

Supporting information

Crizotinib and its enantiomer suppress ferroptosis by decreasing PE-O-PUFA content

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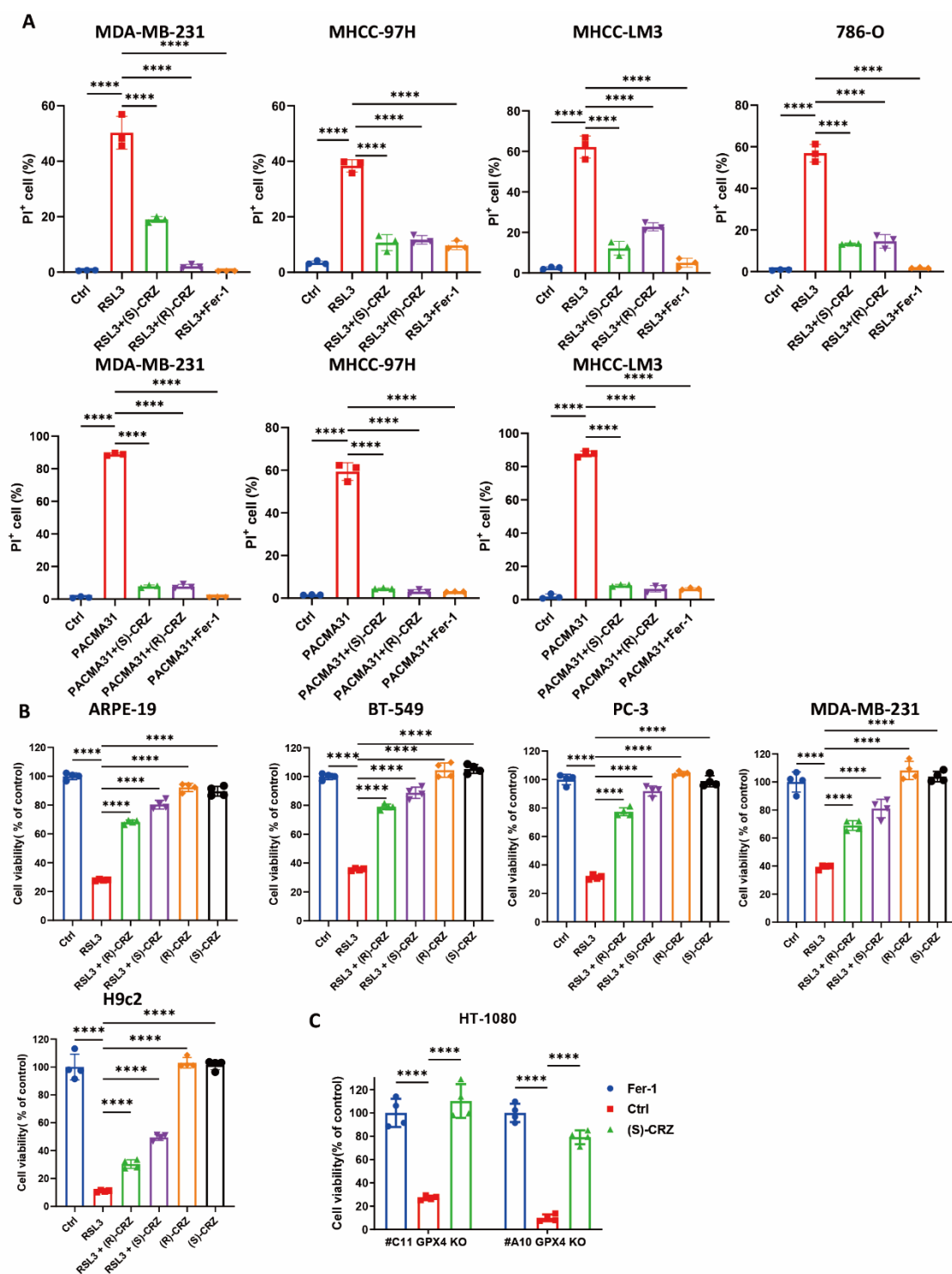
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Supporting information

Supplemental Figures S1-S3: Viability and death of cells with indicated treatment; Gene sequencing result of *AGPAT3* knockout monoclonal cell lines and cell sensitivity to ferroptosis with either *AGPAT3* overexpression or knockout; Cellular lipid-ROS in CD4⁺ T cells from TdLN and nTdLN of tumor-bearing mice.

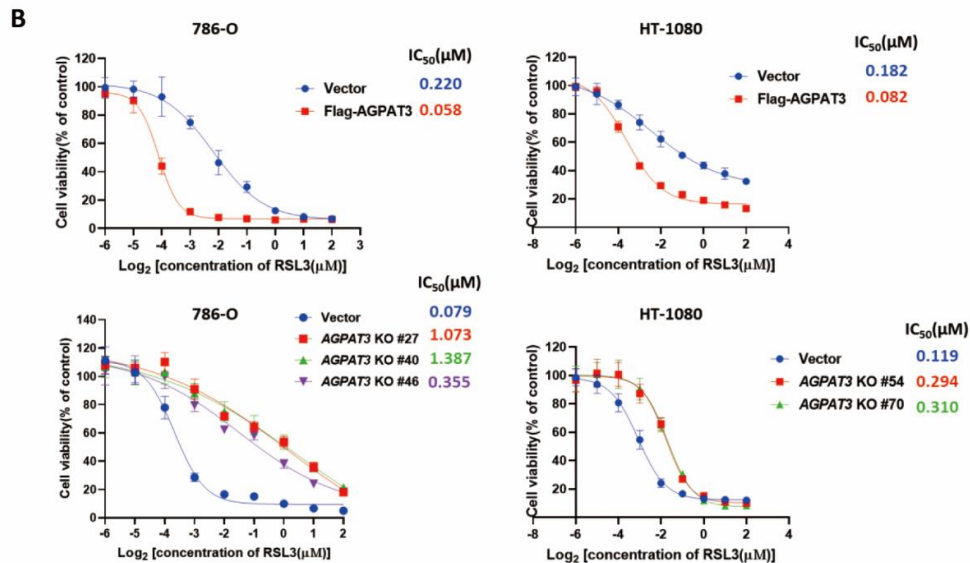
Uncropped original western blots.



Supplemental Figure 1. Enantiomers of CRZ protect cells from ferroptosis

(A-B) (S)-CRZ (1 μ M) and (R)-CRZ (1 μ M) inhibit ferroptosis in multiple cell lines. Cell death (A) and cell viability (B) were detected at appropriate time. ARPE-19, human retinal

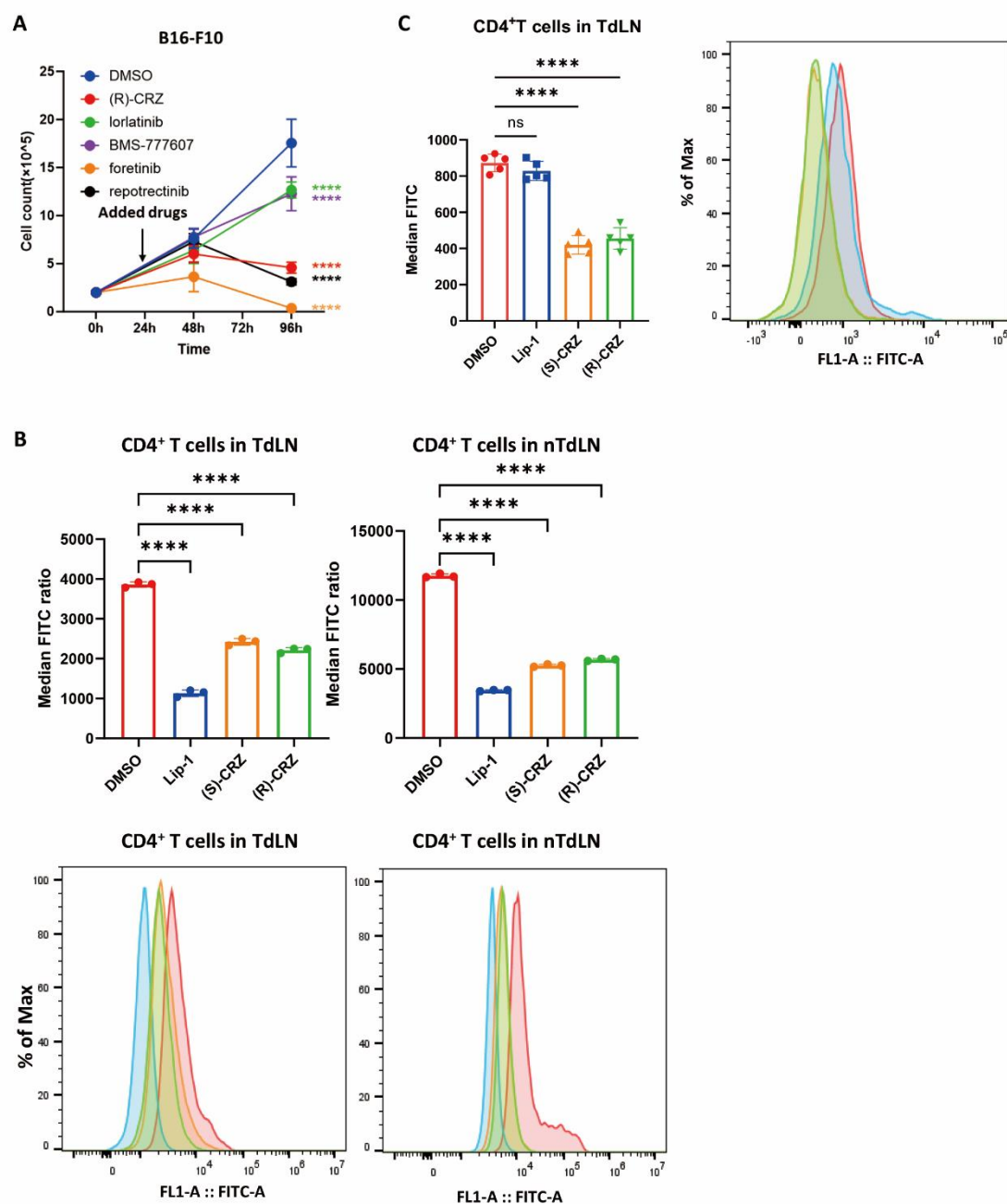
pigmented epithelium; BT-549, epithelial cells from invasive breast ductal tumor; MDA-MB-231, epithelial-like cell of breast adenocarcinoma; PC-3, prostatic adenocarcinoma. (C) (S)-CRZ (3 μ M) inhibits spontaneous ferroptosis in *GPX4* KO monoclonal HT-1080 cell lines. Cell viability was measured after 12 h of treatments. Data in Supplemental Figure 1 were presented as the mean $\pm$ S.D., n = 3 independent repeats in Supplemental 1A, n = 4 independent repeats in Supplemental Figure 1B and 1C.



Supplemental Figure 2. AGPAT3 gene knockout increase the resistance to ferroptosis

(A) Sanger gene sequencing technology was used to identify the knockout effect of the AGPAT3 gene in various monoclonal cell strains. (B) AGPAT3 overexpression increased

cell sensitivity to ferroptosis, while *AGPAT3* knockout rendered cells more resistant to ferroptosis. Cell viability of HT-1080 was measured after 8 h of treatments. Cell viability of 786-O was measured after 10 h of treatments. Data in Supplemental Figure 2B were presented as the mean $\pm$ S.D., n = 3 independent repeats.



Supplemental Figure 3. (S)-CRZ and (R)-CRZ eliminate lipid-ROS in CD4⁺ T cell

(A) The proliferation curves of B16-F10 cells with different drug treatments. Groups were distinguished by color, and statistical significance was assessed by comparing differences with the DMSO group as a control at 96 h. (B) Lip-1 (2 μ M), (S)-CRZ (2 μ M) and (R)-CRZ (2 μ M) treatments reduce lipid-ROS in CD4⁺ T cells isolated from TdLNs and nTdLNs of B16-F10 tumor subcutaneous bearing mice. Lymph cells from lymph nodes

separated from tumor-bearing mice were treated with the indicated compounds for 3 h followed by flow cytometry analysis. Lipid-ROS was detected by fluorescence of C11-BODIPY 581/591 at the FITC channel. Data in Figures S3A-S3B were presented as the mean $\pm$ S.D., n = 3 independent repeats. (C) (S)-CRZ (5 mg/kg) and (R)-CRZ (5 mg/kg) treatment significantly reduce lipid-ROS in CD4⁺ T cells from TdLNs of B16-F10 tumor subcutaneous bearing mice. CD4⁺ T cells were isolated from TdLNs of tumor-bearing mice with intraperitoneal injection of indicated drug for 16 d. Lipid-ROS was detected by fluorescence of C11-BODIPY 581/591 at the FITC channel. Data was presented as the mean $\pm$ S.D., n = 5 independent repeats.

Figure 1F

Original Western blot

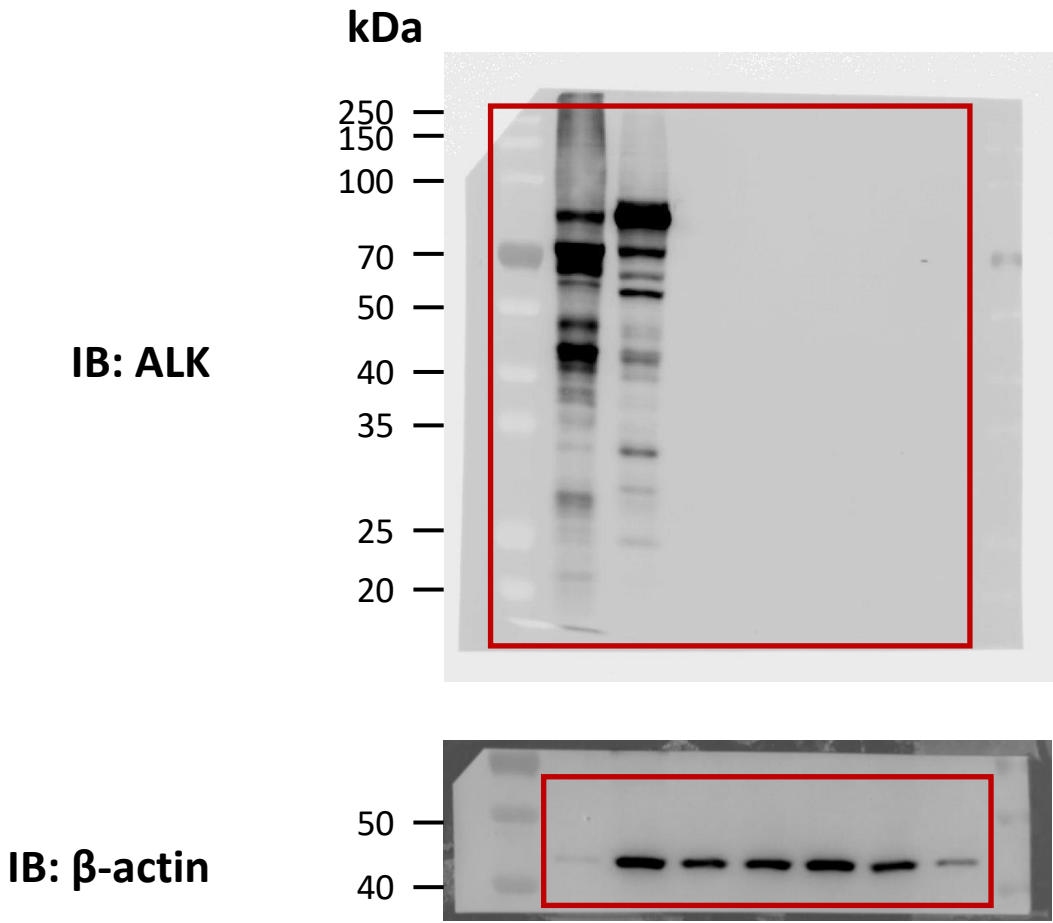
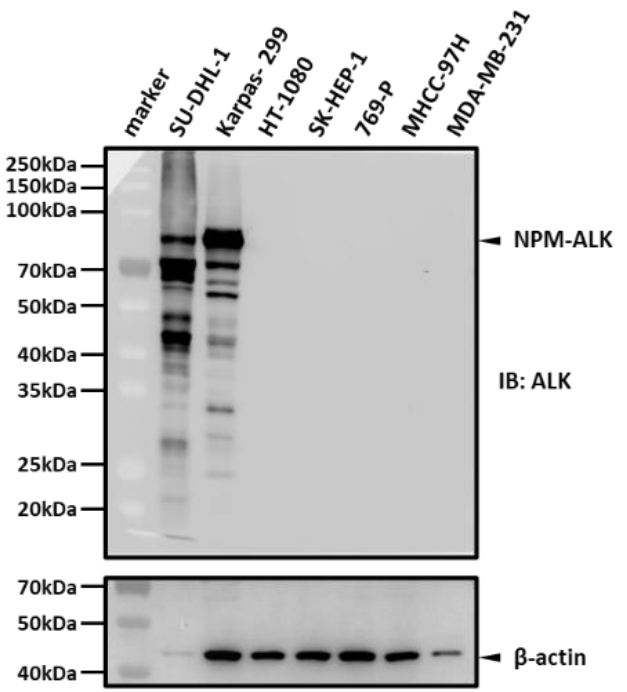


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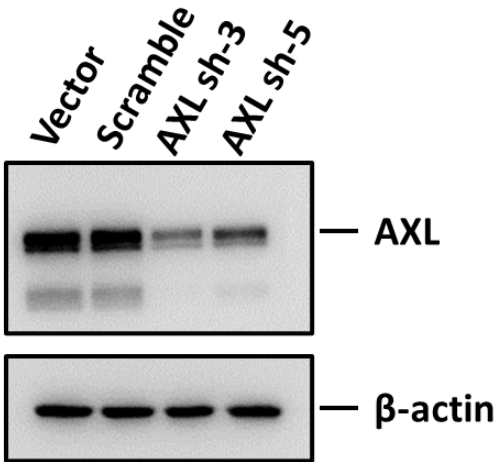
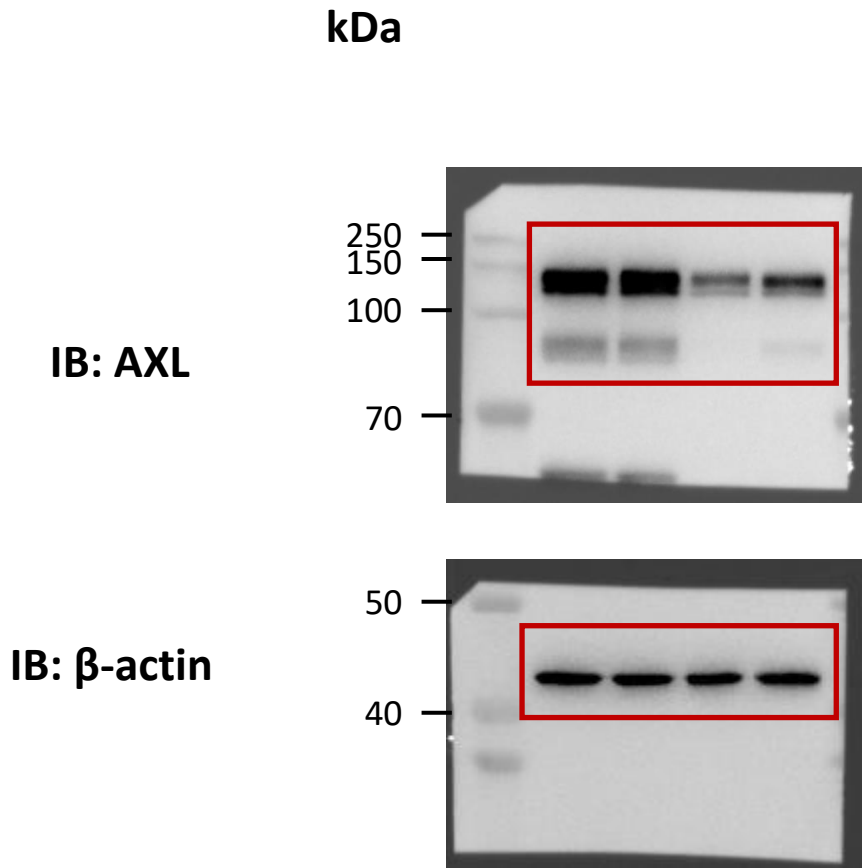


Colorimetric and chemiluminescent were merged.
Red boxes indicate the cropped areas that were used in the figure.

Figure 1H

Original Western blot

Figure presented in manuscript



Membrane used for β -actin detection was cut from the same membrane for AXL.
Colorimetric and chemiluminescent were merged.

Red boxes indicate the cropped areas that were used in the figure.

Figure 1I

Original Western blot

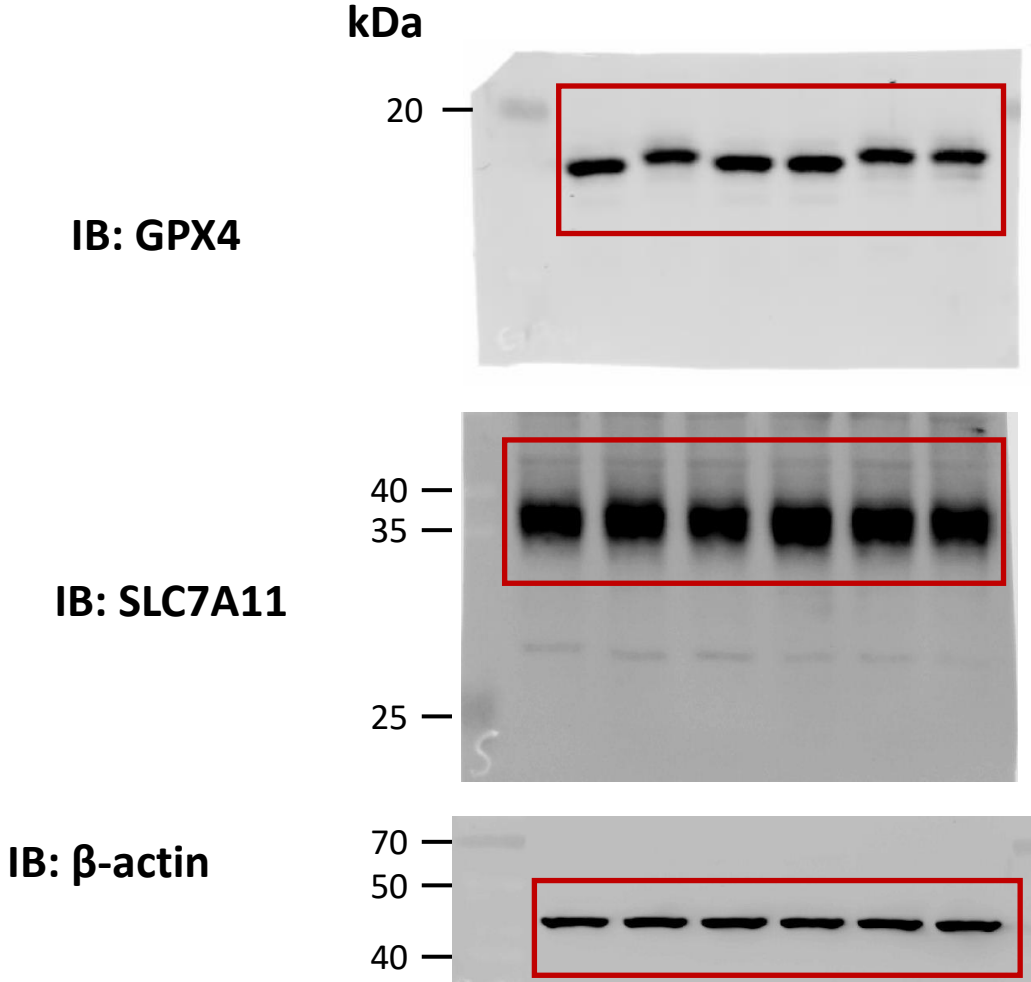
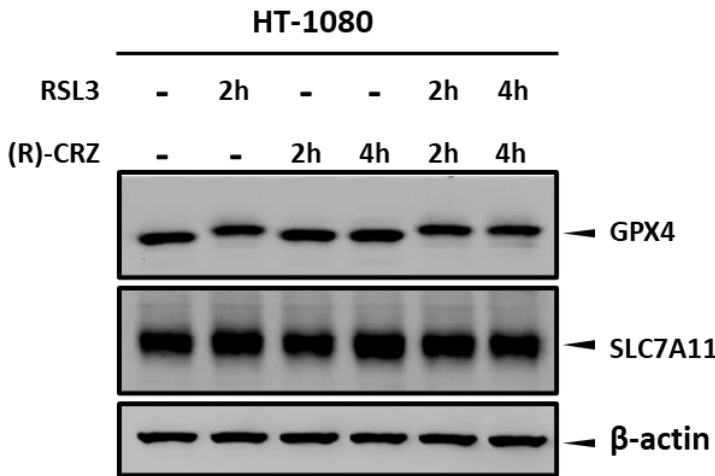


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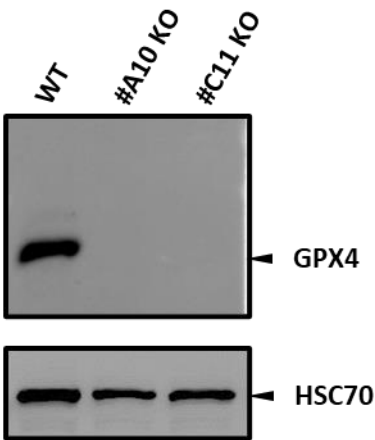
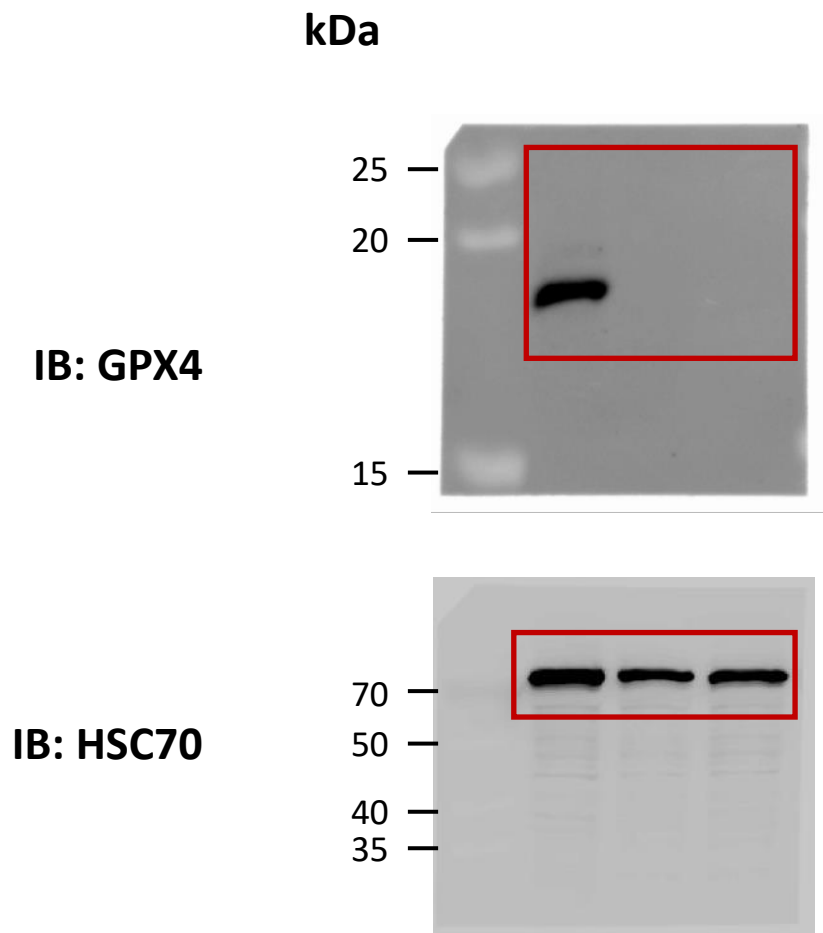


Colorimetric and chemiluminescent were merged.
Red boxes indicate the cropped areas that were used in the figure.

Figure 1J

Original Western blot

Figure presented in manuscript



Colorimetric and chemiluminescent were merged.
Red boxes indicate the cropped areas that were used in the figure.

Figure 3G

Original Western blot

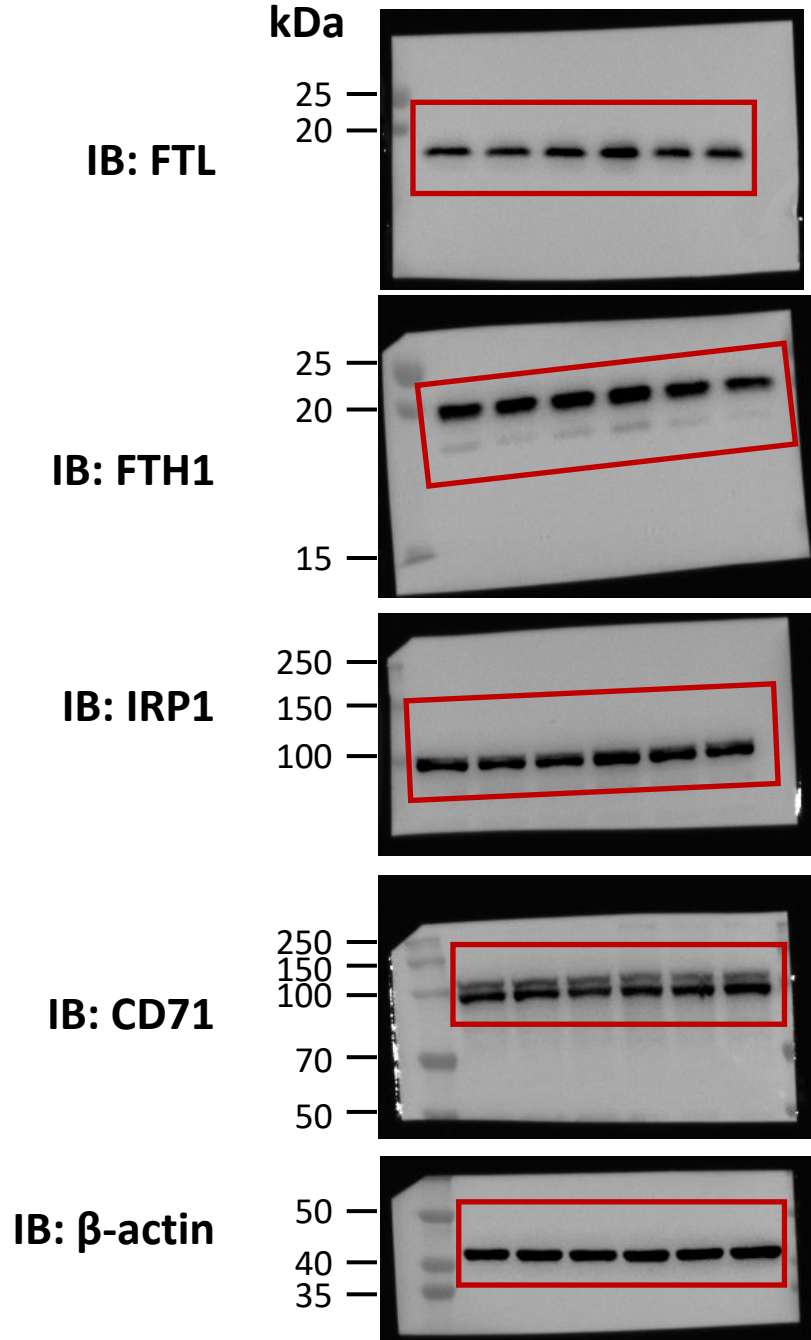
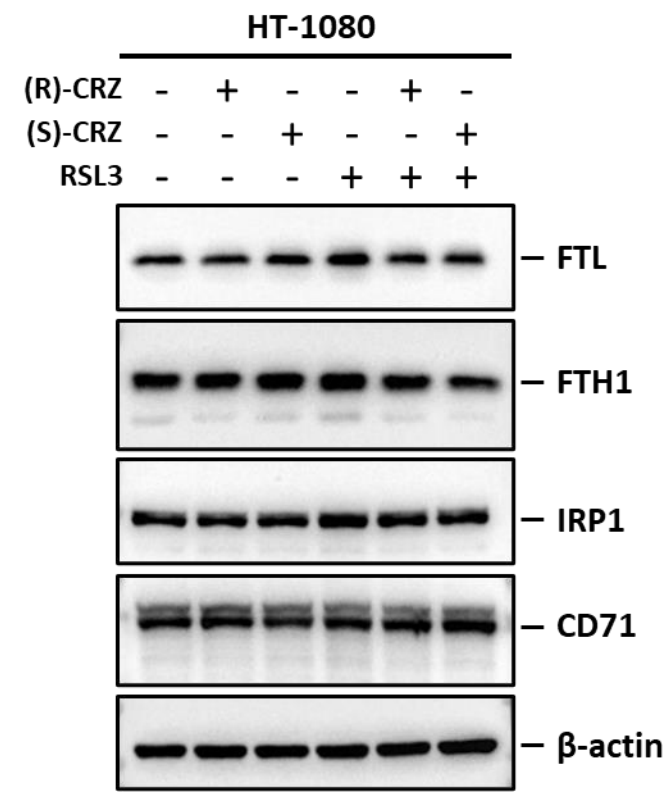
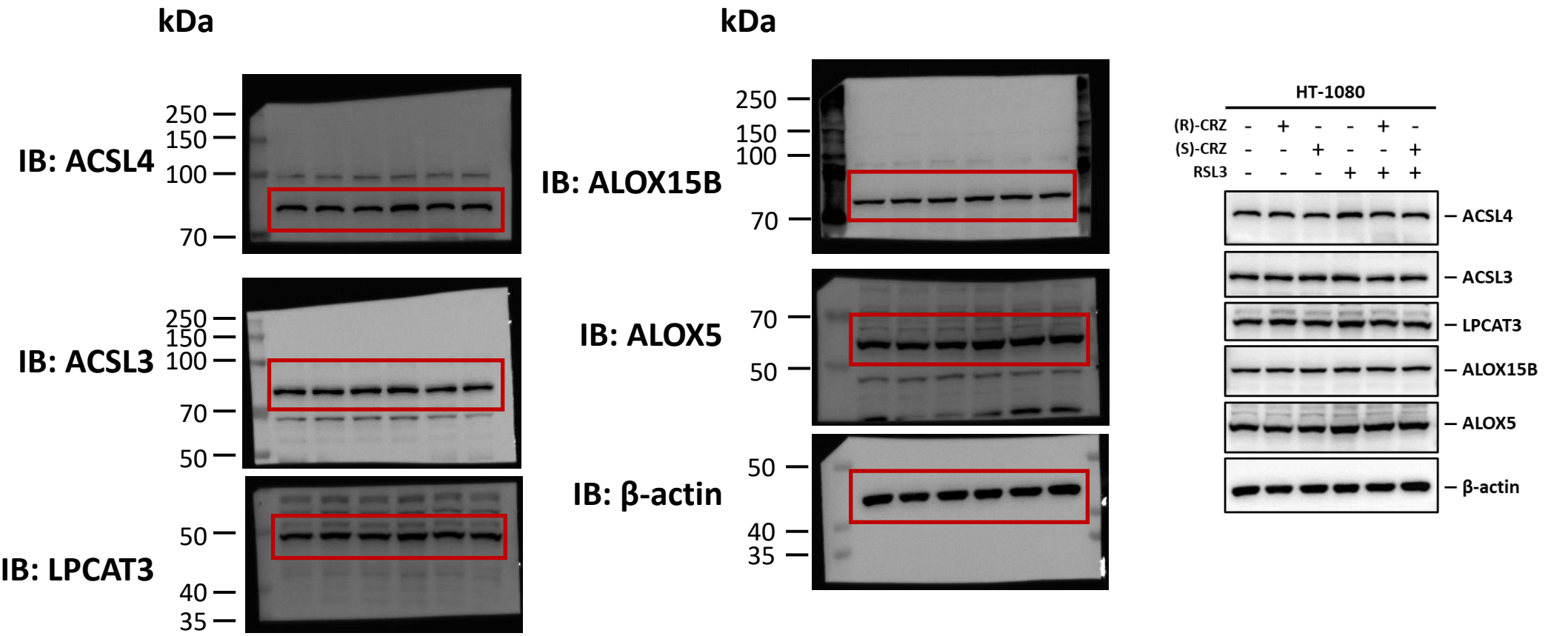


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Colorimetric and chemiluminescent were merged.
Red boxes indicate the cropped areas that were used in the figure.



Colorimetric and chemiluminescent were merged.
Red boxes indicate the cropped areas that were used in the figure.

Figure 4F

Original Western blot

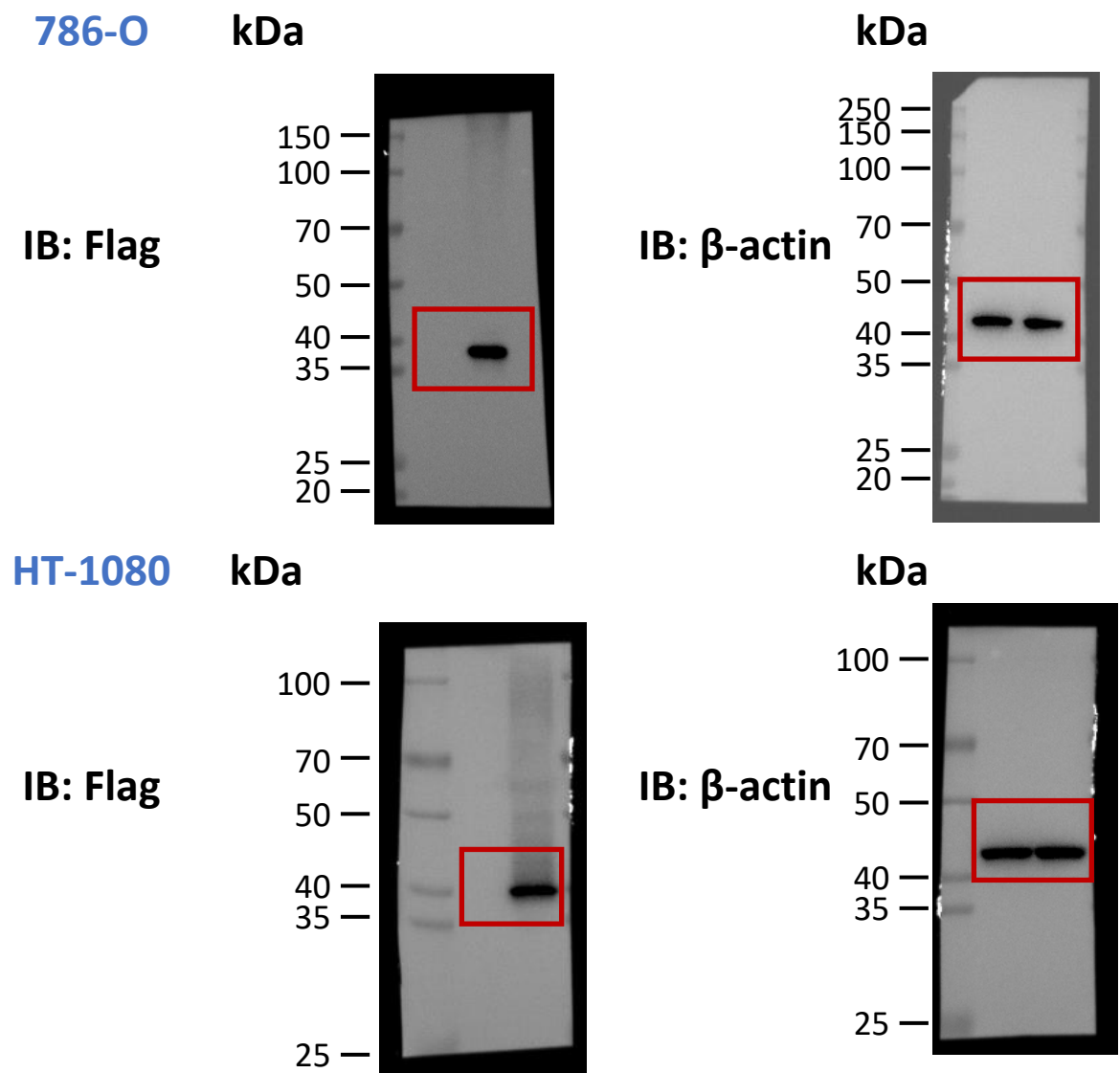
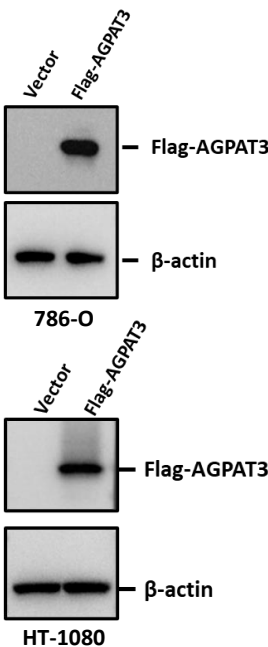


Figure presented in manuscript



Colorimetric and chemiluminescent were merged.
Red boxes indicate the cropped areas that were used in the figure.