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SUPPLEMENTARY INFORMATION

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Two-stage metabolic remodelling in macrophages in response to lipopolysaccharide and interferon-γ stimulation

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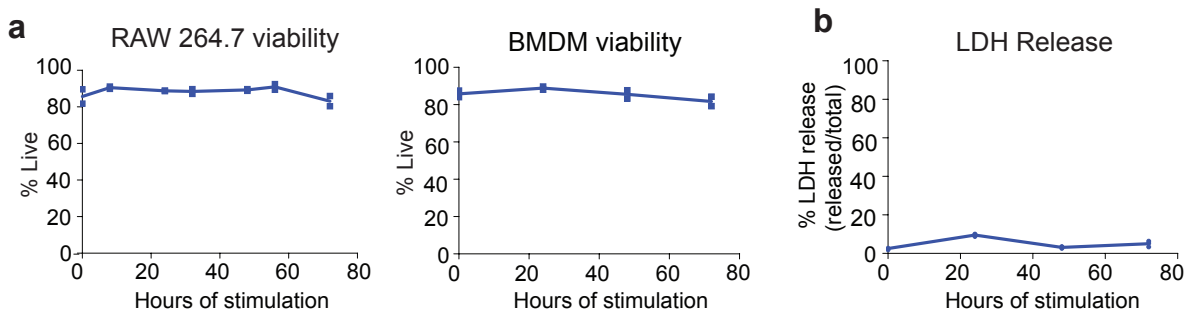
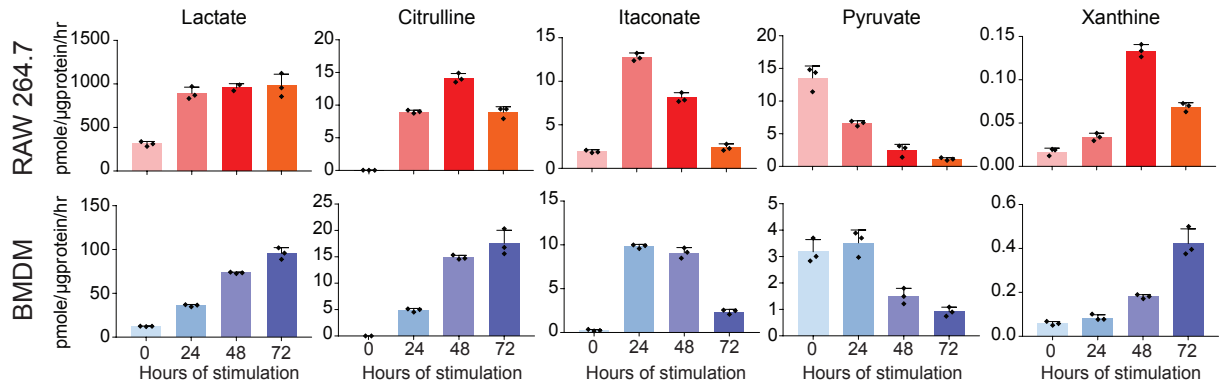


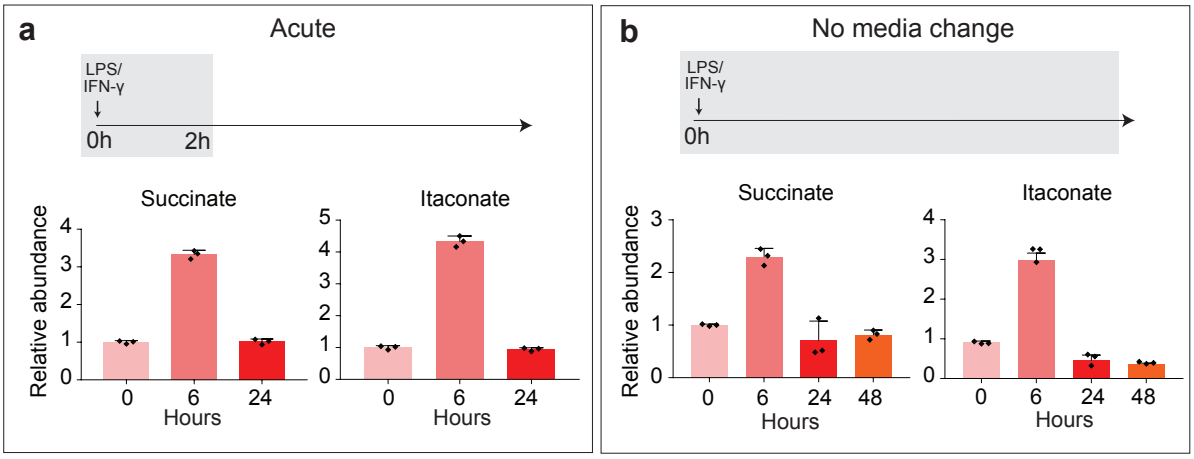
Supplementary Figure 1. Macrophages remain highly viable throughout the time-course of LPS and IFN-γ stimulation.

a. Viability of LPS and IFN-γ stimulated RAW 264.7 cells and BMDMs, as assessed by Trypan blue staining. Center value and error bar represent mean ± SD (n=2 independent samples), dots indicate individual values.

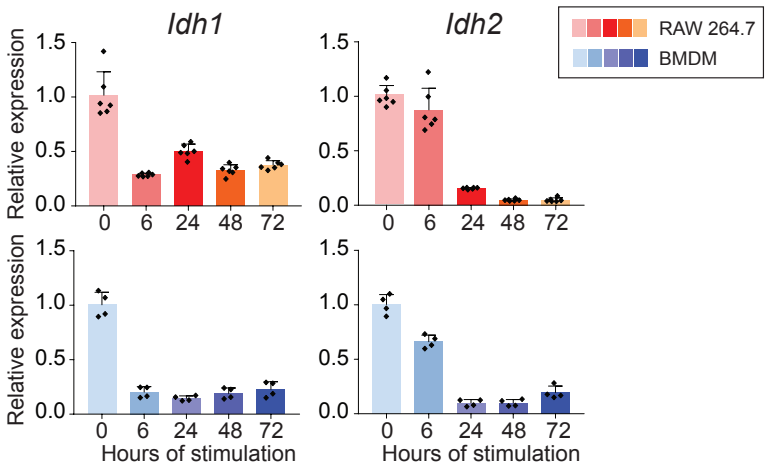
b. Lactate dehydrogenase (LDH) release from LPS and IFN-γ stimulated RAW 264.7 cells. Data are expressed relative to LDH activity after lysing cells (total). Center value and error bar represent mean ± SD (n=3 independent samples), dots indicate individual values.



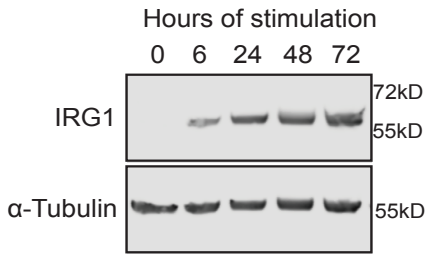
Supplementary Figure 2. Metabolite efflux from LPS and IFN-γ stimulated macrophages. Rate of lactate, citrulline, itaconate, pyruvate and xanthine release in RAW 264.7 cells and BMDMs stimulated with LPS and IFN-γ for indicated time. Rate is normalized to total cellular protein content. Bar graph and error bar represent the mean + SD (n=3 independent samples), dots indicate individual values.



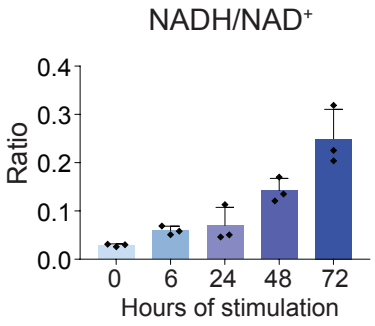
Supplementary Figure 3. Transient increase of succinate and itaconate level in response to LPS and IFN-γ stimulation. In addition to the experiment reported in Figure 1, where macrophages were stimulated with LPS and IFN-γ, and fresh media with stimuli were replaced every 24h throughout the time-course, intracellular succinate and itaconate levels were measured with two additional stimulation protocols: (a) cells were given a dose of LPS and IFN-γ for 2h, then the stimuli were removed completely by washing the cells, and media was replaced with fresh culture media without stimuli (acute); and (b) cells were give a dose of LPS and IFN-γ at time 0, then metabolism was monitored for 48h without changing media for the duration (no media change). Experiments were performed with RAW 264.7 cells. Metabolite abundances were normalized to protein content, then expressed relative to the level in unstimulated macrophages (0h). Bar graph and error bar represent the mean + SD (n=3 independent samples), dots indicate individual values.



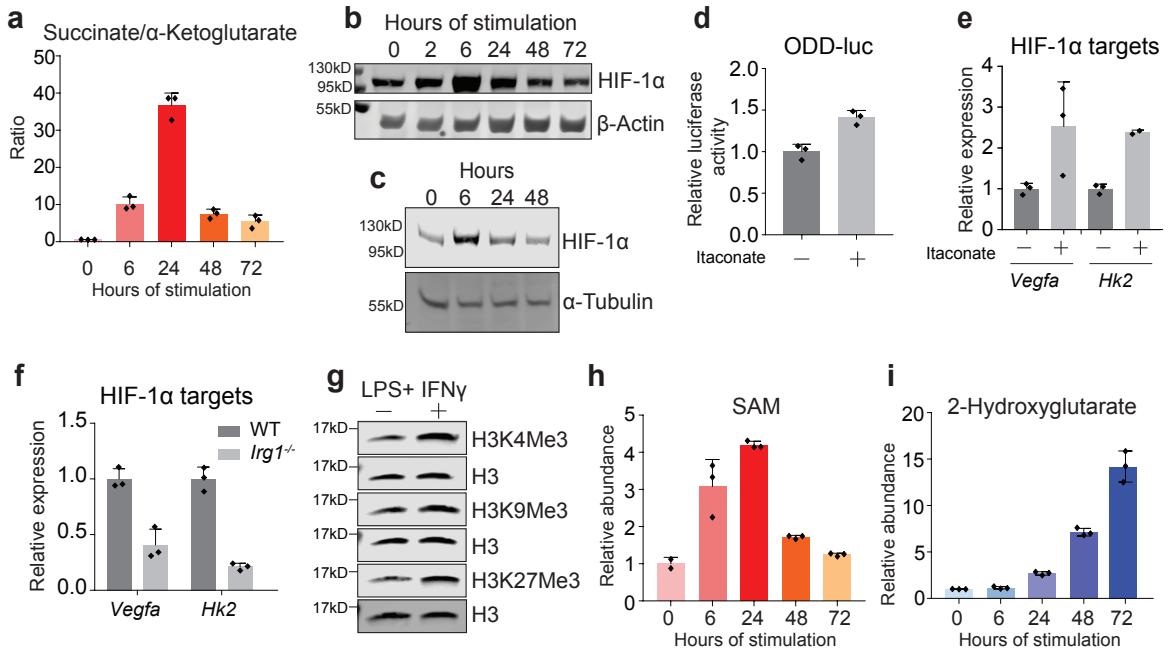
Supplementary Figure 4. IDH is transcriptionally downregulated in response to LPS and IFN-γ. mRNA level of *Idh1* and *Idh2* in LPS and IFN-γ stimulated RAW 264.7 cells and BMDMs. Data were normalized to the expression in unstimulated macrophages (0h). Bar graph and error bar represent the mean + SD (n=6 independent samples for RAW 264.7 cells, n=4 independent samples for BMDMs), dots indicate individual values.



Supplementary Figure 5. IRG1 protein level is elevated throughout time-course of LPS and IFN-γ stimulation. Protein levels of aconitate decarboxylase, IRG1, were determined by western blot in whole cell lysates of RAW 264.7 cells exposed to LPS and IFN-γ stimulation for indicated time. Experiment was repeated in BMDMs with similar results.



Supplementary Figure 6. Ratio of NADH to NAD$^+$ increases with LPS and IFN-γ stimulation in BMDMs. Bar graph and error bar represent the mean + SD (n=3 independent samples), dots indicate individual values.



Supplementary Figure 7. Changes in key metabolite levels correlate with change in HIF-1α protein and histone methylation.

a. Ratio of intracellular succinate to α-ketoglutarate in RAW 264.7 cells stimulated with LPS and IFN-γ for indicated time.

b, c. HIF-1α protein levels in RAW 264.7 cells cultured in media containing LPS+IFN-γ continually (b) and after an acute (2h) stimulation of LPS and IFN-γ (c). Both experiments have been performed three times with similar results.

d. Luciferase activity measured in BMDMs derived from ODD-luc mice after treatment with 5mM itaconate for 48h.

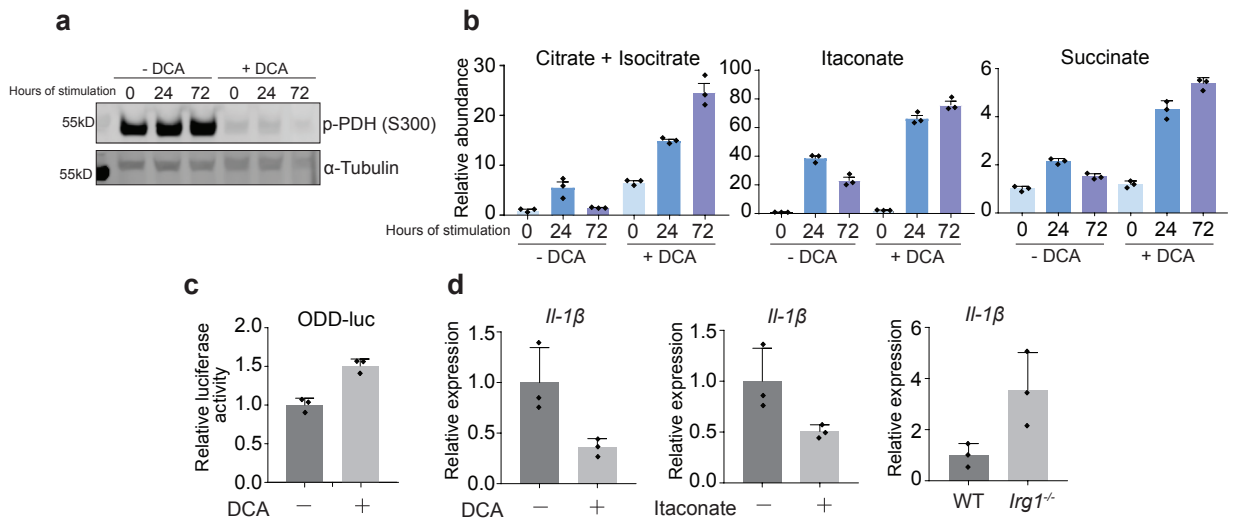
e, f. Relative mRNA level of canonical HIF-1α targets, *Vegfa* and *Hk2*, in LPS and IFN-γ stimulated (24h) RAW 264.7 cells with or without treatment of 5mM itaconate (e), and in LPS and IFN-γ stimulated (24h) wild-type (WT) or *Irg1*$^{-/-}$ BMDMs (f).

g. Histone methylation in RAW 264.7 cells stimulated with LPS and IFN-γ for 24h. Loading control of each H3 trimethylation mark is shown right below the corresponding trimethylation blot (blot was stripped and then reprobed with H3 antibody). Experiment was performed once.

h. Relative abundance of intracellular SAM in RAW 264.7 cells after stimulation with LPS and IFN-γ for indicated time.

i. Relative abundance of intracellular 2-hydroxyglutarate in BMDMs stimulated with LPS+IFN-γ for indicated time.

For a. d. e. f. h. i. Bar graph and error bar represent the mean + SD (n=3 independent samples), dots indicate individual values.



Supplementary Figure 8. Phosphorylation contributes to the dynamic regulation of PDHC flux in LPS and IFN-γ stimulated macrophages.

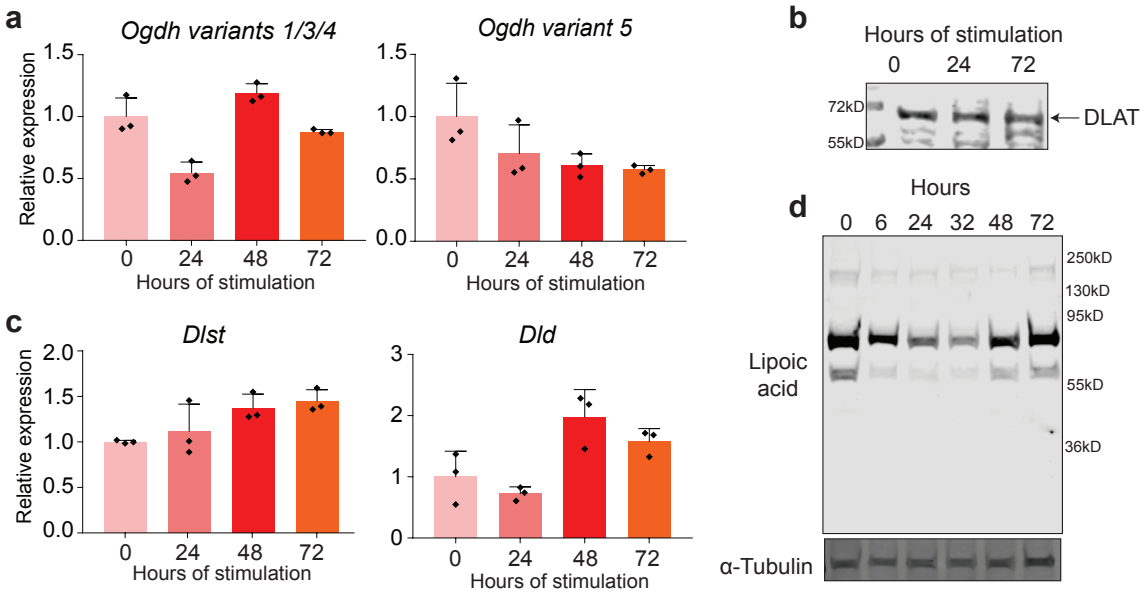
a. Total and phosphorylated PDH (S300) level in BMDMs stimulated with LPS and IFN-γ for indicated time, with or without dichloroacetate (DCA) treatment. Experiments were performed twice independently with similar results.

b. Relative abundance of intracellular citrate, itaconate and succinate in BMDMs stimulated with LPS and IFN-γ for indicated time, with or without DCA treatment.

c. Luciferase activity measured in BMDMs derived from ODD-luc mice after treatment with 10mM DCA for 48h.

d. Relative expression of *Il-1β* in LPS and IFN-γ stimulated (72h) RAW 264.7 cells with or without treatment of 10mM DCA or 5mM itaconate, and in LPS+IFN-γ stimulated (72h) wildtype (WT) or *Irg1*$^{-/-}$ BMDMs.

For b-d, Bar graph and error bar represent the mean + SD (n=3 independent samples), dots indicate individual values.



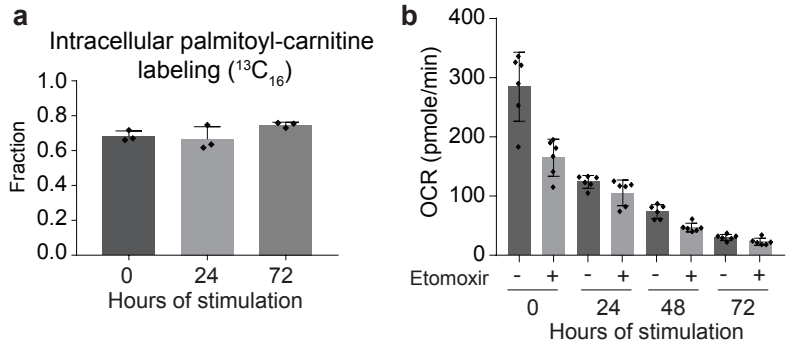
Supplementary Figure 9. Regulation of OGDC and PDHC in LPS and IFN-γ stimulated macrophages.

a. Relative mRNA level of OGDC E1 subunit (*Ogdh*) variants in RAW 264.7 cells stimulated with LPS and IFN-γ for indicated time. Bar graph and error bar represent the mean + SD (n=3 independent samples), dots indicate individual values.

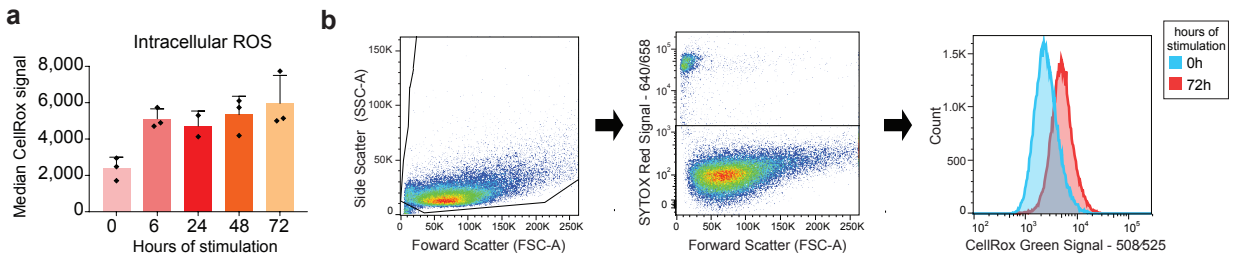
b. Protein levels of DLAT, the E2 subunit of the PDHC complex, in RAW 264.7 cells stimulated with LPS and IFN-γ for indicated time. Experiment performed once.

c. Relative mRNA level of the E2 (*Dlst*) and E3 (*Dld*) subunit of OGDC in RAW 264.7 cells stimulated with LPS and IFN-γ for indicated time. Bar graph and error bar represent the mean + SD (n=3 independent samples), dots indicate individual values.

d. Protein lipoylation in RAW 264.7 cells after an acute (2h) stimulation with LPS and IFN-γ. E2 subunit of PDHC is ~69 kD and E2 subunit of OGDC is ~57 kD. Experiment performed independently twice with similar results.



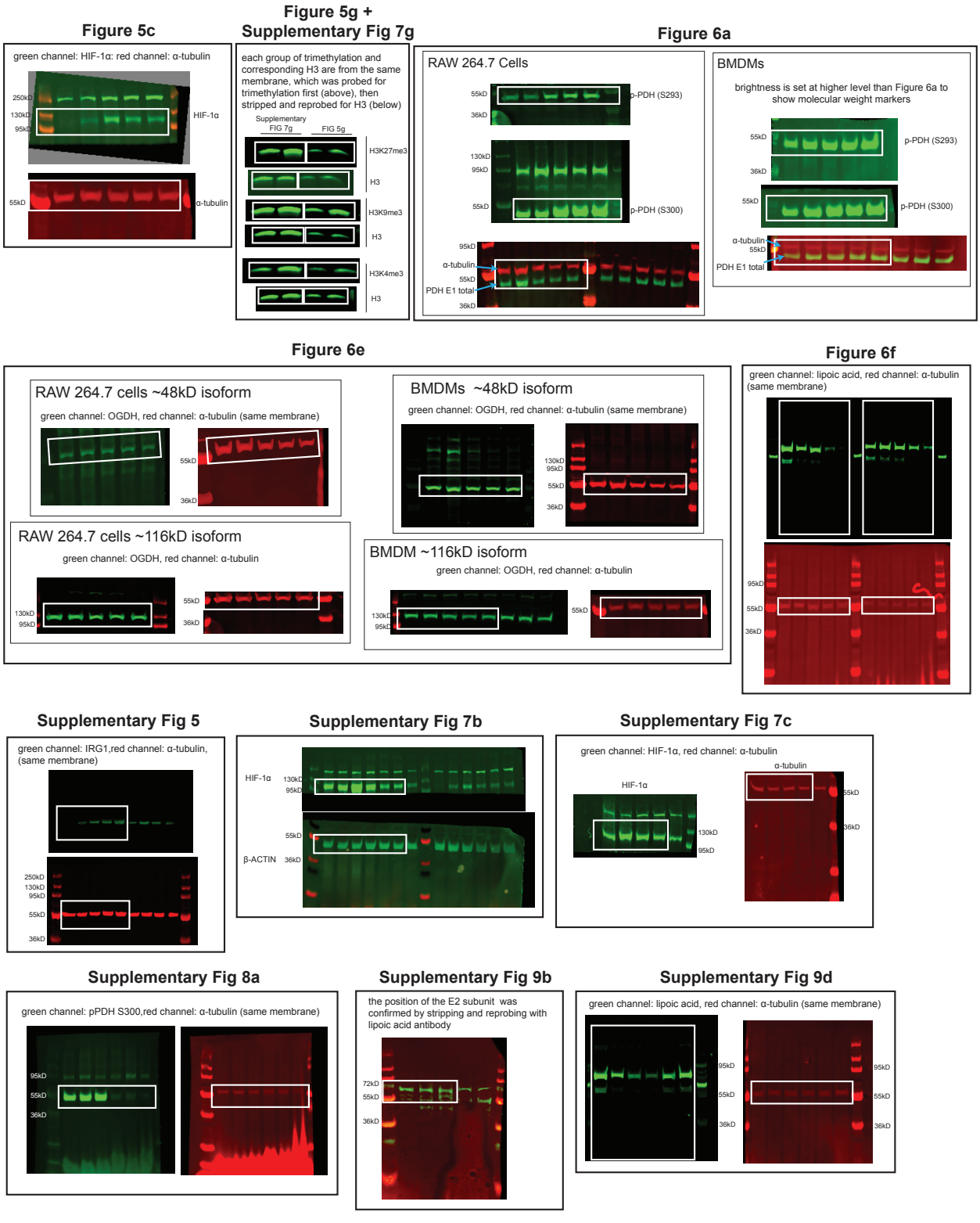
Supplementary Figure 10. Palmitate utilization in LPS and IFN-γ stimulated macrophages.
a. Labeled fraction of intracellular palmitoyl-carnitine in RAW 264.7 cells stimulated with LPS and IFN-γ for indicated time, after 24h incubation with 40μM BSA-conjugated U-^{13}C-palmitic acid. Bar graph and error bar represent the mean + SD (n=3 independent samples), dots indicate individual values.
b. Oxygen consumption rate (OCR) in RAW 264.7 cells stimulated with LPS and IFN-γ for indicated time, with or without the treatment with 40μM etomoxir. Bar graph and error bar represent the mean + SD (n=6 independent samples), dots indicate individual values.



Supplementary Figure 11. Intracellular ROS level increases with LPS and IFN-γ stimulation and remains elevated throughout the time-course.

a. Reactive oxygen species (ROS) in RAW 264.7 cells stimulated with LPS and IFN-γ for indicated time as measured by CellRox Green as specified in Methods. Bar graph and error bar represent the mean + SD (n=3 independent samples; except 24h, n=2 independent samples due to accidental loss of an additional replicate), dots indicate individual values.

b. Gating strategy for measuring CellRox signal. Cell debris were first gated out using SSC and FSC plot. From this population dead cells (SYTOX postitive) cells were gated out, and lastly, the median CellRox signal from this subpopulation was calculated.



Supplementary Figure 12. Uncropped western blot imagines. Regions reported in the corresponding figures or supplementary figures are indicated by box.