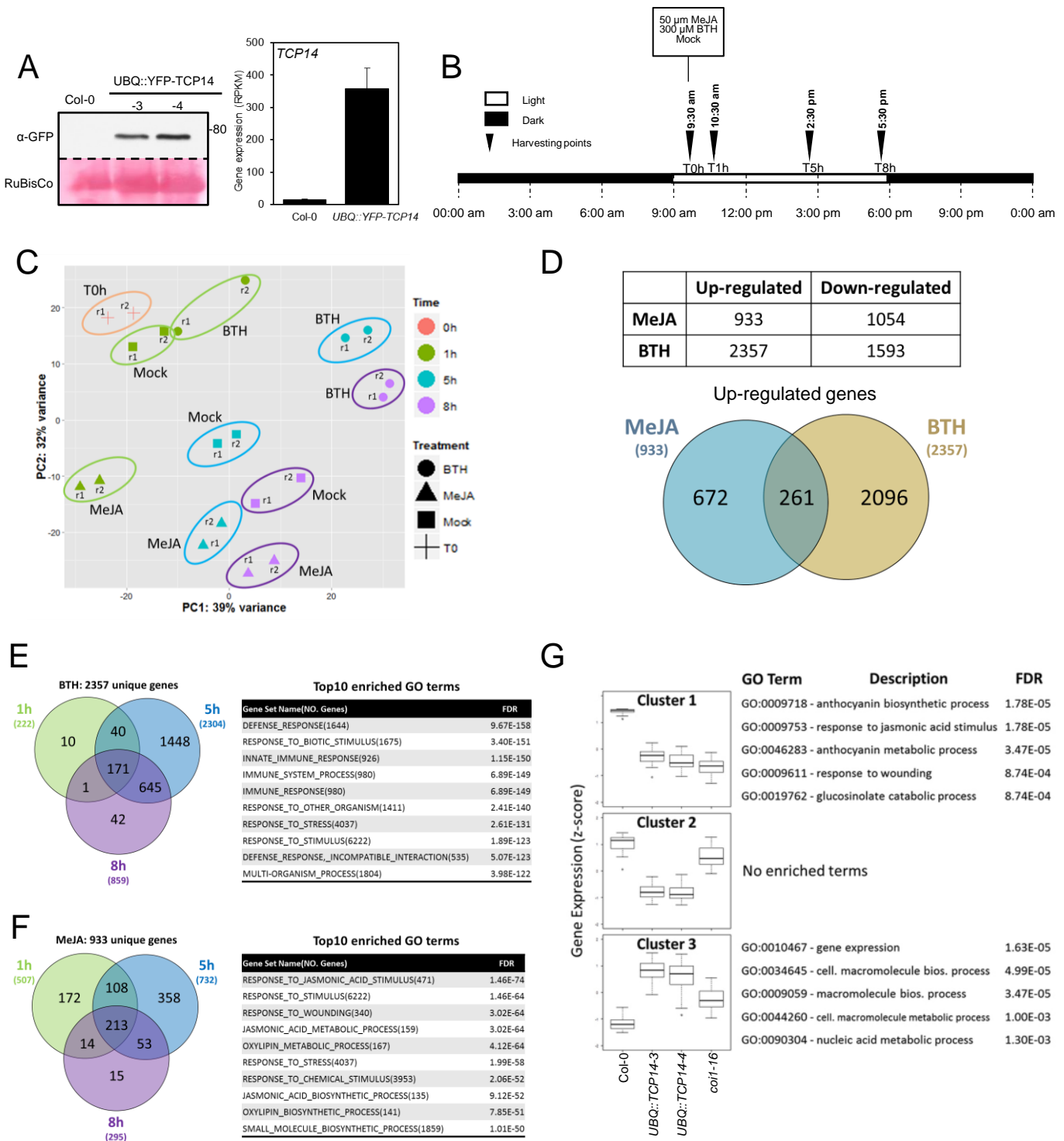
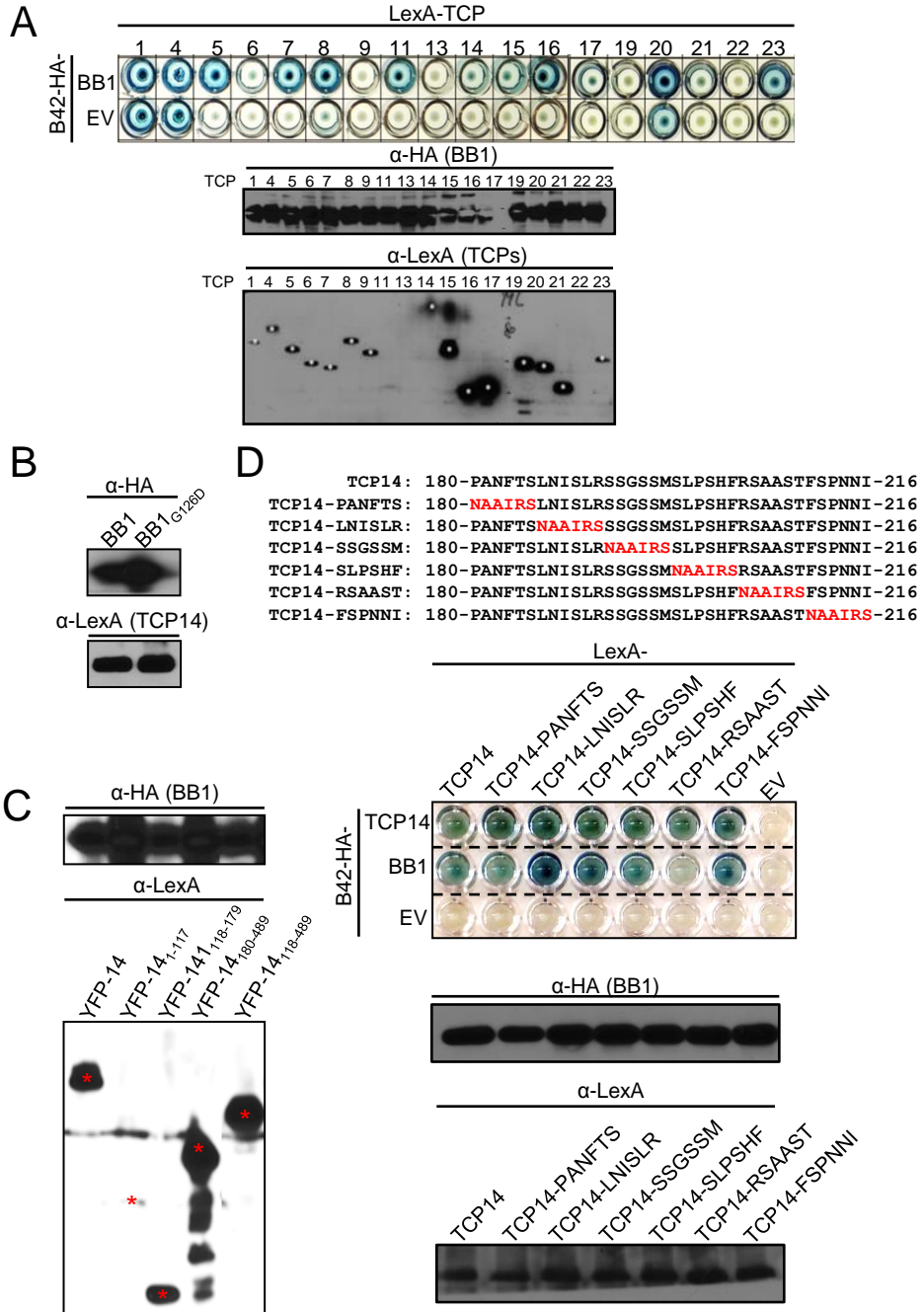


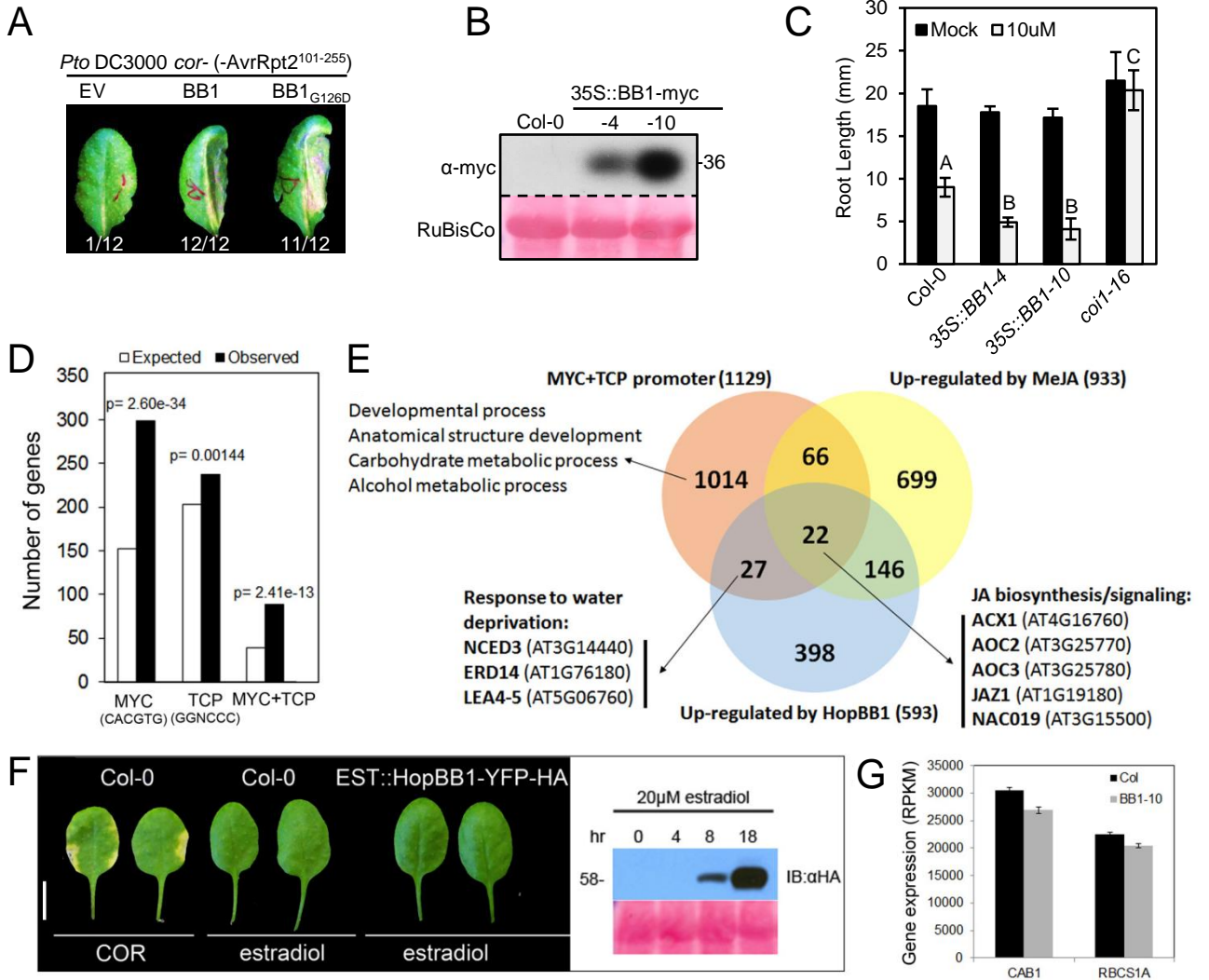
# sFigure 1



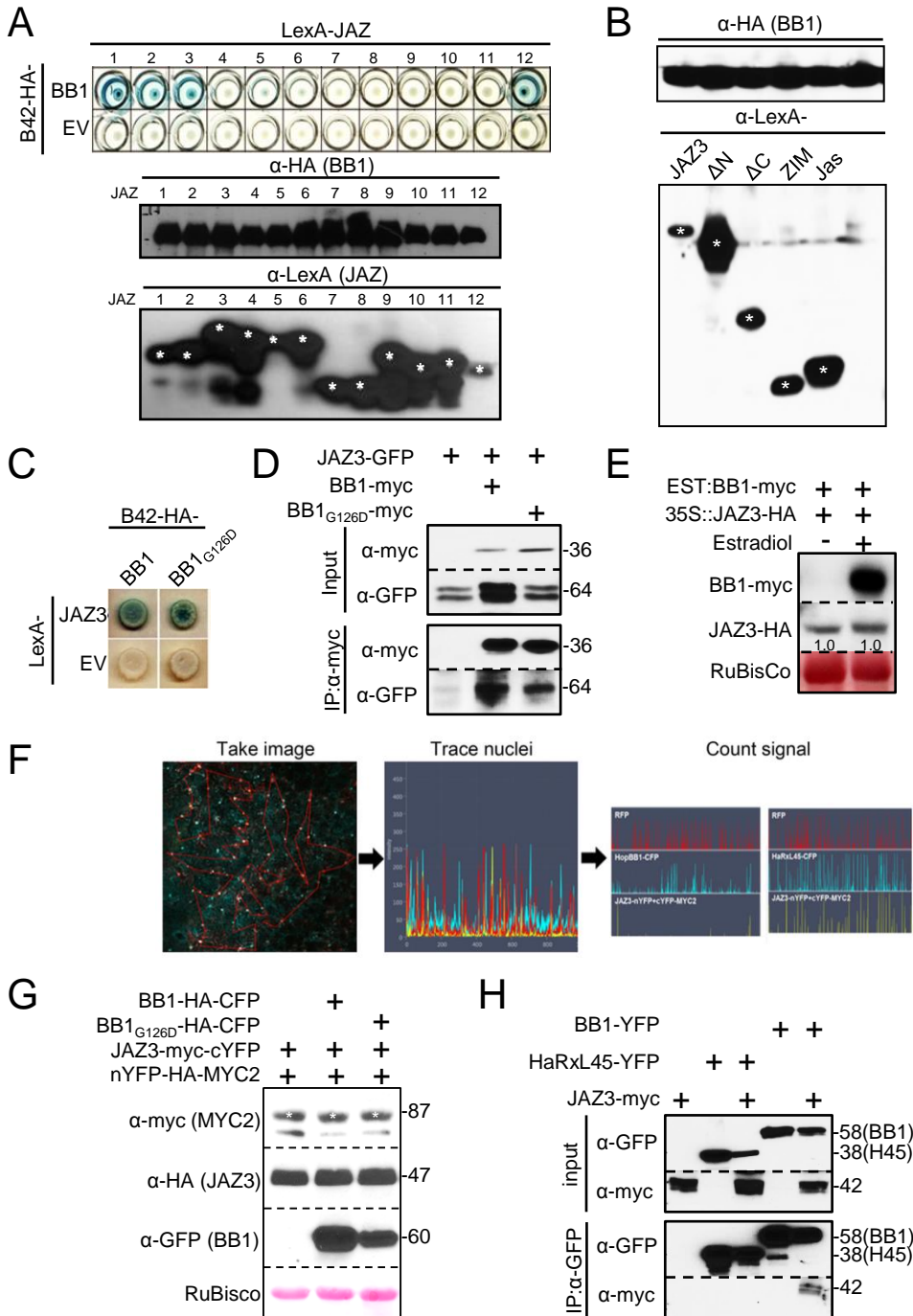
# sFigure 2



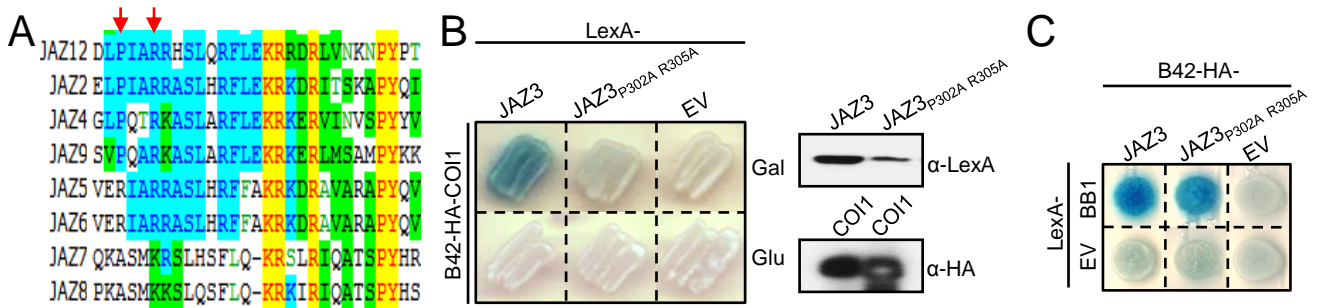
# sFigure 3



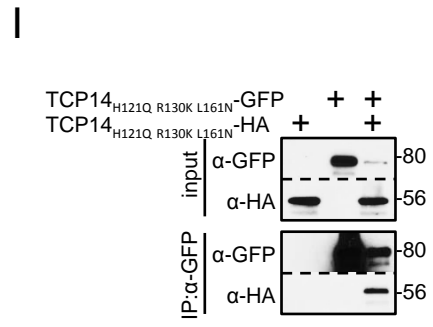
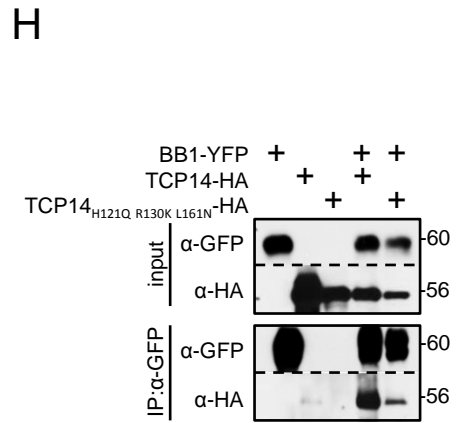
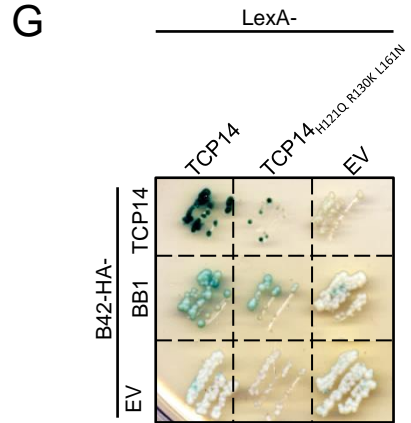
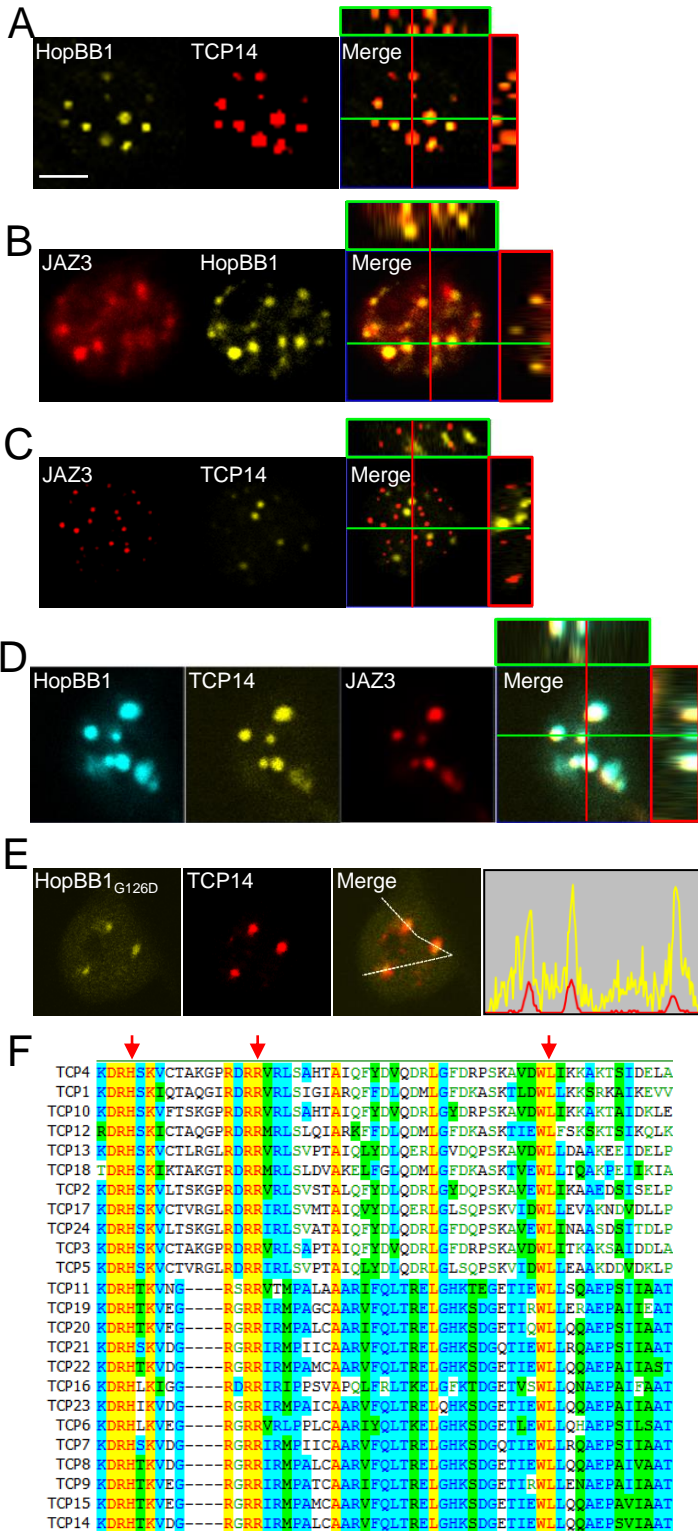
# sFigure 4



# sFigure 5



# sFigure 6



## Figure S1 (Related to Figure 1)

(A) Accumulation of YFP-TCP14 protein (left) and transcripts (right) in transgenic plants.

(B)–(F) Defining JA and SA responsive genes in Col-0. (B) Overview of the experimental conditions used to define genes responsive to MeJA and BTH by RNA-seq. (C) Principal Component Analysis (PCA) showing the overall relationship of the RNA-seq libraries used to define MeJA and BTH/SA markers. Colors represent different time-points and symbols represent treatments. Biological replicates are labeled r1 and r2. (D) Number of genes up- and down-regulated by each treatment defined using the edgeR package ( $FDR \leq 0.01$ ; 1.5 fold-change difference). While 261 genes were up-regulated by both hormones, 672 and 2096 genes were up-regulated specifically by MeJA and BTH, respectively, which define our set of marker genes. Table S1 shows the complete RNA-seq results for all Arabidopsis genes. (E) Overview of genes up-regulated by BTH at each time-point. The table shows Gene Ontology terms (biological processes) enriched in this set of genes. (F) Overview of the genes up-regulated by MeJA at each time-point. The table shows Gene Ontology terms (biological processes) enriched in this set of genes.

(G) Gene Ontology terms (biological processes) enriched in the set of genes identified as differentially expressed in the *UBQ::YFP-TCP14-3* line. The three clusters correspond to the hierarchical clustering analysis presented in Figure 1D.

### Figure S2 (Related to Figure 2)

- (A) Protein interaction between HopBB1 and 18 of 24 Arabidopsis TCP family members in yeast.
- (B) Protein accumulation in Figure 2B.
- (C) Protein accumulation in Figure 2D.
- (D) NAAIRS-scanning mutagenesis in the TCP14<sub>180-216</sub> region. TCP14<sub>RSAAST/NAAIRS</sub> cannot interact with HopBB1.

### Figure S3 (Related to Figure 3)

- (A) HopBB1 and HopBB1<sub>G126D</sub> were delivered into plant cells when expressed in *Pto* DC3000 *cor-*. HopBB1 or HopBB1<sub>G126D</sub> were cloned upstream of AvrRpt2<sup>101-255</sup>. The chimeric proteins were expressed under the control of NPTII promoter (Vinatzer et al., 2005).
- (B) Protein accumulation in transgenic plants expressing HopBB1-myc.
- (C) Plants expressing HopBB1 are hypersensitive to JA-mediated inhibition of root elongation. One-week-old seedlings grown on vertical plates were transferred to mock plates or plates with 10 $\mu$ M MeJA. Root length was measured one week after transfer. Error bars indicate  $\pm$ SD. Statistics were performed using one-way ANOVA test with Tukey-Kramer HSD with 95% confidence. Similar results were obtained from two independent experiments.
- (D) Co-occurrence of consensus MYC (CACGTG) and TCP (GGNCCC) binding sites is enriched in the promoters of MeJA-regulated genes from Figure S1F,



Table S1 (88/933,  $p=2.41e-13$ ; hypergeometric test). We searched for these motifs in the 1kb upstream region relative to the start codon of 27206 nuclear protein-coding genes (TAIR10). The observed number of promoters containing each or both motifs was contrasted to the expected number in each category, given the motif's frequency in the entire genome and tested for over-representation using the hypergeometric test.

- (E) The overlap between genes up-regulated by MeJA and HopBB1, and with the co-occurrence of TCP14 and MYC binding sites in their promoters. We verified that 22 (25%) of the 88 JA-responsive genes containing both MYC and TCP binding sites in their promoters are also up-regulated by HopBB1, which is more than expected by random sampling (2.18%;  $p=1.49e-16$ ; hypergeometric test). Importantly, this list includes genes required for JA biosynthesis and signaling.
- (F) Induced expression of HopBB1 does not trigger chlorosis (left). Four-week old plants were either treated with 50 $\mu$ M of coronatine or 20 $\mu$ M of estradiol for five days. The fifth leaves from three representative plants were photographed. Bar=5mm. The protein accumulation of conditionally expressed HopBB1 is shown in the right panel.
- (G) The expression of representative photosynthesis genes is not altered in HopBB1-myc expressing plants.

**Figure S4 (Related to Figure 5)**

- (A) Protein interaction between HopBB1 and 12 Arabidopsis JAZ family members in yeast. HopBB1 interacts with a subset of JAZ proteins.
- (B) Protein accumulation for Figure 5C.
- (C) HopBB1<sub>G126D</sub> retains interaction with JAZ3 in yeast.
- (D) HopBB1<sub>G126D</sub> retains interaction with JAZ3 in *N. benthamiana*.
- (E) HopBB1 does not promote JAZ3 degradation in Arabidopsis. Conditional expression of HopBB1-myc in transgenic plants expressing 35S::JAZ3-HA does not alter the accumulation of JAZ3.
- (F) Quantification of the HopBB1-mediated disruption of MYC2-JAZ3 association. Proteins were transiently co-expressed in *N. benthamiana*. HaRXL45-CFP, HopBB1-CFP or HopBB1<sub>G126D</sub>-CFP was induced 6 hrs after Agrobacteria infiltration. Microscopy was conducted 18 hrs after induction. Eight to ten confocal images with 1 mm<sup>2</sup> field of view were taken from four randomly sampled leaf discs on each leaf. Images were taken from YFP, CFP and RFP channels. Nuclei were traced only in the RFP channel. Following that, the nucleus signal peaks in each individual channel were counted, and the degree of overlap was compared. Four independent experiments were pooled for the summary presented in Figure 5F.
- (G) HopBB1 does not alter MYC2 level in *N. benthamiana*. Proteins were transiently co-expressed in *N. benthamiana*.
- (H) JAZ3 does not associate with HaRXL45 *in planta*. Proteins were transiently co-expressed in *N. benthamiana* from a constitutive 35S promoter.

**Figure S5 (Related to Figure 6)**

- (A) Alignment of the conserved Jas motifs from 12 Arabidopsis JAZ proteins. The P302 and R305 are highlighted with a red arrow.
- (B) JAZ3<sub>P302A R305A</sub> cannot interact with COI1 in the presence of coronatine. 50µM of coronatine was added to yeast medium. Protein accumulation is shown in the right panel.
- (C) JAZ3<sub>P302A R305A</sub> interacts with HopBB1 in yeast.

**Figure S6 (related to Figure 7)**

- (A) Orthogonal slices of TCP14-RFP and HopBB1-YFP co-localization in sub-nuclear foci. Bar=5 µM. For all orthogonal slices in (A)-(D), the “Merge” panel is the xy plane, right panel (red) is the yz plane, and top panel (green) is the xz plane. The crosshairs indicate the location of the yz and xz planes.
- (B) Orthogonal slices view of JAZ3-RFP and HopBB1-YFP co-localization in sub-nuclear foci.
- (C) Orthogonal slices view of the distinct sub-nuclear localization of TCP14-YFP and JAZ3-RFP in a nucleus.
- (D) Orthogonal slices view of the co-localization of TCP14-YFP, HopBB1-CFP and JAZ3-RFP in sub-nuclear foci.
- (E) HopBB1<sub>G126D</sub> co-localizes with TCP14 when transiently co-expressed in *N.benthamiana*.
- (F) Alignment of the TCP domain from 24 Arabidopsis TCP family members. The mutated H121, R130 and L161 were highlighted with a red arrow. These

residues are conserved in TCP14. Mutation in each individual residue significantly reduced the ability of TCP4 protein to bind DNA (Kosugi and Ohashi, 2002).

(G) TCP14<sub>H121Q R130K L161N</sub> retains the ability to interact with HopBB1 and TCP14 in yeast.

(H) TCP14<sub>H121Q R130K L161N</sub> retains the ability to interact with HopBB1 in *N. benthamiana*.

(I) TCP14<sub>H121Q R130K L161N</sub> homo-dimerizes.

A-E, H-I: Proteins were transiently expressed from the 35S promoter in *N. benthamiana*.

**Table S1 (related to Figure 1):** Transcriptional response to MeJA or BTH in Col-0.

**Table S2 (related to Figure 1):** Transcriptional changes induced by *TCP14* mutation or overexpression.

**Table S3 (related to Figure 3):** Transcriptional changes induced by bacteria delivered HopBB1 or heterologous HopBB1 expression in Arabidopsis.

**Table S4 (related to Figure 3):** Comparison between genes expression altered by HopBB1 and TCP14 expression.

**Table S5 (related to Figure 3):** Distribution of JA-activating virulence factors in 287 *Psy* genomes.

**Table S6 (related to experimental procedures):** Primers, seed stocks, constructs, sequences and core genes for phylogenetic analysis.

## 1 **Extended Experimental Procedures**

### 2 Plants

3 Arabidopsis Col-0, *tcp14-6* (SAIL\_1145\_H03, backcrossed to Col-0 four times)  
4 (Mukhtar et al., 2011), *tcp14-7* (cs108688, backcrossed to Col-0 twice) (Wessling  
5 et al., 2014), *coi1-16* (Ellis and Turner, 2002; He et al., 2012), and all transgenics  
6 were sown and grown as described (Boyes et al., 1998). Primers for genotyping  
7 and constructs for generating transgenic Arabidopsis were listed in Table S6.

8

### 9 Yeast two hybridization

10 HopBB1, JAZ3, TCP14, COI1 and mutant derivatives were cloned into gateway-  
11 compatible pJG4-5 (-Trp) or pEG202 (-His) vectors. pJG4-5 and pEG202  
12 constructs were transformed into competent yeast strains EGY48 and RFY206,  
13 respectively following manufacturer's protocol (Frozen-EZ Yeast Transformation  
14 II <sup>TM</sup>, Zymo Research) and selected on plates with dropout media. Each strain  
15 also carries the GAL4 reporter on psH18-34 (-Ura). Positive colonies were  
16 verified by yeast colony PCR. After mating the strain EGY48 and RFY206, diploid  
17 yeasts were plated on selective medium (-H-W-U) supplied with 100 $\mu$ M X-Gal for  
18 developing blue color from 2-6 days. To measure protein accumulation, yeast  
19 colonies were suspended in 50  $\mu$ l 0.2N NaOH for 10 minutes. Cells were then  
20 collected by centrifugation and re-suspended in 1 x loading buffer. Protein levels  
21 were examined by western blotting.

22

23 RNA sequencing

24 In order to define a comprehensive set of marker genes for the JA and SA  
25 responses, we used RNA-seq to assess the transcriptome of the Arabidopsis  
26 Col-0 ecotype over a time-course hormone treatment (Figure S1B). Two-week-  
27 old seedlings were sprayed with 50  $\mu$ M MeJA (Sigma), 300  $\mu$ M BTH (Actigard  
28 50WG) or a mock solution (0.02% Silwet, 0.1% ethanol). Samples were  
29 harvested 1h, 5h and 8h after spraying. This experiment was repeated twice. The  
30 experiments shown in Figures 1D, 1E and 1F were performed using steady-state  
31 seedlings grown under the same conditions as the ones used in the hormone  
32 treatment experiment. Lines Col-0 (4 replicates), UBQ10::YFP-TCP14-3 (4  
33 replicates), UBQ10::YFP-TCP14-4 (1 replicate), and *coi1-16* (2 replicates) were  
34 used in the experiment presented in Figures 1D and E; whereas Col-0 (3  
35 replicates), *tcp14-6* (3 replicates) and *tcp14-7* (3 replicates) were used in the  
36 experiment shown in Figure 1F. Bacteria-infected plants were used in the  
37 experiments shown in Figures 1G, 1H and 1I. For this, the strain *Pto* DC3000  
38 *cor-* (at OD<sub>600</sub>=0.2 with 10mM MgCl<sub>2</sub> and 0.04% Silwet L-77) was sprayed onto  
39 Col-0 (3 replicates), *tcp14-6* (3 replicates), UBQ10::YFP-TCP14-4 (3 replicates)  
40 and *coi1-16* (3 replicates). Samples were harvested for RNA preparation 24  
41 hours post infection.

42 We also evaluated the effect of bacteria-delivered HopBB1 on the transcriptome  
43 of wild-type plants (shown in Figures 3B, 3C and 3D). For this, two-week-old Col-  
44 0 seedlings were sprayed with a mock solution (10 mM MgCl<sub>2</sub>) or bacteria [*Pto*

45 DC3000 (EV), *Pto* DC3000 *cor-* (EV); *Pto* DC3000 *cor-* (HopBB1); *Pto* DC3000  
46 *cor-* (HopBB1<sub>G126D</sub>)] at OD<sub>600</sub>=0.2 with 10mM MgCl<sub>2</sub> and 0.04% Silwet L-77.  
47 Samples were harvested 24 hours after infection. This experiment included three  
48 biological replicates. The transcriptome of the transgenic line HopBB1-myc-10 (3  
49 replicates) was also compared to the one of Col-0 seedlings at steady-state  
50 conditions (shown in Figure 3G). In all experiments, each biological replicate  
51 corresponds to approximately 30 seedlings grown on the same pot.

52 For RNA isolation, plant tissue was ground to a fine powder using the  
53 Qiashredder tissue homogenizer (Qiagen) and total RNA was extracted using the  
54 RNeasy Plant Mini kit (Qiagen). Illumina-based RNA-seq libraries were prepared  
55 from 1000ng total RNA. Library quality control and quantification were performed  
56 using a 2100 Bioanalyzer instrument (Agilent) and the Quant-iT PicoGreen  
57 dsDNA Reagent (Invitrogen), respectively. The Illumina HiSeq2500 sequencer  
58 was used to generate single-end reads. Raw sequencing data are available at  
59 the NCBI Gene Expression Omnibus accession GSE90606.

60 RNA-seq reads were mapped against the TAIR10 reference genome using  
61 Tophat (Trapnell et al., 2009). Alignment parameters were set to allow only one  
62 mismatch and to discard reads mapping to multiple positions in the reference.  
63 HTSeq (Anders et al., 2015) was then used to count reads mapping to each one  
64 of the 27,206 nuclear protein-coding genes. Differential gene expression  
65 analyses were performed with the edgeR package (Robinson et al., 2010) using  
66 the False Discovery Rate (FDR) method for correction of multiple comparisons  
67 (Benjamini and Hochberg, 1995). Genes with FDR below 0.01 and a fold-change

68 variation greater than 1.5X were considered differentially expressed between  
69 conditions. Gene Ontology enrichment analyses were performed with the  
70 PlantGSEA toolkit (Yi et al., 2013) and with the Cytoscape plugin ClueGO  
71 (Bindea et al., 2009) .

72 We identified a total of 933 and 2357 genes that were significantly up-regulated  
73 ( $FDR \leq 0.01$ ; 1.5 fold-change difference relative to the mock control) in at least  
74 one of the three time-points analyzed after treatment with MeJA or BTH,  
75 respectively (Figure S1D; Table S1). As expected, these sets of genes were  
76 strongly enriched for biological processes related to JA and SA responses  
77 (Figures S1E and S1F). After filtering out the 261 genes upregulated by both  
78 hormones, we defined a set of 672 and 2096 markers of the JA and SA  
79 responses, respectively.

80

### 81 Confocal microscopy

82 Microscopy was conducted 16-24 hours after infiltration using a LSM 7 DUO  
83 (Carl Zeiss). Leaf disc samples were imaged with a 40x water objective. Between  
84 5 and 15 nuclei were observed in each repetition. The confocal images were  
85 edited with Zen 2009 (Zeiss) and Adobe Photoshop CS2. Zen 2009 (Zeiss) and  
86 Excel (Microsoft) were used to create histograms. For the HopBB1-TCP14-JAZ3  
87 co-localization assay, JAZ3-RFP and TCP14-YFP were driven under 35S  
88 promoter, HopBB1-CFP or HopBB1<sub>G126D</sub>-CFP was driven by estradiol-inducible  
89 promoter. Estradiol was applied 6 hours after the co-infiltration of Agrobacteria.



90 The primers and constructs used for confocal analysis are listed in Table S6.  
91 GV3101 carrying 35S promoter-driven p19 protein was co-infiltrated at  
92  $OD_{600}=0.05$  in each experiment to prevent the onset of post-transcriptional gene  
93 silencing and improve the efficiency of transient expression (Lindbo, 2007).

94

95 For transient protein expression in *N. benthamiana*, Agrobacteria at annotated  
96 concentration were suspended in 10mM  $MgCl_2$ , 10mM MES and 100 $\mu$ M  
97 acetosyringone, and hand infiltrated into *N. benthamiana* leaves. For the  
98 HopBB1-mediated disruption of the JAZ3-MYC2 interaction, rBiFC (JAZ3+MYC2)  
99 and EST::HopBB1-CFP-HA, EST::HopBB1<sub>G126D</sub>-CFP-HA or EST::HaRXL45-CFP-  
100 HA were co-inoculated at  $OD_{600}=0.1$  and  $OD_{600}=0.2$ , respectively (Grefen and  
101 Blatt, 2012). Six hours after inoculation, 20 $\mu$ M estradiol was infiltrated. Samples  
102 were collected 20-24 hours after inoculation. 4-6 images of 50-80 cells/per field  
103 were taken in each repetition.

104

#### 105 Disease resistance assay

106 *Pto* DC3000 and *Pto* DC3000 *cor-* were described in (He et al., 2012). *Pto*  
107 DC3000 *cor-* (EV), *Pto* DC3000 *cor-* (HopBB1) and *Pto* DC3000 *cor-*  
108 (HopBB1<sub>G126D</sub>) were generated by transforming *Pto* DC3000 *cor-* with either  
109 pJC531 (empty vector), pJC531 (HopBB1) or pJC531 (HopBB1<sub>G126D</sub>). HopBB1 or  
110 HopBB1<sub>G126D</sub> were expressed from the native promoter. Bacterial growth assays  
111 in *Arabidopsis* were performed by spray or dipping inoculation as described.  
112 Bacterial cultures were resuspended in 10 mM  $MgCl_2$  with 0.04% Silwet L-77.

113 Plants were sprayed or dipped with a bacterial suspension at  $OD_{600}=0.2$ . Results  
114 displayed in Figures 1C, 3A and 3F were performed independently a minimum of  
115 3 times with similar results. Hpa infection was performed as described in  
116 (Mukhtar et al., 2011).

117 Bacterial colony formation units (CFU) were measured after three days. Dashed  
118 line indicates the CFU at day 0. Statistics in Figures 1C, 3A and 3F were  
119 performed using one-way ANOVA test with Tukey-Kramer HSD with 95%  
120 confidence.

121

#### 122 Effector Delivery Assay

123 The coding regions of HopBB1 or HopBB1<sub>G126D</sub> were cloned into pBAV178  
124 (proNPTII:: Gateway cassette-AvrRpt2<sup>101-255</sup>) (Vinatzer et al., 2005). pBAV178  
125 (HopBB1) and pBAV178 (HopBB1<sub>G126D</sub>) were transformed into *Pto* DC3000 *cor*-.  
126 Bacteria were infiltrated into leaves of four-week-old Col-0 plants at  $OD_{600}=0.01$   
127 with 10mM MgCl<sub>2</sub>, and cell death was scored after 16-20 hours.

128

#### 129 Phylogenetic Analysis

130 The phylogenetic history of *Pseudomonas* was inferred by multi-locus alignment  
131 using MUSCLE (Edgar, 2004) to align amino acid sequences of 31 single copy  
132 core genes from the 2681 *Pseudomonas* genomes available for download on the  
133 PATRIC database (Wattam et al., 2014) . Using the resulting tree, *P. syringae*  
134 genomes were identified by selecting the smallest monophyletic group containing

135 all genomes annotated as *P. syringae*. This analysis resulted in a dataset 287 *P.*  
136 *syringae* genomes (Table S5). Pan genome analysis was performed on this  
137 subset using Roary (Page et al., 2015) with default parameters. The nucleotide  
138 sequences of the resulting 84 core genes (Table S6) were used to construct a  
139 new phylogenetic tree. Trees were constructed using a Maximum Likelihood  
140 method (Jones et al., 1992) implemented in MEGA7 (Tamura et al., 2013) with  
141 100 bootstrap iterations. The HopBB1 (from *Pseudomonas syringae* pv. *mori* str.  
142 301020), HopX1 (from *Pseudomonas syringae* pv. *tabaci* str. ATCC 11528),  
143 HopZ1a (from *Pseudomonas syringae* pv. *syringae* strain A2) and the coronatine  
144 biosynthesis pathway genes (from *Pseudomonas syringae* pv. *tomato* str.  
145 DC3000) were used as blast queries to search for homologous proteins in these  
146 287 *P. syringae* genomes. A hit with over 80% protein sequence identity was  
147 considered positive. For HopZ1a, the difference between HopZ1a and HopZ1b  
148 annotated in (Ma et al., 2006) was used as guideline. Each homologue was  
149 manually checked for the integrity of reading frame. The tree was visualized  
150 using iTOL (<http://itol.embl.de/>) (Letunic and Bork, 2007) . A newick file is  
151 available for download and interactive viewing at  
152 <http://itol.embl.de/shared/HopBB1>.

153

154

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