

Supplementary information

**Ciclopirox Targets Cellular Bioenergetics and Activates ER Stress to Induce
Apoptosis in Non-small Cell Lung Cancer Cells**

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Figures

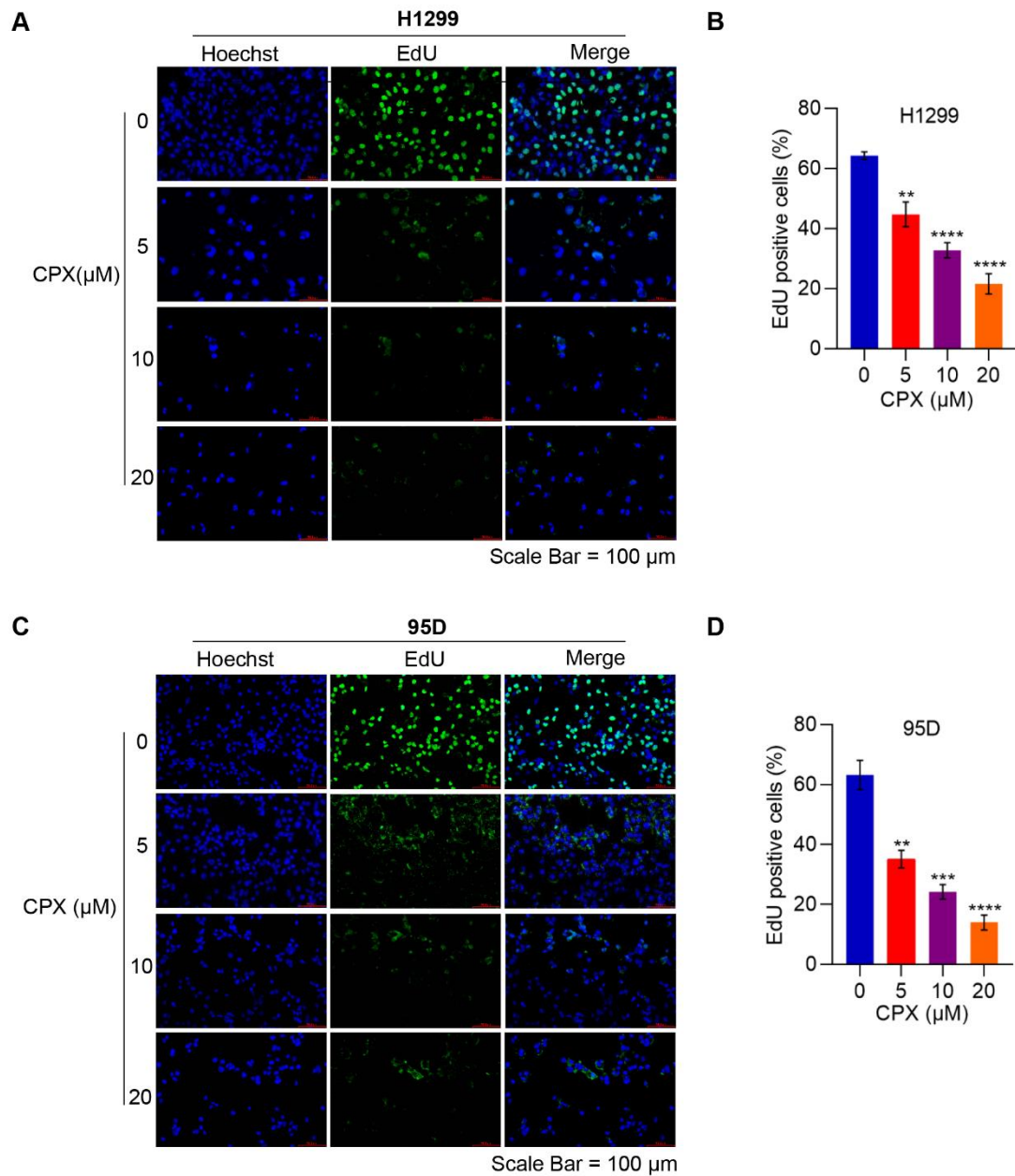


Figure S1 CPX inhibited human NSCLC cell proliferation *in vitro*. Fluorescence micrographs of CPX (0, 5, 10, and 20 μM, 24 h)-treated H1299 and 95D cells with EdU incorporation. Green, EdU-positive cells; Blue, Hoechst 33342 for nuclear staining. Scale bar, 100 μm. (**for $p < 0.01$, ***for $p < 0.001$, ****for $p < 0.0001$).

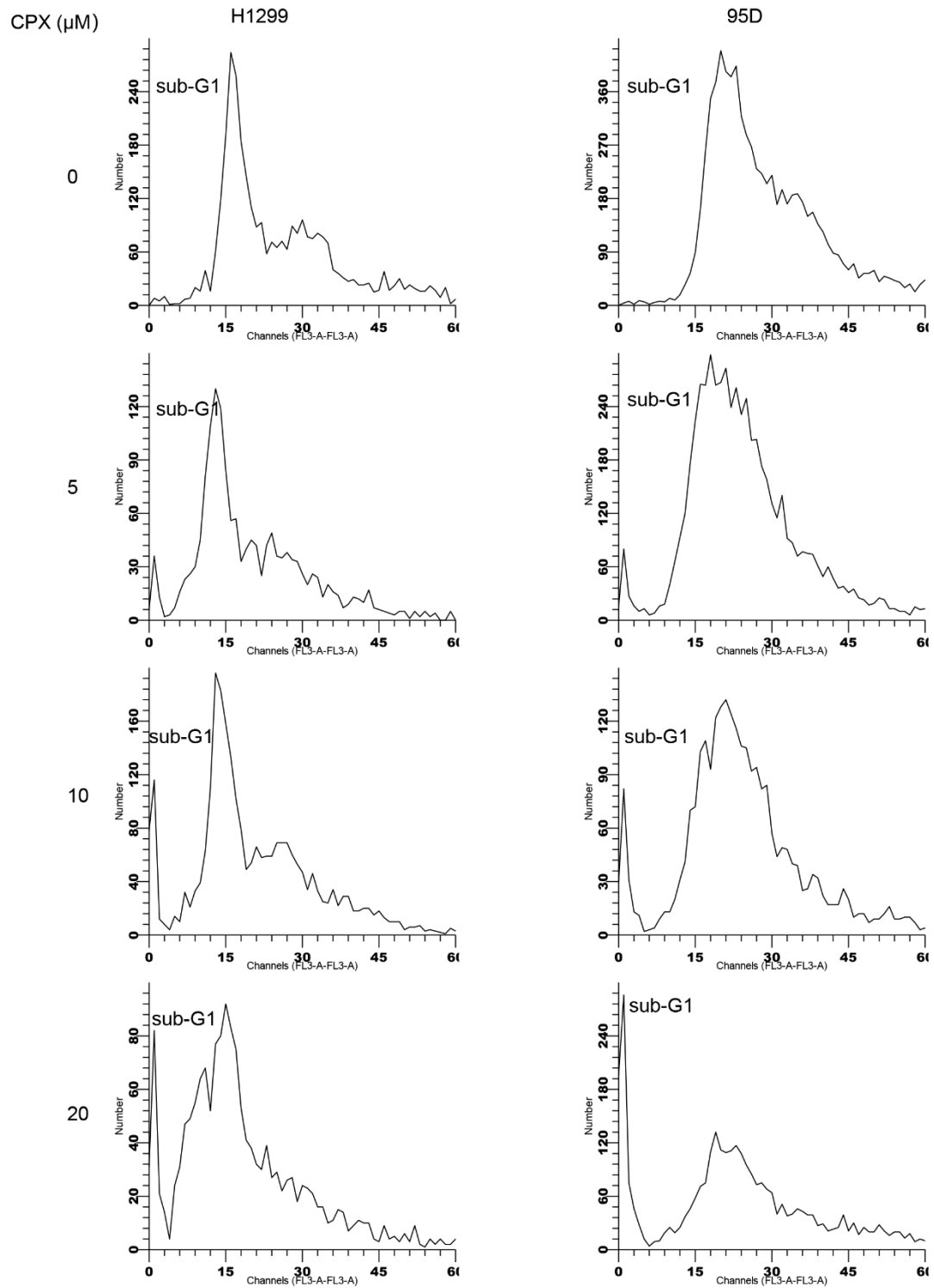


Figure S2 Cell cycle distribution assay. H1299 or 95D cells were incubated in the medium containing DMSO (vehicle control) or CPX (5, 10, 20 μ M) for 48 h. Cell cycle distribution was analyzed by flow cytometry. All cells were included in the analysis.

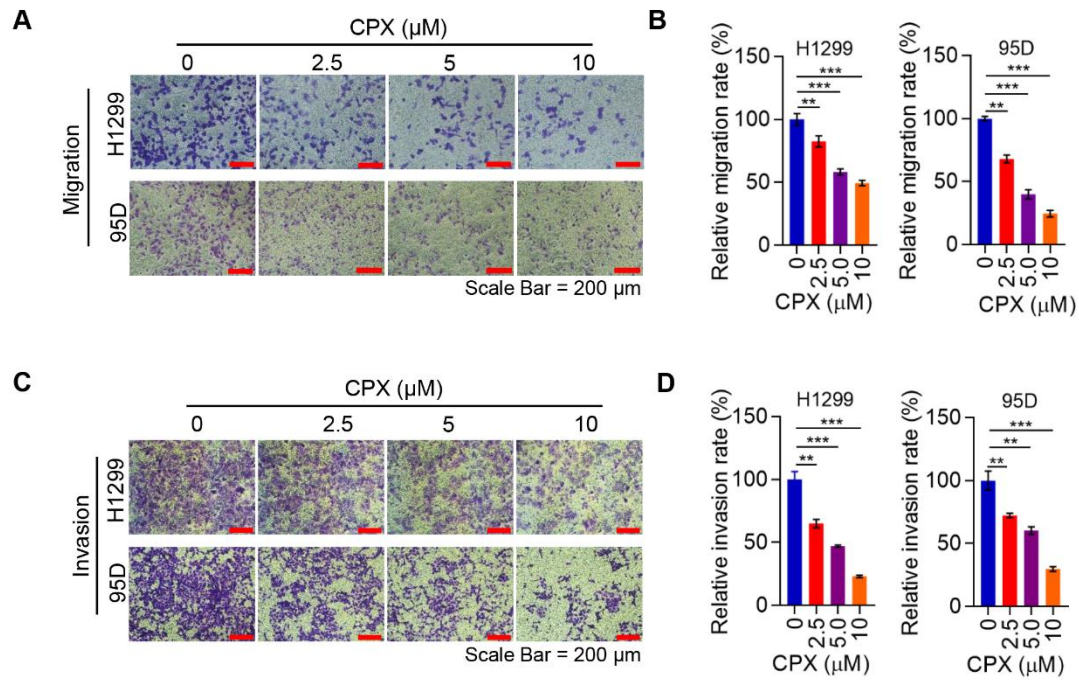


Figure S3 CPX suppresses NSCLC cell migration and invasion. A and B H1299 and 95D cells with the treatment of CPX (0, 2.5, 5, and 10 μM) for 48 h. The migrated H1299 and 95D cells were stained with crystal violet solution and detected using a light microscope. Representative images of transwell migration assay were shown (Scale bar: 200 μm) (A). Cell migration rate quantified with ImageJ Plus (B). C and D H1299 and 95D cells treated with CPX (0, 2.5, 5, and 10 μM) for 48 h. The invaded H1299 and 95D cells were stained with crystal violet solution and detected under a light microscope. Representative images of transwell invasion assay were shown (Scale bar: 200 μm) (C) and cell invasion rate quantified with ImageJ Plus (D). (**for $p < 0.01$, ***for $p < 0.001$).

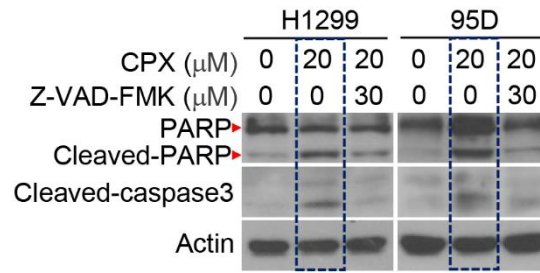


Figure S4 CPX induced apoptosis in NSCLC through Caspase3-PARP pathway. H1299 and 95D cells treated with CPX (0 and 20 μ M), CPX (20 μ M) combined with 30 mM Z-VAD-FMK for 48 h. Western blotting was used to detect the proteins as indicated.

Tables

Table S1 Sequences of primers used in qPCR analysis for selected genes.

| Genes | Forward 5'-3' | Reverse 5'-3' |
|---------------------------------|-----------------------|----------------------|
| <i>ND1</i> | TTCCTAATGCTTACCGAACGA | AGAAGAGCGATGGTGAGAGC |
| <i>COX I</i> | CAGAACGCAACCTCAACAC | TCCGAATGGTAGGATAA |
| <i>β-ACTIN</i> | AGCACAGAGCCTCGCCTTG | AAGCCGGCCTTGACATG |

Table S2 Sequences of primers used in qPCR for mtDNA copy number analysis.

| Genes | Forward 5'-3' | Reverse 5'-3' |
|-----------------|---------------------------------------|---------------------------------------|
| <i>CYT B</i> | CCC CAC AAA CCC CAT TAC TAA ACC CA | TTT CAT CAT GCG GAG ATG TTG GAT GG |
| <i>18S rRNA</i> | TAG AGG GAC AAG TGG CGT TC | CGC TGA GCC AGT CAG TGT |

Table S3 Details of the antibodies used for Western-blot and IHC analyses.

| Antibodies | SOURCE | Cat # |
|--------------------------|---------------------------|--------------|
| Anti-ATF4 | Abcam | ab184909 |
| Anti-COXII | Abcam | ab110258 |
| Anti-Ki67 | Abcam | ab181064 |
| Anti-PFKL | Abcam | ab92742 |
| Anti-PKM2 | Abcam | ab154804 |
| Anti-Cyclin D1 | Cell signaling Technology | 55506 |
| Anti-Caspase 3 | Cell signaling Technology | 9662 |
| Anti-Cleaved-Caspase 3 | Cell signaling Technology | 9664 |
| Anti-Bip | Cell signaling Technology | 3177 |
| Anti-CHOP | Cell signaling Technology | 5554 |
| Anti-p-Rb | Cell signaling Technology | 8516 |
| Anti-N-Cadherin | Cell signaling Technology | 13116 |
| Anti-PDI | Cell signaling Technology | 3501 |
| Anti-PERK | Cell signaling Technology | 5683 |
| Anti-PERK-pT980 | Cell signaling Technology | 12185 |
| Anti-eIF2 α | Cell signaling Technology | 5324 |
| Anti-eIF2 α -pS51 | Cell signaling Technology | 3597 |
| Anti-PARP | Cell signaling Technology | 9532 |
| Anti-Snail | Cell signaling Technology | 3879 |
| Anti-p21 | Cell signaling Technology | 2947 |
| Anti-LDHA | Zen Bioscience | 501146 |
| Anti-HK2 | Zen Bioscience | 220458 |
| Anti-PFKM | Zen Bioscience | 505477 |
| Anti-MMP2 | Santa Cruz Biotechnology | 13595 |
| Anti-CDK4 | Bio-Rad | VMA00520 |
| Anti-Rb | ABclonal | A3618 |
| Anti-ATP5A | Proteintech | 14676-1-AP |
| Anti-PGK1 | Proteintech | 17811-1-AP |
| Anti-MMP9 | Proteintech | 10375-2-AP |
| Anti-SDHA | Proteintech | 14865-1-AP |
| Anti-UQCRC2 | Proteintech | 14742-1-AP |
| Anti- β -actin | Proteintech | 60008-1-Ig |
| Anti-E-Cadherin | BD Biosciences | 610182 |