

1 **SREBP2-activated CNPY3 phase separation promotes colorectal cancer by enhancing**

2 **MDM2-mediated p53 degradation**

3

4 **Supplementary Figures:**

5 Figure S1 to S19

6 **Supplementary Material 1 includes:**

7 Table S1 to S5

8 Supplementary figure legends

9 **Raw images**

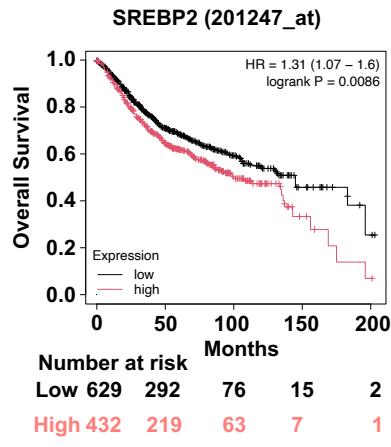
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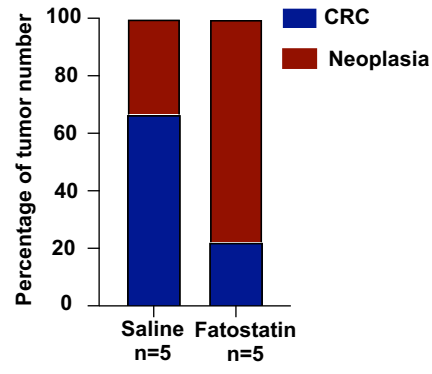
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Figure S1

A



B



C

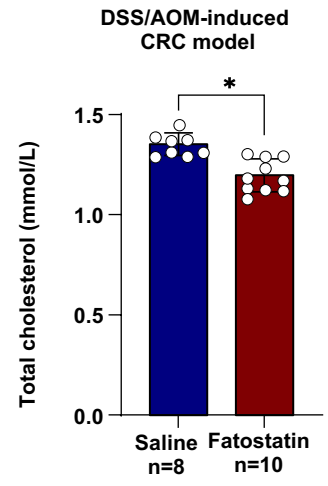
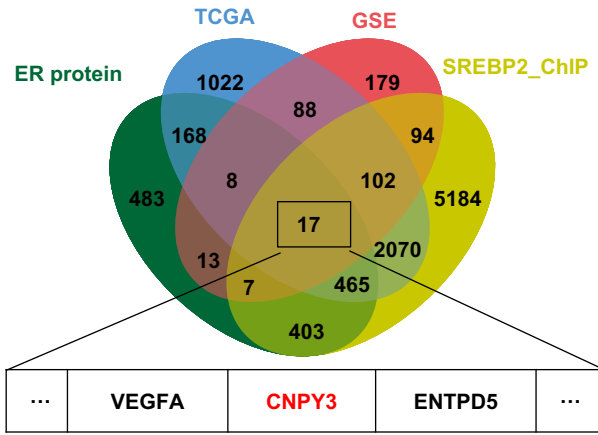
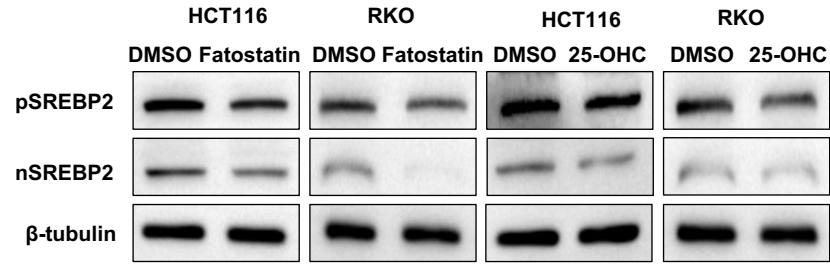


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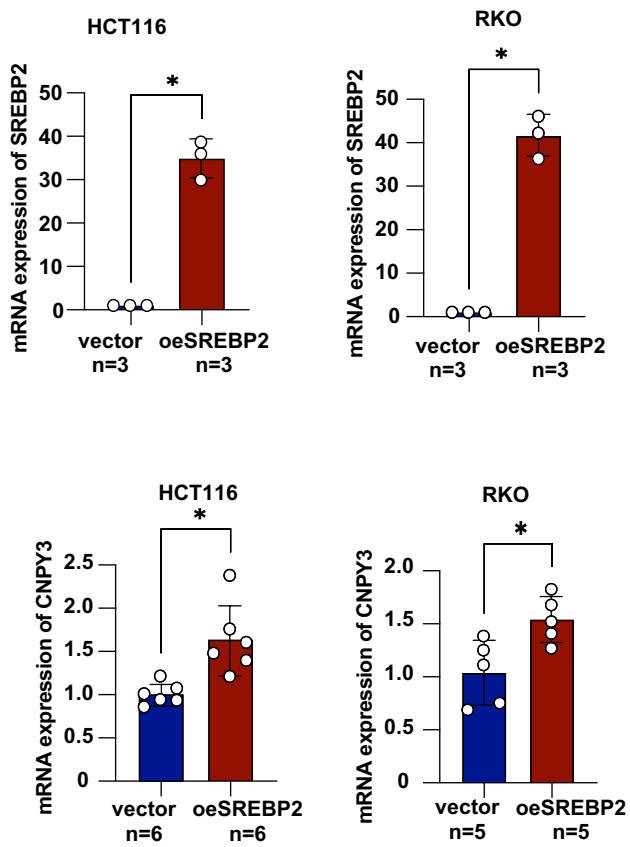
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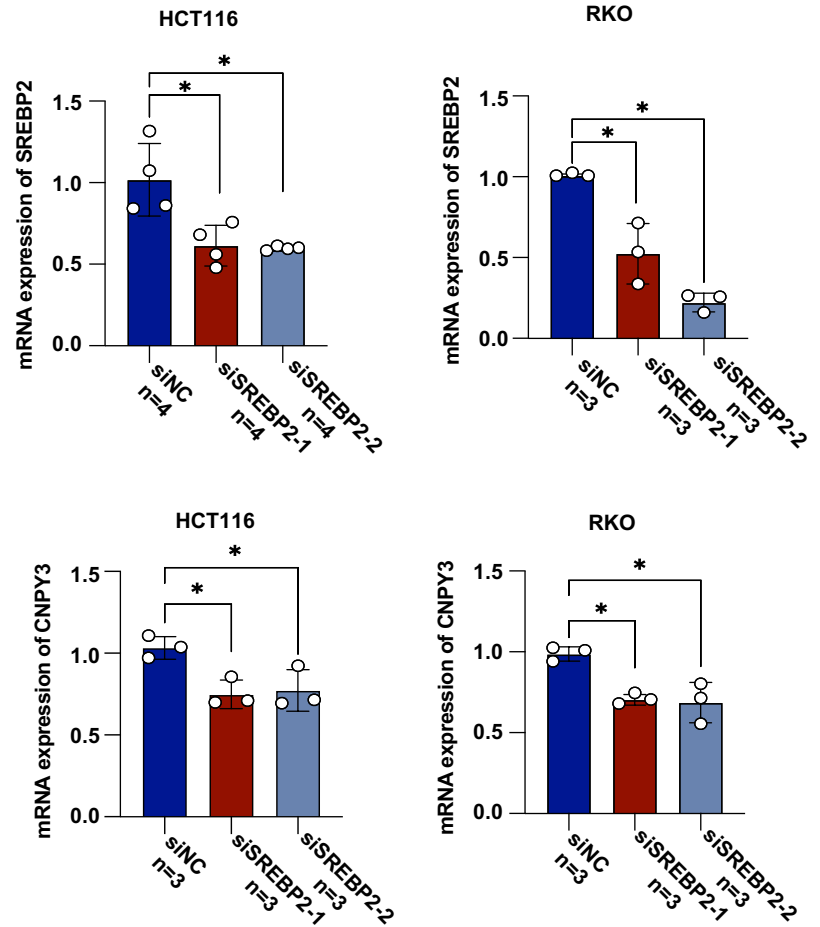
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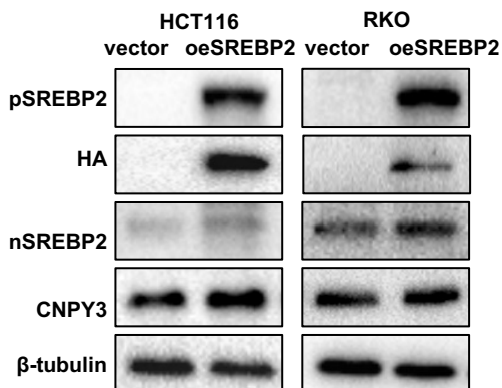
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E



D



F

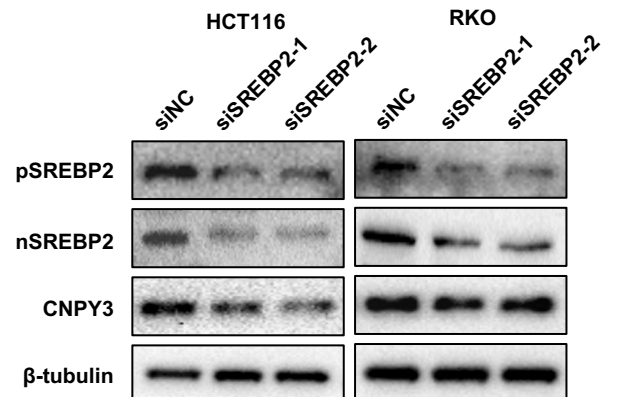


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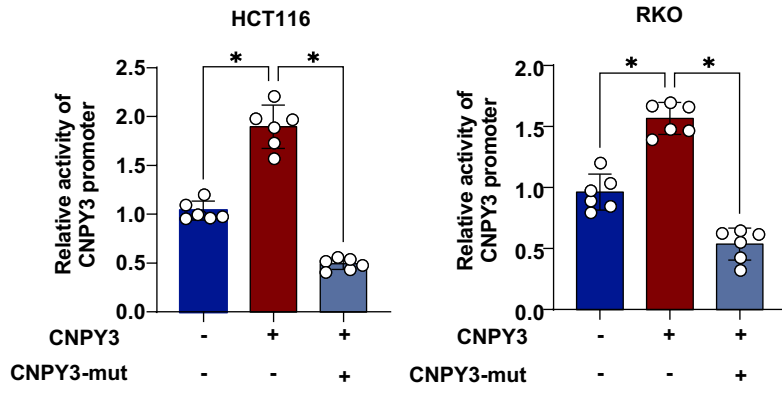
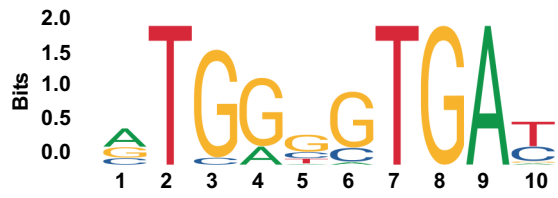
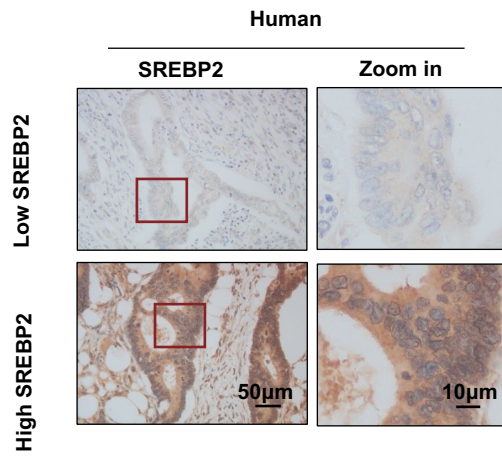


Figure S4

A



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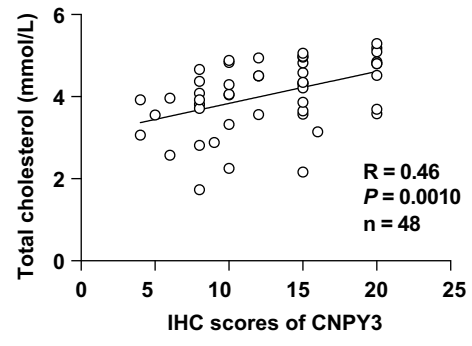
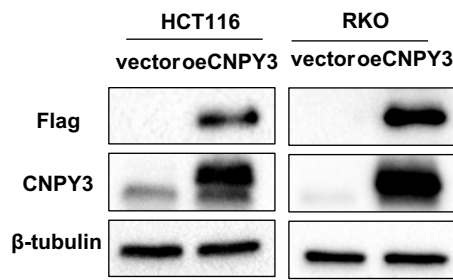
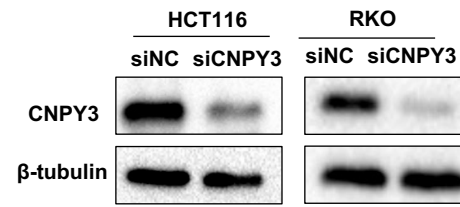


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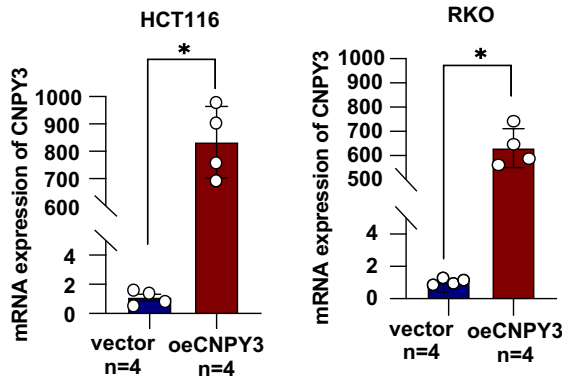
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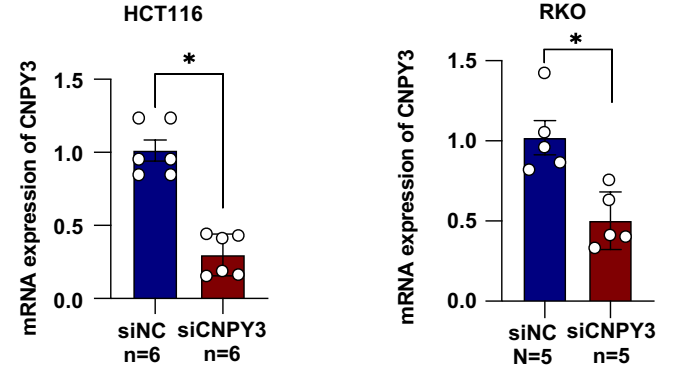
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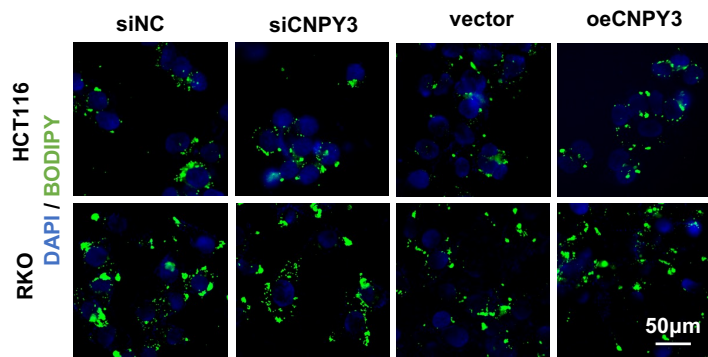
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D



E



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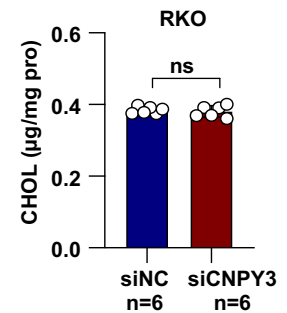
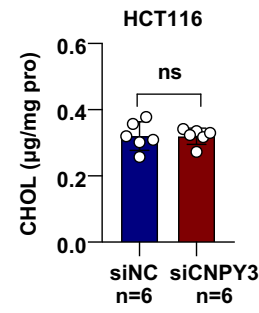
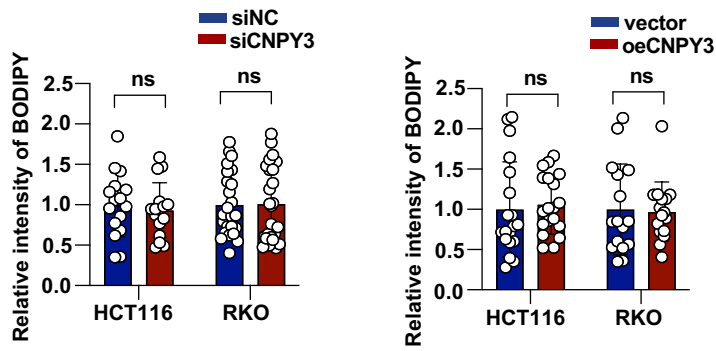
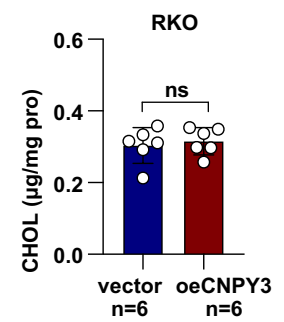
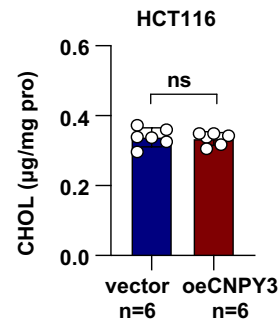
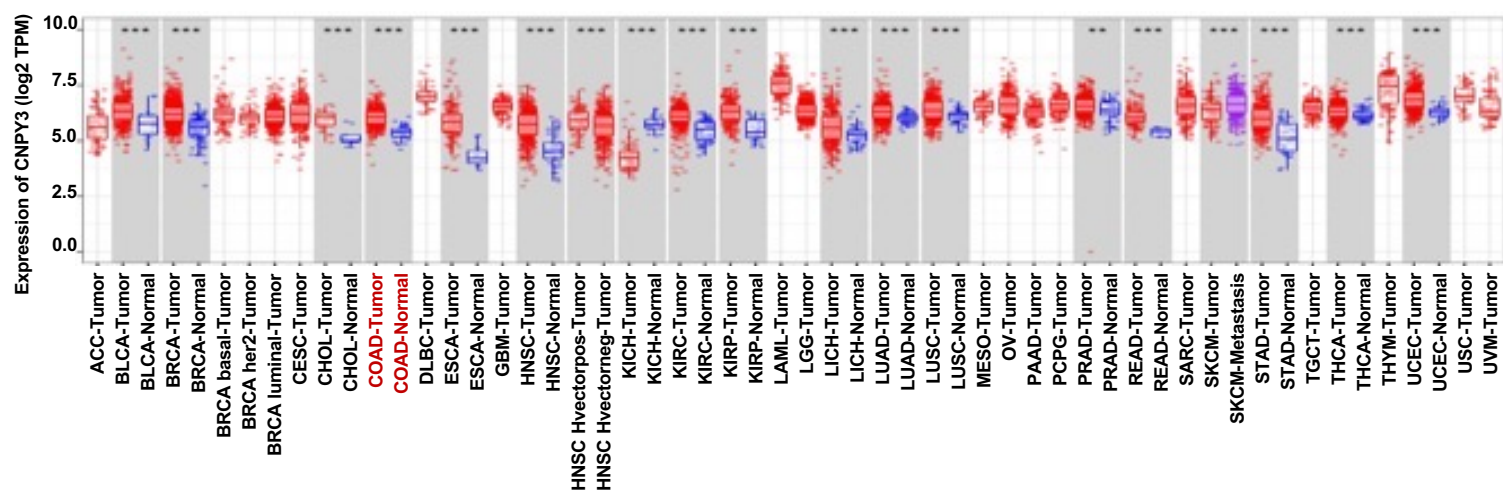
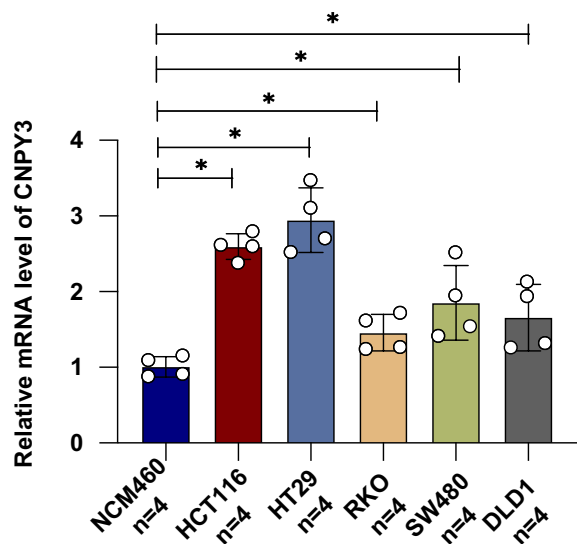


Figure S6

A



B



C

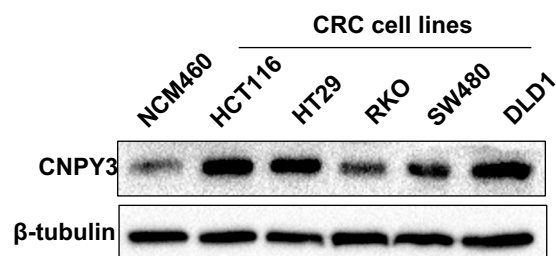
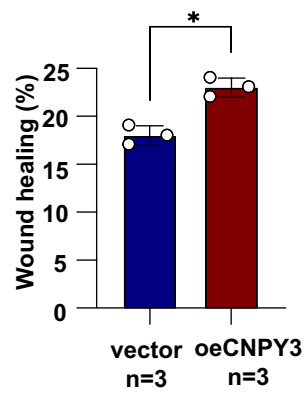
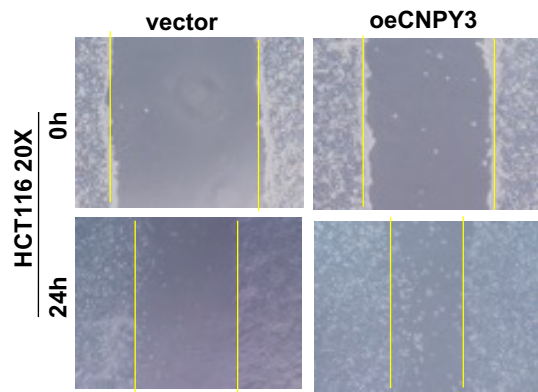


Figure S7

A



B

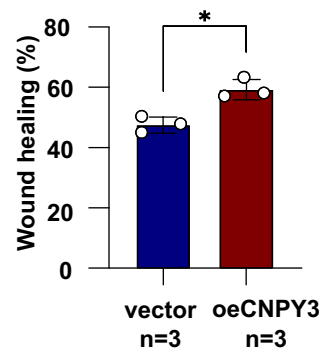
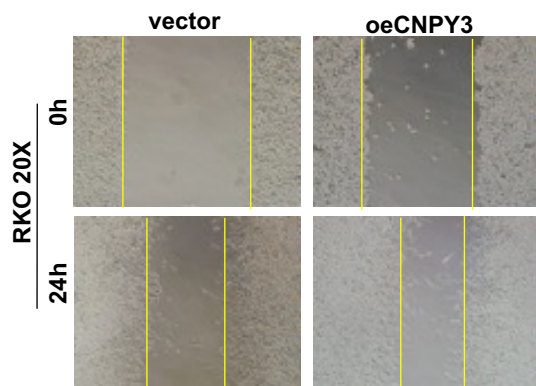
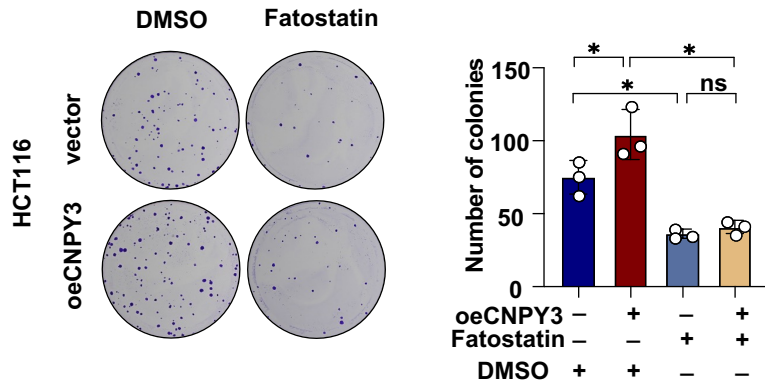


Figure S8

A



B

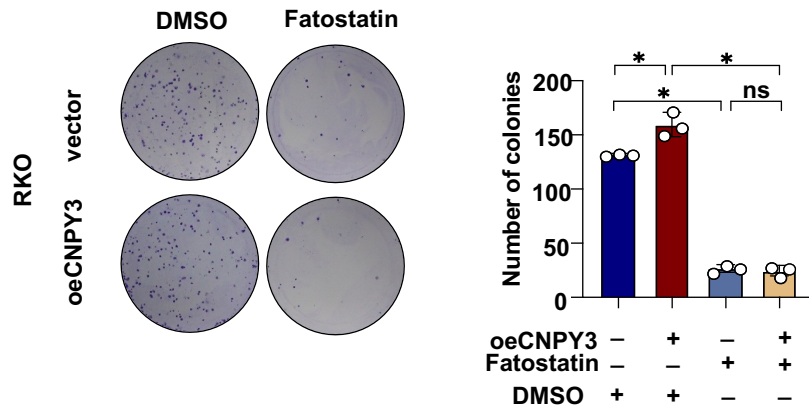
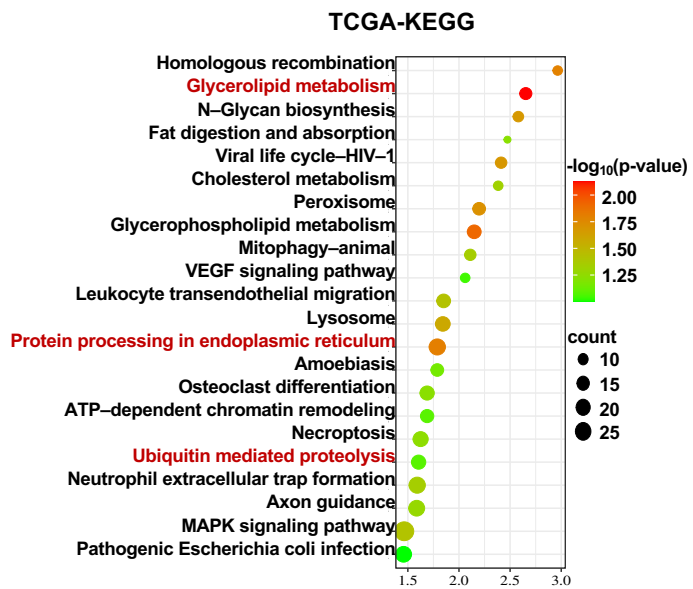


Figure S9

A



B

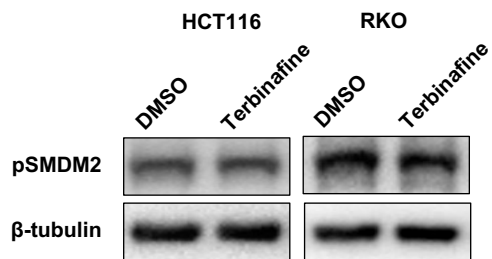


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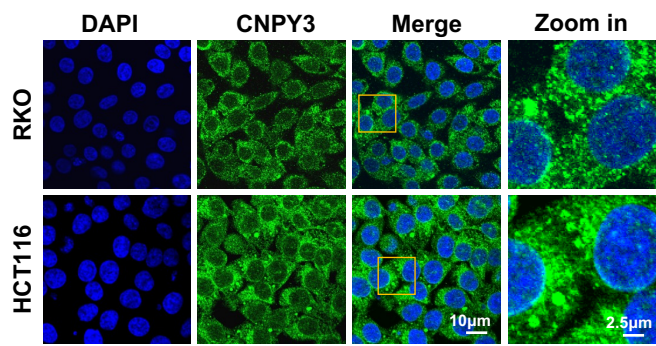
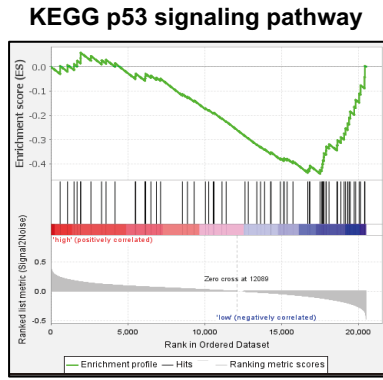


Figure S11

A



B

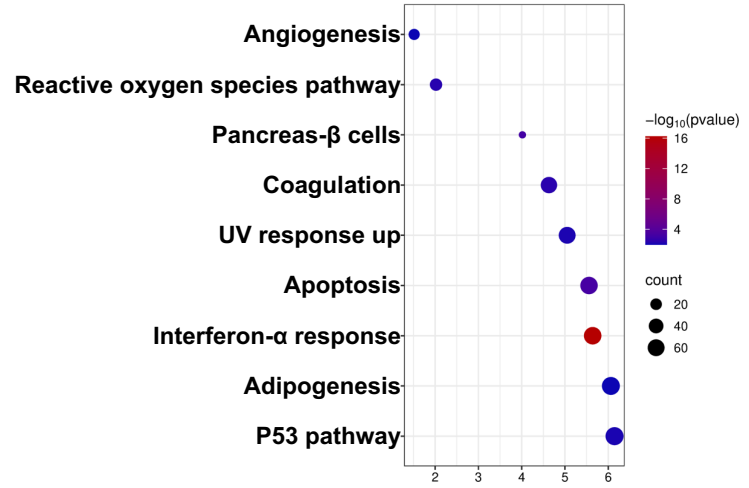


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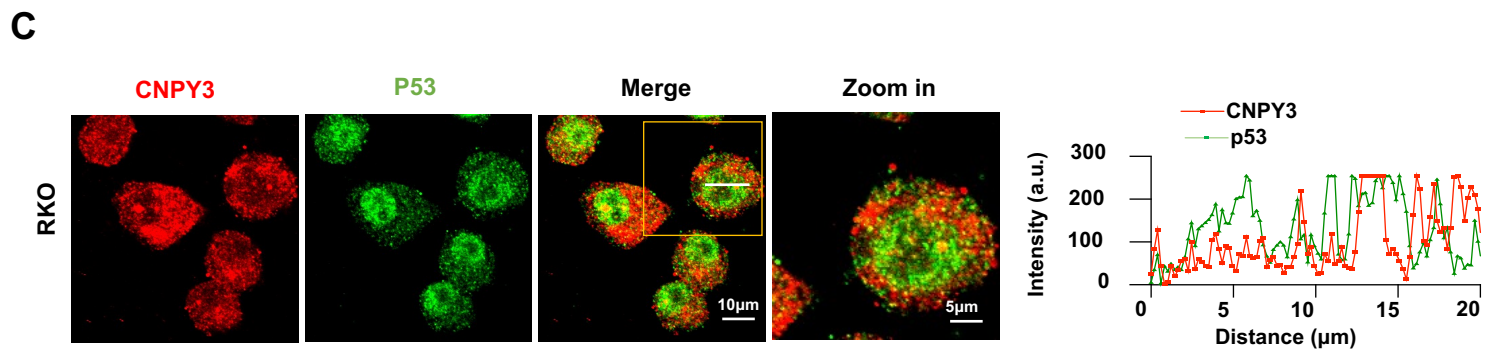
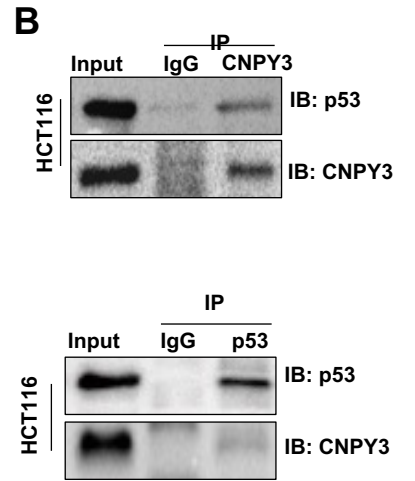
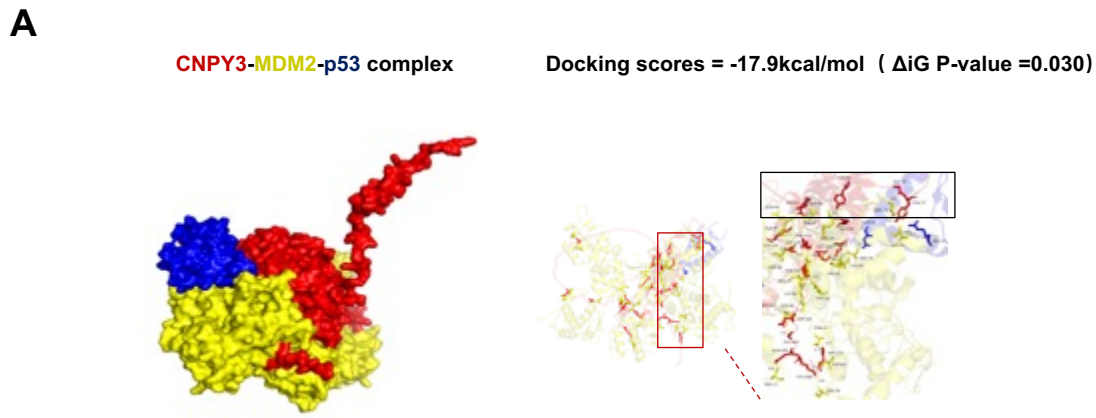
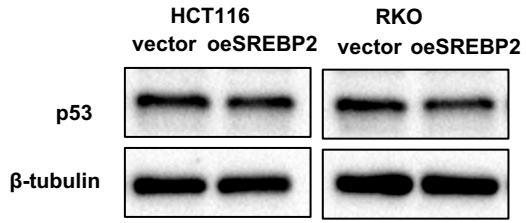
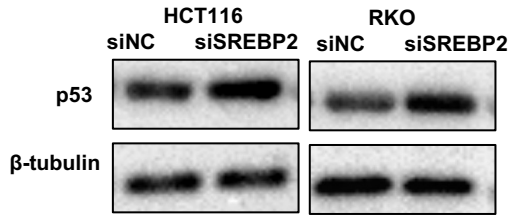


Figure S13

A



B



C

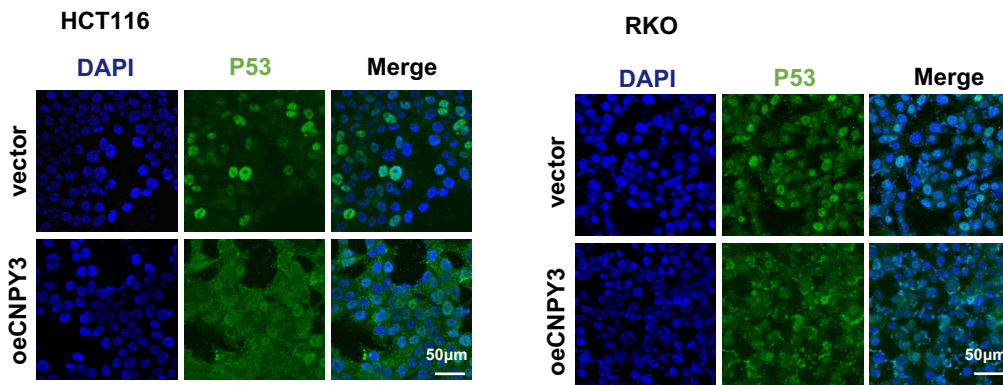
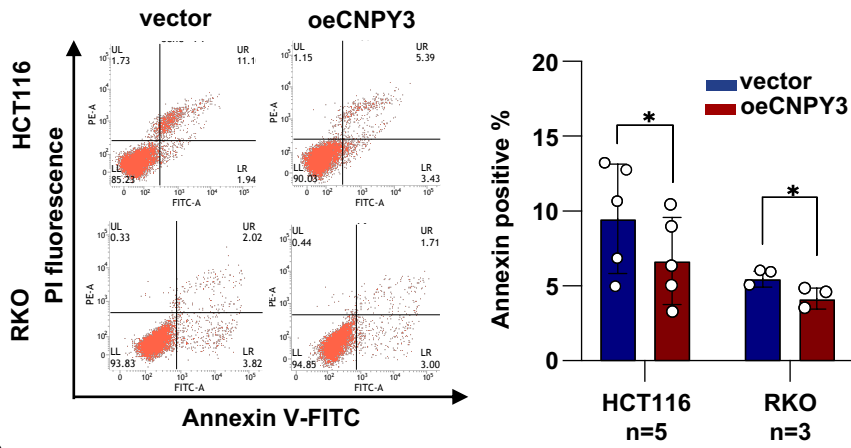


Figure S14

A



B

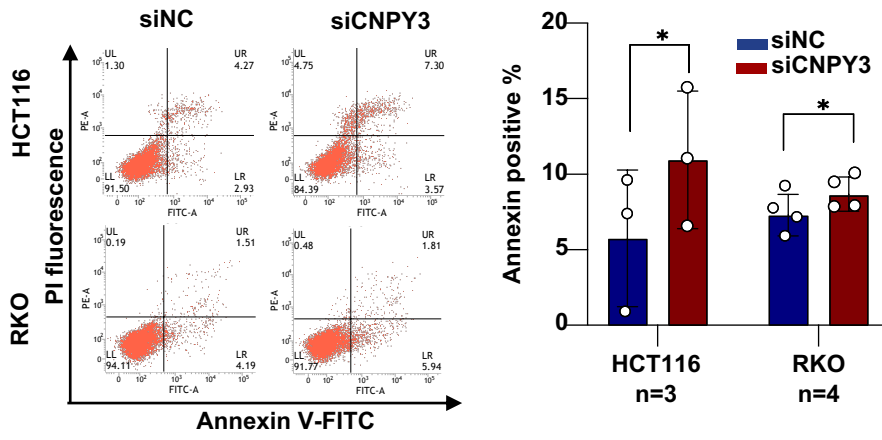


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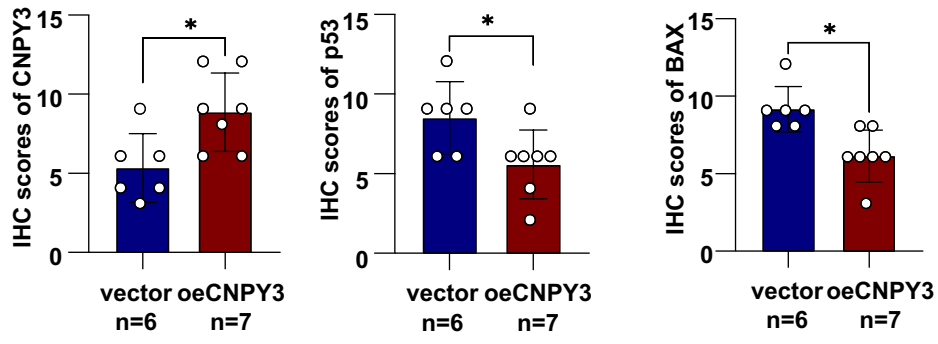
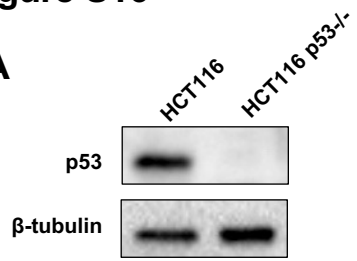


Figure S16

A



B

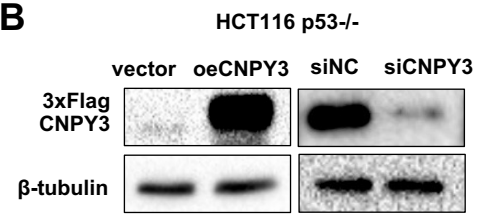


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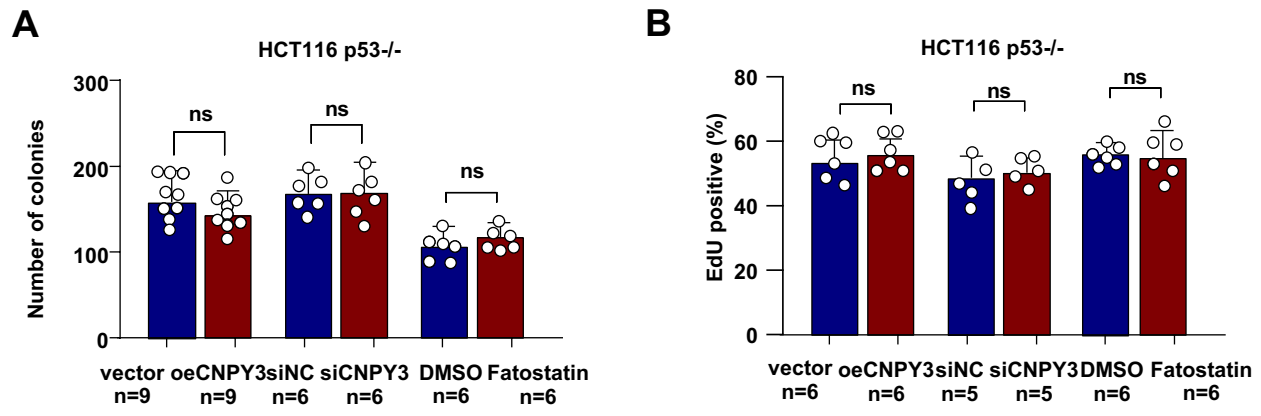


Figure S18

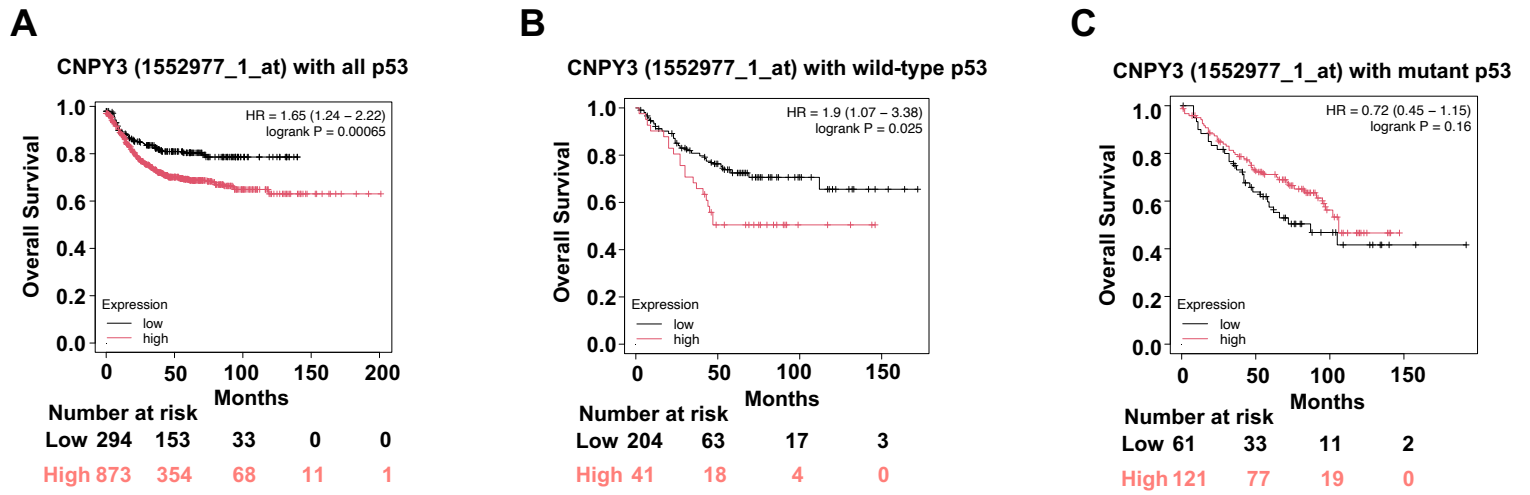
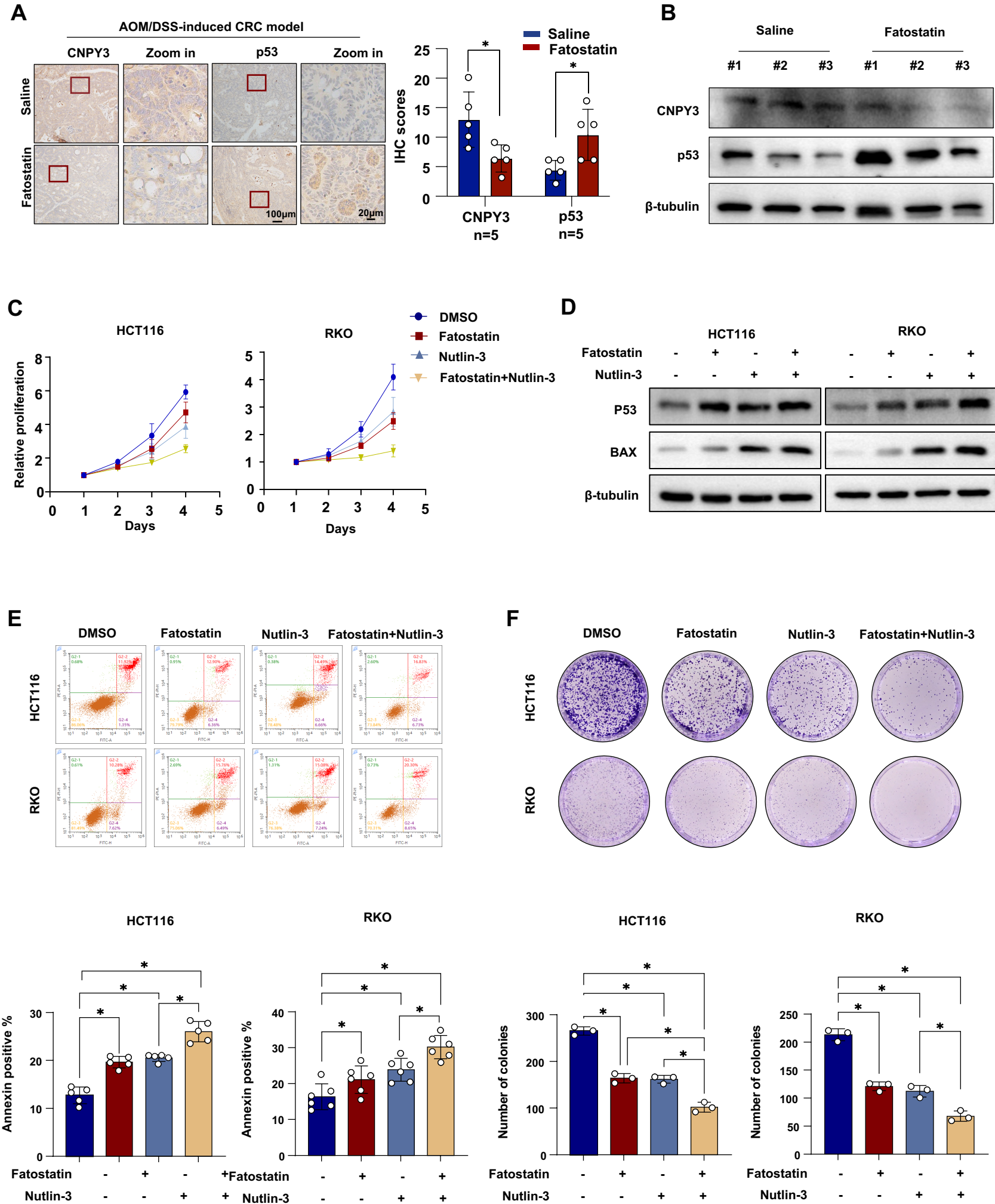


Figure S19



13 **Table S1. The antibodies were applied in this study.**

Antibody name	Company	Catalog No.	Applications
Anti-SREBP2	Abmart	TD7601	IHC (1:100)
Anti-CNPY3	Proteintech	15215-1-AP	WB (1:1000); IHC (1:100); IF (1:100)
Anti-E-cadherin	Cell Signaling Technology	3195	WB (1:1000)
Anti- β -catenin	Cell Signaling Technology	9526	WB (1:1000)
Anti-PCNA	Proteintech	10205-2-AP	WB (1:1000)
Anti- β -tubulin	Proteintech	66240-1-Ig	WB (1:20000)
Anti-SREBP2	Novus	AF7119	ChIP (2 μ g per 0.5 ml of cell lysate); WB (1:500)
Anti-PRAT4a (F-6)	Santa Cruz Biotechnology	sc-515151	Co-IP (2 μ g per 1 ml of cell lysate)
Anti-mouse IgG	Santa Cruz Biotechnology	sc-2025	Co-IP (2 μ g per 1 ml of cell lysate)
Anti-MDM2(SMP14)	Santa Cruz Biotechnology	sc-965	WB (1:100); IF (1:50); Co-IP (2 μ g per 1 ml of cell lysate)
Anti-MDM2	Proteintech	66511-1-Ig	WB (1:1000)
Anti-MDM2 (phospho S166)	Abcam	ab170880	WB (1:50000)
Anti-Flag (DDDDK tag)	Abcam	ab205606	WB (1:2000)
Anti-HA tag	ZSGB	CB051	WB (1:1000)
Anti-His tag	Abmart	M20020S	WB (1:1000)
Anti-GST tag	Abcam	AB58626	WB (1:1000)
Anti-p21	Cell Signaling Technology	2947	WB (1:1000)
Anti-p53	Proteintech	10442-1-AP	WB (1:5000); IHC (1:100)
Anti-BAX	Proteintech	50599-2-Ig	WB (1:2000); IHC

			(1:1000); IF (1:100)
Anti-Bcl2	Proteintech	68103-1-Ig	WB (1:500)
Anti-Ki67	Proteintech	27309-1-AP	IHC (1:5000)
Anti-p53 (DO-1)	Santa Cruz Biotechnology	sc-126	IF (1:100); Co-IP (2 µg per 1 ml of cell lysate)
Anti-ubiquitin	Abcam	ab134953	WB (1:1000)
Anti-Rabbit IgG HRP	Affinity	S0001	WB (1:20000)
Anti-Mouse IgG HRP	Affinity	S0002	WB (1:20000)

14 Note: WB: western blotting; IHC: immunohistochemistry; Co-IP: Co-immunoprecipitation; IF:

15 immunofluorescence staining.

16

17 **Table S2. Primers were verified and used.**

Gene	Forward primer	Reverse primer
CNPY3	5'-CGGAGCTGAGGAGAACGAC-3'	5'-ATAGCCCGTGCCAATCACC-3'
p53	5'-CAGCACATGACGGAGGTTGT-3'	5'-CAGCACATGACGGAGGTTGT-3'
GAPDH	5'-GGAGCGAGATCCCTCCAAAAT-3'	5'-GGCTGTTGTCATACTTCTCATGG-3'
MDM2	5'-CAGTAGCAGTGAATCTACAGGGA-3'	5'-CTGATCCAACCAATCACCTGAAT-3'
CNPY3 pro-1	5'-GGACTTTGCCATAGGTCGCT-3'	5'-AGCCAATAGGCTTTCTGGGG-3'
CNPY3 pro-2	5'-GCCCGGCTATAACAACCACA-3'	5'-GTCCTTTCCCACCCCTAAC-3'

18

19

20 **Table S3. The two clusters of TCGA-COAD population.**

Group 1	Group 2
TCGA-CA-6718-01A-11R-1839-07	TCGA-AA-3549-01A-02R-0821-07
TCGA-AD-6901-01A-11R-1928-07	TCGA-G4-6626-01A-11R-1774-07
TCGA-AA-A00J-01A-02R-A002-07	TCGA-AA-3973-01A-01R-1022-07
TCGA-CM-4752-01A-01R-1410-07	TCGA-AA-3679-01A-02R-0905-07
TCGA-AA-3966-01A-01R-1113-07	TCGA-A6-4107-01A-02R-1410-07
TCGA-AA-3856-01A-01R-0905-07	TCGA-CK-5915-01A-11R-1653-07
TCGA-AZ-4615-01A-01R-1410-07	TCGA-CK-6751-01A-11R-1839-07
TCGA-A6-A566-01A-11R-A28H-07	TCGA-AA-3955-01A-02R-1022-07
TCGA-AA-3877-01A-01R-1022-07	TCGA-AA-3855-01A-01R-1022-07
TCGA-A6-2674-01A-02R-0821-07	TCGA-F4-6856-01A-11R-1928-07
TCGA-AZ-4308-01A-01R-1410-07	TCGA-A6-5659-01B-04R-A277-07
TCGA-AA-A00K-01A-02R-A002-07	TCGA-DM-A280-01A-12R-A16W-07
TCGA-AA-A01P-01A-21R-A083-07	TCGA-A6-2674-01A-02R-A278-07
TCGA-AA-3548-01A-01R-1873-07	TCGA-AA-A00O-01A-02R-A089-07
TCGA-G4-6297-01A-11R-1723-07	TCGA-QG-A5YV-01A-11R-A28H-07
TCGA-AA-3982-01A-02R-1022-07	TCGA-AA-A01V-01A-23R-A083-07
TCGA-AA-3875-01A-01R-0905-07	TCGA-G4-6320-01A-11R-1723-07
TCGA-A6-2671-01A-01R-1410-07	TCGA-DM-A288-01A-11R-A16W-07
TCGA-AA-3977-01A-01R-1022-07	TCGA-NH-A6GA-01A-11R-A37K-07
TCGA-D5-6929-01A-31R-1928-07	TCGA-D5-5540-01A-01R-1653-07
TCGA-AM-5821-01A-01R-1653-07	TCGA-CA-5797-01A-01R-1653-07
TCGA-AZ-6605-01A-11R-1839-07	TCGA-CM-6161-01A-11R-1653-07
TCGA-A6-6780-01A-11R-1839-07	TCGA-AA-3509-01A-01R-1410-07
TCGA-CK-6746-01A-11R-1839-07	TCGA-AA-3502-01A-01R-1410-07
TCGA-CM-5348-01A-21R-1723-07	TCGA-A6-6650-01A-11R-1774-07
TCGA-A6-3807-01A-01R-1022-07	TCGA-AA-A01G-01A-01R-A002-07
TCGA-AA-A01R-01A-21R-A083-07	TCGA-AA-A01I-01A-02R-A089-07
TCGA-AA-3812-01A-01R-0905-07	TCGA-DM-A0XD-01A-12R-A155-07
TCGA-A6-3808-01A-01R-1022-07	TCGA-D5-6532-01A-11R-1723-07
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TCGA-WS-AB45-01A-11R-A41B-07	TCGA-D5-7000-01A-11R-A32Z-07
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TCGA-AA-3553-01A-01R-0821-07	TCGA-CK-4947-01B-11R-1653-07
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TCGA-D5-6928-01A-11R-1928-07	TCGA-A6-2672-01B-03R-2302-07
TCGA-D5-6530-01A-11R-1723-07	TCGA-D5-6540-01A-11R-1723-07
TCGA-A6-2685-01A-01R-1410-07	TCGA-AA-3845-01A-01R-1022-07
TCGA-AA-3994-01A-01R-1113-07	TCGA-A6-6137-01A-11R-1774-07
TCGA-AA-3837-01A-01R-0905-07	TCGA-DM-A1HB-01A-21R-A180-07
TCGA-G4-6299-01A-11R-1774-07	TCGA-AA-3519-01A-02R-0821-07
TCGA-AZ-6600-01A-11R-1774-07	TCGA-CM-6172-01A-11R-1653-07

TCGA-AY-6196-01A-11R-1723-07
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TCGA-CK-5916-01A-11R-1653-07
TCGA-A6-A5ZU-01A-11R-A28H-07
TCGA-A6-A56B-01A-31R-A28H-07
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TCGA-A6-2680-01A-01R-1410-07
TCGA-D5-5538-01A-01R-1653-07
TCGA-AA-3510-01A-01R-1410-07
TCGA-F4-6809-01A-11R-1839-07
TCGA-AA-A03F-01A-11R-A16W-07
TCGA-AA-3552-01A-01R-0821-07
TCGA-AA-3867-01A-01R-1022-07
TCGA-AD-6895-01A-11R-1928-07
TCGA-AA-A01D-01A-01R-A00A-07
TCGA-AA-A01C-01A-01R-A00A-07
TCGA-DM-A28A-01A-21R-A32Y-07
TCGA-AA-3521-01A-01R-0821-07
TCGA-A6-6782-01A-11R-1839-07
TCGA-AD-6890-01A-11R-1928-07
TCGA-D5-6534-01A-21R-1928-07
TCGA-CM-6169-01A-11R-1653-07
TCGA-DM-A1HA-01A-11R-A155-07
TCGA-CA-5796-01A-01R-1653-07
TCGA-AA-A00F-01A-01R-A002-07
TCGA-A6-6650-01B-02R-A277-07
TCGA-AY-A69D-01A-11R-A37K-07
TCGA-CK-6747-01A-11R-1839-07
TCGA-CM-6678-01A-11R-1839-07
TCGA-AD-A5EK-01A-11R-A28H-07
TCGA-DM-A1D6-01A-21R-A155-07
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TCGA-F4-6808-01A-11R-1839-07
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TCGA-4N-A93T-01A-11R-A37K-07
TCGA-AA-3678-01A-01R-0905-07
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TCGA-AY-6197-01A-11R-1723-07
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TCGA-AA-3529-01A-02R-0821-07
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TCGA-CA-6715-01A-21R-1839-07
TCGA-D5-6923-01A-11R-A32Z-07

TCGA-AA-3562-01A-02R-0821-07
TCGA-AD-6548-01A-11R-1839-07
TCGA-AD-6964-01A-11R-1928-07
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TCGA-AA-3543-01A-01R-0826-07
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TCGA-A6-2674-01B-04R-A277-07
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TCGA-5M-AAT4-01A-11R-A41B-07
TCGA-DM-A1D0-01A-11R-A155-07
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TCGA-AA-A02O-01A-21R-A16W-07
TCGA-CM-6676-01A-11R-1839-07
TCGA-AD-A5EJ-01A-11R-A28H-07
TCGA-AA-3560-01A-01R-0821-07
TCGA-CM-6680-01A-11R-1839-07
TCGA-G4-6627-01A-11R-1774-07
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TCGA-G4-6625-01A-21R-1774-07
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TCGA-AA-3496-01A-21R-1839-07
TCGA-AA-3950-01A-02R-1022-07
TCGA-G4-6586-01A-11R-1774-07
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TCGA-CK-4951-01A-01R-1410-07
TCGA-A6-6651-01A-21R-1839-07
TCGA-CK-5913-01A-11R-1653-07
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TCGA-A6-6652-01A-11R-1774-07
TCGA-DM-A28H-01A-11R-A16W-07
TCGA-DM-A1D8-01A-11R-A155-07
TCGA-AA-3819-01A-01R-0905-07
TCGA-A6-2678-01A-01R-0821-07
TCGA-QG-A5YX-01A-11R-A28H-07
TCGA-CK-4952-01A-01R-1723-07
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TCGA-CA-5256-01A-01R-1410-07
TCGA-AA-A01X-01A-21R-A083-07
TCGA-AA-3850-01A-01R-1022-07
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TCGA-AA-3697-01A-01R-1723-07
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TCGA-AZ-4313-01A-01R-1410-07
TCGA-AA-3524-01A-02R-0821-07
TCGA-AA-3860-01A-02R-0905-07
TCGA-G4-6310-01A-11R-1723-07
TCGA-G4-6321-01A-11R-1723-07
TCGA-T9-A92H-01A-11R-A37K-07

TCGA-D5-6926-01A-11R-1928-07
TCGA-CA-6717-01A-11R-1839-07
TCGA-A6-3810-01A-01R-1022-07
TCGA-CA-5254-01A-21R-1839-07
TCGA-QG-A5YW-01A-11R-A28H-07
TCGA-AA-A010-01A-01R-A089-07
TCGA-AD-5900-01A-11R-1653-07
TCGA-AA-A01Q-01A-01R-A002-07
TCGA-AA-A00A-01A-01R-A002-07
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TCGA-NH-A6GC-01A-12R-A41B-07
TCGA-A6-5667-01A-21R-1723-07
TCGA-AA-3851-01A-01R-1022-07
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TCGA-G4-6303-01A-11R-1774-07
TCGA-NH-A8F7-06A-31R-A41B-07
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TCGA-CM-5861-01A-01R-1653-07
TCGA-AZ-5403-01A-01R-1653-07

TCGA-DM-A0X9-01A-11R-A155-07
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TCGA-5M-AATA-01A-31R-A41B-07
TCGA-F4-6463-01A-11R-1723-07
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TCGA-A6-2683-01A-01R-0821-07
TCGA-NH-A6GB-01A-11R-A37K-07
TCGA-AA-3818-01A-01R-0905-07
TCGA-AA-A02F-01A-01R-A089-07
TCGA-A6-5656-01A-21R-A278-07
TCGA-AA-3680-01A-01R-0905-07
TCGA-A6-5665-01B-03R-2302-07
TCGA-A6-5665-01A-01R-1653-07
TCGA-AA-A01S-01A-21R-A083-07
TCGA-CM-5862-01A-01R-1653-07
TCGA-AA-3555-01A-01R-0821-07
TCGA-A6-5666-01A-01R-1653-07
TCGA-G4-6317-01A-11R-1723-07
TCGA-AA-3947-01A-01R-1022-07
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TCGA-A6-5659-01A-01R-A278-07
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TCGA-AA-3666-01A-02R-0905-07
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TCGA-CM-5864-01A-01R-1653-07
TCGA-AA-3841-01A-01R-0905-07
TCGA-A6-3809-01A-01R-A278-07
TCGA-D5-6538-01A-11R-1723-07
TCGA-CM-6677-01A-11R-1839-07
TCGA-AA-3968-01A-01R-1022-07
TCGA-DM-A282-01A-12R-A16W-07
TCGA-AA-3864-01A-01R-1022-07
TCGA-DM-A28C-01A-11R-A32Y-07
TCGA-AZ-4682-01B-01R-A32Z-07
TCGA-D5-6539-01A-11R-1723-07

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23 **Table S4. The gene list of candidate genes.**

Gene symbol
HERPUD1
VAPA
SERPINH1
CYCS
SLMAP
PLOD3
SCD
VEGFA
CNPY3
ENTPD5
SRI
PPP1CB
IFI6
ZDHHC9
PSMA7
TRAP1
NBEAL2

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26 **Table S5. Hydrogen bond between CNPY3 and MDM2.**

CNPY3 (red)	MDM2 (yellow)	Hydrogen bond length (Å)
LYS-229	SER-22	3.4
LYS-229	GLU-25	2.8
LYS-244	SER-78	2.3
GLU-131	HIA-73	3.2
GLU-131	GLU-69	2.0
THR-132	ASP-68	3.2
ASN-135	GLN-71	2.4
TYR-149	ARG-65	2.6
TYR-149	GLY-87	3.4
LYS-124	ALA-43	3.1
SER-61	GLN-44	2.2
GLU-65	THR-47	2.7
HIS-272	THR-47	3.2
HIS-272	THR-47	2.7
GLN-265	SER-17	2.0
GLN-265	SER-78	2.9
GLN-265	ASP-84	2.3
ASP-261	LYS-98	3.3
GLY-263	SER-90	3.3
SER-257	THR-26	3.4
ASP-255	ARG-97	2.6
ASP-255	ARG-97	3.0
ASN-135	ASP-68	3.2
THR-132	GLU-69	2.0
THR-132	GLN-71	2.4
LYS-266	SER-78	2.9
LYS-266	ASP-84	2.3
SER-257	THR-26	3.4

ASP-255	SER-257	2.6
ASP-255	SER-257	3.0
ASP-109	SER-40	2.3
TYR-89	ARG-29	2.9
GLU-98	ARG-29	3.4
GLY-76	SER-78	3.0
TYR-77	SER-78	3.1
SER-241	ASN-79	2.0
SER-241	ASP-84	3.1

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28

29 **Supplementary Figure Legend**

30 **Figure S1. SREBP2 inhibits CRC in vivo**

31 (A) Kaplan-Meier survival curve of overall survival analysis of colon cancer patients stratified by
32 SREBP2 from Kaplan-Meier plotter. (B) The bar graph shows the percentage of CRC and neoplasia in
33 the saline and Fatostatin-treated AOM/DSS induced CRC group. (C) Histograms show the levels of
34 serum total cholesterol in the saline and Fatostatin-treated AOM/DSS induced CRC group.

35 Note: two-tailed $*P < 0.05$ by an unpaired t test (B); data are presented as mean \pm SD.

36 **Figure S2. SREBP2 promotes the expression of CNPY3**

37 (A) The Venn plot show CNPY3 is a candidate gene of SREBP2 from different databases. (B) Western
38 blot showed pSREBP2 and nSREBP2 in HCT116 and RKO cells with Fatostatin or 25-OHC
39 treatment, using β -tubulin as a control. (C) The mRNA expression of SREBP2 (up) and CNPY3 (down)
40 in HCT116 (left) and RKO (right) cells after SREBP2 overexpression, using GAPDH as a control. (D)
41 Western blot showed pSREBP2, nSREBP2 and CNPY3 in HCT116 and RKO cells overexpressed
42 HA-SREBP2 plasmids, using β -tubulin as a control. (E) The mRNA expression of SREBP2 (up) and
43 CNPY3 (down) in HCT116 (left) and RKO (right) cells after SREBP2 knockdown, using GAPDH as a
44 control. (F) Western blot showed pSREBP2, nSREBP2 and CNPY3 in HCT116 and RKO cells with
45 SREBP2 knockdown, using β -tubulin as a control.

46 Note: two-tailed $*P < 0.05$ by an unpaired t test (B, D); 25-OHC, 25-Hydroxycholesterol; data are
47 presented as mean \pm SD.

48 **Figure S3. SREBP2 is a transcriptional regulator of CNPY3**

49 Mutated binding site of the transcription factor SREBP2 to the CNPY3 promoter and promoter activity
50 of CNPY3 and mutant CNPY3.

51 Note: two-tailed $*P < 0.05$ by an unpaired t test; data are presented as mean \pm SD.

52 **Figure S4. CNPY3 expression correlates with cholesterol levels**

53 (A) Representative IHC of SREBP2 in CRC tissues from patients with low and high SREBP2 levels.

54 (B) Spearman's correlation analysis of total cholesterol and CNPY3 in CRC patients.

55 **Figure S5 CNPY3 does not regulate cholesterol biosynthesis**

56 (A-B) Western blot showed CNPY3 in HCT116 and RKO cells with CNPY3 overexpression (A)

57 and knockdown (B), using β -tubulin as a control. (C-D) The mRNA expression of CNPY3 in HCT116

58 (left) and RKO (right) cells after CNPY3 overexpression (C) and knockdown (D), using GAPDH as a

59 control. (E) Representative images (up) and semi-quantitative analysis (down) of lipid droplets in

60 HCT116 and RKO cells after CNPY3 knockdown were determined by BODIPY staining. (F) Histograms

61 showed the concentration of cholesterol within HCT116 (left) and RKO cells (right) after CNPY3

62 overexpression (up) or knockdown (down).

63 Note: two-tailed $*P < 0.05$ and ns by an unpaired *t* test (C, D, E, F); ns, not significant; data are

64 presented as mean \pm SD.

65 **Figure S6. CNPY3 is upregulated in multiple cancers**

66 (A) Boxplot of CNPY3 mRNA expression levels in multiple human cancers and normal tissues from the

67 TCGA database in TIMER. (B-C) The mRNA (B) and protein (C) levels of CNPY3 in NCM460, HCT116,

68 HT29, RKO, SW480, and DLD1 cells.

69 Note: CNPY3, Canopy FGF signaling regulator 3; ACC: Adrenocortical carcinoma; BLCA: Bladder

70 Urothelial Carcinoma; BRCA: Breast invasive carcinoma; CHOL: Cervical and endocervical cancers;

71 CESC: Cholangiocarcinoma; COADREAD: Colon adenocarcinoma and Rectum adenocarcinoma;

72 COAD: Colon adenocarcinoma; DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA:

73 Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and Neck squamous cell

74 carcinoma; KICH: Kidney Chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal
75 papillary cell carcinoma; LAML: Acute Myeloid Leukemia; LGG: Brain Lower Grade Glioma; HCC:
76 Hepatocellular Carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma;
77 MESO: Mesothelioma; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma;
78 PCPG: Pheochromocytoma and Paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum
79 adenocarcinoma; SKCM: Skin Cutaneous Melanoma; STAD: Stomach adenocarcinoma; TGCT:
80 Testicular Germ Cell Tumors; THCA: Thyroid carcinoma; UCEC: Uterine Corpus Endometrial
81 Carcinoma; UCS: Uterine Carcinosarcoma; UVM: Uveal Melanoma; two-tailed $*P < 0.05$ by an
82 unpaired t test (B); data are presented as mean \pm SD.

83 **Figure S7. CNPY3 promotes CRC cell invasion ability**

84 (A-B) Representative images of wound healing assay of HCT116 (A) and RKO (B) cells treated with
85 CNPY3 overexpression (left) and quantitative statistics (right).

86 Note: two-tailed $*P < 0.05$ by an unpaired t test (A, B); data are presented as mean \pm SD.

87 **Figure S8. Fatostatin inhibits the proliferative effects of CNPY3**

88 (A-B) Representative images (left) and quantitative statistics (right) of colony formation assay of
89 HCT116 (A) and RKO (B) cells with CNPY3 overexpression and controls after Fatostatin treatment.

90 Note: two-tailed $*P < 0.05$ by a Tukey's multiple comparisons test (A, B); data are presented as
91 mean \pm SD.

92 **Figure S9. CNPY3 regulates the transcriptional level of MDM2**

93 (A) Bubble chart shows the KEGG pathway analysis from TCGA based on the comparison of CNPY3
94 expression. (B) Western blot showed pSMDM2 in HCT116 and RKO cells with Terbinafine
95 treatment, using β -tubulin as a control.

96 **Figure S10. CNPY3 forms Condensate**

97 Representative immunofluorescence images show the endogenous CNPY3 condensates of RKO and
98 HCT116 cells.

99 **Figure S11. CNPY3 regulates the p53 pathway to promote CRC**

100 (A) GSEA analysis of p53 signaling pathway for KEGG enrichment using TCGA-COAD/TCGA-READ
101 cohorts divided by CNPY3 expression. (B) Bubble chart shows the KEGG pathway analysis based on
102 the comparison of two subtypes of genes related to cholesterol synthesis.

103 **Figure S12. CNPY3 binds to MDM2 to mediate degradation of p53.**

104 (A) CNPY3-MDM2-P53 trimeric molecular docking pattern. (B) Endogenous Co-IP was performed
105 in HCT116 cell lysates using an anti-CNPY3 antibody (up) and anti-p53 antibody (down), compared
106 with control anti-IgG. (C) Representative confocal images of colocalization between CNPY3 and
107 p53 and co-localization analysis in RKO cells.

108 **Figure S13. CNPY3 inhibits p53 expression**

109 (A-B) The expression level of p53 in the HCT116 and RKO cells with SREBP2 overexpression (A)
110 and knockdown (B). (C) Representative IF images exhibited p53 (green) after CNPY3
111 overexpression in HCT116 and in RKO cells.

112 **Figure S14. CNPY3 could inhibit apoptosis in CRC**

113 (A-B) Apoptosis assays (left) and quantitative statistics (right) of HCT116 and RKO with CNPY3
114 overexpression (A) and CNPY3 knockdown (B).

115 Note: two-tailed $*P < 0.05$ by an unpaired t test (A, B); data are presented as mean \pm SD.

116 **Figure S15. Overexpression of CNPY3 inhibits the p53 signaling pathway in vivo**

117 Quantitative statistics of IHC staining of CNPY3 (left), p53 (middle), and BAX (right) expression

118 in HCT116 tumor xenografts.

119 Note: two-tailed $*P < 0.05$ by an unpaired t test; data are presented as mean \pm SD.

120 **Figure S16. Verification of p53 knockout in HCT116 cells**

121 (A) The p53 protein expression in HCT116 and p53^{-/-} HCT116 cells, using β -tubulin as a control. (B)
122 The protein levels of CNPY3 in p53^{-/-} HCT116 cells with CNPY3 overexpression (left) and CNPY3
123 knockdown (right), using β -tubulin as a control.

124 **Figure S17. CNPY3 loses regulation of CRC carcinogenic effects after p53 knockout**

125 (A) Quantitative analysis of colony formation assay of p53^{-/-} HCT116 cells treated with CNPY3
126 knockdown, CNPY3 overexpression, or Fatostatin treatment. (B) Semi-quantitative analysis of of
127 EdU incorporation of p53^{-/-} HCT116 cells treated with CNPY3 knockdown, CNPY3
128 overexpression, or Fatostatin treatment.

129 Note: ns by an unpaired t test (A, B); ns, not significant; data are presented as mean \pm SD.

130 **Figure S18. CNPY3 has a worse prognosis in patients with wild p53 rather than mutant p53**

131 (A) Kaplan-Meier survival curve of overall survival analysis of all colon cancer patients stratified
132 by CNPY3 from Kaplan-Meier plotter. (B-C) Kaplan-Meier survival curve of overall survival
133 analysis of colon cancer patients with wild-type p53 (B) and mutant p53 (C) stratified by CNPY3
134 from Kaplan-Meier plotter.

135 **Figure S19. The combination of Nutlin-3 and Fatostatin increases the inhibitory effect of CRC**

136 (A) Representative images (left) and semi-quantitative analysis (right) of IHC staining of CNPY3
137 and p53 in AOM/DSS-induced CRC model with saline and Fatostatin treatment. (B) The protein
138 levels of CNPY3 and p53 in AOM/DSS-induced CRC model with saline and Fatostatin treatment,
139 using β -tubulin as a control. (C) CCK8 assays of HCT116 (left) and RKO cells (right) with DMSO,

140 Fatostatin, Nutlin-3 and combination of Fatostatin and Nutlin-3, respectively. (D) The protein levels of
141 p53 and BAX in HCT116 (left) and RKO cells (right) with DMSO, Fatostatin, Nutlin-3 and combination
142 of Fatostatin and Nutlin-3, respectively, using β -tubulin as a control. (E-F) Apoptosis assays (E) and
143 colony assays (F) of HCT116 and RKO cells with DMSO, Fatostatin, Nutlin-3 and combination of
144 Fatostatin and Nutlin-3, respectively.

145 Note: two-tailed $*P < 0.05$ by an unpaired t test (A), a Mix-effects analysis (C), and a Tukey's
146 multiple comparisons test (E, F); data are presented as mean \pm SD.

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