Antunes et al.

Overexpression of Branched-Chain Amino Acid Aminotransferases rescues the growth defects of cells lacking the Barth Syndrome related gene *TAZ1*

Supplementary Information

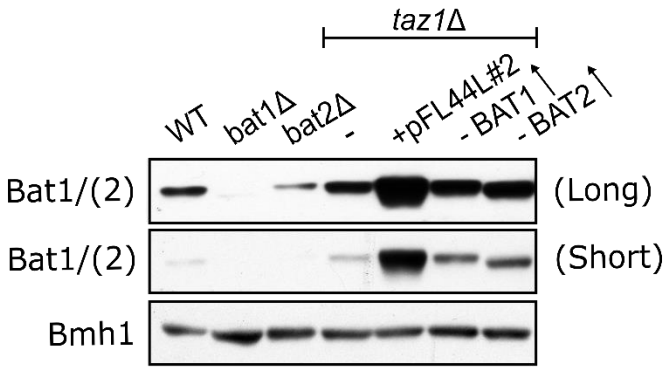

Fig. S1. Steady state levels of Bat1/(2) in relevant cells. Whole cell lysate were obtained from WT, *bat1Δ*, *bat2Δ*, and *taz1Δ* cells transformed with an empty plasmid (-), a plasmid isolated from the screen, or genomically overexpressing *BAT1* or *BAT2*. Samples were analysed by SDS-PAGE and immunodetection with antibodies against Bat1 (long and short exposition) or the cytosolic marker Bmh1.

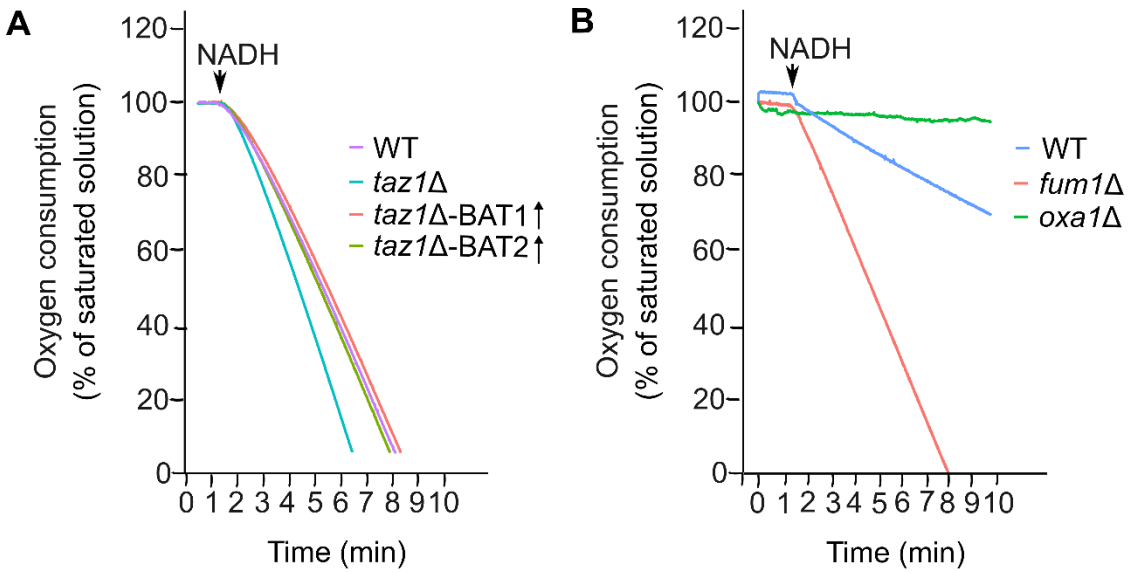

Fig. S2. Overexpression of *BAT1* or *BAT2* restores the altered mitochondrial oxygen consumption of *taz1Δ* cells. (A-B) The rate of oxygen consumption of mitochondria isolated from the indicated strains was measured upon the addition of 7 mM NADH.

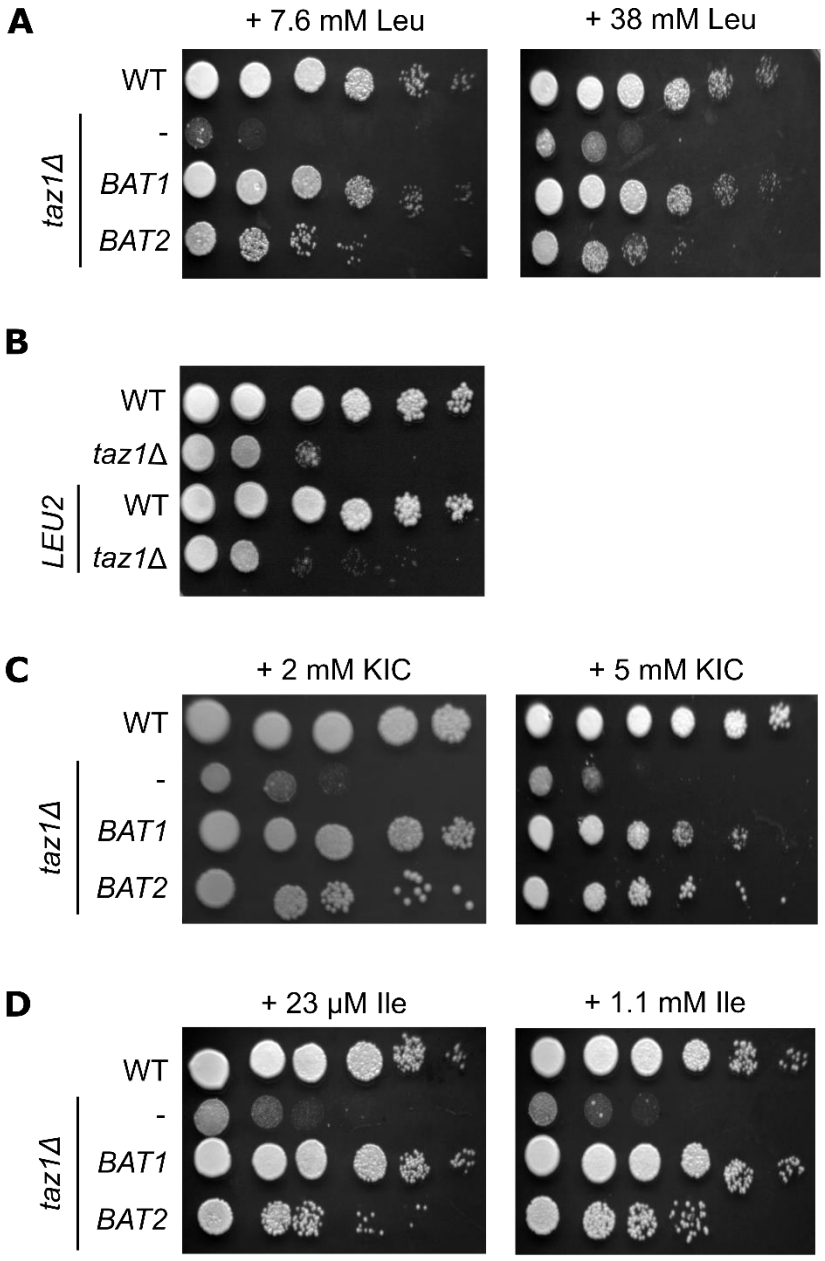

Fig. S3. Supplementation of the branched-chain amino acids leucine and isoleucine has no effect on the growth defect of *taz1Δ* cells. (A) The indicated cells were analysed by drop dilution assay at 30°C on SE medium supplemented with the specified concentrations of leucine (Leu). **(B)** WT and *taz1Δ* cells as well as WT and *taz1Δ* cells genomically overexpressing *LEU2* were analysed by drop dilution assay at 30°C on SE medium. **(C-D)** The growth of the indicated strains was analysed by drop dilution assay at 30°C on SE medium supplemented with the specified concentrations of α-ketoisocaproic acid (KIC) (C), or isoleucine (Ile) (D).

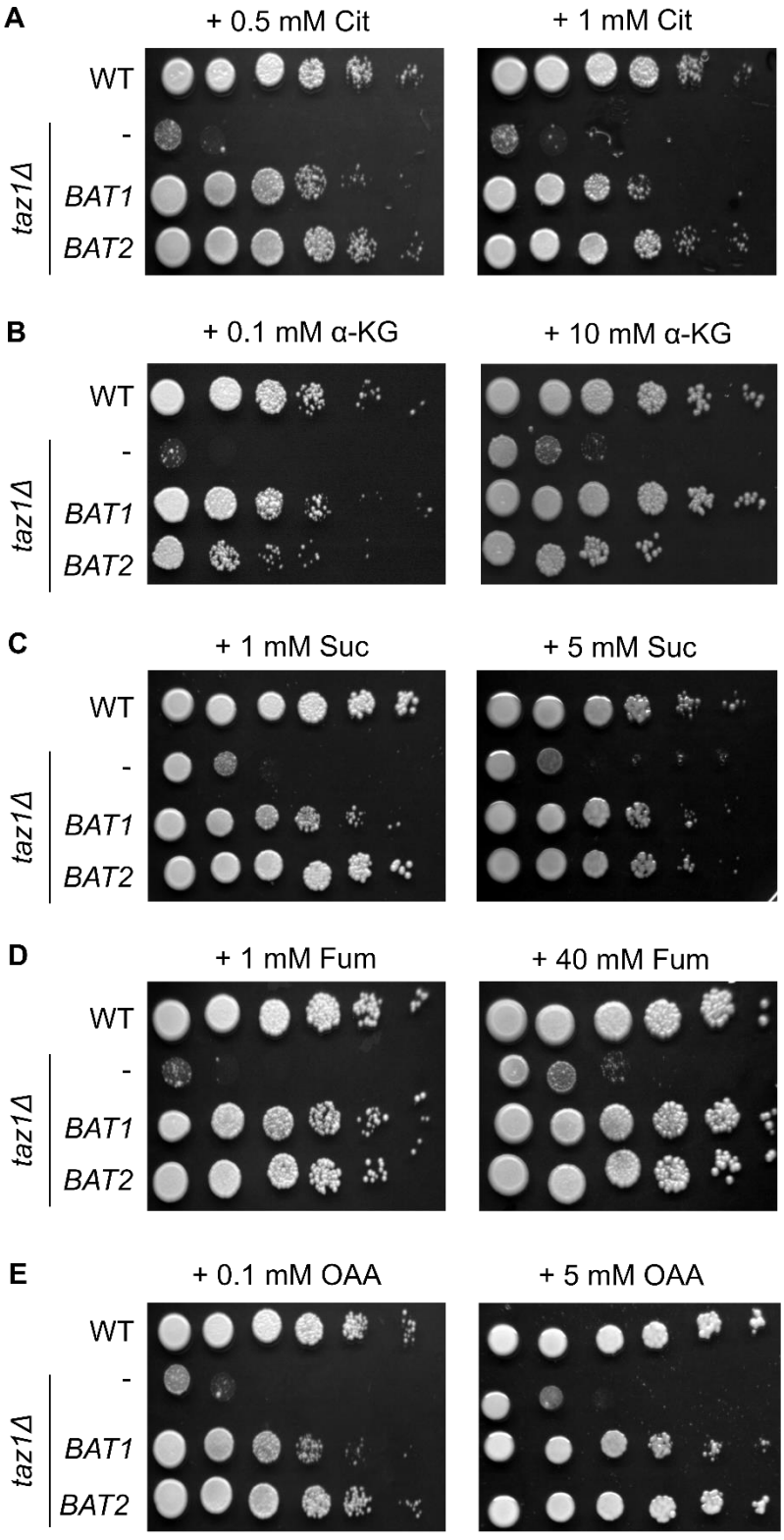

Fig. S4. Supplementation of various TCA cycle metabolites has no effect on the growth of *taz1Δ* cells. (A-E) The specified strains were analysed by drop dilution assay at 30°C on SE medium supplemented with the indicated concentrations of the TCA cycle metabolites citrate (Cit) (A), α-ketoglutarate (α-KG) (B), succinate (Suc) (C), fumarate (Fum) (D), or oxaloacetate (OAA) (E).

Supplementary Table 1. Strains used in this study.

Name	Genotype	Source
WT (W303)	*leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*	N/A
WT (BY4741)	*his3-1 leu2-0 met15-0 ura3-0*	N/A
crd1Δ	*BY4741; crd1Δ::HIS3MX6*	current study
gep4Δ	*BY4741; gep4Δ::KANMX4*	(Sauerwald et al., 2015)
crd1Δ-BAT1	*BY4741; crd1Δ::HIS3MX6 BAT1::natNT2-GPDpr-BAT1*	current study
crd1Δ-BAT2	*BY4741; crd1Δ::HIS3MX6 BAT2::natNT2-GPDpr-BAT2*	current study
gep4Δ-BAT1	*BY4741; crd1Δ::KANMX4 BAT1::natNT2-GPDpr-BAT1*	current study
gep4Δ-BAT2	*BY4741; gep4Δ::KANMX4 BAT2::natNT2-GPDpr-BAT2*	current study
WT-LEU2	*W303α LEU2::natNT2-GPDpr-LEU2*	current study
taz1Δ	W303α; *taz1Δ::HIS3MX6*	current study
bat1Δ	W303α; *bat1Δ::HIS3MX6*	current study
bat2Δ	W303α; *bat2Δ::HIS3MX6*	current study
taz1Δ-BAT1	W303α; *taz1Δ::HIS3MX6 BAT1::natNT2-GPDpr-BAT1*	current study
taz1Δ-BAT2	W303α; *taz1Δ::HIS3MX6 BAT2::natNT2-GPDpr-BAT2*	current study
taz1Δ-LEU2	W303α; *taz1Δ::HIS3MX6 LEU2::natNT2-GPDpr-LEU2*	current study
taz1Δ-AVT1	W303α; *taz1Δ::HIS3MX6 AVT1::natNT2-GPDpr-AVT1*	current study
taz1Δ-AVT4	W303α; *taz1Δ::HIS3MX6 AVT4::natNT2-GPDpr-AVT4*	current study

Supplementary Table 2. Plasmids and primers used in this study.

Plasmids	**Promoter**	**Markers**	**Reference**
pFL44L	-	URA3, AmpR	(Stettler et al., 1993)
pYX142-Taz1	TPI	LEU2, AmpR	current study
pYM-N15	GPD	natNT2, AmpR	(Janke et al., 2004)
Primers for deletion by gene-targeting			
DAFwd012	5'-ATA TTT CAT TTT CAA AAA AAA AAA AAG TAA AGT TTT CCC TAT CAA CGT ACG CTG CAG GTC GAC-3'		
DARev011	5'-GAC CTC ATA CAT GCT AGT ATT TAC ACG AAT TTA ATT GCT TAA ATT ATC GAT GAA TTC GAG CTCG-3'		
DAFwd032	5'-CAC CCT ATA AAC GCA AAA TCA GCT AGA ACC TTA GCA TAC TAA AAC CGT ACG CTG CAG GTC GAC-3'		
DARev034	5'-GTT TTT TTT TTT TGG GGG GGG AGG GGA TGT TTA CCT TCA TTA TCA ATC GAT GAA TTC GAG CTCG-3'		
DAFwd033	5'-TTT AGA AAT TTA AGG GAA AGC ATC TCC ACG AGT TTT AAG AAC GAT CGT ACG CTG CAG GTC GAC-3'		
DARev035	5'-TAT TCT TTT TAA CTT TTA ATT ACT TTA CGT AGC AAT AGC GAT ACT ATC GAT GAA TTC GAG CTCG-3'		
DAFwd039	5'-ACA AGC AGG CCT GGT AGC ATA GTT TGG TCC CTA ATA ATT TAG TCA CGT ACG CTG CAG GTC GAC-3'		
DARev042	5'-CAA AAT GAA AAG TCA GGA CCC TTT TCA AAA AGG ATC GCA ATT ATA ATC GAT GAA TTC GAG CTCG-3'		
Primers for introducing open reading frames into pYX plasmids			
DAFwd015	5'-GGG GAA TTC ATG TCT TTT AGG GAT GTC CTA GAA AGA GGA GAT-3'		
DARev014	5'-GGG AAG CTT TCA ATC ATC CTT ACC CTT TGG TTT ACC CTC TGG A-3'		
Primers for genomic N-terminal overexpression			
BAT1 N' tag pYM F	5'-AAA CGC AAA ATC AGC TAG AAC CTT AGC ATA CTA AAA CAT GCG TAC GCT GCA GGT CGA C-3'		
BAT1 N' tag pYM R	5'-TGA TGG AGA ATT TCC CCA ACT TCA AGG AAT GTC TCT GCA ACA TCG ATG AAT TCT CTG TCG-3'		
BAT2 N' tag pYM F	5'-TTT AAG GGA AAG CAT CTC CAC GAG TTT TAA GAA CGA TAT GCG TAC GCT GCA GGT CGA C-3'		
BAT2 N' tag pYM R	5'-TAG TTA TCT TAA CTT TGG AGG CGT CTA GGG GTG CCA AGG TCA TCG ATG AAT TCT CTG TCG-3'		
COX24 N' tag pYM F	5'-CCA AGA CGA GCA CAC ACG ACA CCA GAA CGA GAT AAA CAT GCG TAC GCT GCA GGT CGA C-3'		
COX24 N' tag pYM R	5'-TAG TAA TGC CCA GCC ACC CAG GTC GCA ATG CCC TTC CTA GCA TCG ATG AAT TCT CTG TCG-3'		
CRG1 N' tag pYM F	5'-CTT CAA AGC CAG TCT TCT GTC AAT GGA AGA ATC CAG AAT GCG TAC GCT GCA GGT CGA C-3'		
CRG1 N' tag pYM R	5'-GAG CAG ATT CAA AAT TTT TGT TTA AAT AAC TAG TTT TAG GCA TCG ATG AAT TCT CTG TCG-3'		
HMX1 N' tag pYM F	5'-GCA TAT ATA CAC ACA CAC ACA TAA AAT AAC CGC AAA AAT GCG TAC GCT GC AGG TCG AC-3'		
HMX1 N' tag pYM R	5'-CGT CAG TGG GTG AGG GTA TGA TTG TAT TGC TAC TGT CCT CCA TCG ATG AAT TCT CTG TCG-3'		
DAFwd038	5'-AAT ATA TAT ATA TAT ATT TCA AGG ATA TAC CAT TCT AAT GCG TAC GCT GCA GGT CGA C-3'		

DARev040	5‘-CGT GGT CAC CTG GCA AAA CGA CGA TCT TCT TAG GGG CAG ACA TCG ATG AAT TCT CTG TCG-3‘
DAFwd044	5‘-GAC TTA CGT ATT CTG TAT AAC TGA TTC CGA GAC GCA AAT GCG TAC GCT GCA GGT CGA C-3‘
DARev046	5‘-AGC GCT TAC GGC CAT TGG GAC TCA ATG GTT CTT GCT CAG GCA TCG ATG AAT TCT CTG TCG-3‘
DAFwd045	5‘-TGG AAT CAA CAT AAC AAT ATC CTA GAA CAC ATC ATC AAT GCG TAC GCT GCA GGT CGA C-3‘
DARev047	5‘-TTC GGA TCC CAA GGT GCT CAC CAT CTC CAT TGT TAG TGA CCA TCG ATG AAT TCT CTG TCG-3‘
Primers for screening PCR of gene-targeting	
DAFwd013	5’-CAG TAT TTC TAT TAC GTT ACT CCA G-3’
DARev012	5’-TTA TAT GGT AGT GTT GCC CAA ACT A-3’
DAFwd014	5’-ATG TCT TTT AGG GAT GTC CTA GAA A-3’
DARev013	5’-GGG CGG GGA GTA GGC TTT TTT TAG C-3’
DAFwd034	5‘-GCG GTT GAT ACT TTG TGC AGA TTT C-3‘
DARev036	5‘-ATA CCT TGG CAA CTA AAT TAC AAG C-3‘
DAFwd035	5‘-ATG TTG CAG AGA CAT TCC TTG AAG T-3‘
DARev037	5‘-AGT GCC AAC ACC TAA ACC CTT GGA T-3‘
DAFwd036	5‘-AAT CTG TAG ATC CGA CTC TTT TTC T-3‘
DARev038	5‘-GAG TTG CTT CTA AGG TAT GTA TGG G-3‘
DAFwd037	5‘-ATG ACC TTG GCA CCC CTA GAC GCC T-3‘
DARev039	5‘-GTA ATA AGG ACC CAC AGG GCA GCA A-3‘
DAFwd042	5’-CGA CAG AGA ATT CAT CGA TG-3’
BAT1 N’tag CHK R	5‘-GTT GGC AAA CAA ATT CTA GC-3‘
BAT2 N’tag CHK R	5‘-TAG TGC CGA TTA ATG TAG GC-3‘
COX24 N’tag CHK R	5‘-GCT TTC TTC TTT CTG CCT TC-3‘
CRG1 N’tag CHK R	5‘-GAA TGC AAA AGT TCC ATC AC-3‘
HMX1 N’tag CHK R	5‘-TGT GTA TGT TTT CGT GGA TG-3‘
DARev041	5‘-ACT TCT GGA ACG GTG TAT TG-3‘
DAFwd040	5’-CGT TTG GCA AAC AAT TAC AGG AAG A-3’
DARev043	5’-TGT TAG ACG ATC TGG TAC TAC GAA C-3’
DAFwd041	5’-ATG ATT CAA ATG GTG CCC ATT TAT T-3‘
DARev044	5’-TAT AAA CAA AGC ACT TAT GGC TAG T-3‘
DARev048	5‘-TTGTTGAATTTTGGATGGAC-3‘
DARev049	5‘-CAGCGCTACTCAGGTTAGTC-3‘

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Supplementary Table 3. Antibodies used in this study.

Antibodies	Dilution	Source
polyclonal rabbit anti-Aco1	1 :7000	Lab stocks
polyclonal rabbit anti-Bmh1	1 :1500	Lab stocks
polyclonal rabbit anti-Bat1	1:3000	Kindly provided by Roland Lill
polyclonal rabbit anti-Cor1	1:2000	Lab stocks
polyclonal rabbit anti-Cox2	1:1000	Lab stocks
polyclonal rabbit anti-Dld1	1:1000	Lab stocks
polyclonal rabbit anti-F1β	1:500	Lab stocks
polyclonal rabbit anti-Fum1	1:10000	Lab stocks
polyclonal rabbit anti-Rip1	1:5000	Lab stocks
polyclonal rabbit anti-Tom40	1:4000	Lab stocks
polyclonal rabbit anti-Tom70	1:2000	Lab stocks

Supplementary References

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