Supplemental Methods

Reagents

Murine IL-10 (Peprotech), neutralizing anti-mouse IFNβ Ab (PBL; 32401-1), neutralizing anti-IL-10 mAb (R&D Systems; clone JES052A5), IgG1 isotype control mAb (R&D systems; clone 43414), Poly (I:C) (Invivogen, tlrl-pic 25μg/ml), lactate assay kit (Abcam; ab65331) and aconitase enzyme activity kit (Abcam; ab109712).

Generation of bone marrow-derived macrophages

For mouse bone marrow-derived macrophage (BMM) preparation, bone marrow cells were flushed from the femur and tibia bones of 8-12-week-old male wild-type (WT) (C57BL/6) or *Ifnar1*$^{-/-}$ mice, as before (1). Briefly, bone marrow cells were isolated and cultured in RPMI 1640 medium, supplemented with 10% heat-inactivated FCS, 2 mM GlutaMax-1, 100 U/ml penicillin, and 100μg/ml streptomycin in the presence of M-CSF (CSF-1) (2000 U/ml) at 37°C in a humidified incubator. On day 4, nonadherent cells were collected and cultured for a further 3d in M-CSF (2000 U/ml) to derive BMM. Adherent BMM were harvested on day 7 and were replated for experiments at 0.5 x 10^6 cells/ml.

Isolation and generation of human macrophages

Human monocytes were purified from buffy coats (Red Cross Blood Bank, Melbourne, VIC, Australia), using RosetteSep Ab mixture (Stem Cell Technologies, Vancouver, BC, Canada), which negatively selects $CD14^+$ monocytes, followed by Ficoll-Paque density gradient centrifugation. Cells were then cultured in RPMI 1640 (supplemented as above) for 5d in M-CSF (2000U/ml) to differentiate them into monocyte-derived macrophages (MDM) (2) at 37°C in a humidified incubator.

Macrophage treatment for metabolomics and U-^{13}C-labeling studies

For metabolomic analyses, BMM (at day 7) were stimulated with LPS (Invivogen, 100ng/ml) or PBS for 24h in RPMI 1640 medium, supplemented with 10% heat-inactivated FCS, 2 mM GlutaMax-1, 100 U/ml penicillin, and 100 μg/ml streptomycin in the presence of M-CSF (2000 U/ml). At completion of an experiment cell lysates were prepared for extraction (see below). For labeling experiments, BMM were treated with LPS (100ng/ml) or PBS for 24h in RPMI 1640 (10% heat-inactivated FCS, 2000U/ml M-CSF and 100 U/ml penicillin, and 100μg/ml streptomycin) containing U-^{13}C-glucose (11mM). At the completion of experiment cell lysates were prepared for extraction (see below). For metabolic and ^{13}C-labeling studies with IFNβ blockade, BMM were preincubated for 1h with 50 units/ml of a neutralizing anti-IFNβ Ab or the equivalent amount of rabbit IgG control antibody, prior to 24h treatment with LPS (100ng/ml). At the completion of the experiment cell lysates were prepared for extraction (see below).

Metabolite extraction and gas chromatography-mass spectrometry (GC-MS) analysis

BMM (1 x 10^6 cells/plate) were washed with Milli-Q water at 37°C then snap-frozen by covering the plate in liquid nitrogen. Metabolites were extracted on ice by addition of 600μl/plate of methanol;chloroform (9:1 v/v), containing the internal standards, U-^{13}C-sorbitol (16.6μM) and $^{13}C_5$,^{15}N-valine (166μM). Cells were scraped and incubated on ice for 10 min. Samples were then centrifuged (5 min, 14,000 rpm, 4°C) to pellet precipitated proteins and the supernatants transferred to fresh Eppendorf tubes. GC-MS analysis was carried out on a Shimadzu GC/MS-TQ8040 system and analyzed in MRM mode using the Shimadzu Smart Metabolites Database containing 470 MRM metabolite targets.

U-^{13}C-stable isotope labelling analysis

U-^{13}C-labelled cells were prepared for GC-MS analysis as described above. Samples were analysed on an Agilent 7890 GC coupled to a 5975 mass selective detector. Conditions for instrument analysis, data processing and isotopic background correction (3).

Seahorse metabolic assay

A Seahorse utility plate (Seahorse Bioscience) containing calibrant medium (200µL/well) together with Seahorse injector port and probe plate were incubated overnight in 37ºC without CO_2. The following day, the medium from Seahorse cell culture plate was replaced with Seahorse XF assay buffer (supplemented with 10mM glucose and 2mM glutamine) and incubated in CO_2-free incubator at 37ºC for at least half an hour. WT and *Ifnar1*$^{-/-}$ BMM (untreated and LPS-stimulated) were then plated at 8 x10^4 cells/well in a 96 well Seahorse cell culture plate (Agilent XFe96) with one well per corner of the plate containing supplemented-medium without cells, as background control. The real-time oxygen consumption (OCR) and extracellular acidification (ECAR) were determined on a Seahorse XF-e96 Bioanalyzer (Agilent) after calibration of the utility plate with injector port plate as per manufacturer's instruction.

Isocitrate dehydrogenase activity assay

Isocitrate dehydrogenase activity was measured using a colorimetric assay kit (Abcam; ab102528), according to the manufacturer's instructions. Briefly, BMM or MDM (1x10^6) were lysed in 200µl of the provided assay buffer. The lysates were centrifuged at 13,000 g for 10 min and the cleared supernatant used for the assay. NAD^+/$NADP^+$ was used as substrate for the IDH assay. Total IDH activity was measured at 37°C with absorbance read at 450 nm in kinetic mode over 30 min on a Varioskan Lux Plate Reader (Thermo Fisher). Activity (mU/ml) is expressed as one unit of IDH that will generate 1µmol of NADH/NADPH per min at pH 8 at 37°C.

Quantitative PCR

Total RNA was extracted using ISOLATE II RNA Mini Kit (Bioline, London, UK) and quantified using a Nanodrop 2000 UV-visible spectrophotometer. cDNA was prepared using SuperScript III reverse transcriptase (Invitrogen), according to manufacturer's instructions. Quantitative PCR (qPCR) was performed using predeveloped Taqman probe/primer combinations for murine *Irg1, Idh1, Idh2, Idh3a, Idh3b and Idh3g* on the QuantStudio 5 Real-Time PCR System (Thermo Fisher). Threshold cycle numbers were transformed to delta-Ct values, and the results were expressed relative to the reference gene, *Hprt*.

Immunoblots

Briefly, macrophages (3×10^6) were lysed with RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, and complete protease inhibitors) at 4°C and whole cell extracts collected. Lysates were clarified by centrifugation at 13,000 × g for 10 min at 4°C and protein concentrations were determined with a Bio-Rad protein assay kit and equal amounts of protein were separated on a NuPAGE Novex (4%-12%) Bis-Tris gel (Thermo Fisher Scientific) and transferred onto PVDF membrane (Bio-Rad). Membranes were probed with antibodies against Irg1 (EPR22066), Idh1 (EPR12296) (abcam) and β-actin (clone AC-74, Sigma-Aldrich). Binding was visualized by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies and chemilluminescence (ECL). Western blots are representative of three independent experiments.

Statistics

Statistical analysis was performed using Prism software (GraphPad Software 7.03). Pairwise comparisons were performed using an unpaired Student t test and multivariate comparisons were performed using one-way ANOVA (Kruskal-Wallis test) with a Tukey's multiple comparisons test or a two-way ANOVA with a Sidak's multiple comparisons test for grouped

analyses. For mass spectroscopy, the data subjected to statistical analysis are the metabolites' abundance measured as area under the observed peak that defines a metabolite. This data was pre-treated before statistical analysis to account for biological, experimental and instrument variations by performing a natural log transformation followed by normalizing the data to the median metabolic abundance of each sample. The resulting data (groups) were then compared using a Student's t-test for unpaired data with alpha = 0.05. In addition, the results of the Student's t-test were controlled for false positives using the Benjamini-Hochberg method (4). The above statistical tests were performed using MetaboAnalyst.

Supplemental References

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2. Lacey DC, Achuthan A, Fleetwood AJ, et al. Defining GM-CSF–and macrophage-CSF–dependent macrophage responses by in vitro models. *The Journal of Immunology.* 2012;188(11):5752-5765.
3. Kowalski GM, De Souza DP, Burch ML, et al. Application of dynamic metabolomics to examine in vivo skeletal muscle glucose metabolism in the chronically high-fat fed mouse. *Biochem Biophys Res Commun.* 2015;462(1):27-32.
4. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *J Roy Stat Soc B Met.* 1995;57(1):289-300.

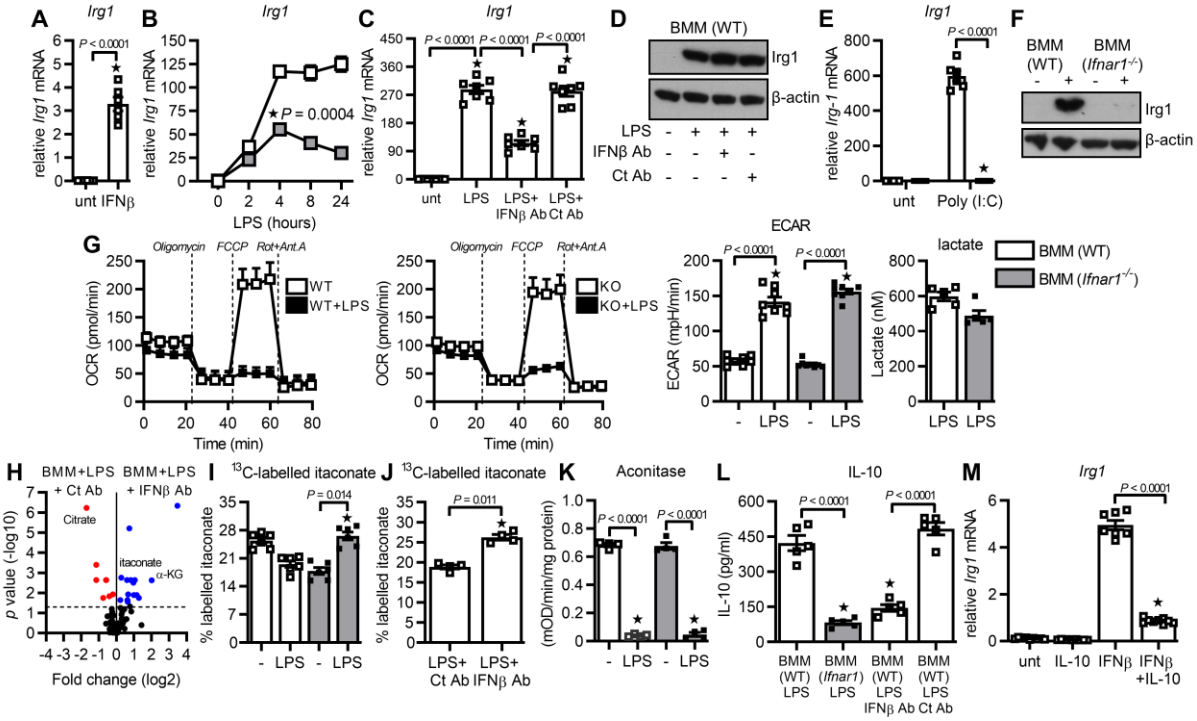

Figure S1. The autocrine type I IFN pathway drives metabolic reprogramming in macrophages. *Irg1* in (A) WT BMM treated ± IFNβ (1000U/ml) for 24h (n=6) or (B) in WT and *Ifnar1*$^{-/-}$ BMM treated with LPS (100ng/ml, 0-24h, n=3). (C) Irg1 gene (n=7) and (D) protein in WT BMM treated with LPS (100ng/ml, 24h) plus anti-IFNβ Ab or Ct Ab. (E) Irg1 gene (n=6) and (F) protein in WT and *Ifnar1*$^{-/-}$ BMM treated with Poly (I:C) (25µg/ml, 24h). (G) Real-time oxygen consumption (OCR), extracellular acidification (ECAR) and lactate secretion in WT and *Ifnar1*$^{-/-}$ BMM treated with LPS (100ng/ml, 24h). (H) Volcano plot of metabolite pools in BMM stimulated with LPS (100ng/ml, 24h) ± anti-IFNβ Ab or Ct Ab. ^{13}C-glucose-labeling of itaconate in (I) BMM (WT and *Ifnar1*$^{-/-}$) treated ± LPS (100ng/ml, 24h) or (J) WT BMM treated with LPS (100ng/ml, 24h) in the presence of anti-IFNβ Ab or Ct Ab. Cells were treated with ^{13}C-glucose (10mM) for 24h and analyzed by GC-MS. The mean percentage labeling ± SEM (n=3-4). (K) Aconitase activity in WT and *Ifnar1*$^{-/-}$ BMM treated with LPS (100ng/ml, 24h, n=4). (L) IL-10 release (by ELISA) from WT and *Ifnar1*$^{-/-}$ BMM stimulated with LPS (100ng/ml, 24h) in the presence of anti-IFNβ Ab or Ct Ab. (M) *Irg1* expression in WT BMM treated with IL-10 (50ng/ml) and/or IFNβ (1000U/ml) for 24h (n=7). Data are mean ± SEM. *P* values by two-way ANOVA (in B), one-way ANOVA (in C/E/G/I/K/L/M) or Student's t-test (in A/G/J).

Table S1. Differentially abundant metabolites (p<0.05) in LPS-stimulated WT BMM vs. LPS-stimulated *Ifnar1*$^{-/-}$ BMM as determined by GC-MS.

Compound	Fold change[a]	BH Adjusted p-value[b]
2-Aminopimelic acid	0.18066	1.04E-09
Citric acid	0.18255	1.42E-09
Galacturonic acid	2.8388	1.49E-06
Monostearin	75.874	9.56E-06
Ribose 5-phosphate	2.9731	2.43E-05
Glutathione	6.9566	2.60E-05
Glucuronic acid	1.8667	2.87E-05
Glucaric acid	1.7706	4.34E-05
2-Ketoglutaric acid	3.2227	5.71E-05
Urea	2.0754	8.44E-05
Galacturonic acid	2.0915	0.00017
Methylsuccinic acid	2.4474	0.000285
Isocitric acid	1.9427	0.00031
4-Hydroxyproline	1.7052	0.000432
Niacinamide	2.3918	0.00056
Cysteine	2.8241	0.000976
Glucose 6-phosphate	1.9841	0.000987
Glucose 6-phosphate	1.9462	0.001153
Aconitic acid	0.53348	0.001208
Mesaconic acid	1.4931	0.001899
Alanine	1.4888	0.002348
Itaconic acid	1.3595	0.003409
Xylitol	1.833	0.005061
Fructose 6-phosphate	1.8435	0.00578
Proline	1.9343	0.006694
Threonic acid	1.3492	0.006927
6-Phosphogluconic acid	1.3965	0.008227
Malic acid	0.78412	0.01915
Batyl alcohol	0.67721	0.030185
1,6-Anhydroglucose	1.3453	0.034766
Citramalic acid	0.74618	0.036148
Serine	1.3884	0.037036
Adenosine	1.6076	0.03807
Pantothenic acid	1.3398	0.039527
Phenylacetic acid	0.79456	0.041506
Octadecanol	0.70965	0.048572

[a]Indicates fold change of metabolite pool size. Values > 1 indicate metabolites increased in LPS-stimulated *Ifnar1*$^{-/-}$ BMM. Values < 1 indicate metabolites increased in LPS-stimulated WT BMM. [b]*p*-value by Student's t test (Benjamin-Hochberg post hoc procedure).

Table S2. Differentially abundant metabolites (p<0.05) in untreated vs. LPS-stimulated WT BMM as determined by GC-MS.

Compound	Fold change[a]	BH Adjusted p-value[b]
Itaconic acid	12.328	5.02E-09
Succinic acid	4.4549	1.66E-07
Citric acid	4.0656	8.47E-06
2-Aminopimelic acid	4.0086	8.47E-06
Isocitric acid	0.17332	8.66E-06
Aspartic acid	0.21465	0.000236
3-Aminoglutaric acid	0.21577	0.000236
2-Ketoglutaric acid	0.23781	0.000447
Lactic acid	2.4434	0.002203
Dihydroxyacetone phosphate	1.5184	0.004944
Batyl alcohol	0.57711	0.005784
Glycerol 3-phosphate	1.4778	0.00615
6-Phosphogluconic acid	0.65324	0.006257
Fructose 6-phosphate-meto	2.6677	0.006739
Inositol	0.67626	0.008901
4-Hydroxyproline	0.55338	0.011011
Cholesterol	0.70139	0.028356
Inositol phosphate	0.68514	0.029179
Glutamic acid	0.42417	0.035944
2-Ketoisocaproic acid	0.54245	0.040095
Caproic acid	0.8087	0.040095
Histidine	0.50897	0.041412
Arabinose	58.825	0.047445
Cysteine	0.44841	0.047445
Glycerol	1.6743	0.047445
Glucaric acid	1.5209	0.047445
Threonic acid	1.4585	0.047445
Mesaconic acid	1.3322	0.047445
2-Hydroxyglutaric acid	1.3262	0.047445

[a]Indicates fold change of metabolite pool size. Values > 1 indicate metabolites increased in LPS-stimulated WT BMM. Values < 1 indicate metabolites increased in untreated WT BMM. [b]*p*-value by Student's t test (Benjamin-Hochberg post hoc procedure).

Table S3. Differentially abundant metabolites (p<0.05) in untreated vs. LPS-stimulated *Ifnar1*$^{-/-}$ BMM as determined by GC-MS.

Compound	Fold change[a]	BH Adjusted p-value[b]
Itaconic acid	109	4.60E-13
Isocitric acid	0.27592	4.89E-10
Glucaric acid	2.6771	6.01E-10
Succinic acid	4.9331	3.41E-09
2-Aminopimelic acid	0.62973	3.07E-08
Aspartic acid	0.21855	5.12E-08
3-Aminoglutaric acid	0.21921	5.17E-08
Glucuronic acid	3.0866	7.35E-08
Citric acid	0.63512	1.58E-07
Mesaconic acid	3.7968	3.06E-07
Galacturonic acid	2.6228	2.46E-06
Methylsuccinic acid	5.465	2.61E-06
Lactic acid	2.653	6.75E-06
Monostearin	61.556	1.51E-05
Fructose 6-phosphate	4.1608	3.53E-05
N-Acetylmannosamine	0.64326	6.82E-05
Arachidonic acid	0.53911	0.000126
Threonic acid	1.7085	0.00017
Glutathione	4.2544	0.000255
Glucose 6-phosphate	2.6436	0.000295
Galacturonic acid	1.9482	0.00058
Niacinamide	2.3488	0.000854
Inositol	0.64737	0.000861
Decanoic acid	0.63409	0.00175
Glutamic acid	0.60159	0.001763
Cholesterol	0.62728	0.001939
Batyl alcohol	0.59299	0.002164
Inositol phosphate	0.68966	0.002208
Acetoacetic acid	0.63764	0.003444
1-Hexadecanol	0.70573	0.003471
Elaidic acid	0.75999	0.003814
Octanoic acid	0.68107	0.0041
Phenylacetic acid	0.72701	0.004279
Octadecanol	0.69975	0.006288
N-Acetylserine	0.73356	0.006434
Urea	1.4987	0.007012

[a]Indicates fold change of metabolite pool size. Values > 1 indicate metabolites increased in LPS-stimulated *Ifnar1*$^{-/-}$ BMM. Values < 1 indicate metabolites increased in untreated *Ifnar1*$^{-/-}$ BMM. [b]*p*-value by Student's t test (Benjamin-Hochberg post hoc procedure).

Table S4. Differentially abundant metabolites (p<0.05) in untreated WT vs. untreated *Ifnar1*$^{-/-}$ BMM as determined by GC-MS.

Compound	Fold change[a]	BH Adjusted p-value[b]
Itaconic acid	0.15376	4.24E-09
Dihydroxyacetone phosphate	1.5727	2.91E-05
Glycerol 3-phosphate	1.5656	3.62E-05
Methylsuccinic acid	0.31782	0.00011366
Mesaconic acid	0.52388	0.00011407
Batyl alcohol	0.65908	0.00035427
N-Acetylserine	1.5392	0.0019112
Glycolic acid	1.2205	0.0089839
Citramalic acid	0.69591	0.0090547
Glucuronic acid	0.7382	0.010357

[a]Indicates fold change of metabolite pool size. Values > 1 indicate metabolites increased in untreated *Ifnar1*$^{-/-}$ BMM. Values < 1 indicate metabolites increased in untreated WT BMM.
[b]*p*-value by Student's t test (Benjamin-Hochberg post hoc procedure).

Table S5. Differentially abundant metabolites (p<0.05) in LPS-treated WT BMM in the presence of a neutralizing anti-IFNβ Ab or an isotype control Ab as determined by GC-MS.

Compound	Fold change[a]	BH Adjusted p-value[b]
2-Aminoheptanedioic acid	0.18166	2.37E-10
Citric acid	0.17962	6.12E-10
Ornithine	31.483	1.21E-08
MG180e/00/00	2.0543	4.80E-07
O-Phosphoethanolamine	0.31837	1.35E-06
Itaconic acid	1.2973	2.80E-05
L-Aspartic acid	0.5533	3.66E-05
3-aminoglutaric acid	0.55814	4.09E-05
Methylsuccinic acid	2.5133	7.87E-05
D-Ribose 5-phosphate	2.7548	0.00010067
Galacturonic acid	2.2434	0.000101
Glucaric acid	1.7999	0.00017393
cis-Aconitic acid	0.65445	0.0012739
Isocitric acid	1.8706	0.001915
L-Cysteine	3.0994	0.0022821
Palmitoleic acid	2.4936	0.0028957
Hydroxylamine	0.4349	0.0032146
Creatinine	1.883	0.0034233
3-Phosphoglyceric acid	3.3864	0.0043679
L-2-Hydroxyglutaric acid	1.2289	0.0045561
D-Fructose	1.8038	0.0064244
Putrescine	2.1499	0.010776
Fumaric acid	0.77484	0.014054
Beta-Alanine	0.75883	0.015253
L-Proline	1.5483	0.016686
Ethanolamine	1.2335	0.023638
4-Hydroxyproline	1.3862	0.023807
Pyruvic acid	2.0286	0.027628
Glycerol	0.70814	0.03795
2-Ketoglutaric acid	1.8149	0.040576

[a]Indicates fold change of metabolite pool size. Values > 1 indicate increased in LPS + anti-IFNβ Ab treated WT BMM. Values < 1 indicate metabolites increased in LPS + isotype control Ab treated WT BMM. [b]*p*-value by Student's t test (Benjamin-Hochberg post hoc procedure).