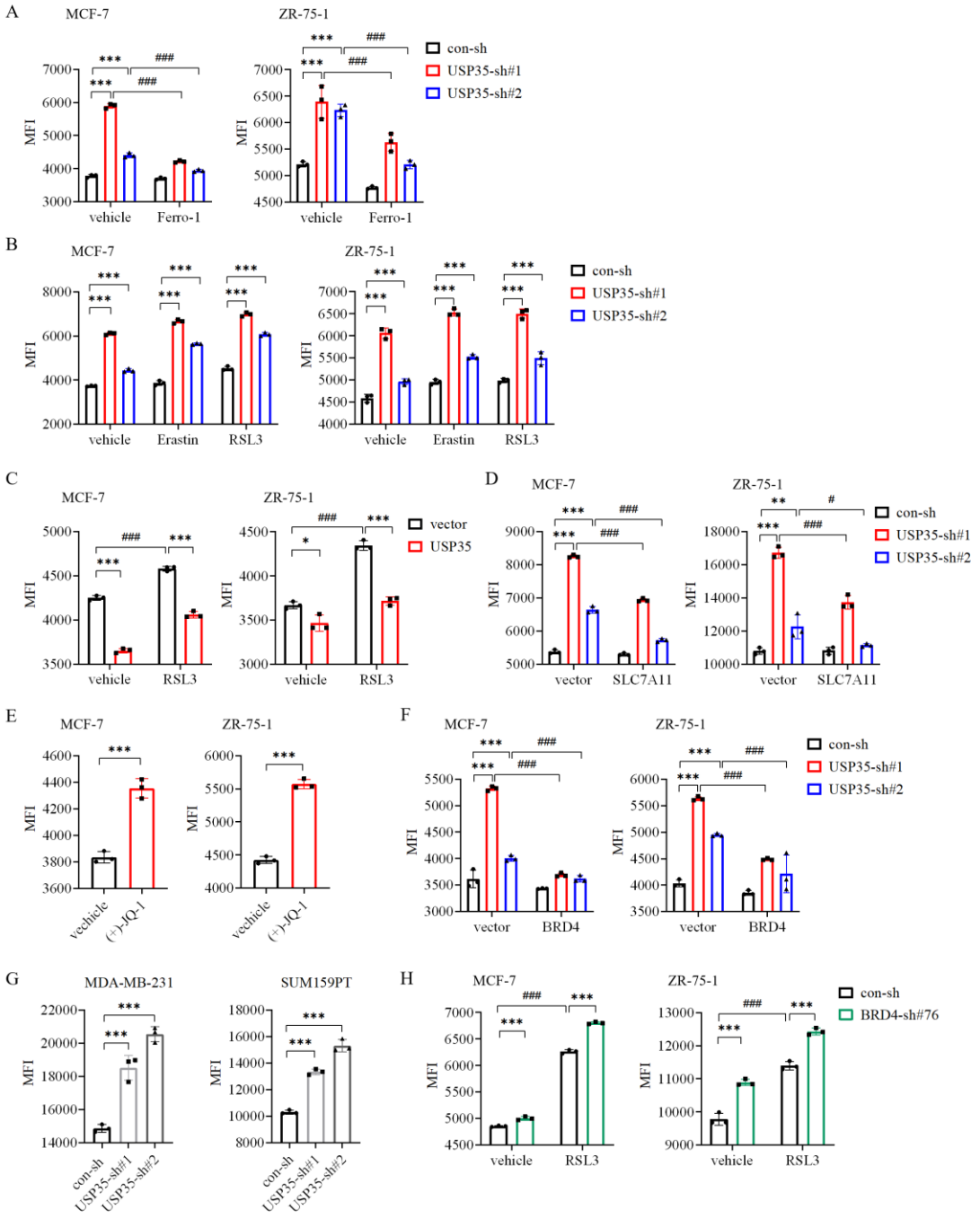
Supplementary Figure

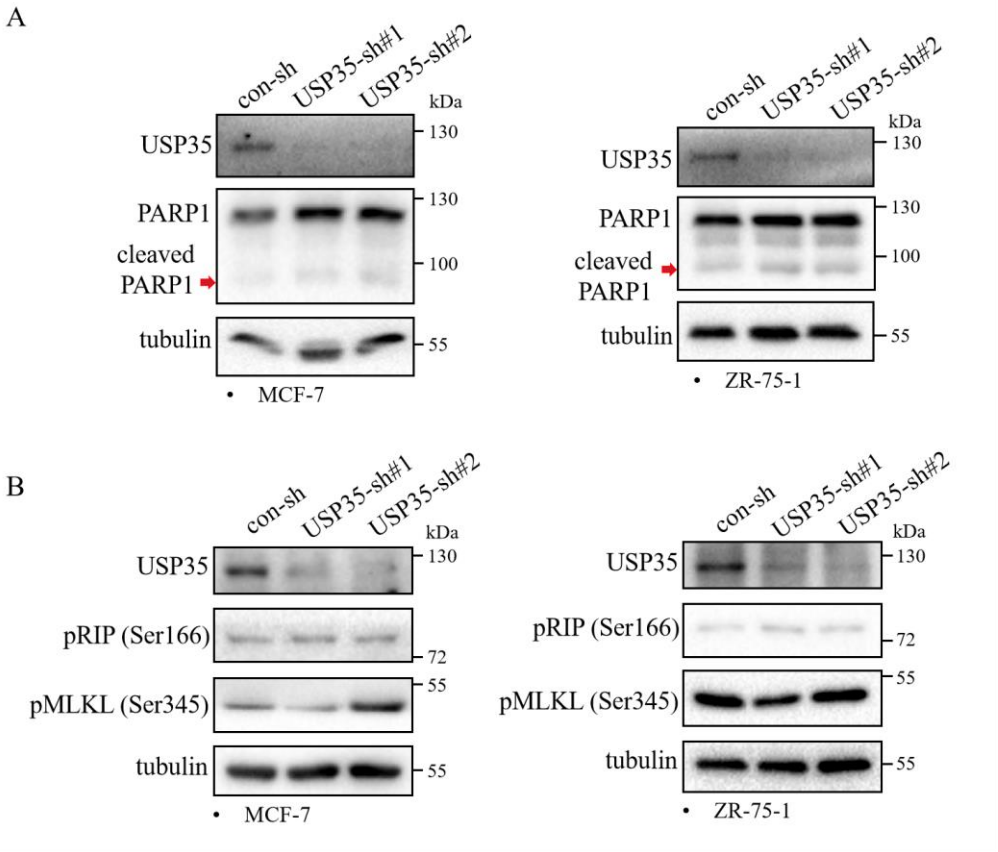


Supplementary Figure 1. The mean fluorescence intensity (MFI) of C11-BODIPY staining for figures in Fig. 1, 2, 3, 5, 6, supplementary 4, and supplementary 8 respectively.

(A) The MFI of MCF-7 and ZR-75-1 cells with control-shRNA and USP35-shRNAs

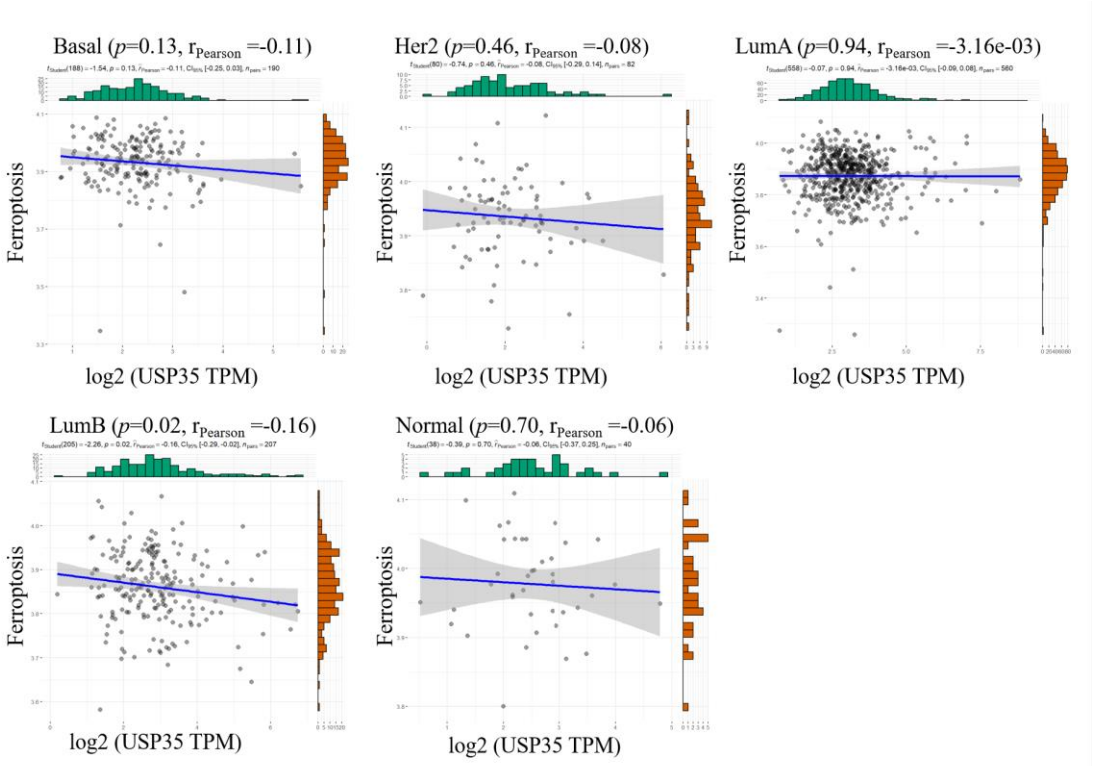
that were treated with Ferro-1 (n=3) for Fig. 1D. MCF-7 and ZR-75-1 cells with USP35 knockdown were seeded in twelve-well plates, treated with vehicle or 5 μM Ferro-1 for 24 h and stained with 5 μM C11-BODIPY followed by flow cytometry analysis (n=3). (B) The MFI of MCF-7 and ZR-75-1 cells with control-shRNA and USP35-shRNAs which were treated with Erastin and RSL3 (n=3) for Fig. 2B. MCF-7 and ZR-75-1 cells with control-shRNA and USP35-shRNAs were treated with Erastin (10 μM) or RSL3 (5 μM) and stained with 5 μM C11-BODIPY followed by flow cytometry analysis after 24-h treatment (n=3). (C) The MFI of MCF-7 and ZR-75-1 cells with USP35 overexpression that were treated with RSL3 for Fig. 2F. MCF-7 and ZR-75-1 cells with vector and USP35 overexpression were treated with RSL3 (5 μM) and stained with 5 μM C11-BODIPY followed by flow cytometry analysis after 24-h treatment (n=3). (D) The MFI of MCF-7 and ZR-75-1 stable cell lines with vector and SLC7A11 overexpression together with control-sh or two different USP35-sh (#1, #2) for Fig. 3D. Cells as indicated were stained with 5 μM C11-BODIPY followed by flow cytometry analysis (n=3). (E) The MFI of MCF-7 and ZR-75-1 cells with (+)-JQ-1 treatment. MCF-7 and ZR-75-1 cells were treated with (+)-JQ-1 (5 μM) for 24 h and stained with 5 μM C11-BODIPY before being subjected to flow cytometry analysis (n=3) for Fig. 5C. (F) The MFI of MCF-7 and ZR-75-1 stable cell lines with vector and BRD4 overexpression together with control-sh or two different USP35-sh (#1, #2) for Fig. 6B. Cells as indicated were subjected to flow cytometry analysis (n=3). (G) The MFI of MDA-MB-231 and SUM159PT cells. MDA-MB-231 and SUM159PT cells with con-shRNA and USP35-shRNAs (#1, #2) USP35 knockdown

were subjected to flow cytometry analysis (n=3) for Fig.S4A. (H) The MFI of MCF-7 and ZR-75-1 cells with control-shRNA and BRD4-shRNA. Cells as indicated were treated with RSL3 (5 μM) and stained with 5 μM C11-BODIPY followed by flow cytometry analysis after 24 h treatment (n=3) for supplementary figure 8A. Data were shown as Mean ± SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, #*P*<0.05, ###*P*<0.001.

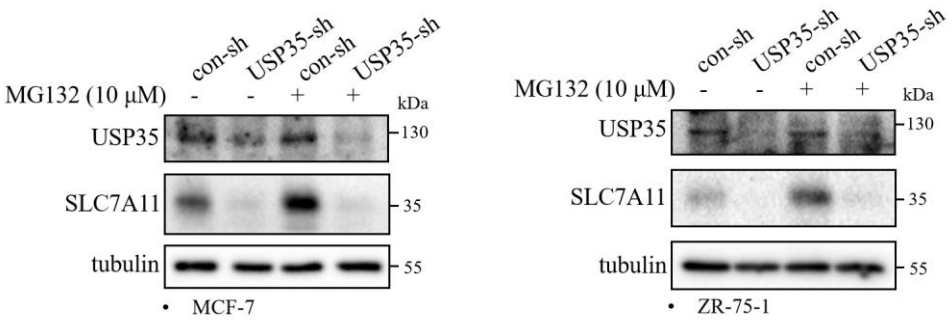


Supplementary Figure 2. Knockdown of USP35 does not induce apoptosis or necroptosis of ER+ breast cancer cells.

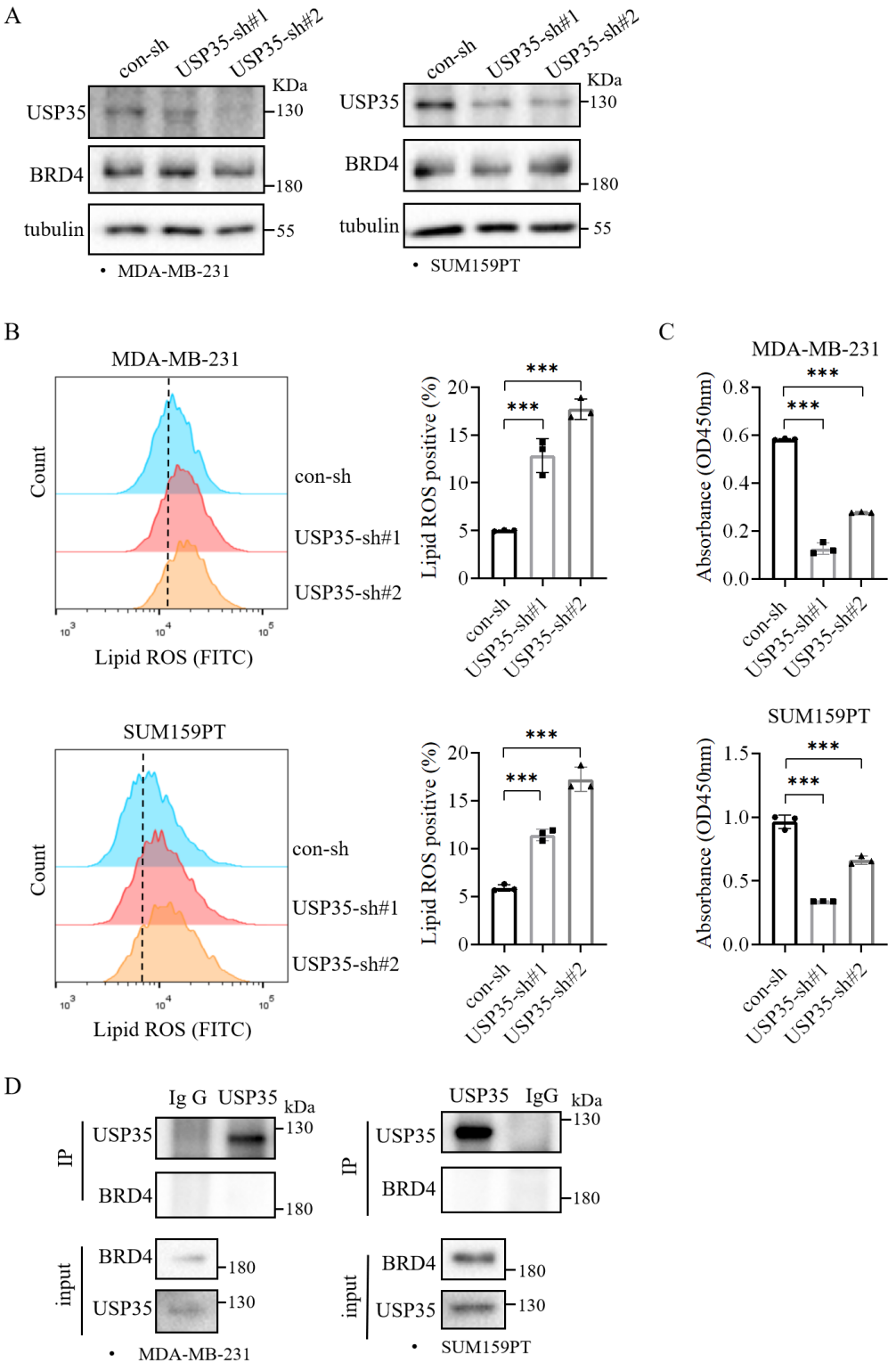
(A) Knockdown of USP35 did not affect apoptosis of ER+ breast cancer cells. MCF-7 and ZR-75-1 cells with control-shRNA and USP35-shRNA (#1, #2) were subjected to western blot analysis using antibodies against USP35, PARP1, and tubulin. (B) Knockdown of USP35 did not affect necroptosis of ER+ breast cancer cells. The same cells as (A) were subjected to western blot analysis using antibodies against USP35, pRIP (Ser166), pMLKL (Ser345), and tubulin.



Supplementary Figure 3. The correlation of USP35 expression and ferroptosis in different subtypes of breast cancer from TCGA database.



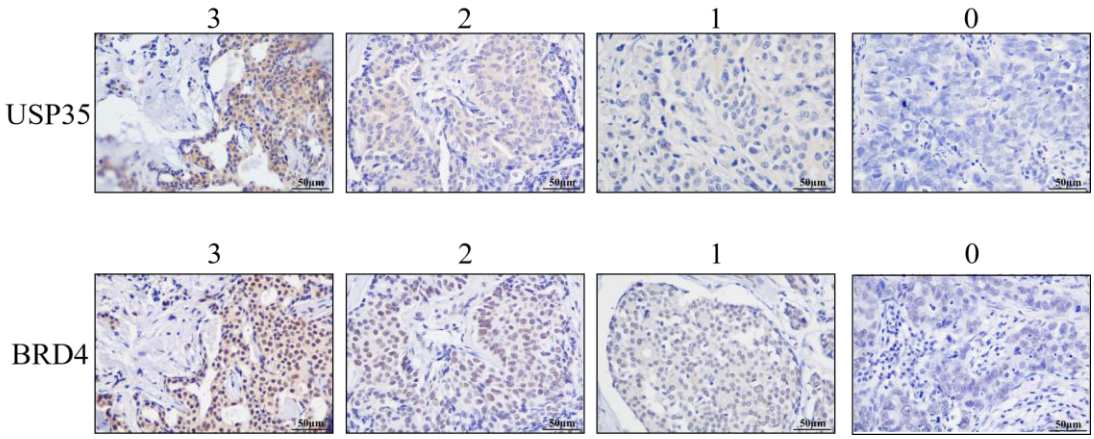
Supplementary Figure 4. MG132 treatment can not rescue SLC7A11 level reduced by USP35 knockdown. MCF-7 and ZR-75-1 cells with con-sh or USP35-sh#1 were treated with MG132 (10 μM) for 12 h, and then were subjected to western blot analysis with the indicated antibodies.



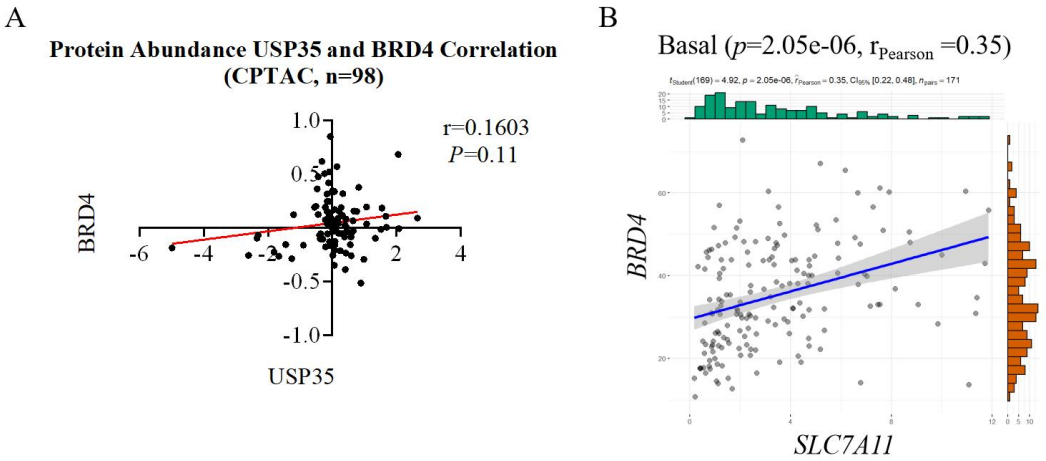
Supplementary Figure 5. Knockdown of USP35 promotes ferroptosis without affecting BRD4 protein level in TNBC cells.

(A) Knockdown of USP35 did not affect BRD4 level in TNBC cells. USP35 and

BRD4 levels were examined by western blot analysis in MDA-MB-231 and SUM159PT cells with USP35 knockdown. (B) Knockdown of USP35 increased Lipid ROS of TNBC cells. MDA-MB-231 and SUM159PT cells with con-shRNA and USP35-shRNAs (#1, #2) USP35 knockdown were subjected to flow cytometry analysis. Quantitation statistical analysis of the data were shown in the right (n=3). (C) Knockdown of USP35 inhibited cell growth of TNBC cells. MDA-MB-231 and SUM159PT cells with USP35 knockdown were subjected to CCK-8 assays (n=3). (D) USP35 did not interact with BRD4 in TNBC cells. MDA-MB-231 and SUM159PT cell lysates were subjected to immunoprecipitation with anti-USP35 antibodies and rabbit IgG (negative control). Data are shown as Mean ± SEM. ***P<0.001

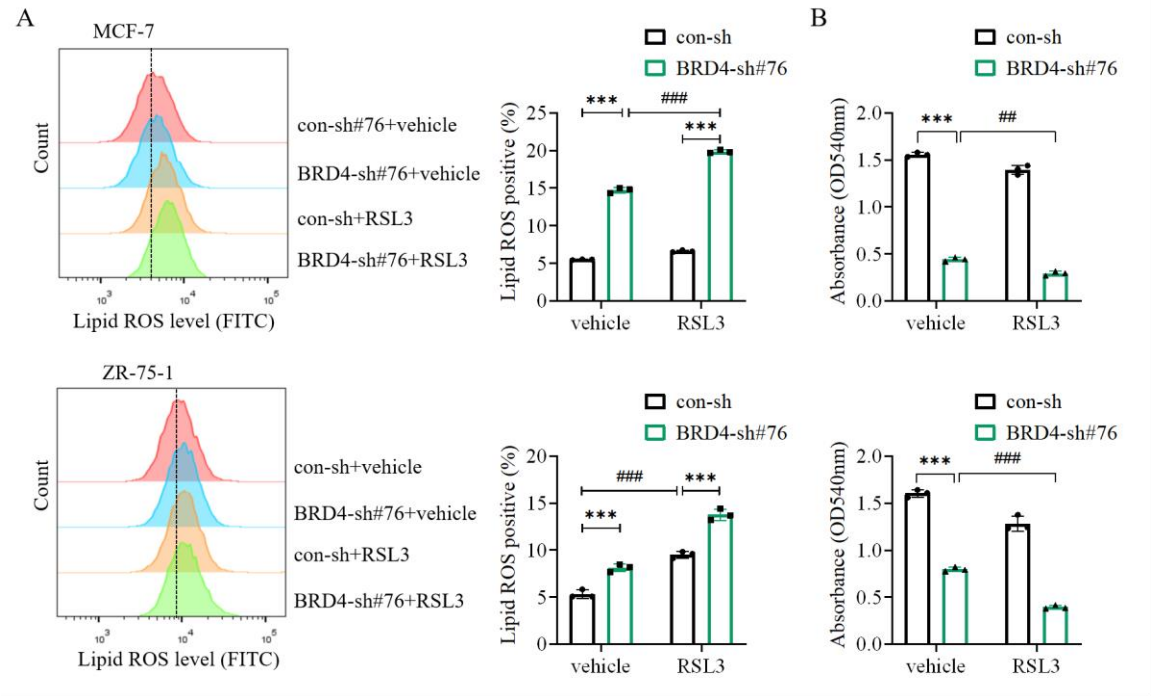


Supplementary Figure 6. The representative images of different scoring grades of IHC staining.



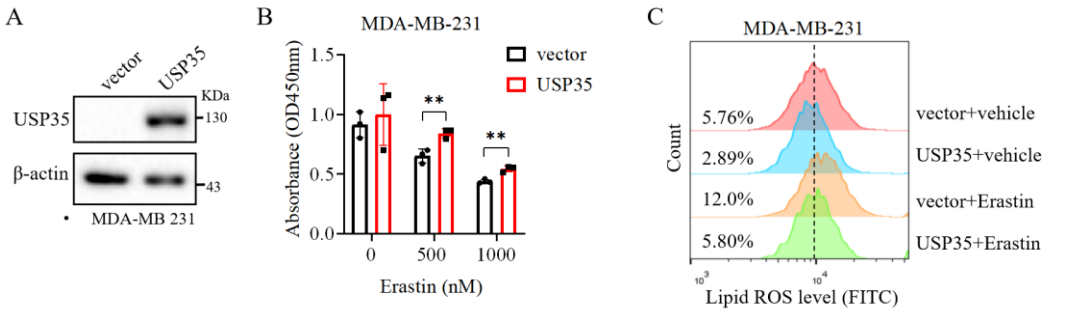
Supplementary Figure 7. The correlation of USP35, BRD4, and SLC7A11 expression in public databases.

(A) The correlation of USP35 and BRD4 protein levels of breast cancer in CPTAC database. (B) The correlation of *BRD4* and *SLC7A11* mRNA levels in TNBC in TCGA database.



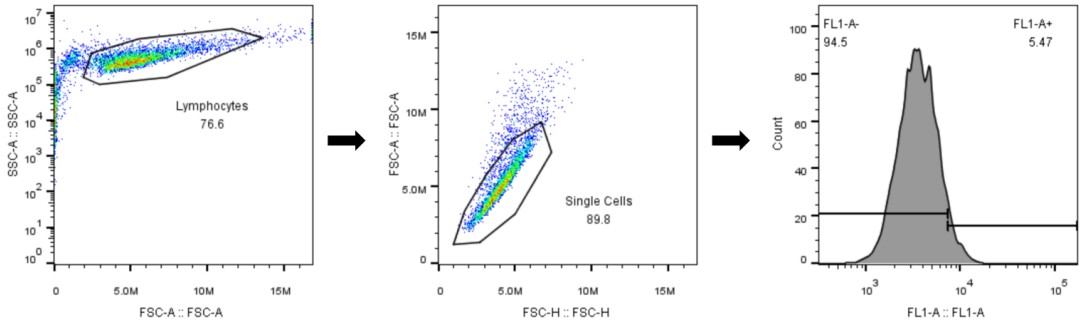
Supplementary Figure 8. Knockdown of BRD4 enhances ferroptosis induced by ferroptosis inducer RSL3.

(A) Knockdown of BRD4 expression enhanced lipid peroxidation induced by RSL3 treatment. MCF-7 and ZR-75-1 cells with control-shRNA and BRD4-shRNA were treated with RSL3 (5 μM) and stained with 5 μM C11-BODIPY followed by flow cytometry analysis after 24 h treatment. Quantitation and statistical analysis of the data were shown on the right (n=3). (B) RSL3 increased the cell growth inhibition caused by BRD4 knockdown. The same cells as (A) subjected to colony formation assay were treated with RSL3 (5 μM) for 3 d (n=3). Data are shown as Mean ± SEM ***P<0.001, ##P<0.01, ###P<0.001.



Supplementary Figure 9. USP35 overexpression partially inhibits ferroptosis induced by ferroptosis inducer Erastin in MDA-MB-231 cells.

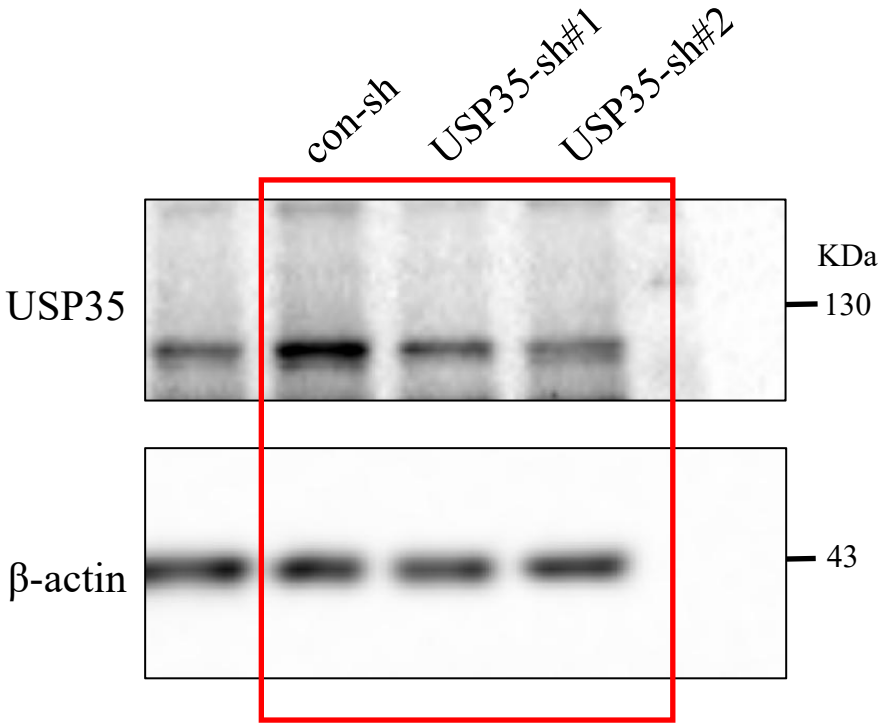
(A) USP35 was overexpressed in MDA-MB-231 cells. (B) USP35 overexpression partially rescued the cell growth inhibited by Erastin. MDA-MB-231 cells with USP35 overexpression were treated with the indicated concentrations of Erastin for 2 d before subjected to the CCK-8 assay (n=3). (C) USP35 overexpression reduced the lipid peroxidation induced by Erastin (1 μM). Data are shown as Mean ± SEM. $^{**}P<0.01$.



Supplementary Figure 10. Gating strategy for flow cytometry in analysis of lipid ROS in breast cancer cells.

Uncropped western blots for all the western blot figures shown in the manuscript

Figure 1A



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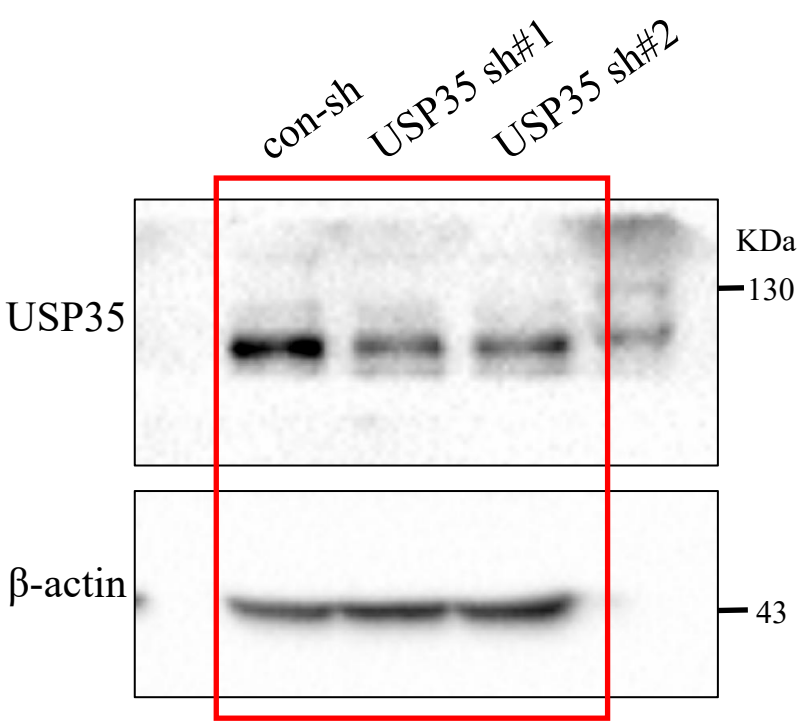

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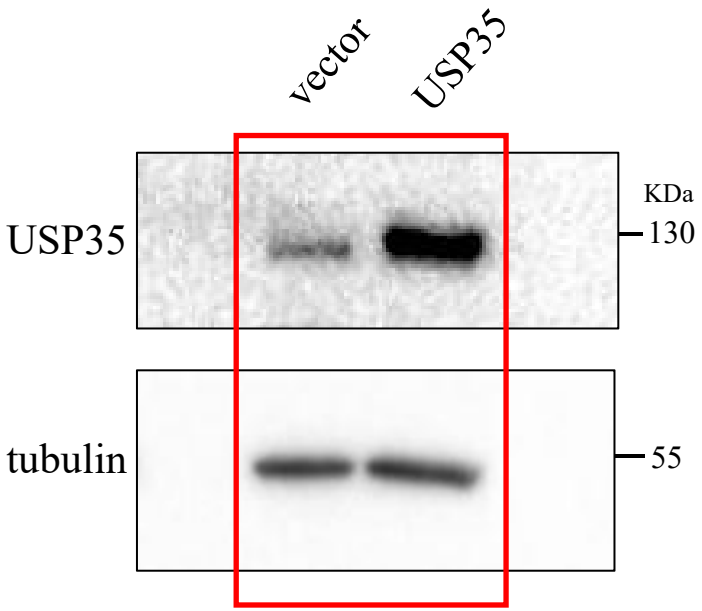
Figure 2D



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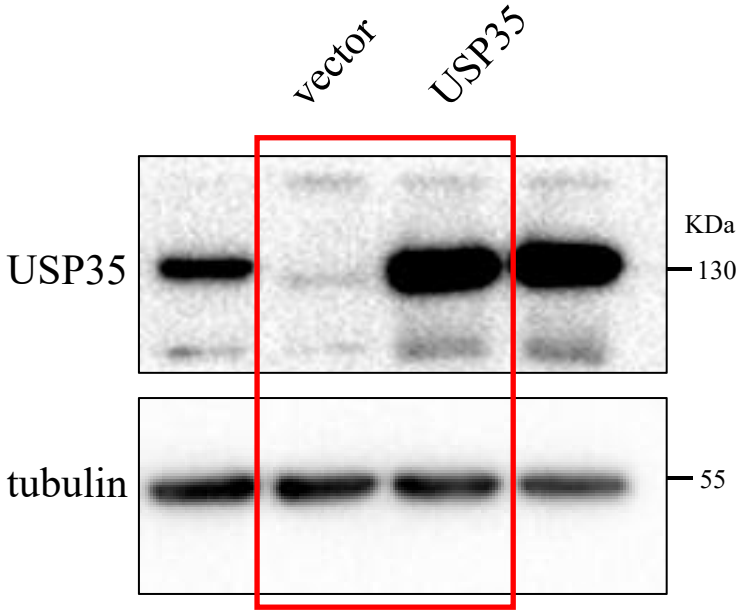

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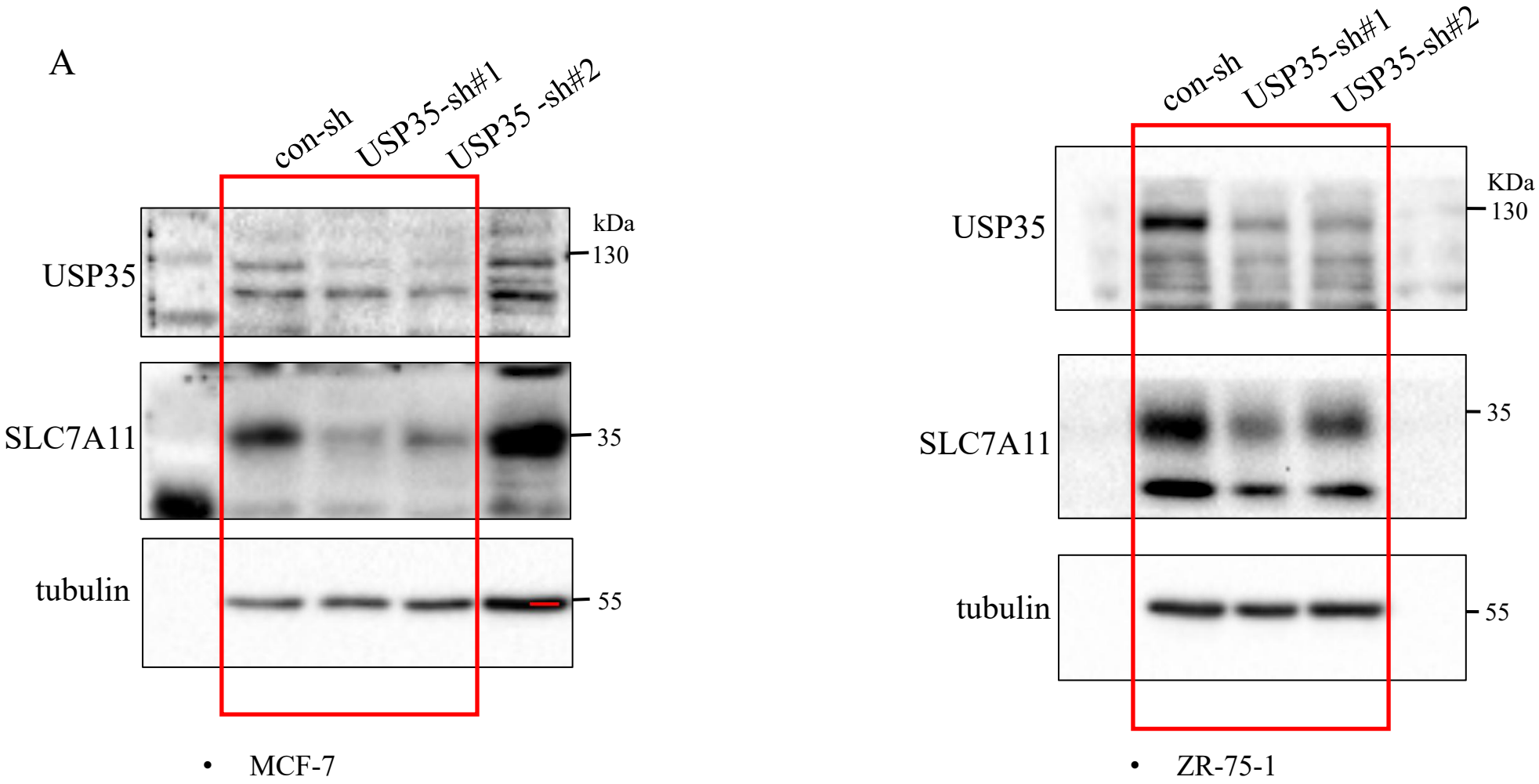
Figure 3A



- MCF-7
- ZR-75-1

Figure 3B

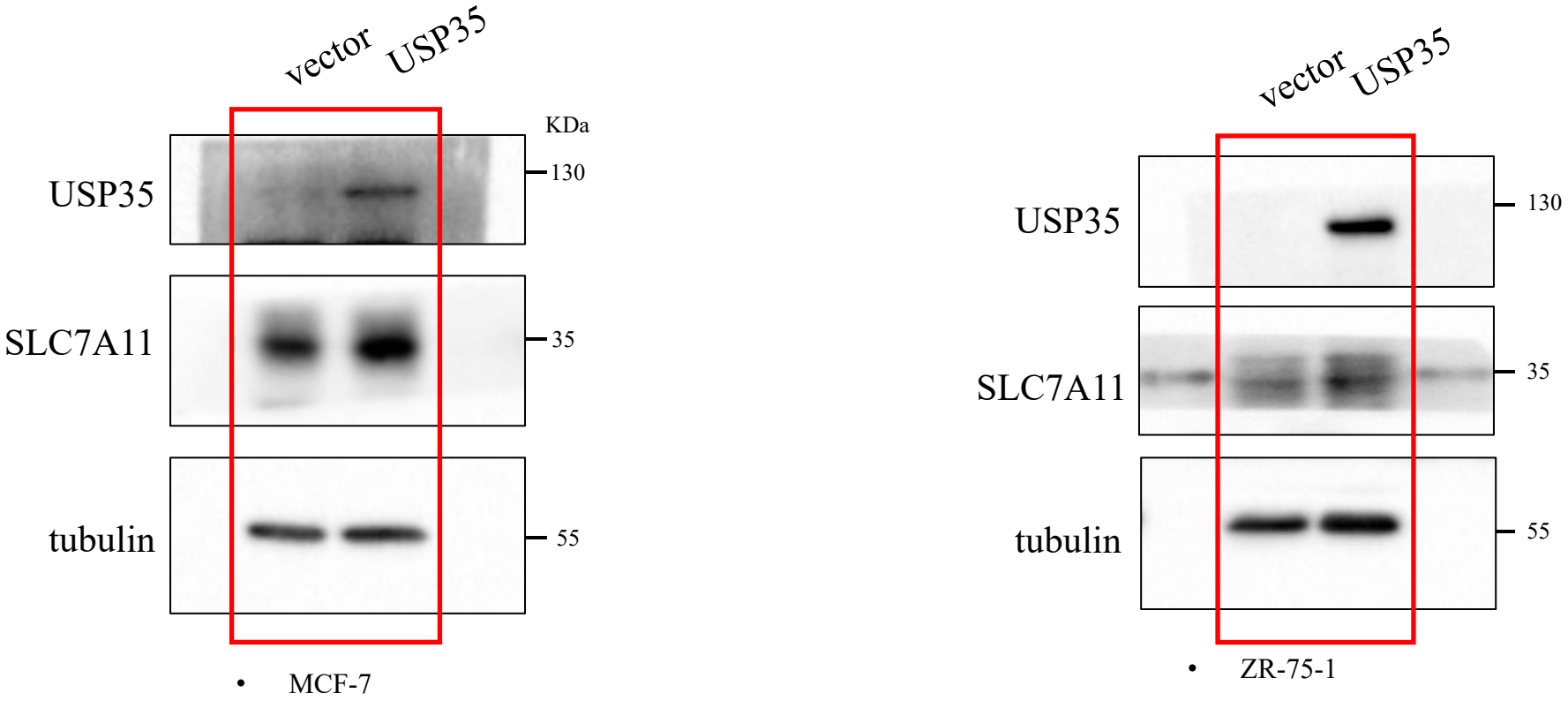

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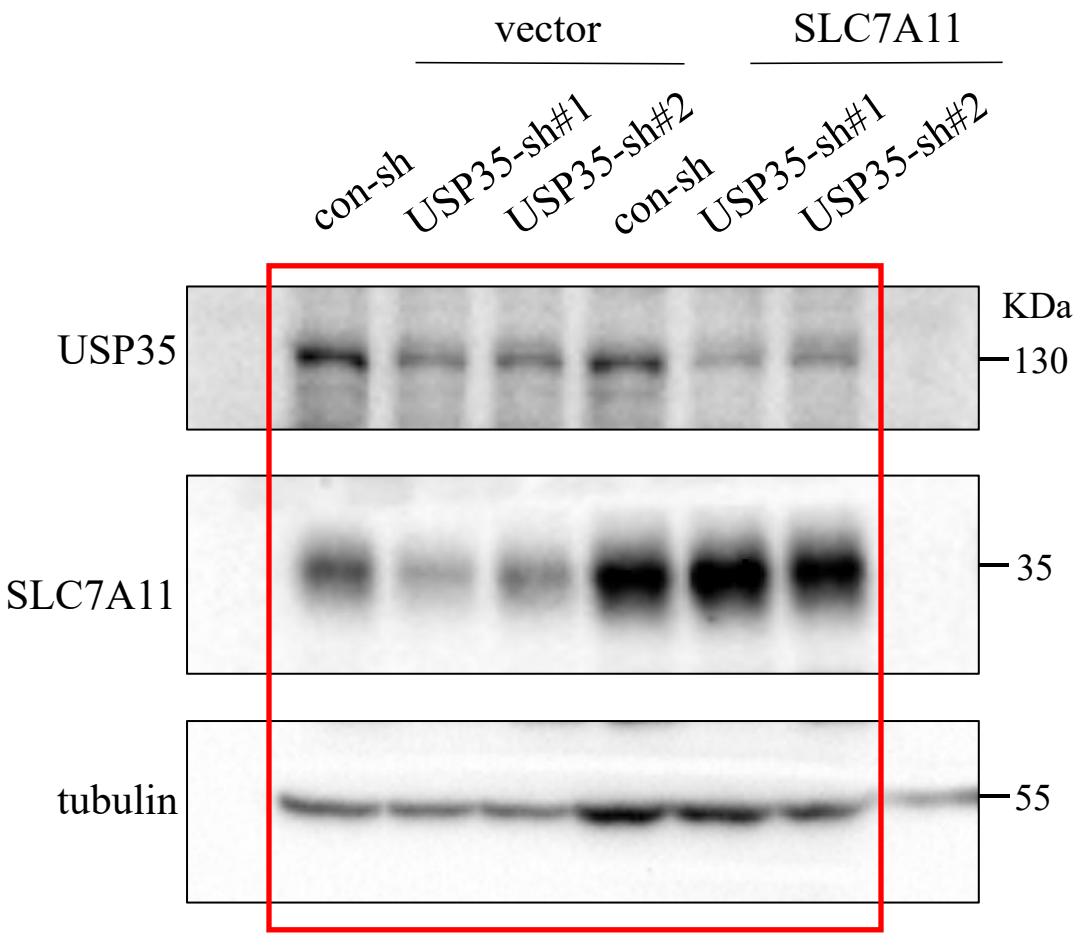
Figure 3C



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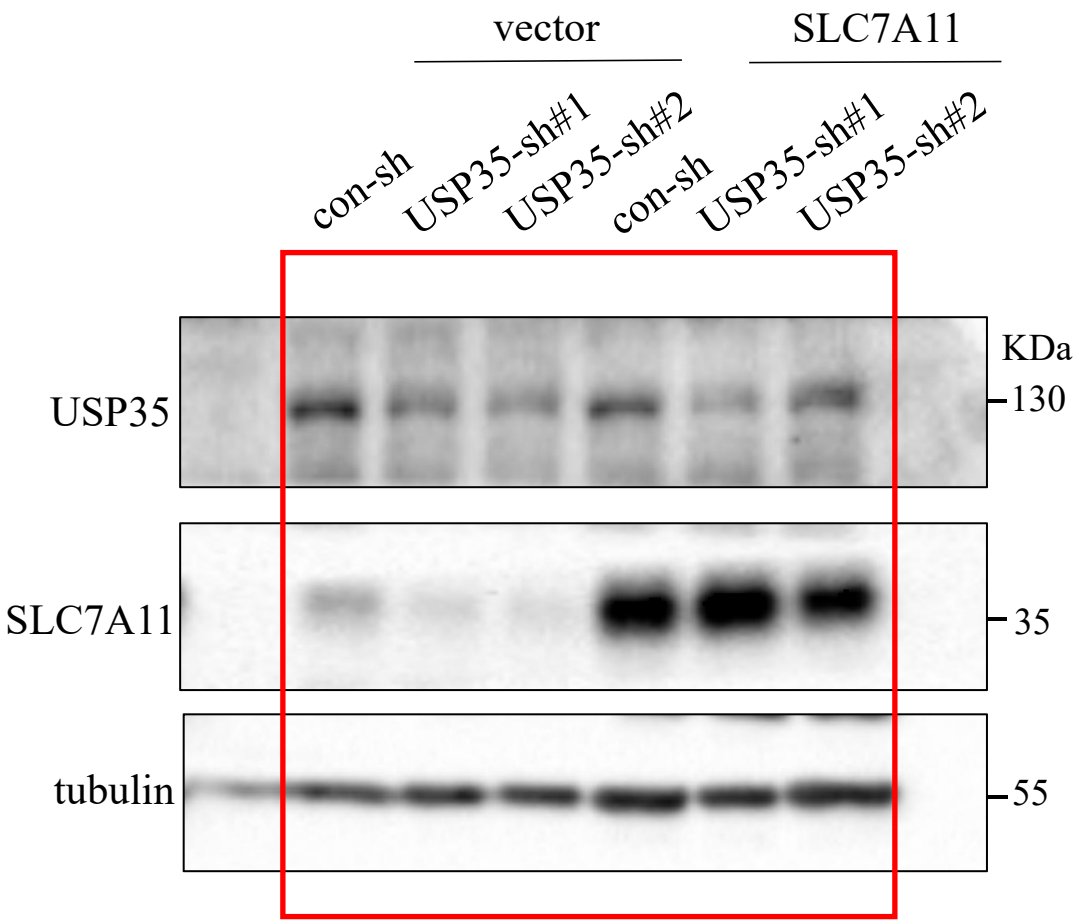

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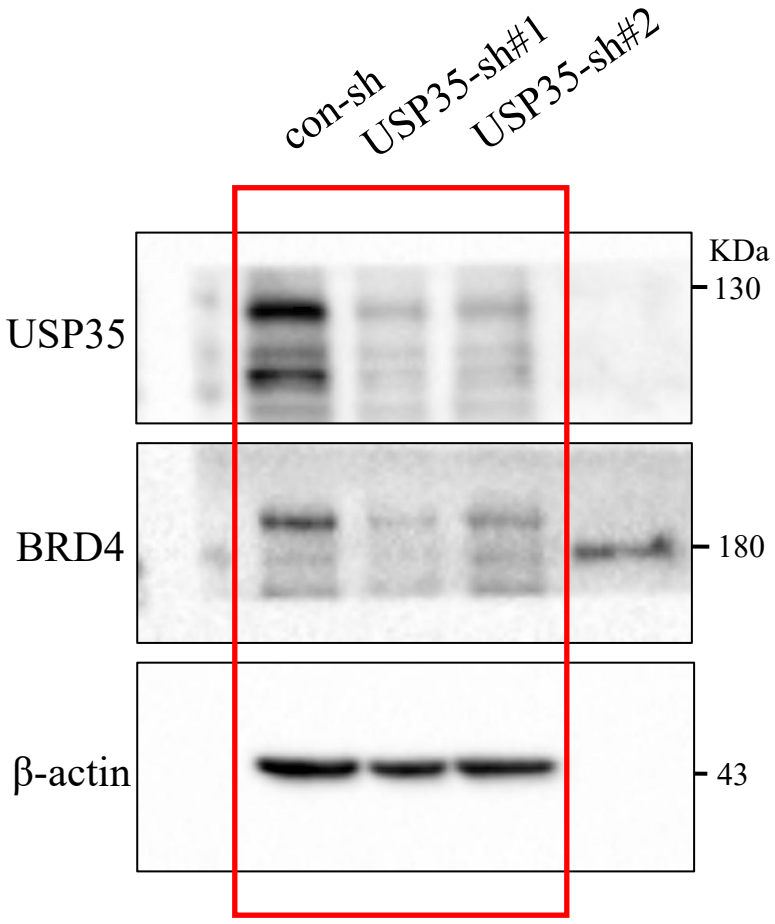

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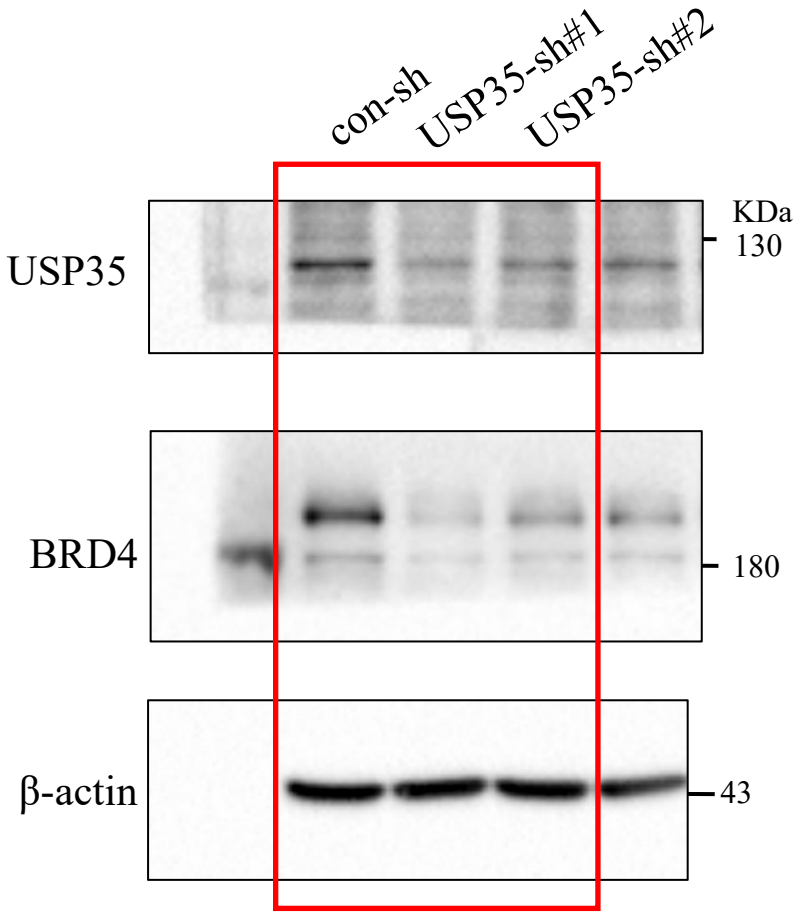

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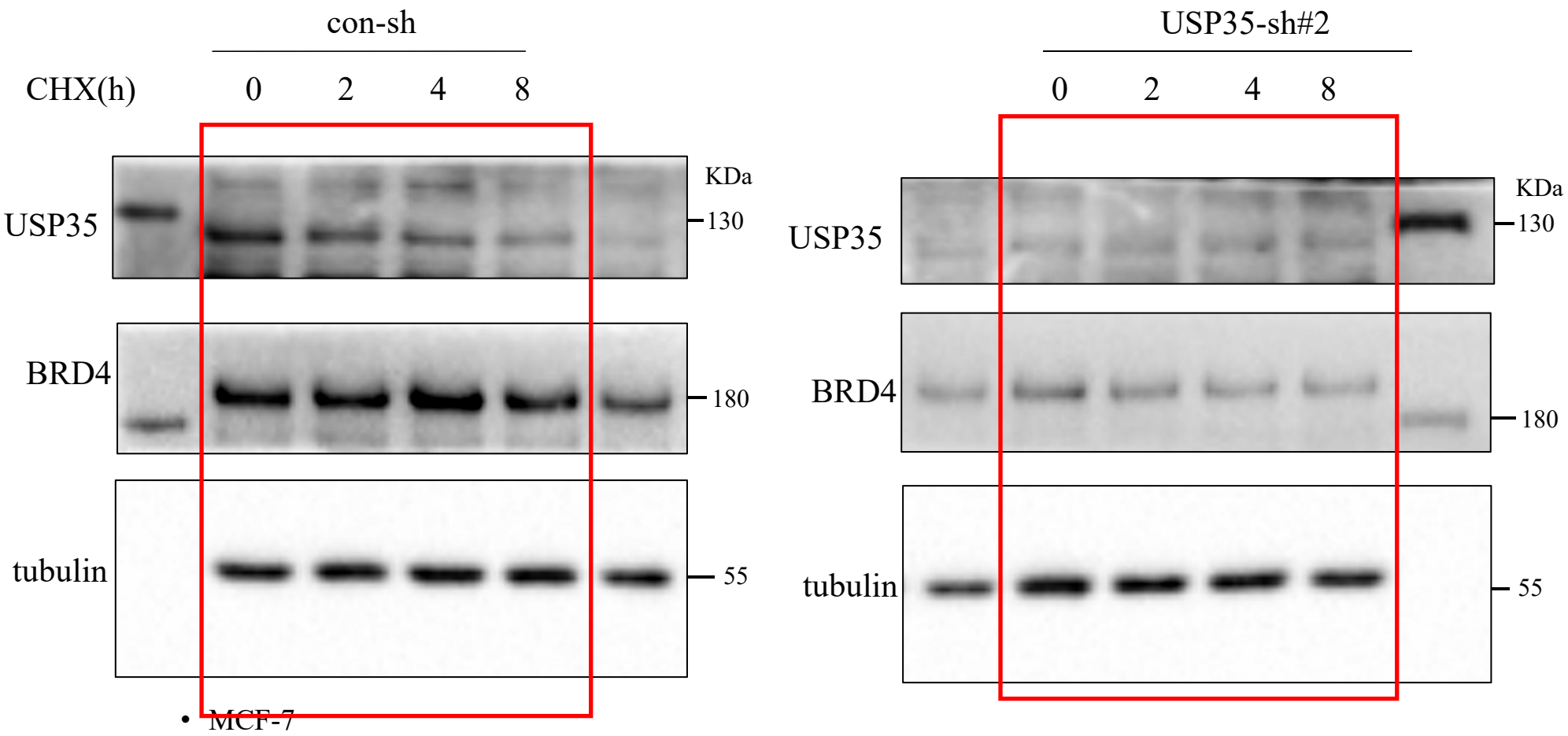
Figure 4B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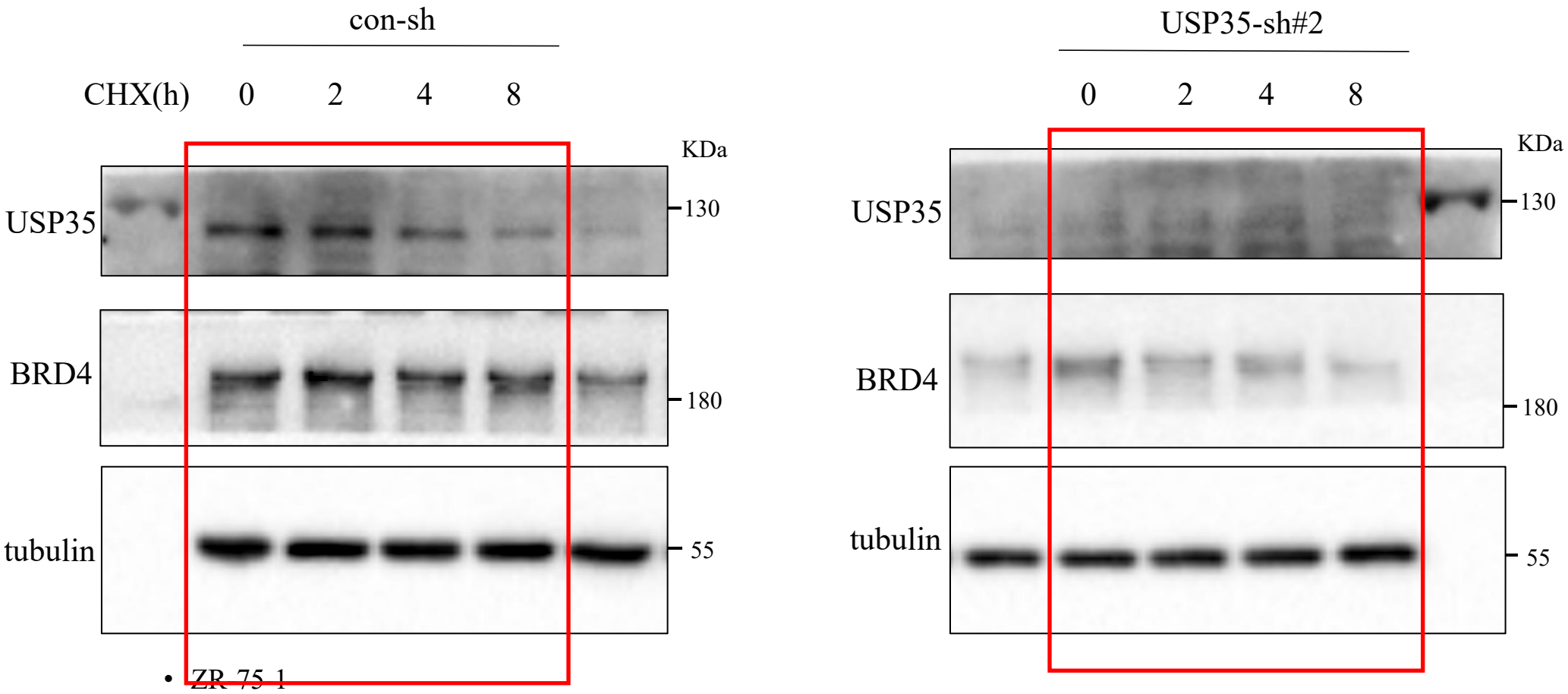

- ZR-75-1

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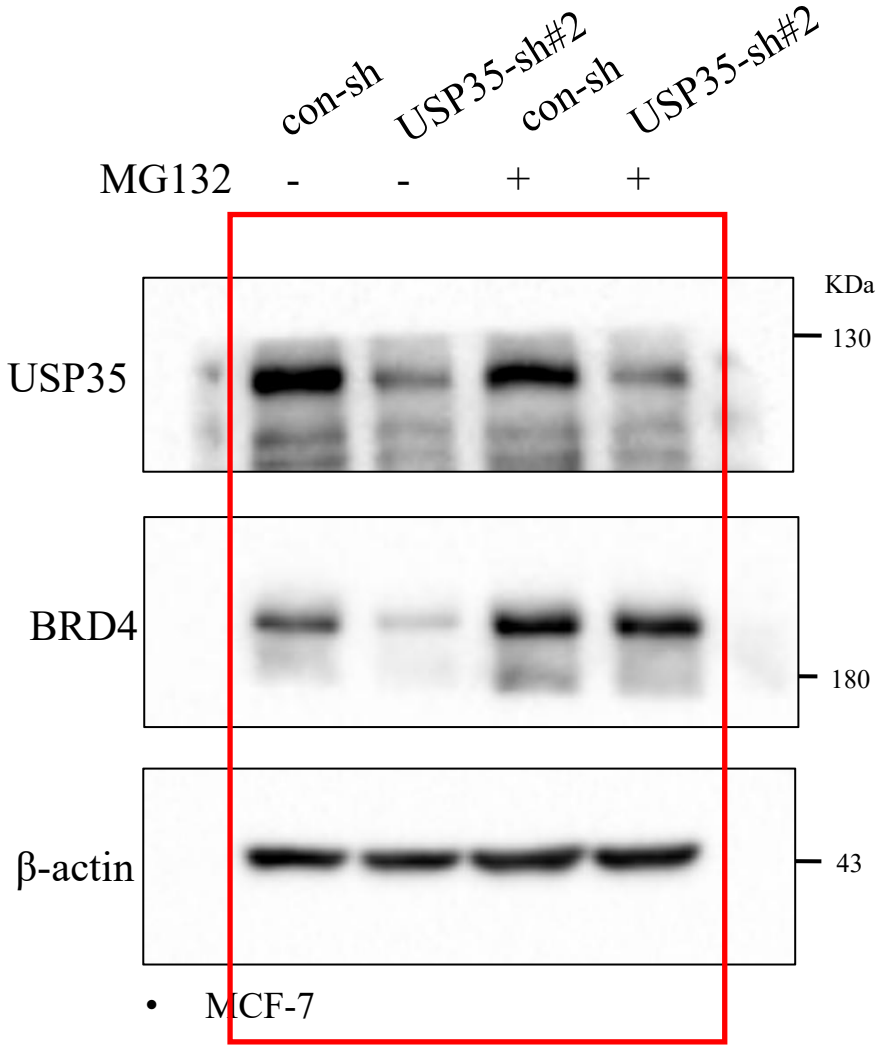
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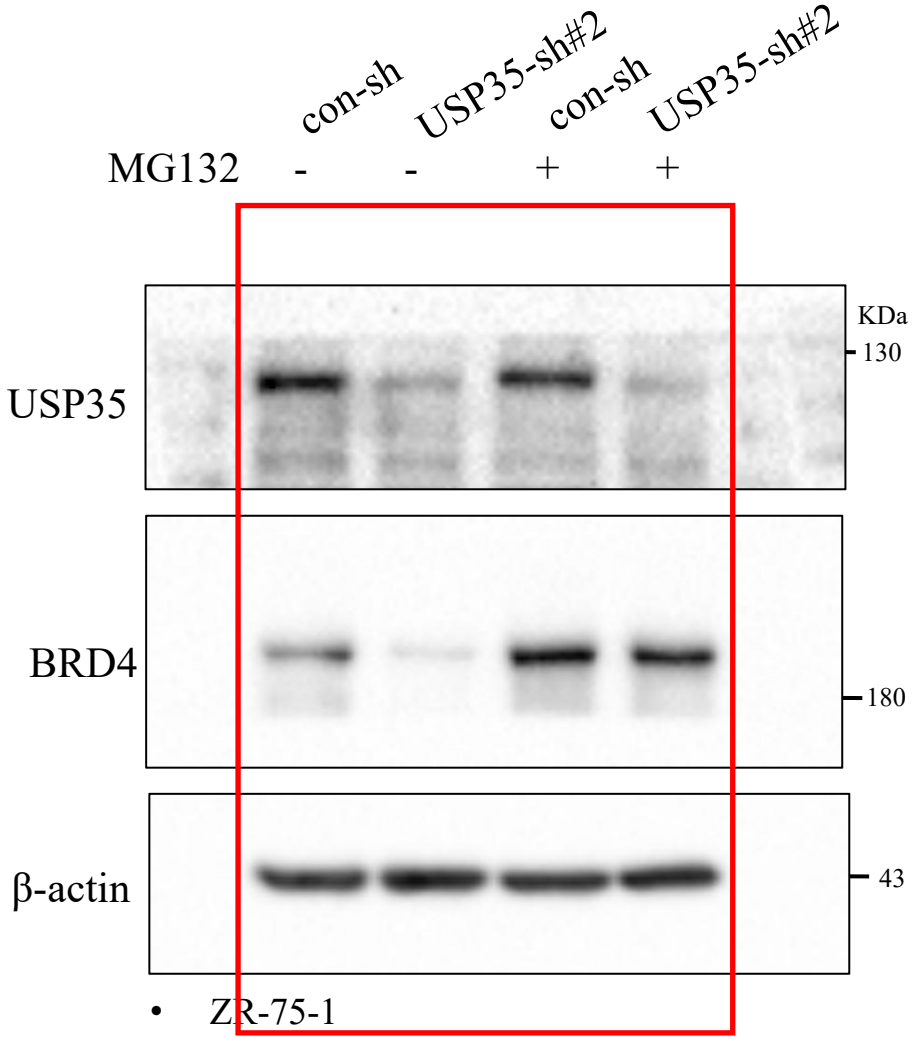
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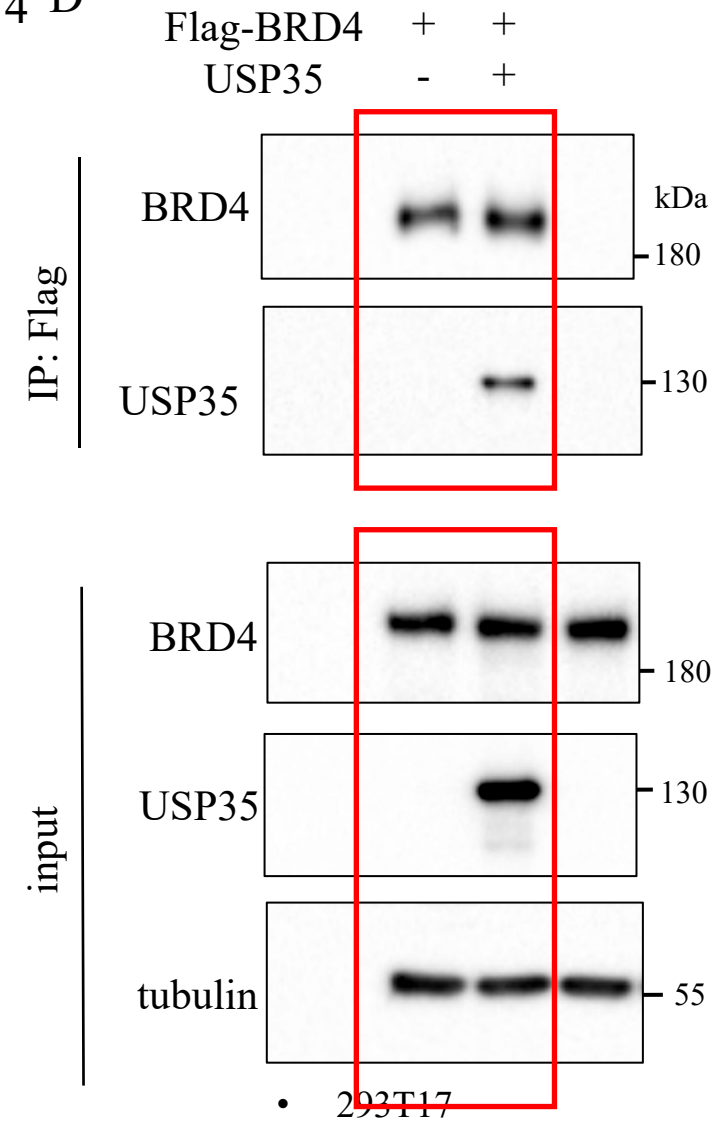
Figure 4 D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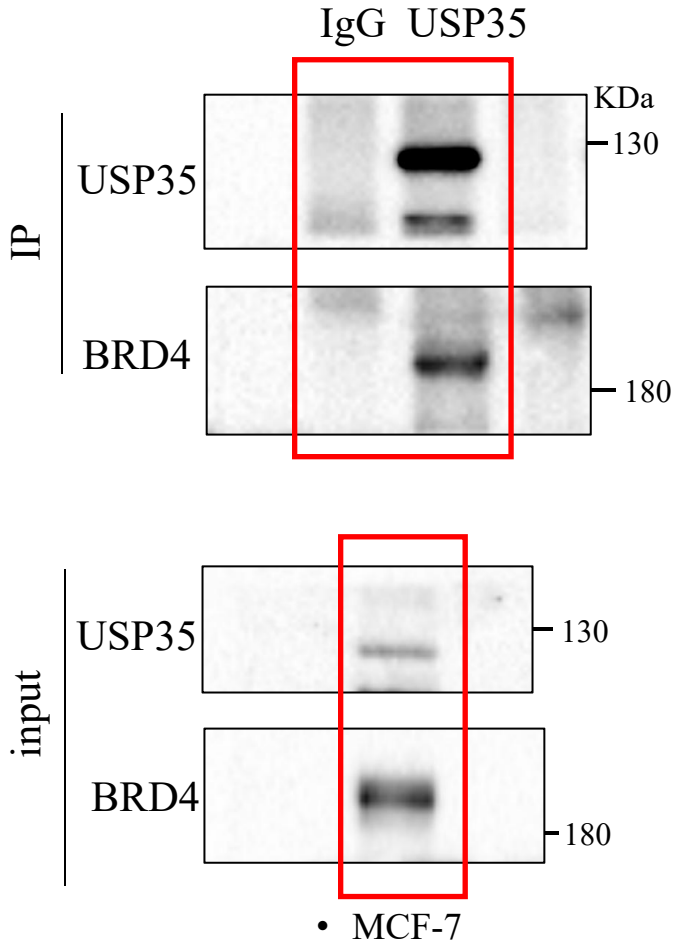
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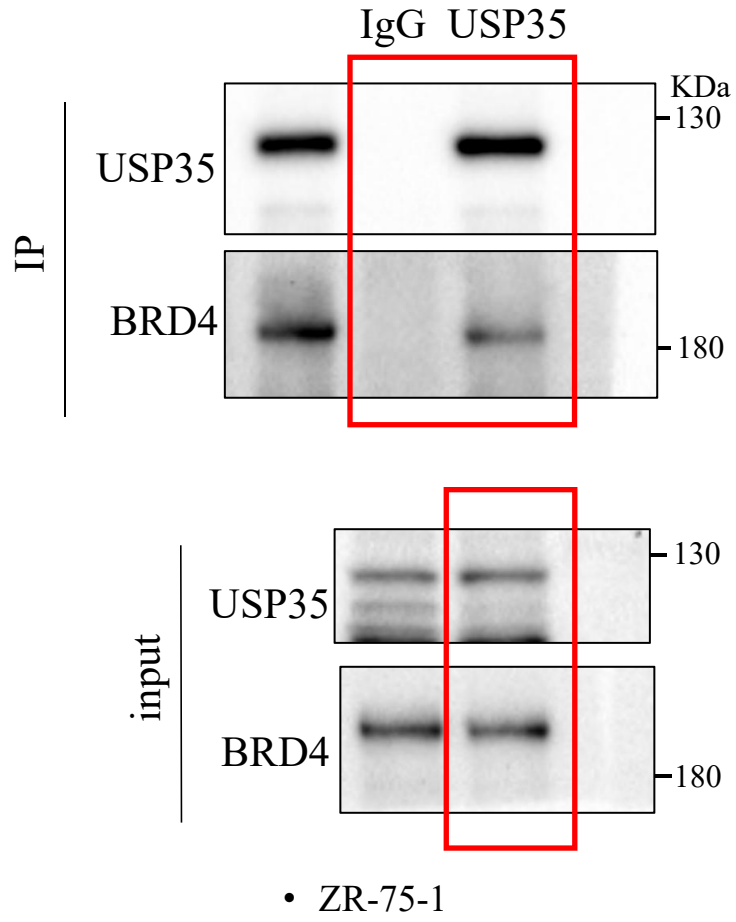
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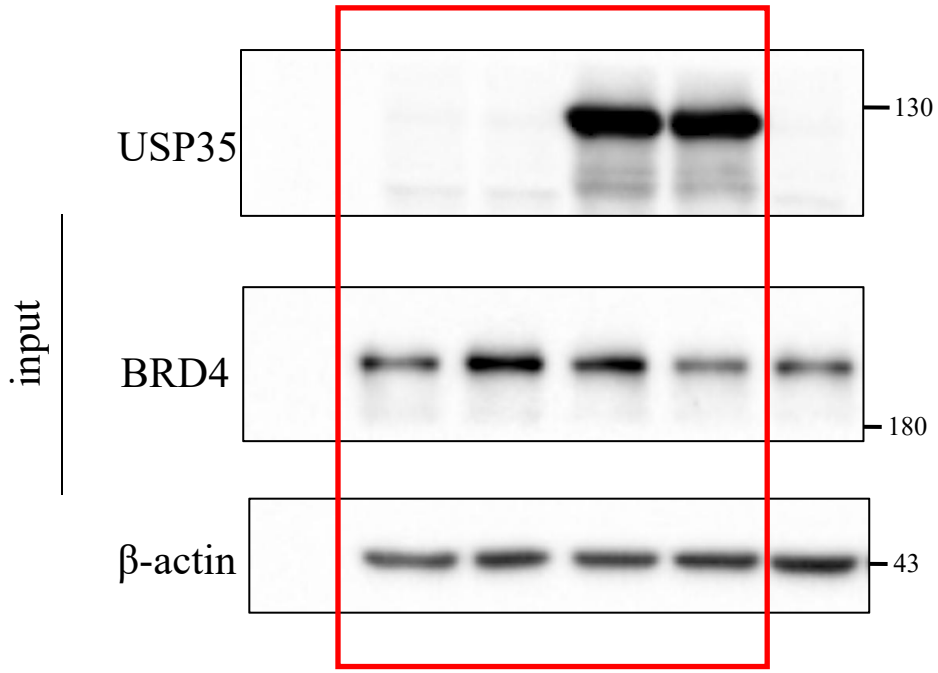
Figure 4F



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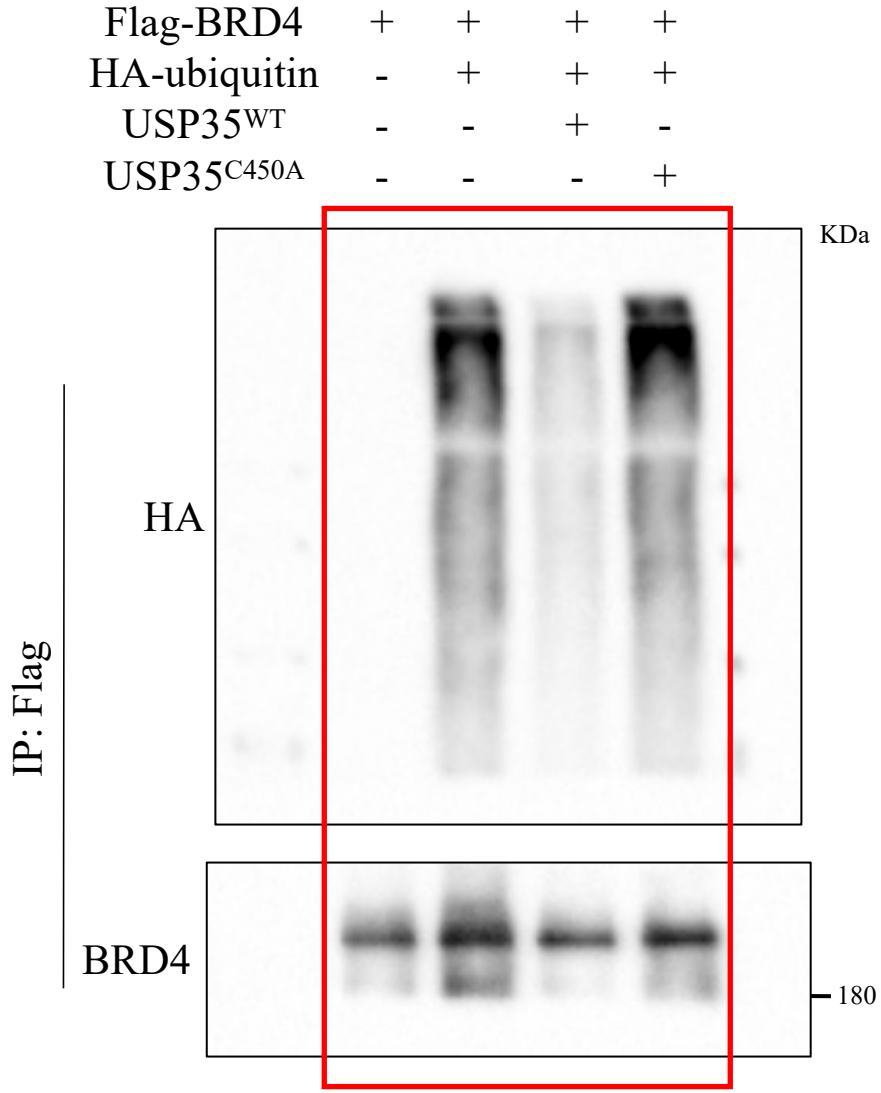

Figure 5

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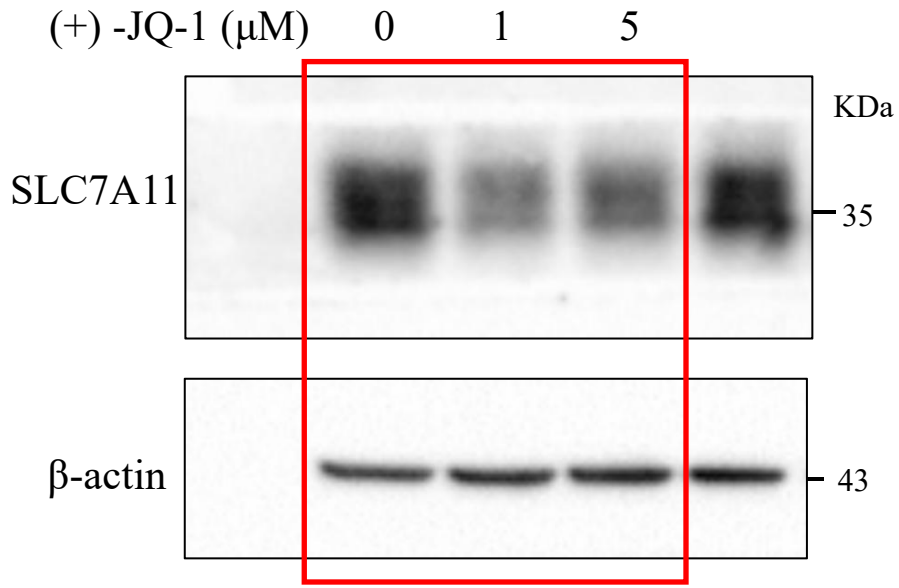

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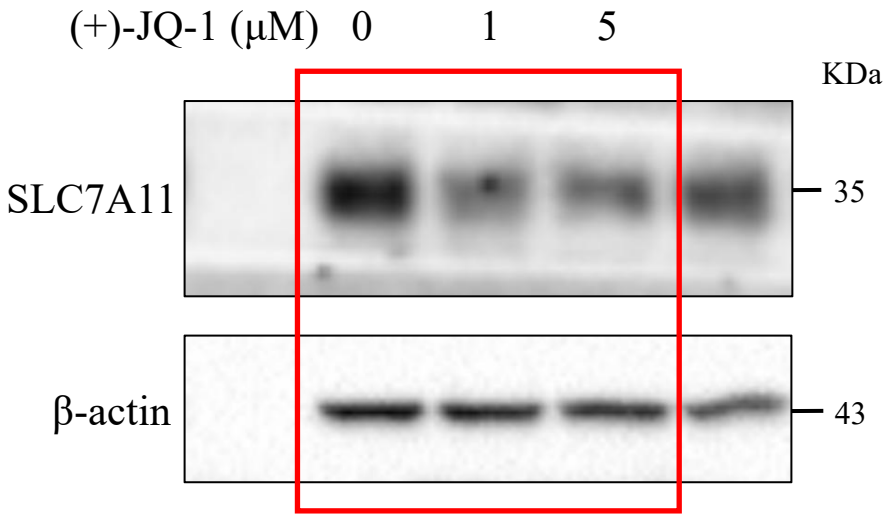

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Figure 5

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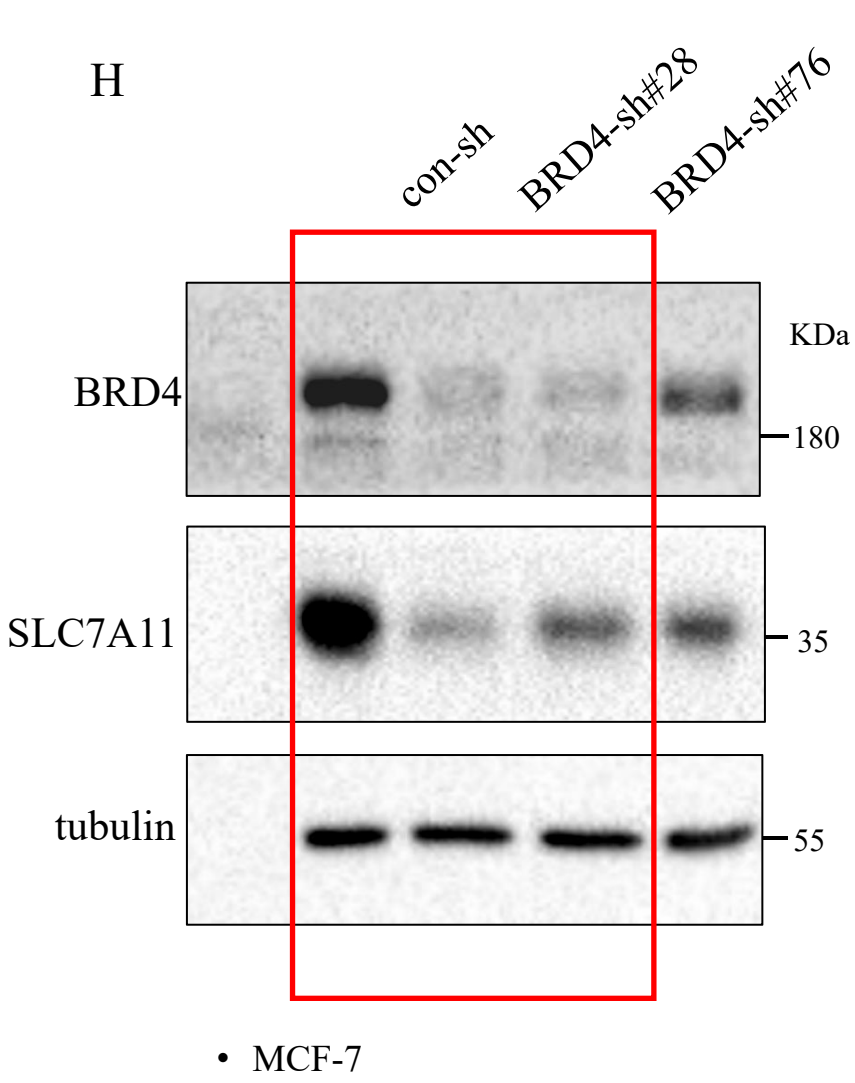

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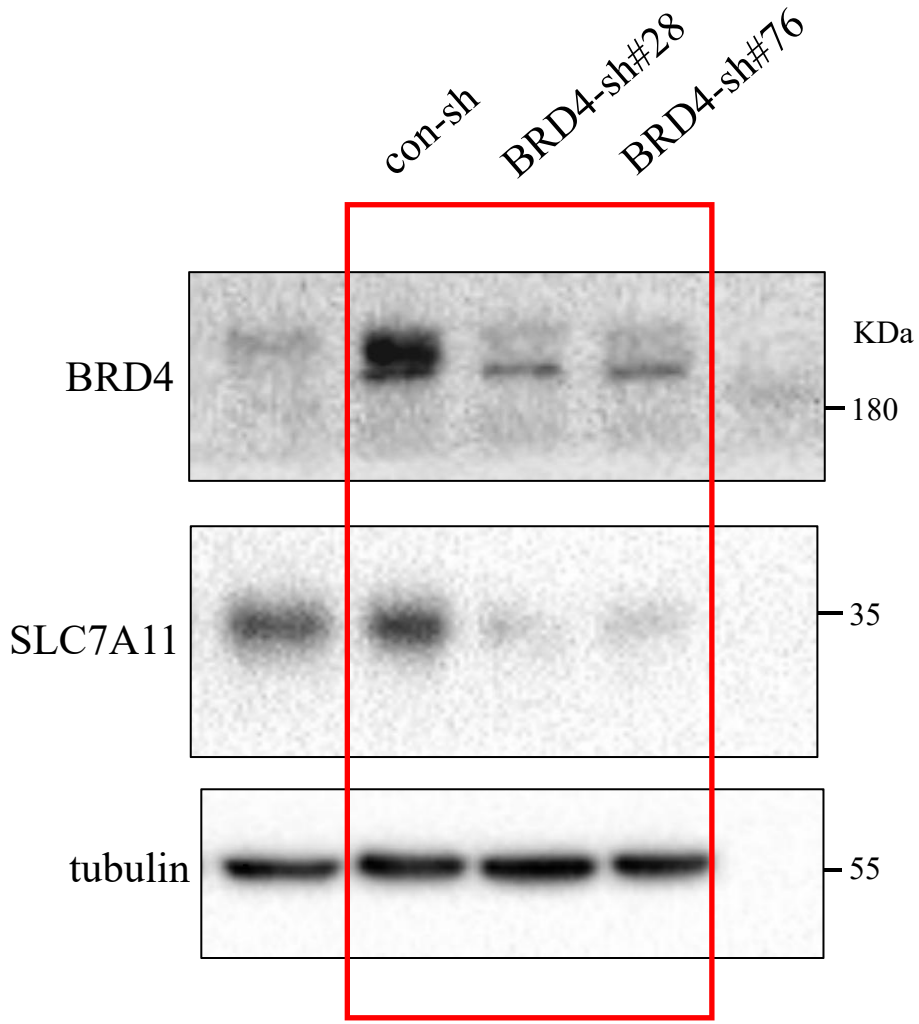

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Figure 5

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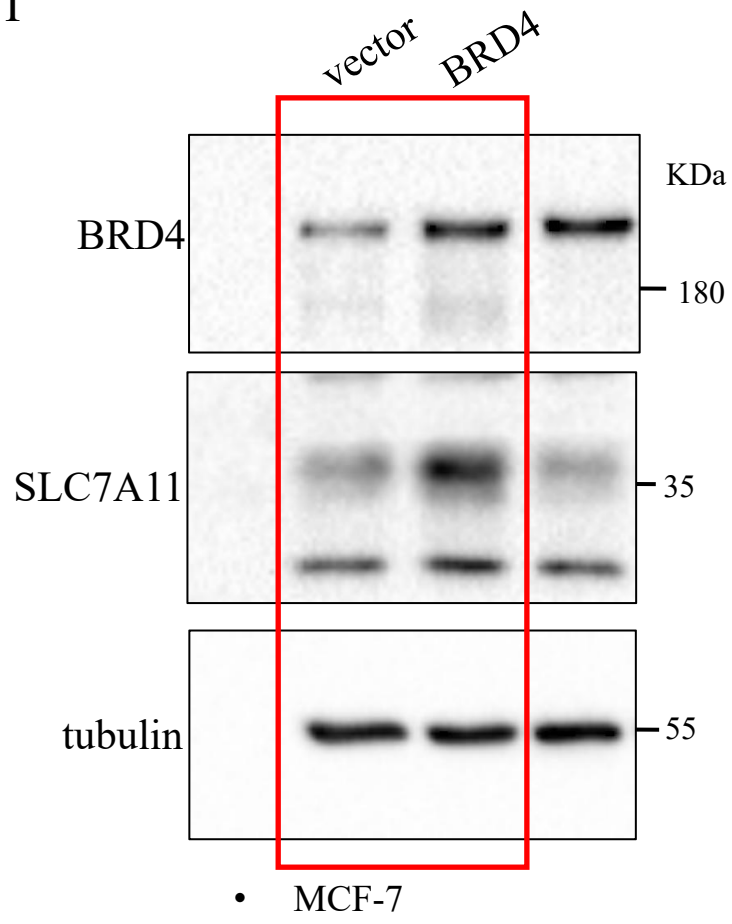

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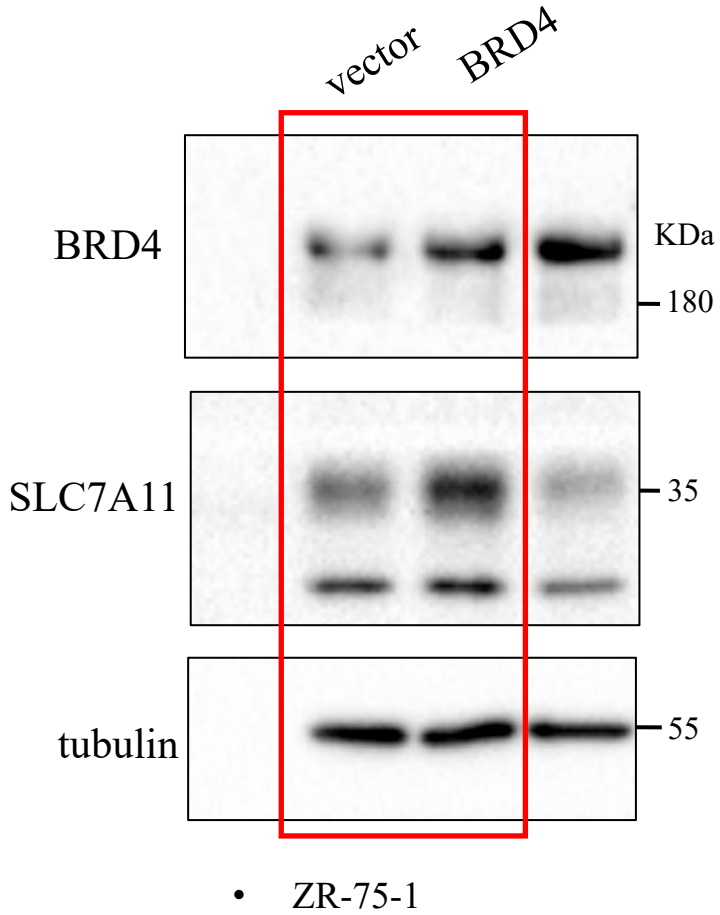

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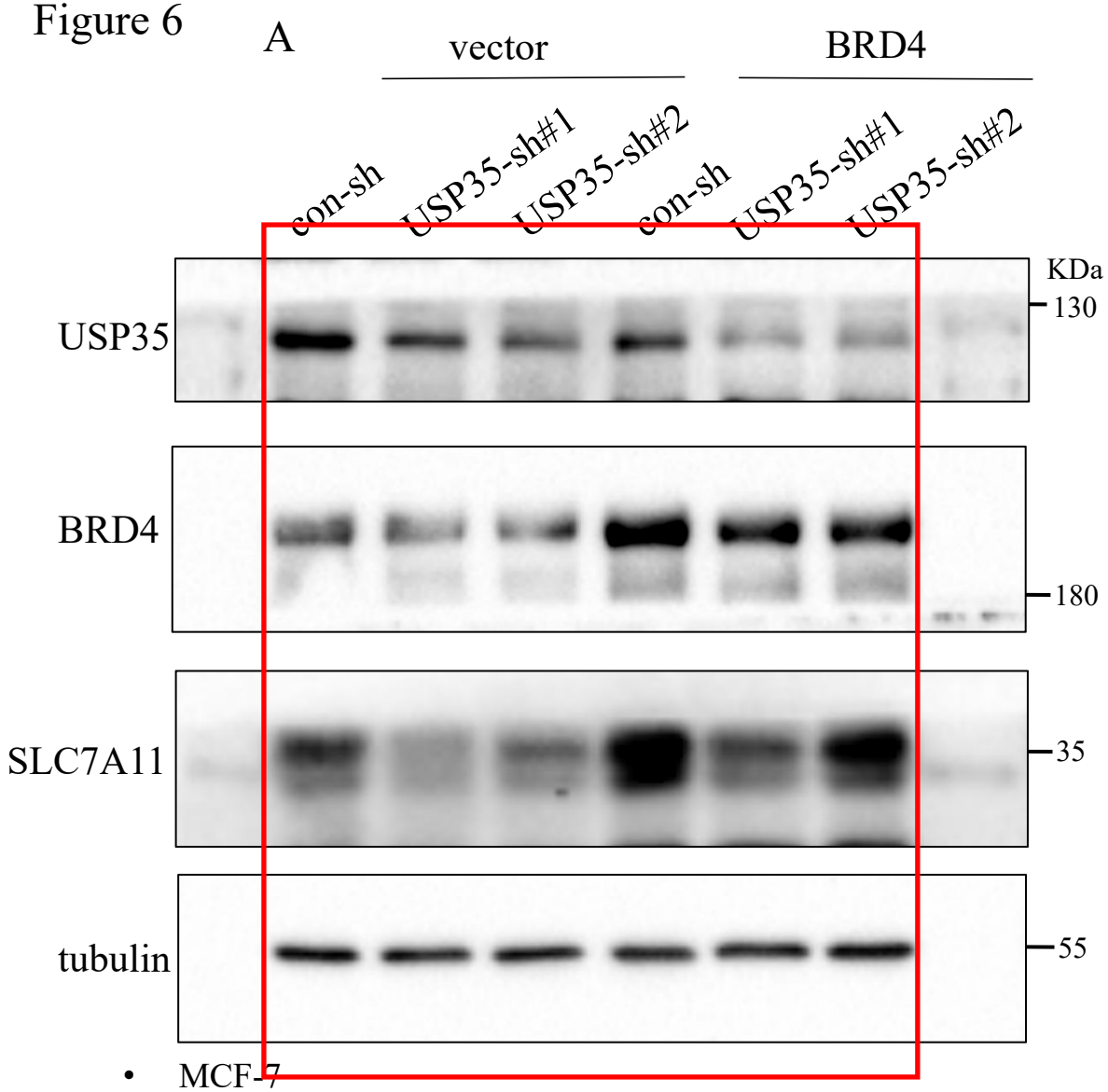
Figure 6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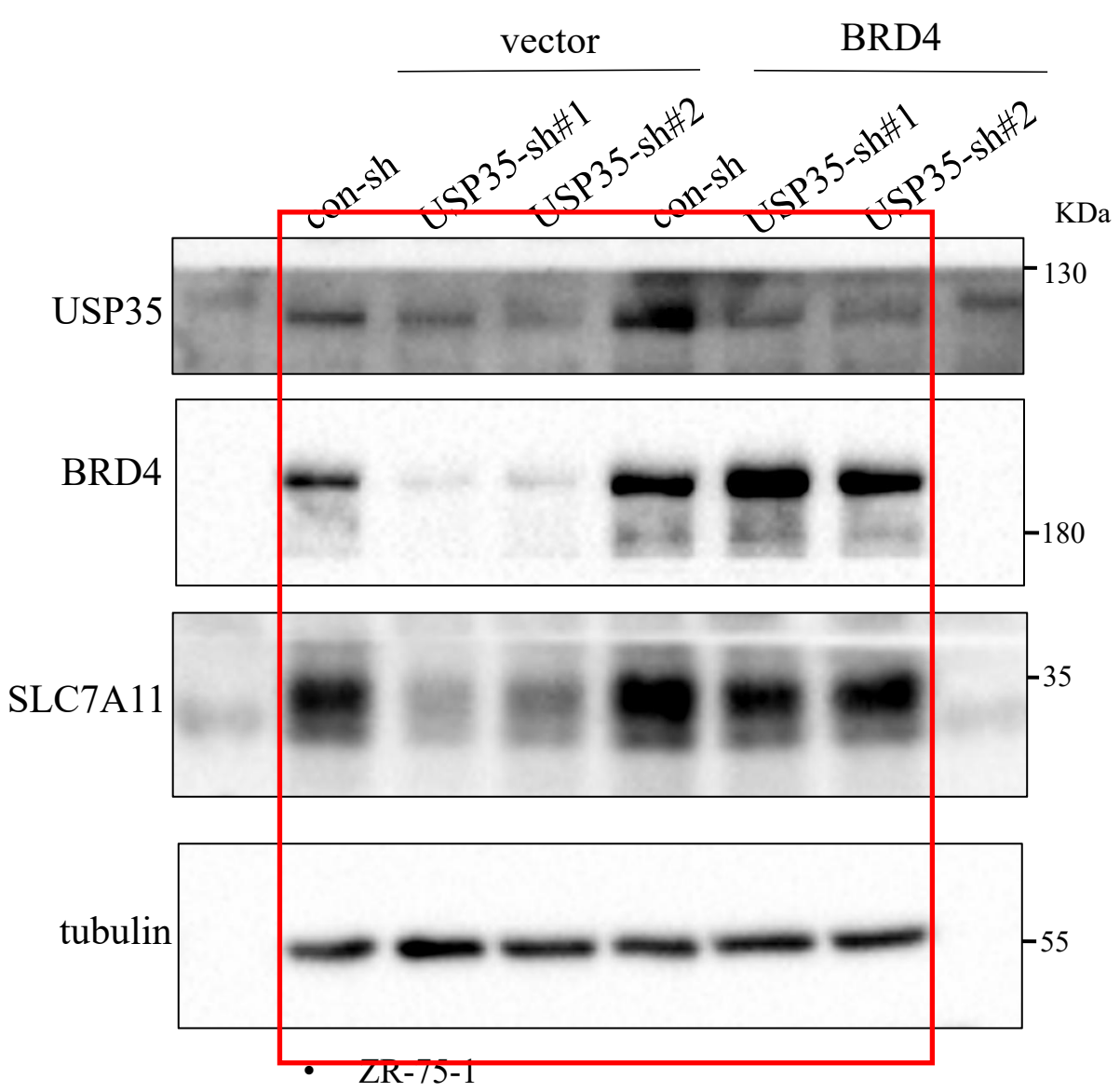
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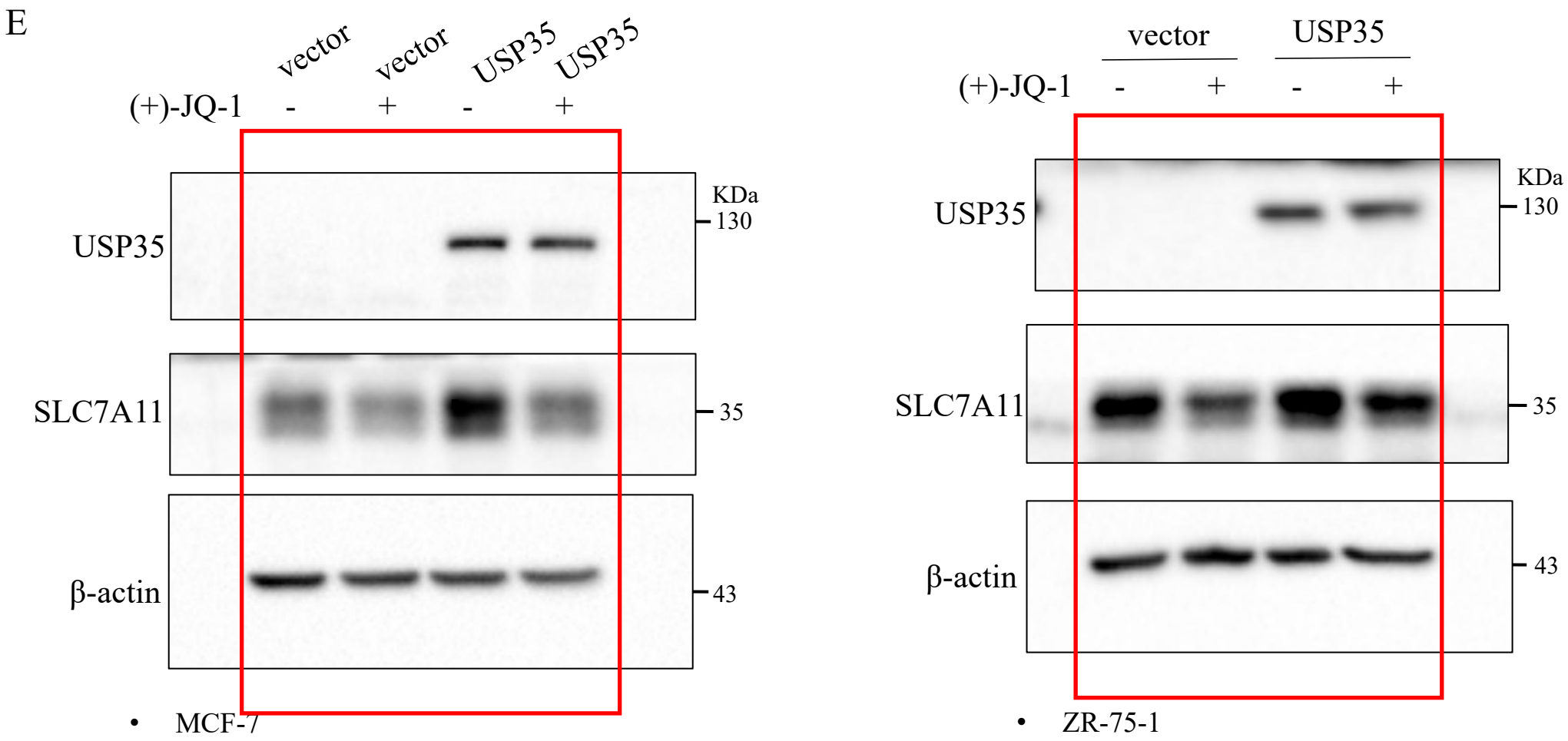
Figure 6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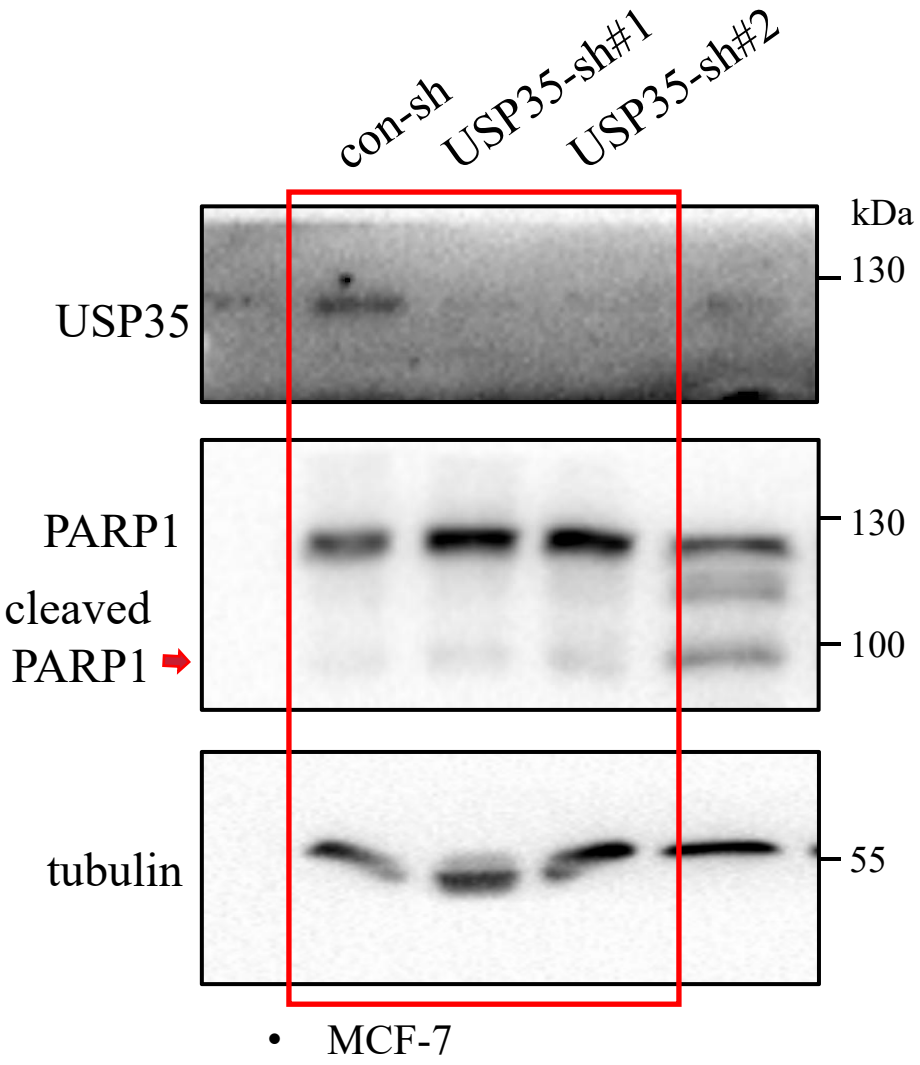
Supplementary Figure 2A



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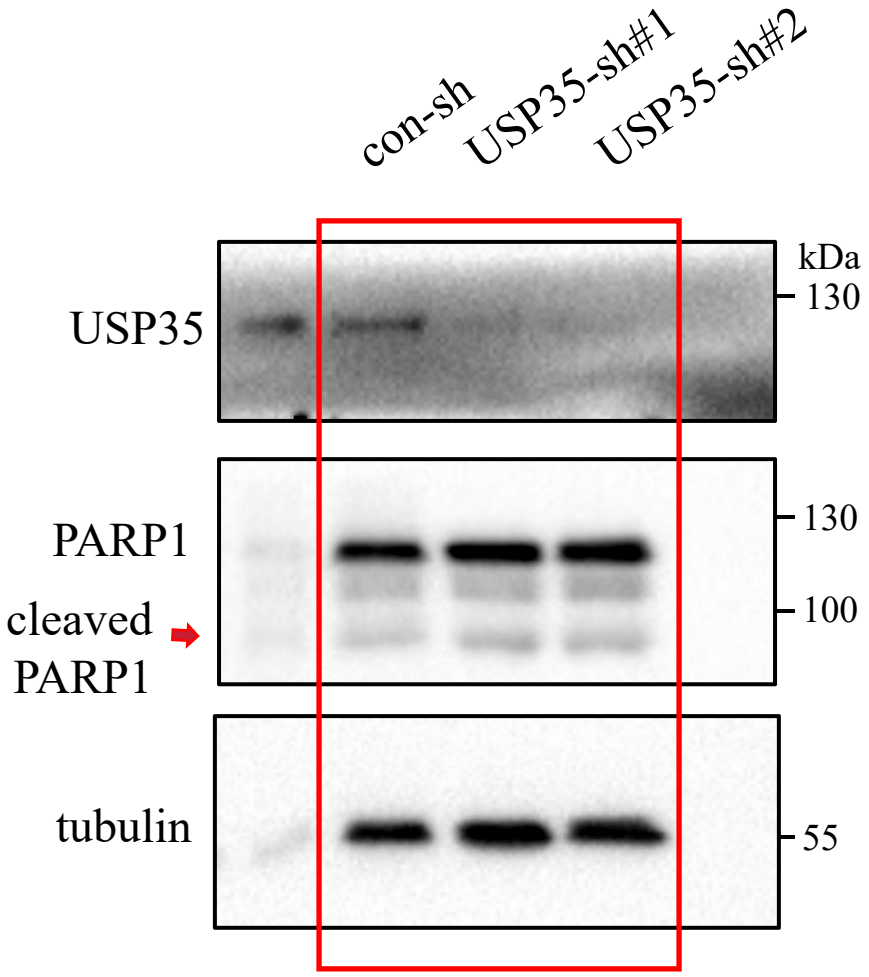
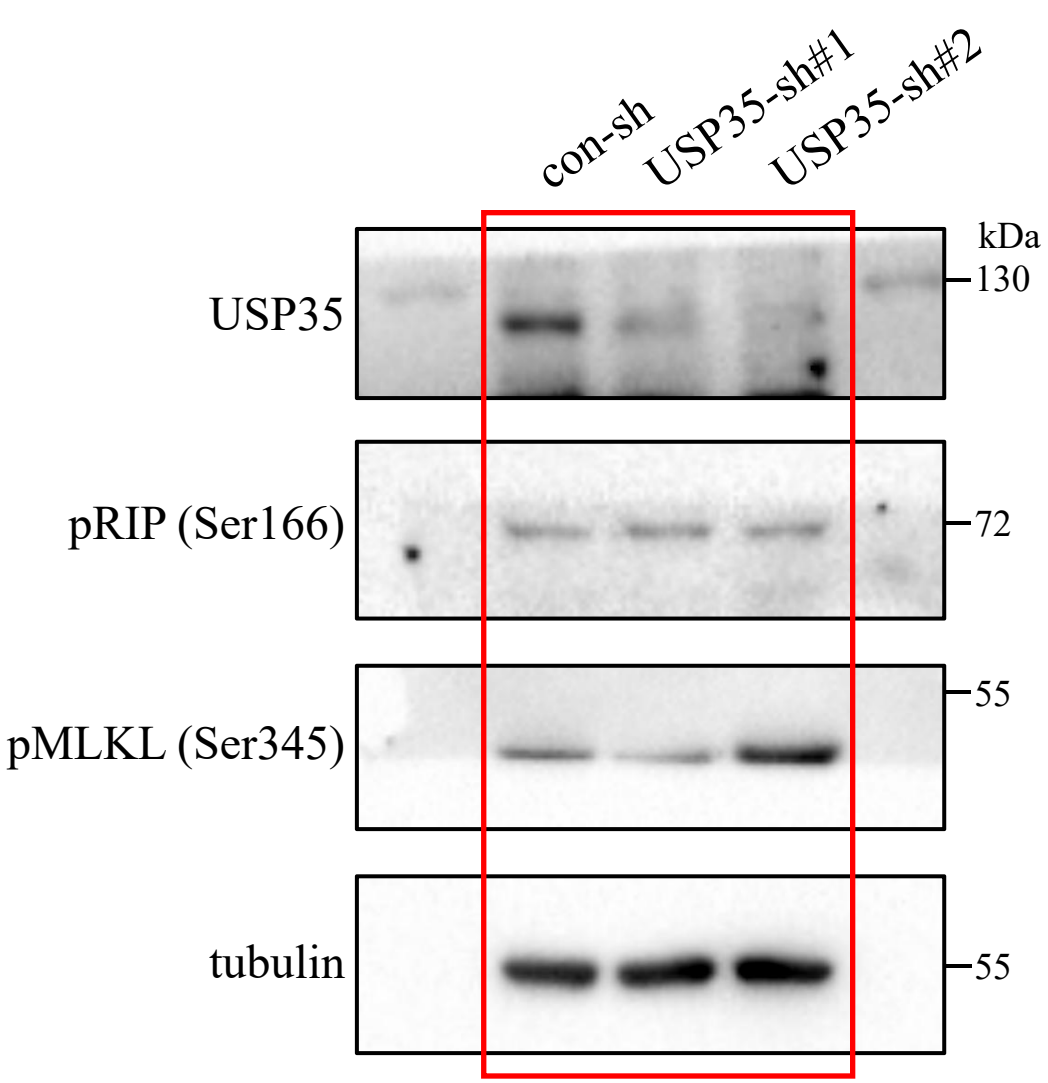
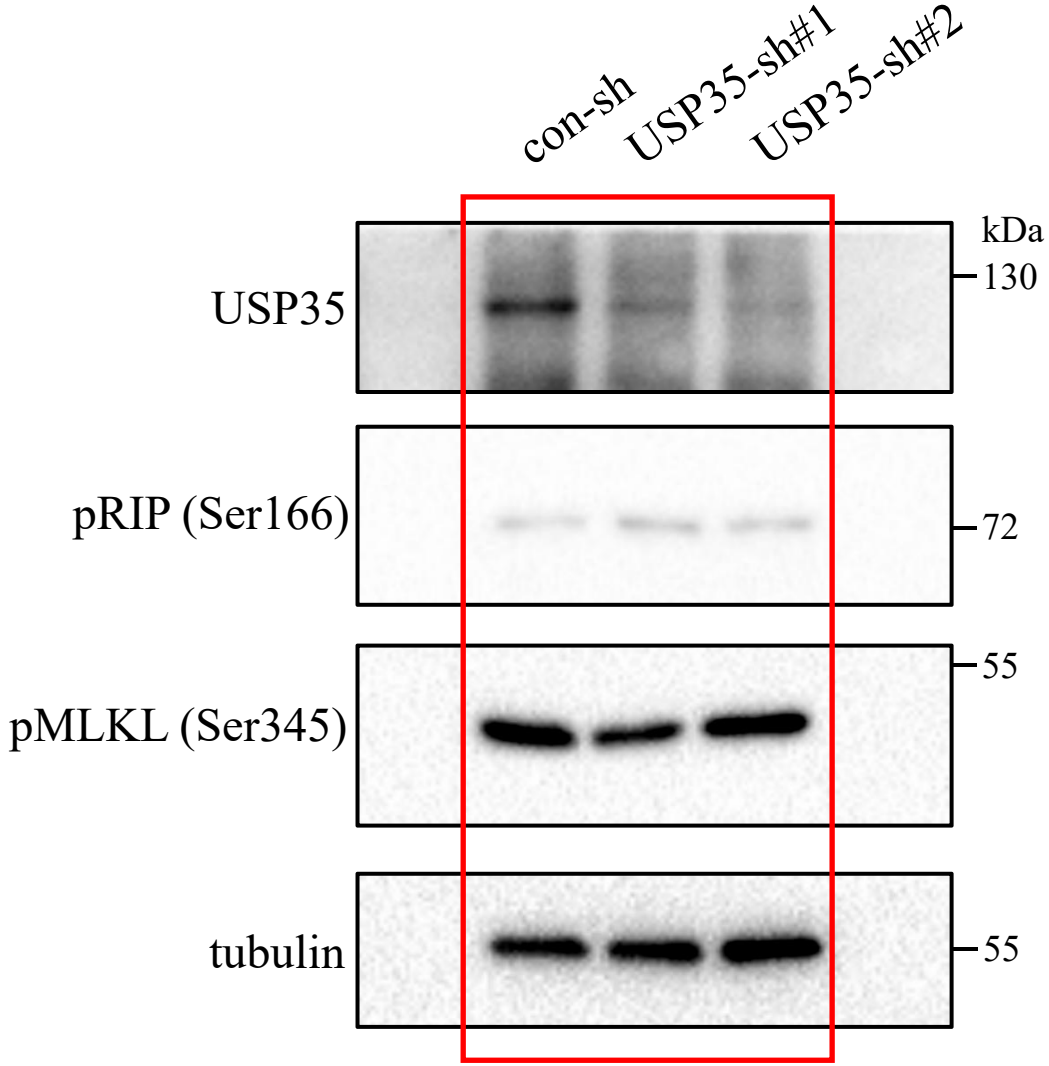


- ZR-75-1

Supplementary Figure 2B

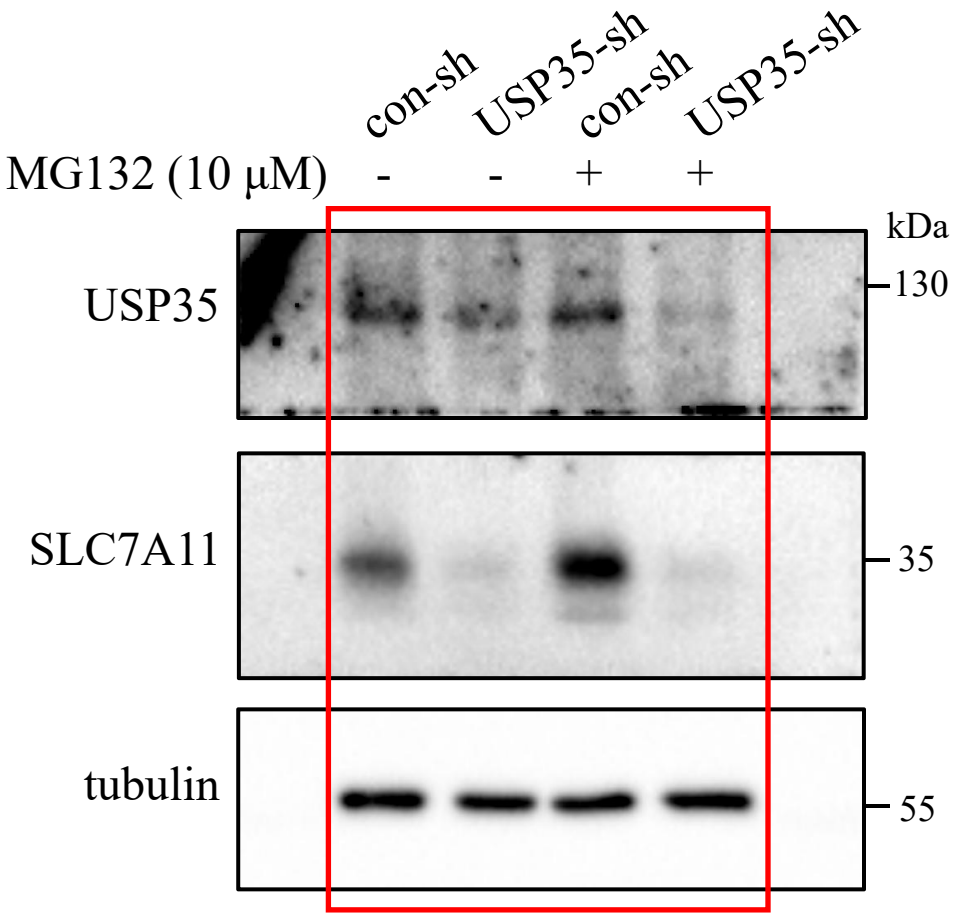


- MCF-7

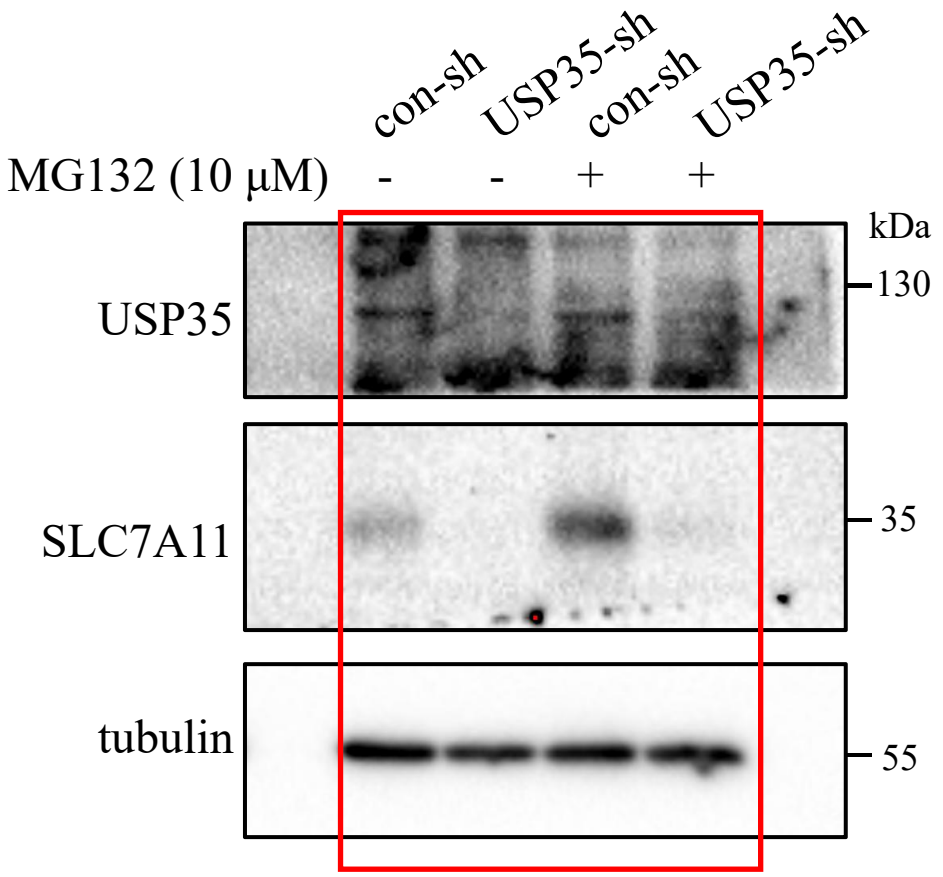


- ZR-75-1

Supplementary Figure 4

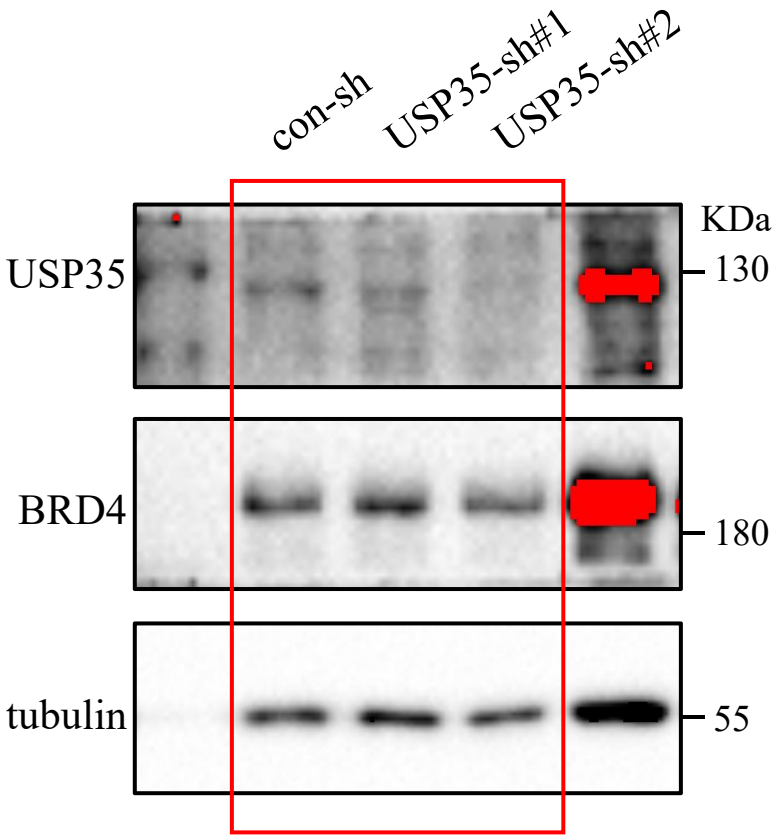


• MCF-7

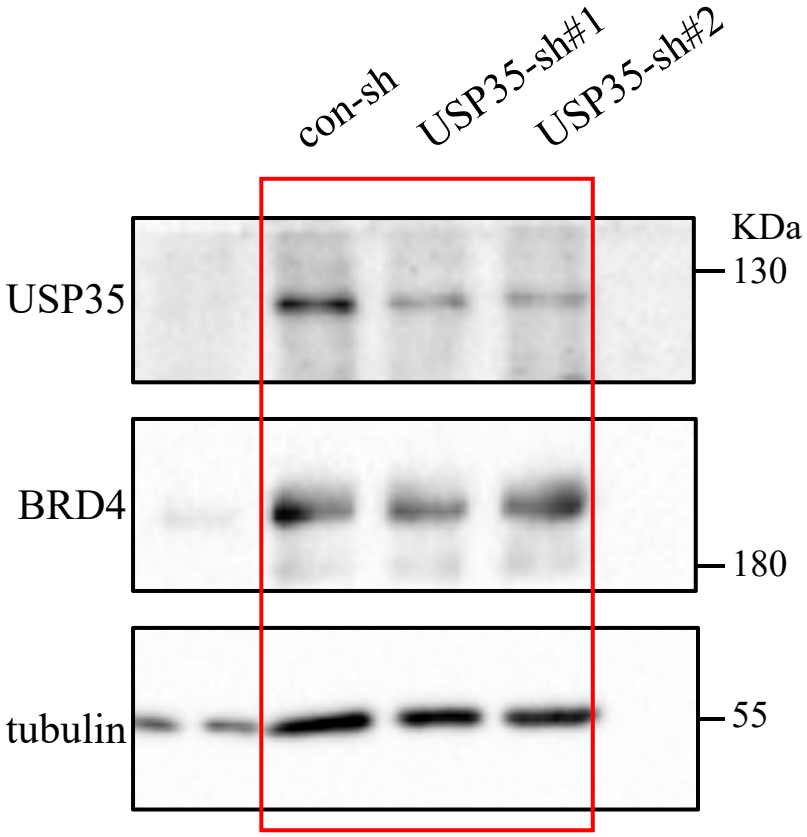


• ZR-75-1

Supplementary Figure 5A

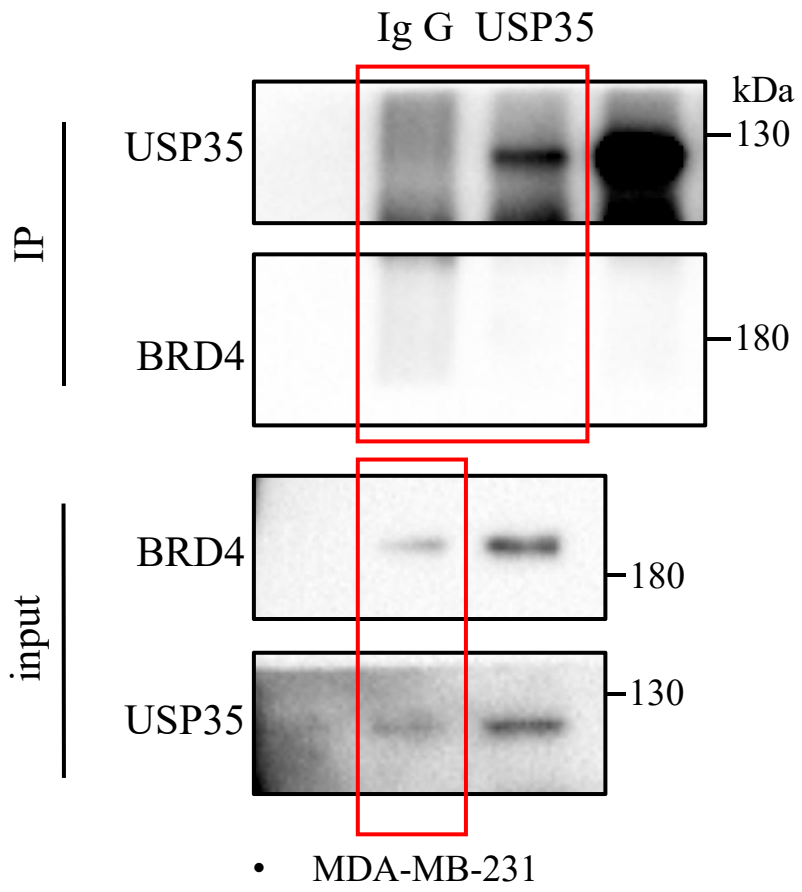


• MDA-MB-231

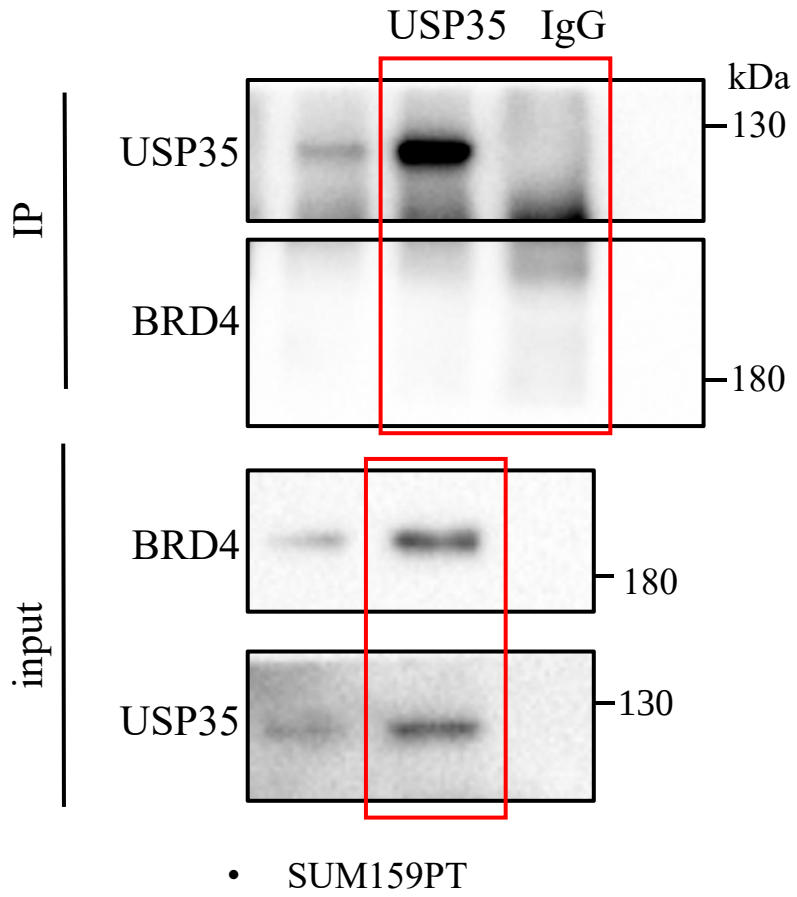


• SUM159PT

Supplementary Figure 5D

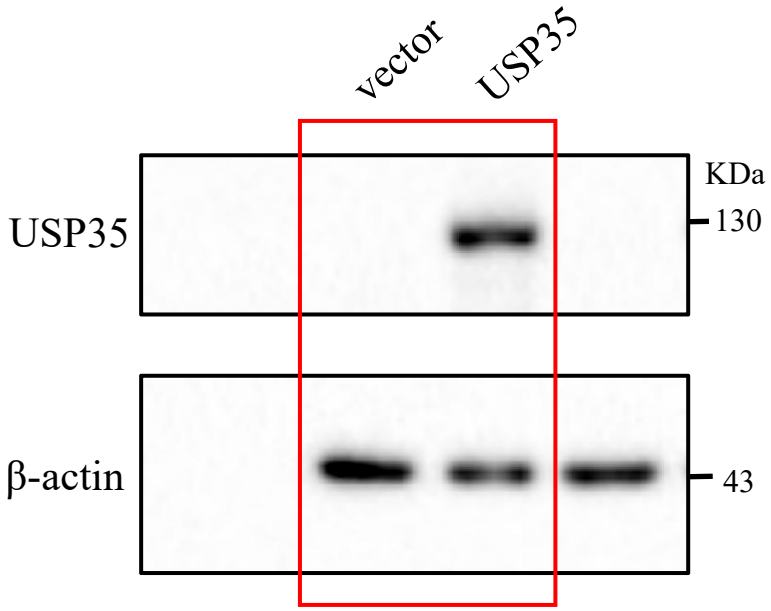


- MDA-MB-231



- SUM159PT

Supplementary Figure 9



- MDA-MB-231