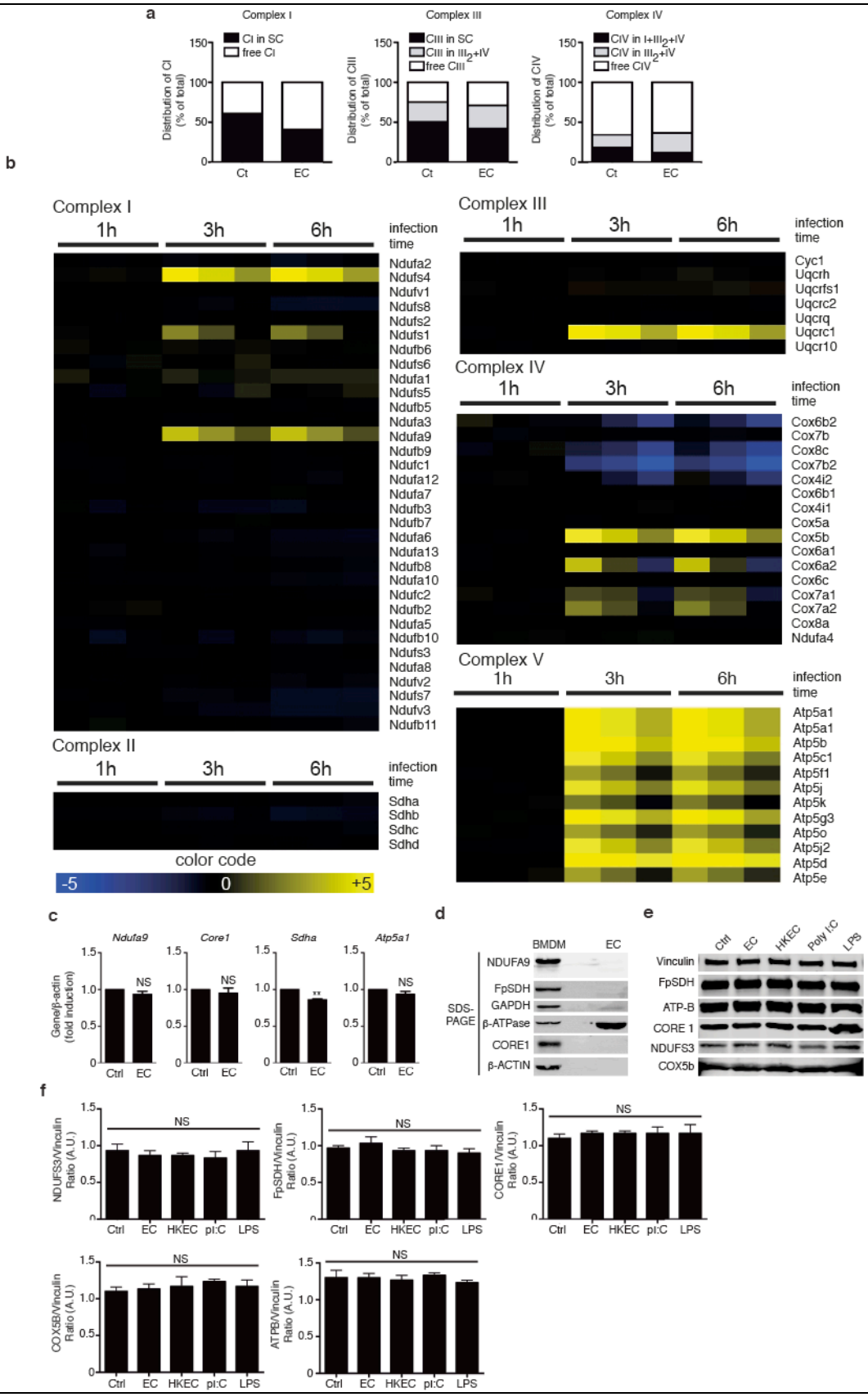
a
Complex I
Complex III
Complex IV
CI in SC
free CI
CIII in SC
CIII in III2+IV
free CIII
CIV in I+III2+IV
CIV in III2+IV
free CIV
Distribution of CI (% of total)
Distribution of CIII (% of total)
Distribution of CIV (% of total)
Ct
EC
b
Complex I
Complex III
Complex IV
Complex V
Complex II
1h
3h
6h
infection time
Ndufa2
Ndufs4
Ndufv1
Ndufs8
Ndufs2
Ndufs1
Ndufb6
Ndufs6
Ndufa1
Ndufs5
Ndufb5
Ndufa3
Ndufa9
Ndufb9
Ndufc1
Ndufa12
Ndufa7
Ndufb3
Ndufb7
Ndufa6
Ndufa13
Ndufb8
Ndufa10
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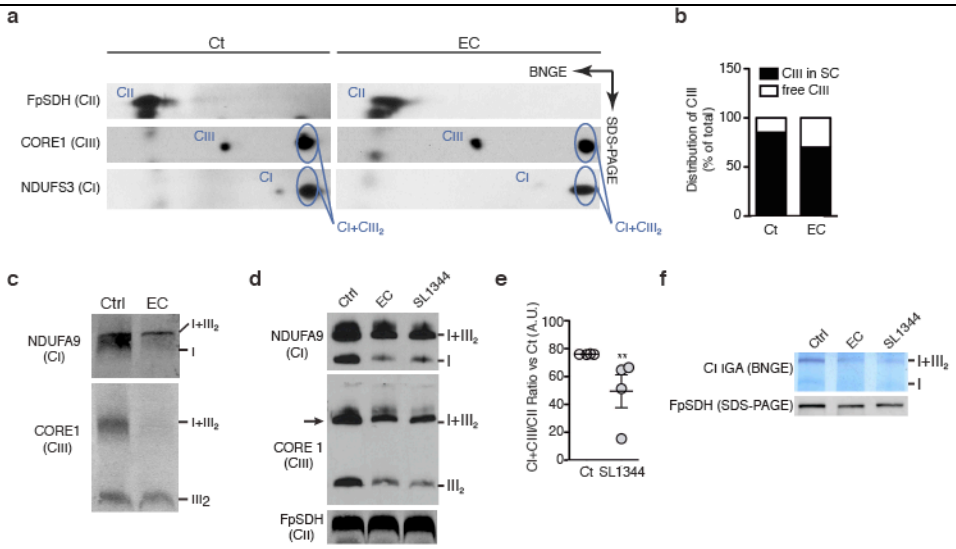
Cyc1
Uqcrh
Uqcrfs1
Uqcrc2
Uqcrq
Uqcrc1
Uqcr10
Cox6b2
Cox7b
Cox8c
Cox7b2
Cox4i2
Cox6b1
Cox4i1
Cox5a
Cox5b
Cox6a1
Cox6a2
Cox6c
Cox7a1
Cox7a2
Cox8a
Ndufa4
Atp5a1
Atp5a1
Atp5b
Atp5c1
Atp5f1
Atp5j
Atp5k
Atp5g3
Atp5o
Atp5j2
Atp5d
Atp5e
Sdha
Sdhb
Sdhc
Sdhd
color code
-5
0
+5
c
Ndufa9
Core1
Sdha
Atp5a1
Gene/β-actin (fold induction)
NS
**
Ctrl
EC
d
BMDM
EC
NDUFA9
FpSDH
GAPDH
β-ATPase
CORE1
β-ACTIN
SDS-PAGE
e
Ctrl
EC
HKEC
Poly I:C
LPS
Vinculin
FpSDH
ATP-B
CORE 1
NDUFS3
COX5b
f
NDUFS3/Vinculin Ratio (A.U.)
FpSDH/Vinculin Ratio (A.U.)
CORE1/Vinculin Ratio (A.U.)
COX5B/Vinculin Ratio (A.U.)
ATPB/Vinculin Ratio (A.U.)
Ctrl
EC
HKEC
pI:C
LPS

Supplementary Figure 1

Effect of *E. coli* challenge on mitochondrial respiratory complex subunit expression in BMDMs.

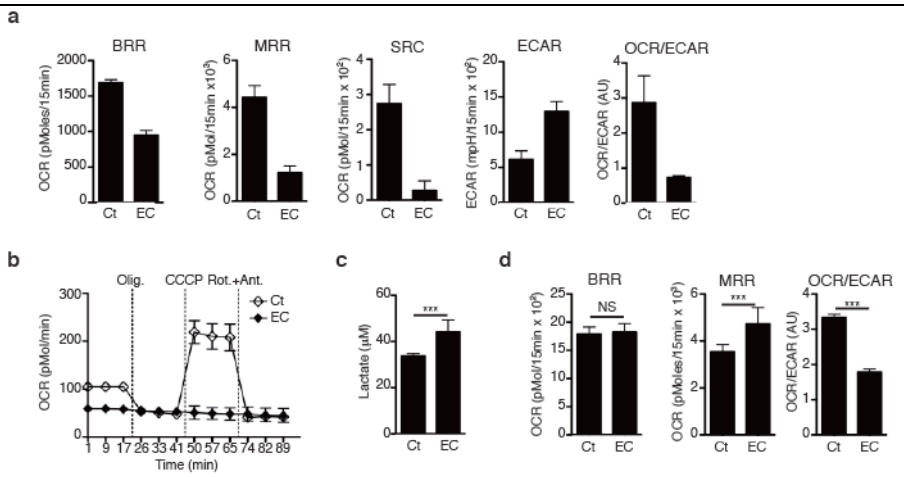
(**a**) Densitometric analysis of the distribution of complex I, III and IV as determined from **Figure 1a**. (**b**) Gene microarray analysis (Affymetrix Microarray data have been previously deposited with the NCBI Gene Expression Omnibus under accession number GSE27960 by Sander L.E. et al., see ref. 19) of C57BL/6J BMDMs treated with *E. coli* for 1, 3 or 6h (3 biological replicates). A heat map of nuclear genome-encoded mitochondrial respiratory complexes subunits is shown. (**c**) Q-PCR analysis of the indicated gene from BMDMs treated with *E. coli* for 1.5h. (**d**) Immunoblot analysis of resting BMDMs and *E. coli*. (**e**) Immunoblot analysis of BMDMs treated as indicated for 1.5h. EC, *E. coli*; HKEC, heat killed-*E. coli*; Poly I:C, polyinosinic:polycytidylic acid; LPS, lipopolysaccharide. (**f**) Quantification analysis of SDS-PAGE as in (**e**). NS, not significant ($P > 0.05$); $^{**}P < 0.01$ (two-tailed unpaired Student's *t*-test). Membranes were probed with the indicated antibodies specific for components of the ETC (**d, e**). Data are from three independent experiments (**c, f**;mean and s.e.m.), one representative of two independent experiments with similar results (**a, d, e**).



Supplementary Figure 2

Detection of Gram-negative bacteria induces ETC rearrangement and is associated with decreased complex I.

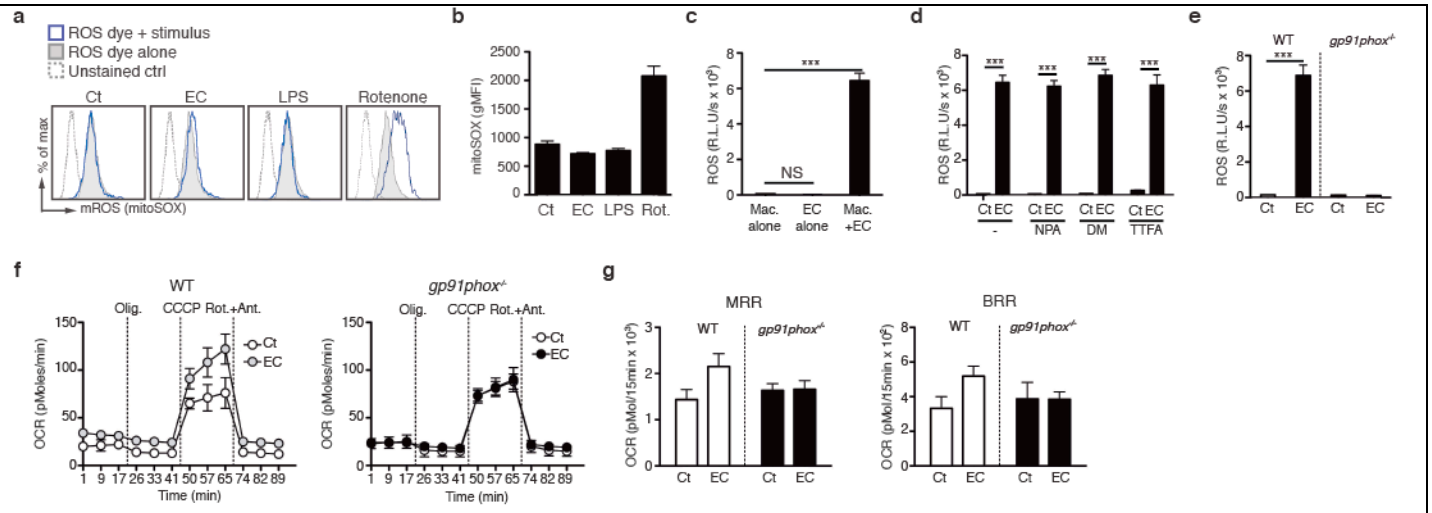
(**a**) Immunoblot of a bi-dimensional gel analysis (First dimension: BN-PAGE, second dimension: SDS-PAGE) of mitochondria isolated from C57BL/6J BMDMs treated or not with EC for 1.5h. (**b**) Quantification from (**a**) of the proportion of free CIII and CIII in super-complex with CI (SC I+III). (**c**) BN-PAGE immunoblot of C57BL/6J thioglycollate-elicited macrophages treated or not with EC. (**d**) BN-PAGE immunoblot of C57BL/6J BMDMs treated with *E. coli* and *S. enterica* Typhimurium for 1.5h. (**e**) Densitometric analysis of BN-PAGE from (**d**) showing CI+CIII SC proportion vs. CII (n=4). $^{**}P < 0.01$ (two-tailed unpaired Student's *t*-test). Data present mean +/- s.e.m. of 4 independent experiments. (**f**) CI IGA of BMDMs treated or not with *E. coli* or *S. enterica* Typhimurium for 1.5h. SDS-PAGE analysis of FpSDH (CII) is shown (lower panel). Data are from two independent experiments (**e**, mean and s.e.m.), one representative of two independent experiments (**a-d, f**).



Supplementary Figure 3

E. coli challenge influences mitochondrial respiration and glycolysis in macrophages.

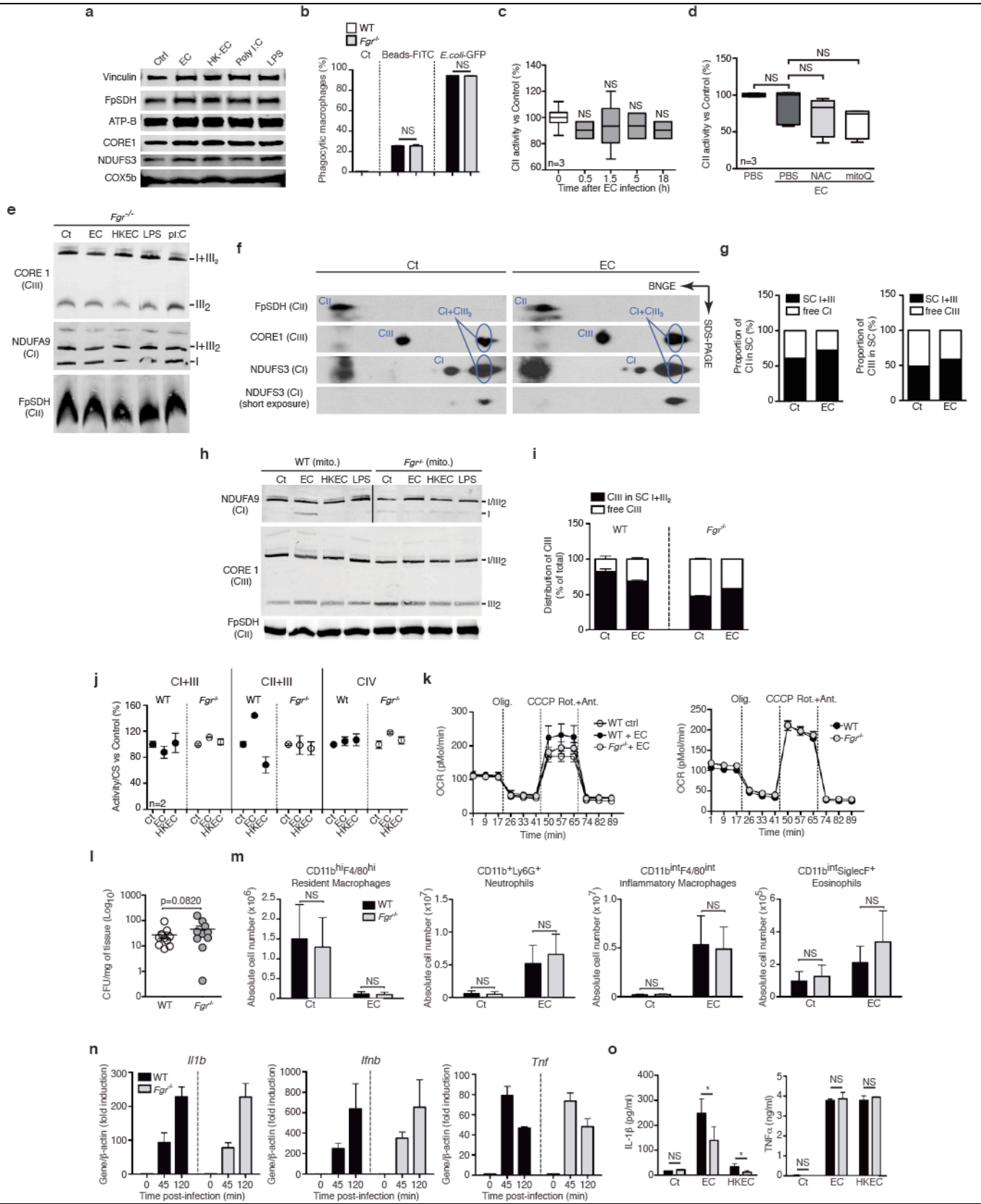
(**a**) Glucose-driven basal respiration rate (BRR), maximum respiration rate (MRR), spare respiration capacity (SRC), basal extracellular acidification rate (ECAR) and oxygen consumption rate (OCR)/ECAR ratio in BMDMs treated or not with EC for 18h. (**b**) Glucose-driven OCR upon sequential treatment of oligomycin (olig.), CCCP, and rotenone+antimycin (Rot.+Ant.) of BMDMs treated or not with EC for 18h. (**c**) Extracellular lactate release by BMDMs treated or not with EC for 18h. (**d**) Glucose-driven BRR, MRR and OCR/ECAR ratio of BMDMs treated or not with EC for 1.5h. NS, not significant, ***P < 0.001 (two-tailed unpaired Student's t-test). Data (means and s.d.) are from one representative of two independent experiments (**a, b**). Data (means and s.e.m.) are from three independent experiments performed in five technical replicates (**c, d**).



Supplementary Figure 4

Bacteria recognition drives mitochondrial respiratory adaptations through phagosomal NADPH oxidase-mediated ROS.

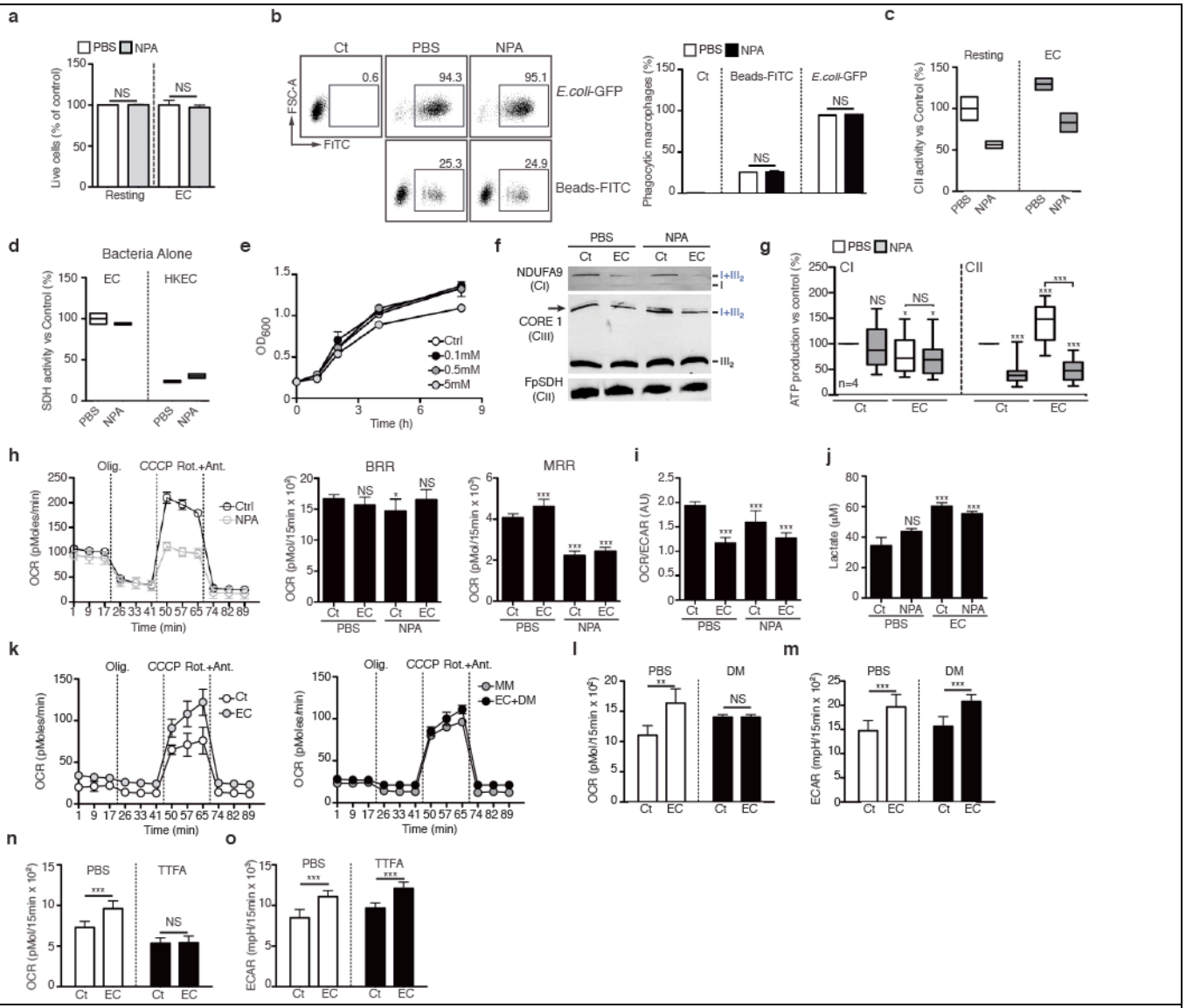
(**a**) Representative histograms of BMDMs treated as indicated for 1.5h, stained with mitoSOX (mROS) and analysed by FACS. Rot., rotenone. (**b**) mROS production by BMDMs treated as in (**a**). One representative experiment performed in triplicate is shown. (**c-e**) ROS production by EC alone (**c**), WT BMDMs (**c-e**) and *Gp91phox*$^{-/-}$ BMDMs stimulated or not with EC for 15min (**e**). ROS production was monitored by chemiluminescence and expressed as relative light units per second (R.L.U./s). In (**d**), BMDMs were treated with the CII inhibitors 3-nitropropionic acid (NPA), dimethyl-malonate (DM) or thenoyltrifluoroacetone (TTFA). (**f**) Glucose-driven OCR upon sequential treatment of oligomycin (olig.), CCCP, and rotenone+antimycin (Rot.+Ant.) of WT and *Gp91phox*$^{-/-}$ BMDMs challenged with EC for 2h. (**g**) Maximum (MRR) and basal (BRR) respiration rate of WT and *Gp91phox*$^{-/-}$ BMDMs challenged with EC for 2h. ***$P <$ 0.001 (two-tailed unpaired Student's *t*-test (**c, d, e**)). Data (mean and s.e.m.) are from three independent experiments performed in duplicates (**c-e**), one representative of three independent experiments. Data are mean and s.d. of one representative of three independent experiments performed in three (**a, b**) and five (**f, g**) technical replicates.



Supplementary Figure 5

Fgr deficiency prevents macrophage ETC adaptations and attenuates innate immunity in response to *E. coli* detection.

(**a**) Immunoblot analysis of BMDMs stimulated as indicated for 1.5h and probed with the indicated antibodies specific for component of the ETC. (**b**) Percentage of phagocytic cells of WT and *Fgr*$^{-/-}$ BMDMs treated with NPA and cultured with GFP-expressing *E. coli* (E. coli-GFP) or FITC-labeled latex beads (Beads-FITC) for 20min. (**c, d**) Spectrophotometric CII activity in permeabilized *Fgr*$^{-/-}$ BMDMs stimulated with EC for 1.5h or the indicated time points and treated or not with N-acetylcystein (NAC) or mitoQ. (**e**) Immunoblot of BN-PAGE analysis of permeabilized *Fgr*$^{-/-}$ BMDMs stimulated as indicated for 1.5h. (**f**) Immunoblot of a bi-dimensional gel analysis (BN-PAGE followed by a SDS-PAGE) of mitochondrial isolated from *Fgr*$^{-/-}$ BMDMs treated or not with EC for 1.5h. (**g**) Quantification from (**f**) of the proportion of CI and CIII as free form or in super-complex (SC I+III). (**h**) Immunoblot of BN-PAGE analysis of mitochondrial isolated from WT and *Fgr*$^{-/-}$ BMDMs stimulated as indicated for 1.5h. Lower panel shows CI in-gel activity (IGA). (**i**) Relative contribution of CIII to SC as determined by BN-PAGE analysis of mitochondria isolated from *Fgr*$^{-/-}$ and WT BMDMs. (**j**) Effect of *E. coli* stimulation on the indicated ETC complex activities in mitochondria isolated from WT and *Fgr*$^{-/-}$ BMDMs. (**k**) Glucose-driven OCR upon sequential treatment of oligomycin (olig.), CCCP, and rotenone+antimycin (Rot.+Ant.) of WT and *Fgr*$^{-/-}$ BMDMs stimulated or not with EC for 2h. (**l**) Splenic bacterial burdens 72h after injection of 1 x 10^8 of viable *E. coli* into the peritoneal cavity of WT and *Fgr*$^{-/-}$ mice. Each symbol represents one mouse. (**m**) Absolute cell numbers determined at 18h by FACS of the indicated cell populations in the peritoneal cavity of WT and *Fgr*$^{-/-}$ mice injected with 1x10^8 viable EC. (**n**) mRNA levels in WT and *Fgr*$^{-/-}$ BMDMs stimulated with EC for the indicated time point. (**o**) Cytokine levels in supernatants of WT and *Fgr*$^{-/-}$ BMDMs stimulated with EC for 18h. NS, not significant; **P* < 0.05, ****P*<0.001 (two-tailed unpaired Student's *t*-test). Data (mean and s.e.m.) are from two to four independent experiments performed in two or five technical replicates (**b-d, j, l-o**). Data (**k**, means and s.d.; **a, e, f, h**) are from one representative of three independent experiments performed in five technical replicates. EC, *E. coli* ; HKEC, heat killed-*E. coli* ; pI:C, polyinosinic:polycytidylic acid ; LPS, lipopolysaccharide.

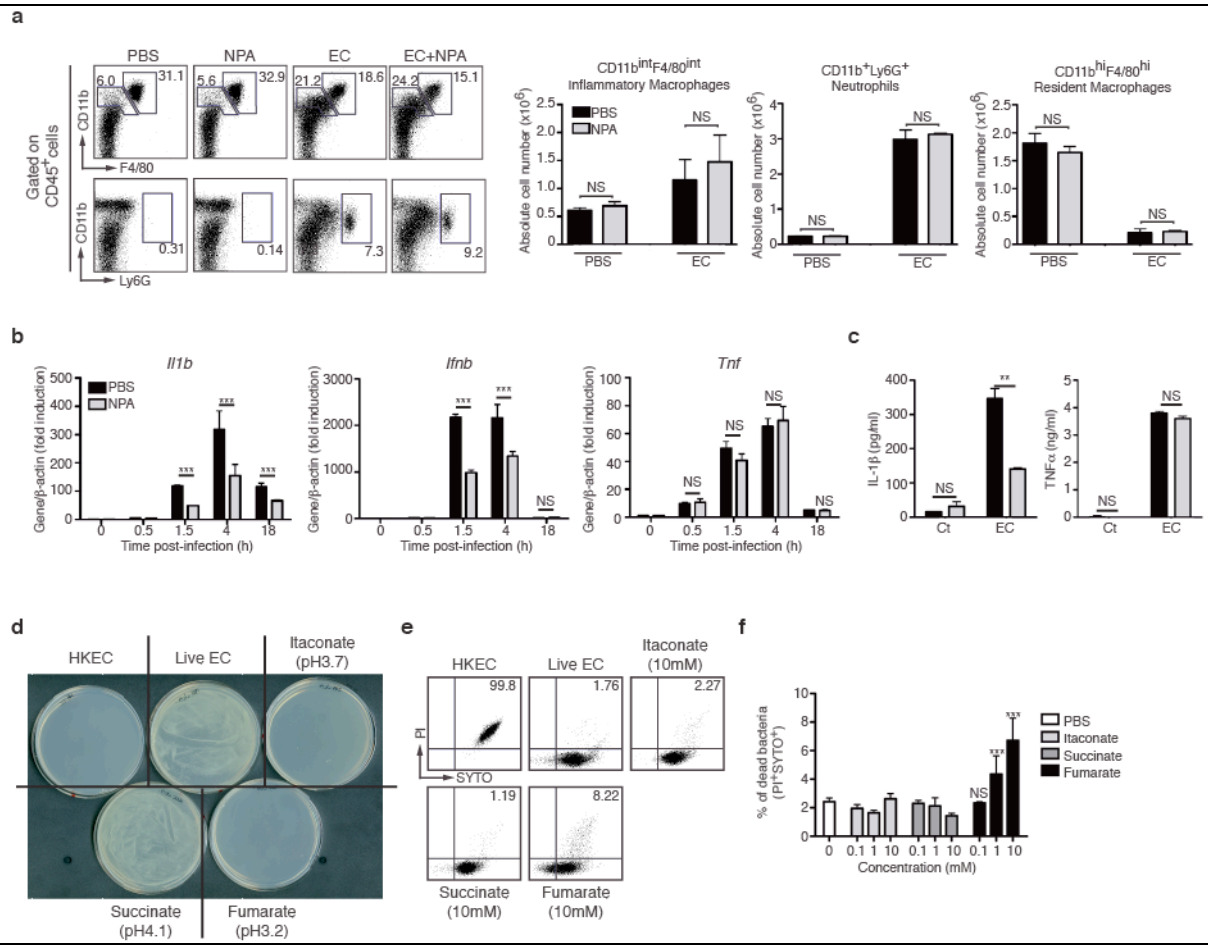


Supplementary Figure 6

Effects of mitochondrial complex II (CII) inhibition on BMDM functions and mitochondrial respiration.

(**a**) Percentage of live BMDMs treated with 0.5mM NPA and challenged with EC for 1.5h. Cells were stained with annexin-V-GFP and 7-aminoactinomycin D (7-AAD) and analyzed by fluorescent-activated cell sorting flow cytometry (FACS). Live cells were defined as annexin-V-/7-AAD- cells. (**b**) Percentage of phagocytic cells (right panel) of BMDMs treated with 0.5mM NPA and cultured with GFP-expressing *E. coli* (*E. coli*-GFP) or FITC-labeled latex beads (Beads-FITC) for 20min. Representative FACS plots (left panel) are shown. (**c**) Spectrophotometric CII activity of BMDMs treated or not with EC in presence of 0.5mM NPA. (**d**) Spectrophotometric SDH activity of log phase EC or heat-killed EC (HKEC) in the presence of NPA. (**e**) *E. coli* growth measured by spectrophotometry (A_{600}) in presence of the indicated concentrations of NPA over a course of 8h. (**f**) BN-PAGE immunoblot or permeabilized C57BL/6J BMDMs treated or not with NPA stimulated with EC for 1.5h. Membranes were stained with the indicated antibodies. (**g**) Glutamate+malate (CI) or succinate (CII)-driven ATP synthesis activity in permeabilized C57BL/6J BMDMs treated or not with NPA stimulated with EC for 1.5h. (**h**) Glucose-driven OCR, BRR and MRR upon sequential treatment of oligomycin (olig.), CCCP, and rotenone+antimycin (Rot.+Ant.) of BMDMs treated or not with NPA and challenged with EC for 2h. (**i**) OCR/ECAR ratio of BMDMs treated or not with NPA and challenged with EC for 2h. (**j**) Extracellular lactate release by BMDMs treated or not with NPA and challenged with EC for 2h. (**k**) Glucose-driven OCR, BRR and MRR upon sequential treatment of oligomycin (olig.), CCCP, and rotenone+antimycin (Rot.+Ant.) of BMDMs treated or not dimethyl-malonate (DM) and challenged with EC for 2h. (**l-o**) SRC (**l, n**) and ECAR (**m, o**) of BMDMs treated or not with dimethyl-malonate (DM) (**l, m**) or thenoyltrifluoroacetone (TTFA) (**n, o**) and challenged with EC for 2h. NS, not significant; **P* < 0.05; ***P* < 0.01; ****P* < 0.001 (two-tailed unpaired Student's *t*-test). Data (mean and s.e.m.) are from two independent experiments performed in two to

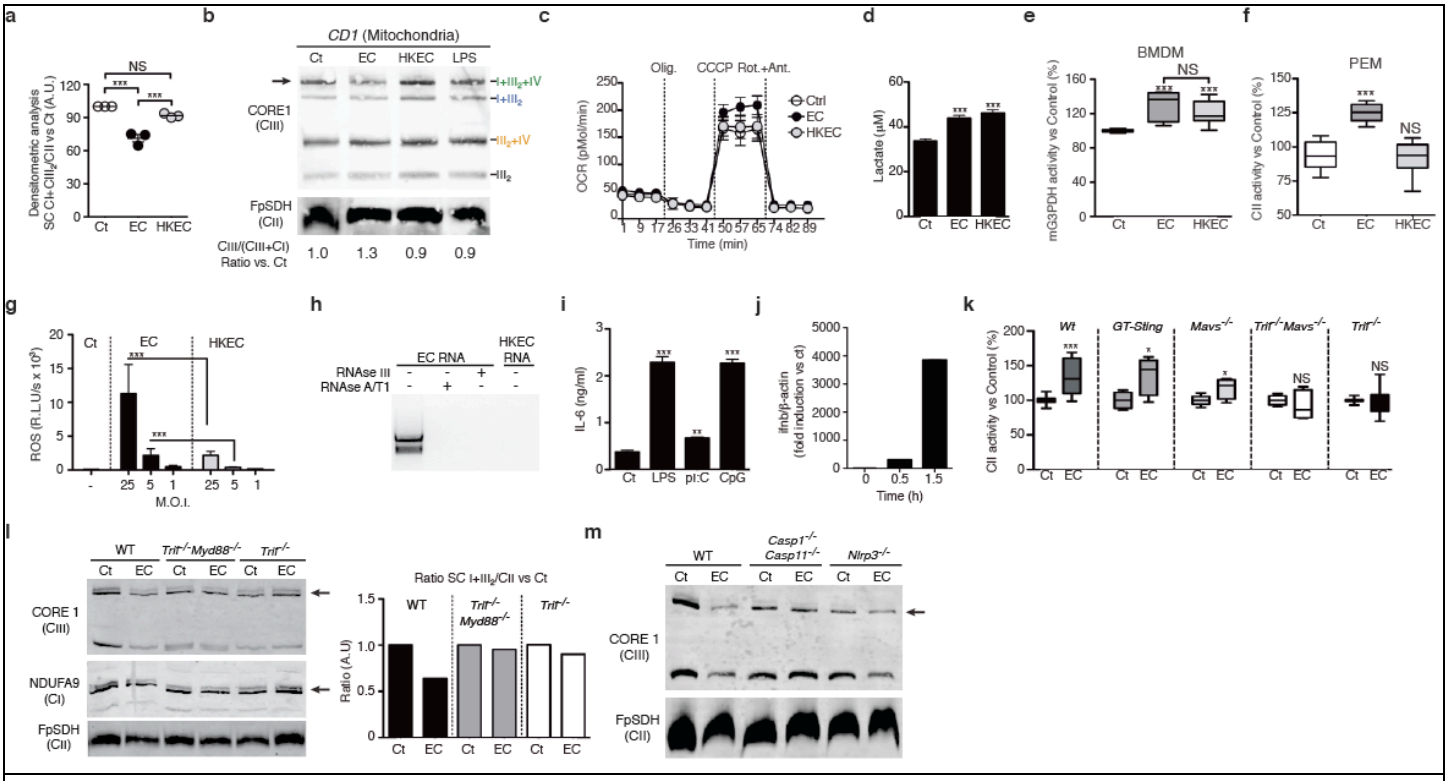
five technical replicates (**a, b,** right**, e, g, i, j, l-o**). Data in (**b,** left**, c, d, f, h, k**) are mean and s.d. of three technical replicates from one representative of three independent experiments.



Supplementary Figure 7

Complex II activity is required for macrophage bactericidal function and CII inhibition by 3-nitropropionic acid (NPA) alters innate immune response to viable *E. coli*.

(**a**) Absolute cell numbers at 18h (right panels) of the indicated cell populations in the peritoneal cavity of C57BL/6J mice treated or not with 50mg/kg NPA and injected with $1x10^8$ viable EC. Representative FACS plots (left panels) are shown. (**b, c**) mRNA (**b**) and cytokine levels (**c**) in BMDMs treated or not with NPA and stimulated with EC for the indicated time points. (**d**) Representative photographs of Petri dishes containing bacteria grown overnight after treatment with the indicated chemicals for 6h. As control, heat-killed *E. coli* (HKEC) were plated. (**e**) Representative flow cytometry plots of *E.coli* treated with the indicated reagents for 6h. (**f**) Percentage of PI+SYTO+ bacteria after 6h treatment with increasing amount of the indicated reagents. NS, not significant; $^{**}P < 0.01$ $^{***}P < 0.001$ (two-tailed unpaired Student's *t*-test). Data (mean and s.e.m. (**a,b,c,f**)) are from two (**c, e, f**) or three (**a, b**) independent experiments performed in triplicates. Data in (**d**) are from one representative of two independent experiments with similar results.



Supplementary Figure 8

Bacteria viability-specific ETC adaptations involves both TLR signaling and the Nlrp3-inflammasome.

(**a**) Densitometry analysis of CI+CIII$_2$/CII signal ratio as observed by BNGE immunoblot of WT BMDMs stimulated or not with EC and HKEC for 1.5h. (**b**) Blue-native gel electrophoresis (BN-PAGE) immunoblot in mitochondria isolated from CD1 BMDMs stimulated with EC, HKEC or lipopolysaccharide (LPS) for 1.5h. (**c**) Glucose-driven OCR upon sequential treatment of oligomycin (olig.), CCCP, and rotenone+antimycin (Rot.+Ant.) of BMDMs treated or not with viable *E. coli* (EC) or heat-killed *E. coli* (HKEC) for 2h. (**d**) Extracellular lactate release by WT BMDMs treated with EC or HKEC for 2h. (**e**) Spectrophotometric mG3PDH activity in WT BMDM stimulated with viable EC or HKEC for 1.5h. (**f**) Spectrophotometric CII activity in permeabilized thioglycollate-elicited peritoneal macrophages (PEM) stimulated with viable EC or HKEC for 1h. (**g**) ROS production by WT BMDMs stimulated with EC or HKEC at the indicated multiplicity of infection (M.O.I.) for 15min. ROS production was monitored by chemiluminescence and expressed as relative light units per second (R.L.U./s). (**h**) Agarose gel electrophoresis of EC and HKEC total RNA before and after treatment with RNases III and A/T1. (**i**) IL-6 cytokine levels in supernatants of WT BMDMs treated with the indicated TLR ligand. (**j**) *Ifnb* mRNA levels in WT BMDMs stimulated with poly(I:C) for the indicated time point. (**k**) Spectrophotometric CII activity in permeabilized WT, *Trif*$^{-/-}$, *Mavs*$^{-/-}$, *Trif*$^{-/-}$*Mavs*$^{-/-}$ and Sting deficient (*Tmem173*^{gt}, here called *GT-Sting*) BMDMs stimulated with viable E. coli (EC) for 1.5h. (**l**) BNGE analysis from permeabilized WT, *Trif*$^{-/-}$, and *Trif*$^{-/-}$*Myd88*$^{-/-}$ BMDMs stimulated with EC for 1.5h. Arrows indicate the main SCs affected. Densitometry analysis of the SCI+III2 vs CII is shown on the right panel. (**m**) Representative BNGE analysis from permeabilized WT, *Caspase1*$^{-/-}$x*Caspase11*$^{-/-}$ (*Casp1*$^{-/-}$*Casp11*$^{-/-}$) and *Nlrp3*$^{-/-}$ BMDMs stimulated with EC for 1.5h. Arrows indicate the main SCs affected. NS, not significant; **P* < 0.05; ****P* < 0.001 (two-tailed unpaired Student's *t*-test or one-way ANOVA followed by Tukey post-test analysis (**a**)). Data are from three (**a, d, i, k**), four (**e, f**) and five (**g**) independent experiments performed in duplicates. Data in (**c**) present mean and s.d. of one representative experiment performed in six technical replicates. Data in (**b, l, j, m**) are from one representative of three independent experiments with similar results.