

Libertellenone T, a Novel Compound Isolated from Endolichenic Fungus, Induces G2/M Phase Arrest, Apoptosis, and Autophagy by Activating the ROS/JNK Pathway in Colorectal Cancer Cells

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Supplementary Table S1

Supplementary Figure S1–S9

Table S1. Nucleotide sequence of EL000327

CACAGCTAGAGGGCAAGGACCATCAGAAGCGAATAAATATCACGCCGAGGTCTCTTGACAG
CTTCTGCTGAAGGATTTAAGAGGGGATCGACCAAGGTTGATCCTCCTCCAGGTCCAGGTGC
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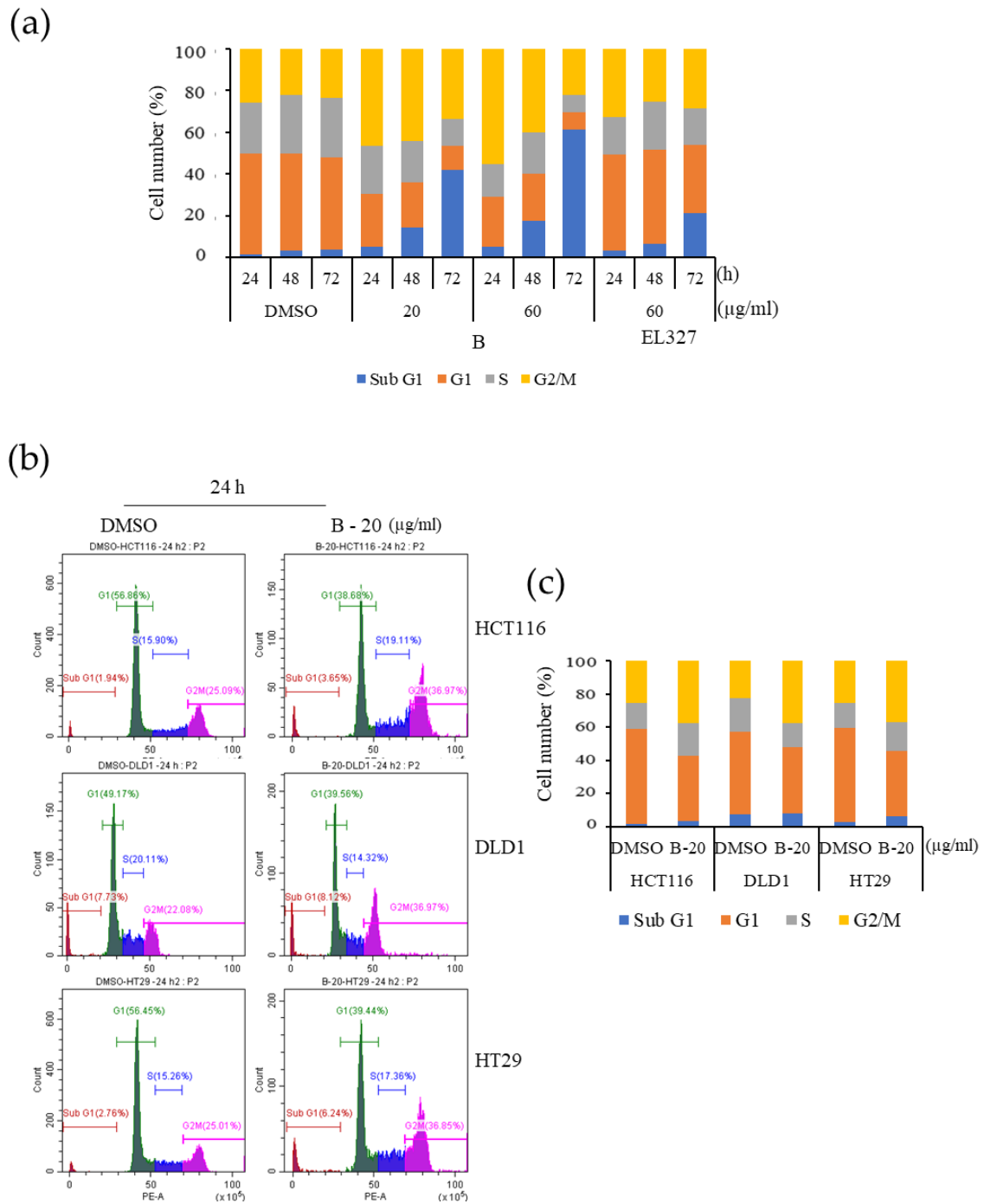


Figure S1. B induces G2/M phase arrest in CRC cells. (a) Quantitative analysis of percent cell distribution in each phase of the cell cycle upon the treatment of 20, 60 µg/ml of **B** and 60 µg/ml of EL000327 for 24, 48 and 72 h. (b) The flow cytometric analysis of the cell-cycle distribution of HCT116, DLD1 and HT29 cells treated with 20 µg/ml of **B** for 24 h. (c) Quantitative analysis of percent cell distribution in each phase of the cell cycle upon the treatment of 20 µg/ml of **B** for 24 h. n=3.

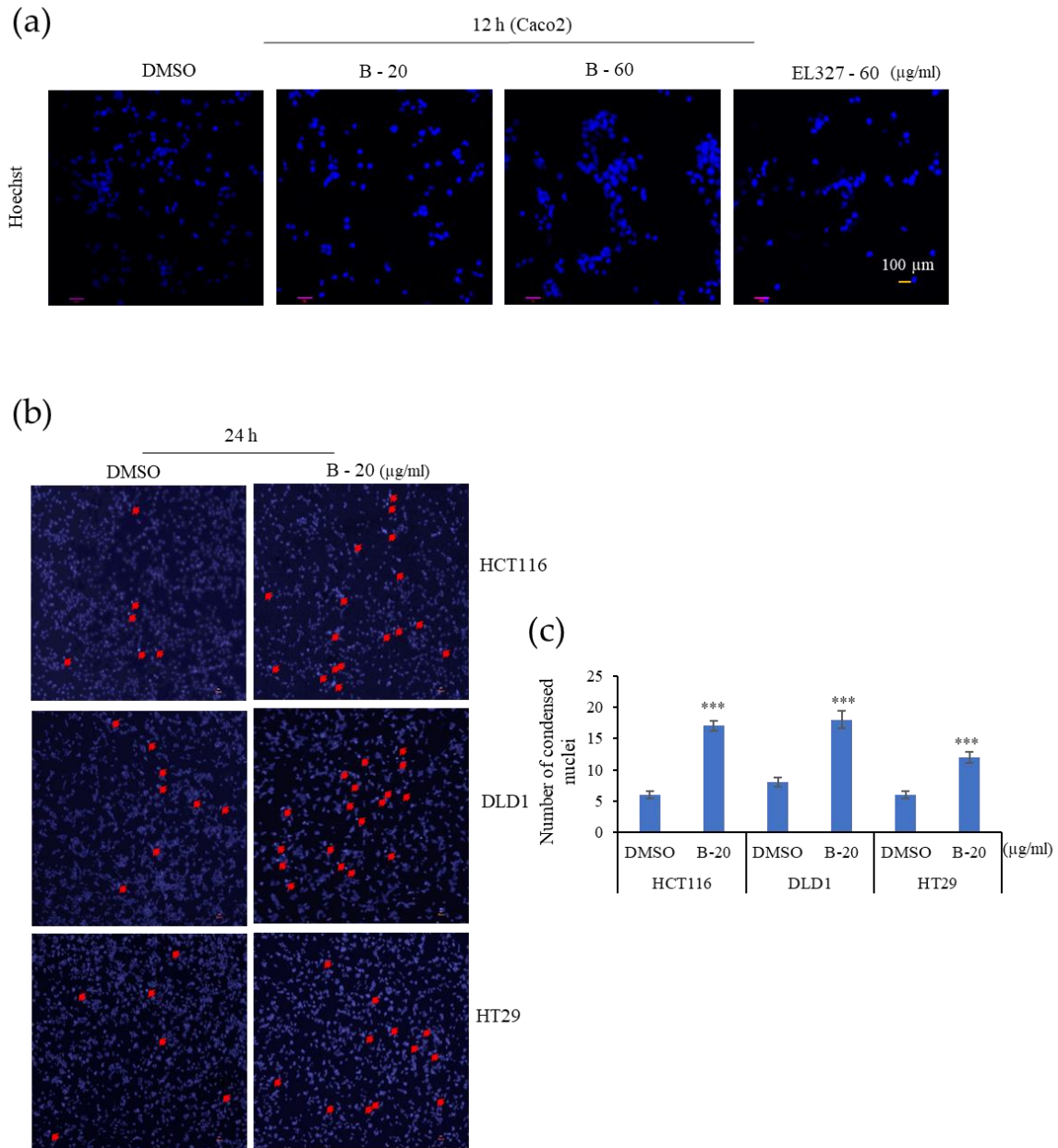


Figure S2. B induces Nuclei condensation in CRC cells. (a) Nuclei condensation of Caco2 cells upon treatment with **B** at the concentration of 20, 60 μg/ml and EL000327 at 60 μg/ml for 12 h, as determined by Hoechst staining. (b) HCT116, DLD1 and HT29 cells upon treatment with **B** at the concentration of 20 μg/ml for 24 h, as determined by Hoechst staining. Arrowheads indicate nuclear condensation in cells. (c) Quantification of condensed nuclei in HCT116, DLD1 and HT29 cells treated with 20 μg/ml of **B** for 24 h. n=3. Data represent mean ± S.D. *** p < 0.001, compared with the DMSO-treated control group.

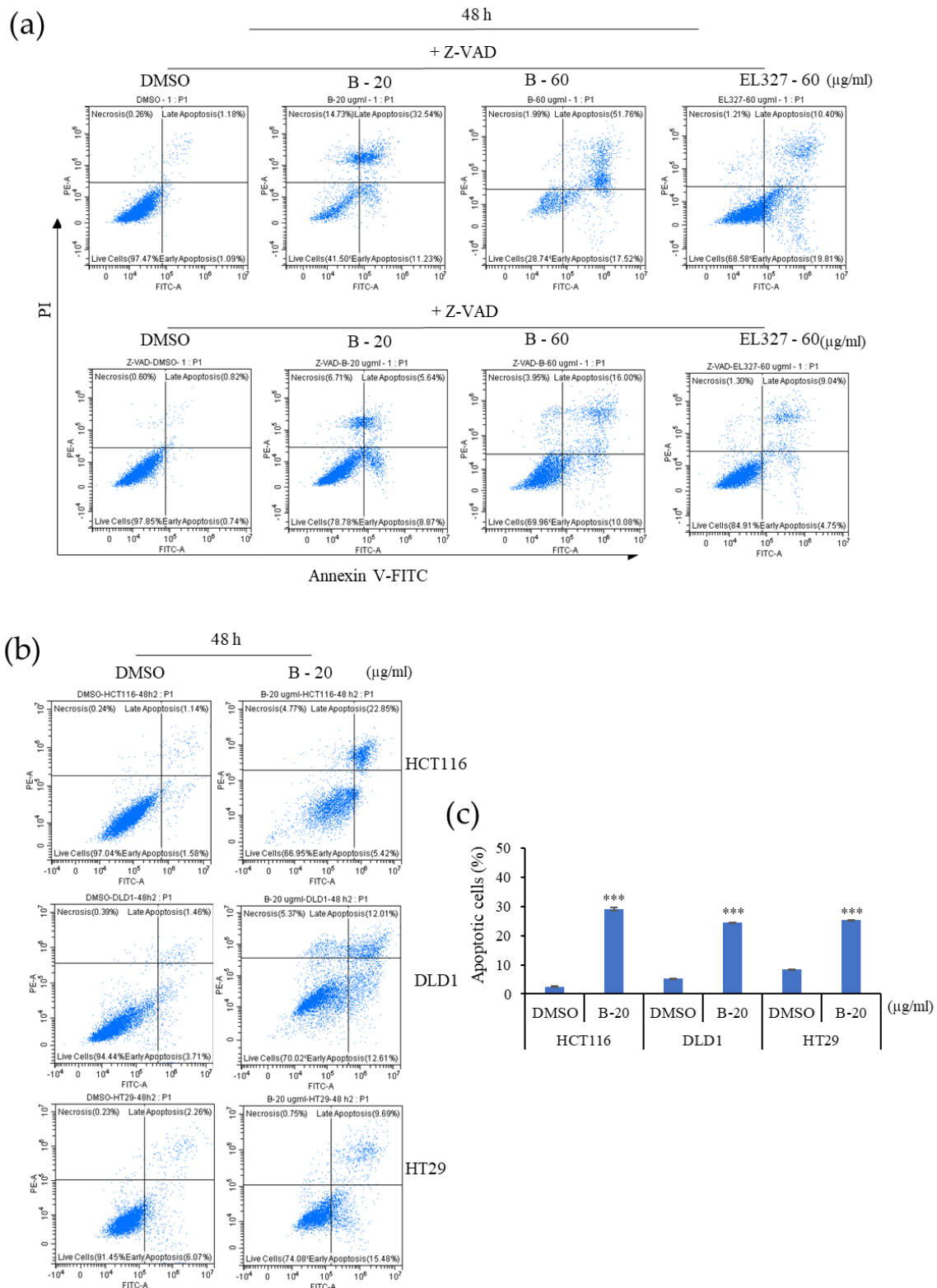


Figure S3. B induces apoptosis in CRC cells. (a) Flow cytometric analysis of dead cells stained by Annexin v-FITC (apoptotic cells) and PI (necrotic cells) upon treatment of **B** (20, 60 μg/ml) or EL000327 (60 μg/ml) for 48 h in presence or absence of Z-VAD-FMK (10 μM). (b) Flow cytometric analysis of apoptosis of HCT116, DLD1 and HT29 cells upon treatment of 20 μg/ml of **B** for 48 h. (c) Quantification of the percentage of apoptotic cells treated with 20 μg/ml of **B** and analyzed by flow cytometry. n=3. Data represent mean ± S.D. *** p < 0.001, compared with the DMSO-treated control group.

(a)

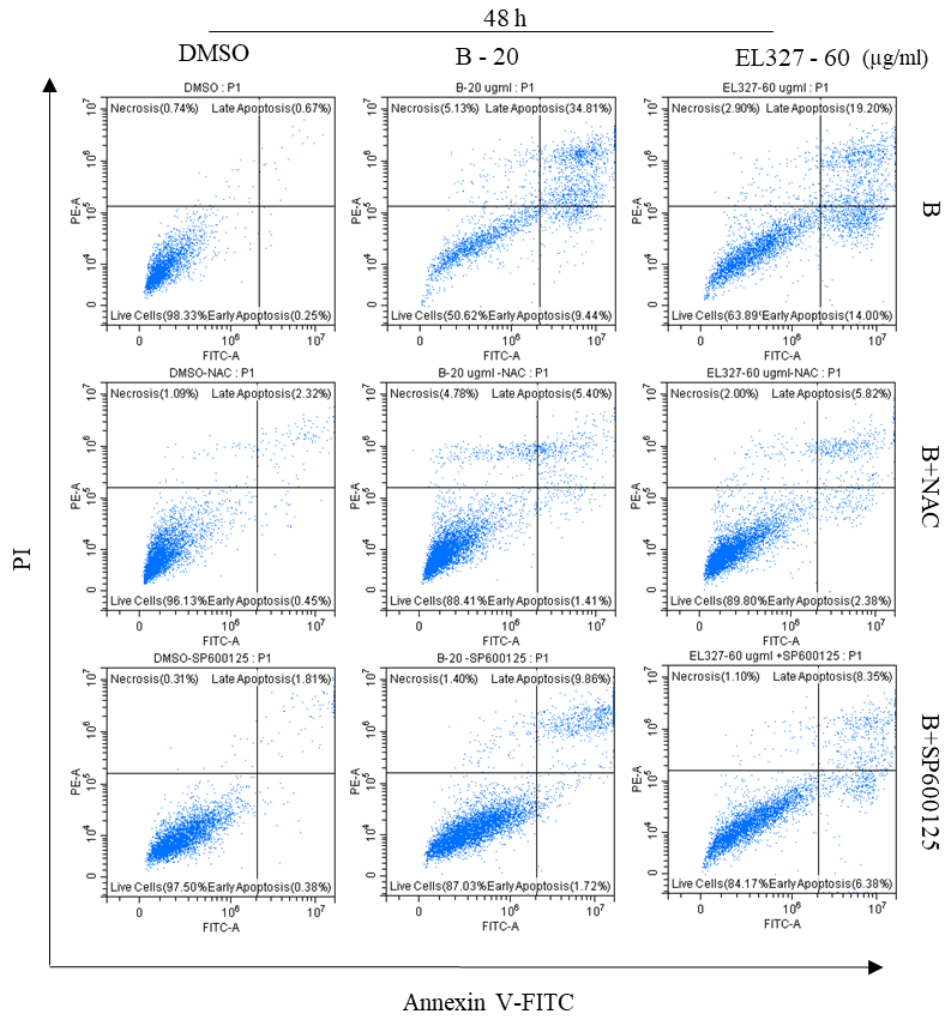
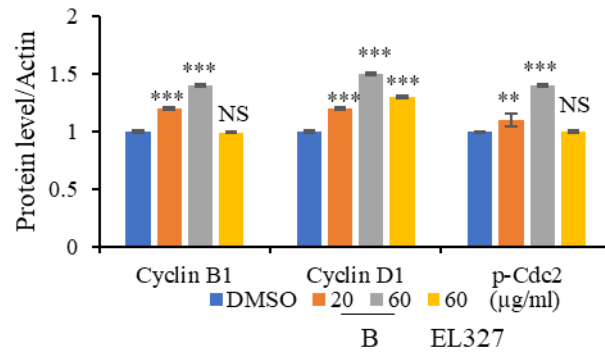


Figure S4. B decreased apoptosis in Caco2 cells in presence of ROS and JNK inhibitors. **(a)** Flow cytometric analysis of apoptotic cells stained by Annexin V-FITC and PI after treatment with **B** (20 µg/ml) or EL000327 (60 µg/ml) for 48 h, with or without NAC (5 mM) or SP600125 (10 µM).

(a)



(b)

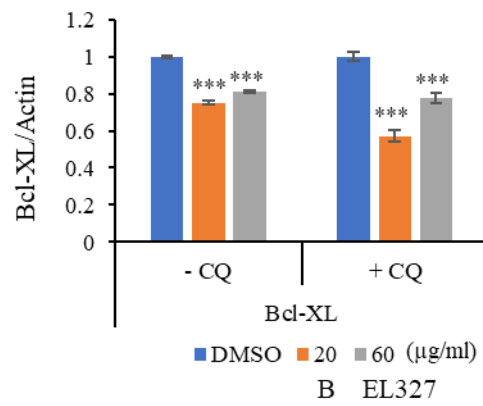


Figure S5. B modulated Cell cycle related protein expressions and anti-apoptotic protein Bcl-XL (a) Quantification of Cyclin B1, Cyclin D1 and p-Cdc2 protein expressions. (b) Quantification of Bcl-XL protein expressions in presence or absence of CQ. Data represent mean \pm S.D. ** $p < 0.01$, *** $p < 0.001$; NS: no significant difference ($p > 0.05$) compared with the DMSO-treated control.

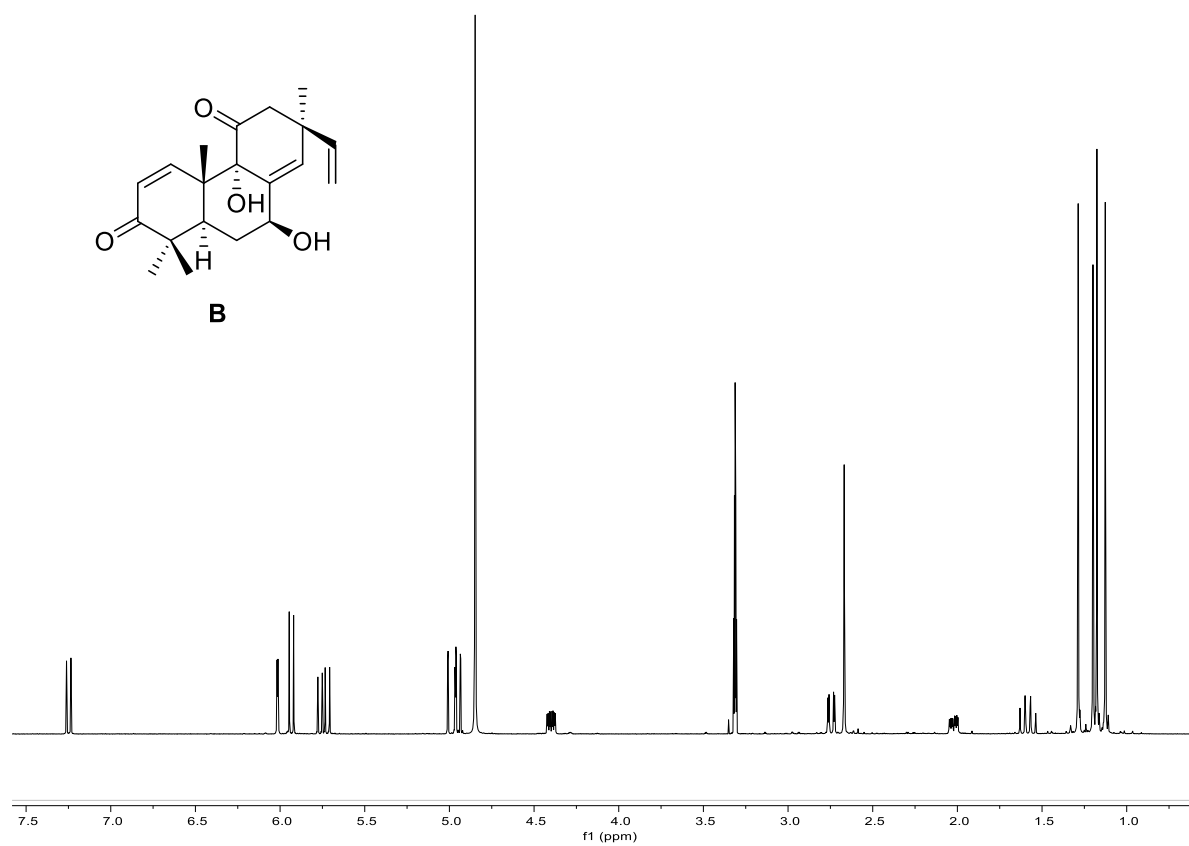


Figure S6. ^1H NMR spectrum of Libertellenone T (**B**) in MeOD

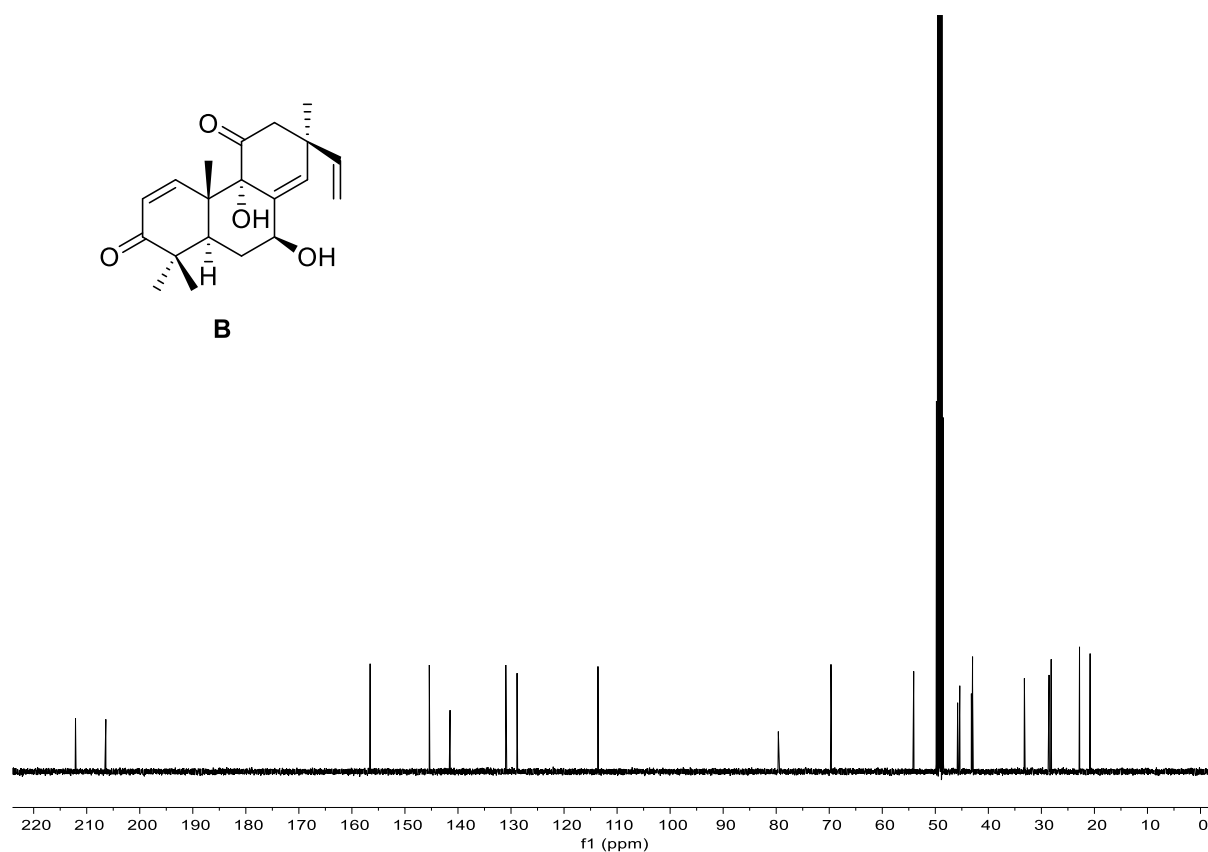


Figure S7. ¹³C NMR spectrum of Libertellenone T (**B**) in MeOD

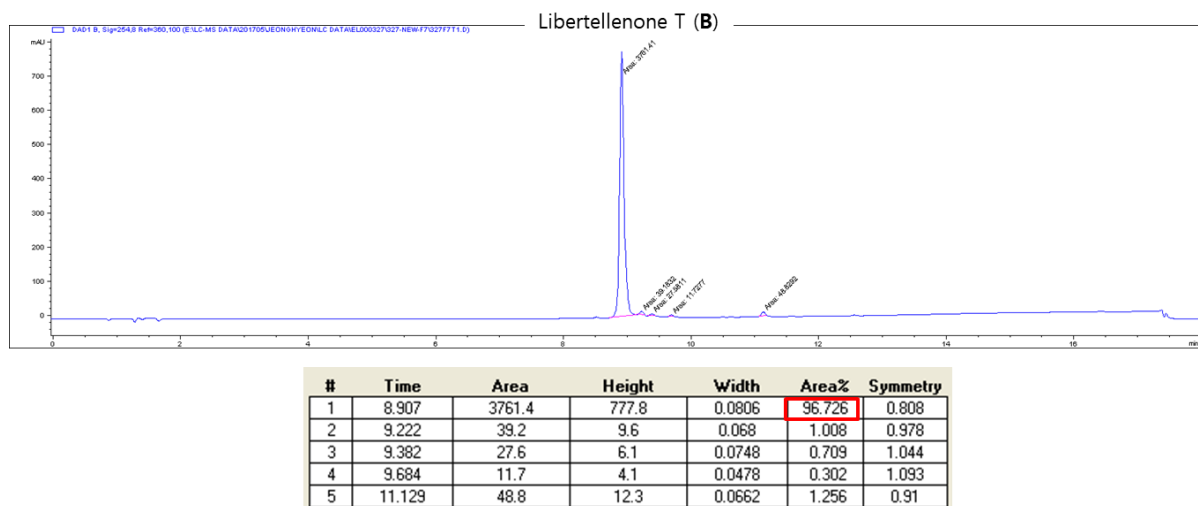
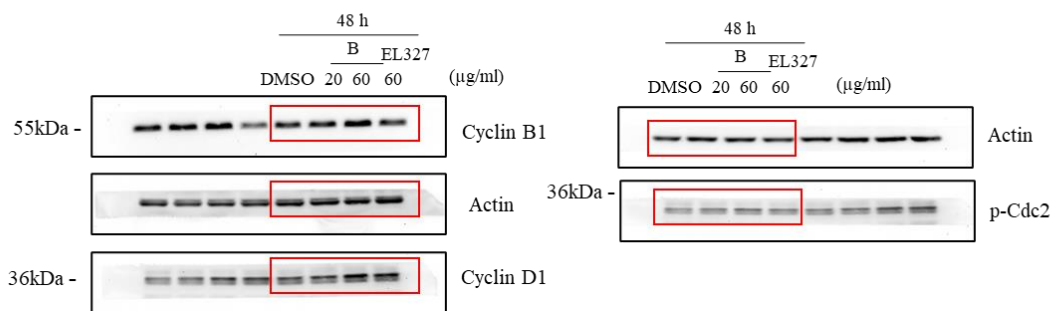
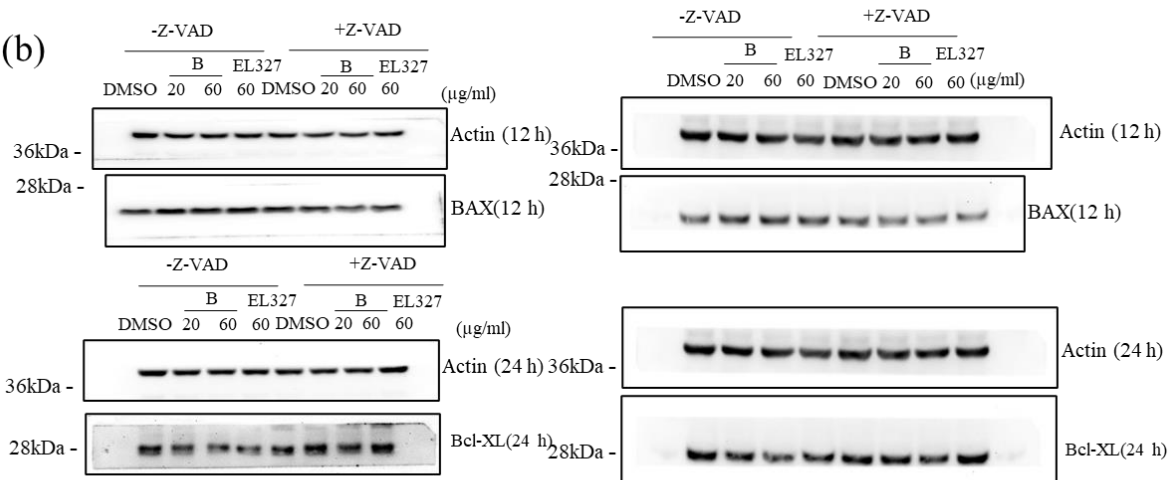


Figure S8. Percentage purity of Libertellenone T (B)

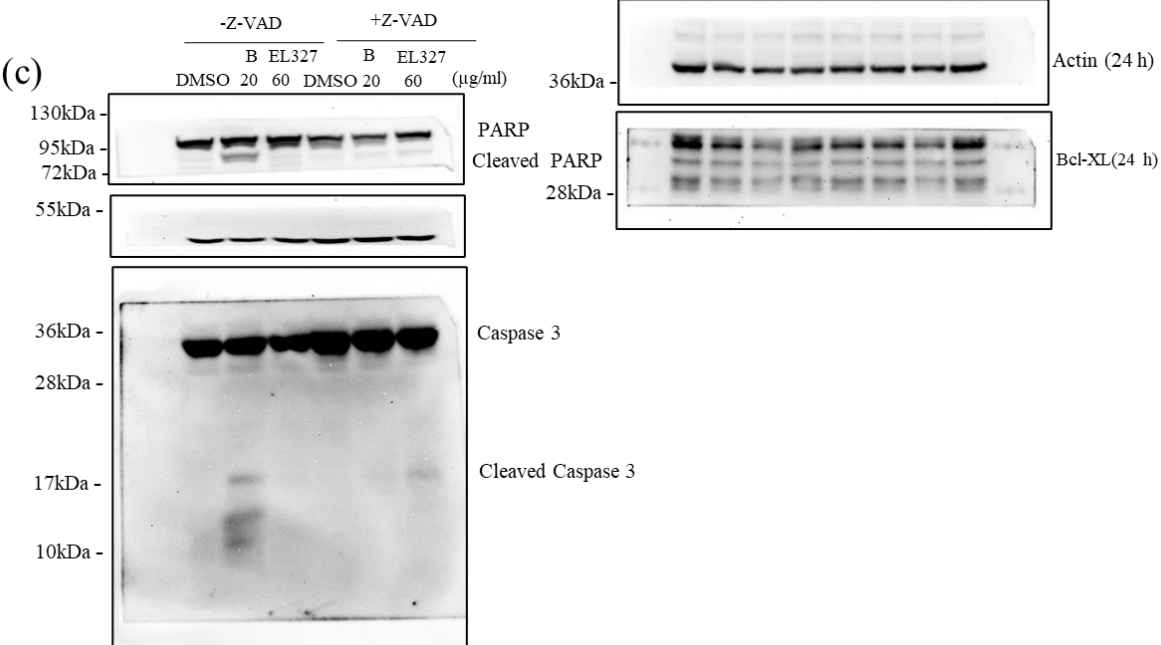
(a)



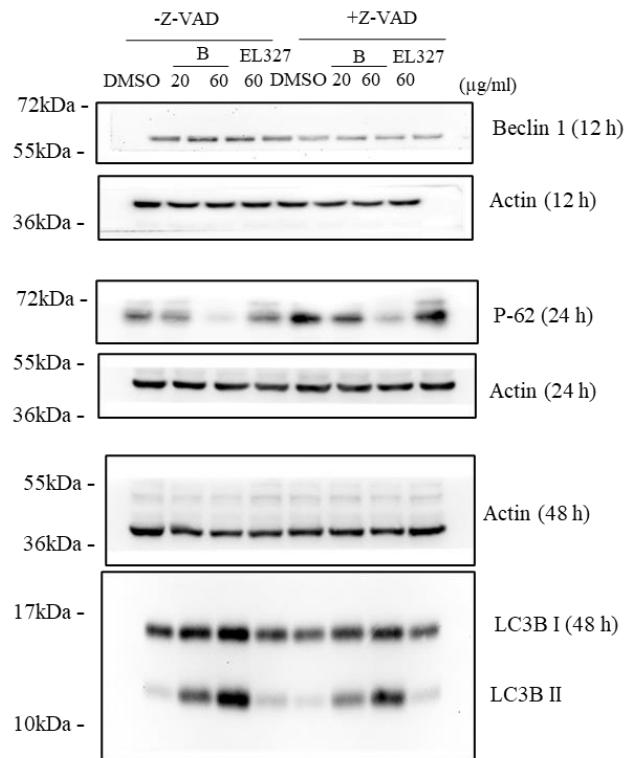
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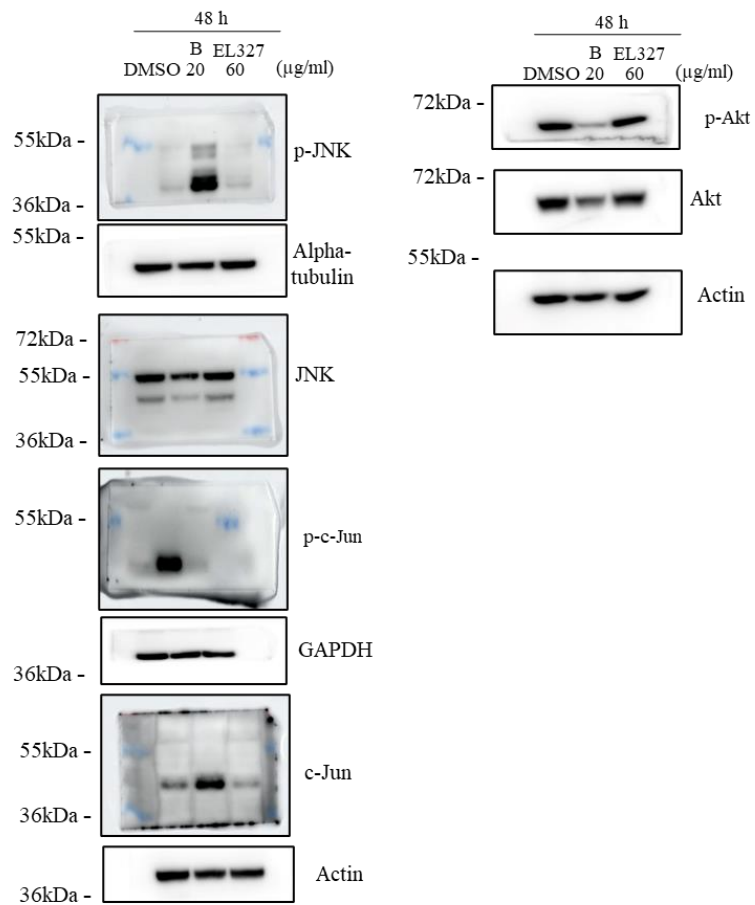
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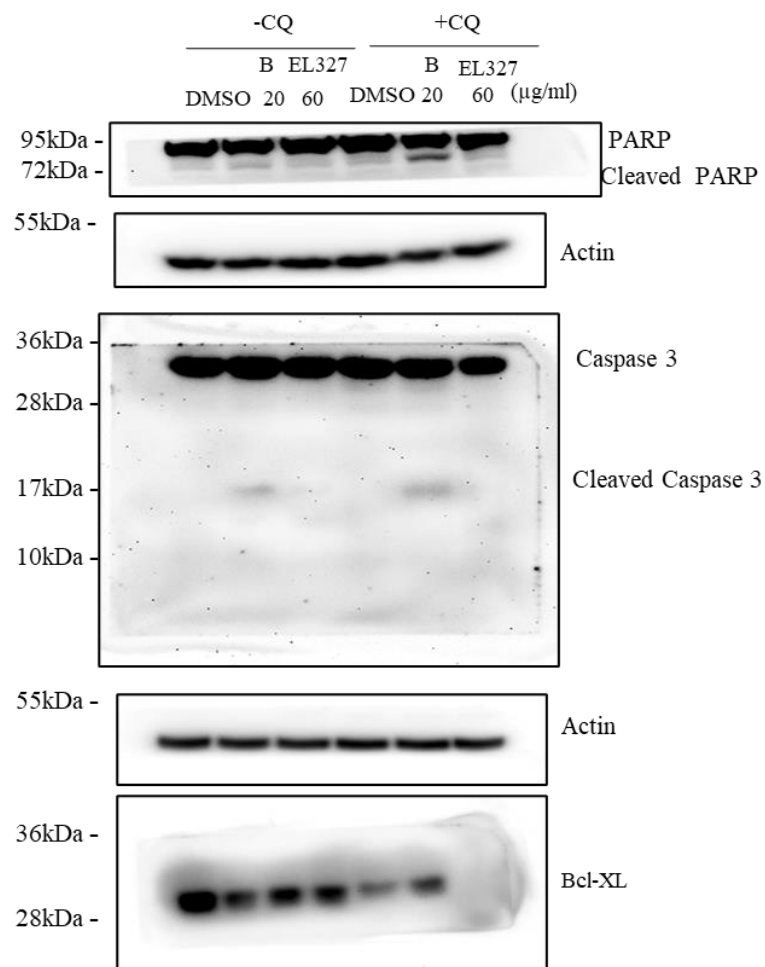
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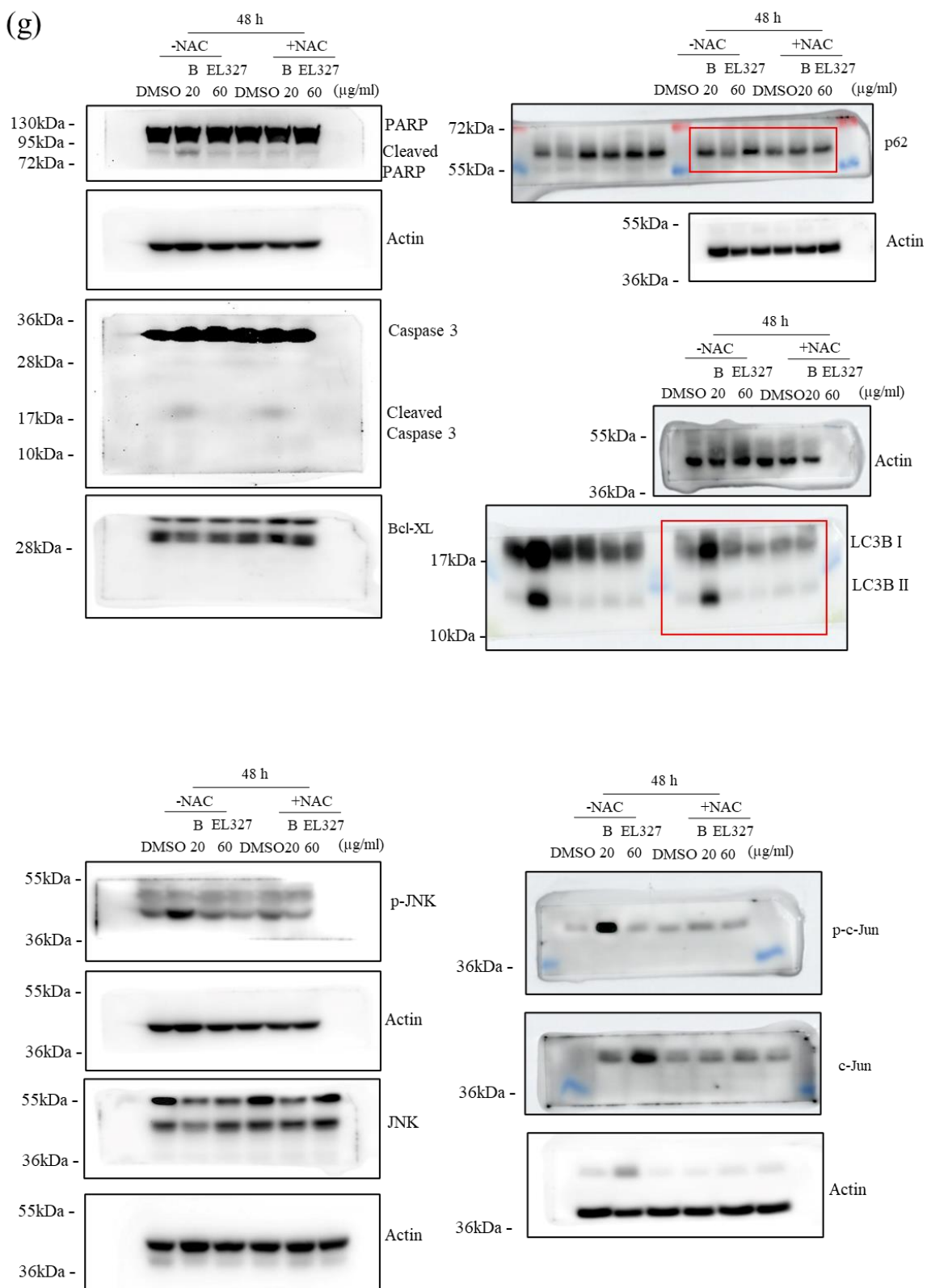
(e)



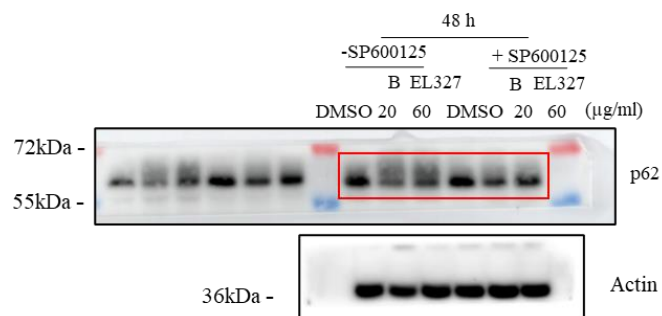
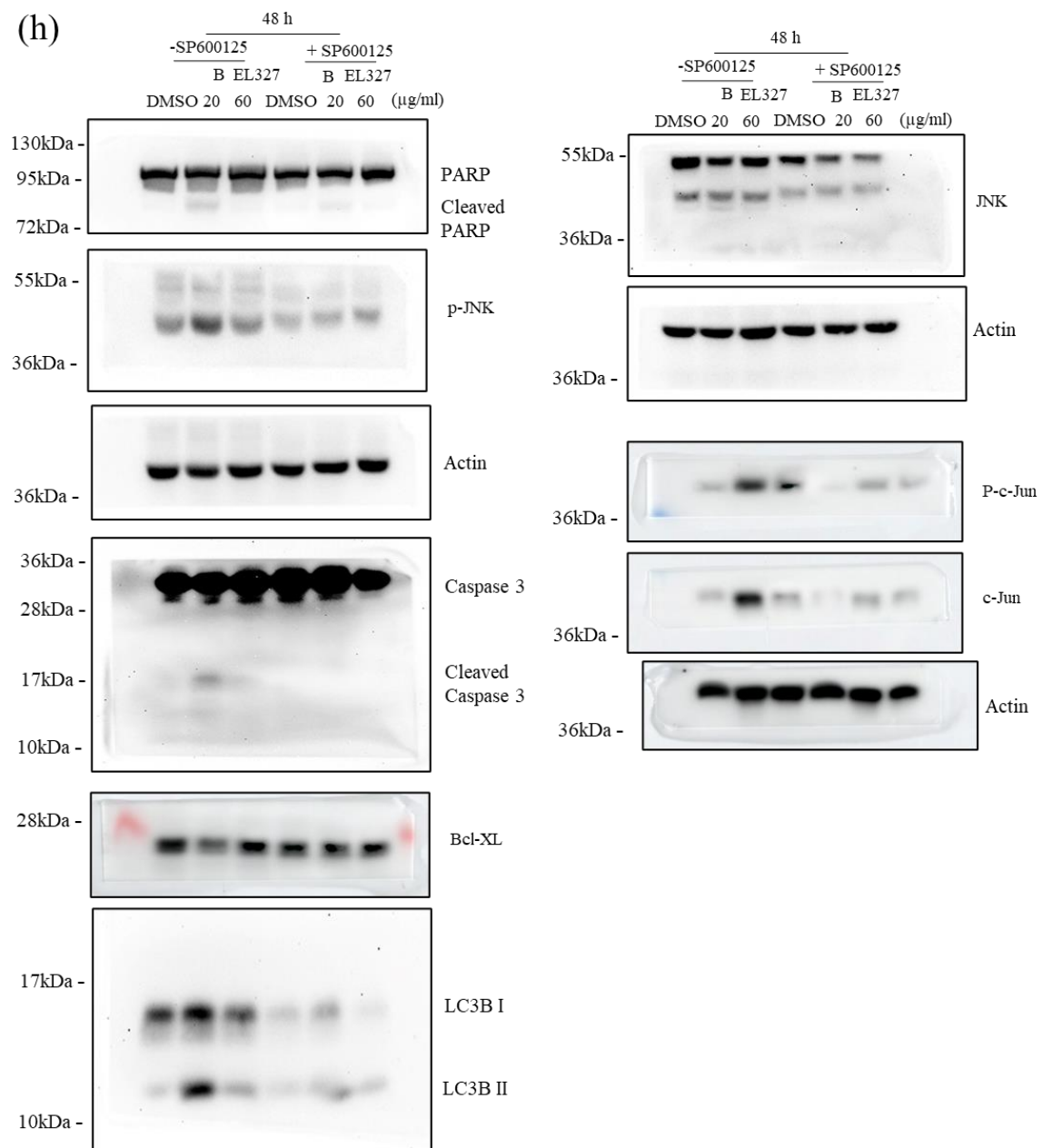
(f)



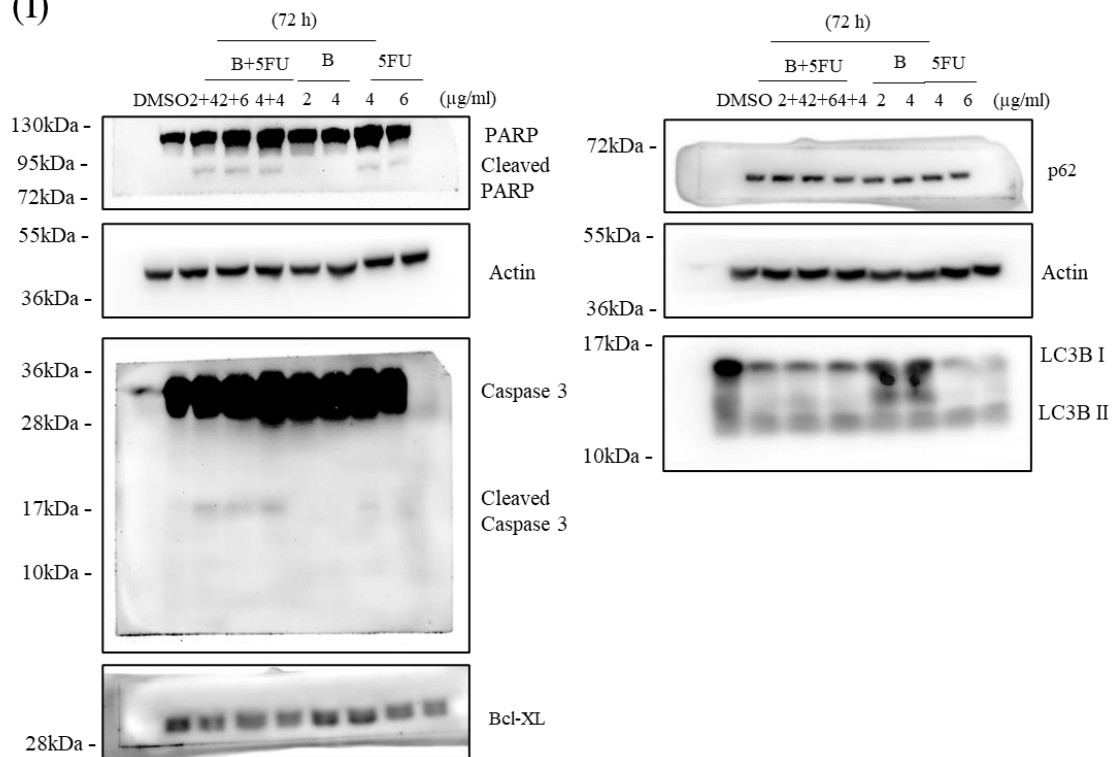
(g)



(h)



(I)



(J)

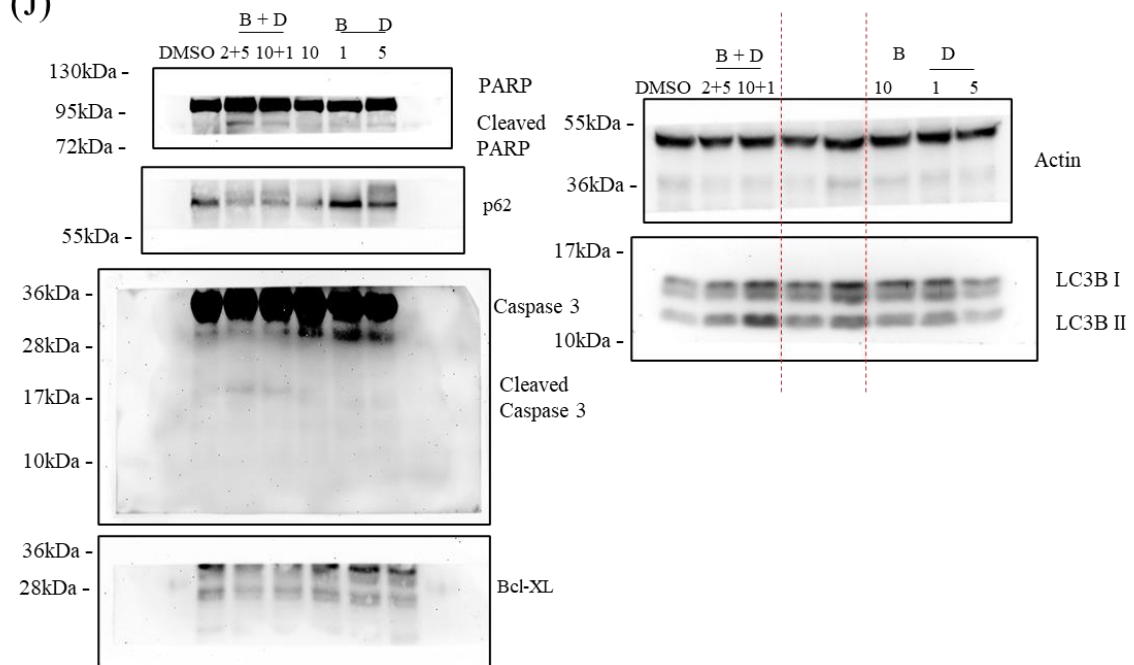


Figure S9. The raw data of western blotting