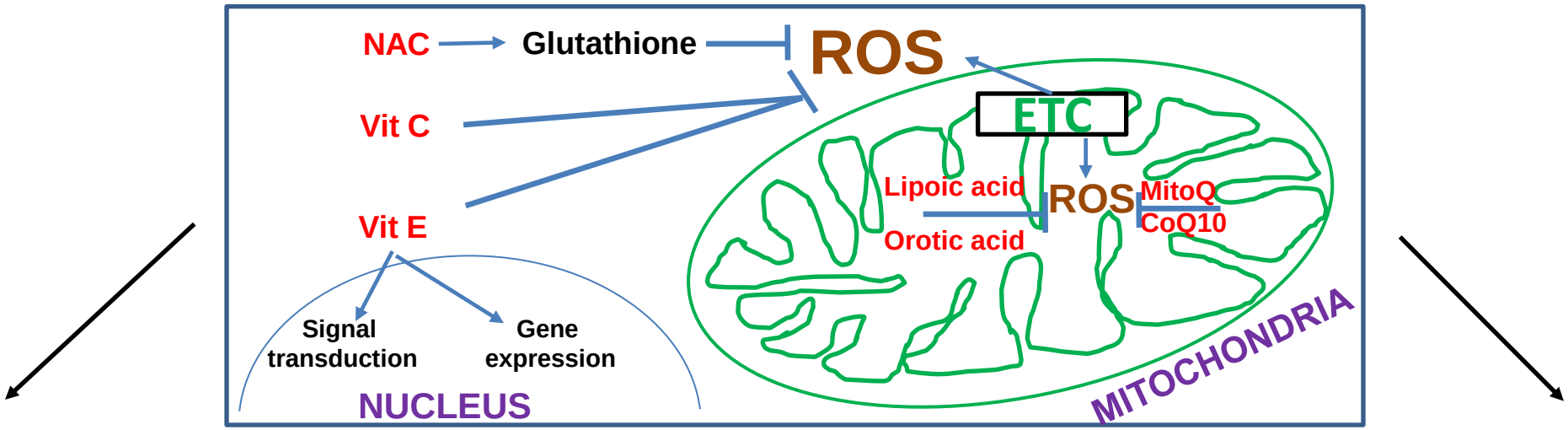
Fig 1

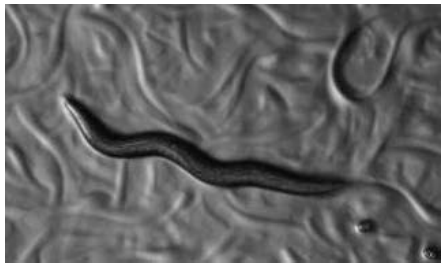
Schema of 7 Antioxidant Drugs' Cellular Sites of Action:



Primary Animal Model for Drug Effect Studies:

C. elegans (nematode)

Complex I *gas-1(fc21)* model



Drugs Studied: NAC, Vit C, Vit E, MitoQ, CoQ10, Lipoate, Orotate

1° Outcome:

- Worm Lifespan

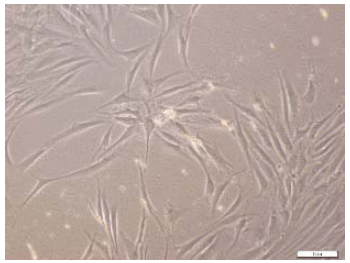
2° Outcomes:

- Mitochondrial physiology (Microscopy)
- Metabolome profiling (HPLC, GC/MS)
- Transcriptome profiling (Microarray+RNAseq)

Validation Models for Drug Effect Studies:

Human Fibroblasts

Complex I genetic disease



Drug Studied: NAC

Outcomes:

- Cell viability (CellGlo)
- Mitochondrial physiology (FACS)

D. rerio (zebrafish)

Complex I inhibition (rotenone)

Drugs Studied: NAC, Vit E

Outcome:

- Brain death (Microscopy)

Fig 2.

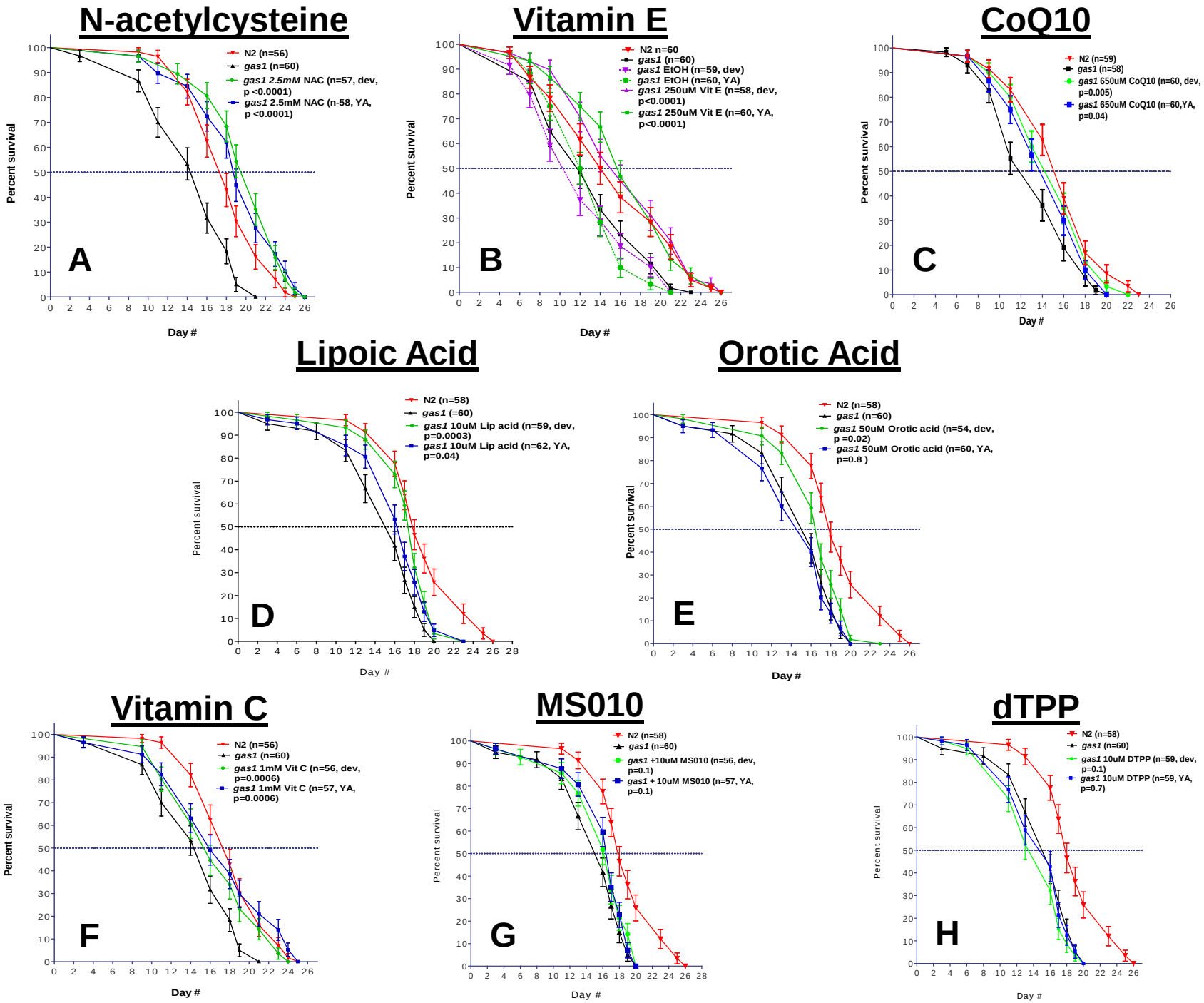

N-acetylcysteine
N2 (n=56)
gas1 (n=60)
gas1 2.5mM NAC (n=57, dev, p <0.0001)
gas1 2.5mM NAC (n-58, YA, p <0.0001)
A
Vitamin E
N2 n=60
gas1 (n=60)
gas1 EtOH (n=59, dev)
gas1 EtOH (n=60, YA)
gas1 250uM Vit E (n=58, dev, p<0.0001)
gas1 250uM Vit E (n=60, YA, p<0.0001)
B
CoQ10
N2 (n=59)
gas1 (n=58)
gas1 650uM CoQ10 (n=60, dev, p=0.005)
gas1 650uM CoQ10 (n=60,YA, p=0.04)
C
Lipoic Acid
N2 (n=58)
gas1 (=60)
gas1 10uM Lip acid (n=59, dev, p=0.0003)
gas1 10uM Lip acid (n=62, YA, p=0.04)
D
Orotic Acid
N2 (n=58)
gas1 (n=60)
gas1 50uM Orotic acid (n=54, dev, p =0.02)
gas1 50uM Orotic acid (n=60, YA, p=0.8)
E
Vitamin C
N2 (n=56)
gas1 (n=60)
gas1 1mM Vit C (n=56, dev, p=0.0006)
gas1 1mM Vit C (n=57, YA, p=0.0006)
F
MS010
N2 (n=58)
gas1 (n=60)
gas1 +10uM MS010 (n=56, dev, p=0.1)
gas1 + 10uM MS010 (n=57, YA, p=0.1)
G
dTPP
N2 (n=58)
gas1 (n=60)
gas1 10uM DTPP (n=59, dev, p=0.1)
gas1 10uM DTPP (n=59, YA, p=0.7)
H
Percent survival
Day #

Fig 3.

Mitochondrial Physiology Effects in *gas-1(fc21) C. elegans*

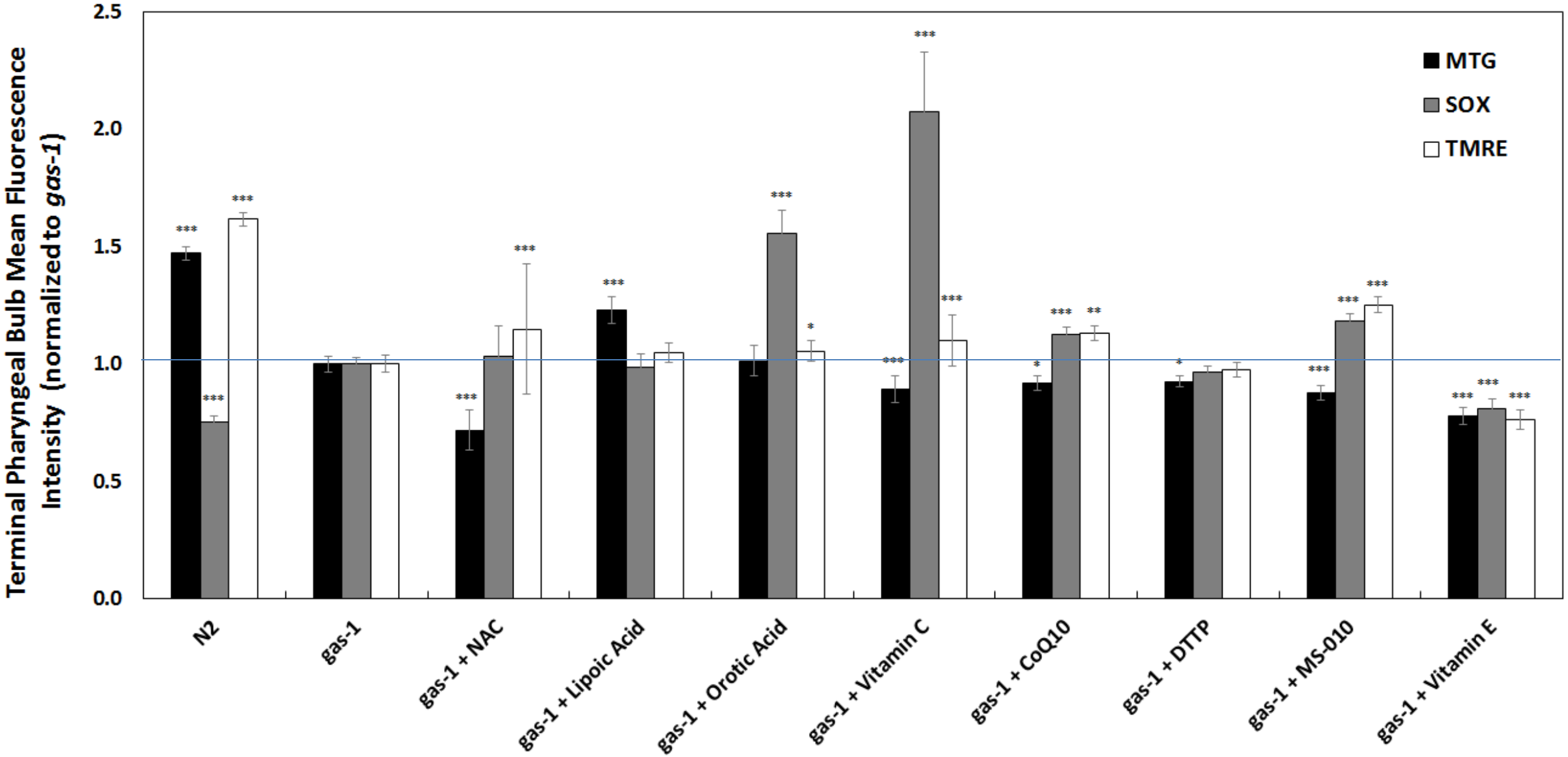


Fig 4.

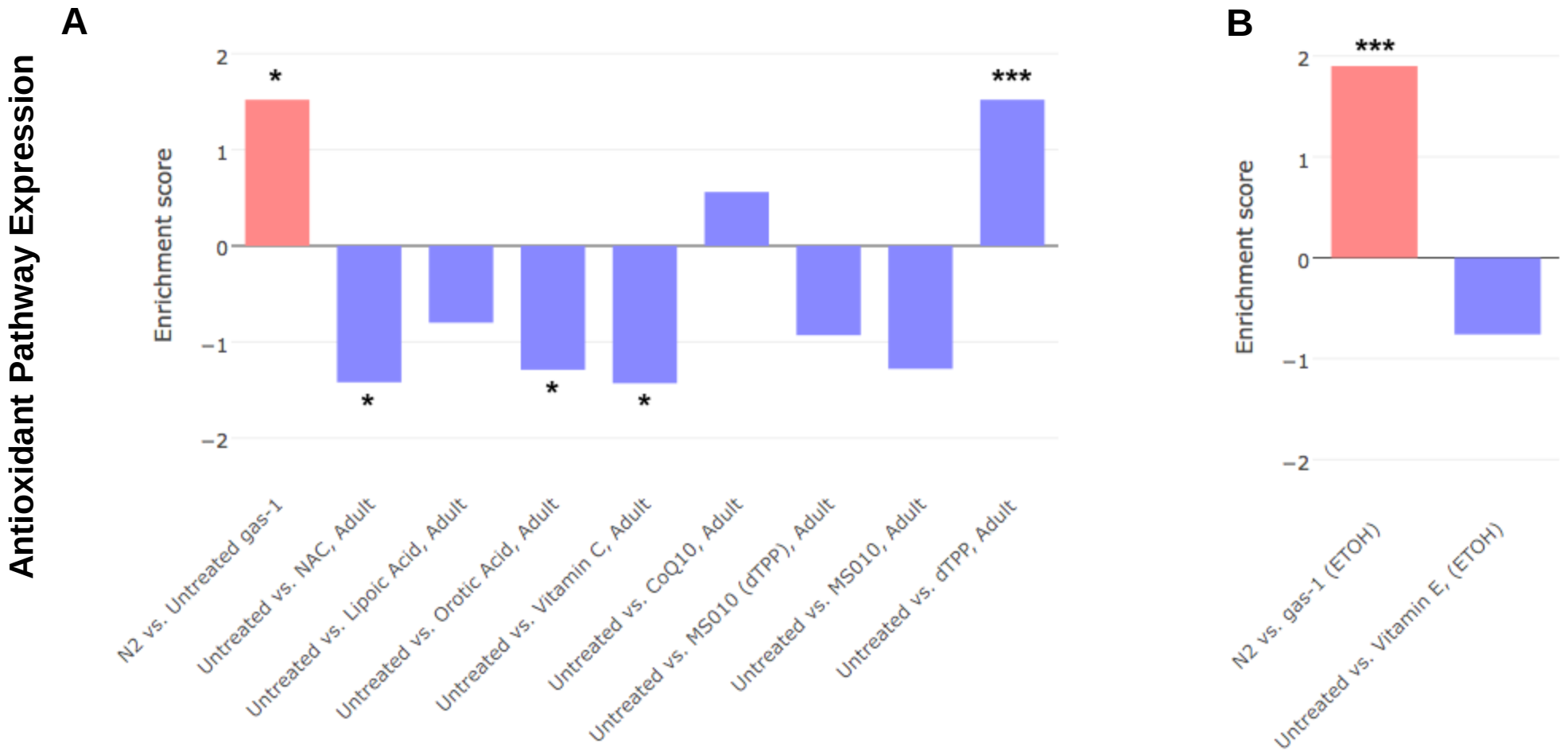
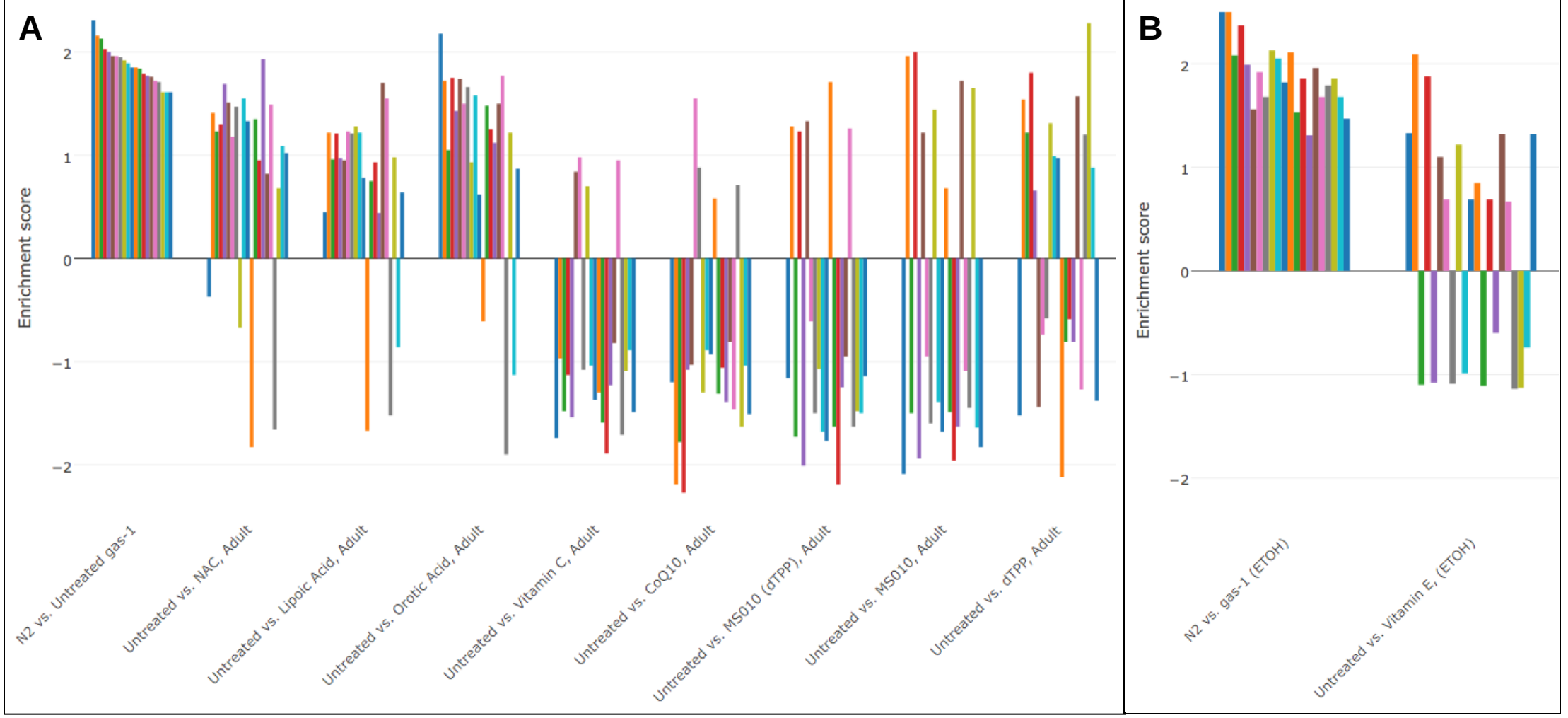
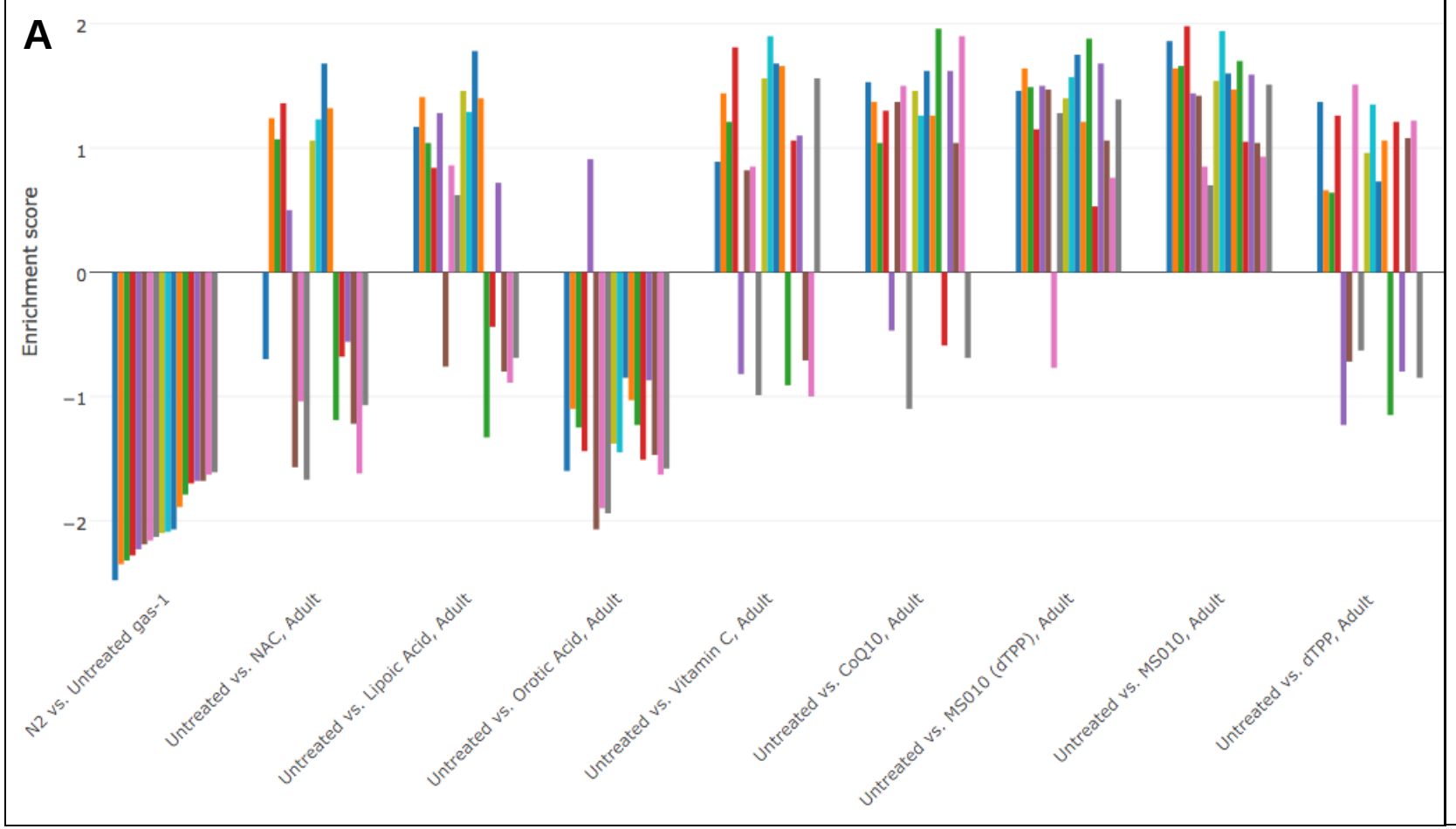

Antioxidant Pathway Expression
A
Enrichment score
2
1
0
−1
−2
*
*
*
*
***
N2 vs. Untreated gas-1
Untreated vs. NAC, Adult
Untreated vs. Lipoic Acid, Adult
Untreated vs. Orotic Acid, Adult
Untreated vs. Vitamin C, Adult
Untreated vs. CoQ10, Adult
Untreated vs. MS010 (dTPP), Adult
Untreated vs. MS010, Adult
Untreated vs. dTPP, Adult
B
Enrichment score
2
1
0
−1
−2
***
N2 vs. gas-1 (ETOH)
Untreated vs. Vitamin E, (ETOH)

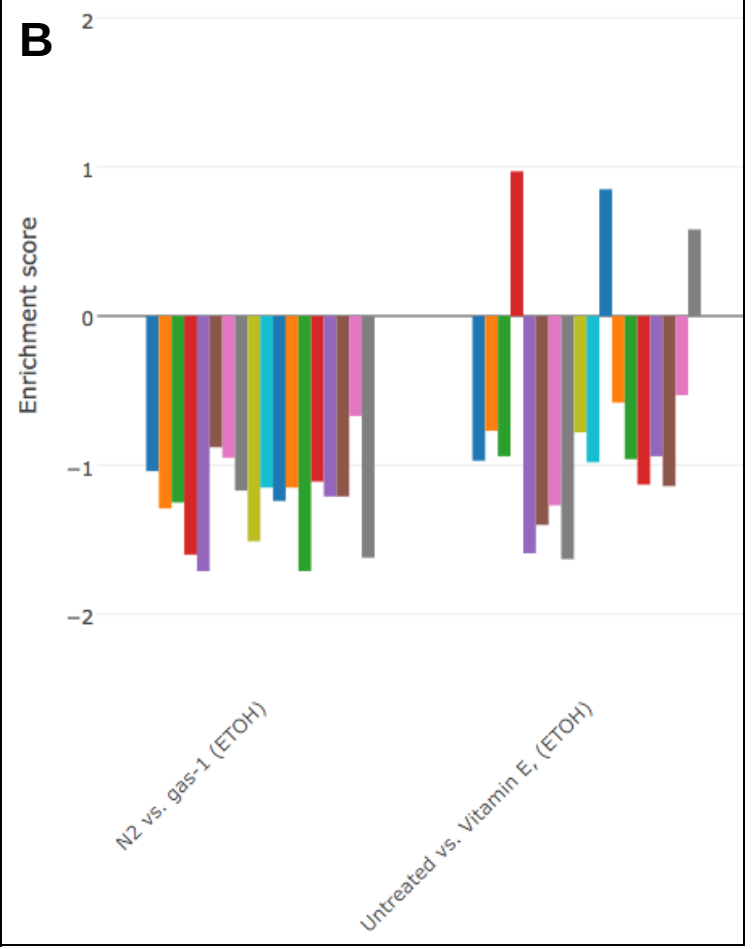
Fig 5.



CEL00190 : OXIDATIVE_PHOSPHORYLATION
CEL00982 : DRUG_METABOLISM
CEL00010 : GLYCOLYSIS_GLUCONEOGENESIS
CEL00980 : METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450
CEL01200 : CARBON_METABOLISM
CEL04146 : PEROXISOME
CEL00260 : GLYCINE_SERINE_AND_THREONINE_METABOLISM
CEL01230 : BIOSYNTHESIS_OF_AMINO_ACIDS
CEL00040 : PENTOSE_AND_GLUCURONATE_INTERCONVERSIONS
CEL01130 : BIOSYNTHESIS_OF_ANTIBIOTICS
CEL00620 : PYRUVATE_METABOLISM
CEL03010 : RIBOSOME
CEL00051 : FRUCTOSE_AND_MANNOSE_METABOLISM
CEL00020 : CITRATE_CYCLE_TCA_CYCLE_
CEL00630 : GLYOXYLATE_AND_DICARBOXYLATE_METABOLISM
CEL00053 : ASCORBATE_AND_ALDARATE_METABOLISM
CEL00350 : TYROSINE_METABOLISM
CEL04010 : MAPK_SIGNALING_PATHWAY
CEL02010 : ABC_TRANSPORTERS
CEL00250 : ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM
CEL01210 : 2_OXOCARBOXYLIC_ACID_METABOLISM

Fig 6.





CEL03040 : SPLICEOSOME
CEL03420 : NUCLEOTIDE_EXCISION_REPAIR
CEL03022 : BASAL_TRANSCRIPTION_FACTORS
CEL03460 : FANCONI_ANEMIA_PATHWAY
CEL03050 : PROTEASOME
CEL03013 : RNA_TRANSPORT
CEL03015 : MRNA_SURVEILLANCE_PATHWAY
CEL04120 : UBIQUITIN_MEDIATED_PROTEOLYSIS
CEL03430 : MISMATCH_REPAIR
CEL03030 : DNA_REPLICATION
CEL03410 : BASE_EXCISION_REPAIR
CEL03440 : HOMOLOGOUS_RECOMBINATION
CEL03020 : RNA_POLYMERASE
CEL04330 : NOTCH_SIGNALING_PATHWAY
CEL00240 : PYRIMIDINE_METABOLISM
CEL03018 : RNA_DEGRADATION
CEL00970 : AMINOACYL_TRNA_BIOSYNTHESIS
CEL00534 : GLYCOSAMINOGLYCAN_BIOSYNTHESIS

Fig 7.

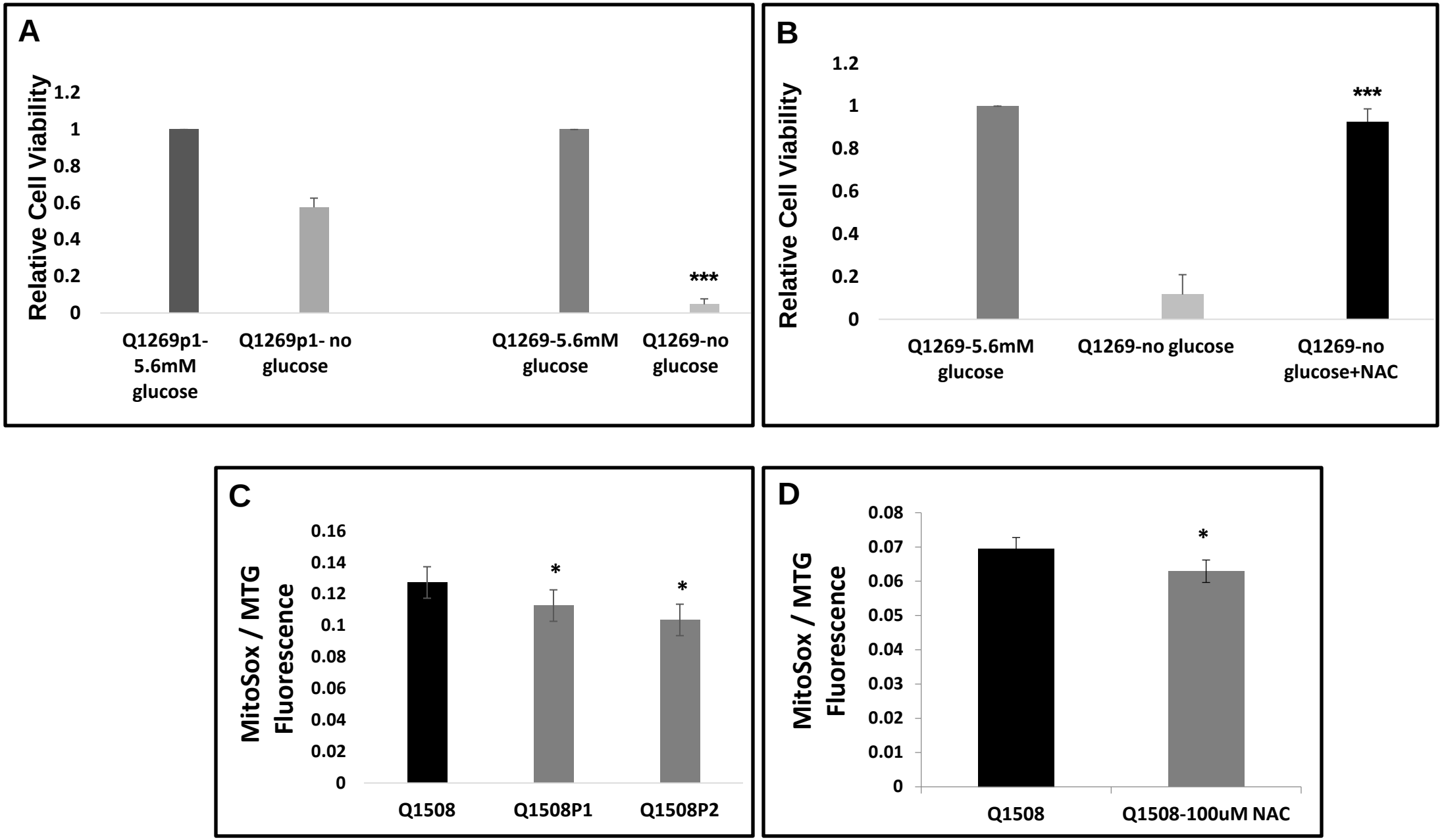
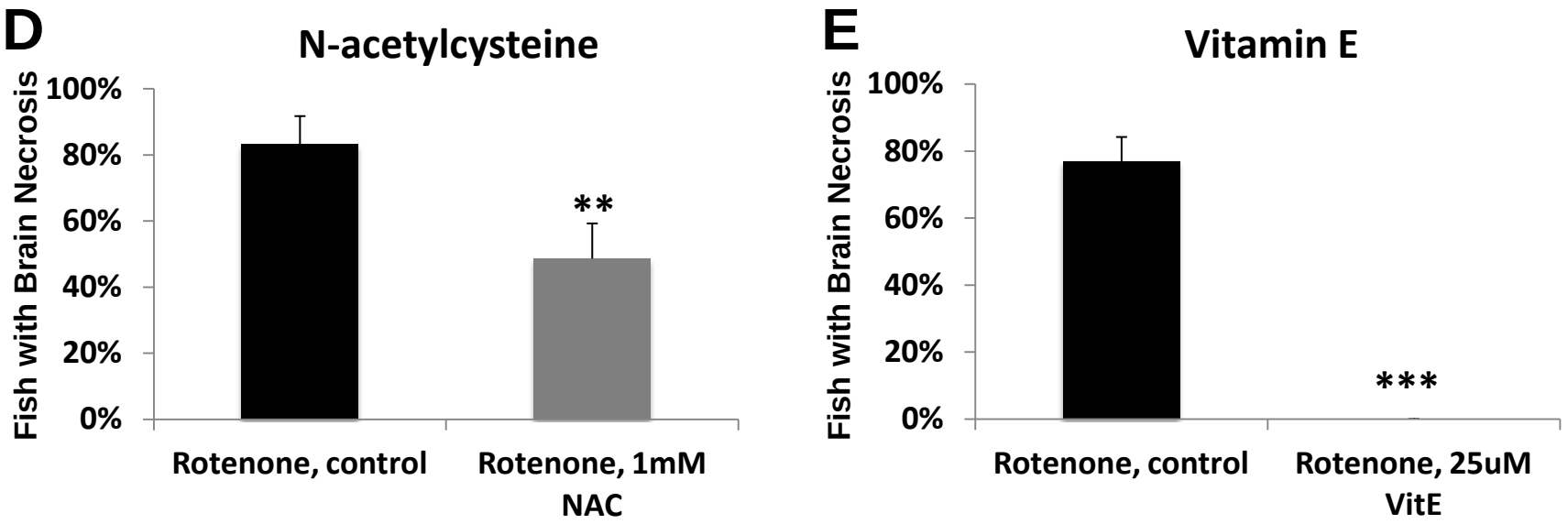

A
Relative Cell Viability
1.2
1
0.8
0.6
0.4
0.2
0
***
Q1269p1-5.6mM glucose
Q1269p1- no glucose
Q1269-5.6mM glucose
Q1269-no glucose
B
Relative Cell Viability
1.2
1
0.8
0.6
0.4
0.2
0
***
Q1269-5.6mM glucose
Q1269-no glucose
Q1269-no glucose+NAC
C
MitoSox / MTG Fluorescence
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
*
*
Q1508
Q1508P1
Q1508P2
D
MitoSox / MTG Fluorescence
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
*
Q1508
Q1508-100uM NAC

Fig 8.


A
Control
B
*
Rotenone
C
Rotenone + NAC


D
N-acetylcysteine
Fish with Brain Necrosis
100%
80%
60%
40%
20%
0%
**
Rotenone, control
Rotenone, 1mM NAC
E
Vitamin E
Fish with Brain Necrosis
100%
80%
60%
40%
20%
0%
***
Rotenone, control
Rotenone, 25uM VitE

SUPPLEMENTAL FILES

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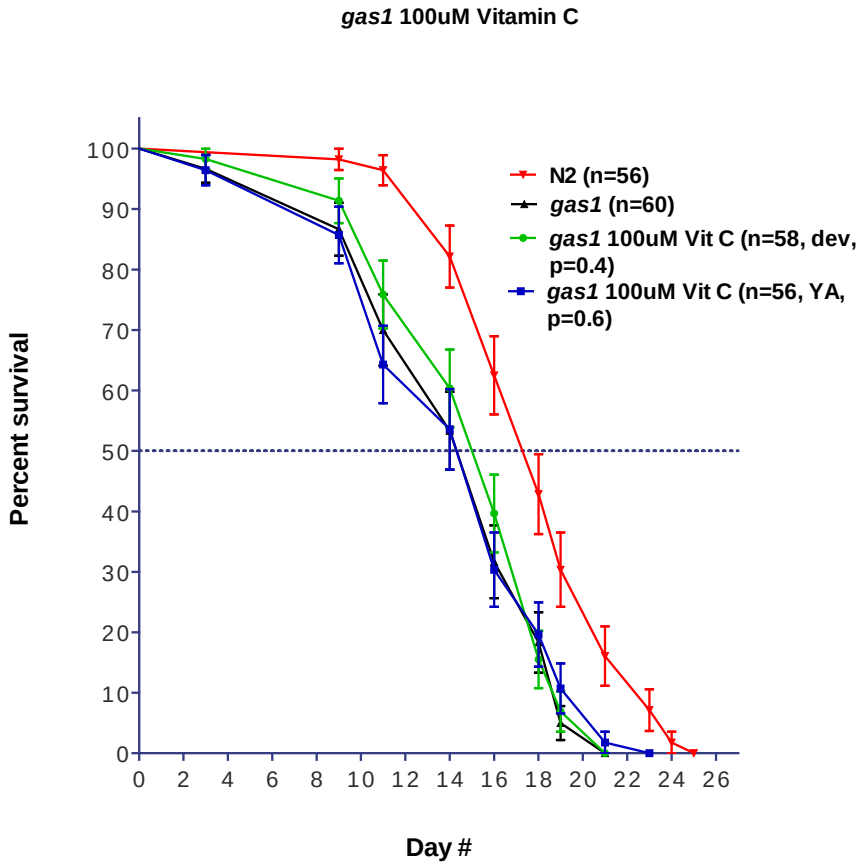
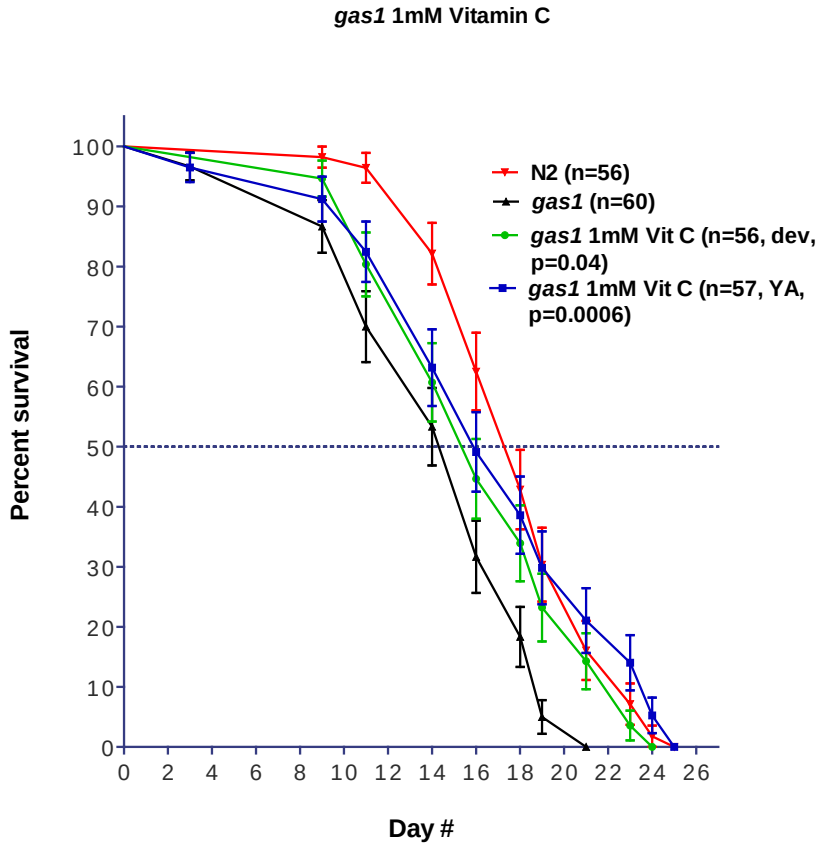
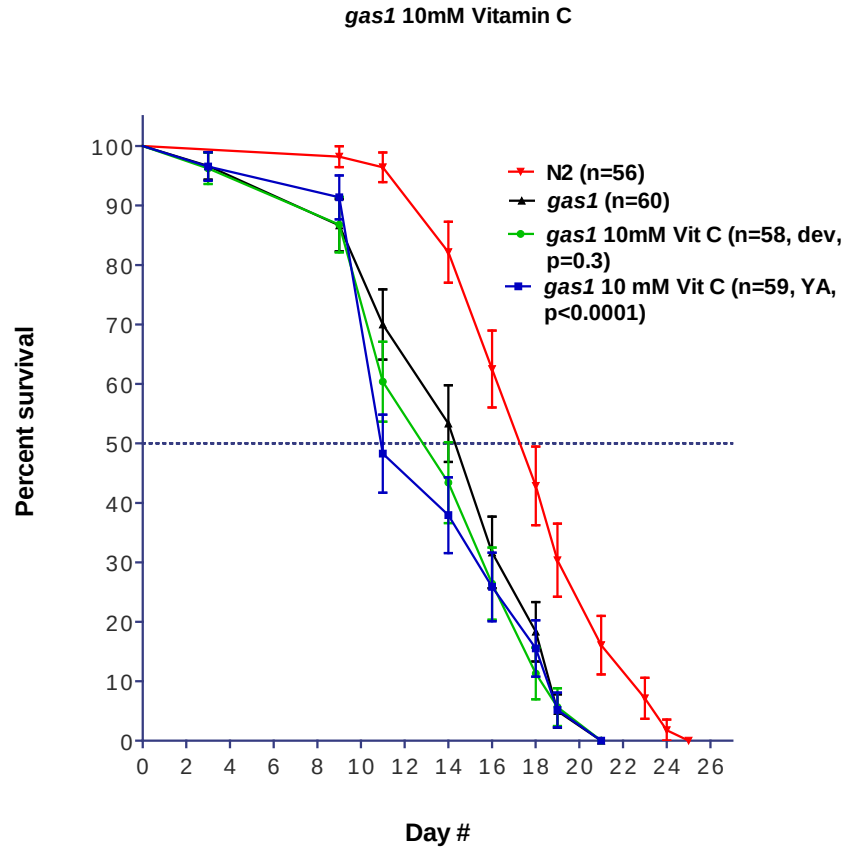
Fig S1.



100 uM Vit C (Ineffective)



1 mM Vit C (Therapeutic)



10 mM Vit C (Ineffective/Toxic)

Fig S2

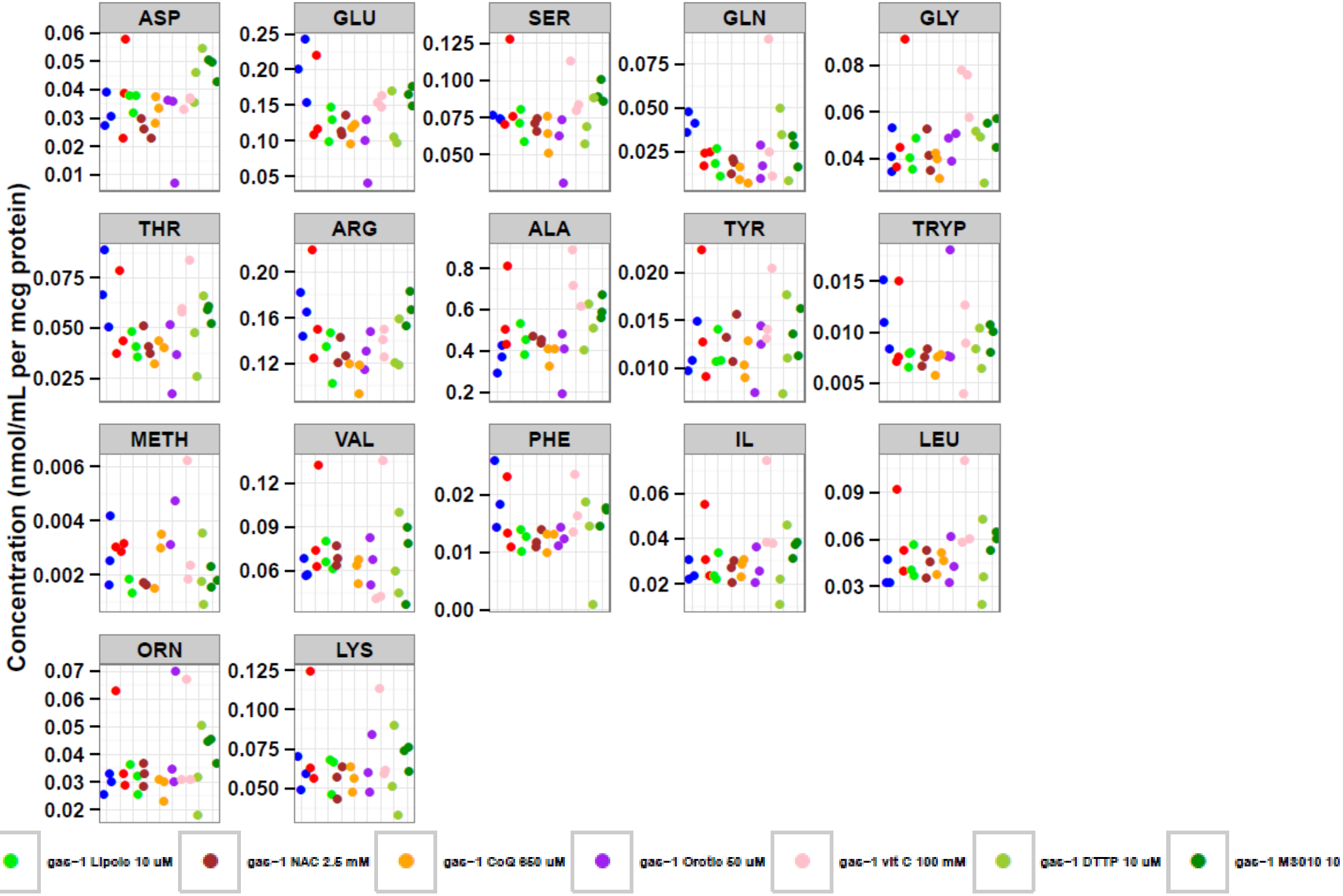

ASP
GLU
SER
GLN
GLY
THR
ARG
ALA
TYR
TRYP
METH
VAL
PHE
IL
LEU
ORN
LYS
Concentration (nmol/mL per mcg protein)
N2
gac-1 H2O
gac-1 Lipoic 10 uM
gac-1 NAC 2.5 mM
gac-1 CoQ 850 uM
gac-1 Orotic 50 uM
gac-1 vit C 100 mM
gac-1 DTTP 10 uM
gac-1 MS010 10

Fig S3

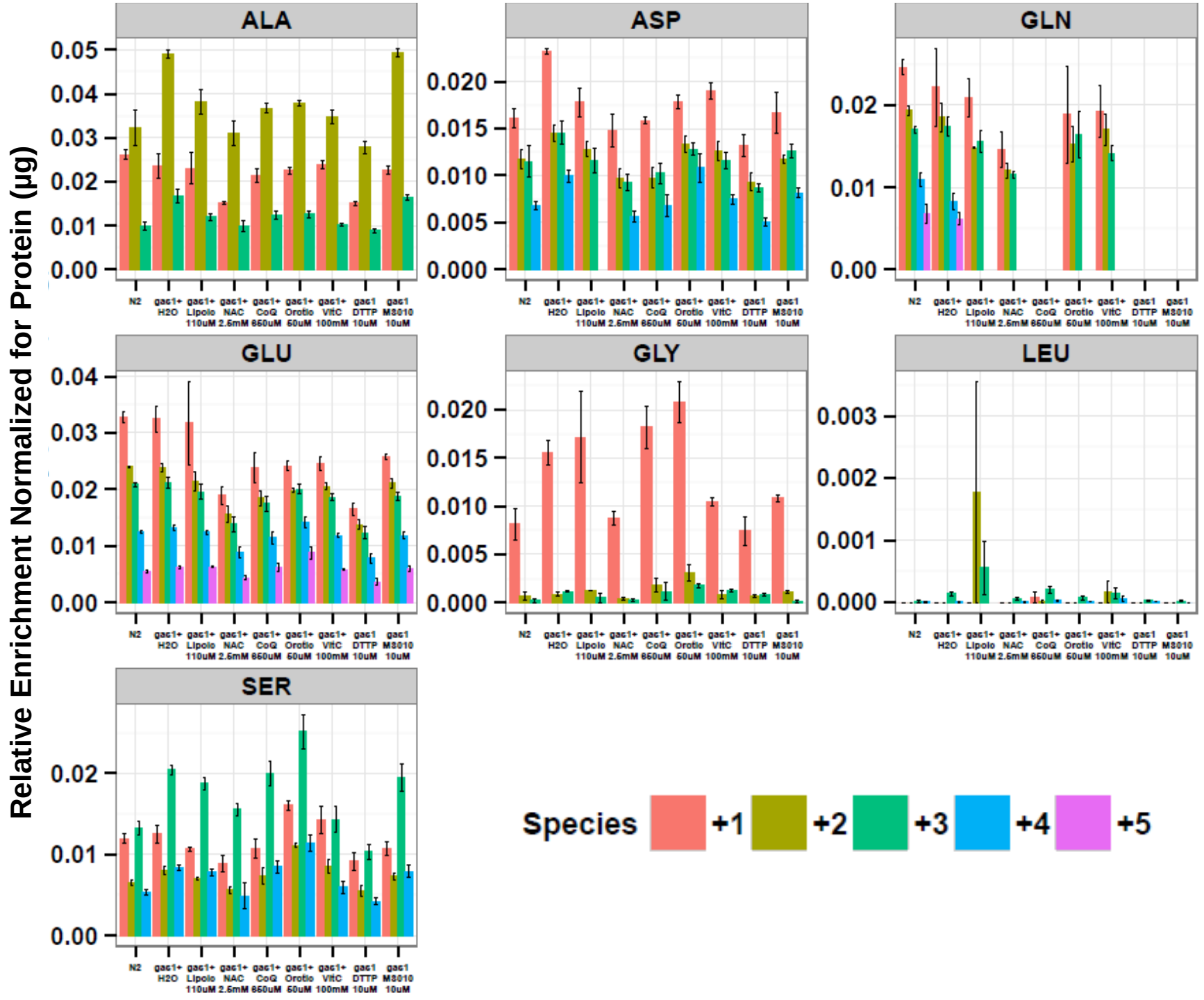

Relative Enrichment Normalized for Protein (µg)
ALA
ASP
GLN
GLU
GLY
LEU
SER
N2
gac1+ H2O
gac1+ LIpoIo 110uM
gac1+ NAC 2.5mM
gac1+ CoQ 860uM
gac1+ Orotic 60uM
gac1+ VitC 100mM
gac1 DTTP 10uM
gac1 M8010 10uM
Species
+1
+2
+3
+4
+5

Fig S4

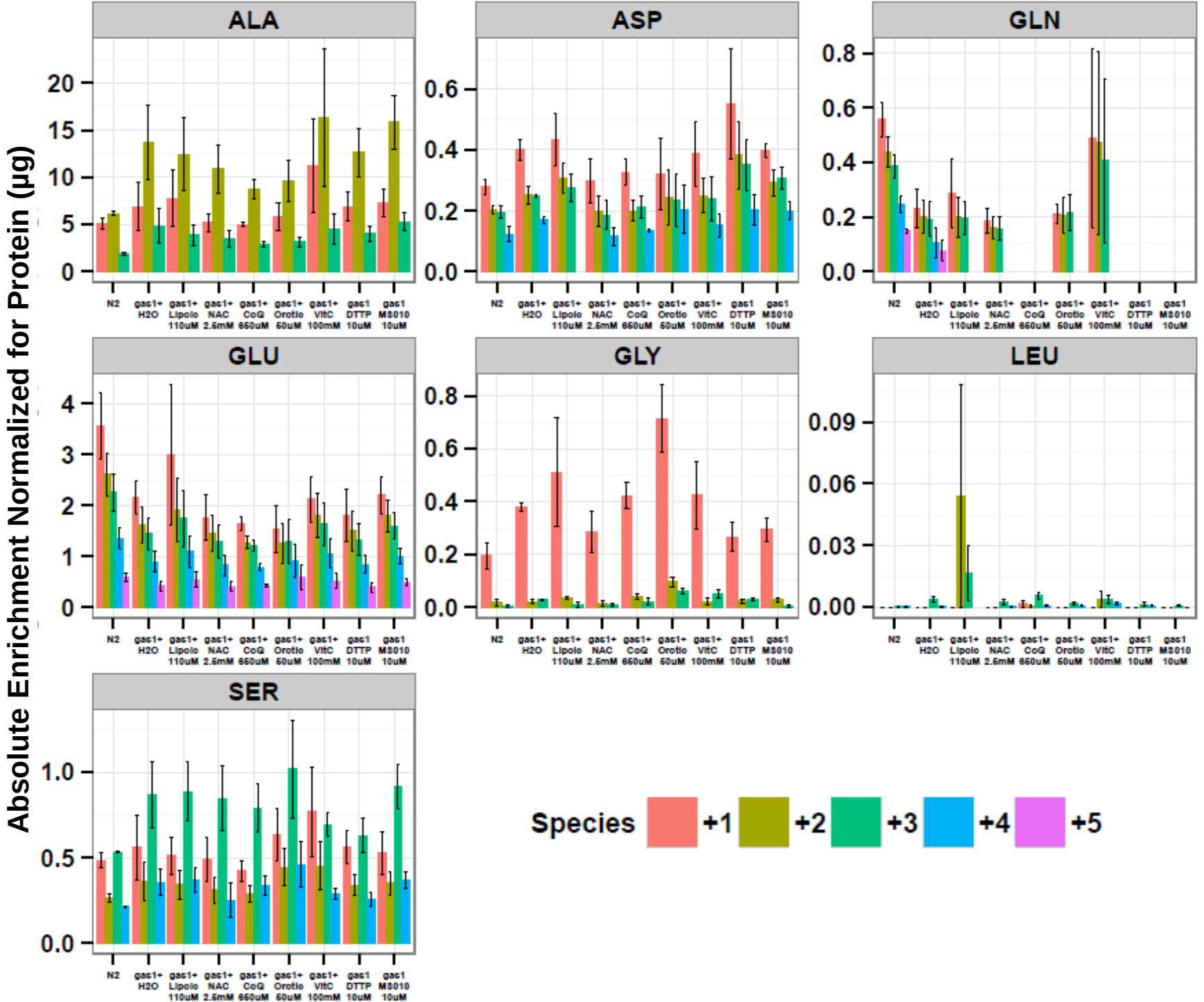

ALA
ASP
GLN
GLU
GLY
LEU
SER
Absolute Enrichment Normalized for Protein (µg)
N2
gac1+ H2O
gac1+ Lipoic 110uM
gac1+ NAC 2.5mM
gac1+ CoQ 650uM
gac1+ Orotic 60uM
gac1+ VitC 100mM
gac1 DTTP 10uM
gac1 M8010 10uM
Species
+1
+2
+3
+4
+5

Fig S5

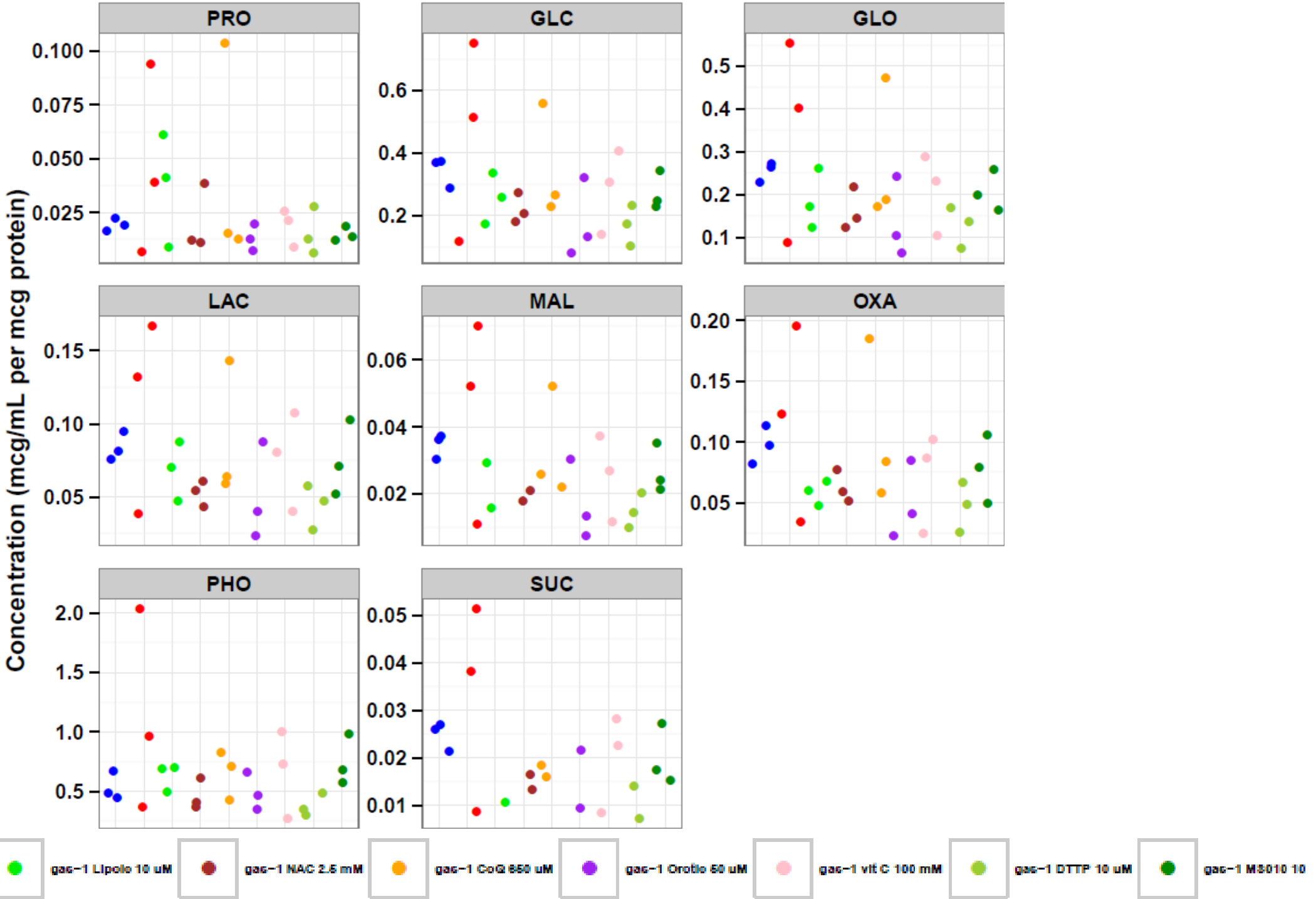

PRO
GLC
GLO
LAC
MAL
OXA
PHO
SUC
Concentration (mcg/mL per mcg protein)
N2
gac-1 H2O
gac-1 Lipoic 10 uM
gac-1 NAC 2.5 mM
gac-1 CoQ 650 uM
gac-1 Orotic 50 uM
gac-1 vit C 100 mM
gac-1 DTTP 10 uM
gac-1 MS010 10

Fig S6

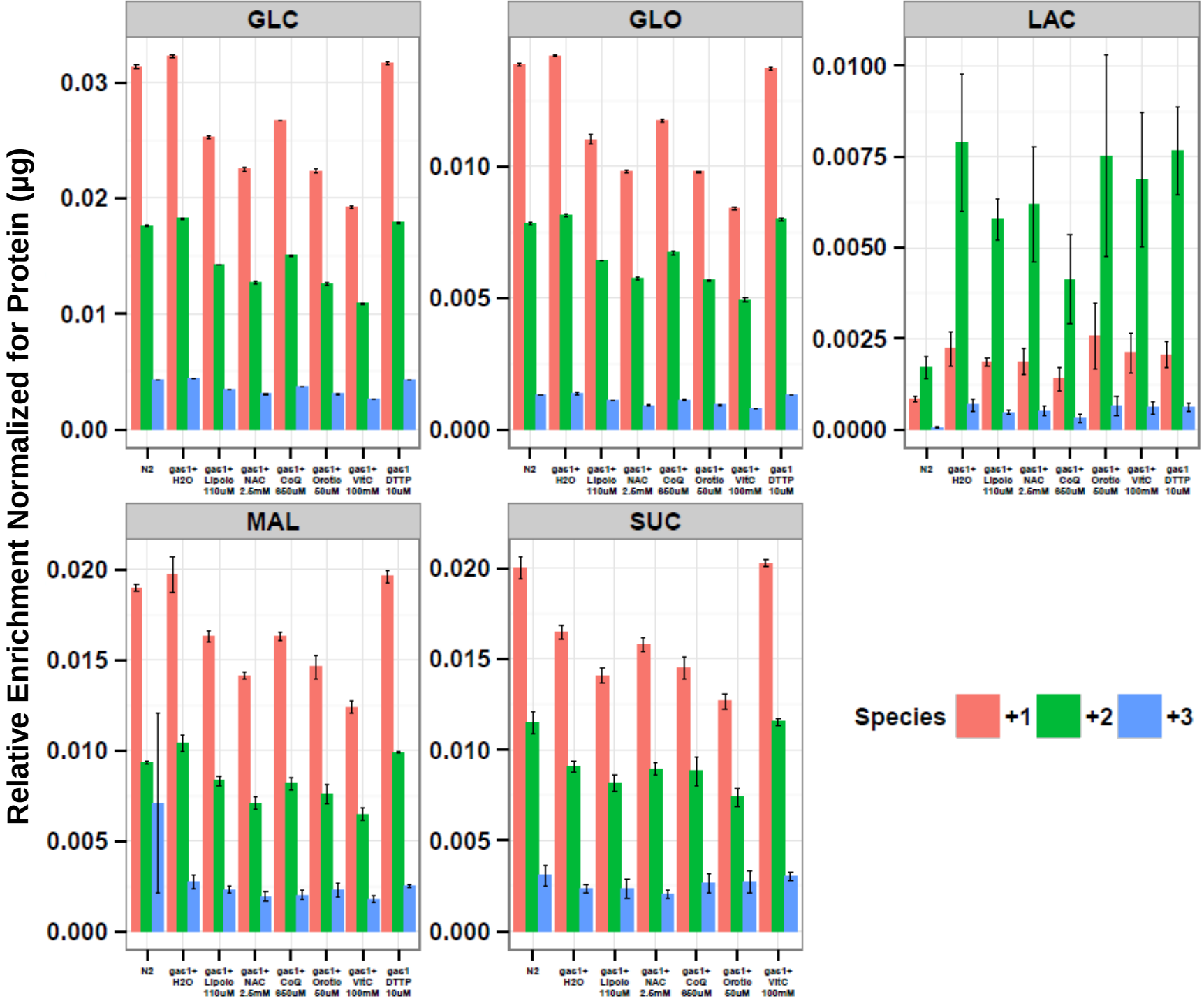

GLC
GLO
LAC
MAL
SUC
Relative Enrichment Normalized for Protein (µg)
N2
gac1+ H2O
gac1+ Lipoic 110uM
gac1+ NAC 2.5mM
gac1+ CoQ 850uM
gac1+ Orotic 50uM
gac1+ VitC 100mM
gac1 DTTP 10uM
Species
+1
+2
+3

Fig S7


GLC
GLO
LAC
MAL
SUC
Absolute Enrichment Normalized for Protein (µg)
N2
gac1+ H2O
gac1+ Lipoic 110uM
gac1+ NAC 2.5mM
gac1+ CoQ 850uM
gac1+ Orotic 50uM
gac1+ VitC 100mM
gac1 DTTP 10uM
gac1 M8010 10uM
Species
+1
+2
+3

Fig S8. **Antioxidant drug effects on <u>upregulated antioxidant pathway</u> in *gas-1(fc21)* worms treated from L1 stage throughout development to 24 hour young adult stage.**

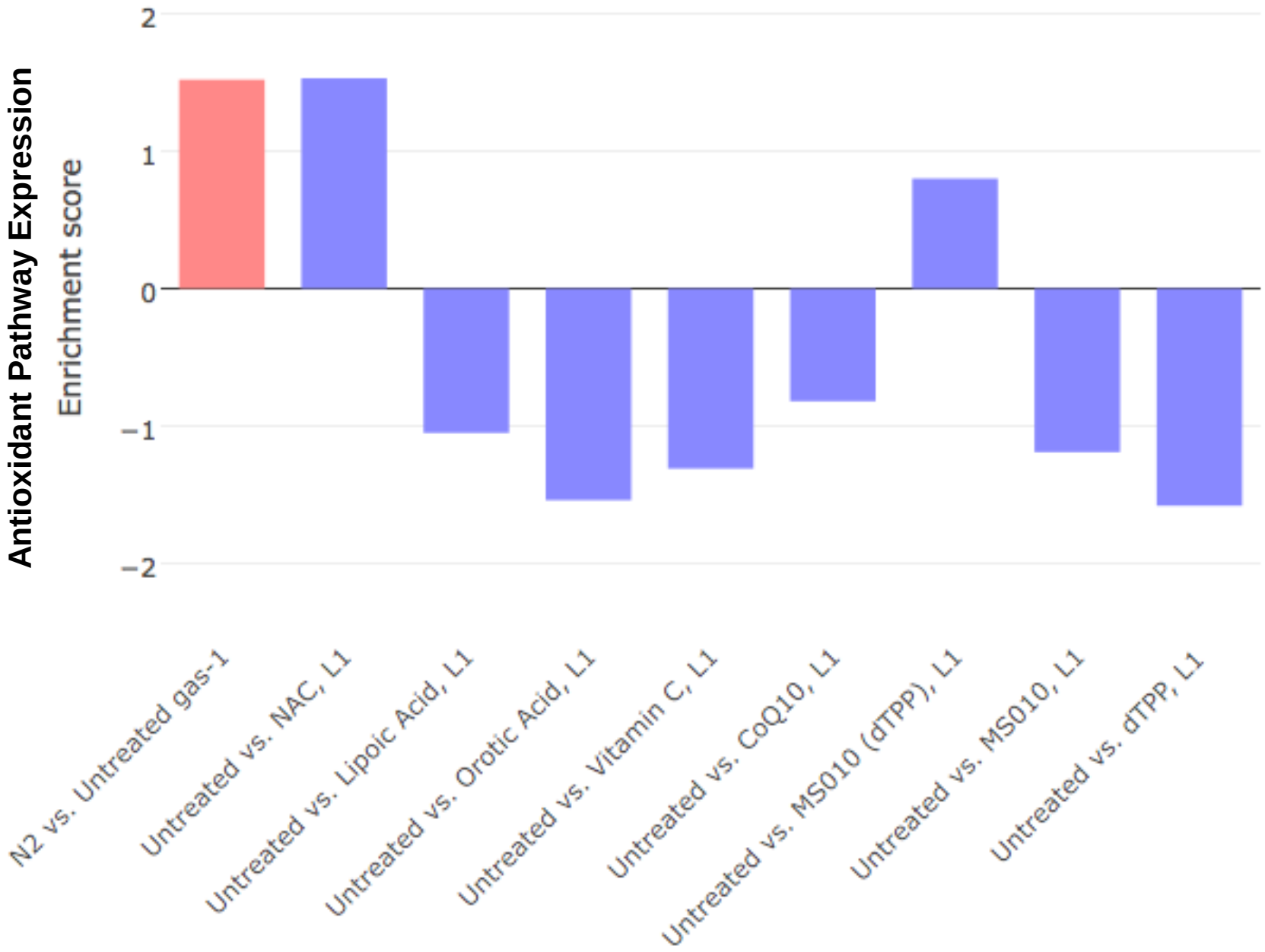
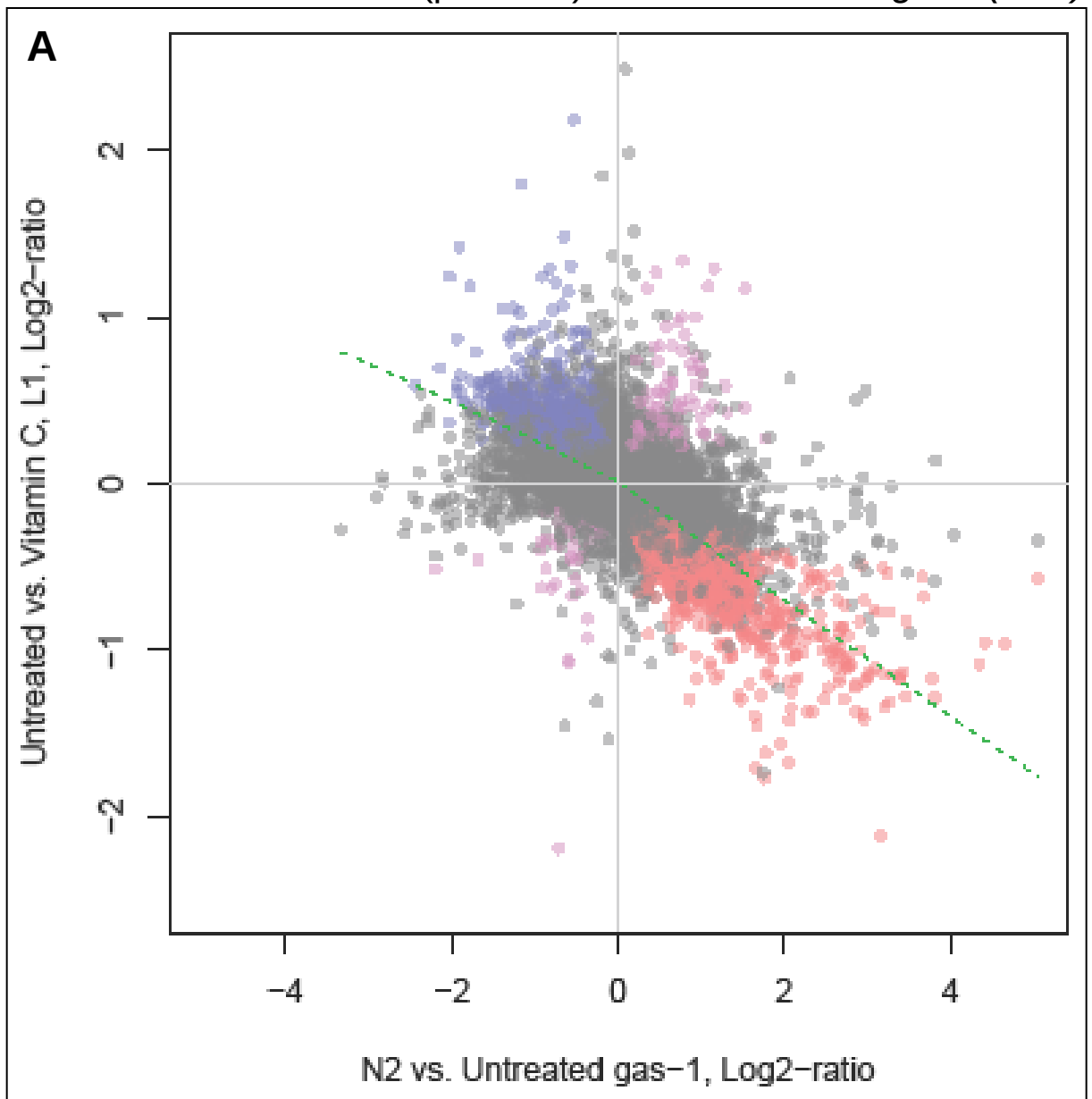


Fig S9. Vitamin C treatment effects on normalizing (A) global gene expression and (B) glutathione pathway expression in *gas-1(fc21)* worms treated from L1 stage throughout development to 24 hour young adult stage. Scatterplot shows the difference of group means, where each dot represents one gene. Colored dots indicate genes with significant changes ($p < 0.05$) in both untreated *gas-1(fc21)* (vs. N2) and *gas-1(fc21)* + vitamin C (vs untreated).



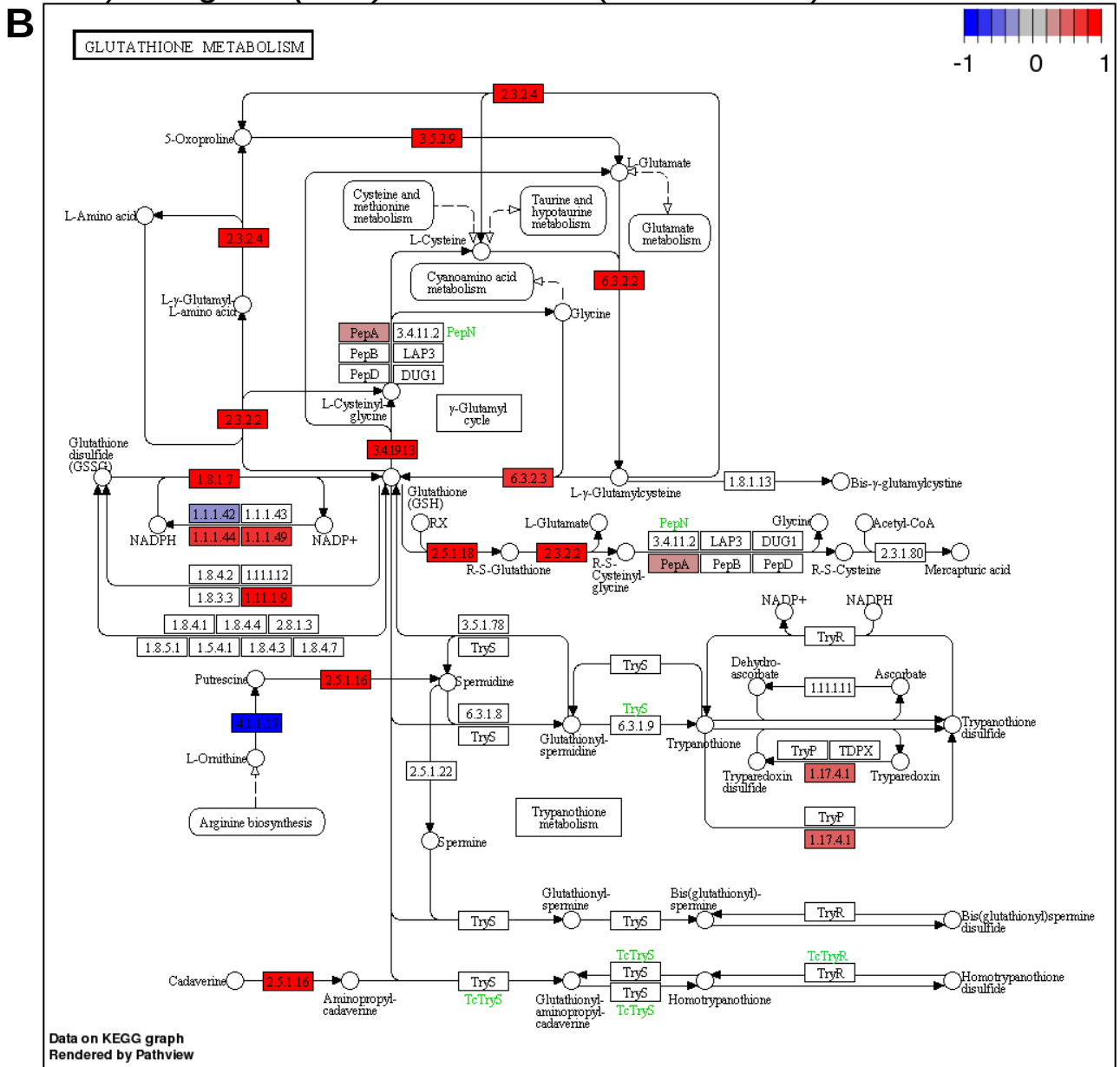


Fig S10. **Antioxidant drug effects on <u>upregulated KEGG pathways</u> in *gas-1(fc21)* worms treated from L1 stage throughout development to 24 hour young adult stage.**

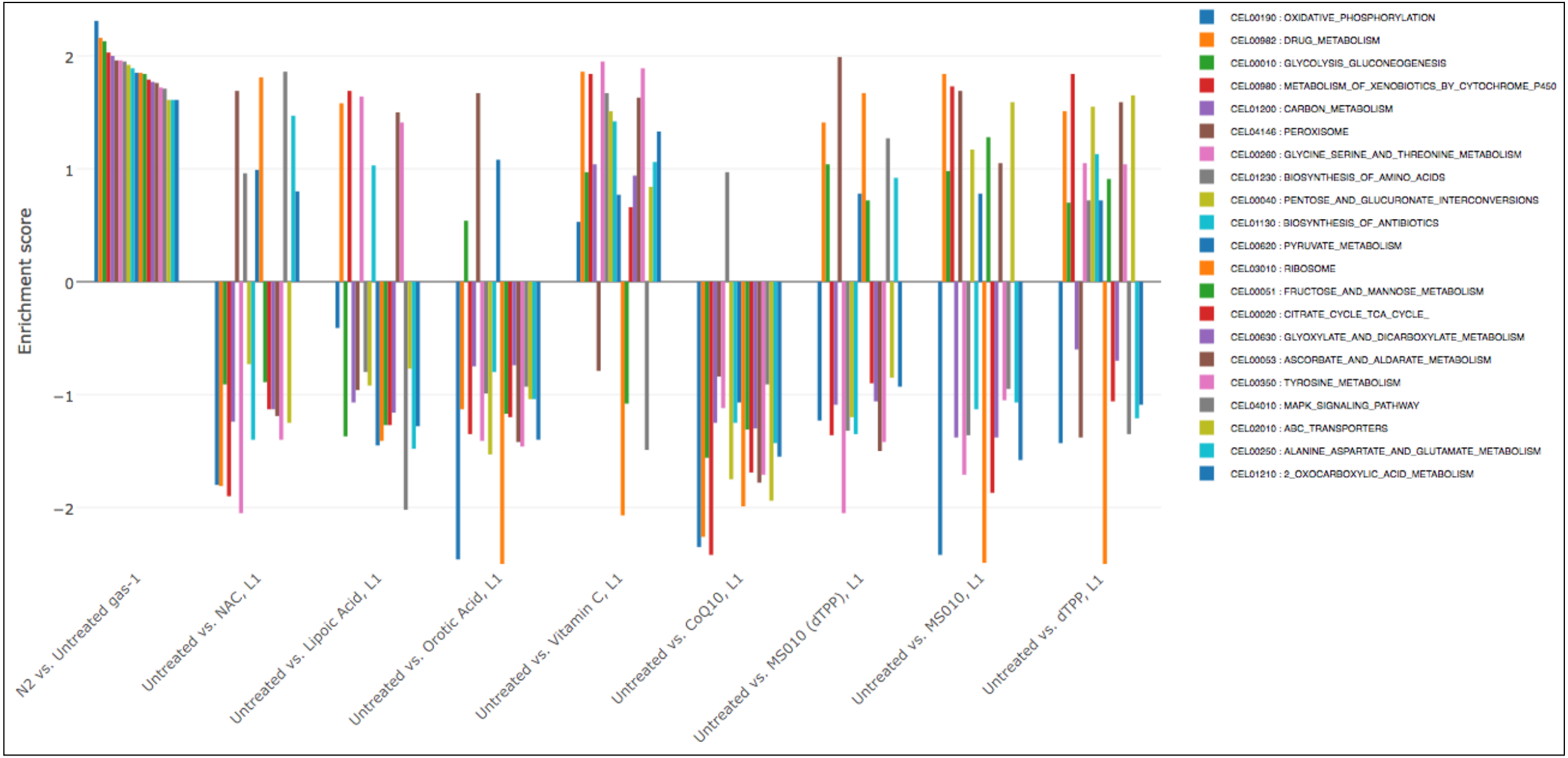


Fig S11. Antioxidant drug effects on <u>downregulated KEGG pathways</u> in *gas-1(fc21)* worms treated from L1 stage throughout development to 24 hour young adult stage.