SUPPLEMENTAL INFORMATION FOR

E1 of α-Ketoglutarate Dehydrogenase Defends *Mycobacterium tuberculosis* Against Glutamate Anaplerosis and Nitroxidative Stress

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Supplementary Figure Legends

Figure S1. HOAS participates in major metabolic and regulatory pathways in Mtb (A) Situation of HOAS and KDHC in Mtb tricarboxylic acid (TCA) cycle. GABA shunt, an alternate route from α-KG to succinate is shown as dotted line. (B) HOAS and KDHC are situated at the node of carbon and nitrogen metabolism. GarA controls glutamate metabolism.

Figure S2. Confirmation of *hoas* deletion and complementation of protein levels. (A) Southern blot design of Mtb genomic region with (I) native *hoas* (II) and the hygromycin resistance cassette after *hoas*' replacement. (B) Southern blot of digested Mtb DNA showing expected bands in Δ*hoas* candidates (Lanes 1-3) compared to WT (Lane 4). (C) Immunoblot analysis of HOAS protein in lysates from WT, Δ*hoas*, Δ*hoas*::*hoas*, Δ*hoas*::*E956Ahoas*, and Δ*hoas*::*E1038Ahoas* using antiserum against KGD (here called HOAS). Antiserum against DlaT was used as a loading control.

Figure S3. Kinetic characterization of HOAS mutants. (A) Michaelis Menten plot for 2-ketoglutarate decarboxylase activity of WT and E956A HOAS in the presence and absence of AcCoA (200 μM) determined by ferricyanide reductase assay described in methods. (B) Effect of allosteric regulator, AcCoA (0-1000 μM), on 2-ketoglutarate decarboxylase activity of WT and E1038A HOAS determined by ferricyanide reductase assay. (Inset) Michaelis-Menten plot for E1038A HOAS. Data points are averages of triplicates and error bars are standard deviation. Results are representative of at least three independent experiments. AcCoA allosteric regulation data were fit to equations described in ref. 16.

Figure S4. HOAS is dispensible for growth in glycerol or acetate. Growth curves of H37Rv WT, deletion mutant (Δ*hoas*), full length complement (Δ*hoa*s::*hoas*), and catalytically inactive mutant (Δ*hoas*::*E956Ahoas*) in a modified Sauton's minimal medium (SMM) supplemented with either (A) glycerol, (B) acetate, each at 0.2%. Data are from one experiment representative of three.

Figure S5. Mtb requires HOAS activity to persist in mice. (A) Pathology of lungs infected with WT, Δ*hoas*, Δ*hoas*::*hoas*, and Δ*hoas*::*E956Ahoas* at 217 days post infection. (B) Bacterial burden in spleens of mice infected with WT, Δ*hoas*, Δ*hoas*::*hoas*, and Δ*hoas*::*E956Ahoas*. Data are means ± SD of for five mice per time point in one experiment representative of two, except that four mice were studied on day 1 and one mouse infected with Δ*hoas*::*hoas* died before day 217.

Figure S6. Metabolism of [U^{13}C, ^{15}N] glutamate. (A) Uptake of [U^{13}C, ^{15}N] glutamate. Data are means ± SD (B) Abundance of ^{13}C-labeled central carbon metabolites. Data are from one experiment representative of at least two**.**

Figure S7. DlaT is essential for growth in glutamate. . (A) Growth of Δ*dlat* in Sauton's minimal medium containing acetate (0.2%), glutamate (0.2%), or glycerol (0.2%). (B) Survival of Δ*dlat* in glutamate (0.2%). All data are means ±SD of triplicates (C) Growth of Δ*pdhC* in Sauton's minimal medium containing acetate (0.2%), glutamate (0.2%), or glycerol (0.2%). Results are representative of at least two independent experiments.

Figure S8. Effect of glutamate on growth of Δ*hoas*::*E1038Ahoas* and Δ*dlat* **Mtb strains.** Mid-growth phase OD of (A) Δ*hoas*::*E1038Ahoas* in Sauton's minimal medium containing glycerol as the sole carbon source with varying amounts of additional glutamate;.(B) Δ*dlat* in Sauton's minimal medium containing glycerol as the sole carbon source with varying amounts of additional glutamate All results are representative of at least two independent experiments.

Figure S9. Rapid Fire Mass spectrometric detection and characterization of KDH reaction. (a) EIC spectra at m/z = 866.12 showing succinyl CoA formation in the KDH reaction progress curve. First 3 sets

are standard curve, next 6 sets are KGDH reaction, next 6 sets are reaction without 2-ketoglutarate, and next 6 sets are reaction without NAD^+. (b) Zoomed in version on the first set of peaks showing time course of reaction progress. First 6 samples are the unactivated and next 6 are the activated reaction. (c) Comparison of natural abundance isotopic distribution of standard and product. (d) EIC at m/z = 866.12 for the Michaelis-Menten kinetics reaction. (e) Zoomed in version on set of peaks (unactivated and activated) obtained with various concentrations of 2-ketoglutarate as indicated.

Figure S10. Kinetic characterization of KDH reaction. (a) Progress curves of activated (open symbols) and unactivated (filled symbols) KDH reaction (circles) and absence of 2-ketoglutarate (triangles) or absence of NAD^+ (squares). (b) Michaelis Menten plot of 2-ketogluatarate for activated (open circles) and unactivated (filled circles) reaction. (c) Michaelis Menten plot of CoA for activated (open circles) and unactivated (filled circles) reaction. (d) Michaelis Menten plot of NAD^+ for activated (open circles) and unactivated (filled circles) reaction. The fitted kinetic parameters are summarized in Table 1. Data points are averages of experimental triplicates and error bars are standard deviation. Solid lines in (a) are spline curves and in (b, c, and d) are regression fit to equation 1. Each experiment was repeated at least 3 times.

Figure S11. Mtb metabolome upon loss of KDHC function under various growth conditions. (A) Quantitation of intracellular metabolites with major changes when exposed to glutamate (0.05%) and acetate (0.2%) in Sauton's minimal medium. Quantitation of secreted metabolites with major changes when Mtb strains were exposed to (B) glycerol (0.2%) or (C) glutamate (0.2%) in Sauton's minimal medium. Quantitation of secreted metabolites with major changes when exposed to (D) acetate (0.2%) (E) glutamate (0.05%) and acetate (0.2%) in Sauton's minimal medium. Means ± SD for triplicate experimental samples. $*p < 0.01$. Results are representative of at least two independent experiments.

Figure S12. SSA inhibits growth of Mtb. OD_{580} at day 10 of Mtb strains in Sauton's minimal medium containing acetate (0.2%) or glycerol (0.2%) as the sole carbon source with varying amounts of additional succinate semialdehyde. Results are representative of at least two independent experiments.

Figure S13. HOAS activity is dispensable for protection against reactive oxygen intermediates, acid, and starvation. Survival of WT, Δ*hoas*, *Δhoas::hoas*, and *Δhoas::E956Ahoas* after (A) 4-hour exposure to H_2O_2 in 7H9 complete medium, and (B) 6 day incubation in 7H9 complete medium at pH 4.5. (C) Survival of the four strains in PBS with 0.05% tyloxapol detergent. Data are means ± SD of triplicates in one experiment representative of three.

Figure S14. Rapid Fire Mass spectrometric detection and characterization of HOAS dependent peroxidase. (a) EIC spectra at m/z = 866.12 showing succinyl CoA formation in the KGDH reaction progress curve. First 3 sets are standard curve, next 6 sets are KGDH reaction, next 6 sets are reaction without 2-ketoglutarate, and next 6 sets are reaction without tBuOOH. (b) Zoomed in version on the first set of peaks showing time course of reaction progress. First 6 samples are the unactivated and next 6 are the activated reaction. (c) EIC at m/z = 866.12 for the Michaelis-Menten kinetics reaction. (d) Zoomed in version on set of peaks (unactivated and activated) obtained with various concentrations of 2-ketoglutarate as indicated.

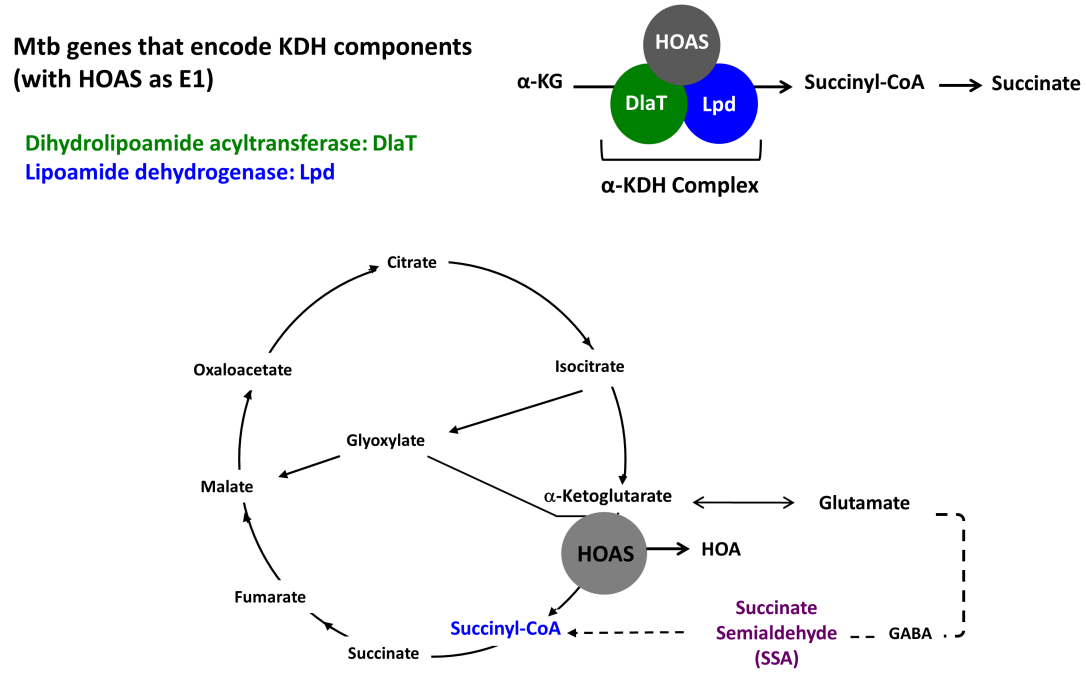
Figure S15. Kinetic characterization of HOAS dependent peroxidase reaction. Michaelis Menten plot of (a) 2-ketogluatarate for activated (open circles) and unactivated (filled circles) reaction (b) CoA for activated (open circles) and unactivated (filled circles) reaction (c) tBuOOH for activated (open circles) and unactivated (filled circles) reaction. The fitted kinetic parameters are summarized in Table 1. Data points are averages of experimental triplicates and error bars are standard deviations. Solid lines are regression fit to equation 1. Each experiment was repeated at least 3 times.

Figure S16. Rapid Fire Mass spectrometric detection and characterization of AceE peroxidase reaction. (a) EIC spectra at m/z = 808.12 showing acetyl CoA formation in the progress curve. First 3 sets are standard curve, next 6 sets are reaction, next 6 sets are reaction without pyruvate, and next 6 sets are reaction without tBuOOH (b) Zoomed in version on the first set of peaks showing time course of reaction progress (c) Comparison of natural abundance isotopic distribution of standard and product. (d) EIC at m/z = 808.12 for the Michaelis-Menten kinetics reaction. (e) Zoomed in version on set of peaks obtained with various concentrations of pyruvate as indicated.

Figure S17. Kinetic characterization of AceE dependent peroxidase reaction. Michaelis Menten plot of (a) pyruvate (b) CoA (c) tBuOOH. The fitted kinetic parameters are summarized in Table 1. Data points are averages of 6 experimental replicates and error bars are standard deviations. Solid lines are regression fit to equation 1. Each experiment was repeated at least 3 times

Figure S1

A.


Mtb genes that encode KDH components
(with HOAS as E1)
Dihydrolipoamide acyltransferase: DlaT
Lipoamide dehydrogenase: Lpd
HOAS
α-KG
DlaT
Lpd
Succinyl-CoA
Succinate
α-KDH Complex
Citrate
Isocitrate
Glyoxylate
Malate
Oxaloacetate
α-Ketoglutarate
Glutamate
HOAS
HOA
Fumarate
Succinyl-CoA
Succinate Semialdehyde (SSA)
GABA
Succinate

B.

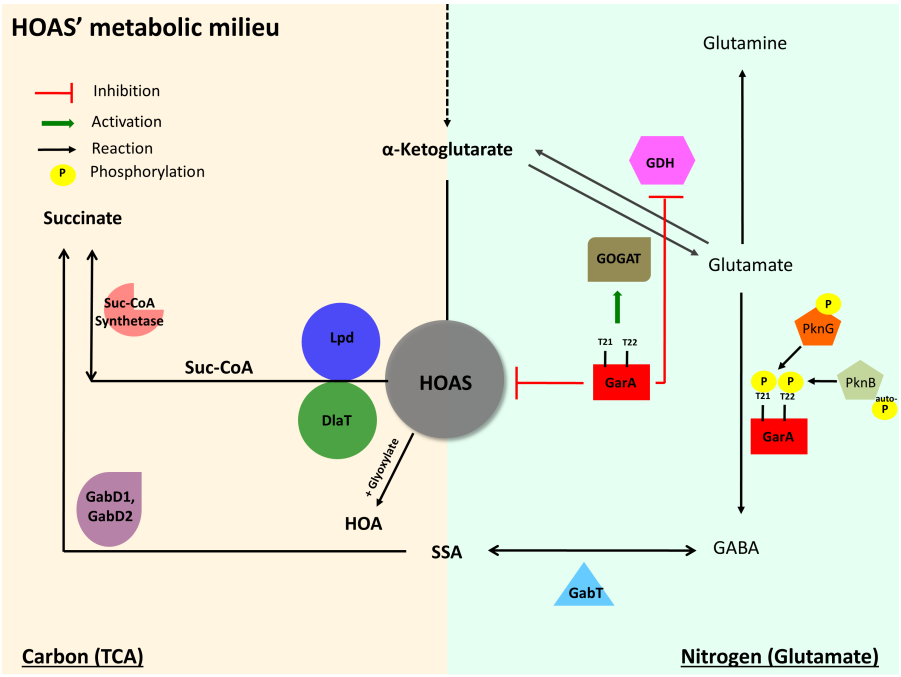
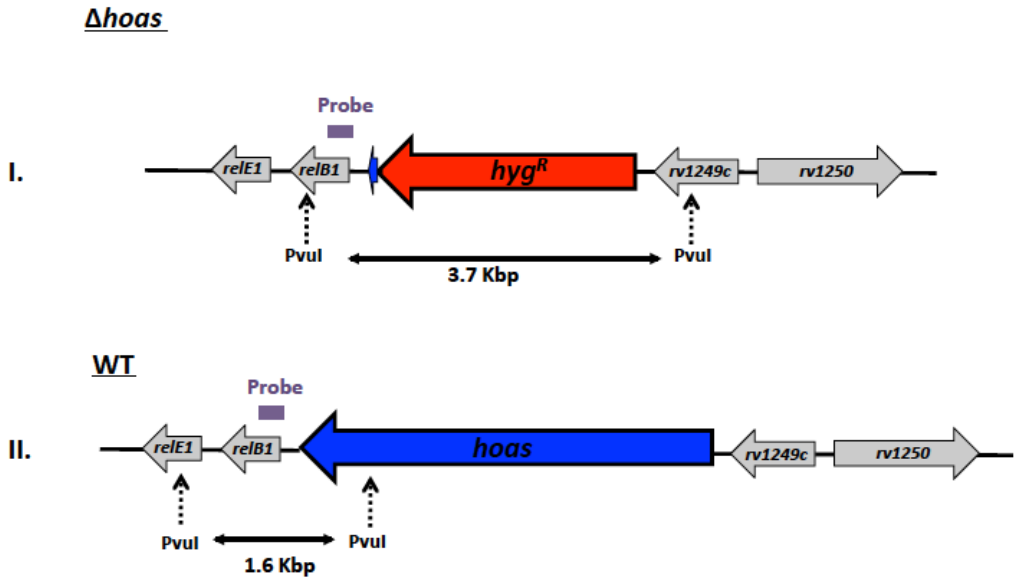

HOAS' metabolic milieu
Inhibition
Activation
Reaction
Phosphorylation
Succinate
Suc-CoA Synthetase
Suc-CoA
Lpd
DlaT
HOAS
GabD1, GabD2
+ Glyoxylate
HOA
SSA
GABA
GabT
α-Ketoglutarate
GDH
Glutamine
Glutamate
GOGAT
T21 T22
GarA
PknG
PknB
auto-P
Carbon (TCA)
Nitrogen (Glutamate)

Figure S2

A.



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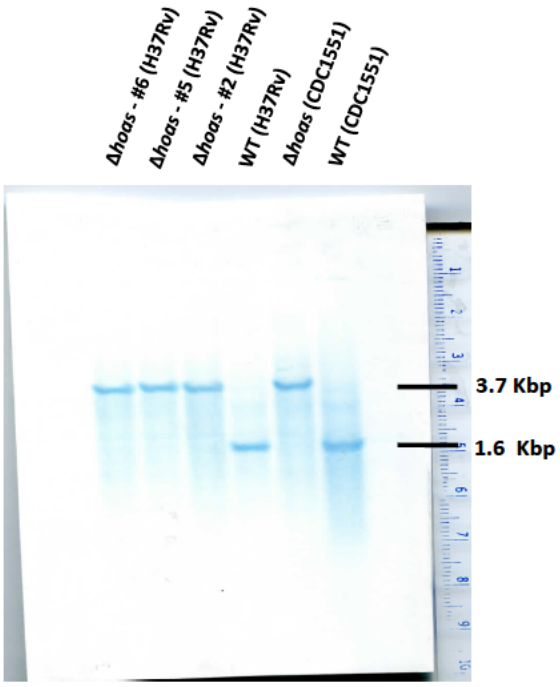


C.

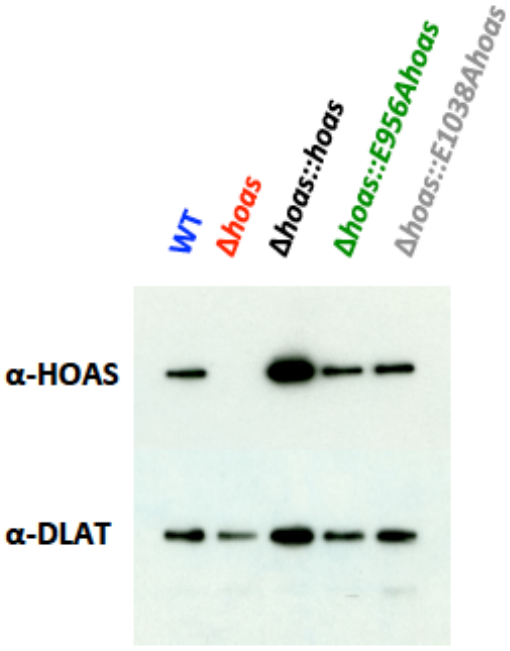
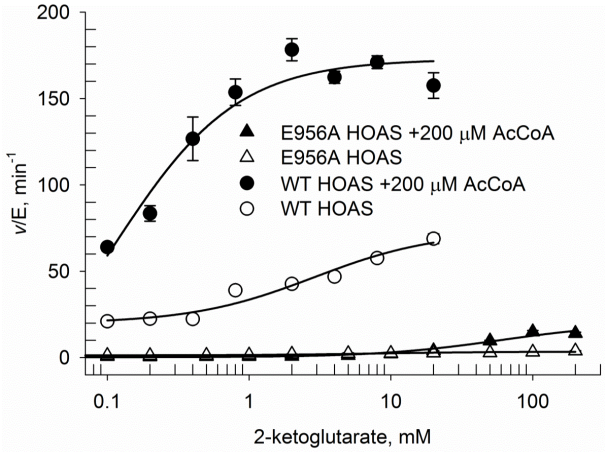


Figure S3

A.


200
150
100
50
0
v/E, min-1
E956A HOAS +200 μM AcCoA
E956A HOAS
WT HOAS +200 μM AcCoA
WT HOAS
0.1
1
10
100
2-ketoglutarate, mM

B.

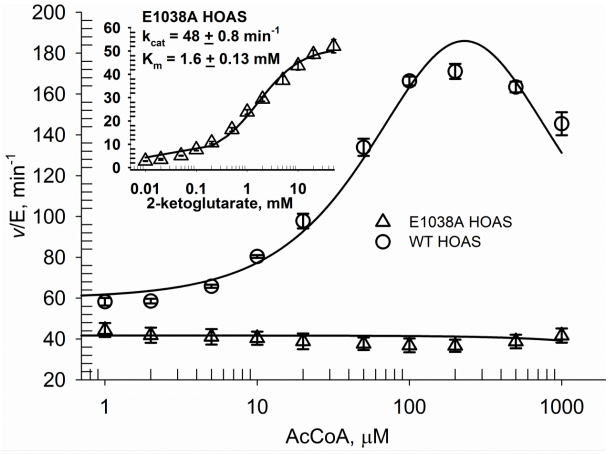
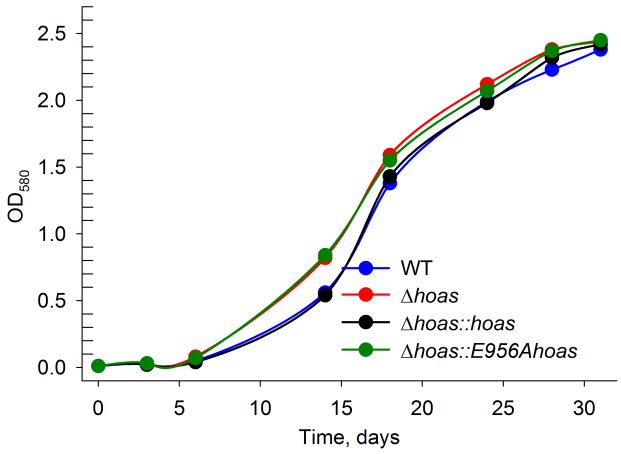

E1038A HOAS
kcat = 48 ± 0.8 min-1
Km = 1.6 ± 0.13 mM
60
50
40
30
20
10
0
0.01
0.1
1
10
2-ketoglutarate, mM
200
180
160
140
120
100
80
60
40
20
v/E, min-1
E1038A HOAS
WT HOAS
1
10
100
1000
AcCoA, μM

Figure S4

A.


2.5
2.0
1.5
1.0
0.5
0.0
OD580
0
5
10
15
20
25
30
Time, days
WT
Δhoas
Δhoas::hoas
Δhoas::E956Ahoas

B.

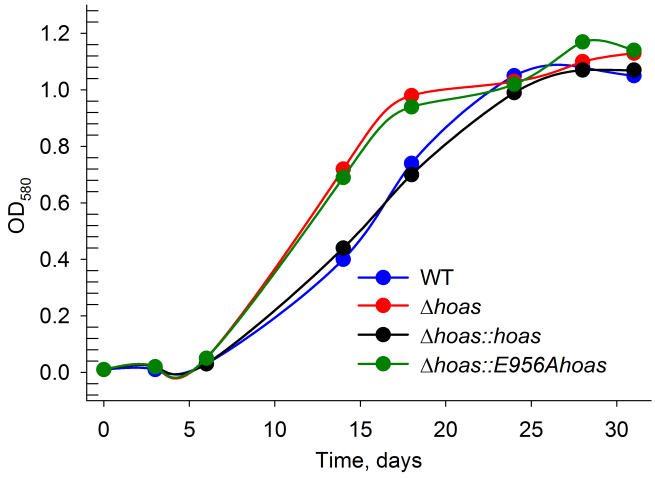
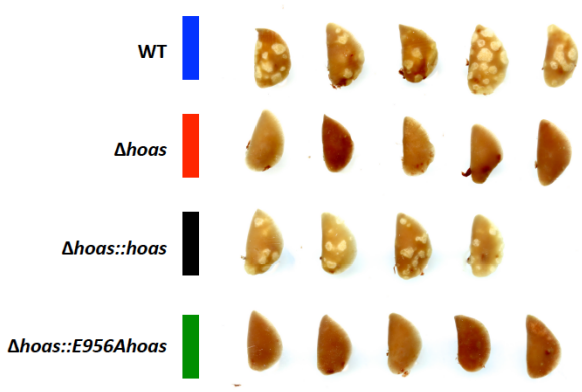

1.2
1.0
0.8
0.6
0.4
0.2
0.0
OD580
0
5
10
15
20
25
30
Time, days
WT
Δhoas
Δhoas::hoas
Δhoas::E956Ahoas

Figure S5

A.


WT
Δhoas
Δhoas::hoas
Δhoas::E956Ahoas

B.

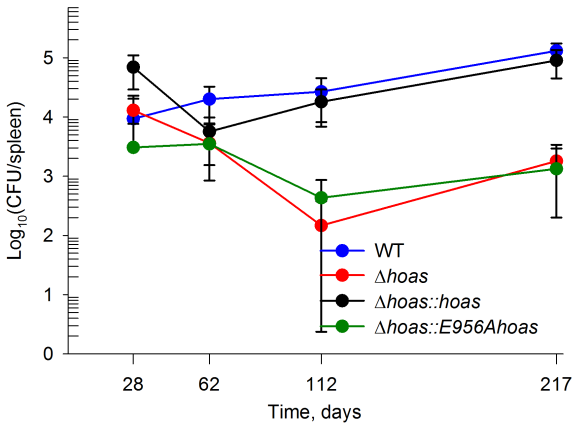
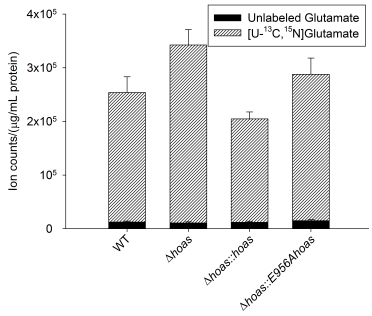

Log10(CFU/spleen)
0
1
2
3
4
5
28
62
112
217
Time, days
WT
Δhoas
Δhoas::hoas
Δhoas::E956Ahoas

Figure S6

A.


Unlabeled Glutamate
[U-13C, 15N]Glutamate
Ion counts/(µg/mL protein)
4x10^5
3x10^5
2x10^5
10^5
0
WT
Δhoas
Δhoas::hoas
Δhoas::E956Ahoas

B.

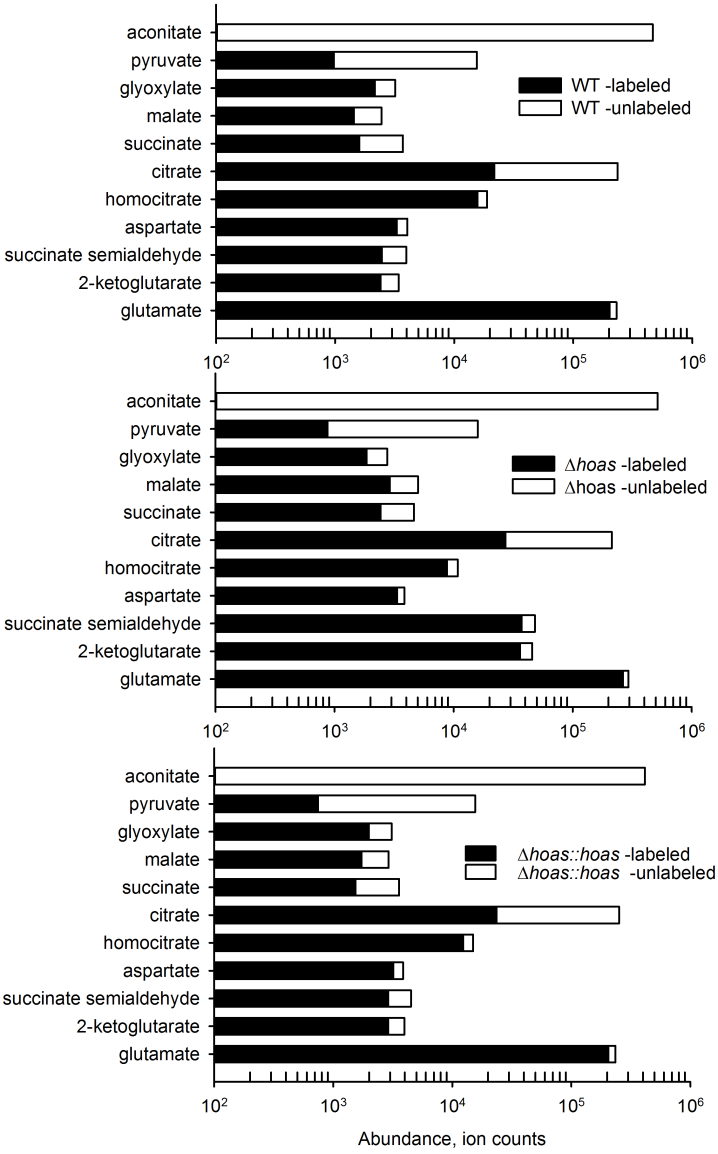
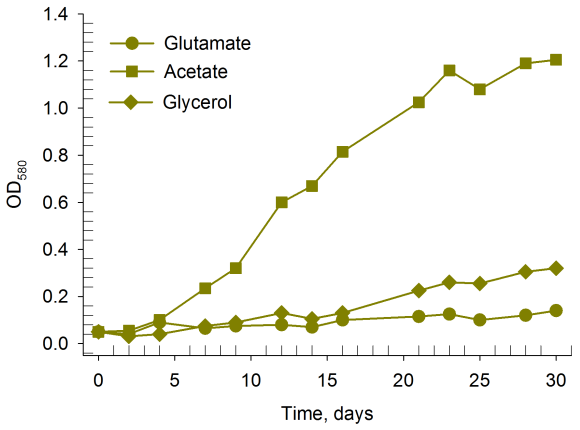
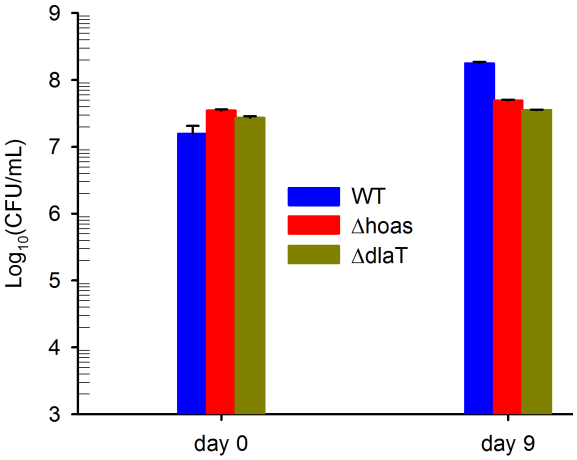

aconitate
pyruvate
glyoxylate
malate
succinate
citrate
homocitrate
aspartate
succinate semialdehyde
2-ketoglutarate
glutamate
WT -labeled
WT -unlabeled
Δhoas -labeled
Δhoas -unlabeled
Δhoas::hoas -labeled
Δhoas::hoas -unlabeled
10^2
10^3
10^4
10^5
10^6
Abundance, ion counts

Figure S7

A.



B.



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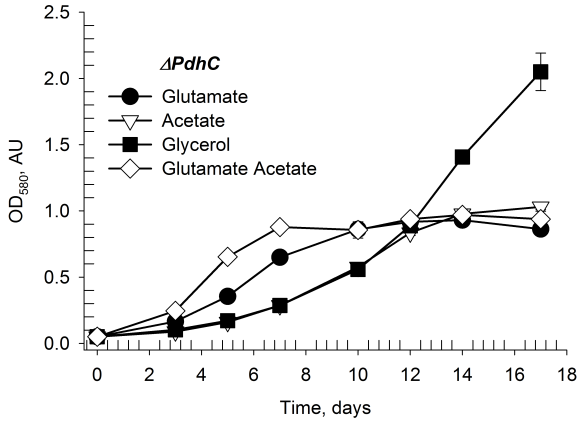
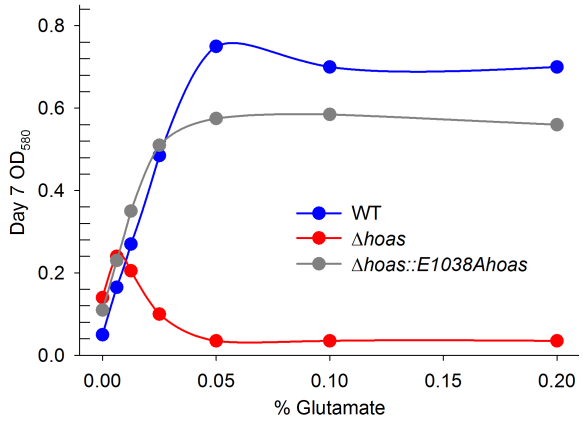


Figure S8

A.


0.8
0.6
0.4
0.2
0.0
Day 7 OD580
WT
Δhoas
Δhoas::E1038Ahoas
0.00
0.05
0.10
0.15
0.20
% Glutamate

B.

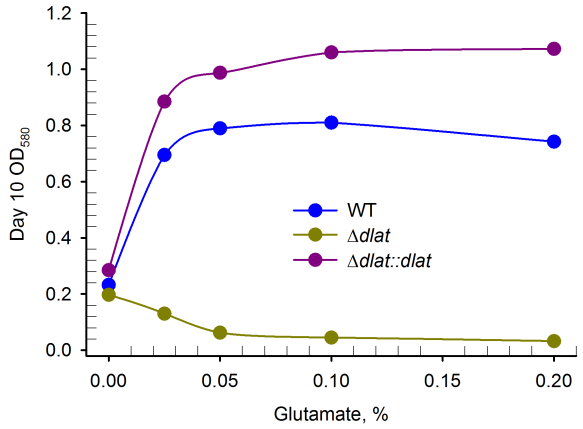

1.2
1.0
0.8
0.6
0.4
0.2
0.0
Day 10 OD580
WT
Δdlat
Δdlat::dlat
0.00
0.05
0.10
0.15
0.20
Glutamate, %

Figure S9

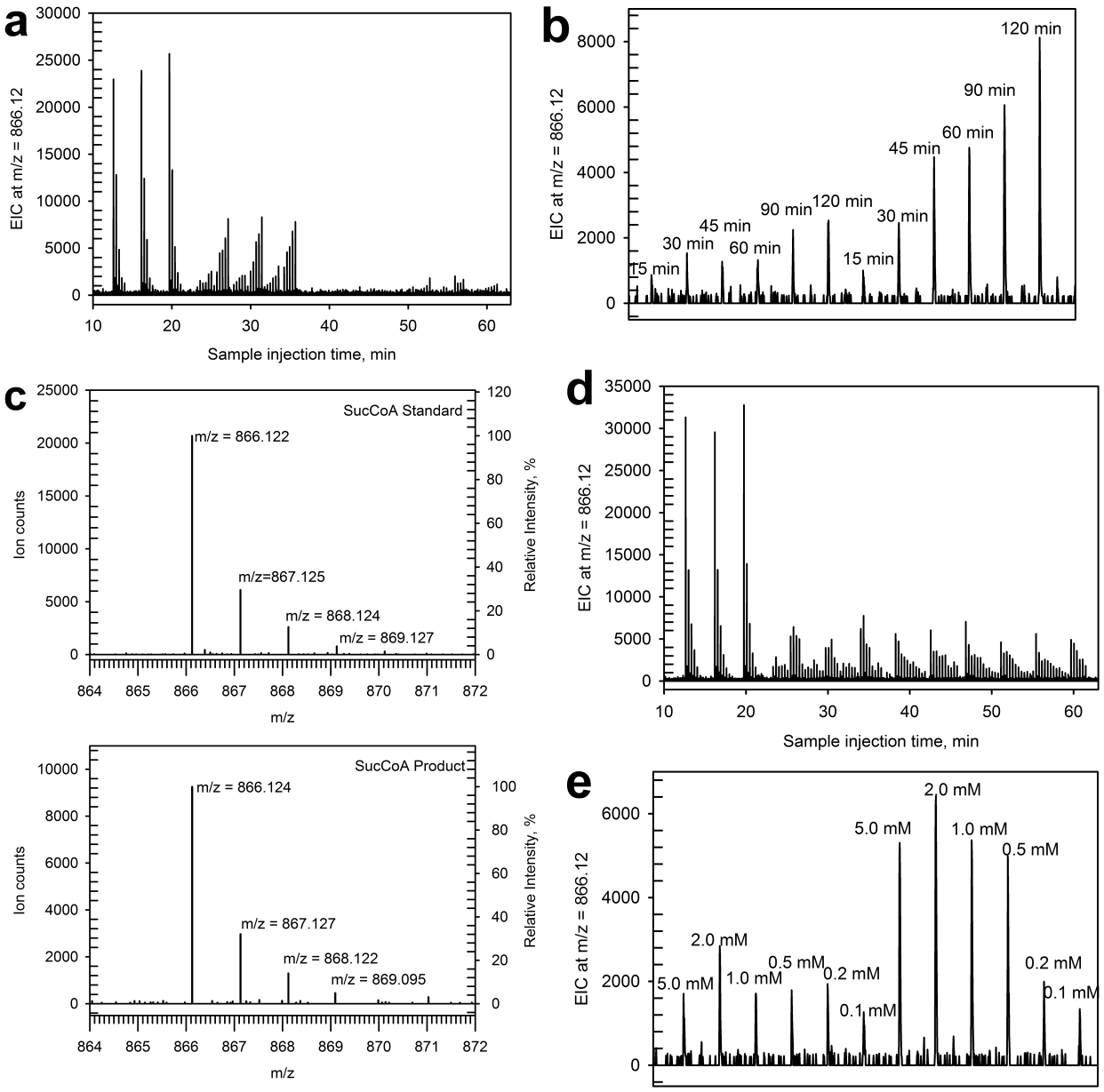

a
EIC at m/z = 866.12
Sample injection time, min
b
EIC at m/z = 866.12
15 min
30 min
45 min
60 min
90 min
120 min
c
SucCoA Standard
m/z = 866.122
m/z=867.125
m/z = 868.124
m/z = 869.127
Ion counts
Relative Intensity, %
m/z
SucCoA Product
m/z = 866.124
m/z = 867.127
m/z = 868.122
m/z = 869.095
d
EIC at m/z = 866.12
Sample injection time, min
e
EIC at m/z = 866.12
5.0 mM
2.0 mM
1.0 mM
0.5 mM
0.2 mM
0.1 mM

Figure S10

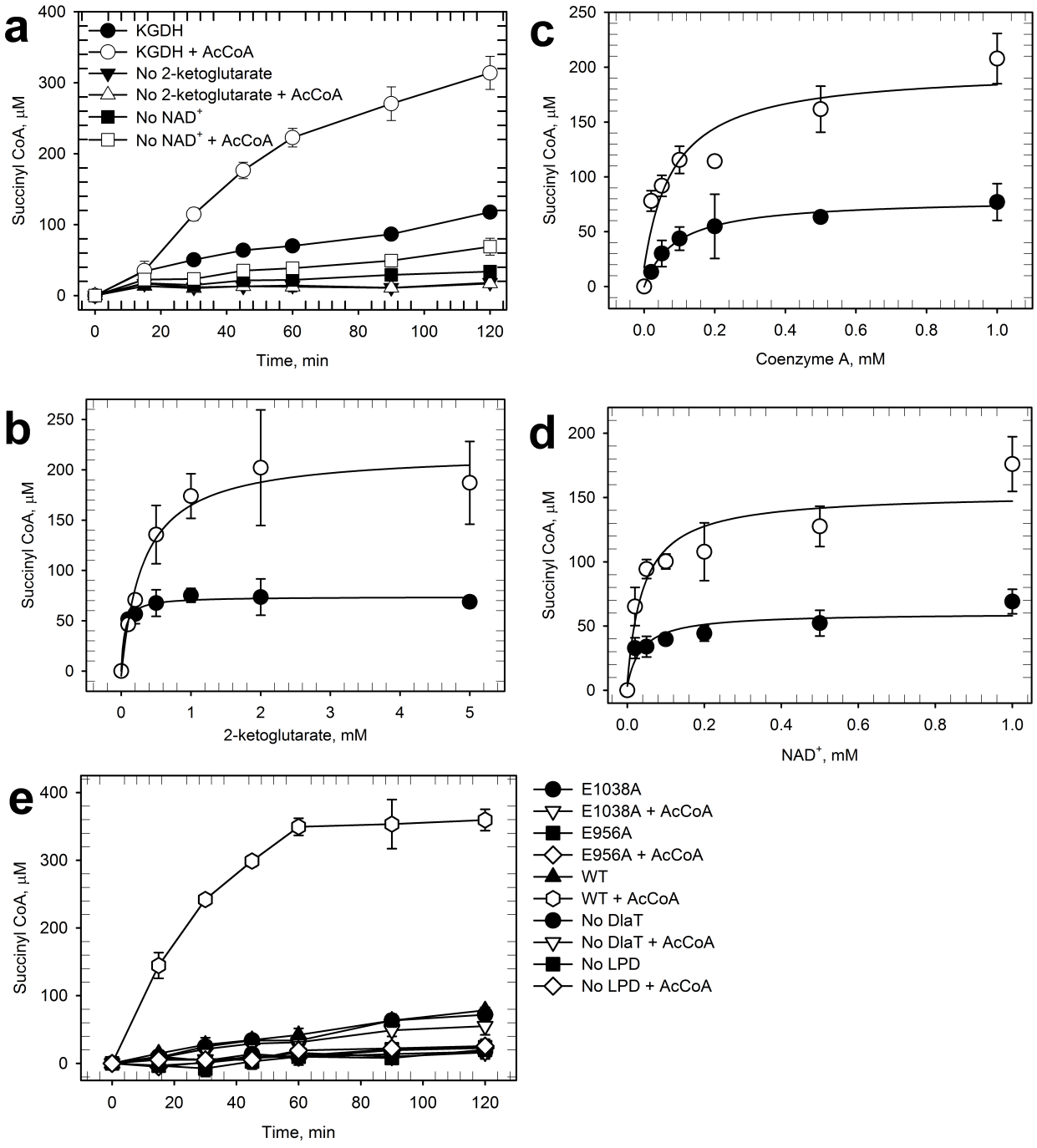

a
KGDH
KGDH + AcCoA
No 2-ketoglutarate
No 2-ketoglutarate + AcCoA
No NAD+
No NAD+ + AcCoA
Succinyl CoA, µM
Time, min
b
Succinyl CoA, µM
2-ketoglutarate, mM
c
Succinyl CoA, µM
Coenzyme A, mM
d
Succinyl CoA, µM
NAD+, mM
e
Succinyl CoA, µM
Time, min
E1038A
E1038A + AcCoA
E956A
E956A + AcCoA
WT
WT + AcCoA
No DlaT
No DlaT + AcCoA
No LPD
No LPD + AcCoA

Figure S11

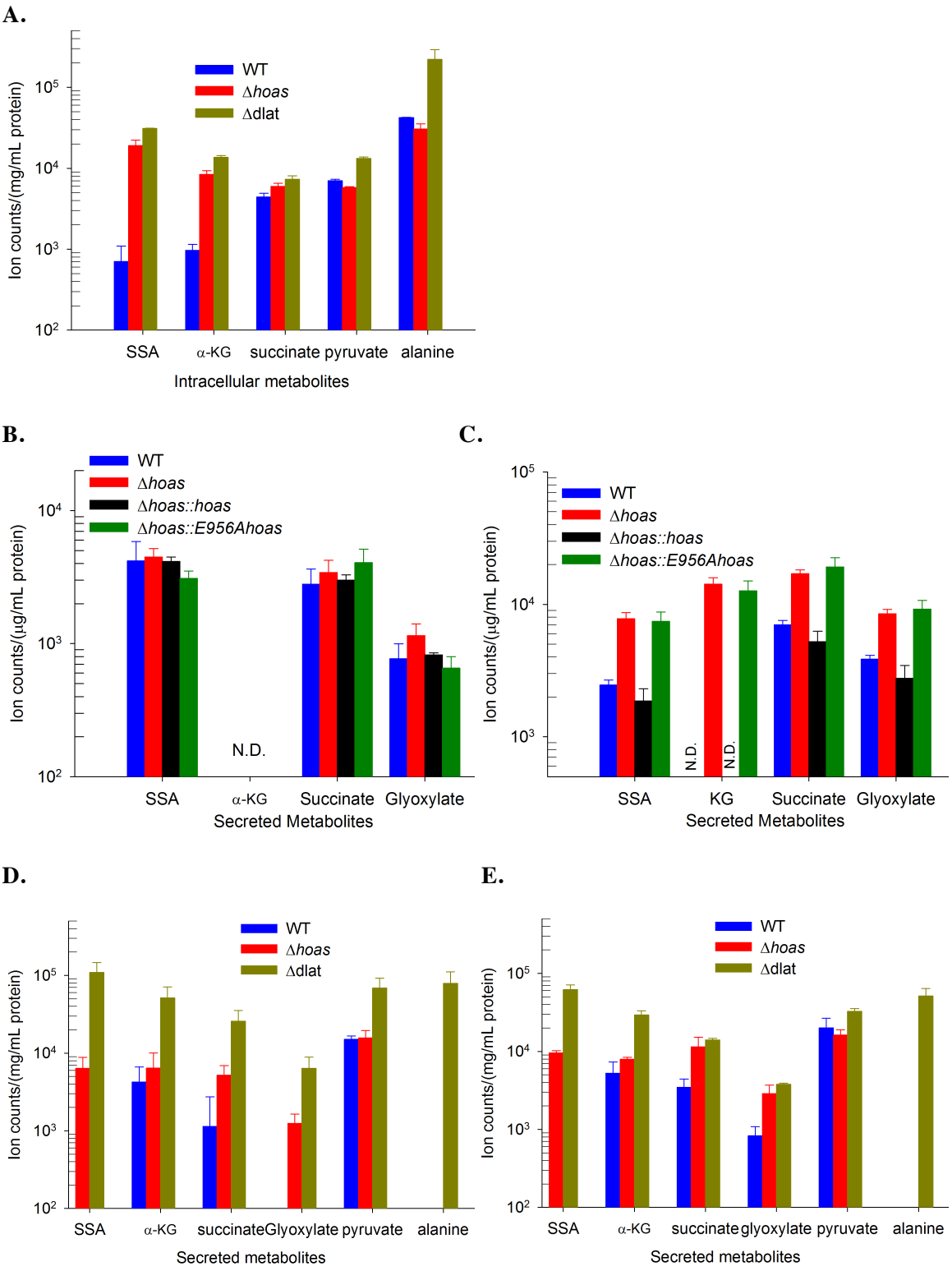

A.
WT
Δhoas
Δdlat
Ion counts/(mg/mL protein)
SSA
α-KG
succinate
pyruvate
alanine
Intracellular metabolites
B.
WT
Δhoas
Δhoas::hoas
Δhoas::E956Ahoas
Ion counts/(μg/mL protein)
N.D.
SSA
α-KG
Succinate
Glyoxylate
Secreted Metabolites
C.
WT
Δhoas
Δhoas::hoas
Δhoas::E956Ahoas
Ion counts/(μg/mL protein)
N.D.
N.D.
SSA
KG
Succinate
Glyoxylate
Secreted Metabolites
D.
WT
Δhoas
Δdlat
Ion counts/(mg/mL protein)
SSA
α-KG
succinate
Glyoxylate
pyruvate
alanine
Secreted metabolites
E.
WT
Δhoas
Δdlat
Ion counts/(mg/mL protein)
SSA
α-KG
succinate
glyoxylate
pyruvate
alanine
Secreted metabolites

Figure S12

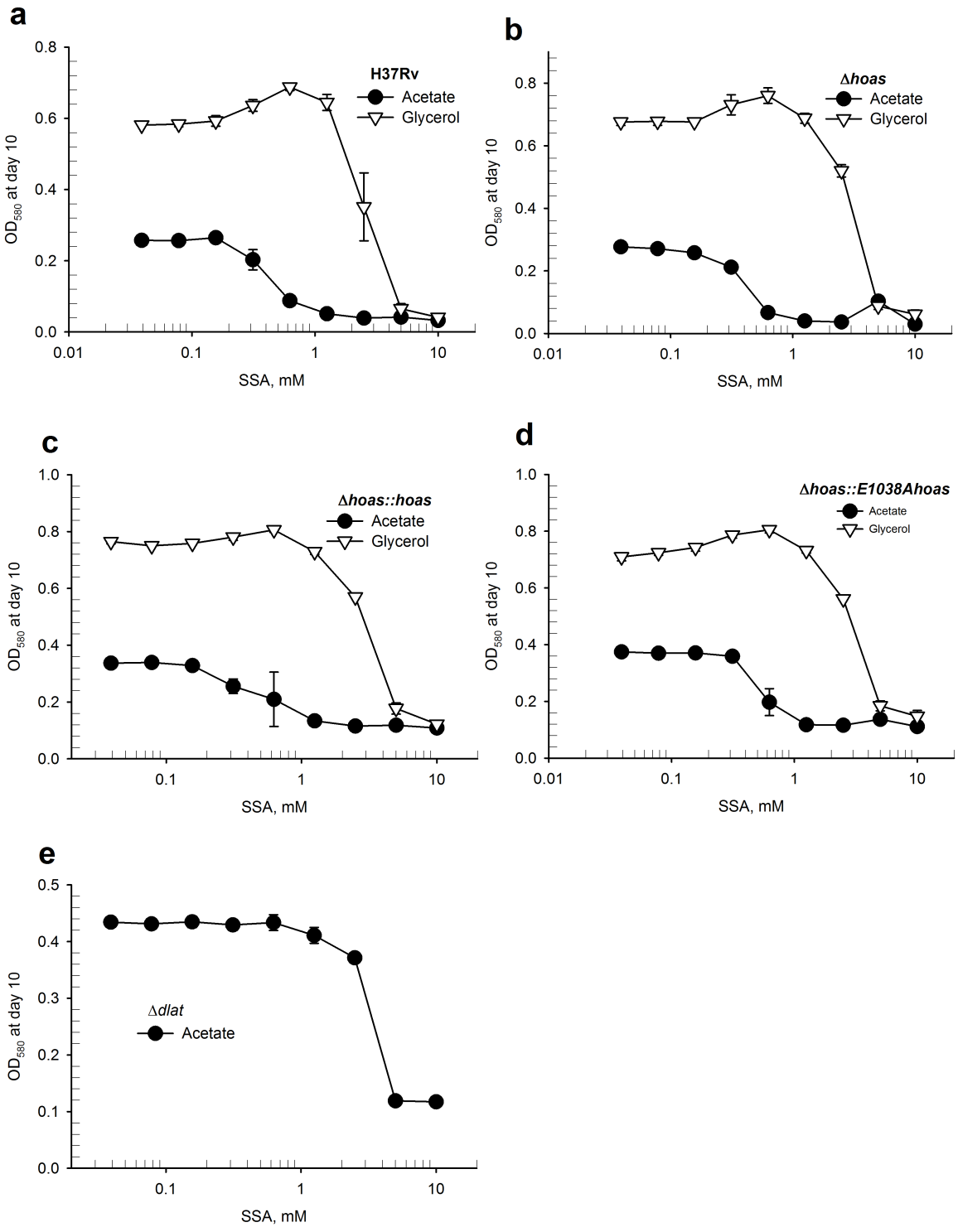
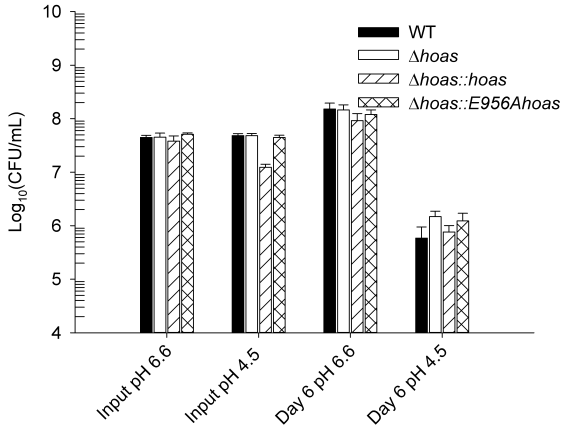
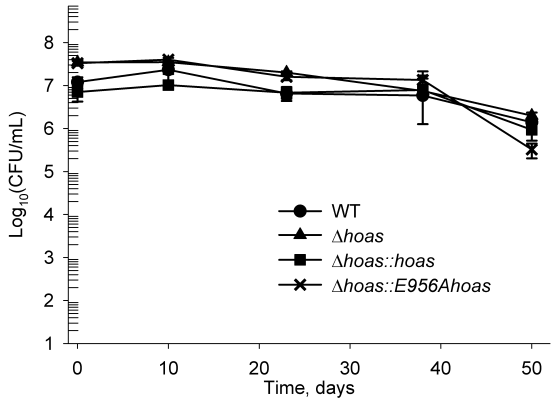

a
H37Rv
Acetate
Glycerol
OD580 at day 10
SSA, mM
b
Δhoas
Acetate
Glycerol
OD580 at day 10
SSA, mM
c
Δhoas::hoas
Acetate
Glycerol
OD580 at day 10
SSA, mM
d
Δhoas::E1038Ahoas
Acetate
Glycerol
OD580 at day 10
SSA, mM
e
Δdlat
Acetate
OD580 at day 10
SSA, mM

Figure S13

A



B



C

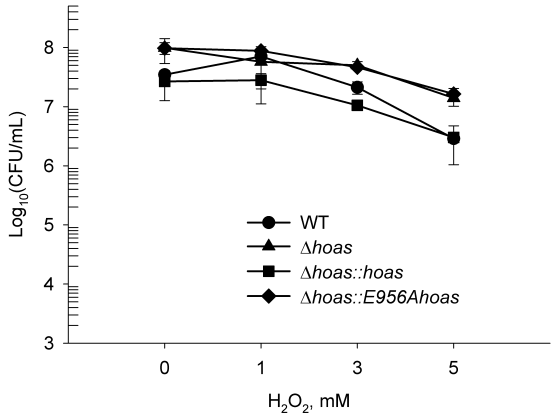


Figure S14

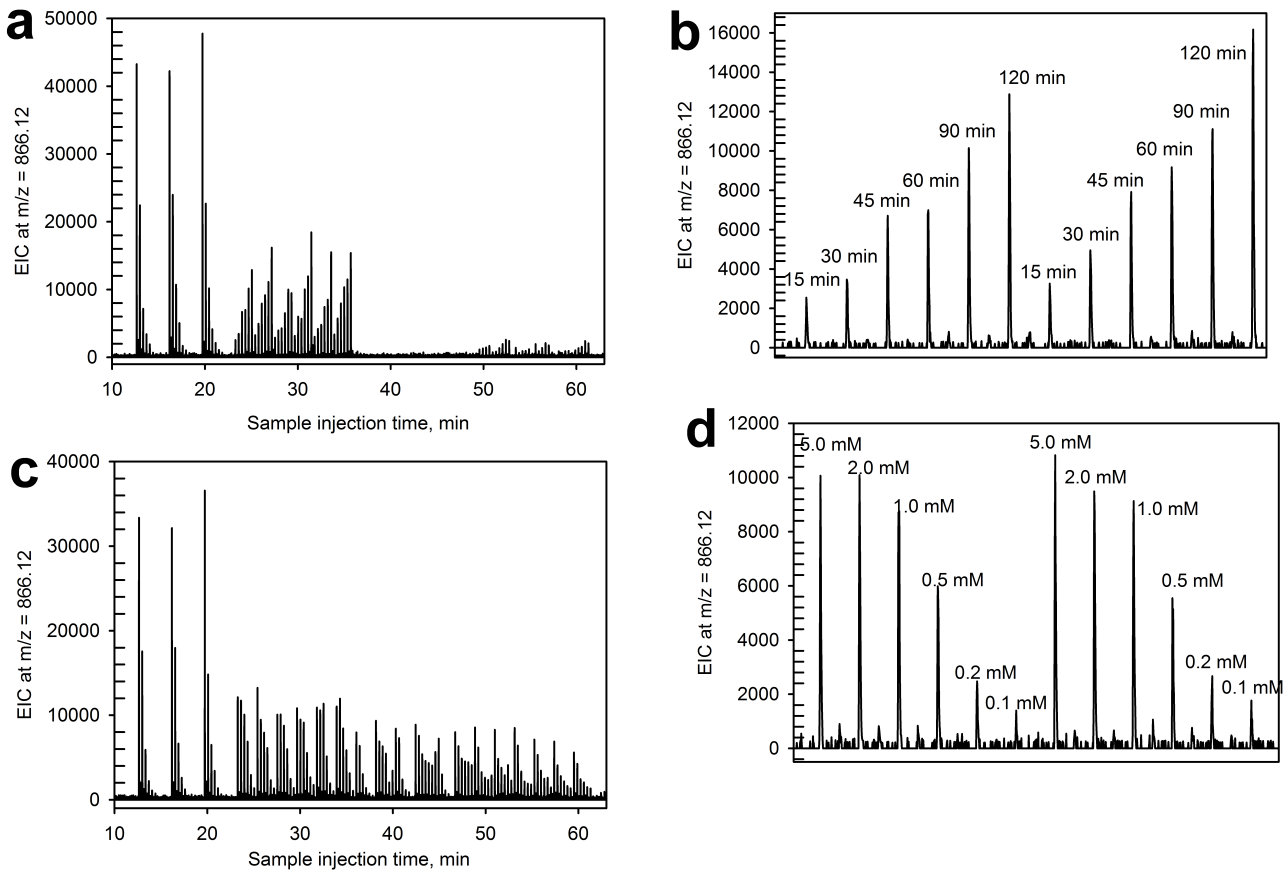

a
EIC at m/z = 866.12
Sample injection time, min
b
EIC at m/z = 866.12
15 min
30 min
45 min
60 min
90 min
120 min
c
EIC at m/z = 866.12
Sample injection time, min
d
EIC at m/z = 866.12
5.0 mM
2.0 mM
1.0 mM
0.5 mM
0.2 mM
0.1 mM

Figure S15

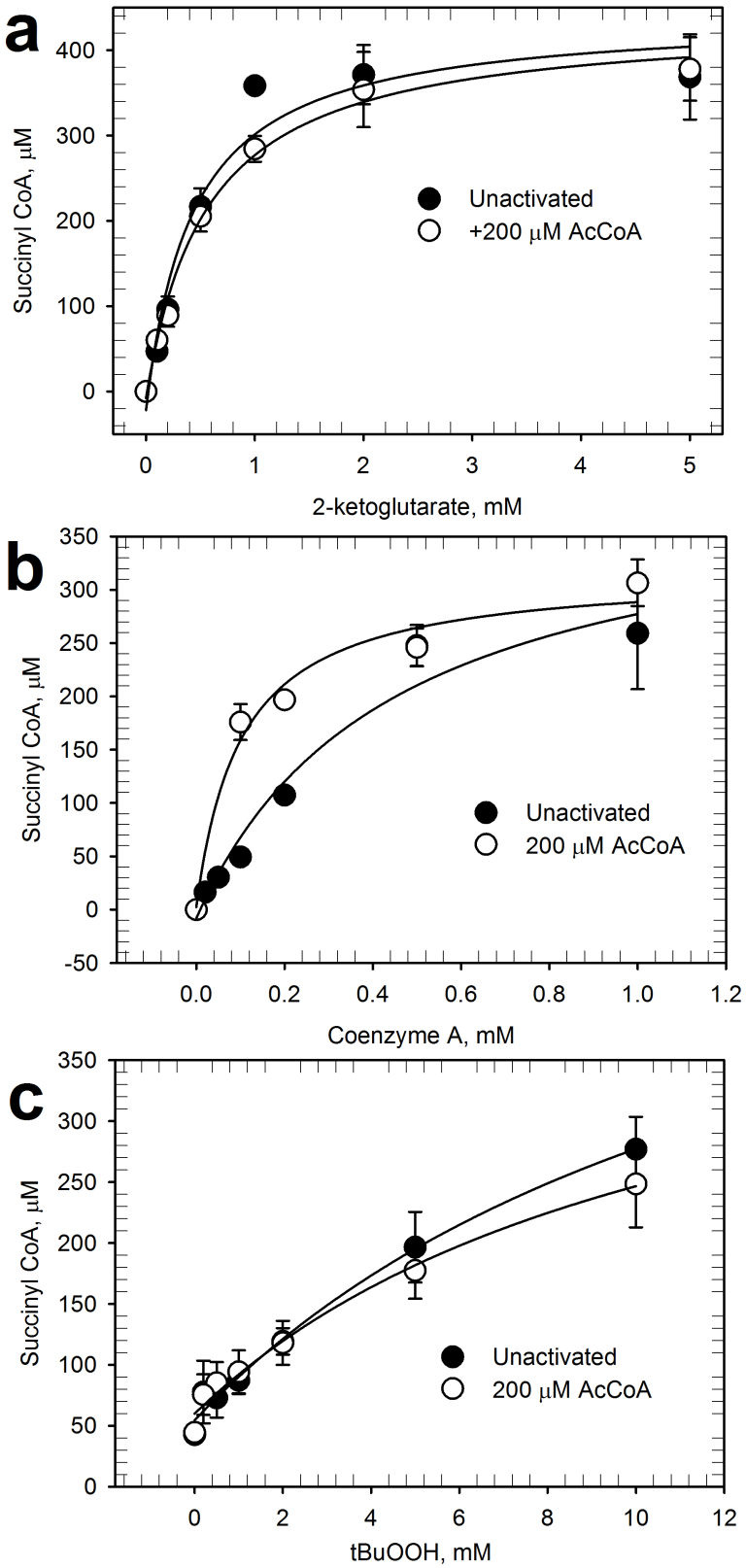

a
Succinyl CoA, μM
400
300
200
100
0
0
1
2
3
4
5
2-ketoglutarate, mM
Unactivated
+200 μM AcCoA
b
Succinyl CoA, μM
350
300
250
200
150
100
50
0
-50
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Coenzyme A, mM
Unactivated
200 μM AcCoA
c
Succinyl CoA, μM
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
tBuOOH, mM
Unactivated
200 μM AcCoA

Figure S16

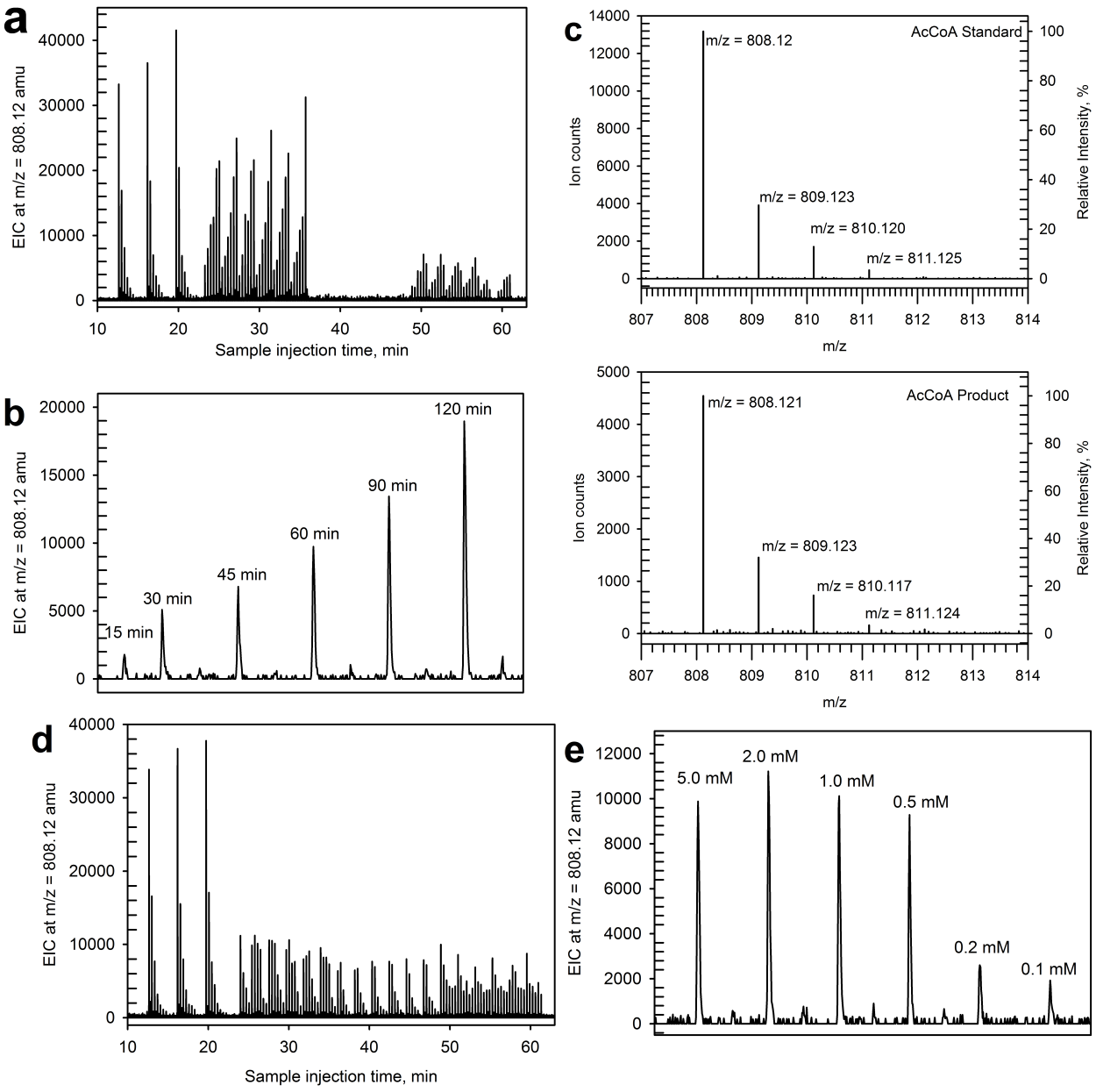
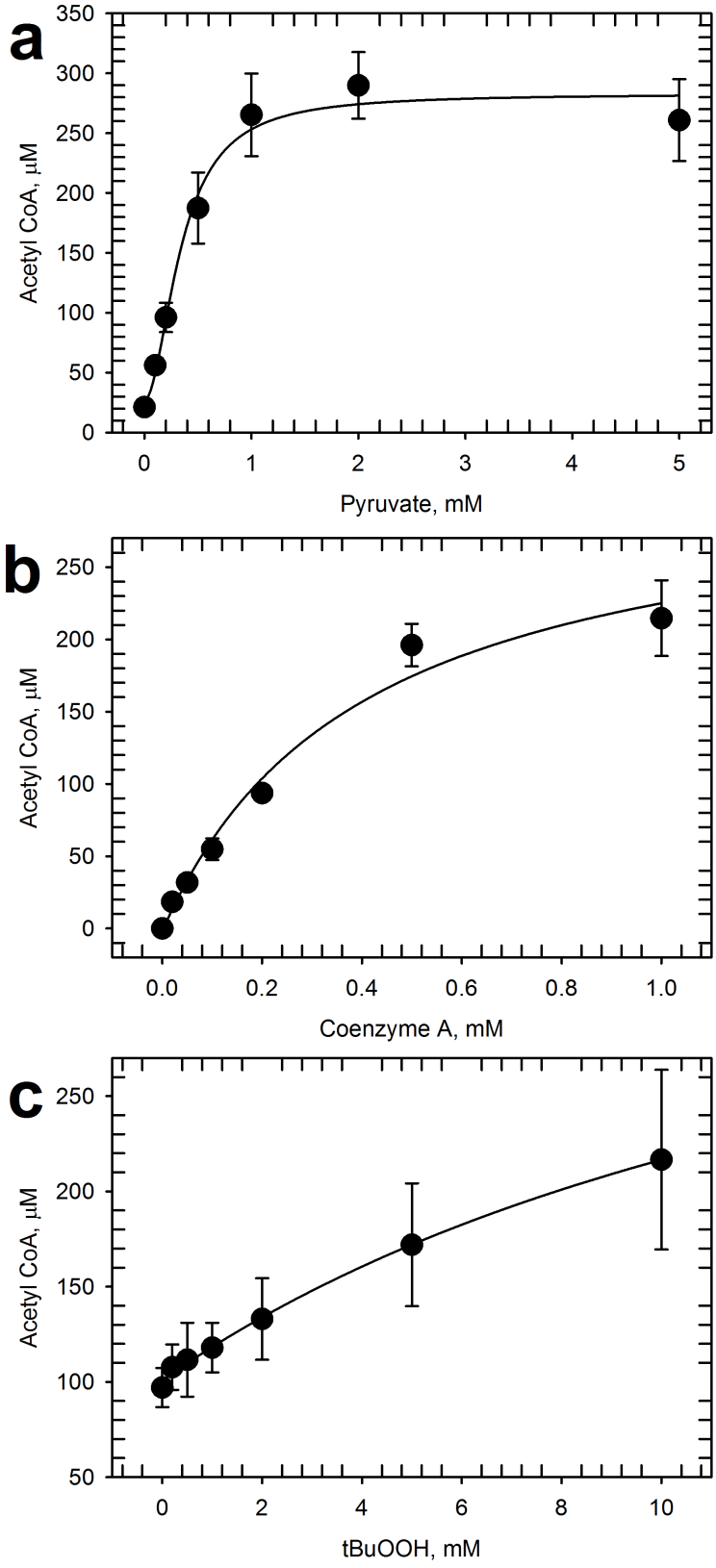

a
EIC at m/z = 808.12 amu
Sample injection time, min
b
15 min
30 min
45 min
60 min
90 min
120 min
c
AcCoA Standard
m/z = 808.12
m/z = 809.123
m/z = 810.120
m/z = 811.125
Ion counts
Relative Intensity, %
m/z
AcCoA Product
m/z = 808.121
m/z = 809.123
m/z = 810.117
m/z = 811.124
d
e
5.0 mM
2.0 mM
1.0 mM
0.5 mM
0.2 mM
0.1 mM

Figure S17


a
Acetyl CoA, µM
Pyruvate, mM
b
Acetyl CoA, µM
Coenzyme A, mM
c
Acetyl CoA, µM
tBuOOH, mM