Cell Reports, Volume *28*

Supplemental Information

Orchestration of Processing Body Dynamics and mRNA Decay in *Arabidopsis* Immunity

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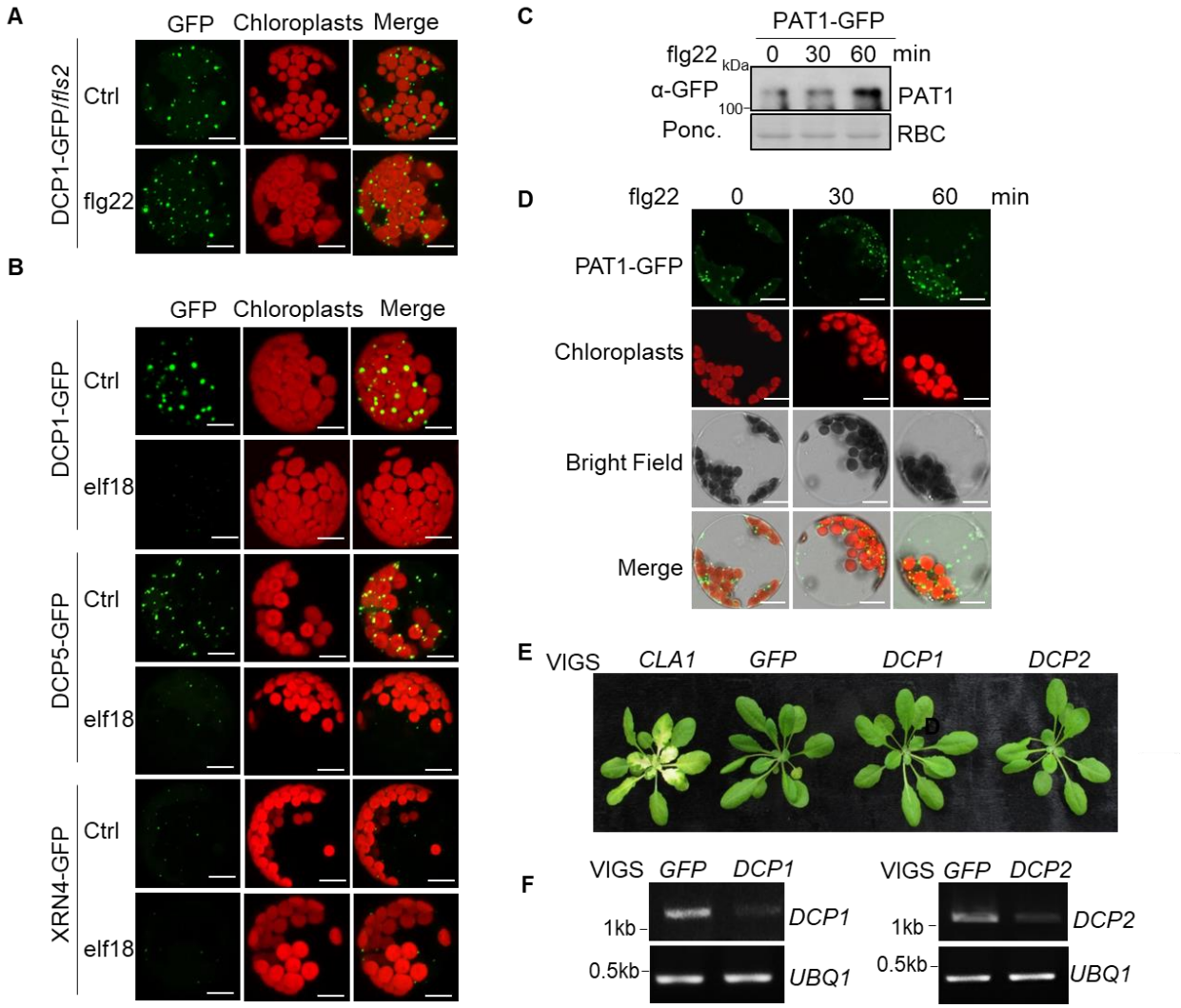

Figure S1. P-body dynamics in plant immunity. Related to Fig 1.

(A) flg22-induced P-body disassembly is blocked in the *fls2* mutant. Protoplasts isolated from *fls2* expressing *DCP1-GFP* were treated with or without 100 nM flg22 for 30 min. Autofluorescence of chloroplasts is shown in the second column. Bar=10 µm. **(B)** Treatment with elf18 triggers P-body disassembly. Protoplasts expressing *DCP1-GFP*, *DCP5-GFP*, or *XRN4-GFP* were treated with or without 100 nM elf18 for 30 min. Bar=10 µm. (**C**) flg22 treatment induces PAT1-GFP protein accumulation. Protoplasts expressing *PAT1-GFP* were treated with or without 100 nM flg22 for 30 min and 60 min. Protein expression was analyzed with an α-GFP immunoblot. (**D**) Confocal microscopy with protoplast expressing *PAT1-GFP* treated with or without 100 nM flg22 for 30 min and 60 min. Bar=10 µm. **(E)** Silencing of *DCP1* or *DCP2* by VIGS does not affect plant growth. Two-week-old soil-grown Col-0 plants were infiltrated with *Agrobacterium* carrying different VIGS vectors and plants were photographed after another 2 weeks. Plants silenced with *CLA1* were used as a visual marker for VIGS efficiency, and plants inoculated with *VIGS-GFP* vector were used as a control. **(F)** VIGS efficiency of *DCP1* and *DCP2*-silenced plants by RT-PCR analysis. Samples were collected two weeks after inoculation with *Agrobacterium*. Full-length *DCP1* and *DCP2* were amplified and *UBQ1* was used as an internal control. The above experiments were repeated three times with similar results.

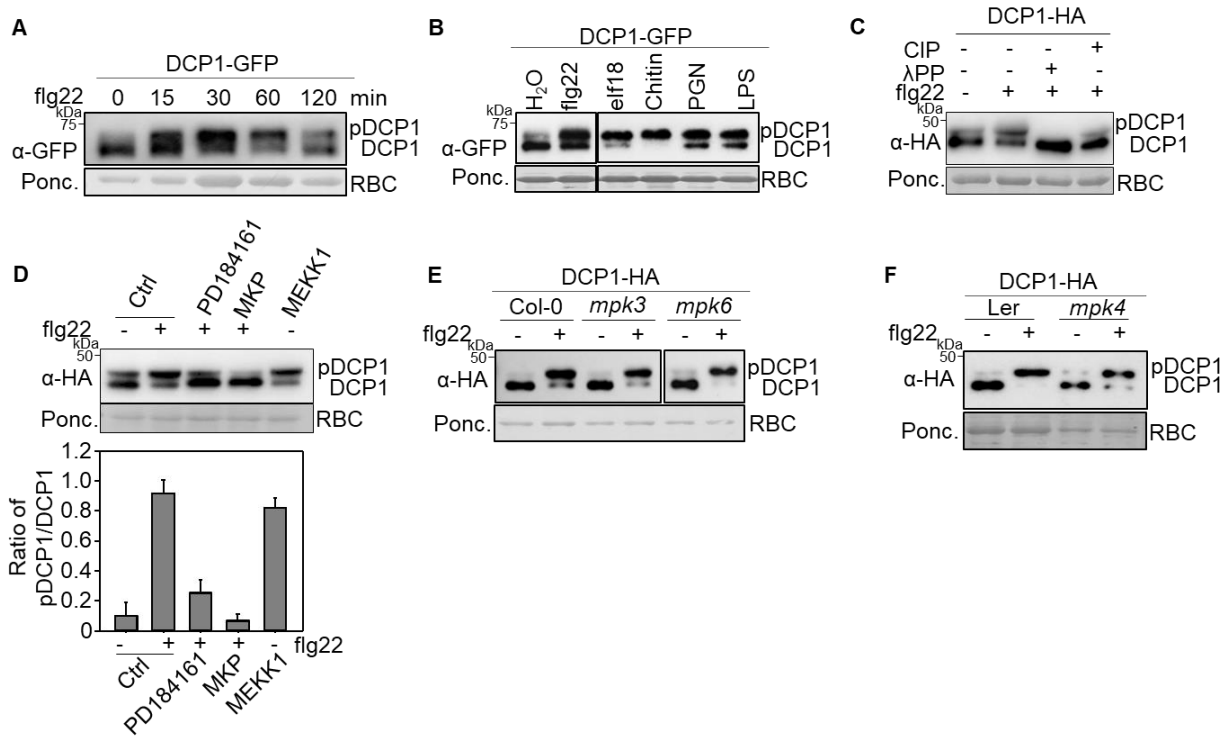

Figure S2. MAMPs induce MAPK-mediated DCP1 phosphorylation. Related to Fig 3.

(A) flg22 treatment induces DCP1 mobility shift in *35S::DCP1-GFP* transgenic plants. Ten-day-old seedlings were treated with 100 nM flg22 for indicated times. Protein loading is shown by Ponceau S staining for RuBisCo (RBC). **(B)** Different MAMPs induce DCP1 mobility shift in *35S::DCP1-GFP* transgenic plants. Ten-day-old seedlings were treated with 100 nM flg22, 100 nM elf18, 50 μg/mL chitin, 50 μg/mL PGN or 50 μg/mL LPS for 15 min. **(C)** Protein phosphatase (CIP or λPP) treatment removes DCP1 mobility shift. Protoplasts expressing *DCP1-HA* were lysed and treated with CIP at 37°C for 1 hr or λPP at 30°C for 1 hr. **(D)** Phosphorylation of DCP1 is dependent on the MAPK cascade. Protoplasts expressing *DCP1-HA* were treated with 7.5 μM PD184161 for 1 hr before 100 nM flg22 treatment for 15 min (third lane). For the last two lanes, protoplasts were co-expressed with *DCP1-HA* and *MKP* or *MEKK1*. The intensity of the phosphorylated and un-phosphorylated bands was quantified by ImageJ software, and the ratio of shifted to the total protein is shown as a bar graph. **(E) & (F)** The *mpk3*, *mpk6* or *mpk4* single mutant does not affect flg22-induced DCP1 phosphorylation. The *mpk3* and *mpk6* mutants are in the Col-0 background (E) and *mpk4* is in the L*er* background (F). Protoplasts expressing *DCP1-HA* were treated with 100 nM flg22 for 15 min. The above experiments were repeated three times with similar results.

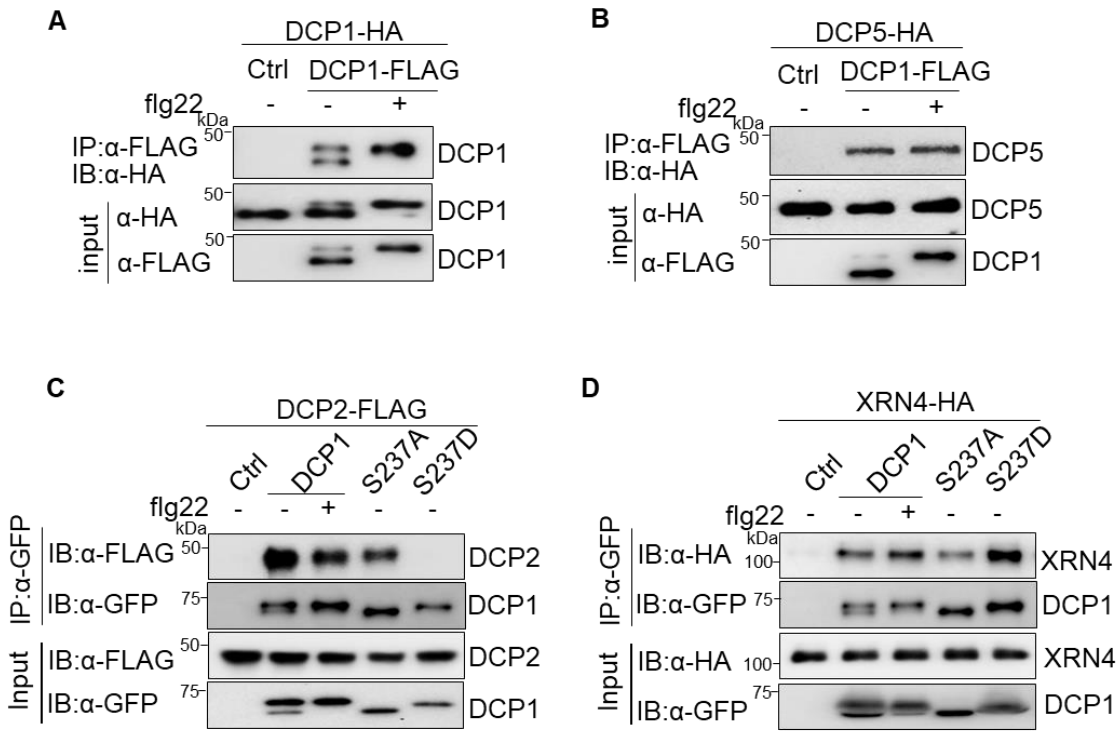

Figure S3. Dynamic interactions of DCP1 with DCP2 and XRN4. Related to Fig 4.

(A) DCP1 homomerizes before and after flg22 treatment. Protoplasts expressing *DCP1-HA* and *DCP1-FLAG* were treated with 100 nM flg22 for 15min, and resulting protein extracts were subjected to IP with α-FLAG (IP: α-FLAG) and IB with α-GFP (top panel). The input control is shown on the bottom two panels. **(B)** DCP1 dimerizes with DCP5 before and after flg22 treatment in *Arabidopsis* protoplasts. **(C)** DCP1^{S237A} shows higher affinity with DCP2 than DCP1^{S237D} in *N. benthamiana*. *Agrobacterium* carrying *35S::DCP2-FLAG* was co-infiltrated with *Agrobacterium* carrying *35S::DCP1-GFP*, *35S::DCP1^{S237A}-GFP, 35S::DCP1^{S237D}-GFP* or an empty vector (Ctrl.) into *N. benthamiana* leaves. For flg22 treatment, 500 nM flg22 was infiltrated at 30 min before harvesting samples. Co-IP was performed with α-GFP (IP: α-GFP), and the proteins were analyzed by IB with α-FLAG and α-GFP (top two panels). The input control is shown on the bottom two panels. **(D)** DCP1^{S237D} shows higher affinity with XRN4 than DCP1^{S237A} in *N. benthamiana*. The experiment was carried out as described in (C). The above experiments were repeated three times with similar results.

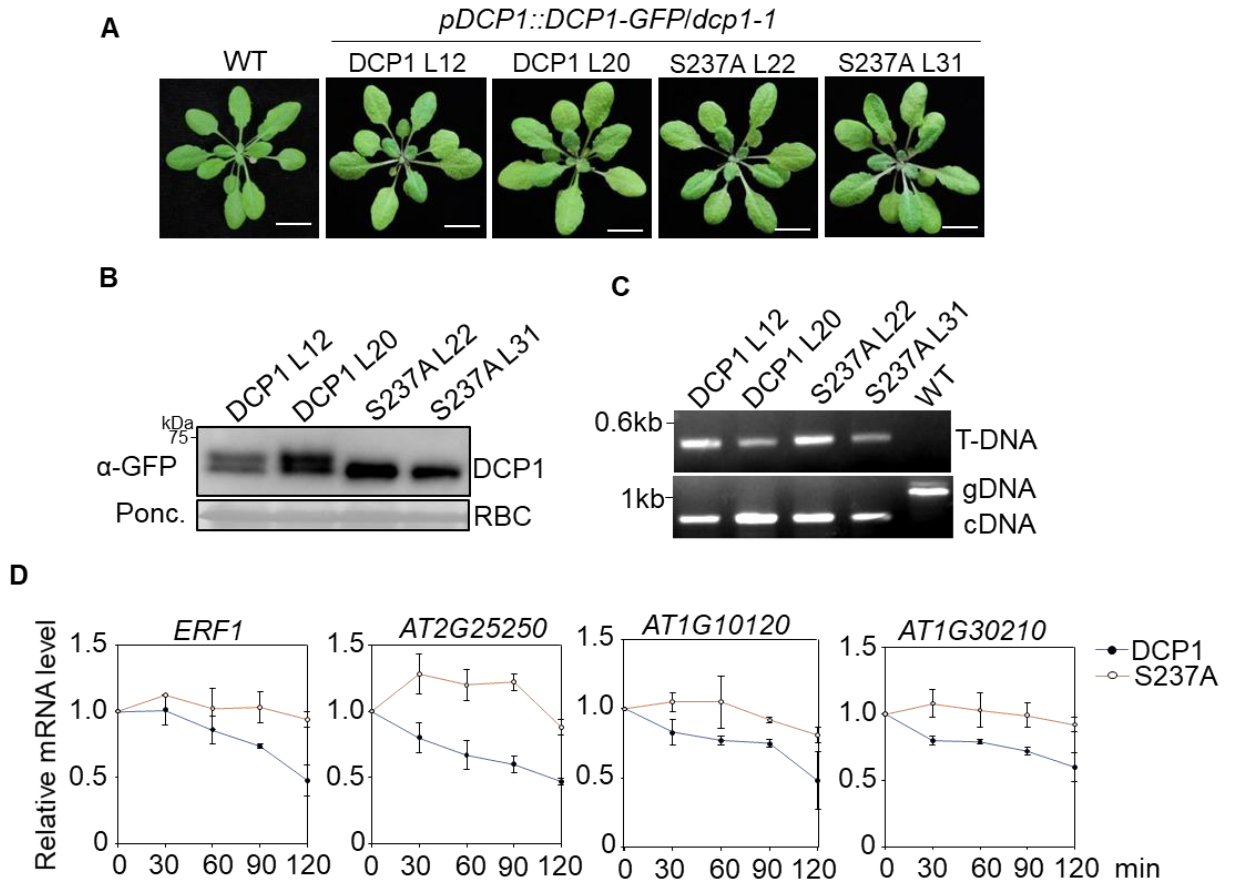

Figure S4. Analysis of DCP1 complementation lines. Related to Fig 5.

(A) Plant growth phenotype of DCP1 complementation lines in *dcp1-1*. Four-week-old soil-grown WT Col-0, *pDCP1::DCP1-GFP/dcp1-1* and *pDCP1::DCP1*^{S237A}*-GFP/dcp1-1* plants were photographed. Bar=1cm. **(B)** Protein expression levels of transgenic plants. Immunoblotting was carried out with an α-GFP antibody and protein loading is shown by RuBisCo (RBC) with ponceau S staining. **(C)** PCR confirmation of transgenic plant genotypes. The primer pair of GABI_LB and *dcp1-1*_RP amplified the T-DNA insertion (top panel) and the primer pair of *dcp1-1*_LP and *dcp1-1*_RP amplified both the genomic DNA and cDNA fragments of DCP1 (bottom panel). **(D)** mRNA decay of XRN4 target genes in *pDCP1::DCP1-GFP/dcp1-1* and *pDCP1::DCP1*^{S237A}*-GFP/dcp1-1* plants. Ten-day-old seedlings were pre-treated with cordycepin for 30 min and samples were harvested at the indicated times for qRT-PCR analysis. Data are shown as means ± SD from two biological repeats.

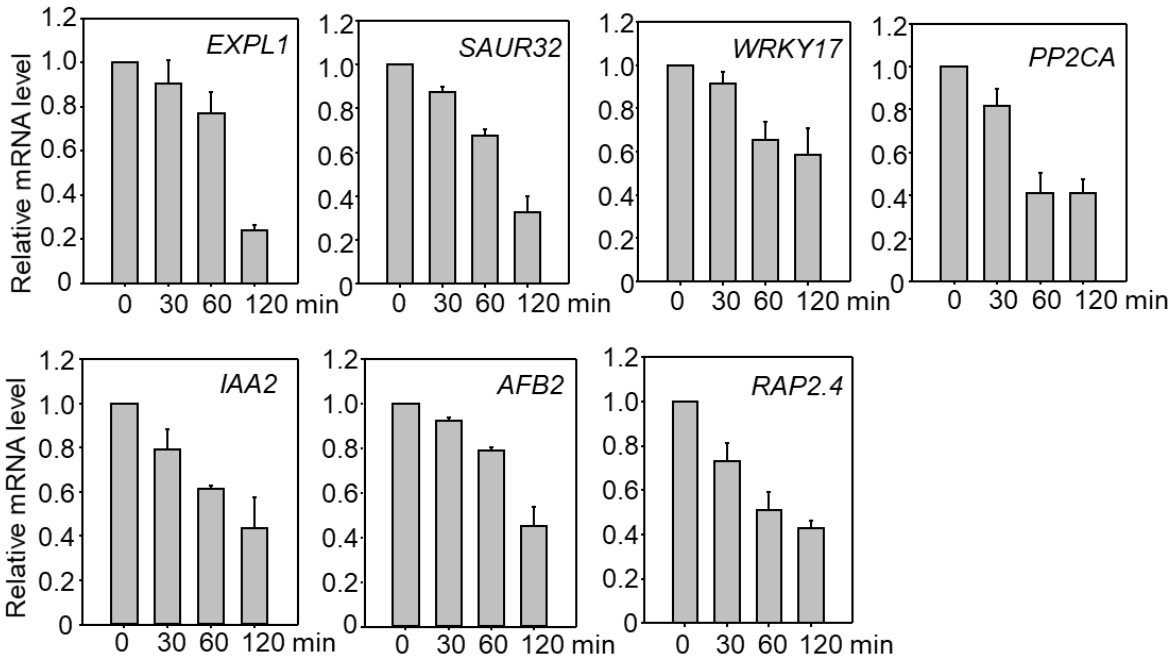

Figure S5. Screen of unstable mRNAs. Related to Fig 6.

Ten-day-old WT Col-0 seedlings were treated with transcription inhibitor cordycepin at 150 μg/ml for 30 min and harvested at the indicated time points. The relative mRNA levels at different time points are shown as the ratio to the mRNA level at 0 min (right after cordycepin treatment), which is set as 1. *ACTIN2* was used as an internal control. Data are shown as means ± SD from two biological replicates.

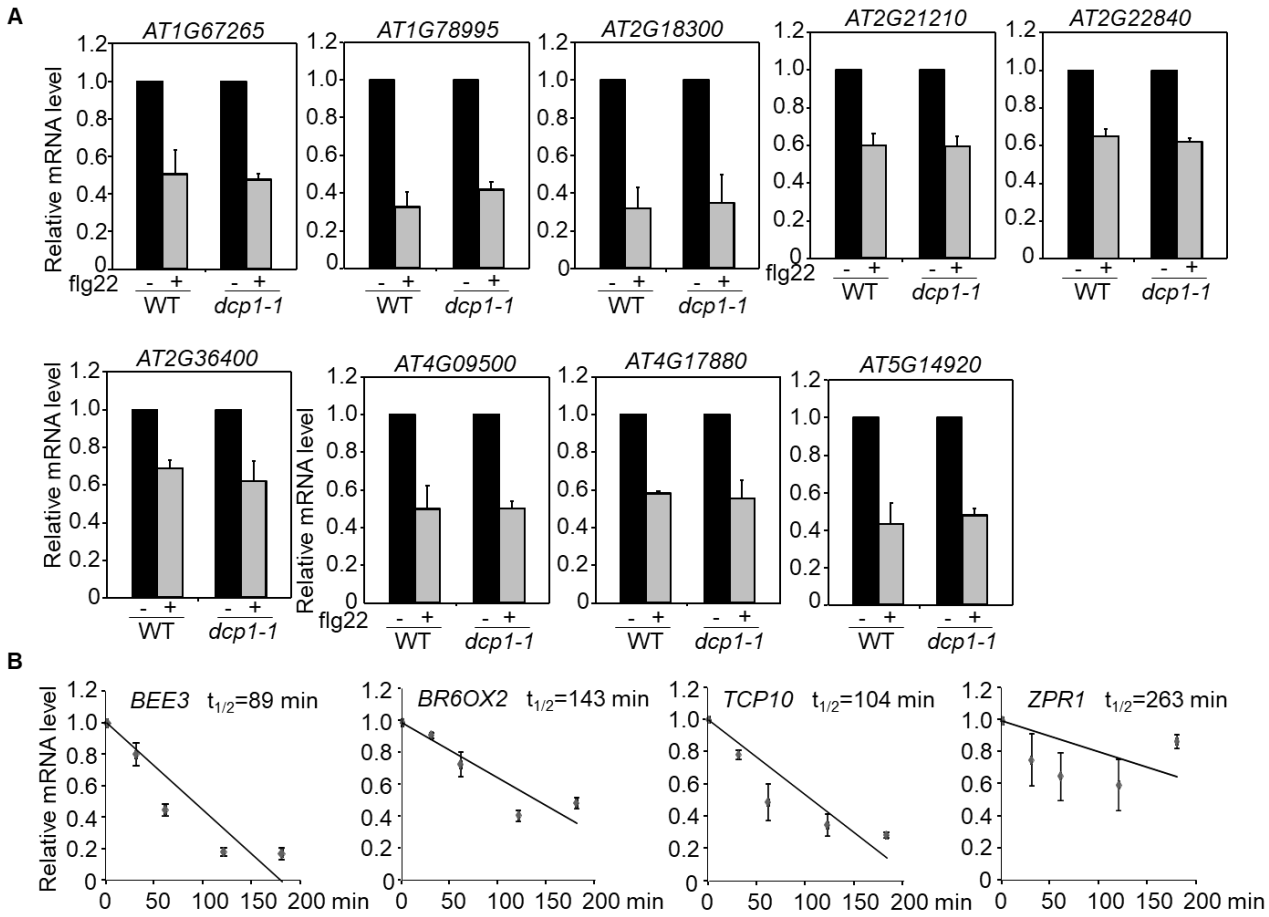

Figure S6. DCP1-independent flg22-downregulated genes. Related to Fig 6.

(**A**) Ten-day-old WT Col-0 and *dcp1-1* seedlings were treated with 100 nM flg22 for 60 min and subjected to qRT-PCR analysis. Gene expression level was normalized with *ACTIN2*. Data are shown as means ± SD from three biological replicates. (**B**) mRNA half-lives of DCP1-dependent flg22-downregulated genes. Ten-day-old WT Col-0 seedlings were pre-treated with 150 µg/ml cordycepin for 30 min and harvested at 0, 30, 60, 120 and 180 min for qRT-PCR analysis. *ACTIN2* was used as an internal control. Data are shown as means ± SD from two independent biological repeats. The percentages of mRNAs at each time point calculated relative to the zero time point value were plotted against time and a regression curve was obtained.

Table S1. Primers used in this study. Related to STAR Methods.

Cloning, point mutation and VIGS primers

Gene	Forward primer	Reverse primer
DCP1	CGGGATCCATGTCTCAAAACGGGAAGA TAATCCC	TCCCCCGGGTTGTTGAAGTGCATTTTG TAAAG
DCP2	CGGGATCCATGTCGGGCCTCCATCG ATCATC	GAAGGCCTAGCTGAATTACCAGATTCC
DCP5	GGACTAGTATGGCGGCTGATAATAC G GG	TCCCCCGGGGGTAGTACGATTTGATAC GCC
XRN4	CGGGATCCATGGGAGTACCGGCGTT CTAC	GAAGGCCTCAAGTTTGCACCTCGATGA CTTG
pDCP1	GCTCTAGAGCTTCACCTACTTAAAATTG G	CGGGATCCCTTTTATAAATCAAAGATCAG
DCP1-VIGS	CGGAATTCCCAATGGAGTCGTAAGG	GGGGTACCGACCCACTCGCAGTGTTC
DCP2-VIGS	CGGAATTCGTCGGGCCTCCATCGATC	GGGGTACCGCACAAGCATGGTCTTC
DCP1-S237A	CCTCCACAGATACAAGCACCACCGCCTC TACAA	TTGTAGAGGCGGTGGTGCTTGTATCTGTGG AGG
DCP1-S237D	CCTCCACAGATACAAGACGCACCGCCT VCTACAA	TTGTAGAGGCGGTGGGTCTTGTATCTGTGG AGG
PAT1	CGGGATCCATGGACGCTTTTGGAATCGG	TCCCCCGGGACTTAATACTGGCTCGGTTTT C

The restriction enzymes are underlined.

Genotyping and RT-PCR primers

Primer	sequence
*dcp1-1*_LP	TTAACCAGAAGCCAAAGGCC
*dcp1-1*_RP	TGTTGAAGTG CATTTTGTAAAG
GABI_LB	TAATAACGCTGCGGACATCTACA
DCP1-F	CGGGATCCATGTCTCAAAACGGGAAGATAATCCC
DCP1-R	TCCCCCGGGTTGTTGAAGTGCATTTTG TAAAG
DCP2-F	CGGGATCCATGTCGGGCCTCCATCG ATCATC
DCP2-R	GAAGGCCTAGCTGAATTACCAGATTCC
UBQ1_FP	ACCGGCAAGACCATCACTCT
UBQ1_RP	AGGCCTCAACTGGTTGCTGT

qRT-PCR primers

Gene	Forward primer	Reverse primer
FRK1	ATCTTCGCTTGGAGCTTCTC	TGCAGCGCAAGGACTAGAG
WRKY30	GCAGCTTGAGAGCAAGAATG	AGCCAAATTTCCAAGAGGAT
At1g07160/PP2C	CGTGTTGGGGATTGATTCG	AGAGCTCGGGCGGTTATG
ACTIN2	GCACCCTGTTCTTCTTACGGA	GTGAGACACACCATCACCAGA
At3g45970/EXPL1	TTATTTCTCCTCTGCCTCTGC	GCCAGCACCGTCTTTGTAG
At2g46690/SAUR32	CCTTGTCACGTGGAGGAGTT	TCAAGCTCTGAAGCATCCAA
At2g24570/WRKY17	TGAACATGACCACTCTGAAGGC	ACTTACCGCCGGTACTCTCAC
At3g11410/PP2CA	CGTAACGGTGTAGCCATTCC	GGCAAGAACTCCAAGAACCC
At3g23030/IAA2	AAGAAGAATCTACACCTCCTAC	GCTCGGGGTAGTTTTTGTATG
At3g26810/AFB2	GGCGGCGCATCCATTCTTGTCC	AGATGCTCTCCATAGCCTTTGC

At1g78080/RAP2.4	TTGGTGATACGGAGGAGGAG	GAAGAATCGAATCCCAATCG
At1g53830/PME2	ATTGAAGGGTCAGGTACACG	CGGTTGTTGTTGTTGGTGAA
At1g73830/BEE3	CCGGATGTTATAAGACAATGG	CACAGCATCAGTCTCCGAGT
At2g01850/XTH27	TGCTTTATCGGAGTGTGTGG	ATCGACTCGGTTCCATCAAC
At2g31070/TCP10	ATCAACCGTCGATGATGACA	GATCCCAAGAACGAAACGAA
At2g45450/ZPR1	TTTTCAGACACACCCACGAG	TTTTCTCTTCCCGCCACAT
At3g30180/BR60X2	ACTCGAGCTGTGATCTTTGAG	TTCTCCAACCTTTTGGGATT
At1g67265/RTGL21	GGTACCAAGAAGAAGACGCC	GCCAACAAATGAGCATGACC
At1g78995	CGATCTGCGGCTGTTCGTATA	CCTGACAGCGTTTGCGGTA
At2g18300/HBI1	AACCCGGAACTAGAGCTTGC	ATCCTTGCTGGTCTAACGGA
At2g21210/SAUR	AAGGACACCTTGCGGTTTAC	ATAGTGAGGCCACCCATTGG
At2g22840/GRF1	AAGAAATGGCGGTGCTCG	GGCAGCATTAGTATTGTGGC
At2g36400/GRF3	ACCGTTCAAGAAAGCCTGTG	GTTGCTGTTGTAGTGGTGGC
At4g09500	TGGTCCAATGTTCCCTGAG	AAACACTACTGACTTCGGCG
At4g17880/MYC4	AATCGAAGGAGCAAACGAGA	CCATCTCCCCAACCTAACAA
At5g14920	CACGCTGCCAACTACTCCTA	CGGTAGTTTGATCGGAGGAG
AT2G25250	GGCTTTGTTTTACGCTGGTC	TAGCGAAGAAAGCCGGTAAA
AT1G10120	AAGCCGAATCACAATGGAAC	TTCCCCTTTGAGGGTCTTCT
AT1G30210	CGTGGGAAAGCAAGAGAGAG	TTGGTCAAAACCACCAGTGA
AT3G23240/ERF1	AGGATGGTTGTTCTCCGGTT	AGACCCCAAAAGCTCCTCAA