Supplemental Table S1

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Supplemental Table S1. Names of all genes used in this study.

Gene name used in this manuscript	GenBank accession ID	Genomic locus at Phytozome	Transcript name at Phytozome	Alias at Phytozome
AtPDK1	AF132742.1	n/a	n/a	n/a
SlPDK1	AAW38936.1	n/a	n/a	n/a
PpPDK1	JN049607	Pp1s217_11V6	Pp1s217_11V6.2	Phypa_144576
n/a	n/a	Pp1s217_11V6	Pp1s217_11V6.1	Phypa_144576
Pp174181	JN049609	Pp1s4_386V6	Pp1s4_386V6.1	Phypa_174181
Pp188412	JN049610	Pp1s118_230V6	Pp1s118_230V6.1	Phypa_188412
Pp2484	JN049608	Pp1s224_73V6	Pp1s224_73V6.1	Phypa_2484

Supplemental Figure S1

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A BLAST AtPDK1-1 protein sequence to P. patens proteome at Phytozome, top hits:

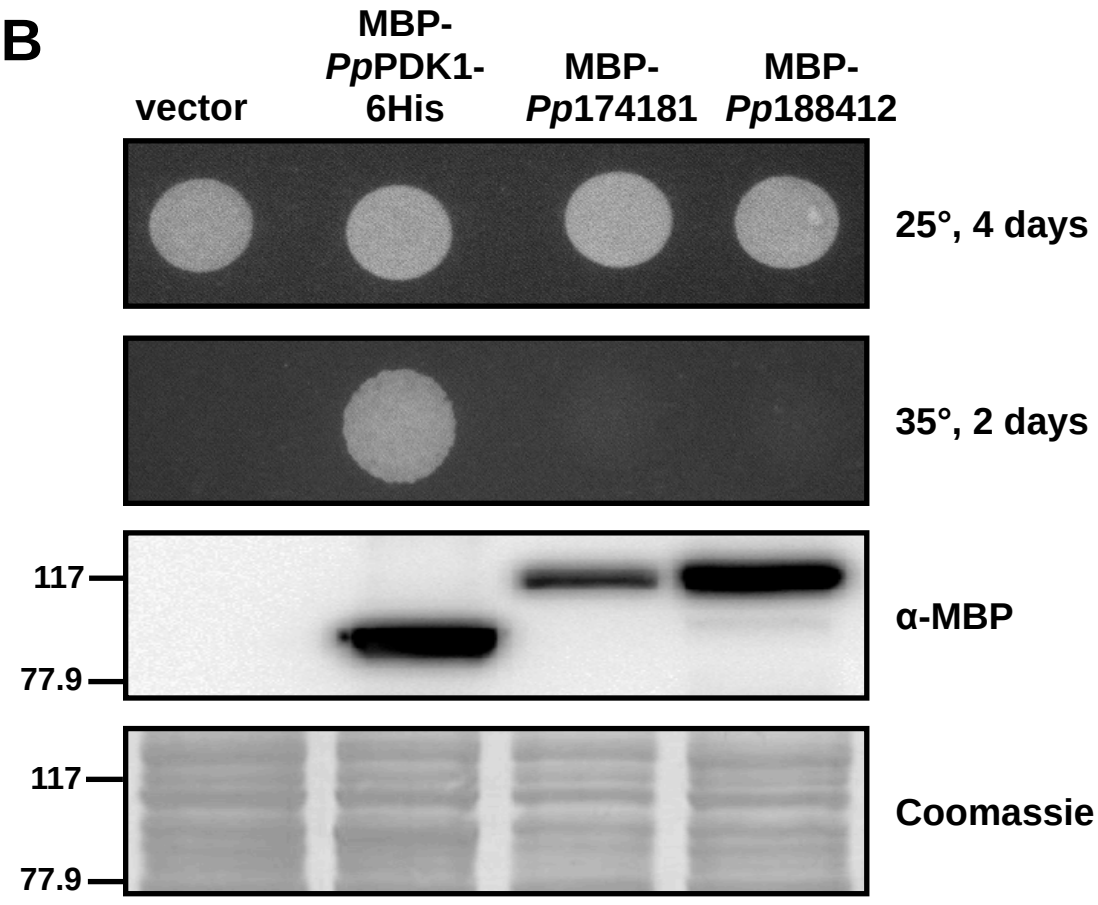
1. Pp1s217_11V6.2 (Phypa_144576), score 369.0, E value 3.0e-102 (1044 nt PpPDK1 transcript)

2. Pp1s217_11V6.1 (Phypa_144576), score 370.5, E value 1.2e-102 (1041 nt PpPDK1 transcript)

3. Pp1s118_230V6 (Phypa_188412), score 224.2, E value 1.2e-58. Annotated as ribosomal S6 protein kinase and related proteins. BLAST of the cDNA below to TAIR10 cDNAs returned AT3G08730.1 (S6K), AT3G08720.2 (S6K2), and AT3G08720.1 (S6K2) as the top hits

4. Pp1s4_386V6.1 (Phypa_174181), score 223.8, E value 1.7e-57. Annotated as ribosomal S6 protein kinase and related proteins. BLAST of the cDNA below to TAIR10 cDNAs returned AT3G08720.2 (S6K2), AT3G08730.1 (S6K), and AT3G08720.1 (S6K2) as the top hits

B



Supplemental Figure S1. Evidence for one *PDK1* in the *P. patens* genome. A, The *At*PDK1-1 protein sequence (GenBank accession AF132742.1) was used in a BLAST search of the *P. patens* proteome at Phytozome (www.phytozome.net). The names of the top 4 hits, as well as the score and E value for each, are ranked by E value. The first two hits are alternately spliced transcripts from the same *PDK1* locus. The first hit is *Pp1s217_11V6.2*, the 1044 nt *PpPDK1* transcript characterized in this manuscript. The second hit is *Pp1s217_11V6.1*, a 1041 nt *PpPDK1* transcript identical to *Pp1s217_11V6.2* except that it lacks a single glutamate from the splice junction between exons 5 and 6. The third hit is *Pp1s118_230V6* (*Pp188412*), a putative ribosomal S6 kinase. The fourth hit is *Pp1s4_38V6.1* (*Pp174181*), another putative ribosomal S6 kinase, suggesting that the *P. patens* genome contains only one *PDK1*. The cDNA sequences of *Pp188412* and *Pp174181* were used in a BLAST search of *A. thaliana* cDNAs at TAIR (www.arabidopsis.org). The names of the top 3 hits are also shown. The top three hits in both cases were *AT3G08730.1* (S6K), *AT3G08720.2* (S6K2), and *AT3G08720.1* (S6K2). B, *Pp*188412 and *Pp*174181 are not able to complement a temperature sensitive allele of *S. cerevisiae PKH1*. Haploid strain INA106-3B, which lacks *PKH2* and contains temperature-sensitive $PKH1^{D398G}$, was transformed with p416GPD containing the indicated constructs under control of the constitutive GPD promoter. Transformed yeast were grown in liquid medium lacking uracil, spotted on plates lacking uracil, and grown at the indicated temperatures and times. Total protein was extracted from cultures grown in liquid medium at 25°C and analyzed by α-MBP western blot to verify expression of each *Pp*PDK1.

Supplemental Figure S2

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A

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TATAAGTGTTGTCCCACGATTTATATACACTATGGATGTCTCCGTTGTGCATTTCCATGTGTTGATATAAGTTAGAATTGCAATTGATTGA
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B

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C

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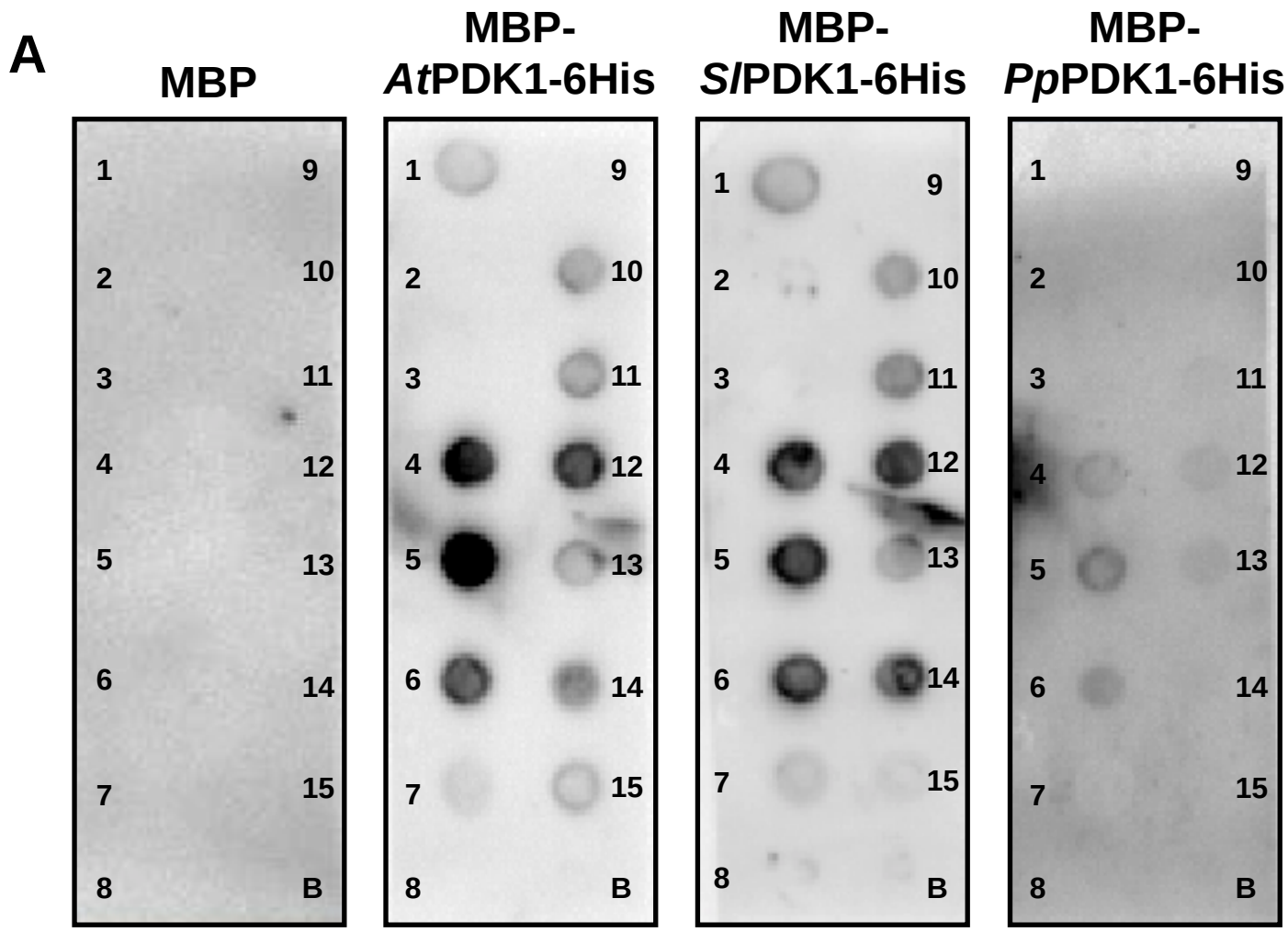
```

Supplemental Figure S2. *PpPDK1* genomic DNA, cDNA, and protein sequences. A, *PpPDK1* genomic DNA sequence of 3941 nt, with 5' and 3' UTRs shown in blue text, exons in black text, and introns in red text. B, *PpPDK1* coding sequence of 1044 nt. The nucleotide at the 3' end of each exon is in red text. C, *Pp*PDK1 protein sequence of 347 amino acids. K67 is highlighted in green and K71, I75, Q106, and L111 are highlighted in yellow.

Supplemental Figure S3

Nelson Dittrich & Devarenne



Supplemental Figure S3. *Pp*PDK1 does not strongly bind phospholipids or sphingolipids. A, Five µg purified MBP, MBP-*At*PDK1-6His, MBP-*Sl*PDK1-6His, or MBP-*Pp*PDK1-6His was incubated with a membrane pre-spotted with 15 common lipids and a solvent blank, and analyzed by α-MBP western blot. Numbers indicate the lipids spotted: 1, lysophosphatidic acid; 2, lysophosphocholine; 3, PtdIns; 4, PtdIns(3)P; 5, PtdIns(4)P; 6, PtdIns(5)P; 7, phosphatidylethanolamine; 8, phosphatidylcholine; 9, sphingosine-1-phosphate; 10, PtdIns(3,4)P_2; 11, PtdIns(3,5)P_2; 12, PtdIns(4,5)P_2; 13, PtdIns(3,4,5)P_3; 14, phosphatidic acid; 15, phosphatidylserine; B, 2 methanol:1chloroform:0.8 water solvent blank. B, Five µg purified MBP, MBP-*Sl*PDK1-6His, or MBP-*Pp*PDK1-6His was incubated with a membrane pre-spotted with 15 common lipids and sphingolipids and a solvent blank, and analyzed by α-MBP western blot. Numbers indicate the lipids spotted: 1, sphingosine; 2, sphingosine-1-phosphate; 3, phytosphingosine; 4, ceramide; 5, sphingomyelin; 6, sphingosyl-phosphatidylcholine; 7, lysophosphatidic acid; 8, myriosine; 9, monosialoganglioside-GM1; 10, disialoganglioside-GD3; 11, 3-sulfogalactosylceramide; 12, psychosine; 13, cholesterol; 14, lysophosphocholine; 15, phosphatidylcholine; B, solvent blank.

Supplemental Figure S4

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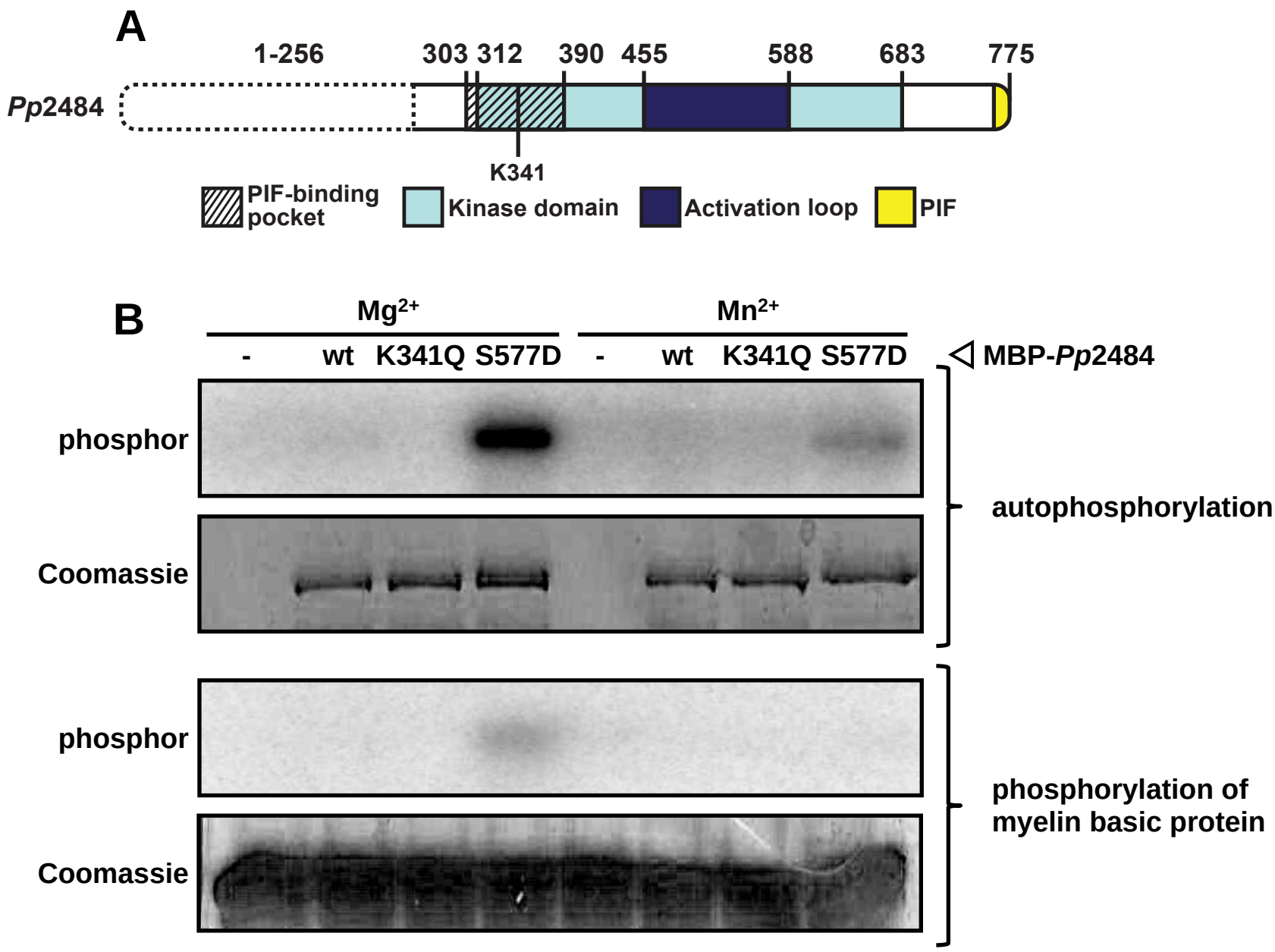

Supplemental Figure S4. Characterization of the kinase activity for the *P. patens* AGC kinase *Pp*2484. A, Diagram showing the protein features of *Pp*2484. B, *Pp*2484 kinase activity is consistent with an AGC kinase that is activated by PDK1 phosphorylation in the conserved activation loop serine. Wild-type (wt), kinase-inactive (K341Q), and kinase-active (S577D) MBP-*Pp*2484 were incubated with myelin basic protein in an *in vitro* kinase assay in the presence of either Mg^{2+} or Mn^{2+}.

Supplemental Figure S5

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A

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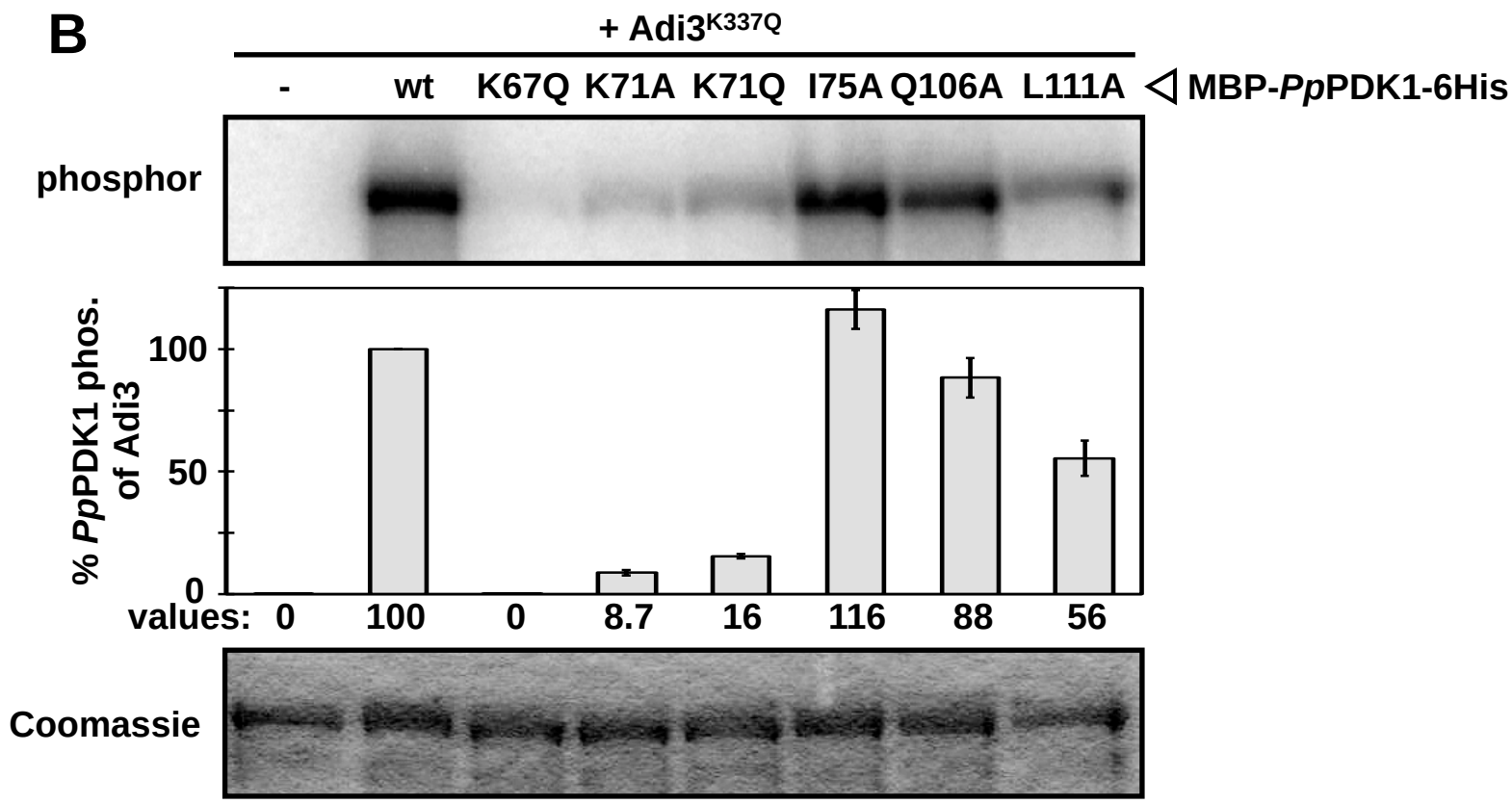
PpPDK1  (30) -QMDFTSNDFLFAKLLGLGSYSKVTKAKRKNTGEIYALKIMNKKHIIRENKVKFVKMERMILDQLDHPGVVKLCFTFQDVHS--LYMGLE (116)
SlPDK1  (40) -QENFTIQDFELGKIYGVGSYSKVVRAKKKDTANVYALKIMDKKFITKENKTAYVKLERIVLDQLDHPGVVRLFFTFQDTFS--LYMALE (126)
HsPDK1  (73) QPRKKRPEDFKFGKILGEGSFSTVVLARELATSREYAIKILEKRHIIKENKVPYVTRERDVMSRLDHPFFVKLYFTFQDDEK--LYFGLS (160)
Adi3   (299) RDGILGMSHFKLLKRLGCGDIGSVYLSELSGTRCYFAMKVMDKASLASRKKLTRAQTEREILQLLDHPFLPTLYTHFETDRFSCLVM--E (386)
```

ATP coordinating lysine
PIF-binding pocket residues mutated in *Pp*PDK1
PIF-binding pocket residues not mutated in *Pp*PDK1

B



Supplemental Figure S5. Phosphorylation of Adi3 by *Pp*PDK1 PIF-binding pocket mutants. A, Alignment of the PIF-binding pockets of PDK1 proteins from *P. patens* (*Pp*), tomato (*Le*), and human (*Hs*), and Adi3 (adapted from Devarenne et al. 2006). Residues that Bind PIF residues is based on the *Hs*PDK1 PIF-binding pocket (Frodin et al., 2002). B, Mutation of *Pp*PDK1 PIF-binding pocket residues reduces phosphorylation of Adi3. Values are reported as the percentage of wt *Pp*PDK1 phosphorylation of Adi3 and are the mean of three independent experiments. Error bars indicate standard error.

Supplemental Figure S6

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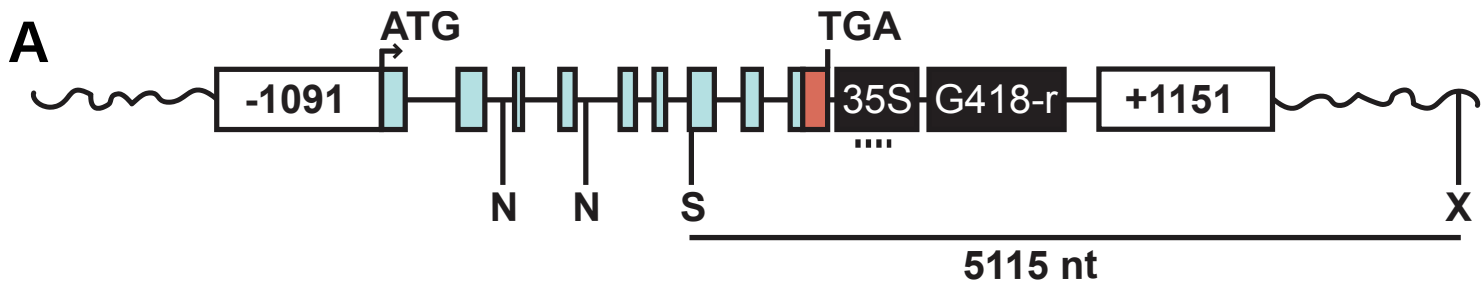

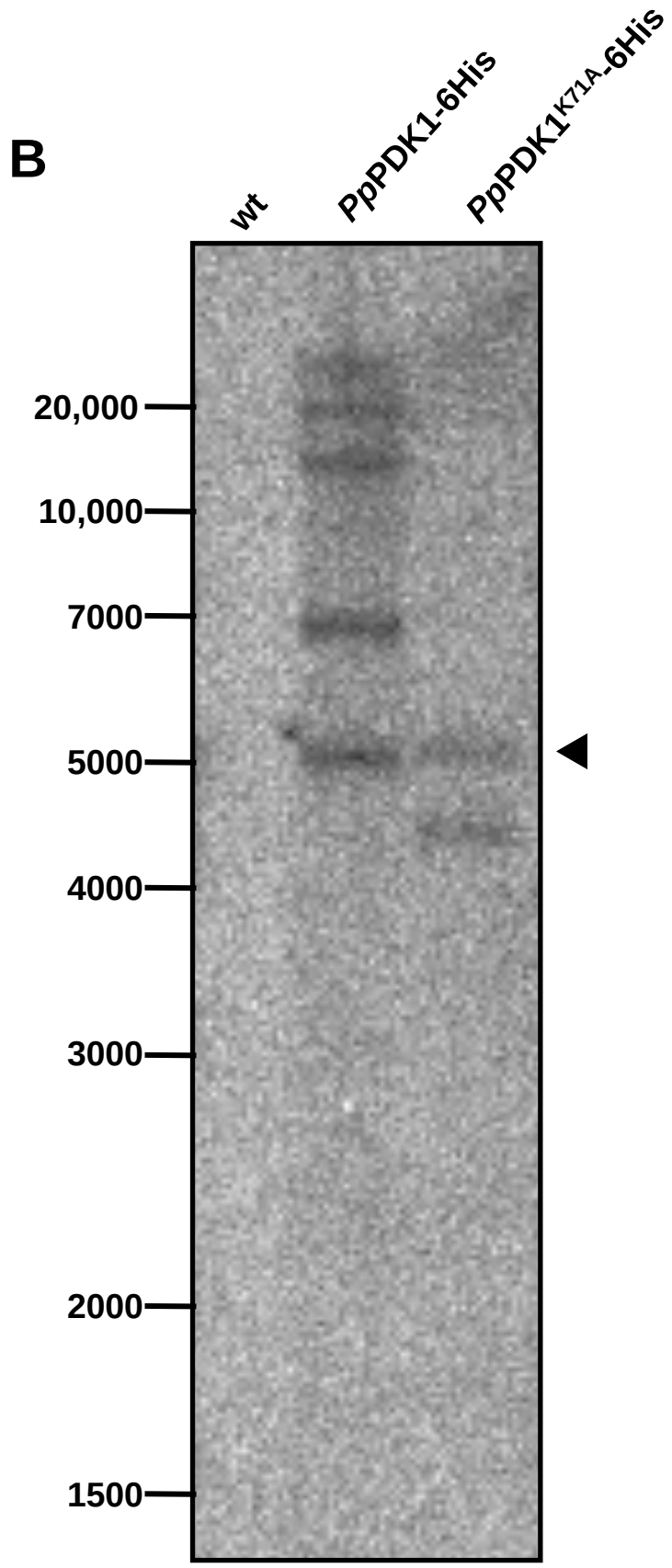

Figure S6. Southern blot analysis of *PpPDK1-6His* and *PpPDK1*^{K71A}*-6His* transformed moss. A, The expected size of fragment detected by a probe based in the 35S promoter is depicted as a solid line below the figure. The location of the probe used in (B) is indicated by a dashed line. *Nde*I, *Sal*I, and *Xba*I cut sites are indicated by N, S, and X respectively. B, Southern blot analysis of genomic DNA digested with *Nde*I, *Sal*I, and *Xba*I from transformants confirms that the *PpPDK1-6His* constructs were integrated into the correct location in the genome, as indicated by the black triangle. Additional integration events, probably into the same genomic locus, also occurred in each transformant. Wild-type *P. patens* genomic DNA was used as a negative control.

Supplemental Figure S7

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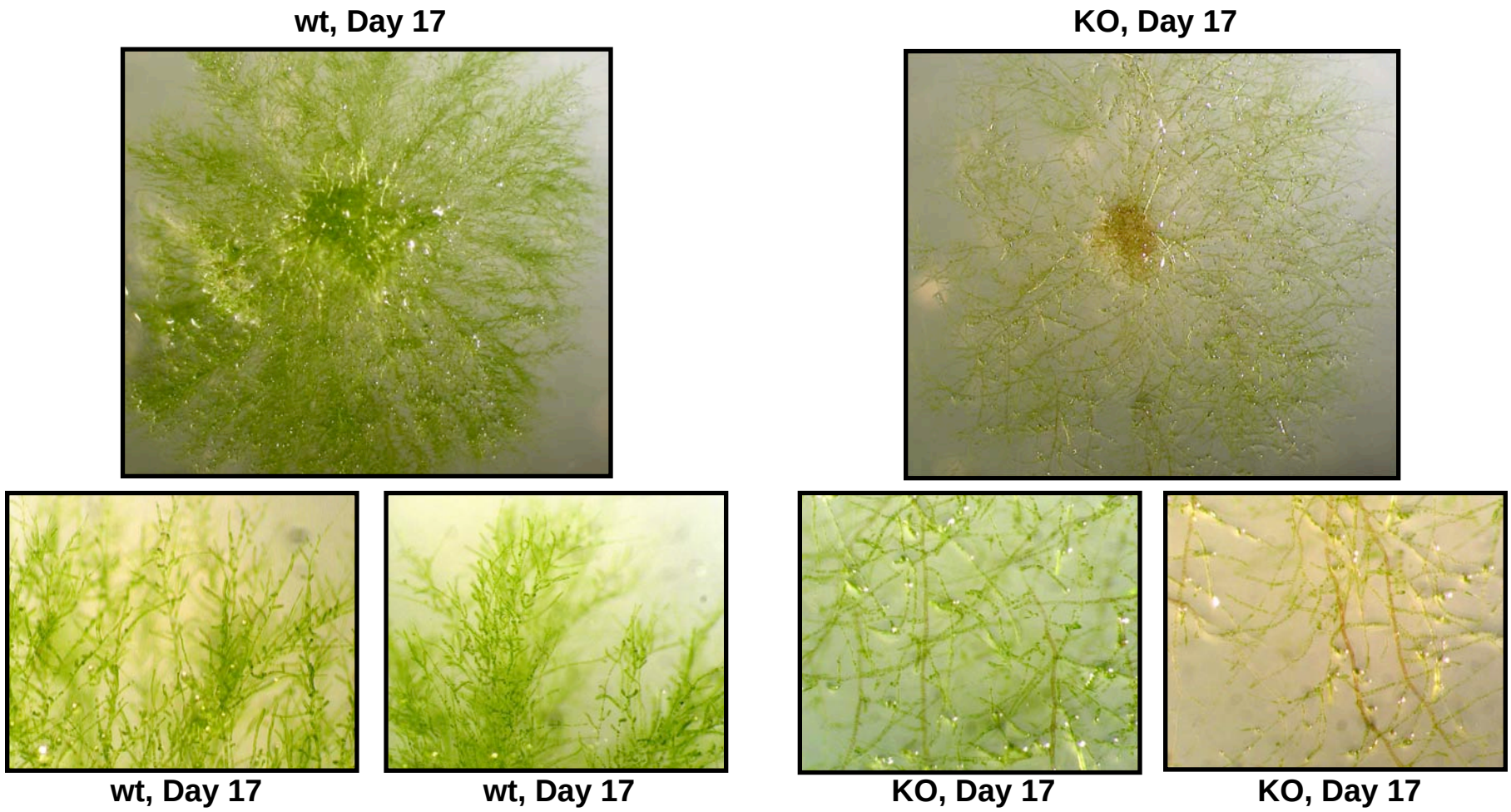

Figure S7. Protonema growth of wild-type and the *pdk1* knockout line at day 17. A, Moss were plated on BCD plates, grown for 17 days, and pictures taken. Pictures of the whole moss colony and close-ups of protonema from two different regions of reach moss colony are shown.

Supplemental Figure S8

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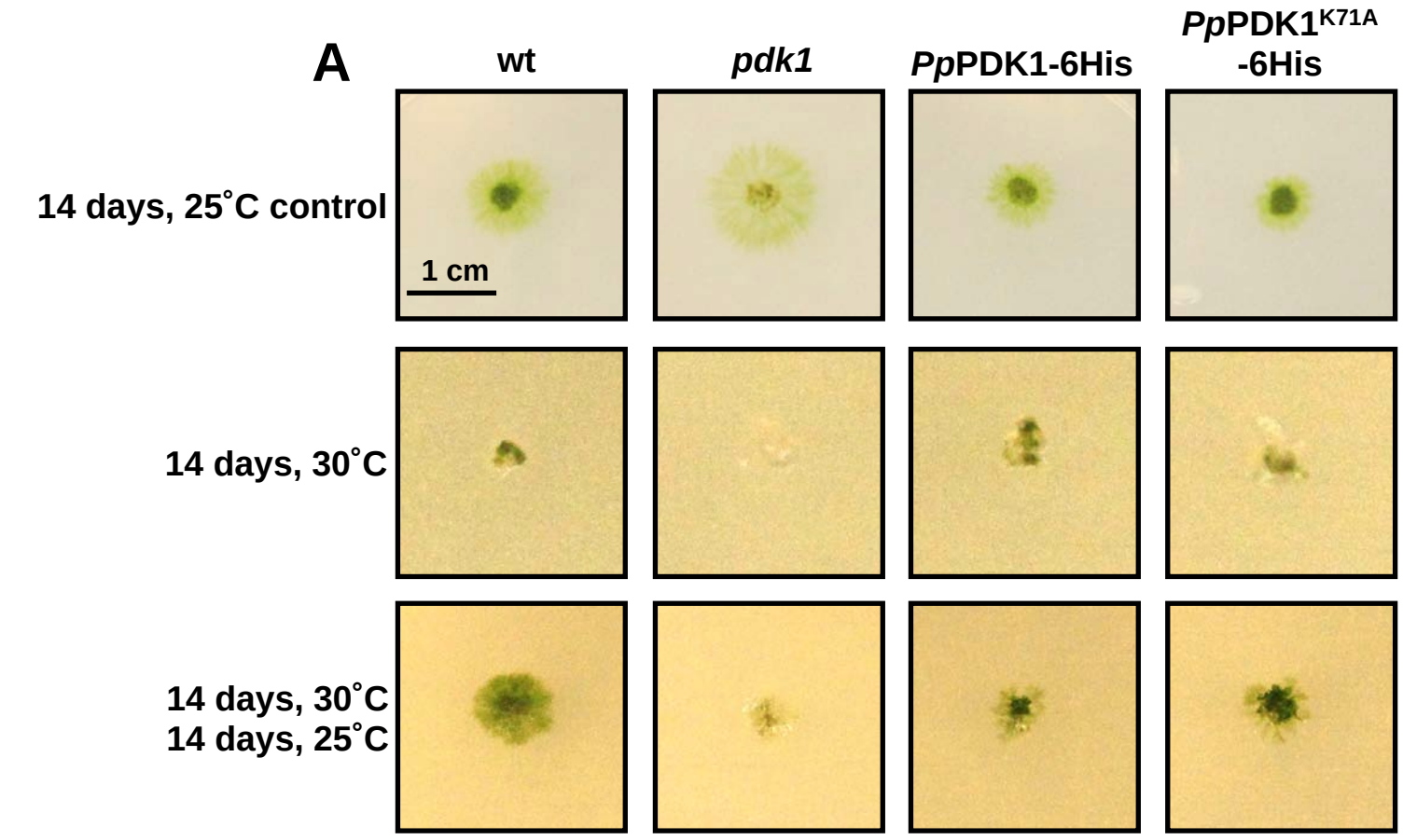

Figure S8. Stress treatment of moss lines. A, Wild-type, *pdk1* knockout, and gene replacement lines were plated on BCD plates, incubated at 30˚C for 14 days, and then moved to 25˚C for 14 days. B, Wild-type, KO, and gene replacement lines were plated on BCD plates containing 0.9M mannitol, grown for 30 days, moved to BCD plates without mannitol, and grown for 14 days.

Supplemental Figure S9

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E.siliculosus          ----------------MRKHTAALLEVKLHEAGDAVEALRRYSSALSHTPDDHLLHSNR
C.merolae              ------------------------------------------------------------
O.lucimarinus          -------------MAEDGDDPGLVSFDELASVGTMRAEDAPRGSSPAANEEDSFGSATS
O.tauri                MATATATATEDGLVNFDALATMESVRERRGRDLRIEKLREEDENSERSERSAEEGSGPGT
O.sativa               ------------------------------------------------------------
S.bicolor              ------------------------------------------------------------
Z.mays1                ------------------------------------------------------------
Z.mays2                ------------------------------------------------------------
A.thaliana1            ------------------------------------------------------------
A.thaliana2            ------------------------------------------------------------
P.trichocarpa1         ------------------------------------------------------------
P.trichocarpa2         ------------------------------------------------------------
R.communis             ------------------------------------------------------------
G.max1                 ------------------------------------------------------------
G.max2                 ------------------------------------------------------------
V.vinifera             ------------------------------------------------------------
S.lycopersicum1        ------------------------------------------------------------
S.lycopersicum2        ------------------------------------------------------------
P.patens               ------------------------------------------------------------
S.moellendorffii       ------------------------------------------------------------
C.hlorella             ------------------------------------------------------------
C.reinhardtii          ------------------------------------------------------------
V.carteri              ------------------------------------------------------------

E.siliculosus          AMALAKLGRWSESEKSAAKAVEASPDFAKGYLRLAKAQLEQAKNAEAVTACDEGLAAERR
C.merolae              ----------------------------------------------------MMSTWRRERR
O.lucimarinus          EGQASPAPTMMRSVLTRKPSVAALKAMLMSERDEEGAGEGSETDEDEGVCSLSEFAMVLN
O.tauri                PTEAAPMRSVLTRKPSVAALKLFSSMDVNESEAESEKMDAEEGDEDVCSLSEFAMVLNVA
O.sativa               ------------------------------------------------------------
S.bicolor              ------------------------------------------------------------
Z.mays1                ------------------------------------------------------------
Z.mays2                ------------------------------------------------------------
A.thaliana1            ------------------------------------------------------------
A.thaliana2            ------------------------------------------------------------
P.trichocarpa1         ------------------------------------------------------------
P.trichocarpa2         ------------------------------------------------------------
R.communis             ------------------------------------------------------------
G.max1                 ------------------------------------------------------------
G.max2                 ------------------------------------------------------------
V.vinifera             ------------------------------------------------------------
S.lycopersicum1        ------------------------------------------------------------
S.lycopersicum2        ------------------------------------------------------------
P.patens               ------------------------------------------------------------
S.moellendorffii       ------------------------------------------------------------
C.hlorella             ------------------------------------------------------------
C.reinhardtii          ------------------------------------------------------------
V.carteri              ------------------------------------------------------------

E.siliculosus          SSLPPSTPVAVAAAGGGGGDGGAPAVAVSKTVRELEMMAATALERLRAQEGAGSRRQSGA
C.merolae              TPSWAVDESWFAELERGIKGGCAAGLPVDTSAKPEPVVCFEDSRDESEKETDQMLPHQPP
O.lucimarinus          AAAWDDASVGALGVEVIVKQLELLRGKIAPEASLSSMLGFDLAEDLSVRANSAGMVSVGA
O.tauri                AWVRDDKLAGQLNVDVIVKQLELLRGTVGTNSSLSGVLGFDVHRDLSVRADTAGFVSIGS
O.sativa               ------------------------------------MAVGGDDD-MERDFAARLRLA--
S.bicolor              ------------------------------------MAVGGDDDSMERDFAARLRLAH-
Z.mays1                ------------------------------------------------MERDFAARLRLAH-
Z.mays2                ------------------------------------MAVGGDDDSMERDFAARLRLAH-
A.thaliana1            ------------------------------------MLA-------MEKEFDSKLVLQG-
A.thaliana2            ------------------------------------MLT-------MDKEFDSKLTLQG-
P.trichocarpa1         ------------------------------------MLE-------MEREFDSKLRIQS-
P.trichocarpa2         ------------------------------------------------MSEHSPSLKCHI-
R.communis             ------------------------------------MLA-------MEKDFDSKLRIQSS
G.max1                 ------------------------------------MLE-------MEKDFDSKLKIQG-
G.max2                 ------------------------------------MLE-------TEKDFDSKLKIQG-
V.vinifera             ------------------------------------------------------------
S.lycopersicum1        ------------------------------------MLALVGGEGDMEQEFDAKLKIQN-
S.lycopersicum2        ------------------------------------------------MEQELDSKLRIEN-
P.patens               ------------------------------------------------MAMDGTSPVSP-
S.moellendorffii       ------------------------------------------------------------
C.hlorella             ------------------------------------------------------------
C.reinhardtii          ------------------------------------------------------------
V.carteri              ------------------------------------------------------------
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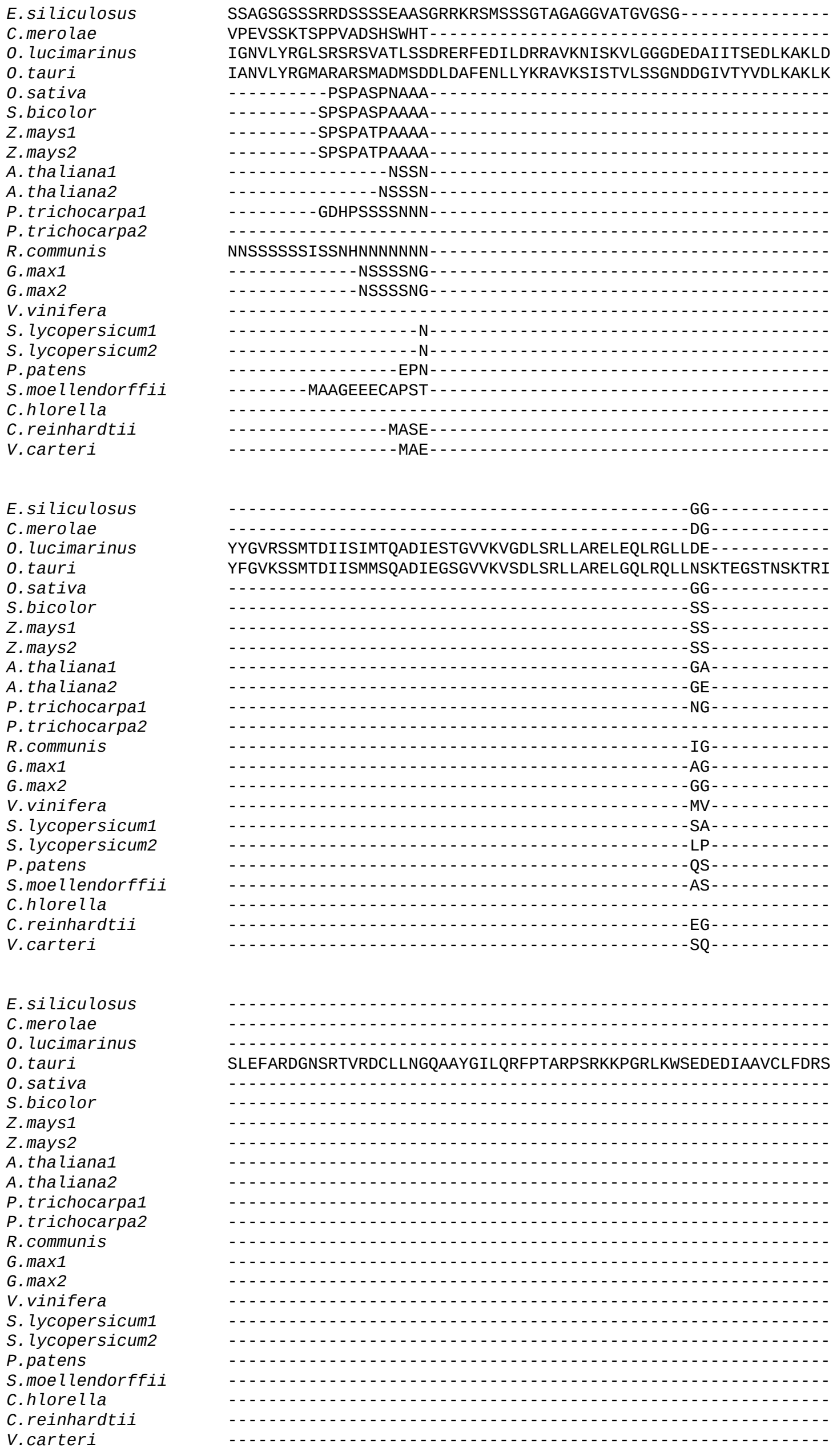
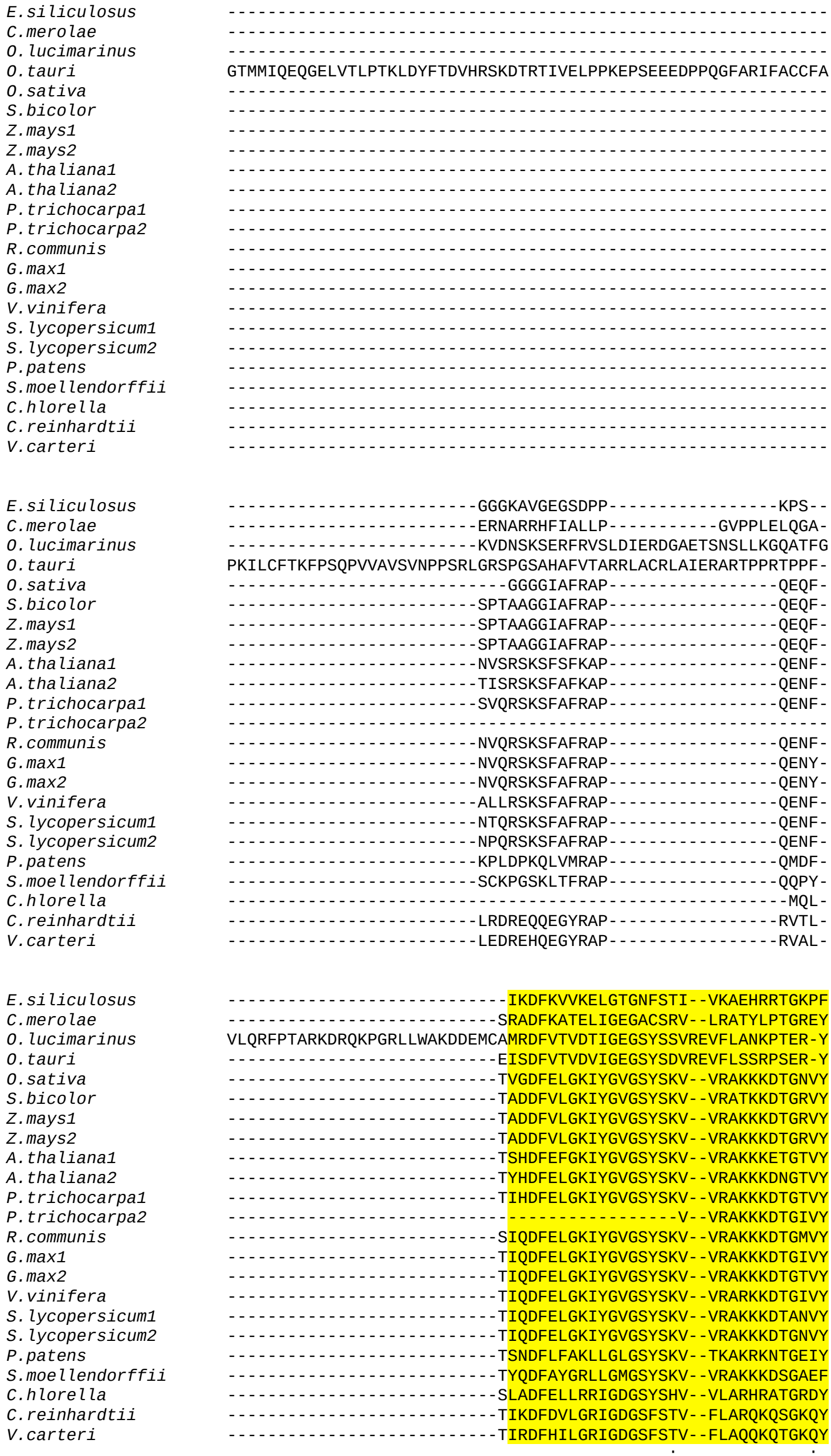
Supplemental Figure S9 contd.

Nelson Dittrich & Devarenne



Supplemental Figure S9 contd.

Nelson Dittrich & Devarenne



Supplemental Figure S9 contd.

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E.siliculosus          ALKMIEKAEVNRLKRRHENVYNEIYMEKRALTK--LSHPNIVRMHSTFQDYSTLYYLLDM
C.merolae              AVKVISKALAEQ-----NEQVLPLRTEQICLQVG-LGHPNIVQLKAILEDENFLYMVIEL
O.lucimarinus          ALKIMDKSHIVR-----EGKARYVATERALLAGRLADCDCVAALRFTFQDTYSLYLGMEL
O.tauri                ALKVMDKAHIVR-----ESKSRYVATERTLLAGRLRECEHVARLMFTFQDTYSLYMGFEL
O.sativa               ALKIMDKKFITK-----ENKISYVKMERIVLDQ--LDHPGVIRLFFTFQDTYSLYMALES
S.bicolor              ALKIMDKKFITK-----ENKISYVKMERIVLDQ--LDHPGVIRLFFTFQDTYSLYMALES
Z.mays1                ALKIMDKKFITK-----ENKISYVKMERIVLDQ--LDHPGVIRLFFTFQDTYSLYMALES
Z.mays2                ALKIMDKKFITK-----ENKISYVKMERIVLDQ--LDHPGVIRLFFTFQDTYSLYMALES
A.thaliana1            ALKIMDKKFITK-----ENKTAYVKLERIVLDQ--LEHPGIIKLYFTFQDTSSLYMALES
A.thaliana2            ALKIMDKKFITK-----ENKTAYVKLERIVLDQ--LEHPGIVKLFFTFQDTQSLYMALES
P.trichocarpa1         ALKIMDKKFITK-----ENKTAYVKLERIVLDQ--LDHPGIVRLFFTFQDNYSLYMALES
P.trichocarpa2         ALKIMDKKFITK-----ENKTAYVKLERIVLDQ--LDHPGIVRLYFTFQDNYSLYMALES
R.communis             ALKIMDKKFITK-----ENKTAYVKLERIVLDQ--LDHPGIVRLFFTFQDSFSLYMALES
G.max1                 ALKIMDKKFITK-----ENKTAYVKLERIVLDQ--LDHPGIVRLYFTFQDSFSLYMALES
G.max2                 ALKIMDKKFITK-----ENKTAYVKLERIVLDQ--LDHPGIVRLYFTFQDSFSLYMALES
V.vinifera             ALKIMDKKFITK-----ENKTAYVKLERIVLDQ--LDHPGIVRLFFTFQDTFSLYMALES
S.lycopersicum1        ALKIMDKKFITK-----ENKTAYVKLERIVLDQ--LDHPGVVRLFFTFQDTFSLYMALES
S.lycopersicum2        ALKIMDKKFITK-----ENKTAYVKLERIVLDQ--LDHPGIVRLFFTFQDTFSLYMALES
P.patens               ALKIMNKKHIIR-----ENKVKFVKMERMILDQ--LDHPGVVKLCFTFQDVHSLYMGLEC
S.moellendorffii       ALKIMDKKHITK-----ENKVAYVKMERLILDH--LDHPGVVRLFFTFQDTHNLYMGLEC
C.hlorella             ALKVIDKQYIMR-----HRVVDYIRKERQILDA--LQYDGIAKLYFTFQDAYSLYLGLEY
C.reinhardtii          AIKMMNKHLVMR-----NKMVEYIKNERFILDK--FDDAGIAKLHFTFQDPDNLYMGMEY
V.carteri              AIKMMNKHLIMR-----NKVVEYIKNERFILDK--LDDAGIAKLHFTFQDPNNLYMGMEY
                      *:*::.*    .          :  *.  *       :  :   ::*   **  ::

E.siliculosus          CDGGEVWKRLTVD-------------------------DKVVGAHP-SLARFWLSEVV
C.merolae              CPHGDLARLLARRRASSETPHPSHRDRFQKREPNGAAPSGGNHVGALSLDAARFYFAEIV
O.lucimarinus          CTGGDLYSQLKRS----------------------------EGEVMTEEKAVFYVSEVT
O.tauri                CPGGDLFWQLKRS----------------------------EEGVMEETKVVFYVSEVL
O.sativa               CEGGELFDQIVRK-----------------------------GRLSEDEARFYAAEIV
S.bicolor              CEGGELFDQIVRK-----------------------------GRLSEDDARFYAAEIV
Z.mays1                CEGGELFDQIVRK-----------------------------GRLSEDDARFYAAEIV
Z.mays2                CEGGELFDQIVRK-----------------------------GRLSEDDARFYAAEIV
A.thaliana1            CEGGELFDQITRK-----------------------------GRLSEDEARFYTAEVV
A.thaliana2            CEGGELFDQITRK-----------------------------GRLSEDEARFYSAEVV
P.trichocarpa1         CEGGELFDQITRK-----------------------------GRLSEDEACFYAAEVV
P.trichocarpa2         CEGGELFDQITRK-----------------------------GRLSEDEARFYAAEVV
R.communis             CEGGELFDQITRK-----------------------------GRLSEDEARFYAAEVV
G.max1                 CEGGELFDQITRK-----------------------------GRLSENEARFYAAEVI
G.max2                 CEGGELFDQITRK-----------------------------GRLSEDEARFYAAEVV
V.vinifera             CEGGELFDQITRK-----------------------------GRLSENEARFYAAEVV
S.lycopersicum1        CEGGELFDQITRK-----------------------------GRLSEDEARFYAAEVV
S.lycopersicum2        CEGGELFDQITRK-----------------------------GRLSEDEARFYAAEVA
P.patens               CTGGELFEQIRRS-----------------------------KRMSEEDTRFYTAEIV
S.moellendorffii       CHGGELFDQIRRK-----------------------------GRLSLEEARFYAAEIV
C.hlorella             CPNGELYDQIRLQ-----------------------------GRLPEATAAAYAGEVV
C.reinhardtii          CAGGELYEQINKR-----------------------------GRLPLEAVRFYAAEVV
V.carteri              CAGGELYEQIKRR-----------------------------GGLPLDAVRFYAAEVV
                     *  *::   :                                      .  : .*:

E.siliculosus          SAMEHMHRRGLVHRDLKPENMMLTMGGHVKLVDFGTCK----DLLETDI-----NG----
C.merolae              SAVDLIHKNGIVHRDLKPHNILIGNKGHCKLADFGVAAILGKTPDDELAGRSPRPQ----
O.lucimarinus          RAVQQCHARGIVHRDVKPENVLIDSTGHVKLCDFGSAL-DLQPVMTSVLTAIAEQAVKKD
O.tauri                VAVQDCHARGVVHRDVKPENVLIDASGHVKICDFGSAL----DLRHEVTSALTALA----
O.sativa               DILEYLHSLGLIHRDVKPENLLLTSDGHIKIADFGSVK----PTKDTPIKVLPNST----
S.bicolor              DILEYLHGVGLIHRDVKPENLLLTSDGHIKIADFGSVK----PTRDTPIKVLPNST----
Z.mays1                DILEYLHGLGLIHRDVKPENLLLTSDGHIKIADFGSVK----PTRDTPIKVLPNST----
Z.mays2                DILEYLHGLGLIHRDVKPENLLLTSDGHIKIADFGSVK----PTRDTPIKVLPNST----
A.thaliana1            DALEYIHSMGLIHRDIKPENLLLTSDGHIKIADFGSVK----PMQDSQITVLPNAA----
A.thaliana2            DALEYIHNMGLIHRDIKPENLLLTLDGHIKIADFGSVK----PMQDSQITVLPNAA----
P.trichocarpa1         DALEYIHSMGLIHRDIKPENLLLTAEGHIKIADFGSVK----PMQDSCITVLPNAA----
P.trichocarpa2         DALEYIHSMGLIHRDIKPENLLFAADGHIKIADFGSVK----PMQDSCITVLPNAA----
R.communis             DALEYIHGMGLIHRDIKPENLLLTADGHIKVADFGSVK----PMQDSRITVLPNAA----
G.max1                 DALEYIHNLGVIHRDIKPENLLLTAEGHIKIADFGSVK----PMQDSQITVLPNAA----
G.max2                 DALEYIHNLGVIHRDIKPENLLLTAEGHIKIADFGSVK----PMQDSQITVLPNAA----
V.vinifera             DALEYIHSLGLIHRDIKPENLLLTADGHIKIADFGSVK----PMQDSLITVLPNAA----
S.lycopersicum1        DALEYIHSMGLIHRDIKPENLLLTSDGHIKIADFGSVK----PMQDSRITVLPNAA----
S.lycopersicum2        DSLEYIHSMGLIHRDIKPENLLLTSDGRIKIADFGSVK----PMQDSRITVLPNAA----
P.patens               DILEYIHSQGIVHRDLKPENILISAEGNLKLCDFGSAKM-FRPLPNG----FFQSE----
S.moellendorffii       DVLEYIHGQGLIHRDLKPENLLLTADGHIKVADFGSAKV-TTPLQNG----LSDAQ----
C.hlorella             LMLRYLRQQGVVHRDLKPENLLLDGEGHLKLIDFGSAKQ-LAPEEEQAAHADAPPD----
C.reinhardtii          LILEYLRKAQVVHRDLKPENLLLSGDGHLKLIDFGSARAFFLPAAEK-------PP----
V.carteri              LILQYLRSAQVVHRDLKPENLLLSADGHLKLIDFGSARASFLPQAEK-------PP----
                        :   .   ::***:** *:::   *. *: ***
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Supplemental Figure S9 contd.

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E.siliculosus          ---GE-----FVGTAQYMSP-QAVAS-EE-QGREADLWALGCCVYQFLVGFT-PFHAPSP
C.merolae              ---DKHRYDSFVGTFAYLAP-EQLRRERPGGGFESDLWALGVVLYQMLCGGELPFRGETD
O.lucimarinus          AKHKKNRCASFVGTAEYVAP-EILEGCAE-TTTAVDLWSIGVMTFQLLTGRV-PFKDKTE
O.tauri                ---SEKRCASFVGTAEYVAP-EILDGCEE-TTTAVDLWSIGIMTFQLLTGRV-PFKGKTD
O.sativa               ---NE-RACTFVGTAAYVPP-EVLNS-AP-PTFGNDLWALGCTLYQLLSGSS-PFKDASE
S.bicolor              ---TE-RACTFVGTAAYVPP-EVLNS-AP-ATFGNDLWALGCTLYQMLSGSS-PFKDASE
Z.mays1                ---TE-RACTFVGTAAYVPP-EVLNS-AP-ATFGNDLWALGCTLYQMLSGSS-PFKDASE
Z.mays2                ---TE-RACTFVGTAAYVPP-EVLNS-AP-ATFGNDLWALGCTLYQMLSGSS-PFKDASE
A.thaliana1            ---SDDKACTFVGTAAYVPP-EVLNS-SP-ATFGNDLWALGCTLYQMLSGTS-PFKDASE
A.thaliana2            ---SDDKACTFVGTAAYVPP-EVLNS-SP-ATFGNDLWALGCTLYQMLSGTS-PFKDASE
P.trichocarpa1         ---SDDKACTFVGTAAYVPP-EVLNS-SP-ATFGNDLWALGCTLYQMLSGTS-PFKDASE
P.trichocarpa2         ---SDDKACTFVGTAAYVPP-EVLNS-SP-ATFGNDLWALGCTLYQMLSGTS-PFKDASE
R.communis             ---SDDKACTFVGTAAYVPP-EVLNS-SP-ATFGNDLWALGCTLYQMLSGTS-PFKDASE
G.max1                 ---SDDKACTFVGTAAYVPP-EVLNS-SP-ATFGNDLWALGCTLYQMLSGTS-PFKDASE
G.max2                 ---SDDKACTFVGTAAYVPP-EVLNS-SP-ATFGNDLWALGCTLYQMLSGTS-PFKDASE
V.vinifera             ---SDDKACTFVGTAAYVPP-EVLNS-SP-ATFGNDLWALGCTLYQMLSGTS-PFKDASE
S.lycopersicum1        ---SDDKACTFVGTAAYVPP-EVLNS-SP-ATFGNDLWALGCTLYQMLSGTS-PFKDASE
S.lycopersicum2        ---SDDKACTFVGTAAYVPP-EVLNS-SP-ATFGNDLWALGCTLYQMLSGFS-PFKDTSE
P.patens               ---EED-SSAFVGTAEYVSP-EVLHG-KS-ASHSVDLWALGCTIYQMLEGRP-PFKAATE
S.moellendorffii       ---ADDKSCTFVGTAEYVSP-EVLNG-HP-VTIGADLWALGCIIYQMLEGRP-PFKGGSE
C.hlorella             ---AAAKAGGAAAKHAAAQPGSILNN-RA-VTCAADLWALGCVVYQMLAGRP-PFKSPSE
C.reinhardtii          ---GKQRATSFVGTAEYVSP-EVLLN-AP-LSYPADLWALGCMIYQMIVGRP-PFKAASE
V.carteri              ---GKNRATSFVGTAEYVSP-EVLLN-QP-LSYPADLWALGCLLYQMIVGRP-PFKAASE
                                  ...     * . :           ***::*   :*:: *   **.  :

E.siliculosus          YLCFLRIKKGVF-RCPEV-LPDDA-TDLVKRLLRRNPSSRLG--AGPLSGTDPAPSPSGE
C.merolae              YLLFQSILKDDV-AFPKSCLPTSSGRDLVEKLLNKDPAQRIT-----------------
O.lucimarinus          YLTMQAVLKGKYVYPPEANVSSAA-KDFIDKLLVREPKKRLG----F-------------
O.tauri                T-----------RLSAN-ISESA-KDFIDSLLTRDPKKRLG----Y-------------
O.sativa               WLIFQRIIARDL-KIPEY-FSDDA-RDLIDKLLDVDPSKRPG--AGP-------------
S.bicolor              WLIFQRIIARDL-KFPEY-FSAEA-RDLIDKLLDVDPSKRPG--AGP-------------
Z.mays1                WLIFQRIIARDL-KFPEY-FSAEA-RDLVDKLLDVDPGKRPG--AGP-------------
Z.mays2                WLIFQRIIARDL-KFPEY-FSAEA-RDLVDKLLDVDPGKRPG--AGP-------------
A.thaliana1            WLIFQRIIARDI-KFPNH-FSEAA-RDLIDRLLDTEPSRRPG--AGS-------------
A.thaliana2            WLIFQRIIARDI-KFPNH-FSEAA-RDLIDRLLDTDPSRRPG--AGS-------------
P.trichocarpa1         WLIFQRIIARDI-RFPDY-FSGEA-RDLIDHLLDIDPSRRPG--AGR-------------
P.trichocarpa2         WLIFQRIIARDL-RFPDY-FSEEA-RDLIDHLLDIDPSRRPG--AGR-------------
R.communis             WLIFQRIIARDI-RFPNY-FSEEA-RDIIDRLLDIDPSRRPG--AGP-------------
G.max1                 WLIFQRIIAREL-RFPDY-FSDEA-RDLIDRLLDLDPSRRPG--AGP-------------
G.max2                 WLIFQRIIARDL-RFPDY-FSDEA-RDLIDRLLDLDPSRRPG--AAP-------------
V.vinifera             WLIFQRIIARDI-RFPNY-FSDEA-RDLIDRLLDTEPSRRPG--AGR-------------
S.lycopersicum1        WLIFQRIIARDI-RFPNY-FSNEA-RDIIDQLLDVDPSRRPG--AGP-------------
S.lycopersicum2        WLIFQRIIARDI-RFPNY-FSNEA-RDLIDQLLDIDPSRRPG--AGP-------------
P.patens               YLTFQKVMAREL-SIPSH-FSPEA-KDLVDSLLNLKPNERLG--V---------------
S.moellendorffii       YLTFQKVLAKDL-VIPSH-FPSAA-KELINKLLNLEPDKRPG--AGP-------------
C.hlorella             YLTFQKIVEADY-ELPEG-GSEAA-ADLVARLLRVEPAQRIG--A---------------
C.reinhardtii          YLTFQKITDRGL-RGPVV-YPDDA-RDLTDRLLTMEPAARIGEWRSA-------------
V.carteri              YLTFQKITDRDF-CYPEEPATAAA-RDLTDRLLAMEPSARIGE-SCA-------------
                                  .    .  :  ::   **  .*  *

E.siliculosus          PGGAAAAGGGAAEGKAEGRGGGRRDGGGGGFEALKGHPFFRGL-EFVGDGQPLPPSVTVP
C.merolae              ------------------------------MRALKLHPFFKGI-DFRHLHRVDASKLLGP
O.lucimarinus          ----------------------------EDETSIRSHPFFASVSDWSTLRARKAPSVL-T
O.tauri                ----------------------------ENETSIRNHPFFAAVDDWSELRSREAPRVL-T
O.sativa               ----------------------------DGYVSLKKHPFFRGI-DWKNIRSTRAPKLA-M
S.bicolor              ----------------------------DGYSSLKAHPFFRGI-DWKNLRKTRPPKLA-F
Z.mays1                ----------------------------DGYSSLKEHPFFRGI-DWKNLRKTRPPKLA-I
Z.mays2                ----------------------------DGYSSLKEHPFFRGI-DWKNLRKTRPPKLA-I
A.thaliana1            ----------------------------EGYVALKRHPFFNGV-DWKNLRSQTPPKLA-P
A.thaliana2            ----------------------------EGYDSLKRHPFFKGV-DWKNLRSQTPPKLA-P
P.trichocarpa1         ----------------------------GGYAELKNHPFFEGV-DWKNLRGETPPKLV-S
P.trichocarpa2         ----------------------------GGYAVLKNHPFFEGV-DWKNLRGETPPKLV-L
R.communis             ----------------------------EGYAALKIHPFFKGI-NWKNLREETPPKLA-L
G.max1                 ----------------------------DGYAILKSHPFFKGV-DWDNLRAQIPPKLA-P
G.max2                 ----------------------------DGYAILKRHPFFKGV-DWDNLRAQIPPKLA-P
V.vinifera             ----------------------------DGYASLKMHPFFNGV-DWKNLRSQTPPKLA-M
S.lycopersicum1        ----------------------------DGYASLKNHPFFSGI-DWENLRLQTPPRLA-M
S.lycopersicum2        ----------------------------DGYASLKNHPFFSGV-DWDNLRLQTPPRLA-A
P.patens               ----------------------------QGYDDIKNHPFFKGF-DWSRLRKMATPKLL-K
S.moellendorffii       ----------------------------AGYTALKSHAFFSGI-EWLKLRQSAAPGLA-P
C.hlorella             ----------------------------ADLAELQAHPFFAGI-DWDTLRSQPAPEFM-P
C.reinhardtii          ----------------------------EDMAELRAHPFFAGV-DWAALRAGPAPPYL-P
V.carteri              ----------------------------EDISELKAHPFFAVI-DWETLRSGPAPSFL-P
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Supplemental Figure S9 contd.

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E.siliculosus       KLYELCVRALAHHMVDYAEFTPLIREPFPDHVDIKRLGAGGEGVPCGTSAGGSTPGGGPA
C.merolae           DAFEKRDFLSFDAASSAGHAAEGFRRVTRAINSWWPRGT--------HAHRRATREPQAL
O.lucimarinus       ATGVGSDATVSESESESD-------------TDDD------------------------
O.tauri             ATGVGSDATVTDSECDHD--------------------------------DG--------
O.sativa            EANA------NEDEDSQD-------------SSW-LSHMGSAPVNQHVSPVGNDGASSSS
S.bicolor           DANA------NEDEDGQD-------------SNW-LAHMGTVAVNQQSNTVGNNGAPSSS
Z.mays1             DANA------NEDEDSQD-------------SNW-LAHMGSVAVNQQSNTVGNNGAPSSS
Z.mays2             DANA------NEDEDSQD-------------SNW-LAHMGSVAVNQQSNTVGNNGAPSSS
A.thaliana1         DPASQT--ASPERDDTHG-------------SPWNLTHIGDSLA---TQNEGHSAPPTSS
A.thaliana2         DPASQS--ASPERD---G-------------SPWNPTHVGDTSV---LQNDGHNGL---S
P.trichocarpa1      EPMVQS----GDSDHDSG-------------SPYNPTRAGDSSL---TQNDGNAGVSSSA
P.trichocarpa2      EPMAQS----GDNDHDPG-------------SPFNPTHIGDSSM---TQNDANVGVSSSA
R.communis          EAMTQS----GDGELDPD-------------ATWNPTHIGDGSA---RQNDGTGGPSSTA
G.max1              EPGTQS----PASDDVHD-------------SSWSPSHIGDGSAASVRQPDGAT----SS
G.max2              EPGTQS----PVADDVHD-------------SSWSPSHIGDGSAASVRQPDGAT----SS
V.vinifera          EAMAHS----SEGDDGQD-------------SWNPAHIGDGSA---RQNDGNSGATSSS
S.lycopersicum1     EPKAPS---THSSGDEQD-------------PSWNPSHIGDGSV---RPNDGNGAAASVS
S.lycopersicum2     EPKGHS---TRTSGEDHD-------------PSWNPSHIGDGSA---RPNDGNGGTPSSS
P.patens            DPNTES----LDEE-----------------EKWQAGII--------------------
S.moellendorffii    SPSSQGGGNGAESDDSEN-------------SDWDLAHLGGRVS-RLDVSDASSPMSSSN
C.hlorella          DI---------DPGEQLV-------------GSWRAAGIPA------------------
C.reinhardtii       PRVPGA--PGSDGGYEEG-------------LDWELTSL--------------------
V.carteri           PTPPGG--AG-DG--EEG-------------WDWELTSL--------------------

E.siliculosus       AAAANAAALKRR-----RGFVERTRHYLRRLRLLQRPKVHRLFHPSAVDAKSLRADPIMR
C.merolae           QDAGAKELFAAH---------EKWRQPARRVLNLASDFTGNPILASAIELVESLLQRIRR
O.lucimarinus       --------------------EEWRA---------------------------------
O.tauri             ----------------TDTDDEWRARV--------NAAA--------------------
O.sativa            EVRSHISRLASI-----DSFDSRWQDFL--------EPGESVVLISKLKKINKLTNKKVQ
S.bicolor           EVRSHISKLSSI-----DSFDSKWQEFL--------DPGESVVLISKLKKINKLANKKVQ
Z.mays1             EVRSHISKLASI-----DSFDSKWQEFL--------DPGESVVLISKLKKINKLANKKIQ
Z.mays2             EVRSHISKLASI-----DSFDSKWQEFL--------DPGESVVLISKLKKINKLANKKIQ
A.thaliana1         ESSGSITRLASI-----DSFDSRWQQFL--------EPGESVLMISAVKKLQKITSKKVQ
A.thaliana2         ESSGSITRLASI-----DSFDSRWQQFL--------EPGESVLMISAVKKLQKITSKKVQ
P.trichocarpa1      EATAHITRLASI-----DSFDSKWQQFL--------DPGESVLMIAMVKKLQKLTSKKVQ
P.trichocarpa2      EATSHIARLASI-----DSFDSKWQQFL--------DPGESVVMISMVKKLQKLTSKKVQ
R.communis          EASGSITRLASI-----DSFDSKWQQFL--------DPGESVLMISMVKKLQKLTSKKVQ
G.max1              EGTGHITRLASI-----DSFDSKWQQFL--------EPGESVLMISMVKKLQKLTSKKVQ
G.max2              EGTGHITRLASI-----DSFDSKWQQFL--------EPGESVLMISMVKKLQKLTSKKVQ
V.vinifera          EAPGSVTRLASI-----DSFDSKWQQFL--------EQGESVLMISMVKKIQKLTNKKVQ
S.lycopersicum1     EAGNSITRLASI-----DSFDSKWKQFL--------DPGESVLMISMVKKLQKLTSKKVQ
S.lycopersicum2     EA-NSVTRLASI-----DSFDSKWKQFL--------EPGESVLMISNVKKIQKLTSKKVQ
P.patens            ----------------DGLDAFVYDV---------------------------------
S.moellendorffii    EPSSPSAVLASAARLLDENVHEPWQKFL--------FEGETILASSRVRKFRKLSVKKRQ
C.hlorella          --------------------TYW-------------------------------------
C.reinhardtii       --------------------------VR--------DAAES------------------
V.carteri           --------------------------VR--------DAADGL-----------------

E.siliculosus       EYLGLDWESQGHWDEPFFFIQIADPQLGMVKADGPV-----------------------
C.merolae           ECMQHRKLPSEEYLFAASQLQSADPRTLTDDHAKIRFWVVLYNVMFIHVRMVHGAPVRSG
O.lucimarinus       ------------------------------------------------------------
O.tauri             ------------------------------------------------------------
O.sativa            ------------------LILTDKPQLICVDPGKMVTK----------------------
S.bicolor           ------------------LILTDKPQLICVDPSKMVAK----------------------
Z.mays1             ------------------LILTDKPQLICVDPSKMVAK----------------------
Z.mays2             ------------------LILTDKPQLICVDPSKMVAK----------------------
A.thaliana1         ------------------LILTNKPKLIYVDPSKLVVK----------------------
A.thaliana2         ------------------LILTNKPRLIYVDPSKLVVK----------------------
P.trichocarpa1      ------------------LILTNKPKLIYVDPSKLVVK----------------------
P.trichocarpa2      ------------------LILTNKPKLIYVDPSKLIVK----------------------
R.communis          ------------------LILTNKPKLIYVDPSKLLVK----------------------
G.max1              ------------------LILTNKPKLIYVDPSKLIVK----------------------
G.max2              ------------------LILTNKPKLIYVDPSKLIVK----------------------
V.vinifera          ------------------LILTNKPKLIYVDPAKLMVK----------------------
S.lycopersicum1     ------------------LILTNKPKLIYVDPSKLVIK----------------------
S.lycopersicum2     ------------------LILTNKPKLIYVDPSKLVVK----------------------
P.patens            ------------------------------------------------------------
S.moellendorffii    ------------------LILTDRPRLFYVHPIKLVFK----------------------
C.hlorella          ------------------------------------------------------------
C.reinhardtii       ------------------------------------------------------------
V.carteri           ------------------------------------------------------------
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Supplemental Figure S9 contd.

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E.siliculosus        -------GGAVWEIEAERLSRVVGIVNRLR---PKFLLVTGDMTHAPPGN-----EYYEG
C.merolae            LRDNRFFGEYFYRVFTLDYCLDDIENGILRVPGPFFRSWERDDPRRELALKHLHPKIVDM
O.lucimarinus        ---------------------------------------------RVNAAT----AALDA
O.tauri              -----------------------------------------------------AALDA
O.sativa             -------GNIMWSDDPSELNVQVSNSSHFRICTPKKVSSFEDAKQRAWQWK----KAIED
S.bicolor            -------GNIIWSDDPSELSVQVSDSSHFRICTPKKVSTFEDAKQRAWQWK----KAIED
Z.mays1              -------GNIIWSDDPSELSVQVSDSSHFRICTPKKVSTFEDAKQRAWQWK----KAIED
Z.mays2              -------GNIIWSDDPSELSVQVSDSSHFRICTPKKVSTFEDAKQRAWQWK----KAIED
A.thaliana1          -------GNIIWSDNSNDLNVVVTSPSHFKICTPKKVLSFEDAKQRASVWK----KAIET
A.thaliana2          -------GNIIWSDNSNDLNVQVSSPSHFKICTPKKVLSFEDAKQRALQWK----KAIET
P.trichocarpa1       -------GNIIWSDNSDDLSVQVTSPSHFKICTPKKVRSFEDAKQRAWQWK----KAIES
P.trichocarpa2       -------GNIIWSDNSDDLSVQVTSPSHFKICTPKKVRSFEDVKQRAWQWK----KAIES
R.communis           -------GNIIWSDNSNDLSVQVSSPSHFKIFTPKKVMSFEDAKQRAWQWK----KAIES
G.max1               -------GNIIWSDNPNDLSIQVASPSNFKICTPKKVMSFEDAKQRAWQWK----KAIEG
G.max2               -------GNIIWSDNPNDLSIQVASPSNFKICTPKKVMSFEDAKQRACQWK----KAIEG
V.vinifera           -------GNIIWSDNPNDLSIQVTSPSHFKICTPKKVMSFEDSKQRAWQWK----KAIEG
S.lycopersicum1      -------GNIIWSDNPNDLSIQVTSPSQFKICTPKKVMSFEDAKNRAQQWK----KAIEA
S.lycopersicum2      -------GNIIWSDNSNDLNIQVISPSQFK---PKKVMSFEDAKQRAMQWK----KAIET
P.patens             ------------------------------------------------------------
S.moellendorffii     -------GEVPWS---RDIYVRVENDLKFCICTPKRTYNLEDTKGQARVWK----ESIEK
C.hlorella           ------------------------------------------------------------
C.reinhardtii        ------------------------------------------------------------
V.carteri            ------------------------------------------------------------
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E.siliculosus         QPRII-------------------------------------------
Cmerolae             LHRIRVSRGMADIPELDHLTETNLLGQAAFEDDGLIETDTERTTLTL
Olucimarinus         L-----------------------------------------------
Otauri               L-----------------------------------------------
Osativa              LQRCQKN-----------------------------------------
Sbicolor             LQRGQRN-----------------------------------------
Zmays1               LQRCQRN-----------------------------------------
Zmays2               LQRCQRN-----------------------------------------
Athaliana1           LQNR--------------------------------------------
Athaliana2           LQNR--------------------------------------------
Ptrichocarpa1        LQNQ--------------------------------------------
Ptrichocarpa2        LQNQ--------------------------------------------
Rcommunis            LQNQ--------------------------------------------
Gmax1                LQNR--------------------------------------------
Gmax2                LQNR--------------------------------------------
Vvinifera            LQNR--------------------------------------------
Slycopersicum1       LQNR--------------------------------------------
Slycopersicum2       LQNR--------------------------------------------
Ppatens              ------------------------------------------------
Smoellendorffii      LVNAK-------------------------------------------
Chlorella            ------------------------------------------------
Creinhardtii         ------------------------------------------------
Vcarteri             ------------------------------------------------
```

Figure S9. Alignment of PDK1 proteins used for phylogenetic analysis. MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/) was used to align protein sequences. Kinase domains are highlighted in yellow, ATP coordinating lysine is highlighted in green, and the PIF-binding pocket residues mutated in *Pp*PDK1 are highlighted in blue.