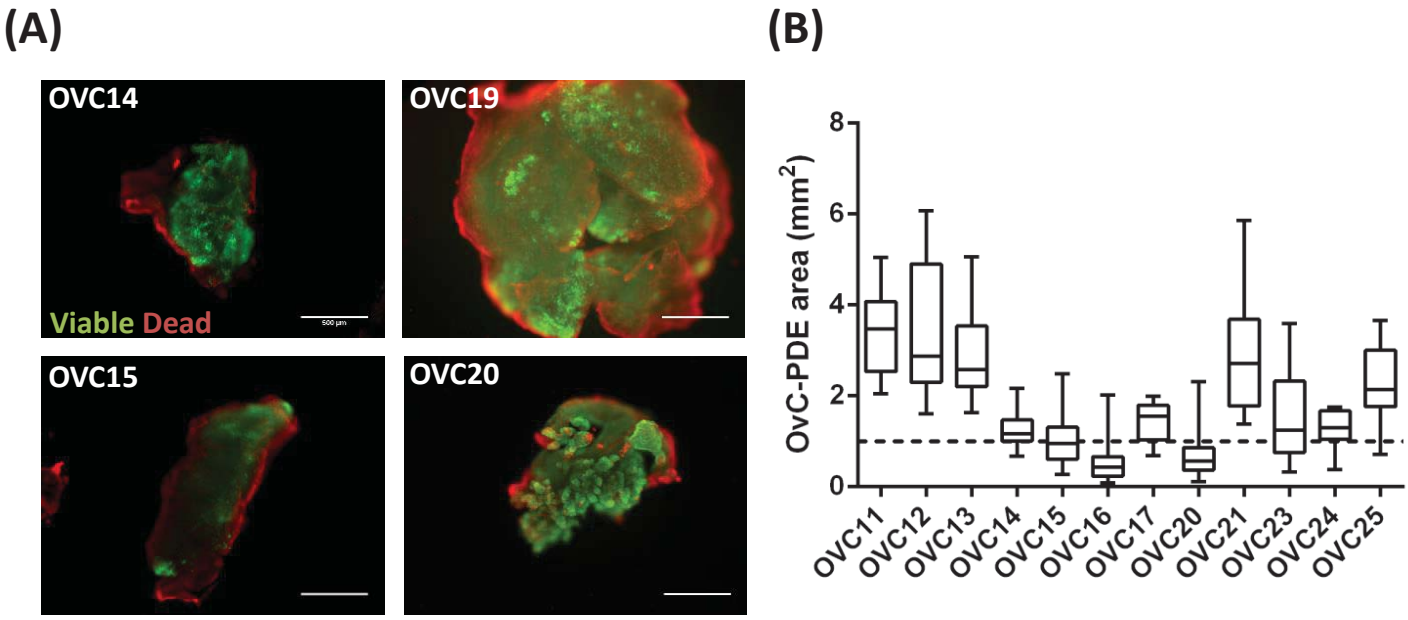
Supplementary Table 1: Clinical pathological annotations of ovarian cancer samples

Case ID	Diagnosis	Age (yrs)	TMN	FIGO
OVC1	High grade serous carcinoma *	60	ypT3bpNx (CRS3)	IIIB
OVC2	Fibroma	63	-	-
OVC3	High grade serous carcinoma	55	pT3CpN0	IIIC
OVC4	Endometrioid carcinoma	59	pT2cpN0	IIC
OVC5	Mucinous borderline tumor	77	pT1a	IA
OVC6	Mucinous carcinoma	79	pT3cpNx	IIIC
OVC7	Endometrioid carcinoma	54	pT1apN0	IA
OVC8	High grade serous carcinoma	71	pT3CpN1	IIIC
OVC9	High grade serous carcinoma	69	pT3cpNx	IIIC
OVC10	High Grade serous carcinoma *	55	yT3c (CRS3)	IIIC
OVC11	Mucinous borderline tumor	58	pT1a	IA
OVC12	High grade serous carcinoma	72	pT3bpN0	IIIB
OVC13	Clear cell carcinoma	69	pT1c2pN0	IC
OVC14	High grade serous carcinoma	79	pT3cpNx	IIIC
OVC15	Carcinosarcoma	62	pT3bpN1	IIIB
OVC16	High grade serous carcinoma	57	pT3cpN1b	IIIC
OVC17	High grade serous carcinoma *	68	ypT3c (CRS –na)	IIIC
OVC18	Endometrioid carcinoma	62	pT1a	IA
OVC19	Undifferentiated carcinoma	80	pT3bpNx	IIIB
OVC20	Low grade serous carcinoma	39	pT3cpN1	IIIC
OVC21	Clear cell carcinoma	72	pT1apN0	IA
OVC23	High grade Serous carcinoma *	67	pT3cpNx	IIIC
OVC24	Endometrioid	78	pT1apN0	IA
OVC25	Mucinous borderline tumor	60	pT1aNx	IA
OVC26	High grade serous carcinoma	77	pT1BNxM1	IIIC
OVC27	High grade serous carcinoma	81	pT3CpNx	IIIC

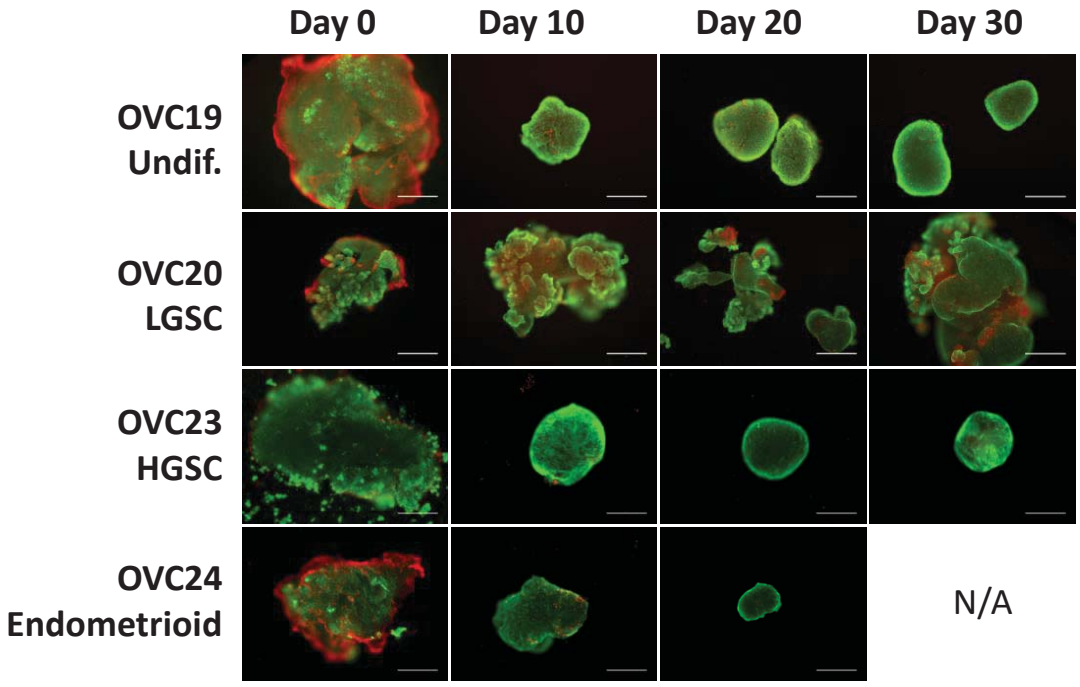
* chemotherapy treatment before surgery

Supplementary Figure 1



Supplementary Figure 2

(A)



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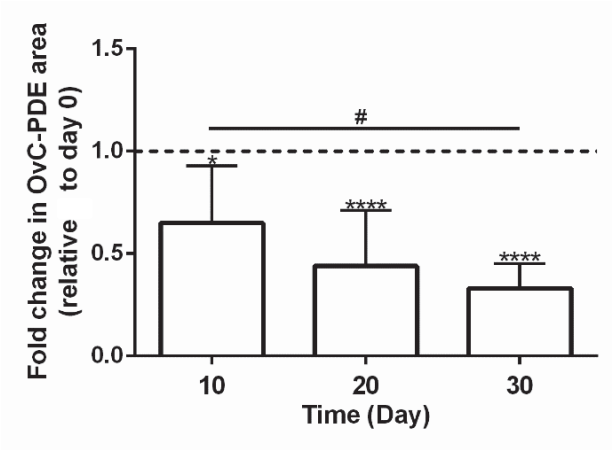
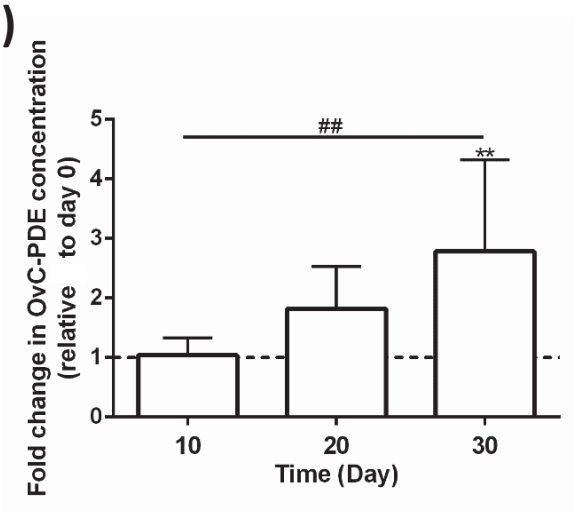
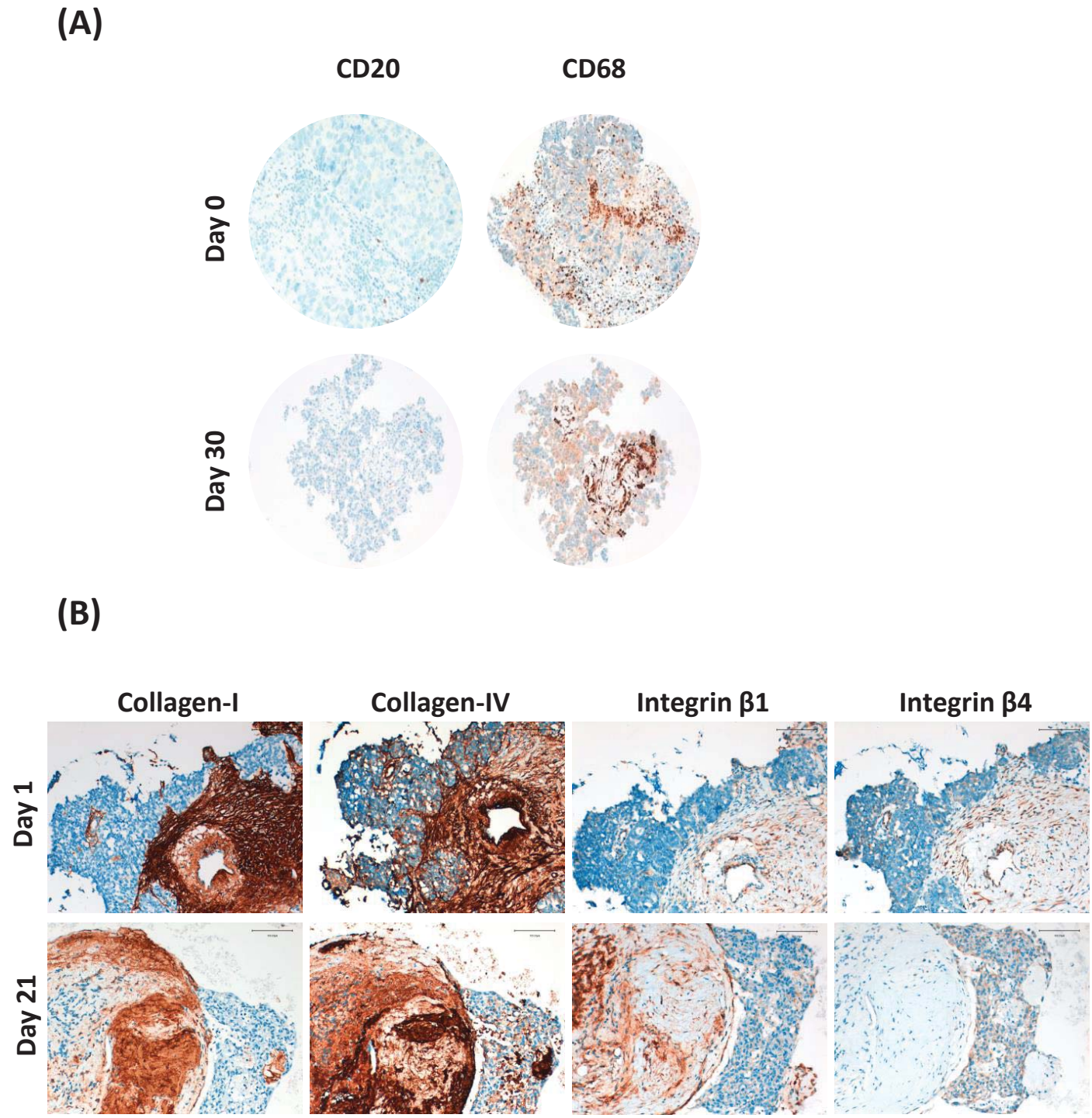


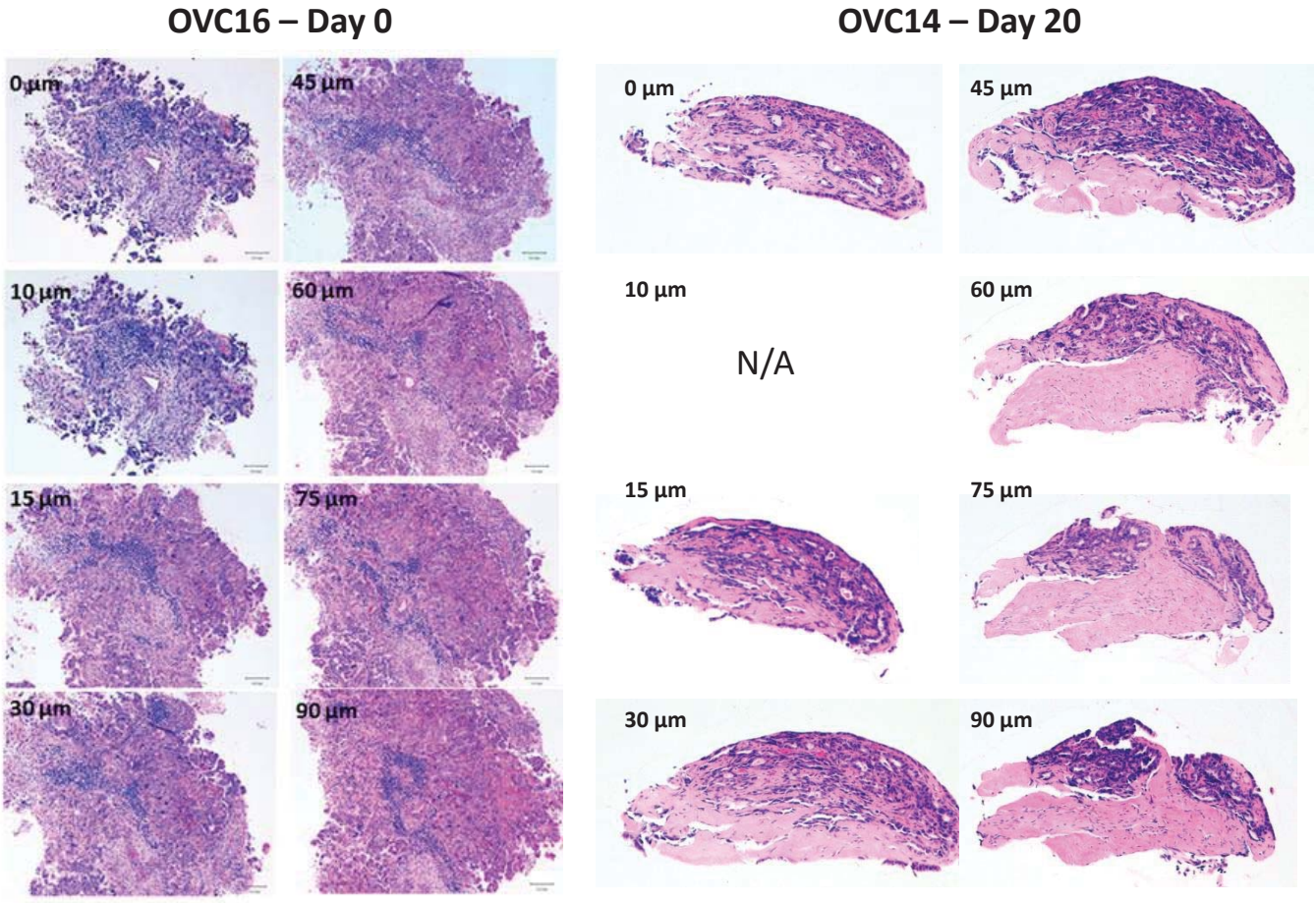
(C)



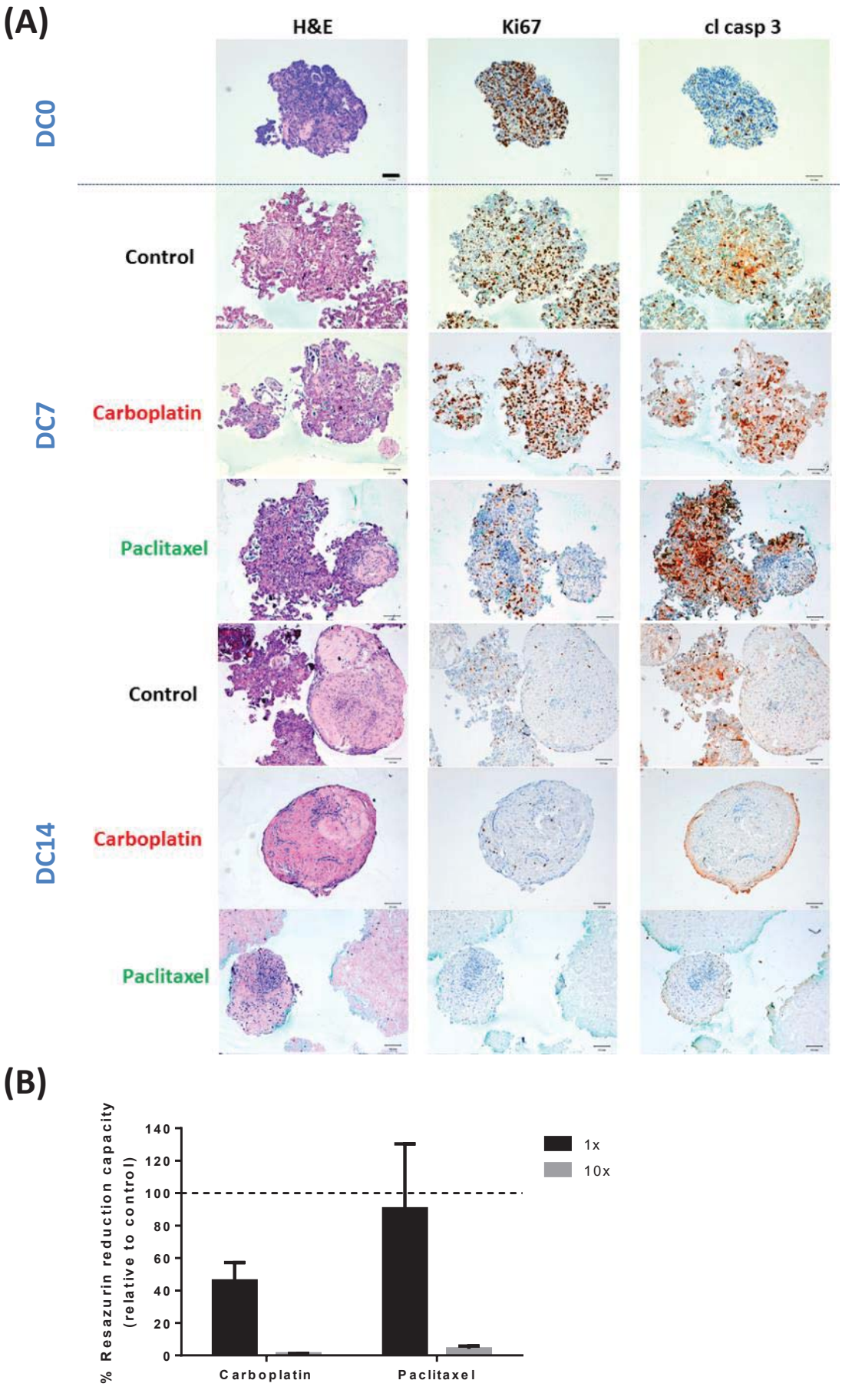
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 1: Processing of ovarian carcinoma samples for the generation of OvC-PDE cultures. (A) Representative images (N=15) of live/dead fluorescent assay of explants, immediately after tissue processing (day 0 of culture): explants were incubated with fluorescein diacetate (FDA, green) for indication of live cells and Propidium Iodide (PI, red) for identification of dead cells. Scale bars represent 500 µm; **(B)** Explant area at Day 0 (immediately after tissue mechanical processing). Data is presented as mean ± SD (N=12, with at least 15 explants counted per tumour).

Supplementary Figure 2: OvC-PDE cultures originated from different subtypes maintained cell viability during 30 days. (A) Representative images (N=15) of live/dead fluorescent assay of OvC-PDE culture along time. Explants were incubated with fluorescein diacetate (FDA, green) for indication of live cells and Propidium Iodide (PI, red) for identification of dead cells. Scale bars represent 500 µm; HGSC – High Grade Serous Carcinoma, LGSC – Low Grade Serous Carcinoma; Undif., Undifferentiated carcinoma. **(B)** Explant area and **(C)** Explant concentration along OvP-PDE culture. Data is presented as mean fold-change ± SD (B, N=8; C, N=6). Two-way ANOVA statistical test (Tukey's multiple comparison test) was applied to compare the mean values at each time-point versus day 0 (*) and along culture time (#). Statistical analysis was carried out using GraphPad Prism 6 Software. # ($p<0.05$), ** or ## ($p<0.01$) and **** ($p<0.0001$).

Supplementary Figure 3: OvC-PDE cultures maintained immune cell populations and extracellular matrix similar to the original tumor. Immunohistochemistry analysis of cross-sections of OvC-PDE cultures from: **(A)** OVC16, at day 0 and 30. Immune markers (CD3 and CD20 for lymphocytes T and B, respectively and CD68 for macrophages). (B) OVC3, at day 1 and 21. Collagen-I, -IV and integrin β1 and β4. Scale bars represent 100 µm. Scale bars represent 100 µm

Supplementary Figure 4: OvC-PDE cultures maintained the original cellular intra-heterogeneity. Haematoxylin & eosin staining of cross-sections at different depths of representative OvC-PDE cultures of HGSC (OVC14 and OVC16) collected at day 0 and 20 of culture, respectively (N=2). Scale bars represent 100 µm

Supplementary Figure 5: OvC-PDE cultures could be challenge with cycles of chemotherapy agents for evaluation drug efficacy. (A) Haematoxylin & eosin staining and immunohistochemical analysis for proliferation (Ki67+ cells) and apoptosis (cleaved caspase 3+ cells) of representative cross-sections of OvC-PDE at day 0, 7 and 14 of drug challenge (DC) (corresponding to day 7, 14 and 21 of culture) with 25 µg/mL carboplatin or 10 µg/mL paclitaxel, N=4 (OVC15, OVC16, OVC23 and OVC24), Scale bars represent 100 µm. **(B)** Measurements of resazurin reduction capacity (in %) of the OvC-PDE along treatment time relative to OvC-PDE control culture, by performance of PrestoBlue assay. Cultures were evaluated at day 0 and 14 of drug challenge (DC) with carboplatin or paclitaxel (corresponding to day 7 and 21 of culture) (1x: 25 µg/mL carboplatin and 10 µg/mL paclitaxel; 10x: 250 µg/mL carboplatin and 100 µg/mL paclitaxel). Data is presented as a mean ± SD (N=2, OVC26 and OVC27).