Supplemental Information

Nitric Oxide Modulates Metabolic Remodeling

in Inflammatory Macrophages through

TCA Cycle Regulation and Itaconate Accumulation

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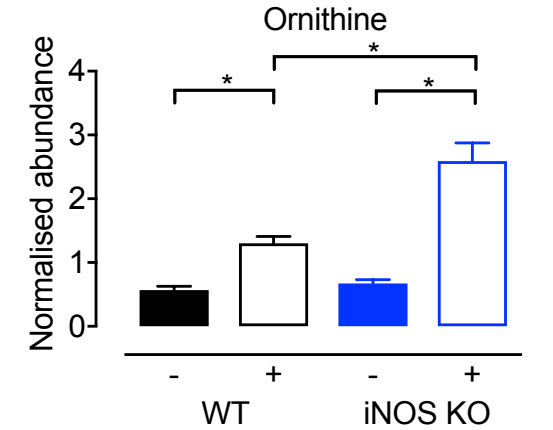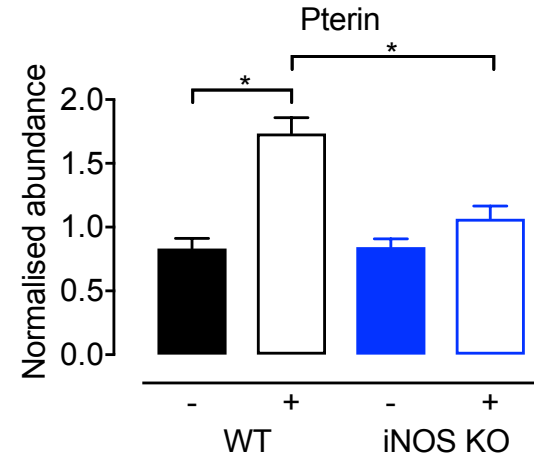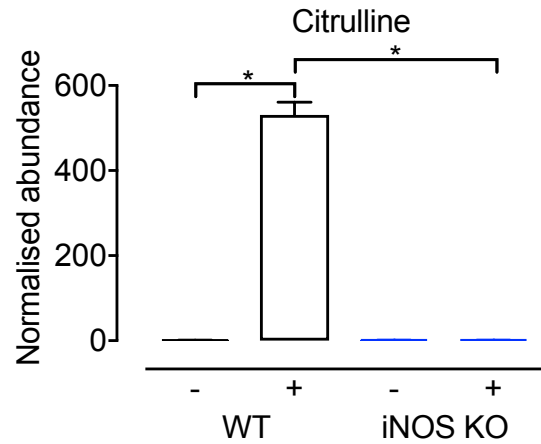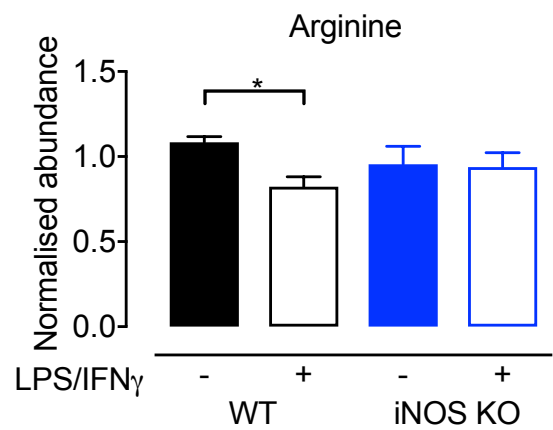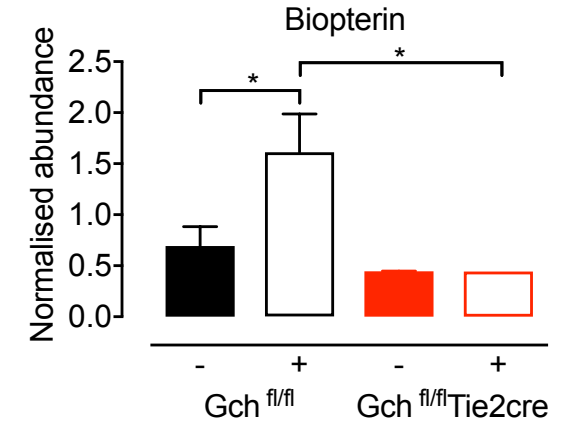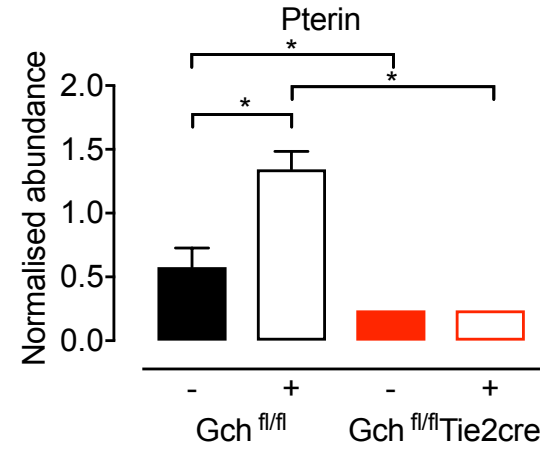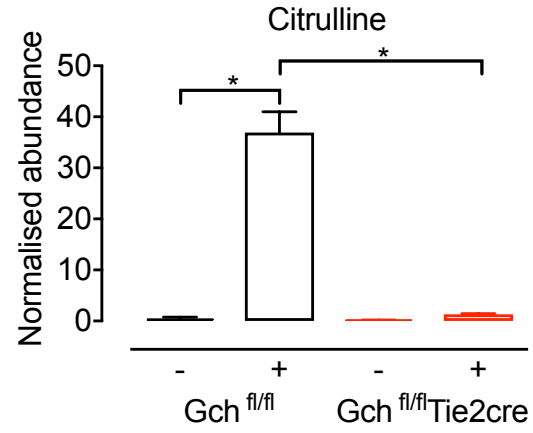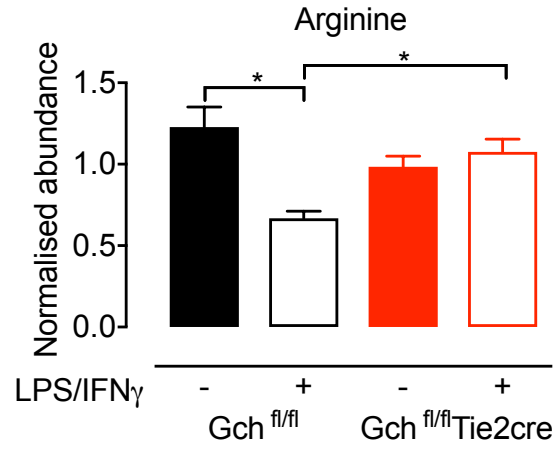
Supplemental figure 1. Quantification of BH4 and NOS related pathway metabolites. Related to Figure 1. Metabolomic measurements of Arginine, Citrulline, Pterin and Biopterin (n=6) Data are mean + SEM. *P* values calculated using 2-way ANOVA with Tukey's post-test (*$P<0.05$).
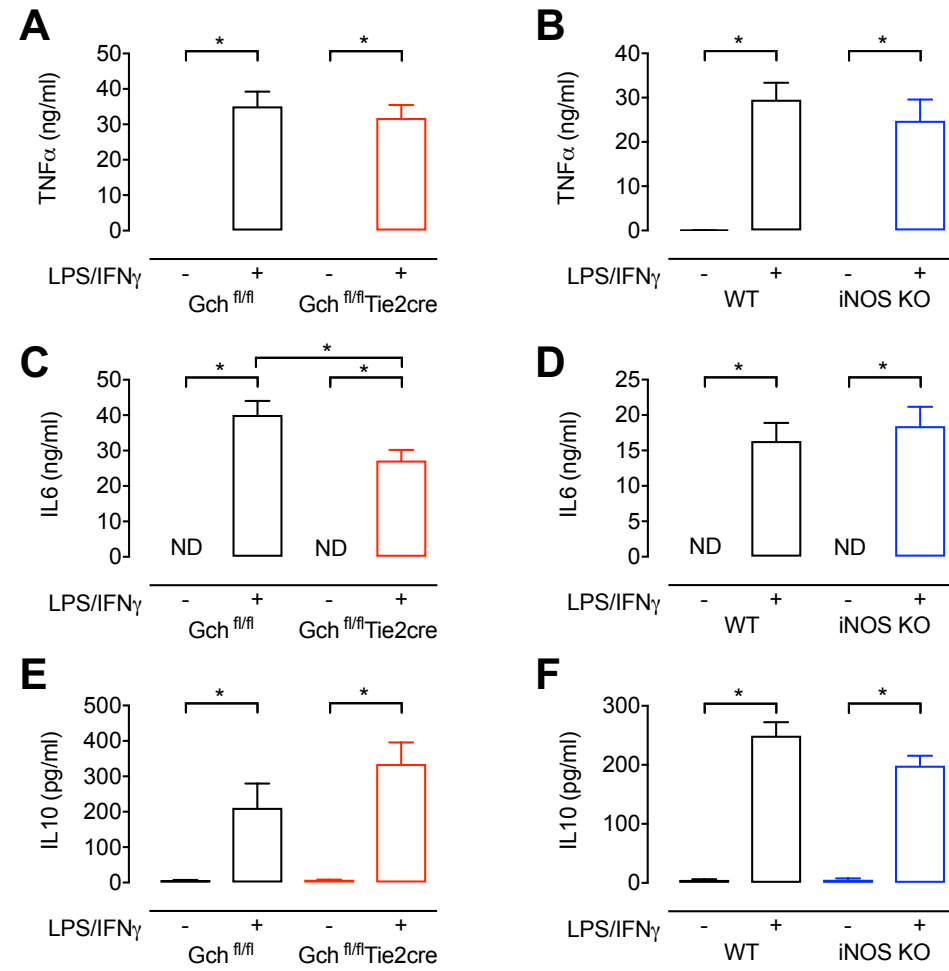
Supplemental figure 2. Inflammatory cytokines are induced in NO-deficient cells. Related to Figure 1. ELISA measurements of TNFα in (A) Gch^{fl/fl}Tie2cre and (B) iNOS KO cells, IL-6 in (C) Gch^{fl/fl}Tie2cre and (D) iNOS KO cells and IL-10 in (E) Gch^{fl/fl}Tie2cre and (F) iNOS KO cells. (n=5) Data are mean + SEM. *P* values calculated using 2-way ANOVA with Tukey's post-test (*$P<0.05$).

A

Unstimulated Gch$^{fl/fl}$ Tie2cre vs Gch$^{fl/fl}$

| Protein	Fold Change
Squalene synthase	2.69
Adenylate kinase 4, mitochondrial	2.25
Thioredoxin interacting protein	1.59
C5a anaphylatoxin chemotactic receptor 1	1.54
Extracellular serine/threonine protein kinase FAM20C	1.49
PTB-containing, cubilin and LRP1-interacting protein	1.42
Beta-1,4 N-acetylgalactosaminyltransferase 1	1.41
6-phosphofructokinase, liver type	1.37
Class II histocompatibility antigen, M alpha chain	-1.36
C-type lectin domain family 6 member A	-1.36
Urokinase-type plasminogen activator	-1.38
MCG21506	-1.41
Interferon regulatory factor 4	-1.41
Methylcytosine dioxygenase TET2	-1.43
Catenin beta-1	-1.64
Peroxisomal acyl-coenzyme A oxidase 3	-1.93
Placenta-expressed transcript 1 protein	-2.22
Cytochrome P450 2S1	-2.39
Arsenite methyltransferase	-3.76
GTP cyclohydrolase 1 (GTPCH)	-4.40

B

LPS/IFNγ treated Gch$^{fl/fl}$ Tie2cre vs Gch$^{fl/fl}$

Protein	Fold Change
R3H and coiled-coil domain-containing protein 1	4.95
Protein Ms4a7	2.90
Serum amyloid A-3 protein	2.30
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12 (NDUFS12)	1.96
Fatty acid desaturase 2	1.89
Protein Gm20547	1.79
Cytochrome P450 4V2	1.77
NADH dehydrogenase [ubiquinone] iron-sulfur protein 6, mitochondrial (NDUFS6)	1.73
Radical S-adenosyl methionine domain-containing protein 2	-1.84
Peroxisomal acyl-coenzyme A oxidase 3	-1.87
DEP domain-containing mTOR-interacting protein	-1.89
Solute carrier family 2, facilitated glucose transporter member 1 (GLUT-1)	-1.97
Platelet-activating factor acetylhydrolase	-1.99
OX-2 membrane glycoprotein	-2.15
Cytochrome P450 2S1	-2.16
Stearoyl-CoA desaturase-3	-2.42
BCL2/adenovirus E1B 19 kDa protein-interacting protein 3	-2.84
Arsenite methyltransferase	-3.07
Isoform 2 of sphingosine kinase 1	-4.67
GTP cyclohydrolase 1 (GTPCH)	-8.96

C
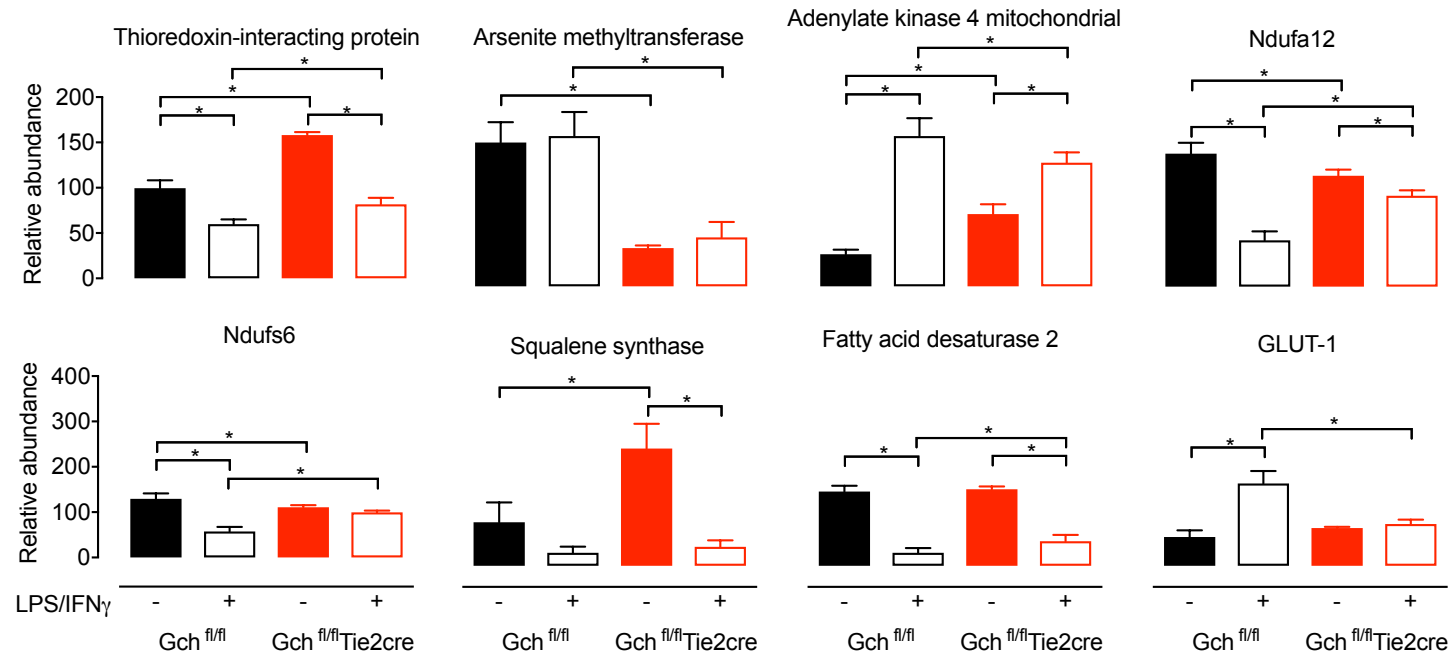
Supplemental figure 3. The proteins with the most changed abundance in Gch$^{fl/fl}$Tie2cre vs Gch$^{fl/fl}$ macrophages. Related to Figure 1. (A-B) The 20 proteins with the most changed abundance in Gch$^{fl/fl}$Tie2cre vs Gch$^{fl/fl}$ macrophages (A) unstimulated or (B) stimulated with LPS and IFNγ. (C) Abundance of selected proteins from A and B. (n=4) Data are mean + SEM (*P<0.05).
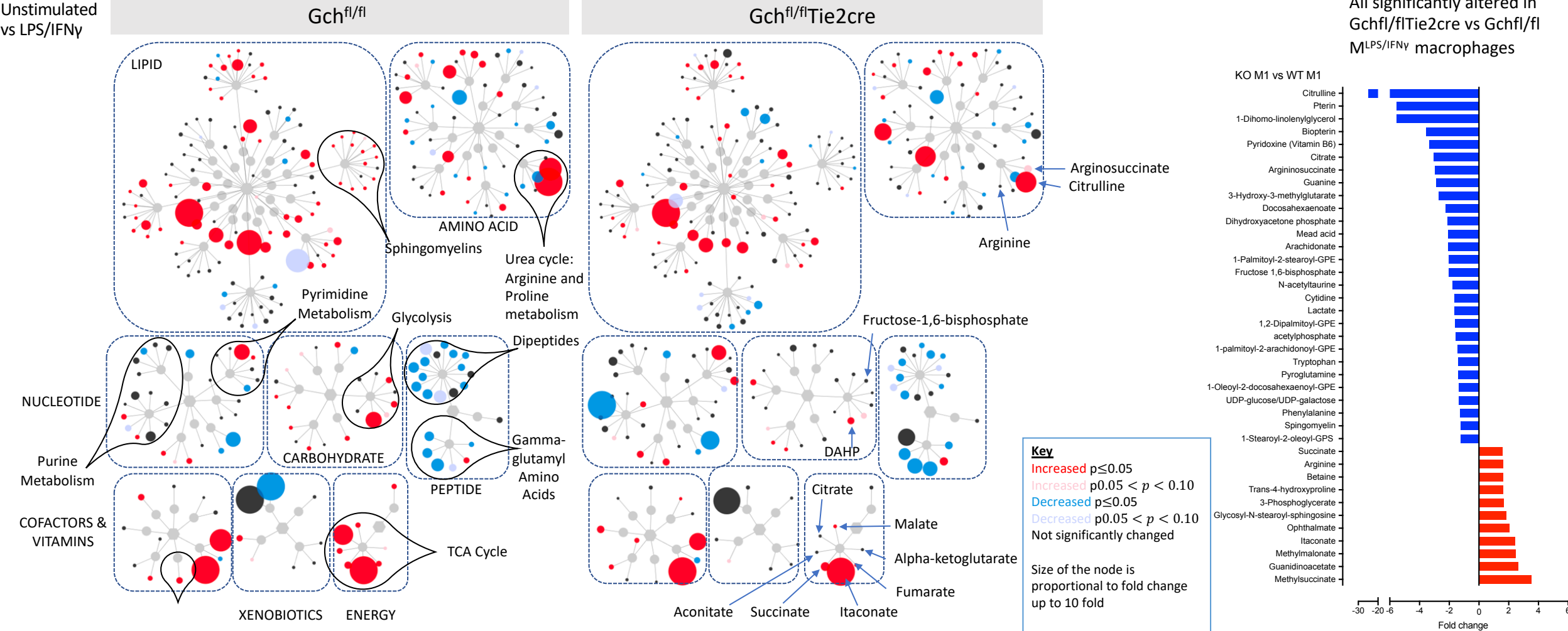
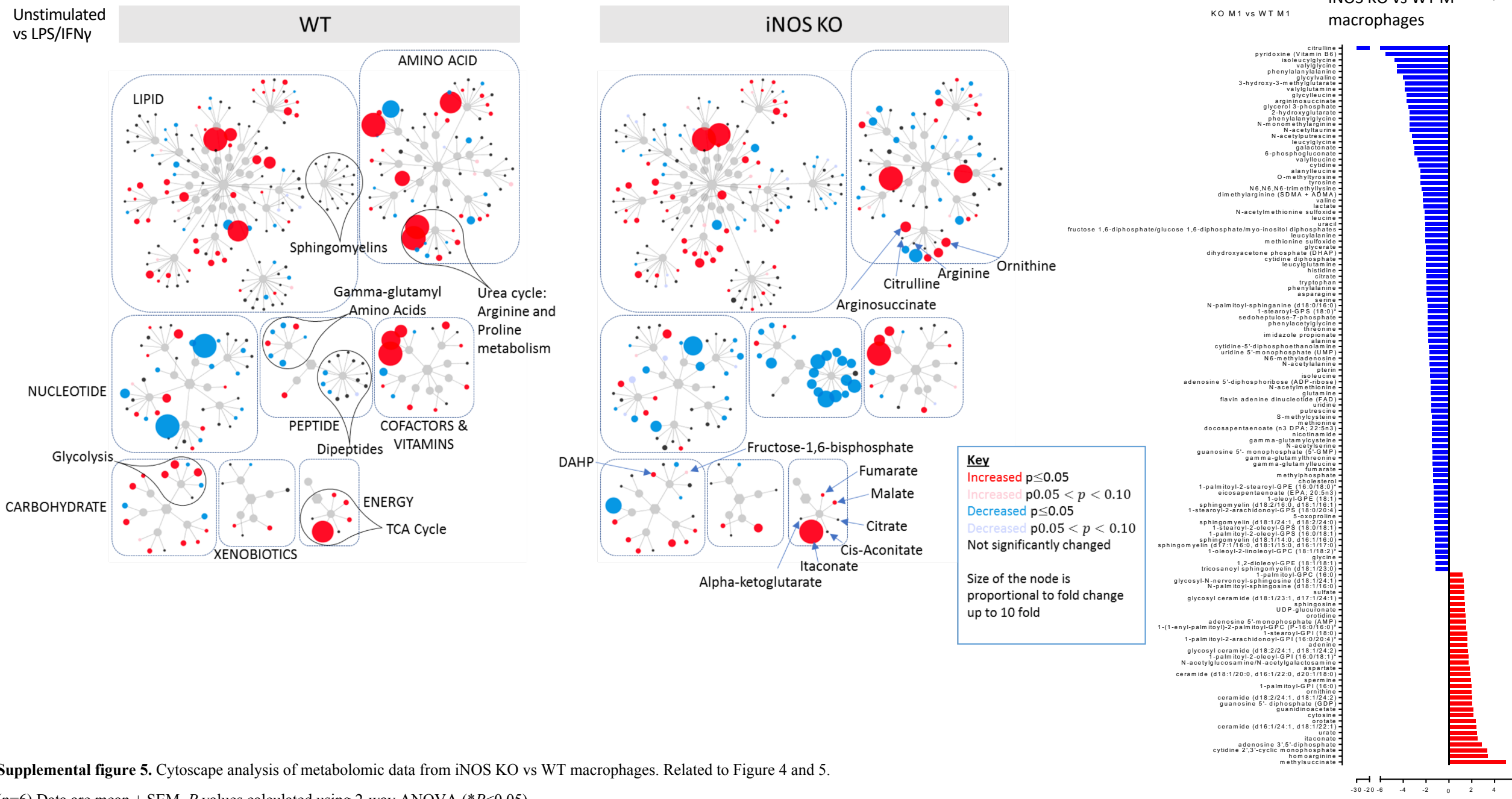**Supplemental figure 4.** Cytoscape analysis of metabolomic data from Gch^fl/fl^Tie2cre vs Gch^fl/fl^ macrophages. Related to Figure 4 and 5.

(n=6) Data are mean + SEM. *P* values calculated using 2-way ANOVA (*P<0.05).

Supplemental figure 5. Cytoscape analysis of metabolomic data from iNOS KO vs WT macrophages. Related to Figure 4 and 5.

(n=6) Data are mean + SEM. *P* values calculated using 2-way ANOVA (*P<0.05).
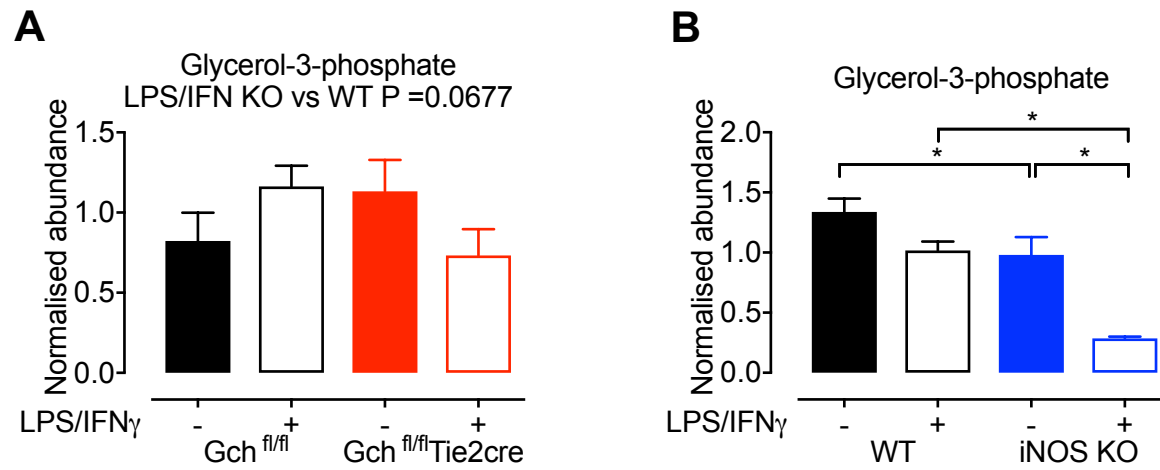
Supplemental figure 6. Glycerol-3-phosphate is decreased in NO deficient activated macrophages. Related to Figure 4. (A-B) Glycerol-3-phosphate abundance measured by metabolomics. (n=6) Data are mean + SEM. P values calculated using 2-way ANOVA (*$P<0.05$).
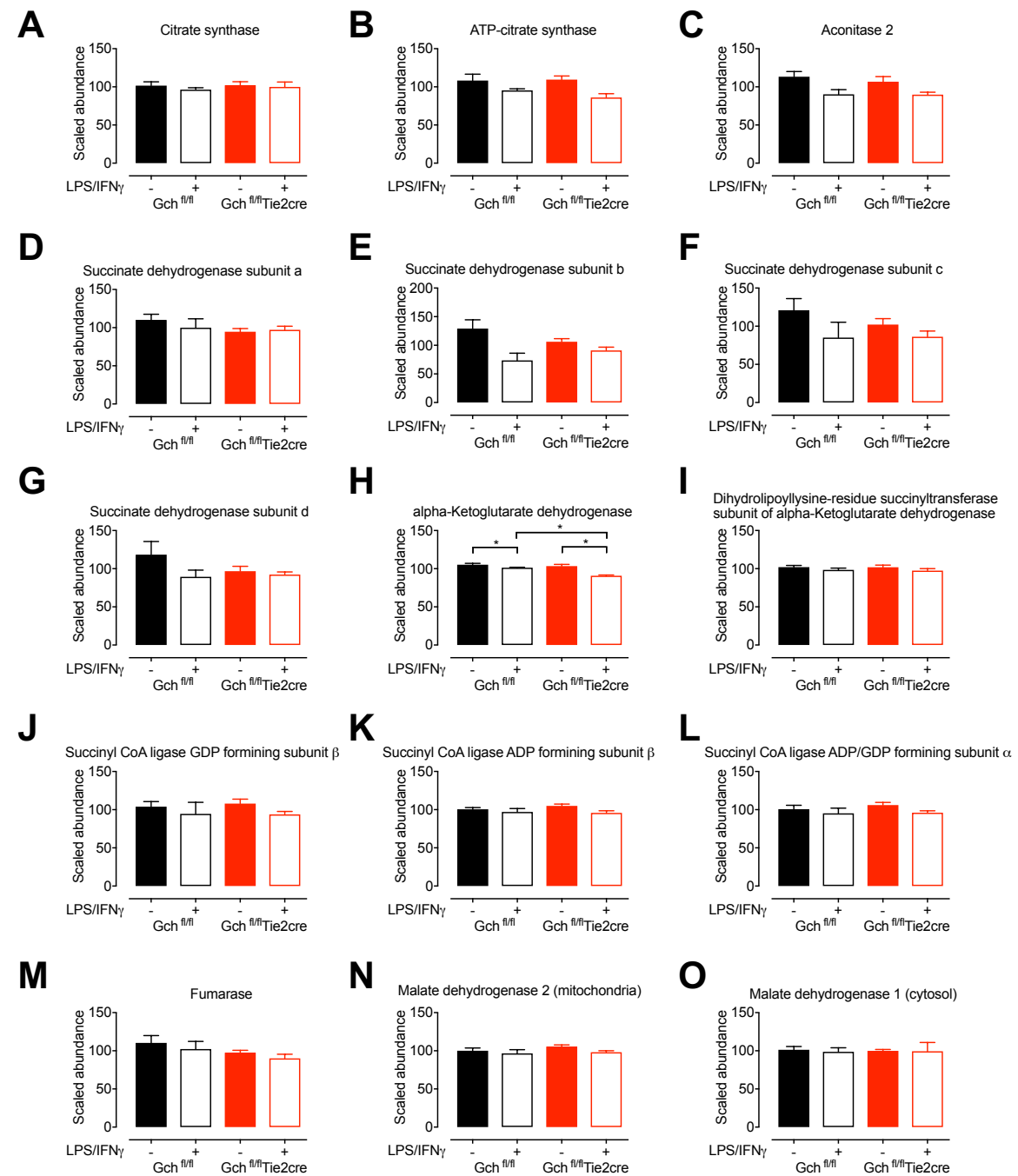
Supplemental figure 7. The abundance of TCA cycle enzymes, measured by metabolomics. Related to Figure 5. (n=6).
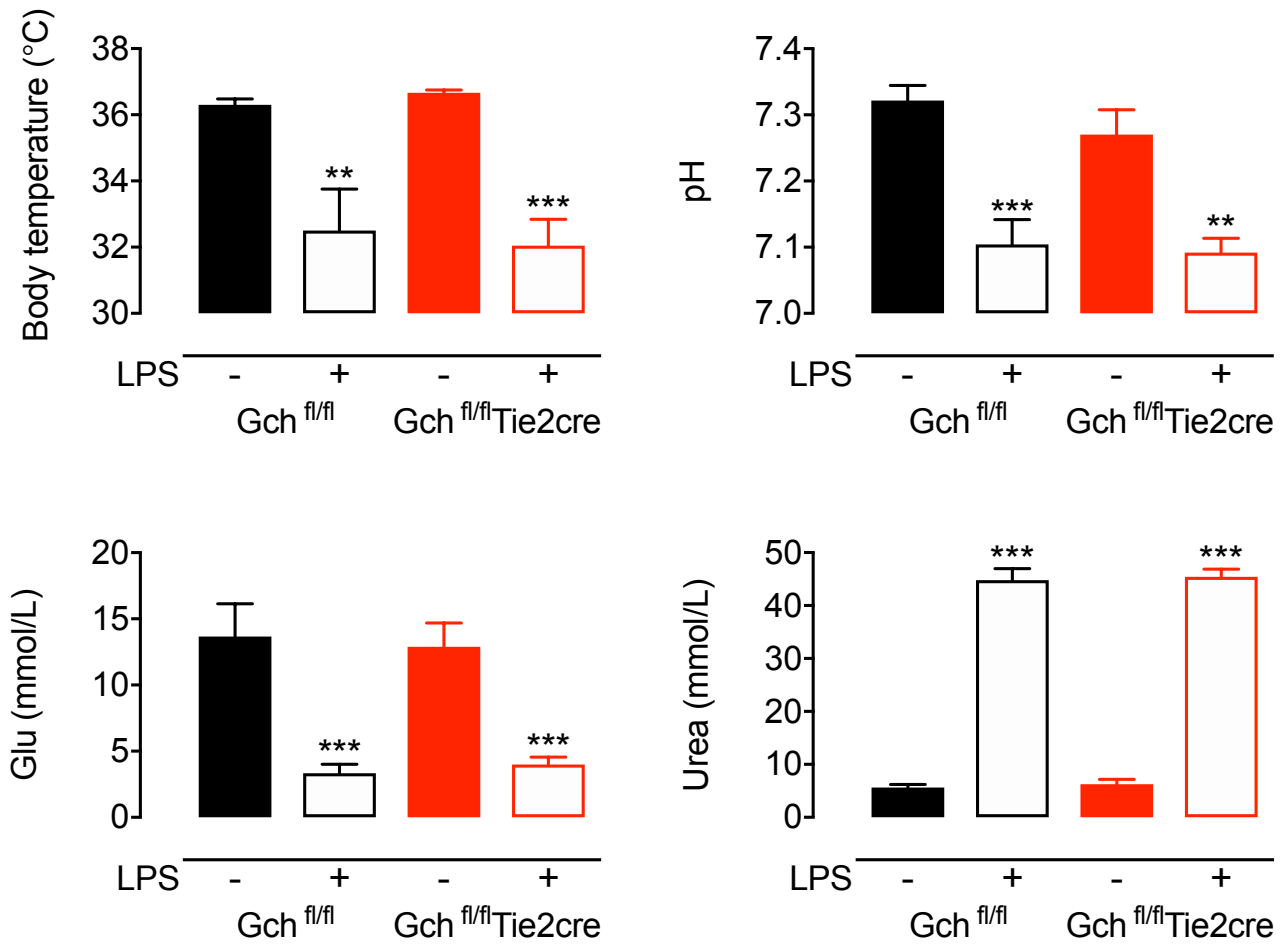
Supplemental figure 8. Physiological responses to acute endotoxaemia, induced by LPS (12.5 mg/kg). Related to Figure 7.

LPS/IFNγ	Gch^fl/fl		Gch^fl/fl Tie2cre	
	-	+	-	+
Citrate	0.378 ± 0.158	2.745 ± 0.337	0.804 ± 0.275	0.915 ± 0.238
cis-Aconitate	0.845 ± 0.368	1.770 ± 0.293	1.278 ± 0.378	1.079 ± 0.238
alpha-Ketoglutarate	0.788 ± 0.084	0.973 ± 0.116	1.223 ± 0.163	1.369 ± 0.155
Succinate	0.521 ± 0.080	1.339 ± 0.223	0.677 ± 0.124	2.122 ± 0.193
Fumarate	0.563 ± 0.148	1.160 ± 0.245	1.115 ± 0.283	1.445 ± 0.180
Malate	0.597 ± 0.130	1.132 ± 0.141	0.942 ± 0.183	1.271 ± 0.106
Itaconate	0.118 ± 0.010	2.390 ± 0.483	0.272 ± 080	5.780 ± 0.315

LPS/IFNγ	WT		iNOS KO	
	-	+	-	+
Citrate	0.873 ± 0.069	1.750 ± 0.147	0.865 ± 0.085	0.896 ± 0.098
cis-Aconitate	0.951 ± 0.074	1.095 ± 0.032	0.952 ± 0.078	1.051 ± 0.125
alpha-Ketoglutarate	0.871 ± 0.132	0.902 ± 0.144	0.914 ± 0.066	0.853 ± 0.144
Succinate	ND	ND	ND	ND
Fumarate	0.892 ± 0.090	1.685 ± 0.182	0.781 ± 0.073	1.200 ± 0.108
Malate	0.806 ± 0.082	1.779 ± 0.0260	0.731 ± 0.054	1.324 ± 0.135
Itaconate	0.192 ± 0.049	1.738 ± 0.139	0.234 ± 0.068	4.362 ± 0.232

Supplemental table 3. Metabolite abundances presented in heat map from Figure 5. Metabolite abundance measured by metabolomics. (n=6) Data are mean + SEM.

Proteins encoded by genes induced by HIF1α	References
GLUT-1	(Chen et al., 2001; Liu et al., 2016; Wang et al., 2017)
Hexokinase-1	(Luo et al., 2006)
Hexokinase-2	(Mathupala et al., 2001; Riddle et al., 2000)
PFKFB3	(Liu et al., 2016; Obach et al., 2004)
Phosphoglucomutase-1	(Hu et al., 2003; Pelletier et al., 2012)
Glycogen synthase	(Pescador et al., 2010)

Supplemental table 4. Glycolytic and glycogen metabolising proteins encoded by genes regulated by HIF1α. Related to Figures 4 and 5.