

Supplemental Figure S1

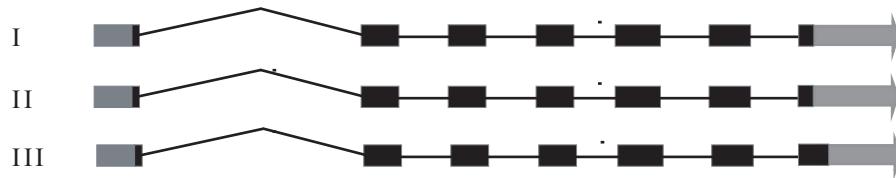
A

AtPDCD5 (I)	MADPELEAIRQRRM QELMARQGM-GKQGNQQNPEQE KQQEDAKREADERRQMM LSQVLSS 59
AtPDCD5 (II)	MADPELEAIRQRRM QELMARQGM-QGKQGNQQNPEQE KQQEDAKREADERRQMM LSQVLSS 60
AtPDCD5 (III)	MADPELEAIRQRRM QELMARQGM-GKQGNQQNPEQE KQQEDAKREADERRQMM LSQVLSS 59
OsPDCD5	MADPELEAIRQRRM QELMAQHGA ---ANPQNAGQQKAQE DAKQEAEERRQMM LAQILSS 56
HsPDCD5	MADEELEALR RQR LAEL QAKHG D-----PGDAAQ QEA KHREA EMR -NSILA QVLDQ 50 *** * * * : * : * : ** * : * : * : * : * : * : * : * : * : * : * : * : * : * : * ..
AtPDCD5 (I)	QARERIARIALVK PEKARGVEDV I LRAAQM GQIVEKVSEERL ITLLEQ INSQTTKQT KVT 119
AtPDCD5 (II)	QARERIARIALVK PEKARGVEDV I LRAAQM GQIVEKVSEERL ITLLEQ INSQTTKQT KVT 120
AtPDCD5 (III)	QARERIARIALVK PEKARGVEDV I LRAAQM GQIVEKVSEERL ITLLEQ INSQTTKQT KVT 119
OsPDCD5	EARERLSRIALVK PKD KARGVEDV LLRAAQS GGISEKVSEERL ISLLEQ INTHTSKQT KVT 116
HsPDCD5	SARARLSNLALVK PEKTKA VENY LIQ MARY GQL SEKV SEQ GLIE I KKV SQ TE KTT TV K 110 . * * : * : * : * : * : * : * : * : * : * : * : * : * : * : * ..
AtPDCD5 (I)	YQRRRG V -----DDD----- 129
AtPDCD5 (II)	YQRRRG V -----DDD----- 130
AtPDCD5 (III)	VSTNGAVGWT TIRKEEYYDVLCISMEISLLC 151
OsPDCD5	IQRRRSVLD-----DDD----- 128
HsPDCD5	F NRRKVMDSD ---EDDDY----- 125 . . : : :

B

	OsPDCD5	HsPDCD5	AtPDCD5 (III)	AtPDCD5 (II)
AtPDCD5 (I)	76.6	43.2	93.0	100
AtPDCD5 (II)	76.6	44.0	92.3	
AtPDCD5 (III)	71.9	40.0		
HsPDCD5	45.9			

C



PDCD5 proteins and different *PDCD5* mRNA spliced forms from *A. thaliana*. (A) Amino acid sequences alignment of the predicted different *AtPDCD5* spliced forms with PDCD5 proteins from *O. sativa* and humans. The sequences were aligned using the Clustal W2 program. Dashes (-) indicate spaces introduced to promote optimal alignment, perfect matches are indicated by an asterisk (*), high amino-acid similarities by double dots (:), and weak similarities by a single dot (.). The proposed double-stranded DNA-binding domain is highlighted in grey. (B) Percentage of amino acid sequence similarity between different *AtPDCD5* spliced forms and PDCD5 proteins from *O. sativa* and humans. (c) Scheme of different *AtPDCD5* mRNA spliced forms. Exons are represented by boxes, grey and black boxes indicate UTR and coding regions, respectively, and black lines represent non-coding regions.

Supplemental Figure S2

Genes	Proteins	dsDNA_bind
PDCD5, <i>H.sapiens</i>	NP_004699.1	-
programmed cell death 5	125 aa	-
PDCD5, <i>P.troglodytes</i>	XP_512563.1	-
programmed cell death 5	125 aa	-
PDCD5, <i>M.mulatta</i>	XP_001086491	-
programmed cell death 5	125 aa	-
PDCD5, <i>C.lupus</i>	XP_00561635.1	-
programmed cell death 5	111 aa	-
PDCD5, <i>B.taurus</i>	NP_001039489	-
programmed cell death 5	125 aa	-
Pcd5, <i>M.musculus</i>	NP_062720.1	-
programmed cell death 5	126 aa	-
Gm3837, <i>M.musculus</i>	XP_00147806.1	-
predicted gene 3837	126 aa	-
Pdcd5, <i>R.norvegicus</i>	NP_001099717	-
programmed cell death 5	125 aa	-
LOC100912106, <i>R. norvegicus</i>	XP_003748886	-
Programmed cell death protein 5-like	125 aa	-
PDCD5, <i>G.gallus</i>	NP_001264515	-
programmed cell death 5	126 aa	-
pdc5, <i>X.tropicalis</i>	NP_001017011	-
programmed cell death 5	125 aa	-
pdc5, <i>D.rerio</i>	NP_957471.1	-
programmed cell death 5	128 aa	-
PDCD5, <i>D.melanogaster</i>	NP_648848.1	-
PDCD5	133 aa	-
AgaP_AGAP005432, <i>A.gambiae</i>	XP_315439.4	-
AgaP_AGAP005432	129 aa	-
D2005.3, <i>C.elegans</i>	NP_492159.2	-
D2005.3	130 aa	-
SPAC23C4.09c, <i>S.pombe</i>	NP_593181.1	-
SPAC23C4.09c	131 aa	-
AT1G29850, <i>A.thaliana</i>	NP_849728.1	-
AT1G29850	130 aa	-
Os05g0547850, <i>O.sativa</i>	NP_001174513	-
Os05g0547850	128 aa	-

Representative scheme of the double strand DNA binding domain conserved in PDCD5 proteins from eukaryotic species. The scheme was obtained using the HomoloGene tool (NCBI) that identifies homologs among the annotated genes of several sequenced eukaryotic genomes. Double-stranded DNA-binding domain is represented by green (pfam01984) and pink (cl00928) boxes.

Supplemental Figure S3

(I) 818pb
 (II) 821pb
 Genomic seq
 (III) 823pb

GGGTTTTGAAGCATTCCCTTTAGAGAAGAGAGCTGTGTTGGTTTT 50
 GGGTTTTGAAGCATTCCCTTTAGAGAAGAGAGCTGTGTTGGTTTT 50
 GGGTTTTGAAGCATTCCCTTTAGAGAAGAGAGCTGTGTTGGTTTT 50
 GGGTTTTGAAGCATTCCCTTTAGAGAAGAGAGCTGTGTTGGTTTT 50

(I) 818pb
 (II) 821pb
 Genomic seq
 (III) 823pb

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 GAGGTAAAAGAACGAGACCAATTTCGATTTGGAGGATAGAGACGAAGAAG 100
 GAGGTAAAAGAACGAGACCAATTTCGATTTGGAGGATAGAGACGAAGAAG 100
 GAGGTAAAAGAACGAGACCAATTTCGATTTGGAGGATAGAGACGAAGAAG 100

(I) 818pb
 (II) 821pb
 Genomic seq
 (III) 823pb

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 AAGACAGCTCCGATGGTGAGTTTTGCTCTAATTCTTACCTTCT 150
 AAGACAGCTCCGATG----- 115

(I) 818pb
 (II) 821pb
 Genomic seq
 (III) 823pb

 TTCATTTCCCTATATACGTTTAGGAACTCGAATTTCCTAGGGT 200

(I) 818pb
 (II) 821pb
 Genomic seq
 (III) 823pb

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(I) 818pb
 (II) 821pb
 Genomic seq
 (III) 823pb

 TGTTGATTGGAAACTGGGTTCGCTGAATTATTTAAGTTGATATCGC 300

(I) 818pb
 (II) 821pb
 Genomic seq
 (III) 823pb

 TTAGAGTTTGAAATTCATGATTGTTACTTAGGGATTTGGATCAGTGGT 350

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 (II) 821pb
 Genomic seq
 (III) 823pb

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(I) 818pb
 (II) 821pb
 Genomic seq
 (III) 823pb

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 Genomic seq
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(I) 818pb
 (II) 821pb
 Genomic seq
 (III) 823pb

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 (II) 821pb
 Genomic seq
 (III) 823pb

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 Genomic seq
 (III) 823pb

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(I) 818pb
 (II) 821pb
 Genomic seq
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 Genomic seq
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 (II) 821pb
 Genomic seq
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 Genomic seq
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 Genomic seq
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(I) 818pb
 (II) 821pb
 Genomic seq
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 Genomic seq
 (III) 823pb

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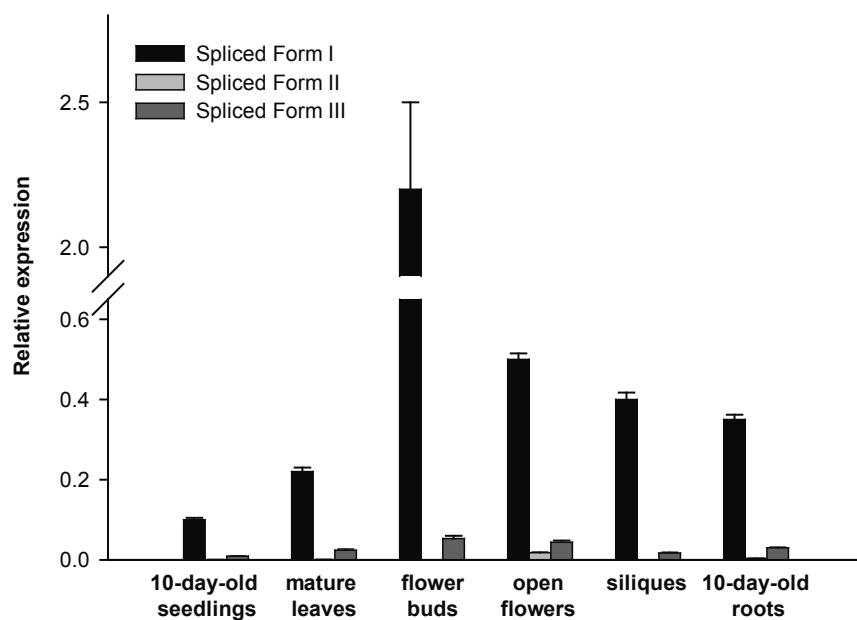
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(I) 818pb (II) 821pb Genomic seq (III) 823pb	CAAGCCCGAGAGAGAA----- 305 CAAGCCCGAGAGAGAA----- 308 CAAGCCCGAGAGAGAAAGTAAGTCTCATCTAAAATGTGTTGGAGTCATC 1350 CAAGCCCGAGAGAGAA----- 305 *****
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(I) 818pb (II) 821pb Genomic seq (III) 823pb	-----TTGCTCGAATTGCTCTGGTCAAAC 329 -----TTGCTCGAATTGCTCTGGTCAAAC 332 TTCTCTTCTTTCTTTTGATAGTTGCTCGAATTGCTCTGGTCAAAC 1450 -----TTGCTCGAATTGCTCTGGTCAAAC 329 *****
(I) 818pb (II) 821pb Genomic seq (III) 823pb	CTGAGAAAGCTAGAGGTGTGGAGGATGTTATTTGAGGGCTGCTCAGATG 379 CTGAGAAAGCTAGAGGTGTGGAGGATGTTATTTGAGGGCTGCTCAGATG 382 CTGAGAAAGCTAGAGGTGTGGAGGATGTTATTTGAGGGCTGCTCAGATG 1500 CTGAGAAAGCTAGAGGTGTGGAGGATGTTATTTGAGGGCTGCTCAGATG 379 *****
(I) 818pb (II) 821pb Genomic seq (III) 823pb	GGACAGATAAGTTGAGAAG----- 397 GGACAGATAAGTTGAGAAG----- 400 GGACAGATAAGTTGAGAAGGTAAGGAAATTCACTTTCAATGTCATA 1550 GGACAGATAAGTTGAGAAG----- 397 *****
(I) 818pb (II) 821pb Genomic seq (III) 823pb	----- ----- GTTCAGAATTCTACACATCATATGGTTCTACTCAAGTGCCGCTGGTAC 1600 -----
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(I) 818pb (II) 821pb Genomic seq (III) 823pb	-----GTTTCTGAGGAACGGCTTATAACACTGTTGAAACAATAAACAGC 442 -----GTTTCTGAGGAACGGCTTATAACACTGTTGAAACAATAAACAGC 445 TACAGGTTCTGAGGAACGGCTTATAACACTGTTGAAACAATAAACAGC 1700 -----GTTTCTGAGGAACGGCTTATAACACTGTTGAAACAATAAACAGC 442 *****
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(I) 818pb (II) 821pb Genomic seq (III) 823pb	-----TACCAA 475 -----TACCAA 478 ATTGAGTGTAAATTGATCTCGGATATTATCTTCACTCGTGTAGTACCAA 1850 -----TACCAA 480 *****
(I) 818pb (II) 821pb Genomic seq (III) 823pb	CGGCGCCGTGGGGTGGACGACGATTAGGAAGGAAGAAGAATATTGATG 525 CGGCGCCGTGGGGTGGACGACGATTAGGAAGGAAGAAGAATATTGATG 528 CGGGGCCGTGGGGTGGACGACGATTAGGAAGGAAGAAGAATATTGATG 1900 CGGGGCCGTGGGGTGGACGACGATTAGGAAGGAAGAAGAATATTGATG 530 *****

(I) 818pb (II) 821pb Genomic seq (III) 823pb	TGCTTTGTATATCTATGGAGATCTCACTGTTATGTTAGTGAGTCACCTA 575 TGCTTTGTATATCTATGGAGATCTCACTGTTATGTTAGTGAGTCACCTA 578 TGCTTTGTATATCTATGGAGATCTCACTGTTATGTTAGTGAGTCACCTA 1950 TGCTTTGTATATCTATGGAGATCTCACTGTTATGTTAGTGAGTCACCTA 580 *****
(I) 818pb (II) 821pb Genomic seq (III) 823pb	AGATCTACTTAAATGTTCTGTTCAATATGTTATTGTGAGTGAATCTTG 625 AGATCTACTTAAATGTTCTGTTCAATATGTTATTGTGAGTGAATCTTG 628 AGATCTACTTAAATGTTCTGTTCAATATGTTATTGTGAGTGAATCTTG 2000 AGATCTACTTAAATGTTCTGTTCAATATGTTATTGTGAGTGAATCTTG 630 *****
(I) 818pb (II) 821pb Genomic seq (III) 823pb	ATTATCTTCCAAACACTTGGGATGAGATTAAGCAATCTGAAACTGAAGC 675 ATTATCTTCCAAACACTTGGGATGAGATTAAGCAATCTGAAACTGAAGC 678 ATTATCTTCCAAACACTTGGGATGAGATTAAGCAATCTGAAACTGAAGC 2050 ATTATCTTCCAAACACTTGGGATGAGATTAAGCAATCTGAAACTGAAGC 680 *****
(I) 818pb (II) 821pb Genomic seq (III) 823pb	TCTACATTTTATTTGGCATTCCTCTGTGTGATCTATCTCCATCTTC 725 TCTACATTTTATTTGGCATTCCTCTGTGTGATCTATCTCCATCTTC 728 TCTACATTTTATTTGGCATTCCTCTGTGTGATCTATCTCCATCTTC 2100 TCTACATTTTATTTGGCATTCCTCTGTGTGATCTATCTCCATCTTC 730 *****
(I) 818pb (II) 821pb Genomic seq (III) 823pb	GTCTCTCTCCCATTGCCTGTATGATTGAATATTATTAGAAAGAAAA 775 GTCTCTCTCCCATTGCCTGTATGATTGAATATTATTAGAAAGAAAA 778 GTCTCTCTCCCATTGCCTGTATGATTGAATATTATTAGAAAGAAAA 2150 GTCTCTCTCCCATTGCCTGTATGATTGAATATTATTAGAAAGAAAA 780 *****
(I) 818pb (II) 821pb Genomic seq (III) 823pb b	TGGTCTGTTCTAAATTGTAATTCTCATAAAATTGATGTTGTTA 818 TGGTCTGTTCTAAATTGTAATTCTCATAAAATTGATGTTGTTA 821 TGGTCTGTTCTAAATTGTAATTCTCATAAAATTGATGTTGTTA 2193 TGGTCTGTTCTAAATTGTAATTCTCATAAAATTGATGTTGTTA 823 *****

Alignment of the *AtPDCD5* genomic sequence with the three *AtPDCD5* mRNA spliced forms. Matches are indicated by an asterisk (*), sequence differences between the mRNA spliced forms are marked in gray.

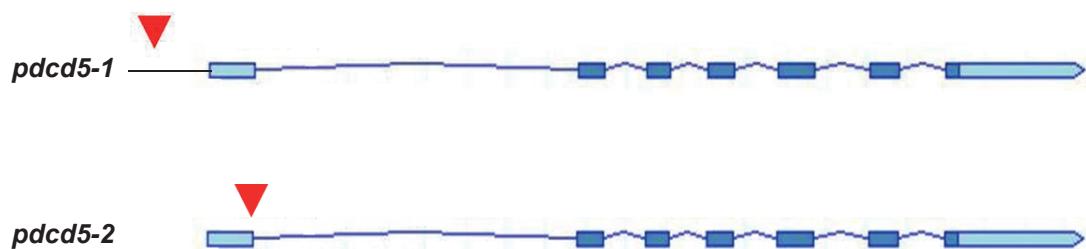
Supplemental Figure S4



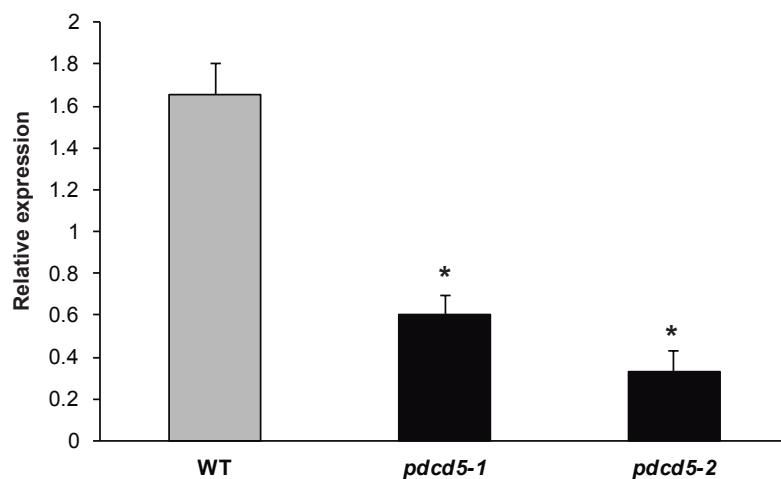
AtPDCD5 spliced forms I, II and III expression analysis in Arabidopsis. *AtPDCD5* spliced forms expression levels in different Arabidopsis tissues determined by qRT-PCR. The relative *AtPDCD5* spliced forms I, II and III transcript abundance was analyzed in seedlings (10-day-old), mature leaves (28-day-old), flower buds, open flowers, siliques and roots (10-day-old). The means of the results obtained using three independent RNAs as a template are shown, the error bars indicate the S.D. of the samples. Each reaction was normalized using the Ct values corresponding to the *UBQ10* mRNA.

Supplemental Figure S5

A

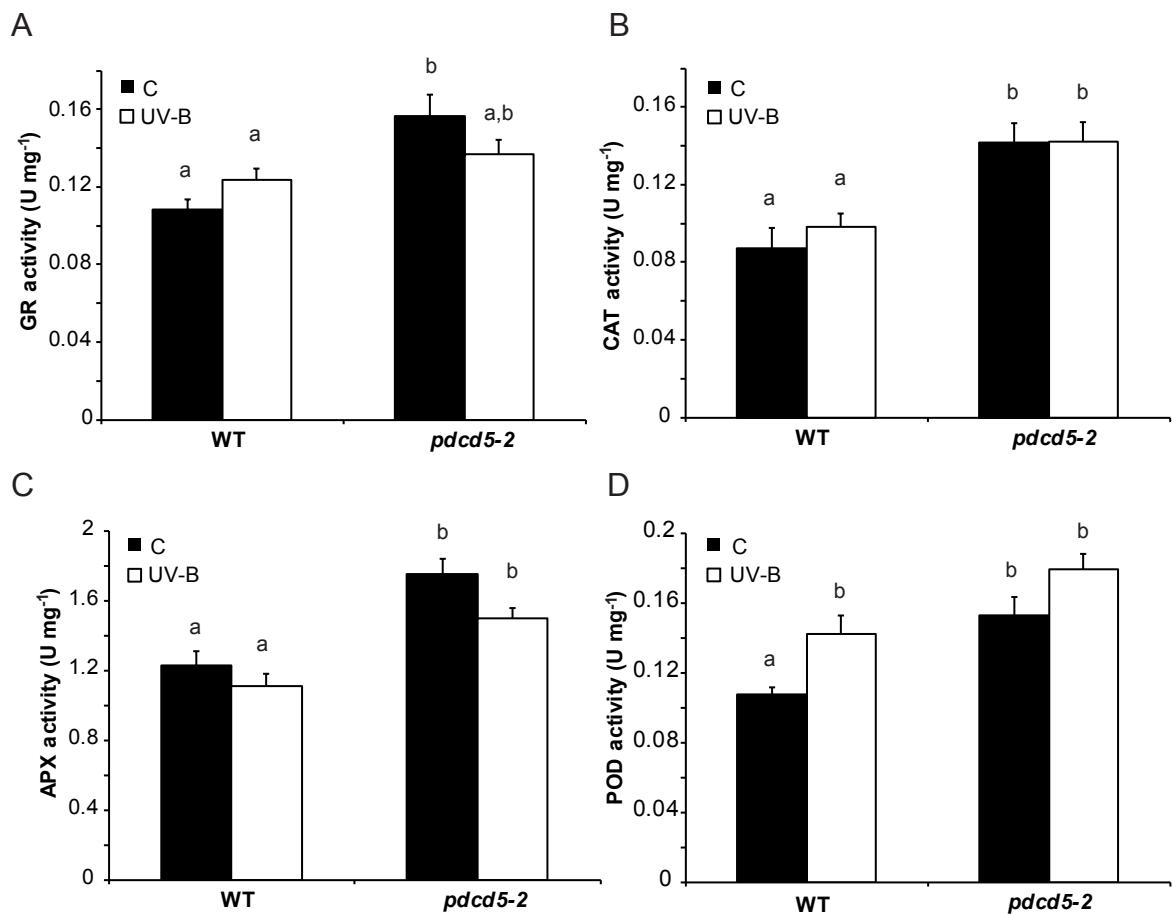


B



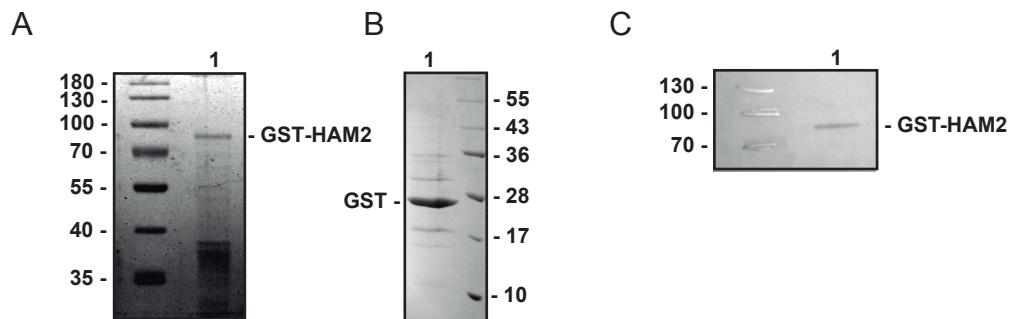
Characterization of *pdc5d* mutant lines. (A) Structure of *A. thaliana* *PDCD5* gene. Boxes indicate exons (coding regions are in blue, while 5' and 3'UTRs are in light blue), and blue and black lines represent introns and promoter regions, respectively. The open triangles show the T-DNA positions. (B) *PDCD5* transcript abundance in T-DNA lines and wild-type (WT) plants analyzed by qRT-PCR. The means of the results obtained using three independent RNAs as a template are shown, the error bars indicate the S.D. of the samples. Each reaction was normalized using the C_t values corresponding to the *UBQ10* mRNA. For each *pdc5d* mutant line analyzed, asterisks over the bars indicate statistically significant differences between wild type and *pdc5d* mutant plants applying Student's *t*-test ($P < 0.05$).

Supplemental Figure S6



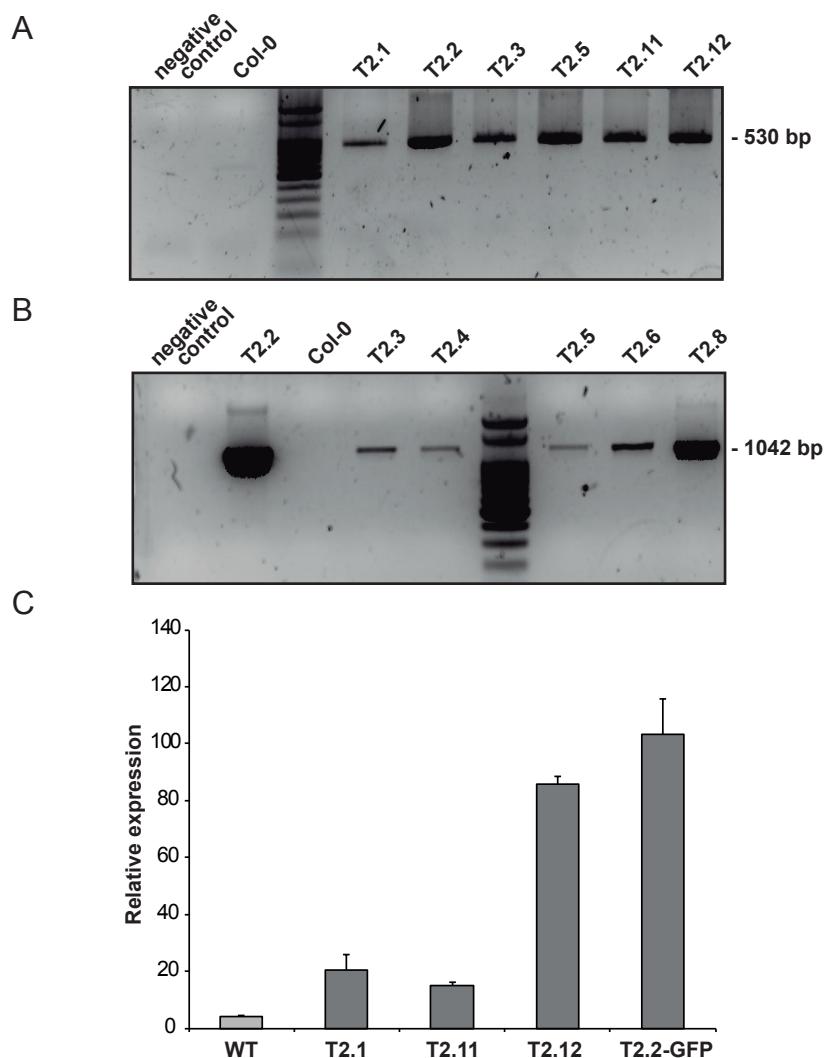
Analysis of antioxidant enzyme activities in wild type (WT, Col-0) and *pdc5d* mutant plants under control conditions and after UV-B treatment. Glutathione reductase (GR) (A), catalase (CAT) (B), ascorbate peroxidase (APX) (C) and peroxidase (POD) (D) activities were determined under control conditions (C) and after a 4h-UV-B treatment at an intensity of 0.2 W m^{-2} . Results represent the average \pm S.E.M. of three independent biological replicates. Statistical significance was analyzed using ANOVA, for each sample analyzed, different letters indicate significant difference with $P < 0.01$.

Supplemental Figure S7



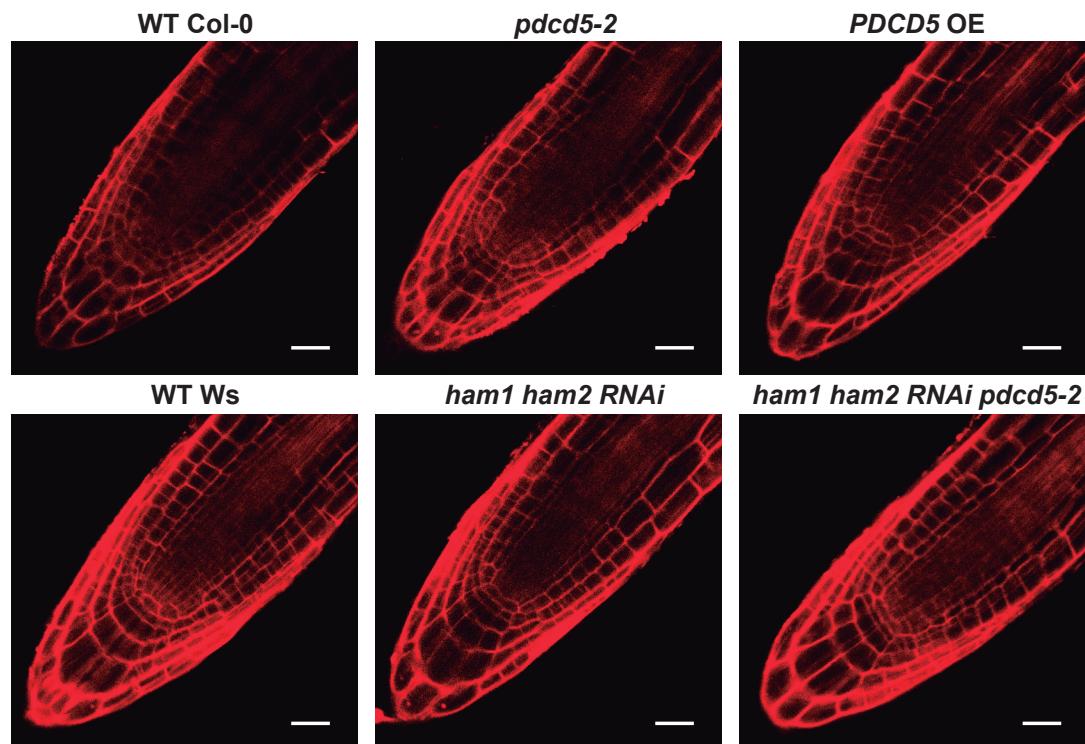
Recombinant expression of GST-AtHAM2 and GST proteins. (A) SDS-PAGE analysis (10%) of the recombinant purified GST-AtHAM2 fusion protein (lane 1, 3 µg). (B) SDS-PAGE analysis (15%) of the recombinant purified GST protein (lane 1, 6 µg). (C) Immunoblot analysis of the recombinant purified GST-AtHAM2 fusion protein (lane 1) using anti-GST antibodies. One µg of the purified protein was loaded. The numbers on the sides of gels indicate the molecular mass in kDa.

Supplemental Figure S8



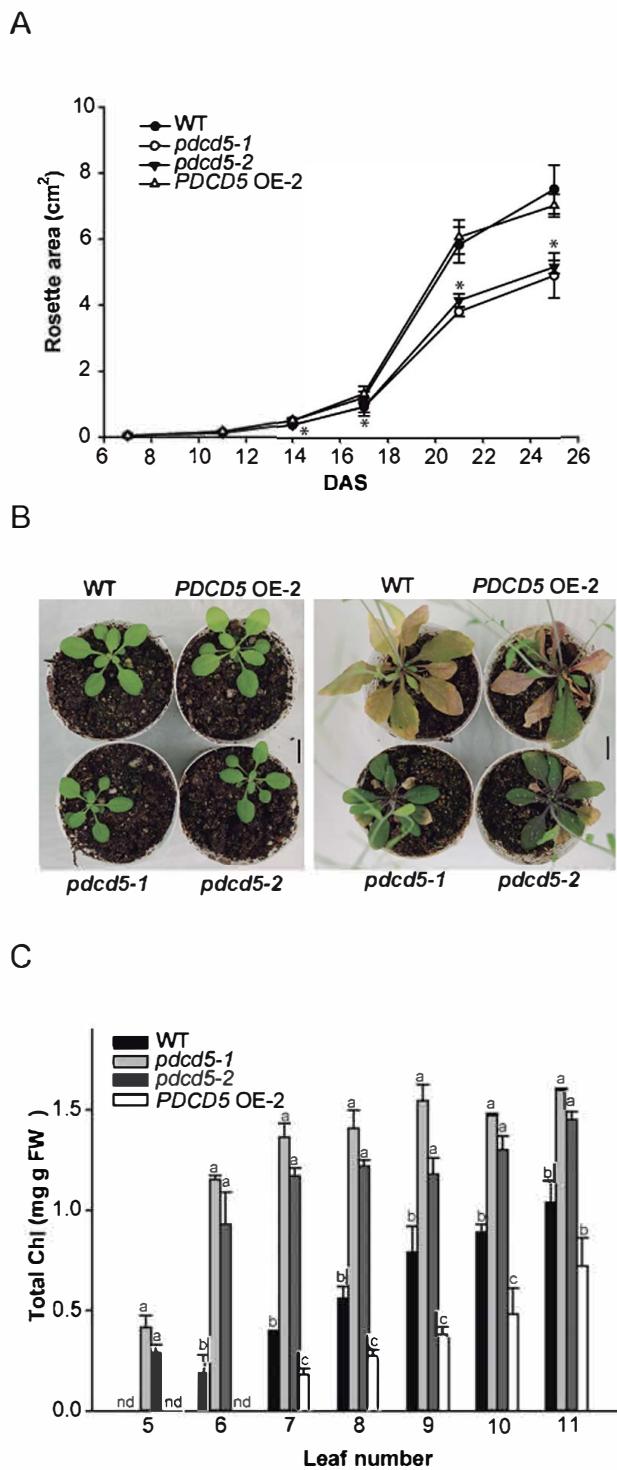
Analysis of Arabidopsis transgenic plants expressing *AtPDCD5-GFP* and *AtPDCD5*. The presence of the *Pro_{35S}:AtPDCD5* (A) and *Pro_{35S}:AtPDCD5-GFP* (B) transgenes was analyzed by PCR on genomic DNA from non-transformed plants (WT, Col-0) and six independent lines (indicated by numbers) for each *Pro_{35S}:AtPDCD5* and *Pro_{35S}:AtPDCD5-GFP* transgenic plants. The negative control was done without genomic DNA. Positive PCR reactions amplified 530 and 1042 bp products for *Pro_{35S}:AtPDCD5* (A) and *Pro_{35S}:AtPDCD5-GFP* (B) transgenic plants, respectively. (C) Transcript levels of *AtPDCD5* in transgenic plants analyzed by qRT-PCR, the expression level of *AtPDCD5* in wild type plants (WT, Col-0) is shown. Each reaction was normalized using the C_t values corresponding to the *UBQ10* mRNA.

Supplemental Figure S9



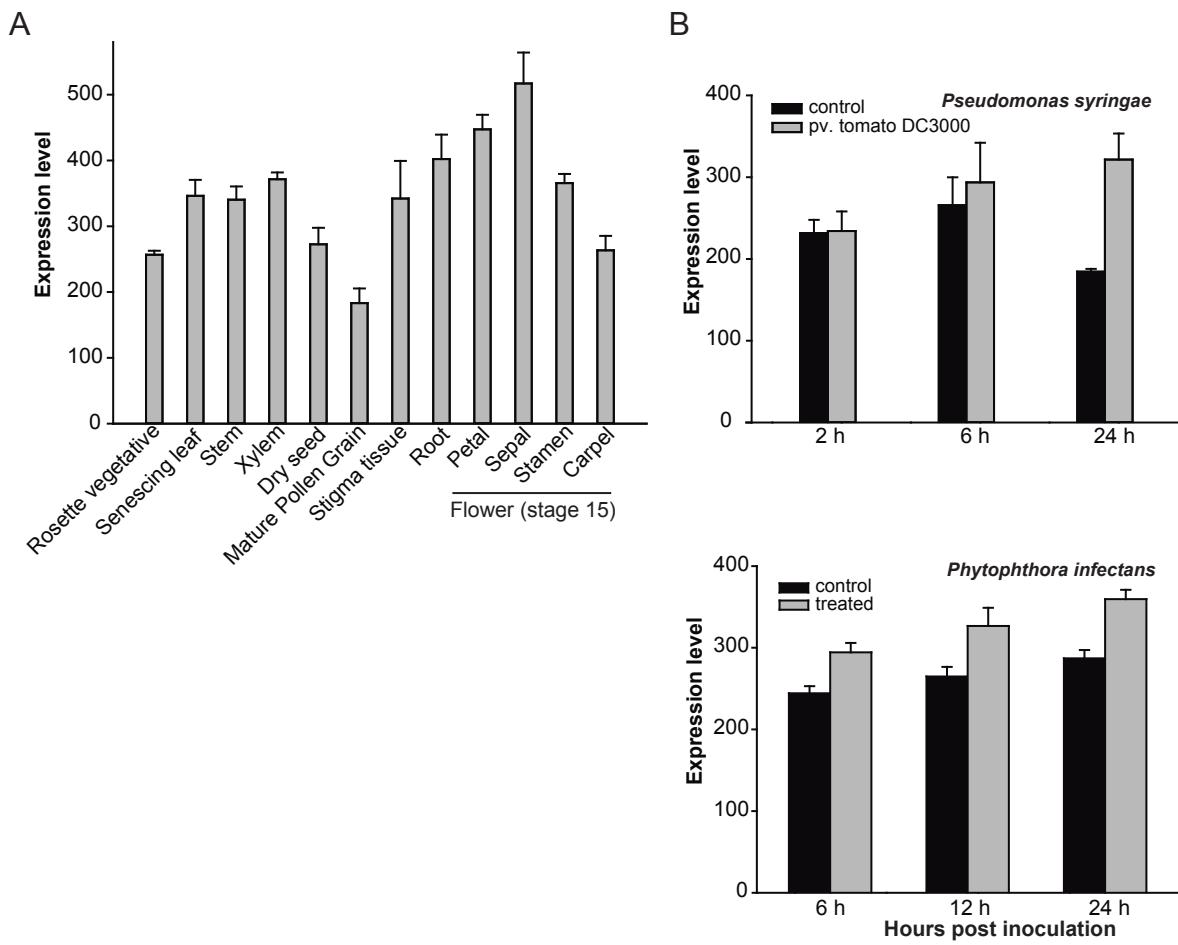
Images of root tips from WT (Col-0, Ws), *pdc5-2*, *ham1ham2 RNAi*, and *ham1ham2 RNAi/pdc5* mutants and *PDCD5* overexpressing plants (*PDCD5* OE) under control conditions (C) without UV-B. Bar corresponds to 50 μ m.

Supplemental Figure S10



Phenotypic analysis of *pdcd5* mutants and transgenic *PDCD5* OE plants. (A) Rosette area of *pdcd5* mutants and transgenic *PDCD5* OE plants measured every 3-4 days from day 7-25 after stratification (DAS). Asterisks indicate statically significant differences ($P < 0.01$, one-way Anova test). (B) Col-0 (WT), *pdcd5-1*, *pdcd5-2* mutants and *PDCD5* OE plants at 21 (left) and 40 (right) DAS. Scale bar: 1 cm. (C) Total chlorophyll content in leaves of Col-0 (WT), *pdcd5-1*, *pdcd5-2* mutants and *PDCD5* OE plants at 40 DAS. For each leaf, statically significant differences between plants are marked with different letters ($P < 0.05$, one-way ANOVA test).

Supplemental Figure S11



Relative expression level of *AtPDCD5* in different tissues (A) and in leaves from plants exposed infected with *Pseudomonas syringae* and *Phytophthora infestans*, B). Microarrays data were retrieved from the Arabidopsis electronic Fluorescent Pictograph (eFP) Browser (Winter et al., 2007).

Supplemental Table S1. Primers used for cloning, RT-qPCR and screening.

Name	Sequence
F-BamHI-PDCD5-OE	5'GAGTACGGATCCATGGCTGATCCTGAACTA3'
R-SalI-PDCD5-OE	5'CGCGCGTCGACTTAAGTGAACTCACAA3'
F-Kpn-PDCD5-GFP	5'GAGTACGGTACCATGGCTGATCCTGAACTA3'
R-BamHI-PDCD5-GFP	5'TATGGATCCATCGTCGTCCACCCCCACGGCG3'
F-AtPDCD5-1	5'GGCTCGACAAGGCATGGG3'
R-AtPDCD5-1	5'ACGGCGCCGTTGGTACGT3'
F-AtPDCD5-2	5'GGCTCGACAAGGCATGCA3'
R-AtPDCD5-2	5'CGGCGCCGTTGGTACTTAC3'
F-AtPDCD5-sc	5'TTCCCTGAGACGAATCCA3'
R-AtPDCD5-sc	5' CGCAGGAACGAAATCAAC3'
35S _{prom}	5'GAGGAGCATCGTGGAAAAAGA3'
R-GFP	5'CTGCAGTCAAGCTTGTATAAGTTCATC3'
F-HindIII-AtPDCD5 _{prom}	5'GCGAAGCTTCCCCTGAAGTCTTAC3'
R-BamHI-AtPDCD5 _{prom}	5'CGGGGATCCAAAAAAACTCACCATC3'
F-AtHAM2-EcoRI	5'CACCAGAATTATGGATCGTCAGCGAACAGAAACCAA3'
R-AtHAM2-Xhol	5'TGTCACTCGAGTTAACTCTGGCCTTGTAAAGGTGTCAA3'
F-AtUBQ10	5'AAGCAGCTTGAGGATGGAC3'
R-AtUBQ10	5'AGATAACAGGAACGGAAACATAGT3'
F-AtCDPK3	5'-CGCTGAGAACCTTCTGAAG-3'
R-AtCDPK3	5'-CCATCTCCATCCATATCAGC-3'
LB-SALK	5'-GTCCGCAATGTGTTATTAAGTTGTC-3'
F-AtUVR2	5'-GACCCGAGTGGATATGTTGG-3'
R-AtUVR2	5'-GAGCTGTTCTTCAGCTTCC-3'
F-AtUVR7	5'-TACATTGGGTCTCTTGCTC-3'
R-AtUVR7	5'-TCCTCGTCTTCTTCAACAGG-3'
F-AtUVH6	5'-CAAAGGCTGATTATGGGATG-3'
R-AtUVH6	5'-CACCAAGTCTCAGCCATCTTC-3'