Supplementary data

VIPP1 has a disordered C-terminal tail necessary for protecting photosynthetic membranes against stress in *Arabidopsis*

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Supplementary movie legends:

Supplementary Movie 1. VIPP1-GFP particles showing movement when chloroplasts isolated from *VIPP1-GFP*/Col underwent 20% hypotonic stress (equivalent to 0.56 osmol/L). Recoding time, 5 sec.

Supplementary Movie 2. VIPP1-GFP particles showing movement when leaf tissue from *VIPP1-GFP*/Col infiltrated with water. Recording time, 7 sec.

Supplementary Movie 3. Small VIPP1-GFP particles began to move when the mesophyll protoplast of *VIPP1-GFP*/Col underwent 20% hypotonic stress (equivalent to 0.56 osmol/L). Recording time, 4 sec.

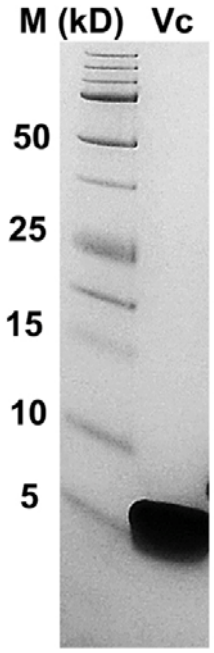
Supplementary Movie 4. Several small VIPP1Δc-GFP particles began to move when the mesophyll protoplast of *VIPP1Δc-GFP*/Col underwent 20% hypotonic stress (equivalent to 0.56 osmol/L). Recording time, 4 sec.

Supplementary Movie 5. Some slightly larger VIPP1-GFP particles of *VIPP1-GFP*/Col start moving in the chloroplast of mesophyll protoplast in the condition of 50% hypotonic stress (equivalent to 0.35 osmol/L). Recording time, 4 sec.

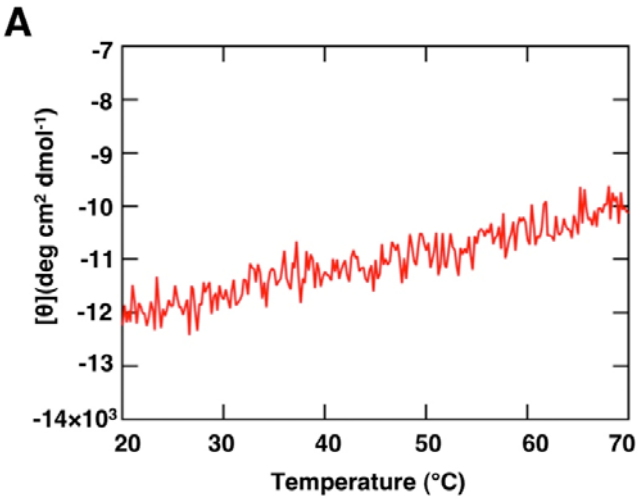
Supplementary Movie 6. Some slightly larger VIPP1Δc-GFP particles of *VIPP1Δc-GFP*/Col start moving in the chloroplast of mesophyll protoplast in the condition of 50% hypotonic stress (equivalent to 0.35 osmol/L). Recording time, 4 sec.

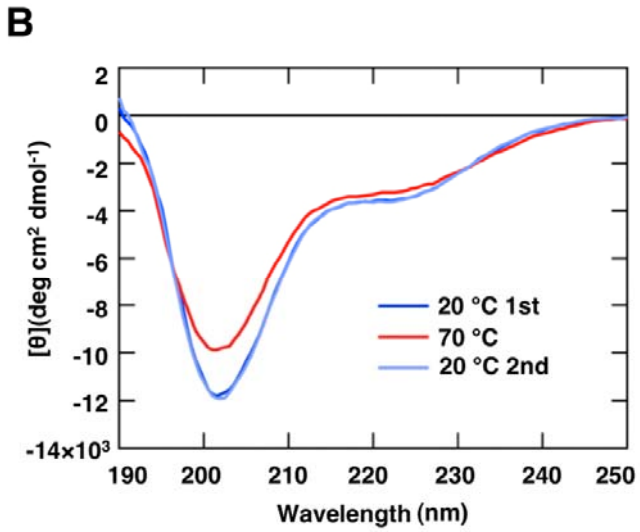
Supplementary Movie 7. VIPP1-GFP remained static in the chloroplast of mesophyll protoplast without hypotonic stress (equivalent to 0.70 osmol/L). Recording time, 4 sec.

Supplementary Movie 8. No movement was observed with VIPP1Δc-GFP particles in the chloroplast of mesophyll protoplast without hypotonic stress (equivalent to 0.70 osmol/L). Recording time, 4 sec.



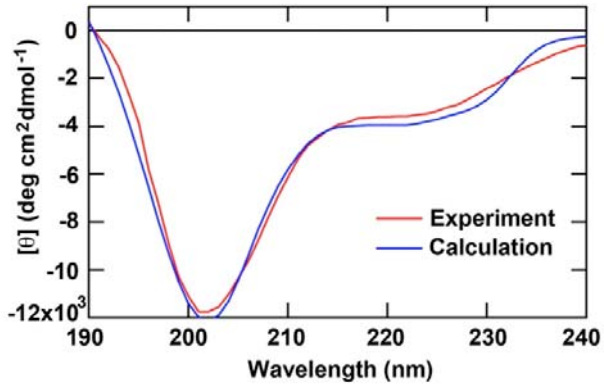
Supplementary Fig. S1 SDS PAGE of synthesized Vc (38 aa).





C

	Helix1	Helix2	Strand1	Strand2	Turns	Unordered	Total
Vc	0.034	0.102	0.158	0.118	0.144	0.444	1



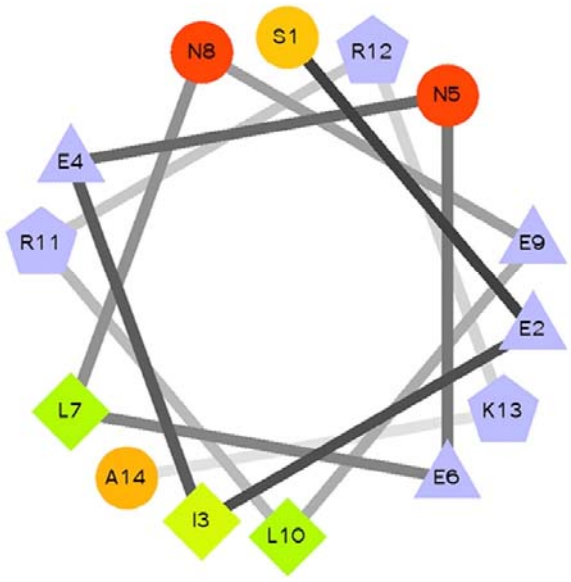
Supplementary Fig. S2

CD analysis of the synthesized Vc.

(**A**) Thermal stability of synthesized Vc monitored upon increased temperature at 202 nm of the CD spectrum.

(**B**) Reversibility of CD spectra measured in denatured (70 °C, red) and room temperature (20 °C, blue) conditions.

(**C**) Secondary structure composition of Vc estimated from the CD spectra shown in Figure 1C using online CD analysis site, DichroWeb (http://dichroweb.cryst.bbk.ac.uk). Upper table illustrates the secondary structure composition of Vc. Helix1, Helix2, Strand1 and Strand2 indicate a regular α-helix, disordered α-helix, regular β-strand and disordered β-strand, respectively. Lower graph shows the calculation result.

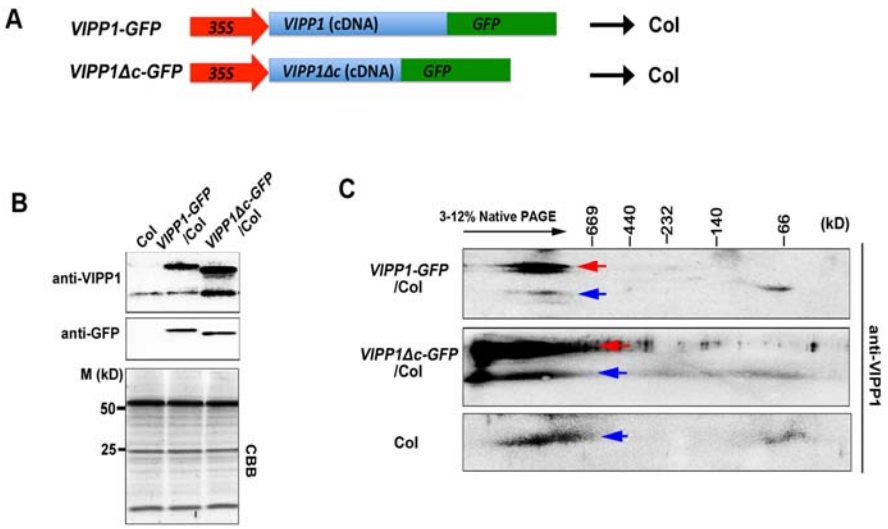


Supplementary Fig. S3 Helical wheel projection of Vc.

The hydrophobic amino acid is shown with green. Hydrophilic residues are coded red.

The prediction was conducted using online software

(http://rzlab.ucr.edu/scripts/wheel/wheel.cgi).

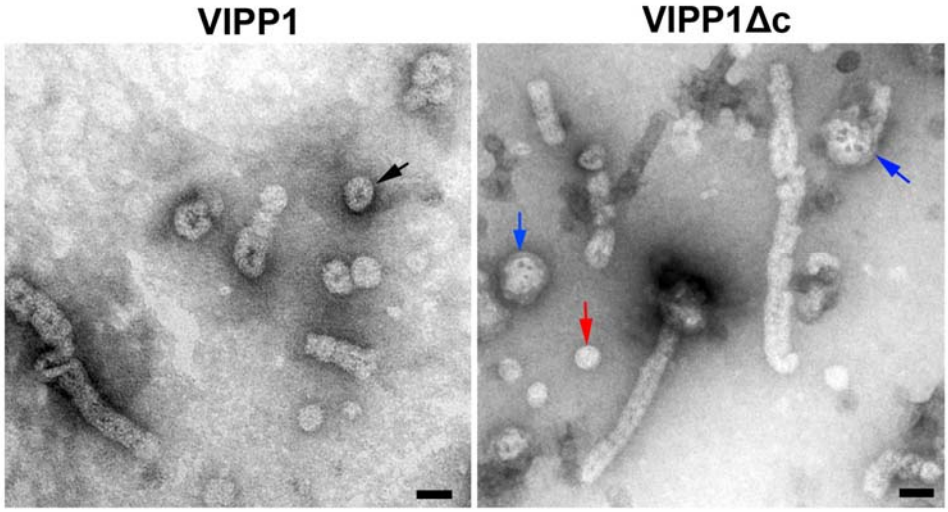


Supplementary Fig. S4 Overexpression of VIPP1-GFP and VIPP1Δc-GFP in *Arabidopsis*.

(**A**) Schematic illustration of *VIPP1-GFP* and *VIPP1Δc-GFP* constructs used for transformation into Col.

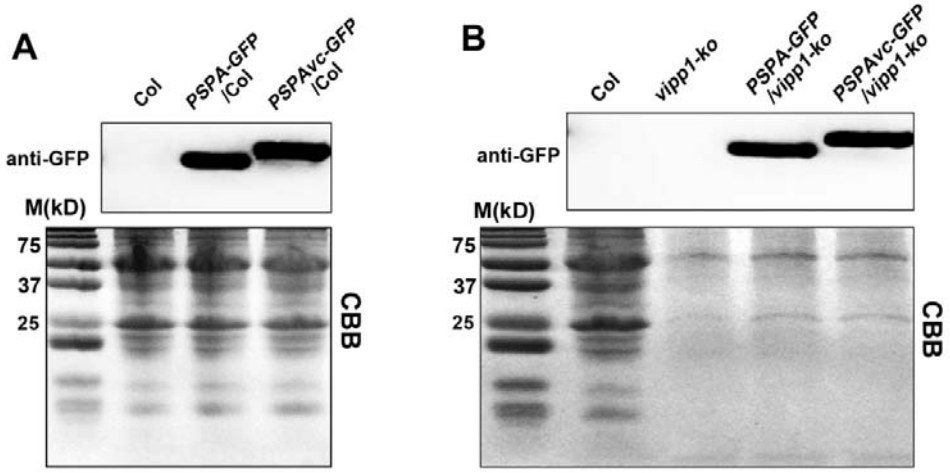
(**B**) Accumulation of VIPP1-GFP and VIPP1Δc-GFP in the overexpression lines confirmed by Western blots.

(**C**) Analysis of VIPP1, VIPP1-GFP and VIPP1Δc-GFP supercomplexes with Native PAGE. 3-12% Native PAGE was used to separate chloroplast proteins of Col, *VIPP1-GFP*/Col and *VIPP1Δc-GFP*/Col isolated as described in Supplemental Methods. Blue arrowhead indicated VIPP1 supercomplexes of different lines. Red arrowhead marked the supercomplexes of VIPP1-GFP (upper panel) and VIPP1Δc-GFP (middle panel), respectively.

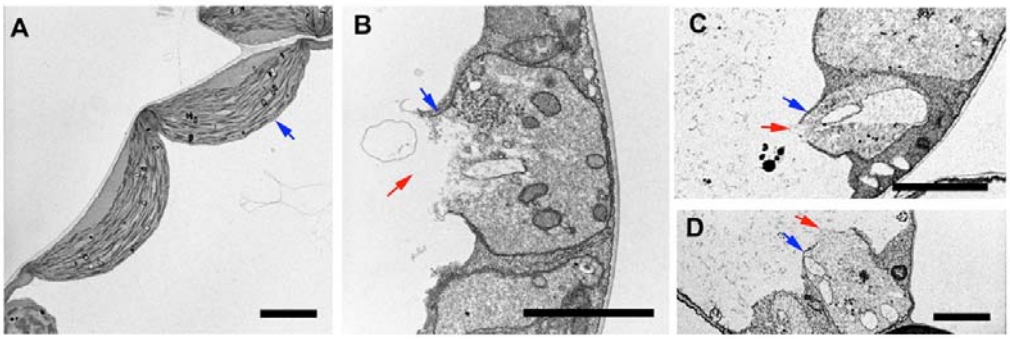


Supplementary Figure S5 Negative stain and TEM observation of VIPP1 and VIPP1Δc in Tris buffer. Recombinant VIPP1 and VIPP1Δc expressed and purified from *E. coli* in Tris buffer were visualized using negative stain and subsequent observation by TEM. Bars = 40 nm. A typical FVP observed in VIPP1 and VIPP1Δc is indicated by black or red arrow, respectively, and variable VIPP1Δc aggregates that look different from those in VIPP1 are indicated by blue arrows.

Supplementary Figure S6 Bright-field still image of the chloroplasts shown in Supplementary Movie 2.



Supplementary Fig. S7 Immunoblot detection of PSPA-GFP and PSPAvc-GFP in *PSPA-GFP/*Col, *PSPAvc-GFP/*Col, *PSPA-GFP/vipp1-ko*, and *PSPAvc-GFP/vipp1-ko*. (**A**) Total leaf protein extracts from Col, *PSPA-GFP/*Col, *PSPAvc-GFP/*Col (normalized based on fresh weight) were probed with anti-GFP antibodies (upper panel). Lower panel indicates CBB-stained SDS-PAGE gel of the same proteins. Molecular size markers (M) are shown on the left.
(**B**) Total leaf protein extracts from Col, *vipp1-ko, PSPA-GFP*/*vipp1-ko*, and *PSPAvc-GFP*/*vipp1-ko* (normalized based on fresh weight) were probed with anti-GFP antibodies (upper panel). Lower panel indicates CBB-stained SDS-PAGE gel of the same proteins. Molecular size markers (M) are shown on the left.



Supplementary Fig. S8 Broken chloroplast/plastids of *vipp1* mutants under transmission electron microscopy (TEM). Chloroplast ultrastructure of Col (*A*), *vipp1-kd* (*B*) and *vipp1-ko* (*C* and *D*) seedlings was examined with TEM. The chloroplast envelope was indicated by blue arrowheads. Damaged sites of chloroplast envelope were marked by red arrowheads. Bars = 2 μm.