Supplemental Information

Ethyl pyruvate modulates murine dendritic cell activation and survival through their immunometabolism

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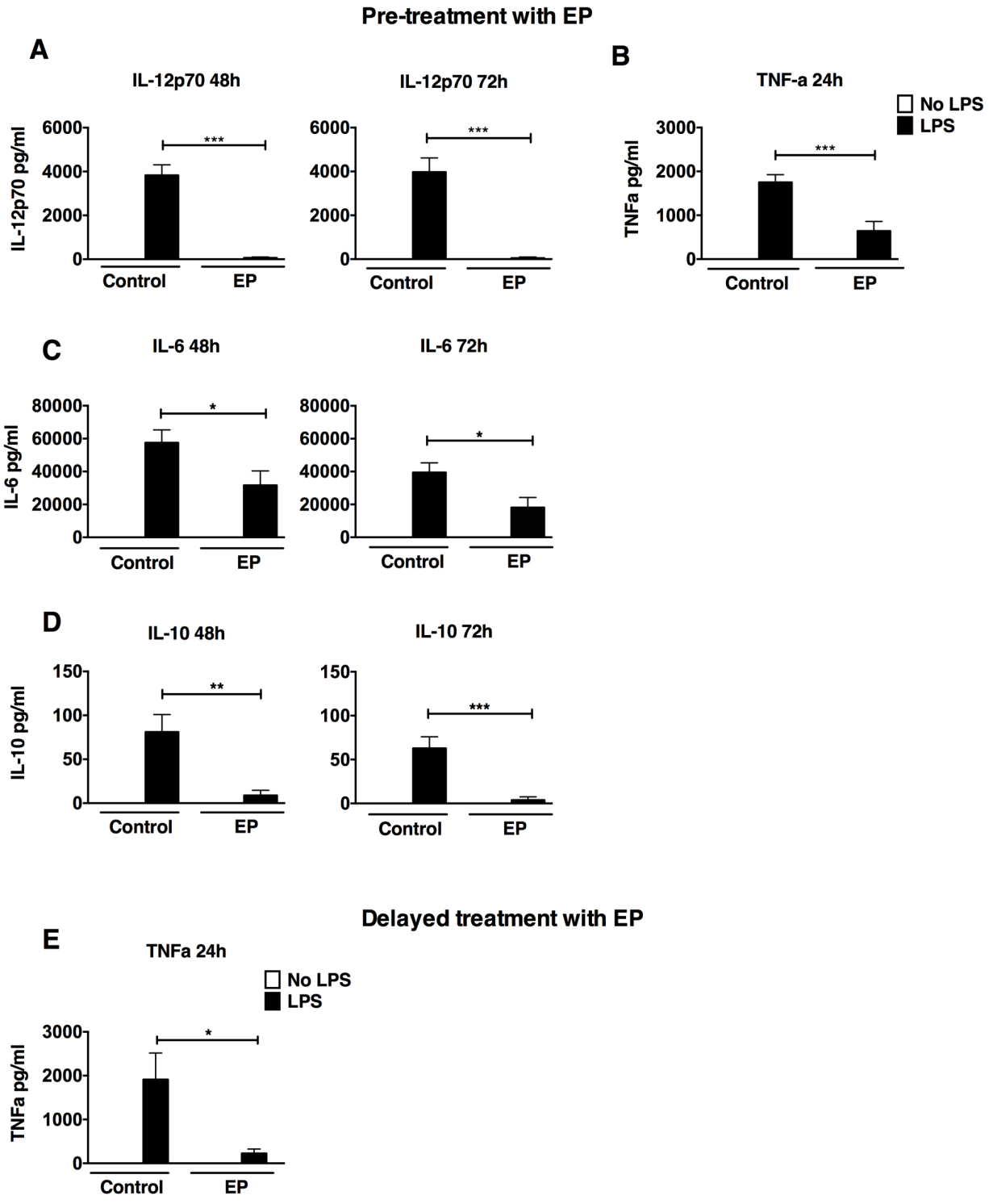
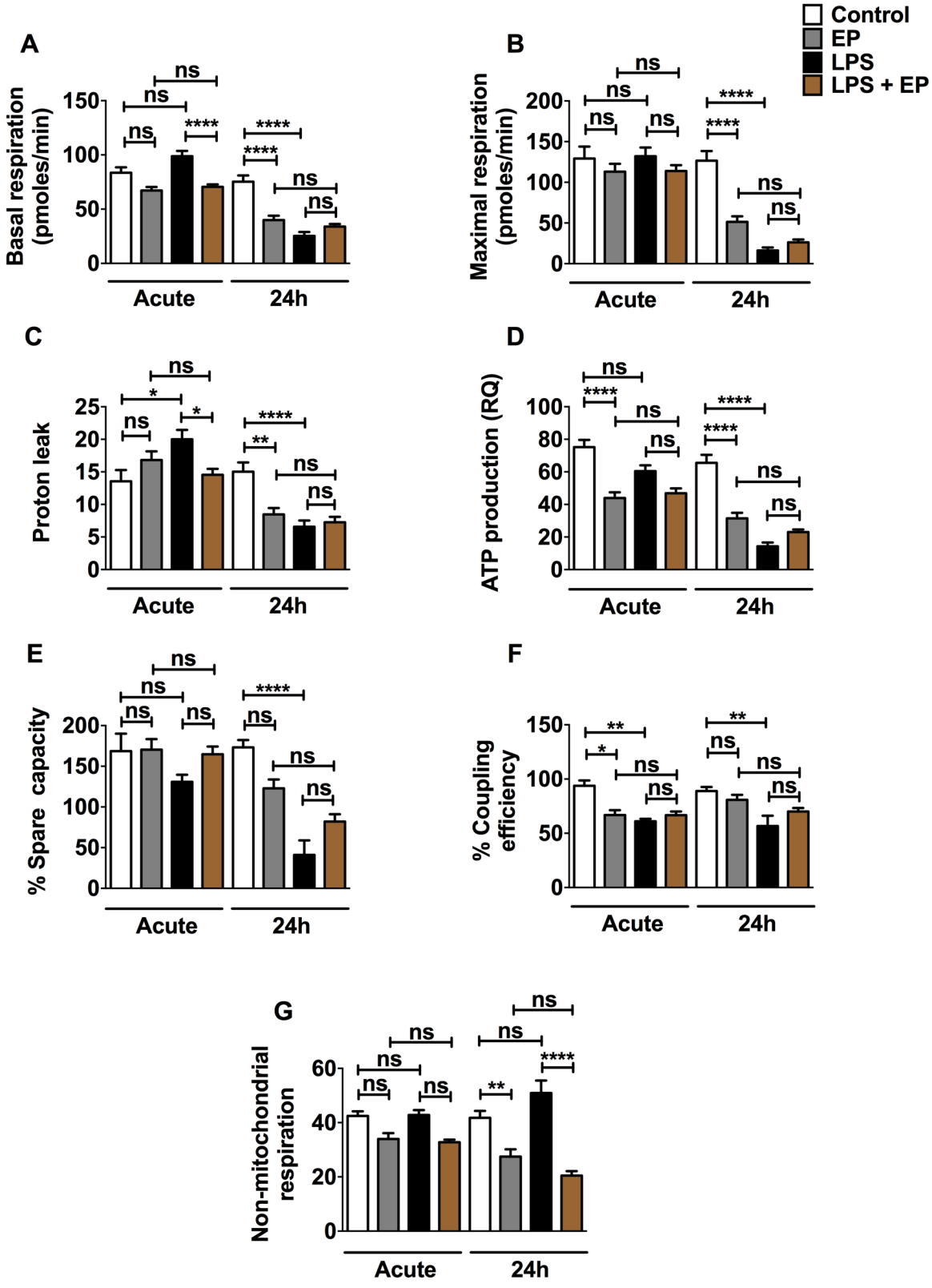
Supplemental Figure 1.



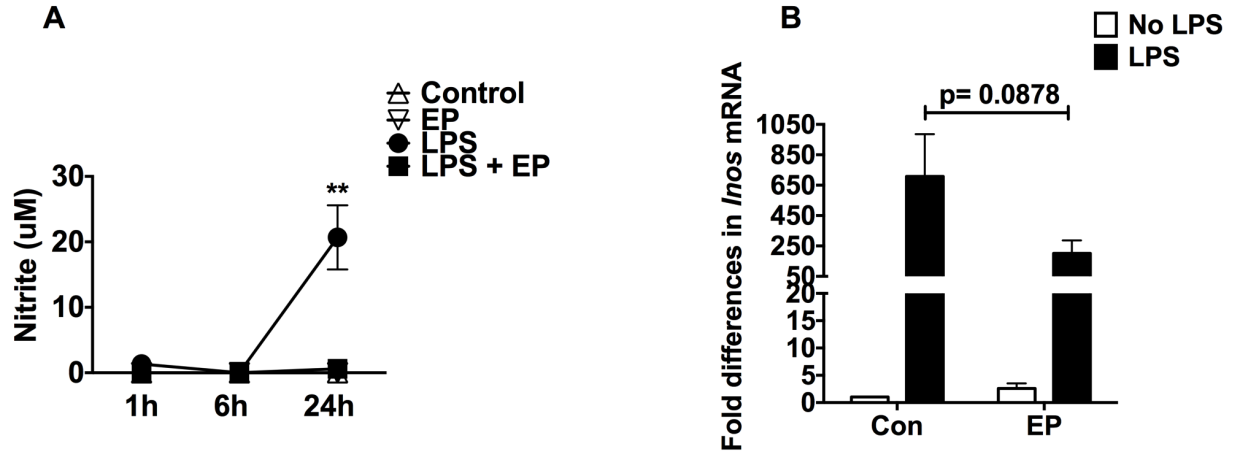
Supplemental Figure 1. Ethyl pyruvate exerts a general level of cytokine production suppression in LPS-stimulated DCs. We cultured DCs in presence of GMCSF for 6-7 days then stimulated them with 10mM EP in presence or absence of 100ng/ml LPS. EP treatments were administered 1h prior **(A-D)** or after **(E)** TLR stimulation. Supernatants were collected at 24h, 48h and 72h post-LPS (**A-D**) and 24h post-EP (**E**) and analyzed for the levels of the indicated cytokines by ELISA. Results are from at least 3 independent experiments (n=3) and are expressed as mean ± SEM. Data was analyzed using unpaired two-tailed Student's t-test with P-values $P<0.05$ considered of statistical significance. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ and **** $P<0.0001$.

Supplemental Figure 2.



Supplemental Figure 2. Ethyl pyruvate alters the LPS-induced shift in metabolism. We performed metabolic assays on DCs pre-treated with EP (10mM) 1 hour before LPS (100ng/ml) stimulation and measured their response to mitochondrial inhibitors: 1μM oligomycin, 1.5μM fluorocarbonyl cyanide phenylhydrazone (FCCP) and 100 nM rotenone plus 1μM antimycin A. Illustrated parameters are **(A)** Basal respiration **(B)** maximal respiration **(C)** proton leak **(D)** ATP production **(E)** percent spare capacity **(F)** percent coupling efficiency and **(G)** non-mitochondrial respiration**.** Results are the average of 4 independent experiments (n=4) and are shown as mean ± SEM. Data was analyzed using One-way ANOVA followed by the Bonferroni multiple comparisons test. P-values $P<0.05$ were considered of statistical significance. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ and **** $P<0.0001$.

Supplemental Figure 3.



Supplemental Figure 3. Delayed ethyl pyruvate treatment inhibits NO production at the later phase of DC activation, thereby attenuating glycolysis. (A) We treated DCs in culture with 100ng/ml LPS followed by 10mM EP an hour later. Supernatants were collected at 1, 6 and 24h post-EP and analyzed for nitrite concentration as a proxy for nitric oxide levels using the Griess reagent assay. (B) Total RNA was extracted from cells to determine *Inos* level of expression by qRT-PCR 6h post-EP using the Ct method. Values were normalized to cyclophillin and expressed as fold difference in mRNA from untreated cells. Results are shown as mean ± SEM and are from 4 independent experiments (n=4). Data was analyzed using unpaired two-tailed Student's t-test with P-values $P<0.05$ considered of statistical significance. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ and **** $P<0.0001$.

STAR Methods

EXPERIMENTAL MODEL AND ANIMAL DETAILS

Mice

Female C57BL/6 (B6) (Cat no. 000664; RRID:IMSR_JAX000664) and C3HeB/FeJ (Cat no. 000658; RRID:IMSR_JAX000658) mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and maintained in our colony in accordance with the guidelines of the Institutional Animal Care and Use Committees of Lewis Katz School of Medicine at Temple University, a member of the American Association for the Accreditation of Laboratory Animal Care-accredited facilities. All mice were housed in autoclaved microisolator cages on ventilated racks and fed sterile rodent chow and water. No more than 5 mice were in the same cage. The facility is a pathogen-free environment with stable temperature, humidity and a 12-hour light and 12-hour dark cycle. Full personal protective equipment was used when handling the animals. Mice were used between 6 and 12 weeks of age. C56BL/6 mice were used to generate DCs from their bone marrow cells. Spleens from C3HeB/FeJ female mice were used as the source of responders in the mixed lymphocyte reaction (MLR) assays.

METHOD DETAILS

In vitro bone marrow-derived DC cultures

Bone marrow precursor cells were flushed from femurs and tibias of B6 mice and differentiated into DCs in presence of granulocyte macrophage-colony stimulating factor (GM-CSF) as previously described (Sriram et al., 2012; Sriram et al., 2014). The cells were seeded as one million per milliliter of complete Iscove's modified Dulbecco's medium (IMDM) (with 10% FBS,

100U/ml penicillin/streptomycin, 200mM L-glutamine and gentamicin) enriched with 1% GM-CSF conditioned medium from a GM-CSF-producing cell line in 24 well plates (GM-CSF collected from the supernatant of a B7H1-GM cell line for GM-CSF conditioned medium). On day 2 of the culture, 1ml of fresh medium was added to each well. Starting day 5 and until the day the culture was used, 1ml per well was replaced with fresh medium every day. The differentiated DCs were stimulated on day 6 or 7 with ethyl pyruvate (EP) 98% (Sigma-Aldrich; Cat no. E47808) diluted in IMDM, and/or 100ng/ml LPS from *Escherichia coli* (Sigma-Aldrich; Cat no. L3755). EP was administered to the cells one hour prior to LPS. In EP dose titration experiments, EP was added to the cells 1h before LPS at the final concentrations of 1mM, 3.4mM, 5mM, 10mM or 20mM. Where indicated, DCs were stimulated with R848 (1ug/ml) (Invivogen; Cat no. tlrl-r848-5) or CpG B (10ug/ml) (Invivogen; Cat no. tlrl-1826) as TLR7 and 9 ligands respectively. In select experiments, EP treatment was delayed and followed LPS stimulation by 1 hour.

Assessment of cell viability by flow cytometry

The generated DCs were harvested at 8, 24, 48 and 72h post-LPS stimulation and stained with Annexin V (BD Bioscience; Cat. no. 556419; RRID:AB_2665412) and 7-AAD (BD Bioscience; Cat no. 559925) to determine cell viability. Briefly, harvested cells were stained with Annexin V in Annexin V-binding buffer for 15 minutes before the addition of 7-AAD. Cells were analyzed on a FACSCanto flow cytometer (BD Bioscience) and FlowJo software was utilized for data analysis (Tree Star, Ashland, OR, USA).

Assessment of dendritic cell activation by flow cytometry

DCs were harvested at 24 and 48h following LPS stimulation and analyzed by flow cytometry for the expression of surface costimulatory markers as well as MHC molecules. Briefly, cells were

incubated with FcγR blocker (purified anti-mouse CD16/CD32, clone 93; Biolegend; Cat no. 101310; RRID:AB_2103871) in PBS with 10% FBS for 15 minutes. They were then stained with fluorochrome-conjugated antibodies against DC surface markers for 30 minutes on ice. The antibodies used were directed against mouse CD11c (N418) (Cat no. 17-0114-82; RRID:AB_469346), CD86 (GL-1) (Cat no. MA1-10300; RRID:AB_11153707) and MHC-II (M5/114.15.2) (Cat no.13-5321-82; RRID:AB_466662) and were all purchased from eBioscience (San Diego, CA). Anti-MHC-II was a biotinylated antibody, hence streptavidin-PerCPCy5.5 (eBioscience, Cat no. 45-4317-82; RRID:AB_10311495) was added to the cells in a secondary step to stain positive DCs. Anti-CD11b (M1/70) (Cat no. 561098; RRID:AB_2033994), anti-CD40 (HM40-3) (Cat no. 553723; RRID:AB_395008) and anti-CD80 (16-10A1) (Cat no. 561955; RRID:AB_10892805) were all from BD Biosciences (San Jose, CA), while anti-MHC-I (H2kb) (28-8-6) (Cat no. 114608; RRID:AB_313599) was from Biolegend (San Diego, CA). Cells were fixed in 2% paraformaldehyde in PBS and analyzed on a FACSCanto flow cytometer (BD Bioscience) with FlowJo software (Tree Star, Ashland, OR, USA). In experiments where EP was added after LPS, flow cytometry was performed 24h after EP treatment.

Measurement of cytokine levels by ELISA

Supernatants were collected from DC cultures at 8, 24, 48 and 72h post-LPS stimulation for the measurement of IL-12p70, TNF-α, IL-6 and IL-10 levels using ELISA kits (BD Pharmingen) according to the manufacturer's protocol. ELISA plates were coated overnight with capture antibody. Plates were blocked with 10% FBS in PBS before cytokine standards and sample supernatants were added in duplicates. Optical densities were measured at 450nm and results analyzed with SoftMax Pro software (Molecular Devices Corporation, Sunnyvale, CA). For

CXCL-10 levels, the R&D ELISA kit was used according to the manufacturer's protocol. For experiments with CpG, R848 and cultures where EP was used post-TLR stimulation, 6h and 24h supernatants post-treatment were collected for ELISA.

Gene expression quantification by q RT- PCR

Gene expression of *in vitro* DCs was analyzed by quantitative reverse transcription PCR (qRT-PCR) using Taqman probes. Total RNA was extracted from cells harvested 1h and 6h after LPS stimulation using the Quick-RNA MiniPrep kit (Zymo Research; Cat no. R1055) according to the manufacturer's protocol. The extracted RNA was reverse transcribed using the High Capacity cDNA RT kit (Applied Biosystems; Cat no. 4368814). Pre-made Taqman primers and probes purchased from Applied Biosystems were used to assess the level of expression of *Ifnb*, *Mx1*, *Isg15*, *Irf7*, *Cxcl10* and *Inos*. Cyclophillin (*Cyc*) was used as the housekeeping gene for normalization. The Ct method of relative quantification of gene expression was used for analysis and the normalized Ct values (against cyclophillin) were calibrated against the control sample (untreated DCs) in each experiment. In experiments where EP was used post-LPS stimulation, qRT-PCR analysis was performed 1 and 6h after EP treatment.

Western blot analysis

Thirty micrograms of denatured total lysate were used in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for Western Blot. Proteins were transferred onto polyvinylidene fluoride (PVDF) membranes and blocked with 5% milk or Bovine serum albumin (BSA) in Tris Buffered Saline +0.1% Tween-20 (TBST). Membranes were incubated overnight at 4°C with primary antibodies against phosphorylated and total ERK 1/2 (p-ERK: Cat no. 4370;

RRID:AB_2315112; Total ERK: Cat no. 9107; RRID:AB_10695739), p38 (p-p38: Cat no. 9211; RRID:AB_331641; Total p38: Cat no. 9212; RRID:AB_330713), JNK 1/2 (p-JNK: Cat no. 9251; RRID:AB_331659; Total JNK: Cat no. 9252; RRID:AB_2250373), AKT (p-AKT: Cat no. 9271; RRID:AB_329825; Total AKT: Cat no. 9272; RRID:AB_329827) as well as total IKBa (Cat no. 4814; RRID:AB_390781) (Cell Signaling) (diluted in 5% BSA in TBST) and primary antibody against total IKBb (Santa Cruz Biotechnology; Cat no. sc-945; RRID:AB_631696) (diluted in 5% milk in TBST). Rabbit anti-mouse (Abcam; Cat no. ab5694; RRID:AB_2223021) and mouse anti-mouse (Santa Cruz Biotechnology; Cat no. sc-8432; RRID:AB_626630) actin were used as loading controls where appropriate. Mouse anti-mouse GAPDH (EMD Millipore; Cat no. MAB374; RRID:AB_2107445) was also used as an extra loading control when probing for ERK proteins. Membranes were then incubated with IR Dye 800 goat anti-rabbit (Cat no. ABIN2169623) and IR Dye 680 donkey anti-mouse (Cat no. ABIN2169633) (LI-COR Biosciences) diluted in blocking buffer plus 0.1% Tween-20. Proteins were visualized by scanning the membranes on an Odyssey Infrared Imaging System (LI-COR Biosciences) in both 700nm and 800nm channels.

Mixed lymphocyte reaction assay

Single cell suspensions were prepared from spleens of C3HeB/FeJ female mice and plated on large petri dishes to allow the Antigen Presenting Cells (APCs) to adhere to the plastic. After 90 minutes of incubation, non-adherent cells were harvested and counted as APC-depleted responders. DCs from B6 female mice were generated in culture as described above and treated on day 6 with or without 10mM EP in the presence or absence of 100ng/ml LPS 1h later. After 24h of stimulation, DCs were washed and added to allogeneic APC-depleted C3HeB/FeJ female splenocytes (800,000 cells per well) in a DC:splenocyte ratio of 1:30. Cells were plated in RPMI-

1640 + 10% FBS+ 0.05mM 2-Mercaptoethanol and 50ug/ml gentamycin. Each condition of DC:splenocyte was run in quadruplicate. B6 DCs alone from each condition and C3HeB/FeJ splenocytes alone served as controls. Plates were incubated at 37°C for 48h. Then cells were pulsed with 1uCi of ^{3}H thymidine for 18 h, harvested and counted with a Tri Carb liquid scintillation counter (PerkinElmer, Waltham, MA) for radioactivity in counts per minute (cpm). Incorporation of ^{3}H thymidine is a measure of splenocyte proliferation post-interaction with the non-syngeneic DCs. Cpm recorded for every condition was subtracted from that of the appropriate control DCs. Stimulation Index was calculated using the formula cpm of a sample / cpm of splenocytes incubated with DCs left untreated in the absence of EP and LPS.

Metabolism assays

Real time analysis of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) was performed using the Seahorse X-96 metabolic extracellular flux analyzer (Seahorse Bioscience). Metabolism assays were run and ECAR and OCR measurements were recorded at an acute (30 min) and 24h time points. For the acute time point, plots were generated based on the values recorded at rate 7 of the assay, which is equivalent to 30 min after addition of LPS or medium for the cells that did not receive LPS. This also coincides with 30 min after the beginning of the Seahorse run. As for the 24h time point, plots were generated based on the values recorded 24h following the addition of LPS or medium for the cells that did not receive LPS. This time point is equivalent to the rate 7 of the assay, coinciding with 30 min after the beginning of the run. For the 24h time point, DCs were generated in culture as previously described and either left untreated, treated with EP (10mM) and/or LPS (100ng/ml) for 24h. The cells were then harvested and plated at a density of 70,000 cells per 70ul per well of XF-96 culture plates previously coated

with poly-d-lysine for adherence. Cells were then washed in XF assay medium (unbuffered RPMI, 10mM glucose, 10% FCS, 100 U/mL penicillin/streptomycin, 2mM L-glutamine, 1% GM-CSF supernatant) and analyzed for OCR and ECAR in response to 1μM oligomycin, 1.5μM fluoro-carbonyl cyanide phenylhydrazone (FCCP) and 100 nM rotenone plus 1μM antimycin A as part of the Seahorse XF Cell Mito Stress Test kit (Agilent, Santa Clara, CA). The use of the Mito Stress Test Kit is an acceptable method for ECAR measurement (Nicholas et al., 2017; Tan et al., 2015; Wang et al., 2013), since it is able to measure the acidification caused by the H^+ ions in the milieu. For the acute time point, DCs were harvested and treated directly in the XF-96 culture plates.

Nitric oxide quantification

Nitrite concentration was measured as a proxy for nitric oxide (NO) in DC culture supernatants collected at 1, 3, 8, 24, 48 and 72h post-LPS stimulation. For experiments with delayed EP treatment, supernatants were analyzed at 1, 6 and 24h after EP treatment. A colorimetric assay using the Griess reagent (Acros Organics; Cat no. AC328670500) was utilized to measure nitrite levels in uM according to the manufacturer's protocol. Absorbance was measured at 550nm and a standard curve was generated.

In vivo EP injection and spleen and lymph node cell staining

C57BL/6 mice were injected i.p. with 80mg/kg of EP in 200ul PBS or PBS vehicle alone, one hour before the injection of 30 ug/mouse of TLR7 ligand R848 in 200ul PBS or PBS vehicle alone. EP was further administered again 4, 8 and 20h after R848 stimulation. Spleens and mesenteric lymph nodes (mLN) were harvested 24h post-R848 stimulation and physically disrupted with a 70um cell strainer to prepare single cell suspensions. Spleens were further digested with DNase I from bovine pancreas (Sigma-Aldrich; Cat no. D4527) and Collagenase Type IV (Worthington

Biochemical Corporation; Cat no. LS004186) treatment followed by Ammonium-Chloride-Potassium (ACK) red blood cell lysis buffer (VWR; Cat no. 118-156-101). Viability of splenic and mLN cells was assessed by either staining with fixable viability dye eFluor780 (eBioscience; Cat no. 65-0865-14) prior to the addition of primary antibody panels or with 7-AAD. Cells were incubated with purified rat anti-mouse CD16/CD32 monoclonal antibody (clone 2.4G2; BD Biosciences; Cat no. 553142; RRID:AB_394657) prior to the addition of fluorochrome-conjugated antibodies against conventional DC/inflammatory monocyte surface markers. Cells were stained with eBioscience rat anti-mouse CD11c (N418), CD86 (GL-1) and Ly-6C (HK1.4) (Cat no. 25-5932-82; RRID:AB_2573503) as well as BD Biosciences rat anti-mouse CD11b (M1/70). Cells not stained with 7-AAD were fixed with 1% paraformaldehyde. Samples were acquired on a FACSCanto Cytometer then analyzed with FlowJo.

QUANTIFICATION AND STATISTICAL ANALYSIS

Prism 6 (GraphPad software, San Diego, CA, USA) was used for data analysis. Means and Standard Errors (Mean ± SEM) were calculated by averaging results from independent experiments. Throughout the manuscript, "n" refers to independent cell cultures from individual mice, when each culture may have two-three biological replicate. Statistical significance was determined using unpaired two-tailed Student's t-test or ratio paired two-tailed Student's t-test for comparison between two groups. One-way ANOVA was used as appropriate for multiple comparisons in one group followed by the Bonferroni multiple comparisons post-hoc correction test. Two-way ANOVA was used as appropriate for multiple comparisons between two groups followed by the Sidak multiple comparisons post-hoc correction test. P-values of $p < 0.05$ (marked

in the figures as * P<0.05, ** P<0.01, *** P<0.001 and **** P<0.0001) were considered significant.

STAR methods:

Key Resource Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
LEAF purified rat monoclonal anti-mouse CD16/CD32 (clone 93)	Biolegend	Cat # 101310; RRID:AB_2103871
APC Armenian hamster monoclonal anti-mouse CD11c (clone N418)	eBioscience	Cat # 17-0114-82; RRID:AB_469346
FITC rat monoclonal anti-mouse CD86 (clone GL-1)	eBioscience	Cat # MA1-10300; RRID:AB_11153707
Biotinylated rat monoclonal anti-mouse MHC-II (I-A/I-E) (clone M5/114.15.2)	eBioscience	**Cat #** 13-5321-82; RRID:AB_466662
Streptavidin-PerCPCy5.5	eBioscience	Cat # 45-4317-82; RRID:AB_10311495
PeCy7 rat monoclonal anti-mouse CD11b (clone M1/70)	BD Biosciences	Cat # 561098; RRID:AB_2033994
FITC Armenian hamster monoclonal anti-mouse CD40 (clone HM40-3)	BD Biosciences	Cat # 553723; RRID:AB_395008
PE Armenian hamster monoclonal anti-mouse CD80 (clone 16-10A1)	BD Biosciences	Cat # 561955; RRID:AB_10892805
PE mouse monoclonal anti-mouse MHC-I (H-2K^{b}/H-2D^{b}) (clone 28-8-6)	Biolegend	Cat # 114608; RRID:AB_313599
Purified rat monoclonal anti-mouse CD16/CD32 (clone 2.4G2)	BD Biosciences	Cat # 553142; RRID:AB_394657
PeCy7 Ly-6C rat monoclonal anti-mouse antibody (clone HK1.4)	eBioscience	Cat # 25-5932-82; RRID:AB_2573503
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) rabbit anti-mouse monoclonal antibody (clone D13.14.4E) XP®	Cell Signaling	Cat # 4370; RRID:AB_2315112

Total p44/42 MAPK (ERK1/2) mouse anti-mouse monoclonal antibody (clone 3A7)	Cell Signaling	Cat # 9107; RRID:AB_10695739
Phospho-SAPK/JNK (Thr183/Tyr185) rabbit anti-mouse polyclonal antibody	Cell Signaling	Cat # 9251; RRID:AB_331659
Total SAPK/JNK rabbit anti-mouse polyclonal antibody	Cell Signaling	Cat # 9252; RRID:AB_2250373
Phospho-p38 MAPK (Thr180/Tyr182) rabbit anti-mosue polyclonal antibody	Cell Signaling	Cat # 9211; RRID:AB_331641
Total p38 MAPK rabbit anti-mouse polyclonal antibody	Cell Signaling	Cat # 9212; RRID:AB_330713
Total IkBa (Amino-terminal antigen) mouse anti-mouse monoclonal antibody (clone L35A5)	Cell Signaling	Cat # 4814; RRID:AB_390781
Total IkBb (C-20) rabbit anti-mouse polyclonal antibody	Santa Cruz Biotechnology	Cat # sc-945; RRID:AB_631696
Phospho-AKT (Ser473) rabbit anti-mouse polyclonal antibody	Cell Signaling	Cat # 9271; RRID:AB_329825
Total AKT rabbit anti-mouse polyclonal antibody	Cell Signaling	Cat # 9272; RRID:AB_329827
Rabbit anti-mouse polyclonal anti-alpha smooth muscle Actin	Abcam	Cat # ab5694; RRID:AB_2223021
Mouse anti-mouse monoclonal Actin (C-2) antibody	Santa Cruz Biotechnology	Cat # sc-8432; RRID:AB_626630
Mouse anti-mouse monoclonal GAPDH antibody (clone 6C5)	EMD Millipore	Cat # MAB374; RRID:AB_2107445
Goat anti-rabbit IgG (heavy and light chain) polyclonal antibody (IRDye800CW)	LI-COR Biosciences	Cat # ABIN2169623
Donkey anti-mouse IgG (heavy and light chain) polyclonal antibody (IRDye680RD)	LI-COR Biosciences	Cat # ABIN2169633
Chemicals, Peptides, and Recombinant Proteins		
FITC Annexin V	BD Biosciences	Cat # 556419; RRID:AB_2665412
Annexin V binding buffer	BD Biosciences	Cat # 556454

7-AAD	BD Biosciences	Cat # 559925
Corning™ Cellgro™ Iscove's modification of DMEM medium	Fisher Scientific	Cat # MT10016CV
Ethyl pyruvate 98%	Sigma-Aldrich	Cat # E47808
LPS from (*Escherichia coli* Serotype O26:B6)	Sigma-Aldrich	Cat # L3755
R848 (Resiquimod)	Invivogen	Cat # tlrl-r848-5
CpG B (ODN 1826)	Invivogen	Cat # tlrl-1826
Dulbecco's Phosphate-Buffered Saline (DPBS), (Corning®)	VWR	Cat # 45000-436
16% Paraformaldehyde (Formaldehyde) Aqueous solution, EM Grade	Thomas Scientific	Cat # 15710
Taqman universal PCR master mix	Applied Biosystems	Cat # 4364338
Bovine serum albumin standard grade	Gemini	Cat # 700-100P
UltraPure™ 1M Tris-HCl, pH 8.0	Thermo Fisher Scientific	Cat # 15568025
Tween-20	Sigma-Aldrich	Cat # P1379-1L
LDS sample buffer 4x NuPAGE	Life Technologies	Cat # NP0007
NuPAGE™ 10% Bis-Tris protein gels, 1.0mm, 12-well	Life Technologies	Cat # NP0302BOX
RPMI 1640, Corning®	VWR	Cat # 45000-396
Gibco™ 2-Mercaptoethanol (55mM)	Fisher Scientific	Cat # 21985023
EMD Millipore™ Poly-D-Lysine solution	Fisher Scientific	Cat # A003E
RPMI 1640 medium, powder (unbuffered)	Thermo Fisher Scientific	Cat # 31800022
D-(+)-Glucose	Sigma-Aldrich	Cat # G8270-100G
DNase I from Bovine pancreas	Sigma-Aldrich	Cat # D4527
Collagenase Type IV	Worthington Biochemical Corporation	Cat # LS004186
Ammonium-Chloride-Potassium (ACK) lysis buffer	VWR	Cat # 118-156-101
Fixable viability dye eFluor780	eBioscience	Cat # 65-0865-14
Critical Commercial Assays		
Mouse IL-12(p70) ELISA Set	BD Biosciences	Cat # 555256
Mouse IL-6 ELISA Set	BD Biosciences	Cat # 555240

Mouse IL-10 ELISA Set	BD Biosciences	Cat # 555252
Mouse TNF (Mono/Mono) ELISA Set	BD Biosciences	Cat # 555268
Mouse CXCL-10/IP-10/CRG-2 DuoSet ELISA kit	R&D Systems	Cat # DY466
Quick-RNA Miniprep kit	Zymo Research	Cat # R1055
High Capacity cDNA Reverse Transcription kit	Applied Biosystems	Cat # 4368814
Griess reagent	Acros Organics	Cat # AC328670500
Seahorse XF Cell Mito Stress Test Kit	Agilent	Cat # 103015-100
Experimental Models: Organisms/Strains		
C57BL/6	Jackson Laboratory	Cat # 000664; RRID:IMSR_JAX000664
C3HeB/FeJ	Jackson Laboratory	Cat # 000658; RRID:IMSR_JAX000658
Softwares and Algorithms		
FlowJo Software	TreeStar	N/A
Prism V 6.0	Graphpad Prism	N/A
SoftMax Pro Software	Molecular Devices Corporation	N/A
Other		
Fetal Bovine Serum (FBS) (A89C05C)	Gemini Bio Products	Cat # 100-106
Penicillin/Streptomycin 100X	VWR	Cat # 45000-652
L-Glutamine liquid	VWR	Cat # 45000-676
Gentamycin	Thermo Fisher Scientific	Cat # 15750-060
cxcl10 Taqman Gene Expression Assay primer	Applied Biosystems	Mm00445235_m1
cyc (ppia) Taqman Gene Expression Assay primer	Applied Biosystems	Mm02342430_g1
ifnb Taqman Gene Expression Assay primer	Applied Biosystems	Mm00439546_s1
inos Taqman Gene Expression Assay primer	Applied Biosystems	Mm00440502_m1
irf7 Taqman Gene Expression Assay primer	Applied Biosystems	Mm00516788_m1
isg15 Taqman Gene Expression Assay primer	Applied Biosystems	Mm01705338_s1
mx1 Taqman Gene Expression Assay primer	Applied Biosystems	Mm00487796_m1
Immun-Blot low fluorescence PVDF membrane	Biorad	Cat # 1620264

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to the Lead Contact Stefania Gallucci (gallucci@temple.edu).