

Supplemental Materials for
p38MAPK Guards the Integrity of Endosomal Compartments through
Regulating Necrotic Death

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Figs. S1 to S44
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Other Supplemental Materials for this manuscript include the following:

Movies S1 to S2
Tables S1 to S2

Legends to Supplemental Movies S1 and S2.

Movie S1

Treatment of BMDMs from MLKL-deficient mice with TM alone triggers no necrotic cell death, as determined by the addition to cell media PI (red). Time-lapsed images of treated cells were obtained with IncuCyte imaging system.

Movie S2

Treatment of BMDMs from MLKL-deficient mice with TM and NPs triggers necrotic-type cell death, as determined by the addition to cell media PI (red). Time-lapsed images of treated cells were obtained with IncuCyte imaging system.

Legends to Supplemental Tables S1 and S2.

Table S1.

The list of 500 genes that most strongly contributed to the divergence on a principal component PC-1 axis between transcriptomes of macrophages treated with tunicamycin alone compared to macrophages treated with a combination of tunicamycin and NP. Please see Methods section for more information on data processing pipeline.

Table S2.

The list of biological processes over-represented in macrophages treated with a combination of tunicamycin and NP compared to macrophages treated with tunicamycin alone based on Gene ontology (GO) biological process over-representation analysis. Please see Methods section for more information on data processing pipeline.

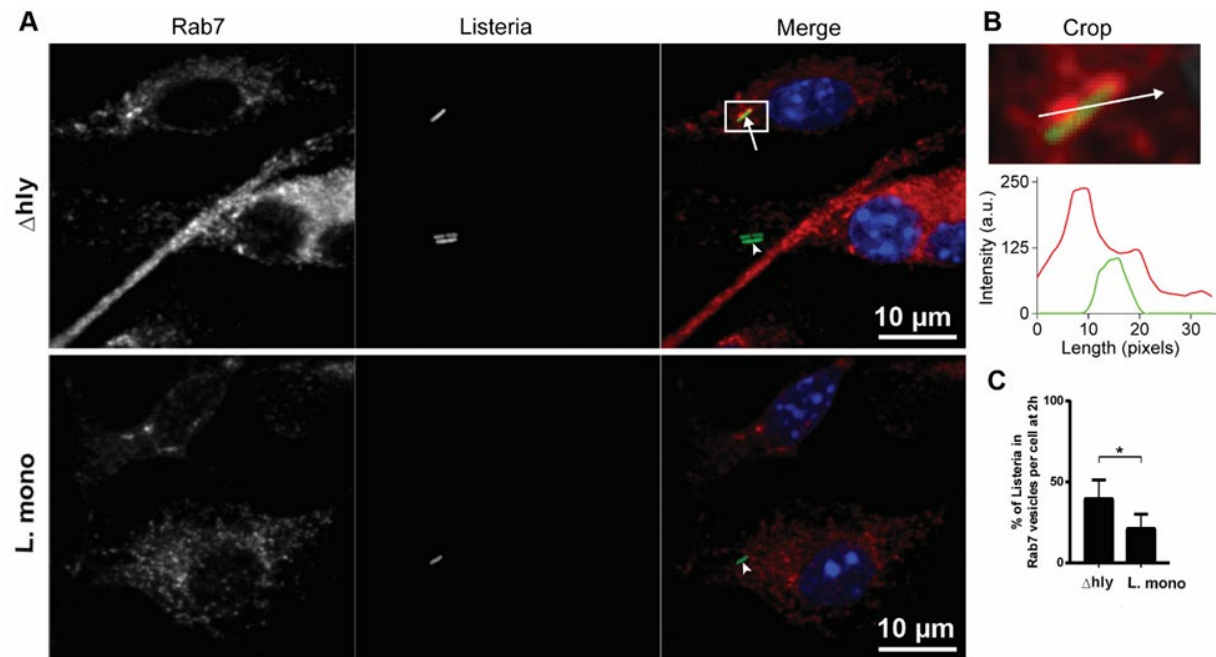


Fig. S1.

Co-localization analysis of mutant and wild-type *L. monocytogenes* or isogenic Δhly strain with Rab7-containing endosomal vesicles. (A) Internalization of mutant (top) or wild-type (bottom) CFDA-labeled *L. monocytogenes* (green) in bone marrow derived macrophages at 2 h. Cells were stained for Rab7 (red) and nuclei (blue). Extended focus images are shown. Scale bar represents 10 μ m. Arrow indicates listeria engulfed in Rab7-containing vesicle, while arrowheads indicate listeria not contained in Rab7-containing vesicles. (B) Enlarged cropped image from the white boxed region from part (A). Intensity profile shows co-localization along the white arrow. (C) Quantification of Mander's overlap coefficient between the bacteria cells and Rab7. Error bars represent 95% confidence intervals. N = 30 cells per group. Asterisks represent P < .005 by Tukey's Multiple Comparisons test.

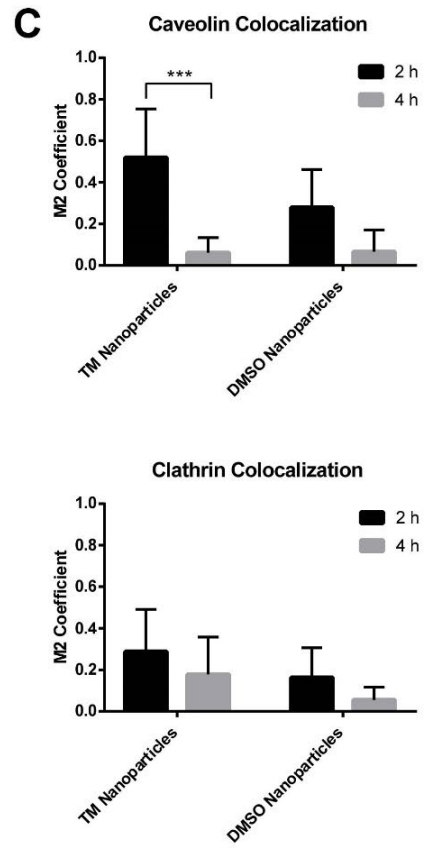
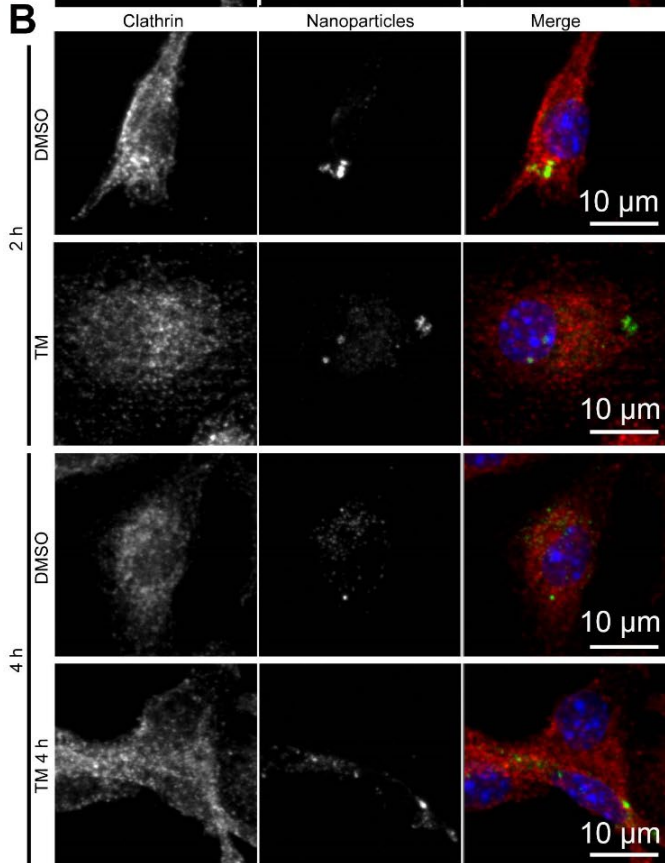
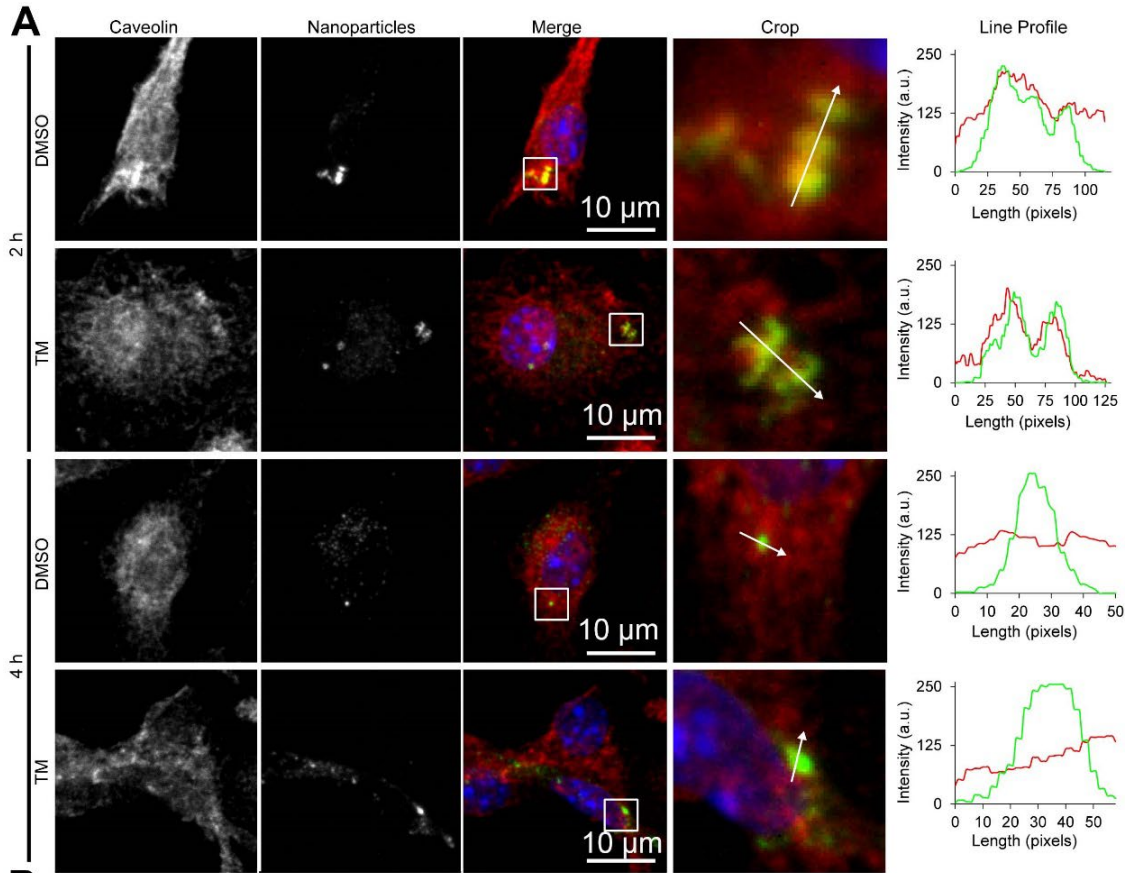


Fig. S2.

Colocalization analysis of nanoparticles with clathrin or caveolin-1. **(A)** Internalization of nanoparticles (green) in bone marrow derived macrophages at 2 h or 4 h treated with either DMSO or tunicamycin (TM). Cells were stained for nuclei (blue) and caveolin-1 (red). Extended focus images are shown. Scale bar represents 10 μm . Cropped images show enlargements from the white boxed images with intensity line profiles drawn along the white arrows. **(B)** Internalization of nanoparticles (green) at 2 h or 4 h treated with either DMSO or tunicamycin (TM). Cells were stained for nuclei (blue) and clathrin light chain (red). **(C)** Quantification of Mander's overlap coefficient between the nanoparticles and caveolin-1 or clathrin light chain as indicated under each treatment condition. Error bars represent 95% confidence intervals. N = 20 cells per group. Asterisks represent $P < .005$ by Tukey's Multiple Comparisons test.

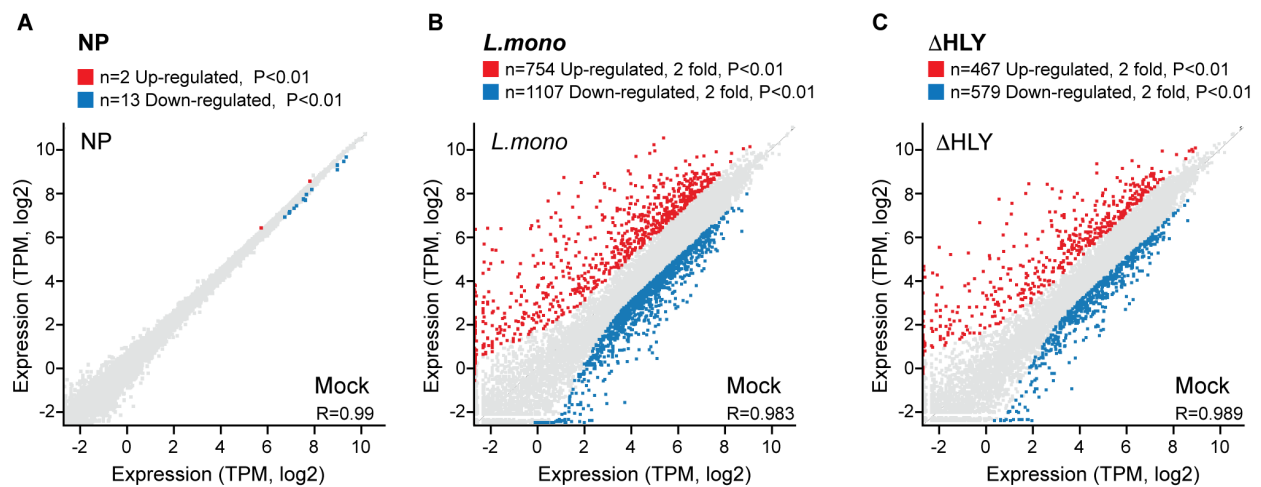


Fig. S3.

Nanoparticle treatment has lesser impact on BMDM transcriptional landscape than *L.monocytogenes* or Δ hly strain. Scatter plots of gene expression in BMDMs treated with (A) nanoparticles, (B) *L.mono*, (C) Δ HLY. The X-axis shows normalized gene expression in the Mock samples, the Y-axis shows the normalized gene expression in the treated samples. The red dots represent the genes that are significantly ($P<0.01$, DeSeq2) upregulated compared to the Mock sample, the blue dots represent the genes that are significantly ($P<0.01$, DeSeq2) downregulated compared to the Mock sample (A), the red and blue dots for (B) and (C) have more strict criteria and additionally have more than two-fold difference in expression compared to Mock. TPM – transcripts per million.

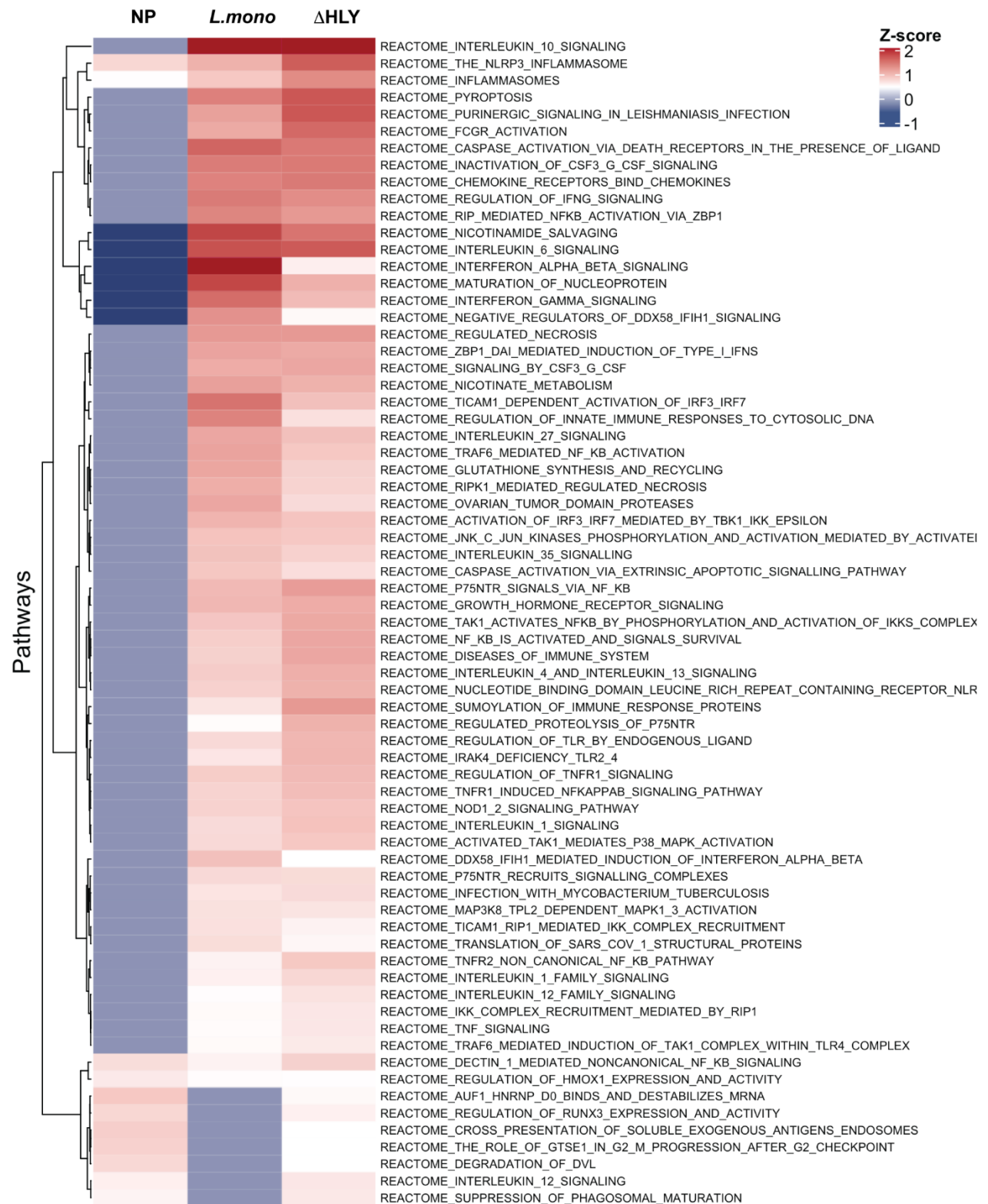


Fig. S4.

Macrophage treatment with bacteria activates broader range of innate immune pathways than nanoparticles. The heatmap of the GSEA z-scores of the REACTOME gene sets that are enriched after BMDM treatment with NP, *L.mono*, or Δ HLY, compared to buffer-treated control. The z-scores in the heatmap were clustered without supervision. The light blue color represents an imputed value of z-scores equal zero and P-values >0.05 , compared to Mock buffer treated controls.

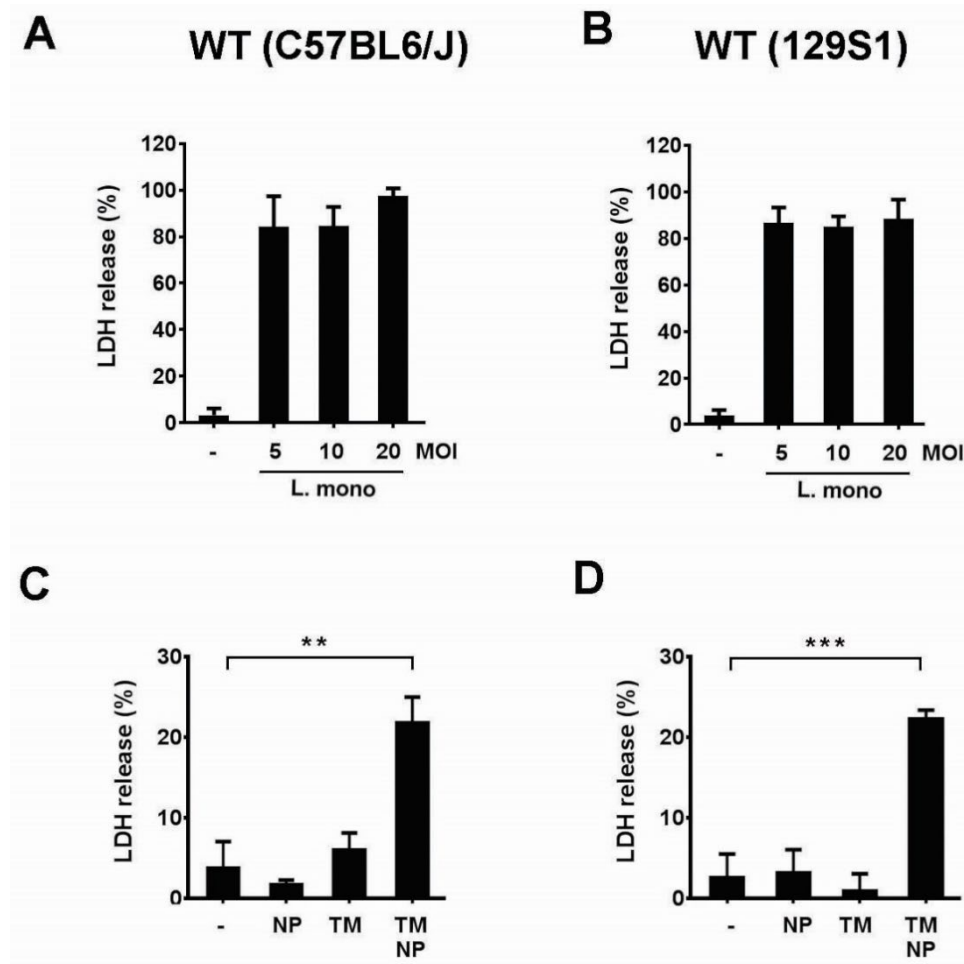


Fig. S5.

BMDM harvested from WT mice of C57BL6/J and 129S1 genetic backgrounds undergo necrotic death after exposure to *L.monocytogenes* (L.mono) or TM+NP treatment. (A) and (B) - BMDM cells from C57BL6/J or 129S1 mice were infected with *L.monocytogenes* at different MOI and cell death was assayed by LDH activity in the media, N=3. (C) and (D) BMDM cells from C57BL6/J or 129S1 mice were treated with TM and NP and cell death was assayed by LDH activity in the media, N=3. ** P=0.0025; * P=0.0004.**

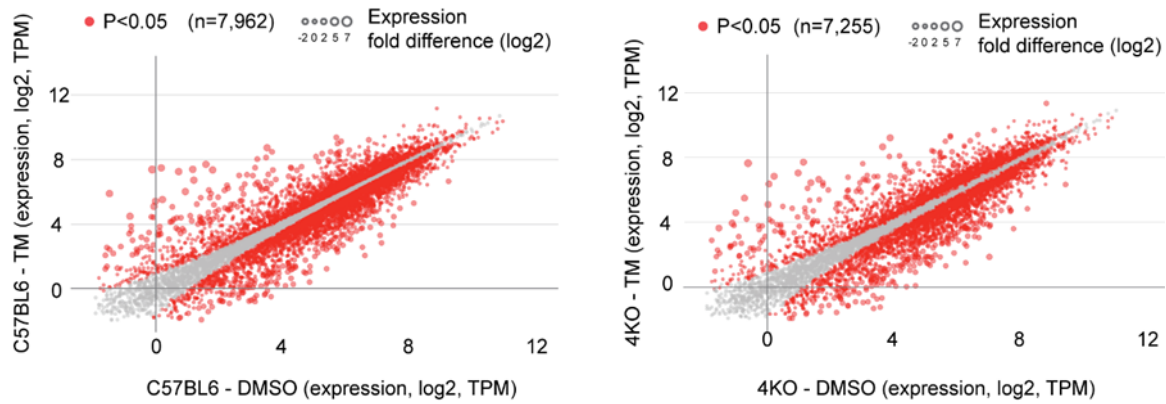


Fig. S6.

Treatment of cells with TM alone triggers global changes in gene expression in both WT and 4KO BMDM. Scatter plots of the expression values of genes in WT and 4KO BMDM treated with DMSO or TM. Genes differentially-expressed between these two treatment conditions with $p < 0.05$, based on DESeq2 analysis, are shown in red. $N=4$ for each condition.

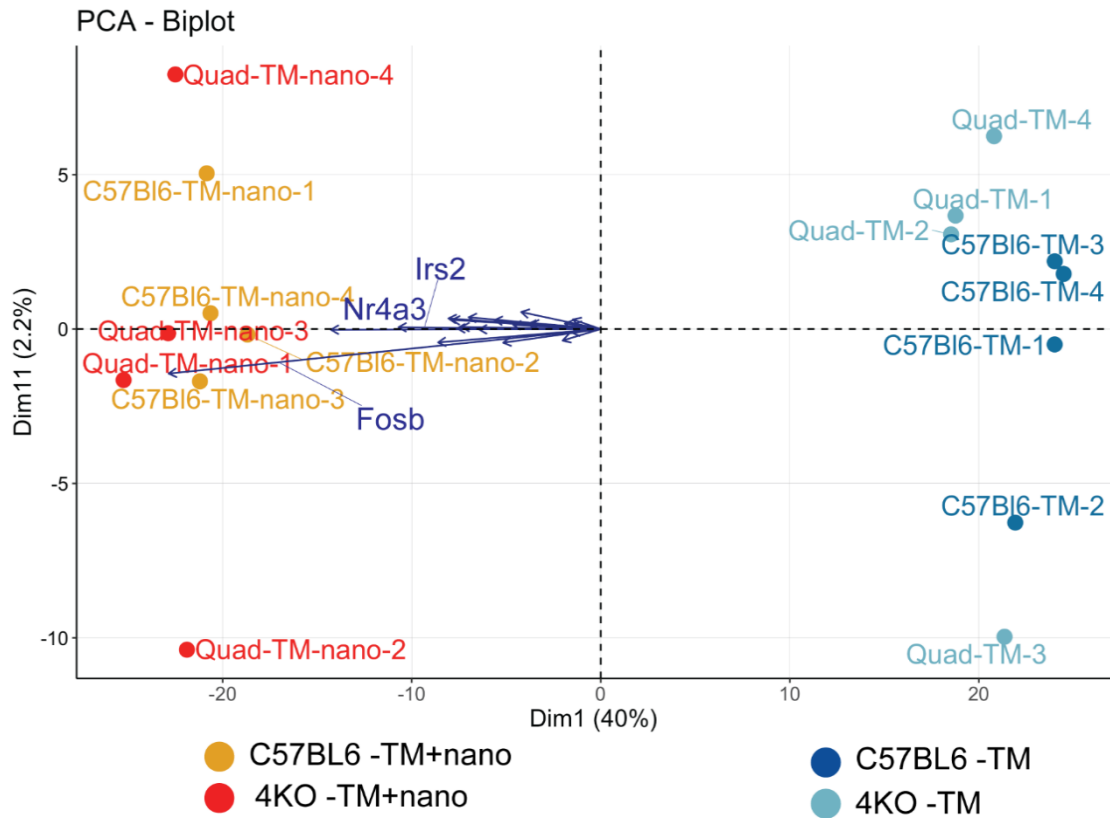


Fig. S7.

The distribution of transcriptomic signatures of BMDMs from WT and 4KO mice treated with TM or TM+NP on a principal component dimension plot. The imputation of as a single variable into the raw global transcriptomics data files for each sample allows for isolation of top genes contributing to the divergence of transcriptional phenotypes between TM+NP and TM-treated cells, and leads to defined sample distribution between these two treatment conditions along PCA Dimension-1 axis. The top 500 genes contributing to separation of samples on PC-1 axis are shown in Table S1. The 22 genes most contributing to sample separation along dimension 1 axis are shown with blue arrows. N=4 for each experimental condition.

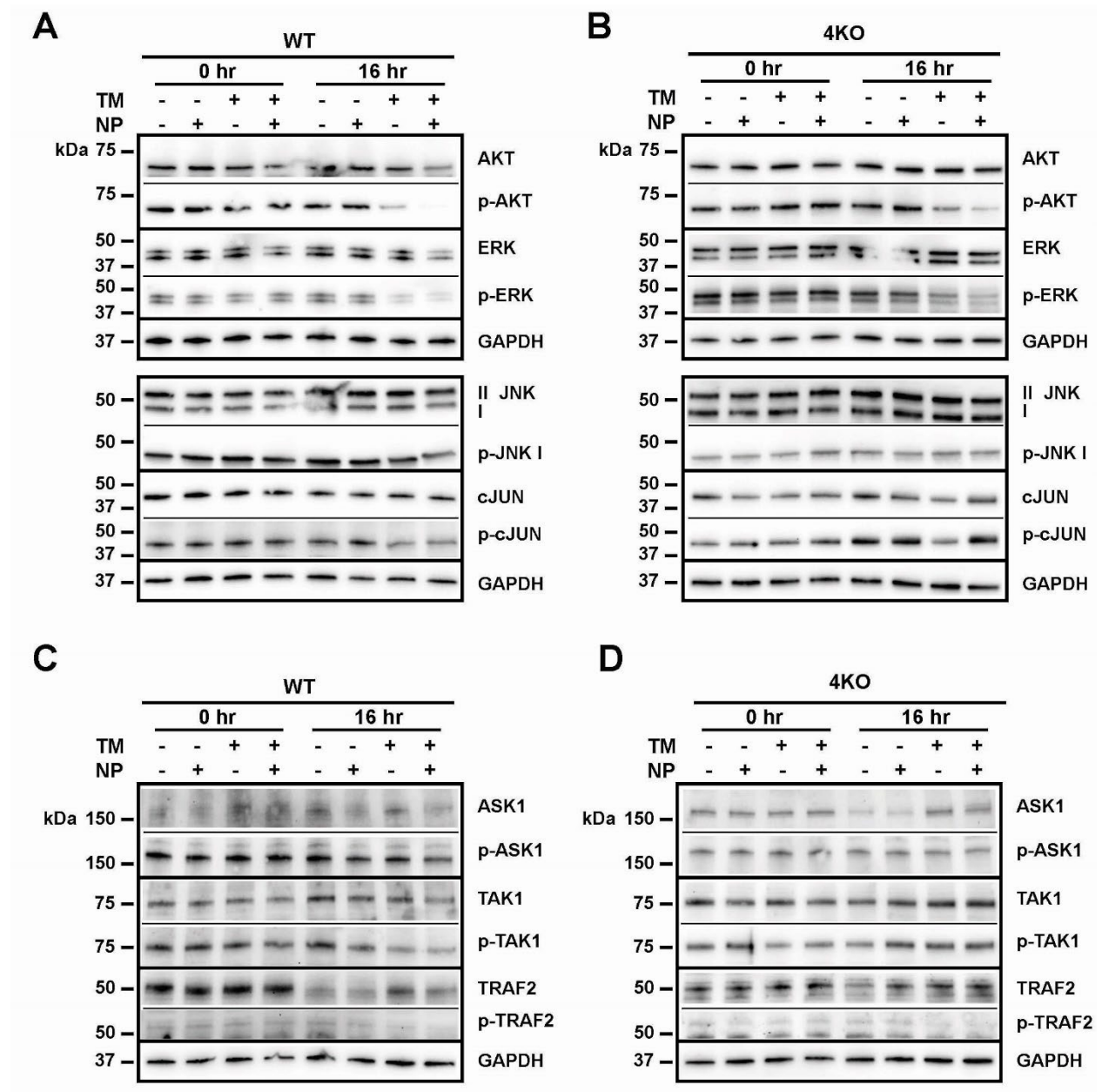


Fig. S8. Western blot analysis of activation of MAPK cascade signaling components at indicated times in WT (A and C) and 4KO (B and D) BMDMs after their treatment with DMSO (-), TM, NP, or TM and NP combination. N= 3.

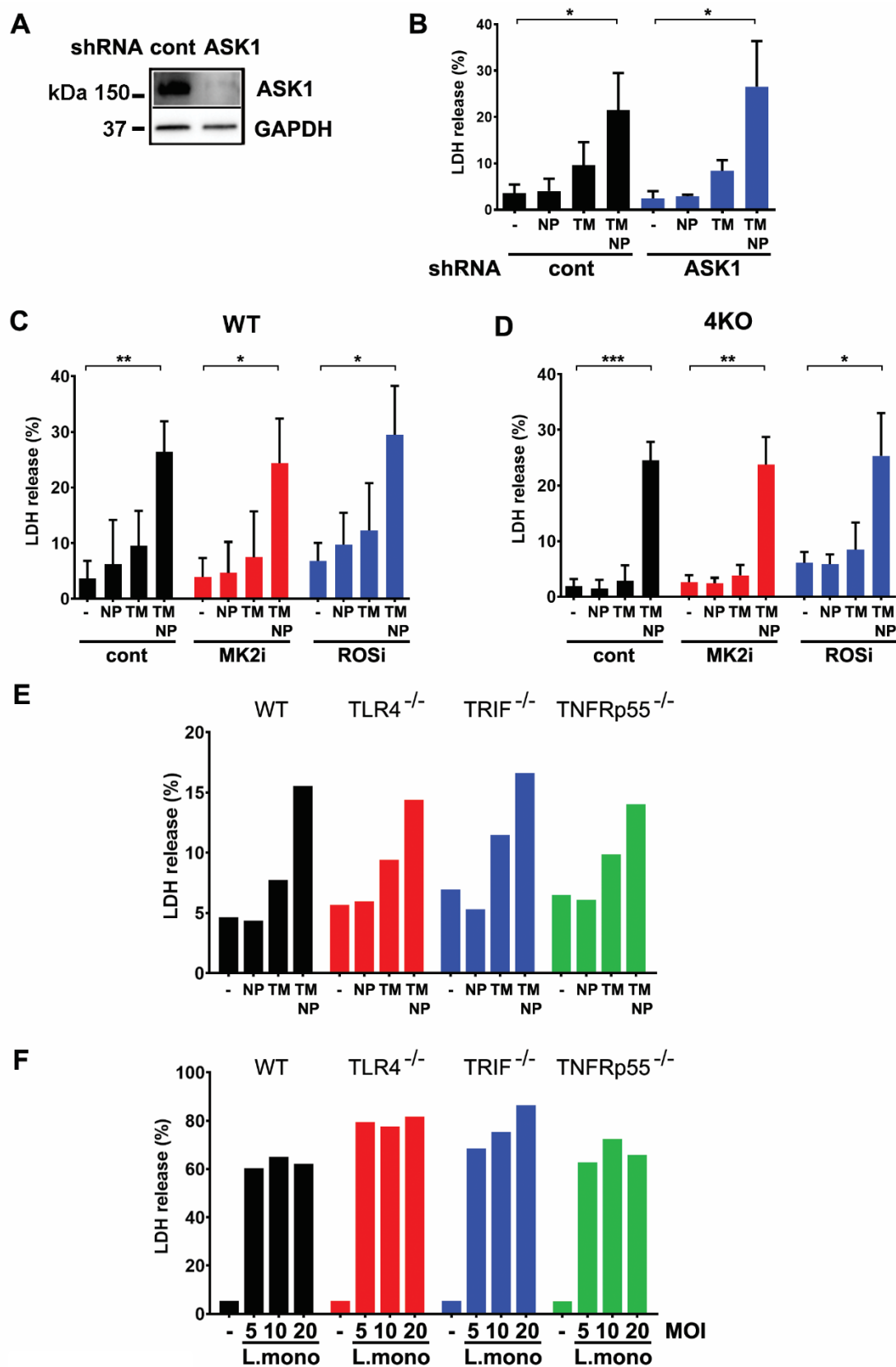


Fig. S9.

(A) Expression of ASK1 was knocked down in BMDM from 4KO mice using lentiviral shRNA. ASK1 protein levels were determined by Western Blotting analysis. **(B)** Necrotic cell death was assessed by LDH activity release in the culture media, N=3. P= 0.0195 (control), P= 0.0137 (ASK1 shRNA). **(C)** Effects of chemicals targeting MK2 activity (MK2i) and ROS generation (ROSi) were assessed in BMDM from WT mice. LDH activity release in the culture media from different conditions was determined. N=3, P= 0.0034(cont); P= 0.0151(MK2i); P= 0.0137 (ROSi). **(D)** Same chemicals as in **(C)** were tested in 4KO BMDM, N=3, P= 0.0004 (cont); P= 0.0020 (MK2i); P= 0.0135 (ROSi). **(E)** BMDM cells from WT mice and mouse strains knockout for TLR4, TRIF, and TNFRp55 were treated with TM and NP and cell death was assayed by LDH activity release in the media. **(F)** BMDM cells from WT mice and mouse strains knockout for TLR4, TRIF, and TNFRp55 were infected with *L. monocytogenes* at different MOI and cell death was assayed by LDH activity in the media.

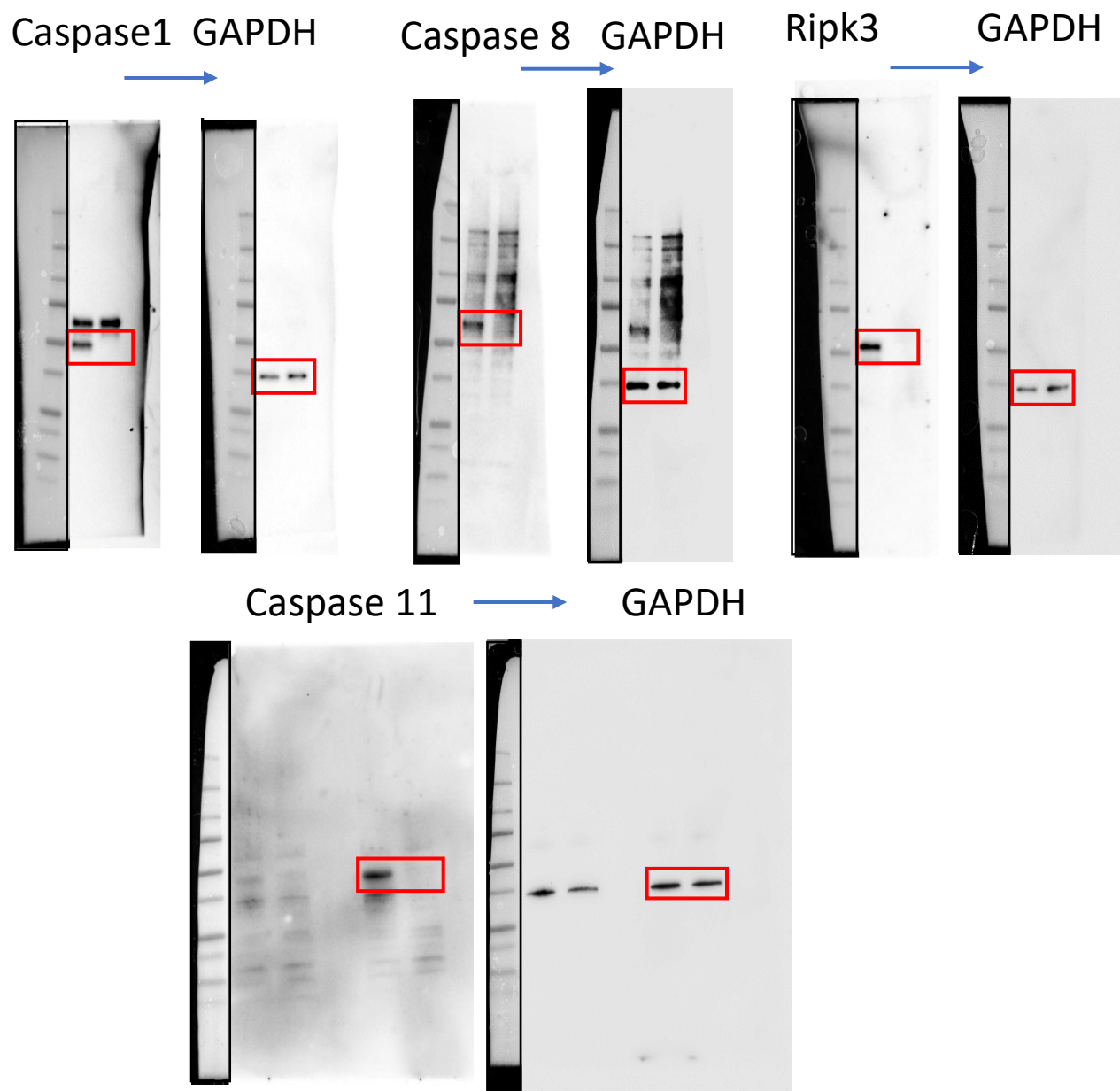


Fig. S10. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 1C in a cropped form. The areas of the membranes that were cropped and presented in Fig. 1C for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 1C. Re-probing sequence for the same membrane is indicated with blue arrow.

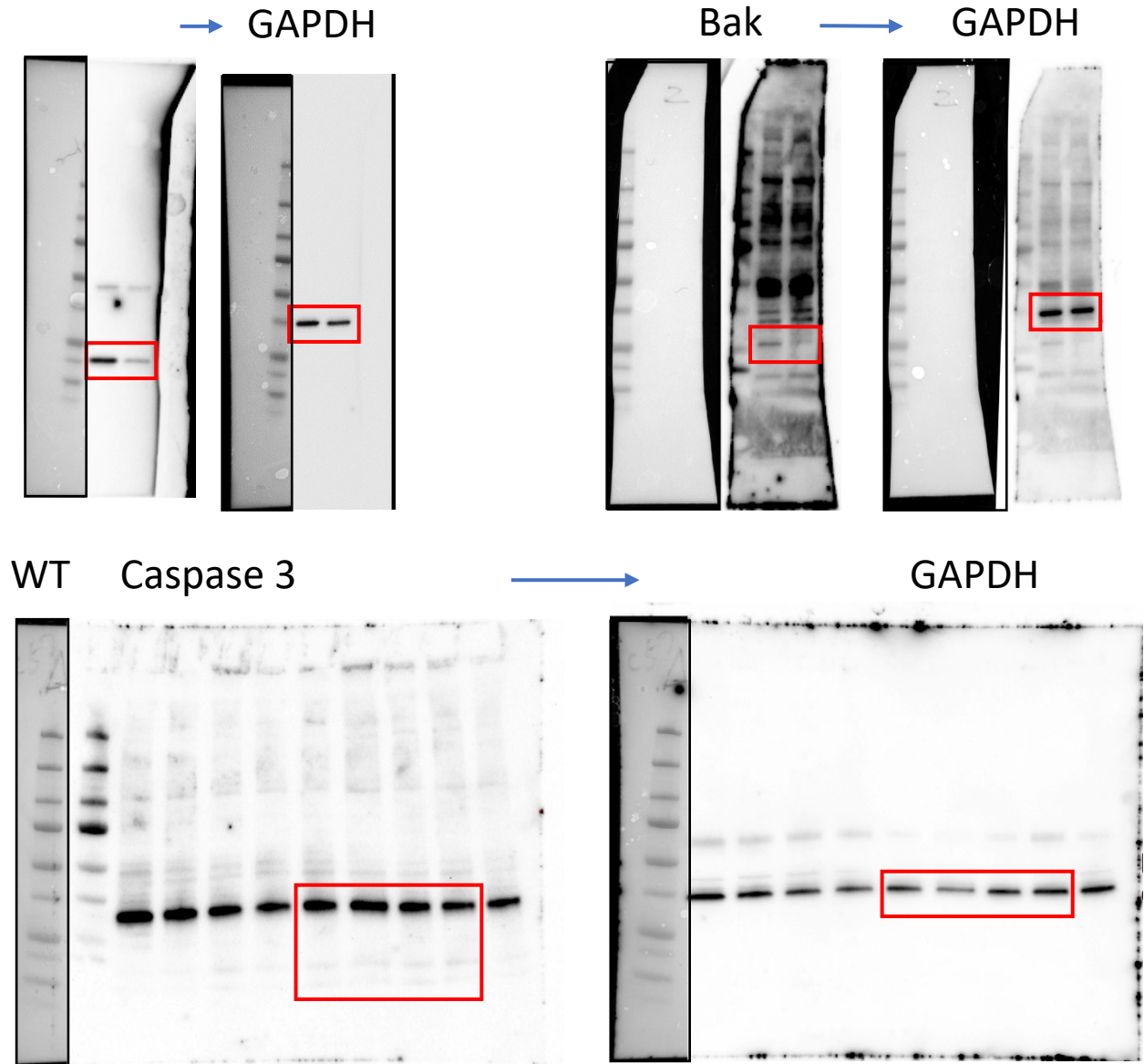


Fig. S11. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 2A and Fig. 2C in a cropped form. The areas of the membranes that were cropped and presented in Fig. 2A and Fig. 2C for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 2A and Fig. 2C. Re-probing sequence for the same membrane is indicated with blue arrow.

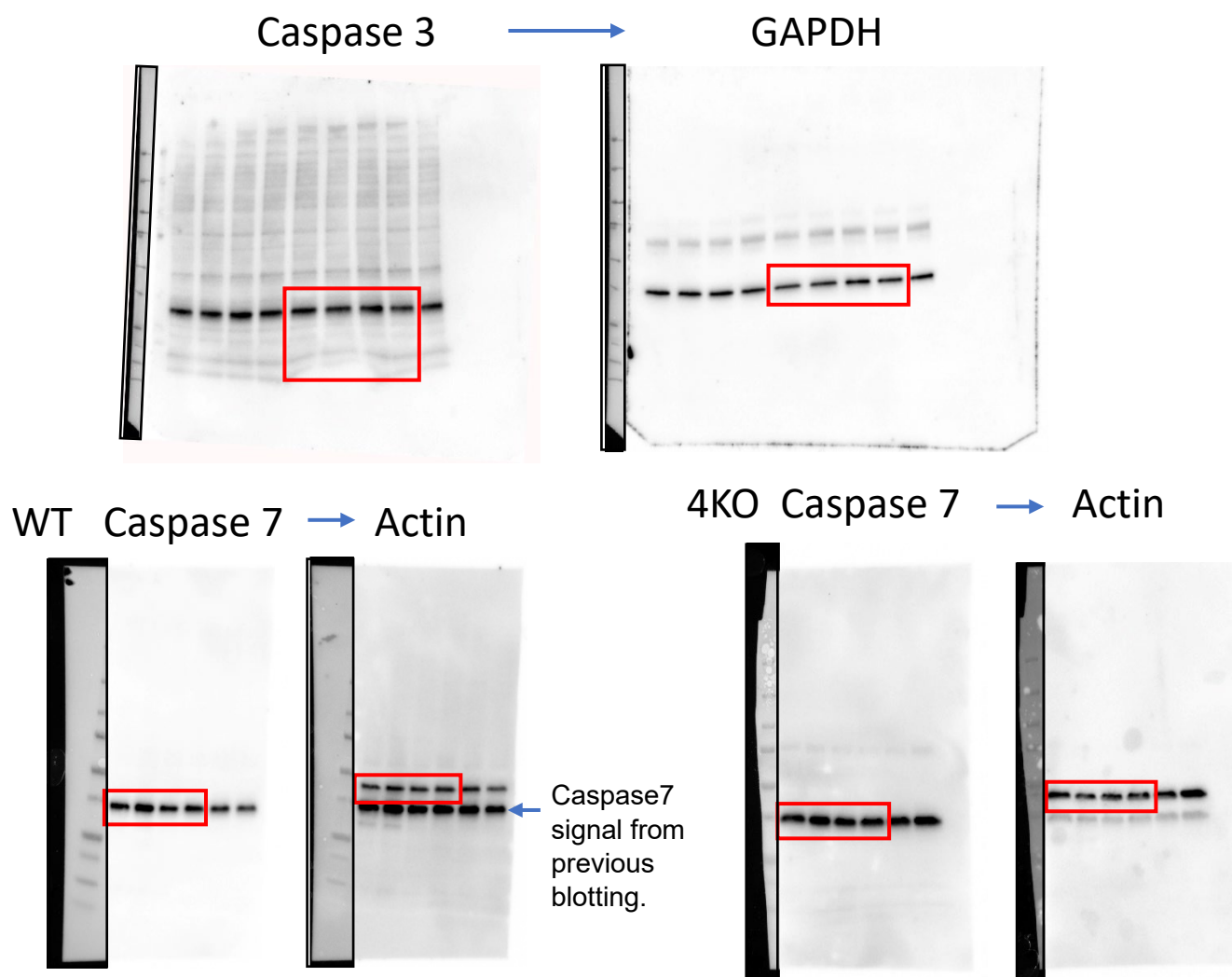


Fig. S12. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 2C and Fig. 2D in a cropped form. The areas of the membranes that were cropped and presented in Fig. 2C and Fig. 2D for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 2C and Fig. 2D. Re-probing sequence for the same membrane is indicated with blue arrow.

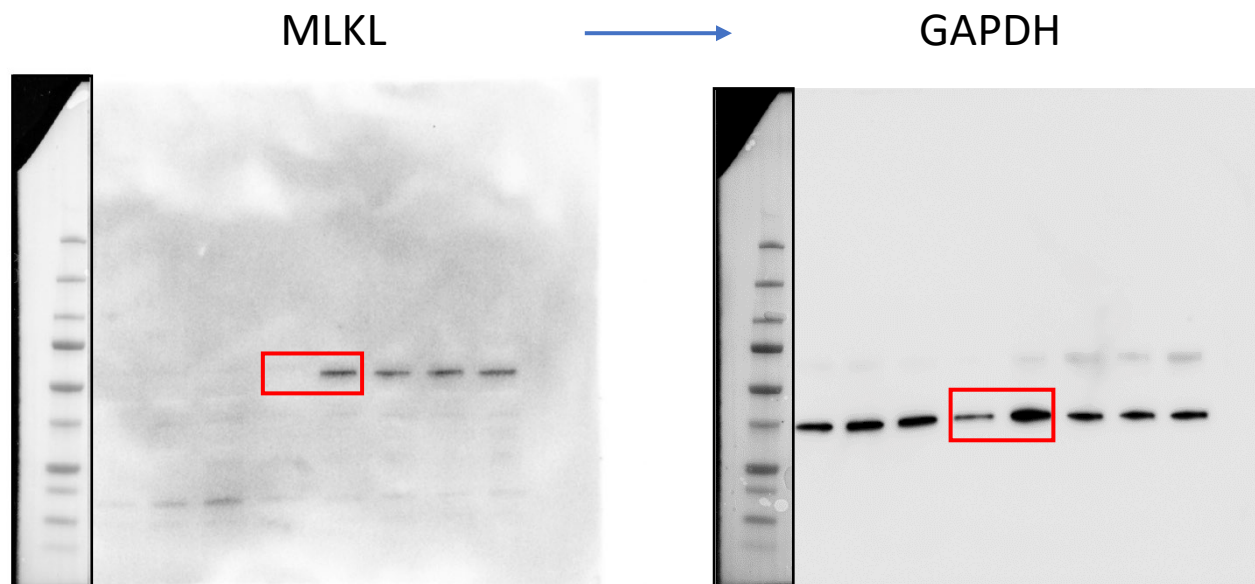


Fig. S13. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 2G in a cropped form. The areas of the membranes that were cropped and presented in Fig. 2G for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 2G. Re-probing sequence for the same membrane is indicated with blue arrow.

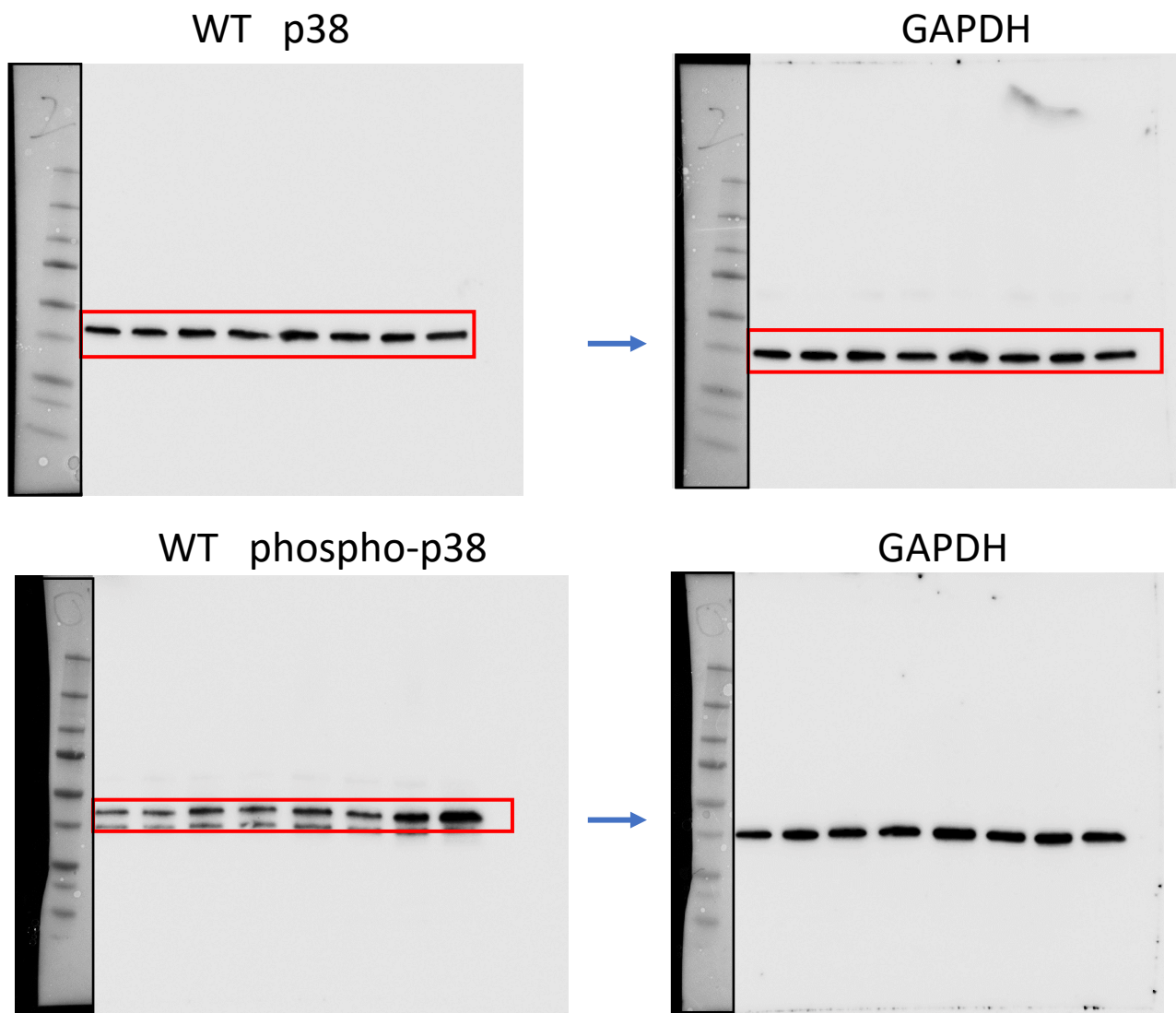


Fig. S14. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 4A in a cropped form. The areas of the membranes that were cropped and presented in Fig. 4A for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 4A. Re-probing sequence for the same membrane is indicated with blue arrow.

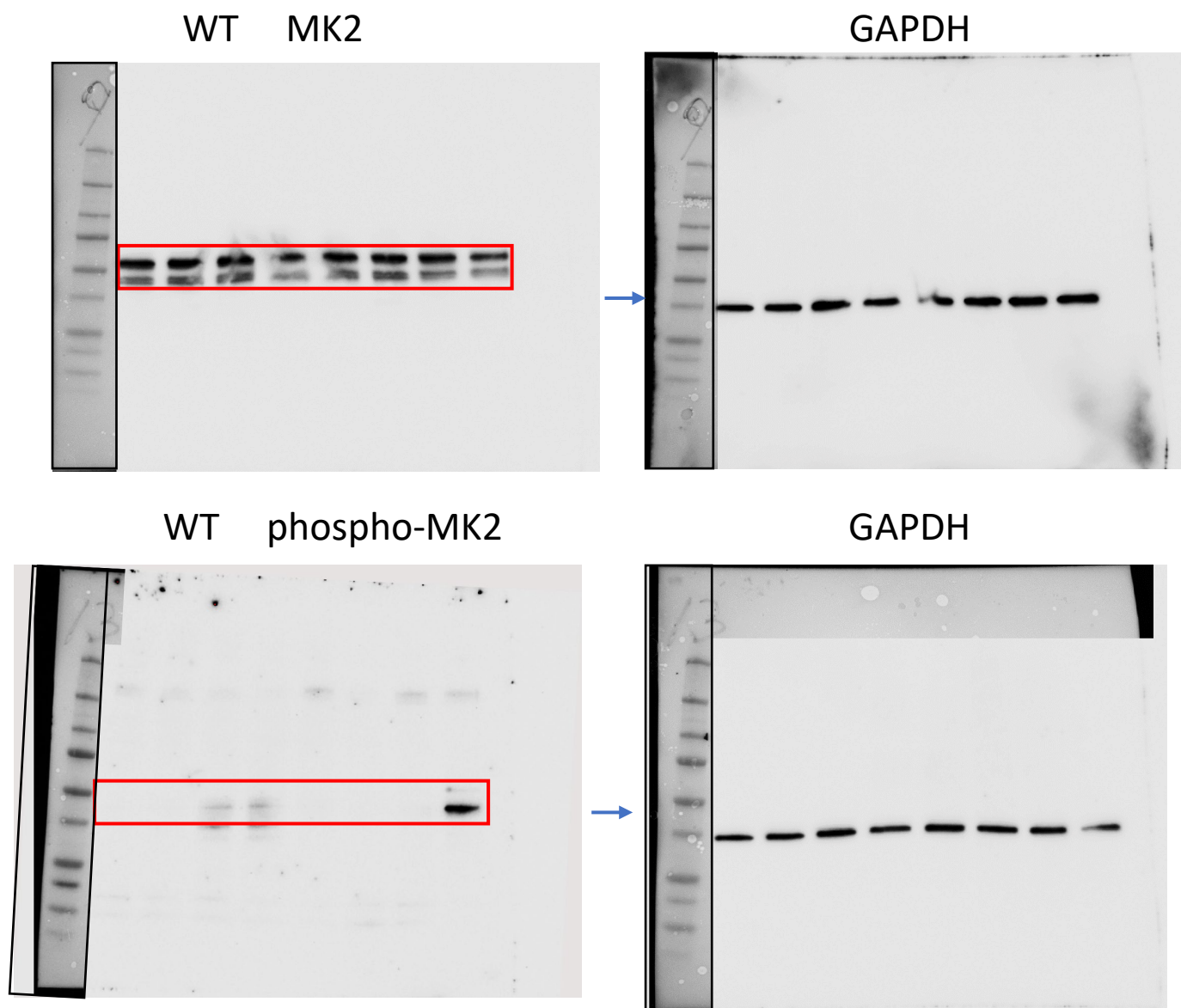


Fig. S15. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 4A in a cropped form. The areas of the membranes that were cropped and presented in Fig. 4A for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 4A. Re-probing sequence for the same membrane is indicated with blue arrow.

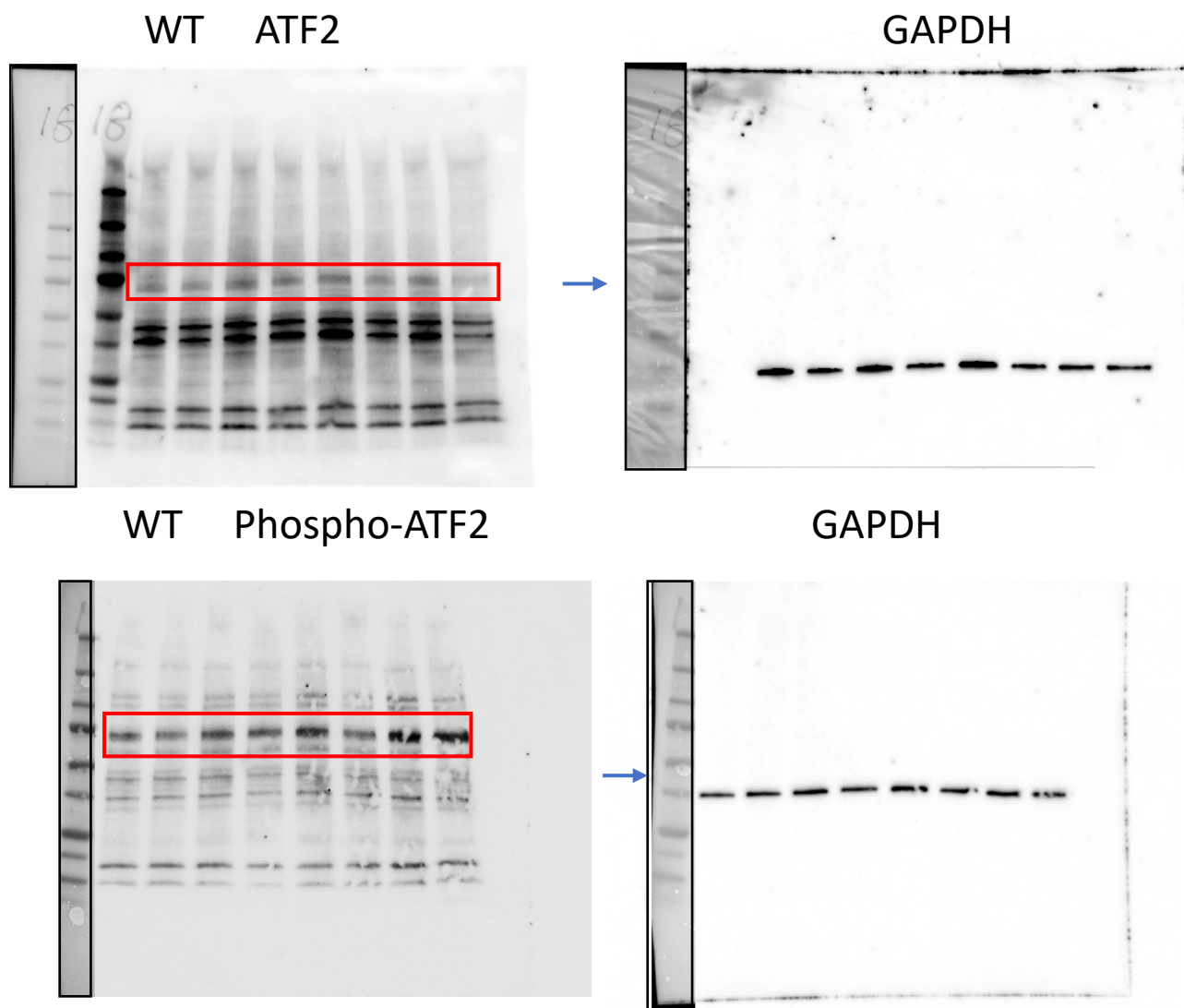


Fig. S16. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 4A in a cropped form. The areas of the membranes that were cropped and presented in Fig. 4A for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 4A. Re-probing sequence for the same membrane is indicated with blue arrow.

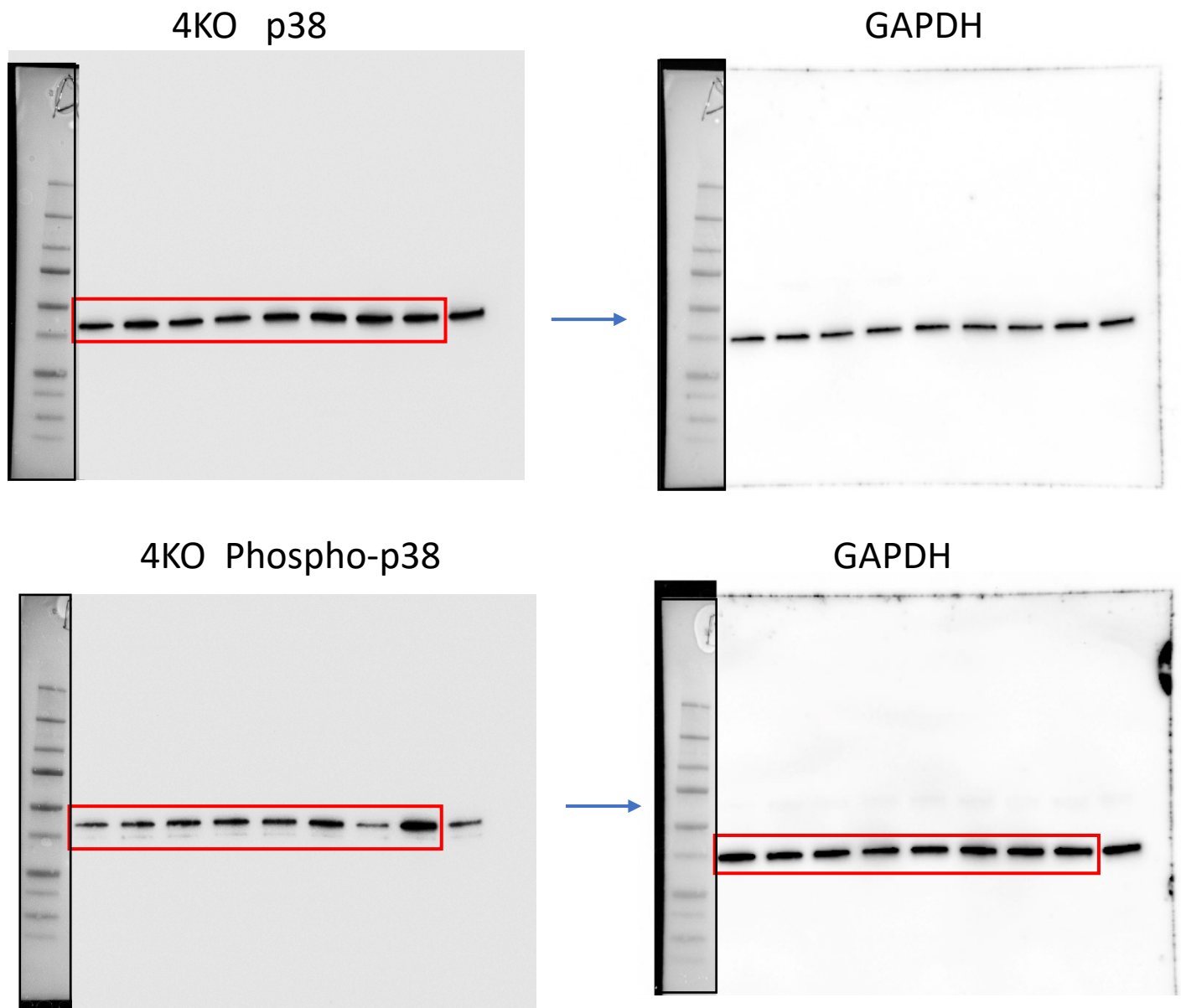


Fig. S17. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 4A in a cropped form. The areas of the membranes that were cropped and presented in Fig. 4A for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 4A. Re-probing sequence for the same membrane is indicated with blue arrow.

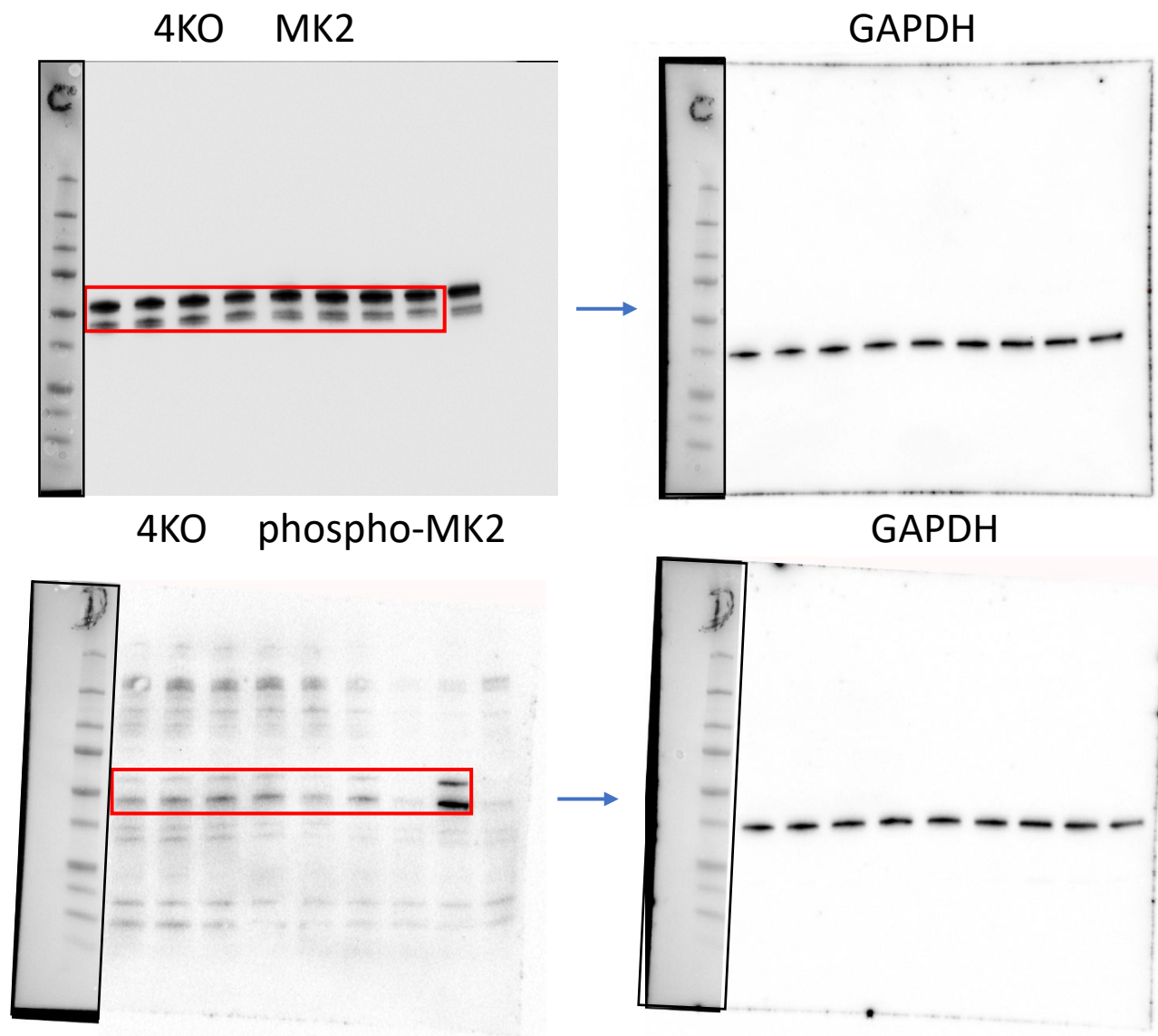


Fig. S18. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 4A in a cropped form. The areas of the membranes that were cropped and presented in Fig. 4A for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 4A. Re-probing sequence for the same membrane is indicated with blue arrow.

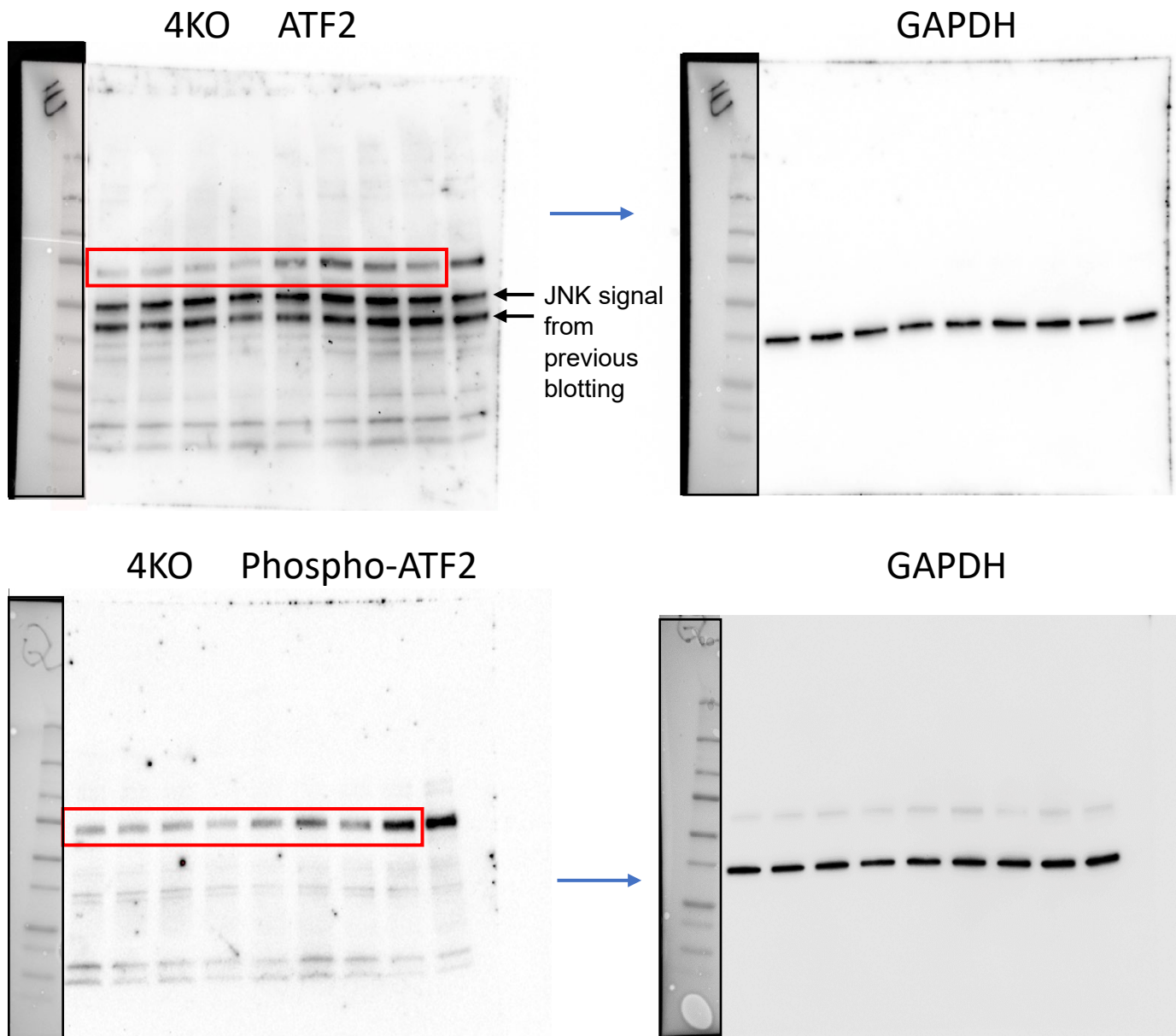


Fig. S19. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 4A in a cropped form. The areas of the membranes that were cropped and presented in Fig. 4A for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 4A. Re-probing sequence for the same membrane is indicated with blue arrow.

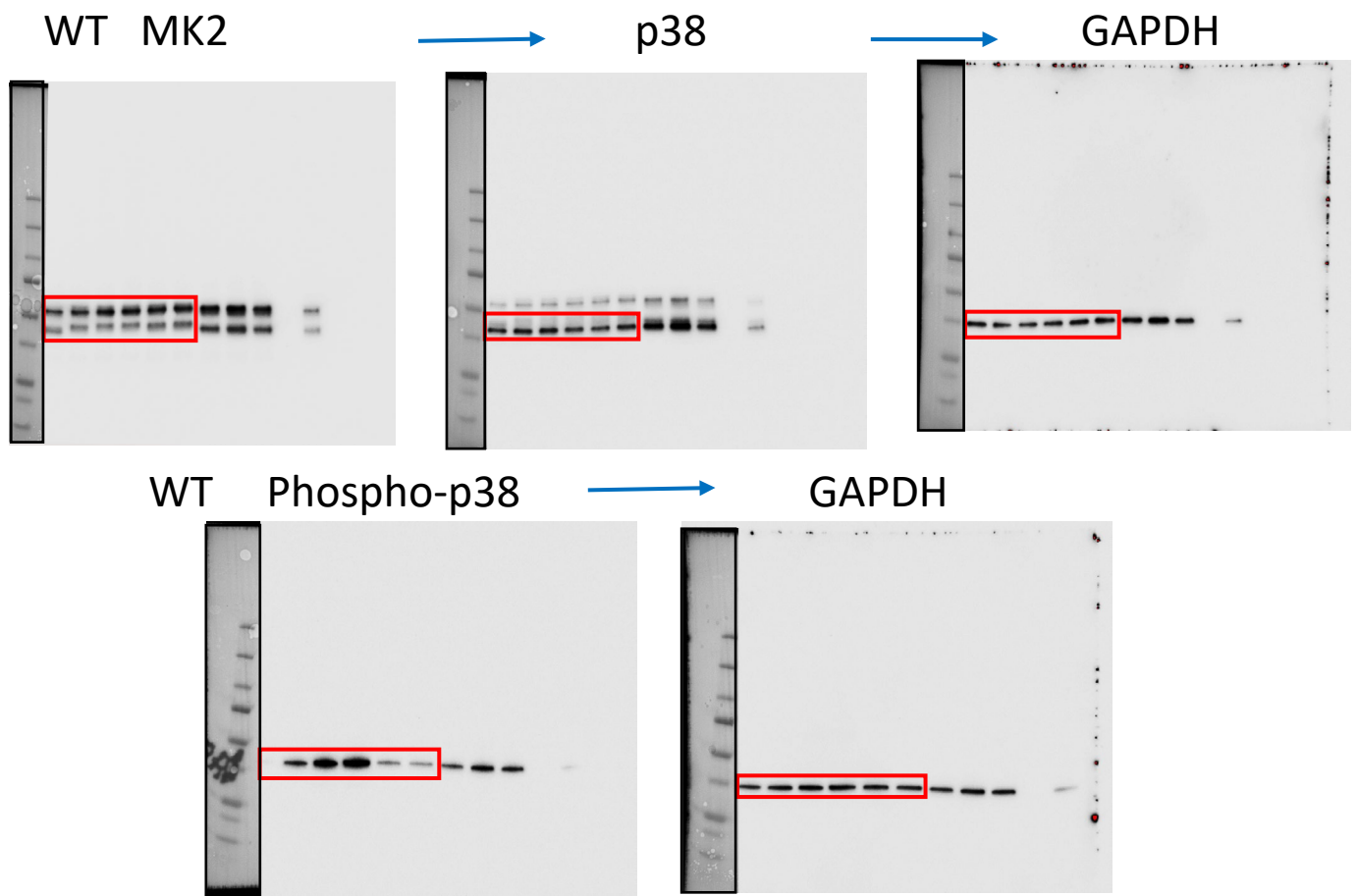


Fig. S20. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 4B in a cropped form. The areas of the membranes that were cropped and presented in Fig. 4B for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 4B. Re-probing sequence for the same membrane is indicated with blue arrow.

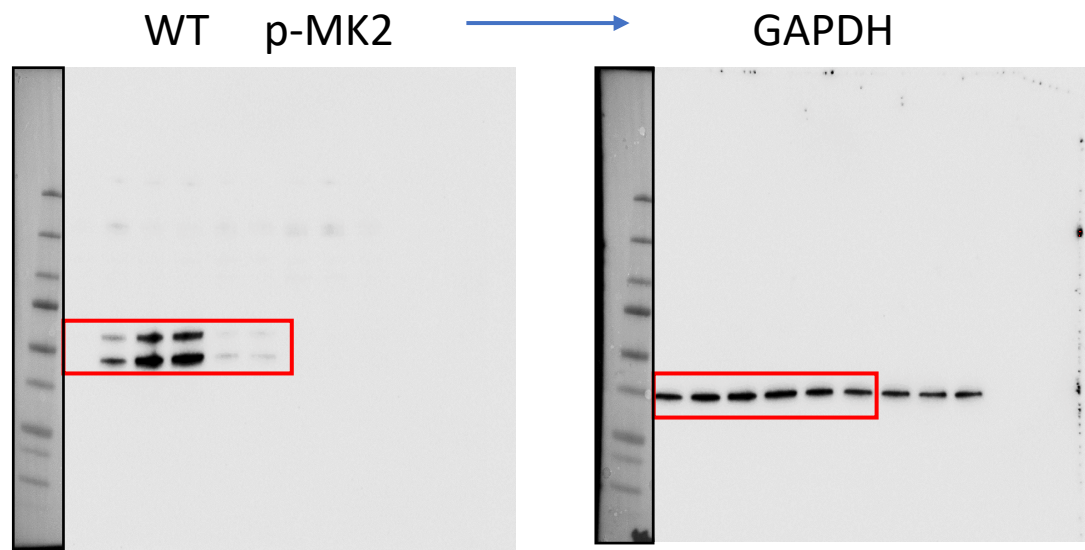


Fig. S21. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 4B in a cropped form. The areas of the membranes that were cropped and presented in Fig. 4B for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 4B. Re-probing sequence for the same membrane is indicated with blue arrow.

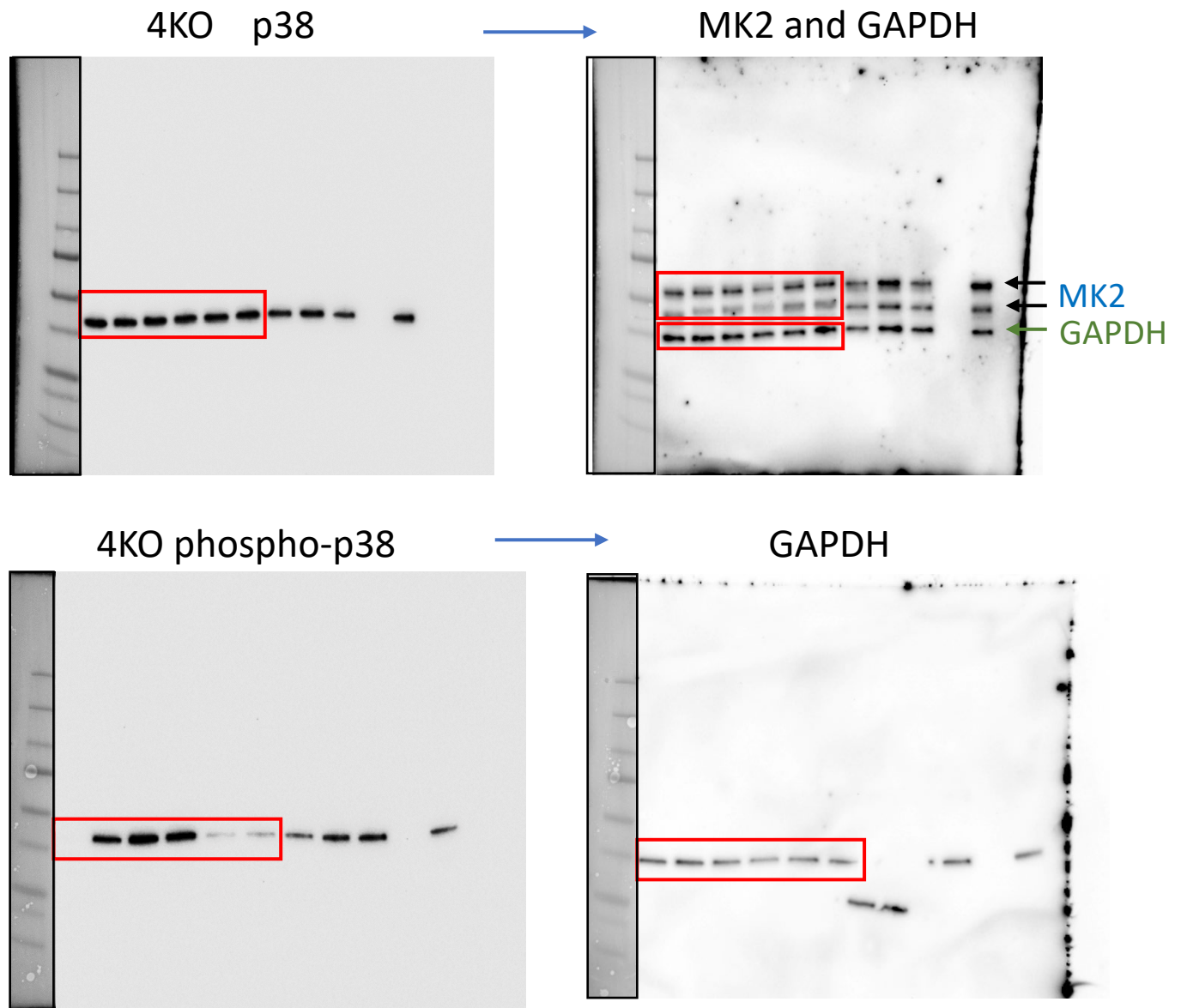


Fig. S22. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 4B in a cropped form. The areas of the membranes that were cropped and presented in Fig. 4B for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 4B. Re-probing sequence for the same membrane is indicated with blue arrow.

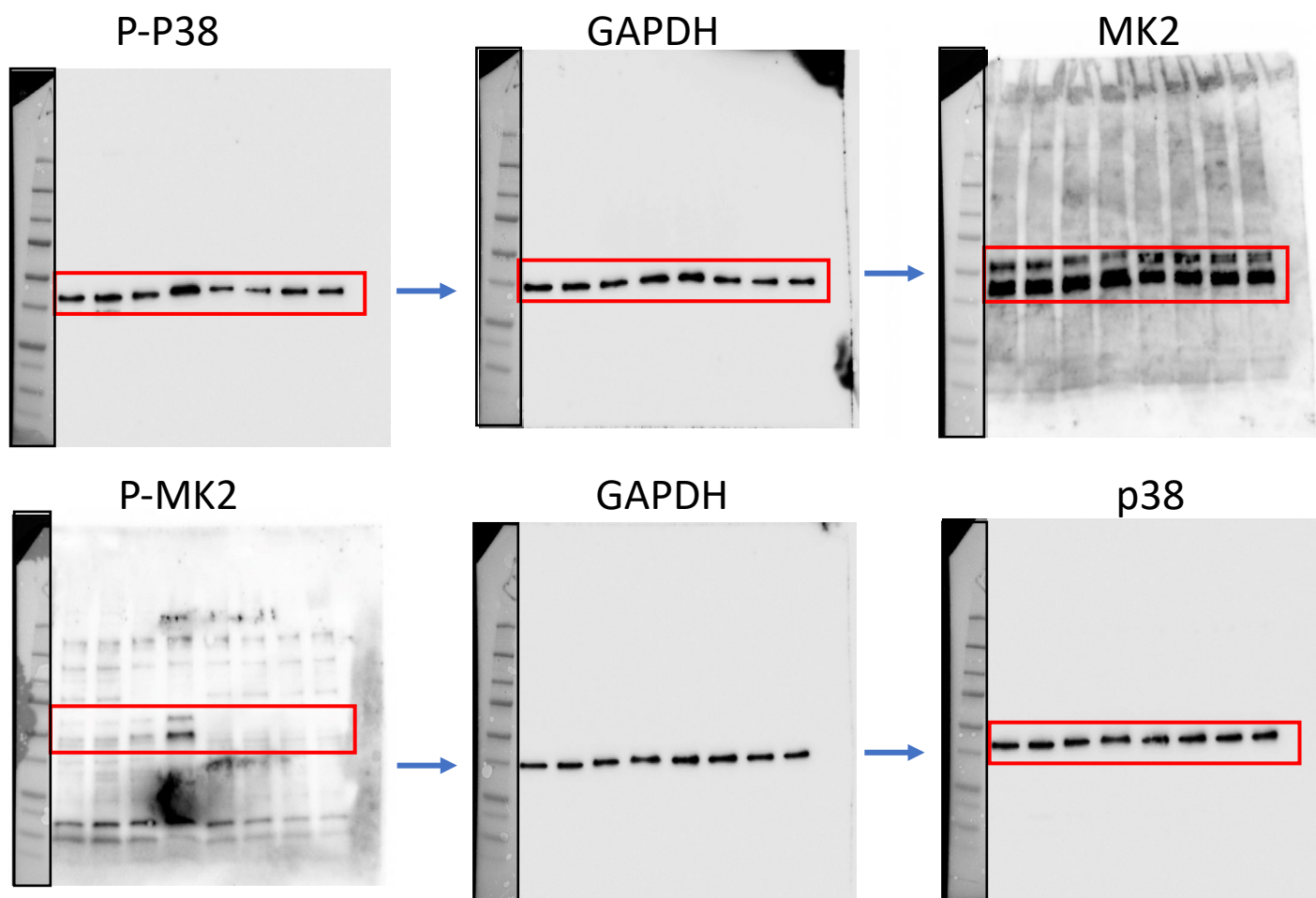


Fig. S23. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 5A in a cropped form. The areas of the membranes that were cropped and presented in Fig. 5A for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 5A. Re-probing sequence for the same membrane is indicated with blue arrow.

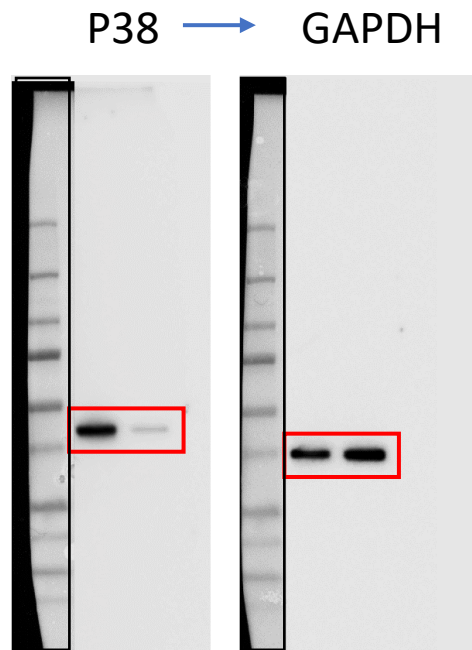


Fig. S24. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 5C in a cropped form. The areas of the membranes that were cropped and presented in Fig. 5C for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 5C. Re-probing sequence for the same membrane is indicated with blue arrow.

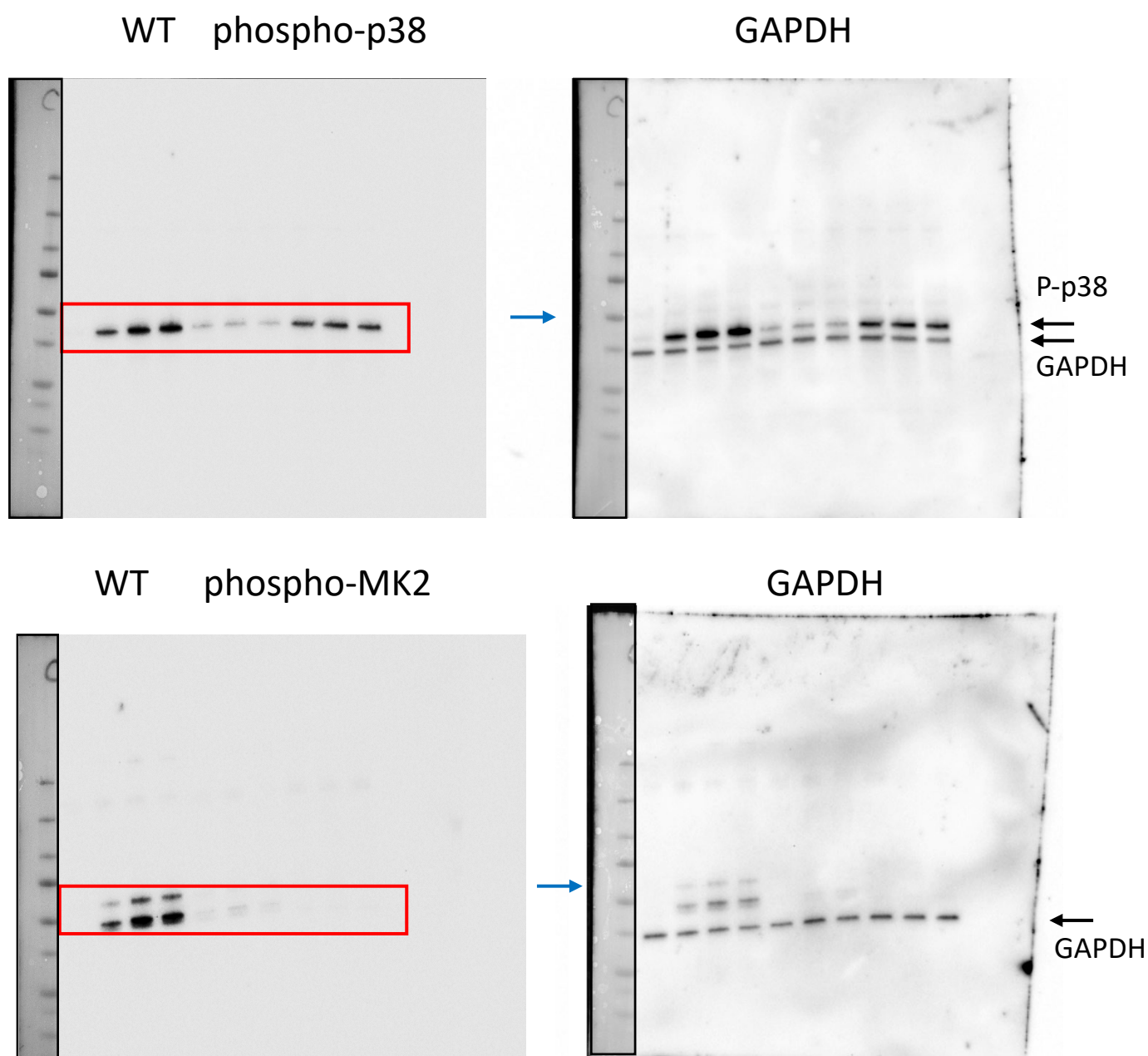


Fig. S25. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 5E in a cropped form. The areas of the membranes that were cropped and presented in Fig. 5E for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 5E. Re-probing sequence for the same membrane is indicated with blue arrow.

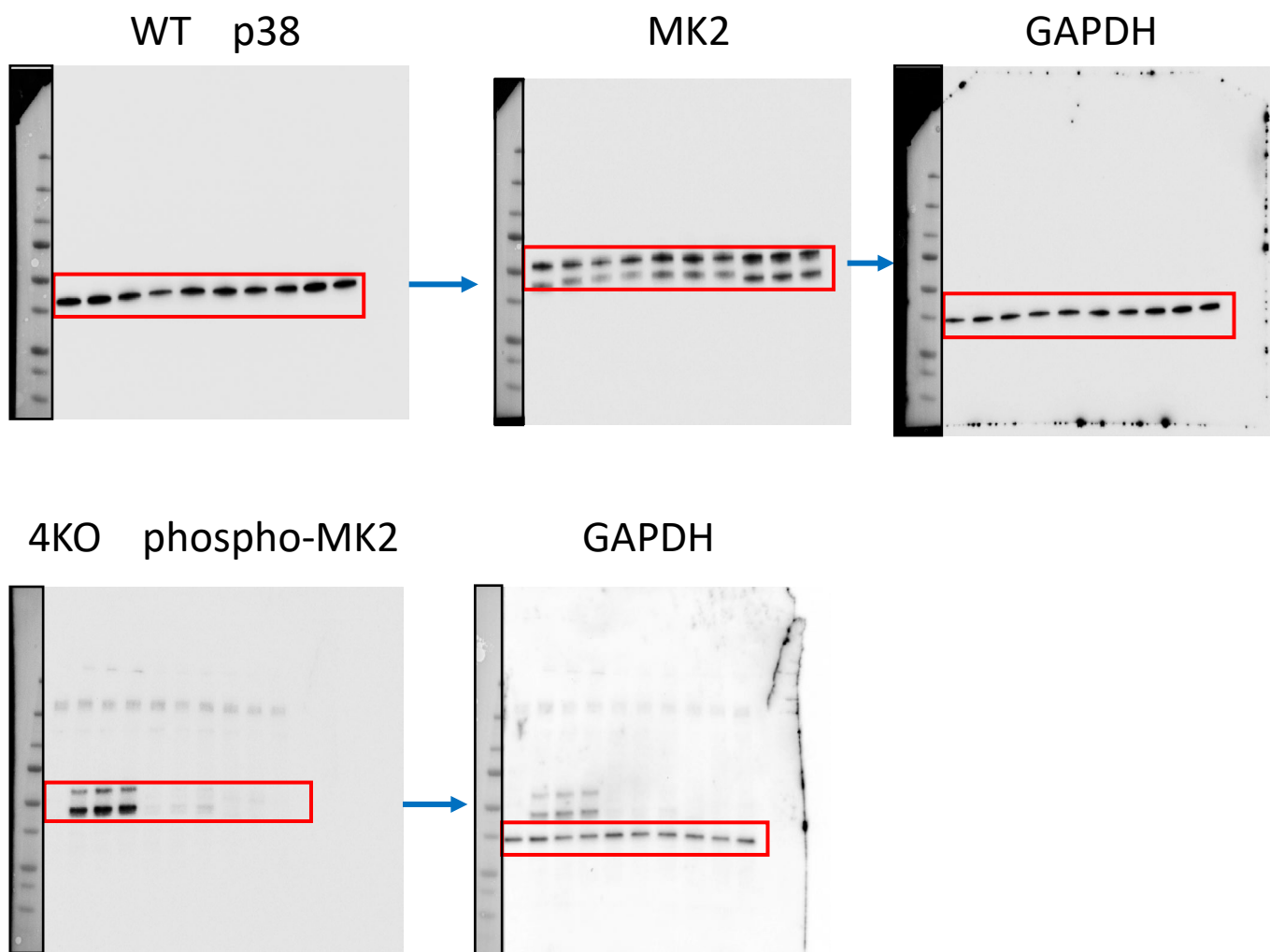


Fig. S26. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 5E in a cropped form. The areas of the membranes that were cropped and presented in Fig. 5E for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 5E. Re-probing sequence for the same membrane is indicated with blue arrow.

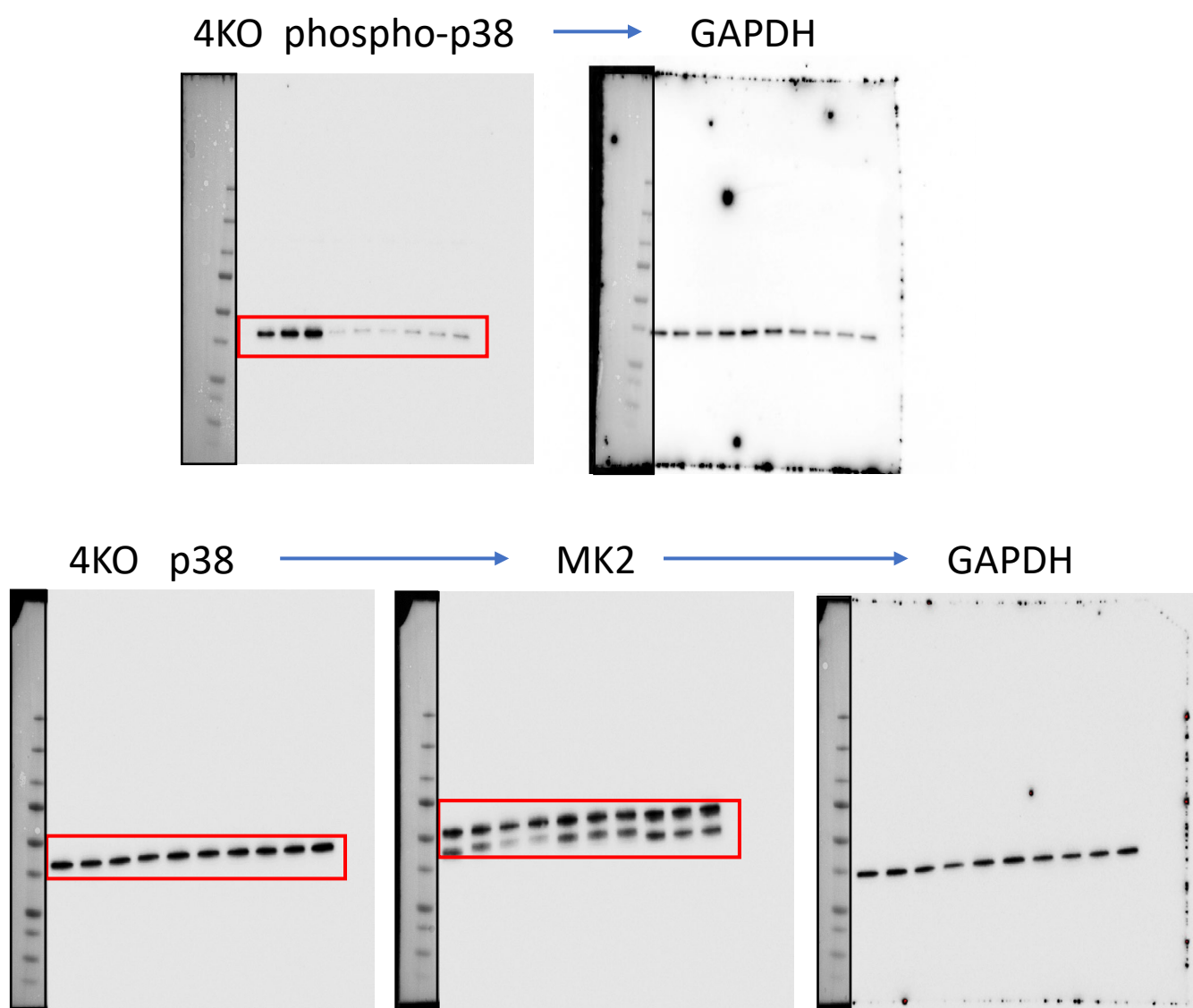


Fig. S27. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 5E in a cropped form. The areas of the membranes that were cropped and presented in Fig. 5E for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 5E. Re-probing sequence for the same membrane is indicated with blue arrow.

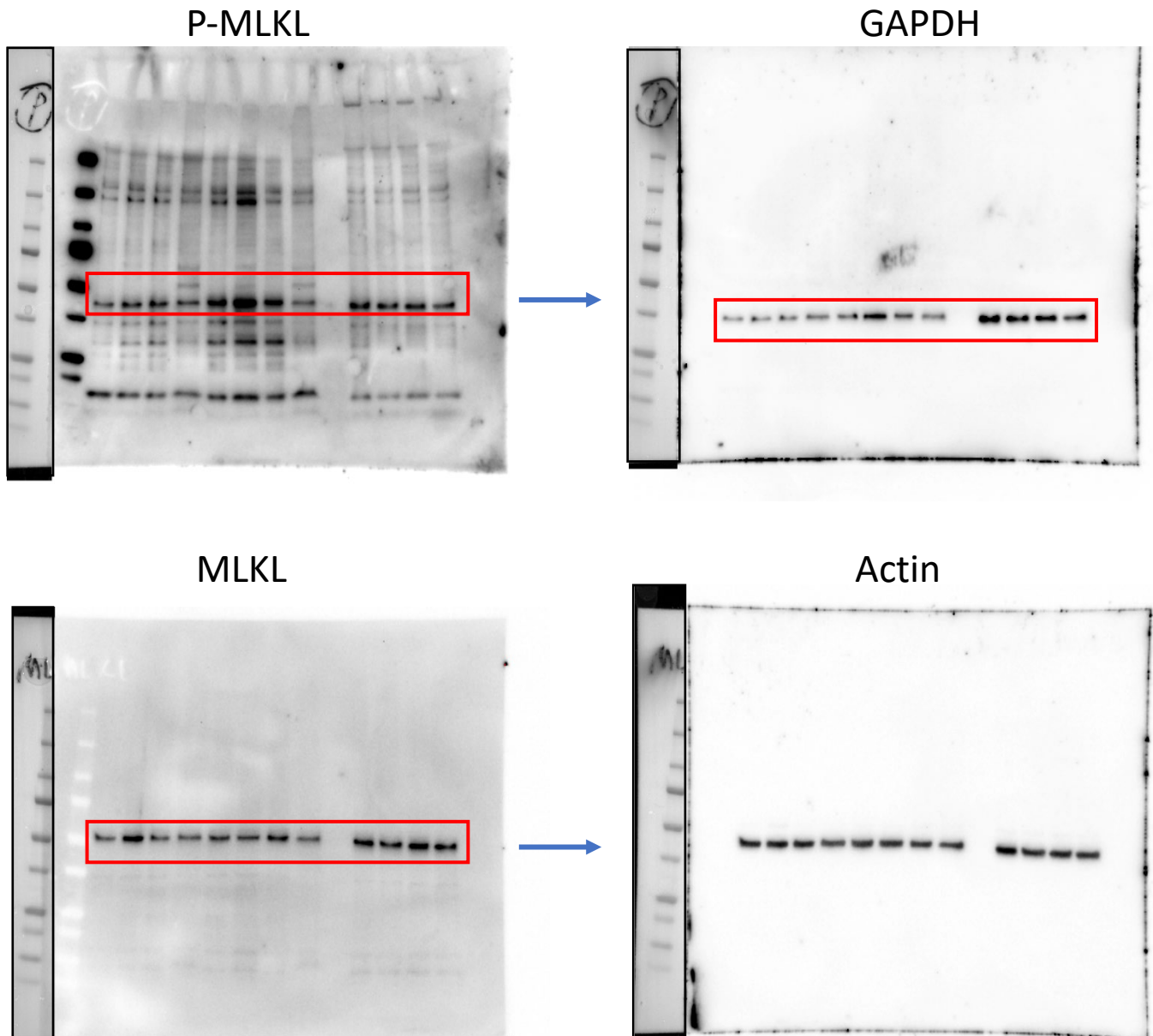


Fig. S28. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 6B in a cropped form. The areas of the membranes that were cropped and presented in Fig. 6B for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 6B. Re-probing sequence for the same membrane is indicated with blue arrow.

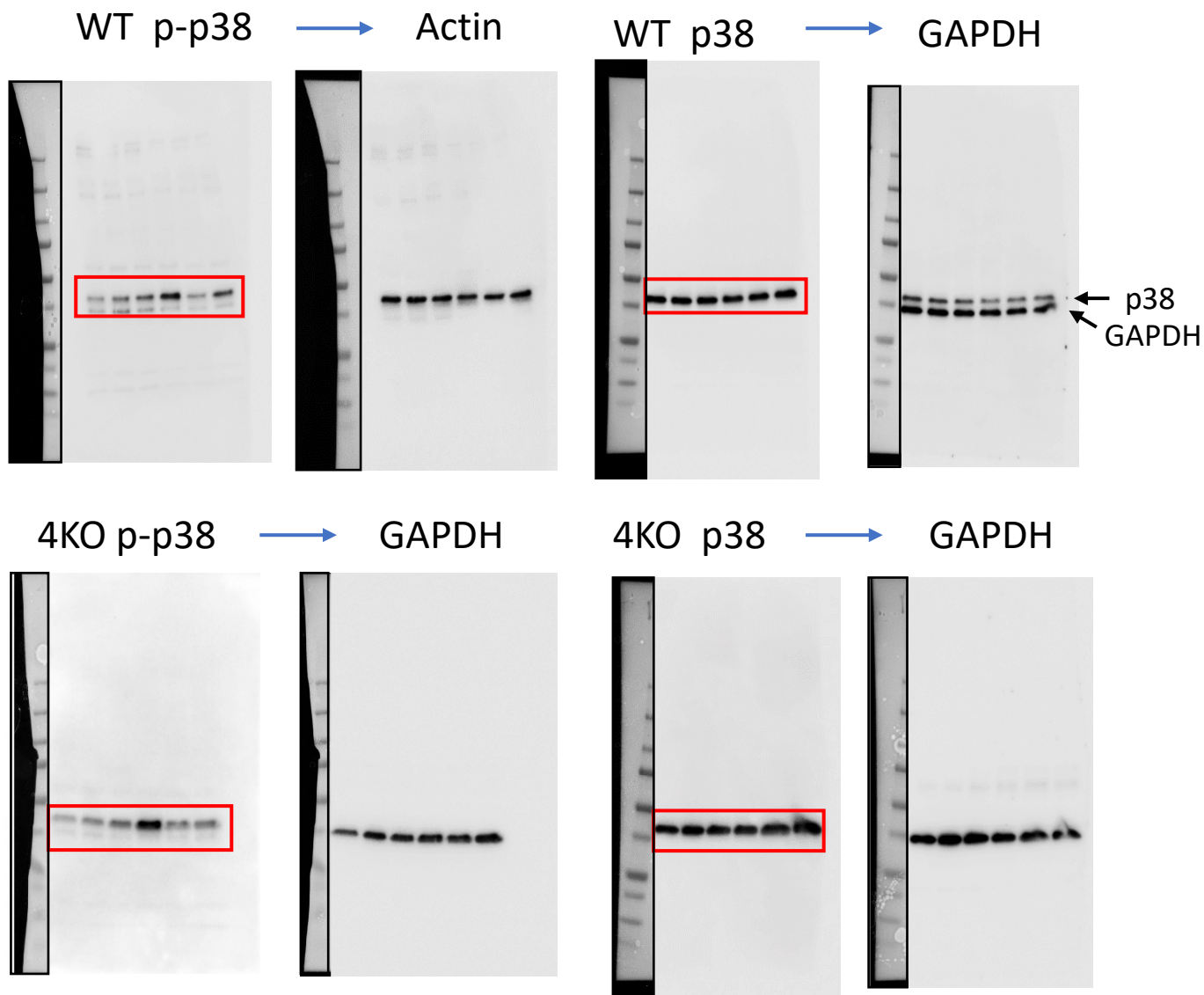


Fig. S29. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 6E in a cropped form. The areas of the membranes that were cropped and presented in Fig. 6E for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 6E. Re-probing sequence for the same membrane is indicated with blue arrow.

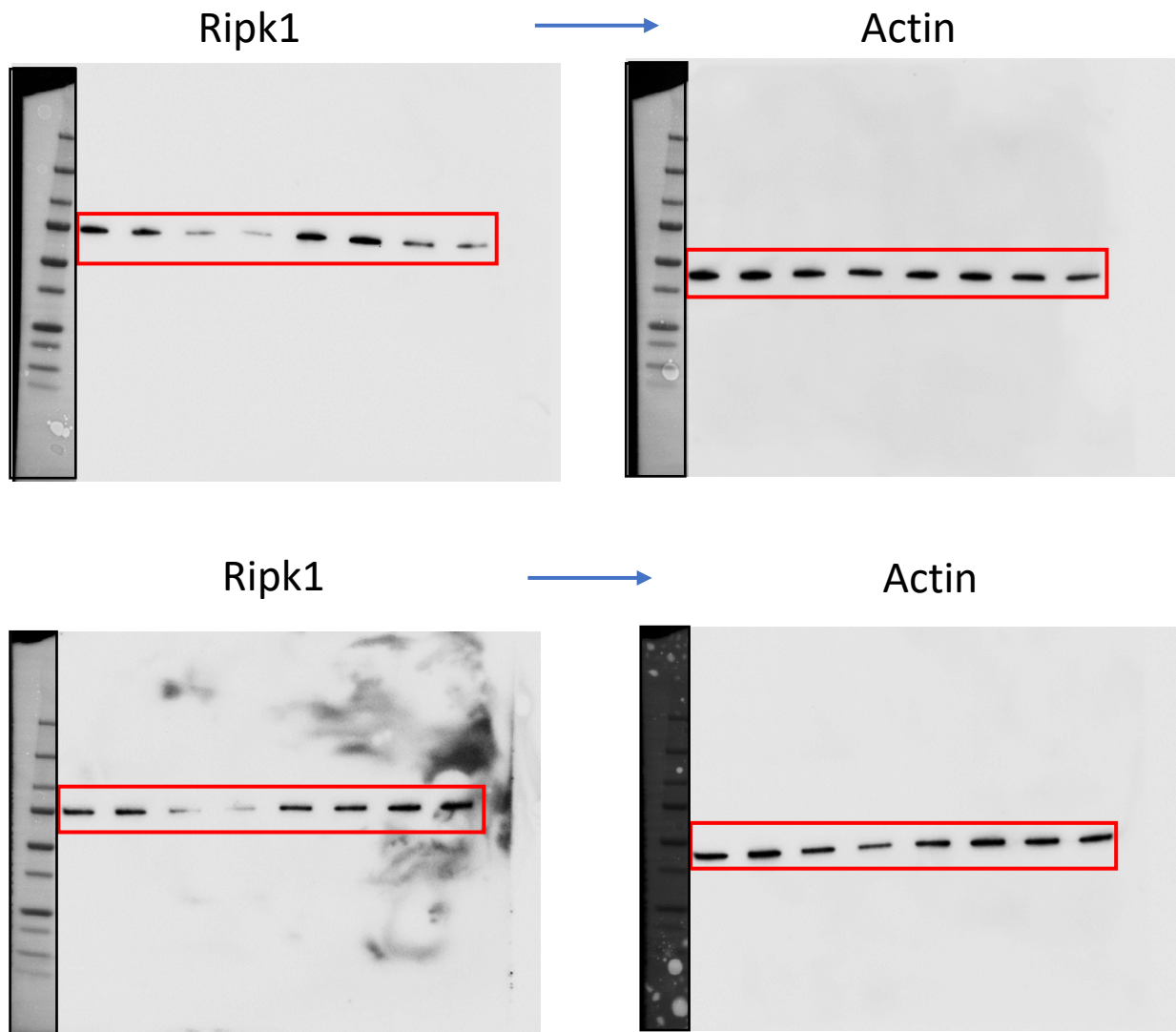


Fig. S30. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Fig. 6I and Fig. 6J in a cropped form. The areas of the membranes that were cropped and presented in Fig. 6I and Fig. 6J for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Fig. 6I and Fig. 6J. Re-probing sequence for the same membrane is indicated with blue arrow.

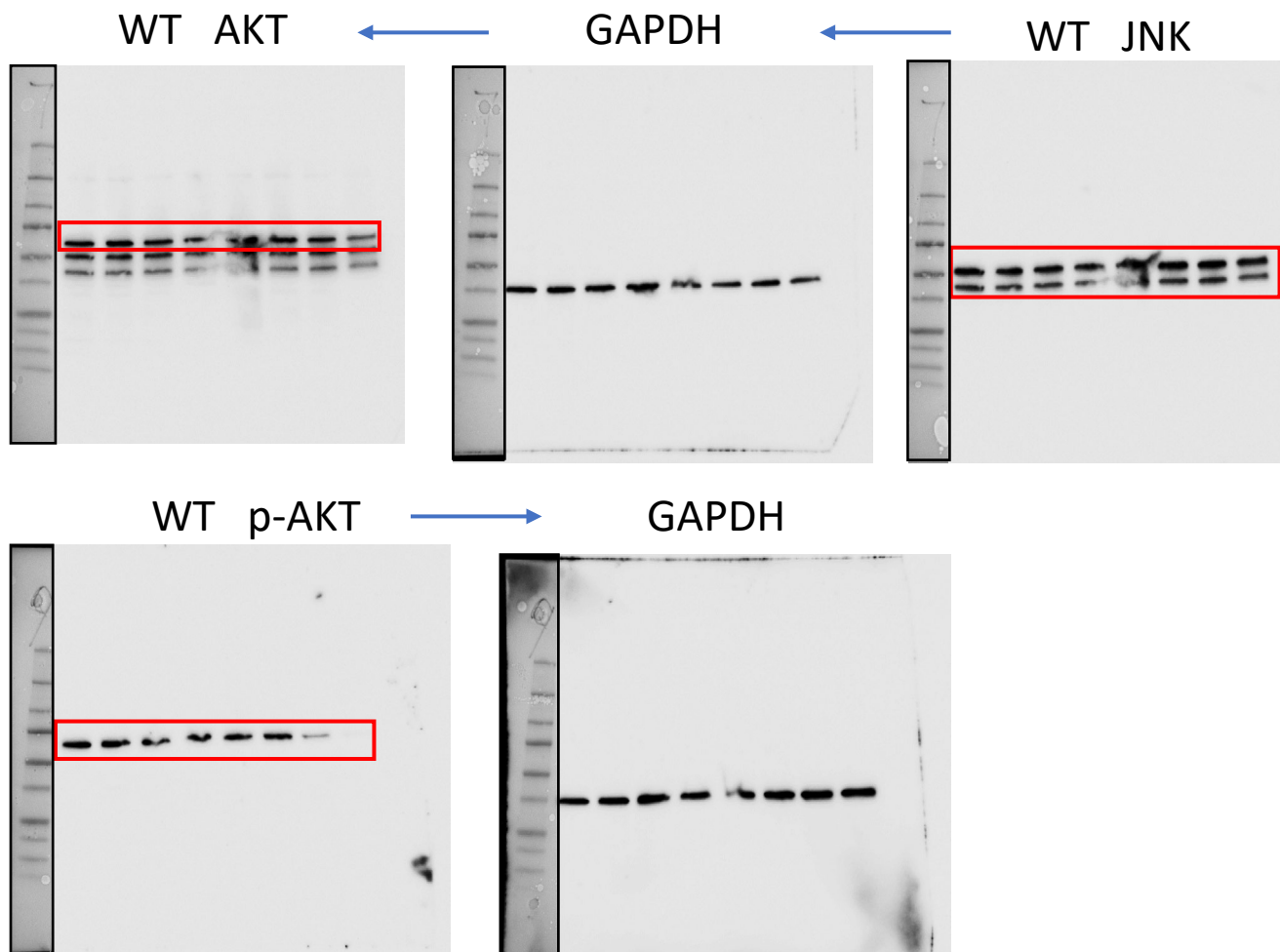


Fig. S31. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8A in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8A for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8A. Re-probing sequence for the same membrane is indicated with blue arrow. The blotting order for p-AKT membrane was P-AKT, GAPDH, and then MK2 for Fig. 4A.

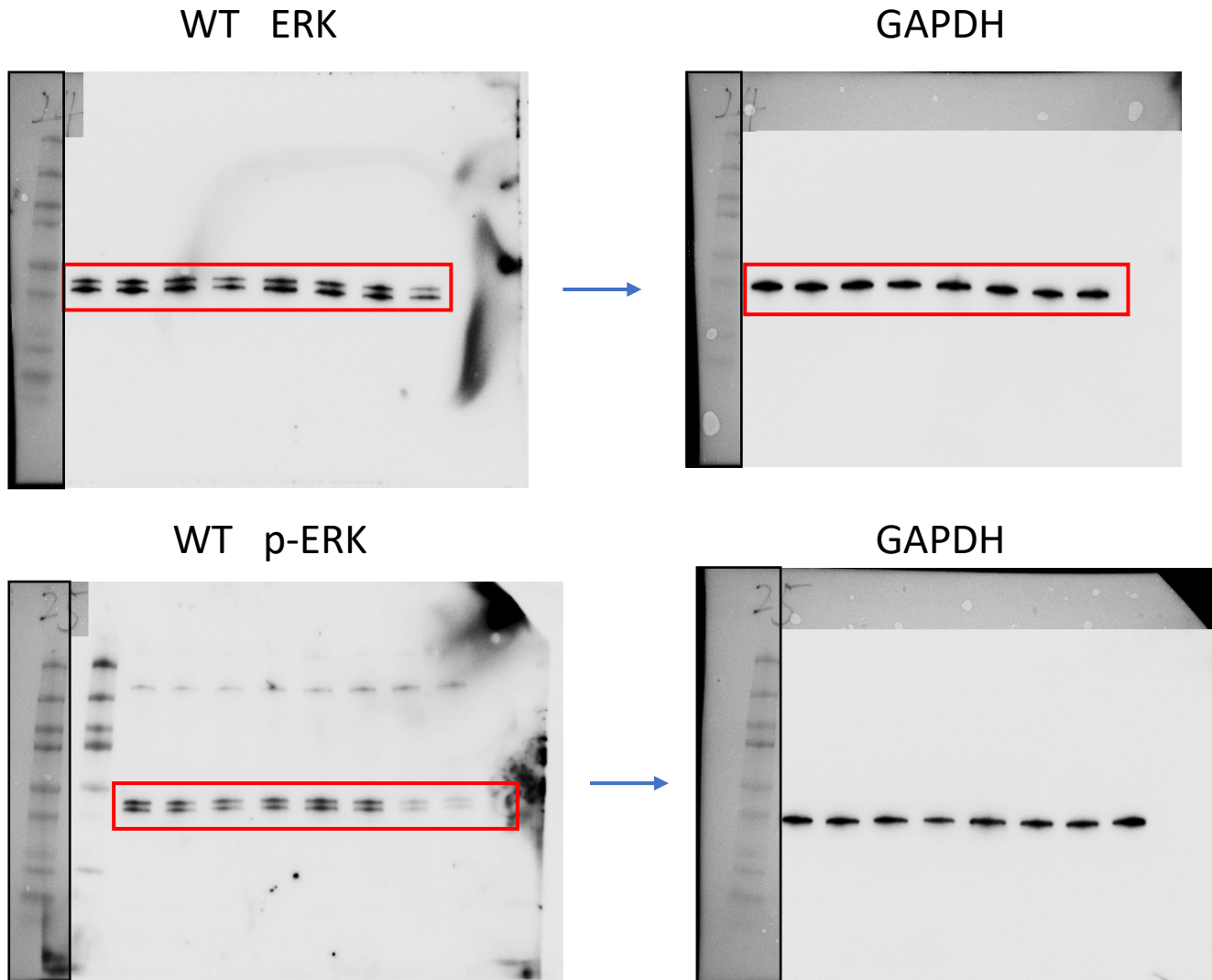


Fig. S32. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8A in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8A for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8A. Re-probing sequence for the same membrane is indicated with blue arrow.

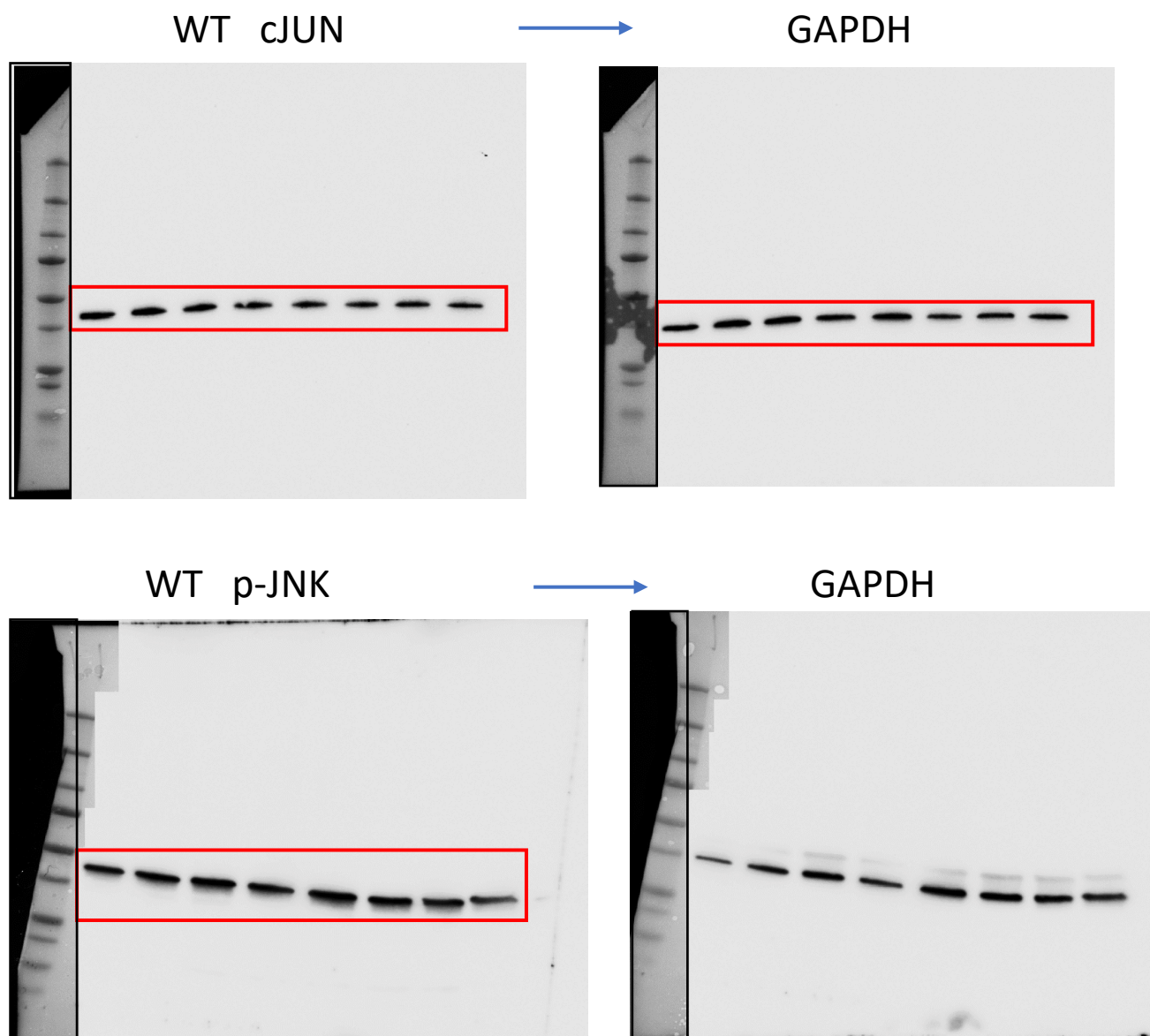


Fig. S33. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8A in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8A for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8A. Re-probing sequence for the same membrane is indicated with blue arrow.

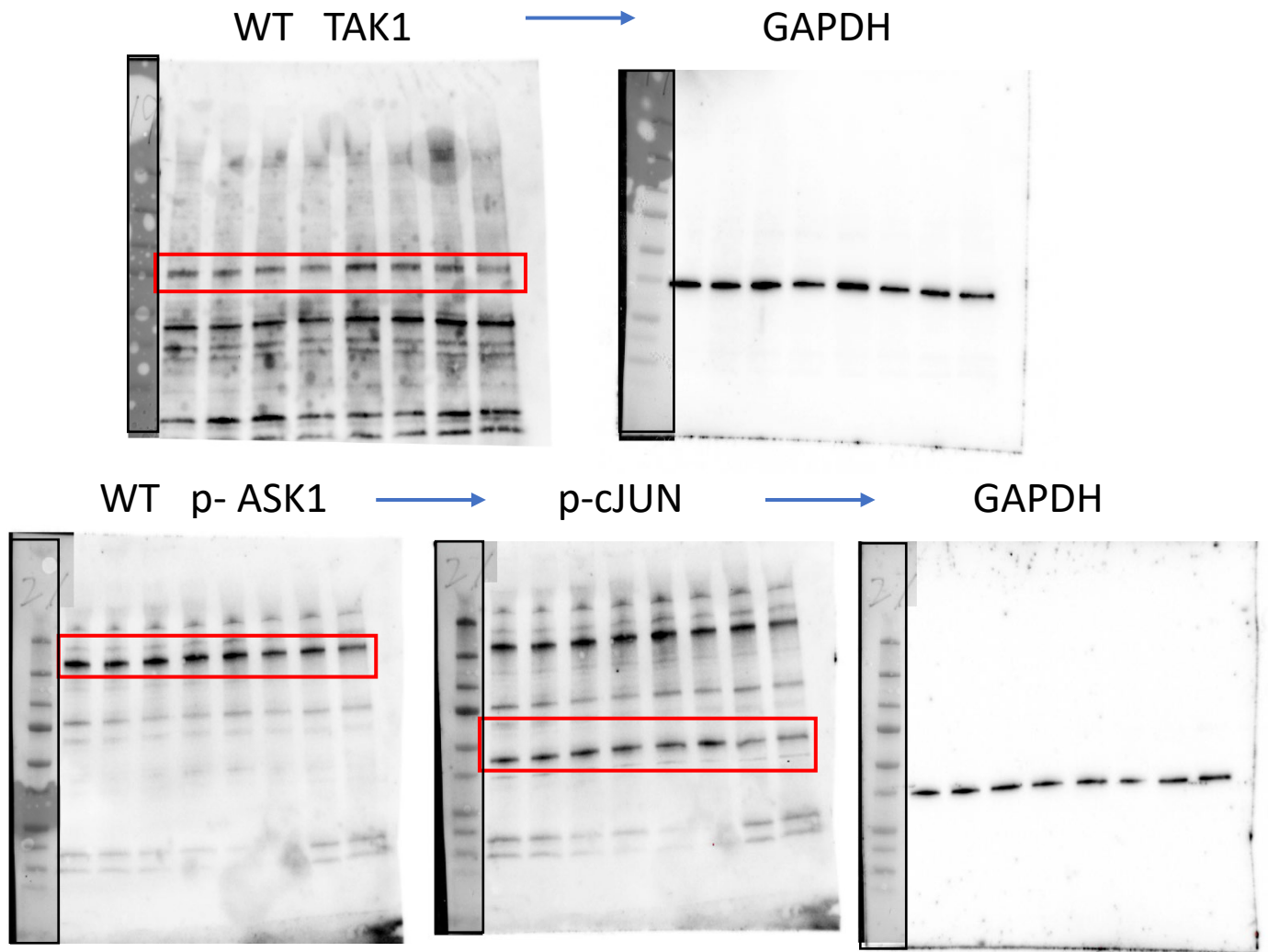


Fig. S34. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8A and Fig. S8C in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8A and Fig. 8C for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8A and Fig. 8C. Re-probing sequence for the same membrane is indicated with blue arrow.

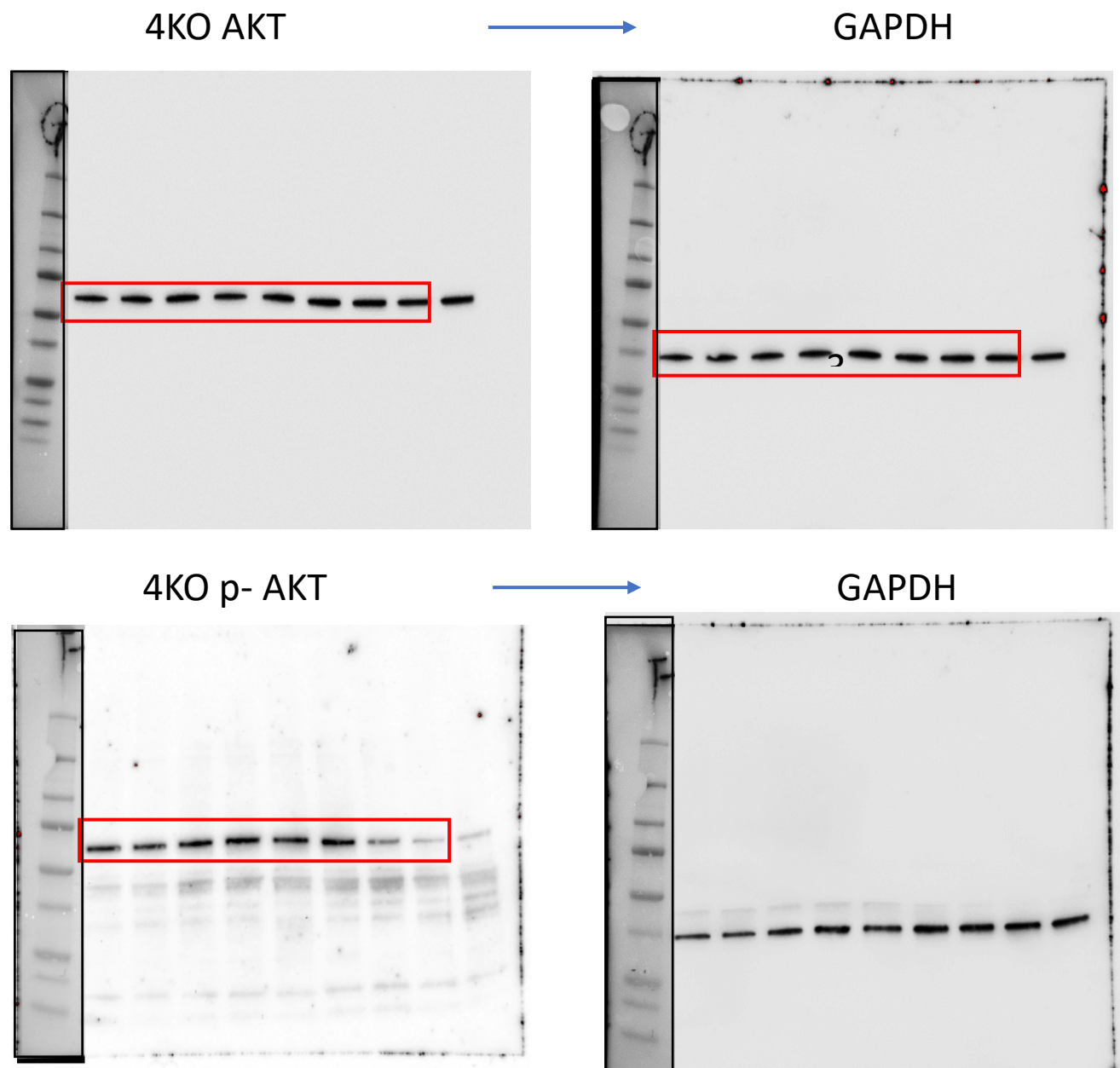


Fig. S35. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8B in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8B for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8B. Re-probing sequence for the same membrane is indicated with blue arrow.

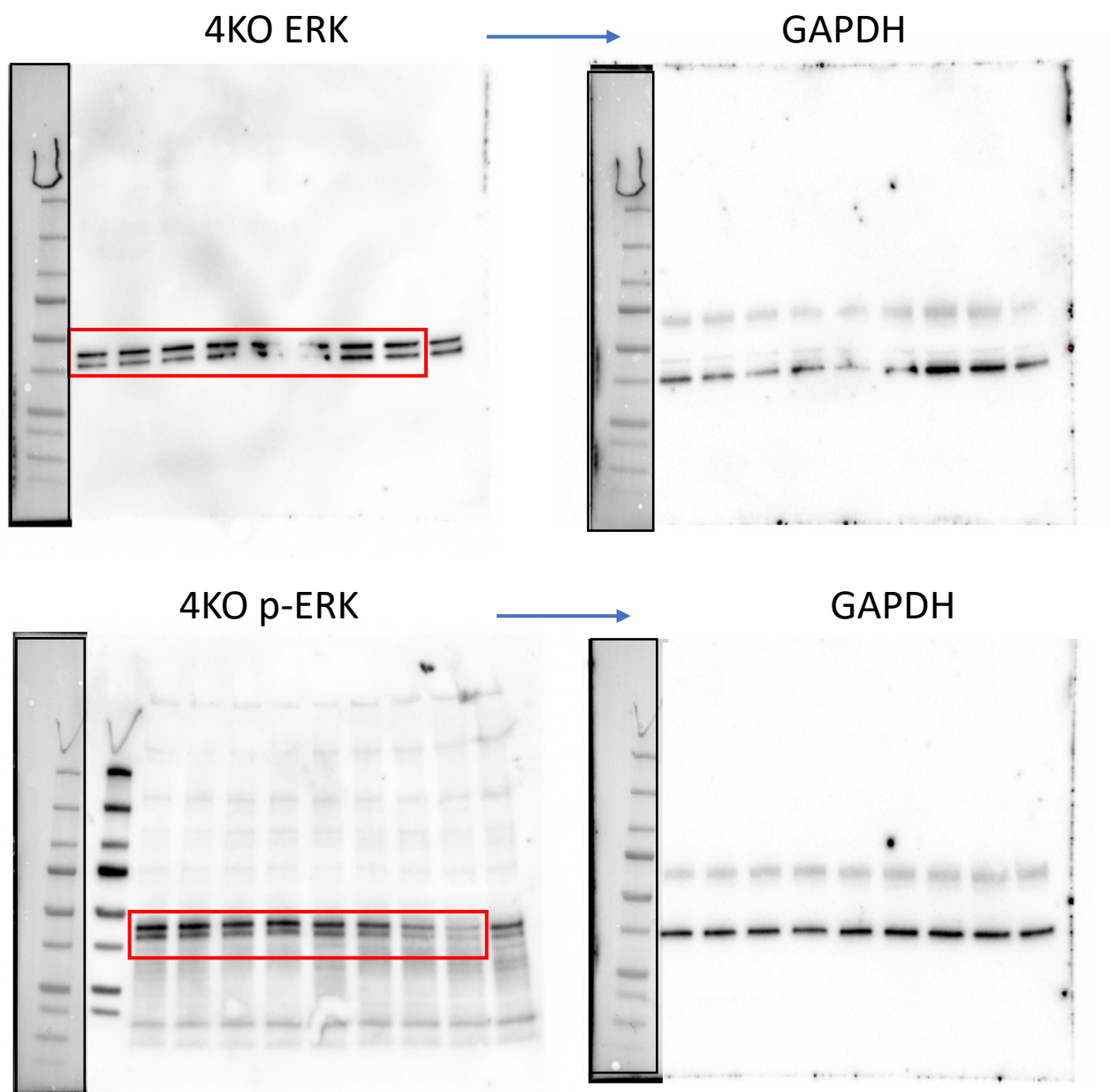


Fig. S36. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8B in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8B for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8B. Re-probing sequence for the same membrane is indicated with blue arrow.

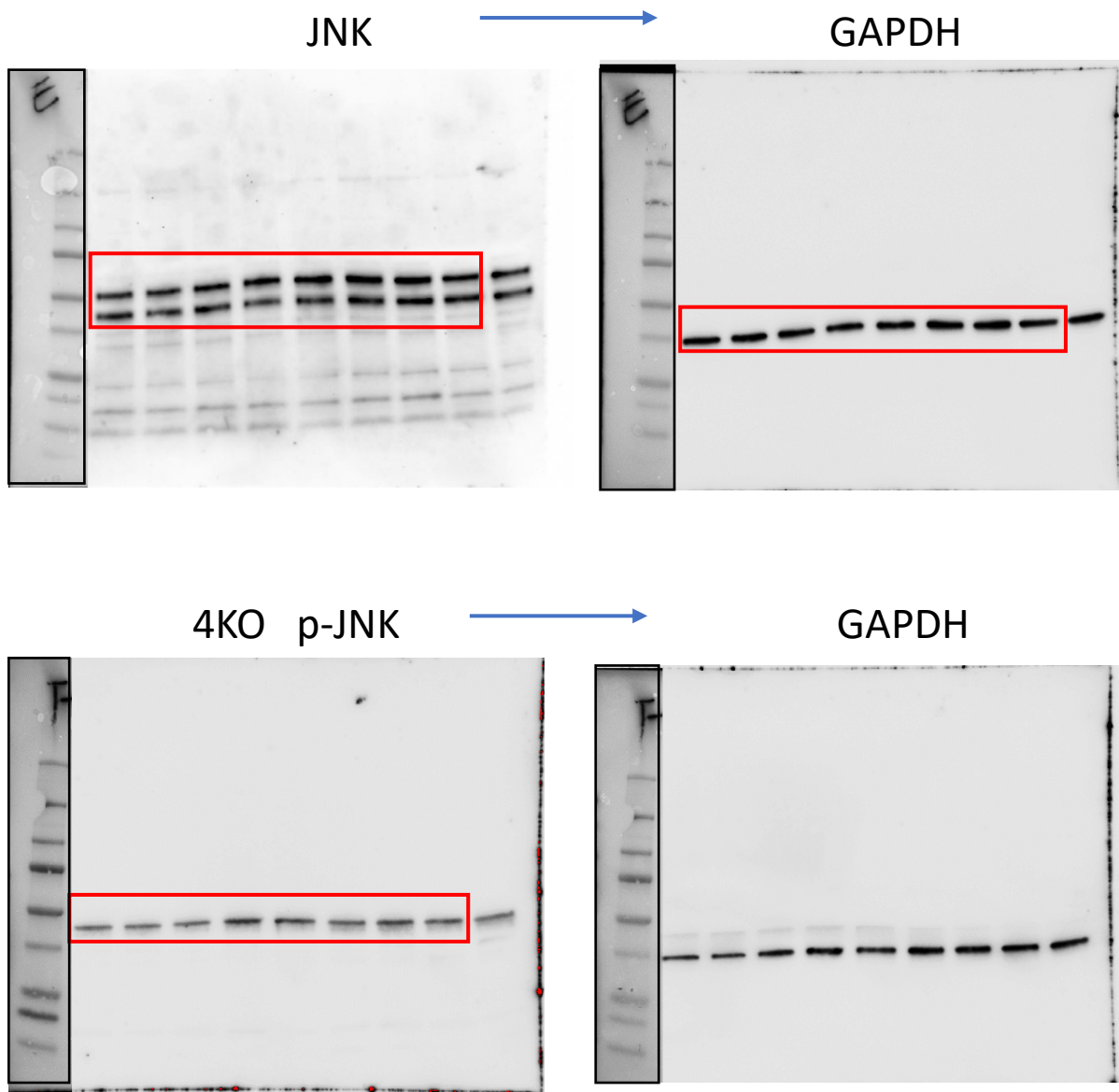


Fig. S37. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8B in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8B for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8B. Re-probing sequence for the same membrane is indicated with blue arrow. The membrane for JNK was also used to analyze expression of ATF2 (Fig. 4A). The probing order was JNK, ATF2, GAPDH. The membrane for p-JNK was also used to analyze expression of p-AKT (S35); the order of processing was p-JNK, p-AKT, GAPDH.

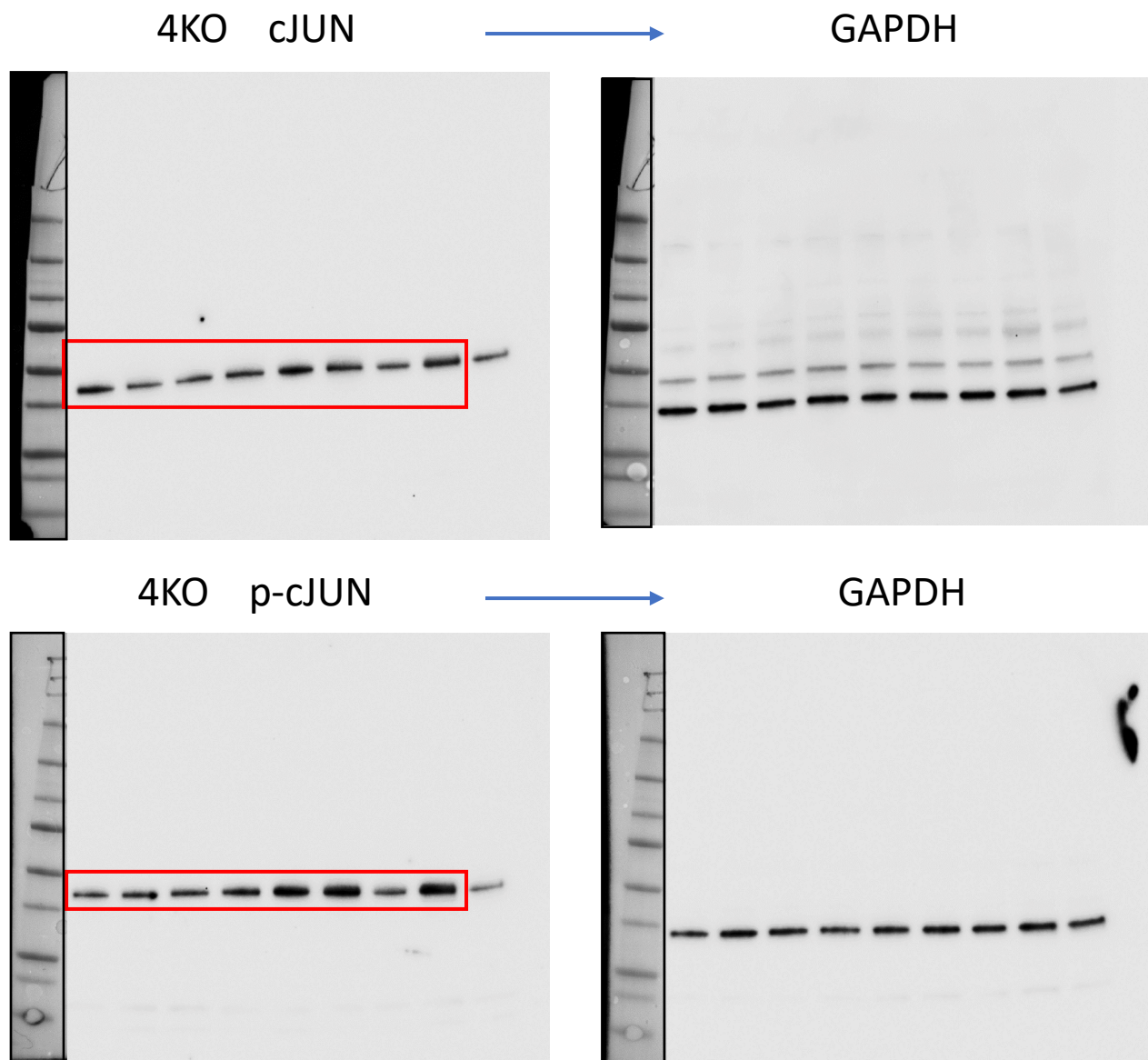


Fig. S38. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8B in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8B for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8B. Re-probing sequence for the same membrane is indicated with blue arrow.

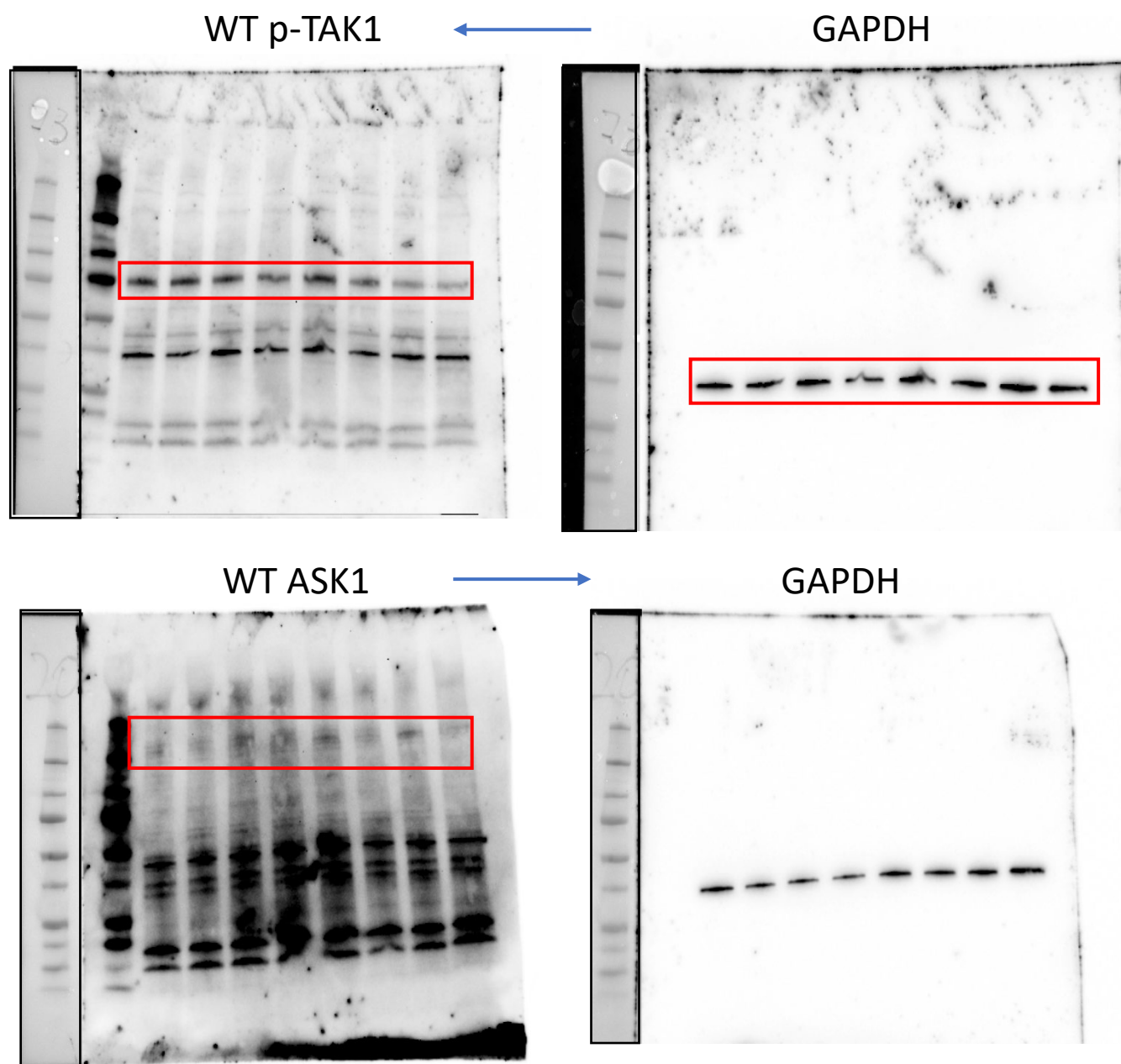


Fig. S39. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8C in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8C for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8C. Re-probing sequence for the same membrane is indicated with blue arrow.

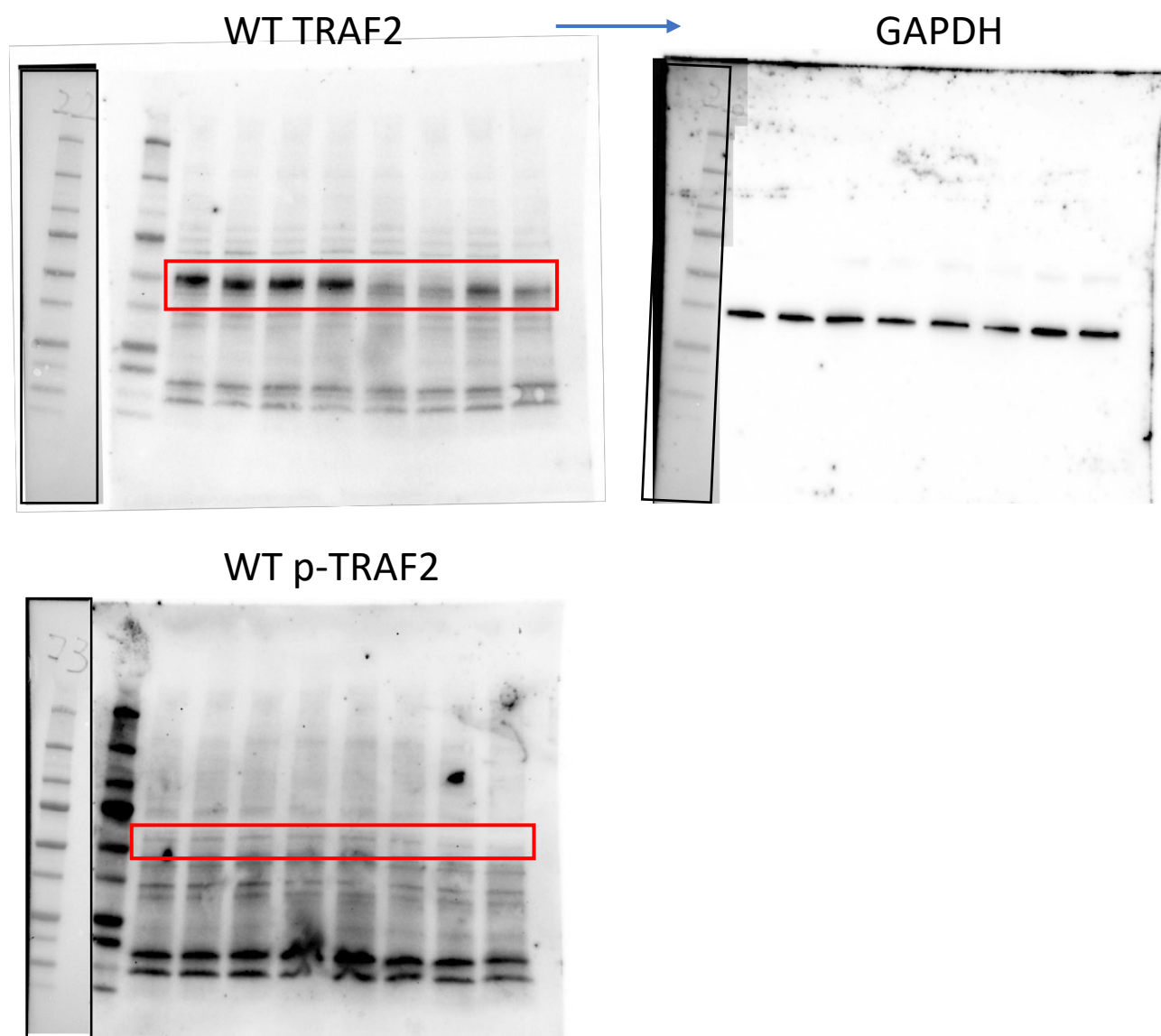


Fig. S40. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8C in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8C for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8C. Re-probing sequence for the same membrane is indicated with blue arrow.

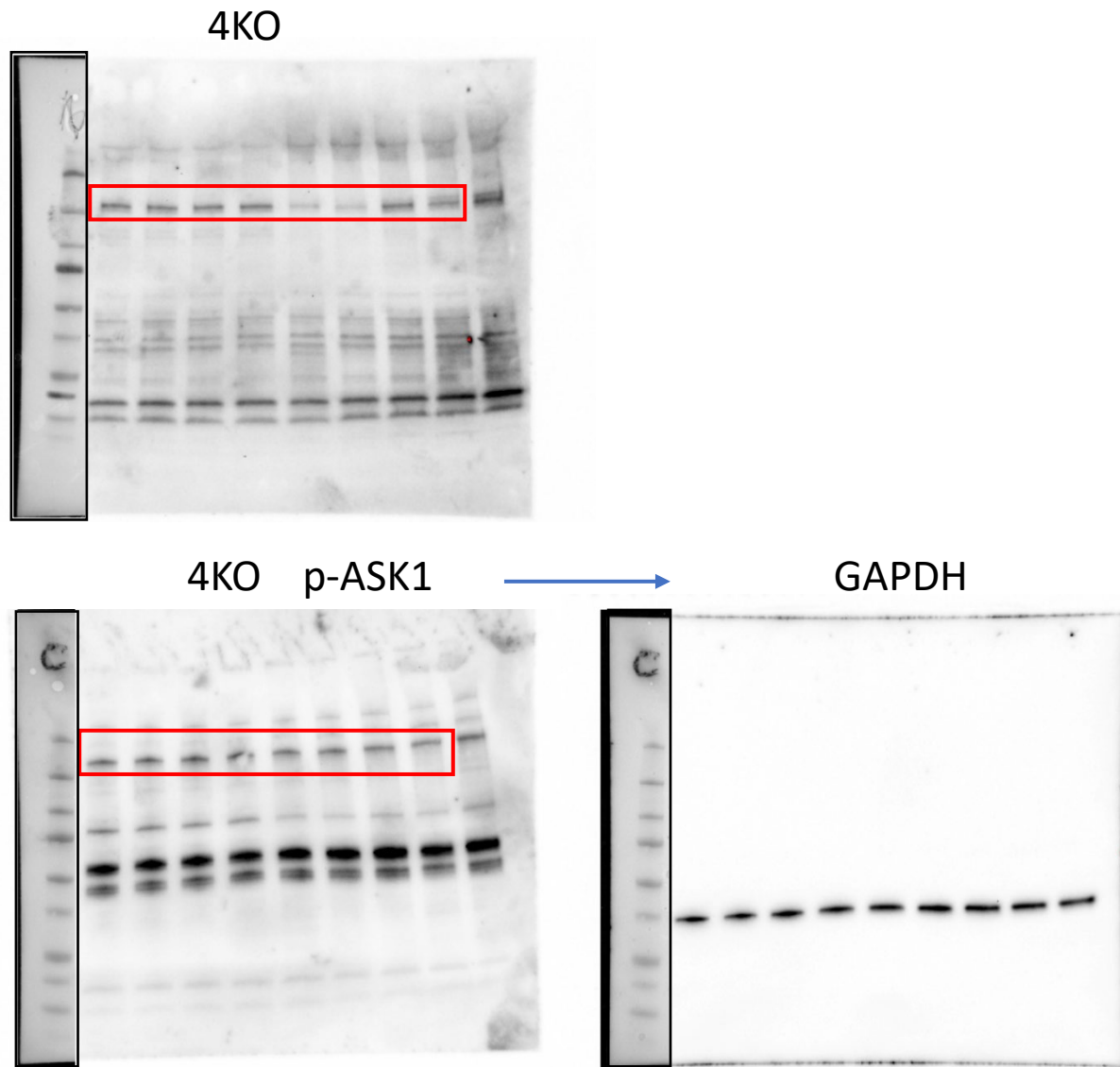


Fig. S41. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8D in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8D for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8D. Re-probing sequence for the same membrane is indicated with blue arrow. The membrane for 4KO p-ASK1 was also used for analyzing MK2 in 4KO cells (Fig4A). The probing order was MK2, pASK1, GAPDH.

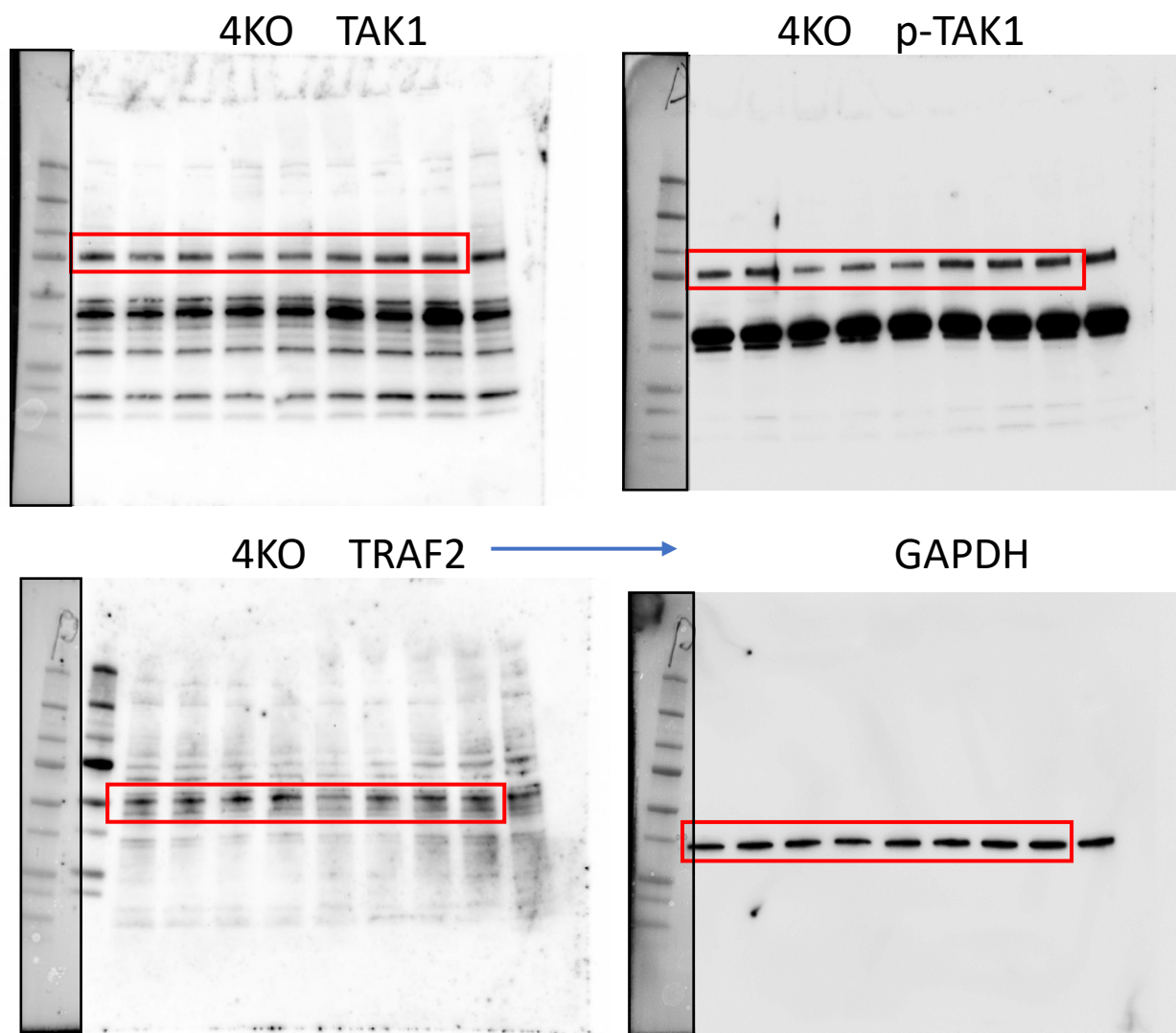


Fig. S42. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8D in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8D for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8D. Re-probing sequence for the same membrane is indicated with blue arrow. The membrane for TAK1 was used to analyze expression of p-p38 first (Fig4A). The membrane for p-TAK1 was used to analyze expression of p38 (Fig4A). The strong low molecular weight bands represent signal post p-p38 and p38 staining, respectively.

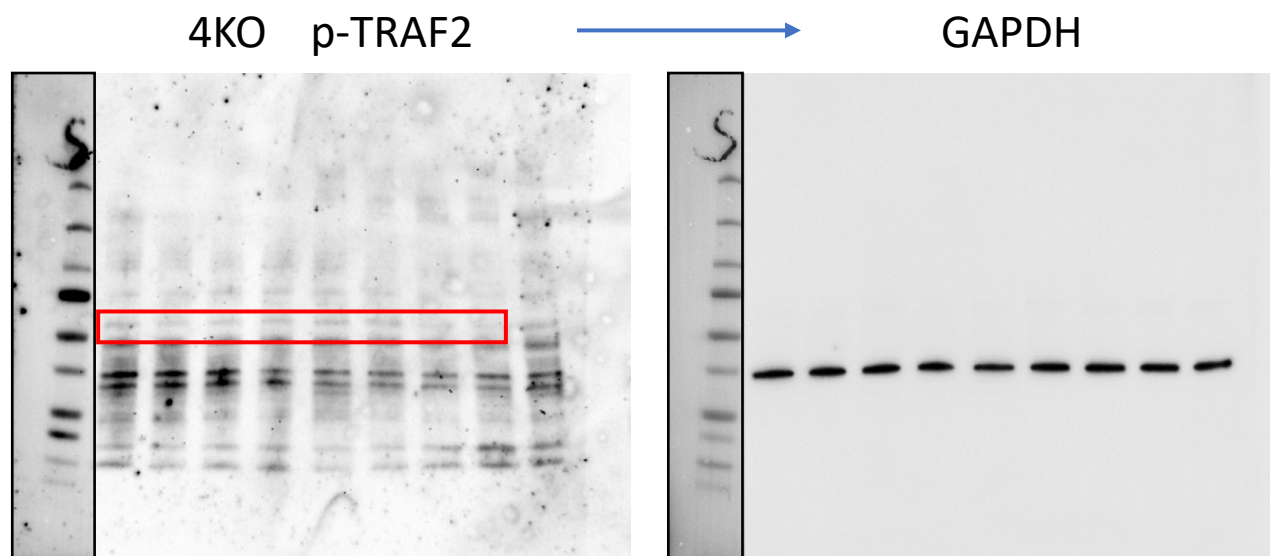


Fig. S43. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8D in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8D for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8D. Re-probing sequence for the same membrane is indicated with blue arrow.

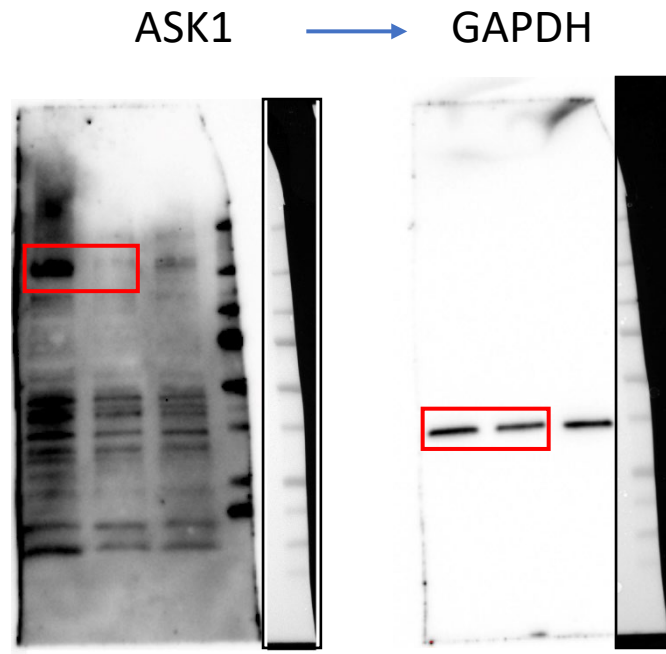


Fig. S44. The full-length images of the original Western blot membranes demonstrating expression of indicated proteins, shown in Supplemental Fig. S8D in a cropped form. The areas of the membranes that were cropped and presented in Supplemental Fig. S8D for each protein are depicted with red rectangles. The image of the molecular weight ladder loaded on the same gel was taken for each original membrane and was juxtaposed with the image of the same membrane after the Western blot processing. The border of the molecular weight ladder image is depicted by the black rectangle. The molecular weights corresponding to specific bands in the ladder are shown in Supplemental Fig. S8D. Re-probing sequence for the same membrane is indicated with blue arrow.

Table S3. List of antibodies used in this study and their providers.

Proteins detected	Cat #	Vendors
Actin	A3853	Sigma
Akt (pan)	#4691	Cell Signaling
phospho-Akt (Ser473)	#4060	Cell Signaling
ASK1	ab45178	abcam
phospho-ASK1 (Thr845)	sc-109911	Santa Cruz
ATF2	#9226	Cell Signaling
phospho- ATF2 (Thr71)	#9221	Cell Signaling
Bak	SAB2500149	Sigma
Bax	ab32503	abcam
Bid	MAB860	R&D
Caspase-1	sc-514	Santa Cruz
Caspase- 3	#9662	Cell Signaling
Caspase -7	ab181579, ab2323	abcam
Caspase- 8	ALX-804-447	Enzo
Caspase- 11	14-9935	affymetrix eBioscience
p44/42 MAPK (Erk1/2)	#9107	Cell Signaling
Phospho-Erk1/2 (Thr202/Tyr204)	#4370	Cell Signaling
GAPDH	MA5-15738	ThermoFisher Scientific
Goat anti-rabbit IgG, HRP	#7074	Cell Signaling
Goat anti-Mouse IgG, HRP	# 62-6520	Invitrogen
Goat Anti-Rat IgG, HRP	ab97057	abcam
JNK	#9252	Cell Signaling
phospho-JNK (Thr183/Tyr185)	sc-6254	Santa Cruz
c-JUN	#9165	Cell Signaling
phospho-c-JUN (Ser73)	#3270	Cell Signaling
MAPKAPK2(MK2)	#12155	Cell Signaling
phospho-MK2 (Thr334)	#3007	Cell Signaling
MLKL	MABC604	Millipore
phospho-MLKL (Ser345)	ab196436	abcam
p38 MAPK	#9212	Cell Signaling
phospho-p38 (Thr180/Tyr182)	#4511	Cell Signaling
Ripk1	ab72139	abcam
Ripk1	#3493	Cell Signaling
Ripk3	#14401, #15828	Cell Signaling
TAK1	#4505	Cell Signaling
phospho-TAK1 (Ser412)	#9339	Cell Signaling
TRAF2	#4724	Cell Signaling
phospho-TRAF2 (Ser11)	#13908	Cell Signaling

Table S4. List of chemicals used in this study and their concentrations.

Chemicals	Targeted proteins/pathways	Final concentration (μm)
BIRB796	pan p38MAPK	10
Cyclosporine A (CsA)	calcineurin, cyclophilin D	1
Deferoxamine mesylate	ferroptosis	100
Ferrostatin-1	ferroptosis	10
GSK843	Ripk3	3
GSK872	Ripk3	3
Liproxstatin-1	ferroptosis	10
N-acetyl-L-cysteine	ROS	3000
Necrostatin-1	Ripk1	20
PF3644022	MK2	10
Z-vad	pan-caspases	25