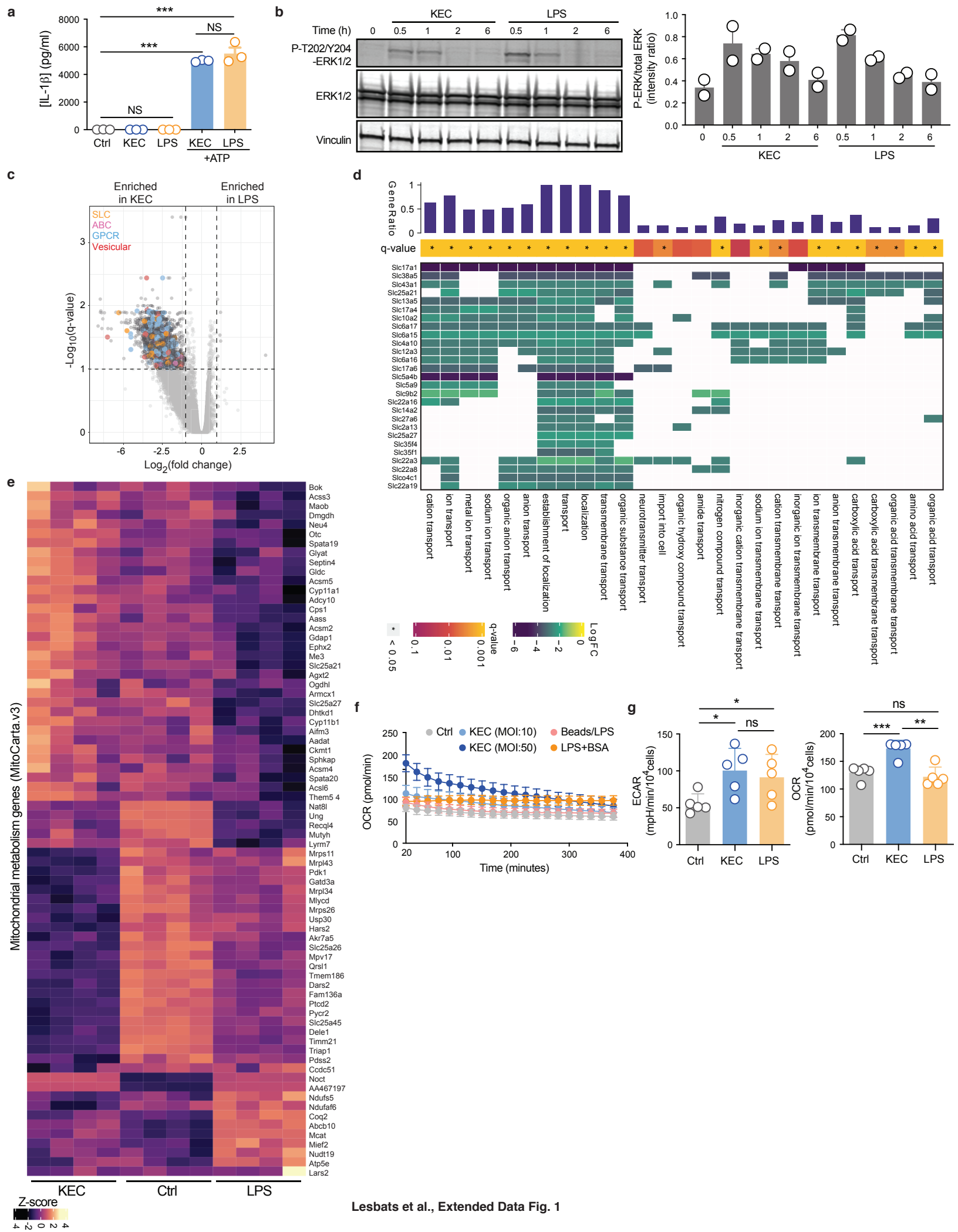
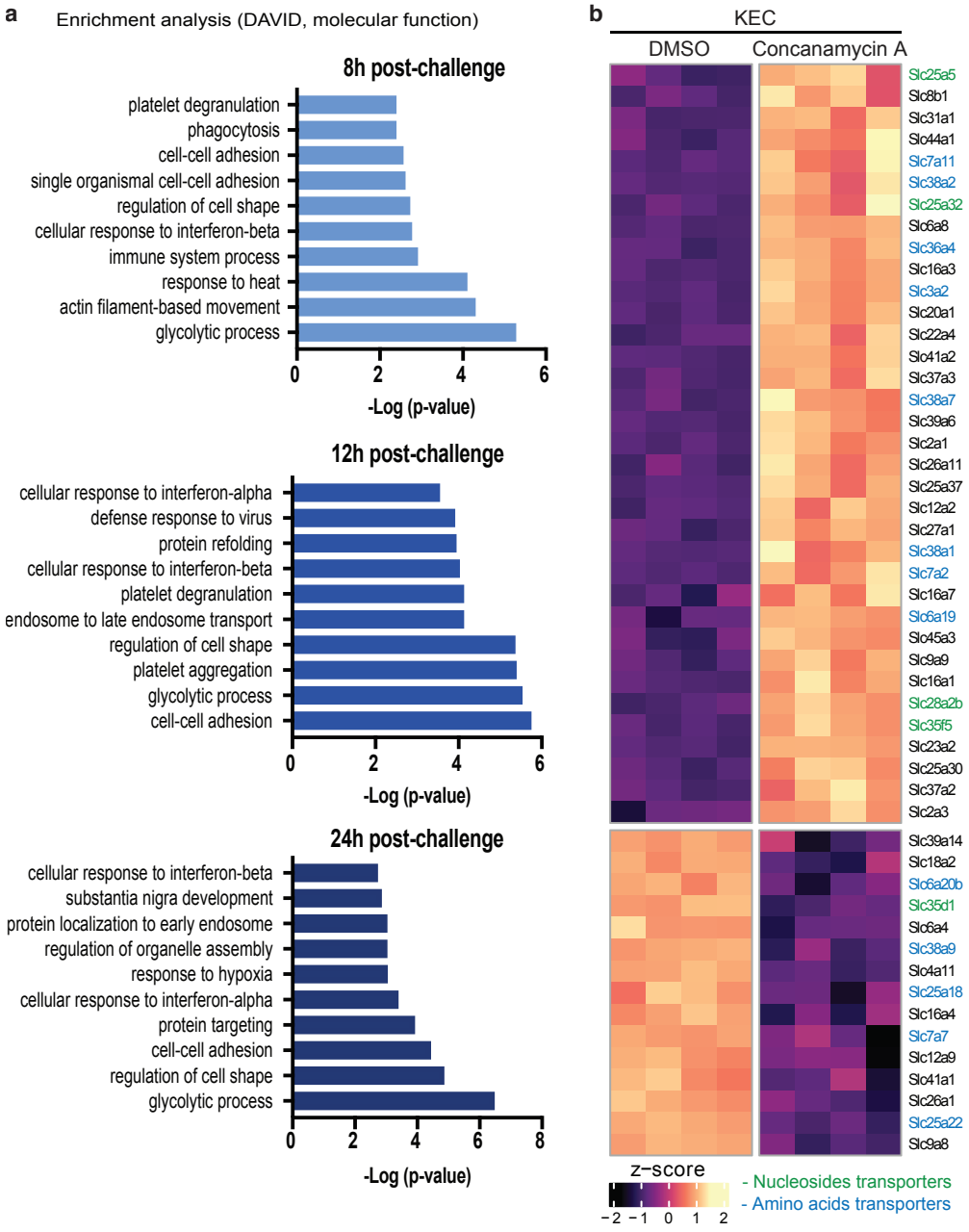


Lesbats et al., Extended Data Fig. 1

Extended Data Fig. 1: Killed E. coli specifically reprograms mitochondria metabolism

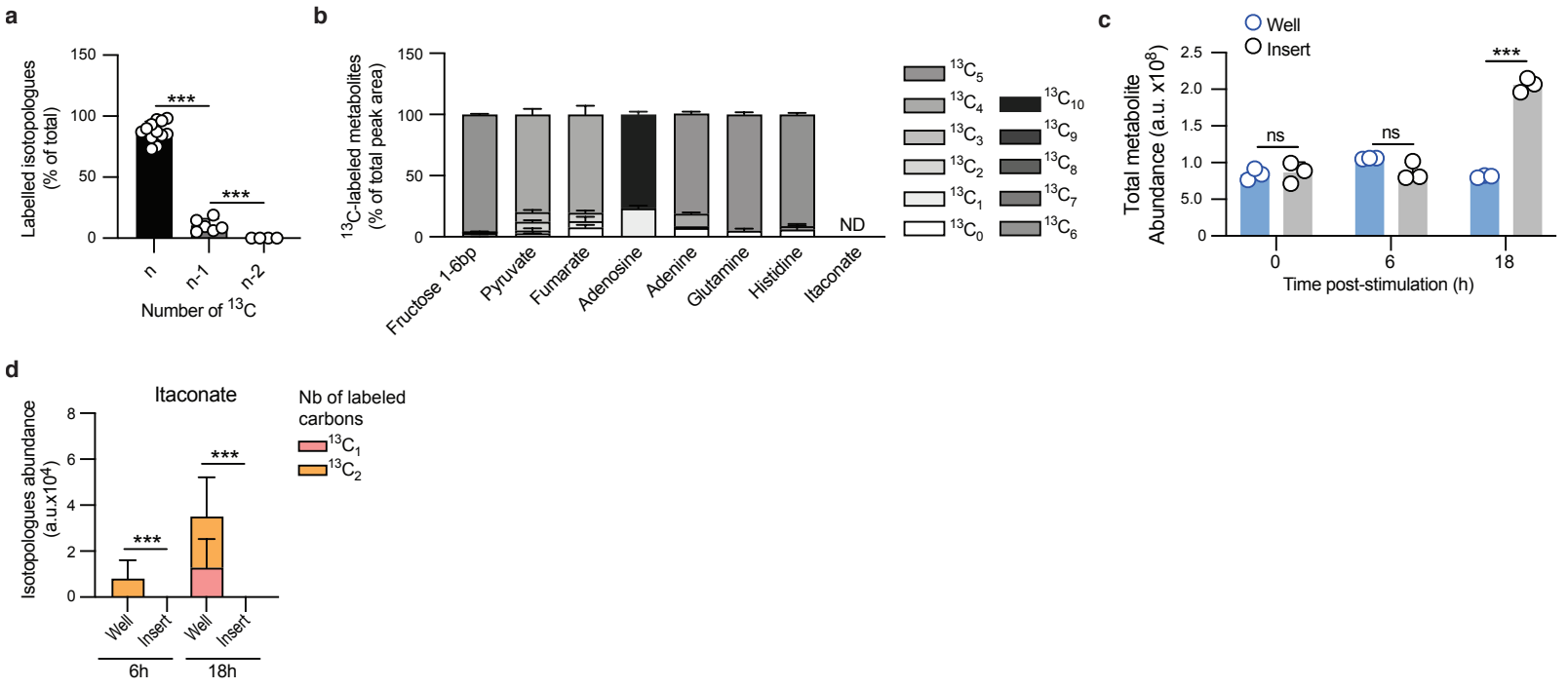
a, Representative ELISA for IL-1β levels in conditioned medium of BMDMs stimulated with KEC or LPS for 4h and subsequently treated or not with ATP for 30min. ***p<0.001. N=3 technical replicates. Data are representative of at least 3 experiments. **b**, Heat map showing the top differentially expressed solute carrier (Slc) genes in BMDMs stimulated 4h with KEC upon treatment with concanamycin A or vehicle (DMSO). **c**, Representative immunoblots of BMDMs stimulated with LPS or KEC for the indicated time. Vinculin is used as a loading control. **d**, Heat maps showing the top differentially expressed solute carrier (Slc) genes and their functions in BMDMs stimulated 4h with KEC as compared to LPS. **e**, Heat maps showing the top differentially expressed mitochondria-associated genes in BMDMs left untreated (Ctrl) or stimulated 4h with KEC or LPS. **f**, oxygen consumption rate (OCR) on BMDMs treated with KEC, LPS covered-latex beads (Beads/LPS) or bovine serum albumin + LPS (LPS+BSA) in a minimal medium supplemented with glucose. Data are mean +/- SD, N= 6 technical replicates. Data are representative of at least 3 experiments. **g**, Extracellular acidification rate (ECAR, left) and oxygen consumption rate (OCR, right) on BMDMs treated for 30min with KEC or LPS in a minimal medium supplemented with glucose. Data are mean +/- s.e.m., N=5 biological replicates (with 6 technical replicates). *p<0.05, ***p<0.001; ns, not significant.



Lesbats et al., Extended Data Fig. 2

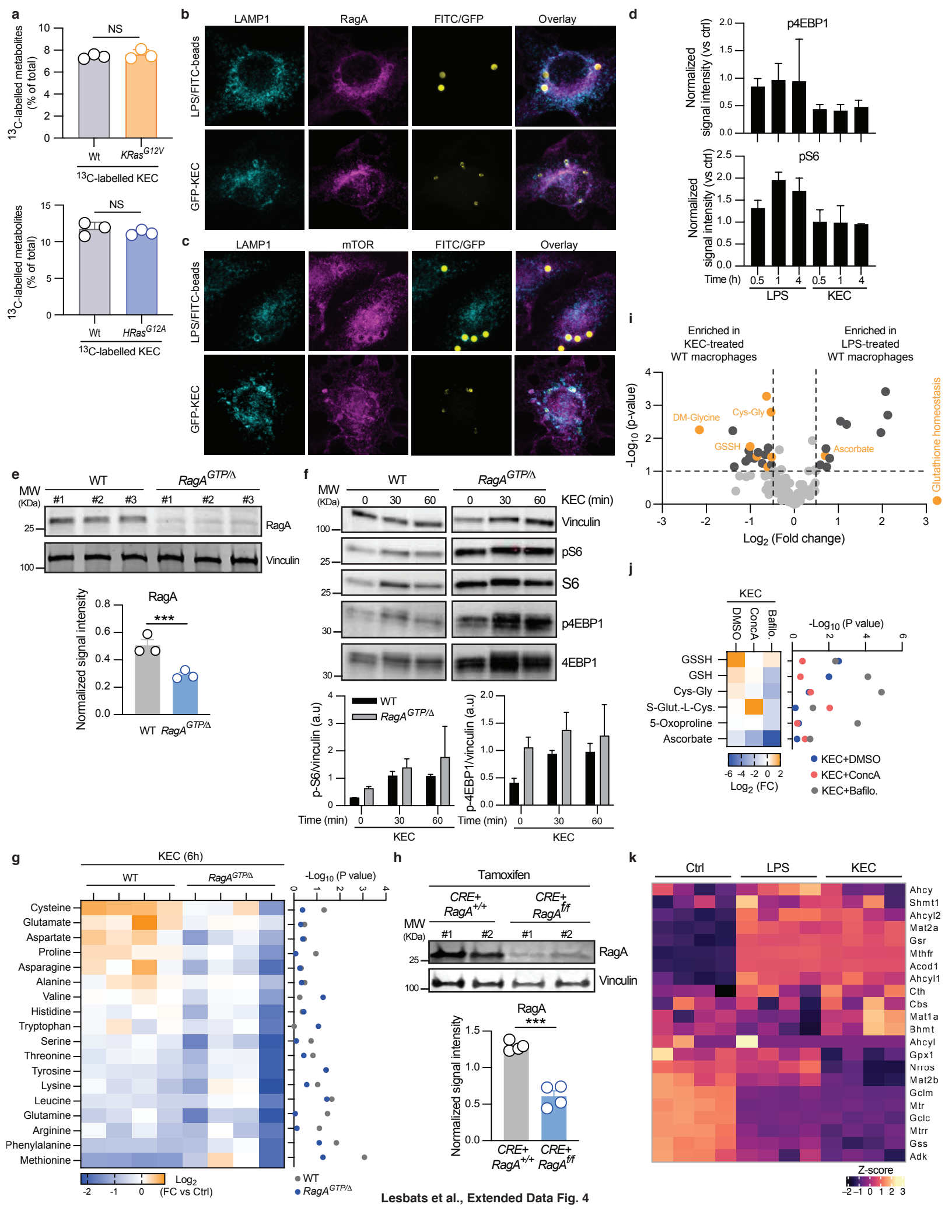
Extended Data Fig. 2: Killed E. coli is a source of amino acids for macrophage upon phagocytosis

a, Top pathways identified by DAVID functional enrichment analysis from heavy proteins identified by LC-MS of BMDMs that have phagocytosed SILAC-KEC for the indicated time points. **b**, Heat map showing the top differentially expressed solute carrier (Slc) genes in BMDMs stimulated 4h with KEC upon treatment with concanamycin A or vehicle (DMSO).


a
Labelled isotopologues (% of total)
150
100
50
0
***
***
n
n-1
n-2
Number of 13C
b
13C-labeled metabolites (% of total peak area)
150
100
50
0
ND
Fructose 1-6bp
Pyruvate
Fumarate
Adenosine
Adenine
Glutamine
Histidine
Itaconate
13C5
13C4
13C3
13C2
13C1
13C0
13C10
13C9
13C8
13C7
13C6
c
Well
Insert
Total metabolite Abundance (a.u. x10^8)
2.5
2.0
1.5
1.0
5.0
0
ns
ns
***
0
6
18
Time post-stimulation (h)
d
Itaconate
Isotopologues abundance (a.u.x10^4)
8
6
4
2
0
***
***
Well
Insert
Well
Insert
6h
18h
Nb of labeled carbons
13C1
13C2

Extended Data Fig. 3: Killed E. coli is a source of metabolites for macrophage upon phagocytosis

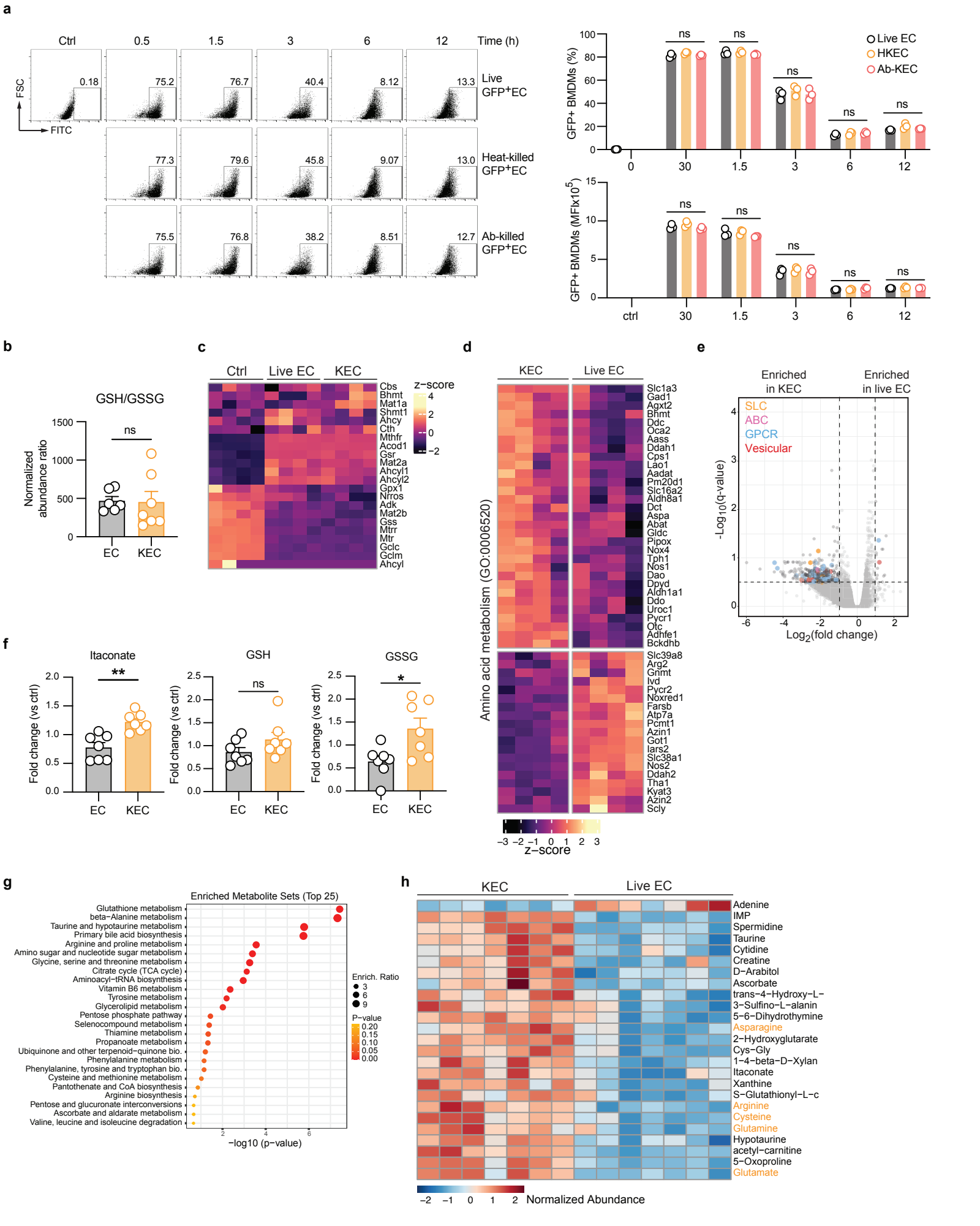
a, Fractional labelling of metabolites in *E. coli* after a 3 day-culture in minimal M9 medium supplemented with U-[^{13}C]glucose. Fraction of isotopologues was determined over 15 metabolites by UHPLC-MS. N=3 biological replicates. Data are expressed as percentage of total pool. ***p<0.001. N is the total number of carbons for an individual metabolite. **b**, Fractional labelling of the indicated metabolites in *E. coli* after 3 day-culture in minimal M9 medium supplemented with U-[^{13}C]glucose as determined by UHPLC-MS. **c**, Total abundance of a pool of 45 metabolites in BMDMs cultured in direct contact (well) or separated (insert, trans-well) from fully-labelled U-[^{13}C]Killed EC for 6h or 18h. N=3 biological replicates. Data are expressed as percentage of total pool. ***p<0.001; ns, not significant. **d**, Fractional labelling of itaconate and glutathione (GSH) in BMDMs cultured in direct contact (well) or separated (insert, trans-well) from fully-labelled U-[^{13}C]Killed EC for 6h or 18h. Fraction of isotopologues was determined by UHPLC-MS. N=3 biological replicates. ***p<0.001.



Lesbats et al., Extended Data Fig. 4

Extended Data Fig. 4: RagA is required for KEC-mediated metabolic reprogramming of macrophages

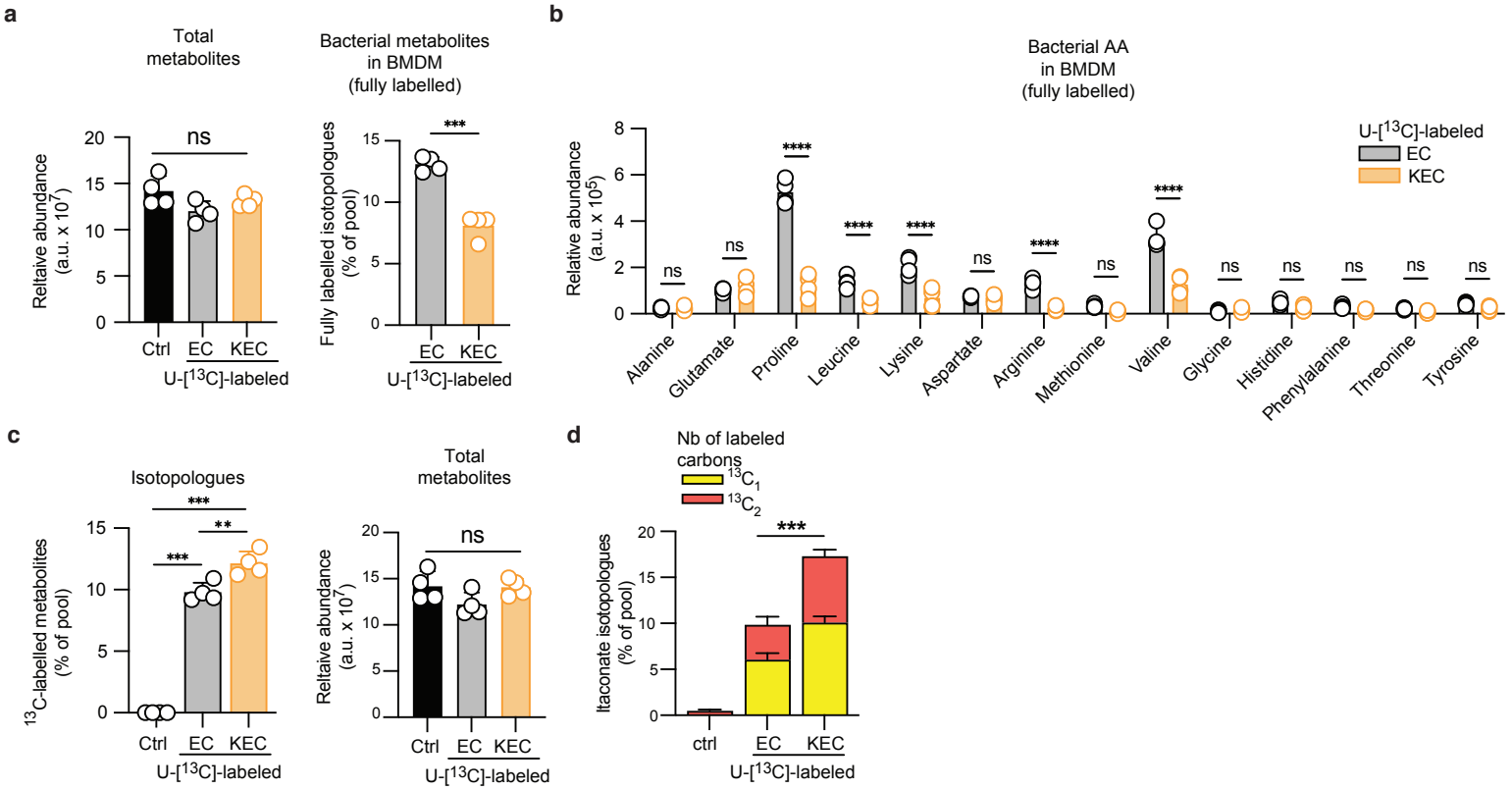
a, Fractional labelling of metabolites in *Kras*^{G12V} (Upper panel) and *Hras*^{G12A} (lower panel) BMDMs treated for 6h with fully-labelled U-[^{13}C]KEC. Fraction of isotopologues was determined over 45 metabolites by LC-MS. N=3 biological replicates. Data are expressed as percentage of total pool. ns, not significant. **b,c**, Representative confocal microscopy images of BMDMs stained with anti-LAMP1 (lysosomes) and anti-RagA (**b**) or mTOR (**c**) antibodies, 20min after stimulation with LPS-covered FITC-latex beads or GFP-expressing KEC. **d**, Normalized signal intensity (normalized to control) of the indicated phosphor-specific 4EBP1 and S6 protein antibodies as determined by western blotting analysis BMDMs treated with LPS or KEC for different time points. Vinculin is used as a loading control. Data are representative of 3 experiments. **e**, Representative immunoblots of WT and *RagA*$^{GTP/\Delta}$ BMDMs. Vinculin is used as a loading control. Three different BMDM preparations were used. Normalized signal ratio to vinculin is shown (lower panel). **f**, Representative immunoblots of WT and *RagA*$^{GTP/\Delta}$ BMDMs stimulated with LPS or KEC for the indicated time. Normalized signal ratio to vinculin is shown (right panel). Vinculin is used as a loading control. **g**, Heat map of fold changes (log_2) of amino acid abundances in WT and *RagA*$^{GTP/\Delta}$ BMDMs stimulated with KEC relative to control (left). *P* values are for comparisons between amino acid abundances shown (right). N=4 biological replicates. **h**, Representative immunoblots of Cre$^+$::*RagA*$^{+/+}$ and Cre$^+$::*RagA*$^{f/f}$ BMDMs treated with tamoxifen and stimulated with LPS or KEC for the indicated time. Normalized signal ratio to vinculin is shown (lower panel). Vinculin is used as a loading control. Four different BMDM preparations were used. **i**, Volcano plot of metabolite levels in BMDMs treated with LPS or KEC determined by LC-MS analysis. Dark grey plots show metabolites that are significantly increased (left) or decreased (right) in KEC-treated versus LPS-treated BMDMs; $Log_2FC>0.5$ and Log_{10}(*P*-value)>1. Orange dots show metabolites associated to GSH metabolism significantly modulated. N=7 biological replicates. **j**, Heat map of fold changes (log_2) of abundances of metabolites of glutathione biosynthetis in WT BMDMs treated with bafilomycin A1 (Bafilo.) or concanamycin A (ConcA) stimulated with KEC relative to control (left). *P* values are for comparisons between amino acid abundances shown (right). N=4 biological replicates. **k**, Heat maps showing differential expression of genes associated with glutathione metabolism in BMDMs stimulated 4h with KEC or LPS. Ctrl, control un-stimulated BMDMs.



Lesbats et al., Extended Data Fig. 5

Extended Data Fig. 5: Sensing of live and dead *E. coli* differentially modulates macrophage metabolism

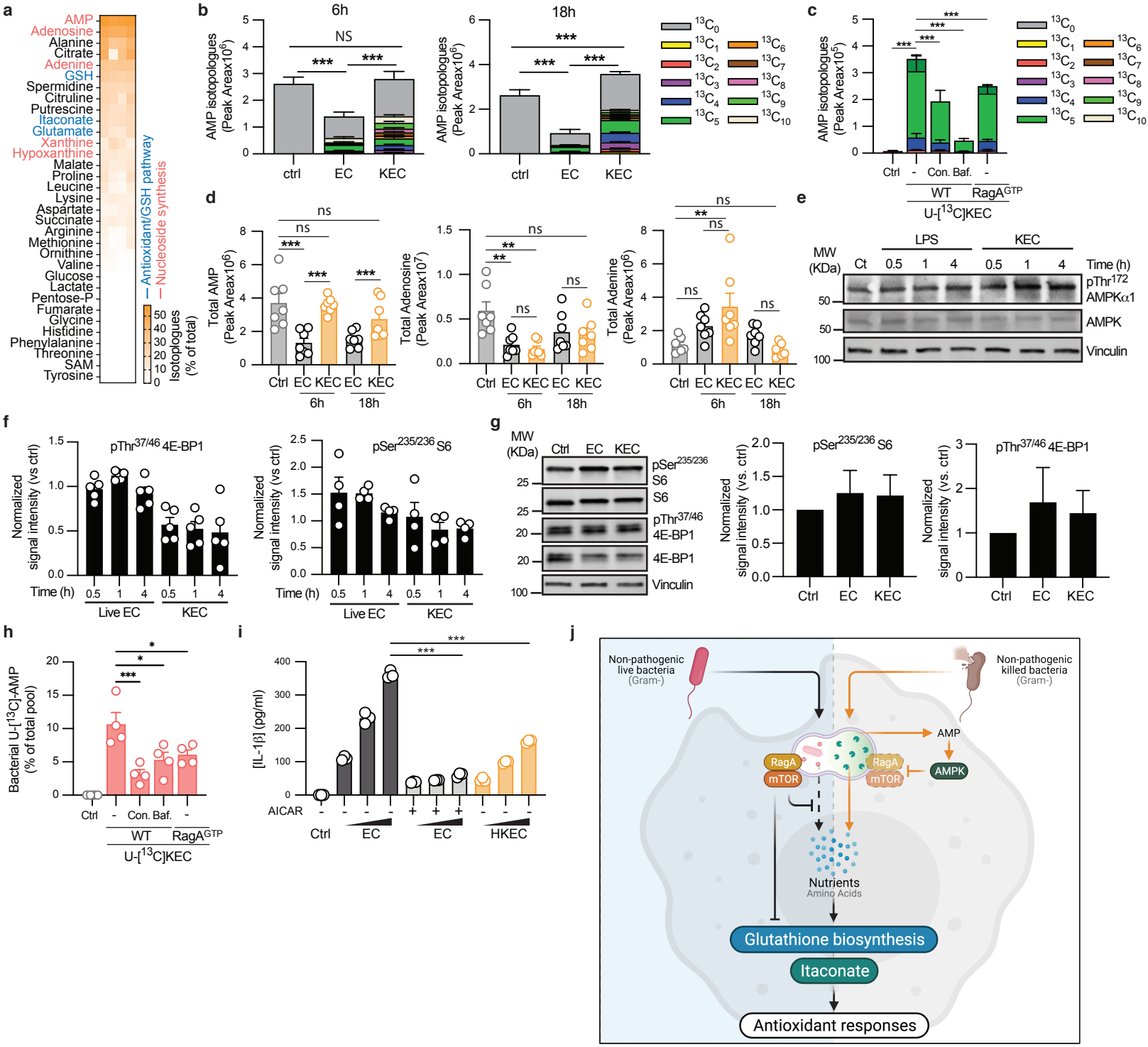
a, Flow cytometry analysis of kinetic engulfment and degradation of GFP-expressing live E. coli (EC) or killed E. coli (KEC) by BMDMs (Right). Quantification of percentages (upper right) and mean fluorescence intensity (lower right) of GFP signal. N=3 per group. ns, not significant. **b**, GSH/GSSG ratio in BMDMs stimulated with live E. coli (EC) or killed EC (KEC) for 6h, determined by LC-MS. ns, not significant. **c,d**, Heat map showing differential expression of genes associated with GSH biosynthesis (**c**) and amino acid metabolism (**d**) between live EC- and KEC-treated BMDMs for 4h. N=4 biological replicates. **e**, Volcano plot of genes expression levels determined by bulk RNA sequencing. Dark grey plots show genes that are significantly increased (right) or decreased (left) in KEC-stimulated macrophages versus live EC-treated macrophages; $Log_2FC>1$ and $Log_{10}(q\text{-value})>1$. Color dots show metabolite transporters of the indicated family. N=4 biological replicates. **f**, Intracellular levels of itaconate, GSH and GSSG detected by LC-MS analysis of BMDMs treated with EC or KEC for 18h. N=7 biological replicates. Data are expressed as fold change compared to resting cells (ctrl). *p<0.05, ***p<0.001. **g**, Metabolite set enrichment analysis (MSEA) of the most altered metabolic pathways in KEC-treated BMDMs versus Live EC-treated BMDMs after 18h of incubation. **h**, Heat map showing relative abundance (normalized peak ion intensity) of the most altered metabolites in BMDMs treated with KEC or Live EC for 18h. N=7 biological replicates.



Lesbats et al., Extended Data Fig. 6

Extended Data Fig. 6: Sensing of live and dead *E. coli* differentially modulates nutrient recycling upon phagocytosis

a, Fractional levels of a pool of 45 *E. coli* fully labeled-metabolites detected by UHPLC-MS in BMDMs, 6h after phagocytosis of live U-[^{13}C]EC (EC) or killed U-[^{13}C]EC (KEC). Data are expressed as percentage of total pool. ***p<0.001. ns, not significant. **b**, Intracellular levels of *E. coli* fully labeled-amino acids detected by UHPLC-MS in BMDMs, 6h after phagocytosis of live U-[^{13}C]EC (EC) or killed U-[^{13}C]EC (KEC). Data are expressed as relative abundance (maximum peak area). *p<0.05, ***p<0.001. ns, not significant. **c**, Fractional labelling of itaconate in BMDMs cultured in direct contact (well) or separated (insert, trans-well) from fully-labelled U-[^{13}C]Killed EC for 6h or 18h. Fraction of isotopologues was determined by LC-MS. N=4 biological replicates. **d,e**, Intracellular levels of isotopologues (**d**) and total metabolites detected (**e**) by UHPLC-MS over 45 selected metabolites in BMDMs, 18h after phagocytosis live U-[^{13}C]EC (EC) or killed U-[^{13}C]EC (KEC). Data are expressed as percentage of total pool (**d**) and relative abundance (maximum peak area) (**e**). *p<0.05, ***p<0.001. ns, not significant.


a
AMP
Adenosine
Alanine
Citrate
Adenine
GSH
Spermidine
Citruline
Putrescine
Itaconate
Glutamate
Xanthine
Hypoxanthine
Malate
Proline
Leucine
Lysine
Aspartate
Succinate
Arginine
Methionine
Ornithine
Valine
Glucose
Lactate
Pentose-P
Fumarate
Glycine
Histidine
Phenylalanine
Threonine
SAM
Tyrosine
Antioxidant/GSH pathway
Nucleoside synthesis
Isotopologues (% of total)
b
6h
18h
NS
AMP isotopologues (Peak Area x10^6)
ctrl
EC
KEC
c
AMP isotopologues (Peak Area x10^5)
Ctrl
Con.
Baf.
WT
RagA^GTP
U-[13C]KEC
d
Total AMP (Peak Area x10^6)
Total Adenosine (Peak Area x10^7)
Total Adenine (Peak Area x10^6)
6h
18h
e
MW (KDa)
LPS
KEC
Ct
Time (h)
pThr172 AMPKα1
AMPK
Vinculin
f
pThr37/46 4E-BP1
pSer235/236 S6
Normalized signal intensity (vs ctrl)
Live EC
KEC
g
pSer235/236 S6
S6
pThr37/46 4E-BP1
4E-BP1
Vinculin
h
Bacterial U-[13C]-AMP (% of total pool)
i
[IL-1β] (pg/ml)
AICAR
HKEC
j
Non-pathogenic live bacteria (Gram-)
Non-pathogenic killed bacteria (Gram-)
RagA
mTOR
AMP
AMPK
Nutrients
Amino Acids
Glutathione biosynthesis
Itaconate
Antioxidant responses

Extended Data Fig. 7: Phagocytosis of killed *E. coli* specifically sustains intracellular AMP pool and activates AMPK in macrophages

a, Heat map of isotopologues levels among various metabolites in BMDMs treated with U-[^{13}C]KEC for 18h. N=4 biological replicates. **b**, Intracellular AMP isotopologue levels in BMDMs treated with v-ATPase inhibitors concanamycin A (Con.) or bafilomycin (Baf.) and stimulated with U-[^{13}C]killed EC for 6h . N=4 biological replicates. Data are mean +/- s.e.m. ***p<0.001; ns, not significant. **c**, Intracellular AMP isotopologues in BMDMs stimulated with live EC of KEC for 6h or 18h. N=7 biological replicates. Data are mean +/- s.e.m. ***p<0.001. **d**, Intracellular AMP, adenosine and adenine levels in BMDMs treated with live EC of KEC for 6h or 18h. N=7 biological replicates. Data are mean +/- s.e.m. **p<0.01, ***p<0.001; ns, not significant. **e**, Representative immunoblots of BMDMs stimulated with LPS or KEC for the indicated time. Vinculin is used as a loading control. **f**, Normalized signal ratio to vinculin from immunoblots shown in (**e**). Each dot represents a separate experiment. Untreated BMDMs were used as control (Ctrl). Data are mean +/- s.e.m. **g**, Representative immunoblots of BMDMs stimulated with live EC or KEC for 18h. Vinculin is used as a loading control. Normalized signal ratio to vinculin from immunoblots (right). Untreated BMDMs were used as control (Ctrl). Data are mean +/- s.e.m. **h**, Intracellular abundance of bacterial AMP in WT or *RagA*^{GTP} BMDMs treated or not with v-ATPase inhibitors concanamycin a (Con.) or bafilomycin (Baf.) and stimulated with U-[^{13}C]killed EC for 6h. Data are mean +/- s.e.m. of percentages of total pool. *p<0.05, ***p<0.001. **i**, Cytokine levels supernatants of BMDMs treated or not with 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) and stimulated for 18h with live EC or KEC. Data are mean +/- SD. Representative of at least 4 experiments. ***p<0.001. **j**, Schematic representation of the metabolic recycling of internalized live and dead bacteria and the signaling pathways mobilized in macrophages.

Supplementary Table 1: List of metabolites analyzed by UHPLC-MS and their associated metabolic pathways.

Compound	CmpdID	Pathway
Alanine	C00041	Amino acids
Arginine	C00062	Amino acids
Asparagine	C00152	Amino acids
Aspartate	C00049	Amino acids
Cysteine	C00097	Amino acids
Glutamate	C00025	Amino acids
Glutamine	C00064	Amino acids
Glycine	C00037	Amino acids
Histidine	C00135	Amino acids
Leucine	C00123	Amino acids
Lysine	C00047	Amino acids
Methionine	C00073	Amino acids
Phenylalanine	C00079	Amino acids
Proline	C00148	Amino acids
Serine	C00065	Amino acids
Threonine	C00188	Amino acids
Tryptophan	C00078	Amino acids
Tyrosine	C00082	Amino acids
Valine	C00183	Amino acids
AMP	C00020	Nucleotides
dAMP	C00360	Nucleotides
Adenosine	C00212	Nucleotides
Adenine	C00147	Nucleotides
GMP	C00144	Nucleotides
CMP	C00055	Nucleotides
Cytidine	C00475	Nucleotides
UMP	C00105	Nucleotides
IMP	C00130	Nucleotides
Inosine	C00294	Nucleotides
Hypoxanthine	C00262	Nucleotides
Xanthine	C00385	Nucleotides
Allantoate	C00499	Nucleotides
5-Hydroxyisourate	C11821	Nucleotides
5-6-Dihydrothymine	C00906	Nucleotides
4-Pyridoxate	C00847	Nucleotides
Nicotinamide	C00153	Nucleotides
UDP-glucose	C00029	Nucleotides
ADP-D-ribose	C01882	Nucleotides
NAD+	C00003	Nucleotides
Phosphate	C00009	Phosphates
Diphosphate	C00013	Phosphates
D-Glucose	C00031	Glycolysis
Hexose phosphate	C02965	Glycolysis
2/3-Phospho-D-glycerate	C00631	Glycolysis
Pyruvate	C00022	Glycolysis
Lactate	C01432	Glycolysis
Maltose	C00208	Other sugars
Mannitol	C00392	Other sugars
D-Arabitol	C01904	Other sugars
Citrate	C00158	TCA cycle
2-Oxoglutarate	C00026	TCA cycle
2-Oxoglutaramate	C00940	TCA cycle
Succinate	C00042	TCA cycle
Fumarate	C00122	TCA cycle
Malate	C00149	TCA cycle
2-Hydroxyglutarate/Citramalate	C02630	Alternative Carboxylic acids
Itaconate		Alternative Carboxylic acids
D-Glucono-1-5-lactone 6-phosphate	C01236	Pentose Phosphate Pathway
Sedoheptulose 1-phosphate	C06222	Pentose Phosphate Pathway
alpha-D-Ribose 1-phosphate	C00620	Pentose Phosphate Pathway
Glutathione	C00051	GSH homeostasis

Glutathione disulfide	C00127	GSH homeostasis
5-Oxoproline	C01879	GSH homeostasis
S-Glutathionyl-L-cysteine	C05526	GSH homeostasis
Cys-Gly	C01419	GSH homeostasis
Ascorbate	C00072	GSH homeostasis
Dehydroascorbate	C05422	GSH homeostasis
gamma-L-Glutamyl-L-cysteine	C00669	Gamma-glutamyls
gamma-Glutamyl-Se-methylselenocysteine	C05695	Gamma-glutamyls
gamma-L-Glutamyl-D-alanine	C03738	Gamma-glutamyls
gamma-Glutamyl-gamma-aminobutyrate	C15767	Gamma-glutamyls
gamma-L-Glutamylputrescine	C15699	Gamma-glutamyls
(5-L-Glutamyl)-L-glutamine	C05283	Gamma-glutamyls
L-Homocysteine	C00155	Serine biosynthesis and one-carbon metabolism
Dimethylglycine	C01026	Serine biosynthesis and one-carbon metabolism
3-Phosphonooxypyruvate	C03232	Serine biosynthesis and one-carbon metabolism
S-Adenosyl-L-methionine	C00019	Serine biosynthesis and one-carbon metabolism
Ornithine	C01602	Urea cycle
L-Citrulline	C00327	Urea cycle
Argininosuccinate	C03406	Urea cycle
Cadaverine	C01672	Polyamines
Putrescine	C00134	Polyamines
Spermidine	C00315	Polyamines
Spermine	C00750	Polyamines
N-Acetylneuraminate	C00270	Aminosugars
N-Glycoloyl-neuraminate	C03410	Aminosugars
alpha-D-Glucosamine 1-phosphate	C06156	Aminosugars
1-4-beta-D-Xylan	C02352	Aminosugars
UDP-N-acetyl-D-glucosamine	C00043	Aminosugars
Creatine	C00300	Arginine and proline metabolism
Creatinine	C00791	Arginine and proline metabolism
4-Acetamidobutanoate	C02946	Arginine and proline metabolism
N-Acetyl-L-citrulline	C15532	Arginine and proline metabolism
N-Acetylornithine	C00437	Arginine and proline metabolism
Guanidinoacetate	C00581	Arginine and proline metabolism
trans-4-Hydroxy-L-proline	C01157	Arginine and proline metabolism
5-Aminopentanoate	C00431	Arginine and proline metabolism
Taurine	C00245	Sulfur metabolism
Hypotaurine	C00519	Sulfur metabolism
3-Sulfino-L-alanine	C00606	Sulfur metabolism
L-Cysteate	C00506	Sulfur metabolism
L-Methionine S-oxide	C02989	Sulfur metabolism
Indole-3-acetate	C00954	Indole and Tryptophan
Indolepyruvate	C00331	Indole and Tryptophan
kynurenine	C00328	Indole and Tryptophan
N-formyl kynurenine	C02700	Indole and Tryptophan
Anthranilate	C00108	Indole and Tryptophan
Picolinic acid	C10164	Indole and Tryptophan
g-Oxalo-crotonate	C03453	Indole and Tryptophan

Glycerol 3-phosphate	C00093	Glycerophospholipid biosynthesis
Ethanolamine phosphate	C00346	Glycerophospholipid biosynthesis
sn-glycero-3-Phosphoethanolamine	C01233	Glycerophospholipid biosynthesis
Acetylcholine	C01996	Glycerophospholipid biosynthesis
acetyl-carnitine	C02571	Carnitine and fatty acid metabolism
propionyl-carnitine	C03017	Carnitine and fatty acid metabolism
butanoyl-l-carnitine	C02862	Carnitine and fatty acid metabolism
O-dodecenoyl-carnitine	HMDB13326	Carnitine and fatty acid metabolism
Hexanoic acid (caproate)	C01585	Saturated Fatty acids
Heptanoic acid	C17714	Saturated Fatty acids
Octanoic acid (caprylate)	C06423	Saturated Fatty acids
Nonanoic acid (pelargonate)	C01601	Saturated Fatty acids
Dodecanoic acid	C02679	Saturated Fatty acids
Tetradecanoic acid	C06424	Saturated Fatty acids
Hexadecanoic acid	C00249	Saturated Fatty acids
Octadecanoic acid (stearic acid)	C01530	Saturated Fatty acids
Tetradecenoic acid	C08322	Monounsaturated Fatty Acids
Hexadecenoic acid	C08362	Monounsaturated Fatty Acids
Octadecenoic acid (oleic acid)	C00712	Monounsaturated Fatty Acids
Linoleate	C01595	Poly-unsaturated Fatty Acids
Octadecatrienoic acid (linolenic acid)	C06427	Poly-unsaturated Fatty Acids
Eicosatetraenoic acid	C00219	Poly-unsaturated Fatty Acids
Eicosapentaenoic acid	C06428	Poly-unsaturated Fatty Acids
Docosahexaenoic acid	C06429	Poly-unsaturated Fatty Acids
(8Z-11Z-14Z)-Icosatrienoic acid	C03242	Essential fatty acids
(5Z-8Z-11Z-14Z-17Z)-Icosapentaenoic acid	C06428	Essential fatty acids
(7Z-10Z-13Z-16Z-19Z)-Docosa-7-10-13-16-19-pentaenoic acid	C16513	Essential fatty acids