

Supplementary description:

Figure S1. Viability of AGS treated with various drug concentrations. A. The AGS cell line was treated with metformin (M: 0.1–9 mM). B. The AGS cell line was treated with lansoprazole (L: 3–270 μ M). C. The AGS cell line was treated with combined drugs L+M (L: lansoprazole 10 μ M; M: metformin 1 mM) for 3 days and then its viability was determined using the WST-1 assay. The data was presented as the mean with SD.

Video S2. Time-lapse video. The AGS cell line was seeded in a 6-well plate (5000 cells/well). The compounds were treated for 3 days. HoloMonitor M4 captured real-time cell images, which were then converted into a video by using the HoloMonitor M4 application program.

The video file can be downloaded from the following URL. <http://tdl.ibms.sinica.edu.tw/download/download.html>

Figure S3. Lansoprazole and metformin exhibited a synergistic effect in AGS. The AGS cell line was treated with metformin (1 mM) and varying concentrations of lansoprazole (3, 10, 30, 90, and 270 μ M), and the data was then analyzed using SynergyFinder to calculate the combination index (CI).

Figure S4. Phase-contrast photographs of the AGS cell line. The cells were treated with metformin (M; 1 mM), lansoprazole (L; 10 μ M), or combined drugs L+M (L: lansoprazole 10 μ M; M: metformin 1 mM) for 3 and 8 days.

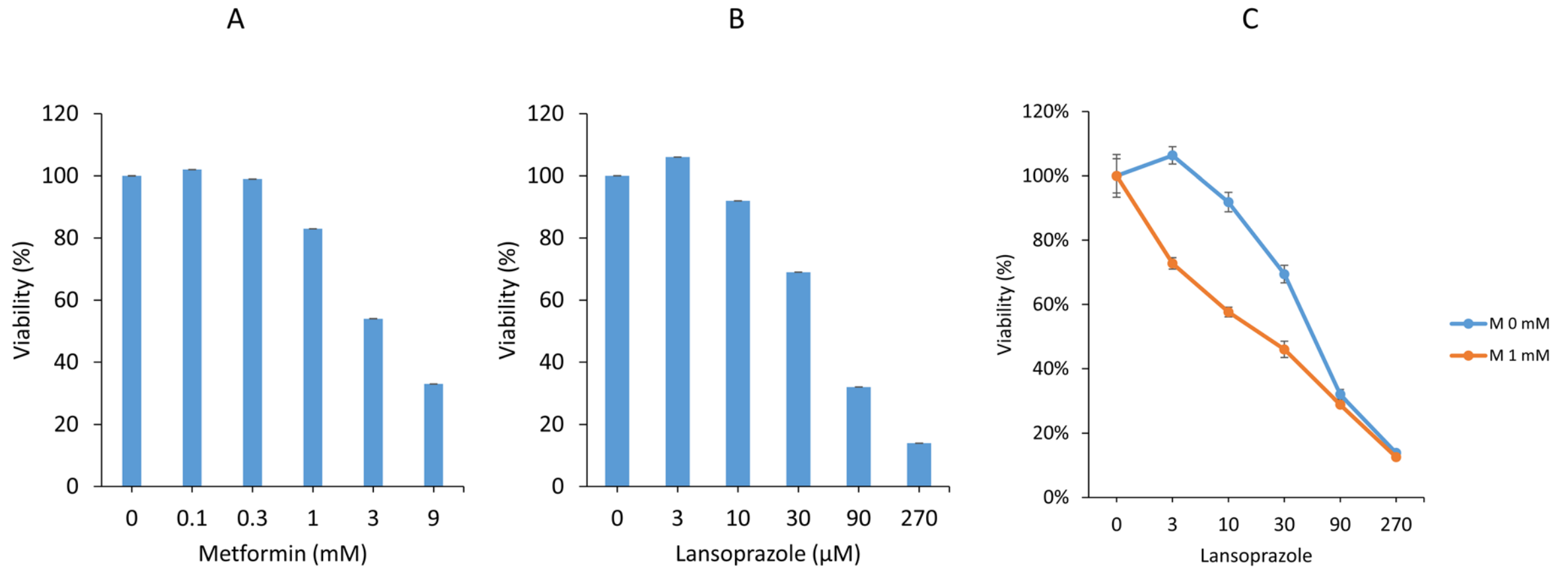
Figure S5. DAPI cell cycle. The AGS cell line was treated with drugs for 3 days, stained with DAPI, and the results were analyzed using the flow cytometer and FlowJo (v7). C: control; L: lansoprazole (10 μ M); M: metformin (1 mM); L+M (L: lansoprazole 10 μ M; M: metformin 1 mM)

Figure S6. AGS cell line was treated with metformin (M: 1mM), lansoprazole (L: 10 μ M) and L+M (L: lansoprazole 10 μ M; M: metformin 1 mM) for 3 days, then using western blot to display the protein level of different drug treatment. Quantification of western-blot bands was done by ImageJ software. The data was presented as the mean with SD.

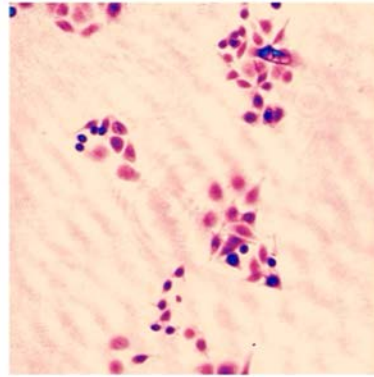
Figure S7. AGS cell line was treated with metformin (M: 1mM),

lansoprazole (L: 10 μ M) and L+M (L: lansoprazole 10 μ M; M: metformin 1 mM) for 3 days, then using western blot to display the protein level of different drug treatment. Quantification of western-blot bands was done by ImageJ software. The data was presented as the mean with SD.

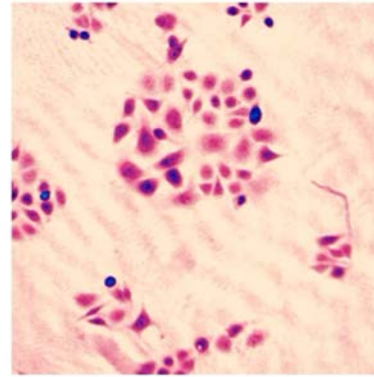
Table S1. Antibodies used in this study



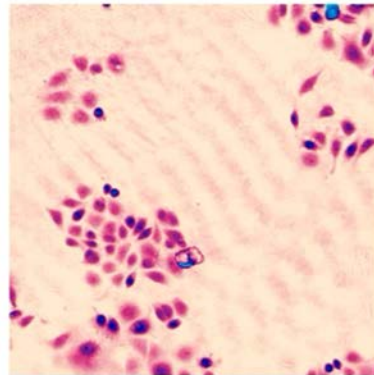
Control



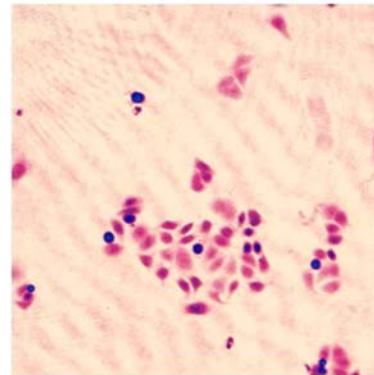
Lansoprazole (10 μ M)



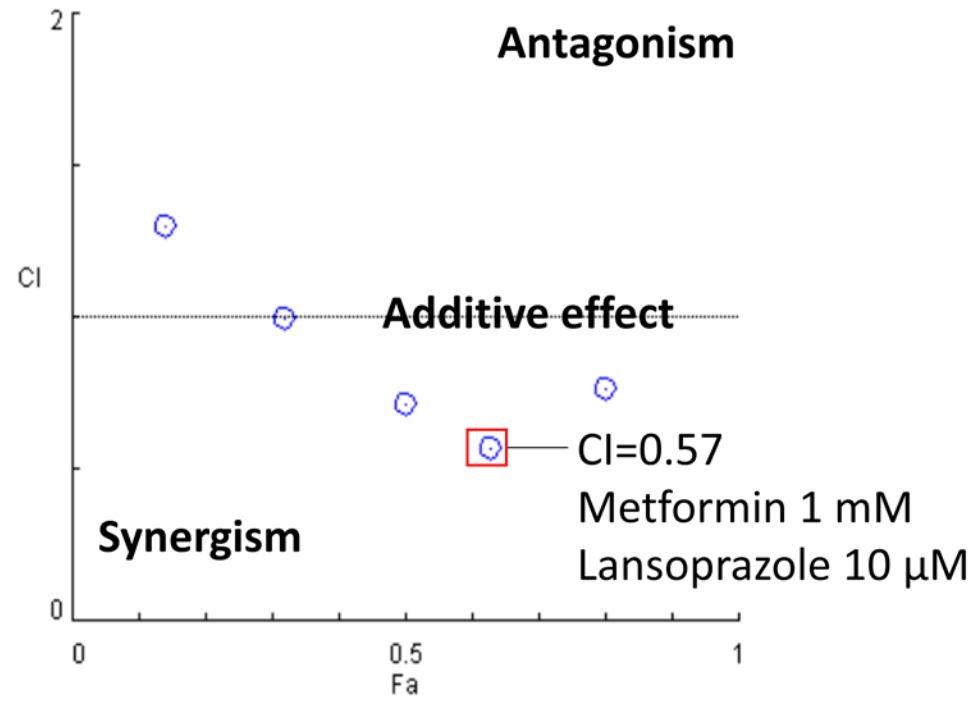
Metformin (1 mM)

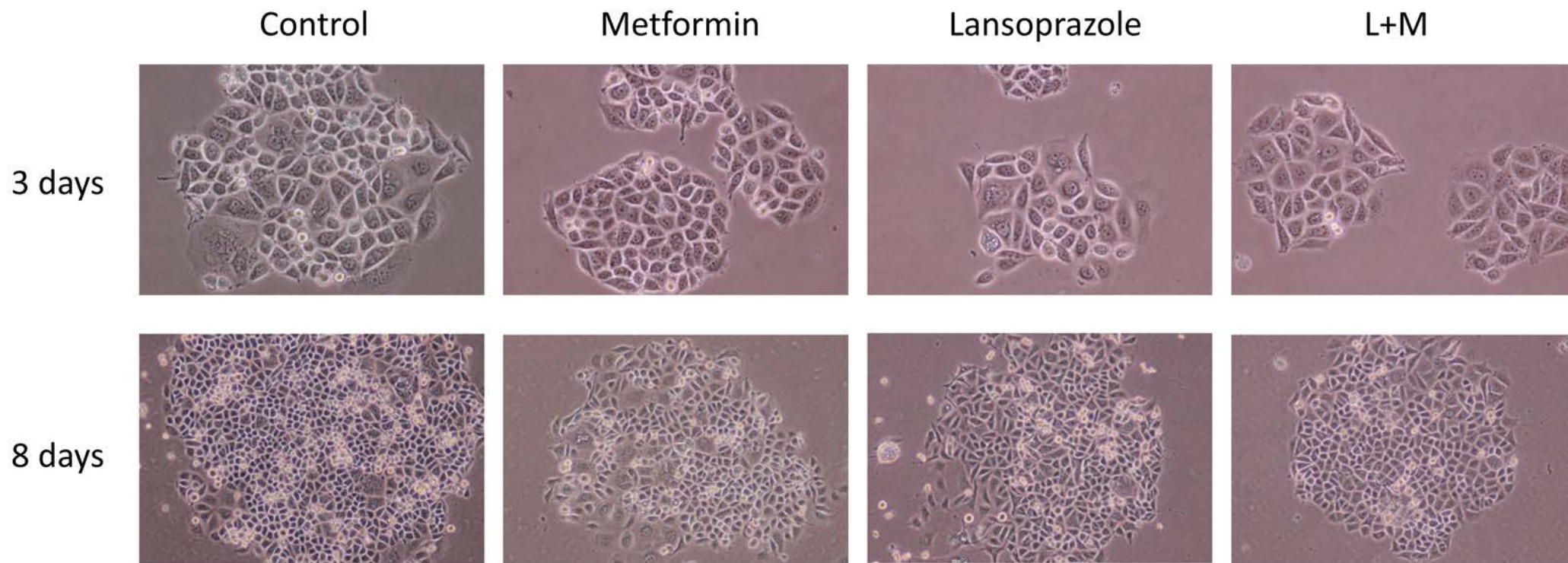


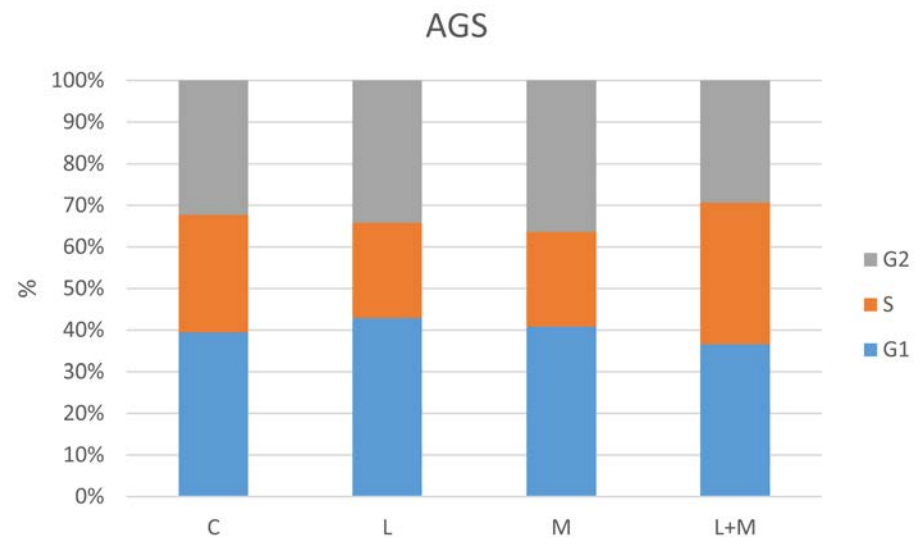
L + M

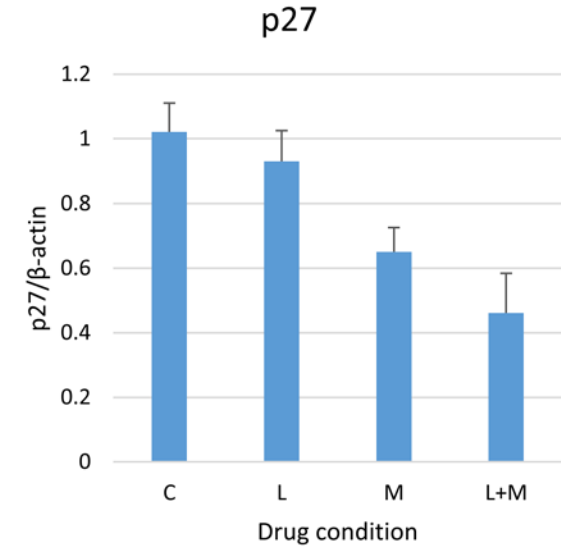
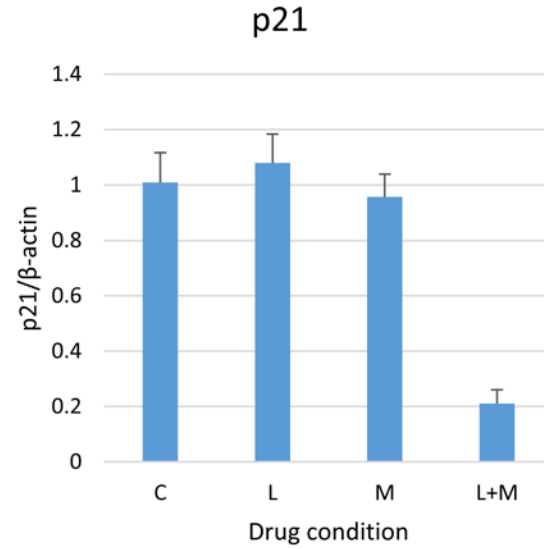
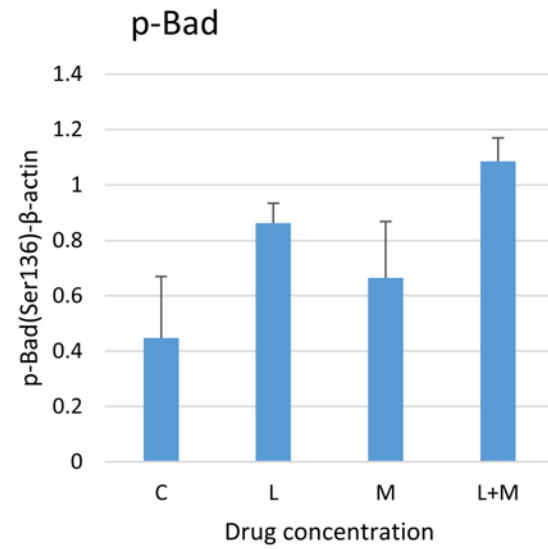
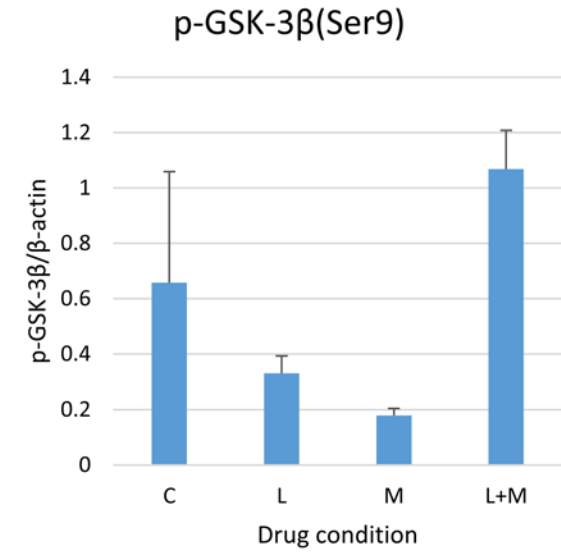
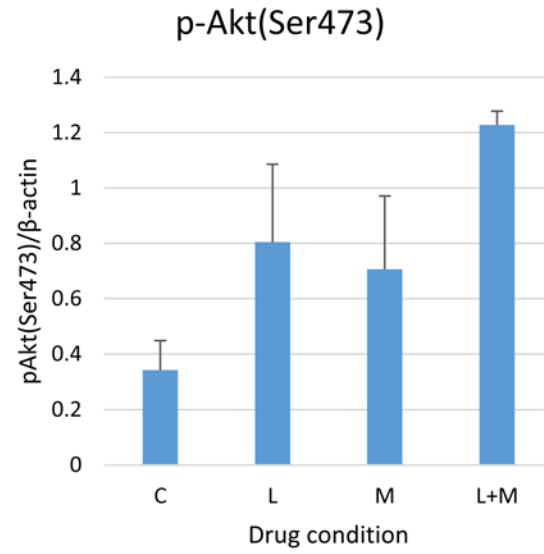
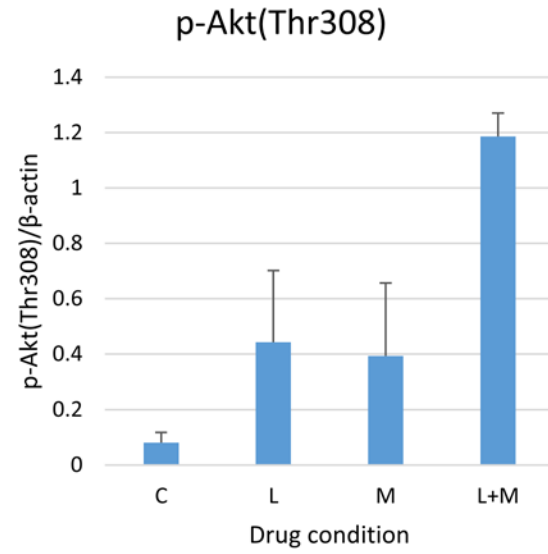


Combination Index Plot









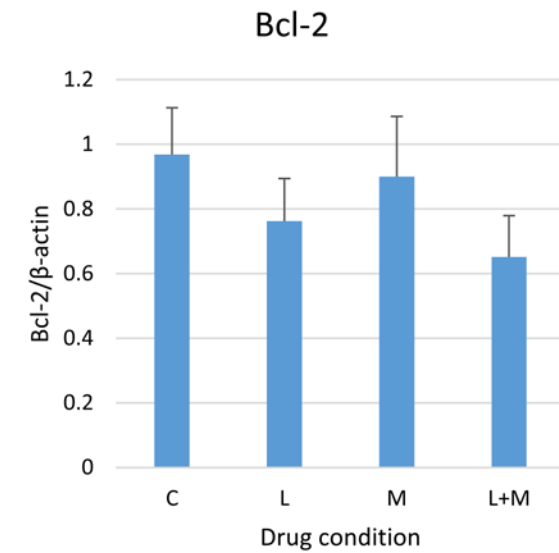
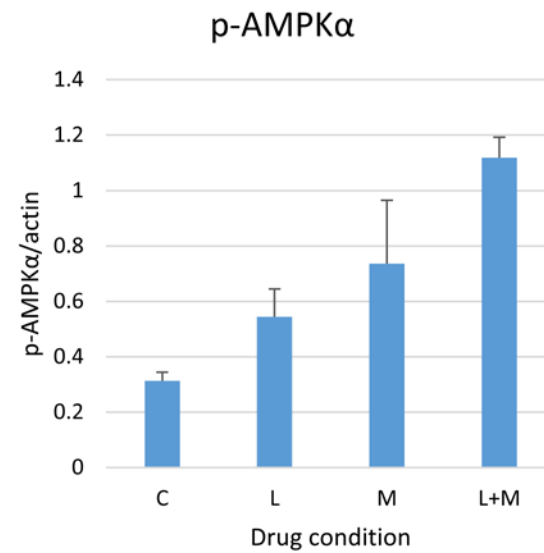
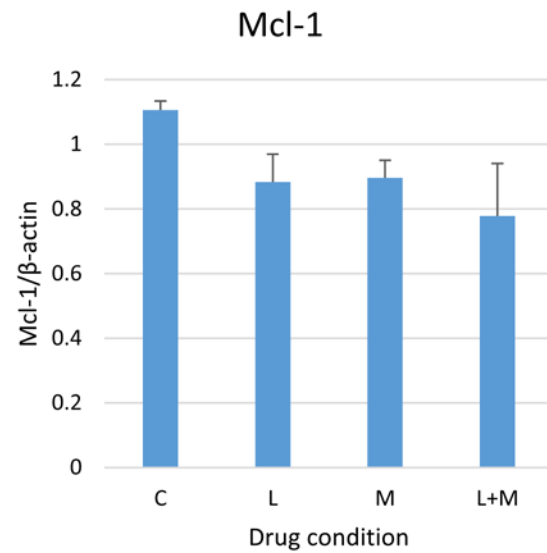
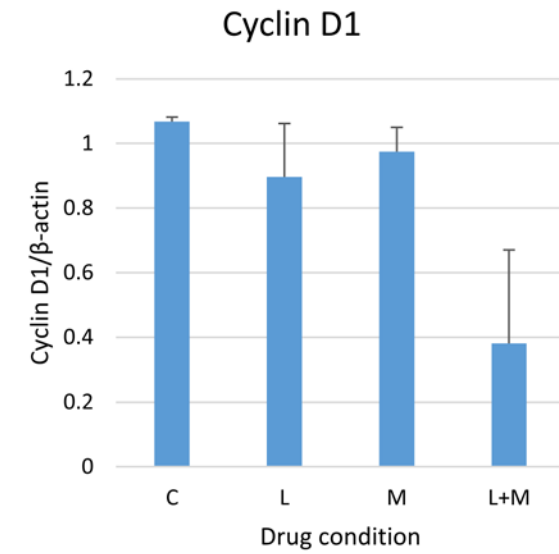
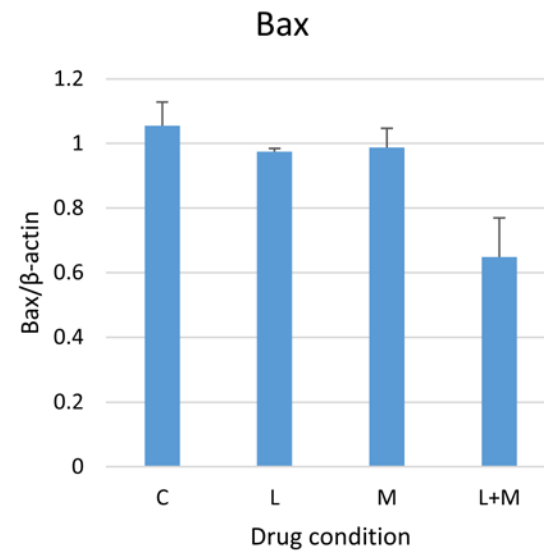
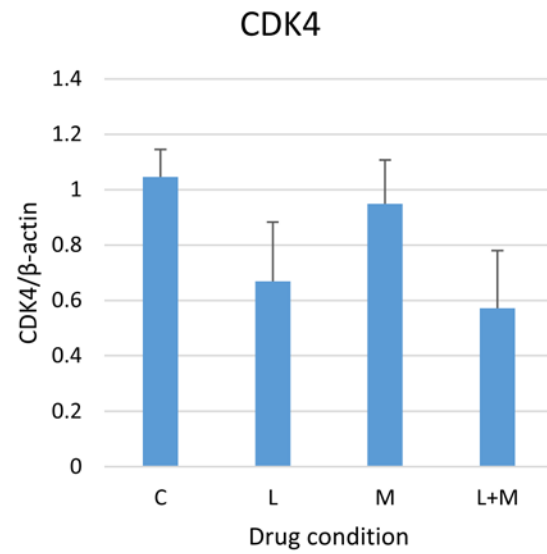


Table S1: Antibodies used in this study

Antibody name	1st Ab Dilution	MW (kDa)	Company	Host	2nd Ab Dilution
CDK1	1:200	34, 27	10762-1-AP, proteintech	Rabbit	1:5000
CDK4	1:1000	34	MA5-12984, ThermoFisher	Mouse	1:5000
CDK2	1:1000	34	MA5-17052, ThermoFisher	Mouse	1:5000
CyclinA2	1:500	56	18202-1-AP, proteintech	Rabbit	1:5000
CyclinB1	1:500	55-60	55004-1-AP, proteintech	Rabbit	1:5000
CyclinD1	1:200	36	MA5-16356, ThermoFisher	Rabbit	1:5000
P21	1:1000	21	#2947, Cell signaling Technology, Inc., USA	Rabbit	1:5000
P27	1:500	27	25614-1-AP, proteintech	Rabbit	1:5000
PI3K	1:1000	85	#4292, Cell signaling Technology, Inc., USA	Rabbit	1:5000
Total-AKT	1:1000	60	#4691, Cell signaling Technology, Inc., USA	Rabbit	1:5000
pAKT(Ser473)	1:1000	60	#4060, Cell signaling Technology, Inc., USA	Rabbit	1:5000
pAKT(Thr308)	1:1000	60	#4056, Cell signaling Technology, Inc., USA	Rabbit	1:5000
GSK-3 β	1:1000	46	#9315, Cell signaling Technology	Rabbit	1:5000
pGSK-3 β (Ser9)	1:1000	46	#9336, Cell signaling Technology	Rabbit	1:5000
Bad	1:1000	23	#9239, Cell signaling Technology, Inc., USA	Rabbit	1:5000
p-Bad (Ser136)	1:1000	23	#4366, Cell signaling Technology, Inc., USA	Rabbit	1:5000
Bcl-2	1:1000	26	#4223, Cell signaling Technology, Inc., USA	Rabbit	1:5000
Bax	1:1000	20	#5023, Cell signaling Technology	Rabbit	1:5000
Mcl-1	1:100	32, 40	sc-819, SANTA CRUZ BIOTECHNOLOGY, INC.	Rabbit	1:5000
β -actin (ACTB)	1:5000	43	MAB1501, Millipore	Mouse	1:5000

*MW: Molecular Weight