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Supplemental information

lncRNA *DDGC* participates in premature ovarian insufficiency through regulating *RAD51* and *WT1*

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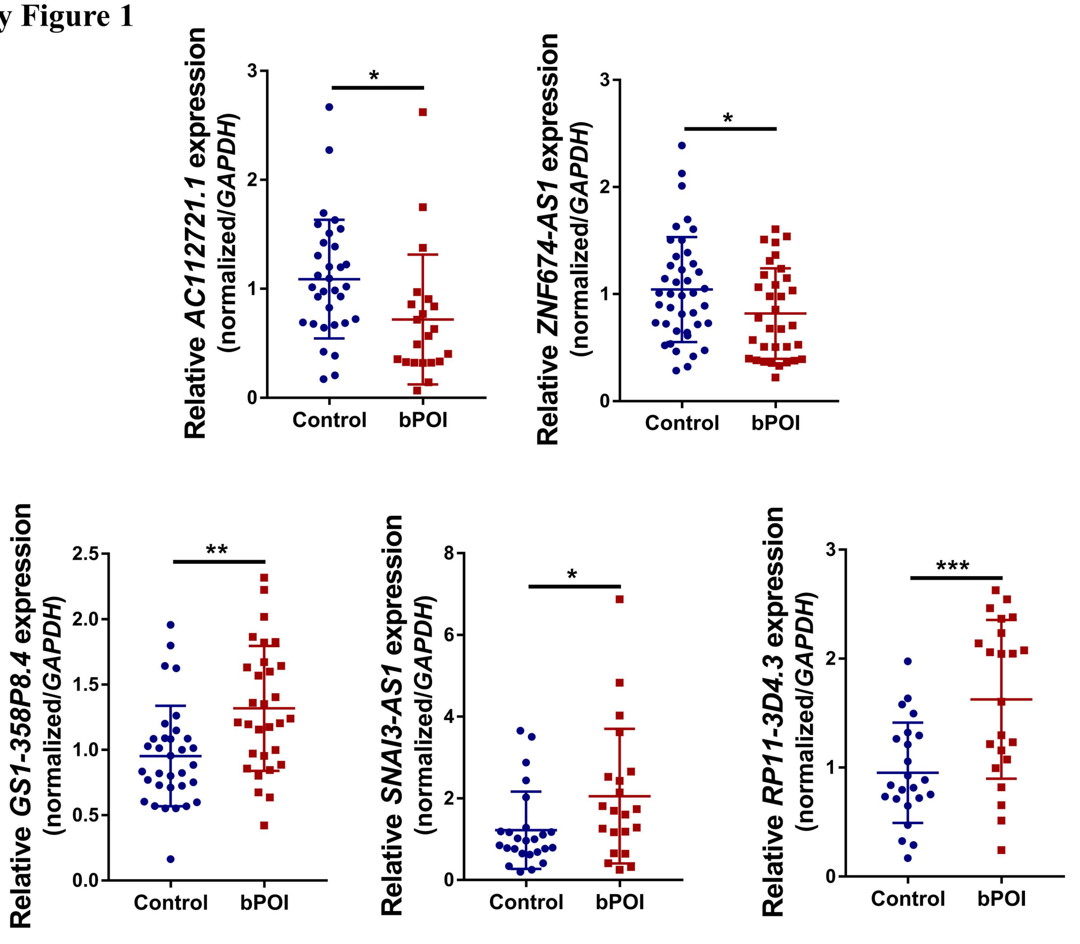
Supplementary Figure 1



Supplementary Figure 1. The expression levels of lncRNAs were validated by qRT-PCR in GCs from controls and bPOI patients. Ct values were normalized to *GAPDH*. Data are presented as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 by two-tailed Student's t-test.

Supplementary Figure 2

A

CPC2

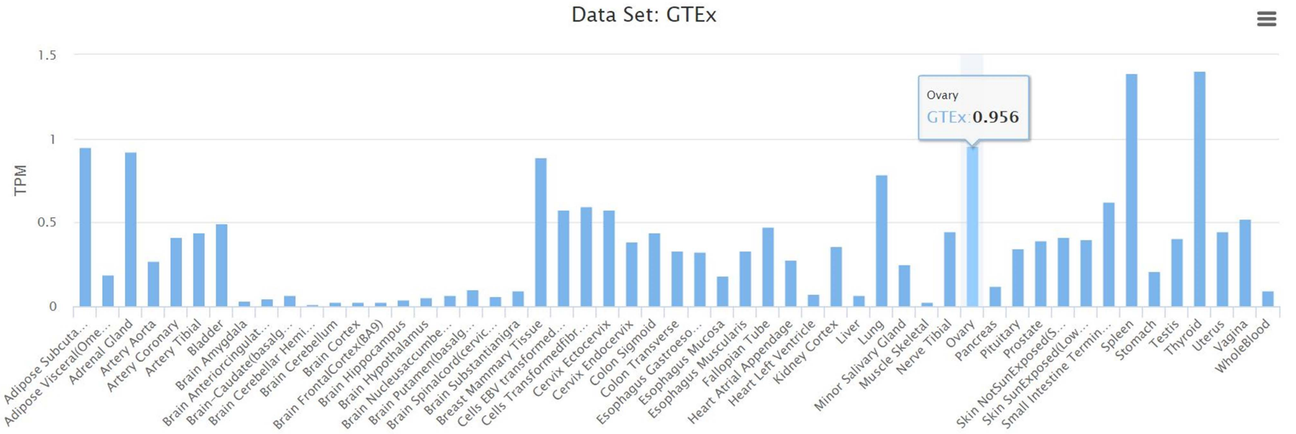
ID	Label	Coding probability	Peptide length(aa)	Fickett score	Isoelectric point	ORF integrity	Details
ENST00000464929.5	noncoding	0.051056	73	0.32494	11.4901733398	complete	View

CPAT

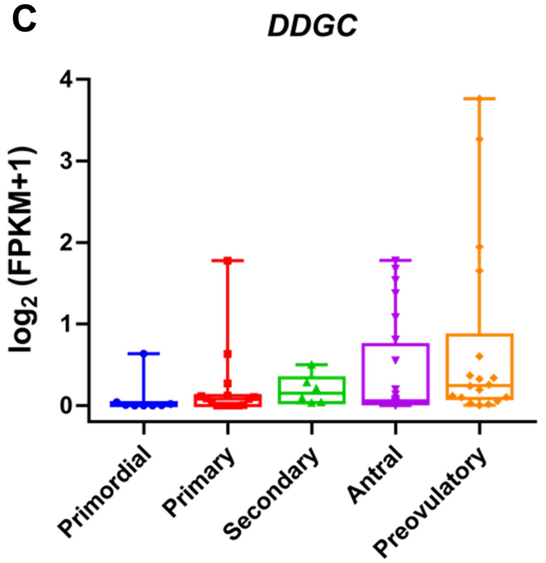
Result for species name : hg19 with job ID :1590583809							
Data ID	Sequence Name	RNA Size	ORF Size	Ficket Score	Hexamer Score	Coding Probability	Coding Label
0	ENST00000464929.5	819	219	0.5972	-0.0726352545543	0.012345255995295	no

B

Maximum (TPM)	Average (TPM)	Median (TPM)	CV	τ-Value	Expression Breadth
1.403	0.368	0.335	0.893	0.752	53

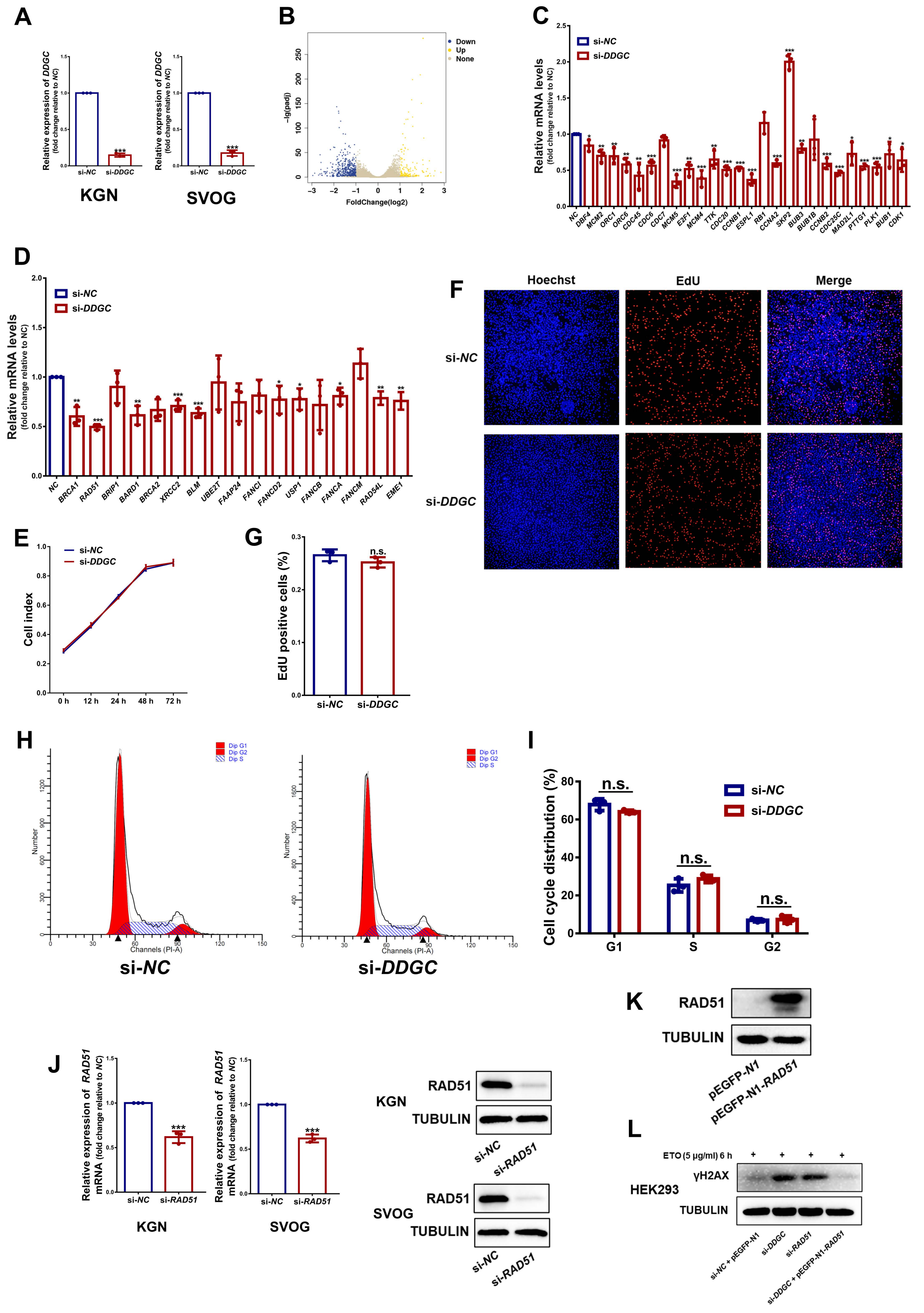


C



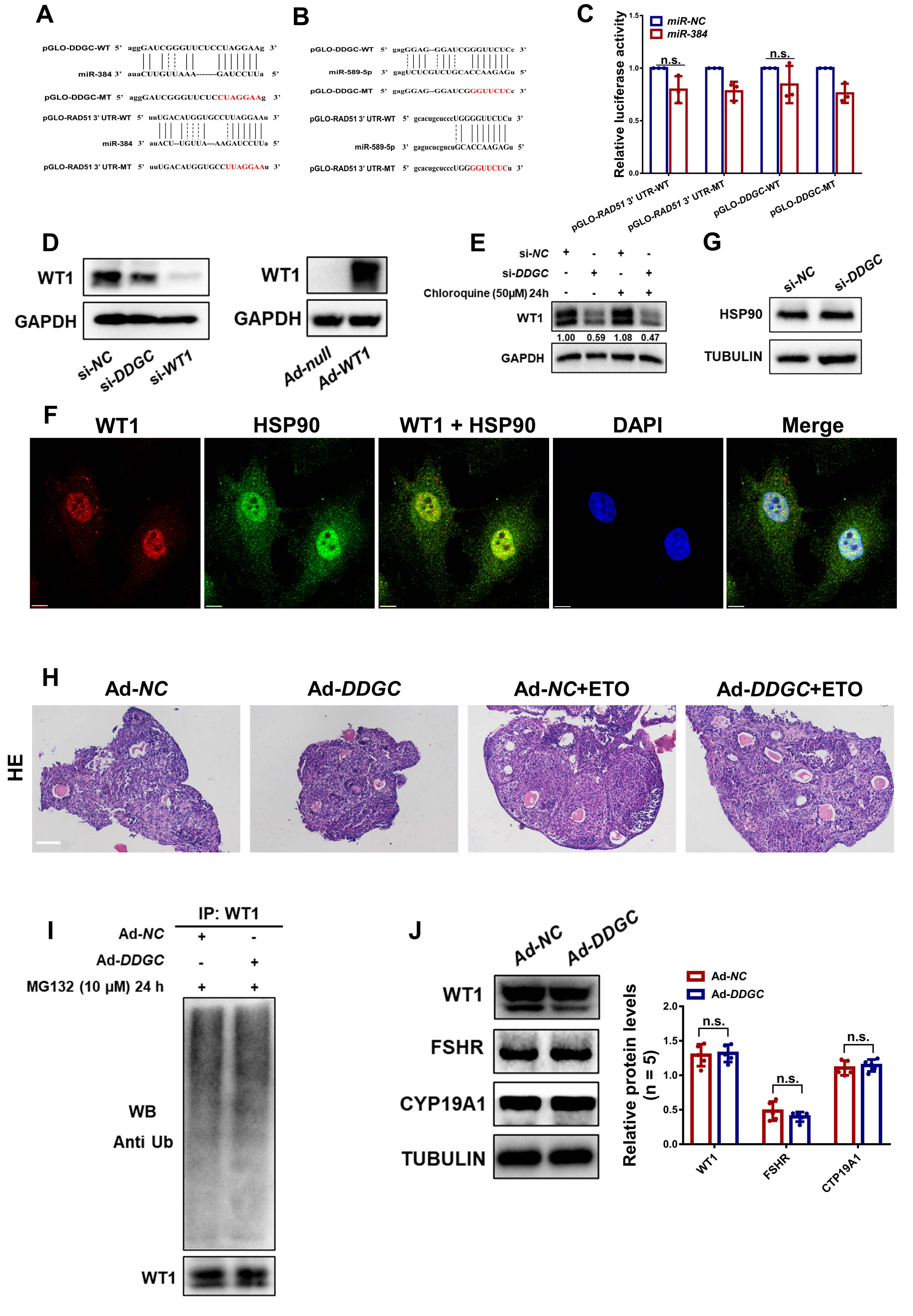
Supplementary Figure 2. (A) The coding probability of *DDGC* was assessed by the Coding Potential Calculator (CPC2) and Coding-Potential Assessment Tool (CPAT). **(B)** The expression levels of *DDGC* in multiple human tissues. Data are based on Genotype-Tissue Expression (GTEx) resources. **(C)** The expression of DDGC in human granulosa cells during folliculogenesis.

Supplementary Figure 3



Supplementary Figure 3. (A) The efficiency of *DDGC* silencing in KGN and SVOG cells was measured by qRT-PCR. **(B)** Volcano plot of differentially expressed mRNAs between *DDGC*-silenced (n = 2) and negative control KGN cells (n = 2). **(C, D)** qRT-PCR was used to validate the expression level of genes involved in cell cycle progression and DNA damage repair in DDGC-silenced and negative control KGN cells. **(E)** Cell viability of *DDGC*-silenced and negative control KGN cells was determined with the CCK8 assay. The results are expressed as the mean ± SD (n = 3). **(F, G)** The proliferative ability of *DDGC*-silenced and negative control KGN cells was measured using an EdU staining assay. The results are expressed as the mean ± SD (n = 3). n.s. = not significant by two-tailed Student's t-test. **(H, I)** The cell cycle distribution of *DDGC*-silenced and negative control KGN cells was determined by flow cytometry analysis. The results are expressed as the mean ± SD (n = 3). Two-tailed Student's t-test. **(J)** *RAD51* mRNA and protein levels in *RAD51*-silenced and negative control KGN and SVOG cells were measured by qRT-PCR and western blot, respectively. **(K)** The RAD51 protein levels in *pEGFP-N1-RAD51* and *pEGFP-N1*-transfected HEK293 cells were measured by western blot. **(L)** The expression of γH2AX in *DDGC*-silenced, *RAD51*-silenced, co-transfected, and negative control HEK293 cells was measured by western blot. All qRT-PCR values were obtained from triplicate experiments and expressed as the mean ± SD (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001, n.s. = no significance by two-tailed Student's t-test.

Supplementary Figure 4



Supplementary Figure 4. (A, B) Luciferase reporter vectors were constructed by cloning the wild-type *DDGC* and *RAD51* mRNA 3' UTR sequences or MT sequences into the pmirGLO plasmid. The red nucleotides represent deleted sequences of the target sites. **(C)** Luciferase activity was analyzed at 48 h after co-transfection of reporter vectors and *miR-384* or negative control into HEK293 cells. Results are expressed as the mean ± SD (n = 3). n.s. = no significance by two-tailed Student's t-test. **(D)** The protein levels of WT1 in *DDGC*-silenced, *WT1* silenced, *WT1*-overexpressing, and negative control KGN cells were measured by western blot. **(E)** The protein levels of WT1 in *DDGC*-silenced and negative control KGN cells was measured by western blot at 24 h after the addition of chloroquine. **(F)** Subcellular localization of WT1 and HSP90 protein was confirmed by immunofluorescence assay. Scale bars = 10 μm. **(G)** The protein levels of HSP90 in *DDGC*-silenced and negative control KGN cells were measured by western blot. **(H)** Representative histological sections of *in vitro* cultured ovaries after *DDGC* over-expression. Scale bar = 50 μm. **(I)** Western blot showing the polyubiquitination level of WT1 in DDGC-overexpressed and negative control in vitro cultured ovaries at 24 h after the addition of MG132. **(J)** The protein levels of WT1, FSHR and CYP19A1 in *DDGC*-overexpressed and negative control mice ovaries were measured by western blot. Relative protein levels are expressed as the mean ± SD (n = 5). n.s. = no significance by two-tailed Student's t-test.

Supplementary Table 1. Fold change of lncRNAs validation

Gene (homo sapiens)	Fold change	P value
AC112721.1	1.59	0.024
ZNF674-AS1	1.27	0.041
DDGC	2.12	0.007
GS1-358P8.4	1.34	0.001
SNAI3-AS1	1.77	0.038
RP11-3D4.3	1.46	<0.001

Supplementary Table 2. List of primers used in this study

Gene (homo sapiens)	Forward (5'-3')	Reverse (5'-3')
AC112721.1	ACAGCAGCCCTAGCAAACTAAC	CCTGAGGAACGTGTAAACTGG
ZNF674-AS1	CCATGCCAGCATTTGCTATTCA	GGGCTTGAGGCTCTAAAGATGT
DDGC	CAGAGCATGGTCATTCAACATT	TCTAGGGAAACCATCTTGGAGA
GS1-358P8.4	TCATTGAATAGTGTGGGCTGCT	CCTTTCCTCCACCTCTGAAACA
SNAI3-AS1	CATCGCTCCCTCTACACGAG	TCCTTCTTGAGGGGTTGAGTTA
RP11-3D4.3	AGGATCAGAGCTTTGCCTTCAA	TATCACCAACTTGGGTGGTCTC
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
DBF4	GGGCAAAAGAGTTGGTAGTGG	ACTTATCGCCATCTGTTTGGATT
MCM2	ATGATCGAGAGCATCGAGAACC	GCCAAGTCCTCATAGTTCACCA
ORC1	ACCGAGATTCACATCCAGATTGG	CGAGCACGTTTCTTAGGAGGA
ORC6	ACAAGGAGACATATCAGAGCTGT	AGTGGCCTGGATAAGTCAAGAT
CDC45	GTGGGCCATCGTTGGACTAAC	TCAAAGGAGATCCGTGTGCAG
CDC6	ACCTATGCAACACTCCCCATT	TGGCTAGTTCTCTTTTGCTAGGA
CDC7	GAGGCGTCTTTGGGGATTCAG	GGTCCTACTTGTAACTGTGCTG
MCM5	ATGTCGGGATTCGACGATCCT	CCAGGTTGTAATGCCGCTTG
E2F1	CGTGGACTCTTCGGAGAACTTT	TGATGGTGGTGGTGACACTATG
MCM4	TGAACCTCTATACATGCAACGAC	CAGGGTAACGGTCAAAGAAGATT
TTK	CGCAGCTTTCTGTAGAAATGGA	GCAACAAACTCAACCAGTCCTC
CDC20	GCACAGTTCGCGTTCGAGA	CTGGATTTGCCAGGAGTTCGG
CCNB1	TTGGGGACATTGGTAACAAAGTC	ATAGGCTCAGGCGAAAGTTTTT
ESPL1	CTTGAAGGAGTTCCTGTCCAACC	TGGGGTAGACACTAAGTAGCCA
RB1	CTCTCGTCAGGCTTGAGTTTG	GACATCTCATCTAGGTCAACTGC
CCNA2	GGATGGTAGTTTTGAGTCACCAC	CACGAGGATAGCTCTCATACTGT
SKP2	GCAAGACTTCTGAACTGCTGTC	TCCCATGAAACACCTGGAAAGT
BUB3	TACATTTGCCACAGGTGGTTCT	TCAAGTACATGGTGACTTGGGT

BUB1B	AAATGACCCTCTGGATGTTTGG	GCATAAACGCCCTAATTTAAGCC
CCNB2	TTGGCTGGTACAAGTCCACTC	TGGGAACTGGTATAAGCATTGTC
CDC25C	AAGTGGCCTATATCGCTCCC	CCCTGGTTAGAATCTTCCTCCA
MAD2L1	GGACTCACCTTGCTTGTAACTAC	GATCACTGAACGGATTTCATCCT
PTTG1	CTGTTCCGCTGTTTAGCTCTTG	TTTGATTGAAGGTCCAGACCCC
PLK1	AAAGAGATCCCGGAGGTCCTA	GGCTGCGGTGAATGGATATTTC
BUB1	TCCTTCAGATGCTTGAAGCCC	GAGGTCACTGTTGTACTCAGCA
CDK1	AAACTACAGGTCAAGTGGTAGCC	TCCTGCATAAGCACATCCTGA
BRCA1	TTGTTACAAATCACCCCTCAAGG	CCCTGATACTTTTCTGGATGCC
RAD51	CAACCCATTTCACGGTTAGAGC	TTCTTTGGCGCATAGGCAACA
RAD54L	TTGAGTCAGCTAACCAATCAACC	GGAGGCTCATACAGAACCAAGG
BRIP1	CTTACCCGTCACAGCTTGCTA	CACTAAGAGATTGTTGCCATGCT
BARD1	TCGCGTTGTACTAACATTCTGA	GACAGCTCATTGTCATGTAGCA
BRCA2	ACAAGCAACCCAAGTGTCAAT	TGAAGCTACCTCCAAAACTGTG
XRCC2	TCACCTGTGCATGGTGATATTCT	AGCTTTGGGATAGTCTGTGCTC
BLM	CAGACTCCGAAGGAAGTTGTATG	TTTGGGGTGGTGTAACAAATGAT
UBE2T	ATCCCTCAACATCGCAACTGT	CAGCCTCTGGTAGATTATCAAGC
FAAP24	GGCCACCAGTAACATGATCTCT	AAAGTCTGGTGTCAAGCCATCC
FANCI	CCACCTTTGGTCTATCAGCTTC	CAACATCCAATAGCTCGTCACC
FANCD2	AAAACGGGAGAGAGTCAGAATCA	ACGCTCACAAGACAAAAGGCA
USP1	ATGCCTGGTGTCATACCTAGT	CAGTCCCACAAATGGTAACAAGT
FANCB	ATGAAGGATGGCCTAAGGGTC	ACACACTAACAACTTTGCCAGT
FANCA	TTTGCTTGAGGTAGAAGGTCCA	CCCGGCTGAGAGAATACCCA
FANCM	TCGTGACGGTGGTTACAACAC	ACAAGACGAATTGGGCTCTTC
MALAT1	AAAGTCCGCCATTTTGCCAC	CTCACAAAACCCCCGGAACT
LMNB1	GAAAAAGACAACTCTCGTCGCA	GTAAGCACTGATTTCCATGTCCA
β-actin	ACAGAGCCTCGCCTTTGCC	GAGGATGCCTCTCTTGCTCTG
FSHR	AAAGCTGCCTACTCTGGAAAAG	GACCCCTAGCCTGAGTCATATAA
CYP19A1	TGGAAATGCTGAACCCGATAC	AATTCCCATGCAGTAGCCAGG
WT1	CACAGCACAGGGTACGAGAG	CAAGAGTCGGGGCTACTCCA
Gene (Mus musculus)	**Forward (5'-3')**	**Reverse (5'-3')**
Rad51	AAGTTTTGGTCCACAGCCTATTT	CGGTGCATAAGCAACAGCC
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Primers for in vitro transcription		
DDGC AS	CTAGCTAGCATTTAAAATAGAAATTTA	ATTTGCGGCCGCGCCACACACCCCGAGCCC

Supplementary Table 3. Oligonucleotides sequences used in this study

siRNAs	Sense(5'-3')
si-*NC*	UUCUCCGAACGUGUCACGUTT
si-*DDGC*	GGAGAGAAACAGGUAGCAATT
si-*RAD51*	CCAACGAUGUGAAGAAAUUTT
si-*WT1*	GCUUACCCAGGCUGCAAUATT
Biotin labelled miRNAs or probes	
Bio-NC	UUCUCCGAACGUGUCACGUTT
Bio-589-5p	UGAGAACCACGUCUGCUCUGAG
Biotin-DDGC-1	CAUACAUCUGUCAUGUAACACU
Biotin-DDGC-2	CAUACUGAAACUUCCUAGGAGA
Biotin-DDGC-3	AGGUAGUAGCUUCUAGGGAAAC
Biotin-DDGC-4	UGCUACCUGUUUCUCUCCUCAAUCA
Biotin-DDGC-5	UUGUAUAUCUGGUUUCUAAGAAUGC
Biotin-NC-probe	GUGUAACACGUCUAUACGCCCA

Supplementary Table 4. List of antibodies used in this study

Antibody	Supplier	Catalog#	Application
Phospho-Histone H2A.X (Ser139)	Cell Signaling Technology	9718S	WB/IF
GAPDH	Proteintech	60004-1-Ig	WB
PARP (Cleaved Asp214, Asp215)	Invitrogen	44-698G	WB
α-Tubulin	Proteintech	66031-1-Ig	WB
BRCA2	Abcam	ab27976	WB
BARD1	Santa Cruz	sc-74559	WB
BRCA1	Cell Signaling Technology	#9010	WB
Rad51 (D4B10) Rabbit mAb	Cell Signaling Technology	8875S	WB
β-Actin	Proteintech	66009-1-Ig	WB
Anti-Argonaute-2 antibody [EPR10411]	Abcam	ab186733	RIP
FSHR	Proteintech	22665-1-AP	WB
Aromatase (D5Q2Y) Rabbit mAb	Cell Signaling Technology	#14528	WB
FoxO1 (C29H4) Rabbit mAb	Cell Signaling Technology	2880T	WB
Anti-Wilms Tumor Protein antibody	Abcam	ab89901	WB/IF
WT1 (F-6)	Santa Cruz	sc-7385	IP
Ubiquitin (P4D1) Mouse mAb	Cell Signaling Technology	3936T	WB
HSP90	Abcam	ab13492	IP/RIP
HSP90 (C45G5) Rabbit mAb	Cell Signaling Technology	#4877	WB/IF
Anti-Lamin B1 antibody	Abcam	ab16048	WB
Anti-Aromatase	Abcam	ab18995	WB