Supplemental Figures

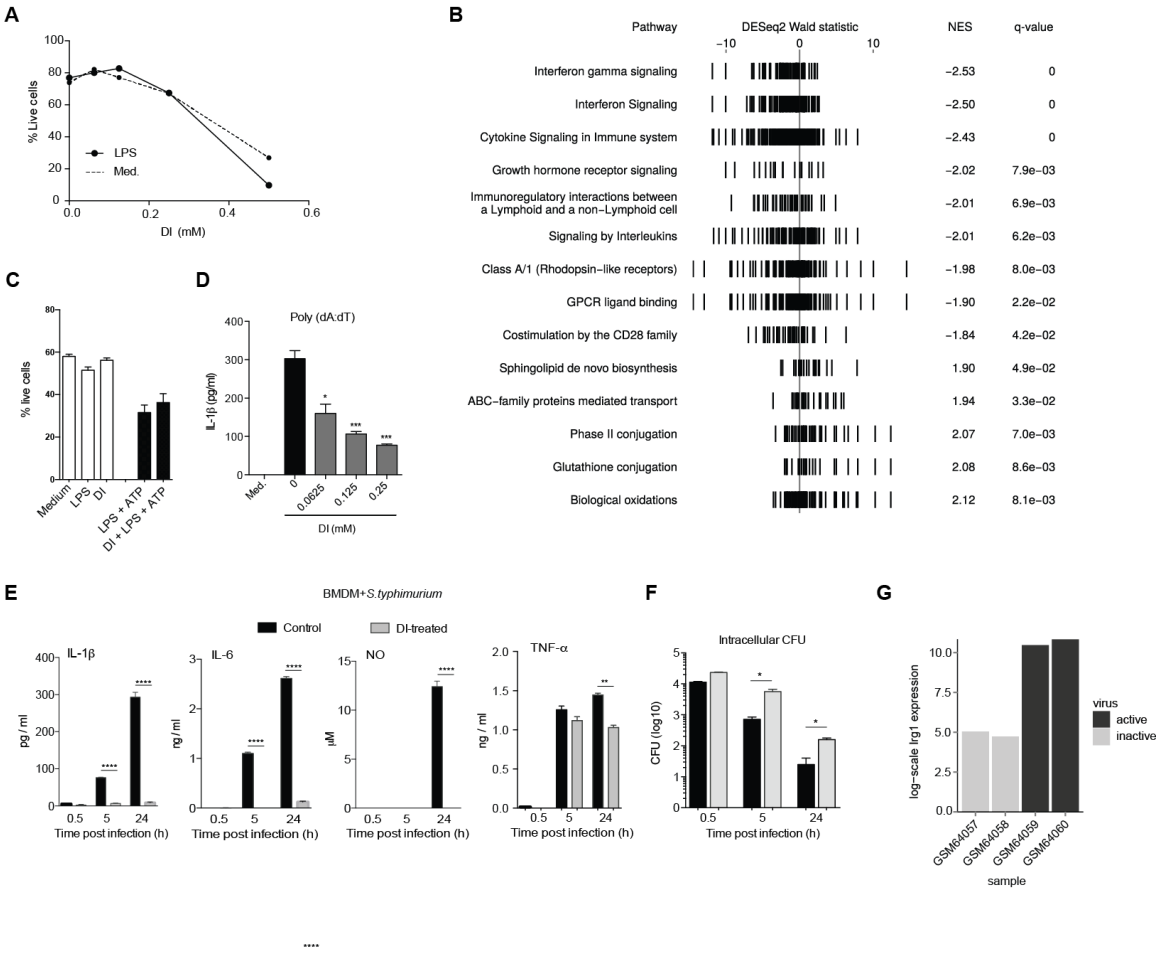

Figure S1, related to Figure 1. Effects of exogenous itaconate on macrophage activation. Percentage of live BMDM determined via flow cytometry after pre-treatment with DI as shown (12 h) alone (Med) or followed by stimulation with LPS for 4 h. Shown are representative data from three independent experiments. (B) Gene Set Enrichment Analysis shows a list of differentially regulated pathways – proinflammatory pathways are downregulated, while Phase II conjugation, glutathione conjugation and biological oxidations are upregulated upon itaconate addition. (C) Percentage of live BMDM determined via flow cytometry after pre-treatment with 0.25 mM DI for 12 h, followed by stimulation with LPS ± ATP. Medium: cells cultured in medium alone; DI: treated with DI in absence of stimulation. Data represent mean ± SEM of values from two independent experiments (n = 4 per condition), (D) mature IL-1β produced by BMDM that were pre-treated with DI as shown and then transfected with poly dA:dT mixed with Xfect polymer Med, indicates cells cultured in medium alone. Data represent mean ± SEM of values from three independent experiments (n = 9 per condition). (E) IL-1β, IL-6, NO, and TNF-α levels in the supernatants of untreated or DI-treated BMDM that were subsequently infected with live *S. typhimurium* for the indicated periods of time. (F), numbers of intracellular bacteria determined in infected cells from E. Data represent mean ± SEM of triplicate cultures per timepoint from one of two experiments. P values were calculated using two-tailed Student's *t*-test (E, F) and one way ANOVA (C; compared to "0" DI). (G) Irg1 expression in macrophages infected with active or inactive Sendai virus as extracted from public data from GSE2935 (Tyner et al., 2005)

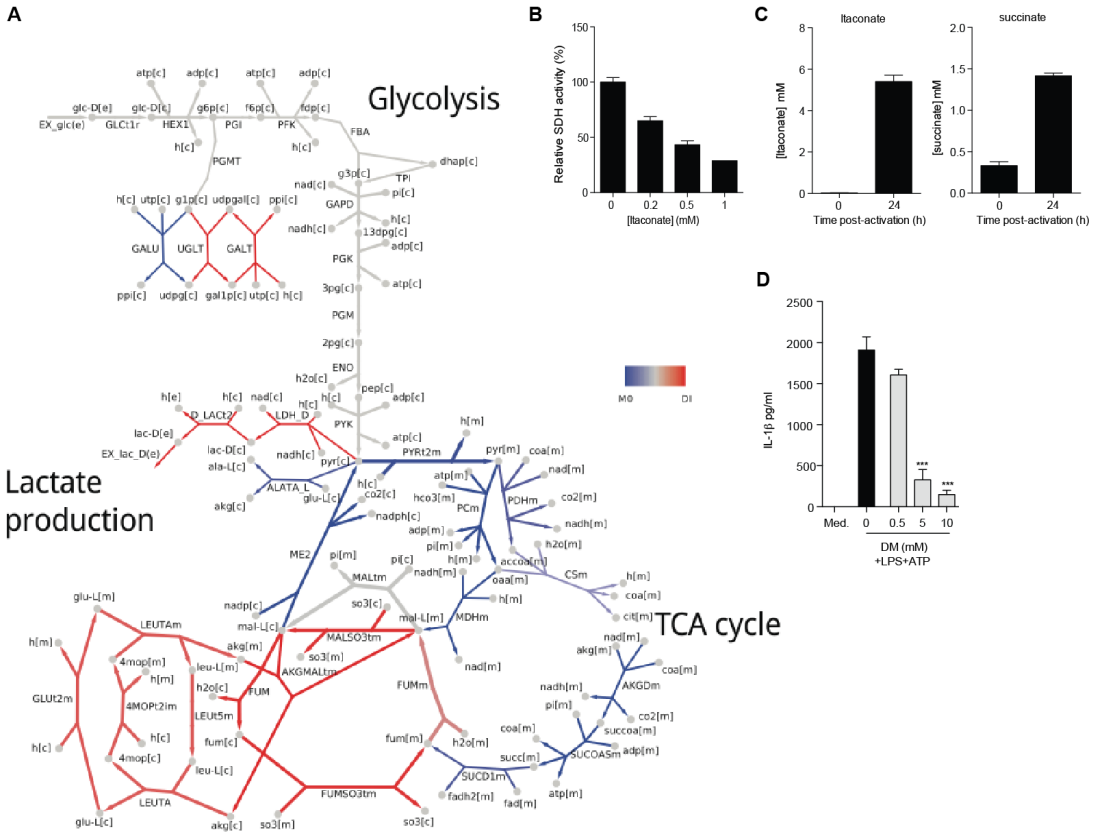

Figure S2, related to Figure 2. Itaconate inhibits activity of macrophage-derived Sdh.

(A) The complete comparative network of flux changes that occurred in resting WT BMDM in response to itaconate treatment. Blue edges indicate decreased fluxes, in red are increased fluxes and in grey fluxes unresponsive to DI treatment. This network includes the fluxes that are highlighted in Figure 2A.

(B) Relative activity of purified Sdh (from a macrophage cell line) in the presence of indicated doses of itaconate (normalizedSdh activity in the absence of itaconate) when 1 mM succinate was used as a substrate. Data shown are mean ± SEM (n=3). (C), Amounts of intracelullar succinate and itaconate in LPS-activated BMDM at 24h. Data shown are mean± SEM (n=3) (D) Mature IL-1b levels in the supernatant of BMDM that were pre-treated with dimethyl malonate (DM) as indicated and then stimulated with LPS+ATP. Data represent mean± SEM from two representative experiments.

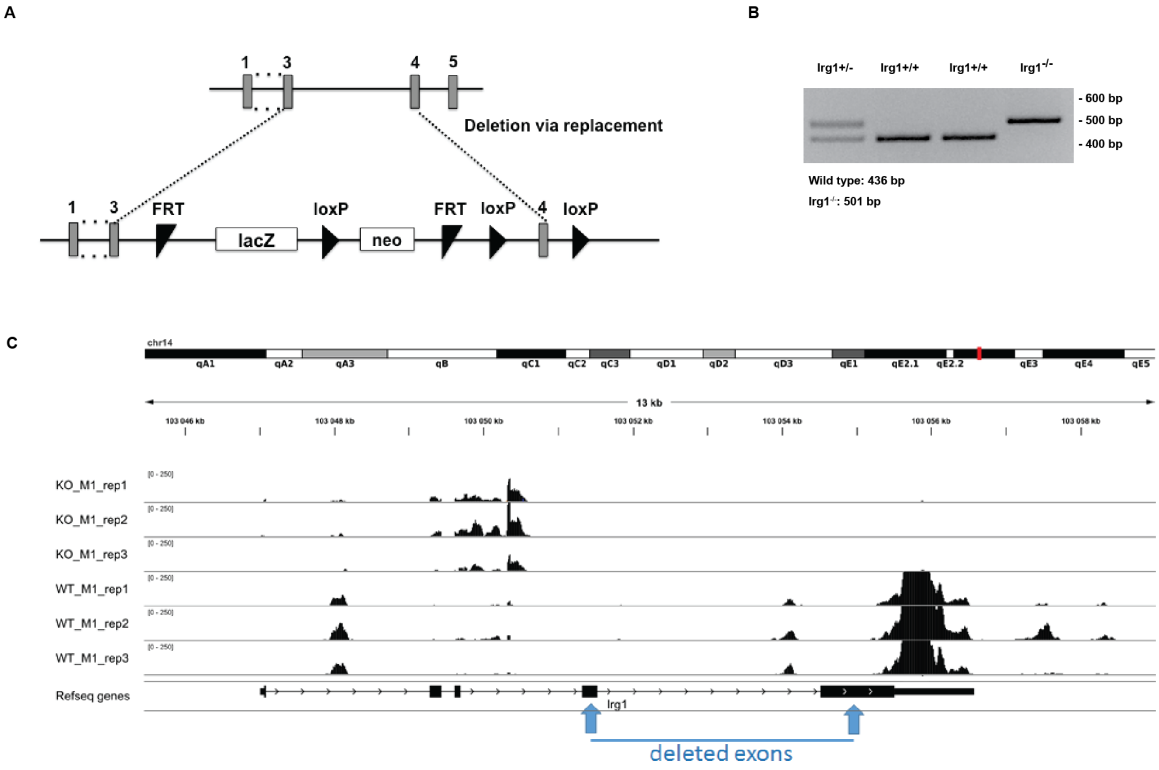

Figure S3. related to Figure 3. Generation and validation of *Irg1*$^{-/-}$ mice. a, Scheme of *Irg1* locus with targeting cassette. Exons are noted in grey and target location for insertion is noted with dashed lines. **b**, *Irg1* gene deletion was verified by PCR by the presence of 501 bp band in the mutant allele, whereas WT *Irg1* manifests as a 436 bp band. **c,** 3' RNA-seq *Irg1* gene coverage plot for WT and *Irg1*$^{-/-}$ macrophages after 24 h stimulation with LPS and IFN-g. Part of *Irg1* mRNA is expressed in *Irg1*$^{-/-}$ cells, but transcription downstream of the exon 4 is prevented.

Supplemental Experimental Procedures

***In vitro* infection with *Salmonella typhimurium*.** This *in vitro* infection protocol was adapted from Weiss et al(Weiss et al., 2004). Twenty-four-well plates were seeded with 2 x 10^5 BMDM/well in 500 µl of RPMI-1640 medium (Gibco) supplemented with 10% FBS (Hyclone), 2 mM L-glutamine (Sigma) and 100 µg/ml kanamycin (Sigma). Cultures were treated with 0.25 mM DI (final concentration) or vehicle (medium). After 12 h, 200,000 CFU *S. Typhimurium* (strain SB100; kanamycin resistant) were added (time zero) and plates were centrifuged for 10 min at 850 × *g*. Gentamicin (100 µg/ml; Sigma) was added at 30 min after infection. Triplicate samples were plated for each timepoint: 30 min, 6 h and 24 h. To determine the number of intracellular bacteria, supernatants was removed and cells were lysed by adding 50 µl of 10% Triton X-100 for 10 min before addition of 450 µl of cold sterile PBS. Appropriate dilutions were made, and samples were plated on Luria agar plates containing 10 µg/ml kanamycin (Sigma). Colonies were counted the next day.

Activation of the AIM2-inflammasome. Untreated of DI-treated BMDM were transfected with poly (dA:dT) (InvivoGen) complexed to Xfect polymer (Clontech laboratories) as previously described (Man et al., 2015); as a control, some cells received polymer alone.

Detailed Flux Balance Analysis. To investigate possible rewiring of the metabolic fluxes we used flux balance analysis framework (FBA). Using the RAW 264.7 macrophage cell line metabolic model (Bordbar et al., 2012) and an algorithm similar to GIMME (Becker and Palsson, 2008) and MADE (Jensen and Papin, 2011), we simulated the fluxes in untreated and itaconate-treated conditions based on their consistency with the obtained RNA-seq data for each condition. First, we updated the RAW 264.7 model as follows: i) added reactions regulated by Irg1, Aco1 and Aco2 and itaconate transport reactions; ii) updated several reaction-gene associations by adding missing homologs for the genes in corresponding Recon 2 reactions; iii) removed dependence of some oxidative phosphorylation reactions on the large gene complexes due to insufficient gene annotation; iv) removed octadecanoate (n-C18:0 and n-C18:1) and tetradecanoate (n-C14:0) production reaction from nothing and v) added ATP, NADH, acetyl coA (AcCoA) usage reactions. ($ATP + H_2O \rightarrow ADP + H^+ + PO_3$, $NADH \rightarrow NAD + H^+$, $AcCoA \rightarrow CoA$). Next, we found the maximal possible biomass production rate by running FBA linear optimization using the sybil R package (Gelius-Dietrich et al., 2013). We added a biomass production of at least 50% of this rate as a constraint to all models used consequently. For each reaction and each of the two conditions (untreated and Itaconate-treated) we calculated an inconsistency penalty per unit of additional flux. Following the MADE algorithm (Jensen et al, 2011), we used differential expression data as an indicator of a gene (and a corresponding reaction) being inactive: a down-regulated gene (p-value < 1e-2) in a given condition (untreated or Itaconate-treated) compared to any other condition (unstimulated macrophages, or with addition of LPS, itaconate, itaconate + LPS) was considered inactive. More formally, a penalty for a gene was calculated as min(-log10(p)+2, 0), where p is the gene minimal p-value among all three comparisons. The reaction penalty scores per unit of flux were calculated by substitution into the corresponding gene-reaction rule "min" and "max" operations instead of "or" and "and" operations and gene penalty instead of a gene. Next, we ran a linear optimization to find the fluxes for untreated and Itaconate-treated conditions with the minimal total inconsistency score and that produced biomass with at least 50% of the optimal rate. Because a reaction inconsistency score depends on the absolute flux, to calculate it we split each reversible reaction into two irreversible reactions corresponding to the forward and reverse directions. In this way, the total flux inconsistency was calculated as a sum of fluxes through individual reactions multiplied by the corresponding penalty score per unit of flux. Also, we used minimal total flux assumption and thus added 0.001 penalty per unit of flux for each reaction. To compare fluxes, we selected reactions that had reaction rates greater than 0.1 unit in any of the two conditions and selected the biggest connected component, ignoring ubiquitous metabolites such as water or ATP. These reactions are shown in **Figures 2A and S2A (**extended network). The width of an edge is proportional to sum of fluxes in M0 and M0 + itaconate conditions with a color displaying relative difference of the fluxes in the conditions. Blue edges correspond

to reactions that have flux only in M0 condition and red edges correspond to reactions that have flux only in M0+Itac conditions. Reactions that have equal predicted fluxes are shown in grey. For the visualization purposes akg[c], akg[m] and glu-L[c] vertices were split in two.

Supplemental References

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