Supplementary information

Vitamin K2 promotes PI3K/AKT/HIF-1α-mediated glycolysis that leads to AMPK-dependent autophagic cell death in bladder cancer cells

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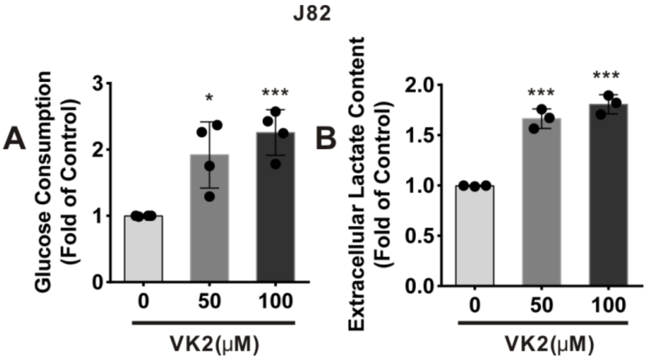
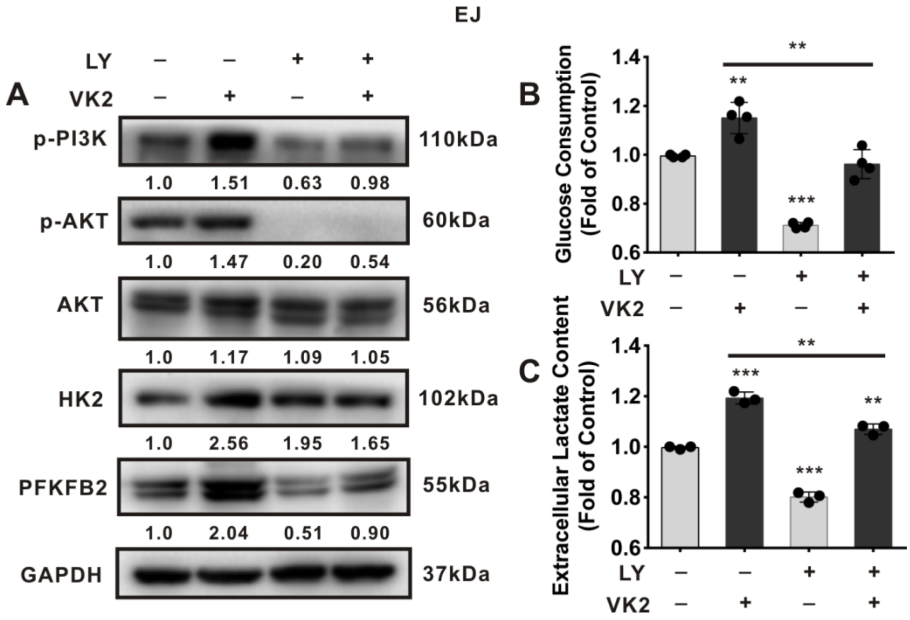
Supplementary Figure 1



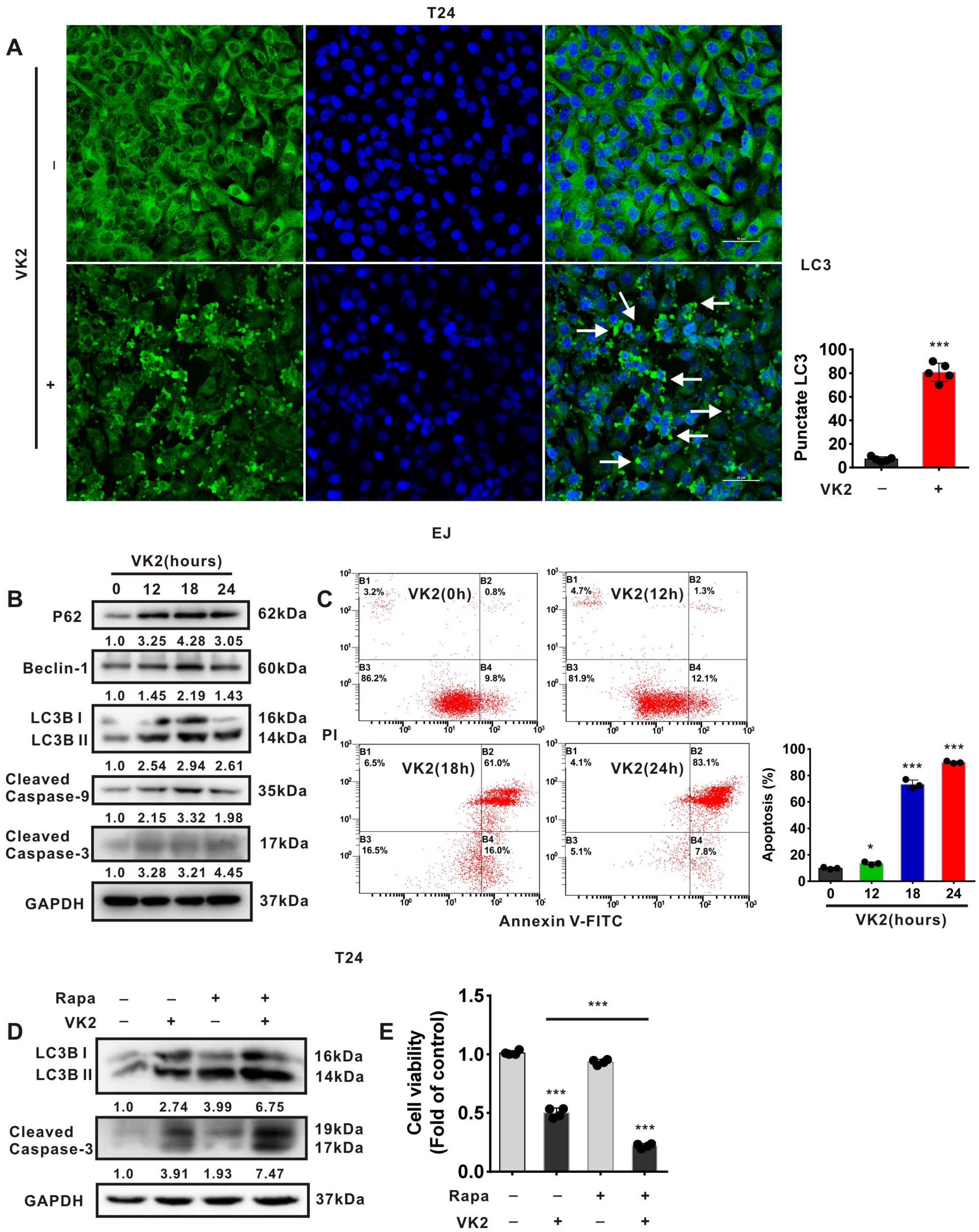
Supplementary Figure 1 Vitamin K2 increases glucose consumption and lactate production in bladder cancer J82 cells. (A) Vitamin K2 dose dependently affected glucose consumption in J82 cells. (B) Vitamin K2 dose dependently affected lactate generation in J82 cells. Data are presented as the mean ± SD of three or four independent experiments. * P<0.05 and *** P<0.001.

Supplementary Figure 2



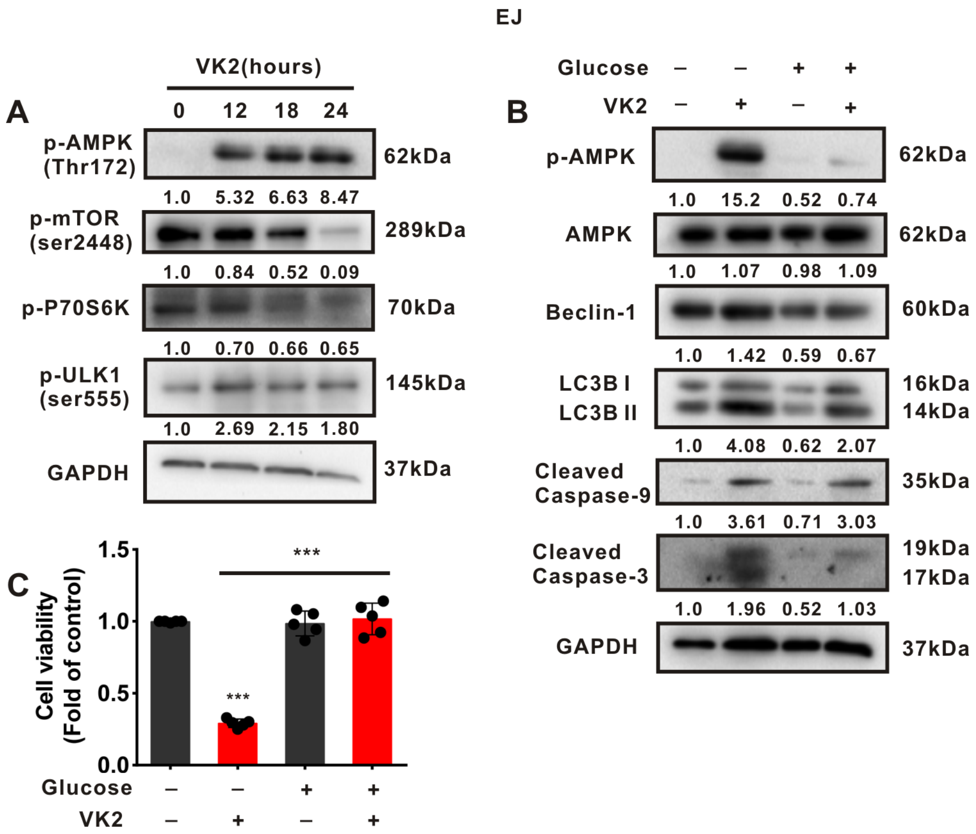
Supplementary Figure 2 Activation of PI3K/AKT is involved in Vitamin K2-induced upregulation of glucose consumption and lactate generation in bladder cancer EJ cells. (A) The effect of 40μM LY294002 (LY, a typical PI3K inhibitor) on the activation of PI3K and AKT as well as the expression of HK2 and PFKFB2 in EJ cells treated with or without 50μM Vitamin K2. (B) and (C) The effect of LY294002 on the glucose consumption and lactate production in EJ cells treated with or without Vitamin K2. Data are presented as the mean ± SD of three or four independent experiments. ** P<0.01 and *** P<0.001.

Supplementary Figure 3



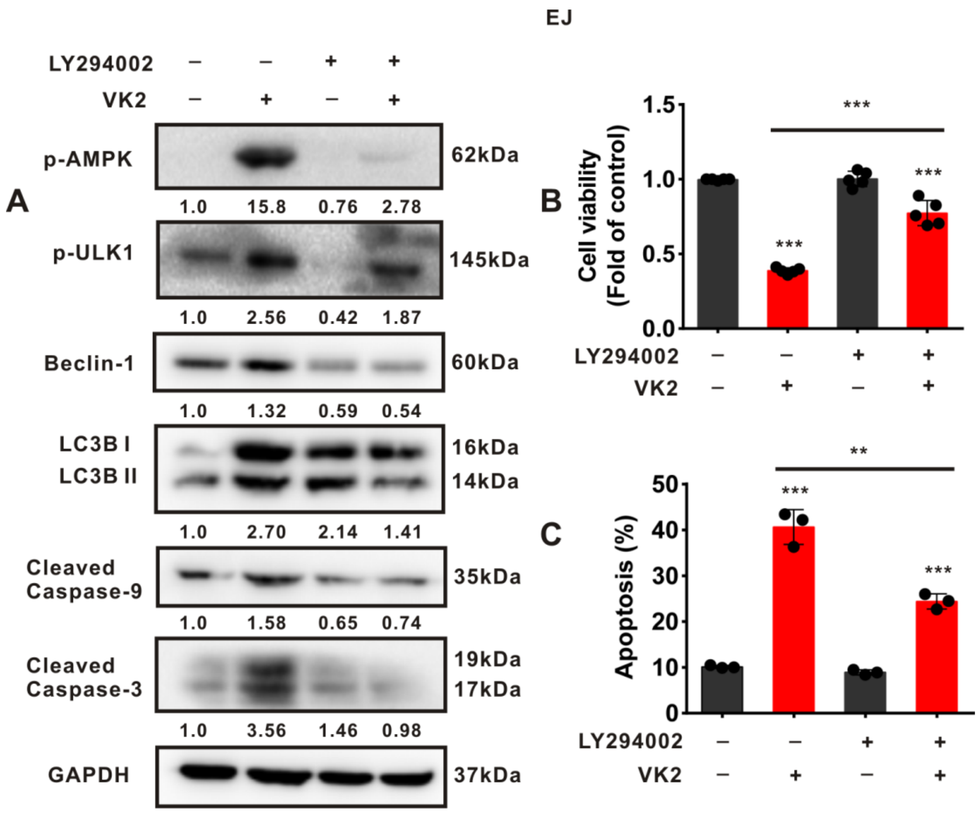
Supplementary Figure 3 Vitamin K2 induces autophagy and apoptosis in bladder cancer cells. (A) Immuno-Fluorescence demonstrated the expression of LC3 in T24 cells treated with or without 50μM Vitamin K2 for 18 hours. Arrow pointed to punctate LC3. Scale bar: 50μm. (B) Western blot demonstrated the effect of Vitamin K2 on the expression of autophagic and apoptotic protein in EJ cells. (C) Flow cytometry demonstrated the apoptotic effect of Vitamin K2 on EJ cells in a time dependent manner. (D) Western blot demonstrated the effect of 20μM Rapamycin on Vitamin K2-induced autophagy and apoptosis in bladder cancer T24 cells. (E) MTS assays showed the effect of Rapamycin on the cell viability of T24 cells treated with or without 50μM Vitamin K2. Data are presented as the mean ± SD of three or four independent experiments. * $P<0.05$ and *** $P<0.001$.

Supplementary Figure 4



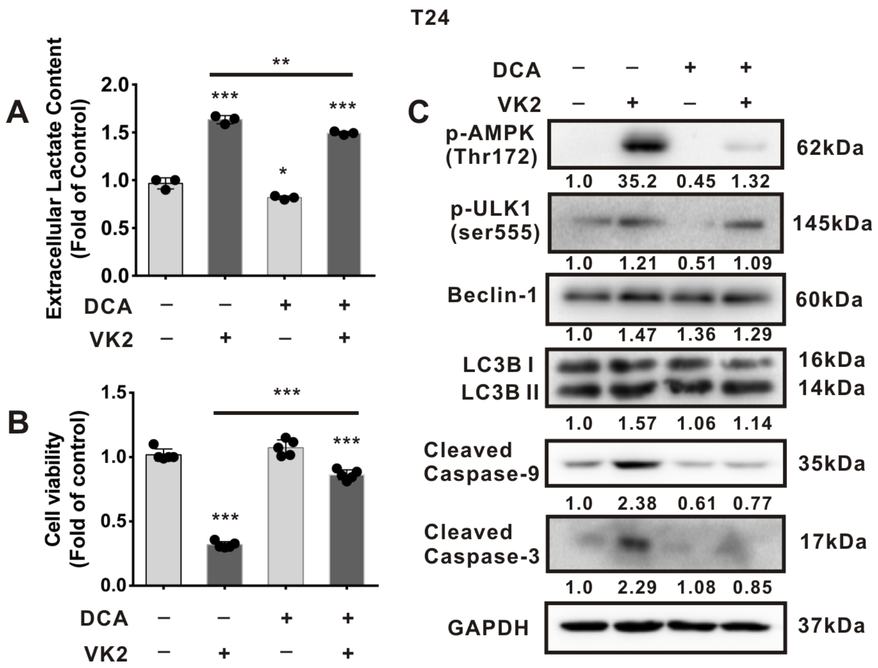
Supplementary Figure 4 Glucose supplementation rescues bladder cancer EJ cells from AMPK-dependent autophagic cell death. (A) Western blot showed the effect of Vitamin K2 on the activation of AMPK and mTORC1 pathway in bladder cancer EJ cells. (B) Western blot showed that 10mM glucose supplementation affected Vitamin K2-triggered AMPK activation, autophagy and apoptosis in EJ cells. (C) MTS assays showed that glucose supplementation affected the cell viability of EJ cells treated with or without 50μM Vitamin K2. Data are presented as the mean ± SD of three or four independent experiments. *** P<0.001.

Supplementary Figure 5



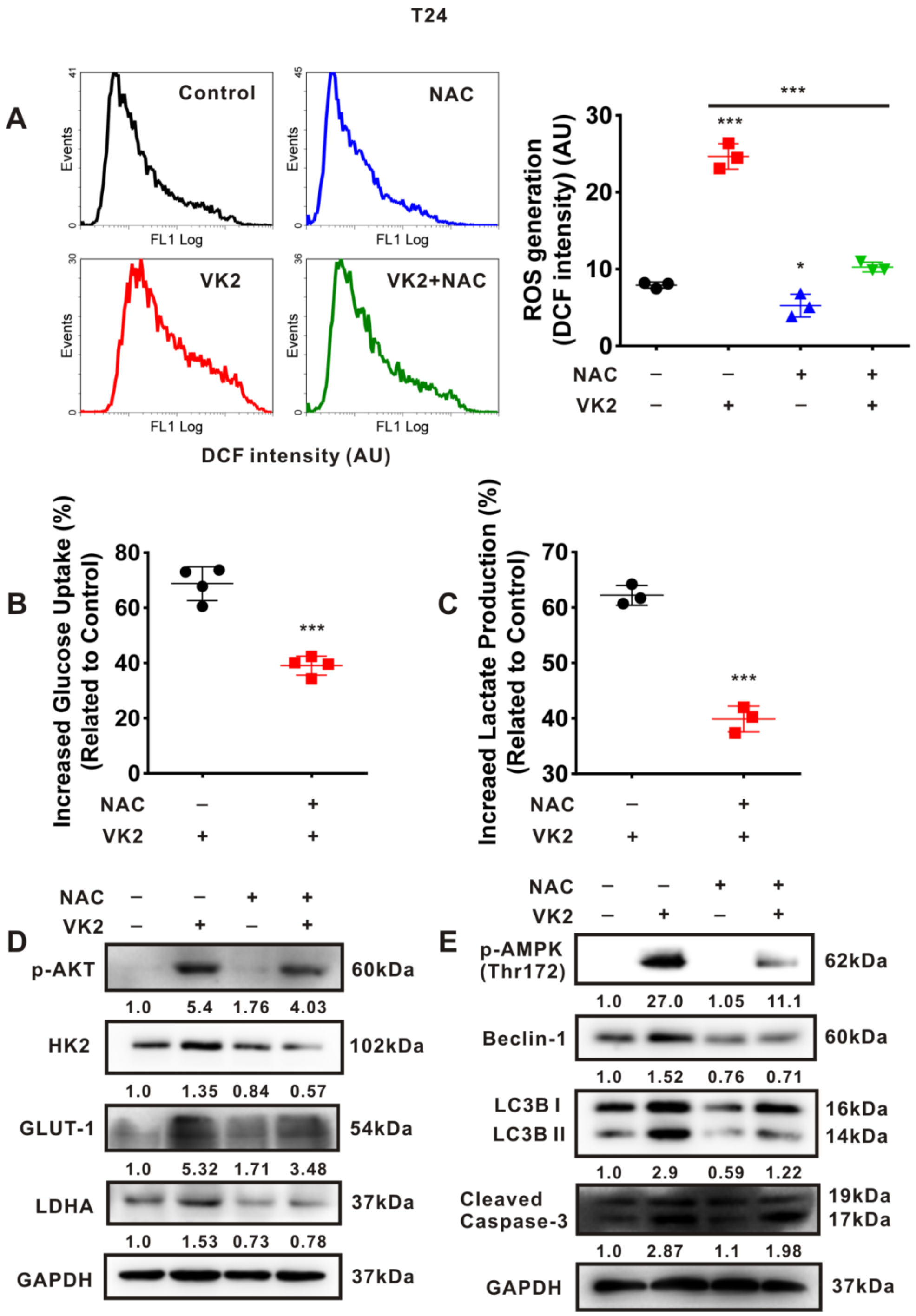
Supplementary Figure 5 Activation of PI3K/AKT is required for Vitamin K2-triggered AMPK dependent autophagic cell death in bladder cancer EJ cells. (A) Western blot showed the effect of 40μM LY294002 (LY, a typical PI3K inhibitor) on AMPK activation, autophagy and apoptosis in Vitamin K2-treated EJ cells. (B) MTS assays showed the effect of LY294002 on EJ cell viability after treatment with or without 50μM Vitamin K2. (C) Flow cytometry showed the effect of LY294002 on Vitamin K2-induced apoptosis in EJ cells. Data are presented as the mean ± SD of at leat three independent experiments. ** P<0.01 and *** P<0.001.

Supplementary Figure 6



Supplementary Figure 6 DCA attenuates the glycolysis and blocks Vitamin K2-induced AMPK dependent autophagic cell death in bladder cancer T24 cells. (A) The effect of 30mM DCA on the lactate generation in T24 cells treated with or without 100μM Vitamin K2. (B) The effect of DCA on the cell viability of T24 cells treated with or without Vitamin K2. (C) Western blot showed that DCA affected Vitamin K2-induced AMPK dependent autophagic cell death in T24 cells. Data are presented as the mean ± SD of three or four independent experiments. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Supplementary Figure 7



Supplementary Figure 7 ROS generation is required for Vitamin K2-upregulated glycolysis and triggered AMPK-dependent autophagic cell death in bladder

cancer T24 cells. (A) Flow cytometry showed the effect of 5mM N-acetyl cysteine (NAC, a ROS scavenger) on Vitamin K2-induced ROS generation in bladder cancer T24 cells. (B) and (C) The effect of NAC on Vitamin K2-elevated glucose uptake and lactate production in bladder cancer T24 cells. (D) Western blot showed the effect of NAC on the expression of glycolytic protein or enzymes in T24 cells treated with or without 50μM Vitamin K2. (E) Western blot showed the effect of NAC on AMPK activation, autophagy and apoptosis in T24 cells treated with or without Vitamin K2. Data are presented as the mean ± SD of three or four independent experiments. * $P<0.05$ and *** $P<0.001$.

The original unprocessed images for western blot and the target panels were marked in red boxes.

T24

Figure 1E

EJ

Figure 1F

Figure 1H

Figure 2A

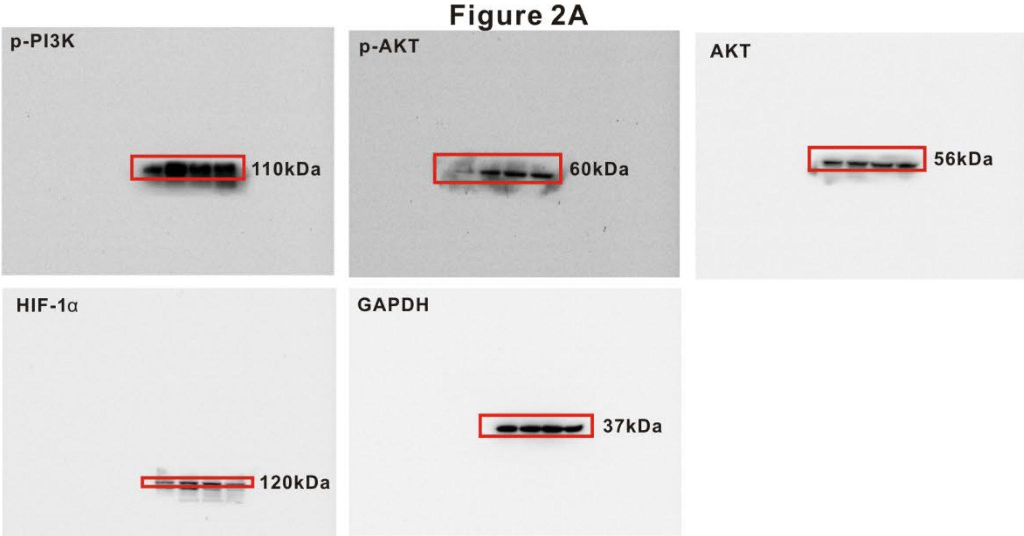
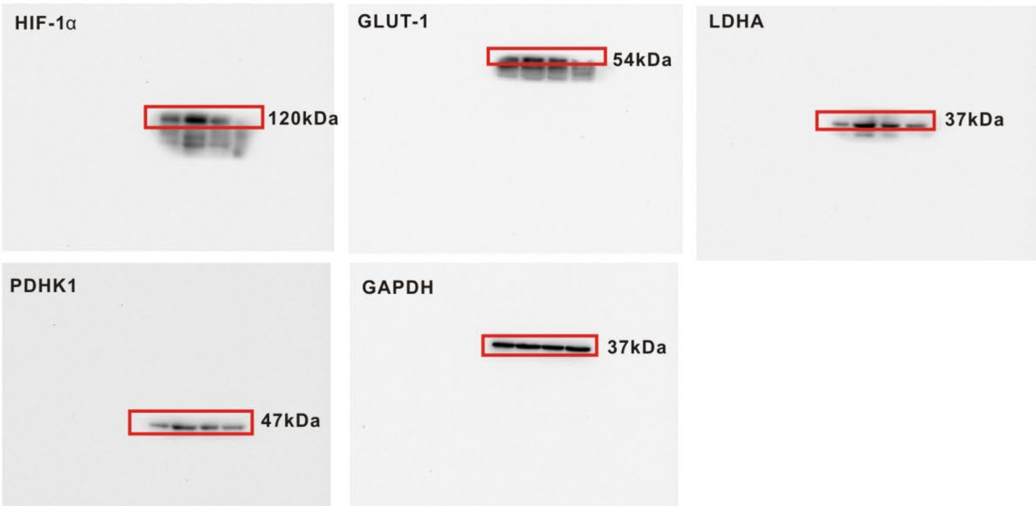


Figure 2B



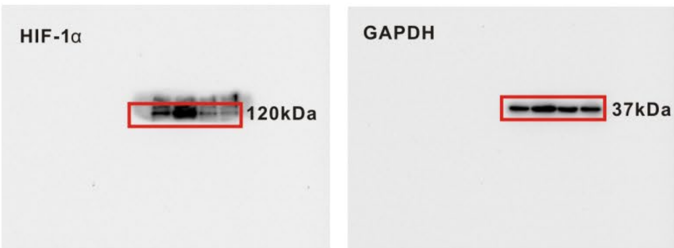


Figure 2G

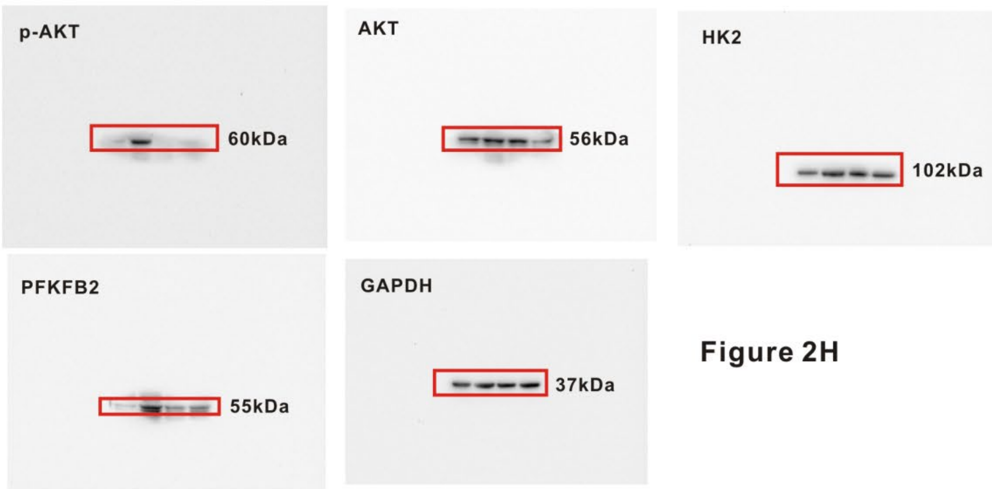


Figure 2H

Figure 2K

Figure 2N

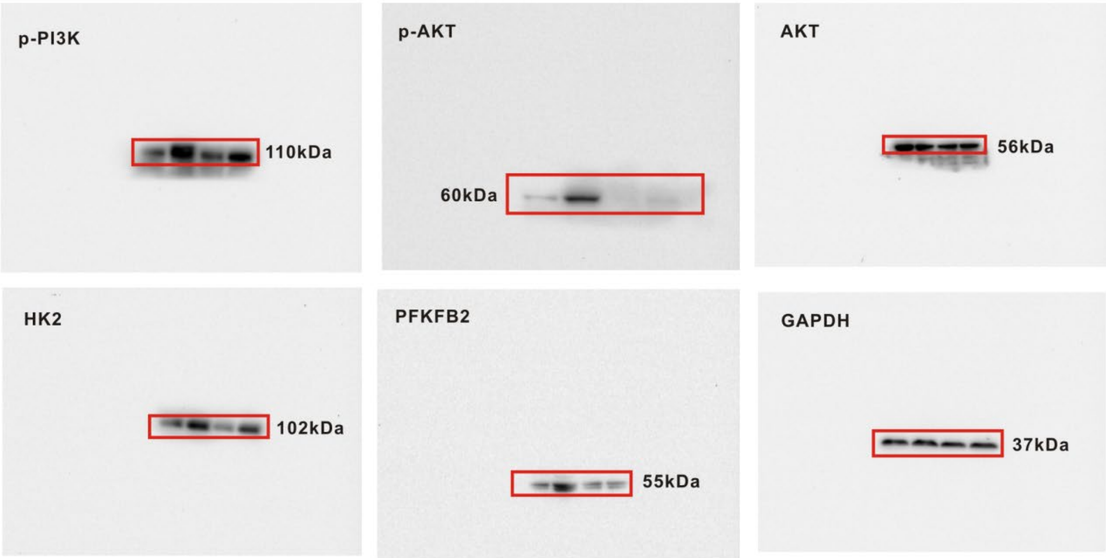
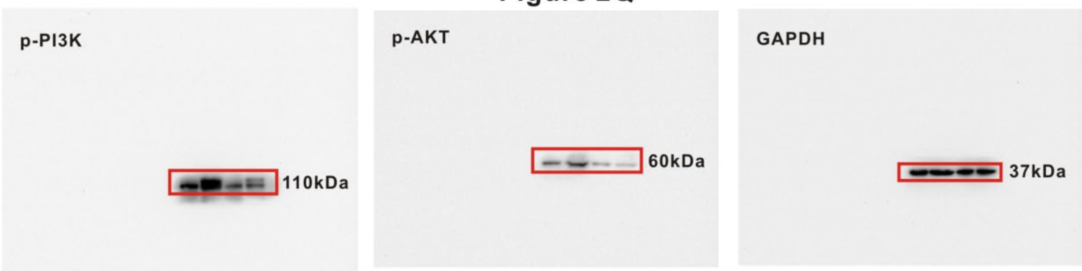


Figure 2Q



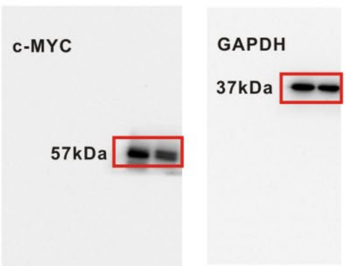


Figure 3D

Figure 4A

Figure 4D

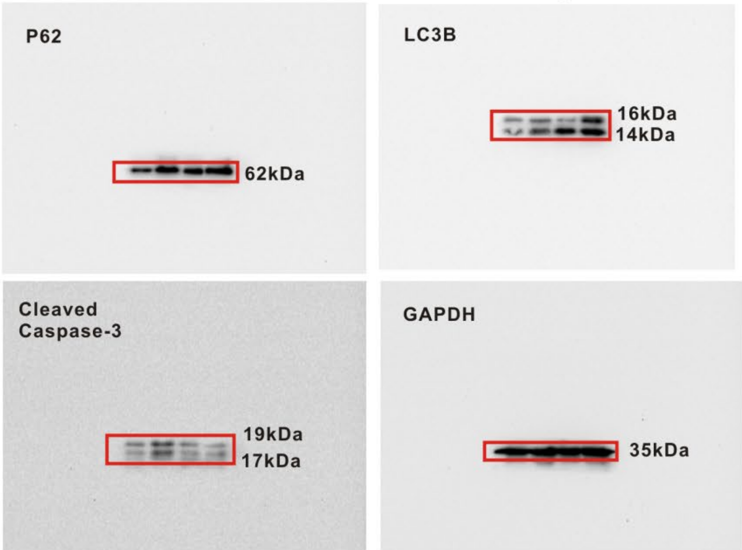


Figure 4E

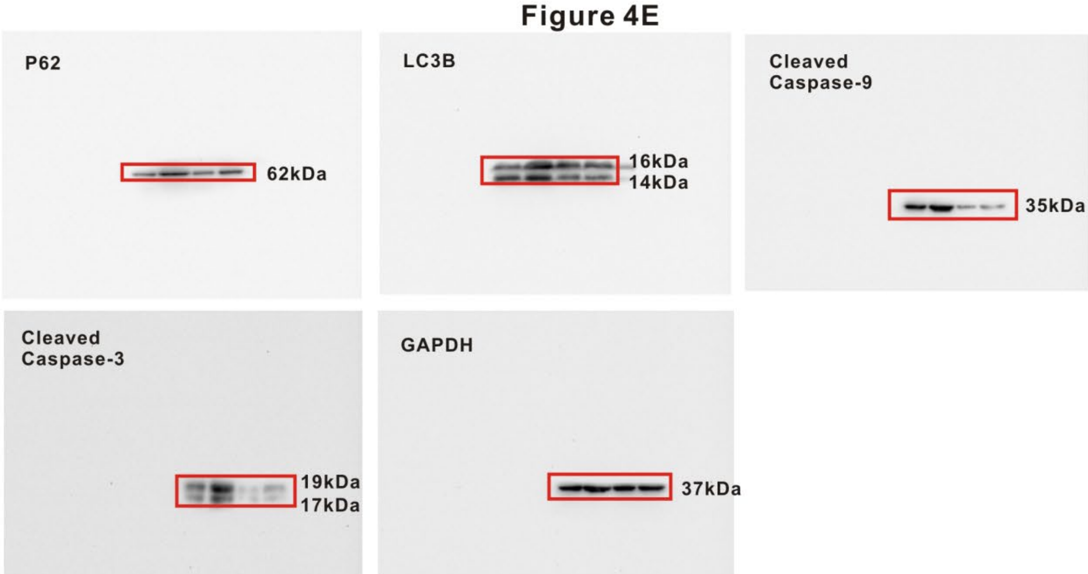
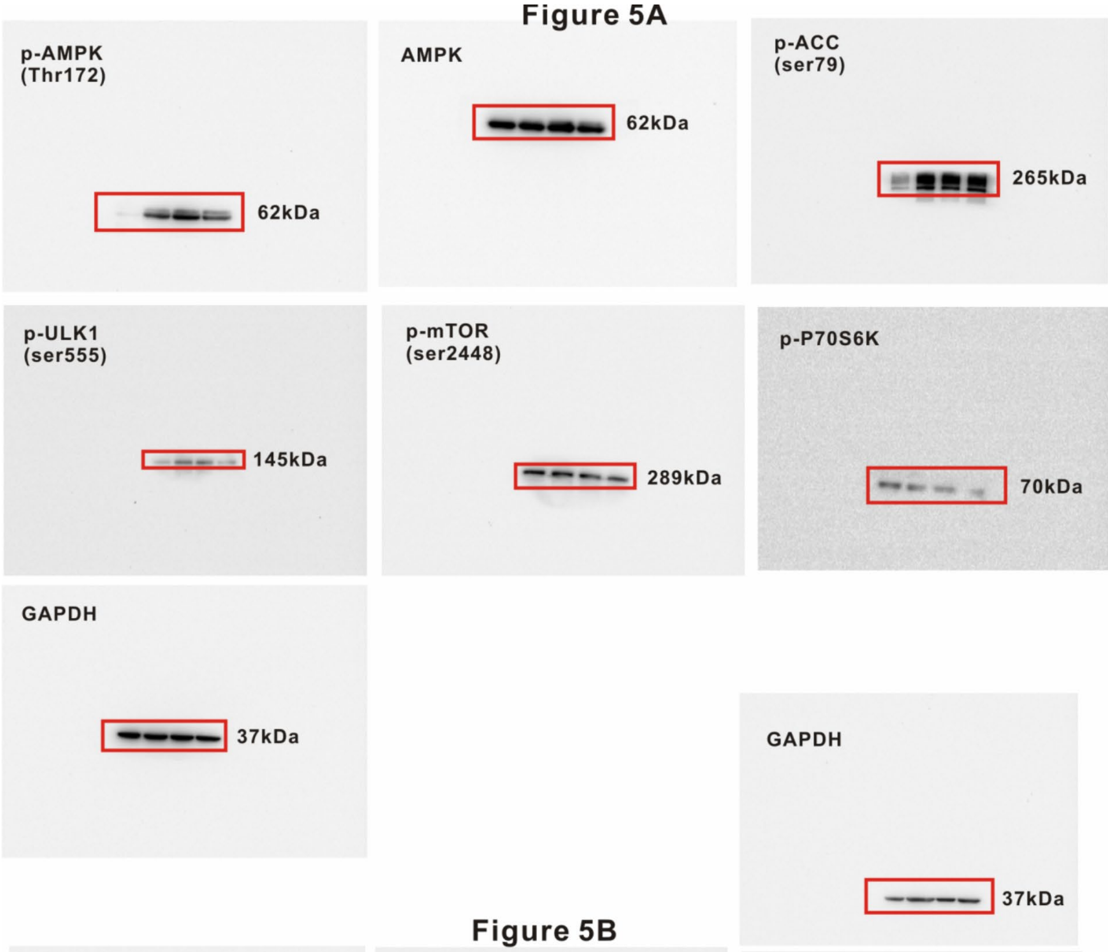


Figure 5A



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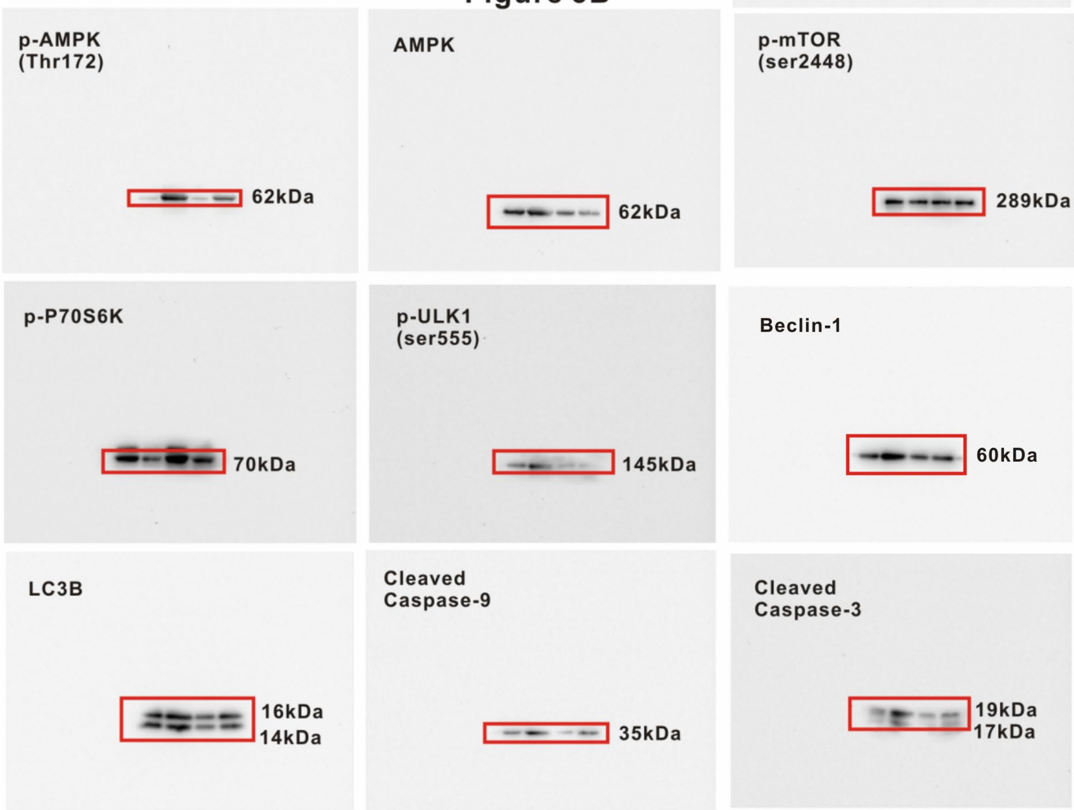


Figure 5C

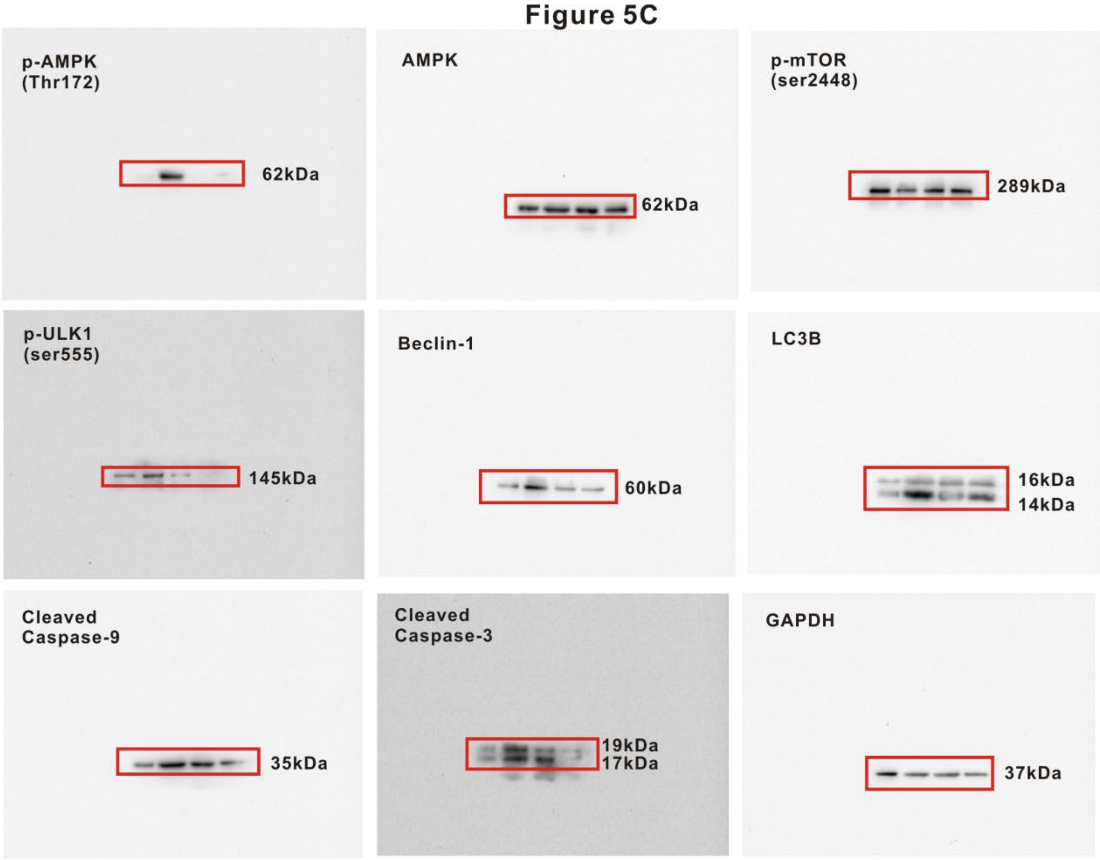


Figure 5F

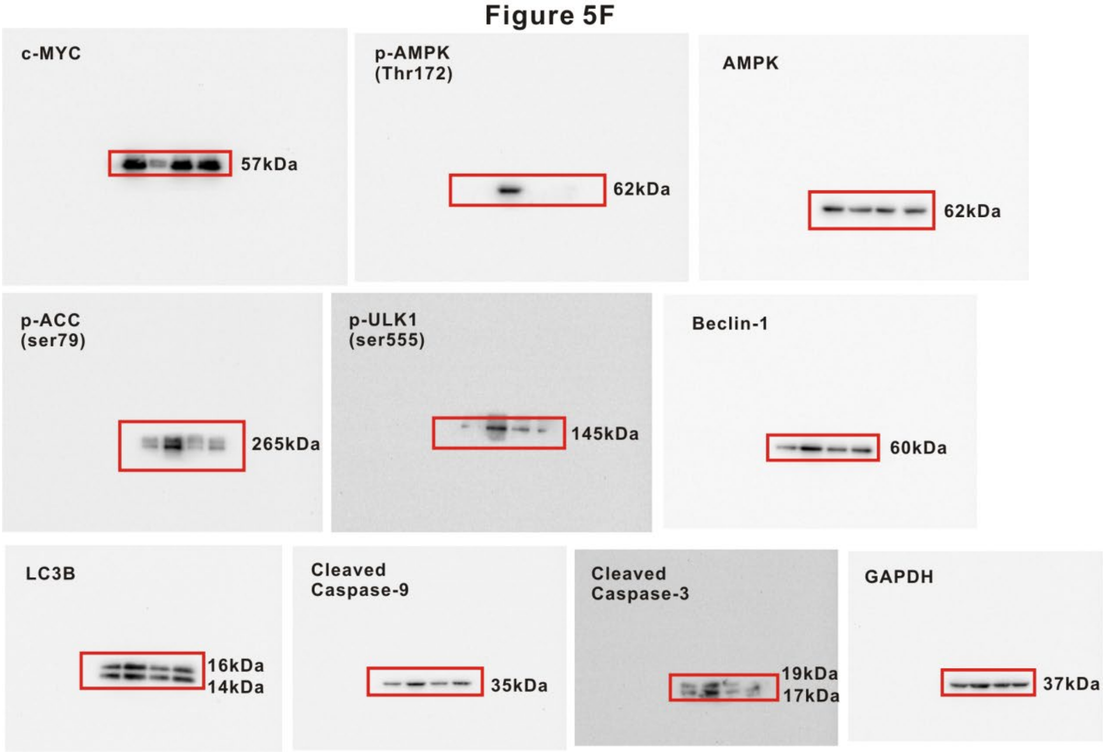
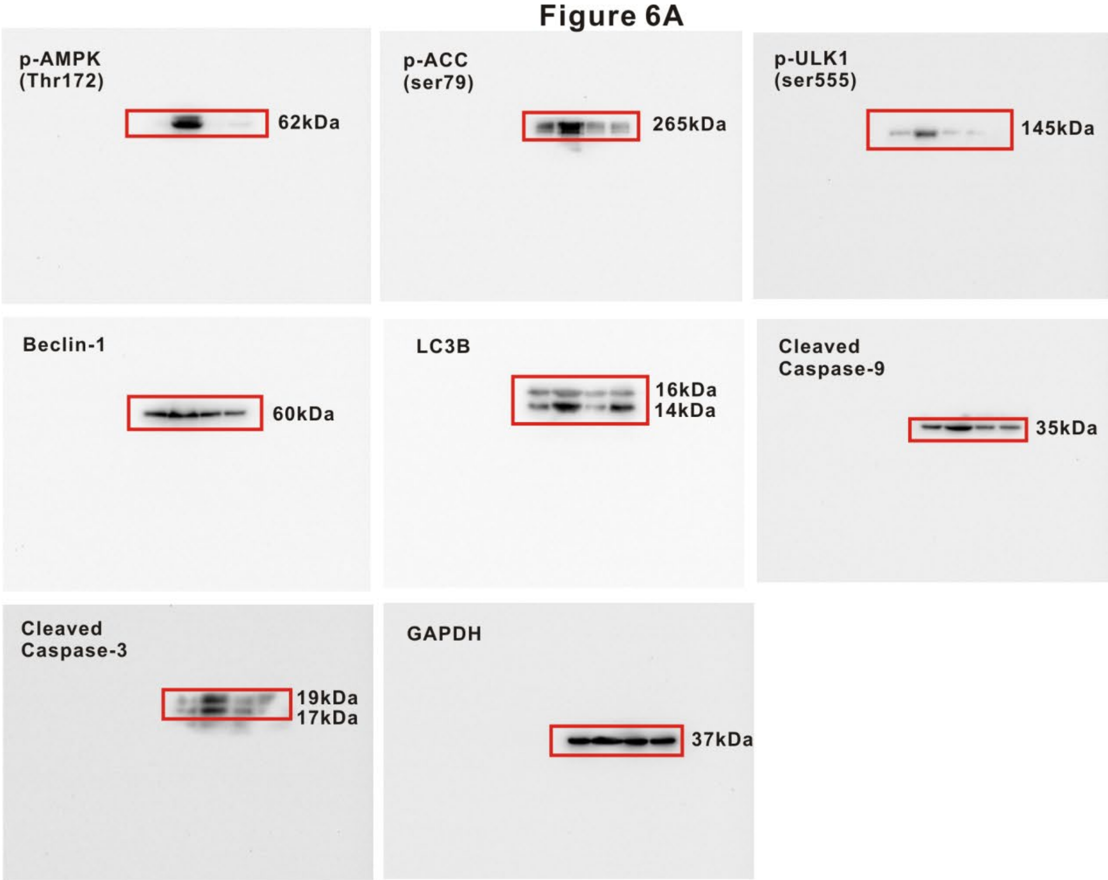


Figure 6A



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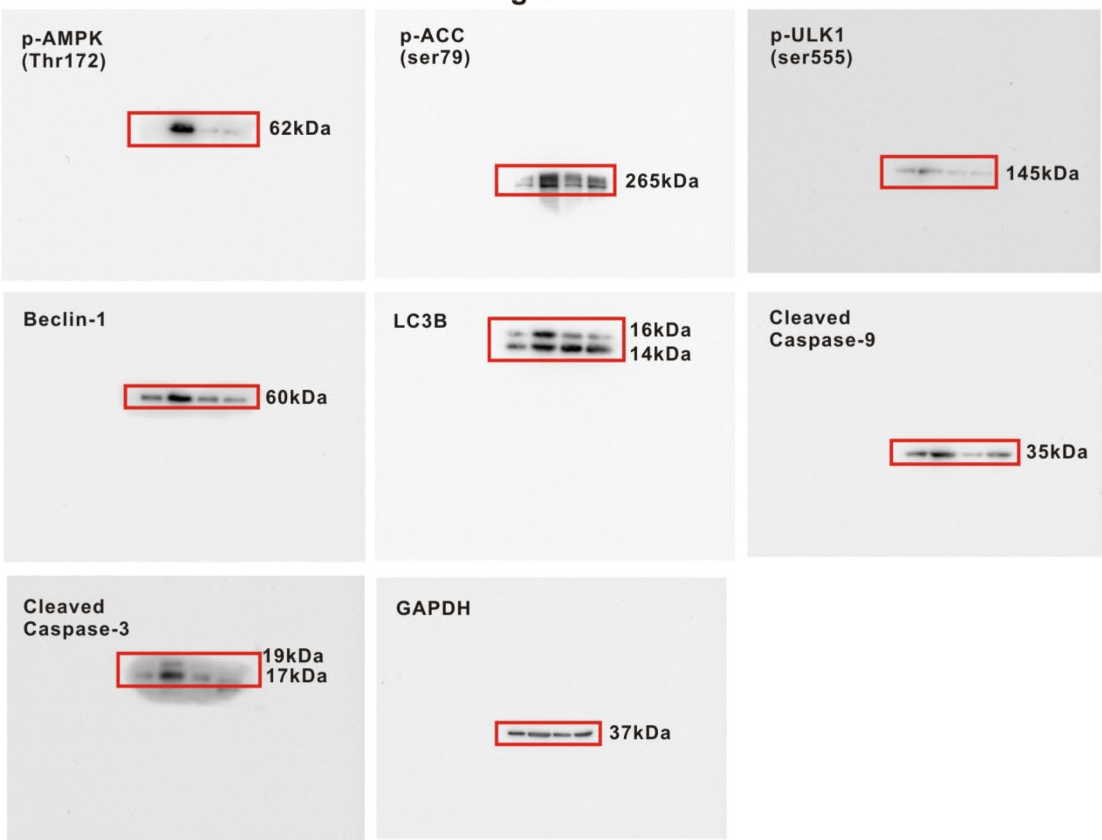
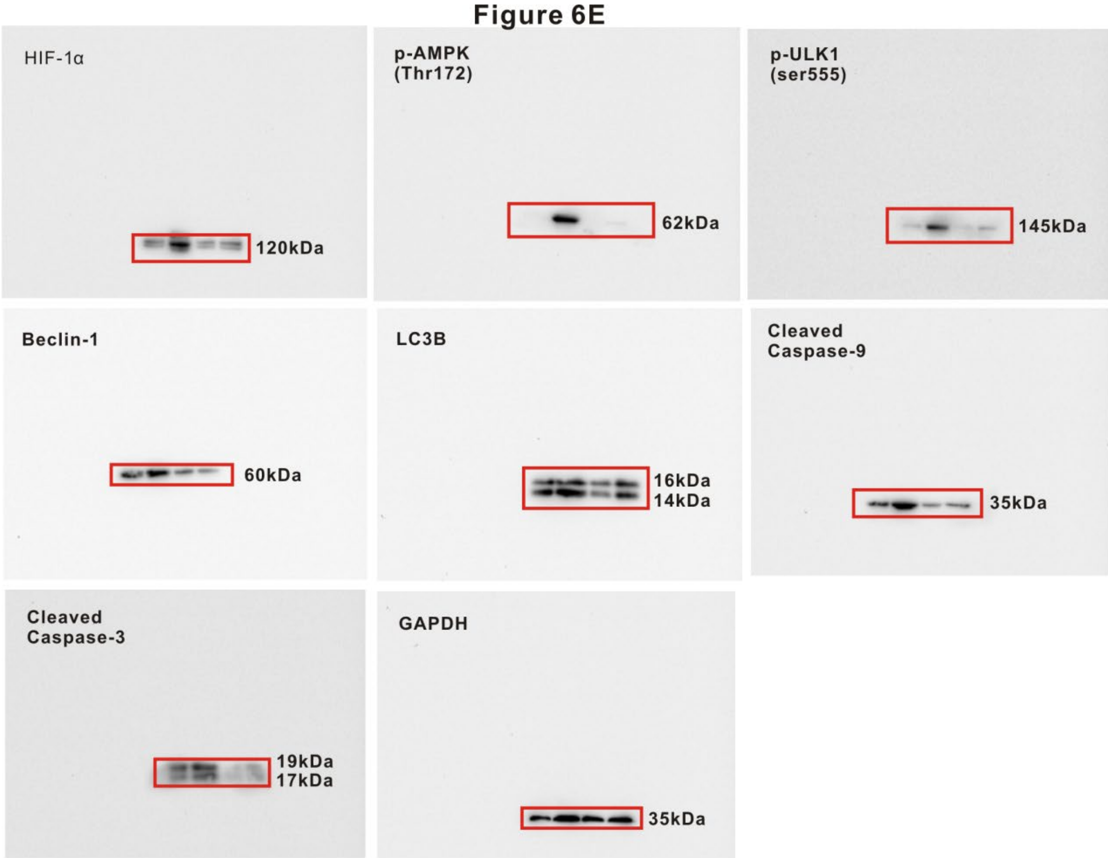


Figure 6E



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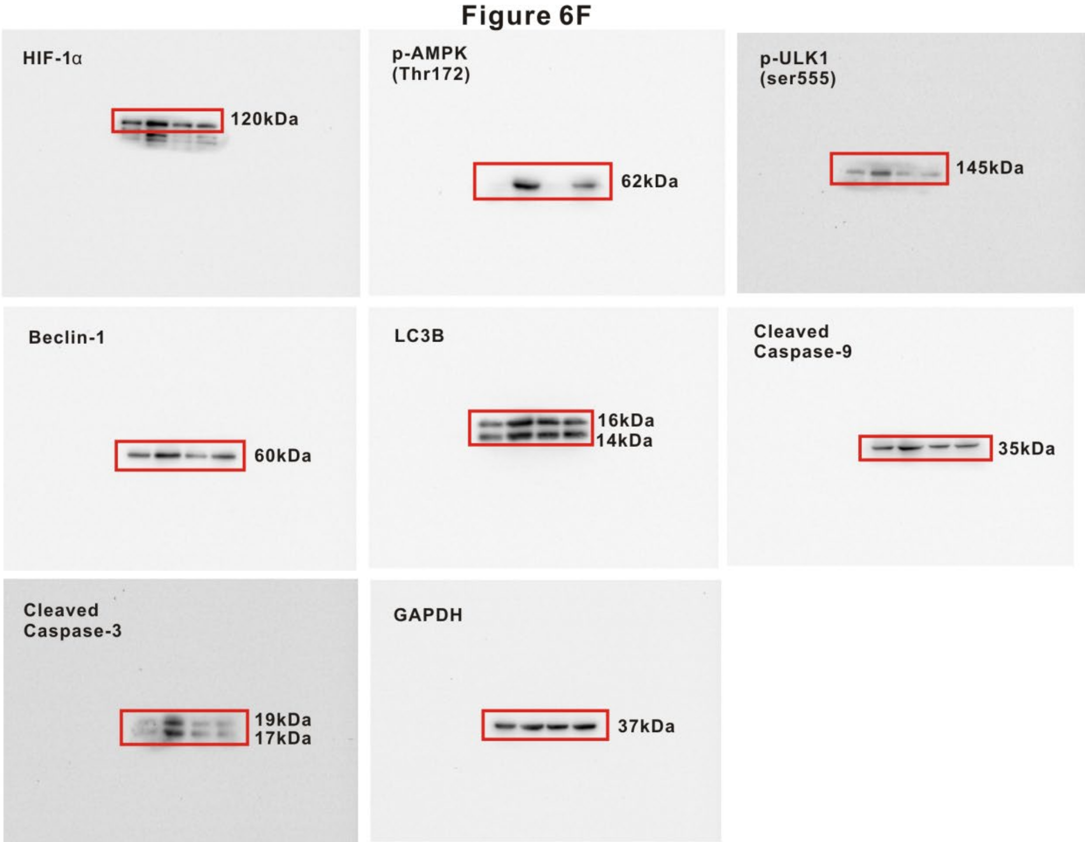


Figure 6I

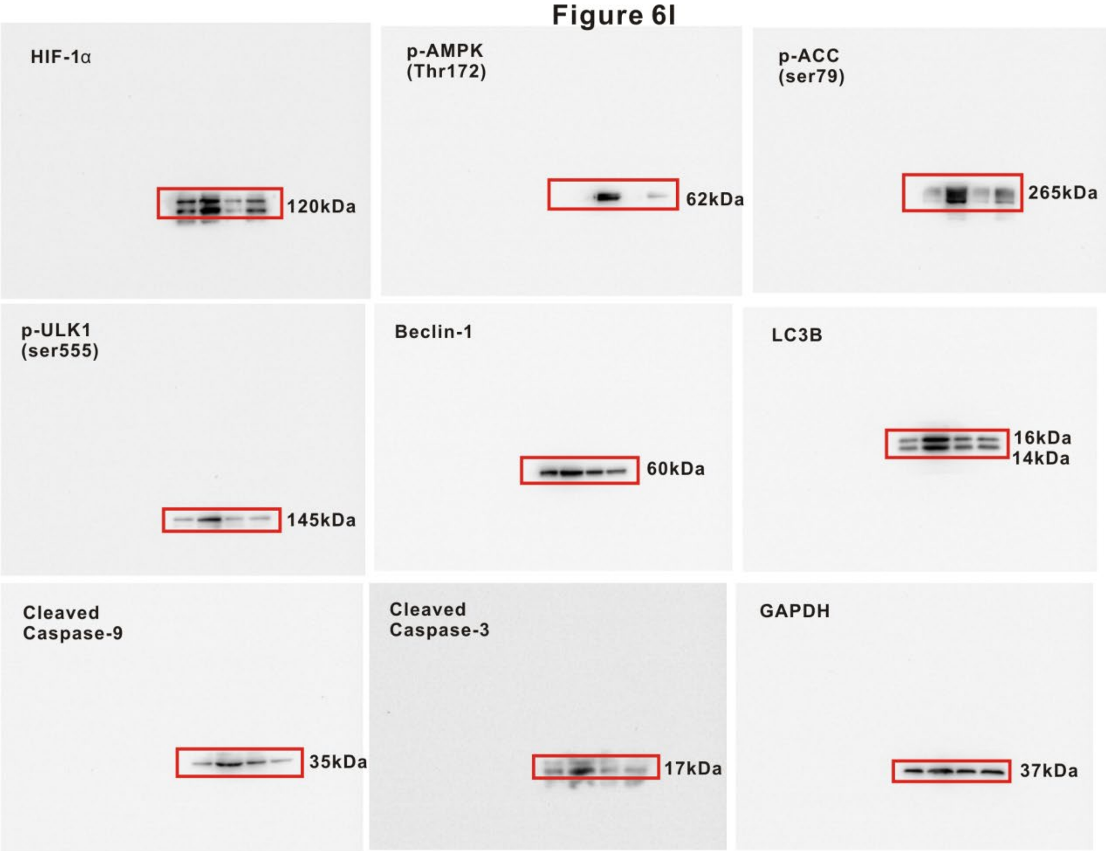
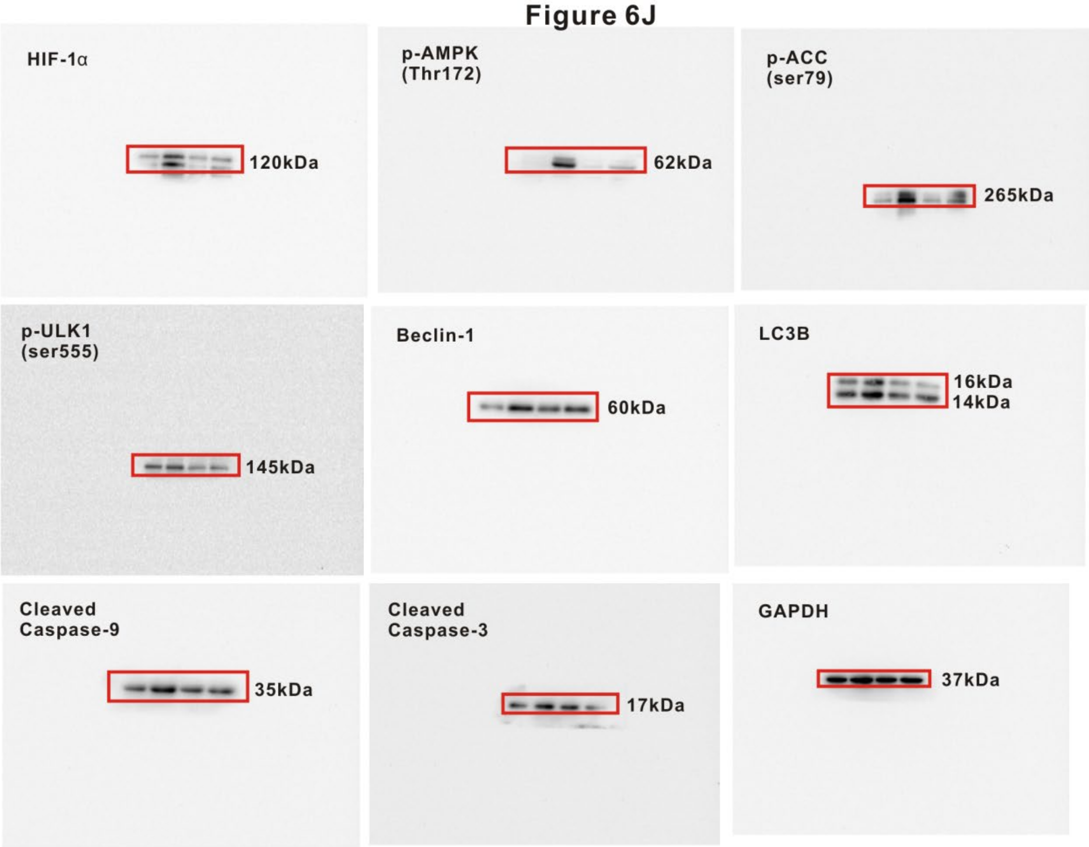
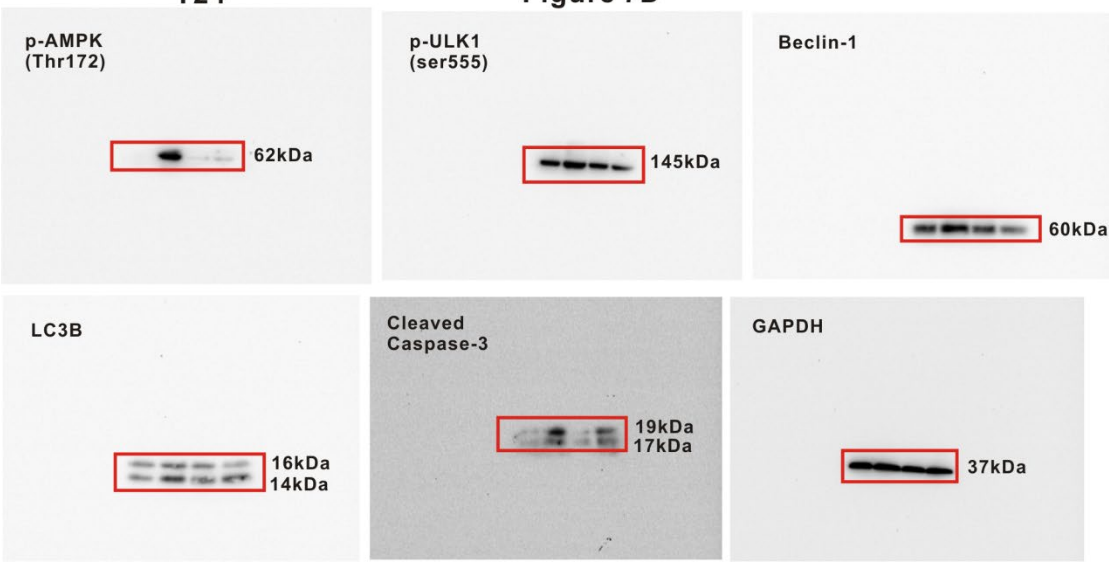


Figure 6J



T24

Figure 7D



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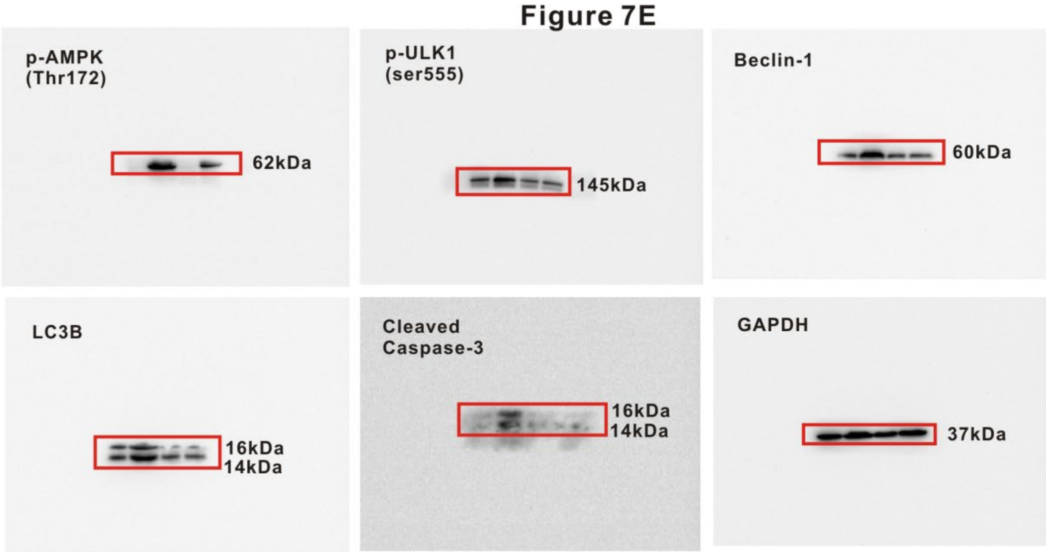
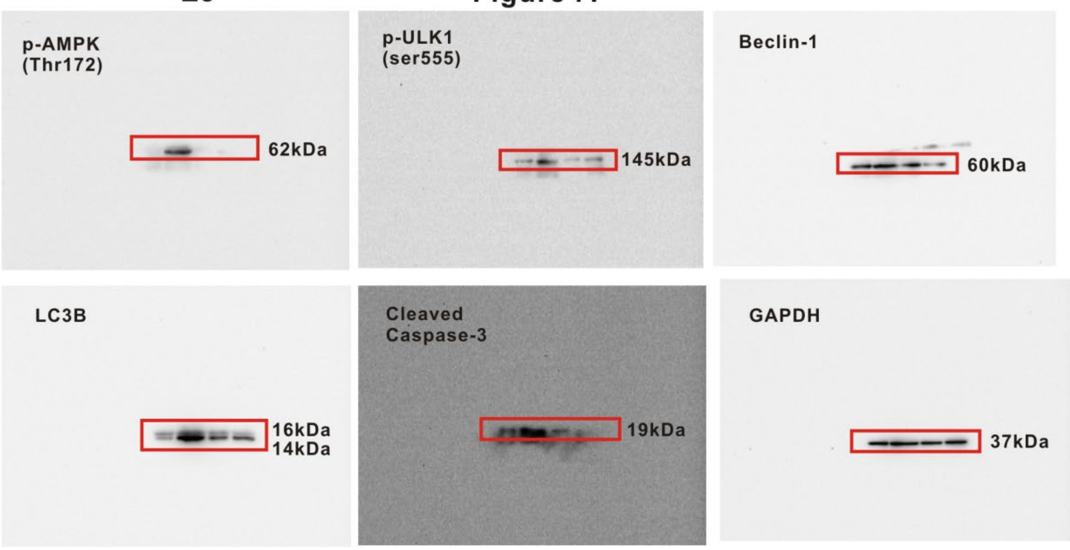
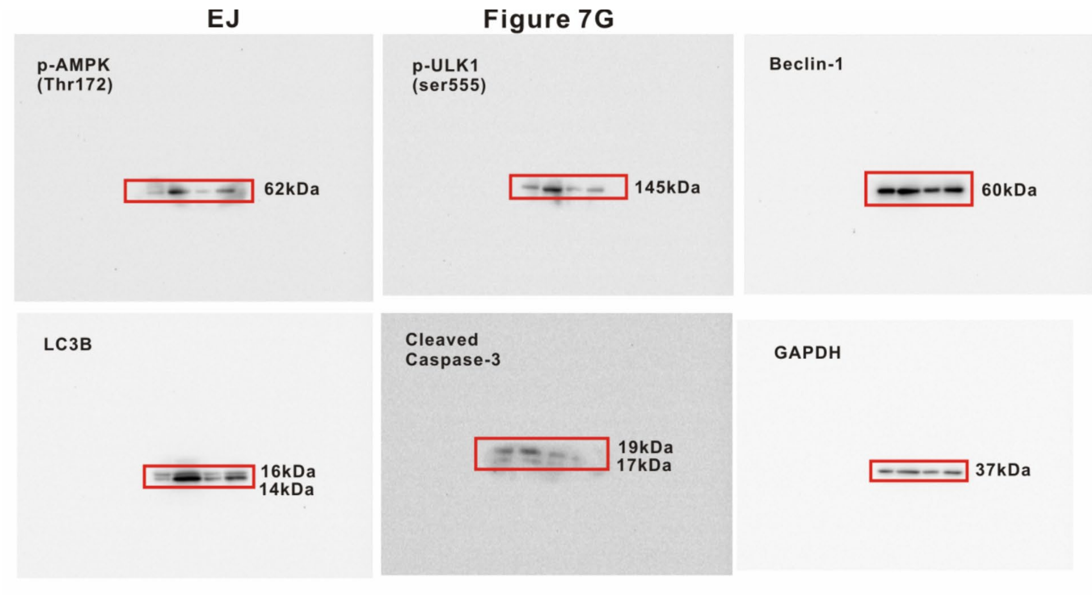
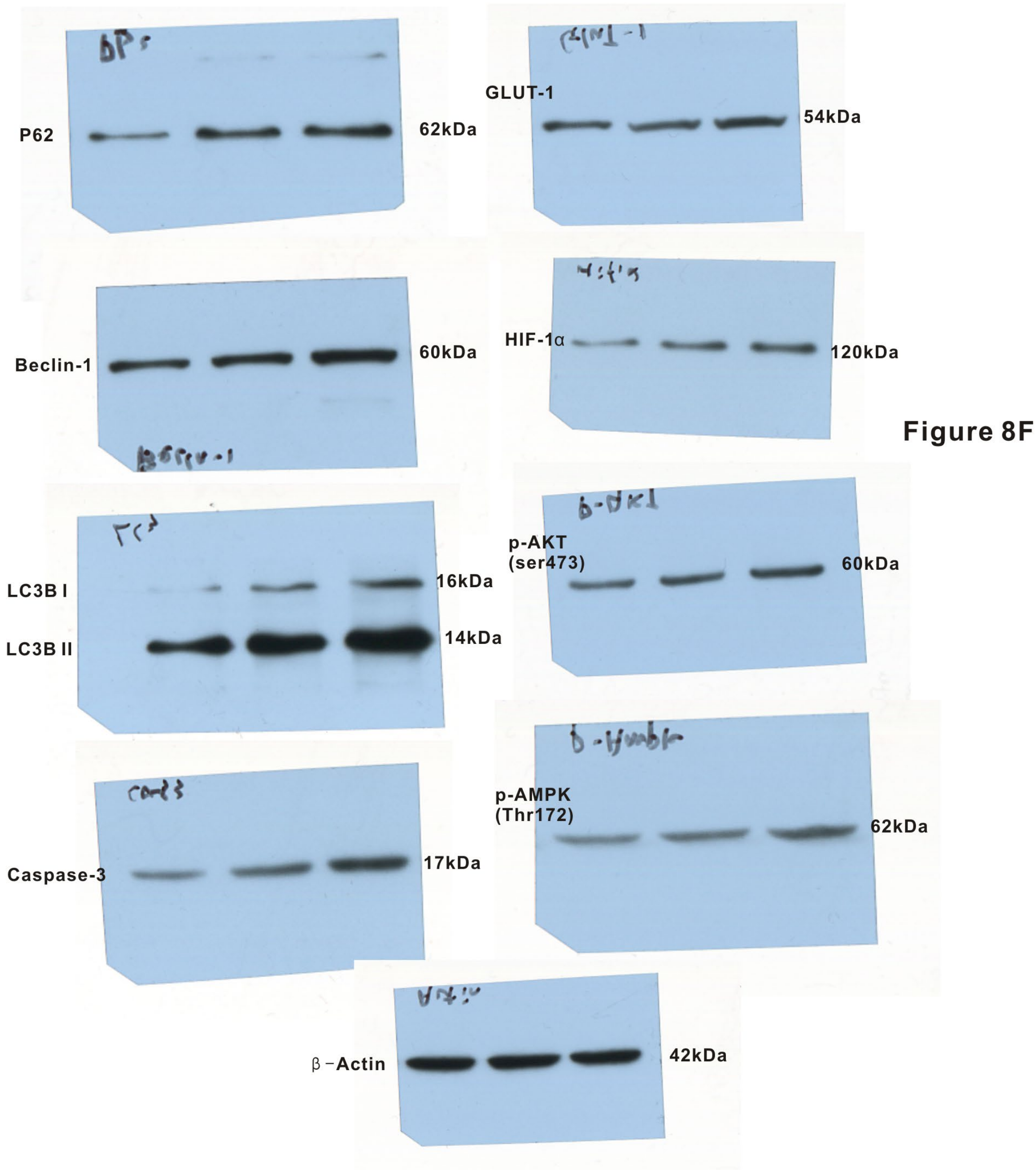


EJ

Figure 7F

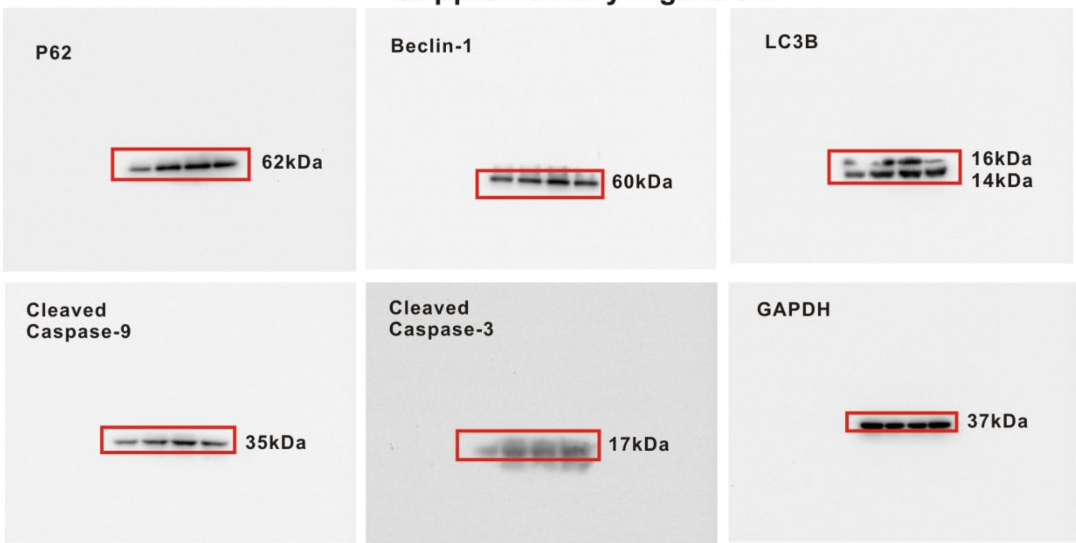



EJ
Figure 7G
p-AMPK
(Thr172)
62kDa
p-ULK1
(ser555)
145kDa
Beclin-1
60kDa
LC3B
16kDa
14kDa
Cleaved
Caspase-3
19kDa
17kDa
GAPDH
37kDa

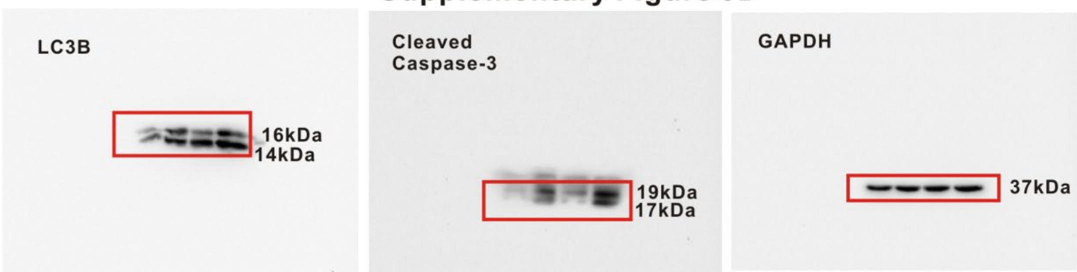

P62
62kDa
GLUT-1
54kDa
Beclin-1
60kDa
HIF-1α
120kDa
Figure 8F
LC3B I
LC3B II
16kDa
14kDa
p-AKT
(ser473)
60kDa
Caspase-3
17kDa
p-AMPK
(Thr172)
62kDa
β-Actin
42kDa

Supplementary Figure 2A

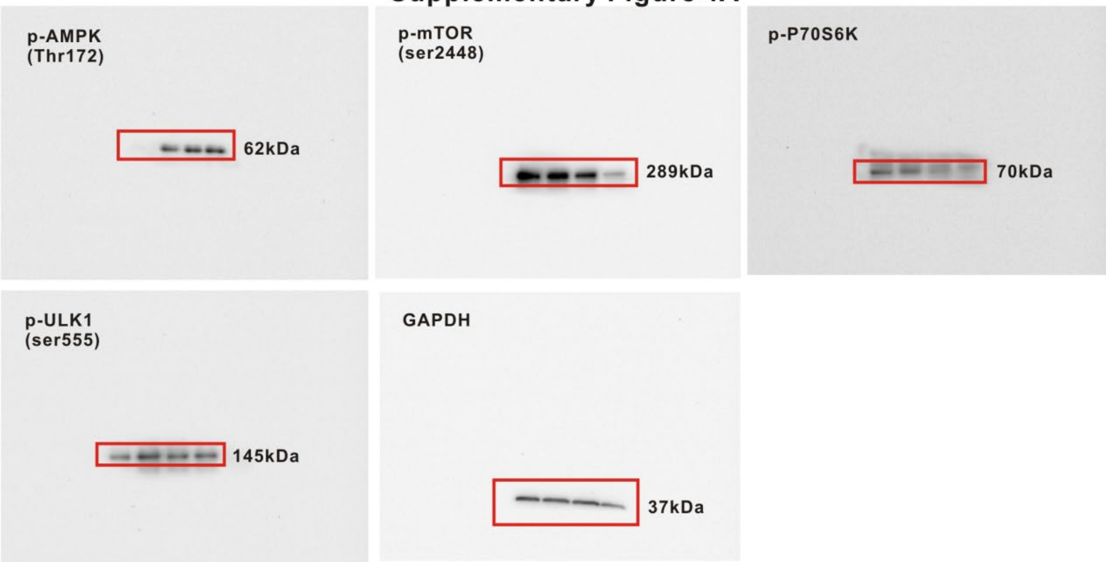
Supplementary Figure 3B



Supplementary Figure 3D



Supplementary Figure 4A



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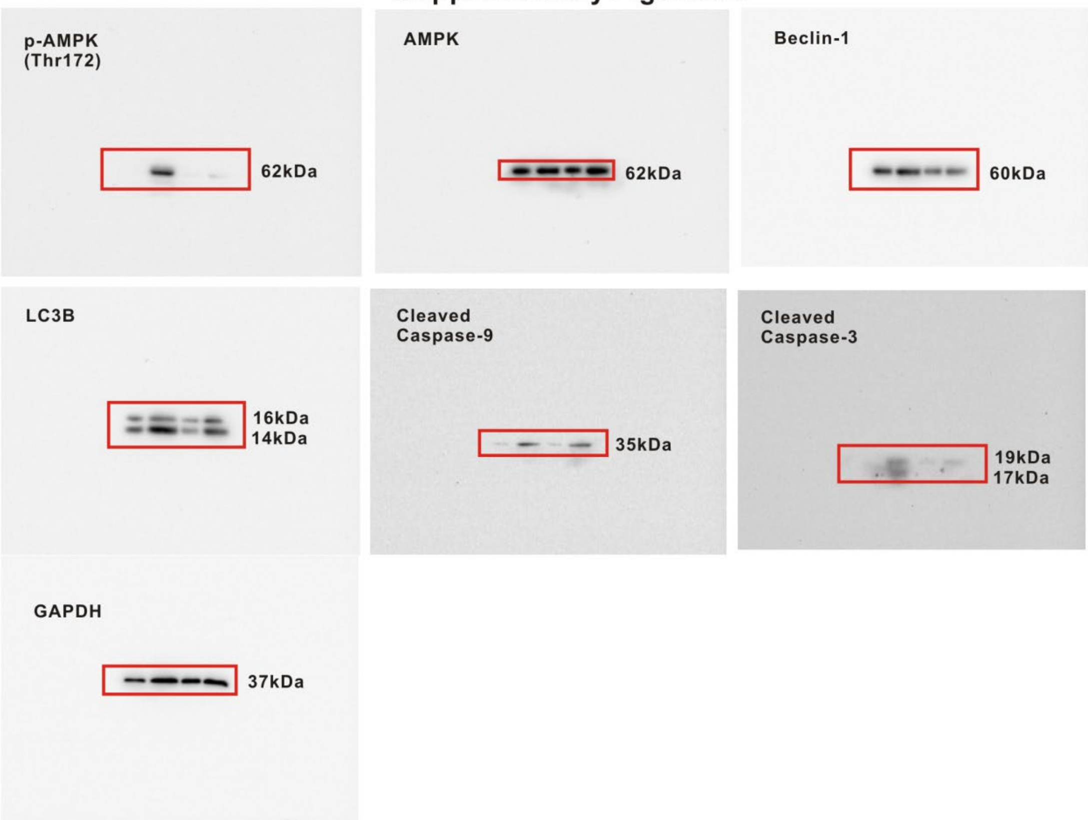
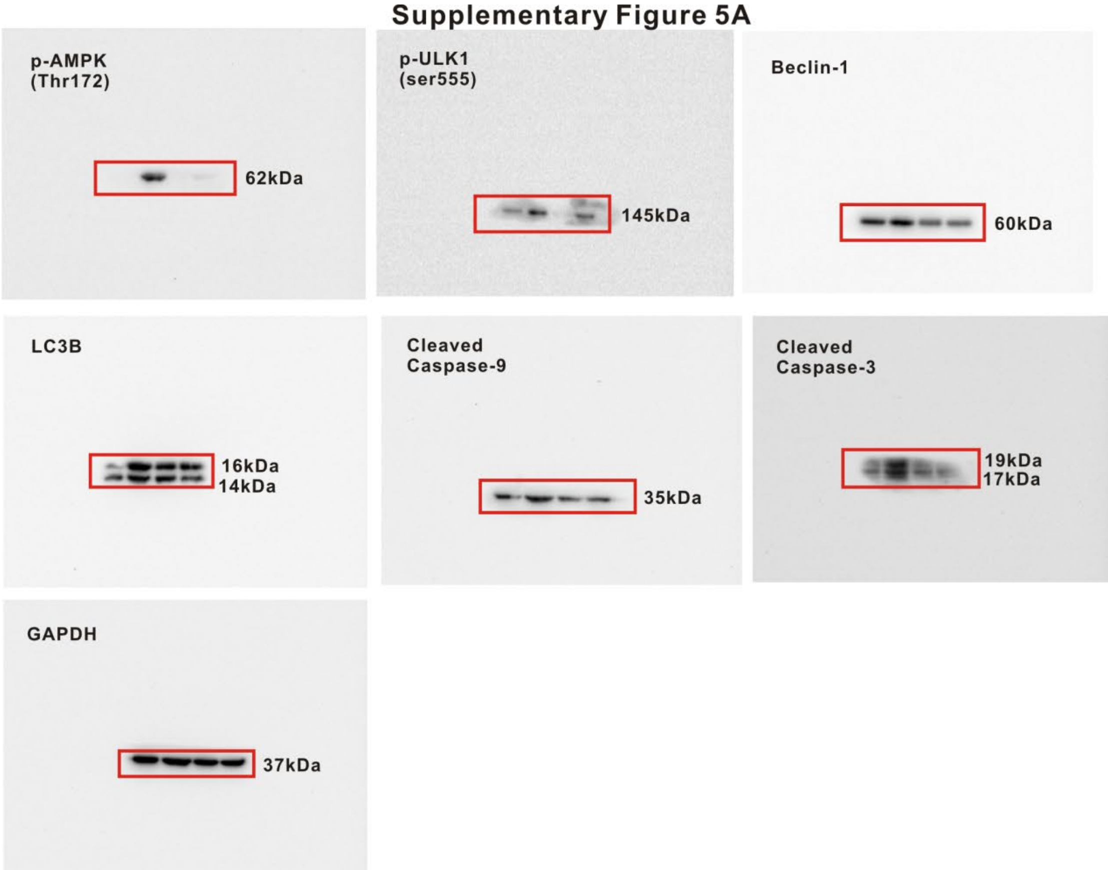
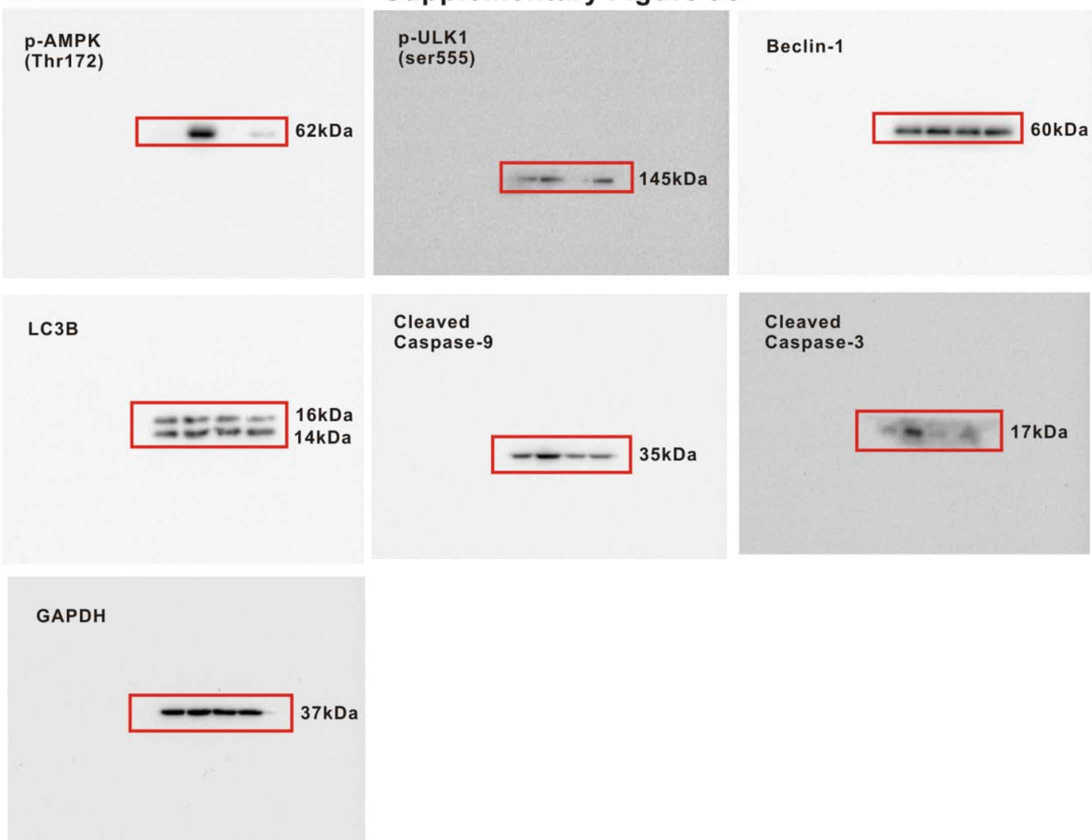


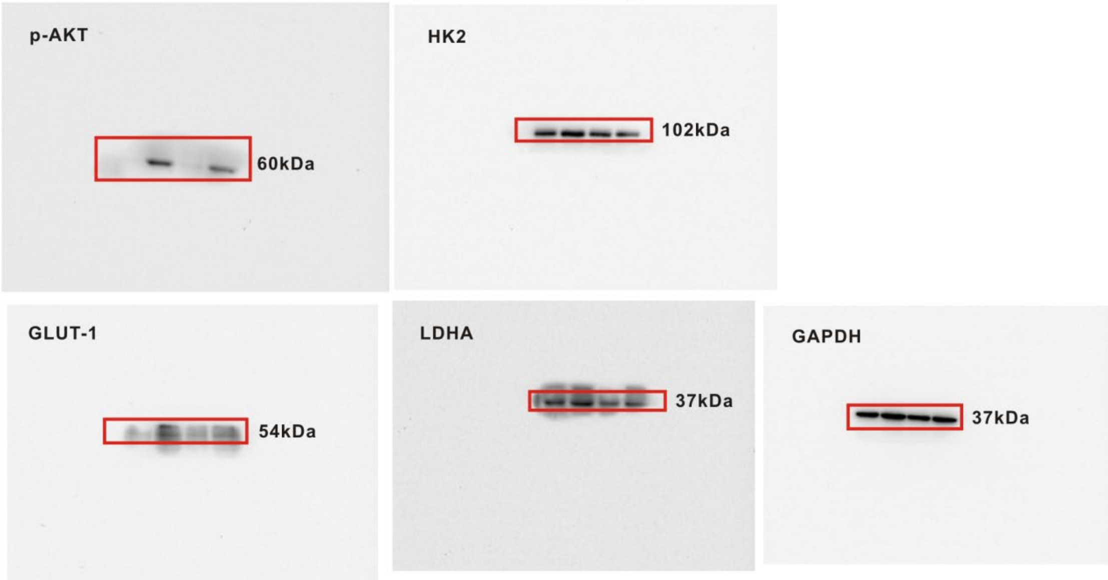
Supplementary Figure 5A



Supplementary Figure 6C



Supplementary Figure 7D



Supplementary Figure 7E