Supplementary materials and methods

Cell proliferation and viability assay

Cell proliferation viability assay was measured by using the Cell Counting Kit-8 (CCK-8 kit, Sigma-Aldrich) as recommended by the manufacturer. In brief, H69 and H82 cells were plated at 1 x104 cells per well in 96-well plates, allowed to recover for 4 hours before exposing to serial concentrations of MLN0128 or AZD1775 or both for 72 hours. CCK-8 reagent was added and incubated for another 4 hours at 37°C and Optical Density (OD) was measured at 450 nm with a microplate reader.

Clonogenic cell survival assay

Clonogenic cell survival assay was performed in complete methylcellulose (MethoCult™ GF M3434, Stem Cell Technologies, Vancouver, Canada) plates according to the manufacturer's protocol. Briefly, H82 (3,000 cells) and H69(50,000 cells) were resuspended in complete methylcellulose. Cell suspensions were incubated with inhibitors(four groups including a. DMSO group; b. MLN018 group; c. AZD1775 group; d. MLM0128+AZD1775 group) in various combinations as indicated. Finally, cell were plated in 35 mm plates and incubated at 37°C for 14 days. Cell clusters consist of ≥40 cells were counted and scored.

Determination of apoptosis by flow cytometry

Apoptotic was detected using Annexin V-FITC apoptosis-detection kit (BD Biosciences; San Jose, CA). Briefly, SCLC cells were plated in 6 well plates (2 × 105 per well) and treated with MLN0128 and/or AZD1775 for 48 hours at indicated concentrations. At the end of incubation, cells were collected and stained with Annexin-V-FITC/Propidium Iodide (PI) followed by FACSAria (BD Biosciences) analysis within an hour of staining. Data was analyzed using FlowJo software.

Cell cycle analysis

For cell cycle analysis, H69 or H82 cells were seeded at a cell density of 2 x105 per well in 6-well plates, treated with MLN0128 and/or AZD1775 for 48 hours. BrdU incorporation was detected using FITC-conjugated anti-BrdU antibody followed by 7-AAD staining per the manufacture's protocol (BD Biosciences). Cell cycle analysis was performed in the FACSAria flow cytometer with FlowJo software.

Lentiviral transduction

Lentivirus vector containing shRNAs specific for CHOP (reagent #1; TRCN0000364328), CHOP (reagent #2; TRCN0000364393) were purchased from Sigma-Aldrich and a firefly luciferase shRNA was used as a control (5'- GCTTACGCTGAGTACTTCGA-3'). shRNA-encoding recombinant lentiviruses were packaged in 293T cells to generate viral. Viral supernatants were collected 48 hours after transfection. Viral particles were concentrated with Lenti-X™ Concentrator (Clontech, CA, USA) and resuspended in PBS. SCLC cells were transduced with viral supernatant for 48 hours in fibronectin-coated 6-well plates in the presence of 8 mg/mL polybrene after spinoculation at 800 x g, 32°C for 30 minutes. Cells were then selected in culture media containing 2 mg/mL puromycin for at least 48 hours before subjecting to MLN0128 and/or AZD1775 treatments.

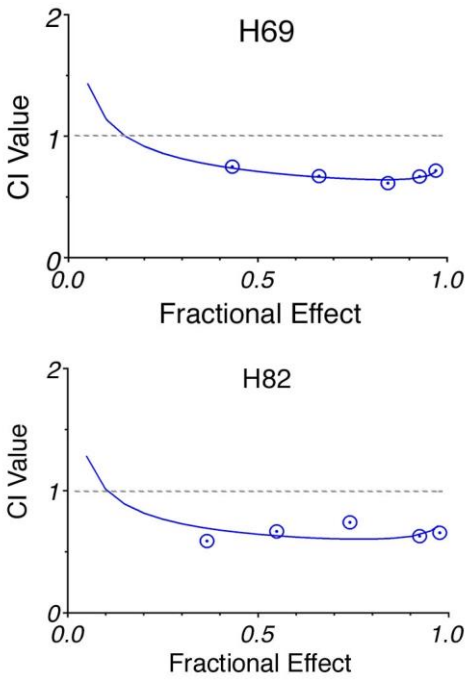
Western blot

Briefly, 10 to 30 µg of total protein was subjected to 8% or 12% SDS-PAGE gels and transferred electrophoretically onto PVDF membranes by a semidry blotting system (Bio-Rad, Hercules, CA). Membranes were blocked in 5% fat-free milk/Tris–buffered saline for 1 hour at RT and incubated with primary antibodies at 4°C for overnight, followed by secondary antibodies conjugated with horseradish peroxidase. Immunoblotting analysis was carried out after treating with ECL detection reagent. In the case of xenografts, tumor tissue was harvested and homogenized in lysis buffer and then processed for Western blot as described above.

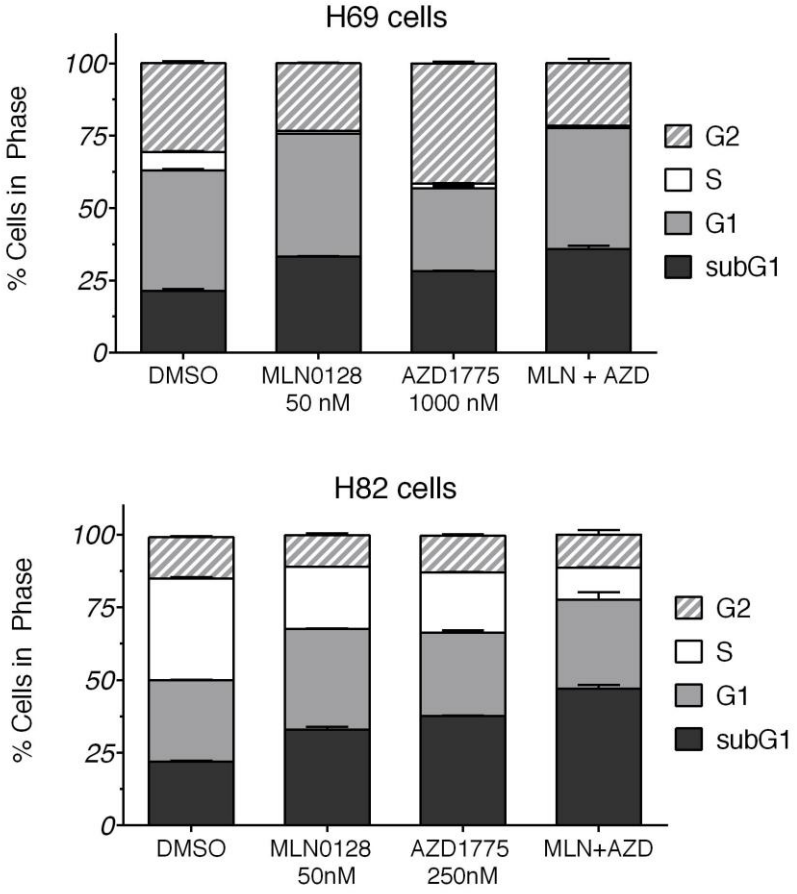
Immunohistochemistry

Xenograft tumors were fixed overnight in 10% neutral buffered formalin and paraffin-embedded by routine histology procedure. Five micrometer tissues sections were made and slides were incubated with primary antibodies for p-mTOR(1:100), cleaved caspase-3(1:100) p-Histone3 (1:500), and Ki67(1:2000) at 4°C overnight, respectively. Goat anti-rabbit-secondary antibody was used and followed by developing with horseradish peroxidase detection system. Slides were viewed on an Olympus BX51 Research System Microscope and images were captured using a high-resolution interline CCD camera at 400x magnification. Positively stained cells were quantified and 5 randomly chosen fields per slide and three slides per group were quantified for each staining. The data were presented as the proportion of positively stained cells over the total number of cells.

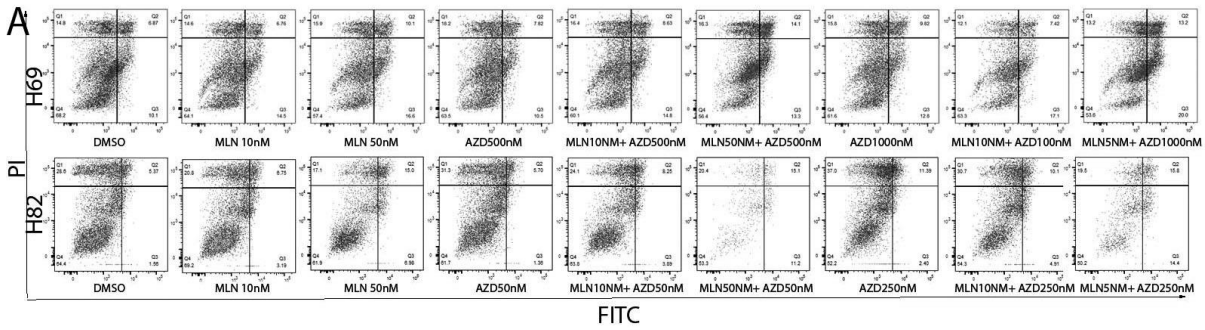
Supplementary Figure 1.

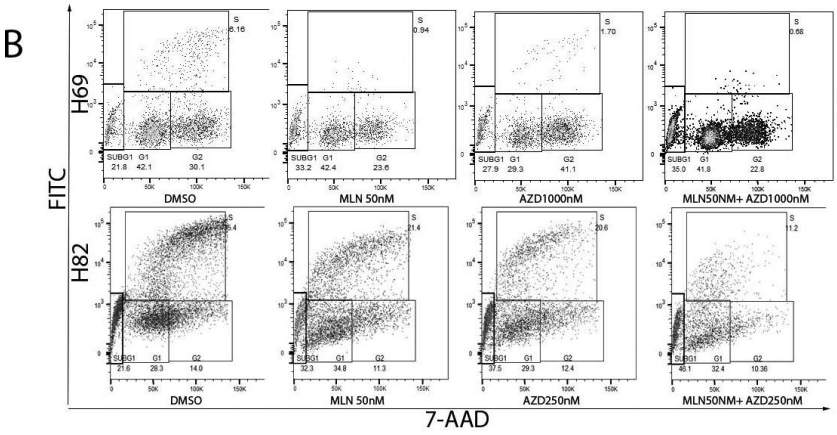


Supplementary Figure 2.



Supplementary Figure 3.





Supplementary Figure 4.

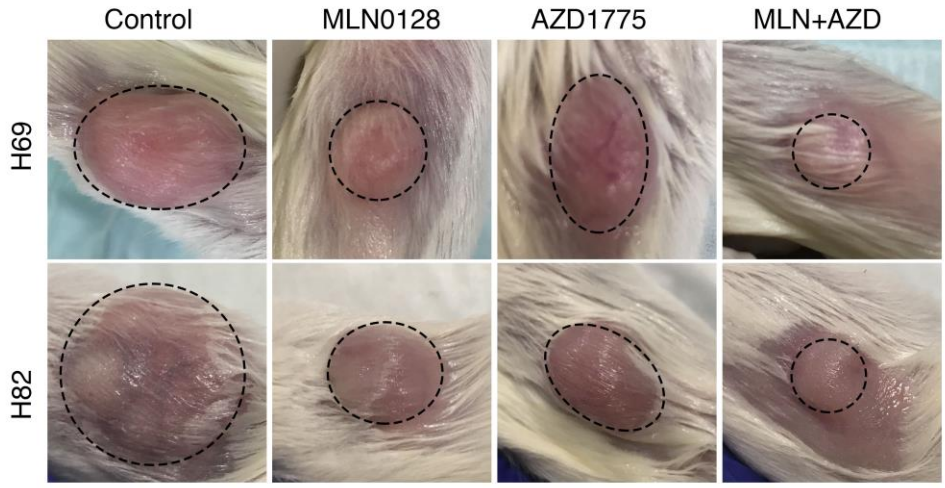


Figure Legends.

Supplementary Figure 1.

Combination of AZD1775 and MLN0128 treatment leads to more profound inhibition of SCLC cell viability. SCLC cells were cultured with inhibitors at various combinations for 72 hours followed by CCK-8 assay, the combination index (CI) method was used and analyzed by CompuSyn software. The CI values were between 0.5-0.8 at all doses tested, indicating that MLN0128 and AZD1775 were synergistic in suppressing cell viability in both H69 and H82 cells..

Supplementary Figure 2.

AZD1775 /MLN0128/ combination induces SCLC cell arrest. H69 or H82 treated with AZD1775 (1000 nM for H69, 250 nM for H82), MLN0128 (50nM) for 48 hours or AZD1775+MLN0128 , followed by BrdU and 7-AAD staining and flow cytometry analysis. Stacked bars show percentage of SCLC cell population in sub-G1, G1, S, and G2 cell cycle phases. Data are presented as the mean ± SEM of quadruple

experiments. (n=3)

Supplementary Figure 3.

MLN0128, AZD1775 or combination treatment induces SCLC cell death and cell cycle arrest. A. Representative FACS histograms show the Annexin V- FITC and PI staining after H69 or H82 cells were treated with MLN0128, AZD1775 or combination for 48 hours. B. Representative FACS histograms showing the distribution of SCLC cells in cell cycle after MLN0128, AZD1775 or combination treatment. H69 and H82 cells were treated with MLN0128, AZD1775 or combination for 48 hours and stained with Brdu-FITC and 7-AAD followed by flow-cytometry analysis.

Supplementary Figure 4.

MLN0128, AZD1775 or combination treatment suppresses SCLC xenograft growth in vivo. Representative examples of xenograft tumors treated with MLN0128, AZD1775 or MLN0128+ AZD1775 from SCLC tumor-bearing NSG mice.