

Supplementary Figure S1. H₂S does not increase the Alcohol dehydrogenase activity two hours after stimulation. Yeast cell cultures at exponential phase were treated with NaHS 0.1 and 0.25 mM. Two hours later whole cell extracts were used to measure Alcohol dehydrogenase activity *in vitro* at 37°C.

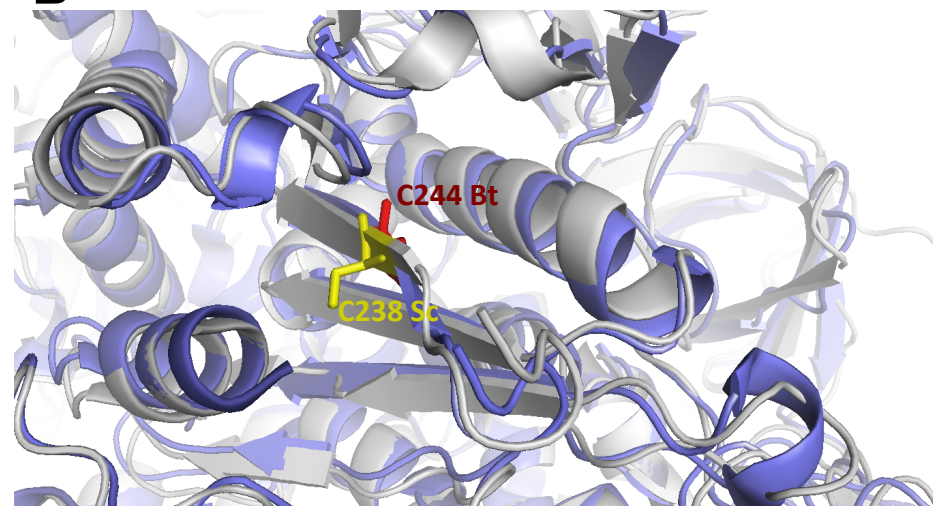
A

sp	P07251	ATPA_YEAST	MLAR---TAAIRSLR-----TLINSTKAARPAALASTRLASTKAQPTVEVSILE	50
sp	P25705	ATPA_HUMAN	MLSVRVAAAVRALPRRAGLVSRNALGSSFI---AARNPHASNTLQKTGTAEVMSILE	56
sp	P19483	ATPA_BOVIN	MLSVRVAAAVRALPRRAGLVSRNALGSSFI---AARNLHASNRLQKTGTAEVMSILE	56
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sp	P07251	ATPA_YEAST	ERIKGVSDKANLNETGRVLAVGDGIARVFLNNIQAEELVEFSSGVKGMALNLEPGQVGI	110
sp	P25705	ATPA_HUMAN	ERILGADTSVDLEETGRVLSIGDGIARVHGLRNVQAEEVMEVFSSGLKMSLNLEPDNVGV	116
sp	P19483	ATPA_BOVIN	ERILGADTSVDLEETGRVLSIGDGIARVHGLRNVQAEEVMEVFSSGLKMSLNLEPDNVGV	116
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sp	P07251	ATPA_YEAST	VLFSGDRVLKEGELVKRTGNIVDPVPGGLLGRVVDALGNPIDGKGPIDAGRSRAQVKA	170
sp	P25705	ATPA_HUMAN	VVFGNDKLIKEGDIVKRTGAIVDVPVGEELLGRVVDALGNAIDGKGPISKTRRRVGLKA	176
sp	P19483	ATPA_BOVIN	VVFGNDKLIKEGDIVKRTGAIVDVPVGEELLGRVVDALGNAIDGKGPISKARRVGLKA	176
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sp	P07251	ATPA_YEAST	PGILPRRSVHEPVQTGLKAVDALVPIGRGQRELIIGDRQTKTAVALDITLNQKRWNGS	230
sp	P25705	ATPA_HUMAN	PGIIPRISVREPMQTGKAVDSLVPVIGRGQRELIIGDRQTKTSIAIDTIINQKRFNDGS	236
sp	P19483	ATPA_BOVIN	PGIIPRISVREPMQTGKAVDSLVPVIGRGQRELIIGDRQTKTSIAIDTIINQKRFNDGT	236
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sp	P07251	ATPA_YEAST	DESKKLYCYVVAVGQKRSTVAQLVQTLQHDAMKYSIIVAATASEAAPLQYLAPFTAAIS	290
sp	P25705	ATPA_HUMAN	DEKKKLYCYVVAIGQKRSTVAQLVKRLTDADAMKYTIIVSATASDAAPLQYLAPYSGQSM	296
sp	P19483	ATPA_BOVIN	DEKKKLYCYVVAIGQKRSTVAQLVKRLTDADAMKYTIIVSATASDAAPLQYLAPYSGQSM	296
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sp	P07251	ATPA_YEAST	GEWFRDNGKHALIYDDLSKQAVAYRQLSLLRRPPGREAYPGDVFLHSRLLEAAKLS	350
sp	P25705	ATPA_HUMAN	GEYFRDNGKHALIYDDLSKQAVAYRQMSLLRRPPGREAYPGDVFLHSRLLEAAKMN	356
sp	P19483	ATPA_BOVIN	GEYFRDNGKHALIYDDLSKQAVAYRQMSLLRRPPGREAYPGDVFLHSRLLEAAKMN	356
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sp	P07251	ATPA_YEAST	EKEGSGSLTALPVIEITQGGDVSAIPTNVISITDGGIFLEAEIFYKGIKPAINVGLSVSR	410
sp	P25705	ATPA_HUMAN	DAFGGSGSLTALPVIEITQAGDVSAIPTNVISITDGGIFLETELFYKGIKPAINVGLSVSR	416
sp	P19483	ATPA_BOVIN	DAFGGSGSLTALPVIEITQAGDVSAIPTNVISITDGGIFLETELFYKGIKPAINVGLSVSR	416
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sp	P07251	ATPA_YEAST	VGSAAQVKALKQVAGSLKFLAQYREVAFAQFGSDLDASTKQTLVGRERLTQLLKQNGY	470
sp	P25705	ATPA_HUMAN	VGSAAQTRAMKQVAGTMKLELAQYREVAFAQFGSDLDAAQQLLSRGVRLTELLKQGGY	476
sp	P19483	ATPA_BOVIN	VGSAAQTRAMKQVAGTMKLELAQYREVAFAQFGSDLDAAQQLLSRGVRLTELLKQGGY	476
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sp	P07251	ATPA_YEAST	SPLATEEQVPLIYAGVNGHLDGIELSRIGEFESSFLSYLKSNNHLLTEIREKGELSKEL	530
sp	P25705	ATPA_HUMAN	SPMAIEEQVAVIYAGVRGYLDKLEPSKITKFENAFLSHVVSQHQALLGTIRADGKISEQS	536
sp	P19483	ATPA_BOVIN	SPMAIEEQVAVIYAGVRGYLDKLEPSKITKFENAFLSHVVSQHQALLSKIRTDGKISEES	536
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sp	P07251	ATPA_YEAST	LASLKSATESFVATF--	545
sp	P25705	ATPA_HUMAN	DAKLKEIVTNFLAGFEA	553
sp	P19483	ATPA_BOVIN	DAKLKEIVTNFLAGFEA	553
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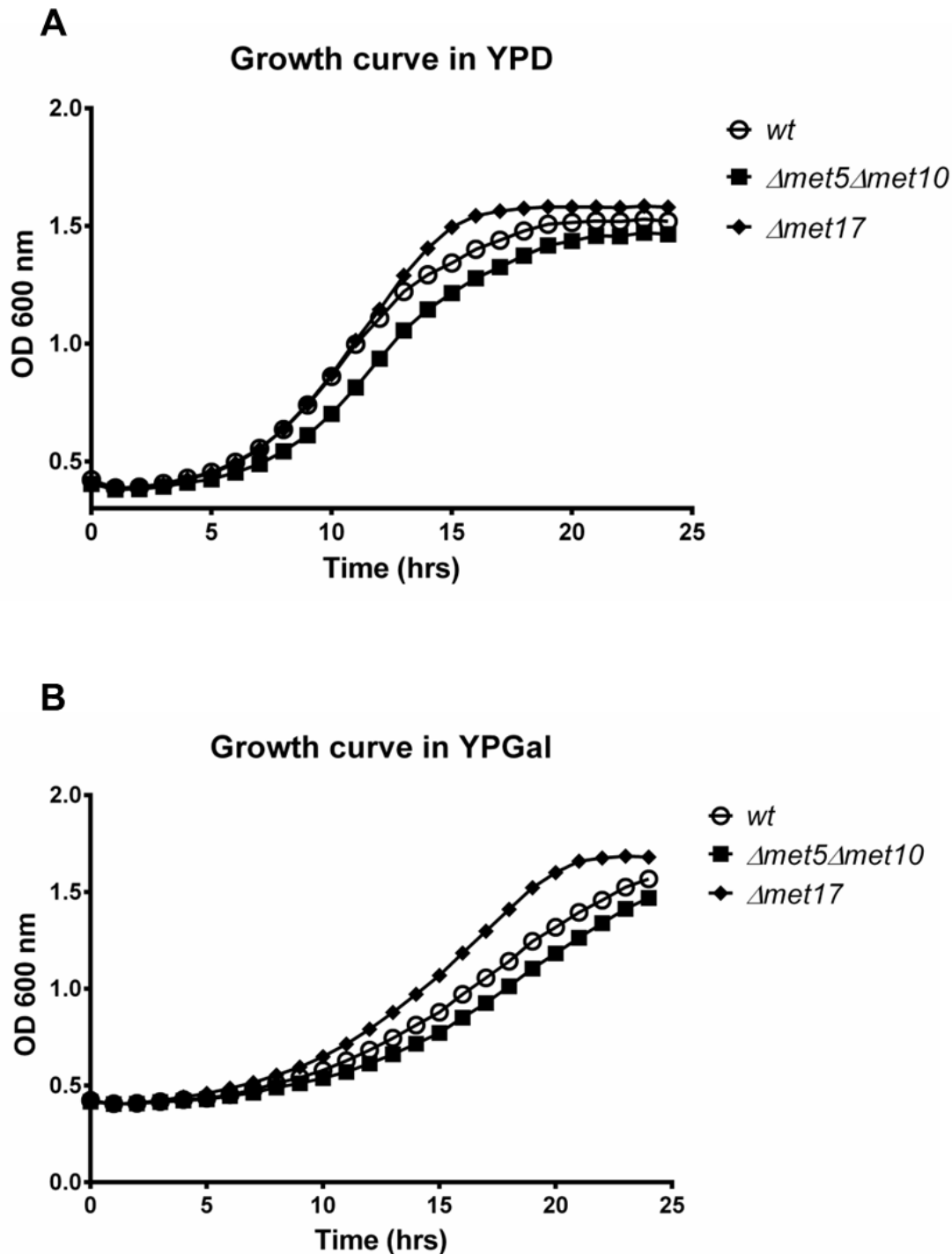
Percentage identity: 70.79%

Conserved cysteine residue Unconserved cysteine residue

B



Supplementary Figure S2. ATP synthase cysteine residue conservation. (A) Sequence alignment of ATP synthases from *Saccharomyces cerevisiae*, *Homo sapiens* and *Bos taurus*. Mammal C244 and yeast C238 conserved residues are highlighted in red box. Mammal C294 residue is not conserved in yeast, also highlighted by green box. Lineal alignments were made using Clustal Omega. (B) Zoom from the structural superimposition of bovine ATP1A1 (blue, PDB code 5ARA) and yeast ATP5 (gray, PDB code 6CP6). Cysteine 238 from yeast (yellow, C238 Sc) and cysteine 244 from bovine (red, C244 Bt) are in the same sheet with similar orientation. Nearly all secondary structures from local vicinity are conserved between mammals and yeast. Structural superimposition were made using PyMOL.



Supplementary Figure S3. H₂S overproducer strain grows faster on fermentable culture medium. *S. cerevisiae* BY4742 (*wt*) and derived mutants were precultured in YPD medium for 24 h at 30°C under constant agitation. Next, cells were inoculated at OD₆₀₀=0.03 in A) YPD or B) YPGal for 24 hrs and OD₆₀₀ nm in a Bioscreen C microplate absorbance reader were measured.

Supplementary Table S1

Yeast strains

Name	Genotype	Source
BY4741	<i>MATα; his3Δ 1; leu2Δ 0; met17Δ 0; ura3Δ</i>	Euroscarf
BY4742	<i>MATα; his3Δ 1; leu2Δ 0; lys2Δ 0; ura3Δ</i>	Euroscarf
GNO13	<i>MATα; his3Δ 1; leu2Δ 0; lys2Δ 0; ura3Δ; met5Δ:KanMx; met10Δ:ClonNAT</i>	This study
PM01	<i>MATα; his3Δ 1; leu2Δ 0; lys2Δ 0; ura3Δ; met17Δ:KanMx</i>	This study
<i>Kluyveromyces marxianus</i>	Isolated from mezcal production	Estrada-Ávila 2022
<i>Meyerozyma guilliermondii</i>	Isolated from mezcal production	Estrada- Ávila 2022

Oligonucleotide primers

Name	Secuence
met5F	ATAAAGTAACAGTAGGGAACGGGAACCAGAGAAAAACAAAAGATTGGCGAcagctgaagcttcgtacgc
met5R	AATGTGGGAAGAAAACCCAATAGTATGTCCTACTATGTCATATGCTATCAcatagggcactagtggatc
met10F	ATAGTTTTTTTCAACTGCTTTTCCTCGAGGTCACCCAAATATACAACGAGcagctgaagcttcgtacgc
met10R	TTCAAGACAGGTTCAATAAATAGATATTTAGTTTTTATTACTATATTAATcatagggcactagtggatc
met17F	TACAGGGTCGTCAGATACATAGATACAATTCTATTACCCCATCCATACacagctgaagcttcgtacgc
met17R	TTGTGAGAGAAAGTAGGTTTATACATAATTTTACAACCTCATTACGCACACcatagggcactagtggatc
met5A	TTCATCACGTGCGTATTATCTCTTA
met5D	TTTATTCTTCACCTCGTTTTTCATTC
met10A	GCAGTAGTTCTCGAGACCACC
met10D	TTAGTATAATGTGATGGTTAGTTTT
met17A	AGGGTTCGAATCCCTTAGC
met17D	ACAGCTTTATAGGAGGCGT

Supplementary Table S2

YPGal-Control			
Accession	Protein Name	Unused Score	Coverage (%)
ACAC_YEAST	Acetyl-CoA carboxylase	59.56	44.42
PYC1_YEAST	Pyruvate carboxylase 1	30.46	46.10
SYEC_YEAST	Glutamate--tRNA ligase cytoplasmic	16.73	47.18
ARC1_YEAST	tRNA-aminoacylation cofactor ARC1	10.27	39.10
RS17B_YEAST	40S ribosomal protein S17-B	8.00	75.00
PYC2_YEAST	Pyruvate carboxylase 2	2.00	42.03
YJ41B_YEAST	Transposon Ty4-J Gag-Pol polyprotein	2.00	16.75
RS25B_YEAST	40S ribosomal protein S25-B	2.00	55.56
YPGal- SSH			
Accession	Protein Name	Unused Score	Coverage (%)
ARC1_YEAST	tRNA-aminoacylation cofactor ARC1	18.28	37.23
ADH1_YEAST	Alcohol dehydrogenase 1	14.00	27.01
ALF_YEAST	Fructose-bisphosphate aldolase	12.01	30.36
EF1A_YEAST	Elongation factor 1-alpha	11.53	29.69
ENO2_YEAST	Enolase 2	10.63	47.60
HSP71_YEAST	Heat shock protein SSA1	10.20	40.81
PGK_YEAST	Phosphoglycerate kinase	8.02	37.50
KPYK1_YEAST	Pyruvate kinase 1	8.01	40.00
RL27B_YEAST	60S ribosomal protein L27-B	4.54	38.24
RS3A1_YEAST	40S ribosomal protein S1-A	4.48	38.04
ACAC_YEAST	Acetyl-CoA carboxylase	4.38	15.63
RS8B_YEAST	40S ribosomal protein S8-B	4.15	38.99
TPIS_YEAST	Triosephosphate isomerase	4.13	29.44
RL10_YEAST	60S ribosomal protein L10	4.00	53.39
GAL1_YEAST	Galactokinase	4.00	16.67
G3P3_YEAST	Glyceraldehyde-3-phosphate dehydrogenase 3	4.00	28.92
PDC1_YEAST	Pyruvate decarboxylase isozyme 1	4.00	11.90
PMG1_YEAST	Phosphoglycerate mutase 1	4.00	27.13
RL3_YEAST	60S ribosomal protein L3	2.00	39.02
VDAC1_YEAST	Mitochondrial outer membrane protein porin 1	2.00	32.86
RS4B_YEAST	40S ribosomal protein S4-B	2.00	19.92
RL6A_YEAST	60S ribosomal protein L6-A	2.00	26.70
HSP74_YEAST	Heat shock protein SSA4	1.46	33.02

Supplementary Table S2

YPD-Control			
Accession	Protein Name	Unused Score	Coverage (%)
ACAC_YEAST	Acetyl-CoA carboxylase	132.24	70.67
PYC1_YEAST	Pyruvate carboxylase 1	48.95	70.08
SYEC_YEAST	Glutamate--tRNA ligase cytoplasmic	47.71	70.20
ARC1_YEAST	tRNA-aminoacylation cofactor ARC1	31.70	76.60
DUR1_YEAST	Urea amidolyase	21.94	54.55
RS17B_YEAST	40S ribosomal protein S17-B	14.00	95.59
COPA_YEAST	Coatomer subunit alpha	11.51	46.63
PYC1_YEAST	Pyruvate carboxylase 1	6.35	56.53
RL37A_YEAST	60S ribosomal protein L37-A	4.18	86.36
RL17A_YEAST	60S ribosomal protein L17-A	4.07	81.52
RL37B_YEAST	60S ribosomal protein L37-B	4.01	87.50
RS16B_YEAST	40S ribosomal protein S16-B	3.21	94.41
RL35B_YEAST	60S ribosomal protein L35-B	2.34	71.67
RL43B_YEAST	60S ribosomal protein L43-B	2.13	69.57
RS30B_YEAST	40S ribosomal protein S30-B	2.01	87.30
RS15_YEAST	40S ribosomal protein S15	2.00	57.04
RS19B_YEAST	40S ribosomal protein S19-B	2.00	66.67
IRC5_YEAST	Uncharacterized ATP-dependent helicase IRC5	1.96	32.94
RGT1_YEAST	Glucose transport transcription regulator RGT1	1.42	18.38
RL13B_YEAST	60S ribosomal protein L13-B	1.33	80.40
YPD-SSH			
Accession	Protein Name	Unused Score	Coverage (%)
ARC1_YEAST	tRNA-aminoacylation cofactor ARC1	26.13	67.02
KPYK1_YEAST	Pyruvate kinase 1	24.17	63.40
EF1A_YEAST	Elongation factor 1-alpha	24.03	55.90
ADH1_YEAST	Alcohol dehydrogenase 1	18.61	54.60
ACAC_YEAST	Acetyl-CoA carboxylase	18.51	43.48
ALF_YEAST	Fructose-bisphosphate aldolase	14.03	37.88
ENO2_YEAST	Enolase 2	12.09	55.38
RS11B_YEAST	40S ribosomal protein S11-B	11.05	83.33
HSP71_YEAST	Heat shock protein SSA1	10.59	32.87
G3P3_YEAST	Glyceraldehyde-3-phosphate dehydrogenase 3	8.06	49.10
PGK_YEAST	Phosphoglycerate kinase	8.00	54.09
HSP75_YEAST	Heat shock protein SSB1	6.58	32.79
RL3_YEAST	60S ribosomal protein L3	6.15	60.98
RL27B_YEAST	60S ribosomal protein L27-B	6.10	76.47
RS8B_YEAST	40S ribosomal protein S8-B	6.09	53.50
RS16B_YEAST	40S ribosomal protein S16-B	6.03	70.63
PDC1_YEAST	Pyruvate decarboxylase isozyme 1	6.00	22.56
PST2_YEAST	Protoplast secreted protein 2	6.00	36.36
RL20B_YEAST	60S ribosomal protein L20-B	5.70	56.40
RL6B_YEAST	60S ribosomal protein L6-B	4.80	38.07
RS3_YEAST	40S ribosomal protein S3	4.70	63.33
RS4B_YEAST	40S ribosomal protein S4-	4.63	60.54
EF2_YEAST	Elongation factor 2	4.48	31.71
RL10_YEAST	60S ribosomal protein L10	4.11	44.80
RS3A2_YEAST	40S ribosomal protein S1-B	4.09	52.16

Supplementary Table S2

TKT1_YEAST	Transketolase 1	4.03	31.32
RL4A_YEAST	60S ribosomal protein L4-A	4.00	67.68
PMG1_YEAST	Phosphoglycerate mutase 1	4.00	54.25
RS20_YEAST	40S ribosomal protein S20	4.00	71.07
TPIS_YEAST	Triosephosphate isomerase	4.00	25.81
CYPH_YEAST	Peptidyl-prolyl cis-trans isomerase	4.00	25.31
RL7A_YEAST	60S ribosomal protein L7-A	3.50	72.54
RL13B_YEAST	60S ribosomal protein L13-B	2.89	70.35
RL15B_YEAST	60S ribosomal protein L15-B	2.86	76.47
PYR1_YEAST	Protein URA2	2.50	21.54
RL30_YEAST	60S ribosomal protein L30	2.16	50.48
MPG1_YEAST	Mannose-1-phosphate guanylttransferase	2.11	30.75
RS24B_YEAST	40S ribosomal protein S24-B	2.05	68.15
RS19B_YEAST	40S ribosomal protein S19-B	2.01	64.44
RL6A_YEAST	60S ribosomal protein L6-A	2.00	47.16
CG13_YEAST	G1/S-specific cyclin CLN3	2.00	18.79
YCP4_YEAST	Flavoprotein-like protein YCP4	2.00	34.01
HAL9_YEAST	Halotolerance protein 9	1.34	13.20

Supplementary Table S3

Protein activity values

GAPDH 2 h

NaHS mM	Media \pm SD
0	0.2364 \pm 0.01
0.1	0.3324 \pm 0.03
0.25	0.3505 \pm 0.02

GAPDH 7 h

NaHS mM	Media \pm SD
0	0.2832 \pm 0.05
0.1	0.3716 \pm 0.02
0.25	0.2837 \pm 0.02

PK 2 h

NaHS mM	Media \pm SD
0	0.1216 \pm 0.02
0.1	0.2909 \pm 0.02
0.25	0.3398 \pm 0.09

PK 7 h

NaHS mM	Media \pm SD
0	0.3686 \pm 0.02
0.1	0.4577 \pm 0.06
0.25	0.3286 \pm 0.05