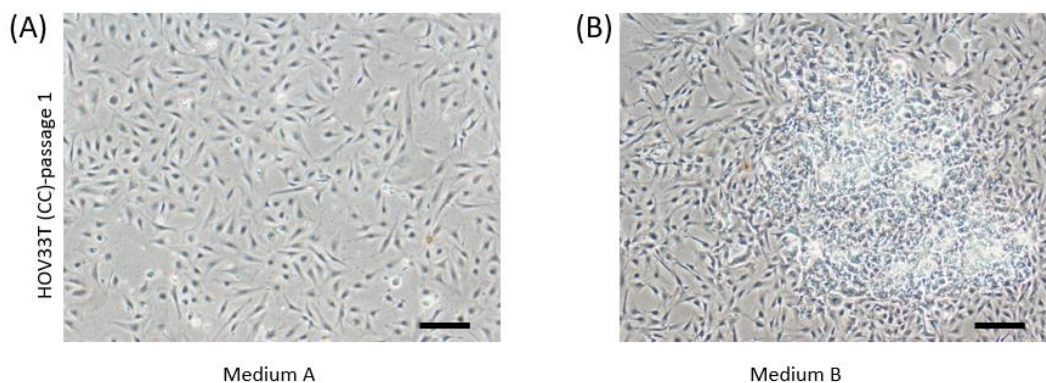
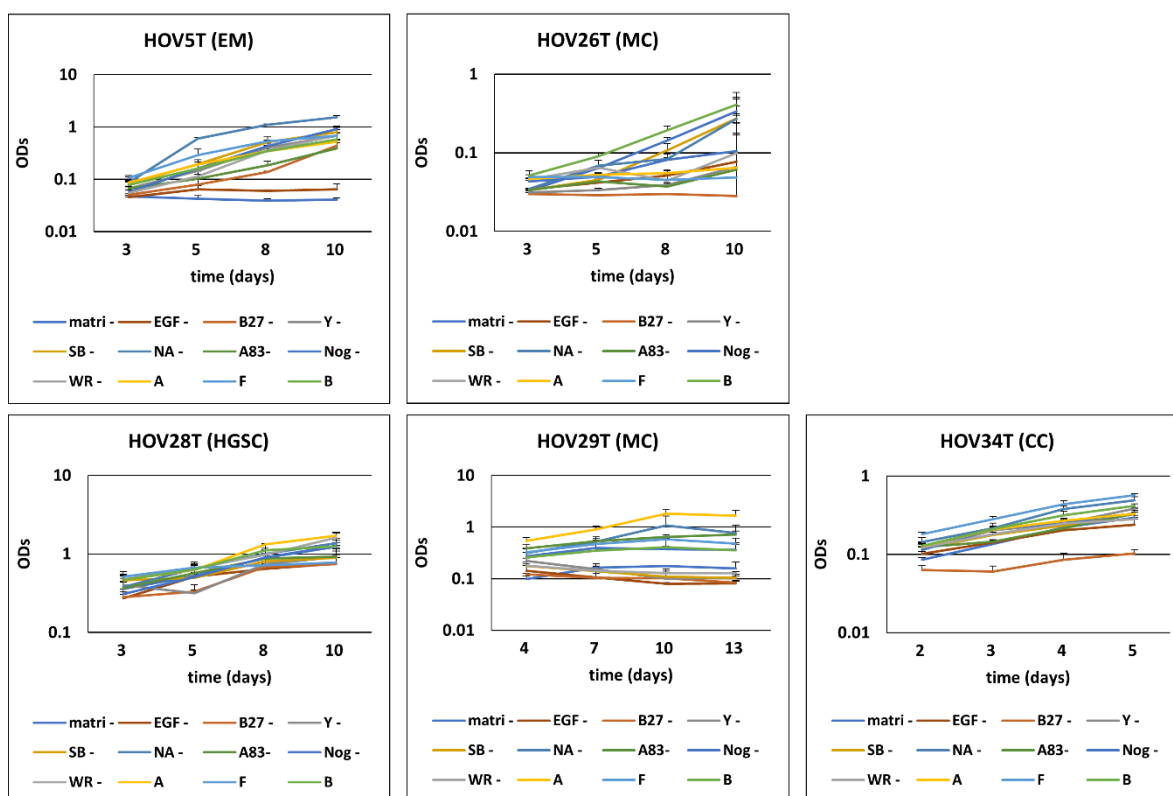


# Supplementary: An *Ex-Vivo* Culture System of Ovarian Cancer Faithfully Recapitulating the Pathological Features of Primary Tumors



**Figure S1.** Different proliferation pattern in medium A and B. Representative images of passage 1 HOV33T cells in (A) medium A and (B) Medium B were taken under a phase-contrast microscope. Scale bar 200  $\mu\text{m}$ .



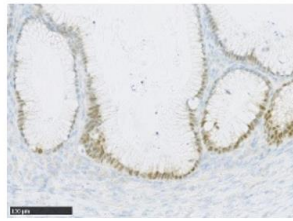
Doubling time (hours) of the cells in each condition

Cell line	HOV5T	HOV26T	HOV28T	HOV29T	HOV34T
observation period (day)	3-8	3-8	3-8	4-10	2-5
matrigel-coating(-)	-430	56	91	175	40
medium B -EGF	299	207	96	-177	59

medium B -B27	83	8359	94	-570	101
medium B -Y27632	46	380	136	-128	42
medium B -SB202190	45	72	152	-209	49
medium B -Nicotinamide	33	95	86	81	41
medium B -A83-01	81	805	96	191	51
medium B -Noggin	48	131	77	315	46
medium B -Wnt3a/RSPO1	41	-2237	114	-304	60
medium B	56	63	96	221	37
medium A	44	485	80	82	53
F-medium	60	-1069	252	172	44

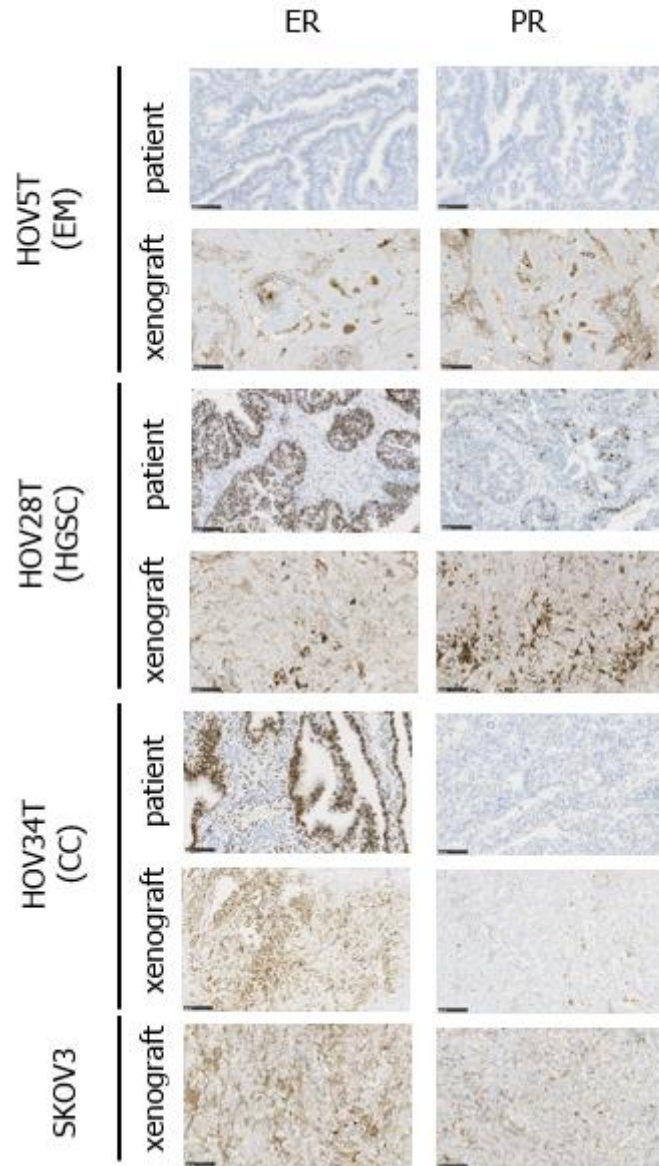
**Figure S2. Cell growth curve of HOV lines in different culture conditions.** HOV5T ( $1 \times 10^3$  cells/well), HOV26T ( $1 \times 10^3$  cells/well), HOV28T ( $1 \times 10^3$  cells/well), HOV29T ( $2 \times 10^3$  cells/well) and HOV34T ( $1 \times 10^3$  cells/well) cells were seeded on matrigel-coated or uncoated (matri-) 96 well plates with the complete medium B. Next day, the complete medium B was replaced with either medium A (A), F-medium, complete medium B (B) or medium B without one component of the supplements, EGF, B27, Y27632 (Y), SB202190 (SB), nicotinamide (NA), A83-01 (A83), Noggin conditioned medium (Nog), or Wnt3A and RSPO1 conditioned medium (WR). Cells were fixed at the indicated time points, and stained by acid red to measure the relative number of cells. The calculated doubling time in each condition is also presented in the table. Minus value means the cell number rather decreased in the condition. Comparison of individual conditions was summarized in Table 2 as a heatmap.

HOV35T (MC borderline with micro invasion)

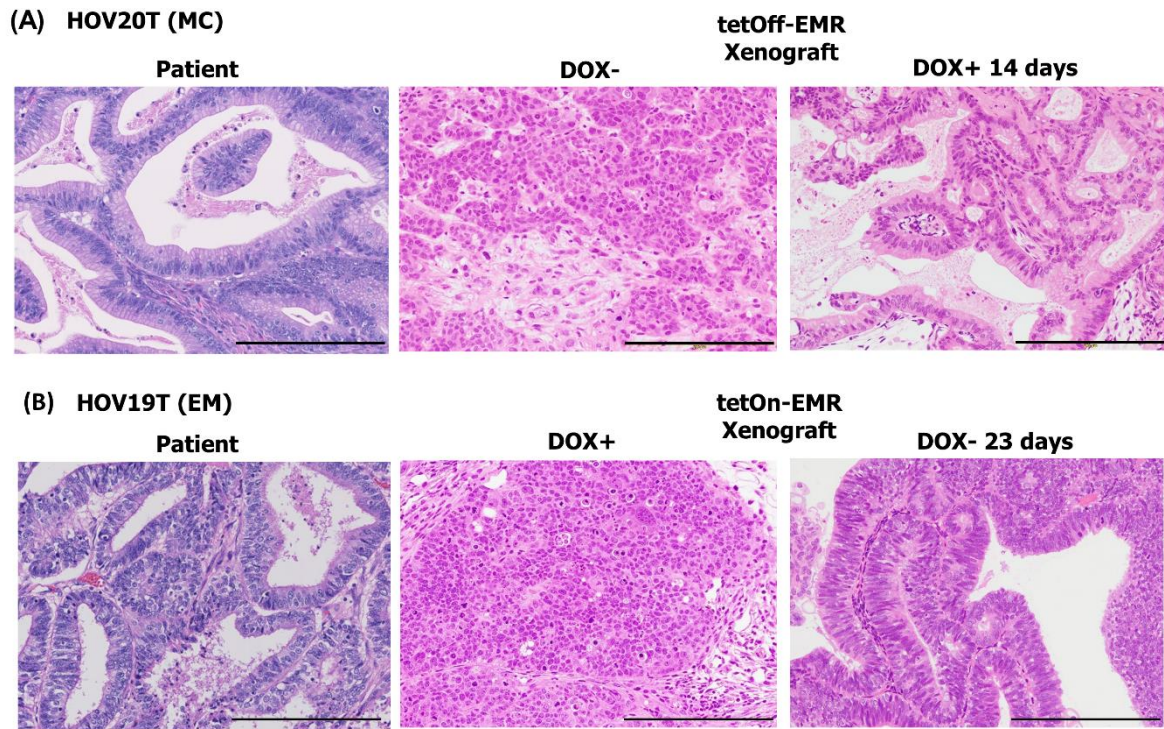


PAX8

**Figure S3. PAX8 immunostaining of original patient tissue of HOV35T.** FFPE section of HOV35T original patient tissue was stained for PAX8 as described in Materials and Methods. Scale bar 100  $\mu$ m.



**Figure S4. Histotype-specific marker expression fidelity of HOV tumors.** FFPE sections of patient tumor tissue, HOV xenograft and SKOV3 xenograft were stained with ER (estrogen receptor) and PR (progesterone receptor). Scale bar 100  $\mu$ m.



Scale bar, 200  $\mu$ m

**Figure S5.** Generation of tumors and recapitulation of original tumor tissues by temporal expression of additional oncogenes in MC and EM type HOV lines. (A) Histopathology of HOV29T/tetOFF/EMR xenograft and original tumor of HOV20T and (B) HOV19T/tetOne-EMR xenograft and original tumor.

**Table S1.** List of antibodies used for western blotting.

<b>Antibody</b>	<b>Company</b>	<b>Catalog</b>
ARID1A/BAF250A (D2A8U)	cell signaling	12354S
HNF 1B	Sigma	HPA002083-100U
PAX8	Proteintech	10336-1-AP
Estrogen Receptor $\alpha$ (62A3)	Cell Signaling	2512
GAPDH	Ambion	AM4300
E-cadherin	BD Transduction Lab	BD-610181
Estrogen receptor (ER alfa) (F-10)	Santa Cruz	sc-8002
Cytokeratin 7 (RCK105)	Santa Cruz	sc-23876
WilmsTumor protein	abcam	ab89901
p16INK4A	BD Pharmingen	550834
TP53	Calbiochem (Oncogene Science) Ab6	OP43-100UG
HPV16 E7(716-325)	Santa Cruz	sc-51951
MYC	Santa Cruz	sc-42
pan RAS (C-4)	Santa Cruz	sc-166691
HPV16 E6 (46A4)	Home-made, mouse monoclonal, clone 46A4	Raised against N-terminal 16 amino acid peptide of HPV16 E6
MCM7 (141.2)	Santa Cruz	sc-9966

**Table S2.** List of antibodies, antigen retrieval methods and detection systems used for immunohistochemistry.

Antibody	Animal	Company name	Catalog no	Antigen retrieval			Detection	
				Solution	Temperature	Time	Name	Company
PAX8	mouse	abcam	ab53490	TRSpH9	98°C	40min	Envision™ Detection System	DAKO
WT1	mouse	DAKO	M3561	TRSpH9	98°C	40min	Envision™ Detection System	DAKO
P16	mouse	BD-Phamingen	550834	Cit.B	121°C	10min	Envision™ Detection System	DAKO
HNF1B	goat	Santa Cruz	sc-7411	TRSpH9	98°C	40min	Simple stain-PO(G)	Nichirei
ARID1A	rabbit	SIGMA	HPA005456	TRSpH9	98°C	40min	Envision™ Detection System	DAKO
CK7	mouse	DAKO	M7018	Cit.B	121°C	10min	Envision™ Detection System	DAKO
E-cadherin	mouse	DAKO	M3612	TRS	121°C	10min	Envision™ Detection System	DAKO
P53	mouse	DAKO	M7001	TRSpH9	98°C	40min	Envision™ Detection System	DAKO
PR	rabbit	Roche	518-102333				ultraViewDAB Universal Kit	Roche
ER	rabbit	Roche	518-107925				ultraViewDAB Universal Kit	Roche