Supplementary Methods

1. Cell viability assay

Cells were seeded in 96-well plates at a density of 2-8×10^3 cells per well, incubated overnight at 37℃, and then exposed to various concentrations of AZD1775 and/or AZD0156 for 3 days. No treatment was a control. A 50 μL aliquot of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) solution (Sigma-Aldrich, St. Louis, MO) was added to each well and the incubation was continued at 37℃ for 4 hours. The medium was removed and 150 μL of dimethyl sulfoxide (DMSO) was added to each well and mixed. The absorbance at 540 nm was measured with a VersaMax Microplate Reader (Molecular Devices, Sunnyvale, CA). The experiments were performed three times.

2. Colony-forming assay

Cells (0.5-4×10^3) were seeded in 6-well plates and exposed to various concentrations of AZD1775 for 10 days. The colonies were then stained with Coomassie Brilliant Blue for 2 hours and counted using Gel Doc system software (Bio-Rad, Hercules, CA). Each experiment was repeated three times.

3. Western blot analysis

Cells (1×10^6) were seeded in 100-mm dishes and treated with AZD1775 and/or AZD0156 for 24 or 72 hours. The cells were harvested and lysed in RIPA buffer containing protease inhibitors on ice for 30 minutes. Samples of lysate containing equal amounts of protein were resolved by SDS-PAGE and transferred to membranes for western blotting. Primary antibodies against the following molecules were purchased from Cell Signaling Technology (Beverley, MA): ATR (#2790), p-ATR-Ser428 (#2853), caspase-7 (#9492), CDC2 (#9112), p-CDC2-Tyr15 (#9111), WEE1 (#4936), p-WEE1-Ser642 (#4910), c-Myc (#5605), p-NF-κB p65-Ser536 (#3033), NF-κB (#8242), MCL-1 (#4572), CtIP (#9201), MMP-9 (#3852), MMP-2 (#4022), PD-L1 (#13684), STAT-1 (#9172), and p-STAT-1-Tyr701 (#9167). Anti-β-actin antibody was from Sigma-Aldrich; antibodies against p-ATM-

Ser1981 (#ab81292), ATM (#ab78), CXCR-2 (#ab217314), and PA32/RPA2 (#ab2175) were from Abcam Bioscience (Cambridge, UK); anti-p-RPA32 S4/S8 (#A300-245A) was from Bethyl Laboratories (Montgomery, TX); anti-γH2AX antibody (#05-636) was from Millipore (Billerica, MA); anti-Rad51 (#sc-8349) and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH; #sc-25778) were from Santa Cruz Biotechnology (Dallas, TX); and anti-CMTM-6 (#PA5-34747) and secondary antibodies were from Thermo Fisher Scientific (Waltham, MA).

4. Immunoprecipitation

Cells were seeded in 150 mm dishes at a density of 2.5×10^6 cells/dish, treated with inhibitors for 72 hours. Anti–PD-L1 antibody (#13684), anti-CMTM6 (#PA5-34747), anti-IgG antibody (#ab133470, Abcam Bioscience) and Protein A/G PLUS agarose (#sc-2003, Santa Cruz Biotechnology) were used. Finally, samples were analyzed by western blotting as described above.

5. Cell cycle analysis

Cells (2×10^5) were seeded in 60-mm dishes and treated with or without 1μM AZD1775 for 24 hours. The cells were then harvested, and fixed with 70% ethanol at −20°C for 2 days. An aliquot of 7 μL of 20 mg/mL RNase A (Invitrogen, Carlsbad, CA) was added to each well and the plates were incubated for 10 minutes at 37℃. Finally, propidium iodide (PI; Sigma-Aldrich) was added to each well and the cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ). Each experiment was repeated three times.

6. Annexin V/PI apoptosis assay

Cells (2×10^5) were seeded in 60-mm dishes and treated with or without 1μM AZD1775 for 48h. Apoptosis was measured by double-staining the cells with Annexin V-FITC and PI (#556547, BD Biosciences) according to the manufacturer's protocol. The cells were then analyzed on a FACSCalibur flow cytometer. Cells in early and late apoptosis were defined as Annexin V-FITC-positive/PI-negative and Annexin V-FITC-positive/PI-positive, respectively. The results are presented

as the means of three independent experiments.

7. Comet assay

Cells (2×10^5) were treated with 1μM AZD1775 and/or AZD0156 for 24 hours, resuspended at 1×10^5 cells/mL in ice-cold phosphate-buffered saline, and mixed with molten LMAgarose at a ratio of 1:10. Aliquots were placed on comet slides and incubated at 4℃ in the dark for 40 minutes. The slides were immersed in precooled lysis solution (#4250-050-01, Trevigen Inc., Gaithersburg, MD) at 4℃ for 40 minutes and then in freshly prepared alkaline unwinding solution (200 mM NaOH, 1mM EDTA, pH > 13) for 30 minutes at room temperature in the dark. The slides were subjected to electrophoresis for 30 min and dried at room temperature overnight. Diluted SYBR Green staining solution (100 μL) was placed onto each circle of agarose and the samples were covered with a coverslip. Tail moment (migration of DNA fragments) and intensity (DNA content) were measured using the Comet Assay IV program (Andor Technology, Belfast, UK). Each condition was analyzed in three independent experiments.

8. Transwell migration assay

Migration assays were conducted using 6.5-mm Transwell insert chambers with 8 μm-pore polycarbonate membranes (#CLS3422, Sigma-Aldrich). Cells ($0.3\text{-}2\times10^5$) were seeded into the upper chamber in 200 μL medium containing 0.1% FBS, and 500 μL medium containing 10% FBS was added to the lower chamber. AZD1775 and/or AZD0156 were added to the upper chamber and the plates were incubated at 37℃ for 24 hours. The non-migrated cells remaining on the upper side of the membranes were removed with cotton swabs, and the filters were fixed with 4% paraformaldehyde (#P2031, Biosesang, Seongnam, Korea) for 20 min at room temperature. The membranes were then incubated in 1% crystal violet solution (#V5265, Sigma-Aldrich) for 10 minutes. The cells were visualized using microscope and photographed, and the cells were then dissolved by incubating the membranes in 300μL of 33% acetic acid (#1.00063.2511, Merck, Darmstadt, Germany) for 10 minutes. The liquid was collected and the absorbance at 573 nm was measured using a microplate

reader.

9. Immunohistochemistry (IHC)

Sections of paraffin-embedded xenograft tumor tissues were deparaffinized and dehydrated. Proliferating cells were detected by staining with anti-Ki-67 antibody (GeneTex Inc.) at a dilution of 1:100. Apoptosis was detected by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) using an ApopTag In Situ Apoptosis Detection Kit (EMD Millipore) according to the manufacturer's protocol. Anti–PD-L1 (#13684, Cell Signaling) and anti-CD163 antibodies (#93498, Cell Signaling) were used at dilutions of 1:100.