**ROS release by *PPAR*β/δ-null fibroblasts reduces tumor load through epithelial antioxidant response.**

Eddie Han Pin TAN[1], Ming Keat SNG[1,2], Ivan Shun Bo HOW[1], Jeremy Soon Kiat CHAN[1], Jiapeng CHEN[2], Chek Kun TAN[2], Walter WAHLI[2,3,4] and Nguan Soon TAN[1,2,5,6]

[1]School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551.
[2]Lee Kong Chian School of Medicine, Nanyang Technological University, Novena Campus, 11 Mandalay Road, Singapore 308232.
[3]INRA ToxAlim, UMR1331, Chemin de Tournefeuille, Toulouse Cedex 3, France.
[4]Center for Integrative Genomics, University of Lausanne, Le Genopode, Lausanne, Switzerland.
[5]Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Agency for Science Technology & Research, Singapore 138673.
[6]KK Research Centre, KK Women's and Children Hospital, 100 Bukit Timah Road, Singapore 229899.

**Correspondences to:**

**EHPT,** Email: HTAN024@e.ntu.edu.sg; **NST (Senior Corresponding Author):** Email: nstan@ntu.edu.sg, Tel: +65 6316 2941; Fax: +65 6791 3856

**Supplementary Information**

## Supplementary Materials and Methods

*Generation of FSPCre-Pparb/d$^{-/-}$ mice*

Fsp1Cre transgenic mice were from Jackson Laboratory, Maine, USA (stock number 012641). The FspCre transgenic mice express Cre recombinase under the control of Fsp1 promoter. The *Pparb/d*$^{fl/fl}$ transgenic mice were obtained from Prof Walter Wahli (University of Lausanne, Lausanne, Switzerland). The transgenic mice carry alleles of the PPARβ/δ gene where the exon 4, which encodes the DNA-binding domain, was flanked by loxP sites [4]. FSPCre-*Pparb/d*$^{-/-}$ mice were generated in-house by crossing *Pparb/d*$^{fl/fl}$ and FspCre mice progenies, and backcrossed with *Pparb/d*$^{fl/fl}$ in C57BL/6 background for at least 6 generations.

*Generation of mice on APC$^{min}$ x FSPCre-Pparb/d$^{-/-}$ background*

APC$^{min/+}$ mice were purchased from The Jackson Laboratory (The Jackson Laboratory, Maine, USA) and maintained as heterozygous min/+ mice. APC$^{min/+}$ mice were crossed with FSPCre-*Pparb/d*$^{-/-}$ mice to generate mice with mixed APC$^{min/+}$ and FSPCre-*Pparb/d*$^{-/-}$ backgrounds.

*Mouse genotyping*

Small ear biopsies (< 0.5 cm) were taken from 3-week old mice and harvested for DNA as instructed in the KAPA Express Extract kit (Kapa Biosystems, Massachusetts, USA). PCR was performed with 2X KAPA2G Fast Genotyping Mix (Kapa Biosystems, Massachusetts, USA) with appropriate primers (Primer list attached).

*Chemical-induced murine colorectal cancer and colitis*

To chemically induce colonic tumors, mice were intraperitoneally injected with 10 mg/kg body weight azoxymethane (AOM; Sigma-Aldrich Co. LLC, Missouri, USA) dissolved in sterile 0.9 % saline. One week after injection, mice were fed 2% (w/v) dextran sulfate sodium (DSS; MP Biomedicals LLC, California, USA) dissolved in drinking water for 7 days *ad libitum*. To induce colitis, $APC^{min}$ mice were given 2% (w/v) DSS. Mice were then returned to normal drinking water until sacrifice. For anti-oxidant treatment, mice were administered with 400 µL of 40 mM N-acetylcysteine (NAC, Sigma-Aldrich Co. LLC, Missouri, USA) intra-gastrically for 5 consecutive days prior to DSS treatment. Mice were maintained at the facility under a 12-h dark-light cycle at 23°C ± 2°C and given standard mouse chow and water ad libitum. The DAI of treated mice were determined by combining scores of stool consistency, gross bleeding and gait changes (Supp. Table 2) [7]. Power analysis was used to estimate sample size. Double blinded randomization was used. All protocols were approved by the Institutional Animal Care and Use Committees (IACUC) in Nanyang Technological University (NTU), Singapore (Reference number: ARF-SBS/NIE-A0217) and SingHealth, Singapore (Reference number: IACUC #2013/SHS/866).

*Colonic fibroblast explant*

Colons were dissected longitudinally and washed for 30 min in ice cold phosphate buffered solution (PBS) containing 30% antimycotic/antibiotic solution (AA) (Thermo Fisher Scientific Inc., Massachusetts, USA). Specimens were further dissected to 1-2 $mm^2$ pieces and placed into empty culture plates for 10-15 min to promote attachment. Next, DMEM-10 medium supplemented with 10% FBS and 10% AA was added. The explants were incubated in a humidified incubator at 37°C and 5% $CO_2$. Colonic fibroblasts migrate out of the

explants after 72 h of explant incubation. Colonic fibroblasts were cultured for another 48 h before harvest.

*Cell Culture*

Human colon adenocarcinoma cell lines (HCT116; ATCC No. CCL-247), human immortalized skin-derived fibroblasts (BJ1; ATCC No. CRL-2522) and human colon myofibroblasts (CCD18Co, ATCC No. CRL-1459) were obtained from American Type Culture Collection (ATCC) and cultured in DMEM supplemented with 10% FBS (HyClone, Utah, USA). SV40-immortalized human colon epithelial cells were procured from Applied Biological Materials Canada (iCECs; T0570; ABM, Vancover, Canada) and cultured in Prigrow III Medium (ABM Canada) in collagen-coated flasks. The siRNA knockdown against PPARβ/δ was accomplished using Dharmacon ON-TARGETplus siRNA according to the manufacturer's protocol (Thermo Fisher Scientific Inc., Massachusetts, USA). Colon epithelial cells were seeded in 24-well transwell plates (Corning Inc, New York, USA) and were either incubated with conditioned media of explanted fibroblasts Pparb/$d^{fl/fl}$ and FSP-Pparb/$d^{-/-}$ mice for 24 hours, co-cultured with BJ1 or CCD18co in the top chamber of a transwell 0.4 μm pore size membrane insert for 48 hours, or treated with 1μM $H_2O_2$ with or without 5mM NAC (Sigma-Aldrich Co. LLC), Missouri, USA.

*Laser capture microdissection for FFPE tissue collection*

Tissue sections were mounted onto MembraneSlide 1.0 PEN (Carl Zeiss AG, Oberkochen, Germany) and histologically stained. Laser capture microdissection was performed using the PALM MicroBeam system (Carl Zeiss AG, Oberkochen, Germany) as previously described [1, 2].

*Real-time qPCR*

Total RNA was extracted from cells or tissues using TRIzol Reagent (Invitrogen, California, USA) followed by E.Z.N.A Total RNA Kit (Omega Bio-Tek, Georgia, USA). The purity and quantity of total RNA were recorded using Nanodrop ND1000 (Thermo Fisher Scientific Inc., Massachusetts, USA). Reverse transcription and real-time quantitative PCR (qPCR) were performed as previously described [5, 6]. The primers and their respective sequences are found in the attached primer list.

*Microarray Analysis*

LCM-collected tissues were processed for total RNA using the Ambion RecoverAll Total Nucleic Acid Isolation Kit for FFPE (Life Technologies, California, USA). The quantity of the collected RNA was determined using Bioanalyzer and processed for array hybridization using the GeneChip® WT Pico Kit (Affymetrix, California, USA) [8]. Microarray and data analysis were performed by Denova Science Pte Ltd (Singapore). The GeneChip® Mouse Gene 1.0 ST Array gene chips (Affymetrix, California, USA) were used. The data were normalized and analyzed using the Partek Genomics Suite v6.6 (Partek Inc., Missouri, USA). FSPCre-*Pparb/d*$^{-/-}$ and Pparb$^{fl/fl}$ samples were compared using multi-way ANOVA analysis in the Partek Genomics Suite. Gene ontology (GO) analysis of gene subsets was performed using the Ingenuity Pathway Analysis software (Ingenuity Systems Inc., California, USA). The microarray protocols and data have been deposited in NCBI's Gene Expression Omnibus (GEO) database accession number (GSE97473).

*Flow cytometry*

Fresh tissue biopsies were dissociated using the gentleMACS Dissociator (Miltenyi Biotec, Bergisch Gladbach, Germany). Homogenates were strained, washed and processed for

staining with the respective fluorophore-conjugated antibodies on ice, washed and resuspended in PBS. Flow cytometry was performed using Accuri C6 Flow Cytometer (BD Biosciences, New Jersey, USA) and analyses were completed on FlowJo v10.0.7 (FlowJo LLC, Oregon, USA).

Production of $H_2O_2$ in cultured cells was measured using 5-&-6-carboxy-2', 7'dichlorodihydrofluorescein diacetate (CM-$H_2$DCFDA; Sigma-Aldrich Co. LLC, Missouri, USA), as per the manufacturer's protocol. The oxidation of CM-$H_2$DCFDA to dichlorofluorescein (DCF) by $H_2O_2$ is representative of cellular ROS generated. Production of $H_2O_2$ in dissociated intestinal tissue was measured using CellROX Green oxidative stress detection kit (Thermo Fisher Scientific Inc., Massachusetts, USA), as per the manufacturer's protocol.

*Protein extraction and western blot.*

Fresh tissues were homogenized and lysed in mammalian protein extraction reagent (M-PER; Thermo Fisher Scientific Inc., Massachusetts, USA) supplemented with complete protease inhibitor mix (Roche Applied Science, Penzberg, Germany). Proteins from different subcellular compartment were extracted using Bio Basic's membrane, nuclear and cytoplasmic protein extraction kit (#BSP002; Bio Basic Inc, Ontario, Canada). Far-infrared immunoblotting was performed as previously described [3]. Antibodies used were PPAR β/δ (#sc-1987), NRF2 (#sc-722), PCNA (#sc-56) and β-Tubulin (#sc-9104) from Santa Cruz Biotechnology Inc., Texas, USA.

*Cytokine detection by multiplex microbead immunoassay*

Colon biopsies were processed and lysed in mammalian protein extraction reagent (M-PER; Thermo Fisher Scientific Inc., Massachusetts, USA) supplemented with complete protease inhibitor mix (Roche Applied Science, Penzberg, Germany). Samples were processed using a multiplex immunoassay service (Discovery Assay; Eve Technologies, Alberta Canada). The multiplex immunoassay was performed using the Milliplex mouse cytokine kit (EMD Millipore, Massachusetts, USA) and reading were obtained using the BioPlex 200 system (Bio-Rad Laboratories, Inc., California, USA). The assay examined 31 cytokines, namely Eotaxin, G-CSF, GM-CSF, IFNgamma, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, KC, LIF, LIX, MCP-1, M-CSF, MIG, MIP-1alpha, MIP-1beta, MIP-2, RANTES, TNFalpha, VEGF. The sensitivities of the assay ranged from 0.1 to 33.3 pg/mL.

*Statistical analysis*

Statistical analysis was performed using two-tailed non-parametric tests, either Mann-Whitney test (for 2 variables) or Kruskal-Wallis test (for > 2 variables). All statistical tests were performed using GraphPad Prism software (version 5; GraphPad Software Inc, California, USA), with p values of <0.05 considered significant. (*P < 0.05; **P < 0.01; ***P < 0.001; n.s., not significant.)

**Supplementary Figure Legends**

**Suppl Figure 1.**

**(A - D)** Relative fold change in mRNA levels of Vilin (A, C) and vimentin (B, D) in Colon (A, B) and Ileum (C, D) in laser microdissected mesenchyme (mes), and mucosal villi (villi) samples, compared to whole undissected intestine (whole). The results show the enrichment of villin expressing tissue in the villi samples and vimentin expressing tissue in the mesenchyme samples.

**(E, F)** Mean DAI score of DSS-treated mice over 5 weeks. FSPCre-*Pparb/d*$^{-/-}$ mice (n=14) displayed a lower DAI after withdrawal of DSS at week 2 when compared with *Pparb/d*$^{fl/fl}$ littermates (n=13) (E). Pretreatment with NAC before DSS results in FSPCre-*Pparb/d*$^{-/-}$ mice (n=5) and *Pparb/d*$^{fl/fl}$ littermates (n=5) (F). Data are represented as mean ± S.E.M.

**(G)** Representative images of colons from vehicle-, DSS- or NAC/DSS-treated *Pparb/d*$^{fl/fl}$ and FSPCre-*Pparb/d*$^{-/-}$ mice in APC$^{min/+}$ background at indicated weeks after treatment. APC$^{min/+}$ mouse spontaneously develops tumors after 25 weeks. Colons from APC$^{min/+}$FSPCre-*Pparb/d*$^{-/-}$ mice showed fewer tumors than APC$^{min/+}$*Pparb/d*$^{fl/fl}$ mice. Pretreatment with NAC before DSS greatly reduces tumor formation in *Pparb/d*$^{fl/fl}$. Scale: 1 cm

**(H, K, N)** Percentage survival of APC$^{min/+}$FSPCre-*Pparb/d*$^{-/-}$ and APC$^{min/+}$*Pparb/d*$^{fl/fl}$ mice (n=13-14) that were either developed tumors spontaneously for 25 weeks (H), given DSS treatment for 5 weeks (K) or pretreated with NAC and then DSS (N). In untreated and DSS-treated models, APC$^{min/+}$FSPCre-*Pparb/d*$^{-/-}$ mice maintained higher survivability than APC$^{min/+}$*Pparb/d*$^{fl/fl}$ (n=13-15). Pretreatment with NAC before DSS challenge improved the survival for APC$^{min/+}$*Pparb/d*$^{fl/fl}$ mice and poorer survival for APC$^{min/+}$FSPCre-*Pparb/d*$^{-/-}$ mice.

**(I, L, O)** Bar graph depicting total colonic tumor numbers per mouse. In untreated and DSS-treated models, colons of APC$^{min/+}$ FSPCre-*Pparb/d*$^{-/-}$ mice (n=14-15) showed 50% fewer tumors than cognate APC$^{min/+}$*Pparb/d*$^{fl/fl}$ colons (n=13-14). NAC pretreatment of APC$^{min/+}$ FSPCre-*Pparb/d*$^{-/-}$ mice (n=5) and cognate APC$^{min/+}$*Pparb/d*$^{fl/fl}$ colons (n=5). Values are mean ± S.E.M.

**(J, M, P)** Box-and-whisker plot showing distribution of tumor number and size in APC$^{min/+}$FSPCre-*Pparb/d*$^{-/-}$ and APC$^{min/+}$*Pparb/d*$^{fl/fl}$ mice that either spontaneously developed tumors (J), were treated with DSS (M) or were pretreated with NAC and then DSS.

**Suppl Figure 2.**

**(A - D)** FACS analysis of immune cells in colons from FSPCre-*Pparb/d*$^{-/-}$ and *Pparb/d*$^{fl/fl}$ mice treated with vehicle or DSS, gated for neutrophils (A), T-lymphocytes (B) and Macrophages (C, D). As expected, DSS treatment resulted in an increase immune cell numbers compared with vehicle treatment. No significant difference in infiltrated neutrophils and T-lymphocytes was observed between the two genotypes (A, B), but FSPCre-*Pparb/d*$^{-/-}$ colons showed higher macrophage numbers in the vehicle treated control (C). The macrophages were found to be the anti-inflammatory M2 subtype, agreeing with our earlier observations that FSPCre-*Pparb/d*$^{-/-}$ colons contained more anti-inflammatory cytokines. Values are mean ± S.E.M. (n=4).

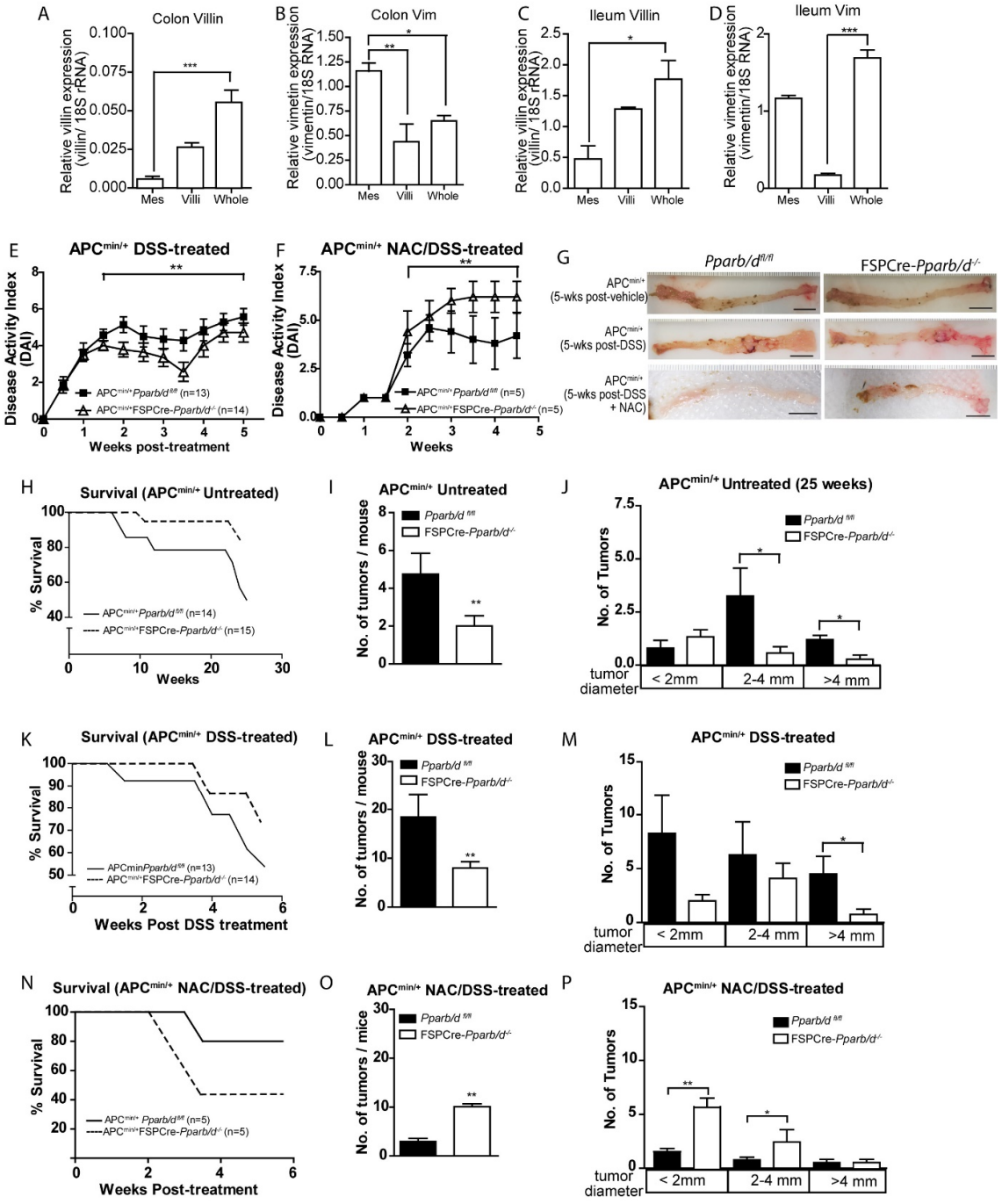
**(E, F)** Relative fold change in mRNA levels of 13 genes associated with oxidative stress response in ICEC (E, F) cultured in conditioned medium of fibroblasts from *Pparb/d*$^{fl/fl}$ and FSPCre-*Pparb/d*$^{-/-}$ (E) or treated with NAC and $H_2O_2$ (F). The expression of epithelial NRF2 and associated anti-oxidant genes were elevated in colon cancer cells cultured in conditioned medium from PPARβ/δ -deficient fibroblasts. Treatment of cells with $H_2O_2$ resulted in increased expression of antioxidant genes over vehicle-treated controls. Data are represented

as mean ± S.E.M. from 4 independent experiments. * P<0.05, ** P<0.01, n.d. expression below assay thresholds.

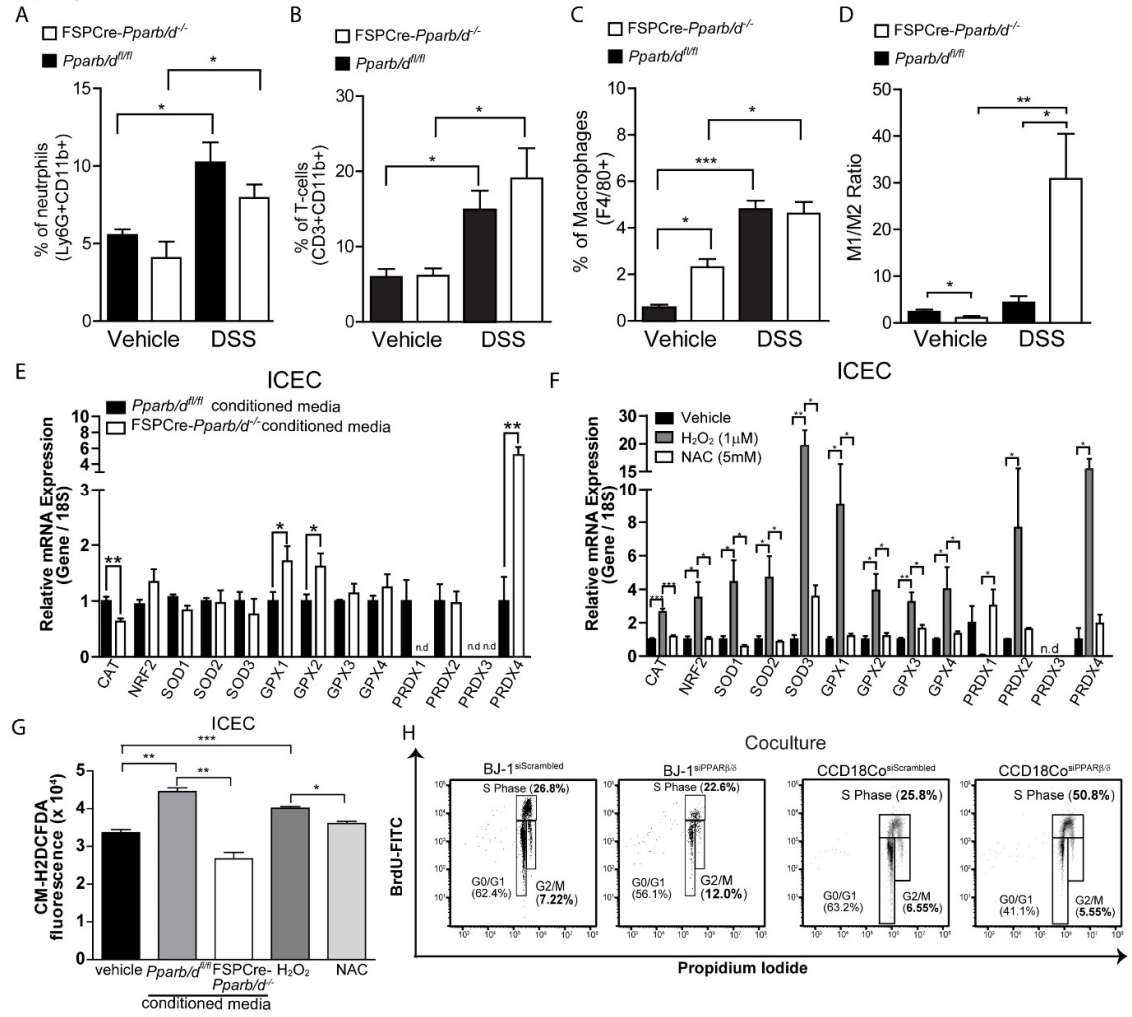
**(G)** Intracellular oxidative stress levels in ICEC cells either cultured in condition medium of fibroblasts from *Pparb/d*$^{fl/fl}$ and FSPCre-*Pparb/d*$^{-/-}$ or treated with $H_2O_2$ or NAC. Cells with no treatment served as cognate controls. Similar to prior results in HCT116, we observed a significant reduction in intracellular ROS level of ICEC cells cultured in conditioned medium from or co-cultured with PPARβ/δ -deficient fibroblasts as determined by CM-H2DCFDA assay. While $H_2O_2$-treated cells were significantly differed from untreated and NAC-treated ICECs, NAC-treatment did not produce a significant reduction in intracellular stress. Values are mean ± S.E.M. (n=4).

**(H)** Cellular proliferation assay by BrDU-FITC and PI staining of HCT116 cells co-cultured with normal BJ fibroblasts (BJ-1$^{siScrambled}$) and CCD-18co myofibroblasts (CCD18Co$^{siScrambled}$) or ablated of PPARβ/δ expression (BJ-1$^{siPPARβ/δ}$ and CCD18Co$^{siPPARβ}$). Values in bold denote percentage of proliferating cells in S phase and G2/M phase from cells in cell cycle arrest at G1/0 phase.

Suppl Fig. 1


A
Colon Villin
Relative villin expression (villin/ 18S rRNA)
Mes
Villi
Whole
B
Colon Vim
Relative vimetin expression (vimentin/18S RNA)
C
Ileum Villin
D
Ileum Vim
E
APCmin/+ DSS-treated
Disease Activity Index (DAI)
Weeks post-treatment
APCmin/+Pparb/d fl/fl (n=13)
APCmin/+FSPCre-Pparb/d-/- (n=14)
F
APCmin/+ NAC/DSS-treated
Weeks
APCmin/+Pparb/d fl/fl (n=5)
APCmin/+FSPCre-Pparb/d-/- (n=5)
G
Pparb/d fl/fl
FSPCre-Pparb/d-/-
APCmin/+ (5-wks post-vehicle)
APCmin/+ (5-wks post-DSS)
APCmin/+ (5-wks post-DSS + NAC)
H
Survival (APCmin/+ Untreated)
% Survival
APCmin/+Pparb/d fl/fl (n=14)
APCmin/+FSPCre-Pparb/d-/- (n=15)
Weeks
I
APCmin/+ Untreated
No. of tumors / mouse
Pparb/d fl/fl
FSPCre-Pparb/d-/-
J
APCmin/+ Untreated (25 weeks)
No. of Tumors
tumor diameter
< 2mm
2-4 mm
>4 mm
K
Survival (APCmin/+ DSS-treated)
APCminPparb/d fl/fl (n=13)
APCmin/+FSPCre-Pparb/d-/- (n=14)
Weeks Post DSS treatment
L
APCmin/+ DSS-treated
M
APCmin/+ DSS-treated
N
Survival (APCmin/+ NAC/DSS-treated)
APCmin/+ Pparb/d fl/fl (n=5)
APCmin/+FSPCre-Pparb/d-/- (n=5)
Weeks Post-treatment
O
APCmin/+ NAC/DSS-treated
No. of tumors / mice
P
APCmin/+ NAC/DSS-treated

Suppl Fig. 2


A
FSPCre-Pparb/d-/-
Pparb/dfl/fl
% of neutrphils (Ly6G+CD11b+)
Vehicle
DSS
B
% of T-cells (CD3+CD11b+)
C
% of Macrophages (F4/80+)
D
M1/M2 Ratio
E
ICEC
Pparb/dfl/fl conditioned media
FSPCre-Pparb/d-/- conditioned media
Relative mRNA Expression (Gene / 18S)
CAT
NRF2
SOD1
SOD2
SOD3
GPX1
GPX2
GPX3
GPX4
PRDX1
PRDX2
PRDX3
PRDX4
n.d
F
Vehicle
H2O2 (1μM)
NAC (5mM)
G
CM-H2DCFDA fluorescence (x 10⁴)
vehicle
Pparb/dfl/fl
FSPCre-Pparb/d-/-
H2O2
NAC
conditioned media
H
Coculture
BJ-1siScrambled
BJ-1siPPARβ/δ
CCD18CosiScrambled
CCD18CosiPPARβ/δ
S Phase (26.8%)
S Phase (22.6%)
S Phase (25.8%)
S Phase (50.8%)
G0/G1 (62.4%)
G2/M (7.22%)
G0/G1 (56.1%)
G2/M (12.0%)
G0/G1 (63.2%)
G2/M (6.55%)
G0/G1 (41.1%)
G2/M (5.55%)
BrdU-FITC
Propidium Iodide

**Suppl. Table 1: Cytokine levels in colon extracts of *FSP-Pparb/d* $^{-/-}$ mice vs *Pparb/d* $^{fl/fl}$**

| Cytokine | *Pparb/d* $^{fl/fl}$ | *FSP-Pparb/d* $^{-/-}$ |
|---|---|---|
| Eotaxin | 1.345 ± 0.195 | 1.361 ± 0.273 |
| G-CSF | 0.03 ± 0.01 | 0.026 ± 0.005 |
| **GM-CSF** | **0.013 ± 0.005** | **0.003 ± 0.003** |
| **IFNy** | **0.004 ± 0.002** | **0.002 ± 0.002** |
| IL-1a | 0.248 ± 0.036 | 0.346 ± 0.112 |
| IL-1B | 0.022 ± 0.007 | 0.035 ± 0.009 |
| IL-2 | 0.048 ± 0.008 | 0.05 ± 0.016 |
| **IL-3** | **0.001 ± 0.001** | **0.002 ± 0.001** |
| IL-4 | 0.001 ± 0.001 | 0.002 ± 0.001 |
| IL-5 | 0.023 ± 0.005 | 0.01 ± 0.004 |
| IL-6 | 0.006 ± 0.002 | 0.01 ± 0.001 |
| IL-7 | 0.073 ± 0.007 | 0.051 ± 0.01 |
| **IL-9** | **0.124 ± 0.013** | **0.279 ± 0.083** |
| **IL-10** | **0.018 ± 0.002** | **0.044 ± 0.013** |
| IL-12 (p40) | 0.012 ± 0.005 | 0.01 ± 0.003 |
| **IL-12 (p70)** | **0.027 ± 0.007** | **0.012 ± 0.006** |
| IL-13 | 0.004 ± 0.001 | 0.002 ± 0.001 |
| IL-15 | 0.196 ± 0.035 | 0.22 ± 0.076 |
| IL-17 | 0.01 ± 0.003 | 0.007 ± 0.002 |
| **IP-10** | **0.443 ± 0.07** | **0.115 ± 0.026** |
| **KC** | **0.104 ± 0.034** | **0.21 ± 0.047** |
| LIF | 0.019 ± 0.001 | 0.029 ± 0.003 |
| **LIX** | **0.028 ± 0.022** | **0.006 ± 0.006** |
| MCP-1 | 0.09 ± 0.019 | 0.074 ± 0.017 |
| **M-CSF** | **0.015 ± 0.004** | **0.036 ± 0.017** |
| **MIG** | **2.282 ± 1.16** | **0.694 ± 0.415** |
| **MIP-1a** | **0.203 ± 0.022** | **0.034 ± 0.013** |
| **MIP-1B** | **0.368 ± 0.127** | **0.051 ± 0.032** |
| MIP-2 | 0.399 ± 0.055 | 0.63 ± 0.177 |
| **RANTES** | **1.883 ± 0.659** | **0.116 ± 0.045** |
| TNFa | 0.02 ± 0.004 | 0.012 ± 0.002 |
| **VEGF** | **0.047 ± 0.006** | **0.136 ± 0.042** |

Highlighted **Bold** - indicates difference above 2-fold.
Cytokine in colon extracts (pg /mg protein s.e.m. )

**Suppl Table 2: Disease activity index (DAI) scoring matrix for DSS treatment**

| DAI Score | Stool Consistency | Presence of bleeding | Mouse appearance |
|---|---|---|---|
| 0 | Normal | None | Normal |
| 1 | Moist or sticky | Visible blood traces in stool or around anus | Ruffled fur or altered gait |
| 2 | Soft | Gross bleeding | Lethargic or moribund |
| 3 | Diarrhea | | |

**Primer List**

**List of genotyping primers**

| | |
|---|---|
| APCmin/+ | |
| Forward primer 1 (Apcwt) | 5'-GCCATCCCTTCACGTTAG-3' |
| Forward primer 2 (Apcmin) | 5'-TTCTGAGAAAGACAGAAGTTA-3' |
| Common reverse primer (ApcR) | 5'-TTCCACTTTGGCATAAGGC-3' |
| PPARβ/δ floxed | |
| Forward primer (Pbx10) | 5'-GCAGCTGCTCAGCTGCCTGC-3' |
| Reverse Primer 1 (Ab008) | 5'-GGACCCCGTAGTGGAAGCCCGAG-3' |
| Reverse Primer 2 (Ab021) | 5'-ATGCCGAGTGCCAGGCACTTCTG-3' |
| Fsp1Cre | |
| Forward primer (fspcreF) | 5'-GCGGTCTGGCAGTAAAAACTATC-3' |
| Reverse primer (fspcreR) | 5'-GTGAAACAGCATTGCTGTCACTT-3' |

**List of mouse qPCR primers**

| | |
|---|---|
| PPARβ/δ | |
| Forward | 5'- TGCAGATGGGCTGTGATGGG-3' |
| Reverse | 5'- CTCGAGCTTCATGCGGATTGTC-3' |
| Mouse catalase | |
| Forward | 5'- CCCCTATTGCCGTTCGATTCT-3' |
| Reverse | 5'- TTCAGGTGAGTCTGTGGGTTT-3' |
| Mouse nuclear factor erythroid-derived factor-2 (mNRF2) | |
| Forward | 5'-CCTAGTTCTCCGCTGCTGGG-3' |
| Reverse | 5'-CCATGTCCTGCTGGGACTGTA-3' |
| Mouse superoxide dismutase 1 | |
| Forward | 5'-ATGGCGATGAAAGCGGTGT-3' |
| Reverse | 5'-CCTTGTGTATTGTCCCCATACTG-3' |
| | |
| Mouse superoxide dismutase 2 | |
| Forward | 5'-CAGACCTGCCTTACGACTATGG-3' |
| Reverse | 5'-CTCGGTGGCGTTGGATTGTT-3' |
| Mouse superoxide dismutase 3 | |
| Forward | 5'-ACCGGCTTGGTTCTCTTCC-3' |
| Reverse | 5'-CTCCATCGGGTTGTAGTGCG-3' |
| Mouse glutathione peroxidase 1 | |
| Forward | 5'-AATGTCGCGTCTCTCTGAGG-3' |
| Reverse | 5'-TCCGAACTGATTGCACGGG-3' |
| Mouse glutathione peroxidase 2 | |
| Forward | 5'-GAGCTGCAATGTCGCTTTCC-3' |
| Reverse | 5'-TGGGTAAGACTAAAGGTGGG -3' |
| Mouse glutathione peroxidase 3 | |
| Forward | 5'-TTTGTGCCTAATTTCCAGCTCTT-3' |

| | |
|---|---|
| Reverse | 5’-GTCCATCTTGACGTTGCTGA-3’ |
| Mouse glutathione peroxidase 4 | |
| Forward | 5’-GATGGAGCCCBTTCCTGAACC-3’ |
| Reverse | 5’-CCCTGTACTTATCCAGGCAGA-3’ |

**List of human qPCR Primers**

| | |
|---|---|
| Human catalase | |
| Forward | 5’- TCATCAGGGATCCCATATTGTT-3’ |
| Reverse | 5’- CCTTCAGATGTGTCTGAGGATTT-3’ |
| Human nuclear factor erythroid-derived factor-2 | |
| Forward | 5’-ACACGGTCCACAGCTCATC-3’ |
| Reverse | 5’-TGCCTCCAAAGTATGTCAATCA-3’ |
| Human superoxide dismutase 1 | |
| Forward | 5’-TCATCAATTTCGAGCAGAAGG-3’ |
| Reverse | 5’-GCAGGCCTTCAGTCAGTCC-3’ |
| Human superoxide dismutase 2 | |
| Forward | 5’-AAGTACCAGGAGGCGTTGG-3’ |
| Reverse | 5’-TGAACTTCAGTGCAGGCTGA-3’ |
| Human superoxide dismutase 3 | |
| Forward | 5’-GGTGCAGCTCTCTTTTCAGG-3’ |
| Reverse | 5’-AACACAGTGGCGCCAGCATGGCC-3’ |
| Human glutathione peroxidase 1 | |
| Forward | 5’-TCTGTTGCTCGTAGCTGCTG-3’ |
| Reverse | 5’-CTATGGATCACCGGGATTTTG-3’ |
| Human glutathione peroxidase 2 | |
| Forward | 5’-CTGGTGGTCCTTGGCTTC-3’ |
| Reverse | 5’-TGTTCAGGATCTCCTCATTCTG-3’ |
| Human glutathione peroxidase 3 | |
| Forward | 5’-GGGGACAAGAGAAGTCGAAGA-3’ |
| Reverse | 5’-TGTTCAGGATCTCCTCATTCTG-3’’ |
| Human glutathione peroxidase 4 | |
| Forward | 5’-TTTCCGTGTAACCAGTTCG-3’ |
| Reverse | 5’-TGCCTCCAAAGTATGTCAATCA-3’ |
| Human peroxiredoxin 1 | |
| Forward | 5’-AATGCAAAAATTGGGTATCCTGC-3’ |
| Reverse | 5’-CCGTGGGACACACAAAAGTAAA-3’ |
| Human peroxiredoxin 2 | |
| Forward | 5’-GGTAACGCGCAAATCGGAAAG-3’ |
| Reverse | 5’-TCCAGTGGGTAGAAAAAGAGGT-3’ |
| Human peroxiredoxin 3 | |
| Forward | 5’-GTCTCGACGACTTTAAGGGAAAA-3’ |
| Reverse | 5’-ACTGAAACTGCAACTACTTCACA-3’ |
| Human peroxiredoxin 4 | |

| | |
|---|---|
| Forward | 5’-CTCAAACTGACTGACTATCGTGG-3’ |
| Reverse | 5’-CGATCCCCAAAAGCGATGATTTC-3’ |
| Human NADPH oxidase 1 | |
| Forward | 5’-AAGGATCCTCCGGTTTTACC-3’ |
| Reverse | 5’-TTTGGATGGGTGCATAACAA-3’ |
| Human NADPH oxidase 3 | |
| Forward | 5’-ACGGGTCGGATTGTTCGAGG-3’ |
| Reverse | 5’-ACGGTCGGATTGTTCGAGG-3’ |
| Human NADPH oxidase 4 | |
| Forward | 5’-TGCCTGCTCATTTGGCTGT-3’ |
| Reverse | 5’-CCGGCACATAGGTAAAAGGATG-3’ |

**List of mouse and human reference gene qPCR primers**

| | |
|---|---|
| 18S Ribosomal RNA (18S rRNA) | |
| Forward | 5’-GTAACCCGTTGAACCCCATT-3 |
| Reverse | 5’-CCATCCAATCGGTAGTAGCG-3’ |
| 60S Ribosomal protein L27 (RPL27) | |
| Forward | 5’-GCTGGTTATCGGGAGTTGG-3’ |
| Reverse | 5’-ACTGGCCTGGTGTCCTAGAG-3’ |

**List of siRNA sequences used in the study**

| | |
|---|---|
| PPARβ/δ siRNA (J-003435-06) | 5’-GACCUGGCCCUAUUCAUUG-3’ |
| PPARβ/δ siRNA (J-003435-07) | 5’-UAUCAUUGAGCCUAAGUUU-3’ |
| PPARβ/δ siRNA (J-003435-08) | 5’-GAGCGCAGCUGCAAGAUUC-3’ |
| PPARβ/δ siRNA (J-003435-09) | 5’-CCACUACGGUGUUCAUGCA-3’ |

**References**


1 Chan JS, Tan MJ, Sng MK, Teo Z, Phua T, Choo CC *et al*. Cancer-associated fibroblasts enact field cancerization by promoting extratumoral oxidative stress. Cell Death Dis 2017; 8: e2562.

2 Chong HC, Tan MJ, Philippe V, Tan SH, Tan CK, Ku CW *et al*. Regulation of epithelial-mesenchymal IL-1 signaling by PPARbeta/delta is essential for skin homeostasis and wound healing. J Cell Biol 2009; 184: 817-831.

3 Chong HC, Chan JS, Goh CQ, Gounko NV, Luo B, Wang X *et al*. Angiopoietin-like 4 stimulates STAT3-mediated iNOS expression and enhances angiogenesis to accelerate wound healing in diabetic mice. Mol Ther 2014; 22: 1593-1604.

4 Iglesias J, Barg S, Vallois D, Lahiri S, Roger C, Yessoufou A *et al*. PPARbeta/delta affects pancreatic beta cell mass and insulin secretion in mice. J Clin Invest 2012; 122: 4105-4117.

5 Lam CR, Tan MJ, Tan SH, Tang MB, Cheung PC, Tan NS. TAK1 regulates SCF expression to modulate PKBalpha activity that protects keratinocytes from ROS-induced apoptosis. Cell Death Differ 2011; 18: 1120-1129.

6 Lam CR, Tan C, Teo Z, Tay CY, Phua T, Wu YL *et al*. Loss of TAK1 increases cell traction force in a ROS-dependent manner to drive epithelial-mesenchymal transition of cancer cells. Cell Death Dis 2013; 4: e848.

7 Maxwell JR, Brown WA, Smith CL, Byrne FR, Viney JL. Methods of inducing inflammatory bowel disease in mice. Curr Protoc Pharmacol 2009; Chapter 5: Unit5 58.

8 Phua T, Sng MK, Tan EH, Chee DS, Li Y, Wee JW *et al*. Angiopoietin-like 4 Mediates Colonic Inflammation by Regulating Chemokine Transcript Stability via Tristetraprolin. Sci Rep 2017; 7: 44351.