Supplemental Figure Legend

Fig. S1 ER stress mediates the apoptotic effect of sulindac sulfide in HCT116 cells. (A) RT-PCR analysis of mRNA expression of indicated genes in HCT116 cells treated with 120 μM sulindac sulfide (SUS) for 8 or 24 hr. **(B)** Representative immunofluorescence staining pictures showing the induction of CHOP and BiP upon treatment of HCT116 cells with 120 μM SUS for 24 hr. Scale bars: 10 μm. **(C)** Representative Immuno-TEM pictures showing the surface expression of BiP (gold particles 6 nm) upon SUS treatment as in (B). Scale bars: 400 nm. **(D)** RT-PCR analysis of *DR5* mRNA expression in HCT116 cells transfected with indicated siRNA and treated with SUS as in (B). **(E)** Western blotting of indicated proteins in wildtype (WT) and *BID* KO HCT116 cells treated as in (B). **(F)** Western blotting of indicated proteins in WT and *DR5* KO HCT116 cells treated as in (B). **(G)** Apoptosis in WT and *DR5* KO HCT116 cells treated with 120 μM SUS for 48 hr was analyzed by counting condensed and fragmented nuclei after nuclear staining with Hoechst 33258. Results in (A), (D) and (G) were expressed as means + SD of three independent experiments. *P < 0.05; ***P < 0.001.

Fig. S2 ER stress mediates the apoptotic effect of sulindac sulfide and indomethacin in RKO and HT29 cells. (A) Western blotting of indicated proteins in RKO and HT29 cells treated with 200 μM sulindac sulfide (SUS) for 24 hr. *, non-specific bands. **(B)** Western blotting of indicated proteins in RKO and HT29 cells transfected with control scrambled or *ATF4* siRNA were treated with SUS as in (A). **(C)** Western blotting of indicated proteins in RKO and HT29 cells transfected with control scrambled or *CHOP* siRNA were treated with SUS as in (A). **(D)** Apoptosis in cells transfected and treated as in (B) and (C) was analyzed by counting condensed and fragmented nuclei after nuclear staining with Hoechst 33258. **(E)** Western blotting of indicated proteins in WT and *DR5* KO RKO cells treated as in (A). **(F)**

Apoptosis in WT and *DR5* KO RKO cells treated as in (A) was analyzed as in (D). **(G)** Western blotting of indicated proteins in RKO and HT29 cells treated with 500 μM indomethacin (Indo) for 24 hr. Results in (D) and (F) were expressed as means + SD of three independent experiments. **P < 0.01; ***P < 0.001.

Fig. S3 Analysis of immunogenic cell death in colon cancer cells treated with sulindac sulfide. (A) RKO cells pretreated with Salubrinal (1.0 μM) for 1.5 hr were treated with 200 μM sulindac sulfide (SUS) for 12 hr. CRT translocation was examined by immunostaining followed by flow cytometry. Means + SD are shown. **P < 0.01. **(B)**, **(C)** HCT116 cells with WT or KO of *BID* or transfection of *CHOP* or *ATF4* siRNA were treated with 120 μM sulindac sulfide (SUS) for 12 hr. After treatment, HCT116 cells and dendritic cells prepared from human blood in healthy donors were labelled with red (Far Red) and green (CFSE) fluorescence, respectively, and co-incubated at 1:1 ratio. DC phagocytosis was analyzed by detecting fused cells by fluorescence microscopy and quantified by flow cytometry. **(B)** Representative pictures of fluorescence microscopy showing early and late DC phagocytosis in indicated boxes. Scale bars: 10 μm. **(C)** Representative flow cytometry results. Black: HCT116 cells; green: DCs; red: fused HCT116 and phagocytic DC cells. **(D)** Analysis of DC phagocytosis in NCM356 cells transfected with control scrambled or *APC* siRNA and treated with SUS as in (B) and (C).

Fig. S4 ER stress inhibition and *BID* KO suppresses NSAID-mediated immune cell infiltration. Ten-week-old $APC^{Min/+}$ mice with WT or KO of *BID* were treated with sulindac +/- salubrinal as in Fig. 5A. Sections of small intestine were analyzed by immunostaining for indicated immune cell markers. **(A)** Representative pictures of CD3 staining. **(B)** Representative pictures and **(C)** quantification of CD11c staining. Scale bars: 25 μm. Results in (C) were expressed as means + SD; n=5. ***P < 0.001.

Fig. S5 Lymphocyte infiltration in advanced adenomas from patients treated with NSAIDs. Advanced adenomas from 16 patients taking NSAIDs and 14 patients not taking NSAIDs were analyzed for lymphocyte infiltration by CD3 immunostaining. *Left*: representative staining pictures; scale bars: 200 μm. *Right*: mean numbers + SEM of CD3 positivity.

Figure S1

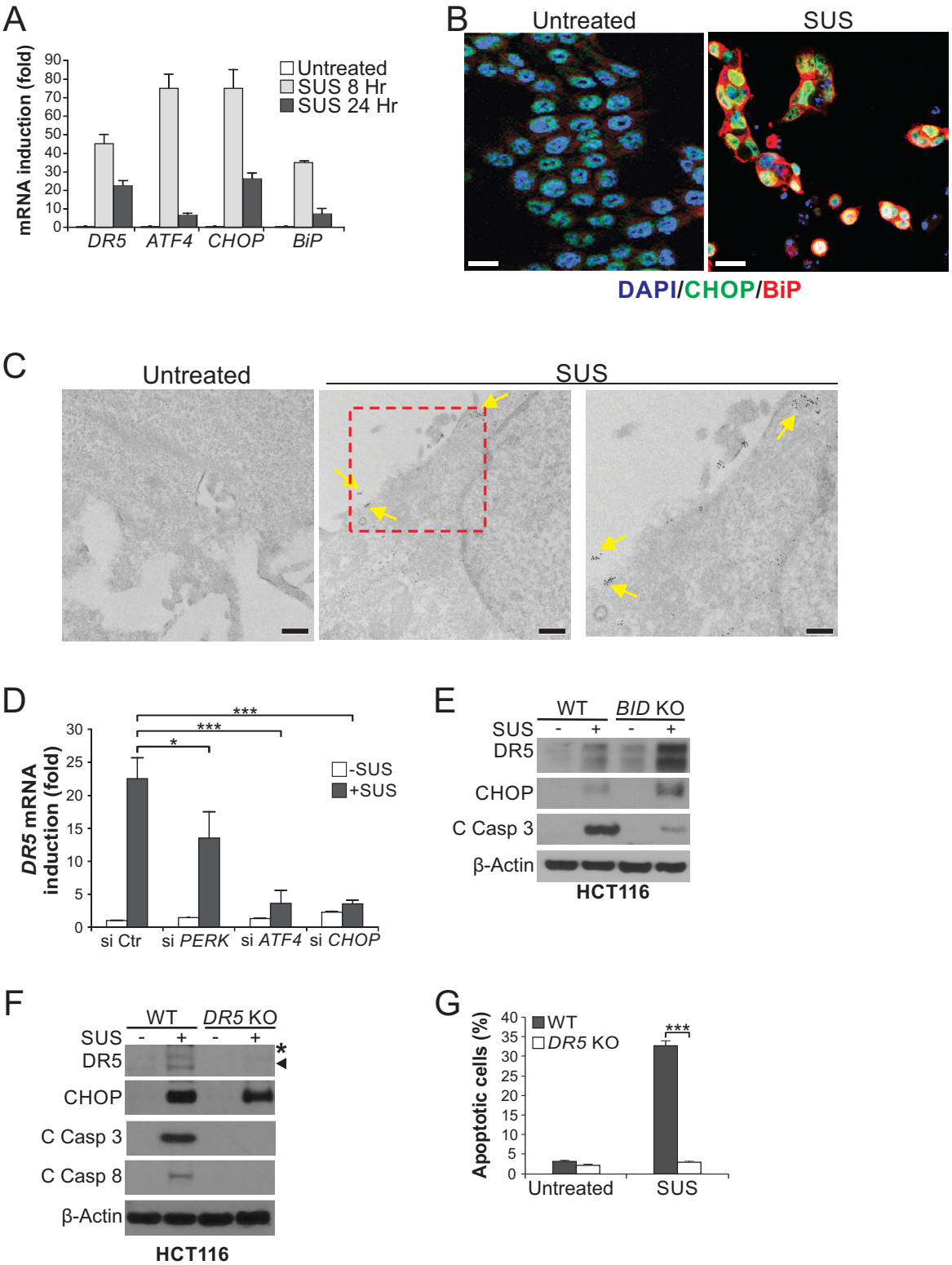

A
mRNA induction (fold)
90
80
70
60
50
40
30
20
10
0
Untreated
SUS 8 Hr
SUS 24 Hr
DR5
ATF4
CHOP
BiP
B
Untreated
SUS
DAPI/CHOP/BiP
C
Untreated
SUS
D
DR5 mRNA induction (fold)
30
25
20
15
10
5
0
***
***
*
-SUS
+SUS
si Ctr
si PERK
si ATF4
si CHOP
E
WT
BID KO
SUS
-
+
-
+
DR5
CHOP
C Casp 3
β-Actin
HCT116
F
WT
DR5 KO
SUS
-
+
-
+
DR5
CHOP
C Casp 3
C Casp 8
β-Actin
*
HCT116
G
Apoptotic cells (%)
40
35
30
25
20
15
10
5
0
WT
DR5 KO
***
Untreated
SUS

Figure S2

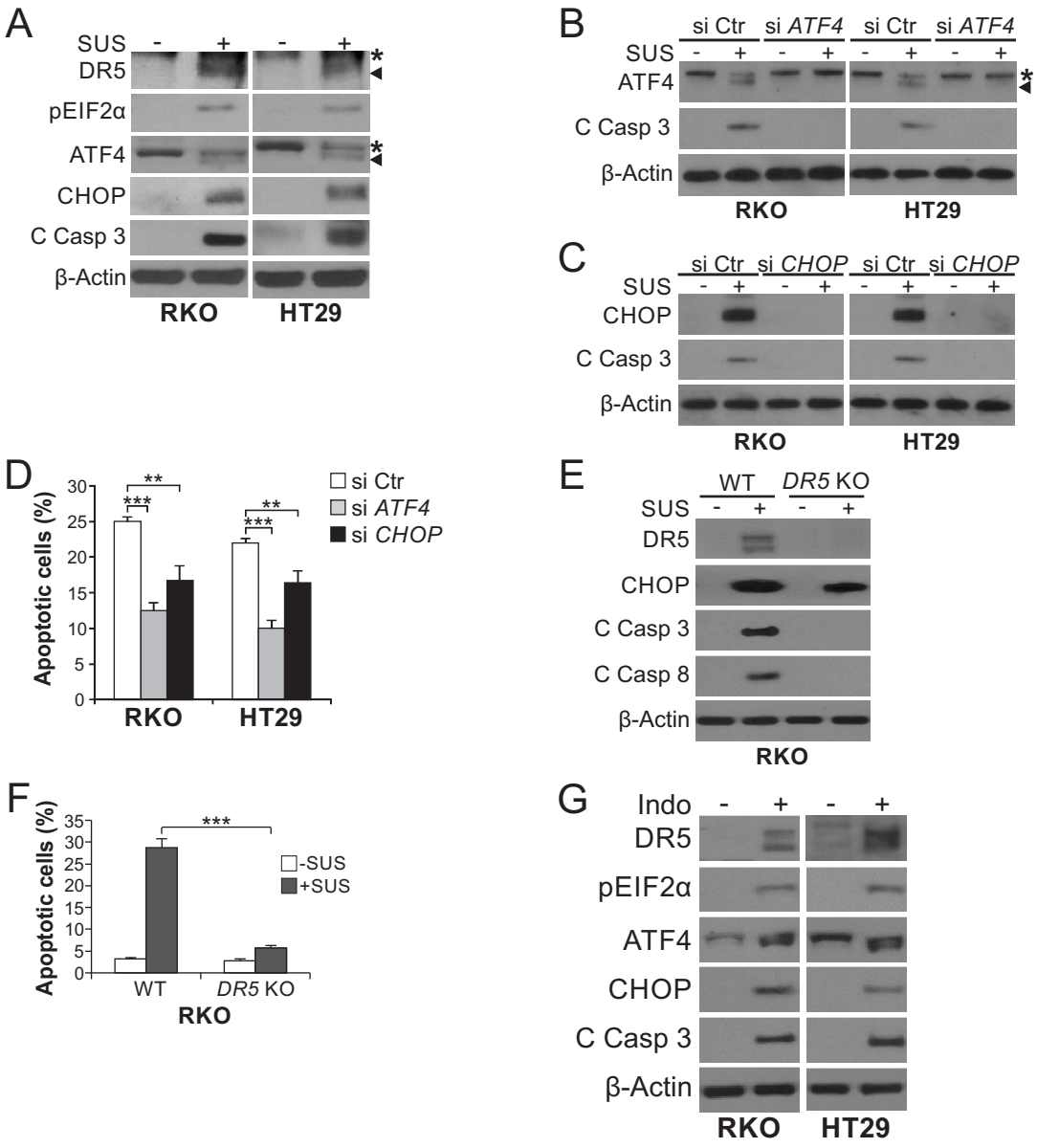
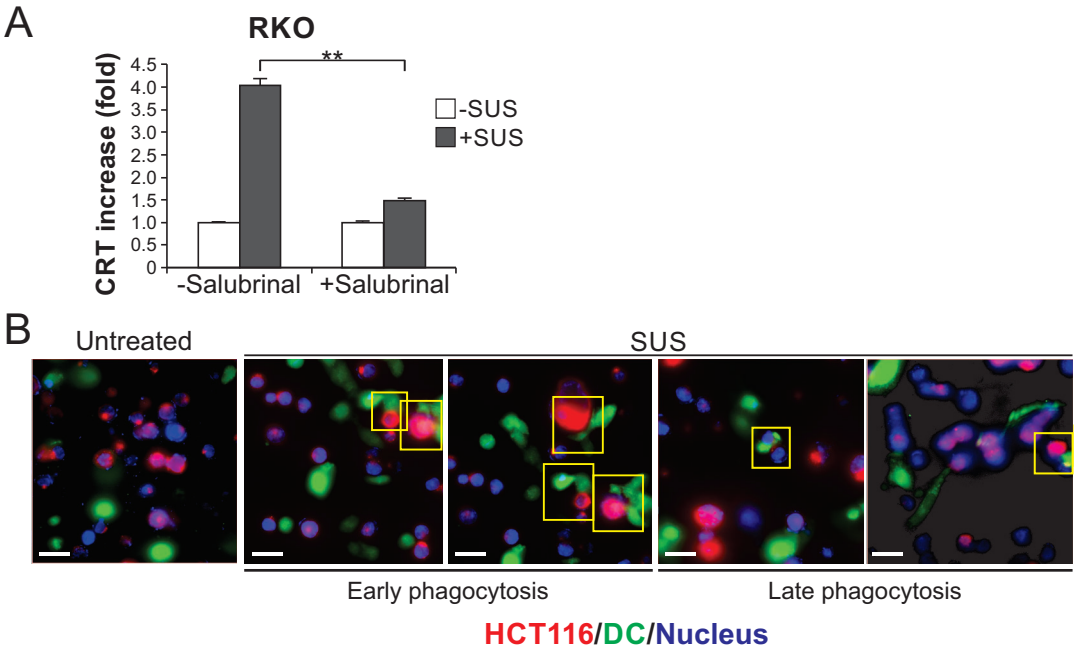
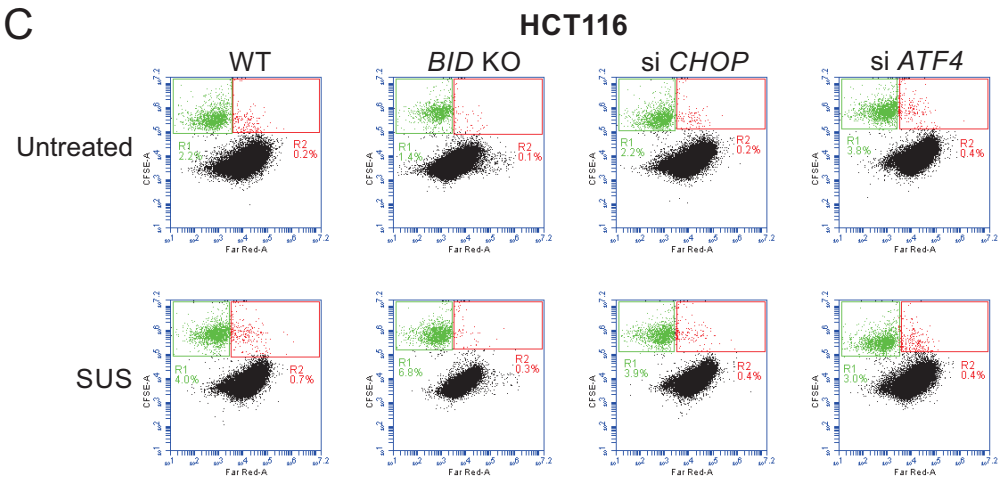

A
SUS - + - +
DR5
pEIF2α
ATF4
CHOP
C Casp 3
β-Actin
RKO HT29
B
si Ctr si ATF4 si Ctr si ATF4
SUS - + - + - + - +
ATF4
C Casp 3
β-Actin
RKO HT29
C
si Ctr si CHOP si Ctr si CHOP
SUS - + - + - + - +
CHOP
C Casp 3
β-Actin
RKO HT29
D
Apoptotic cells (%)
si Ctr
si ATF4
si CHOP
RKO HT29
E
WT DR5 KO
SUS - + - +
DR5
CHOP
C Casp 3
C Casp 8
β-Actin
RKO
F
Apoptotic cells (%)
-SUS
+SUS
WT DR5 KO
RKO
G
Indo - + - +
DR5
pEIF2α
ATF4
CHOP
C Casp 3
β-Actin
RKO HT29

Figure S3


A
RKO
CRT increase (fold)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
**
-SUS
+SUS
-Salubrinal
+Salubrinal
B
Untreated
SUS
Early phagocytosis
Late phagocytosis
HCT116/DC/Nucleus


C
HCT116
WT
BID KO
si CHOP
si ATF4
Untreated
SUS
CFSE-A
Far Red-A
R1
R2
2.2%
0.2%
1.4%
0.1%
2.2%
0.2%
3.8%
0.4%
4.0%
0.7%
6.8%
0.3%
3.9%
0.4%
3.0%
0.4%

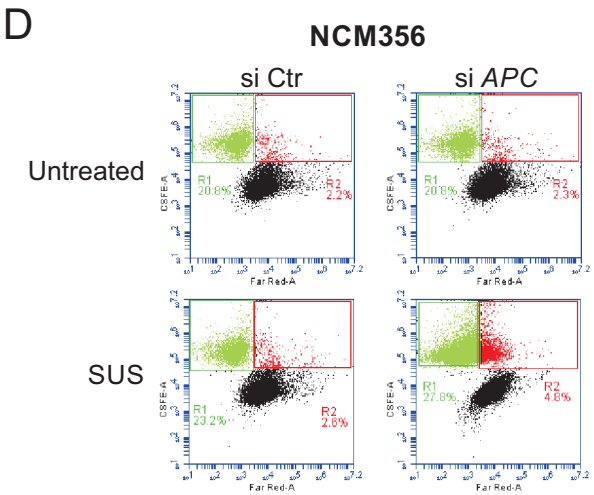

D
NCM356
si Ctr
si APC
Untreated
SUS
CSFE-A
Far Red-A
R1
R2
20.8%
2.2%
20.8%
2.3%
23.2%
2.8%
27.8%
4.8%

Figure S4

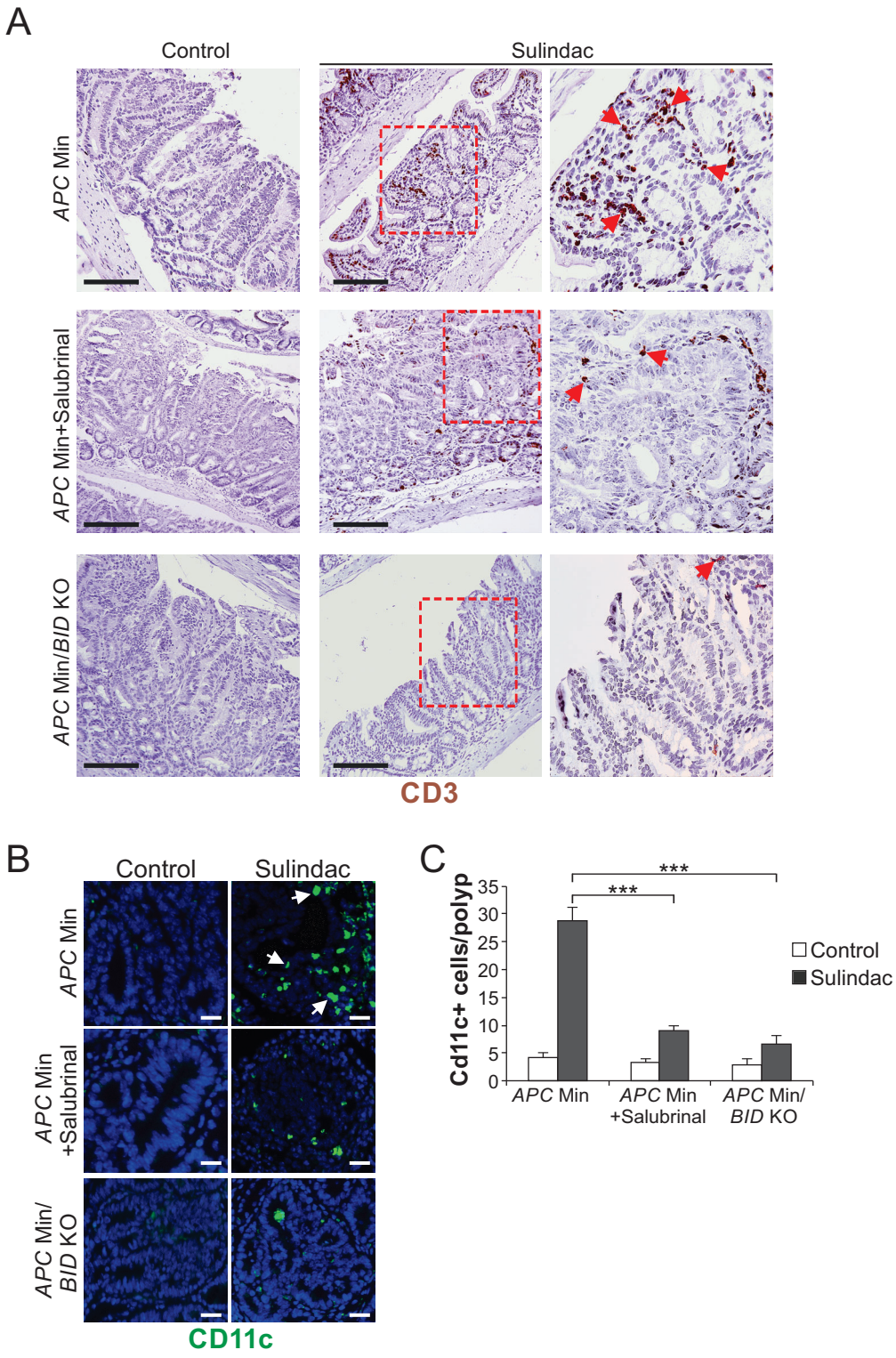
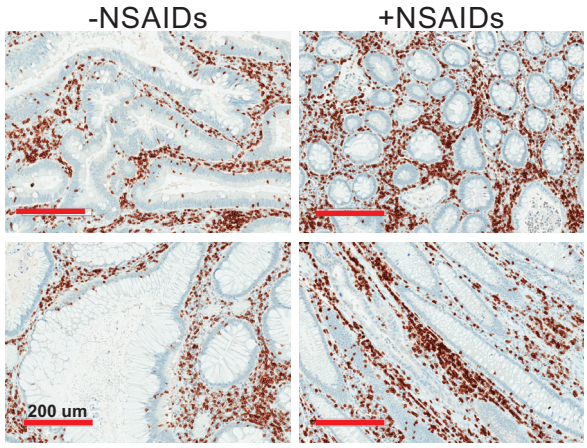

A
Control
Sulindac
APC Min
APC Min+Salubrinal
APC Min/BID KO
CD3
B
Control
Sulindac
APC Min
APC Min +Salubrinal
APC Min/ BID KO
CD11c
C
Cd11c+ cells/polyp
35
30
25
20
15
10
5
0
***
***
Control
Sulindac
APC Min
APC Min +Salubrinal
APC Min/ BID KO

Figure S5


-NSAIDs
+NSAIDs
200 um

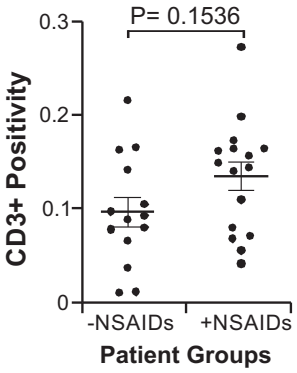

P= 0.1536
0.3
0.2
0.1
0
CD3+ Positivity
-NSAIDs
+NSAIDs
Patient Groups

Table S1- Key resources

Reagent or Resource	Source	Identifier
Chemicals		
Sulindac sulfide	Merk	N/A
Indomethacin	Sigma	I7378
Celecoxib	Sigma	Z0008
Acetaminophen	Sigma	A7085
Metformin	Cayman Chemical Company	*13118*
Naproxen sodium	Apex Bio	B5984
Diclofenac (sodium salt)	Cayman Chemical Company	*70680*
Sodium salicylate	Sigma	S3007
Salubrinal	Apex Bio	B2025
DMSO	Sigma	D2650-5X 10ML
Antibodies		
Western Blotting		
Rabbit polyclonal, Anti-cleaved caspase 3	Cell Signaling	#9661
Rabbit polyclonal, Death receptor 5	Abcam	#ab8416
Rabbit polyclonal, CREB-2 (ATF4)	Santa Cruz	#sc-200
Rabbit polyclonal, pEIF2α	Cell Signaling	#3398
Rabbit polyclonal, EIF2α	Santa Cruz	#sc-11386
Rabbit polyclonal, PERK	Cell Signaling	#5683P
Rabbit polyclonal, pPERK	Santa Cruz	#sc-32577
Rabbit polyclonal, Bid	Cell Signaling	#2002
Rabbit monoclonal, PDI	Cell Signaling	#3501P
Rabbit monoclonal, IRE1a	Cell Signaling	#3294P
Mouse monoclonal, β-Actin	Sigma	#A5441
Mouse monoclonal, CHOP	Cell Signaling	#2895s
Mouse monoclonal, APC	Calbiochem	#OP44
Mouse monoclonal, C-Myc	Santa Cruz	#sc-40
Goat polyclonal,caspase-8 p18	Santa Cruz	sc-6136
IHC/IF		
Rat monoclonal, CD8	eBioscience	#14-0195-82
Rabbit monoclonal, CD3	Novus Biologicals	#NB600-1441
Rabbit polyclonal, active caspase 8	Novus Biologicals	#NB100-56116
Mouse monoclonal, CHOP	ThermoFisher	#MA1-250
Rabbit polyclonal, BiP	Abcam	ab21685
Rabbit polyclonal, BiP	Abcam	ab21685
Rabbit polyclonal, CD69	Abcam	ab202909
Rabbit monoclonal, CD11c	Cell Signaling	97585
Rat monoclonal, MHCII	Abcam	ab25333

Goat anti-rabbit secondary antibodies, AlexaFluor 594	Invitrogen	A11012
Goat anti-mouse secondary antibodies, AlexaFluor 488	Invitrogen	A11001
Biotinylated goat anti-rabbit	Pierce	#31822
Biotinylated goat anti-rat	Pierce	#31830
Flow Cytometry		
Rabbit polyclonal, CRT	Abcam	ab2907
Goat anti-rabbit secondary antibodies, AlexaFluor 488	Invitrogen	A11008
Rabbit Ig	Jackson ImmunoResearch Laboratories	011-000-002
APC anti-human CD14	Biolegend	367117
FITC anti-human CD86	Biolegend	374203
PE anti-human HLA-DR	Biolegend	327007
PE/Cyanine7 anti-human CD83	Biolegend	305325
DC Preparation and Phagocytosis		
Healthy human buffy coats	Central Blood Bank, Pittsburgh, PA or BioIVT	N/A
Human CD14 MicroBeads	Miltenyi Biotec	130-050-201
Cell Trace Far Red Cell Proliferation Kit	ThermoFisher	C34564
CellVue Claret Far Red Kit	Sigma	MINCLARET-1KT
AIM V medium (Gibco)	ThermoFisher	12055091
Recombinant Human IL-4	PeproTech	200-04
Recombinant Human GM-CSF	PeproTech	300-03
Critical Commercial Assays		
Mini RNA Isolation II Kit	ZYMO Research	R1055
ABC Kit	Vector Laboratories	PK-6100
DAB	Vector Laboratories	SK-4100
VectaShield + DAPI	Vector Laboratories	H-1500
Experimental Models		
Cell Lines and Reagents		
HCT116, HT29 and RKO	American Type Culture Collection	N/A
McCoy's 5A medium (Gibco)	ThermoFisher	16600082
NCM356	INCELL	N/A
M3 media	INCELL	M300A-500
Animal Models and Diet		
APC$^{Min/+}$mice	Jackson Laboratory	002020
C57BL6J *BID*$^{-/-}$ (*BID* KO)	Jackson Laboratory	8887
AIN-93G diets	Dyets Inc	N/A
AIN-93G diets- 200 ppm sulindac	Dyets Inc	N/A

Table S2-Details for siRNA

siRNA	Sequence /Catalogue number	Vendor
APC-157	5'-GGAAGUAUUGAAGAUGAAG-3'	Dharmacon
APC-8993	5'-GCUGUGAAAUUCACAGUAAUA-3'	Dharmacon
CHOP	5'GCACAGCUAGCUGAAGAGA-3'	Dharmacon
ATF4-89	5'-GCCUAGGU UCUUAGAUGA-3'	Dharmacon
PERK	sc-36213	Santa Cruz

Table S3- PCR primers

Human Primers	Sequence
DR5 Forward	5'-AAGACCCTTGTGCTCGTTGT-3'
DR5 Reverse	5'AGGTGGACACAATCCCTCTG-3'
CHOP Forward	5'-GGTCCTGTCTTCAGATGAAAATG-3'
CHOP Reverse	5'-CAGCCAAGCCAGAGAAGCA-3'
BiP Forward	5'-CCAAGAGAGGGTTCTTGAATCTCG-3'
BiP Reverse	5'-ATGGGCCAGCCTGGATATACAACA-3'
APC Forward	5'-GGAAGCAGAGAAAGTACTGGA-3'
APC Reverse	5'-CTGAAGTTGAGCGTAATACCA-3'
ATF4 Forward	5'-GTTCTCCAGCGACAAGGCTA-3
ATF4 Reverse	5'-GTGTCATCCAACGTGGTCAG-3
β-Actin Forward	5'-GACATTAAGGAGAAGCTGTGCTATGTT-3'
β-Actin Reverse	5'-GCCTAGAAGCATTTGCGGTGGACGA-3'