

Supporting Information

Compartmentalized proteomic profiling outlines the crucial role of the Classical Secretory Pathway during recombinant protein production in Chinese hamster ovary cells

Author List

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Figure S1. Profiles of pH and ions during culture of CRL-12444 and CRL-12445 cells.

Concentration of sodium, potassium and calcium, and pH, were measured in supernatants by using BioProfile FLEX2 Automated Cell Culture Analyzer during batch cultures of CHO cells. Standard deviation was calculated from three biological replicates.

Figure S2. Affinity purification and biological activity of mAbs.

Purified antibody from CRL-12444 (Lane 1) or CRL-12445 (Lane 2) cells was subjected to reducing 12% (A) and non-reducing 7.5% (B) SDS-PAGE. Expression of human IL-8 in *E. coli* was verified by reducing 15% SDS-PAGE (Lane 2, panel C). IL-8 was recognized by purified antibody from CRL-12444 (Lane 2, panel D) and CRL-12445 (Lane 2, panel E) cells by western blot assay. Non-induced cells were used as a negative control of expression (Lane 1, panel C) and antibody binding (Lane 1, panels D and E). Images were representative of two biological replicates. Red, blue and green arrows indicated IL-8 bands of 10-15, 15-25 and around 35 kDa. MW: molecular weight.

Figure S3. Intracellular concentration of mAb light chain.

Intracellular concentration of anti IL-8 mAb light chain was determined in ER containing compartments C1, C2 and C3, by adding its protein sequence to the search database. An asterisk indicates statistical significance ($p < 0.05$, t test).

Figure S4. Correlation between Loess-G normalized replicates from CRL-12444 cells.

A Pearson's correlation test was done between each pair of replicates by each subcellular compartment (C1-C10). Correlation coefficient (R) and the corresponding p-value were determined.

Figure S5. Correlation between VSN-G or RLR-G normalized replicates from CRL-12444 cells.

A Pearson's correlation test was done between each pair of replicates by each subcellular compartment (C1-C10). Correlation coefficient (R) and the corresponding p-value were determined.

Figure S6. Correlation between Loess-G normalized replicates from CRL-12445 cells. A Pearson's correlation test was done between each pair of replicates by each subcellular compartment (C1-C10). Correlation coefficient (R) and the corresponding p-value were determined.

Figure S7. Correlation between VSN-G or RLR-G normalized replicates from CRL-12445 cells A Pearson's correlation test was done between each pair of replicates by each subcellular compartment (C1-C10). Correlation coefficient (R) and the corresponding p-value were determined.

Figure S8. PANTHER classification of shared altered proteins by functional classification analysis. Shared upregulated (A, C, E, G, I) and downregulated (B, D, F, H, J) proteins were classified using functional classification analysis based on biological process (A, B), cellular component (C, D), molecular function (E, F), pathway (G, H) and protein class (I, J). CC: cellular component, MOP: multicellular organismal process, TRA: transcription regulator activity, TCA: tricarboxylic acid.

Figure S9. PANTHER classification of all altered proteins by functional classification analysis. Upregulated (A, C, E, G, I) and downregulated (B, D, F, H, J) proteins were classified based on biological process (A, B), cellular component (C, D), molecular function (E, F), pathway (G, H) and protein class (I, J). ACR 13: acetylcholine receptor 1 and 3, AG: axon guidance, AGII-SS: angiotensin II-stimulated signaling, AH: adenine and hypoxanthine, BA: beta-arrestin, CC-SP: chemokine and cytokine signaling pathway, CC: cellular component, CCKR: cholecystokinin receptor, CR: cytoskeletal regulation, EGFR: epidermal growth factor receptor, FGF: fibroblast growth factor , FTHF: formyltetrahydroformate, GP: G proteins, GR: glutamate receptor, GRHR: gonadotropin-releasing hormone receptor, HGP-SP Gq alpha and Go alpha MP: heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway, HHR: histamine H1 receptor, MGR: metabotropic glutamate receptor, MOP: multicellular organismal process, NACR: nicotinic acetylcholine receptor, PDGF: platelet derived growth factor, PP: presenilin pathway, SP: signaling pathway, TCA:

tricarboxylic acid, TF: transcription factor, TR: transcription regulation, TRA: transcription regulator activity, VEGF: vascular endothelial growth factor.

Figure S10. Classification of shared differentially expressed proteins by DAVID. Shared upregulated (A, C, E) and downregulated (B, D, F) proteins were classified using functional annotation tool based on biological process (A, B), cellular component (C, D) and molecular function (E, F). MP: metabolic process, ASM: anatomical structure morphogenesis, CSA: cell-substrate adhesion, SADCS: substrate adhesion-dependent cell spreading, MO: matrix organization, SO: structure organization, LS: large subunit, LSP: large subunit precursor, TPCP: transferring phosphorus-containing groups.

Figure S11. KEGG mapping of all differentially expressed proteins. Upregulated (A) and downregulated (B) proteins were mapped to pathways by using Annotate sequence interface of BlastKoala. EIP: environmental information processing, PF: protein families, GIP: genetic information processing, cof.: cofactors, vit.: vitamins, Sig.: signaling, UC: unclassified.

Figure S12. Differential proteomics analysis of glycolysis. All upregulated (red) or downregulated (green) proteins in CRL-12445 cells in comparison to CRL-12444 cells were mapped to glycolysis resulting in accumulation/enhancement (red rectangles) or deprivation/impairment (green rectangles) of certain products or cellular functions. Circled triangle represents a metabolite who favors ongoing enzymatic reaction. Aldehyde dehydrogenase (ALDH), aldo-keto reductase family 1 member A1 (AKR1A1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphofructokinase 1 (PFK1), pyruvate kinase (PK).

Figure S13. Differential proteomics analysis of lipid synthesis. All upregulated (red) or downregulated (green) proteins in CRL-12445 cells in comparison to CRL-12444 cells were mapped to synthesis of prostaglandins and fatty acids and their derivatives resulting in

accumulation/enhancement (red rectangles) or deprivation/impairment (green rectangles) of certain products or cellular functions. Circled triangle represents a metabolite who favors ongoing enzymatic reaction. Alkylglycerone phosphate synthase (AGPS), choline (Ch), cytidine diphosphate (CDP), cytidine monophosphate (CMP), cytidine triphosphate (CTP), cytochrome B5 reductase (CYB5R), ethanolamine (ETA), fatty acyl-CoA reductase (FAR), glycerol-3-phosphate dehydrogenase (GPDH), leukotriene A4 hydrolase (LTA4H), leukotriene B4 (LTB4), malate dehydrogenase 1 (MDH1), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phospholipid phosphatase (PLPP), phosphorylcholine transferase (PCYT1), prostaglandin E synthase 3 (PTGES3), prostaglandin E2 (PGE2), prostaglandin H2 (PGH2), reduced glutathione (GSH), S-adenosyl methionine (SAM), S-adenosyl-L-homocysteine (SAH), serine (Ser), serine palmitoyltransferase (SPT), sterol carrier protein 2 (SCP2), triacylglyceride (TAG).

Figure S14. Differential proteomics analysis of pentose phosphate pathway. All upregulated (red) or downregulated (green) proteins in CRL-12445 cells in comparison to CRL-12444 cells were mapped to pentose phosphate pathway (PPP) resulting in accumulation/enhancement (red rectangles) of certain products or cellular functions. Adenine phosphoribosyltransferase (APRT), adenosine monophosphate deaminase (AMPD), adenylate kinase 3 (AK3), aspartate (Asp), CTP synthase (CTPS), cytidine triphosphate (CTP), formylglycinamide ribonucleotide (FGAR), glutamate (Glu), glutamine (Gln), inosine monophosphate (IMP), nicotinate phosphoribosyltransferase (NAPRT), phosphogluconate dehydrogenase (6PGD), phosphoribosyl pyrophosphate (PRPP), phosphoribosyl pyrophosphate synthetase (PRPS).

Figure S15. Differential proteomics analysis of tricarboxylic acid (TCA) cycle and synthesis of cholesterol and proline. All upregulated (red) or downregulated (green) proteins in CRL-12445 cells in comparison to CRL-12444 cells were mapped to TCA cycle and synthesis of docosahexaenoic acid, cholesterol and their derivatives, and proline, resulting in accumulation/enhancement (red rectangles)

or deprivation/impairment (green rectangles) of certain products or cellular functions. A red triangle represents a metabolite who favors the indicated enzymatic reaction. Aminoacids feeding the cycle were highlighted in blue. 3-hydroxyisobutyrate dehydrogenase (HIBADH), acetyl-CoA acetyltransferase 2 (ACAT2), acetyl-CoA synthetase (ACSS), aldehyde dehydrogenase 18 family member A1 (ALDH18A1), alpha-ketoglutarate dehydrogenase (α -KGDH), asparagine (Asn), branched chain aminoacid transaminase (BCAT), citrate synthase (CS), dihydrolipoamide dehydrogenase (DLD), fumarylacetoacetase (FAH), glutamine (Gln), isocitrate dehydrogenase (IDH), isoleucine (Ile), leucine (Leu), methylsterol monooxygenase 1 (MSMO1), omega-amidase NIT2 (NIT2), phenylalanine (Phe), pyruvate dehydrogenase (PDH), sterol carrier protein 2 (SCP2), sterol O-acyltransferase (SOAT), succinyl-CoA synthetase (SCS), tyrosine (Tyr), valine (Val).

Figure S16. Differential proteomics analysis of fatty acids degradation. All downregulated (green) proteins in CRL-12445 cells in comparison to CRL-12444 cells were mapped to fatty acid degradation pathways resulting in deprivation/impairment (green rectangles) of certain products or cellular functions. Discontinuous brown lines represent different cellular compartments. Acetyl-CoA acetyltransferase 1 (ACAT1), acetyl-CoA acyltransferase 1 (ACAA1), acyl-CoA thioesterase (ACOT), aldehyde dehydrogenase (ALDH), alpha-methylacyl-CoA racemase (AMACR), carnitine O-palmitoyltransferase 2 (CPT2), enoyl-CoA delta isomerase 2 (ECI2), hydroxymethylglutaryl-CoA lyase (HMGL).

Figure S17. Differential proteomics analysis of one carbon metabolism. All upregulated (red) or downregulated (green) proteins in CRL-12445 cells in comparison to CRL-12444 cells were mapped to the methionine and folate cycles resulting in accumulation/enhancement (red rectangles) or deprivation/impairment (green rectangles) of certain products or cellular functions. Adenosylhomocysteinase (AHCY), dihydrolipoamide dehydrogenase (DLD), glycine cleavage system protein H (GCSH), methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1 like (MTHFD1L),

methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2 (MTHFD2), serine hydroxymethyltransferase (SHMT), tetrahydrofolate (TH4).

Table S2. Identification of samples from proteomics. The identity of samples from Table S1 is provided. Sample numbers are linked to their respective band number in the sucrose gradient, biological replicate (experiment) and cell line.

Table S16. Reconstruct KEGG pathways of differentially regulated proteins. Shared or all upregulated and downregulated proteins were mapped to pathways by using Annotate sequence interface of BlastKoala tool from KEGG database and reconstructed. *Cricetidae* family (337677) was used as reference database.

Table S17. Differential enrichment of Biological processes by GSEA. Defined sets of genes from biological processes were statistically compared between both cell lines using prebuilt gene sets of biological processes available in GO2MSIG. Collapse dataset to gene symbols and metric for ranking genes were set to “false” and “diff of classes”, respectively. FDR < 0.25 and p < 0.05 were cut-off values for statistical significance. Downregulated (green) and upregulated (red) processes were shown.

Table S18. Differential enrichment of Cellular components by GSEA. Defined sets of genes from cellular components were statistically compared between both cell lines using prebuilt gene sets of cellular component available in GO2MSIG. Collapse dataset to gene symbols and metric for ranking genes were set to “false” and “diff of classes”, respectively. FDR < 0.25 and p < 0.05 were cut-off values for statistical significance. Downregulated (green) and upregulated (red) processes were shown.

Table S19. Differential enrichment of Molecular functions by GSEA. Defined sets of genes from molecular functions were statistically compared between both cell lines using prebuilt gene sets of molecular functions available in GO2MSIG. Collapse dataset to gene symbols and metric for ranking genes were set to “false” and “diff of classes”, respectively. FDR < 0.25 and $p < 0.05$ were cut-off values for statistical significance. Downregulated (green) and upregulated (red) processes were shown.

Supporting Discussion. A supplementary discussion that focuses on processes and organelles other than the secretory pathway is provided. Consumption of glucose and glutamine, nucleic acids and proteins, cytoskeleton organization, cell signaling and metabolism of carbon, nitrogen, cofactors and vitamins were addressed in this section.

Figure S1

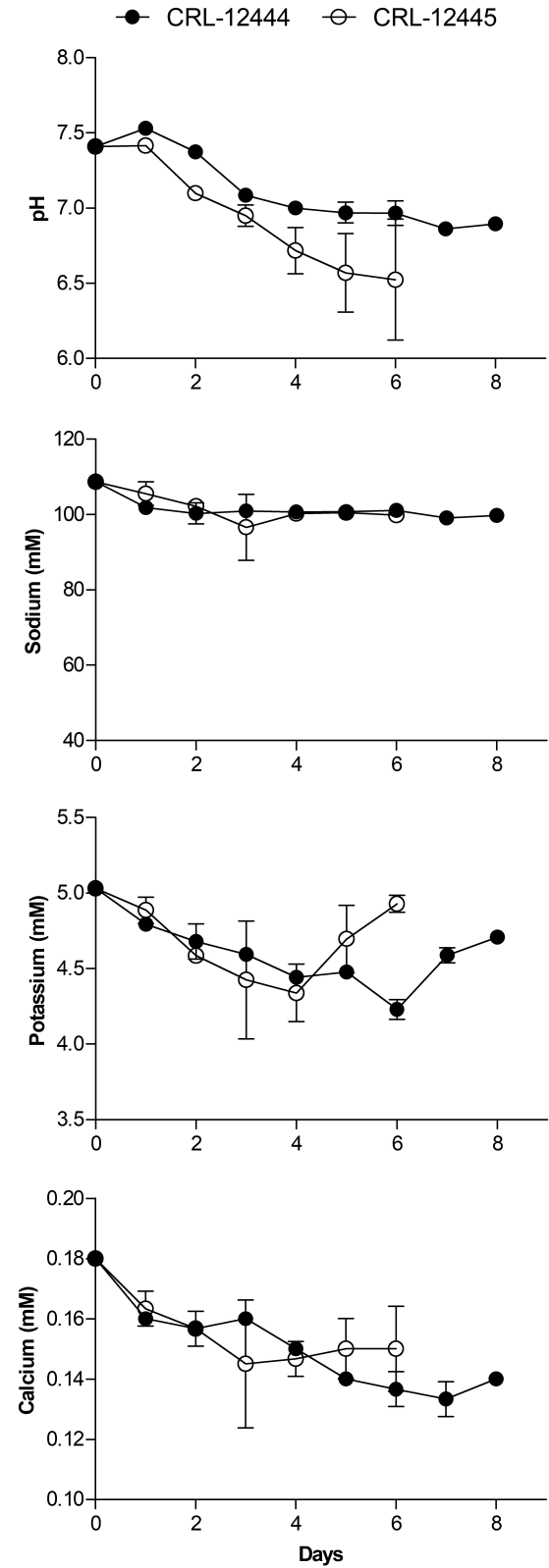


Figure S2

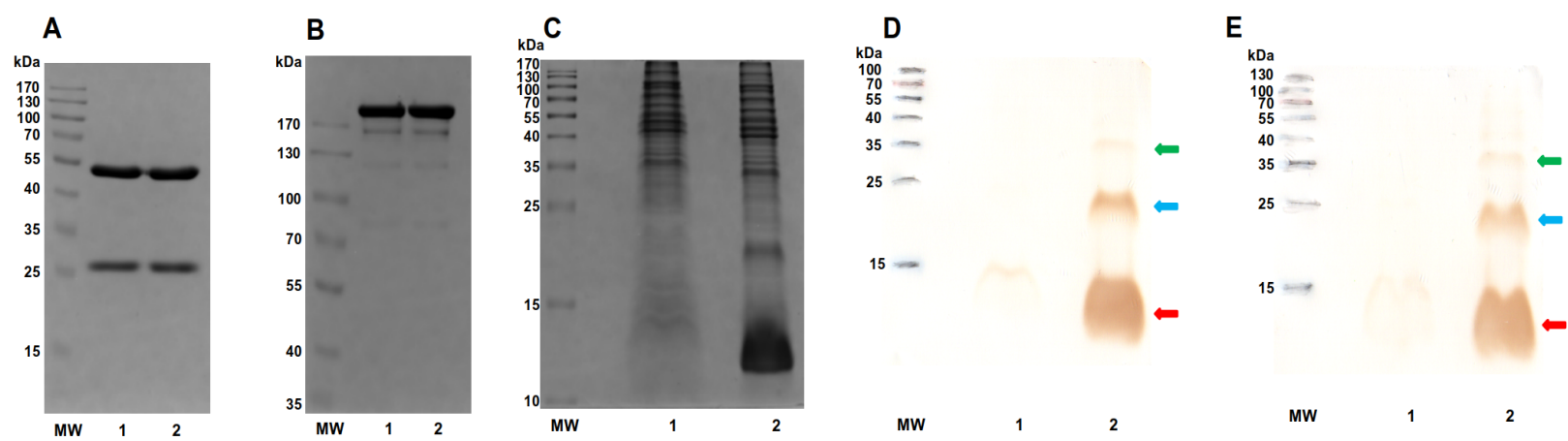


Figure S3

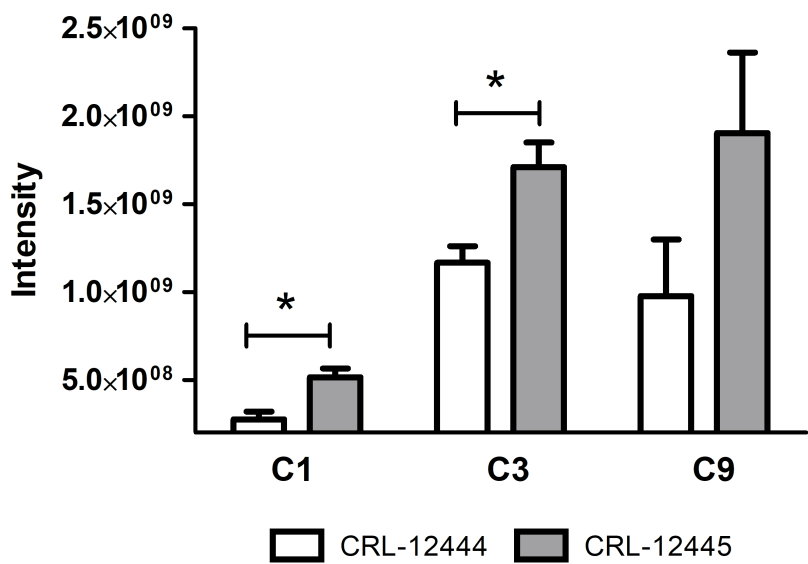


Figure S4

CRL-12444 Biological replicates – Data normalized by Loess-G

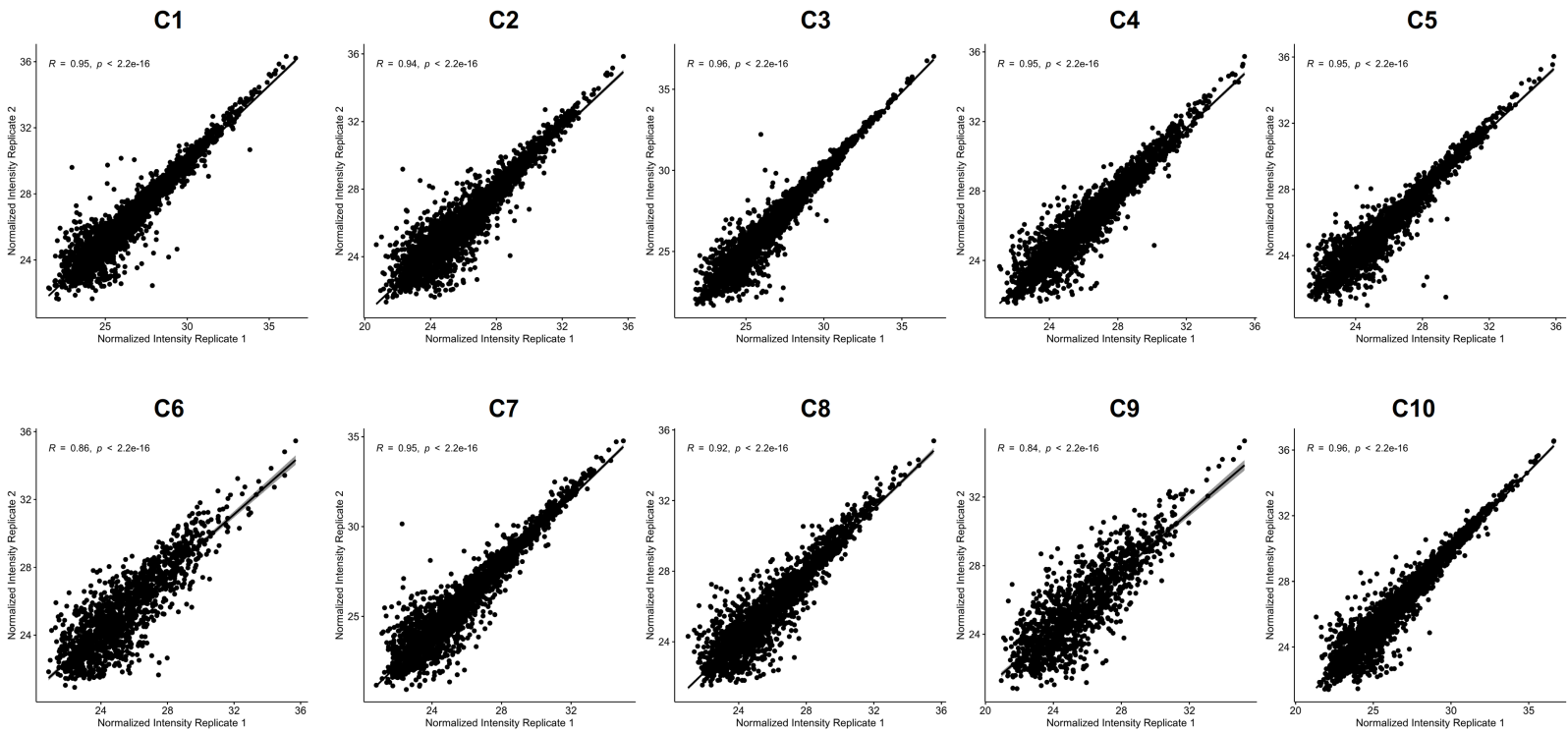


Figure S5

CRL-12444 Biological replicates – Data normalized by VSN-G or RLR-G

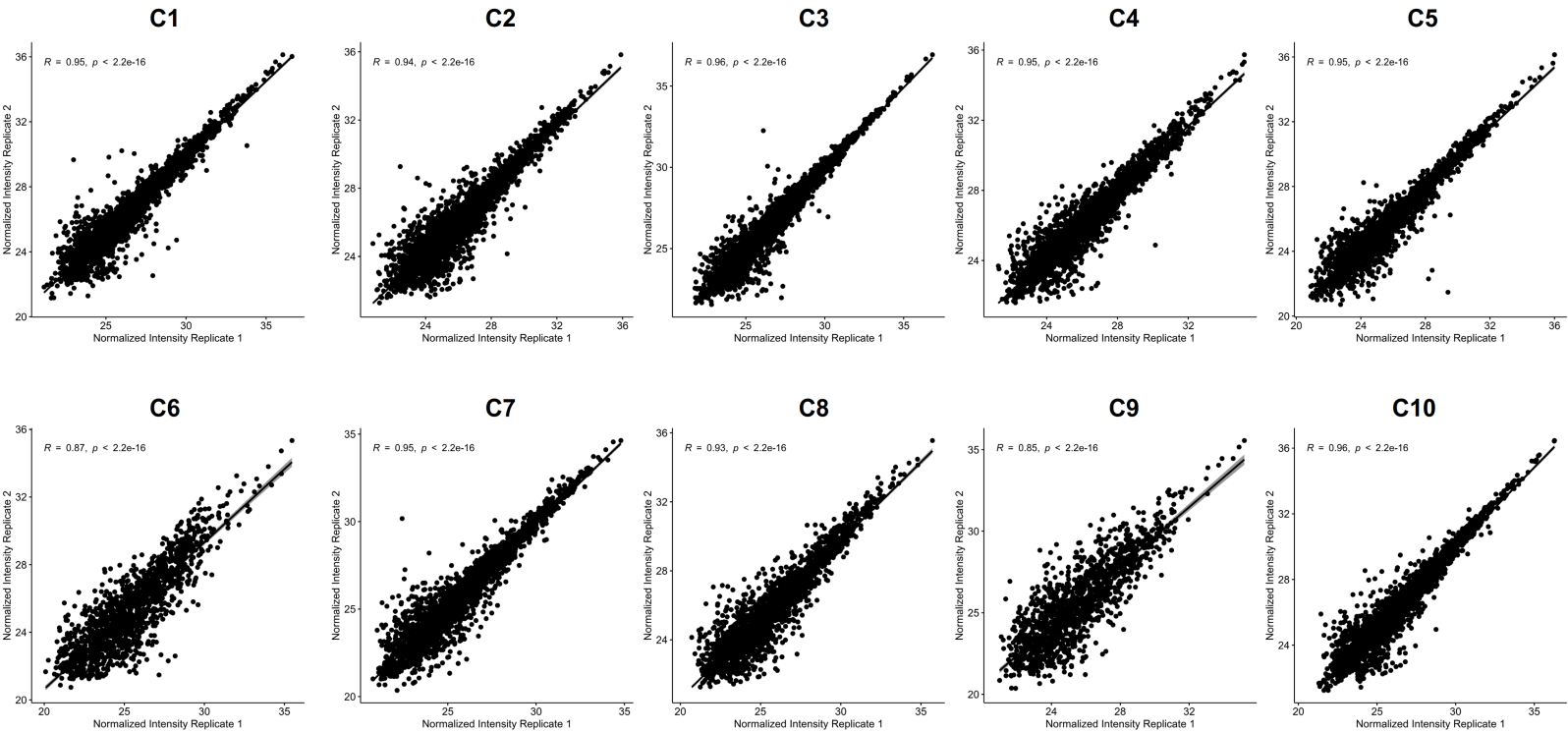


Figure S6

CRL-12445 Biological replicates – Data normalized by Loess-G

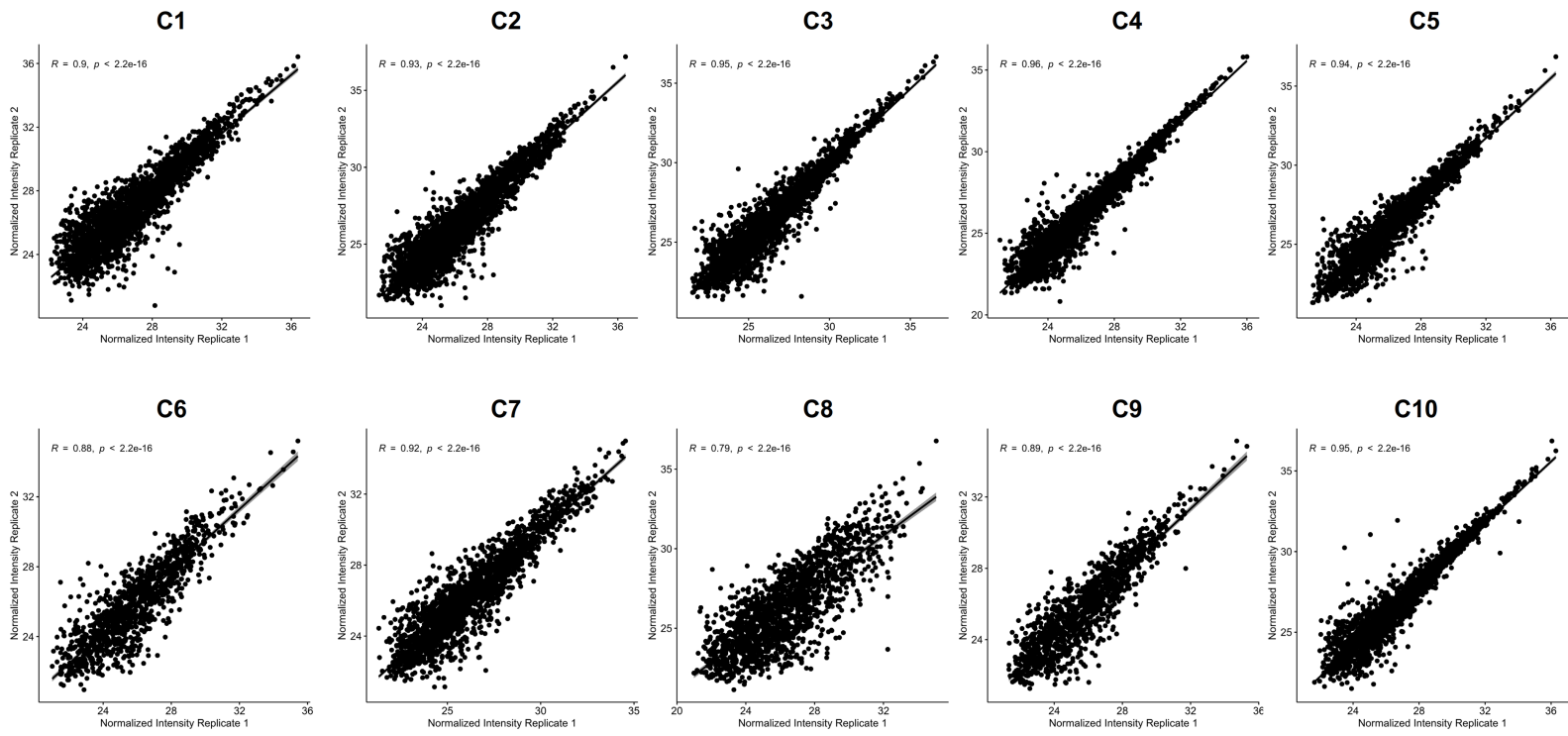


Figure S7

CRL-12445 Biological replicates – Data normalized by VSN-G or RLR-G

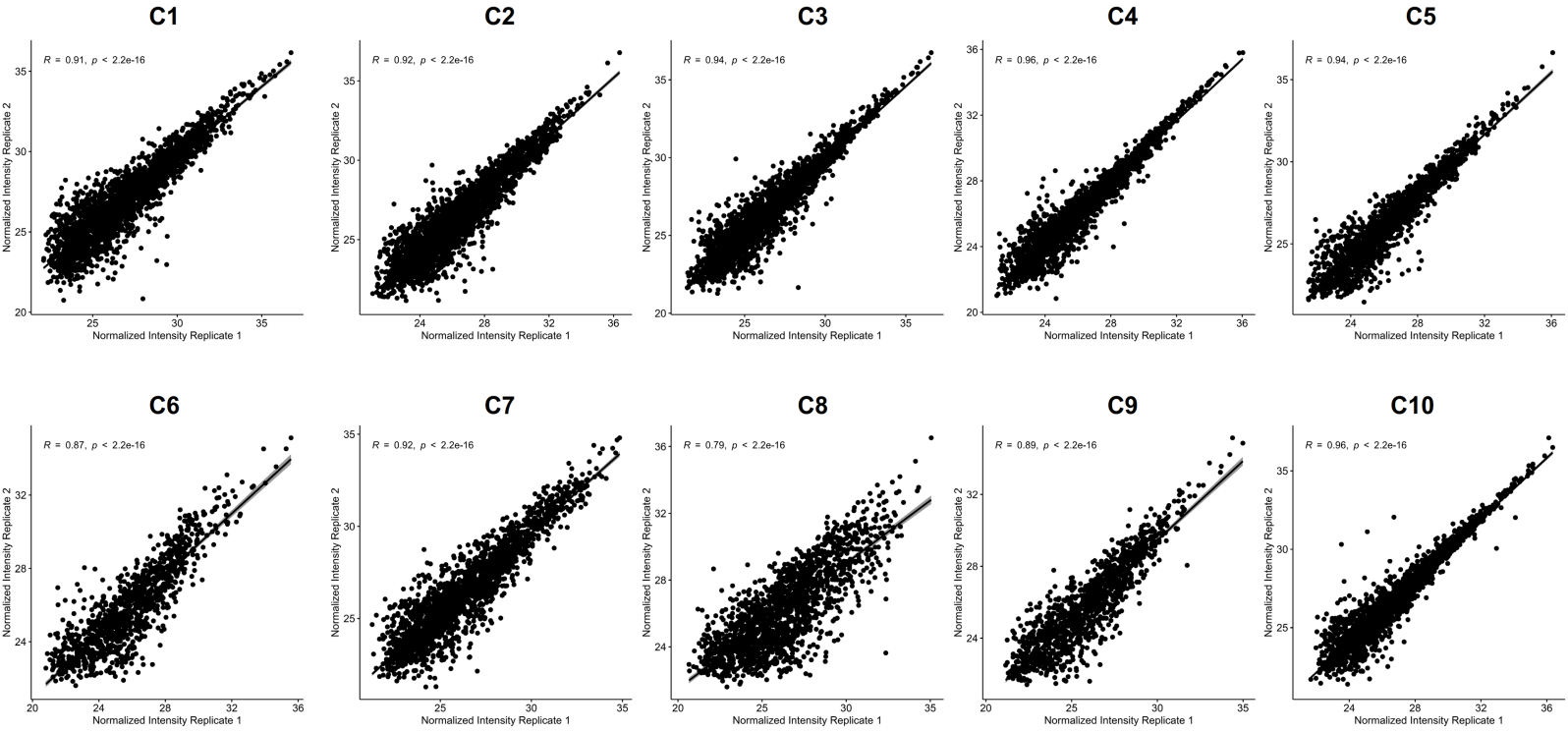
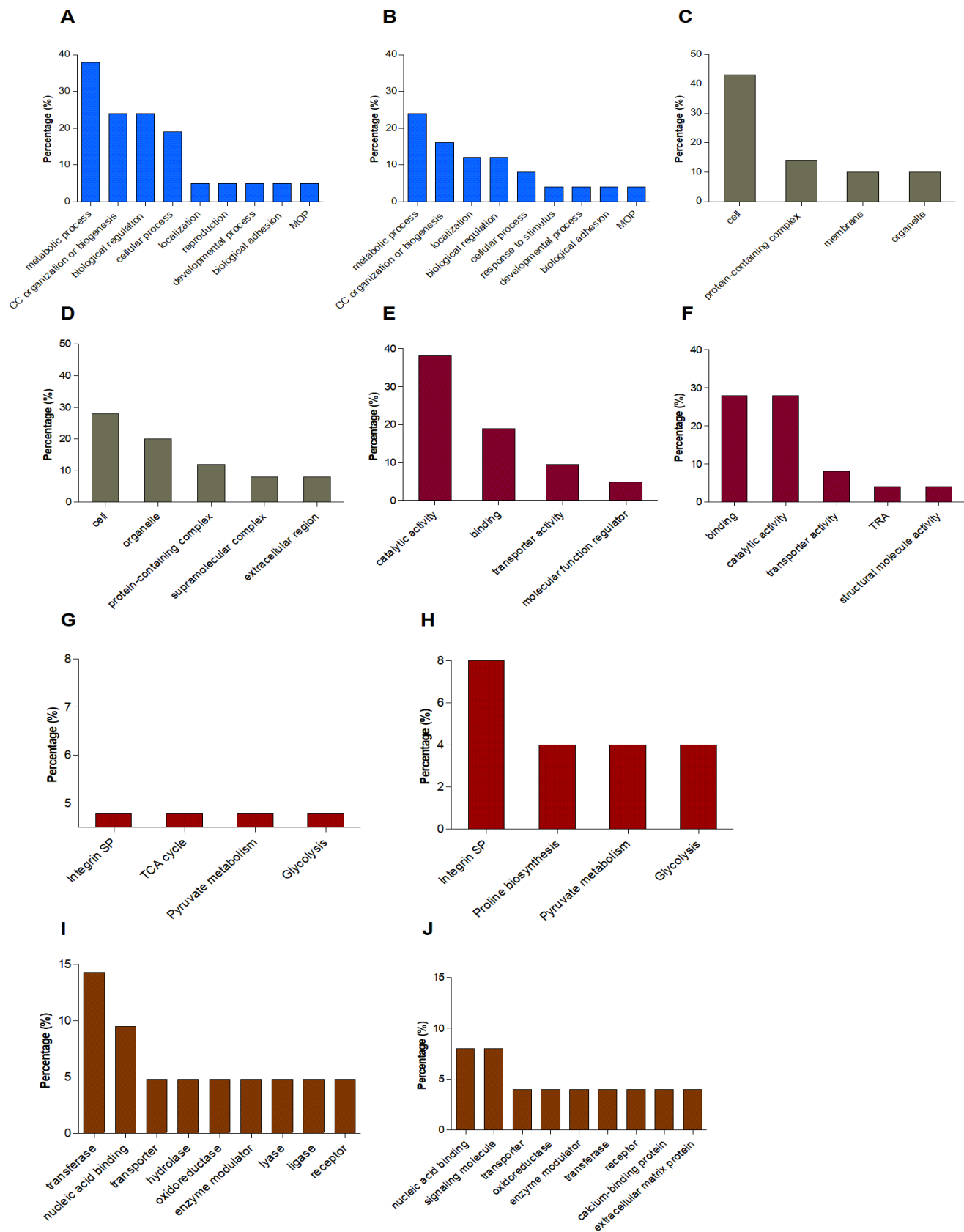


Figure S8



A

GO Term	Percentage (%)
metabolic process	40
biological regulation	21
CC organization or biogenesis	19
cellular process	18
developmental process	15
response to stimulus	12
immune system process	7
MOP	5
reproduction	4
signaling	2
locomotion	1

B

GO Term	Percentage (%)
metabolic process	30
biological regulation	16
localization	15
cellular process	14
response to stimulus	9
developmental process	4
MOP	3
biological adhesion	3
cell proliferation	2
growth	1
locomotion	1
immune system process	1

C

GO Term	Percentage (%)
cell	45
organelle	16
protein-containing complex	13
membrane	10
extracellular region	2
synapse	1
supramolecular complex	1

D

GO Term	Percentage (%)
cell	35
organelle	16
protein-containing complex	10
membrane	8
extracellular region	3
supramolecular complex	1
cell junction	1
synapse	1

E

GO Term	Percentage (%)
catalytic activity	40
binding	35
molecular function regulator	8
transporter activity	4
structural molecule activity	2
molecular transducer activity	2
translation regulator activity	1
cargo receptor activity	1

F

GO Term	Percentage (%)
catalytic activity	30
binding	28
structural molecule activity	4
transporter activity	4
molecular function regulator	3
molecular transducer activity	1
translation regulator activity	1

G

GO Term	Percentage (%)
Integrin SP	2.4
Huntingtin disease	2.4
Ubiquitin proteasome pathway	2.4
GRIN1 pathway	1.8
JAK-STAT signaling pathway	1.8
P53 pathway	1.8
Arts salvage pathway	1.2
T cell activation	1.2
IGF1R signaling pathway	1.2
Parkinson	1.2
TGF-beta signaling pathway	1.2
TCR signaling pathway	1.2
Notch signaling pathway	1.2
PGC signaling pathway	1.2
IL-6 signaling pathway	1.2
CCR4 signaling pathway	1.2
AG-mediated pathway	1.2
TGF-beta group II pathway	1.2
De novo purine biosynthesis	0.6
lysosomal degradation	0.6
Alzheimer disease PP	0.6

H

GO Term	Percentage (%)
Integrin SP	1.9
Wnt signaling pathway	1.9
GRIN1 pathway	1.5
Apoptosis MP	1.5
Alzheimer disease PP	1.2
Inflammation mediated by CC-SP	0.9
HGP-SP	0.9
Alpha adrennergic	0.9
Huntington	0.9
Viral cycle	0.6
TRK signaling pathway	0.6
TCR signaling pathway	0.6
Endothelial dysfunction	0.6
Parkinson disease	0.6
GR signaling pathway	0.6
Pathway for drug synthesis	0.6
Myogenic CCR15 SP	0.6
AGI-SS through PP and BA	0.6

I

GO Term	Percentage (%)
hydrolase	14.5
enzyme modulator	9.0
nucleic acid binding	8.5
transferase	5.5
cytoskeletal protein	5.5
transcription factor	4.5
signaling molecule	4.5
ligase	4.0
membrane traffic protein	3.5
cell adhesion molecule	3.0
calcium-binding protein	3.0
lyase	2.5
receptor	2.5
chaperone	2.5
transfer/canier protein	2.0
cell junction protein	1.0
structural protein	0.5

J

GO Term	Percentage (%)
nucleic acid binding	11.5
oxido-reductase	7.5
hydrolase	7.0
transferase	6.5
enzyme modulator	6.5
transporter	6.0
cytoskeletal protein	4.0
transferrin carrier protein	3.0
calcium-binding protein	2.5
receptor	2.5
ligase	2.0
chaperone	2.0
signaling molecule	1.5
transcription factor	1.5
extracellular matrix protein	1.0
R-regulatory/adaptor protein	1.0
defense/immunity protein	0.5
cell adhesion molecule	0.5
structural protein	0.5

Figure S10

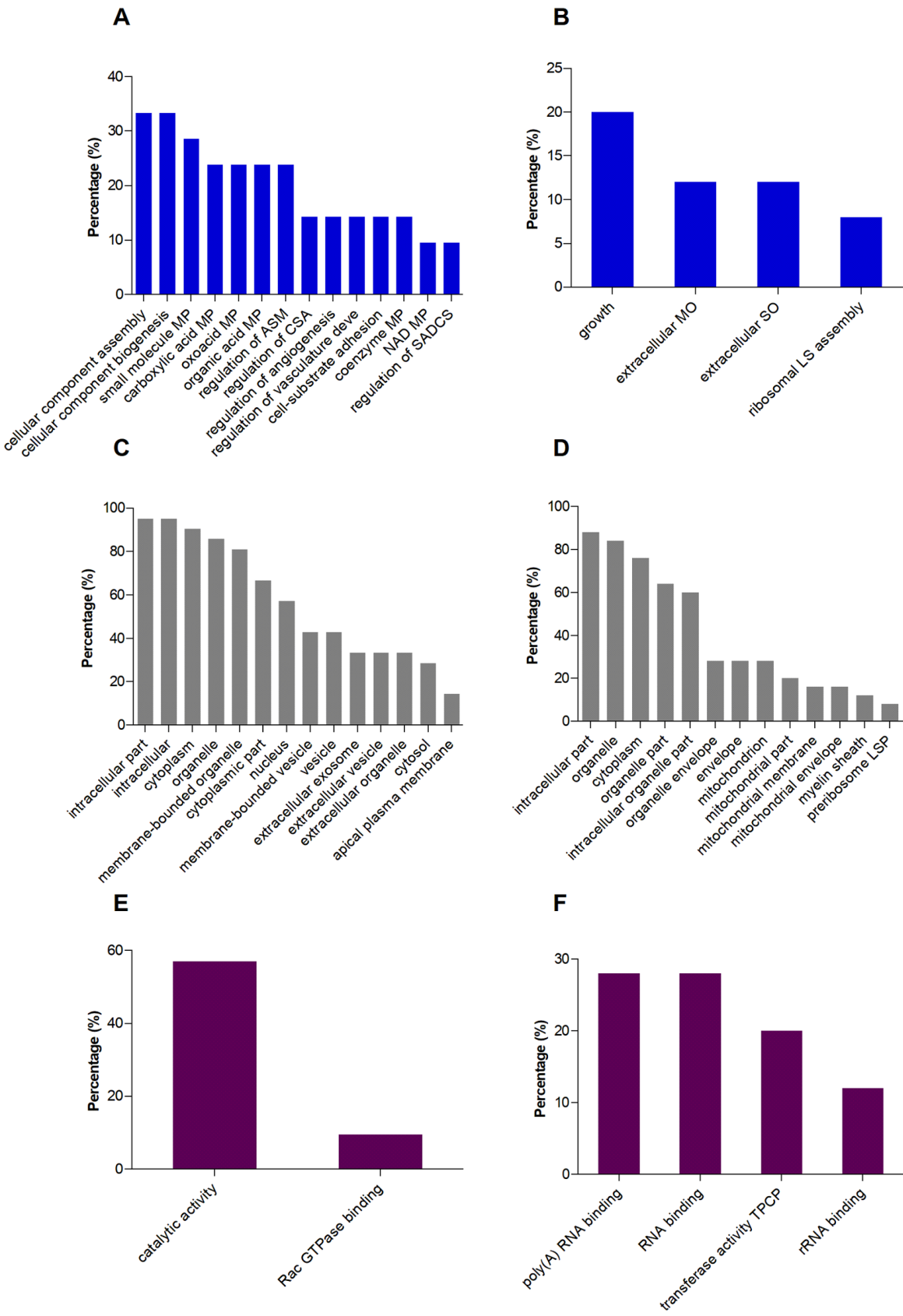


Figure S11

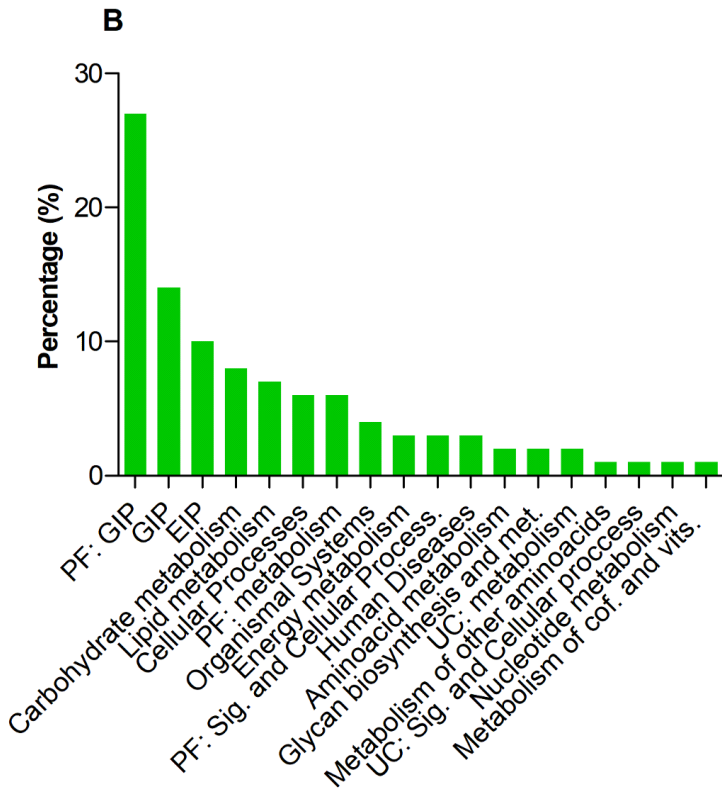
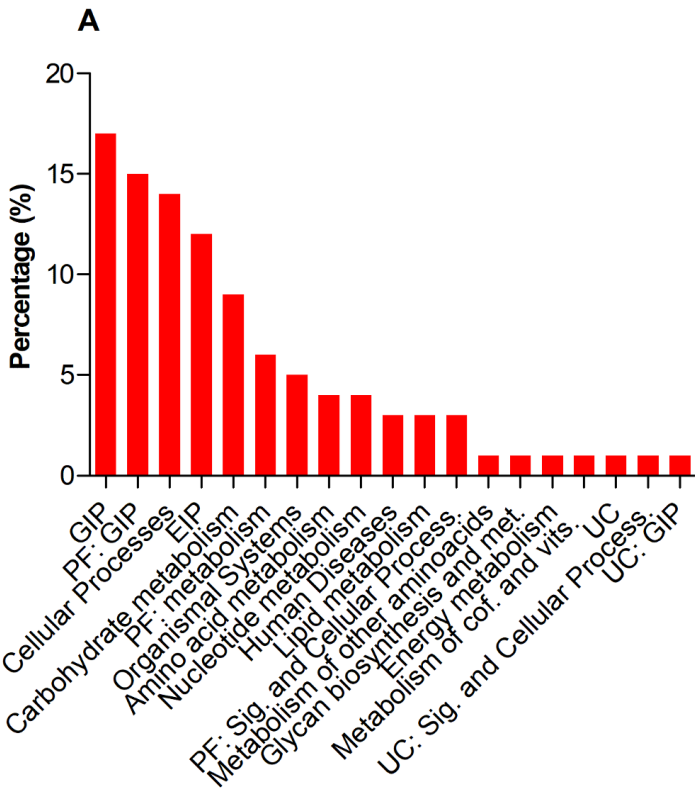


Figure S12

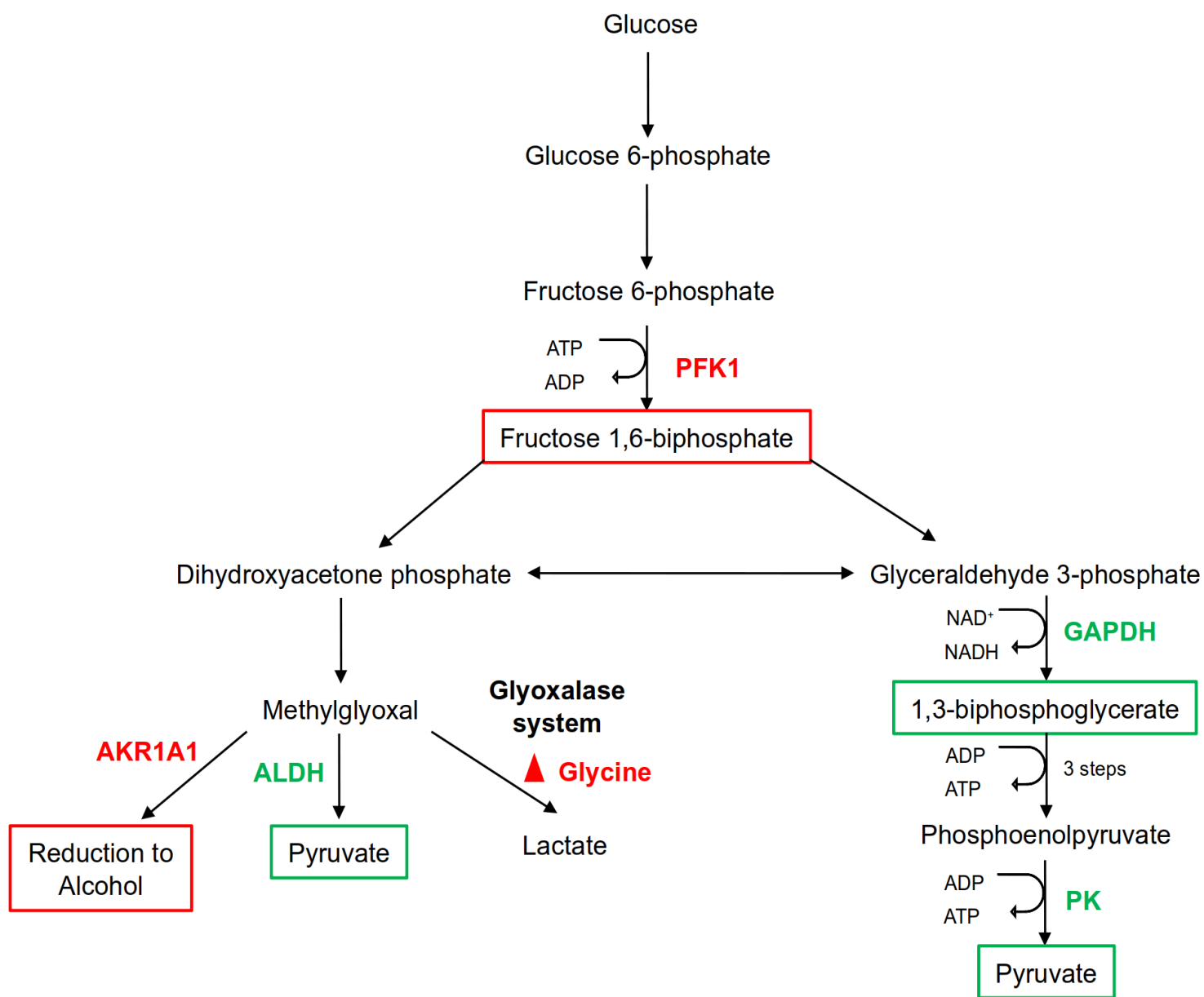


Figure S13

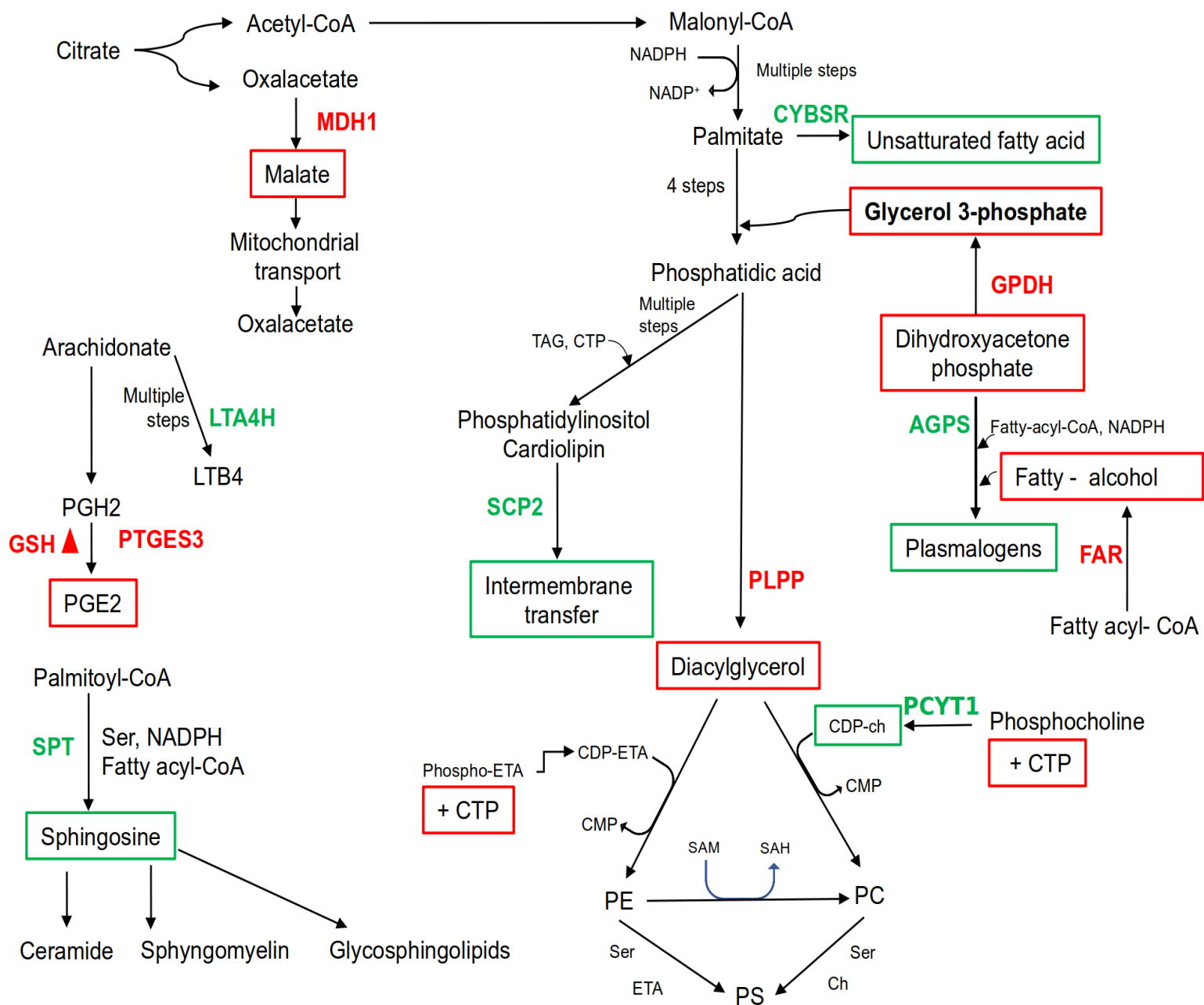


Figure S14

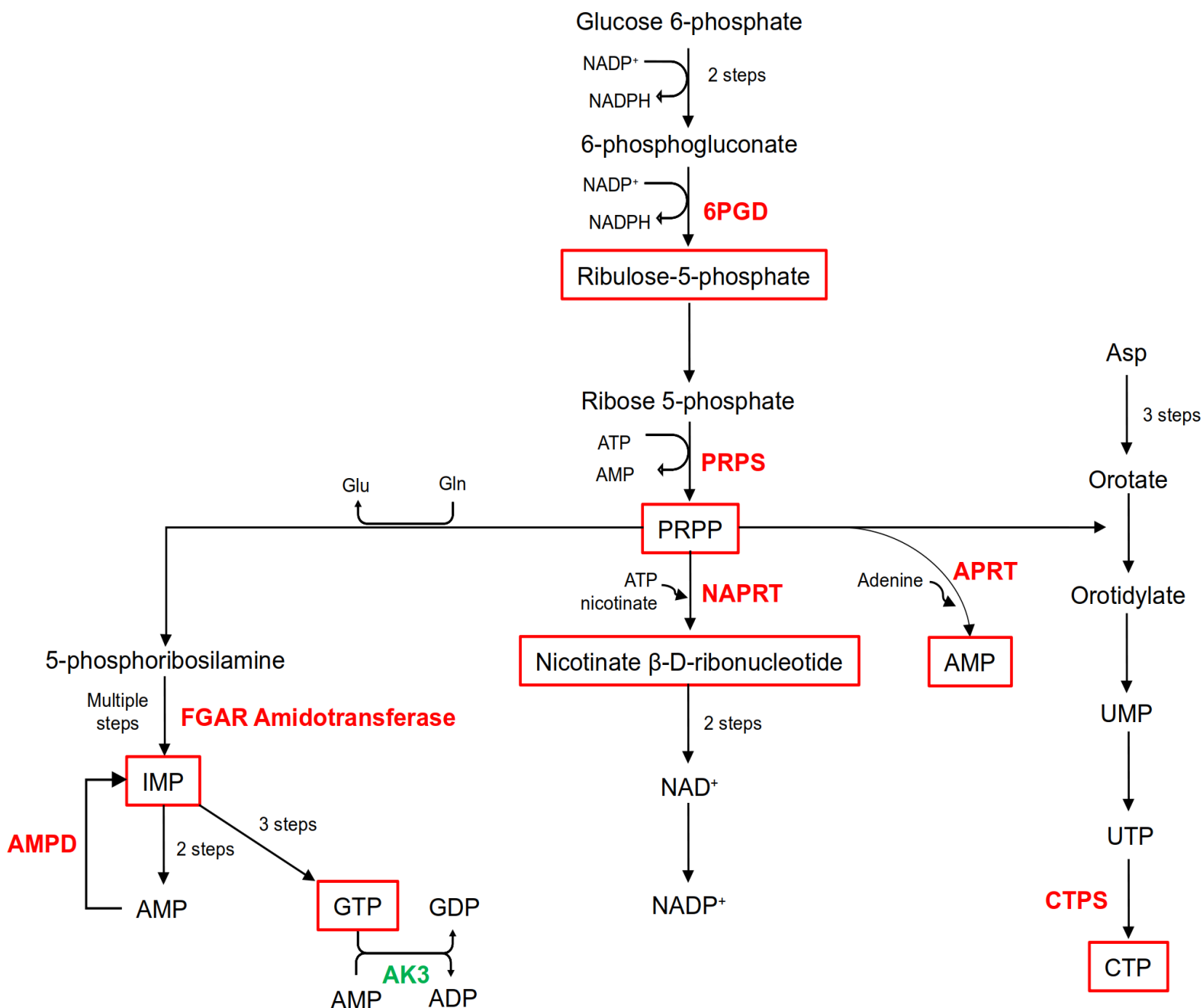


Figure S15

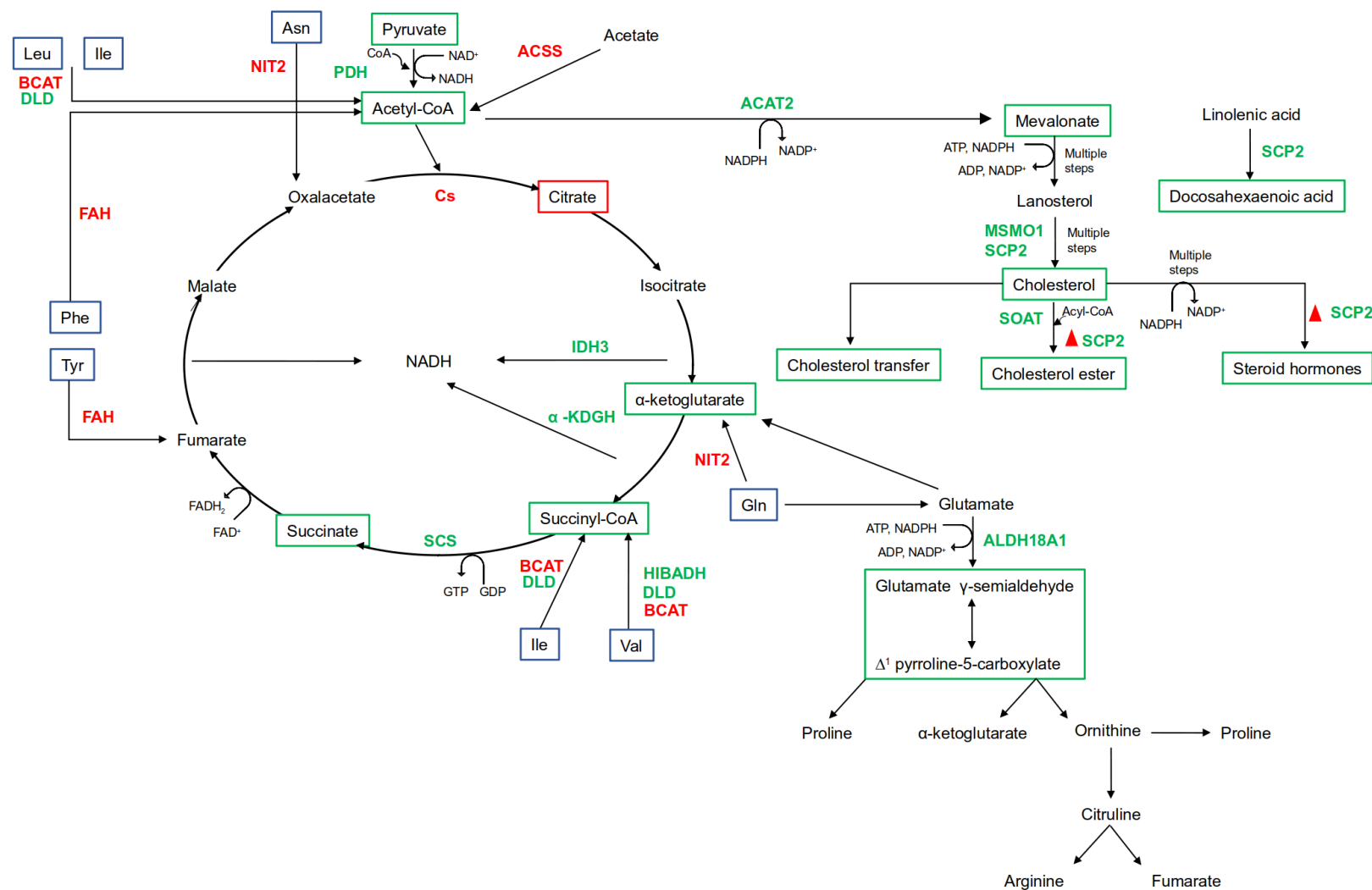


Figure S16

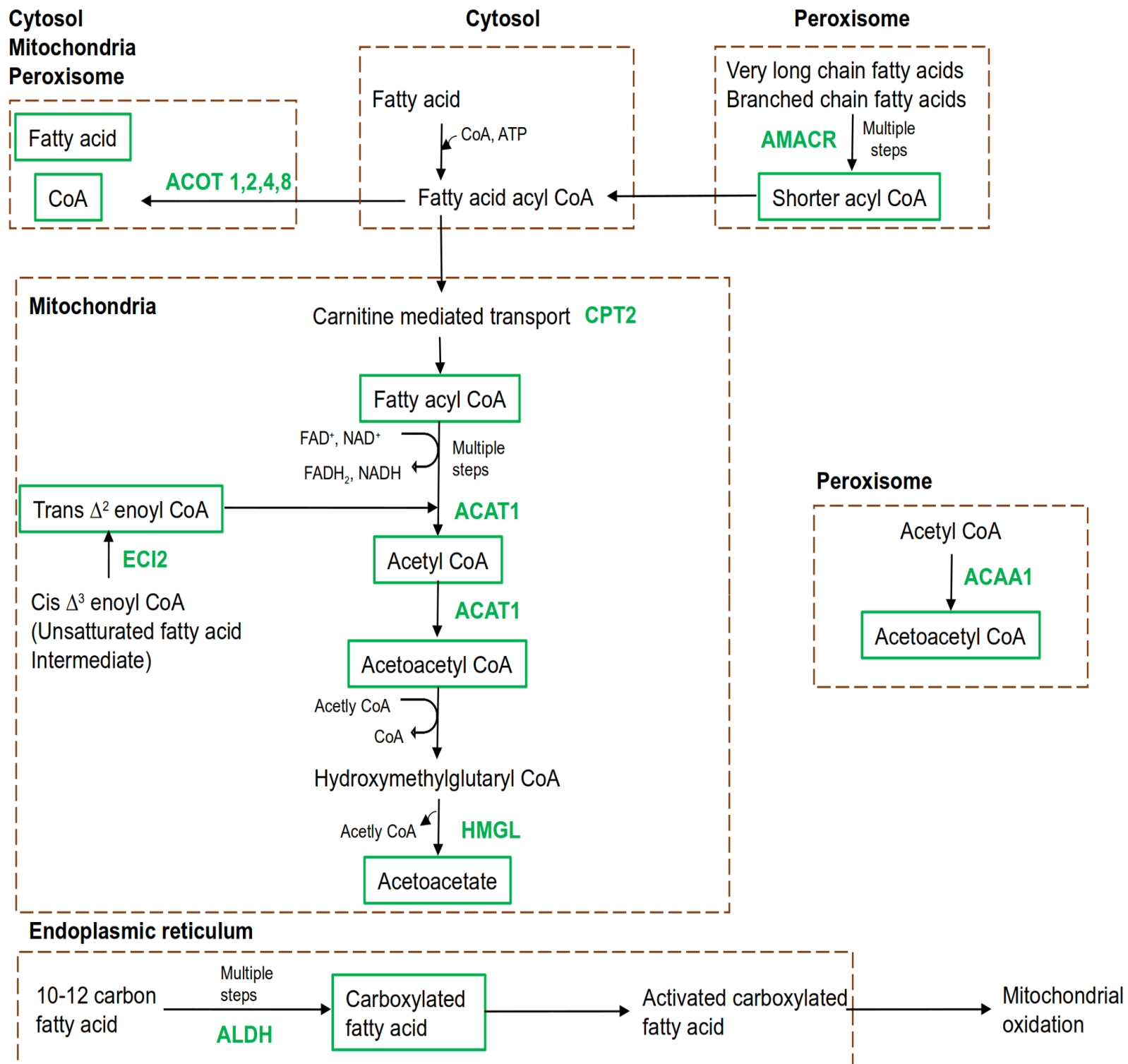


Figure S17

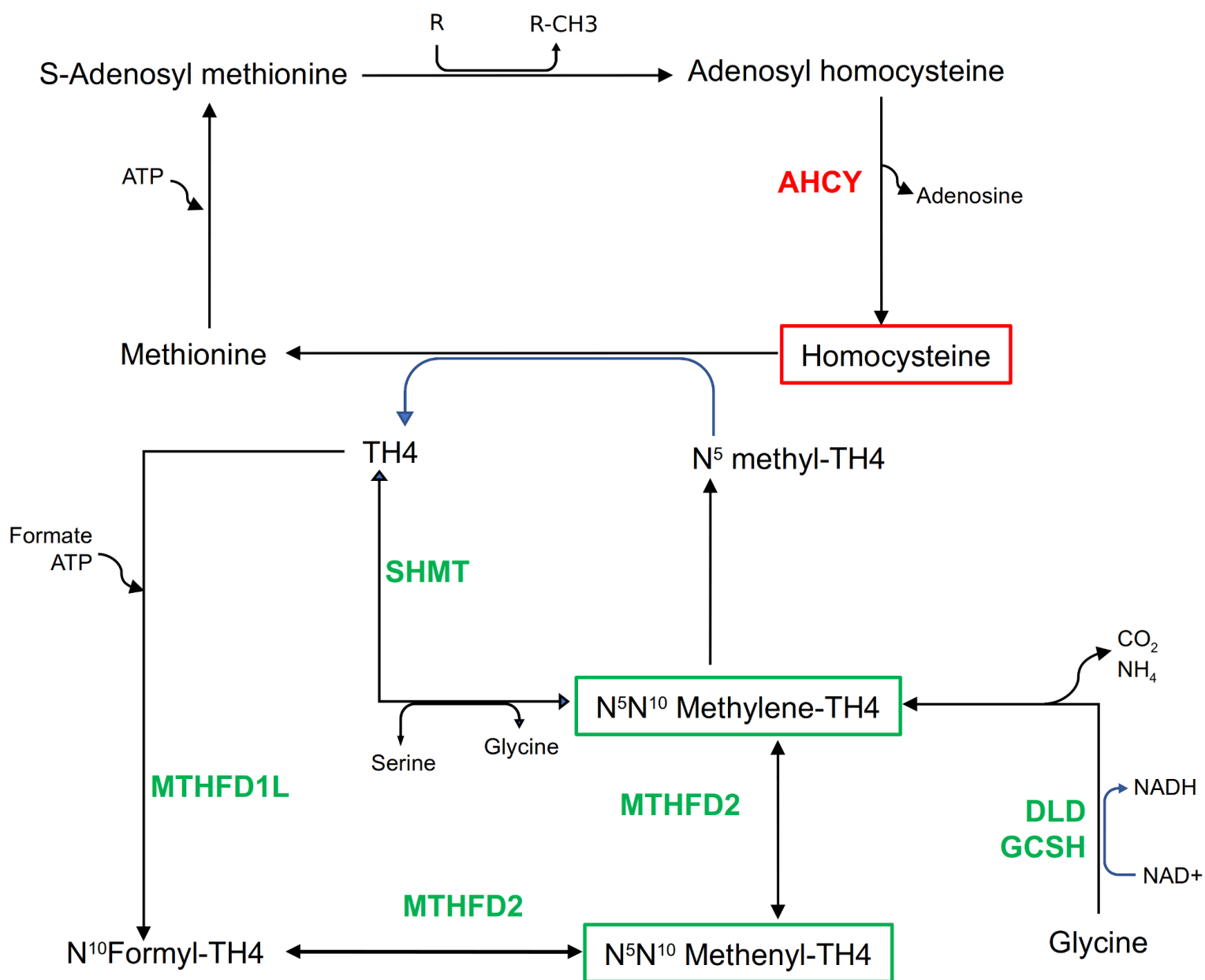


Table S2

Sample number	Gradient	Band number	Experiment	Cell line
1	Nuclear	1	1	CRL-12 444
2	Nuclear	1	2	CRL-12 444
3	Nuclear	2	1	CRL-12 444
4	Nuclear	2	2	CRL-12 444
5	Nuclear	3	1	CRL-12 444
6	Nuclear	3	2	CRL-12 444
7	Mitochondrial	1	1	CRL-12 444
8	Mitochondrial	1	2	CRL-12 444
9	Mitochondrial	2	1	CRL-12 444
10	Mitochondrial	2	2	CRL-12 444
11	Mitochondrial	3	1	CRL-12 444
12	Mitochondrial	3	2	CRL-12 444
13	Microsomal	1	1	CRL-12 444
14	Microsomal	1	2	CRL-12 444
15	Microsomal	2	1	CRL-12 444
16	Microsomal	2	2	CRL-12 444
17	Microsomal	3	1	CRL-12 444
18	Microsomal	3	2	CRL-12 444
19	Cytosol		1	CRL-12 444
20	Cytosol		2	CRL-12 444
23	Nuclear	1	1	CRL-12 445
24	Nuclear	1	2	CRL-12 445
25	Nuclear	2	1	CRL-12 445
26	Nuclear	2	2	CRL-12 445
27	Nuclear	3	1	CRL-12 445
28	Nuclear	3	2	CRL-12 445
29	Mitochondrial	1	1	CRL-12 445
30	Mitochondrial	1	2	CRL-12 445
31	Mitochondrial	2	1	CRL-12 445
32	Mitochondrial	2	2	CRL-12 445
33	Mitochondrial	3	1	CRL-12 445
34	Mitochondrial	3	2	CRL-12 445
35	Microsomal	1	1	CRL-12 445
36	Microsomal	1	2	CRL-12 445
37	Microsomal	2	1	CRL-12 445
38	Microsomal	2	2	CRL-12 445
39	Microsomal	3	1	CRL-12 445
40	Microsomal	3	2	CRL-12 445
41	Cytosol		1	CRL-12 445
42	Cytosol		2	CRL-12 445

Table S16

Shared upregulated proteins	Shared downregulated proteins	All upregulated proteins	All downregulated proteins
Metabolism Global and overview maps 01100 Metabolic pathways (5) 01110 Biosynthesis of secondary metabolites (3) 01120 Microbial metabolism in diverse environments (3) 01130 Biosynthesis of antibiotics (4) 01200 Carbon metabolism (2) 01210 2-Oxocarboxylic acid metabolism (1) 01230 Biosynthesis of amino acids (2) 01220 Degradation of aromatic compounds (1) Carbohydrate metabolism 00010 Glycolysis / Gluconeogenesis (2) K00002 tr G3GYP7 G3GYP7_CRIGR K00850 tr G3IDJ8 G3IDJ8_CRIGR 00020 Citrate cycle (TCA cycle) (1) 00030 Pentose phosphate pathway (1) 00040 Pentose and glucuronate interconversions (1) 00051 Fructose and mannose metabolism (1) 00052 Galactose metabolism (1) 00520 Amino sugar and nucleotide sugar metabolism (1) 00630 Glyoxylate and dicarboxylate metabolism (1)	Metabolism Global and overview maps 01100 Metabolic pathways (7) 01110 Biosynthesis of secondary metabolites (4) 01120 Microbial metabolism in diverse environments (2) 01130 Biosynthesis of antibiotics (2) 01200 Carbon metabolism (2) 01230 Biosynthesis of amino acids (2) Carbohydrate metabolism 00010 Glycolysis / Gluconeogenesis (2) 00020 Citrate cycle (TCA cycle) (1) 00620 Pyruvate metabolism (2) Energy metabolism 00190 Oxidative phosphorylation (1) Lipid metabolism 00062 Fatty acid elongation (1) 00564 Glycerophospholipid metabolism (1) 01040 Biosynthesis of unsaturated fatty acids (1) Nucleotide metabolism 00230 Purine metabolism (2) 00240 Pyrimidine metabolism (1) Amino acid metabolism 00330 Arginine and proline metabolism (1) Metabolism of other amino acids 00440 Phosphonate and phosphinate metabolism (1)	Metabolism Global and overview maps 01100 Metabolic pathways (27) 01110 Biosynthesis of secondary metabolites (15) 01120 Microbial metabolism in diverse environments (10) 01130 Biosynthesis of antibiotics (12) 01200 Carbon metabolism (8) 01210 2-Oxocarboxylic acid metabolism (2) 01230 Biosynthesis of amino acids (4) 01220 Degradation of aromatic compounds (2) Carbohydrate metabolism 00010 Glycolysis / Gluconeogenesis (4) 00020 Citrate cycle (TCA cycle) (2) 00030 Pentose phosphate pathway (3) 00040 Pentose and glucuronate interconversions (1) 00051 Fructose and mannose metabolism (2) 00052 Galactose metabolism (1) 00500 Starch and sucrose metabolism (1) 00520 Amino sugar and nucleotide sugar metabolism (2) 00620 Pyruvate metabolism (2) 00630 Glyoxylate and dicarboxylate metabolism (3) 00640 Propanoate metabolism (1)	Metabolism Global and overview maps 01100 Metabolic pathways (50) 01110 Biosynthesis of secondary metabolites (23) 01120 Microbial metabolism in diverse environments (15) 01130 Biosynthesis of antibiotics (19) 01200 Carbon metabolism (13) 01210 2-Oxocarboxylic acid metabolism (2) 01212 Fatty acid metabolism (4) 01230 Biosynthesis of amino acids (6) Carbohydrate metabolism 00010 Glycolysis / Gluconeogenesis (6) 00020 Citrate cycle (TCA cycle) (8) 00040 Pentose and glucuronate interconversions (1) 00052 Galactose metabolism (1) 00053 Ascorbate and aldarate metabolism (2) 00520 Amino sugar and nucleotide sugar metabolism (1) 00620 Pyruvate metabolism (5) 00630 Glyoxylate and dicarboxylate metabolism (4) 00640 Propanoate metabolism (4) 00650 Butanoate metabolism (2) 00562 Inositol phosphate metabolism (1)

Energy metabolism 00680 Methane metabolism (1) Lipid metabolism 00561 Glycerolipid metabolism (1) Metabolism of cofactors and vitamins 00760 Nicotinate and nicotinamide metabolism (1) Xenobiotics biodegradation and metabolism 00930 Caprolactam degradation (1) Genetic Information Processing Translation 00970 Aminoacyl-tRNA biosynthesis (1) Folding, sorting and degradation 03018 RNA degradation (2) Environmental Information Processing Membrane transport 02010 ABC transporters (1) Signal transduction 04010 MAPK signaling pathway (1) 04068 FoxO signaling pathway (1) 04024 cAMP signaling pathway (1) 04152 AMPK signaling pathway (2) Signaling molecules and interaction 04512 ECM-receptor interaction (1) Cellular Processes Transport and catabolism 04145 Phagosome (1) Cellular community - eukaryotes	Genetic Information Processing Transcription 03020 RNA polymerase (1) Translation 03010 Ribosome (1) Folding, sorting and degradation 04141 Protein processing in endoplasmic reticulum (1) Environmental Information Processing Signal transduction 04151 PI3K-Akt signaling pathway (1) Signaling molecules and interaction 04512 ECM-receptor interaction (1) 04514 Cell adhesion molecules (CAMs) (1) Cellular Processes Transport and catabolism 04144 Endocytosis (1) Cellular community - eukaryotes 04510 Focal adhesion (1) Organismal Systems Immune system 04611 Platelet activation (1) Endocrine system 04922 Glucagon signaling pathway (1) 04926 Relaxin signaling pathway (1) Digestive system 04974 Protein digestion and absorption (1) Excretory system 04962 Vasopressin-regulated water	00562 Inositol phosphate metabolism (1) Energy metabolism 00190 Oxidative phosphorylation (1) 00710 Carbon fixation in photosynthetic organisms (1) 00720 Carbon fixation pathways in prokaryotes (1) 00680 Methane metabolism (4) Lipid metabolism 00071 Fatty acid degradation (1) 00073 Cutin, suberine and wax biosynthesis (1) 00561 Glycerolipid metabolism (2) 00564 Glycerophospholipid metabolism (2) 00565 Ether lipid metabolism (1) 00600 Sphingolipid metabolism (1) 00590 Arachidonic acid metabolism (1) Nucleotide metabolism 00230 Purine metabolism (5) 00240 Pyrimidine metabolism (2) Amino acid metabolism 00250 Alanine, aspartate and glutamate metabolism (1) 00270 Cysteine and methionine metabolism (3) 00280 Valine, leucine and isoleucine degradation (1) 00290 Valine, leucine and isoleucine biosynthesis (1) 00330 Arginine and proline metabolism (1) 00350 Tyrosine metabolism (2) Metabolism of other amino acids 00480 Glutathione metabolism (3) Glycan biosynthesis	Energy metabolism 00190 Oxidative phosphorylation (9) 00710 Carbon fixation in photosynthetic organisms (1) 00720 Carbon fixation pathways in prokaryotes (2) 00680 Methane metabolism (1) Lipid metabolism 00062 Fatty acid elongation (1) 00071 Fatty acid degradation (5) 00072 Synthesis and degradation of ketone bodies (2) 00100 Steroid biosynthesis (2) 00120 Primary bile acid biosynthesis (3) 00140 Steroid hormone biosynthesis (1) 00561 Glycerolipid metabolism (1) 00564 Glycerophospholipid metabolism (2) 00565 Ether lipid metabolism (1) 00600 Sphingolipid metabolism (1) 00590 Arachidonic acid metabolism (2) 00592 alpha-Linolenic acid metabolism (1) 01040 Biosynthesis of unsaturated fatty acids (3) Nucleotide metabolism 00230 Purine metabolism (4) 00240 Pyrimidine metabolism (2) Amino acid metabolism 00260 Glycine, serine and threonine metabolism (3) 00280 Valine, leucine and isoleucine degradation (6) 00310 Lysine degradation (6)
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04510 Focal adhesion (1) Cell motility 04810 Regulation of actin cytoskeleton (2) Organismal Systems Immune system 04640 Hematopoietic cell lineage (1) Endocrine system 04922 Glucagon signaling pathway (1) 04920 Adipocytokine signaling pathway (1) 03320 PPAR signaling pathway (1) Digestive system 04976 Bile secretion (1) 04975 Fat digestion and absorption (1) 04979 Cholesterol metabolism (1) Human Diseases Cancers: Overview 05230 Central carbon metabolism in cancer (1) Endocrine and metabolic diseases 04931 Insulin resistance (1) Infectious diseases: Bacterial 05131 Shigellosis (1) 05100 Bacterial invasion of epithelial cells (1) Infectious diseases: Parasitic 05144 Malaria (1) Drug resistance: Antineoplastic 01523 Antifolate resistance (1)	reabsorption (1) Environmental adaptation 04714 Thermogenesis (1) Human Diseases Cancers: Overview 05200 Pathways in cancer (1) 05230 Central carbon metabolism in cancer (1) 05231 Choline metabolism in cancer (1) 05203 Viral carcinogenesis (1) Cancers: Specific types 05222 Small cell lung cancer (1) Neurodegenerative diseases 05020 Prion diseases (1) Endocrine and metabolic diseases 04930 Type II diabetes mellitus (1) 04933 AGE-RAGE signaling pathway in diabetic complications (1) Infectious diseases: Viral 05165 Human papillomavirus infection (2) Infectious diseases: Parasitic 05146 Amoebiasis (2) 05145 Toxoplasmosis (1)	and metabolism 00531 Glycosaminoglycan degradation (1) 00511 Other glycan degradation (1) Metabolism of cofactors and vitamins 00740 Riboflavin metabolism (1) 00760 Nicotinate and nicotinamide metabolism (1) 00770 Pantothenate and CoA biosynthesis (1) 00830 Retinol metabolism (1) 00860 Porphyrin and chlorophyll metabolism (1) Xenobiotics biodegradation and metabolism 00625 Chloroalkane and chloroalkene degradation (1) 00643 Styrene degradation (1) 00930 Caprolactam degradation (1) 00626 Naphthalene degradation (1) 00980 Metabolism of xenobiotics by cytochrome P450 (2) 00982 Drug metabolism - cytochrome P450 (2) 00983 Drug metabolism - other enzymes (1) Genetic Information Processing Transcription 03040 Spliceosome (2) Translation 03010 Ribosome (1) 00970 Aminoacyl-tRNA biosynthesis (2) 03013 RNA transport (6) 03015 mRNA surveillance pathway (3) 03008 Ribosome	00330 Arginine and proline metabolism (3) 00340 Histidine metabolism (1) 00380 Tryptophan metabolism (3) Metabolism of other amino acids 00410 beta-Alanine metabolism (1) 00440 Phosphonate and phosphinate metabolism (1) 00450 Selenocompound metabolism (2) 00460 Cyanoamino acid metabolism (1) 00480 Glutathione metabolism (3) Glycan biosynthesis and metabolism 00510 N-Glycan biosynthesis (2) 00513 Various types of N-glycan biosynthesis (2) 00515 Mannose type O-glycan biosynthesis (1) 00514 Other types of O-glycan biosynthesis (4) 00532 Glycosaminoglycan biosynthesis - chondroitin sulfate / dermatan sulfate (1) 00534 Glycosaminoglycan biosynthesis - heparan sulfate / heparin (1) 00533 Glycosaminoglycan biosynthesis - keratan sulfate (1) 00601 Glycosphingolipid biosynthesis - lacto and neolacto series (1) Metabolism of cofactors and vitamins 00670 One carbon pool by folate (3) 00830 Retinol metabolism (1) 00860 Porphyrin and chlorophyll metabolism (1)
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	biogenesis in eukaryotes (1) Folding, sorting and degradation 03060 Protein export (2) 04141 Protein processing in endoplasmic reticulum (1) 04130 SNARE interactions in vesicular transport (1) 04120 Ubiquitin mediated proteolysis (4) 03050 Proteasome (1) 03018 RNA degradation (2) Replication and repair 03030 DNA replication (1) Environmental Information Processing Membrane transport 02010 ABC transporters (1) Signal transduction 04014 Ras signaling pathway (2) 04015 Rap1 signaling pathway (2) 04010 MAPK signaling pathway (4) 04013 MAPK signaling pathway - fly (1) 04011 MAPK signaling pathway - yeast (1) 04012 ErbB signaling pathway (2) 04310 Wnt signaling pathway (2) 04330 Notch signaling pathway (1) 04350 TGF-beta signaling pathway (1) 04390 Hippo signaling pathway (2) 04391 Hippo signaling pathway - fly (1) 04370 VEGF signaling pathway (1) 04371 Apelin signaling pathway (1)	00130 Ubiquinone and other terpenoid-quinone biosynthesis (1) Metabolism of terpenoids and polyketides 00900 Terpenoid backbone biosynthesis (2) 00981 Insect hormone biosynthesis (1) 00903 Limonene and pinene degradation (1) 00281 Geraniol degradation (1) Xenobiotics biodegradation and metabolism 00362 Benzoate degradation (1) 00625 Chloroalkane and chloroalkene degradation (1) 00980 Metabolism of xenobiotics by cytochrome P450 (1) 00982 Drug metabolism - cytochrome P450 (2) 00983 Drug metabolism - other enzymes (1) Genetic Information Processing Transcription 03020 RNA polymerase (2) 03022 Basal transcription factors (2) 03040 Spliceosome (5) Translation 03010 Ribosome (8) 03013 RNA transport (6) 03015 mRNA surveillance pathway (1) 03008 Ribosome biogenesis in eukaryotes (5) Folding, sorting and degradation 03060 Protein export (1) 04141 Protein processing in endoplasmic
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		04630 JAK-STAT signaling pathway (2) 04064 NF-kappa B signaling pathway (1) 04668 TNF signaling pathway (1) 04066 HIF-1 signaling pathway (1) 04068 FoxO signaling pathway (2) 04020 Calcium signaling pathway (1) 04070 Phosphatidylinositol signaling system (1) 04072 Phospholipase D signaling pathway (2) 04071 Sphingolipid signaling pathway (2) 04024 cAMP signaling pathway (3) 04022 cGMP-PKG signaling pathway (4) 04151 PI3K-Akt signaling pathway (3) 04152 AMPK signaling pathway (5) Signaling molecules and interaction 04060 Cytokine-cytokine receptor interaction (1) 04512 ECM-receptor interaction (1) 04514 Cell adhesion molecules (CAMs) (1) Cellular Processes Transport and catabolism 04144 Endocytosis (5) 04145 Phagosome (3) 04142 Lysosome (4) 04146 Peroxisome (2) 04140 Autophagy - animal (2) 04138 Autophagy - yeast (2) 04136 Autophagy - other (1) Cell growth and death 04111 Cell cycle -	reticulum (5) 04120 Ubiquitin mediated proteolysis (2) 03018 RNA degradation (1) Replication and repair 03030 DNA replication (2) 03410 Base excision repair (2) 03420 Nucleotide excision repair (3) 03430 Mismatch repair (1) 03440 Homologous recombination (1) Environmental Information Processing Membrane transport 02010 ABC transporters (1) Signal transduction 02020 Two-component system (1) 04014 Ras signaling pathway (2) 04015 Rap1 signaling pathway (2) 04010 MAPK signaling pathway (3) 04013 MAPK signaling pathway - fly (2) 04012 ErbB signaling pathway (1) 04330 Notch signaling pathway (1) 04350 TGF-beta signaling pathway (2) 04390 Hippo signaling pathway (3) 04391 Hippo signaling pathway - fly (4) 04392 Hippo signaling pathway - multiple species (2) 04370 VEGF signaling pathway (1) 04371 Apelin signaling pathway (1) 04668 TNF signaling pathway (2) 04066 HIF-1 signaling pathway (2) 04068 FoxO
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	yeast (1) 04113 Meiosis - yeast (3) 04114 Oocyte meiosis (4) 04210 Apoptosis (2) 04214 Apoptosis - fly (1) 04217 Necroptosis (6) 04218 Cellular senescence (2) Cellular community - eukaryotes 04510 Focal adhesion (4) 04520 Adherens junction (2) 04530 Tight junction (4) 04540 Gap junction (1) Cellular community - prokaryotes 02026 Biofilm formation - Escherichia coli (1) Cell motility 04810 Regulation of actin cytoskeleton (6) Organismal Systems Immune system 04640 Hematopoietic cell lineage (1) 04611 Platelet activation (2) 04621 NOD-like receptor signaling pathway (1) 04625 C-type lectin receptor signaling pathway (2) 04650 Natural killer cell mediated cytotoxicity (1) 04612 Antigen processing and presentation (1) 04660 T cell receptor signaling pathway (1) 04658 Th1 and Th2 cell differentiation (3) 04659 Th17 cell differentiation (3)	signaling pathway (1) 04020 Calcium signaling pathway (2) 04070 Phosphatidylinositol signaling system (1) 04071 Sphingolipid signaling pathway (1) 04022 cGMP-PKG signaling pathway (2) 04151 PI3K-Akt signaling pathway (5) 04152 AMPK signaling pathway (3) 04150 mTOR signaling pathway (1) Signaling molecules and interaction 04060 Cytokine- cytokine receptor interaction (1) 04512 ECM-receptor interaction (2) 04514 Cell adhesion molecules (CAMs) (2) Cellular Processes Transport and catabolism 04144 Endocytosis (6) 04145 Phagosome (1) 04142 Lysosome (2) 04146 Peroxisome (10) 04140 Autophagy - animal (4) 04138 Autophagy - yeast (3) 04136 Autophagy - other (2) 04137 Mitophagy - animal (1) Cell growth and death 04110 Cell cycle (5) 04111 Cell cycle - yeast (2) 04113 Meiosis - yeast (1) 04114 Oocyte meiosis (2) 04210 Apoptosis (4) 04214 Apoptosis - fly (5)
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		04657 IL-17 signaling pathway (1) 04662 B cell receptor signaling pathway (1) 04666 Fc gamma R-mediated phagocytosis (4) 04670 Leukocyte transendothelial migration (2) 04062 Chemokine signaling pathway (2) Endocrine system 04911 Insulin secretion (1) 04910 Insulin signaling pathway (3) 04922 Glucagon signaling pathway (4) 04923 Regulation of lipolysis in adipocytes (1) 04920 Adipocytokine signaling pathway (2) 03320 PPAR signaling pathway (2) 04917 Prolactin signaling pathway (2) 04921 Oxytocin signaling pathway (3) 04918 Thyroid hormone synthesis (1) 04919 Thyroid hormone signaling pathway (1) 04924 Renin secretion (1) 04925 Aldosterone synthesis and secretion (1) Circulatory system 04260 Cardiac muscle contraction (1) 04261 Adrenergic signaling in cardiomyocytes (4) 04270 Vascular smooth muscle contraction (1) Digestive system 04970 Salivary secretion (1) 04971 Gastric acid secretion (1) 04972 Pancreatic secretion (1) 04976 Bile secretion (2)	04215 Apoptosis - multiple species (1) 04216 Ferroptosis (1) 04217 Necroptosis (2) 04115 p53 signaling pathway (1) 04218 Cellular senescence (2) Cellular community - eukaryotes 04510 Focal adhesion (6) 04530 Tight junction (2) 04540 Gap junction (1) Cell motility 04810 Regulation of actin cytoskeleton (2) Organismal Systems Immune system 04610 Complement and coagulation cascades (1) 04611 Platelet activation (2) 04621 NOD-like receptor signaling pathway (2) 04622 RIG-I-like receptor signaling pathway (1) 04623 Cytosolic DNA-sensing pathway (2) 04625 C-type lectin receptor signaling pathway (2) 04660 T cell receptor signaling pathway (1) 04666 Fc gamma R-mediated phagocytosis (1) 04670 Leukocyte transendothelial migration (1) 04062 Chemokine signaling pathway (2) Endocrine system 04922 Glucagon signaling pathway (3) 03320 PPAR signaling pathway (3) 04912 GnRH signaling pathway (3)
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		04973 Carbohydrate digestion and absorption (1) 04974 Protein digestion and absorption (1) 04975 Fat digestion and absorption (2) 04979 Cholesterol metabolism (1) 04978 Mineral absorption (2) Excretory system 04962 Vasopressin-regulated water reabsorption (2) 04960 Aldosterone-regulated sodium reabsorption (1) 04961 Endocrine and other factor-regulated calcium reabsorption (1) 04964 Proximal tubule bicarbonate reclamation (2) Nervous system 04724 Glutamatergic synapse (2) 04728 Dopaminergic synapse (4) 04720 Long-term potentiation (2) 04730 Long-term depression (1) 04721 Synaptic vesicle cycle (1) 04722 Neurotrophin signaling pathway (2) Sensory system 04750 Inflammatory mediator regulation of TRP channels (1) Development 04360 Axon guidance (2) 04380 Osteoclast differentiation (2) Aging 04211 Longevity regulating pathway (1) 04212 Longevity regulating pathway - worm (2) 04213 Longevity regulating pathway - multiple species (1)	04915 Estrogen signaling pathway (2) 04914 Progesterone-mediated oocyte maturation (1) 04921 Oxytocin signaling pathway (1) 04926 Relaxin signaling pathway (2) 04918 Thyroid hormone synthesis (3) 04919 Thyroid hormone signaling pathway (1) 04928 Parathyroid hormone synthesis, secretion and action (2) 04924 Renin secretion (1) 04925 Aldosterone synthesis and secretion (1) 04927 Cortisol synthesis and secretion (1) Circulatory system 04260 Cardiac muscle contraction (3) 04270 Vascular smooth muscle contraction (2) Digestive system 04970 Salivary secretion (1) 04971 Gastric acid secretion (1) 04972 Pancreatic secretion (1) 04976 Bile secretion (1) 04974 Protein digestion and absorption (1) 04979 Cholesterol metabolism (2) Excretory system 04962 Vasopressin-regulated water reabsorption (2) 04961 Endocrine and other factor-regulated calcium reabsorption (1) Nervous system 04724 Glutamatergic synapse (2) 04725 Cholinergic
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		Environmental adaptation 04710 Circadian rhythm (1) 04714 Thermogenesis (2) Human Diseases Cancers: Overview 05200 Pathways in cancer (5) 05230 Central carbon metabolism in cancer (1) 05231 Choline metabolism in cancer (2) 05205 Proteoglycans in cancer (2) 05204 Chemical carcinogenesis (2) 05203 Viral carcinogenesis (4) Cancers: Specific types 05225 Hepatocellular carcinoma (1) 05221 Acute myeloid leukemia (2) 05220 Chronic myeloid leukemia (2) 05211 Renal cell carcinoma (1) 05224 Breast cancer (1) 05223 Non-small cell lung cancer (2) Immune diseases 05322 Systemic lupus erythematosus (1) Neurodegenerative diseases 05010 Alzheimer disease (3) 05012 Parkinson disease (2) 05014 Amyotrophic lateral sclerosis (ALS) (1) 05016 Huntington disease (1) Substance dependence 05031 Amphetamine addiction (2) 05034 Alcoholism (2) Cardiovascular diseases 05418 Fluid shear stress and atherosclerosis	synapse (1) 04728 Dopaminergic synapse (1) 04726 Serotonergic synapse (1) 04720 Long-term potentiation (1) 04730 Long-term depression (1) 04723 Retrograde endocannabinoid signaling (6) 04721 Synaptic vesicle cycle (1) 04722 Neurotrophin signaling pathway (1) Sensory system 04745 Phototransduction - fly (1) 04750 Inflammatory mediator regulation of TRP channels (2) Development 04360 Axon guidance (1) Aging 04212 Longevity regulating pathway - worm (4) 04213 Longevity regulating pathway - multiple species (2) Environmental adaptation 04713 Circadian entrainment (1) 04714 Thermogenesis (10) Human Diseases Cancers: Overview 05200 Pathways in cancer (6) 05230 Central carbon metabolism in cancer (1) 05231 Choline metabolism in cancer (1) 05202 Transcriptional misregulation in cancer (2) 05206 MicroRNAs in cancer (3) 05205 Proteoglycans in cancer (5) 05204 Chemical carcinogenesis (1)
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		(1) 05410 Hypertrophic cardiomyopathy (HCM) (1) Endocrine and metabolic diseases 04932 Non-alcoholic fatty liver disease (NAFLD) (1) 04931 Insulin resistance (4) 04933 AGE-RAGE signaling pathway in diabetic complications (2) Infectious diseases: Bacterial 05130 Pathogenic Escherichia coli infection (1) 05132 Salmonella infection (2) 05131 Shigellosis (2) 05134 Legionellosis (2) 05152 Tuberculosis (1) 05100 Bacterial invasion of epithelial cells (2) Infectious diseases: Viral 05166 Human T-cell leukemia virus 1 infection (5) 05170 Human immunodeficiency virus 1 infection (3) 05162 Measles (3) 05161 Hepatitis B (2) 05160 Hepatitis C (1) 05168 Herpes simplex infection (1) 05163 Human cytomegalovirus infection (1) 05167 Kaposi sarcoma-associated herpesvirus infection (2) 05169 Epstein-Barr virus infection (1) 05165 Human papillomavirus infection (3) Infectious diseases: Parasitic 05146 Amoebiasis (2) 05144 Malaria (2) 05142 Chagas	05203 Viral carcinogenesis (6) Cancers: Specific types 05212 Pancreatic cancer (1) 05225 Hepatocellular carcinoma (1) 05226 Gastric cancer (1) 05214 Glioma (1) 05221 Acute myeloid leukemia (1) 05220 Chronic myeloid leukemia (2) 05218 Melanoma (1) 05211 Renal cell carcinoma (1) 05224 Breast cancer (1) 05222 Small cell lung cancer (3) 05223 Non-small cell lung cancer (1) Immune diseases 05322 Systemic lupus erythematosus (1) Neurodegenerative diseases 05010 Alzheimer disease (10) 05012 Parkinson disease (10) 05014 Amyotrophic lateral sclerosis (ALS) (1) 05016 Huntington disease (12) 05020 Prion diseases (1) Substance dependence 05034 Alcoholism (1) Cardiovascular diseases 05418 Fluid shear stress and atherosclerosis (1) 05410 Hypertrophic cardiomyopathy (HCM) (1) 05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC) (2) 05414 Dilated cardiomyopathy (DCM) (1) 05416 Viral myocarditis (1)
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		disease (American trypanosomiasis) (1) Drug resistance: Antimicrobial 01503 Cationic antimicrobial peptide (CAMP) resistance (1) Drug resistance: Antineoplastic 01524 Platinum drug resistance (1) 01523 Antifolate resistance (1)	Endocrine and metabolic diseases 04930 Type II diabetes mellitus (2) 04932 Non-alcoholic fatty liver disease (NAFLD) (9) 04931 Insulin resistance (1) 04933 AGE-RAGE signaling pathway in diabetic complications (3) 04934 Cushing syndrome (2) Infectious diseases: Bacterial 05133 Pertussis (1) 05134 Legionellosis (1) 05150 Staphylococcus aureus infection (1) 05100 Bacterial invasion of epithelial cells (3) Infectious diseases: Viral 05170 Human immunodeficiency virus 1 infection (4) 05162 Measles (2) 05164 Influenza A (3) 05161 Hepatitis B (2) 05160 Hepatitis C (2) 05168 Herpes simplex infection (2) 05163 Human cytomegalovirus infection (3) 05167 Kaposi sarcoma-associated herpesvirus infection (2) 05169 Epstein-Barr virus infection (3) 05165 Human papillomavirus infection (6) Infectious diseases: Parasitic 05146 Amoebiasis (3) 05145 Toxoplasmosis (3) Drug resistance: Antineoplastic 01523 Antifolate resistance (1)
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Table S17

Summary of highlighted biological processes from CRL-12444 (downregulated) and CRL-12445

(upregulated) cells

CRL-12444	CRL-12445
actin_filament_organization actin_polymerization_or_depolymerization blood_vessel_morphogenesis brain_development carboxylic_acid_metabolic_process catabolic_process cell_cycle_checkpoint cell_matrix_adhesion cell_migration cell_motility&localization_of_cell cell_substrate_adhesion cellular_catabolic_process cellular_component_assembly_involved_in_morphogenesis cellular_lipid_catabolic_process cellular_lipid_metabolic_process cellular_macromolecular_complex_assembly cellular_protein_complex_assembly cellular_response_to_nitrogen_compound cellular_response_to_oxygen_containing_compound central_nervous_system_development central_nervous_system_neuron_differentiation chromatin_organization epithelial_cell_development epithelial_cell_differentiation erythrocyte_homeostasis establishment_of_protein_localization_to_organelle establishment_of_vesicle_localization exocytosis fatty_acid_metabolic_process forebrain_development gland_development gliogenesis head_development hindbrain_development innate_immune_response intracellular_receptor_signaling_pathway lipid_catabolic_process lipid_metabolic_process macromolecular_complex_assembly macromolecular_complex_subunit_organization membrane_organization mitochondrion_organization monocarboxylic_acid_metabolic_process monovalent_inorganic_cation_transport mRNA_metabolic_process mRNA_processing	activation_of_immune_response alcohol_metabolic_process alpha_amino_acid_metabolic_process ameboidal_type_cell_migration amino_acid_transport anatomical_structure_homeostasis angiogenesis anion_transport anterior_posterior_pattern_specification antigen_processing_and_presentation appendage_development&limb_development axon_development axonogenesis behavior blood_vessel_development blood_vessel_morphogenesis bone_development branching_morphogenesis_of_an_epithelial_tube carbohydrate_derivative_biosynthetic_process carbohydrate_derivative_catabolic_process carbohydrate_derivative_metabolic_process carbohydrate_metabolic_process carboxylic_acid_metabolic_process carboxylic_acid_transport catabolic_process cation_homeostasis cation_transmembrane_transport cation_transport cell_cell_junction_organization cell_cell_signaling cell_cycle_phase_transition cell_cycle_process cell_cycle cell_growth cell_maturation cell_migration cell_morphogenesis_involved_in_differentiation cell_morphogenesis_involved_in_neuron_differentiation cell_motility&localization_of_cell cell_projection_morphogenesis cell_recognition cell_substrate_adhesion cellular_amino_acid_metabolic_process cellular_carbohydrate_metabolic_process cellular_catabolic_process cellular_component_disassembly

mRNA_splicing_via_spliceosome&RNA_splicing_via_t	cellular_lipid_catabolic_process
ransesterification_reactions_with_bulged_adenosine_a	cellular_macromolecular_complex_assembly
s_nucleophile	cellular_protein_catabolic_process
muscle_cell_differentiation	cellular_protein_complex_disassembly
muscle_organ_development	cellular_response_to_endogenous_stimulus
muscle_structure_development	cellular_response_to_external_stimulus
muscle_tissue_development	cellular_response_to_extracellular_stimulus
ncRNA_metabolic_process	cellular_response_to_growth_factor_stimulus
negative_regulation_of_cell_proliferation	cellular_response_to_hormone_stimulus
negative_regulation_of_cellular_biosynthetic_process	cellular_response_to_lipid
negative_regulation_of_cellular_component_organization	cellular_response_to_nutrient_levels
negative_regulation_of_cellular_macromolecule_biosynthetic_process	cellular_response_to_starvation
negative_regulation_of_cellular_protein_metabolic_process	cellular_response_to_stress
negative_regulation_of_immune_system_process	cerebral_cortex_development
negative_regulation_of_macromolecule_biosynthetic_process	chemical_homeostasis
negative_regulation_of_nitrogen_compound_metabolic_process	chromosome_segregation
negative_regulation_of_nucleic_acid_templated_transcription	cognition
negative_regulation_of_nucleobase_containing_compound_metabolic_process	cytokine_mediated_signaling_pathway
negative_regulation_of_protein_metabolic_process	developmental_growth_involved_in_morphogenesis
negative_regulation_of_protein_modification_process	developmental_maturation
negative_regulation_of_protein_phosphorylation	divalent_inorganic_cation_homeostasis
negative_regulation_of_RNA_biosynthetic_process	DNA_repair
negative_regulation_of_transcription_DNA_templated	endocytosis
negative_regulation_of_transcription_from_RNA_polymerase_II_promoter	endomembrane_system_organization
neurological_system_process	ensheathment_of_neurons&axon_ensheathment
neuron_development	enzyme_linked_receptor_protein_signaling_pathway
neuron_migration	epithelial_tube_formation&embryonic_epithelial_tube_formation
neuron_projection_development	epithelial_tube_morphogenesis
organelle_assembly	establishment_of_organelle_localization
organic_acid_catabolic_process&carboxylic_acid_catabolic_process	establishment_of_protein_localization_to_organelle
organic_acid_metabolic_process	establishment_of_protein_localization
organic_substance_catabolic_process	establishment_of_vesicle_localization
oxoacid_metabolic_process	establishment_or_maintenance_of_cell_polarity
pallium_development	exocytosis
peptidyl_lysine_modification	extracellular_matrix_organization
positive_regulation_of_cell_cycle_process	extracellular_structure_organization
positive_regulation_of_cell_cycle	fat_cell_differentiation
positive_regulation_of_cellular_component_biogenesis	G_protein_coupled_receptor_signaling_pathway
positive_regulation_of_cellular_component_organization	germ_cell_development
positive_regulation_of_intracellular_signal_transduction	glycoprotein_biosynthetic_process
positive_regulation_of_intracellular_transport	glycoprotein_metabolic_process
positive_regulation_of_neurogenesis	glycosylation
positive_regulation_of_secretion	Golgi_vesicle_transport
positive_regulation_of_signal_transduction	hair_follicle_development
positive_regulation_of_transport	homeostasis_of_number_of_cells
	I-kappaB_kinase/NF-kappaB_signaling
	immune_effector_process
	immune_response_activating_signal_transduction
	immune_response_regulating_signaling_pathway
	inflammatory_response
	innate_immune_response
	inorganic_ion_homeostasis
	intracellular_transport
	ion_homeostasis
	ion_transmembrane_transport

protein_complex_biogenesis&protein_complex_assembly	ion_transport
protein_complex_subunit_organization	JNK_cascade
protein_modification_by_small_protein_conjugation_or_removal	learning_or_memory
protein_modification_by_small_protein_conjugation	learning
protein_polymerization	leukocyte_apoptotic_process
protein_processing	leukocyte_homeostasis
protein_targeting	leukocyte_mediated_immunity
proteolysis_involved_in_cellular_protein_catabolic_process	leukocyte_proliferation
regulation_of_actin_cytoskeleton_organization	lipid_localization
regulation_of_actin_filament_length	lipid_metabolic_process
regulation_of_actin_polymerization_or_depolymerization	lipid_transport
regulation_of_cell_migration	locomotory_behavior
regulation_of_cell_motility	lymphocyte_mediated_immunity
regulation_of_cellular_component_biogenesis	macromolecular_complex_disassembly
regulation_of_cellular_component_movement	maintenance_of_location
regulation_of_cellular_component_size	MAPK_cascade
regulation_of_cellular_protein_catabolic_process	meiotic_cell_cycle_process
regulation_of_exocytosis	meiotic_cell_cycle
regulation_of_locomotion	meiotic_nuclear_division
regulation_of_microtubule_based_process	membrane_organization
regulation_of_nervous_system_development	metal_ion_homeostasis
regulation_of_protein_catabolic_process	mitotic_cell_cycle_phase_transition
regulation_of_proteolysis_involved_in_cellular_protein_catabolic_process	mitotic_cell_cycle_process
regulation_of_proteolysis	mitotic_cell_cycle
regulation_of_secretion	mitotic_nuclear_division
regulation_of_transport	molting_cycle_process&hair_cycle_process
regulation_of_vesicle_mediated_transport	mononuclear_cell_proliferation&lymphocyte_proliferation
response_to_endoplasmic_reticulum_stress	monosaccharide_metabolic_process
response_to_organonitrogen_compound	morphogenesis_of_a_branching_epithelium
response_to_organonitrogen_compound	morphogenesis_of_a_branching_structure
response_to_oxygen_containing_compound	multi_multicellular_organism_process
response_to_peptide_hormone	multi_organism_reproductive_process
response_to_peptide	multicellular_organism_reproduction
response_to_wounding	multicellular_organismal_homeostasis
ribosome_biogenesis	multicellular_organismal_reproductive_process
RNA_processing	muscle_cell_differentiation
RNA_splicing_via_transesterification_reactions	myelination
RNA_splicing	myeloid_cell_differentiation
secretion_by_cell	myeloid_cell_homeostasis
secretion	myeloid_leukocyte_differentiation
sensory_perception	myotube_differentiation
single_organism_catabolic_process	negative_regulation_of_cell_communication
single_organism_cellular_localization	negative_regulation_of_cell_death
single_organism_membrane_organization	negative_regulation_of_homeostatic_process
skeletal_muscle_organ_development	negative_regulation_of_immune_system_process
skeletal_muscle_tissue_development	negative_regulation_of_locomotion
small_molecule_catabolic_process	negative_regulation_of_neuron_death
striated_muscle_cell_development	negative_regulation_of_programmed_cell_death
striated_muscle_cell_differentiation	negative_regulation_of_protein_processing
striated_muscle_tissue_development	negative_regulation_of_proteolysis
telencephalon_development	negative_regulation_of_response_to_stimulus
	negative_regulation_of_transport
	neural_precursor_cell_proliferation
	neural_tube_closure
	neurological_system_process

	neuromuscular_process_controlling_balance neuromuscular_process neuron_apoptotic_process neuron_death neuron_development neuron_neuron_synaptic_transmission neuron_projection_development neuron_projection_morphogenesis neurotransmitter_transport nitrogen_compound_transport nuclear_division nucleobase_containing_small_molecule_metabolic_process nucleoside_phosphate_metabolic_process nucleotide_metabolic_process organelle_fission organelle_localization organic_acid_metabolic_process organic_acid_transport organic_anion_transport organic_hydroxy_compound_metabolic_process organic_hydroxy_compound_transport organic_substance_catabolic_process organonitrogen_compound_catabolic_process organonitrogen_compound_metabolic_process organophosphate_biosynthetic_process oxidation_reduction_process oxoacid_metabolic_process pallium_development pattern_specification_process peptidyl_tyrosine_modification peptidyl_tyrosine_phosphorylation phagocytosis plasma_membrane_organization positive_regulation_of_catabolic_process positive_regulation_of_catalytic_activity positive_regulation_of_cell_activation positive_regulation_of_cell_development positive_regulation_of_cell_differentiation positive_regulation_of_cell_migration positive_regulation_of_cell_morphogenesis_involved_in_differentiation positive_regulation_of_cell_motility positive_regulation_of_cell_projection_organization positive_regulation_of_cell_proliferation positive_regulation_of_cellular_catabolic_process positive_regulation_of_cellular_component_movement positive_regulation_of_cellular_component_organization positive_regulation_of_cellular_protein_metabolic_process positive_regulation_of_cytokine_production positive_regulation_of_defense_response positive_regulation_of_GTPase_activity positive_regulation_of_homeostatic_process positive_regulation_of_immune_response
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	positive_regulation_of_immune_system_process positive_regulation_of_kinase_activity positive_regulation_of_leukocyte_activation positive_regulation_of_locomotion positive_regulation_of_lymphocyte_activation positive_regulation_of_MAP_kinase_activity positive_regulation_of_MAPK_cascade positive_regulation_of_mitotic_cell_cycle positive_regulation_of_molecular_function positive_regulation_of_nervous_system_development positive_regulation_of_neurogenesis positive_regulation_of_neuron_differentiation positive_regulation_of_neuron_projection_developmen t positive_regulation_of_phosphorus_metabolic_proces s&positive_regulation_of_phosphate_metabolic_proce ss positive_regulation_of_phosphorylation positive_regulation_of_protein_kinase_activity positive_regulation_of_protein_metabolic_process positive_regulation_of_protein_modification_process positive_regulation_of_protein_phosphorylation positive_regulation_of_protein_serine_threonine_kinas e_activity positive_regulation_of_protein_transport positive_regulation_of_response_to_external_stimulus positive_regulation_of_transferase_activity positive_regulation_of_transport primary_neural_tube_formation protein_complex_disassembly protein_depolymerization protein_glycosylation&macromolecule_glycosylation protein_import_into_nucleus&protein_targeting_to_nuc leus&single_organism_nuclear_import protein_import protein_localization_to_plasma_membrane protein_processing proteolysis_involved_in_cellular_protein_catabolic_pro cess proteolysis purine_nucleotide_metabolic_process purine_ribonucleotide_metabolic_process Rab_protein_signal_transduction reactive_oxygen_species_metabolic_process receptor_mediated_endocytosis regionalization regulation_of_anatomical_structure_morphogenesis regulation_of_anatomical_structure_size regulation_of_catabolic_process regulation_of_catalytic_activity regulation_of_cell_cycle_process regulation_of_cell_migration regulation_of_cell_morphogenesis_involved_in_differ entiation regulation_of_cell_morphogenesis regulation_of_cell_motility
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	regulation_of_cellular_catabolic_process regulation_of_cellular_component_movement regulation_of_cellular_localization regulation_of_cellular_protein_catabolic_process regulation_of_cellular_protein_localization regulation_of_defense_response regulation_of_developmental_growth regulation_of_DNA_metabolic_process regulation_of_endocytosis regulation_of_establishment_of_protein_localization regulation_of_growth regulation_of_GTPase_activity&Ran_protein_signal_transduction&Arf_protein_signal_transduction&regulation_of_Arf_protein_signal_transduction&regulation_of_Ran_protein_signal_transduction&regulation_of_Rab_protein_signal_transduction&Ral_protein_signal_transduction&regulation_of_Ral_protein_signal_transduction&regulation_of_Rap_protein_signal_transduction&Cdc42_protein_signal_transduction&regulation_of_Cdc42_protein_signal_transduction regulation_of_homeostatic_process regulation_of_I-kappaB_kinase/NF-kappaB_signaling regulation_of_immune_effector_process regulation_of_immune_response regulation_of_immune_system_process regulation_of_inflammatory_response regulation_of_intracellular_protein_transport regulation_of_intracellular_signal_transduction regulation_of_ion_transmembrane_transport regulation_of_kinase_activity regulation_of_leukocyte_differentiation regulation_of_leukocyte_proliferation regulation_of_locomotion regulation_of_lymphocyte_activation regulation_of_MAP_kinase_activity regulation_of_MAPK_cascade regulation_of_mitotic_cell_cycle regulation_of_mononuclear_cell_proliferation&regulation_of_lymphocyte_proliferation regulation_of_nervous_system_development regulation_of_neuron_projection_development regulation_of_neurotransmitter_levels regulation_of_nucleocytoplasmic_transport regulation_of_peptidyl_tyrosine_phosphorylation regulation_of_phosphate_metabolic_process regulation_of_phosphorus_metabolic_process regulation_of_phosphorylation regulation_of_protein_kinase_activity regulation_of_protein_localization regulation_of_protein_processing regulation_of_protein_serine_threonine_kinase_activity regulation_of_protein_transport regulation_of_Rac_protein_signal_transduction regulation_of_response_to_external_stimulus regulation_of_response_to_stress
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	regulation_of_response_to_wounding regulation_of_stress_activated_protein_kinase_signaling_cascade&regulation_of_stress_activated_MAPK_cascade regulation_of_synaptic_plasticity regulation_of_synaptic_transmission regulation_of_transferase_activity regulation_of_transmembrane_transport regulation_of_transmembrane_transporter_activity regulation_of_transport regulation_of_transporter_activity regulation_of_vesicle_mediated_transport reproduction response_to_abiotic_stimulus response_to_bacterium response_to_biotic_stimulus response_to_endogenous_stimulus response_to_extracellular_stimulus response_to_growth_factor response_to_hormone response_to_inorganic_substance response_to_light_stimulus response_to_lipid response_to_lipopolysaccharide response_to_metal_ion response_to_molecule_of_bacterial_origin response_to_nitrogen_compound response_to_nutrient_levels response_to_organic_cyclic_compound response_to_organonitrogen_compound response_to_other_organism&response_to_external_biotic_stimulus response_to_oxygen_containing_compound response_to_reactive_oxygen_species response_to_starvation response_to_transforming_growth_factor_beta&cellular_response_to_transforming_growth_factor_beta_stimulus response_to_wounding ribose_phosphate_metabolic_process secretion sensory_organ_morphogenesis signal_transduction_by_phosphorylation single_organism_behavior single_organism_biosynthetic_process single_organism_carbohydrate_metabolic_process single_organism_cellular_localization single_organism_membrane_organization skeletal_system_development skeletal_system_morphogenesis skin_development skin_epidermis_development small_GTPase_mediated_signal_transduction small_molecule_catabolic_process small_molecule_metabolic_process steroid_metabolic_process
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	stress_activated_MAPK_cascade stress_activated_protein_kinase_signaling_cascade synaptic_transmission synaptic_vesicle_localization synaptic_vesicle_transport&establishment_of_synaptic_vesicle_localization tissue_homeostasis tissue_remodeling translational_initiation transmembrane_receptor_protein_serine_threonine_kinase_signaling_pathway transmembrane_receptor_protein_tyrosine_kinase_signal transmembrane_receptor_protein_tyrosine_kinase_signaling_pathway transmembrane_transport tube_closure tube_development tube_morphogenesis vacuole_organization vasculature_development vesicle_localization vesicle_mediated_transport
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Table S18

Summary of highlighted cellular components from CRL-12444 (downregulated) and CRL-12445 (upregulated) cells

CRL-12444	CRL-12445
adherens_junction anchoring_junction basement_membrane bounding_membrane_of_organelle cell_cell_junction cell_junction cell_leading_edge chromatin chromosomal_part chromosomal_region chromosome contractile_fiber_part contractile_fiber cortical_cytoskeleton early_endosome endoplasmic_reticulum_membrane endoplasmic_reticulum_part endoplasmic_reticulum endosome envelope extracellular_matrix_component extracellular_matrix extracellular_region_part intracellular_organelle_lumen lamellipodium membrane_enclosed_lumen microbody&peroxisome microtubule microtubule_cytoskeleton mitochondrial_envelope mitochondrial_inner_membrane mitochondrial_membrane mitochondrial_part myelin_sheath myofibril nuclear_body nuclear_chromatin nuclear_chromosome_part nuclear_chromosome nuclear_lumen nuclear_outer_membrane_endoplasmic_reticulum_me mbrane_network nuclear_part nucleolus nucleoplasm_part nucleoplasm organelle_envelope	actin_cytoskeleton actomyosin adherens_junction anchoring_junction apical_part_of_cell axon basolateral_plasma_membrane cell_body cell_cortex_part cell_cortex cell_projection_membrane cell_substrate_adherens_junction cell_substrate_junction cell_surface chromatin chromosomal_part chromosome ciliary_part coated_vesicle condensed_chromosome contractile_fiber_part contractile_fiber cortical_cytoskeleton cytoplasmic_membrane_bounded_vesicle cytoplasmic_ribonucleoprotein_granule cytoplasmic_vesicle_part cytoplasmic_vesicle cytoskeletal_part cytoskeleton cytosol dendrite early_endosome endosome external_side_of_plasma_membrane extracellular_region_part extracellular_region extracellular_space extrinsic_component_of_membrane Golgi_apparatus integral_component_of_membrane integral_component_of_plasma_membrane intrinsic_component_of_membrane intrinsic_component_of_plasma_membrane late_endosome lytic_vacuole(6)&lysosome membrane_bounded_vesicle membrane_raft

organelle_inner_membrane	membrane_region
organelle_lumen	microtubule_cytoskeleton
organelle_membrane	myofibril
plasma_membrane_region	neuron_part
postsynaptic_density	neuron_projection
proteasome_accessory_complex	neuronal_cell_body
proteasome_complex	nuclear_chromosome_part
proteinaceous_extracellular_matrix	nuclear_chromosome
ribonucleoprotein_complex	nuclear_envelope
sarcomere	nuclear_periphery
secretory_granule	perinuclear_region_of_cytoplasm
synapse_part	plasma_membrane_region
synapse	proteasome_accessory_complex
transcription_factor_complex	proteasome_complex
Z_disc	proteasome_core_complex
	proteinaceous_extracellular_matrix
	ribonucleoprotein_granule
	sarcolemma
	secretory_granule
	side_of_membrane
	somatodendritic_compartment
	sperm_part
	vacuole
	vesicle_membrane
	vesicle

Table S19

Summary of highlighted molecular functions from CRL-12444 (downregulated) and CRL-12445 (upregulated) cells

CRL-12444	CRL-12445
anion_binding cation_transmembrane_transporter_activity core_promoter_binding cytoskeletal_protein_binding GTPase_regulator_activity identical_protein_binding inorganic_cation_transmembrane_transporter_activity microtubule_binding nucleic_acid_binding_transcription_factor_activity&sequence_specific_DNA_binding_transcription_factor_activity nucleoside_triphosphatase_regulator_activity Poly_A_RNA_binding&mRNA_binding protein_binding_transcription_factor_activity protein_dimerization_activity protein_heterodimerization_activity protein_homodimerization_activity regulatory_region_nucleic_acid_binding®ulatory_region_DNA_binding&transcription_regulatory_region_DNA_binding RNA_binding sequence_specific_DNA_binding_RNA_polymerase_II_transcription_factor_activity sequence_specific_DNA_binding structural_molecule_activity transcription_coactivator_activity transcription_cofactor_activity transcription_factor_binding_transcription_factor_activity transcription_factor_binding transcription_regulatory_region_sequence_specific_DNA_binding tubulin_binding	actin_binding anion_binding anion_transmembrane_transporter_activity carbohydrate_derivative_binding cation_transmembrane_transporter_activity chromatin_binding cytoskeletal_protein_binding endopeptidase_activity GTP_binding GTPase_activity guanyl_nucleotide_binding guanyl_ribonucleotide_binding hydrolase_activity ion_binding ion_transmembrane_transporter_activity lipid_binding molecular_transducer_activity nucleoside_binding&purine_nucleoside_binding&ribonucleoside_binding&purine_ribonucleoside_binding nucleoside_triphosphatase_activity nucleotide_binding&nucleoside_phosphate_binding oxidoreductase_activity_acting_on_CH_OH_group_of_donors oxidoreductase_activity_acting_on_the_CH_OH_group_of_donors_NAD_or_NADP_as_acceptor peptidase_activity_acting_on_l_amino_acid_peptides peptidase_activity protein_complex_binding purine_nucleotide_binding purine_ribonucleoside_triphosphate_binding purine_ribonucleotide_binding receptor_activity receptor_binding ribonucleotide_binding signal_transducer_activity signaling_receptor_activity small_molecule_binding substrate_specific_transmembrane_transporter_activity substrate_specific_transporter_activity transmembrane_signaling_receptor_activity transmembrane_transporter_activity transporter_activity

Supporting Discussion

Glucose consumption and glutamine addiction were associated with a poor productive phenotype

In addition to the differential proteomic comparison between CRL-12444 (lower producer) and CRL-12445 (higher producer) cells, the metabolism of both cell lines was characterized from the concentration of metabolites in supernatants and growth curves (Figure 2 and Table 1). The higher producer cells slowed down glucose consumption (q_{Glc}) and lactate production (q_{Lac}), parameters that were coupled to an increase in efficiency of carbon transformation, represented by a lower $Y'_{\text{Lac/Glc}}$ (Table 1). This reduced yield that translates into a more oxidative metabolic state has been formerly identified as a common marker of higher producer cells ¹⁻³. In fact, despite the 14.2% reduction in q_{Glc} in the most productive clone, there is a 20% increase in the carbon flux that would enter at the TCA cycle level. The supplementary energy provided by the oxidative metabolism in these cells could be diverted to increase culture biomass ^{4,5} or q_p and to maintain cellular viability ⁶. Glutamine consumption was also reduced in the higher producer CRL-12445 cells, as suggested by a lower glutamate (q_{Glu}) and ammonium ($q_{\text{NH}_4^+}$) production (Figure 2E-F and Table 1) and the residual glutamine from day 4 onwards (Figure 2D). This lower glutamine consumption could mitigate the generation of deleterious metabolites such as lactate and ammonium ⁷⁻¹⁸ without impairing synthesis of fatty acids, nucleotides, aminoacids and pyruvate. The mutual downregulation of glycolysis and glutamine import to the cell has proved to favor the cellular growth and product titer ^{19,20}, a fact that matches well enough with the observed metabolic results in our study, where a more efficient carbon flux renders more energy for RP production and decreases the concentration of lactate and ammonium in supernatants.

A higher protein production was linked to changes in metabolism of nucleic acids and proteins, cytoskeleton organization and cell signaling

In addition to proteins belonging to the CSP, other targets with a differential expression were also examined and classified into the following categories for a better understanding of how the processes in which they participate are modified depending on q_p : metabolism of nucleic acids and proteins, cytoskeleton arrangement, signaling pathways and metabolism of carbon, nitrogen, cofactors and vitamins.

With respect to nucleic acids metabolism, an enrichment in DNA replication associated proteins was observed for upregulated targets in CRL-12445 cells by PANTHER over-representation test (Table S5), even though these cells showed a lower growth rate ($p < 0.01$) (Table 1). In agreement with previous studies ^{21–24}, the lower growth rate of CRL-12445 cells was associated with their higher antibody productivity, indicating that the upregulated proteins involved in DNA replication likely exerted a beneficial effect on q_p through an unknown mechanism. At the same time, an enhanced DNA repair in CRL-12445 cells (Table S17) could be intimately related to a more active DNA replication ²⁵ or arisen as a compensatory mechanism for reduced levels of STRAD α to maintain genome stability ^{26,27}. The downregulation of STRAD α and overexpression of PMRT1 in CRL-12445 cells could confer some tumoral properties to these higher producer cells. STRAD α is a pseudokinase that in complex with MO25 binds to and activates LKB1 ^{28,29}, a tumor suppressor gene involved in DNA repair, cell cycle arrest, genome stability and apoptosis ^{27,30–32}, while PRMT1 is a arginine methyltransferase overexpressed in cancer cells where it drives tumor growth, survival and metastasis ^{33–36}. Therefore, the changes in cellular concentration of STRAD α and PMRT1 could lead to a higher viability, apoptosis resistance and cell cycle progression in CRL-12445 cell line. Other targets from nucleic acid-related processes such as transcription and RNA metabolism were negatively regulated at a higher q_p , accompanied by lower levels of proteins belonging to metabolism of non-coding RNAs, assembling of ribonucleic particles, ribosomes and splicing machinery (Tables S14-15, S16, S17 and S19). This differential expression of proteins and genes participating in transcription ^{21,37} and the downregulation of those participating in the splicing and transport of RNA ^{22,26} have been observed before in comparative transcriptomic and proteomic studies, in agreement with our results. However,

given that ribosomal proteins can be increased ³⁸ or decreased ²¹ as shown in previous studies, the downregulation of these targets in the present work appears to be clone dependent.

Protein metabolism was apparently activated at higher q_p , as showed by the upregulation of many targets that participates during protein translation (Tables S4-S8), aminoacyl-tRNA biosynthesis, ubiquitin-mediated proteolysis (Table S16), protein degradation processes and glycoprotein biosynthesis (Table S17), in consonance with the key role played previously by synthesis, folding and catabolism of proteins during production of a chimeric protein ²¹. In spite of the plausible lower ribosome availability in CRL-12445 cells, translation initiation appeared to be remarkably favored (Tables S4-S8) and could be positively associated with a higher producer phenotype, as demonstrated by other proteomic studies ^{21,26,37,38}.

Cytoskeleton re-arrangements have been previously linked to changes in q_p of CHO cells ^{21,22,24,26,38}, as in our work where differential analysis insinuated a lower number of stress fibers, with the consequent reduction of focal adhesions, integrin signaling and substrate adhesion in the higher producer cells (Figures S8 and S10, Tables S16-S17). The lower representation of calcium-binding proteins (Figure S8J and Table S14) could impair stress fiber formation and focal adhesion maturation through inactivation of myosin ³⁹⁻⁴³, a phenotype reinforced by the activation of Rac (Figure S10E) and repression of Rho (Figure S9H and downregulated RHOC from Table S21) GTPases ⁴⁴⁻⁴⁶. This apparent downregulated calcium signaling was probably the result of decreased calcium-calmodulin binding proteins, ER located inositol 1,4,5-trisphosphate (IP3) receptor and HOMER proteins, inactivation of G-protein coupled receptors or receptor tyrosine kinases, and loss of PI supply to PM ⁴⁷. Microtubules cytoskeleton could also be changed by the downregulation of STRAD α /LKB1 complex which regulates MARK (MAP/microtubule affinity regulating kinase) family of kinases that in turns phosphorylates microtubules ⁴⁸. The loss of substrate adhesion and related signaling pathways could result in resource savings that can be used during RP production, while the modification of cytoskeleton assembly could aid in vesicle transport along the CSP to increase protein traffic.

Cell signaling occupied an important position among the identified pathways (Figure S9 and Tables S16, S17 and S19). Signaling through VEGF was likely reduced (Figure S9H) whereas EPHA2 (Table S20) and PDGF (Figure S9G) seemed to be activated. Although members from EGFR signaling were identified as both up and downregulated, this pathway was probable inactive in CRL-12445 cells due to TAOK3 kinase was overexpressed (Table S20) and the activity of this enzyme is abolished by EGF stimulation ⁴⁹. Given that VEGF ⁵⁰, PDGF ⁵¹ and EGFR ⁵² signaling pathways promote proliferation, migration and survival, as well as EPHA2 in a ligand-independent manner ⁵³, their functions could be redundant in such a way that probably the most advantageous ones for cell survival and compatible with high productivity were selected in the higher producer cells. Ionotropic receptors were over-represented to the detriment of metabotropic receptors, possibly regulating in this way cation transport through plasma membrane ⁵⁴, while signaling through Gq alpha associated receptors and alpha adrenergic receptors seemed abrogated (Figure S9G,H) presumably to preserve ER-luminal calcium and avoid ROS generation ⁵⁵.

Signaling proteins belonging to TGF- β and Hippo pathways were reduced while those from JAK/STAT, NF- κ B and Notch1 were activated (Figure S9 and Table S16). Although the effects of TGF- β depends on ligands, cell type and cellular context ⁵⁶, its signaling in wild type CHO cells leads to inhibition of DNA synthesis and growth ⁵⁷, similarly to Hippo pathway whose activation repress proliferation and promotes apoptosis ⁵⁸. On the contrary, NF- κ B activation, that could be reinforced by downregulation of inhibitory HDAC1,2 proteins (Table S21) ⁵⁹, is positively associated with proliferation and anti-apoptotic responses in breast cancer ⁶⁰, and can be triggered by insulin stimulation on CHO cells ⁶¹. On the same page, constitutively activated Notch1 cooperates in formation and proliferation of adult T-cell leukemia tumoral cells ⁶² and aberrant JAK/STAT activation stimulates cancer progression of different malignancies ⁶³. Hence, the differential regulation of these pathways in the higher producer cells could be part of a generalized cellular response that favors DNA replication and survival.

Apoptosis (Figure S9G,H and Tables S16-S17) and response to ROS (Glutathione metabolism in Tables S16-S17) were also identified as affected processes associated to changes in q_p . Cell death

appears to be delayed in the higher producer cells by the downregulation of pro-apoptotic proteins AIF, PPIF ^{64–68}, HTRA2 ^{69–74} and STRAD α ^{27–32,75,76} (Table S21), and the differential activation of the aforementioned signaling pathways. In respect to the maintenance of cellular redox homeostasis, the upregulation of glutathione related proteins (PGD, GST, LAP3, GSR, ADH5, ESD in Table S20) indicates the paramount importance of these antioxidant defenses during protein expression. PGD participates in NADPH synthesis ⁷⁷, GST in detoxification reactions ^{78,79} and LAP3, a cytosolic aminopeptidase, could be involved in processing and regular turnover of many glutathione related enzymes based on protein-protein interactions by STRING resource ⁸⁰. GSR aids in maintaining oxidative balance ⁸¹ and ADH5 and ESD recycle reduced glutathione ^{82,83}. Overexpression of proteins participating in cellular defense against ROS has been reported in previous differential proteomic studies of CHO cells for thioredoxin ³⁸, SOD ³⁷ and enzymes from glutathione metabolism ²², while intracellular GSH content positively correlates with growth rate and product titer in a mAb producer CHO cell line ⁸⁴. These evidences strongly suggest that the stimulation of ROS responses to overcome oxidative stress associated to an enhanced oxidative metabolism and protein production is a molecular trait of higher producer cells.

Metabolism of carbon, nitrogen, cofactors and vitamins was probably modified to accommodate a higher protein expression

Profound changes in this category were systematically detected by all tools used for protein classification (Figures S8-S11 and Tables S4, S6-S7, S12, S14-S15, S16-S17). In agreement with consumption or production rates of metabolites (Table 1), the higher producer cells appeared to constrain carbon flux through glycolysis accompanied by a reduced metabolic over flow and lactate production (Figure S12). At the same time, overexpressed PFK1 presumably redirected the glycolytic flux towards the production of fructose 1,6 bi-phosphate, dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (G3P), in correspondence with the fact that PFK1 overexpression in CHOK1 cells increases fructose 1,6 bi-phosphate levels without any effect on the glycolytic flux ⁸⁵,

relaying the decrease of q_{Glc} in the higher producer cells on the downregulation of other glycolytic enzymes (Figure S12).

The higher levels of DHAP enhance methylglyoxal formation^{86–88}, a deleterious metabolite^{86–94} that could be eliminated by upregulating AKR1A1, a reductase that participates in detoxification reactions and activation of pentose phosphate pathway (PPP) and glutathione synthesis⁹⁴. The supposed higher production of DHAP, G3P and fructose 1,6 bi-phosphate in CRL-12445 cells could be diverted to lipid synthesis (Figure S13), PPP feeding and phosphoribosyl pyrophosphate (PRPP) generation⁹⁵. The augmented expression of several enzymes downstream from PRPP (Figure S14) indicated that the higher the q_p , the greater the activation of purine salvage pathway, NAD/NADP biosynthesis and *de novo* biosynthesis of pyrimidines and purines, which are essential for cell survival⁹⁶ and have a positive impact on cell growth and product titer⁹⁷.

The biosynthesis of acetyl-CoA by PDH enzyme, a feeding metabolite for tricarboxylic acid (TCA) cycle, appeared to be decreased (Figure S15), in line with glycolysis reduction. However, the upregulation of other enzymes participating in acetyl-CoA generation (Figure S15) could maintain the necessary levels of this intermediate for an efficient TCA cycle and lipid biosynthesis (Figure S13). Although some TCA enzymes could be decreased, the cleavage of certain amino acids and the upregulation of citrate synthase would aid to replenish the pools of their respective intermediates and to maintain an active TCA cycle (Figure S15), highlighting the importance of amino acid balance during bioprocesses^{98–101}. Intimately linked to the TCA cycle is the electron transport chain and oxidative phosphorylation. In the former, various subunits from different respiratory complexes, principally from complex I, were underexpressed coupled to the modification of subunit composition of ATPase. The changes in this metabolic pathway could respond to maintaining an adequate coupling between the TCA cycle, electron transport chain and oxidative phosphorylation to produce the necessary amount of ATP without increasing ROS generation.

Acetyl-CoA, together with DHA and cytidine triphosphate (CTP), probably augmented the synthesis of palmitate, phosphatidic acid, diacylglycerol, activated fatty acids, fatty alcohols,

phosphatidylethanolamine and phosphatidylserine (Figure S13). However, enzymes that participate in synthesis of other lipids such as unsaturated fatty acids, leukotrienes, plasmalogens, phosphatidylcholine, sphingosine and cholesterol, were under-represented (Figures S13 and S15). Cholesterol depletion in CHO cells translates into membrane stiffness and cell detachment ¹⁰², enhancement of cell mechano-sensitivity, regulatory volume decrease ¹⁰³ and thermal survival ¹⁰⁴ and a less ROS generation and cell death ¹⁰⁵. Although these effects can be advantageous for the higher producer cells, the suggested cholesterol deficiency could be also linked to PM permeability in order to favor the diffusion of oxygen ¹⁰⁶ and accordingly the oxidative metabolism and protein production. Given that increasing the intracellular cholesterol concentration by genetic approaches ¹⁰⁷ or culture supplementation ^{108,109} have not shown the same effects, and even have led to contradictory effects on q_p , product titer, μ and viable cell concentration, the advantages of cholesterol on culture performance and protein production appear to depend on the clone. As expected from a mutual negative cross-regulation between synthesis and catabolism of lipids, proteins participating in lipid oxidation in mitochondria, peroxisome and ER were downregulated (Figure S16). Proteomic studies together with metabolomics help to elucidate which metabolites are actively consumed or produced by recombinant CHO cells in such a way that they dictate the most adequate cell culture supplementation that can boost cell growth, viability and product expression ^{110–113}. As a result of our proteomic study, it is expected that the higher producer cells could benefit from addition of lipid supplements enriched in triglycerides and saturated fatty acids during mAb production.

With the exception of AHCY, most of the enzymes of tetrahydrofolate cycle were under-represented (Figure S17), presumptively resulting in the maintenance of glycine levels, homocysteine accumulation and a low methylation capacity. The global hypomethylation pattern of these cells expected from this downregulated cycle has been confirmed before, and resulted in activation of gene expression, energy metabolism and cell cycle control, and suppression of neurological processes, cellular adhesion and calcium ion homeostasis ¹¹⁴, in congruence with our results.

CHO cells are proline auxotroph due to blocking of glutamate conversion to proline (glutamate pathway) and a weakened ornithine pathway (low expression of ornithine transaminase)^{115,116}. Thus, the downregulated ALDH (Figure S12) could reinforce cellular dependence on proline for growth and maintenance and aid preserve cellular resources for protein production.

Abbreviations

Cytidine triphosphate (CTP), dihydroxyacetone phosphate (DHAP), glyceraldehyde 3-phosphate (G3P), inositol 1,4,5-trisphosphate (IP3), MAP/microtubule affinity regulating kinase (MARK), pentose phosphate pathway (PPP), phosphoribosyl pyrophosphate (PRPP), tricarboxylic acid (TCA).

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