SUPPLEMENTAL INFORMATION

Aspirin cooperates with p300 to activate the acetylation of H3K9 and promote FasL-mediated apoptosis of cancer stem-like cells in colorectal cancer

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INVENTORY OF SUPPLEMENTAL INFORMATION

Figure S1:related to Figure 1. It provides evidence that expression of Cancer Stem Cell Molecular Markers decrease after Aspirin treatment.

Figure S2: related to Figure 2. It provides evidence that aspirin induces more apoptosis in CSCs compared to non-CSCs.

Figure S3: related to Figure 3. It provides additional information that Aspirin induces *FasL*-mediated apoptosis in a COX-2-independent manner.

Figure S4: related to Figure 4. It provides evidence that Aspirin induced FasL expression instead of other NSAID drugs.

Figure S5: related to Figure 5. It provides additional information on the synthesis of biotinylated Aspirin and light-inducible epigenetic modification system based on CRISPR technology.

Figure S6: related to Figure 6. It demonstrates the important role played by p300 in Aspirin-induced *FasL* expression.

Table S1: It provides the characteristic of patients included in this study.

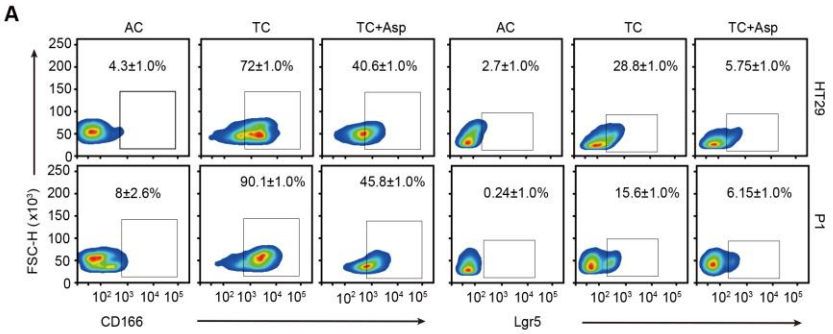
Table S2: related to Figure 1. It shows the increasing frequency of CSCs in tumorsphere cells (TCs) compared to adherent cells (ACs), Aspirin significantly decreases the frequency of CSCs.

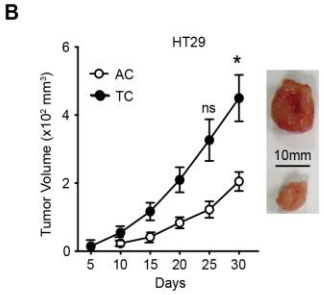
Table S3: related to Figure 1. It shows the 50% inhibitory concentration (IC50) of Oxa in ACs, TCs, and TCs treated with 5mM Aspirin.

Extended Experimental Procedures: Due to the length limitation of the main text, the detailed information on the reagents and experimental methods is provided in this section.

SUPPLEMENTAL FIGURES

Figure S1





C

Aspirin treatment and Lymphode status			
	Lymphode positive	Lymphode negative	P Value
CRC Patients without Aspirin treatment	11	9	0.041
CRC Patients with Aspirin treatment	4	14	

D

Aspirin treatment and AJCC stage			
	Ⅰ - Ⅱ	Ⅲ - Ⅳ	P Value
CRC Patients without Aspirin treatment	6	14	0.007
CRC Patients with Aspirin treatment	15	3	

Figure S1. Expression of cancer stem cell molecular markers after Aspirin treatment; supplement of Figure 1. (**A**) Expression of cancer stem cell markers (CD166, Lgr5) in ACs, TCs, or TCs with Aspirin treatment of HT29 cell lines analyzed via FCM assays. ALDH1 immunoreactive cells and their average percentages in the total cell counts are indicated in each panel (mean ± SEM, n=3). (**B**) Tumor growth curve from xenograft assays following subcutaneous injection of $1x10^5$ ACs or TCs of the HT29 cell line into 6-week-old female nude mice (mean ± SEM, n=6). (**C**) The relationship between Asp treatment and lymph node status. (**D**) The relationship between Asp treatment and clinical stage (AJCC stage).

Figure S2

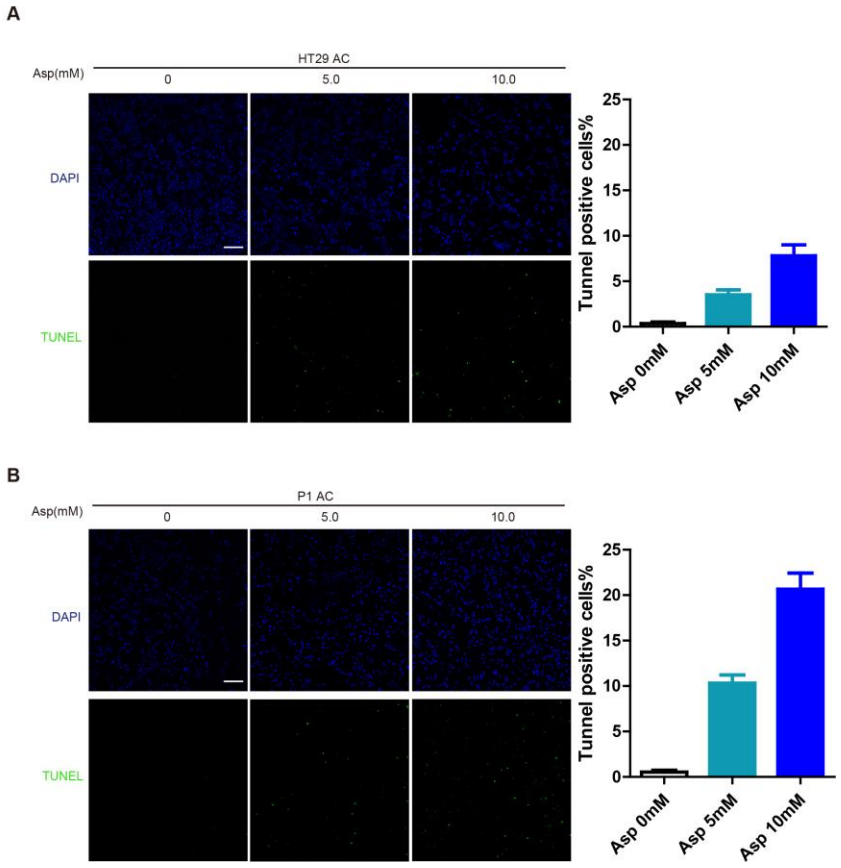


Figure S2. Aspirin induces the apoptosis in normal cancer cells. (**A**)Representative images and qualification of apoptotic cells analyzed with TUNEL assay from HT29 ACs following 2-day treatment with Asp at the indicated concentrations. (**B**)Representative images and qualification of apoptotic cells analyzed with TUNEL assay from P1 ACs following 2-day treatment with Asp at the indicated concentrations. Scale bar indicates 100 μm.

Figure S3

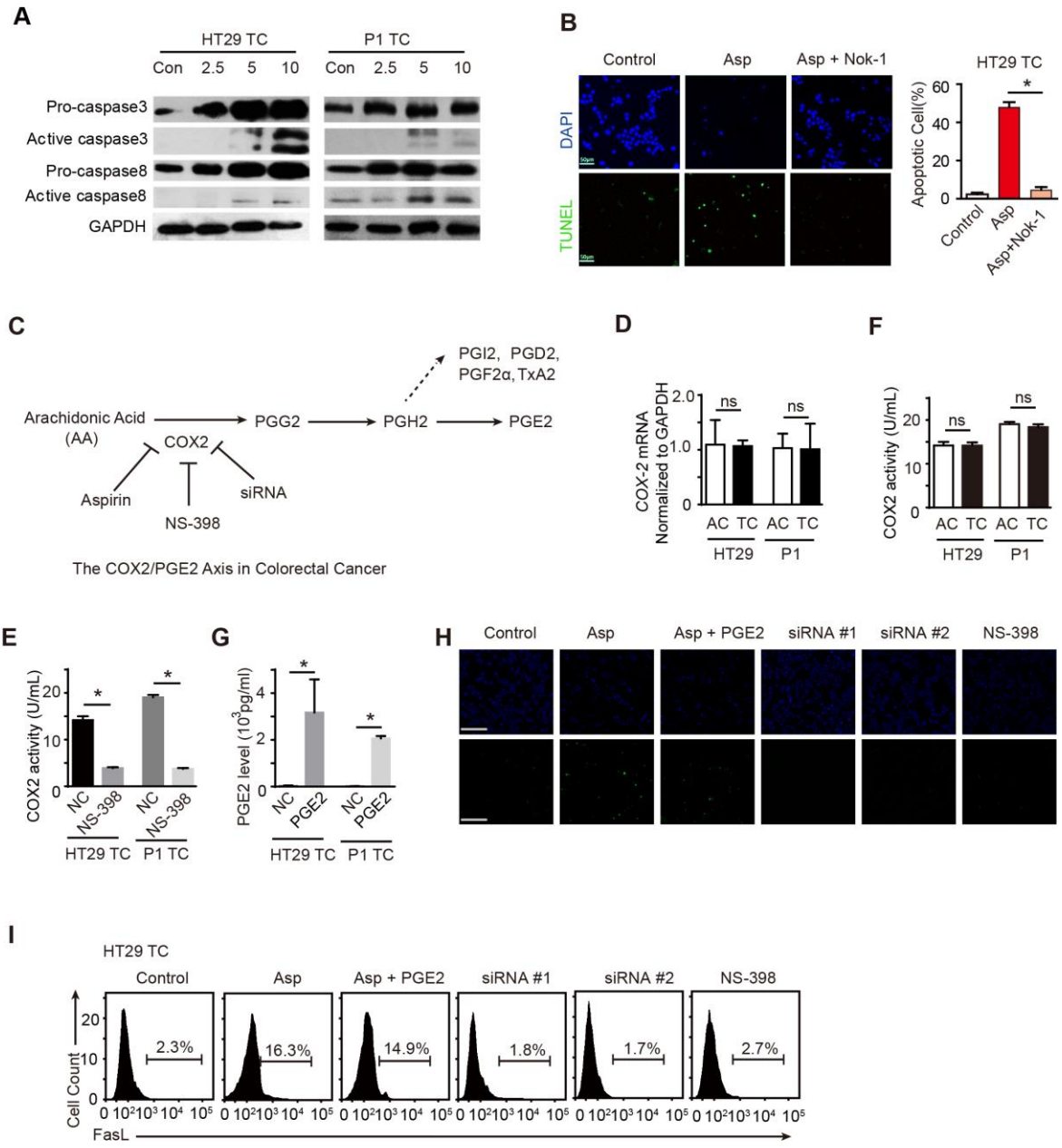


Figure S3. Aspirin induces apoptosis of CSCs in a COX2-independent manner. (**A**) Western blots of cell lysates of TCs after 48 h treatment with Asp with antibodies against caspase-3, caspase-8, and Gapdh. (**B**) Representative results and qualification of apoptotic cells analyzed with TUNEL assay from HT29 TCs following 48 h exposure to 5 mM Asp with control antibody or 2 μg/mL Nok-1. (**C**) Schematic of the COX-2-PGE2 signaling pathway. (**D**) qPCR results of *COX-2* mRNA (mean ±SEM, n=3, normalized to

Gapdh mRNA expression). (**E**) ELISA of COX-2 enzyme activity both in ACs and TCs. (**F**) ELISA of COX-2 enzyme activity after NS-398 treatment. (**G**) PGE2 levels after PGE2 treatment. (**H**) TUNEL assay results of HT29 TCs treated with saline (control), Asp (5 mM), Asp (5 mM) and PGE2 (1 μM), siRNAs (#1 and #2) against *COX-2*, or the COX-2 antagonist NS-398 (75 μM). Scale bar indicates 100 μm. (**I**) Expression of FasL based on FCM assay of HT29 TCs treated as in (H).

Figure S4

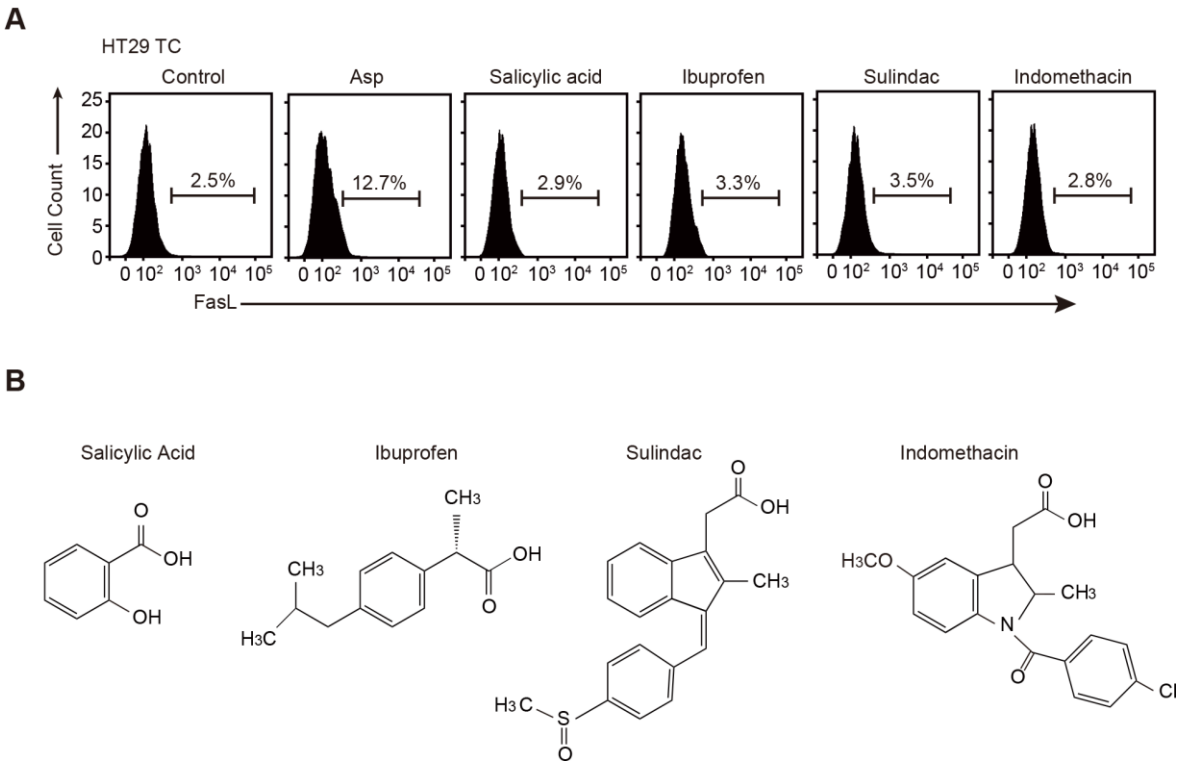


Figure S4. Aspirin induced FasL expression. (**A**) Chemical structure of non-steroidal anti-inflammatory drugs (NSAIDs), including salicylic acid, ibuprofen, sulindac, and indomethacin. (**B**) Expression of FasL based on FCM assay of HT29 TCs treated with saline (Control), Asp (5 mM), salicylic acid (5 mM), ibuprofen (1 mM), sulindac (100 μM), or indomethacin (600 μM).

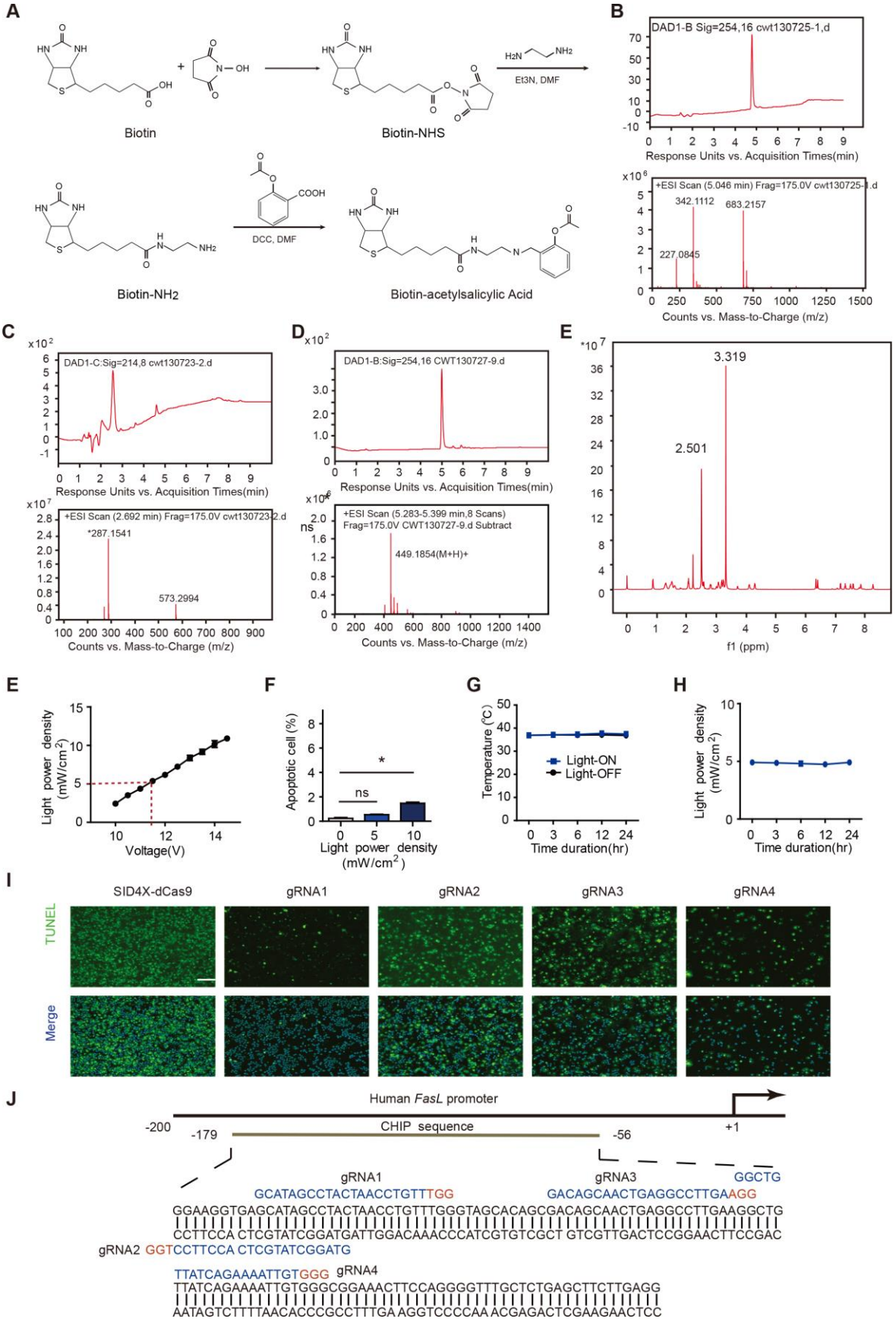

A
Biotin
Biotin-NHS
Et3N, DMF
Biotin-NH2
DCC, DMF
Biotin-acetylsalicylic Acid
B
DAD1-B Sig=254,16 cwt130725-1,d
Response Units vs. Acquisition Times(min)
+ESI Scan (5.046 min) Frag=175.0V cwt130725-1.d
342.1112
683.2157
227.0845
Counts vs. Mass-to-Charge (m/z)
C
DAD1-C:Sig=214,8 cwt130723-2.d
Response Units vs. Acquisition Times(min)
+ESI Scan (2.692 min) Frag=175.0V cwt130723-2.d
*287.1541
573.2994
Counts vs. Mass-to-Charge (m/z)
D
DAD1-B:Sig=254,16 CWT130727-9.d
Response Units vs. Acquisition Times(min)
+ESI Scan (5.283-5.399 min,8 Scans)
Frag=175.0V CWT130727-9.d Subtract
449.1854(M+H)+
ns
Counts vs. Mass-to-Charge (m/z)
E
3.319
2.501
f1 (ppm)
E
Light power density (mW/cm2)
Voltage(V)
F
Apoptotic cell (%)
*
ns
Light power density (mW/cm2)
G
Temperature (℃)
Light-ON
Light-OFF
Time duration(hr)
H
Light power density (mW/cm2)
Time duration(hr)
I
SID4X-dCas9
gRNA1
gRNA2
gRNA3
gRNA4
TUNEL
Merge
J
Human FasL promoter
-200
-179
CHIP sequence
-56
+1
gRNA1
GCATAGCCTACTAACCTGTTTGG
gRNA3
GACAGCAACTGAGGCCTTGAAGG
GGCTG
GGAAGGTGAGCATAGCCTACTAACCTGTTTGGGTAGCACAGCGACAGCAACTGAGGCCTTGAAGGCTG
CCTTCCA CTCGTATCGGATGATTGGACAAACCCATCGTGTCGCT GTCGTTGACTCCGGAACTTCCGAC
gRNA2 GGTCCTTCCA CTCGTATCGGATG
TTATCAGAAAATTGTGGG gRNA4
TTATCAGAAAATTGTGGGCGGAAACTTCCAGGGGTTTGCTCTGAGCTTCTTGAGG
AATAGTCTTTTAACACCCGCCTTTGA AGGTCCCCAA ACGAGACTCGAAGAACTCC

Figure S5. Synthesis of biotinylated aspirin and light-inducible and gene-specific histone modification system. (**A**) Schematic of the procedures used to synthesize biotinylated acetylsalicylic acid (Biotin-Aspirin). (**B**) Synthesis of biotinyl-N-hydroxysuccinimide ester (Biotin-NHS). The final yield of the product was 85%. HPLC-TOF MS: Calculated for (C14H19N3O5S+H)+: 342.1124; actual: 342.1112. (**C**) Synthesis of N-(2-aminoethyl)biotinamide (Biotin-NH2). The final yield of the product was 75%. HPLC-TOF MS: Calculated for (C12H22N4O2S+H)+: 287.1542; actual: 287.1541. (**D**) Synthesis of Biotin-Aspirin. The final yield of the product was 60%. HPLC-TOF MS: Calculated for (C21H28N4O5S+H)+: 449.1859; actual: 449.1854. 1H NMR spectroscopy analysis of the structure of Biotin-Aspirin. 1H NMR (500 MHz, DMSO) δ 8.26 (s, 1H), 7.85 (s, 1H), 7.60 (d, J=7.5 Hz, 1H), 7.51 (t, J=7.5 Hz, 1H), 7.33 (t, J=7.5Hz, 1H), 7.17 (d, J=8.0 Hz, 1H), 6.40 (s, 1H), 6.34 (s, 1H), 4.29 (m, 1H), 4.11 (m, 1H), 3.24-3.17 (m, 4H), 3.07 (m, 2H), 2.82-2.79 (m, 1H), 2.22 (s, 3H), 2.08-2.05(m, 2H), 1.51 (m, 3H), 1.31 (m, 3H). (**E**) Correlation of voltage and LED light power density. (**F**) Quantification of apoptotic TCs under increasing light intensity. TUNEL-positive cells were analyzed after 24 h illumination, and 5 mW/cm^2 was chosen for the experiments in Figure 6. The results are presented as the mean ± SEM (n=3). *, $p<0.01$, one-way ANOVA. (**G**) The effects of 5 mW/cm^2 light illumination on temperature changes in cell dishes. The results are presented as the mean ± SEM (n=3). (**H**) Stability of light illumination over 24 h. The results are presented as the mean ± SEM (n=3). (**I**) Representative results of apoptotic TCs stained with TUNEL following transfection with the indicated plasmids (SID4X-dCas9 vector, gRNA1-4 in SID4X-dCas9). Scale bar indicates 100 μm. (**J**) Schematic of gRNA target sites and ChIP sequence in the human *FasL* promoter region locus. Each gRNA target sequence is in blue, and the PAM sequence in red.

Figure S6

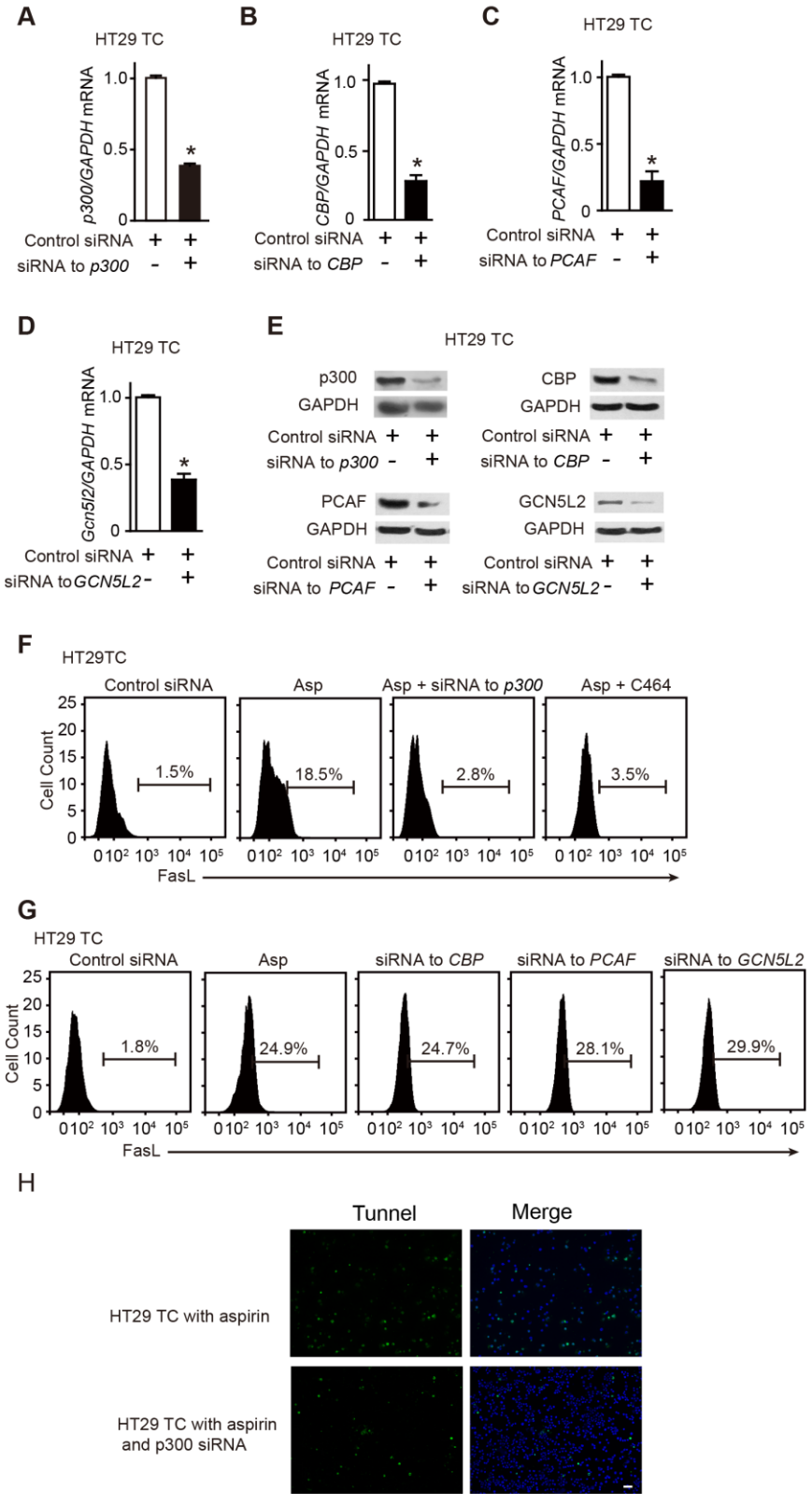

A
HT29 TC
p300/GAPDH mRNA
1.0
0.5
0
*
Control siRNA + +
siRNA to p300 - +
B
HT29 TC
CBP/GAPDH mRNA
1.0
0.5
0
*
Control siRNA + +
siRNA to CBP - +
C
HT29 TC
PCAF/GAPDH mRNA
1.0
0.5
0
*
Control siRNA + +
siRNA to PCAF - +
D
HT29 TC
Gcn5l2/GAPDH mRNA
1.0
0.5
0
*
Control siRNA + +
siRNA to GCN5L2 - +
E
HT29 TC
p300
GAPDH
Control siRNA + +
siRNA to p300 - +
CBP
GAPDH
Control siRNA + +
siRNA to CBP - +
PCAF
GAPDH
Control siRNA + +
siRNA to PCAF - +
GCN5L2
GAPDH
Control siRNA + +
siRNA to GCN5L2 - +
F
HT29TC
Control siRNA
Asp
Asp + siRNA to p300
Asp + C464
Cell Count
25
20
15
10
5
0
1.5%
18.5%
2.8%
3.5%
0 10² 10³ 10⁴ 10⁵
FasL
G
HT29 TC
Control siRNA
Asp
siRNA to CBP
siRNA to PCAF
siRNA to GCN5L2
Cell Count
25
20
15
10
5
0
1.8%
24.9%
24.7%
28.1%
29.9%
0 10² 10³ 10⁴ 10⁵
FasL
H
Tunnel
Merge
HT29 TC with aspirin
HT29 TC with aspirin and p300 siRNA

Figure S6. p300 is required for aspirin-induced FasL expression. (**A**-**D**) qPCR results (mean ±SEM, n=3, normalized to of Gapdh mRNA expression) and immunoblots (**E**) of HT29 TCs transfected with siRNAs targeting p300, CBP, PCAF, or GCN5L2. *, $p<0.01$, unpaired t-test. (**F**) Expression of FasL based on FCM assay of HT29 TCs treated with scrambled control siRNA, Asp (5 mM), Asp (5 mM) and siRNA of *p300*, or Asp (5 mM) and the p300 inhibitor C464 (10 μM). (**G**) Expression of FasL based on FCM assay of HT29 TCs treated with scrambled control siRNA, Asp (5 mM), Asp (5 mM) and siRNA of *CBP*, *PCAF*, or *GCN5L2*. (**H**) Representative results of TUNEL assay on HT29 TCs treated with 5mM aspirin or siRNA against p300 plus 5mM aspirin. Scale bar indicates 50 μm.

Table S1. The characteristics of patients included in this study; supplement of Figure 1.

The characteristic of the patients

Patient numer	Sex	Age	Pathology	TNM	AJCC stage	Aspirin treatment	the time of Aspirin treatment
1	M	80	colon	T2N0M0	I	yes	2 years
2	M	71	colon	T3N0M0	II	yes	1.3 years
3	F	77	colon	T3N2M0	III	yes	1 month
4	M	63	rectal	T3N0M0	II	yes	10 years
5	F	82	colon	T4N1MO	III	yes	17 years
6	M	65	colon	T3N2MO	III	yes	7 months
7	M	78	colon	T3NOMO	II	yes	2 years
8	M	88	colon	T3N0M0	II	yes	3 years
9	M	76	rectal	T3NOMO	II	yes	7 years
10	M	74	colon	T3NOMO	II	yes	1 year
11	M	85	colon	T3NOMO	II	yes	20 years
12	M	78	rectal	T2N0M0	I	yes	14 years
13	M	77	rectal	T2N0M0	I	yes	1 year
14	F	77	colon	T3NOMO	II	yes	5 years
15	M	66	colon	T2N0M0	I	yes	6 years
16	M	82	colon	T3N1MO	III	yes	1 year
17	M	76	colon	T2N0M0	I	yes	1 year
18	M	72	rectal	T2N0M0	I	yes	1 year
19	M	84	colon	T4NOMO	II	no	
20	F	61	colon	T4N1MO	III	no	
21	M	77	rectal	T4NOM1	IV	no	
22	M	56	colon	T4N2M0	III	no	
23	F	76	colon	T4N0M0	II	no	
24	M	61	colon	T4N0M1	IV	no	
25	F	43	colon	T4N0M0	II	no	
26	M	76	colon	T4N0M0	II	no	
27	F	47	colon	T4N1M1	IV	no	
28	F	69	rectal	T4N1M0	III	no	
29	F	65	colon	T4N2M0	III	no	
30	M	81	colon	T2N0M0	I	no	
31	M	83	colon	T3N2M0	III	no	
32	M	59	colon	T3N2M0	III	no	
33	M	62	colon	T3N2M0	III	no	
34	F	65	colon	T3N2M0	III	no	
35	F	55	colon	T3N2M0	III	no	
36	M	68	colon	T3N0M0	II	no	
37	F	73	colon	T4N0M1	IV	no	
38	M	54	colon	T3N1M1	IV	no	

Table S2: *in vitro* Limiting Dilution Assay, related to Figure 1.

	Sphere LDA (CC-IC frequency, 1/colony #)		
	Lower	Estimate	Upper
HT29	1396	646	299
HT29 TCs	139.4	64.4	29.8
HT29 TCs with 2.5mM Asp	932	433	201
HT29 TCs with 5mM Asp	28690	12052	5063
P1	200	88.8	39.4
P1 TCs	83.9	38.6	17.7
P1 TCs with 2.5mM Asp	652	303	141
P1 TCs with 5mM Asp	93386	43429	20196
P2	28.36	11.87	4.97
P2 TCs	4.9	1.9	0.7
P2 TCs with 2.5mM Asp	10015	4447	1975
P2 TCs with 5mM Asp	65371	30400	14137

Table S3: IC50 of Oxaliplatin, related to Figure 1.

	IC50 of Oxa		
	ACs	TCs	TCs treated with 5mM Asp
HT29	31.73	100.2	44.25
P1	9.2	178.8	9.37

Note: IC50: 50% inhibitory Concentration; Oxa: Oxaliplatin, Asp: Aspirin.

Extended Experimental Procedures

Cell viability assay

TCs (10000/well in 96-well plates) were incubated with different doses of Asp in triplicate. The viable cells were evaluated via Cell Titer 96AQueous Non-230 Radioactive Cell Proliferation Assays (MTS, Promega) according to manufacturer's instructions.

Limited dilution assay (LDA)

LDA was performed as previously described (O'Brien et al., 2012). A defined number of cells were plated per well in an ultralow-attachment 96-well plate in serum-free medium. The cell concentration gradient was 100,000 to 1 cell per well in a fixed volume of 200 µL. Experiments were performed using octuplicate wells for each cell concentration. At the end of the experiment, a binary output was scored, *i.e.*, the wells were read as positive or negative. A positive well was defined as having at least one sphere and a negative well was defined as having no spheres based on visual inspection. The sphere-initiating cell frequency was then calculated using ELDA website software (http://bioinf.wehi.edu.au/software/elda/index.html).

Apoptosis assay

CSCs were treated with different doses of aspirin and digested by accutase (eBioscience) at 37° C for 5 min and then filtered through a 40-µm strainer into single cells, washed twice in 1x phosphate-buffered saline (PBS), and fixed in chilled 70% ethanol. The cells were stained with 5 µg/mL propidium iodide at room temperature for 10 min and analyzed using a BD Aria II flow cytometer. The cell cycle profile was determined with Modfit LT software. Apoptotic cells were confirmed via TUNEL staining using an *in situ* Cell Death Detection Kit FITC (Roche, Germany).

Quantitative real-time RT-PCR analysis

CSCs were homogenized and lysed in 350 µL buffer RLT, and total RNA was extracted using an RNeasy kit (Qiagen, Hilden, Germany) with column DNA digestion according to the standard protocol provided by the manufacturer. The mRNA was reverse transcribed into cDNA using an iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA). All PCRs were carried out using a StepOnePlus™ PCR system (Applied Biosystems, Foster City, California, USA). Following an activation step at 95° C for 10 min, determination of mRNA expression was performed over 40 cycles with denaturation for 15 sec at 95° C

and annealing/extension/data acquisition at 60° C for 60 sec using a Power SYBR Green PCR kit (Applied Bio systems). Relative fold mRNA expression levels were determined using the 2 (-ΔΔCt) method. All reactions were performed in triplicate, and the results are presented as the mean values and standard errors. The following primers were used:

ALDH1A1 forward ATGTCATCCTCAGGCACG
ALDH1A1 reverse ATCCTCCTTATCTCCTTCTTCTA
COX-2 forward CTGGCGCTCAGCCATACAG
COX-2 reverse CGCACTTATACTGGTCAAATCCC
18s rRNA forward GACTCAACACGGGAAACCTCAC
18s rRNA reverse CCAGACAAATCGCTCCACCAAC
Bcl-2 forward CGCCCTGTGGATGACTGAGTA
Bcl-2 reverse GGGCCGTACAGTTCCACAAAG
Bax forward CCCTTTTGCTTCAGGGTTTCATCCA
Bax reverse CTTGAGACACTCGCTCAGCTTCTTG
Fas forward GTTGGTGGACCCGCTCAGTA
Fas reverse AATCTAGCAACAGACGTAAGAACCAG
FasL forward GCAGCAGCCCTTCAATTACCCAT
FasL reverse CACAGAGGTTGGACAGGGAAGAA
caspase-3 forward GACAGACAGTGGTGTTGATGATGAC
caspase-3 reverse GCATGGCACAAAGCGACTGGAT
caspase-8 forward GCTGGAGTGCAGTGGCGTGAT
caspase-8 reverse GGGAGGCTGAGGCAGGAGAA
caspase-9 forward GCGAACTAACAGGCAAGCAGCAA
caspase-9 reverse CTCAAGAGCACCGACATCACCAAA
p300 forward AGCCAAGCGGCCTAAACTC
p300 reverse TCACCACCATTGGTTAGTCCC
KAT2A forward CTCTGCCTTAACTACTGGAAGC
KAT2A reverse GCCATCTGGTGTAATTGACCTTG
CREBBP forward CAACCCCAAAAGAGCCAAACT
CREBBP reverse CCTCGTAGAAGCTCCGACAGT
KAT2B forward CGAATCGCCGTGAAGAAAGC
KAT2B reverse CTTGCAGGCGGAGTACACT
Nanog forward CTCTCCTCTTCCTTCCTCCAT
Nanog reverse TTGCGACACTCTTCTCTGC
Oct4 forward CCATCTGCCGCTTTGAGG
Oct4 reverse ACGAGGGTTTCTGCTTTGC

Sox2 forward CAGCCCATGCACCGCTACGACG
Sox2 reverse CACCGAACCCATGGAGCCAAGAGC
Notch1 forward CACCCATGACCACTACCCAGTT
Notch1 reverse CCTCGGACCAATCAGAGATGTT
Bmi-1 forward AACTCGAGATCACAAATAGGAC
Bmi-1 reverse TTATACGTAGCTTGTTGCTCTTA
(Shan et al., 2012)

Flow cytometric analysis

For Fas or FasL labeling, 1×10^6cells were resuspended in fluorescence-activated cell sorting (FACS) buffer (2% FBS containing Ca^{2+} and Mg^{2+}-free PBS). Cells were stained with APC conjugated anti-Fas (DX-2) (eBioscience) or PE conjugated anti-FasL (Nok-1) (eBioscience) primary antibody at 37° C for 30 min. Cells were washed twice in wash buffer and finally resuspended in 500 μL FACS buffer. Fluorescently labeled cells were examined using a BD Aria II flow cytometer.

RNA interference

Oligonucleotides (GenePharma Tech, Shanghai, China) were transfected into TCs from HT29 and P1 cells using Lipofectamine® RNAiMAX (Invitrogen) according to the manufacturer's instructions. Cells were harvested and analyzed at 72 hr post-transfection. The siRNAs used to target p300, CBP, GCN5L2, PCAF and COX-2 were as follows:

p300 siRNA sense 5'-CAGAGCAGUCCUGGAUUACTT-3'
anti-sense 5'-GUAAUCCAGGACUGCUCUGTT-3'
GCN5L2 siRNA sense 5'-GCUCUACACAACCCUCAAATT-3'
anti-sense 5'-UUUGAGGGUUGUAGAGCTT-3'
COX-2 siRNA 1# sense 5'-GGAACGUUGUGAAUAACAUTT-3'
anti-sense 5'-AUGUUAUUCACAACGUUCCTT-3'
COX-2 siRNA 2# sense 5'-GUGGCUAUCACUUCAAACUTT-3'
anti-sense 5'-AGUUUGAAGUGAUAGCCACTT-3'
COX-2 siRNA 3# sense 5'-CUGCCUGACACCUUUCAAATT-3'
anti-sense 5'-UUUGAAAGGUGUCAGGCAGTT-3'
CBP siRNA sense 5'-AAUCCACAGUACCGAGAAAUGUUTT-3'
anti-sense 5'-AACAUUUCUCGGUACUGUGGAUUTT-3'
PCAF siRNA sense5'- UCGCCGUGAAGAAAGCGCATT-3'
anti-sense 5'-UGCGCUUUCUUCACGGCGATT-3'

(Love et al., 2012)

Synthesis of biotinylated acetylsalicylic acid

D-biotin (2.0 g, 8.19 mM) and N-hydroxysuccinimide (0.94 g, 8.19 mM) were dissolved into hot anhydrous DMF (60 mL, 70°C) in a 100-mL round-bottom flask while stirring. DCC (2.19 g, 10.65 mM) was added, and the solution was stirred overnight at room temperature. The formed solid was filtered off, and the residue was precipitated by excess ether. The white precipitate was filtered and washed with ether three times to yield a white powder of biotinyl-N-hydroxysuccinimide ester (Biotin-NHS). While stirring the solution of Biotin-NHS (0.682 g, 2 mM) in DMF (2 mL), ethylenediamine (1.3 mL, 20 mM) was added at 0°C. The reaction was continued for 2 hr. The precipitated solid was filtered off, and the excess ether was added to the residue. The precipitated white solid was filtered off and washed with ether to yield a white solid, N-(2-aminoethyl)biotinamide (Biotin-NH2). Then, ethylamine (211 µl, 1.5 mM) and isobutyl chloroformate (175 µL, 1.5 mM) were added to a solution of acetylsalicylic acid (270 mg, 1.5 mM) in DMF (5 mL) at 0°C for 30 min. A solution of Biotin-NH2 (357 mg, 1.2 mM) in DMF (15 mL) was added to the above mixture. Then, the reaction mixture was stirred overnight. The reaction mixture was precipitated by adding excess ether. The precipitated white solid was filtered off. Finally, the white solid was dissolved in excess acetone, which was evaporated to yield biotinylated acetylsalicylic acid as a white solid.

IP and western blotting

Cells were lysed in buffer A (150 mM NaCl, 50 mM Tris-HCl [pH 7.4], 0.5% [vol/vol] Nonidet P-40, and protease inhibitor cocktail) as indicated and quickly frozen on dry ice. The lysates were thawed, centrifuged at 14,000 rpm for 10 min, and cleared by incubation with 30 mL of a protein A/protein G mixture. Antibodies were incubated with 50 µL of 50% (vol/vol) protein A-protein G-Sepharose for 2 h and added to the lysates. After incubation overnight, the beads were extensively washed with the same buffer, and bound proteins were resolved by SDS-PAGE. For western blot analysis, proteins were transferred to PVDF membranes (Millipore) and probed with the indicated antibody for 1 hr. Reactive proteins were developed with secondary antibodies conjugated to horseradish peroxidase and visualized using an enhanced chemiluminescence detection system.

ChIP assay

ChIP assays were performed according to the manufacturer’s instructions (Pierce).

Briefly, CSCs ($1x10^6$) were administered with different doses of Asp. Then, cells were fixed with 37% formaldehyde, lysed with MNase and immunoprecipitated with anti-Ac-H3 and anti-p300. DNA isolated from the immunoprecipitated sample was amplified by PCR using the *FasL* primers 5'-GGAAGGTGAGCATAGCCTACTAACC-3' (forward) and 5'-CCTCAAGAAGCTCAGAGCAAACCC-3' (reverse). PCR was carried out using a Power SYBR Green PCR kit (Applied Biosystems) as follows: activation at 95° C for 10 min, determination of mRNA expression for over 40 cycles with 15 sec of denaturation at 95° C and annealing/extension/data acquisition at 60° C for 60 sec. The PCR product of the *FasL* promoter was 123 bp.

Supplemental References

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