

**Table S1.** Accumulation of Cd in *A. thaliana*. Plants were grown hydroponically for two weeks before transferring to a fresh medium containing the indicated concentrations of CdCl<sub>2</sub> for the indicated time. Plants were harvested at the bolting stage. Roots and shoots were collected and Cd content was analysed by ICP-MS. Shown are arithmetic means ± S.D (*n* = 2-4)

| Growth conditions                  | Cadmium Accumulation (µg g <sup>-1</sup> dry biomass) |                |
|------------------------------------|---|----------------|
|                                    | Root  | Shoot          |
| 25 µM CdCl <sub>2</sub> ; 3 days   | 2212.1 ± 174.3  | 1565.6 ± 145.1 |
| 1 µM CdCl <sub>2</sub> ; 10 days   | 789.9 ± 59.4  | 291.7 ± 14.0   |
| 2.5 µM CdCl <sub>2</sub> ; 10 days | 1259.8 ± 62.7   | 715.9 ± 1.1    |

**Table S2.** Cloning primers used in this study

| Name                 | 5' → 3'   |
|----------------------|---|
| <i>COPT2-F</i>       | GCCGCCTCGAGCATTAGTATCATGGATCATGATCAC                              |
| <i>COPT2-R</i>       | GCGGCGAATTCACAAACGCAGCCTGAAGAC                                    |
| <i>attB-COPT2-F</i>  | TCGTCTGGGGACAACCTTTGTACAAAAAAGTTGGATTAGTATCATGGATCATGATCAC<br>ATG |
| <i>attB-COPT2-R</i>  | GGCGGCCGCACAACCTTTGTACAAGAAAGTTGGGTTGTTCAACAAACGCAGCCT            |
| <i>COPT2-M111A-F</i> | GGTGATGCTCGCTGTTGCTTCCTTTAACGCAGGTGT                              |
| <i>COPT2-M111A-R</i> | ACACCTGCGTTAAAGGAAGCAACAGCGAGCATCACC                              |
| <i>COPT2-1-LP</i>    | CTGTGTCGTGAGGTTTTGAGG   |
| <i>COPT2-1-RP</i>    | TCTTGAGTGTGTACACAGCGG   |
| <i>COPT2-2-LP</i>    | GAGACAGAGAGCGTACATGCC   |
| <i>COPT2-2-RP</i>    | TTTATGGGGAATTCCCAAAG  |
| <i>COPT1-LP</i>      | TCCTCCTCCTCACATTCACAC   |
| <i>COPT1-RP</i>      | CCTACATTACCCGATTTGCTG   |
| <i>LBb1.3</i>        | ATTTTGCCGATTTTCGGAAC  |

**Table S3.** qPCR primers used in this study

| <b>Gene</b>        | <b>5' → 3'</b>            |
|--------------------|---------------------------|
| <i>Actin 2-F</i>   | GACCTTTAACTCTCCCGCTA      |
| <i>Actin 2-R</i>   | GGAAGAGAGAAACCCCTCGTA     |
| <i>COPT1-F</i>     | CATGTCGTTTAACGCCGGTGTGTT  |
| <i>COPT1-R</i>     | CCGGAAAGTTTGGCTTCCGAACAA  |
| <i>COPT2-F</i>     | TGGTGATGCTCGCTGTTATGTCCT  |
| <i>COPT2-R</i>     | TCTGGTCATCGGAGGGTTTCTTGA  |
| <i>COPT3-F</i>     | AATGTATTGGGTCTGTCTCGCCGT  |
| <i>COPT3-R</i>     | GCCACGAAGACTCCTCCATTGAA   |
| <i>COPT4-F</i>     | AGACCGTCACTGTTACACCCAACA  |
| <i>COPT4-R</i>     | AGTGCATACATCCCACGGTCAGAA  |
| <i>COPT5-F</i>     | ATCAATACCTCGAGAATCGCCGCA  |
| <i>COPT5-R</i>     | AGCTGCAAGCATCAGCAAGTAACC  |
| <i>COPT6-F</i>     | ACACTCAAGACAGGCCTT        |
| <i>COPT6-R</i>     | CGAAGAGCATGAAACCCAC       |
| <i>SPL7-F</i>      | GAGCTGGAGGGCTATATCCG      |
| <i>SPL7-R</i>      | GGAAGAGGCTCGATGACTGT      |
| <i>miR398b/c-F</i> | GGATCTCGACAGGGTTGATATG    |
| <i>miR398b/c-F</i> | AAGAGCTCAGCAGGGGTGACCTG   |
| <i>FSD1-F</i>      | ACTTACAGCTTCCCAAGACAC     |
| <i>FSD1-R</i>      | TGCTGTGAATCCCCTTG TG      |
| <i>CSD1-F</i>      | TTCTGGCCTTAAGCCTGGTC      |
| <i>CSD1-R</i>      | CGACATGCTGGTGATCTAGG      |
| <i>CSD2-F</i>      | CATGACACACGGAGCTCCAG      |
| <i>CSD2-R</i>      | GAGCTGGAGGGCTATATCCG      |
| <i>F-box-F</i>     | TTTCGGCTGAGAGGTTTCGAGT    |
| <i>F-box-R</i>     | GATTCCAAGACGTAAAGCAGATCAA |

## SUPPLEMENTAL FIGURE LEGENDS

**Figure S1.** Basic characterization of *copt2-1* and *copt2-2* alleles. **A.** Genomic structure of *COPT2* (start codon indicated by horizontal black arrow on the black box labeled *COPT2*). Note that *COPT2* does not contain introns. Triangles indicate T-DNA insertions located 89 and 455 bp in the genomic region upstream of *COPT2* ORF in the *copt2-1* (*copt2-1*) and *copt2-2* (*copt2-2*) alleles, respectively. Positions of primers used for genotyping the *copt2-1* allele are indicated as arrows above and for the *copt2-2* allele below the schematic representation of the genomic structure of *COPT2*. Note that the T-DNA insertion for *copt2-1* is oriented towards the *COPT2* coding region, while the T-DNA insertion within the *copt2-2* allele is oriented in the opposite direction. Scale bar = 500 bp. **B.** Comparison of PCR products using both LBb1.3 + RP1/2 and LP1/2 + RP1/2 primer combinations and genomic DNA (gDNA) isolated from *copt2-1* (*copt2-1*) and *copt2-2* (*copt2-2*) mutant plants, respectively, shows that both mutants bear homozygous T-DNA insertions, in contrast to wild-type plants (Wt). LBb1.3 + RP1/2 primers used to detect T-DNA insertions produce a ~0.6 kb product for *copt2-1* and *copt2-2*, suggesting a presence of T-DNA inserts. Primer pairs LP1/RP1 and LP2/RP2 detect genomic fragments lacking the T-DNA insert and thus, PCR products of the indicated size are present in the gDNA from the wild-type but not from homozygous *copt2-1* or *copt2-2* alleles. **C.** RT-PCR detection of full-length *COPT2* transcripts (482 bp, 27 cycles) using *COPT2*-F and *COPT2*-R (Table S2) in roots of 14-day old Wt, *copt2-1* and *copt2-2* seedlings show the lack of the detectable *COPT2* transcript, in contrast to wild-type plants. *Actin2* was used as a loading control and detected using qPCR primers (141 bp Table S3). Note: 30 cycles show a faint band in *copt2-1* seedlings (not shown).

**Figure S2. A.** Genomic structure of *COPT1* (start codon indicated by horizontal black arrow). Note that *COPT1* does not contain introns. Gray arrowheads indicate a T-DNA insertion with a predicted location 93 bp in the promoter region of *COPT1* in the *copt1-1* (*copt1-1*) mutant, according to the flanking sequence associated with the SALK\_067183 allele. Arrowheads seen above the genomic structure indicate LP (**LP**), RP (**RP**), LBb1.3 (**LBb1.3**) primer positions. Scale bar = 500 bp. **B.** Comparison of PCR products using both LBb1.3 + RP and LP + RP primer combinations in gDNA isolated from *copt1-1* (*copt1-1*) mutant plants indicate that the *copt1-1* allele contains homozygous T-DNA insertions, in contrast to wild-type plants (**Wt**).

LBb1.3 + RP primers used to detect T-DNA produce a ~0.8 kb product while LP + RP primers used to detect non-T-DNA bearing plants result in a 1.2 kb product.

**Figure S3.** RT-PCR comparison of the transcript abundance of *COPT1*, *COPT2* and *COPT6* in the wild-type (*Wt*) and a triple *copt1-1copt2-1copt6-1* mutant (*copt1copt2copt6*) of *A. thaliana*. Plants were grown on solid ½ MS medium for 10 days prior RNA extraction and cDNA synthesis. *COPT1* (***COPT1***), *COPT2* (***COPT2***) and *COPT6* (***COPT6***) transcripts were detected using qPCR primer pairs (Table S3) and *Actin2* (***ACTIN2***) as a loading control. Reactions were run for 27 cycles before detection.

Figure S1

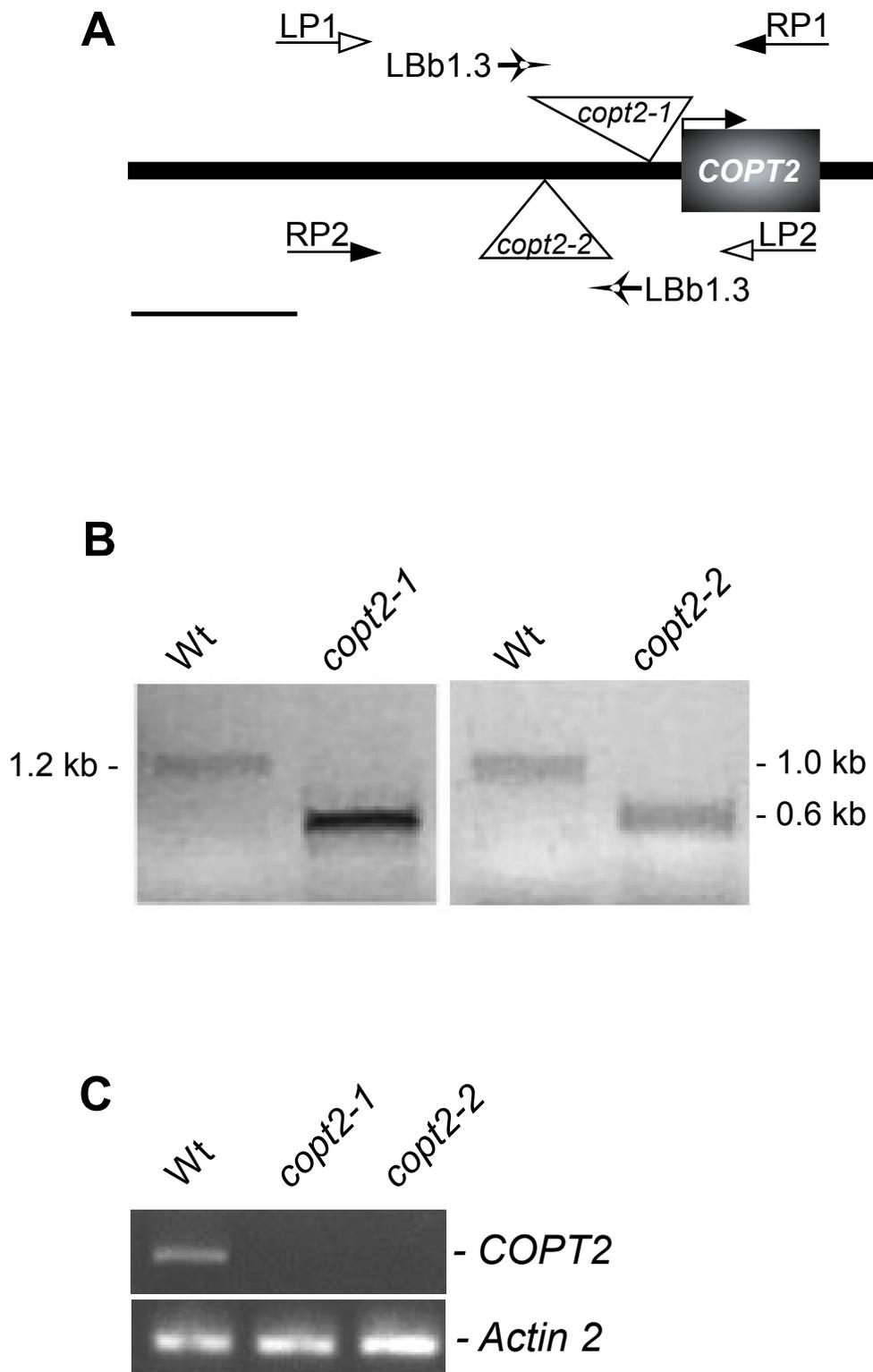
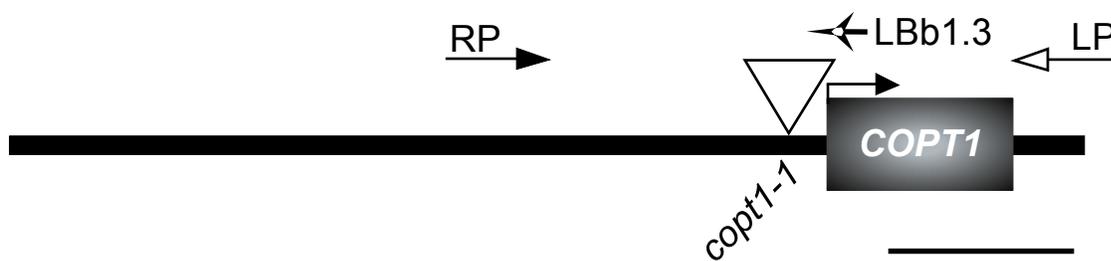
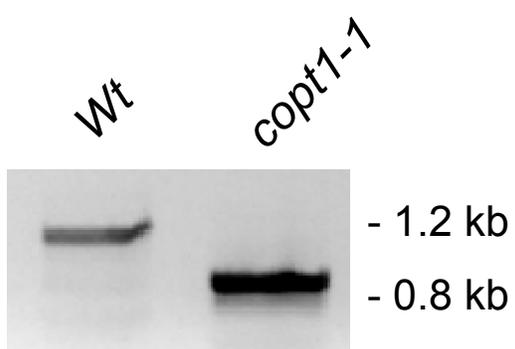


Figure S2

**A**



**B**



**C**

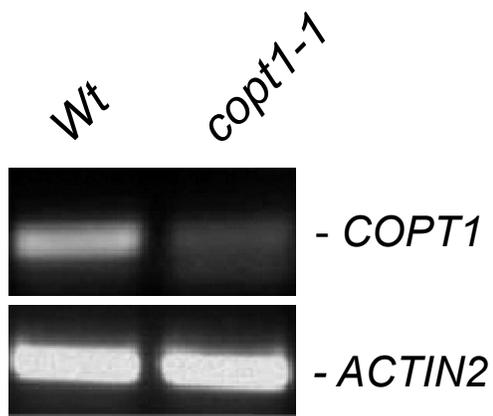


Figure S3

