Figure S1

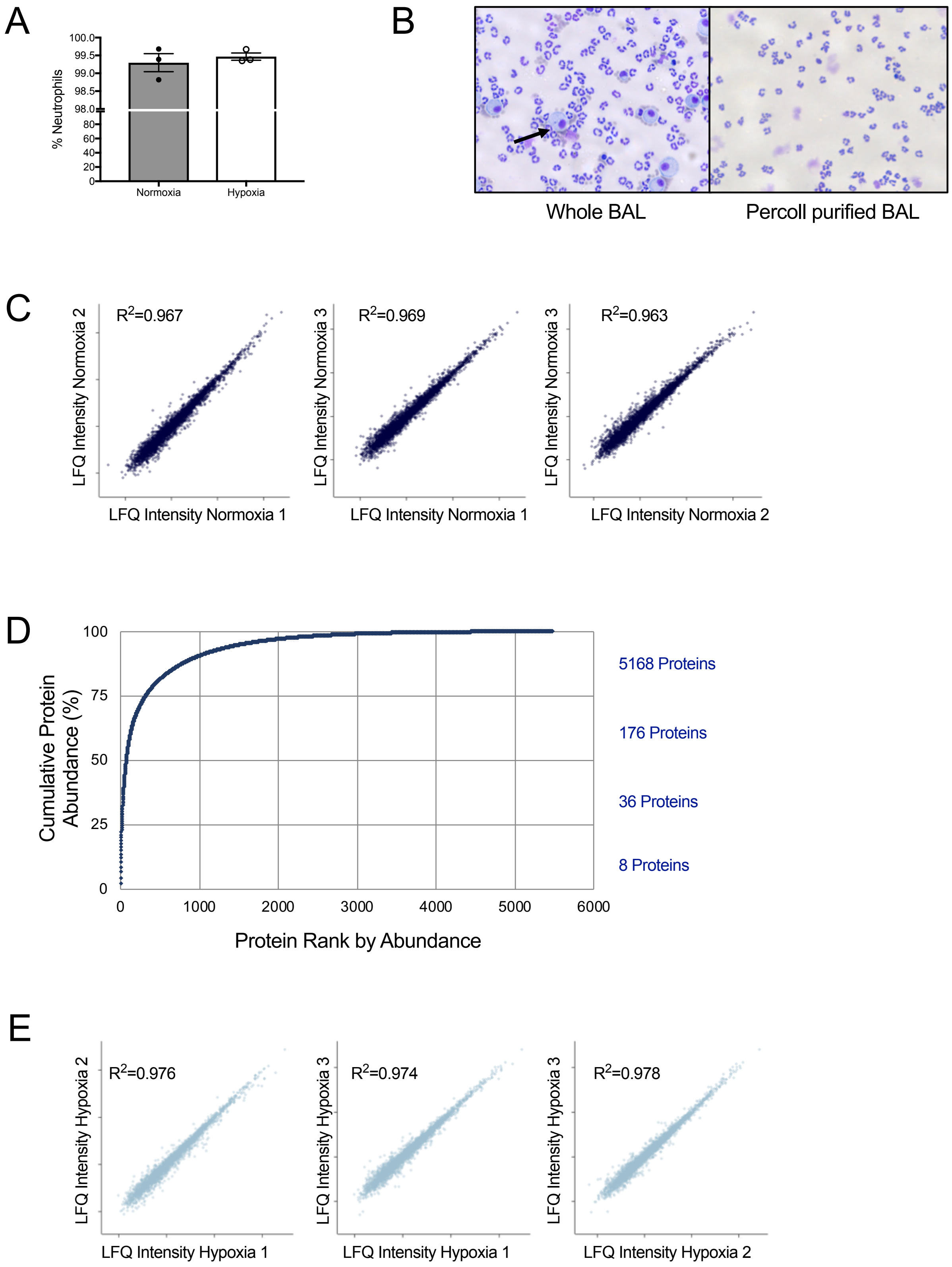

A
% Neutrophils
100.0
99.5
99.0
98.5
98.0
80
60
40
20
0
Normoxia
Hypoxia
B
Whole BAL
Percoll purified BAL
C
R^2=0.967
LFQ Intensity Normoxia 2
LFQ Intensity Normoxia 1
R^2=0.969
LFQ Intensity Normoxia 3
LFQ Intensity Normoxia 1
R^2=0.963
LFQ Intensity Normoxia 3
LFQ Intensity Normoxia 2
D
Cumulative Protein Abundance (%)
100
75
50
25
0
0
1000
2000
3000
4000
5000
6000
Protein Rank by Abundance
5168 Proteins
176 Proteins
36 Proteins
8 Proteins
E
R^2=0.976
LFQ Intensity Hypoxia 2
LFQ Intensity Hypoxia 1
R^2=0.974
LFQ Intensity Hypoxia 3
LFQ Intensity Hypoxia 1
R^2=0.978
LFQ Intensity Hypoxia 3
LFQ Intensity Hypoxia 2

Figure S1:

(A) BAL neutrophils isolated 24 hours post- nebulised LPS from normoxic or hypoxic mice were purified by Percoll gradient to generate samples of >98.8% purity (n=3 per condition). (B) Representative cytospin of BAL cells with contaminating alveolar macrophages seen prior to purification (black arrow). Correlation of LFQ intensity for three biological replicates from normoxic (C) and hypoxic (E) samples, with R^2 value shown for each comparison. (D) The contribution of each protein to the total protein mass was calculated using the copy number and molecular mass for each of the identified proteins, the number of proteins accounting for percentage of total protein mass is shown for each condition.

Figure S2

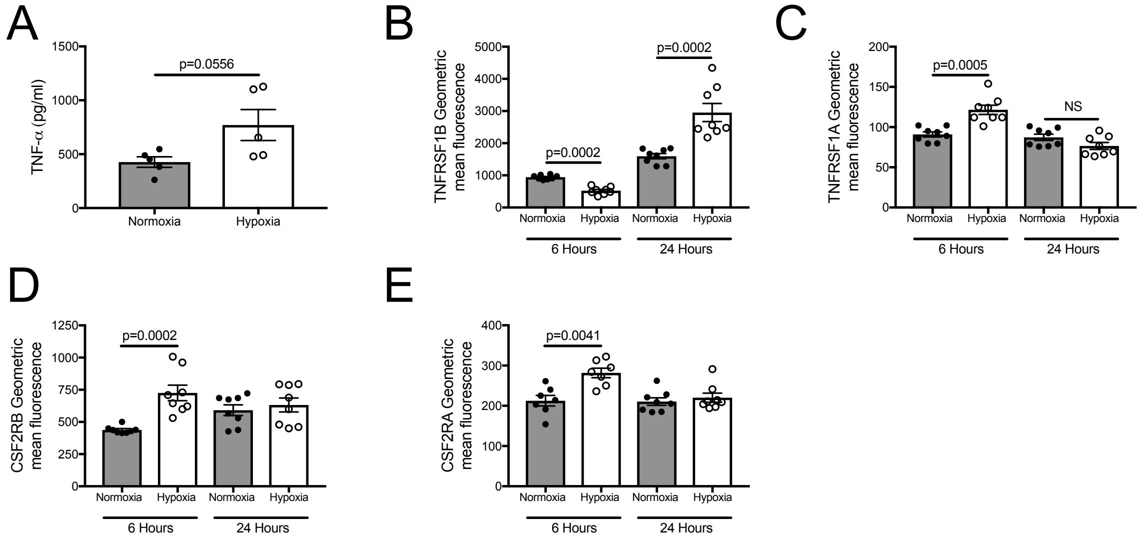


Figure S2:

(A) BAL supernatant TNF-α concentration measured by multiplex cytokine assay, (n=5 over two epxeirments). Flow cytometric analysis of surface expression of (B) TNFRSF1B and (C) TNFRSF1A on BAL neutrophils from normoxic and hypoxic mice, 6 and 24 hours post-LPS (n=8 over two experiments). Surface expression of (D) CSF2RB and (E) CSF2RA on BAL neutrophils, from normoxic and hypoxic mice, 6 and 24 hours post-nebulised LPS, n=7-8 over three experiments. All data analysed by Mann-Whitney test of significance and represents individual values and mean ± SEM.

Figure S3

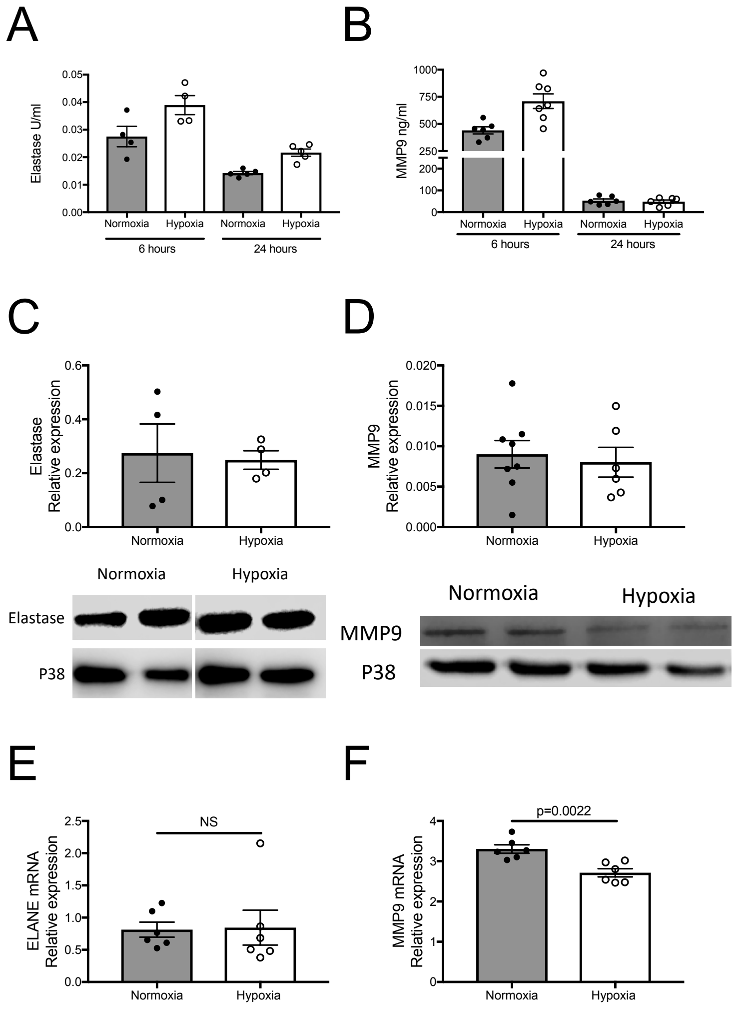
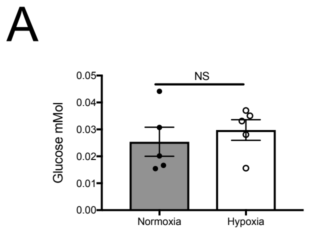


Figure S3:

(A) Elastase activity (n=4-5 over two experiments) and (B) MMP-9 concentrations (n=6-7 over two experiments) in BAL supernatant from normoxic and hypoxic mice 6 and 24 hours post-LPS. Intracellular expression of Elastase and MMP-9 protein was analysed by western blot (C)&(D), representative blots (including p38 loading control) shown, n≥4 over two to three experiments. Levels of Elastase (E) and MMP9 (F) transcript were measured by RT-PCR (data shown relative to β-actin gene expression), n=5-6 over two experiments. (C)-(F) all represent data from highly pure BAL neutrophils isolated from mice 24 hours post-LPS, housed in either normoxia or hypoxia. All data analysed by Mann-Whitney test of significance and represents individual values and mean ± SEM.

Figure S4



B

	Normoxia 1	Normoxia 2	Normoxia 3	Hypoxia 1	Hypoxia 2	Hypoxia 3
Slc38a2	0	0	0	0	0	0
Slc38a9	0	0	2253200	0	0	0
Slc38a10	2677900	0	0	0	0	0
Rank by abundance	4145/4333	N/A	4474/4980	N/A	N/A	N/A

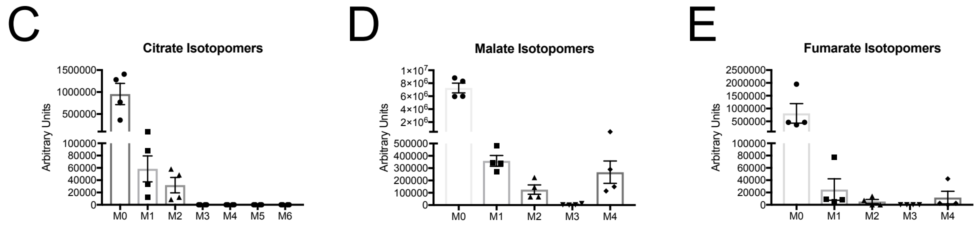


Figure S4:

(A) BAL supernatant glucose concentration from normoxic and hypoxic mice, 24 hours post-LPS, n=5 over 2 experiments, Mann-Whitney test of significance. (B) Expression of SLC38 family of glutamine transporters in the BAL neutrophil proteome (LFQ and rank shown). (C)-(E) Breakdown of isotopomers identified in neutrophils cultured with $^{13}C_5$ Glutamine labelled HEK cell lysates (summarised in Fig 5B-D).

Figure S5

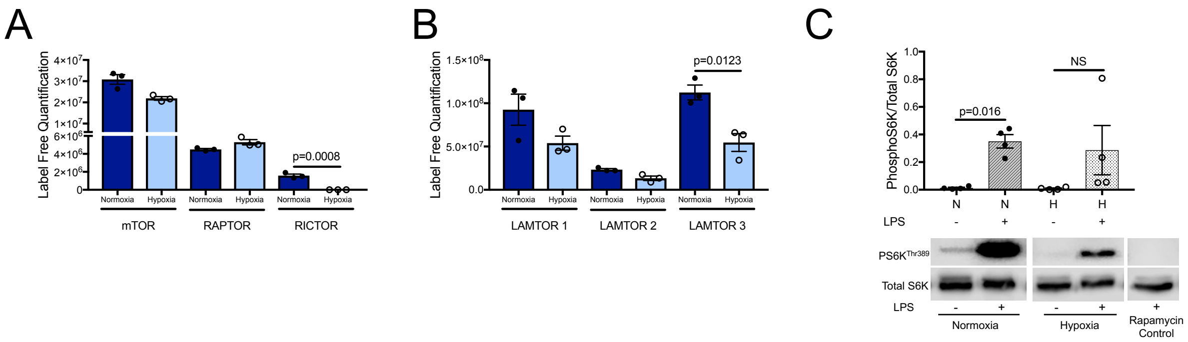


Figure S5:

Label free quantification of (A) mTORC components and (B) LAMTOR proteins involved in localising mTORC to the lysosome in highly pure BAL neutrophils from normoxic and hypoxic mice 24 hours post-nebulised LPS, n=3. (C) Ratio of phosphorylated to total S6K measured by western blot in unstimulated and LPS treated human peripheral blood neutrophils cultured in normoxia or hypoxia, representative blot shown including rapamycin control (n=4 over two experiments).

Table S1.

KEGG Pathway Term	**Count**	**P Value**	**Fold Enrichment**
Pentose phosphate pathway	27	1.9E-08	2.463
Amino sugar and nucleotide sugar metabolism	44	1.3E-13	2.457
Fructose and mannose metabolism	29	5.3E-08	2.334
Citrate cycle (TCA cycle)	27	2.7E-07	2.309
Galactose metabolism	25	1.0E-05	2.138
Pentose and glucuronate interconversions	28	2.8E-06	2.128
Propanoate metabolism	21	7.9E-05	2.128
Carbon metabolism	90	5.7E-19	2.123
mTOR signaling pathway	45	2.7E-09	2.087
Biosynthesis of amino acids	56	9.4E-10	1.965
Pyruvate metabolism	28	3.3E-05	1.965
Fatty acid metabolism	35	1.1E-05	1.878
Insulin signaling pathway	90	5.2E-11	1.759
Glutathione metabolism	35	1.2E-04	1.741
Biosynthesis of unsaturated fatty acids	17	1.3E-02	1.723
Glucagon signaling pathway	62	4.9E-07	1.697
Cysteine and methionine metabolism	24	5.4E-03	1.642
Starch and sucrose metabolism	19	1.8E-02	1.625
AMPK signaling pathway	75	4.2E-07	1.616
Oxidative phosphorylation	76	1.8E-05	1.496

Caption overleaf

Table S2.

KEGG Pathway Term	**Count**	**P Value**	**Fold Enrichment**
Fc gamma R-mediated phagocytosis	65	1.1E-13	2.118
Bacterial invasion of epithelial cells	56	9.4E-10	1.965
Lysosome	87	1.7E-14	1.952
NOD-like receptor signaling pathway	39	1.8E-06	1.906
VEGF signaling pathway	41	1.9E-06	1.870
Endocytosis	185	1.2E-24	1.821
Phagosome	105	2.6E-10	1.651
TNF signaling pathway	63	1.1E-05	1.582
Toll-like receptor signaling pathway	56	1.7E-04	1.517
HIF-1 signaling pathway	57	2.2E-04	1.500
NF-kappa B signaling pathway	49	5.8E-03	1.382
Antigen processing and presentation	41	1.5E-02	1.368
MAPK signaling pathway	124	4.2E-05	1.341
Chemokine signaling pathway	89	9.0E-03	1.243
Focal adhesion	90	3.1E-02	1.190

Table S3.

KEGG Pathway	**Count**	**P Value**	**Fold Enrichment**
Spliceosome	125	5.3E-44	2.57200961
RNA transport	126	2.9E-23	2.02831703
Protein processing in endoplasmic reticulum	116	1.6E-17	1.88956972
Ribosome	85	1.0E-07	1.60422447

Tables S1-S3

Neutrophils show enrichment for metabolic, inflammatory and biosynthetic pathways.

The genes encoding the 5398 proteins identified in the neutrophil proteome were compared with the whole mouse genome using DAVID software to identify pathway enrichment. Enriched KEGG pathway terms with their corresponding gene counts, significance and fold enrichment are shown. Table one shows selected metabolic pathways, table two shows selected metabolic pathways and table three shows selected biosynthetic pathways.

Table S4.

Protein names	**Gene names**	**Fold change**	**P value**
Phosphatidylinositol-glycan-specific phospholipase D	Gpld1	7.8093	0.0004
V-type proton ATPase 116 kDa subunit a isoform 2	Atp6v0a2	1.9209	0.0085
Epididymal secretory protein E1	Npc2	1.9188	0.0171
Solute carrier family 15 member 3	Slc15a3	1.8297	0.0019
CSC1-like protein 1	Tmem63a	1.6388	0.0100
Arylsulfatase B	Arsb	1.6018	0.0021
Sortilin	Sort1	1.5990	0.0133
N-acetylgalactosamine-6-sulfatase	Galns	1.5236	0.0093
Di-N-acetylchitobiase	Ctbs	1.4789	0.0076
Beta-hexosaminidase subunit beta	Hexb	1.4192	0.0001
Sphingosine kinase 2	Sphk2	1.4077	0.0093
Beta-hexosaminidase subunit alpha	Hexa	1.3850	0.0028
Beta-mannosidase	Manba	1.3549	0.0052
Adenosine deaminase	Ada	1.3432	0.0010
Annexin A6	Anxa6	0.6900	0.0011
Serum paraoxonase/arylesterase 2	Pon2	0.6839	0.0064
V-type proton ATPase subunit d 1	Atp6v0d1	0.6362	0.0119
Enhancer of mRNA-decapping protein 3	Edc3	0.6235	0.0185
N-acylethanolamine-hydrolyzing acid amidase	Naaa	0.6145	0.0190
Guanine nucleotide-binding protein subunit beta-1	Gnb1	0.6101	0.0120

Table S4: Upregulation of the lysosome in hypoxic neutrophils

Significantly up- and down-regulated lysosomal proteins as detailed in Figure 6B.

Significantly upregulated proteins are in green and down regulated in red, n=3, significance defined as $p<0.05$, $S_0=0.1$. Corrected for multiple hypothesis testing using permutation-based FDR=0.05, p value and fold change shown.

Data files: Table S5 is included as a separate Microsoft Excel File

Table S6: Flow cytometry antibodies

Lung Digest Flow Cytometry Antibody Panel

Antigen	Fluorophore	Concentration in mastermix	Source	Product code
CD11b	BV 421	1:400	Biolegend	101235
Ly6C	FITC	1:200	Biolegend	128005
Ly6G	PE	1:200	Biolegend	127607
SiglecF	PE CF594	1:800	BD	562757
CD64	APC	1:200	Biolegend	139305
CD45	AF 700	1:200	Biolegend	103128

Mouse Whole Blood Flow Cytometry Antibody Panel

Antigen	Fluorophore	Concentration in mastermix	Source	Product code
Ly6G	V450	1:200	Biolegend	127612
CD3	PE	1:200	Biolegend	100205
CD19	PE	1:200	Biolegend	152407
SiglecF	PE CF594	1:800	BD	562757
CD115	APC	1:200	Biolegend	135510
CD45	AF 700	1:200	Biolegend	103128
CD11B	APC Cy7	1:200	Biolegend	101225
CD62L	PE Cy7	1:200	Biolegend	104417

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Additional Flow Cytometry Antibodies

Antigen	Fluorophore	Concentration in mastermix	Source	Product code
CSF2Ra	APC	7.5µl per test	Bio-techne	FAB6130A
CSF2Rb	PE	5µl per test	Bio-techne	FAB5492P
TNFRSF1	APC	1:100	Biolegend	113005
TNFRSF2	PE	1:100	Biolegend	113405