



**Figure S1** Rosette (green bars) and root (gray bars) dry weight (mg) of *Arabidopsis thaliana* plants grown under control conditions (0  $\mu\text{M}$  CdSO<sub>4</sub>, light bars) or exposed to 5  $\mu\text{M}$  CdSO<sub>4</sub> (dark bars) during 2 h, 4 h, 6 h and 24 h after 3 weeks of growth. For each time point, data represent the mean  $\pm$  S.E. of 5 biological independent replicates. No significant differences (t-test:  $p < 0.05$ ) were observed between control and exposed plants, within each time point.

**Table S1** Percentage reduced glutathione (GSH) in leaves and roots of *Arabidopsis thaliana* plants grown under control condition (0  $\mu\text{M}$  CdSO<sub>4</sub>) or exposed to 5  $\mu\text{M}$  CdSO<sub>4</sub> during 2 h, 4 h, 6 h and 24 h after 3 weeks of growth. For each time point, data represent the mean  $\pm$  S.E. of 4 biological replicates. Significant differences (t-test:  $p < 0.05$ ) between control and exposed plants, within each time point, are indicated with an asterisk (\*).

[CdSO <sub>4</sub> ]	Reduced GSH (%)			
	2 h	4 h	6 h	24 h
<i>Leaf</i>				
0 $\mu\text{M}$	97.80 $\pm$ 0.20	97.62 $\pm$ 0.43	91.70 $\pm$ 0.00	97.20 $\pm$ 0.15
5 $\mu\text{M}$	97.97 $\pm$ 0.25	95.56 $\pm$ 0.28	91.71 $\pm$ 0.00	99.07 $\pm$ 0.10*
<i>Root</i>				
0 $\mu\text{M}$	97.15 $\pm$ 0.27	97.13 $\pm$ 0.81	94.15 $\pm$ 1.02	97.97 $\pm$ 0.54
5 $\mu\text{M}$	98.32 $\pm$ 0.33*	99.71 $\pm$ 0.41	98.24 $\pm$ 0.48*	98.97 $\pm$ 0.19

**Table S2** Sequence of forward and reverse primers used for gene expression analyses (RT-qPCR) of genes of interest. UTR: untranslated region; x2: primer concentration of 600 nM instead of 300 nM, x3: primer concentration of 900 nM instead of 300 nM.

Genes of interest					
Gene	Locus	Forward Primer (5'-3')	Reverse Primer (5'-3')	Primer Location	Amplicon Size
<i>GSH1</i>	AT4G23100	CCCTGGTGAAGTGCCTTCA	CATCAGCACCTTCATCTCCA	Exon 10 and 11	101 bp
<i>GSH2</i>	AT5G27380	GGACTCGTCGTTGGTGACAA	TCTGGGAATGCAGTTGGTAGC	Exon 1 and 2	101 bp
<i>GR1</i>	AT3G24170	CTCAAGTGTGGAGCAACCAAAG	ATGCGTCTGGTCACACTGC	Exon 15 and 16	101 bp
<i>GGT1</i>	AT4G39640	TAGATGTTCCACCACCAGCA	GGCAATAATGTTGGCTCCTC	Exon 4 and 5	140 bp
<i>ZAT6</i>	AT5G04340	TGACCTGCCTTCTTCTTCGT	GTCCACCAAGAGCCTGGTAA	No introns	113 bp
<i>RBOHC</i>	AT5G51060	TCACCAGAGACTGGCACAATAAA	GATGCTCGACCTGAATGCTC	Exon 6 and 7	101 bp
<i>RBOHD</i>	AT5G47910	AACTCTCCGCTGATTCCAACG	TGGTCAGCGAAGTCTTTAGATTCTT	Exon 1 and 2	91 bp
<i>RBOHF</i>	AT1G64060	GGTGTCAATGAACGAAGTTGCA	AATGAGAGCAGAACGAGCATCA	Exon 11 and 12	105 bp
<i>AT1G05340</i>	AT1G05340	TCGGTAGCTCAGGGTAAAGTGG	CCAGGGCACAACAGCAACA	Exon 2 and 3 op compl	91 bp
<i>AT1G19020</i>	AT1G19020	GAAAATGGGACAAGGGTTAGACAAA	CCCAACGAAAACCAATAGCAGA	No introns	92 bp
<i>AT1G57630</i>	AT1G57630	ACTCAAACAGGCGATCAAAGGA	CACCAATTCGTCAAGACAACACC	No introns	91 bp
<i>AT2G21640</i>	AT2G21640	GACTTGTTTCAAAAACACCATGGAC	CACTTCCTTAGCCTCAATTTGCTTC	Exon 1 and 2	91 bp
<i>AT2G43510</i>	AT2G43510	ATGGCAAAGGCTATCGTTTCC	CGTTACCTTGCCTTCTATCTCC	Exon 1 and 2	91 bp
<i>ZAT12</i>	AT5G59820	GTGCGAGTCACAAGAAGCCTAACA	GCGACGACGTTTTACCTTCTTCA	No introns	72 bp
<i>RRTF1</i>	AT4G34410	CGGAGCAAGAGCTTTCAGTT	GCGCTTATCACTGTGCTGTC	No introns	109 bp
<i>ACS2</i>	AT1G01480	CATGTTCTGCCTTGCGGATC	ACCTGTCCGCCACCTCAAGT	Exon 3 and 4	91 bp
<i>ACS6</i> <sup>x3</sup>	AT4G11280	TTAGCTAATCCCGGCGATGG	ACAAGATTCACTCCGGTTCTCCA	Exon 3 and 4	92 bp
<i>ACO2</i>	AT1G62380	TCTACGTTTCGTCACCTCCCTCA	CTCTTACCAAAGTCTTTCATGGCC	Exon 2 and 3	91 bp
<i>ACO4</i>	AT1G05010	CTCCGATGTCCCTGATCTCG	ATCCAGTAGCTCCTCCGACAACCT	Exon 2 and 3 compl	91 bp
<i>ERF1</i> <sup>x2</sup>	AT3G23240	TCCTCGGCGATTCTCAATTTT	CAACCGGAGAACAACCATCCT	No introns	91 bp
<i>OXII</i>	AT3G25250	TAGAGGATCGAACCGGAAAG	GACCCTTGATTTCTCAACG	Exon 2	149 bp
<i>MPK3</i>	AT3G45640	GACGTTTGACCCCAACAGAA	TGGCTTTTGACAGATTGGCTC	Exon 5 and 6	103 bp
<i>MPK6</i>	AT2G43790	TAAGTTCCTGACAGTGCATCC	GATGGGCCAATGCGTCTAA	Exon 5 and 6	101 bp
<i>WRKY33</i>	AT2G38470	TCATCGATTGTCAGCAGAGACG	CCATCCCACCATTTGTTTCAT	Exon 3 and 4	92 bp

**Table S3** Sequence of forward and reverse primers used for gene expression analyses (RT-qPCR) of selected reference genes. Exon jn: exon junction UTR: untranslated region

Reference genes					
Gene	Locus	Forward Primer (5'-3')	Reverse Primer (5'-3')	Primer Location	Amplicon Size
<i>Root</i>					
<i>UBC21</i>	AT5G25760	CTGCGACTCAGGGAATCTTCTAA	TTGTGCCATTGAATTGAACCC	Exon jn 3-4 and exon 4	61 bp
<i>PPR</i>	AT5G55840	AAGACAGTGAAGGTGCAACCTTACT	AGTTTTTGAGTTGTATTTGTCAGAGAAAG	UTR	59 bp
<i>YSL8</i>	AT5G08290	TTACTGTTTCGGTTGTCTCCATT	CACTGAATCATGTTCCAAGCAAGT	UTR	61 bp
<i>Leaf</i>					
<i>ACT2</i>	AT3G18780	CTTGACCAAGCAGCATGAA	CCGATCCAGACACTGTACTTCCTT	Exon 2	68 bp
<i>PPR</i>	AT5G55840	AAGACAGTGAAGGTGCAACCTTACT	AGTTTTTGAGTTGTATTTGTCAGAGAAAG	UTR	59 bp
<i>MON1</i>	AT2G28390	AACTCTATGCAGCATTGATCCACT	TGATTGCATATCTTTATCGCCATC	Exon 13 and 14	61 bp
<i>FBOX</i>	AT5G15710	TTTCGGCTGAGAGGTTTCGAGT	GATTCCAAGACGTAAAGCAGATCAA	Exon 1	63 bp
<i>UBQ10</i>	AT4G05320	GGCCTTGATAATCCCTGATGAATAAG	AAAGAGATAACAGGAACGGAAACATAGT	UTR	61 bp

**Table S4** Reverse transcription quantitative PCR (RT-qPCR) parameters according to the Minimum Information for publication of Quantitative real-time PCR Experiments (MIQE) guidelines based on Bustin *et al.* (2009).

<b>Sample - Template</b>	
<i>SOURCE</i>	three-weeks-old <i>Arabidopsis thaliana</i> roots and leaves grown in hydroponics
<i>METHOD OF PRESERVATION</i>	snap frozen in N <sub>2</sub> , long-term storage at -80°
<i>STORAGE TIME</i>	< 6 months
<i>HANDLING</i>	frozen
<i>EXTRACTION METHOD</i>	silica-columns: RNAqueous™ Kit (Ambion, Thermo Fisher Scientific, Waltham, MA, USA)
<i>RNA; DNA-FREE</i>	turbo DNA-free™ Kit (Ambion, Thermo Fisher Scientific, Waltham, MA, USA) use of intron-spanning primers if possible verification of amplicon-specificity via dissociation curve
<i>CONCENTRATION</i>	NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Ma, USA)
<b>Assay optimisation – validation</b>	
<i>ACCESSION NUMBER</i>	see Supplementary Table S1 & S2
<i>AMPLICON DETAILS</i>	see Supplementary Table S1 & S2
<i>PRIMERS SEQUENCE</i>	see Supplementary Table S1 & S2
<i>IN SILICO</i>	TAIR primer-BLAST <a href="http://www.arabidopsis.org/Blast/index.jsp">http://www.arabidopsis.org/Blast/index.jsp</a>
<i>EMPIRICAL</i>	primer concentration of 300 nM unless stated otherwise (Table S1 & S2) annealing temperature of 60°C
<i>PRIMING CONDITIONS</i>	combinations of random hexamers and oligo-dT primers
<i>PCR EFFICIENCY</i>	dilution curves of pooled samples (slope, deviation)
<i>LINEAR DYNAMIC RANGE</i>	samples are within the range of the efficiency curve
<b>RT and qPCR</b>	
<i>PROTOCOLS</i>	Turbo DNA-free™ Kit (Ambion, Thermo Fisher Scientific, Waltham, MA, USA) PrimeScript™ RT Reagent Kit (Perfect Real Time, Takara Bio Inc., Kusatsu, Japan) Qauntinova™ SYBR® Green PCR kit (Qiagen, Hilde, Germany) as described in the Materials and Methods section
<i>REAGENTS</i>	as described in the Materials and Methods section
<i>NTC</i>	verification based on Cq-value and dissociation curve
<b>Data analysis</b>	
<i>SPECIALIST SOFTWARE</i>	7500 Fast System Sequence Detection Software, version 1.4.0 (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA)

*STATISTICAL JUSTIFICATION*

specified in Materials and Methods section and legends of Figures and Tables

*NORMALISATION*

a selection of at least three reference genes based on the GrayNorm algorithm (Remans *et al.* 2014)

see Materials and Methods section

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