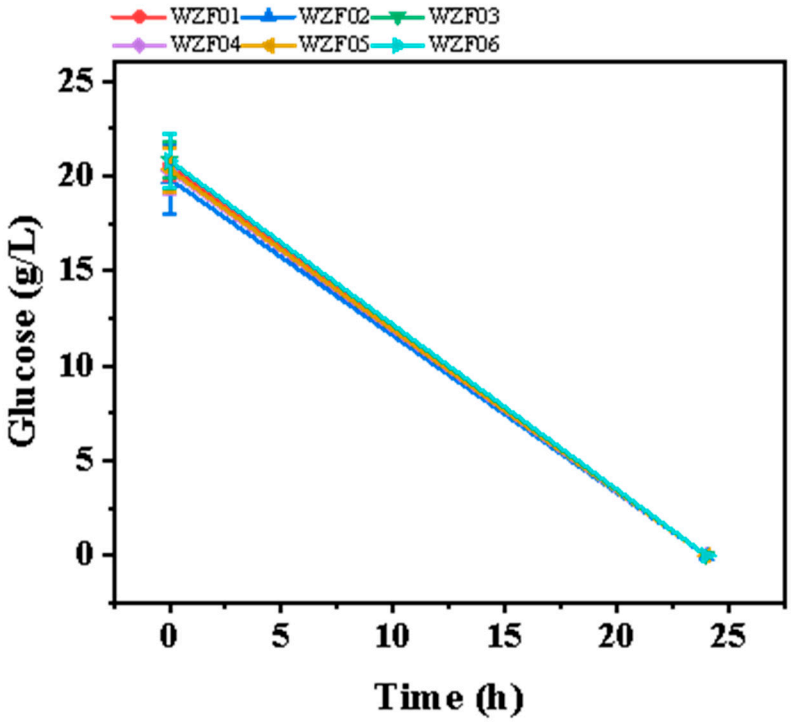

Fig. S1 Glucose consumption in the context of FDH expression's impact on strain metabolism with the addition of formate. The culture medium was MM medium, supplemented with 20 g/L glucose, 5 g/L formate, and 10 g/L acetate. The fermentation process spanned 96 hours. All data were presented as the mean ± s.d., with error bars denoting standard error (n=3).

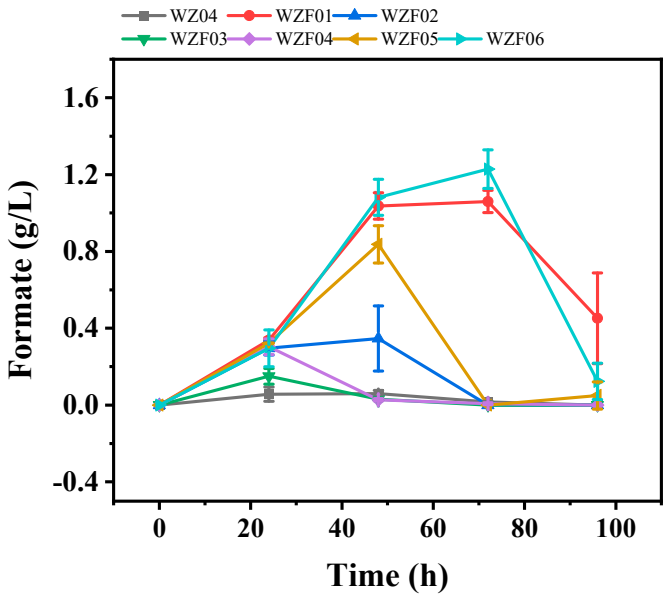

Fig. S2 Formate production in the context of FDH expression's impact on strain metabolism without exogenous formate addition. The culture medium was MM medium, supplemented with 20 g/L glucose, 5 g/L formate, and 10 g/L acetate. The fermentation process spanned 96 hours. All data were presented as the mean ± s.d., with error bars denoting standard error (n=3).

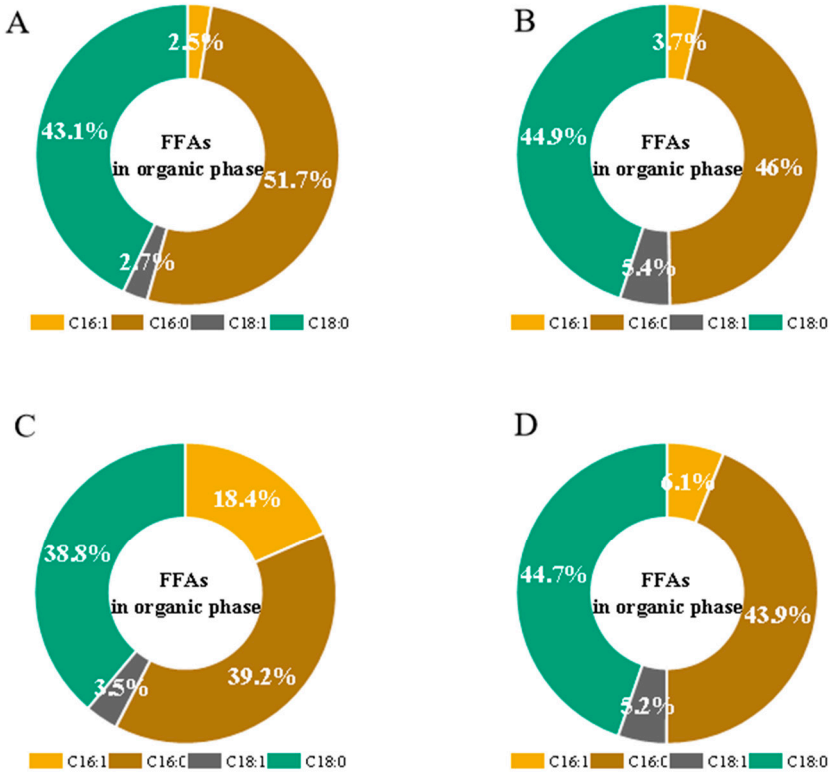

Fig. S3 . The proportion of FFAs in the organic phase. ABCD conditions were consistent with Figure 5 in the main text. All data were presented as the mean ± s.d., with error bars denoting standard error (n=3).

Table S1. Plasmids used in this study[a]

Plasmids	Genotype or characteristic	Resource
pSP-GM2	2 μm, AmpR, *URA3*, TEF1p, CYC1t, PGK1p, ADH1t	Laboratory preservation
pIY-C04	2-micron, AmpR, HIS3, TEF1p, ADHt, PGK1p,CYCt	Laboratory preservation
pFDH3	pSP-GM2-(TEF1p-**FDH3**-CYC1t)	Laboratory preservation
pFDH4	pSP-GM2-(TEF1p-**FDH4**-CYC1t)	Laboratory preservation
pFDH5	pSP-GM2-(TEF1p-**FDH5**-CYC1t)	Laboratory preservation
pFDH34	pSP-GM2-(TEF1p-**FDH3**-CYC1t)- (PGK1p-**FDH4**-ADH1t)	Laboratory preservation
pFDH45	pSP-GM2-(TEF1p-**FDH4**-CYC1t)- (PGK1p-**FDH5**-ADH1t)	Laboratory preservation
pFDH345	pSP-GM2-(TEF1p-**FDH5**-CYC1t)- (PGK1p-**FDH4**-ADH1t)- (PGI1p-**FDH3**-PYK1t)	Laboratory preservation
pScACS1	pIY-C04-(TEF1p-**ScACS1**-ADH1t)	This study
pScACS2	pIY-C04-(TEF1p-**ScACS2**-ADH1t)	This study
pYlACS1	pIY-C04-(TEF1p-Yl**ACS1**-ADH1t)	This study
PYlACS2	pIY-C04-(TEF1p-Yl**ACS2**-ADH1t)	This study
pSeACS^{L641D}	pIY-C04-(TEF1p-**SeACS^{L641D}**-ADH1t)	This study

[a] The bold text in the table indicates the name of the overexpressed gene.

Table S2. *S. cerevisiae* strains used in this study

Strain	Genotype or characteristic	Resource
YJZ08	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ*	[1]
WZ01	YJZ08 with pScACS1	This study
WZ02	YJZ08 with pScACS2	This study
WZ03	YJZ08 with pYlACS1	This study
WZ04	YJZ08 with pYlACS2	This study
WZ05	YJZ08 with pSeACS^{L641D}	This study
WZF01	WZ04 with pFDH3	This study
WZF02	WZ04 with pFDH34	This study
WZF03	WZ04 with pFDH345	This study
WZF04	WZ04 with pFDH4	This study
WZF05	WZ04 with pFDH5	This study
WZF06	WZ04 with pFDH45	This study

Table S3. Primers used in this study

Primer No.	Name	Sequence (5'-3')
P1	SCACS1-F	AAGCGGCCGCATGACAATCAAGGAACATAAAGTAGT
P2	SCACS1-R	GTCAACAACGTTTATTTCTTTTTTTGAGAGAAAAATTGGTTC
P3	SCACS2-F	AAGCGGCCGCATGTCGCCCTCTGCCGTA
P4	SCACS2-R	TGTCAACAACGTTTACAACTTGACCGAATCAATTAGATGTCT
P5	YLACS1-F	ACAAGCGGCCGCATGGAGTATTATTGTGGGGTAAACCAGGT
P6	YLACS1-R	AGAATTGTTAATTAATTAGAGCTTGGAAGAGCTGCC
P7	YLACS2-F	TACAAGCGGCCGCATGACAGTCAACTCCACTTTTAGATCG
P8	YLACS2-R	AATTGTTAATTAACTACTGTTCAACCAGATCAGGATACAG
P9	$SEACS^{L641D}$-F	CAAGCGGCCGCATGTCTCAGACGCATAAGCACG
P10	$SEACS^{L641D}$-R	GTCAACAACGTTTAAGATGGCATAGCAATAGCCTG

Table S4. The exogenous genes used in this study.

Synthesized genes	Sequence (5'-3')
ScACS1	ATGACAATCAAGGAACATAAAGTAGTTTATGAAGCTCACAACGTAAAGGCTCTTAAGGC TCCTCAACATTTTTACAACAGCCAACCCGGCAAGGGTTACGTTACTGATATGCAACATTA TCAAGAAATGTATCAACAATCTATCAATGAGCCAGAAAAATTCTTTGATAAGATGGCTAA GGAATACTTGCATTGGGATGCTCCATACACCAAAGTTCAATCTGGTTCATTGAACAATGG TGATGTTGCATGGTTTTTGAACGGTAAATTGAATGCATCATACAATTGTGTTGACAGACA TGCCTTTGCTAATCCCGACAAGCCAGCTTTGATCTATGAAGCTGATGACGAATCCGACAA CAAAATCATCACATTTGGTGAATTACTCAGAAAAGTTTCCCAAATCGCTGGTGTCTTAAA AAGCTGGGGCGTTAAGAAAGGTGACACAGTGGCTATCTATTTGCCAATGATTCCAGAAG CGGTCATTGCTATGTTGGCTGTGGCTCGTATTGGTGCTATTCACTCTGTTGTCTTTGCTGG GTTCTCCGCTGGTTCGTTGAAAGATCGTGTCGTTGACGCTAATTCTAAAGTGGTCATCACT TGTGATGAAGGTAAAAGAGGTGGTAAGACCATCAACACTAAAAAAATTGTTGACGAAGG TTTGAACGGAGTCGATTTGGTTTCCCGTATCTTGGTTTTCCAAAGAACTGGTACTGAAGGT ATTCCAATGAAGGCCGGTAGAGATTACTGGTGGCATGAGGAGGCCGCTAAGCAGAGAAC TTACCTACCTCCTGTTTCATGTGACGCTGAAGATCCTCTATTTTTATTATACACTTCCGGTT CCACTGGTTCTCCAAAGGGTGTCGTTCACACTACAGGTGGTTATTTATTAGGTGCCGCTTT AACAACTAGATACGTTTTTGATATTCACCCAGAAGATGTTCTCTTCACTGCCGGTGACGT CGGCTGGATCACGGGTCACACCTATGCTCTATATGGTCCATTAACCTTGGGTACCGCCTC AATAATTTTCGAATCCACTCCTGCCTACCCAGATTATGGTAGATATTGGAGAATTATCCA ACGTCACAAGGCTACCCATTTCTATGTGGCTCCAACTGCTTTAAGATTAATCAAACGTGT AGGTGAAGCCGAAATTGCCAAATATGACACTTCCTCATTACGTGTCTTGGGTTCCGTCGG TGAACCAATCTCTCCAGACTTATGGGAATGGTATCATGAAAAAGTGGGTAACAAAAACT GTGTCATTTGTGACACTATGTGGCAAACAGAGTCTGGTTCTCATTTAATTGCTCCTTTGGC AGGTGCTGTCCCAACAAAACCTGGTTCTGCTACCGTGCCATTCTTTGGTATTAACGCTTGT ATCATTGACCCTGTTACAGGTGTGGAATTAGAAGGTAATGATGTCGAAGGTGTCCTTGCC GTTAAATCACCATGGCCATCAATGGCTAGATCTGTTTGGAACCACCACGACCGTTACATG GATACTTACTTGAAACCTTATCCTGGTCACTATTTCACAGGTGATGGTGCTGGTAGAGAT CATGATGGTTACTACTGGATCAGGGGTAGAGTTGACGACGTTGTAAATGTTTCCGGTCAT AGATTATCCACATCAGAAATTGAAGCATCTATCTCAAATCACGAAAACGTCTCGGAAGCT GCTGTTGTCGGTATTCCAGATGAATTGACCGGTCAAACCGTCGTTGCATATGTTTCCCTAA AAGATGGTTATCTACAAAACAACGCTACTGAAGGTGATGCAGAACACATCACACCAGAT AATTTACGTAGAGAATTGATCTTACAAGTTAGGGGTGAGATTGGTCCTTTCGCCTCACCA AAAACCATTATTCTAGTTAGAGATCTACCAAGAACAAGGTCAGGAAAGATTATGAGAAG AGTTCTAAGAAAGGTTGCTTCTAACGAAGCCGAACAGCTAGGTGACCTAACTACTTTGGC CAACCCAGAAGTTGTACCTGCCATCATTTCTGCTGTAGAGAACCAATTTTTCTCTCAAAA AAAGAAATAA
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