

Supplemental Figure 1. Representative HBI1-binding and HBI1-regulated genes with known functions in various developmental and cellular processes.

Genes induced or repressed by HBI1 are in red or blue, respectively. Direct HBI1-binding target genes are underlined. Genes induced or repressed by flg22 are in yellow or gray background, respectively.



Supplemental Figure 2. Growth-promoting and growth-inhibiting HLH factors are directly repressed and induced by HBI1, respectively.

(A) Quantitative RT-PCR analysis of the expression of *PRE1*, *KDR*, *IBH1*, *AIF1*, *PAR1* and *PAR2*. *PP2A* was used as the internal control. The wild-type and *HBI1-Ox* plants were grown in half-strength MS medium under constant light for five days. Error bars indicate SD from three biological repeats. Asterisk indicates significant difference from wild-type (*t* test; *P<0.05).

(B) Quantitative ChIP-PCR analysis of HBI1 binding to the promoter of selected genes. The chromatin of *pHBI1::HBI1-YFP* and *35S::YFP* transgenic plants was immunoprecipitated with anti-YFP antibody, and the precipitated DNA was quantified by qPCR. Enrichment of DNA was calculated as the ratio between *pHBI1::HBI1-YFP* and *35S::YFP*, normalized to that of the *PP2A* coding region. Error bars indicate SD of three biological repeats. Asterisk indicates significant difference from control gene *PP2A* (*t* test; *P<0.05).



Supplemental Figure 3. HBI1 and PIF have overlapping and distinct functions

(A) GO analyses of HBI1- and PIF-regulated genes. Numbers indicate the percentage of genes belonging to each GO category. Asterisk indicates significant difference from genomic random (Fisher exact test; *P<0.05).

(B) Growth of wild-type, *pifq*, *HBI1-Ox* and *HBI1-Ox/pifq* in the dark for 6 days. Bottom graph shows the quantification of hypocotyl lengths. Asterisk indicates significant difference from wild-type (*t* test; *P<0.05). Error bars indicate SD from three biological repeats.



Supplemental Figure 4. HBI1 negatively regulates elf18- and flg22-mediated PTI responses.

(A) Table shows the overlaps between gene sets regulated by HBI1 and elf18.

(B) Scatter plot of log2 fold change values of the genes co-regulated by HBI1 and elf18.

(C) Quantitative RT-PCR analysis of RNA levels of *HBI1* and its homologs in 4-week old plants treated with mock (M) or 1μ M elf18 for 3 hours. *PP2A* was used as the internal control. Error bars indicate SD from three biological repeats. Asterisk indicates significant difference from Col with mock treatment. (*t* test; *P<0.05).

(D) Elf18-dependent inhibition of wild-type (Col) and *HBI1-Ox* seedling growth. Seedlings were grown in half-strength MS medium for 5 days, then transferred to liquid half-strength MS medium without (M) or with 1 μ M elf18 for another 8 days. Growth is represented relative to untreated plants. Error bars indicate SD from three biological repeats. Asterisk indicates significant difference from wild-type (*t* test; *P<0.05).

(E) Growth of wild-type (Col) and two independent lines of Ox-*HBI1-4MH* in the absence (M) or presence of flg22 (20 and 100 nM). Growth is represented as fresh weight relative to untreated plants. Error bars indicate SD from three biological repeats.

(F) Oxidative burst triggered by 1µM elf18 in leaf discs of wild-type and *HBI1-Ox* plants. Error bars indicate SD from twelve biological repeats.



Supplemental Figure 5. Activation of HBI1 results in the suppression of PTI marker gene expression, but not MAPK activation.

(A-D) Quantitative RT-PCR analyses of flg22 effects on the expression of defense-related genes in wild type and *HBI1-Ox*. The wild-type and *HBI1-Ox* plants were grown in half-strength MS medium under constant light for 2 weeks, and then treated with mock (M) or 1 μ M flg22 for 3 hours. *PP2A* was used as the internal control. Error bars indicate SD from three biological repeats. Asterisk indicates significant difference from flg22 treatment between wild-type and *HBI1-Ox* (*t* test; *P<0.05).

(E) Immunoblot analysis of flg22-induced (1 uM) MAPK phosphorylation in 2-week-old Col and *HBI1-Ox* seedlings. Arrowheads indicate phosphorylated MPK3 and MPK6. Blot stained with ponsceau S is presented to show equal loading. Immunoblots were probed with anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibody (Cell signaling).



Supplemental Figure 6. Quantitative RT-PCR analyses of flg22 effects on the expression of HBI1 homologous genes.

The wild-type plants were grown in half-strength MS medium under constant light for 2 weeks, and then treated with mock or 1 μ M flg22 for 3 hours. *PP2A* was used as the internal control. Error bars indicate SD from three biological repeats. Asterisks indicate significant difference from the mock treatment (*t* test; *P<0.05).

Gene	HBI1-Ox vs WT	
	RT-qPCR	RNA-Seq
At1g26380	-8.25 ±0.54*	-8.83
At1g26420	-3.96 ±0.06*	-5
WRKY33	-2.65 ±0.05*	-2.5
At4g26120	-2.22 ±0.27*	-2.69
VQ28	-1.91 ±0.08*	-2.24
MPK11	-2.35 ±0.12*	-3.12
KDR	-1.78 ±0.1*	-1.63
BEE1	-1.44 ±0.08*	-1.29
IBH1	0.79 ±0.04*	1.04
AIF1	0.80 ±0.03*	1.02
EXP8	1.00 ±0.05*	1.34
EXP1	1.06 ±0.1*	1.04
PIF4	1.04 ±0.07*	1.24

Supplemental Table 1. Quantitative RT-PCR validation of the RNA-Seq data.

Seedlings of wild-type (WT) and *HBI1-Ox* plants were grown in half-strength MS medium under constant light for 5 days. The RT-qPCR data were normalized to PP2A, and the average ratio (in log2) between *HBI1-Ox* and WT and the standard deviation were calculated from three biological repeats. *Significant difference between *HBI1-Ox* and WT (p<0.05). RNA-Seq data are from Supplemental Table 2.

RI-PCR primers		
CODDTE		
DWF4RTF	catagagetetteanteacha	
DWF4RTR	catcatettettetteetaa	
BR60X1RTF	TCGGGTTACGAAACTGTCTCTACG	
BR6OX1RTR	CCTGAATGCCAAATGCTCAGCTC	
FRK1RTF	ATCTTCGCTTGGAGCTTCTC	
FRK1RTR	TGCAGCGCAAGGACTAGAG	
At2g17740 RTF	TGCTCCATCTCTTTGTGC	
At2g17749 RTR	ATGCGTTGCTGAAGAAGAGG	
PR1RTF	AAGGGTTCACAACCAGGCAC	
PR1RTR	CACTGCATGGGACCTACGC	
JAZ6RTF	cgtggtgattcccgatcttaacgag	
JAZ6RTR	tgggaggaagatgactaccgtgttg	
HBI1RTF	TGCCTGGATGCAATAAGGTCACAG	
HBI1RTR	TGGAGCTTCGATAGATGTCGTTTGG	
BEE2RTF	CAGTTTCAGGCTTACTTCACAGGT	
BEE2RTR	CTTTAGCGCCGAGAGATGTGGT	
BEE1RTF	TGGTGCCCGGATGTTATAAGGC	
BEE1RTR	GCTGCAGTGAGTTTCATCGAGAGG	
BEE3RTF	TGCTGTGGAATCCATGCAGAAG	
BEE3RTR	AGGGTCCACGATGATGAATGGAAG	
CIB1RTF	aggccggattttgatatggatgac	
CIB1RTR		
CIB5RTE		
CIB5RTR		
AGEIRIK		
	acgagaaaacggcatgtgaaattgg	
DWF4ChIPR	gatactttgggcacgtccatgtgttc	
CPDChIPF	gagagacatgtgaagtgttcatag	
CPDChIPR	gttcgatgaatgtagtcagtgttagattc	
BR6OX1ChIPF	accgtaatcatggttatgcctagagc	
BR6OX1ChIPR	tgccttgtgatgattgagcgcacg	

Supplemental Table 2. Oligonucleotide sequences used in this paper