A

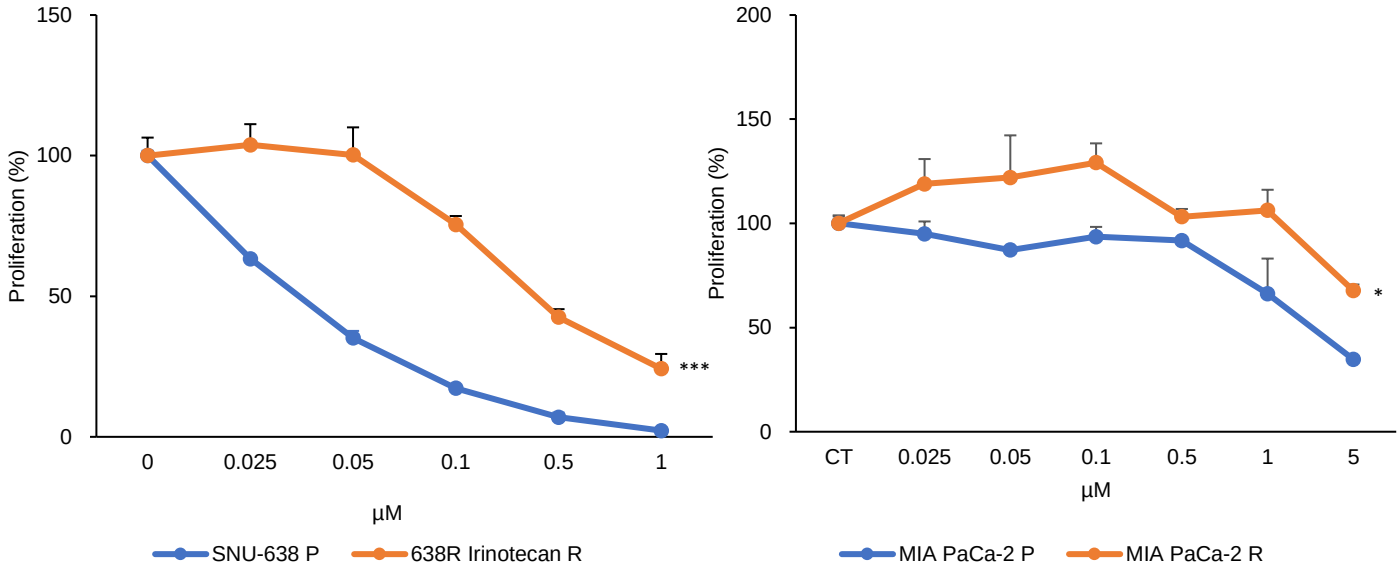


Figure S1. Irinotecan-resistant cancer cells were established through long-term treatment.
The effect of irinotecan on irinotecan-resistant SNU-638 and MIA PaCa-2 cells after 48 h was determined by SRB assay (n=3). Each bar represents the mean + s.d. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

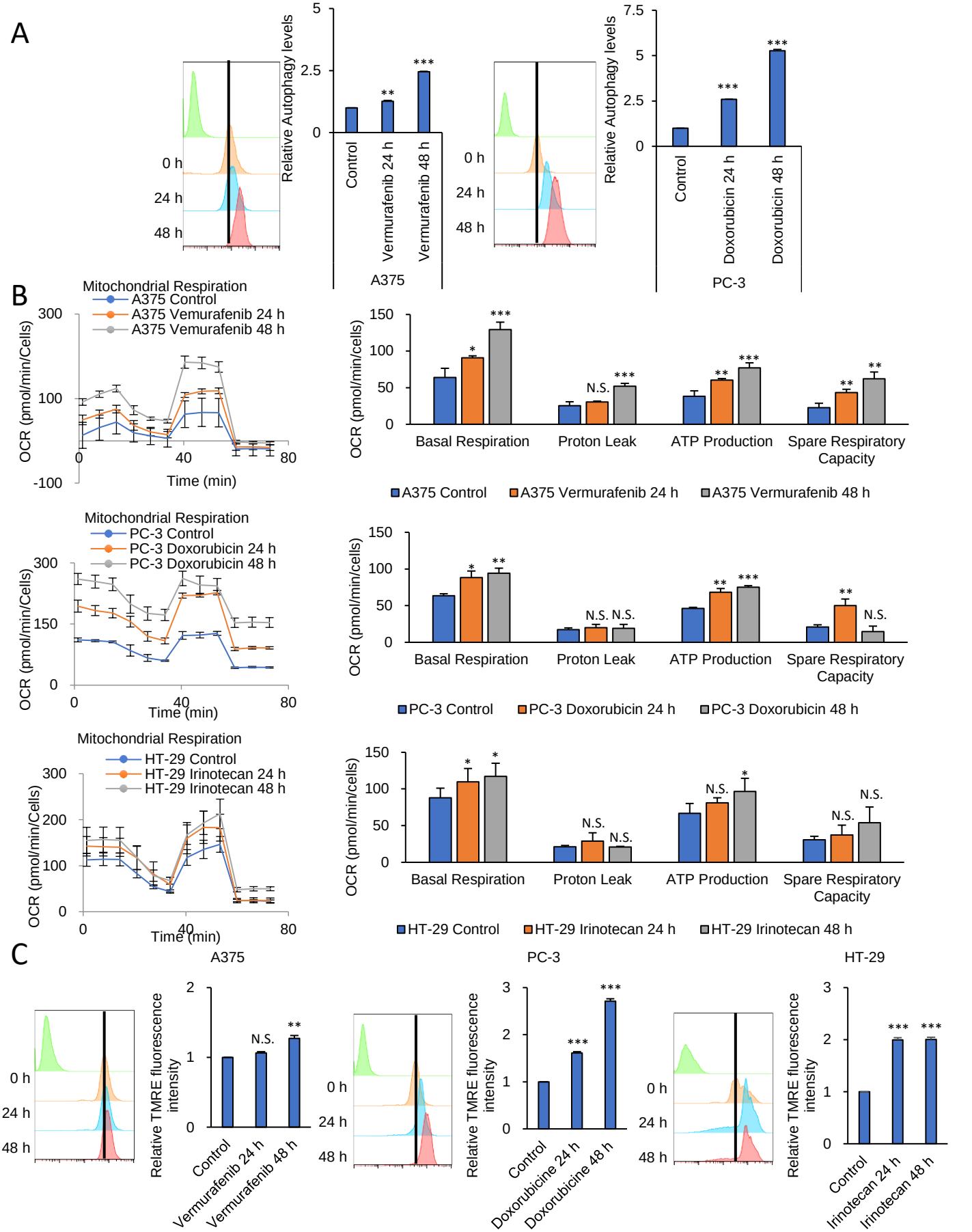
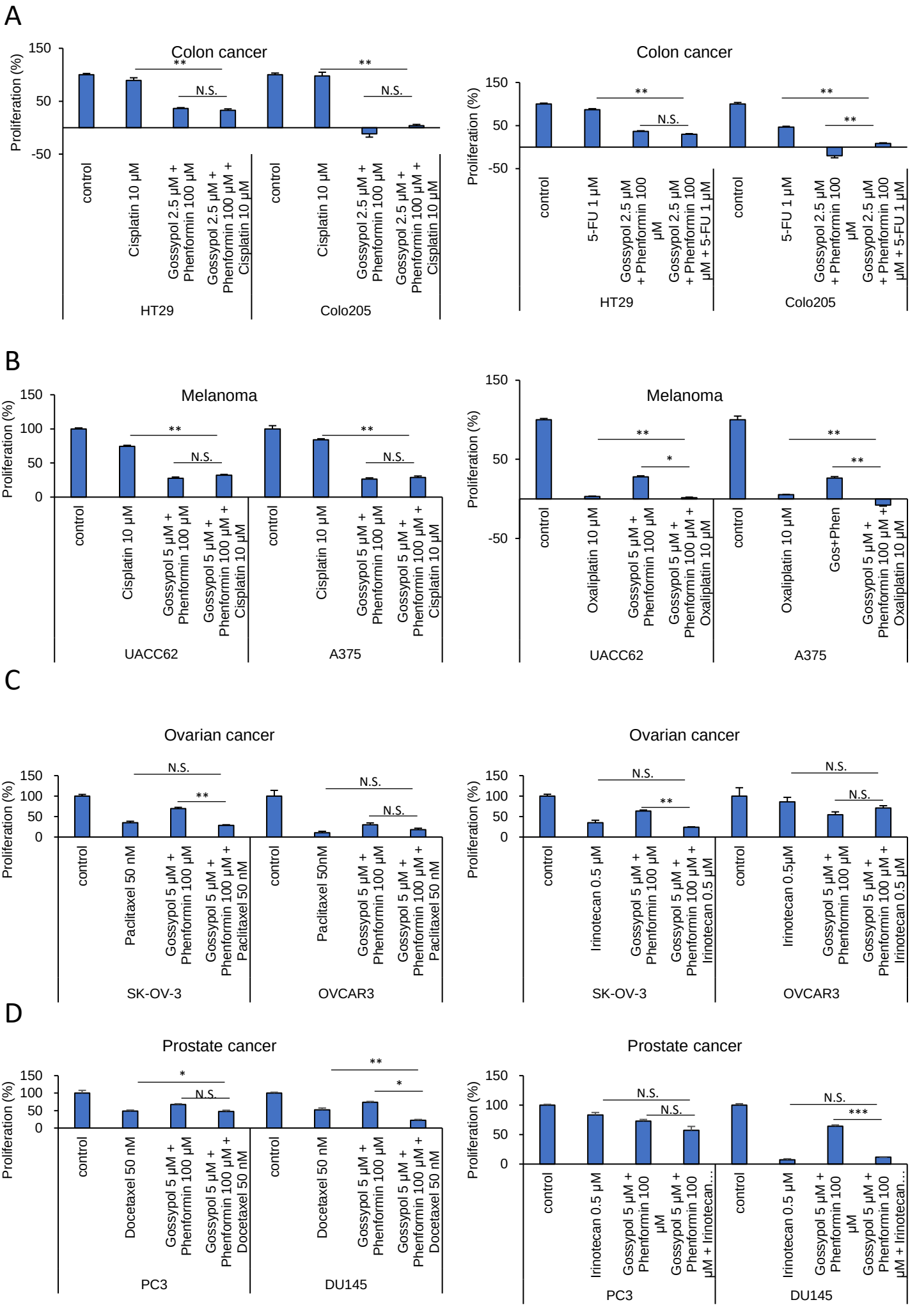


Figure S2. Anti-cancer drug treatment induces autophagy and Oxygen Consumption Rate.
(A) Autophagy levels were analyzed by Cyto-ID autophagy detection kit in anti-cancer drug treatment cancer cells and non-treatment cancer cells (Vermurafenib; 0.5 μM and Doxorubicin; 1 μM treatment). (B) Oxygen Consumption Rates and respiration parameters were analyzed in A375, PC-3 and HT-29 after treatment anti-cancer drug (Vermurafenib; 0.5 μM, Doxorubicin; 1 μM and Irinotecan; 0.5 μM) treatment for 24 h and 48 h (n=4). (C) Mitochondrial membrane potential was Analyzed by staining TMRE. Mitochondrial membrane potential was increased in anti-cancer drug treatment cancer cells compared to non-treatment cancer cells (n=3). Each bar represents the mean + s.d. *p < 0.05, **p < 0.01, ***p < 0.001.


A
Colon cancer
Proliferation (%)
150
50
-50
**
N.S.
**
N.S.
control
Cisplatin 10 µM
Gossypol 2.5 µM + Phenformin 100 µM
Gossypol 2.5 µM + Phenformin 100 µM + Cisplatin 10 µM
control
Cisplatin 10 µM
Gossypol 2.5 µM + Phenformin 100 µM
Gossypol 2.5 µM + Phenformin 100 µM + Cisplatin 10 µM
HT29
Colo205
Colon cancer
Proliferation (%)
150
50
-50
**
N.S.
**
**
control
5-FU 1 µM
Gossypol 2.5 µM + Phenformin 100 µM
Gossypol 2.5 µM + Phenformin 100 µM + 5-FU 1 µM
control
5-FU 1 µM
Gossypol 2.5 µM + Phenformin 100 µM
Gossypol 2.5 µM + Phenformin 100 µM + 5-FU 1 µM
HT29
Colo205
B
Melanoma
Proliferation (%)
150
100
50
0
**
N.S.
**
N.S.
control
Cisplatin 10 µM
Gossypol 5 µM + Phenformin 100 µM
Gossypol 5 µM + Phenformin 100 µM + Cisplatin 10 µM
control
Cisplatin 10 µM
Gossypol 5 µM + Phenformin 100 µM
Gossypol 5 µM + Phenformin 100 µM + Cisplatin 10 µM
UACC62
A375
Melanoma
Proliferation (%)
150
50
-50
**
*
**
**
control
Oxaliplatin 10 µM
Gossypol 5 µM + Phenformin 100 µM
Gossypol 5 µM + Phenformin 100 µM + Oxaliplatin 10 µM
control
Oxaliplatin 10 µM
Gos+Phen
Gossypol 5 µM + Phenformin 100 µM + Oxaliplatin 10 µM
UACC62
A375
C
Ovarian cancer
Proliferation (%)
150
100
50
0
N.S.
**
N.S.
N.S.
control
Paclitaxel 50 nM
Gossypol 5 µM + Phenformin 100 µM
Gossypol 5 µM + Phenformin 100 µM + Paclitaxel 50 nM
control
Paclitaxel 50nM
Gossypol 5 µM + Phenformin 100 µM
Gossypol 5 µM + Phenformin 100 µM + Paclitaxel 50 nM
SK-OV-3
OVCAR3
Ovarian cancer
Proliferation (%)
150
100
50
0
N.S.
**
N.S.
N.S.
control
Irinotecan 0.5 µM
Gossypol 5 µM + Phenformin 100 µM
Gossypol 5 µM + Phenformin 100 µM + Irinotecan 0.5 µM
control
Irinotecan 0.5µM
Gossypol 5 µM + Phenformin 100 µM
Gossypol 5 µM + Phenformin 100 µM + Irinotecan 0.5 µM
SK-OV-3
OVCAR3
D
Prostate cancer
Proliferation (%)
150
100
50
0
*
N.S.
**
*
control
Docetaxel 50 nM
Gossypol 5 µM + Phenformin 100 µM
Gossypol 5 µM + Phenformin 100 µM + Docetaxel 50 nM
control
Docetaxel 50 nM
Gossypol 5 µM + Phenformin 100 µM
Gossypol 5 µM + Phenformin 100 µM + Docetaxel 50 nM
PC3
DU145
Prostate cancer
Proliferation (%)
150
100
50
0
N.S.
N.S.
N.S.
***
control
Irinotecan 0.5 µM
Gossypol 5 µM + Phenformin 100 µM
Gossypol 5 µM + Phenformin 100 µM + Irinotecan…
control
Irinotecan 0.5 µM
Gossypol 5 µM + Phenformin 100 µM
Gossypol 5 µM + Phenformin 100 µM + Irinotecan…
PC3
DU145

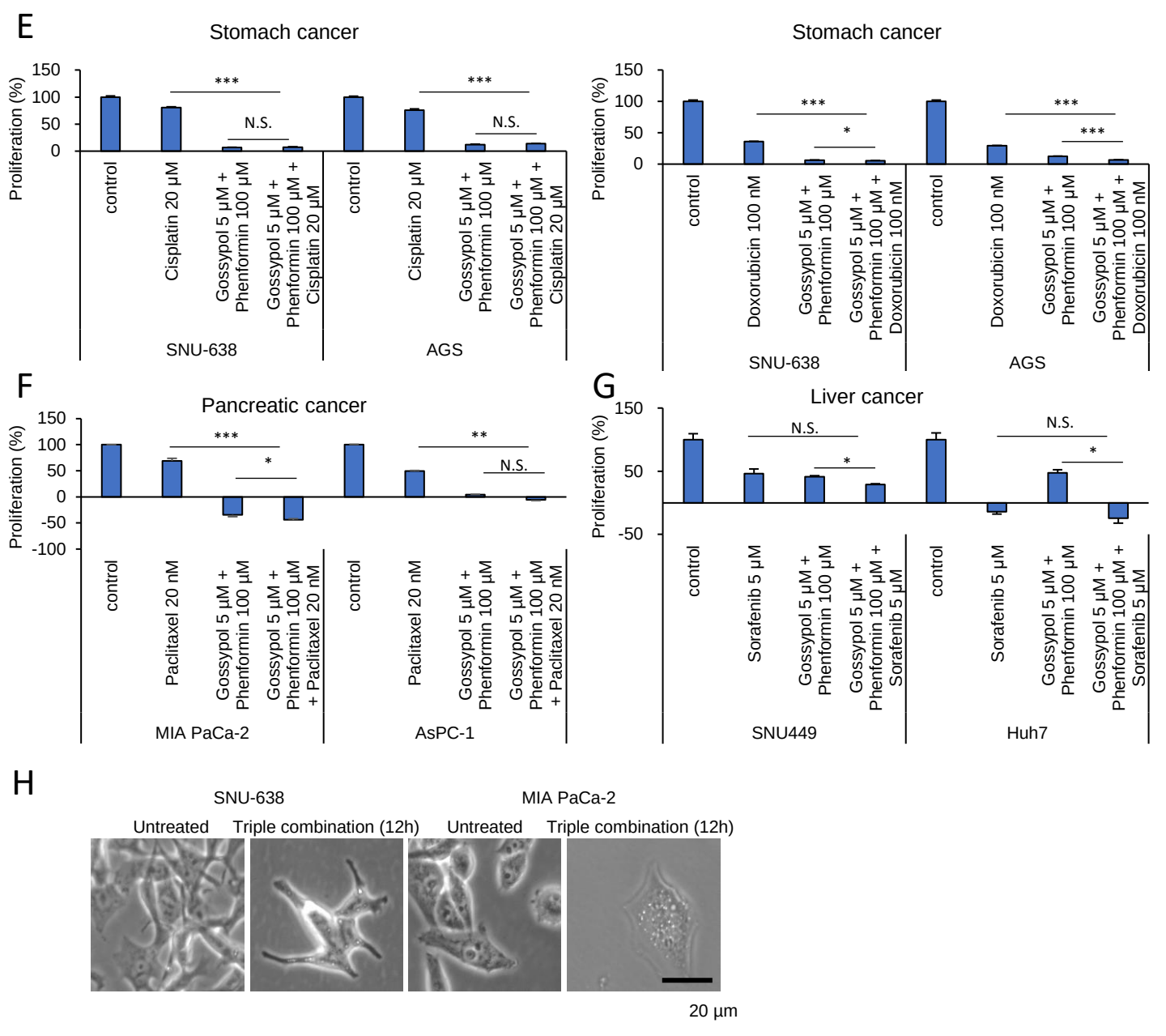


Figure S3. The cell death induced by triple combination treatment showed a Autophagic cell death morphology. (A-G) Non-synergistic effect of triple-combined treatment of 2.5 μM or 5 μM gossypol, 100 μM phenformin and indicated anti-cancer drug after 48 h on cell proliferation was determined by SRB assay. (H) MIA PaCa-2 and SNU-638 cells were treated with triple-combination of 5 μM gossypol, 100 μM phenformin and irinotecan (SNU-638; 0.5 μM and MIA PaCa-2 ; 1 μM) for indicated times, and then cells were imaged by means of a microscope. Scale bar = 20 μm.
Each bar represents the mean + s.d. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

A

Cell line	Colo 205
Mice	BALB/c-nude (Orient)
N (Head)	3
Drug delivery	Gossypol, Phenformin: PO Irinotecan: IP
Treatment on/off	Gossypol, Phenformin: On (6) Off (1) Irinotecan: On (1) Off (6)
Week	3
Drug dose	Gossypol: 80 mg/kg Phenformin: 100 mg/kg Irinotecan: 40 mg/kg
Drug vehicle	Gossypol and Phenformin: 5 % DMSO, 5 % cremophor and 90 % PBS Irinotecan: 5 % DMSO and 95 % water
IRB Number	NCC-18-435

B

Cell line	A375
Mice	BALB/c-nude (Orient)
N (Head)	7
Drug delivery	Gossypol, Phenformin and Vemurafenib: PO
Treatment on/off	Gossypol, Phenformin: On (6) Off (1) Vemurafenib: On (5) Off (2)
Week	3
Drug dose	Gossypol: 80 mg/kg Phenformin: 100 mg/kg Vemurafenib: 30 mg/kg
Drug vehicle	Gossypol and Phenformin: 5 % DMSO, 5 % cremophor and 90 % PBS Vemurafenib: 4 % DMSO + 30 % PEG300 + 5 % Tween 80 + 61 % water
IRB Number	NCC-18-435

C

Cell line	SK-OV-3
Mice	BALB/c-nude (Orient)
N (Head)	4
Drug delivery	Gossypol, Phenformin: PO Cisplatin: IP
Treatment on/off	Gossypol, Phenformin: On (6) Off (1) Cisplatin: On (2) Off (5)
Week	6
Drug dose	Gossypol: 40 mg/kg Phenformin: 100 mg/kg Cisplatin: 4 mg/kg
Drug vehicle	Gossypol and Phenformin: 5 % DMSO, 5 % cremophor and 90 % PBS Cisplatin: water
IRB Number	NCC-18-435

D

Cell line	PC-3
Mice	BALB/c-nude (Orient)
N (Head)	4
Drug delivery	Gossypol, Phenformin: PO Doxorubicine: PO
Treatment on/off	Gossypol, Phenformin: On (6) Off (1) Doxorubicin: On (5) Off (2)
Week	2
Drug dose	Gossypol: 80 mg/kg Phenformin: 100 mg/kg Doxorubicin: 5 mg/kg
Drug vehicle	Gossypol and Phenformin: 5 % DMSO, 5 % cremophor and 90 % PBS Doxorubicin: 5 % DMSO and 95 % water
IRB Number	NCC-18-435

E

Cell line	SNU-638
Mice	BALB/c-nude (Orient)
N (Head)	5
Drug delivery	Gossypol, Phenformin: PO Irinotecan: IP
Treatment on/off	Gossypol, Phenformin: On (6) Off (1) Irinotecan: On (1) Off (6)
Week	5
Drug dose	Gossypol: 80 mg/kg Phenformin: 100 mg/kg Irinotecan: 10 mg/kg
Drug vehicle	Gossypol and Phenformin: 5 % DMSO, 5 % cremophor and 90 % PBS Irinotecan: 5 % DMSO and 95 % water
IRB Number	NCC-20-558

F

Cell line	MIA PaCa-2
Mice	BALB/c-nude (Orient)
N (Head)	5
Drug delivery	Gossypol, Phenformin: PO Irinotecan: IP
Treatment on/off	Gossypol, Phenformin: On (6) Off (1) Irinotecan: On (1) Off (6)
Week	4
Drug dose	Gossypol: 80 mg/kg Phenformin: 100 mg/kg Irinotecan: 40 mg/kg
Drug vehicle	Gossypol and Phenformin: 5 % DMSO, 5 % cremophor and 90 % PBS Irinotecan: 5 % DMSO and 95 % water
IRB Number	NCC-20-558

Table S1. Xenograft mouse models conditions table.