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Supporting Information for

HDAC7 is an immunometabolic switch triaging danger signals for engagement of antimicrobial versus inflammatory responses in macrophages

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Supplementary Materials and Methods

Chemicals and Reagents

The glycolytic inhibitor 2-Deoxy-D-glucose (2-DG) (Cat – D8375, Sigma-Aldrich) was dissolved in DMSO and used at a final concentration of 5 mM. The PKM2 activator DASA (Cat #550602) was purchased from Calbiochem, resuspended in DMSO and used at a final concentration of 10 mM. The NOX2 inhibitor GSK2795039 (Cat - HY-18950), as well as the PPP inhibitors 6-Aminonicotinamide (6-AN) and Polydatin (Cat - HY-W010342 and Cat - HY-N0120A) were purchased from MedChemExpress, dissolved in DMSO and used at the indicated concentrations. The metabolites ribulose-5-phosphate and 6-phosphogluconate (Cat – 83899 and Cat – P7877) were purchased from Sigma-Aldrich, dissolved in water and used at the concentrations indicated in individual figure legends. The TLR4 agonist LPS (from *Salmonella enterica* serotype Minnesota) was purchased from Sigma Aldrich (Cat – L2137) and dissolved in complete RPMI media. The TLR1/2 agonist Pam_3CSK_4 was purchased from Merck (Cat – 506350) and dissolved in water before use at the desired concentration.

Preparation of inhibitors of Class IIa HDACs

The selective class IIa HDAC inhibitor TMP195 (1) was synthesized in-house. Purity was determined by HPLC (> 98%), with post characterization and confirmation by ^{1}H and ^{13}C NMR spectroscopy and high-resolution mass spectrometry. TMP195 was used in cell culture experiments at concentrations of up to 10 μM, as indicated in individual figure legends.

Preparation of LPS-coated latex beads

Polystyrene latex beads (3 mm mean particle size) (Cat – LB30, Sigma Aldrich) were incubated with 1 μg/ml LPS (or media alone) overnight at 4 ^{0}C. The beads were then washed with media 15 times and resuspended in media for further use as latex-coated beads.

Bacterial culture

All bacterial strains were cultured at 37 ^{0}C on solid or liquid Luria-Bertani (LB) medium. The non-pathogenic *E. coli* K12 strain MG1655 (2), MG1655_mCherry and EC958, a representative strain of the globally-disseminated multidrug-resistant ST131 clone isolated from the urine of a patient with a urinary tract infection (3, 4), were used in this study, as was a mutant of EC958 that is susceptible to oxidative stress (Δ*katG*). This EC958 mutant was generated using l-Red-mediated homologous recombination, as previously described (5, 6). The primers used to delete *katG* from EC958 are listed in Table S3. Overnight cultures of *E. coli* MG1655 were routinely grown under shaking conditions, while cultures were grown statically to induce type I fimbriae production as previously described (3). Before infection, bacteria were washed twice and resuspended in macrophage infection medium (described below). Optical density at 600 nm (OD_{600}) was measured and bacterial suspensions were diluted to OD_{600} = 0.6, which corresponds to ~10^9 cfu/ml for both MG1655 and EC958. To assess the effect of inhibitors of class IIa HDACs on bacterial growth, bacteria were incubated with the indicated concentration of inhibitors for 5-12 h at 37 ^{0}C. To assess the effect of RL5P and 6PG on UPEC growth, *E. coli* strain EC958 was incubated with the indicated concentrations of these metabolites with or without the indicated concentrations of H_2O_2 for 12 h at 37 ^{0}C. Bacterial growth was assessed by monitoring OD_{600} using a POLARstar Omega (BMG Labtech) at 20-30 min intervals.

Mammalian cell culture

Bone marrow-derived macrophages (BMM) were generated from indicated strains of mice, as previously described (7). Bone marrow cells were cultured for 6 days in RPMI-1640 media (Thermo Fisher Scientific) supplemented with 10% FCS, 50 U/ml penicillin, 50 mg/ml streptomycin and 2 mM L-glutamine, in the presence of recombinant human colony-stimulating factor 1 (CSF-1), at either 1 x 10^4 U/ml (Chiron) or 150 ng/ml (The University of Queensland Protein Expression Facility). On day 6, BMM were harvested and replated at an appropriate density with fresh CSF-1, with cells being used in experiments on the following day. CD14+ human monocytes, which were purified from buffy coats provided by the Australian Red Cross Blood Service with approval from the University of Queensland Human ethics committee (2013001519), were differentiated into human monocyte-derived macrophages (HMDM) by culturing for 7 days in IMDM containing 10% FCS, 50 U/ml penicillin, 50 mg/ml streptomycin, 2 mM L-glutamine and recombinant human CSF-1 (as above). No donor identity was provided, and it is presumed that there were equal or similar numbers of male and female donors. Each data point in figure panels displaying HMDM experiments represents a different donor. THP-1 cells (ATCC, TIB-202) were cultured in RPMI-1640 media supplemented with 10% FCS, 50 U/ml penicillin, 50 mg/ml streptomycin, 2 mM L-glutamine, 1% HEPES (Thermo Fischer Scientific) and 1% sodium pyruvate. RAW 264.7 cells stably transfected with a mammalian expression vector encoding murine HDAC7 (8) were cultured in RPMI-1640 media supplemented with 5% FCS, 50 U/ml penicillin, 50 mg/ml streptomycin and 2 mM L-glutamine. PlatE cells, used for generating retrovirus as well has HEK293T cells, were cultured in DMEM media supplemented with 10% FCS, 50 U/ml penicillin, 50 mg/ml streptomycin. For all infection assays (see below), cells were cultured in IMDM (Thermo Fisher Scientific) supplemented with 10% FCS. All cells were cultured at 37 ^{0}C and 5% CO_2, unless otherwise indicated.

Cytokine analysis

BMM (5 x 10^5 cells/ml) or HMDM (5 x 10^5 cells/ml) were treated for 4 h with TLR agonists and/or phagocytic stimuli or infected for 4 h with EC958 (MOI 100), as indicated in individual figure legends. Where necessary, specific pharmacological reagents (2-DG, DASA, Polydatin, 6-Aminonicotinamide, GSK2795039, RL5P or TMP195) were added 1 h prior to macrophage stimulation (or otherwise specified), as indicated in individual figure legends. Supernatants were collected and assessed for secreted TNF. To assess secreted IL-1β by ELISA, cells were further treated with nigericin for 1 h (2 h for HMDMs). Supernatants were collected and analysed for levels of IL-1β. ELISA was performed on culture supernatants as per manufacturer's instructions (human IL-1β: Cat – DY201 and mouse IL-1β: DY401; R&D Systems, human TNF: Cat – 88-7346-77; Thermo Fisher Scientific, mouse TNF: Cat – 558534; BD OptEIA, human IL-6: Cat – 554543 and 554546 and mouse IL-6: Cat – 554400 and 554402; BD Pharmingen). For experiments comparing macrophages derived from mice of different genotypes, MTT assays were performed in parallel to control for any differences in plating densities.

Inflammasome activation assays

Indicated BMM populations (5 x 10^5 cells/ml) were plated in low-serum containing Opti-MEM media. Cells were treated with LPS (0.5 ng/ml) or infected with EC958 (MOI 100) for 4 h, followed by 1 h treatment with nigericin (5 μg/ml) (Cat – N7143, Sigma Aldrich). Percentage cell death was assessed using LDH assays, as described below. Secreted proteins from culture supernatants were mixed with methanol and chloroform (3:1) and centrifuged at maximum speed to isolate the middle protein layer. Proteins were further precipitated using 1.6 times methanol followed by centrifugation for 10 m at maximum speed. Total cell lysates were also

prepared in RIPA buffer containing a cocktail of protease and phosphatase inhibitors. Protein from both lysates and supernatants were then immunoblotted for pro- and cleaved IL-1β (Cat – 2275-PC-100, R&D Systems).

_In_ vitro infection assays

In vitro bacterial infections of primary macrophages and cell lines were carried out as previously described (9). Briefly, 4 x 10^5 cells were seeded overnight in antibiotic-free IMDM media. At 1 h prior to bacterial challenge, macrophages were treated with or without the indicated inhibitors. An MOI of 100 was used for both *E. coli* MG1655 and EC958, unless otherwise stated. At 1 h post-infection, cells were washed and maintained in medium containing 200 µg/ml gentamicin to exclude any extracellular bacteria for 1 h, after which, cells were washed with media again and maintained in medium containing 20 µg/ml gentamicin. At appropriate time points, cells were washed twice with PBS before being lysed in PBS containing 0.01% Triton X100. Diluted lysates were plated onto LB agar and incubated overnight at 37 ^{0}C. Numbers of colonies were counted to determine intracellular CFU.

6PGD enzyme activity assays

For loss-of-function assays, BMM (4 x 10^5 cells/ml) from indicated genotypes were infected with EC958 (MOI 100) for 2 h. Alternatively, HMDM or BMM were treated with or without the class IIa HDAC inhibitor, TMP195 (5 or 10 mM) for 1 h prior to infection. Cell lysates were then used to assess the activity of 6PGD, as per the manufacturer's instructions (Cat – ab241016, Abcam). Enzyme activity was normalized to the total protein present in cell lysates. For gain-of-function assays, HEK293T cells were transiently transfected with 1:1 molar ratio of each of the indicated constructs using Lipofectamine 2000 reagent. The total amount of DNA used per transfection was kept constant at 2 mg through the addition of empty vector. At 24 h post-transfection, cell lysates were prepared and 6PGD activity assays were performed, as described above.

Quantification of oxygen consumption rates and oxidative stress

A Seahorse Xfe96 Extracellular Flux Analyser (Agilent) was used for real time analysis of oxygen consumption rates (OCR) in cells. Briefly, 1 x 10^5 indicated BMM populations were seeded in XF96 cell culture plates overnight. The cells were washed and left for 1 h in unbuffered seahorse XF DMEM media containing glucose (10 mM), glutamine (2 mM) and pyruvate (1 mM) at 37 ^{0}C. Changes in OCR were measured post-*E. coli* addition (MOI 100) for a period of 1 h. To account for any differences in plating densities, MTT assays were performed in parallel and OCR values were normalized to MTT data. For quantifying oxidative stress as a read-out of ROS production, primary macrophages or cell lines (5 x 10^5 cells) were incubated overnight in antibiotic-free IMDM media. On the following day, cells were treated for 30 min with 10 µM CM-H_2DCFDA (Cat – C6827, Thermo Fisher Scientific), then challenged with *E. coli* (MOI 100) for 30 min. Cells were washed twice with PBS and then analysed by flow cytometry using a FACSCanto II (BD Biosciences).

Quantification of phagocytic uptake of bacteria

Phagocytic uptake of bacteria was assessed using fluorescently-labelled *E. coli* (Cat – P35366, Thermo Fisher Scientific), as previously described (10). Briefly, primary macrophages (5 x 10^5 cells) were pre-treated for 30 min with or without the indicated concentration of the class IIa HDAC inhibitor, TMP195. 100 µg of pHrodo bacterial particles were then added to the respective wells and incubated for 1 h, after which bacterial uptake was assessed by flow cytometry (FACSCanto II or LSRFortessa^{TM} Cell Analyser, BD Biosciences). Data were collected using FACSDiva software (BD Biosciences) and analysed using FlowJo (Version 10.1). As a negative control for phagocytosis, cells were pre-treated with 10 µM cytochalasin

D (Cat – PHZ1063, Thermo Fisher Scientific) to confirm that the signal was due to phagocytosis. Alternatively, primary macrophages were spin-fected with either *E. coli* MG1655 or EC958 for 5 min at 500 g at 35 ^{0}C, using a multiplicity of infection (MOI) of 100. The cells were then rested for 5 min before removing extracellular bacteria with gentamicin (200 μg/ml). Bacterial uptake at 20 min post-infection was then assessed by counting colony forming units (CFU), as described before.

Quantification of cell death by lactate dehydrogenase release assays

To assess cell death resulting from infection or inhibitor treatment, lactate dehydrogenase (LDH) release was measured colorimetrically using the CytoTox 96 Non-Radioactive Cytotoxicity Assay kit (Cat – G1780, Promega), as per the manufacturer's instructions. To determine total LDH release, cells were lysed using 0.1% Triton X-100, while spontaneous cell death was assessed in supernatants from untreated cells. Percentage cell death was determined as % cell death = [(Sample LDH release – Spontaneous LDH release)/(Total LDH release – Spontaneous LDH release)] x 100.

Gene silencing

THP-1 cells were differentiated with phorbol 12-myristate 13-acetate (PMA) (30 ng/ml) (Cat – P1585, Sigma Aldrich) for 48 h, then incubated at 2.5 x 10^6 cells/cuvette in 350 μl complete media supplemented with 10 mM HEPES and indicated siRNAs (Life Technologies) at a final concentration of 1 μM. Cells were electroporated at 260 V, 1000 μF and ∞Ω (Bio-Rad), rested for 5 min, then plated overnight at 37 ˚C. On the following day, 2 h prior to infection with indicated bacteria, media was removed, and cells were supplemented with fresh antibiotic-free IMDM media. Phagocytosis assays using pHrodo *E. coli* bioparticles were performed as described earlier, and qPCR was performed in parallel (see below) to confirm effective gene silencing. To silence *Pgd*, BMM were electroporated with a pool of siRNAs (Life Technologies) at a final concentration of 1 μM, using methods described above. At 24 h post-electroporation, cells were treated with LPS or infected with EC958 (MOI 100) for 4 h, followed by an additional 1 h of nigericin treatment. Supernatants were then assessed for cytokines by ELISA, as described earlier.

Retroviral transduction

Retroviral expression constructs for murine HDAC7 and enzyme-dead HDAC7ΔH649A (both with a C-terminal V5 tag), as well as PKM2 and PKM2-K433R (both with a N-terminal Flag tag), have previously been described (7). Murine PGD on pCMV3 backbone with a C-term Flag tag was purchased from Sino Biological (Cat – MG53165) and subsequently cloned into pMIGR backbone. Retroviral overexpression of target genes in primary macrophages was performed as previously described (7). Briefly, 2 x 10^6 PlatE cells were transfected with 40 μg of the respective retroviral expression vectors using Lipofectamine 2000 (Thermo Fisher Scientific). At 24 h post-transfection, media was replaced and cells were incubated at 32 ^{0}C for 48 h for optimal virus production. Viral supernatants were filtered through a 0.45 μM Millex-HV PVDF syringe filter (Merck) and supplemented with 1 M HEPES, 10 μg/ml polybrene (Cat – TR-1003-G, Merck) and CSF-1. Bone marrow progenitors treated with CSF-1 for 2 days were spin-infected (1000 *g*, 2 h, 35 ^{0}C) with viral supernatants in 6-well non-tissue culture plates. The cells were immediately supplemented with complete RPMI media, then incubated at 37 ^{0}C for 48 h. At 48 h post-transduction, cells were supplemented with complete RPMI media. On day 6 of macrophage differentiation, adherent cells were collected and plated for subsequent experiments.

Immunofluorescence microscopy
2 x 10^5 Mac-Hdac7 BMM on coverslips were spin-fected with *E. coli* MG1655_mCherry (MOI 100) for 5 min at 500 *g* at 35 ^{0}C, after which the cells were maintained at 37 ^{0}C for different timepoints. The cells were washed twice with PBS and then fixed using 4% paraformaldehyde, washed in PBS then permeabilised and incubated in blocking buffer (0.5% BSA in PBS). Primary antibody against V5 (Cat - MCA1360, Bio-Rad/AbD Serotec), DAPI (Sigma) and wheat-germ agglutinin-Alexa Fluor 647 (Invitrogen, W32466) were used to detect HDAC7-V5, DNA and cell boundary respectively. Coverslips were mounted on slides using IMBiol mounting media (made in-house) and cells were imaged at 63X using oil immersion with Carl Zeiss Meta Inverted LSM 510 microscope (Carl Zeiss) and processed using ImageJ. Excitation of DAPI, mCherry and Alexa-647 was achieved through laser emission at a wavelength of 405 nm, 561 nm, and 647 nm respectively. Manual quantification of bacterial numbers was performed across multiple images (minimum 26 cells per timepoint, per experiment) utilising Image J.

Gene expression analysis
Total RNA was extracted using RNA purification kits (Qiagen), as per the manufacturer's instructions. RNA was reverse transcribed to cDNA using Superscript III (Invitrogen) and oligo dT. Levels of specific mRNAs were quantified by qPCR using SyBR Green-PCR mix (Invitrogen) in the Applied Biosystems Viia 7 RT-PCR system. Appropriate negative controls with no Superscript III were included for all experiments. Data were expressed relative to the housekeeping gene hypoxanthine phosphoribosyltransferase (*HPRT*, human) using the ΔCt method (11).

Class IIa HDAC enzyme assays
BMM (1 x 10^5 cells/ml) were challenged with the *E. coli* strain EC958 (MOI 100) for the indicated periods of time. Cells were then lysed using a mild lysis buffer (50 mM Tris HCl, 150 mM NaCl, 1 mM EDTA, 1% NP40), without protease inhibitors. The class IIa HDAC-specific substrate (Ac-Leu-Gly-Lys(TFAc)-AMC) (12) (200 μM) was then added, after which samples were incubated for 30 min at 37 ˚C. A stop solution containing 20 mM SAHA (manufactured in house) and 1 mg/ml Trypsin (Sigma) was then used to quench reactions. Fluorescence was measured after excitation and emission at 350 nm and 460 nm, respectively, using TECAN plate reader.

Co-immunopreciptation and immunoblotting
HEK293T cells were transiently transfected with the indicated constructs using Lipofectamine 2000 reagent (Cat – 11668019, Thermo Fisher Scientific) as previously described (7). At 24 h post-transfection, whole cell lysates were prepared in RIPA buffer, containing a cocktail of 1X protease inhibitors (Roche) and 1X PhosSTOP phosphatase inhibitors (Sigma). V5-tagged proteins were immunoprecipitated and bound fractions, as well as whole cell lysates, were then immunoblotted for FLAG-tagged 6PGD or FLAG-tagged PKM2.

Rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME)
A detailed protocol for RIME has been described by others (13). RIME was performed as previously described (7). Briefly, Mac-Hdac7 BMM were cross-linked with formaldehyde before lysing the cells and subjecting the lysate to immunoprecipitation using Protein G magnetic beads coupled to anti-V5 antibody. Sequencing grade trypsin (Promega) was used to perform in-solution digest of bound proteins. Peptides were vacuum dried, reconstituted in 0.1% formic acid and analysed using LC-MS/MS (Shimadzu) coupled to a Triple TOF 5600 mass spectrometer (ABSCIEX). Protein Pilot v4.5 was used to identify the proteins against a

UniProt database. HDAC7-interacting partners were considered positive if at least one peptide was identified with over 99% confidence and was not present in the MacBlue control lysates.

Cell-free protein expression using the *Leishmania tarentolae* expression (LTE) system and protein-protein interaction analysis by AlphaLISA assay

AlphaLISA was performed as previously described (7). Briefly, N-terminal HDAC7 (23-504), C-terminal HDAC7 (498-938), full length 6PGD and full length PKM2 were sub-cloned into the Gateway cloning compatible LTE expression vectors (14). Each N-terminal and C-terminal truncated form of HDAC7 (labelled with C-terminal mCherry) was co-translated with N-terminally eGFP-tagged 6PGD or PKM2 in the LTE expression system. The cell-free co-expressed proteins were diluted 25 x in assay buffer (25 mM HEPES, 50 mM NaCl, 0.1% BSA and 0.01% Nonidet P-40) and incubated with the mCherry nanobody and AlphaLISA anti-GFP acceptor beads (Perkin Elmer) for 30 min at room temperature, followed by addition of streptavidin donor beads (Perkin Elmer). The samples were incubated for 30 min at room temperature under subdued light. The AlphaLISA signal was detected with a microplate reader (Tecan Spark ®). As a negative control for the assay, the FK506-binding protein (FKBP) (as a non-interacting partner of PKM2 or 6PGD) was co-translated with either N-term HDAC7-mCherry or C-term HDAC7-Cherry.

Analysis of central carbon metabolites

Central carbon metabolites were analyzed as previously described (15). 1 x 10^7 BMM from *Hdac7*$^{flox/flox}$/*LysM*^{Cre} (*Hdac7*$^{-/-}$) and *Hdac7*$^{flox/flox}$ littermate control mice (*Hdac7*$^{+/+}$) were infected with UPEC strain EC958 (MOI 100) for 30 min or 2 h. Cells were washed in cold PBS, then polar metabolites were extracted with 50% acetonitrile, using repeat cycles of freeze-thaw and sonication in a cold-water bath. Supernatants were collected after centrifugation at 4 ^{o}C for 10 min at 16,000 *g*. The supernatants were then diluted to 25% acetonitrile and frozen prior to freeze drying. Metabolites were then resuspended in 100 ml of aqueous 2% acetonitrile containing 5 μM a*zidovudine*. Central carbon metabolites were analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS). In brief, analyses were performed using a Dionex Ultimate 3000 HPLC system coupled to an ABSciex 4000 QTRAP mass spectrometer. Liquid chromatography was performed using a 50 min gradient with 300 ml/min flowrate, on a Phenomenex Gemini-NX C18 column (150 x 2 mm, 3 μm, 110 A), with a guard column (SecurityGuard Gemini-NX C18, 4 x 2 mm) and column temperature of 55 °C. The mobile phases used were: 7.5 mM aqueous tributylamine (Sigma-Aldrich) with pH adjusted to 4.95 (±0.05) using acetic acid (Labscan) for Solvent A, and acetonitrile (Merck) for Solvent B. Details of the chromatography gradient include 100% Solvent A (i.e. 0% B) from 0-8 min, 0-20% B from 8-20 min, 20-27 % B from 20-30 min, 27-100% B from 30-31 min, held at 100% B until 33 min, then back to 0% B from 34-50 min. Samples were kept at 4 °C in the autosampler and 10 μl were injected for analyses. The HPLC was controlled by Chromeleon 6.80 software (Dionex). Mass spectrometry was performed using a scheduled multiple reaction monitoring (sMRM) method on the negative ionisation mode. Other hardware parameter values were as follows: ion spray voltage -4500 V, ion source nebuliser (GS1), ion source auxiliary (GS2), curtain (CUR) and collision (CAD) gases were 60, 60, 20 and medium (arbitrary units), respectively, using ultra-high purity liquid nitrogen (BOC). The auxiliary gas temperature was kept at 350 °C. The mass spectrometer was controlled by Analyst 1.6.3 software (AB Sciex). Amounts obtained for each metabolite detected were based on standard curves from serial dilutions of analytical standards (Sigma), where L1 = 200000, L2 = 100000, L3 = 50000, ... L20 = 0.38 nM. This number of standard dilutions guaranteed that the standard curves consisted of at least five data points. Standard mix and pooled samples were regularly injected along the

run sequence for quality control. Collected data were processed using MultiQuant 2.1 (AB Sciex).

In parallel, gentamicin exclusion assays were performed to assess the number of intracellular bacteria at the different time points. To account for central carbon metabolites contributed from bacteria, 1 x 10^5 *E. coli* strain EC958 *E. coli* strain (equivalent to the approximate number of surviving intracellular bacteria, as determined by gentamicin exclusion assays) were also assessed for their metabolites. The absolute values of these metabolites were used for background subtraction to calculate the concentrations of metabolites derived from macrophages.

Statistical analysis

Statistical analyses were performed on data combined from three or more independent experiments for cell culture experiments (taking averages from replicates within each experiment) or two independent trials for *in vivo* studies (each mouse used as an individual data point). Statistical analyses were performed using Prism 8 software (Graph-Pad) with error bars indicating the standard error of the mean (SEM) or inter-quartile range. Data were analysed for normality using the Shapiro-Wilk normality test. Statistical analyses on normally distributed data sets were performed using parametric tests (t-test or ANOVA), whereas data sets with non-normal distributions were analyzed using nonparametric tests (Mann-Whitney t-test, Kruskal-Wallis' test or Friedman test). For experiments analysing matched samples or data, repeated measures (RM) ANOVA was performed. For data sets with three or more variables, a one-way or two-way analysis of variance (ANOVA) was performed followed by Tukey's, Dunn's, Dunnett's or Sidak's multiple comparison test. For data with two variables, unpaired t-tests were performed, with appropriate corrections (Giesser Greenhouse correction for ANOVA, and Welch's correction for t-test). Statistical tests used for individual experiments are described in the figure legends. Differences with confidence values of 95% ($P < 0.05$) were considered statistically significant.

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SUPPLEMENTARY FIGURES

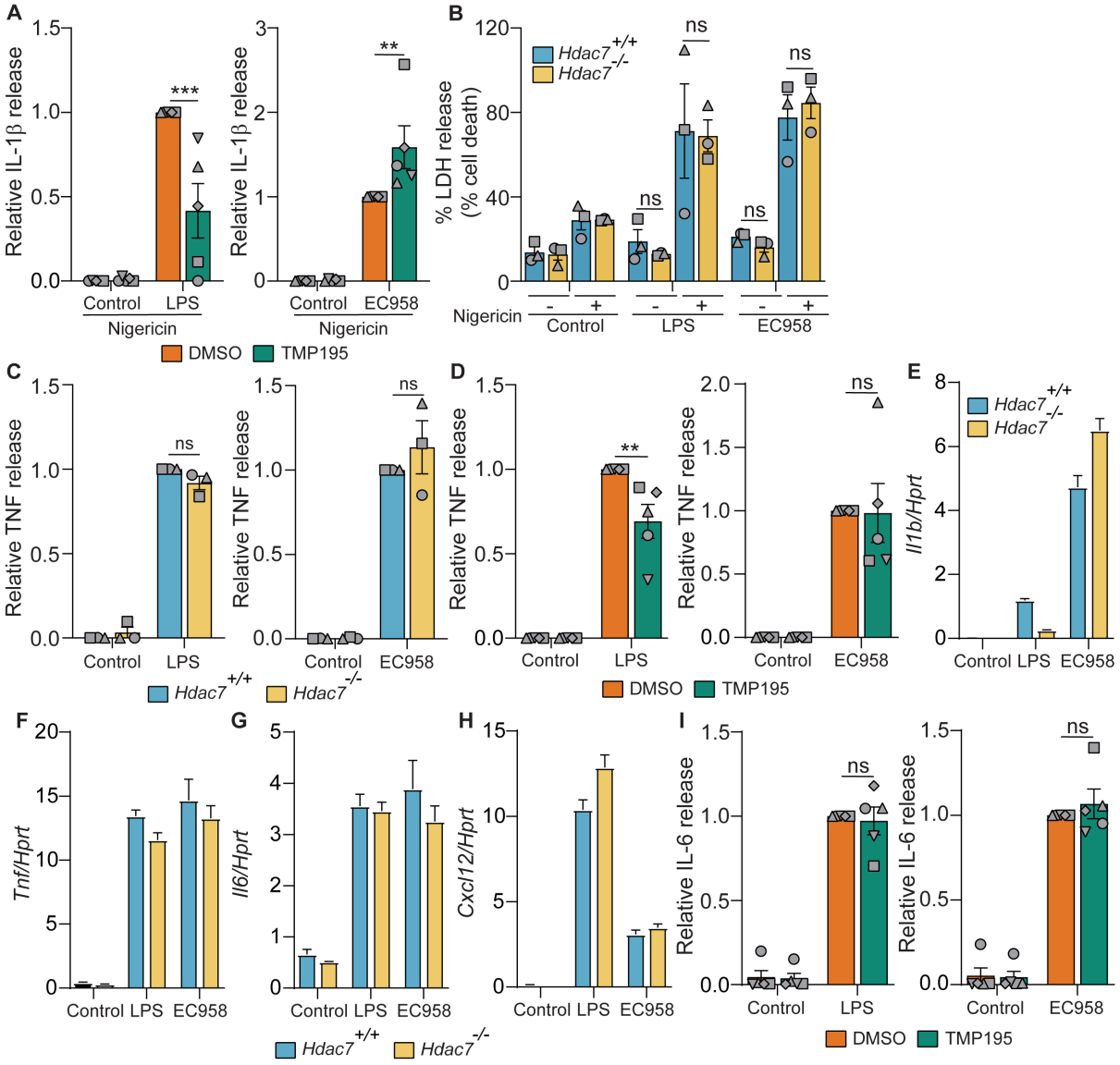

Fig. S1: Selective effect of HDAC7 in controlling IL-1β production in macrophages responding to LPS versus EC958 infection.

(**A**) BMM were pre-treated with TMP195 (1-2 μM) for 1 h, after which they were either treated with LPS (10 ng/ml; **left**) or infected with EC958 (MOI 10; **right**) for 4 h, followed by an additional treatment with nigericin (5 μg/ml) for 1 h. Supernatants were then assessed for secreted IL-1β by ELISA. (**B**) The indicated BMM populations were treated with either LPS (0.5 ng/ml) or infected with EC958 (MOI 10) for 4 h, followed by an additional treatment with nigericin (5 μg/ml) for 1 h. Supernatants from the infected cells were then assessed for released LDH, as a measure of cell death. (**C**) The indicated BMM populations were treated with either LPS (0.5 ng/ml; **left**) or infected with EC958 (MOI 10; **right**) for 4 h. Supernatants were then assessed for secreted TNF by ELISA. (**D**) BMM were pre-treated with TMP195 (1-2 μM) for 1 h, after which they were either treated with LPS (10 ng/ml; **left**) or infected with EC958 (MOI 10; **right**) for 4 h. Supernatants were then assessed for secreted TNF by ELISA. (**E-H**) The indicated BMM populations were treated with either LPS (0.5 ng/ml) or infected with EC958 (MOI 10) for 4 h. Total RNA was extracted and mRNA levels of *Il1b* (**E**), *Tnf* (**F**), *Il6*

(**G**) and *Cxcl2* (**H**) were measured by qPCR. Data are from a single experiment (mean+SEM, experimental triplicates), with similar findings being apparent in two independent experiments. (**I**) BMM were pre-treated with TMP195 (1-2 μM) for 1 h, after which they were either treated with LPS (10 ng/ml; **left**) or infected with EC958 (MOI 10; **right**) for 4 h. Supernatants were then assessed for secreted IL-6 by ELISA. With the exception of (**E-H**), all graphical data (mean±SEM, n=3-5) are combined from at least three independent experiments performed in duplicates and are normalized to the LPS-treated and EC958-infected samples (**A, D** and **I**) or *Hdac7*$^{+/+}$ LPS-treated and EC958-infected samples (**C**). Statistical significance was determined using two-way ANOVA followed by Sidak's multiple comparison test (ns – not significant; ** $p<0.01$; *** $p<0.001$).

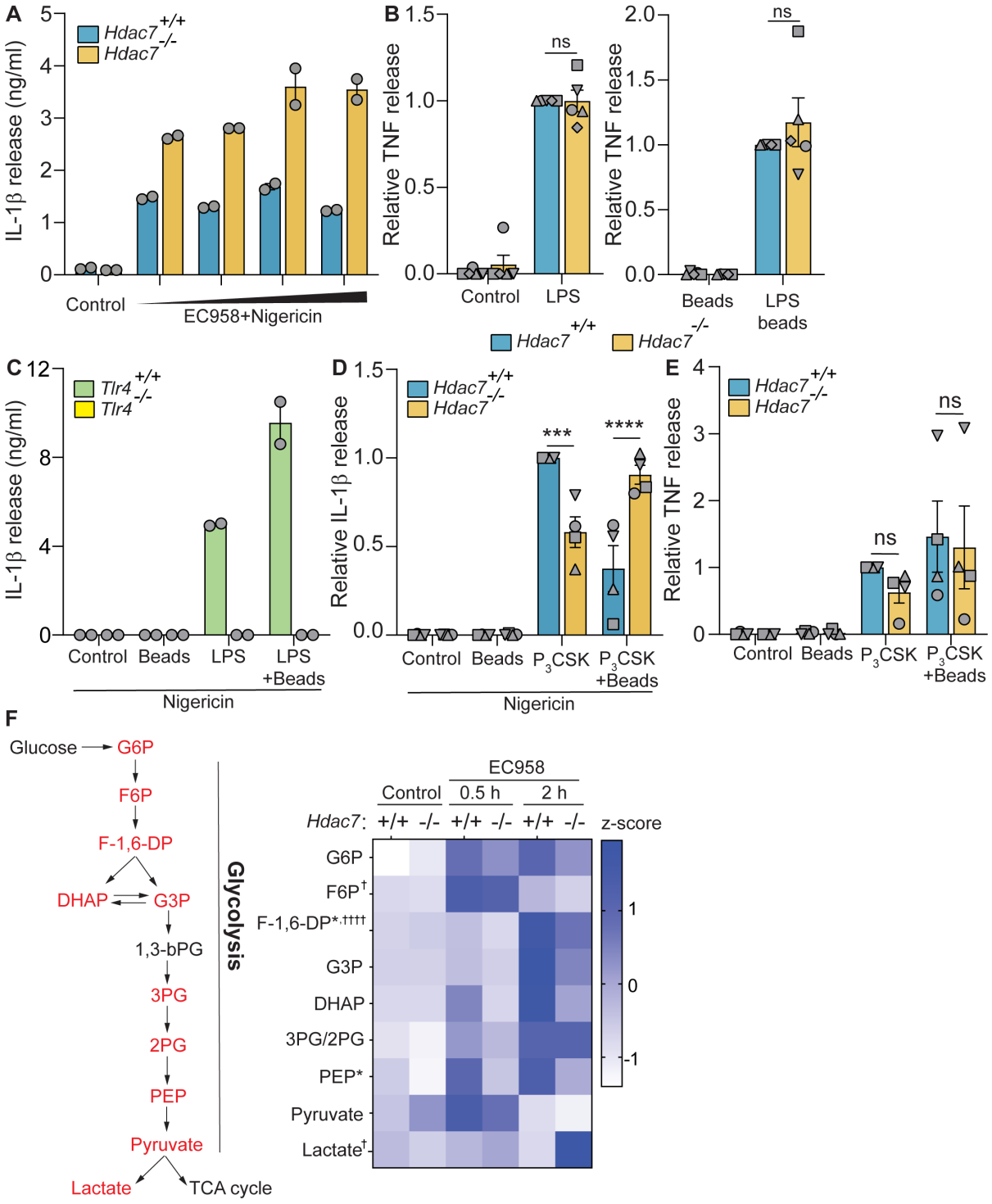

Fig. S2: Selective effects of HDAC7 in controlling inflammatory and metabolic responses in macrophages.

(**A**) The indicated BMM populations were infected with EC958 over an MOI range (3, 10, 30, 100) for 4 h, followed by an additional treatment with nigericin (5 µg/ml) for 1 h. Supernatants were then assessed for secreted IL-1β by ELISA. Data are representative of two independent experiments. (**B**) The indicated BMM populations were treated with either LPS (0.5 ng/ml; **left**) or LPS-coated latex beads (**right**) for 4 h. Supernatants were then assessed for secreted TNF by ELISA. (**C**) The indicated BMM populations were treated with either LPS (100 ng/ml) or co-treated with LPS and latex beads for 4 h, followed by an additional treatment with

nigericin (5 μg/ml) for 1 h. Supernatants were then assessed for secreted IL-1β by ELISA. Data are representative of two independent experiments. (**D-E**) The indicated BMM populations were treated with either Pam_3CSK_4 (15 ng/ml) or co-treated with Pam_3CSK_4 and latex beads for 4 h, followed by an additional treatment with nigericin (5 μg/ml) for 1 h. Supernatants were then assessed for secreted IL-1β (**D**) and TNF (**E**) by ELISA. (**F**) A heatmap showing relative levels of glycolytic pathway metabolites (highlighted in red) in lysates from wild type (*Hdac7*$^{+/+}$) and *Hdac7*$^{-/-}$ BMM infected with EC958 (MOI 100, 30 min and 2 h). * indicates statistical comparison between *Hdac7*$^{+/+}$ and *Hdac7*$^{-/-}$ BMM at 30 min post-infection, and † or †††† indicates statistical comparison between *Hdac7*$^{+/+}$ and *Hdac7*$^{-/-}$ BMM at 2 h post-infection. With the exception of **A** and **C**, all graphical data (mean±SEM, n=3-5) are combined from at least three independent experiments performed in duplicates. Data are normalized to *Hdac7*$^{+/+}$ LPS-treated and LPS-coated latex bead samples (**B**) or *Hdac7*$^{+/+}$ Pam_3CSK_4-treated sample (**D-E**). Statistical significance was determined using two-way ANOVA followed by Sidak's multiple comparison test (ns – not significant; * $p<0.05$; *** $p<0.001$; **** $p<0.0001$; † $p<0.05$; †††† $p<0.0001$).

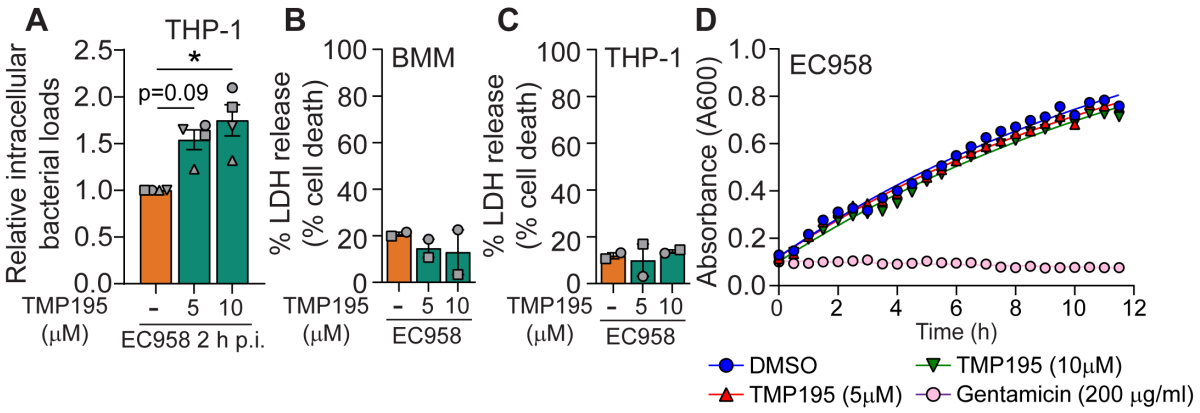

Fig. S3: Inhibition of HDAC7 compromises host defence in macrophages.

(**A**) PMA-differentiated THP-1 were pre-treated with the indicated concentrations of TMP195 for 1 h prior to challenge with EC958 (MOI 100). Relative intracellular bacterial loads at 2 h p.i. were quantified using gentamicin exclusion assays. (**B-C**) BMM (**B**) and PMA-differentiated THP-1 (**C**) were pre-treated with the indicated concentrations of TMP195 for 1 h prior to challenge with EC958. Supernatants from the infected cells were then assessed for released LDH, as a measure of cell death. (**D**) UPEC strain EC958 was cultured ± the indicated concentrations of TMP195 for 12 h, during which growth was assessed (A_{600} at the indicated time points). Graphical data (mean±SEM, n=4 for **A** or mean±range, n=2 for **B-C**) are combined from at least two independent experiments performed in duplicate. Statistical significance was determined using Kruskal-Wallis test followed by Dunn's multiple comparison test (**A)** (* $p<0.05$).

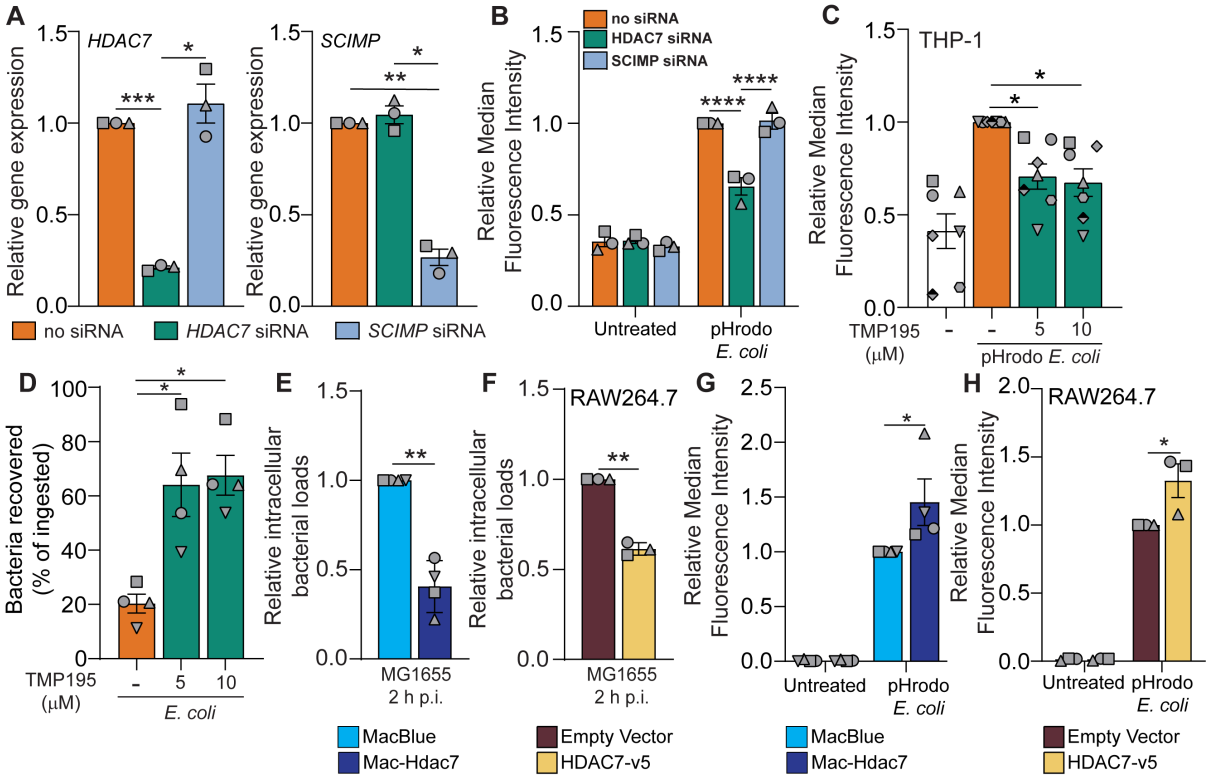

Fig. S4: HDAC7 promotes phagocytic uptake and killing of E. coli by macrophages.

(**A-B**) PMA-differentiated THP-1 were electroporated with the indicated siRNAs. Total RNA was extracted and mRNA levels of indicated genes were measured by qPCR (**A**). Degree of phagocytosis in THP-1 cells after silencing of indicated genes was assessed using pHrodo *E. coli* median fluorescence intensity (**B**). (**C**) PMA-differentiated THP-1 were pre-treated with the indicated concentrations of TMP195 for 30 min prior to treatment with pHrodo *E. coli*. The degree of phagocytosis was assessed using pHrodo *E. coli* fluorescence. (**D**) BMM were pre-treated with the indicated concentrations of TMP195 for 1 h. Cells were then spin-fected with *E. coli* strain MG1655 for 5 min, rested for a further 5 min, then cultured in the presence of gentamicin. Intracellular bacterial loads were then assessed at 20 min p.i. (indicative of phagocytic uptake of bacteria) and 2 h p.i. (indicative of bacterial killing) by gentamicin exclusion assays. Data plotted show % viable bacteria recovered at 2 h p.i. versus 20 min p.i. (**E-F**) The indicated macrophage populations (Mac-Hdac7 or Macblue control BMM: **E** or RAW264.7-Hdac7 or Empty vector RAW264.7 control cells: **F**) were challenged with *E. coli* strain MG1655. Relative intracellular bacterial loads at 2 h p.i. were quantified using gentamicin exclusion assays. (**G-H**) The indicated macrophage populations were treated with pHrodo *E. coli* for 1 h. Phagocytic uptake of pHrodo *E. coli* was assessed by flow cytometry. Graphical data (mean±SEM, n=3-7) are combined from at least three independent experiments performed in duplicates and are normalized to the no siRNA control (**A**), no siRNA pHrodo-treated samples (**B**), no TMP195 control (**C**), MacBlue (**E, G**) or empty vector control (**F, H**). Statistical significance was determined using Kruskal-Wallis test followed by Dunn's multiple comparison test (**A, C-D**) or two-way (**B, G-H**) ANOVA followed by Sidak's or Tukey's multiple comparison test or unpaired t-test with Welch's correction (**E-F**) (ns – not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$).

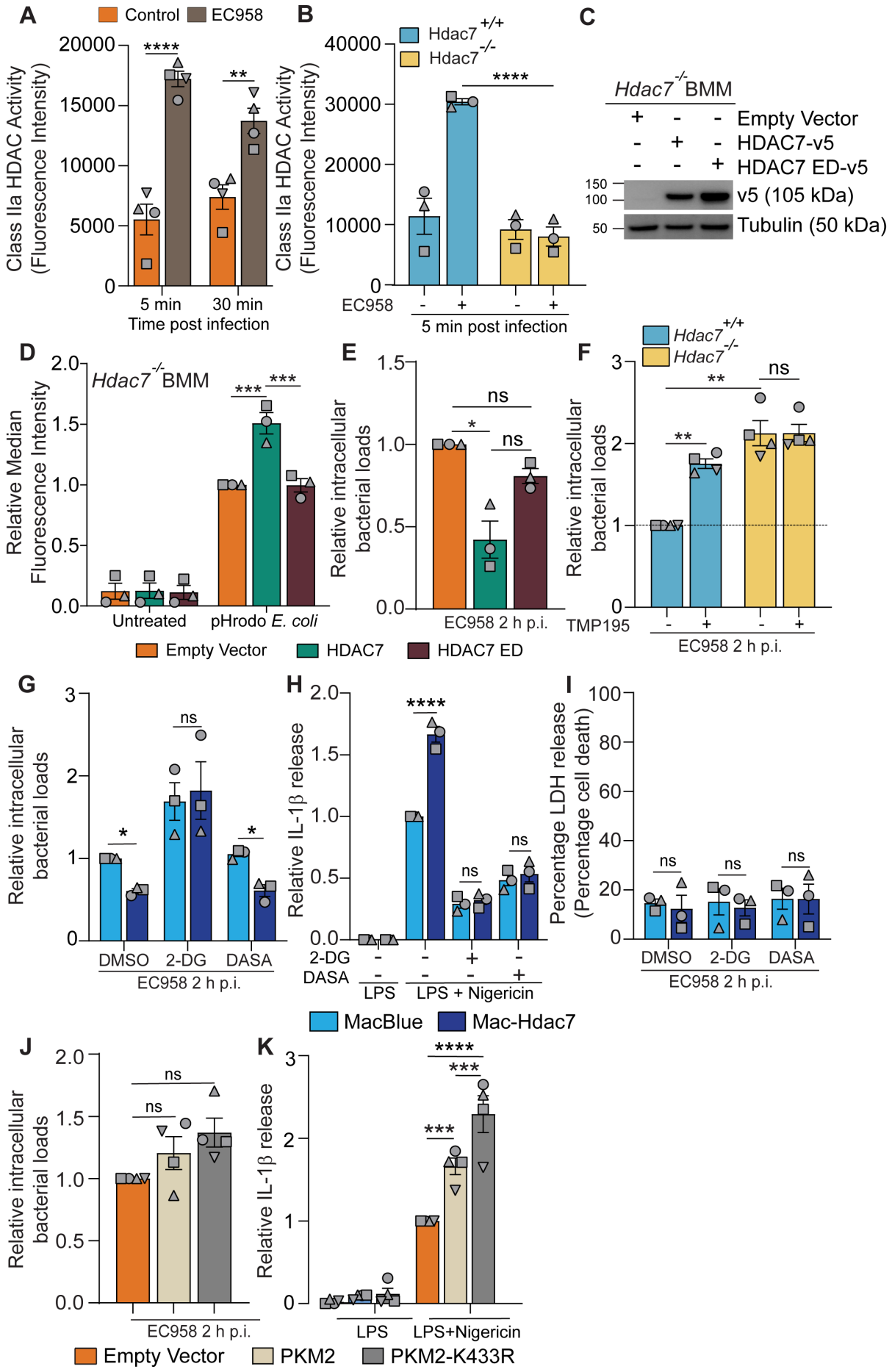
A
Control
EC958
Class IIa HDAC Activity (Fluorescence Intensity)
20000
15000
10000
5000
0
****
**
5 min
30 min
Time post infection
B
Hdac7 +/+
Hdac7 -/-
Class IIa HDAC Activity (Fluorescence Intensity)
40000
30000
20000
10000
0
****
EC958
- + - +
5 min post infection
C
Hdac7 -/- BMM
+ - - Empty Vector
- + - HDAC7-v5
- - + HDAC7 ED-v5
150
100
50
v5 (105 kDa)
Tubulin (50 kDa)
D
Hdac7 -/- BMM
Relative Median Fluorescence Intensity
2.0
1.5
1.0
0.5
0.0
***
***
Untreated
pHrodo E. coli
Empty Vector
HDAC7
HDAC7 ED
E
Relative intracellular bacterial loads
1.5
1.0
0.5
0.0
ns
*
ns
EC958 2 h p.i.
F
Hdac7 +/+
Hdac7 -/-
Relative intracellular bacterial loads
3
2
1
0
**
**
ns
TMP195
- + - +
EC958 2 h p.i.
G
Relative intracellular bacterial loads
3
2
1
0
*
ns
*
DMSO
2-DG
DASA
EC958 2 h p.i.
H
Relative IL-1β release
2.0
1.5
1.0
0.5
0.0
****
ns
ns
2-DG
DASA
LPS
LPS + Nigericin
MacBlue
Mac-Hdac7
I
Percentage LDH release (Percentage cell death)
100
80
60
40
20
0
ns
ns
ns
DMSO
2-DG
DASA
EC958 2 h p.i.
J
Relative intracellular bacterial loads
2.0
1.5
1.0
0.5
0.0
ns
ns
EC958 2 h p.i.
K
Relative IL-1β release
3
2
1
0
****
***
***
LPS
LPS+Nigericin
Empty Vector
PKM2
PKM2-K433R

Fig. S5: Antimicrobial effects of HDAC7 require its enzymatic activity and are independent of glycolysis-PKM2.

(**A-B**) BMM from C57BL/6J mice (**A**) or the indicated strains (**B**) were infected with the UPEC strain EC958 for either 5 min or 30 min. Class IIa HDAC activity in cell lysates was then measured. (**C**) Lysates from *Hdac7*$^{-/-}$ BMM, retrovirally transduced with the indicated constructs, were immunoblotted for v5-tagged proteins and tubulin (loading control). The displayed immunoblot is representative of three independent experiments. (**D**) BMM from *Hdac7*$^{-/-}$ mice were retrovirally-transduced with the indicated constructs, after which transduced cells were treated with pHrodo *E. coli* for 1 h. Phagocytic uptake of pHrodo *E. coli* was assessed by flow cytometry. (**E**) The indicated retrovirally-transduced BMM were infected with EC958, after which relative intracellular bacterial loads at 2 h p.i. were assessed using gentamicin exclusion assays. (**F**) The indicated BMM populations were pre-treated with TMP195 (1 μM) for 1 h, after which they were infected with EC958. Relative intracellular bacterial loads at 2 h p.i. were then determined using gentamicin exclusion assays. The dotted line indicates baseline relative bacterial loads in control cells. (**G**) The indicated BMM populations were either pre-treated with 2-DG (5 mM) or DASA (10 μM) for 1 h, before infecting them with EC958. Relative intracellular bacterial loads at 2 h p.i. were then assessed using gentamicin exclusion assays. (**H-I**) The indicated BMM populations were either pre-treated with 2-DG (5 mM) or DASA (10 μM) for 1 h, before treating them with LPS (100 ng/ml) for 4 h, followed by an additional treatment with nigericin (5 μg/ml) for 1 h. Supernatants were then assessed for secreted IL-1β by ELISA (**H**). Alternatively, the BMM were infected with UPEC strain EC958 for 2 h. Supernatants from the infected cells were then assessed for released LDH, as a measure of cell death (**I**). (**J-K**) BMM were retrovirally-transduced with the indicated constructs, after which relative intracellular survival of EC958 at 2 h p.i. infection was assessed using gentamicin exclusion assays (**J**). Additionally, the transduced BMM were treated with LPS (100 ng/ml) for 4 h followed by nigericin (5 μg/ml) for an additional 1 h. Secreted IL-1β in culture supernatants was assessed by ELISA (**K**). All graphical data (mean±SEM, n=3-5) are combined from at least three independent experiments and are normalized to empty vector control (**D-E**), *Hdac7*$^{+/+}$ control (**F**), MacBlue control (**G-H**) or empty vector control (**J-K**). Statistical significance was determined using two-way ANOVA (**A-B, D, F-I**, **K**) followed by Tukey's or Sidak's multiple comparison test or non-parametric Kruskal-Wallis test followed by Dunn's multiple comparison test (**E, J**) (ns – not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$, **** $p<0.0001$).

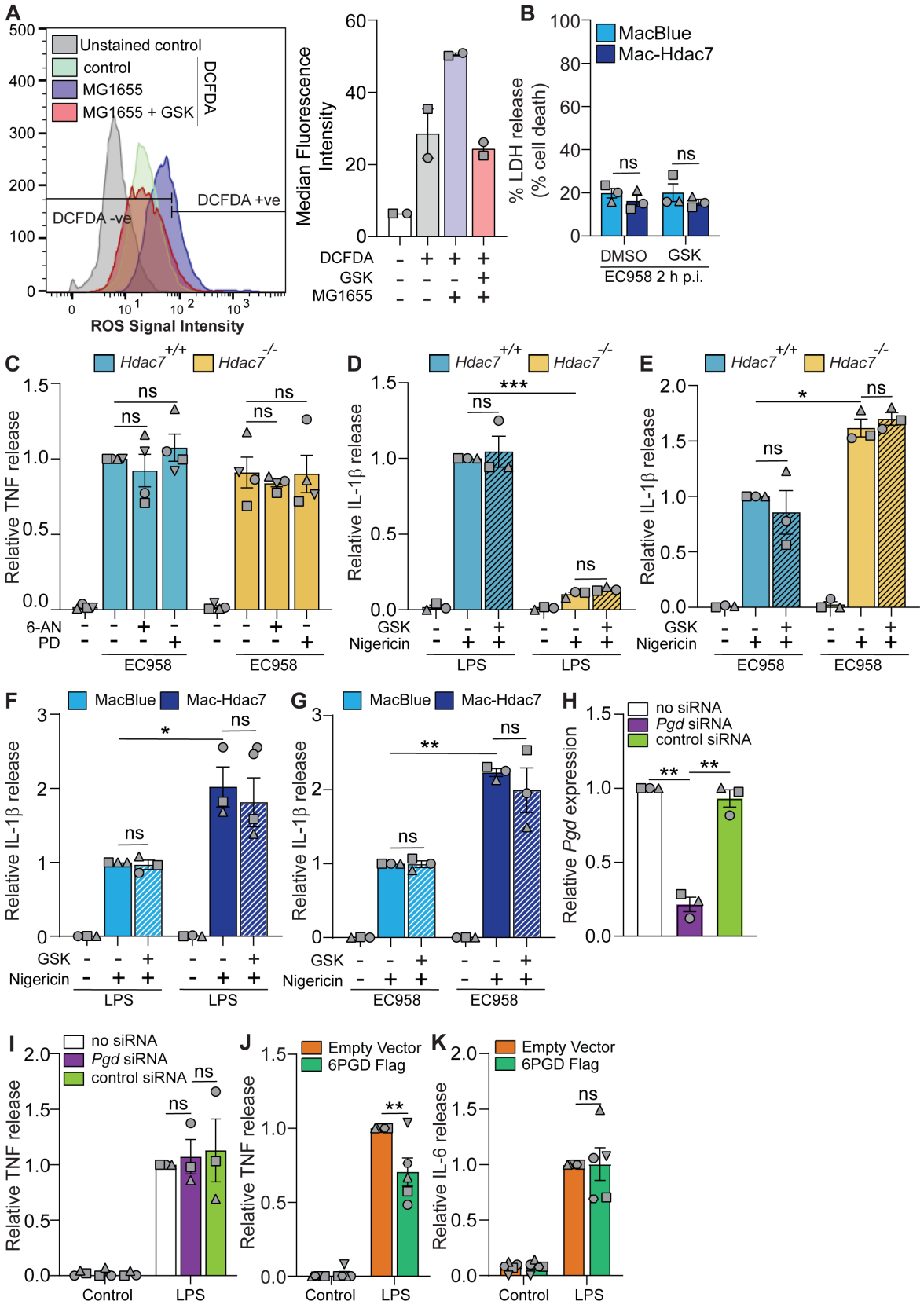

Fig S6: Mechanisms by which the PPP suppresses macrophage inflammatory responses.

(**A**) BMM were either left untreated or were treated with the NADPH oxidase inhibitor, GSK2795039 (GSK, 10 μM) for 1 h, after which they were challenged with MG1655 (MOI 100) for 30 min. Total cellular ROS levels were then quantified by DCFDA fluorescence.

Representative flow cytometry plot (**left**), as well as relative median fluorescence intensity (MFI) data (**right**), are shown. Data are combined from two independent experiments. (**B**) The indicated BMM populations were pre-treated with GSK2795039 (GSK, 10 μM), before being infected with EC958 (MOI 100) for 2 h. Supernatants from the infected cells were then assessed for released LDH, as a measure of cell death. (**C**) The indicated BMM populations were pre-treated with the PPP inhibitors 6-aminonicotinamide (6-AN) (0.5 μM) or polydatin (PD) (0.5 μM) for 1 h, after which they were infected with EC958 (MOI 100) for 4 h. Supernatants were then assessed for secreted TNF by ELISA. (**D-G**) The indicated BMM populations were pre-treated with GSK2795039 (GSK, 10 μM), before treating them with LPS (0.5 ng/ml (**D**) or 100 ng/ml (**F**)) or infected with EC958 (MOI 100 (**E, G**)) for 4 h, followed by an additional treatment with nigericin (5 μg/ml) for 1 h. Supernatants were then assessed for secreted IL-1β by ELISA. (**H-I**) BMM were electroporated with the indicated siRNAs. Levels of knockdown were confirmed by qPCR (**H**). Electroporated BMMs were treated with LPS (0.5 ng/ml) for 4 h. Supernatants were then assessed for secreted TNF by ELISA (**I**). (**J-K**) BMM were retrovirally transduced with the indicated constructs. Transduced BMMs were treated with LPS (0.5 ng/ml) for 4 h. Supernatants were then assessed for secreted TNF (**J**) and IL-6 (**K**) by ELISA. All graphical data (mean±range for n=2; mean±SEM for n=3-5) are combined from two (**A**) or at least three (**B-K**) independent experiments unless specified and are normalized to the *Hdac7*$^{+/+}$ sample (**C-E**), MacBlue sample (**F-G**), no siRNA control (**H**), LPS-treated no siRNA control (**I**) or LPS-treated empty vector control (**J-K**). Statistical significance was determined using two-way ANOVA followed by Sidak's multiple comparison test (ns – not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$).

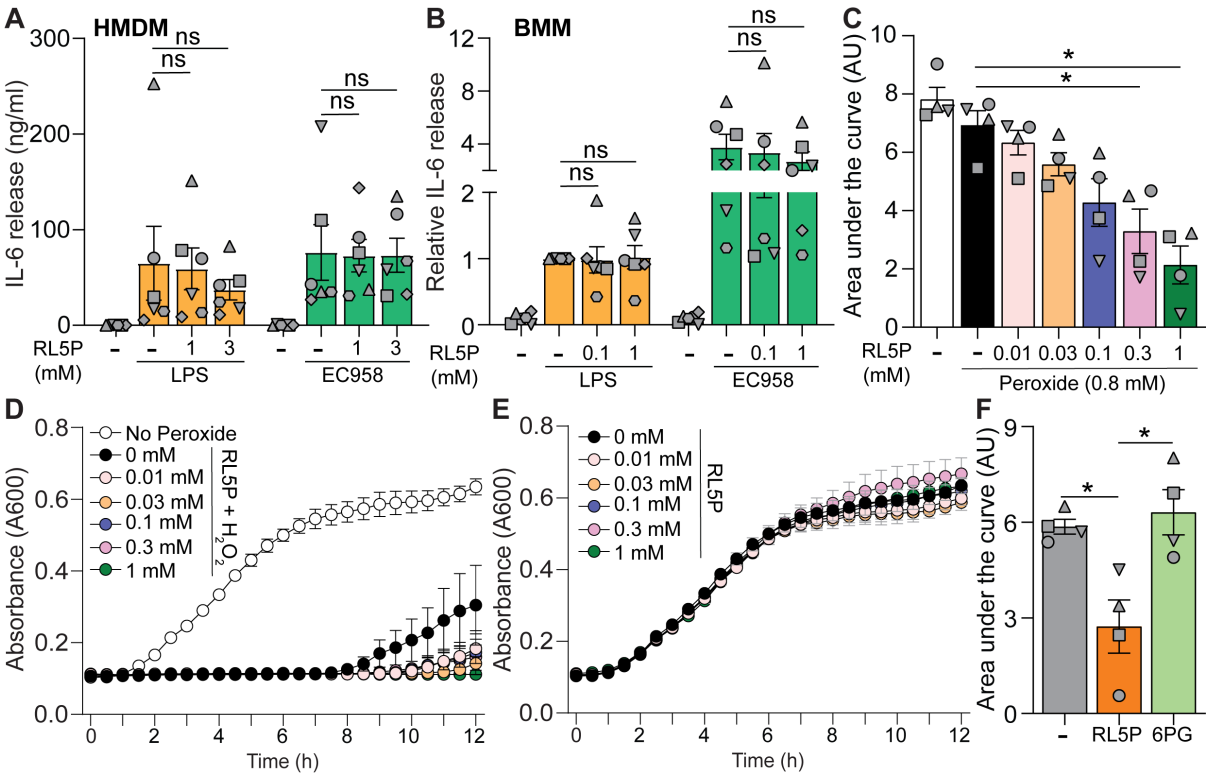

Fig. S7: Effects of RL5P on inflammatory responses in macrophages and E. coli growth.

(**A-B**) HMDM and BMM were pre-treated with the indicated concentrations of RL5P for 16 h, after which they were either stimulated with LPS (10 ng/ml) or infected with EC958 (MOI 100) for 4 h. Supernatants were then assessed for secreted IL-6 by ELISA. (**C**) EC958 was cultured ± H_2O_2 (0.8 mM) in the presence of the indicated concentrations of RL5P for 12 h, during which growth was assessed (A_{600} at the indicated time points). Area under the curve analysis for the 12 h period was calculated thereafter. Each symbol represents a different experiment. (**D**) EC958 was cultured ± H_2O_2 (1 mM) in the presence of the indicated concentrations of RL5P for 12 h, during which growth was assessed (A_{600} at the indicated time points). (**E**) EC958 was cultured in the presence of the indicated concentrations of RL5P for 12 h, during which growth was assessed (A_{600} at the indicated time points). (**F**) EC958 was cultured ± H_2O_2 (0.8 mM) in the presence of the indicated concentration of RL5P or 6PG for 12 h, during which growth was assessed (A_{600} at the indicated time points). Area under the curve analysis for the 12 h period was calculated thereafter. Each symbol represents a different experiment. All graphical data (mean±SEM, n=3-6) are combined from three to six independent experiments (or donors) unless otherwise specified and are normalized to LPS alone treated BMMs (**B**). Statistical significance was determined using repeated measure two-way ANOVA followed by Dunnett's multiple comparison test (**A-B**) or non-parametric Kruskal Wallis test followed by Dunn's multiple comparison test (**C, F**) (ns – not significant; * $p<0.05$).

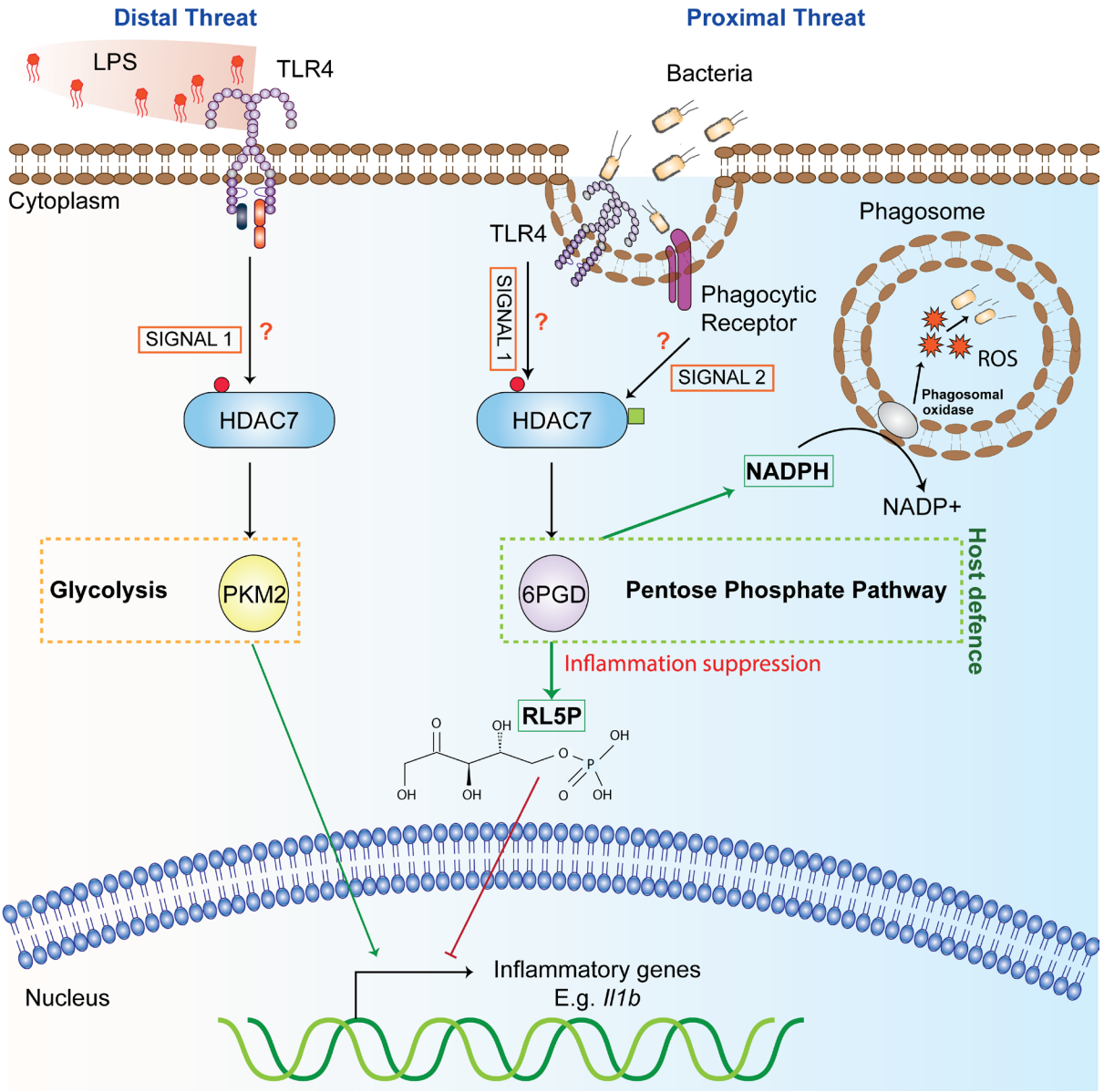

Fig. S8: Proposed model of HDAC7 involvement in context-dependent danger sensing and immunometabolic reprogramming in macrophages.

In response to soluble danger signals such as LPS that are indicative of distal danger, TLRs activate HDAC7 for engagement of the glycolytic enzyme PKM2 and production of pro-inflammatory IL-1β. In contrast, proximal threats such as bacteria engage both TLRs and phagocytic receptors on macrophages. This may lead to signal integration and reprogramming of HDAC7 functions, such that it engages the PPP enzyme 6PGD for production of both NADPH and RL5P. The former fuels the phagocyte oxidase for antimicrobial ROS generation, while the latter selectively suppresses inflammatory responses. This mechanism may enable HDAC7 to focus cellular resources on immediate antimicrobial responses for effective host defence.

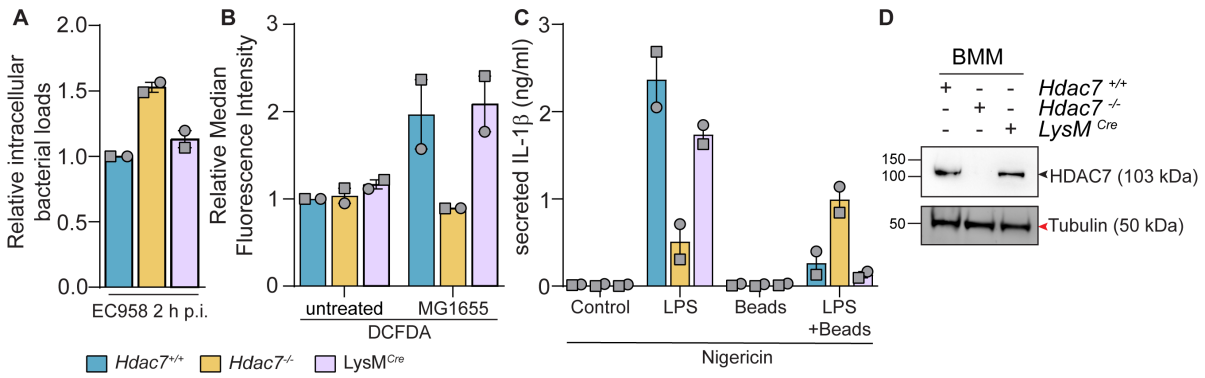

Fig. S9: Comparison of functional outputs between Hdac7$^{+/+}$, Hdac7$^{-/-}$ and LysM^{Cre} BMMs.

(**A**) The indicated BMM populations were infected with EC958 (MOI 100) for 2 h. Relative intracellular bacterial survival was assessed by gentamicin exclusion assay. (**B**) The indicated BMM populations were infected with MG1655 (MOI 100) for 30 min. Inducible oxidative stress was quantified using DCFDA (10 µM) by flow cytometry. (**C**) The indicated BMM populations were either treated with LPS (0.5 ng/ml) or co-treated with LPS and latex beads for 4 h, followed by an additional 1 h of nigericin treatment (5 µg/ml). Secreted levels of IL-1β were measured by ELISA. (**D**) HDAC7 immunoblot of BMM lysates to confirm *Hdac7* deletion from *Hdac7*$^{-/-}$ BMM. The indicated immunoblot is from one experiment and is representative of two independent experiments. All graphical data (mean±range, n=2) are compiled from two independent experiments.

Table S1

Central Carbon Metabolite Analysis
(Related to Figure 1G and SI Appendix Figure S2F)

BMMs from *Hdac7*$^{+/+}$ and littermate *Hdac7*$^{-/-}$ were either uninfected (control) or infected with EC958 (MOI-100) for 0.5 h and 2 h. Raw Data are in μM.

Raw Data – Pentose Phosphate Pathway Intermediates

	Hdac7 +/+			***Hdac7 -/-***		
	Expt 1	*Expt 2*	*Expt 3*	*Expt 1*	*Expt 2*	*Expt 3*
	6-Phosphoglycerate (6PG)					
Control	28.012	17.015	16.4137	29.698	18.463	15.7611
0.5 h	33.156	19.983	17.6996	31.079	18.611	16.4975
2 h	30.029	18.446	29.4753	30.247	19.927	18.3844
	Ribulose-5-Phosphate (RL5P)					
Control	0**	0.4412	3.61435	0**	0.4448	3.20664
0.5 h	4.9938	0.5492	8.14389	3.5699	0.4425	4.92781
2 h	0.1243	0.4682	5.00631	0.1016	0.4594	4.1387
	Ribose-5-Phosphate (R5P)					
Control	0.1931	0.0495	0.48348	0.205	0.0498	0.46406
0.5 h	1.0209	0.0516	0.86111	0.8219	0.0509	0.70257
2 h	0.2235	0.0572	0.59581	0.2194	0.056	0.50866
	Xylulose-5-Phosphate (X5P)					
Control	0**	1.6572	1.8951	0**	0.3623	0.687
0.5 h	29.338	10.1764	15.3698	12.659	5.876	4.1714
2 h	14.7218	34.5645	33.1803	0.2934	14.0615	22.1525
	Erythrose-4-Phosphate (E4P)					
Control	0**	1.7971	2.1643	0**	2.5622	0.9551
0.5 h	5.5808	4.2362	6.2587	2.7559	1.4564	2.8824
2 h	0.6876	7.0029	12.335	0.0056	2.5005	5.7616
	**** - not detected**					

Relative Data (to EC958 infection at 0.5 h)

	Hdac7 +/+			***Hdac7 -/-***		
	Expt 1	*Expt 2*	*Expt 3*	*Expt 1*	*Expt 2*	*Expt 3*
	6-Phosphoglycerate (6PG)					
Control	0.8449	0.8515	0.9273	0.8957	0.9239	0.8905
0.5 h	1.0000	1.0000	1.0000	0.9374	0.9313	0.9321
2 h	0.9057	0.9231	1.6653	0.9123	0.9972	1.0387

	Ribulose-5-Phosphate (RL5P)					
Control	0**	0.8034	0.4438	0**	0.8099	0.3937
0.5 h	1.0000	1.0000	1.0000	0.7149	0.8057	0.6051
2 h	0.0249	0.8525	0.6147	0.0203	0.8365	0.5082
	Ribose-5-Phosphate (R5P)					
Control	0.1891	0.9593	0.5615	0.2008	0.9651	0.5389
0.5 h	1.0000	1.0000	1.0000	0.8051	0.9864	0.8159
2 h	0.2189	1.1085	0.6919	0.2149	1.0853	0.5907
	Xylulose-5-Phosphate (X5P)					
Control	0**	0.1628	0.1233	0**	0.0356	0.0447
0.5 h	1.0000	1.0000	1.0000	0.4315	0.5774	0.2714
2 h	0.5018	3.3965	2.1588	0.0100	1.3818	1.4413
	Erythrose-4-Phosphate (E4P)					
Control	0**	0.4242	0.3458	0**	0.6048	0.1526
0.5 h	1.0000	1.0000	1.0000	0.4938	0.3438	0.4605
2 h	0.1232	1.6531	1.9709	0.0010	0.5903	0.9206
	**** - not detected**					

Raw Data – Glycolytic Intermediates

	Hdac7 +/+			***Hdac7 -/-***		
	Expt 1	*Expt 2*	*Expt 3*	*Expt 1*	*Expt 2*	*Expt 3*
	Glucose-6-Phosphate (G6P)					
Control	5.4148	2.1603	2.5160	6.3362	8.5110	4.1150
0.5 h	41.3250	11.5966	12.5210	38.3330	6.9705	7.6570
2 h	24.5810	26.9437	20.3360	8.9793	27.7300	15.3487
	Fructose-6-Phosphate (F6P)					
Control	3.3367	3.4812	4.1552	2.9162	2.9449	3.1158
0.5 h	26.7612	25.8668	18.1158	23.6017	25.2052	16.2257
2 h	11.6214	5.5676	8.2296	5.1055	4.3864	5.2298
	Fructose-1,6-Diphosphate (F1,6DP)					
Control	9.2762	6.291781	18.1294	8.2158	7.076376	22.3124
0.5 h	11.121	10.15474	22.8979	8.7868	5.2964	16.1188
2 h	50.587	42.71895	26.6182	41.835	33.31062	9.1047
	Glyceraldehyde-3-Phosphate (G3P)					
Control	0.9258	1.27193	0.6455	1.3255	0.94288	0.4489
0.5 h	0.94573	2.30815	2.5217	0.5269	1.32641	1.2241
2 h	22.428	4.68794	3.4462	10.9948	2.68058	1.2356
	Dihydroxyacetone Phosphate (DHAP)					
Control	0.3316	1.0302	2.8106	0.3508	1.3936	2.4135
0.5 h	0.3317	4.5003	3.6316	0.2995	1.0895	2.8662
2 h	4.6562	3.2384	5.2689	2.8352	2.6842	1.5470

	3/2 Phosphoglycerate (3/2 PG)					
Control	26.392	3.6308	53.9276	27.287	3.8933	33.3552
0.5 h	57.381	3.9537	102.51	69.25	3.5016	57.7062
2 h	98.422	3.4256	128.65	134.97	4.2838	90.8787
	Phosphoenol Pyruvate (PEP)					
Control	2.6979	0.9528	17.1538	2.5975	1.2685	7.8940
0.5 h	4.8545	2.5934	35.0495	4.3888	1.1335	16.7606
2 h	5.2530	3.4089	38.1313	4.2839	2.8874	20.3956
	Pyruvate					
Control	142.2100	11.0329	60.6258	140.1900	76.1390	66.1526
0.5 h	215.4400	98.8537	84.2558	181.0600	73.4460	81.2251
2 h	120.4300	13.4543	42.2256	115.5000	9.1198	20.2235
	Lactate					
Control	361.0200	91.3932	181.5893	319.5200	93.3221	185.3325
0.5 h	175.4800	234.1150	231.9635	264.3400	192.1230	215.2398
2 h	245.7800	126.5730	208.2269	361.5400	253.9430	277.8819
	**** - not detected**					

Relative Data (to EC958 infection at 0.5 h)

	Hdac7 +/+			***Hdac7 -/-***		
	Expt 1	*Expt 2*	*Expt 3*	*Expt 1*	*Expt 2*	*Expt 3*
	Glucose-6-Phosphate (G6P)					
Control	0.1310	0.1863	0.2009	0.1533	0.7339	0.3286
0.5 h	1.0000	1.0000	1.0000	0.9276	0.6011	0.6115
2 h	0.5948	2.3234	1.6242	0.2173	2.3912	1.2258
	Fructose-6-Phosphate (F6P)					
Control	0.1247	0.1346	0.2294	0.1090	0.1138	0.1720
0.5 h	1.0000	1.0000	1.0000	0.8819	0.9744	0.8957
2 h	0.4343	0.2152	0.4543	0.1908	0.1696	0.2887
	Fructose-1,6-Diphosphate (F1,6DP)					
Control	0.8341	0.6196	0.7917	0.7388	0.6969	0.9744
0.5 h	1.0000	1.0000	1.0000	0.7901	0.5216	0.7039
2 h	4.5488	4.2068	1.1625	3.7618	3.2803	0.3976
	Glyceraldehyde-3-Phosphate (G3P)					
Control	0.9789	0.5511	0.2560	1.4016	0.4085	0.1780
0.5 h	1.0000	1.0000	1.0000	0.5571	0.5747	0.4854
2 h	23.7150	2.0310	1.3666	11.6257	1.1614	0.4900
	Dihydroxyacetone Phosphate (DHAP)					
Control	0.9997	0.2289	0.7739	1.0576	0.3097	0.6646
0.5 h	1.0000	1.0000	1.0000	0.9029	0.2421	0.7892
2 h	14.0374	0.7196	1.4508	8.5475	0.5964	0.4260

	3/2 Phosphoglycerate (3/2 PG)					
Control	0.4599	0.9183	0.5261	0.4755	0.9847	0.3254
0.5 h	1.0000	1.0000	1.0000	1.2068	0.8857	0.5629
2 h	1.7152	0.8664	1.2550	2.3522	1.0835	0.8865
	Phosphoenol Pyruvate (PEP)					
Control	0.5558	0.3674	0.4894	0.5351	0.4891	0.2252
0.5 h	1.0000	1.0000	1.0000	0.9041	0.4371	0.4782
2 h	1.0821	1.3145	1.0879	0.8825	1.1134	0.5819
	Pyruvate					
Control	0.6601	0.1116	0.7195	0.6507	0.7702	0.7851
0.5 h	1.0000	1.0000	1.0000	0.8404	0.7430	0.9640
2 h	0.5590	0.1361	0.5012	0.5361	0.0923	0.2400
	Lactate					
Control	2.0573	0.3904	0.7828	1.8208	0.3986	0.7990
0.5 h	1.0000	1.0000	1.0000	1.5064	0.8206	0.9279
2 h	1.4006	0.5406	0.8977	2.0603	1.0847	1.1980

**** Statistical analysis performed on relative data using repeated measure two-way ANOVA with Sidak's multiple comparison test.

Table S2

in vivo infection - Serum levels of proinflammatory cytokines
(Related to Figure 3)

		Hdac7$^{+/+}$			*Hdac7*$^{-/-}$			
Rank	**Cytokine**	**Mean**	**SEM**	**N**	**Mean**	**SEM**	**N**	**Significance/p value**
1	CCL2	359.27	152.69	10	1480.41	385.88	10	YES, p=0.0146
2	TNF	26.376	10.829	10	64.215	13.781	10	YES, p=0.0446
3	IFN-γ	37.111	29.639	10	190.102	79.201	10	NO, p=0.0872
4	IL-27	33.669	5.94	10	24.053	1.984	10	NO, p=0.1421
5	IFN-β	4.476	1.73	10	2.169	0.309	10	NO, p=0.2058
6	IL-10	254.796	78.548	10	468.148	146.17	10	NO, p=0.2148
7	IL-6	1400.96	973.83	10	2776.28	700.3	10	NO, p=0.2666
8	IL-1α	50.245	33.021	10	18.982	4.529	10	NO, p=0.3607
9	IL-23	11.291	4.283	10	9.703	2.235	10	NO, p=0.7461
10	GMCSF	9.718	1.093	10	9.344	0.755	10	NO, p=0.7815
11	IL-17a	32.855	6.355	10	30.484	7.561	10	NO, p=0.8130
12	IL-1β	21.123	2.135	10	20.938	0.807	10	NO, p=0.9363
13	IL-12p70	7.077	2.157	10	7.289	2.684	10	NO, p=0.9516

Table S3

List of primers used to generate an EC958 oxidative stress mutant

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')	Comment
katG	GGCGTGGATGGCACAAAT	GGAATAGGAACTAAGG AGGAACACAGCGTTAG AGAGAAG	To generate 500 bp homology region for 3-way PCR to the **left** of deletion site in EC958*katG*
	CCTACACAATCGCTCAAGA CATGCCCACGAGAAGTTTG GAGAAACAACGCGGGAA	GAGAAACAACGCGGC GAA	To generate 500 bp homology region for 3-way PCR to the **right** of deletion site in EC958*katG*
	GGCAGGCATTGATGTGGA	CCACGGCTGCAACACC AAA	**To screen site of deletion in EC958*katG***

List of real time qPCR primers used in this study

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
Human *HDAC7*	TCAGAGGCGTCACAGATGGC	GGATTTGATGCTGCTGAGGG
Human *SCIMP*	TACTTTCACAGTTCAGGATTC	ACAGACACAGTACAGGATGAG
Human *HPRT*	TCAGGCAGTATAATCCAAAGAT	AGTCTGGCTTATATCCAACACT
Mouse *Pgd*	TGAAGGGTCCTAAGGTGGTCC	CCGCCATAATTGAGGGTCCAG
Mouse *Hprt*	GCAGTACAGCCCCAAAATGG	AACAAAGTCTGGCCTGTATCCA
Mouse *Il1b*	GAAGTTGACGGACCCCAAAA	GCCTGCCTGAAGCTCTTGTT
Mouse *Il6*	CTGCAAGAGACTTCCATCCAGTT	GAAGTAGGGAAGGCCGTGG
Mouse *Tnf*	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC
Mouse *Cxcl12*	GGAGGATAGATGTGCTCTGGAAC	AGTGAGGATGGAGACCGTGGTG

List of siRNA used in this study

Gene	Sequence
Human HDAC7	GAGCCCAUGAGGCUCUCCAUGGAC
	GCACCCUCAGGUGUUGCUCUGGGA
	GAGCCCAACCUGAAGCUGCGCUAU
Human SCIMP	GAAACACAAGCAAGUAGAUGAAGA
	CCGCUACAUACUCACUGGUAAAUAA
	UCUUAAUGAGUCGCCAGUUCAAUUA
Mouse PGD	CCCAAGGCUUUAUGCUGCUCAGACA
	CCGGGAGAAUUUAUCCACACCAACU
	CAUCAUCGAUGGAGGAAAUUCUGAA
Mouse Dnm3 (control siRNA)	AGCAGAGGAGCAGUCAGGUUCACAA