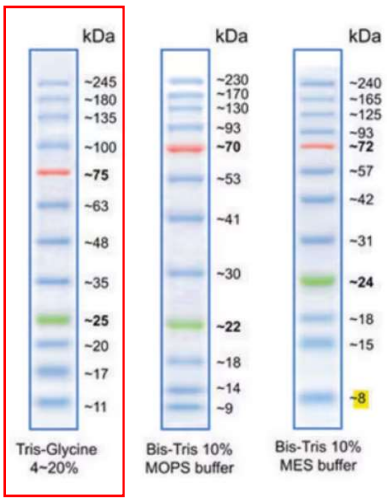
Supplement: Original images of Western Blots

This study used the BLU12 (BLUeye) Pre-stained Protein Ladder (Cat. 1BHC-PMB12-0500, Bio-Helix, Taiwan) as the protein marker, run on a Tris–glycine SDS-PAGE system, with the molecular weights presented below.



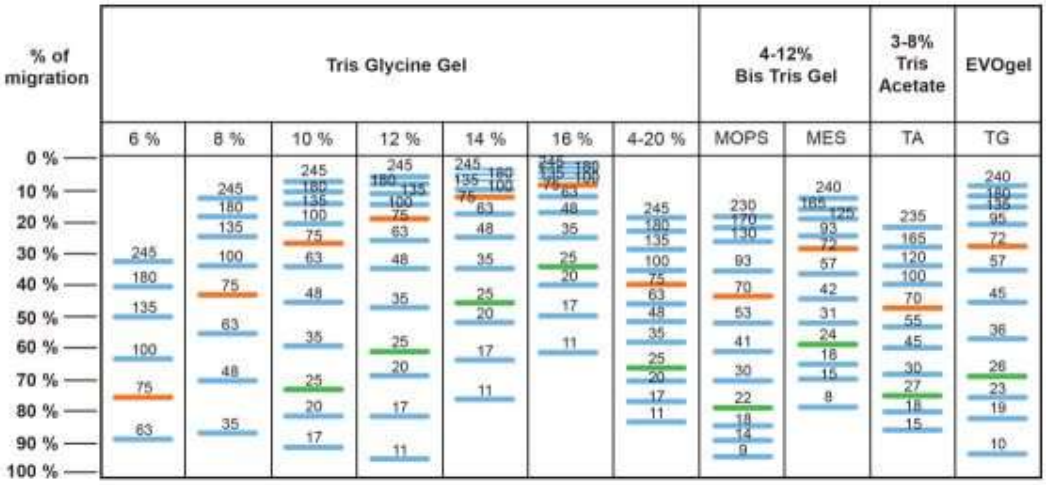


Figure S0. Molecular weight ladder of BLU12 (BLUeye) Pre-stained Protein Ladder.

To identify the optimal induction period, a time-course study was conducted, as the expression levels of signaling proteins may vary over time. The concentrations of essential signaling proteins peaked at 30 minutes following induction, after the cells had been stimulated with LPS at time points of 0, 0.5, 1, 2, 4, 6, 12, 18, and 24 hours. This condition was subsequently identified as the optimal time point for signaling pathway analysis (Figure S1).

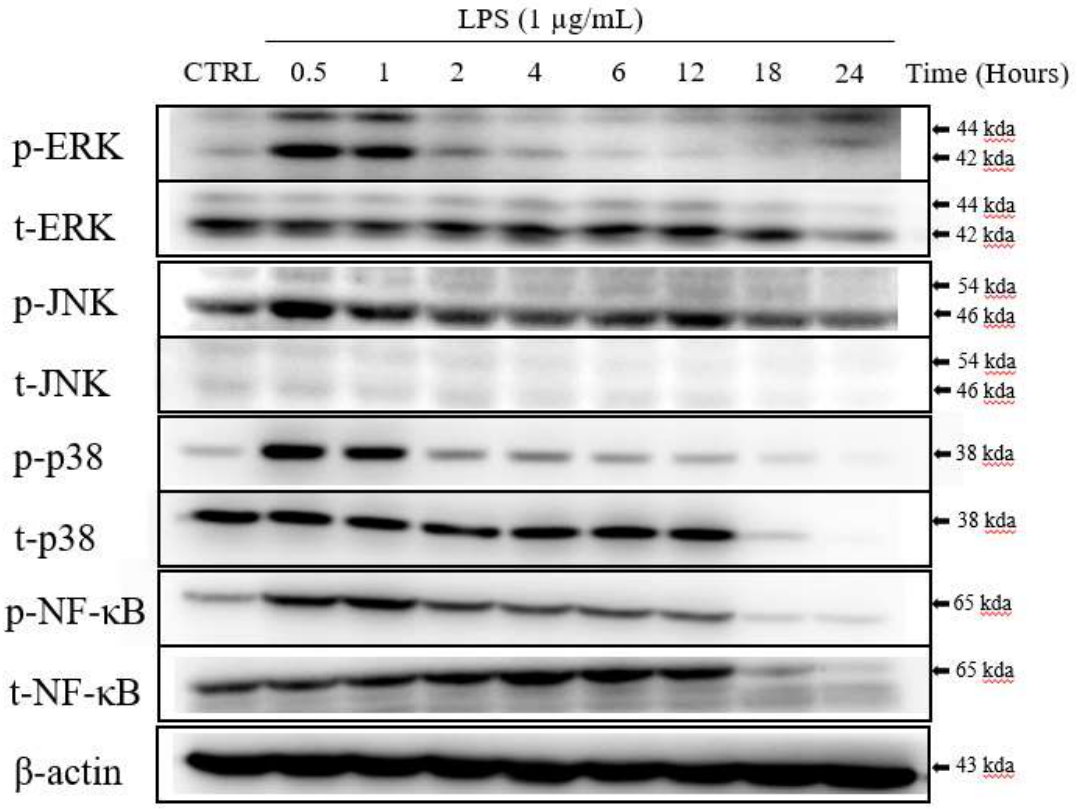


Figure S1. Time-course study of cell signaling changes in RAW 264.7 cells exposed to LPS.

The original Western blot bands from Figure S1 are presented in Figures S2 and S3. The membrane was sectioned at about 75 kDa and 35 kDa for p-JNK, JNK, and their β-actin, resulting in three distinct membranes. Membranes 1 and 3 were eliminated due to the absence of probed protein; thus, only membrane 2 was utilized for probing and examining the time-course of p-JNK, JNK, and their β-actin (Figure S2). For NF-κB, p38, and ERK, we conducted separate protein analyses and utilized the same membrane, which was segmented at around 100 kDa and above 48 kDa, resulting in three membrane fragments, the membrane 1 was excluded due to the absence of detecting protein. The membrane 2 was used to ascertain the temporal progression of p-NF-κB and NF-κB. Membrane 3 was utilized to assess p-p38 and p38 using probing, stripping, and reprobing. Subsequent to detection, the membrane was stripped and reprobed with p-ERK, ERK, and β-actin, respectively (Figure S3). For this time period, we conducted just one replication to identify the increases in these proteins and the optimization employed to detect the effects of SDSPs on these signaling proteins.

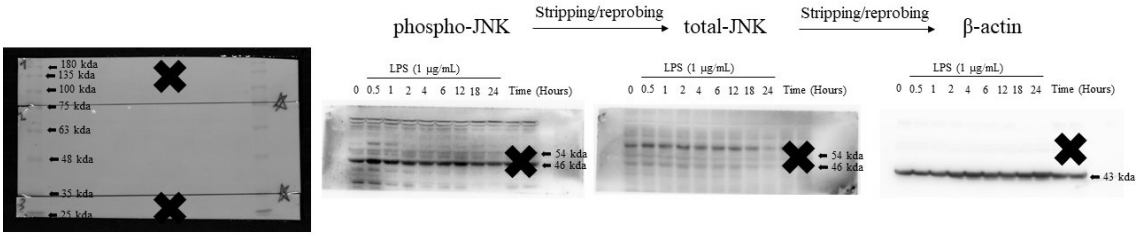


Figure S2. Workflow for the preparation and detection and original Western blot of phosphorylated and total time-course p-JNK, JNK, and β-actin from Figure S1.

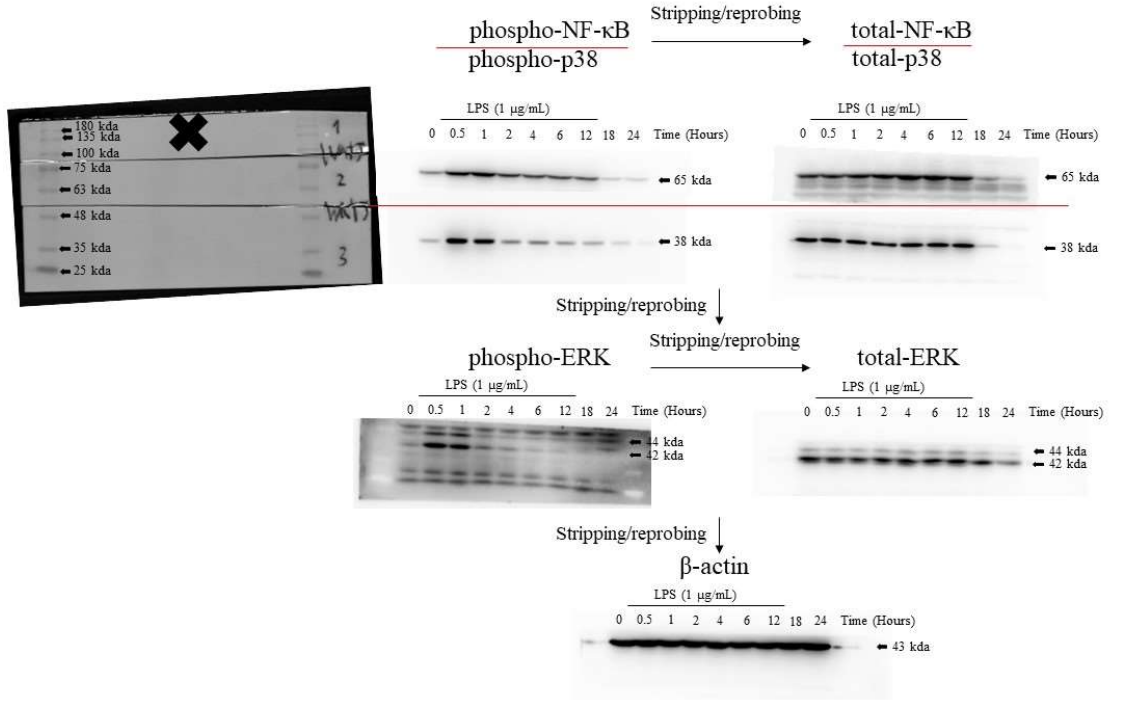


Figure S3. Workflow for the preparation and detection and original Western blot of phosphorylated and total time-course p-NF-κB, NF-κB, p-p38, p38, p-ERK, ERK, and β-actin from Figure S1

In FigureS5 and Figure 6, the membranes were cut at approximately below 63 kDa; the upper portion was used to probe p-NF-κB and NF-κB, while the lower portion was used for p-JNK, JNK, p-p38, p38, pERK, ERK, and β-actin. Proteins on both membranes (p-NF-κB/NF-κB on the upper, and p-JNK/JNK, p-p38/p38, p-ERK/ERK, and β-actin on the lower) were sequentially probed using stripping and reprobing, as descriped below.

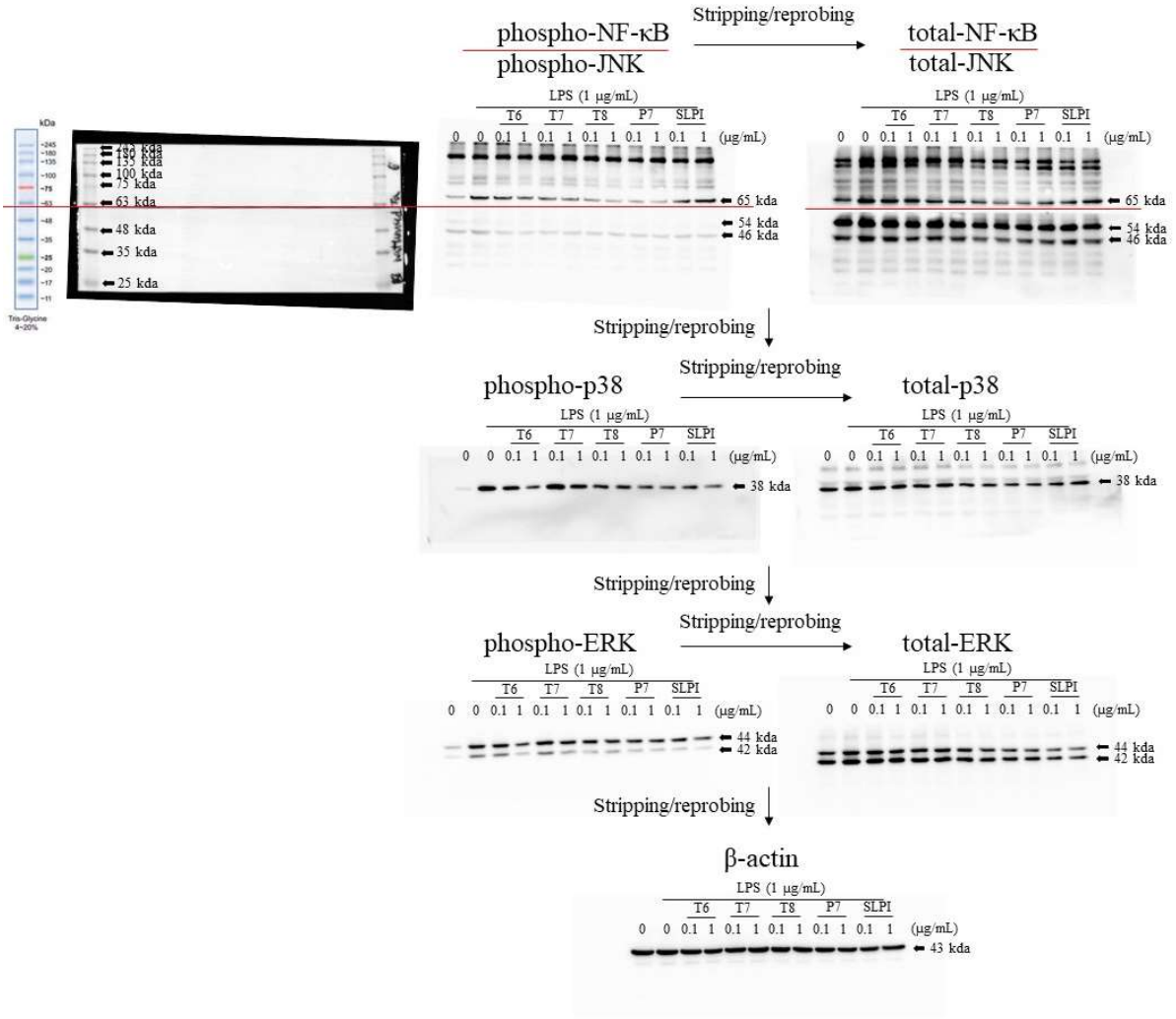


Figure S4. Workflow for the preparation and detection of phosphorylated and total MAPK/NF-κB proteins. This figure illustrates the sequential stripping and reprobing process used to determine p-NF-κB, total NF-κB, p-JNK, JNK, p-p38, p38, p-ERK, ERK, and β-actin for Figure S5 and Figure 6.

The representative Western blot bands shown in Figure 6 were obtained by cropping the raw blot in Figure S5 and arranging the panels for clarity. Data quantification was performed using three independent experimental replicates. The uncropped raw blots used for densitometric analysis are provided as Figure S5 (replicate N1), Figure S6 (replicate N2), and Figure S7 (replicate N3).

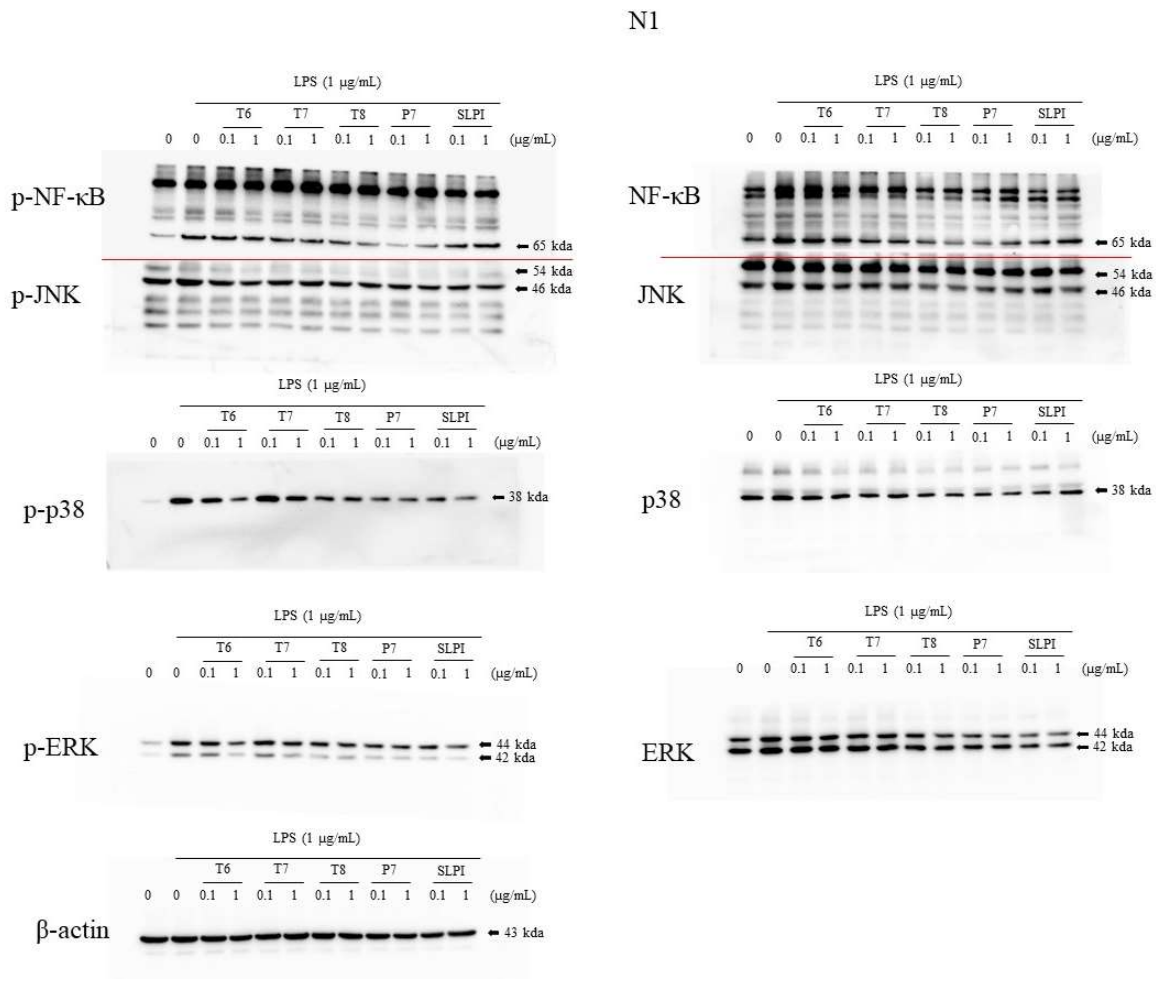


Figure S5. Raw Western blot image for representation and quantification from replicate N1 used in Figure 6.

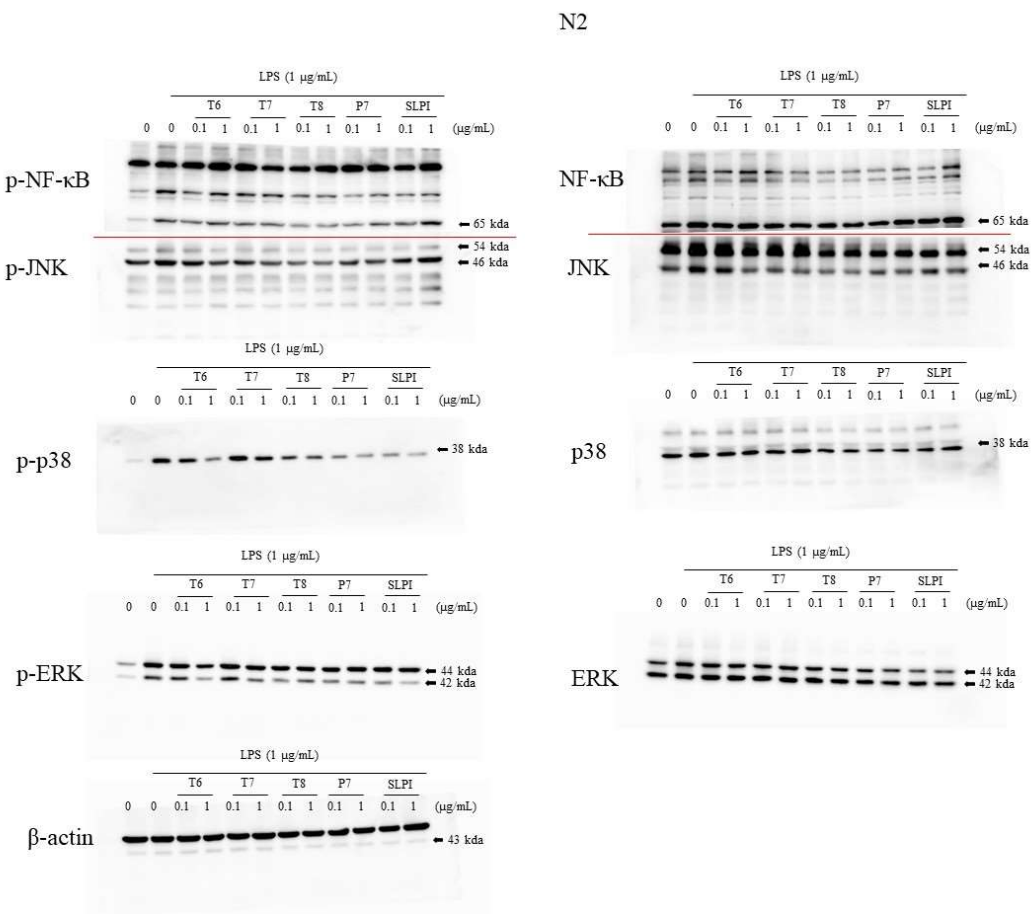


Figure S6. Raw Western blot image for quantification from replicate N2 used in Figure 6.

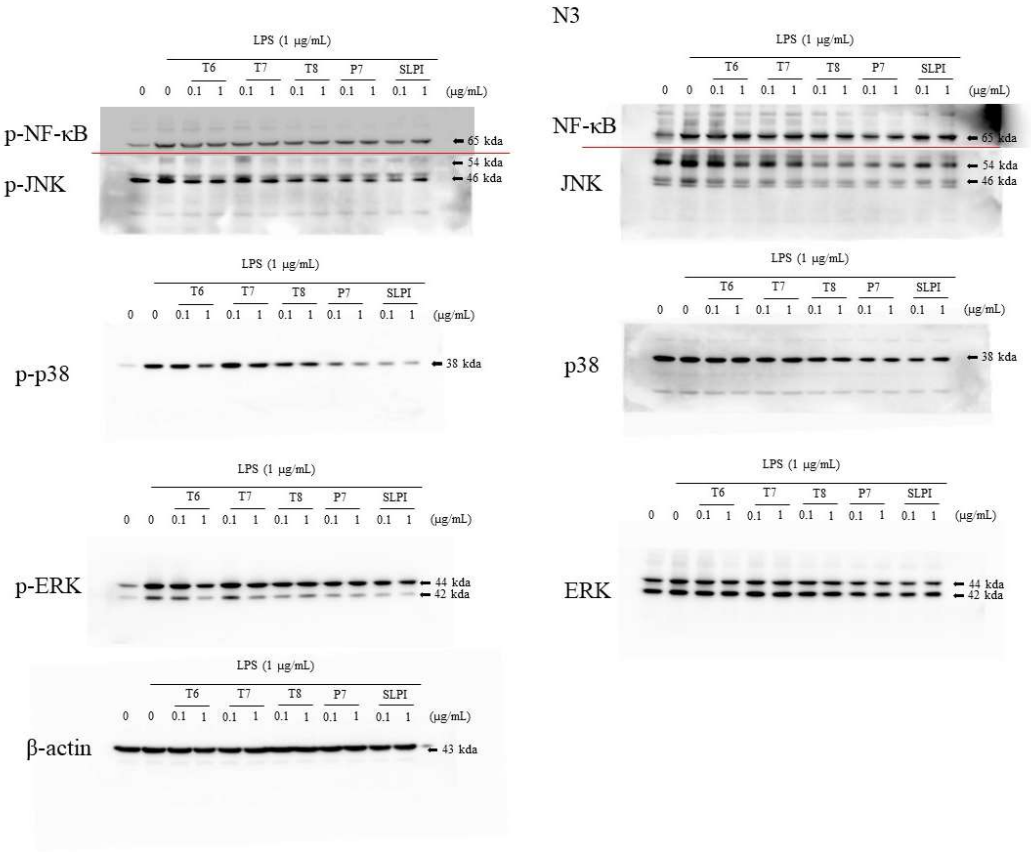


Figure S7. Raw Western blot image for quantification from replicate N3 used in Figure 6.

The original images were processed by the gel documentation imaging system, which automatically applied uniform adjustments (such as autofocus and background enhancement) to the entire image during capture. Subsequent to this stage, the images were cropped to encompass just the relevant bands and lanes, and organized aesthetically for figure presentation. The final layout and configuration were performed in Microsoft PowerPoint, where the photographs were only positioned and arranged with graphical components. No additional modifications—such as alterations to contrast, brightness, intensity, color, or other enhancements—were executed in Microsoft PowerPoint; the software was exclusively utilized of cropping Fiand layout functions. The step-by-step adjustment process is shown below.

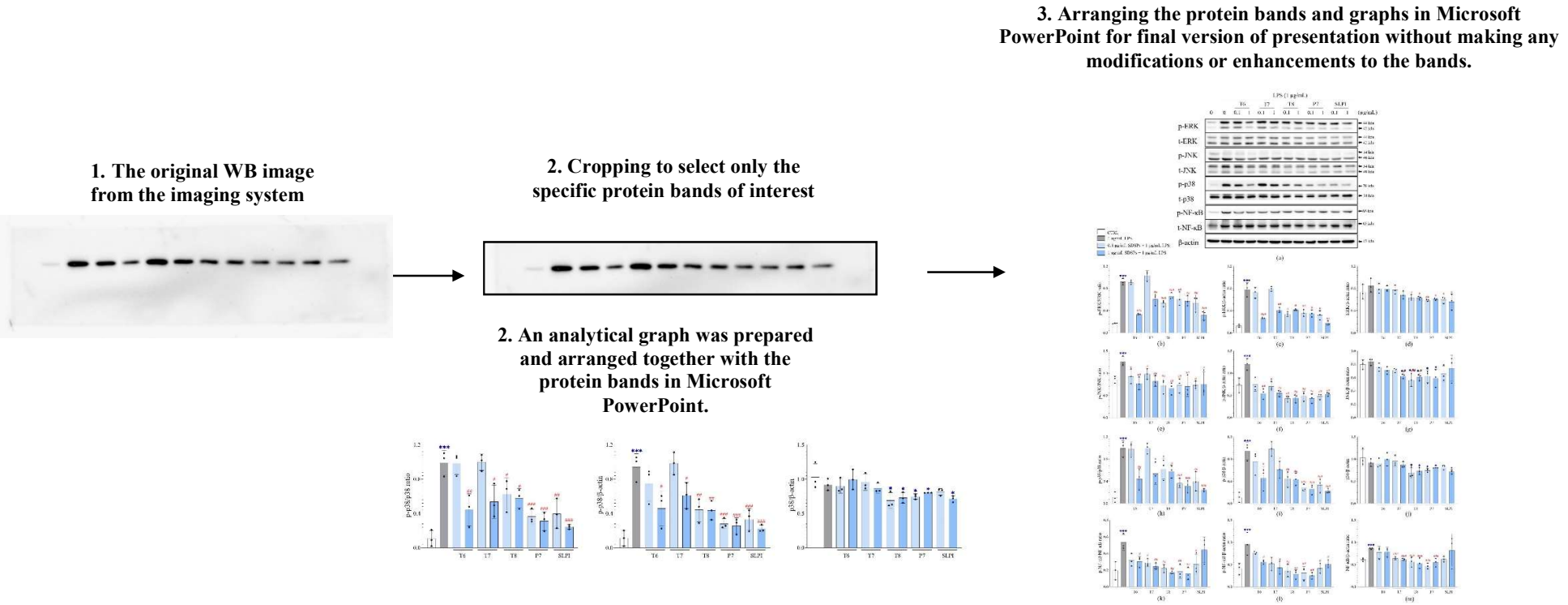


Figure S8. Image-processing workflow for western blot preparation used in this study