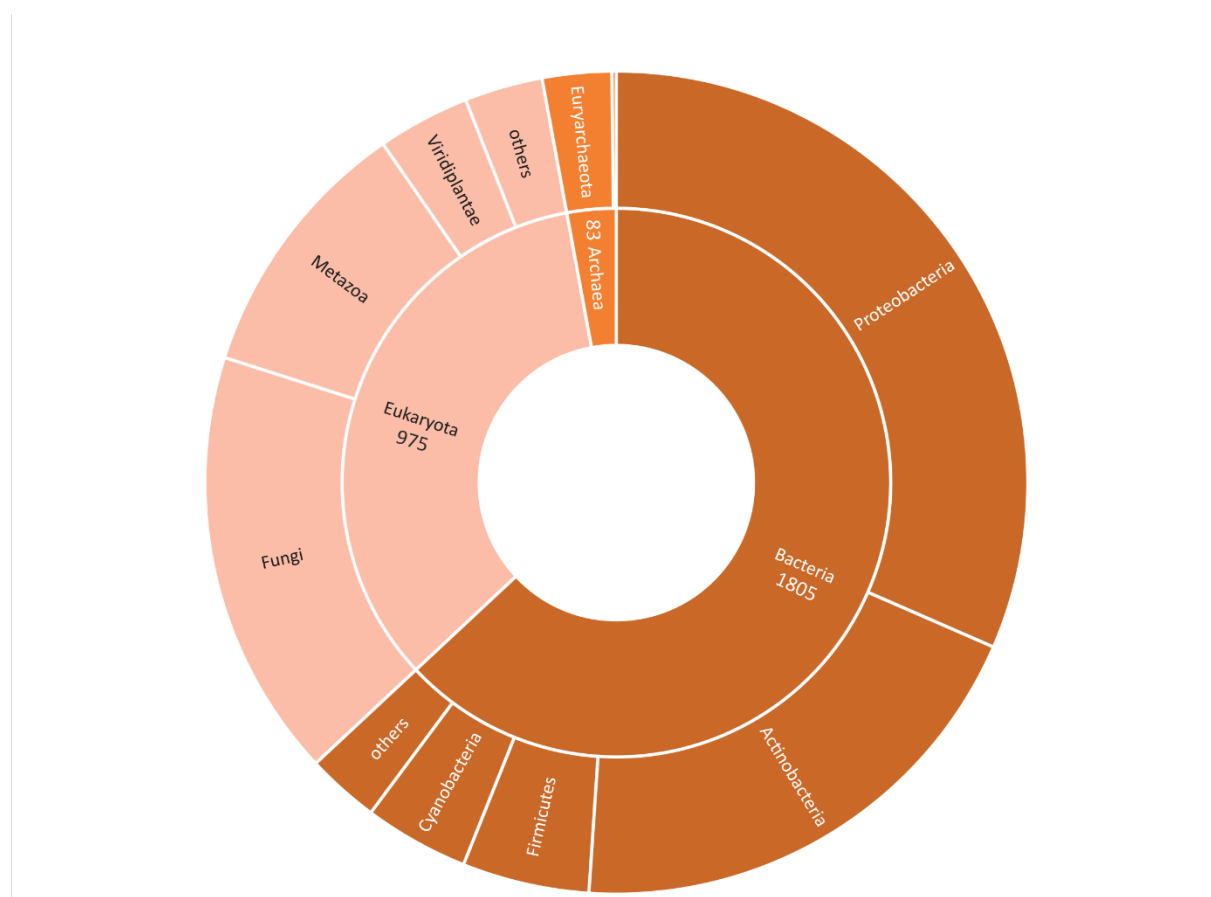


Supplemental Figures and Tables



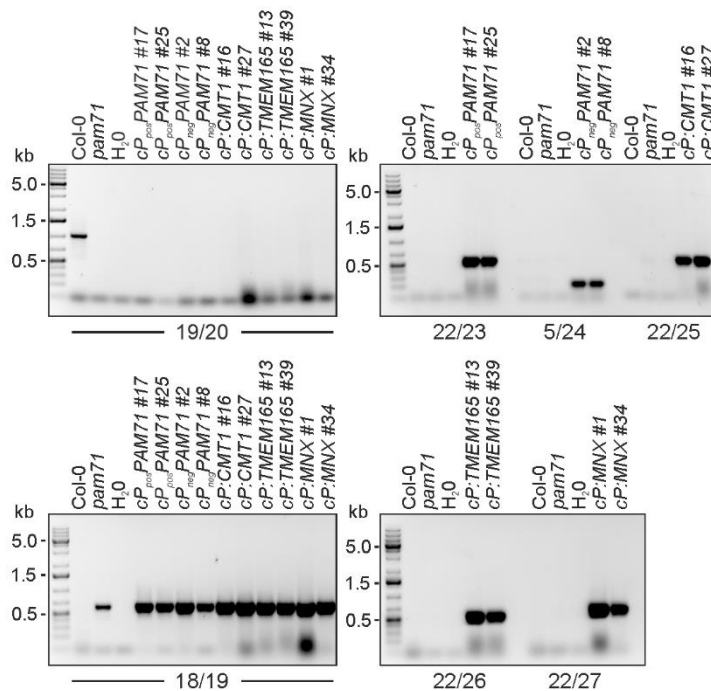
**Figure S1.** Distribution of UPF0016 sequences among Eukaryotes, Bacteria and Archaea. Data were retrieved from the protein family database at <http://pfam.xfam.org/> accessed on 01. March 2021. The three superkingdoms including currently collected number of species within the Eukaryote, Bacteria and Archaea domains are shown. The eukaryotic domain is divided into kingdoms (Fungi, Metazoa, Viridiplantae and others) and bacterial and archaeal domains are divided into phyla.



**A**

| plant line                       | T <sub>1</sub> plant<br>Fv/Fm | T <sub>2</sub> plants<br>segregation<br>(+ : -) | assumption<br>3:1 model |
|----------------------------------|-------------------------------|-------------------------------------------------|-------------------------|
| <i>cP<sub>pos</sub>PAM71</i> #17 | 0,82                          | 27 : 9                                          | $\chi^2 = 0$            |
| <i>cP<sub>pos</sub>PAM71</i> #25 | 0,83                          | 30 : 6                                          | $\chi^2 = 1,33$         |
| <i>cP<sub>neg</sub>PAM71</i> #2  | 0,58                          | 25 : 10                                         | $\chi^2 = 0,24$         |
| <i>cP<sub>neg</sub>PAM71</i> #8  | 0,57                          | 27 : 8                                          | $\chi^2 = 0,09$         |
| <i>cP:CMT1</i> #16               | 0,82                          | 23 : 13                                         | $\chi^2 = 2,37$         |
| <i>cP:CMT1</i> #27               | 0,82                          | 28 : 8                                          | $\chi^2 = 0,15$         |
| <i>cP:TMEM165</i> #13            | 0,78                          | 28 : 8                                          | $\chi^2 = 0,15$         |
| <i>cP:TMEM165</i> #39            | 0,78                          | 30 : 6                                          | $\chi^2 = 1,33$         |
| <i>cP:MNX</i> #1                 | 0,79                          | 30 : 6                                          | $\chi^2 = 1,33$         |
| <i>cP:MNX</i> #34                | 0,80                          | 28 : 8                                          | $\chi^2 = 0,15$         |

**B**

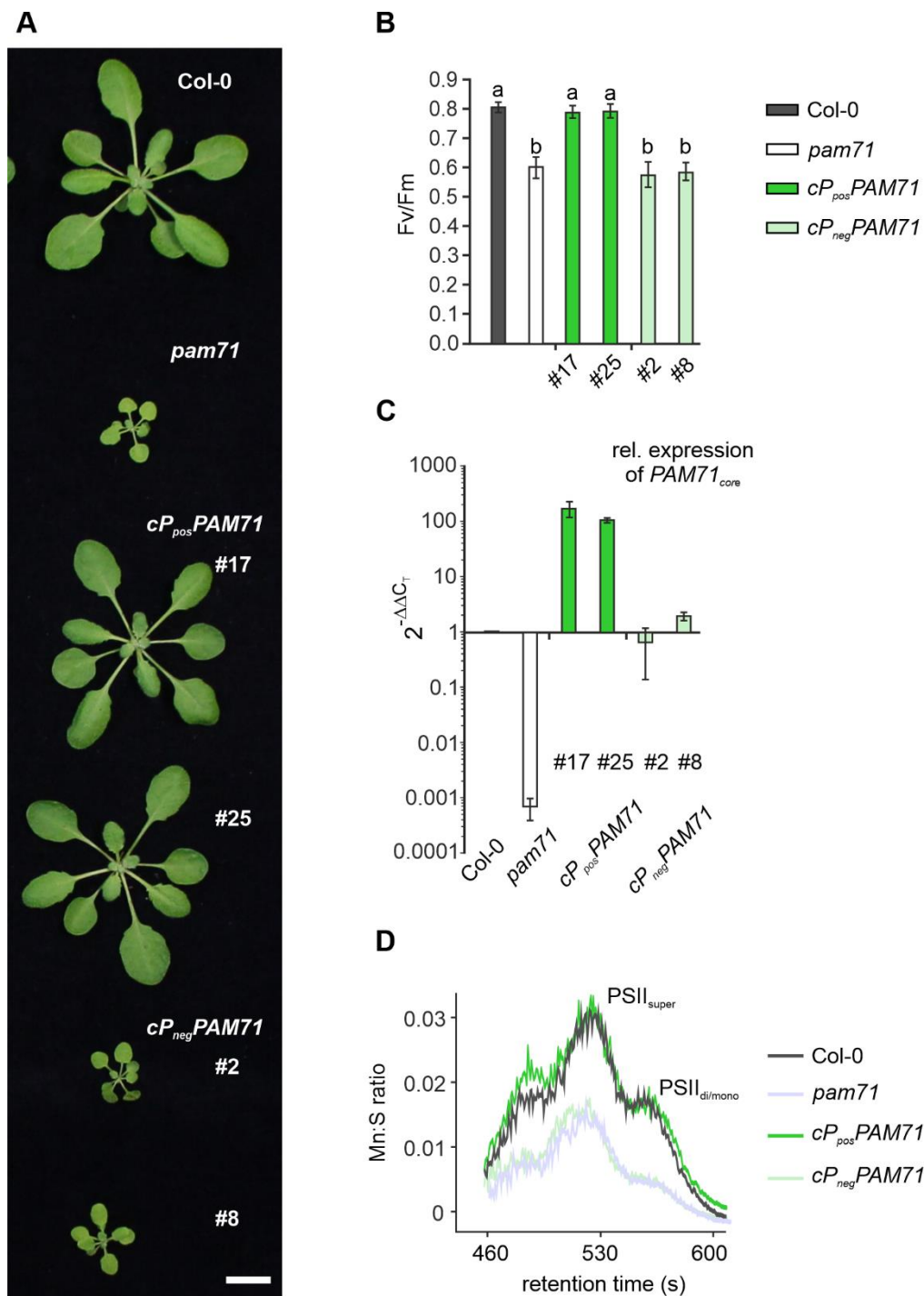


**Figure S3.** Selection of individual transgenic lines with *pam71* genetic background.

(A) The maximum quantum yield of PSII (Fv/Fm) of selected T<sub>1</sub> plants is shown in the left panel. Selected T<sub>1</sub> plants were allowed to self-pollinate and the presence (+) and absence (-) of transgenes in the following generation (T<sub>2</sub>) determined (middle panel). A  $\chi^2$  test was applied to judge if the null-hypothesis of a 3:1 segregation can be rejected or not (right panel). For values below 3,84 (degree of freedom: 1) the null-hypothesis cannot be rejected, which means it probably applies ( $\alpha = 0,05$ ).

(B) The indicated transgenic plant lines were subjected to PCR analysis using three primer combinations amplifying the wild-type allele (19/20, Table S1), the *pam71* mutant allele

(18/19, Table S1) and the specific constructs (right panels, for primer sequences see Table S1) for verification. The primer combinations (given in the right panels) were used for the analysis in (A).



**Figure S4.** Comparison of *cP<sub>pos</sub>PAM71* and *cP<sub>neg</sub>PAM71* to wild-type and *pam71* plants.

(A) Rosette phenotype of the indicated genotype. Plants were grown for 3,5 weeks in a 12 h-12 h light-dark cycle. Scale bar = 1 cm.

(B) Photosynthetic activity of the indicated genotypes. The maximum quantum yield of PSII (Fv/Fm) is shown as mean values  $\pm$  SD ( $n \geq 17$ ). Plants were grown for 4 weeks in a 12 h-

12 h light-dark cycle at 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Different letters indicate statistical significance according to ANOVA ( $p < 0.05$ , Tukey's HSD test).

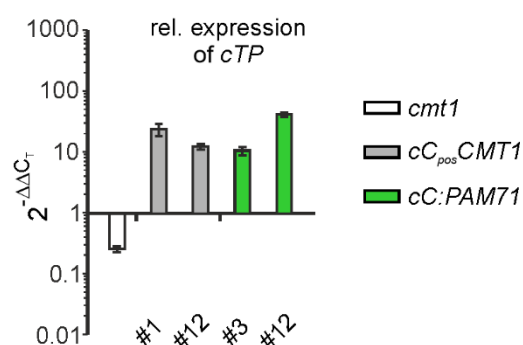
(C) Expression analysis of *PAM71<sub>core</sub>*. Quantitative real-time PCR (qRT-PCR) was performed using primer combination qRT\_PAM71\_fwd/qRT\_PAM71\_rev (Table S1). Expression levels are relative values based on the expression levels in Col-0 (=1), and *Actin2* as reference gene. Mean values  $\pm$  SD are based on three biological replicates.

(D) Quantification of the Mn:S stoichiometric ratios in the indicated genotypes. Mn:S ratios are shown in fractionated photosynthetic complexes. PSII = Photosystem II; different assembly states are indicated. Analysis was performed with two independent experiments.

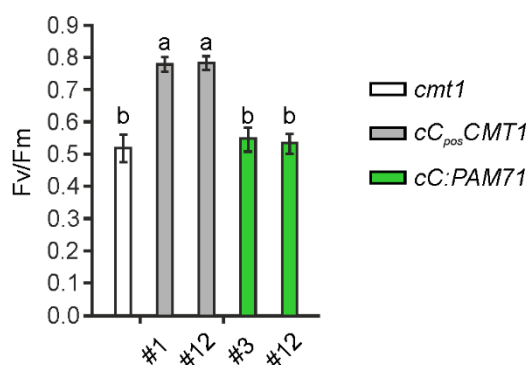
**A**

| plant line                      | T <sub>1</sub> plant<br>Fv/Fm | T <sub>2</sub> plants<br>segregation<br>(+ : -) | assumption<br>3:1 model |
|---------------------------------|-------------------------------|-------------------------------------------------|-------------------------|
| <i>cmt1</i>                     | --                            | --                                              | --                      |
| <i>cC<sub>pos</sub>CMT1</i> #1  | 0,81                          | 27 : 8                                          | $\chi^2 = 0,09$         |
| <i>cC<sub>pos</sub>CMT1</i> #12 | 0,80                          | 24 : 10                                         | $\chi^2 = 0,24$         |
| <i>cC:PAM71</i> #3              | 0,62                          | 23 : 13                                         | $\chi^2 = 2,37$         |
| <i>cC:PAM71</i> #12             | 0,57                          | 25 : 11                                         | $\chi^2 = 0,59$         |

**B**



**C**



**Figure S5.** Selection of individual transgenic lines with *cmt1* as background.

(A) The maximum quantum yield of PSII (Fv/Fm) of selected T<sub>1</sub> plants in comparison to *cmt1* is shown in the left panel. Selected T<sub>1</sub> plants were allowed to self-pollinate and the presence (+) and absence (-) of transgenes in the following generation (T<sub>2</sub>) determined (middle panel). A  $\chi^2$  test was applied to judge if the null-hypothesis of a 3:1 segregation can be rejected or not (right panel). For values below 3,84 (degree of freedom: 1) the null-hypothesis cannot be rejected, which means it probably applies ( $\alpha = 0,05$ ).

(B) Expression analysis in transgenic lines in comparison to *cmt1*. Quantitative real-time PCR (qRT-PCR) was performed using primer combination

qRT\_CMT1cTP\_fwd/qRT\_CMT1cTP\_rev (Table S1) for expression of *cTP<sub>CMT1</sub>*. Expression levels are relative values based on the expression levels in Col-0 (=1), and Actin2 as reference gene. Mean values  $\pm$  SD are based on three biological replicates.

(C) Photosynthetic activity of transgenic lines in comparison to *cmt1*. The maximum quantum yield of PSII (Fv/Fm) of selected transgenic lines is shown as mean values  $\pm$  SD ( $n \geq 17$ ) in comparison to *cmt1* plants. Plants were grown for 4 weeks in a 12 h-12 h light-dark cycle at 100  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Different letters indicate statistical significance according to ANOVA ( $p < 0.05$ , Tukey's HSD test).



**Table S1:** Oligonucleotides used in this study. Vector sequences and restriction sites are underlined. Primers 10 to 15 were used in Gibson assembly (GA) cloning, GK-LB is the abbreviation for Gabi-Kat-Left-Border, GT stands for genotyping.

|    | Primer name      | Sequence (5'-3')                                                   | Application                       |
|----|------------------|--------------------------------------------------------------------|-----------------------------------|
| 1  | PAM71cTP_F       | <u>CAC</u> CAT GCT AAG TTT GAA TCT CTC G                           | Generation of chimeric constructs |
| 2  | PAM71cTP_R       | TTT <u>CTC GAG</u> GTA ACA AGC CGC                                 |                                   |
| 3  | PAM71core-F      | TTT <u>CTC GAG</u> AAA GCT GAG TCT GAA G                           |                                   |
| 4  | PAM71core-R      | TTA CGT AAC GAT CTC AGC CAC C                                      |                                   |
| 5  | PAM71neg-F       | <u>CAC</u> CAT GCT CGA GAA AGC TG                                  |                                   |
| 6  | CMT1core-F       | AAC <u>CTC GAG</u> GGG CCA TTT C                                   |                                   |
| 7  | CMT1core-R       | TCA GAA CAC TCC AAA GAA TGT GG                                     |                                   |
| 8  | TMEM165-F        | TTT <u>CTC GAG</u> GCG GCC GCG GCT CCA GGG AAC GGC                 |                                   |
| 9  | TMEM165-R        | TTA AAA ACC AGA ATC AGG GCT TAT                                    |                                   |
| 10 | GA_PAM71N-F      | <u>CAT TAC AAT TTA CTA TTC TAG TCG AAT</u> GCT AAG TTT GAA TCT CTC |                                   |
| 11 | GA_PAM71N-R      | TAA AAG CGG TCA GAA TAT CTC CAA GGT CTC C                          |                                   |
| 12 | GA_MNX-F         | CCT TGG AGA TAT TCT GAC CGC TTT TAC TGC C                          |                                   |
| 13 | GA_MNX-R         | <u>ATT TTT GCG GAC TCT AGC ATG GCC GCT</u> ATG CAA TCT TGG TCC AC  |                                   |
| 14 | GA_pB2GW7-F      | <u>CGG CCA TGC TAG AGT CCG CAA AAA TCA C</u>                       |                                   |
| 15 | GA_pB2GW7-R      | <u>TCG ACT AGA ATA GTA AAT TGT AAT G</u>                           |                                   |
| 16 | CMT1cTP_F        | <u>CAC</u> CAT GAA GCT CAC AAG CTT G                               | Genotyping                        |
| 17 | CMT1cTP_R        | TTT <u>CTC GAG</u> GCT TTC TGA TGA AGT G                           |                                   |
| 18 | GK-LB            | ATA TTG ACC ATC ATA CTC ATT GC                                     |                                   |
| 19 | 1g64150-F        | AAT TCT CCT GAA ACT TGG AAG ACA                                    |                                   |
| 20 | 1g64150-R        | TTC GGT CTT TAG AAC ACA CTC TCT                                    |                                   |
| 21 | GT-CMT1cTP-F     | CAG ATG CTG GTG TTG GAT C                                          |                                   |
| 22 | GT-PAM71cTP-F    | GTC TCC GCT TAT CCA ATT CC                                         |                                   |
| 23 | GT-PAM71-R1      | GAC ATA GTG GAA AGT GCG TC                                         |                                   |
| 24 | GT-PAM71-R2      | CAA GGT CTC CAA AAG AAG TGA CA                                     |                                   |
| 25 | GT-CMT1-R        | CAC CAT TCT TGG CTT CCA C                                          |                                   |
| 26 | GT-TMEM165-R     | CAT GTC ATT AGT CCC AAG GC                                         |                                   |
| 27 | GT-MNX-R         | CTG CAT CTT CCA TTT CCT CC                                         |                                   |
| 28 | qRT_actin_fwd    | CTC TTT CTT TCC AAG CTC ATA AAA AAT G                              | qRT-PCR                           |
| 29 | qRT_actin_rev    | CAG CAC AAT ACC GGT TGT ACG AC                                     |                                   |
| 30 | qRT_PAM71cTP_fwd | GTC GCT TCG CAT TCC ATT CC                                         |                                   |
| 31 | qRT_PAM71cTP_rev | TAA CAA GCC GCA TCG TTT CG                                         |                                   |
| 32 | qRT_PAM71_fwd    | CAA GAA GAG AGA TTC AGA GAG TTC                                    |                                   |
| 33 | qRT_PAM71_rev    | GAA ACA GAA CTC CTC CGA CAT AAG                                    |                                   |
| 34 | qRT_CMT1_fwd     | TAA TTC CAC AGC AAC AGC AG                                         |                                   |
| 35 | qRT_CMT1_rev     | ATT TTC CCC AGA GCA AAT CC                                         |                                   |

**Table S2.** Proteomic analysis of envelope and thylakoid membrane fractions isolated from wild-type (Col-0) and *cP<sub>neg</sub>PAM71* #8 plants.

| UniProt ID    | protein name                                 | Col-0         |              | <i>cP<sub>neg</sub>PAM71</i> |              |
|---------------|----------------------------------------------|---------------|--------------|------------------------------|--------------|
|               |                                              | thylakoid (%) | envelope (%) | thylakoid (%)                | envelope (%) |
| <b>Q94AX5</b> | PAM71                                        | n.d.          | n.d.         | n.d.                         | n.d.         |
| <b>Q9T0H9</b> | CMT1 (Arabidopsis)                           | n.d.          | 100,00       | 19,92                        | 80,08        |
| <b>Q9HC07</b> | TMEM165 (human)                              | n.d.          | n.d.         | n.d.                         | n.d.         |
| <b>P52876</b> | MNX (Synechocystis)                          | n.d.          | n.d.         | n.d.                         | n.d.         |
|               | <b>envelope marker:</b>                      |               |              |                              |              |
| <b>Q9ZTZ7</b> | K(+) efflux antiporter 1                     | 12,88         | 87,12        | 25,66                        | 74,34        |
| <b>O65272</b> | K(+) efflux antiporter 2                     | 14,73         | 85,27        | 5,60                         | 94,40        |
| <b>Q9LXV3</b> | Dicarboxylate transporter 1                  | 10,40         | 89,60        | 31,60                        | 68,40        |
| <b>Q9FMF7</b> | Dicarboxylate transporter 2.1                | 7,31          | 92,69        | 6,28                         | 93,72        |
| <b>Q9FMF8</b> | Dicarboxylate transporter 2.2                | n.d.          | 100,00       | n.d.                         | 100,00       |
| <b>Q9ZSR7</b> | Triose phosphate/phosphate translocator      | 20,88         | 79,12        | 6,61                         | 93,39        |
| <b>Q8RXN3</b> | Phosphoenolpyruvate/phosphate translocator 1 | 16,72         | 83,28        | 23,05                        | 76,95        |
| <b>Q9SZC9</b> | Copper-transporting ATPase PAA1              | n.d.          | 100,00       | n.d.                         | 100,00       |
|               | <b>thylakoid marker:</b>                     |               |              |                              |              |
| <b>Q9SHR7</b> | Chlorophyll a-b binding protein 2.1          | 100,00        | n.d.         | n.d.                         | n.d.         |
| <b>P83755</b> | Photosystem II protein D1                    | 84,53         | 15,47        | 62,95                        | 37,05        |
| <b>P56761</b> | Photosystem II protein D2                    | 82,97         | 17,03        | 57,29                        | 42,71        |
| <b>P56778</b> | Photosystem II CP43 reaction center protein  | 86,43         | 13,57        | 60,42                        | 39,58        |
| <b>P56777</b> | Photosystem II CP47 reaction center protein  | 91,87         | 8,13         | 82,27                        | 17,73        |
| <b>B9DFX7</b> | Copper-transporting ATPase PAA2              | 64,83         | 35,17        | 36,16                        | 63,84        |
| <b>Q9M0Z3</b> | K(+) efflux antiporter 3                     | 73,62         | 26,38        | 63,89                        | 36,11        |

**Table S3.** Proteomic analysis of thylakoid and envelope membrane fractions isolated from *cP<sub>pos</sub>PAM71* #25 plants. Experiments were derived from three independent grown batches of plants.

| <i>cP<sub>pos</sub>PAM71</i> |                                              | experiment 1  |              | experiment 2  |              | experiment 3  |              |
|------------------------------|----------------------------------------------|---------------|--------------|---------------|--------------|---------------|--------------|
| UniProt ID                   | protein name                                 | thylakoid (%) | envelope (%) | thylakoid (%) | envelope (%) | thylakoid (%) | envelope (%) |
| <b>Q94AX5</b>                | PAM71                                        | 51,96         | 48,04        | 50,48         | 49,52        | 33,13         | 66,87        |
| <b>Q9T0H9</b>                | CMT1 (Arabidopsis)                           | 36,52         | 63,48        | 6,92          | 93,08        | 15,62         | 84,38        |
| <b>Q9HC07</b>                | TMEM165 (human)                              | n.d.          | n.d.         | n.d.          | n.d.         | n.d.          | n.d.         |
| <b>P52876</b>                | MNX (Synechocystis)                          | n.d.          | n.d.         | n.d.          | n.d.         | n.d.          | n.d.         |
| <b>envelope marker:</b>      |                                              |               |              |               |              |               |              |
| <b>Q9ZTZ7</b>                | K(+) efflux antiporter 1                     | 15,18         | 84,82        | 4,60          | 95,40        | 6,76          | 93,24        |
| <b>O65272</b>                | K(+) efflux antiporter 2                     | 8,95          | 91,05        | 1,46          | 98,54        | 2,09          | 97,91        |
| <b>Q9LXV3</b>                | Dicarboxylate transporter 1                  | 4,93          | 95,07        | 8,61          | 91,39        | 12,37         | 87,63        |
| <b>Q9FMF7</b>                | Dicarboxylate transporter 2.1                | 8,43          | 91,57        | 5,16          | 94,84        | 6,41          | 93,59        |
| <b>Q9FMF8</b>                | Dicarboxylate transporter 2.2                | n.d.          | 100,00       | n.d.          | 100,00       | n.d.          | 100,00       |
| <b>Q9ZSR7</b>                | Triose phosphate/phosphate translocator      | 4,86          | 95,14        | 0,73          | 99,27        | 25,73         | 74,27        |
| <b>Q8RXN3</b>                | Phosphoenolpyruvate/phosphate translocator 1 | 8,15          | 91,85        | 19,25         | 80,75        | 23,85         | 76,15        |
| <b>Q9SZC9</b>                | Copper-transporting ATPase PAA1              | 11,44         | 88,56        | n.d.          | 100,00       | n.d.          | 100,00       |
| <b>thylakoid marker:</b>     |                                              |               |              |               |              |               |              |
| <b>Q9SHR7</b>                | Chlorophyll a-b binding protein 2.1          | 100,00        | n.d.         | 100,00        | n.d.         | 100,00        | n.d.         |
| <b>P83755</b>                | Photosystem II protein D1                    | 76,18         | 23,82        | 78,25         | 21,75        | 67,08         | 32,92        |
| <b>P56761</b>                | Photosystem II protein D2                    | 76,36         | 23,64        | 66,97         | 33,03        | 64,04         | 35,96        |
| <b>P56778</b>                | Photosystem II CP43 reaction center protein  | 68,99         | 31,01        | 60,89         | 39,11        | 63,50         | 36,50        |
| <b>P56777</b>                | Photosystem II CP47 reaction center protein  | 85,02         | 14,98        | 78,43         | 21,57        | 67,24         | 32,76        |
| <b>B9DFX7</b>                | Copper-transporting ATPase PAA2              | 46,39         | 53,61        | n.d.          | 100,00       | 14,29         | 85,71        |
| <b>Q9M0Z3</b>                | K(+) efflux antiporter 3                     | 100,00        | n.d.         | n.d.          | 100,00       | n.d.          | 100,00       |

**Table S4.** Proteomic analysis of thylakoid and envelope membrane fractions isolated from *cP:MNX* #1 plants. Experiments were derived from three independent grown batches of plants.

| <i>cP:MNX</i> |                                              | experiment 1  |              | experiment 2  |              | experiment 3  |              |
|---------------|----------------------------------------------|---------------|--------------|---------------|--------------|---------------|--------------|
| UniProt ID    | protein name                                 | thylakoid (%) | envelope (%) | thylakoid (%) | envelope (%) | thylakoid (%) | envelope (%) |
| <b>Q94AX5</b> | PAM71                                        | n.d.          | n.d.         | n.d.          | n.d.         | n.d.          | n.d.         |
| <b>Q9T0H9</b> | CMT1 (Arabidopsis)                           | 22,63         | 77,37        | 21,29         | 78,71        | 9,98          | 90,02        |
| <b>Q9HC07</b> | TMEM165 (human)                              | n.d.          | n.d.         | n.d.          | n.d.         | n.d.          | n.d.         |
| <b>P52876</b> | MNX (Synechocystis)                          | 24,46         | 75,54        | 44,77         | 55,23        | 59,05         | 40,95        |
|               | <b>envelope marker:</b>                      |               |              |               |              |               |              |
| <b>Q9ZTZ7</b> | K(+) efflux antiporter 1                     | 16,62         | 83,38        | 23,43         | 76,57        | 8,26          | 91,74        |
| <b>O65272</b> | K(+) efflux antiporter 2                     | 6,94          | 93,06        | 9,43          | 90,57        | 5,08          | 94,92        |
| <b>Q9LXV3</b> | Dicarboxylate transporter 1                  | 4,21          | 95,79        | 16,17         | 83,83        | 15,25         | 84,75        |
| <b>Q9FMF7</b> | Dicarboxylate transporter 2.1                | 9,04          | 90,96        | 15,92         | 84,08        | 2,52          | 97,48        |
| <b>Q9FMF8</b> | Dicarboxylate transporter 2.2                | n.d.          | 100,00       | n.d.          | 100,00       | n.d.          | 100,00       |
| <b>Q9ZSR7</b> | Triose phosphate/phosphate translocator      | 4,69          | 95,31        | 17,18         | 82,82        | 7,17          | 92,83        |
| <b>Q8RXN3</b> | Phosphoenolpyruvate/phosphate translocator 1 | 11,35         | 88,65        | 24,44         | 75,56        | 22,76         | 77,24        |
| <b>Q9SZC9</b> | Copper-transporting ATPase PAA1              | n.d.          | 100,00       | n.d.          | n.d.         | 29,76         | 70,24        |
|               | <b>thylakoid marker:</b>                     |               |              |               |              |               |              |
| <b>Q9SHR7</b> | Chlorophyll a-b binding protein 2.1          | 79,55         | 20,45        | 69,08         | 30,92        | 90,17         | 9,83         |
| <b>P83755</b> | Photosystem II protein D1                    | 69,07         | 30,93        | 71,18         | 28,82        | 57,92         | 42,08        |
| <b>P56761</b> | Photosystem II protein D2                    | 69,99         | 30,01        | 64,70         | 35,30        | 56,54         | 43,46        |
| <b>P56778</b> | Photosystem II CP43 reaction center protein  | 73,88         | 26,12        | 67,37         | 32,63        | 59,29         | 40,71        |
| <b>P56777</b> | Photosystem II CP47 reaction center protein  | 92,50         | 7,50         | 80,17         | 19,83        | 58,38         | 41,62        |
| <b>B9DFX7</b> | Copper-transporting ATPase PAA2              | 47,96         | 52,04        | 48,57         | 51,43        | 27,46         | 72,54        |
| <b>Q9M0Z3</b> | K(+) efflux antiporter 3                     | 68,93         | 31,07        | 23,36         | 76,64        | n.d.          | 100,00       |

**Table S5.** Proteomic analysis of envelope and thylakoid membrane fractions isolated from *cP:TMEM165* #39 plants. Experiments were derived from three independent grown batches of plants.

| <i>cP:TMEM165</i>        |                                              | experiment 1  |              | experiment 2  |              | experiment 3  |              |
|--------------------------|----------------------------------------------|---------------|--------------|---------------|--------------|---------------|--------------|
| UniProt ID               | protein name                                 | thylakoid (%) | envelope (%) | thylakoid (%) | envelope (%) | thylakoid (%) | envelope (%) |
| <b>Q94AX5</b>            | PAM71                                        | n.d.          | n.d.         | n.d.          | n.d.         | n.d.          | n.d.         |
| <b>Q9T0H9</b>            | CMT1 (Arabidopsis)                           | 30,77         | 69,23        | 17,96         | 82,04        | 18,62         | 81,38        |
| <b>Q9HC07</b>            | TMEM165 (human)                              | 16,97         | 83,03        | 30,40         | 69,60        | 16,87         | 83,13        |
| <b>P52876</b>            | MNX (Synechocystis)                          | n.d.          | n.d.         | n.d.          | n.d.         | n.d.          | n.d.         |
| <b>envelope marker:</b>  |                                              |               |              |               |              |               |              |
| <b>Q9ZTZ7</b>            | K(+) efflux antiporter 1                     | 12,66         | 87,34        | 15,07         | 84,93        | 12,74         | 87,26        |
| <b>O65272</b>            | K(+) efflux antiporter 2                     | 6,59          | 93,41        | 6,83          | 93,17        | 6,87          | 93,13        |
| <b>Q9LXV3</b>            | Dicarboxylate transporter 1                  | 35,29         | 64,71        | 3,25          | 96,75        | 22,15         | 77,85        |
| <b>Q9FMF7</b>            | Dicarboxylate transporter 2.1                | 5,88          | 94,12        | 31,21         | 68,79        | 2,33          | 97,67        |
| <b>Q9FMF8</b>            | Dicarboxylate transporter 2.2                | 10,80         | 89,20        | n.d.          | 100,00       | n.d.          | 100,00       |
| <b>Q9ZSR7</b>            | Triose phosphate/phosphate translocator      | 32,10         | 67,90        | 25,84         | 74,16        | 13,19         | 86,81        |
| <b>Q8RXN3</b>            | Phosphoenolpyruvate/phosphate translocator 1 | 12,30         | 87,70        | 20,73         | 79,27        | 22,26         | 77,74        |
| <b>Q9SZC9</b>            | Copper-transporting ATPase PAA1              | 20,02         | 79,98        | n.d.          | 100,00       | n.d.          | 100,00       |
| <b>thylakoid marker:</b> |                                              |               |              |               |              |               |              |
| <b>Q9SHR7</b>            | Chlorophyll a-b binding protein 2.1          | 100,00        | n.d.         | 100,00        | n.d.         | 100,00        | n.d.         |
| <b>P83755</b>            | Photosystem II protein D1                    | 68,46         | 31,54        | 81,18         | 18,82        | 66,24         | 33,76        |
| <b>P56761</b>            | Photosystem II protein D2                    | 73,07         | 26,93        | 75,92         | 24,08        | 63,46         | 36,54        |
| <b>P56778</b>            | Photosystem II CP43 reaction center protein  | 79,22         | 20,78        | 74,47         | 25,53        | 73,39         | 26,61        |
| <b>P56777</b>            | Photosystem II CP47 reaction center protein  | 93,74         | 6,26         | 83,35         | 16,65        | 68,08         | 31,92        |
| <b>B9DFX7</b>            | Copper-transporting ATPase PAA2              | 41,16         | 58,84        | 60,33         | 39,67        | 46,31         | 53,69        |
| <b>Q9M0Z3</b>            | K(+) efflux antiporter 3                     | 40,76         | 59,24        | 64,83         | 35,17        | 23,38         | 76,62        |

**Table S6.** Proteomic analysis of envelope and thylakoid membrane fractions isolated from *cP:CMT1* #27 plants. Experiments were derived from three independent grown batches of plants.

| <i>cP:CMT1</i> |                                              | experiment 1  |              | experiment 2  |              | experiment 3  |              |
|----------------|----------------------------------------------|---------------|--------------|---------------|--------------|---------------|--------------|
| UniProt ID     | protein name                                 | thylakoid (%) | envelope (%) | thylakoid (%) | envelope (%) | thylakoid (%) | envelope (%) |
| <b>Q94AX5</b>  | PAM71                                        | n.d.          | n.d.         | n.d.          | n.d.         | n.d.          | n.d.         |
| <b>Q9T0H9</b>  | CMT1 (Arabidopsis)                           | 55,61         | 44,39        | 45,50         | 54,50        | 11,16         | 88,84        |
| <b>Q9HC07</b>  | TMEM165 (human)                              | n.d.          | n.d.         | n.d.          | n.d.         | n.d.          | n.d.         |
| <b>P52876</b>  | MNX (Synechocystis)                          | n.d.          | n.d.         | n.d.          | n.d.         | n.d.          | n.d.         |
|                | <b>envelope marker:</b>                      |               |              |               |              |               |              |
| <b>Q9ZTZ7</b>  | K(+) efflux antiporter 1                     | 15,20         | 84,80        | 18,48         | 81,52        | 3,71          | 96,29        |
| <b>O65272</b>  | K(+) efflux antiporter 2                     | 8,01          | 91,99        | 9,83          | 90,17        | 2,73          | 97,27        |
| <b>Q9LXV3</b>  | Dicarboxylate transporter 1                  | 9,49          | 90,51        | 24,95         | 75,05        | 10,64         | 89,36        |
| <b>Q9FMF7</b>  | Dicarboxylate transporter 2.1                | 20,36         | 79,64        | 24,30         | 75,70        | 8,24          | 91,76        |
| <b>Q9FMF8</b>  | Dicarboxylate transporter 2.2                | n.d.          | 100,00       | n.d.          | n.d.         | n.d.          | 100,00       |
| <b>Q9ZSR7</b>  | Triose phosphate/phosphate translocator      | 24,12         | 75,88        | 49,28         | 50,72        | 12,27         | 87,73        |
| <b>Q8RXN3</b>  | Phosphoenolpyruvate/phosphate translocator 1 | 23,96         | 76,04        | 41,97         | 58,03        | 10,75         | 89,25        |
| <b>Q9SZC9</b>  | Copper-transporting ATPase PAA1              | n.d.          | 100,00       | 22,07         | 77,93        | n.d.          | 100,00       |
|                | <b>thylakoid marker:</b>                     |               |              |               |              |               |              |
| <b>Q9SHR7</b>  | Chlorophyll a-b binding protein 2.1          | 96,66         | 3,34         | 79,86         | 20,14        | 88,01         | 11,99        |
| <b>P83755</b>  | Photosystem II protein D1                    | 67,53         | 32,47        | 91,97         | 8,03         | 64,80         | 35,20        |
| <b>P56761</b>  | Photosystem II protein D2                    | 69,32         | 30,68        | 90,42         | 9,58         | 73,55         | 26,45        |
| <b>P56778</b>  | Photosystem II CP43 reaction center protein  | 83,18         | 16,82        | 91,81         | 8,19         | 83,50         | 16,50        |
| <b>P56777</b>  | Photosystem II CP47 reaction center protein  | 87,81         | 12,19        | 84,22         | 15,78        | 87,80         | 12,20        |
| <b>B9DFX7</b>  | Copper-transporting ATPase PAA2              | 65,93         | 34,07        | 78,44         | 21,56        | n.d.          | n.d.         |
| <b>Q9M0Z3</b>  | K(+) efflux antiporter 3                     | 57,18         | 42,82        | 91,25         | 8,75         | 72,41         | 27,59        |

**Table S7.** Proteomic analysis of envelope and thylakoid membrane fractions isolated from *cP:CMT1* #27 plants, which were grown supplementary to experiments 1-3. Median and mean absolute deviation (MAD) were calculated from data given in Tables S2 to S7.

|               |                                                     | experiment 4     |                 | Median ± MAD<br>of exp 1-4 comprising<br>genotype <i>cP:CMT1</i><br><br>N = 4 |                 | Median ± MAD<br>of exp 1-3 comprising<br>genotypes<br><i>cP<sub>pos</sub>:PAM71</i> ,<br><i>cP:TMEM165</i> ,<br><i>cP:MNX</i> , and Col-0<br>and <i>cP<sub>neg</sub>:PAM71</i><br>N = 11 |                 |
|---------------|-----------------------------------------------------|------------------|-----------------|-------------------------------------------------------------------------------|-----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| UniProt ID    | protein name                                        | thylakoid<br>(%) | envelope<br>(%) | thylakoid<br>(%)                                                              | envelope<br>(%) | thylakoid<br>(%)                                                                                                                                                                         | envelope<br>(%) |
| <b>Q9T0H9</b> | CMT1 (Arabidopsis)                                  | 34,09            | 65,91           | 39,80<br>±13,97                                                               | 60,20<br>±13,97 | 18,62<br>±7,37                                                                                                                                                                           | 81,38<br>±7,37  |
| <b>Q9ZT27</b> | <b>envelope marker:</b><br>K(+) efflux antiporter 1 | 15,80            | 84,20           | 15,50<br>±4,79                                                                | 84,50<br>±4,79  | 12,88<br>±4,73                                                                                                                                                                           | 87,12<br>±4,73  |
| <b>O65272</b> | K(+) efflux antiporter 2                            | 9,18             | 90,82           | 8,60<br>±2,35                                                                 | 91,40<br>±2,35  | 6,83<br>±2,38                                                                                                                                                                            | 93,17<br>±2,38  |
| <b>Q9LXV3</b> | Dicarboxylate transporter 1                         | 12,30            | 87,70           | 11,47<br>±5,30                                                                | 88,53<br>±5,30  | 12,37<br>±8,33                                                                                                                                                                           | 87,63<br>±8,33  |
| <b>Q9FMF7</b> | Dicarboxylate transporter 2.1                       | 13,90            | 86,10           | 17,13<br>±5,63                                                                | 82,87<br>±5,63  | 6,41<br>±5,25                                                                                                                                                                            | 93,59<br>±5,25  |
| <b>Q9FMF8</b> | Dicarboxylate transporter 2.2                       | n.d.             | 100,00          | n.d.                                                                          | 100<br>±0       | 0<br>±1,79                                                                                                                                                                               | 100<br>±1,79    |
| <b>Q9ZSR7</b> | Triose phosphate/phosphate<br>translocator          | 32,08            | 67,92           | 28,10<br>±11,24                                                               | 71,90<br>±11,24 | 13,19<br>±8,99                                                                                                                                                                           | 86,81<br>±8,99  |
| <b>Q8RXN3</b> | Phosphoenolpyruvate/phosphate<br>translocator 1     | 34,22            | 65,78           | 29,09<br>±10,37                                                               | 70,91<br>±10,37 | 20,73<br>±4,72                                                                                                                                                                           | 79,27<br>±4,72  |
| <b>Q9SZC9</b> | Copper-transporting ATPase PAA1                     | n.d.             | 100,00          | 0<br>±8,28                                                                    | 100<br>±8,28    | 10,01<br>±12,45                                                                                                                                                                          | 89,99<br>±12,45 |