Supplementary Figures Information

S1. (a) Representative TEM sections of liver, ileum and lung from *mdr1a*$^{-/-}$ and WT mice (n=6) Scale bar 2μm. (b) TEM sections of colons from *il10*$^{-/-}$ and WT-following 3 days of 3% DSS colitis. Orange scale bar is 2μm and white scale bar is 0.5μm. Yellow asterisks – denoting abnormal mitochondria.

S2. (a) Western blot analyses of p62, Parkin, PINK, LC3I-II in isolated mouse CECs (n=3/group). (b) Quantitative PCR of mitochondria DNA normalised to nuclear *18S* in CECs (n=6/group). (c) Immunohistochemistry of Ki67 in colon sections (n=6/group). Quantification in right panel, black scale bar 100μm (d) TUNEL assay (green: quantification in right panel), white scale bar 100μm (n=6/group). WT – wild-type. All data represent mean± SEM.

S3. (a) (b) Quantitative PCR of *MDR1* gene (triplicate) and representative Western blotting of MDR1 (C219) in T84 *shMDRI* and *shCtrl*. (c) Basal OCR of T84 *shMDRI* and *shCtrl* (5 replicates). (d) Western blotting of p62 and SOD2 and, (e) LC3I/II in untreated or bafilomycin (100nM for 2 hours) treated *shMDR1* vs. *shCtrl* CECs (representative of 2 independent experiments). All data represent mean± SEM.

S4. (a) Representative H&E colon sections following colonic treatment with rotenone at 1, 10 and 100μM (3x/week and harvested at Day 7; n=4/group) in WT mice. (b) Representative H&E colon sections and (c) histology colitis scores of *mdr1a*$^{-/-}$ and WT mice (n=5 in 0.25% DSS groups and n=6 in rotenone groups), black scale bar 100μm, yellow arrows indicating leukocyte infiltration. (d) Representative TUNEL staining in *mdr1a*$^{-/-}$ and WT mice (n=3/group) and (e) quantification of TUNEL+ve CECs (green: quantification in right panel), white scale bar 100μm. (e) qPCR analyses of isolated CECs in rotenone vs. vehicle treated *mdr1a*$^{-/-}$ and untreated WT mice (n=6/group). *p<0.05 comparing vehicle vs. rotenone treatment in *mdr1a*$^{-/-}$ mice. All data represent mean± SEM.

S5. (a) Western blotting of PARP and cleaved PARP products in T84 *shMDR1* vs. *shCtrl* treated with rotenone (10μM), CCCP (10μM), Cisplatin 10ng/ml for 12 hours in T84 *shMDR1* vs. *shCtrl*. (b) Live cell imaging of T84 *shMDR1* vs. *shCtrl* treated with rotenone (5μM) at 0 and 24 hours. Loss of viability – rounded appearance, increased lucency and decreased adherence. (c) Rate of transepithelial electrical resistance (TEER) loss in T84 *shMDR1* vs. *shCtrl* grown on 12-well transwell plates following culture with rotenone 5μM, (n=3). (d) IL-8 ELISA of T84 *shMDR1* vs. *shCtrl* 12 hours following co-culture with rotenone (10μM), flagellin (10ng/ml) and bacterial CpG (2μM) (n=2/treatment group, representative of 2 independent experiments), R=rotenone, F=flagellin. All data represent mean± SEM.

S6. (a) Representative H&E staining of rotenone vs. vehicle vs. MQ vs. non-colitic *mdr1a*$^{-/-}$ colons. (b) Representative H&E staining of MQ vs. vehicle following 7-day 2% DSS colitis protocol in WT and *mdr1a*$^{-/-}$ colons, (c) Representative H&E staining of MQ vs. vehicle treated WT colons following 5-day 2% DSS and 5-days recovery, scale bar 50μm.

Supplementary Material and Methods

Immunohistochemistry, Western blotting and qPCR

Formalin-fixed paraffin-embedded sections were stained according to standard immunohistochemistry protocols and Ab dilutions are available on request. Cell death was assessed by TdT-mediated dUTP nick end labelling (TUNEL) of formalin-fixed paraffin-embedded slides using *in situ* TUNEL cell death detection kit (Roche). We enumerated total TUNEL^{+} and Ki67^{+} IECs in entire colonic slide sections and data

expressed as percentage of total IECs (>3 slides per subject). For Western blotting, cells were lysed with RIPA buffer, protein quantification by Bradford protocol, 20-40 µg of total protein was applied to each lane and subjected to SDS-PAGE and Western blotting via enhanced chemiluminescence (Luminata Classico, Millipore). Total RNA was isolated from cells using RNeasy Mini kit (QIAGEN) and qPCR performed with SYBR Green RT-PCR Kit (Qiagen Cat # 204243) on ABI 7900 system. Experimental groups were compared using ΔΔCt values. Mouse *KC* 5-GGCTGGGATTCACCTCAAGAA-3, 5-CTTGGGGACACCTTTTAGCATC-3; *il-6* 5-GATGGATGCTACCAAACTGGA-3, 5-GGAAATTGGGGTAGGAAGGA-3; *tnf-α* 5-CTGGGACAGTGACCTGGACT-3, 5-GCACCTCAGGGAAGAGTCTG-3; mouse *18S* Housekeeping FORWARD, 5-TAGAGGGACAAGTGGCGTTC-3; REVERSE 5-CGCTGAGCCAGTCAGTGT-3.

Chemicals

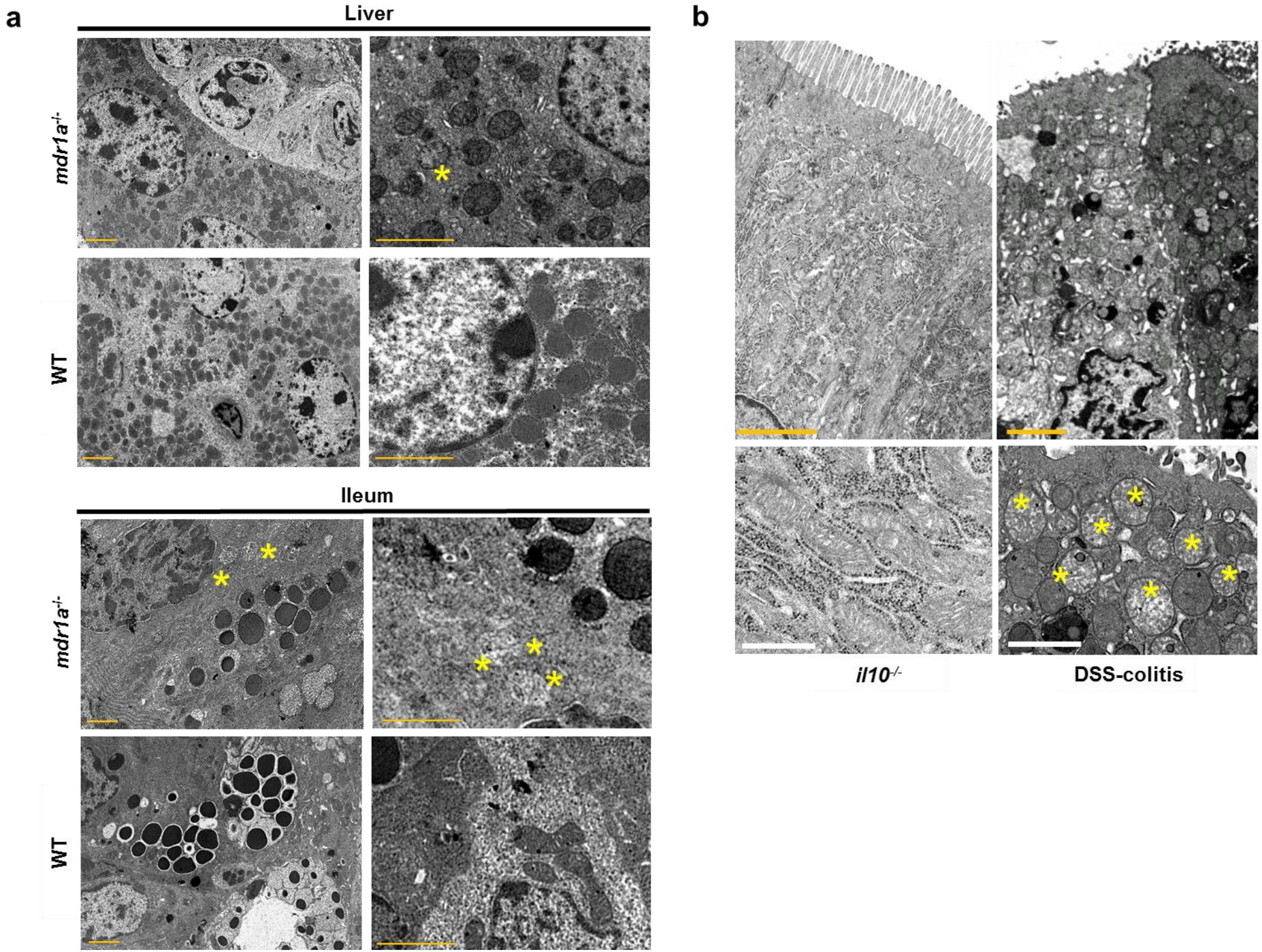
Anti-p62/SQSTM1 (1:1000 IHC, MBL), monoclonal anti-SOD2 (ab68155), polyclonal anti-SOD2 (1:50, PA5-30604 Thermoscientific), anti-VDAC (Abcam ab18988), PARP (Cell signalling), anti-PINK-1 (1:100 IHC, Abcam ab23707), anti-Parkin (Abcam ab81153), anti-Ki67 (Novo castra NCL-Ki67p), anti-TLR9 (abcam ab52967). Antimycin A (A8674-Sigma), Rotenone (R8875-Sigma), Carbonyl-cyanide 4-(trifluoromethoxy) phenylhydrazone, CCCP (C2920 – Sigma), Bafilomycin (B1793-Sigma) TLR9 antagonist ODN TTAGGG (Invivogen), Lipofectamine (ThermoFisher). $MitoQ_{10}$ was a kind gift from Dr Mike Murphy, University of Cambridge. Mitotracker Green and MitoSOX (Molecular Probes). GFP-LC3 plasmid was kindly provided by Dr C Stevens, University of Edinburgh. IL-8 and IL-6 ELISA (Cambridge Bioscience).

Mouse genotyping primers

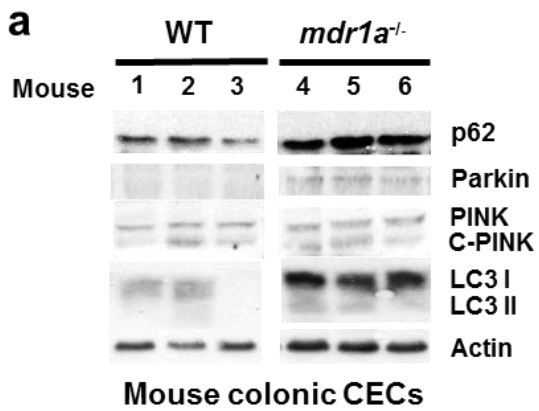
SOD2: LoxNeo 5-AGCTTGGCTGGACGTAA-3, *MnSOD#42* 5-CGAGGGGCATCT AGTGGAGAA-3; *P2* 5-TTAGGGCTCAGGTTTGTCCAGAA-3; Flox-allele 358 bp, wild-type 500 bp; 95C for 5 mins, 32 cycles of 94C for 1 min, 58C for 1 min, 72C for 2 mins and finally 72C for 10 mins. IMR 1878 5-GTGTGGGACAGAGAACAAACC-3, IMR 1879 5-ACATCTTCAGGTTCTGCGGG-3; 94C for 3 mins, 35 cycles of 94C for 30 seconds, 64C for 1 min, 72C for 1.5 min and finally 72C for 2 mins; villin-Cre transgene 1100 bp.

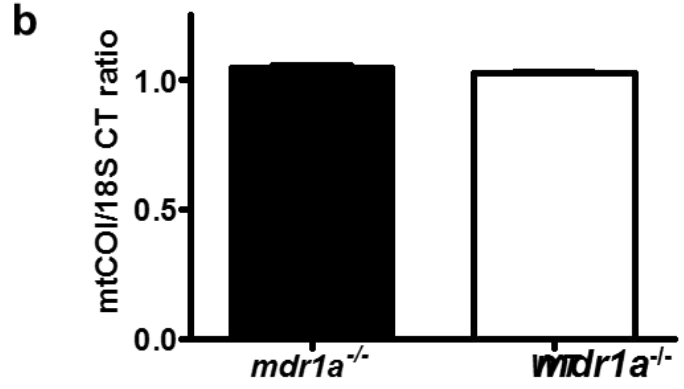
Table S6: Genes involved in mitochondria function (using gene-set defined by term GO:0005739) within subset of 574 genes within 100 kb of loci with genome wide statistical significant association with inflammatory bowel disease ($p<5 \times 10^{-8}$).

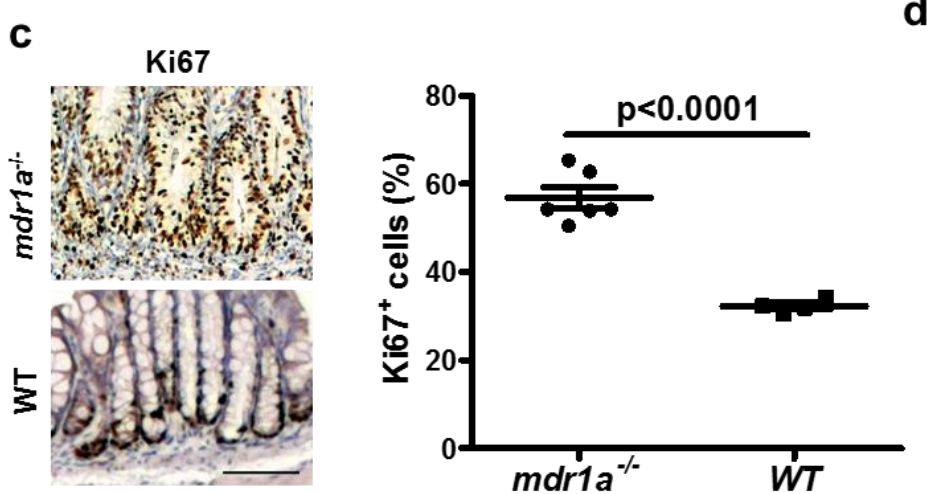
Gene	Gene ID	P-value	Protein Name
SLC25A28	81894	1.70×10^{-26}	Mitoferrin-2
VARS	7407	4.83×10^{-26}	Valine--tRNA ligase
RNF5	6048	9.47×10^{-24}	E3 ubiquitin-protein ligase RNF5
HSPA1A	3303	1.88×10^{-23}	Heat shock 70 kDa protein 1A/1B
HSPA1B	3304	1.88×10^{-23}	Heat shock 70 kDa protein 1A/1B
HSPA1L	3305	1.88×10^{-23}	Heat shock 70 kDa protein 1-like
TAP1	6890	2.78×10^{-21}	Antigen peptide transporter 1
GPX1	2876	2.40×10^{-20}	Glutathione peroxidase 1
SLC22A4	6583	7.07×10^{-17}	Solute carrier family 22 member 4
PLA2G2A	5320	2.31×10^{-16}	Phospholipase A2, membrane associated
MCCD1	401250	4.56×10^{-16}	Mitochondrial coiled-coil domain protein 1
STARD3	10948	1.35×10^{-15}	StAR-related lipid transfer protein 3
LRRK2	120892	3.18×10^{-15}	Leucine-rich repeat serine/threonine-protein kinase 2
DLD	1738	4.50×10^{-14}	Dihydrolipoyl dehydrogenase, mitochondrial
STAT3	6774	2.16×10^{-12}	Signal transducer and activator of transcription
PTRF	284119	2.16×10^{-12}	Polymerase I and transcript release factor
GPX4	2879	2.37×10^{-11}	Phospholipid hydroperoxide glutathione peroxidase, mitochondrial
TUFM	7284	3.86×10^{-10}	Elongation factor Tu, mitochondrial
PARK7	11315	7.36×10^{-10}	Protein DJ-1
NDUFAF3	25915	7.92×10^{-10}	NADH dehydrogenase [ubiquinone]1 alpha subcomplex assembly factor 3
SLC25A20	788	1.06×10^{-9}	Mitochondrial carnitine/acylcarnitine carrier protein
ATG5	9474	1.26×10^{-9}	Autophagy protein 5
TRIM39	56658	1.46×10^{-9}	E3 ubiquitin-protein ligase TRIM39
C6orf136	221545	2.77×10^{-9}	Uncharacterized protein C6orf136
TRIM31	11074	1.02×10^{-8}	E3 ubiquitin-protein ligase TRIM31
SDHC	6391	1.08×10^{-8}	Succinate dehydrogenase cytochrome b560 subunit, mitochondrial
UQCR10	29796	4.54×10^{-8}	Cytochrome b-c1 complex subunit 9
VARS2	57176	1.21×10^{-9}	Valine--tRNA ligase, mitochondrial

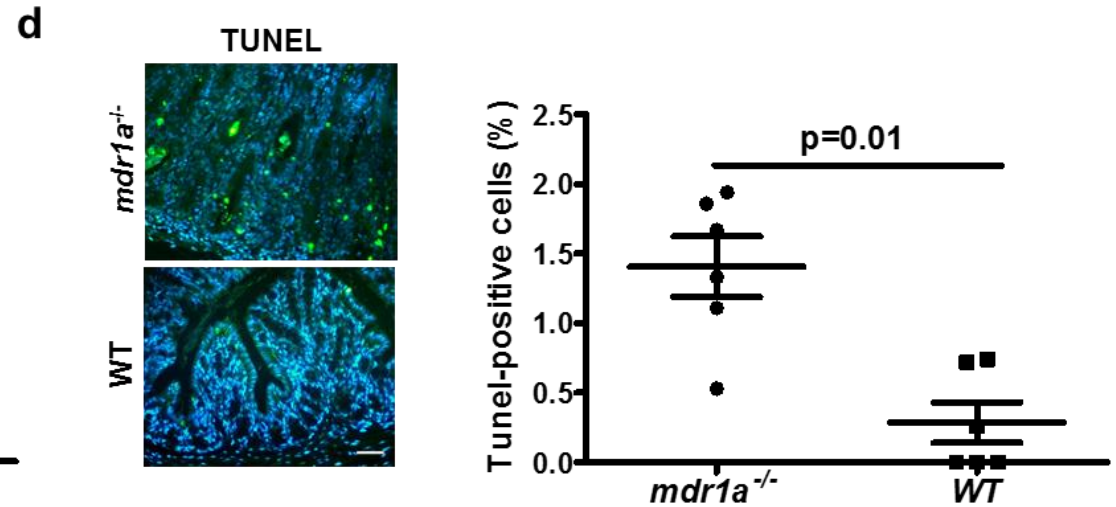

a
Liver
mdr1a-/-
WT
Ileum
mdr1a-/-
WT
b
il10-/-
DSS-colitis

SI 2

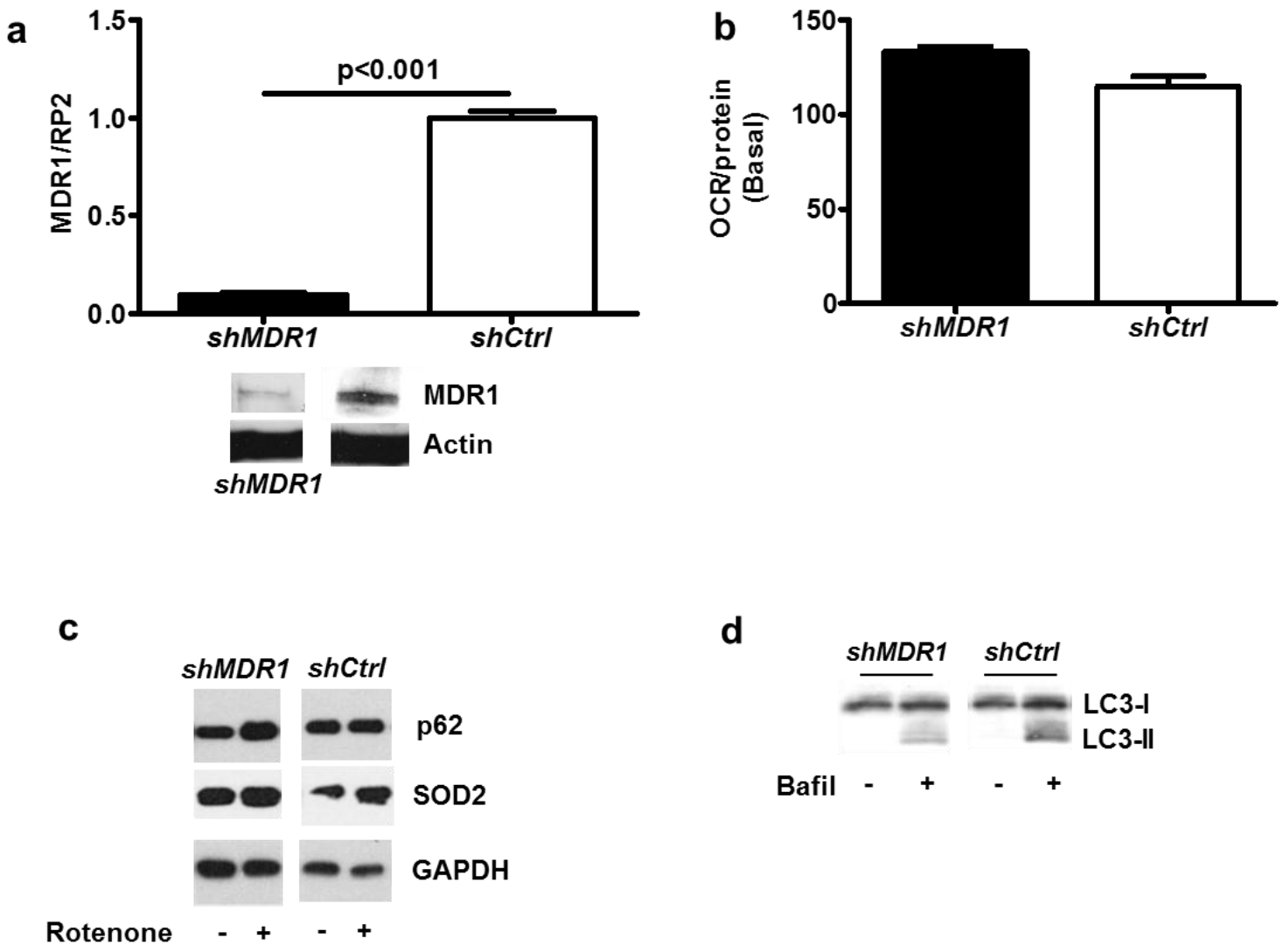

a
WT
mdr1a-/-
Mouse
1 2 3 4 5 6
p62
Parkin
PINK
C-PINK
LC3 I
LC3 II
Actin
Mouse colonic CECs


b
mtCOI/18S CT ratio
1.0
0.5
0.0
mdr1a-/-
wtdr1a-/-

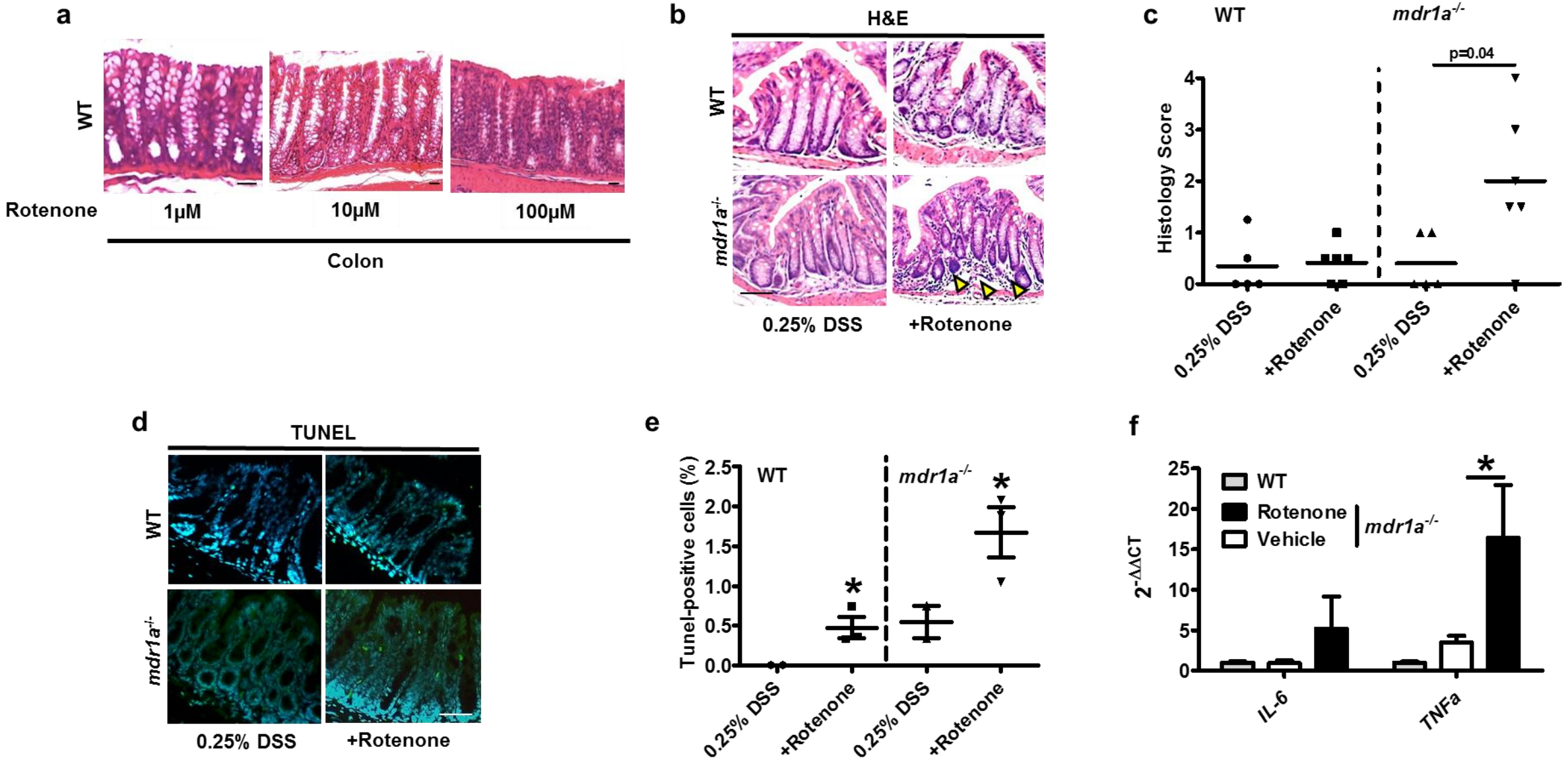

c
Ki67
mdr1a-/-
WT
Ki67+ cells (%)
80
60
40
20
0
p<0.0001
mdr1a-/-
WT


d
TUNEL
mdr1a-/-
WT
Tunel-positive cells (%)
2.5
2.0
1.5
1.0
0.5
0.0
p=0.01
mdr1a-/-
WT

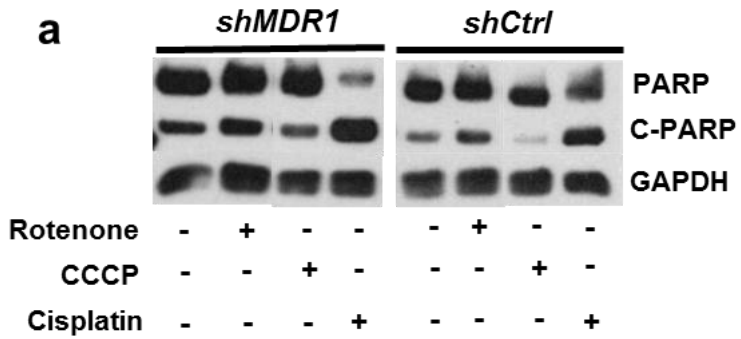
SI 3

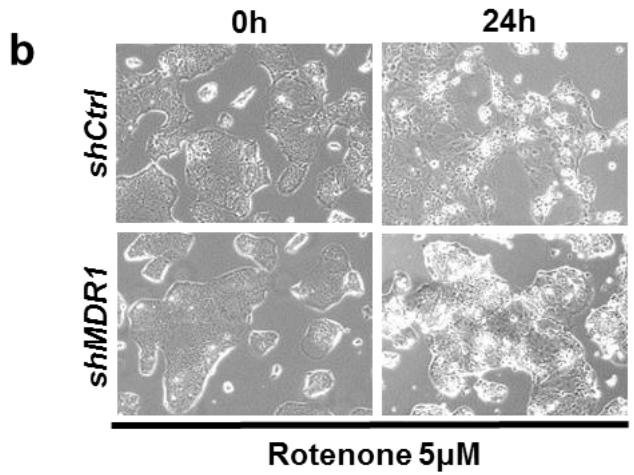

a
1.5
1.0
0.5
0.0
MDR1/RP2
p<0.001
shMDR1
shCtrl
MDR1
Actin
shMDR1
b
150
100
50
0
OCR/protein (Basal)
shMDR1
shCtrl
c
shMDR1
shCtrl
p62
SOD2
GAPDH
Rotenone - + - +
d
shMDR1
shCtrl
LC3-I
LC3-II
Bafil - + - +

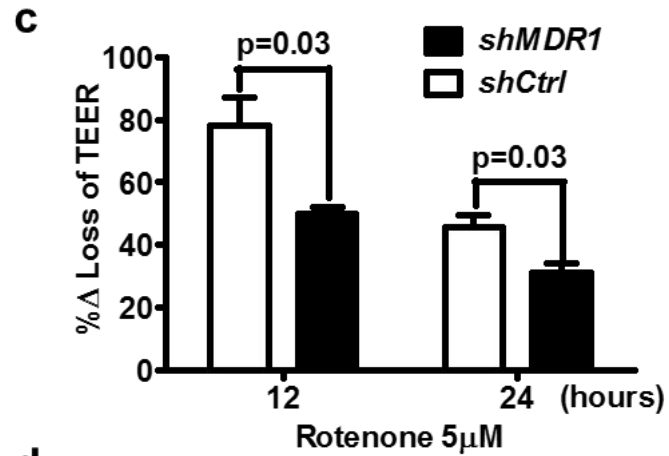
SI 4

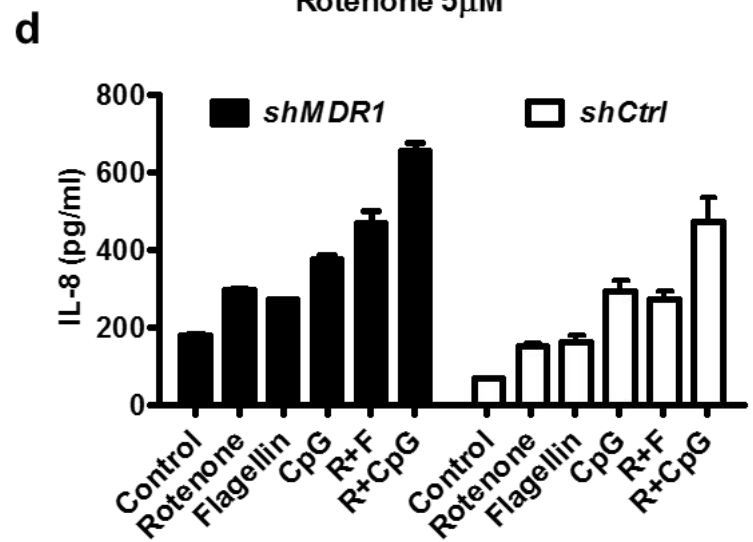

a
WT
Rotenone
1μM
10μM
100μM
Colon
b
H&E
WT
mdr1a-/-
0.25% DSS
+Rotenone
c
WT
mdr1a-/-
p=0.04
Histology Score
0
1
2
3
4
0.25% DSS
+Rotenone
0.25% DSS
+Rotenone
d
TUNEL
WT
mdr1a-/-
0.25% DSS
+Rotenone
e
Tunel-positive cells (%)
0.0
0.5
1.0
1.5
2.0
2.5
WT
mdr1a-/-
*
*
0.25% DSS
+Rotenone
0.25% DSS
+Rotenone
f
2-ΔΔCT
0
5
10
15
20
25
WT
Rotenone
Vehicle
mdr1a-/-
*
IL-6
TNFa

SI 5

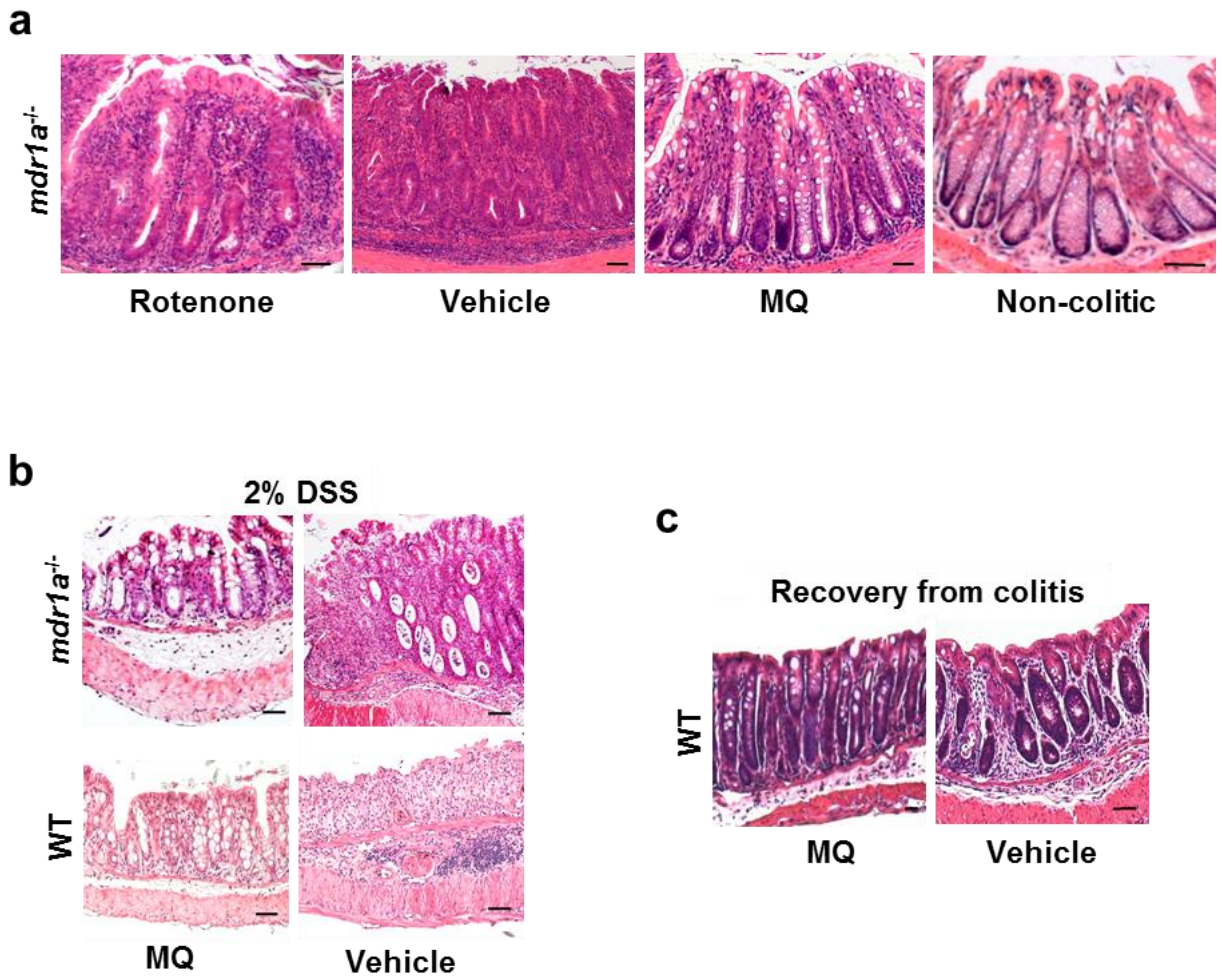

a
shMDR1
shCtrl
PARP
C-PARP
GAPDH
Rotenone - + - - - + - -
CCCP - - + - - - + -
Cisplatin - - - + - - - +


b
0h
24h
shCtrl
shMDR1
Rotenone 5μM


c
p=0.03
p=0.03
shMDR1
shCtrl
%Δ Loss of TEER
100
80
60
40
20
0
12
24 (hours)
Rotenone 5μM


d
shMDR1
shCtrl
IL-8 (pg/ml)
800
600
400
200
0
Control
Rotenone
Flagellin
CpG
R+F
R+CpG
Control
Rotenone
Flagellin
CpG
R+F
R+CpG

SI 6


a
mdr1a-/-
Rotenone
Vehicle
MQ
Non-colitic
b
2% DSS
mdr1a-/-
WT
MQ
Vehicle
c
Recovery from colitis
WT
MQ
Vehicle