

Supplemental Table S1 Forward and reverse primer sequences used for gene expression analyses (RT-qPCR) of genes of interest. Ex jn: exon junction x2: primer concentration of 600 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	CATCAGCACCTCTCATCTCCA	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	GGTGTCATGAACGAAGTTGCA	AATGAGAGCAGAACGAGCATCA	Exon 11 and 12	105 bp
<i>AT1G05340</i>	AT1G05340	TCGGTAGCTCAGGGTAAAGTGG	CCAGGGCACAACAGCAACA	Exon 2 and 3	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> ^{x2}	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	91 bp
<i>ERF1</i> ^{x2}	AT3G23240	TCCTCGGCGATTCTCAATTTT	CAACCGGAGAACAACCATCCT	No introns	91 bp
<i>OXI1</i>	AT3G25250	TAGAGGATCGAACCGGAAAG	GACCCTTGATTTCCTCAACG	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	CCATTCCCACCATTTGTTTCAT	Exon 3 and 4	92 bp
<i>APX1</i>	AT1G07890	TGCCACAAGGATAGGTCTGG	CCTTCCTTCTCTCCGCTCAA	Exon 5 and 6	101 bp
<i>APX2</i>	AT3G09640	TTGCTGTTGAGATCACTGGAGGA	TGAGGCAGACGACCTTCAGG	Exon 3 and 4	91 bp
<i>CAT1</i>	AT1G20630	AAGTGCTTCATCGGGAAGGA	CTTCAACAAAACGCTTCACGA	Ex jn 5, 6 and exon 7	103 bp
<i>CAT2</i>	AT4G35090	AACTCCTCCATGACCGTTGGA	TCCGTTCCCTGTCGAAATTG	Exon 2 and 3	76 bp

Supplemental Table S2 Forward and reverse primer sequences used for gene expression analyses (RT-qPCR) of selected reference genes. Ex jn: exon junction; UTR: untranslated region

Reference genes					
Gene	Locus	Forward Primer (5'-3')	Reverse Primer (5'-3')	Primer Location	Amplicon Size
<i>Leaf</i> 2 h					
<i>PPR</i>	AT5G55840	AAGACAGTGAAGGTGCAACCTTACT	AGTTTTTGAGTTGTATTTGTCAGAGAAAG	UTR	59 bp
<i>MON1</i>	AT5G55840	AACTCTATGCAGCATTTGATCCACT	TGATTGCATATCTTTATCGCCATC	Exon 13 and 14	61 bp

<i>EF1α</i>	AT5G60390	TGAGCACGCTCTTCTTGCTTTCA	GGTGGTGGCATCCATCTTGTTACA	Exon 1 and ex jn 1,2	76 bp
24 h					
<i>UBC21</i>	AT5G25760	CTGCGACTCAGGGAATCTTCTAA	TTGTGCCATTGAATTGAACCC	Ex jn 3,4 and exon 4	61 bp
<i>PPR</i>	AT5G55840	AAGACAGTGAAGGTGCAACCTTACT	AGTTTTTGAGTTGTATTTGTCAGAGAAAG	UTR	59 bp
<i>MON1</i>	AT5G55840	AACTCTATGCAGCATTTGATCCACT	TGATTGCATATCTTTATCGCCATC	Exon 13 and 14	61 bp
72 h					
<i>ACT2</i>	AT3G18780	CTTGACCAAGCAGCATGAA	CCGATCCAGACACTGTACTTCCTT	Exon 2	68 bp
<i>FBOX</i>	AT5G15710	TTTCGGCTGAGAGGTCGAGT	GATTCCAAGACGTAAAGCAGATCAA	Exon 1	63 bp
<i>UBQ10</i>	AT4G05320	GGCCTTGTATAATCCCTGATGAATAAG	AAAGAGATAACAGGAACGGAAACATAGT	Exon 1 and ex jn 1,2	76 bp
<i>Root</i>					
2 h					
<i>MON1</i>	AT5G55840	AACTCTATGCAGCATTTGATCCACT	TGATTGCATATCTTTATCGCCATC	Exon 13 and 14	61 bp
<i>RHIP1</i>	AT4G26410	GAGCTGAAGTGGCTTCCATGAC	GGTCCGACATACCCATGATCC	Ex jn 7, 8 and exon 8	81 bp
<i>EF1α</i>	AT5G60390	TGAGCACGCTCTTCTTGCTTTCA	GGTGGTGGCATCCATCTTGTTACA	Exon 1 and 1,2	76 bp
24 h					
<i>UBC21</i>	AT5G25760	CTGCGACTCAGGGAATCTTCTAA	TTGTGCCATTGAATTGAACCC	Exon 3,4 and 4	61 bp
<i>MON1</i>	AT5G55840	AACTCTATGCAGCATTTGATCCACT	TGATTGCATATCTTTATCGCCATC	Exon 13 and 14	61 bp
<i>TIP41-like</i>	AT4G34270	GTGAAAACGTGTGGAGAGAAGCAA	TCAACTGGATACCCTTTCGCA	Exon 7 and Exon 7,8	61 bp
<i>YSL8</i>	AT5G08290	TTACTGTTTCGGTTGTCTCCATT	CACTGAATCATGTTCTGAAGCAAGT	UTR	61 bp
<i>UBQ10</i>	AT4G05320	GGCCTTGTATAATCCCTGATGAATAAG	AAAGAGATAACAGGAACGGAAACATAGT	Exon 1 and ex jn 1,2	76 bp
<i>EF1α</i>	AT5G60390	TGAGCACGCTCTTCTTGCTTTCA	GGTGGTGGCATCCATCTTGTTACA	Exon 1 and ex jn 1,2	76 bp
72 h					
<i>UBC21</i>	AT5G25760	CTGCGACTCAGGGAATCTTCTAA	TTGTGCCATTGAATTGAACCC	Exon 3,4 and 4	61 bp

UBQ10	AT4G05320	GGCCTTGTATAATCCCTGATGAATAAG	AAAGAGATAACAGGAACGGAAACATAGT	Exon 1 and 1,2	76 bp
EF1 α	AT5G60390	TGAGCACGCTCTTCTTGCTTTCA	GGTGGTGGCATCCATCTTGTTACA	Exon 1 and 1,2	76 bp

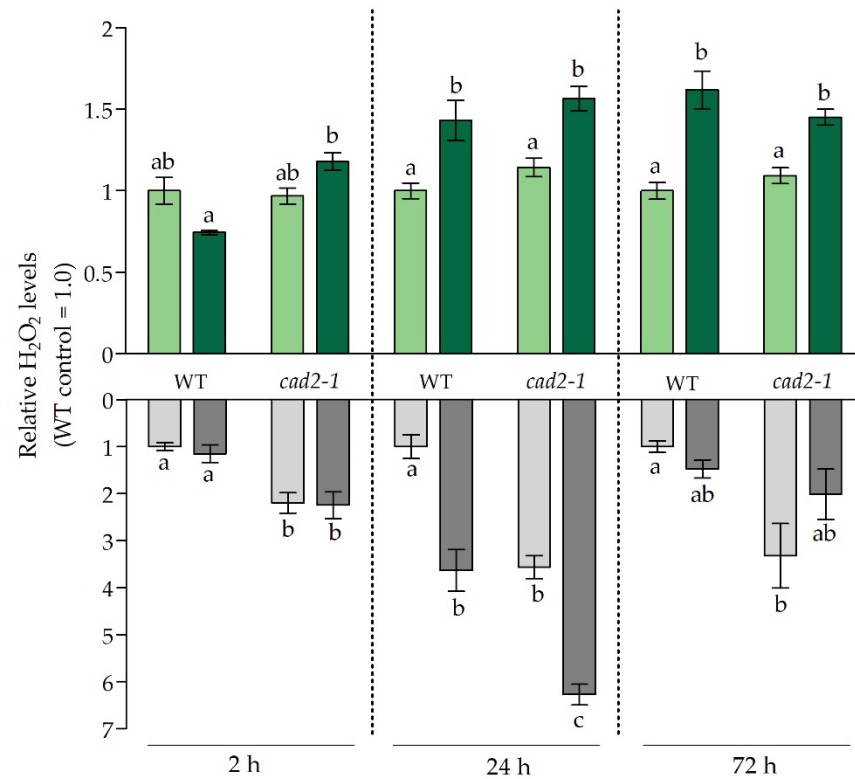
Table S3 Reverse transcription quantitative PCR (RT-qPCR) parameters according to the Minimum Information for publication of Quantitative real-time PCR Experiments (MIQE) guidelines based on Bustin *et al.* (2009).

Sample - Template	
SOURCE	roots and leaves of three-weeks-old <i>Arabidopsis thaliana</i> plants grown in hydroponics
METHOD OF PRESERVATION	snap frozen in N ₂ , long-term storage at -80°
STORAGE TIME	< 6 months
HANDLING	frozen
EXTRACTION METHOD	silica-columns: RNAqueous™ Kit (Ambion, Thermo Fisher Scientific, Waltham, MA, USA)
RNA; DNA-FREE	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
CONCENTRATION	NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Ma, USA)
Assay optimisation – Validation	
ACCESSION NUMBER	see Supplementary Table S1 & S2
AMPLICON DETAILS	see Supplementary Table S1 & S2
PRIMERS SEQUENCE	see Supplementary Table S1 & S2
IN SILICO	TAIR primer-BLAST http://www.arabidopsis.org/Blast/index.jsp
EMPIRICAL	primer concentration of 300 nM unless mentioned otherwise (Table S1 & S2) annealing temperature of 60°C
PRIMING CONDITIONS	combinations of oligo-dT and random hexamer primers
PCR EFFICIENCY	dilution curves of pooled samples (slope, deviation)
LINEAR DYNAMIC RANGE	samples are within the range of the efficiency curve
RT and qPCR	
PROTOCOLS	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
REAGENTS	as described in the Materials and Methods section
NON TEMPLATE CONTROL	verification based on Cq-value and dissociation curve
Data analysis	
SOFTWARE	QuantStudio™ Design & Analysis Software (Applied Biosystems, Thermo Fisher Scientific, Foster City, CA, USA)
STATISTICAL ANALYSIS	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 <i>et al.</i> 2014)
	see Materials and Methods section

Supplemental Table S4 Percentage reduced glutathione (GSH) deduced from oxidised GSSG levels and total GSH levels in leaves and roots of 3 weeks old wild-type (WT) and *cad2-1* mutant *Arabidopsis thaliana* plants grown under control conditions (0 μ M CdSO₄, light bars) or exposed to 5 μ M CdSO₄ (dark bars) during 2 h, 24 h and 72 h. For each time point, data presented as the mean \pm S.E. of 4 biological replicates. Significant differences (2-way ANOVA: $p < 0.05$) within each time point are indicated with different letters. L.O.D.: limit of detection for oxidised GSSG.

Reduced GSH (%)							
		2 h		24 h		72 h	
	[CdSO ₄]	WT	<i>cad2-1</i>	WT	<i>cad2-1</i>	WT	<i>cad2-1</i>
Leaf	0 μ M	96.87 \pm 9.01 ^a	97.98 \pm 1.74 ^a	96.94 \pm 0.30 ^a	96.11 \pm 0.75 ^a	90.82 \pm 0.81 ^a	94.37 \pm 0.46 ^b
	5 μ M	97.56 \pm 7.43 ^a	98.06 \pm 1.23 ^a	98.92 \pm 0.22 ^b	98.84 \pm 0.07 ^{ab}	96.56 \pm 1.08 ^c	98.08 \pm 0.43 ^c
Root	0 μ M	95.95 \pm 7.38	96.92 \pm 1.91	97.97 \pm 0.39 ^a	98.05 \pm 0.24 ^a	97.00 \pm 0.92 ^a	96.89 \pm 1.33 ^a
	5 μ M	99.96 \pm 0.22	L.O.D.	97.34 \pm 0.70 ^{ab}	95.52 \pm 0.09 ^b	98.06 \pm 0.11 ^a	96.77 \pm 1.03 ^a



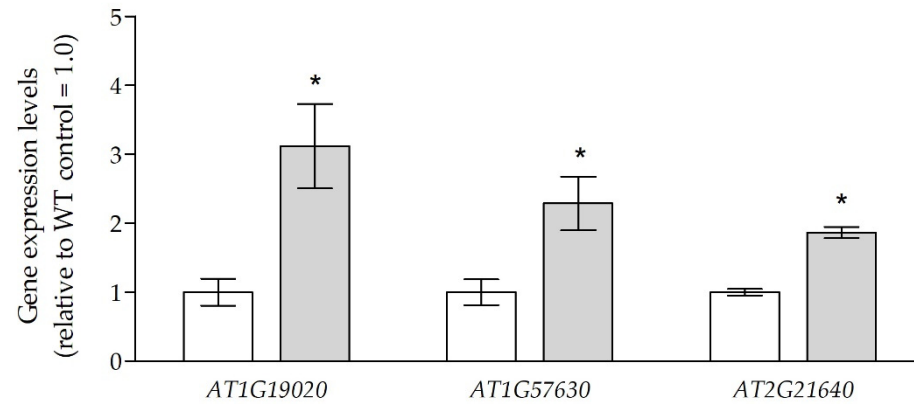
Supplemental Figure S1 Relative hydrogen peroxide (H₂O₂) levels in leaves (green bars) and roots (grey bars) of 3 weeks old wild-type (WT) and *cad2-1* mutant *Arabidopsis thaliana* plants grown under control conditions (0 μM CdSO₄, light bars) or exposed to 5 μM CdSO₄ (dark bars) during 2 h, 24 h and 72 h. Data are presented as the mean ± S.E. of at least three biological independent replicates relative to the control of the WT set at 1.00. Significant differences (2-way ANOVA: $p < 0.05$) within each time point are indicated with different letters.

Supplemental Table S5 Relative gene expression levels of antioxidative enzymes in leaves and roots of 3 weeks old wild-type (WT) and *cad2-1* mutant *Arabidopsis thaliana* plants grown under control conditions (0 μ M CdSO₄, light bars) or exposed to 5 μ M CdSO₄ (dark bars) during 2 h, 24 h and 72 h. Data are presented as the mean \pm S.E. of at least three biological independent replicates relative to the control of the corresponding genotype set at 1.00. Significant differences (2-way ANOVA: $p < 0.05$) between control and Cd-exposed plants of the same genotype, within each time point are marked in colour (upregulated: , downregulated:). Significant differences between Cd-induced responses of both genotypes within each time point are indicated with an asterisk (*). APX: ascorbate peroxidase , CAT: catalase.

Antioxidative enzymes							
Gene	CdSO ₄	2 h		24 h		72 h	
		WT	<i>cad2-1</i>	WT	<i>cad2-1</i>	WT	<i>cad2-1</i>
<i>Leaf</i>							
<i>APX1</i>	0 μM	1.00 ± 0.08	1.00 ± 0.08	1.00 ± 0.02	1.00 ± 0.08	1.00 ± 0.02	1.00 ± 0.02
	5 μM	1.04 ± 0.12	0.65 ± 0.04*	2.13 ± 0.18	1.25 ± 0.27	1.41 ± 0.36	0.44 ± 0.00
<i>APX2</i>	0 μM	1.00 ± 0.09	1.00 ± 0.15	1.00 ± 0.21	1.00 ± 0.35	1.00 ± 0.12	1.00 ± 0.29
	5 μM	0.65 ± 0.09	0.85 ± 0.18	4.21 ± 1.41	0.41 ± 0.20*	4.02 ± 0.75	0.22 ± 0.00
<i>CAT1</i>	0 μM	1.00 ± 0.07	1.00 ± 0.14	1.00 ± 0.04	1.00 ± 0.14	1.00 ± 0.18	1.00 ± 0.22
	5 μM	1.10 ± 0.14	0.62 ± 0.05*	3.48 ± 0.41	1.20 ± 0.36*	1.12 ± 0.34	0.91 ± 0.03
<i>CAT2</i>	0 μM	1.00 ± 0.03	1.00 ± 0.12	1.00 ± 0.10	1.00 ± 0.10	1.00 ± 0.01	1.00 ± 0.06
	5 μM	0.89 ± 0.11	0.71 ± 0.05	0.30 ± 0.04	0.72 ± 0.03*	1.02 ± 0.22	0.66 ± 0.10
<i>Root</i>							
<i>APX1</i>	0 μM	1.00 ± 0.01	1.00 ± 0.11	1.00 ± 0.05	1.00 ± 0.03	1.00 ± 0.14	1.00 ± 0.09
	5 μM	1.10 ± 0.04	1.16 ± 0.05	1.04 ± 0.05	1.75 ± 0.21	0.98 ± 0.05	0.75 ± 0.05
<i>APX2</i>	0 μM	1.00 ± 0.19	1.00 ± 0.04	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.17	1.00 ± 0.30
	5 μM	2.25 ± 0.27	14.95 ± 1.61*	9.02 ± 1.29	157.47 ± 36.74*	0.26 ± 0.07	6.99 ± 0.62*
<i>CAT1</i>	0 μM	1.00 ± 0.05	1.00 ± 0.02	1.00 ± 0.07	1.00 ± 0.04	1.00 ± 0.14	1.00 ± 0.06
	5 μM	0.90 ± 0.08	0.89 ± 0.04	3.74 ± 0.25	4.16 ± 0.07	1.81 ± 0.02	2.64 ± 0.33*
<i>CAT2</i>	0 μM	1.00 ± 0.15	1.00 ± 0.08	1.00 ± 0.04	1.00 ± 0.08	1.00 ± 0.15	1.00 ± 0.07
	5 μM	0.78 ± 0.12	0.78 ± 0.00	0.38 ± 0.01	1.05 ± 0.04*	0.64 ± 0.02	0.36 ± 0.05*

Supplemental Table S6 Relative gene expression levels of ROS-generating NADPH oxidases in leaves and roots of 3 weeks old wild-type (WT) and *cad2-1* mutant *Arabidopsis thaliana* plants grown under control conditions (0 μ M CdSO₄, light bars) or exposed to 5 μ M CdSO₄ (dark bars) during 2 h, 24 h and 72 h. Data are presented as the mean \pm S.E. of at least three biological independent replicates relative to the control of the corresponding genotype set at 1.00. Significant differences (2-way ANOVA: $p < 0.05$) between control and Cd-exposed plants of the same genotype, within each time point are marked in colour (upregulated: , downregulated:). Significant differences between Cd-induced responses of both genotypes within each time point are indicated with an asterisk (*). *RBOH*: respiratory burst oxidase homologue.

Gene	CdSO ₄	ROS-generating NADPH oxidase					
		2 h		24 h		72 h	
		WT	<i>cad2-1</i>	WT	<i>cad2-1</i>	WT	<i>cad2-1</i>
<i>Leaf</i>							
<i>RBOHC</i>	0 μ M	1.00 \pm 0.32	1.00 \pm 0.33	1.00 \pm 0.14	1.00 \pm 0.46	1.00 \pm 0.15	1.00 \pm 0.38
	5 μ M	0.47 \pm 0.06	0.74 \pm 0.34	79.81 \pm 6.35	0.63 \pm 0.11*	0.58 \pm 0.14	1.42 \pm 0.42
<i>RBOHD</i>	0 μ M	1.00 \pm 0.02	1.00 \pm 0.04	1.00 \pm 0.16	1.00 \pm 0.23	1.00 \pm 0.05	1.00 \pm 0.06
	5 μ M	1.37 \pm 0.02	1.17 \pm 0.04*	2.45 \pm 0.09	1.46 \pm 0.23	2.25 \pm 0.28	1.93 \pm 0.19
<i>RBOHF</i>	0 μ M	1.00 \pm 0.30	1.00 \pm 0.19	1.00 \pm 0.08	1.00 \pm 0.13	1.00 \pm 0.10	1.00 \pm 0.14
	5 μ M	0.72 \pm 0.13	0.58 \pm 0.07	6.74 \pm 0.82	0.66 \pm 0.11*	3.99 \pm 0.58	1.20 \pm 0.01*
<i>Root</i>							
<i>RBOHC</i>	0 μ M	1.00 \pm 0.01	1.00 \pm 0.17	1.00 \pm 0.06	1.00 \pm 0.01	1.00 \pm 0.16	1.00 \pm 0.10
	5 μ M	1.41 \pm 0.19	1.18 \pm 0.03	1.04 \pm 0.06	0.87 \pm 0.06	0.68 \pm 0.02	0.69 \pm 0.04
<i>RBOHD</i>	0 μ M	1.00 \pm 0.10	1.00 \pm 0.14	1.00 \pm 0.04	1.00 \pm 0.05	1.00 \pm 0.11	1.00 \pm 0.14
	5 μ M	1.56 \pm 0.01	2.43 \pm 0.13*	4.50 \pm 0.60	3.90 \pm 0.21	2.51 \pm 0.28	2.38 \pm 0.45
<i>RBOHF</i>	0 μ M	1.00 \pm 0.03	1.00 \pm 0.03	1.00 \pm 0.03	1.00 \pm 0.04	1.00 \pm 0.08	1.00 \pm 0.06
	5 μ M	1.28 \pm 0.03	1.28 \pm 0.05	2.20 \pm 0.08	1.92 \pm 0.05	1.98 \pm 0.25	1.78 \pm 0.23



Supplemental Figure S2 Relative gene expression levels of a subset of oxidative stress markers in roots after 24 h under control conditions (0 μ M CdSO₄) in 3 weeks old wild-type (WT, white bars) and *cad2-1* mutant (grey bars) *Arabidopsis thaliana* plants. Data are represented as the mean \pm S.E. of at least three biological independent replicates relative to the control of the WT set at 1.00. For each gene, significant differences (t-test; $p < 0.05$) between the two genotypes under control conditions are indicated with an asterisk (*).