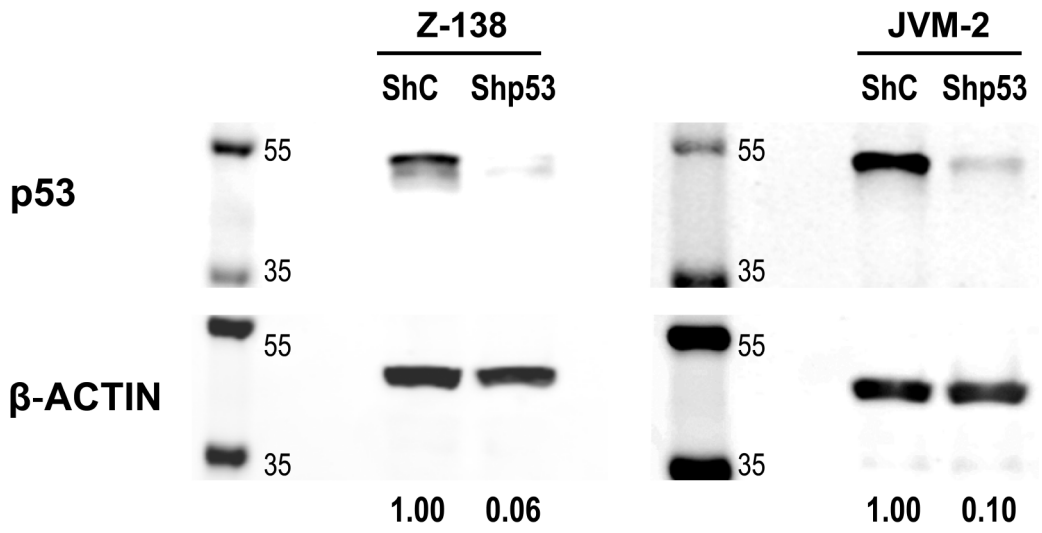
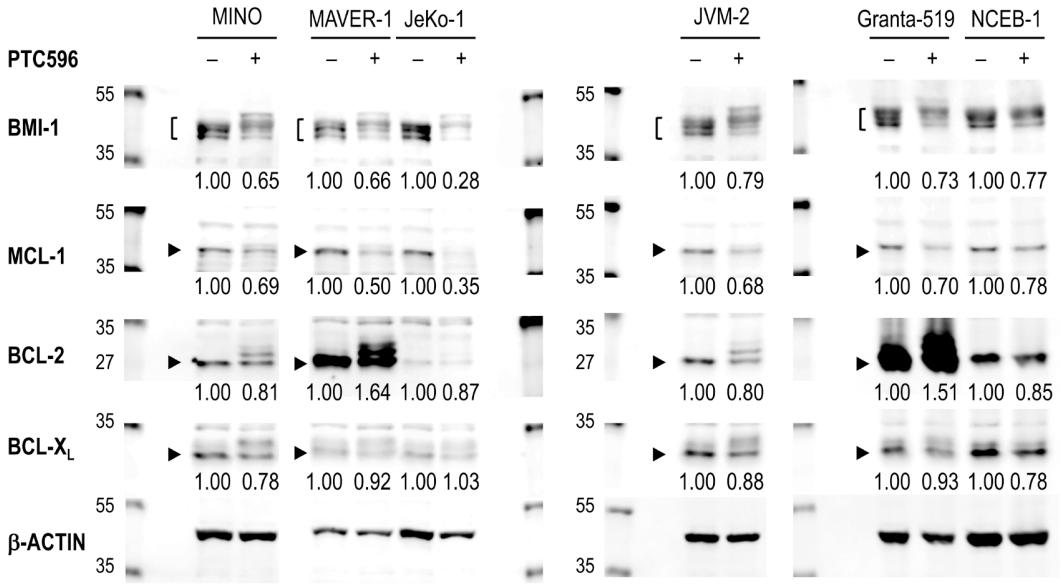
Targeting of BMI-1 expression by the novel small molecule PTC596 in mantle cell lymphoma

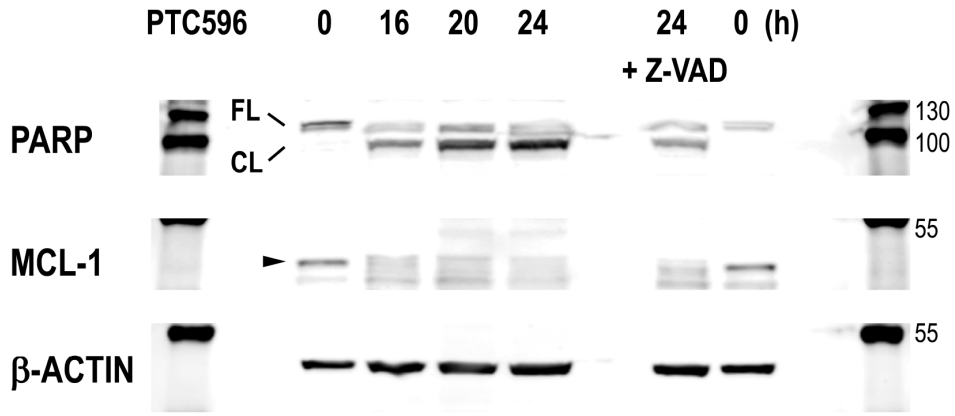
SUPPLEMENTARY MATERIALS



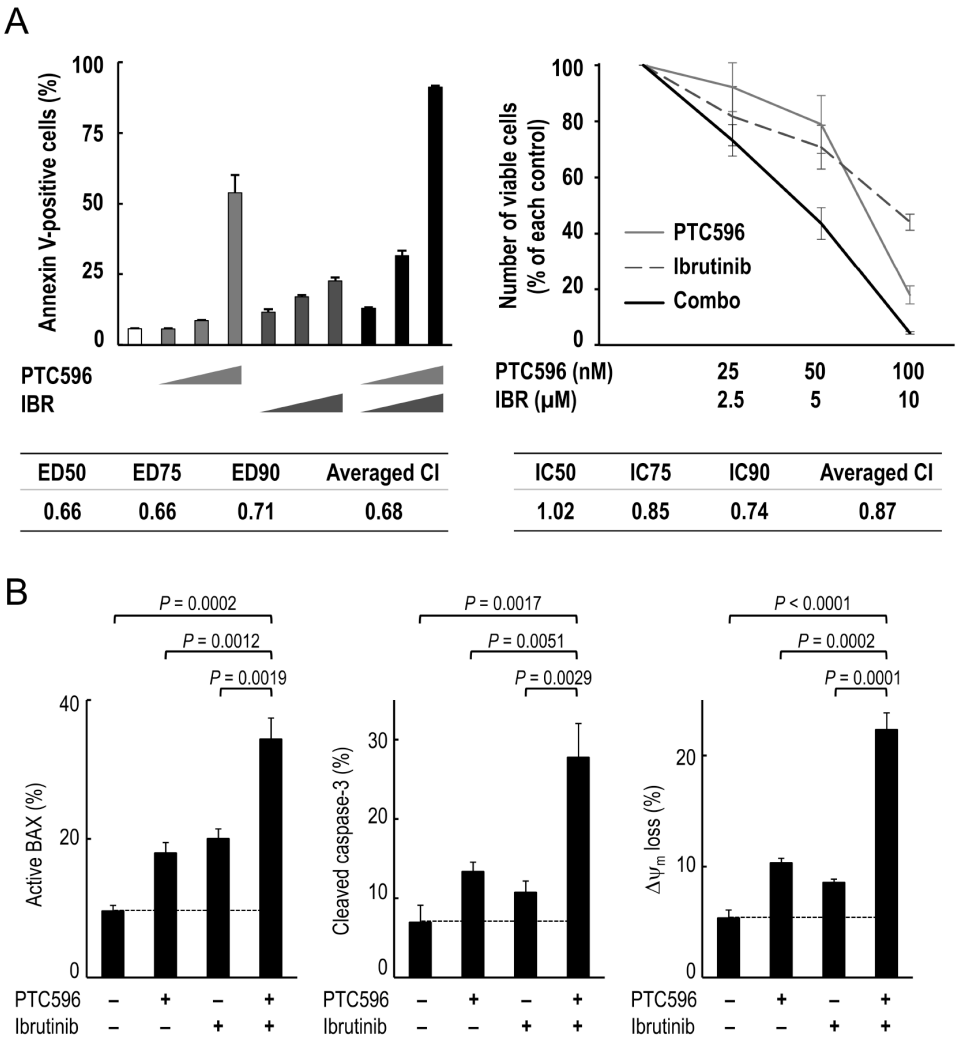
Supplementary Figure 1: *TP53*-shRNA reduced p53 basal levels by ~ 90% in MCL cell lines. p53 expression was determined in Z-138 and JVM-2, which were transduced with retroviruses encoding either scrambled shRNA (shC) or *TP53*-specific shRNA (shp53). Signal intensity was quantified and the relative intensity compared with β-ACTIN was calculated. Results are representative of three independent experiments.



Supplementary Figure 2: MCL cells were treated for 24 hours with PTC596 (1 μM in relatively insensitive JVM-2 cells and 300 nM in other cells), after which BCL-2, BCL-X_L and MCL-1 protein levels were determined. The intensities of immunoblot signals were quantified and normalized to those of β-ACTIN. Levels in untreated cells were set at 1.0. Results are representative of three independent experiments.



Supplementary Figure 3: Z-138 cells were treated with 300 nM PTC596 for the indicated times, in the absence or presence of 10 μM Z-VAD. Comparable results were obtained in two other independent experiments. FL, full length PARP. CL, cleaved PARP.



Supplementary Figure 4: PTC596 and ibrutinib cooperatively induce mitochondrial apoptosis in mantle cell lymphoma cells. (A) Z-138 cells were treated for 72 hours with PTC596 and ibrutinib (IBR) either as individual agents or in combination, after which the annexin V-positive fractions and the numbers of viable cells were determined. **(B)** BAX conformational changes, caspase-3 cleavage and Δψm loss were determined by flow cytometry in Z-138 cells after 24-h exposure to 100 nM PTC596 and 10 μM ibrutinib, either as individual agents or in combination. The results are expressed as the mean ± SD.