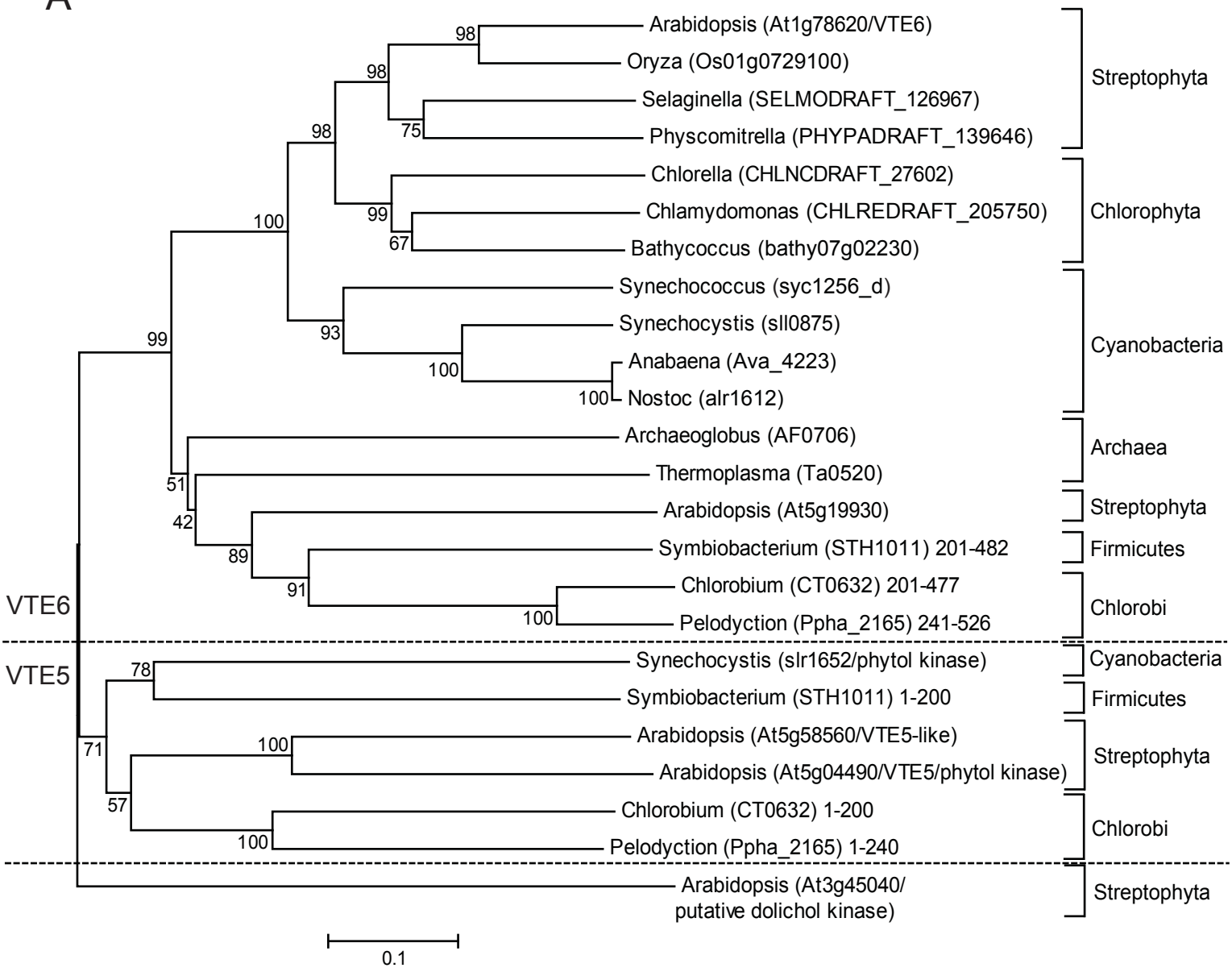
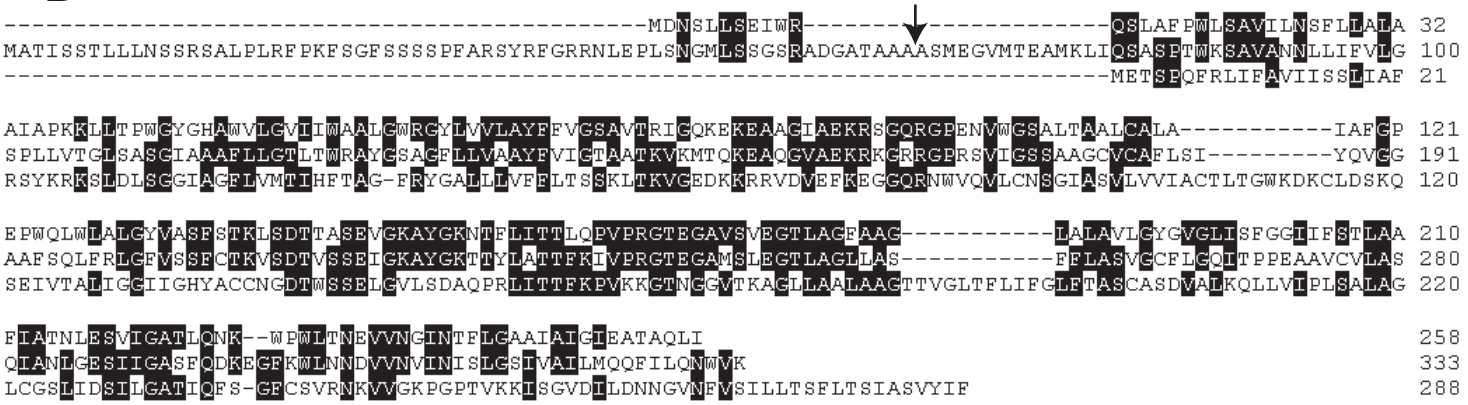
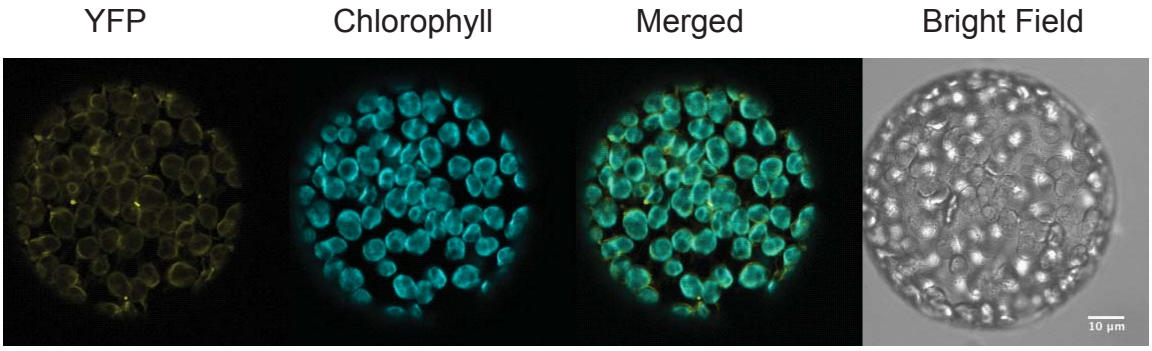
A



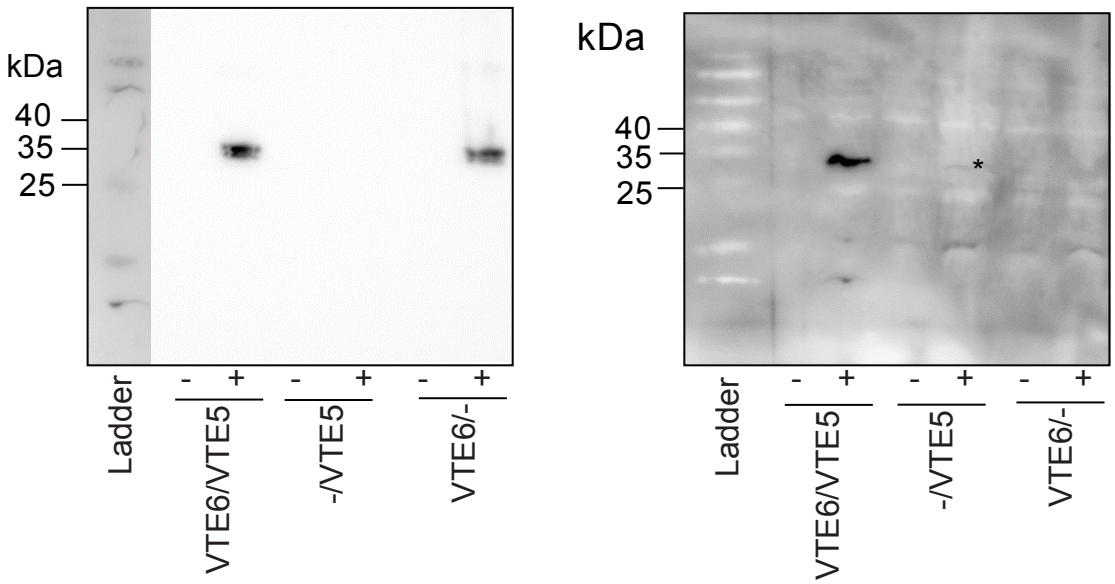
B



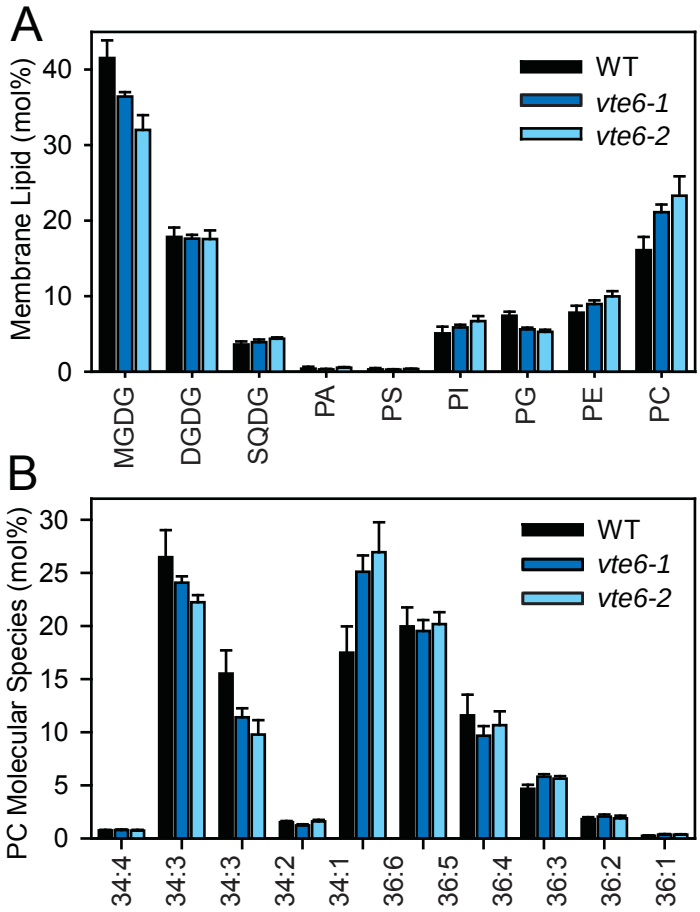
Supplemental Figure 1. Phylogenetic Relationship of COG1836-Like Sequences.
(**A**) Phylogenetic analysis of phytol kinase (VTE5) and phytyl-P kinase (VTE6) sequences from plants and bacteria using MEGA6 (Tamura et al., 2013). Protein sequences from *Arabidopsis*, *Oryza sativa*, *Physcomitrella patens*, *Selaginella moellendorfii*, *Chlorella*, *Chlamydomonas reinhardtii*, *Bathycoccus*, *Synechococcus*, *Synechocystis*, *Anabaena*, *Nostoc*, *Archeoglobus*, *Thermoplasma*, *Symbiobacterium*, *Chlorobium* and *Pelodyction* were obtained from GenBank. The phylogenetic analysis was done using the neighbor-joining-method. In the x-dimension, branch length represents evolutionary distance (number of amino acid differences per site). Bootstrap values were calculated from 1000 replicates.
(**B**) Alignment of COG1836-like sequences from *Arabidopsis* and *Synechocystis*.
The *Arabidopsis* VTE6 sequence (At1g78620, middle) was aligned with the COG1836 sequence from *Synechocystis* (sll0875, top) and At5g19930 (bottom) using ClustalW (MEGA6). The arrow indicates the predicted transit peptide cleavage site (position 65/66; ChloroP1.1).



Supplemental Figure 2. Subcellular localization of VTE6.
The fusion protein of YFP:VTE6 (VTE6 C-terminally fused to yellow fluorescent protein) was transiently expressed in *N. benthamiana* leaves and fluorescence in isolated protoplasts observed by confocal microscopy.



Supplemental Figure 3. Heterologous expression of VTE5 and VTE6 in *E. coli*.
After ligation into pETDuet-1, His:VTE6 and/or VTE5:S fusion proteins were expressed in *E. coli* Rosetta for 4 h. Total protein extracts were isolated from cells before (-) and after (+) induction with IPTG, and expression of fusion proteins was demonstrated by immunodetection using antibodies against His- and S-tag, respectively. The asterisk indicates the weak signal of VTE5:S. Molecular masses of His:VTE6 and VTE5:S are 35 kDa.



Supplemental Figure 4. Membrane Glycerolipids in Leaves of the *vte6* Mutant.
(**A**) Glycerolipids are presented in mol%.
(**B**) Molecular species composition of phosphatidylcholine (PC) in mol%.
Lipids were measured in leaves extracts of WT, *vte6-1* and *vte6-2* by direct infusion mass spectrometry (Gasulla et al., 2013). Data are mean and SD of 4 measurements. *Significantly different to WT; $p < 0.05$; Student's t test. Molecular species are indicated as X:Y, where X represents the number of the carbon atoms, and Y the number of double bonds, in the tow acyl chains. DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidyl-glycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SQDG, sulfoquinovosyldiacylglycerol.

Supplemental Table 1: Oligonucleotides Used in this Study		
Primer	Sequence	Gene target
bn130	TCCGTTCCGTTTTCGTTTTTTAC	Transposon primer Ds5-2a G-edge
bn232	CCGGATCGTATCGGTTTTCG	Transposon primer Ds3-2a H-edge
bn233	ATGGCAACGATTTCGTCAACTC	VTE6 forward (*vte6-1*, *vte6-2*)
bn234	GTCCAGCCAATGTTCCTTCAAG	VTE6 reverse (vte6-1, vte6-2)
bn771	ACTGTACCTCCGTCAATCGCC	VTE5 forward (*vte5-2*)
bn772	AAGCTTAAGACAAGCGCGTATG	VTE5 reverse(*vte5-2*)
bn78	ATTTTGCCGATTTCGGAAC	Left border primer SALK
bn358	GCCATCCAAGCTGTTCTCTC	Forward RT-PCR primer *ACT2*
bn359	GAACCACCGATCCAGACACT	Reverse RT-PCR primer *ACT2*
bn577	GCAACGAAGGTTAAAATGACGC	Forward RT-PCR primer *VTE6*
bn578	GAAGCACCTATTATGCTCTCTC	Reverse RT-PCR primer *VTE6*
bn410	CAAACTCAGTTCCCTCCGTC	Forward RT-PCR primer *VTE5*
bn411	GTCCGTTAATAACAAGCCTTAAG	Reverse RT-PCR primer *VTE5*
bn327	CCTAGGATGGCAACGATTTCGTCAACTC	Forward VTE6 overexpression
bn328	GTCGACTTACTTGACCCAGTTCTGGAG	Reverse VTE6 overexpression
CB157	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGGCAACGATTTCGTCAACTC	YFP::VTE6 expression, forward
CB158	AGAAAGCTGGGTGCTTGACCCAGTTCTGGAGTATA	YFP::VTE6 expression, reverse
CB1	AAGGATCCGATGGCAACGATTTCGTCAACTC	VTE6 expression in *E. coli*, forward
CB2	AAGCGGCCGCTTACTTGACCCAGTTCTGGAGT	VTE6 expression in *E. coli*, reverse
CB3	AACATATGATGGCAGCAACCTTACCTCTAT	VTE5 expression in *E. coli*, forward
CB4	AAGGCCGGCCCGATATCCGAAACTTAAATAAGCAGCTA	VTE5 expression in *E. coli*, reverse