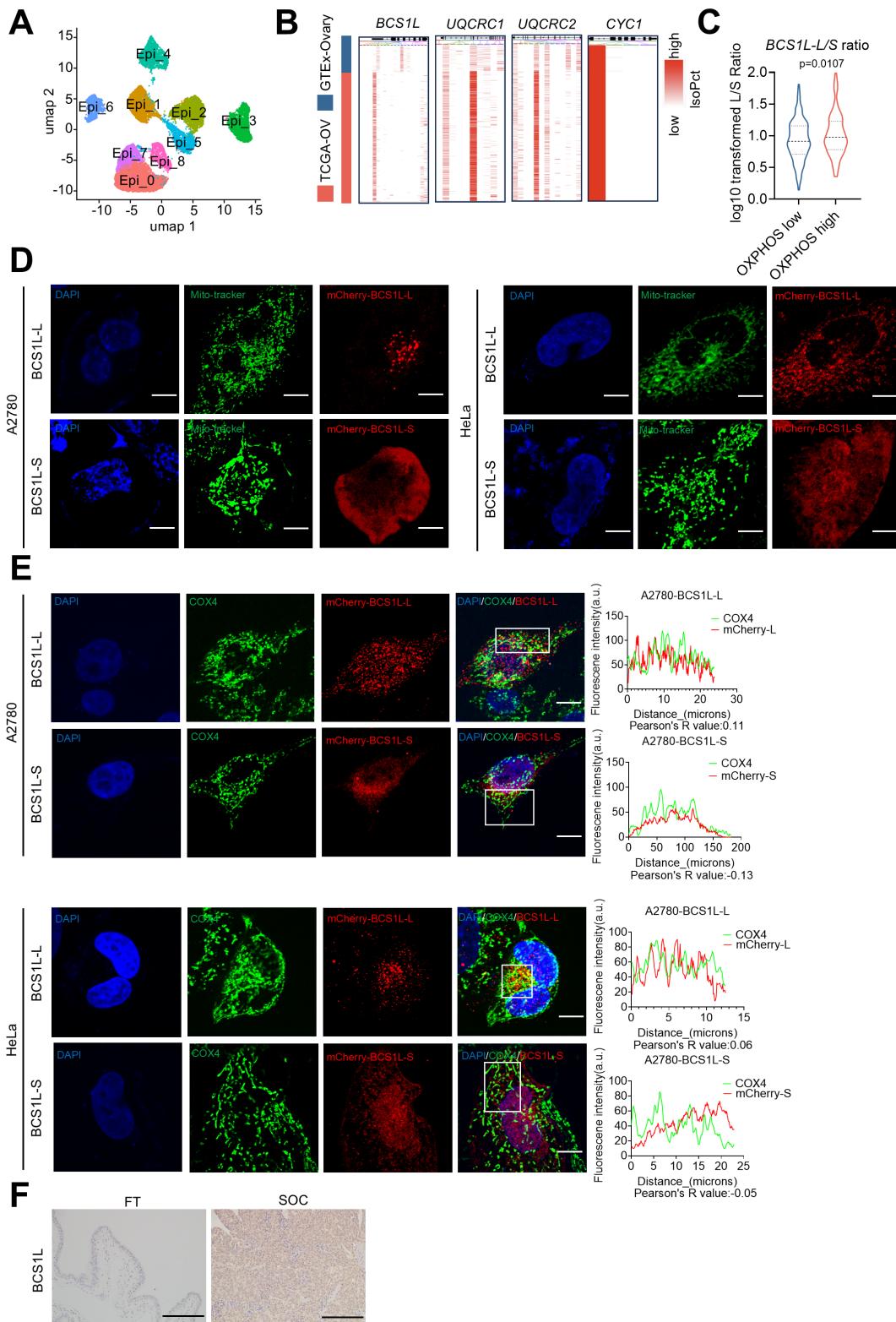


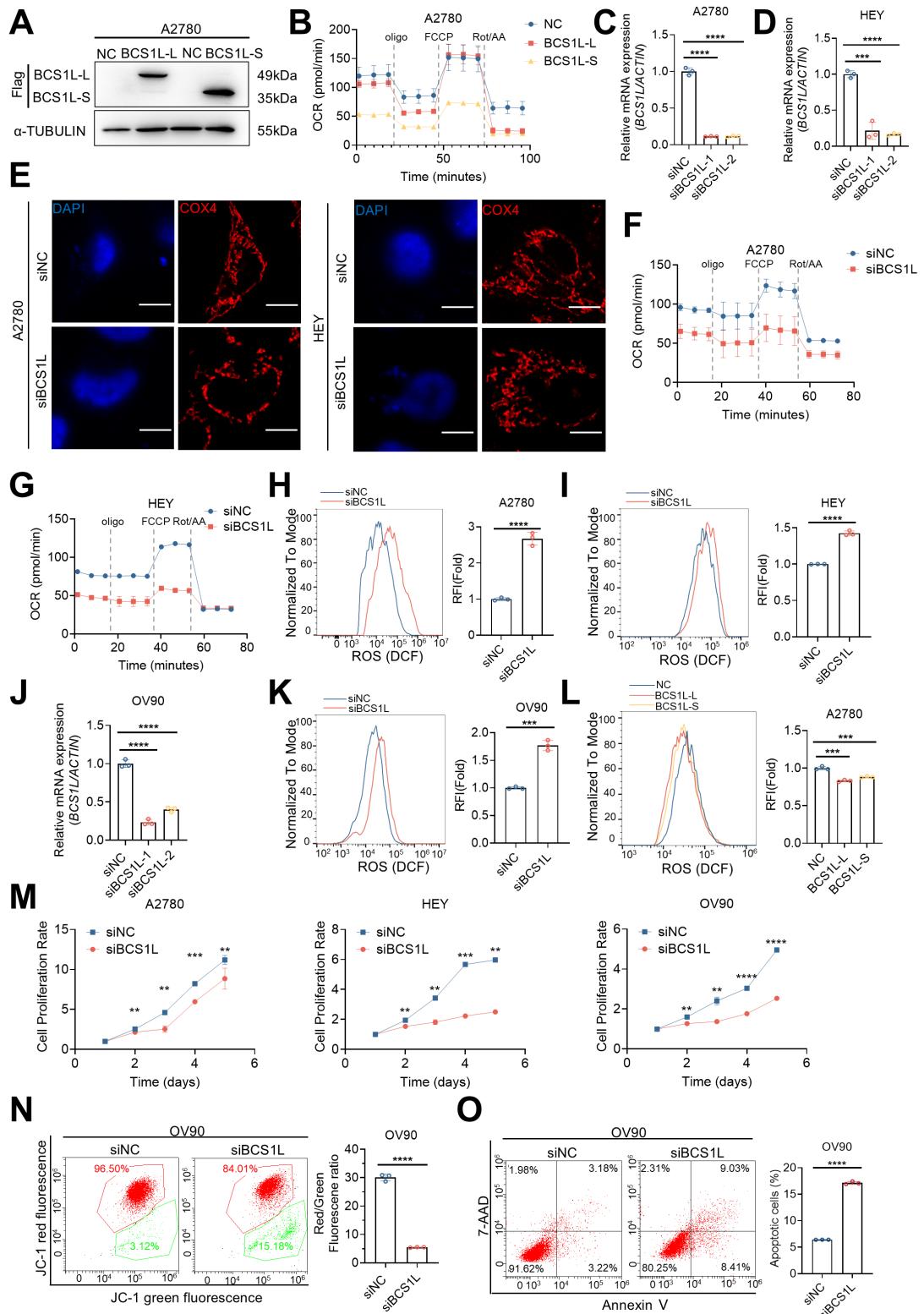
# Supplemental Information



**Figure S1. *BCS1L-L* or *BCS1L-S* expression difference and subcellular localization.**

(A) UMAP plot illustrated nine epithelial subclusters after dimension reduction colored by subclusters. (B) The main isoforms expression of mitochondrial complex III subunit BCS1L,

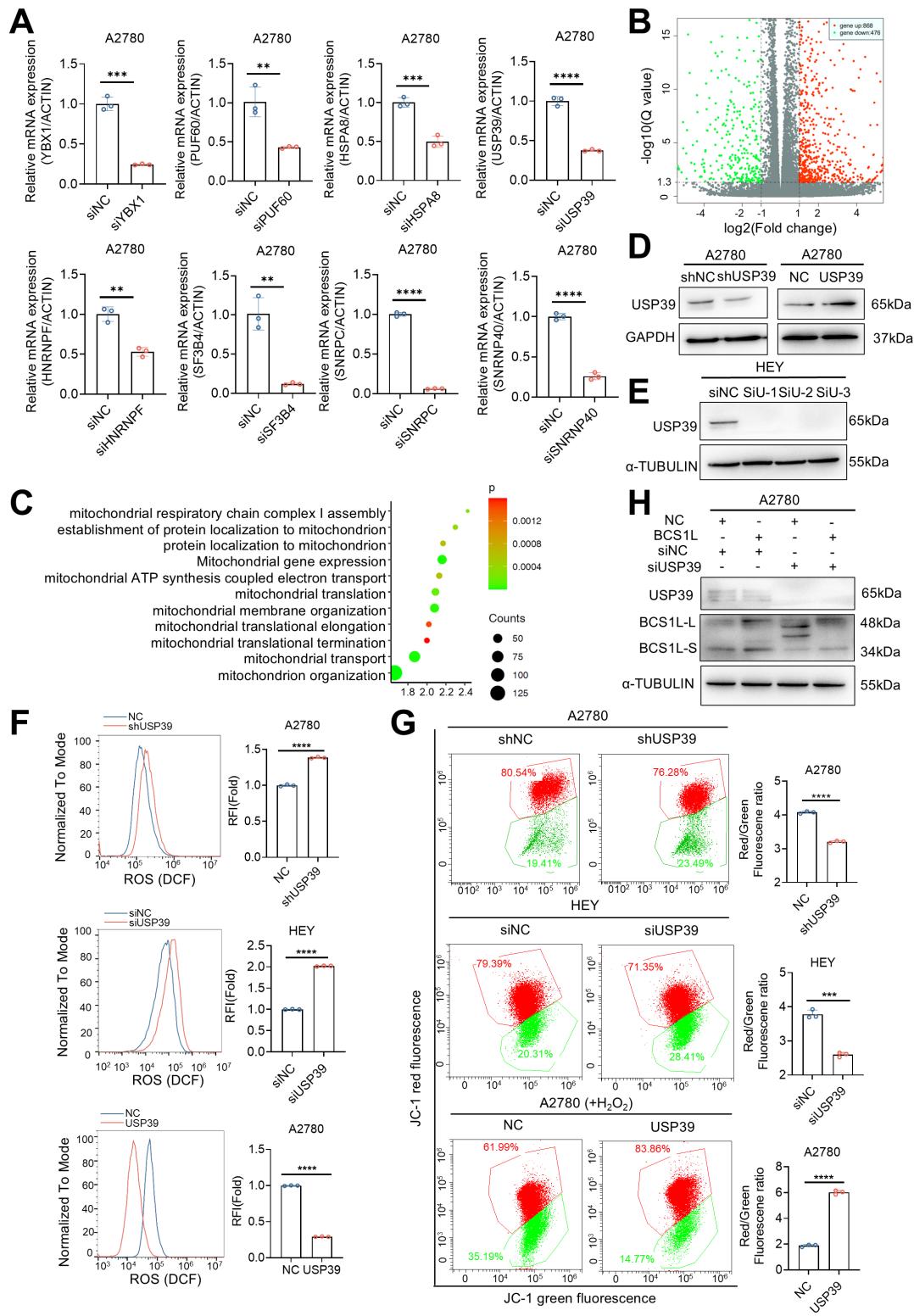
UQCRC1, UQCRC2 and CYC1 in GTEx-ovary and TCGA-OV datasets. **(C)** The BCS1L-L/BCS1L-S value transformed to log10 in OXPHOS low or OXPHOS high group from TCGA-OV,  $p = 0.0107$ . **(D)** Immunofluorescence image of A2780 and HeLa live cells transfected with pUltra-hot-BCS1L-L and pUltra-hot-BCS1L-S plasmids respectively for 72 h showing localization of BCS1L-L and BCS1L-S (red). Mito-tracker (green) indicated the mitochondria localization and DAPI (blue) was used to visualize nuclei. Scale bars, 10  $\mu$ m. **(E)** Colocalization of BCS1L-L or BCS1L-S protein labeled by mCherry (red) with mitochondria marked by COX4 (green) in control and pUltra-hot-BCS1L-L or pUltra-hot-BCS1L-S plasmids transfection for 72 h in A2780 and HeLa. DAPI (blue) was used to visualize nuclei. The quantification of co-localization was performed with image J Coloc 2 and Plot Profile. Scale bars, 10  $\mu$ m. **(F)** Representative images of IHC staining of BCS1L in FT and SOC tissues. Scale bars, 200  $\mu$ m.



**Figure S2. BCS1L knockdown impairs mitochondrial homeostasis in ovarian cancer cells.**

(A) The protein level of BCS1L-L and BCS1L-S in A2780 with or without Flag-BCS1L-L or Flag-BCS1L-S overexpression vectors. (B) The OCR curve of A2780 with BCS1L-L or BCS1L-S overexpression and control for 72 h measured by Agilent Seahorse XFe24 after the oligomycin, FCCP and rotenone/antimycin injections. (C) ROS level in A2780 cell transfected

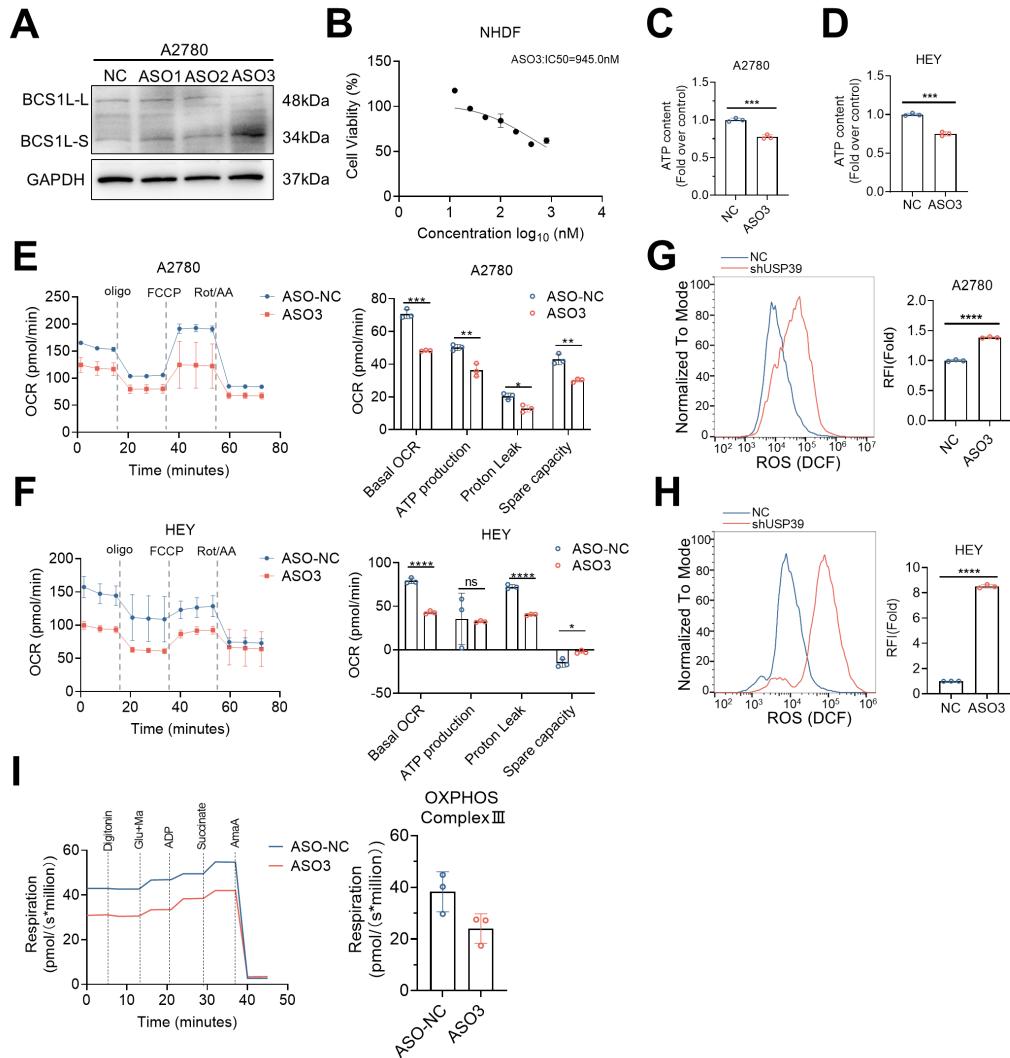
with control and BCS1L-L or BCS1L-S vectors for 48 h was detected by ROS probe DCFH-DA on a flow cytometer. **(D-E)** The efficiency of a siRNA suit silencing BCS1L in A2780 and HEY cell lines. **(F)** Confocal image of COX4 (red) and DAPI (blue) in A2780 and HEY cells with BCS1L knockdown and control showing mitochondria morphology. DAPI was used to visualize nuclei. Scale bars, 10  $\mu$ m. **(G-H)** The OCR curve of A2780 and HEY with BCS1L knockdown and control measured by Agilent Seahorse XFe96 after the oligomycin, FCCP and rotenone/antimycin injections. **(I)** The efficiency of a siRNA suit silencing BCS1L in OV90 cell line. **(J-L)** ROS level was detected by flow cytometry in A2780, HEY and OV90 cells with BCS1L depletion for 48 h. **(M)** Mitochondrial membrane potential analyzed by flow cytometry using JC-1 staining in OV90 cells with BCS1L knockdown for 72 h. **(N)** Cell proliferation was measured by the MTT assay in ovarian cancer cells (A2780, HEY, OV90) with BCS1L knockdown compared to corresponding controls ( $n = 3$  biologically independent experiments for the cell proliferation assay). Absorbance at 570 nm at each time point was compared to the initial value on the first day. **(O)** Apoptotic cells analyzed by flow cytometry using Annexin V/7-AAD staining in OV90 cells with BCS1L knockdown for 72 h. All results are presented as the mean  $\pm$  SD. The p-value was obtained by unpaired two-sided Student's t-test. \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.



**Figure S3. USP39-BCS1L axis mediates mitochondrial oxidative phosphorylation in ovarian cancer cells.**

**(A)** The knockdown efficiency of all the siRNA (siYBX1, siPUF60, siHSPA8, siUSP39, siHNRNPF, siSF3B4, siSNRPC, siSNRNP40) compared with control in A2780 cells was demonstrated by qPCR. **(B)** Volcano plot of differentially expressed genes (DEGs) in A2780 cells with USP39 knockdown. The cutoff was set as  $q\text{-value} < 0.05$  and  $|\text{fold change (FC)}| > 1$ .

868 genes were upregulated, and 476 genes were downregulated. **(C)** GO biological process enrichment analysis according to DEGs in the RNA-seq data from A2780 cells after USP39 stable knockdown. **(D)** USP39 protein level in stably USP39-depleted A2780 cell lines and stably USP39-overexpressed A2780 cell lines. **(E)** The efficiency of a siRNA suit silencing USP39 was verified with western blot. **(F)** ROS level of A2780 and HEY with USP39 depletion and A2780 with USP39 overexpression were detected by ROS probe DCFH-DA on a flow cytometer. RFI, relative fluorescence intensity. **(G)** Flow cytometry analysis of A2780 and HEY with USP39 knockdown and A2780 overexpressing USP39 stained with JC-1, indicating the level of mitochondrial membrane potential. Three biological replicates were conducted in all functional experiments. **(H)** Western blot analysis of BCS1L and USP39 protein expression in A2780 cells. The p values were obtained by a two-tailed unpaired Student's t-test **(A, F, G, H, I)**, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Data are mean  $\pm$  SD.



**Figure S4. Antisense oligonucleotides targeting BCS1L induce mitochondrial dysfunction specifically in ovarian cancer cells.**

**(A)** The BCS1L isoform expression in A2780 cells transfected with three ASOs with 100nM for 48h detected by western blot. **(B)** The IC<sub>50</sub> of ASO3 was measured in Normal Human Dermal Fibroblasts (NHDF) at 945.0 nM. **(C-D)** The total ATP content of whole cells was detected in A780 cells with 200 nM ASO3 and HEY with 100 nM ASO3 for 48 h compared with the corresponding control. **(E-F)** Mitochondrial basal OCR, Maximal OCR, Proton leak and spare capacity were analyzed by Agilent Seahorse XFe96 after oligomycin, FCCP, and rotenone/antimycin injection in A2780 and HEY cells treated with ASO3 100 nM and 50 nM for 24 h. **(G-H)** ROS level was analyzed by flow cytometry followed by DCFH-DA staining in A2780 treated with 200 nM and HEY treated with 100 nM for 48 h. **(I)** ETC Complex III activities in A2780 cells transfected with 100 nM was evaluated by substrate-uncoupler-inhibitor-titration (SUIT) high-resolution Oxygraph-2k respirometer,  $p = 0.0631$ . The  $p$  values were obtained by a two-tailed unpaired Student's t-test (**C, D, E, F, G, H, I**),  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ ,  $****p < 0.0001$ . ns: not significant. Data are mean  $\pm$  SD.