

Supplemental Figures

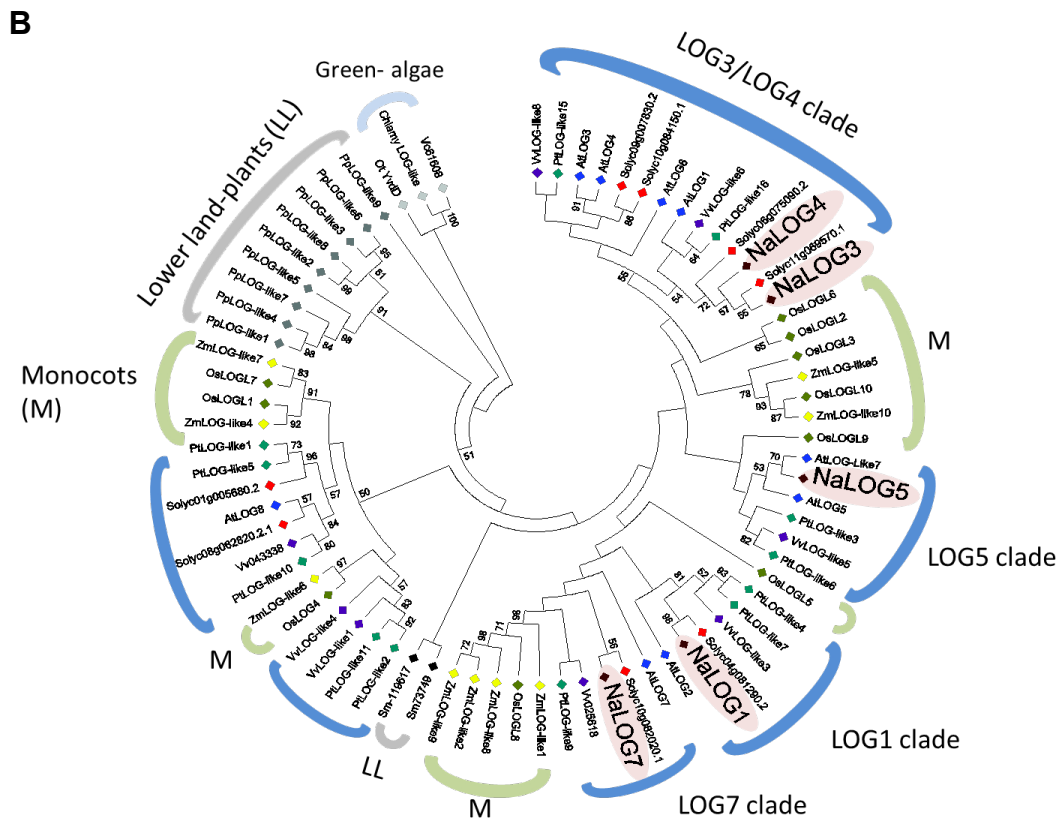
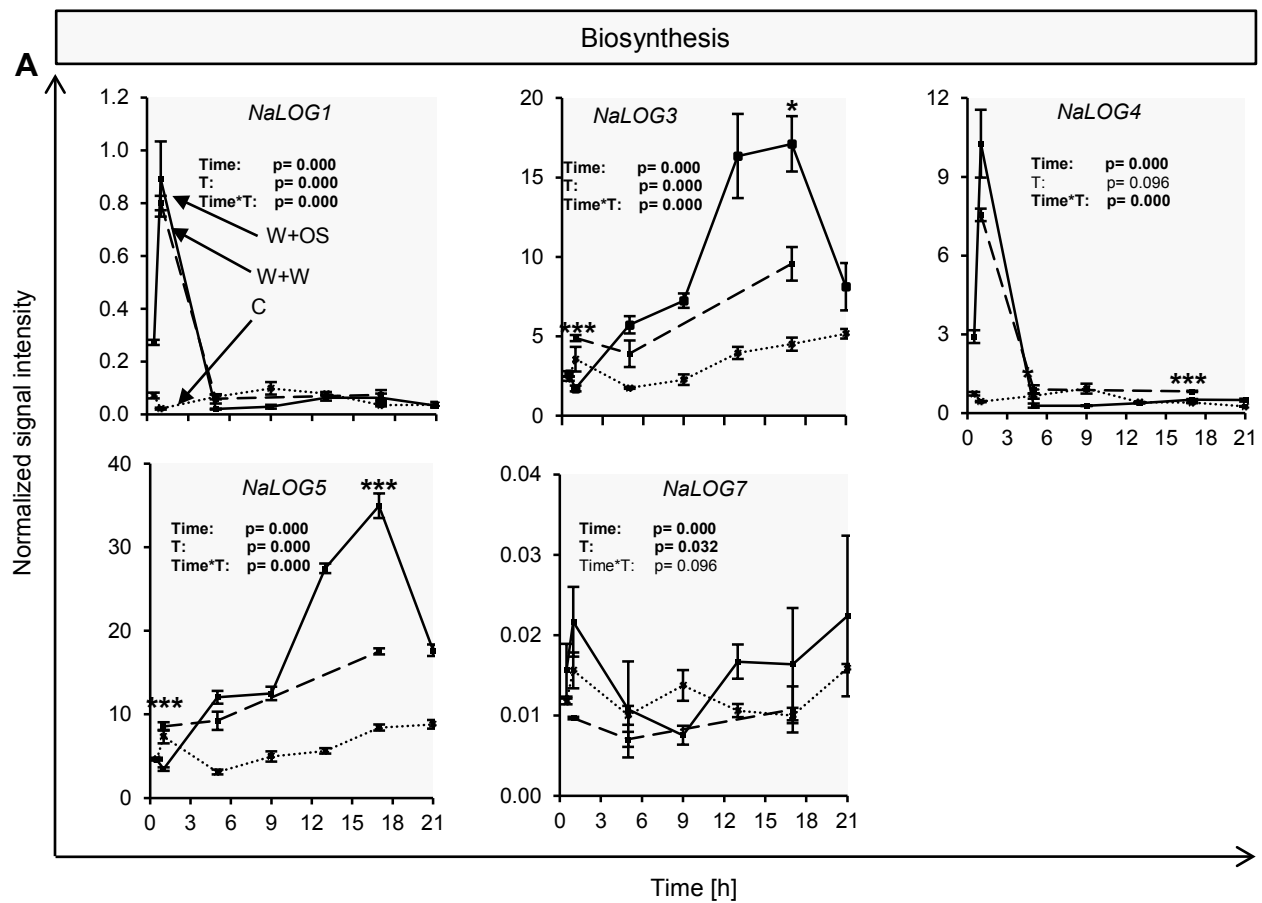


Figure S1. Wounding and herbivory regulate the expression of LOG-family genes.

(A) Relative transcript levels of cytokinin nucleoside 5' -monophosphate phosphoribohydrolases (LOG) were measured in leaves of *N. attenuata* at different time points after wounding and application of water (W+W; dashed line; 1, 5 and 17 h) or *M. sexta* oral secretions (W+OS; solid line; 0.5, 1, 5, 9, 13, 17 and 21 h) to the puncture wounds, as well as in untreated control leaves (C; dotted line; 0.5, 1, 5, 9, 13, 17 and 21 h). Time and treatment (C and W+OS; T) effects and their interaction (Time*T) were analyzed by univariate ANOVA, except for *NaLOG3*, *NaLOG4* and *NaLOG7* data which were analyzed by a generalized least squares model. Asterisks indicate significant differences between W+W and W+OS-treated samples at the same time point (independent samples *t* test: * $P \leq 0.05$, *** $P \leq 0.001$). Error bars are standard errors (N=3). Data are obtained from microarray kinetic analysis.

(B) Phylogenetic analysis of these cytokinin related genes and their homologs in the following species groups (in parentheses the abbreviations and label colors): green algae [*Volvox carteri* (Vc), *Ostreococcus tauri* (Ot), *Chlamydomonas reinhardtii* (Chlamy); all in grey]; lower-land plants [*Physcomitrella patens* (Pp, dark grey) and *Selaginella mollendorffii* (Sm, black)]; monocots [*Oryza sativa* cv. *Japonica* (Os, light green) and *Zea mays* (Zm, yellow)] and dicots [*Vitis vinifera* (Vv, purple), *Solanum lycopersicum* (Solyc, red), *Arabidopsis thaliana* (At, blue) and *Populus trichocarpa* (Pt, dark green)]. For *N. attenuata* we used the abbreviation Na the label color brown and light red shading.

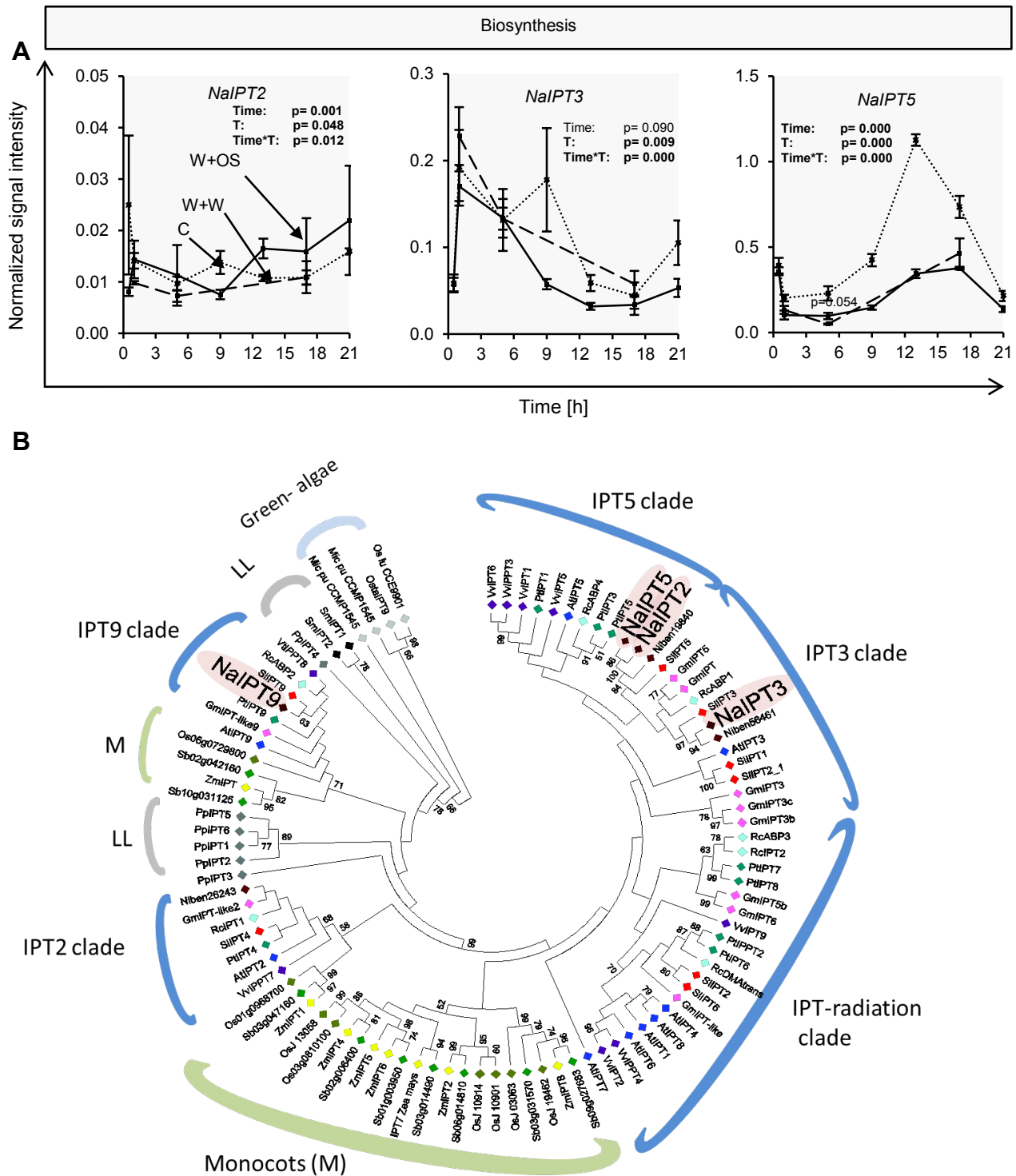


Figure S2. Wounding and herbivory regulate the expression of IPT-family genes.

(A) Relative transcript levels of isopentenyltransferases (IPT) were measured in leaves of *N. attenuata* at different time points after wounding and application of water (W+W; dashed line; 1, 5 and 17 h) or *M. sexta* oral secretions (W+OS; solid line; 0.5, 1, 5, 9, 13,

17 and 21 h) to the puncture wounds, as well as in untreated control leaves (C; dotted line; 0.5, 1, 5, 9, 13, 17 and 21 h). Time and treatment (C and W+OS; T) effects and their interaction (Time*T) were analyzed by univariate ANOVA, except for *NaIPT1* data which were analyzed by a generalized least squares model. Error bars are standard errors (N=3). Data are obtained from microarray kinetic analysis.

(B) Phylogenetic analysis of these cytokinin related genes and their homologs in the following species groups (in parentheses the abbreviations and label colors): green algae [*Micromonas pusilla* (MicPu), *Ostreococcus lucimarinus* (Oslu) and *Ostreococcus tauri* (Osta), all in grey]; lower-land plants [*Physcomitrella patens* (Pp, dark grey) and *Selaginella mollendorffii* (Sm, black)]; monocots [*Oryza sativa* cv. *Japonica* (Os, light green), *Sorghum bicolor* (Sb, bright green) and *Zea mays* (Zm, yellow)] and dicots [*Vitis vinifera* (Vv, purple), *Solanum lycopersicum* (Sl, red), *Arabidopsis thaliana* (At, blue), *Glycine max* (Gm, pink), *Ricinus communis* (Rc, light blue) and *Populus trichocarpa* (Pt, dark green)]. For *N. attenuata* we used the abbreviation Na(light brown, light red shading) and for *N. benthamiana* Niben(dark brown).

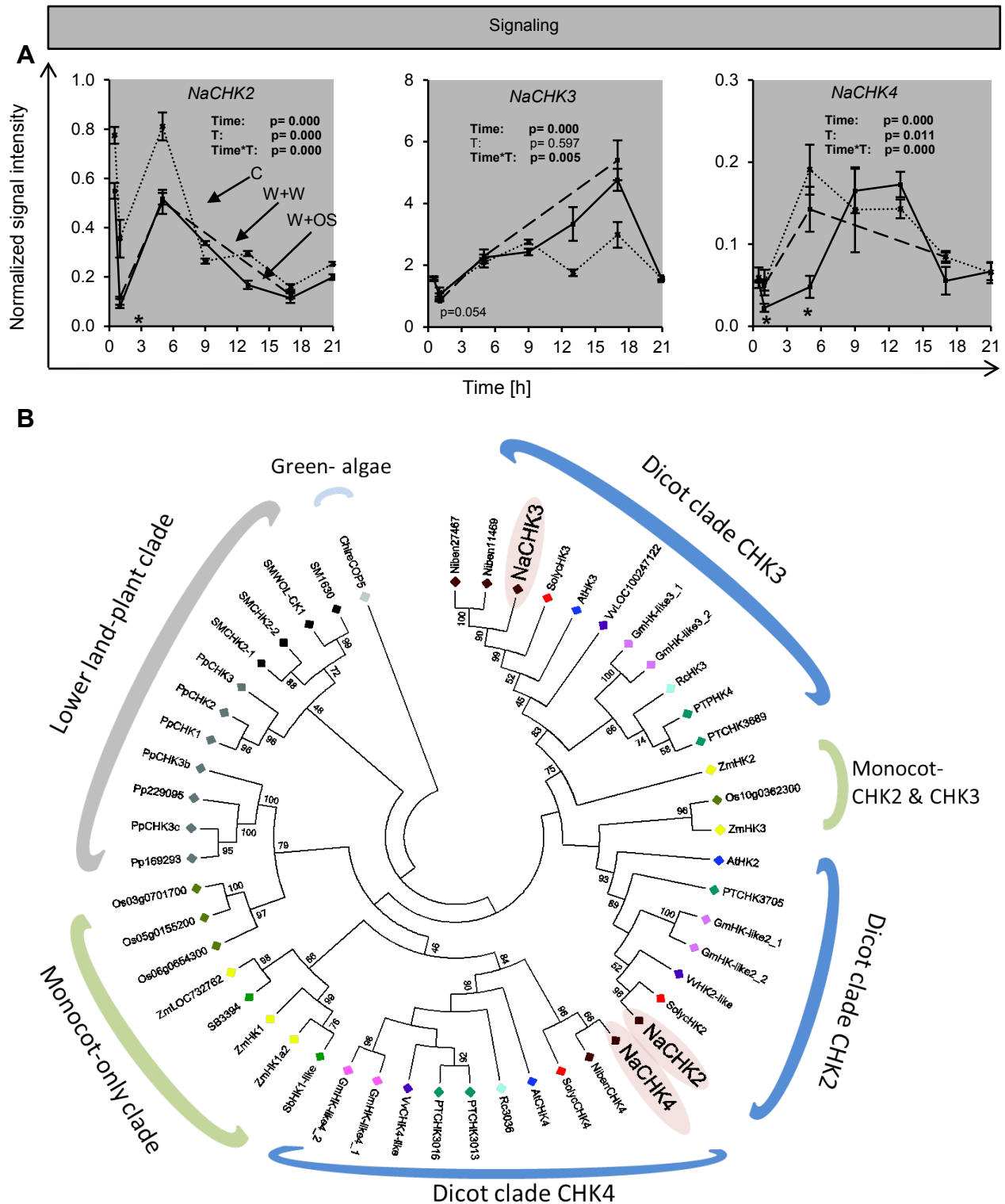


Figure S3. Wounding and herbivory regulate the expression of cytokinin receptor genes.

(A) Relative transcript levels of the corresponding histidine kinases were measured in leaves of *N. attenuata* at different time points after wounding and application of water

(W+W; dashed line; 1, 5 and 17 h) or *M. sexta* oral secretions (W+OS; solid line; 0.5, 1, 5, 9, 13, 17 and 21 h) to the puncture wounds, as well as in untreated control leaves (C; dotted line; 0.5, 1, 5, 9, 13, 17 and 21 h). Time and treatment (C and W+OS; T) effects and their interaction (Time*T) were analyzed by univariate ANOVA, except for *NaCHK2* and *NaCHK3* data which were analyzed by a generalized least squares model. Asterisks indicate significant differences between W+W and W+OS-treated samples at the same time point (independent samples *t* test: * $P \leq 0.05$). Error bars are standard errors (N=3). Data are obtained from microarray kinetic analysis.

(B) Phylogenetic analysis of these cytokinin related genes and their homologs in the following species groups (in parentheses the abbreviations and label colors): the green alga *Chlamydomonas reinhardtii* (Chlre), (light grey); lower-land plants [*Physcomitrella patens* (Pp, dark grey) and *Selaginella mollendorffii* (Sm, black)]; monocots [*Oryza sativa* cv. *Japonica* (Os, light green), *Sorghum bicolor* (Sb, bright green) and *Zea mays* (Zm, yellow)] and dicots [*Vitis vinifera* (Vv, purple), *Solanum lycopersicum* (Solyc, red), *Arabidopsis thaliana* (At, blue), *Glycine max* (Gm, pink), *Ricinus communis* (Rc, light blue) and *Populus trichocarpa* (Pt, dark green)]. For *N. attenuata* we used the abbreviation Na(light brown, light red shading) and for *N. benthamiana* Niben (dark brown).

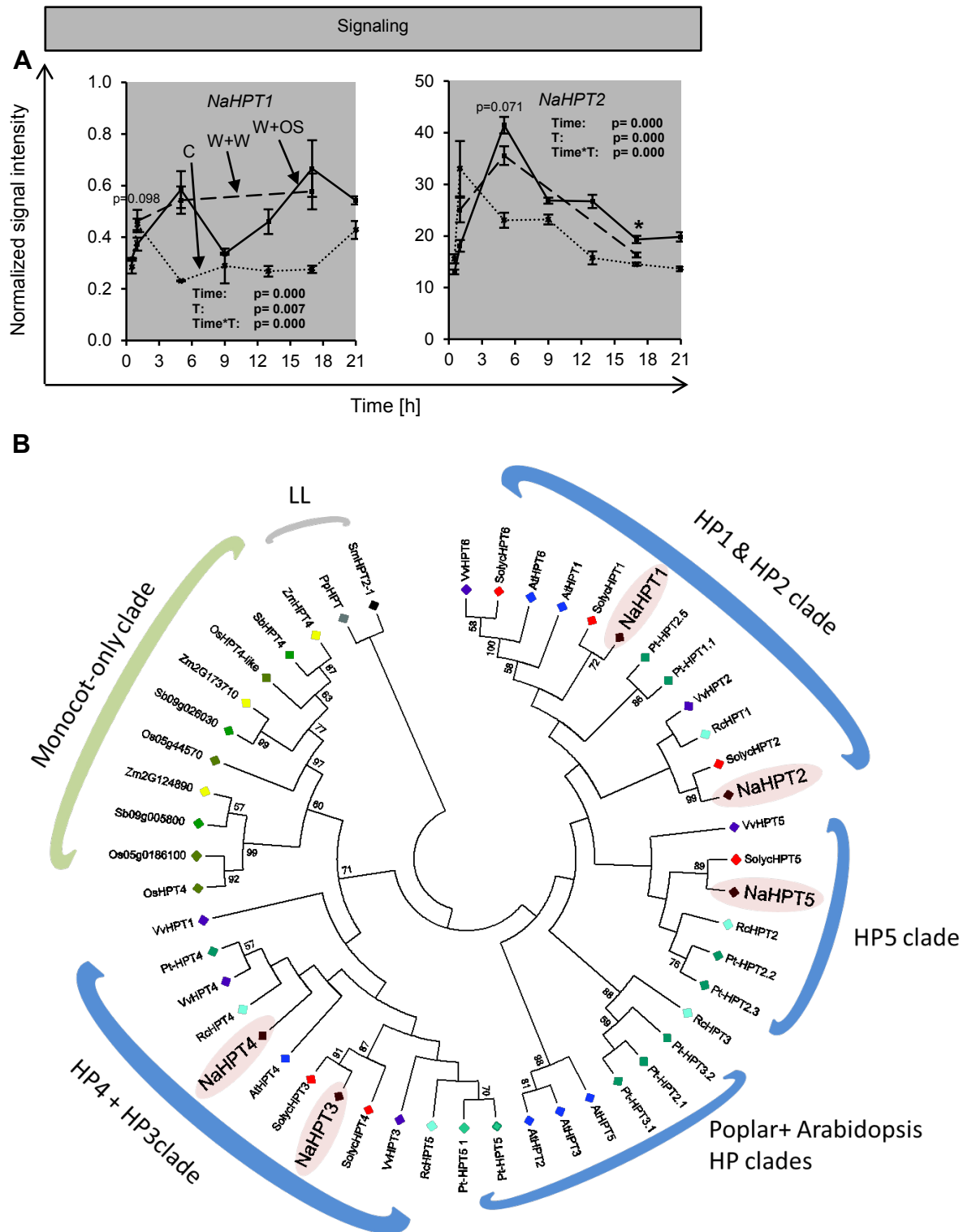
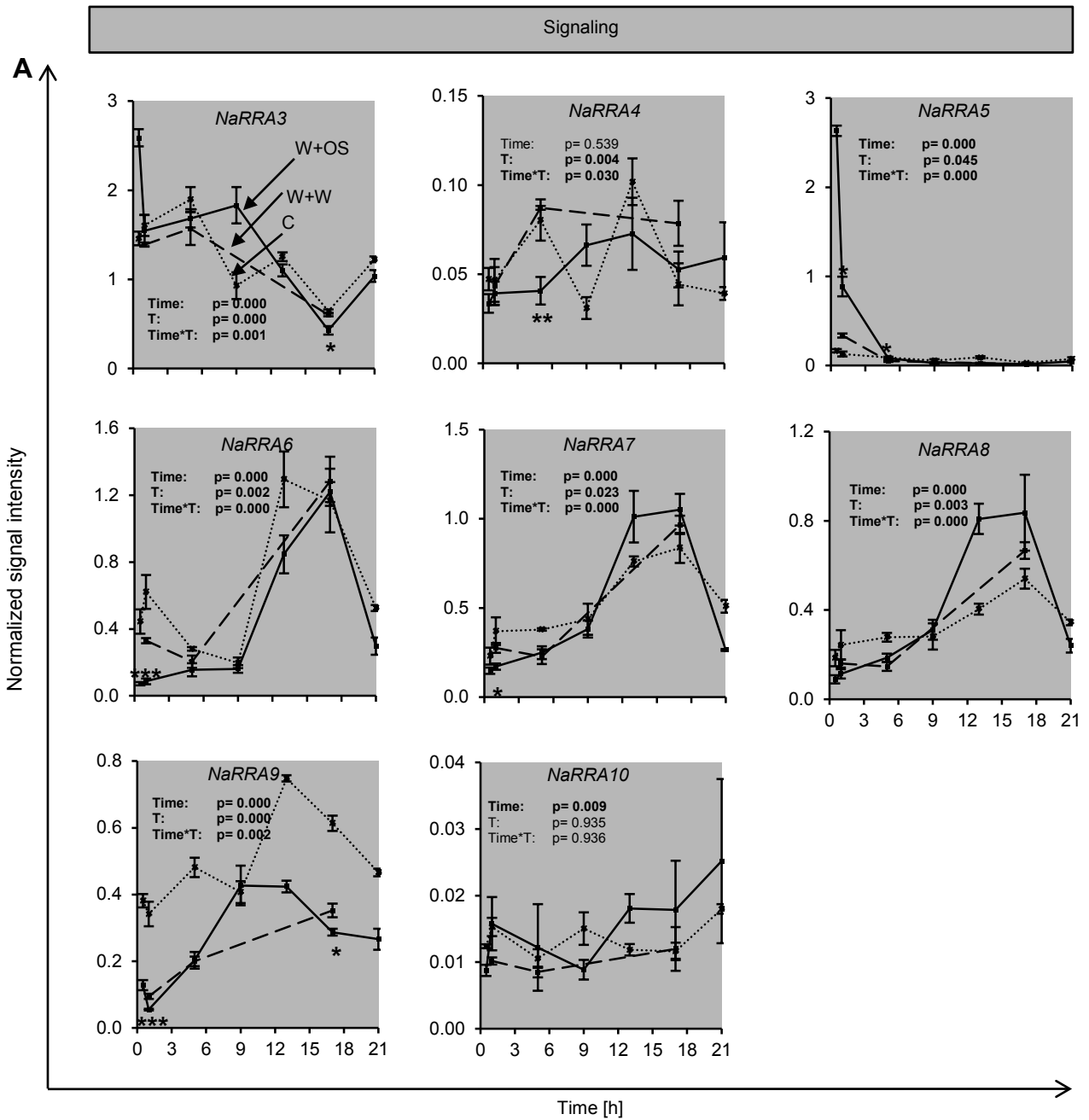


Figure S4. Wounding and herbivory regulate the expression of HPT-family genes. (A) Relative transcript levels of histidine phosphotranfer proteins (HPT) were measured in leaves of *N. attenuata* at different time points after wounding and application of water (W+W; dashed line; 1, 5 and 17 h) or *M. sexta* oral secretions (W+OS; solid line; 0.5, 1,

5, 9, 13, 17 and 21 h) to the puncture wounds, as well as in untreated control leaves (C; dotted line; 0.5, 1, 5, 9, 13, 17 and 21 h). Time and treatment (C and W+OS; T) effects and their interaction (Time*T) were analyzed by a generalized least squares model. Asterisks indicate significant differences between W+W and W+OS-treated samples at the same time point (independent samples *t* test: * $P \leq 0.05$). Error bars are standard errors (N=3). Data are obtained from microarray kinetic analysis.

(B) Phylogenetic analysis of these cytokinin related genes and their homologs in the following groups of species (in parentheses the abbreviations and label colors): lower-land plants [*Physcomitrella patens* (Pp, dark grey) and *Selaginella mollendorffii* (Sm, black)]; monocots [*Oryza sativa* cv. *Japonica* (Os, light green), *Sorghum bicolor* (Sb, bright green) and *Zea mays* (Zm, yellow)] and dicots [*Vitis vinifera* (Vv, purple), *Solanum lycopersicum* (Solyc, red), *Arabidopsis thaliana* (At, blue), *Ricinus communis* (Rc, light blue) and *Populus trichocarpa* (Pt, dark green)]. For *N. attenuata* we used the abbreviation Na (light brown) and light red shading.



B

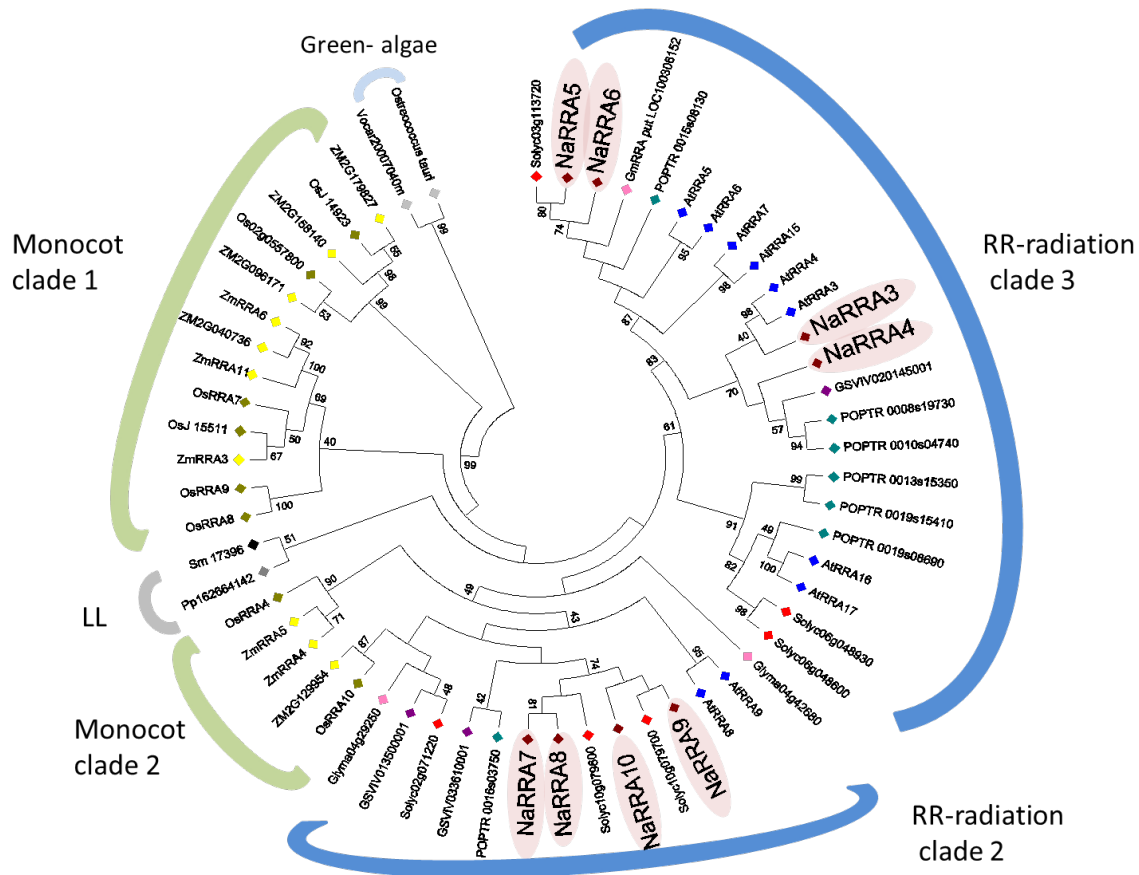


Figure S5. Wounding and herbivory regulate the expression of type-A RR-family genes.

(A) Relative transcript levels of type-A response regulators (RRA) were measured in leaves of *N. attenuata* at different time points after wounding and application of water (W+W; dashed line; 1, 5 and 17 h) or *M. sexta* oral secretions (W+OS; solid line; 0.5, 1, 5, 9, 13, 17 and 21 h) to the puncture wounds, as well as in untreated control leaves (C; dotted line; 0.5, 1, 5, 9, 13, 17 and 21 h). Time and treatment (C and W+OS; T) effects and their interaction (Time*T) were analyzed by univariate ANOVA, except for *NaARRA3*, *NaARRA5*, *NaARRA6*, *NaARRA7*, *NaARRA8* and *NaARRA9* data which were analyzed by a generalized least squares model. Asterisks indicate significant differences between W+W and W+OS-treated samples at the same time point (independent samples *t* test: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$). Error bars are standard errors (N=3). Data are obtained from microarray kinetic analysis.

(B) Phylogenetic analysis of these cytokinin related genes and their homologs in the following species groups (in parentheses the abbreviations and label colors): green algae [*Volvox carteri* (Vocar) and *Ostreococcus tauri*; all in grey]; lower-land plants [*Physcomitrella patens* (Pp, dark grey) and *Selaginella mollendorffii* (Sm, black)]; monocots [*Oryza sativa* cv. *Japonica* (Os, light green) and *Zea mays* (Zm, yellow)] and dicots [*Vitis vinifera* (GSV, purple), *Solanum lycopersicum* (Solyc, red), *Arabidopsis thaliana* (At, blue), *Glycine max* (Gm/Glyma, pink) and *Populus trichocarpa* (POPTR, dark green)]. For *N. attenuata* we used the abbreviation Na (light brown) and light red shading.

Figure S6. Wounding and herbivory regulate the expression of type-B RR-family genes.

(A) Relative transcript levels of type-B response regulators (RRB) were measured in leaves of *N. attenuata* at different time points after wounding and application of water (W+W; dashed line; 1, 5 and 17 h) or *M. sexta* oral secretions (W+OS; solid line; 0.5, 1, 5, 9, 13, 17 and 21 h) to the puncture wounds, as well as in untreated control leaves (C; dotted line; 0.5, 1, 5, 9, 13, 17 and 21 h). Time and treatment (C and W+OS; T) effects and their interaction (Time*T) were analyzed by univariate ANOVA, except for *NaRRB2* and *NaRRB2b* data which were analyzed by a generalized least squares model.

Asterisks indicate significant differences between W+W and W+OS-treated samples at the same time point (independent samples *t* test: ** $P \leq 0.01$). Error bars are standard errors (N=3). Data are obtained from microarray kinetic analysis.

(B) Phylogenetic analysis of these cytokinin related genes and their homologs in the following species (in parentheses the abbreviations and label colors): green algae [*Volvox carteri* (Vc), *Ostreococcus lucimarinus* (Oslu), *Ostreococcus tauri* (Osta), *Micromonas pusilla* (MicPu), all in grey]; lower-land plants [*Physcomitrella patens* (Pp, dark grey), *Selaginella mollendorffii* (Sm, black)]; monocots [*Oryza sativa* cv. *Japonica* (Os, light green), *Sorghum bicolor* (Sb, bright green) and *Zea mays* (Zm, yellow)] and dicots [*Vitis vinifera* (Vv, purple), *Arabidopsis thaliana* (At, blue), *Glycine max* (Gm, pink), *Ricinus communis* (Rc, light blue) and *Populus trichocarpa* (Pt, dark green)]. For *N. attenuata* we used the abbreviation Na(light brown) and for *N. tabacum* Nt (dark brown).

application of water (W+W; dashed line; 1, 5 and 17 h) or *M. sexta* oral secretions (W+OS; solid line; 0.5, 1, 5, 9, 13, 17 and 21 h) to the puncture wounds, as well as in untreated control leaves (C; dotted line; 0.5, 1, 5, 9, 13, 17 and 21 h). Time and treatment (C and W+OS; T) effects and their interaction (Time*T) were analyzed by univariate ANOVA, except for *NaCKX5* and *NaCKX7* data which were analyzed by a generalized least squares model. Asterisks indicate significant differences between W+W and W+OS-treated samples at the same time point (independent samples *t* test: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$). Error bars are standard errors (N=3). Data are obtained from microarray kinetic analysis.

(B) Phylogenetic analysis of these cytokinin related genes and their homologs in the following species (in parentheses the abbreviations and label colors): monocots [*Oryza sativa* cv. *Japonica* (Os, light green) and *Zea mays* (Zm, yellow)] and dicots [*Solanum lycopersicum* (Solyc, red), *Arabidopsis thaliana* (At, blue), *N. attenuata* (Na, light brown) and *N. benthamiana* (Niben, dark brown)]. The outgroup is a bacterial species (*Rhodococcus fascians*), as no homolog was found in another green plant outside flowering plants.

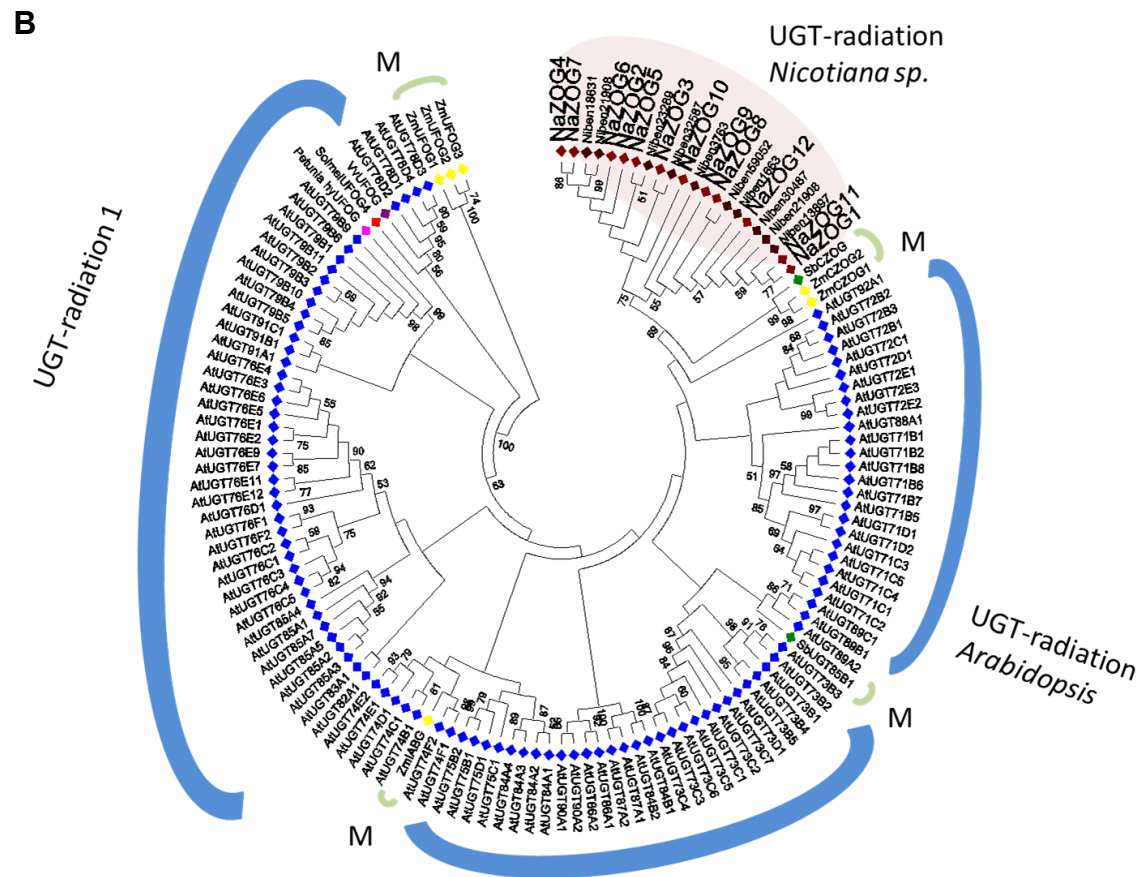
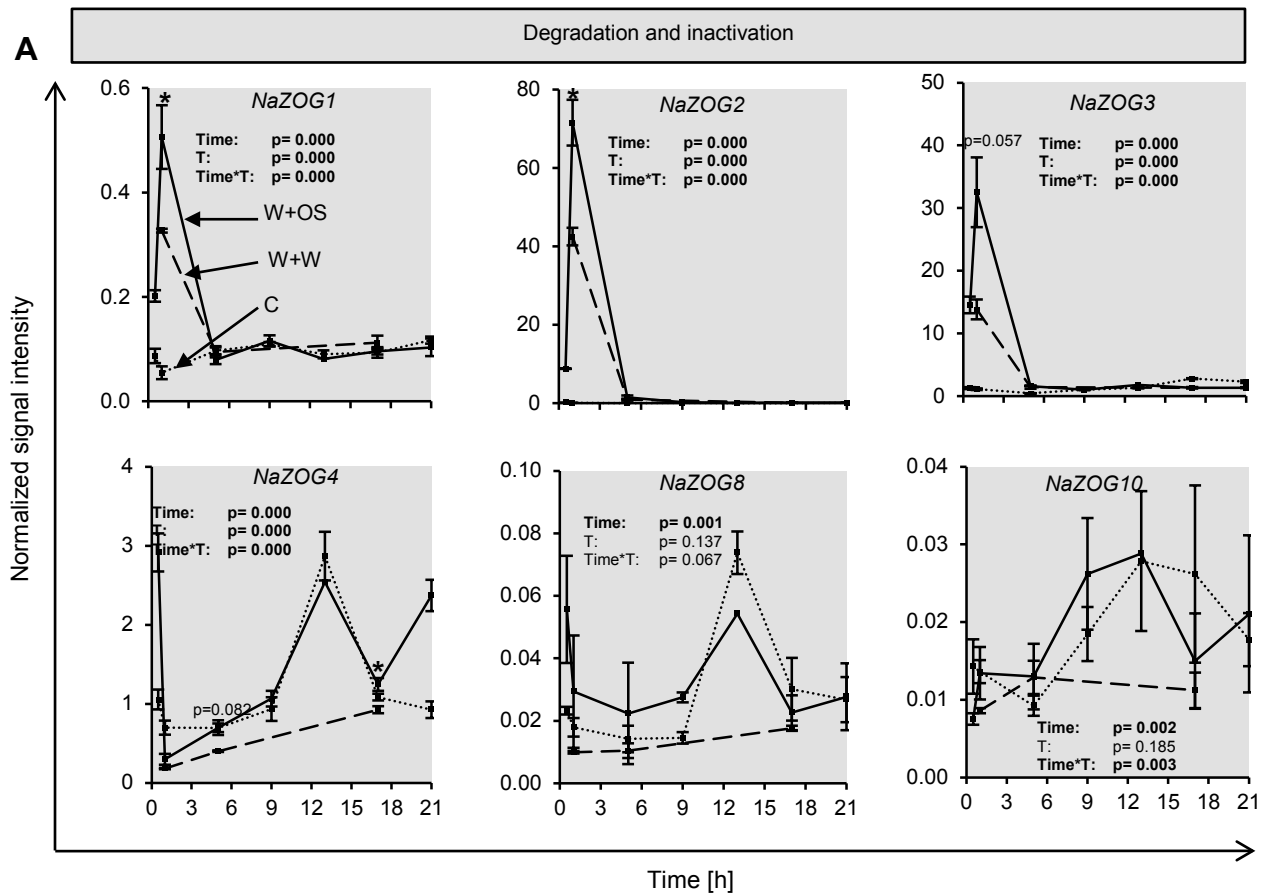


Figure S8. Wounding and herbivory regulate the expression of ZOG-family genes.

(A) Relative transcript levels of zeatin O-glucosyltransferase (ZOG) were measured in leaves of *N. attenuata* at different time points after wounding and application of water (W+W; dashed line; 1, 5 and 17 h) or *M. sexta* oral secretions (W+OS; solid line; 0.5, 1, 5, 9, 13, 17 and 21 h) to the puncture wounds, as well as in untreated control leaves (C; dotted line; 0.5, 1, 5, 9, 13, 17 and 21 h). Time and treatment (C and W+OS; T) effects and their interaction (Time*T) were analyzed by univariate ANOVA, except for *NaZOG2*, *NaZOG8* and *NaZOG10* data which were analyzed by a generalized least squares model. Asterisks indicate significant differences between W+W and W+OS-treated samples at the same time point (independent samples *t* test: * $P \leq 0.05$). Error bars are standard errors (N=3). Data are obtained from microarray kinetic analysis. Only expression data of genes that are differentially regulated by treatments are presented.

(B) Phylogenetic analysis of these cytokinin related genes and their homologs in the following species (in parentheses the abbreviations and label colors): Monocots [*Sorghum bicolor* (Sb, bright green) and *Zea mays* (Zm, yellow)], dicots [*Vitis vinifera* (Vv, purple), *Solanum spp.* (Sol, red), *Arabidopsis thaliana* (At, blue) and *Petunia spp.* (pink)]. For *N. attenuata* we used the abbreviation Na(light brown), for *N. benthamiana* Niben(dark brown). No ZOG genes were identified in either lower-land plants or green algae.

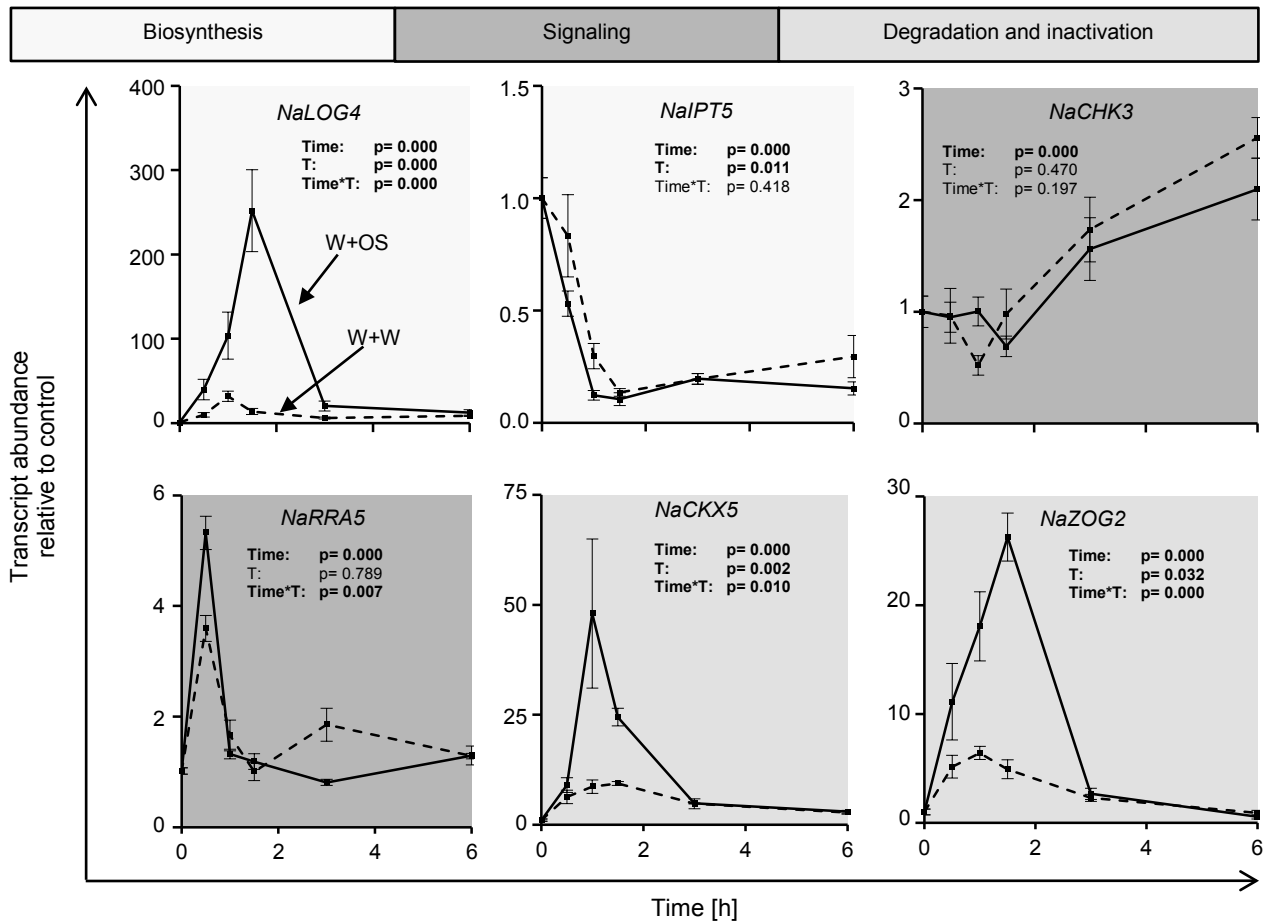


Figure S9. Confirmation of microarray expression data.

Relative transcript levels of cytokinin-related genes were measured in leaves of *N. attenuata* at different time points after wounding and application of water (W+W, dashed line) or *M. sexta* oral secretions (W+OS, solid line) to the puncture wounds by quantitative PCR. Time and treatment (C, W+W and W+OS; T) effects and their interaction (Time*T) were analyzed by univariate ANOVA, except for *NaRRA5* and *NaZOG2* data which were analyzed by a generalized least squares model. Error bars are standard errors ($N \geq 3$).

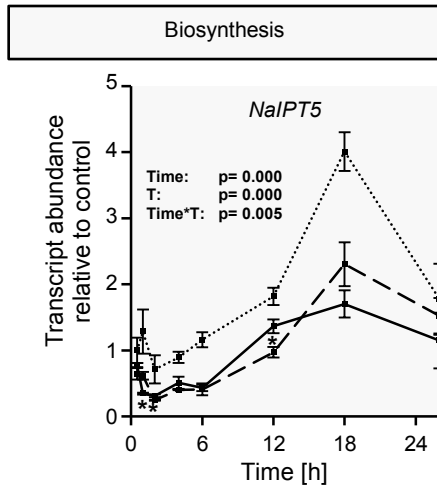


Figure S10. Confirmation of *NaIPT5* microarray data.

Relative transcript levels of isopentenyltransferases 5 (*NaIPT5*) were measured in leaves of *N. attenuata* at different time points (0.5, 1, 2, 4, 6, 12, 18 and 26 h) after wounding and application of water (W+W, dashed line) or *M. sexta* oral secretions (W+OS, solid line) to the puncture wounds, as well as in untreated control leaves (C; dotted line) by quantitative PCR. Time and treatment (C and W+OS; T) effects and their interaction (Time*T) were analyzed by a generalized least squares model. Asterisks indicate significant differences between W+W and W+OS-treated samples at the same time point (independent samples *t* test: * $P \leq 0.05$). Samples from five plants per time and treatment were pooled and three technical replicates were measured. Error bars are standard errors (N=3).

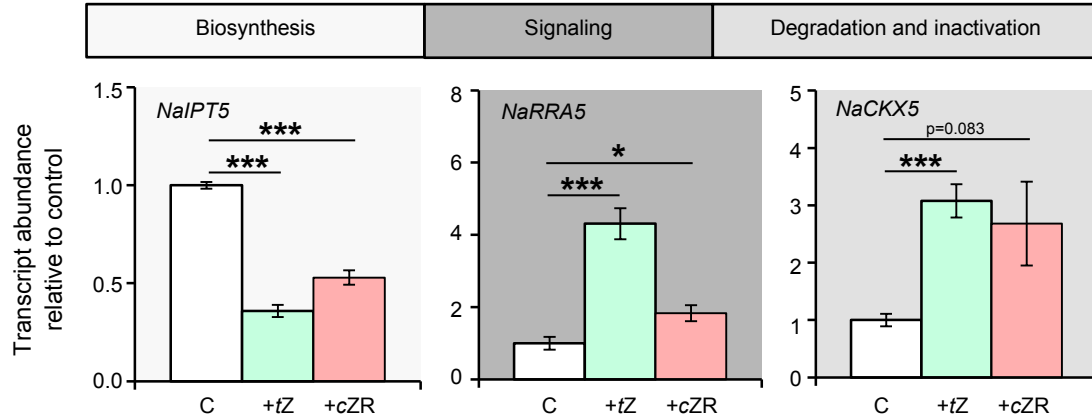


Figure S11. Cytokinin-spraying changes *NaIPT5*, *NaRRA5* and *NaCKX5* transcript accumulation.

Relative transcript accumulations of *NaIPT5*, *NaRRA5* and *NaCKX5* were measured in leaves of *N. attenuata* after three days of *trans*-zeatin (*tZ*; 1 μ M) or *cis*-zeatin riboside (*cZR*; 5 μ M)-spraying or spraying of the buffer control (C). Spraying was performed three times per day.

Asterisks indicate significant differences between *tZ/cZR*-sprayed samples compared to the control treatment (independent samples *t* test: * $P \leq 0.05$, *** $P \leq 0.001$). Error bars are standard errors (N=5).

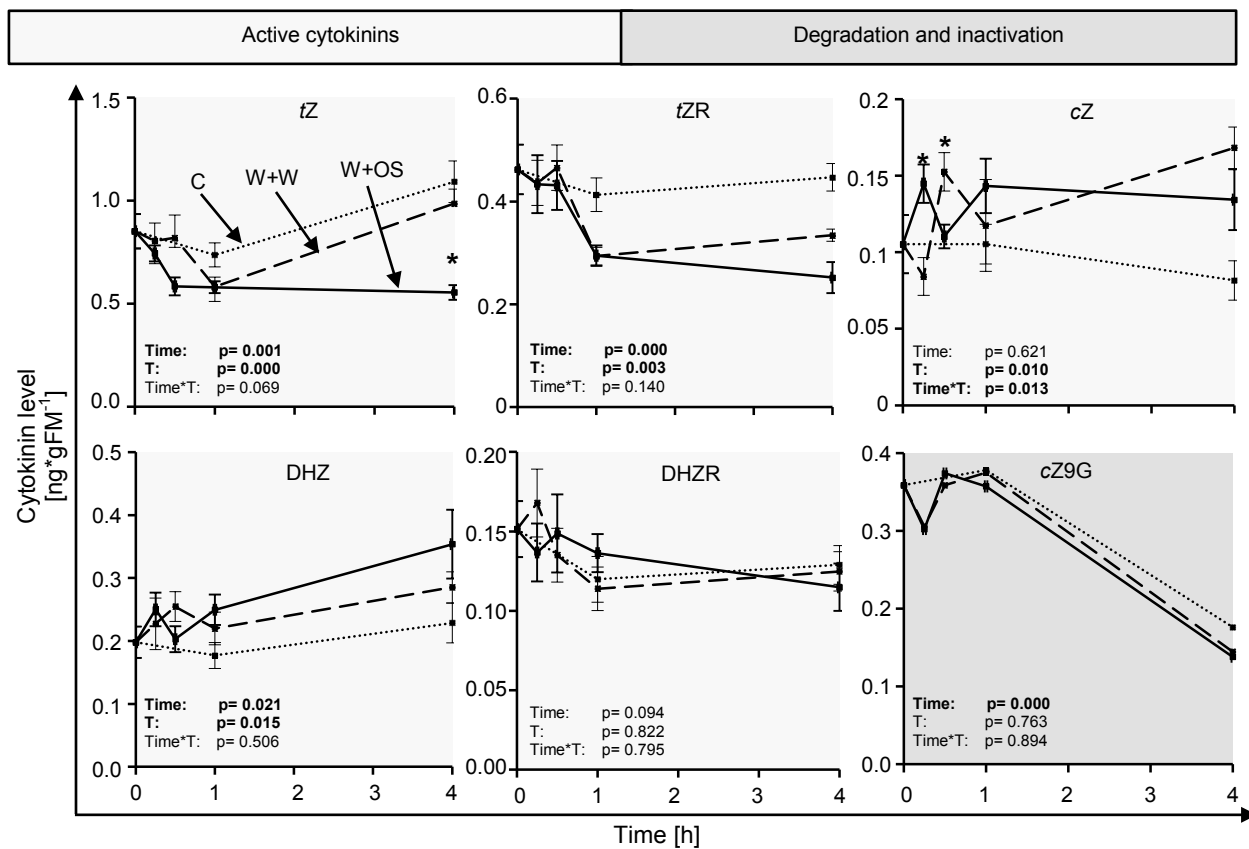


Figure S12. Wounding and herbivory-induced changes in cytokinin levels.

trans-Zeatin (*tZ*), *trans*-zeatin riboside (*tZR*), *cis*-Zeatin (*cZ*), dihydrozeatin (DHZ), dihydrozeatin riboside (DHZR) and *cis*-zeatin 9-glucoside (*cZ9G*) levels in leaves of *N. attenuata* at different time points after wounding and application of water (W+W, dashed line) or *M. sexta* oral secretions (W+OS, solid line) to the puncture wounds, as well as in untreated control leaves (C, dotted line). Time and treatment (C, W+W and W+OS; T) effects and their interaction (Time*T) were analyzed by univariate ANOVA, except for *tZR* and *cZ9G* data which were analyzed by a generalized least squares model. Asterisks indicate significant differences between W+W and W+OS-treated samples at the same time point (independent samples *t* test: * $P \leq 0.05$). Error bars are standard errors (N=5). FM, fresh mass.

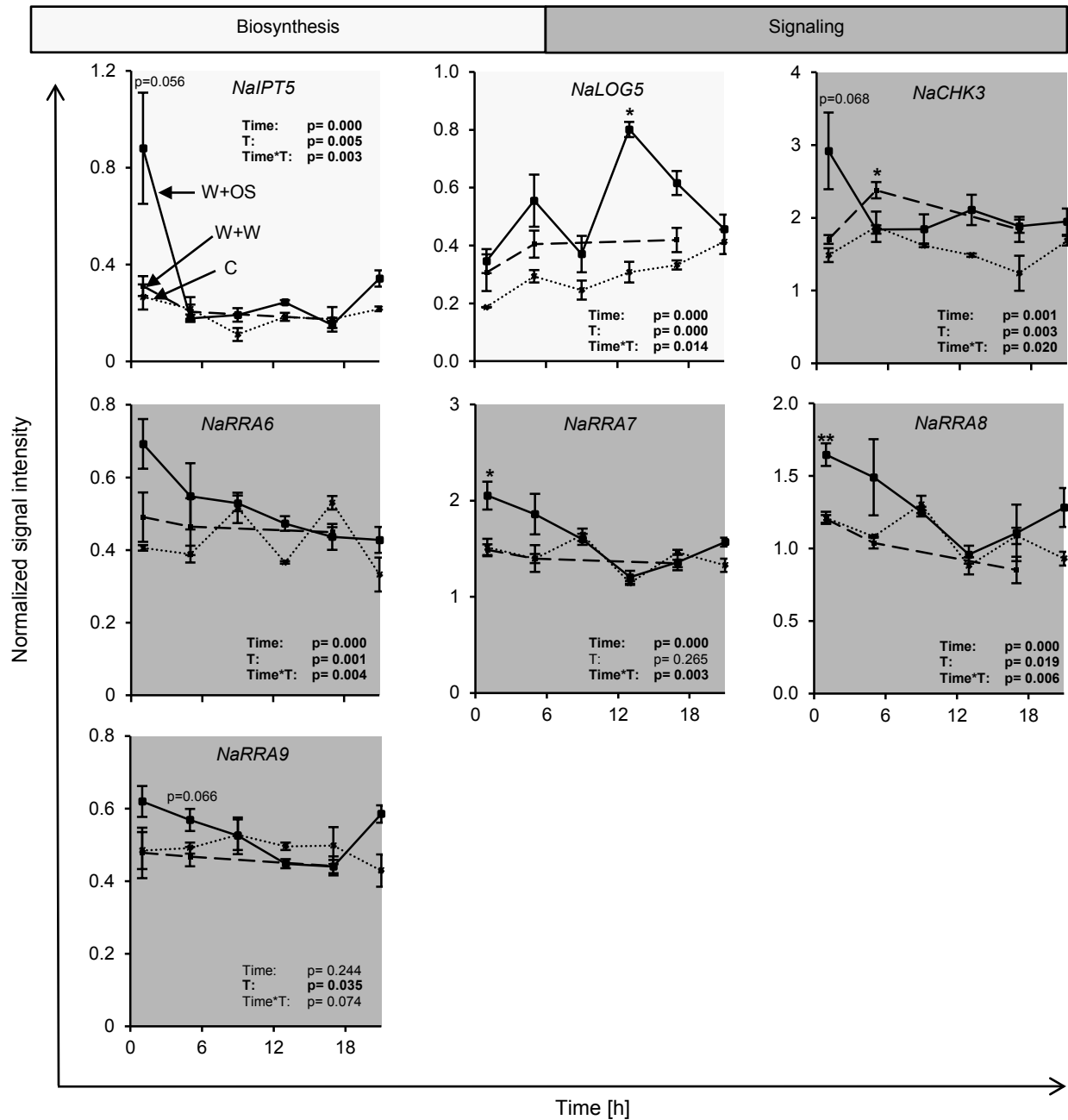


Figure S13. Wounding and herbivory regulate transcript accumulation of cytokinin-related genes in the root.

Transcript accumulation was measured in roots and systemic leaves of *N. attenuata* at different time points after wounding and application of water (W+W; dashed line; 1, 5 and 17 h) or *M. sexta* oral secretions (W+OS; solid line; 1, 5, 9, 13, 17 and 21 h) to the puncture wounds, as well as in untreated control leaves (C; dotted line; 1, 5, 9, 13, 17 and 21 h). Data are obtained from kinetic analysis conducted with microarrays.

Time and treatment (C and W+OS; T) effects and their interaction (Time*T) were analyzed for *NaRRA9* by univariate ANOVA; the other transcript data were analyzed by a generalized least squares model. Asterisks indicate significant differences between W+W and W+OS-treated samples at the same time point (independent samples *t* test: * $P \leq 0.05$, ** $P \leq 0.01$). Error bars are standard errors (N=3).

Biosynthesis	Signaling	Degradation and inactivation
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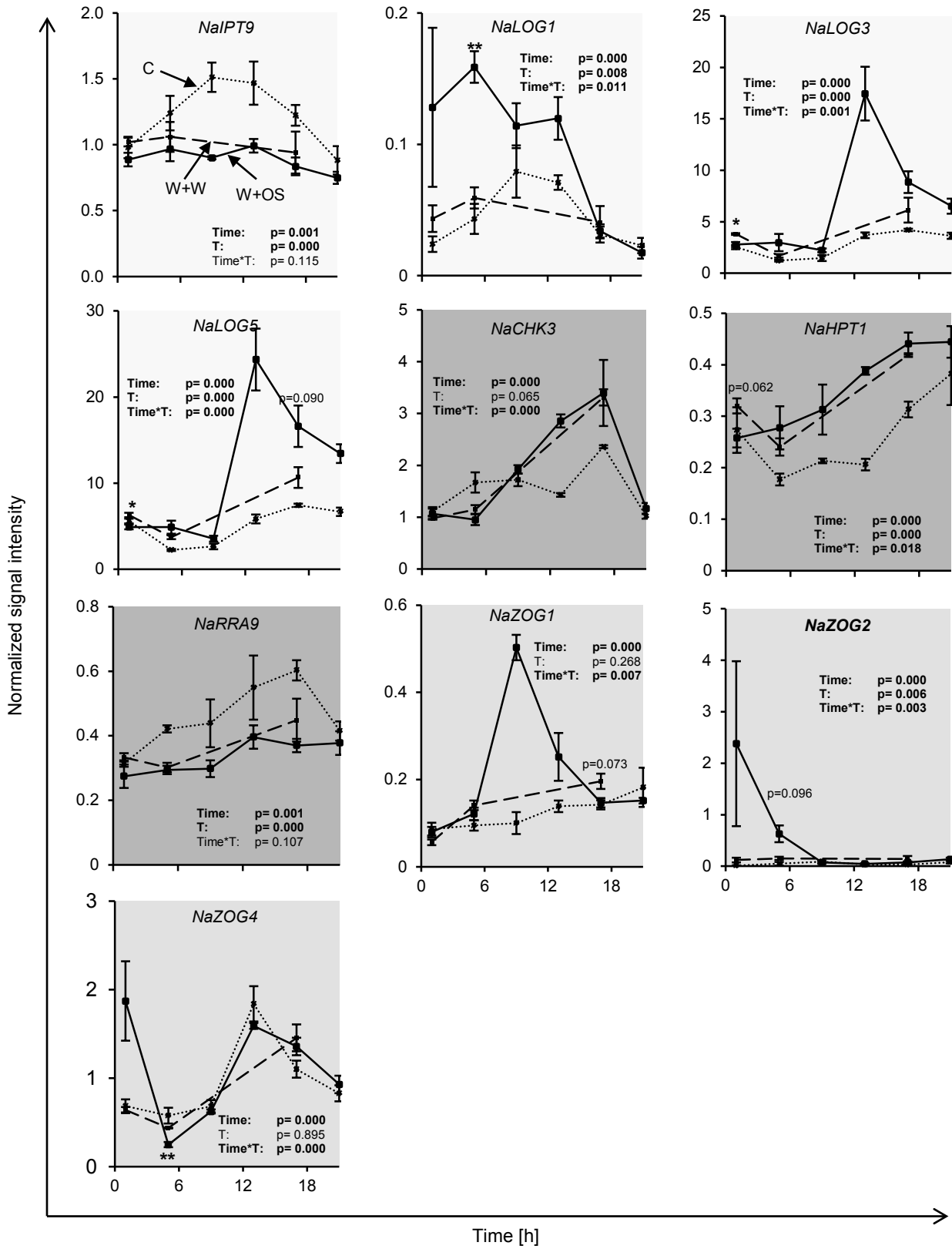


Figure S14. Wounding and herbivory regulate transcript accumulation of cytokinin-related genes in systemic leaves.

Transcript accumulation was measured in roots and systemic leaves of *N. attenuata* at different time points after wounding and application of water (W+W; dashed line; 1, 5 and 17 h) or *M. sexta* oral secretions (W+OS; solid line; 1, 5, 9, 13, 17 and 21 h) to the puncture wounds, as well as in untreated control leaves (C; dotted line; 1, 5, 9, 13, 17 and 21 h). Data are obtained from kinetic analysis conducted with microarrays.

Time and treatment (C and W+OS; T) effects and their interaction (Time*T) were analyzed for *NalIPT9* by univariate ANOVA; the other transcript data were analyzed by a generalized least squares model. Asterisks indicate significant differences between W+W and W+OS-treated samples at the same time point (independent samples *t* test: ** $P \leq 0.01$). Error bars are standard errors (N=3).

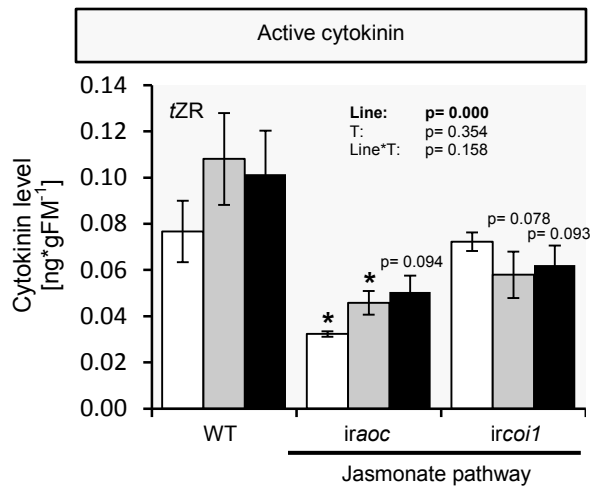


Figure S15. *Trans*-zeatin riboside levels in jasmonic acid pathway impaired transgenic plants.

Trans-zeatin riboside (*tZR*) levels in leaves of *N. attenuata* 30 min after wounding and application of water (W+W, grey bars) or *M. sexta* oral secretions (W+OS, solid bars) to the puncture wounds and in untreated control leaves (C, open bars). Measurements were performed in leaves of wild-type (WT) plants and RNAi lines silenced in *AOC* or *COI1* expression.

Line and treatment (C, W+W and W+OS; T) effects and their interaction (Line*T) were analyzed by univariate ANOVA. Asterisks indicate significant differences between same treatments from RNAi lines and WT plants (independent samples *t* test: * P≤0.05). Error bars are standard errors (N≥3). FM, fresh mass.

Supplemental Tables

Table S1. Abbreviations

Abbr.	Definition	Abbr.	Definition
AOC	Allene oxide cyclase	IP	Isopentenyladenine
CHASE	Cyclase/histidine kinase extracellular associated sensing	IPR	Isopentenyladenosine
CHK	CHASE domain-containing histidine kinase	IPT	Isopentenyltransferase
CIG2	Cytokinin-induced gene 2	JA	Jasmonic acid
CK	Cytokinin	JA-Ile	Jasmonic acid-isoleucine conjugate
CKX	Cytokinin oxidase/dehydrogenase	JAZ	Jasmonate zim-domain
COI1	Coronatine insensitive 1	MeJA	Methyl-jasmonate
CRK1	Cytokinin-regulated kinase 1	OS	Oral secretion
<i>cZ</i>	<i>Cis</i> -zeatin	OS _{GH}	Grasshopper oral secretion
<i>cZ9G</i>	<i>Cis</i> -zeatin <i>N</i> ⁹ -glucoside	RR	Response regulator
<i>cZR</i>	<i>Cis</i> -zeatin riboside	RRA	Type-A response regulator
<i>cZROG</i>	<i>Cis</i> -zeatin riboside <i>O</i> -glucoside	RRB	Type-B response regulator
DHZ	Dihydrozeatin	<i>tZ</i>	<i>Trans</i> -zeatin
DHZR	Dihydrozeatin riboside	<i>tZ7G</i>	<i>Trans</i> -zeatin <i>N</i> ⁷ -glucoside
FAC	Fatty acid-amino acid conjugate	<i>tZR</i>	<i>Trans</i> -zeatin riboside
HAMP	Herbivore-associated molecular pattern	<i>tZROG</i>	<i>Trans</i> -zeatin riboside <i>O</i> -glucoside
HPT	Histidine-containing phosphotransfer protein	W+OS	Wounding and application of oral secretion
		W+W	Wounding and water
		ZOG	Zeatin <i>O</i> -glucosyltransferase

Table S2. Sequences of primers used for qPCR.

Gene	forward primer	reverse primer
<i>NaActin</i>	5'ggcgtaccaccggtattgtg3'	5'gtcaagacggagaatggcatg3'
<i>NaLOG4</i>	5'ctcagctcacaagctcttcacg3'	5'ccattaagccaacactccacc3'
<i>NaIPT5</i>	5'tcagccacttattaattccgagag3'	5'tggctagatcaatggatagtctag3'
<i>NaRRA5</i>	5'agatgagttgcatgttcttgctgt3'	5'tcaatccccacagaggcttct3'
<i>NaCKX5</i>	5'tgtcggcttattgtaaccgctc3'	5'gtaagaactgccatcggtc3'
<i>NaZOG2</i>	5'agtcatgcaagtcaatttaagagctc3'	5'aggaaattgggaagaagggtgaag3'
<i>NaCHK3</i>	5'tgctctccggagaggaagatc3'	5'ttagaaggaagatcggtttgtaaact3'

Table S3. Multi-reaction-monitoring settings for cytokinin quantification in positive ionization mode.

Analyte	Q1 [m/z] →	Q3 [m/z]	DP	CE	CXP	Internal Standard
<i>t</i> Z	220.2	136.3	26	25	16	[² H5] <i>t</i> Z
<i>t</i> ZR	352.2	220.3	76	25	30	[² H5] <i>t</i> ZR
<i>t</i> ZROG	514.1	382.1	96	25	16	[² H5] <i>t</i> ZROG
<i>t</i> Z7G	382.1	220.2	71	31	16	[² H5] <i>t</i> Z7G
<i>c</i> Z	220.2	136.3	26	25	16	[² H5] <i>t</i> Z
<i>c</i> ZR	352.2	220.3	76	25	30	[² H5] <i>t</i> ZR
<i>c</i> ZROG	514.1	382.1	96	25	16	[² H5] <i>t</i> ZROG
<i>c</i> Z9G	382.1	220.0	71	31	16	[² H5] <i>t</i> Z9G
IP	204.1	136.0	81	23	16	[² H6]IPR ^a
IPR	336.1	204.3	61	23	26	[² H6]IPR
DHZ	222.0	136.0	76	27	20	[² H5] <i>t</i> Z ^b
DHZR	354.2	220.1	101	29	18	[² H5] <i>t</i> ZR ^c
[² H5] <i>t</i> Z	225.2	136.3	26	25	16	
[² H5] <i>t</i> ZR	357.2	225.3	76	25	30	
[² H5] <i>t</i> ZROG	519.1	387.1	96	25	16	
[² H5] <i>t</i> Z7G	387.1	225.0	71	31	16	
[² H5] <i>t</i> Z97G	387.1	225.0	71	31	16	
[² H6]IPR	342.0	210.1	61	23	26	

^a Reference factor → $m_{IP} = 8.772 \times m_{D6-IPR}$

^b Reference factor → $m_{DHZ} = 0.6029 \times m_{D5-tZ}$

^c Reference factor → $m_{DHZR} = 0.5525 \times m_{D5-tZR}$