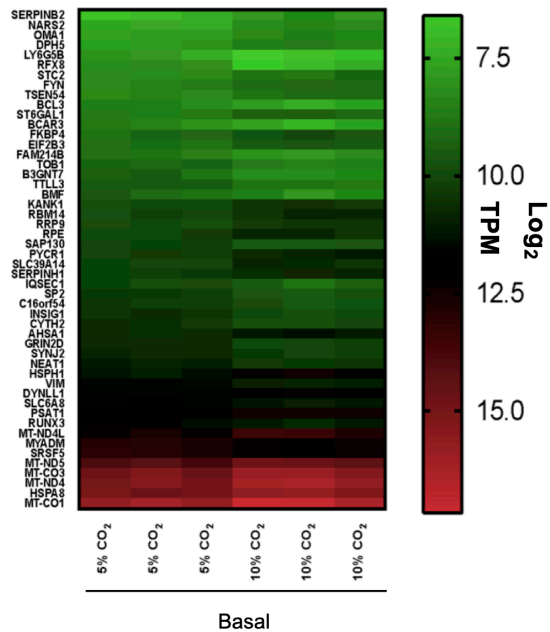
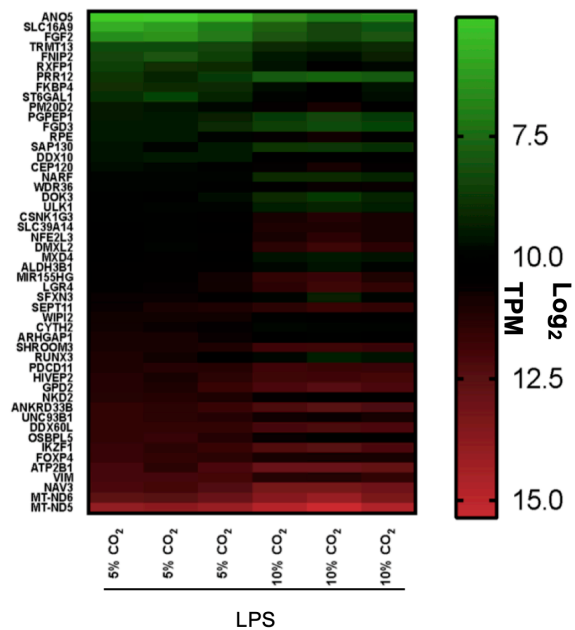


## Supplementary Figure 1

(a)

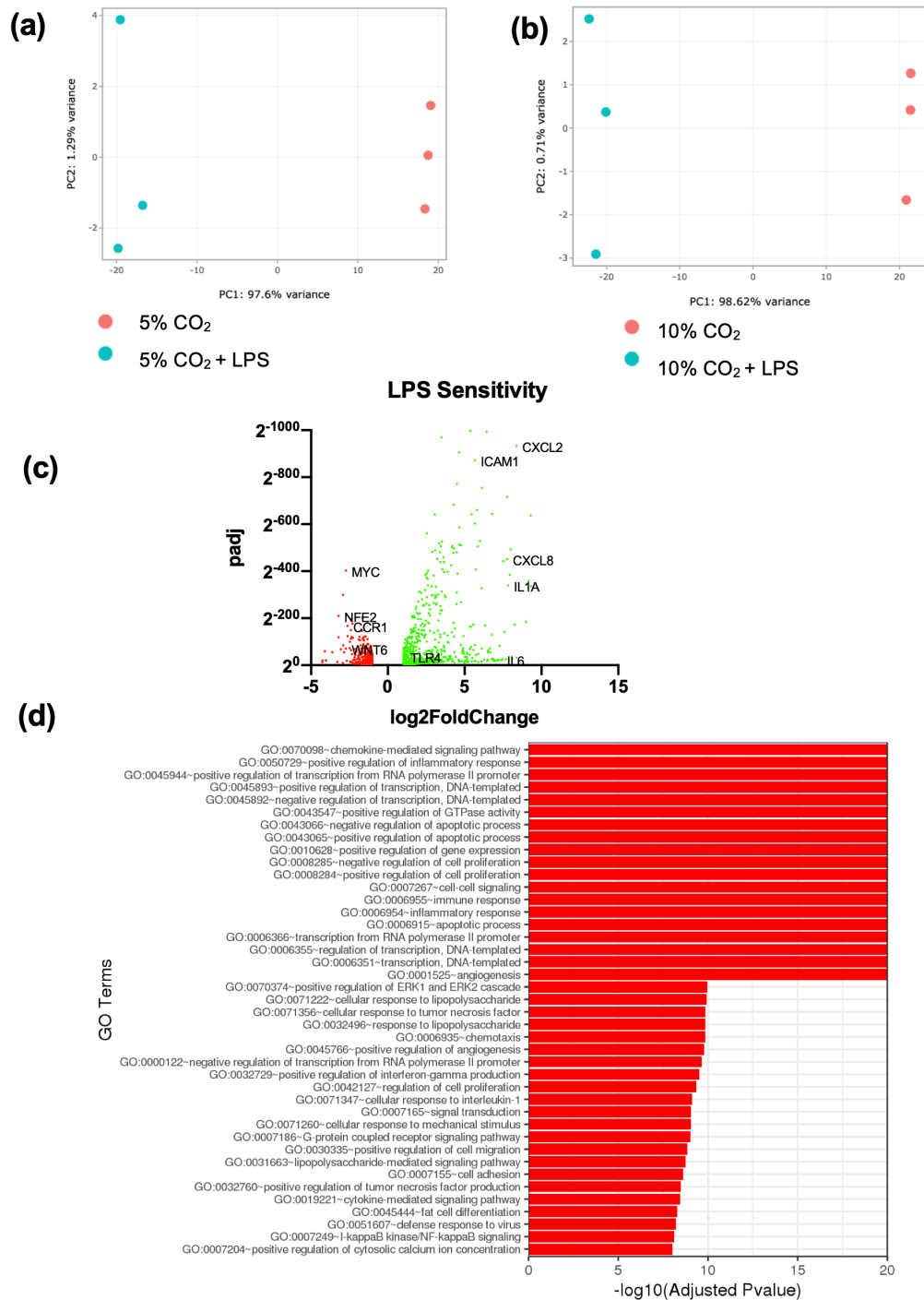


(b)



**Hypercapnia regulates gene expression in THP-1 monocytes in the basal and LPS -stimulated states.** Heat map of the top 50 differentially expressed genes between 5% and 10% CO<sub>2</sub> in the basal state for 4h. The top 50 genes were selected by significance (p-adj) and then ranked by expression level (Log<sub>2</sub> of TPM values) for visualisation. All genes had a p-adj <0.05. Data are representative of n=3 experiments (a). Heat map of the top 50 differentially expressed genes between 5% and 10% CO<sub>2</sub> in an LPS stimulated state. The top 50 genes were selected by significance (p-adj) and then ranked by expression level (Log<sub>2</sub> of TPM values) for visualisation. All genes had a p-adj <0.05. Data are representative of n=3 experiments (b).

## Supplementary Figure 2

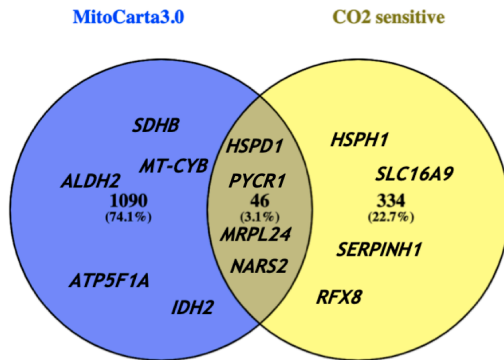


### LPS affects gene expression in monocytes.

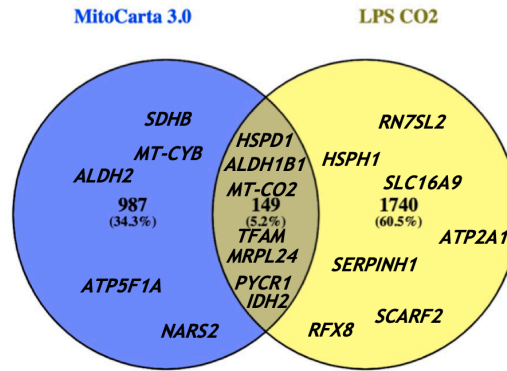
PCA plots between 5% CO<sub>2</sub>, and 5% CO<sub>2</sub> + LPS treated cells **(a)** and 10% CO<sub>2</sub> and 10% CO<sub>2</sub> +LPS treated cells **(b)**. Volcano plot of differential expression between control and LPS treated (2.5µg/ml for 2h) cells at 5% CO<sub>2</sub>. The cut offs employed are  $\log_2$ FoldChange >1 or <-1,  $p_{adj}$  <0.05. Upregulated genes are shown in green, downregulated genes are shown in red. Data is representative of n=3 experiments **(c)**. The top gene ontology terms associated with differentially expressed genes between control and LPS treated (2.5µg/ml for 2hrs) cells at 5% CO<sub>2</sub>. The cut offs employed are  $\log_2$ FoldChange >1 or <-1,  $p_{adj}$  <0.05. Terms are ranked by fold enrichment. Data is representative of n=3 experiments **(d)**.

### Supplementary Figure 3

(a)



(b)



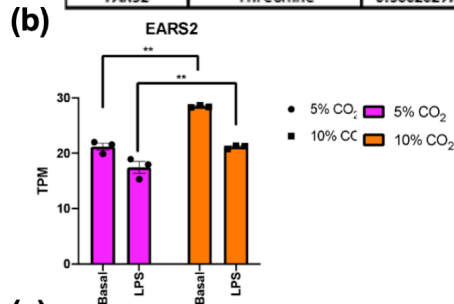
### Supplementary Figure 3.

**Hypercapnia regulates mitochondrial gene expression in monocytes in the basal and LPS-stimulated states.** Venn diagram of mitochondrial genes associated with the MitoCarta 3.0 database (blue) and significantly differentially expressed genes in hypercapnia in the basal state (a) and in the LPS stimulated state (b) ( $p\text{-adj} \leq 0.05$ ) (yellow). Image generated using Venny <https://bioinfogp.cnb.csic.es/tools/venny/index.html>

## Supplementary Figure 4

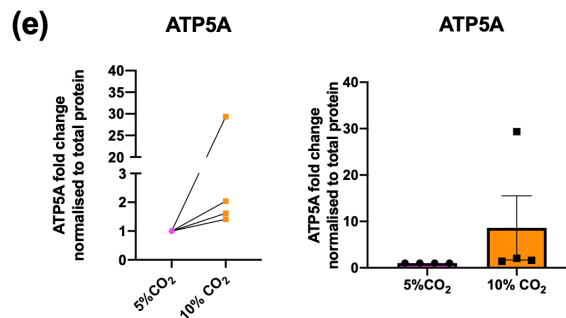
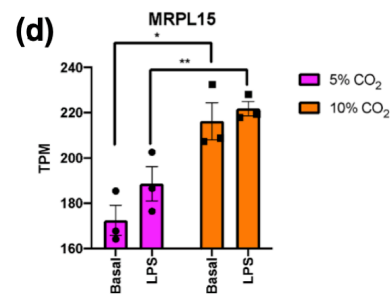
(a)

Significant Aminoacyl tRNA Synthetases					
5% CO <sub>2</sub> vs 10% CO <sub>2</sub>					
Gene	Amino Acid	Basal		LPS-Stimulated	
		LOG2FC	p-adj	LOG2FC	p-adj
AARS2	Alanine	0.380711939	0.027938545	0.387630666	9.08E-03
DARS2	Aspartic Acid	0.199970335	0.506343522	0.380817037	0.020711511
EARS2	Glutamic Acid	0.508974692	1.54E-03	0.407725889	8.62E-03
NARS2	Asparagine	0.82522484	1.29E-04	0.417230486	0.072824704
TARS2	Threonine	0.506262977	0.014313262	0.286736561	0.122851793



(c)

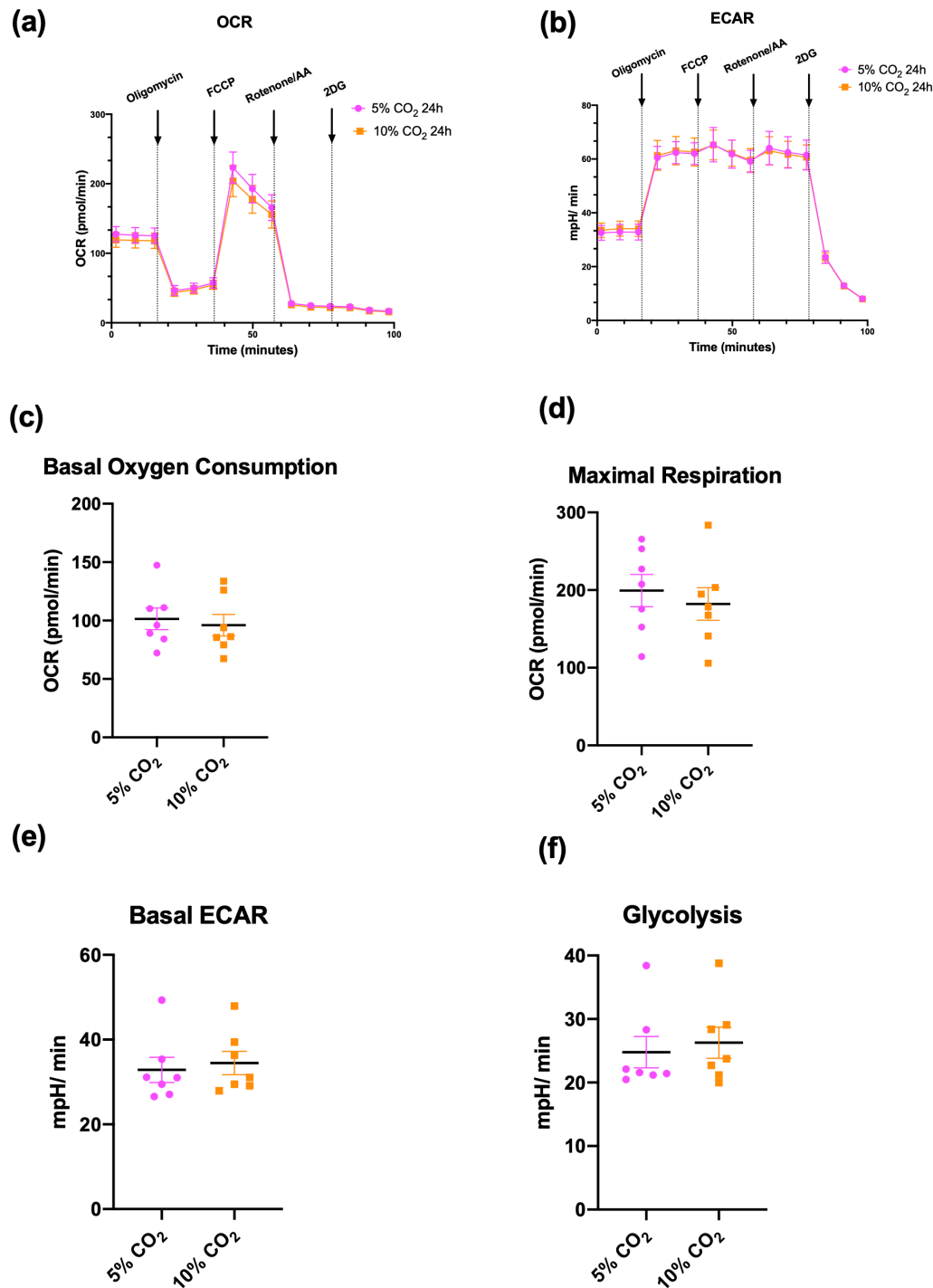
Significant Mitochondrial Proteins					
5% CO <sub>2</sub> vs 10% CO <sub>2</sub>					
Gene	Mitochondrial Subunit	Basal		LPS-Stimulated	
		LOG2FC	p-adj	LOG2FC	p-adj
MRPL1	Large	0.37888066	0.07801416	0.328665701	0.047536476
MRPL3	Large	0.310227446	0.078118385	0.362502101	0.00417056
MRPL14	Large	0.354490701	0.11771385	0.296950399	0.044087516
MRPL15	Large	0.4082036	0.012493083	0.357019223	0.009155445
MRPL17	Large	0.236049359	0.241395756	0.341530765	0.036013238
MRPL19	Large	0.25788655	0.348276434	0.345414273	0.033591935
MRPL24	Large	0.398561208	0.025756164	0.300253692	0.034916625
MRPL30	Large	0.214612429	0.433994076	0.399991861	0.003175403
MRPL32	Large	0.298130613	0.219816173	0.35213712	0.040208417
MRPL33	Large	0.402198645	0.034044735	0.389587789	0.011632126
MRPL34	Large	0.408325717	0.069635359	0.428819521	0.012387402
MRP525	Small	0.323401866	0.1244148	0.397665513	0.006021671
MRP530	Small	0.236256695	0.202768379	0.272607033	0.026304776
MRP533	Small	0.19612769	0.458115946	0.343005972	0.037356545



### Monocyte nuclear encoded mitochondrial genes in hypercapnia

List of significant differentially expressed aminoacyl tRNA synthetase genes in THP-1 cells exposed to 5% or 10% CO<sub>2</sub> for 4h +/- LPS (2.5µg/ml) for 2h. Non-significant results are shown in grey (a). Raw TPM values extracted from RNA-seq data for *EARS2* (b), and *MRPL15* (d) in THP-1 cells in the basal (unstimulated) and LPS stimulated (2.5µg/ml for 2h) state in both 5% (pink) and 10% (orange) CO<sub>2</sub> conditions (4h). Data shown as mean +/- SEM for n=3 independent experiments. Significant differential expression from individual comparisons using DESeq2 analysis is denoted by (\*). List of significant differentially expressed mitochondrial ribosomal protein genes in hypercapnia in basal and LPS stimulated states. Non-significant results in the basal state are shown in grey (c). Protein quantification of ATP5A protein expression in mitochondrial extracts from THP-1 cells exposed to 5% or 10% CO<sub>2</sub> for 24h was performed using Empiria Studio, and ATP5A fold change normalised to total protein (Revert stain) was plotted (n=4) (e).

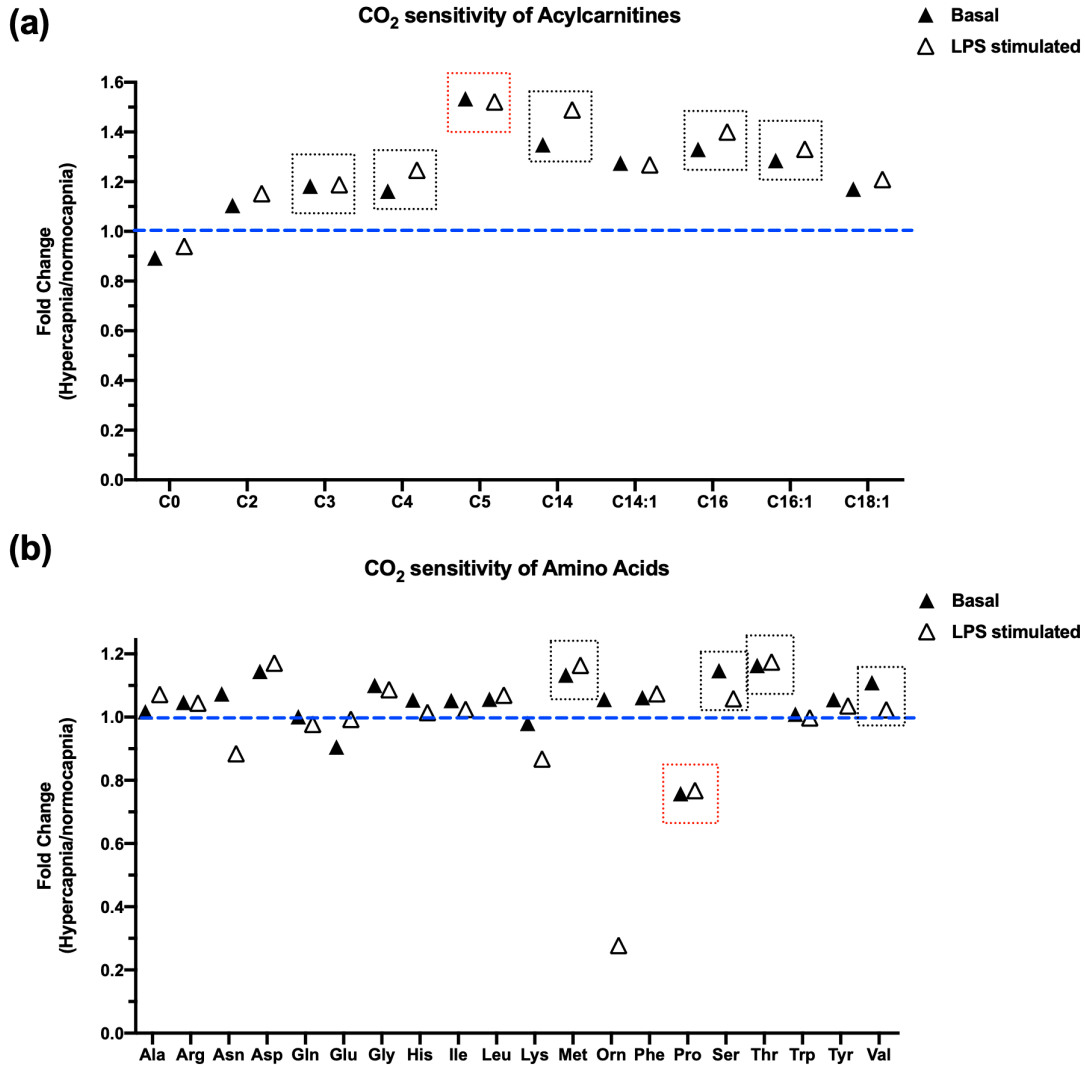
## Supplementary Figure 5



**Hypercapnia does not significantly affect oxygen consumption rate (OCR) or extracellular acidification rate (ECAR) in THP-1 monocytes.**

Real time cell metabolic analysis was performed on THP-1 cells pre-exposed to 5% or 10% CO<sub>2</sub> for 24h prior to Seahorse XF Cell Mito Stress Tests performed at 0.04% CO<sub>2</sub>. OCR (a) and ECAR (b) were measured for the duration of the experiment. Basal oxygen consumption (c), maximal respiration (d), basal ECAR (e) and glycolysis (f) were calculated using raw OCR and ECAR values. Data are mean  $\pm$  SEM for n=7 individual experiments. No statistically significant differences were observed between 5% CO<sub>2</sub> and 10% CO<sub>2</sub>.

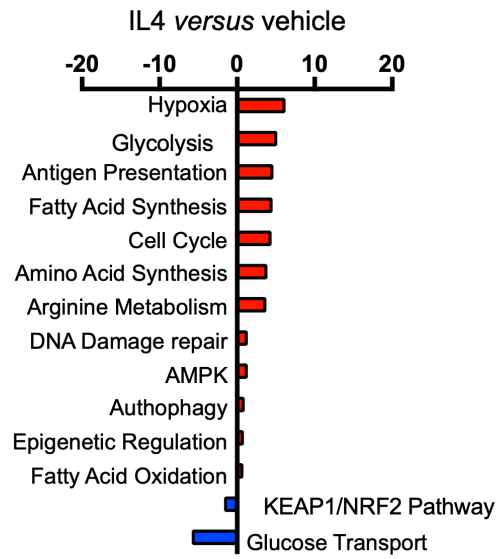
## Supplementary Figure 6



### Hypercapnia affects acylcarnitine and amino acid expression in THP-1 monocytes.

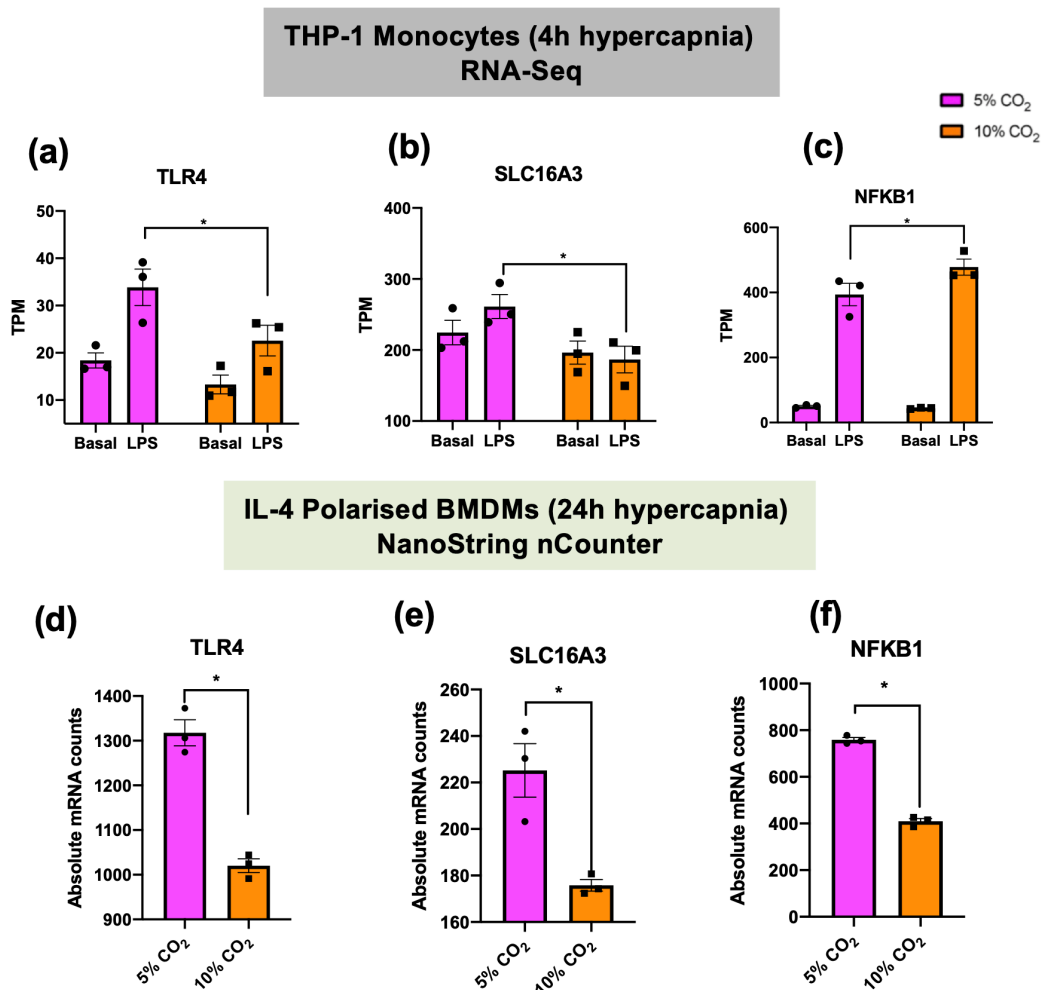
LC-MS/MS analysis of metabolites from THP-1 cells exposed to 5% or 10% CO<sub>2</sub> for 24h +/- LPS (2.5µg/ml for 2h). Data shown are ratio of hypercapnia/normocapnia for specific acylcarnitines **(a)** and amino acids **(b)** normalised to total metabolites in the basal state (filled triangles) and in the presence of LPS (clear triangles). Boxes indicate metabolites that were deemed statistically significantly different in response to 10% CO<sub>2</sub> when statistical analysis of metabolites (n=10) was compared using one-way ANOVA followed by a Fisher's LSD post-hoc test. Red boxes indicate the most statistically significantly different metabolite in each case in response to 10% CO<sub>2</sub>.

## Supplementary Figure 7



**IL4- stimulation promotes BMDM polarisation.** Bone marrow-derived macrophages (BMDMs) were polarised using IL4 (100ng/ml). Absolute mRNA levels and differential gene expression were assessed by a metabolic and inflammatory gene panel on the NanoString platform. Gene set enrichment analysis is presented.

## Supplementary Figure 8



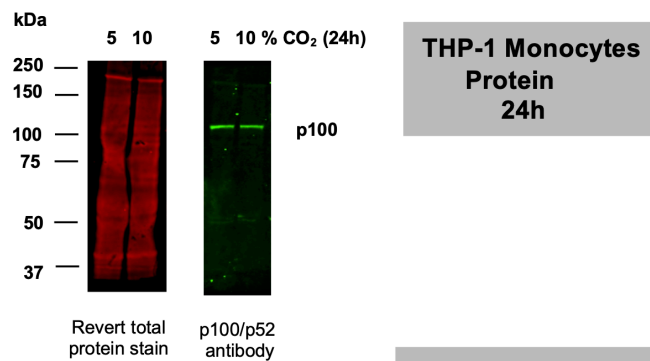
### Hypercapnia affects gene expression in both THP-1 monocytes and IL-4 polarised BMDMs.

Raw TPM values extracted from RNA-seq data for *TLR4* (a), *SLC16A3* (b) and *NFKB1* (c) in THP-1 cells in the basal (unstimulated) and LPS stimulated (2.5µg/ml for 2h) state in both 5% (pink) and 10% (orange) CO<sub>2</sub> conditions (4h). Data shown as mean +/- SEM for n=3 independent experiments. Significant differential expression from individual comparisons using DESeq2 analysis is denoted by (\*). Bone marrow-derived macrophages (BMDMs) were exposed to 5% (pink) or 10% CO<sub>2</sub> (orange) for 6hrs followed by IL4 (100ng/ml) stimulation for an additional 18h. Absolute mRNA levels were measured for *TLR4* (d), *SLC16A3* (e) and *NFKB1* (f). Data shown as mean +/- SEM for n=3 independent experiments. Significant differential expression from individual comparisons as determined by NanoString nCounter analysis, P-value <= 0.05 (\*).

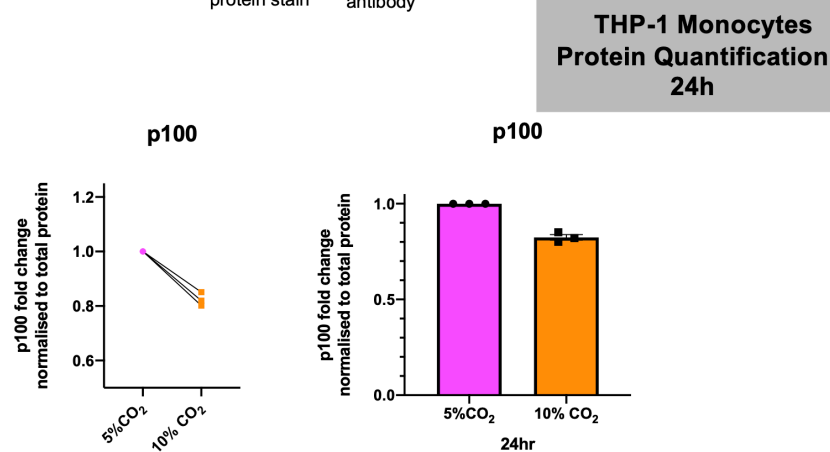


## Supplementary Figure 9

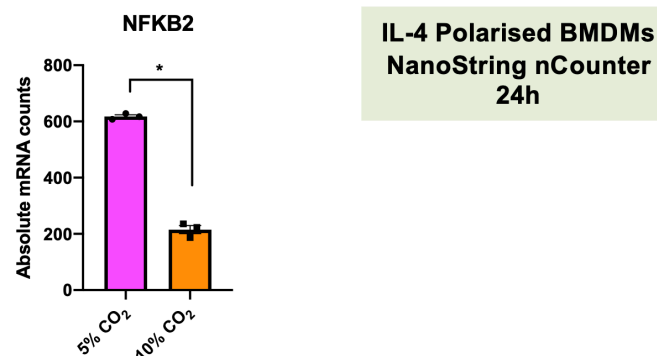
(a)



(b)



(c)

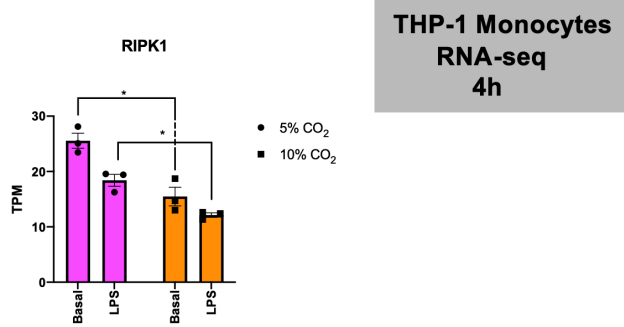


### Hypercapnia reduces p100 protein and NFKB2 mRNA in THP-1 cells and IL-4 primed BMDMs respectively.

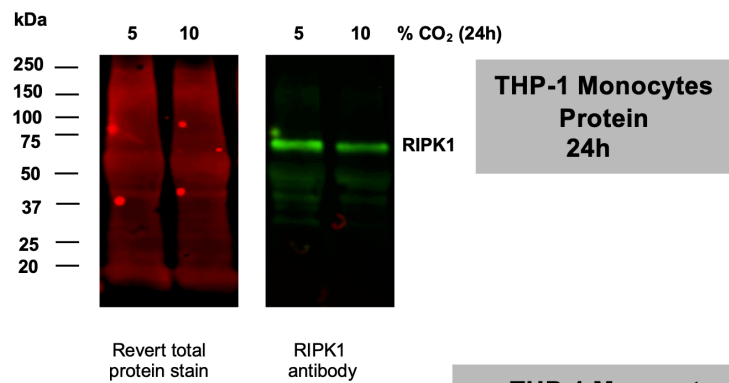
Western blot analysis of whole cell lysates from THP-1 cells exposed to 5% or 10% CO<sub>2</sub> for 24h. Lysates were probed using a total Revert stain in the 700nm channel (red) or incubated with p100/p52 primary antibody followed by a fluorescent secondary mouse antibody and imaged in the 800nm channel (green) on a Li-COR imaging system. Image is representative of n=3 independent experiments (a). Protein quantification was performed using Empiria Studio and p100 fold change normalised to total protein (Revert stain) plotted (b). Bone marrow-derived macrophages (BMDMs) were exposed to 5% or 10% CO<sub>2</sub> for 6h followed by IL4 (100ng/ml) stimulation for an additional 18h. Absolute mRNA levels and differential gene expression of NFKB2 were assessed by a metabolic and inflammatory gene panel on the NanoString. Data shown are mean +/- SEM for n=3 independent experiments. Significant differential expression from individual comparisons as determined by NanoString nCounter analysis, P-value  $\leq 0.05$  (\*) (c).

## Supplementary Figure 10

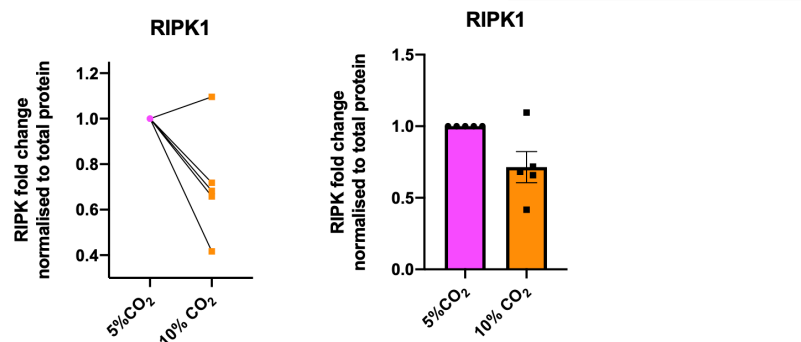
(a)



(b)



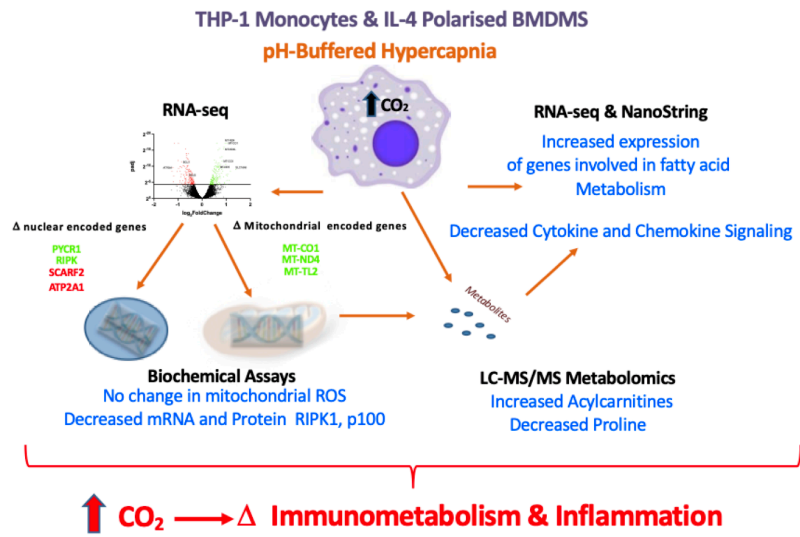
(c)



### Hypercapnia reduces RIPK1 mRNA and protein in THP-1 cells

Raw TPM values extracted from RNA-seq data for *RIPK1* (a) in THP-1 cells in the basal (unstimulated) and LPS stimulated (2.5µg/ml for 2h) state in both 5% (pink) and 10% (orange) CO<sub>2</sub> conditions (4h). Data shown are mean +/- SEM for n=3 independent experiments. Significant differential expression from individual comparisons using DESeq2 analysis is denoted by (\*). Western blot analysis of whole cell lysates from THP-1 cells exposed to 5% or 10% CO<sub>2</sub> for 24h. Lysates were probed using a total Revert stain in the 700nm channel (red) or incubated with RIPK1 primary antibody followed by a fluorescent secondary mouse antibody and imaged in the 800nm channel (green) on a Li-COR imaging system. Image is representative of n=5 independent experiments (b). Protein quantification was performed using Empiria Studio and RIPK fold change normalised to total protein (Revert stain) plotted (c).

## Supplementary Figure 11



Graphical abstract summarising key findings.