

Role of GPX3+ Astrocytes in Breast Cancer Brain Metastasis Activated by Circulating Tumor Cell Exosomes

Running Title: Novel GPX3+ Astrocyte Activation in BMBC

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Supplementary Table 1. Transfection plasmid sequence

Targets	Sequences
sh-NC	5'-CCTAAGGTTAAGTCGCCCTCG-3'
sh-HIF-1 α -1	5'-GGGATTAACTCAGTTTGA ACT-3'
sh-HIF-1 α -2	5'-GGAAATGAGAGAAATGCTTAC-3'

Supplementary Table 2. Flow cytometry antibody manufacturer information

Name	Article number	Concentration	Manufacturer
Anti CD45 antibody	ab210224	1: 500	Abcam
Anti CD4 antibody	ab252149	1: 100	Abcam
Anti CD8 antibody	ab237364	1: 500	Abcam
Anti FOXP3 antibody	ab218773)	1: 500	Abcam
Anti IL-17A antibody	53-7177-81	0.5 µg/test	Thermo Fisher
Anti CD19 antibody	ab270176	1: 100	Abcam
Anti CD11b antibody	ab24874	1: 150	Abcam
Anti F4/80 antibody	ab237331	1: 500	Abcam
Anti Ly6C antibody	ab25025	1: 200	Abcam
Anti Ly6G antibody	ab25024	1: 100	Abcam

Supplementary Table 3. Immunofluorescence staining experiment manufacturer information of the first and second antibodies

Name	Article number	concentration	Manufacturer
Anti-GPX3 antibody	ab256475	1: 1000	Abcam
Anti-IL-1 beta antibody	MA5-23691	8-25 µg/mL	Thermo Fisher
Anti-GFAP antibody	ab302644	1: 250	Abcam
Anti-HIF-1 α antibody	MA1-516	1: 20-1: 200	Thermo Fisher
Anti-EpCAM antibody	ab213500	1: 100	Abcam
Anti-alpha Tubulin antibody	ab195889	1: 150-1: 250	Abcam
Donkey anti-rabbit IgG	ab7084	1: 400-1: 2000	Abcam
Donkey anti-goat IgG	ab150129	1: 200-1: 1000	Abcam
Goat anti-rabbit IgG	ab6722	1: 200-1: 1000	Abcam
Goat anti-mouse IgG	ab150115	1: 200-1: 1000	Abcam

Supplementary Table 4. Western blot experiment All primary antibody manufacturer information

Name	Article number	concentration	Manufacturer
Anti-GPX3 antibody	ab256475	1: 1000	Abcam
Anti-IL-1 beta antibody	MA5-23691	1 µg/mL	Thermo Fisher
Anti-Fn-1 antibody	ab268020	1: 1000	Abcam
Anti-S100A8 antibody	ab92331	1: 1000	Abcam
Anti-S100A9 antibody	ab242945	1: 1000	Abcam
Anti-MMP9 antibody	ab283575	1: 1000	Abcam
Anti-IL-17A antibody	ab218013	1: 1000	Abcam
Anti-IL-17F antibody	ab187059	1:500-1:2000	Abcam
Anti-IL-23 antibody	ab189300	1 - 2 µg/mL	Abcam
Anti-HIF-1 α antibody	MA1-516	1: 2000	Thermo Fisher

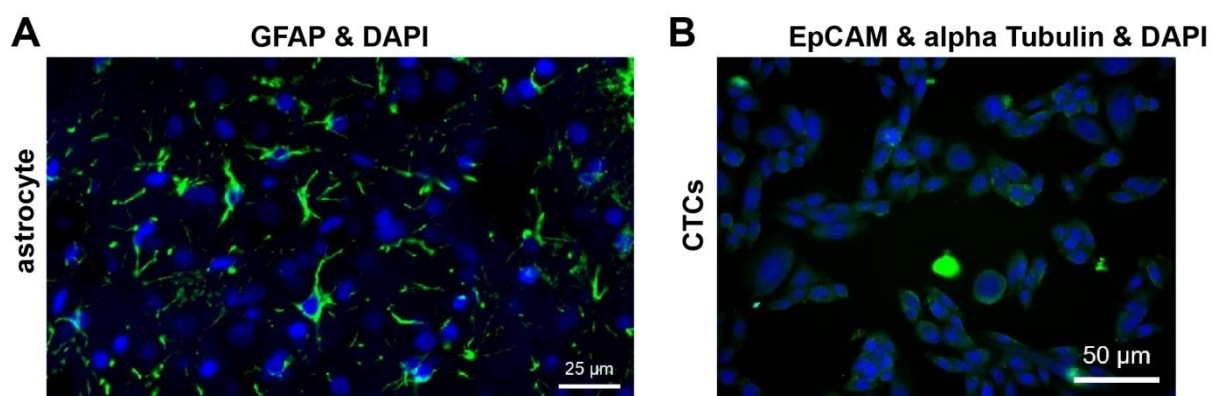
Supplementary Table 5. RT qPCR primer sequence (mouse)

	Forward Primer (5'-3')
IL-17A	F: 5'-GGC CCT CAG ACT ACC TCA AC-3' R: 5'-TCT CGA CCC TGA AAG TGA AGG-3'
IL-1 β	F: 5'-AAG AAG AGC CCA TCC TCT GTG-3' R: 5'-TGT TCA TCT CGG AGC CTG TAG-3'
MMP9	F: 5'-AAACCTCCAACCTCACGGAC-3' R: 5'-CTGAAGCATCAGCAAAGCCG-3'
Fn-1	F: 5'-GACCCCCTTCATCACCAACC-3' R: 5'-TGTCCGCCTAAAGCCATGTT-3'
S100A8	F: 5'-CCATGCCCTCTACAAGAATGACT-3' R: 5'-ATCACCATCGCAAGGAACTC-3'
S100A9	F: 5'-AGGAAGGAAGGACACCCTGA-3' R: 5'-TGTGTCCAGGTCCTCCATGA-3'
IL-17F	F: 5'-ACGTGAATTCCAGAACCGCT-3' R: 5'-TGATGCAGCCTGAGTGTCTG-3'
GPX3	F: 5'-CATCCTGCCTTCTGTCCCTG-3' R: 5'-CGATGGTGAGGGCTCCATAC-3'
IL-23	F: 5'-AAAGGATCCGCCAAGGTCTG-3' R: 5'-GGAGGTGTGAAGTTGCTCCA-3'
HIF-1 α	F: 5'-AGGATGAGTTCTGAACGTCGAAA-3' R: 5'-GGGGAAGTGGCAACTGATGA-3'
GAPDH	F: 5'-GGAGAGTGTTTCCTCGTCCC-3' R: 5'-ATGAAGGGGTCGTTGATGGC-3'

Note: F: Forward; R: Reverse

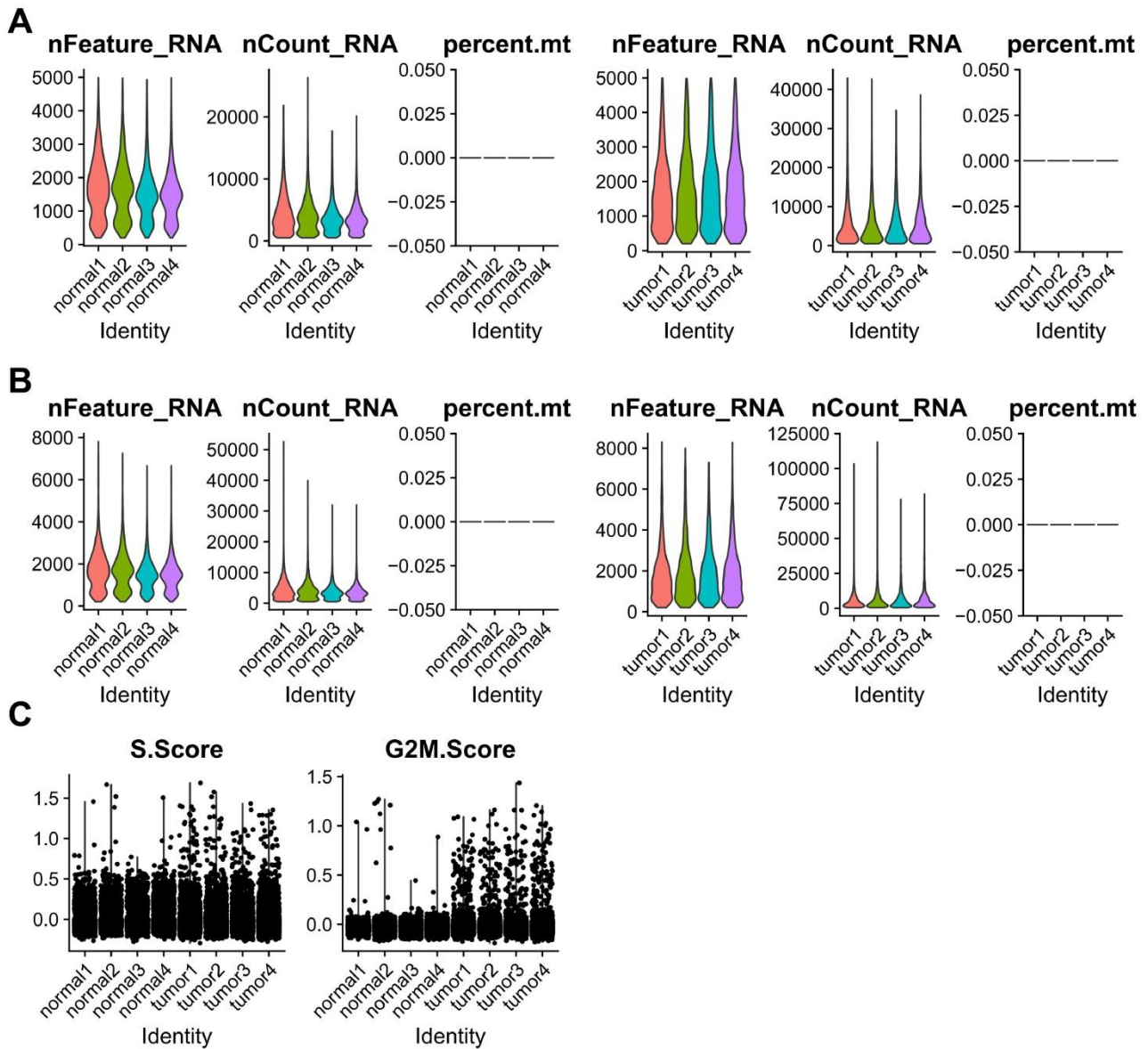
Supplementary Table 6. Transcription factor HIF-1 α And IL-1 β Binding sites of promoter regions

TF Name	Gene Name	Start	End	Predicted sequence
MA0259.1.ARNT::HIF1A	IL-1 β	323	330	GGGCGTGG
MA0259.1.ARNT::HIF1A	IL-1 β	359	366	CAGCGTGA
MA0259.1.ARNT::HIF1A	IL-1 β	1294	1301	GCAAGTGC
MA0259.1.ARNT::HIF1A	IL-1 β	1649	1656	TGACGTGG



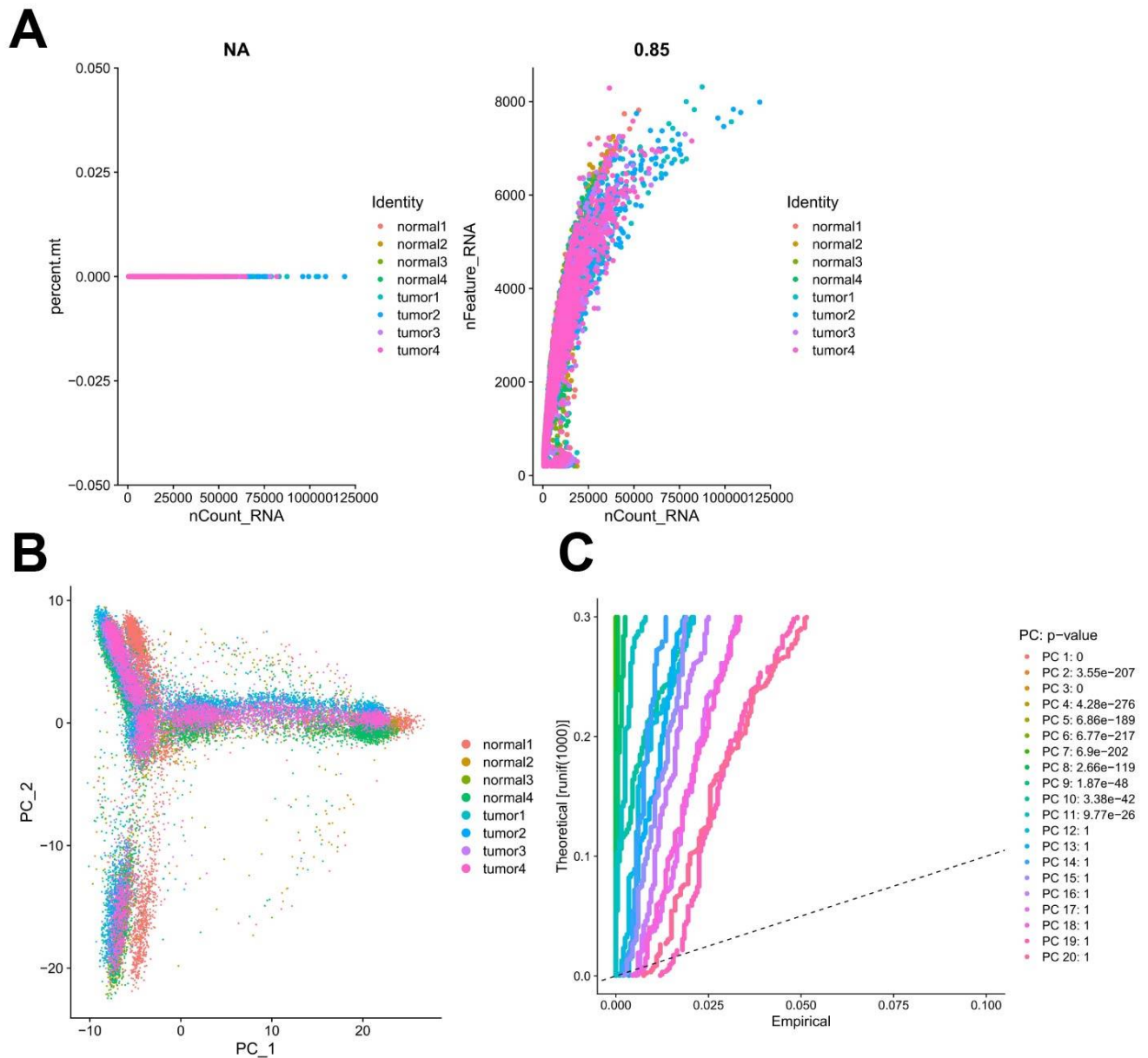
Supplementary figure 1. Identification of astrocytes and CTCs.

Note: (A) Immunofluorescence analysis of GFAP expression in mouse astrocytes (scale bar: 25 μm), green fluorescence represents GFAP, blue fluorescence represents DAPI; (B) Immunofluorescence analysis of EpCAM expression in mouse CTCs (scale bar: 50 μm), green fluorescence represents EpCAM, red fluorescence represents alpha Tubulin, blue fluorescence represents DAPI; cell experiments were repeated 3 times.



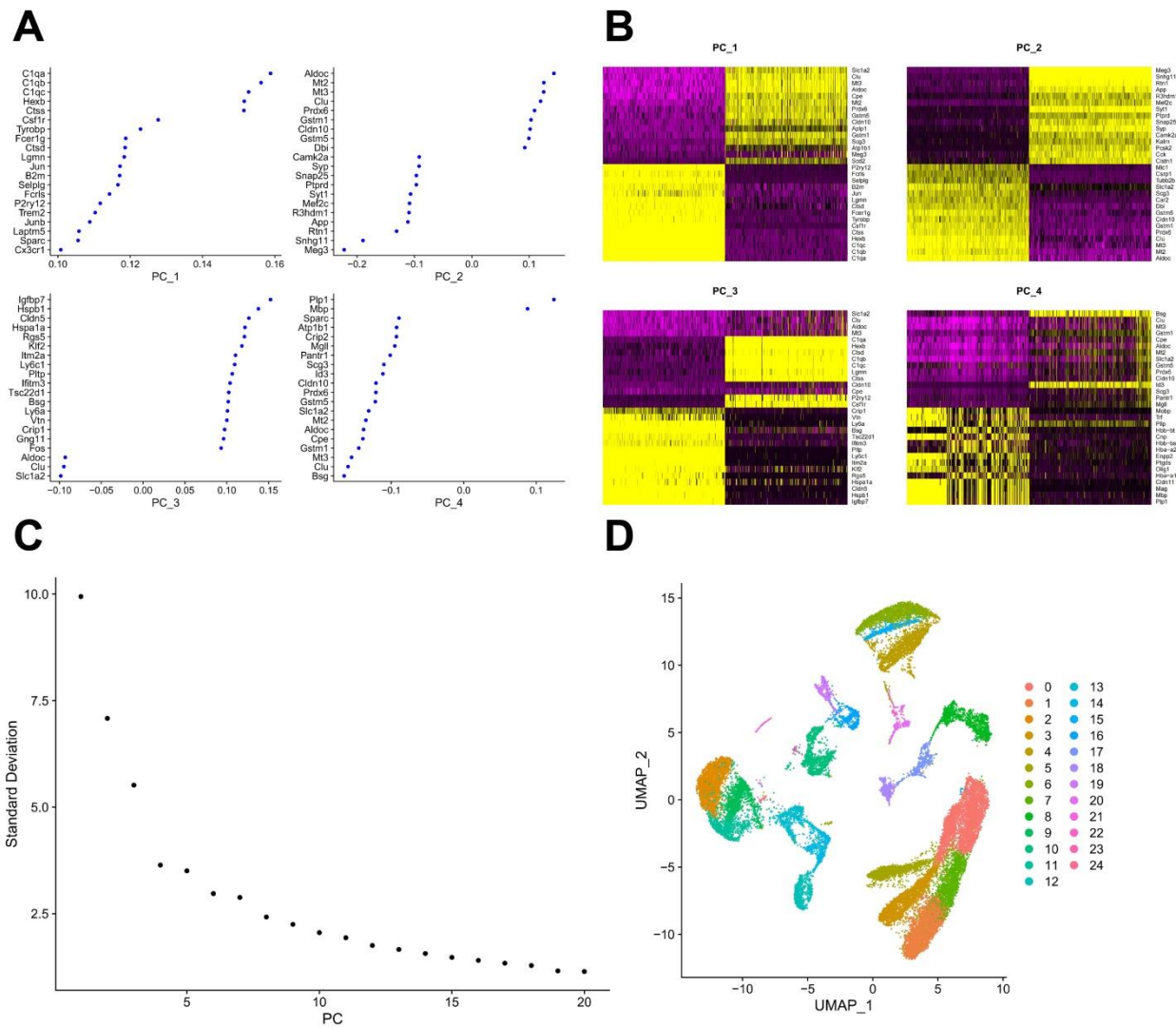
Supplementary figure 2. Quality Control of scRNA-seq Data.

Note: (A) Violin plots showing the distribution of gene counts per cell (nFeature_RNA), mRNA molecule counts per cell (nCount_RNA), and the percentage of mitochondrial genes (percent.mt) for each cell before quality control in the tumor group (Tumor) and normal group (Normal); (B) Violin plots showing the distribution of gene counts per cell (nFeature_RNA), mRNA molecule counts per cell (nCount_RNA), and the percentage of mitochondrial genes (percent.mt) for each cell after quality control in the Tumor and Normal groups; (C) Cell cycle status of each cell in the scRNA-seq data, with S.Score indicating the S phase and G2M.Score indicating the G2M phase.



Supplementary figure 3. Cell Clustering and Annotation in scRNA-seq Data.

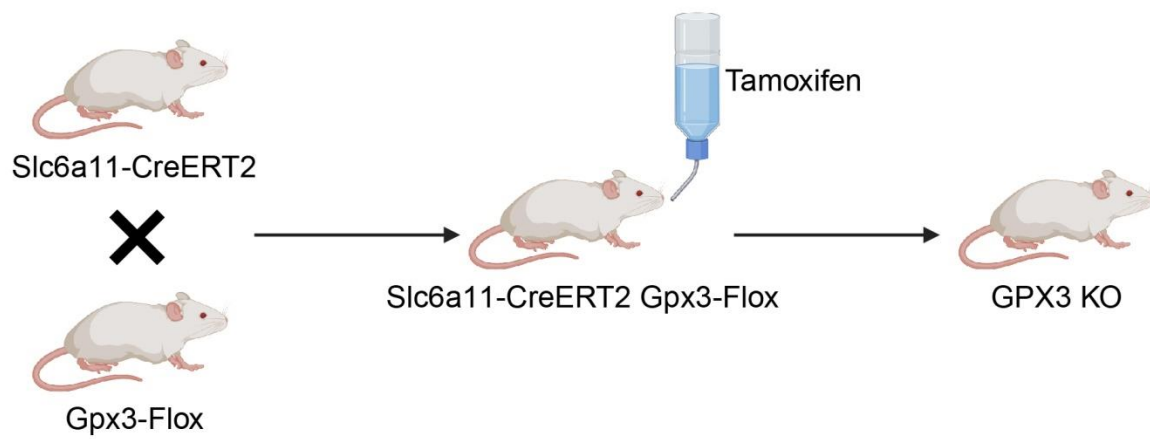
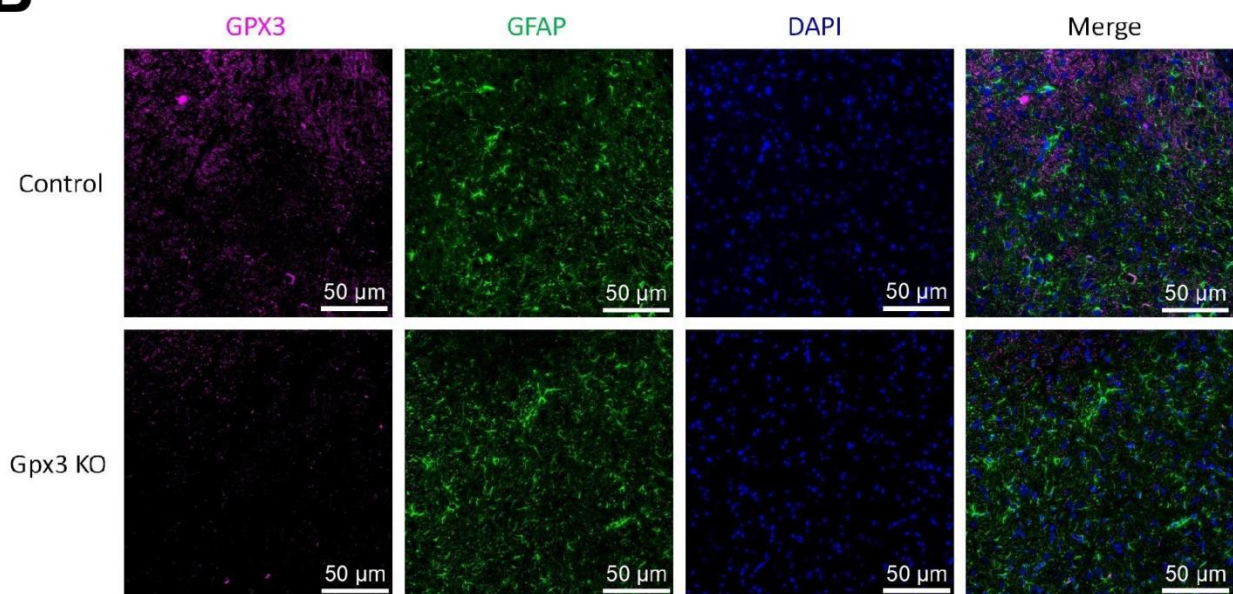
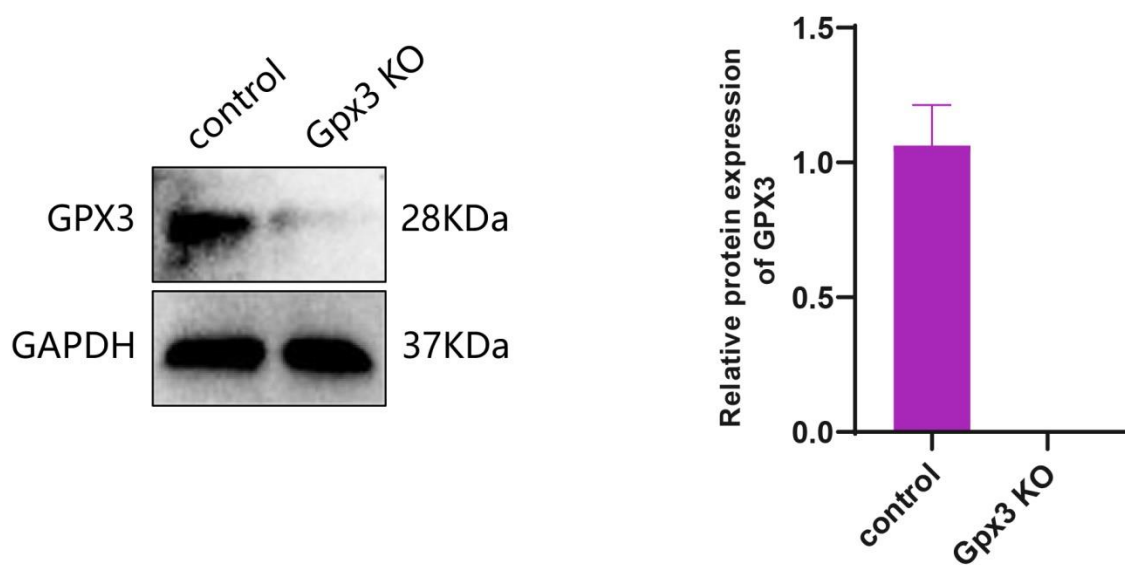
Note: (A) Scatter plot of the correlation between post-quality control data nCount_RNA and percent.mt, as well as nCount_RNA and nFeature_RNA; (B) PCA analysis results of cells from various sample sources (each point in the plot represents a cell, with different colors indicating different samples); (C) p-values of the 20 PCs obtained from PCA analysis.



Supplementary figure 4. Dimensionality Reduction and Clustering of scRNA-seq Data.

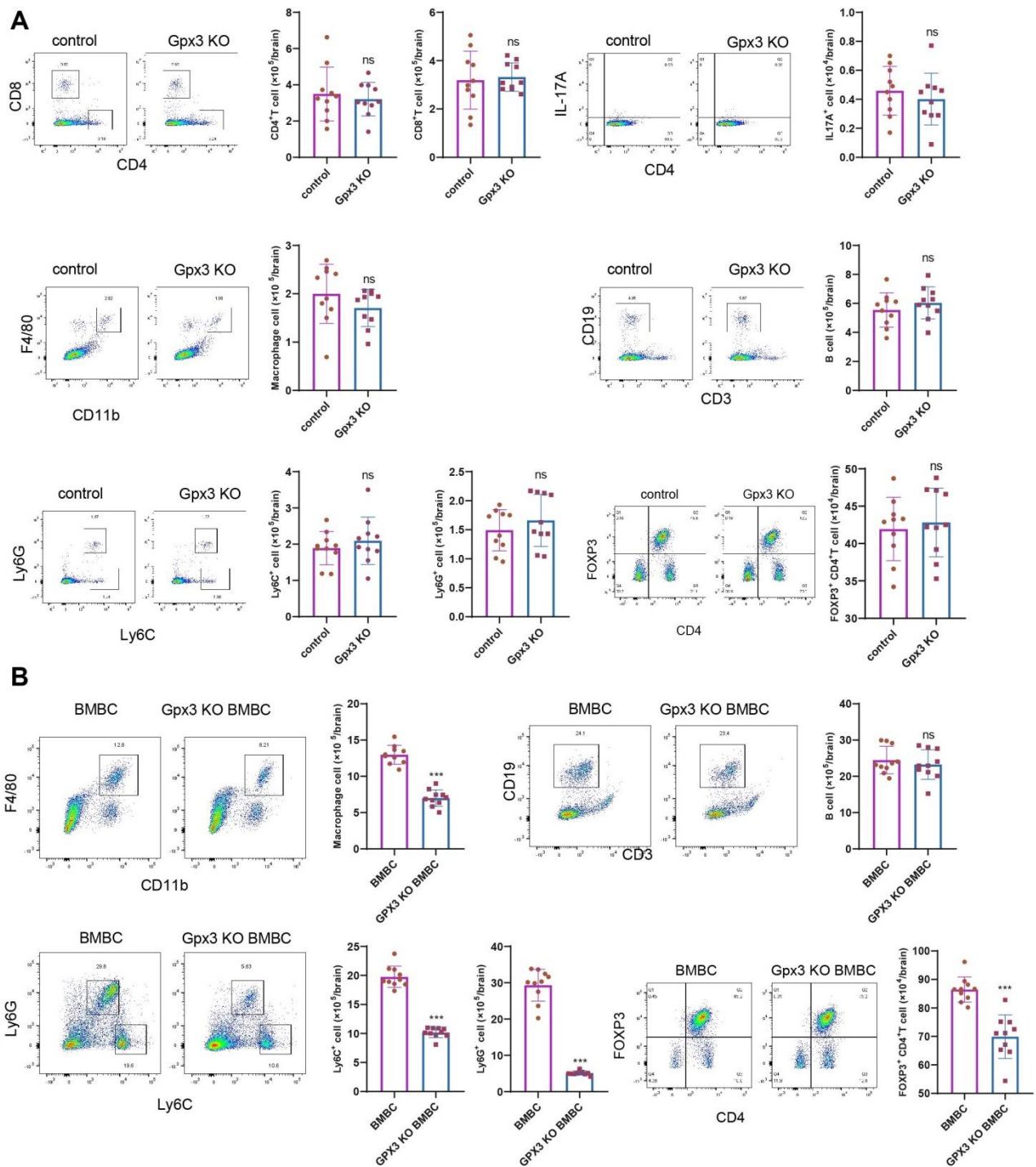
Note: (A-B) Heatmaps showing the expression of feature genes and their expression levels in the top 4 principal components (PCs) before performing the principal component analysis (PCA) (yellow represents high expression, purple represents low expression); (C) Scree plot depicting the variance contribution of each principal component; (D) UMAP clustering analysis dividing cells into 25 cell clusters.

Note: (A-B) Heatmaps showing the expression of feature genes and their expression levels in the top 4 PCs before performing PCA (yellow represents high expression, purple represents low expression); (C) p-values for the top 20 PCs obtained from PCA analysis; (D) Scree plot depicting the variance contribution of each principal component; (E) Heatmap showing the top 10 marker gene expression for each cell cluster.

A**B****C**

Supplementary figure 6. Generation of GPX3 Conditional Knockout (KO) Mice in Astrocytes.

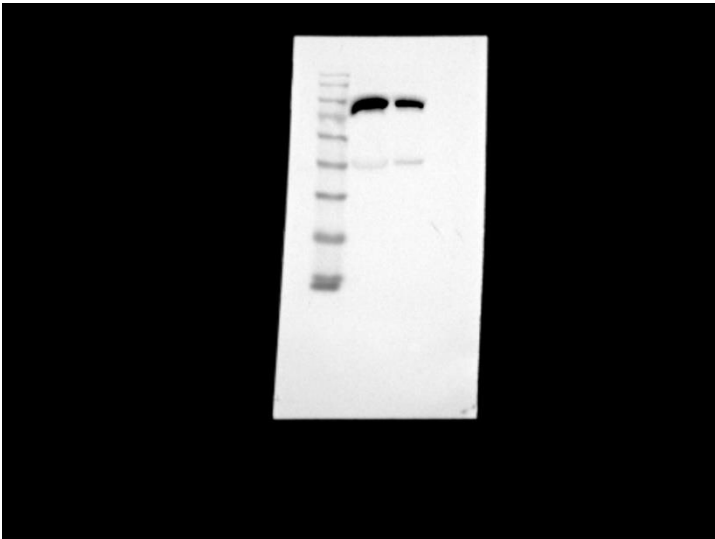
Note: (A) Schematic representation of the experimental model for conditional knockout of GPX3 in astrocytes by crossing astrocyte-specific Slc6a11-CreERT2 mice with Gpx3-Flox mice (Created with BioRender.com); (B) Immunofluorescence staining showing GPX3 protein levels in brain tissue of each mouse group (scale bar: 50 μ m); (C) Western blot analysis of GPX3 protein levels in brain tissue of each mouse group; n=5.



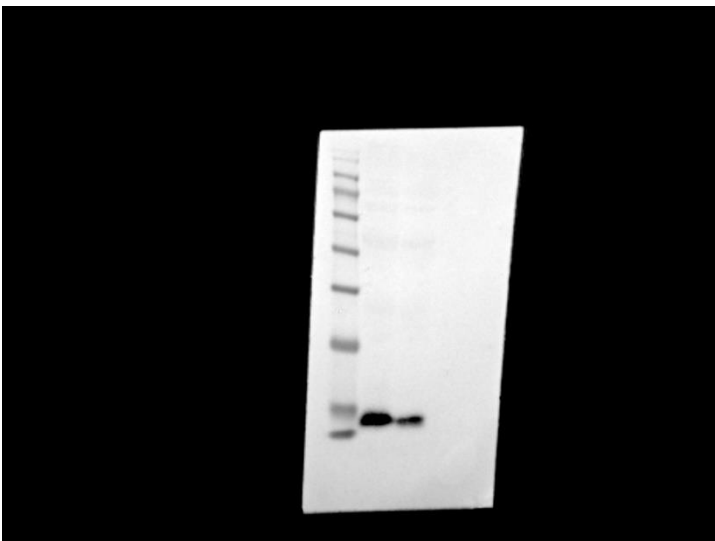
Supplementary figure 7. Differences in Immune Cell Subpopulations in Mouse Brain Tissue Before and After Tumor Implantation.

Note: (A) Flow cytometry analysis of the number of CD4⁺ T cells, CD8⁺ T cells, Th17 cells, macrophages, B cells, Ly6C⁺ Ly6G⁻ monocytes, Ly6Cint Ly6G⁺ neutrophils and FOXP3⁺ CD4⁺ T cells (regulatory T cells) in brain tissue of each mouse group before tumor implantation; (B) Flow cytometry analysis of the number of macrophages, B cells, Ly6C⁺ Ly6G⁻ monocytes, Ly6Cint

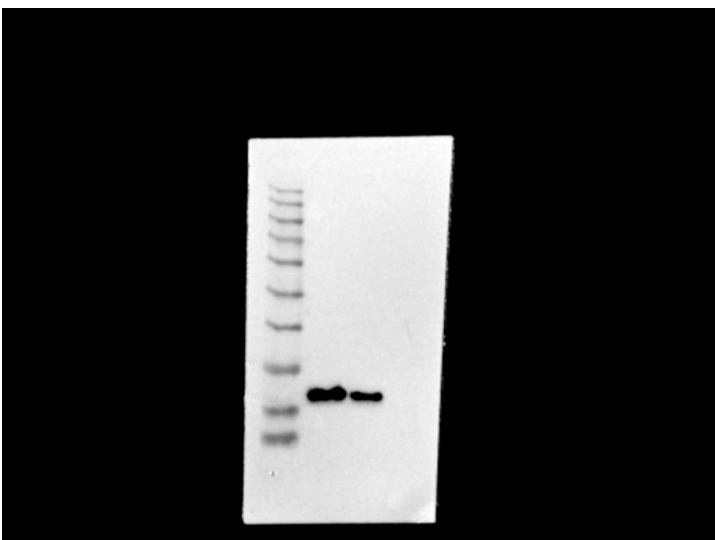
Ly6G⁺ neutrophils and FOXP3⁺ CD4⁺ T cells (regulatory T cells) in brain tissue of each mouse group after tumor implantation; Counting data were presented as Mean $\pm$ SD. Compared to the control group or BMBC group; ns, no difference, ** $P < 0.01$; *** $P < 0.001$; n=10.



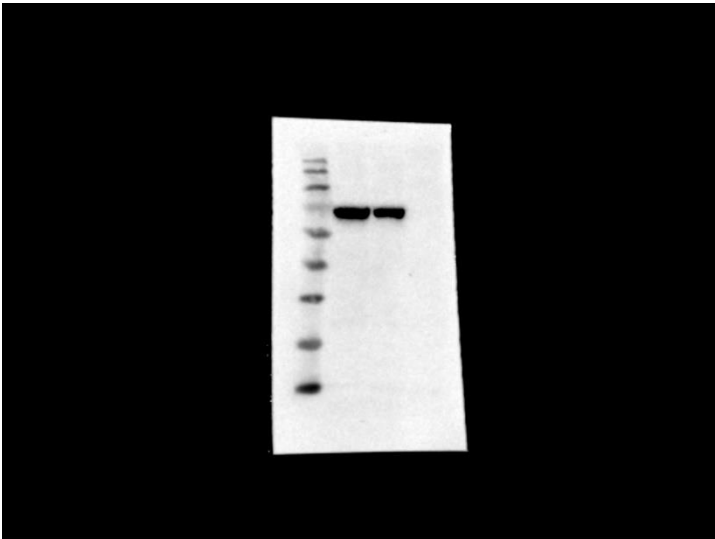
Supplementary Figure 8: Original un-cropped image for figure 4F-1



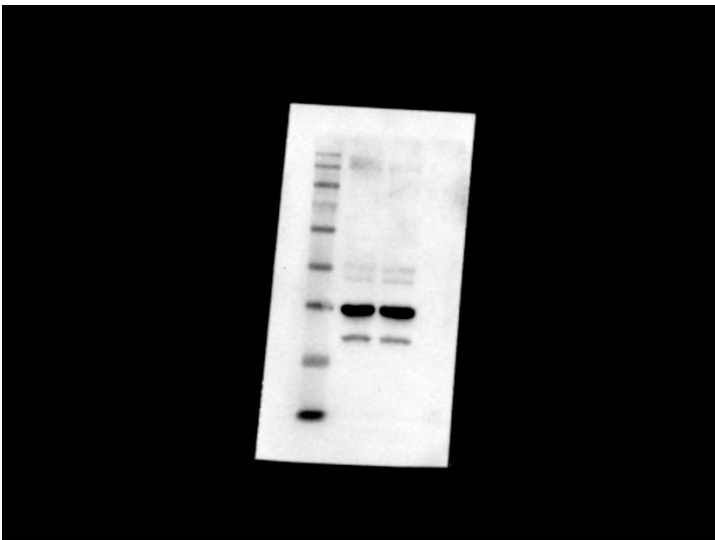
Supplementary Figure 9: Original un-cropped image for figure 4F-2



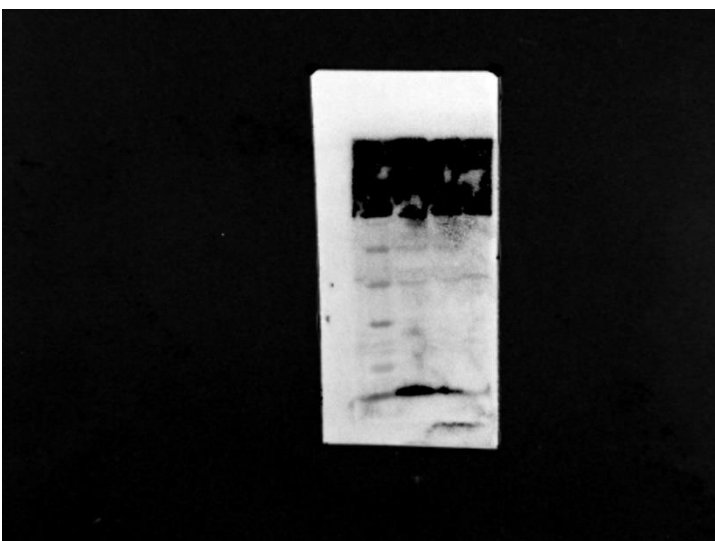
Supplementary Figure 10: Original un-cropped image for figure 4F-3



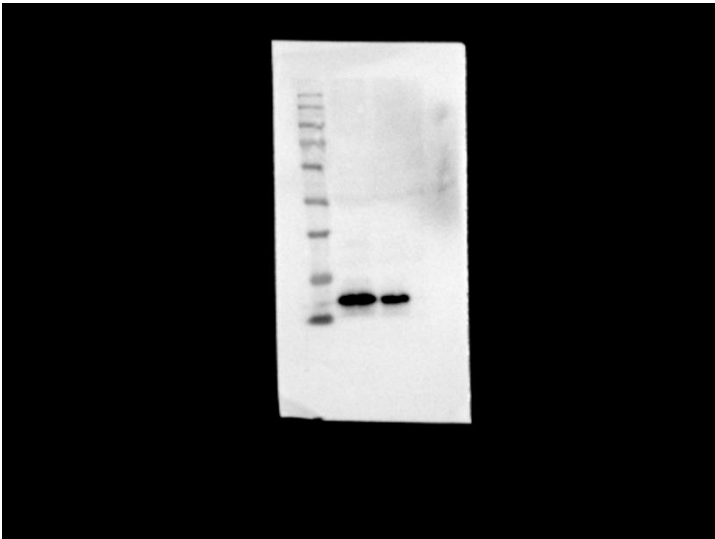
Supplementary Figure 11: Original un-cropped image for figure 4F-4



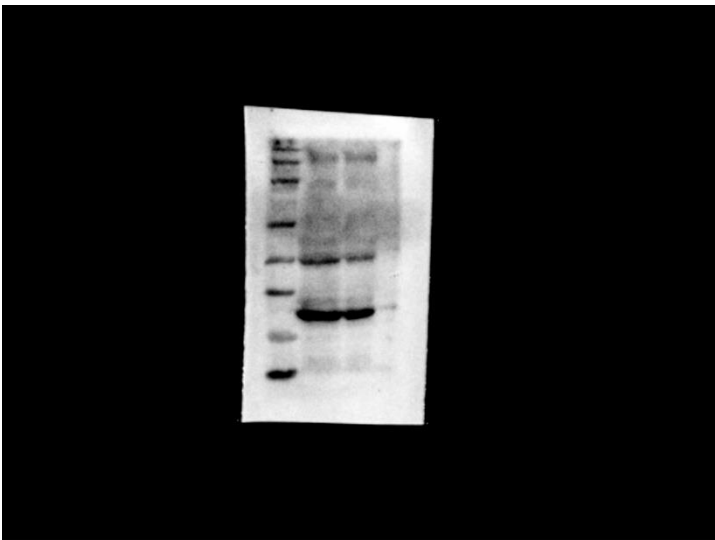
Supplementary Figure 12: Original un-cropped image for figure 4F-5



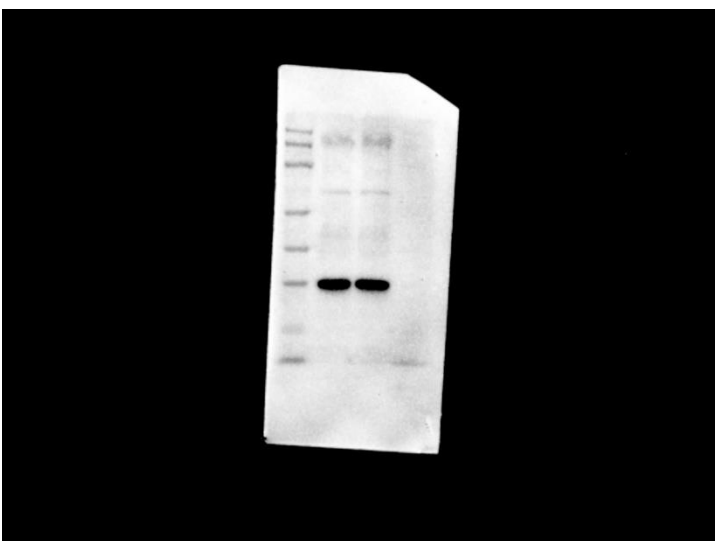
Supplementary Figure 13: Original un-cropped image for figure 5D-1



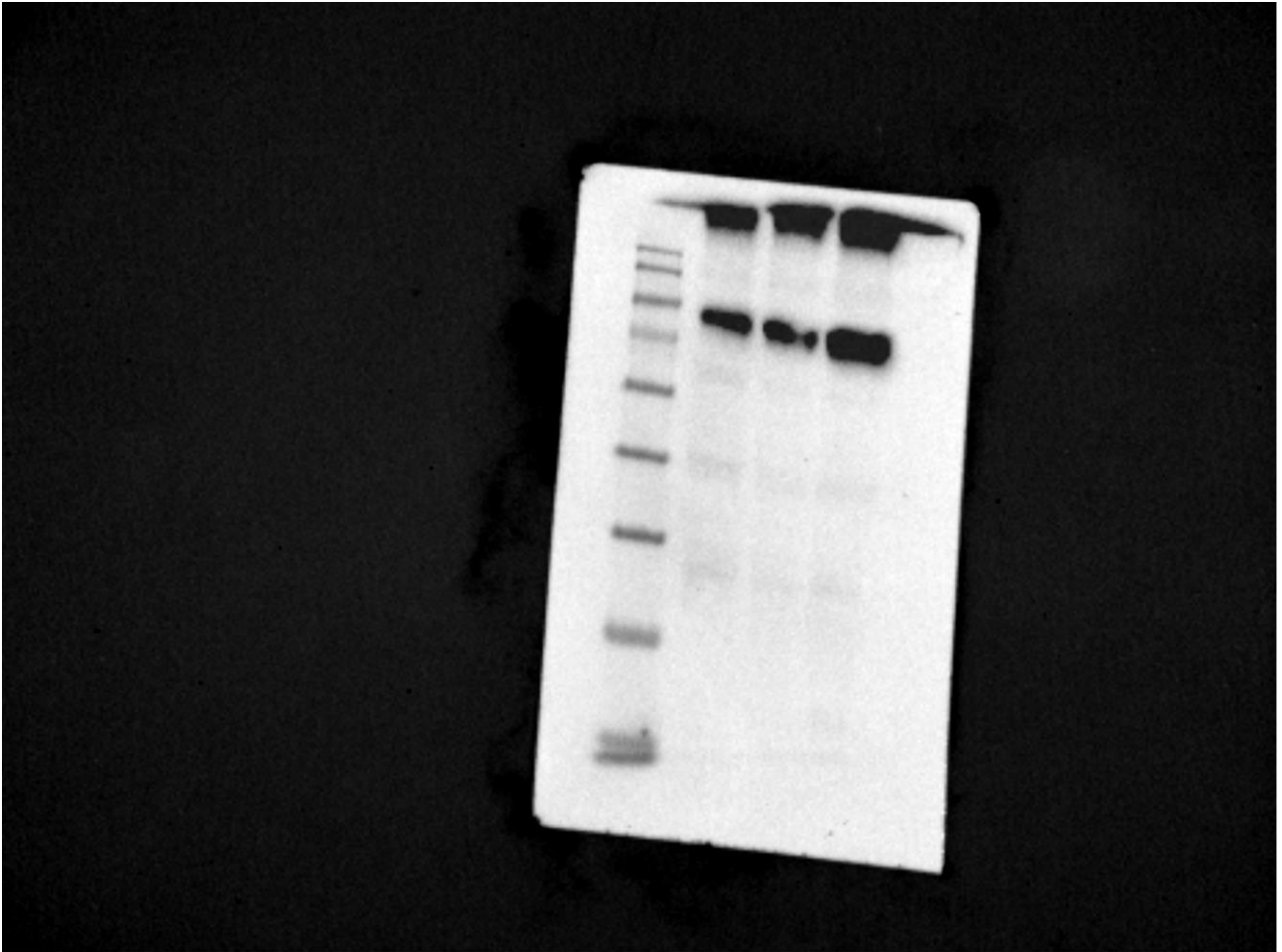
Supplementary Figure 14: Original un-cropped image for figure 5D-2



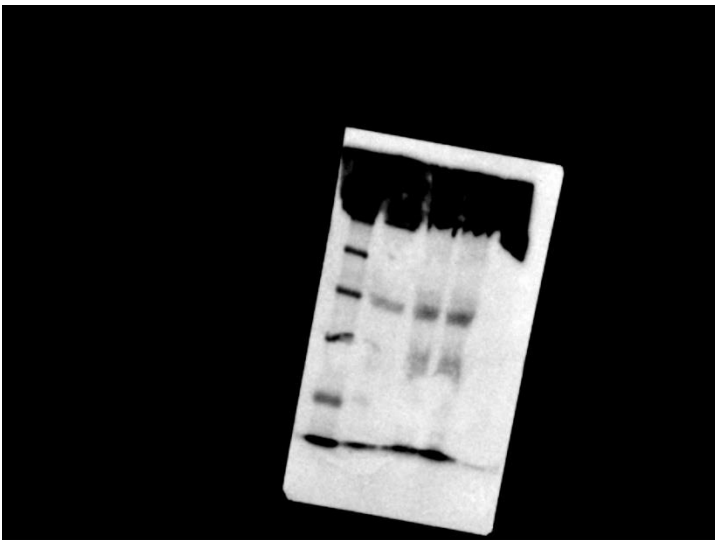
Supplementary Figure 15: Original un-cropped image for figure 5D-3



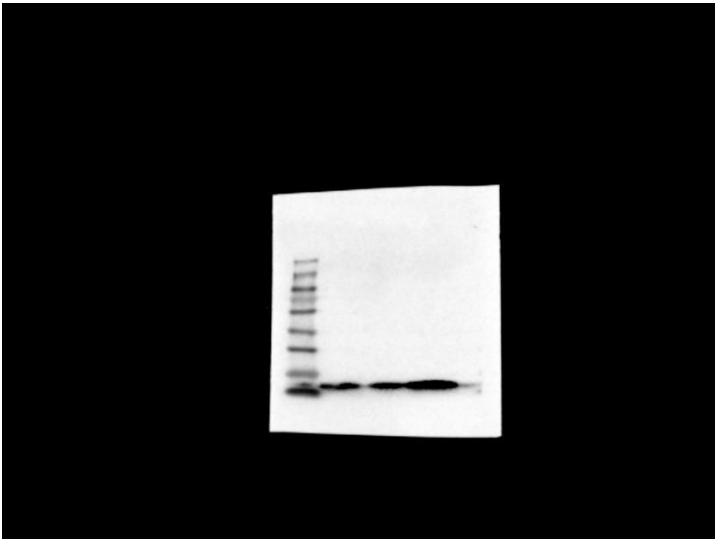
Supplementary Figure 16: Original un-cropped image for figure 5D-4



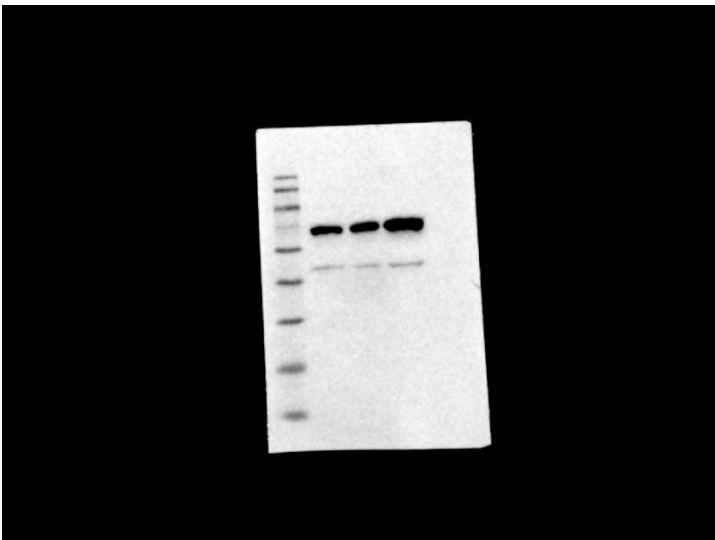
Supplementary Figure 17: Original un-cropped image for figure 5H-1



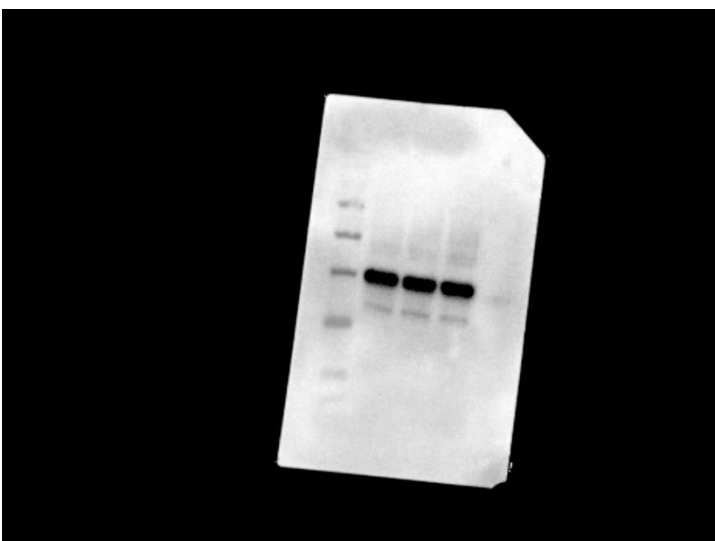
Supplementary Figure 18: Original un-cropped image for figure 5H-2



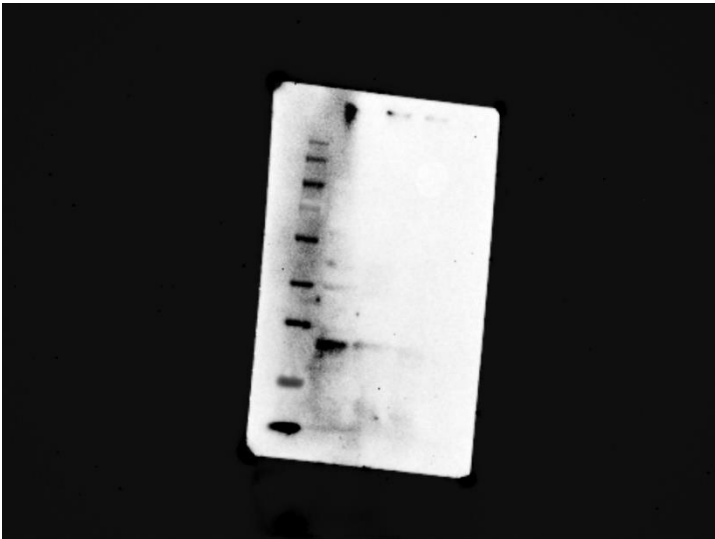
Supplementary Figure 19: Original un-cropped image for figure 5H-3



Supplementary Figure 20: Original un-cropped image for figure 5H-4



Supplementary Figure 21: Original un-cropped image for figure 5H-5



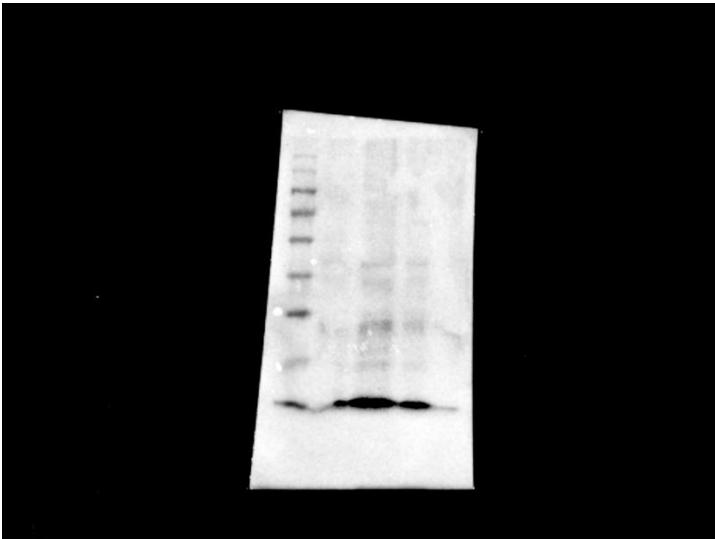
Supplementary Figure 22: Original un-cropped image for figure 6C-1



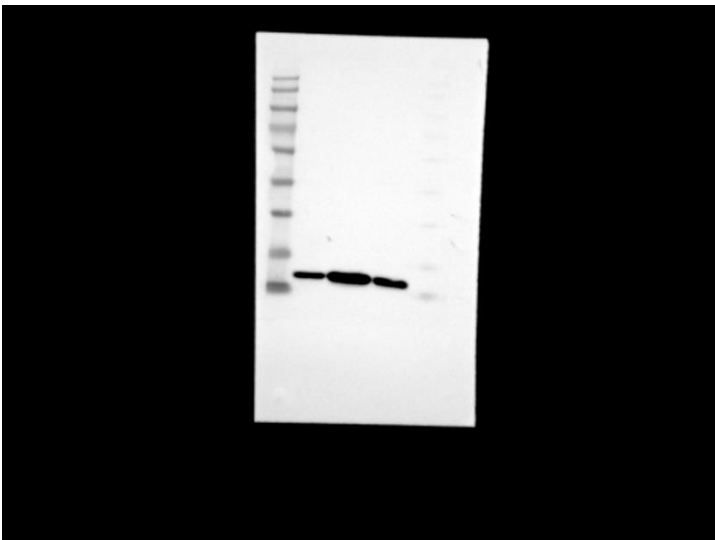
Supplementary Figure 23: Original un-cropped image for figure 6C-2



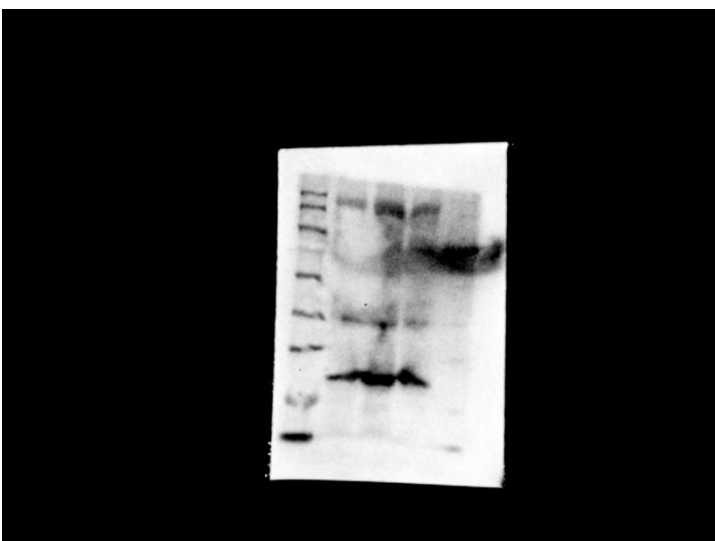
Supplementary Figure 24: Original un-cropped image for figure 6C-3



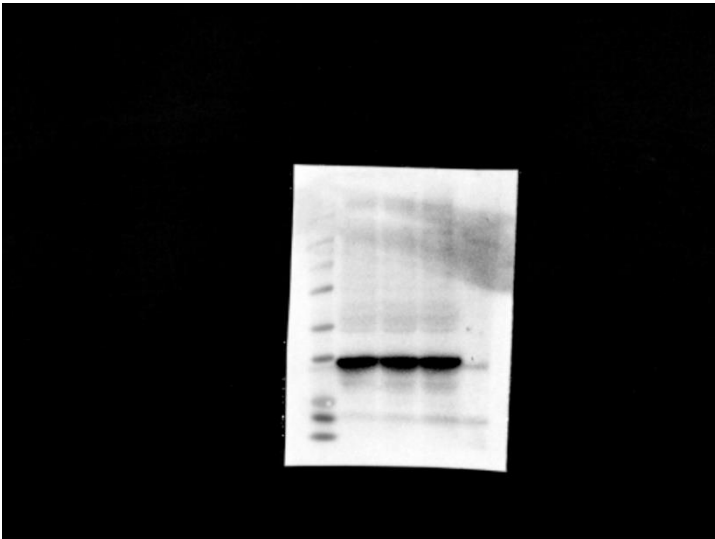
Supplementary Figure 25: Original un-cropped image for figure 6G-1



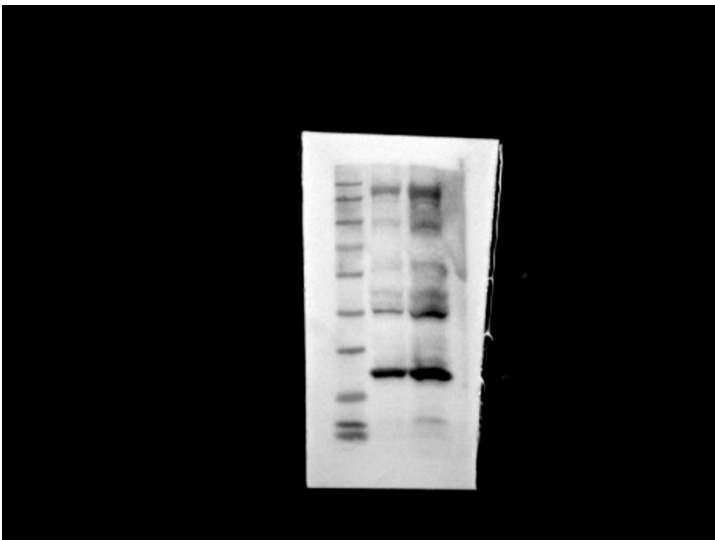
Supplementary Figure 26: Original un-cropped image for figure 6G-2



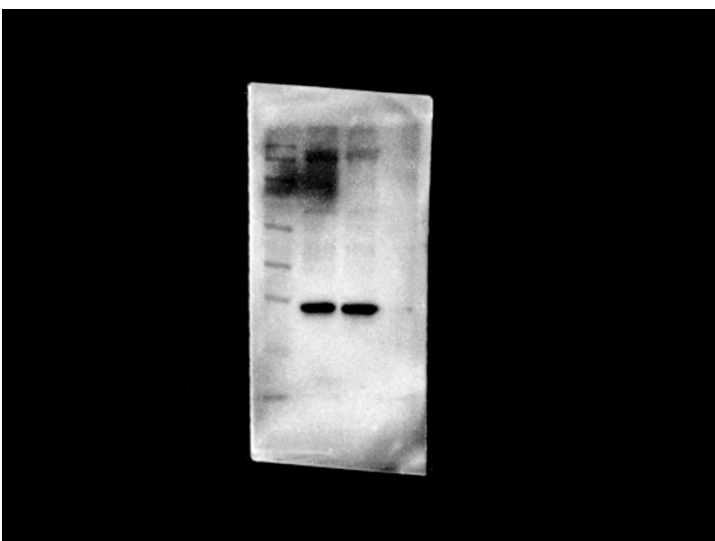
Supplementary Figure 27: Original un-cropped image for figure 6G-3



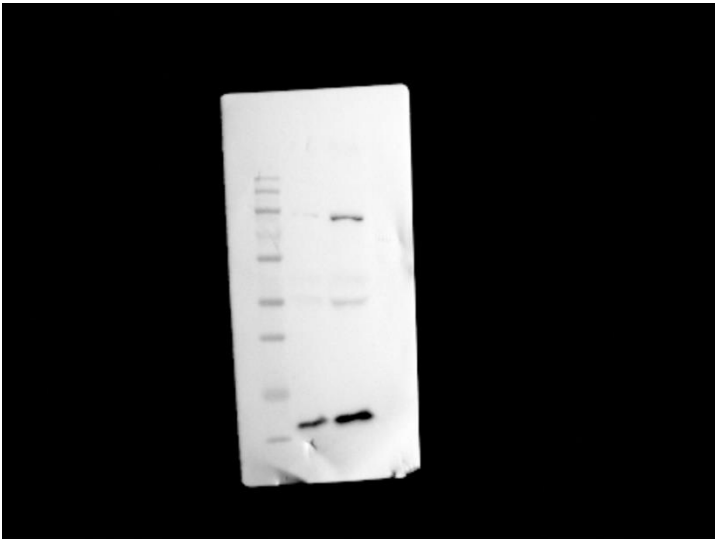
Supplementary Figure 28: Original un-cropped image for figure 6G-4



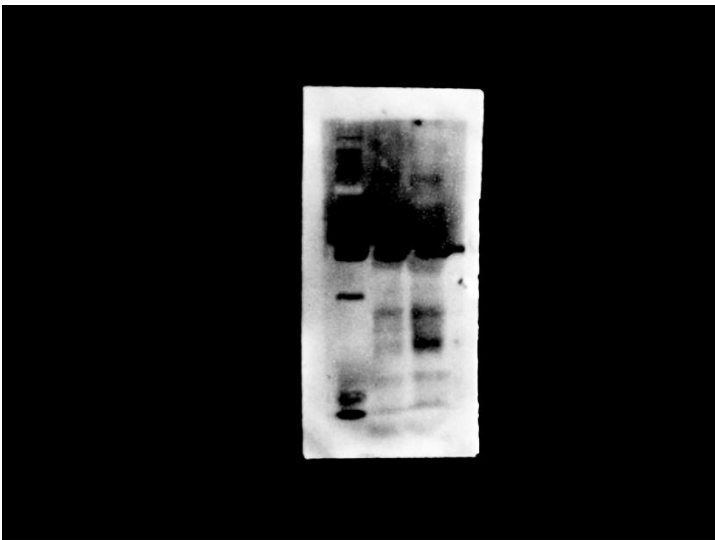
Supplementary Figure 29: Original un-cropped image for figure 7A-1



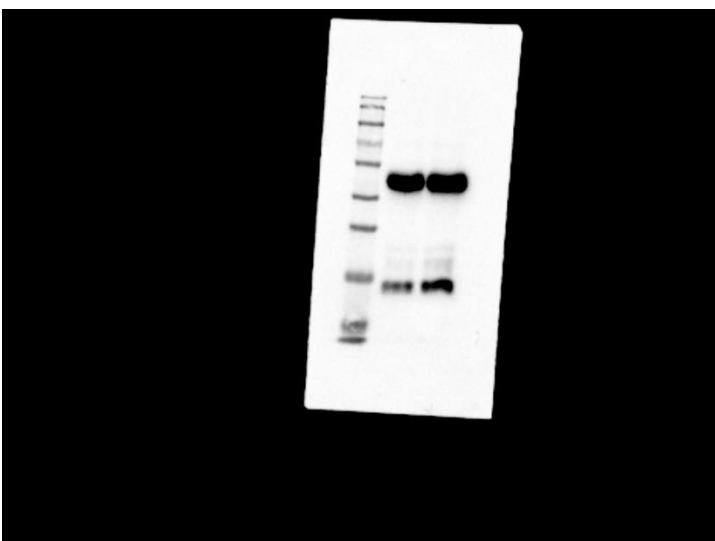
Supplementary Figure 30: Original un-cropped image for figure 7A-2



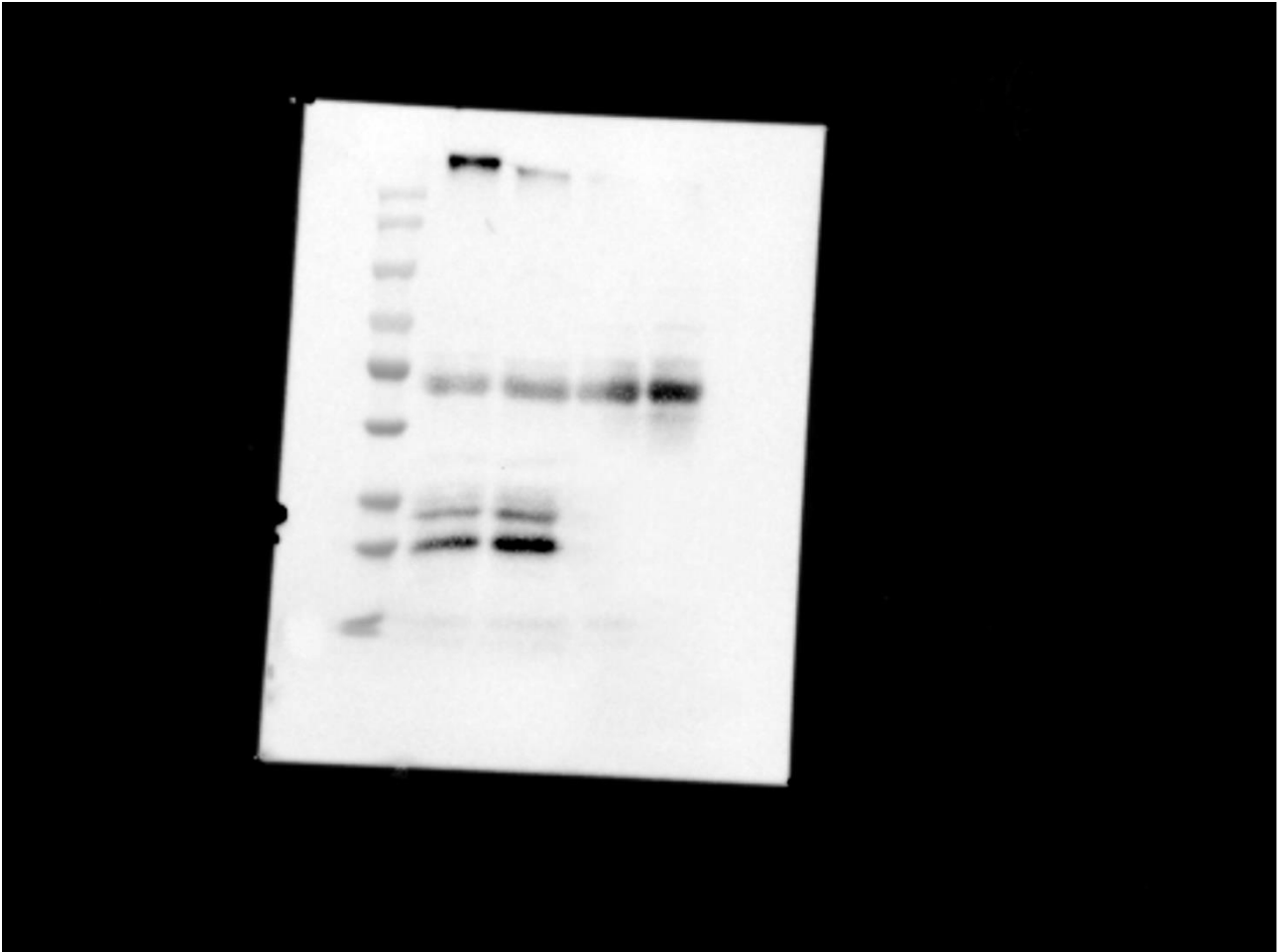
Supplementary Figure 31: Original un-cropped image for figure 7D-1



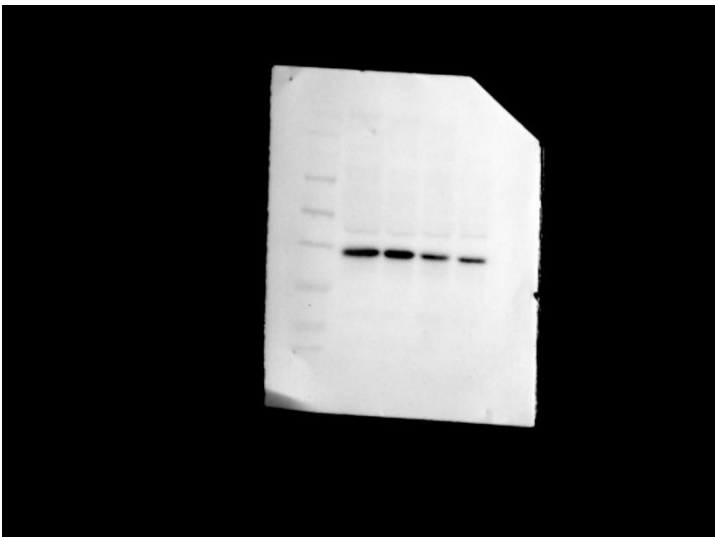
Supplementary Figure 32: Original un-cropped image for figure 7D-2



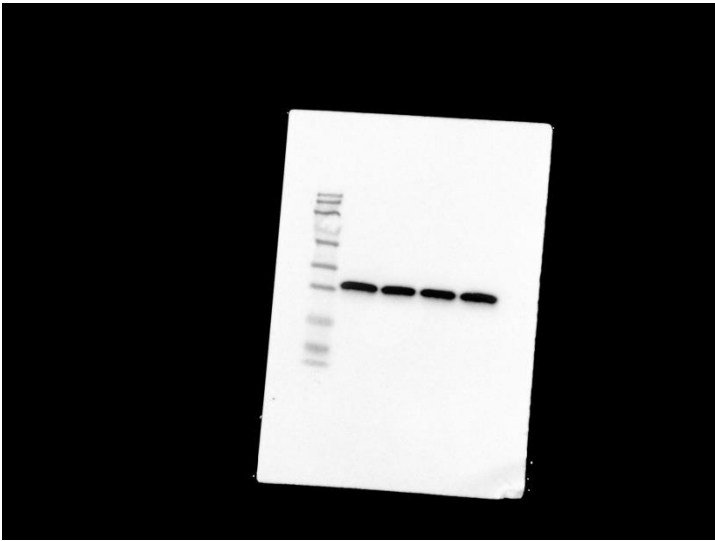
Supplementary Figure 33: Original un-cropped image for figure 7D-3



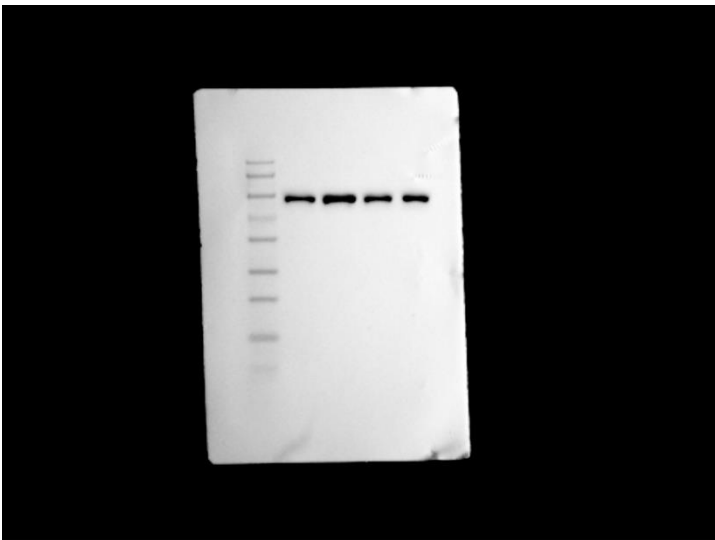
Supplementary Figure 34: Original un-cropped image for figure 7E-1



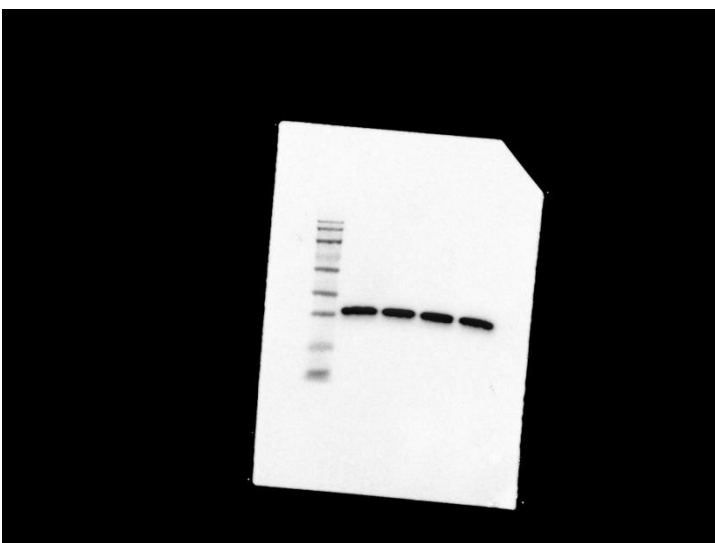
Supplementary Figure 35: Original un-cropped image for figure 7E-2



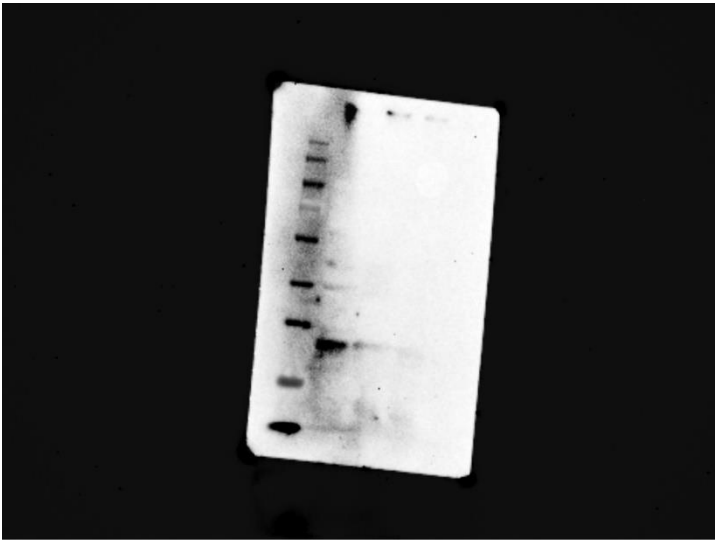
Supplementary Figure 36: Original un-cropped image for figure 7E-3



Supplementary Figure 37: Original un-cropped image for figure 8H-1



Supplementary Figure 38: Original un-cropped image for figure 8H-2



Supplementary Figure 39: Original un-cropped image for supplementary figure 6C-1



Supplementary Figure 40: Original un-cropped image for supplementary figure 6C-2