RSL3 Induces Ferroptosis by Activating the NF-κB Signalling Pathway to Enhance the Chemosensitivity of Triple-negative Breast Cancer Cells to Paclitaxel

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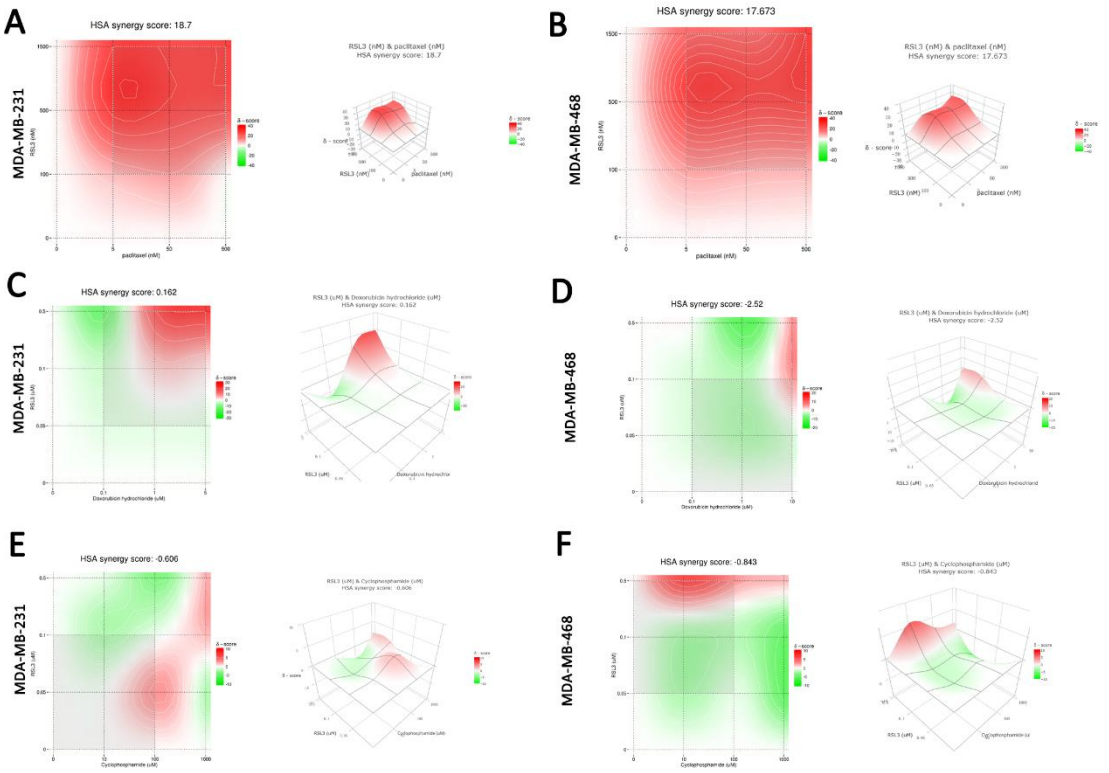


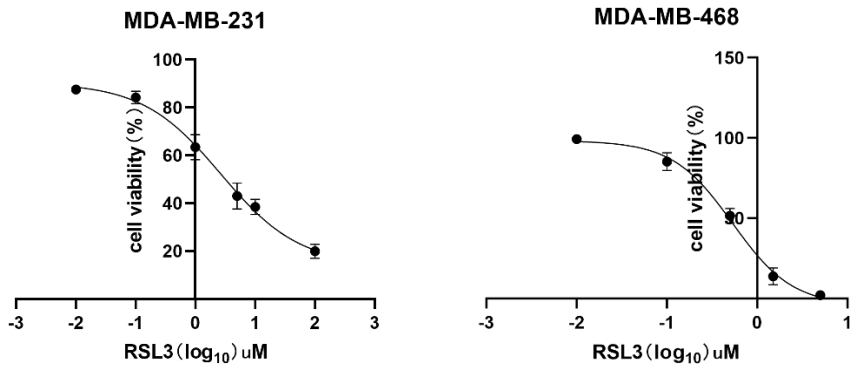
Supplementary Figure 1

The synergistic effect of the drug combination was evaluated via the SynergyFinder software, where an HSA score greater than 10 indicates a synergistic sensitization effect of the two drugs. (A, B) The HSA scores of paclitaxel combined with RSL3 were 18.7 for MDA-MB-231 and 17.673 for MDA-MB-468. (C, D) The HSA scores of doxorubicin combined with RSL3 were 0.162 for MDA-MB-231 and -2.52 for MDA-MB-468. (E, F) The HSA scores of cyclophosphamide combined with RSL3 were -0.606 for MDA-MB-231 and -0.843 for MDA-MB-468.

IL-1β	Forward Primer	5’ -TTCGACACATGGGATAACGAGG-3’
	Reverse Primer	5’ -TTTTTGCTGTGAGTCCCGGAG-3’
CXCL8	Forward Primer	5’ -TTTTGCCAAGGAGTGCTAAAGA-3’
	Reverse Primer	5’ -AACCCTCTGCACCCAGTTTTC-3’
CXCL2	Forward Primer	5’ -GAAAGCTTGTCTCAACCCCG-3’
	Reverse Primer	5’ -TGGTCAGTTGGATTTGCCATTTT-3’
IL-6	Forward Primer	5′-ACTCACCTCTTCAGAACGAATTG-3′
	Reverse Primer	5′-CCATCTTTGGAAGGTTCAGGTTG-3′
EGR1	Forward Primer	5′-GGTCAGTGGCCTAGTGAGC-3′
	Reverse Primer	5′-GTGCCGCTGAGTAAATGGGA-3′

Supplementary Table 1

The primer sequences for the qRT-PCR of the genes examined.



Supplementary Figure 2

We examined the antiproliferative effect of RSL3 in TNBC cells. The IC50 values of RSL3 were calculated by fitting curves using Graphpad prism8 software, revealing that the IC50 of RSL3 in MDA-MB-231 was 2.668 μM, while that in MDA-MB-468 was 0.5329 μM. We selected the drug concentration at 20% of the IC50 of RSL3 for subsequent sensitization experiments.

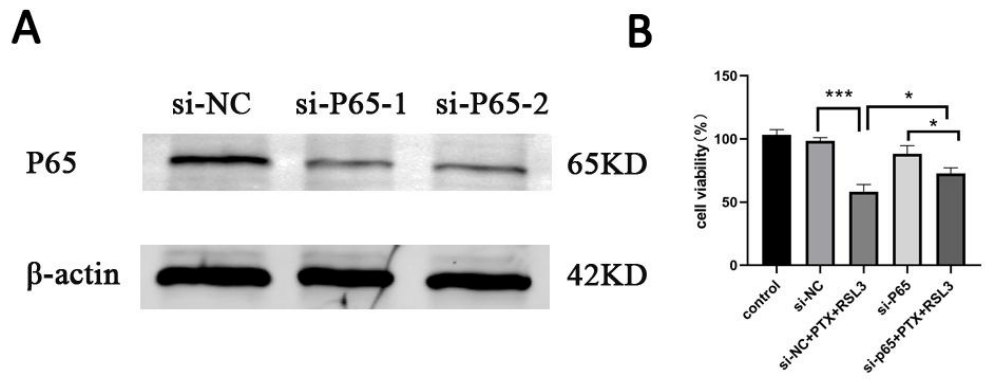
RNAi Treatment

MDA-MB-231 cells were transfected with the indicated small interfering RNAs (siRNAs) using GP-transfect-Mate for a duration of 96 hrs, at which point they were harvested. The precise sequences of the utilized RNAs (siRNAs) were as follows:

siRNA-p65-1: forward 5′-GGACAUAUGAGACCUUCAATT-3′,
reverse 5′-UUGAAGGUCUCAUAUGUCCTT-3′;

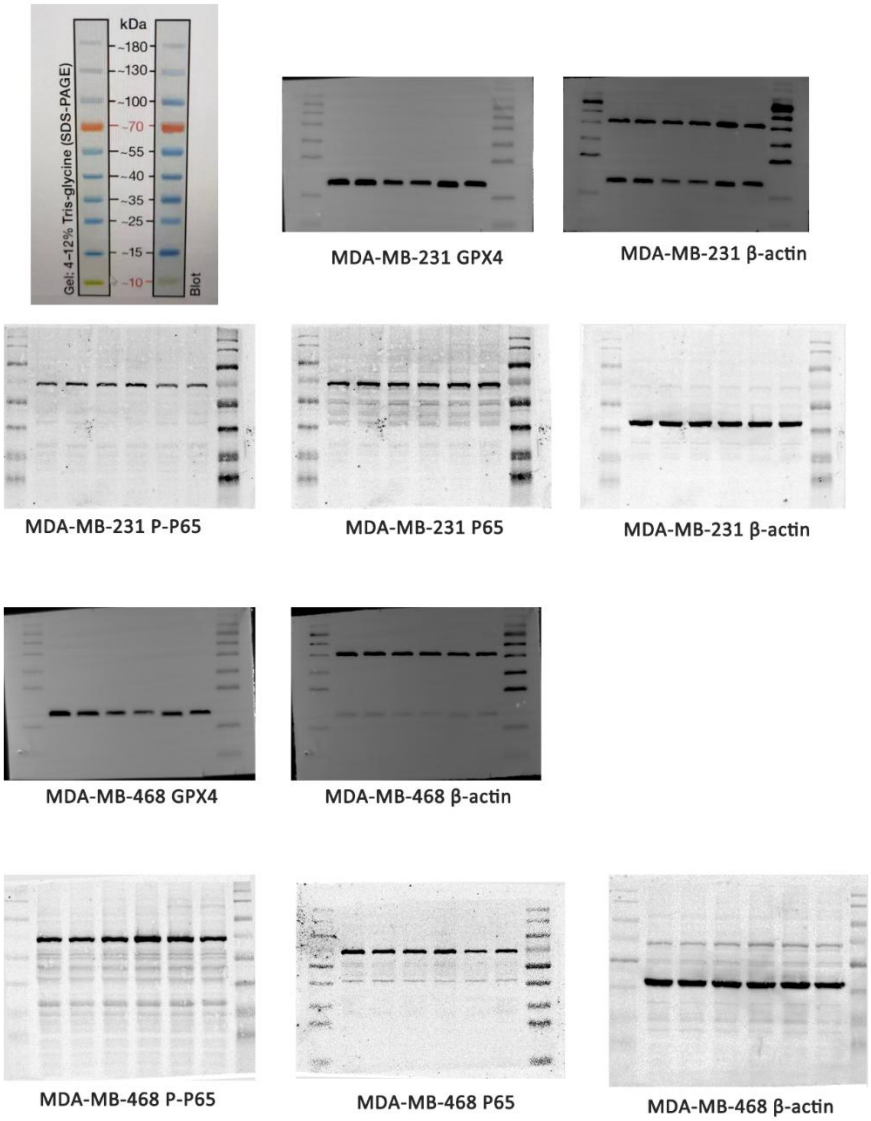
siRNA-p65-2: forward 5′-UCUUCCUACUGUGUGACAATT-3′,
reverse 5′-UUGUCACACAGUAGGAAGATT-3′;

We evaluated the impact of P65 knockdown on the proliferative capacity of MDA-MB-231 cells. The results indicated that compared to the si-NC group, the si-NC+PTX+RSL3 group exhibited decreased cell proliferation. Furthermore, in comparison to the si-NC + PTX + RSL3 group, cell viability is increased in the si-P65 + PTX + RSL3 group. Additionally, when compared to the si-P65 group, cell viability decreases in the si-P65+PTX+RSL3 group. (Supplementary Fig. 3).



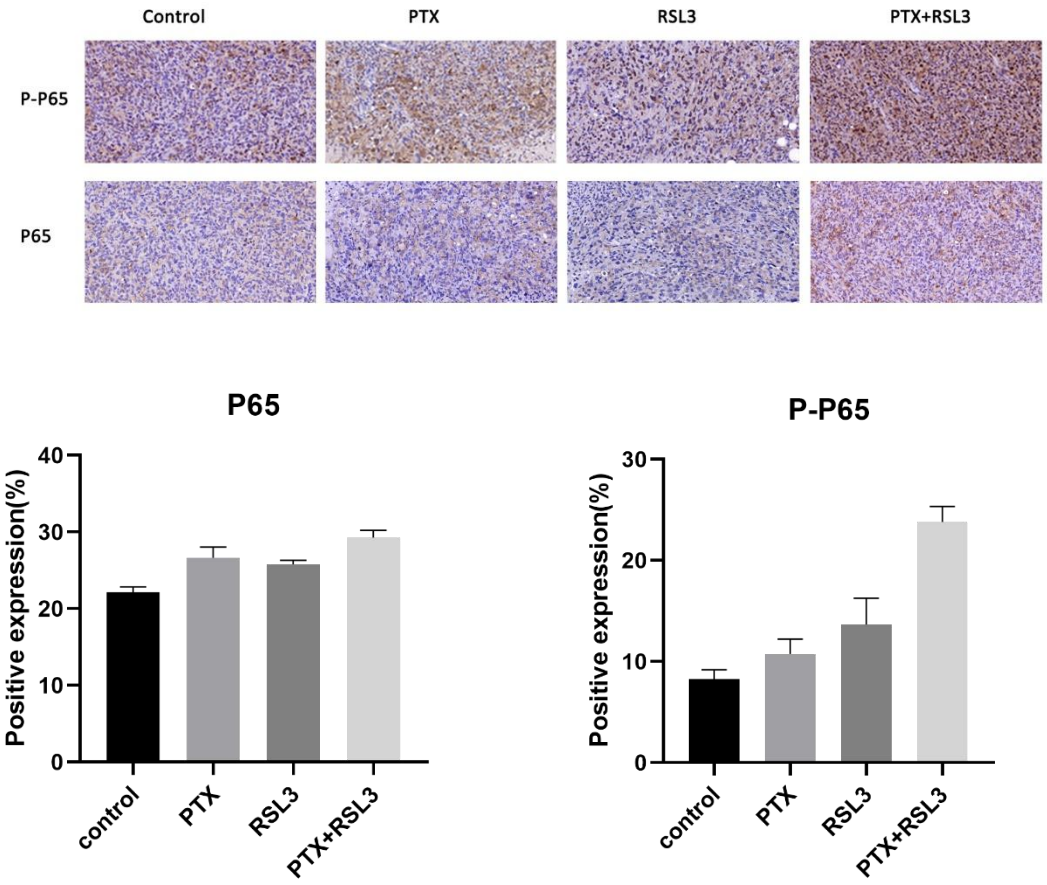
Supplementary Figure 3

(A) MDA-MB-231 cells transfected with the indicated small interfering RNAs (siRNAs) were analyzed by western blot. **(B)** Cell proliferation was measured by CCK-8 assay.



Supplementary Figure 4D

Western blotting, full-length/uncropped gels and blots, (lanes 1, 2, 3, 4, 5 and 6, corresponding to Control, Paclitaxel (PTX), RSL3, PTX+RSL3, BAY11-7082 and PTX+RSL3+BAY11-7082) all of them.



Supplementary Figure 5

The expression of P65 and P-P65, detected by immunohistochemical staining, scale bar: 20μm.

In P65 immunohistochemical staining, the positive expression in the PTX + RSL3 group exhibited a notable increase of 32.2% compared with the control group, 10.0% compared to the PTX group, and 13.7% compared to the RSL3 group. Similarly, in the P-P65 immunohistochemical staining, the positive expression in the PTX + RSL3 group also demonstrated an significant increase, with a rise of 187.7% over the control group, 121.5% over the PTX group, and 74.2% over the RSL3 group. Notably, although both P-P65 and P65 expressions increased in the PTX+RSL3 group compared to the other three groups, the increase in P-P65 expression was more pronounced.