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Supplemental information

Arabidopsis ABCG14 forms a homodimeric transporter for multiple cytokinins and mediates long-distance transport of isopentenyladenine-type cytokinins

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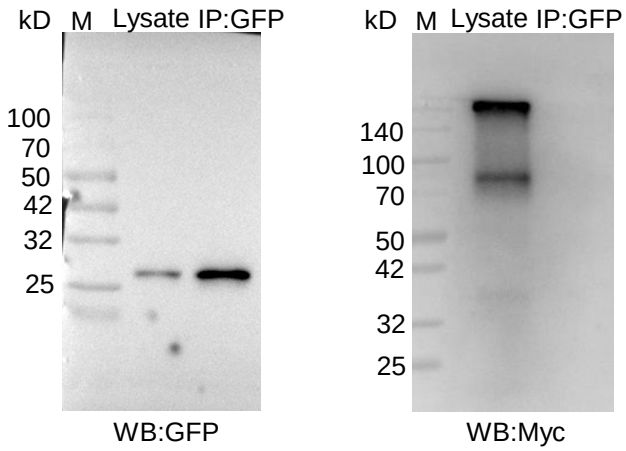
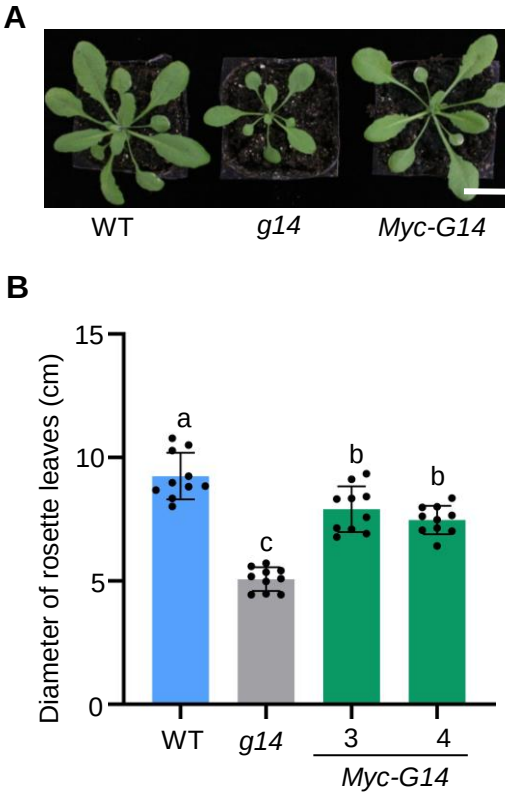


Supplemental Figure 1. Co-IP assay of free GFP and Myc-AtABCG14 expression in tobacco leaves. Total protein was extracted from tobacco leaves with co-expression of free GFP and Myc-AtABCG14.



Supplemental Figure 2. Phenotypes of *atabcg14* mutant plants complemented by *Myc-AtABCG14* fusion gene. (A) Phenotypes of 20-DAG *atabcg14* plants complemented with *Myc-AtABCG14* gene driven by 35S promoter. Scale bar, 2 cm. (B) Rosette leaf diameters of the plants in (A). The data are means ± SD, n = 10. Groups marked by different letters are significantly different ($P < 0.05$, ANOVA). *G14* represents *AtABCG14*.

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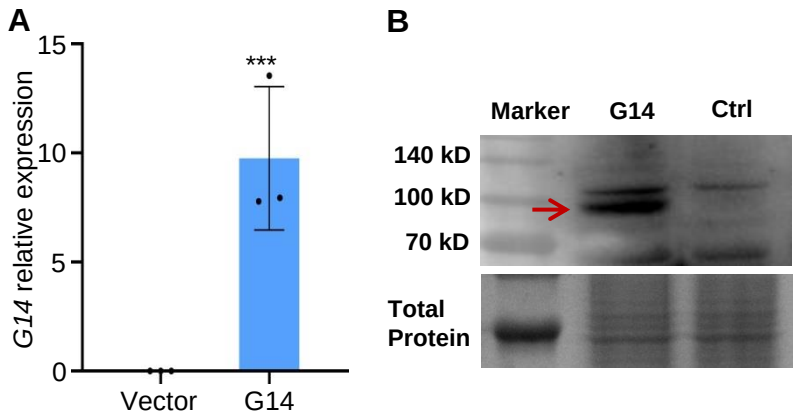
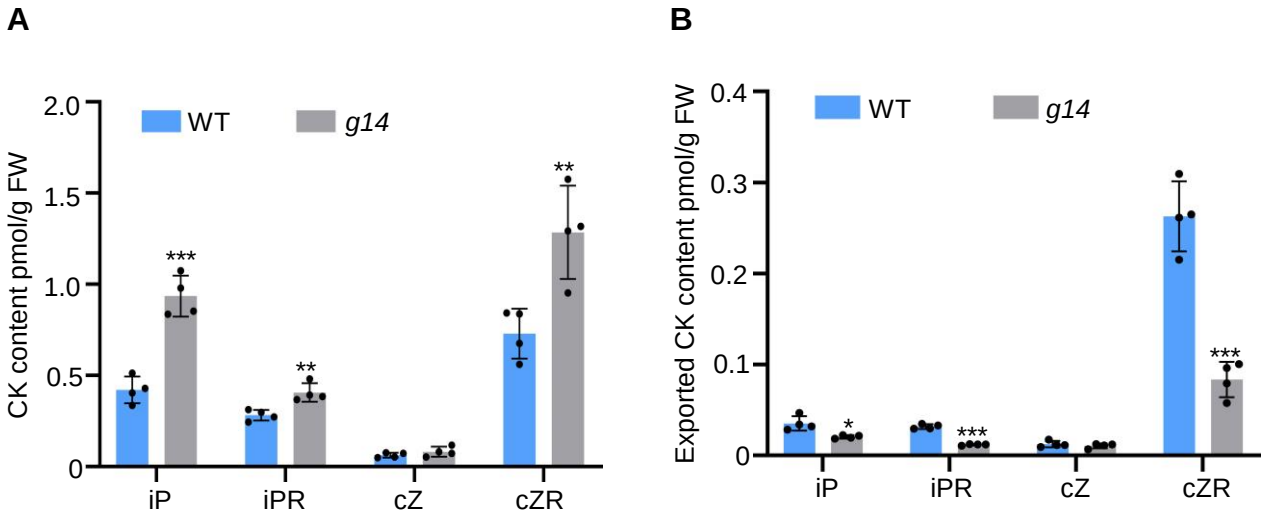
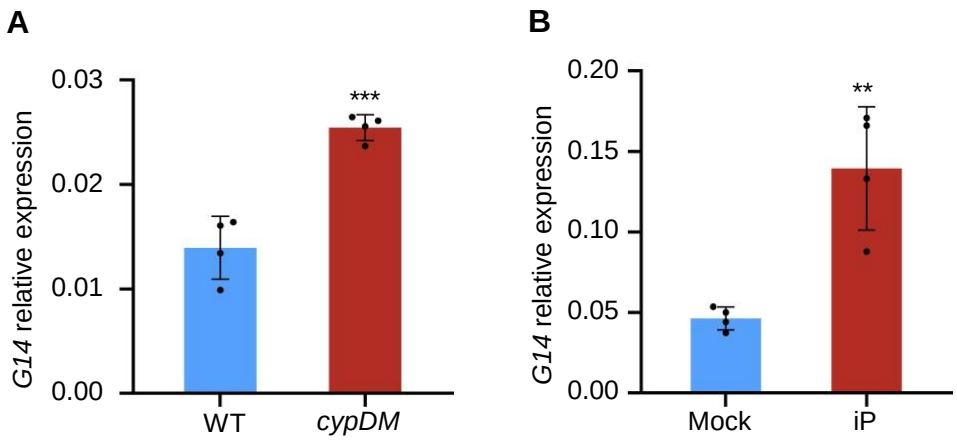


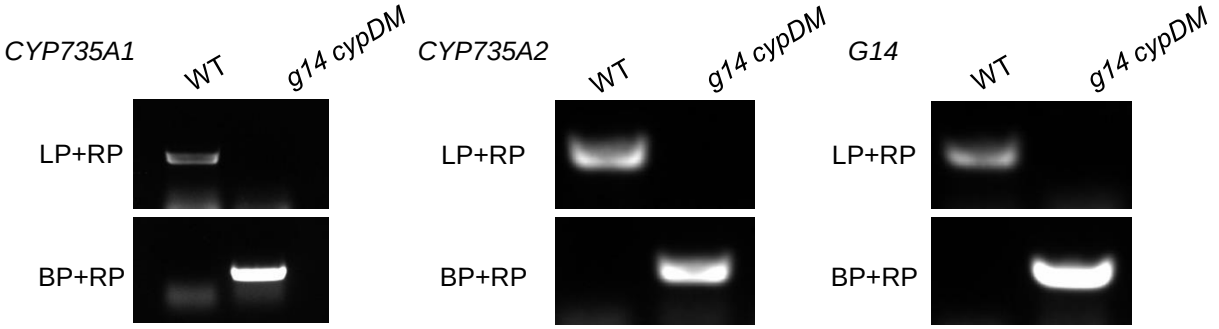
Supplemental Figure 3. The expression of *AtABCG14* in BY-2 cells transformed with *35S::GFP-AtABCG14*. (A) Stable expression of empty vector *35S::GFP* (RCS2) and *35S::GFP-AtABCG14* in BY-2. The transcript levels of the target genes were normalized to that of *ACTIN2*. Data are means ± SD (n = 4). (B) Detection of GFP-AtABCG14 fusion protein expression in BY-2 by immunity blotting. The red arrow indicates GFP–AtABCG14 protein. ***P < 0.001 (Student's t-test). G14 represents AtABCG14.



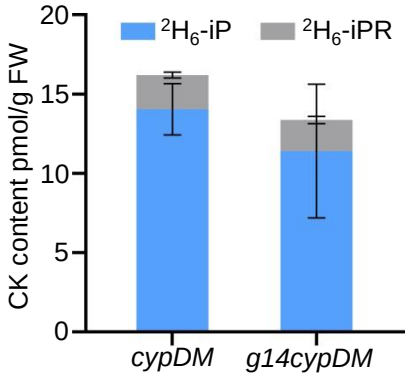
Supplemental Figure 4. Less CKs exported from *atabcg14* mutant. (A) Quantification of iP, iPR, *c*Z and *c*ZR in WT *Arabidopsis* cells and *atagcg14* mutant cells 5 d after subculture. Data are means ± SD (n = 4). (B) Quantification of the exported iP, iPR, *c*Z and *c*ZR in the B5 medium culturing WT *Arabidopsis* cells and *atagcg14* mutant cells at 4 hours. Data are means ± SD (n = 4). *, ** and *** indicate P-values of $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively, from Student's t-test.



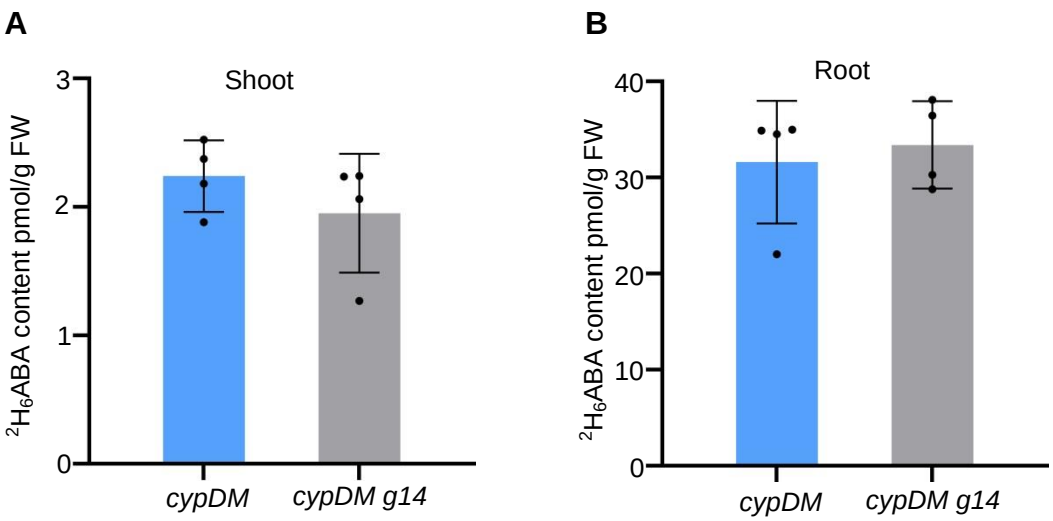
Supplemental Figure 5. Expression of *AtABCG14* was induced by iP. (A) RT-qPCR analysis of *AtABCG14* expression in 8-DAG seedlings of WT and *cypDM*. Data are means ± SD (n = 4). (B) RT-qPCR analysis of *AtABCG14* expression of 8-DAG seedlings of *cypDM* treated with 1 μM iP for 4 h. Data are means ± SD (n = 4). $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's t-test). G14 represents AtABCG14.



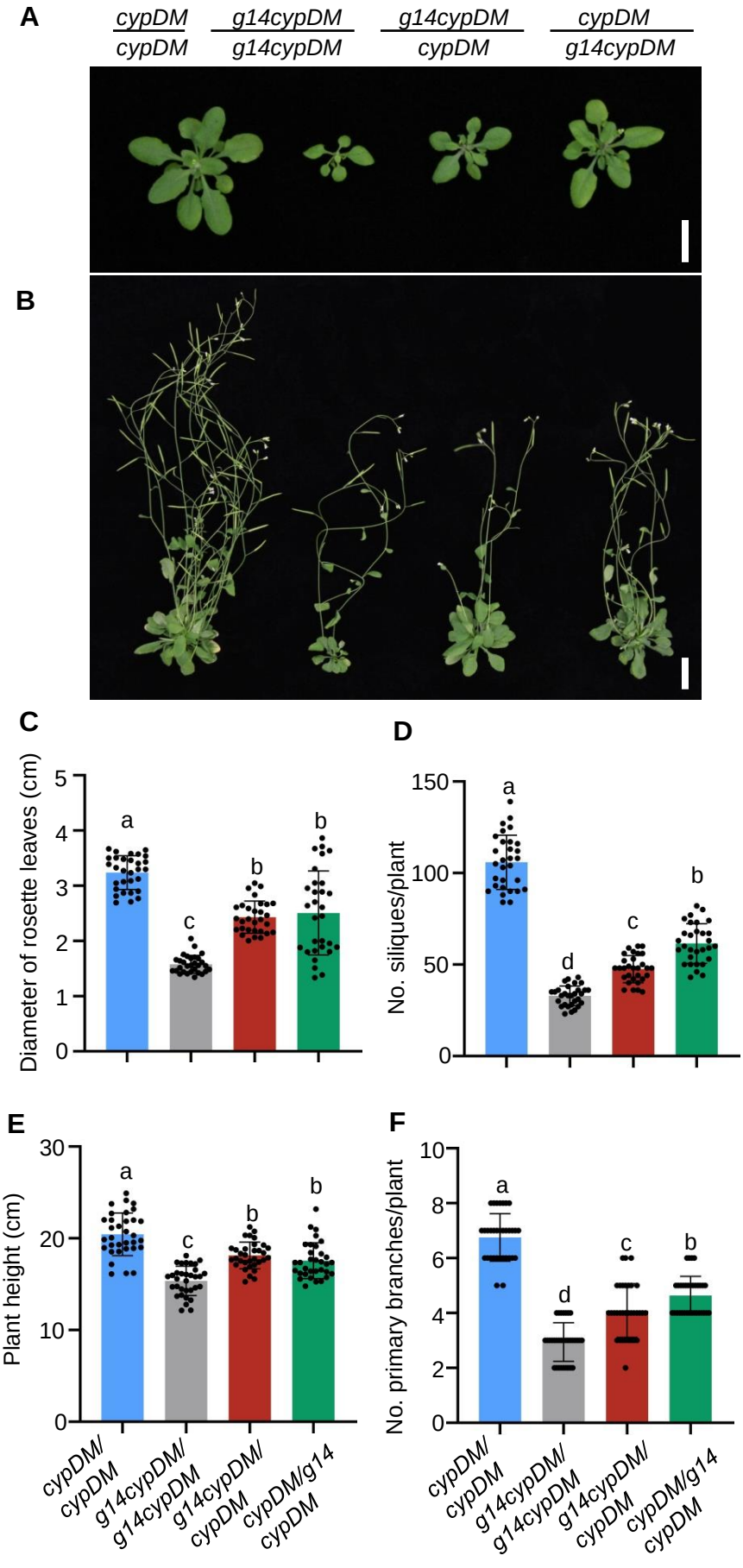
Supplemental Figure 6. Characterization of *atabcg14 cypDM* triple mutant. *abcg14 cypDM* triple mutant lines were obtained by crossing *atabcg14* with double mutant *cypDM*. Sepecific primers CYP735A1-P1, P2 and T-DNA border primer LB1.3 were used for identifying T-DNA insertion of *cyp735a1* mutant. Sepecific primers CYP735A2-P1, P2 and T-DNA border primer LB1.3 were used for identifying T-DNA insertion of *cyp735a2* mutant. Sepecific primers AtABCG14-P7, -P8 (amplifying genomic DNA fragment of *AtABCG14*) and pSKTAIL, AtABCG14-P11 (amplifying T-DNA fragment) were used for identifying T-DNA insertion of *atabcg14* mutant. *g14cypDM, atabcg14 cypDM*.



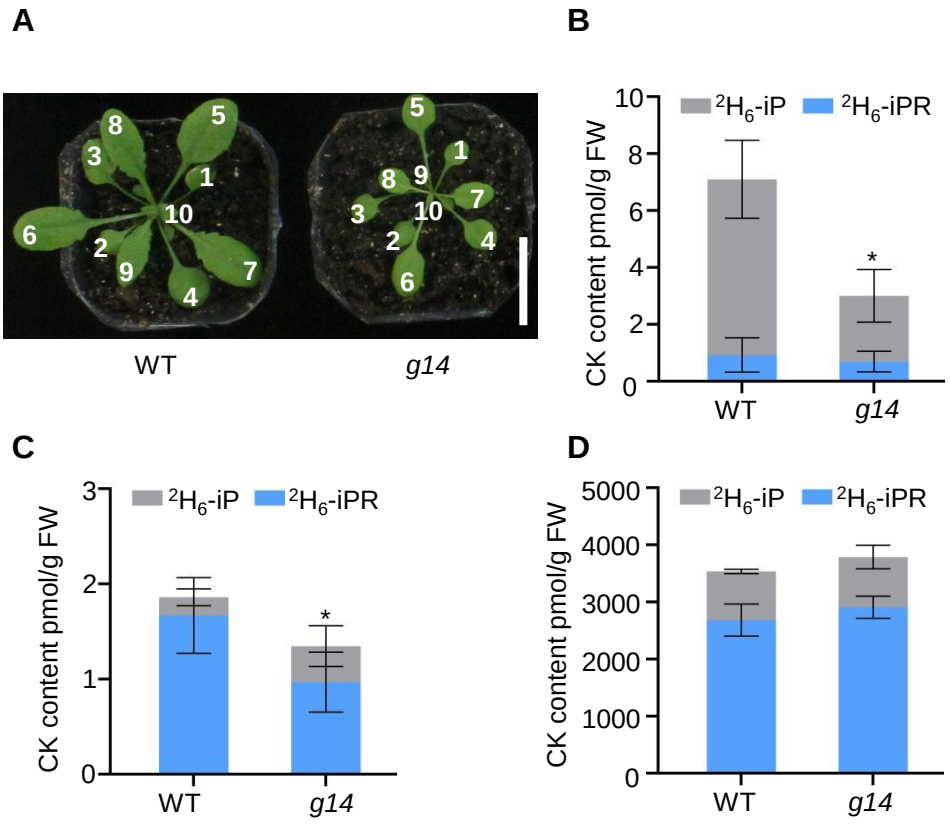
Supplemental Figure 7. ^{2}H-labled iP and iPR in *atabcg14 cypDM* roots. Roots of the 8-DAG *cypDM* and *atabcg14 cypDM* seedlings were incubated with 50 nM $^2H_6$-iP for 1 h. The roots were harvested for CK quantification.



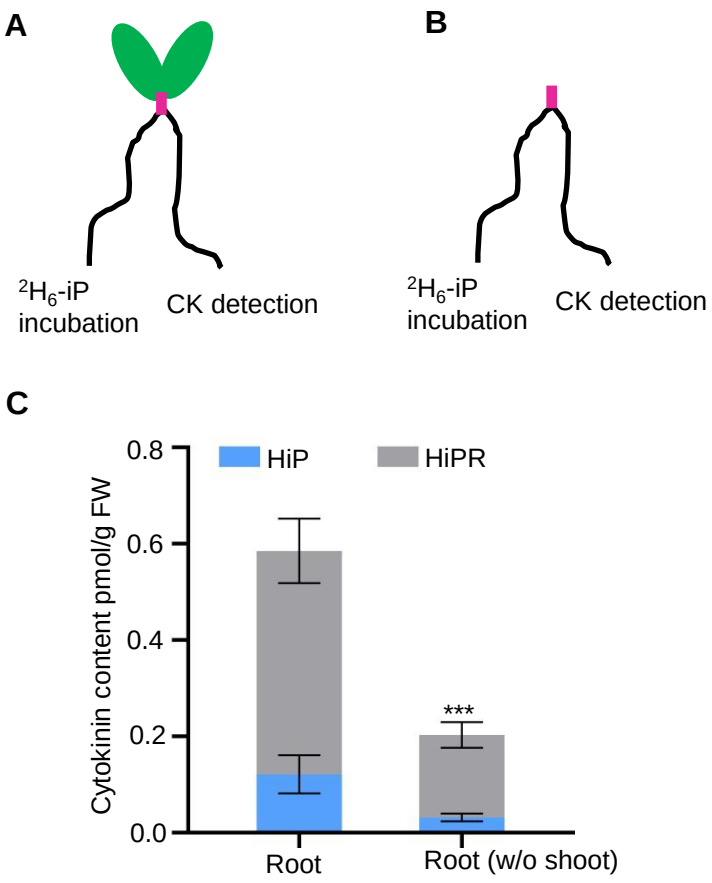
Supplemental Figure 8. Translocation of $^2H_6$-ABA in *atabcg14 cypDM*. Roots of the 8-DAG *cypDM* and *atabcg14 cypDM* seedlings were incubated with 50 nM $^2H_6$-ABA for 1 h. The shoots (A) and roots (B) were harvested for $^2H_6$-ABA quantification. Data are means ± SD, n = 4.


A
cypDM/cypDM
g14cypDM/g14cypDM
g14cypDM/cypDM
cypDM/g14cypDM
B
C
Diameter of rosette leaves (cm)
0
1
2
3
4
5
a
c
b
b
D
No. siliques/plant
0
50
100
150
a
d
c
b
E
Plant height (cm)
0
10
20
30
a
c
b
b
F
No. primary branches/plant
0
2
4
6
8
10
a
d
c
b
cypDM/cypDM
g14cypDM/g14cypDM
g14cypDM/cypDM
cypDM/g14cypDM

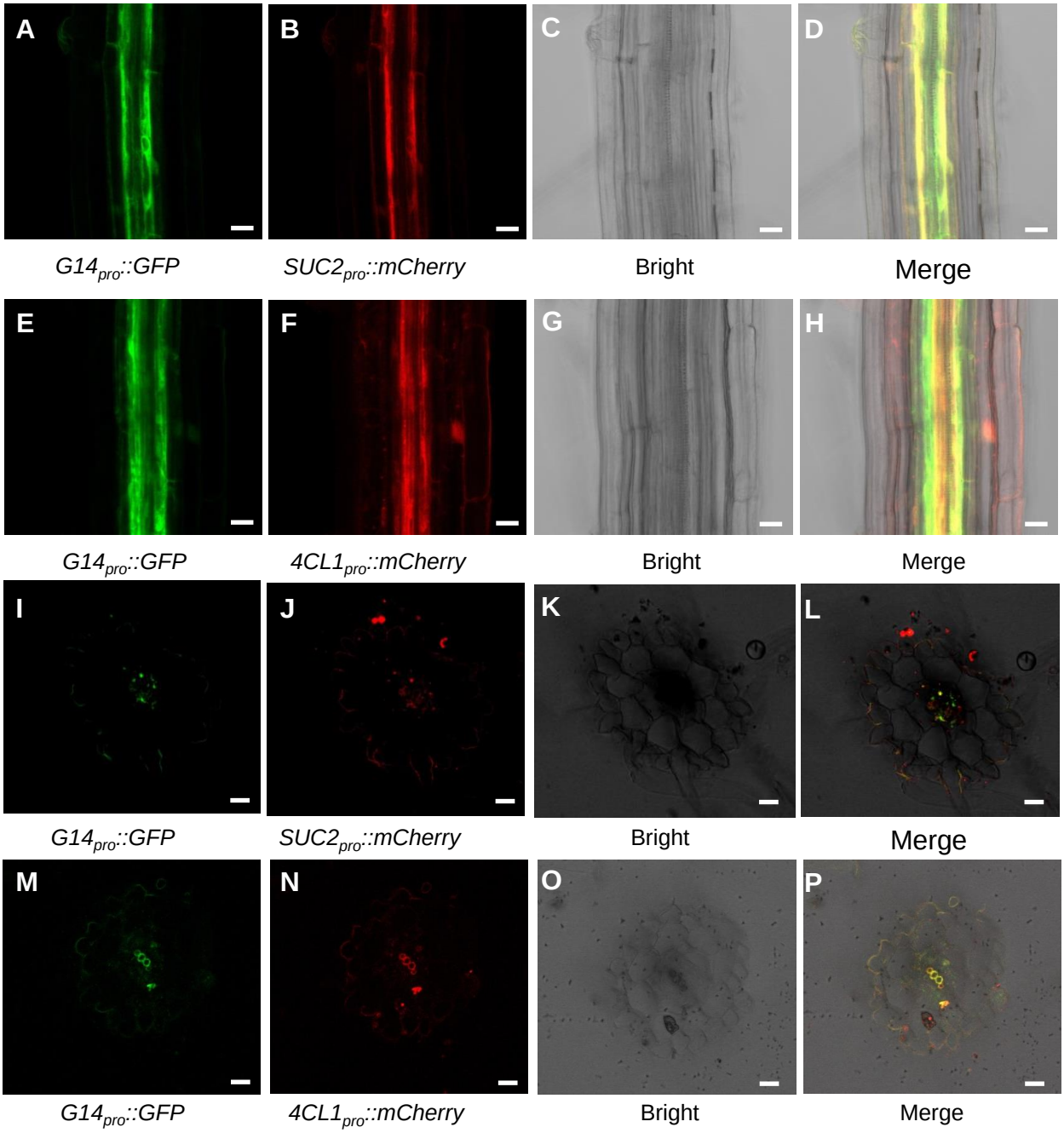
Supplemental Figure 9. The phenotypes of the grafted plants between *cypDM* and *atabcg14 cypDM*. (A) Morphological phenotypes of 25-DAG *cypDM/cypDM*, *atabcg14 cypDM/atabcg14 cypDM, atabcg14 cypDM /cypDM, cypDM /atabcg14 cypDM* grown on soil. Scale bar, 1.5 cm. (B) Phenotypes of 35-DAG *cypDM/cypDM*, *atabcg14cypDM/atabcg14cypDM, atabcg14cypDM/cypDM, cypDM /atabcg14cypDM*. Scale bar, 1.5 cm. (C) Quantification of rosette diameter in (A). Data are means ± SD (n = 30). (D–F) Quantification of number of siliques per plant (D), plant height (E) and number of primary rosette branches per plant (F) in (B). Data are means ± SD (n = 30). Groups marked by different letters are significantly different ($P < 0.05$, ANOVA). *g14cypDM, atabcg14 cypDM*.



Supplemental Figure 10. Phloem unloading of iP-type CKs was medicated by AtABCG14. (A) Morphological phenotypes of 21-DAG WT and *atabcg14* plants. The 3rd and 4th leaves of plants were dripped with 5 ppm $^2H_6$-iP (7 μL/cm^2 leaf area) and incubated for 6 h. Then the roots (B), the younger (from the 5th to 10th) leaves (C) and the 3rd and 4th leaves (D) were harvested for quantification of $^2H_6$-iP and $^2H_6$-iPR. Data are means ± SD (n = 3), *P < 0.5 (Student's t-test). Scale bars, 2 cm for (A). *g14* represents *atabcg14*. FW, fresh weight.



Supplemental Figure 11. Circulated transport of iP-type CKs in *Arabidopsis* seedlings. (A) and (B) The WT plants were incubated under split-root with shoot (A) and without shoot (B) conditions with or without $^2H_6$-labeled iP. (C) Quantification of $^2H_6$-labeled iP and iPR contents in the root (without feeding $^2H_6$-labeled iP) after feeding with $^2H_6$- iP for 4 h in (A and B). Data are means ± SD, $n = 4$. ***$P < 0.001$ (Student's *t*-test). FW, fresh weight.



Supplemental Figure 12. Co-expression of *AtABCG14*, *SUC2*, and *4CL1* in the root steles of *Arabidopsis* seedlings. (A), (E), (I) and (M), GFP fluorescence. (B), (F), (J) and (N), mCherry fluorescence. (C), (G), (K) and (O), Bright field. (D), (H), (L) and (P), merged signals. Scale bars = 20 μm. The seedlings for fluorescence observation are *AtABCG14*$_{pro}$*::GFP* and *SUC2*$_{pro}$*::mCherry* or *AtABCG14*$_{pro}$*::GFP* and *4CL1*$_{pro}$*::mCherry* double-transgenic plants. *G14*$_{pro}$*::GFP*, *SUC2*$_{pro}$*::mCherry* and *4CL1*$_{pro}$*::mCherry* indicate transgenic plants of WT transformed with GFP and mCherry under *AtABCG14* promoter and *4CL1* promoter, and *SUC2* promoter.

Supplemental Table S1. Primers used in this study.

Primer name	Primer sequences (5'-3')	Restriction site (with underline)	Purpose
AtABCG14-P1	GCAGGCTCCGAATTCATCCCATCGATTTAAAGCTATG	EcoR Ⅰ	For constructing Myc-and Flag-AtABCG14
AtABCG14-P2	AAGCTGGGTCGAATTCTTACCGCAACTTCACCCGATGCAG	EcoR Ⅰ	expression in Arabidopsis
AtABCG14-P3	CGAGCGGCCGCGGATCCATGGTGAGCAAGGGCGAGGA	BamH Ⅰ	For constructing AtABCG14-pDR195 expression in
AtABCG14-P4	AGTCCAAAGCTGGATCCTTACCGCAACTTCACCCGATG	BamH Ⅰ	yeast
ACTIN-F	GGTAACATTGTGCTCAGTGGTGG		The standard gene for qRT-PCR
ACTIN-R	CTCGGCCTTGGAGATCCACATC		
AtABCG14-P5	TCGGTGCTCTGCTTATGAAC		For AtABCG14 qRT-PCR
AtABCG14-P6	ACGATGAAGGGAGGAATTTG		
LB1.3	ATTTTGCCGATTTCGGAAC		SALK T-DNA insert line order primer (BP)
AtABCG14-P7	TGCTGAGCTTTTGAGTTGTACC		For *atabcg14* homozygote identification (LP+RP)
AtABCG14-P8	CACAATGCGATGAGCAGTGG		
AtCYP735A1-P1	AGGTGATCGATGGACATATGC		For *cyp735a1* homozygote identification (LP+RP)
AtCYP735A1-P2	AGAAGCACTTCGCATGATCAC		
AtCYP735A2-P1	TCAAAAATGACTGAAATGGCC		For *cyp735a2* homozygote identification (LP+RP)
AtCYP735A2-P2	ATTATGAGCAAGGAGCATG		
AtABCG14-P9	ATGCCTCAGAACTGCATAGCACCAAGGCCT		For pCS2 vector construction
AtABCG14-P10	GCATCTAGACCGCAACTTCACCCGATGCAG	Xba Ⅰ	
pSKTAIL	ATACGACGGATCGTAATTTGTCG		T-DNA insert line order primer for *abcg14* (BP)
AtABCG14-P11	ATGCCTCAGAACTGCATAGCAC		For *atabcg14* homozygote identification (RP)