

Figure S1

Rat peritoneal inflammatory cells

Fe(II)
Low High

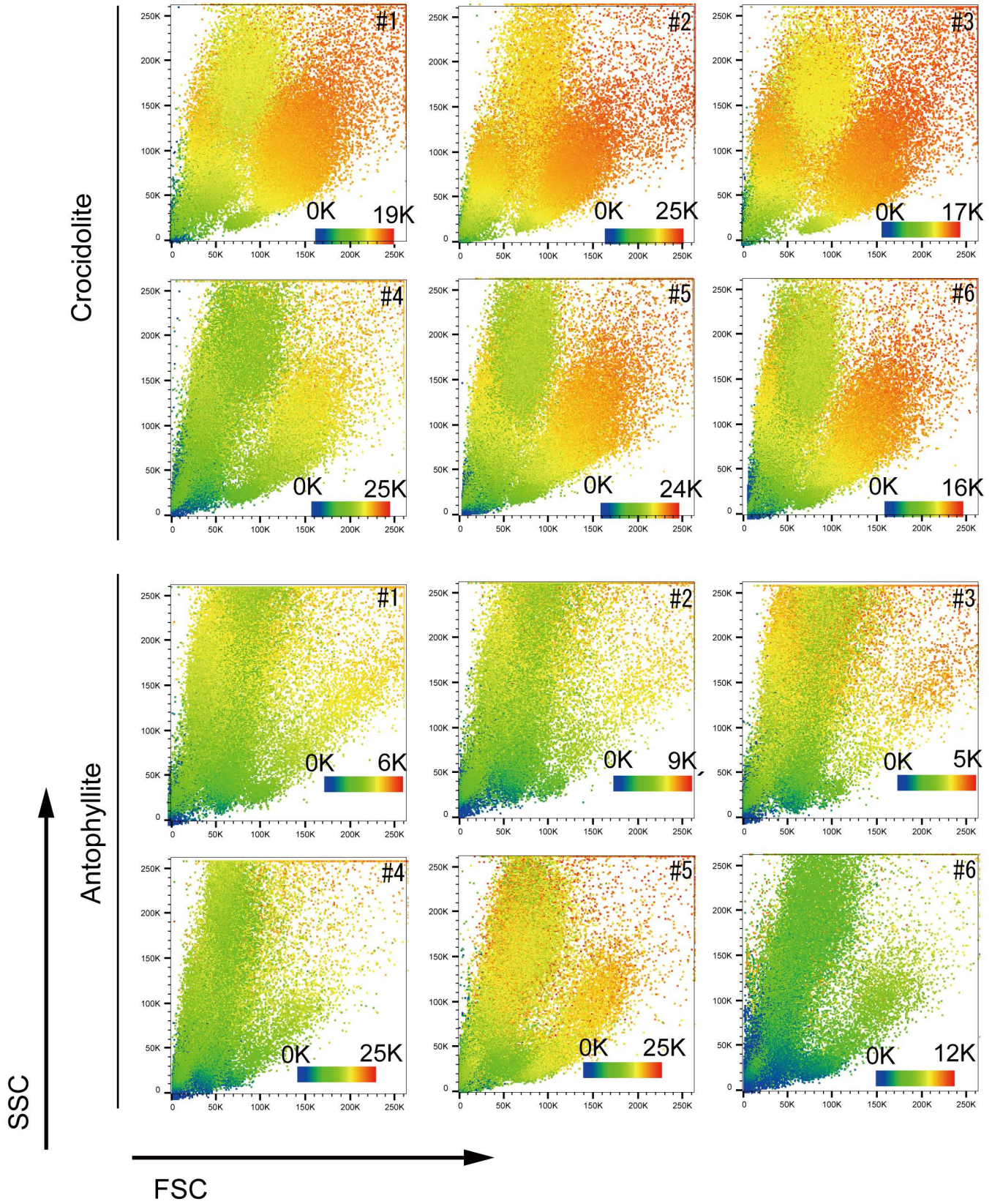
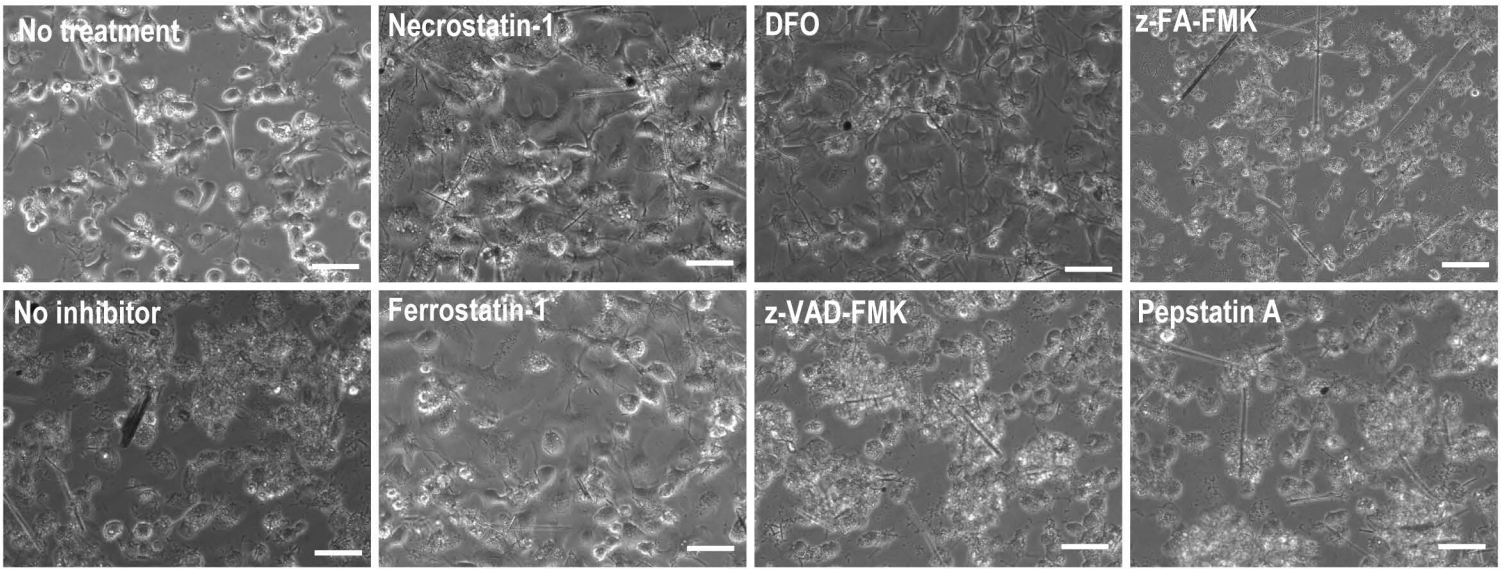


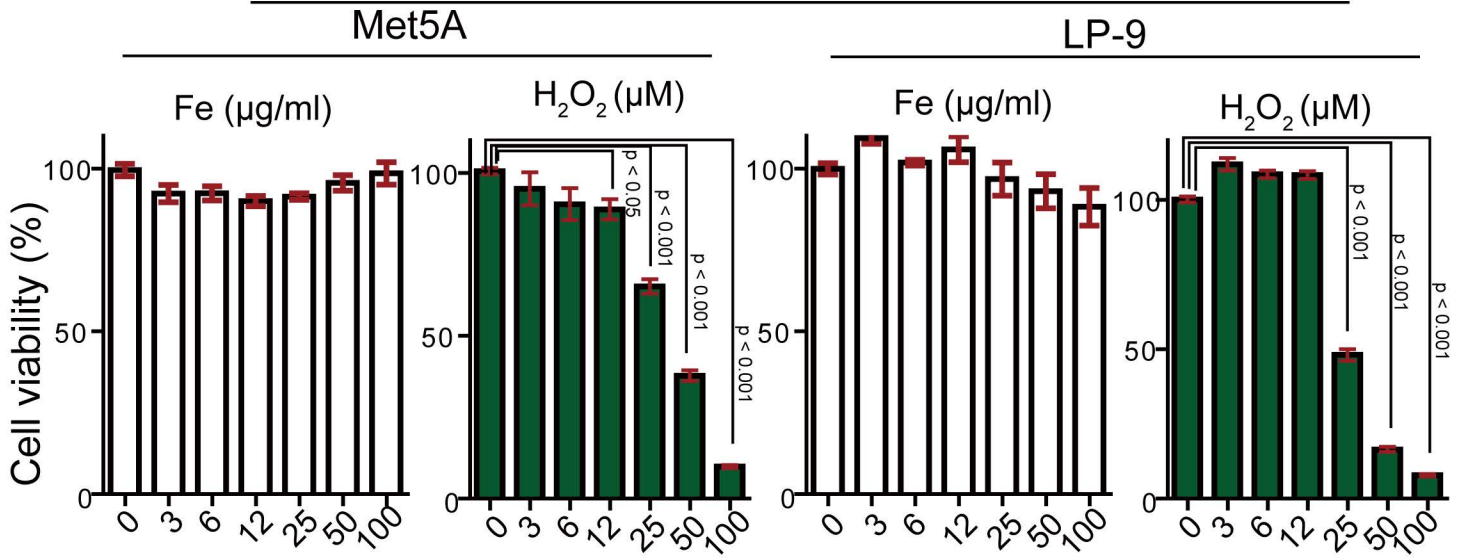
Figure S2

a

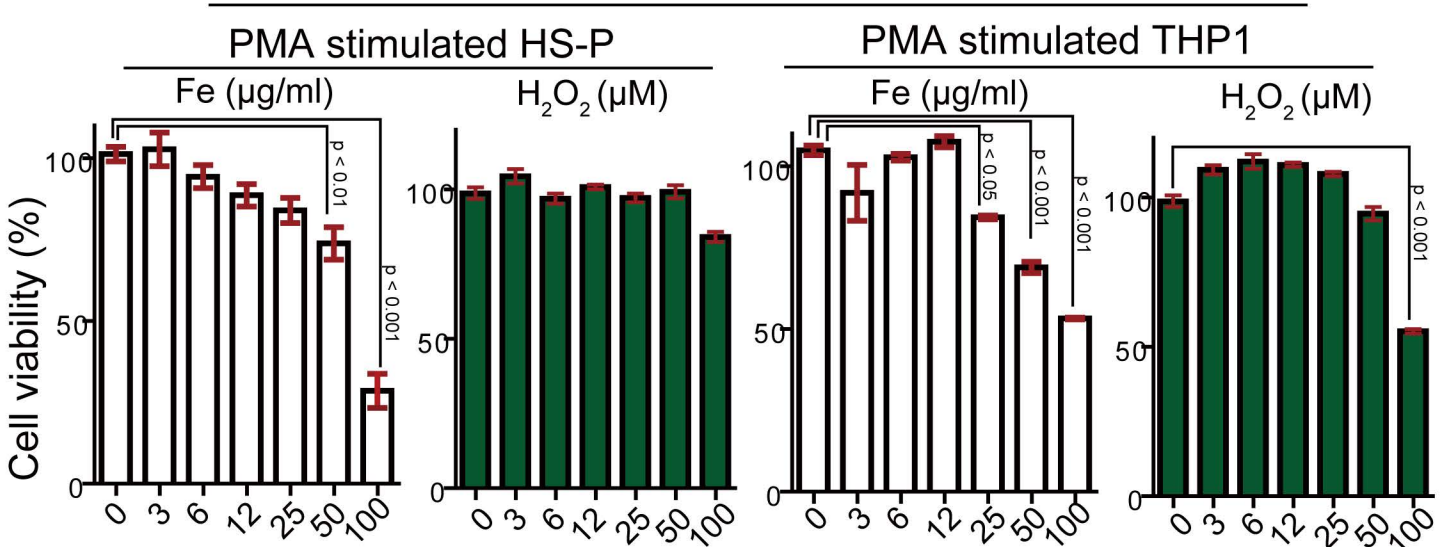
M2a THP1 (96 h)

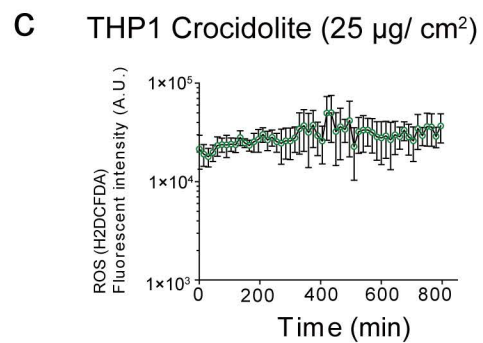
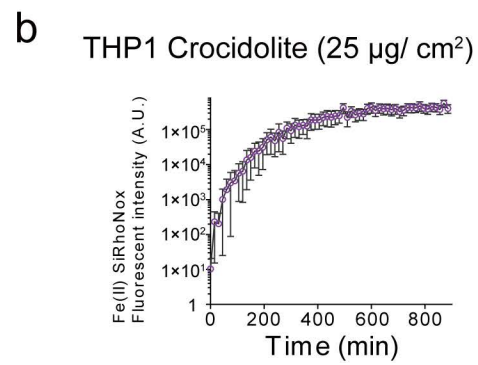
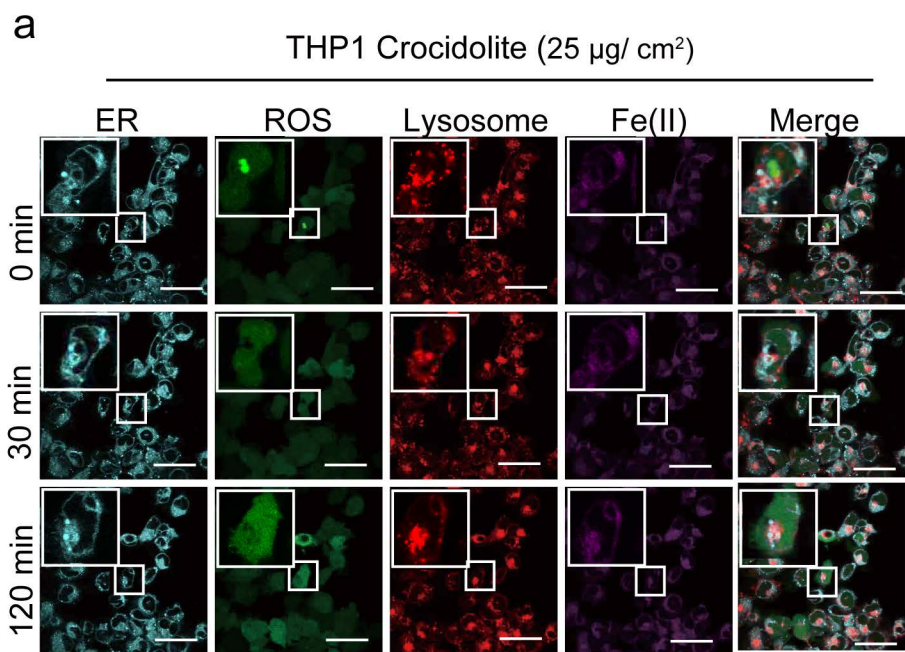
**b**

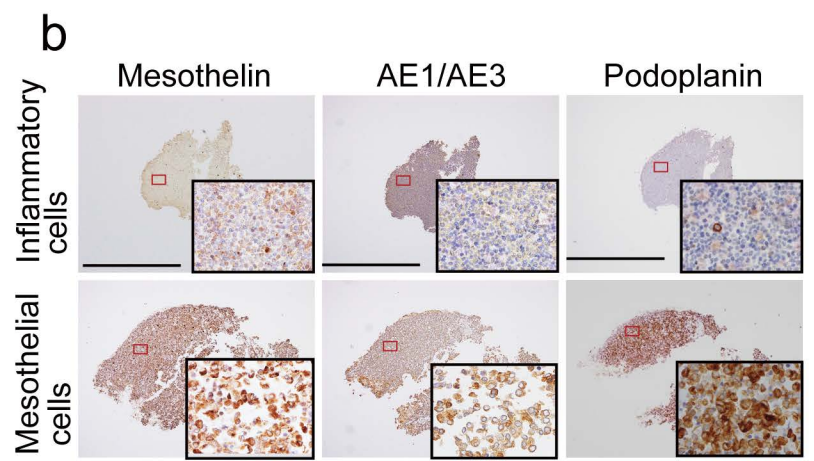
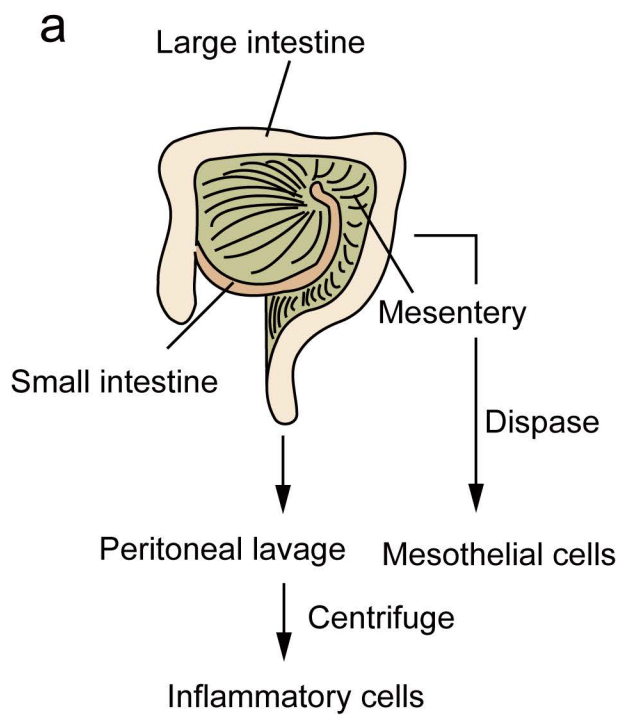
Mesothelial cell (72 h)

**c**

Macrophage cells (72 h)







c

Mesothelial cells

	Mesothelin	AE1/AE3	Podoplanin
Mean (%)	88.58	90.24	85.09
SEM	3.04	2.51	3.67

Figure Legends for Supplementary Figures

Figure S1. H₂O₂ production by macrophage after asbestos exposure does not depend only on NOX2.

(a, b) Immunohistochemical analysis for the polarization of peritoneal and granuloma macrophages reveals M1-subtype for peritoneal macrophages (M1 marker: iNOS) and M2-subtype for granuloma macrophages, (M2 marker: CD163 and MHC II) both of which express NOX2 (NADPH oxidase 2). (c) Scheme of asbestos-induced tissue remodeling in the somatic cavity. After intraperitoneal asbestos injection, macrophages present a spectrum of phase-specific phenotypes. Asbestos exposure (0 day) induces intraperitoneal macrophages to recognize and migrate to asbestos. At macrophage trapping phase (1-2 days), asbestos causes cell death to macrophages (M1) and mesothelia. At granuloma formation phase (1 wk ~), mesothelia have completely recovered, when macrophages engulf asbestos with M2 differentiation in the granuloma. (d) Immunoblot analysis for NOX2 protein in PMA-stimulated THP1 macrophage reveals no remarkable change between anthophyllite and crocidolite 24 h after exposure. (e) qRT-PCR analysis of *Nox2* in a macrophage cell line, THP1, after polarization shows no remarkable effect of crocidolite. M0, PMA 5 ng/ml; M1, 20 ng/ml IFN- γ + 10 ng/ml LPS; M2a: 20 ng/ml IL-4; M2b, IgG-coated wells + 100 ng/ml LPS; M2c, 0.5 ng/ml activated TGF- β . (f) Extracellular H₂O₂ (Amplex Red™) in the media of THP1 cells was higher after 24 h-exposure to crocidolite than to anthophyllite. The cells were differentiated into each subtype by stimulation with PMA (10 μ M), IFN- γ (100 ng/ml), IL-4 (20 ng/ml), coated IgG and LPS (100 ng/ml) or TGF- β (0.5 mg/ml), (means \pm SEM; N \geq 7, scale: 100 μ m).

Figure S2. Peritoneal macrophages accumulate catalytic Fe(II) after exposure to crocidolite but not to anthophyllite.

FACS analysis for catalytic Fe(II) in rat peritoneal inflammatory cells. Catalytic Fe(II) was detected by SiRhoNox-1 (633 nm) and heatmaps are shown according with catalytic Fe(II) concentration. The upper panels are 6 independent analyses of peritoneal lavage from rats intraperitoneally injected with crocidolite (4 wks); the lower 6 panels are from rats injected with anthophyllite (4 wks).

Figure S3. Necrotic feature of macrophages after exposure to crocidolite and its partial

rescue by various inhibitors for specific cell death.

(a) Phase contrast images of IL-4-stimulated THP-1 macrophage cells exposed to crocidolite ($15 \mu\text{g}/\text{cm}^2$; NT, no-treatment; NI, no-inhibitor; necrostatin-1, $40 \mu\text{M}$, necroptosis inhibitor; ferrostatin-1, 500 nM , ferroptosis inhibitor; DFO, $400 \mu\text{M}$, redox-inactive iron chelator; z-VAD-FMK, $184 \mu\text{M}$, apoptosis inhibitor; z-FA-FMK, $20 \mu\text{M}$, lysosome-associated cell death inhibitor; pepstatinA, $200 \mu\text{g}/\text{ml}$, autophagic cell death inhibitor; bar = $50 \mu\text{m}$). (b, c) Cell viability (MTT assay) for iron and H_2O_2 toxicity reveals iron-resistance/ H_2O_2 sensitivity in mesothelial cells and iron sensitivity/ H_2O_2 resistance in macrophages. FAC (ferric ammonium acetate) and H_2O_2 were exposed at different concentrations (0 to $100 \mu\text{M}$; means \pm SEM; $N \geq 4$).

Figure S4. Propagation of ROS from lysosome to the entire cytoplasm in THP1 macrophage cells after exposure to crocidolite.

(a) Time-lapse imaging of M0-subtype THP1 macrophage exposed to crocidolite ($25 \mu\text{g}/\text{cm}^2$) 24 h after differentiation reveals H_2DCFDA -positive ROS, starting from single granule in lysosome to propagate to the entire cytoplasmic area (magenta, SiRhoNox-1, catalytic $\text{Fe}[\text{II}]$; red, LysoTracker, lysosome; green, H_2DCFDA , ROS; cyan, ER tracker, ER; bar = $10 \mu\text{m}$). (b, c) Time-lapse imaging of $\text{Fe}(\text{II})$ and ROS (H_2DCFDA) quantification in M0 THP1 ($10 \mu\text{M}$ PMA stimulated, 6 h; 0 to 13 h; means \pm SEM; $N \geq 4$).

Figure S5. Methods for collection and confirmation of rat mesothelial cells.

(a) A scheme of the collection of peritoneal lavage and mesothelial cells from rats. Dissected mesentery was incubated with dispase ($1,000 \text{ pU}/\text{ml}$) at $37 \text{ }^\circ\text{C}$ for 30 min. (b, c) Immunohistochemical confirmation of inflammatory cells and mesothelial cells (bar = 1 mm).

Table S1: KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
β -catenin	Abcam	Cat# ab32572
p β -catenin (Ser 675)	CST	Cat# 9567S
c-myc	Abcam	Cat# ab32072
γ H2AX (Ser139)	Merck	Cat# 05-636
53BP1	Abcam	Cat# ab36823
4HNE	JaICA	Cat# MHN-100P
8OHdG	JaICA	Cat# MOG-100P
Ki67	Abcam	Cat# ab16667
BrdU	Abcam	Cat# ab152095
GSK3B	SANTA CRUZ	Cat# sc-9166
TfR1	Thermo Fischer	Cat# 13-6800
FtL	Abcam	Cat# ab69090
FtH	SANTA CRUZ	Cat# sc-25617
β -actin	Sigma aldrich	Cat# A1978-200UL
LaminB1	Abcam	Cat# ab16048
Lamp2	SANTA CRUZ	Cat# 66301-1
Mesothelin	IBL	Cat# 28001
D2-40 (podoplanin)	Sigma Aldrich	Cat# P2120
AE1/AE3	Thermo Fischer	Cat# MS-343-PO
p15 (D-12)	SANTA CRUZ	Cat# sc-271791
p16 (C-3)	SANTA CRUZ	Cat# sc-166760
Necroptosis Antibody Sampler Kit	CST	Cat# 98110
PARP1	Proteinthech	Cat# 13371-1-AP
GPX4	Abcam	Cat# ab125066
Cathepsin B	NBP	Cat# NBP1-86048
CD68	BioRad	Cat# MCA341R
iNOS	BD Transduction Laboratories™	Cat# N32020/L20
MHCII	Abcam	Cat# ab23990
CD163	Abcam	Cat# ab182422
CCRII	Abcam	Cat# ab227015
NOX2/gp91 phox	Abcam	Cat# ab131083
anti-mouse HRP	Dako	Cat# P0260
anti-rabbit HRP	Dako	Cat# P0448

anti-mouse Alexa 488	Invitrogen	Cat# A11029
anti-Rabbit Alexa 546	Invitrogen	Cat# A11003
anti-Rabbit Alexa 633	Invitrogen	Cat# A21070
anti-Digoxigenin/Digoxin Dylight 594	Vector	Cat# SA-5001

Chemicals, Peptides, and Recombinant Proteins

PMA	Wako	Cat# 162-23591
recombinantHuman IL-4	PEPROTECH	Cat# 200-04
recombinant human IFN- γ	PEPROTECH	Cat# 300-02
recombinant human TGF- β 1	R &D system	Cat# 240-B-002
recombinat human TNF- α	CST	Cat# 8922SC
Human Gamma Globulin	Jakson ImmunoResearch	Cat# 009-000-002
recombinant mouse IL-4	PEPROTECH	Cat# 214-14
recombinant human Wnt1	PEPROTECH	Cat# 120-17
LiCl	Sigma Aldrich	Cat# 203637-100G
LPS	Sigma Aldrich	Cat# L4391-1MG

Cell death inducer and inhibitor

Ferrostatin 1	Sigma Aldrich	Cat# SML0583-5MG
Erastin	Sigma Aldrich	Cat# E7781
z-FA-FMK	SANTA CRUZ	Cat# sc-201303
Pepstatin A	TOCRIS	Cat# 26305-03-3
Necrostatin-1	Sigma Aldrich	Cat# N9037
z-VAD-FMK	R &D system	Cat# FMK001
Desferal for injection 500mg	Novaltis	Cat# 873929

Commercial Assays

Cell Count Reagent SF	Nacalai Tesque	Cat# 07553-15
Cytotoxicity Detection Kit	Sigma Aldrich	Cat# 11644793001
Amplite Red hydrogen peroxide assay	AAT Bioquest	Cat# 11011
Cellular Glutathione Detection Assay Kit	CST	Cat# 13859

Recombimnant DNA

pcDNA3-EGFP	Addgene	Cat# 13031
pLKO.1 puro	Addgene	Cat# 8453
pFucci-G1 Orange	MBL	Cat# AM-V9003M
pFucci-S/G2/M Green (N+C)-Hyg	MBL	Cat# AM-V9030M
pLH-stsgRNA3.1	Addgene	Cat# 64118

pHAGE-TO-nls-st1dCas9-3nls-3XTagBFP2	Addgene	Cat# 64512
Experimental Models: Cell Lines		
Human: HEK293T	RIKEN CELL BANK	RCB2202
Human: Met5A	ATCC	ATCC CRL-9444
Human: LP-9	CORIELL INSTITUTE	AG07086
Human: THP-1	RIKEN CELL BANK	RCB-1189
Mouse: RAW 264	RIKEN CELL BANK	RCB0535
Rat: HS-P	RIKEN CELL BANK	RCB1757
Cell culture experiments		
Fetal bovine serum	Nualille	Cat# S1780
trypsin	Nacalai Tesque	Cat# 35555-54
Antibiotic-antimycotic	Nacalai Tesque	Cat# 02892-54
RPMI1640 with PR	Wako	Cat# 187-02025
RPMI1640 without PR	Gibco	Cat# 11835-030
DMEM	Wako	Cat# 043-30085
FluoroBrite DMEM	Gibco	Cat# A18967-01
PEI	Polysciences, Inc.	Cat# 23966-1
FerroOrange	GORYO Chemical, Inc	Cat# GC904-01
FerroFarRed™	GORYO Chemical, Inc	Cat# GC903-01
LysoTracker Green DND-26	Thermo Fisher Scientific	Cat# L7526
LysoTracker Red DND-99	Thermo Fisher Scientific	Cat# L7528
ERTracker Blue-white DPX	Thermo Fisher Scientific	Cat# E12353
MitoTracker™ Green FM	Thermo Fisher Scientific	Cat# M7514
MitoTracker™ Deep Red FM	Thermo Fisher Scientific	Cat# M22426
Transmission electron microscope		
Gluthalaldehyde	TAAB	Cat# 3032
Epon-812	TAAB	Cat# R3244
DDSA EM	TAAB	Cat# R3252
MNA	TAAB	Cat# R3282
DMP-30	TAAB	Cat# R3268
Software		
ImageJ	https://imagej.nih.gov/ij/	RRID:SCR_003070
GraphPad Prism 5	Graph Pad Software	RRID:SCR_002798
Multiple experimental Viewer (MeV)	http://mev.tm4.org/#/welcome	N/A
GSEA	http://software.broadinstitute.org/gsea/index.jsp	2005 , PNAS 102 , 15545-15550

GSEA <http://software.broadinstitute.org/gsea/index.jsp> [2003, Nat Genet 34, 267-273](#)

Ape – A plasmid Editor <https://jorgensen.biology.utah.edu/wayned/ape/> RRID:SCR_014266

Table S2 Primer used for RT-PCR assay.

Primer Name	Primer Sequence
<i>β-catenin</i>	Fwd: (5'-GAAGGTGCTGTCTGTCTGCT-3') Rev: (5'-TAGTCGCTGCATCGGACAAG-3')
<i>MET</i>	Fwd: (5'-CAGTGACCTGCTGAAGCTGA-3') Rev: (5'-ACAGGAAGAGCCCGGATACT-3')
<i>EGFR</i>	Fwd: (5'-CAACACCCTGGTCTGGAAGT-3') Rev: (5'-GCCCTTCTGGTTGTTGACAT-3')
<i>TGFRB1</i>	Fwd: (5'-ACCTTCTGATCCATCCGTTG-3') Rev: (5'-AGCTGTCAGCCTAGCTGCTC-3')
<i>GAPDH</i>	Fwd: (5'-TGACTCTACCCACGGCAAGTTCAA-3') Rev: (5'-ACGACATACTCAGCACCAGCATCA-3')

Table S3 Oligo used for knockdown assay.

gene	shRNA Oligo sequence
<i>β-catenin</i>	Fwd: (5'-CCGTTGTTATCAGAGGACTAAATACTCGAGTATTTAGTCCTCTGATAACAATTTTTG-3') Rev: (5'-AATCCTTGTTATCAGAGGACTAAATACTCGAGTATTTAGTCCTCTGATAACAATTTTTG-3')
Scramble	Fwd: (5'-CCGTAATAGCGACTAAACACATCAATTCAAGAGATTGATGTGTTTAGTCGCTATTTTTTTG-3') Rev: (5'-AATCAAAAAAATAGCGACTAAACACATCAATCTTGAATTGATGTGTTTAGTCGCTATTG-3')

Table S4 Oligo used for CasFISH assay.

gene	sgRNA Oligo sequence	sgRNA vector	Cas9
<i>p16</i>	Fwd: (5'-ACGGATCGGGGATGTCTGCAGAGGGCAGAAG-3')	addgene #64118	st dCad9
	Rev: (5'-AGACCTGGCCCTAGCCTCCATGTAGCAGAAA-3')	addgene #64118	st dCad9