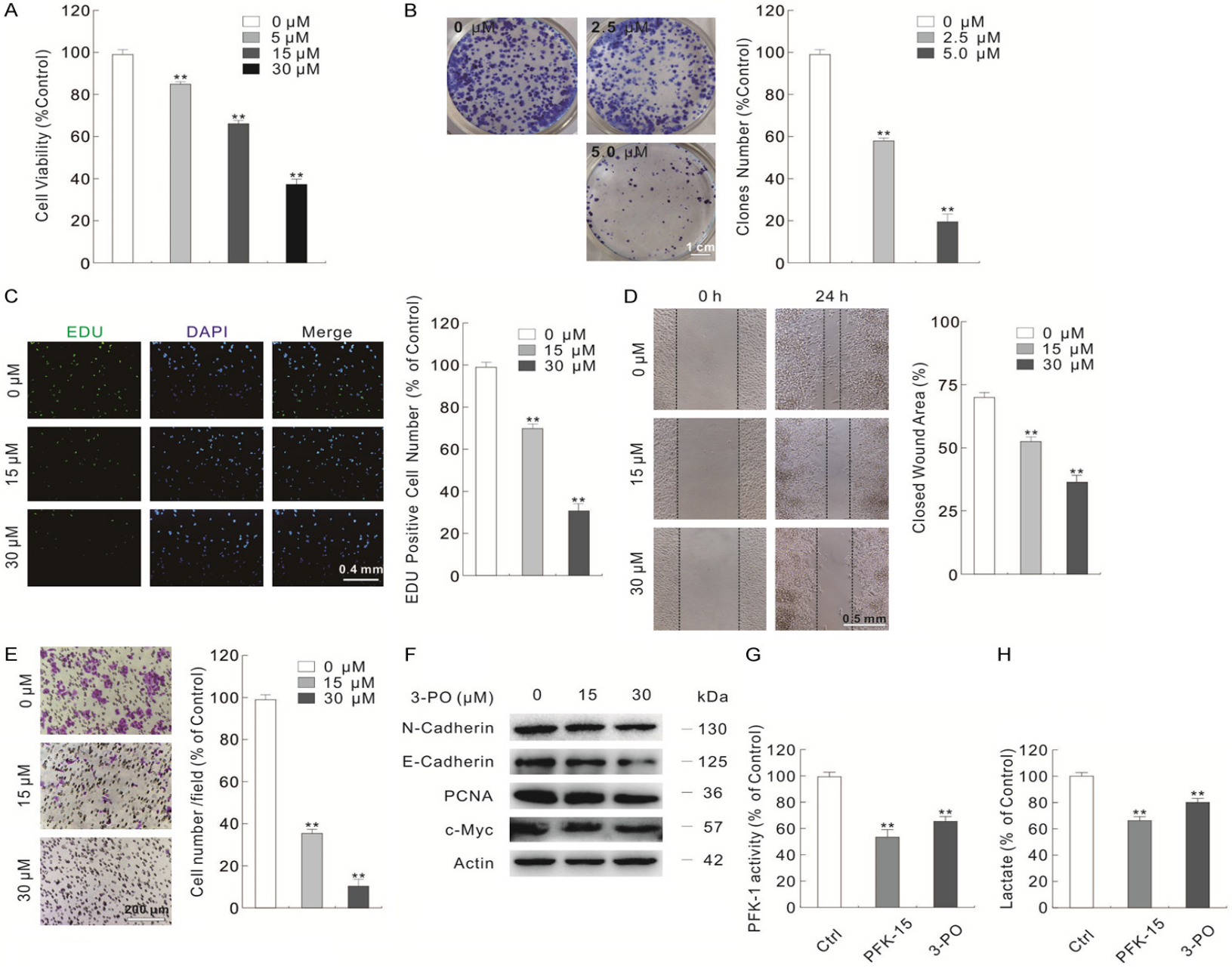
A
Cell Viability (%Control)
0 μM
5 μM
15 μM
30 μM
B
0 μM
2.5 μM
5.0 μM
1 cm
Clones Number (%Control)
0 μM
2.5 μM
5.0 μM
C
EDU
DAPI
Merge
0 μM
15 μM
30 μM
0.4 mm
EDU Positive Cell Number (% of Control)
0 μM
15 μM
30 μM
D
0 h
24 h
0 μM
15 μM
30 μM
0.5 mm
Closed Wound Area (%)
0 μM
15 μM
30 μM
E
0 μM
15 μM
30 μM
200 μm
Cell number /field (% of Control)
0 μM
15 μM
30 μM
F
3-PO (μM)
0
15
30
kDa
N-Cadherin
E-Cadherin
PCNA
c-Myc
Actin
130
125
36
57
42
G
PFK-1 activity (% of Control)
Ctrl
PFK-15
3-PO
H
Lactate (% of Control)
Ctrl
PFK-15
3-PO

Figure S1. 3-PO reduces cell viability in SW480 cells. A. Cell viability was measured in SW480 cells with indicated dose of 3-PO. B. Colony growth assay was performed with different dose of 3-PO. C. EDU staining assay was carried in SW480 cells with treatment of 3-PO for 6 h. D and E. Cell migration following 3-PO treatment for 24 h was monitored by wound healing assay and transwell assay. F. Following treatment of the cells with 3-PO for appropriated periods, cell lysates were prepared and analyzed by immunoblotting with presented antibodied. G and H. After indicated treatment for 24 h, SW480 cell pellets were performed PFK-1 activity assay (Comin, PFK-2-Y) and cell culture media were performed lactate assay (Megazyme, K-DATE) following the manufacturer's instructions. The scale bars were shown in the figure. For histogram results, data were presented as mean + S.D. and were representatives of three independent experiments (**P < 0.01 vs. control).

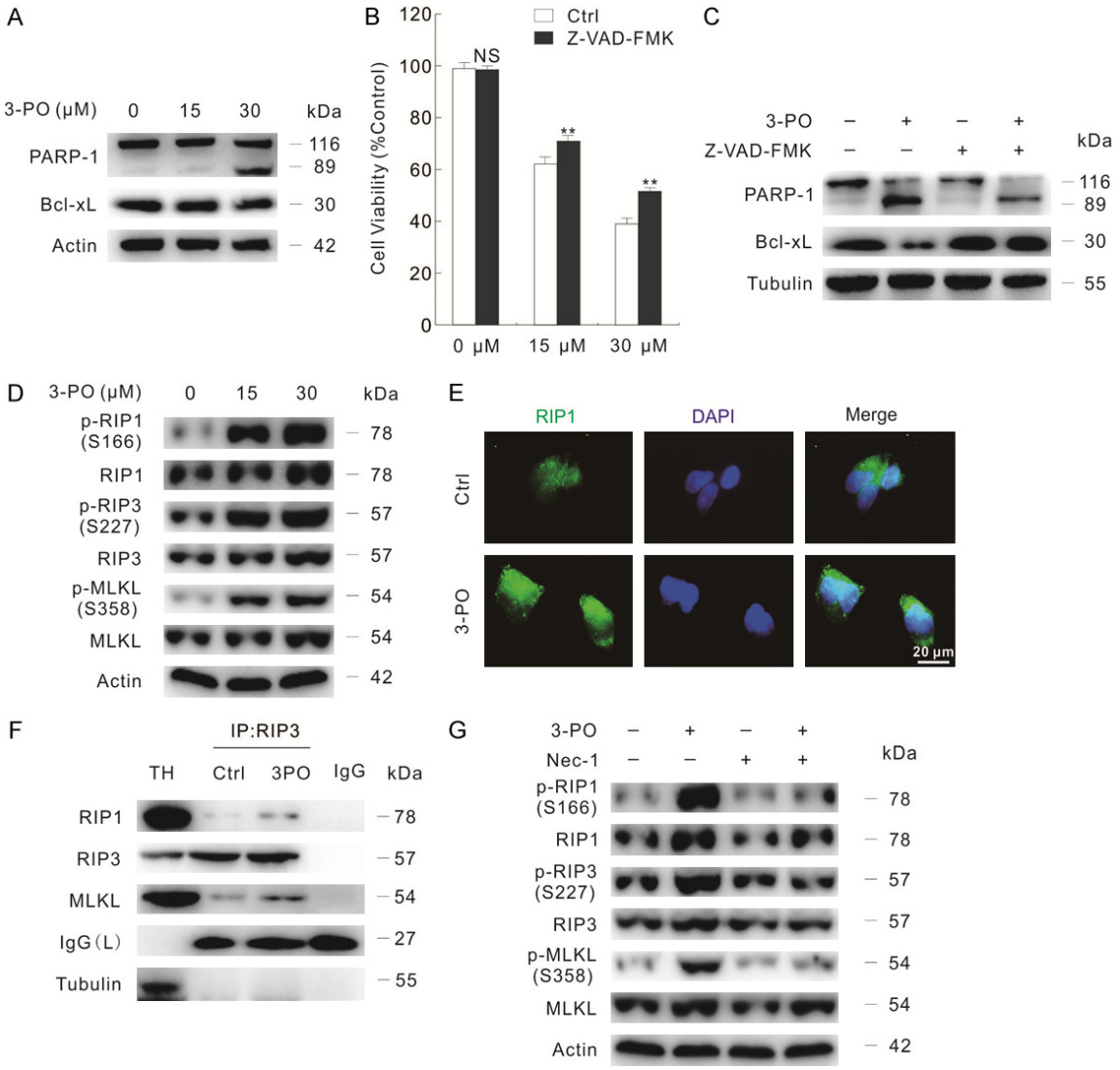

Figure S2. 3-PO induces both apoptosis and necroptosis in SW480 cells. A. Cell lysates were subjected to immunoblotting with indicated antibodies following treatment with 3-PO for 24 h. B. Cells were treated with different dose of 3-PO in the presence or absence of Z-VAD-FMK (20 μM; unless otherwise indicated), and then the cell viability was analyzed by MTS assay. C and D. Cell lysates were subjected to immunoblotting after treatment with 3-PO (30 μM; unless otherwise indicated) in the presence or absence of Z-VAD-FMK for 24 h. E. Immunofluorescence using the RIP1 antibody was performed following treatment with 3-PO treatment for 6 h. F. Immunoprecipitation was peformed using RIP3 antibody with or without 3-PO treatment for 24 h, and the immunoprecipitates were resolved by electrophoresis and probed by immunoblotting with the indicated antibodies. G. Cell lysates were collected and subjected to immunoblotting following treatment with 3-PO in the presence or absence of Nec-1 (30 μM; unless otherwise indicated) for 24 h. **P < 0.01 versus control, and NS indicated of none significant. The scale bars were shown in the figure.

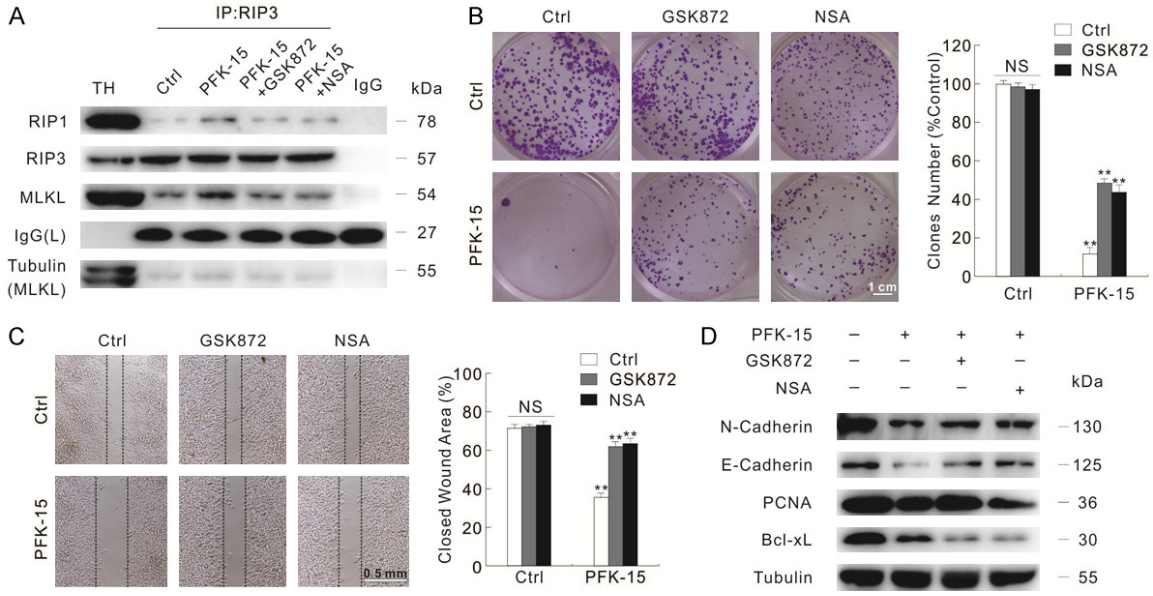

Figure S3. GSK872 and NSA attenuate the effect of PFK-15 on cell proliferation and migration in SW480 cells. (A) Immunoprecipitation was peformed using RIP3 antibody with indicated treatment for 24 h, and the immunoprecipitates were resolved by electrophoresis and probed by immunoblotting with the indicated antibodies. (B and C) Colony growth assay and wound healing assay were performed with PFK-15 (B: 1.5 μM; C: 6 μM) in the presence or absence of GSK872/NSA. (D) Following indicated treatment for 24 h, cell lysates were prepared and subjected to immunoblotting with indicated antibodies. For histogram graph data, **P < 0.01 versus control, and NS indicated of none significant. The scale bars were shown in the figure.

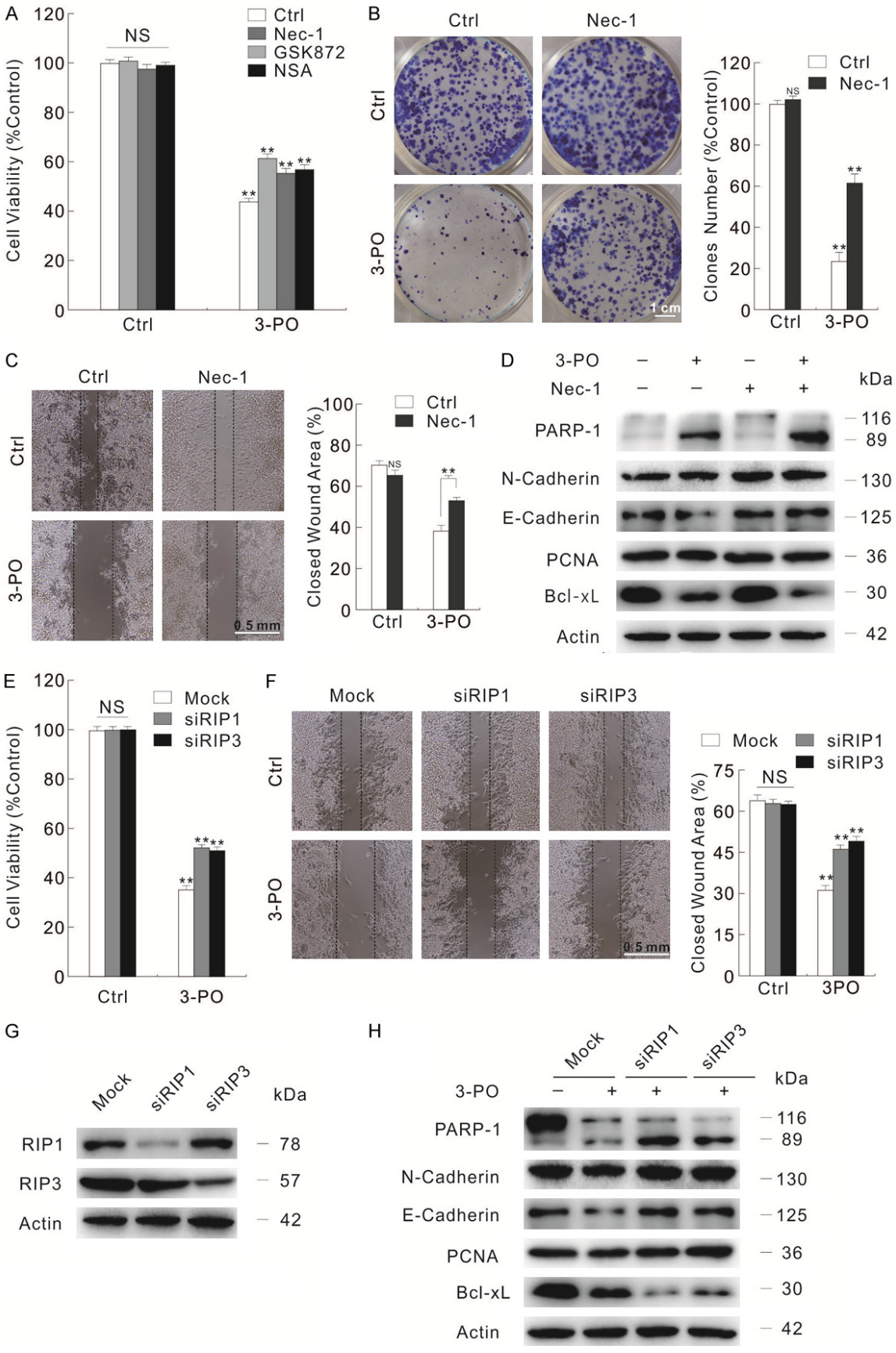
A
Cell Viability (%Control)
NS
Ctrl
Nec-1
GSK872
NSA
Ctrl
3-PO
B
Ctrl
Nec-1
Ctrl
3-PO
1 cm
Clones Number (%Control)
C
Ctrl
Nec-1
Ctrl
3-PO
0.5 mm
Closed Wound Area (%)
D
3-PO
Nec-1
kDa
PARP-1
N-Cadherin
E-Cadherin
PCNA
Bcl-xL
Actin
116
89
130
125
36
30
42
E
Cell Viability (%Control)
NS
Mock
siRIP1
siRIP3
Ctrl
3-PO
F
Mock
siRIP1
siRIP3
Ctrl
3-PO
0.5 mm
Closed Wound Area (%)
Ctrl
3PO
G
Mock
siRIP1
siRIP3
kDa
RIP1
RIP3
Actin
78
57
42
H
Mock
siRIP1
siRIP3
3-PO
kDa
PARP-1
N-Cadherin
E-Cadherin
PCNA
Bcl-xL
Actin
116
89
130
125
36
30
42

Figure S4. Inhibition of necroptosis attenuates the cell viability loss aroused by 3-PO. (A) MTS assays were performed in SW480 cells exposed to 3-PO treatment with or without necroptosis inhibitors (30 μM Nec-1, 3 μM GSK872, 3 μM NSA). (B and C) Colony growth assay and wound healing assay were performed with 3-PO (B: 2.5 μM; C: 30 μM) in the presence or absence of Nec-1. (D) Following indicated treatment for 24 h, cell lysates were prepared and subjected to immunoblotting with indicated antibodies. (E-H) Cells were transfected with the RIP1, RIP3 target siRNAs or control siRNA for 48 h. Cell viability was analyzed by MTS assay following the treatment with 3-PO for 24 h (E). Wound healing assay were performed with 3-PO (F). Following 3-PO treatment for 24 h, cell lysates were prepared and subjected to immunoblotting with indicated antibodies (G and H). For histogram graph data, **P < 0.01 versus control, and NS indicated of none significant. The scale bars were shown in the figure.

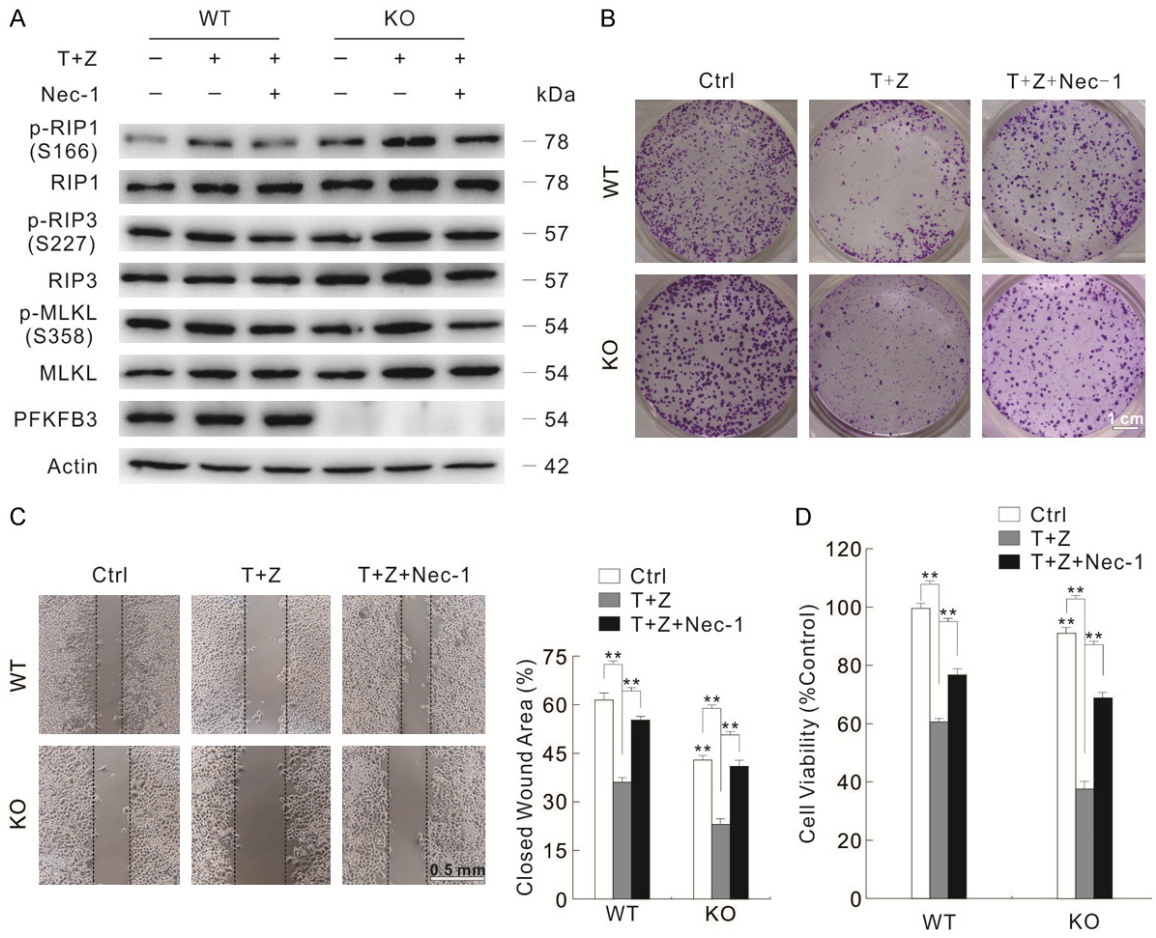

Figure S5. Knockout of PFKFB3 promotes the cell viability loss aroused by TNFα+Z-VAD-FMK. PFKFB3 wild type (WT) and knockout (KO) HT29 cell lines were cultured and adjusted to same concentration. A. Cell lysates were subjected to immunoblotting following treatment of cells with T+Z (10 ng/mL hTNFα+Z-VAD-FMK) in the presence or absence of Nec-1 for 24 h. B and C. Colony growth assay and wound healing assay were performed with T+Z in the presence or absence of Nec-1. D. Cell viability was measured following indicated treatment for 24 h by MTS assay. *P < 0.05 vs. control, and **P < 0.01 vs. control. The scale bars were shown in the figure.

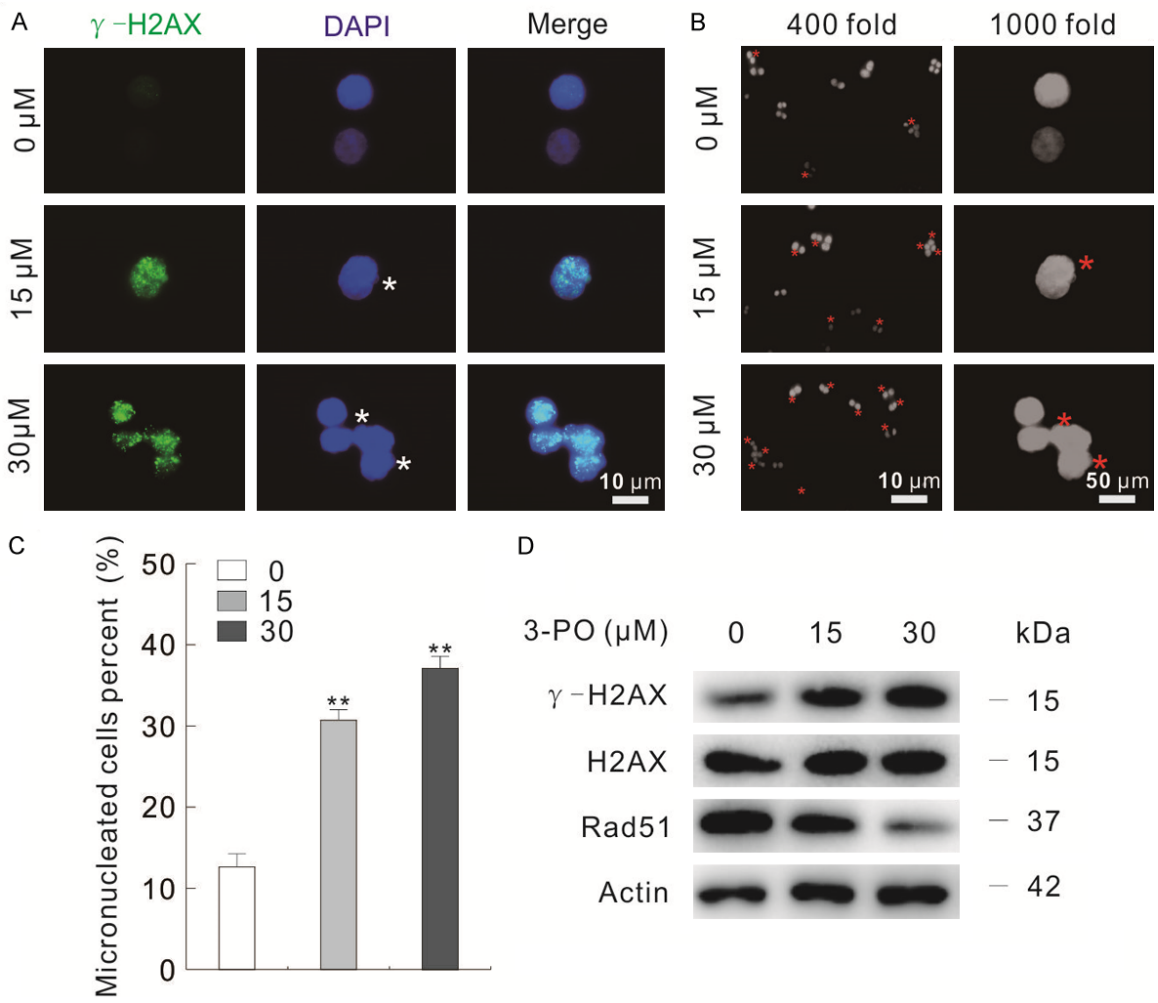

Figure S6. 3-PO stimulates genome instability. (A) Immunofluorescence using the antibody of γ-H2AX were performed following treatment with 3-PO for 6 h in SW480 cells. (B and C) Cells were treated with indicated dose of 3-PO for 6 h, and then the images were obtained by fluorescence microscopy after DAPI staining with both 400 and 1000 magnification. The percentages of micronuclei were analyzed and shown in (C), and the data were presented as mean + S.D. in graphs, **P < 0.01 versus control. At least 50 cells were included for each group. (D) Following treatment of the cells with 3-PO for appropriated periods, cell lysates were subjected to immunoblotting with the antibodies indicated. The scale bars were shown in the figure. Asterisk indicated micronuclei.

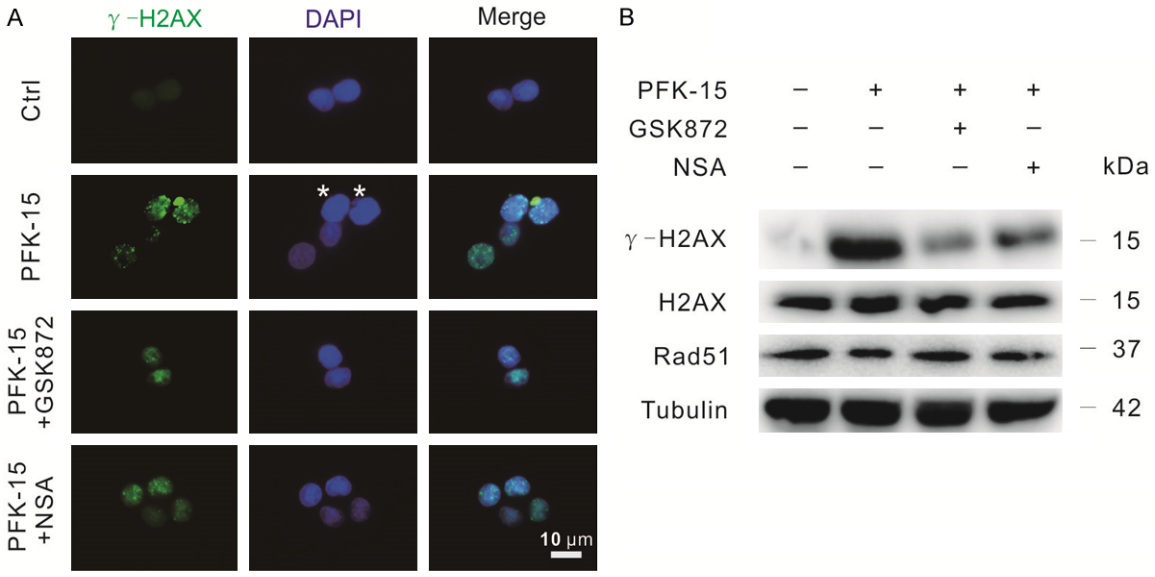

Figure S7. GSK872 and NSA depress the genome instability induced by PFK-15. (A) Cells were treated with indicated drugs for 6 h, and then the images were obtained by fluorescence microscopy after DAPI and γ-H2AX staining. (B) Following similarly treatment as (A), cell lysates were subjected to immunoblotting with the antibodies indicated. The scale bars were shown in the figure. Asterisk indicated micronuclei.

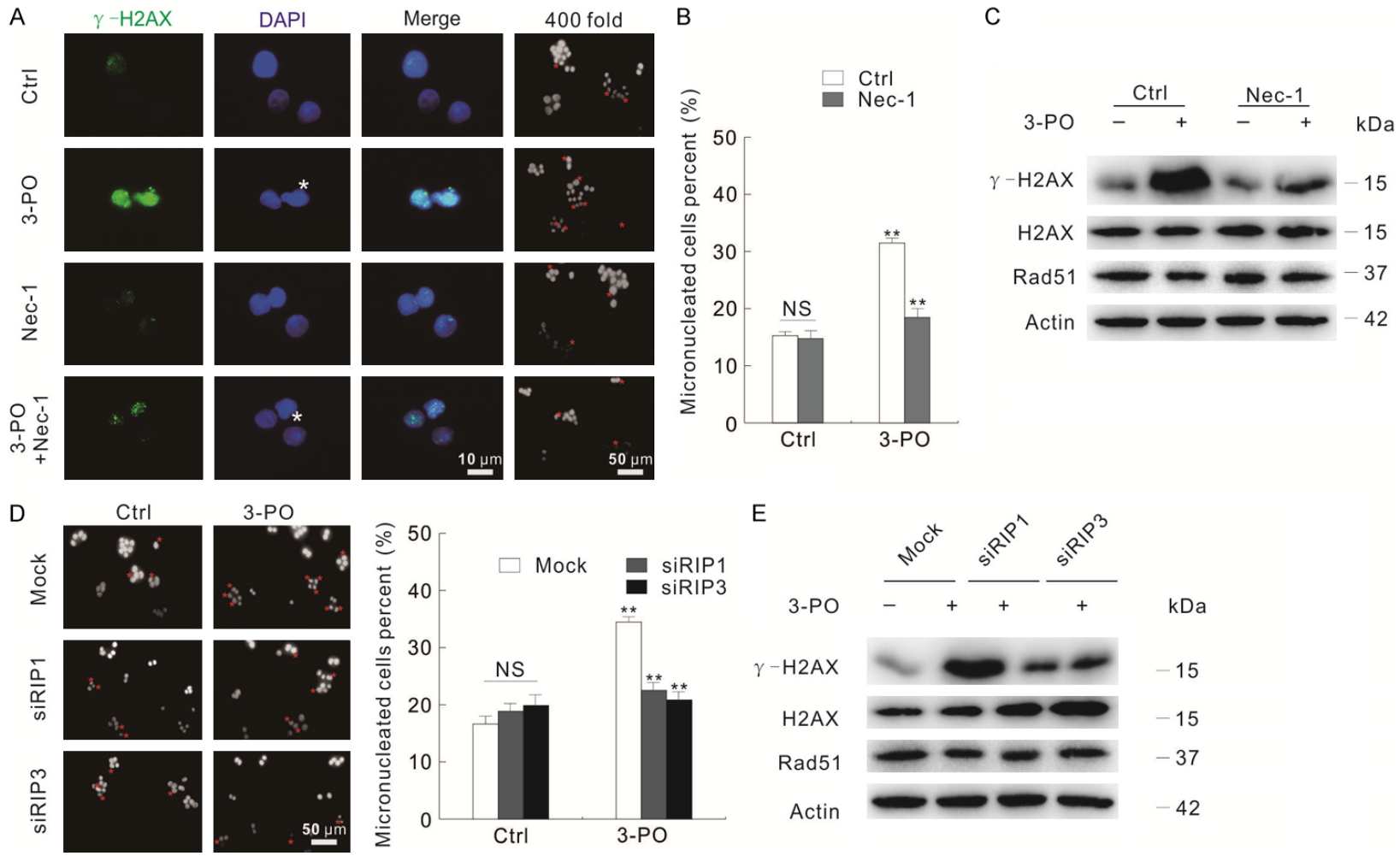

Figure S8. Necroptosis silence attenuats the genome instability induced by 3-PO in SW480 cells. (A and B) Immunofluorescence using the antibody of γ-H2AX was performed following treatment with 3-PO in the presence or absence of Nec-1 for 6 h. The percentages of micronuclei were analyzed and shown in (B). (C) Following 3-PO treatment with or without Nec-1 for 6 h, cell lysates were subjected to immunoblotting with the antibodies indicated. (D and E) Cells were transfected with the RIP1, RIP3 target siRNAs or control siRNA for 48 h. Following treatment with 3-PO for 6 h, the images were obtained by fluorescence microscopy after DAPI staining with 400 magnification (D); cell lysates were prepared and subjected to immunoblotting after exposure to 3-PO for 6 h (E). **P < 0.01 versus control, and NS indicated of none significant, and at least 50 cells were included for each group. The scale bars were shown in the figure. Asterisk indicated micronuclei.