Table S1. Primers used for cloning of *PAA1* and *PAA2*

AGI	Gene	Sequence (5' to 3')	Sense
At4g33520	*PAA1 Nco*l	catgccatgGAGAGTGGTGATTCCAAGTCAAAACTGGG	Forward
	*PAA1 Bam*Hl	cgcggatccCTCGCGGCCACTCTCTTTTAAGCG	Reverse
At5g21930	*PAA2 Nco*l	catgccatgGAATCTTCAATCGAATCTGTGAAATCCATT	Forward
	*PAA2 Bam*Hl	cgcggatccACGGTTCCTGCTCTTAACAAGCAA	Reverse

qRT-PCR primers

AGI	Gene	Sequence (5' to 3')	Sense
At5g08290	Mitosis	TTACTGTTTCGGTTGTTCTCCATTT	Forward
	protein *YLS8*	CACTGAATCATGTTCGAAGCAAGT	Reverse
At2g28390	*SAND* family	AACTCTATGCAGCATTTGATCCACT	Forward
		TGATTGCATATCTTTATCGCCATC	Reverse
At5g21930	*PAA2*	AATCTAGCGTGGGCAATTGCG	Forward
		CTCCAAAATAGATGTGGAGCCG	Reverse
At4g33520	*PAA1*	ACGACAAAAATGTCAAACCGG	Forward
		CCCTCTCAAGACCAAGAGC	Reverse
At1g12520	*CCS*	AGATCAAACTGGCACAGAGCC	Forward
		GCTCTCCCGATAAGGTCTGC	Reverse

miRNA Stem-loop qRT-PCR primers

AGI	Gene	Sequence (5' to 3')	Sense
At2g47015	*miR408* RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCCAGG	Reverse
	miR408 SL	GTGTGATGCACTGCCTCTTC	Reverse
At3g63375	*miR167* RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTAGATC	Reverse
	*miR167*SL	TCGCGTGAAGCTGCCAGCAT	Reverse
-	miRUniversal	CCAGTGCAGGGTCCGAGG	Forward

***miR408* mutant line verification**

AGI	Gene	Sequence (5' to 3')	Sense
At2g47015	*miR408F*	AGAGGGTTAGGAATACAGTTGG	Forward
	miR408R	CATTTGTTTCACCCACTACGC	Reverse
T-DNA	LBb1.3	ATTTTGCCGATTTCGGAAC	Forward

Table S2. Cellular Cu content

Cu content was measured for protoplasts and chloroplasts of wild-type (Col-0), *paa1-3*, and *ccs* plants grown *in vitro* for 4 weeks in the presence of 0.05 μM or 5 μM $CuSO_4$. Values for protoplasts (cells) and chloroplasts of each line and treatment are given in ng Cu/ mg chlorophyll and represent averages of three measurements ± SD. The ratio represents the fraction of cellular Cu found in the chloroplasts.

	Col-0		*paa1-3*		*ccs*	
$CuSO_4$	0.05 μM	5 μM	0.05 μM	5 μM	0.05 μM	5 μM
Protoplast	**333** ± 70	**500** ± 98	**385** ± 68	**580** ± 162	**312** ± 63	**523** ± 145
Chloroplast	**234** ± 38	**440** ± 163	**137** ± 41	**247** ± 75	**265** ± 8	**382** ± 174
Ratio	**0.7**	**0.88**	**0.36**	**0.43**	**0.85**	**0.73**

SUPPLEMENTARY FIGURES

FIGURE S1. PAA1 and PAA2 antibody specificity
(a,b) Immunoblot analysis of PAA2, PAA1 and cFBPase in rosette leaves of wild-type (Col-0), *paa2-1* and *paa1-1* seedlings grown *in vitro* for 18 days on agar solidified half-strength MS. *paa1-1* and *paa2-1* were used as negative controls for the respective antigens. *****indicates an unspecific band.

FIGURE S2. Root-length of metal treated wild-type (Col-0) plants
Root length of 11 days old Col-0 seedlings grown vertically in the absence (Ø) or presence of different metals. Results represent the average ± SD ($n \geq 13$ seedlings per treatment).

FIGURE S3. *miR408* knock-out line characterization
(a) Map of the putative *miR408* targeting site within the *PAA2* coding sequence. The schematic drawing furthermore indicates the location of the T-DNA insertion within the *miR408* coding sequence. The primer binding sites for verification of the T-DNA insert are indicated. Exons are represented by black boxes and introns by black lines. (b) The presence of the T-DNA insert was analyzed by PCR using the primers listed in Table S1. (c) Expression levels of mature *miR408* measured via stem-loop qRT-PCR in the wild-type (Col-0) and *miR408* knock out background. Plants were grown in the presence of 0.05 µM $CuSO_4$ (left, black bars) or 5 µM $CuSO_4$ (right, grey bars). Results represent the average (± SD) of three biological replicates including two technical replicates each. n.d. means not detectable.

FIGURE S4. Intactness of isolated protoplasts and chloroplasts
Immunoblot analysis of isolated protoplasts (P) and chloroplasts (C) from Col-0, *paa1-3* and *ccs* plants, grown in the presence of the indicated $CuSO_4$ concentrations for 4 weeks. Protein extract, equivalent to 1.7 µg chlorophyll was loaded into each lane. Blots were probed for PC and CCS using specific antibodies. cFBPase serves as a cytosolic marker and ferredoxin (FDX) as stromal marker.

Figure S1

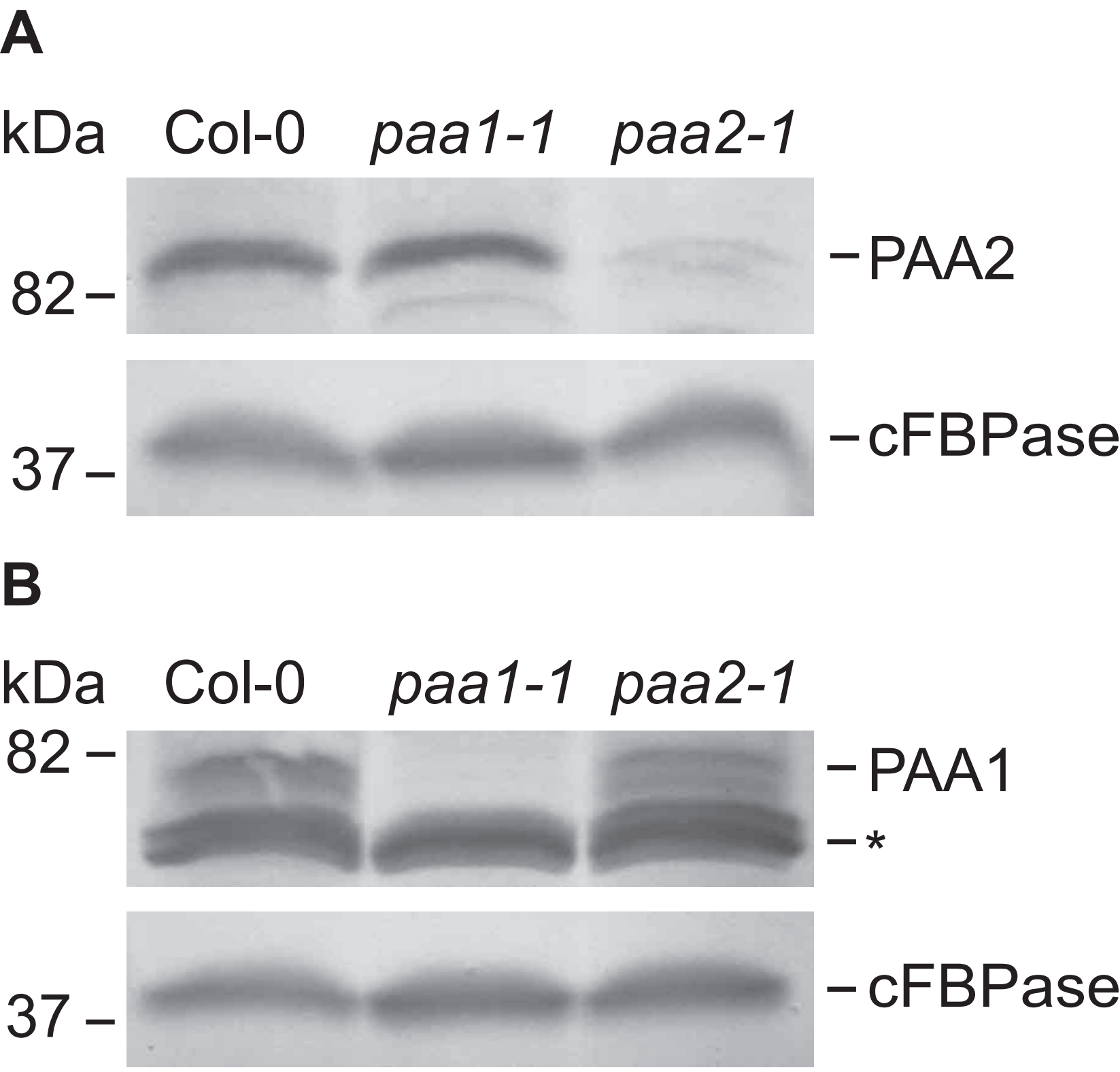

A
kDa
Col-0
paa1-1
paa2-1
82 –
– PAA2
37 –
– cFBPase
B
kDa
Col-0
paa1-1
paa2-1
82 –
– PAA1
– *
37 –
– cFBPase

Figure S2

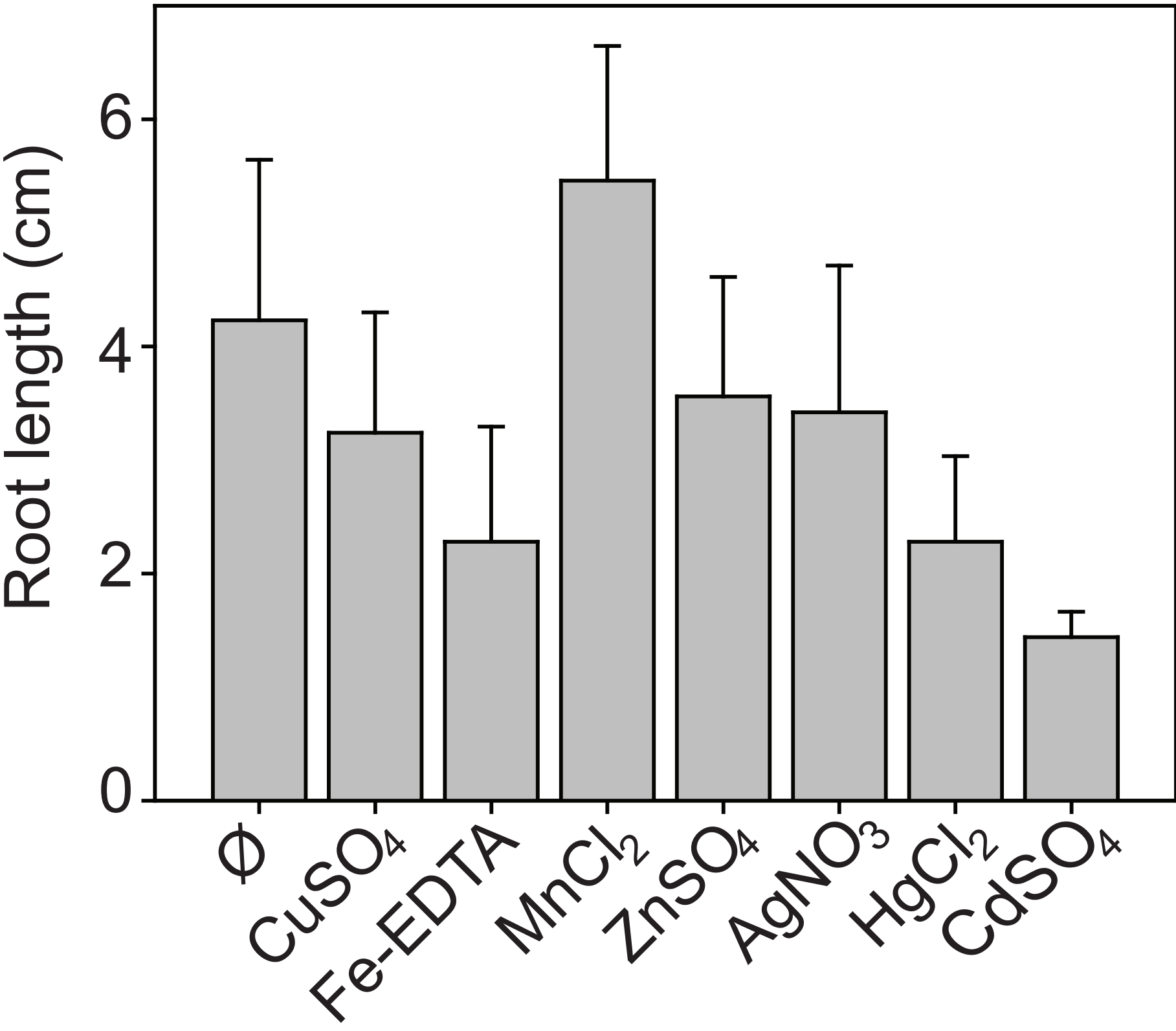
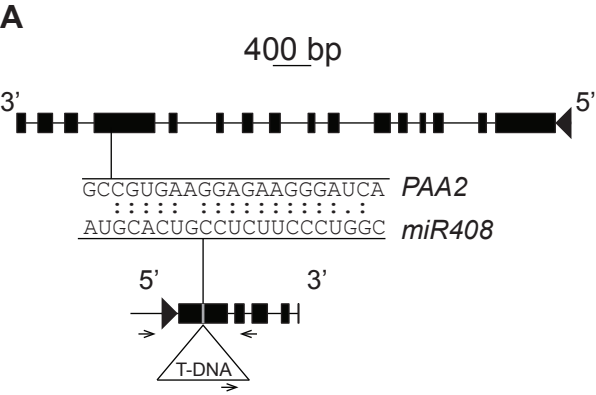
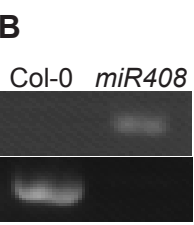

Root length (cm)
0
2
4
6
Ø
CuSO4
Fe-EDTA
MnCl2
ZnSO4
AgNO3
HgCl2
CdSO4

Figure S3


A
400 bp
3'
5'
GCCGUGAAGGAGAAGGGAUCA PAA2
AUGCACUGCCUCUUCCCUGGC miR408
5'
3'
T-DNA


B
Col-0 miR408
LBb1.3 + miR408R
miR408F + miR408R

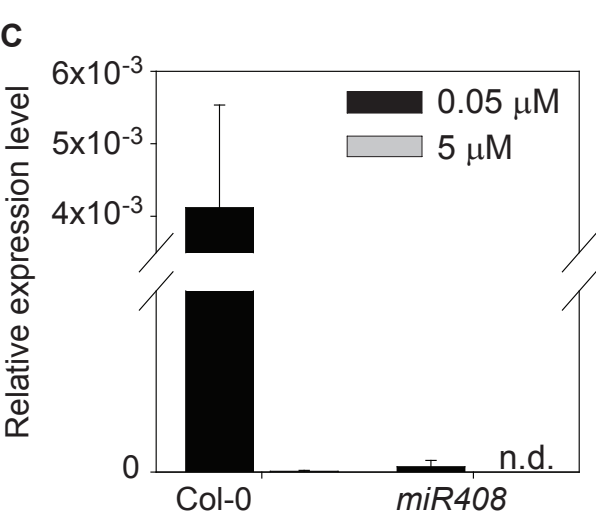
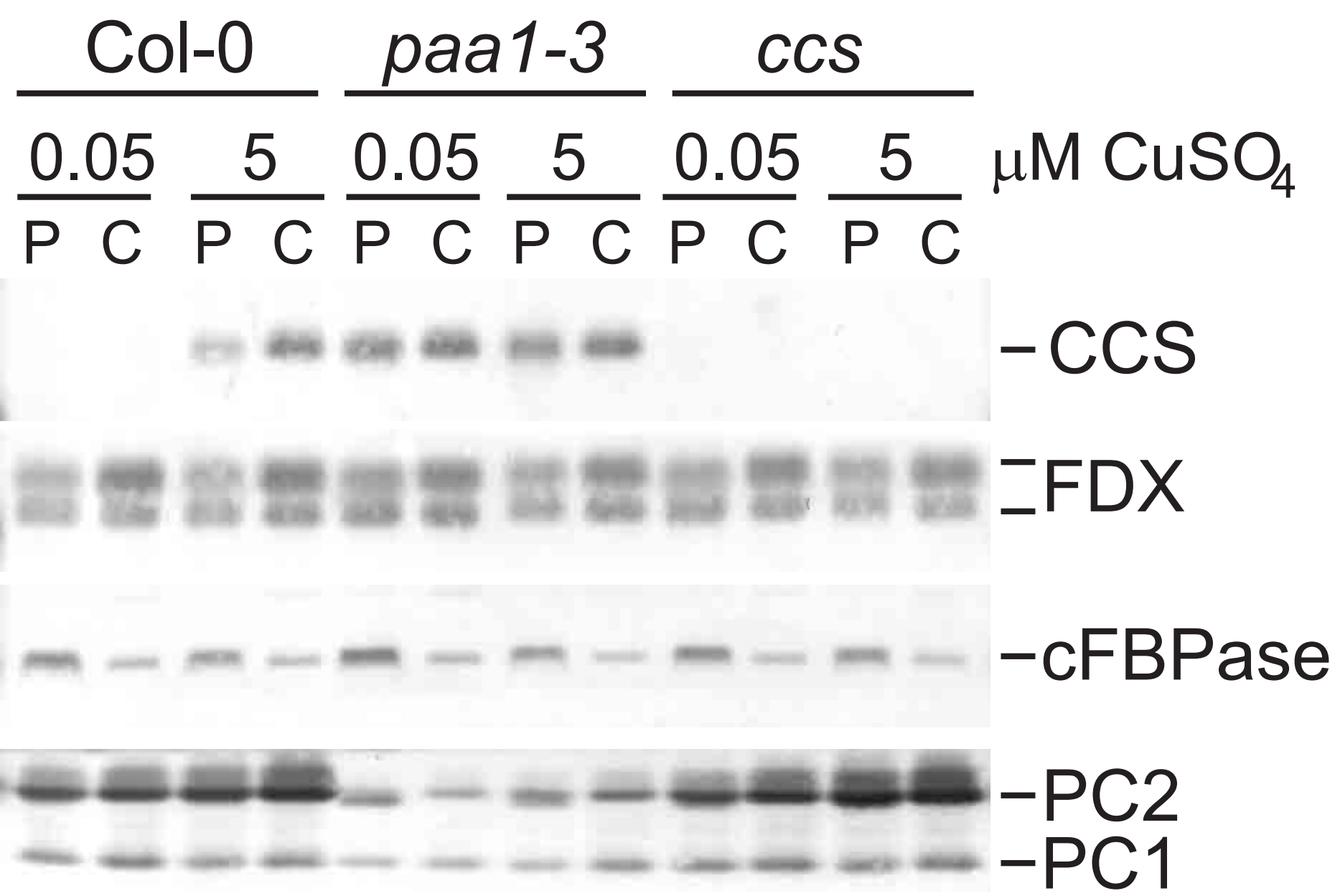

C
Relative expression level
6x10-3
5x10-3
4x10-3
0
0.05 μM
5 μM
n.d.
Col-0
miR408

Figure S4


Col-0
paa1-3
ccs
0.05
5
0.05
5
0.05
5
μM CuSO4
P C P C P C P C P C P C
–CCS
–FDX
–cFBPase
–PC2
–PC1