

## **Manipulation of the precursor supply for high-level production of longifolene by metabolically engineered *Escherichia coli***

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### **Supplementary figure captions**

**Figure S1** Codon optimization of the longifolene synthase gene. wlgfs, wild-type longifolene synthase gene; olgfs, codon-optimized longifolene synthase gene. The identical codons are shown in dark blue and the optimized codons are shown in light blue. The amino acid encoded by each codon is shown as its abbreviation.

**Figure S2** Time-course profiles for cell density (OD<sub>600</sub>) and mevalonate production during shake-flasks culture of strain BL21/pA-mvaES. Cultures were performed in 500 ml shake flasks containing 50 ml liquid M9 mineral medium supplemented with 1 mM MgSO<sub>4</sub> and 20 g/L glucose. Error bars represent the range of three independent fermentations.

**Figure S3** Schematic diagram of the expression constructs containing the genes encoding longifolene biosynthesis pathway. Igfs, longifolene synthase; ispA, *E. coli* FPP synthase; isoA, *B. trispora* FPP synthase; erg20, *S. cerevisiae* FPP synthase; mvaE, acetoacetyl-CoA synthase/HMG-CoA reductase; mvaS, HMG-CoA synthase gene; erg19, mevalonate kinase; erg8, phosphomevalonate kinase; erg12, mevalonate pyrophosphate decarboxylase; idi1, IPP isomerase.



# Figure S1

wlgfS	ATGGCTCAGATTTCTAAATGTTCTGTCACCTGTC CGCCGAGT TGAACGAATCCTCCATCATC	60
olgfS	ATGGCTCAGATCAGTAAATGCTCTTCTCTGTCTGCTGAAC TGAACGAATCTCTATCATC	60
wlgfS	TCTCATCATCATGGAATCTGTGGGACGACGAT TTCATACAATCTCTTAAAGTCGTCCAAT	120
olgfS	TCTCACCACCCACGGTAACTGTGGGACGACGACTTCATC CAGTCTCTGAAATCTCTTAACT	120
wlgfS	GGGGCACCTCAAATATCATGAACGCGCTGCCGAAACTT GTTGAAGAAATCAAAGAAATTTAGTT	180
olgfS	GGTGTCTCCGCAGTACCACGAACGTGCTGCTAAACTG GTTGAAGAAATCAAAGAACTTGGTT	180
wlgfS	GTGAGCGAGATGAAAGAT TGCAACGATGATTTAATCAGACGTCTT CAGATGGTTGACAT	240
olgfS	GTTTCTGAAATGAAAGACTGCAACGACGACCTGATCCGT CGTCTGCAGATGGTTGACATC	240
wlgfS	TTTGAATGTTCTAGGAATTGATCGGCACCTT CAGCATGAAATA CAAGTTGCTCTTGATTAC	300
olgfS	TTCCGAATGCCTGGTATCGACCGTCACTTCCAGCACGAAATC CAGGTTGCTCTCGACTAC	300
wlgfS	GTGTACAGATAT TGGAACCAGCTGGAAGGCATCGGTAT TGGATCAAGAGATTCCTTATC	360
olgfS	GTCTACCGCTACTGGAACCAGCTGGAAGGTATCGGTATCGGT TCTCGTGACTCTCTGATC	360
wlgfS	AAAGATTTCAAATGCTACAGCTT TGGGATTTCTGGGCTCTCCGACTGCATCGATATAACGTT	420
olgfS	AAAGACTTCAAACGCTACCGCTCTGGGTTTCCGTGCTCTGCGTCTGCACCGTTACAACGTT	420
wlgfS	TCCTCAGATGCTTGGAGAATTTCAAGAACGAAAACGGG CAGTTCTTCTGCAGTTCCAG	480
olgfS	TCTTCTGACGTTCTGGAAAACTTCAAAAACGAAAACGGT CAGTTCTTCTGCTCTTCTTACC	480
wlgfS	GTTGAAGAAAAAGAAGT GAGATGCATGTTGACGT TATTTCGAGCTTCAGAAATTTCAATTT	540
olgfS	GTTGAAGAAAAAGAAGTTCGTGTCATGCTGACCCTGTTCCGTGCTTCTGAAATCTCTTTCT	540
wlgfS	CCCGGAGAAAAAGT GATGGACGAGGCAAGGCGTTTACACAACA GAATATCTAACC AAAAGTT	600
olgfS	CCGGGTGAAAAAGTTATGGACGAAGCTAAAAGCTTTCACACCGAATACCTGACC AAAAGTT	600
wlgfS	TTAACGGGAGTGGATGTAACG GACGTCATCAAAGCCTTTTGAGAGAAGTGAAGTATGCC	660
olgfS	CTGACCGGTGTTGACGTTACCGACGTTAAC CAGTCTCTGCTGCGTGAAGTTAAATACGCT	660
wlgfS	CTGGAGTTTCCATGGCATTGCAGTTTGCCGAGATGGGAGGCAAGGAGCTTTATCGAAATA	720
olgfS	CTGGAATTCCTCCGTGGCACTGCTCTCTGCCGCGTGGGAAGCTCGTTCTTTATCGAAATC	720
wlgfS	TGTGGACAAAACGATTCATGGCTCAAGTGGATATGAACAAACGAGTTT TAGAGTTGGGG	780
olgfS	TGCGGT CAGAACGACTCTGGCTGAAATCTATCATGAACAAACGTTTCTGGAAC TGGCT	780
wlgfS	AAATGGACTTCAAATATTTTGCAATGGGCACATCATAGAGA ACTACAGCTTCTGTCAAGT	840
olgfS	AAACTGGACTTCAACATCTGCAAGTGGGCTCACACCGTGAACTGCAGCTGCTGTCTTCT	840
wlgfS	TGGTGGTCACAAATCGGATATAGCGCAGCAGAA TTCTATCGGAAAGCGTCACGTTGAATTT	900
olgfS	TGGTGGTCTCAGTCTGACATCGCTCAGCAGAACTTCTACCGTAAAAGCGTCACGTTGAATTC	900
wlgfS	TACTTATGGGTGTTATAGGCACGTTTCGAGCCGGAGTTT TCAACATGCAGAATCACCTTT	960
olgfS	TACCTGTGGGTGTTATCGGTACCTTCGAAACCGGAATTCCTTACCTGCGTATCACCTTC	960
wlgfS	GCAAAAATCTCTACACTGATGACTATTTCTAGATGACCTCTACGATAC TACGGAACGTTG	1020
olgfS	GCTAAAATCTCTACCCTGATGACCATCTTGACGACCTGTACGACACCCACGGTACCTG	1020
wlgfS	GAACAACCTCAAATCTTCACAGAGGGAGTGAACGATGGGATCTTTCGTTAGTAGACCGC	1080
olgfS	GAACAGCTGAAAATCTTCACCGAAGGTGTTAAACGTGGGACCTGTCTCTGGTTGACCGT	1080
wlgfS	CTTCCAGACTACATAAAGATTACATTCGAATTCCTTCTCAACACATCTAAATGAATTGAT	1140
olgfS	CTGCCGACTACATCAAATCACCTTCGAATTCCTTCTCAACACCTCTAACGAAC TGATC	1140
wlgfS	GCTGAAGTTGCGAAAACGCAAGAGCGGGATATGTCAGCCTACATA CGAAAACATGGGAA	1200
olgfS	GCTGAAGTTGCTAAAACCCAGGAACGTGACATGTCGCTTACATCCGTAAAACCTGGGAA	1200
wlgfS	CGATACCTTGAAGCTTATTTGCAAGAAGCGGAATGGATAGCAGCTCGACACGTTCCCTAGG	1260
olgfS	CGTTACCTTGAAGCTTACCTGCAGGAAGCTGAATGGATCGCTGCTCGTCACGTTCCGACC	1260
wlgfS	TTTGATGAGTACATGAAGAATGGCATATCCAGTTCTGGGATGTGTATACTAAATTTGTAT	1320
olgfS	TTTCGACGAAATACATGAAAACGGTATCTCTTCTTCTGGTATGTGCATCTGAACCTGTAC	1320
wlgfS	TCGCTTCTGTTAATGGGGCAACTTCTACCTGACGACGTTCTGGAGCAAATACACTCTCCA	1380
olgfS	TCTCTGCTGCTGATGGGTGAGCTGCTGCCGGACGACGTTCTGGAAACAGATCACTCTCCG	1380
wlgfS	TCCAAGATCCACGAGCTTGTAGAAATGACGGCCAGACTGGTAGACGACTCAAAGGATTTT	1440
olgfS	TCTAAAATCCACGAACTGGTGAACCTGACCGCTCGTCTGGTTGACGACTCTAAAGACTTC	1440
wlgfS	GAGACGAAGAAAGTTGGTGGGGAGTTAGCTTTCAGGTATAGAGTGT TACGTGAAAGACAAC	1500
olgfS	GAAACCAAAAAAGTTGGTGGTGAACCTGCTTCTGGTATGAATGCTACGTTAAAGACAAC	1500
wlgfS	CCTGAATGTACACTGGAAGATGCTTCAAATCATCTAAATGGCCTCCTTGATCTTACCGTT	1560
olgfS	CCGGAATGCACCCTGGAAGACGCTTCTAACACACCTGAACGGTCTGCTGGACCTGACCGTT	1560
wlgfS	AAGGAATGAAATGGGAGTTTGTAAAGCATGACAGCGTGGCCTGTGTGTTTCAAGAAAGTTC	1620
olgfS	AAAGAACTGAACTGGGAATTCGTTCCTCACGACTCTGTGCTCTGTGCTTCAAAAAATTC	1620
wlgfS	GCCTTCAACGTTGCACGAGGCCTCCGACTTATCTACAAATACAGAGACGGCTTTGACGTT	1680
olgfS	GCTTTC AACGTTGCTCGTGGTCTGCTCTGATCTACAAATACCGTGACGGTTTCGACGTT	1680
wlgfS	TCTAACCAGGAGATGAAAACCCACATATTCAAATCTCATCGATCCACTTACTTAG	1737
olgfS	TCTAACCAGGAAATGAAAACCCACATCTTCAAATCTGATCGACCCGCTGACCTAA	1737



Figure S2

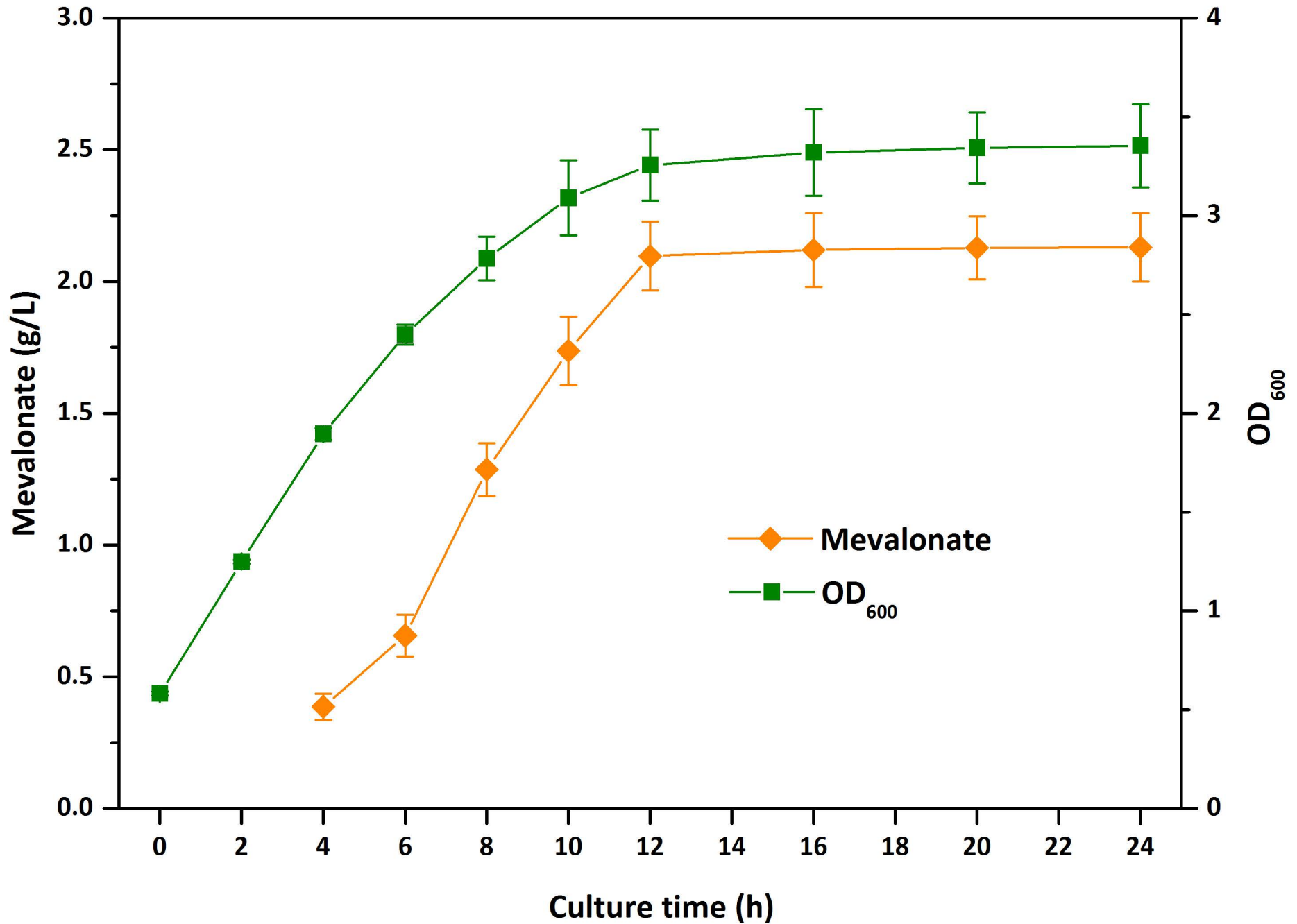


Figure S3

