Supplementary materials

Heterologous biosynthesis of prenylated resveratrol through multiplex metabolic engineering in *Escherichia coli*

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Table S1 Primers used in this study.

Name	Seuqence	Description
pJ-F1	ATGAGCGAATTACGTGCATTCAGC	Primers used to amplify the fragments for pJBEI6410-1
pJ-R1	GTATATCTCCTTCTTAAAAGATCCTTAATTCTGACGAAATG	
pJ-F2	GACAGTAATTAAAGCCGCCATTGAACG	
pJ-R2	ACGTAATTCGCTCATATGATCTCCCT	
pJ-F3	GCTTTAATTACTGTCGCCCCCAGG	
pJ-R3	AAGAAGGAGATATACGGATCCAAACTCGAGTAAGGATCTCC	
pJ-F4	CACAACGGCACGATCAACACG	Primers used to amplify the fragments for pJBEI6410-2 and pJBEI6410-3
pJ-R4	CTCGAGTTTGGATCCGTATATCTCCTTCTTAAAAGATCCTGAAATTGT	
pJ-F5	GTCCCGGTAAATGTTGTCACTCG	
pJ-R5	GATCGTGCCGTTGTGGCTGTCTTTC	
pJ-F6	GGATCCAAACTCGAGTAAGGATCTCC	
pJ-R6	AACATTTACCGGGACGATTTCGG	
pJ-R7	GTATATCTCCTTCTTAAAAGATCCTGAAATTGT	
IacE-F2	AAGAAGGAGATATACATGGCTATCTCAACCCCTTCCAAC	
IacE-R2	CTCGAGTTTGGATCCTTAGTAGTAGAACTCG	
4CL-BamHI-F	ACCATCATCACCACAGCCAGGATCCGATGGCGCCGCAGGAACAG	Primers used to insert At4CL and VvSTS into pCDFDuet-1 for pCDFRESa.
4CL-NotI-R	GTTCGACTTAAGCATTATGCGGCCGCCAGACCGTTCGCCAGTTTCG	
STS-NdeI-F	GTATAAGAAGGAGATATACATATGGCGTCTGTTGAAGAA	
STS-XhoI-R	GCAGCGGTTTCTTTACCAGATTAGTTGGTAACGGTCGGA	
4CL-fusion-R	TTTCGCCGCCGCTTCCAGACCGTTCGCCAGTTTCG	Primers used for the fusion of At4CL-VvSTS, used with 4CL-BamHI-F and STS-XhoI-R to construct pCDFRESf.
STS-fusion-F	GAAGCGGCGGCGAAAATGGCGTCTGTTGAAGAA	
Plac-pET28-F	TGCGTCCGGCGTAGAGGATCGTCTAGAGACTGGAAAGCGGGCAGTG	Primers for anti-sense RNA expressing plasmids
Plac-R	CCTCCATGGTGAAATTGTTATCCGCTCACAATTCC	
asgltA-lac-F	CGGATAACAATTTCACCATGGAGGAGGAATTAACCATGCAGTGGTGGTGGTGGT	

	GGTGCACATCCAGTTCAACAGCTG	
asgltA-R	GCTGTCCACCAGTCATAGGAGGAATTA ACCATGCAGTGGTGGTGGTGGTGGTGG GTTTTCCCCTCTTTCACAG	
lac-asacpS-F	GATAACAATTTCACCATGGAGGAGGAA TTAACCATGCAGTGGTGGTGGTGGTGG TGCATTCGTTATCGCTTAATACGC	
asacpS-R	GCTGTCCACCAGTCATAGGAGGAATTA ACCATGCAGTGGTGGTGGTGGTGGTGC CATCCCTGAGATGCATG	
lac-asacpT-F	GGATAACAATTTCACCATGGAGGAGGA ATTAACCATGCAGTGGTGGTGGTGGTG GTGATAGATGATCTCCGGTAGCG	
ascapT-R	GCTGTCCACCAGTCATAGGAGGAATTA ACCATGCAGTGGTGGTGGTGGTGGTGA AAGGCGGTAGCAAACTG	
Target-accA-F	GTAACTGGAGTTTGACTAATACGTTTT AGAGCTAGAAATAGCAAGTT	Primers used for sgRNA expressing plasmids pTargetF-accA, pTargetF-accB, and pTargetF-ACS.
Target-accA-R	GTATTAGTCAAACTCCAGTTACTAGTA TTATACCTAGGACTGAGC	
Target-accB-F	ATCACACTAAACAAAGAGTAGTTTTAG AGCTAGAAATAGCAAGTT	
Target-accB-R	TACTCTTTGTTTAGTGTGATACTAGTAT TATACCTAGGACTGAGC	
Target-acs-F	CATATTATTAACATCCTACAGTTTTAGA GCTAGAAATAGCAAGTT	
Target-acs-R	CTGTAGGATGTTAATAATATGACTAGT ATTATACCTAGGACTGAGC	
119-C-F	TTGACAGCTAGCTCAGTCCTAGGTATA ATGCTAGCTAATAGAAATAATTTTGTT TAACT	For amplification of J23119 promoter with an artificial RBS and a 6His tag.
119-C-R	TGATGCATTCTTGCCTCTTAACTTTAAA GTTAAACAAAATTATTTCTATTAGCTA GCAT	
accA-119-Dn-F	TAAGAGGCAAGAATGCATCATCACCAT CACCACATGAGTCTGAATTTCCTTGATT TTGA	For amplification of *accA* homologous arms
accA-119-Dn-R	GACGATAGCTTTATCGTCTGC	
accA-119-UP-F	GCTTGCTATCTCGCTGAC	
accA-119-UP-R	GGACTGAGCTAGCTGTCAACACCGCCA GAGATAATTAGTC	
119-accB-Dn-F	TAAGAGGCAAGAATGCATCATCACCAT CACCACATGAGTCTGAATTTCCTTGATT TTGA	For amplification of *accB* homologous arms

119-accB-Dn-R	GACGATAGCTTTATCGTCTGC	
119-accB-UP-F	GCTTGCTATCTCGCTGAC	
119-accB-UP-R	GGACTGAGCTAGCTGTCAACACCGCCAGAGATAATTAGTC	
119-acs-Dn-F	TAAGAGGCAAGAATGCATCATCACCATCACCACATGAGCCAAATTCACAAACACA	For amplification of *ACS* homologous arms
119-acs-Dn-R	GCTGATATGTTTGCTCTGGC	
119-acs-UP-F	CACGACAGTAACCGCACC	
119-acs-UP-R	GGACTGAGCTAGCTGTCAATGGCATAAGCGTTAAATGTAGG	

Table S2 DNA sequences

Name	Seuqence	Description
lac promoter	TCTAGAgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcaCCATGG	Amplified from pUC19 with XbaI/NcoI added to either ends.
asgltA	aggaggaattaaccatgcagtggtggtggtggtggtg**CACATCCAGTTCAACAGCTGTGTCCCCGTTGAGGGTGAGTTTTGCTTTTGTATCAGCCATTTAAGGTCTCCTTAGCGCCTTATTGCGTAAGACTGCCGGAACTTAAATTTGCCTTCGCACATCAACCTGGCTTTACCCGTTTGTTATTTGGCTCGCCGCTCTGTGAAAGAGGGGAAAACC**caccaccaccaccaccactgcatggttaattcctcct	Anti-sense RNA for *gltA*. Underlined letters are post trascription reverse complementary structures, bolded letters are target mRNA complementary sequences.
asacpS	aggaggaattaaccatgcagtggtggtggtggtggtg**CATTCGTTATCGCTTAATACGCGGCGTGCCAGGCGATCACCGGATCGGGCGATCACCGCTTCGATGCGAGCGATCTCCACAATATCCGTGCCTAAACCTAATATTGCCATTAGCCACGCGCTTCCAGCATCAGACGCTTCATTTCTGCCACCGCATCTTTCAGTCCGGTCATCACTGCACGACCAATAATGGCATGACCGATATTCAGTTCATGCATCTCAGGGATGG**caccaccaccaccaccactgcatggttaattcctcct	Anti-sense RNA for *acpS*. Underlined letters are post trascription reverse complementary structures, bolded letters are target mRNA complementary sequences.
asacpT	aggaggaattaaccatgcagtggtggtggtggtggtg**ATAGATGATCTCCGGTAGCGGGGAAAGCGTGTGCGAAAGCAATGCACGCCCCGCCAGCCAGCGTTCGCGTCGTGGACCTTGCGGTGCTTGCTCGCGTAAACCCGGTGGCAGTGGAGCTGCGCTTAAGGTCGAAACTTTCCCCAGAACTATCCGATACATATCAGGGCCAACGTTTAATGGAAAATGAAAGTGCGTATCGTATCACTTGTCGCCTCATCCCGGTAACCGACTTTTCGGTCTGCCCGGCCCCAGTAAAATCGCCAGTTTGCTACCGCCTTT**ccaccaccaccaccactgcatggttaattcctcct	Anti-sense RNA for *acpS*. Underlined letters are post trascription reverse complementary structures, bolded letters are target mRNA complementary sequences.
J23119 promoter region	ttgacagctagctcagtcctaggtataatgctagc*TAATAGAAAATAATTTTGTTTAACTTTAAAGTTAAGAGGCAAGA***ATGCATCATCACCATCACCAC**	Underlined letters are J23119 promoter sequence, italic letters are artificial RBS sequence, bolded letters are His tag sequence.
sgRNA for *accA*	AACTGGAGTTTGACTAATAC	

sgRNA for *accB*	ATCACACTAAACAAAGAGTA	
sgRNA for *acs*	CATATTATTAACATCCTACA	
DNA fragment used for *accA* promoter editing	GCTTGCTATCTCGCTGACGGACAGGCAAA TTGATGACCAGCTTTTAAACCGACTCCGTC AGTCTCTGGAACCCCACCGCTCTGGGACA ATTCCAGTACATCTCTACTATCAGAGGGC GGATGCACGCGCGCGGTTGCGTTTTGGCG CGACGTGGCGTGTCTCTCCGAGCGATCGT TTATTAAACGATCTCCGTGGCCTCATTGGT TCGGAGCAGGTGGAACTGGAGTTTGACTA ATTATCTCT*TTGACAGCTAGCTCAGTCCTAG GTATAATGCTAGCTAATAGAAATAATTTTGTTT AACTTTAAAGTTAAGAGGCAAGAATGCATCAT CACCATCACCAC***ATGAGTCTGAATTTCCT TGATTTTGAACAGCCGATTGCAGAGCT GGAAGCGAAAATCGATTCTCTGACTGC GGTTAGCCGTCAGGATGAGAAACTGGA TATTAACATCGATGAAGAAGTGCATCGT CTGCGTGAAAAAAGCGTAGAACTGACA CGTAAAATCTTCGCCGATCTCGGTGCAT GGCAGATTGCGCAACTGGCACGCCATC CACAGCGTCCTTATACCCTGGATTACGT TCGCCTGGCATTTGATGAATTTGACGAA CTGGCTGGCGACCGCGCGTATGCAGAC GATAAAGCTATCGTC***	Underlined letters are upstream and downstream homologous arms, italic letters are J23119 promoter region, bold letters are part of the coding region of *accA* gene.
DNA fragment used for *accBC* promoter editing	GCTGTTGATTATCTTCCCTGATAAGACCAG TATTTAGCTGCCAATTGCTACGAAATCGTT ATAATGTGCGACCTCGTCCTCCCTGACGC AGTTTTTGCGCTGCGGAAAAGGTGACATT GGCGCAACGAAGGTATATTTTGTTTTTTGC CGGAGGATAGCAGCAGATCGCTGCACAAT GTCCGTCAAGTCTAACATTGACACTCTGG GGCAAAATAGACCGGCGTCCCGGCCTGCT GGAATTTATCGCTATGCATACAGCTGTCG GGGCATACGCTTTACAGACGGCGGTGAAA CGCCTGTCACA*TTGACAGCTAGCTCAGTCCT AGGTATAATGCTAGCTAATAGAAATAATTTTG TTTAACTTTAAAGTTAAGAGGCAAGAATGCAT CATCACCATCACCAC***ATGGATATTCGTAA GATTAAAAAACTGATCGAGCTGGTTGA AGAATCAGGCATCTCCGAACTGGAAAT TTCTGAAGGCGAAGAGTCAGTACGCAT TAGCCGTGCAGCTCCTGCCGCAAGTTT CCCTGTGATGCAACAAGCTTACGCTGC***	Underlined letters are upstream and downstream homologous arms, italic letters are J23119 promoter region, bold letters are part of the coding region of *accB* gene.

	ACCAATGATGCAGCAGCCAGCTCAATC TAACGCAGCCGCTCCGGCGACCGTTCC TTCCATGGAAGCGCCAGCAGCAGCGGA AATCAGTGGTCACATCGTACGTTCCCCG ATGGTTGGTACTTTCTACCGCACCCCAA GCCCGGACGCAAAAGCGTTCATCGAAG TGGGTCAGAAAGTCAACGTGGGCGATA CCCTGTGCATCGTTGAAGCCATGAAAAT GATGAACCAGATCGAAGC	
DNA fragment used for *acs* promoter editing	CACGACAGTAACCGCACCTACACTGTCAT GACATTGCTCGCCCCTATGTGTAACAAAT AACCACACTGTGAATGTTGTCTTTAATCA ATTGTAAGTGCATGTAAAATACCACTTTA GAGTTAGTTAGTATCTTCCTCTTTTTCAAC AGCATGCATAACTGCATGTTCCTCAAAGA ATTAATCAACTTTTGTTGCTGACCTTCAAA AATTACCCTGCCGTTTATTTGCACAATTCT ACTTTTGCGTGATCTGTCGCCCAAATACTA AACAAAACTGCCAATACCCCTACATTTAA CGCTTATGCCA*TTGACAGCTAGCTCAGTCCT AGGTATAATGCTAGCTAATAGAAATAATTTTG TTTAACTTTAAAGTTAAGAGGCAAGAATGCAT CATCACCATCACCAC***ATGAGCCAAATTCA CAAACACACCATTCCTGCCAACATCGCA GACCGTTGCCTGATAAACCCTCAGCAG TACGAGGCGATGTATCAACAATCTATTA ACGTACCTGATACCTTCTGGGGCGAAC AGGGAAAAATTCTTGACTGGATCAAAC CTTACCAGAAGGTGAAAAACACCTCCTT TGCCCCCGGTAATGTGTCCATTAAATGG TACGAGGACGGCACGCTGAATCTGGCG GCAAACTGCCTTGACCGCCATCTGCAA GAAAACGGCGATCGTACCGCCATCATC TGGGAAGGCGACGACGCCAGCCAGAGC AAACATATCAGC**	Underlined letters are upstream and downstream homologous arms, italic letters are J23119 promoter region, bold letters are part of the coding region of *acs* gene.

Information of genes used in this study:

Gene: *At4CL*

GenBank: KX817185.1

Codon optimized DNA sequence:

ATGGCGCCGCAGGAACAGGCGGTTTCTCAGGTTATGGAAAAACAGTCTAA
CAACAACAACTCTGACGTTATCTTCCGTTCTAAACTGCCGGACATCTACAT
CCCGAACCACCTGTCTCTGCACGACTACATCTTCCAGAACATCTCTGAATT
CGCGACCAAACCGTGCCTGATCAACGGTCCGACCGGTCACGTTTACACCT
ACTCTGACGTTCACGTTATCTCTCGTCAGATCGCGGCGAACTTCCACAAAC
TGGGTGTTAACCAGAACGACGTTGTTATGCTGCTGCTGCCGAACTGCCCG
GAATTCGTTCTGTCTTTCCTGGCGGCGTCTTTCCGTGGTGCGACCGCGACC
GCGGCGAACCCGTTCTTCACCCCGGCGGAAATCGCGAAACAGGCGAAAG
CGTCTAACACCAAACTGATCATCACCGAAGCGCGTTACGTTGACAAAATC
AAACCGCTGCAGAACGACGACGGTGTTGTTATCGTTTGCATCGACGACAA
CGAATCTGTTCCGATCCCGGAAGGTTGCCTGCGTTTCACCGAACTGACCCA
GTCTACCACCGAAGCGTCTGAAGTTATCGACTCTGTTGAAATCTCTCCGGA
CGACGTTGTTGCGCTGCCGTACTCTTCTGGTACCACCGGTCTGCCGAAAGG
TGTTATGCTGACCCACAAAGGTCTGGTTACCTCTGTTGCGCAGCAGGTTGA
CGGTGAAAACCCGAACCTGTACTTCCACTCTGACGACGTTATCCTGTGCGT
TCTGCCGATGTTCCACATCTACGCGCTGAACTCTATCATGCTGTGCGGTCT
GCGTGTTGGTGCGGCGATCCTGATCATGCCGAAATTCGAAATCAACCTGC
TGCTGGAACTGATCCAGCGTTGCAAAGTTACCGTTGCGCCGATGGTTCCG
CCGATCGTTCTGGCGATCGCGAAATCTTCTGAAACCGAAAAATACGACCT
GTCTTCTATCCGTGTTGTTAAATCTGGTGCGGCGCCGCTGGGTAAAGAACT
GGAAGACGCGGTTAACGCGAAATTCCCGAACGCGAAACTGGGTCAGGGT
TACGGTATGACCGAAGCGGGTCCGGTTCTGGCGATGTCTCTGGGTTTCGC
GAAAGAACCGTTCCCGGTTAAATCTGGTGCGTGCGGTACCGTTGTTCGTA
ACGCGGAAATGAAAATCGTTGACCCGGACACCGGTGACTCTCTGTCTCGT
AACCAGCCGGGTGAAATCTGCATCCGTGGTCACCAGATCATGAAAGGTTA
CCTGAACAACCCGGCGGCGACCGCGGAAACCATCGACAAAGACGGTTGG
CTGCACACCGGTGACATCGGTCTGATCGACGACGACGACGAACTGTTCAT
CGTTGACCGTCTGAAAGAACTGATCAAATACAAAGGTTTCCAGGTTGCGC
CGGCGGAACTGGAAGCGCTGCTGATCGGTCACCCGGACATCACCGACGTT
GCGGTTGTTGCGATGAAAGAAGAAGCGGCGGGTGAAGTTCCGGTTGCGTT
CGTTGTTAAATCTAAAGACTCTGAACTGTCTGAAGACGACGTTAAACAGT
TCGTTTCTAAACAGGTTGTTTTCTACAAACGTATCAACAAAGTTTTCTTCA
CCGAATCTATCCCGAAAGCGCCGTCTGGTAAAATCCTGCGTAAAGACCTG
CGTGCGAAACTGGCGAACGGTCTGTAA

Gene: *VvSTS*

GenBank: NM_001281010.1

Codon optimized DNA sequence:

ATGGCGTCTGTTGAAGAATTCCGTAACGCGCAGCGTGCGAAAGGTCCGGC
GACCATCCTGGCGATCGGTACCGCGACCCCGGACCACTGCGTTTACCAGT
CTGACTACGCGGACTACTACTTCCGTGTTACCAAATCTGAACACATGACC
GAACTGAAAAAAAAATTCAACCGTATCTGCGACAAATCTATGATCAAAAA
ACGTTACATCCACCTGACCGAAGAAATGCTGGAAGAACACCCGAACATCG
GTGCGTACATGGCGCCGTCTCTGAACATCCGTCAGGAAATCATCACCGCG
GAAGTTCCGCGTCTGGGTCGTGACGCGGCGCTGAAAGCGCTGAAAGAATG
GGGTCAGCCGAAATCTAAAATCACCCACCTGGTTTTCTGCACCACCTCTGG
TGTTGAAATGCCGGGTGCGGACTACAAACTGGCGAACCTGCTGGGTCTGG
AAACCTCTGTTCGTCGTGTTATGCTGTACCACCAGGGTTGCTACGCGGGTG
GTACCGTTCTGCGTACCGCGAAAGACCTGGCGGAAAACAACGCGGGTGCG
CGTGTTCTGGTTGTTTGCTCTGAAATCACCGTTGTTACCTTCCGTGGTCCGT
CTGAAGACGCGCTGGACTCTCTGGTTGGTCAGGCGCTGTTCGGTGACGGT
TCTTCTGCGGTTATCGTTGGTTCTGACCCGGACGTTTCTATCGAACGTCCG
CTGTTCCAGCTGGTTTCTGCGGCGCAGACCTTCATCCCGAACTCTGCGGGT
GCGATCGCGGGTAACCTGCGTGAAGTTGGTCTGACCTTCCACCTGTGGCC
GAACGTTCCGACCCTGATCTCTGAAAACATCGAAAAATGCCTGACCCAGG
CGTTCGACCCGCTGGGTATCTCTGACTGGAACTCTCTGTTCTGGATCGCGC
ACCCGGGTGGTCCGGCGATCCTGGACGCGGTTGAAGCGAAACTGAACCTG
GAAAAAAAAAAACTGGAAGCGACCCGTCACGTTCTGTCTGAATACGGTAA
CATGTCTTCTGCGTGCGTTCTGTTCATCCTGGACGAAATGCGTAAAAAATC
TCTGAAAGGTGAAAAAGCGACCACCGGTGAAGGTCTGGACTGGGGTGTTC
TGTTCGGTTTCGGTCCGGGTCTGACCATCGAAACCGTTGTTCTGCACTCTG
TTCCGACCGTTACCAACTAA

Gene: *IacE*

GenBank: XM_007832623.1

DNA sequence:

ATGGCTATCTCAACCCCTTCCAACGGCGTAAGCCATGTCGCAAAGCCTCT
ACCCAACCTGAAAGAGGTCAATAAAGGCATTGAGACAGACAGTGAAGAC
CGAGCCTTCTGGTGGGGTGCCTTGTCTGAGCCTTTGGCCTCGCTCCTCGAG
GCAAATCACTACACCAAGGAAGTGCAACTTCACTACCTCCGTTGGTTTTAC
CAATGGATTCTACCGGCTCTGGGGCCACGACCTCTCGACGGCAAACCCTA
TTACGGGTCTTGGATTACTCATGATTTGTCGCCCTTTGAGTACAGTCTCAA
TTGGAAAGAAAAGAGTTCCAAACAGACCATCCGTTTCACTATTGAGGCGG

TTACGAAGCAGTCTGGCACAGCCAGCGATCCTATCAACCAGCTCGGCGCA
AAGGAGTTCCTGGAAGCAGTCTCCAAGGATGTTCCTGGTATGGATCTCAC
GCGATTCAACCAGTTTCTTGAGGCAACCAACGTACCCAACGACTGCGTAG
ATGATGCCATTGCAAAGCACCCAGCACACTTCCCTCGCAGCCGCGTTTGG
ATCGCTTTTGACTTGGAGCACTCTGGCAACCTTATGGCCAAGTCATACTTC
CTGCCGCATTGGCGTGCGATCCAGAGCGGTATCTCGGCCAATACCATCAT
TGGCGATACGGTCAAGGAGTGCAACAAGGCGGACGGTTCGTCGTACGAC
GGGTCGCTGAACGCCATTGAGTCGTACCTGGCAACGTTCACGCGACCGGA
GGAGGCGCCGCAGATGGGACTGCTGTCAAATGACTGCGTGGCCGAAACG
CCCGGCTCGCGCCTCAAGGTCTATTTCCGCTCCTCGGCGGACACGCTCGCC
AAGGCCAAGGACATGTACAACTTGGGTGGCCGCCTGAAGGGCCCCAAGA
TGGATGCCAGCCTCAAGGGTATCAGTGATTTCTGGTACCACCTGTTTGGCC
TCGACAGCTCCGACCCGGCCTCTGATGACAAGGTCTGTATCGGCAACCAC
AAGTGCATTTTCGTCTACGAGATGCGCTCCTCGCAAGGCTCGGAGCCCGA
TATTGATGTCAAGTTCCACATCCCAATGTGGCAGCTTGGTAAGACGGATG
GGCAGATTAGCGAGCTGCTGGCTTCTTGGTTCGAGAGCCATGGTCACCCT
GATCTCGCCTCGAGGTATAAGTCTGACTTGGGCACGGCATTTCCCAAGCA
CAATATTACGGGCAAGAGTGTAGGAACCCATACTTACATCTCCATCACTC
ACACACCCAAGACGGGATTGTACATGACCATGTATCTCAGCCCGAAACTG
CCCGAGTTCTACTACTAA

Gene: *accA*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence:

ATGAGTCTGAATTTCCTTGATTTTGAACAGCCGATTGCAGAGCTGGAAGC
GAAAATCGATTCTCTGACTGCGGTTAGCCGTCAGGATGAGAAACTGGATA
TTAACATCGATGAAGAAGTGCATCGTCTGCGTGAAAAAAGCGTAGAACTG
ACACGTAAAATCTTCGCCGATCTCGGTGCATGGCAGATTGCGCAACTGGC
ACGCCATCCACAGCGTCCTTATACCCTGGATTACGTTCGCCTGGCATTTGA
TGAATTTGACGAACTGGCTGGCGACCGCGCGTATGCAGACGATAAAGCTA
TCGTCGGTGGTATCGCCCGTCTCGATGGTCGTCCGGTGATGATCATTGGTC
ATCAAAAAGGTCGTGAAACCAAAGAAAAAATTCGCCGTAACTTTGGTATG
CCAGCGCCAGAAGGTTACCGCAAAGCACTGCGTCTGATGCAAATGGCTGA
ACGCTTTAAGATGCCAATCATCACCTTTATCGACACCCCGGGGGCTTATCC
GGGCGTGGGCGCAGAAGAGCGCGGTCAGTCTGAAGCCATTGCACGCAAC
CTGCGTGAAATGTCTCGCCTTAGCGTACCGACTATTTGTACCGTTATCGGT
GAAGGTGGTTCTGGCGGCGCGCTGGCGATTGGCGTGGGCGATAAAGTGAA
TATGCTGCAATACAGCACCTATTCCGTTATCTCGCCGGAAGGTTGTGCGTC
CATTCTGTGGAAGAGCGCTGATAAAGCGCCGCTGGCGGCTGAAGCGATGG
GTATCATCGCTCCGCGTCTGAAAGAACTAAAACTGATCGACTCCATCATC

CCAGAACCGCTGGGTGGTGCTCACCGTAACCCGGAAGCGATGGCGGCATC
GTTGAAAGCGCAACTGTTGGCGGATCTGGCCGATCTCGACGTGTTAAGCA
CTGAAGATTTAAAAAATCGTCGTTATCAGCGCCTGATGAGCTACGGTTAC
GCGTAA

Gene: *accBC*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence (the AccB coding sequence was underlined, AccC coding sequence was

in bold):

ATGGATATTCGTAAGATTAAAAAACTGATCGAGCTGGTTGAAGAATCAGG
CATCTCCGAACTGGAAATTTCTGAAGGCGAAGAGTCAGTACGCATTAGCC
GTGCAGCTCCTGCCGCAAGTTTCCCTGTGATGCAACAAGCTTACGCTGCAC
CAATGATGCAGCAGCCAGCTCAATCTAACGCAGCCGCTCCGGCGACCGTT
CCTTCCATGGAAGCGCCAGCAGCAGCGGAAATCAGTGGTCACATCGTACG
TTCCCCGATGGTTGGTACTTTCTACCGCACCCCAAGCCCGGACGCAAAAG
CGTTCATCGAAGTGGGTCAGAAAGTCAACGTGGGCGATACCCTGTGCATC
GTTGAAGCCATGAAAATGATGAACCAGATCGAAGCGGACAAATCCGGTA
CCGTGAAAGCAATTCTGGTCGAAAGTGGACAACCGGTAGAATTTGACGAG
CCGCTGGTCGTCATCGAGTAACGAGGCGAAC**ATGCTGGATAAAATTGTT**
ATTGCCAACCGCGGCGAGATTGCATTGCGTATTCTTCGTGCCTGTAAA
GAACTGGGCATCAAGACTGTCGCTGTGCACTCCAGCGCGGATCGCGA
TCTAAAACACGTATTACTGGCAGATGAAACGGTCTGTATTGGCCCTGC
TCCGTCAGTAAAAAGTTATCTGAACATCCCGGCAATCATCAGCGCCG
CTGAAATCACCGGCGCAGTAGCAATCCATCCGGGTTACGGCTTCCTC
TCCGAGAACGCCAACTTTGCCGAGCAGGTTGAACGCTCCGGCTTTAT
CTTCATTGGCCCGAAAGCAGAAACCATTCGCCTGATGGGCGACAAAG
TATCCGCAATCGCGGCGATGAAAAAAGCGGGCGTCCCTTGCGTACCG
GGTTCTGACGGCCCGCTGGGCGACGATATGGATAAAAACCGTGCCAT
TGCTAAACGCATTGGTTATCCGGTGATTATCAAAGCCTCCGGCGGCG
GCGGCGGTCGCGGTATGCGCGTAGTGCGCGGCGACGCTGAACTGGC
ACAATCCATCTCCATGACCCGTGCGGAAGCGAAAGCTGCTTTCAGCA
ACGATATGGTTTACATGGAGAAATACCTGGAAAATCCTCGCCACGTC
GAGATTCAGGTACTGGCTGACGGTCAGGGCAACGCTATCTATCTGGC
GGAACGTGACTGCTCCATGCAACGCCGCCACCAGAAAGTGGTCGAAG
AAGCGCCAGCACCGGGCATTACCCCGGAACTGCGTCGCTACATCGGC
GAACGTTGCGCTAAAGCGTGTGTTGATATCGGCTATCGCGGTGCAGG
TACTTTCGAGTTCCTGTTCGAAAACGGCGAGTTCTATTTCATCGAAAT
GAACACCCGTATTCAGGTAGAACACCCGGTTACAGAAATGATCACCG
GCGTTGACCTGATCAAAGAACAGCTGCGTATCGCTGCCGGTCAACCG

CTGTCGATCAAGCAAGAAGAAGTTCACGTTCGCGGCCATGCGGTGGA
ATGTCGTATCAACGCCGAAGATCCGAACACCTTCCTGCCAAGTCCGG
GCAAAATCACCCGTTTCCACGCACCTGGCGGTTTTGGCGTACGTTGG
GAGTCTCATATCTACGCGGGCTACACCGTACCGCCGTACTATGACTC
AATGATCGGTAAGCTGATTTGCTACGGTGAAAACCGTGACGTGGCGA
TTGCCCGCATGAAGAATGCGCTGCAGGAGCTGATCATCGACGGTATC
AAAACCAACGTTGATCTGCAGATCCGCATCATGAATGACGAGAACTT
CCAGCATGGTGGCACTAACATCCACTATCTGGAGAAAAAACTCGGTC
TTCAGGAAAAATAA

Gene: *acs*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence:

ATGAGCCAAATTCACAAACACACCATTCCTGCCAACATCGCAGACCGTTG
CCTGATAAACCCTCAGCAGTACGAGGCGATGTATCAACAATCTATTAACG
TACCTGATACCTTCTGGGGCGAACAGGGAAAAATTCTTGACTGGATCAAA
CCTTACCAGAAGGTGAAAAACACCTCCTTTGCCCCCGGTAATGTGTCCATT
AAATGGTACGAGGACGGCACGCTGAATCTGGCGGCAAACTGCCTTGACCG
CCATCTGCAAGAAAACGGCGATCGTACCGCCATCATCTGGGAAGGCGACG
ACGCCAGCCAGAGCAAACATATCAGCTATAAAGAGCTGCACCGCGACGTC
TGCCGCTTCGCCAATACCCTGCTCGAGCTGGGCATTAAAAAAGGTGATGT
GGTGGCGATTTATATGCCGATGGTGCCGGAAGCCGCGGTTGCGATGCTGG
CCTGCGCCCGCATTGGCGCGGTGCATTCGGTGATTTTCGGCGGCTTCTCGC
CGGAAGCCGTTGCCGGGCGCATTATTGATTCCAACTCACGACTGGTGATC
ACTTCCGACGAAGGTGTGCGTGCCGGGCGCAGTATTCCGCTGAAGAAAAA
CGTTGATGACGCGCTGAAAAACCCGAACGTCACCAGCGTAGAGCATGTGG
TGGTACTGAAGCGTACTGGCGGGAAAATTGACTGGCAGGAAGGGCGCGA
CCTGTGGTGGCACGACCTGGTTGAGCAAGCGAGCGATCAGCACCAGGCGG
AAGAGATGAACGCCGAAGATCCGCTGTTTATTCTCTACACCTCCGGTTCTA
CCGGTAAGCCAAAAGGTGTGCTGCATACTACCGGCGGTTATCTGGTGTAC
GCGGCGCTGACCTTTAAATATGTCTTTGATTATCATCCGGGTGATATCTAC
TGGTGCACCGCCGATGTGGGCTGGGTGACCGGACACAGTTACTTGCTGTA
CGGCCCGCTGGCCTGCGGTGCGACCACGCTGATGTTTGAAGGCGTACCGA
ACTGGCCGACGCCTGCCCGTATGGCGCAGGTGGTGGACAAGCATCAGGTC
AATATTCTCTATACCGCGCCCACGGCGATTCGCGCGCTGATGGCGGAAGG
GGATAAAGCGATTGAAGGCACCGACCGATCGTCGCTGCGCATTCTCGGTT
CCGTGGGCGAGCCAATCAACCCGGAAGCGTGGGAGTGGTACTGGAAAAA
AATCGGCAACGAGAAATGTCCGGTGGTCGATACCTGGTGGCAGACCGAA
ACTGGCGGTTTCATGATCACGCCGCTGCCTGGCGCTACCGAGCTGAAAGC
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Gene: *gltA*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence:

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Gene: *acpS*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence:

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Gene: *acpT*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence:

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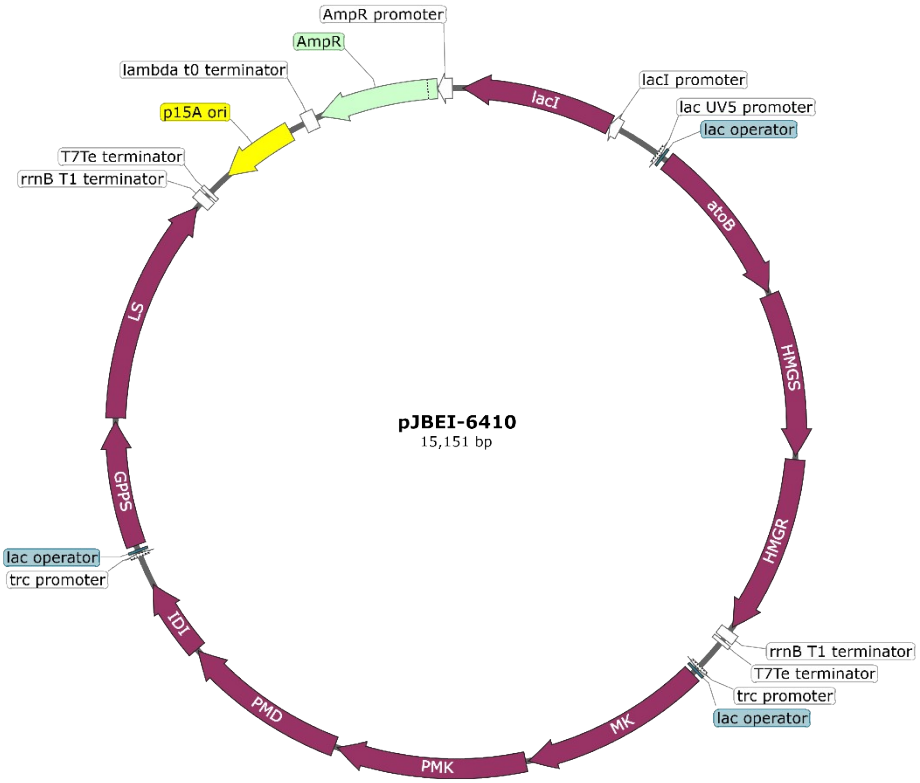

Fig. S1 The structure illustration of plasmid pJBEI-6410. The MTSA operon containing genes for the conversion of acetyl CoA to mevalonate was expressed by lac UV5 promoter. AtoB: acetoacetyl-CoA synthase from *E. coli*; HMGS: HMG-CoA synthase from *Staphylococcus aureus* codon optimized for *E. coli*; HMGR: HMG-CoA reductase from *S. aureus* codon optimized for *E. coli*. MBI operon containing genes for the conversion of mevalonate to isoprenoid precursors (IPP and DMAPP) was expressed by trc promoter. MK: Mevalonate Kinase, PMK: Phosphomevalonate Kinase, and PMD: Phosphomevalonate Decarboxylase were from *Saccharomyces cerevisiae*; IDI: isopentenyl diphosphate isomerase from *E. coli*. GPPS: Geranyl pyrophosphate synthase from *Abies grandis* and codon optimized for *E. coli*; LS: Limonene synthase from *Mentha spicata*, a truncated version without plastidial targeting sequence and codon optimized for *E. coli*[29].

Plasmid sequence of pJBEI-6410:

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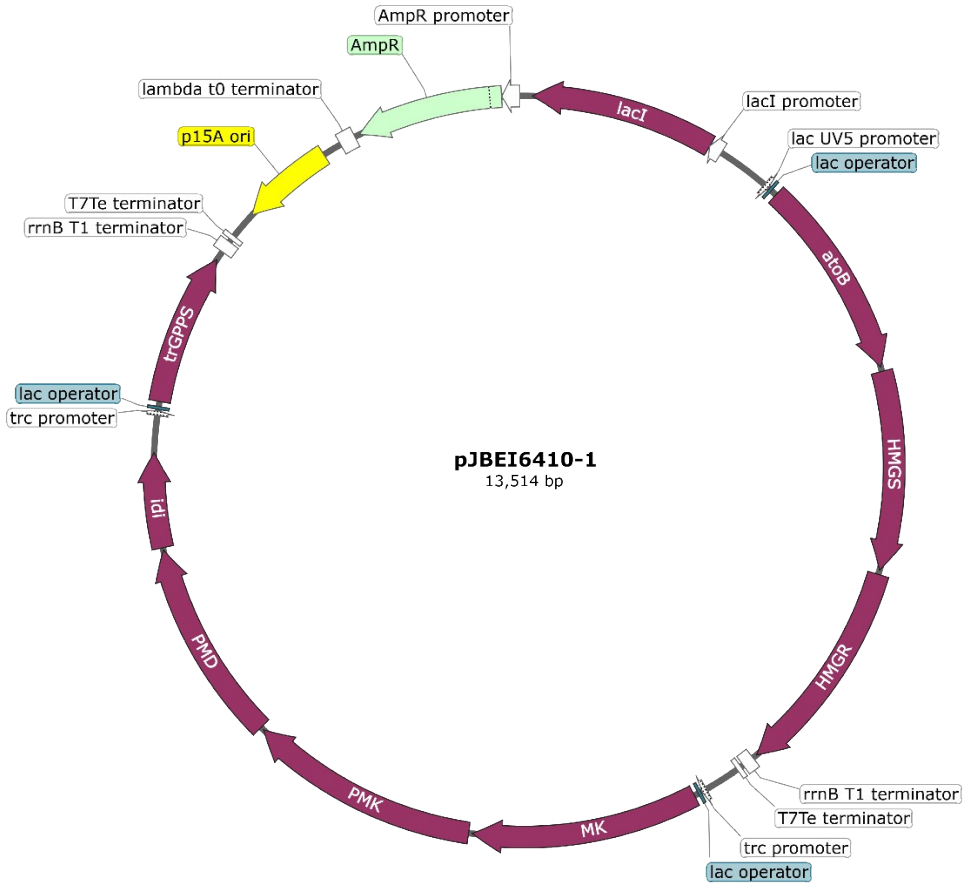

Fig. S2 The structure illustration of plasmid pJBEI6410-1. In this plasmid, limonene synthase gene was deleted for GPP supplementation.

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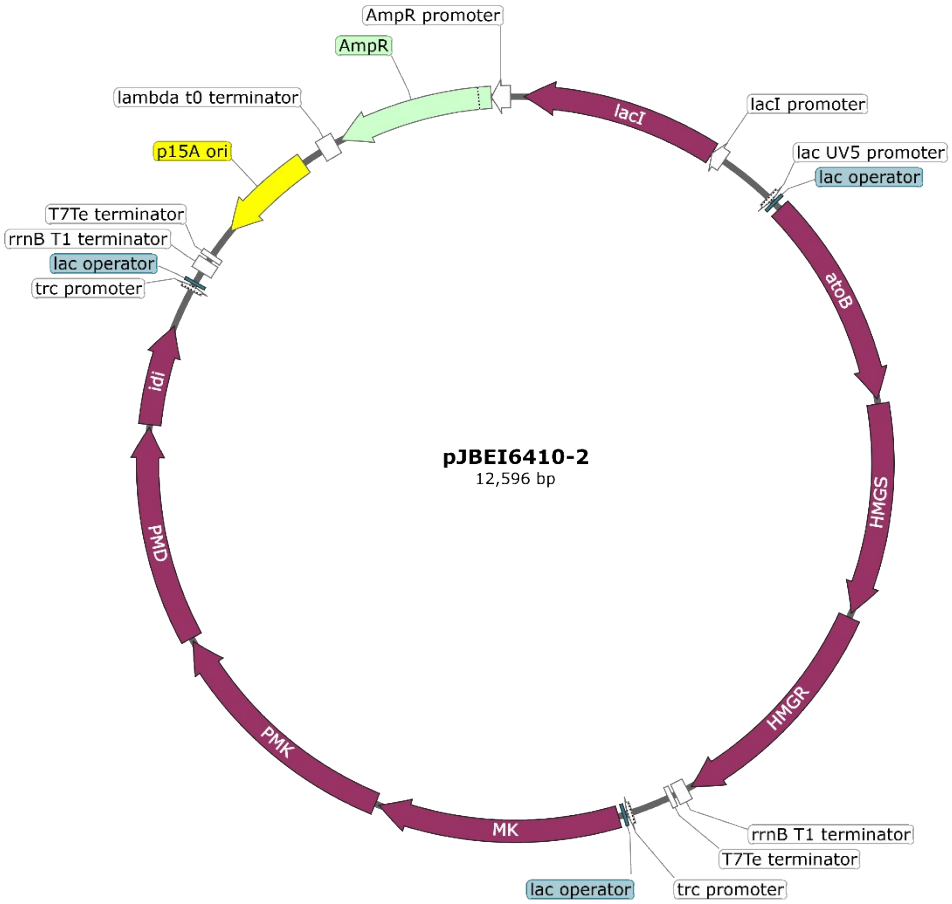

Fig. S3 The structure illustration of plasmid pJBEI6410-2. In this plasmid, the geranyl pyrophosphate synthase gene was further deleted for DMAPP supplementation.

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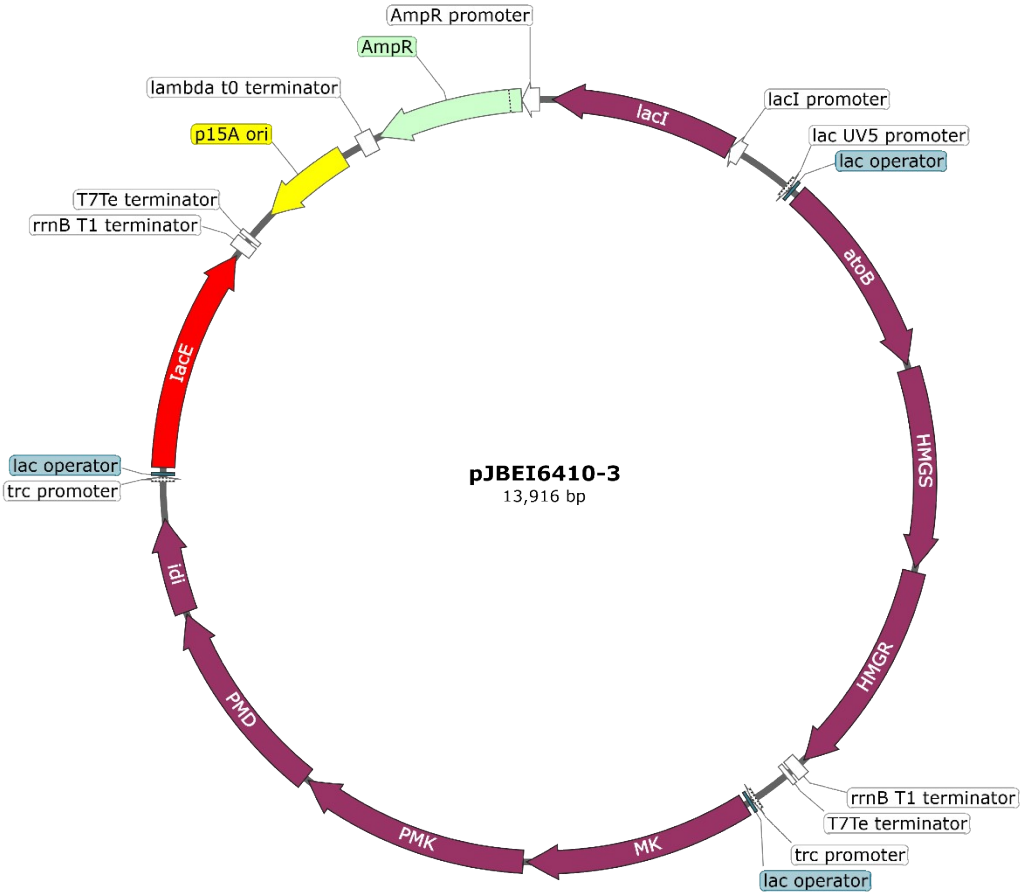

Fig. S4 The structure illustration of plasmid pJBEI6410-4. In this plasmid, the prenyltransferase IacE was inserted after the trc promoter.

Plasmid sequence of pJBEI6410-3:

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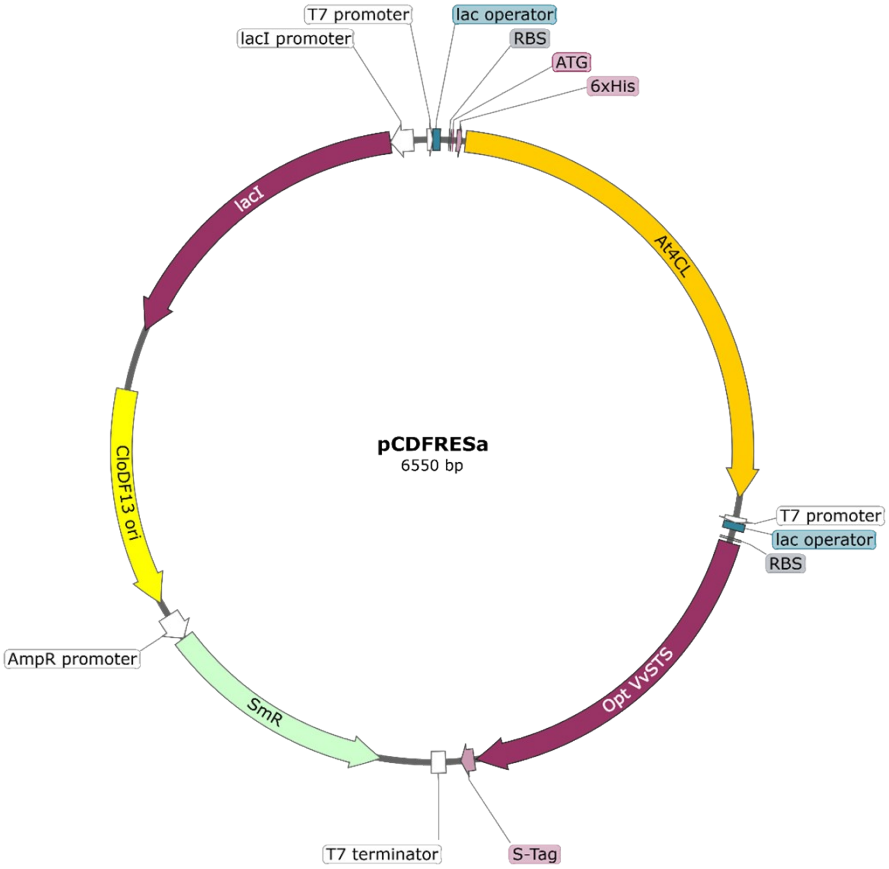

Fig. S5 The structure illustration of plasmid pCDFRESa. The codon optimized At4CL was inserted between restriction sites BamHI and NotI, so that it was expressed with a 6 × His tag at the N-terminal. The codon optimized VvSTS was inserted between restriction sites NdeI and XhoI.

Plasmid sequence of pCDFRESa:

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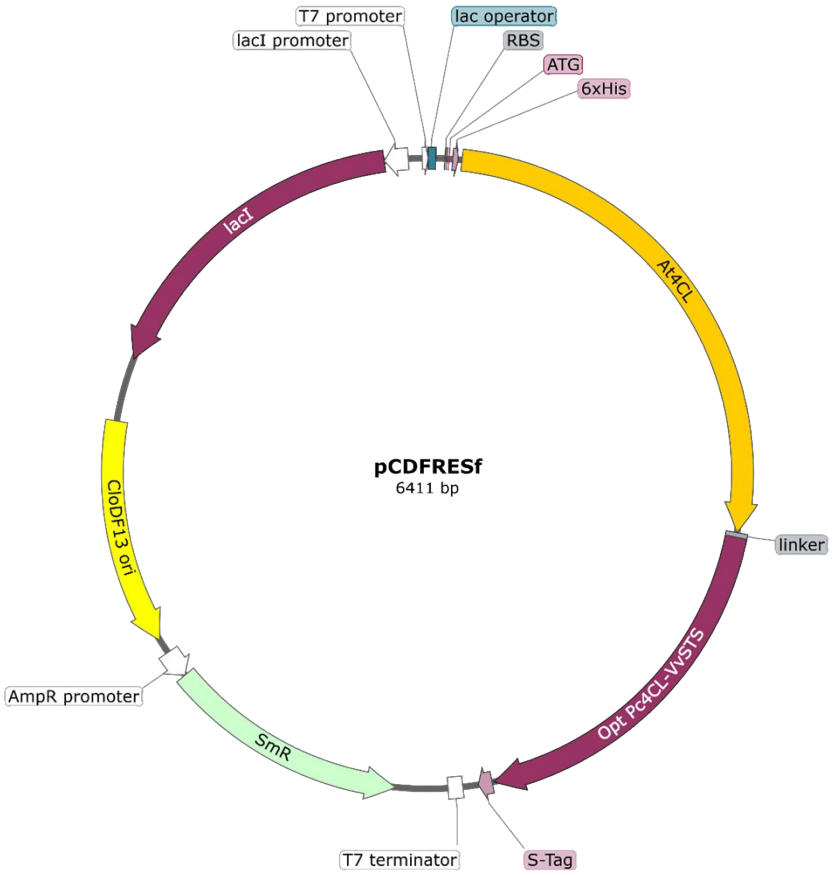

Fig. S6 The structure illustration of plasmid pCDFRESf. The codon optimized At4CL and VvSTS were expressed as a fusion protein, linked with a rigid linker (EAAAK) between the C-terminal of At4CL and the N-terminal of VvSTS. The fusion protein was inserted between restriction sites BamHI and NotI, so that it was expressed with a 6 × His tag at the N-terminal.

Plasmid sequence of pCDFRESf:

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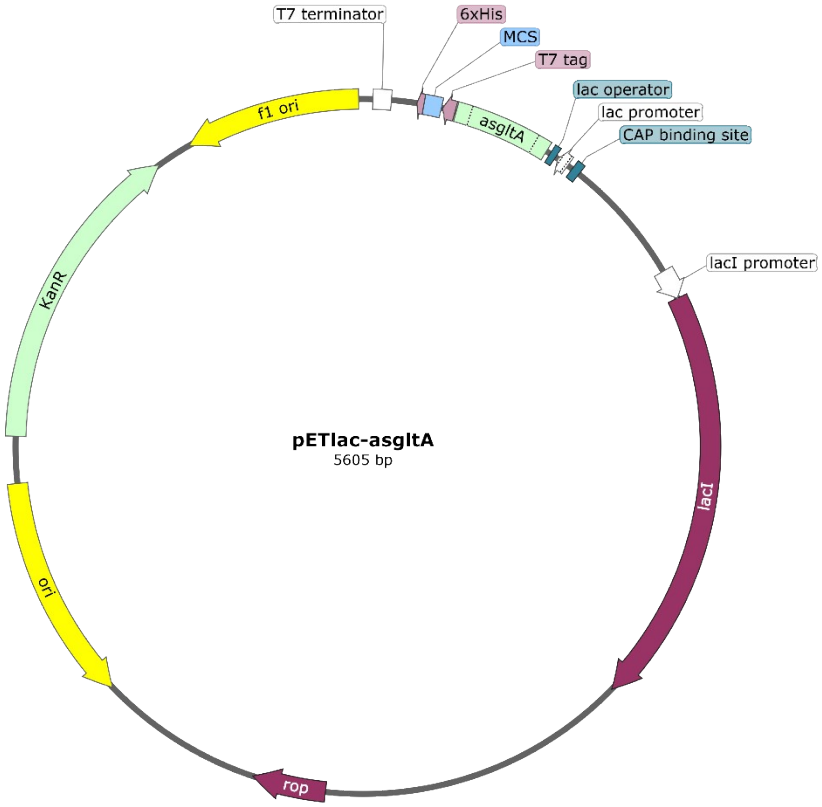

Fig. S7 The structure illustration of plasmid pETlac-asgltA. The T7 promoter was replaced by lac promoter amplified from pUC19, followed with the anti-gltA RNA frame.

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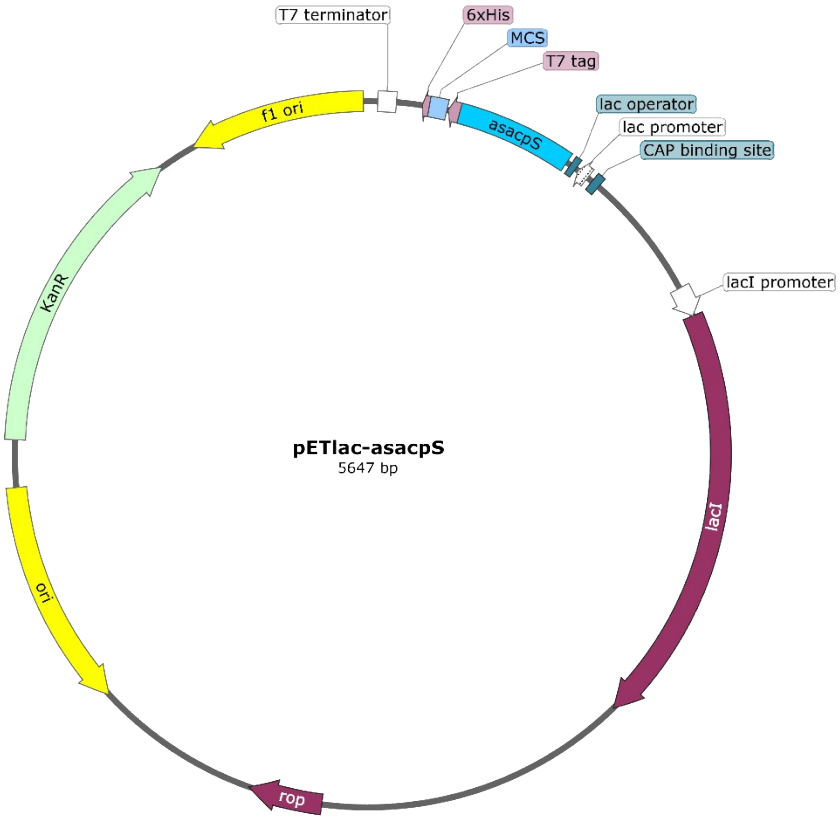

Fig. S8 The structure illustration of plasmid pETlac-asacpS. The T7 promoter was replaced by lac promoter amplified from pUC19, followed with the anti-gltA RNA frame.

Plasmid sequence of pETlac-asacpS:

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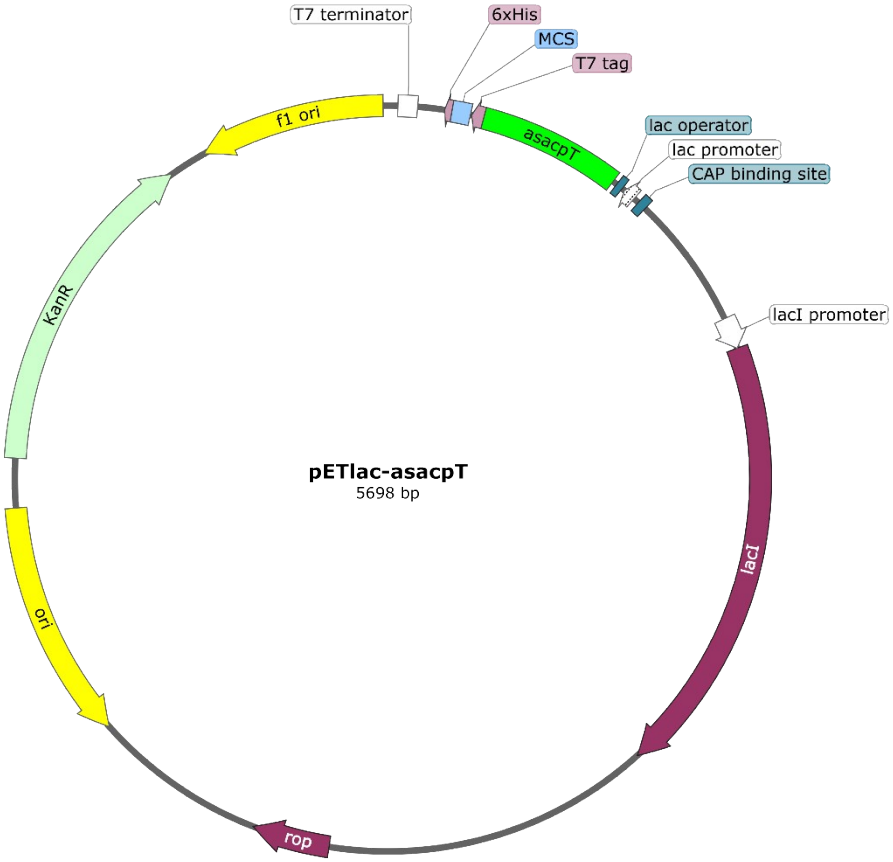

Fig. S9 The structure illustration of plasmid pETlac-asacpT. The T7 promoter was replaced by lac promoter amplified from pUC19, followed with the anti-gltA RNA frame.

Plasmid sequence of pETlac-asacpT:

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gatttagagcttgacggggaaagccggcgaacgtggcgagaaaggaagggaagaaagcgaaaggagcgggcgctagggcgctggcaa
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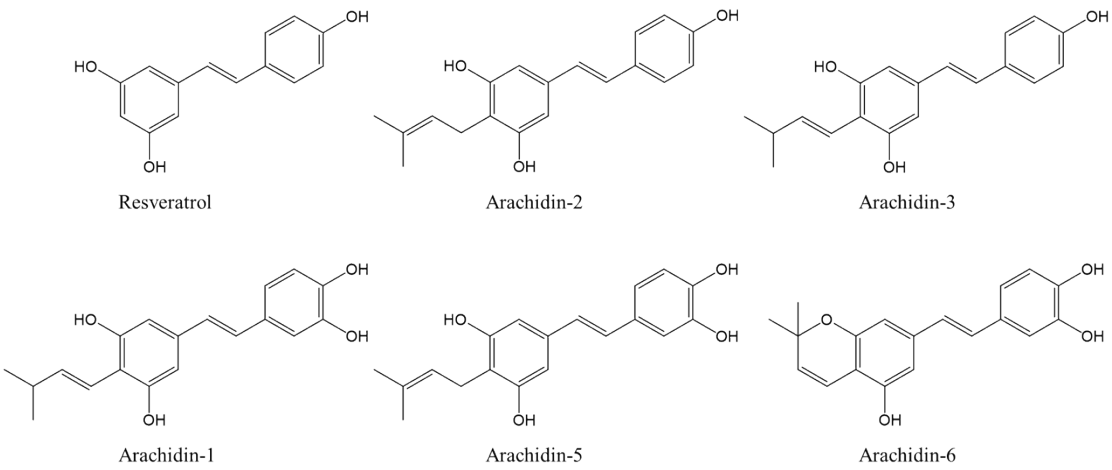

Fig. S10 Resveratrol and the prenylated stilbenoids isolated from the elicited hairy root cultures and seedlings of peanut[38-40].

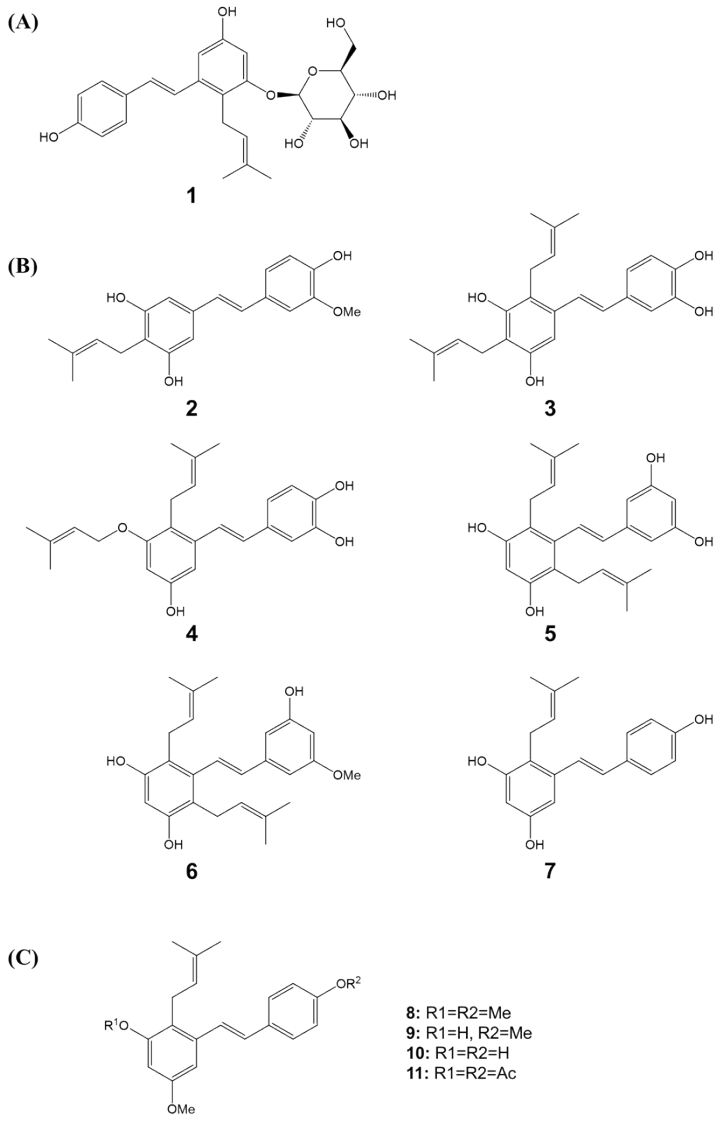

Fig. S11 2-C-prenyl resveratrol and its derivatives. (A) Vulpinoideol A (**1**) from *Carex vulpinoidea*[42]. (B) (*E*)-4-(3-methyl-2-buten-1-yl)-3,4′,5-trihydroxy-3′-methoxystilbene (**2**), (*E*)-2,4-bis(3-methyl-2-buten-1-yl)-3,3′,4′,5-tetrahydroxystilbene (**3**), (*E*)-2-(3-methyl-2-buten-1-yl)-3-(3-methyl-2-butenyloxy)-3′,4′,5-trihydroxystilbene (**4**), (*E*)-2,6-bis(3-methyl-2-buten-1-yl)-3,3′,5,5′-tetrahydroxystilbene (**5**) and (*E*)-2,6-bis(3-methyl-2-buten-1-yl)-3,4′,5-trihydroxy-3′-methoxystilbene (**6**), and 2-C-prenyl resveratrol (**7**) isolated from Kangaroo Island propolis[41]. (C) 2-prenyl-3,5,4'-trimethoxystilbene (**8**), 2-prenyl-3-hydroxy-5,4'-dimethoxystilbene (**9**) and 2-prenyl-3,4'-dihydroxy-5-methoxystilbene (**10**) and its corresponding acetate (**11**) isolated from *Scirpus holoschoenus* L.[43].

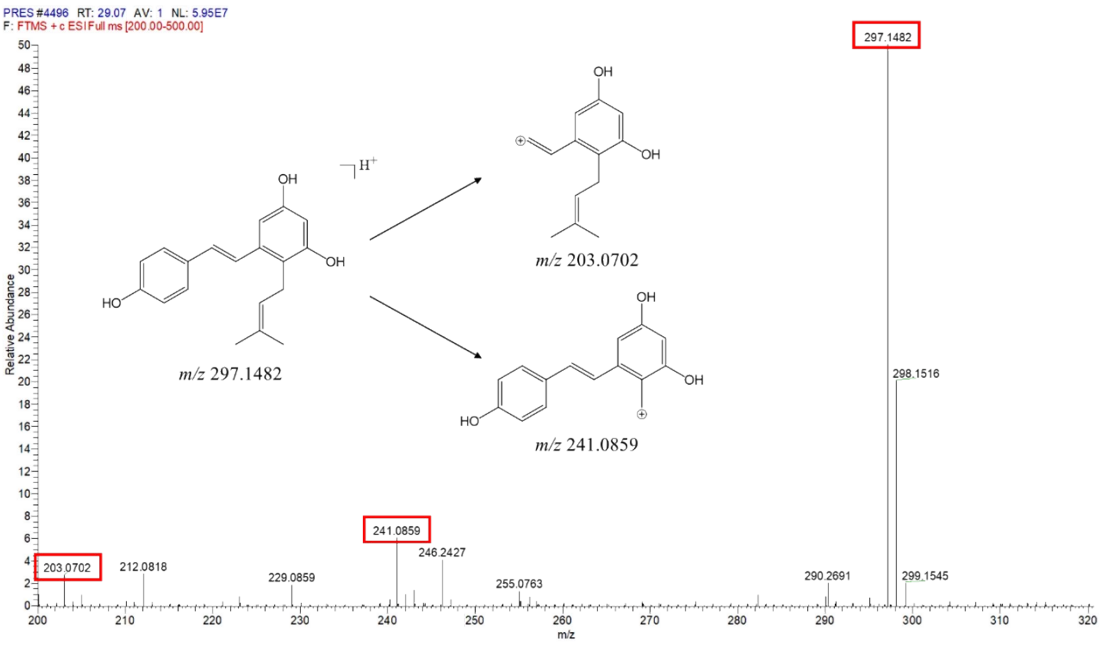

Figure S12. The ESI-MS spectrum and putative fragmentation pathway of the product 2-C-prenyl resveratrol.

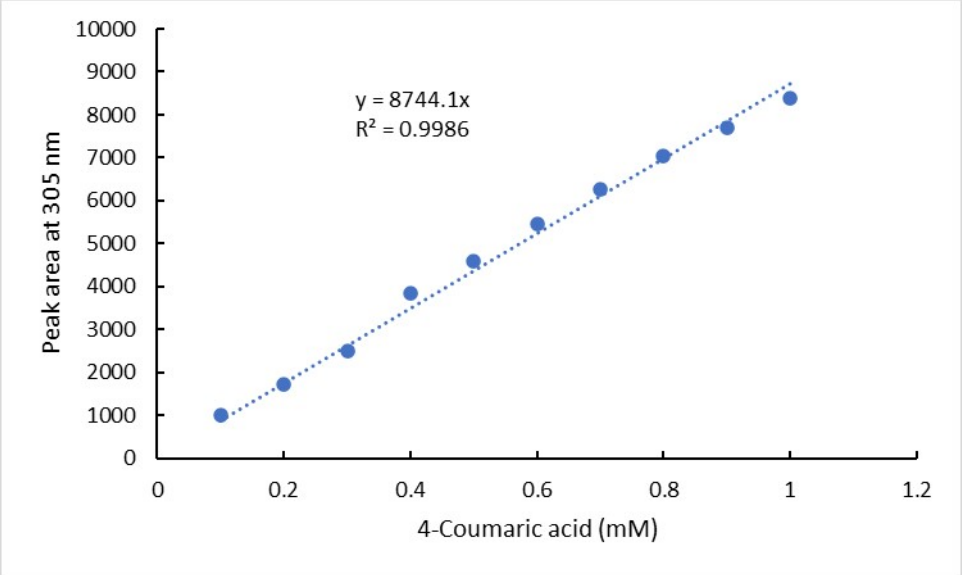

Fig. S13 Standard curve for 4-coumaric acid.

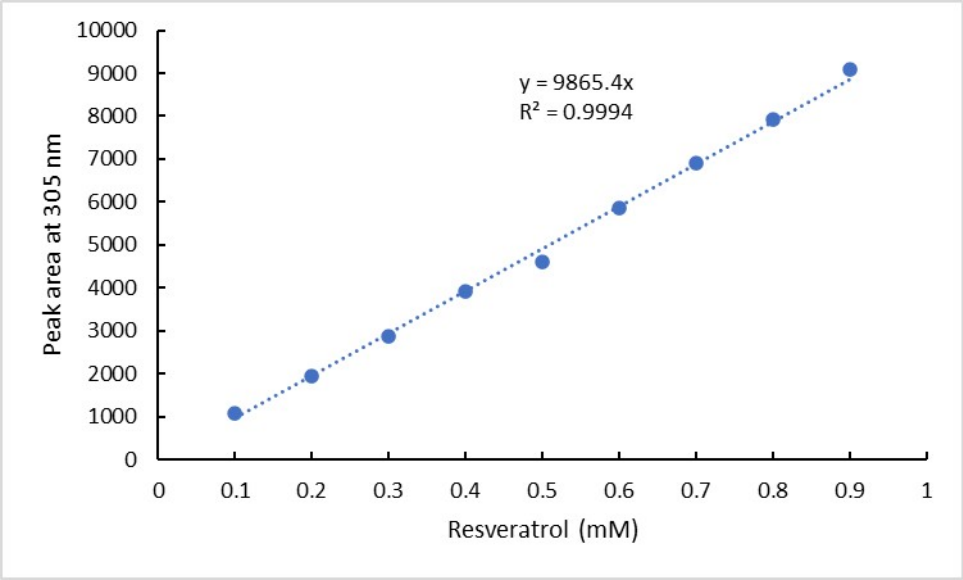

Fig. S14 Standard curve for resveratrol.

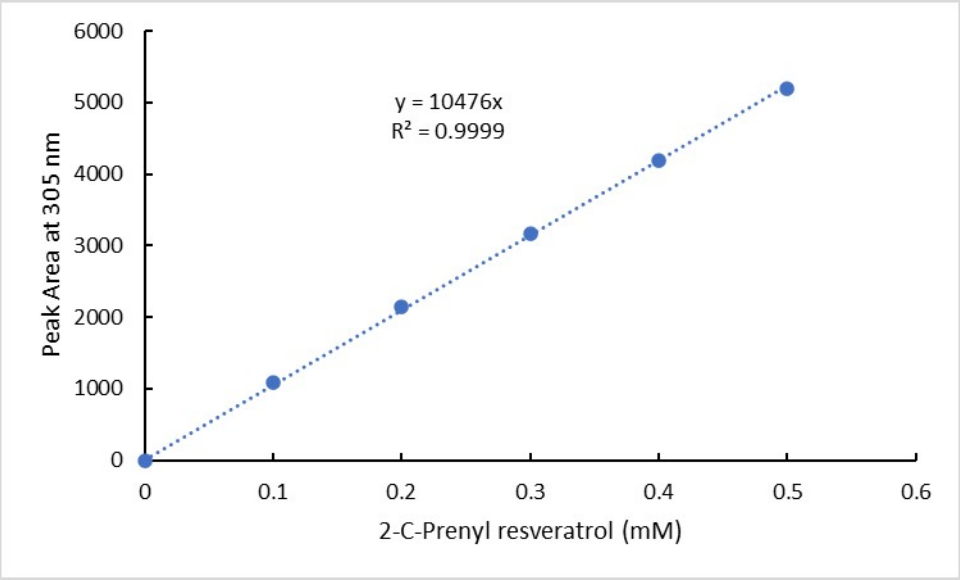

Fig. S15 Standard curve for 2-C-prenyl resveratrol.

Media compositions:

LB: tryptone 10 g/L, sodium chloride 10 g/L, yeast extract 5 g/L, sterilized at 121 ℃ for 20 min.

Minimal M9: disodium hydrogen phosphate 6.8 g/L, potassium dihydrogen phosphate 3.0 g/L, sodium chloride 0.5 g/L, ammonium chloride 1.0 g/L, calcium chloride 0.01 g/L, sterilized at 121 ℃ for 20 min. The medium was supplemented with glucose 10 g/L, magnesium sulfate 0.12 g/L before use. Glucose and magnesium sulfate were sterilized separately at 115 ℃ for 30 min.

TB: tryptone 12 g/L, yeast extract 24 g/L, glycerol 4 mL/L, dipotassium phosphate 12.54 g/L, potassium dihydrogen phosphate 2.31 g/L, sterilized at 121 ℃ for 20 min.

EZ Rich Defined Medium was purchased from Coolaber (Beijing) Technology (product No. MK0106) containing 10×MOPS mixture, 10×ACGU, 5×Supplement EZ, diluted with sterilized water and supplemented with 0.23 g/L sterilized dipotassium phosphate and 2 g/L glucose.

Mineral medium: diammonium hydrogen phosphate 4 g/L, potassium dihydrogen phosphate 13.54 g/L, citric acid 1.7 g/L, yeast extract 5 g/L, sterilized at 121 ℃ for 20 min. The medium was supplemented with glucose 10g/L, magnesium sulfate 0.7 g/L, 200×trace elements 5ml/L before use. Glucose and magnesium sulfate were sterilized separately at 115 ℃ for 30 min. 200×trace elements were sterilized through filtration with 0.22 μm filter.

200×trace elements: ferrous sulfate 11 g/L, calcium chloride 4 g/L, copper sulfate 1.28 g/L, zinc sulfate 2.47 g/L, ammonium heptamolybdate 0.19 g/L, manganese sulfate 0.68 g/L, sodium tetraborate 0.02 g/L, dissolved in 1 M HCl filtered with 0.22 μm filter.