Supplemental Methods

Cell Cycle Analysis: Following the designated treatments, cells were harvested by centrifugation, washed twice with 1X PBS and fixed in 70% ethanol overnight. Fixed cells were washed twice with 1X PBS and re-suspended in 1X PBS with 0.1% Triton X-100, RNAse A and propidium iodide for 15 minutes at 37°C in the dark. Cell cycle data were collected on a flow cytometer with a 488 nM laser and analyzed with ModFit 3.0, as previously described (34).

Assessment of percentage non-viable cells: Following designated treatments, cells were stained with trypan blue (Sigma, St. Louis, MO). The numbers of non-viable cells were determined, as previously described (29,30,34).

RNA interference: Small interfering RNAs against EZH2 were obtained from Dharmacon. JeKo-1 cells were transfected with siRNA to a final concentration of 100 nM using an Amaxa Nucleofector. Cells were incubated for 48-72 hours for detection of EZH2 knockdown. Lentiviral shRNAs against EZH2 were obtained from Sigma-Aldrich (St. Louis, MO). JeKo-1 cells were transduced with lentivirus and incubated for 48 hours, then the cells were washed with complete media and incubated an additional 24 hours for immunoblot analyses or 72 hours to observe effects on cell proliferation, as previously described (34,36).

RNA isolation and Reverse Transcription-Polymerase Chain Reaction: RNA was extracted from the cultured cells using an RNaqueous-4PCR kit (Applied Biosystems, Foster City, CA). Purified total RNA was quantified and quantitative real time PCR analyses for EZH2, SUZ12, EED, Cyclin D2, SMARCA2, TCF4 and EIF3A were performed on cDNA using TaqMan probes from Applied Biosystems (Foster City, CA), as previously described (33,34).

Detection and analysis of hsa-miR-101 in MCL cells: For detection of hsa-miR-101 in JeKo-1 and MO2058 cells, microRNAs were isolated with a kit from Applied Biosystems (Foster City, CA). Enriched RNA was reverse transcribed with a stem loop primer included in the TaqMan hsa-miR101 microRNA assay following the manufacturer's protocol (Applied Biosystems, Foster City, CA).

Expression of hsa-miR101 was detected by qPCR with a TaqMan probe specific to hsa-miR-101. Relative expression of hsa-miR101 was normalized against expression of 18S RNA.

Chromatin immunoprecipitation and Polymerase Chain Reaction: JeKo-1 cells were treated with DZNep for 16 hours. Following drug exposure, the chromatin in the cells was cross-linked with formaldehyde for 10 minutes at 37°C. The cross-linking reaction was quenched with 1/20 volume of 2.5 M glycine for 5 minutes at room temperature, then the cells were washed twice for 5 minutes in ice-cold 1X PBS, as previously described (34). Cell lysis, sonication and chromatin immunoprecipitation for EZH2 was performed according to the manufacturer's protocol (Millipore, Billerica, MA). For quantitative assessment of HOXA9, RUNX3, and WNT1 promoters in the chromatin immunoprecipitates, a SYBR Green Mastermix from Applied Biosystems was used (Foster City, CA). Relative enrichment in the chromatin immunoprecipitates was normalized against HOXA9, RUNX3, and WNT1 promoter DNA in the input samples, as previously described (28,34,37).

Assessment of DNA methylation in MCL cells: MO2058 cells were treated with DZNep or decitabine for 24 hours. At the end of treatment, total genomic DNA was isolated with a kit from Qiagen. One microgram of total DNA was subjected to sodium bisulfite conversion utilizing a kit from Millipore and following the manufacturer's protocol. The bisulfite converted DNA was subjected to PCR with primers specific for the p16 promoter. After 40 cycles of PCR (95°C [30 seconds], 55°C [30 seconds] and 72°C [30 seconds]), the resulting products were resolved on a 2.0 % agarose gel and visualized with a UV trans-illuminator.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Treatment with DZNep depletes expression of PRC2 proteins in MCL MO2058 and Z-138 cells. A. MO2058 and Z-138 cells were treated with the indicated concentrations of DZNep for 24 hours. Cell lyates were prepared and immunoblot analyses were performed for DNMT1, EZH2, SUZ12, EED, CyclinD1, Cyclin E, FBXO32, p27, and p21. The expression levels of β-actin in the lysates served as the loading control. **B.** MO2058 and JeKo-1 cells were treated with 2.0 μM of DZNep and/or 100 nM of bortezomib (BZ) for 8 hours. Then, cell lysates were prepared and immunoblot analyses were performed for EZH2 and SUZ12. The expression levels of β-actin in the lysates served as the loading control. **C.** JeKo-1 cells were treated with the indicated concentrations of DZNep for 4 hours. After treatment, CDK4 was immunoprecipitated from the total cell lysates and immunoblot analyses were performed for Cyclin D1 and CDK4 on the immunoprecipitates. Two separate antibodies (a rabbit polyclonal and a mouse monoclonal) were used to confirm the specificity of the interaction.

Supplemental Figure 2. Treatment with DZNep upregulates mRNA expression of EZH2 target genes in MCL cells.**A-C.** JeKo-1 cells were treated with 2 μM of DZNep for 8 and 24 hours. Total RNA was isolated and reverse transcribed. Quantitative real time PCR analyses were performed with TaqMan probes for SMARCA2, EIF3A and TCF4. Relative expression of each target was normalized against GAPDH.

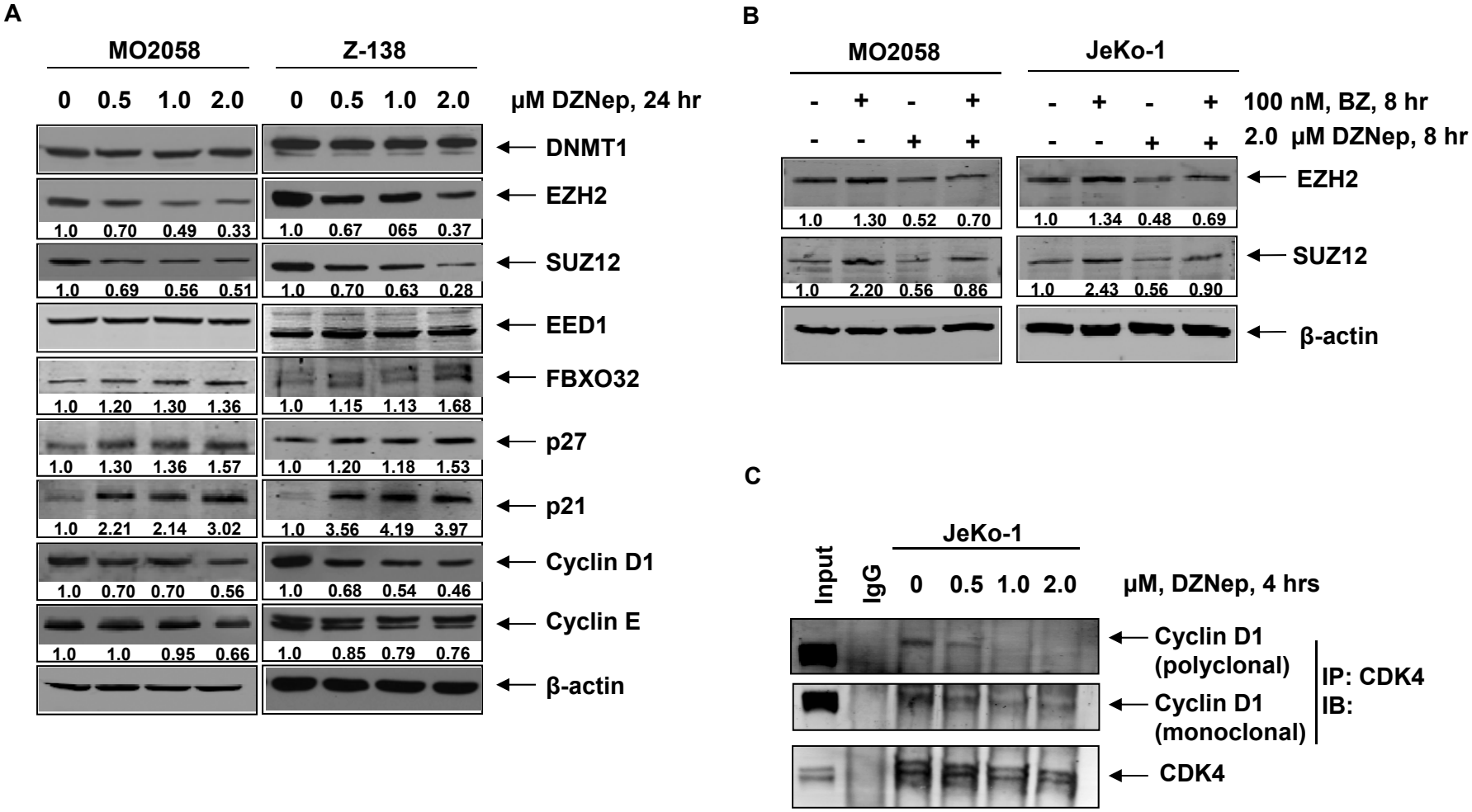
Supplemental Figure 3. Treatment with DZNep does not alter hsa-miR-101 expression in MCL cells. A. Representative agarose gel demonstrating expression of pre-miR-101 in MO2058 and JeKo-1 cells. **B**. MO2058 and JeKo-1 cells were treated with the indicated concentrations of DZNep for 16 hours. Total RNA was isolated and reverse transcribed with a stem loop primer for hsa-miR-101. Following reverse transcription, qPCR for hsa-miR-101 was performed and expression of hsa-miR-101 was normalized against 18S RNA expression. **C.** MO2058 cells were treated with the indicated concentrations of DZNep or decitabine (DAC) for 24 hours. Genomic DNA was isolated and bisulfite converted. Methylation specific PCR was performed for the p16 promoter.

Supplemental Figure 4. Effects of treatment with DZNep or PS in MCL Z-138 cells. A. Z-138 cells were treated with the indicated concentrations of DZNep as indicated. At the end of treatment, cells were washed with 1X PBS and stained with annexin V and TOPRO3 iodide. The percentages of annexin V-positive, apoptotic cells were determined by flow cytometry. Columns represent the mean of three experiments; Bars represent the standard error of the mean. **B.** Z-138 cells were treated with the indicated concentrations of DZNep for 24 hours. Then, cell lysates were prepared and immunoblot analyses were performed for PARP. The expression levels of β-actin in the lysates served as the loading control. **C.** Z-138 cells were treated with the indicated concentrations of PS for 24 hours. After treatment, total cell lysates were prepared and immunoblot analyses were performed for DNMT1, EZH2, SUZ12, EED, BMI1, Cyclin D1 and Acetyl α-tubulin. The expression levels of β-actin in the lysates served as the loading control.

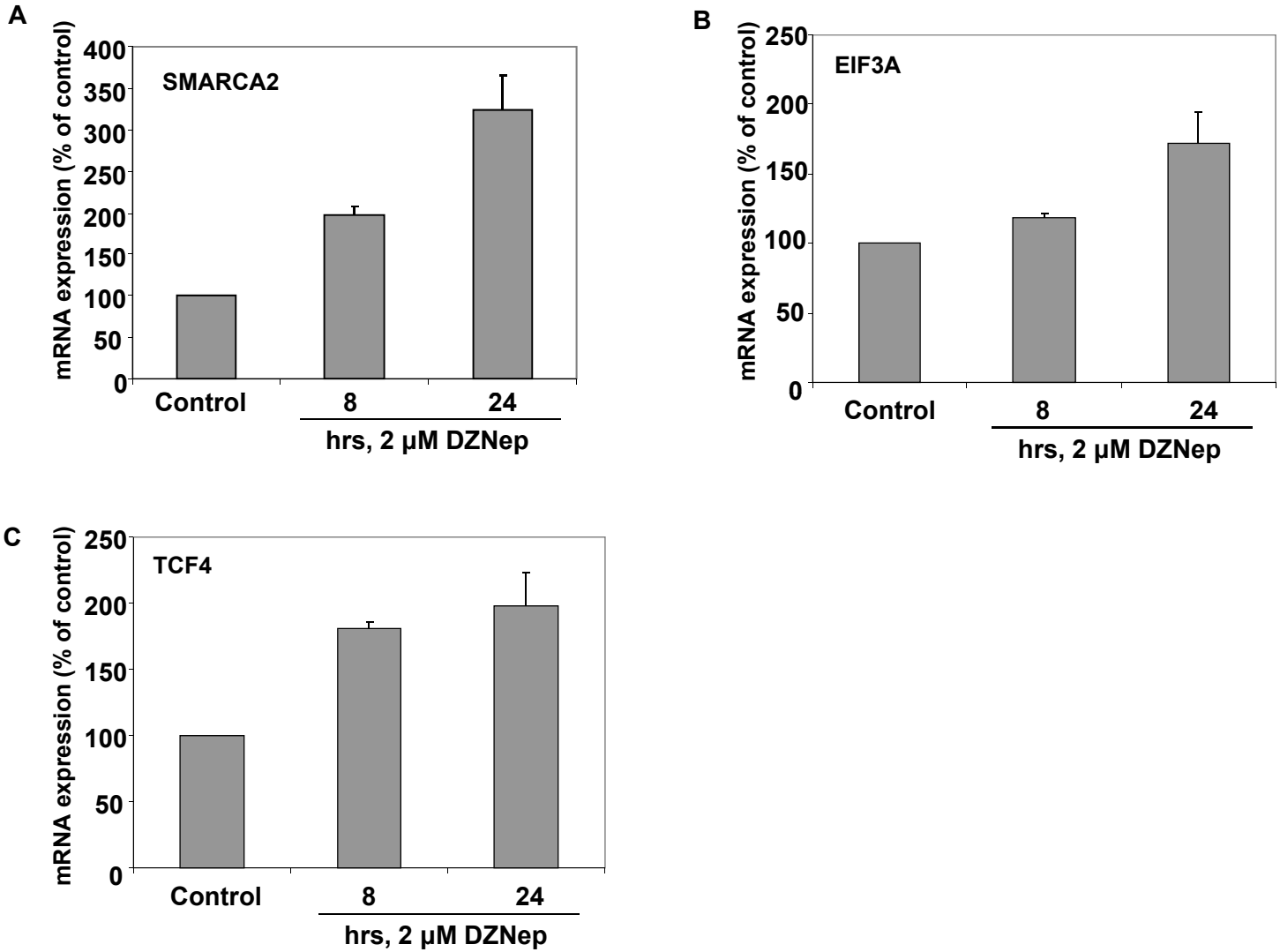
Supplemental Figure 5. Treatment with PS significantly enhances the anti-MCL activity of DZNep in JeKo-1 and Z-138 cells. A. JeKo-1 cells were treated with the indicated concentrations of DZNep and/or PS for 24 hours. Cell lysates were prepared and immunoblot

analyses were performed for DNMT1, EZH2, SUZ12, EED, 3Me K27H3, 3Me K4H3, FBXO32, Cyclin D1, p21 and p27. The expression levels of β-actin in the lysates served as the loading control. **B**. Z-138 cells were treated with the indicated concentrations of DZNep and/or PS for 24 hours. Cell lysates were prepared and immunoblot analyses were performed for DNMT1, EZH2 and SUZ12. The expression levels of β-actin in the lysates served as the loading control. **C.** Z-138 cells were treated with the indicated concentrations of DZNep and/or PS for 48 hours. Following treatment, cells were washed with 1X PBS and stained with annexin V and TOPRO3 iodide. The percentages of annexin V-positive, apoptotic cells were determined by flow cytometry. Columns represent the mean of three experiments; Bars represent the standard error of the mean. * indicates values significantly greater ($p < 0.05$) in the combination, compared to treatment with either agent alone.

Supplemental Figure S1



Supplemental Figure S2



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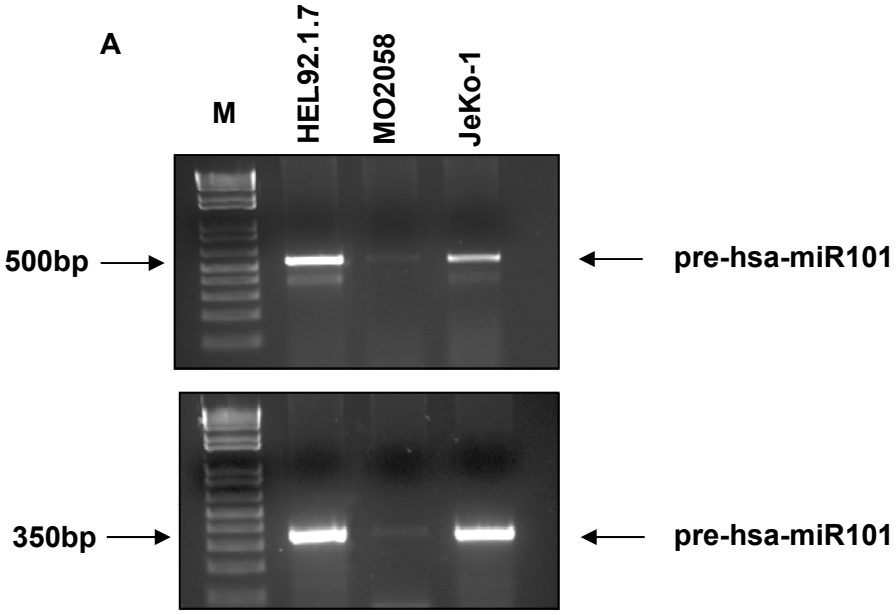
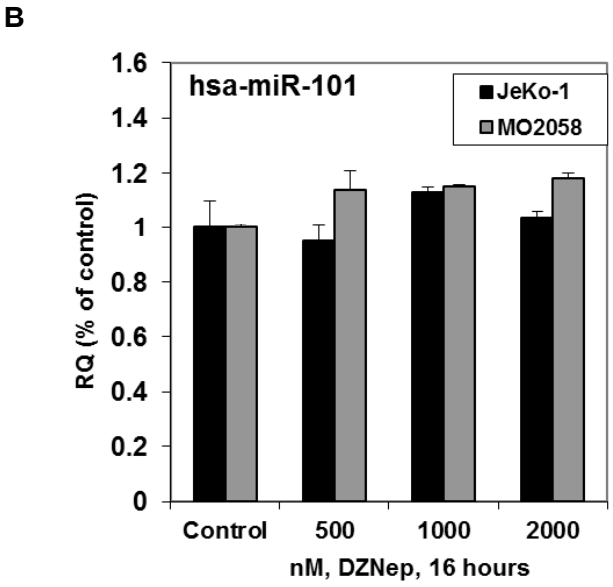
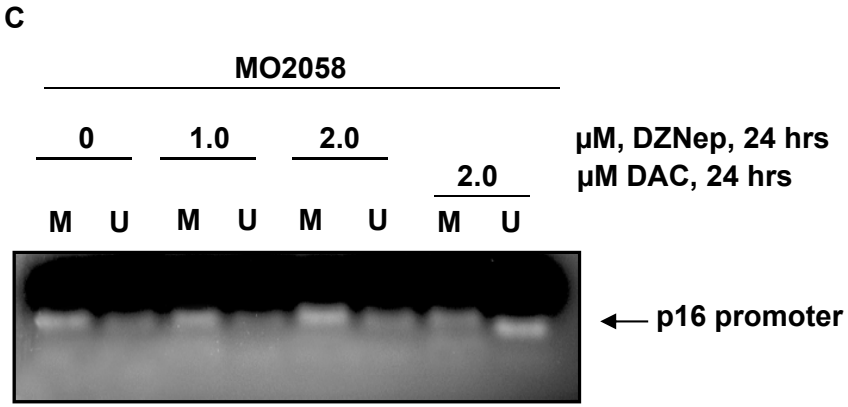
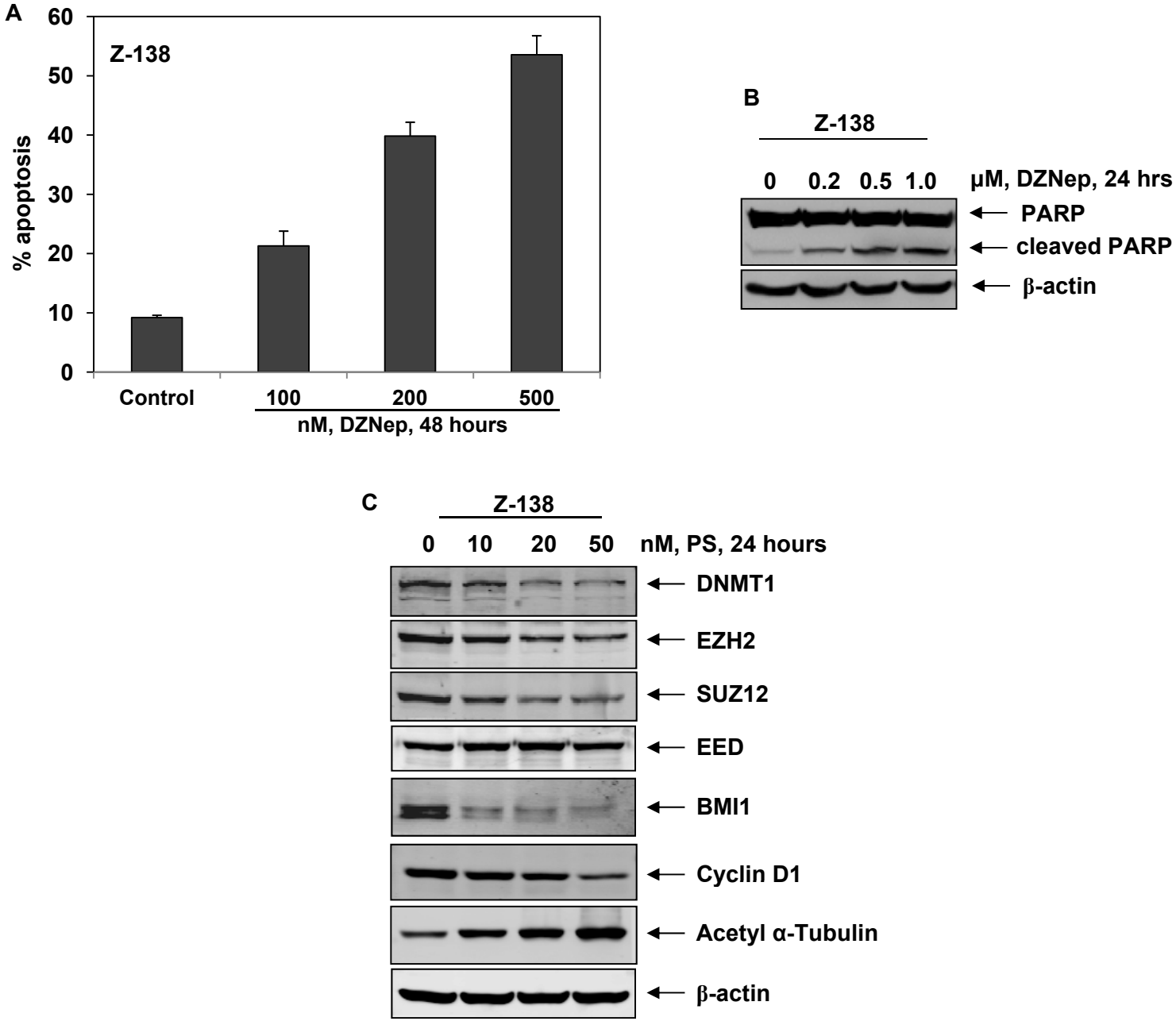






Supplemental Figure S4



Supplemental Figure S5