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Supporting Information

Sulforaphane is Synergistic with CB-5083 and Inhibits Colony Formation of CB-5083-Resistant HCT116 Cells

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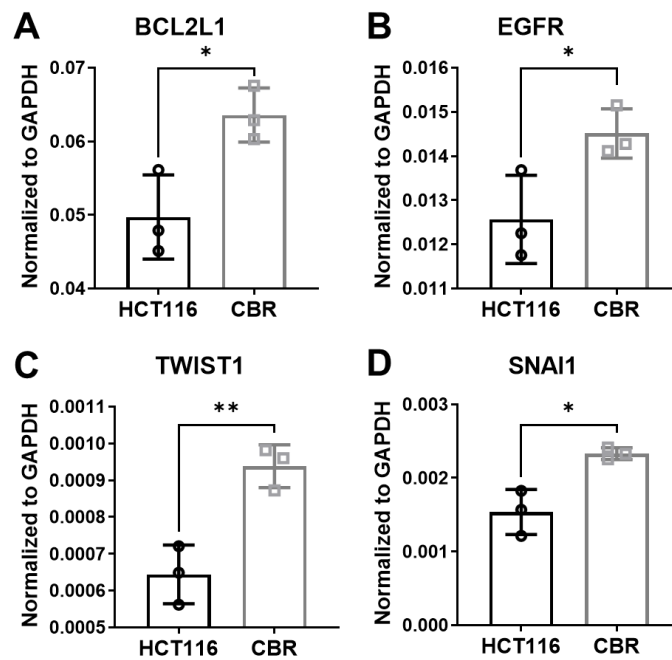# Table of Contents

Supplemental Figures

Figure S1. qPCR analysis on the basal levels of NF-κB target genes, BCL2L1 (A), EGFR (B), TWIST1 (C) and SNAI1 (D) in parental HCT116 cells and CBR cells, n=3. Data are presented as the mean ± SD. *, $p < 0.05$; **, $p < 0.01$; according to unpaired t test.
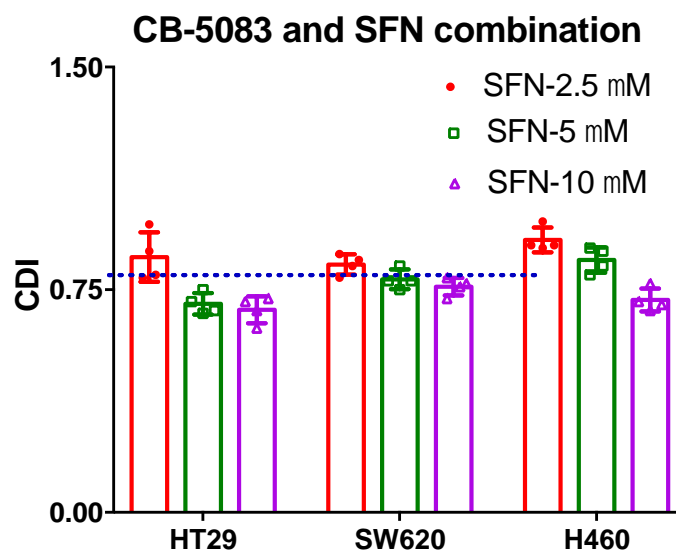

Figure S2. The combination of CB-5083 and SFN displayed synergistic enhancement of anti-proliferation effects on multiple human cancer cell lines. Cells were treated with 0.5 μM CB-5083 alone or 0.5 μM CB-5083 plus different concentration of SFN for 96 hrs, n=4. Data are presented as the mean ± SD.
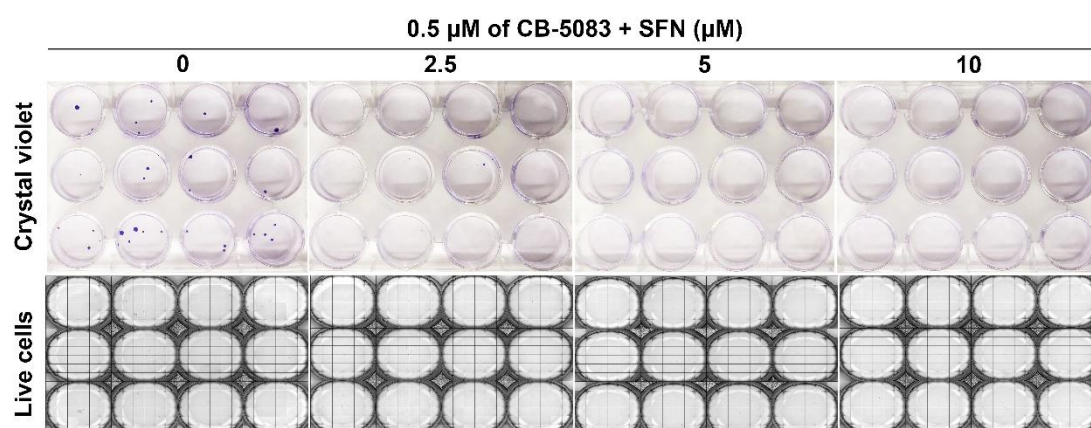
Figure S3. SFN inhibits the formation and proliferation of CB-5083 resistant HCT116 cells. 2*10^5 cells were seeded on one well of 12 well plate. 24 hrs after seeding, cells were treated with 0.5 μM CB-5083 alone or 0.5 μM CB-5083 plus different concentration of SFN. Images were taken after 10 days of treatment, n=12.

Supplemental Tables

Table S1. Plasmids used in this study.

Plasmid Number	Plasmid name	Vector	Source and Reference
TCB-197	Human p97 pET15_T	pET15b_TEV linker	Chou, 2014 [1]
TCB-599	Human D649A/A659T p97 pET15_T	pET15b_TEV linker	This study

Table S2. PCR and Sequencing primers used in this study.

Primer name	Primer sequence
PCR Primer 1: VCP-Cloning F	5'- CAG CGT TGT TCG CCC -3'
PCR Primer 2: VCP-Cloning R	5'- ACC CCC AGG GAA CAA G -3'
Sequencing Primer 1	5'- CAG CGT TGT TCG CCC -3'
Sequencing Primer 2	5'- GCA TCC AGC CAT GCC CTG ATG TG -3'
Sequencing Primer 3	5'- GAC CCT GAT TGC TCG AGC TG -3'
Sequencing Primer 4	5'- GAA CAG GTA GCC AAT GAG ACT -3'
Sequencing Primer 5	5'- GAC AAA TTC CTG AAG TTT GGC -3'
Sequencing Primer 6	5'- CTA ACC TGC GCA AGT CCC AGT TG -3'

Table S3. SYBR green qPCR primers used in this study.

Primer name	Primer sequence
human GAPDH F	5'- GAAGGTGAAGGTCGGAGTC -3'
human GAPDH R	5'- GAAGATGGTGATGGGATTTC -3'
human BCL2L1 F	5'- GGAGAACGGCGGCTGGGATA -3'
human BCL2L1 R	5'- GGCCACAGTCATGCCCGTCA -3'
human EGFR F	5'- AACACCCTGGTCTGGAAGTACG -3'
human EGFR R	5'- TCGTTGGACAGCCTTCAAGACC -3'
human SNAI1 F	5'- ATGCACATCCGAAGCCACA -3'
human SNAI1 R	5'- GAGGGTCAGCGGGGACATC -3'
human TWIST1 F	5'- GGAGTCCGCAGTCTTACGAG -3'
human TWIST1 R	5'- TCTGGAGGACCTGGTAGAGG -3'

Table S4. TaqMan qPCR probes used in this study.

Target	Catalog number
GAPDH	Hs02786624_g1
p97	Hs00997642_m1
CHOP	Hs00358796_g1

Table S5. Primary and secondary antibodies used in this study.

Antibodies	Source	Catalog number	RRID
Anti-p62	MBL	M162-3	AB_1279299
Anti-GAPDH	CST	2118	AB_561053
Anti-Lamin B1	Proteintech	66095-1-Ig	AB_11232208
Anti-k48	Boston Biochem	A-101	AB_10699867
Anti-p97	Thermo Fisher	MA3-004	AB_2214638
Anti-ATF4	Santa Cruz	sc-200	RRID:AB_2058752
Anti-CHOP	CST	2895	RRID:AB_2089254
Anti-IkBa	CST	9242S	RRID:AB_331623
Anti-phos-IkBa	CST	5209S	RRID:AB_10829358
Anti-p65	CST	8242	RRID:AB_10859369
Goat Anti-Rabbit IgG-HRP Conjugate	BIO-RAD	170-6515	AB_11125142
Goat Anti-Mouse IgG-HRP Conjugate	BIO-RAD	170-6516	AB_11125547

Reference

[1]   T. F. Chou, Bulfer, S. L., Weihl, C. C., Li, K., Lis, L. G., Walters, M. A., Schoenen, F. J., Lin, H. J., Deshaies, R. J., and Arkin, M. R., *J. Mol. Biol.* **2014**, *426*(15), 2886-2899.