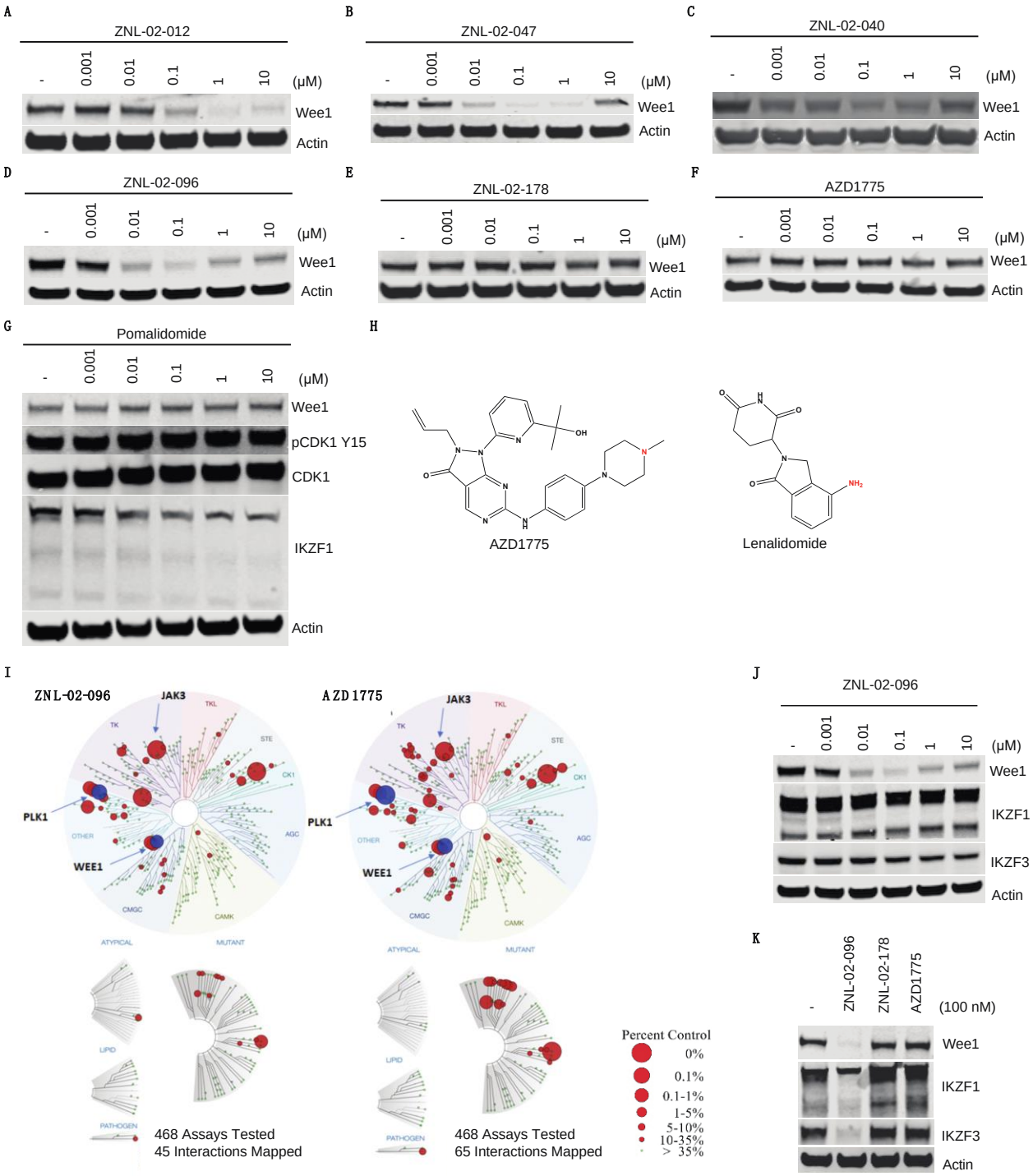


Figure S1. Characterization of potency and selectivity of Wee1 degradation. *Related to Figure 1 and Figure 2.* Immunoblot analysis of MOLT4 cells treated for 5 hrs with the indicated concentrations of ZNL-02-012 (**A**), ZNL-02-047 (**B**), ZNL-02-040 (**C**), ZNL-02-096 (**D**), ZNL-02-178 (**E**), AZD1775 (**F**), or Pomalidomide (**G**); (**H**) Atom selection of AZD1775 and lenalidomide used for the Rosetta pairwise distance calculation is highlighted in red; (**I**) Kinase trees represent kinome-wide selectivity of ZNL-02-096 and AZD1775 through Kinomescan profiling at 1 μM. Full data set is available in Supplementary Data Set 1; (**J**) Immunoblot analysis of MOLT4 cells after a 5-hr treatment with the indicated concentrations of ZNL-02-096; (**K**) Immunoblot analysis of MOLT4 cells after a 24-hr treatment with 100 nM of ZNL-02-096, ZNL-02-178, or AZD1775. Degradation of IKZF1 and IKZF3 is apparent after a 24-hr treatment, but not after a 5-hr treatment.

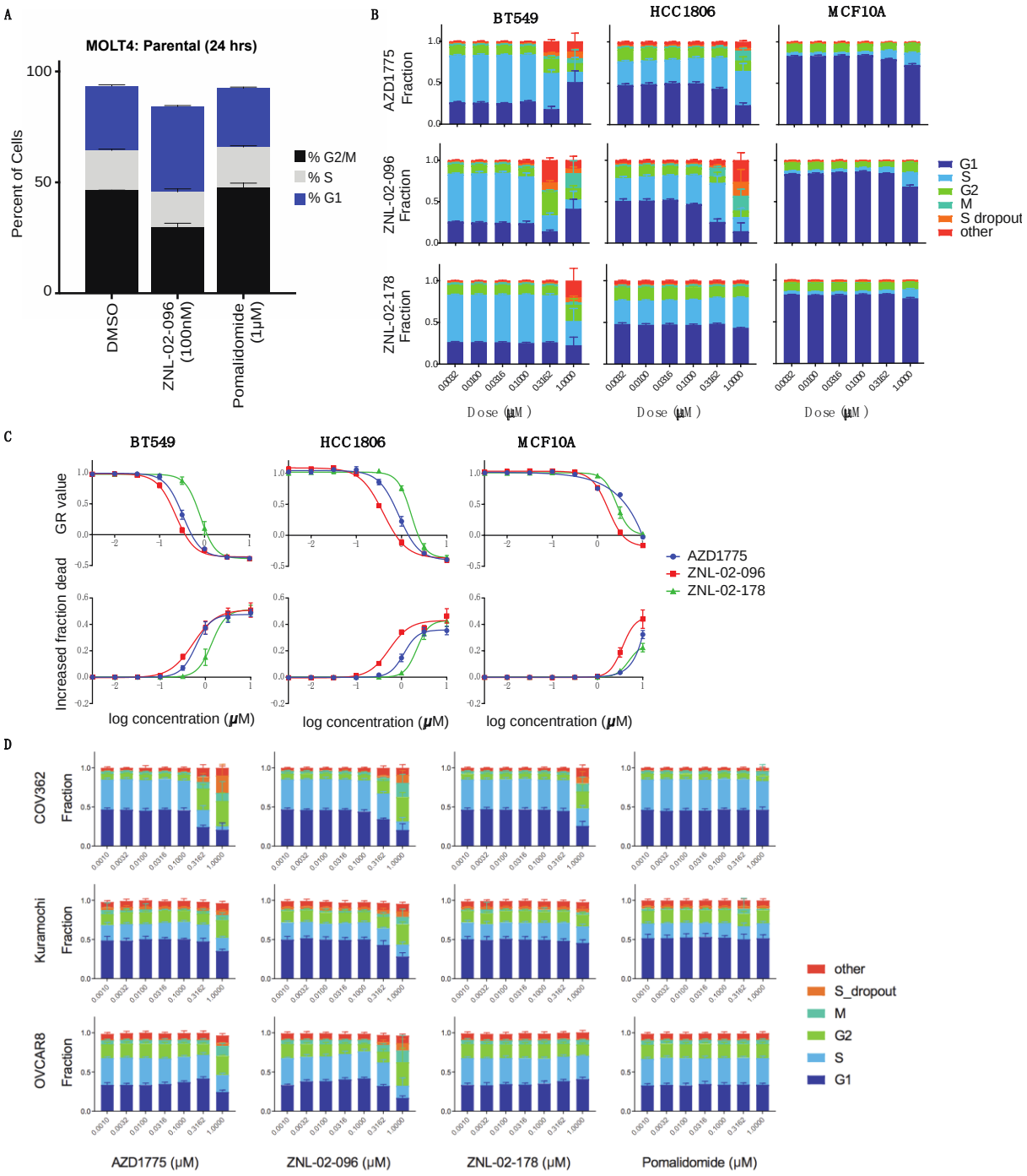


Figure S2. ZNL-02-096 induces G2/M accumulation in MOLT4, breast, and ovarian cancer cells. *Related to Figure 3*. (**A**) MOLT4 cells treated with DMSO, ZNL-02-096 (100 nM), or Pomalidomide (1 μM) for 24-hrs and stained with propidium iodide. Error bars represent standard deviation from the mean for technical triplicates from one biological replicate; (**B**) Microscopy-based cell cycle analysis using Hoechst 33342, EdU, and phospho-histone H3 in BT549, HCC1806, and MCF10A cells 72 hrs after treatment with AZD1775, ZNL-02-096, or ZNL-02-178. (**C**) In the same experiment as (B), live cell counts (classified by LIVE/DEAD Far Red Dead Cell Stain (LDR) signal) were normalized to DMSO-treated controls on the same plate to yield normalized growth rate inhibition (GR) values. Error bars represent standard deviation of the mean for technical triplicates from one biological replicate. (D) Microscopy-based cell cycle analysis using Hoechst 33342, EdU, and phospho-histone H3 in COV362, Kuramochi, and OVCAR8 cells or 72 hrs after treatment with AZD1775, ZNL-02-096, or ZNL-02-178. Error bars represent standard deviation of the mean for technical triplicates from one biological replicate.

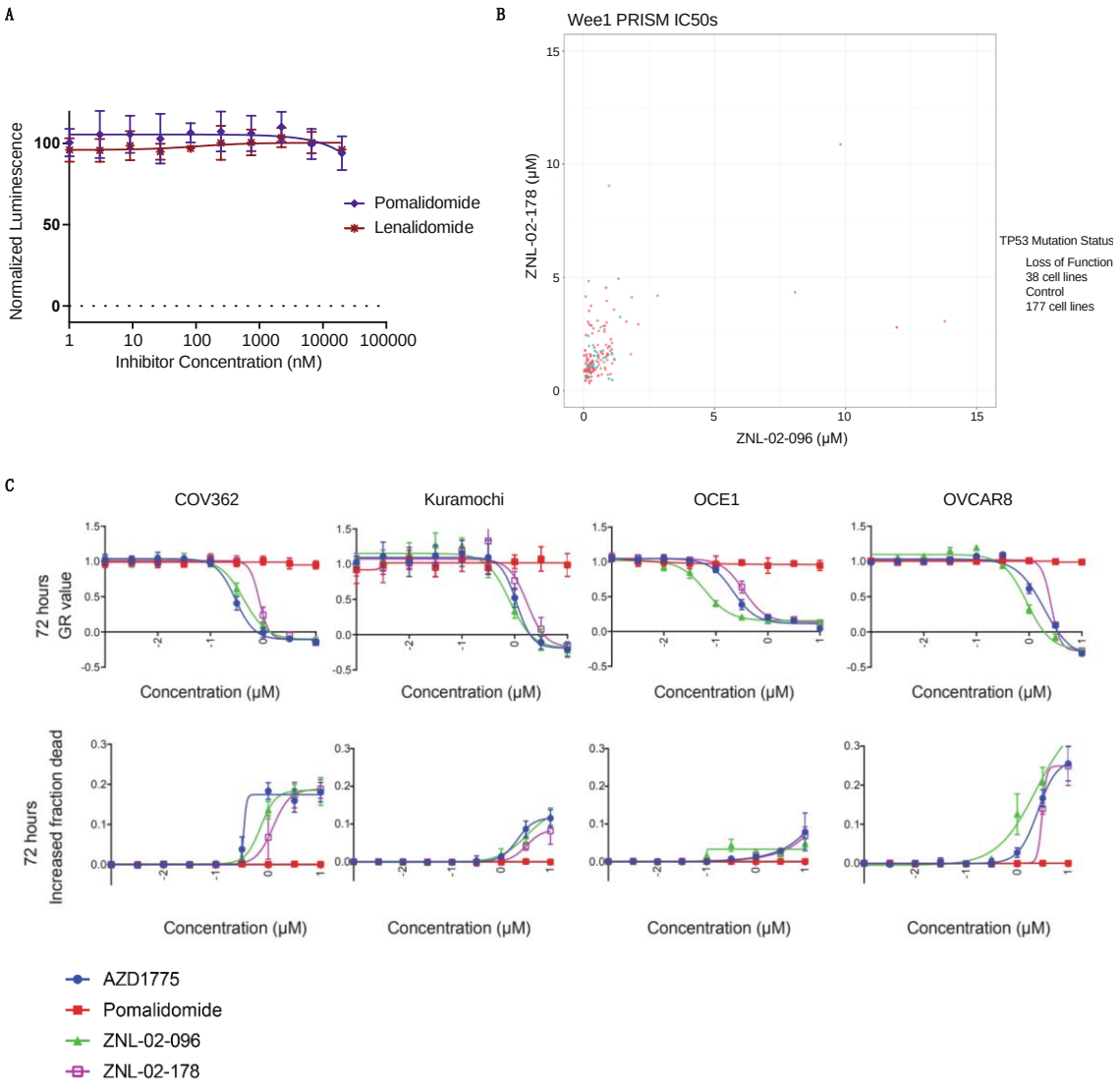
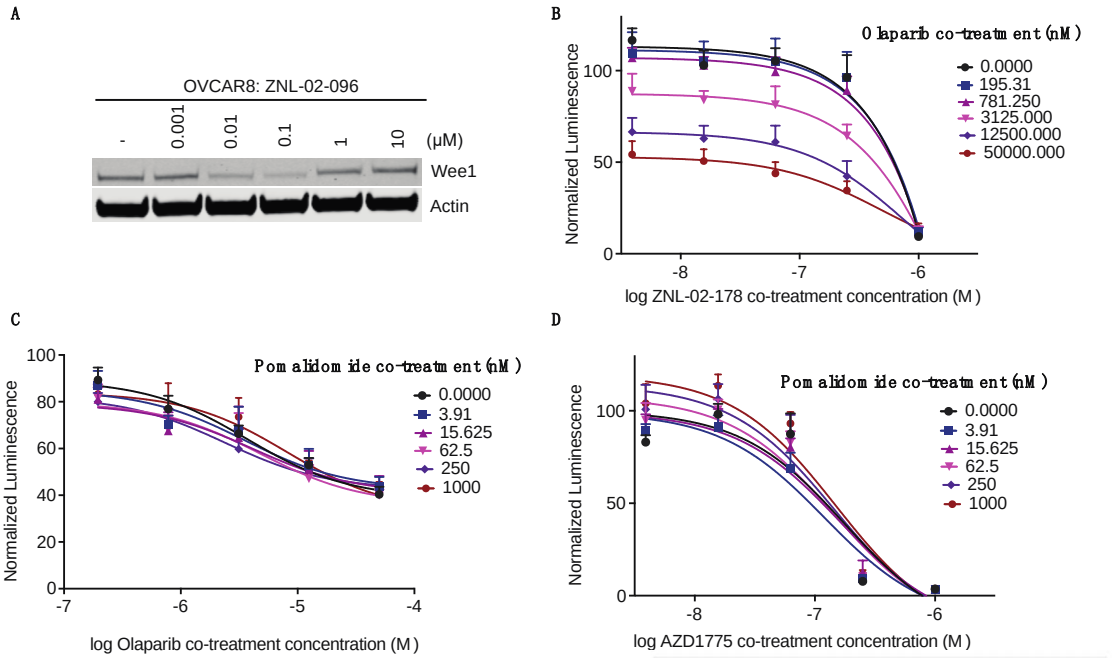
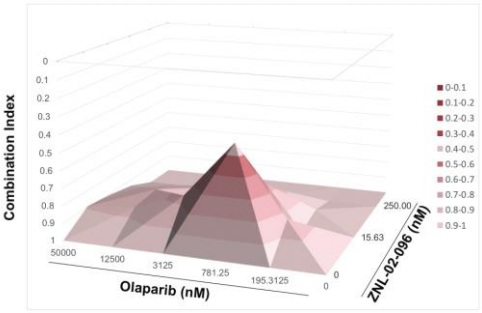


Figure S3. ZNL-02-096 exhibits cancer cell line-dependent cytotoxicity that is independent of p53 mutational status. *Related to Figure 4*. (**A**) Antiproliferative effects of pomalidomide and lenalidomide in MOLT4 WT cells after a 72-hr treatment, as approximated by CellTiter-Glo. Data points are plotted as the average of three replicates ± SEM. (**B**) Cell line p53 status was obtained from DepMap (Broad Institute), and merged with the PRISM data, yielding 215 cell lines for which p53 status was recorded and screening data was available. The sensitivities to ZNL-02-096 and ZNL-02-178 were plotted as a function of their p53 status (the plot provided). Linear regression using normalized IC_{50} values revealed that p53 status was not predictive of sensitivity based on this model (data not shown). All analysis was performed in R. (**C**) Live cell counts (classified by LIVE/DEAD Far Red Dead Cell Stain (LDR) signal) were normalized to DMSO-treated controls on the same plate to yield normalized growth rate inhibition (GR) values and fraction dead in COV362, Kuramochi, OVCAR8, and OCE1 cells following a 72-hr treatment with the indicated concentrations of AZD1775, ZNL-02-096, or ZNL-02-178. Error bars represent standard deviation of the mean for technical triplicates from one biological replicate.



E

ZNL-02-096 (μM) \ Olaparib (μM)	0.195	0.781	3.125	12.5	50
0.004	0.83	0.43	0.76	0.86	0.89
0.016	1.09	0.71	0.93	0.85	0.91
0.063	0.96	0.86	0.88	0.90	0.93
0.25	1.01	0.99	1.01	1.01	1.01
1	1.01	1.01	1.02	1.02	1.02



F

AZD1775 (μM) \ Olaparib (μM)	0.195	0.781	3.125	12.5	50
0.004	1.22	0.73	0.99	1.04	1.00
0.016	0.86	0.71	0.97	0.96	0.95
0.063	0.45	0.42	0.63	0.78	0.79
0.25	0.98	0.98	0.99	1.02	1.04
1	1.00	1.00	1.00	1.02	1.02

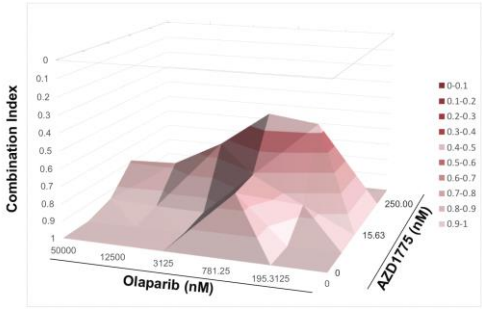


Figure S4. ZNL-02-096 synergizes with Olaparib in OVCAR8 cells. *Related to Figure 4.* **(A)** Immunoblot analysis of OVCAR8 cells treated for 5 hrs with the indicated concentrations of ZNL-02-096; (**B**) Cell viability of OVCAR8 cells co-treated with ZNL-02-178 and Olaparib, (**C**) Pomalidomide and Olaparib, or (**D**) Pomalidomide and AZD1775 at the indicated concentrations for 72 hours, as approximated by CellTiter-Glo. Data points are plotted as the average of three replicates ± SEM. (**E**) Combination Index (CI) for ZNL-02-096 and Olaparib co-treatment, or (**F**) for AZD1775 and Olaparib co-treatment, for 72-hours in OVCAR8 cells, where CI = [A+B – A*B]/AB. Synergistic effect (CI <1), additive (CI = 1), antagonism (CI > 1).

Table S1. Enzymatic IC_{50} of Described Compounds. *Related to Figure 1.*

Compound ID	IC50s (nM)	
	Wee1[a]	PLK1[b]
AZD1775	1.52±0.17	212±12
ZNL-02-012	7.28±0.67	118±6
ZNL-02-040	4.46±0.95	23.6±4.0
ZNL-02-047	3.18±0.52	285±14
ZNL-02-096	3.58±0.36	102±6
ZNL-02-178	7.38±0.47	43±4
Pomalidomide	>10000	>10000

[a] IC50s against Wee1 was obtained with LanthaScreen Binding activity assays and reported as the average of two replicates ± SD. [b] IC50s against PLK1 was obtained with ZLYTE activity assay and reported as the average of two replicates ± SD.