

Supplementary material:

A transcriptional hub integrating gibberellin-brassinosteroid signals to promote seed germination in *Arabidopsis*

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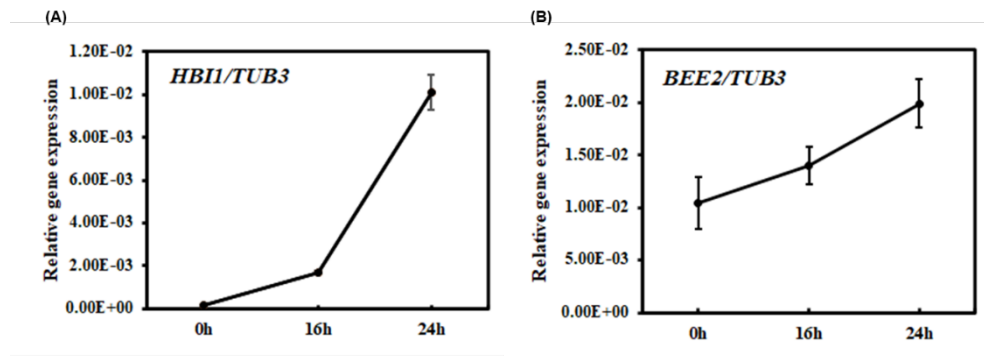


Fig. S1 Relative gene expression of *HBI1* (A) and *BEE2* (B) during seed germination, 0 h was marked as the timepoint when seeds were exposed to light after 3 days stratification. The transcript levels were normalized to *TUB3*.

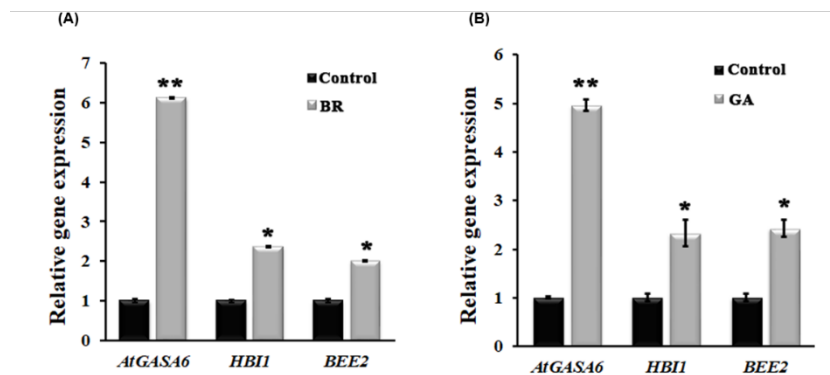


Fig. S2 Relative gene expression of *HBI1*, *BEE2*, and *GASA6* in response to BR (A) and GA (B). Two-week-old seedlings were treated with 1 μ M BR or 100 μ M GA₃ for 2 h. The transcript levels were normalized to *TUB3*. One-way ANOVA was used to analyze the significant difference. * $P < 0.05$; ** $P < 0.01$.

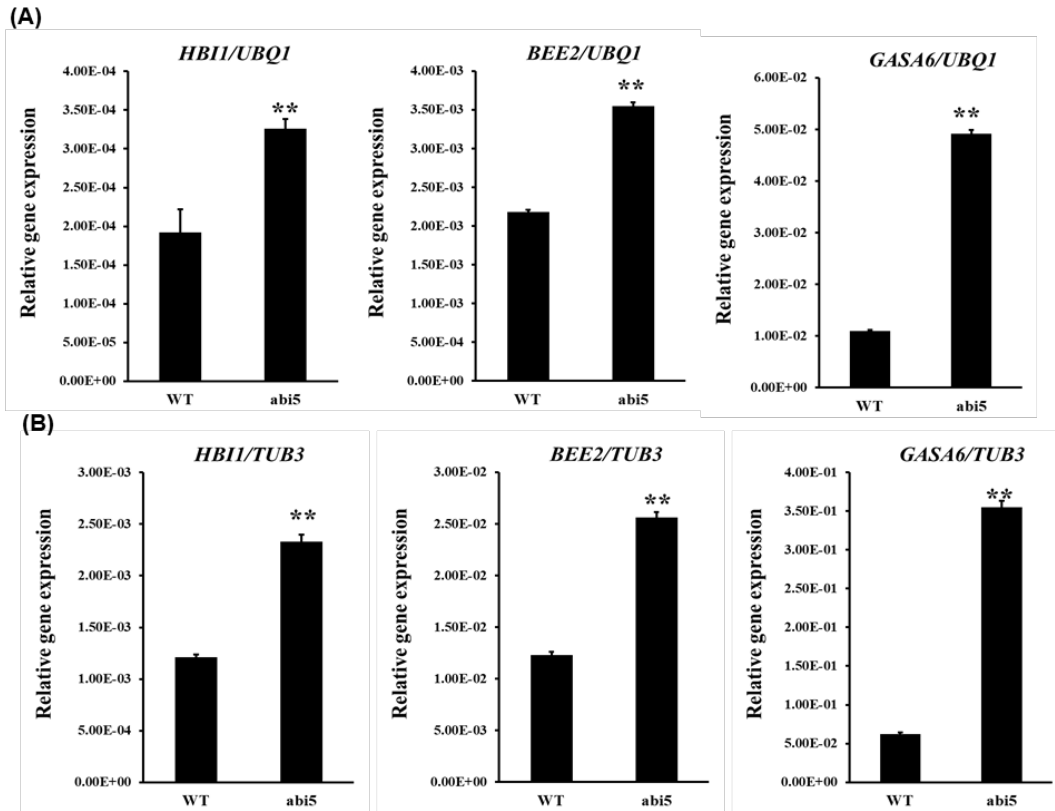


Fig. S3 Relative expression of *GASA6*, *HBI1* and *BEE2* in *abi5* mutant seeds.

Wild-type Arabidopsis (Ws) was used as the control. The transcript levels were normalized to *UBQ1* (A) or *TUB3* (B) gene expression. One-way ANOVA was used to analyze the significant difference. ** $P < 0.01$.

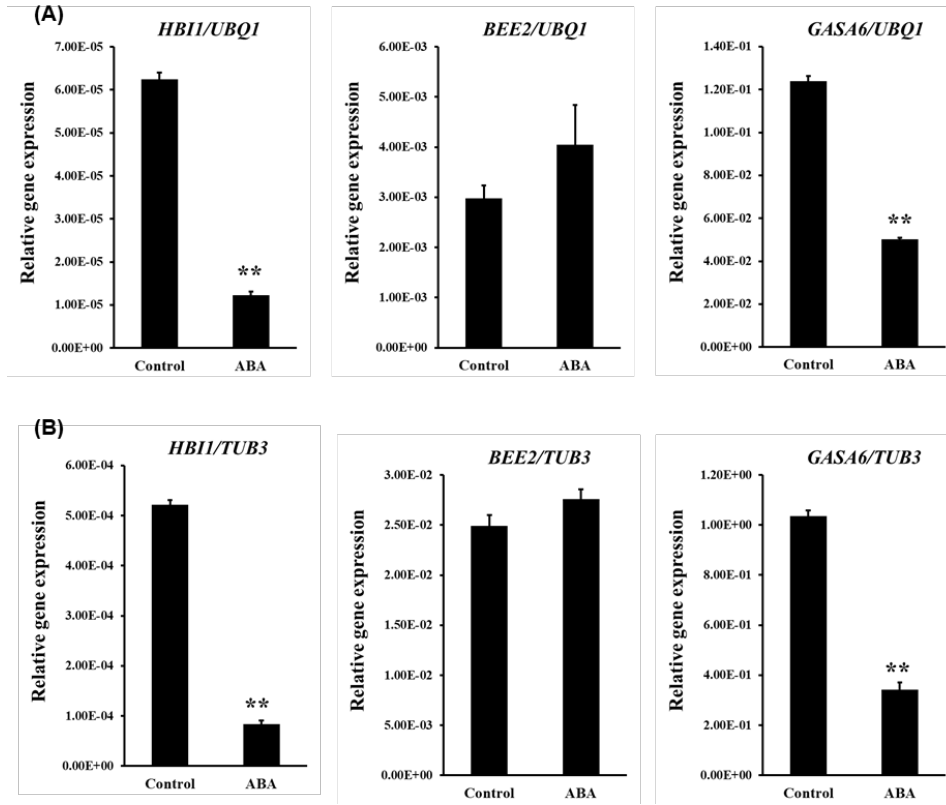


Fig. S4 Relative gene expression of *HBII*, *BEE2*, and *GASA6* in response to ABA.

Germinating seeds were treated with 100 μ M ABA for 2 h. The transcript levels were normalized to *UBQ1* (A) or *TUB3* (B) gene expression. One-way ANOVA was used to analyze the significant difference. ** $P < 0.01$.

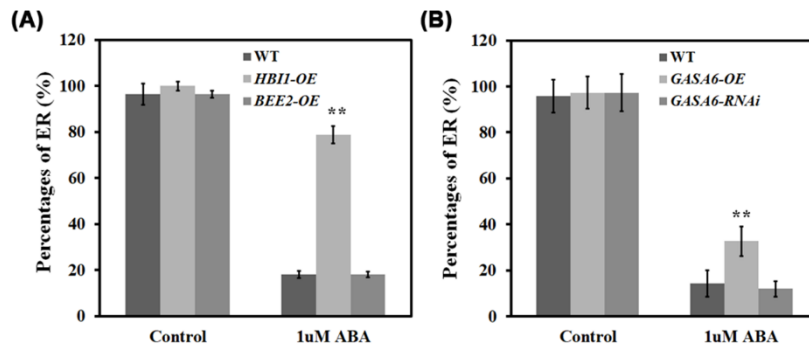


Fig. S5 Effect of ABA on ER of WT, *HBII-OE* or *BEE2-OE* seeds.

(A) Percentage of ER in WT, *HBII-OE* or *BEE2-OE*, seeds were treated with 1 μ M ABA for 36 h. (B) Percentage of ER in WT, *GASA6-OE* and *RNAi* seeds treated with 1 μ M ABA for 36 h. Seeds germinated on $\frac{1}{2}$ basal MS was used as all of the control. The asterisks indicate significant differences compared with WT. one-way ANOVA was used to analyze the significant difference. ** $P < 0.01$.

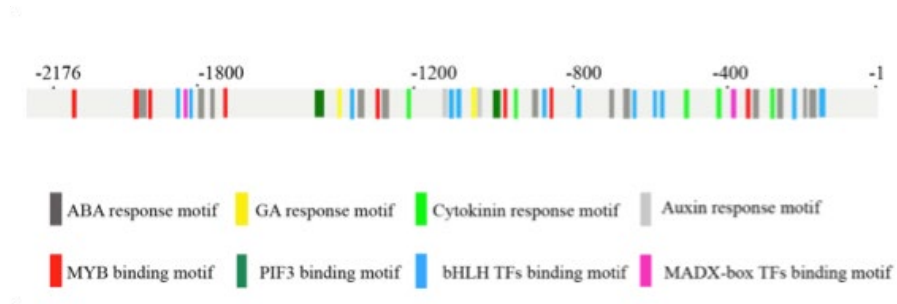


Fig. S6 *Cis*-motif analysis of *AtGASA6* promoter. Distribution of ABA-, GA-, CK- and Auxin-response elements, and MYB, PIF, bHLH and MADX-box transcription factors binding elements in *AtGASA6* promoter. *Cis*-motifs were identified by using online software PlantPAN (<http://plantpan2.itps.ncku.edu.tw/>), PLACE (<http://www.dna.affrc.go.jp/PLACE/>) and AGRIS (<http://arabidopsis.med.ohio-state.edu/>). Promoter of *AtGASA6* 2176 bp from ATG was used for analysis.

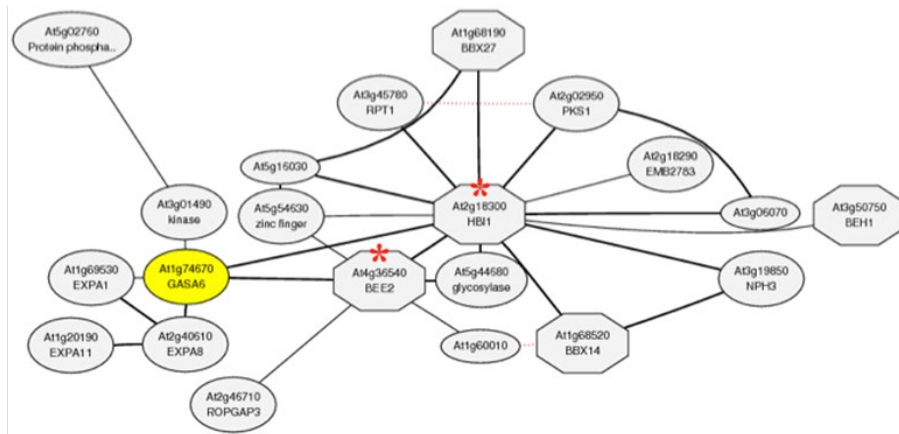


Fig. S7. The co-expression network of *AtGASA6* in Arabidopsis analyzed by the ATTED-II network drawer (http://atted.jp/top_draw.shtml#NetworkDrawer). The yellow oval indicated the query gene. The red asterisks indicate the closet genes.

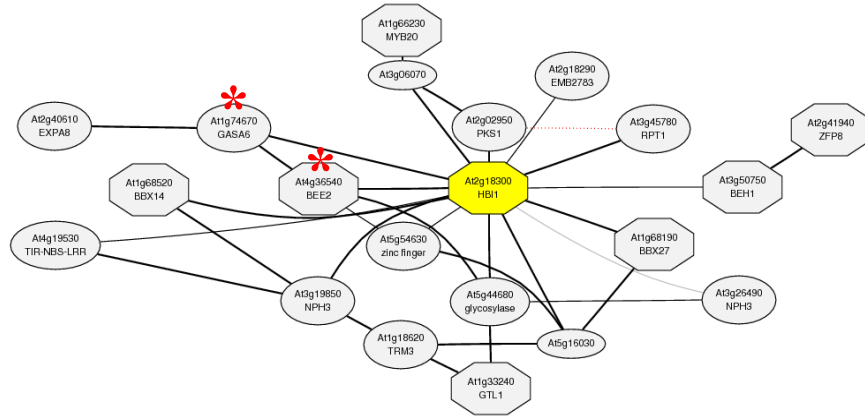


Fig. S8. The co-expression network of *HBI1* in Arabidopsis analyzed by the ATTED-II network drawer. The yellow oval indicated the query gene. The red asterisks indicate the closet genes.



Fig. S9. The co-expression network of *BEE2* in Arabidopsis analyzed by the ATTED-II network drawer. The yellow oval indicated the query gene. The red asterisks indicate the closet genes.

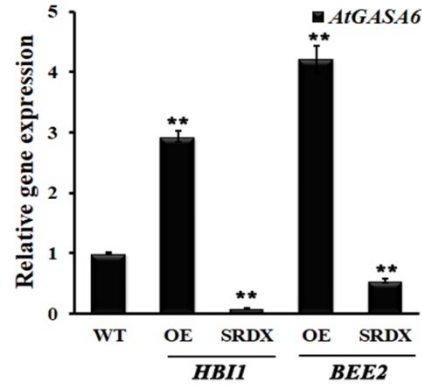


Fig. S10 Relative gene expression of *GASA6* in *HBI1*- or *BEE2*-OE and SRDX lines. Col0 was used as WT. The transcript levels were normalized to *TUB3*. One-way ANOVA was used to analyze the significant difference. ** $P < 0.01$.

Table S1 List of gen-specific primer sequences

Primer name	Sequence (5' to 3')
<i>GASA6</i> -FW-QPCR	CAATGCACAAGGAGATGTAGCAAC
<i>GASA6</i> -RV-QPCR	AGGGACACAAAGGCATTTAGCA
<i>HBI1</i> -FW-QPCR	TGTGCCTGGATGCAATAAGG
<i>HBI1</i> -RV-QPCR	ATACATCTTCCACGGCAAGC
<i>BEE2</i> -FW-QPCR	ACAGAGCCAAGCATGAAAGG
<i>BEE2</i> -RV-QPCR	TTGCCCTCTCTGCTAAGCTATG
<i>UBQ1</i> -FW-QPCR	GGCCAAGATCCAAGACAAAG
<i>UBQ1</i> -RV-QPCR	GTTGACAGCTCTTGGGTGAA
TUB3-FW-QPCR	GTGACTGCCTCCAAGGGTTC
TUB3-RV-QPCR	CGTTGTAAGGCTCCACCACA
<i>PP2A</i> -ChIP-F	ATGGCTGCGATTCTAGAGATCG
<i>PP2A</i> -ChIP-R	TAATCTGGAGTCTTGCGAGTGG
<i>GASA6</i> -P1-ChIP-F-2	GAATGACTTAAACAATCAAGTGGCAG
<i>GASA6</i> -P1-ChIP-R-2	GTGTGTGTAAGCTTCCTCATTAC
<i>GASA6</i> -P2- ChIP-F-2	GACACTATTAGTATATTATTGGACCCG
<i>GASA6</i> -P2- ChIP-R-2	CTACAGGCAGAAATTATTTTACATGTG

<i>GASA6</i> -P3- ChIP-F-1	TGGTCCCAATCACTTGCCT
<i>GASA6</i> -P3- ChIP-R-1	GTCACATTTGGGACTACACCC
<i>GASA6</i> -P4- ChIP-F-1	GTGTATCCACAAAATGCAAAGATAGA
<i>GASA6</i> -P4- ChIP-R-1	GAGAGACAAAAGGGTAGAGGCA
<i>GASA6</i> -P5- ChIP-F-1	GAATTGTGAGTGTTCGTAACAAGAT
<i>GASA6</i> -P5- ChIP-R-1	TTGGTCGTCCAATAAAAATAGCATT
<i>GASA6</i> -P6- ChIP-F-1	CATCTCCATCAATGGTTTCTCATTG
<i>GASA6</i> -P6- ChIP-R-1	ACAAGTGTTCTTATGGGTTTCTCAG
<i>pGASA6</i> -1.4k-LUC-XhoI-F	CCGCTCGAGCTTTGAATTGTGAGTGTCTG
<i>pGASA6</i> -1.4k-LUC-PstI-R	CAACTGCAGGACTAGCTCTTGAGAGAGAGAG
<i>pGASA6</i> -1.2k-LUC- XhoI -F	CCGCTCGAGCCACAAAATGCAAAGATAGAA
<i>pGASA6</i> -1.1k-LUC- XhoI -F	CCGCTCGAGAACTGCCTCTACCCTTTTGTCT
<i>pGASA6</i> -0.9k-LUC- XhoI -F	CCGCTCGAGTATTTGTTGGTTGGTCCCAATC
<i>pGASA6</i> -1.4k-mut1-LUC-F	CACAAAAACTATTTTATTGGACG
<i>pGASA6</i> -1.4k-mut1-LUC-R	ATTAAAAATTGTTTGTGTTTAACG
<i>pGASA6</i> -1.4k-mut2-LUC-F	CCATAAAAGT GCACATGGAGTT
<i>pGASA6</i> -1.4k-mut2-LUC-R	GAGACATATATCTATCTAATGCTTTC
<i>pGASA6</i> -1.4k-mut3-LUC-F	CCATGTGAGTGCACAAAGAGTT
<i>pGASA6</i> -1.4k-mut3-LUC-R	The same sequence as <i>pGASA6</i> -1.4k-mut2-LUC-R
HBI1-FW-PBS-BamHI	GTACGGATCCATGTTGGAAGGTCTTGTCTCTCA
HBI1-RV-PBS-PstI	ATGCTGCAGTTAGTAATGAAAACCGAGGCTAG
BEE2-FW-PBS-BamHI	AGCTGGATCCATGGACTTGTCTGTACTTGATAGGC
BEE2-RV-PBS-XbaI	GTA CTCTAGATTACTTGAGGCTGAAGAAATTGGA
IBH1-FW-PBS-BamHI	ATCGGGATCCATGGCCTCTGCAGACAAACT
IBH1-RV-PBS-XbaI	ATCGTCTAGAATCATTGGGAGATAAGCCATCA
HBI1-pAD/BD-EcoRI-F	ACCGGAATTCATGTTGGAAGGTCTTGTCTCTCA
HBI1-pAD-XhoI-R	CACGCTCGAGTTAGTAATGAAAACCGAGGCTAG
HBI1-pBD-PstI-R	CCAACTGCAGTTAGTAATGAAAACCGAGGCTAG

BEE2-pAD-BamHI-F	ACGCGGATCCATATGGACTTGTCTGTACTTGATAGGC
BEE2-pAD-XhoI-R	CACGCTCGAGTTACTTGAGGCTGAAGAAATTG
BEE2-pBD-NcoI-F	CACGCCATGGAGATGGACTTGTCTGTACTTGATAGGC
BEE2-pBD-BamHI-R	ACGCGGATCCTTACTTGAGGCTGAAGAAATTG
IBH1-pAD/pBD-EcoRI-F	ACCGGAATTCATGGCCTCTGCAGACAAACT
IBH1-pAD/pBD-BamHI-R	ACGCGGATCCTCATTGGGAGATAAGCCATCA
HBI1-BiFC-EcoRI-F	ACCGGAATTCTATGTTGGAAGGTCTTGTCTCTCA
HBI1-BiFC-BamHI-R	CGCGGATCCTTAGTAATGAAAACCGAGGCTAG
BEE2-BiFC-XhoI-F	CCGCTCGAGCTATGGACTTGTCTGTACTTGATAGGC
BEE2-BiFC-BamHI-R	The same sequence as BEE2-pBD-BamHI-R
IBH1-BiFC-EcoRI-F	ACCGGAATTCTATGGCCTCTGCAGACAAACT
IBH1-BiFC-BamHI-R	The same sequence as IBH1-pAD/pBD-BamHI-R
BEE2-pAD-N1-BamHI-F	ACGGGATCCATATGGACTTGTCTGTACTTGATAGGC
BEE2-pAD-N1-XhoI-R	TCCGCTCGAGTTCTGGATTTATGACAGAGAGTTTCA
BEE2-pAD-N2-BamHI-F	The same sequence as BEE2-pAD-N1-BamHI-F
BEE2-pAD-N2-XhoI-R	TCCGCTCGAGGGCTTCACCTCGTCTAGCC
BEE2-pAD-C1-BamHI-F	ACGCGGATCCATACCGACAGACATAGCTTAGCAG
BEE2-pAD-C1-XhoI-R	TCCGCTCGAGTTACTTGAGGCTGAAGAAATTGGA
BEE2-pAD-C2-BamHI-F	CGCGGATCCATCTTGAGTGTGCATATCGATGATTT
BEE2-pAD-C2-XhoI-R	The same sequence as BEE2-pAD-C1-XhoI-R
<i>ProGASA6</i> -GUS-a-F	GCGTCGACGGTAAAGGGTTTATCAAGTAAG
<i>ProGASA6</i> -GUS-b-F	GCGTCGACAGTCGTTATGTTTGTGAAAGC
<i>ProGASA6</i> -GUS-c-F	ATGTCGACTATTTGTTGGTTGGTCCCAATCAC
<i>ProGASA6</i> -GUS-d-F	TCGTCGACTGCCTGTAGTCTTTTAATCACTG
<i>ProGASA6</i> -GUS-R	TACCCGGGCATGACTAGCTCTTGAGAGAGAG
Probe -1	CAATTTTAAATCACAAATGCTATTTTATTGGACGACC
Probe 2	GTCTCCCATGTGAGTGACATGGAGTTATG
