

Supplementary Figures Legends

Figure S1. PA alters expression of cytokines and chemokines in PRR-activated DC and macrophages. Related to Figure 1.

(A-B) Expression of *Il6* and *Il12a* mRNAs in GM-DC activated by indicated TLR ligands during 24 hr in fatty acid-free medium (Control) or in the presence of PA. n = 3 per group. (C) Expression of cytokine and chemokine genes in GM-DC treated as in (A-B), analyzed by microarray. Data are shown as heat map with relative expression values (z-score). (D) Expression of *Il23a* mRNA in GM-DC treated with curdlan, furfuran, TNF or IL-1 β in fatty acid-free medium (Control) or in the presence of PA for 24 hr. n = 4 per group. (E) Expression of *Il23a* mRNA in BMDC treated with IMQ or LPS in fatty acid-free medium (Control) or in the presence of PA for 24 hr. n = 4 per group. Data are shown as mean \pm SEM. *P < 0.05, **P < 0.01, ***P < 0.001 (unpaired Student's t test).

Figure S2. PA-mediated regulation of IL-23 expression in DC and macrophages. Related to Figure 1.

(A) IL-23p19 secretion by Tlr4^{+/+} and Tlr4^{-/-} GM-DC activated by IMQ or LPS during 24 hr in the presence of 0.5 mM PA. n = 3-4 per group. (B) *Il23a* mRNA expression in GM-DC activated with IMQ and PA for 2 or 24 hr with/without NF- κ B inhibitory peptide SN50. n = 3 per group. (C) WB analysis of JNK and p-JNK in GM-DC treated with IMQ and PA for 24 hr. n = 3 per group. (D) *Mapk8*, *Mapk9*, and *Il23a* mRNA expression in GM-DC transfected with siRNA against *Mapk8* and *Mapk9* and 48 hr later activated with IMQ and PA for 24 hr. n = 4 per group. (E) IL-23p19 secretion by GM-DC activated with IMQ in the presence of PA or OA for 24 hr. n = 4 per group. (F) *Il23a* mRNA expression, IL-23p19 secretion, and lactate secretion by GM-DC activated by IMQ during 24 hr in fatty acid-free medium (Control) or in the presence of indicated concentrations of PA. n = 3 per group. (G) Lactate secretion from GM-DC treated as in Figure S1D. (H-I) Real time changes of ECAR (H) and OCR (I) measured by the extracellular flux analyzer in GM-DC incubated in fatty acid-free medium in the presence on glucose after administration of IMQ alone or IMQ plus PA. Time of injection of compounds during the test is indicated by arrow. n = 4 per group. (J) Lactate secretion from BMDM treated as in Figure S1E. (K-L) GM-DC activated by IMQ in fatty acid-free medium or in the presence of PA during 24 hr. Representative flow cytometric analysis of 2-NBDG uptake (K), mitochondrial content measured by staining with Mitotracker Green (L). Data from one of three reproducible independent experiments are shown. (M) Activity of electron transport chain complexes in permeabilized cells after addition of indicated substrates and inhibitors measured as oxygen consumptions by Oxygraph-2 k high-resolution respirometry in GM-DC activated by IMQ in fatty acid-free medium or in the presence of PA and/or etomoxir during 24 hr. n = 4 per group. (N) Relative ATP production and ATP/ADP ratio in GM-

DC activated by IMQ in fatty acid-free medium or in the presence of PA during 24 hr. n = 4 per group. (O) Hexokinase activity in GM-DC treated with IMQ for 24 hr and after that incubated with 0.5 mM of indicated FA for 2 hr. PA, palmitic acid (C16:0); 2Me-PA, 2-methylpalmitic acid (2Me-C16:0); OA, oleic acid (C18:1); LA, linoleic acid (C18:2); OCTA, octanoic acid (C8:0). n = 4 per group. (P) WB analysis of hexokinase 1 (HK1) protein in total cell lysates and isolated mitochondria and the cytosol from GM-DC activated with IMQ and PA for 24 hr. n = 3 per group. (Q) ECAR in GM-DC activated with IMQ with/without triacsin C for 24 hr and injected with BSA or PA during the “Seahorse” assay. n = 4 per group. Data are shown as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 (unpaired Student’s t test or one-way ANOVA). NS – not significant.

Figure S3. FAO-independent IL-23 expression in DC. Related to Figure 1.

(A-C) Cpt1a (A), Il-23a (B) mRNAs expression, and HK activity (C) in Cpt1a^{fl/fl} and Cpt1a^{tgax/tgax} GM-DC activated with IMQ and PA for 24 hr. (D-F) Cpt1a (D), Cpt2 (E), and Il23a (F) mRNA expression GM-DC transfected with control siRNA or siRNAs against Cpt1a and Cpt2, treated with or without 3 μM etomoxir (Eto) and activated with IMQ and PA for 24 hr. (G) Cpt1a mRNA expression in BMDC activated with IMQ and PA for indicated time. (H) ¹⁴CO₂ production and accumulation of ¹⁴C in the acid-soluble metabolite fraction from GM-DC treated with IMQ and PA for 20 hr and incubated with ¹⁴C-PA for consecutive 4 hr. (I) Relative abundance of intracellular PA measured by GC-MS in GM-DC treated with or without IMQ for 20 hr and incubated with PA for consecutive 4 hr. n = 3-6 per group. Data are shown as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 by t-test.

Figure S4. Interaction between IMQ and PA results in rewiring of glycolysis and TCA cycle metabolism in DC. Related to Figures 1-3.

(A-O) GM-DC activated by IMQ in fatty acid-free medium or in the presence of PA during 24 hr. Relative expression of genes controlling glycolysis (A) and TCA cycle metabolism (G) analyzed by microarray. Relative abundance of metabolites implicated in glycolysis (B-F) and TCA cycle (H-O) measured by LC-MS and GC-MS. (P) Schematic diagrams of gene and metabolite alterations in GM-DC activated by IMQ with/without PA. Up-regulated genes/metabolites are shown as red, down-regulated – blue. G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; R5P, ribose 5-phosphate; S7P, sedoheptulose 7-phosphate; F1,6BP, fructose 1,6-bisphosphate; G3P, glyceraldehyde 3-phosphate; 1,3BPG, 1,3-bisphosphoglyceric acid; 3PG, 3-phosphoglyceric acid; 2PG, 2-phosphoglyceric acid; PEP, phosphoenolpyruvic acid. Results from n = 4-6 per group. (Q) Il23a mRNA expression in Acod1^{+/+} and Acod1^{-/-} GM-DC treated with IMQ and PA for 24 hr. n = 4 per group. Data are shown as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 (unpaired Student’s t test).

Figure S5. Effects of PA to glycolysis and the PPP in DC. Related to Figure 3.

(A-B) ECAR (A) and OCR (B) in GM-DC activated by IMQ with/without mitoTEMPO for 24 hr and injected with PA during the “Seahorse” assay. n = 4 per group. (C-F) Lactate secretion (C), HK activity (D), GAPDH activity (E), G6PDH activity (F) in GM-DC activate by IMQ and PA with/without mitoTEMPO for 24 hr. n = 3-4 per group. (G-K) Relative level of ribose-5-phosphate (G), carbon flux from 1,2-¹³C-glucose into ribose-5-phosphate (H), relative level of sedoheptulose-7-phosphate (I), carbon flux from 1,2-¹³C-glucose into sedoheptulose-7-phosphate (J) and schematic representation of the flux (I) in GM-DC treated with IMQ and PA for 24 hr. n = 3-6 per group. (L) NADP⁺/NADPH ratios in GM-DC treated with IMQ and PA for indicated time. n = 4 per group. Data are shown as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 (unpaired Student's t test or one-way ANOVA).

Figure S6. HFD feeding exacerbates psoriasis-like inflammation through accumulation of IL-23⁺ cDC in iLN and increased IL-23 signaling in the skin. Related to Figures 2 & 4.

(A-G) Male mice were treated daily by application of vehicle or IMQ to shaved abdominal skin during 5 days and fed CD or HFD. Schematic design of this experiment (A). Concentration of non-esterified fatty acids (NEFAs) in plasma after day 5 of treatment (B). Representative MGG staining of abdominal skin sections (scale bar 50 μm) (C). Average epidermal thickness of abdominal skin (D). GSEA of genes from KEGG OXPHOS pathway in cDC sorted from iLN and analyzed by microarray (E). Proportion of mitoSOX⁺ cDC in iLN (F). Volcano plots with log₂ fold change versus log₁₀ P-value of all transcripts (GSE69750, gray dots), including 1288 significantly up-regulated transcripts after intradermal injection of IL-23 (GSE50400, fold change (FC) > 2, q-value < 0.05) (red dots). Numbers of up- and down-regulated among the 1288 transcripts, and statistical significance levels of up-regulation of this entire IL-23-dependent set of transcripts are shown (G). (H) Mice were treated daily by application of IMQ to shaved abdominal skin during 5 days, injected IP with 3 mg anti-IL-23p19 neutralizing antibody or control IgG at 1st and 4th days, and fed CD or HFD. Average epidermal thickness in abdominal skin. Data are pooled from two independent experiments and shown as mean ± SEM. n = 4-12 mice per group. (I-K) Irradiated C57BL/6 mice reconstituted with bone marrow from Cpt1a^{+/+} or Cpt1a^{Zbtb46/Zbtb46} mice and fed 6 weeks post-irradiation with CD or HFD and epicutaneously treated with vehicle- or IMQ-containing cream for 5 days. Macroscopic aspect of lesions and disease activity score (I). MGG staining of lesions (J). Numbers and per cent IL-23⁺ cDC (K) in iLN. n = 2-8 mice per group. Data are shown as mean ± SEM or box plots with median ± first-third quartiles. (L-M) GM-DC treated with IMQ and PA in the presence or absence of metformin during 24 hr. Oxygen consumption rate (OCR) and mitochondrial respiration (L), *Prkaa1*, *Prkaa2*, and *Ii23a* mRNA

expression and IL-23p19 protein secretion (**M**). (**N**) mRNA expression of indicated genes in the skin from mice treated as in Figure 2D. n = 2-4 mice per group. (**O**) Pfkf, Pfkp, Il23a mRNA expression and lactate secretion in GM-DC transfected with siRNA against Pfkf and Pfkp or control siRNA and 48 hr later treated with IMQ and PA for 24 hr. n = 4 per group. Data are shown as mean \pm SEM. *P < 0.05, **P < 0.01, ***P < 0.001 (unpaired Student's t test or two-way ANOVA with Tukey's *post-hoc* test). NS – not significant.

Figure S7. PA increases the UPR in IMQ-activated DC. Related to Figures 5-7.

(**A**) Heat-map with relative expression of genes from GO:0030968 “Endoplasmic reticulum unfolded protein response” analyzed by microarray analysis in GM-DC activated by IMQ in fatty acid-free medium or in the presence of PA during 24 hr. n = 4 per group. (**B**) Quantification of WB from Figure 5A. n = 5 per group. (**C**) Heat-map with relative expression of genes implicated in the UPR analyzed by microarray analysis in cDC isolated from iLN from mice treated with IMQ and fed CD or HFD. n = 4 mice per group. (**D-E**) GM-DC differentiated from the ER stress activated indicator (ERAI) mice. Representative flow cytometric analysis (**D**) and proportion (**E**) of Xbp1s-venus⁺ GM-DC treated with IMQ and PA during 24 hr. n = 2 per group. #P < 0.05 vs. control groups and *P < 0.05 vs. IMQ groups. (**F**) Glycolytic and mitochondrial activities in Xbp1^{fl/fl} and Xbp1^{CD11c/CD11c} GM-DC activated with IMQ for 24 hr. n = 5 per group. (**G**) Atf4 and Il23a mRNA expression in GM-DC transfected with siRNA against Atf4 or control siRNA and 48 hr later activated by IMQ and PA for 24 hr. n = 4 per group. (**H**) Xbp1^{fl/fl}, Xbp1^{CD11c/CD11c}, and Ddit3^{-/-}Xbp1^{CD11c/CD11c} GM-DC activated by IMQ in fatty acid-free medium or in the presence of PA during 24 hr. Deletion-specific qPCR for Xbp1 and Ddit3 mRNA expression. n = 4 per group. (**I**) Il6 mRNA expression in GM-DC activated by IMQ with or without PA during 24 hr in the presence of rotenone or mitoTEMPO. n = 5 per group. (**J**) Il6 mRNA expression in Xbp1^{fl/fl}, Xbp1^{CD11c/CD11c} and Ddit3^{-/-}Xbp1^{CD11c/CD11c} GM-DC activated by IMQ with or without PA during 24 hr. n = 4 per group. (**K**) Expression of genes from the mitochondrial UPR transcriptional signature in GM-DC. (**L**) WB analysis and quantification of ATF5 in isolated mitochondria from GM-DC. n = 2 per group. (**M-N**) Il23a, Atf5, and Atf3 mRNA expression in GM-DC transfected with control siRNA or siRNAs against Atf5 or Atf3 and 48 hr later activated by IMQ and PA for 24 hr. n = 4 per group. Data are shown as mean \pm SEM. ND – not detected. *P < 0.05, **P < 0.01, ***P < 0.001 (unpaired Student's t test), #P < 0.05 vs. PA groups (unpaired Student's t test).

Supplemental Table Legends

Supplemental Table S1. Sequences for primers used in RT-qPCR experiments. Related to Figures 1-3 & 5-6 and STAR Methods.

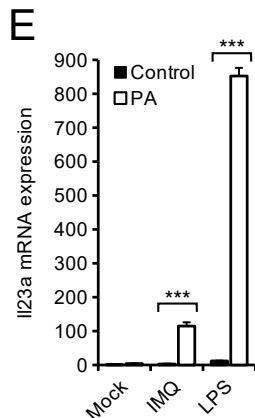
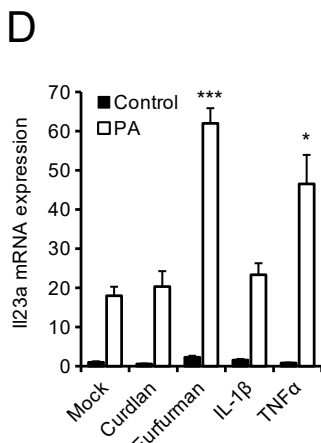
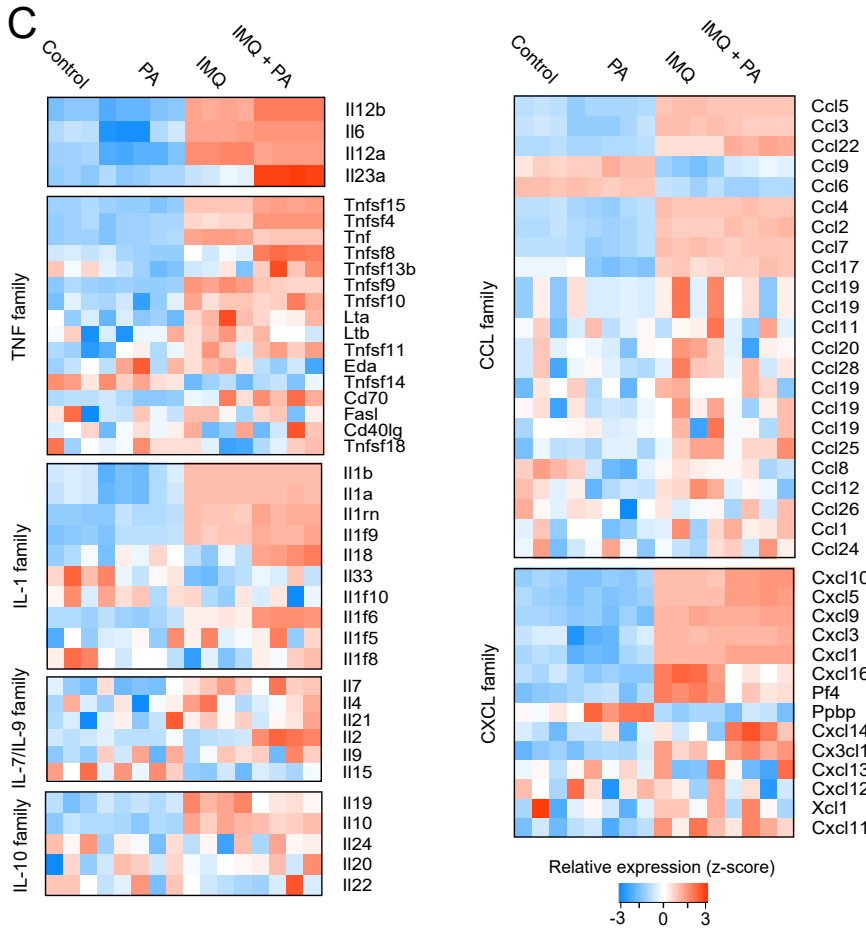
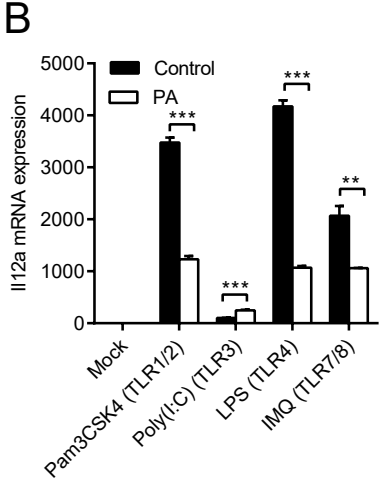
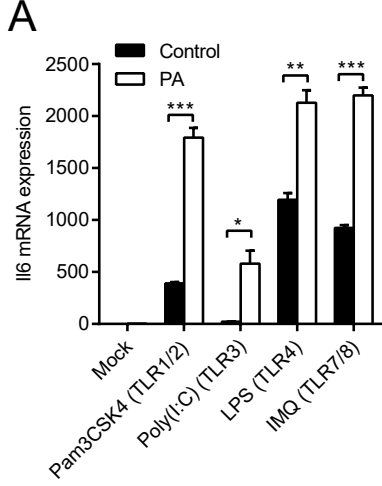
Supplemental Table S2. Sequences for primers used in ChIP–qPCR experiments. Related to Figure 6 and STAR Methods.

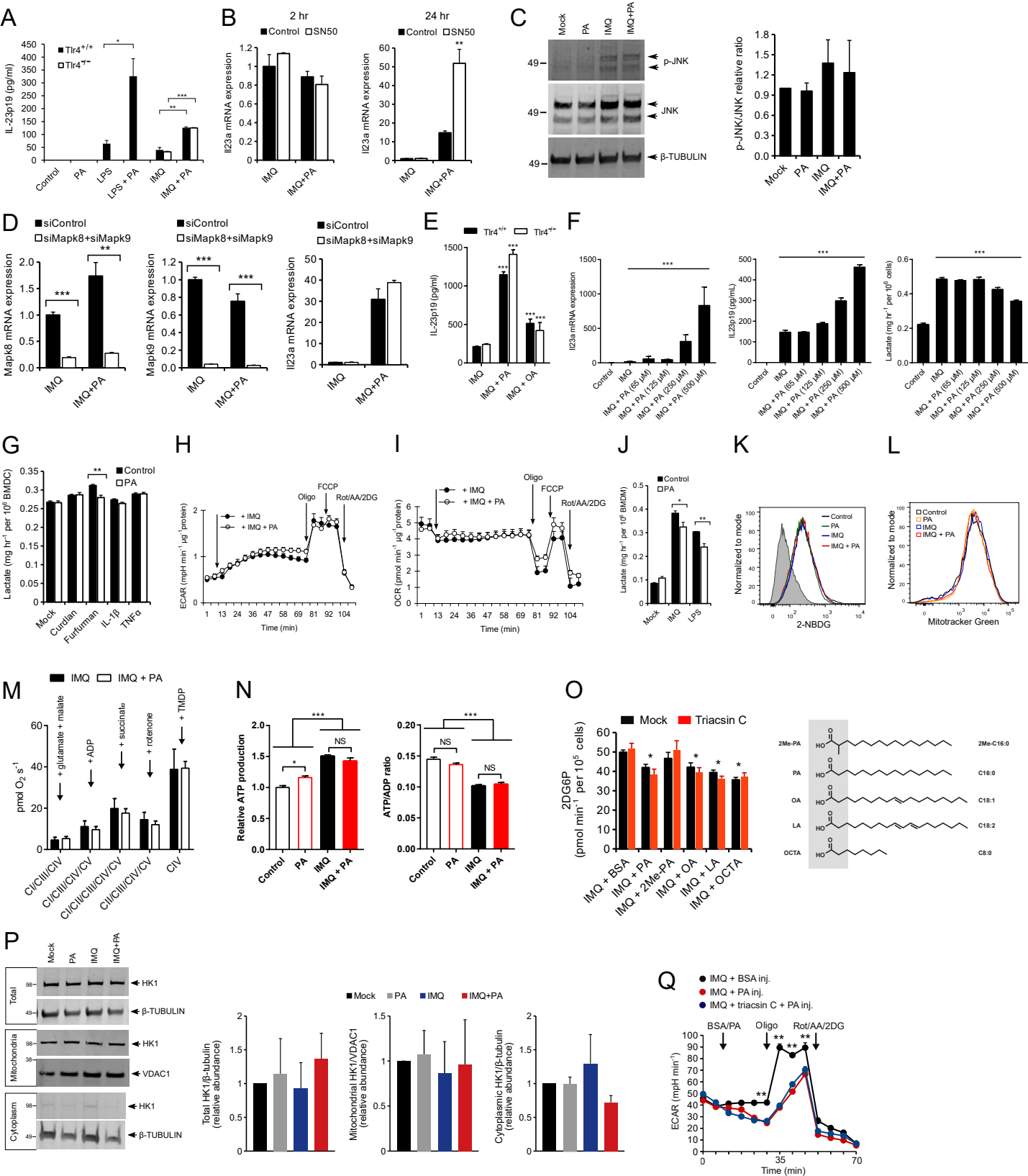
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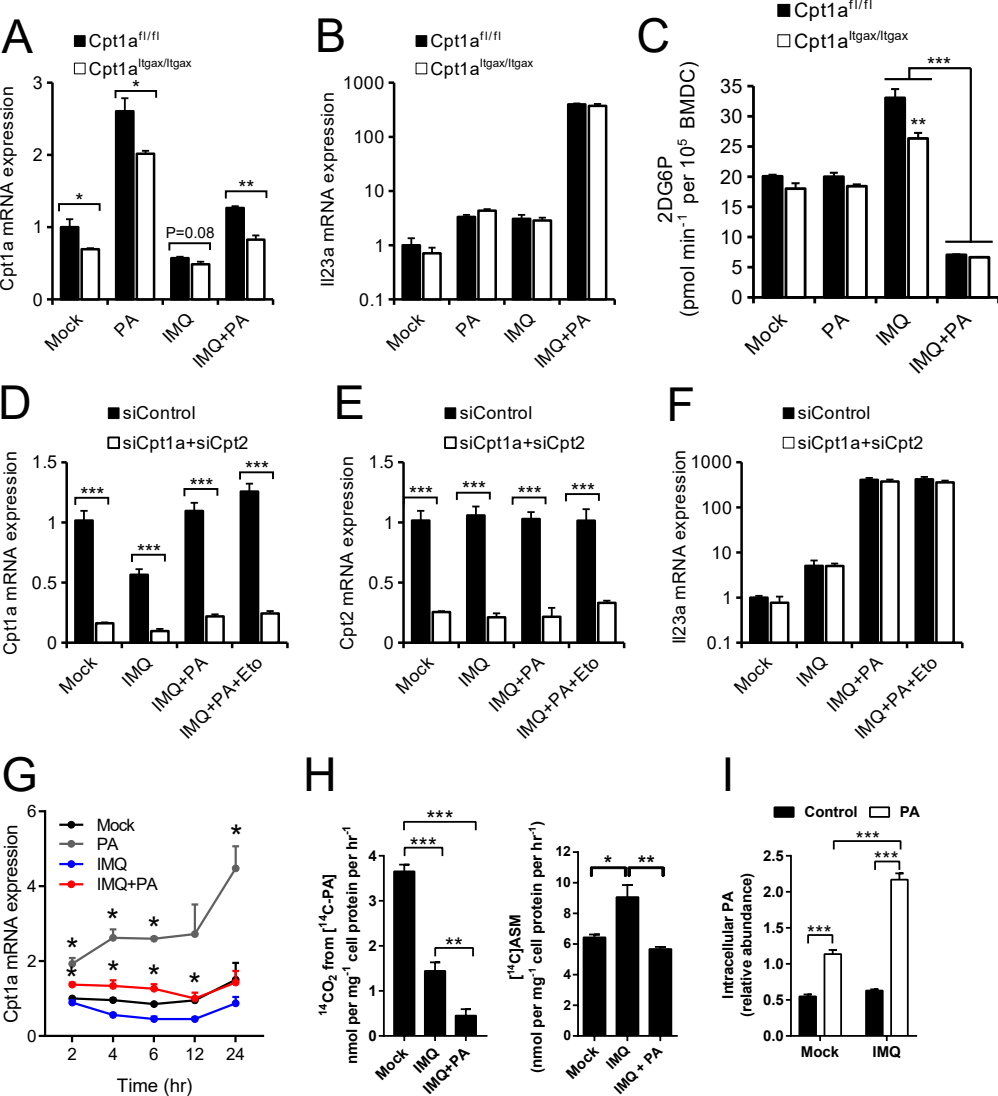
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Il23a	CCAGCAGCTCTCTCGGAATC	AAGCAGAACTGGCTGTTGTC
Rpl4	CTGAACCCTTACGCCAAGAC	CCTTCTCGGATTTGGTTGCC
Pfkfb3	CCGCTCTCATCTGTCTCGTG	CCCACAGGATCTGGGCAAC
Hspa5	TGCAGCAGGACATCAAGTTC	TACGCCTCAGCAGTCTCCTT
Ddit3	CCTAGCTTGGCTGACAGAGG	CTGCTCCTTCTCCTTCATGC
Xbp1	CCTGAGCCCGGAGGAGAA	CTCGAGCAGTCTGCGCTG
Xbp1s	GAGTCCGCAGCAGGTG	GTGTCAGAGTCCATGGGA
Il6	CCACGGCCTTCCCTACTTCA	CCACGATTTCCAGAGAACATG
Il12a	CAAACCAGCACATTGAAGA	AGCTCCCTCTTGTTGTGGAA
Hif1a	CGCCTCTGGACTTGTCTCTT	TCGACGTTCAGAACTCATCCT
Prkaa1	ACCTGAGAACGTCCTGCTTGATGC	CACCTCGGGCCTGCGTACA
Prkaa2	TGCTGGATGCCAGATGAACGC	CCTCAGGTGCTGCATAATTTGGCG
Gls	TGCGAACATCTGATCCCAGG	TGAATTTGGCCAGCTGAGGA
Gls2	CTCGACTTGGTGACCTGCTT	GCACAATGTTGCTGCTCACA
Mapk8	GTCATTCTCGGCATGGGCTA	CTGGGAACAAAACACCACCTT
Mapk9	CTGGGCATGGGCTACAAAGA	TGAACTCTGCGGATGGTGTT
Cpt1a	ACGTTGACGAATCGGAACA	CCATGCAGCAGAGATTTGGC
Cpt2	CAACTCGTATACCCAAACCCAGTC	GTTCCCATCTTGATCGAGGACATC
Pfkl	CCGCTGTAAGGCCTTCACTACGAG	TTATCGATGGAGCCCACCAGACC
Pfklp	CCCATGGTTATGGTTCCTGCT	GTCCCACTGGCTGACTGTTT
Atf4	GTGGCCAAGCACTTGAAACC	GGAAAAGGCATCCTCCTTGC
Atf5	GGGCACGGCTAGAAGGAAAT	AGGCAGCGTGGAAAGATTGTT
Atf3	CTTCCCCAGTGGAGCCAATC	TCATTTTGCTCCAGTCTTCGC
Krt16	CGGCCCACTGAGATCAAAGA	AGCTCATTCTCGTACTTGGTCC
Sprr1b	CCAGCGACCACACTACCTGT	TTGTTGCTCATGCAACTGTGG
S100a8	CCGTCTTCAAGACATCGTTTGA	GTAGAGGGCATGGTGATTTCCCT
S100a9	AATGGTGAAGCACAGTTGG	CTGGTTTGTGTCCAGGTCCTC
Il1f9	TACAGCTTGGGGAAGGGAACA	TAGAGCAGACAGCGATGAACC
Lce3c	TCAGCACAGCCTTCTTCTCC	GCACTTTGGGGAGCACTTTG

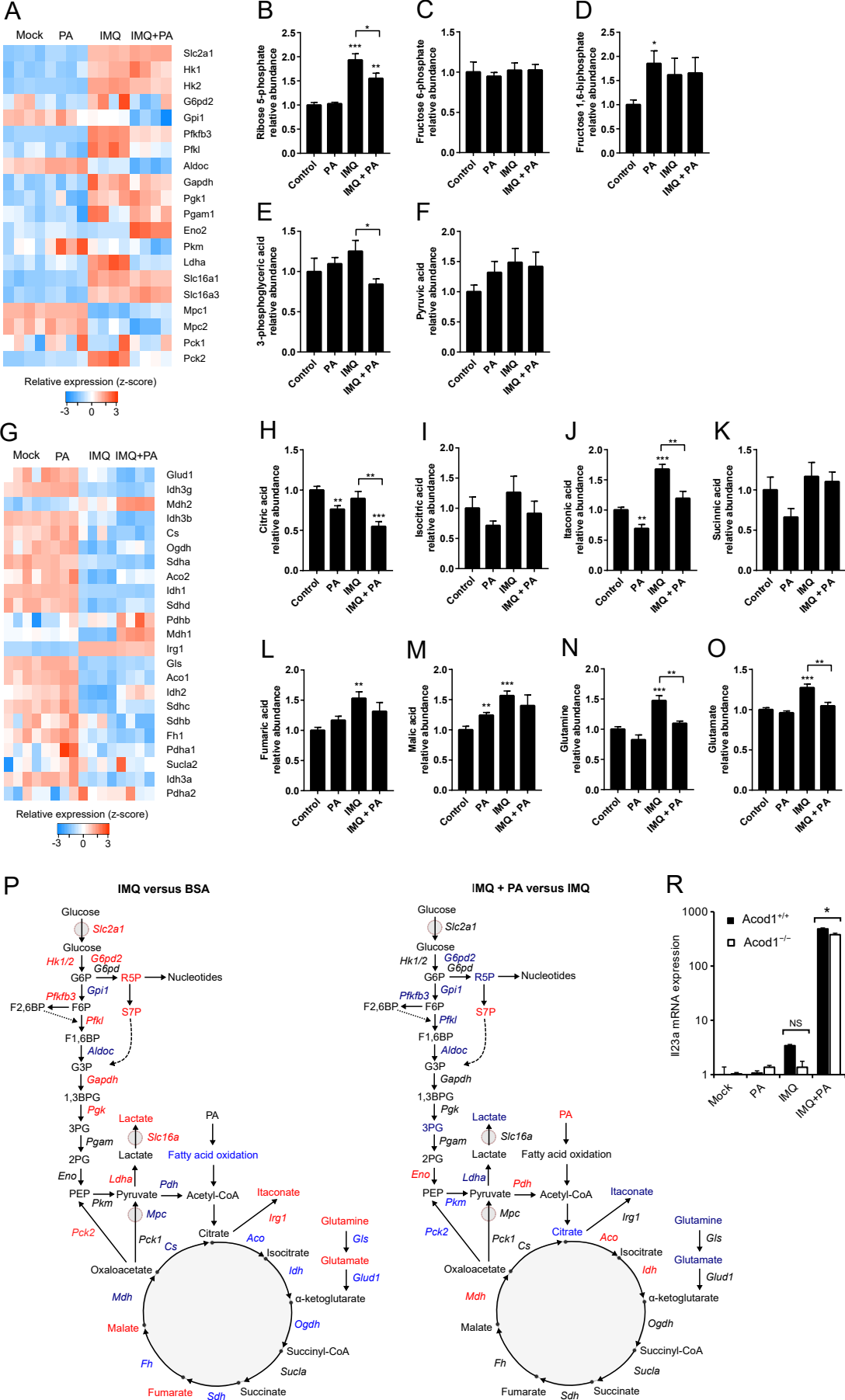
Supplemental Table S2

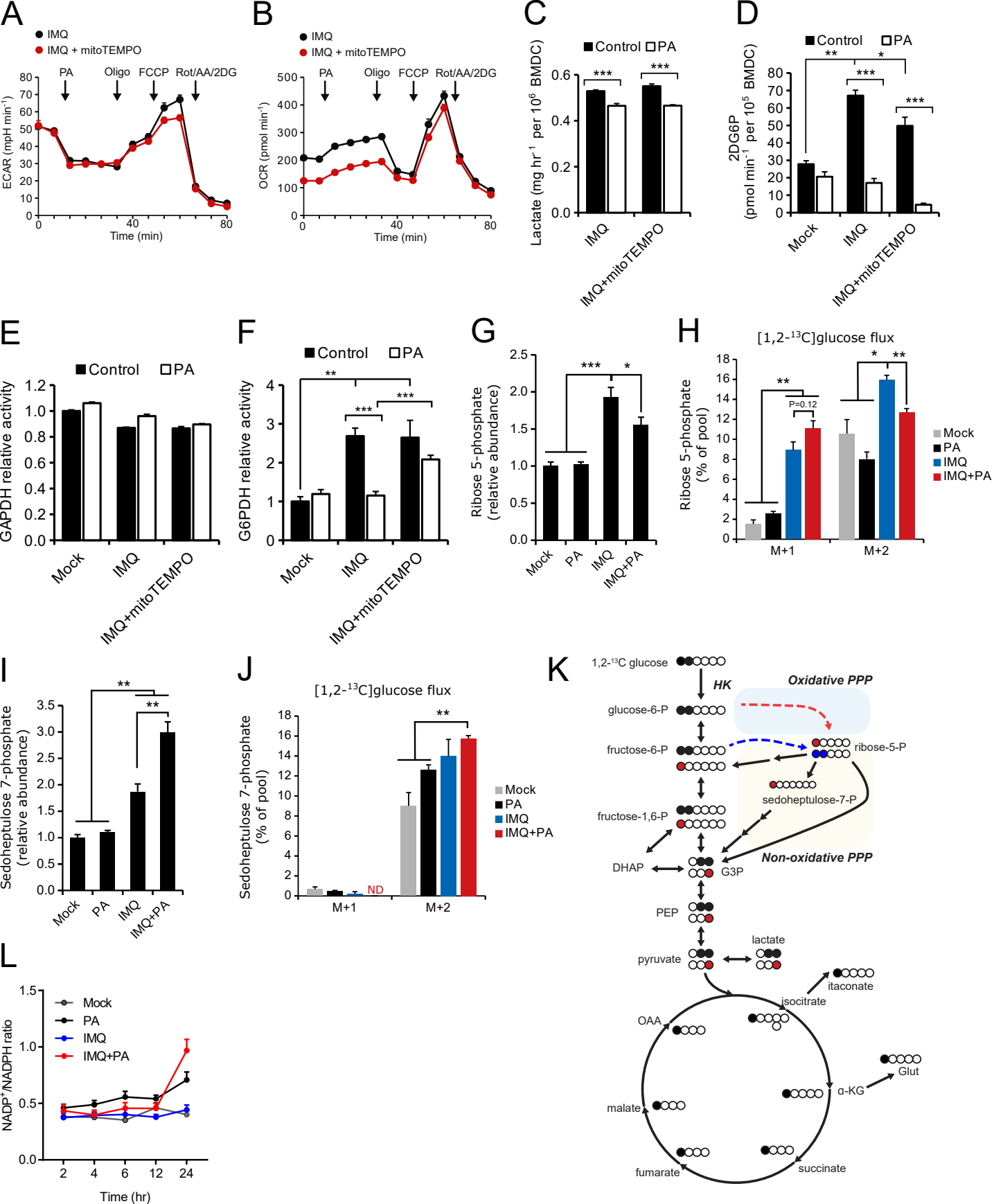
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Il23a-ChIP-SiteB	CCTCTAGCCACAACAACCTC	CCTTCACACTAGCAGGTGACT
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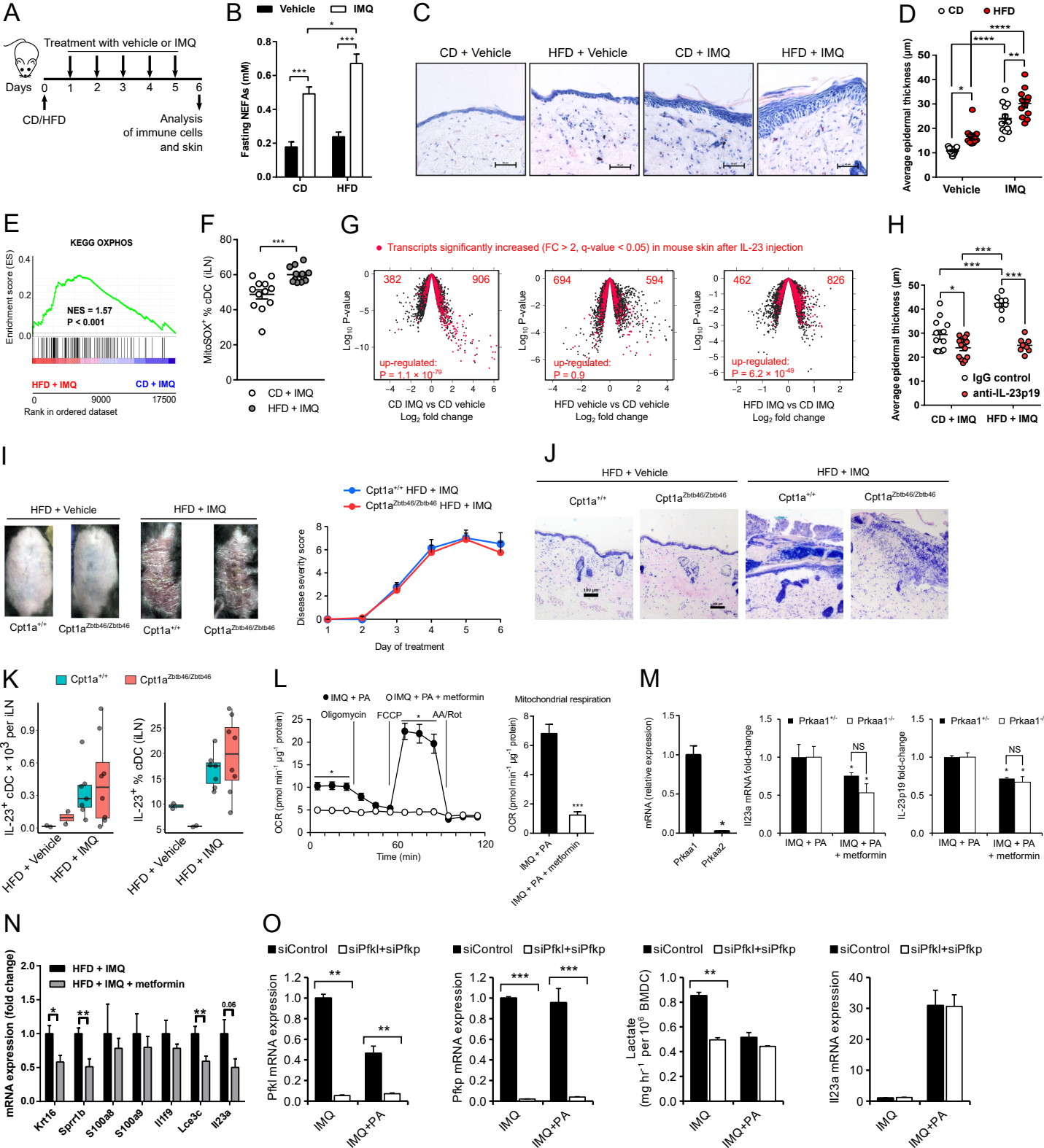


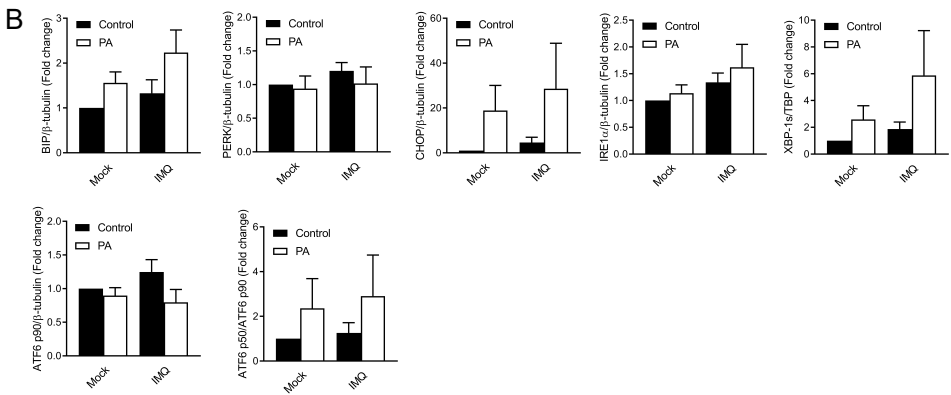
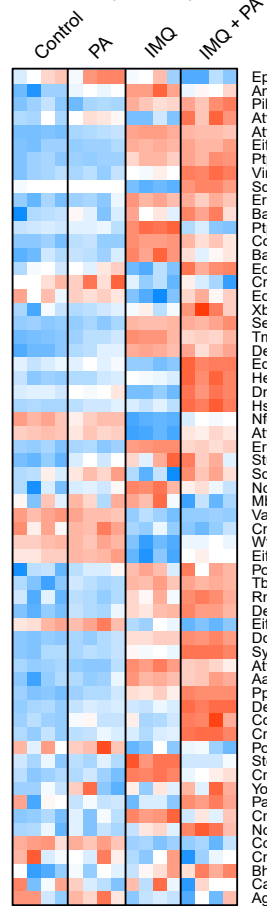




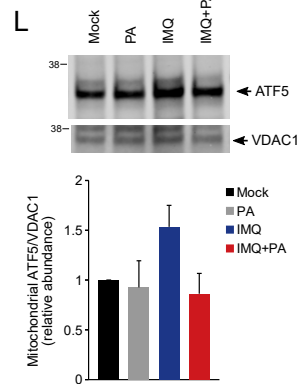
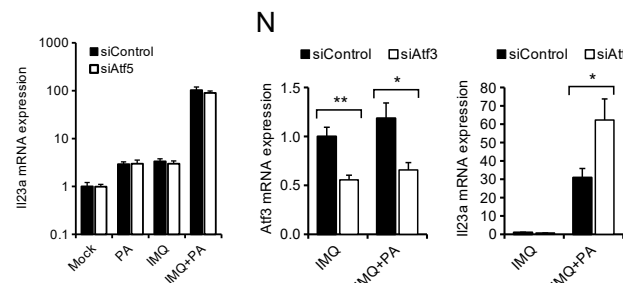
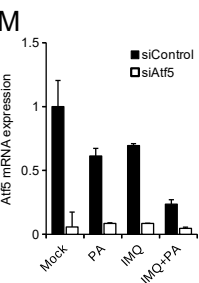
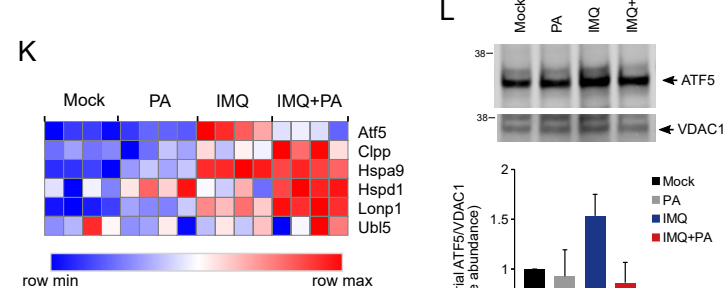
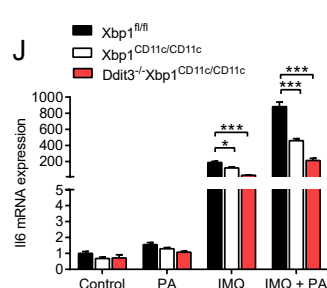
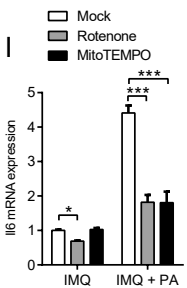
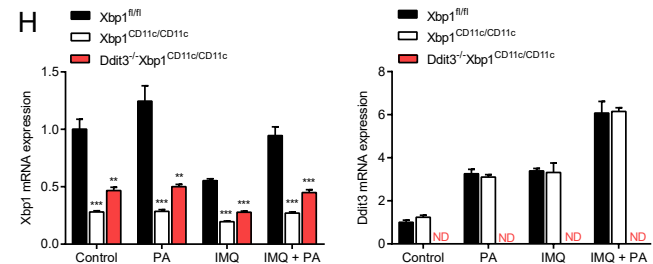
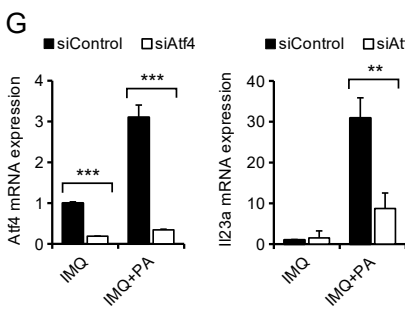
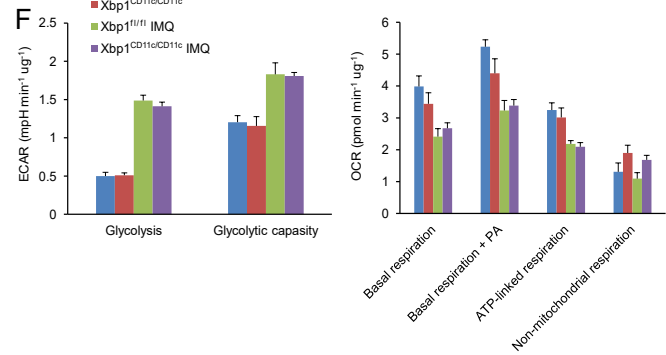
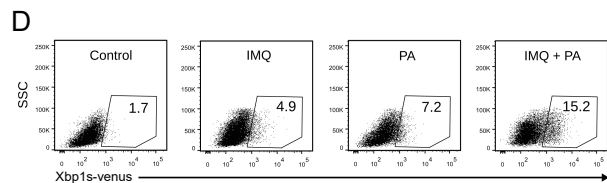
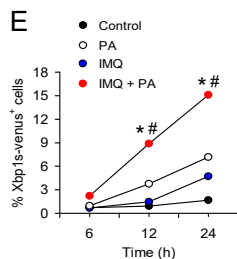
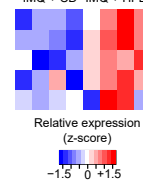








C IMQ + CD IMQ + HFD



STAR METHODS

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, David Dombrowicz (david.dombrowicz@pasteur-lille.fr).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice

Wild-type male C57BL/6 mice (8 weeks of age) were purchased from Charles River Laboratories (France). Tlr4^{-/-} mice were a gift from Jean-Claude Sirard (Inserm U1019, Institut Pasteur de Lille, France). Acod1^{-/-} mice were a gift from Eik Hoffmann (CILL, Institut Pasteur de Lille, France). Prkaa1^{+/-} and Prkaa1^{-/-} mice (Jørgensen et al., 2004) were from Institut Cochin, Université Paris Descartes, Paris, France. Hif1a^{Vav/Vav} mice (Vukovic et al., 2016) and Hif1a^{Tie2/Tie2} mice were from MRC Centre for Regenerative Medicine, University of Edinburgh, UK. Hif1a^{fl/fl}Rosa26CreER mice were from Department of Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Sweden. Ddit3^{-/-} mice (Oyadomari et al., 2001) were from Division of Molecular Biology, Institute for Genome Research, Tokushima University, Japan. Xbp1^{CD11c/CD11c} mice (Cubillos-Ruiz et al., 2015; Osorio et al., 2014) were from VIB Center for Inflammation, Gent University, Belgium or Department of Medicine, and Weill Cornell Medical College, New York, USA. Ddit3^{-/-}Xbp1^{CD11c/CD11c} mice (Tavernier et al., 2017) and ERAI Xbp1-venus reporter mice (Iwawaki et al., 2004) were from VIB Center for Inflammation, Gent University, Belgium. Cpt1a^{CD11c/CD11c} mice (Divakaruni et al., 2018) were from Institute of Infection Immunology, Hannover, Germany. Cpt1a^{Zbtb46/Zbtb46} mice were from William Harvey Research Institute, London, UK. Mice were maintained in pathogen-free environment (12:12 hr light/dark cycle, 21°C-24°C) at the Institut Pasteur de Lille. 8-12-week-old male mice were used for all experiments with *ad libitum* access to water and food. During the experiments, food and bedding was changed daily to prevent any accumulation of food in the cages. Mice were randomized into the different treatment and diet groups equilibrated for body weight and age. Mice were fed a control diet (CD, standard rodent chow, 5% kcal fat) or high fat diet (HFD, 60% kcal fat) for the indicated period of time. Mice with fight wounds at the skin were excluded from analyses. All experiments were performed following approval by the Ethics Committee for Animal Experimentation from Nord-Pas de Calais Region (CEEA75-n°01-2002R and APAFIS#7160-2017040313471173).

Cell culture

Bone marrow-derived dendritic cells differentiated in the presence of GM-CSF (GM-DC) were obtained

from femurs of WT, *Tlr4*^{-/-}, *Prkaa1*^{+/-}, *Prkaa1*^{-/-}, *Hif1a*^{Tie2/Tie2}, *Hif1a*^{Vav/Vav}, *Ddit3*^{-/-}, *Xbp1*^{CD11c/CD11c}, *Ddit3*^{-/-}*Xbp1*^{CD11c/CD11c}, *Cpt1a*^{CD11c/CD11c}, and ERAI *Xbp1*-venus reporter mice (C57BL/6 background, 8-12 weeks of age), cultured in DMEM media containing 10% fetal calf serum (FCS), 10 μ M 2-mercaptoethanol, 25 mM HEPES, 10 μ g/ml gentamycin and 20 ng/ml GM-CSF. Medium was refreshed every 3 days. GM-DC were used for experiments on day 14th of culture (mature GM-DC expressed CD11c⁺MHCII⁺ in > 80-90% cells). Bone marrow-derived macrophages differentiated in the presence of M-CSF (BMDM) were obtained from femurs of WT mice (C57BL/6 background, 8 weeks of age), cultured in RPMI media containing 10% FCS, 10 μ M 2-mercaptoethanol, 10 μ g/ml gentamycin and 20 ng/ml M-CSF and used at day 6. Cell viability was tested by 7AAD or trypan blue assays and was between 70-90% of live cells depending on type of treatment.

METHOD DETAILS

IMQ-induced psoriasis-like skin inflammation

IMQ-induced psoriasis-like inflammation was induced as previously described (Fits et al., 2009). Mice were treated with a daily topical dose (62.5mg) of IMQ cream (Aldara 5%) or control “Lanette” cream, unless stated otherwise, on the shaved abdominal skin for 5 days and sacrificed 24 hr later. Mice were fed HFD or control chow diet for the treatment duration. In some experiments, mice were treated by metformin in drinking water (0.5g/l) (Lien et al., 2014), starting at 3 days before and during treatment with IMQ. At the time of sacrifice, skin samples were directly frozen in liquid N₂ for RNA isolation or fixed in Immunohistofix for histological analysis.

For bone marrow chimeras, 8 – 10 week old C57BL/6 male mice were gamma-irradiated twice with 5 Gy 3 hr apart. Mice were reconstituted 3 hr later by intravenous (i.v.) injection with marrow cells (3 $\times 10^6$ cells) harvested from the femurs and tibiae of *Cpt1a*^{Zbtb46/Zbtb46} mice and control *Cpt1*^{+/-} WT littermates. Mice were maintained on acidified water containing Baytril (Enrofloxacin) during the critical 3-week reconstitution period. Six weeks after reconstitution, animals were fed a HFD or CD and psoriasis-like inflammation was induced by IMQ application as described.

Isolation of cells from iLN

Mouse iLN were mechanically homogenized, passed through 70 μ m filter and centrifugated at 400 g for 10 min. Cell pellets were washed two times with cold PBS and used for cell sorting or flow cytometry. For IL-23p19 staining, cells were incubated in DMEM containing brefeldin A (1 μ g/ml) at +37°C, 5% CO₂ for 4 hr.

Flow cytometry

Cells were stained with Zombie UV viability reagent during 15 min at +20°C, washed two times with cold PBS + 0.5% BSA, incubated with Fc block and antibodies mentioned in the figure legends and the Key Resources Table in PBS + 0.5% BSA during 30 min at +4°C, washed two times with cold PBS + 0.5% BSA, fixed with 1% PFA and used for flow cytometry. Flow cytometry analyses were performed on BD LSRFortessa X-20 flow cytometer. Results were acquired with the Diva software and analyzed using FlowJo software. cDC were defined as CD3⁻CD19⁻CD64⁻Ly6G⁻CD11c⁺MHCII⁺ cells.

Sorting of cDCs from iLN

CD3⁻CD19⁻CD64⁻Ly6G⁻CD11c⁺MHCII⁺ DCs were sorted from mouse iLN using BD Influx Cell Sorter (BD Biosciences). 5-8 × 10⁵ sorted cDC were used for RNA isolation with Arcturus Picopure RNA isolation Kit.

Activation of GM-DC

GM-DC (5 × 10⁵ per ml) were incubated in DMEM containing 10% FCS, 2 mM L-glutamine, 4.5 g/L glucose, and 10 µg/ml gentamycin and treated with 100 ng/ml Pam3CSK4, 10 µg/ml Poly(I:C), 100 ng/ml LPS, and 3 µg/ml Imiquimod, 100 µg/ml curdlan, 1 µg/ml furfuran, 50 ng/ml TNF, 50 ng/ml IL-1β with or without PA or other FA conjugated with BSA (0.065-0.5mM, molar ratio PA:BSA 6:1, OA:BSA 6:1) in the presence of 3 µM etomoxir, 5 mM 2-deoxyglucose, 1 µM rotenone, 5 mM metformin, 0.5 mM MitoTEMPO, 10 µM 4µ8C, 2 µM GSK2606414, 1 µM tunicamycin, 100 µg/ml SN50, 5 mM dichloroacetate (DCA), 10 µM triacsin C, 10 µM or 20 µM MitoPQ as indicated in figure legends. Reagents were added to cells simultaneously with IMQ or LPS, unless stated otherwise. SN50, MitoTEMPO, GSK2606414, 4µ8C, etomoxir, triacsin C and DCA were added 1 hr before other reagents. GM-DC were activated during 24 hr unless otherwise indicated.

siRNA transfection

5 × 10⁵ GM-DC per biological replicate were transfected with siRNA using Mouse Dendritic Cell Nucleofector Kit and Nucleofector II/2b device according to manufacturer's instructions. Cells were incubated during 48 hr in DMEM + 10% FCS and treated with IMQ and PA as described in TLR activation of GM-DC. Used siRNAs are mentioned in the figure legends and the Key Resources Table.

2-NBDG uptake

5 × 10⁵ GM-DC per biological replicate were incubated in DMEM without glucose during 1 hr following by incubation in the presence of 0.1 mM 2-NBDG during 10 min, washed two times with PBS and analyzed by flow cytometry.

Analysis of mitochondrial content

5×10^5 GM-DC per biological replicate were incubated in DMEM in the presence of 1 μ M MitoTracker Green FM during 15 min, washed two times with PBS and analyzed by flow cytometry.

Mitochondrial ROS production

To detect mitochondrial ROS, 5×10^5 GM-DC or cells isolated from iLN per biological replicate were incubated with 5 μ M MitoSOX Red mitochondrial superoxide indicator at 37°C for 20 min in DMEM, washed two times with PBS, stained with surface antibody if indicated, and then analyzed with flow cytometry.

Mitochondrial respiration assay

4×10^6 GM-DC per biological replicate were placed into the O2K chambers (Oroboros Instruments) and filled with MiR05 as described on the Oroboros website (<http://www.bioblast.at/index.php/MiR05>). After digitonin permeabilization for 10 min, mitochondrial respiration was studied at 25°C by adding sequentially the following compounds: glutamate (10 mM) and malate (2 mM), ADP (2.5 mM), succinate (10 mM), rotenone (0.5 μ M), antimycin A (2.5 μ M), TMPD / Ascorbate (0.5 mM / 2 mM), cytochrome c (10 μ M) and sodium azide (100 mM).

Chromatin Immunoprecipitation (ChIP)

5×10^6 GM-DC per biological replicate were cross-linked with 1% PFA at room temperature for 10 min, lysed, nuclei were isolated and resuspended in 0.4 ml nuclear lysis buffer containing 0.5% SDS. Nuclei were sonicated using a Bioruptor (Diagenode) according to the manufacturer's protocol, and chromatin was immunoprecipitated with antibodies against CHOP, XBP-1, and control IgG overnight at 4°C, followed by 4 hr incubation in the presence of Protein A/G beads. After washing, bead-bound chromatin was subject to decrosslinking for 4 hr at 65°C. DNA was purified using Agencourt AMPure beads. Relative DNA enrichment was quantified and normalized to input DNA by qPCR using SYBR Green Master Mix. Primers used for ChIP are listed in Table S2.

RNA isolation

Total RNA was isolated from GM-DC using Trizol reagent. Total RNA from sorted cDC was isolated using RNeasy Micro Kit.

Reverse transcription and real-time PCR

500 ng of total RNA isolated from GM-DC was treated with DNase I and used to generate cDNA with High-capacity cDNA reverse transcription kit. Gene expression was measured by SybrGreen based qPCR. Results were normalized to the housekeeping genes *Hprt1* and *Rpl4*, and the $\Delta\Delta C_t$ method was employed for all real-time PCR analyses. Primers used for real-time PCR are listed in TableS1.

Microarray analysis

200 ng RNA from GM-DC was amplified with GeneChip WT PLUS Reagent Kit, labeled with GeneChip WT Terminal Labeling Kit. 5 ng RNA from sorted cDC was amplified with Ovation Pico WTA Systems V2, labeled with GeneChip WT Terminal Labeling Kit. The resulting complementary RNAs were hybridized on the GeneChip Mouse Gene 2.0 ST Array (Affymetrix) according to the manufacturer's protocol. Microarray data were normalized by the Robust Multi-Average method (Irizarry et al., 2003) by using affy R package (Gautier et al., 2004). Transcripts associated with annotated genes were selected for analysis. The expression dataset was collapsed to gene levels using a max-median approach following selection of top 15000 genes with maximal average expression levels among all experimental groups using Phantasus. Differentially expressed genes were identified by using limma R package (Smyth, 2005), which uses an empirical Bayesian approach to estimate variances in moderated t tests. Raw P values were adjusted for multiple testing using the Benjamini–Hochberg procedure.

Protein Analysis

Whole cell lysate from 1×10^6 GM-DC was extracted using RIPA lysis buffer supplemented with complete protease inhibitor cocktail and PhosSTOP phosphatase inhibitors. Proteins were diluted in NuPAGE LDS sample buffer, heated at 65°C for 5 min, and loaded on 4–12% NuPAGE Bis-Tris Gel. Proteins were transferred to nitrocellulose membrane using iBlot 2 Transfer stacks and blotted with commercial antibodies mentioned in the figure legends and the Key Resources Table.

Seahorse assays

2.5×10^5 GM-DC per well were seeded in a XF24 plate and analyzed in a Seahorse XFe24 Analyzer. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured in DMEM with 25 mM glucose and 2 mM glutamine with or without of 0.5 mM PA, before and after the sequential injection of 0.75 μ M oligomycin, 1.5 μ M FCCP, 1 μ M of rotenone/antimycin A, and 50 mM 2-deoxyglucose. Mixing, waiting, and measurement times were 4, 2, and 2 min (3, 1, and 1 min in some experiments), respectively. Measures were normalized by total protein. Basal and maximal respiration

values were calculated by subtraction of OCR value after treatment of cells with rotenone and antimycin A (which corresponds to non-mitochondrial respiration) from OCR values in cells treated with glucose or with oligomycin and FCCP, respectively. Glycolysis and glycolytic capacity values were calculated by subtraction of ECAR value after treatment of cells with 2-deoxyglucose (which corresponds to non-glycolytic acidification) from ECAR values in cells treated with glucose or with oligomycin, respectively.

Measurement of fatty acid oxidation

Fatty acid oxidation was measured as described previously (Haas et al., 2012). Briefly, 5×10^5 GM-DC per biological replicate were pre-treated for 21 hr with IMQ or IMQ + 500 μ M PA. Cells were then switched to oxidation media containing 1mM carnitine, 500 μ M PA, and 1 μ Ci/mL 14 C-PA for 3 hr. Media was acidified with 70% perchloric acid and CO₂ was captured with 1N NaOH. Complete oxidation (captured CO₂) and incomplete oxidation (acid soluble metabolites, ASMs) were calculated by counting the NaOH and cleared, acidified media.

Metabolomics

Metabolite extraction of GM-DC was performed on 2.5 million per well using 70°C aqueous 70% ethanol as described previously (Jha et al., 2015). Prior to collection, cells were treated with BSA (control), 500 μ M PA, IMQ or IMQ + 500 μ M PA for 24 hours. At collection, cells were placed immediately on ice, the media was removed and cells were washed three times with ice-cold PBS to remove residual media. Intracellular metabolites were extracted twice with hot ethanol using 10 μ M norvaline as an internal control. For LCMS, samples were dried under nitrogen flow and reconstituted in a milliQ water/acetonitrile (1:1) mixture for injection using a UPLC Acquity (Waters) separation system coupled with a Xevo G2 ToF (Waters) as described (Paglia et al., 2012) with slight modification. Compounds were ionized using an electrospray ionization source in negative mode. Data processing was performed in MATLAB (Mathworks, Inc.) using a custom made in-house protocol. Compound identification was performed using both retention time of authentic standards and accurate mass with an accepted deviation of 0.005 Da. For GCMS, samples were derivatized with methyl chloroformate as described (Smart et al., 2010) with slight modifications. Analysis was performed using GC (7890B, Agilent) coupled to a quadrupole detector (59977B, Agilent) and controlled by ChemStation software (Agilent). Raw data was converted to netCDF format using Chemstation (Agilent), before processing in Matlab R2014b (Mathworks, Inc.) using PARADISE software as described (Johnsen et al., 2017). In both cases, samples were randomized prior to injection. All MS sample processing and analysis were performed by MS-Omics, Inc. (Copenhagen, Denmark).

Histology

Mouse skin samples were fixed in ImmunoHistoFix and embedded in ImmunoHistoWax at 37°C. 5µm sections were stained with May-Grünwald Giemsa for measurement of epidermal thickness by using a Nikon Eclipse Ti-E microscope with NIS-Elements imaging software. Average epidermal thickness was determined as a mean of 10 measures calculated for each skin sample. We did blind investigators during epidermal thickness measurement.

QUANTIFICATION AND STATISTICAL ANALYSES

Statistical analyses of biological data

Data are presented as mean \pm SEM. We tested whether data were normally distributed by Shapiro-Wilk test and examined quantile-quantile plots. Levene's test was used to analyze homogeneity of variances. For a two-group comparison two-sided Student's *t*-test was used. For more than two groups data were analyzed by ANOVA, or two-way ANOVA, followed by Tukey's (when we compared each group with every other group) or Sidak (when we compared groups within separate time points in repeated measures ANOVA) *post-hoc* tests for multiple comparisons. In case of data showing differences compared to normal distribution we applied two-sided Mann-Whitney U-test. Statistical analyses were performed with Prism 6 or R software. $p < 0.05$ was considered to be statistically significant and is presented as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, or **** $p < 0.0001$.

DATA AND SOFTWARE AVAILABILITY

The accession numbers for microarray datasets reported in this paper are publicly available at the NCBI Gene Expression Omnibus: GSE68750, GSE110962, and GSE110963.