Supplementary materials and methods

Reagents and antibodies.

The following compounds were used: DMSO, 2,4-Dinitrophenol (2,4-DNP); N-acetyl-L-cysteine (NAC); rotenone; antimycin A; echinomycin; 3-methyladenine A (3-MA); carbonyl cyanide m-chlorophenyl hydrazone (CCCP); LPS (*Salmonella enterica* serotype Typhimurium and *Escherichia coli* O55:B5) were obtained from Sigma-Aldrich. Apigenin; 4,5,6,7-tetrabromobenzotriazole (TBB); Mdivi-1 were purchased from Tocris (Bio-Techne). Lipoteichoic acid (LTA); Pam3CSK4; Pam2CSK4; R848 (Resiquimod); Poly(I:C) LMW; CpG ODN D-SL01 were obtained from InvivoGen. mitoTEMPOL and mitoParaquat from Abcam. The following reagents were used: recombinant mouse IFN-γ was purchased from Biolegend. Cyto-ID autophagy detection kit was obtained from Enzo Lifesciences. Luminex-based multiplex assays (Milliplex) were from Merck. Fluorescent red latex beads (carboxylate-modified polystyrene); 2µm mean particle size were obtained from Sigma-Aldrich. Mitotracker green FM; Mitosox; JC-1 and TMRM were obtained from Molecular Probes (ThermoFisher Scientific). JetPEI macrophages were purchased from Polyplus Transfection. Mouse mitochondria isolation kit was obtained from Miltenyi Biotec. All consumables for western blotting were from Biorad. Precast gels (mini protean TGX, stain free, 4-20%) and PVDF membranes were used. Trizol reagent was from ThermoFisher Scientific. iScript Reverse Transcription Supermix was from BioRad. SYBR green reagent was purchased from Applied Biosystems. Antibodies were used as follows: anti-PINK1 (ab75487, Abcam); anti-parkin (ab15954, Abcam); anti-DJ1 (5933, CST); anti-VDAC (4661, CST); anti-DRP1 (8570, CST); anti-β-actin (A1978, Sigma-Aldrich); anti-Tom20 (sc-11415, Santa Cruz). For mouse studies, flow cytometry reagents and antibodies were from Miltenyi Biotec: mouse FcR blocking reagent, anti-mouse CD45-Viogreen (Clone REA737); anti-mouse CD115-PE (clone AFS98); anti-mouse Ly6G-VioBlue (Clone REA526); anti-mouse Ly6C-APC (clone REA 796); anti-mouse CD64-APC (clone REA286); anti-mouse CD80-PE (clone 1610A1); anti-mouse CD86-PE (clone PO3.3); anti-mouse F4/80-PerCP-Vio700 (clone REA126); anti-mouse CD11b-

APC-Vio770 (clone M1/70.15.11.5). For human studies, anti-human CD33-PE-Vio770 (Miltenyi Biotec, clone REA775), anti-human CD16-BV 421 (BD biosciences, clone 3G8), anti-human CD14-BV 605 (BD biosciences, clone M5E2), anti-human CD66b-APC-Vio770 (Miltenyi Biotec, clone REA306) were used for flow cytometry experiments of human blood samples. The following
5 plasmids were used: mitochondrial mKeima (mt-mKeima) expression vector was a kind gift from Dr. A. Miyawaki (Brain Science Institute, RIKEN, Saitama, Japan) {Katayama, 2011 #264}. pMito-LSSmOrange (human cytochrome C oxidase subunit VIII fused LSSmOrange) was purchased from Addgene. The following bacteria: *Escherichia coli* strain CIP 54.8 and *Bacillus subtilis* strain CIP 52.65 were obtained from CRBIP. GFP-expressing *Escherichia coli* (*E. coli*-GFP) was
10 obtained from Addgene. *E. coli*, *E. coli*-GFP and *Bacillus subtilis* were grown aerobically in TSB for 24 h at 37°C. Bacterial cells were washed twice in sterile PBS and resuspended at 10^9 CFU per mL in PBS. Recombinant T. Castaneum PINK1 was purchased from R&D Systems. Human recombinant active caspase 1 and caspase 4/11 were purchased from Enzo Life Sciences.

In vitro cell stimulation

15 Except for LPS, IFNγ and TLR agonists, prepared in PBS, all compounds were dissolved in DMSO at 1000X concentration. Control cells were treated with an equivalent volume of vehicle. To stimulate macrophages, unless specified, cells were incubated with LPS (*Salmonella enterica* serotype Typhimurium, 100ng/mL) in combination with recombinant mouse IFNγ (20ng/mL) for 24h or at indicated duration. For dose studies, LPS was used at 100, 250 and 500ng/mL for 24h.
20 Stimulation with TLR agonists was performed for 24h with LTA (10, 100, 1000ng/mL); Pam2CSK4 (1, 10, 100ng/mL); Pam3CSK4 (1, 10, 100ng/mL); R848 (1, 10, 50µg/mL); Poly(I:C) LMW (0.1, 1, 10µg/mL); CpG ODN D-SL01 (10, 100, 1000nmol/L). The ROS scavengers NAC (1, 5mM) or MitoTEMPOL (50, 100µM) were used for 24h alone or in combination with LPS/IFNγ. TBB, Apigenin and Mdivi-1 were used at 50µM for 24h. 3-MA was used at 1mM for 24h. Rotenone was
25 used at 1, 5, 10µM for 24h. For dose studies, antimycin A was used at 33, 66 and 100µM for 6h. Otherwise, antimycin A was used at 66 µM for 6h alone or in combination. Echinomycin was used

at 1, 2.5 and 5nM for 24h. Mitochondrial uncouplers CCCP and 2,4-DNP were used for 24h at 500nM and 1 μM, respectively. Mdivi-1 {Cassidy-Stone, 2008 #321}, 3-Methyladenine (3-MA) {Zhou, 2011 #221}, Apigenin and 4,5,6,7-Tetrabromobenzotriazole (TBB) {Duncan, 2008 #262;Nelson, 2017 #261} were used to inhibit mitophagy. Apigenin and TBB were chosen because they are inhibitors of Casein Kinase 2 (CK2). Deficiency in CK2 was shown to result in the inhibition of mitophagy {Kanki, 2013 #258}.

Transmission electron microscopy

Cells were fixed for 1 h in 4% formaldehyde/2.5% glutaraldehyde in 0.1 M Sörensen phosphate buffer, then washed in the same buffer at room temperature, and post-fixed for 1 h in 1% osmium tetroxide. The samples were dehydrated in increasing concentrations of ethanol (30%, 50%, 70%;95%; 100%) and were embedded in Epon 812 (EMS). Contrasted ultrathin sections (produced with an ultramicrotome (Reichert UltracutE)) were observed at 80 kV on a transmission electron microscope (Hitachi, H75500).

Quantitative PCR

Total RNA were isolated with Trizol reagent according to the manufacturer's instructions. Reverse transcription was performed with iScript Reverse Transcription Supermix. PCR was performed using intron-spanning gene-specific primers and SYBR green reagent on a StepOne machine (ThermoFisher Scientific). The expression levels of target genes were normalized to 18S rRNA and *Gapdh*. Sequences of the primers used : TNFα, 5'-ACATTCGAGCCAGTGAATTCGG and 5'-GGCAGGTCTACTTTGGAGTCATTGC; iNOS, 5'-TCGCTCAAGTTCAGCTTGGT and 5'-GGCAAACCCAAGGTCTACGTT; IL-1β 5'- TGCCCATCAGAGGCAAGGAGGA and 5'-CCTCGGCCAAGACAGGTCGC; IL-12α 5'-TTTCTCTGGCCGTCTTCACC and 5'-CTCAGTTTGGCCAGGGTCAT; IL-6 5'-GTCACCAGCATCAGTCCCAA and 5'-GGAGCCCACCAAGAACGATA.

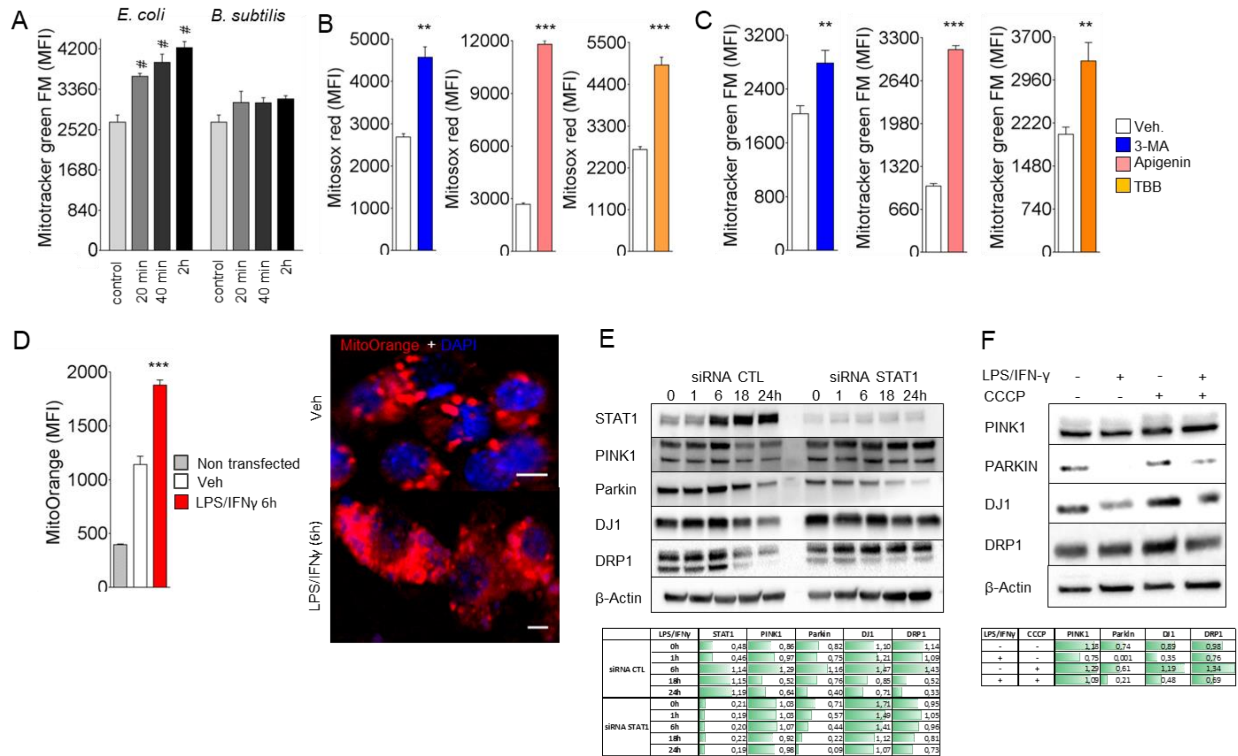
Gentamycin Protection assay

Raw 264.7 macrophages were exposed to 3-MA, 2,4-DNP or CCCP only prior to gentamycin protection assay. *E. coli*-GFP were washed and prepared at 10^9 CFU per mL in PBS. *E. coli*-GFP were added at a multiplicity of infection (MOI) of 10 into the medium of raw 264.7 cells and incubated for 1h at 37°C. Cells were washed with PBS supplemented with gentamycin (100ng/mL). A subset of cells was collected and maintained on ice (time point 0h). Fresh culture medium supplemented with gentamycin (100ng/mL) was added on remaining cells. Cells were incubated for 1h at 37°C. Cells were washed with PBS supplemented with gentamycin (100ng/mL). A subset of cells was collected and maintained on ice (time point 1h). Fresh culture medium supplemented with gentamycin (20ng/mL) was added on remaining cells. Cells were incubated for 5h at 37°C. Cells were washed with PBS supplemented with gentamycin (20ng/mL). A subset of cells was collected and maintained on ice (time point 6h). The same procedure was renewed for additional 18h. Cells were then analyzed by flow cytometry with a BD LSRII flow cytometer. Mean fluorescence intensity (MFI) corresponding to *E. coli*-GFP was analyzed with FlowJo V10. The decline in MFI in raw 264.7 cells translates an increase bactericidal activity.

Western blot analysis

For protein-extract collection, 5 to 10 x 10^6 cells were lysed in RIPA buffer supplemented with protease and phosphatase inhibitor cocktails (Roche). Proteins were quantified with Pierce BCA protein assay kit (Pierce) and subsequently boiled for 5 min at 95 °C in Laemmli buffer. Protein lysates were separated on 4–20% precast gels. Proteins were transferred to PVDF membranes using trans-blot turbo transfer system (BioRad). Membranes were then blocked for 1h with 5% bovine serum albumin (BSA) or 5% semi-skimmed milk in TBST (Tris-buffered saline plus 0.1% Tween 20) and probed with antibodies at 4°C overnight. Membranes were then incubated with appropriate secondary antibody conjugated to horseradish peroxidase (HRP). Chemiluminescent detection was performed with ECL substrate and visualized with ChemiDoc Imaging system (BioRad). Images were analyzed with Image Lab 5.1 software (BioRad).

Supplementary figures and legends



	LPS/IFNγ	STAT1	PINK1	Parkin	DJ1	DRP1
siRNA CTL	0h	0,48	0,86	0,82	1,10	1,14
	1h	0,46	0,97	0,75	1,21	1,09
	6h	1,14	1,29	1,16	1,47	1,43
	18h	1,15	0,52	0,76	0,85	0,52
	24h	1,19	0,64	0,40	0,71	0,33
siRNA STAT1	0h	0,21	1,03	0,71	1,71	0,95
	1h	0,19	1,03	0,57	1,49	1,05
	6h	0,20	1,07	0,44	1,41	0,96
	18h	0,22	0,92	0,22	1,12	0,81
	24h	0,19	0,98	0,09	1,07	0,73

LPS/IFNγ	CCCP	PINK1	Parkin	DJ1	DRP1
-	-	1,18	0,74	0,89	0,98
+	-	0,75	0,001	0,35	0,76
-	+	1,29	0,61	1,19	1,34
+	+	1,09	0,21	0,48	0,69

Figure S1. Macrophages activation is associated with the early inhibition of mitophagy. **A.** Flow cytometry assessment mitochondrial density in BMDMs exposed to bacteria (Multiplicity Of Infection=10) for indicated duration (n=3 per condition). **b-c**. Flow cytometry assessment mitochondrial ROS production (**B**) or mitochondrial density (**C**) in raw 264.7 macrophages exposed to vehicle (Veh., DMSO 0.1%) or mitophagy inhibitors (Apigenin, TBB, 50µM; 3-MA, 1mM) for 24h (n=3 per condition). **D**. Mitochondrial density assessed by flow cytometry or fluorescent microscopy in raw 264.7 macrophages transfected with LSSmOrange-cytochrome C oxidase subunit VIII expression vector (MitoOrange) exposed to LPS/IFNγ (6h) 24h post-transfection (n=3 per condition) Scale bars, 10µm. **E-F**. Immunoblots of mitophagy and mitochondrial fission checkpoints on protein lysates from Raw 264.7 macrophages (**E**) transfected with STAT1 siRNA or Control siRNA and exposed to LPS/IFNγ for indicated duration or (**F**) exposed for 24h to LPS/IFNγ alone or in combination with a mitophagy inducer (CCCP, 500nM) (Densitometry: ratio to β-actin is presented above the immunoblots). Bar graphs represent mean ± s.e.m.; $^{\#}$ *P<0.05* determined by ANOVA corrected for multiple comparison; ** *P<0.01,* *** *P<0.001* determined by *Student's t-test* with Welch's correction.

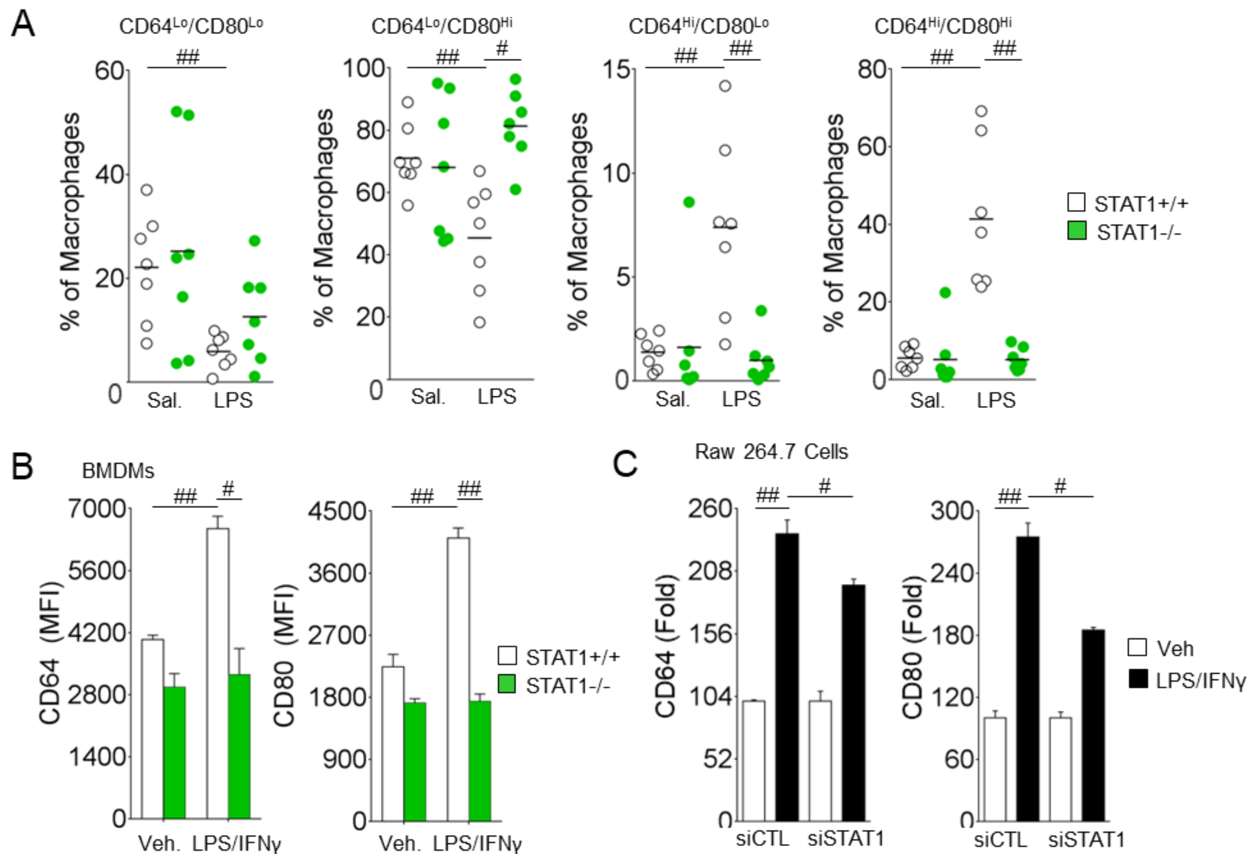


Figure S2. LPS triggers the classical activation of macrophages through STAT1 signaling. **A.** Flow cytometry assessment of peritoneal macrophages subpopulation in peritoneal fluid of *stat1*$^{+/+}$ or *stat1*$^{-/-}$ mice after intraperitoneal injection of saline (sal.) or LPS (0.5mg/kg, 24h) (n= 5-7 sal.; n=5-7 LPS). **B-C.** Flow cytometry assessment of macrophage activation with (**B**) *stat1*$^{+/+}$ and *stat1*$^{-/-}$ BMDMs or (**C**) raw 264.7 macrophages targeted with control (siCTL) or stat1 siRNA (siSTAT1) then exposed to vehicle (veh.) or LPS/IFNγ for 24h (n=3 per condition). Graphs with plots represent mean plus individual values. Bar graphs represent mean ± s.e.m.; $^{\#}$ *P<0.05,* $^{\#\#}$ *P<0.01* determined by ANOVA corrected for multiple comparison.

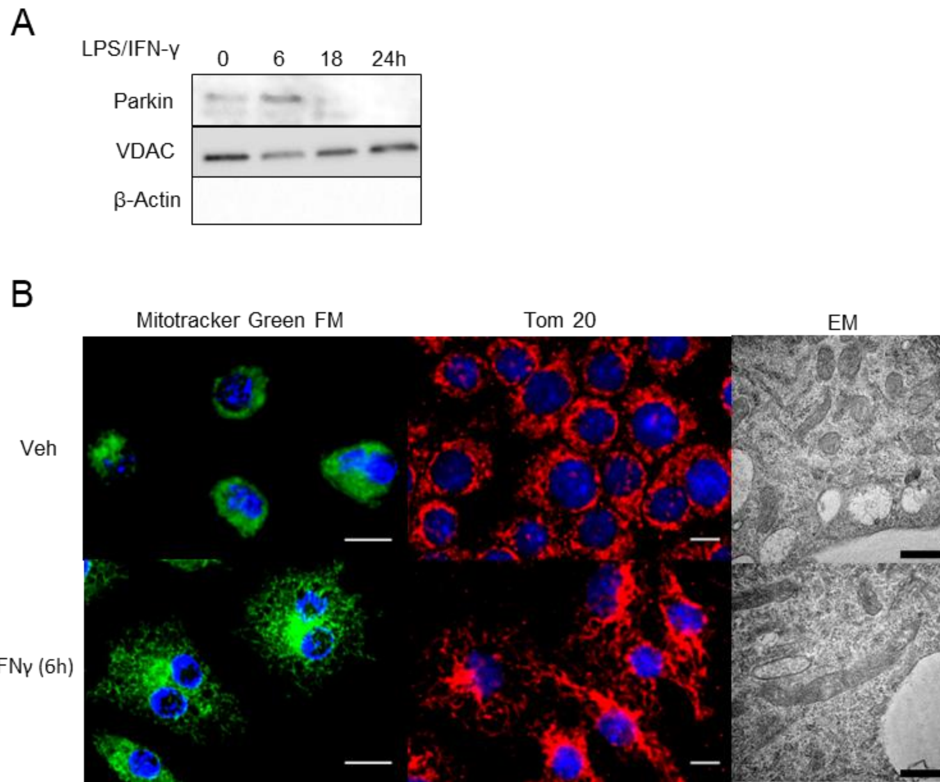


Figure S3. Macrophages activation is associated with the early inhibition of mitophagy.
A. Immunoblots of Parkin on immuno-purified mitochondria from raw264.7 macrophages exposed
5 to LPS/IFNγ. **B.** Mitochondrial network in 264.7 macrophages after exposure to vehicle or LPS/IFNγ (6h) assessed by Mitotracker Green FM (scale bar: 10μM), Tom 20 immunostaining (scale bar: 10μM) or transmission electron microscopy (scale bar: 100nm).

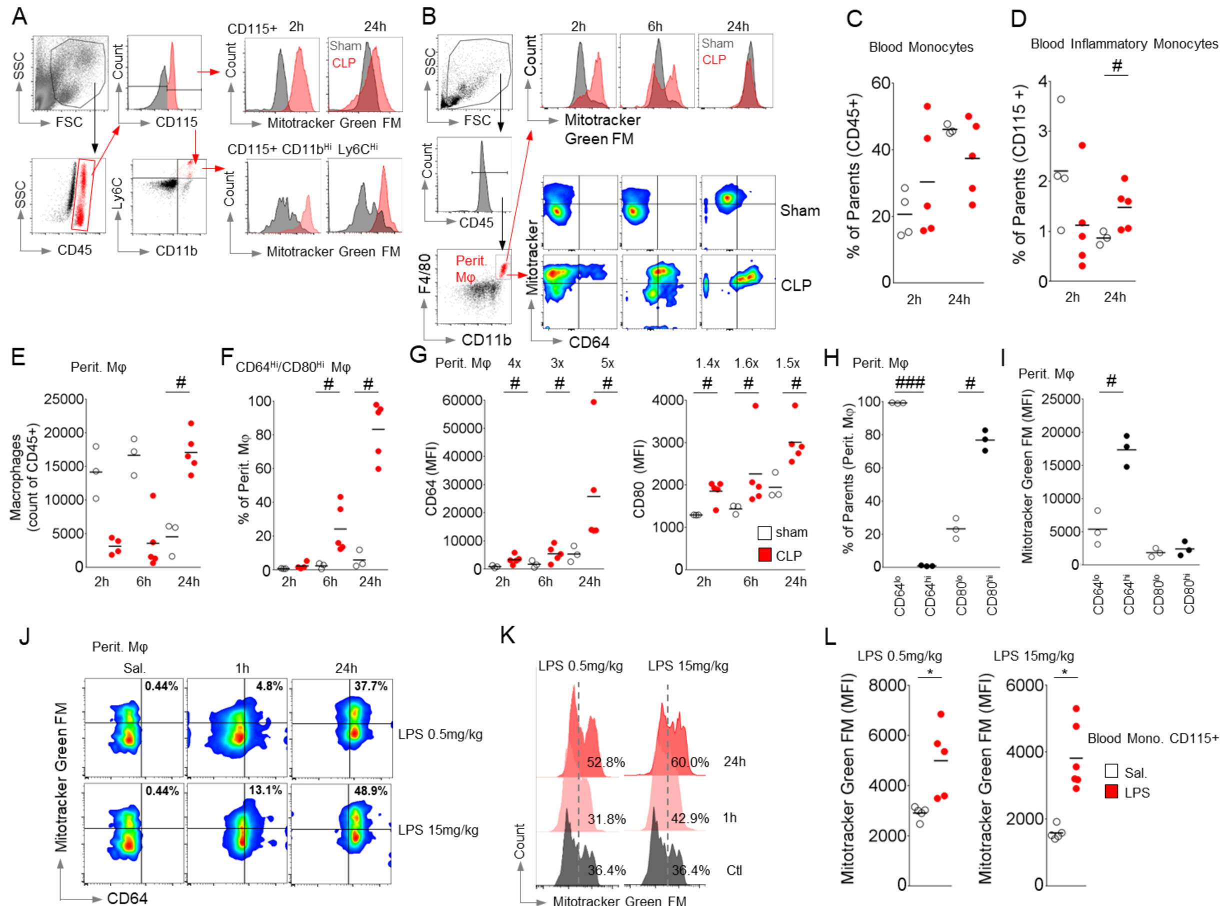
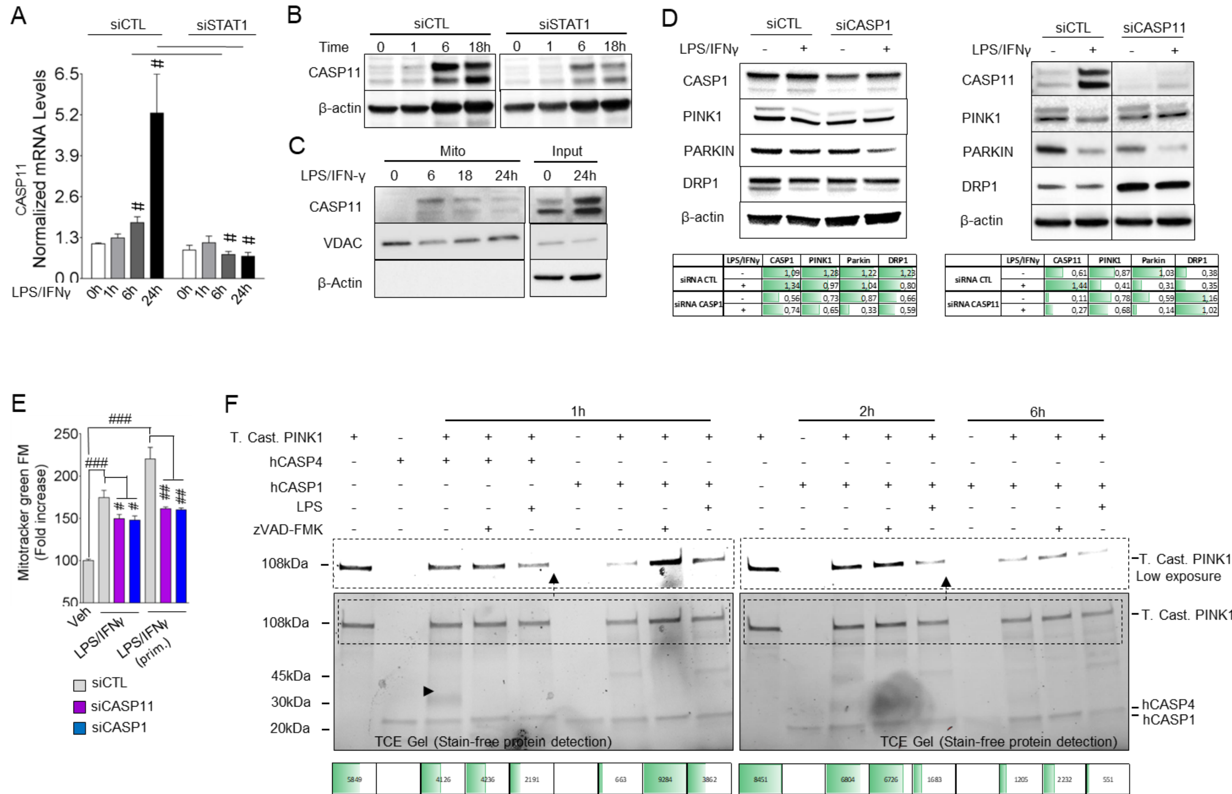


Figure S4. Endotoxemia triggers the early inhibition of mitophagy in myeloid cells.
A. Gating strategy for the assessment of mitochondrial density in blood monocytes ($CD115^+$) or in $Ly6C^{Hi}$ inflammatory blood monocytes ($CD115^+CD11b^{Hi}Ly6C^{Hi}$) in C57BL6/J mice submitted to sham- or cecal ligation and puncture (CLP) surgery. **B.** Gating strategy for the assessment of mitochondrial density in peritoneal macrophages ($F4/80^{Hi}$ $CD11b^{Hi}$) or in $CD64^{Hi}$ $Mitotracker^{Hi}$ subpopulation of peritoneal macrophages in C57BL6/J mice submitted to sham- or cecal ligation and puncture (CLP) surgery. **C-D.** Flow cytometry assessment of the proportion of (**C**) total monocytes ($CD45^+$ $CD115^+$) and (**D**) inflammatory ($CD45^+$ $CD115^+$ $ly6C^{Hi}$) in the blood of C57BL6/J mice after sham- or cecal ligation and puncture (CLP) surgery (n=3-4 sham; n=5 CLP). **E-F.** Flow cytometry assessment of the proportion of (**E**) total macrophages ($CD45^+$ $F4/80^{Hi}$ $CD11b^{Hi}$) and of (**F**) $CD64^{Hi}/CD80^{Hi}$ macrophages in peritoneal fluid of mice operated as in **C-D.** **G.** Flow cytometry assessment of the level of activation marker in peritoneal macrophages of mice operated as in **C-D. H.** Percentage of $CD64^{Hi}$, $CD64^{Lo}$, $CD80^{Hi}$, $CD80^{Lo}$ peritoneal macrophages in non-infected C57BL6/J mice (n=3). **I.** Flow cytometry assessment of mitochondrial density in $CD64^{Hi}$, $CD64^{Lo}$, $CD80^{Hi}$, $CD80^{Lo}$ peritoneal macrophages in non-infected C57BL6/J mice (n=3). **J-L.** Flow cytometry assessment of mitochondrial density in (**J**) $CD64^{Hi}$ $Mitotracker^{Hi}$ subpopulation of peritoneal macrophages or (**K**) peritoneal macrophages ($F4/80^{Hi}$ $CD11b^{Hi}$) of C57BL6/J mice submitted to an intraperitoneal injection of saline (sal.) or LPS at indicated dose for indicated duration. **L.** Flow cytometry assessment of mitochondrial density in monocytes of C57BL6/J mice submitted to an intraperitoneal injection of saline (Sal.) or LPS (n=5-6 per condition). Graphs with plots represent mean plus individual values; # *P<0.05,* ### *P<0.001* determined by ANOVA corrected for multiple comparison; * *P<0.05* determined by *Student's t-test* with Welch's correction.



	LPS/IFNγ	CASP1	PINK1	Parkin	DRP1
siRNA CTL	-	1,09	1,28	1,22	1,23
	+	1,34	0,97	1,04	0,80
siRNA CASP1	-	0,56	0,73	0,87	0,66
	+	0,74	0,65	0,33	0,59

	LPS/IFNγ	CASP11	PINK1	Parkin	DRP1
siRNA CTL	-	0,61	0,87	1,03	0,38
	+	1,44	0,41	0,31	0,35
siRNA CASP11	-	0,11	0,78	0,59	1,16
	+	0,27	0,68	0,14	1,02

5849		4126	4236	2191		663	9284	3862	8451		6804	6726	1683		1205	2232	551

Figure S5. LPS/IFNγ inhibits mitophagy in macrophages through the STAT1-dependent regulation of caspase 1 and caspase 11.

A-B. *Caspase 11* expression in raw 264.7 macrophages targeted with control (siCTL) or STAT1 siRNA (siSTAT1) exposed to vehicle or LPS/IFNγ for indicated duration assessed by (**A**) qPCR or (**B**) immunoblotting. **C.** Assessment by immunoblotting of caspase 11 expression in immuno-purified mitochondria from raw 264.7 macrophages exposed to LPS/IFNγ at indicated duration. **D**. Assessment of LPS/IFNγ-dependent inhibition of mitophagy by (**D**) immunoblotting or (**E**) flow cytometry in raw 264.7 macrophages targeted with control (siCTL) or *Caspase 1* or *11* siRNA (siCasp1/siCasp11) then exposed to vehicle or LPS/IFNγ for 18h (Densitometry: ratio to β-actin is presented above the immunoblots). **F.** Proteolytic activity of recombinant human caspase 1 (hCASP1) or human caspase 4/11 (hCASP4) against recombinant Tribolium Castaneum PINK1 (T. Cast. PINK1) in the presence of zVAD-FMK (50μM) and LPS (100ng/mL) alone or in combination. Protein levels were assessed with 2,2,2-trichloroethanol (TCE) gels after UV trans-illumination (table above presents densitometry data). Bar graphs represent mean ± s.e.m.; # *P<0.05,* ## *P<0.01,* ### *P<0.001* determined by ANOVA corrected for multiple comparison.

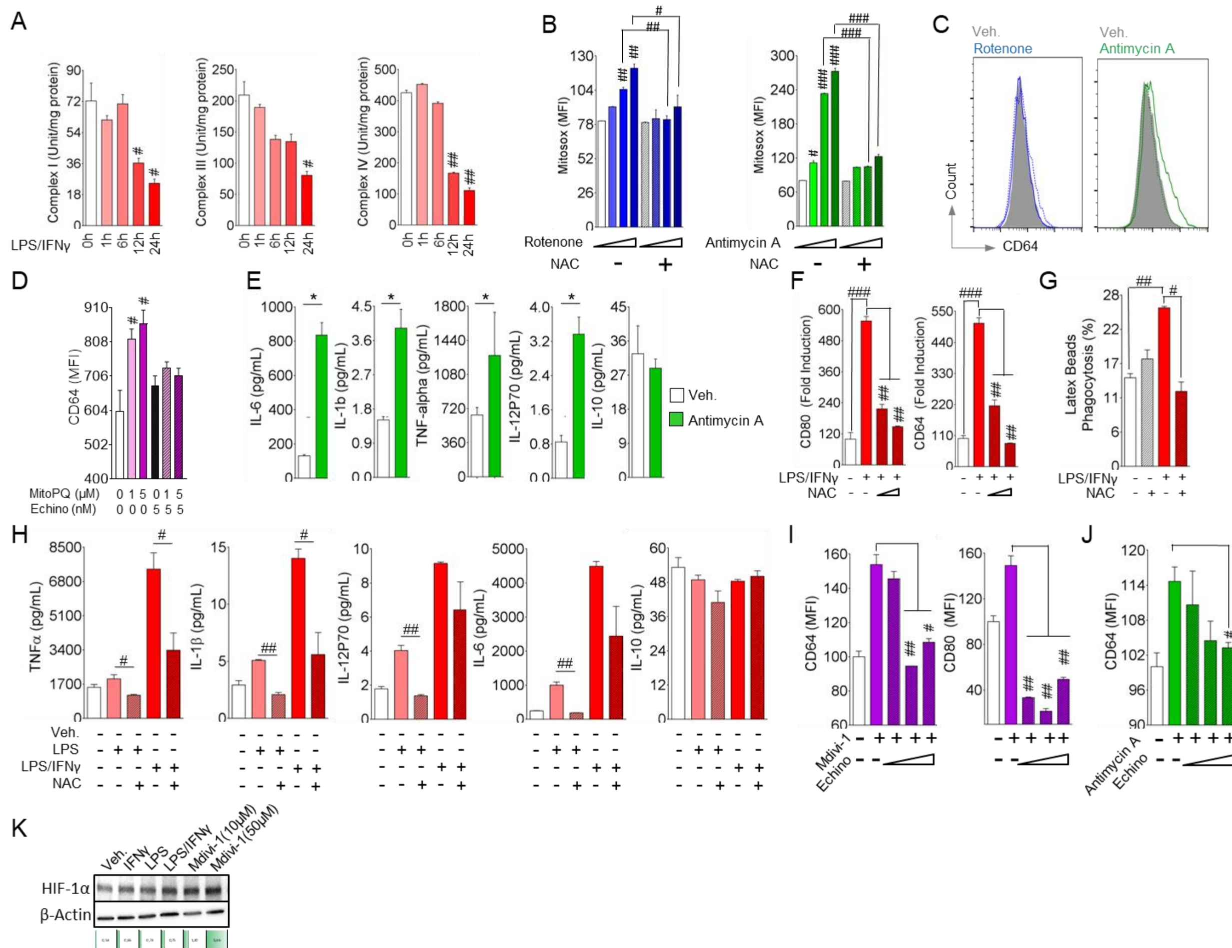


Figure S6. The inhibition of mitophagy triggers classical activation of macrophages through mitochondrial ROS.

A. Respiratory chain complexes activity in raw 264.7 macrophages exposed to LPS/IFNγ for indicated duration (n=3 per time point). **B-C**. Flow cytometry assessment of (**B**) mitochondrial ROS production and (**C**) macrophage activation in raw 264.7 macrophages exposed to rotenone (1-10µM, 24h) or antimycin A (33-100µM, 6h) alone or (**B**) in combination with N-acetylcysteine (NAC, 5mM) (**B.** n=3 per condition). **D.** Assessment of macrophage activation with flow cytometry in BMDMs treated for 24h with vehicle (DMSO 0.1%) or indicated concentrations of mitoParaquat (mitoPQ) and echinomycin (Echino) alone or in combination. **E.** Cytokines release in raw 264.7 macrophages exposed to vehicle (veh., DSMO 0.1%) or antimycin A (100µM, 6h) (n=3 per condition). **F-H**. Assessment of macrophage activation with (**F**) flow cytometry, (**H**) cytokines release and (**G**) flow cytometry assessment of phagocytosis of latex beads in raw 264.7 macrophages exposed to LPS or LPS/IFNγ alone or in combination with N-acetylcysteine (NAC, 1-5mM) (n=3 per condition). **I-J**. Flow cytometry assessment of macrophage activation in raw 264.7 macrophages incubated with vehicle or (**I**) mitochondrial division inhibitor 1 (mdivi-1, 50µM, 24h) or (**J**) antimycin A (100µM, 6h) alone or in combination with echinomycin (Echino, 1-5nM, 24h) (n=3 per condition). **K.** Assessment by immunoblotting of HIF-1α expression in raw 264.7 macrophages exposed for 24h to LPS (100ng/mL) or IFNγ (20ng/mL) alone or in combination or to mdivi-1 at indicated concentrations (Densitometry: ratio to β-actin is presented above the immunoblots). Bar graphs represent mean ± s.e.m.; # *P<0.05,* ## *P<0.01,* ### *P<0.001* determined by ANOVA corrected for multiple comparison. * *P<0.05* determined by *Student's t-test* with Welch's correction.

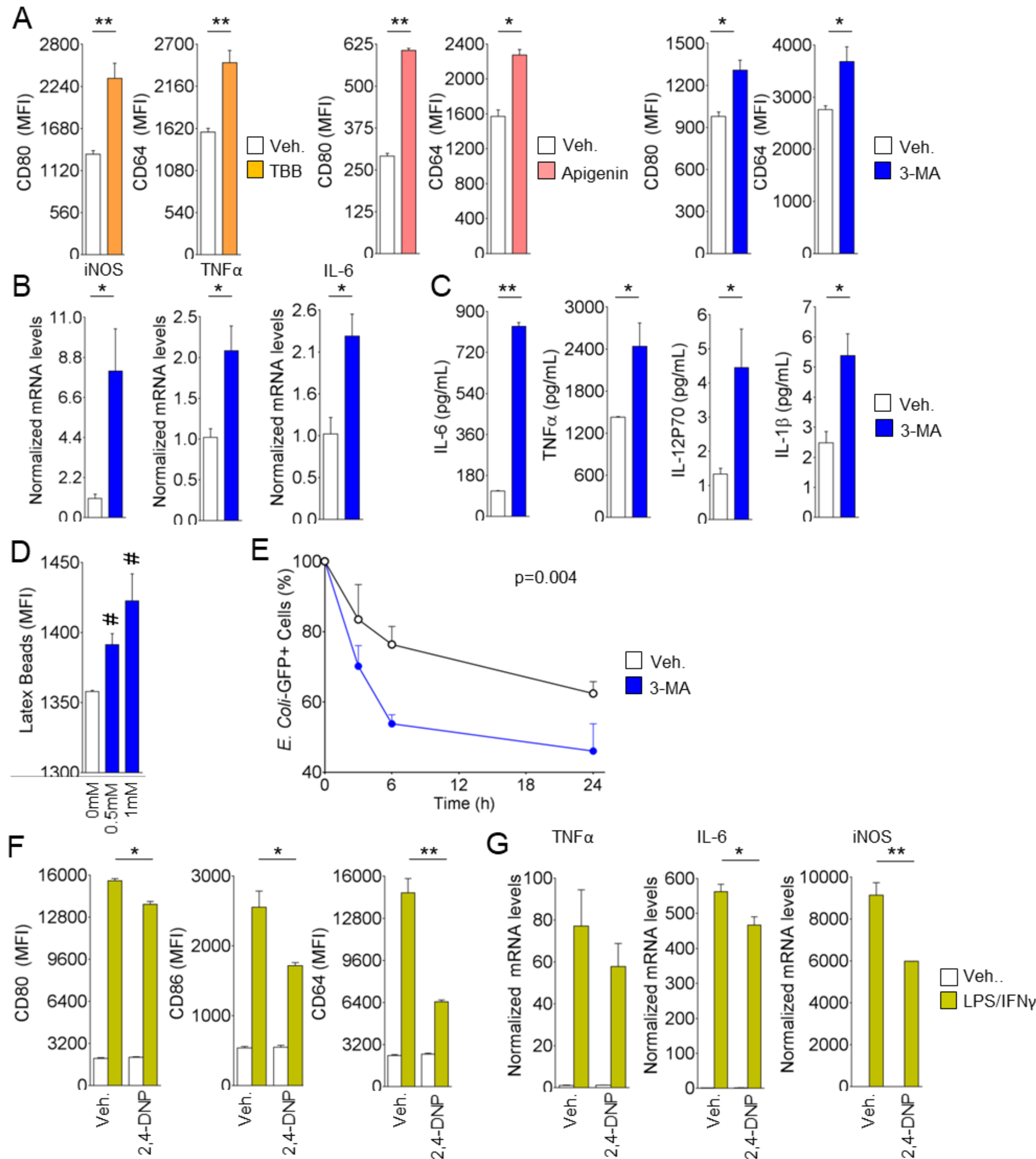


Figure S7. Pharmacological inhibition of mitophagy promotes the classical activation of macrophages.

A. Flow cytometry assessment of classical activation of macrophages in raw 264.7 cells exposed for 24h to vehicle (DMSO, 0.1%) or 4,5,6,7-tetrabromobenzotriazole (TBB, 50μM) or apigenin (50μM) or 3-methyl adenine (3-MA, 1mM) (n=3 per condition). **B-E**. Assessment of macrophage activation profile by (**B**) gene expression, (**C**) cytokine release, flow cytometry assessment of (**D**) phagocytosis of latex beads and (**E**) bactericidal activity (gentamycin protection assay) in raw 264.7 cells incubated for 24h with 3-MA (0.5-1mM) (n=3 per condition). **F-G**. Assessment of macrophage activation profile by (**F**) flow cytometry and (**G**) gene expression in raw 264.7 cells incubated for 24h with LPS/IFNγ alone or in combination with 2,4 dinitrophenol (2,4-DNP, 1μM) (n=3 per condition). Bar graphs represent mean ± s.e.m.; # *P<0.05* determined by ANOVA corrected for multiple comparison; * *P<0.05,* ** *P<0.01,* *** *P<0.001* determined by *Student's t-test* with Welch's correction.

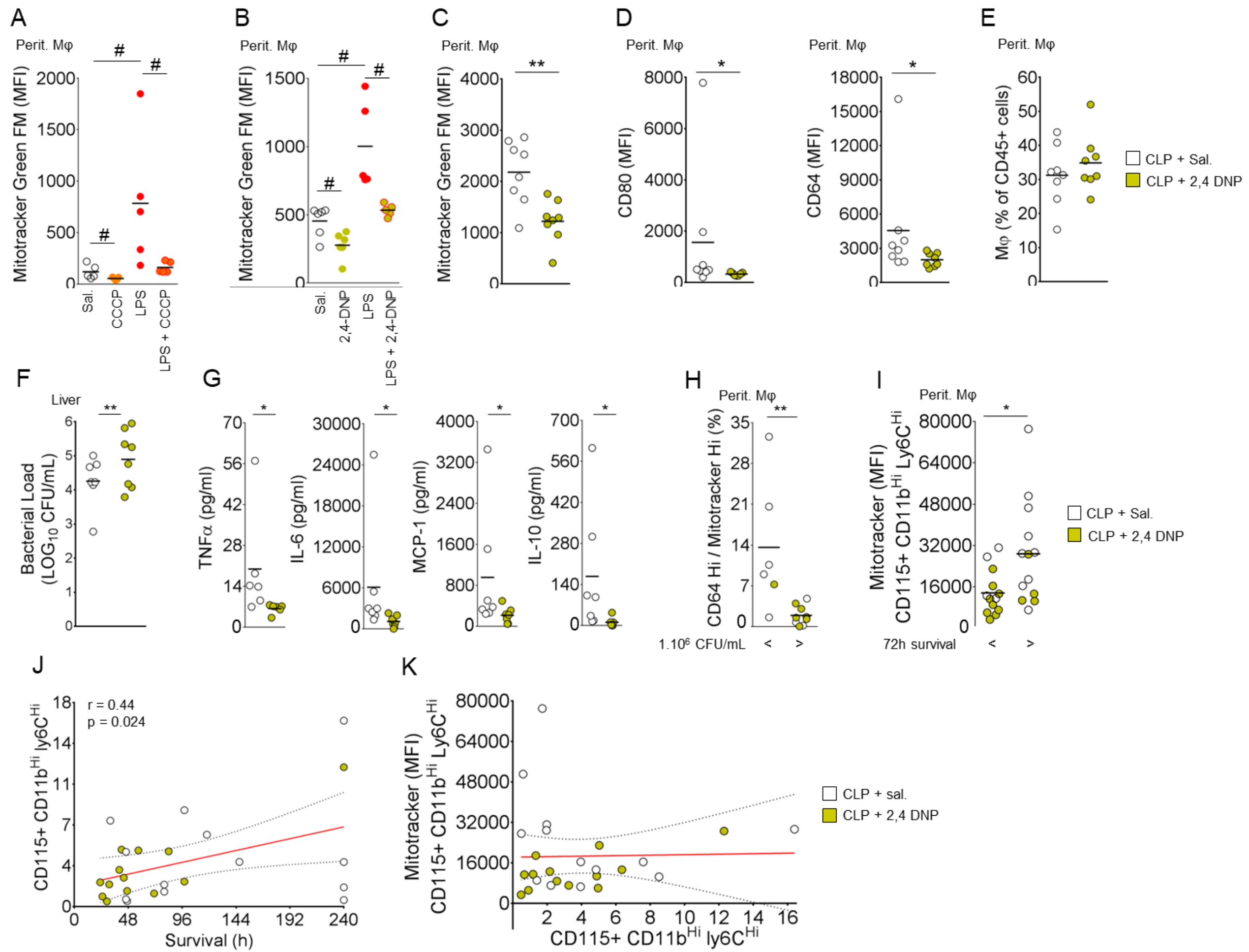


Figure S8. Pharmacological induction of mitophagy in myeloid cells reduces macrophages activation and favors bacterial infection.

A-B. Flow cytometry assessment of mitochondrial density in peritoneal macrophages from peritoneal fluid of C57BL6/J mice submitted to a 24h intraperitoneal injection of (**A**) LPS (0.5 mg/kg) and carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 5mg/Kg) alone or in combination (n=5-6 per condition) or (**B**) LPS (10mg/kg) and 2,4 dinitrophenol (2,4-DNP, 10mg/Kg) alone or in combination (n=5-6 per condition). **C-D.** Flow cytometry assessment of (**C**) mitochondrial density and (**D**) levels of activation markers in peritoneal macrophages ($CD11b^{Hi}$ $F4/80^{Hi}$) in C57BL6/J mice treated with saline (sal.) or 2,4 dinitrophenol 2,4-DNP (10mg/kg) (n=8 per condition) 24h prior to cecal ligation and puncture (CLP). **E-F.** (**E**) Percentage of peritoneal macrophages ($CD11b^{Hi}$ $F4/80^{Hi}$) and (**F**) bacterial load in the liver of mice treated as in **C-D** (n=8 per condition). **G.** Plasma levels of sepsis-induced cytokines in C57BL6/J mice treated as in **C-D** (n=7 per condition). **H.** Extent of the bacterial load according to the levels of $CD64^{Hi}$ $Mitotracker^{Hi}$ macrophages in the peritoneal cavity in mice treated as in **C-D** (n=16). **I.** Extent of the survival according to the levels of $Ly6C^{Hi}$ blood monocytes 2h after CLP surgery. Mice treated as in **C-D** (n=26). **J-K.** Correlation of $ly6C^{Hi}$ blood monocytes 2h after CLP surgery ($CD115^{+}CD11b^{Hi}Ly6C^{Hi}$) versus (**I**) mouse survival or (**J**) mitochondrial density in $ly6C^{Hi}$ blood monocytes 2h after CLP surgery (n=26). *R* and *P values* were determined by *Spearman's rank correlation*. Graphs with plots represent mean plus individual values; # *P<0.05* determined by ANOVA corrected for multiple comparison; * *P<0.05,* ** *P<0.01* determined by *Student's t-test* with Welch's correction.

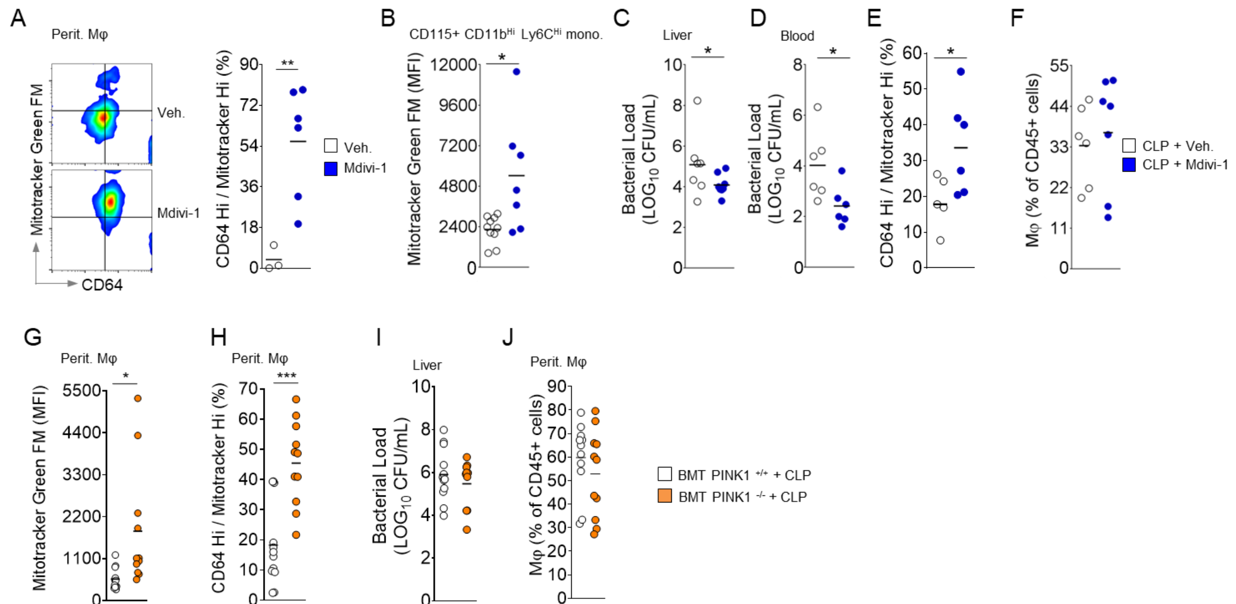


Figure S9. Pharmacologically and genetically-induced inhibition of mitophagy in myeloid cells protects against bacterial infection.

A. Flow cytometry assessment of CD64^{Hi} Mitotracker^{Hi} peritoneal macrophages subpopulation in mice treated with vehicle (Veh.: saline + 1% DMSO, n=3) or mdivi-1 (50mg/kg, n=6) for 24h. **B.** Flow cytometry assessment of mitochondrial density in blood inflammatory monocytes (CD45^{+} CD115^{+} CD11b^{Hi} Ly6C^{Hi}) in C57BL6/J mice treated with vehicle or mdivi-1 for 24h prior to CLP surgery. (CLP + Veh., n=10 ; CLP + mdivi-1, n=7). **C-D**. Bacterial load in (**m**) the liver and (**n**) the blood of mice treated as in **b**. **e-f**. Flow cytometry assessment of the proportion of (**e**) CD64^{Hi}/Mitotracker^{Hi} subpopulation of peritoneal macrophages and of (**f**) the proportion of total macrophages in the peritoneal fluid of mice treated and operated as in **b**. **g**. Flow cytometry assessment of mitochondrial density in peritoneal macrophages of mice transplanted with *Pink1*$^{+/+}$ (BMT *Pink1*$^{+/+}$) or *Pink1*$^{-/-}$ bone marrow (BMT *Pink1*$^{-/-}$) 5 weeks prior to CLP surgery (n=11-12 per group). **H.** Flow cytometry assessment the percentage of CD64^{Hi} Mitotracker^{Hi} macrophage subpopulation (% of CD11b^{Hi} F4/80^{Hi} macrophages) in the peritoneal cavity of mice that underwent transplantation and surgery as in **G** (n=11-12 per group). **I.** Bacterial load in the liver of C57BL6/J mice that underwent transplantation and surgery as in **G** (n=11-12 per group). **J.** Flow cytometry assessment of the percentage of macrophages (% of CD45+ cells) in the peritoneal cavity of mice transplanted and operated as in **G**. (n=11-12 per group). Graphs with plots represent mean plus individual values; * *P<0.05,* ** *P<0.01;* *** *P<0.001* determined *Student's t-test* with Welch's correction.

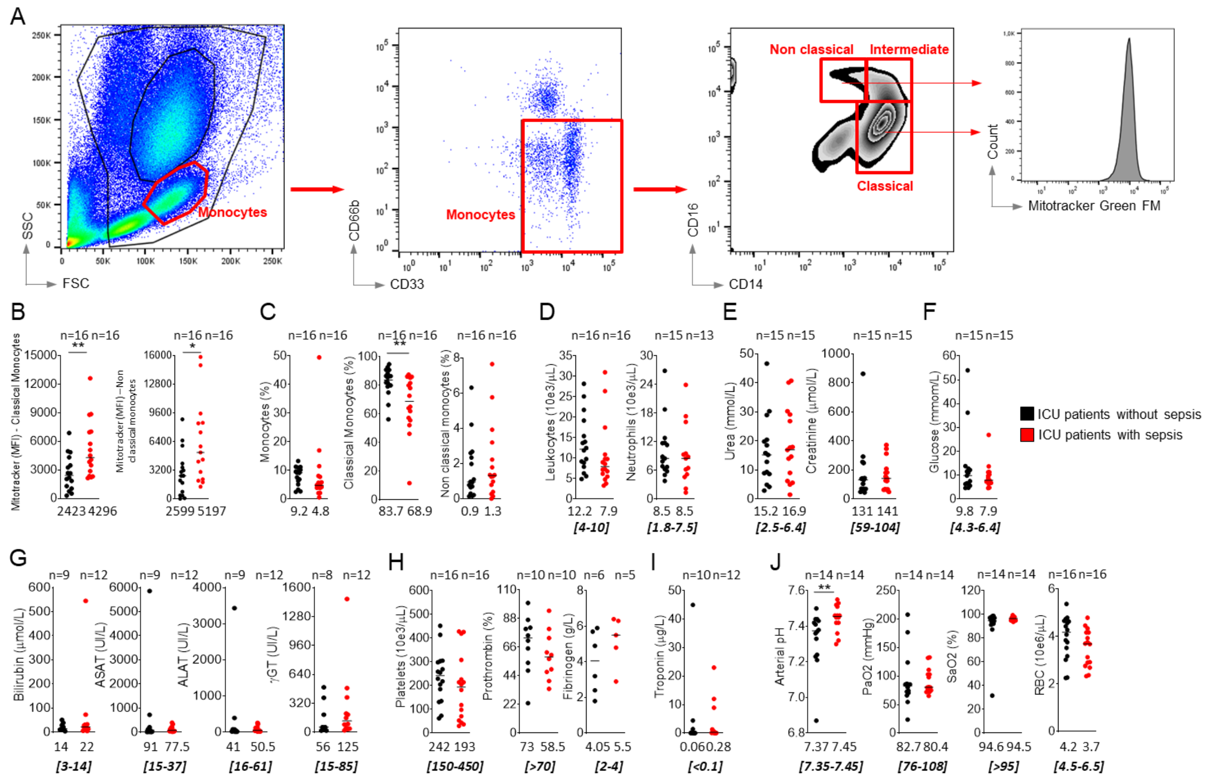


Figure S10. Blood profile of enrolled critically ill patients.
A. Gating strategy for the assessment of mitochondrial density in blood monocytes ($CD66b^-CD33^+$), and their subsets (classical ($CD66b^-CD33^+CD14^{++}CD16^-$), intermediate ($CD66b^-CD33^+CD14^{++}CD16^+$), non classical ($CD66b^-CD33^+CD14^+CD16^{++}$)). **B.** Flow cytometry assessment of mitochondrial density in classical and non classical blood monocytes of critically ill patients (Intensive Care Unit (ICU) patients) without (n=16) or with sepsis (n=16) according to sepsis-3 task force criteria (within 8h after admission in ICU). **C-D.** Flow cytometry assessment of percentage of total monocytes and monocyte subpopulations (**C**) and leukocytes and neutrophil count (**D**) in the blood of ICU patients as described in **B**. **E-J.** Levels of plasma biomarkers of (**E**) renal function, (**F**) glucose metabolism, (**G**) liver function, (**H**) coagution, (**I**) heart function, (**G**) blood oxygenation and hemodynamics in the blood of ICU patients as described in **B**. Graphs represent median plus individual values. Median value of each group is presented at the bottom of each graph. Normal values of healthy patients are presented in bold italic enclosed in square brackets. Data (**D-J**) were collected from patient medical records and were not available for all patients (n are indicated above graphs). Graphs represent median plus individual values. Median value of each group is presented at the bottom of each graph. Normal range of healthy patients are presented in bold italic enclosed in square brackets. * *P<0.05,* ** *P<0.01,* *** *P<0.001* determined by *Student's t-test* with Welch's correction.

Table S1. Characteristics of enrolled critically ill patients with sepsis status as defined by sepsis-3 task force.

Gender	Age	Pathology	Sepsis	Documented Infection	CRP (mg/L)	PCT (µg/L)	Immunodeficiency
F	32	Liver transplanted patient with hepatic thrombosis	No	No	30,0	0,26	Yes
F	49	Pneumoniae	No	Yes	ND	ND	No
F	59	Hematemesis	No	No	10,0	0,37	No
F	60	Status epilepticus	No	No	46,0	0,05	No
M	24	Acute respiratory distress syndrome + HIV	No	No	40,4	0,34	Yes
M	38	Severe acute asthma	No	No	2,9	0,05	No
M	40	Pancreatitis	No	No	400,0	ND	No
M	49	Non documentated pneumopathy	No	No	523,0	ND	No
M	50	Cardiogenic shock	No	No	2,9	ND	No
M	63	Diabetic acidocetosis	No	No	3,0	0,34	No
M	66	Acute respiratory failure due to pleurisy	No	No	ND	ND	No
M	73	Interstital pneumopathy	No	No	ND	0,16	Yes
M	78	Cardiogenic shock	No	No	65,0	0,96	No
M	79	Cardiogenic shock + renal failure	No	No	ND	0,13	No
M	82	Hyperkaliemia	No	No	44,7	0,20	No
M	89	Hemorrhagic shock	No	No	ND	0,16	No
F	76	Pyelonephritis	Yes	No	ND	13,20	No
F	70	Cardiogenic shock + Infectious pneumopathy	Yes	No	155,0	2,13	No
F	41	Pyelonephritis	Yes	Yes	148,0	ND	No
M	32	Infectious pneumopathy	Yes	Yes	ND	ND	No
M	40	Pyelonephritis	Yes	Yes	149,0	ND	No
M	49	Colitis + Pneumopathy	Yes	No	ND	0,41	No
M	50	Infectious pneumopathy	Yes	Yes	ND	1,39	No
M	56	Septic shock due to pyelonephritis	Yes	Yes	149,0	17,00	No
M	56	Infectious pneumopathy	Yes	Yes	ND	0,18	No
M	65	Renal transplanted patient + Pneumocytosis	Yes	No	102,0	0,56	Yes
M	69	Pyelonephritis	Yes	Yes	ND	1,27	No
M	74	Sepsis + Leukemia	Yes	No	479,0	46,00	Yes
M	77	Infectious pneumopathy	Yes	No	134,0	12,40	No
M	79	Pyelonephritis	Yes	Yes	177,0	11,40	No
M	79	Infectious pneumopathy	Yes	Yes	60,0	0,35	No
M	82	Angiocholitis	Yes	No	ND	40,00	No

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