Supplementary online material for:

A MOLECULAR MECHANISM FOR CHRONOLOGICAL AGING IN YEAST

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This file includes

Supplemental Table

	Initial	0mM	100mM	200mM	300mM	400mM	500mM
Acetic Acid	3.0	3.0	2.5	2.5	2.5	2.5	2.5
Citric Acid	3.0	3.0	2.5	2.2	1.6	1.6	1.6
Malic Acid	3.0	3.0	2.5	2.2	2.2	2.2	1.9

	Initial	0mM	1mM	2mM	3mM	4mM	5mM
HCl	3.0	3.0	2.5	2.5	2.5	2.5	2.2

Supplemental Table 1. The pH of transient acid treatments in **Figure 5** was measured using litmus paper to verify the acidity of the treatment after addition of acid. The addition of citric, malic, or hydrochloric acid decreased the pH of the media to a degree similar to that of an equal addition of acetic acid.

Supplemental Figures

Figure S1. **Growth in DR media increases respiratory metabolism through transcriptional upregulation of respiratory genes.** SC 2% and SC 0.05% cells were harvested in log phase (OD = ~0.4) and expression analysis was performed by qPCR on several genes that are induced upon catabolite de-repression (*CYT1*, *ALD2*, *CIT1*, *FBP1*, and *ACS1*) as well as the transcriptional activator *HAP4*. Error bars indicate the standard deviation of two biological replicates.

Figure S2. Initial glucose concentration affects medium pH in exponentially growing yeast cultures. pH of **(A)** BY4743, **(B)** BY4742, **(C)** W303AR and **(D)** DBY746 was recorded using a pH meter at 0, 24, 48, 72, 96, and 144 hours of growth in the indicated media. In all cases, the acidification observed in standard SC 2% medium was abolished by growth in low glucose (SC 0.5% or SC 0.05%), by growth in SG 3%, or by transfer to water at day 2. Growth in SC 2% + 18% sorbitol acidified the medium similar to SC 2% alone. Error bars indicate the standard deviation of three biological replicates.

Figure S3. Buffering with 0.1M MES pH 6 increases CLS similarly to buffering with a citrate phosphate buffer. (A) BY4743 and **(B)** W303AR5 were inoculated and aged SC 2% medium containing 0.1M MES adjusted to pH 6.0. Similar life span results were obtained by buffering with the low salt MES buffer, as with a citrate phosphate buffer.

Figure S4. Representative chromatograms from HPLC analysis of cell-free culture supernatant. Chromatogram of 48 hour SC 2% **(A)**, SC 0.05% **(B)**, and SG 3% **(C)** cell-free culture medium (blue), superimposed with a standard identifying six organic acids (red).

Figure S5. Fermentation results in the accumulation ethanol, and ethanol results in the accumulation of acetic acid in the culture medium. (A) BY4743 and **(B)** DBY746 were grown under standard SC 2% aging conditions, and ethanol was monitored in the culture medium. ~ 200mM ethanol was depleted from the culture medium by 3 days of aging. **(C)** Yeast transferred to water supplemented with 200mM ethanol utilize the ethanol over a 48hr period. **(D)** Acetic acid is detected in the culture supernatant which persists 48 hours after addition of ethanol.

Figure S6. Growth curves for BY4742 and isogenic *sch9Δ*. The doubling time, and hence the growth rate, for the long lived sch9Δ cells is shorter than the parental strain, as evidenced by the slope of the growth curve. Error bars indicate the standard deviation of three technical replicates.

Figure S7. Deletion of ADH2 increases CLS. (A) The chronological life span of BY4743 yeast is increased by deleting *ADH2*, the alcohol dehydrogenase which shunts ethanol back into respiratory metabolism. The increase is significant by calculating a survival integral, or the area under the curve. Error bars indicate the standard deviation

of three biological replicates. **(B)** By either overexpressing *ADH1* or deleting *ADH2*, the production of ethanol is favored over the production of acetic acid. Both genetic manipulations increase the CLS, supporting the hypothesis of acetic acid induced chronological aging.

Figure S8. Acetic acid is rapidly consumed by yeast. Acetic acid was maintained throughout the duration of the acetic acid add-back experiment due to the consumption of the acid from cells **(A)** transferred to water or **(B)** pre-grown in SC 0.05%. **(C)** The culture OD increased with the addition of acetic acid only in cultures that were maintained in spent SC 0.05% medium. In cases where treatment resulted in a change in culture density, the life span curve was normalized to the fold increase.

Figure S1

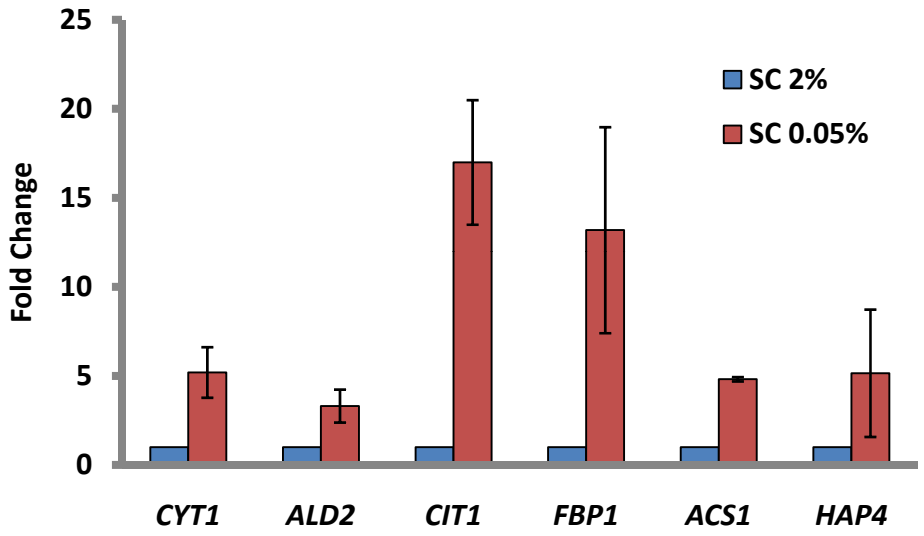

25
20
15
10
5
0
Fold Change
SC 2%
SC 0.05%
CYT1
ALD2
CIT1
FBP1
ACS1
HAP4

Figure S2

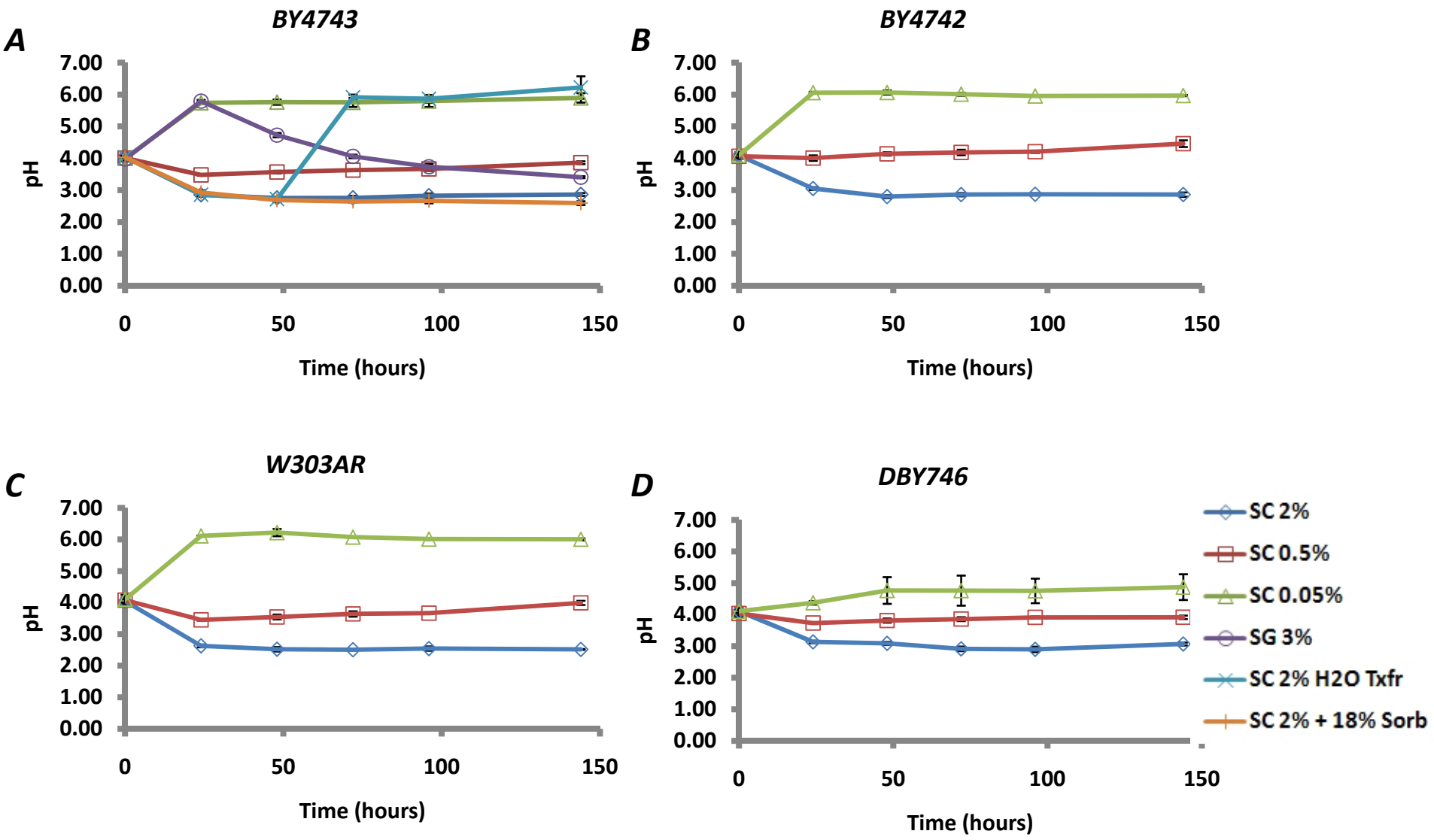

A
BY4743
pH
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
50
100
150
Time (hours)
B
BY4742
pH
Time (hours)
C
W303AR
pH
Time (hours)
D
DBY746
pH
Time (hours)
SC 2%
SC 0.5%
SC 0.05%
SG 3%
SC 2% H2O Txfr
SC 2% + 18% Sorb

Figure S3

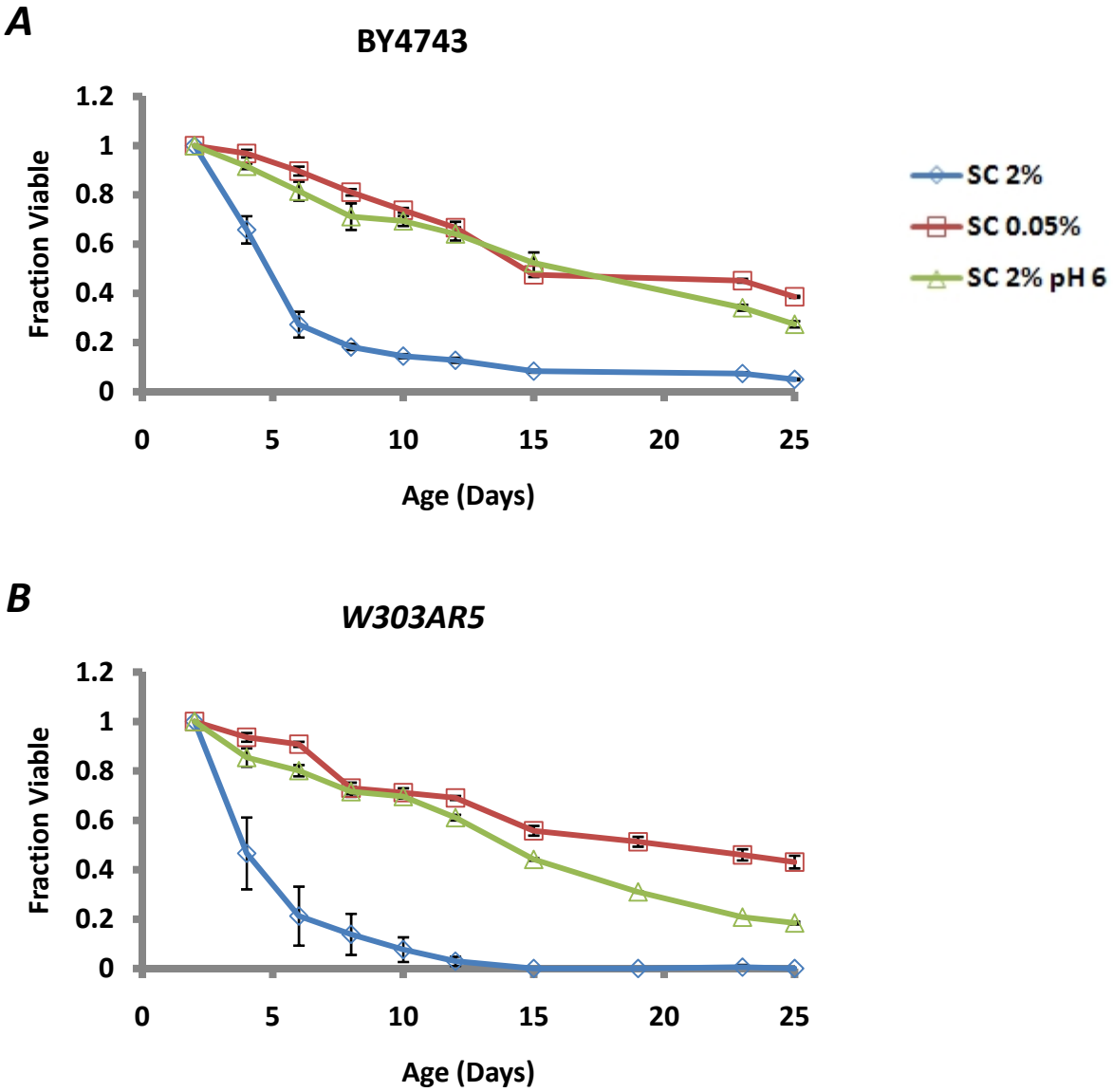

A
BY4743
Fraction Viable
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
Age (Days)
SC 2%
SC 0.05%
SC 2% pH 6
B
W303AR5
Fraction Viable
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
Age (Days)

Figure S4A

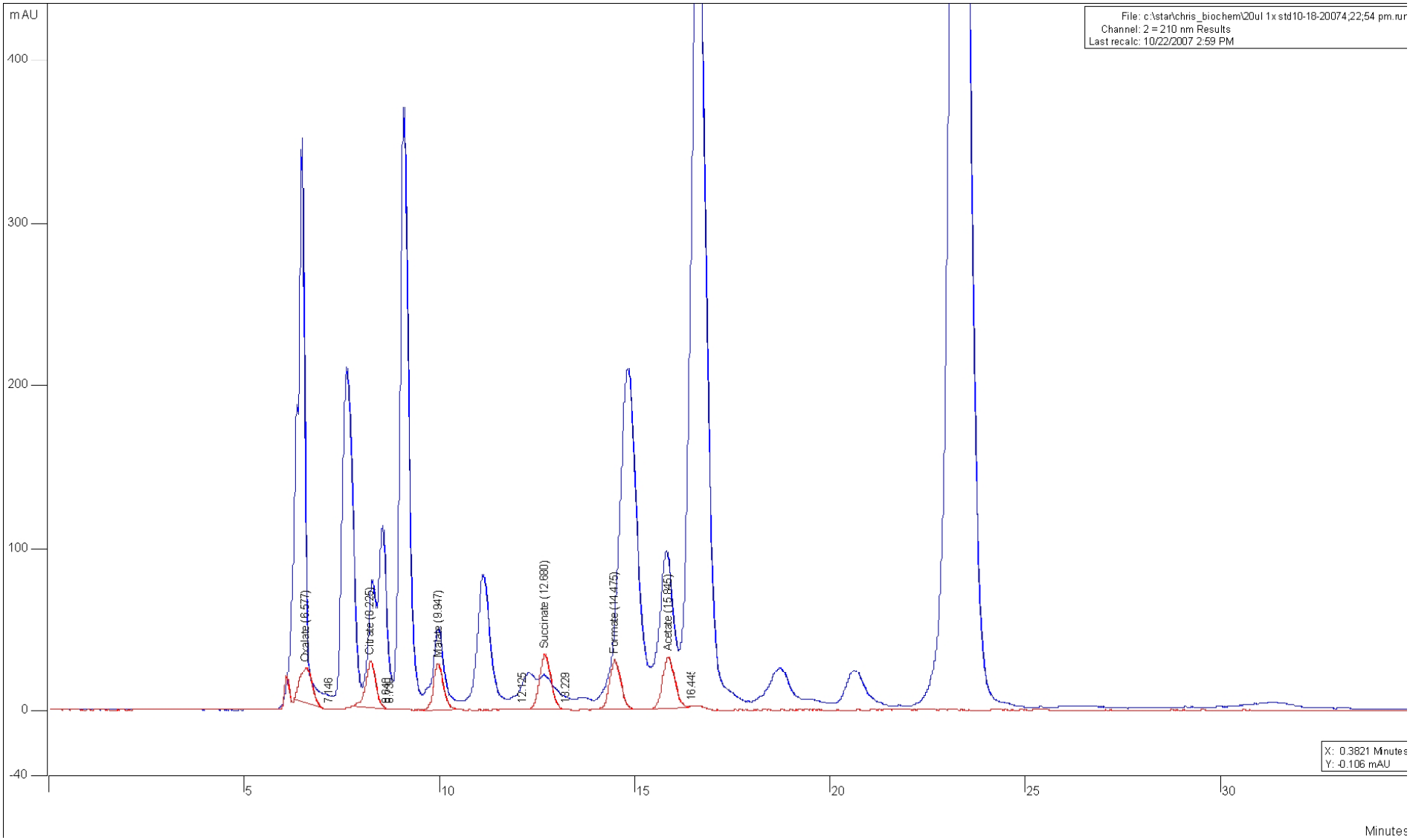

mAU
400
300
200
100
0
-40
File: c:\star\chris_biochem\20ul 1x std10-18-20074;22;54 pm.rur
Channel: 2 = 210 nm Results
Last recalc: 10/22/2007 2:59 PM
Oxalate (6.577)
7.146
Citrate (8.225)
8.840
8.930
Malate (9.947)
12.125
Succinate (12.680)
13.229
Formate (14.475)
Acetate (15.845)
16.44
X: 0.3821 Minutes
Y: -0.106 mAU
5
10
15
20
25
30
Minutes

Figure S4B

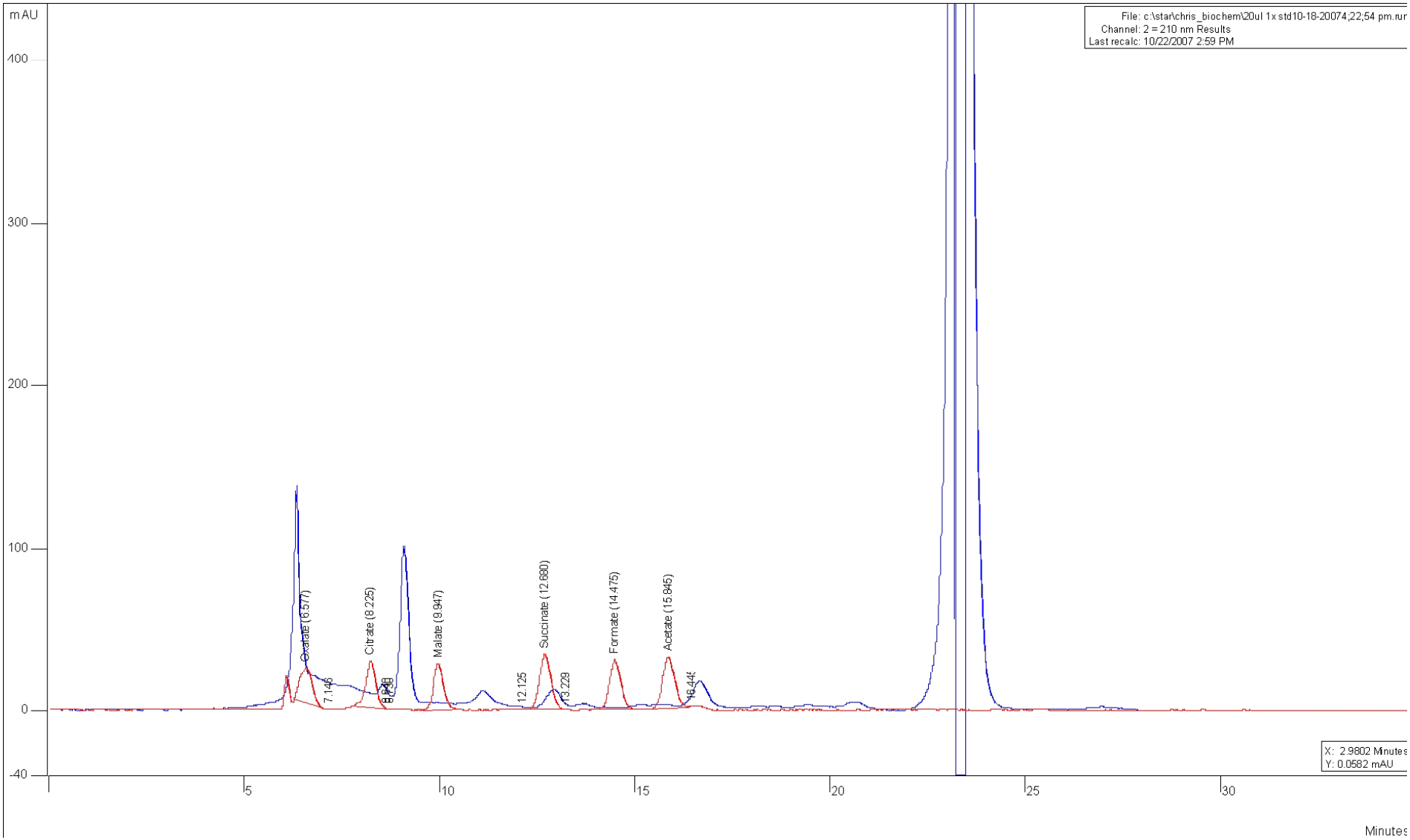

mAU
400
300
200
100
0
-40
File: c:\star\chris_biochem\20ul 1x std10-18-20074;22;54 pm.rur
Channel: 2 = 210 nm Results
Last recalc: 10/22/2007 2:59 PM
Oxalate (6.577)
7.146
Citrate (8.225)
Malate (9.947)
12.125
Succinate (12.680)
13.229
Formate (14.475)
Acetate (15.845)
X: 2.9802 Minutes
Y: 0.0582 mAU
5
10
15
20
25
30
Minutes

Figure S4C

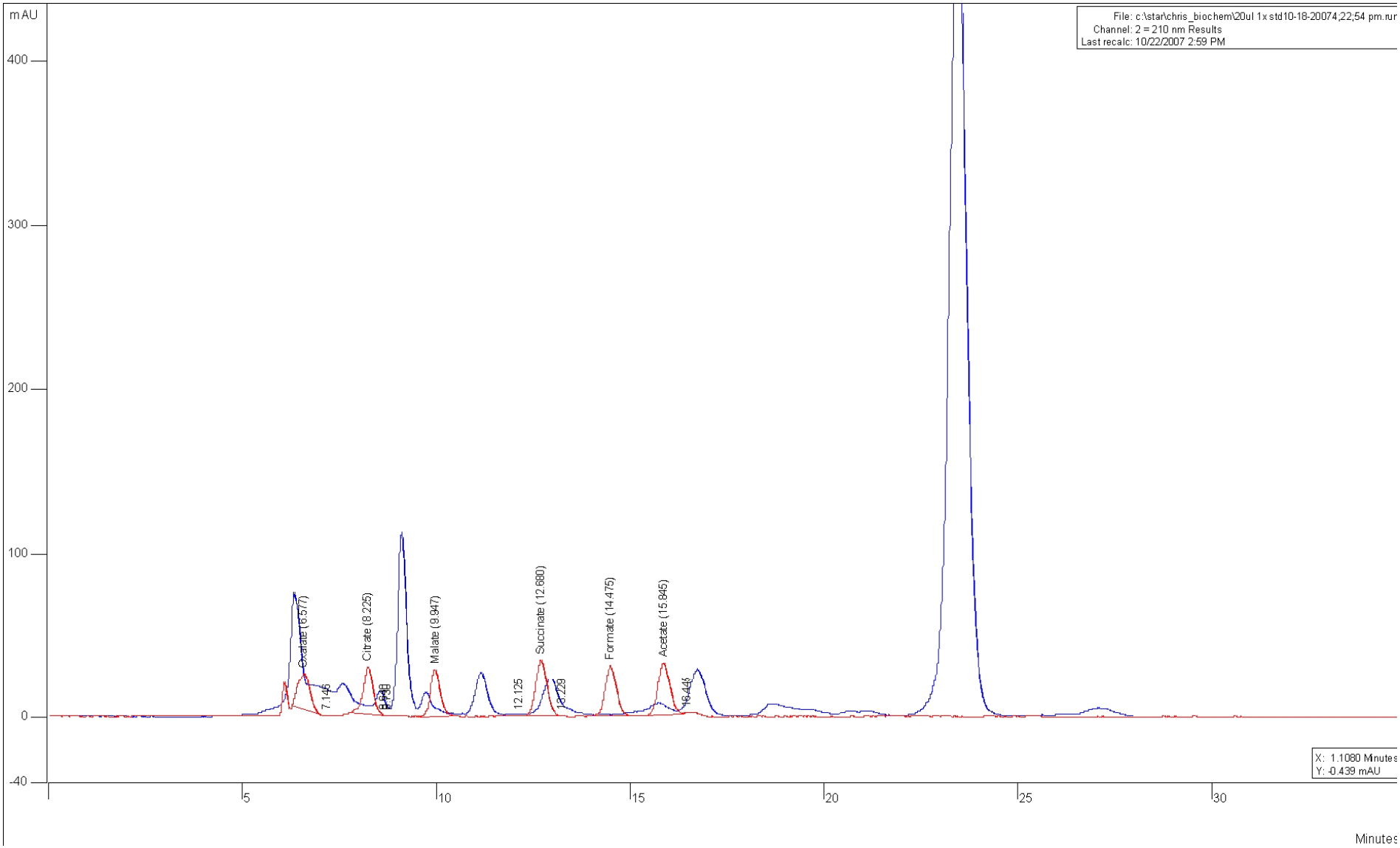

mAU
400
300
200
100
0
-40
File: c:\star\chris_biochem\20ul 1x std10-18-20074;22;54 pm.rur
Channel: 2 = 210 nm Results
Last recalc: 10/22/2007 2:59 PM
Oxalate (6.577)
7.146
Citrate (8.225)
8.838
8.938
Malate (9.947)
12.125
Succinate (12.880)
13.228
Formate (14.475)
Acetate (15.845)
16.446
X: 1.1080 Minutes
Y: -0.439 mAU
5
10
15
20
25
30
Minutes

Figure S5

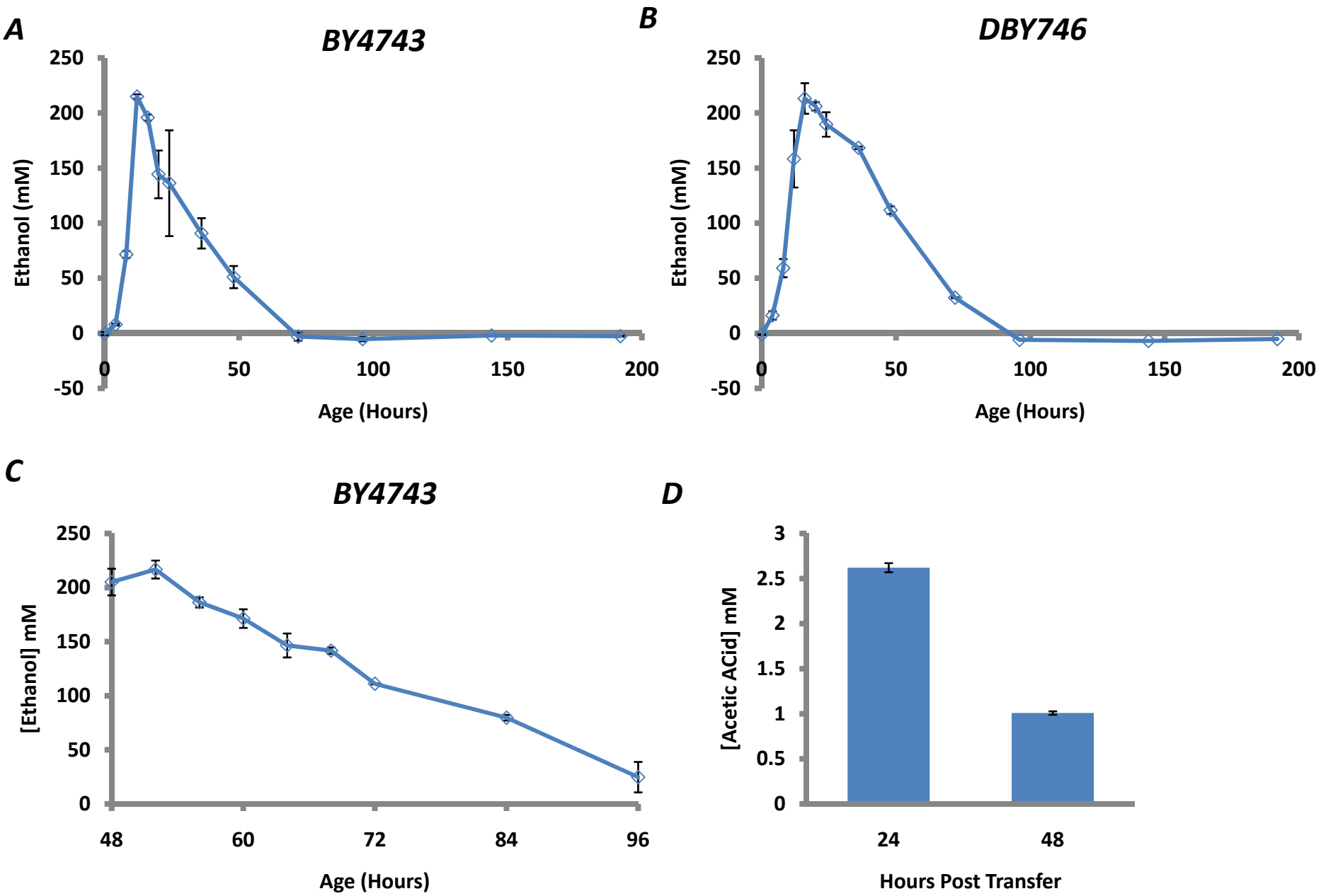

A
BY4743
Ethanol (mM)
250
200
150
100
50
0
-50
0
50
100
150
200
Age (Hours)
B
DBY746
Ethanol (mM)
250
200
150
100
50
0
-50
0
50
100
150
200
Age (Hours)
C
BY4743
[Ethanol] mM
250
200
150
100
50
0
48
60
72
84
96
Age (Hours)
D
[Acetic ACid] mM
3
2.5
2
1.5
1
0.5
0
24
48
Hours Post Transfer

Figure S6

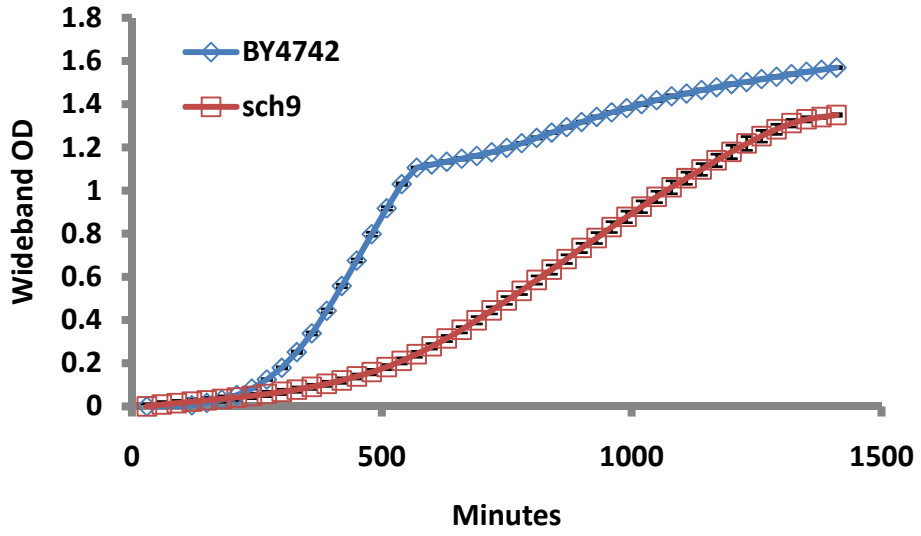
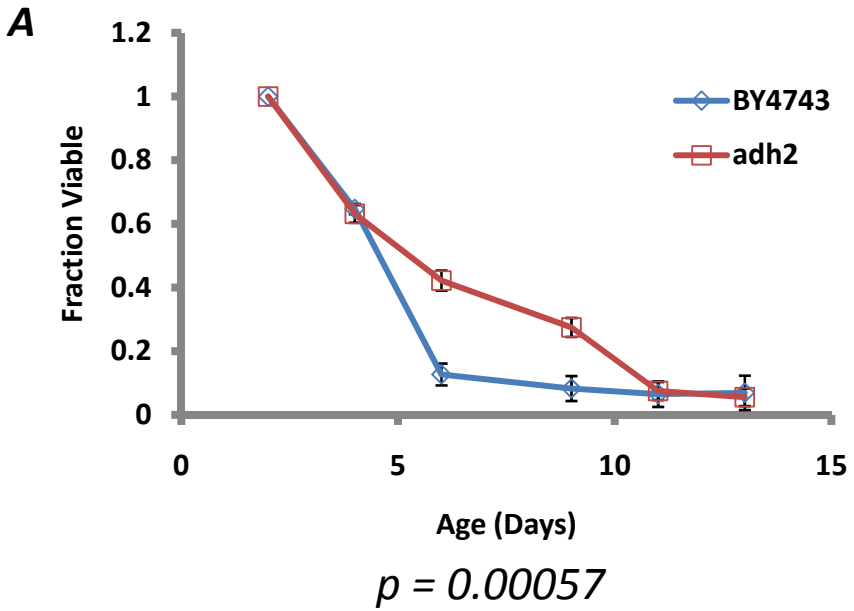

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Wideband OD
BY4742
sch9
0
500
1000
1500
Minutes

Figure S7


A
1.2
1
0.8
0.6
0.4
0.2
0
Fraction Viable
0
5
10
15
Age (Days)
BY4743
adh2
p = 0.00057

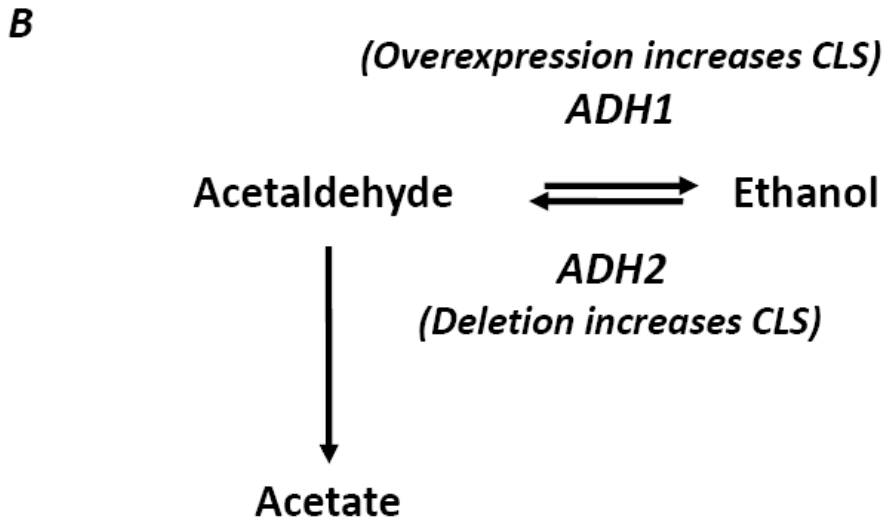
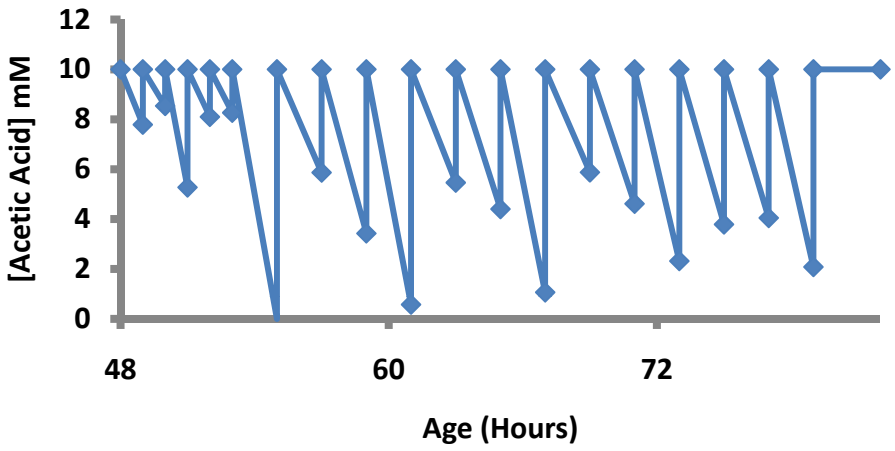

B
(Overexpression increases CLS)
ADH1
Acetaldehyde
Ethanol
ADH2
(Deletion increases CLS)
Acetate

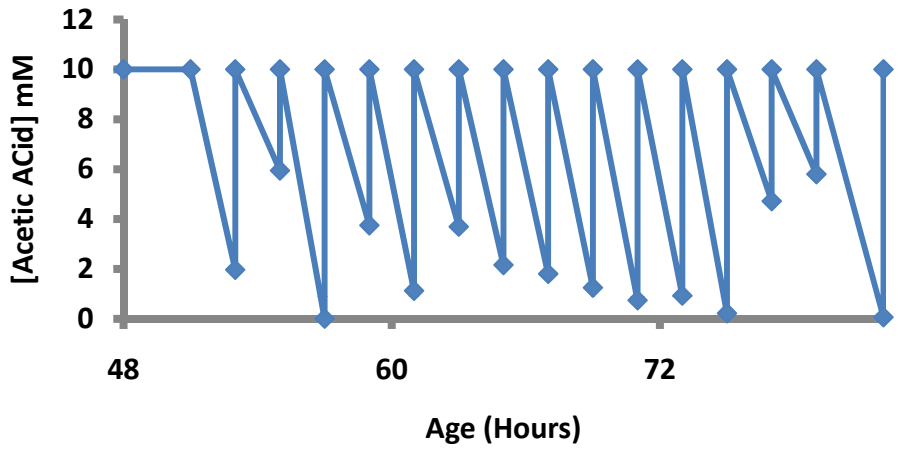
Figure S8

A



Total Added = 108.6 mM

B



Total Added = 125.9 mM

C

Sample	Culture OD (Day 6)	Ratio
SC 2% H2O Txfr pH 2.8	12.1 ± 0.4	1.0
SC 2% H2O Txfr pH 2.8 + Acetic Acid	12.4 ± 0.4	
SC 0.05% pH 2.8	2.6 ± 0.1	2.2
SC 0.05% pH 2.8 + Acetic Acid	5.6 ± 0.6	