**Figure legend**

**Figure 1. circPLPP4 expression is increased in CDDP-resistant OC cells and**

**tissues**

(A, B) Unsupervised hierarchical clustering and volcano plot analysis of the circRNAs differentially expressed in Platinum resistant OC tissues and Platinum sensitive OC tissues. (C) qRT-PCR analysis of *circPLPP4* expression in a 20-case cohort of freshly collected human OC samples with Platinum resistance and 20-case cohort of Platinum sensitive OC samples. The nonparametric Mann–Whitney U-test was used. (D) qRT-PCR analysis of *circPLPP4* in two CDDP resistant OC cells and its corresponding parent cell. (E) Schematic illustration demonstrated the circularization of exons 2-4 of *PLPP4* forms *circPLPP4* by “head-to-tail” junction and the upper black arrow represents the splicing sites. (F, G) *circPLPP4* expression in A2780-CDDP and SKOV3-CDDP cells examined by RT-PCR. Agarose gel electrophoresis demonstrated that divergent primers amplified *circPLPP4* in cDNA rather than genomic DNA (gDNA). GAPDH acted as a negative control. (H). RT–qPCR analysis for the expression of *circPLPP4* and *PLPP4* mRNA after treatment with RNase R in A2780-CDDP and SKOV3-CDDP cells. Data represent mean ± S.D. from three independent experiments; The P value was determined by a two-tailed unpaired Student’s t test. (I, J) qRT–PCR analysis for the expression of *circPLPP4* and *PLPP4* mRNAs after Actinomycin D treatment at the indicated time in A2780-CDDP and SKOV3-CDDP cells. Data represent mean ± S.D. from three independent experiments; The P value was evaluated by a two-way ANOVA. (K, L) Cytoplasmic and Nuclear mRNA Fractionation experiment indicating that *circPLPP4* mainly localized in the cytoplasm. β-actin and U3 were applied as positive controls in the cytoplasm and nucleus, respectively. Data represent mean ± S.D. from three independent experiments; The P value was evaluated by a two-tailed unpaired Student’s t test. (M, N). FISH for *circPLPP4*. Nuclei were stained with DAPI.

**Figure 2.** **CircPLPP4 expression level is associated with poor prognosis in OC patients and silencing of circPLPP4 facilitate CDDP sensitivity of OC cells**

(A). Representative sections of *circPLPP4* in 166 OC tissues using in situ hybridization. (B). The distribution of *circPLPP4* in Platinum resistant and Platinum sensitive patient specimens detected by in situ hybridization (ISH). χ2 test was used. (C). *circPLPP4* is remarkably relevant with the response status of chemotherapy. χ2 test was used. (D) Kaplan–Meier analysis of 5-year Relapse-free survival (RFS) in OC patients stratifed by low and high *circPLPP4* levels (n= 166, log-rank test). HR, hazard ratio. (E). Multivariate Cox regression analysis to assess the significance of the correlation between *circPLPP4* expression signature and OS in the presence of other important clinical variables. (G, H) ISH analysis of *circPLPP4* expression in OC tissues (92 Platinum sensitive OC patients and 72 Platinum resistant OC patients). (I) Staining index of *circPLPP4* in 166 OC tissues (92 Platinum sensitive OC patients and 72 Platinum resistant OC patients). (J) qRT-PCR analysis of *circPLPP4* and linear PLPP4 expression in the indicated OC cells. GAPDH act as a control. (K) MTT cell viability assay of CDDP in the indicated cells. (K, L) Representative images and quantification of colony number of the indicated cells. (M) Western blot analysis shows apoptotic proteins in the indicated cells (GAPDH was selected as the loading control). (N) Western blotting analysis of level of γ-H2AX and BRCA1 in the indicated cells. (GAPDH was acted as the loading control). (O) FACS analysis of Annexin V / PI staining (left) and quantification (right) of indicated cells treated with vehicle or CDDP (5μM) after 24 hours. (P) Representative images (left) and quantification (right) of γ-H2AX in the indicated OC cells treated with CDDP (5μM) after 24 hours.

**Figure 3. circPLPP4 exerts its function by sponging miR-136**

(A) Ago2-RNA RIP assay for *circPLPP4* expression in the indicated cells. (B) Schematic illustration indicating potential target miRNAs of *circPLPP4* as predicted by CircInteractome. (C, D) Gel electrophoresis and qRT-PCR were used to validated the specificity and efficiency of the *circPLPP4* probe in A2780-CDDP and SKOV3-CDDP cells. (E, F) qRT-PCR analysis of the expression of thirteen potential target miRNAs in A2780-CDDP and SKOV3-CDDP cells. MiR-136 was stably pulled down by *circPLPP4* in both A2780-CDDP and SKOV3-CDDP cells. (G) Biotinylated miRNA pull-down (WT or mut) and qRT-PCR assays indicating the levels of *circPLPP4* in the indicated OC cells. GAPDH was acted as the negative control. (H) Schematic illustration of *circPLPP4*-wt and *circPLPP4*-mut luciferase reporter vectors; the binding ability between *circPLPP4* and MiR-136 was measured by dual-luciferase reporter assay in A2780-CDDP and SKOV3-CDDP cells. (I, J) The luciferase activities of the *circPLPP4* luciferase reporter vector (WT or mut) assessed after transfection with miR-136 mimics or mimic NC into A2780-CDDP and SKOV3-CDDP cells. (K, L). FISH showing the expression of *circPLPP4* and miR-136 in A2780-CDDP and A2780 cells. Nuclei were stained with DAPI. (M) Platinum-resistant or Platinum-sensitive OC

tissues from patients. FISH scores of *circPLPP4* and miR-136 were further evaluated in 30 Platinum-resistant and 19 Platinum-sensitive patient tissues. Nuclei were stained with DAPI.

**Figure 4. circPLPP4 enhanced PIK3R1 expression by sponging miR-136 in EOC cells**

(A) RNA-seq data of the top 20 up-regulated mRNAs in A2780 CDDP cells and A2780 CDDP-ASO#1 cells are showed as heatmaps. (B)Venn diagram demonstrated 4 genes that are putative miR-136 targets computationally predicted by four algorithms (miRanda, RNAhybrid, miRWalk and TargetScan) within the top 20 upregulated genes. (C) The expression of PIK3R1 was analyzed using RT-qPCR and western blot in A2780 CDDP, SKOV3 CDDP, A2780 and SKOV3 cells. (D) The expression of PIK3R1 was assessed using RT-qPCR and western blot in A2780 CDDP, SKOV3 CDDP, A2780 and SKOV3 cells. (E) Schematic of PIK3R1 3’UTR wild-type (WT) and mutant (Mut) luciferase reporter vectors is displayed (Top). The relative luciferase activities were examined in A2780CDDP cells co-transfected with miR-136 mimics or miR-NC and luciferase reporter vectors PIK3R1 3’UTR (WT) or PIK3R1 3’UTR (Mut) (Bottom). (F, G) The expression of PIK3R1 was detected by RT-qPCR in A2780 CDDP and SKOV3 CDDP cells were transfected with miR-126 mimic or co-transfected with the indicated vectors. (H) The expression levels of PIK3R1 were detected using RT-qPCR. A2780 cells were transfected with the indicated vectors and miR-136 mimics. (I) The expression levels of PIK3R1 were analyzed using RT-qPCR. A2780 CDDP cells were transfected with ASO-circPLPP4#1 alone or co-transfected the inhibitors. (J)The expression of PIK3R1, γ-H2AX，BRCA1, p-AKT, AKT and cleaved caspased 3 was examined by western blot . A2780 CDDP and SKOV3 CDDP cells were transfected with miR-136 mimic or co-transfected with the indicated vectors. (K). The IC50 was examined by the MTT assay. A2780CDDP cells were transfected with miR-136 mimic alone or cotransfected with the indicated vectors with CDDP treatment (5 μM) for 48 h. (L) The apoptosis percentages of A2780 CDDP cells transfected with miR-136 mimic alone or cotransfected with the indicated vectors upon CDDP exposure (5 μM) for 48 h. (M) The levels of PIK3R1 and apoptosis markers, γ-H2AX, BRCA1 and cleaved-caspased 3 were detected using western blotting in A2780 CDDP cells and SKOV3 CDDP cells transfected with ASO-circPLPP4#1alone or co-transfected with the inhibitor after CDDP treatment (5 μM). (N)Three-dimensional scatter plot of circPLPP4, miR-136 and PIK3R1 levels in 25 OC tissues. The results are displayed as the mean ± SEM. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

**Figure 5. circPLPP4 is modulated by m6A methylation**

(A) Predicted m6A sites in *circPLPP4* from a N6-methyladenosine (m6A) modification predictor (SRAMP), which based on sequence. (B) Flow diagram of m6A-specific immunoprecipitation (MeRIP) assays. (C) MeRIP assays for detecting m6A-modified circPLPP4 in A2780 CDDP cells and SKOV3 CDDP cells. (D) m6A RIP-qPCR analysis of circPLPP4 in A2780, SKOV3, A2780CDDP and SKOV3CDDP cells. Error bars represent the mean ± SD of three experiments. (E) Heat map profiling the expression of m6A WERs in 40 OC tissues (including 20 platinum resistant OC tissues and 20 platinum sensitive OC tissues). (F, G) METTL3 and IGF2BP1 were examined by RNA pulldown assays and western blotting using *circPLPP4* probe. (H) METTL3 expression was evaluated by western blotting in the indicated OC cells. GAPDH acted as the loading control. (I) m6A RIP-qPCR analysis of the m6A level in *circPLPP4* in the indicated cells. Error bars represent the mean ± SD of triplicate experiments. (J) qRT-PCR analysis of *circPLPP4* expression in the indicated cells. Error bars represent the mean ± SD of triplicate. (K) Correlation analysis demonstrated the correlation between *METTL3* and *circPLPP4* in OC tissues obtained from SYSUCC. Statistical analyses were performed by Spearman correlation coefficient. (L, N) Control or *METTL3*-knockdown or *IGF2BP1*-knockdown A2780CDDP cells were treated with actinomycin D (5mg/mL) for the indicated times. Total RNA was extracted and then analyzed using RT-qPCR to assess the half-lives of *circPLPP4*. Error bars represent the mean ± SD of three experiment. (M, P) RIP analysis indicating that the enrichment of *circPLPP4* on *IGF2BP1* in the indicated cells. Error bars represent the mean ± SD of triplicate experiment. (P) The putative wild- type m6A sites and designed mutant m6A sites in circPLPP4. (Q) qRT-PCR analysis of *circPLPP4* expression in the *circPLPP4* Wt or circPLPP4-Mut A2780CDDP cells with or without METTL3 or IGF2BP1 silencing. Error bars represent the mean ± SD of triplicate experiments. (R) The luciferase activities of different mutated *circPLPP4* reporter in the indicated groups. Error bars represent the mean ± SD of three experiments.

**Figure 6. Targeting circPLPP4 in vivo** **retards CDDP resistant OC**

(A-C) In vivo luminescent imaging of intraperitoneally implanted A2780 CDDP -luci or A2780 CDDP-luci-ASO-*circPLPP4#*1 cells in 4–6 weeks-old female BALB/c nude mice treated with PBS or CDDP (5 mg/kg) for three times per week upon the luminescence signal reached 2×107 p/sec/cm2/sr. Luminescence intensity ranges from low (blue) to high (red). Tumor burdens were quantified by total photon flux (p/s). (D) PIK3R1, γH2AX, cleaved caspase-3 and BRCA1 expression levels are examined in representative xenograft tumors by IHC (Left). Quantification of the IHC scores of PIK3R1, γH2AX, cleaved caspase-3 and BRCA1 expression levels (Right). (E-H) Flow Chart of A2780-CDX-CR (CDDP resistant) model construction. The first CDX generation was constructed in 4–6 weeks-old female BALB/c nude mice and treated with CDDP (5 mg/kg, three times per week). Twelve weeks later, the most resistant xenograft was disaggregated and implanted subcutaneously into 4–6 weeks-old female BALB/c nude mice as the second CR -CDX. Four weeks after implantation, the second CR -CDX mice were treated with CDDP (5 mg/kg, three times per week) and injected via tail vein with ASOs-targetting *circPLPP4* or its negative control twice a week. Mice were euthanized when the experiments were finished. The subcutaneous tumor size was measured and recorded every 3 days using the Vernier caliper as follows: tumor volume (mm3) = (L × W2)/2, where L is the long axis and W the short axis.

**Figure 7. circPLPP4 acts as a therapy target in EOC pre-clinical models**

(A) Schematic treatment administration (in vivo-optimized *circPLPP4* inhibitor or control) in PDX models. (B) Representative images of *circPLPP4* ISH analysis of OC tissue samples from OC patients. (C, D) Tumor weight and volume were examined in PDX-2 after 4-week treatment. (E) Tumor volumes were measured at the indicated time points in PDX-2. (F, G) Tumor weight and volume were assessed in PDX-1 after 4-week treatment. (H) Tumor volumes were measured at the indicated time points in PDX-1. (I, J) Body weights of tumor-bearing mice (PDX-1 and PDX-2) treated with CDDP combined with in vivo-optimized *circPLPP4* inhibitor or control. (K-M) Decreased PIK3R1 (K), increased γ-H2AX level (L) and apoptosis (M) were showed in PDX-2 tumors after CDDP combined with in vivo-optimized *circPLPP4* inhibitor treatment. Left, representative images of indicated staining. Right, quantification result according to its corresponding criteria . (N) Representative images showing high or low expression of *circPLPP4*, METTL3, IGF2BP1 and PIK3R1 in OC tumor specimens. (O) Correlation between *circPLPP4* and METTL3 IGF2BP1or PIK3R1in 166 OC tumor specimens. (P) qRT-PCR and western blotting analysis of *circPLPP4* and METTL3, IGF2BP1 or PIK3R1 expression in five OC tumor specimens. *circPLPP4* levels were normalized to that *circPLPP4* expression of case 1. GAPDH was acted as loading controls. (Q) Graphical abstract for *circPLPP4* function in CDDP resistance in OC. METTL3-mediated m6A modification for *circPLPP4* acts as a sponge for miR-136 to promote OC CDDP resistance via regulating PIK3R1 signaling.

**Supplemental Figure 1. CircPLPP4 expression level is relevant with poor prognosis in OC patients**

(A). Correlation analysis between *circPLPP4* expression and patient vital status. (B). (D) Kaplan–Meier analysis of Overall survival (OS) in OC patients stratifed by low and high *circPLPP4* levels (n= 166, log-rank test). HR, hazard ratio.

**Supplemental Figure 2. circPLPP4 knockdown increased CDDP sensitivity of CDDP-resistant OC cells**

(A) RT-qPCR analysis of *circPLPP4* and PLPP4 expression in the ASO-ctrl or *circPLPP4*-ASO#1, *circPLPP4*-ASO#2 cells. (B) MTT cell viability assay of CDDP in the indicated OC cells. (C, D) Quantification of colony number of the indicated cells. (E) FACS analysis of Annexin V/PI staining of indicated cells treated with vehicle or CDDP (5μM) after 24 hours. (F) Western blotting analysis of level of caspased 3, cleaved-caspased 3, PARP and cleaved-PARP in the indicated cells.(G) Western blotting analysis of level of γ-H2AX and BRCA1 in the indicated cells. (H) Immunofluorescence staining and quantification of of γ-H2AX in the indicated cells treated with CDDP (5μM) after 24 hours.

**Supplemental Figure 3. circPLPP4 confers CDDP resisitance of CDDP in OC cells**

(A) qRT-PCR analysis of circPLPP4 and PLPP4 expression in the indicated OC cells. (B, C) MTT cell viability assay of CDDP in the indicated cells. (D, E) Quantification of colony number of the indicated cells. (F) Western blotting analysis of level of caspased 3, cleaved-caspased 3, PARP and cleaved-PARP in the indicated cells.(G) Western blotting analysis of level of γ-H2AX and BRCA1 in the indicated cells. (H, I) FACS analysis of Annexin V/PI staining of indicated cells with vehicle or CDDP (5μM) treatment after 24 hours. (J, K) Quantification of γ-H2AX in the indicated OC cells with CDDP (5μM) treatment after 24 hours.

**Supplemental Figure 4**

(A-C) The expression of SLAMF7, UGT1A4 and PRDM8 were analyzed using qRT-PCR in A2780 CDDP, SKOV3 CDDP, A2780 and SKOV3 cells. Error bars represent the mean ± SD of three experiments. Statistical analyses were performed by unpaired Student’s t-test. \*P < 0.05, ns indicates no significance.

**Supplemental Figure 5. circPLPP4 enhanced PIK3R1 expression by sponging miR-136 in OC cells**

(A) The expression levels of PIK3R1 were examined using qRT-PCR in the indicated cells .(B) The expression levels of PIK3R1 were analyzed using qRT-PCR. SKOV3 cells were transfected with the indicated vectors and miR-136 mimics. (C) The expression levels of PIK3R1 were analyzed using qRT-PCR in the indicated cells. (D, E). The proteins levels of PIK3R1, apoptosis markers, γH2AX, BRCA1 were detected using western blotting in A2780 and SKOV3 cells transfected with the indicated vectors and miR-136 mimics after CDDP treatment (5μM). (F) The IC50 was detected by the MTT assay. SKOV3 CDDP cells were transfected with miR-136 mimic alone or co-transfected with the indicated vectors upon CDDP exposure (5 μM) for 48 h. (G) The apoptosis rates of SKOV3CDDP cells transfected with miR-136 mimic alone or co-transfected with the indicated vectors upon CDDP treatment (5 μM) for 48 h. The results are presented as the mean ± SEM. \*P < 0.05, \*\*P < 0.01.

**Supplemental Figure 6. m6A modification contributes to the upregulation of circPLPP4 in OC.**

(A) qRT-PCR analysis of circPLPP4 expression in the indicated cells with or without treatment of 5-zaz-dC. (B) RT-qPCR analysis of circPLPP4 expression in the indicated cells with or without treatment of SAHA or NaB. (C) qRT-PCR analysis of mRNA expression in 20 OC tissues and 20 normal ovary tissues. (D) Western blotting of ALKBH5 expression in the indicated cells. GAPDH served as the loading control. (E). qRT-PCR analysis of circPLPP4 in the indicated cells. (F) RIP analysis showing the enrichment of circPLPP4 on several proteins in the indicated cells. (G) qRT-PCR analysis of circPLPP4 expression in the indicated cells. (H) Nuclear–cytoplasmic fractionation assays revealing circPLPP4 expression in cytoplasm and nucleus of control or METTL3-knockdown the indicated OC cells. U3 and GAPDH were used as positive controls in the nucleus and cytoplasm, respectively. (I, J) Control or METTL3-knockdown or IGF2BP1-knockdown A2780 CDDP cell with actinomycin D (5mg/mL) treatment. Total RNA was extracted in the indicated times and then analyzed using qRT-PCR to examine the half-lives of circPLPP4. Error bars represent the mean ± SD of triplicate experiment.

**Supplemental Figure 7 Clinical relevance of m6A/ circPLPP4 /PIK3R1 axis in OC**

(A-C) Correlation analysis showing the correlation between circPLPP4 and METTL3 (A), IGF2BP1 (B) or PIK3R1 (C) in OC specimens . Statistical analyses were performed by Spearman correlation coefficient.