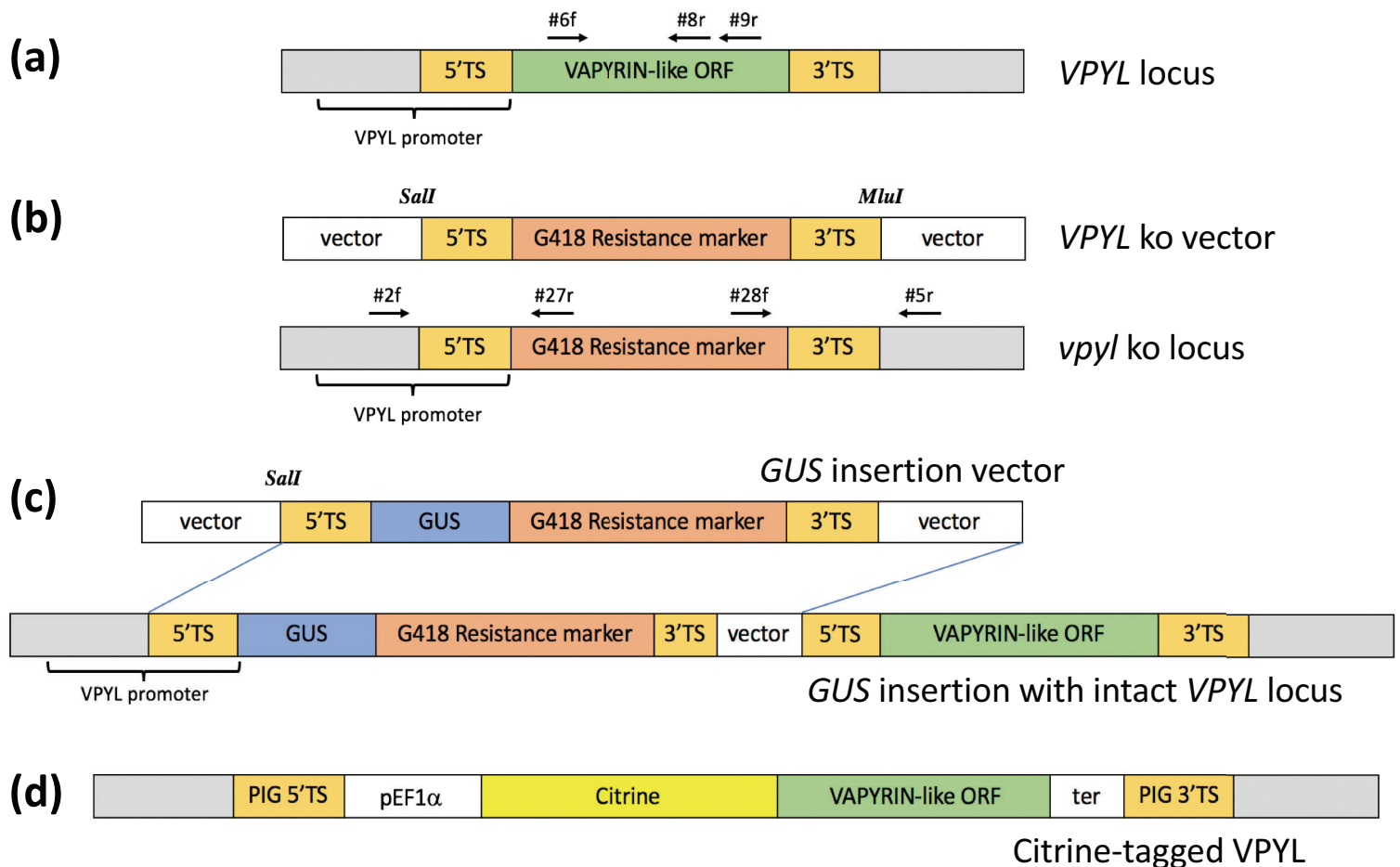


**Figure S1. Full-size phylogenetic tree of the VAPYRIN gene family in land plants (compare with Fig. 1).** Maximum likelihood tree (model JTT+R8) of VAPYRIN and VAPYRIN-like. The tree was rooted using closely related sequences (outgroup). Branches of the VAPYRIN and VAPYRIN-like clades are colored in red and black, respectively. The ability of each species to form arbuscular mycorrhiza is indicated by the color of the label: blue for the mycorrhizal species and black for non-mycorrhizal ones. Colors in the inner circle indicate the following clades: purple, bryophytes; cyan, lycophytes; green, monilophytes; blue, gymnosperms; yellow, basal angiosperms; red, monocots; orange, eudicots. The position of the *P. patens* VAPYRIN-like sequence is indicated by a purple triangle.





**Figure S3. Vector cloning strategy and genetic constitution of the *PpVPYL* locus in various transgenic lines**

(a) Natural *PpVPYL* locus with primers indicated for the *PpVPYL* open reading frame (ORF). 5'TS and 3'TS represent the targeting sequences upstream of the ATG start codon, and downstream of the stop codon, respectively.

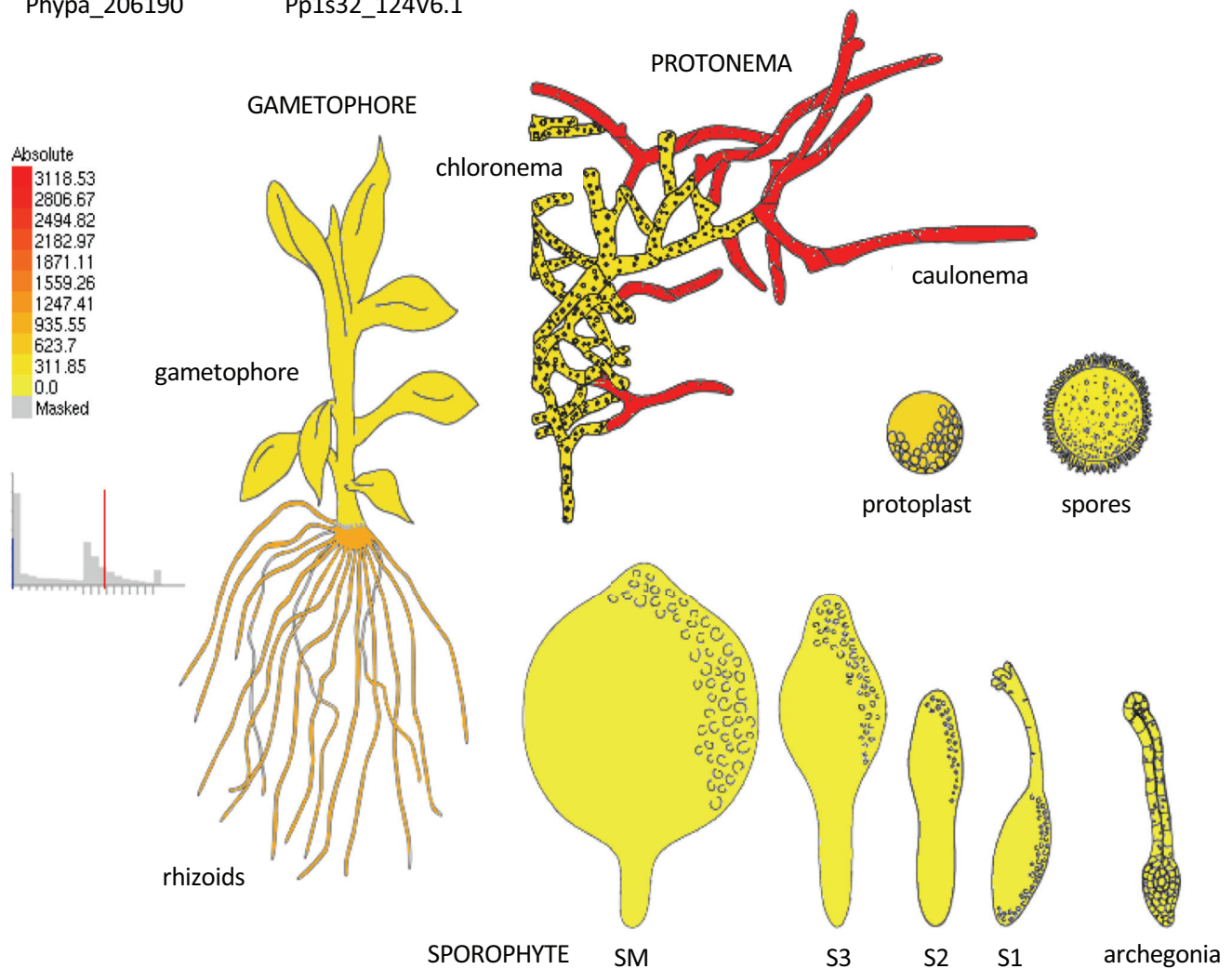
(b) Gene replacement strategy by homologous recombination. Primers indicated span the two targeting sequences for the detection of successful homologous recombination events. Restriction sites for linearization are indicated (*SalI*, *MluI*).

(c) Strategy for the generation of a *pPpVPYL::GUS* reporter line. In a first step, the *UidA* coding sequence was inserted between the 5'TS and the resistance marker in the vector used for gene replacement (see in (b)). In a second step, this construct was inserted at the *PpVPYL* by recombination at the 5'TS, resulting in an insertion without gene replacement. (d) The *PIG1* locus after insertion of the Citrine-VAPYRIN construct. The *VPYL* coding sequence was inserted in frame downstream of the *Citrine* ORF. The fusion gene is under the control of the constitutive promoter of the elongation factor1 alpha (pEF1 $\alpha$ ).

Grey boxes represent upstream and downstream genomic sequences of the *PpVPYL* locus (a)-(c), or the *PIG1* locus (d).

Phypa\_206190

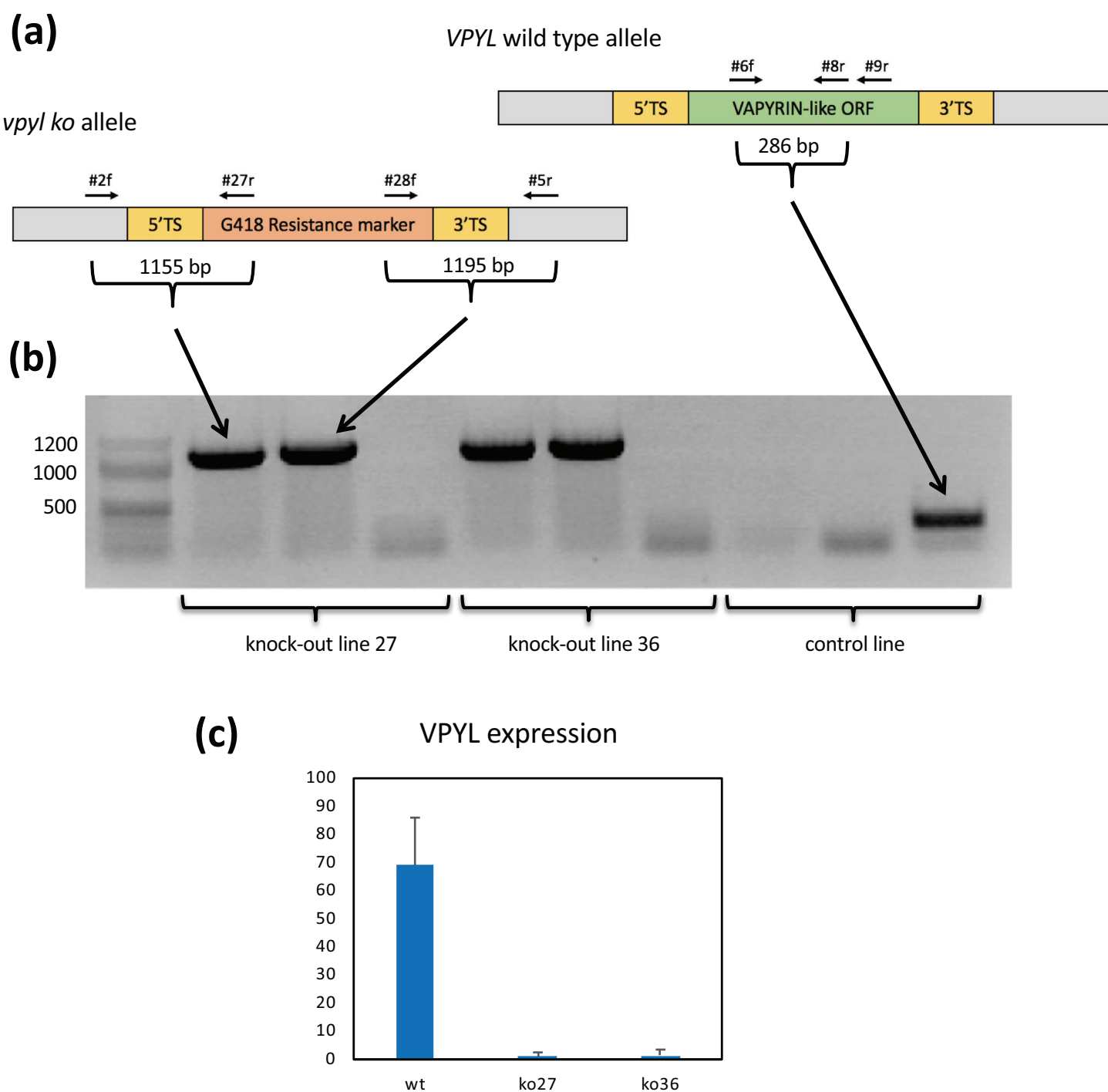
Pp1s32\_124V6.1



Physcomitrella eFP Browser at [bar.utoronto.ca](http://bar.utoronto.ca) (Ortiz-Ramirez et al., Molecular Plant, 2016)  
 The different tissue types were isolated from wild-type *Physcomitrella patens* (Gransden) grown in controlled conditions at 25°C with 16h light and 50% humidity. Induction of gametangia and sporophyte development was conducted in short day conditions at 17°C and 50% humidity. Tissues were sampled in triplicate and processed for hybridization on NimbleGen v1.6 *P. patens* 135k arrays (32741 probe sets). Expression data were normalized by RMA. Drawings by Marcela H. Coronado. Image under CC BY 4.0 license  
**Waese, J., Fan, J., Pasha, A., Yu, H., Fucile, G., Shi, R., Cumming, M., Kelley, L. A., Sternberg, M. J., Krishnakumar, V., et al. (2017). ePlant: visualizing and exploring multiple levels of data for hypothesis generation in plant biology. Plant Cell 29, 1806-1821. doi: 10.1105/tpc.17.00073.**

#### Figure S4. VPYL expression pattern in *P. patens* according to the eFP browser

The eFP browser ([http://bar.utoronto.ca/efp\\_physcomitrella/cgi-bin/efpWeb.cgi](http://bar.utoronto.ca/efp_physcomitrella/cgi-bin/efpWeb.cgi)) was searched for VPYL (Gene ID: Pp1s32\_124V6.1).



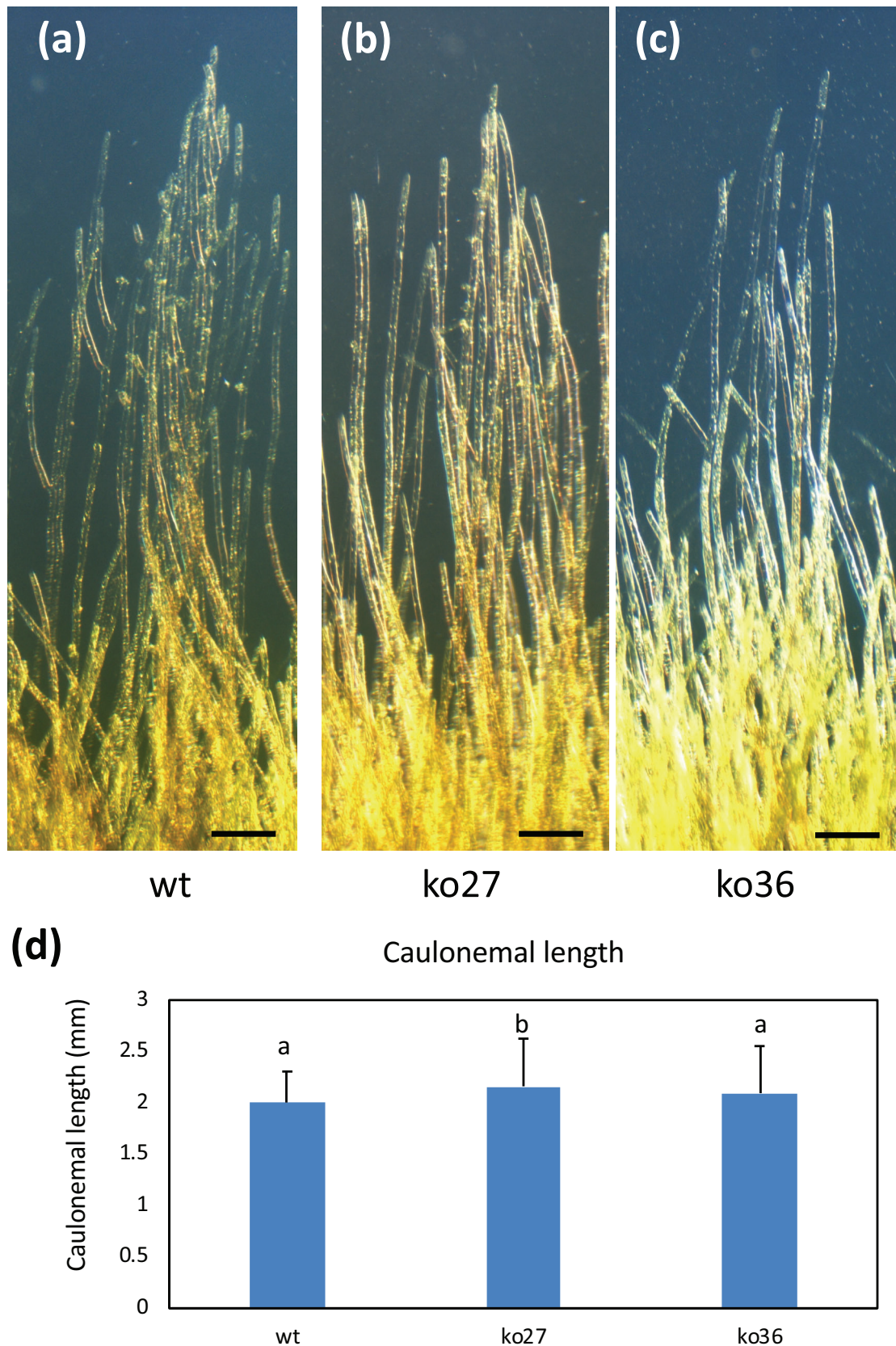
**Figure S5. Molecular analysis of transformants from gene replacement**

**(a)** Genomic DNA of antibiotic-resistant regenerants from protoplast transformation, and of a wild type strain, were subjected to PCR with *VPYL* ORF primers #6f and #8r, and with the primers #2f and #27r, or with #28f and #5r, to detect the 5' and 3' recombination sites, respectively (see Fig. S3).

**(b)** Agarose gel electrophoresis reveals the respective amplicons in wild type, *ko27*, and *ko36*. *Ko27* and *ko36* had lost the *VPYL* ORF. PCR with primers flanking the 5'TS and 3'TS confirmed that *ko27* and *ko36* had undergone gene replacement and lack the *VPYL* coding sequence.

**(c)** Quantitative real-time RT-PCR (qPCR) analysis with primers for the *VPYL* ORF (#51f and #52r) and of Tubulin A as reference gene (Primers: TUA1f and TUA1r) reveals transcript in wild type, but not in *ko27* and *ko36*.

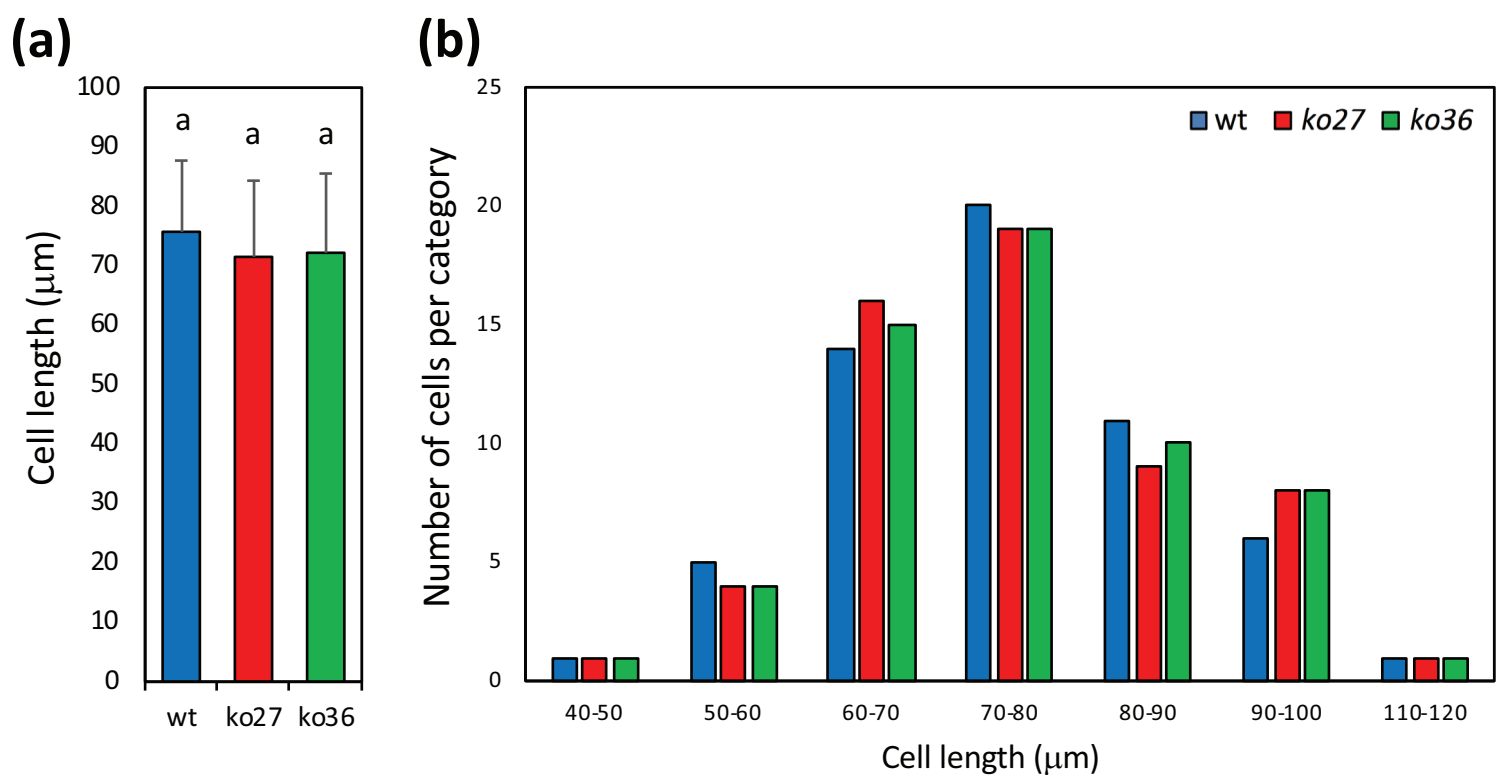




**Figure S6. Caulonema growth phenotype in wild type and *Ppvp1* mutants**

**(a-c)** Caulonema growth after transfer of *P. patens* cultures to unilateral light in the wild type (a), *ko27* (b), and *ko36* (c).

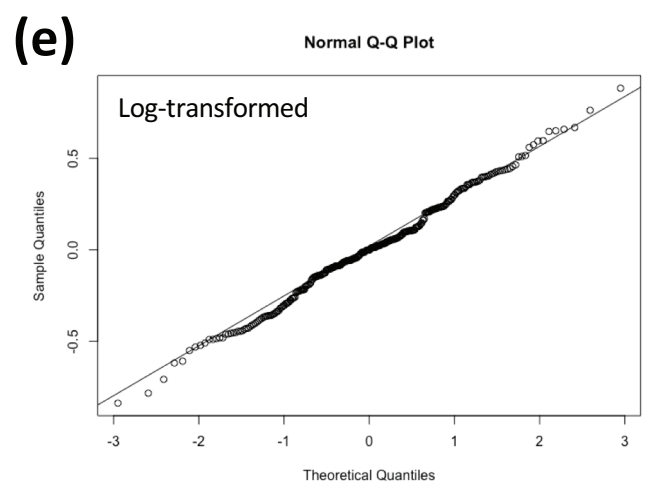
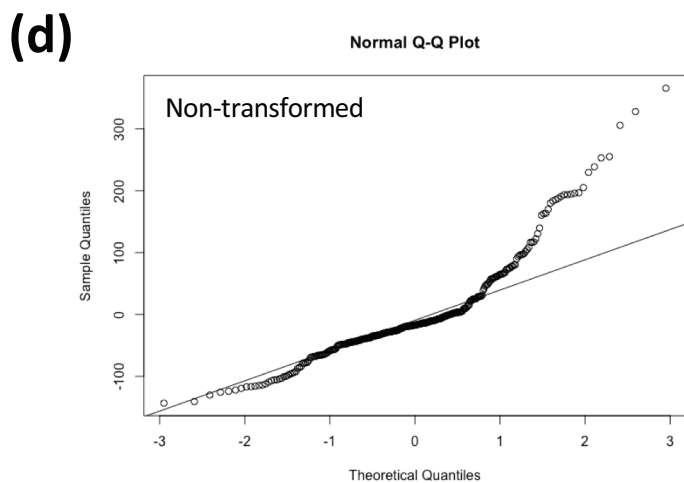
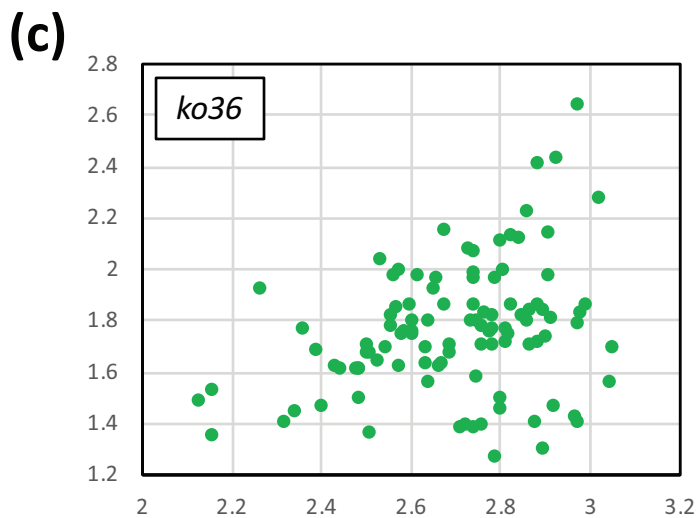
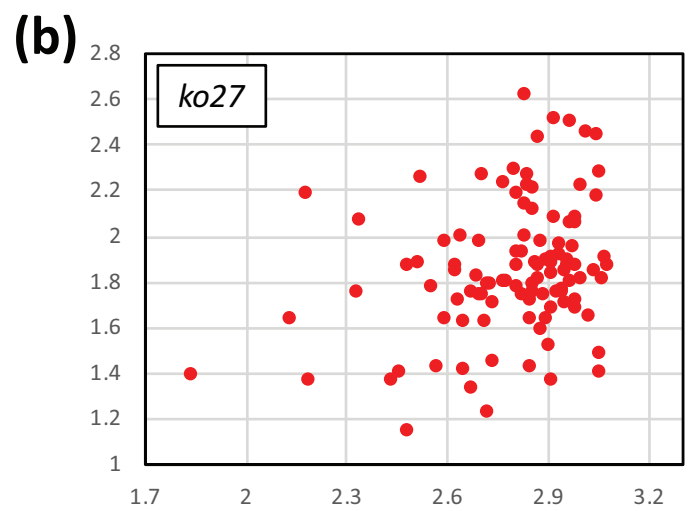
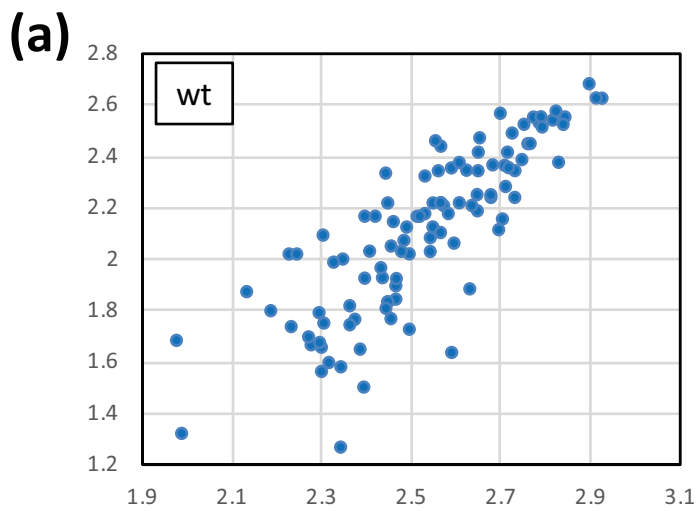
**(d)** Measurement of caulonema growth length after treatment for 6 days with dim unilateral light. Columns represent the average of 4 cultures for each genotype, of which three pictures were taken each, and 5 caulonemata each were measured (n=60 per genotype). Bars represent the mean + s.d., significant changes are indicated by letters (T-test). Size bars, 250  $\mu$ m.



**Figure S7. Cell size phenotype of *Ppvpvl* ko mutants**

(a) Average cell length of protonemal cells of wild type (wt), and the *vpvl* mutants *ko27* and *ko36*. Protonema was stained with Calcofluor White as described (Kofuji et al., 2018), and analyzed by confocal microscopy. Bars represent the mean (n=100) + s.d.. Significance of differences was tested by two-way ANOVA.

(b) Cell length distribution of 58 protonemal cells of wild type (wt), and the *vpvl* mutants *ko27* and *ko36*. Only cells 2-5 from the tip in protonemal cell files were considered.

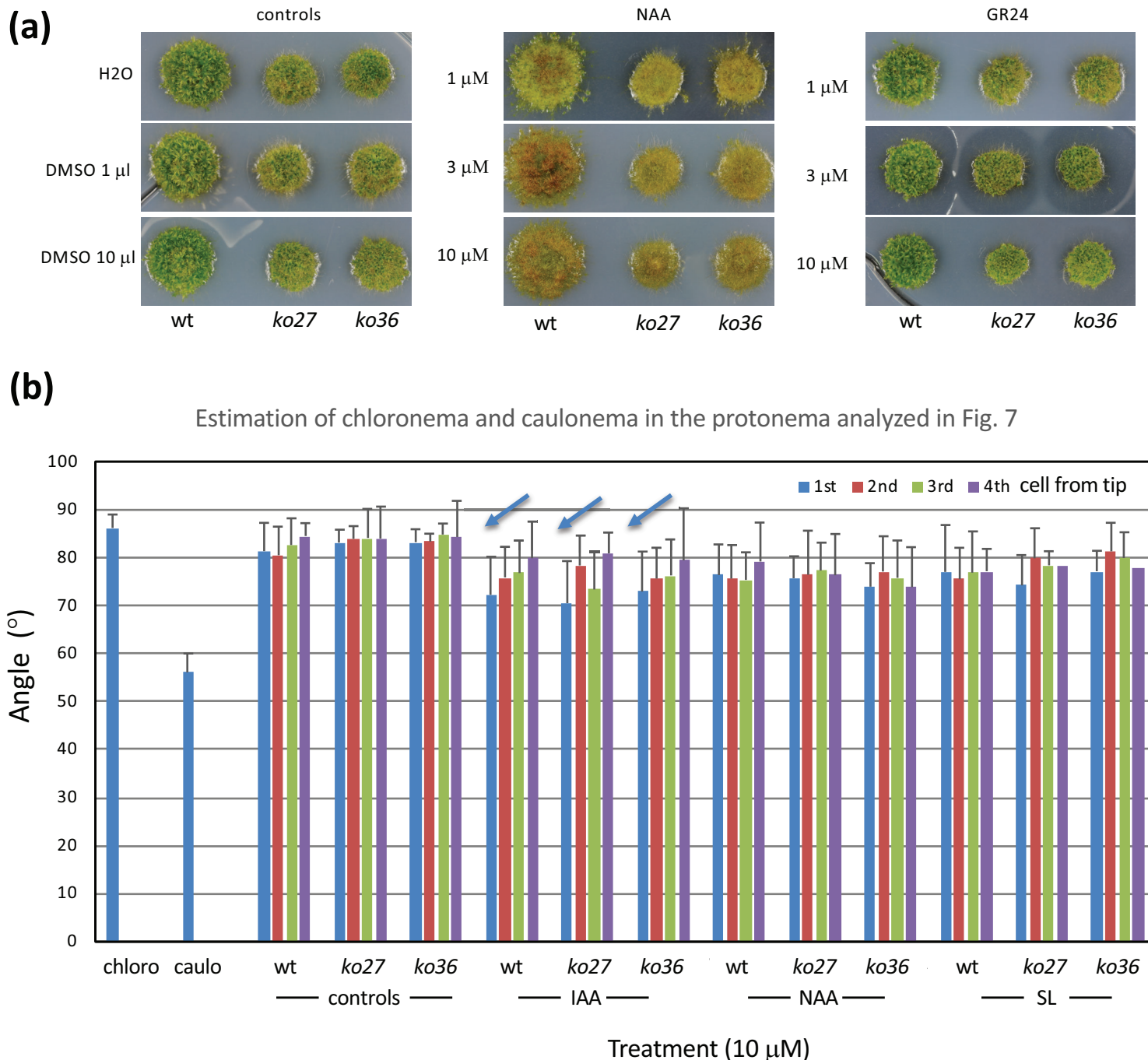


**Figure S8. Log10-normal characteristics of branch length distribution**

(a-c) Data shown in Fig. 5 was log10 transformed;

(d-e) Normal Q-Q plotting of the residues revealed that the log10 transformed data was normally distributed (e), compared to the non-transformed data (d). See Fig. 5 for additional information.

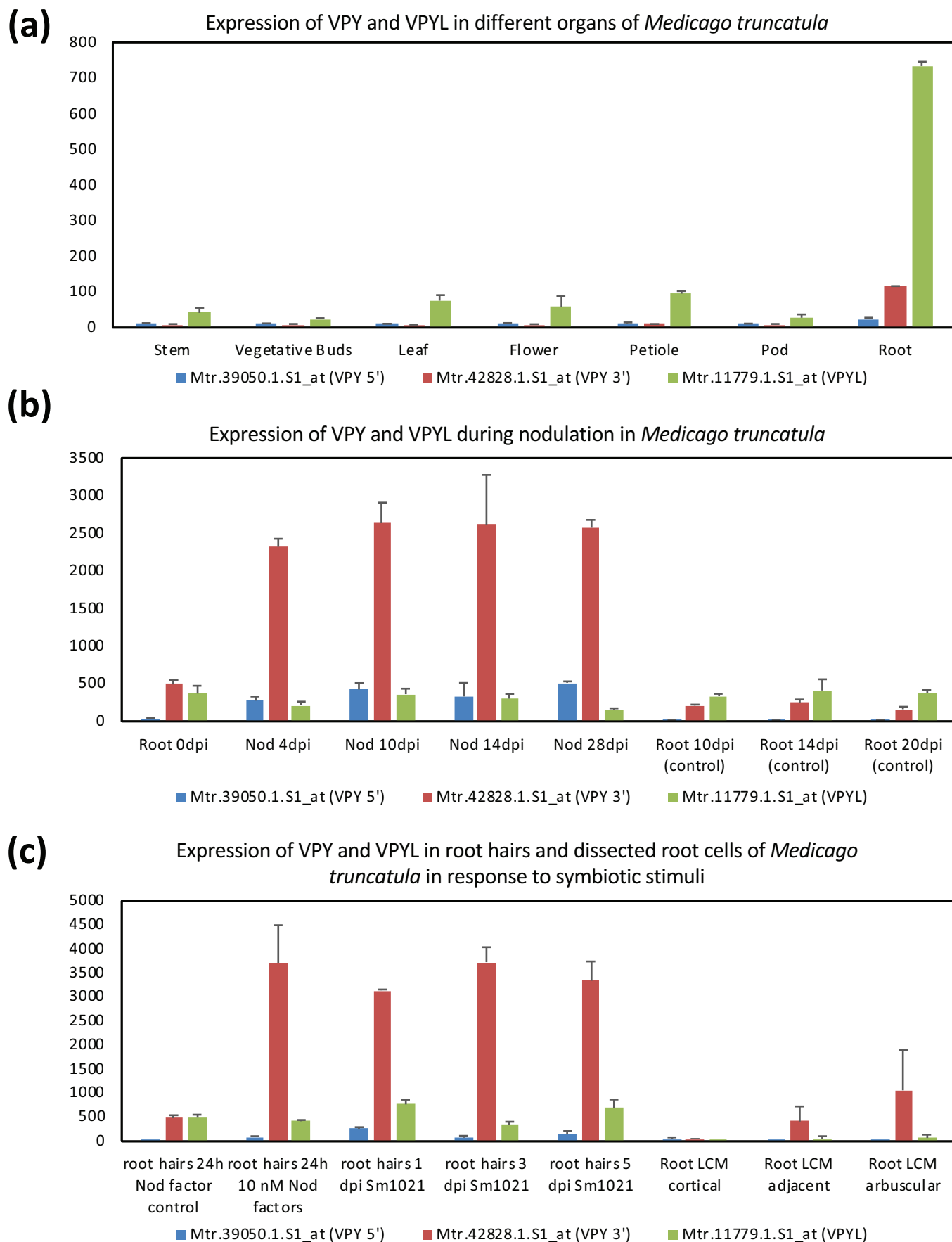




**Figure S9. Growth phenotype and protonemal identity after hormonal treatments of wild type and *vpyI* mutants**

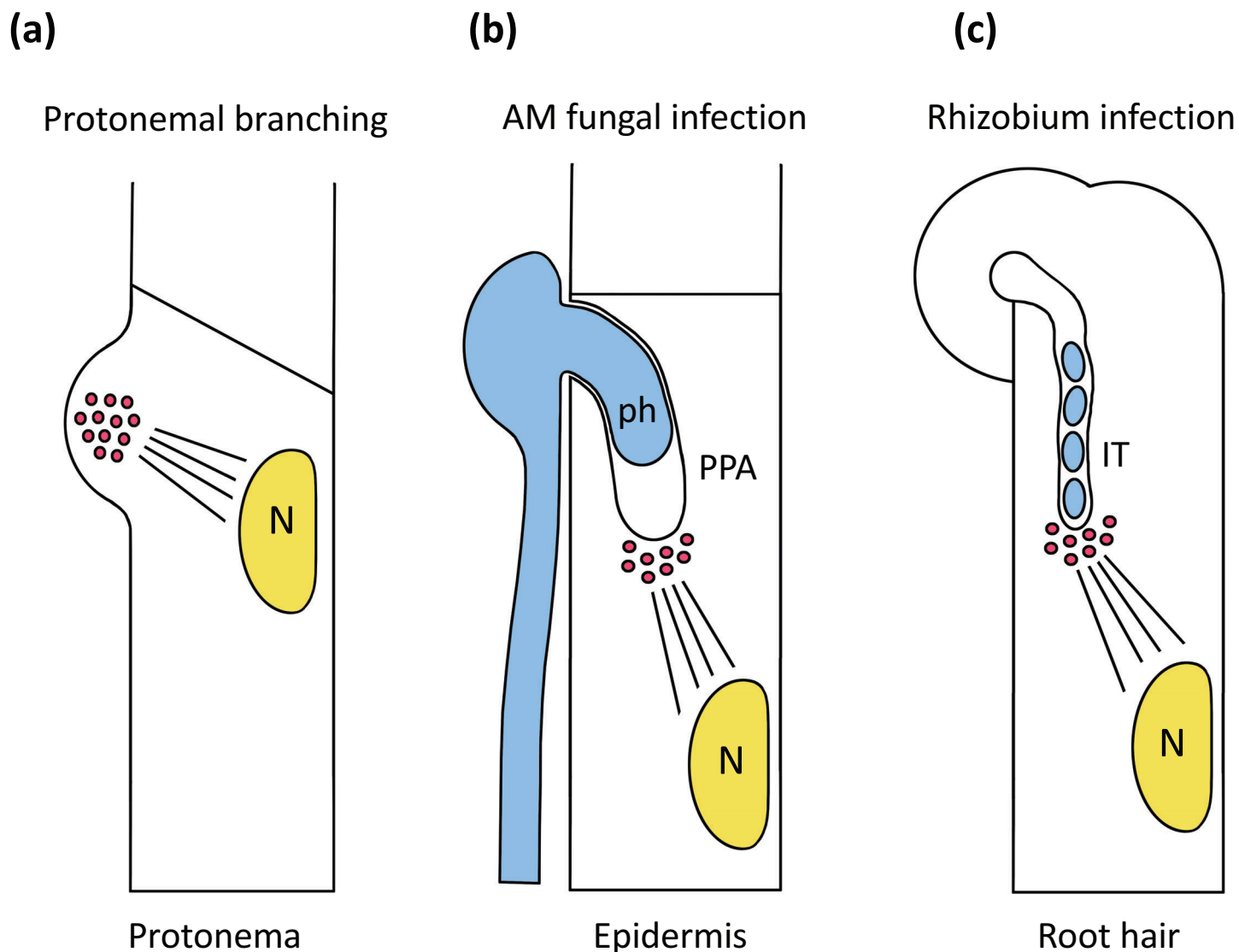
(a) *P. patens* wild type, *ko27*, and *ko36* were grown as in Fig. 3a, with the indicated additions of hormones for one week. The only macroscopic effect was the color change (reduction in chlorophyll) observed after NAA treatments.

(b) Protonemal identity determined by the angle of the cross walls in protomal cell files for cell 1 (1<sup>st</sup>) to cell 4 (4<sup>th</sup>). Pure chloronemal cultures (chloro) and caulonemal cultures (caulo) were used to calibrate the method. Control protonemata exhibited largely chloronemal identity (angles >80°). Hormonal treatments caused a tendency towards caulonemal identity, in particular of the youngest cells in the files (1<sup>st</sup>). The effect is most prominent in the case of IAA treatments, which caused gradually smaller angles towards the protonemal tip (arrows) indicative of the developmental transition towards caulonemal identity in the youngest cells. Note that the patterns were indistinguishable between the genotypes. Bars represent the mean (n=22) + s.d.



**Figure S10. Expression pattern of VPY and VPYL in *M. truncatula***

Publicly available data sets from microarray experiments accessible at the *M. truncatula* Gene Expression Atlas (MtGEA) were extracted for different plant organs (a), roots during nodulation at different stages (b), and from root hairs or LCM micro-dissected cortex cells exposed to nod factor, rhizobia, or AM fungi, respectively, as indicated (c). Note that VPY is represented by a 5' probe (blue), and a 3' probe (red), with the latter yielding consistently stronger signals. VPYL is represented by only one probe (green). Bars represent the mean (n=3) + s.d. For more information see: <https://mtgea.noble.org>.



**Figure S11. Parallels in protonemal branching with intracellular accommodation of microbes in plant symbioses.**

(a) Branches in a moss protonema are initiated as bulges at the apical end of cells in a protonemal file, opposite to the nucleus.

(b) Infection of root epidermal cells by AM fungi depends on repositioning of the nucleus and formation of an infection structure, the pre-penetration apparatus (PPA) that guides the penetration hypha (ph).

(c) Infection of root hairs by rhizobia involves the formation of an infection thread (IT), through which the bacteria pass through the cell towards the base.

All three phenomena require repositioning of the nucleus to the site of action, transport of large amounts of organelles, vesicles and other cytoplasmic constituents (red circles) to establish a new tip-growing center (a), a PPA (b), and an IT (c), respectively.

Table S1: List of species used for the different phylogenetic analyses (See protein sequences in Supplementary Material & Methods, and phylogenetic trees in Fig. 1, Fig. S1 and Fig. S2).

All sequences are available at: <http://www.polebio.lrsv.ups-tlse.fr/symdb/web/>

\* 1=AM-competent species; 0=non-AM species; y=sequence found in the genome; n=sequence not found in the genome; nd=VPYL sequence was not searched for (Fig. S2 with only VPY analyzed)

[Click here to Download Table S1](#)

## Supplementary Material and Methods

### Primers for cloning and molecular analysis of *P. patens* transformants

Red : Restriction sites for XbaI (TCTAGA), XhoI (CTCGAG), SpeI (ACTAGT), MluI (ACGCGT)

Primer	Sequence
#2f	AGGGCGAATGAAATCAAGT
#5r	AGCGAGGAGGACGAGAAGAC
#6f	GGGTGCTACGGATTCTTC
#8r	CAAGGAGCACTACCAGCACA
#9r	TGACCACTCTCCACTGCAAGCAGCTC
#27r	GTGTCGTGCTCCACCATG
#28f	CGCTGAAATCACCAGTCTCTCT
#19f	ACATGCTCGAGATGTTACGTCCTGTAGAAACCCCAACC
#20r	TGTACTIONGAGAGTAACATAGATGACACCGCGCGC
#Fk6	GACAGGCTTGTATGCTTAGATGTG
#RevO1	CTAGATGAGGATTC CTTACCCTTGAGGG
KO-5'F	ATGTCTAGAGTGTGATAGAGGTGAAAGCCA
KO-5'R	CATCTCGAGGTGCAAGGGATTCCTTTCTA
KO-3'F	ATGACTAGTGGAGCGCTTCGGATGGGGCGTTTCATG
KO-3'R	ATGACGCGTCAGACTTCTTCAGCATCACCC
#51	CAAGCGATCGGGAGGGATAC
#52	GGGATTCAACCGCAATGTCG
TUA1f	AAGGCGTACCACGAGCAG
TUA1r	GTCCTTCGGCACCATC



## PpVAPYRIN-like genomic locus (Accession number MN971578)

Coding region shaded in grey

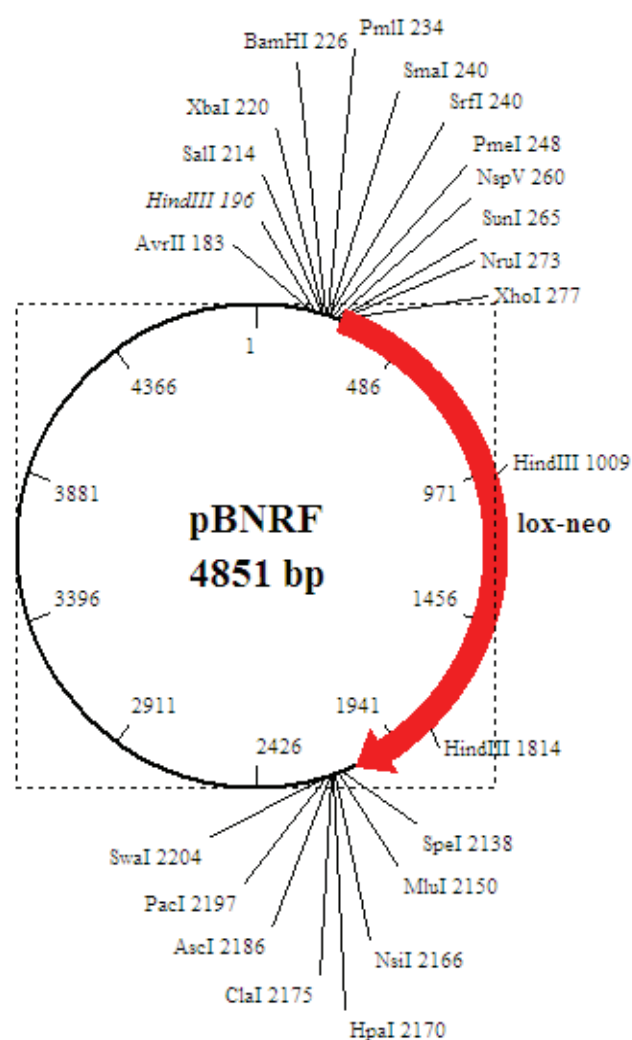
5' and 3' targeting sequences shaded in blue

Start and stop codons shaded in red

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## ***Physcomitrella patens* transformation vector pBNRF**



Vector map

## ***Physcomitrella patens* transformation vector pBNRF** **(complete sequence)**

2 LoxP sites

Selectable resistance cassette 35S nptII CaMV ter EcoRI

>pBNRF

```
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AAAACCTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATA
GTGACTCTTGTTCCAACTGGAACAACACTCAACCTATCTCGGTCTATTCTTTTGATTATAAGGGATTTTGCCGATTTTCGGCTATTGG
TTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTT
```

### PpVAPYRIN-like knockout vector (PpVPYL-KO)

5' and 3' targeting sequences shaded in blue

2 LoxP sites

Selectable resistance cassette 35S nptII CaMV ter **EcoRI**

## Cloning sites for targeting sequences

>PpVAPYRIN\_ko

[illegible]

## Protein sequences used for phylogenetic analysis (See Fig. 1, Fig. S1, Fig. S2)

(see also Supplementary Table S1 that lists all species used for searching VPY and VPYL homologues)

>Ausspi\_2010622\_BTTS\_Conifers.p1

MDRLVAVDPLEMRIDFKRGQKAHARFRLQNLMTMSVAFKVLATEPKKYGVPRGMAIIPPLGKIWVEVTMAAQAEPLSLITVSEDKLFVKSLMLPTGKATDSELNRLFSSNHHIFNDATLKVIFTGSLILKNLVANGSPREVKEALLKYSVDNGKYEQDLTLHIAVFRGNSEIVQTLSDSGALVDVNKSSQKSPLEAAFYGHVEIVKNLLSAGANTETADARGYTALHFAASGNCVQILISLLD SGAQINARDIEGKTPLHVSVIAGHLDVSVQLLAGAGANVDADKDKVGTCTAIHCAASCGYLDIIFLLESGNKYIRNNDGKNALEALDNGHQHLLDVLHLGDMQLQKTSKE\*

>Ausspi\_2078762\_BTTS\_Conifers.p1

MDRLLAVEPKEVAIRFEQGRCSGSVELRNVMYTMPVAFRVHPMKHKKYSVKPSCGIISPLRATSVEIILQPQGELPEVFPHSDDRFVIQSVVVPGGKETETVSLDWFRTARKKQVFEDSGLKVMFTGGAILSKLVADGSGVEIREVLEKDEESVNSCDEQGRSLLHMAVASRRPDLVQVLIEFKVDMEARNKMGQTALQVVAIQGEGLIAELLNLANGANAGAKDSSGWQA AHFAAAGGHVEVLRVLVQKGVSGGDINGAVSDGRTALHMTVEGGYRNGLKVLLVAGADVDDARADDGDTALHRAAARADGSMVRLLMQKGANKDIRNKQKGTPTYDLAADTGDSALLDILRLDGLRRAARRGDVRSVQRCLCEQGAANGKDDQHGWTALHRAAFKGWLEIVKALVDKGADLDGKDDGYTALHCAAESGHMDLVELLKKGGDVNARSKRGVTPQLQIAISLHYSVGVARLLEGGGAVRDNINNTKSETLIVYDITPLPAKKISRRNEGVKKPSPSPFRVQYGRATA\*

>Pruand\_2009248\_EGLZ\_Conifers.p1

MDRLVAVDPLEVSIIHFNRGQRACARIRLHNLMTMSVAFKVLPTPEPKRYGVPRCLAILPPLGEILVEVAMAAQSELPELIEPCGDKFFVKSLMLPTGKATDSELNDFSRNNHIFTDATLKVXXXXXXXSXXXXXXXXSRDVKEVILRCTDVNSRYEDDRTPHIAAIRGSEIVQTLVASGAIVDAKNSTHQTPLLEAAFNHVEVVKFLISNGADQEMVDARGYTALHVAASCNRVQVLISILDSGAKIDAQDIEGKTPLHVSVIARHLESVQLLTGAGANIDSKDKDGWTAIHYAAANGYLDIMKFLLEGGNKYARNNDGKTALGLAMDNGHQHLLDALHLGDMQLQKTAREDNLVSLKSLAQGALVDGSDQHGWWTALHRAAFKGHLDNVKLLYEHGADINSLDDAGFTPLHCAAETGHKDVVEYLIKHDADIKASIKGETPIHLASSMGYSGIVRLLQEQLVEQMHRNRYSSDLFSHINHSTCSQLKNKSVNSVQSP\*

>Pruand\_2010717\_EGLZ\_Conifers.p1

MDRLLDPEPKEVAIRLEPGQKCSGHVELRNVMYTMPVAFRVYPAKQKKYSVKPTCGIISPLGYITVEITMPPQAEPLPESYPHSDDLFIQSVVVPGGRETENVSLWFSAARKKQVFEDSGLRVMFTGGCILARLIADGSGVEIREVLERDGTVDASDDVGRSLLHVLAVQSRPELVQVLLEFRADVEAKDRMGRTALHVAAGDGEALITELLANGASAGGTDPXPSGWQAVHVAAGGHVEALKLILAKGADVDDAVSDGRTALHIAVDGAHRDCLKVLLVAGARVDARADDGDTALHRAAARGDGHMARVLLQKGANKDIRNRNGKTSYDLAADSGDSPLLDILRLDGLRRAARRGDVRSVQRCLCEQGAANGRDQHGWTALHRAAFKGCLDIVKSLLDKGADLDSRDDEGYSVLHSAAESGHMDIVELLKKGADV NARSKRGVSPLQIAASLHYTGIVRLEGGGA AKDSGNKIHHLNNSNMTDGLAAEKVKYLNSSSLTEGFVAEKVKYDIPAAPVKKNPRRPEGLKKPPAFPVQHGRTLVA\*

>Piluvi\_2010340\_ETCJ\_Conifers.p1

MDRLVAVDPVEIRIDFKKGQKAHSTFRLRNLMTMSVAVKISTIEPKKYAVRPGLAIIISPLCEILVEVTAAQAEPLSSIPVSEDSFVVRSLMLPTGKATDSELNELFSKNDHVFTDANKVVYTGNLILRSLVENGSSNDVKEVLLRCKDVNCKYEHDLTPHIAAIRGNSDIVQYLLDSGATVDVNKSAQKSPLEAASC GHVETVKTLSSGANTEIADARGHTALHVAASANNLQILTSLLDSGANINVVDIEGKTPLYSSVIGRHLESVQLLVGAGANIDAKDKDGWTAIHCAAACGYLDTINVLLSEGNKHARTNDGKTALGLALDKGHKHLDDALHLGDMQLQKTSREGDIATLKSCLSQGGAMVNGKDQHGWSALHRAAFKGHLDTVKLLHQQGAEINSLDSDGFTPLHCASETGRKDVVEYLIKHGADVNAAASIKGETPLQLASSMGYSGIVRLLQEQLVENMHGNCFSADRFFNIHTKASSRLKSKLIRCIACK\*

>Piluvi\_2010341\_ETCJ\_Conifers.p1

MDRLVAVDPVEIRIDFKKGQKAHSTFRLRNLMTMSVAVKISTIEPKKYAVRPGLAIIISPLCEILVEVTAAQAEPLSSIPVSEDSFVVRSLMLPTGKATDSELNELFSKNDHVFTDANKVVYTGNLILRSLVENGSSNDVKEVLLRCKDVNCKYEHDLTPHIAAIRGNSDIVQYLLDSGATVDVNKSAQKSPLEAASC GHVETVKTLSSGANTEIADARGHTALHVAASANNLQILTSLLDSGANINVVDIEGKTPLYSSVIGRHLESVQLLVGAGANIDAKDKDGWTAIHCAAACGYLDTINVLLSEGNKHARTNDGKTALGLALDKGHKHLDDALHLGDMQLQKTSREGDIATLKSCLSQGGAMVNGKDQHGWSALHRAAFKGHLDTVKLLHQQGAEINSLDSDGFTPLHCASETGRKDVVEYLIKHGADVNAAASIKEEQI\*

>Piluvi\_2064370\_ETCJ\_Conifers.p1

MDRLLLEVPEKVVIRFEHGQRCSGFVELRNVMYTMPVAFRVHPMVKKYTVRPSCGIISPLGKASVEITMQPQTEIPELPHSDDQFIIQSVVVPGGKETETVSLDWFSAARKKQVFEDSGLKVMFTGGAILTKLVADGSGVEIREVLEKDEGTVNSCDEQGRSLLHMAVASRRADLVQVLIEFKVDMEVRNKKGQTALHAAKGEGLIAELLNLANGAKSGAKDSSWQALHYAAAAGHVDVLRQVANKGAAEENVSVSDGRTALHMAVEGGHRDCLKVLLAGANVDARSDDGDTPLHRAAARGDGS MVRLLMQKGATKDIRNKHGKTSYDLAVDTGDSGLDILRLDGLRRAARRGDFRSLQRCLCEQGAANGKDDQHGWTALHRAAFKGWIEIVKALLDKGADLDSRDDEGYSILHCATESGHMELVELLLKKGADVNGRSKRGVTPQLQIAISLHYSGIVRLLLEVGGAAKENISILKTKSDKNLKSETFRHYELPLPAKKVSRRLQGLKNPPAVSVQYGRATA\*

>Taxdis\_2013665\_FHST\_Conifers.p1

MDRLVAVDPVEIMIDFKRGQKAQNTFRVRNLMTMSVAFKVVSTTEPKKYAVRPGLAIIISPLCEILVEVTAAQAEPLSSIPVSEDKFVVKSLMLPTGKATDSELNDSFSRNTHIFTDATLKVITYTGNLILRSLVENGSSKDVKEVLLRCKDVNRKYEHDLTPHIAAIRGNSEIVQDLSDSGALVDVNKSTQKSPLEAVFHGHVETVKTLSSGANTEITDARGHTALHVAASGNYVQILNFFLFGGANINAQDIEGKTPLYLSVIDRHLESVQLLVGAGANVDADKDKDGWTVIHYAAACGYLDIIVLLESGNKYARTNDGKTALGLALDKGHKHLDDALHLGDMQLQKTSREGDVVTLKSCLAQGA MVNGKDQHGWSALHRAAFKGHLDTVKFLHQQGAEINSVDDTGFTPLHCASETGRKDVVEYLIKHGADVNATSIKGETPLQLASSMGYSGIVRLLQEQLVENTHGNCFSA DRFFNIHTK

>Taxdis\_2063267\_FHST\_Conifers.p1

MQPQTEFPEFFPHSDDQFLVQSVVVPGGKETETVSLWFSAARKKQVFEDNGLKVMFTGGTVLTKLVADGSGVEIREVLEKDEGTVNSCDEQGRSLLHMAVANRRPDLVQLLIEFKVDMEARNKKQTALHVAAAKGEALIAELLNLANGAKVGA DLSSWQALHFAAAGGHVDVLRQVVLKGADENVSVSDGRSALHMAVEGGHRDCLKVLLVAGANVDARSDDGTAVHRAAARGDGS MVRLLMQKGANKDIRNKQKGTPTYDLAADSGDSALLDILRLDGLRRAARRGDVRSVQRCLCEQGAANGKDDQHGWTALHRAAFKGWLEIVKALLDKGADLDSRDDEGYSILHCATESGHMELVELLLKKGADV NARSKRGVTPQLQIAASLHYS\*

>Cephar\_2011946\_GJTI\_Conifers.p1

MDKLLSVEPKEVAIRFEQGRCSGFVELRNVMYTMPVAFRIHPMKHKKYSVKPSCGIISPLGTASVEIIMQPQSELPESFPHSNDHFVIQSVVVPGGKETETVSLDWFRTARKKQVFEDSGLKVMFTGGSILTKLVADGSGVEIREVLEKDEGTVDSCDEQGRSLLHMAVSSRRPDLVQVLIEFKADMEGRNRNMGQTALHVASAQGEGLIAELLNLANGANTQAKDSFGWQAVHFAAAGGHVEVLRVLVQKGANSDINGAVIDGRTALHMTVEGGHRDCLKVLLVAGANVDARSDDGTAVHRAAARGDGS MVRLLMQKGANKDIRNKQKGTPTYDLAADSGDSALLDILRLDGLRRAARRGDVRSVQRCLCEQGAANGKDDQHGWTALHRAAFKGWLEIAKALVEKGADLDSRDDGYTVLHCAAESGHMDVVELLLKKGADV NARSKRGVTALQIATSLHYSVGVARLLEGGGATRDNFTKNNKSETPIVYDIPPTPAKKISRRLEGLKKASFPVPVQYGRATA\*

>Cephar\_2061825\_GJTI\_Conifers.p1

MDRLVAVDPLEIKIDFRRGQKAHTRRLRLQNLMTMSVAFKVLATEPEKYGVKPSLAIIIPPLGEIWVEVTMAAQAEPLSLIPVSEDKFFVKSLMRPTGKATDSELNELFSSNHHIFNDATLKVIFTGNLILRSLVANGSPREVKEVLLRCSVDNGKYEQDLTLHIAVFRGNSEIVQALLDSGALVDVNKSSQKSPLEAAFYGHVEIVKILLSAGANTETADARGYTALHFAALGNYVQVLISLLDSGQINAQDIEGKTPLHVSVIARHLESVQLLVGAGANVDVKDKDGWTAIHSAAAYGHLDIIFLLESGNKYARNNDGKTALGLALDNGHQHLLDVLHLGDMQLQKTSKEDNLVTLKSCLAQG\*



>Disarc\_2010781\_GKCZ\_Conifers.p1  
MDRLVAVDPVEIRIDFKKGQKAHSTFRLRNLMTMSVAVKISTVVPKKYAVRPLAIISPLGEILVEVTTAAQADLPSSIPVSEDSFVVRSLMLPTGKATDFELNELFSKNHHVFTDANL  
KVYYTGNLILRSLVENGSSNDVKEVLLRCKDVNCKYEHDLTPLHIAAIRGNSEIVQYLLDSGAIVDIKNSTQKSPILLEAAFCGHVETVKTLSSSGANTEIADARGNTALHVAASANNVQIL  
TSLDSGANKNVQDIEGKTPLYLSVIGRHLESVQLLVGAGANIGAKDKDGWTAIHCAAACGYLDIISILLESNGKHARTNDGKTALGLALDKGHHLLDALHLGDMQLKTSREGDVVTL  
KSCLSQGAMVNGKDQHGWSALHRAAFKGHLDSVKLLHQQGAEINSLDSDGFTPLHCASETGRKDIVEYLIKHGADVNATSIKEEQI\*

>Disarc\_2010782\_GKCZ\_Conifers.p1  
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FSADRRFFNIKTSSLLKSLVRCIAXXXXXXLYFAICMRYCNCYSMLLLAILTERVVAKAFNMSCENAILCLYC\*

>Disarc\_2012351\_GKCZ\_Conifers.p1  
ETETVSLDWFSARKKQVFEDSGLKVMFTGGAILAKLVPDGSVEEIREVLEKDEGTVNSCDEQGRSLLHMAVASRRADLVQVLIEFKVDMEVRNKKGQTVLHLLAAKGEGLIAELLAN  
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KRGVTPQLIAISLHYSGVVRLLEASGAAKENTSILKTSKDTLKSEIFRHYEMPPPLAKKISRRLQGHKNSPA VSVQHGRTVVA\*

>Cephar\_2019092\_NVZ\_Conifers\_2\_samples\_combined.p1  
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GLKVMFTGGSGILTKLVADGSEVEIREVLEKDEGTVDSCDEQGRSLLHMAVSSRRPDLVQVLIEFKADMEGRNRNMGQTALHVASAQGEGLIAELLANGANTQAKDSFGWQAVHFA  
AAAGHVEVLRVLVAQKGANSDDINGAVIDGR TALHMTVEGGHRDCLKVLVAGANVDARSDDGDTAVHRAAARGDGS MVRLLMQKGANKDIRNKKQKTPYDLAADSGDSALLDI  
LRLGDGLRRAARRGDVRSVQRCLEQGAANGKDKQHGWTALHRAAFKGWLEIAKALVEKGADLDSRDDEGYTVLHCAAESGHMDVVELLLKKGADVNARSKRGVTALQIATSLHYS  
GVARLLEGGGATRDNFTKNNKSETPIVYDIPPTPAKKISRRLGLELKKASPFVQYGR TAVA\*

>Cephar\_2112894\_NVZ\_Conifers\_2\_samples\_combined.p1  
MDRLVAVDPLEIKIDFRRGQKAHTRLRLQNLMTMSVAFKVLATEPEKYGVKPSLAIIPPLGEIIVWEVTMAAQAEPLSIPVSEDKFFVKSLMRPTGKATDSELNELFSSNHIFNDAT  
LKVIFTGNLILRSLVANGSPREVKEVLLRCKDVNGKYEQDLTLLHIAVFRGNSEIVQALLDSGALVDVKNSSQKSPILLEAAFYGHVEIVKILLSAGANTETADARGYTALHFAALGNVYQV  
LISLLDSGAQINAQDIEGKTSLHVSVIARHLESVQLLVGAGANVDVKDKDGWTAIHSAAYGHLDIIFLLESNGKYARNNDGKTALGLALDNGHQHLLDVLHLGDMQLKTSKEDNLV  
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>Taicry\_2066441\_QSNJ\_Conifers.p1  
ITMQPQTELEPFFPHSDDQFVIQSVVVPGGKETETVSLWFSAARKKQVFEDSGLVKLTGGSVLTKLVADGSEVEIREVLEKDEGTVDSCDEQGRSLLHMAVASHRPDLVQVLIEFKVD  
VEARNKKGQTALHLAAAKGEGLIAELLANGANYGATDLSGRQALHFAAAGGHVEVLRVLVKGDDVNSVVIDGRSALHMAVEGGHRDCLKVLVAGANVDARAEDGDTPLHK  
AAARGDASMVRLLMQKGATKDIRNKKQKTSYDLAVDTGDSALLDMLRLGDGLRRAARRGDLRSLQRCLEQGAANGKDKQHGWTALHRAAFKGWIEIVKALLDKGADLDSRDDE  
GYTILHCATESGHMELVELLLKKGADVDARSKRGVTPLQIATSLHYSGIARLLEAGGAARDNINTINTKSETLLYDIPSLSAKKISRHRDGLKKPPTFSMQYGR TAF A\*

>Taicry\_2069042\_QSNJ\_Conifers.p1  
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VYTGNLILRSLVENGSPCDVKEILLRCKDINSKDEHDLTPLHVAAIRGNSEIVQDLLDSGATVDVKNSSQKSPILLEAFNGHIEIVKMILLSSSGANTEIADARGHTALHVAASRNYEQILISL  
LDSGANINAQDIEGKTPLYLSVIGRHLESVQLLVGSGANVDAKDKAGWTVIHCAAACGYLDIIKVLLESNGKYARTNDGKTALGLALDKGHHLLDALHLGDMQLKQTSMEGNIATLKS  
CLAQGAMVNGKDQHGWSALHRAAFKGHLDAVKLLHQHGAEINSLDDAGFTPLHCASETGRKD VVEYLIKHGADV KATNFKGETPLLASFMGYSGIVRQLQEQLVENMHGNCF  
SADRFLNIHTKTSSRLKSLVRCIAQST\*

>Cephar\_2089635\_WYAJ\_Conifers.p1  
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SSQKSPILLEAAFYGHVEIVKIISAGANTETADARGYTALHFAALGNVYQVVISLLDSGAQINAQDIEGKTSLHVSVIARHLESVQLLVGAGANVDVKDKDGWTAIHSAAYGHLDIIFL  
LESNGKYARNNDGKTALGLALDNGHQHLLDVLHLGDMQLKTSKEDNLVTLKSCLAQGAMVNGKDQHGWSALHRAAFKGHLDNVKLLHQHGADINSLDDAGFTPLHCAVETGRK  
DVVEYLIKHGADVNTTSIKGETPFHIASSMGYGIVRLLKEKQVVEKMHGNYLSAGLFLNIHKTSSQLKSNSVR\*

>Auschi\_2071080\_YYPE\_Conifers.p1  
MDRLLVPEPKEVVIRFEHQRCSGFVELRNVMTMPVAFRVHPMQQKKYTVRPSCGIISPLGKASVEITMQPQTDIPEFFPHSDDQFIIQSVVVPGGKETETVSLDWFSARKKQVFE  
DSGLKVMFTGGAILTKLAVADGSEVEIREVLEKDEGTVNSCDEQGRSLLHMAVASRRADLVQVLIEFKVDMEGRNKKGQTALHLAAAKGEGLIAELLANGAKSGAKDSSSWQALHYA  
AAAGHVDVLRLQVAHKGTEEANYVSDGR TALHMAVEGGHRDCLKVLVLAGANVDARSDDGDTPLHRAAARGDGS MVRLLMQKGATKDIRNKHGKTSYDLAVDTGDSALLDLRL  
LGDGLRRAARRGDFRSLQRCLEQGAANGKDKQHGWTALHRAAFKGWIEIVKALLDKGADLDSRDDEGYSLHCATESGQMELVELLLKKGADVNARSKRGVTPQLIAISLHYSGIVRL  
LEVGGAAKENVSILKTSKDTLKSETFRHYEMSP LPIKKISRRLQGLKNLPA VSVQYGR TAVA\*

>Auschi\_2071697\_YYPE\_Conifers.p1  
MDRLVAVDPVEIRIDFKKGQKAHSTFRLRNLMTMSVAVKISTIEPKKYAVRPLAIISPLYEILVEVTIAAQAEPLSIPVSEDSFVVRSLMLPTGKATDSELNELFSKNHHVFTDANLKV  
VYTGNLILRSLVENGSSNDVKEVLLRCKDVNCKYEHDLTPLHIAAIRGNSEIVQYLLDSGAIVDVKNSSQKSPILLEAAFCGHVETVKTLSSSGANTEIAHARGHTALHVAASANNVQILTS  
LLDSGANINVDIEGKTPLYLSVIGRHLESVQLLVSAGANIDAKDKDGWTAIHCAAACGYLDIINILLESNGKHARTNDGKTALGLALDKGHHLLDALHLGDMQLKTSREGDVVTLKS  
CLSQGAMVNGKDQHGWSALHRAAFKGHLDTVKLLHQQGAEINSLDSDGFTPLHCASETGRKD VVEYLIKHGADV NATSIKGETPLQLASSMGYSGLVRLQLQEKQLVENMHGNCFS  
ADRRFFNIHTKTSSRLMSKLVRCIAKCP\*

>Anetom\_2008299\_CQPW\_Leptosporangiate\_Monilophytes.p1  
RKKHVYNDATLKVILTGPLVLCRLAVNGGSSDSIREVL RDTLVNARDSQDR TALFLATLKGNLDAVQTLIERKADVNIKDRMGRTPLEAVLAGHSEIVKALLGAGAAIKVKDSRGWTS  
LHAAAASNRTDIISLLDAGAGIDAATGRERTPLHEAAVAGHFKAVALLVKRGADLEKRCINGTTALHIAASSGFGDIAEFLLQEGSDIHARSYEGKTPYDLARANGHSR FLESSYLQEVL  
QKASRKNNLQTVQDCLLQGAATDASDQHGWTALHRASFKEYIDVVKLLKHQFDVDAKDENG YTPLHCAVEAGHKEVVELLHQHGADVSM TANKGGLTPLALAFTRKYSRIRQLIE  
EAKPGYCKCK\*

>Anetom\_2097813\_CQPW\_Leptosporangiate\_Monilophytes.p1

MDRLLIIEPKELIMHFVEVCEQSHASIRLKNVMHTMPVAFMVQATPQKNYTIKPSHGIIPLGLVAHVEITRCPQTELPDFIPYSKDQFLVNSVVVPGGGNNGSVSLDWFTAKKKQVVS  
DRGMRLILIGGGILRRLVALGSMENIREVVELDERLVNSQDEKGRTPMHIAVASRRPELVQLLEMMADLEIKNAFGQTALHEAASQGEALIVELLARGAQANSRNASGCTPLHNA  
VMGNHAKVVHFLVENGADANAVMEDGRTALHVAVEQGNIECIALLKEGAWPDIPWGEDAETPLHIAASKGYDKALELLLRHGASRDVQNRGQTPRDVAAALEQKQILFLLNE  
LILGDELRAARKGKVEDVELCLRKGALVDGTDEHGW TALHCAAFKGHMGVVSHLVKEGADLT SRDCTGYTPLHCAIEAAQKDVVRLLIKHGAPVDACNDSGLSAFDLACRHKYM  
ENIQMLLDYGDGEDCKLEKVVKAEEQGVSGMDISYAFSSPLD\*

>Osmsp.\_2013284\_UMY\_Leptosporangiate\_Monilophytes\_gametophyte.p1

MDRLLIIEPCELVLKFELGQKSSATVQLKNVMFTMPVAFEVQPTAPARYSIKPSGGIISPLGVVSFEIVLNPQSQLPCSPFPFSDDKFLVKSVVVPGGSDNEAVCLEWFTAKKKQVFS DHG  
MRVVFIGGGILRRLVADGSMEDIREVLERDEKLVDSKDEQGR TAMHIAVASGRPELVQLLLEFKADVEIKSRSGQTALQEASGGETLMVELLLAKGARADAKNPSGWTALHYAVM  
GGHADVVKLLLEKGAQVDSAVQDGR TALHMAVAEGRQGCELELLQEGANVDLRSSDGEAPLHKAALRGDVATVELLLKYGANQDIRNSKGKRPFCALPSGKQKLLDILSLGDDL  
RAARKGQLKDVQQLTGGASANGRDQHGW TALHRAAFKGYVEVVGFLENGADLHSHKDEAGYTVLHCATEAGQKDVVQLLLKKGADINARRSNSGGTALDLATHLNYSGIIRLL  
EWGVSKDAAVHHQGRTAFDKLEKDYRISPLKSPSESVIADSSLRHPQGDGLCMMIPVSKHSNARNVF\*

>Osmsp.\_2080824\_UMY\_Leptosporangiate\_Monilophytes\_gametophyte.p1

MDRLLVVTPREVRLNFETGKKVFAKIRLHNLMTMPVAFKVLTTAPRKYSVHPEALIIPRGEHLEVVMTAQKNFPECFFPSADKLYIKSLMVPTGKATEAELNLWFSTRRKHV FND  
ASLKVVFTGSQILWLSAASGSWESVKEVLRD TDVNAKDSQDR TALYIAAVQGNYGAMKALIEGGAKLVNKNTEQTPLFEAALAGHSEIVKILLSAGADTEAKNSRGWTPLHAAAA  
WNQGEIVRSLLEAGSDKNAKTDKGRTP LHEAAMAGHVKT VALLVQSGADIEKRCFDGSTALHIAASSGFQDVVELLLEAGANRNVNRNHKGKTPRDSAFENGHNQLDSLHLQQM  
VQKAARKNDVGTVNDCLILGASTEGRDQHGW TALHRAASFKGHLDTVKMLLKKHINVNSKDDEGYTPLHCAVETGQKEVVDLLQH GADMNARINKGSTPLALALTMKYLGILKLL  
EEKKPGQNVYENKSSVEM\*

>Osmjav\_2076565\_VIBO\_Leptosporangiate\_Monilophytes.p1

MDRLLIIEPTLVLFELGLKSSATVQLKNVMFTMPVAFEVQPTAPTRYSIQPSGGIISPLGVVSFEIVLNPQTQLPCSPFPFSDDKFLVKSVVVPGGSDNEAVCLEWFTAKKKQVFS DHG  
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GGHADVVKLLLEKGAQVDSAVQDGR TALHMAVAEGRQGCELELLQEGANVDLRSSDGEAPLHKAASRGDVATVELLLNYGANQDIRNSKGKRPFDALPSGKQKLLDVLSLGDDL  
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>Osmjav\_2078330\_VIBO\_Leptosporangiate\_Monilophytes.p1

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>Anacom\_Aco008773.1

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>Anacom\_Aco007073.1 outgroup

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>Aradur\_Aradu.7VM84

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DKEGRTP LHLAASKGLLGSGKALIGAGA QVDARSKDGR TALFRAAANGDC E M A K M L V E M G A D P T L T E L H L R R S A L D I A R A K G H G V V V K I L E R G E A V L H A A R H G D V Q L L E T L L E K G A T T  
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>Vigrad\_Vradi08g09860.1  
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>Vigrad\_Vradi0007s00890.1  
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>Vigrad\_Vradi01g08090.1  
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>Zeamay\_Zm00008a004787  
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>Marpol\_Mapoly0060s0009.1

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RVGVLRI LLKAGANENAPDMDFRTP LHAAVTEGHTRAA LILLQH GADLDVQSIDGRTP LHCAALQGHL SVIELLIDWGAQKEIKDCEGKTPFELAVESGHTYVLDPLLGGQFLHKAAREG  
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>Sellep\_maker-Contig\_3-snap-gene-11.119-mRNA-1

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VDAKDANGWTALHHA VDKRVLDVVGVLISKGANVDARGNDGRTP LHVAVITGDLGCVHSLLRHGADVGAATLDGTTPLHLAATQGNEVGIARMLLDWGANRAYDEAVEMGQEKL  
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>Sellep\_snap\_masked-Contig\_8-abinit-gene-5.35-mRNA-1

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>Sphfal\_Sphfalx0093s0059.1

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EGNSEAVQRYLEQGAHVNGIDQHGF TTLHRAAFKGHVAVATILLDRHASIDPEQEDGYTPLHCASMAGHKAVVQ LLLQRGANVNARCKKGSTPLHVAAAMD SLGIVQ LLLRNGARR  
DLQTNDGR TAFDISRGLANPD LSSLRPQQPPSPPPSFGDDPHD PHLLTTSFSGLKV\*

p-values of results in Fig. 3, Fig. 4, Fig. 6, and Fig. 7

<b>Fig. 3C</b>					
1w					
	p values	signif			
KO36 - KO27	0.111		wt	KO27	KO36
wt - KO27	<0.001	***	b	a	ab
wt - KO36	0.137				
2w	p values	signif			
KO36 - KO27	0.006797	**	wt	KO27	KO36
wt - KO27	< 0.0001	***	c	a	b
wt - KO36	0.000675	***			
3w	p values	signif			
KO36 - KO27	0.00499	**	wt	KO27	KO36
wt - KO27	< 0.0001	***	c	a	b
wt - KO36	< 0.0001	***			
4w	p values	signif			
KO36 - KO27	0.0161	*	wt	KO27	KO36
wt - KO27	< 0.0001	***	c	a	b
wt - KO36	< 0.0001	***			

<b>Fig. 3D</b>					
18 days	p values	signif			
KO36 - KO27	0.9902		wt	KO27	KO36
wt - KO27	0.0253	*	a	b	b
wt - KO36	0.033	*			
23 days	p values	signif			
KO36 - KO27	0.7941		wt	KO27	KO36
wt - KO27	0.1262		a	a	b
wt - KO36	0.0381	*			
28 days	p values	signif			
KO36 - KO27	0.78374		wt	KO27	KO36
wt - KO27	0.01144	*	b	a	a
wt - KO36	0.00304	**			

<b>Fig. 3E</b>					
18 days	p values	signif			
KO36 - KO27	0.96564		wt	KO27	KO36
wt - KO27	0.00177	**	a	b	b
wt - KO36	0.00287	**			
23 days	p values	signif			
KO36 - KO27	0.0534		wt	KO27	KO36
wt - KO27	< 0.0001	***	a	b	b
wt - KO36	< 0.0001	***			
28 days	p values	signif			
KO36 - KO27	0.73782		wt	KO27	KO36
wt - KO27	0.01924	*	a	b	b
wt - KO36	0.00453	**			

<b>Fig. 4C</b>					
	p values	signif			
KO36 - KO27	0.9996		wt	KO27	KO36
wt - KO27	0.0212	*	a	b	b
wt - KO36	0.0173	*			
<b>Fig. 4D</b>					
<10					
	p values	signif			
KO36 - KO27	0.994		wt	KO27	KO36
wt - KO27	<0.001	***	a	b	b
wt - KO36	<0.001	***			
<15					
	p values	signif			
KO36 - KO27	0.979		wt	KO27	KO36
wt - KO27	0.875		a	a	a
wt - KO36	0.953				
<20					
	p values	signif			
KO36 - KO27	0.987		wt	KO27	KO36
wt - KO27	< 0.0001	***	a	b	b
wt - KO36	< 0.0001	***			
>20					
	p values	signif			
KO36 - KO27	0.426		wt	KO27	KO36
wt - KO27	< 0.0001	***	a	b	b

<b>Fig. 6D</b>					
Inner					
	p values	signif			
KO36 - KO27	0.99939		wt	KO27	KO36
wt - KO27	<0.001	***	c	a	a
wt - KO36	<0.001	***			
Middle					
	p values	signif			
KO36 - KO27	0.99217		wt	KO27	KO36
wt - KO27	<0.001	***	c	a	a
wt - KO36	<0.001	***			
Outer					
	p values	signif			
KO36 - KO27	0.89061		wt	KO27	KO36
wt - KO27	<0.001	***	d	b	b
wt - KO36	<0.001	***			
<b>Fig. 6E</b>					
Inner					
	p values	signif			
KO36 - KO27	1		wt	KO27	KO36
wt - KO27	1		b	b	b
wt - KO36	1				
Middle					
	p values	signif			
KO36 - KO27	1		wt	KO27	KO36
wt - KO27	0.992		b	b	b
wt - KO36	0.925				
Outer					
	p values	signif			
KO36 - KO27	1		wt	KO27	KO36
wt - KO27	1		a	a	a
wt - KO36	1				
<b>Fig. 6F</b>					
length					
	p values	signif			
KO36 - KO27	1		wt	KO27	KO36
wt - KO27	0.0138	*	c	b	b
wt - KO36	0.0103	*			
width					
	p values	signif			
KO36 - KO27	0.9999		wt	KO27	KO36
wt - KO27	1		a	a	a
wt - KO36	0.9999				

<b>Fig. 7</b>					
control					
	p values	signif			
KO36 - KO27	0.671		wt	KO27	KO36
wt - KO27	<0.01	***	b	c	c
wt - KO36	<0.01	***			
IAA					
	p values	signif			
KO36 - KO27	0.943		wt	KO27	KO36
wt - KO27	0.987		a	a	a
wt - KO36	1				
NAA					
	p values	signif			
KO36 - KO27	1		wt	KO27	KO36
wt - KO27	1		a	a	a
wt - KO36	1				
SL					
	p values	signif			
KO36 - KO27	1		wt	KO27	KO36
wt - KO27	1		a	a	a
wt - KO36	1				