Supplementary Materials

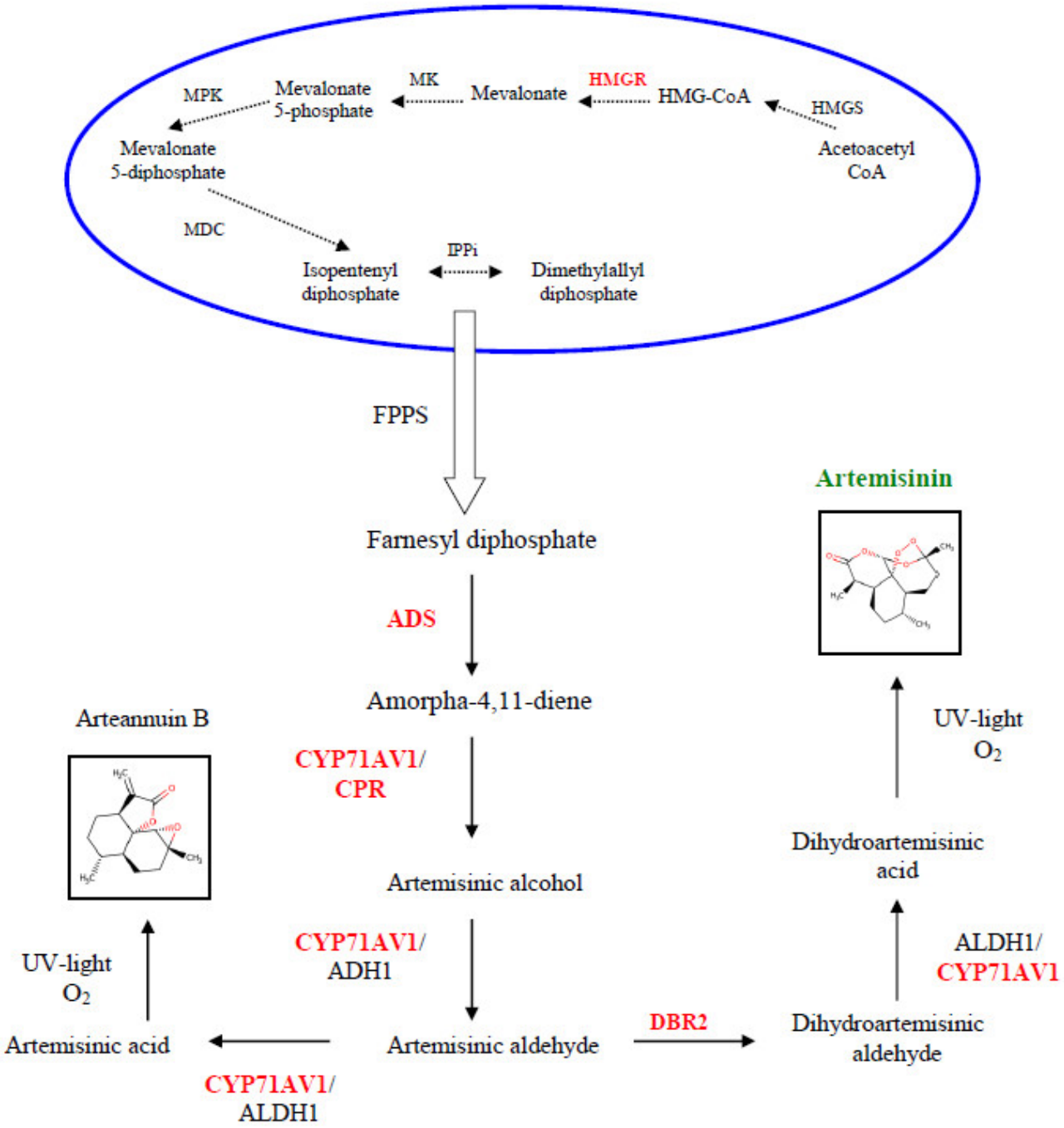

Figure S1. Biosynthetic pathway of artemisinin. The mevalonic acid (MVA) pathway is shown in a blue frame. Artemisinin pathway: ADS - amorpha-4,11-diene synthase; CYP71AV1 - amorpha-4,11-diene monooxygenase; CPR - cytochrome P450 reductase; ADH1 - alcohol dehydrogenase; DBR2 - artemisinic aldehyde Δ11(13) reductase; ALDH1 - aldehyde dehydrogenase. MVA pathway: HMGS - 3-hydroxy-3-methylglutaryl-CoA synthase; HMGR - 3-hydroxy-3-methylglutaryl-CoA reductase; MK - mevalonate kinase; MPK - mevalonate 5-phosphate kinase; MDC - mevalonate 5-diphosphate decarboxylase; IPPi - isopentenyl diphosphate isomerase; FPPS, farnesyl diphosphate synthase. Artemisinin biosynthesis genes cloned in p1240 vector are shown in red.

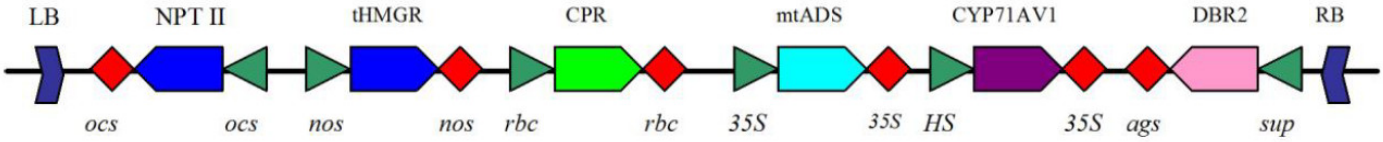

Figure S2. Schematic depiction of the expression cassette of vector p1240 to artemisinin production in chrysanthemum. tHMGR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase, CPR: cytochrome P450 reductase, ADS: mitochondria tar-

geted amorpha-4,11-diene synthase, CYP71AV1: amorpha-4,11-diene monooxygenase, DBR2: artemisinic aldehyde Δ11(13) reductase. Green triangles indicate promoters, red rhombus - terminators; ocs: octopine synthase; nos: nopaline synthase; rbc: rubisco; 35S: cauliflower mosaic virus (CaMV) 35S; HS: hps18.1 promoter; sup: superpromoter; ags: agrocinopine synthase. LB and RB: left and right T-DNA borders, respectively.

Table S1. PCR regimes and nucleotide sequences of used primers.

Target Gene	Primer Sequence, 5′ → 3′	Sequence Annealing	Amplified Fragment, bp	Annealing Temperature, °C	Extension Time, sec
		PCR			
mtADS	F ctatccttcgcaagacccttcc	35S CaMV promoter	591	55	40
	R tgatcaatctcccgttcaaagtga	ADS			
DBR2	F gctggtcaatcccattgcttttga	Sup promoter	931	61	60
	R caggatacgtgccataggttgcat	DBR2			
CPR	F atgcaatcaacaacttccgttaagttat	CPR gene, full-length	2115	60	120
	R ttaccatacatcacggagatatcttcc				
tHMGR	F acacctaataagtccaacatgg	tHMGR	1011	56	60
	R tgatactacgagagcggttg				
CYP71AV1	F atgaagagtatactaaaagcaatggcactc	CYP71AV1	1488	60	90
	R ctagaaacttggaacgagtaacaactca				
		RT-PCR			
ADS	F ggaagagctcagccatgtgt	mtADS	496	60	20
	R caggttagcaccgccagtaa				
tHMGR	F gcctataacaccaacgggca	tHMGR	229	60	20
	R cgctgccttggttattcacg				
CYP71AV1	F accctccactacccttggtt	CYP71AV1	242	60	20
	R ggctccaggacacatccttc				
CPR	F gtggagactggggctctttc	CPR	154	60	20
	R ctttggcatcaccgcaaaca				
DBR2	F aaccacgttacacggctgat	DBR2	136	60	20
	R ctagtgtaaccaccgcagca				
Actin of *C. morifolium*	F tggacgtgacttgaccgatg	actin	228	60	20
	R cacctgaacctctcagcacc				

F- forward primer, *R*- reverse primer

PCR amplification of the target fragments was performed using DreamTaq polymerase (Thermo Fisher Scientific, USA) in a manufacturer recommended buffer. The reaction mix contained genomic DNA (200 ng), forward and reverse primers (0.5 μM each) and 1.0 U DreamTaq polymerase in total volume of 25 μL. The reaction was performed under the following conditions: initial denaturation in 95°C 5 min, denaturation was carried out by 94°C 30 s, primer annealing in temperature described in the table, 30 s and extension at 72°C in the extension time in the table with 32 cycles.

First strand cDNA synthesis for RT-PCR analysis was performed using of M-MuLV reverse transcriptase (Thermo Fisher Scientific, USA) in a manufacturer recommended buffer. The three microgram of the total RNA and 100 pmol of oligo $d(T)_{16}$ primer were added into reaction buffer and first incubated at 42° C 90 min. Amplification of the target fragments was performed using DreamTaq polymerase (Thermo Fisher Scientific, USA) in a manufacturer recommended buffer. The reaction mix contained of 2 μL of the RT reaction mix, forward and reverse primers (0.5 μM each) and 1.0 U DreamTaq polymerase in total volume of 25 μL. The reaction was performed under the following conditions: initial denaturation in 95° C 5 min, denaturation was carried out by 94° C 30 s, primer annealing at 60° C 30 s and extension at 72°C in the extension time in the table with 35 cycles.

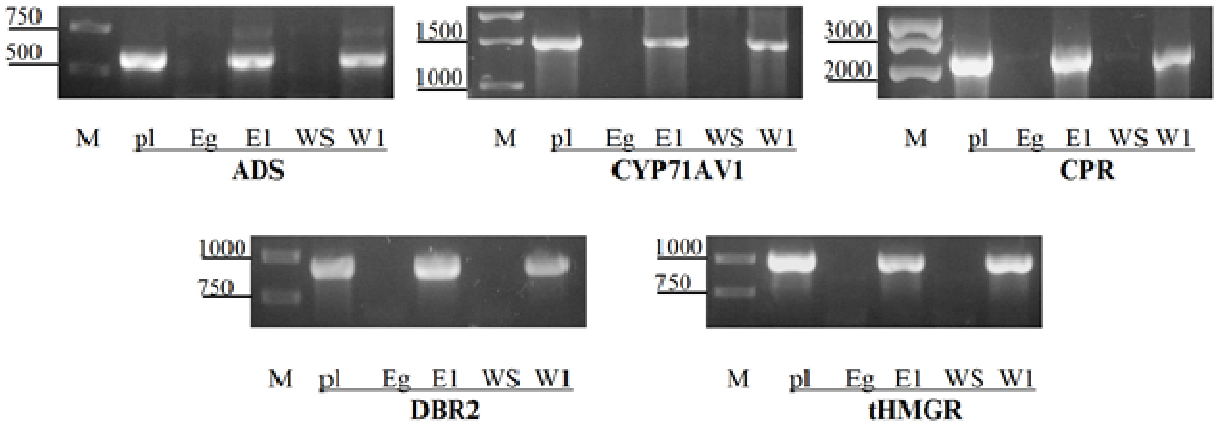

Figure S3. PCR analysis of transgenic chrysanthemum lines. Eg and WS: non-transformed plants cvs. Egyptianka and White Snowdon, respectively, negative control; E1 and WS1: transgenic lines cvs. Egyptianka and White Snowdon, respectively. ADS: amorpha-4,11-diene synthase (the expected amplified fragment length is 591 bp); CYP71AV1: amorpha-4,11-diene monooxygenase (1488 bp); CPR: cytochromeP450 reductase (2115 bp); DBR2: artemisinic aldehyde Δ11(13) reductase (931 bp); tHMGR:3-hydroxy-3-methylglutaryl-coenzyme A reductase (1011 bp). pl: DNA of plasmid p1240, positive control. M: molecular size marker.