SUPPLEMENTAL MATERIAL

Supplemental Methods

Bacterial infection

BMDM were seeded in 24-well culture plates, cultivated in RPMI medium (11875085, Thermo Fisher Scientific), and infected with *E. coli* HB101 (MOI 100) [17]. One hour post-infection, cells were washed twice with PBS and incubated in medium containing 100 µg/mL gentamicin (Sigma; #G1264) for two additional hours. Where indicated, 40 mM NaCl or mitochondrial inhibitors were added to the medium. Subsequently, the infected cells were lysed in PBS containing 0.1% Triton-X (VWR; 28880.293) and 0.05% Tween 80 (Merck; #8.22187.0500). Bacterial survival was assessed by plating serial dilutions on Müller-Hinton-II agar plates and counting colony-forming units (CFUs) after one day of incubation.

Griess Assay

Griess Assay was performed with supernatants of BMDM. Supernatants were diluted with Griess Reagents I (1% (w/v) sulfanilamide (S9251, Sigma by Merck) and 5% (v/v) H3PO4 in water) and II (1mg/mL N-(α-naphthyl-)ethylenediamine dihydrochloride (N9125, Sigma by Merck) in water) at a ratio of 2:1:1. Absorbance was measured at 550 nm on a Spectra Fluor plate reader (Tecan). NO2- concentrations were quantified by parallel measurement of a NaNO2 standard curve.

RNA Purification, Preparation of cDNA and Quantitative Real-Time Polymerase Chain Reaction

Total RNA was isolated from BMDM with QIAzol lysis reagent (79306, Qiagen) and the RNeasy Mini Kit (74106, Qiagen). Samples were treated with DNase (79254, Qiagen), according to the manufacturer's instructions, and eluted in RNase/DNase-free water. RNA quality and concentration were measured with the NanoDrop Spectrophotometer ND-1000 from PeqLab. The High Capacity cDNA Reverse Transcription Kit (4368813, Applied Biosystems by Thermo Fisher Scientific) was used to reverse transcribe RNA into cDNA according to the manufacturer's protocol.

Quantitative real-time polymerase chain reaction (qPCR) was performed as TaqMan assay (4367846, Applied Biosystems by Thermo Fisher Scientific) with sequence specific DNA probes or as SYBR green assay (4385614, Applied Biosystems by Thermo Fisher Scientific). Progression of qPCR was detected with the QuantStudio 3 Real-Time PCR System (Applied Biosystem by Thermo Fisher Scientific) and analysed with the QuantStudio Design & Analysis Software v1.4.3 (Applied Biosystems by Thermo Fisher Scientific). Gene expression was normalized to expression levels of the ribosomal RNA gene 18S. Species specific primers and probes were purchased from BioTeZ Berlin-Buch GmbH and are listed below:

18S
F: ACATCCAAGGAAGGCAGCAG
R: TTTTCGTCACTACCTCCCCG
P: CGCGCAAATTACCCACTCCCGAC
Arg1
F: CCACAGTCTGGCAGTTGGAA

R: GCATCCACCCAAATGACACA

P: TGGCCACGCCAGGGTCCAC

Ccl5

F: GCAGTCGTGTTTGTCACTCGAA

R: GATGTATTCTTGAACCCACTTCTTCTC

P: AACCGCCAAGTGTGTGCCAACCC

Chil3

F: TCCTACTGGAAGGACCATGGAGCA

R: TCCTGGTGGGCCAGTACTAATTGT

Cox2

F: CAGGTCATTGGTGGAGAGGTGTA

R: GGATGTGAGGAGGGTAGATCATCT

P: CCCCCCACAGTCAAAGACACTCAGGT

Il6

F: GTTGCCTTCTTGGGACTGATG

R: GGGAGTGGTATCCTCTGTGAAGTCT

P: TGGTGAGAACCACGGCCTTCCC

Irf4

F: CGGGCAAGCAGGACTACAA

R: TCGGAACTTGCCTTTAAACAATG

Irf5

F: CTTGGCCCATGGCTCCTGCC

R: AGCAACCGGGCTGCAACAGG

Ldha

F: ATGAAGGACTTGGCGGATGA

R: ATCTCGCCCTTGAGTTTGTCTT

Mgl2

F: GAGACAGACTTGAAGGCCTTGAC

R: GCCACTTCCGAGCCATTG

Mrc1

F: AATACCTTGAACCCATTTATCATTCC

R: GCATAGGGCCACCACTGATT

P: CGATGTGCCTACCGGCTGCCC

Nlrp3

F: GGAGTCTAGCAGACCTGATTGTCA

R: GGCTTGCGCAGGATCTTG

P: CTGCTGGCCTGACCCAAACCCA

Nos2

F: GGGCAGCCTGTGAGACCTT

R: TGCATTGGAAGTGAAGCGTTT

P: TCCGAAGCAAACATCACATTCAGATCCC

Pcx
F: TTGCCAAGCAGGTAGGCTAT
R: TGGATCTGAGCATGGACCAG
Pdha1
F: ATGGTGCTGCTAATCAGGGT
R: CATGCCATAGCGGTTGTTCT
Retnla1
F: CGTGGAGAATAAGGTCAAGGAACT
R: CACTAGTGCAAGAGAGAGTCTTCGTT
P: TTGCCAATCCAGCTAACTATCCCTCCACTG
Slamf1
F: TGGCTAATGGATCCCAAAGGA
R: CCATCACACCTCCACCTGTT
Sdha
F: TGCCTTGCCAGGACTTAGAA
R: GCAACAGGTGCGTATCTCTC
Tnf
F: GGTCCCCAAAGGGATGAGAA
R: TGAGGGTCTGGGCCATAGAA
P: TTCCCAAATGGCCTCCCTCTCATCA

Human macrophages were lyzed in RLT buffer (79216, Qiagen) containing β-mercaptoethanol and RNA was isolated from the lysates using the RNeasy micro Kit (74004, Qiagen). RNA was reversely transcribed using the qScript cDNA SuperMix (95048 Quanta Biosciences). Quantitative real-time PCR was performed with the Taqman system (Applied Bioscience) according to manufacturer's instructions. Samples were measured on the Step ONE Plus RT-PCR machine (Applied Biosciences). The following probes were used: GAPDH: Hs99999905_m1, IL-8: Hs00174103_m1, TNFα: Hs00174128_m1, Ccl18: Hs00268113_m1, ALOX15: HS00993765_g1, Ccl17: Hs00171074_m1, IL-6: Hs00174131_m1.

Determination of Intracellular ATP

Quantification of intracellular ATP of BMDM, human blood-derived monocytes and human macrophages was performed using the ATPlite Luminescence Assay System (6016941, PerkinElmer). Cell supernatant was discarded, cells lysed and luminescence measured according to the manufacturer's protocol with a FLUOstar OPTIMA reader (BMG Labtech) or a Spectra Fluor plate reader (Tecan).

Determination of Mitochondrial Membrane Potential

Mitochondrial membrane potential was determined by using the TMRE-Mitochondrial Membrane Potential Assay Kit (ab113852, Abcam).

BMDM and PBMC-derived monocytes were cultivated (and activated) as described above. During the last hour of stimulation, cells were incubated for 60 min at 37°C with MitoTracker Green FM (M7514, Invitrogen by Thermo Fisher Scientific) at a final concentration of 100nM and Hoechst (B2261, Sigma

by Merck) at a final concentration of 10µg/mL, both directly into cell culture medium. TMRE staining was performed during the last 20 min at 37°C at a concentration of 40nM into cell culture medium. Cells were then washed with PBS, detached (in case of BMDM) with 2mM EDTA in PBS on ice and analysed by flow cytometry (BD LSR-Fortessa using FACSDiva and FlowJo).

Mitochondrial membrane potential was also determined in isolated mitochondria. Therefore, 1x10e7 BMDM were lysed in the Miltenyi Biotec Mitochondrial Lysis buffer, supplemented with 5% (v/v) cOmplete Protease Inhibitor Cocktail (04693132001, Roche by Merck). Cells were lysed with a 30G needle on ice, magnetically labelled and mitochondria isolated using the Mitochondria Isolation Kit (130-096-946, Miltenyi Biotec) and the QuadroMacs Separator according to the manufacturer's instructions. Isolated mitochondria were re-suspended in a mitochondrial respiration buffer containing 180 mM sucrose (7651, Merck), 0.1 mM EDTA (E-4884, Sigma by Merck), 60 mM KCl (4936.1000, Merck), 10 mM K3PO4 (A442903, Merck), 12 mM K2CO3 (P5833-500G), 2mM K-lactate (60389-250ML-F), 2 mM Na-pyruvate (11360.039, Gibco), 2 mM K3-citrate (1548225-1G), 2mM K-2-oxo-glutaric acid (K2000-5G), 1 mM Na2-fumarate (8205840100), 2 mM Na2-succinate (W327700), 1 mM malate (46940-U), 1 mM glutamine (25030-081, Gibco by Thermo Fisher Scientific), 0.035 mM ADP (A5285-1G), 1 mM glycine, 1 mM alanine, 1 mM asparagine, 1 mM aspartic acid, 1 mM glutamate, 1 mM proline, and 1 mM serine (non-essential amino acids, 11140050, Gibco by ThermoFisher Scientific). They were then stained and analysed as described above for cells.

Human macrophages were analysed by fluorescence plate reading. In brief, macrophages were cultivated and stimulated in black 96 well plates with transparent bottom (655097, Greiner). After washing the cells, TMRE was applied at 80nM solved in 0.2% BSA/PBS for 30 minutes at 37°C. Wells were washed twice and measured at the FLUOstar OPTIMA reader (BMG Labtech) with the following settings: Ex-540nm, Em-590.

Intracellular Sodium Measurement

Human monocytes (10^6 cells) were stimulated with HS for 60 min, harvested and washed with iso-osmolal sucrose solution. The pellet was lysed as described earlier [Neubert et al. PlosONE 2020] and total Na^+ was determined by atomic absorption spectrometry (Thermo Scientific, iCE 3000 Series).

Transmission Electron Microscopy

BMDM were grown and stimulated as indicated above, washed with PBS, detached with 2mM EDTA in PBS and gentle scraping, and cell pellet fixed with 2.5% glutaraldehyde (G5882, Sigma by Merck) in PBS for 2h at 4°C. Tonicity during washes, detachment, and fixation was adjusted to +40mM NaCl for the respective high salt groups. The pellets were rinsed several times in the same buffer and postfixed in 1% osmium tetroxide (201030, Sigma by Merck) buffered in the respective buffer (normal salt or high salt) for 30 min at 4°C.

The material was subsequently dehydrated in an acetone series and embedded in epoxy resin using the EMbed-812 kit (E14121, Science Services). Series of silver to grey interference color ultra-thin sections (50-65 nm) were made with a diamond knife (Diatome Ultra 45°) mounted on a Leica Ultracut S ultramicrotome and placed on formvar covered single-slot grids. The sections were stained with 2% uranyle acetate (E22400, Science Services) and 2.6% lead citrate (E17810, Science Services) after

Reynolds in an automated TEM stainer (QG-3100, Boeckeler Instruments). Sections were examined and imaged under a ZEISS EM10CR with DITABIS imaging plates. Series of 20 to 30 sections of a single cell from each sample were recorded at higher magnification (12500 to 20000 times) and used for partial reconstruction of mitochondria. Images were aligned using IMOD and IMOD-align (Boulder Laboratories). Aligned TEM images were then used to reconstruct the mitochondrial network within a ±1.5µm thick segment of each cell using the software Amira (6.5.0).

Determination of Intracellular FAD

Quantification of intracellular FAD of BMDM and human monocytes was performed using the Flavin Adenine Dinucleotide (FAD) Assay Kit (ab204710, Abcam). Cells were cultivated and activated as described above. After washing with ice cold PBS, cells were lysed in the supplied FAD Assay Buffer, centrifuged for 5 min at 10000xg and 4°C, and supernatant deproteinized by filtering through 10kDa spin columns (Nanosep Centrifugal Devices with Omega Membrane 10K, OD010C34, Pall Life Science). Samples were then treated according to the kit's instructions and absorbance at 570 nm measured in kinetic mode on a Spectra Fluor plate reader (Tecan).

Electron Transport Chain Complex Assays

The activity of mitochondrial electron transport chain complexes was detected by using different kits.

Complex I activity was determined with the colorimetric Complex I Enzyme Activity Microplate Assay Kit (ab109721, Abcam). Cell supernatant was discarded, cells lysed with the supplied lysis buffer and protein concentration determined with the NanoDrop Spectrophotometer ND-1000 from PeqLab. Protein concentration was adjusted to 5mg/mL and 200 µL per well of the supplied 96-well plate were added and incubated for 3h at room temperature. Complex I NADH dehydrogenase was thereby bound to the plate. After thorough washing, the supplied reaction mix as well as increasing concentrations of NaCl (4mM, 1mM, and 0.25mM NaCl) were added to the plate. Finally, NADH was added to the mix and absorbance at 450 nm was measured in kinetic mode on a Spectramax 190 plate reader.

Similarly, Complex II activity was determined by immunoprecipitation with the Complex II Enzyme Activity Microplate Assay Kit (ab109908, Abcam). Cell and mitochondrion independent Complex II activity was assessed by incubating with succinate and ubiquinone according to the manufacturer's instructions and measuring absorbance at 600 nm in kinetic mode on a Spectra Fluor plate reader (Tecan). For high salt conditions, 4mM, 1mM, and 0.25mM NaCl were added to the reaction.

Complex II/III activity was assessed using the MitoTox Complex II + III OXPHOS Activity Assay Kit (ab109905, Abcam) according to the manufacturer's instructions. Activity solution was mixed with increasing concentrations of NaCl (serial dilutions from 64mM to 0.0625mM NaCl) or Antimycin A as positive control (serial dilutions from 352nM to 0.3438nM). Bovine heart mitochondria were added and absorbance at 550nm was measured in kinetic mode on a Spectramax 190 plate reader. Complex II/III activity was calculated relative to the solvent control (water or DMSO, respectively). Opposed to the Complex I and Complex II assays, this assay was performed in intact (bovine) mitochondria.

Complex IV activity was assessed with the MitoTox Complex IV OXPHOS Activity Assay Kit (ab109906, Abcam). Intact (bovine) mitochondria were incubated with reduced cytochrome C and absorbance at

550nm was measured in kinetic according to the manufacturer's instructions on a Spectramax 190 plate reader.

Determination of Intracellular NAD and NADH

Quantification of intracellular total NAD and NADH of BMDM was performed using the NAD/NADH colorimetric assay kit from Abcam (ab65348). Cell supernatant was discarded, cells lysed and lysate split into two equal parts. One part was heated at 60°C for 30 min, in order to decompose all NAD+. Absorbance of both parts (incubated with the provided reaction mix) was measured at 450 nm according to the manufacturer's protocol on a Spectramax 190 plate reader. The assay is based on the conversion of NAD+ to NADH by the developer added. Thereby, NADH is detected in the heated half of the lysate (where no NAD+ is present), whereas total NAD is detected in the untouched half. The amount of NAD+ can be calculated as the difference between total NAD and NADH.

Transwell assay

Human macrophages were seeded in 24 well plates and stimulated as indicated for 24 hours. Transwell inserts with a pore size of 3 µm (662630, Greiner) were inserted and 2x10e5 CD4+ T cells loaded in the insert. After 24h incubation time cells were harvested and subjected to flow cytometry analysis. CD4+ T cells were sorted by using the EasySep CD4+ T cell isolation kit (17952, Stemcell Technologies) according to manufacturer's protocol.

Human PBMC killing

PBMCs were obtained from healthy donors using density gradients and blood-derived monocytes were isolated as described above (ethical approval 20-1707-101; Ethikkommission an der Universität Regensburg). Cells were seeded in 24 well plates and infected with *E. coli* HB101 (MOI 100; as described above) for 1h ± 40 mM NaCl. Subsequently, extracellular bacteria were removed by washing with PBS and cells were incubated for 2h in 100 µg/ ml Gentamicin. Cells were lysed and serial dilutions containing intracellular bacteria were plated on Müller-Hinton II agar plates. CFU were counted and normalized to the mean of the normal salt group.

Supplemental Tables

Table I: Antibodies, bacteria, biological samples, chemicals, commercial assays, experimental models, and software used for this study, listed with their respective source and identifier.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
FITC-CD11b anti-mouse	BD Biosciences	553310
FITC-CD16, anti-human	Miltenyi biotec	130-106-703
PB-F4/80, anti-mouse	eBioscience by Thermo Fisher Scientific	48-4801-80
PE-Vio 770 CD14, anti-human	Miltenyi biotec	130-110-579
PerCP-Vio700 HLA-DR	Miltenyi biotec	130-095-291
Bacterial and Virus Strains		
E. coli HB101	[55]	N/A
Biological Samples		
Human peripheral blood mononucleated cells	In-house	N/A
Murine bone marrow cells	In-house	N/A
Chemicals, Peptides, and Recombinant Proteins		
Ammonium chloride	Merck	1145.0500
Adenosine diphosphate	Sigma by Merck	A5285
Adult horse serum	Cell Concepts	S-HEU03-I
Antimycin A	Sigma by Merck	A8674
BD FACSClean	BD Biosciences	340345
BD FACSFlow	BD Biosciences	342003
BD FACSRinse	BD Biosciences	340346
BD FACSSheath solution	BD Biosciences	336911
BD FACSShutdown solution	BD Biosciences	334224
Beta-mercaptoethanol	Sigma by Merck	M3148
bisBenzimide H 33342 trihydrochloride (Hoechst)	Sigma by Merck	B2261
Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP)	Sigma by Merck	C2920
Chloroform	Merck	102445
CM-H2 DCFDA	Invitrogen by Thermo Fisher Scientific	C6827
Complete protease inhibitor cocktail	Roche Diagnostics GmbH	1836145
Dimethyl malonate	Sigma by Merck	136441
DMEM, high glucose, with sodium pyruvate and L-glutamine	Gibco by Thermo Fisher Scientific	41966-029

DMEM, without glucose and sodium pyruvate, with L-glutamine	Gibco by Thermo Fisher Scientific	11966-025
DMEM, without glucose, L-glutamine and sodium pyruvate	Gibco by Thermo Fisher Scientific	A14430-01
Dimethylsulfoxide (DMSO)	Sigma by Merck	D4540
Ethylenediaminetetraacetic acid (EDTA)	Sigma by Merck	E-4884
Fast SYBR Green Master Mix	Applied Biosystems by Thermo Fisher Scientific	4385614
Fetal bovine serum	Biochrom by Merck	S0615
Fetal bovine serum	Biowest	S1400
Gentamicin	Sigma by Merck	G1264
Glucose (D(+)-)	Carl Roth	X997.2
Glucose (U-13C6 D-)	Cambridge Isotope Laboratories	CLM-1396-PK
Glutamine (L-)	Gibco by Thermo Fisher Scientific	25030-081
Glutamine (13C5 L-)	Cambridge Isotope Laboratories	CLM-1822-H-PK
Glutaraldehyde	Sigma by Merck	G5882
HEPES 1M	Gibco by Thermo Fisher Scientific	15630-056
IFNγ, human	Peprotech	300-02
IL-4, human	Immunotools	113040045
IL-4, mouse	R/D Systems by bio-techne	404-ML-010
IL-13, mouse	Invitrogen by Thermo Fisher Scientific	PMC0134
Isofluran CP	cp-pharma	1214
Lead citrate	Science Services	E17810
LPS from *E. coli* o111:B4	Sigma by Merck	L3024
LPS, ultrapure	Invivogen	tlrl-3pelps
M-CSF	Peprotech	300-25
Malic acid	Sigma by Merck	46940-U
Methanol	Carl Roth	HN41.2
Methoxyamine hydrochloride	Sigma by Merck	M6524
MitoTracker Green FM	Invitrogen by Thermo Fisher Scientific	M7514
N5,N6-bis(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (BAM15)	Sigma by Merck	SML1760-5MG

N-(α-naphthyl-)ethylenediamine dihydrochloride	Sigma by Merck	N9125
N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA)	Macherey-Nagel	701270.201
Non-essential amino acids	Sigma by Merck	M7145
Oligomycin A	Sigma by Merck	75351
Osmium tetroxide	Sigma by Merck	201030
PBS without $CaCl_2$ and $MgCl_2$	Sigma by Merck	D8537
Penicillin/Streptomycin	Gibco by Thermo Fisher Scientific	15140-122
Potassium 2-oxo-glutaric acid	Sigma by Merck	K2000
Potassium carbonate	Sigma by Merck	P5833
Potassium chloride	Carl Roth	6781.1
Potassium citrate	Sigma by Merck	1548225
Potassium lactate	Sigma by Merck	60389
Potassium phosphate	Sigma by Merck	P5629
Pyridine	Carl Roth	9729.3
QIAzol Lysis Reagent	Qiagen	79306
Rotenone	Sigma by Merck	R8875
RPMI medium	Gibco by Thermo Fisher Scientific	11875085
Seahorse XF base medium	Agilent	102363-100
Sodium bicarbonate	Merck	017 K13779929
Sodium chloride	Carl Roth	9265.1
Sodium Dodecylsulfate	Sigma by Merck	L4509
Sodium fumarate	Sigma by Merck	8205840100
Sodium pyruvate	Gibco by Thermo Fisher Scientific	11360070
Sodium succinate	Sigma by Merck	W327700
Sucrose	Sigma by Merck	S0389
Sulfanilamide	Sigma by Merck	S9251
TaqMan fast universal PCR master mix 2x	Applied Biosystems by Thermo Fisher Scientific	4367846
Trans-Cinnamic acid	Sigma by Merck	133760
Tris	Carl Roth	4855.2
Triton X-100	Sigma by Merck	T9284
Triton X-100	VWR	28880.293
Trypan blue 0.5%	PromoCell	PK-CA902-1209
Trypsin/EDTA	Sigma by Merck	59417C
Tween 80	Merck	8.22187.0500
Uranyl acetate	Science Services	E22400

X-VIVO 15 medium	Lonza	BE02-060F
Commercial Assays		
ATPlite Luminescence Assay System	PerkinElmer	6016941
Complex I Enzyme Activity Microplate Assay Kit	Abcam	ab109721
Complex II Enzyme Activity Microplate Assay Kit	Abcam	ab109908
EMbed-812 Kit	Science Services	E14121
EasySep CD14 Positive Selection kit II	Stemcell Technologies	17858
Flavin Adenine Dinucleotide (FAD) Assay Kit	Abcam	ab204710
High Capacity cDNA Reverse Transcription Kit	Applied Biosystems by Thermo Fisher Scientific	4368813
LIVE/DEAD Fixable Aqua Dead Cell Stain kit, for 405 nm excitation	Invitrogen by Thermo Fisher Scientific	L34966
Mitochondria Isolation Kit	Miltenyi Biotec	130-096-946
MitoTox Complex II + III OXPHOS Activity Assay Kit	Abcam	ab109905
MitoTox Complex IV OXPHOS Activity Assay Kit	Abcam	ab109906
NAD/NADH Assay Kit (Colorimetric)	Abcam	ab65348
Pan Monocyte Isolation Kit human	Miltenyi Biotec	130-096-537
qScript cDNA SuperMix	Quanta Biosciences	95048
RNase-free DNase Set	Qiagen	79254
RNeasy micro Kit	Qiagen	74004
RNeasy Mini Kit	Qiagen	74106
Seahorse XFe96 FluxPak	Agilent	102416-100
TMRE-Mitochondrial Membrane Potential Assay Kit	Abcam	ab113852
Experimental Models: Cell Lines		
L-929, NCTC clone 929, strain C3H/An	ATCC by LGC Standards	ATCC CCL-1
Experimental Models: Organisms/Strains		
C57BL/6J mice, wild type, 9 to 12-week old, male	Harlan Laboratories	
C57BL/6J mice, wild type, 9 to 12-week old, male	Charles River	
Software and Algorithms		
BD FACSDiva	BD Biosciences	
ChromaTOF 5.0	LECO Corporation	
FlowJo 10.5.3	Tree Star by BD Biosciences	

MAUI-SILVIA	Proteomics and Metabolomics Platform, Max-Delbrück Center for Molecular Medicine Berlin	
Primer Express version 3.0	Applied Biosystems by Thermo Fisher Scientific	
Prism Version 8	GraphPad	
QuantStudio Design&Analysis Software v1.4.3	Applied Biosystems by Thermo Fisher Scientific	
R 3.5.1	GNU General Public License	
RStudio	GNU General Public License	
Wave 2.6.0.31	Agilent Technologies	

Supplemental Figures

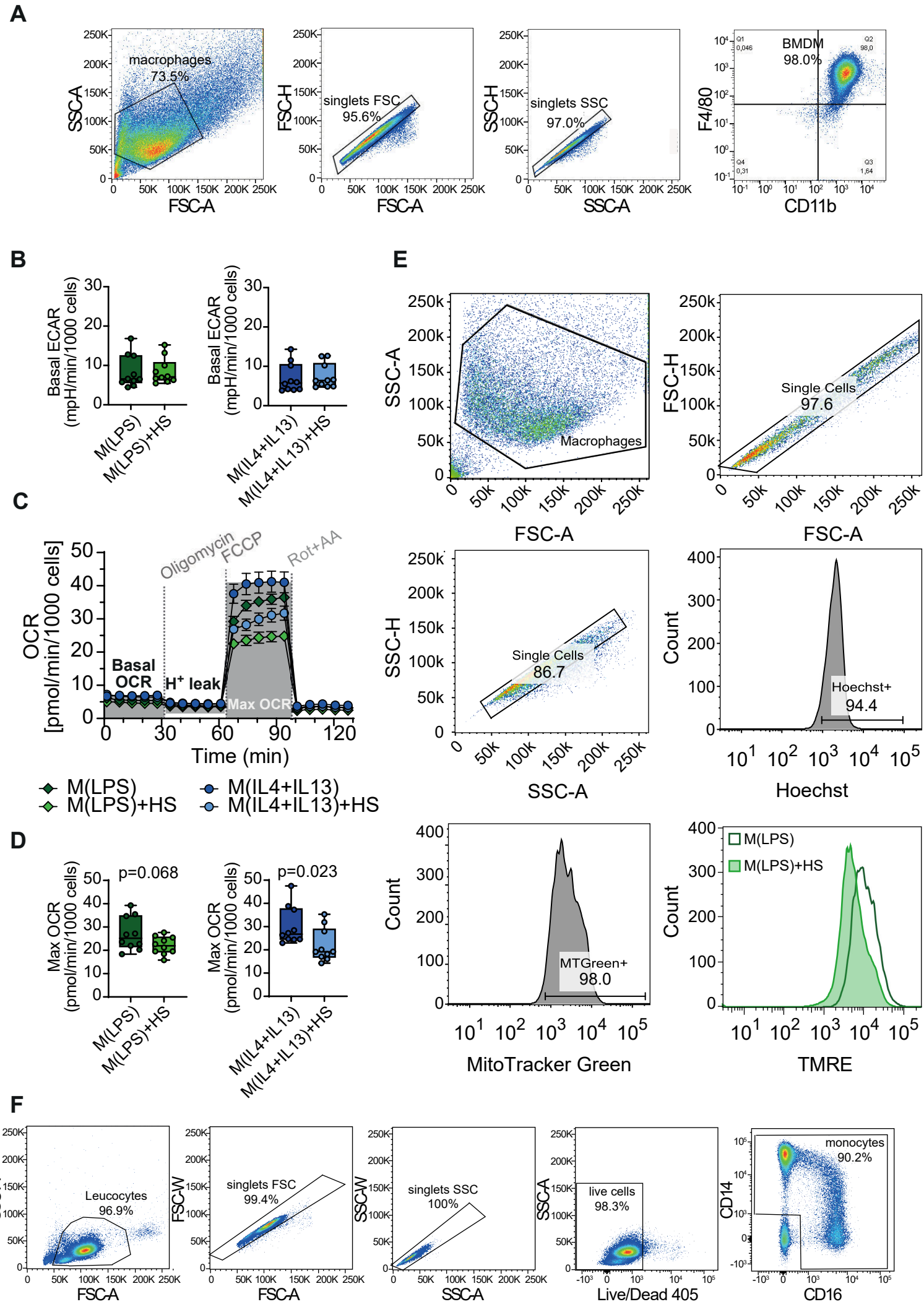

Figure I, A Representative flow cytometric staining as purity control for F4/80^{+}, CD11b^{+} murine BMDM. **B-D,** BMDM were treated for 3h with LPS or IL4+IL13, under normal salt (NS) or HS conditions. **B,** Basal extracellular acidification rate (ECAR) (pooled data n=10 from 3 independent experiments). Data is depicted as box and whisker with min to max. Significance was analyzed by Mann-Whitney test. **C,** Representative mitochondrial stress test, showing oxygen consumption rate (OCR) over time. **D,** Maximal OCR as in (**B**). Significance was analyzed by unpaired two-tailed t-test for M(LPS) and by Mann-Whitney test for M(IL4+L13). **E,** Representative flow cytometric staining of TMRE in MitoTracker Green^{+}, Hoechst^{+} murine BMDM. **F,** Representative flow cytometric staining as purity control for CD14^{high}CD16^{low}, CD14^{high}CD16^{high}, and CD14^{low}CD16^{high} live human monocytes.

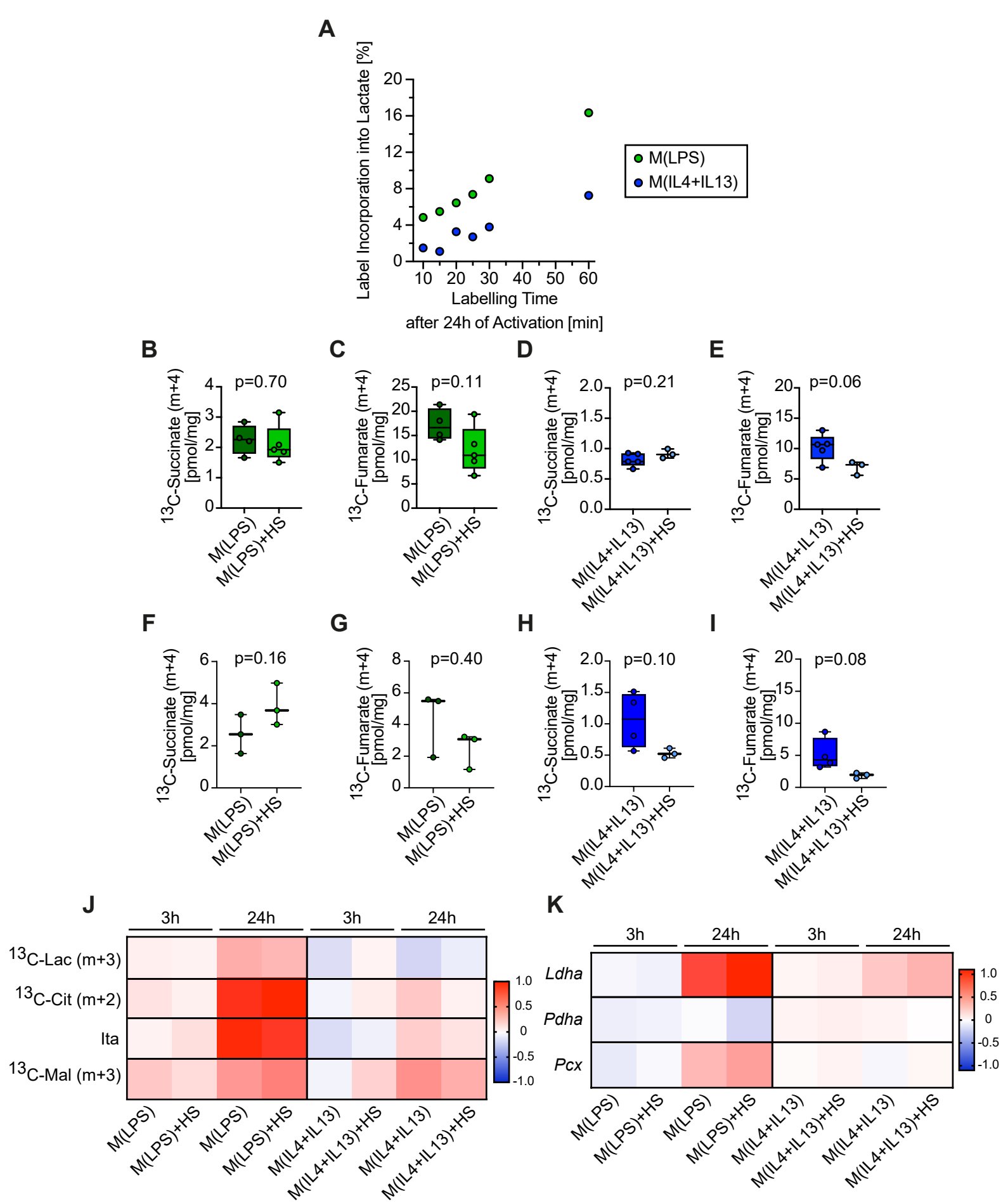
A
Label Incorporation into Lactate [%]
Labelling Time
after 24h of Activation [min]
M(LPS)
M(IL4+IL13)
B
C
D
E
F
G
H
I
13C-Succinate (m+4) [pmol/mg]
13C-Fumarate (m+4) [pmol/mg]
p=0.70
p=0.11
p=0.21
p=0.06
p=0.16
p=0.40
p=0.10
p=0.08
M(LPS)
M(LPS)+HS
M(IL4+IL13)
M(IL4+IL13)+HS
J
K
3h
24h
13C-Lac (m+3)
13C-Cit (m+2)
Ita
13C-Mal (m+3)
Ldha
Pdha
Pcx

Figure II, A Glucose-derived ^{13}C-label incorporation into Lac in 24h-treated M(LPS) and M(IL4+IL13) macrophages in dependency of the U13C6-Glc labelling duration (10-60 min). **B-I,** BMDM were treated for 3h (**B-E**) or 24h (**F-I**) with LPS or IL4+IL13, under normal salt (NS) or HS conditions. ^{13}C-glutamine labeling was performed during the last hour of activation. Data (n=5 each) is depicted as box and whisker with min to max. Significance was analyzed by unpaired, two-tailed *t*-test for B-F, H, I or Mann-Whitney test for G. **B, D, F, H,** ^{13}C-glutamine derived succinate (m+4). **C, E, G, I,** ^{13}C-glutamine derived fumarate (m+4). **J, K,** Heatmaps showing relative quantities of ^{13}C-glucose derived ^{13}C-Lac, ^{13}C-Cit, total Ita, and ^{13}C-Mal (**J**), as well as relative gene expression of *Ldha*, *Pdha*, and *Pcx* (**K**) in BMDM activated for 3h and 24h with LPS or IL4+IL13, under NS or HS conditions, in relation to untreated M0 macrophages (n=6 for each time point and group). ^{13}C-glucose labeling was performed during the last hour of activation.

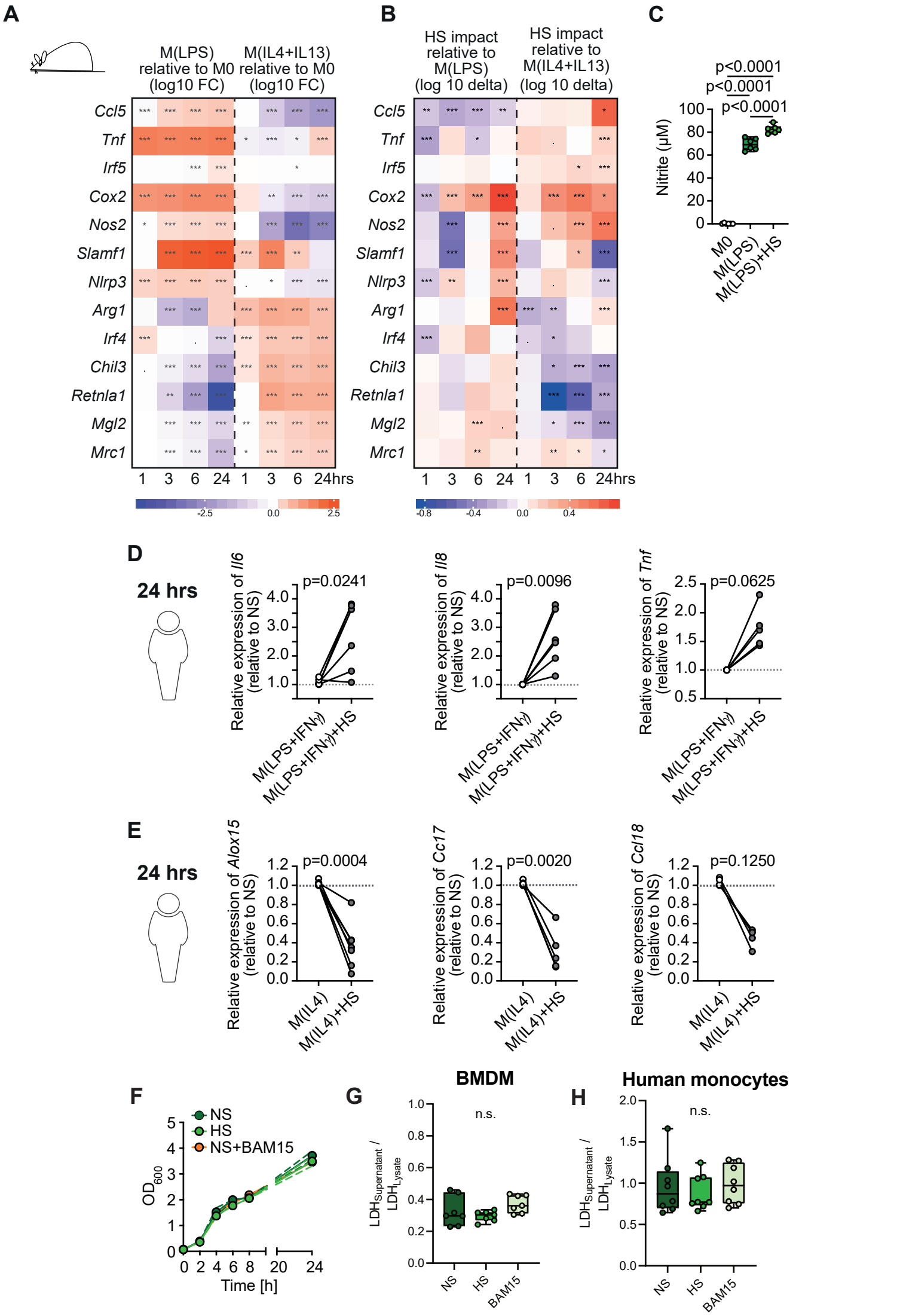
A
M(LPS) relative to M0 (log10 FC)
M(IL4+IL13) relative to M0 (log10 FC)
Ccl5
Tnf
Irf5
Cox2
Nos2
Slamf1
Nlrp3
Arg1
Irf4
Chil3
Retnla1
Mgl2
Mrc1
1 3 6 24 1 3 6 24hrs
-2.5 0.0 2.5
B
HS impact relative to M(LPS) (log 10 delta)
HS impact relative to M(IL4+IL13) (log 10 delta)
1 3 6 24 1 3 6 24hrs
-0.8 -0.4 0.0 0.4
C
p<0.0001
p<0.0001
p<0.0001
Nitrite (µM)
M0
M(LPS)
M(LPS)+HS
D
24 hrs
Relative expression of Il6 (relative to NS)
p=0.0241
M(LPS+IFNγ)
M(LPS+IFNγ)+HS
Relative expression of Il8 (relative to NS)
p=0.0096
Relative expression of Tnf (relative to NS)
p=0.0625
E
24 hrs
Relative expression of Alox15 (relative to NS)
p=0.0004
M(IL4)
M(IL4)+HS
Relative expression of Cc17 (relative to NS)
p=0.0020
Relative expression of Ccl18 (relative to NS)
p=0.1250
F
NS
HS
NS+BAM15
OD600
Time [h]
G
BMDM
n.s.
LDHSupernatant / LDHLysate
NS
HS
BAM15
H
Human monocytes
n.s.
LDHSupernatant / LDHLysate
NS
HS
BAM15

Figure III, A, B, relative gene expression of M1 marker genes *Ccl5*, *Tnf*, *Irf5*, *Cox2*, *Nos2*, *Slamf1*, *Nlrp3*, and M2 marker genes *Arg1*, *Irf4*, *Chil3*, *Retnla1*, *Mgl2*, *Mrc1* normalized to *18S* in murine BMDM activated for 1h, 3h, 6h, and 24h with LPS or IL4+IL13 under NS or HS conditions (pooled data n=12 from 2 independent experiments). **A,** heatmap displaying the impact of LPS or IL4+IL13 activation over time relative to untreated M0 BMDM (as logarithmic fold change (FC)). A nested model test, treating HS treatment as covariate, was performed. **B,** heat map displaying the impact of HS relative to the NS equivalent at the same time point (as logarithmic delta). Kruskal-Wallis test with Mann-Whitney U post-hoc test, comparing normal salt to HS to each time point separately for each activation group was performed. FDR-correction was performed via Benjamini-Hochberg procedure, with · for q<0.1, * for q<0.05, ** for q<0.01, and *** for q<0.001. **C,** Nitrite in the supernatant of murine BMDM activated for 24h with LPS under NS or HS conditions, as well as untreated M0. Data (n=6, one out of two independent experiments) is depicted as box and whisker with min to max. Significance was analyzed by one-way ANOVA with Tukey's post-hoc test. **D,** Relative gene expression of *Il6*, *Il8*, and *Tnf* in human macrophages activated for 24h with LPS+IFNγ under NS or HS conditions (n=6 each for *IL6* and *Il8*, n=5 each for *Tnf*). **E,** Relative gene expression of *Alox15*, *Cc17*, and *Ccl18* in human macrophages activated for 24h with IL4 under NS or HS conditions (n=7 each for *Alox15*, n=5 each for *Ccl17* and *Ccl18*). Data in **D, E,** is shown as donor-paired mean and was analyzed by paired, two-tailed t-test (*Il6, Il8, Alox15, Ccl17*) or Wilcoxon matched-pairs signed rank two-tailed test (*Tnf, Ccl18*). **F**, Representative *E. coli* growth under NS, HS, and NS+BAM15 treatment. **G, H,** Representative cell viability of murine BMDM (**G**) and human monocytes (**H**) under NS, HS, and NS+BAM15 treatment, determined by the ratio of LDH in the supernatant and intracellular LDH.

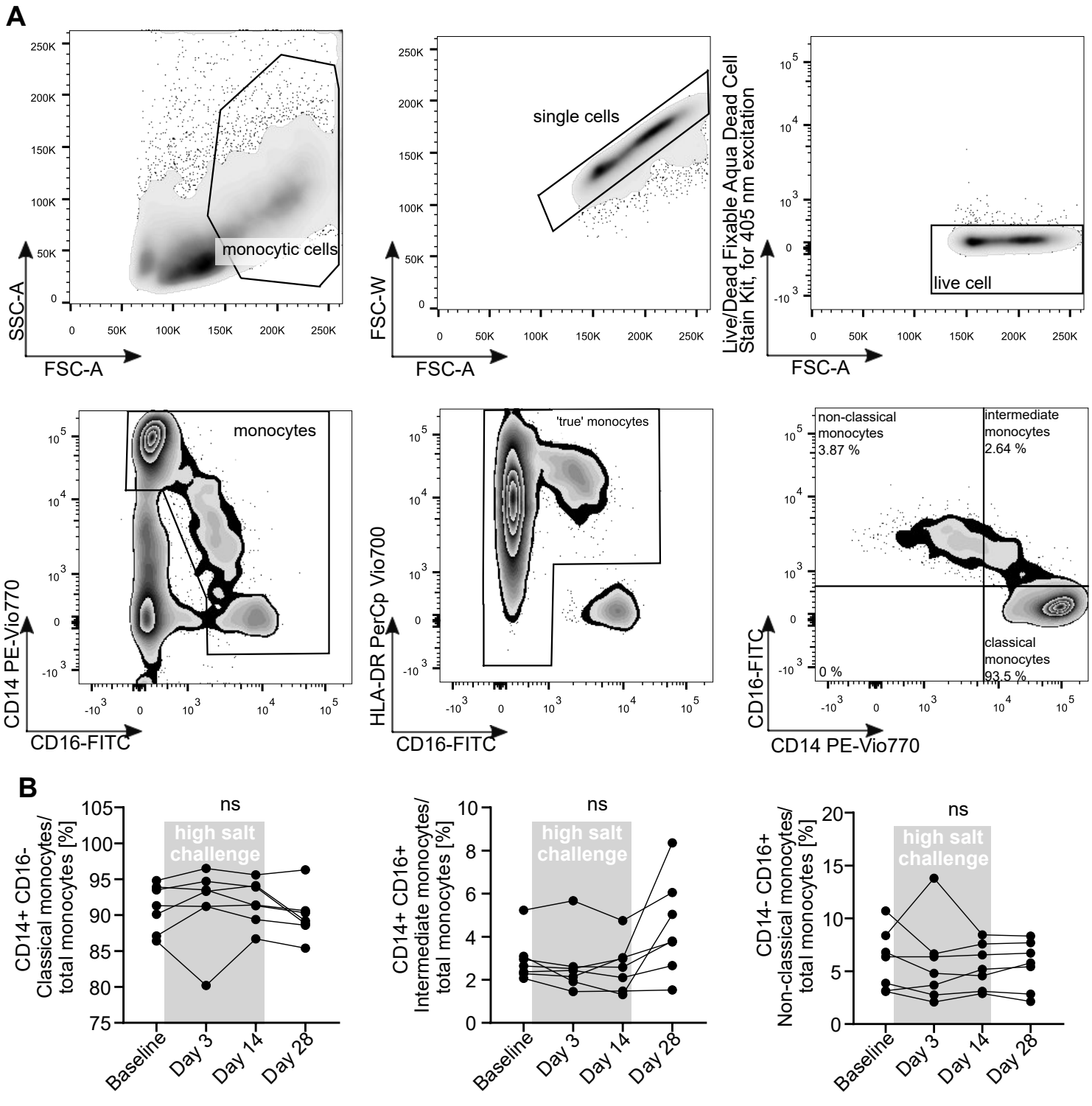
A
SSC-A
FSC-A
monocytic cells
FSC-W
single cells
Live/Dead Fixable Aqua Dead Cell Stain Kit, for 405 nm excitation
live cell
CD14 PE-Vio770
CD16-FITC
monocytes
HLA-DR PerCp Vio700
'true' monocytes
non-classical monocytes 3.87 %
intermediate monocytes 2.64 %
classical monocytes 93.5 %
0 %
B
CD14+ CD16- Classical monocytes/ total monocytes [%]
CD14+ CD16+ Intermediate monocytes/ total monocytes [%]
CD14- CD16+ Non-classical monocytes/ total monocytes [%]
ns
high salt challenge
Baseline
Day 3
Day 14
Day 28

Figure IV, A, B, Healthy volunteers underwent an intervention study and consumed 6g of salt additionally to their habitual diet for 14 days. Monocytes were analyzed by flow cytometry at four different time points: baseline, after 3 days and 14 days on high salt diet, and 14 days after the end of the intervention (day 28). **A,** representative gating strategy in PBMCs for the different monocyte subsets (identified by CD14, CD16, and HLA-DR). **B,** monocyte subsets analyzed are classical monocytes ($CD14^+$ $CD16^-$), intermediate monocytes ($CD14^+$ $CD16^+$) and non-classical monocytes ($CD14^-$ $CD16^+$). No significant (ns) differences were detected by repeated measures ANOVA.