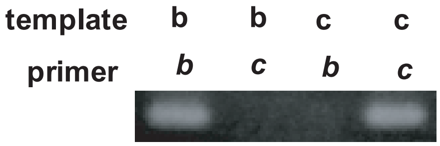
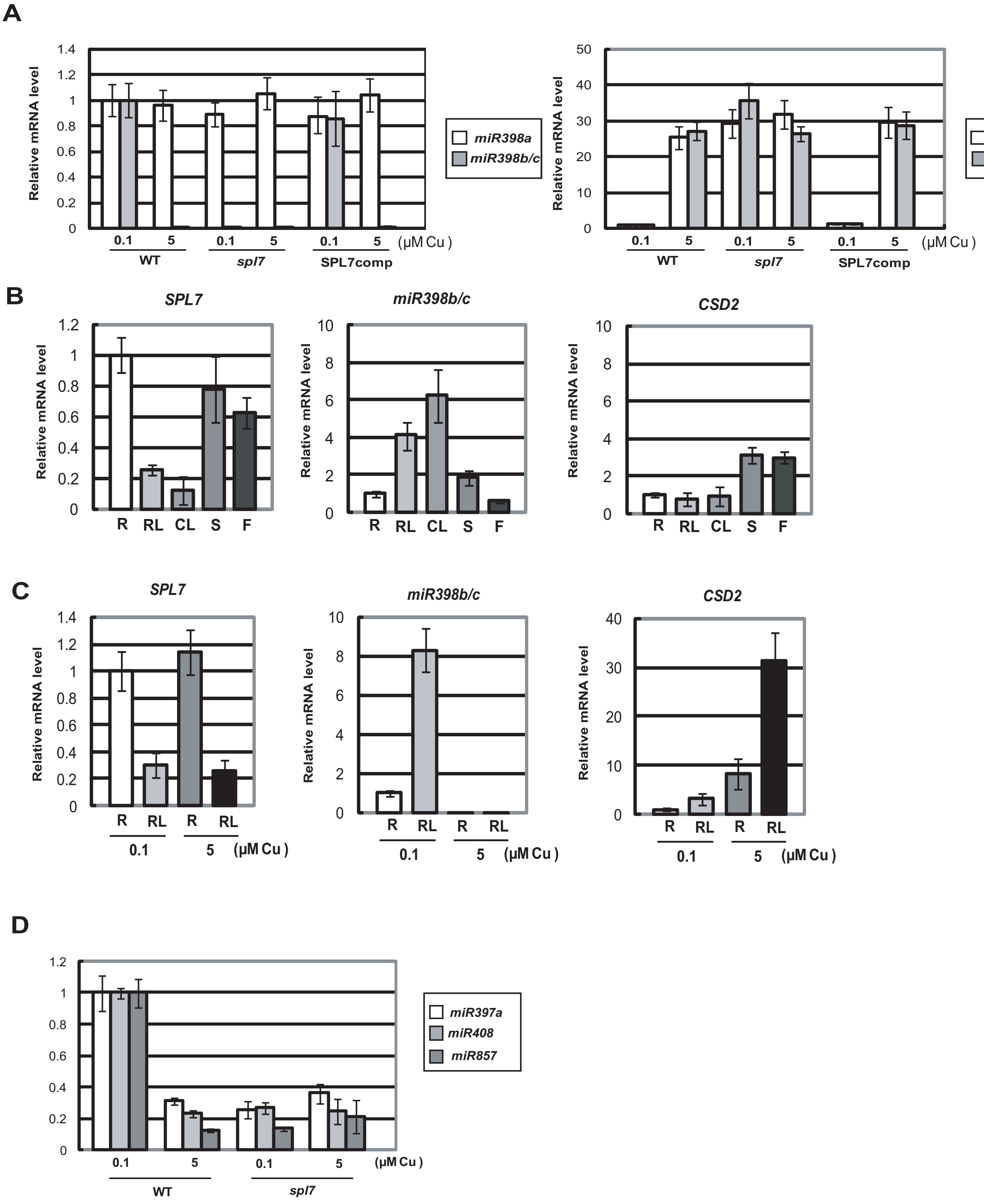


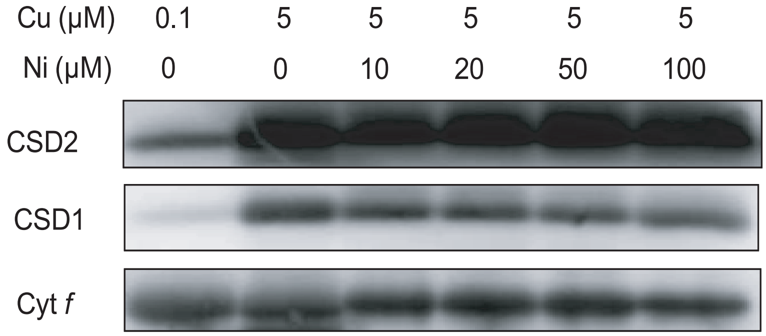
Specific amplification of *miR398b* and *miR398c*. The genomic region of *miR398b* and *miR398c* was amplified by using specific primer sets, *miR398b*-FW and *miR398b*-RV for *miR398b* (b), and *miR398c*-FW and *miR398c*-RV for *miR398c* (c). Diluted PCR products were used as template and were amplified by specific primers *miR398b*-dtF and *miR398b*-dtR for *miR398b* (*b*), and *miR398c*-dtF and *miR398c*-dtR for *miR398c* (*c*) The annealing temperature was 65 ℃ the primer sequences are described in Supplemental table 5.

Supplemental Figure 1



Quantification of gene expression by real-time PCR. (A) Relative mRNA level of *miR398a*, *miR398b/c*, *CSD1* and *CSD2*. The plant growth conditions were the same as Figure 4A. **(B,C)** Relative mRNA level of *SPL7*, *miR398b/c* and *CSD2*. Plant growth conditions were the same as Figure 6A (B) and Figure 6B (C). **(D)** Relative mRNA level of *miR397a*, *miR408* and *miR857*. The plant growth conditions were the same as Figure 7A.

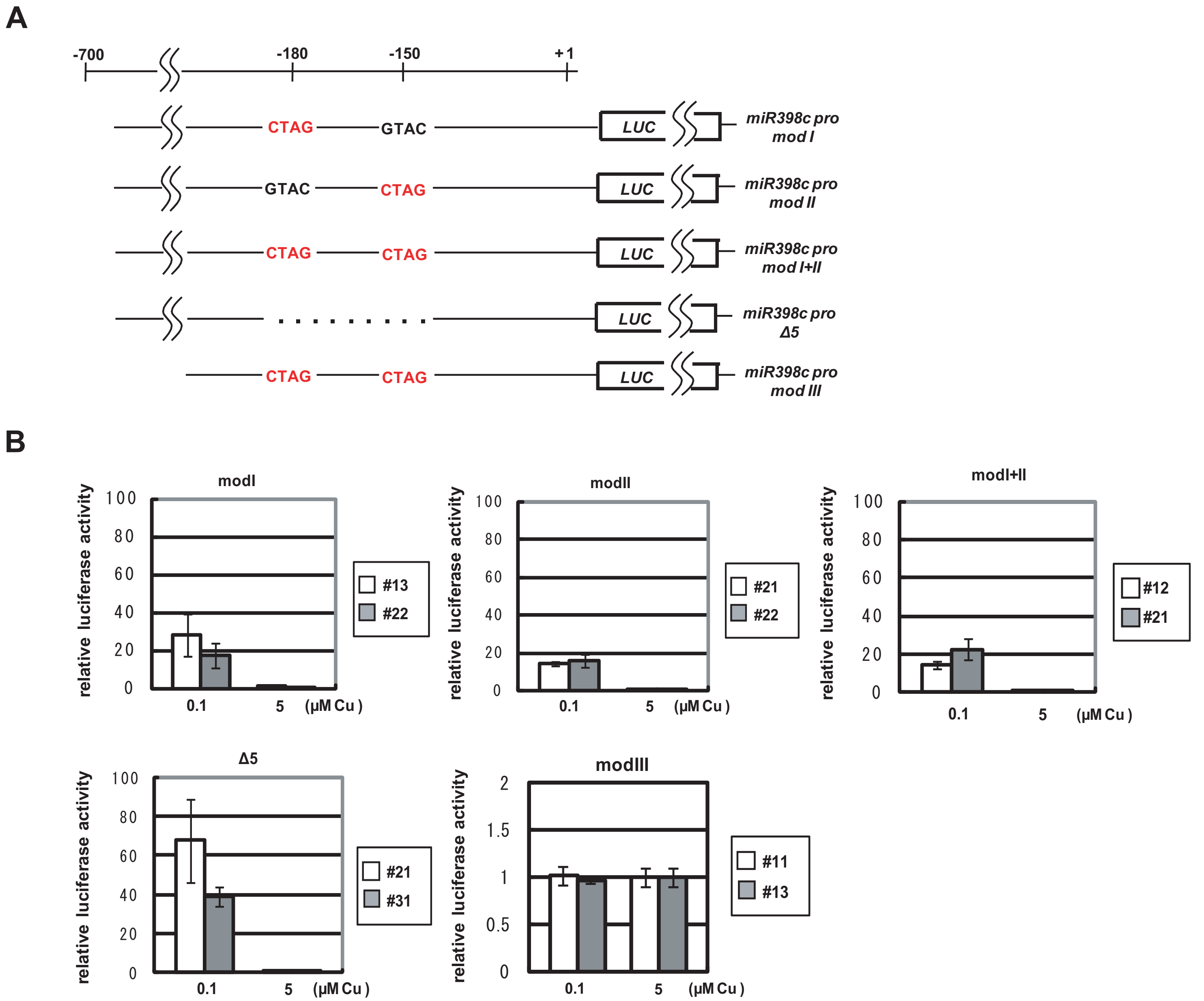
Supplemental Figure 2



Excess nickel does not disturb copper regulation under copper sufficiency.

Immunoblot analysis of CSDs and Cyt*f* in the seedlings grown for 3 weeks on MS media containing indicated concentrations of copper and nickel. Cyt f is shown as a loading control.

Supplemental Figure 3



Identification of cis-elements in the promoter region of *miR398c*. (A) Construction of mutant virsions of *miR398c pro* :: *LUC*. Red characters indicate base substitutions and a dotted line indicates a truncated region. **(B)** Relative luciferase activity in 2-week-old seedlings of transgenic lines. Each graph shows relative levels of luminescence in two independent transgenic lines. Data are average of four independent seedlings with standard deviations. The scales of the *y*-axes differ among panels.

Supplemental Figure 4

Supplemental Table 1

Microarray data for genes detected in RT-PCR. The results shown are the means ±SD of three biological replicates.

			signal intensity			
Gene name	**AGI code**	**fold change WT Cu0.1/ *spl7* Cu 0.1**	**WT Cu 0.1**	**WT Cu 5**	***spl7* Cu 0.1**	***spl7* Cu 5**
COPT1	At5g59030	3.01	7380±290	2150±70	2450±70	2960±50
COPT2	At3g46900	4.36	4190±500	1310±160	961±25	1150±80
ZIP2	At5g59520	7.23	410±30	64.7±20.3	56.7±3.0	50.0±2.4
ZIP4	At1g10970	0.945	811±169	744±60	858±66	620±24
FRO3	At1g23020	2.61	8530±560	3210±120	3260±100	5400±250
CCH	At3g56240	4.53	49300±4000	10100±1100	10900±1100	8320±530
ATX1	At1g66240	0.914	19700±2300	15400±2600	21500±1600	13400±700

Supplemental Table 2

A list of genes up-regulated more than 20 times under copper deficiency in the wild-type.
The results shown are the means ±SD of three biological replicates.

	Gene name	AGI code	fold change WT Cu0.1/ *spl7* Cu 0.1	WT Cu 0.1	WT Cu 5	*spl7* Cu 0.1	*spl7* Cu 5
1	FSD1	At4g25100	24000	68100±10300	136±40	2.83±0.89	3.53±1.09
2	bHLH protein	At1g71200	277	686±94	2.51±0.44	2.47±0.27	2.40±0.41
3	hypothetical protein	At1g33840	55.6	211±35	2.74±0.25	3.79±0.14	2.63±0.26
4	unknown protein	At1g14880	54.3	828±103	12.6±4.9	15.2±3.0	21.7±2.2
5	TAT3	At2g24850	39.9	2910±370	36.2±4.8	73.0±3.4	42.4±4.1
6	AAA-type ATPase family protein	At3g28510	38.6	319±29	18.1±1.2	8.26±4.95	6.09±2.89
7	unknown protein	At4g21840	38.1	516±46	19.9±4.3	13.5±3.5	16.6±2.6
8	expressed protein	At3g48640	35.5	109±21	25.0±3.5	3.08±0.72	4.44±2.95
9	WRKY type transcription factor	At5g22570	33.4	186±25	5.57±2.79	5.57±1.72	9.55±1.06
10	cytochrome P450 family protein	At3g25180	26.9	258±65	11.8±1.9	9.62±2.34	11.2±2.1
11	lyase	At1g61120	25.7	470±106	24.2±10.8	18.2±7.0	3.86±1.72
12	unknown protein	At5g52720	25.4	58.2±11.1	3.75±2.64	2.28±0.32	3.16±1.93
13	glycosyl hydrolase family 17	At3g57260	24.4	4990±560	413±41	203±7	273±40
14	oxidoreductase	At4g10500	23.8	392±47	20.6±3.5	16.4±1.4	18.8±4.9
15	leucine-rich repeat family protein	At3g24954	22.5	503±51	17.7±4.4	22.3±1.5	12.8±5.3
16	wall-associated kinase	At1g21240	21.7	71.9±26.7	8.94±5.68	3.31±0.78	3.31±0.64
17	mannitol dehydrogenase	At4g37990	21	373±63	21.4±1.0	17.7±2.5	13.8±1.1
18	expressed protein	At2g44240	20.2	169±44	14.0±5.7	8.37±0.93	7.47±1.77
19	glutaredoxin family protein	At1g28480	20	771±98	55.9±1.8	38.6±2.9	71.4±5.0

Supplemental Table 3

A list of genes down-regulated under copper deficiency to less than 5 percent of normal transcript levels in the wild-type compared to *spl7*. The results shown are the means ±SD of three biological replicates.

				signal intensity			
	Gene name	**AGI code**	**fold change WT Cu0.1/ *spl7* Cu 0.1**	**WT Cu 0.1**	**WT Cu 5**	***spl7* Cu 0.1**	***spl7* Cu 5**
1	LEA protein	At1g52690	0.006	163±34	774±17	26100±1200	238±34
2	alternative NADH-dehydrogenase	At2g20800	0.01	4.25±2.40	11.2±3.3	417±22	9.71±2.01
3	glycosyl hydrolase family 38	At5g66150	0.01	2.53±0.18	3.77±1.91	250±34	55.2±5.6
4	LEA domain-containing protein	At3g17520	0.013	76.8±20.2	139±14	5720±350	114±13
5	MutT/nudix family	At5g19470	0.017	11.3±0.9	5.46±2.80	643±48	7.94±2.86
6	lipid transfer protein 3	At5g59320	0.017	23.3±2.0	49.3±5.7	1360±240	80.5±16.8
7	expressed protein	At4g12735	0.02	3.19±0.79	6.35±3.11	1580±10	2.47±0.12
8	expressed protein	At5g54550	0.021	5.47±2.53	4.16±1.45	259±18	8.77±1.00
9	LEA domain-containing protein	At5g44310	0.024	3.68±1.17	12.2±2.6	148±51	11.0±1.2
10	CSD2	At2g28190	0.027	2480±700	108000±9000	88700±9700	104000±6000
11	CSD1	At1g08830	0.031	1500±100	43800±3200	47200±1600	41500±5100
12	peroxiredoxin	At1g48130	0.035	2.91±0.76	3.64±2.65	81.4±2.0	39.5±7.6
13	AAA-type ATPase family protein	At2g18193	0.038	79.5±19.2	189±4	2060±370	82.8±3.8
14	transferase family protein	At1g65450	0.038	47.4±8.5	19.0±3.2	1240±90	602±57
15	expressed protein	At3g12960	0.038	4.28±1.51	8.69±1.12	111±29	5.40±1.75
16	Plantacyanin	At2g02850	0.043	57.8±7.6	625±19	1340±110	680±116
17	heat shock transcription factor	At5g43840	0.045	205±104	407±203	4560±870	262±64
32	CCS	At1g12520	0.14	6540±100	59900±3700	46500±4600	49600±3500

Supplemental Table 4

The primer sequences used for plasmid construction. The underline indicates the base substitution.

Name	Sequence
miR398c pro-F	5'-GGCGTAGATCTCAAGGGAGAGTAATTAC-3'
miR398c pro-R	5'-GTCCTTTTATCCATGGTTTAGAAGATAG-3'
miR398c pro-F1	5'-GAGGAGATCTTTGGAACACTTCC-3'
miR398c pro-F2	5'-TGTAGATCTGTTTCGCAGTACAC-3'
miR398c pro-F3	5'-AATGCAACAGATCTTTGGCCCGTTTG-3'
miR398c pro-F4	5'-CACAATCCTTTCATCATTAGATCTGTTGTG-3'
miR398c modI-R	5’-CAGTTTCGCACATGACAATTCTG-3’
miR398c modI-F	5’-TTCGCACATGACAATTCTGTAGG-3’
miR398 c modII-R	5’-GGTTTTTTTTATTCATGTAAAGAA-3’
miR398 c modII-F	5’-TTTTATTCATGTAAAGAAAACG-3’
pBI121-F	5'-TATAGTCCTCTCGAGTTTCGC-3'
pBI121-R	5'-CGTTATCCCCAGATCTTGTGG-3'
miR398c proHindIII-F	5'-ATGTTGATTTTTTTGTAAATAAGCTTCGC-3'
miR398c pro SalI-R	5'-GAAAACGTCGACGAATGCAACAAATCG-3'

Supplemental Table 5

The primer sequences used for RT-PCR analysis.

Name	Sequence	Name	Sequence
miR397a -F	5'-GAATGAACATCATTGAGTGCAG-3'	CCH-F	5'-GTTGGTATGTCATGCCAAGGC-3'
miR397a -R	5'-GCGTTGCGCTCAATTATGTTT-3'	CCH-R	5'-GCTGATGTTGAACCAAAAGCC-3'
miR398b -FW	5'-TGGGGACTAGGGACCATAGAG-3'	ATX1-F	5'-TCTTTCAAGCCTTGTCGGTGG-3'
miR398b -RV	5'-GAACTTCCTGGCGAAGAGGAA-3'	ATX1-R	5'-GAAGCTGAAGGTGAAACTGCT-3'
miR398c -FW	5'-AGAAAGAAATAGAGGTGCTGG-3'	SPL7-F (A)	5'-TTGAAGACTCTCAGATGTCTTCTCTG-3'
miR398c -RV	5'-GATGAAGGATCTACTTTAATTTTC-3'	SPL7-R (A)	5'-CTTCCGAAGAAGAAACGGGTT-3'
miR398b -dtF	5'-CGAGTAATCAACGGCTGTAAT-3'	SPL7-F (B)	5'-AGCTCTACGATTGGAATCCAG-3'
miR398c -dtF	5'-CGAGCAATCAACGGCTATAAC-3'	SPL7-R (B)	5'-GGACAAAACCTTCTGCAACCTAAAT-3'
miR408 -F	5'-AAGCGGTAATGAGAGAGAGAC-3'	FSD1-F	5'-CAAACTCTGGAGTTTCACTG-3'
miR408 -R	5'-GGAATTTTATTGCTACTGCTAAC-3'	FSD1-R	5'-GCTGTAAGTGCCAGACTTGA-3'
miR857 -F	5'-CGACTCCTACAACAAACTTTC-3'	YSL2-F	5'-GTACCTCTTCGAAAGGTGATG-3'
miR857 -R	5'-CGTCGAGCATTTGAATTTCTAGA-3'	YSL2-R	5'-GCACTGATCTTAGGAGATGGG-3'
COPT1-F	5'-TGATCACATGCATGGAATGCC-3'	bHLH-F	5'-TGTCTTCTCAACCGAATCATC-3'
COPT1-R	5'-GAATACCTCCGATGACCGGAA-3'	bHLH-R	5'-GGTCCTTCTATTCGAGCAATC-3'
COPT2-F	5'-TGATCACATGCATGACATGCC-3'	TAT3-F	5'-AACCAAGCCATAGAGATCGTG-3'
COPT2-R	5'-CTTTCTCTTTGGGAGCACTAC-3'	TAT3-R	5'-GGGGAAGTTTGCATCAATAGC-3'
ZIP2-F	5'-TTCCTCTGTTTCTCCTTGATC-3'	At1g14880-F	5'-TCATGCTCAAGGAGAATGGTC-3'
ZIP2-R	5'-CATGTTAGCAGCCGCTGGATA-3'	At1g14880-R	5'-CAGGGAACGCAGAGAAACAAC-3'
ZIP4-F	5'-GACAATGGCTTCTTCTACCAC-3'	At4g21840-F	5'-TGCAGTTCCATCAAGTGGATC-3'
ZIP4-R	5'-GTTCTTTGCAATGGTTGCTGC-3'	At4g21840-R	5'-CTCTACTCCAACCGATCAACG-3'
FRO3-F	5'-CAAGGCTGGATTCAATAGCAG-3'	CCS-F	5'-ATTCTCAGGTCAGTGGCAACG-3'
FRO3-R	5'-GCTCTTCTTTGTCTTCCACGT-3'	CCS-R	5'-GGTACTGTCATATGGGAAGCT-3'

Supplemental Table 6

The primer sequences used for quantitative RT-PCR analysis.

Name	Sequence
miR398a q-F	5'-TCAAAGGAGTGGCATGTGAACAC-3'
miR398a q-R	5'-GTTCTCAGGTCACCCCTTTGAATC-3'
miR398bc q-F	5'-GGATCTCGACAGGGTTGATATG-3'
miR398bc q-R	5'-CAGGTCACCCCTGCTGAGCTCTT-3'
CSD1 q-F	5'-TTCTGGCCTTAAGCCTGGTC-3'
CSD1 q-R	5'-CGACATGCTGGTGATCTAGG-3'
CSD2 q-F	5'-CATGACACACGGAGCTCCAG-3'
CSD2 q-R	5'-GGATGACCTCGGAAAGGGTG-3'
SPL7 q-F	5'-GAGCTGGAGGGCTATATCCG-3'
SPL7 q-R	5'-GGAAGAGGCTCGATGACTGT-3'
miR397a -F	5'-GAATGAACATCATTGAGTGCAG-3'
miR397a -R	5'-GCGTTGCGCTCAATTATGTTT-3'
miR408 q-F	5'-CAGGGAACAAGCAGAGCATG-3'
miR408 -R	5'-GGAATTTTATTGCTACTGCTAAC-3'
miR857 q-F	5'-CGACTCCTACAACAAACTTTC-3'
miR857 -R	5'-CGTCGAGCATTTGAATTTCTAGA-3'