

A MADS-box gene *NtSVP* regulates pedicel elongation by directly suppressing a *KNAT1*-like KNOX gene *NtBPL* in tobacco (*Nicotiana tabacum* L.)

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Supplementary figure legends

Supplementary Fig. S1. Protein sequence analysis and tissue-specific expression of *NtSVP*.

(A) Alignment of SVP-like protein sequences. The MADS-box, I domain, K domain and C-terminal domain are marked. Amino acids in black shading and grey shading indicate identical or similar amino acids among the proteins.

(B) An unrooted phylogenetic tree of SVP-clade MADS-box proteins from some species. The scale indicates branch lengths. Accession numbers are as follows: OsMADS22 (Os02g0761000), OsMADS47 (Os03g0186600), OsMADS55 (Os06g0217300), HvBM1 (AJ249142), HvBM10 (EF043040), Brassica campestris (Chinese cabbage) BcSVP (DQ922945), and CaSVP (AFI49342), Eucalyptus grandis EgrSVP (AY263809), NtSTMADS11 (mRNA_51421 in SOL Genomics Network), INCO (CAG27846), JOINTLESS (Solyc11g010570), SVP (At2g22540), AGL24 (At4g24540), Ta-VRT2(DQ022679), SOC1 (At2g45660).

(C) Tissue-specific expression of *NtSVP* in wild-type plants.

Supplementary Fig. S2. Phenotypes of the *NtSVP* transgenic plants.

(A) DNA fragment used for *NtSVP*-RNAi construct.

(B) The morphology of abscission zones in wild-type and *NtSVP*-RNAi transgenic tobacco indicated by white arrows.

(C) The morphology of flower and pedicel in the five developmental stages as described (Zhu *et al.*, 2013).

(D) Quantitative measurement of the pedicel lengths of the WT and two *NtSVP*-RNAi lines from S1 to S5 (N = 10 X 5, 50 pedicels from five individual plants per genotype). Values are means \pm SE.

(E) The whole plant morphology of wild-type and *NtSVP* transgenic plants at the reproductive stage.

(F) Quantitative measurement of plant height of WT, *NtSVP*-RNAi and *NtSVP*-OE plants at anthesis stage. (N=5, 5 plants for each genotype). Values are means \pm SE.

Supplementary Fig. S3. Protein sequence analysis and tissue-specific expression of *NtBPL*.

(A) Alignment of the NtBPL protein sequence with its homologs in tobacco, tomato and Arabidopsis. KNOX I and KNOX II subdomain, ELK domain, homeobox domain are marked. Amino acids in black shading and grey shading indicate identical or similar amino acids among the proteins.

(B) An unrooted phylogenetic tree of KNOX proteins in tobacco, tomato and Arabidopsis. The scale

indicates branch lengths. Accession numbers are as follows: TKN1 (Solyc04g077210.2), TKN2 (Solyc02g081120.2), NTH20 (BAA76904), BP (At4g08150), STM (At1g62360), NTH22 (BAA76905), NTH201 (BAF95776), KNAT2 (At1g70510), KNAT6 (At1g70510), KNAT5 (At4g32040), KNAT7 (At1g62990), KNAT4 (At5g11060), and KNAT3 (At5g25220), ATH1 (At4g32980).

(C) Tissue-specific expression of *NtBPL* in wild-type plants.

Supplementary Fig. S4. Developmental-specific expression of *NtSVP* and *NtBPL* in pedicels of wild-type plants.

Supplementary Fig. S5. Phenotypes of the *NtBPL* transgenic plants.

(A) The whole plant morphology of wild-type and *NtBPL* transgenic plants at the reproductive stage.

(B) Distribution of the angles between the pedicels and the inflorescences in WT, *NtBPL*-OE and *NtBPL*-RNAi plants. N= 10 X 5, 50 pedicels from five plants were measured as a replicate, and three replications were performed. Values are means \pm SE.

(C) Longitudinal sections of the pedicels of WT and *NtBPL* transgenic plants at anthesis stage. Asterisks pointed to cortex cells. Bars = 100 μ m.

(D) Cell numbers in the longitudinal cortex file of pedicels in WT and *NtBPL* transgenic plants at anthesis (N= 3 X 5, 15 sections from five individual plants per genotype were used). Values are means \pm SE.

(E) qRT-PCR detection of *NtSVP* expression in pedicels of WT and *NtBPL* transgenic plants. *NtACTIN9* was used as an internal control.

Supplementary Fig. S6. The promoter sequence of *NtBPL*. The CArG-box and initiation codon were highlighted by black boxes.

Supplementary Fig. S7. Confirmation of microarray data. Four differently expressed genes *NtGRAS1*, *NtEREBP5*, *NtWRKY3*, and *NtSBP2* were randomly selected for qRT-PCR detection compared with those of microarray results. *NtACTIN9* was used as an internal control.

Supplementary Fig. S8. GO enrichment analysis of differentially expressed genes in *NtBPL*-RNAi pedicels and their MapMan classification.

(A) Gene ontology analysis of 427 down-regulated genes in *NtBPL*-RNAi pedicels compared with those in the wild type. Colors indicate significance level of each over-represented terms (FDR \leq 0.01).

(B) Gene ontology analysis of 230 up-regulated genes in *NtBPL*-RNAi pedicels compared with those in the wild type plant. Colors indicate significance level of each over-represented terms ($FDR \leq 0.01$).

(C) MapMan classification of differentially expressed genes in *NtBPL*-RNAi pedicels.

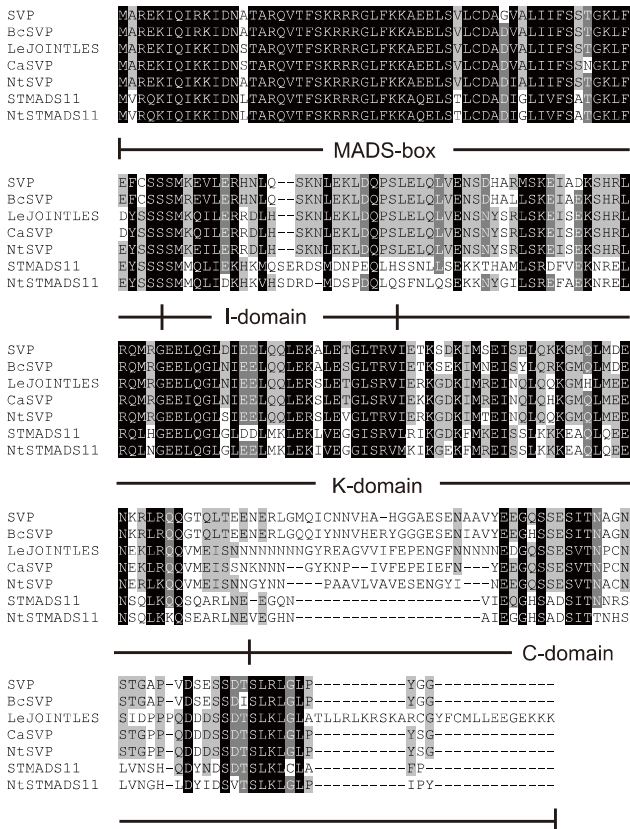
Supplementary Table S1. Hormone related genes that are differentially expressed due to down-regulation of *NtBPL*.

Supplementary Table S2. Primers used in this work.

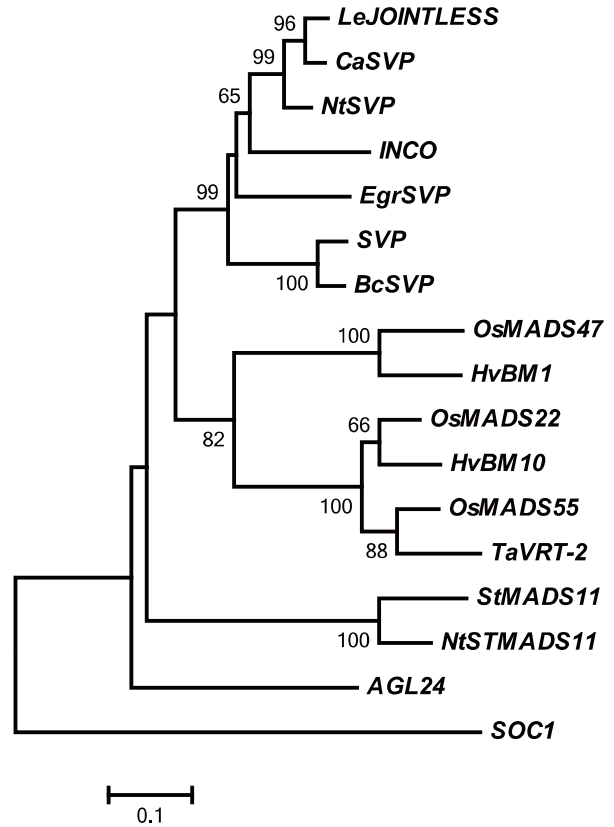
Supplementary dataset 1. List of differentially expressed genes between the wild type and *NtBPL*-RNAi pedicels.

Fig. S1

A



B



C

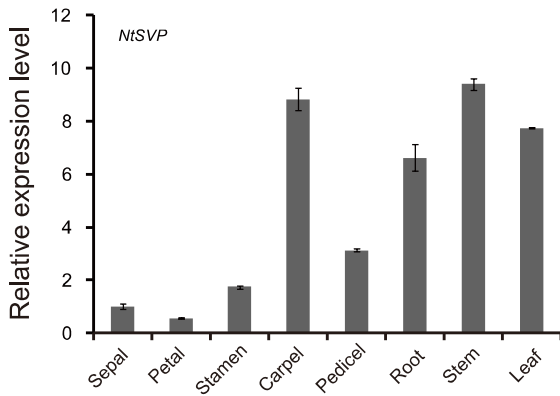
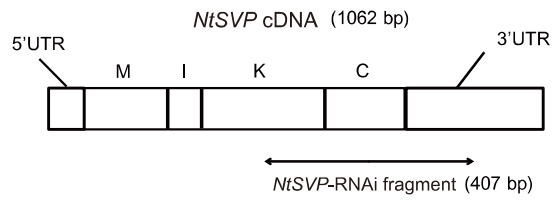
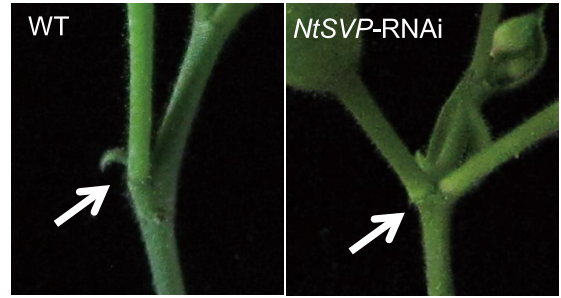


Fig. S2

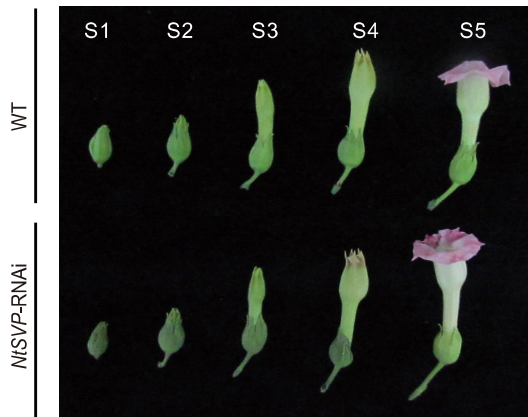
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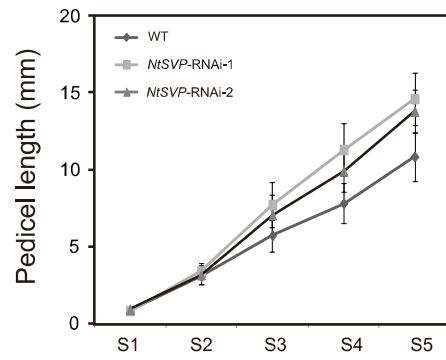
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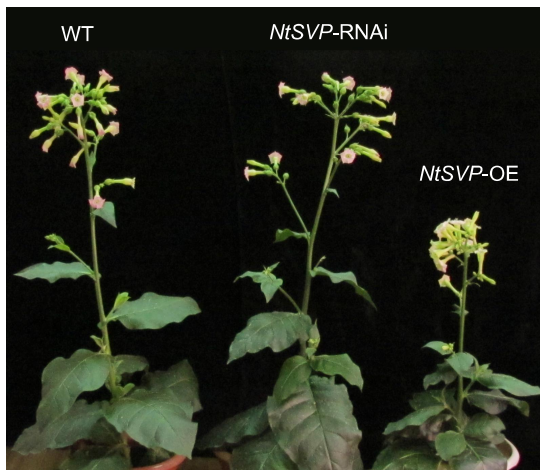
C



D



E



F

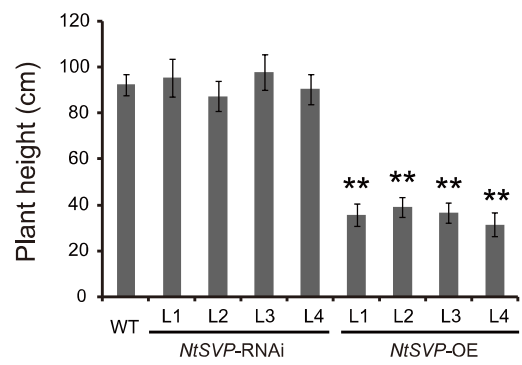


Fig. S3

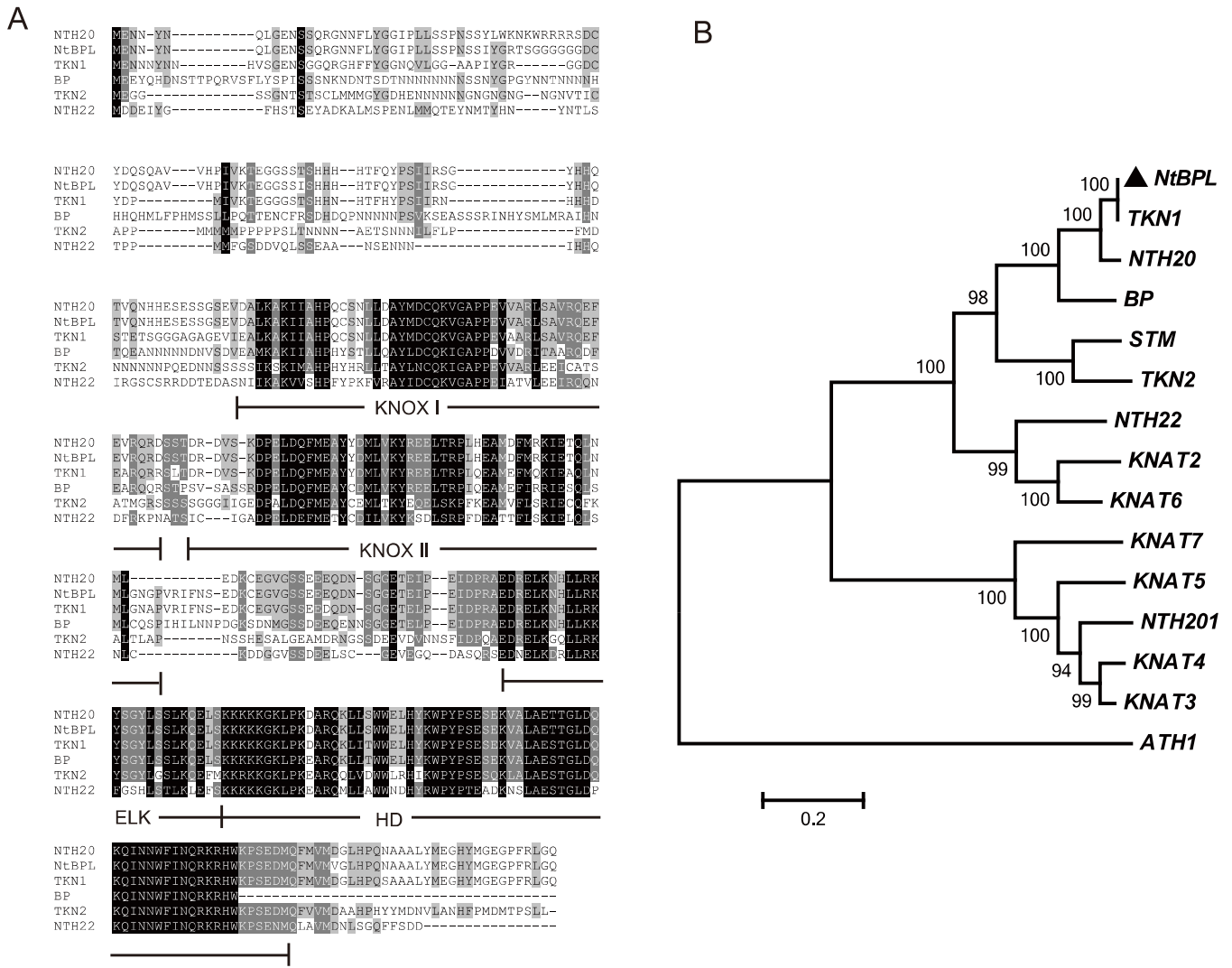


Fig. S4

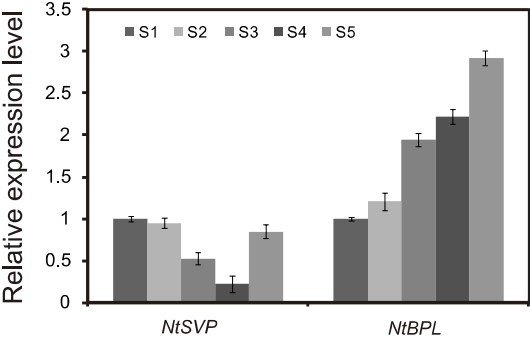
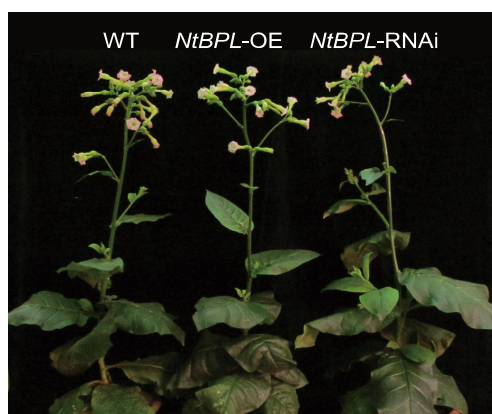
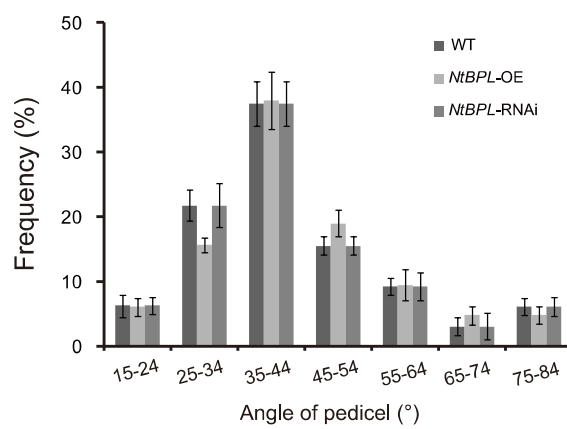


Fig. S5

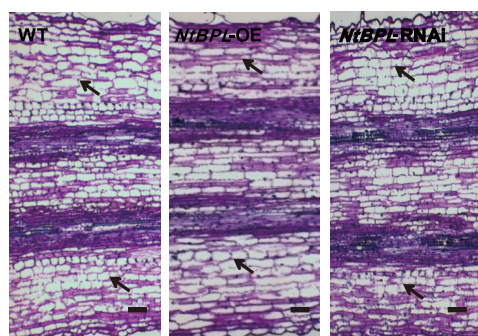
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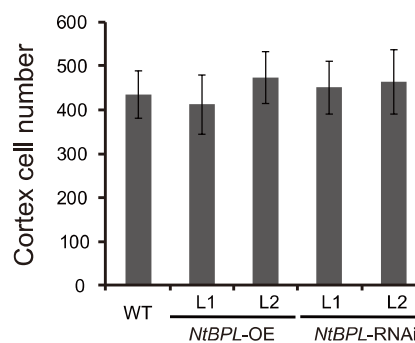
B



C



D



E

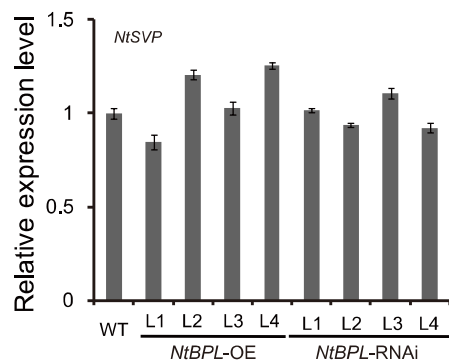


Fig. S6

GGCCTACTAA TAGGGGGCGA TTGAGCTGCC CTTAGCTTCG CTTCTTCTAT CAATTCCTTG
CCATCATCCT CTTTGATAAT GTAAACAATT GAACCAATTT ATATAACTCA ATTTTTCTAT
CAATTATAAG AACTAACTT TTCCAAGGTA GTGAAATAAA TAATCAATAG AAAAAGAAAG
ACCTCACAAA AGGAAAAAGA AAATGAAAAG GGCTTCCCAT GGAAGTAATA GGAAAAGCAA
CArG-box
AAGAATACAA AAAGAAAGAG AAAGTTAAAA GATCTAACTA ACTAACTCTA TATATATACA
TATATATATA TTGAATAAAA TAATTATGCT TTCTTTATAA TTGAGTGGTT TTCTCTGCAT
AGGATTATAG GGGGTATAGT TAAACTAAAG ACTAATATTT TCCAATACAT TTCCTTATTC
TTCAACGTAA GAACGTCTTG TCATCTACGA AGCCGCTACC AGGAGCGGCC ACTAGCTTAC
CCCTTCCCTT CCTTTCCTTT CCTTTTTTCT TTGTTACAG CCACTATCTT CTATGTTGTC
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TCCGCTCACA ATTCCACACA ACATACGAGC CGGAAGCATA AAGTGTAAG CCTGGGGTGC
TAATGAGTGA GCTAACTCAC ATTATT**ATG** AGAATAATTA
Initiation Codon

Fig. S7

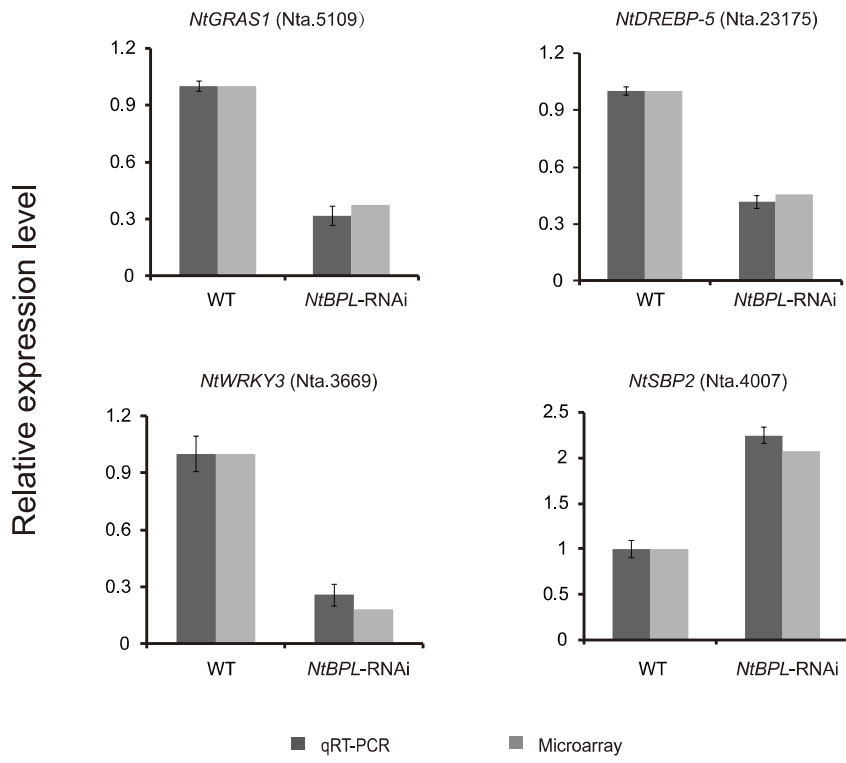
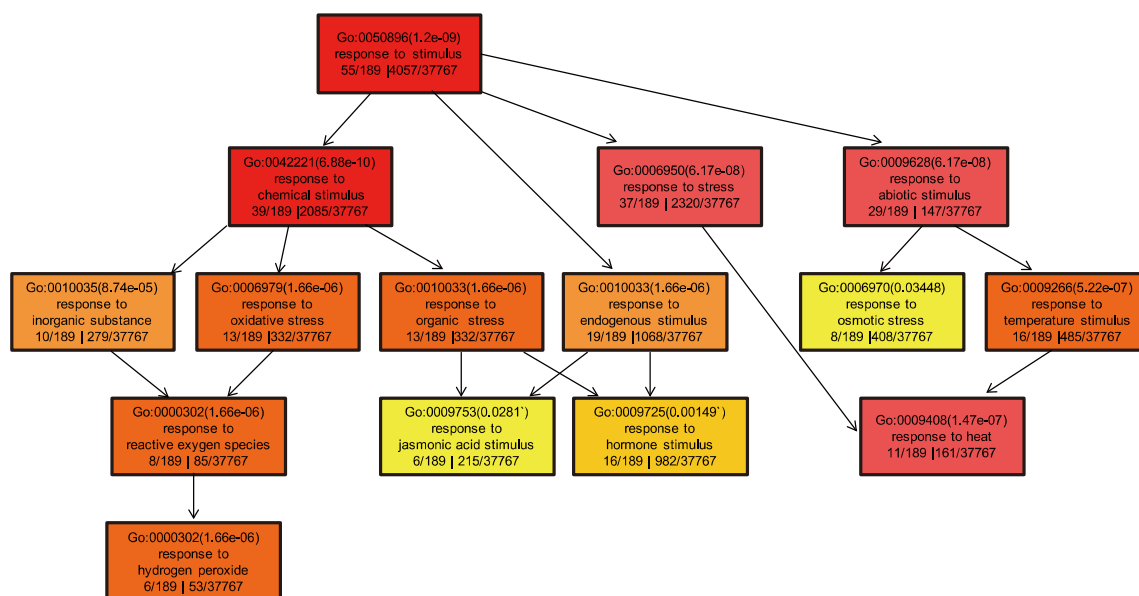
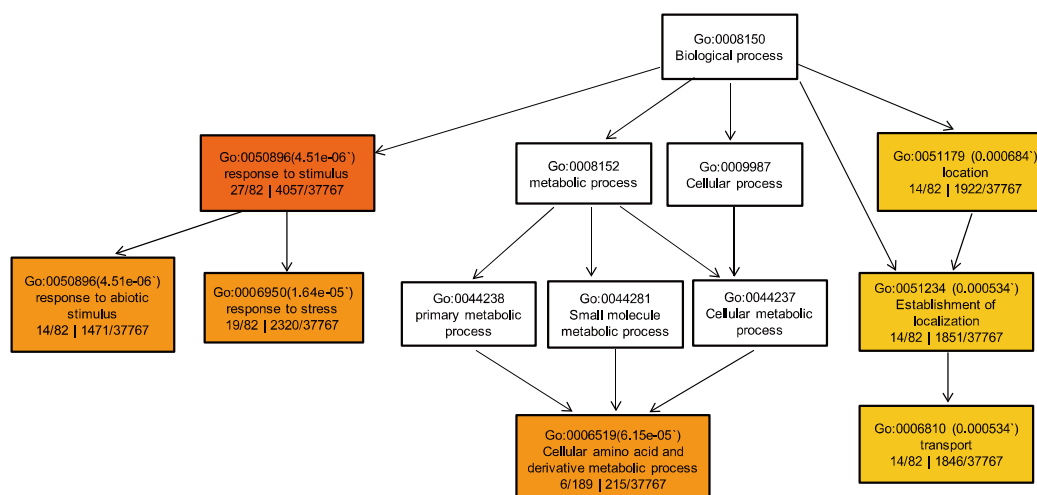


Fig. S8

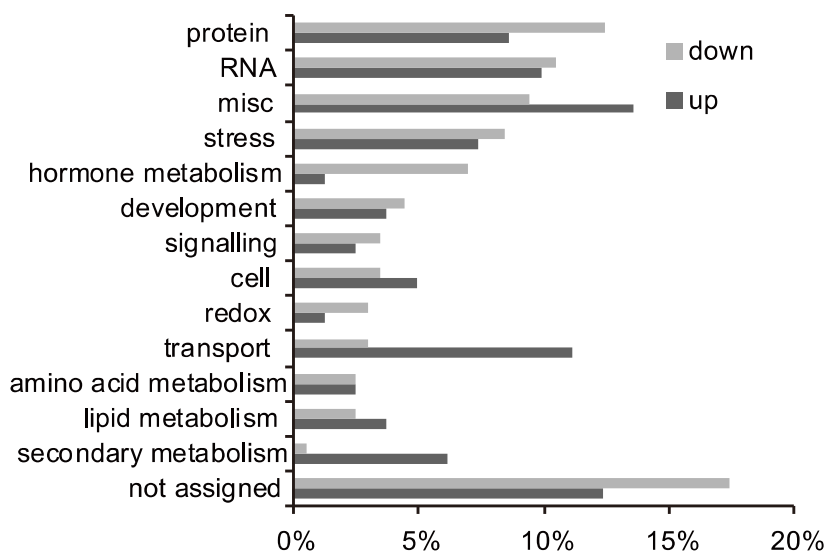
A



B



C



Supplementary Table S1. Hormone related genes that are differentially expressed due to down-regulation of *NiBPL*.

	Arabidopsis homologs	Description	Foldchange	UniGene ID
<i>Up regulated</i>				
auxin.induced-regulated-responsive-activated	at5g54510	GH3.6, DFL1 DFL1 (DWARF IN LIGHT 1)	2.3	Nta.23121
gibberelin.induced-regulated-responsive-activated	at3g02885	GASA5	6.01	Nta.8147
<i>Down regulated</i>				
auxin.induced-regulated-responsive-activated	at2g45210	Auxin-responsive protein-related	0.24	Nta.3096
auxin.induced-regulated-responsive-activated	at5g35735	Auxin-responsive family protein	0.297	Nta.8872
auxin.induced-regulated-responsive-activated	at4g27450	Unknown protein	0.42	Nta.801
brassinosteroid.synthesis-degradation.BRs.metabolic regulation	at2g26710	BAS1, CYP734A1, CYP72B1	0.48	Nta.15867
ethylene.synthesis-degradation.1-amino cyclopropane-1-carboxylate synthase	at5g65800	ACS5, CIN5, ETO2, ATACS5	0.35	Nta.14789
ethylene.synthesis-degradation.1-amino cyclopropane-1-carboxylate oxidase	at2g19590	ACO1	0.15	Nta.12328
gibberelin.synthesis-degradation.copalyl diphosphate synthase	at4g02780	GA1, ABC33, ATCPS1	0.22	Nta.4073
gibberelin.synthesis-degradation.ent-kaurene synthase	at1g79460	GA2, KS, ATKS	0.38	Nta.23734
gibberelin.synthesis-degradation.ent-kaurenoic acid hydroxylase/oxygenase	at1g05160	CYP88A3, ATKA01, KAO1	0.47	Nta.4075
gibberelin.synthesis-degradation.GA3 oxidase	at1g15550	GA4, ATGA3OX1, GA3OX1	0.33	Nta.3870
gibberelin.synthesis-degradation.GA3 oxidase	at1g80340	GA4H, ATGA3OX2, GA3OX2	0.18	Nta.9957
gibberelin.induced-regulated-responsive-activated	at2g14900	GASA7	0.23	Nta.3268

Supplementary Table S2. Primers used in this work

Sequence (5'-3')

For transgenic plants

<i>NiSVP</i> RNAi-F	GTCATAGAGA GAAAG
<i>NiSVP</i> RNAi-R	GCCAAACATG AATCTGA
<i>NiSVP</i> OE-F	CGATGAGCTCATGGCTAGAGAGAAAATT
<i>NiSVP</i> OE-R	CGTAGGTACCTCAACCTGAGTAGGGCAG

For RACE

<i>NiSVP</i> gstWR	TTTCTCTATGACGCGGGTCAATCCAG
<i>NiSVP</i> -F1	TCCAATAATGGATAACAACAACCCAG
<i>NiSVP</i> -F2	GCAGCAGTATTAGTAGCAGTTGAAT
Gene Racer 5' primer	CGACTGGAGCACGAGGACACTGA

For TAIL-PCR

<i>NiBPL</i> -SP1	CCGCTTGTTCTCCATAAATAG
<i>NiBPL</i> -SP2	TATGGGATGTACCACTGCTTG
<i>NiBPL</i> -SP3	GATGAGTTCTCCCCAGTTGAT

For real-time PCR

<i>NiACTIN9</i> -F	CTATTCTCCGCTTTGGACTTGGCA
<i>NiACTIN9</i> -R	AGGACCTCAGGACAACGGAAACG
<i>NiSVP</i> -F	TGTGATGCTGATGTTGCTCTC
<i>NiSTMADS11</i> -F	AGAGCTGCAAGGACTTGGCTT
<i>NiSTMADS11</i> -R	TGATGGAGTCTGCGGAATGTC
<i>NTH20</i> -Fw	TACAATCAACTGGGGGAGAAC
<i>NTH20</i> -Rv	CTACGAATAATCGAAGGGTA
<i>NTH22</i> -F	AGTTTGGGAGTCACCTAAGTAC
<i>NTH22</i> -R	AGCTAACTGCATATTCTCTGAC
<i>NiSVP</i> -R	GTCTTTCTCCCCTCATTTGTC
<i>NiBPL</i> -F	AGCAGTGGTACATCCCATAGT
<i>NiBPL</i> -R	CTCACTTCAAACCTTGCCGT
<i>NiCPS</i> -F	TCAAAGAGAAGATGGATCTTGGG
<i>NiCPS</i> -R	AGGTGAAATCTGATCCTTGTATC
<i>NiKS</i> -F	GTCATGGGCCAAGAATGGTG
<i>NiKS</i> -R	CGCTGAAAATATGATGCCAC
<i>NiKAO</i> -F	TGGGGATGGTATAACCATTAATCAT
<i>NiKAO</i> -R	TGAATCTTCTGGTCATATTACATG
<i>NiGA3ox1</i> -F	GCCTTTTTTCGCTTCCAATGC
<i>NiGA3ox1</i> -R	GCTTTTCCATTTCCCTTTCGT
<i>NiGA3ox2</i> -F	GAGGAAGCTGGAAAACGACTT
<i>NiGA3ox2</i> -R	AGCATCAGCCACATCAGTCTT
<i>NiAS1</i> -F	AGAGGAGCAGCGTCTTGTAT
<i>NiAS1</i> -R	GACACTCCGCTCTTTCACAAT
<i>NiAS2</i> -F	TCTACCGTGGCTGATCCATAC
<i>NiAS2</i> -R	AGAAACCTCGAACCATCCTTG
<i>NiER</i> -F	AAACAGCTGGAACCTGTAAC
<i>NiER</i> -R	CGCACATCAAAAATACCACAGG
<i>NiCRM</i> -F	CTGTTTCAGGCTAATGTGGTAT
<i>NiCRM</i> -R	GGGATGATGGACAAAACAAGAG
<i>NiYUCC5</i> -F	AGTTTCTCAGATAACGATTTCTTTG
<i>NiYUCC5</i> -R	GTCTCTTTGTAGGGTACTCAGGGTAG

For yeast one hybrid

<i>NiSVP</i> -F	<u>aagcagtgatcaacgcagagtgccattatgccccATGGCTAGAGAGAAAATTCAGATCA</u>
<i>NiSVP</i> -R	<u>AGATCTCCGGCTCCGCGGCTGTACACCTGAGTAGGGCAGCCCCAAGCTTG</u>
CArG-box	<u>GAGCTCGCAATTAAGACGCTATTAAGACGCTATTAAGACCTCGA</u>
CArG-box(m)	<u>GAGCTCGCAAGGAAAAGACGCTATTAAGACGCTATTAAGACCTCGA</u>

Probes for EMSA

mu-pro <i>NiBPL</i> (+)	actcaatTTTTctatGGATGCATCCaaactaaactttcca
mu-pro <i>NiBPL</i> (-)	TGGAAAAGTTAGTTTGGATGCATCCATAGAAAAATTGAGT
wt-pro <i>NiBPL</i> (+)	ACTCAATTTT TCTATCAATT ATAAGAACT AACTTTTCCA
wt-pro <i>NiBPL</i> (-)	TGGAAAAGTTAGTTTCTTATAATTGATAGAAAAATTGAGT
BamHI- <i>NiSVP</i> Fw	CGAagatccATGGCTAGAGAGAAAATTCAG
XhoI- <i>NiSVP</i> Rv	CGCctcgagACCTGAGTAGGGCAGCCC

For dual-luciferase assay

<i>Sal</i> I-pro <i>NiBPL</i> -Fw	caGTCGACgcttegettctctatcaatc
Bam HI-pro <i>NiBPL</i> -Rv	cgGGATCCCTAATTACCCTAACAAGATATGGT
m1CArG-R	TACCTTGAAAAGTTAGTTTATTATAATTATAGAAAAATTGAG
m1CArG-F	ACTCAATTTTCTATAAATTATAATAAACTAACTTTTCCAAGGT
m2CArG-R	ACCTTGAAAAGTTAGTTTATTAGCATTTATAGAAAAATTGAGT
m2CArG-F	ACTCAATTTTCTATAAATGCTAATAAACTAACTTTTCCAAGGT