Preservation of stemness in high-grade serous ovarian cancer organoids requires low Wnt environment

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Supplementary Figure Legends

Supplementary Figure 1. Pathology of HGSOC tumor samples and drug response of HGSOC organoids. A) Diagnosis of ovarian cancer in tissue fragments used for organoids generation were confirmed by experienced pathologists to be of HGSOC phenotype, being marked by strong EpCAM, Pax8 and p16 expression. Depicted are three examples of the pathology stainings. B) Three HGSOC organoid lines were tested for their response to the drug carboplatin showing slightly different responses as determined by cell viability assay. Data represent mean ±SD of technical triplicates.

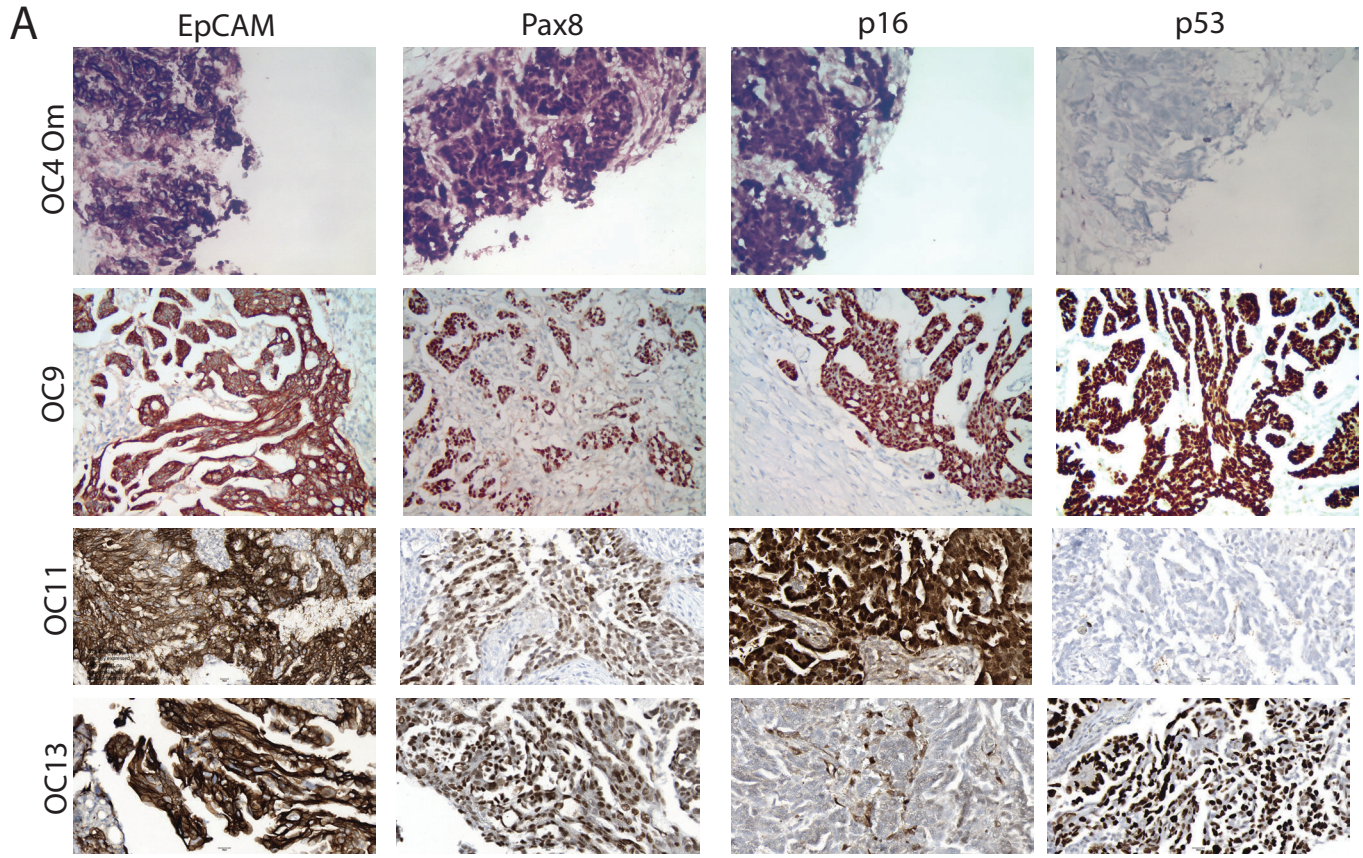
Supplementary Figure 2. Expression profile of HGSOC organoids. A) Representative western blot indicating loss of BRCA1 protein expression in sample OC4 but WT level expression in OC7. B) Nuclear p53 expression in different cancer organoids was confirmed by IF stainings. C) Spearman correlation map based on single-color microarray data indicates stronger similarity in expression patterns between the healthy organoids vs tumor organoids**.** D) Protein expression profiling confirmed frequent CCNE1 overexpression in established HGSOC organoid lines.

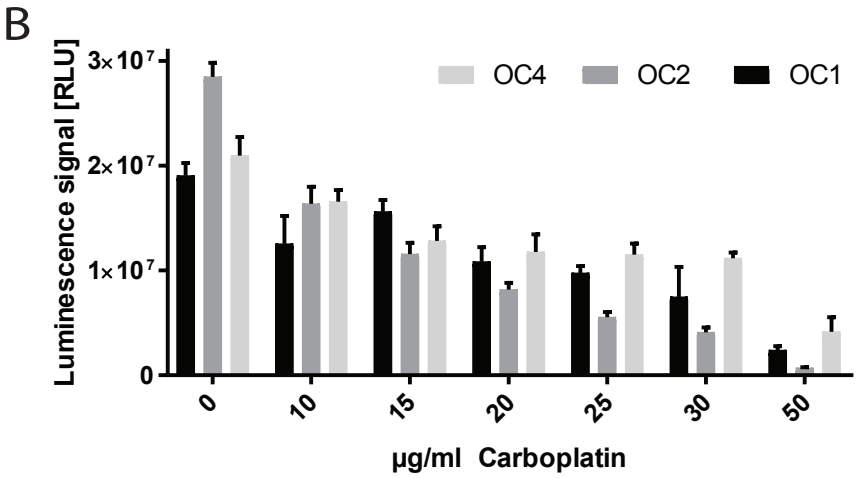
Supplementary Figure 3. Effects of p53, PTEN and Rb knockdown in FT organoids. A) Gating strategy to sort GFP/mCherry double-positive single cells derived from transduced FT organoids. B) Activation of the AKT pathway as indicated by increased levels of phospho Akt protein proves functional PTEN knockdown. C) P53 KD organoids are resistant to Nutlin3A treatment, confirming functional disruption of p53 signaling. D) Summary table and representative phase contrast images of the 7 triple KD organoid lines that were generated from different donors, all of which showed a premature growth arrest.

Supplementary Figure 4. Confirmation of DNA damage and apoptosis as well as activation of Wnt target genes. A) Growth curves of a second biological replicate showing loss of growth capacity and premature growth arrest of KD organoids compared to the controls when grown in FTM. B) Phase contrast images of 3 engineered organoid lines (p53/PTEN/RB knockdown) differentially treated with FTM and OCM confirm differences in organoid formation and long-term growth. Scale bar: 500 µm. C) Western blot from donor FT268 as independent biological replicate of the effects shown in Fig. 4C. Increase in DNA damage (γH2AX, PARP1 and cleaved CC3) in triple KD organoids is not significantly affected by the medium change. D) Wnt target genes were significantly downregulated upon change from FTM to OCM as revealed by microarray analysis. Differential expression determined by dual-color microarray for 2 biological replicates was significant for all genes with $p < 0.05$.

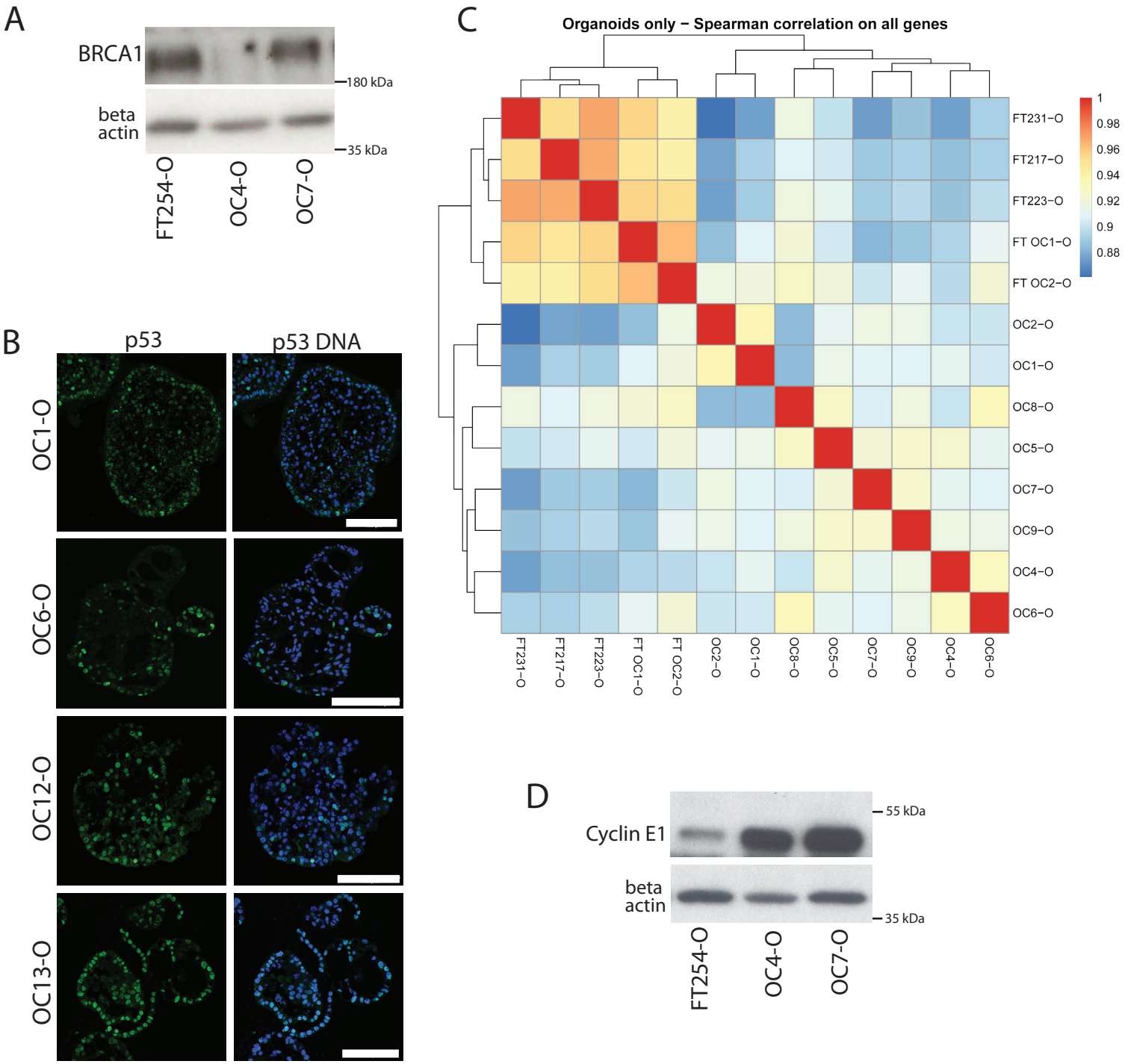
Supplementary Figure 5. HGSOC organoids respond to exogenous Wnt signals with changes in gene expression and growth pattern. A) The percentage of CD133+ cells was determined by flow cytometry in knockdown and control organoids. B) Gating strategy for CD133+ cells in HGSOC organoids. C) Canonical Wnt target gene AXIN2 is significantly upregulated in HGSOC organoids treated with Wnt3a/Rspo1 conditioned medium as confirmed by qRT-PCR. AXIN2 expression levels were normalized to GAPDH and calculated as mean ±SEM from technical triplicates.

Supplementary Figure 1. Pathology of HGSOC tumor samples and drug response of HGSOC organoids.



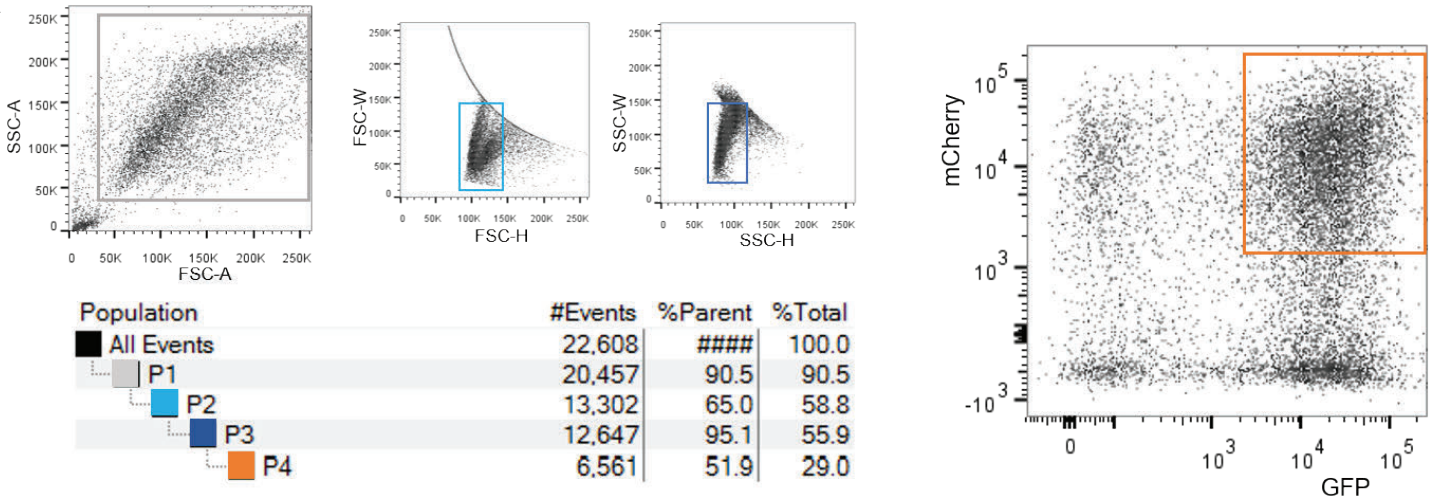


Supplementary Figure 2. Expression profile of HGSOC organoids.



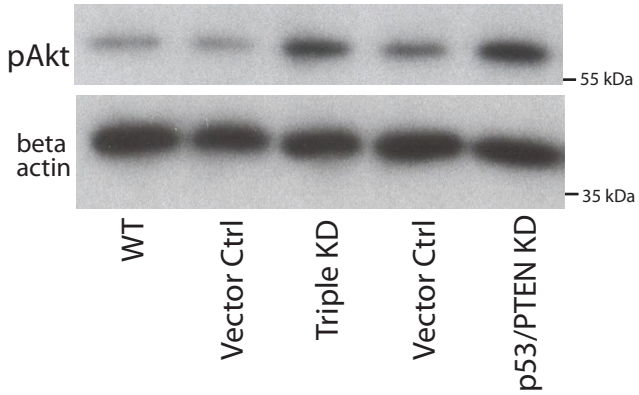
Supplementary Figure 3. Effects of p53, PTEN and Rb knockdown in FT organoids.

A

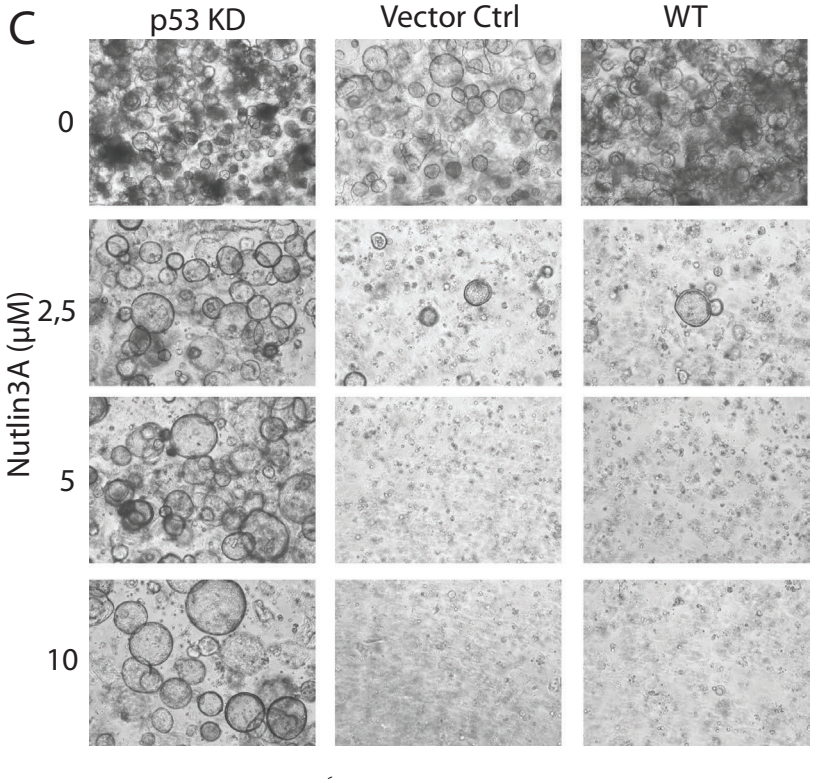


Population	#Events	%Parent	%Total
All Events	22,608	####	100.0
P1	20,457	90.5	90.5
P2	13,302	65.0	58.8
P3	12,647	95.1	55.9
P4	6,561	51.9	29.0

B

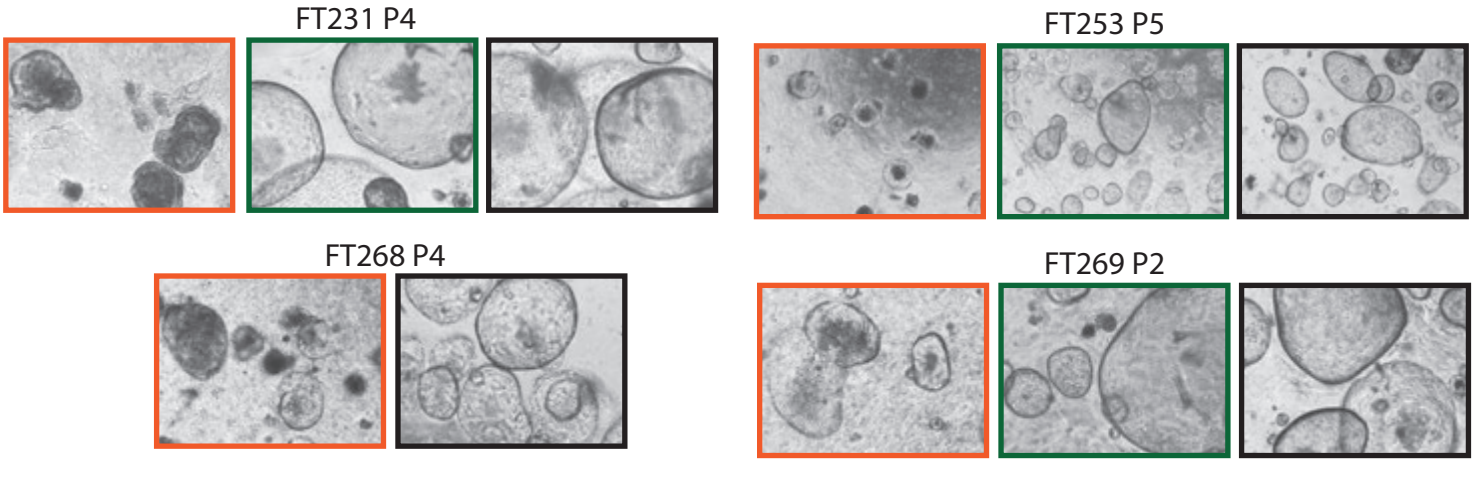


C

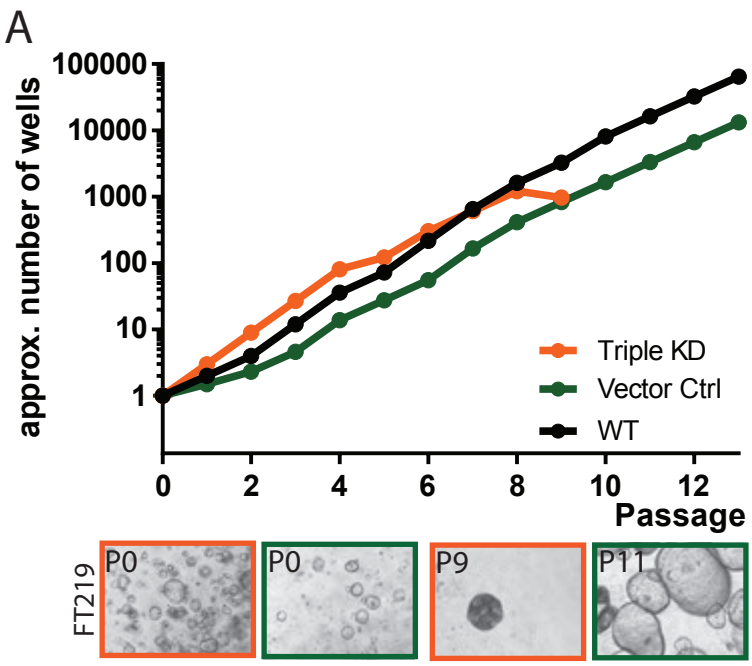


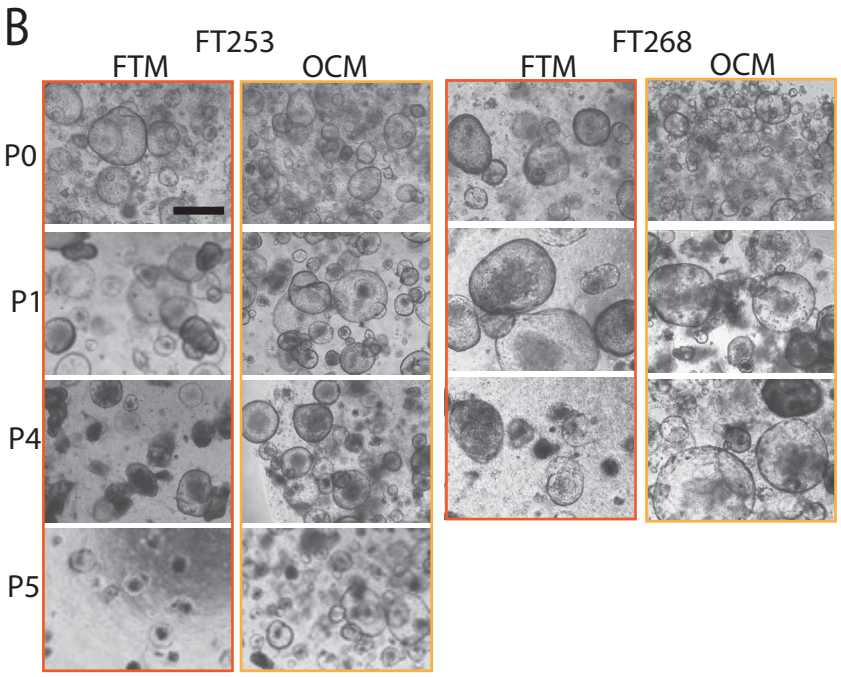
D

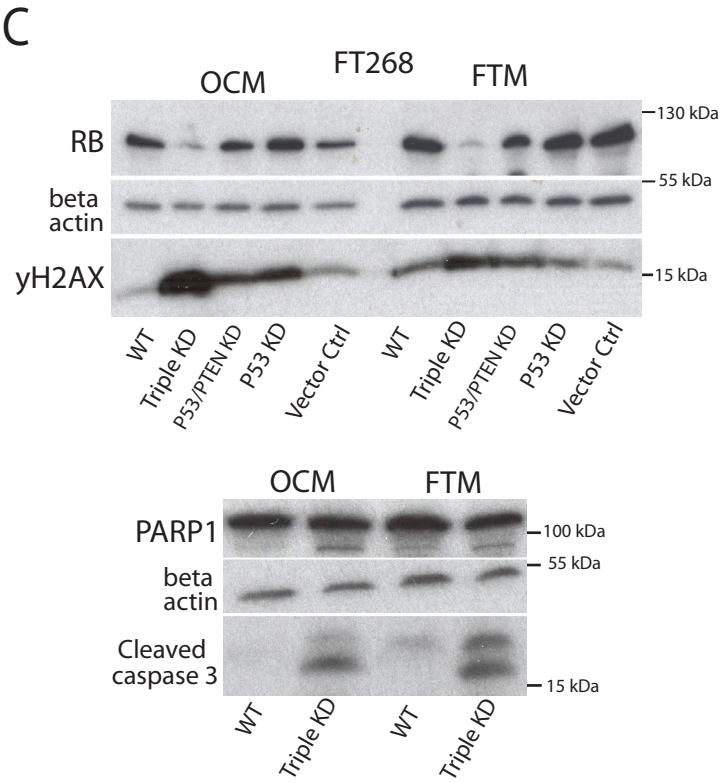
KD organoid line	Passage / time in culture
FT219	p9 / 7 months
FT231	p4 / 3.5 months
FT234	p7 / 5 months
FT253	p5 / 4 months
FT254	p3 / 3 months
FT268	p4 /3 months
FT269	p2 / 1.5 months

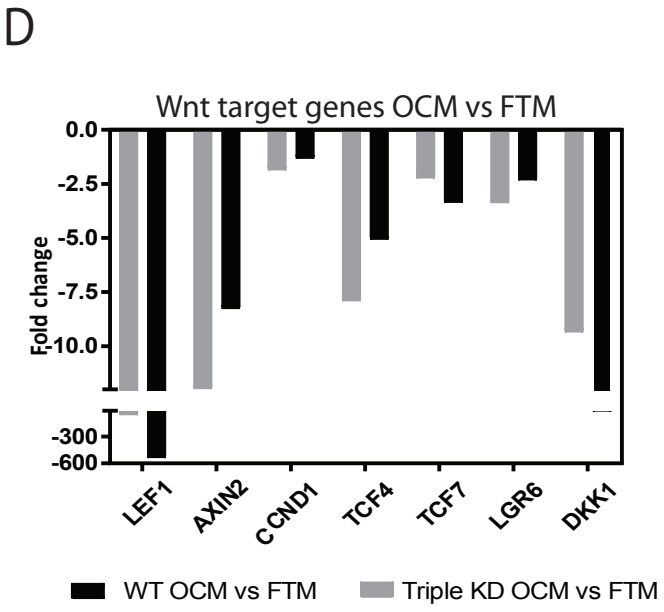


Supplementary Figure 4. Cofirmation of growth arrest, DNA damage and apoptosis as well as activation of Wnt target genes.

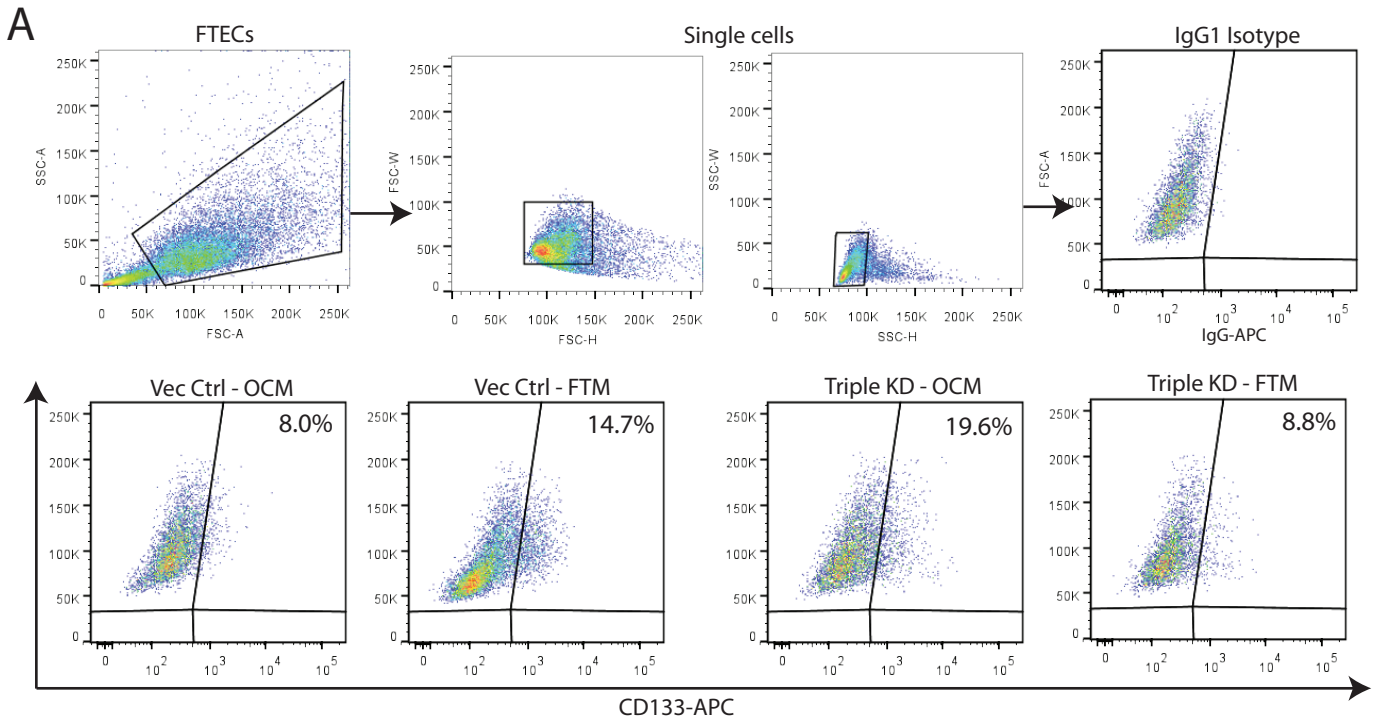


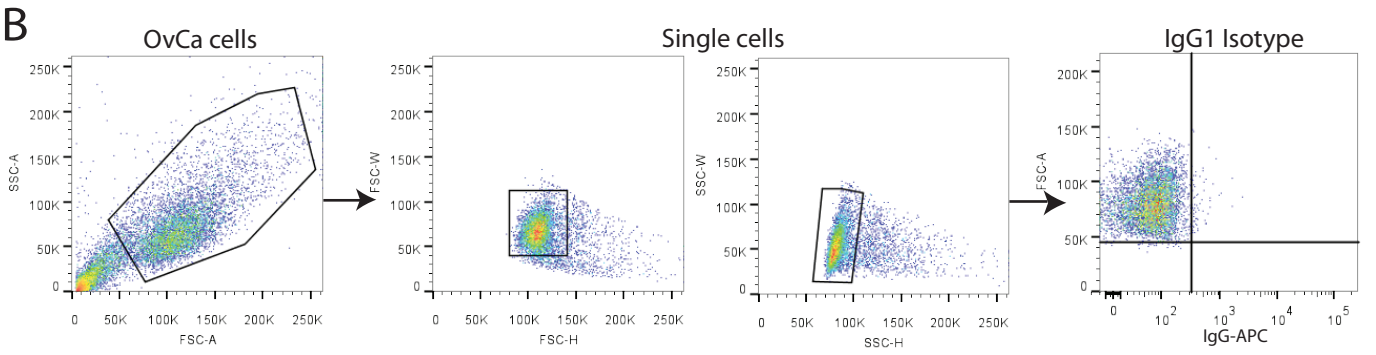


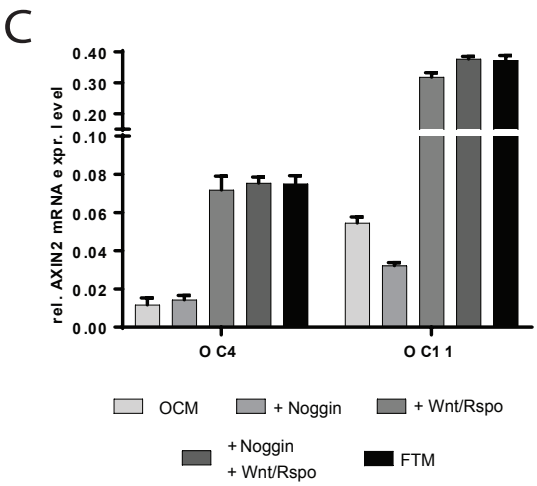




Supplementary Figure 5. HGSOC organoids respond to exogenous Wnt signals with changes in gene expression and growth pattern.







Supplementary Table 1 – Patient data of HGSOC samples

Sample ID	Tumor site	Diagnosis	Histopathology	Age at first disease	FIGO stage	TNM stage	Ascites	Residual mass after surgery	CA125 serum levels	Adjuvant chemotherapy	Cycles	Response
OC1	Omentum	Primary Ovarian cancer	HGSOC	74	IIIC	T3cN1M0	>500ml	>=2cm	54	Carboplatin/Gemcitabine	10	CR (Platinum resistant)
OC2	Peritoneum	Primary Ovarian cancer	HGSOC	69	IIIC	T3cN1M0	>500ml	<0.5cm	900	-		
OC3	Peritoneum	Primary Ovarian cancer, Interval surgery	HGSOC	71	IVb	T3bN1M1	No ascites	<0.5cm	34	Carboplatin/Paclitaxel/ Avastin	9	PR (Platinum resistant)
OC4	Omentum/Peritoneur	Primary Ovarian cancer	HGSOC	55	IIIC	T3cN1M0	>500ml	<1cm	2800	Carboplatin/Paclitaxel/ Avastin	6	SD (Platinum resistent)
OC5	Omentum/Peritoneur	Primary Peritoneal carcinor	HGSOC	59	IIIC	T3cN1M0	>500ml	No residual mass	447	-		
OC6	Peritoneum	Primary Ovarian Cancer	HGSOC	29	IIIb	T3bN1M0		<0.5cm	631	-	6	CR (Platinum sensitiv)
OC7	Peritoneum	Primary Ovarian cancer	HGSOC	86	IIIC	T3cN1M0		<2cm		-		
OC8	Peritoneum	Primary Ovarian cancer	HGSOC	53	IIIC	T2bN1M0	<500ml	No residual mass	982	Carboplatin/Paclitaxel/ Avastin or Olaparib	6	CR (Platinum sensitiv)
OC9	Peritoneum	Primary Ovarian cancer	HGSOC	58	IIIC	T3cN1M0	>500ml	No residual mass		Carboplatin/Paclitaxel/ Avastin	6	CR (Platinum sensitiv)
OC10	Laparoscopy	Primary Ovarian cancer	HGSOC	71	IVb	T3cN0M1	No ascites	No residual mass	84	Carboplatin/Paclitaxel	6	CR (Platinum sensitiv)
OC11	Peritoneum	Primary Ovarian cancer	HGSOC	65	IVb	T3bN0M1	>500ml	<2cm	897	-		
OC12	Omentum	Primary Ovarian cancer	HGSOC	80	IIIb	T3bN0M0	<500ml	<0.5cm	91	Carboplatin/Paclitaxel/ Avastin	6	CR (Platinum sensitiv)
OC13	Omentum	Primary Ovarian cancer	HGSOC	63	IVa	T3cN1bM1	>500ml	0cm	1995	Carboplatin/Paclitaxel/ Avastin	6	PR (Platinum resistant)

T- Size of tumor	CR- Complete Response
N - Number of lymphnodes	PR- Partial Response
M- Number of metastase	SD- Stable Disease

FIGO Stage	TNM stage	Description
I	T1-N0-M0	Tumor confined to ovaries or fallopian tube(s)
IA	T1a-N0-M0	Tumor limited to 1 ovary (capsule intact) or fallopian tube; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings
IB	T1b-N0-M0	Tumor limited to both ovaries (capsules intact) or fallopian tubes; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings
IC1	T1c1-N0-M0	Tumor limited to 1 or both ovaries or fallopian tubes, with surgical spill
IC2	T1c2-N0-M0	Tumor limited to 1 or both ovaries or fallopian tubes, with capsule ruptured before surgery or tumor on ovarian or fallopian tube surface
IC3	T1c3-N0-M0	Tumor limited to 1 or both ovaries or fallopian tubes, with malignant cells in the ascites or peritoneal washings
II	T2-N0-M0	Tumor involves 1 or both ovaries or fallopian tubes with pelvic extension (below pelvic brim) or peritoneal cancer
IIA	T2a-N0-M0	Extension and/or implants on uterus and/or fallopian tubes and/or ovaries
IIB	T2b-N0-M0	Extension to other pelvic intraperitoneal tissues
III	T1/T2-N1-M0	Tumor involves 1 or both ovaries or fallopian tubes, or peritoneal cancer, with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lym
IIIA1	T3a2-N0/N1-M0	Positive retroperitoneal lymph nodes only (cytologically or histologically proven)
IIIA2	T3a2-N0/N1-M0	Microscopic extrapelvic (above the pelvic brim) peritoneal involvement with or without positive retroperitoneal lymph nodes
IIIB	T3b-N0/N1-M0	Macroscopic peritoneal metastasis beyond the pelvis up to 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes
IIIC	T3c-N0/N1-M0	Macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes
IV	Any T, any N, M1	Distant metastasis excluding peritoneal metastases
IVA	Any T, any N, M1	Pleural effusion with positive cytology
IVB	Any T, any N, M1	Parenchymal metastases and metastases to extra-abdominal organs

According to the FIGO Cancer Report 2018

Supplementary Table 2 – HGSOC Medium composition

Sample	Deposit	Medium	Time in culture
OC1	Om	Basic +EFR	p17 (12.5 months)
OC2	Peri	Basic +EFR	p20 (13 months)
OC3	Peri	Basic +E	p15
OC4-1	Peri	Basic +E	p26 (16 months)
OC4-2	Om	Basic +EFNR	p26
OC5-1	Peri	Basic +E	p8
OC5-2	Om	Basic +E	p8
OC6	Peri	Basic +E	p8
OC7	Peri	Basic +EB	p26 (17 months)
OC8	Peri	Basic +EFN	p10; limited expansion in later passage
OC9	Peri	Basic +E	p6
OC10	L	Basic +EB	p23 (14 months)
OC11	Peri	Basic +EB	p23 (12.5 months)
OC12	Peri	Basic +EB	p17
OC13	Peri	Basic +EB	p17

	active BMP signaling, low Wnt
	active BMP, high Wnt
	BMP inhibition

Om - Omentum	p - Passage
Peri - Peritoneum	
L - Laparoscopy	
Basic = Nicotinamide, B27, N2, ROCKi, TGF-β inhibitor	
Fallopian tube medium = Basic +EGF +Noggin +FGF +RSPO1 +Wnt3a	
Ovarian Cancer Medium = Basic +EGF +BMP2	
B	BMP2
E	EGF
F	FGF
N	Noggin
R	R-Spondin1

Supplementary Table 3 – Target genes for sequencing

Target genes	
ABL1	KIT
ALK	KRAS
APC	LEF1
ARID1A	LRP5
ATM	MAML1
ATR	MAML2
BACH1	MAML3
BARD1	MAP3K7
BRAF	MAPK2K1
BRCA1	MET
BRCA2	MLH1
BRD4	MMP7
BRF2	MPL
BRIP1	MSH2
CCNE1	MSH6
CDH1	MYC
CDK12	NBN
CDKN2A	NF1
CHD8	NFAT5
CHEK2	NFATC3
CREBBP	NOTCH1
CSF1R	NOTCH3
CSMD3	NPM1
CSNK2A1	NRAS
CTBP2	PALB2
CTNNB1	PDGFRA
CUL1	PDGFRB
DAAM2	PIK3CA
DDR2	PIK3R1
DSN1	PMS2
EGFR	PPP2R1A
EMSY	PPP3P1
EP300	PRICKLE1
ERBB2	PRICKLE2
ERBB4	PRKACG
EZH2	PTEN
FANCA	PTPN11
FANCI	RAD50
FAT3	RAD51B
FBXW7	RAD51C
FGFR1	RAD51D
FGFR2	RAD51L1
FGFR3	RAD51L3
FLT3	RAD52
FZD1	RAD54L
FZD3	RB1
GABRA6	RET
GNA11	ROCK1
GNAQ	ROCK2
GNAS	SENP2
HNF1A	SMAD4
HRAS	SMARCB1
IDH1	SMO
IDH2	SRC
IGF1R	STK11
JAK1	TCF7L2
JAK2	TGFBR2
JAK3	TP53
KDR	TPX2
	VANGL1
	VANGL2
	VHL

Supplementary Table 4 – Allelic frequencies of mutations identified by targeted sequencing in tissue and organoids

Gene	Mutation (HGVS.c)	Effect	rare in healthy	OC2 -T	OC2 - O	OC4 P- T	OC4 P -O	OC4 Om -T	OC4 Om - O	OC5 - T	OC5 -O	OC6 - T	OC6 -O	OC7 - T	OC7 -O	OC8 -T	OC8 -O	OC10 -T	OC10 - O	OC11 -T	OC11 -O
ATM	c.4534G>A	missense_variant	yes, <1% of population																	0,452	0,514
ATM	c.2119T>C	missense_variant	yes, <1% of population	0,461	0,491																
ATR	c.1517A>G	missense_variant	yes, <1% of population									0,603	0,627								
ATR	c.4576A>G	missense_variant	yes, <1% of population									0,589	0,636								
BRCA1	c.4900A>G	missense_variant	no							0,774	0,987			0,988	0,993	0,24		0,911	0,984		
BRCA1	c.3113A>G	missense_variant	no							0,784	0,991			0,982	0,989	0,208		0,909	0,992		
BRCA1	c.2612C>T	missense_variant	no							0,774	0,998			0,997	0,998	0,207		0,921	0,999		
CREBBP	c.5933A>G	missense_variant	yes, <1% of population	0,377	0,459																
EGFR	c.1562G>A	missense_variant	no							0,992	0,988					0,36	0,303				
EP300	c.6288T>G	missense_variant	yes, <1% of population	0,547	0,486																
ERBB2	c.1963A>G	missense_variant	no	0,602	0,473											0,763	0,995				
FANCA	c.1238G>T	missense_variant	yes, <1% of population											0,453	0,465						
FAT3	c.10552G>T	missense_variant	no	0,515	0,479					0,997	0,997					0,416	0,47	0,548	0,353		
FZD9	c.434G>A	missense_variant	yes, <1% of population																	0,355	0,28
KIT	c.1621A>C	missense_variant	no							0,497	0,535	0,481	0,511								
LRP5	c.2335G>A	missense_variant	yes, <1% of population													0,485	0,495				
MAML1	c.1526T>G	missense_variant	yes, <1% of population									0,485	0,479								
MLH1	c.655A>G	missense_variant	no			0,512	0,498	0,516	0,75	0,62	0,763	0,602	0,631	0,568	0,341			0,48	0,487		
MSH6	c.472C>T	missense_variant	no									0,861	0,966	0,574	0,626			0,454	0,694		
NOTCH3	c.3399C>A	missense_variant	no	0,502	0,482																
PTEN	c.464A>G	missense_variant	yes, <1% of population														0,454				
ROCK2	c.1313A>G	missense_variant	yes, <1% of population											0,579	0,673						
ROCK2	c.1292C>A	missense_variant	no	0,995	0,999	0,491	0,481	0,501	0,242	0,997	0,997			0,409	0,322	0,998	0,997	0,989	0,996	0,29	0,318
TCF7L2	c.1535C>G	missense_variant	yes, <1% of population	0,624	0,591																
TP53	c.524G>A	missense_variant	yes, <1% of population																0,986		
TP53	c.501dupG	frameshift_variant	yes, <1% of population							0,541	0,958										
TP53	c.283delT	frameshift_variant	yes, <1% of population											0,304	0,956						
TP53	c.660T>G	stop_gained	yes, <1% of population																	0,58	0,994
TP53	c.993+1G>C	splice_donor_variant&	yes, <1% of population	0,22																	
TP53	c.517G>A	missense_variant	yes, <1% of population									0,711	0,983								
TP53	c.560-1G>A	splice_acceptor_varia	yes, <1% of population			0,418	0,993	0,693	0,995												
TP53	c.817C>T	missense_variant	yes, <1% of population													0,515	0,987				

O - Organoid
T - Tissue

Supplementary Table 5 – Protein expression in OC organoids

	p53	CyclinE1	BRCA1	RB
OC1-O				
OC2-O				
OC3-O				
OC4-O				
OC5-O				
OC6-O				
OC7-O				
OC8-O				
OC9-O				
OC10-O				
OC11-O				
OC12-O				
OC13-O				

	unchanged
	gain
	partial loss/down-reg.
	complete loss
	not tested

Supplementary Table 6 – Microarray data of Wnt target genes

	WT FTM vs Triple KD FTM					Triple KD FTM vs Triple KD OCM			
Gene	**Fold Change**	**P-value**	**Intensity1**	**Intensity2**		**Fold Change**	**P-value**	**Intensity1**	**Intensity2**
LEF1	-6,22	1,68E-27	4824	710		-54,44	7,93E-20	624	9
AXIN2	-1,41	8,62E-07	142	101		-6,15	0	97	16
CCND1	-1,76	0,00189	4362	2443		-1,86	0,00001	2685	1477
TCF4	-1,41	0,00501	3034	2087		-7,90	0	1649	210
DKK3	2,94	0,00	14,52	39,20		2,86	0,00	36,79	104,88
KREMEN2	3,44	1,24E-31	43	145		-1,26	0,00298	131	103