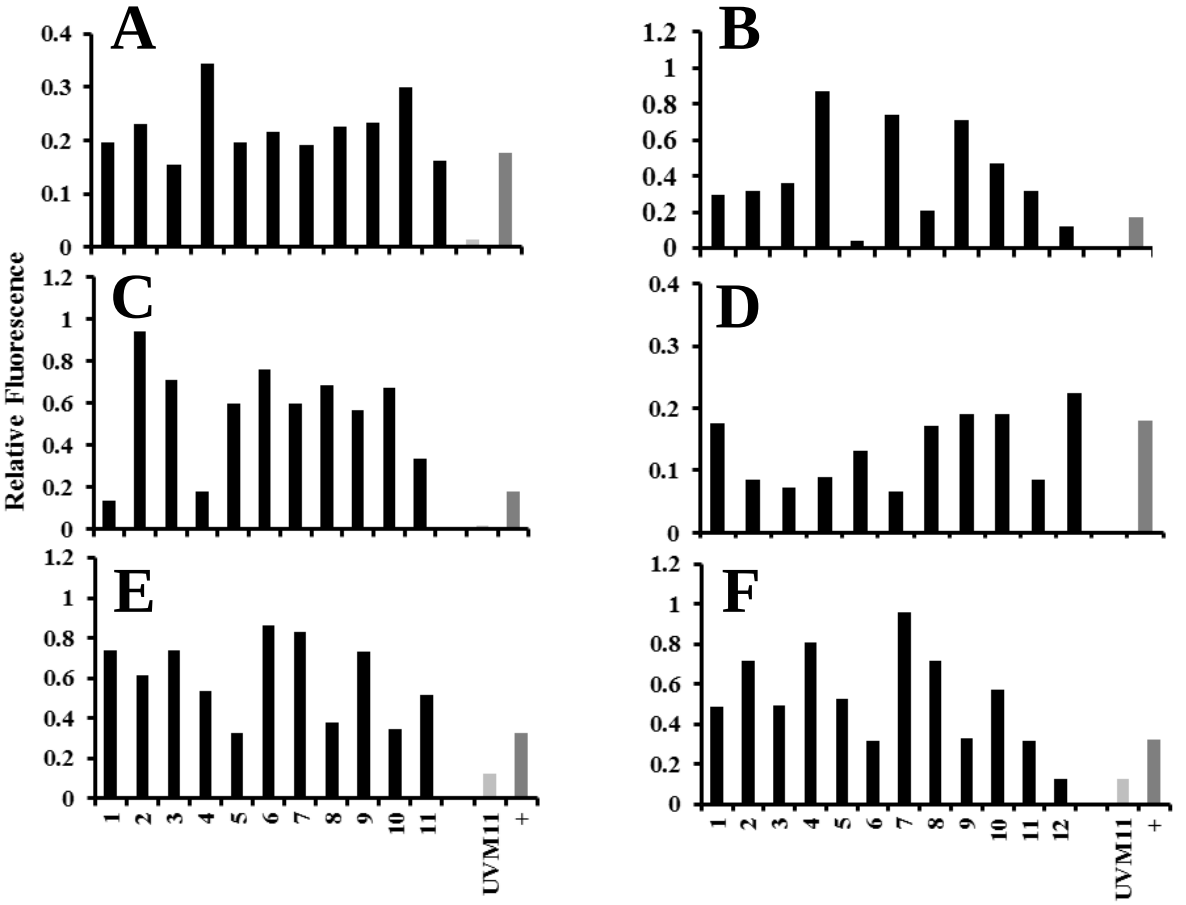
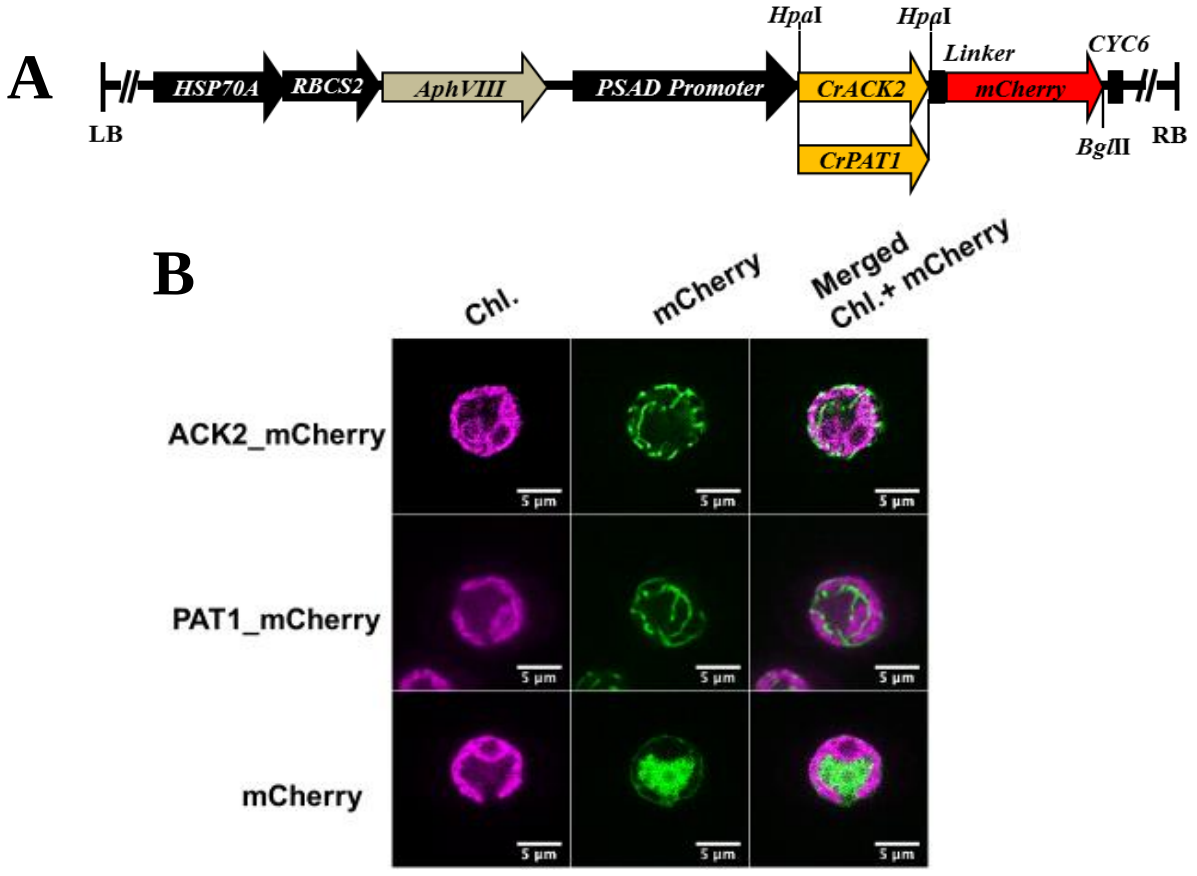


Supplemental Figure 1. Relative Fluorescence Intensity of Chlamydomonas Cells Expressing Fluorescent Fusion Proteins.

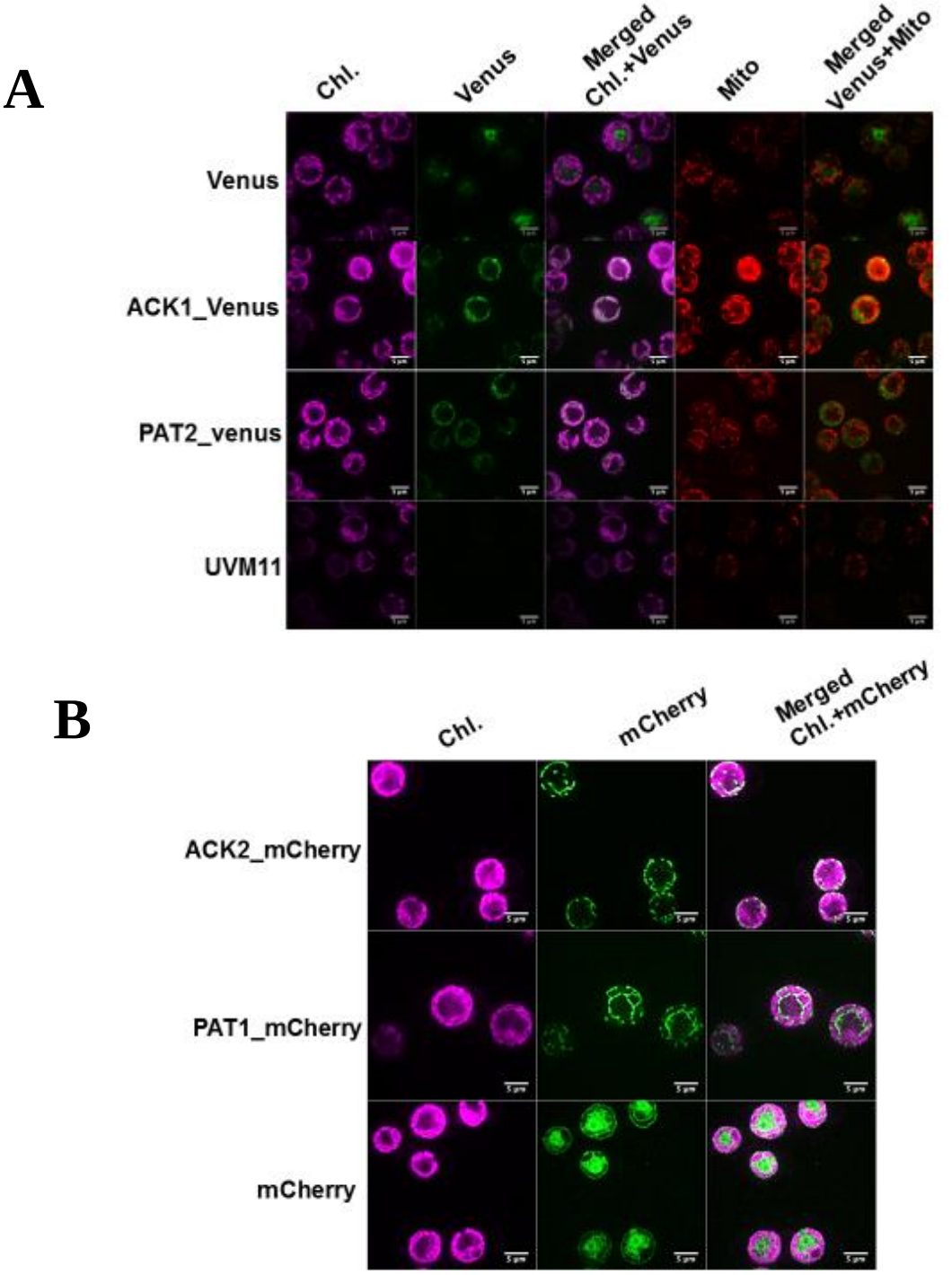
Fluorescence of the Venus **(A-D**: ACK1, ACK2, PAT1, and PAT2**)** and mCherry **(E-F**: ACK2 and PAT1**)** fusion proteins from Chlamydomonas transformants (from 1-12), visualized using a TECAN microtiter-plate reader. The UVM11 strain was used as a negative control while pLM004_Venus and pLM004_mCherry were used as positive controls (indicated with '+' on the x-axis). The y-axis represents the relative fluorescence compared to the UVM11 strain, and the x-axis represents individual transformants. Fluorescence from the cells was monitored at 3 different cell densities; the results for the different densities were similar.



Supplemental Figure 2. Localization of ACK2 and PAT1 Using mCherry Fusion Proteins.

(A). Schematic of the pLM004_mCherry with the *ACK2* or *PAT1* gene fusions. The black arrows represent the promoters, including the tandem *HSP70* and *RBCS2* promoter driving expression of the *AphVIII* gene, and the *PSAD* promoter driving expression of the genes of interest. The *ACK2* and *PAT1* cDNAs are highlighted in yellow; the *mCherry* gene is in red. The black box in front of the mCherry gene represents a small linker region and the black box after the *mCherry* gene represents the *Cyc6* terminator.

(B). Localization of ACK2 and PAT1 proteins. For all panels, column 1 shows the auto-fluorescence of chlorophyll (Chl.), column 2 the images of the mCherry fluorescence signal (mCherry), and column 3 the merged images of column 1 and column 2. Scale bars are 5 µm.

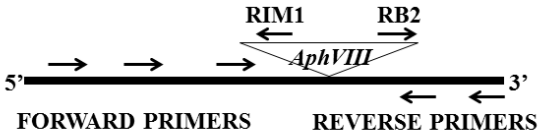


Supplemental Figure 3. Localization of ACK 1/2 and PAT 1/2 in Multiple Cells.

(A). Localization of ACK1 and PAT2 Venus fusion proteins. Scale bars are 5 μm.

(B). Localization of ACK2 and PAT1 mCherry fusion proteins. Scale bars are 5 μm. The different columns are the same as in **Figure 3B**.

A



B

Primers	ACK1-R1/RB2	ACK1-F1/RIM1	ACK1-F1/ACK1-R1
WT	-	-	819bp
ack1	491bp	793bp	-
Primers	**ACK2-F6/RB2**	**ACK2-R6/RIM1**	**ACK2-F6/ACK2-R6**
WT	-	-	1053 bp
ack2	951bp	569bp	-
Primers	**PAT2-R5/RB2**	**PAT2-F5/RIM1**	**PAT2-F5/PAT2-R5**
WT	-	-	696bp
pat2-1	537bp	688bp	-
Primers	**PAT2-R6/RB2**	**PAT2-F6/RIM1**	**PAT2F6/PAT2-R6**
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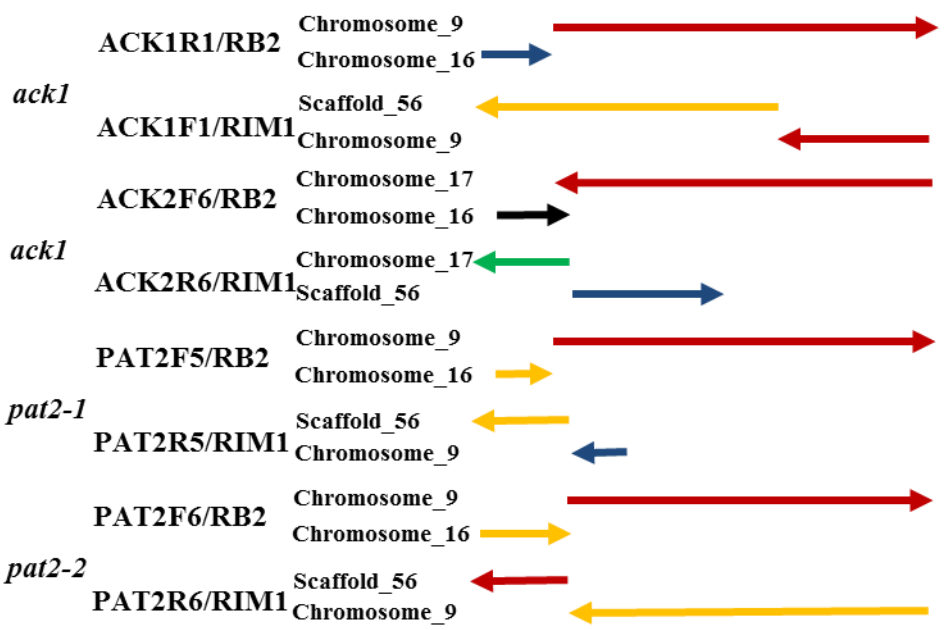
C



Supplemental Figure 4. Genetic Analyses of *ack* and *pat* Single and Double Mutants.

(A). Schematic showing the positions of the RB2 and RIM1 primers at the borders of the *AphVIII* insertion with representations of primers within the *PAT*/*ACK* target gene.

(B). Amplicon sizes from WT and the different *PAT* and *ACK* mutants by PCR.

(C). Junction analyses of the four single mutants by PCR amplification and sequencing. The amplicons described in (B), generated with RB2 and primers for PAT or ACK sequences represent chimeric products containing the *Cyc6* sequence of the 3' end of the insertion cassette and a region of the *PAT* or *ACK* gene contiguous to the 3' end of the cassette. The amplicons generated with RIM1 and primers for *PAT* or *ACK* represent chimeric products containing the *PSAD* promoter at the 5'end of the insertion cassette and a region of the *PAT* or *ACK* gene contiguous to the 5'end of the cassette. Different colors of arrows represent the lengths of the alignment (the matches are exact). Black: less than 40 nucleotides. Blue: 40-49 nucleotides. Green: 50-79 nucleotides. Orange: 80-199 nucleotides. Red: more than 200 nucleotides.

Supplemental Figure 5. Genomic and CDS Sequences of *ack1*, *ack2*, *pat2-1* and *pat2-2* Mutants.
Sequences from the genes are in uppercase while the sequences of the *PSAD* promoter, *AphVIII* gene, and *Cyc6* terminator are in lowercase. Blue nucleotides represent UTR, red nucleotides exons, and black nucleotides introns. The forward and reverse primers for the PCR are highlighted in green, while the RB2 and RIM1 primers are highlighted in pink and yellow, respectively. Newly introduced nucleotides are shown in bold.

***ack1* GENOMIC SEQUENCE**

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ack1 **CDS SEQUENCE**

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pat2-1 **GENOMIC SEQUENCE**

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***pat2-2* CDS SEQUENCE**

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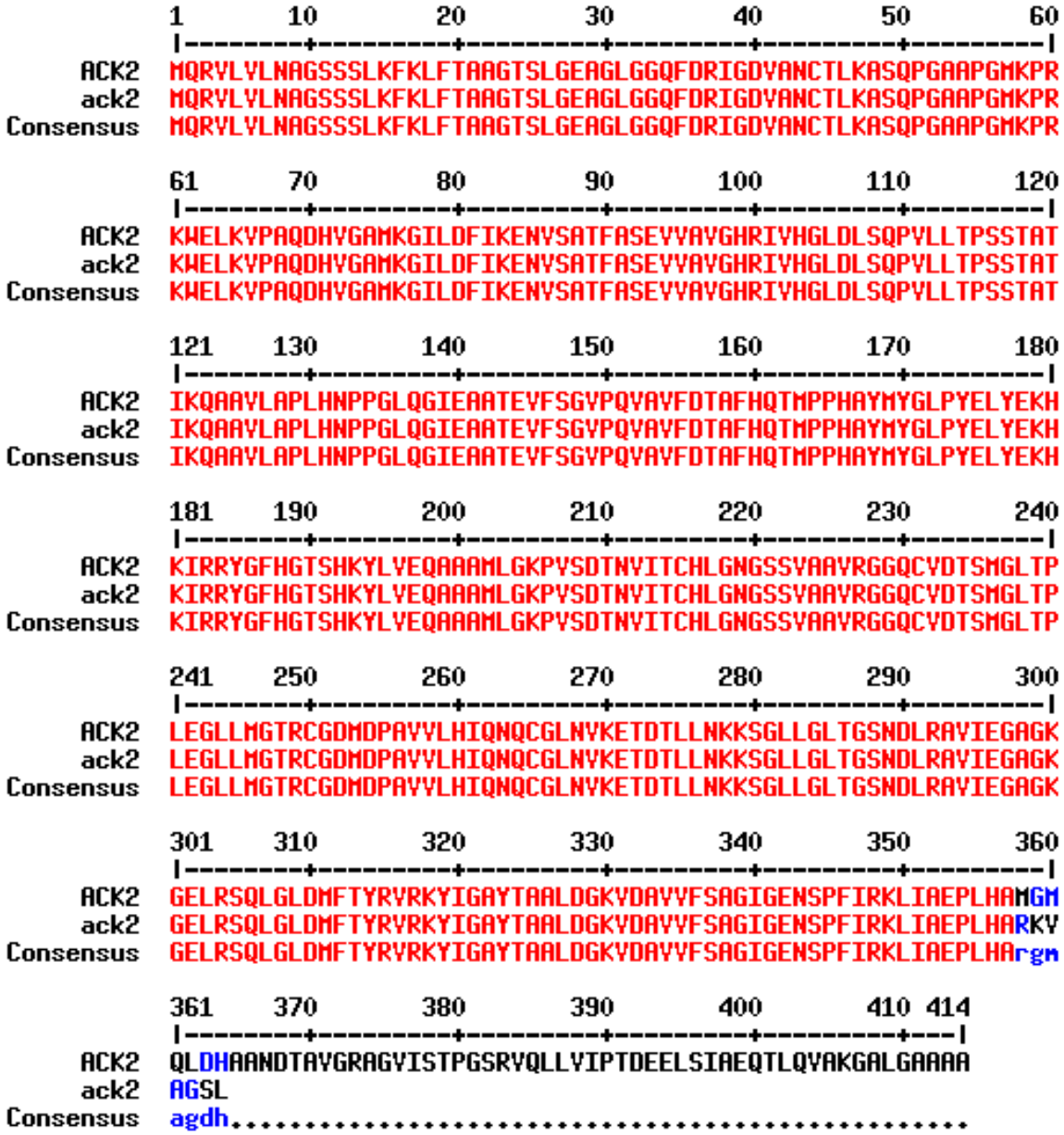
Supplemental Figure 6. Alignments of WT and Truncated ACK and PAT Proteins.
(A). Alignment of amino acid sequences of WT ACK1 and the truncated protein of the *ack1* mutant. **(B).** Alignment of amino acid sequences of WT ACK2 and the truncated protein of the *ack2* mutant. **(C).** Alignment of amino acid sequences of WT PAT2 and the truncated protein of the *pat2-1* mutant. Identical or highly conserved amino acids in all positions are given in red letters (high consensus region), a low consensus amino acid position is given in blue letters, while black letters indicate an overall lack of strong consensus. Alignments were performed using http://multalin.toulouse.inra.fr/multalin/.

(A). Alignment of amino acid sequences of WT ACK1 and the truncated protein of the *ack1* mutant.

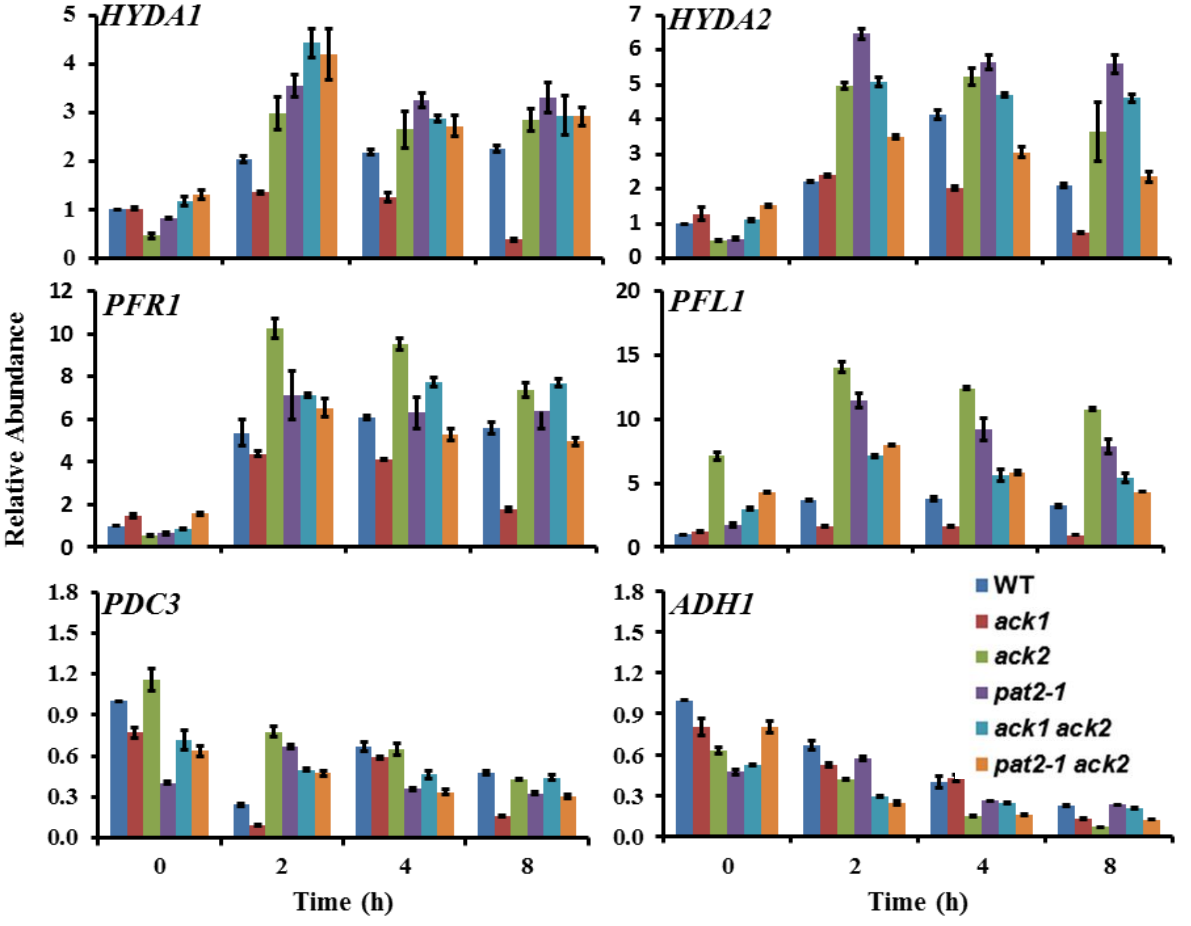
```
           1       10        20        30        40        50        60
           |--------+---------+---------+---------+---------+---------|
ACK1       MLAGKVPVGSAVPPRNTAPSGKGSKVLVYNAGSSSIKFKIFTAETLLPGAAGLFDRIGDP
ack1       MLAGKVPVGSAVPPRNTAPSGKGSKVLVYNAGSSSIKFKIFTAETLLPGAAGLFDRIGDP
Consensus  MLAGKVPVGSAVPPRNTAPSGKGSKVLVYNAGSSSIKFKIFTAETLLPGAAGLFDRIGDP

           61      70        80        90        100       110       120
           |--------+---------+---------+---------+---------+---------|
ACK1       ANCVLKASVPDDGGKPKKFEKKIPAKDHLTAMQDIMSFIDENVSSTFASEVCAVGHRIVH
ack1       ANCVLKASVPDDGGKPKKFEKKIPIVTRALVRNTSPPRSPSHGRPRSPFALS
Consensus  ANCVLKASVPDDGGKPKKFEKKIPakdralar#dimpridengrprfafalc.........

           121     130       140       150       160       170       180
           |--------+---------+---------+---------+---------+---------|
ACK1       GLDMSQPKLLTPDVIRCIQDASMFAPLHNPPHLQGIYASQKVFGPDVPQVAVFDTAFHQT
ack1
Consensus  ............................................................

           181     190       200       210       220       230       240
           |--------+---------+---------+---------+---------+---------|
ACK1       MPPESYVYGLPYDMYEKHKIRRYGFHGTSHKYLVDQAAAMLGKKVSETNVITCHLGNGSS
ack1
Consensus  ............................................................

           241     250       260       270       280       290       300
           |--------+---------+---------+---------+---------+---------|
ACK1       VAAVRGGQCVDTSMGMTPLEGLLMGTRCGDIDPAVVLHIQNQLGLSASETDTLLNKKSGL
ack1
Consensus  ............................................................

           301     310       320       330       340       350       360
           |--------+---------+---------+---------+---------+---------|
ACK1       LGLTGSNDLRTVIEGAGKGEPRSQLGLDMFVRRVRKYIGAYMAMLDGQVDAVVFSAGIGE
ack1
Consensus  ............................................................

           361     370       380       390       400       410       420
           |--------+---------+---------+---------+---------+---------|
ACK1       NSALVRGLICDRMQALGIAVDKDANAAAVGGKQADISGPGARIRALVIPTDEELSIAQQT
ack1
Consensus  ............................................................

           421     431
           |--------+|
ACK1       LQVVRGAALAA
ack1
Consensus  ...........
```

(B). Alignment of amino acid sequences of WT ACK2 and the truncated ACK2 protein of the *ack2* mutant.



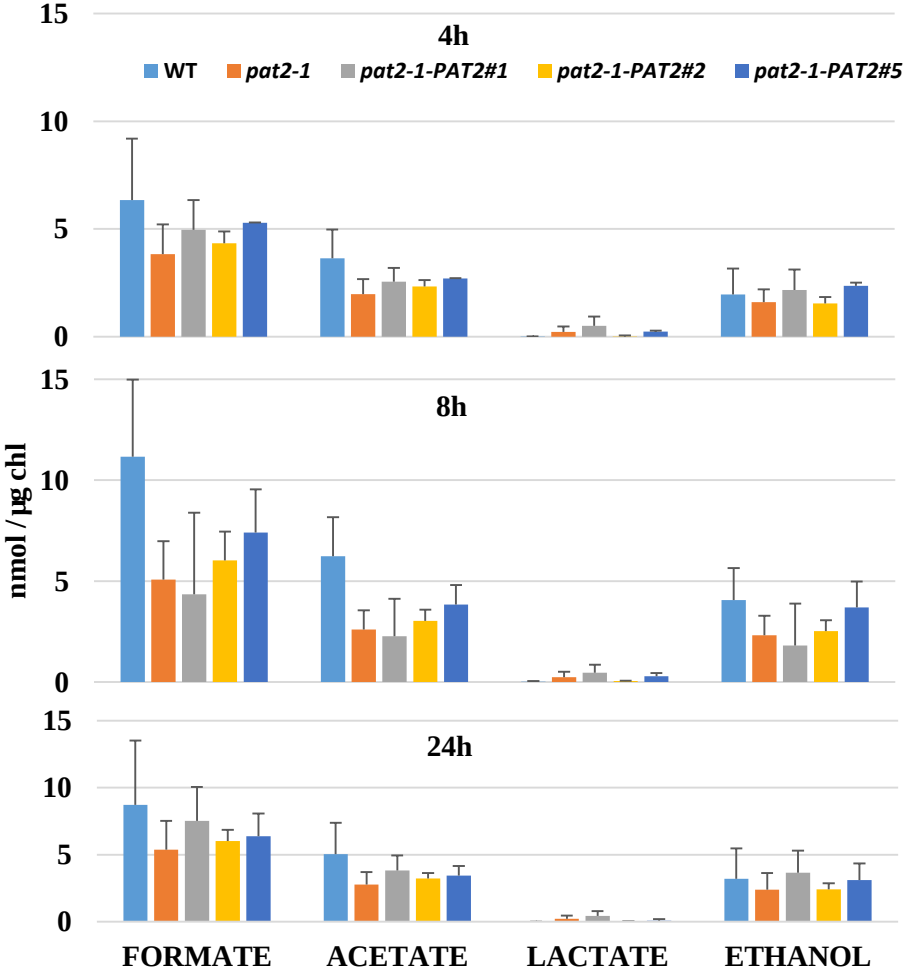
(C). Alignment of amino acid sequences of WT PAT2 and truncated PAT2 protein of the *pat2-1* mutant.

```
           1       10        20        30        40        50        60        70        80
           |--------+---------+---------+---------+---------+---------+---------+---------|
PAT2       MSLNSSTMSRRQAVAGAPAVAPFRHAGLFPRVRLCANRRVARVAPKAAGNGNIAQGEQGFDTLFLSDISLVGQRTPLLLG
pat2-1     MSLNSSTMSRRQAVAGAPAVAPFRHAGLFPRVRLCANRRVARVAPKAAGNGNIAQGEQGFDTLFLSDISLVGQRTPLLLG
Consensus  MSLNSSTMSRRQAVAGAPAVAPFRHAGLFPRVRLCANRRVARVAPKAAGNGNIAQGEQGFDTLFLSDISLVGQRTPLLLG

           81      90        100       110       120       130       140       150       160
           |--------+---------+---------+---------+---------+---------+---------+---------|
PAT2       FFNYFERHLPHVGFFEPIAAEALASSELRIDRHVELVYKVFNLKGDVRAMTGVQDAEAARMIANGQHSELLDKIYSQYAS
pat2-1     FFNYFERHLPHVGFFEPIAAEALASSELRIDRHVELVYKVFNLKGDVRAMTGVQDAEAARMIANGQHSELLDKIYSQYAS
Consensus  FFNYFERHLPHVGFFEPIAAEALASSELRIDRHVELVYKVFNLKGDVRAMTGVQDAEAARMIANGQHSELLDKIYSQYAS

           161     170       180       190       200       210       220       230       240
           |--------+---------+---------+---------+---------+---------+---------+---------|
PAT2       YKEGQDLVLVEGPGPLMGGTELDAQIAAALNAPVLMTMTGQPNATVADYYNRAMVKRQVFLDHHVEVLGLVMNGLPRQSH
pat2-1     YKEGQDLVLVEGPGPLMGGTELDAQIAAALNAPVLMTMTGQPNATVADYYNRAMVKRQVFLDHHVEVLGLVMNGLPRQSH
Consensus  YKEGQDLVLVEGPGPLMGGTELDAQIAAALNAPVLMTMTGQPNATVADYYNRAMVKRQVFLDHHVEVLGLVMNGLPRQSH

           241     250       260       270       280       290       300       310       320
           |--------+---------+---------+---------+---------+---------+---------+---------|
PAT2       AILSGQLRDKFAAAGLPFAGAIPTDIMLRNVRLDEVQTAMGAQRLYGDSLLTDVEFDDVVVASQRLEELLEILAERPMGR
pat2-1     AILSGQLRDKFAAAGLPFAGAIPTDIMLRPSVWNCERITISHRKQL
Consensus  AILSGQLRDKFAAAGLPFAGAIPTDIMLRnsrl#ceriaigarrql.....................................

           321     330       340       350       360       370       380       390       400
           |--------+---------+---------+---------+---------+---------+---------+---------|
PAT2       PLVVTSADRLDIVLGLLAAQLSVSGPGVAGILLTQAGSARSGRNYARDTIDRIFAGLSSSGLYKGSLLPVLVTDMPLRDA
pat2-1
Consensus  ................................................................................

           401     410       420       430       440       450       460       470       480
           |--------+---------+---------+---------+---------+---------+---------+---------|
PAT2       IRKLDNLDAAILPSSTRKISQCKRLFEQYVDANAVVARLQNMVRPNRMTPKMFMHTLKSMCNATPQHIVLPESEDKRVLA
pat2-1
Consensus  ................................................................................

           481     490       500       510       520       530       540       550       560
           |--------+---------+---------+---------+---------+---------+---------+---------|
PAT2       AAADVVQRGLAKITLLGDPTTILAEAAKLGLDLSGCNIHNPNTSDRFDKYVDMLVEARKKKGMTREVAADTLHGDVNFFA
pat2-1
Consensus  ................................................................................

           561     570       580       590       600       610       620       630       640
           |--------+---------+---------+---------+---------+---------+---------+---------|
PAT2       TMMIVAGDADGMVSGAVHTTASTVRPALQVLKSPDTPLVSSVFIMCLPDRVVVYGDCAVNVNPSAADLAQIAITSNDTAA
pat2-1
Consensus  ................................................................................

           641     650       660       670       680       690       700       710       720
           |--------+---------+---------+---------+---------+---------+---------+---------|
PAT2       AFGIEPRVAMLSYSTLGSGSGPDVQKVSEAVAIVKQRRPDIKVEGPIQYDAAIDPKVAAVKVQGLSEVAGKATVFIFPDL
pat2-1
Consensus  ................................................................................

           721     730       740       750       760       770       780       79@92
           |--------+---------+---------+---------+---------+---------+---------+-|
PAT2       NTGNNTYKAVQQSTGAIAMGPVMQGLLRPVNDLSRGCTVPDIINTICVTSIQASRMSSAARAAAAKAAVAAV
pat2-1
Consensus  ............................................................................
```



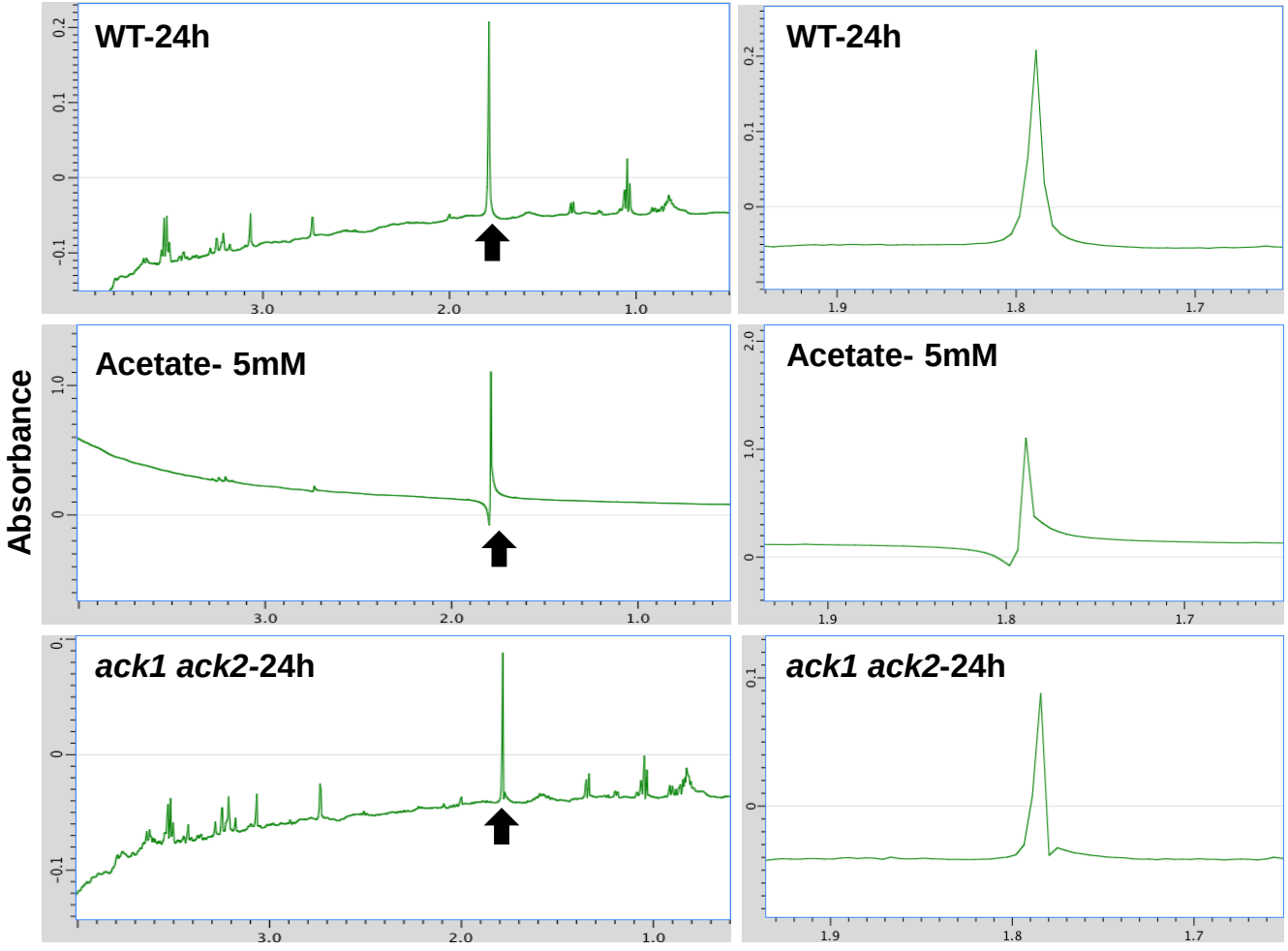
Supplemental Figure 7. Changes in Abundance of Transcripts Encoding Fermentative Enzymes in *pat* and *ack* Mutants.

RT-qPCR was used to determine transcript levels from the indicated fermentative genes in WT and single and double mutant strains under dark anoxic conditions. There were no significant differences between the values based on *t*-tests. Errors bars represent SD.



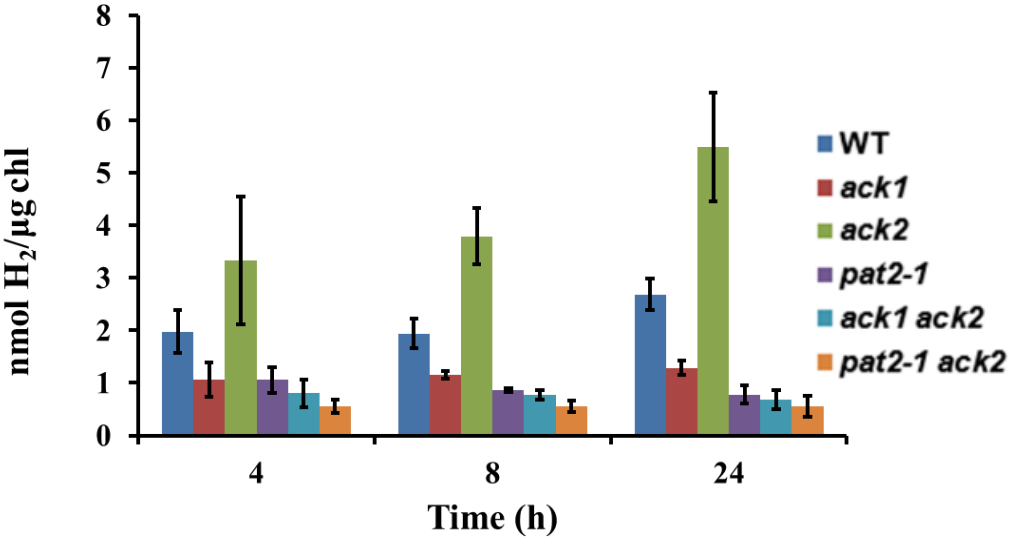
Supplemental Figure 8. Accumulation of External Metabolites in the Complemented Strains of *pat2-1*.

HPLC quantitation of extracellular metabolites secreted by the indicated mutants after 4, 8 or 24 h of dark anoxic acclimation. Errors bars represent SEM.



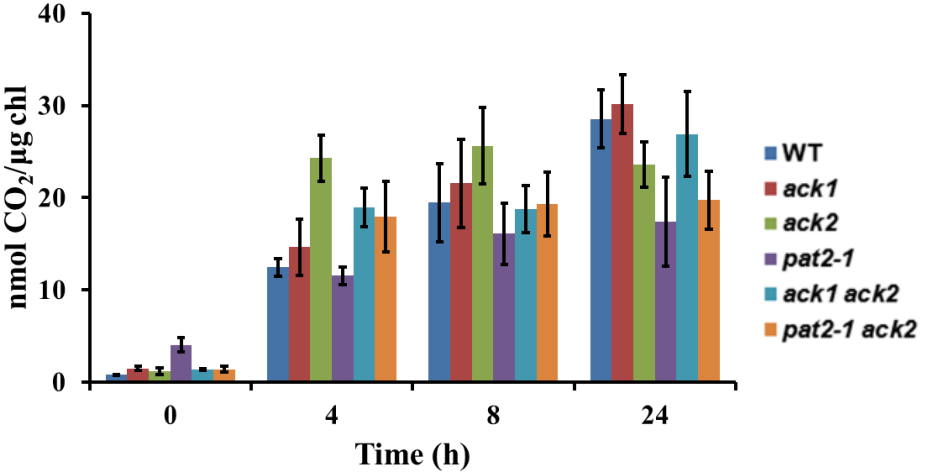
Supplemental Figure 9. Nuclear Magnetic Resonance (NMR) to Confirm the Acetate Production in *ack1 ack2* Double Mutants.

NMR confirmation of acetate secreted by the WT and *ack1 ack2* mutant strains after 24 h of dark anoxic acclimation. 5 mM acetate was applied as positive controls. Arrows represent the acetate absorbance peak. The right pics are the close-up pic of the acetate absorbance peaks.



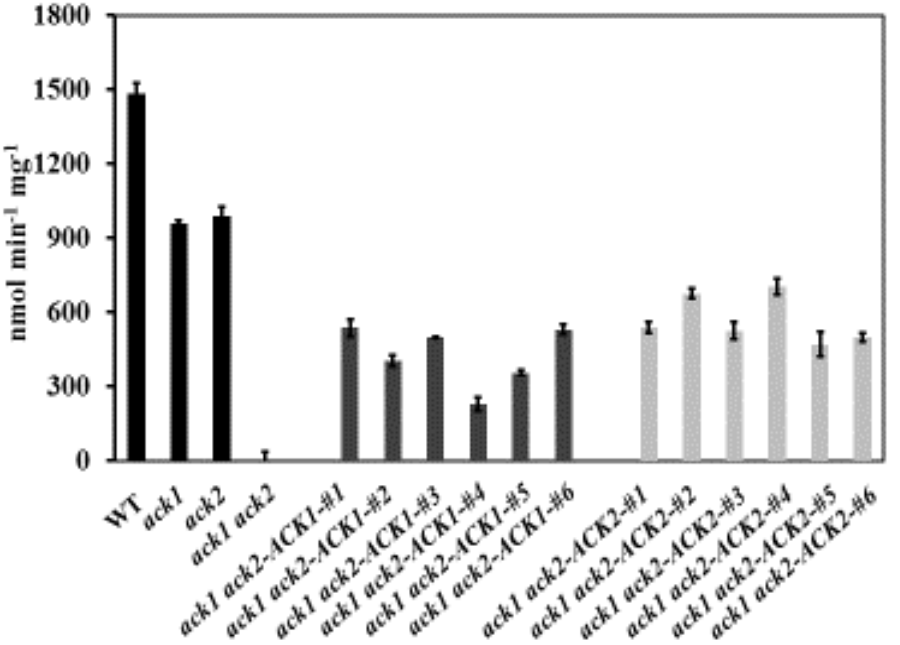
Supplemental Figure 10. Fermentative H_2 Production by Various Mutants.

Fermentative H_2 production at the indicated times of dark anoxic acclimation determined using gas chromatography from cultures of the indicated mutants. There were no significant differences between the values based on *t*-tests. Errors bars represent SD.



Supplemental Figure 11. Fermentative CO_2 Production by Various Mutants.

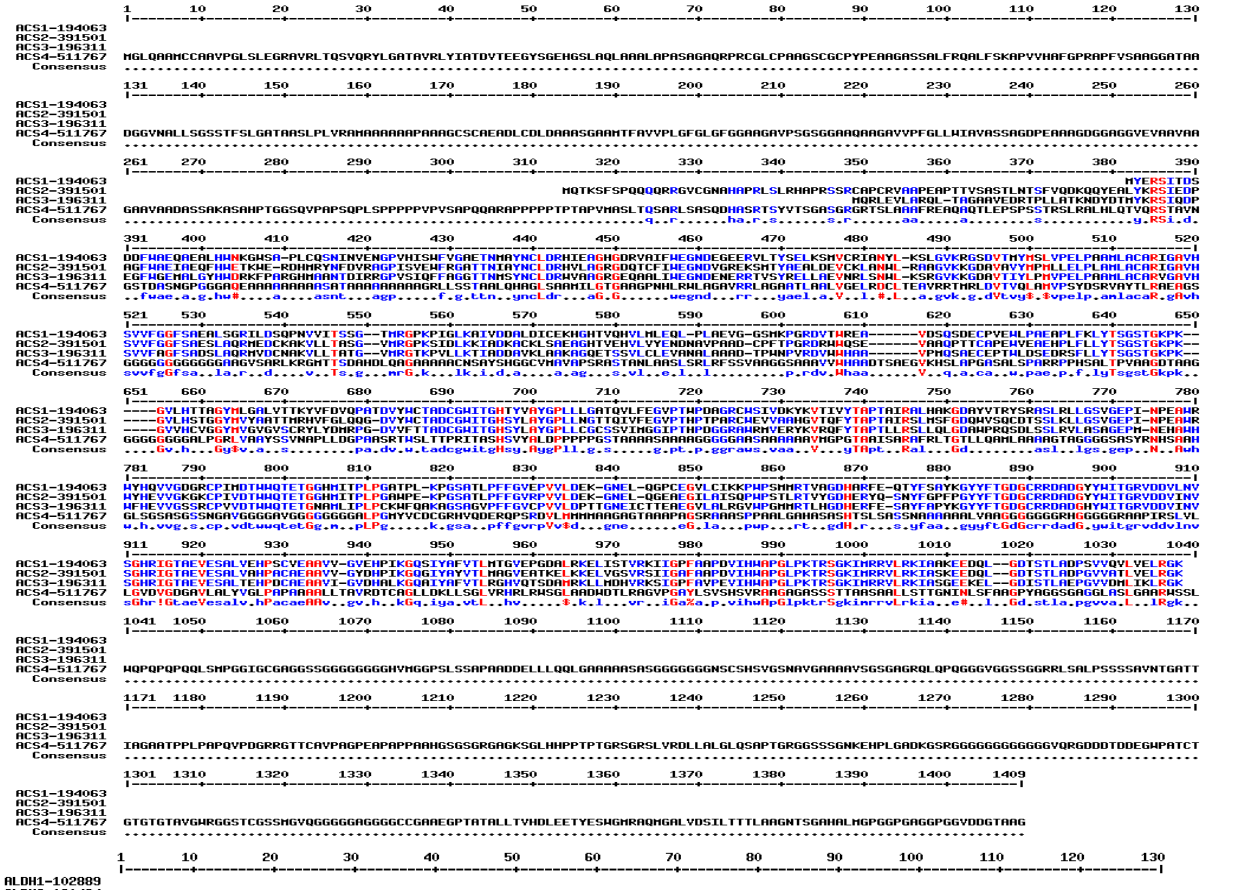
Fermentative CO_2 production at indicated times of dark anoxic acclimation determined using gas chromatography from cultures of the indicated mutants. There were no significant differences between the values based on *t*-tests. Errors bars represent SD.



Supplemental Figure 12. *In Vitro* Acetate Kinase Activity in Mutant and Complemented Strains.

Acetate kinase activity was measured in the *ack1*, *ack2* and *ack1 ack2* mutants. The *ack1 ack2-ACK1-#1* to #6 lines are the *ack1 ack2* double mutants transformed with *ACK1*, while *ack1 ack2-ACK2-#1* to #6 are *ack1 ack2* double mutants transformed with *ACK2*. Error bars represent SD.

A



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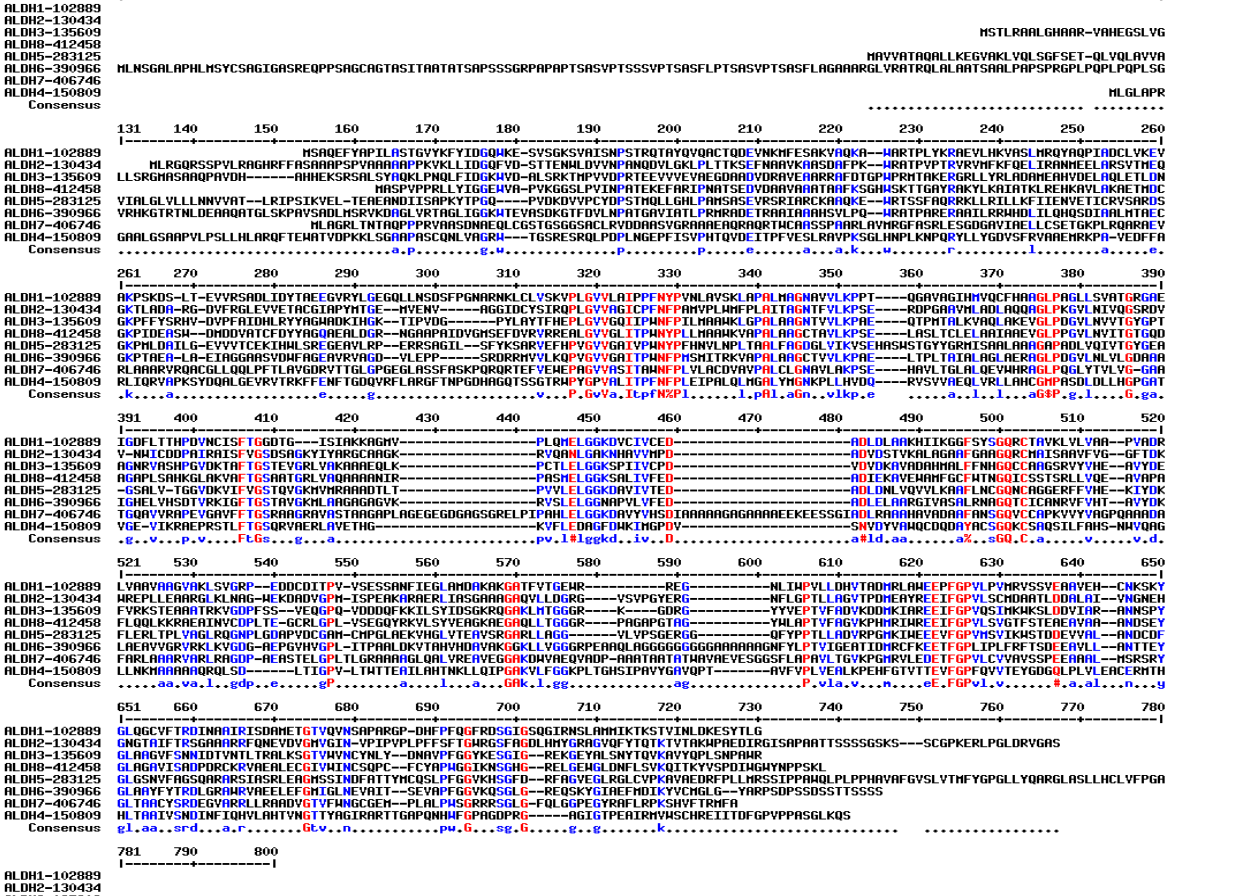
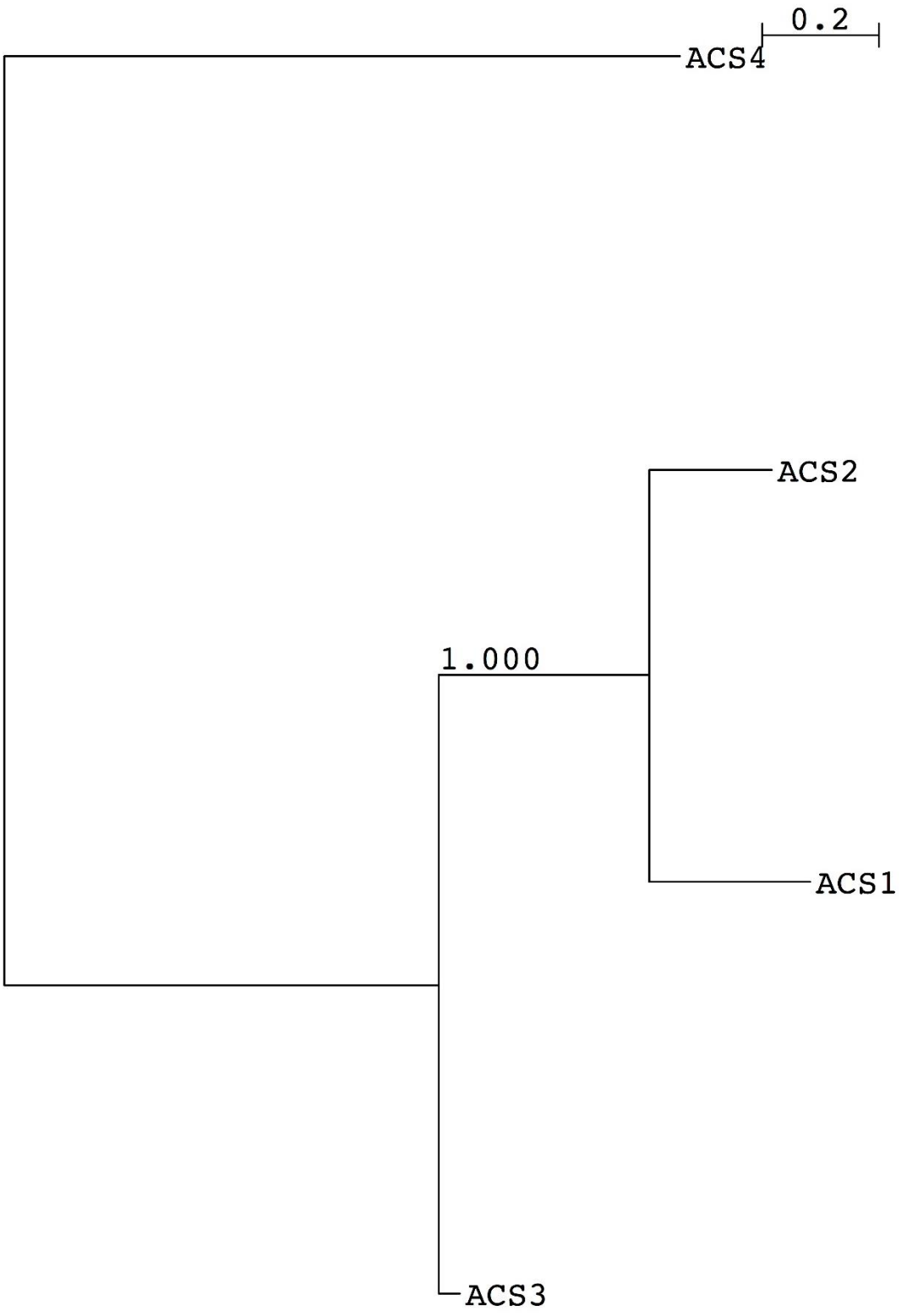
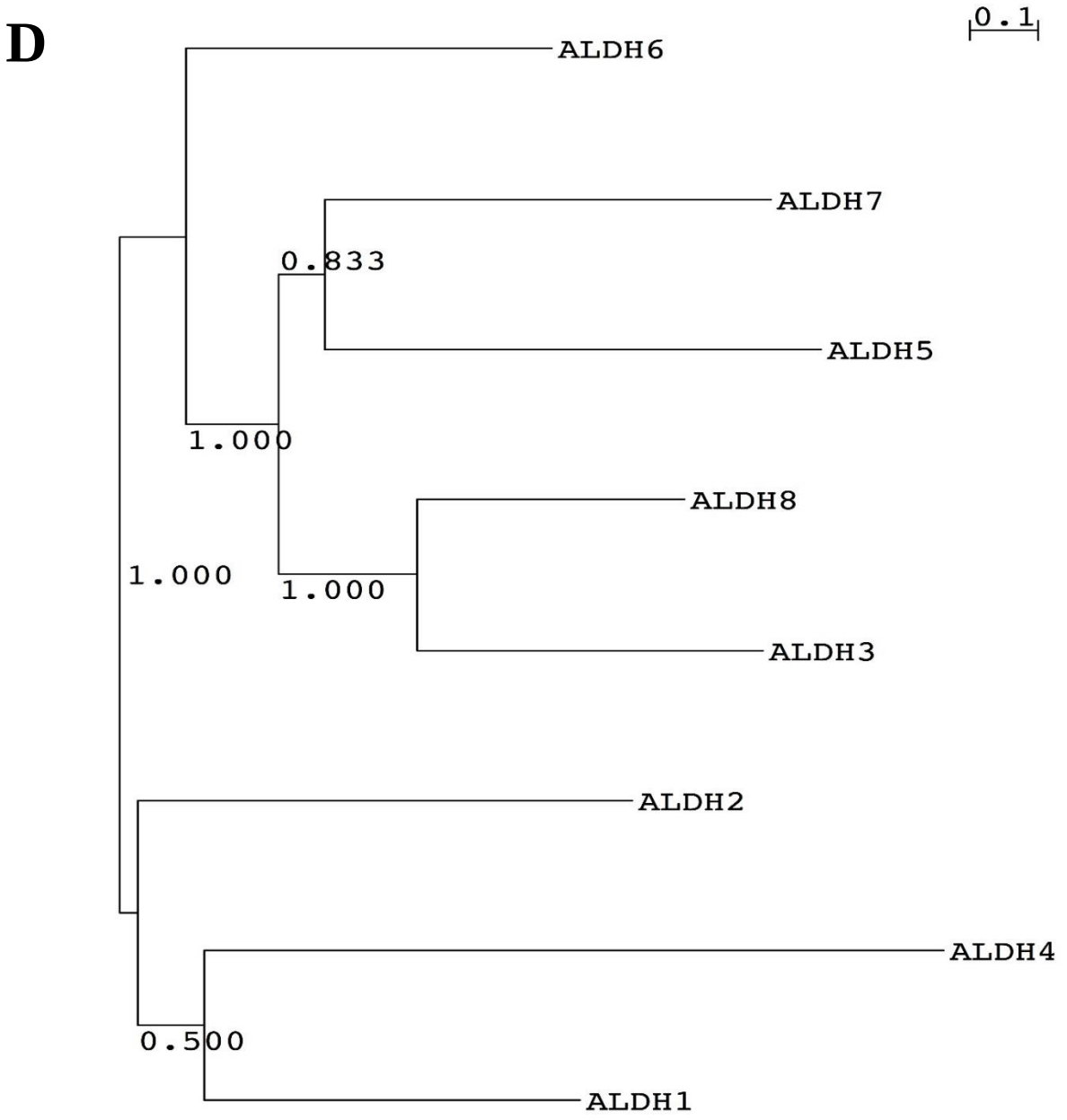


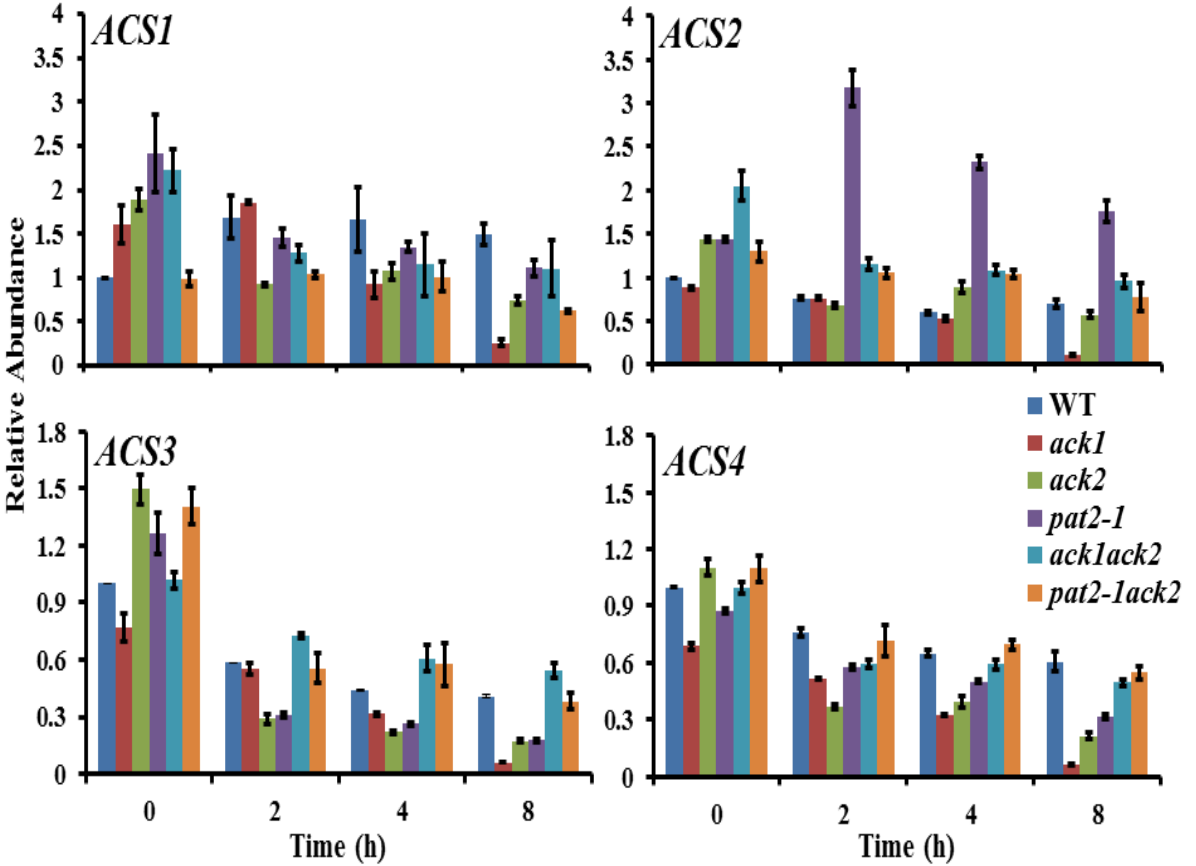
C





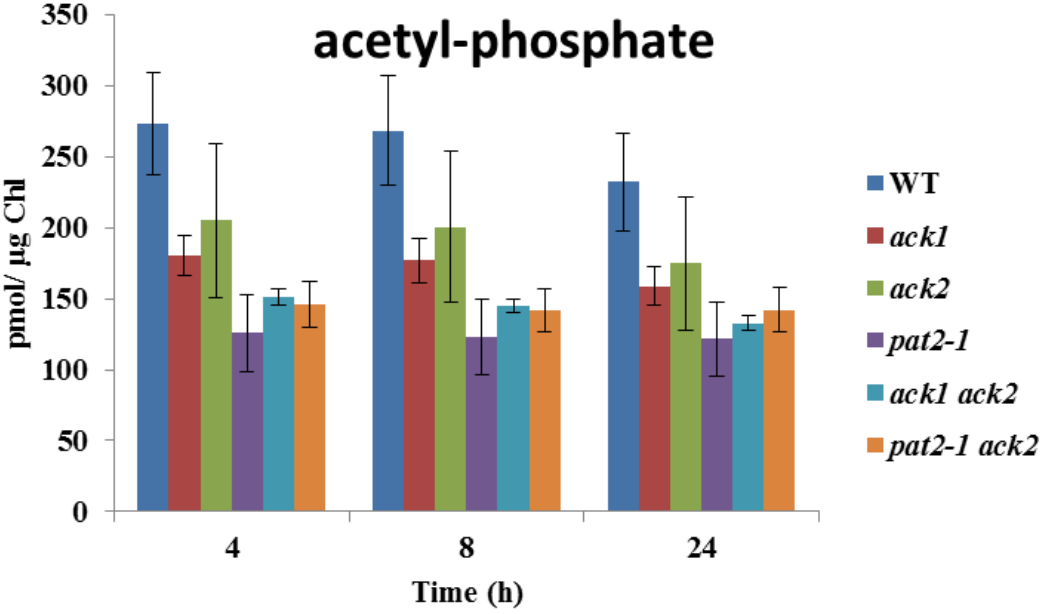
Supplemental Figure 13. Alignments and Phylogenetic Analyses of Chlamydomonas ACS and ALDH Proteins.

Alignments of 4 ACS **(A)** and 8 ALDH **(B)** protein homologs along with phylogenetic analysis (**C** and **D** for ACS and ALDH, respectively). Protein IDs are given on the right. Alignments was performed using the online software http://multalin.toulouse.inra.fr/multalin/ (Corpet, 1988). Identical or highly conserved amino acids in all positions are given in red letters (high consensus region), a low consensus amino acid position is given in blue letters, while black letters indicate an overall lack of strong consensus. The settings for the alignment were: Symbol comparison table: blosum62; Gap weight: 12; Gap length weight: 2; Consensus levels: high=90% low=50%. MrBAYES (via the Cipres Portal) was used to generate the trees (https://www.phylo.org/portal2/) (Miller et al., 2010). Newicktops (via the Mobyle Portal) was used to draw the trees http://mobyle.pasteur.fr/) (Bertrand et al., 2009). The numbers associated with the phylogenies (**C** and **D**) are boot strap values.



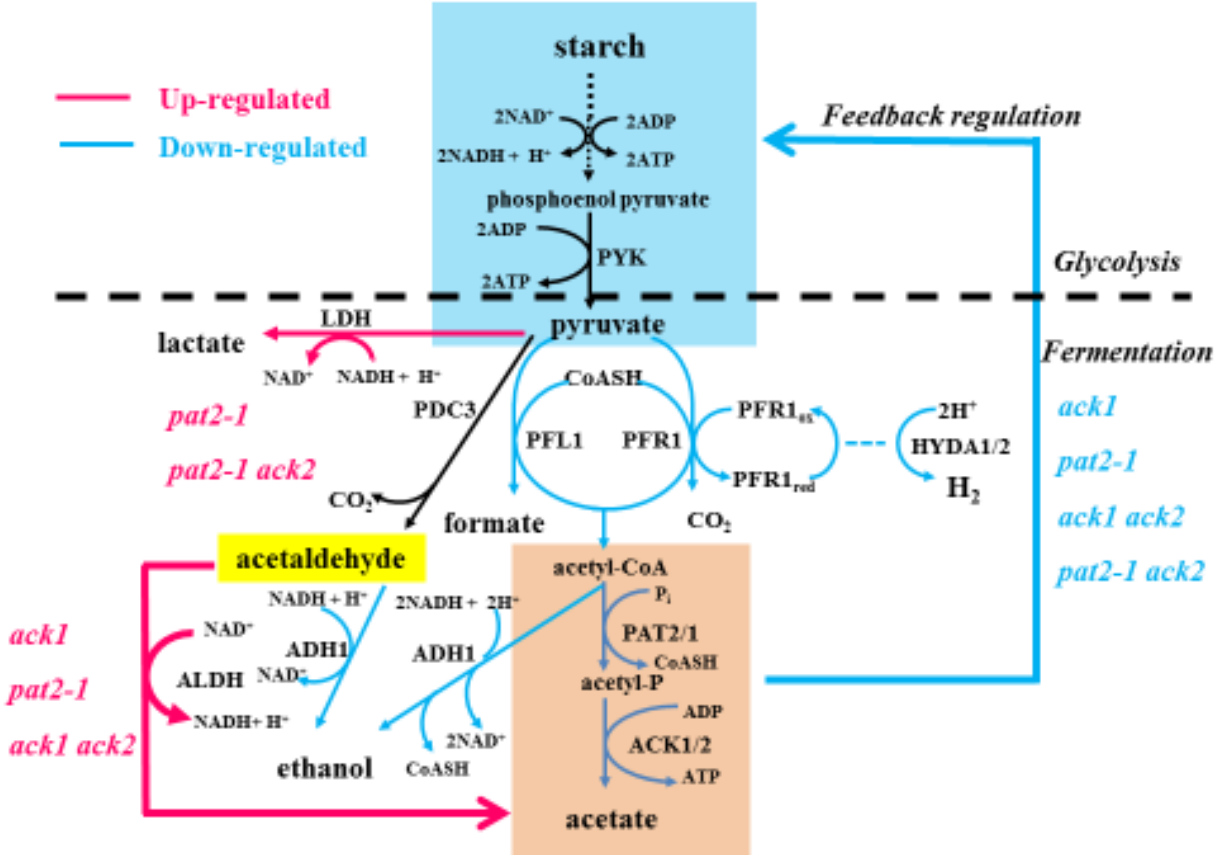
Supplemental Figure 14. Changes in Levels of Putative Acetyl-CoA Synthetase Transcripts.

RT-qPCR was used to assess transcript levels of 4 *ACS* genes in the indicated mutants. Errors bars represent SD.



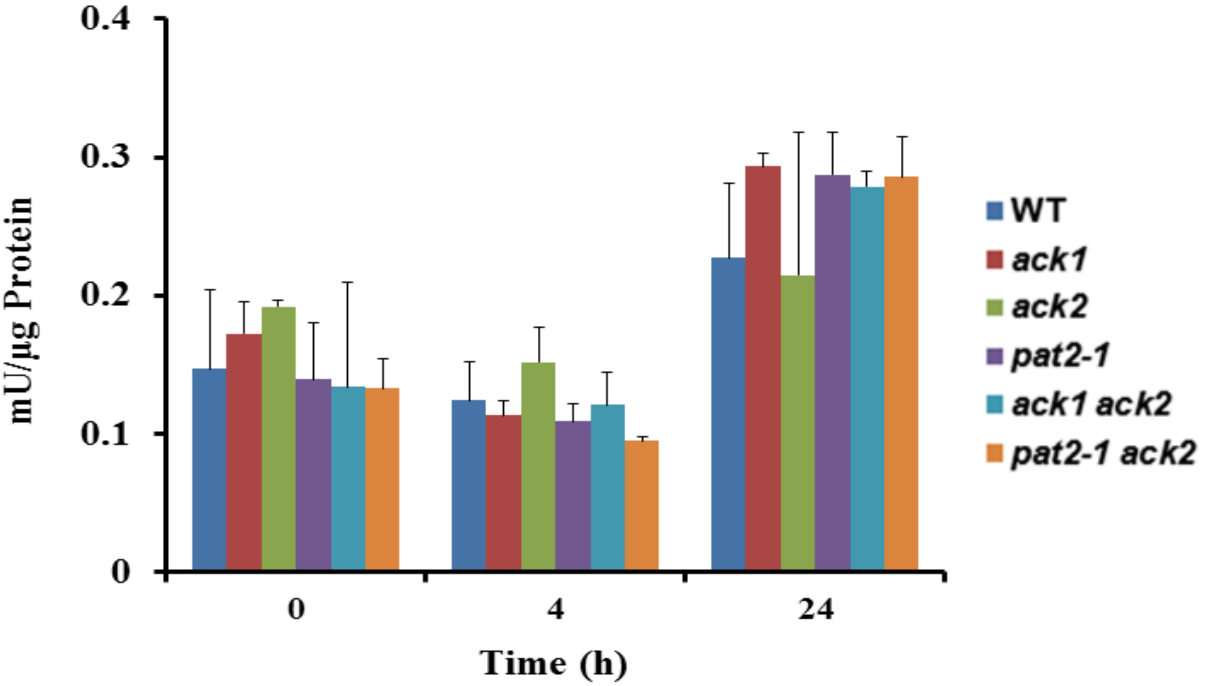
Supplemental Figure 15. Accumulation of Intracellular Acetyl-Phosphate.

Acetyl-phosphate was measured in the indicated mutants after 4, 8 or 24 h of dark anoxic acclimation. Errors bars represent SD.



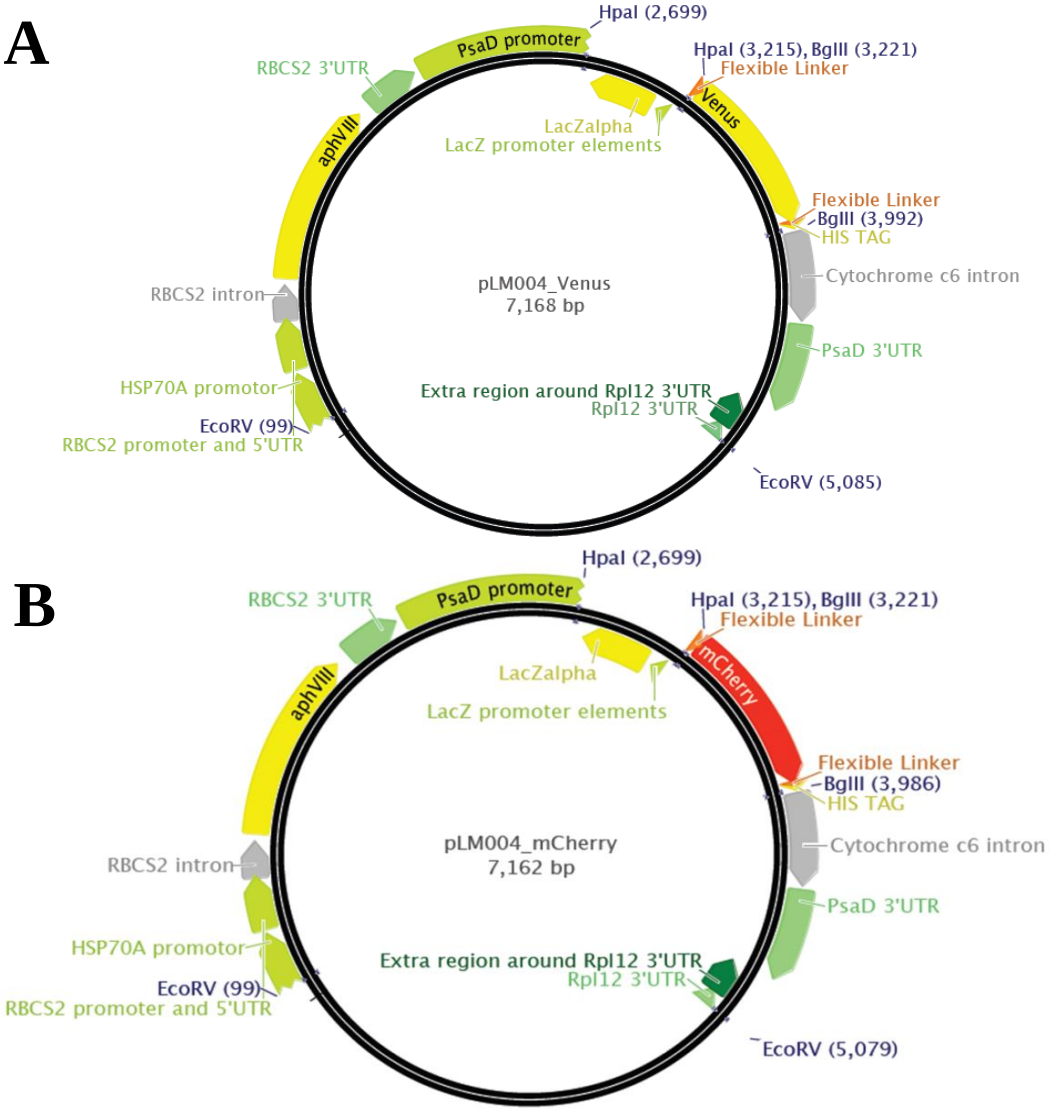
Supplemental Figure 16. Proposed Anoxic Metabolisms in Single and Double Mutants.

The light blue background highlights the glycolytic breakdown of starch, and the orange-brown background represents the acetate-producing pathways by the chloroplast and mitochondrial PAT-ACK enzymes. The blue arrows represent reactions and pathways that appear to be suppressed in a subpopulation of mutants (as indicated), while the red arrows represent pathways that appear to be more active in a subpopulation of mutants (as indicated). The black dashed line separates glycolysis from the fermentative pathways of metabolism. The blue dashed line represents omitted electron transfer components involved in hydrogenase reduction (e.g. ferredoxin). The black dashed arrow represents the omitted pathways from starch to phosphoenol pyruvate. Acetaldehyde is highlighted (yellow) to emphasize that the ALDH pathway may be active in some of the mutant strains.



Supplemental Figure 17. *In Vitro* Aldehyde Dehydrogenase Activity.

Aldehyde dehydrogenase activity in WT and the indicated mutants after acclimation to dark, anoxic conditions. Errors bars represent SD. One unit (U) is the amount of enzyme that will generate 1.0 mol of NADH per min at pH 8 at room temperature.



Supplemental Figure 18. Maps of Plasmids used for Protein Subcellular Localization.

Schematic representations of the pLM004_Venus (**A**) and pLM004_mCherry (**B**) plasmids, which were used to generate fluorescent fusion proteins for subcellular localization of the ACK and PAT enzymes. Additional information provided in the Methods.

Supplemental Table 1. Intracellular Pyruvate Levels.

Intracellular concentrations of pyruvate during a transition from photoautotrophic growth to fermentative metabolism. Values are presented as fg pyruvate cell^{-1}. Values shown are the range of biological duplicates. Two replicate samples of each biological duplicate were analyzed. Single values represent the technical average of a single biological replicate.

Time (h)	WT	*ack1*	*ack2*	*ack1ack2*	*pat2-1*	*pat2-1ack2*
4	1.3 – 1.4	0.9 – 2.5	1.7 – 1.8	2.0 – 3.8	2.1 – 4.2	2.8
8	1.1 – 1.3	0.2 – 0.5	0.6 – 1.8	2.3 – 5.1	1.9	0.7 – 0.9
24	0.3 – 0.6	0.6 – 0.6	0.6 – 0.6	0.4 – 0.9	0.9 – 1.1	0.4 – 0.7

Supplemental Table 2. Primers Used in this Study.

(A). Primers used for the genetic screen to identify the mutants, to amplify the genes for complementation, and for genotyping progenies of the genetic crosses.

(B). Primers for generating the constructs used for subcellular localization.

(C). Primers used for qRT-PCR.

Gene	Use of Primers	Orientation	Sequence	Tm
A. Primers used for the genetic screen to identify the mutants, to amplify the genes for complementation, and for genotyping progenies of the genetic crosses				
ACK1-F1	Mutant Screen	forward	5'- ATACCTAGAATGCTGGCTGGC -3'	60.6 ℃
ACK1-R1	Mutant Screen	reverse	5'- GCATGTCACAGCGAGGAGT -3'	60.0 ℃
ACK1-F2	Mutant Screen	forward	5'- ACTCCTCGCTGTGACATGC -3'	60.0 ℃
ACK1-R2	Mutant Screen	reverse	5'- AAGCAGGAGGAAGGAGAAGG -3'	60.0 ℃
ACK1-F3	Mutant Screen	forward	5'- CCTTCTCCTTCCTCCTGCTT -3'	60.0 ℃
ACK1-R3	Mutant Screen	reverse	5'- CACCACCCACCTGACTTCTT -3'	60.0 ℃
ACK1-F4	Mutant Screen	forward	5'- AAGAAGTCAGGTGGGTGGTG -3'	60.0 ℃
ACK1-R4	Mutant Screen	reverse	5'- GCAGCTGCCGTAGATCTGTA -3'	59.2 ℃
ACK1-F5	Mutant Screen	forward	5'- TACAGATCTACGGCAGCTGC -3'	59.2 ℃
ACK1-R5	Mutant Screen	reverse	5'- TGTAGGTCCCTTCTCCATCG -3'	60.0 ℃
ACK2-F1	Mutant Screen	forward	5'- GTATCGCGAGAAACGATGC -3'	59.4 ℃
ACK2-R1	Mutant Screen	reverse	5'- CAACAGGTGGAGGTGTGATG -3'	60.0 ℃
ACK2-F2	Mutant Screen	forward	5'- CATCACACCTCCACCTGTTG -3'	60.0 ℃
ACK2-R2	Mutant Screen	reverse	5'- CAAGCAGATGAGGACAAGCA -3'	60.1 ℃
ACK2-F3	Mutant Screen	forward	5'- TGCTTGTCCTCATCTGCTTG -3'	60.1 ℃
ACK2-R3	Mutant Screen	reverse	5'- TACAATGCCTTGCACGAGAG -3'	60.0 ℃
ACK2-F4	Mutant Screen	forward	5'- CTCTCGTGCAAGGCATTGTA -3'	60.0 ℃
ACK2-R4	Mutant Screen	reverse	5'- CTGATGACCGGGCAGAGTAT -3'	60.0 ℃
ACK2-F5	Mutant Screen	forward	5'- ATACTCTGCCCGGTCATCAG -3'	60.0 ℃
ACK2-R5	Mutant Screen	reverse	5'- GAGGGAACCCATCACAACTG -3'	60.4 ℃
ACK2-F6	Mutant Screen	forward	5'- CAGTTGTGATGGGTTCCCTC -3'	60.4 ℃
ACK2-R6	Mutant Screen	reverse	5'- CACCTGCAGGGTCTGCTC -3'	60.6 ℃
PAT2-F1	Mutant Screen	forward	5'- ATCGAATACTTTCGGCTCGA -3'	59.8 ℃
PAT2-R1	Mutant Screen	reverse	5'- ACCGTATGGAACGCAGGA -3'	60.1 ℃
PAT2-F2	Mutant Screen	forward	5'- TCCTGCGTTCCATACGGT -3'	60.1 ℃
PAT2-R2	Mutant Screen	reverse	5'- TCACCGCACGGTTGTAGTAG -3'	59.8 ℃
PAT2-F3	Mutant Screen	forward	5'- CTACTACAACCGTGCGGTGA -3'	59.8 ℃
PAT2-R3	Mutant Screen	reverse	5'- TCTCTACGGGGGCTAAAAGC -3'	60.7 ℃
PAT2-F4	Mutant Screen	forward	5'- GCTTTTAGCCCCCGTAGAGA -3'	60.7 ℃
PAT2-R4	Mutant Screen	reverse	5'- TTTCCCCTCTACAGCTTCCA -3'	59.8 ℃
PAT2-F5	Mutant Screen	forward	5'- TGGAAGCTGTAGAGGGGAAA -3'	59.8 ℃
PAT2-R5	Mutant Screen	reverse	5'- GTGCTCCGCTTCGTCTACTC -3'	60.2 ℃
PAT2-F6	Mutant Screen	forward	5'- GAGTAGACGAAGCGGAGCAC -3'	60.2 ℃

PAT2-R6	Mutant Screen	reverse	5'- CTATGCCACCACAGCATGTC -3'	60.1 ℃
PAT2-F7	Mutant Screen	forward	5'- GACATGCTGTGGTGGCATAG -3'	60.1 ℃
PAT2-R7	Mutant Screen	reverse	5'- CGTCACACACACAGGTGACA -3'	60.3 ℃
PAT2-F8	Mutant Screen	forward	5'- TGTCACCTGTGTGTGTGACG -3'	60.3 ℃
PAT2-R8	Mutant Screen	reverse	5'- GAGAGCGTGTGTGCATTTGT -3'	60.0 ℃
PAT2-F9	Mutant Screen	forward	5'- ACAAATGCACACACGCTCTC -3'	60.0 ℃
PAT2-R9	Mutant Screen	reverse	5'- AGCCCAAACAAGGGGTGT -3'	60.4 ℃
PAT2-F10	Mutant Screen	forward	5'- ACACCCCTTGTTTGGGCT -3'	60.4 ℃
PAT2-R10	Mutant Screen	reverse	5'- CCACAACCACGCAAGACA -3'	60.3 ℃
PAT2-F11	Mutant Screen	forward	5'- TGTCTTGCGTGGTTGTGG -3'	60.3 ℃
PAT2-R11	Mutant Screen	reverse	5'- GACAGTGACATTGCCGTTTG -3'	60.2 ℃
PAT2-F12	Mutant Screen	forward	5'- CAAACGGCAATGTCACTGTC -3'	60.2 ℃
PAT2-R12	Mutant Screen	reverse	5'- CGCCCTACACAACCAAGAG -3'	59.3 ℃
RB1	Mutant Screen	forward	5'- ATGGGGCGGTATCGGAGGAAAAG-3'	60.0 ℃
RB2	Mutant Screen	forward	5'- TACCGGCTGTTGGACGAGTTCTTCTG -3'	60.0 ℃
RIM1	Mutant Screen	reverse	5'- GCTGGCACGAGTACGGGTTG -3'	58.0 ℃
MID-UP	Mating type	forward	5'- ATGGCCTGTTTCTTAGC -3'	61.6 ℃
MID-LOW	Mating type	reverse	5'- CTACATGTGTTTCTTGACG-3'	52.0 ℃
FUS-UP	Mating type	forward	5'- ATGCCTATCTTTCTCATTCT-3'	52.0 ℃
FUS-LOW	Mating type	reverse	5'- GCAAAATACACGTCTGGAAG-3'	52.0 ℃
Paro-up	Southern Blot	forward	5'- ATGGGGCGGTATCGGAGGAAAAG -3'	60.0 ℃
Paro-dw	Southern Blot	reverse	5'- TACCGGCTGTTGGACGAGTTCTTCTG -3'	60.0 ℃
ACK1-COM-F1	Complementation	forward	5'- GAATTCCAAAGACGCAAACATATACG-3'	56.2 ℃
ACK1-COM-R1	Complementation	reverse	5'- TAGGATCCAATTATCCGAGCTACACACT-3'	56.8 ℃
ACK1-COM-F2	Complementation	forward	5'- GCACTAGCCACAAGTACCTGG-3'	60.0 ℃
ACK2-COM-F1	Complementation	forward	5'- GAATTCCCGTAGGGGGTATTGATATACAA G-3'	59.9 ℃
ACK2-COM-R1	Complementation	reverse	5'- TAGGATCCCCCTGTCACATTGACTCACG-3'	60.2 ℃
PAT2-COM-F1	Complementation	forward	5'- GAATTCATGTCTCTGAACAGTAGCACTAT G-3'	58.5 ℃
PAT2-COM-R1	Complementation	reverse	5'- GGATCCGGTGGGCATTGACAGACAA-3'	59.1 ℃
PAT2-COM-F2	Complementation	forward	5'- CGACTACTACAACCGTGCGA-3'	59.9 ℃
PAT2-COM-F3	Complementation	forward	5'- ATCACGCTGCTGGGAGAC-3'	59.9 ℃
B. Primers for generating the constructs used for subcellular localization				
ACK1-Loc-F	Localization	forward	5'- GTTAACATGCTGGCTGGCAAAGTGCCTGTC GGGT -3'	60.0 ℃
ACK1-Loc-R	Localization	reverse	5'- GGTTAACAGCGGCCAGCGCCGCGCC -3'	60.0 ℃
ACK2-Loc-F	Localization	forward	5'- GGTTAACATGAAGCCGCGCAAATGGGAGC -3'	60.0 ℃
ACK2-Loc-R	Localization	reverse	5'- GGTTAACTGCCGCTGCGGCTCCCAA -3'	60.0 ℃
PAT1-Loc-F	Localization	forward	5'- GGTTAACATGGCCTTCGCGAGCAGCAGCAT -3'	60.0 ℃
PAT1-Loc-R	Localization	reverse	5'- GGTTAACCTTGGGCGCTGCTGCCGC -3'	60.0 ℃

PAT2-Loc-F	Localization	forward	5'- GGTTAACATGTCTCTGAACAGTAGCACTATGT -3'	60.0 ℃
PAT2-Loc-R	Localization	reverse	5'-GGTTAACGAACGGCCGCGACGGCAGC -3'	60.0 ℃
C. Primers used for qRT-PCR				
PFL1	qRT-PCR	forward	5'- ATGTACGCGAACACCATGAA-3'	54.6 ℃
PFL1	qRT-PCR	reverse	5'- GTCACCTGGGCGTACTTGAT-3'	57.1 ℃
PFR1	qRT-PCR	forward	5'- CGCGCAGGGCACAATCACAC-3'	62 ℃
PFR1	qRT-PCR	reverse	5'- ATCAGACGGCGCCACAAACACA-3'	61.9℃
ACK1-B	qRT-PCR	forward	5'- CAACTGCGTGTTGAAGGCTA-3'	57.4 ℃
ACK1-B	qRT-PCR	reverse	5'- AAGGTGCTCGACACGTTCTC-3'	55.2 ℃
ACK1-A	qRT-PCR	forward	5'- GCCGTACGACATGTACGAGA-3'	60.0 ℃
ACK1-A	qRT-PCR	reverse	5'- AGGTGATGACGTTGGTCTCC-3'	60.0 ℃
ACK2-B	qRT-PCR	forward	5'- GCAGCAGCTCGCTTAAGTTT-3'	56.2 ℃
ACK2-B	qRT-PCR	reverse	5'- GCACTTTGAGCTCCCATTTG-3'	54.6 ℃
PAT1	qRT-PCR	forward	5'- ATCAACTTCTTCGGCACCAT-3'	55.2 ℃
PAT1	qRT-PCR	reverse	5'- ATGGAGGACACGAGGCTAGA -3'	54.8 ℃
PAT2-B	qRT-PCR	forward	5'- ACCCTCTTCCTCTCGGACAT-3’	57.4 ℃
PAT2-B	qRT-PCR	reverse	5’- GTCGCCCTTGAGGTTAAACA-3'	55.2 ℃
PAT2-A	qRT-PCR	forward	5'- GTTCATGCACACGCTCAAGT -3'	60.0 ℃
PAT2-A	qRT-PCR	reverse	5'- CTCCCAGCAGCGTGATCT -3'	60.0 ℃
HYDA1	qRT-PCR	forward	5'- CGGGAACGTGGGTAGCATTTAGG-3'	59.8 ℃
HYDA1	qRT-PCR	reverse	5'- CGCCAAGGGTCCACATCAGAGT-3'	61.5 ℃
HYDA2	qRT-PCR	forward	5'- GCGGGCAAGTTCTTCAATCT-3'	61.6 ℃
HYDA2	qRT-PCR	reverse	5'- AGGCCGAATGACTCAGCAAT-3'	62.0 ℃
PDC3	qRT-PCR	forward	5'- TACTCCACTGCCGGCTACTC-3'	58.6 ℃
PDC3	qRT-PCR	reverse	5'- AGAGCCATGCGCTTGTAGAT-3'	61.6 ℃
LDH	qRT-PCR	forward	5’- AGTTAGCCAAGGCGGGTGTG-3’	55.5 ℃
LDH	qRT-PCR	reverse	5’- TGTTGAGCGCGAAGATGAGC-3’	61.6 ℃
ADH1	qRT-PCR	forward	5'- GCACCTGCTGAACATCAAGA-3'	55.6 ℃
ADH1	qRT-PCR	reverse	5'- AGGATGTGGGTGACCTTGTC-3'	56.9 ℃
ACS1	qRT-PCR	forward	5'- TCCAACATCAACGTGGAGAA -3'	60.1 ℃
ACS1	qRT-PCR	reverse	5'- GTCATTGCCCTCCCAAAATA-3'	59.8 ℃
ACS2	qRT-PCR	forward	5'- GTGCTGGTGTACGAGAACGA -3'	59.9 ℃
ACS2	qRT-PCR	reverse	5'- CGAGGTGTACAGCAGGAACA -3'	59.9 ℃
ACS3	qRT-PCR	forward	5'- ACTCATCTGGGAGGGCAAC -3'	60.1 ℃
ACS3	qRT-PCR	reverse	5'- AGGTAGATGGTCACGGCATC -3'	60.0 ℃
ACS4	qRT-PCR	forward	5'- GACGAGAAGGGCAACGAGT-3'	60.4 ℃
ACS4	qRT-PCR	reverse	5'- GAAGTAGTAGCCGGGGAAGG-3'	60.1 ℃
ALDH1	qRT-PCR	forward	5'- CGCAGACCTGATCGACTACA -3'	60.0 ℃
ALDH1	qRT-PCR	reverse	5'- GTTGACCGGGTAGTTGAAGG -3'	59.5 ℃
ALDH2	qRT-PCR	forward	5'- GGTCATCGTTTCTTCGCTTC -3'	59.8 ℃

ALDH2	qRT-PCR	reverse	5'- AACTCGGATTTGGTGGTCAG -3'	60.0 ℃
ALDH3	qRT-PCR	forward	5'- CAGCTGGAGACACTGGACAA -3'	60.0 ℃
ALDH3	qRT-PCR	reverse	5'- AAGGTGTAGGCCAGGTAGGG -3'	60.0 ℃
ALDH4	qRT-PCR	forward	5'- CTACCGTCCTTGTTGCACCT -3'	60.1 ℃
ALDH4	qRT-PCR	reverse	5'- TCTCATCCACCTGTGTGTGC -3'	60.8 ℃
ALDH5	qRT-PCR	forward	5'- ATCTGGGAGGAGGAGGTGTT -3'	59.6 ℃
ALDH5	qRT-PCR	reverse	5'- ATGTAGGTGGTGGCGAAGTC -3'	60.0 ℃
ALDH6	qRT-PCR	forward	5'- GACAAAGGCACCTTCGATGT -3'	60.1 ℃
ALDH6	qRT-PCR	reverse	5'- TGATGCTGCAGAATGAGGTC -3'	60.0 ℃
ALDH7	qRT-PCR	forward	5'- CGAAGGTGCTGAAAGCCTAC -3'	60.0 ℃
ALDH7	qRT-PCR	reverse	5'- GCCCTAGGCCAGTCGTAACT -3'	60.7 ℃
ALDH8	qRT-PCR	forward	5'- CCTACCGCGCTAAGTACCTG -3'	60.0 ℃
ALDH8	qRT-PCR	reverse	5'- GACCCGCGTAGTAGTCGAAG -3'	60.0 ℃

Supplemental Table 3. Constructs Used in this Study

Use	Construct	Plasmid name	Insert or PCR product	Primers	Plasmid backbone	Cloning method
Localization	Venus	pLM004_Venus	-	-	pUC19	-
	Venus:ACK1	pLM004ACK1_Venus	ACK1CDS	ACK1-Loc-F/ ACK1-Loc-R1	pUC19	HpaI/HpaI
	Venus:ACK2	pLM004ACK2_Venus	ACK2CDS	ACK2-Loc-F/ ACK2-Loc-R1	pUC19	HpaI/HpaI
	Venus:PAT1	pLM004PAT1_Venus	PAT1CDS	PAT1-Loc-F/ PAT1-Loc-R1	pUC19	HpaI/HpaI
	Venus:PAT2	pLM004PAT2_Venus	PAT2CDS	PAT2-Loc-F/ PAT2-Loc-R1	pUC19	HpaI/HpaI
	mCherry	pLM004_mCherry	-	-	pUC19	-
	mCherry:ACK2	pLM004ACK2_mCherry	ACK2CDS	ACK2-Loc-F/ ACK2-Loc-R1	pUC19	HpaI/HpaI
	mCherry:PAT1	pLM004PAT1_mCherry	PAT1CDS	PAT1-Loc-F/ PAT1-Loc-R1	pUC19	HpaI/HpaI
Complementation	PSAD:ACK1	pJM43Ble_ACK1	ACK1 cDNA	ACK1-COM-F1/ ACK1-COM-R1	pSP124	EcoRI/BamHI
	PSAD:ACK2	pJM43Ble_ACK2	ACK2 cDNA	ACK2-COM-F1/ ACK2-COM-R1	pSP124	EcoRI/BamHI
	PSAD:PAT2	pJM43Ble_ACK2	PAT1 cDNA	PAT2-COM-F1/ PAT2-COM-R1	pSP124	EcoRI/BamHI