

Supplementary Material: Metformin and ICG-001 Act Synergistically to Abrogate Cancer Stem Cells-Mediated Chemoresistance in Colorectal Cancer by Promoting Apoptosis and Autophagy

Souvick Roy, Yinghui Zhao, Yate-Ching Yuan and Ajay Goel

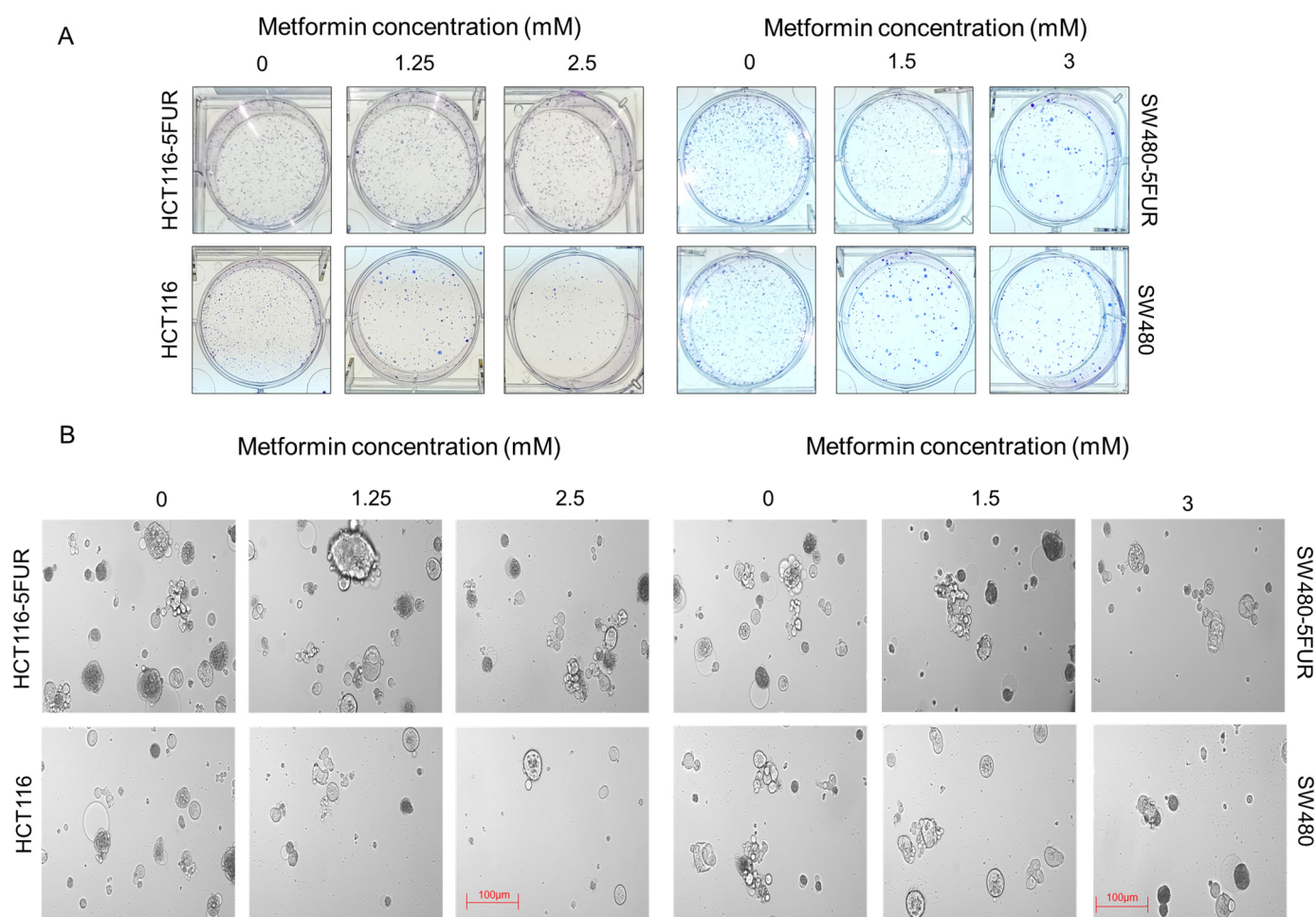
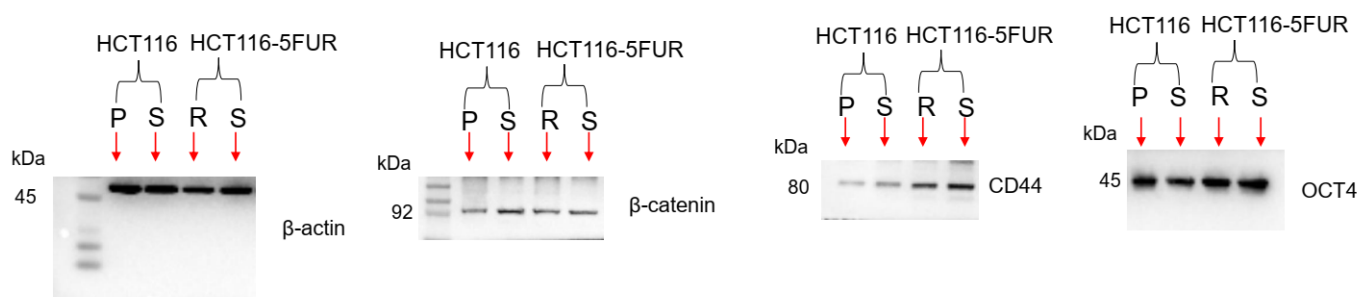
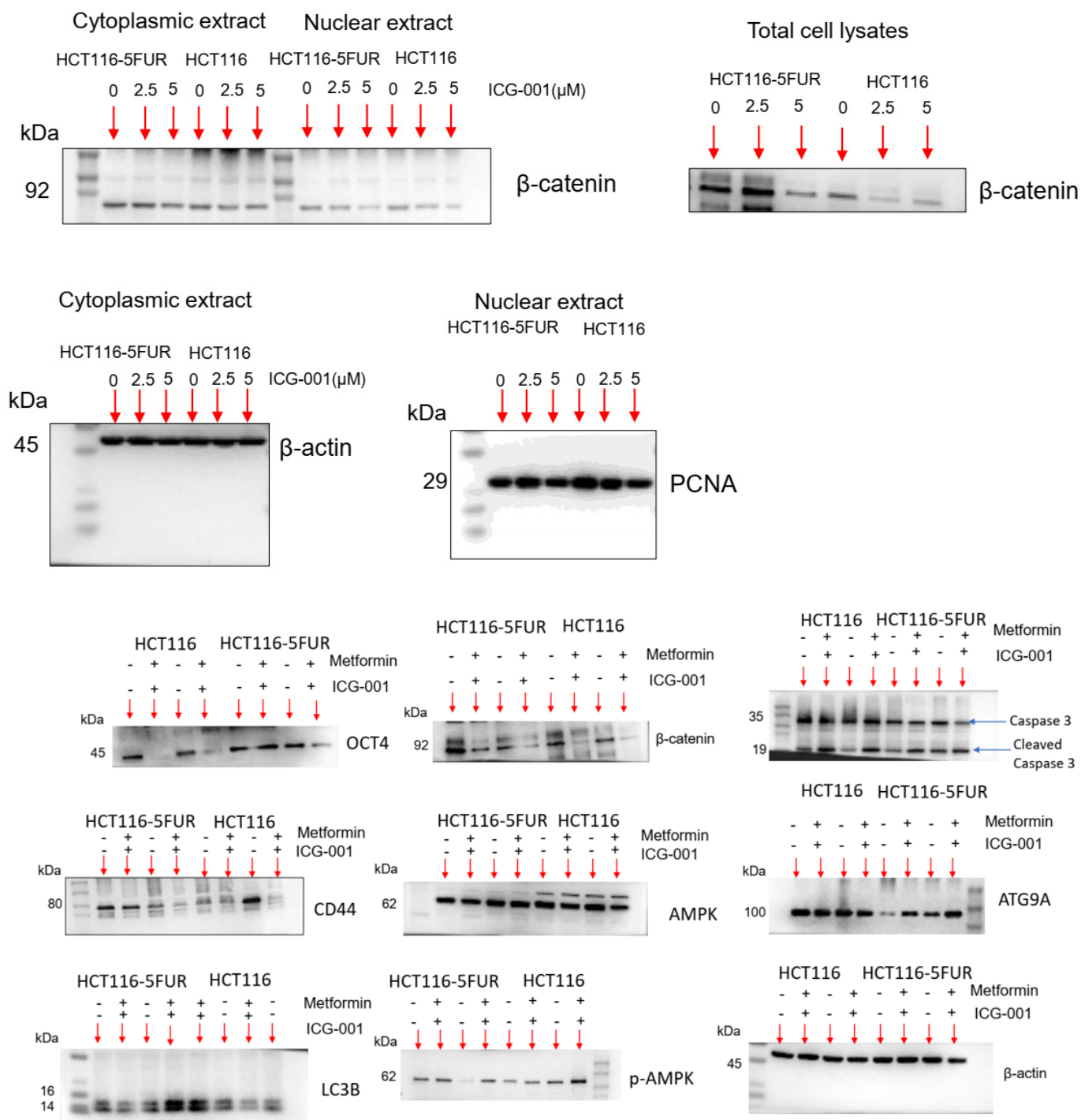


Figure S1. (A) Colony forming assay and (B) spheroid forming assay after treatment with different concentrations of metformin for 48 hours in HCT116 and SW480-5FUR and parental cells.





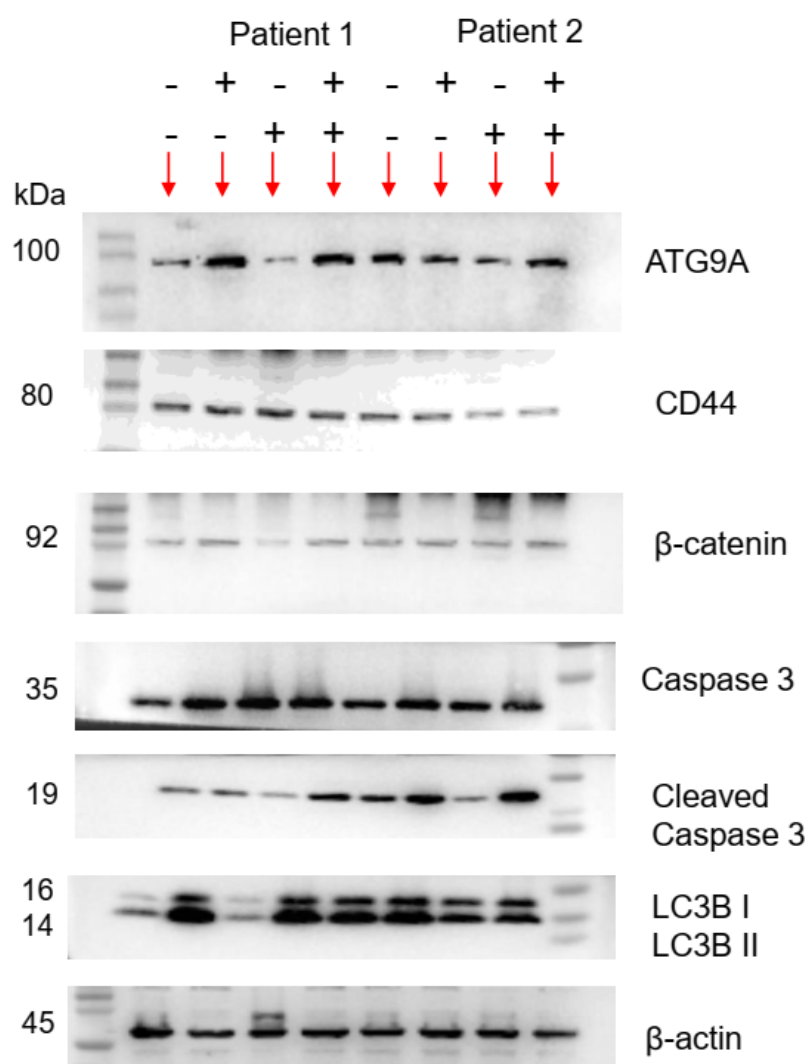


Figure S2. Original blots.

Table S1. Clinical details of the CRC patients used for tumor organoid generation.

SI No	Patient ID	Age	Gender	Ethnicity	Location of Tumor	TNM Stage	Pathological Stage
1	Patient 1	67	Female	Caucasian	Cecum	pT4b N1b	Moderate
2	Patient 2	40	Male	Caucasian	Rectosigmoid	pT3 N0	Moderate

Table S2. List of primer sequences.

Genes	Forward Primer	Reverse Primer
β-catenin	5'-GCTTTCAGTTGAGCTGACCA-3'	5'-CAAGTCCAAGATCAGCAGTCTC-3'
CD44	5'-CGGACACCATGGACAAGTTT-3'	5'-CCGTCCGAGAGATGCTGTAG-3'
OCT4	5'-CCTGAAGCAGAAGAGGATCA-3'	5'-CCGCAGCTTACACATGTTCT-3'
Caspase 3	5'-CAAACCTTTTTCAGAGGGGATCG-3'	5'-GCATACTGTTTCAGCATGGCAC-3'
LC3B	5'-CGGAAAGCAGCAGTGTACCA-3'	5'-GGCAGAAGGGAGTGTGTCTGA-3'
β-actin	5'-TCACCCACACTGTGCCCATCTACGA-3'	5'-CAGCGGAACCGCTCATTGCCAATG-3'

Table S3. IC₅₀ value of metformin and ICG-001 in different CRC cells.

Drug	Cell Line	IC ₅₀
Metformin	HCT116	2.5 ± 0.3
	SW480	3.0 ± 0.4
	HCT116-5FUR	5.0 ± 0.5
	SW480-5FUR	7.6 ± 0.4
	SW620	7.4 ± 0.5
	RKO	8.0 ± 0.6
	HT29	5.9 ± 0.4
ICG-001	HCT 116	4.4 ± 0.5
	SW480	4.7 ± 0.3
	HCT 116 5FUR	6.5 ± 0.5
	SW480 5FUR	5.6 ± 0.3

Table S4. List of top 10 upregulated and downregulated genes between 5FU-resistant and parental colorectal cancer cells.

Upregulated Genes		Downregulated Genes	
Gene symbol	Log ₂ Fc	Gene symbol	Log ₂ Fc
SPARC	8.03	PSG1	−10.91
ADD2	7.16	KRT5	−9.24
OASL	6.30	TRIM29	−8.95
IFIT2	6.29	NELL2	−8.67
IFIT1	5.95	CLDN1	−8.59
CCL5	5.73	MAP7D2	−8.55
IFIT3	5.50	NRIP1	−8.32
CHRM1	5.32	CSMD3	−8.24
LONRF2	5.23	CBS	−8.19
FEV	5.10	ARHGEF38	−8.15
Log ₂ Fc= 5FU-resistant vs Parental			