

Table S1. Clinicopathological characteristics of the high serous ovarian cancer TMA cohort.

Serous ovarian carcinomas (n=144)		
Age at diagnosis (years)	Median (range)	61 (24-87)
Histological grade	Moderate	22
	Poor	122
FIGO stage	Stage II	2
	Stage III	134
	Stage IV	8
Residual disease after surgery	No	21
	Yes	81
	Unknown	42
FSHR immunoreactive score	≤2	39
	≥3	73
	Lost/unstained cores	32
LHCGR immunoreactive score	≤2	82
	≥3	38
	Lost/unstained cores	24
Recurrence	No	30
	Yes	99
	Unknown	15
Cause of death	Ovarian cancer	87
	Other cause	14
	Alive	41
	Lost to follow-up	2

Table S2. Clinicopathological characteristics of benign and high grade serous ovarian cancer cohort used in the Fluidigm qRT-PCR

Benign serous cystadenomas (n=17)		
Age	Median (range)	60 (25-75)
High grade serous ovarian carcinomas (n=29)		
Age at Diagnosis	Median (range)	59 (38-84)
Histological Grade	Moderate	1
	Poor	28
FIGO stage	Stage I	4
	Stage II	8
	Stage III	17

Table S3. Summary of FSHR and LHCGR antibodies used for immunohistochemistry and western blotting

Antibody	Source	Immunogen	Clonality	Dilution (IHC)	Dilution (WB)	Description
FSHR 323	In-house ^{26, 29}	Fusion protein amino acids 172-358	Monoclonal	1/300	1/600	IgG, mouse
FSHR (H-190)	Santa Cruz (sc-13935)	Peptide amino acids 1-190	Polyclonal	-	1/400	IgG, rabbit
LHCGR (H-50)	Santa Cruz (sc-25828)	Peptide amino acids 28-77	Polyclonal	1/200	1/400	IgG, rabbit
LHCGR (LS-C334599)	Sapphire Bioscience	Recombinant protein of human <i>LHCGR</i>	Polyclonal	-	1/1000	IgG, rabbit

Table S4. *FSHR* and *LHCGR* siRNA used for knockdown studies

siRNA (Ambion), 10nM	ID#	Lot#	Target exons
<i>FSHR</i> A	s5377	AS02B123	7/8
<i>FSHR</i> B	s5379	AS02B124	7/8
<i>LHCGR</i> A	s8163	AS02B125	2
<i>LHCGR</i> B	s8164	AS02BBRO	11

Table S5. Relationship between FSHR and LHCGR expression with clinicopathological parameters

	Low FSHR IR <3	High FSHR IR ≥3	Low LHCGR IR <3	High LHCGR IR ≥3
Age				
<55	13/39 (33%)	26/39 (67%)	30/42 (71%)	12/42 (29%)
≥55	24/71 (34%)	47/71 (66%)	53/77 (69%)	24/77 (31.0%)
Chi-squared test ^a	<i>p</i> = 1.000		<i>p</i> = 0.837	
Tumor stage				
FIGO STAGE II	1/2 (50%)	1/2 (50%)	1/2 (50%)	1/2 (50%)
FIGO stage III	35/106 (33%)	71/106 (67%)	78/114 (68%)	36/114 (32%)
FIGO stage IV	3/4 (75%)	1/4 (25%)	4/5 (80%)	1/5 (20%)
Chi-squared test	<i>p</i> = 0.202		<i>p</i> = 0.732	
Tumor grade				
Moderate	7/17 (41%)	10/17 (59%)	16/19 (84%)	3/19 (16%)
Poor	32/95 (34%)	63/95 (66%)	67/102 (66%)	35/102 (34%)
Chi-squared test ^a	<i>P</i> = 0.587		<i>P</i> = 0.177	
Residual disease				
No	4/16 (25%)	12/16 (75%)	9/19 (47%)	10/19 (53%)
Yes	20/70 (29%)	50/70 (71%)	58/74 (78%)	16/74 (22%)
Chi-squared test ^a	<i>P</i> = 0.837		<i>P</i> = 0.01	

Fishers's exact test

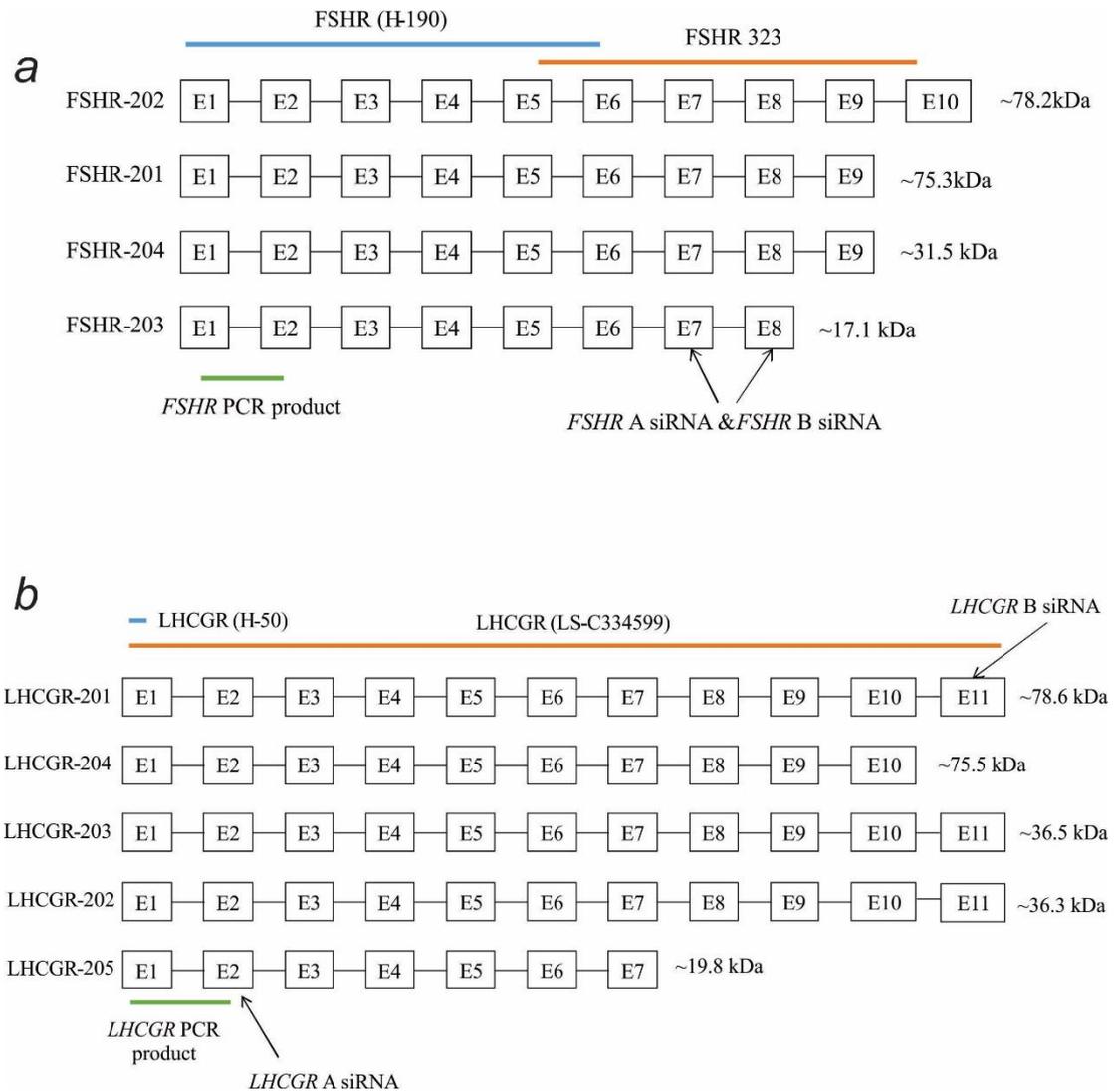


Figure S1. Human *FSHR* and *LHCGR* splice variants. E# refers to the exon number, not drawn to scale. (a) *FSHR* siRNA target exons indicated by arrows. *FSHR* H-190 antibody target region shown by blue line. *FSHR* 323 antibody target region shown by red line. *FSHR* (Hs00174865_m1) qRT-PCR primer location shown by green line. *FSHR* sequences from *FSHR* Ensembl and NCBI reference sequence NM_000145.3. *FSHR*-203 is not protein coding (nonsense mediated decay) (b) *LHCGR* siRNA target exons indicated by arrows. *LHCGR* H-50 antibody target region shown by blue line. *LHCGR* LS-C334599 antibody target region shown by red line. *LHCGR* (Hs00896336_m1) qRT-PCR primer location shown by green line. *LHCGR* sequences from *LHCGR* Ensembl and NCBI reference sequence NM_000233.3. (<https://asia.ensembl.org/index.html>)

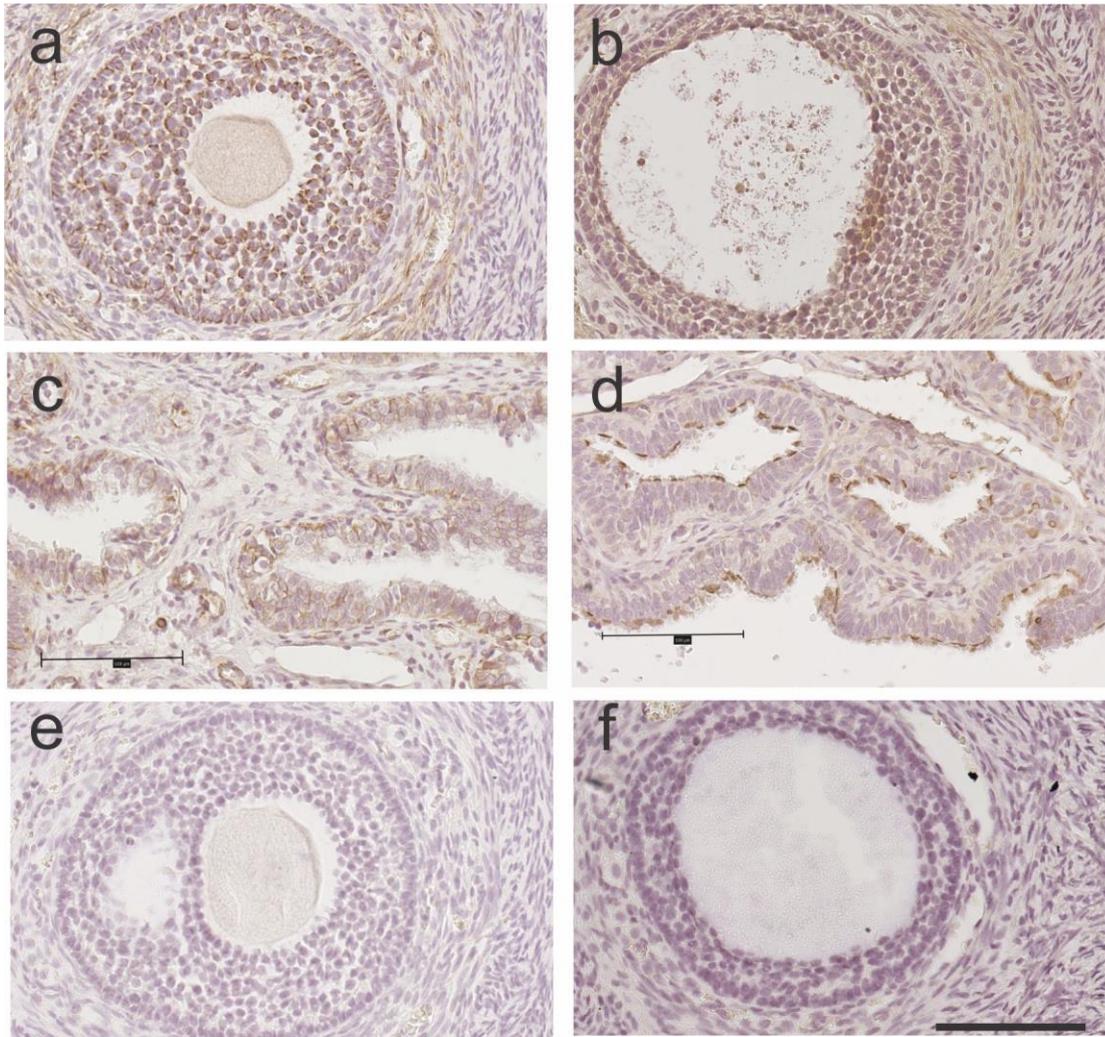


Figure S2. Follicle-stimulating hormone receptor (FSHR) and luteinising hormone receptor (LHCGR) expression in human ovary. (a) FSHR in normal ovary (FSHR 323 antibody, 1/300 obtained from Prof Ghinea [26]). (b) LHCGR in normal ovary and Fallopian tube (LHCGR H-50, 1/200, Santa Cruz). (c) FSHR in Fallopian tube (FSHR 323 antibody, 1/300). (d). LHCGR in Fallopian tube (LHCGR H-50, 1/200, Santa Cruz). (e) ovary with mouse IgG (3 μ g/ml) and (f) ovary with rabbit IgG (1 μ g/ml). Scale bar=100 μ m (all images the same magnification).

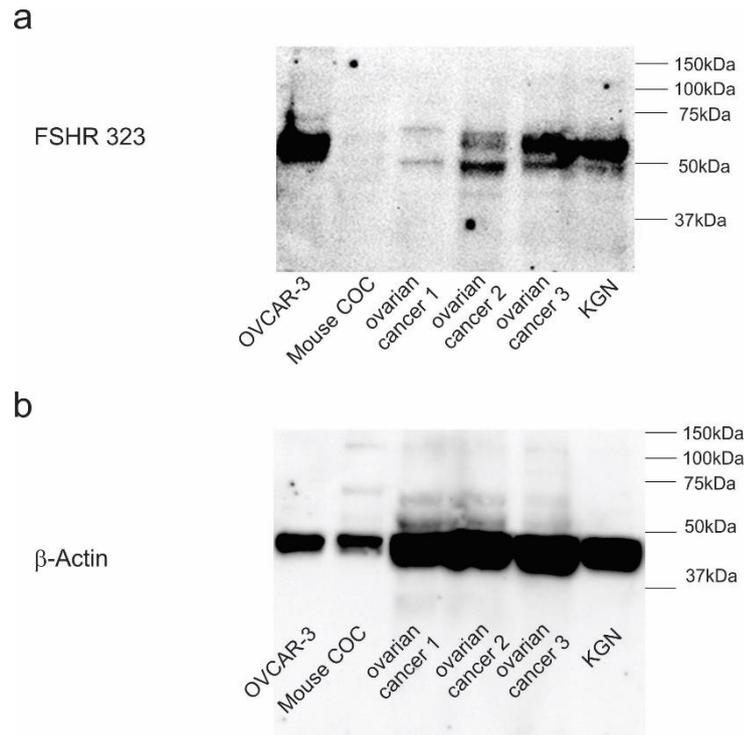


Figure S3. Western blot using FSHR323 antibody a) Protein extracts from cell lines (OVCAR3 and KGN, $\sim 40\mu\text{g}$) and ovarian cancer tissue extracts ($\sim 5\mu\text{g}$) were electrophoresed and immunoblotted with FSHR323 (1/600) antibody. b) β -actin (1/2000, Abcam) was used as a loading control. FSHR bands were detected at $\sim 50\text{kDa}$ and $\sim 65\text{kDa}$.

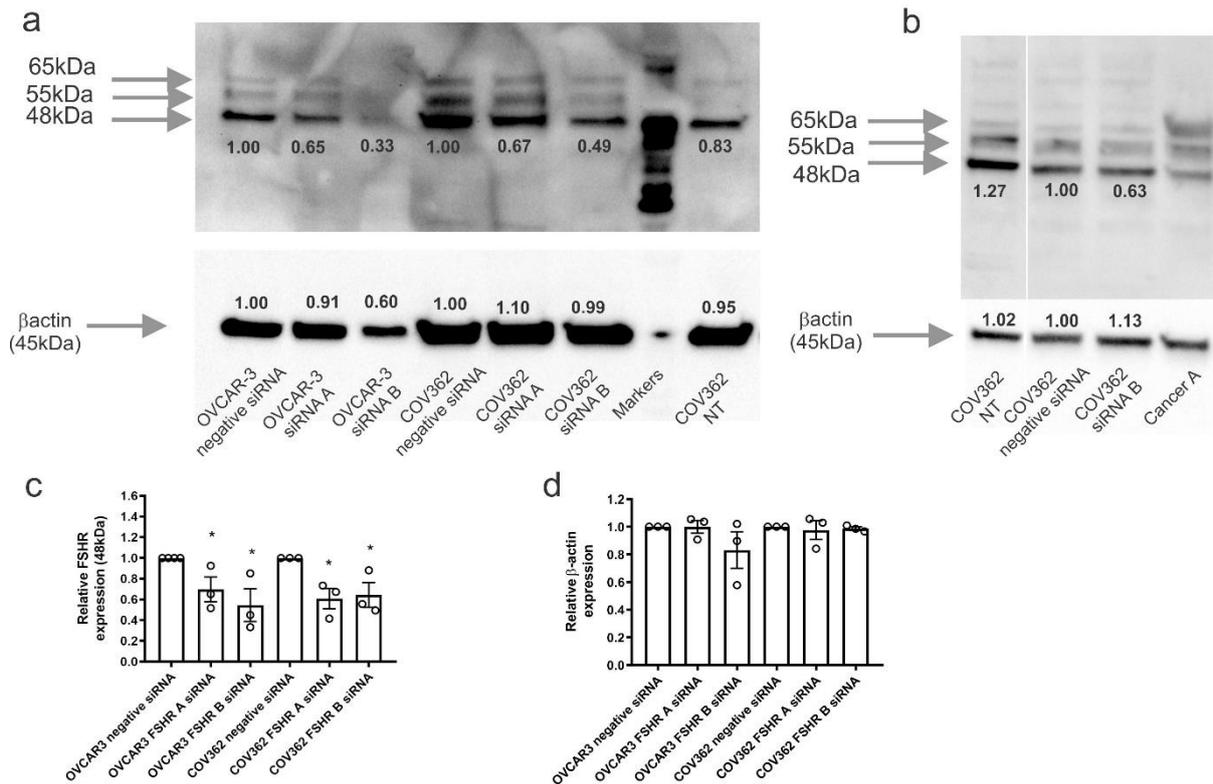


Figure S4. Effect of follicle-stimulating hormone receptor (FSHR) siRNA treatment on FSHR protein expression in OVCAR3 and COV362 cells. (a) Western blot following FSHR A and B siRNA treatment with FSHR (H-190) and β -actin antibodies in OVCAR3 and COV362 cells. $\sim 40\mu\text{g}$ of protein for cell lines and $\sim 5\mu\text{g}$ protein from ovarian cancer tissue extract were run on a 4-20% TGX gel and incubated with rabbit polyclonal antibodies FSHR H-190 (1/400, Santa Cruz) and β -actin (1/2,000, Abcam cat Ab8227). Numbers below protein bands are fold changes relative to the negative siRNA control treatment. **(b)** FSHR quantitation (48kDa) in FSHR A and B siRNA treated OVCAR3 and COV362 cells compared to the negative siRNA control treated cells. Data is from 3 independent experiments. Statistical significance was determined using the Student's t-test for the experiments on COV362 cells, $*p < 0.05$. **(c)** FSHR and **(d)** β -actin expression in FSHR A and B siRNA treated OVCAR3 and COV362 cells compared to the negative siRNA control treated cells

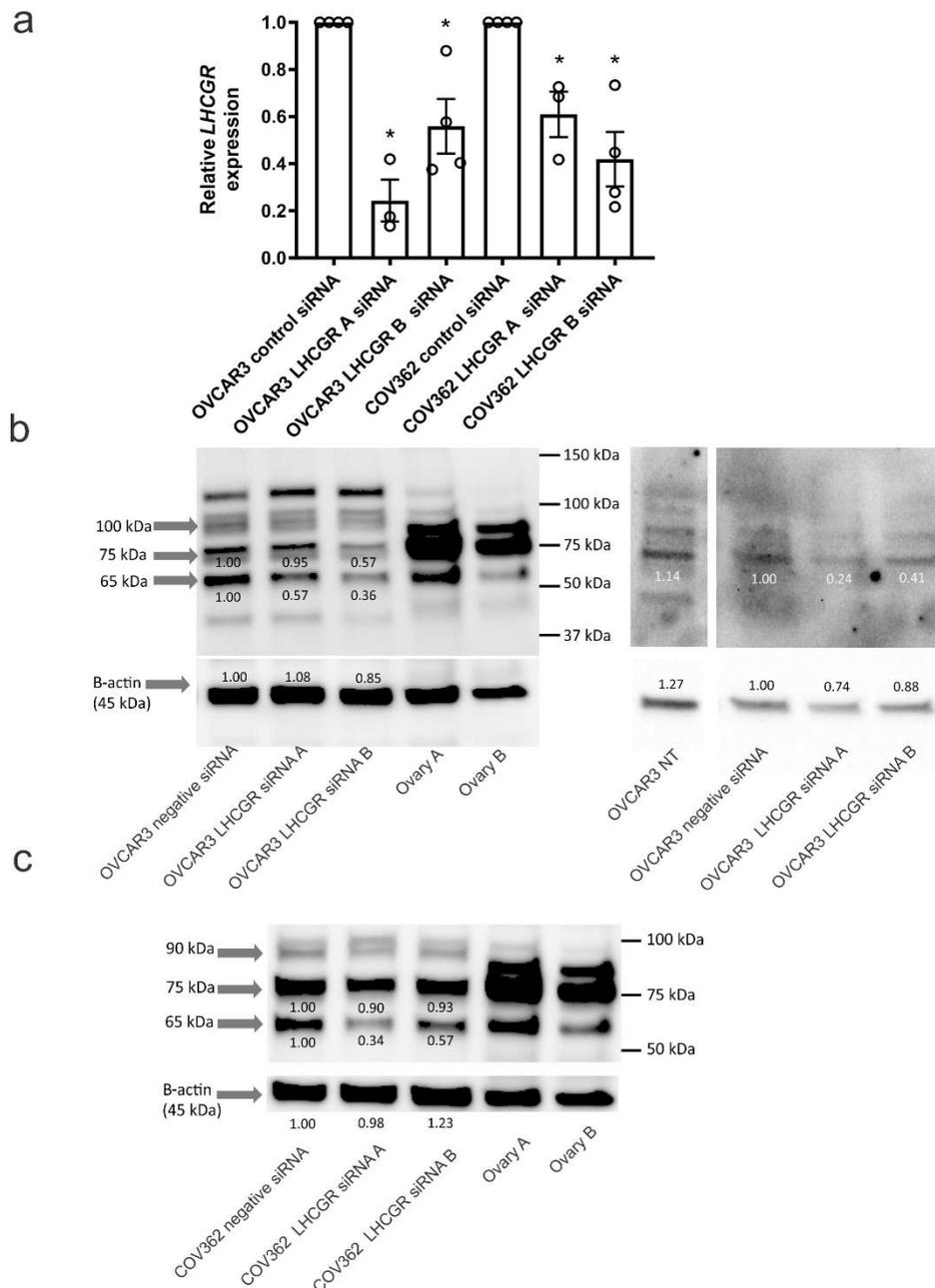


Figure S5. Effect of luteinising hormone receptor (LHCGR) siRNA treatment on LHCGR mRNA and protein expression in OVCAR3 and COV362 cells. (a) LHCGR expression was quantified using the $2^{-\Delta\Delta CT}$ method and normalised to housekeeping gene β -actin using negative siRNA control treatment as a calibrator. Data is from 3 to 4 independent experiments performed in triplicate ($n=9$ to $n=12$). Statistical significance from negative siRNA control treatment was determined using the Student's t-test, $*p<0.05$. Western blots for LHCGR expression following LHCGR A and B siRNA treatment in **(b)** OVCAR3 cells and **(c)** COV362 cells. Protein extracts ($\sim 40\mu\text{g}$ for OVCAR3 and COV362 & $\sim 2\mu\text{g}$ for the normal ovary tissues) were run on a 4-20% TGX gel and incubated with rabbit polyclonal antibodies LHCGR LS-C334599 (1/1000, Sapphire Bioscience) and β -actin (1/2,000, Abcam cat Ab8227). Human ovary extracts were from pre-menopausal women. Numbers below protein bands are fold changes relative to the negative siRNA control treatment.