

Figure S1

Figure S1 The artemisinin biosynthesis pathway in *A. annua*. Branch pathway genes are marked in blue, and artemisinin biosynthesis genes are marked in red. ADS, amorpha-4,11-diene synthase; ALDH1, aldehyde dehydrogenase 1; BFS, β -farnesene synthase; CPS, β -caryophyllene synthase; CYP71AV1, amorphadiene-12-hydroxylase; DBR2, artemisinic aldehyde Δ 11(13) reductase; FDS, farnesyl diphosphate synthase; GAS, germacrene A synthase; HMGR, 3-hydroxy-3-methyl-glutaryl coenzyme A reductase; HMGS, 3-hydroxy-3-methyl-glutaryl coenzyme A synthase; SQS, squalene synthase; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase.

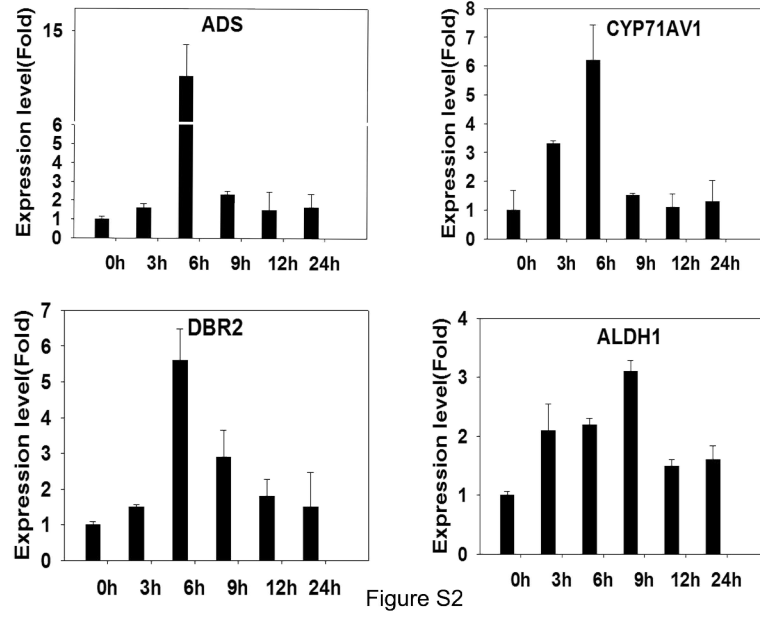


Figure S2 Expression levels of *ADS*, *CYP71AV1*, *DBR2* and *ALDH1* under SA treatment. β -*ACTIN* was used as an endogenous reference gene. Error bars indicate SE (n = 3).

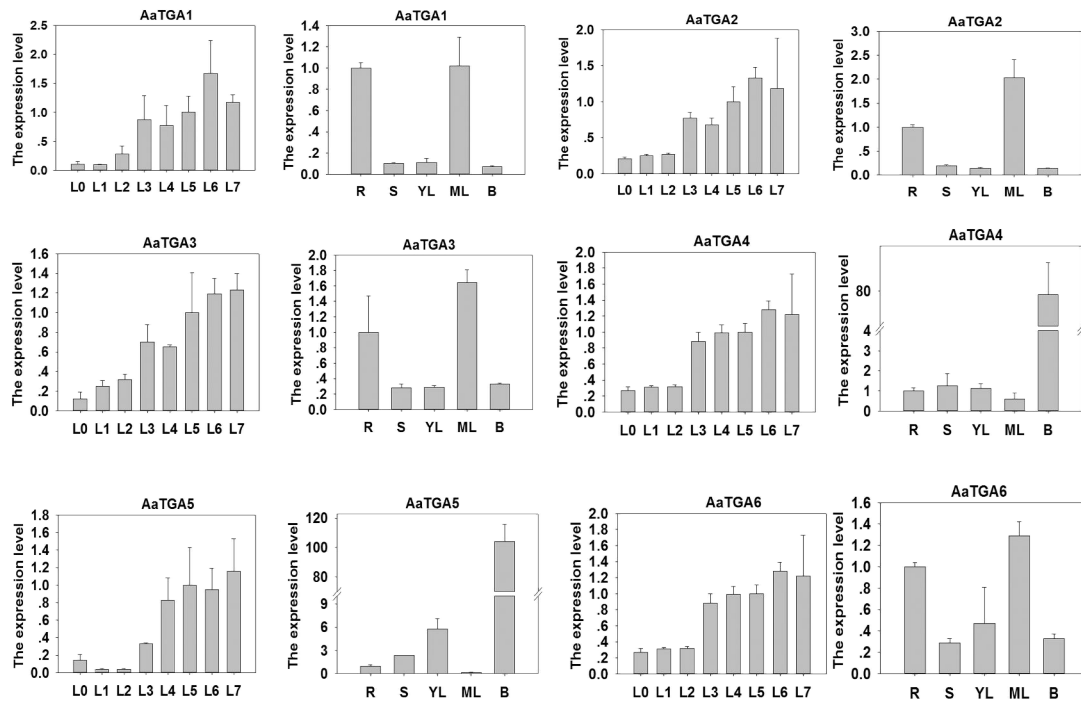


Figure S3

Figure S3 The expression levels of *AaTGA1*~*AaTGA6* in the leaf0~leaf7(L0~L7) and roots (R), stems (S), leaves (L) and buds (B). β -*ACTIN* was used as endogenous reference gene. Error bars indicate SE (n = 3).

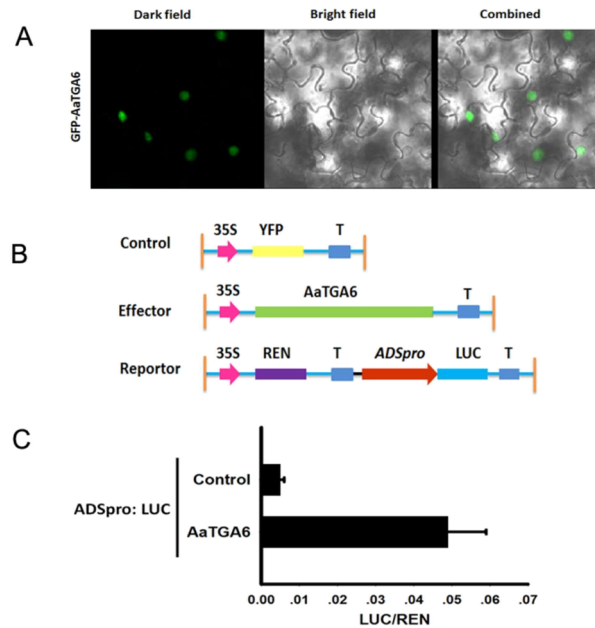


Figure S4

Figure S4 Subcellular location and transient dual-LUC assay of AaTGA6.

(A) Subcellular location assay indicates that AaTGA6 is located in the nuclear. AaTGA6 localizes to the nucleus. The ORF of AaTGA6 was fused with the C-terminal of YFP in YFP-PHB vector, and the AaTGA6 was driven by 2×35S promoter. The plasmids were delivered into tobacco leaves by *A. tumefaciens* infiltration. (B) Schematic illustration of the reporter and effector constructs used in the transient dual-LUC assays. (C) Transient dual-LUC analysis shows AaTGA6 activation of the transcription level of *ADS*. LUC/REN represents luciferase/Renilla ratio, n = 3 independent experiments.

>pro-AaERF1

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Figure S5 The promoter sequences of *AaERF1*, *AaERF2* and *AaORA*.

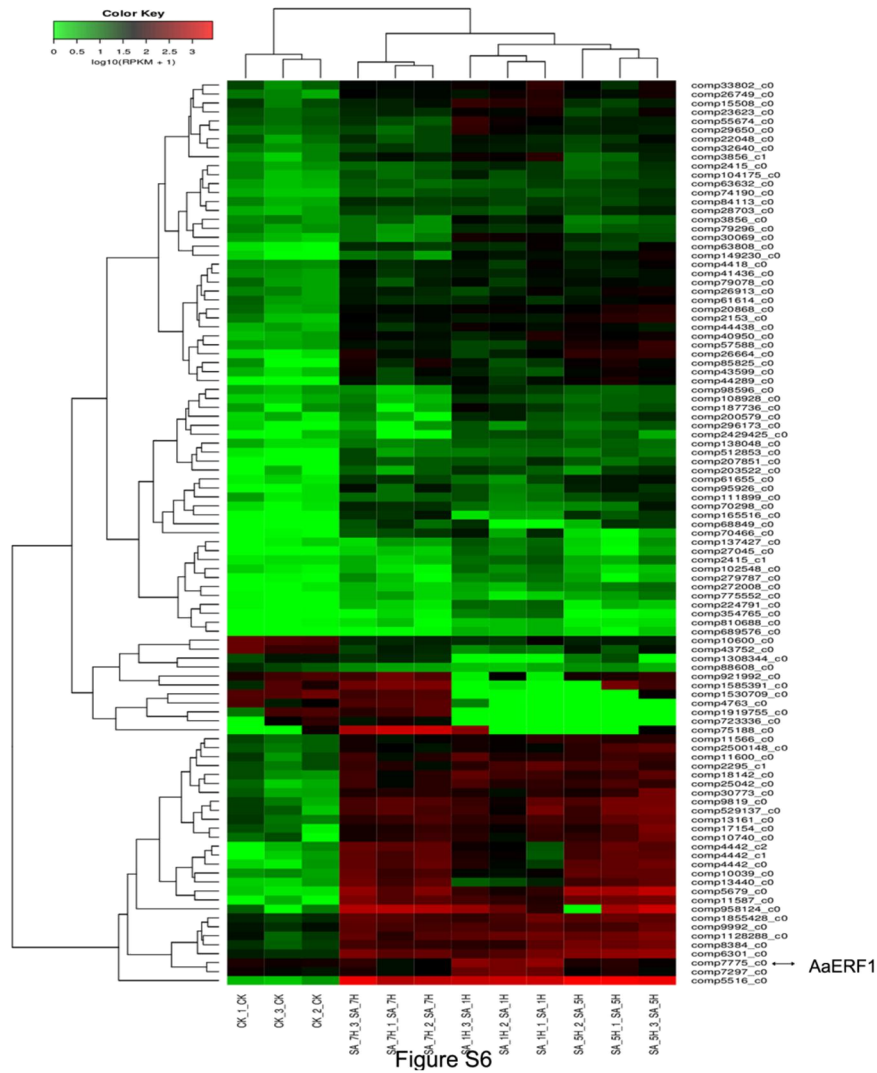


Figure S6 Expression heat map of SA-related genes in *A. annua*. The heat map shows the RPKM change of 101 genes expressed in 30-day-old *A. annua* under the treatment of SA (100 μ M) at the time points of 1 h, 5 h and 7 h. The color scale at the top represents the value of RPKM (red represents high expression and green represents low expression).

A

C-box3	caagcc	A	<u>TGACGTCA</u>	ccctaatacctaataat
Mutant1	caagcc	A	<u>TGACGTC</u> g	ccctaatacctaataat
Mutant2	caagcc	A	<u>TGACGT</u> a A	ccctaatacctaataat
Mutant3	caagcc	g	<u>TGACGTCA</u>	ccctaatacctaataat

B

AaTGA6	+	+	+	+
C-box3	+	-	-	-
Mutant1	-	+	-	-
Mutant2	-	-	+	-
Mutant3	-	-	-	+

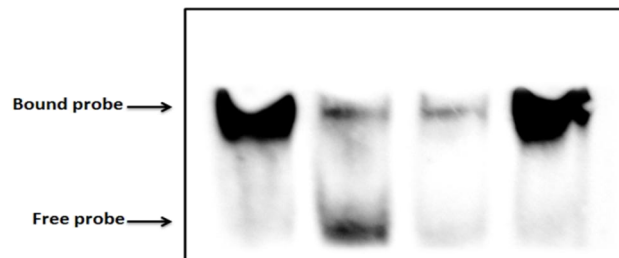


Figure S7

Figure S7 EMSA assay to test the binding of AaTGA6-His to the mutant “TGACG” motifs in the promoter of the *AaERF1* gene. (A) The mutant “TGACG” sequences. (B) The binding of AaTGA6-His to the mutant “TGACG” sequences.

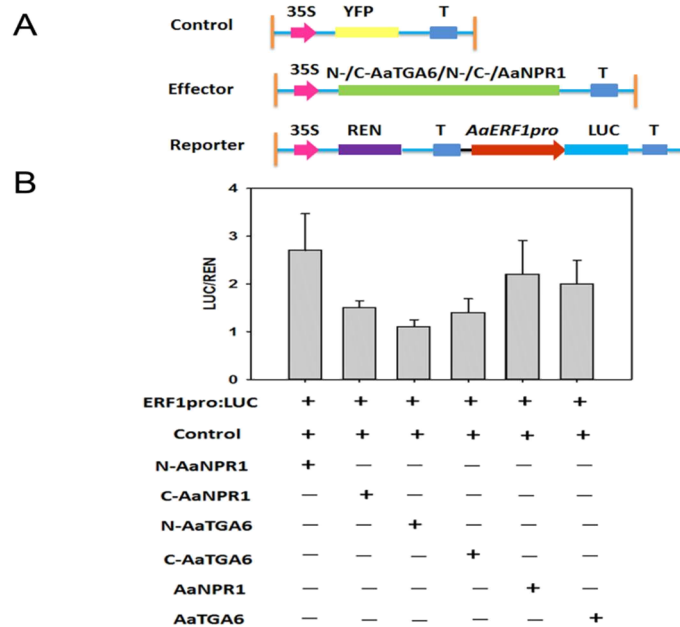


Figure S8

Figure S8 N-AaNPR1 can activate the promoter of *AaERF1*.

(A) Schematic illustration of the reporter and effector constructs used in the transient

dual-LUC assays. (B) Transient dual-LUC analysis showed that N-AaNPR1 can activate the promoter of *AaERF1*. LUC/REN represents the luciferase/Renilla ratio, n=4 independent experiments. *P<0.05.



Figure S9

Figure S9 The phenotype of *A. annua* transgenic plants. The phenotype of three-month-old seedlings of wild type (WT), RNAi (AaTGA6-phellsgate) and overexpression (2×35S::AaTGA6) *A. annua* plants.

Table S1 Sequence of primers

primers	Primers sequence(5'-3')
AaTAG1-F	atggatactagatcatttgg
AaTGA1-R	ttattgtattgtttatctctctgc
AaTAG2-F	atgaattcatgtccactcag
AaTGA2-R	tcacaaagccggttcac
AaTAG3-F	atggcatcaacatctacacaa
AaTGA3-R	ctagctcatgcgttcacgag
AaTAG4-F	atgacaggcacaactagtgtt
AaTGA4-R	ctaagtagcgtccctttttg
AaTAG5-F	atgaccaccaacaacaataat
AaTGA5-R	ctataaagttaaaatgtttgc
AaTAG6-F	atgggtagcaaaagcttgaag
AaTGA6-R	ttactctgtgggcgagcaag
AaNPR1-F	atggatgtagaaatgggttt
AaNPR1-R	ctatnttgggattgggaactg

AaNPR2-F	atgttaaaaatgcagcgacgc
AaNPR2-R	tcacgagtatttccgagatctg
AaNPR3-F	atgctactgaagattcattg
AaNPR3-R	ctaatactcatgatgagaatg
AaNPR4-F	cttgaaaattgttatgattg
AaNPR4-R	ctagagagtctaataggccaa
AaNPR5-F	atggaatacttagccatggaa
AaNPR5-R	gcgctttattctttccacca
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AaNPR1-BD-R	cgctgcaggtcgacggatccctatttgggattgggaactg
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AaTGA6-AD-R	cgc g gatcc t ttactcttgtggcgagcaag
AaTAG6-42AD-F	ccg gaattc atggtagcaaaagctgaag
AaTGA6-42AD-R	agctcgag ttactcttgtggcgagcaag
PAaERF1-F	cgtgtaccacgtccatgcaa
PAaERF1-R	ttttaaataatgggtttttatgtt
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pADS-R-KpnI	gcggtaccgaaagacagtagcacactcaata
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Q- AaERF1-PR	taaacggagtccatccaaca
Q-AaTAG1-F	gaggtccagcacagaa
Q-AaTGA1-R	accagaagcccataaa
Q-AaTAG2-F	tctggctgacgctgtg
Q-AaTGA2-R	ccgctgaataacaagtc
Q-AaTAG3-F	gccacaagactgaat
Q-AaTGA3-R	taagtaaaccgcata
Q-AaTAG4-F	tctcagccgcaaatca
Q-AaTGA4-R	tccacaagaacgcatc
Q-AaTAG5-F	ctaaccgagtccta
Q-AaTGA5-R	ttgaatccaccaatcc
Q-AaTAG6-F	aactgggaggattga
Q-AaTGA6-R	ttgaagttggtggaa
ox- AaTGA6 - BamH1-f	cgcgatccatgggtagcaaaagctgaag
ox- AaTGA6-xba1-r	tgctctagattactcttgtggcgagcaag
Q-AaNPR1-PF	GTTTGGATAAGCCTGGAA
Q-AaNPR1-PR	CAGCATAGGCGACAGC
Q-AaNPR2-PF	CCAAGTGAAGCGAAAC

Q-AaNPR2-PR	AATGAGGGAAGAAACG
Q-AaNPR3-PF	CAGACCCTAATGTTTCG
Q-AaNPR3-PR	GAGTTCAAGGCAAAGC
Q-AaNPR4-pF	ATAAGTGGTTGTGGGAGT
Q-AaNPR4-pR	CATCTGTGGGTTCTGC
Q-AaNPR5-PF	GCCCAAACAACCTCCG
Q-AaNPR5-PR	CGCCTACCCATCTCCA
EMSA-F	aatgatgegtcaagccaTGACGtcaccctaatacctaaat
EMSA-R	atttaggattaggggtgacgtcatggcttgacgcatcatt