Glucose feeds the TCA cycle via environmental ethanol in fermenting yeast

Authors

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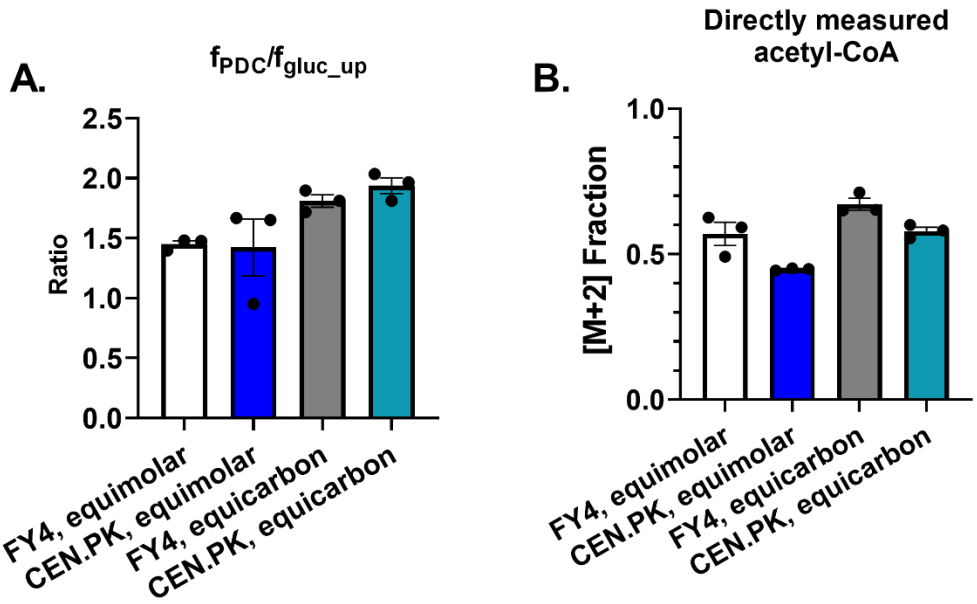
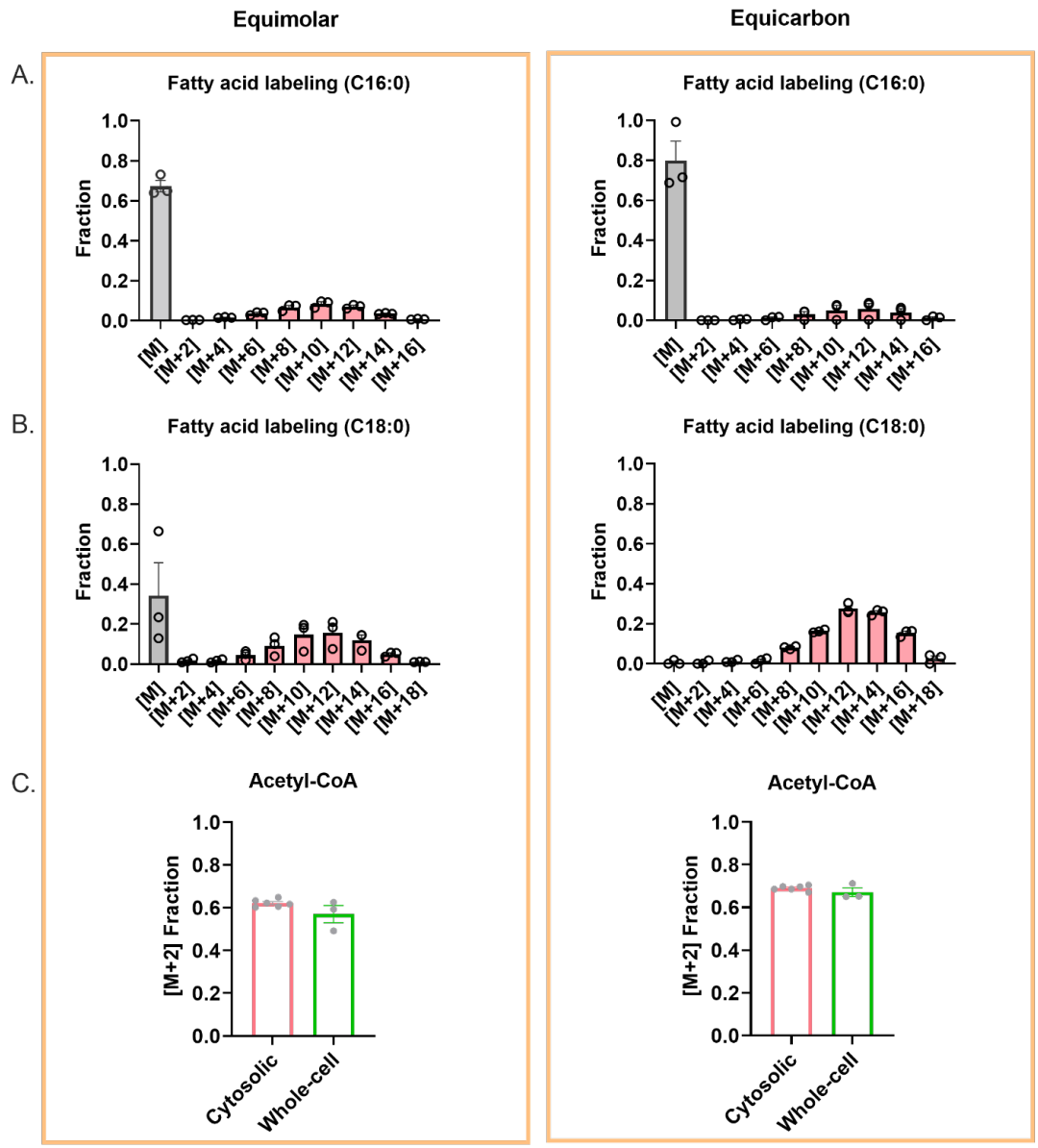


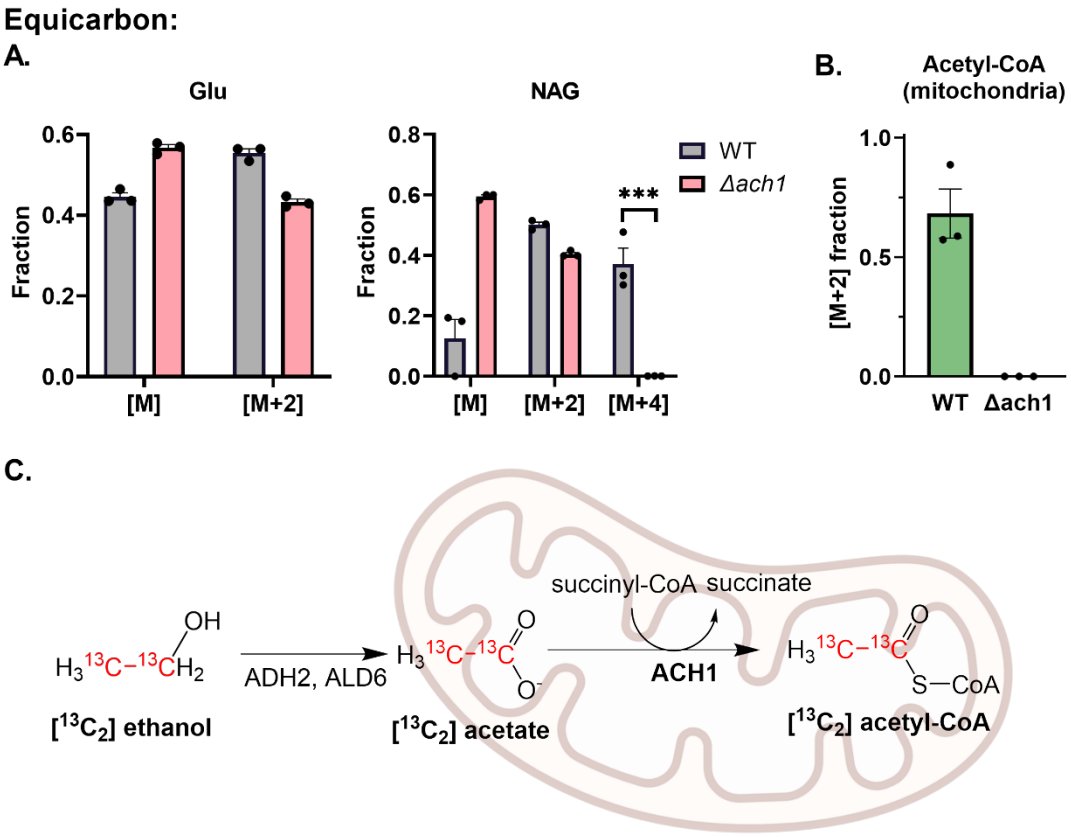
Supplementary Figure 1. Environmental ethanol consistently provides acetyl units across two *S. cerevisiae* strains

- **A.** The ratios between glucose uptake flux and ethanol excretion flux are consistent between the two strains tested and the two carbon sources conditions tested (mean, SE, n=3 (biological replicates)).
- **B.** The [M+2] labeled acetyl-CoA fraction from the cell (mixture of cytosolic and mitochondrial origins) is directly measured by LC-MS (mean, SE, n=3 (biological replicates)).



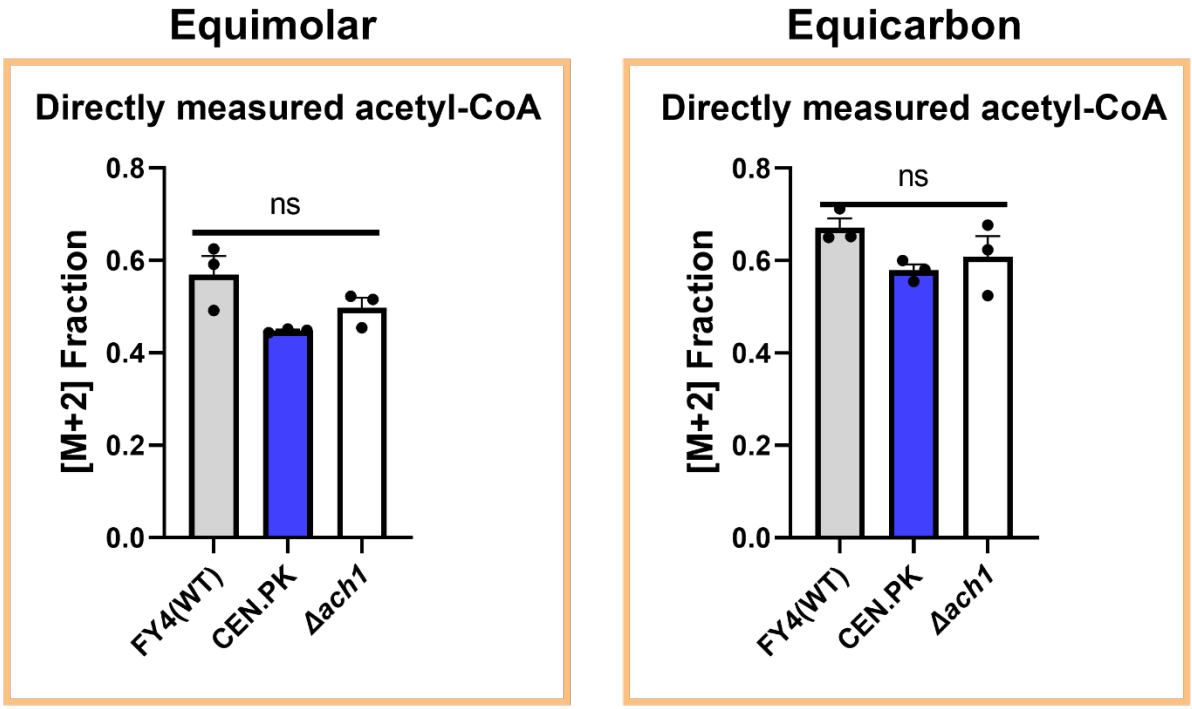
Supplementary Figure 2. Enviromental ethanol feeds fatty acid synthesis to a similar extent across the equimolar and equicarbon conditions relative to glucose

- **A.** ^{13}C isotope labeling pattern of palmitic acids from *S. cerevisiae* FY4(S288c) switched to and incubated in minimal media (YNB) with equimolar or equicarbon glucose: [$^{13}C_2$]ethanol (mean, SE, n=3 (biological replicates)).
- **B.** ^{13}C isotope labeling pattern of stearic acids from the same experiments as shown in **A** (mean, SE, n=3 (biological replicates)).
- C. The cytosolic acetyl-CoA ^{13}C [M+2] fraction derived from fatty acids labeling and directly whole-cell acetyl-CoA are similar (mean, SE, n=3 (biological replicates); for the 'Cytosolic' column, results from both C16:0 and C18:0 are plotted, resulting a total n=6). The directly measured cellular acetyl-CoA is plotted from the same data as shown in figure 1**F**.



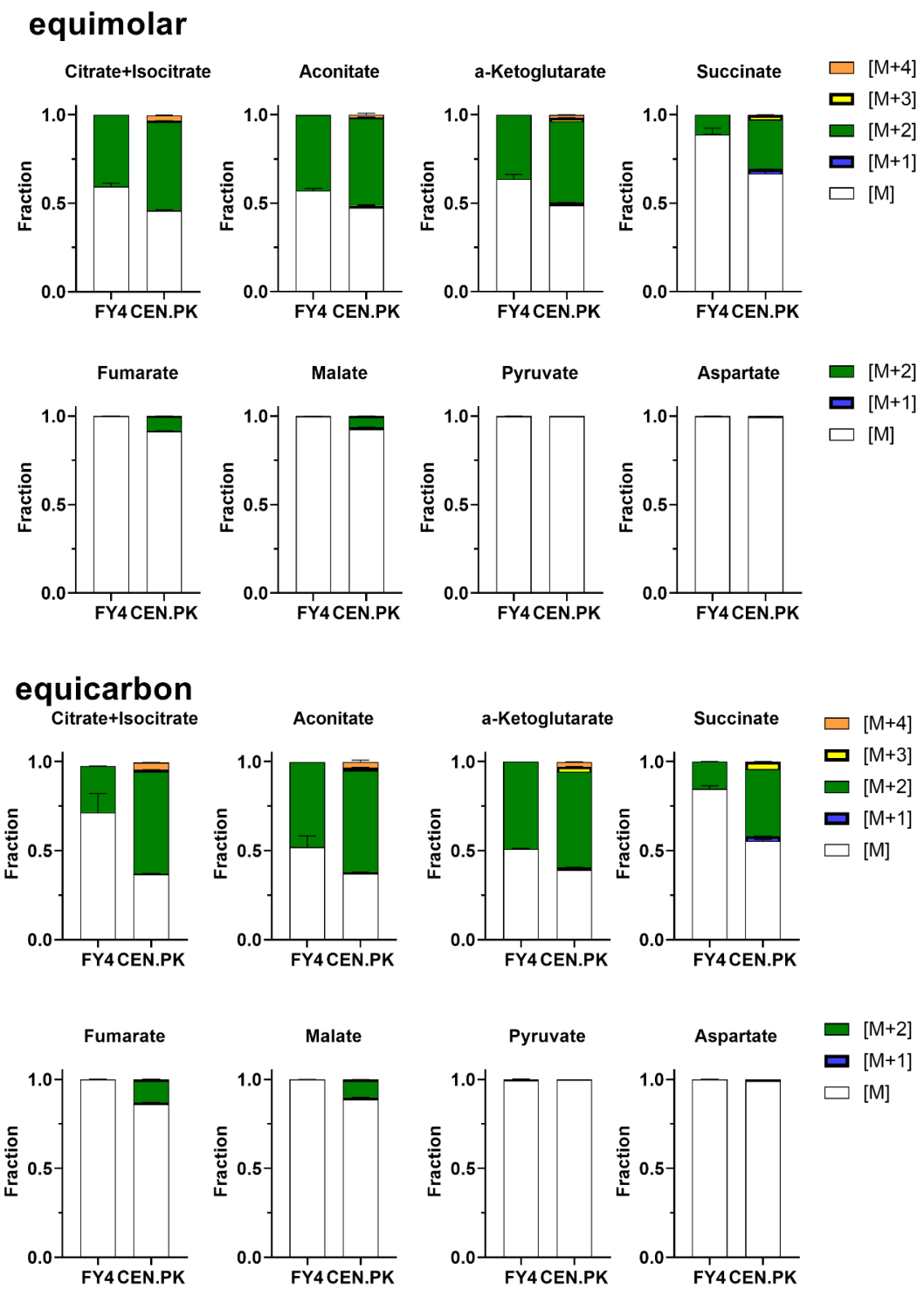
Supplementary figure 3. Ethanol contribution to mitochondrial acetyl-CoA is blocked by knocking out ACH1

- **A.** Glu and NAG labeling from the experiment in **Figure 1D** with equicarbon glucose: [$^{13}C_2$]ethanol (mean, SE, n=3 (biological replicates); ***, p<.001, by t-test). Briefly, while Glu is labeled up to [M+2], NAG [M+4] arises from the reaction of [M+2] Glut with mitochondrial [M+2] acetyl-CoA, which depends on Ach1.
- **B.** Mitochondrial acetyl-CoA [M+2] fraction fitted from glutamate and NAG labeling in **A** (mean, SE, n=3 (biological replicates)).
- **C.** Schematic showing ACH1 as the exclusive point of entry for carbons from ethanol or acetate into mitochondrial acetyl-CoA (*Created with Biorender*).



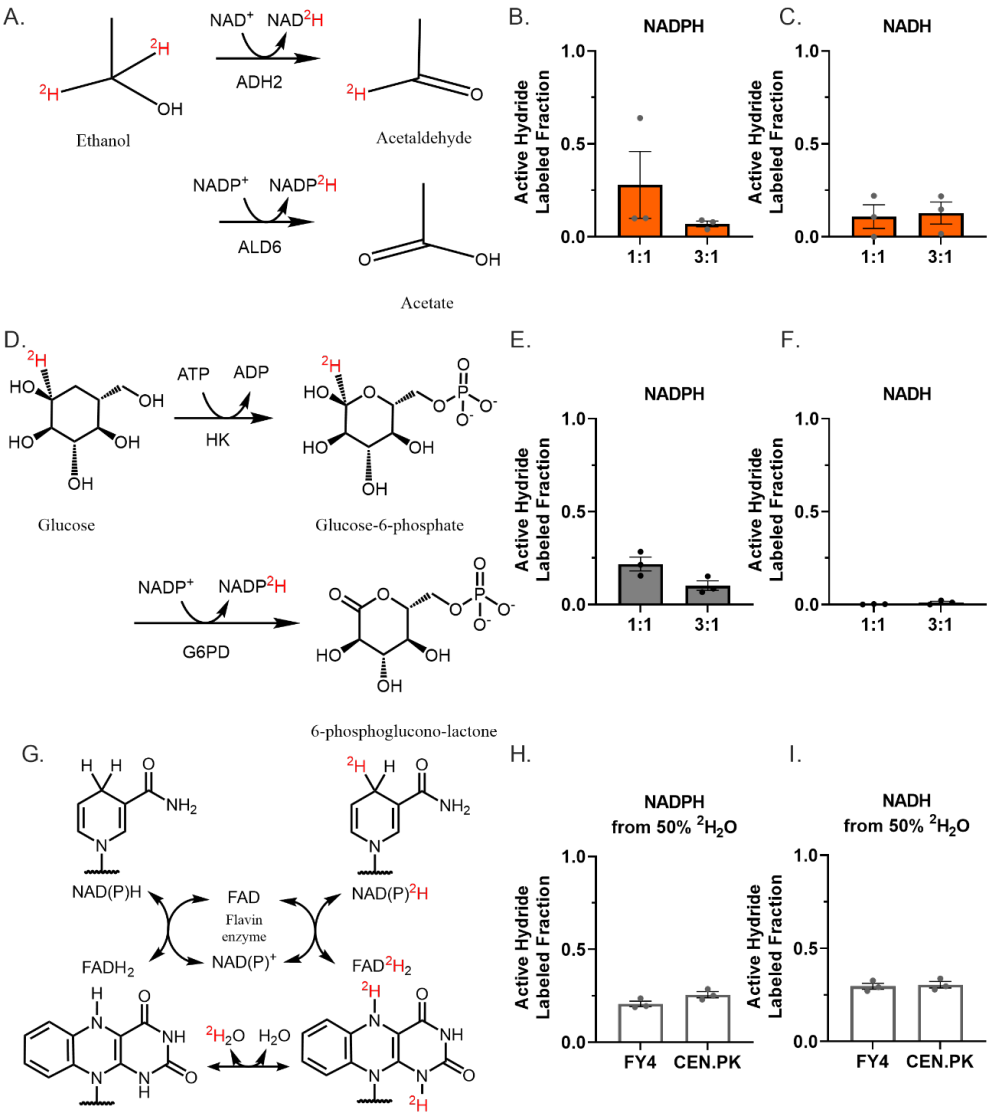
Supplementary Figure 4. Knocking out ACH1 does not affect ethanol contribution to whole-cell acetyl-CoA

Directly measured cellular acetyl-CoA [M+2] labeled fraction is similar across media conditions or strains including ACH1 knockout strain (mean, SE, n=3 (biological replicates)). FY4 is isogenic to S288c and Δ*ach1* is from S288c, while CEN.PK is derived from ENY.WA-1A and MC996A.



Supplementary Figure 5. Carbons from ethanol feed into TCA intermediates consistently across media conditions and strains, with a modestly greater contribution in CEN.PK.

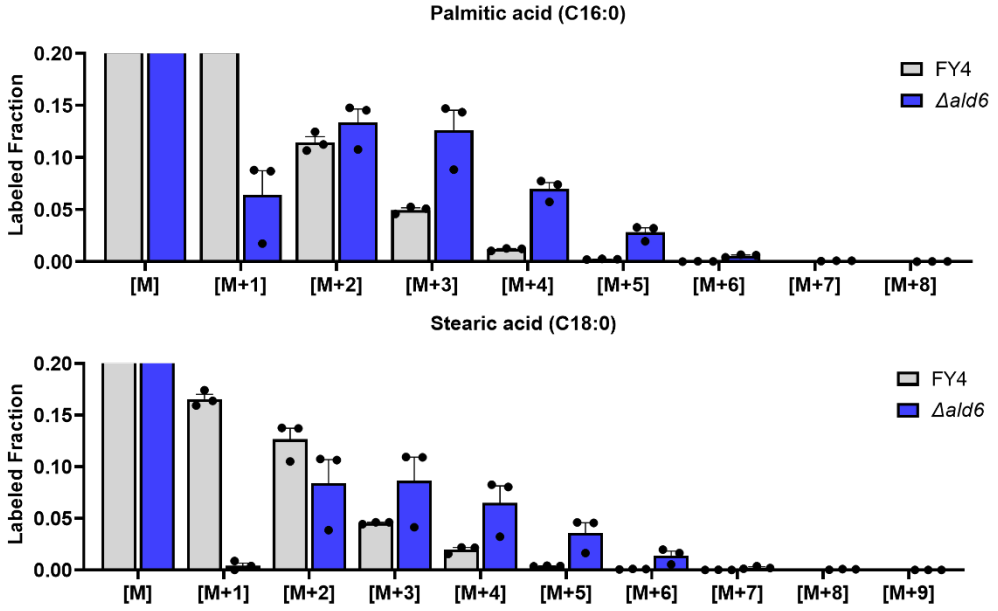
(mean, SE, n=3 (biological replicates))



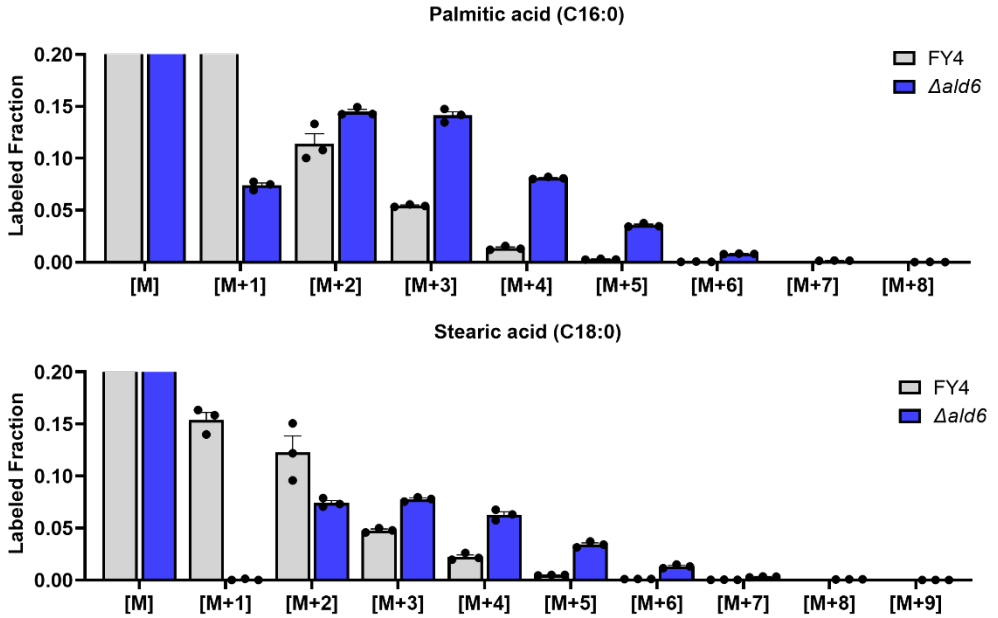
Supplementary Figure 6. Deuterium tracing into NAD(P)H is similar across media conditions and strains.

- **A.** Scheme depicting NADP^{2}H and NAD^{2}H production from [1,1-$^2H_2$] ethanol isotope tracer.
- **B.** Labeled fractions of NADPH active hydride with the tracer in **A**. The values are computed from matrix decompositions of labeling distributions of NAD(P)H and NAD(P)$^+$ that are directly measured by LC/MS (mean, SE, n=3 (biological replicates)).
- **C.** As in **B,** for NADH (mean, SE, n=3 (biological replicates)).
- **D.** Chemical scheme depicting NADP^{2}H production from [1-^{2}H] glucose via the first step of oxPPP.
- **E.** Labeled fractions of NADPH active hydride with the tracer in **D** (mean, SE, n=3 (biological replicates)).
- **F.** As in **D**, for NADH (mean, SE, n=3 (biological replicates)).
- **G.** Chemical basis of D_2O active hydride exchange with NAD(P)H[16].
- **H.** The fraction of NADPH hydride exchanged with water, as measured in media swap experiment with 50% D_2O (mean, SE, n=3 (biological replicates)).
- **I.** As in **H**, for NADH (mean, SE, n=3 (biological replicates)).

A. equimolar



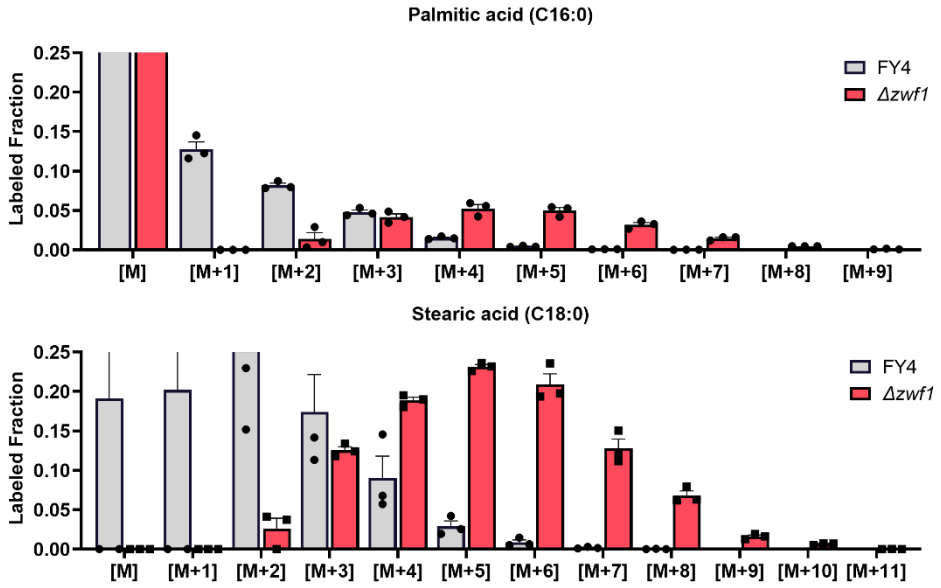
B. equicarbon



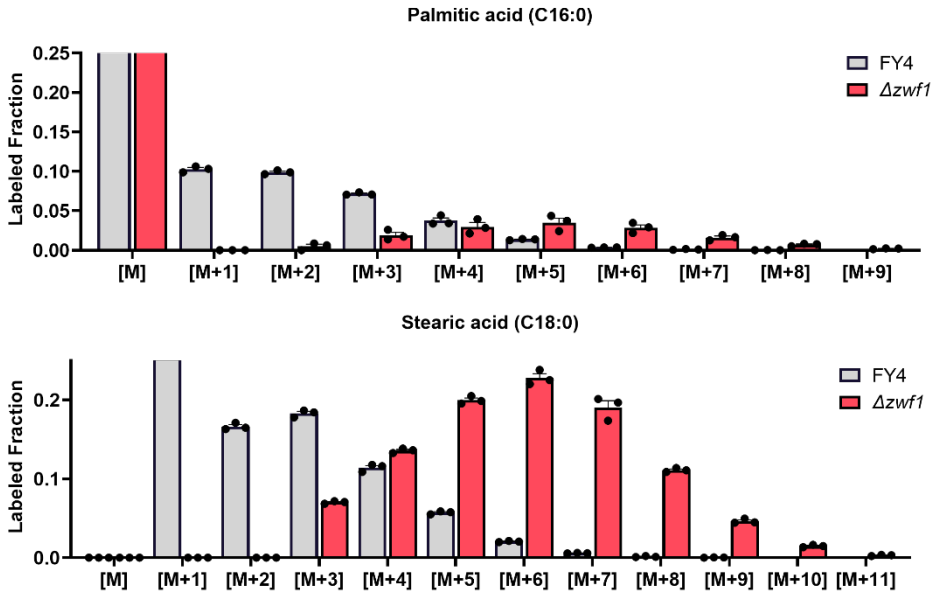
Supplementary Figure 7. Knocking out ALD6 increases cytosolic NADPH (and thus fatty acid) hydride labeling from the oxidative pentose phosphate pathway.

- **A.** ^{2}H isotope labeling pattern of palmitic and stearic acids from *S. cerevisiae* FY4 and Δald6 swapped into equimolar [1-^{2}H]glucose: ethanol for 1 h (mean, SE, n=3 (biological replicates)).
- **B.** As in **A,** for equicarbon [1-^{2}H]glucose: ethanol (mean, SEM, n=3 (biological replicates)).

A. equimolar

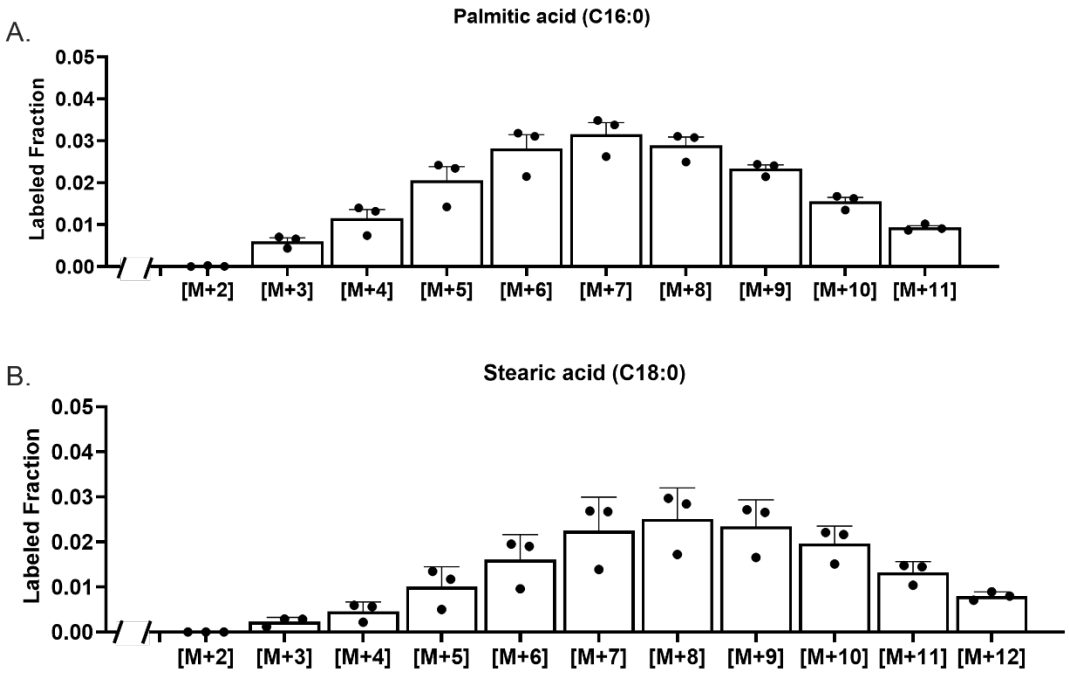


B. equicarbon



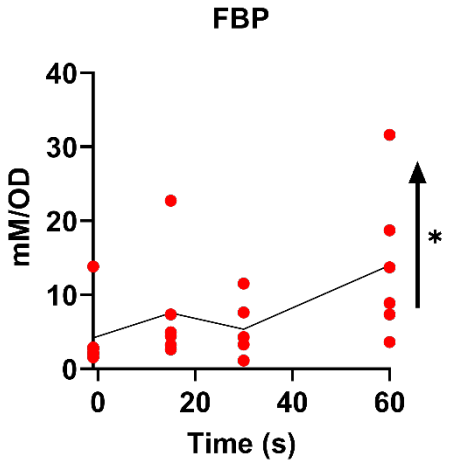
Supplementary Figure 8: Knocking out ZWF increases cytosolic NADPH (and thus fatty acid) hydride labeling from [1,1-$^{2}H_2$]ethanol via ALD6

- **A.** ^{2}H isotope labeling pattern of palmitic and stearic acids from *S. cerevisiae* FY4 and Δald6 swapped into equimolar glucose: [1,1-$^{2}H_2$]ethanol for 1 h (mean, SE, n=3 (biological replicates)).
- **B.** As in **A,** for equicarbon glucose: [1,1-$^{2}H_2$]ethanol (mean, SE, n=3 (biological replicates)).



Supplementary Figure 9: 50% D_2O labeling of fatty acids

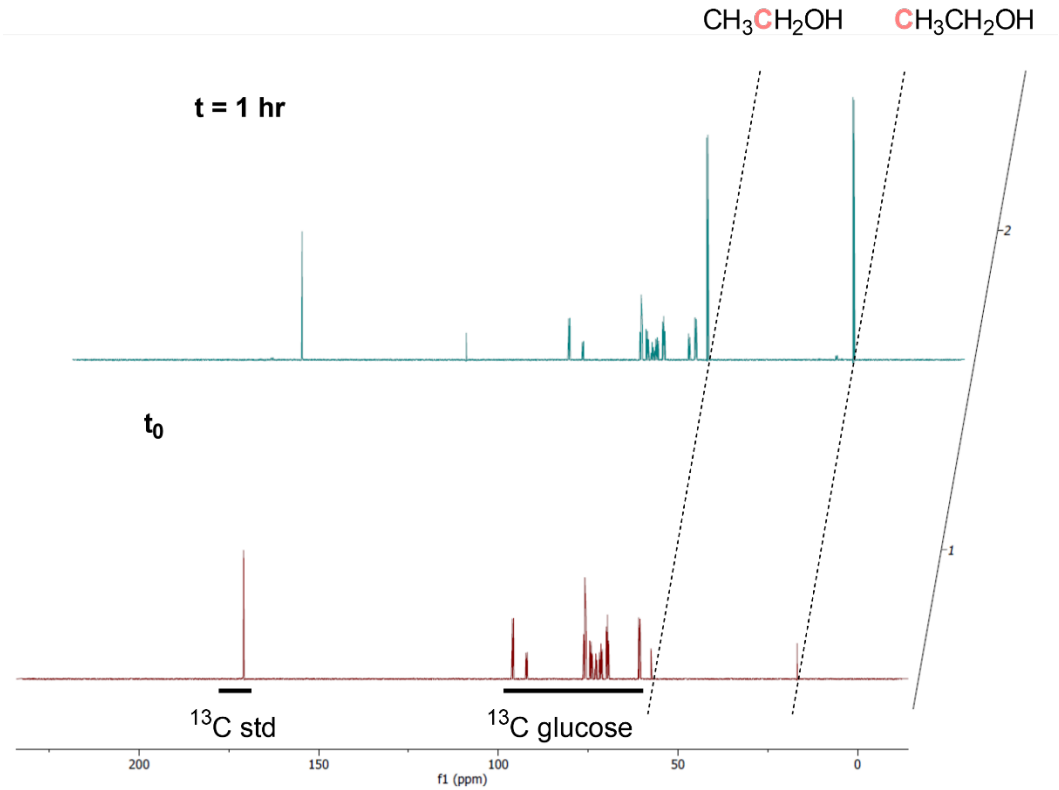
^2H isotope labeling pattern of palmitic acid (A) and stearic acid (B) *S. cerevisiae* FY4 swapped into regular glucose media with 50% D_2O for 1 h (mean, SE, n=3 (biological replicates)).



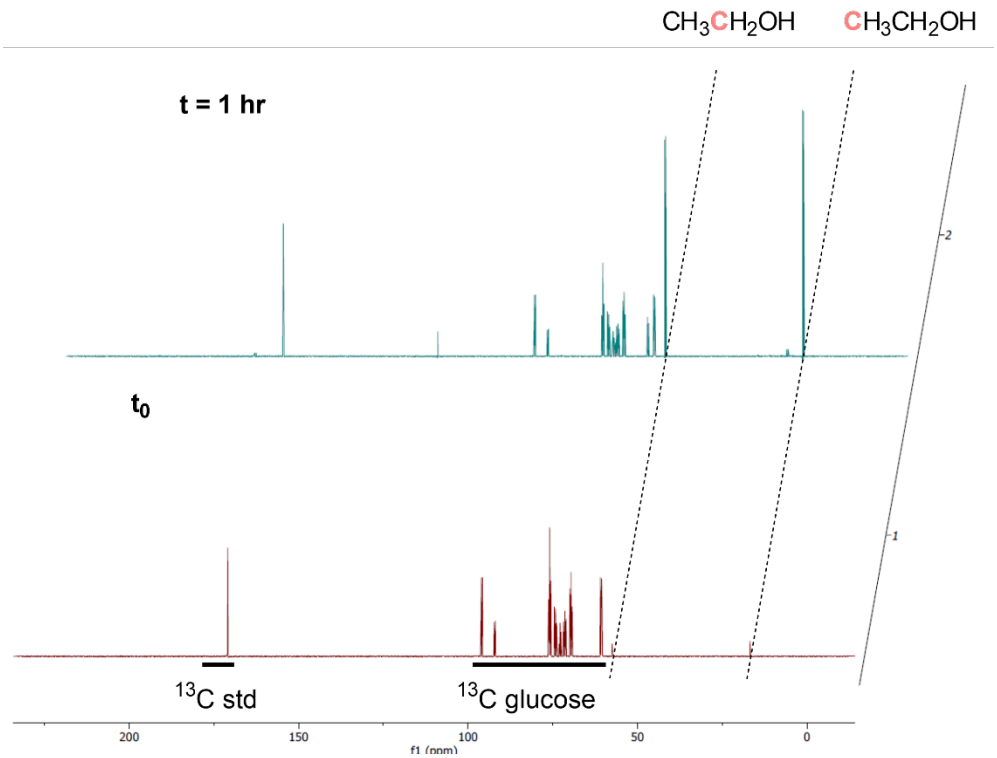
Supplementary Figure 10: Increase of FBP pool size upon adding H_2O_2

FBP pool size, after treatment with 20 mM hydrogen peroxide in *S. cerevisiae* FY4 grown in glucose+ethanol. The increase in FBP pool size after the oxidative stress is statistically significant (mean, SE, n=6 (biological replicates), positive linear trend (slope=2.7), p=.019(*)).

^{13}C NMR spectrum of media samples: equicarbon



^{13}C NMR spectrum of media samples: equimolar



Supplementary figure 11 (for method): Examples of q^{13}C NMR spectra