Additional file 1

Targeted Inhibition of the PI3K/AKT/mTOR Pathway by (+)-Anthrabenzoxocinone Induces Cell Cycle Arrest, Apoptosis, and Autophagy in Non-Small Cell Lung Cancer

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Content of Supplementary Data

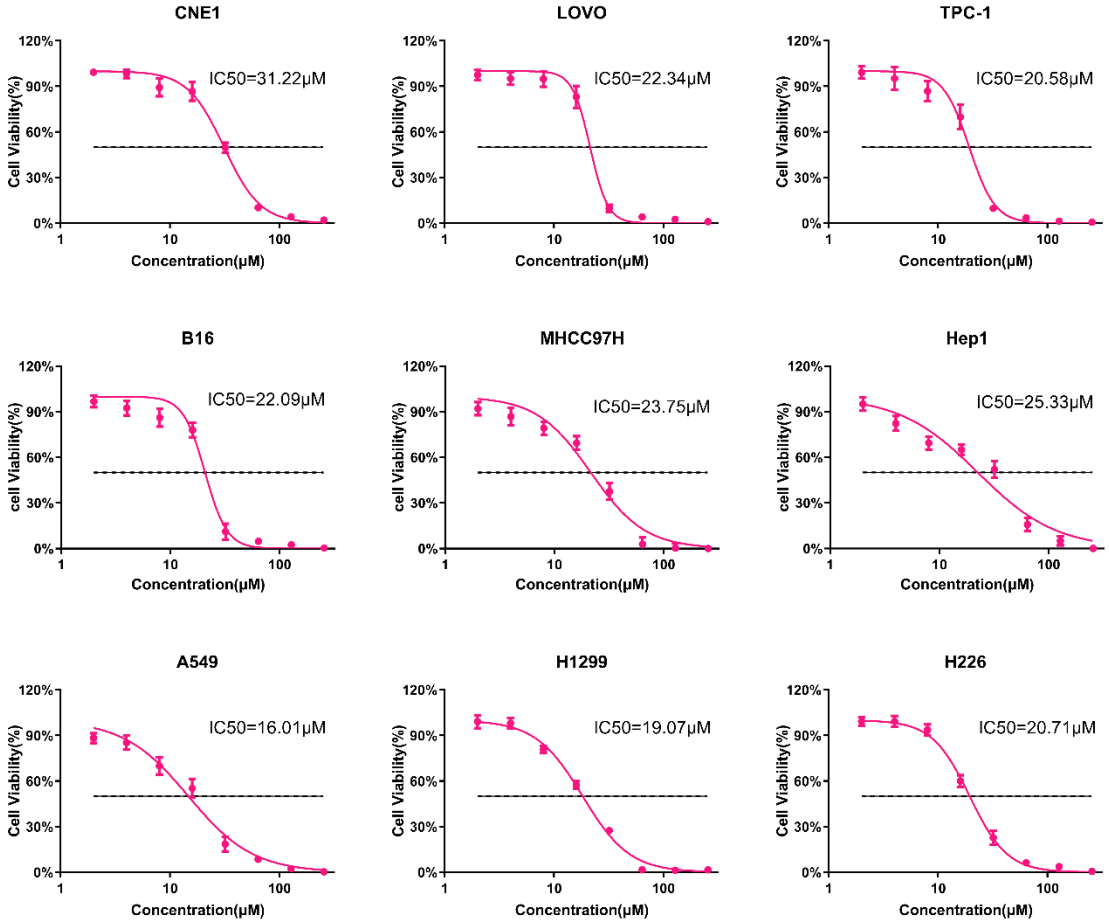


Fig. S1. Antitumor activity screening results of (+)-ABX. The screening range includes nasopharyngeal carcinoma cells (CNE1), colorectal cancer cells (LOVO), thyroid cancer cells (TPC-1), melanoma cells (B16), liver cancer cells (MHCC97H, Hep1), and lung cancer cells (A549, H1299, and H226). The IC50 values were determined using the CCK-8 method.

Table S1. The primer sequences in qRT-PCR assay.

Gene	Primer name	Sequence (5' to 3')
Bcl2	Bcl2-F	GGAGGATTATGGCCTTCTTTG
	Bcl2-R	GCAGGCATGTTGACTTCACTTG
BAX	BAX -F	TTTGCTTCAGGGTTTCATCC
	BAX -R	GCCACAAAGATGGTCACGGT
Caspase-9	Caspase-9-F	GTGGTGGGGAGCAGAAAGAC
	Caspase-9-R	TGCAAGATAAGGCAGGGTGAG
β-actin	β-actin -F	AGACCTTCAACACCCCAG
	β-actin -R	CACGATTTCCCTCTCAGC

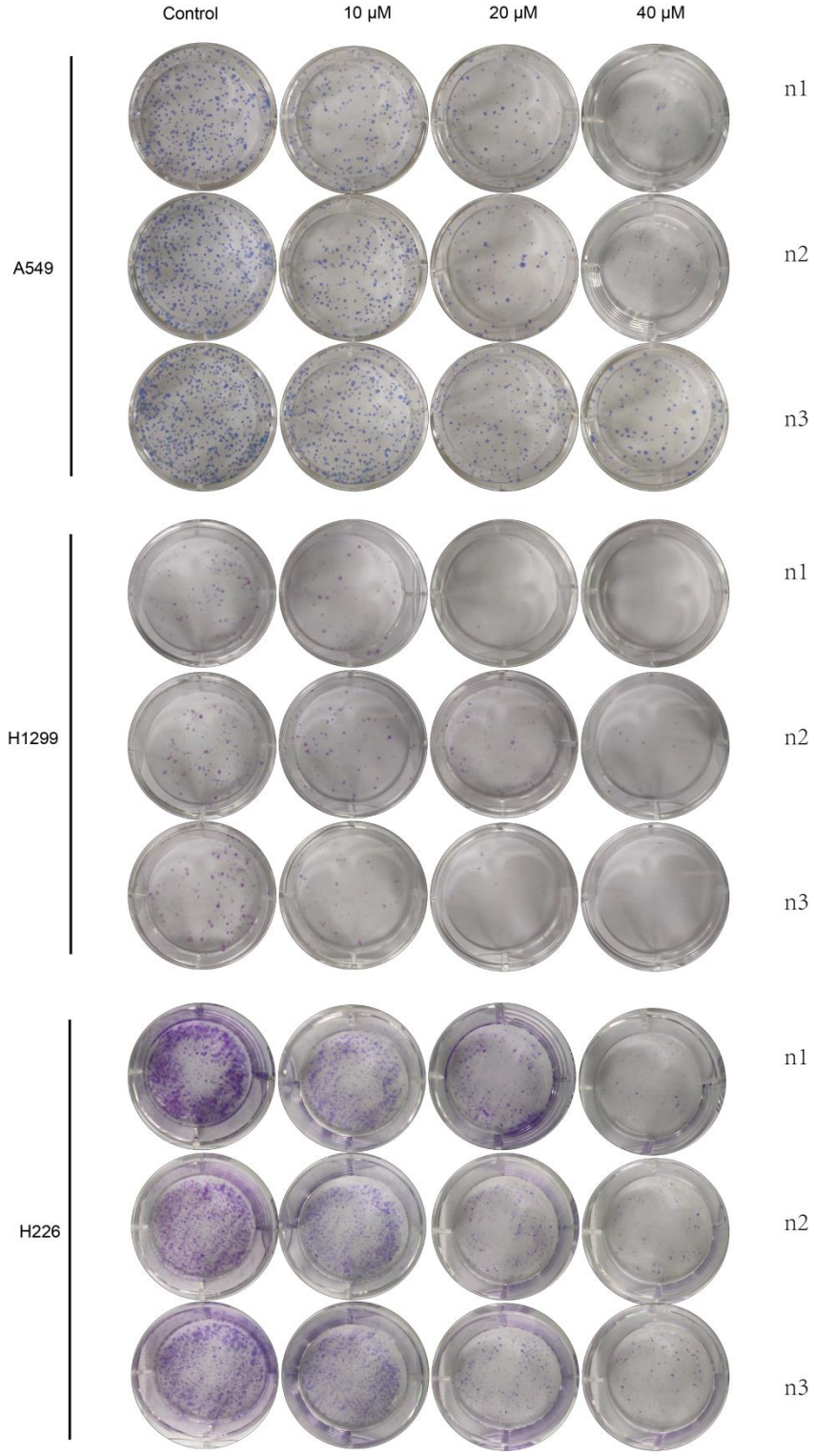


Fig. S2. Effects of different concentrations of (+)-ABX on the colony formation of three NSCLC cell lines.

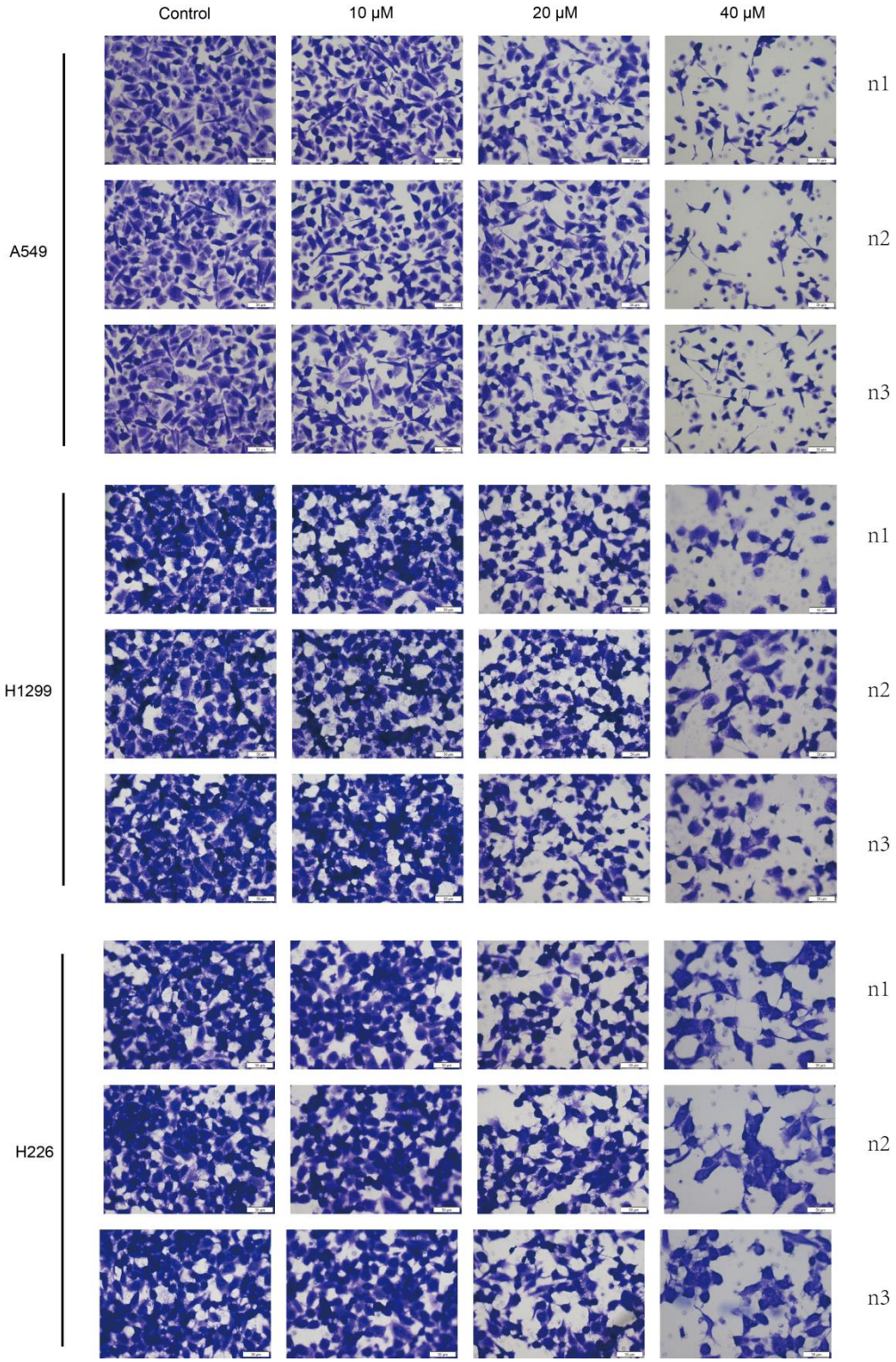


Fig. S3. Effects of different concentrations of (+)-ABX on migration of three NSCLC cell lines (magnification ×200, scale bar = 50 μm).

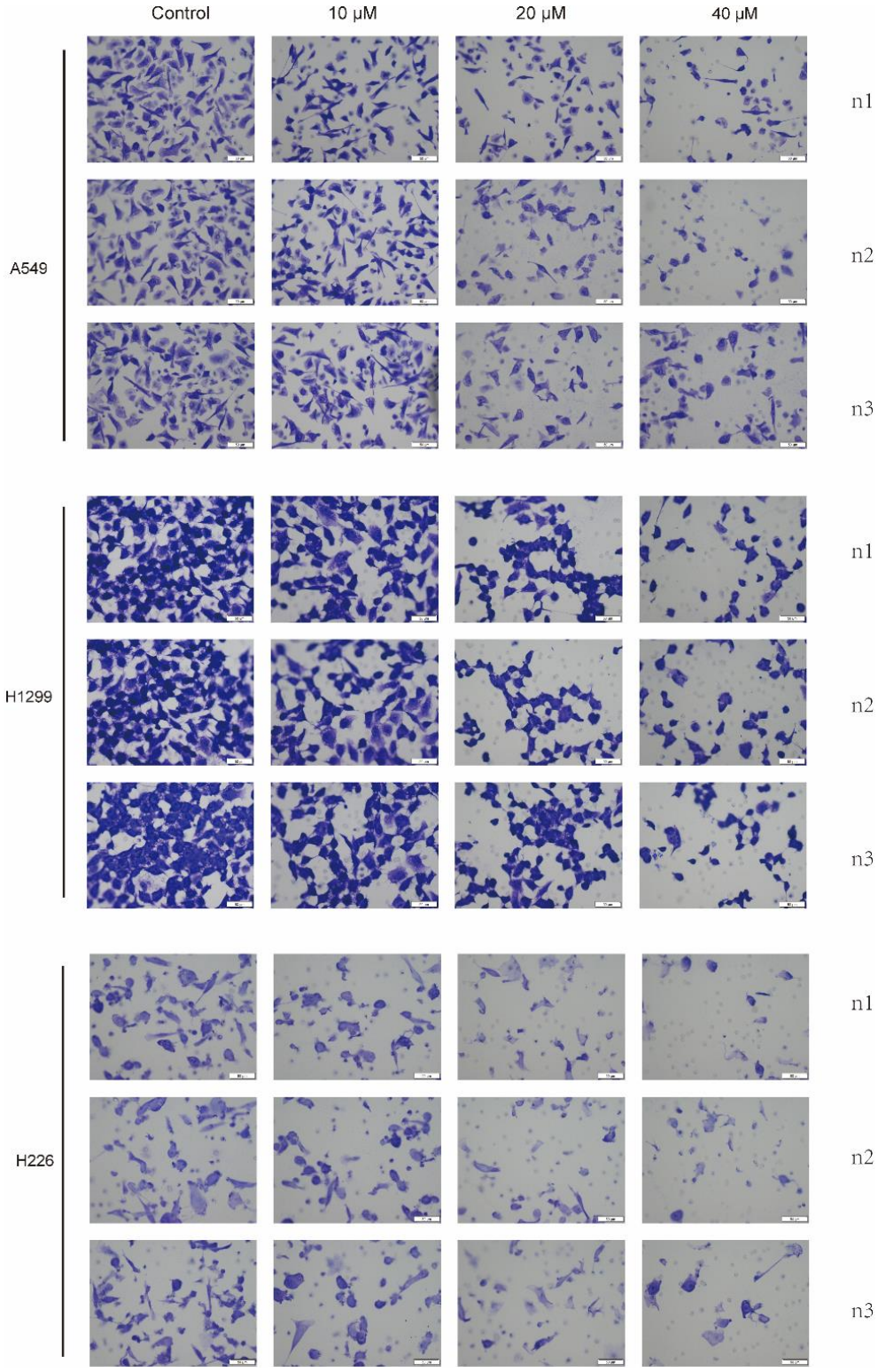


Fig. S4. Effects of different concentrations of (+)-ABX on invasion of three NSCLC cell lines (magnification ×200, scale bar = 50 μm).

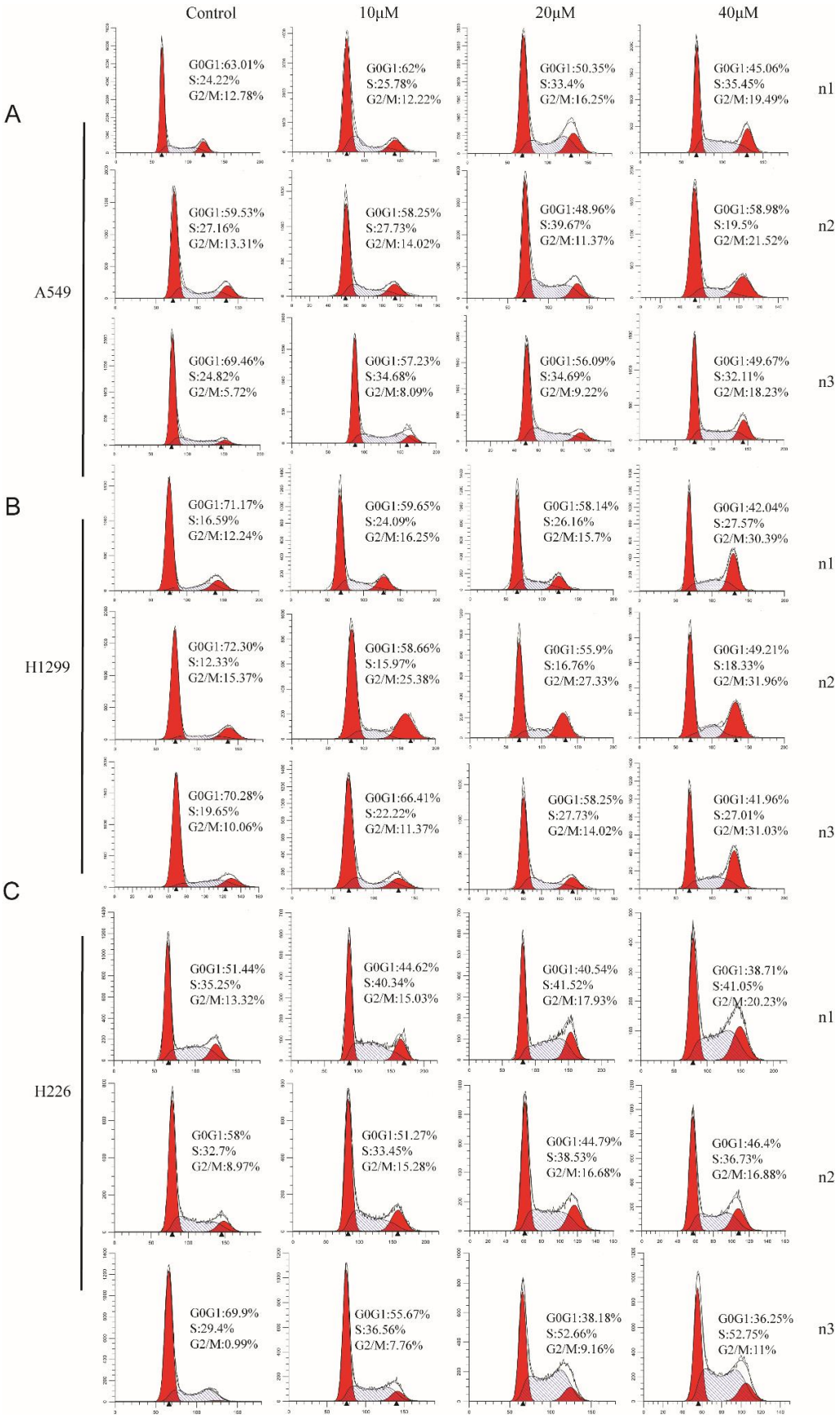


Fig. S5. Effects of different concentrations of (+)-ABX on the cell cycle distribution of three NSCLC cell lines.

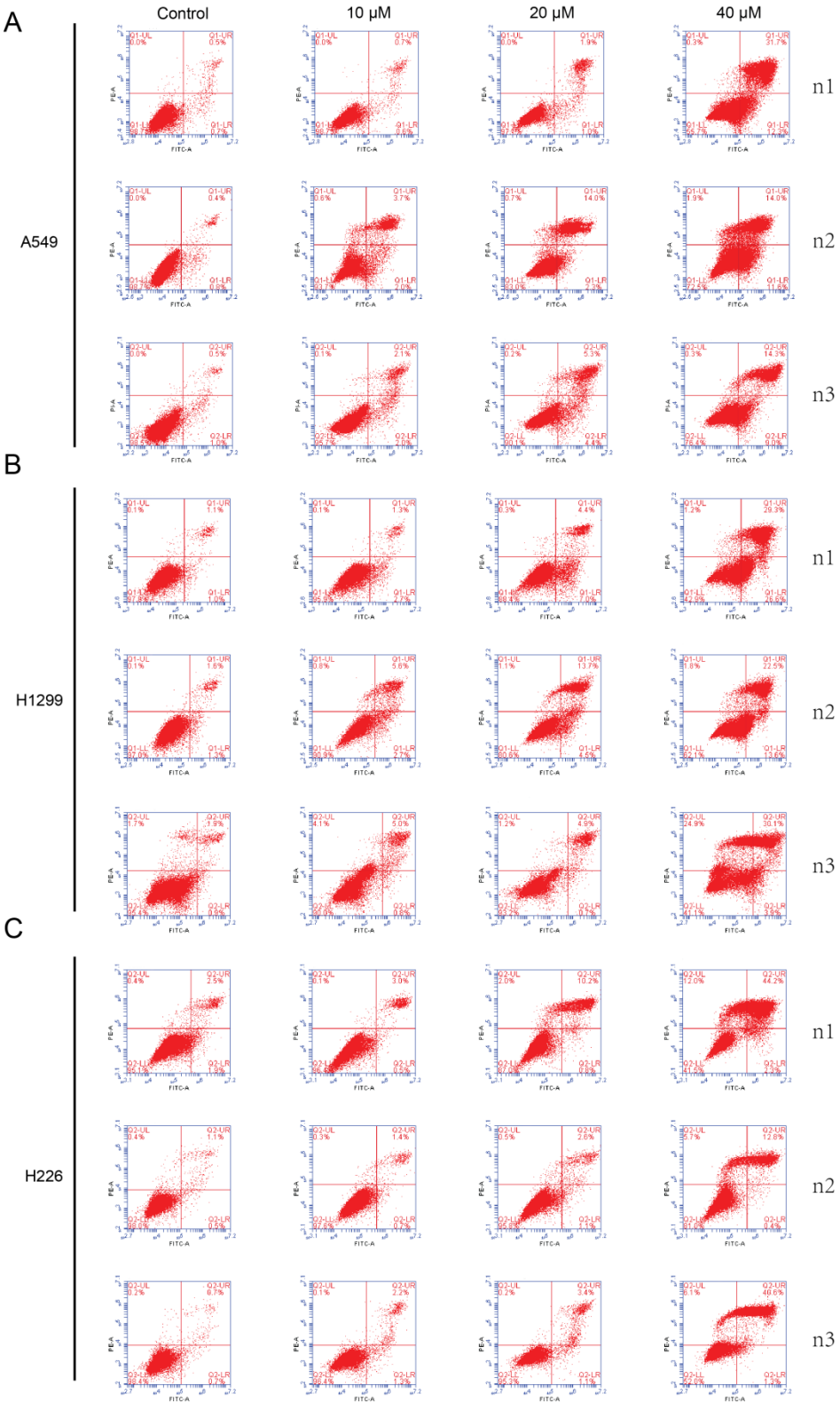


Fig. S6. Flow cytometry analysis of apoptosis rates in three NSCLC cell lines treated with different concentrations of (+)-ABX.

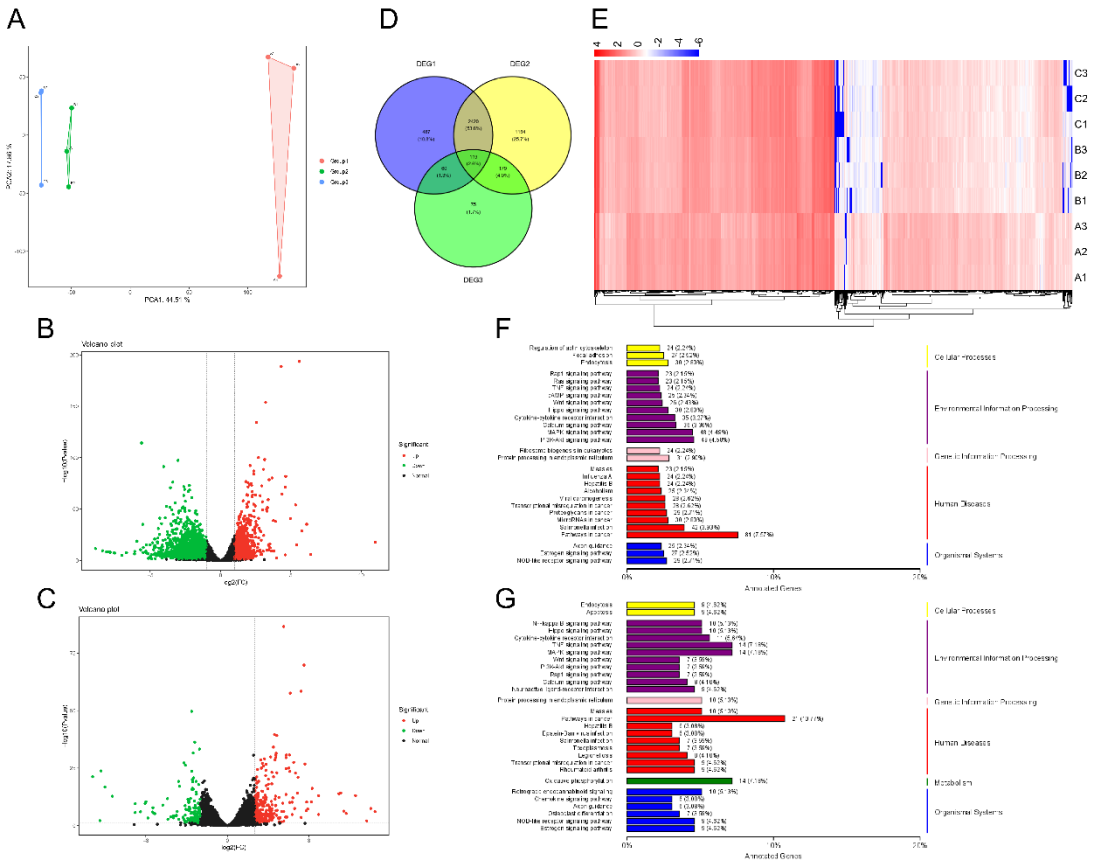
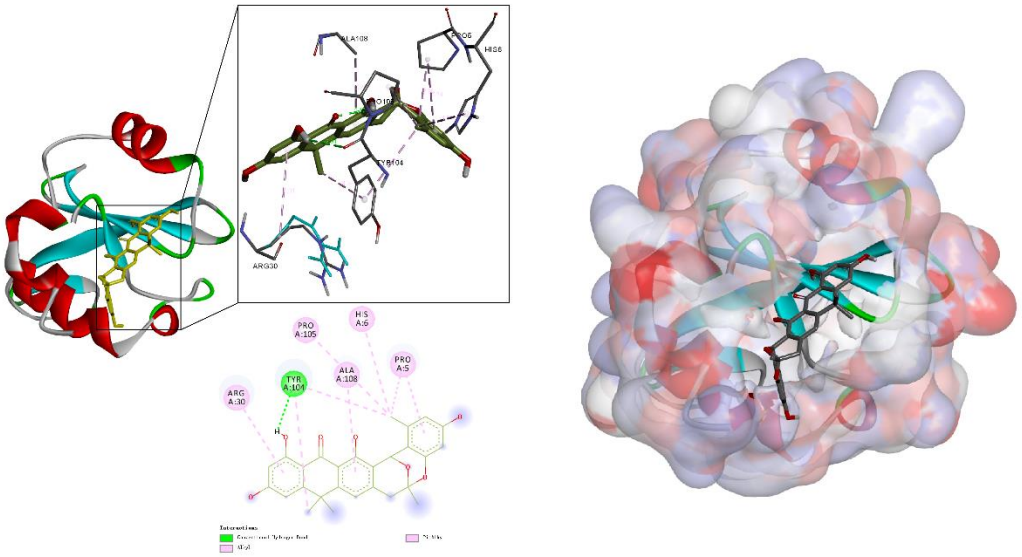


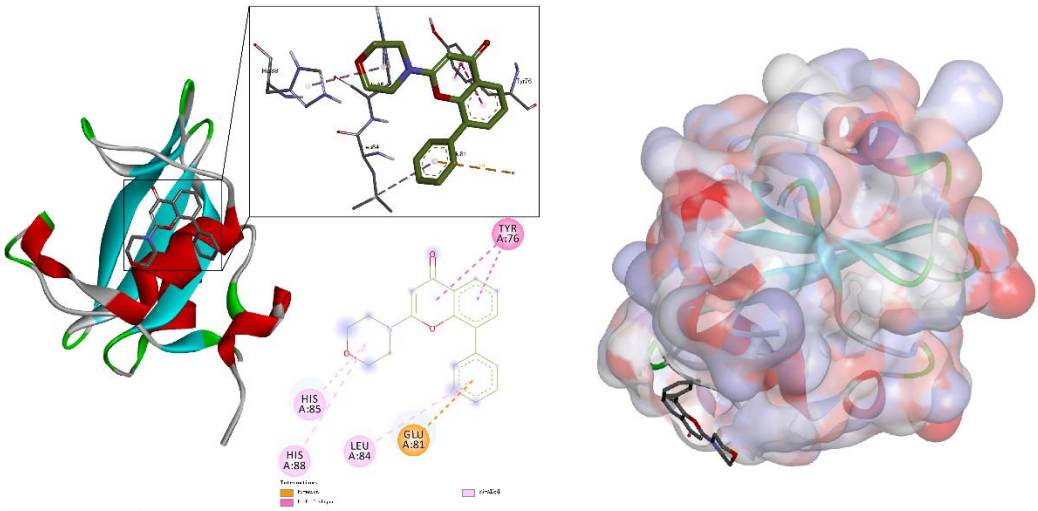
Fig. S7. Transcriptomic analysis. Group A: untreated group ; Group B: 20 μM (+)-ABX treated group ; Group C: 40 μM (+)-ABX treated group. (A) Principal Component Analysis (PCA). (B) Volcano plot of differentially expressed genes (A vs B). (C) Volcano plot of differentially expressed genes (B vs C). (D) Venn diagram of differentially expressed genes. (E) Hierarchical clustering heatmap of differentially expressed genes in three groups. (F) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (A vs B). (G) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (B vs C).

A



Ligand	Protein	PDB ID	Affinity (kcal/mol)	Dist from RMSD 1. b.	Best mode RMSD u. b.
(+)-ABX	PI3K	1H9O	-8.4	0	0

B



Ligand	Protein	PDB ID	Affinity (kcal/mol)	Dist from RMSD 1. b.	Best mode RMSD u. b.
LY294002	PI3K	1H9O	-6.7	0	0

Fig. S8. Differences in the binding sites between (+)-ABX and LY294002 with the PI3K protein. (A) Docking results of (+)-ABX with PI3K. (B) Docking results of LY294002 with PI3K.

Table S2. The images of immunohistochemistry staining in three repetitions

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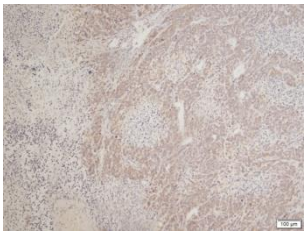
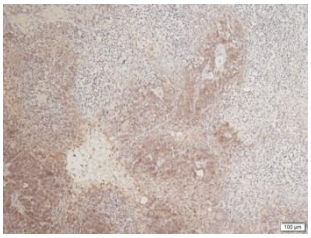
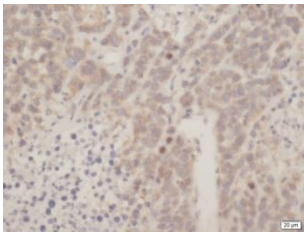
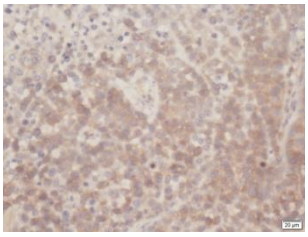
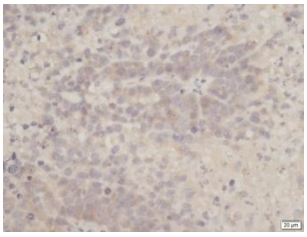
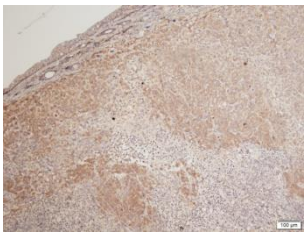
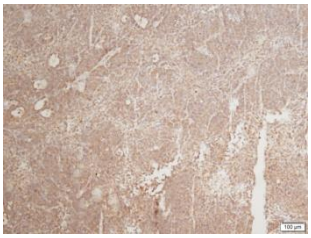
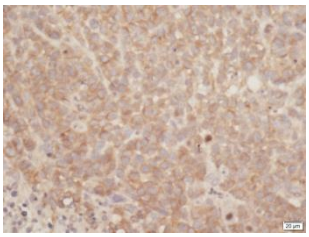
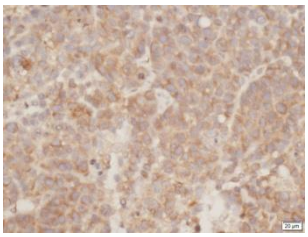
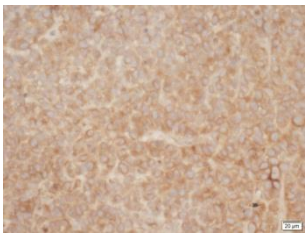
Control ×100 (100μm)			
Control ×400 (20μm)			
Treated ×100 (100μm)			
Treated ×400 (20μm)			
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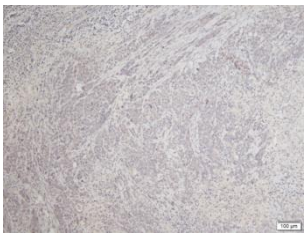
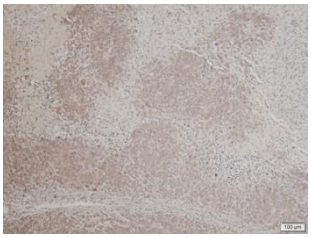
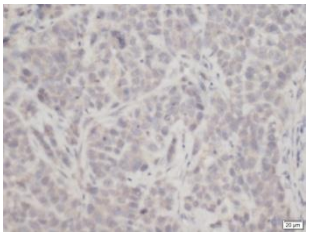
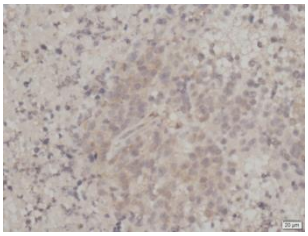
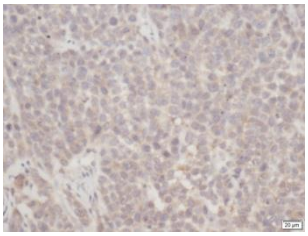
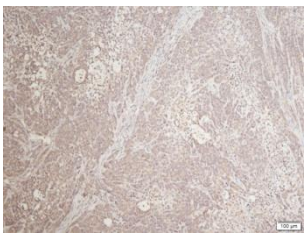
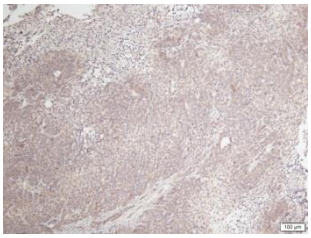
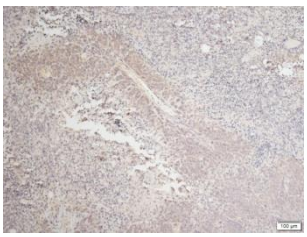
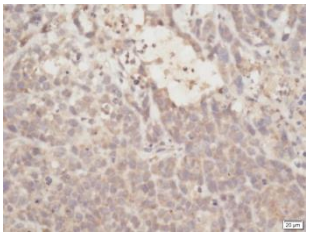
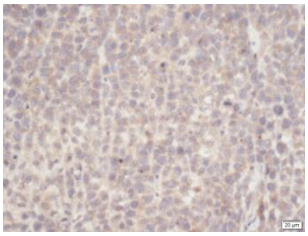
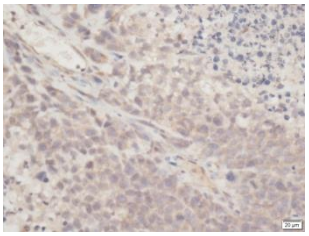
Control ×100 (100μm)			
Control ×400 (20μm)			

Treated ×100 (100μm)			
Treated ×400 (20μm)			
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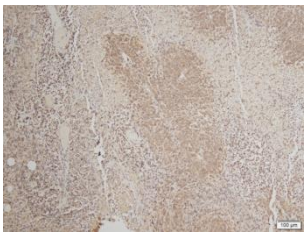
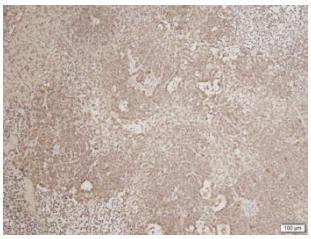
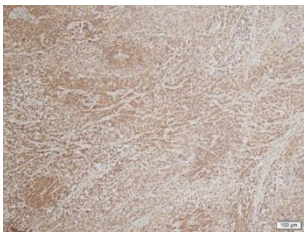
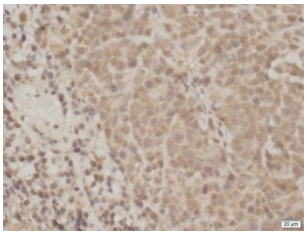
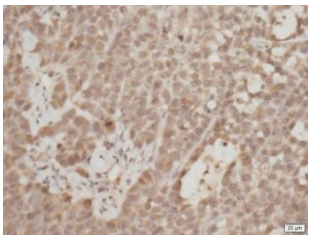
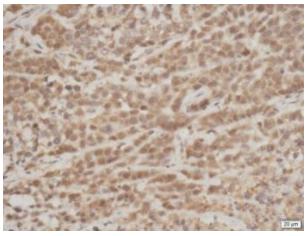
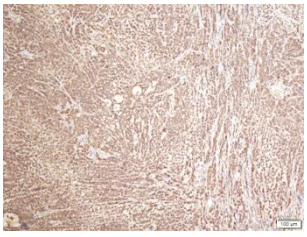
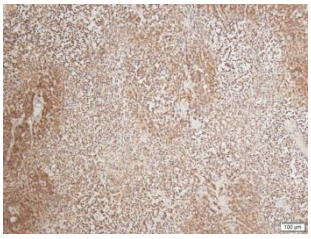
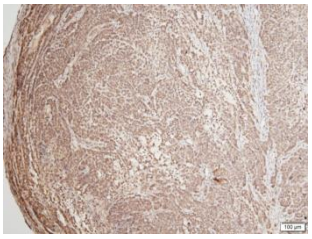
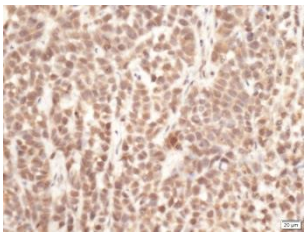
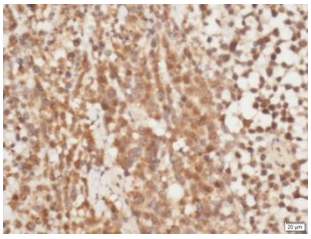
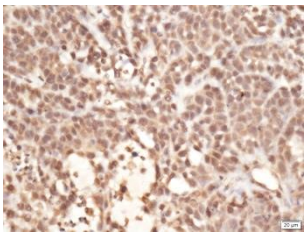
Control ×100 (100μm)			
Control ×400 (20μm)			
Treated ×100 (100μm)			
Treated ×400 (20μm)			
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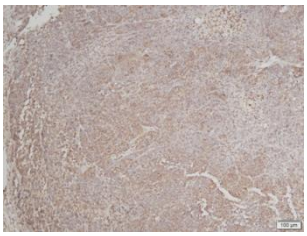
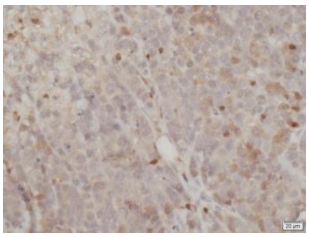
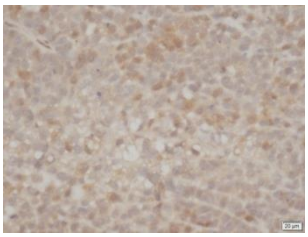
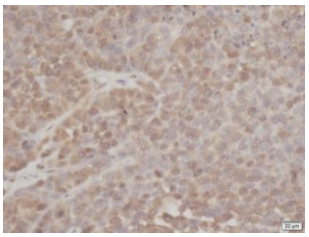
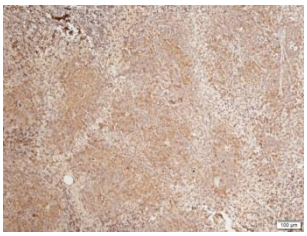
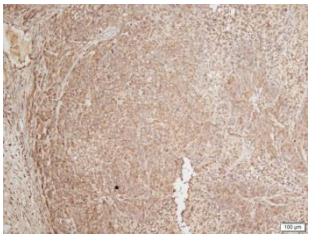
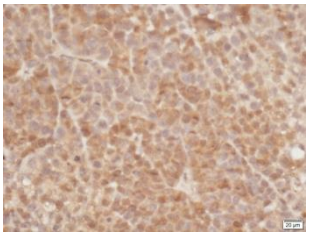
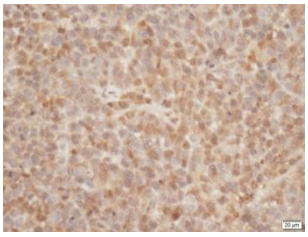
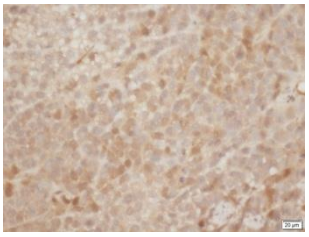
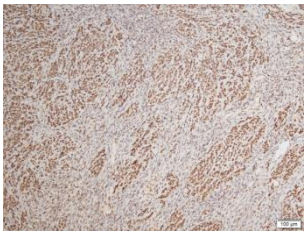
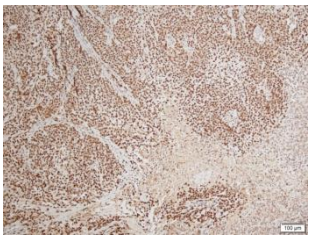
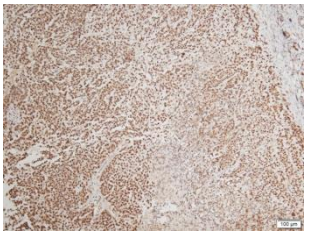
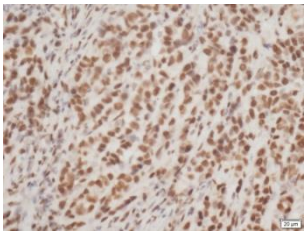
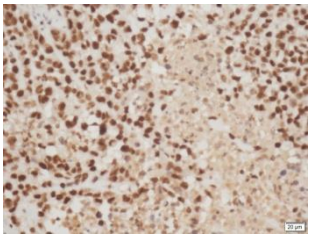
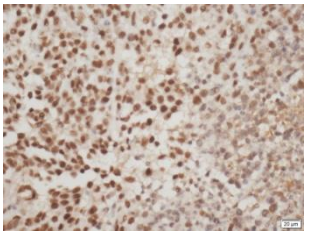
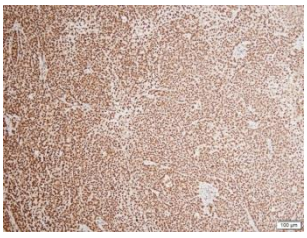
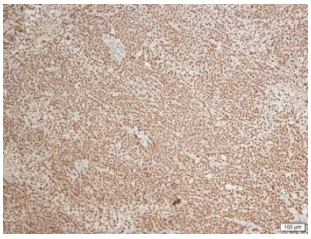
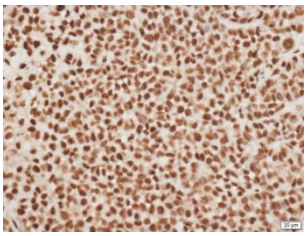
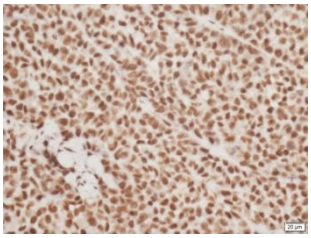
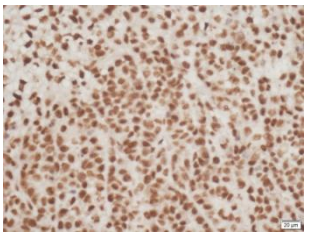
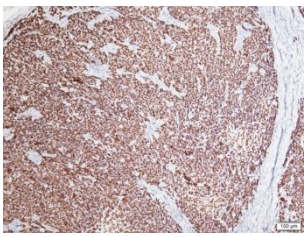
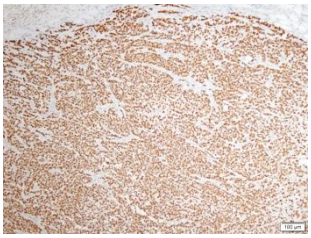
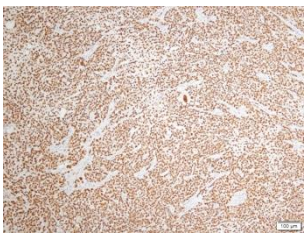
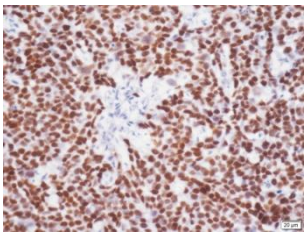
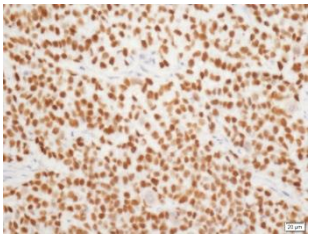
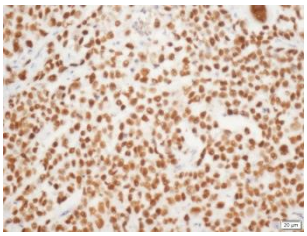
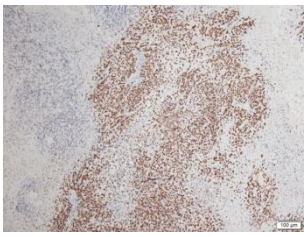
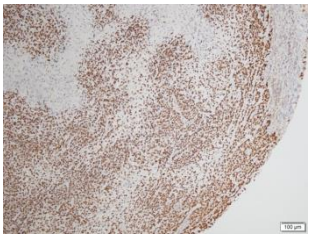
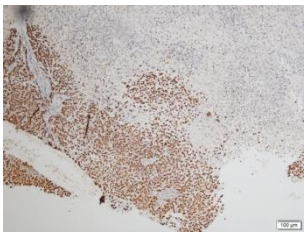
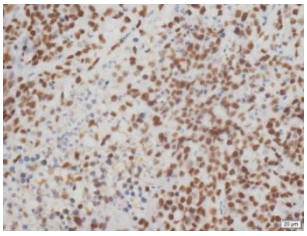
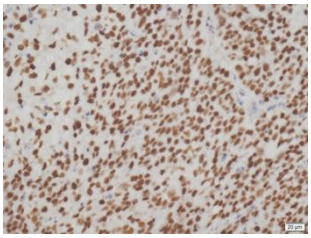
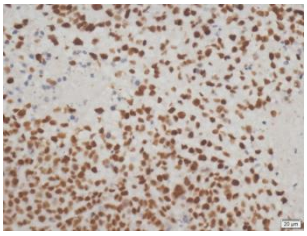
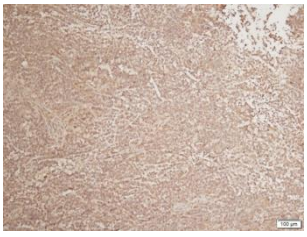
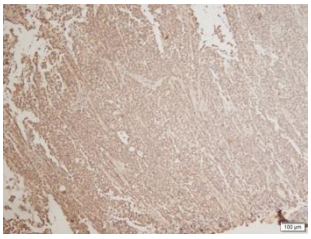
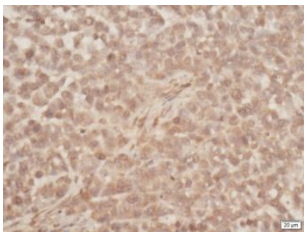
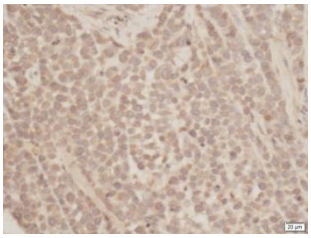
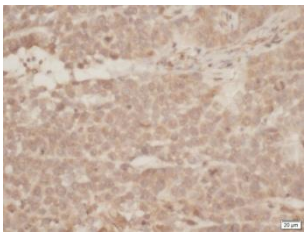
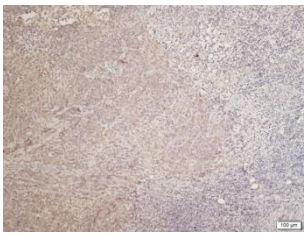
Control ×100 (100μm)			

Control ×400 (20μm)			
Treated ×100 (100μm)			
Treated ×400 (20μm)			
Caspase 9	n1	n2	n3
Control ×100 (100μm)			
Control ×400 (20μm)			
Treated ×100 (100μm)			
Treated ×400 (20μm)			
Ki67	n1	n2	n3

Control ×100 (100μm)			
Control ×400 (20μm)			
Treated ×100 (100μm)			
Treated ×400 (20μm)			
LC3A	n1	n2	n3
Control ×100 (100μm)			
Control ×400 (20μm)			
Treated ×100 (100μm)			

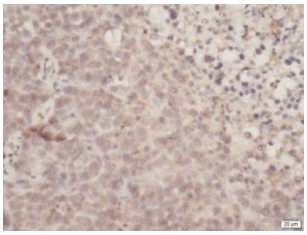
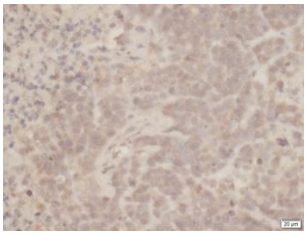
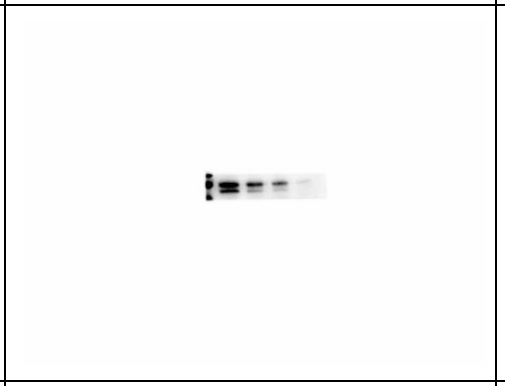
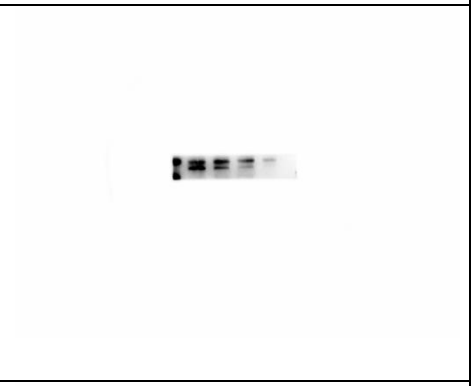
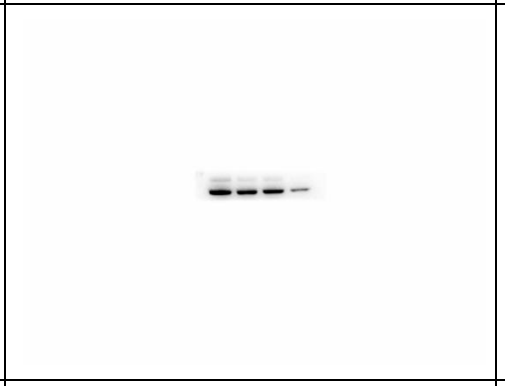
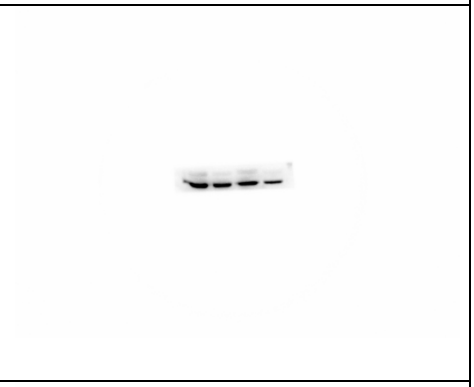
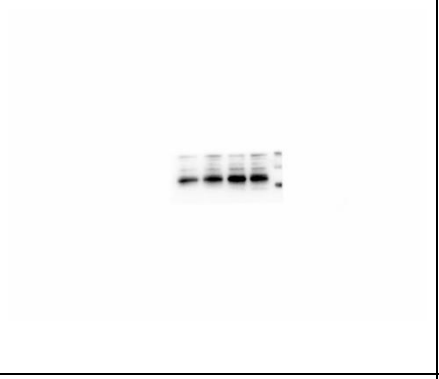
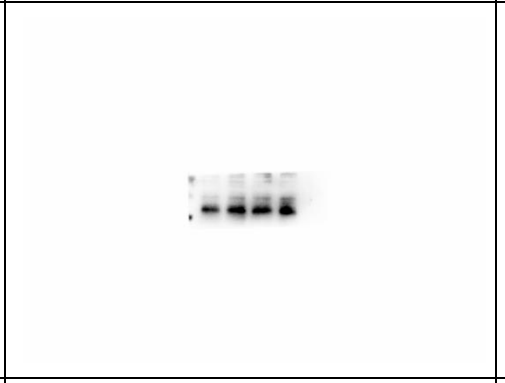
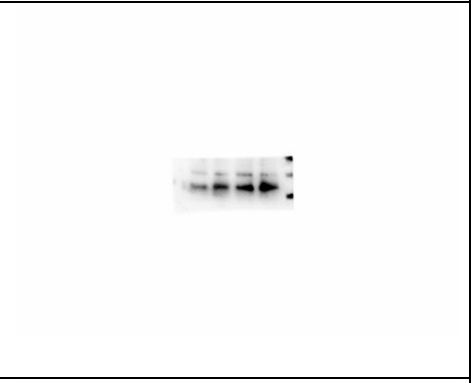
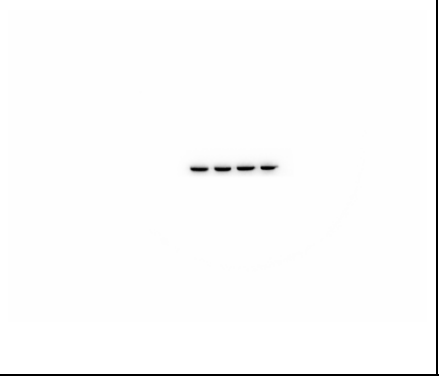
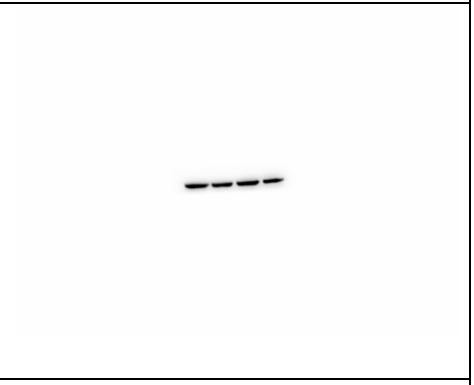
Treated ×400 (20μm)			

Table S3. The images of the original western blots in three repetitions

Figure 2D			
Proteins	n1	n 2	n 3
CDK1			
Cyclin B1			
P21			
β-actin			
Figure 2E			

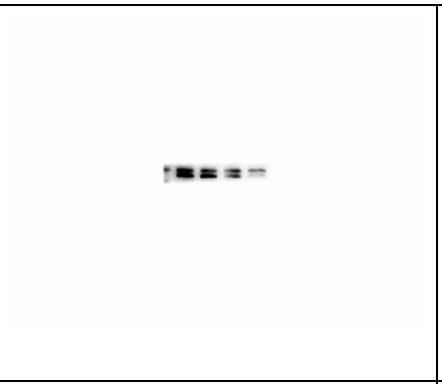
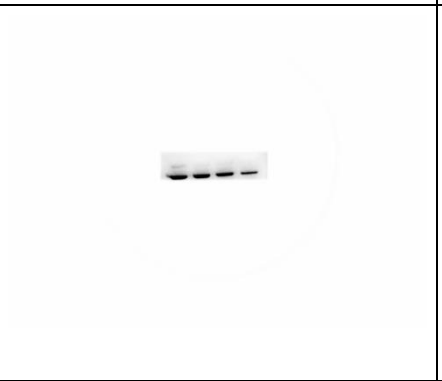
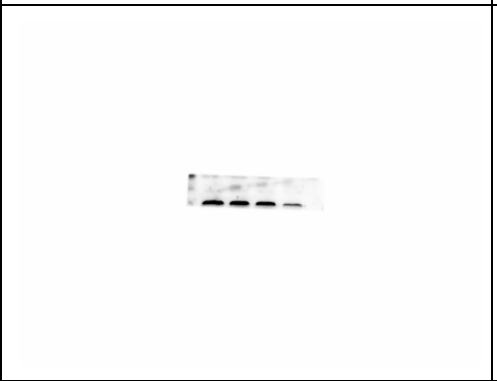
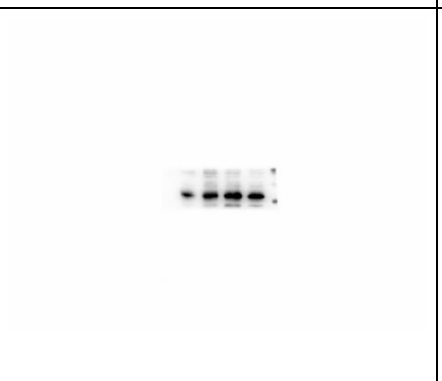
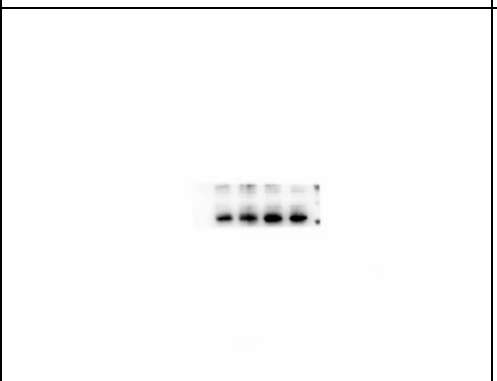
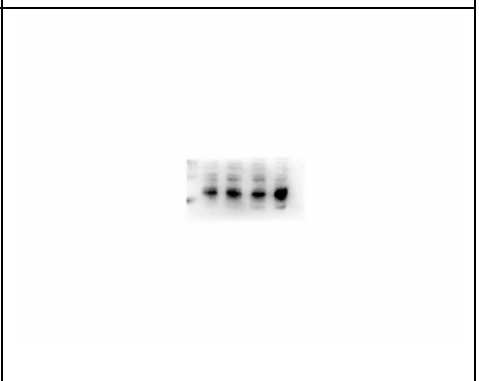
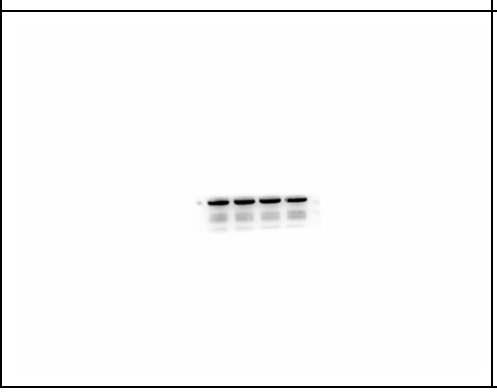
CDK1			
Cyclin B1			
P21			
β-actin			

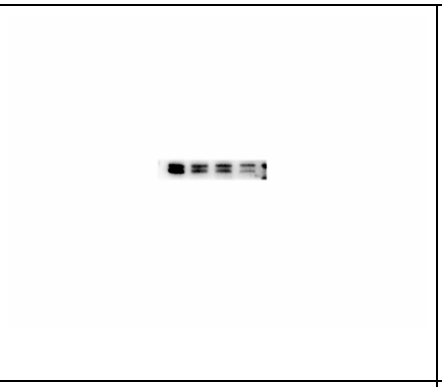
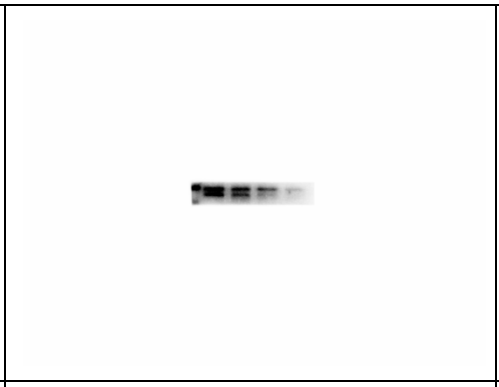
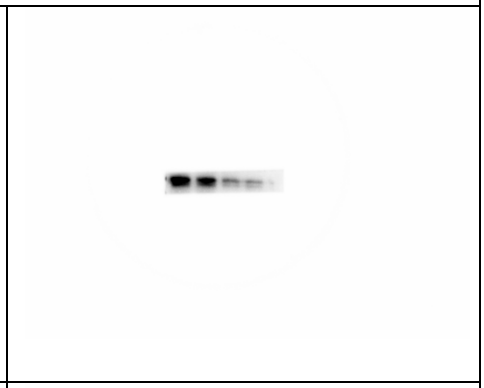
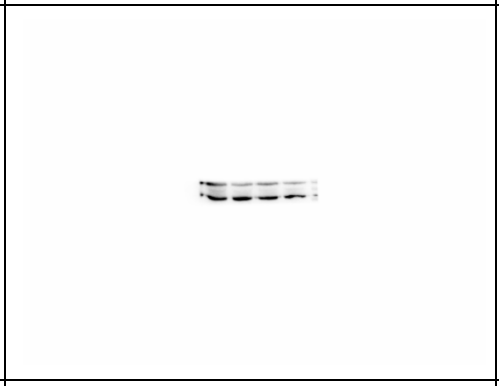
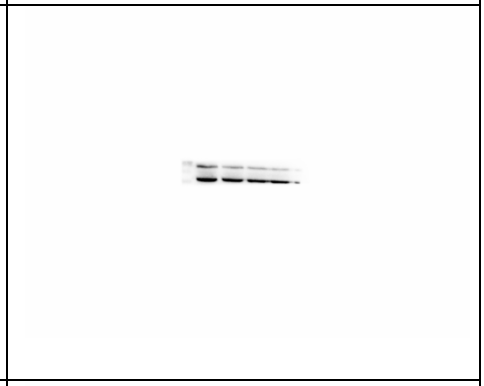
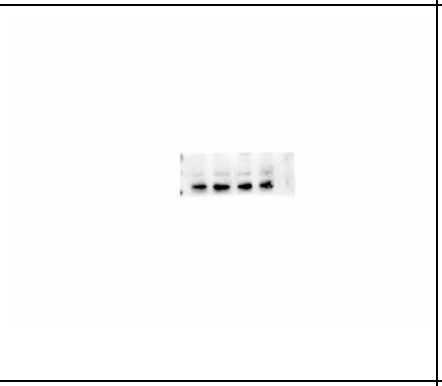
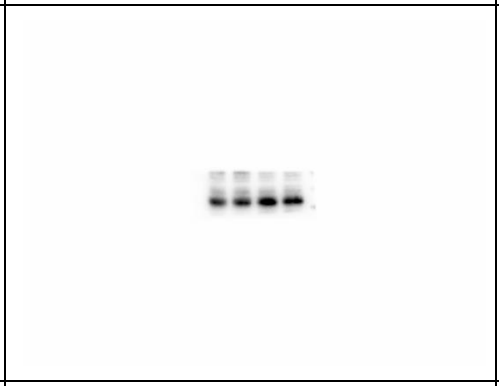
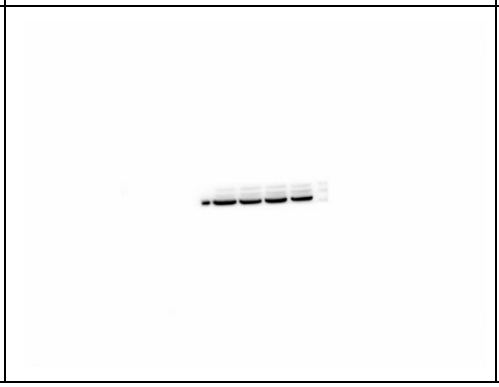
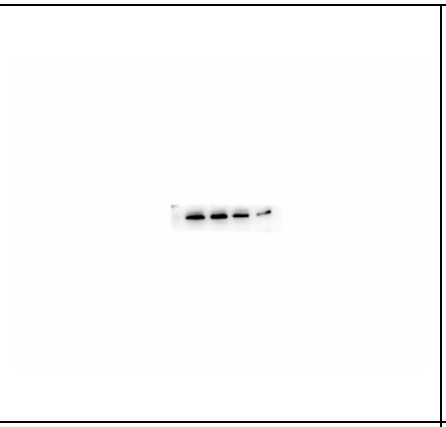
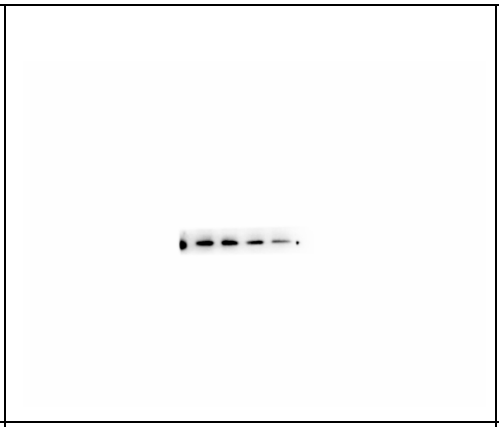
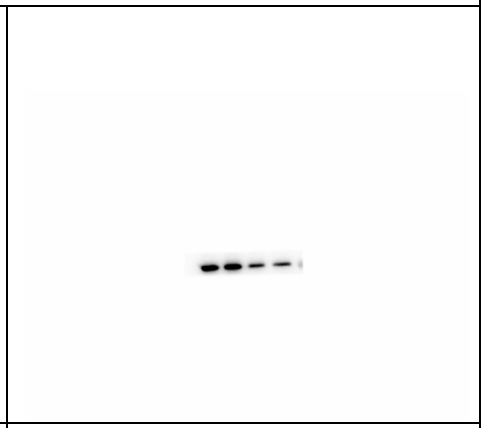
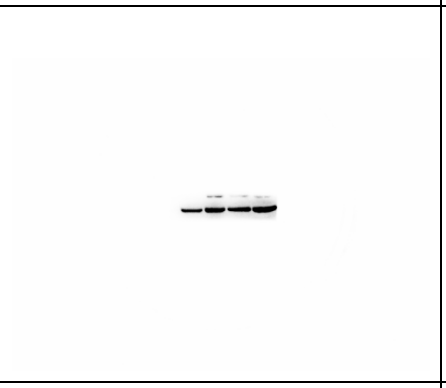
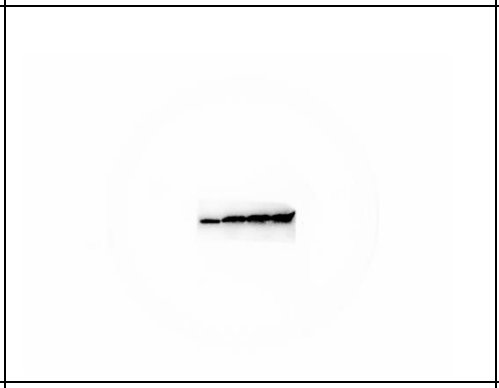
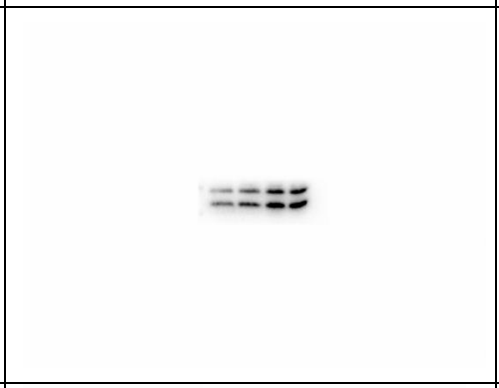
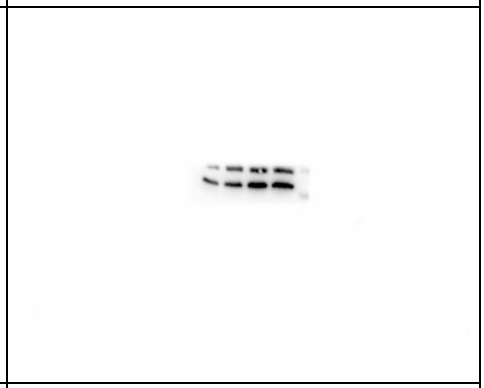
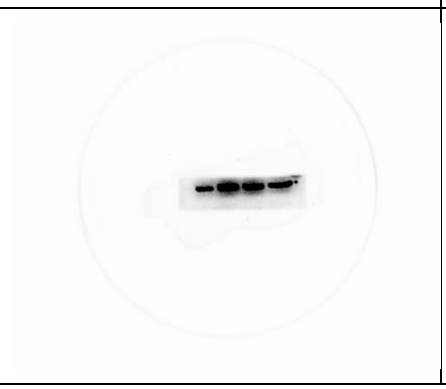
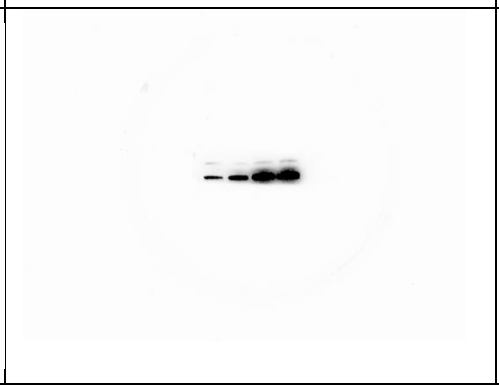
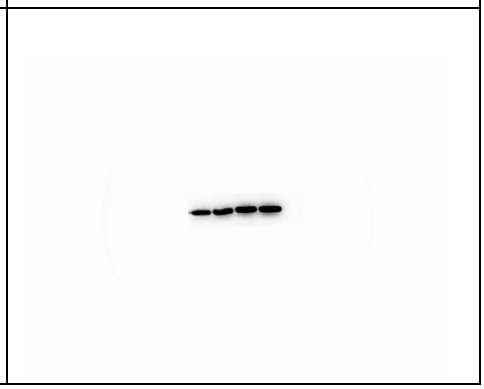
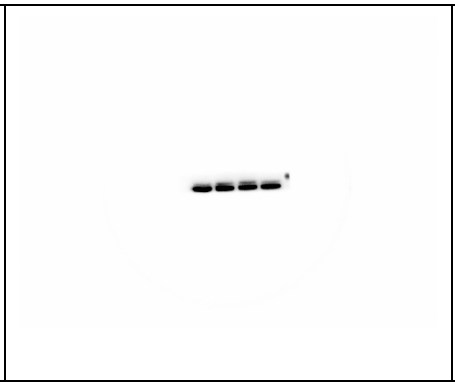
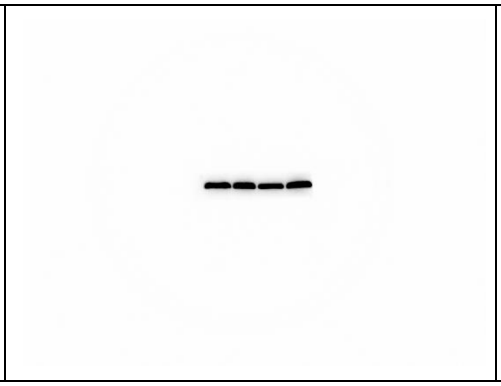
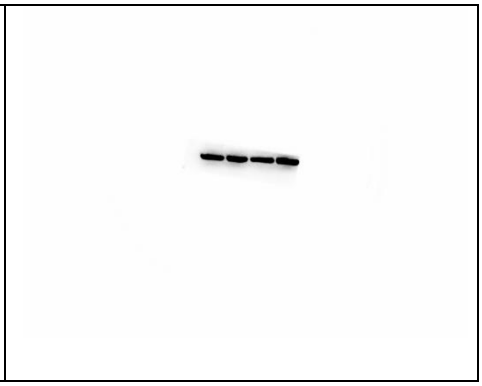
Figure 2F			
CDK1			
Cyclin B1			
P21			
β-actin			

Figure 3E (A549)			
Bcl2			
Bax			
Cle-caspase3			
Cle-caspase9			

β-actin			

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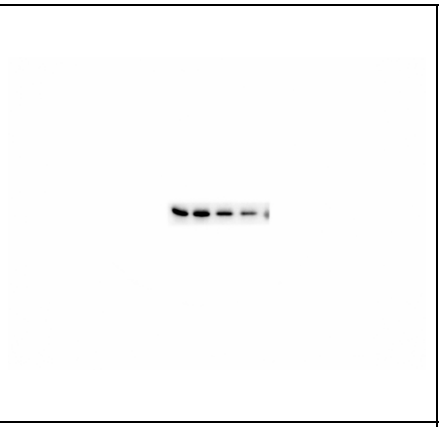
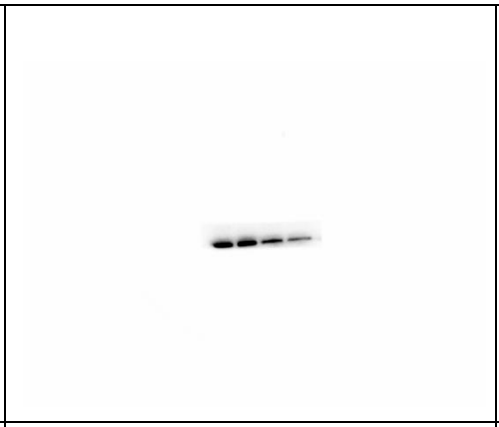
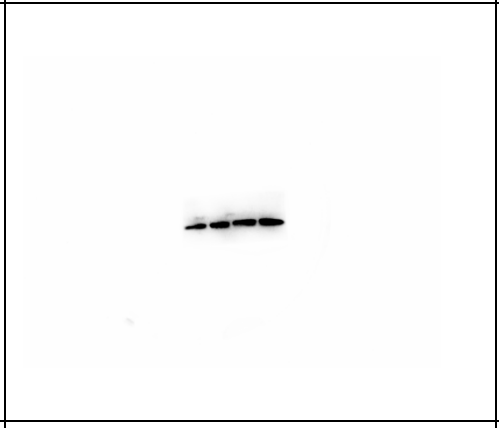
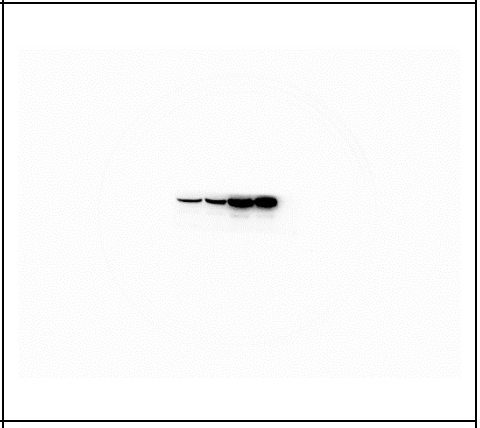
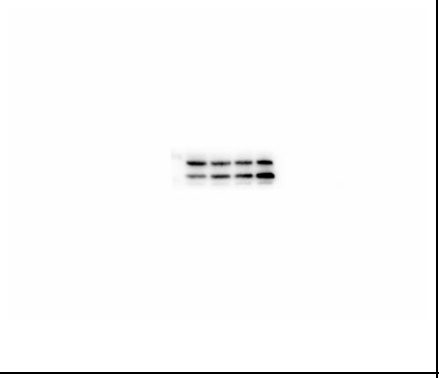
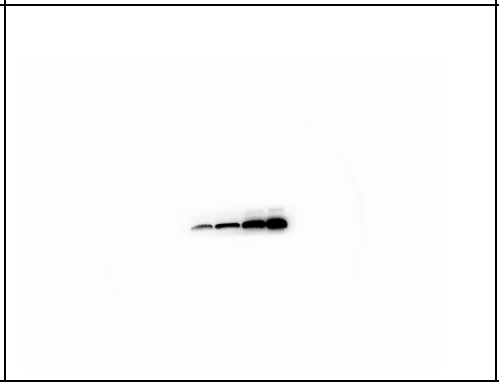
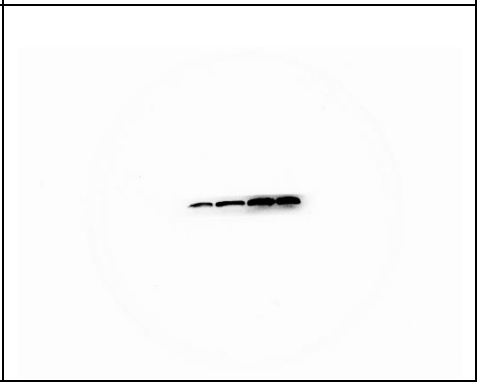
Bcl2			
Bax			
Cle-caspase3			
Cle-caspase9			

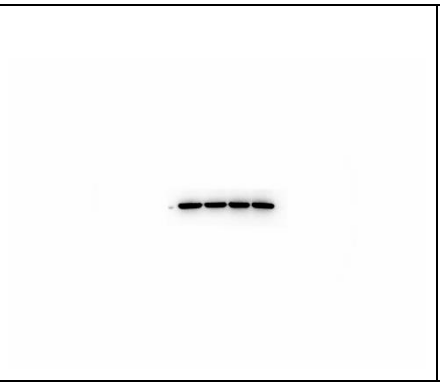
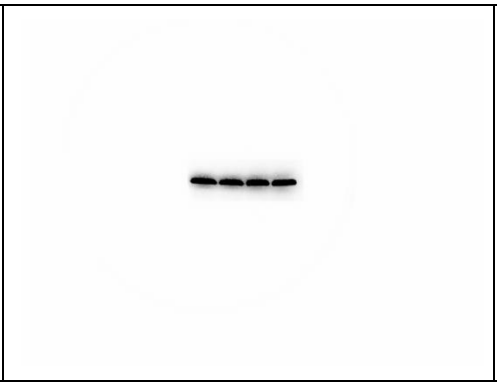
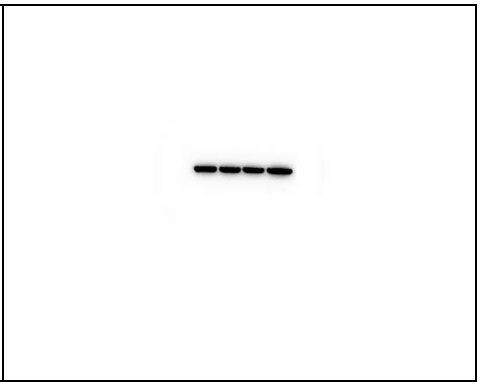
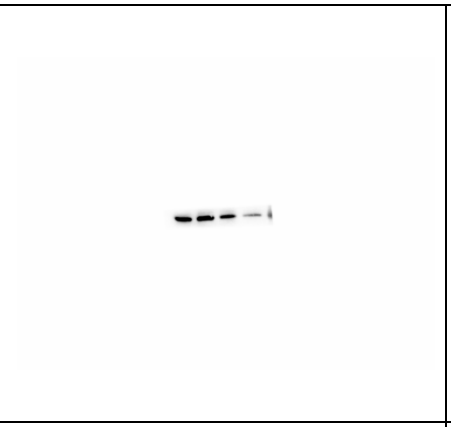
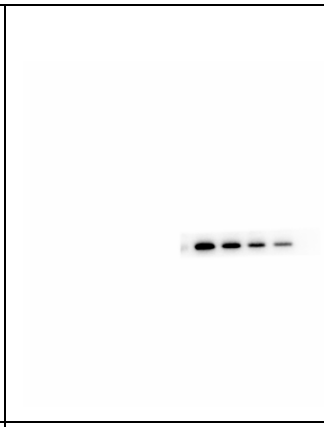
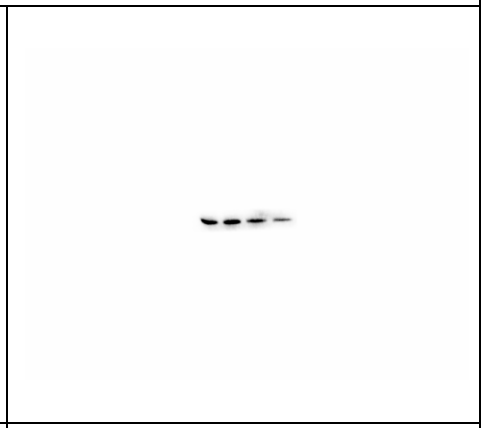
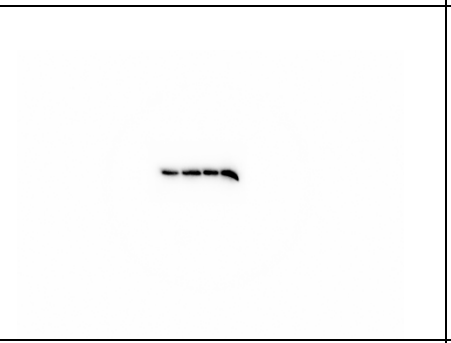
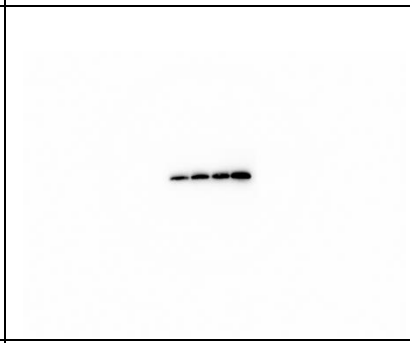
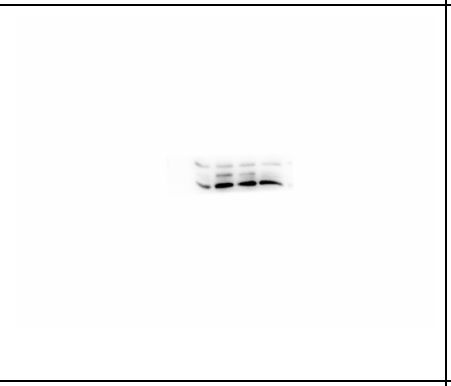
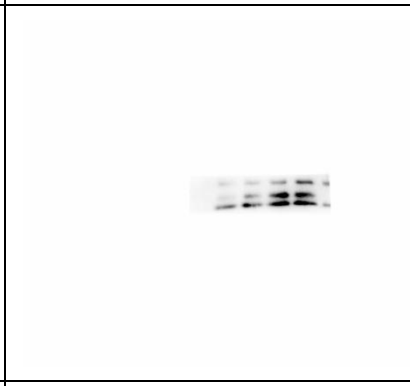
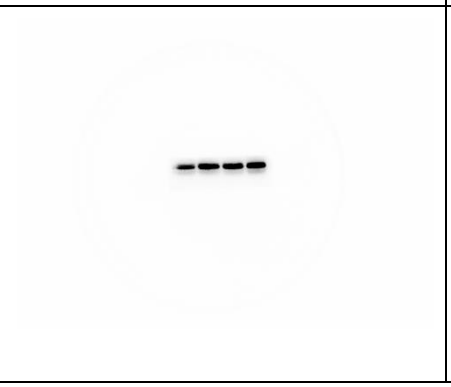
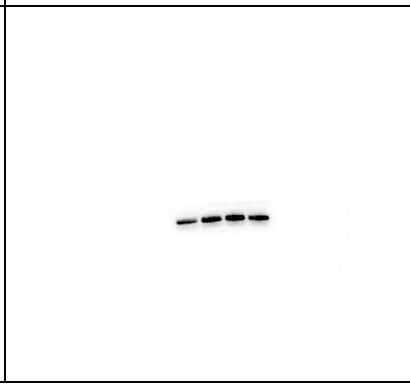
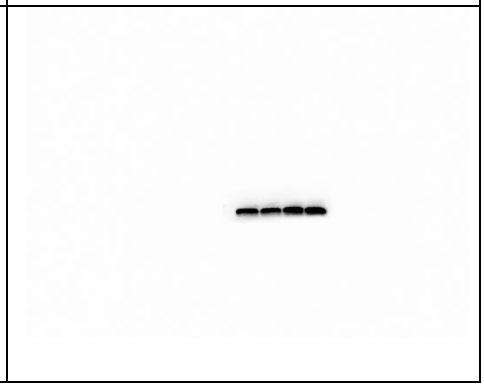
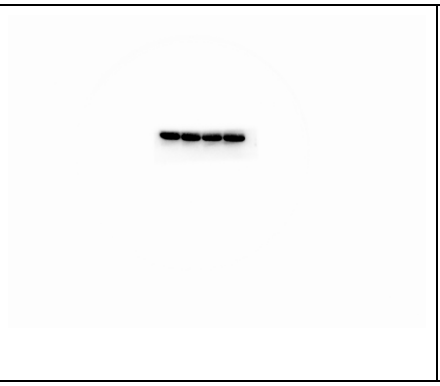
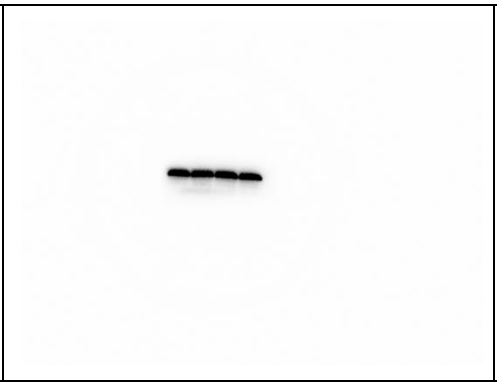
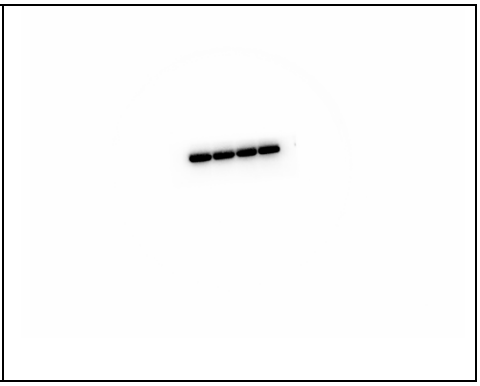
β-actin			

Figure 3E (H226)			
Bcl2			
Bax			
Cle-caspase3			
Cle-caspase9			

β-actin			

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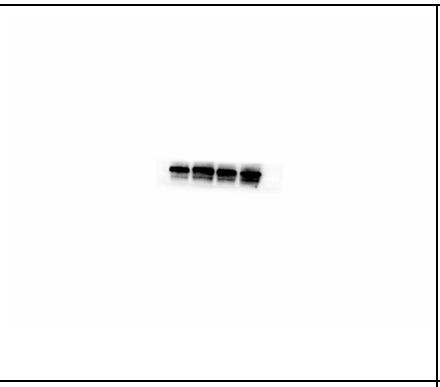
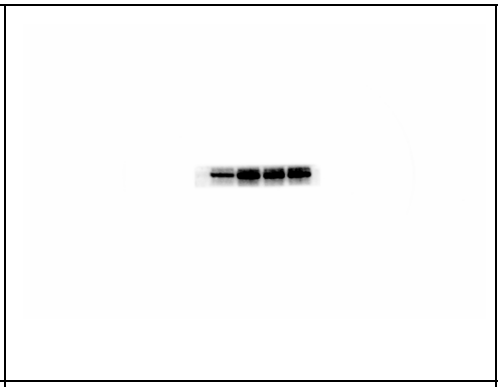
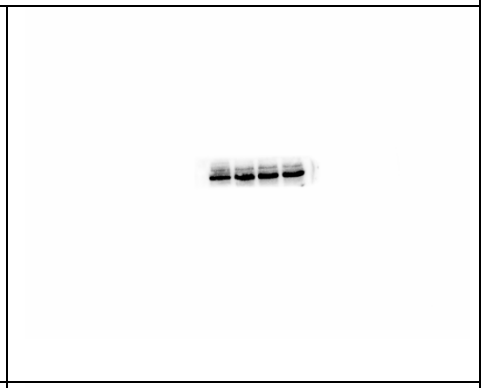
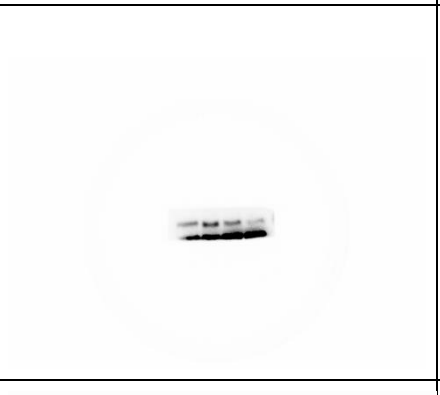
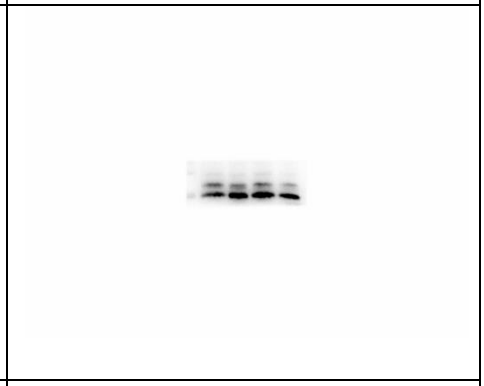
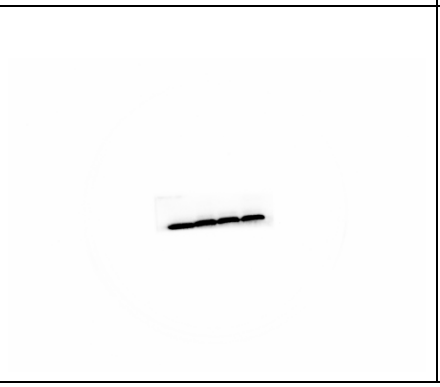
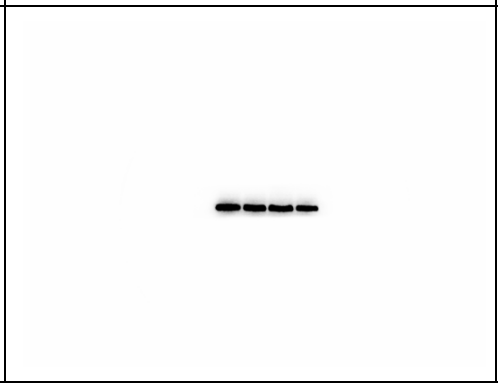
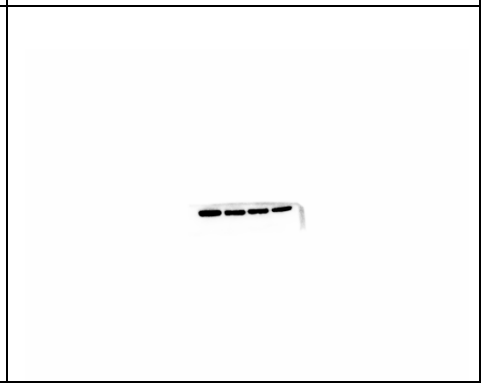
Beclin1			
Lc3			
P62			
β-actin			

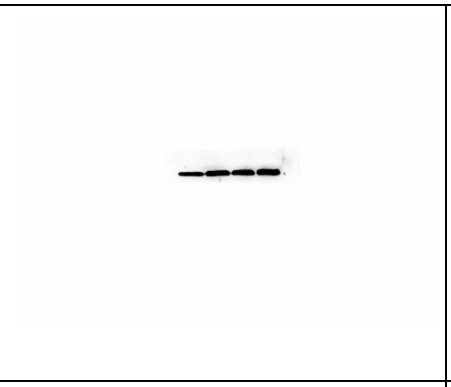
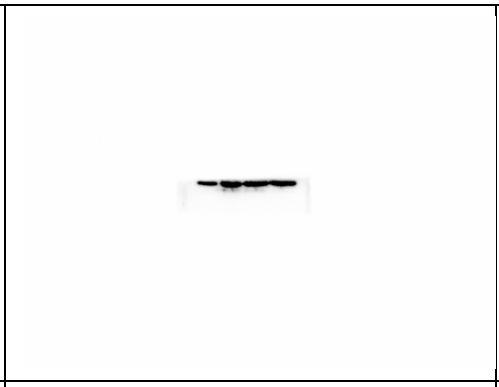
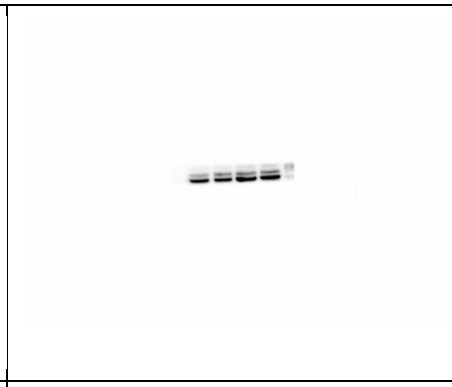
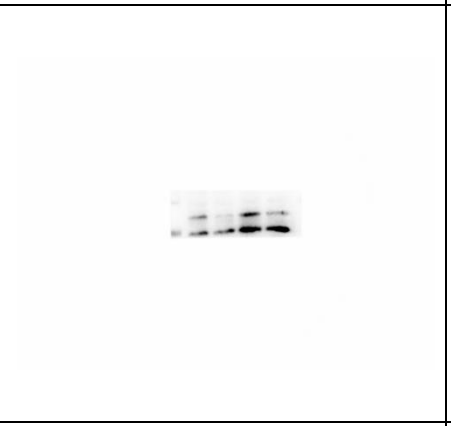
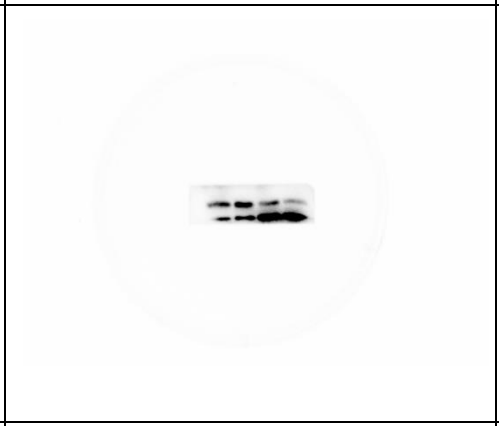
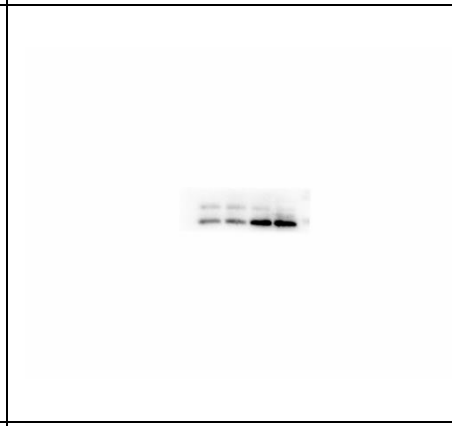
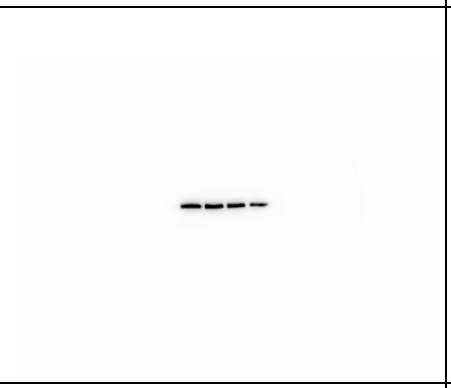
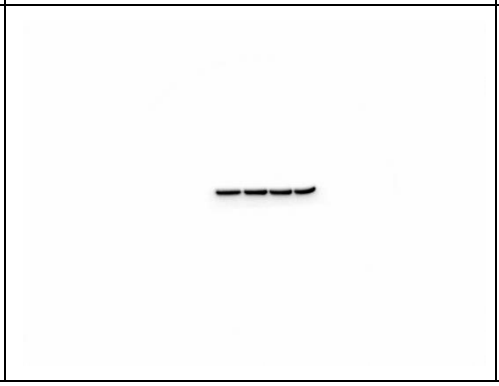
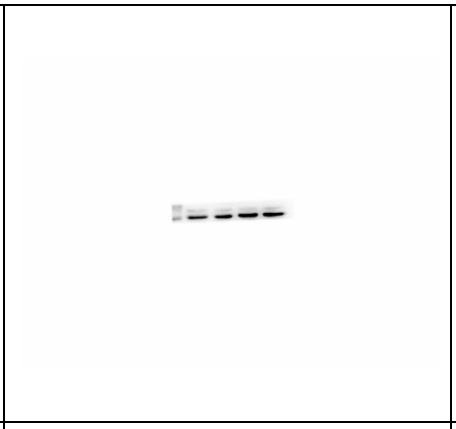
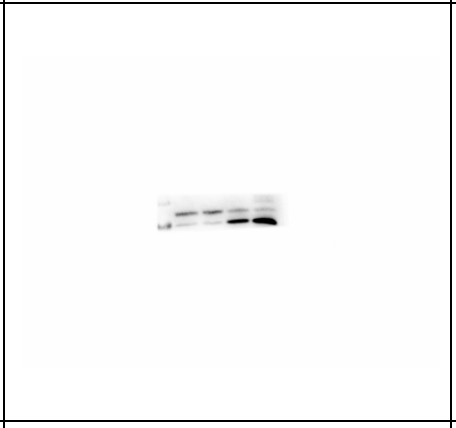
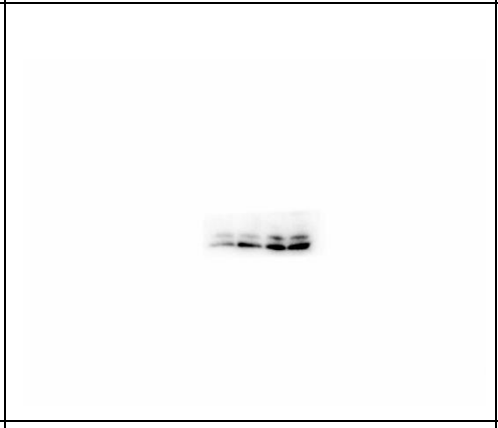
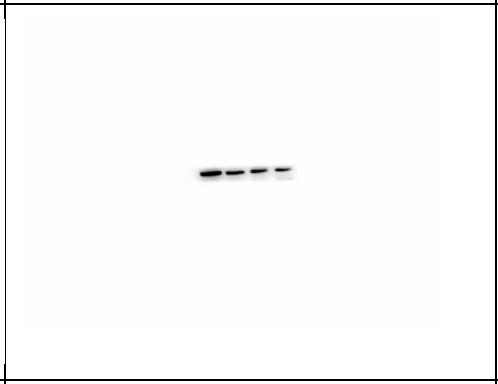
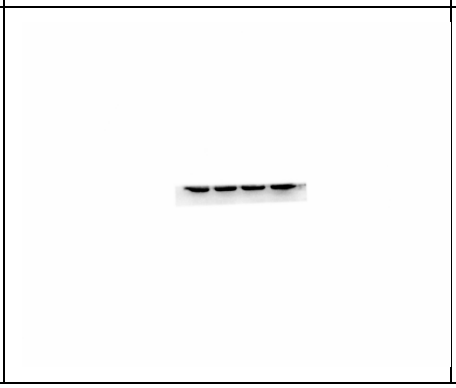
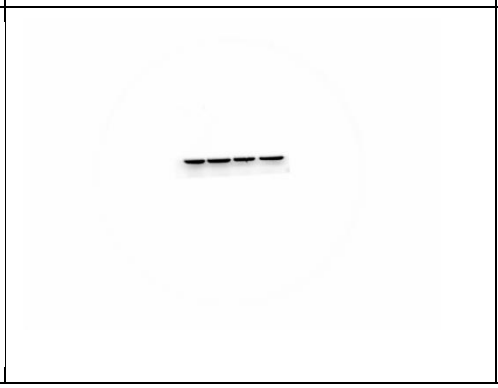
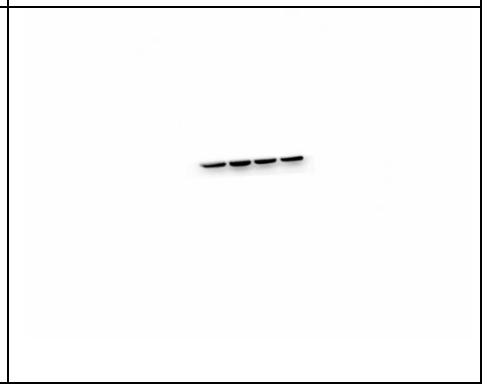
Figure 4B (H1299)			
Beclin1			
Lc3			
P62			
β-actin			

Figure 4B (H226)			
Beclin1			
LC3			
P62			
β-actin			

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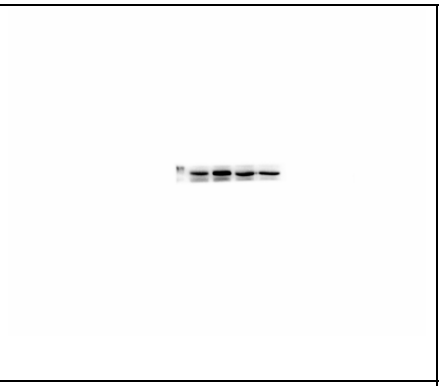
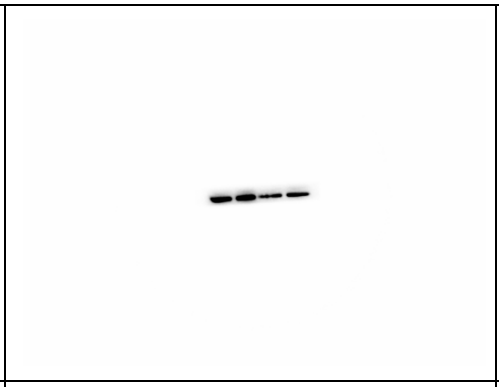
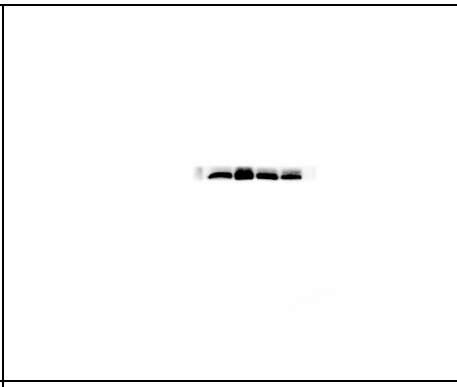
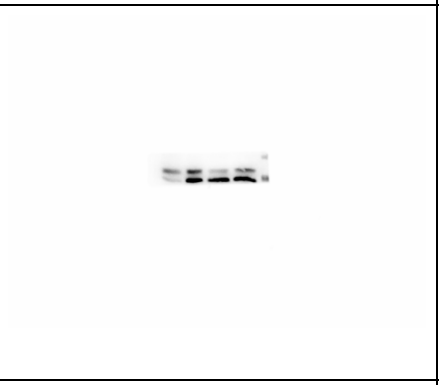
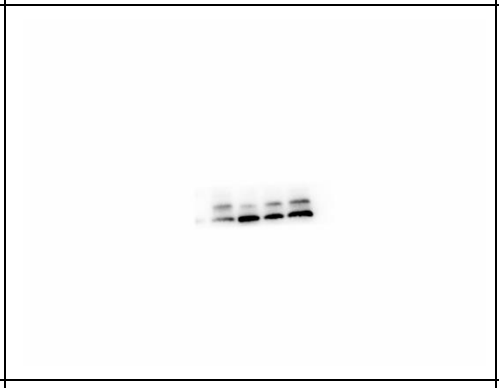
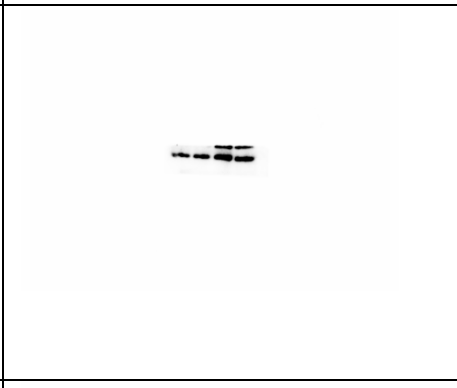
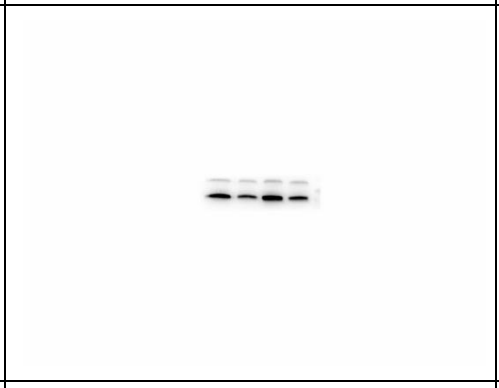
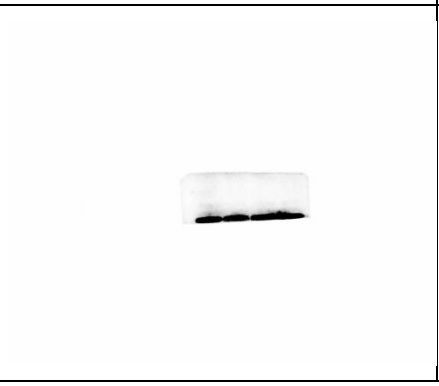
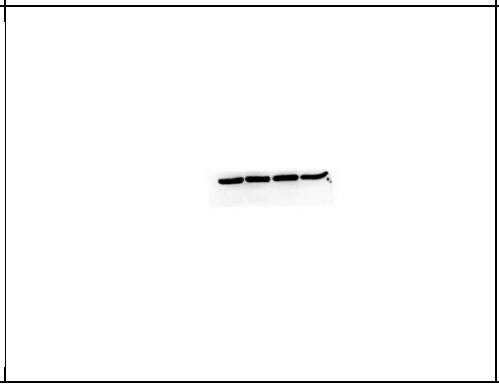
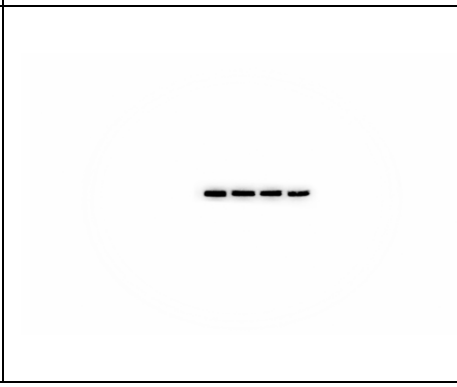
Beclin1			
Lc3			
P62			
β-actin			

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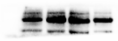
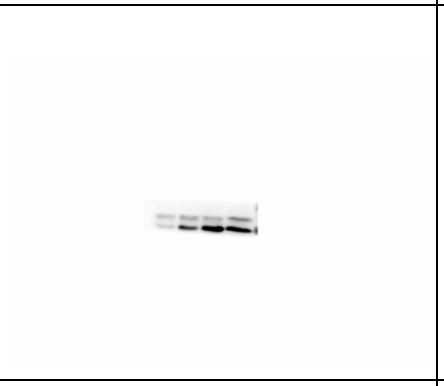
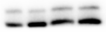
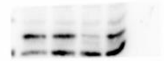
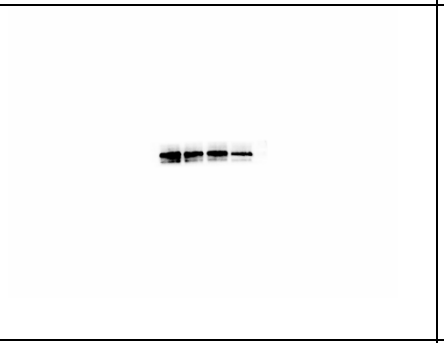
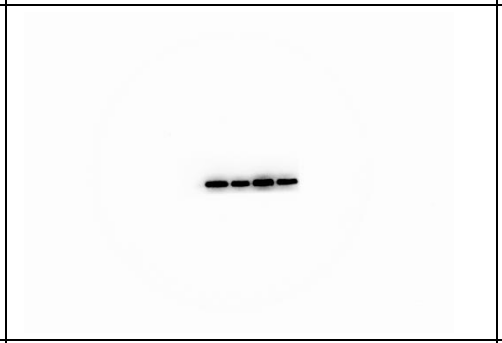
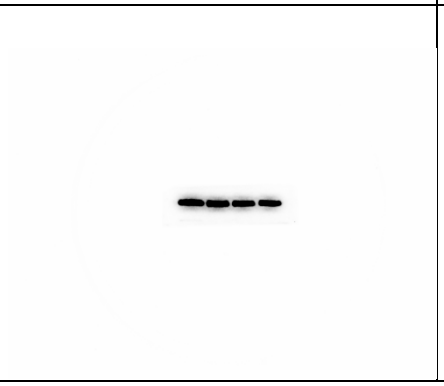
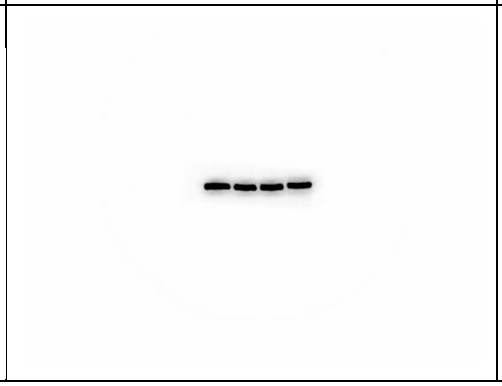
Beclin1			
Lc3			
P62			
β-actin			

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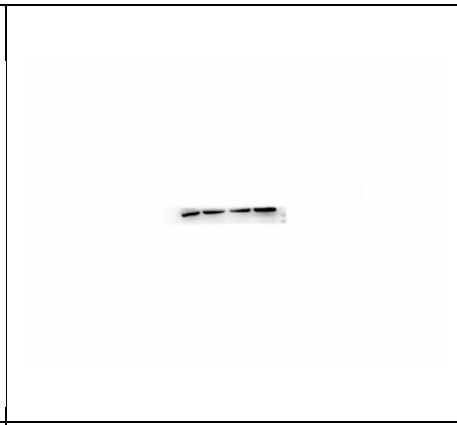
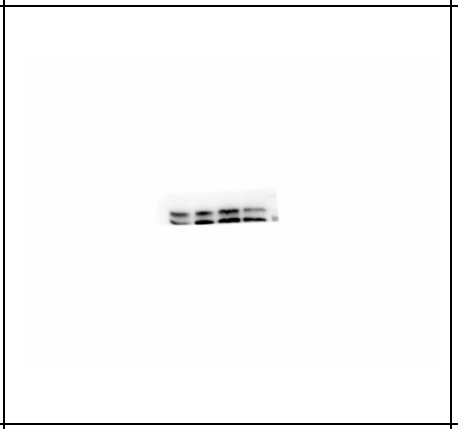
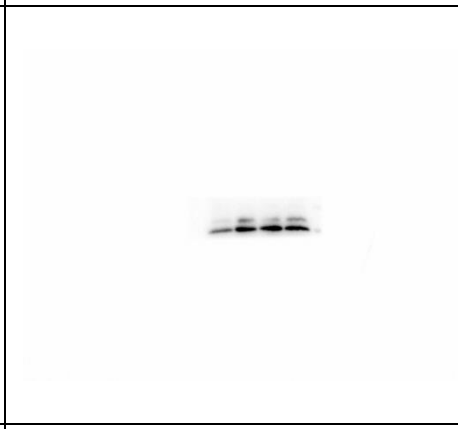
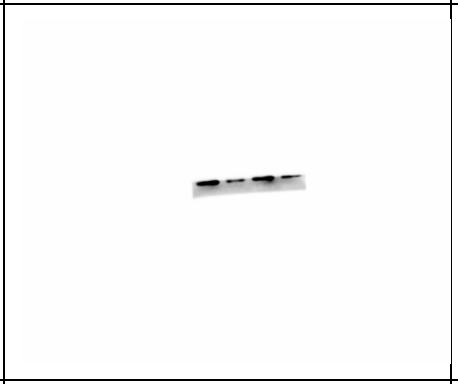
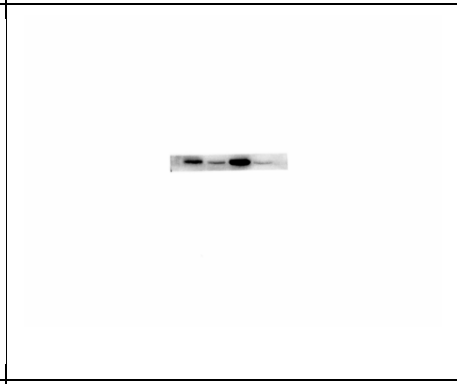
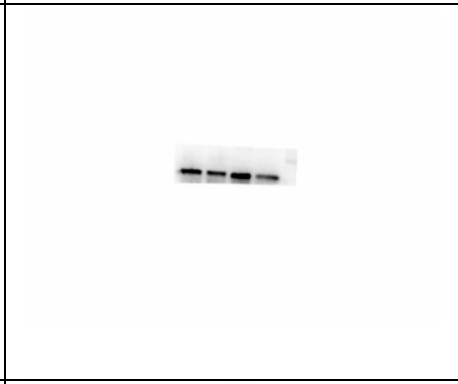
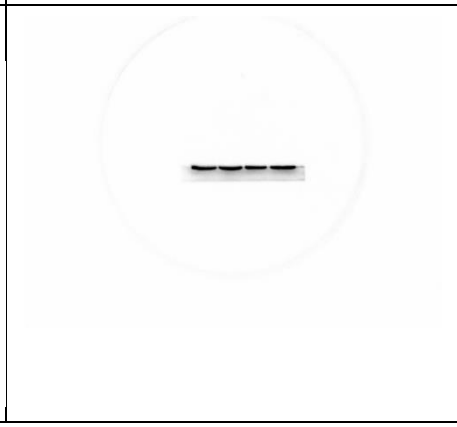
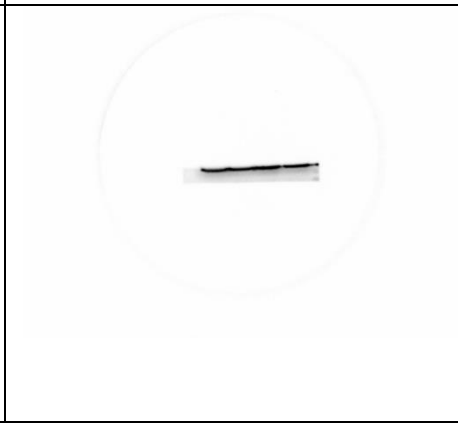
Beclin1			
LC3			
P62			
β-actin			

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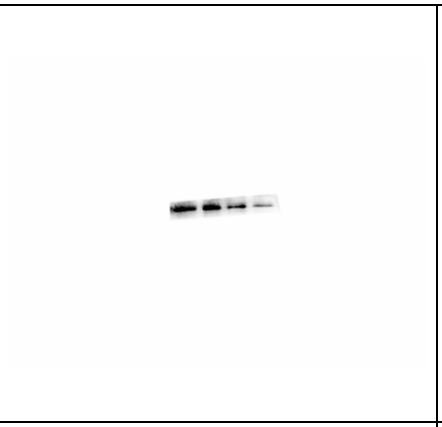
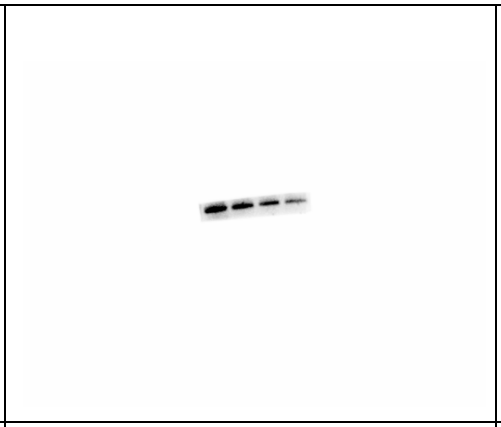
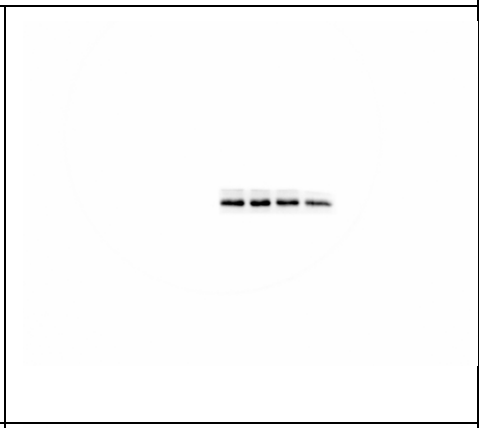
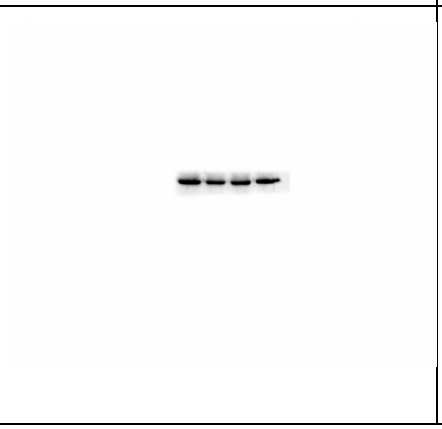
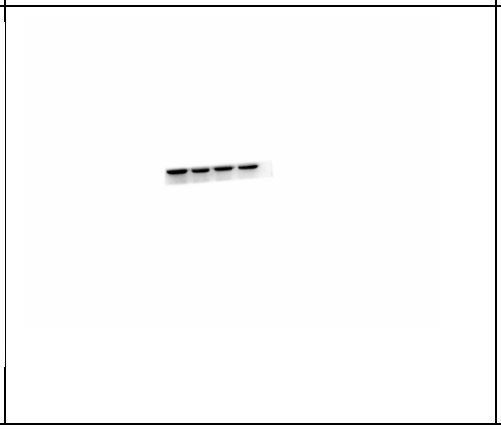
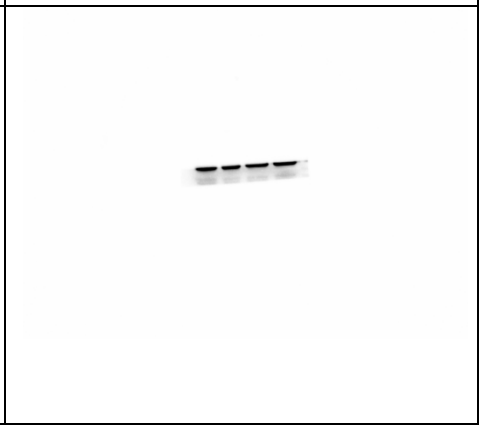
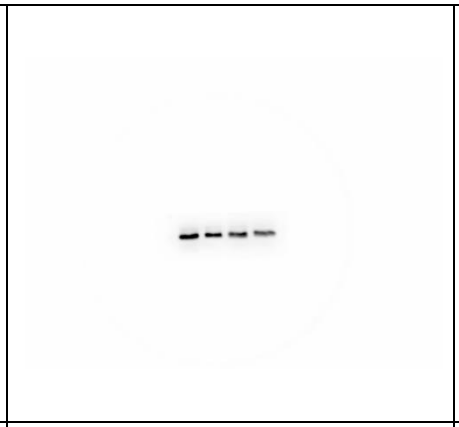
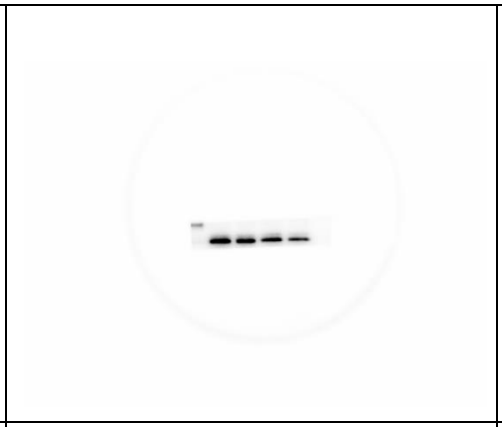
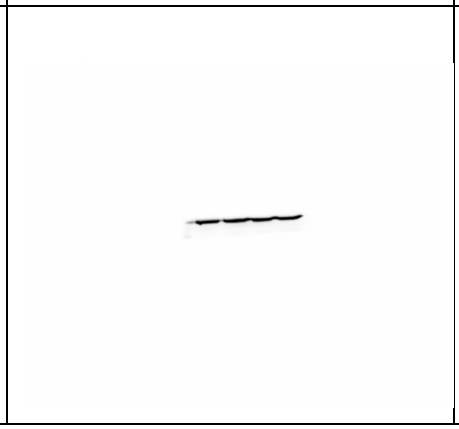
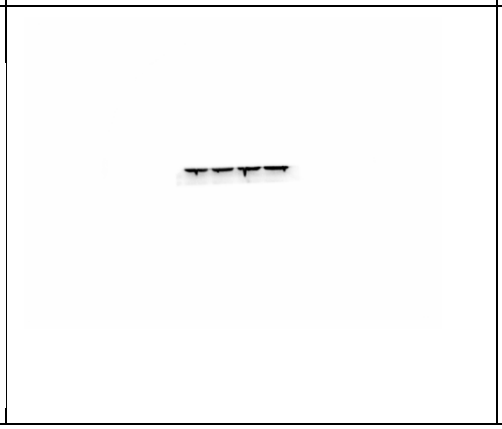
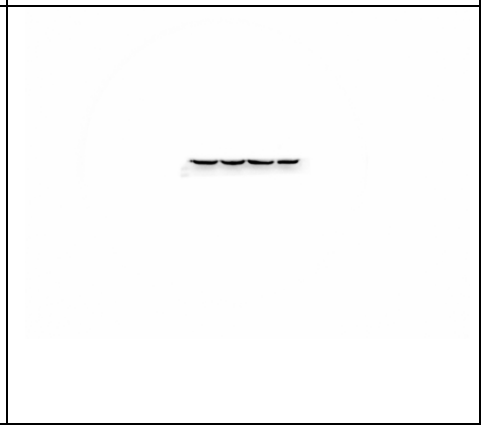
Bcl2			
β-actin			

Figure 4F (H1299)			
Bcl2			
β-actin			

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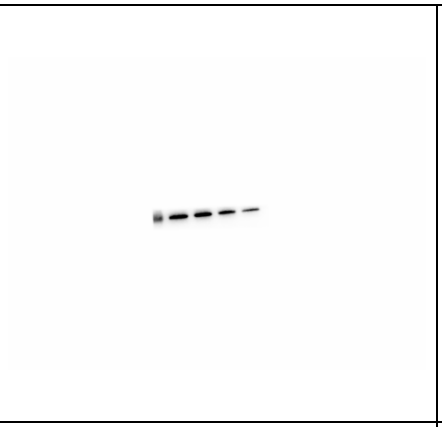
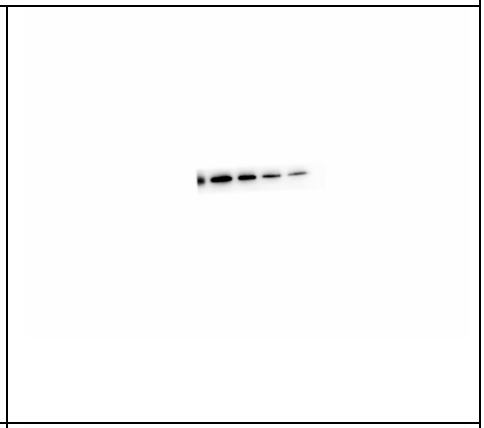
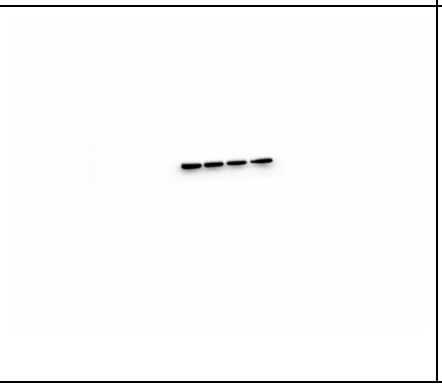
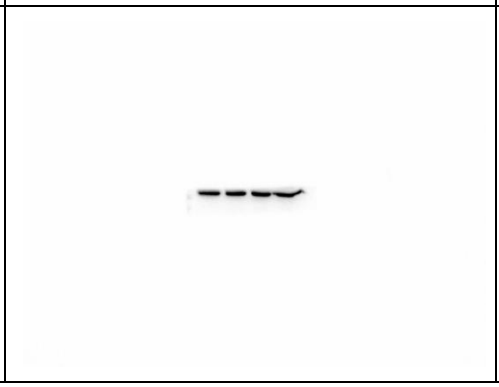
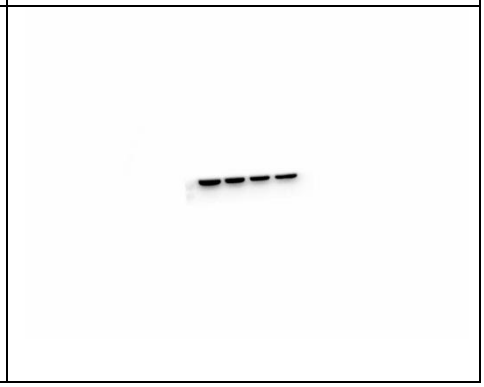
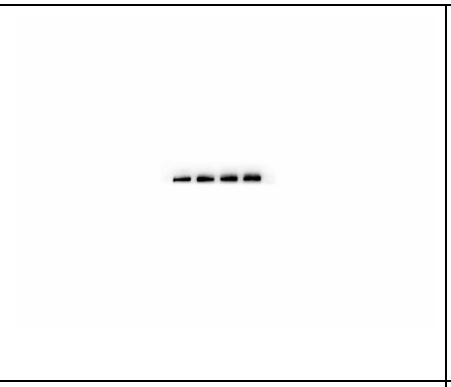
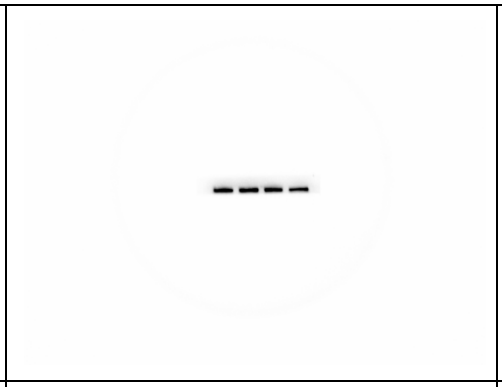
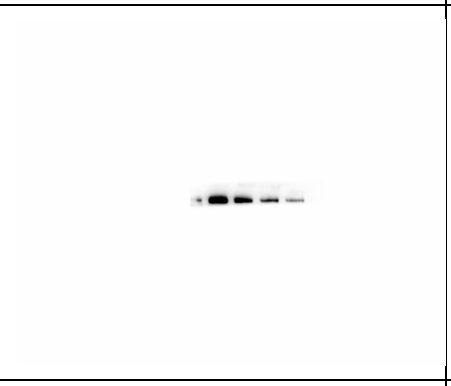
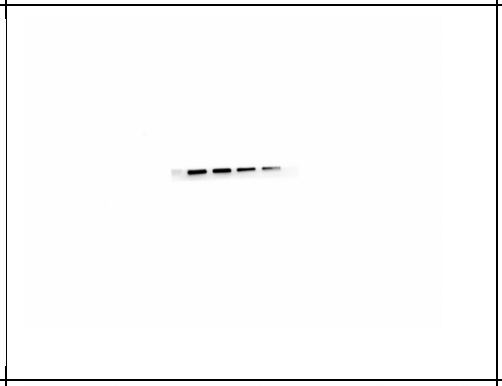
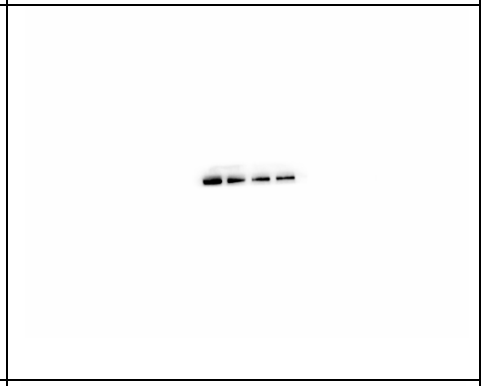
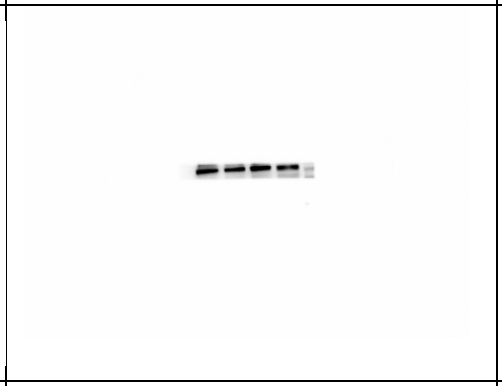
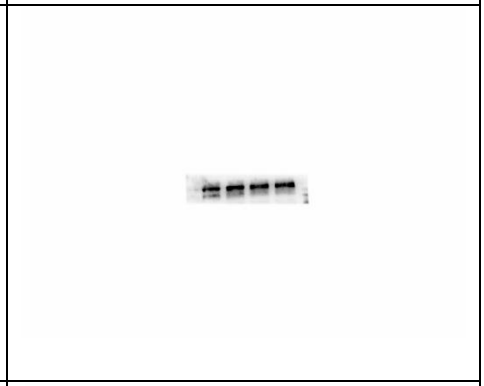
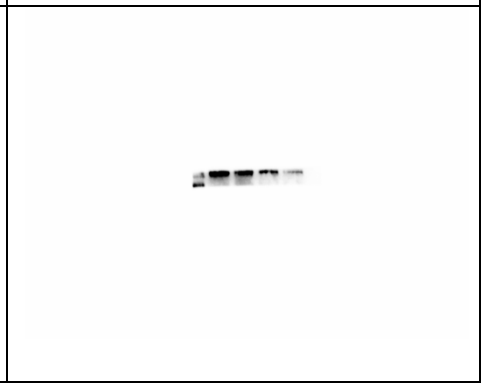
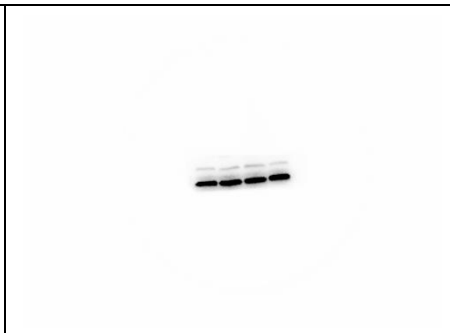
Bcl2			
β-actin			

Figure 6A (A549)			
mTOR			
p-mTOR			
PI3K			
p-PI3K			

AKT			
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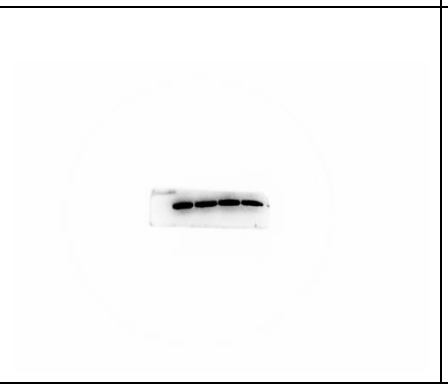
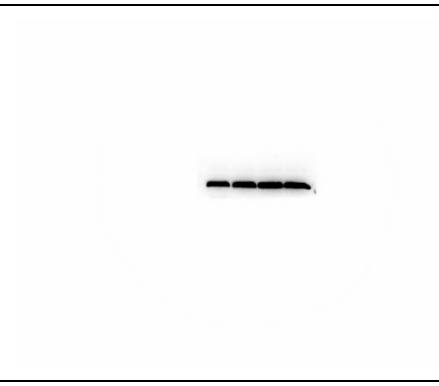
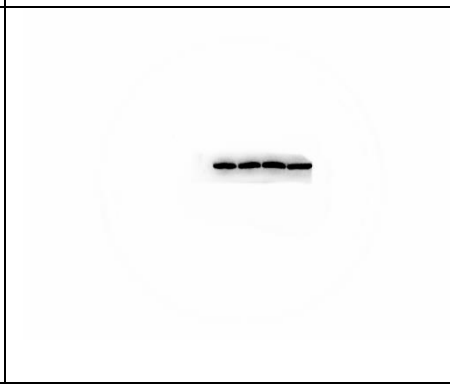
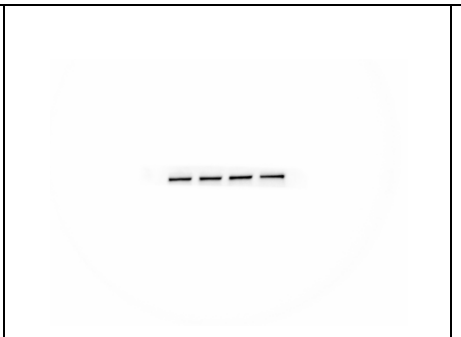
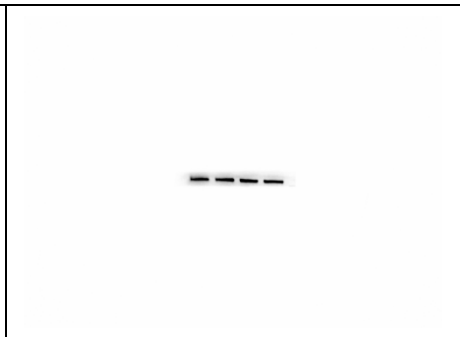
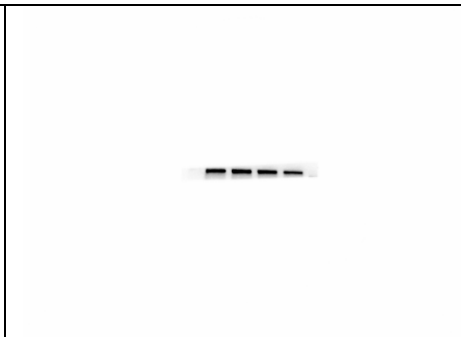
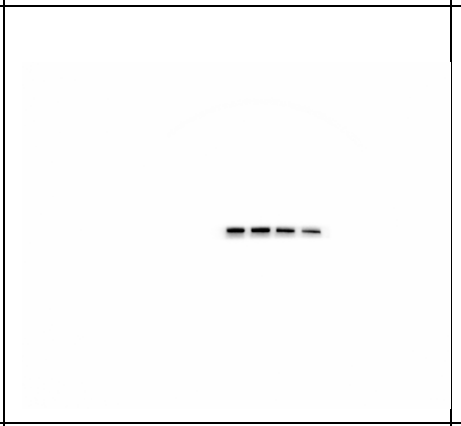
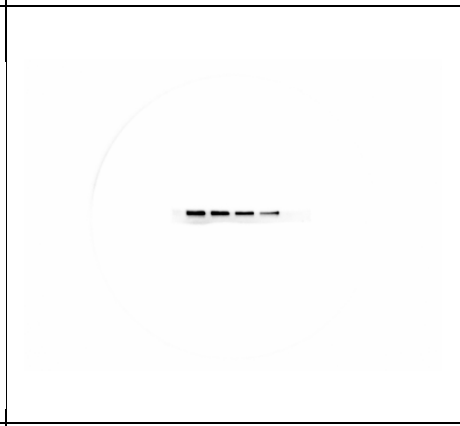
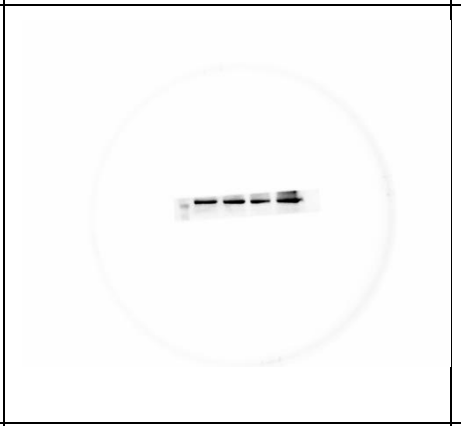
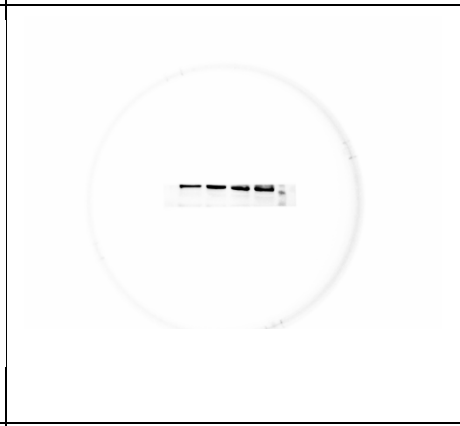
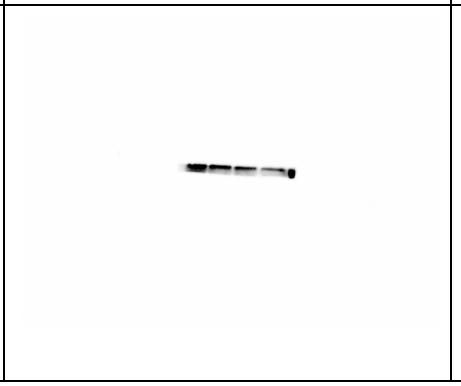
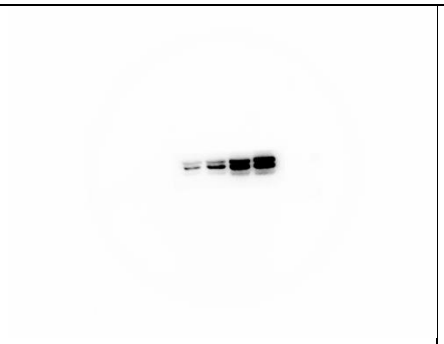
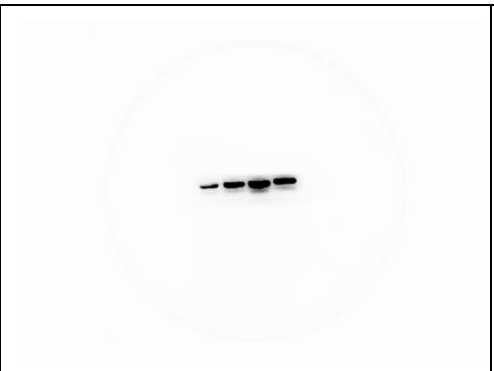
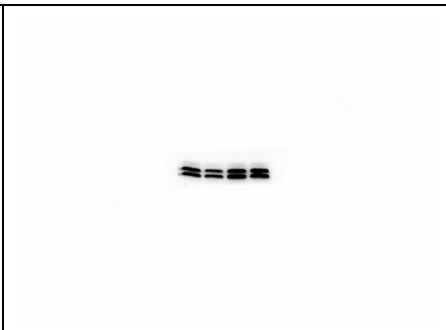
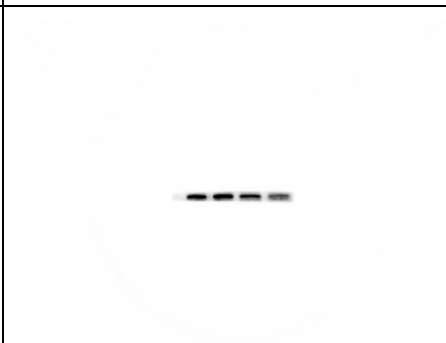
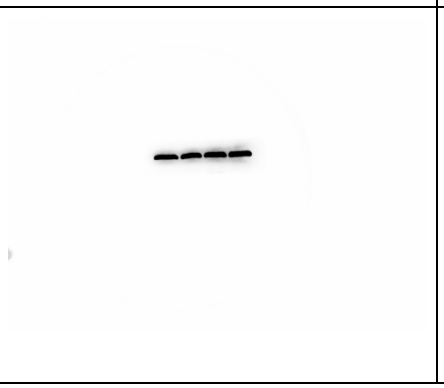
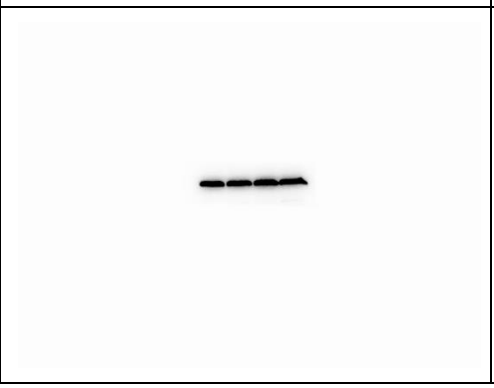
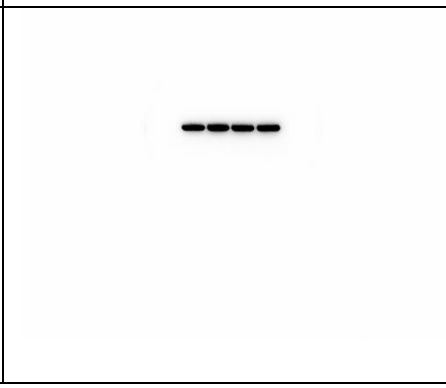
β-actin			

Figure 6A (H1299)			
mTOR			
p-mTOR			
PI3K			
p-PI3K			

AKT			
p-AKT			
β-actin			

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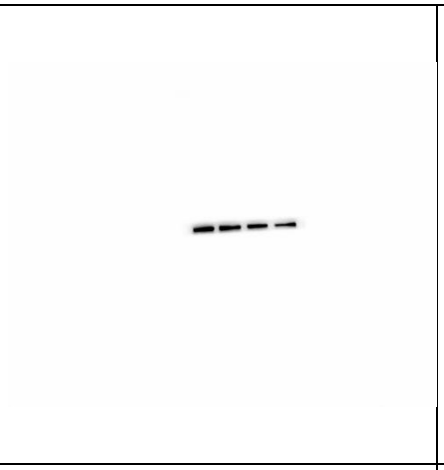
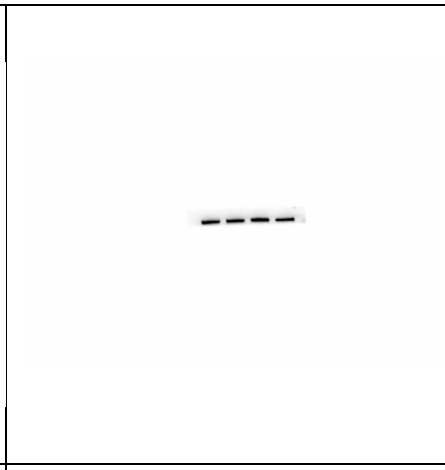
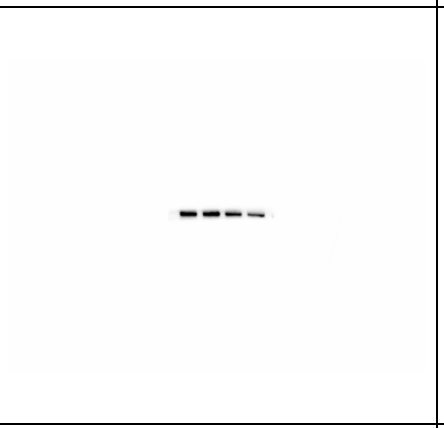
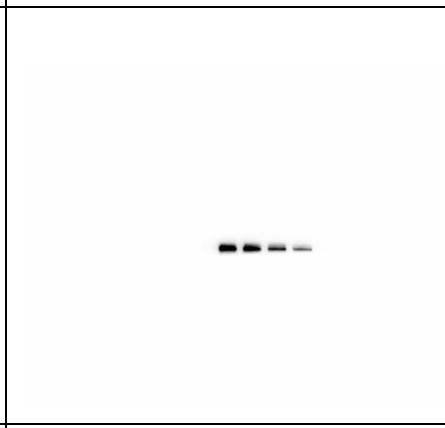
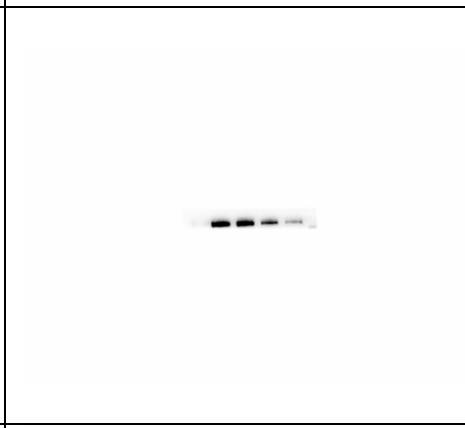
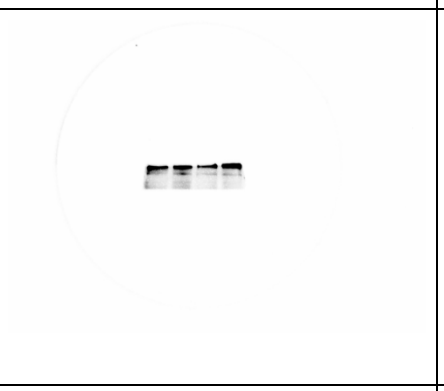
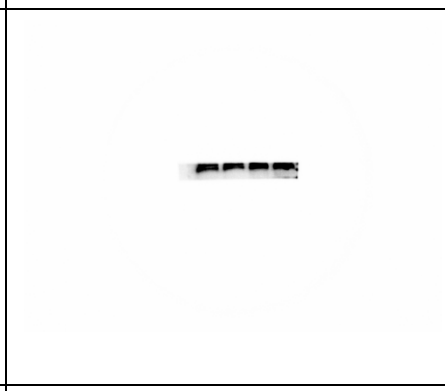
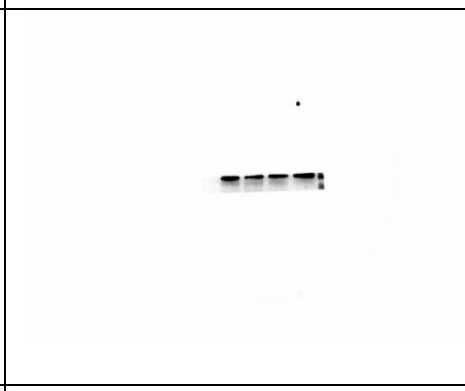
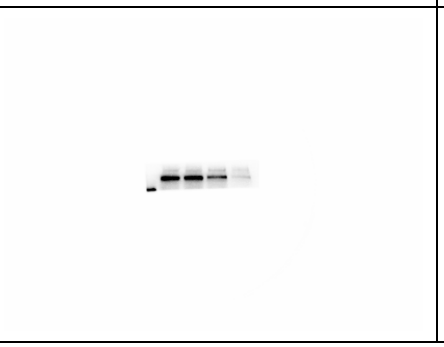
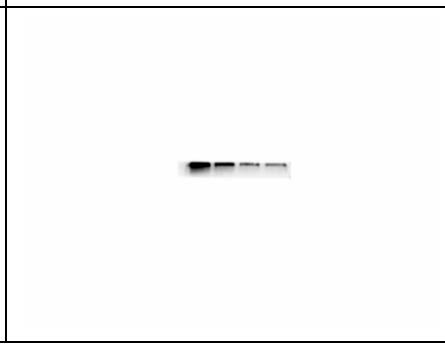
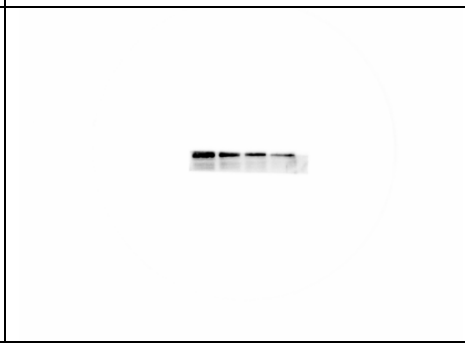
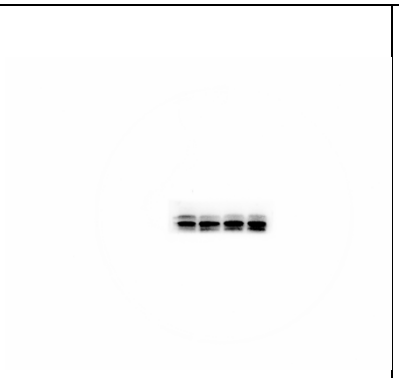
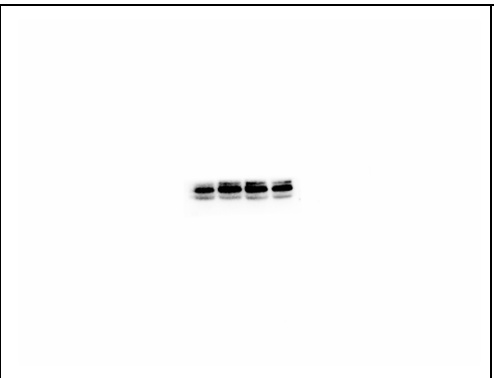
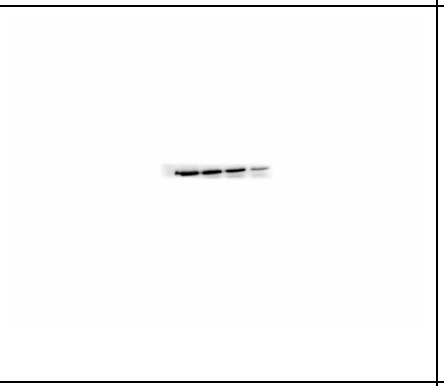
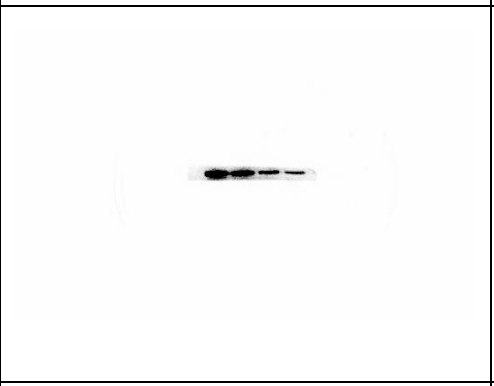
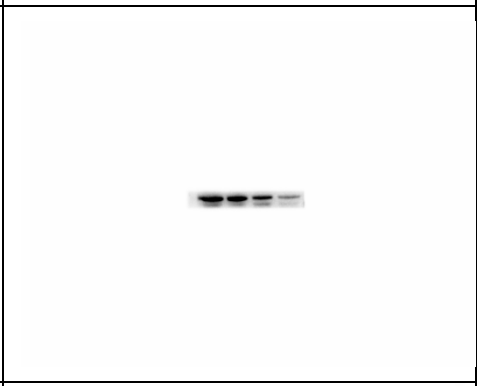
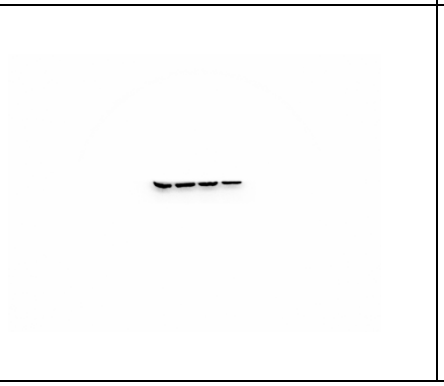
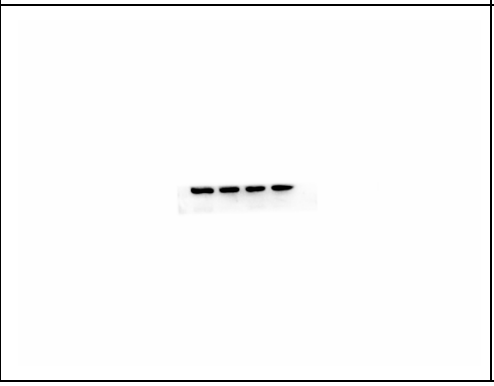
mTOR			
p-mTOR			
PI3K			
p-PI3K			

AKT			
p-AKT			
β-actin			

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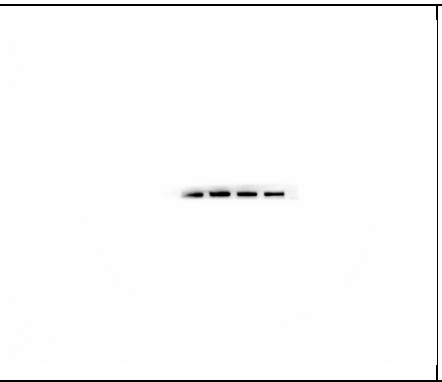
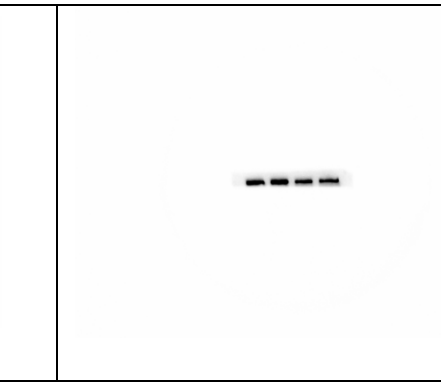
mTOR			
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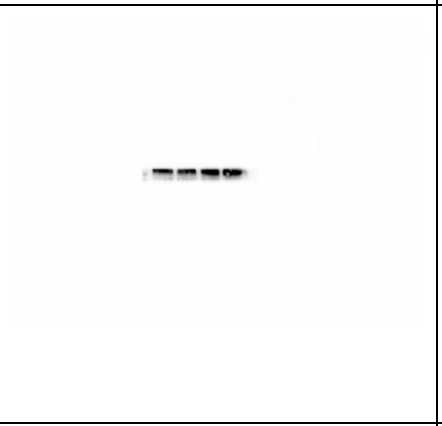
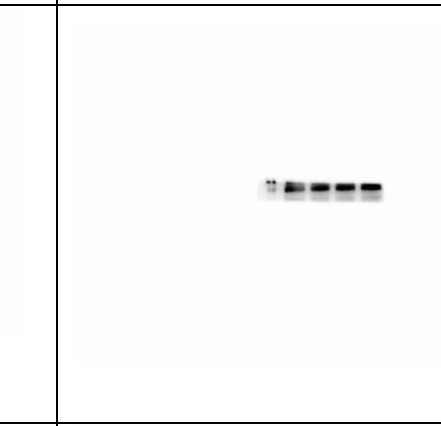
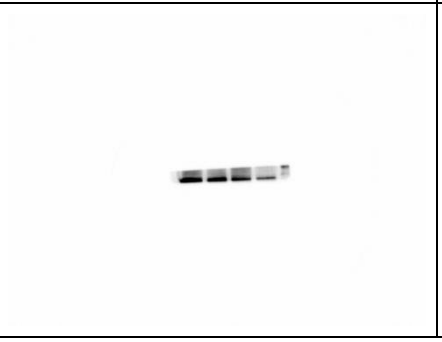
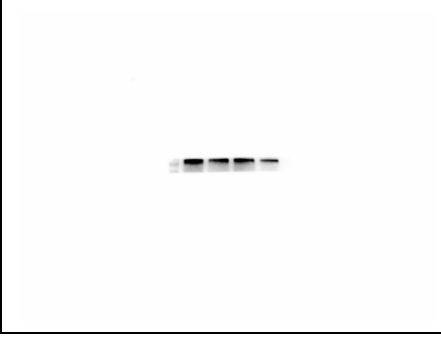
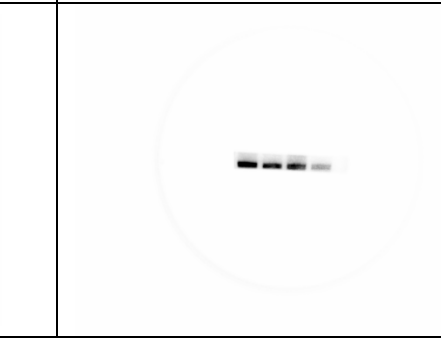
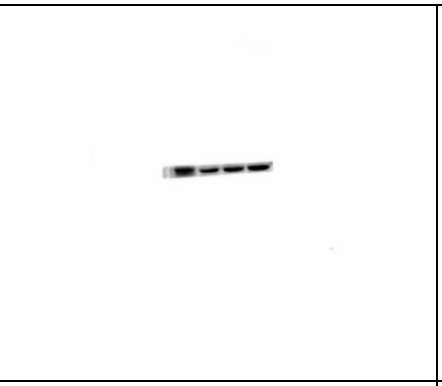
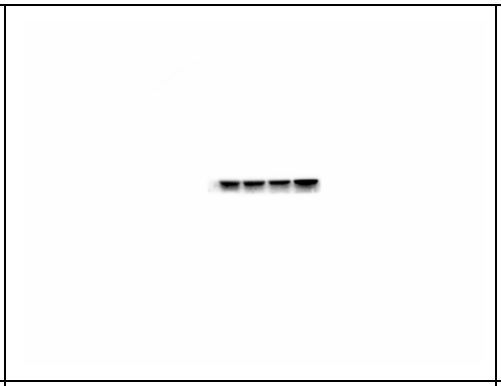
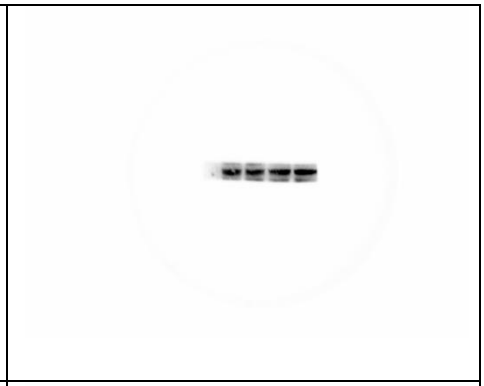
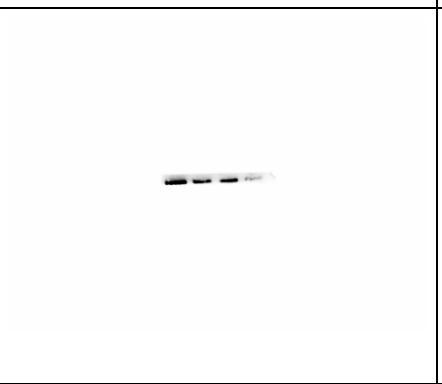
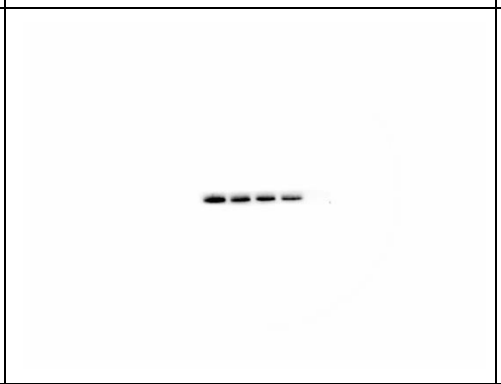
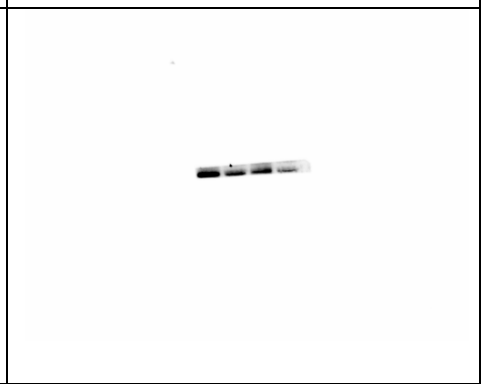
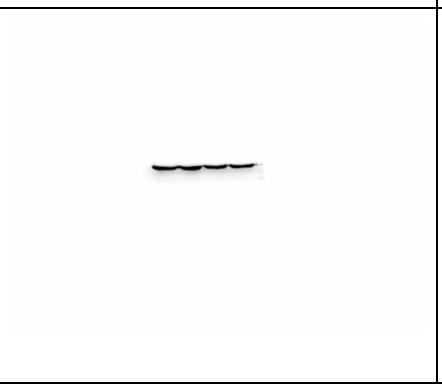
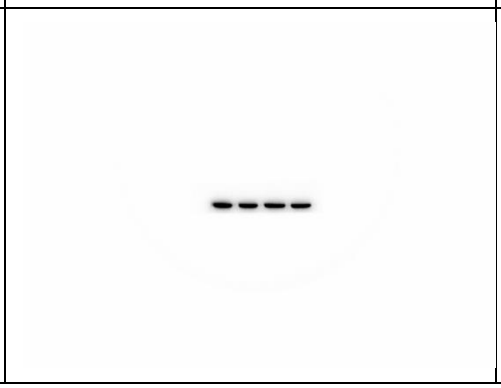
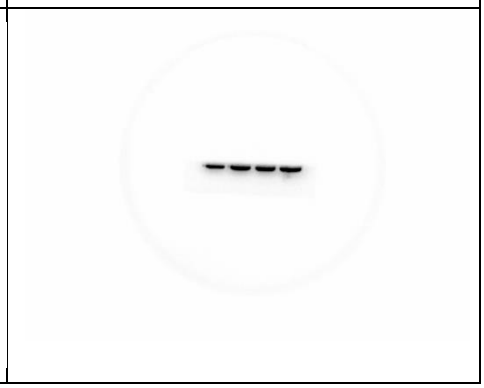
PI3K			
p-PI3K			

AKT			
p-AKT			
β-actin			

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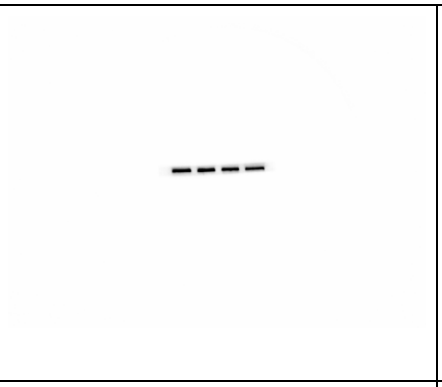
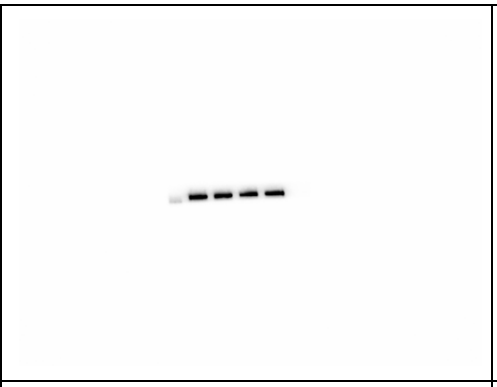
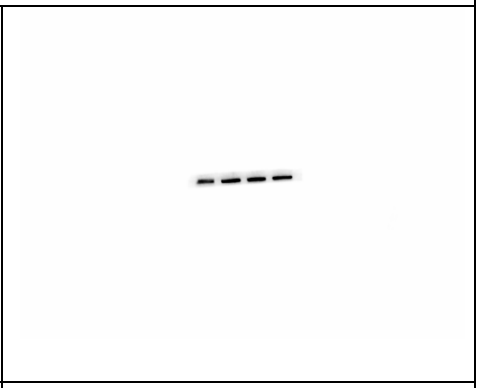
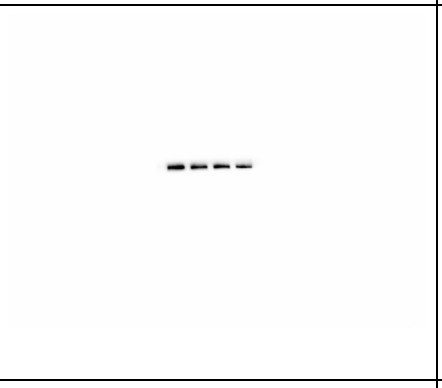
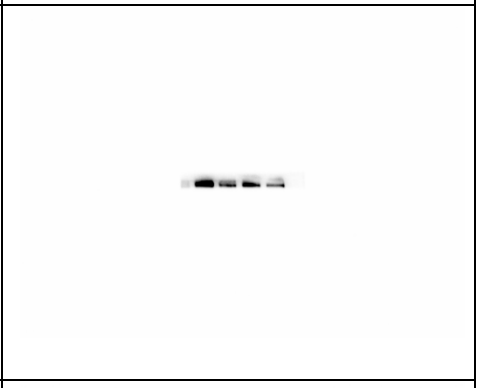
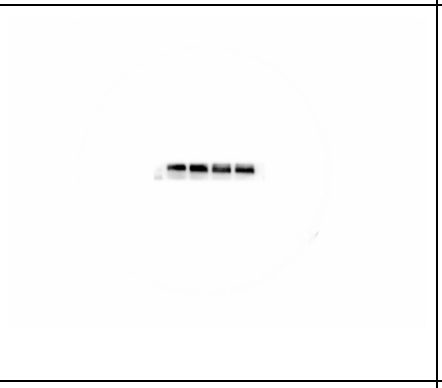
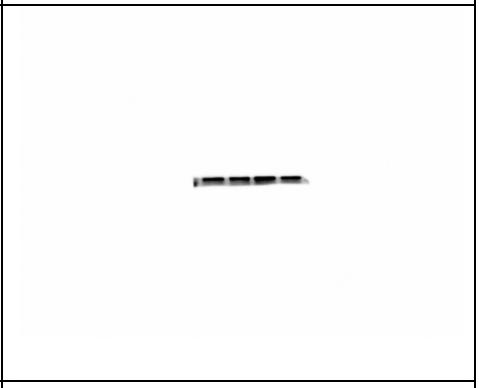
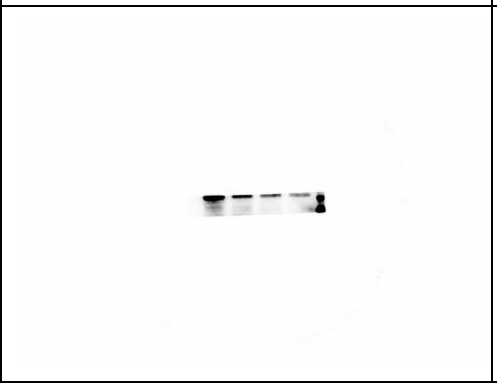
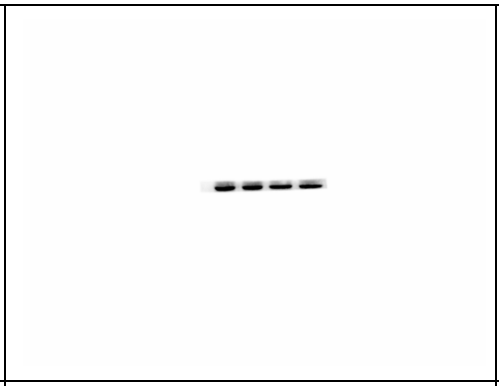
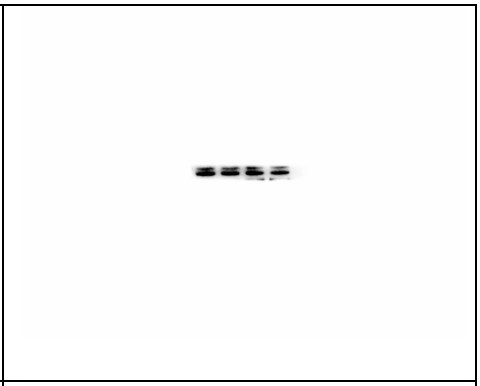
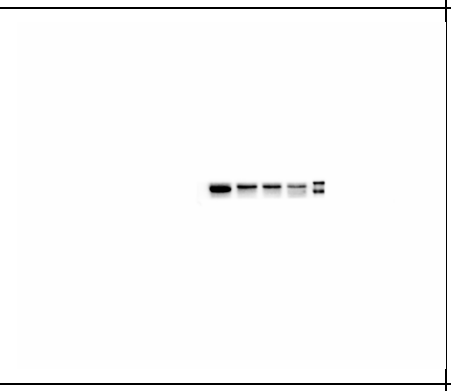
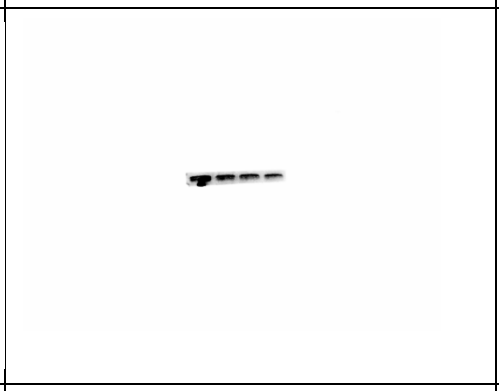
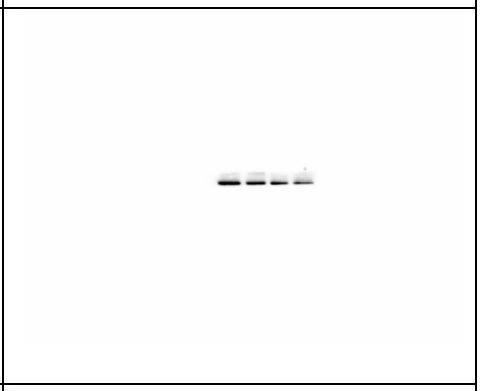
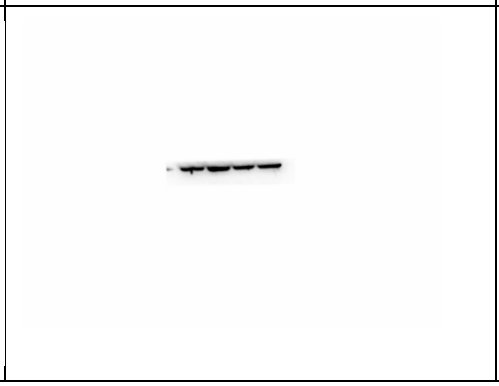
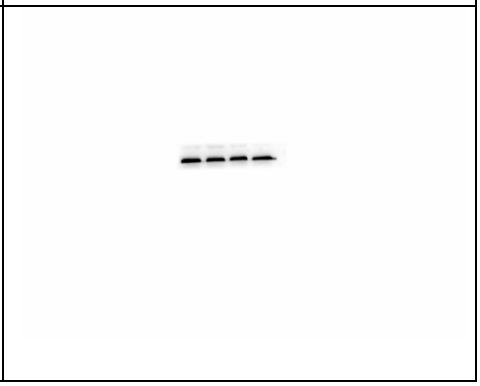
mTOR			
p-mTOR			
PI3K			
p-PI3K			

AKT			
p-AKT			
β-actin			

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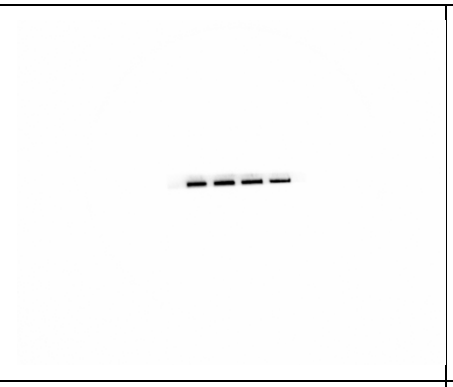
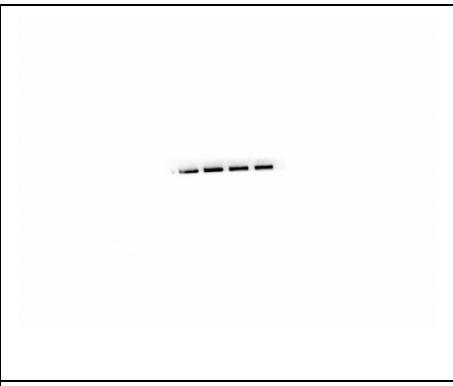
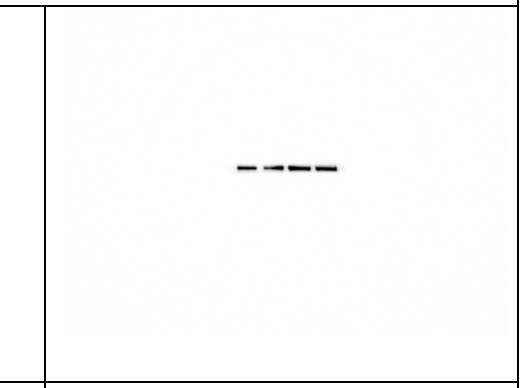
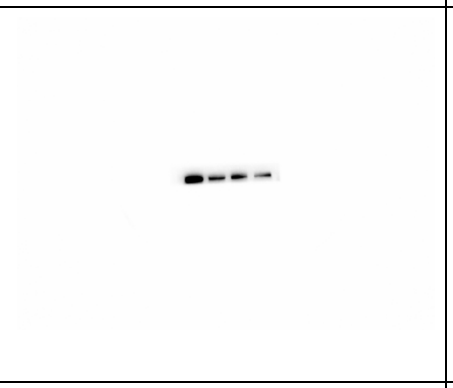
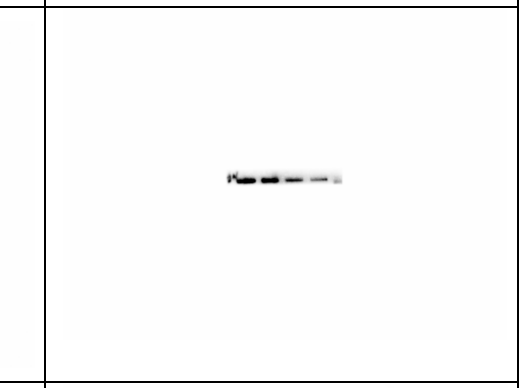
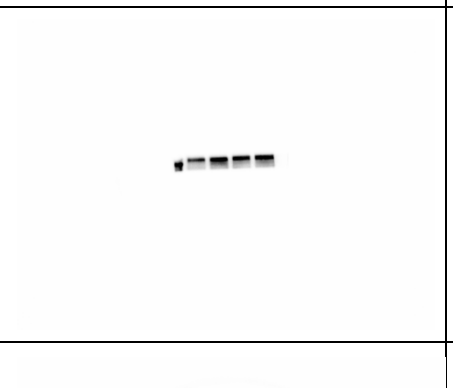
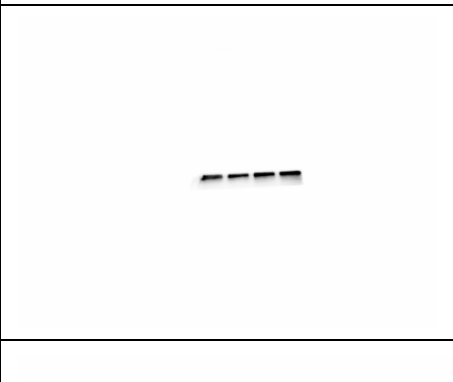
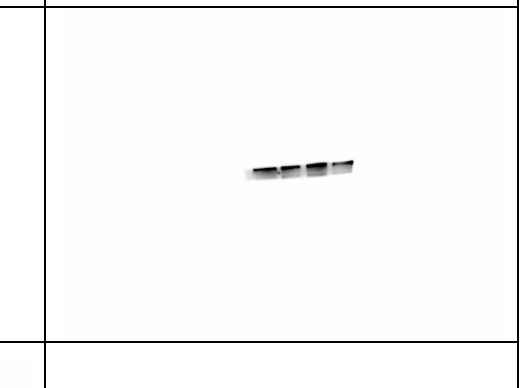
mTOR			
p-mTOR			
PI3K			
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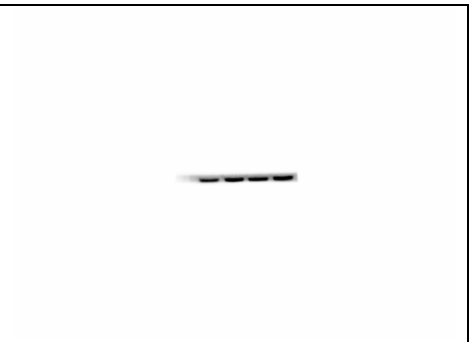
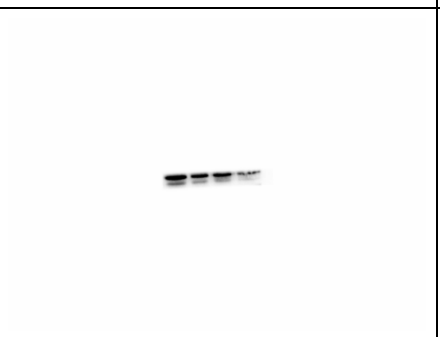
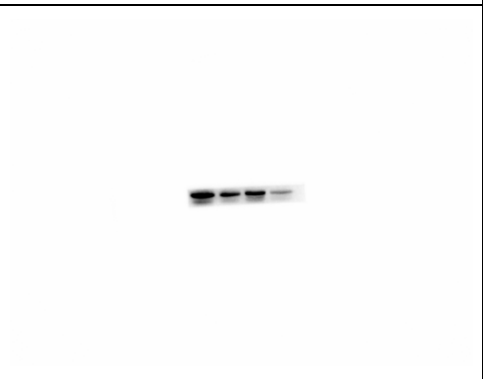
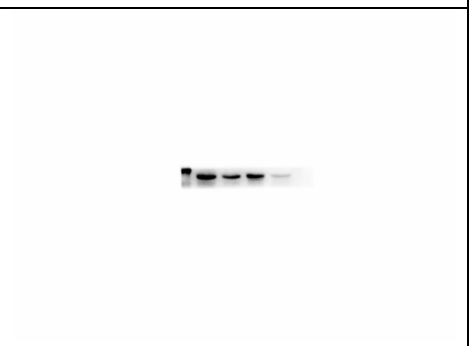
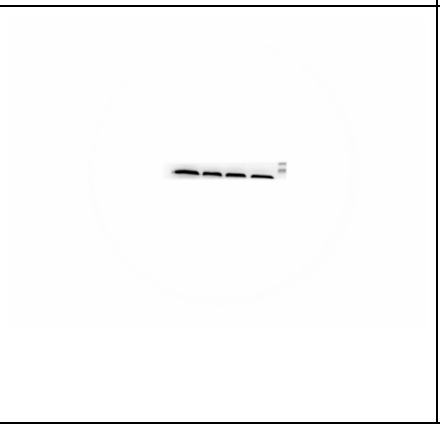
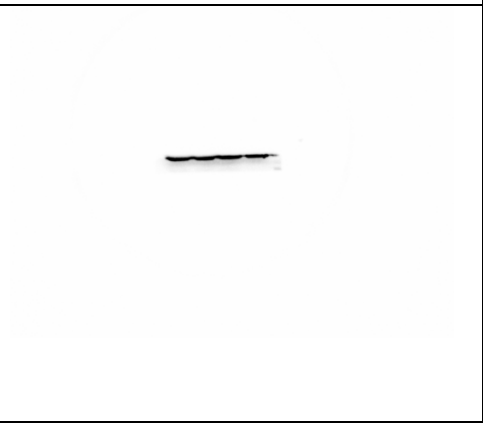
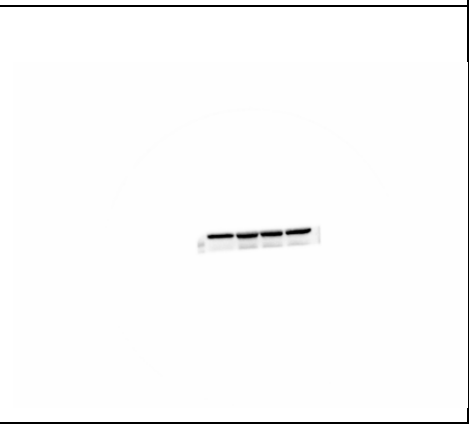
AKT			
p-AKT			
β-actin			

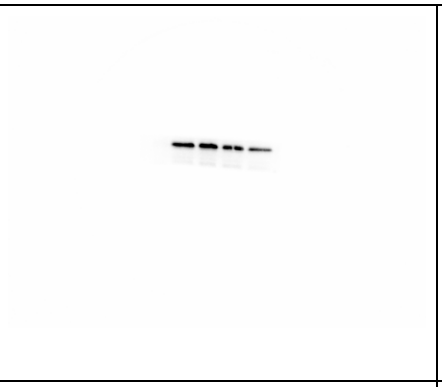
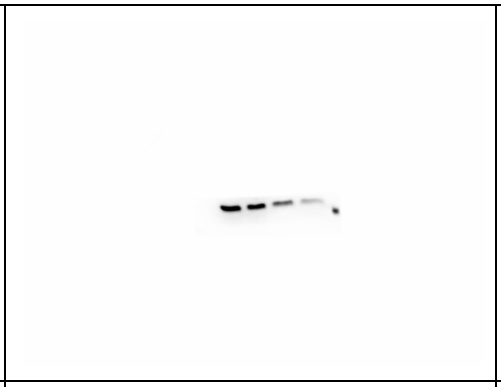
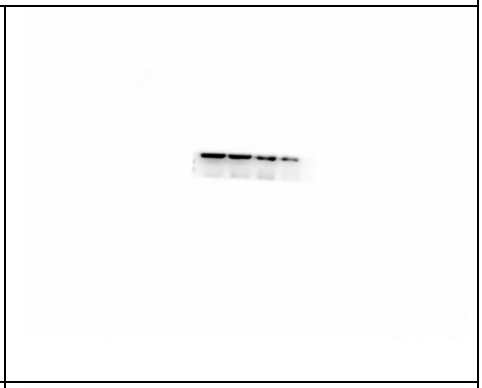
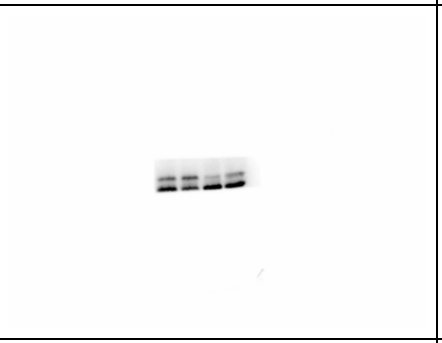
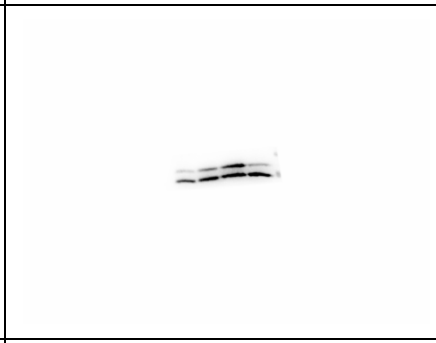
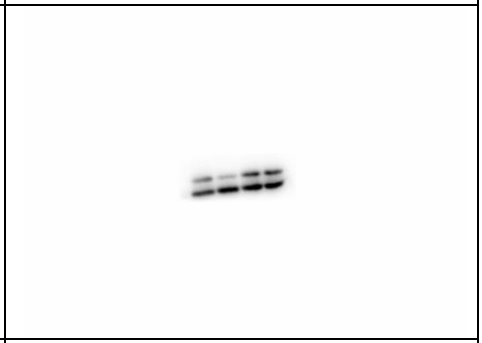
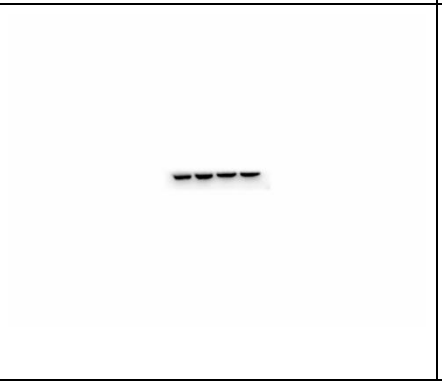
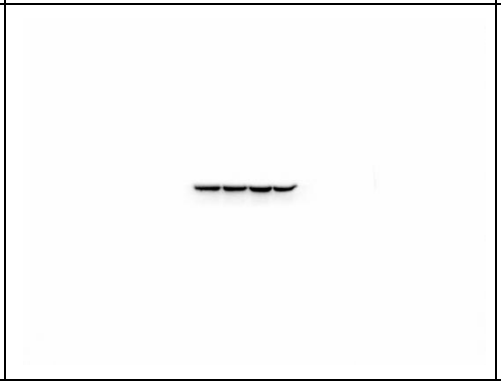
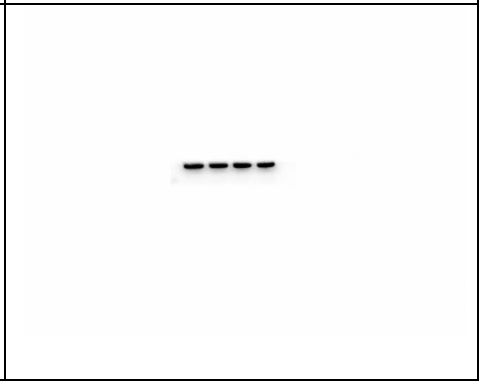
Figure 6E (A549)			
Bcl2			
LC3			
β-actin			

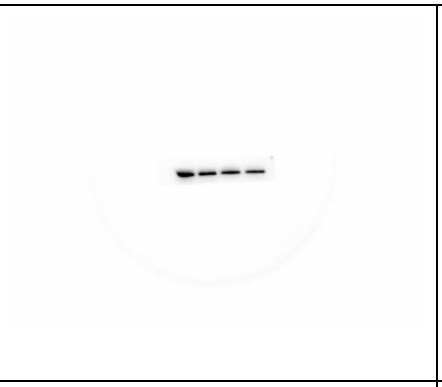
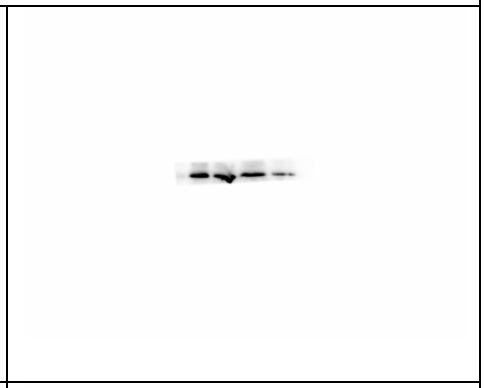
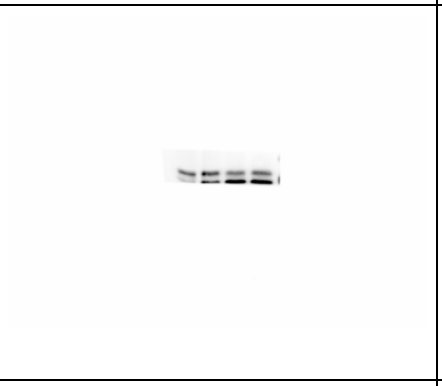
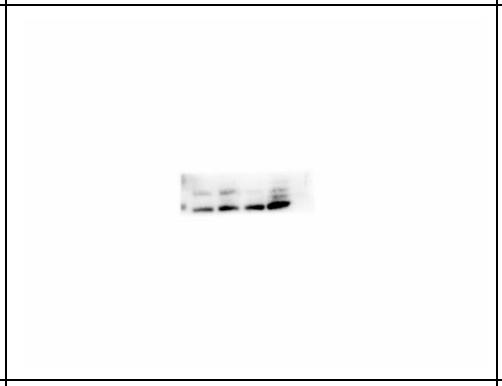
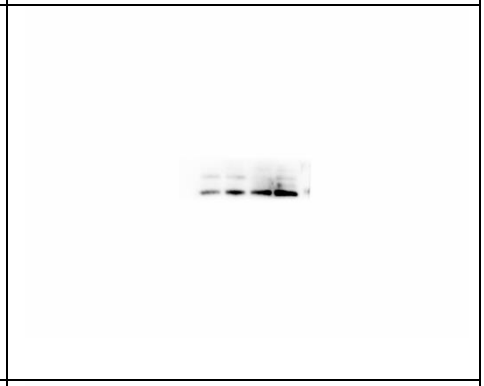
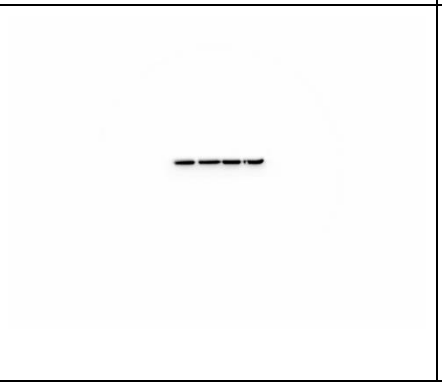
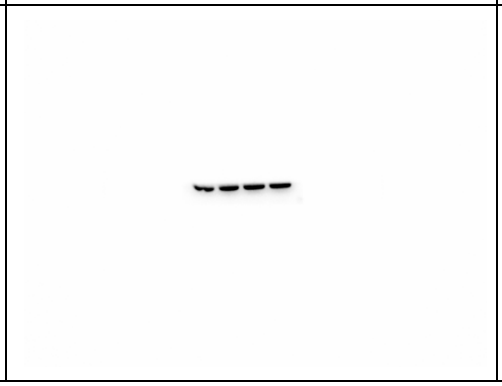
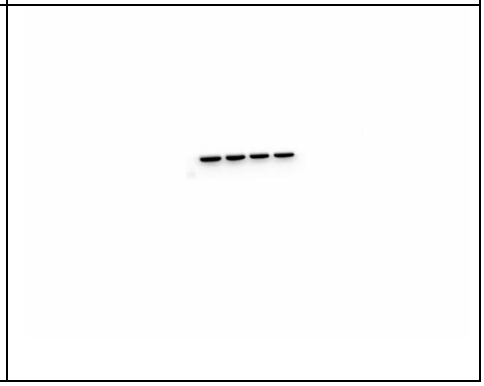
Figure 6E (H1299)			
Bcl2			
LC3			
β-actin			

Figure 6E (H226)			
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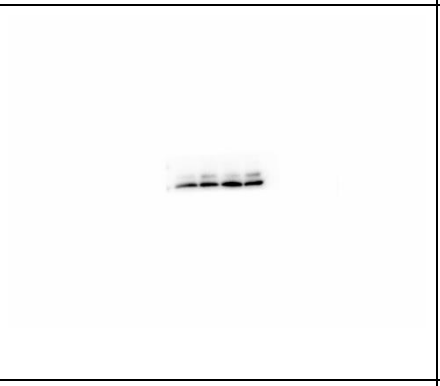
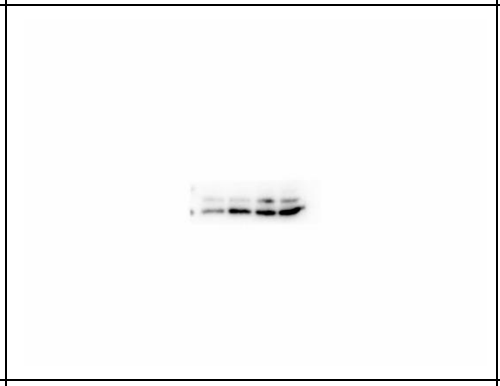
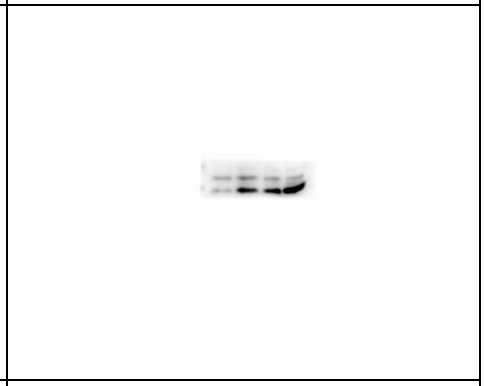
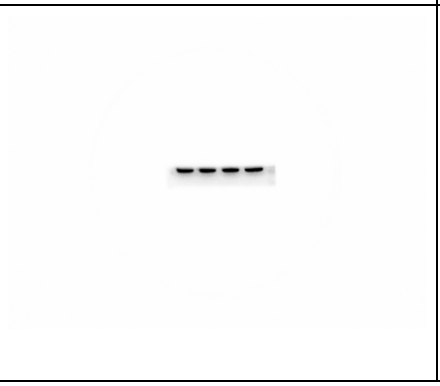
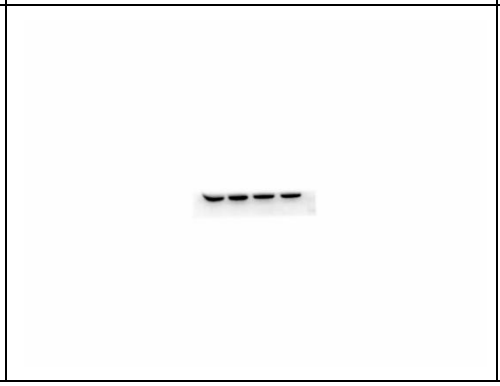
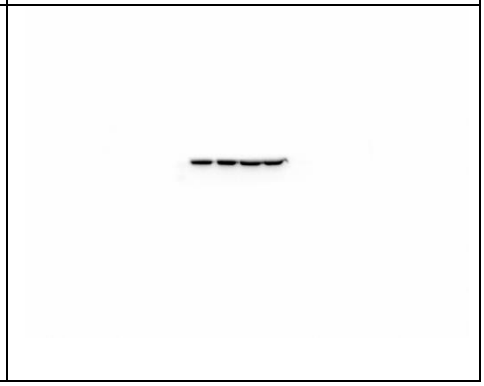
LC3			
β-actin			

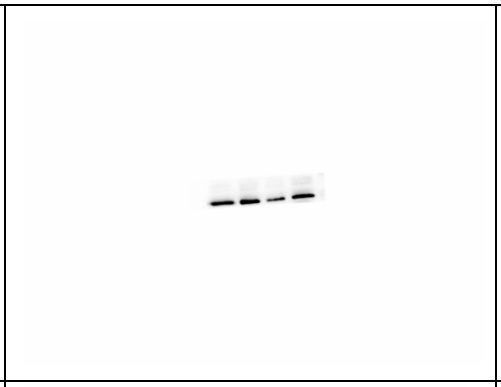
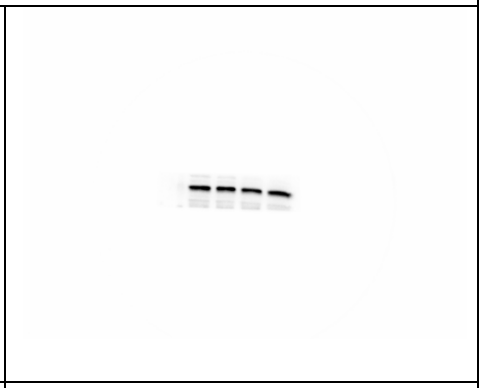
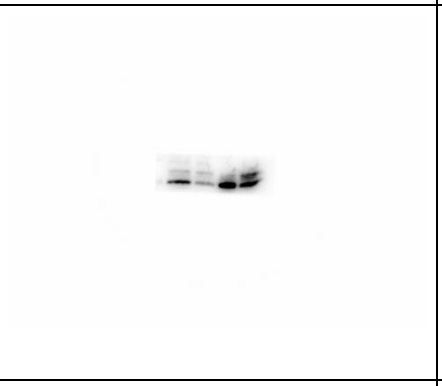
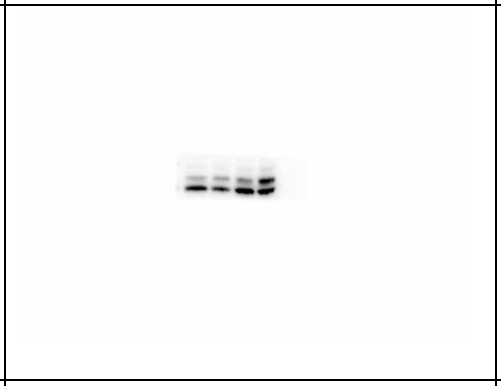
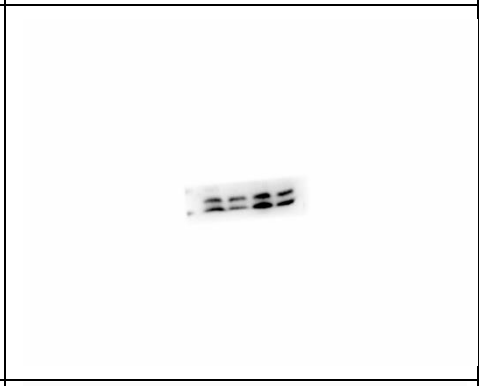
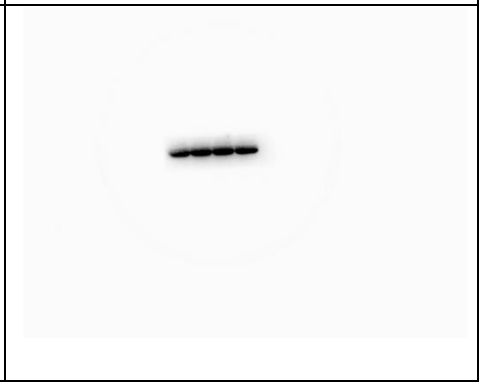
Figure 7D (A549)			
Bcl2			
LC3			
β-actin			

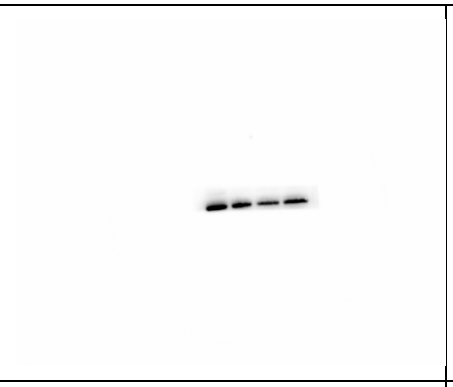
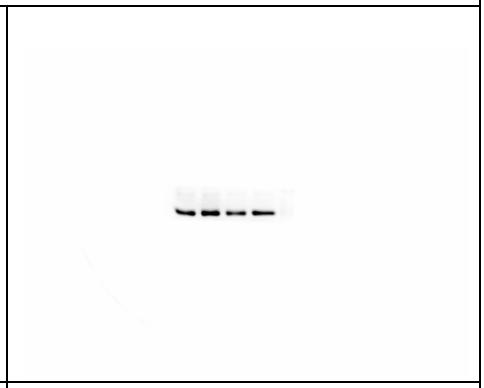
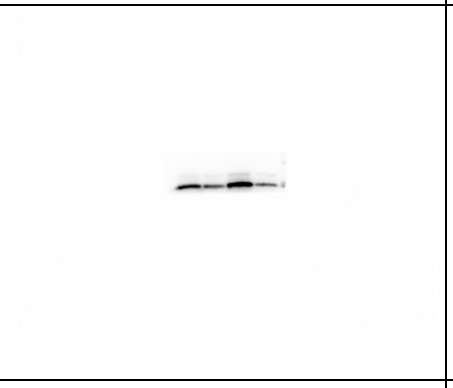
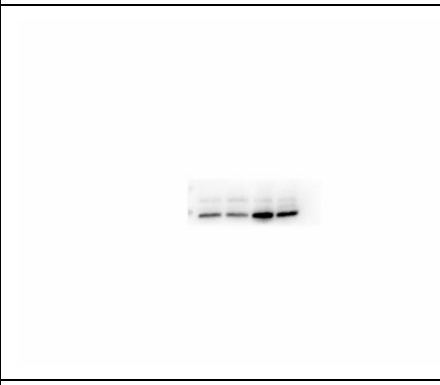
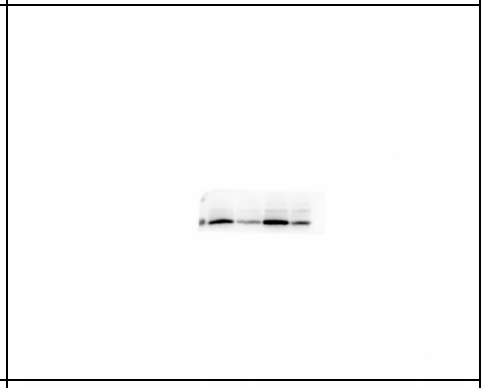
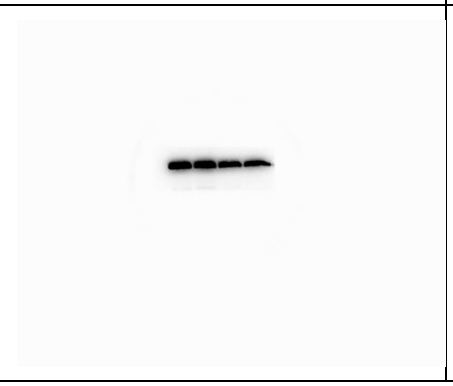
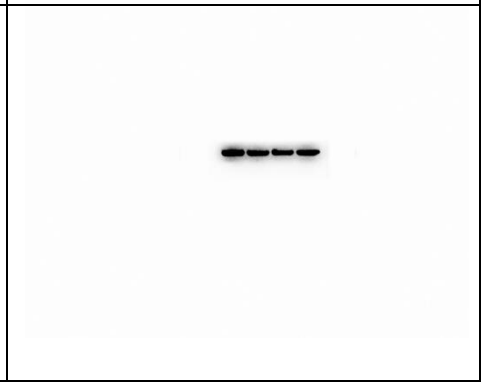
Figure 7D (H1299)			
Bcl2			
LC3			
β-actin			

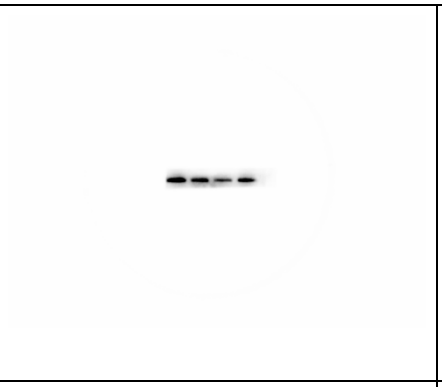
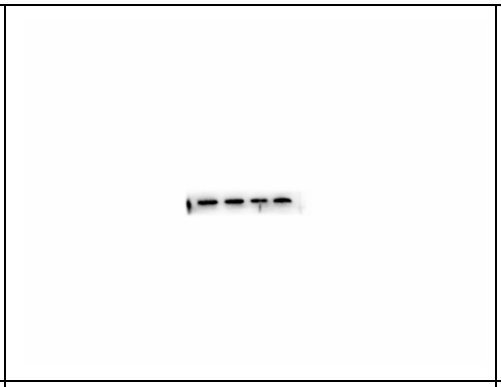
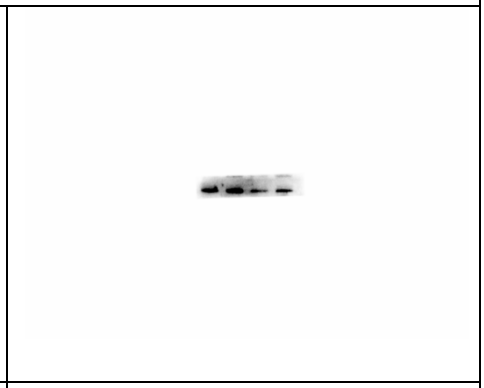
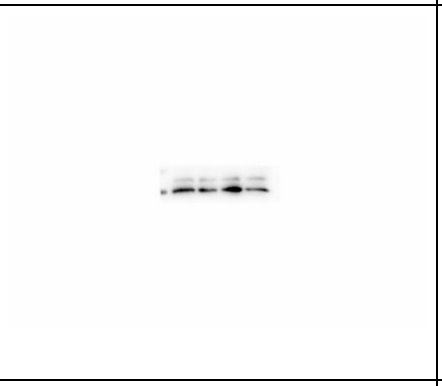
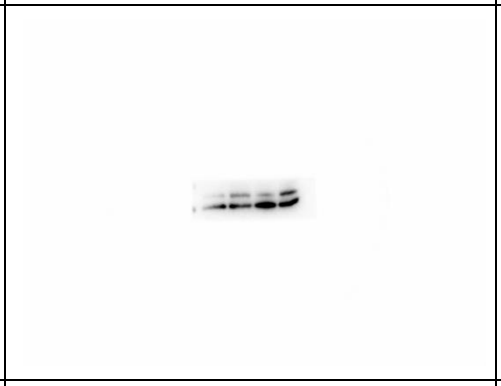
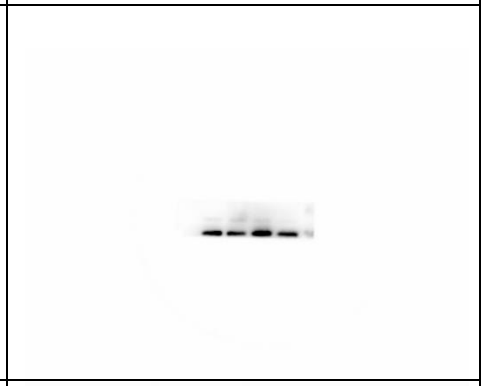
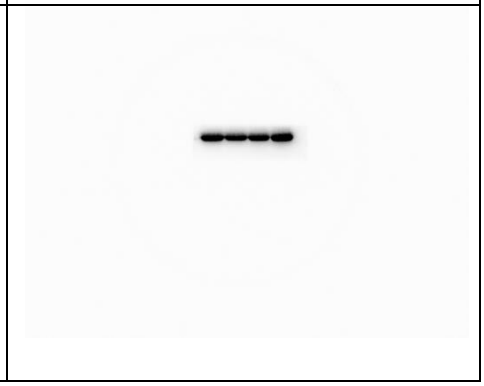
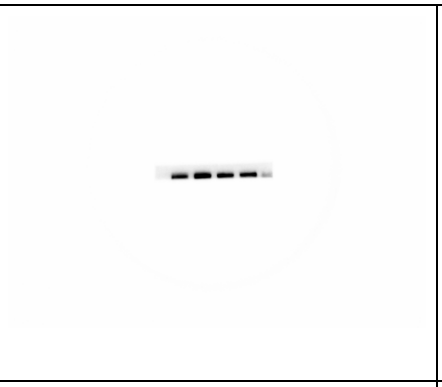
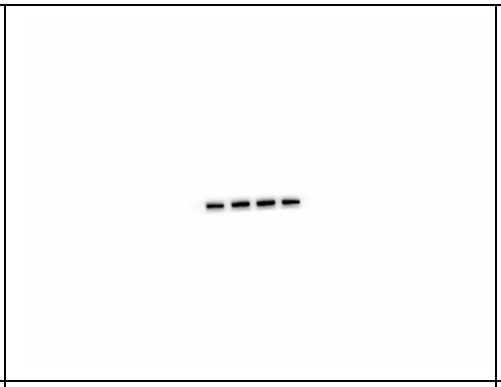
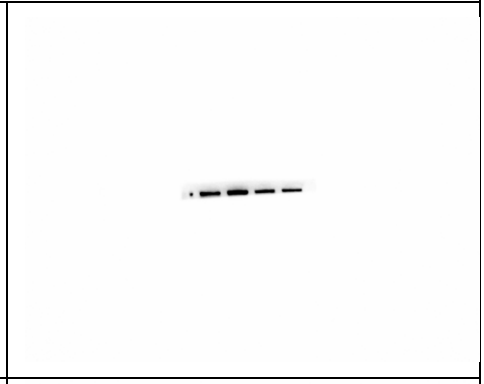
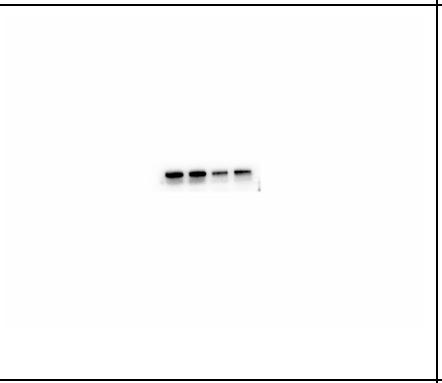
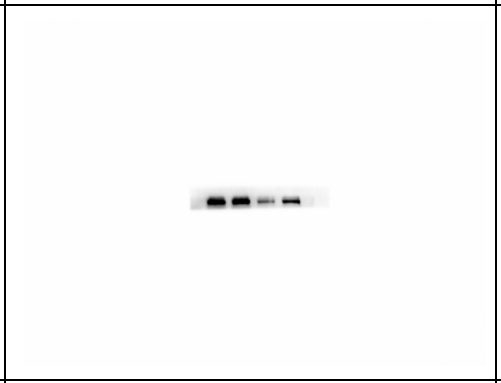
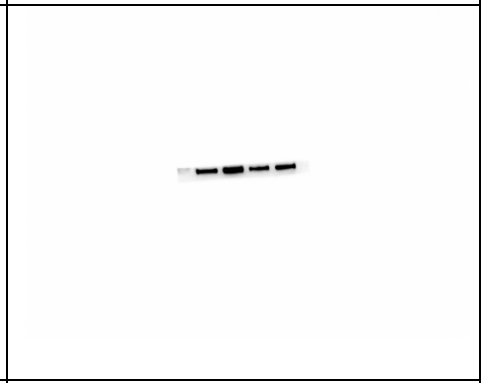
Figure 7D (H226)			
Bcl2			
LC3			
β-actin			

Figure 7F (A549)			
mTOR			
p-mTOR			
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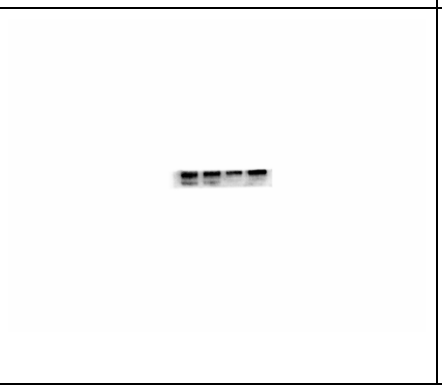
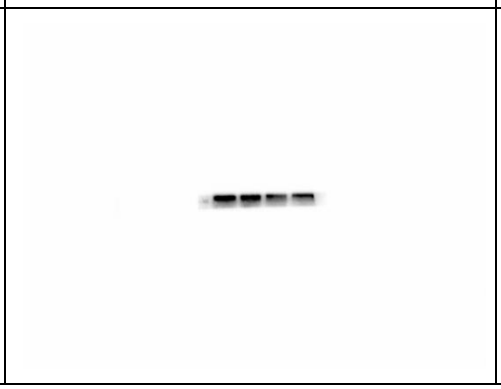
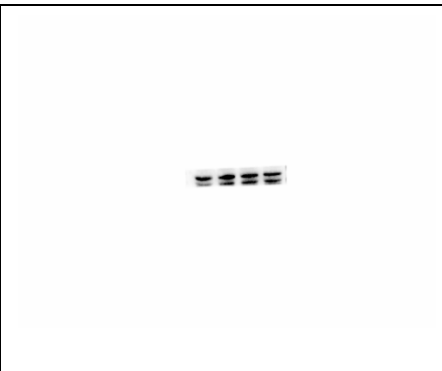
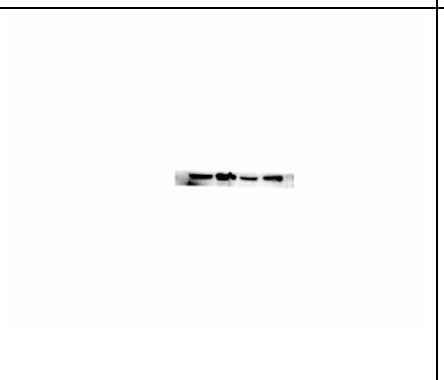
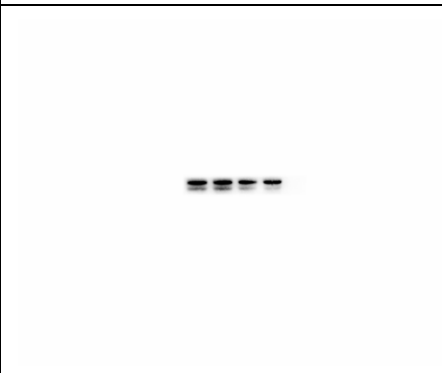
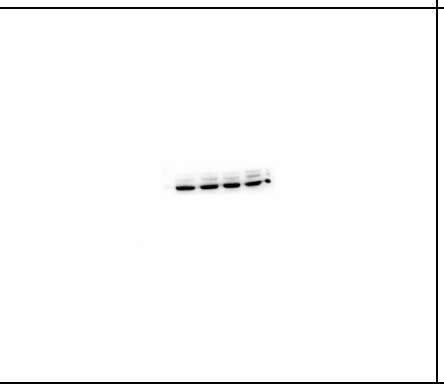
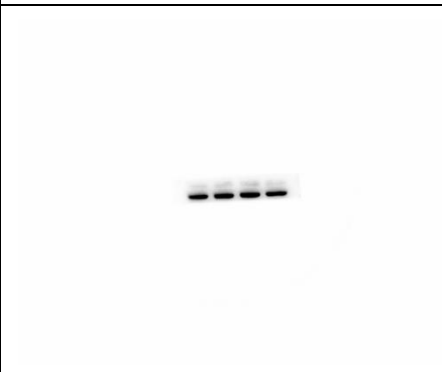
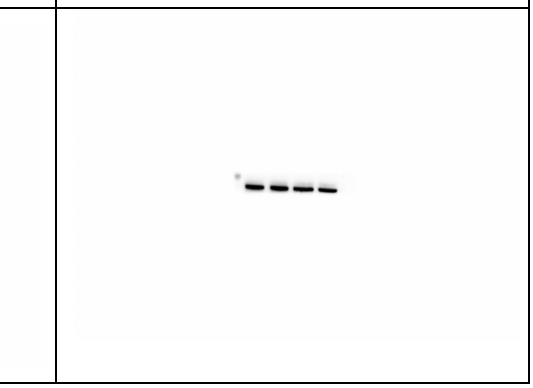
p-PI3K			

AKT			
p-AKT			
β-actin			

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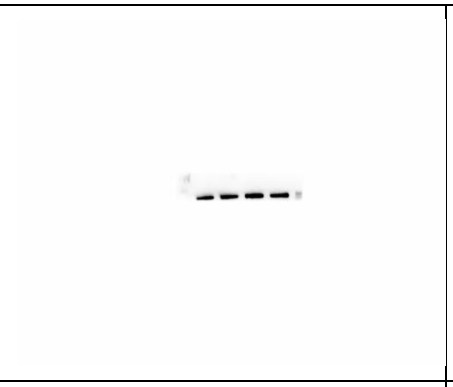
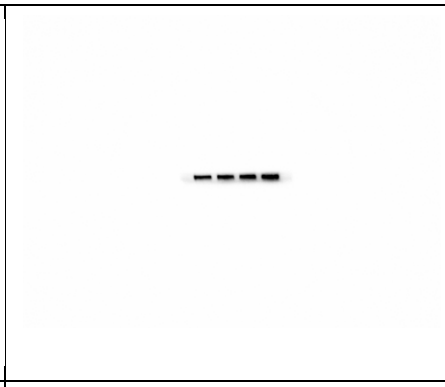
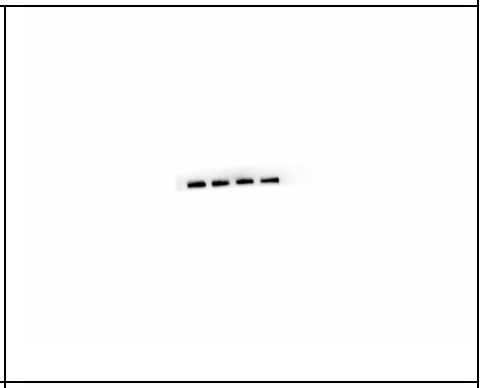
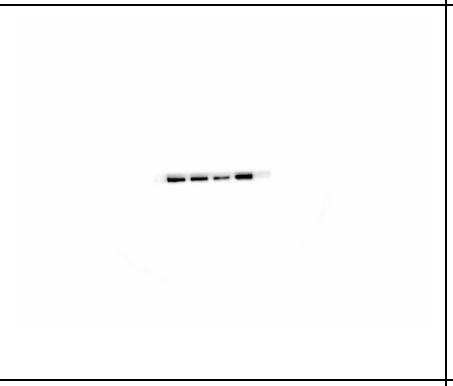
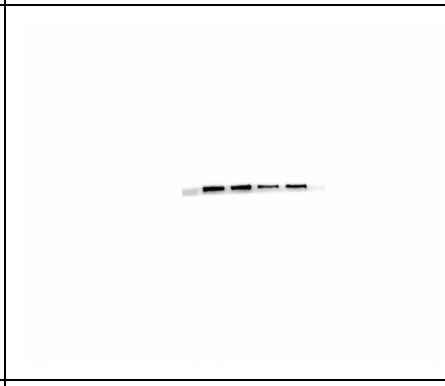
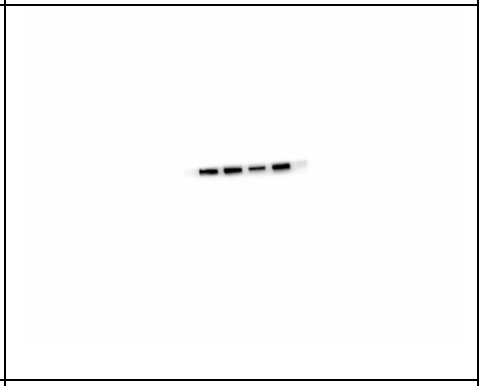
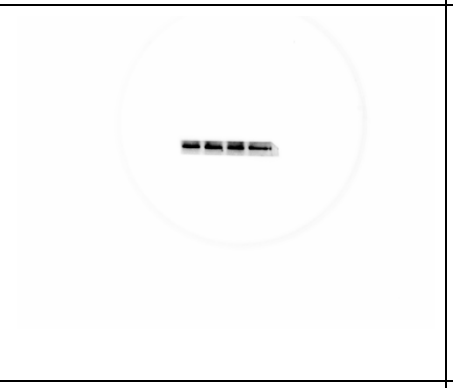
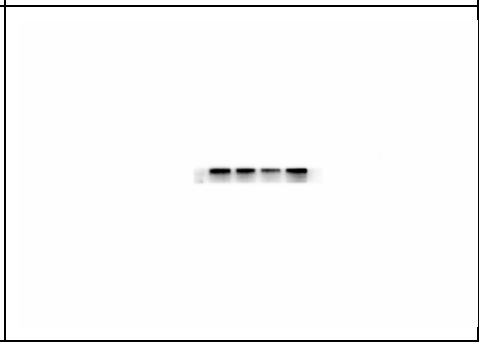
mTOR			
p-mTOR			
PI3K			
p-PI3K			

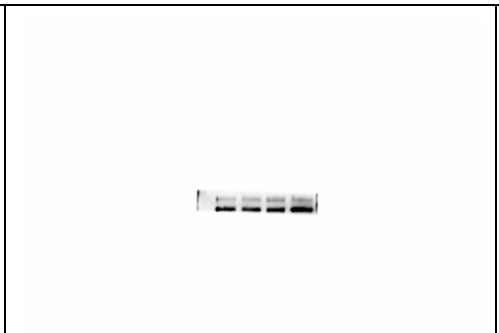
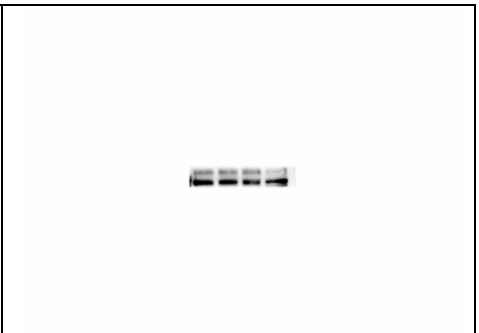
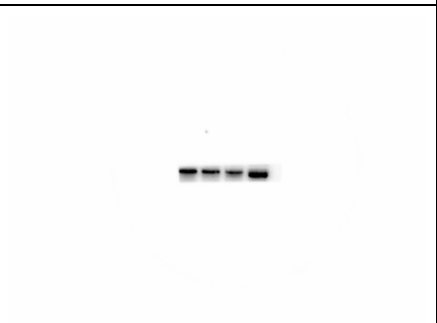
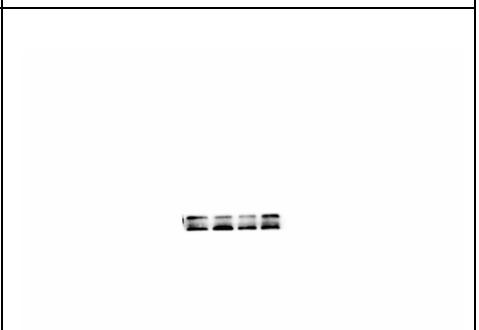
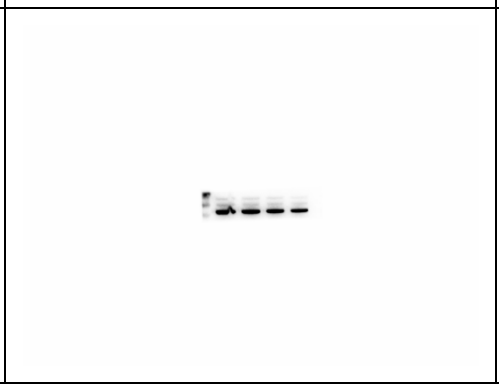
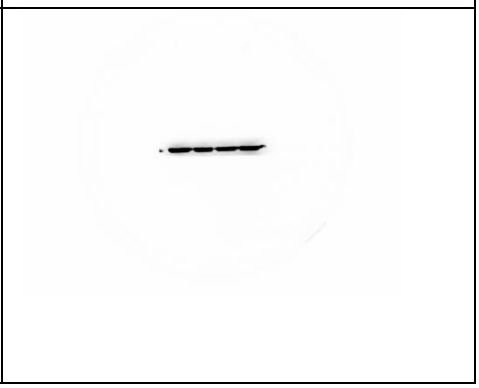
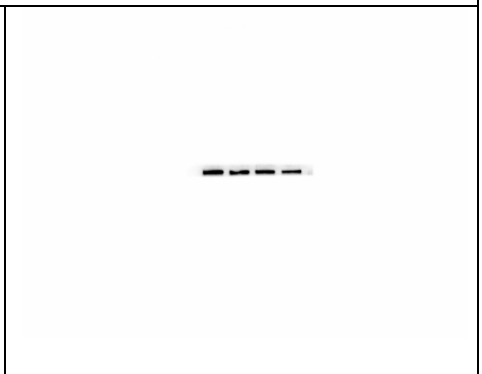
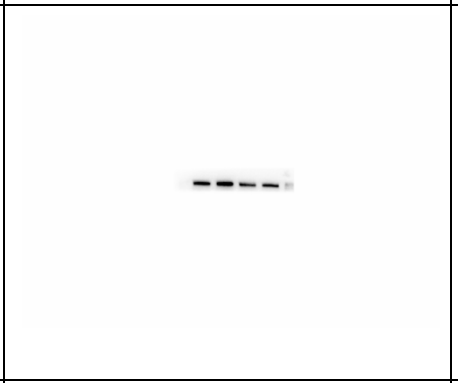
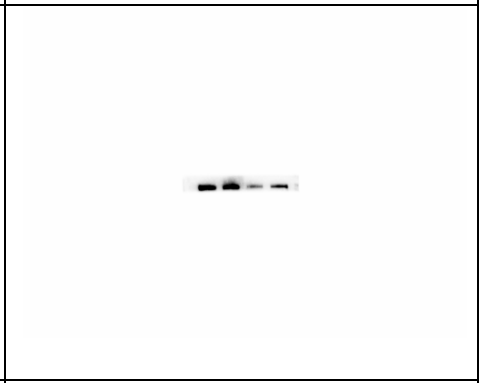
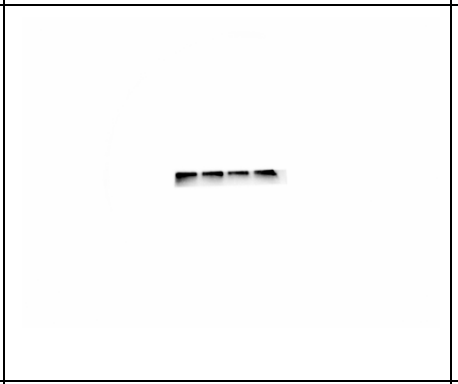
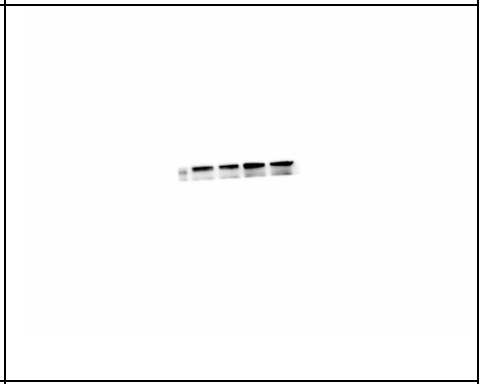
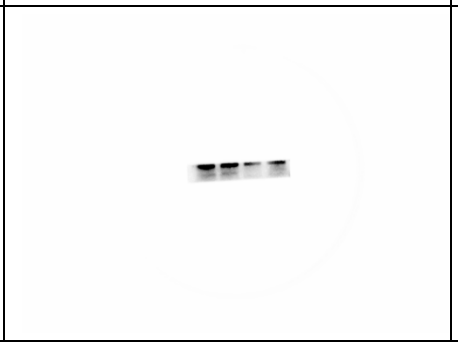
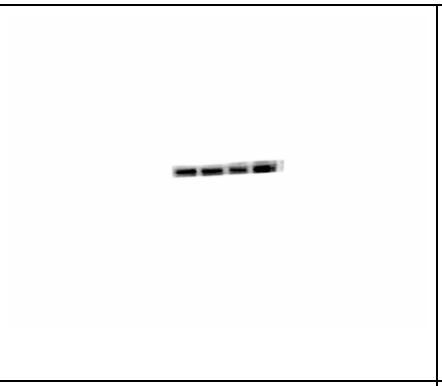
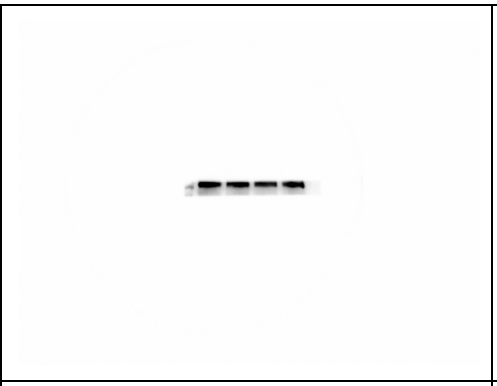
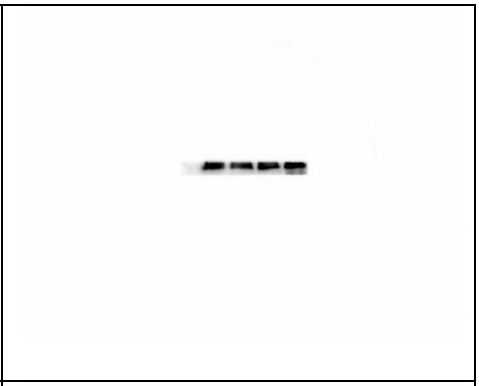
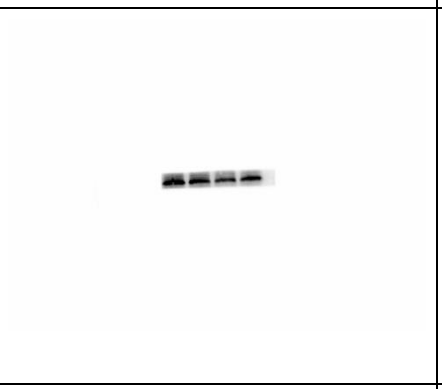
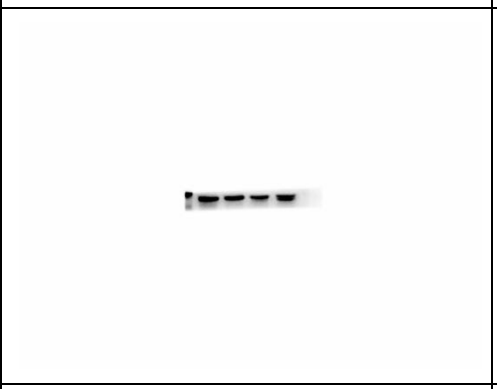
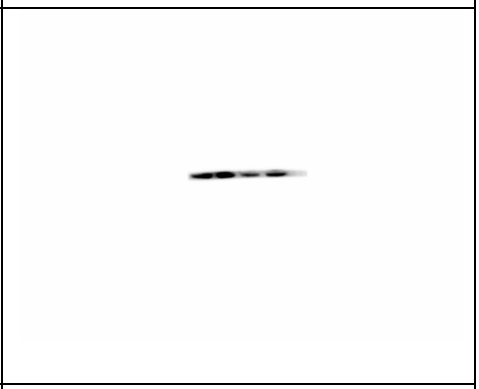
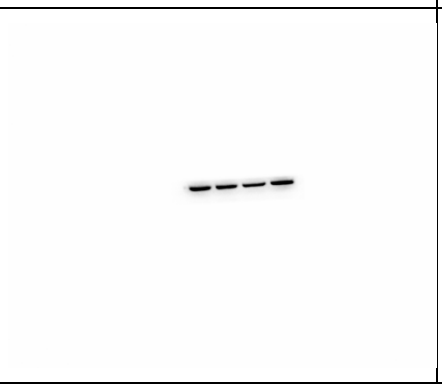
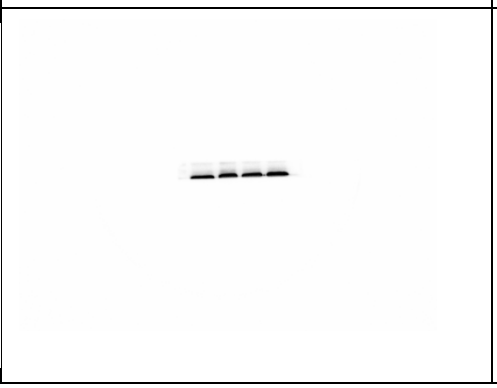
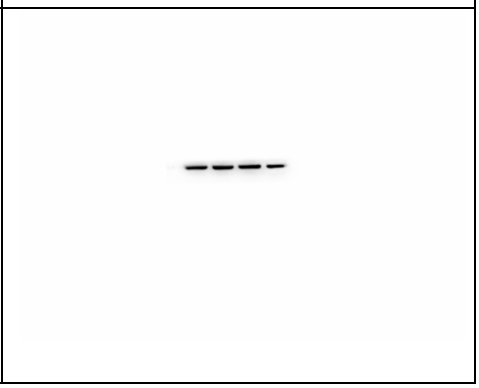
AKT			
p-AKT			
β-actin			

Figure 7F (H226)			
mTOR			
P-mTOR			
PI3K			
p-PI3K			

AKT			
p-AKT			
β-actin			

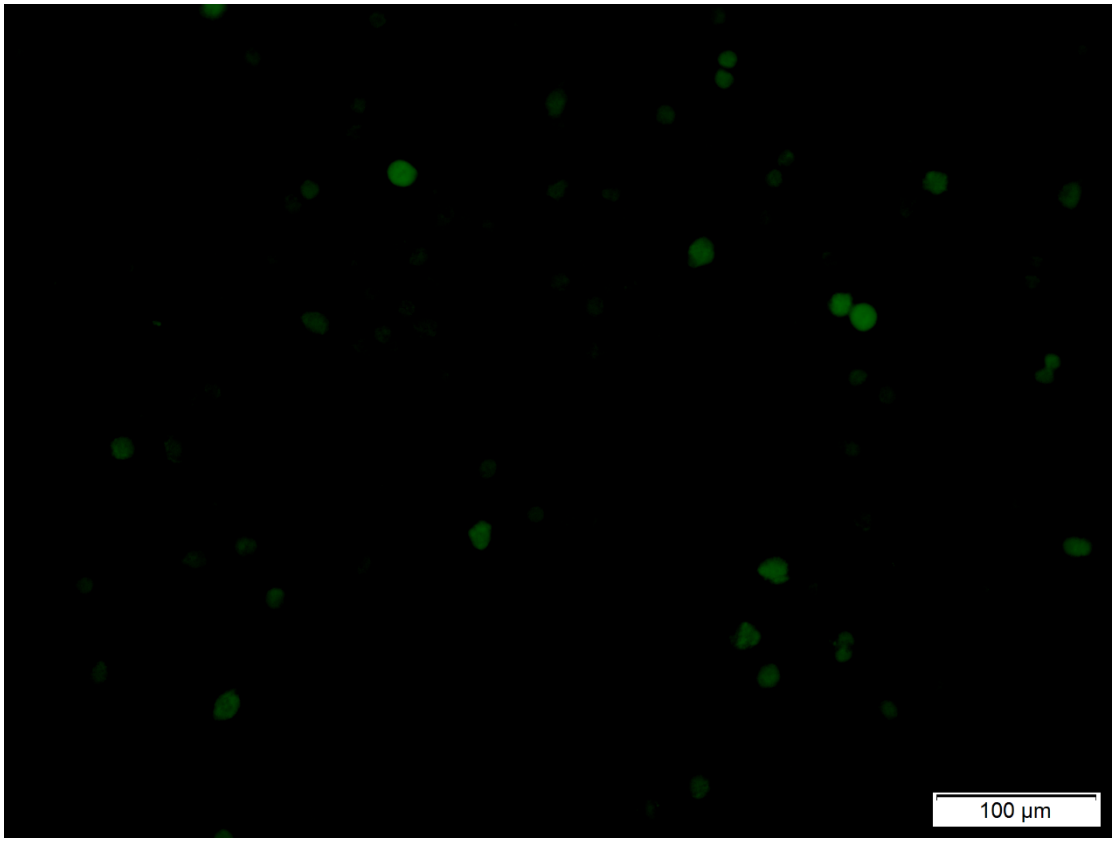


Fig. 7A_A549_control

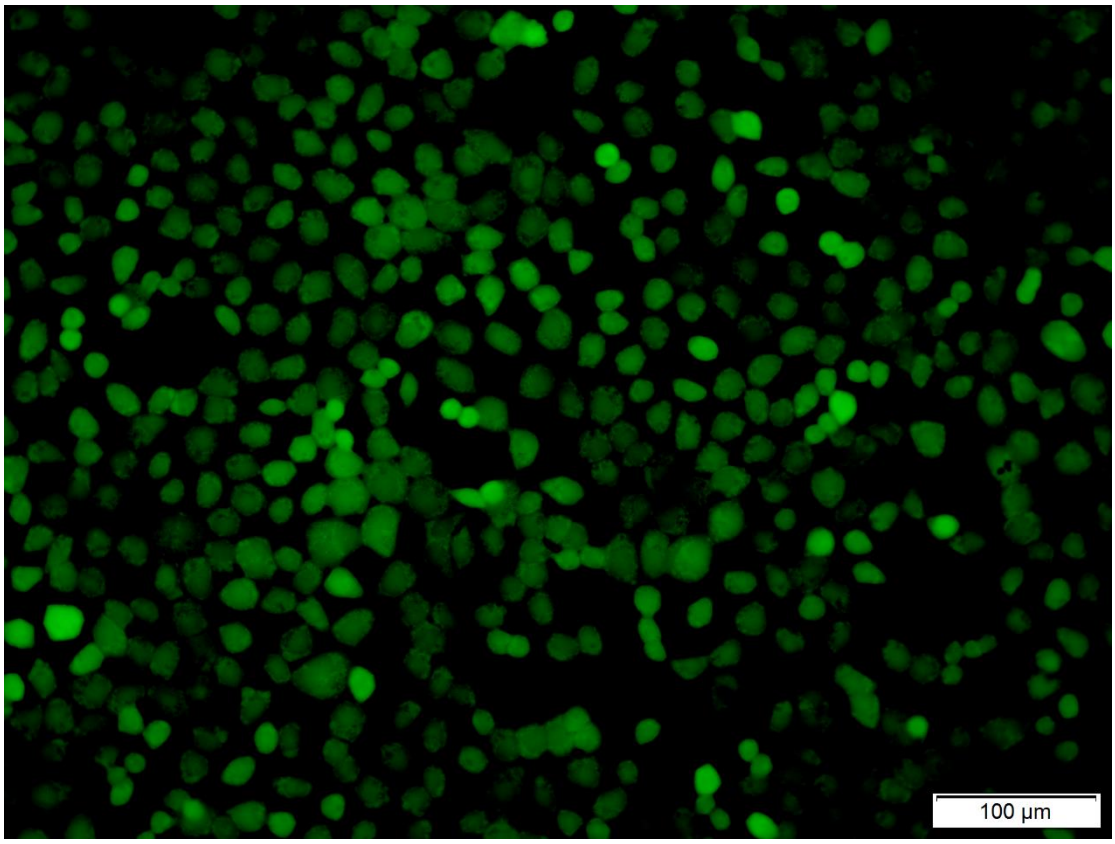


Fig. 7A_A549_ABX_10µM

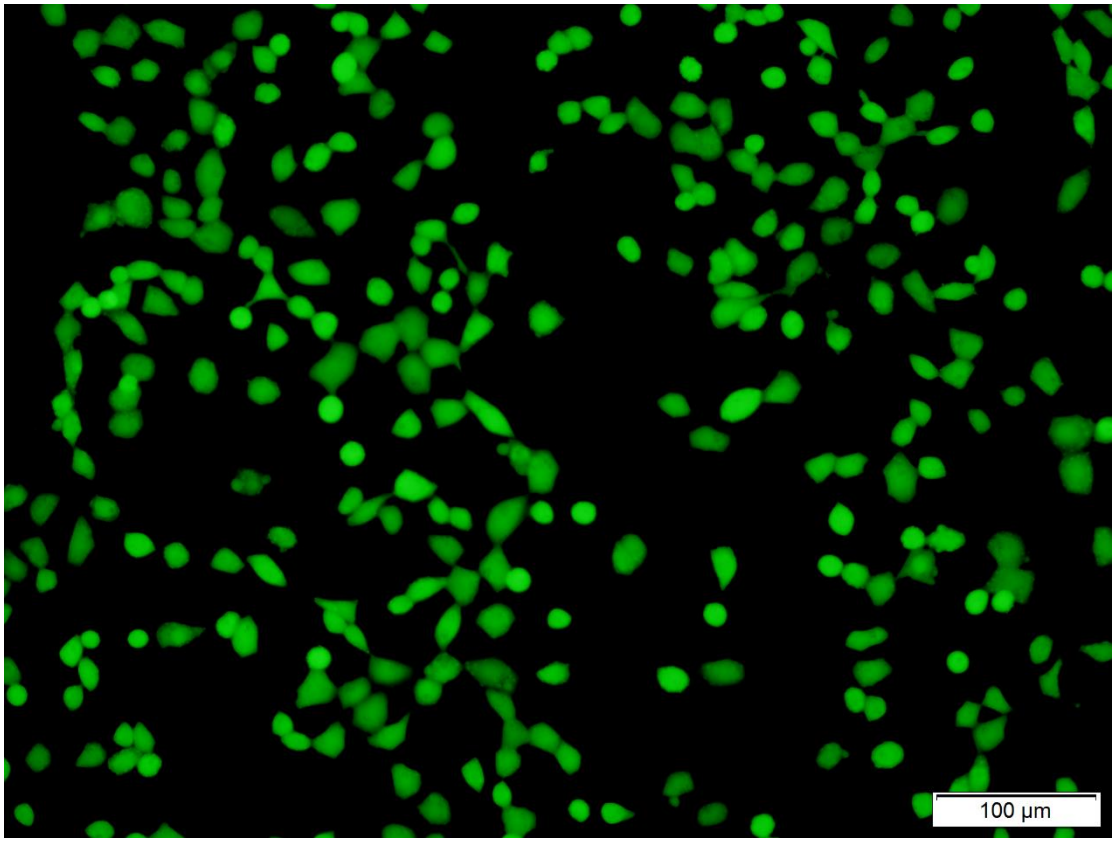


Fig. 7A_A549_ABX_20µM

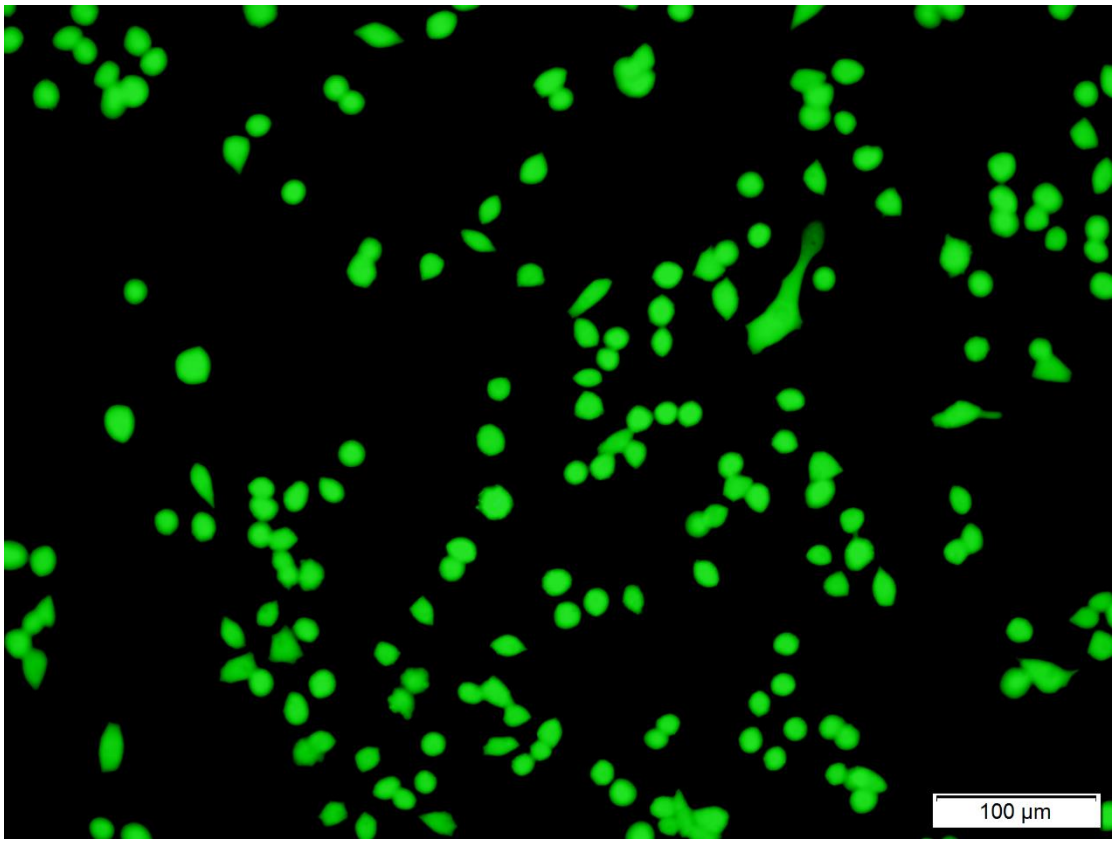


Fig. 7A_A549_ABX_40µM

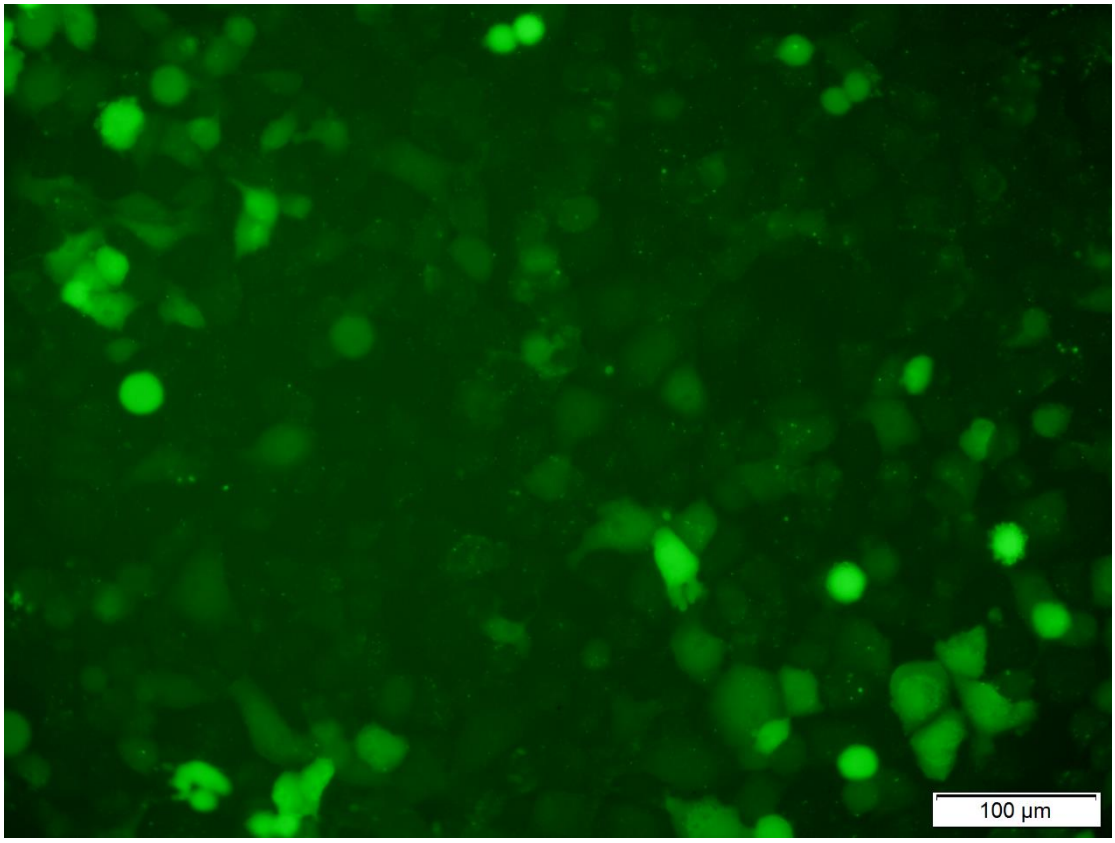


Fig. 7A_H1299_control

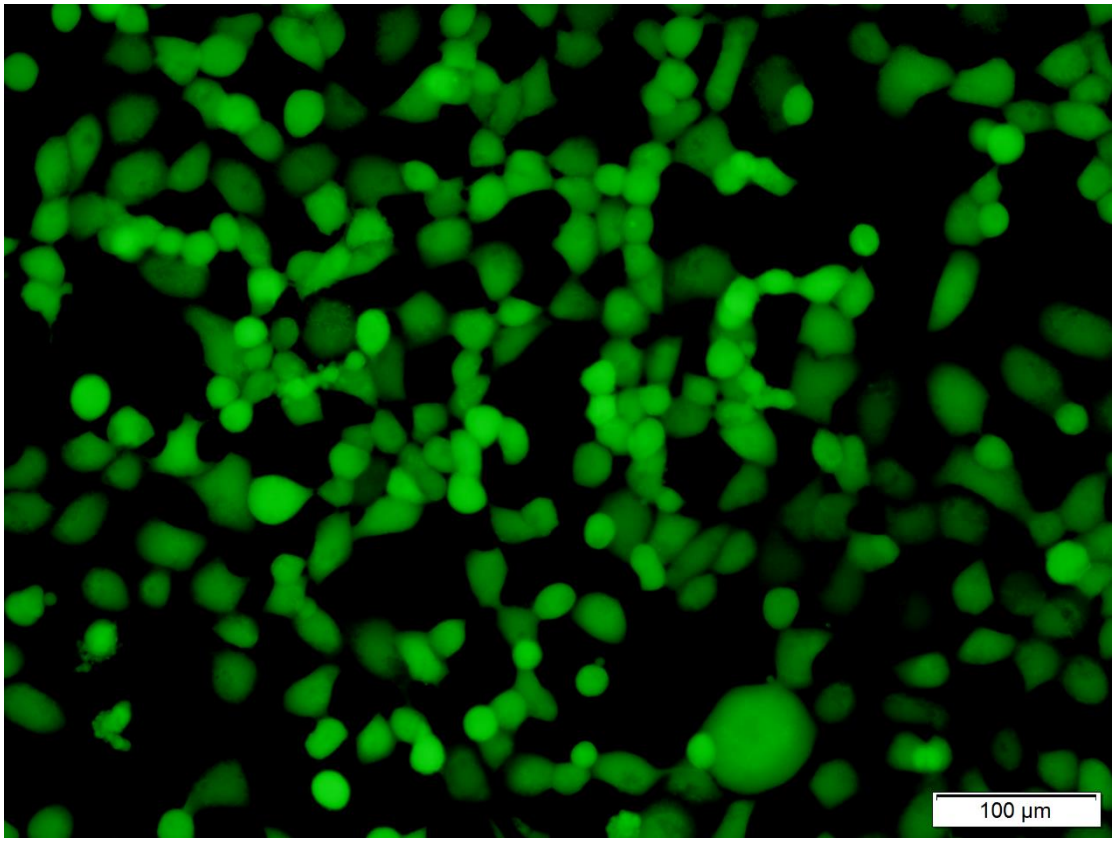


Fig. 7A_H1299_ABX_10µM

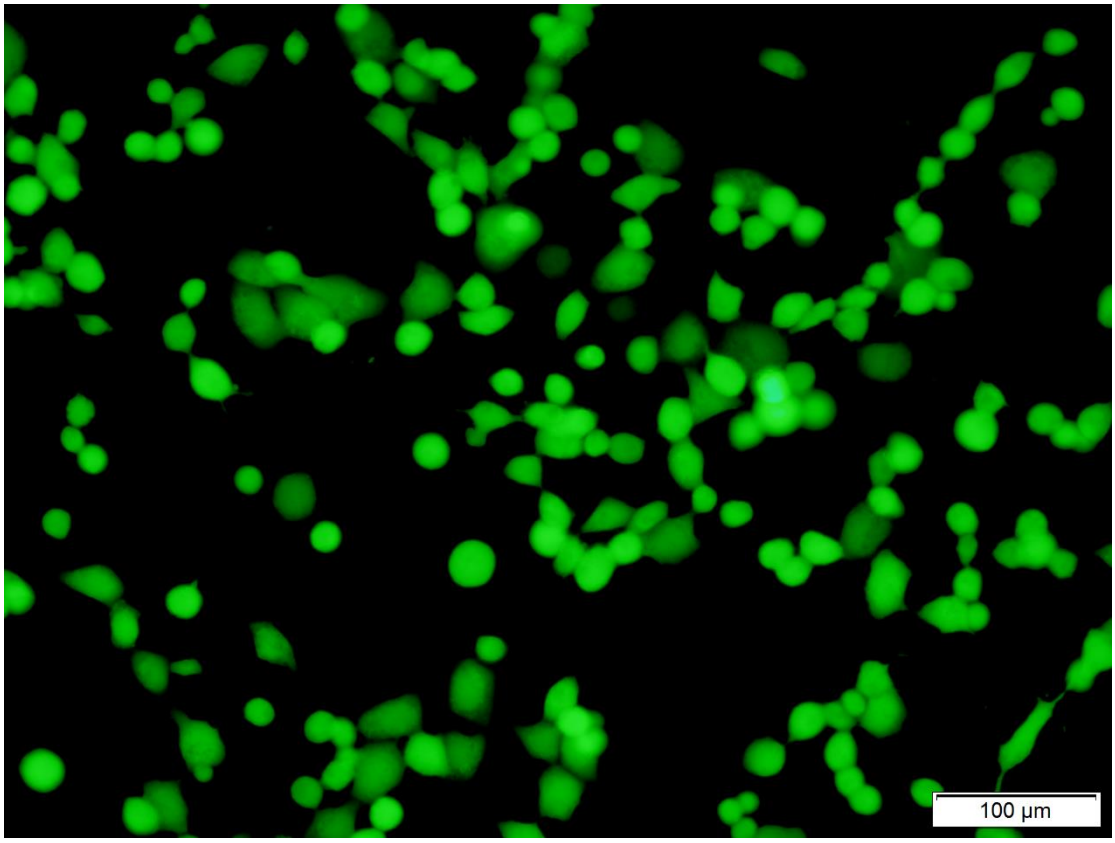


Fig. 7A_H1299_ABX_20µM

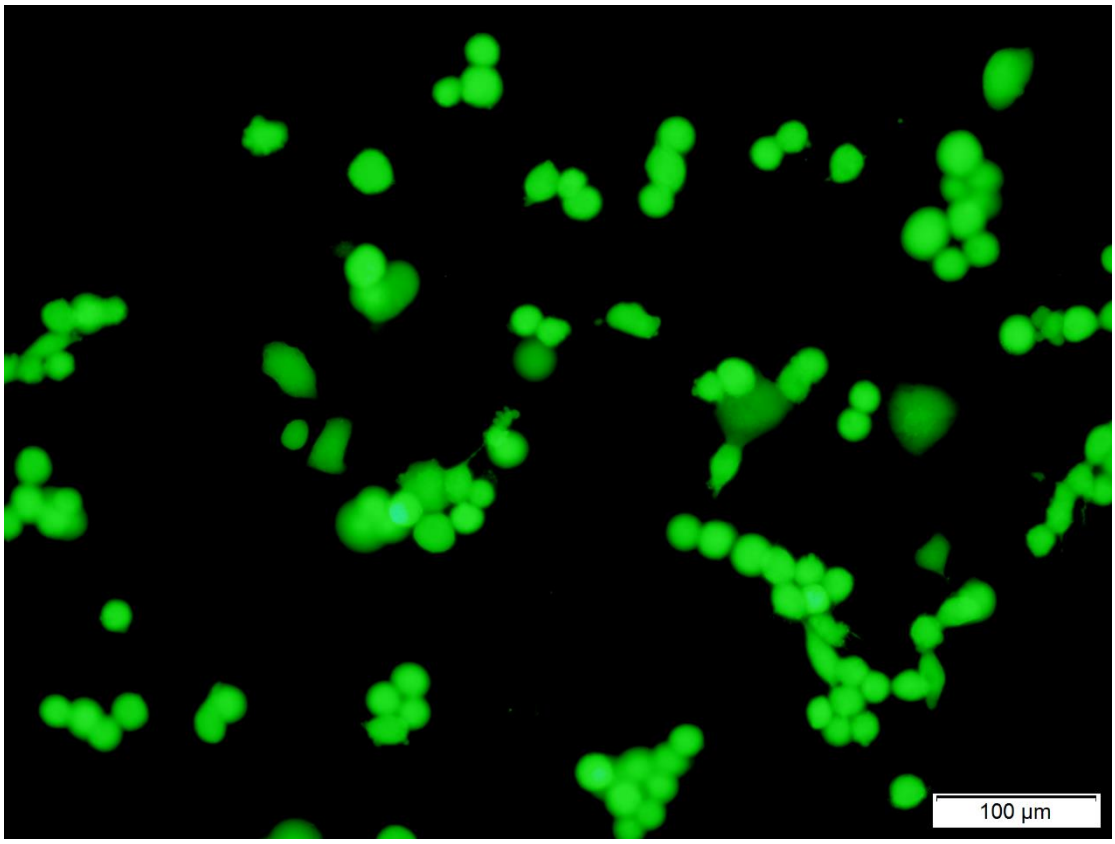


Fig. 7A_H1299_ABX_40µM

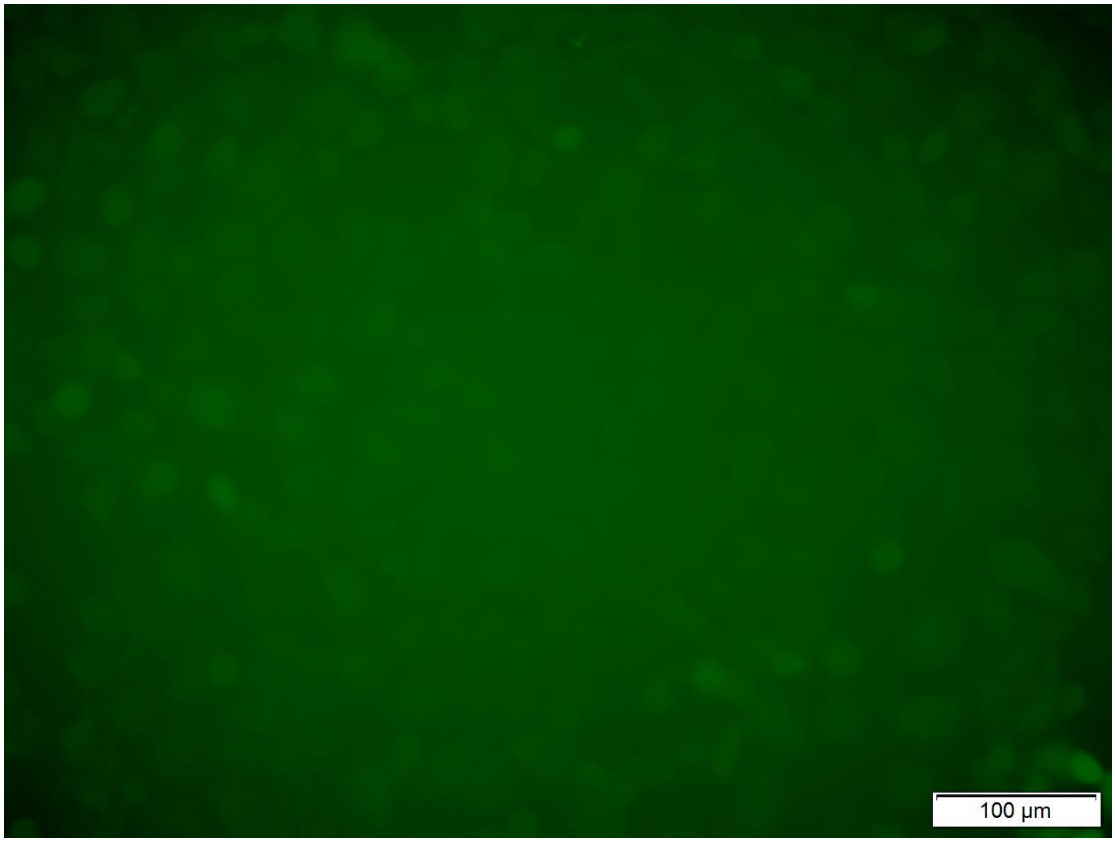


Fig. 7A_H226_control

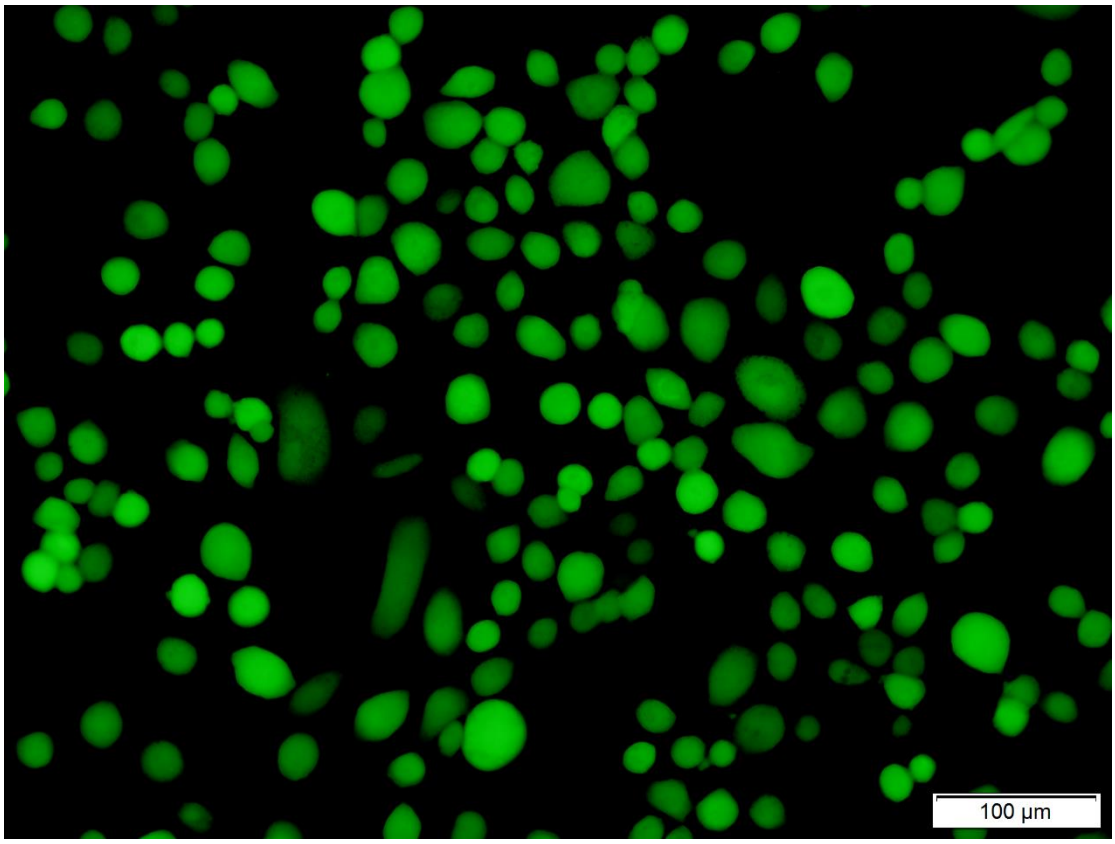


Fig. 7A_H226_ABX_10µM

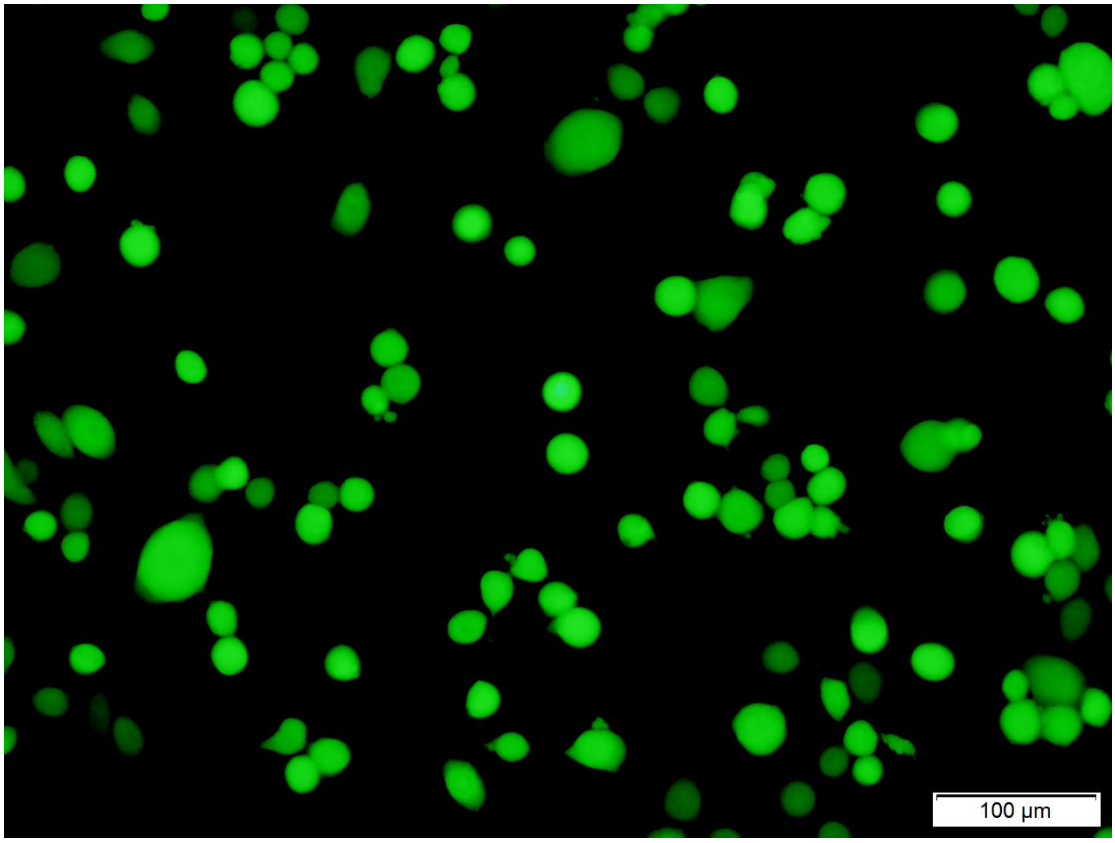


Fig. 7A_H226_ABX_20µM

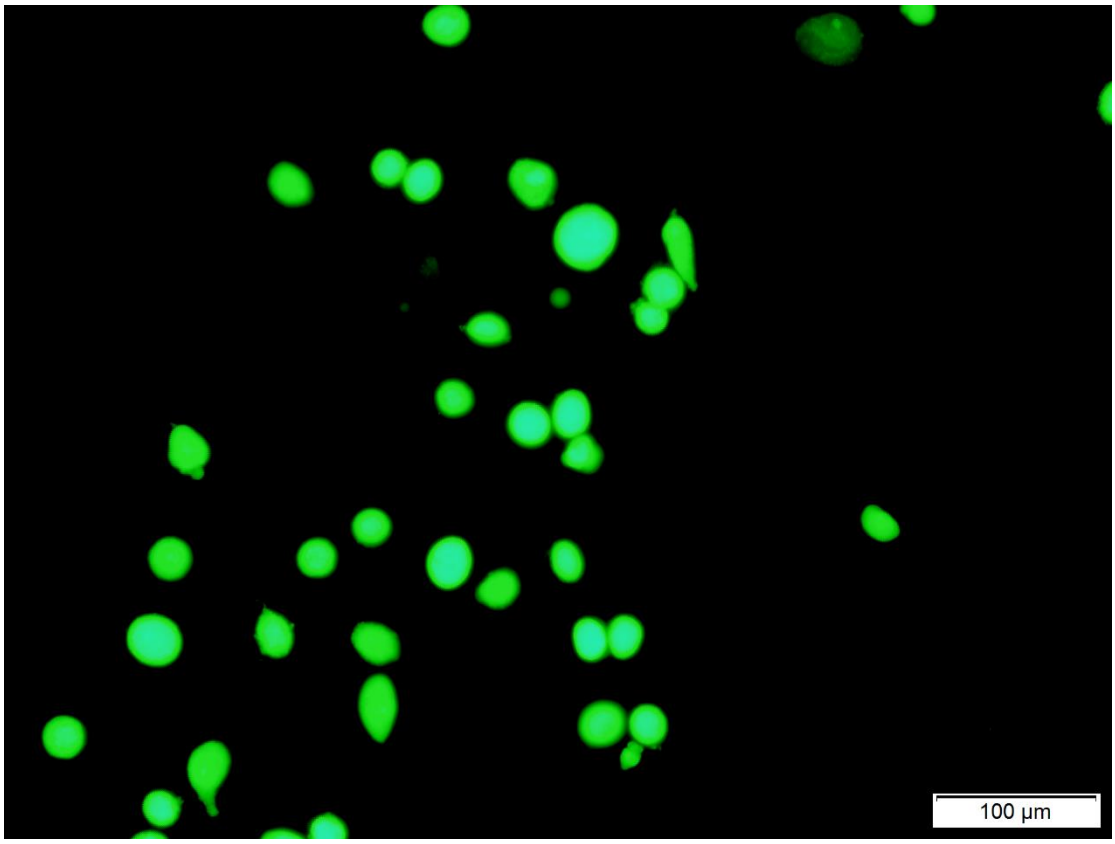


Fig. 7A_H226_ABX_40µM

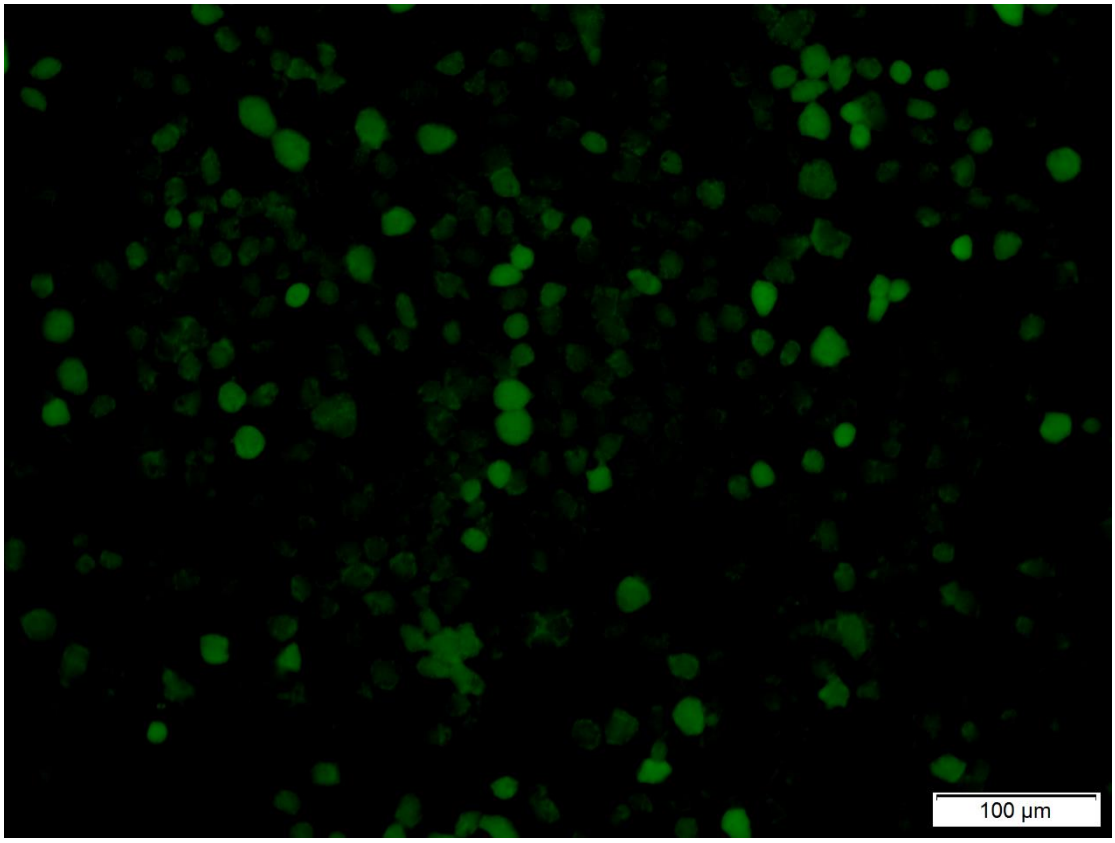


Fig. 7B_A549_control

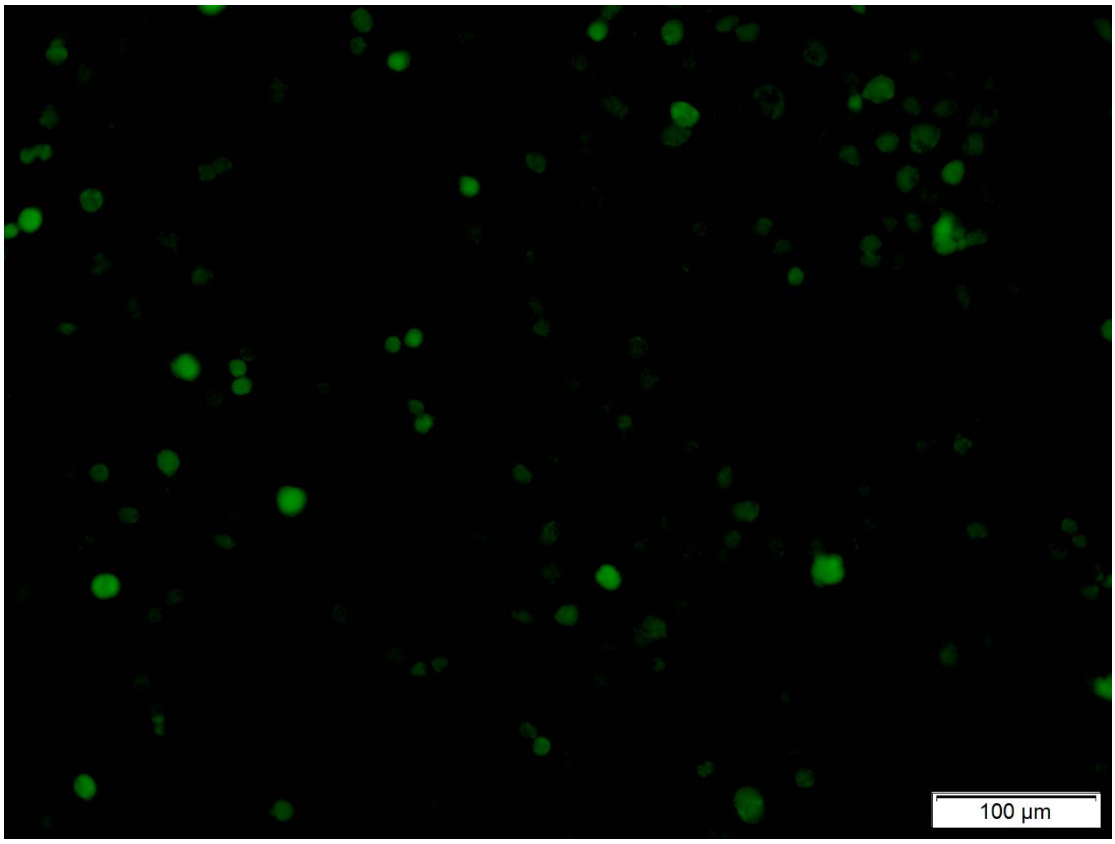


Fig. 7B_A549_NAC_10µM

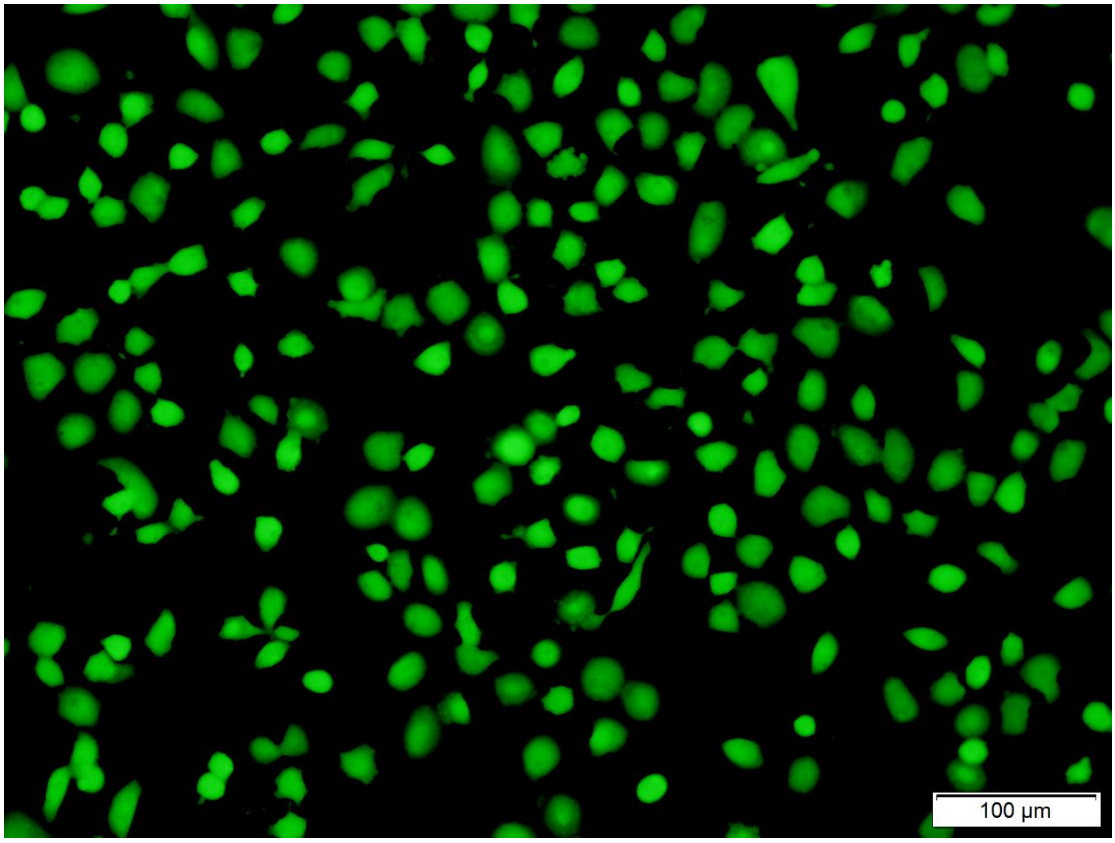


Fig. 7B_A549_ABX_20µM

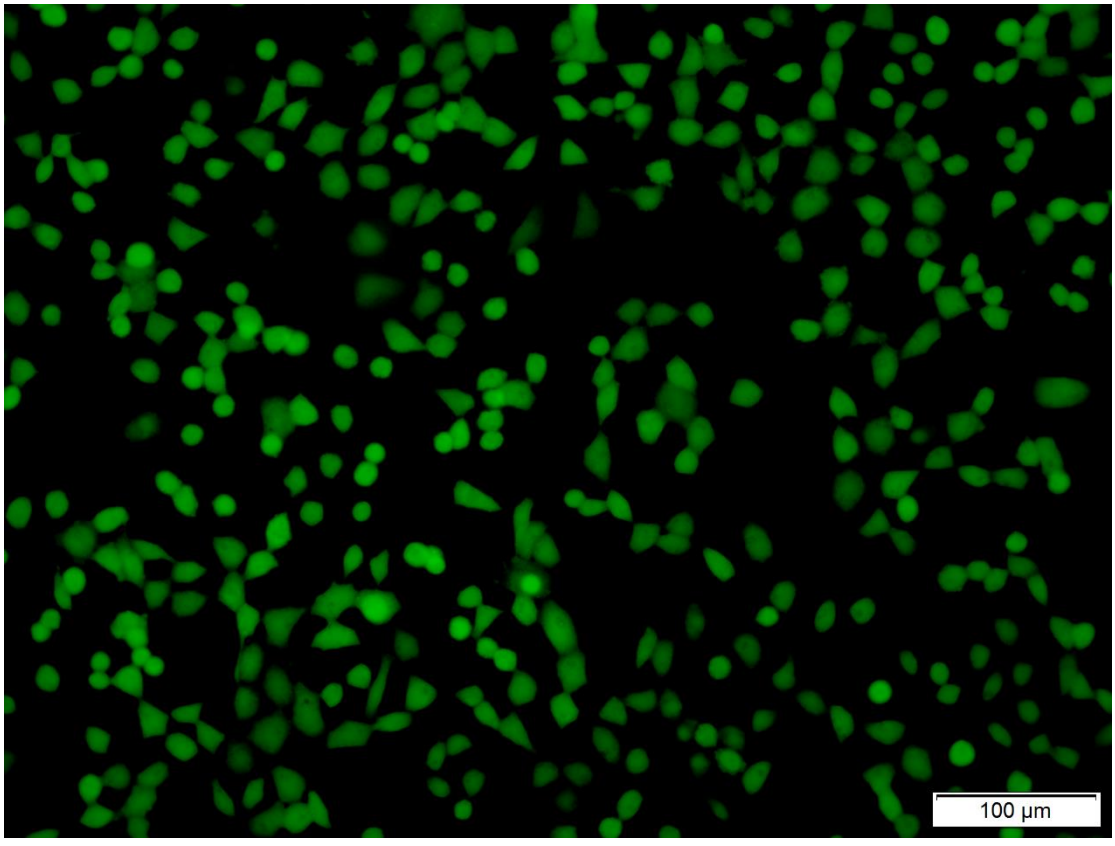


Fig. 7B_A549_ABX+NAC

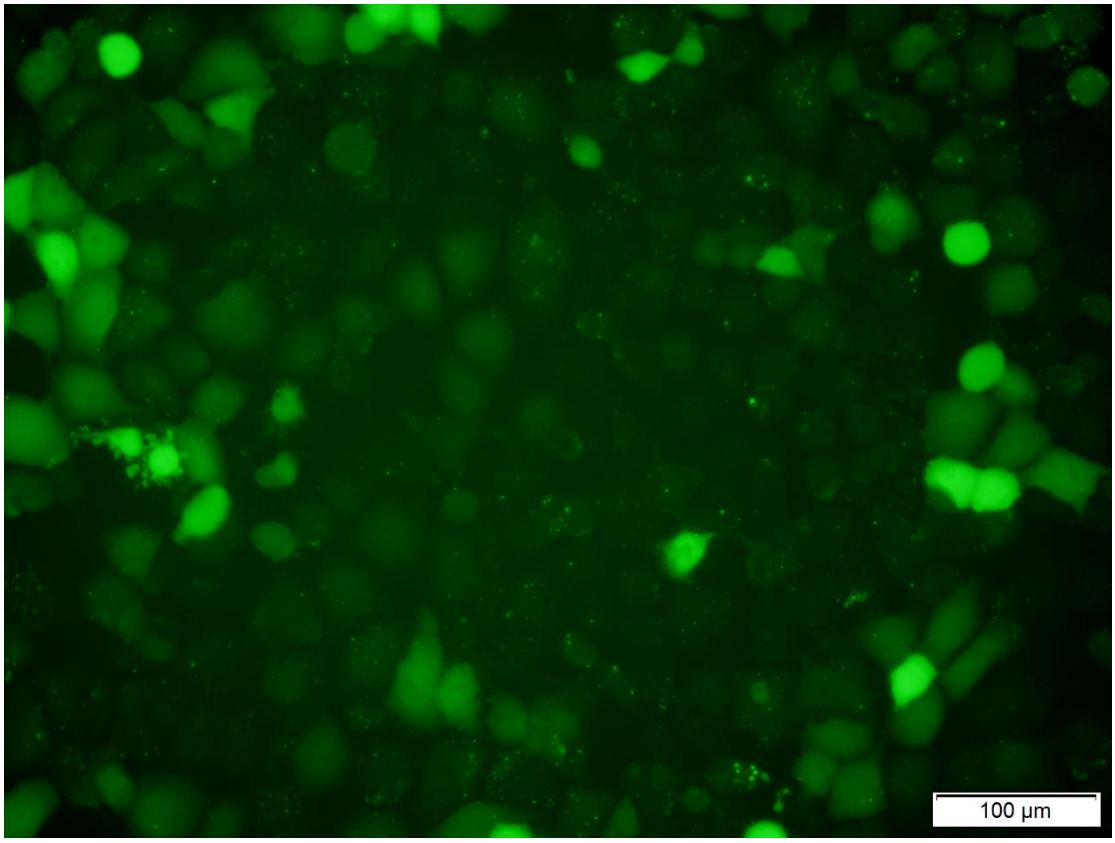


Fig. 7B_H1299_control

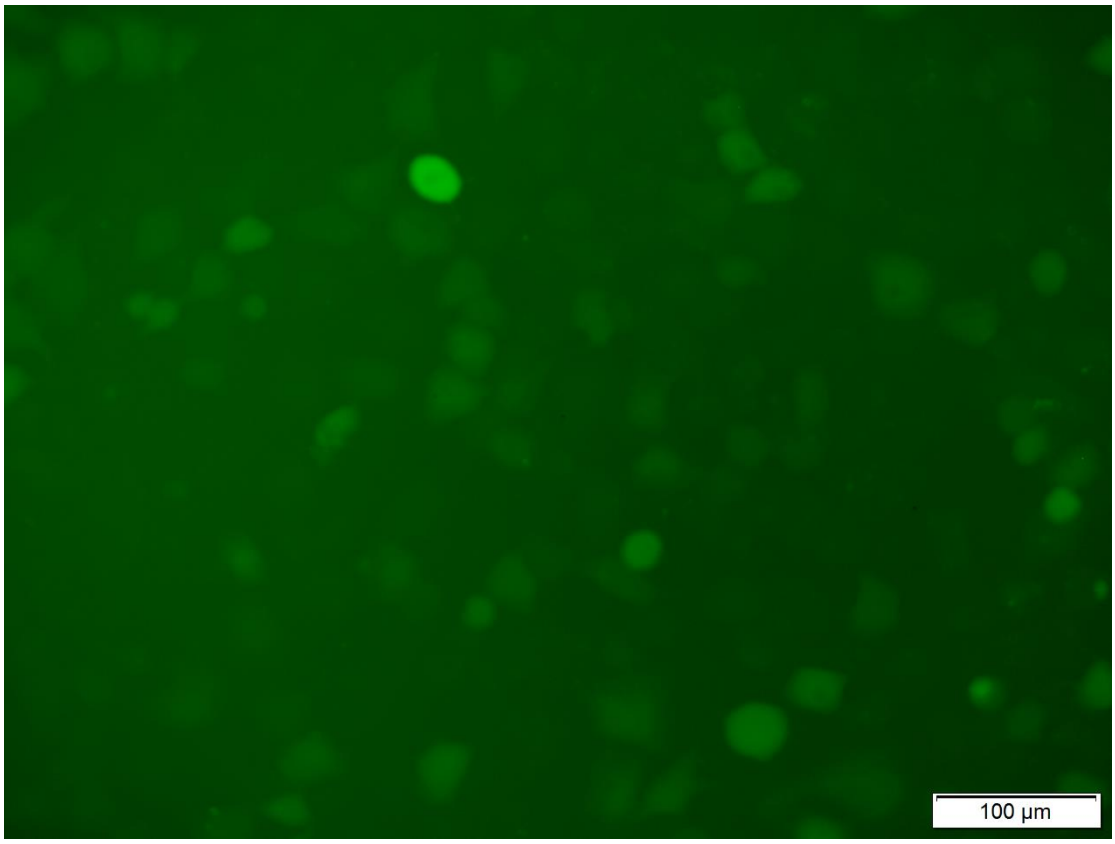


Fig. 7B_H1299_NAC_10µM

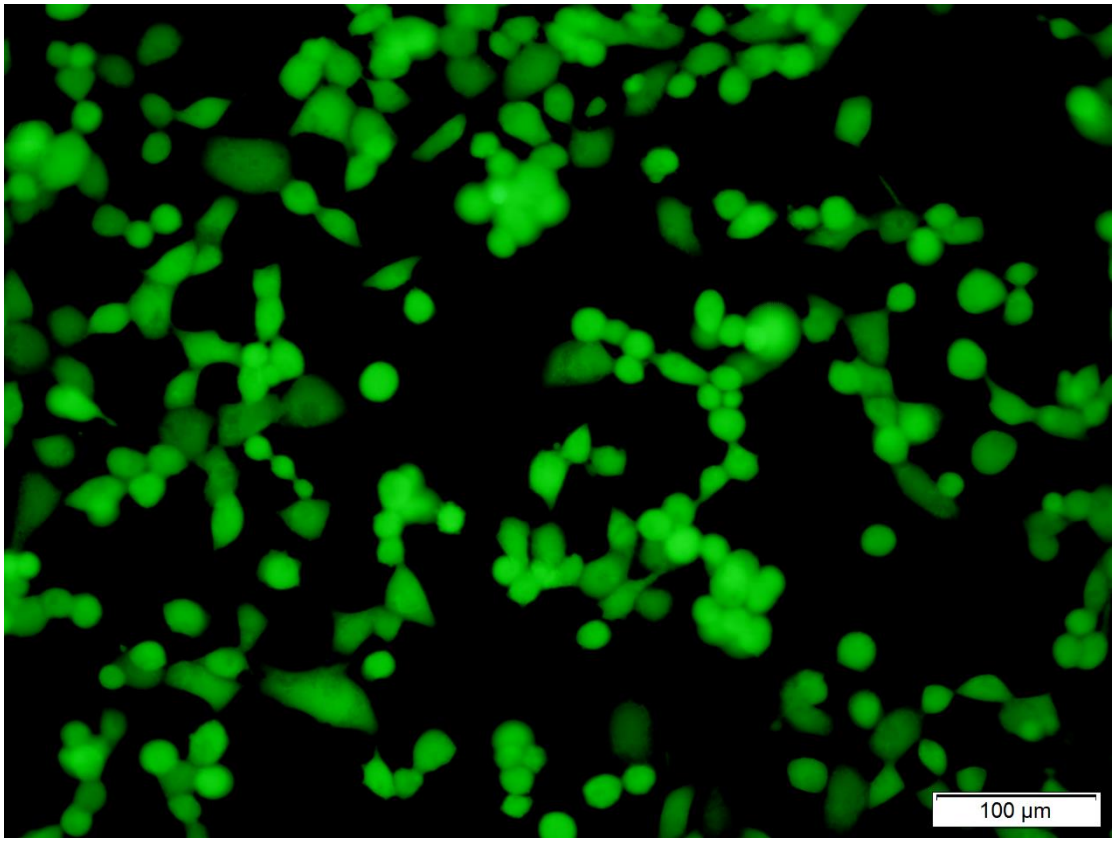


Fig. 7B_H1299_ABX_20µM

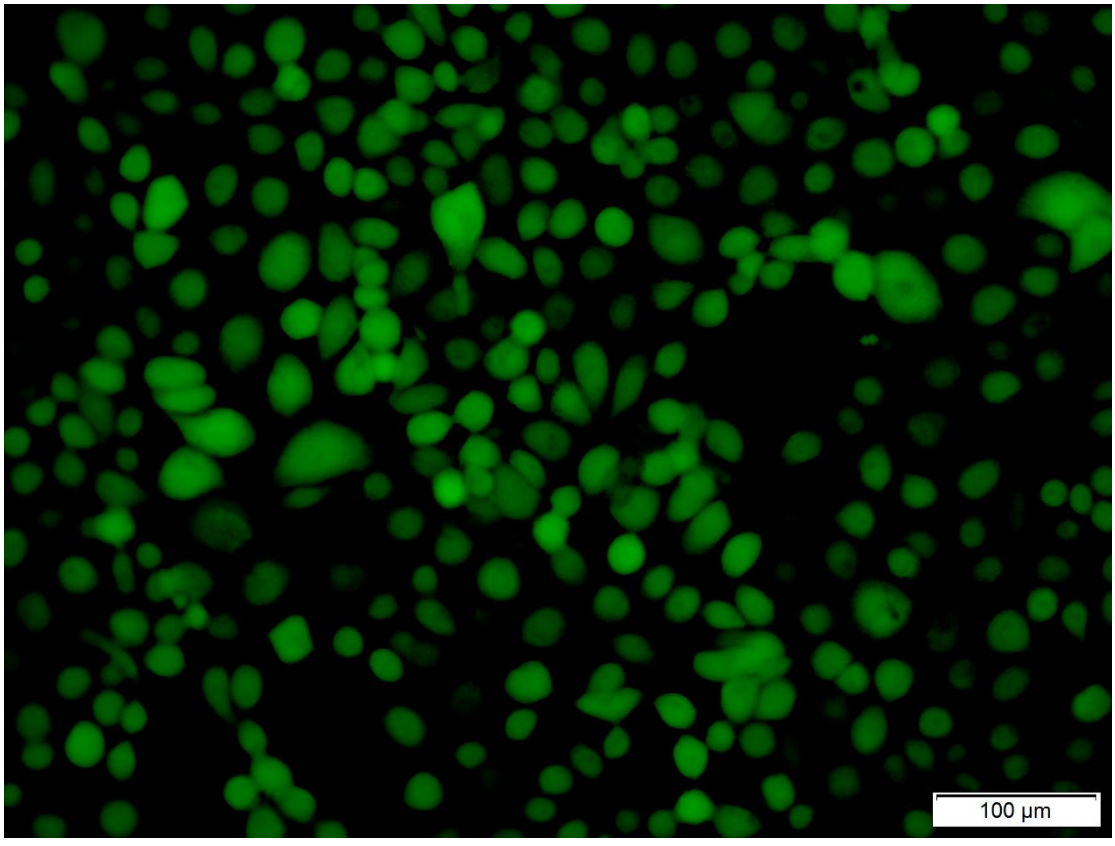


Fig. 7B_H1299_ABX+NAC

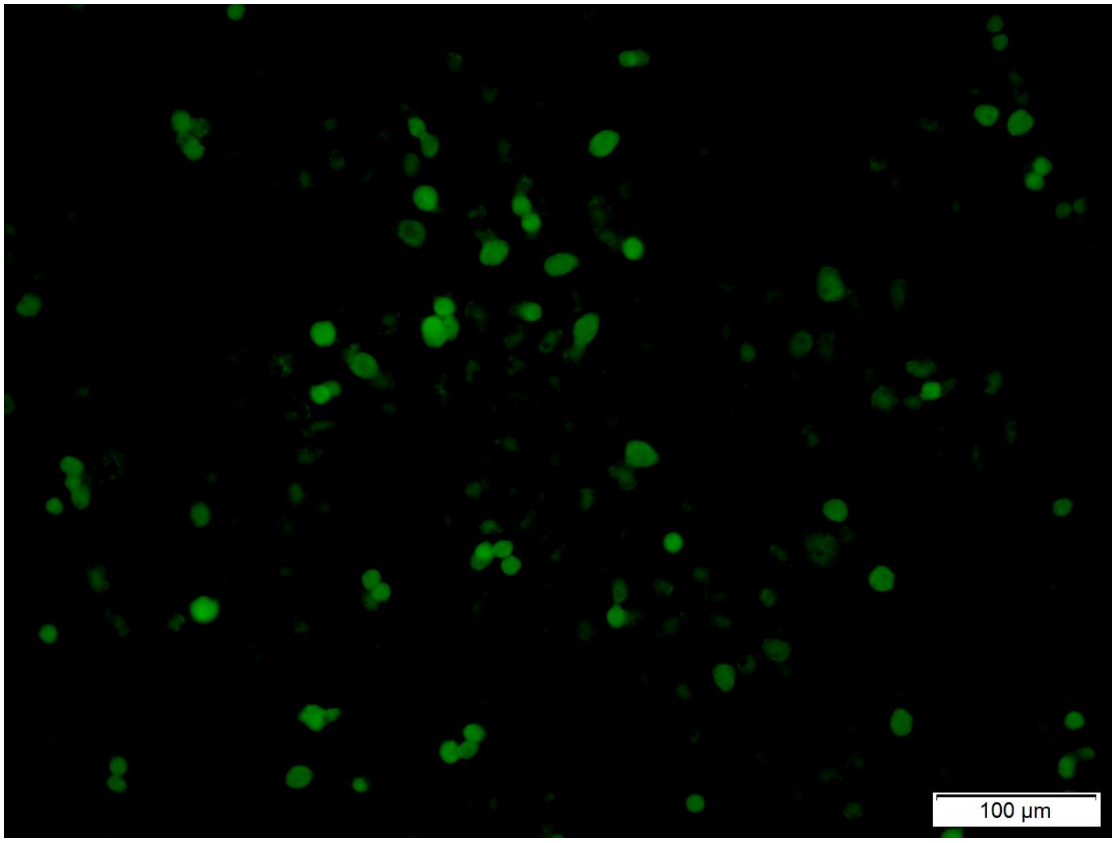


Fig. 7B_H226_control

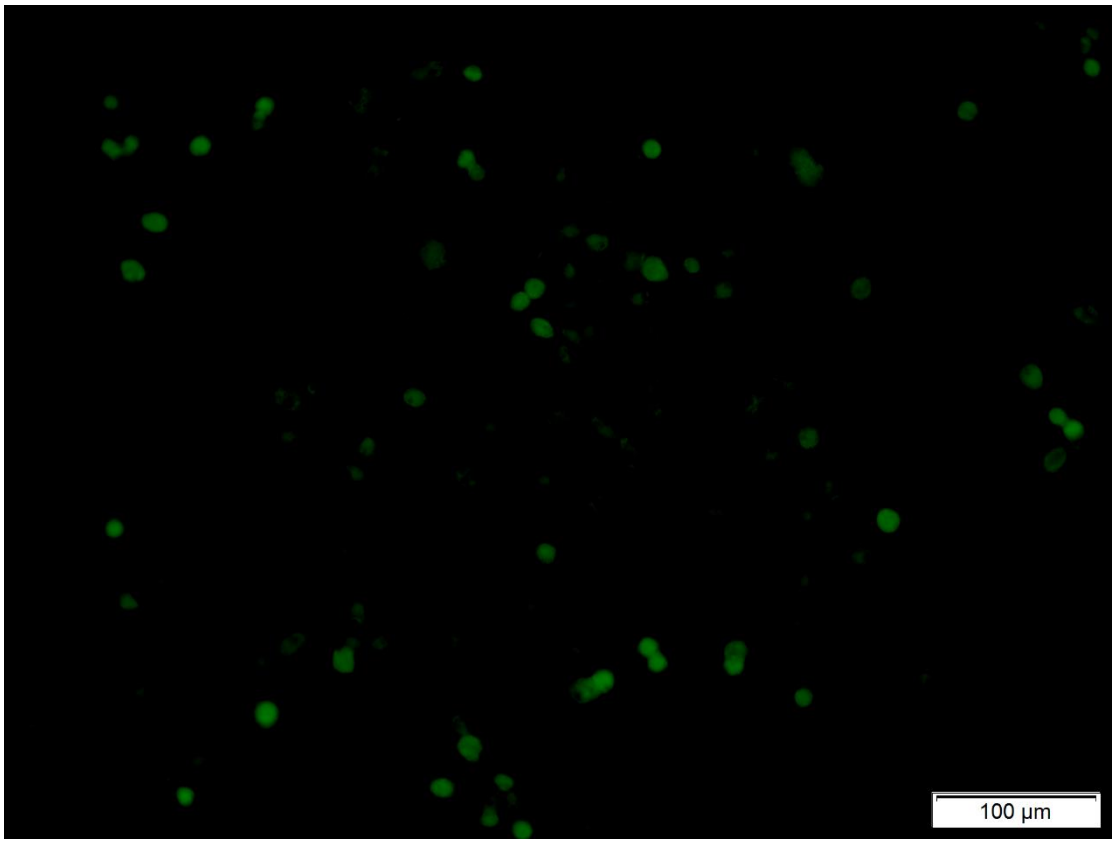


Fig. 7B_H226_NAC_10µM

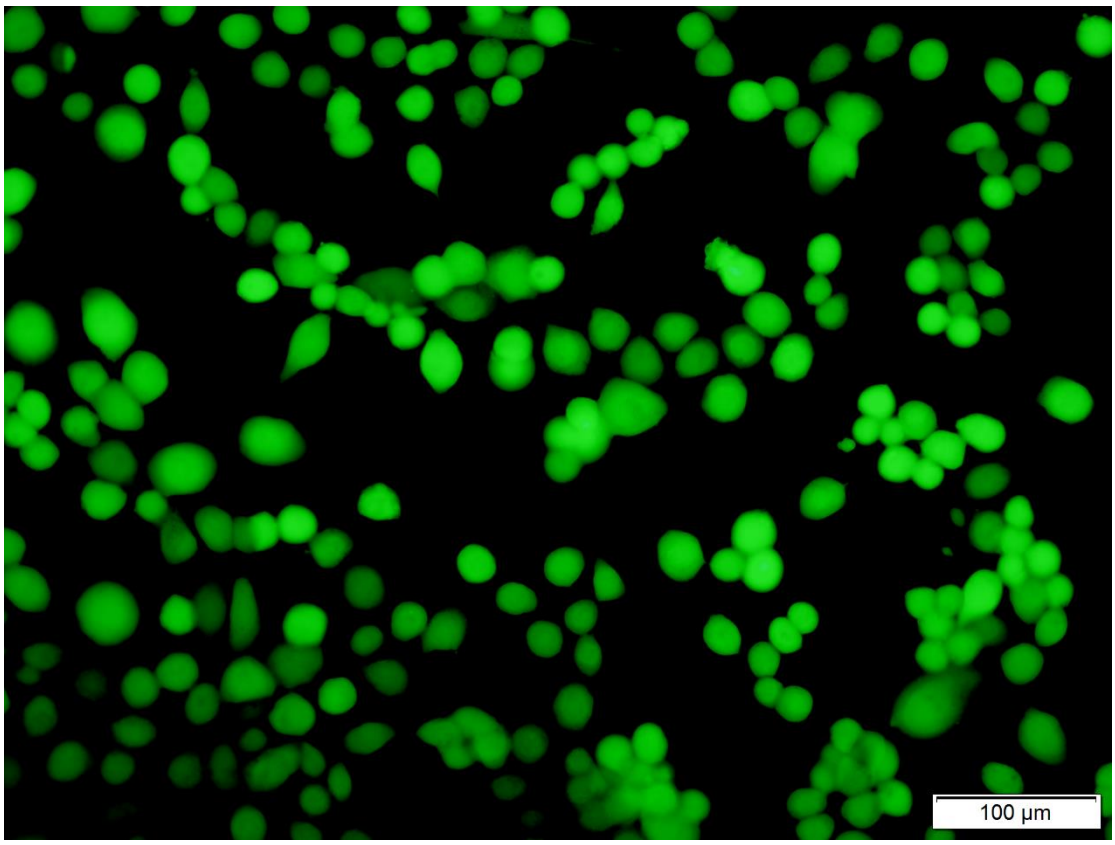


Fig. 7B_ H226_ABX_20µM

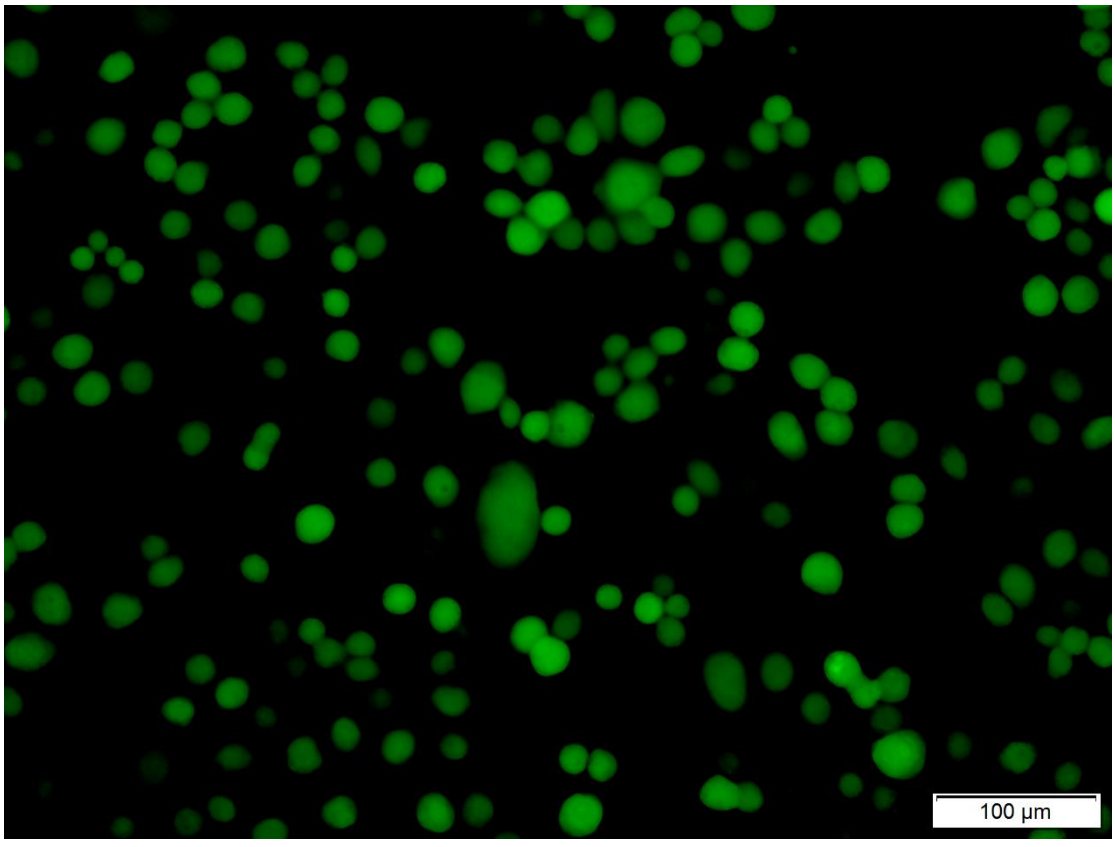


Fig. 7B_ H226_ABX+NAC