SUPPLEMENTARY INFORMATION

Concerted upregulation of aldehyde/alcohol dehydrogenase (ADHE) and starch in *Chlamydomonas reinhardtii* increases survival under dark anoxia

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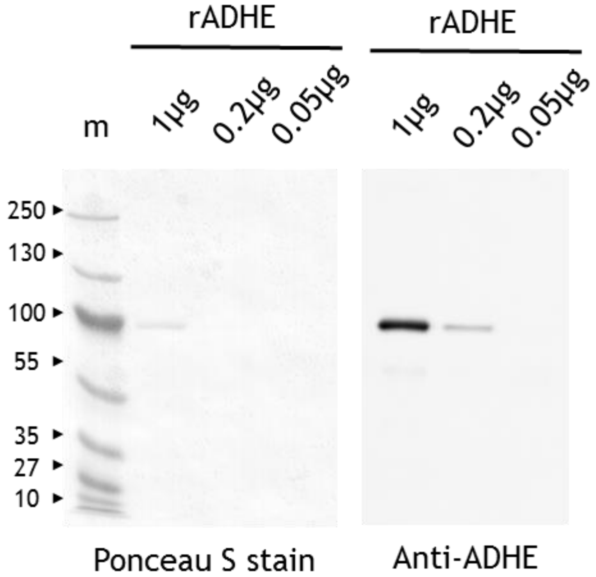
Supplemental Data Set 1. Species used for the phylogenetic analysis presented in Figure 2
Supplemental Data Set 2. Multiple sequence alignment of ADHEs.

FIGURE S1. Sensitivity and specificity of the anti-ADHE serum.

- Proteins were separated on urea/SDS-PAGE (6M urea/5-12% acrylamide) and transferred to nitrocellulose membrane. rADHE, recombinant ADHE. Left: membrane was stained with Ponceau red S; right: membrane was probed for ADHE, using the anti-ADHE antibodies at a dilution of 1:2,500.



- Proteins were separated on SDS-PAGE (5-12% acrylamide) and transferred to nitrocellulose membrane. *Lane 1*, recombinant ADHE (0.15 µg); *lane 2*, cell extract from *C. reinhardtii* wild-type strain CC-124 (40 µg proteins); *lane 3*, cell extract from *C. reinhardtii* mutant strain 10-6C (40 µg proteins). Left: membrane was stained with Ponceau red S; right: membrane was probed for ADHE, using the anti-ADHE antibodies as above.

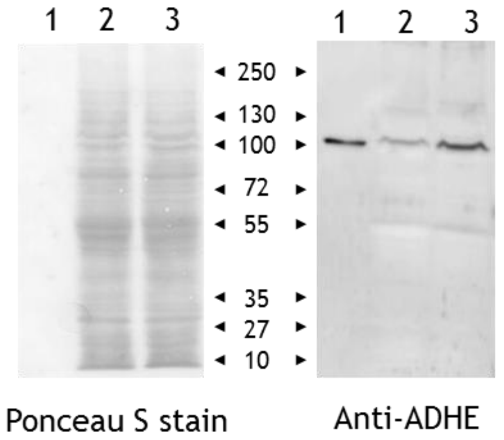
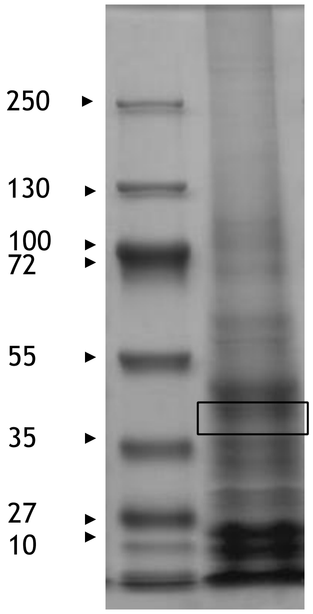


FIGURE S2. Mutant RBCL in *C. reinhardtii* strain 10-6C: identification by mass spectrometry.

- Chloroplasts from strain 10-6C were loaded on a urea/SDS-PAGE. The gel piece in the 40-kDa region was subjected to nanoLC-MS/MS analysis.



- **Sequence alignment of wild-type and mutant RBCLs.** Tryptic peptides that match mutated RBCL protein are indicated, including the peptide that covers the mutation (Gly -> Asp).

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Wild-type     MVPQTETKAGAGFKAGVKDYRLTYYTPDYVVRDTDILAAFRMTPQPGVPPEECGAAVAAE
Mutant        MVPQTETKAGAGFKAGVKDYRLTYYTPDYVVRDTDILAAFRMTPQPGVPPEECGAAVAAE
              ************************************************************

Wild-type     SSTGTWTTVWTDGLTSLDRYKGRCYDIEPVPGEDNQYIAYVAYPIDLFEEGSVTNMFTSI
Mutant        SSTGTWTTVWTDGLTSLDRYKGRCYDIEPVPGEDNQYIAYVAYPIDLFEEGSVTNMFTSI
              ************************************************************

Wild-type     VGNVFGFKALRALRLEDLRIPPAYVKTFVGPPHGIQVERDKLNKYGRGLLGCTIKPKLGL
Mutant        VGNVFGFKALRALRLEDLRIPPAYVKTFVGPPHGIQVERDKLNKYGRGLLDCTIKPKLGL
              ************************************************** *********

Wild-type     SAKNYGRAVYECLRGGLDFTKDDENVNSQPFMRWRDRFLFVAEAIYKAQAETGEVKGHYL
Mutant        SAKNYGRAVYECLRGGLDFTKDDENVNSQPFMRWRDRFLFVAEAIYKAQAETGEVKGHYL
              ************************************************************

Wild-type     NATAGTCEEMMKRAVCAKELGVPIIMHDYLTGGFTANTSLAIYCRDNGLLLHIHRAMHAV
Mutant        NATAGTCEEMMKRAVCAKELGVPIIMHDYLTGGFTANTSLAIYCRDNGLLLHIHRAMHAV
              ************************************************************

Wild-type     IDRQRNHGIHFRVLAKALRMSGGDHLHSGTVVGKLEGEREVTLGFVDLMRDDYVEKDRSR
Mutant        IDRQRNHGIHFRVLAKALRMSGGDHLHSGTVVGKLEGEREVTLGFVDLMRDDYVEKDRSR
              ************************************************************

Wild-type     GIYFTQDWCSMPGVMPVASGGIHVWHMPALVEIFGDDACLQFGGGTLGHPWGNAPGAAAN
Mutant        GIYFTQDWCSMPGVMPVASGGIHVWHMPALVEIFGDDACLQFGGGTLGHPWGNAPGAAAN
              ************************************************************

Wild-type     RVALEACTQARNEGRDLAREGGDVIRSACKWSPELAAACEVWKEIKFEFDTIDK
Mutant        RVALEACTQARNEGRDLAREGGDVIRSACKWSPELAAACEVWKEIKFEFDTIDK
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FIGURE S3. Identification of *C. reinhardtii* ADHE by mass spectrometry

●**List of the tryptic peptides that match the ADHE sequence (Phytozome accession number g18056).** Peptides were identified in 3 independent nanoLC-MS/MS analyses: *gel band*: chloroplast proteins in 100-kDa region; *stacking 1,* chloroplast soluble proteins; and *stacking 2*, whole cells.

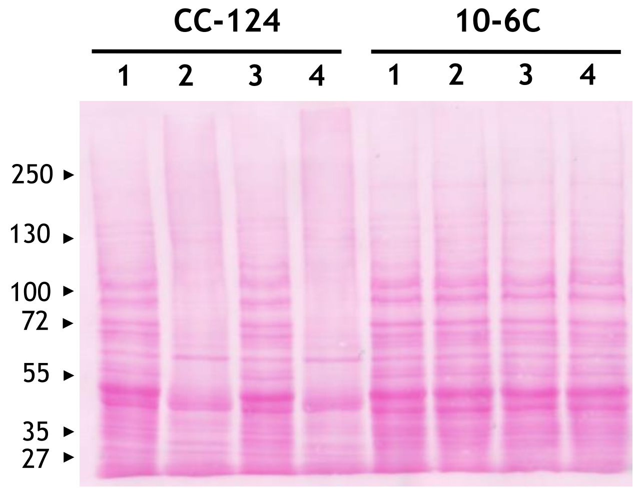
Peptide sequence	Position		gel band		stacking 1		stacking 2	
	Start	Stop	Spectral Count	Highest score	Spectral Count	Highest score	Spectral Count	Highest score
AEAAAPVAAAPATPHAEVK	54	72	1	40.63	1	37.91		
AEAAAPVAAAPATPHAEVKK	54	73	2	41.53	1	46.61	1	25.64
ERAPATDEALTELK	74	87	1	69.03	2	62.75	2	63.27
APATDEALTELK	76	87	1	77.70	1	79.12	1	53.21
RAQTAQAQYSTYTQEQVDEIFR	92	113			1	53.60		
AQTAQAQYSTYTQEQVDEIFR	93	113	1	136.06	2	104.36	2	119.00
NHFASEFIYNK	146	156	1	61.34	1	60.90	2	54.80
TCGVIEHDPAGGIQK	162	176	2	88.75	2	96.77	2	83.08
VAEPVGVIAGIVPTTNPTSTAIFK	177	200	2	71.88	2	58.46	1	32.24
NALVLCPHPR	209	218					1	44.21
AAYSSGNPSLGVGAGNTPALIDETADVAMAVSSILLSK	278	315	1	25.53	1	33.28		
TFDNGVICASEQSVVVVAK	316	334			1	98.25	1	91.52
RGAYFLTEDDKVK	346	358	1	47.37			1	40.16
GAYFLTEDDKVK	347	358	1	73.87	1	51.59	1	48.91
LNPNIVGQSIPK	369	380	1	83.04	1	69.30	1	72.70
LAALFGIK	381	388	1	55.48	1	50.57	2	43.48
VLIGEVEK	395	402					1	36.45
VLIGEVEKIGPEEALSQEK	395	413	1	39.44				
IGPEEALSQEK	403	413	1	59.36	2	40.93	3	64.23
LCPILAMYR	414	422	2	31.23				
MACELIMYGGAGHTSVLYTNPLNNAHIQQYQSAVK	432	466					3	27.58
ENMLWFR	525	531	1	35.84	1	36.86	1	26.61
GGCLEVALTDLR	540	551	1	94.51	2	95.03	2	86.84
AFIVTDKPLFDMGYADK	556	572			1	34.71	4	83.15
VTHILDSINVHHQVFYHVTPDPTLACIEAGLK	573	604					1	23.46
EILEFKPDVIIALGGGSPMDAAK	605	627					2	80.22
IMWLMYECPDTR	628	639	2	78.58	2	74.95	1	63.07
FDGLAMR	640	646	1	41.84	1	38.04		
VYEVPELGK	654	662			1	29.54		
KATMVCIPTTSGTGSEVTPFSVVTDER	663	689			1	42.89		
ATMVCIPTTSGTGSEVTPFSVVTDER	664	689	1	47.29	3	94.16	2	79.97
YPLADYALTPSMAIVDPQLVLNMPK	694	718	5	72.20	3	91.93	3	94.95
EAISLLFK	751	758	1	46.66	1	46.76	1	46.54
AYANGSNDYLAR	763	774					1	58.43
LGAAYHVPHGLANAALISHVIR	803	824	2	39.67				
YNATDMPAK	825	833	1	47.89	1	50.74	1	57.09
QAAFPQYEYPTAK	834	846	1	55.49	1	62.15	1	58.91
QDYADLANMLGLGGNTVDEK	847	866	3	117.60	4	133.25	3	100.77
LIEAVEELK	870	878					1	44.04
LIEAVEELKAK	870	880	2	62.52	1	45.87		
VDIPPTIK	881	888	1	25.51				
EIFNDPKVDADFLANVDALAEDAFDDQCTGANPR	889	922			1	48.55		
VDADFLANVDALAEDAFDDQCTGANPR	896	922	2	111.57	3	114.74	1	111.70
YPLMADLK	923	930	2	33.37			1	27.48
QLYLDAHAAPILPVK	931	945	2	64.38			2	54.53
TLEFFSK	946	952	1	29.82				

●**Tryptic peptides that match the ADHE sequence**. Peptides are highlighted in blue. Residues highlighted in black are those which differ between the phytozome sequence (here; T, V) and the sequence in PubMed (accession number: CAF04128; residues A, T). The identification of tryptic peptide “VLIGEVEK” confirms the phytozome sequence. The catalytic center in the ALDH as well as the signatures for iron-binding in the ADH domain (See Figure 1) are underlined.

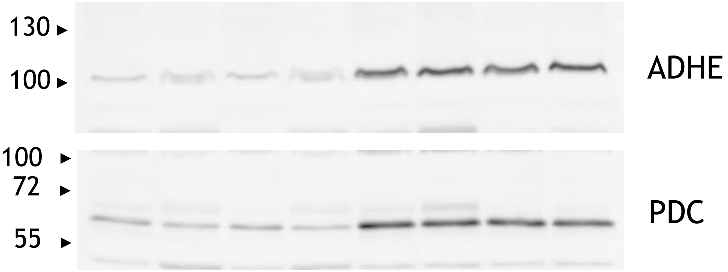
1 MMSSSLVSGKRVAVPSAAKPCAAVPLPRVAGRRTAARVVCEAAPSGAAPASPKAEAAAPV
61 AAAPATPHAEVKKERAPATDEALTELKALLKRAQTAQAQYSTYTQEQVDEIFRAAAEAAN
121 AARIPLAKMAVEETRMGVAEDKVVKNHFASEFIYNKYKHTKTCGVIEHDPAGGIQKVAEP
181 VGVIAGIVPTTNPTSTAIFKSLLSLKTRNALVLCPHPRAAKSTIAAARIVRDAAVAAGAP
241 PNIISWVETPSLPVSQALMQATEINLILATGGPAMVRAAYSSGNPSLGVGAGNTPALIDE
301 TADVAMAVSSILLSKTF**DNGVICASE**QSVVVVAKAYDAVRTEFVRRGAYFLTEDDKVKVR
361 AGVVVDGKLNPNIVGQSIPKLAALFGIKVPQGTKVLIGEVEKIGPEEALSQEKLCPILAM
421 YRAPDYDHGVKMACELIMYGGAGHTSVLYTNPLNNAHIQQYQSAVKTVRILINTPASQGA
481 IGDLYNFHLDPSLTLGCGTWGSTSVSTNVGPQHLLNIKTVTARRENMLWFRVPPKIYFKG
541 GCLEVALTDLRGKSRAFIVTDKPLFDMGYADKVTHILDSINVHHQVFYHVTPDPTLACIE
601 AGLKEILEFKPDVIIALGGGSPMDAAKIMWLMYECPDTRFDGLAMRFMDIRKRVYEVPEL
661 GKKATMVCIPTTSGTGSEVTPFSVVTDERLGAKYPLADYALTPSM**AIVDPQLVLNMPKKL**
721 **TAWGGIDALTHALE**SYVSICATDYTKGLSREAISLLFKYLPRAYANGSNDYLAREKVHYA
781 ATIAGMAFANAFL**GICHSMAHKLGAAYHVPHGLA**NAALISHVIRYNATDMPAKQAAFPQY
841 EYPTAKQDYADLANMLGLGGNTVDEKVIKLIEAVEELKAKVDIPPTIKEIFNDPKVDADF
901 LANVDALAEDAFDDQCTGANPRYPLMADLKQLYLDAHAAPILPVKTLEFFSKIN

FIGURE S4. Immunoblot analysis of cells incubated in dark anoxia in presence of sodium hypophosphite, an inhibitor of PFL.

Proteins (40 µg) from dark anoxic *C. reinhardtii* cells were separated on urea/SDS-PAGE and transferred to nitrocellulose membrane. Detection of ADHE and PDC was performed by Western blotting. *Lane 1,* incubation for 6 h in AIB; *lane 2,* incubation for 24 h in AIB; *lane 3,* incubation 6 h in AIB supplemented with 10 mM $NaPO_2H_2$; *lane 4,* incubation 24 h in AIB supplemented with 10 mM $NaPO_2H_2$.



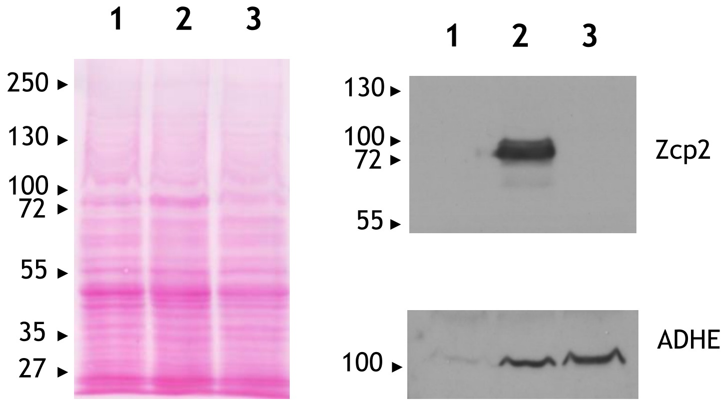
Ponceau S stain



Immunoblots

FIGURE S5. ADHE abundance in conditions of zinc-deficiency.

Protein analysis of *C. reinhardtii* cell extracts (40 µg). *Lane 1*, strain CC-124 grown on TAP medium; *lane 2*, strain CC-124 grown on zinc-deficient TAP medium; *lane 3*, strain 10-6C grown on TAP medium.



Ponceau S stain

Immunoblots

FIGURE S6. Production of antibodies to *C. reinhardtii* phosphotransacetylases.

●**Protein Overexpression and Antibody Production-** A partial sequence of *C. reinhardtii PTA1* cDNA (coding for His^{492}-Asn^{778}; tPTA1) was amplified by PCR using oligonucleotide primers containing the EcoRI and HindIII restriction sites (underlined) as follows: 5′-GACGAATTCCACATCGTGCTGCCCGAGTC -3′, and 5′-GTCAAGCTTGTCGTTCACCGGCTTGAGCAG -3′. The PCR product was cloned in pGEM-T Easy (Promega) and recloned in the BamHI/HindIII sites of the overexpression vector pET24a (Novagen). The resultant construct was introduced in *Escherichia coli* strain BL21 to produce the recombinant protein. His-tagged protein was purified under denaturing conditions using Ni-NTA matrix (Qiagen), as recommended by the supplier. Antibodies against tPTA1 were produced at Eurogentec (Leuven, Belgium).

●**Specificity of the produced antibodies-** In a fraction enriched in *C. reinhardtii* chloroplasts, the anti-PTA1 serum recognized two bands, which likely correspond to the two phosphotransacetylases PTA1 and PTA2 present in the alga.

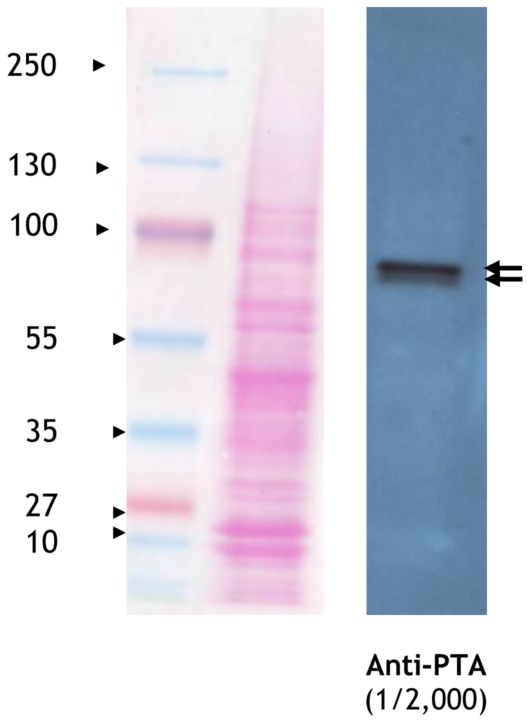
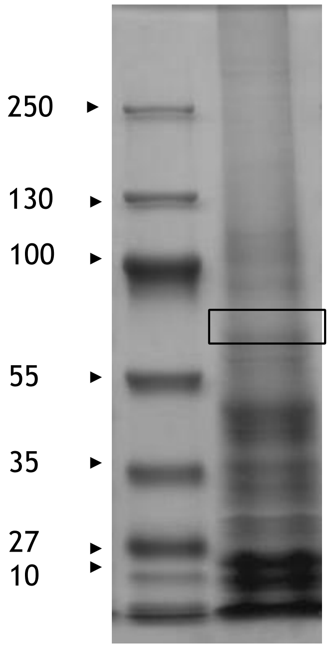


FIGURE S7. Identification of *C. reinhardtii* PTAs by mass spectrometry.

- **Urea/SDS-PAGE loaded with a fraction enriched in chloroplasts.** Chloroplasts were isolated from strain 10-6C and loaded on a urea/SDS-PAGE. The gel piece that contained the protein detected by the anti-PTA serum (see Supplemental Fig. S6) was subjected to nanoLC-MS/MS analysis.



- **Identification of PTAs by mass spectrometry**

	accession	score	mass	coverage	#peptides	emPAI
PTA2	Cre09.g396650.t1.1	2215,23	85020,14	54,58	29	3,56
PTA1	Cre17.g699000.t1.1	1977,00	86071,30	50,12	28	2,24

- Tryptic peptides that match *C. reinhardtii* PTAs. Peptides that match chloroplast PTA2 protein are highlighted in green; tryptic peptides that match mitochondrial PTA1 protein are highlighted in yellow. The underlined sequence correspond to tPTA1 used for antibody production.

```
PTA2       MSLNSSTMSRRQ---A-VAGAPAVAPFRHAGLFPRVRLCANRRVARVAP-KAAGNGNIAQ
PTA1       MAFASSSMAALSRPLAAVSSGLGSALSRASQLLTSGSLSSSPTASHSSTRRFISDG-TVG
           *:: **:*:  .   * *:.. . *  * : *:    *.:.  .:: :  :  .:*  .

PTA2       GEQGFDTLFLSDISLVGQRTPLLLGFFNYFERHLPHVGFFEPIAAEALASSELRIDRHVE
PTA1       SKGRPDSLFLSDISMSGHRAPLLLGWLNYLERHLPHVGFFEPIGGRALAGSELSVDRHVE
           .:   *:*******: *:*:*****::**:*************...***.*** :*****

PTA2       LVYKVFNLKGDVRAMTGVQDAEAARMIANGQHSELLDKIYSQYASYKEGQ--DLVLVEGP
PTA1       LMYRVFNMKGDATRMTGISDTEAAQLIASGKQSEVLDRIYAAYMAYKAGGELDLCLVEGP
           *:*:***:***.  ***:.*:***::**.*::**:**:**: * :** *   ** *****

PTA2       GPLMGGTELDAQIAAALNAPVLMTMTGQPNATVADYYNRAMVKRQVFLDHHVEVLGLVMN
PTA1       GPLMGGTELDAQIAAALNAPVLMAMSGRPNATANDYYNKAMVKRQVFADHKVDVLGVVIN
           ***********************:*:*:****. ****:******** **:*:***:*:*
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PTA2      GLPRQSHAILSGQLRDKFAAAGLPFAGAIPTDIMLRNVRLDEVQTAMGAQRLYGDSLLTD
PTA1      GLPREHHAILSSQLRDKLERAGLPFAGALPEDPVLSSVRLDEVRTALGATQLYGETWLGD
          ****: *****.*****:  ********:* * :* .******:**:** :***:: * *

PTA2      VEFDDVVVASQRLEELLEILAERPMGRPLVVTSADRLDIVLGLLAAQLSVSGPGVAGILL
PTA1      VEFDEVVVGSQRLEELLETLVERPMGRPLVVTSADRLDIVLGLLAAQLSVRGPSVAGVLL
          ****:***.********* *.***************************** **.***:**

PTA2      TQAGSARSGRNYARDTIDRIFAGLSS--------------------SGLYKGSLLPVLVT
PTA1      TQAGASRITRSYAKSAVDNIFAGLSNNTGASGGGPDGAAAANGSAQGSLYRGALLPVLST
          ****::*  *.**:.::*.******.                    ..**:*:***** *

PTA2      DMPLRDAIRKLDNLDAAILPSSTRKISQCKRLFEQYVDANAVVARLQNMVRPNRMTPKMF
PTA1      DKHLAEALAVIGRMDASILPTSIRKVTQCKMLFDKYIDANAVVTGLQK-SRPTRVTPKMF
          *  * :*:  : .:**:***:* **::*** **::*:******: **:  **.*:*****

PTA2      MHTLKSMCNATPQHIVLPESEDKRVLAAAADVVQRGLAKITLLGDPTTILAEAAKLGLDL
PTA1      QHTMKAMCRASPQHIVLPESVDKRVLAAAADVTARGLARVTLLGDPTTVQAEAKKLGLDL
           **:*:**.*:********* ***********. ****::********: *** ******

PTA2      SGCNIHNPNTSDRFDKYVDMLVEARKKKGMTREVAADTLHGDVNFFATMMIVAGDADGMV
PTA1      SGCHIHNPNSSDRFDKYVDMLVEARKKKGMTREAAADTLHGDINFFGTMMVAAGDADGMV
          ***.*****:***********************.********:***.***:.********

PTA2      SGAVHTTASTVRPALQVLKSPDTPLVSSVFIMCLPDRVVVYGDCAVNVNPSAADLAQIAI
PTA1      SGAIHTTASTIRPALQMLKNPASSLVSSIFFMCLPDRVLVYGDCAVNVSPSAADLAAIAT
          ***:******:*****:**.* : ****:*:*******:*********.******* **

PTA2      TSNDTAAAFGIEPRVAMLSYSTLGSGSGPDVQKVSEAVAIVKQRRPDIKVEGPIQYDAAI
PTA1      TSADTAAAFGIEPRVAMLSYSTLGSGAGPDVQKVTEAVALVKQQRQDIKVEGPIQYDAAI
          ** ***********************:*******:****:***:* **************

PTA2      DPKVAAVKVQGLSEVAGKATVFIFPDLNTGNNTYKAVQQSTGAIAMGPVMQGLLRPVNDL
PTA1      DPAVAAVKVKGGSEVAGRATVFVFPDLNTGNNTYKAVQQSTGAIAMGPVMQGLLKPVNDL
          ** ******:* *****:****:*******************************:*****

PTA2      SRGCTVPDIINTICVTSIQASRMSSAARAAAAKAAVAAV*
PTA1      SRGCTVPDIVNTICVTSIQAMQFKQRTQAAVAAAAAPK*-
          *********:********** ::.. ::**.* **.
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