

Supplementary Material

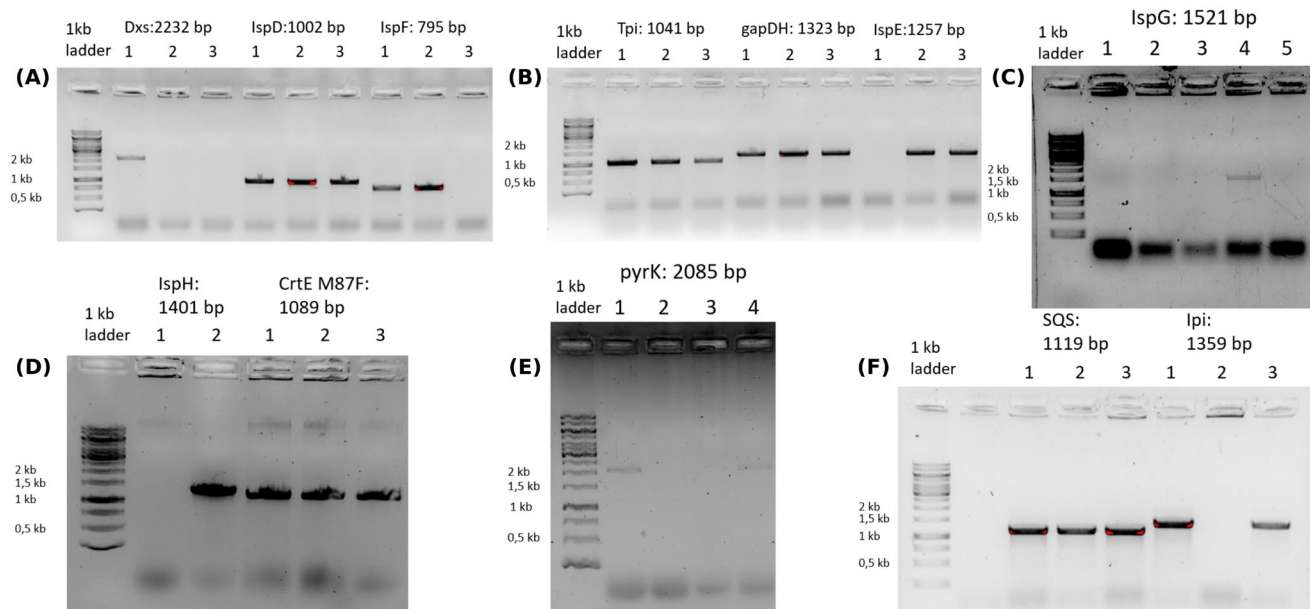
Supplementary Table 1. Plasmids used in this study and information regarding their origin.

Plasmid name	Source
pSHDY rhaS	(Behle <i>et al.</i> 2020)
pEERM4	(Englund <i>et al.</i> 2015)
pEERM4 Prha dxs	This study
pEERM4 Prha ispD	This study
pEERM4 Prha ispE	This study
pEERM4 Prha ispF	This study
pEERM4 Prha ispG	This study
pEERM4 Prha ispH	This study
pEERM4 Prha idi	This study
pEERM4 Prha sqs	This study
pEERM4 Prha crtE	This study
pEERM4 Prha gap2	This study
pEERM4 Prha pyrK	This study
pEERM4 Prha tpi	This study

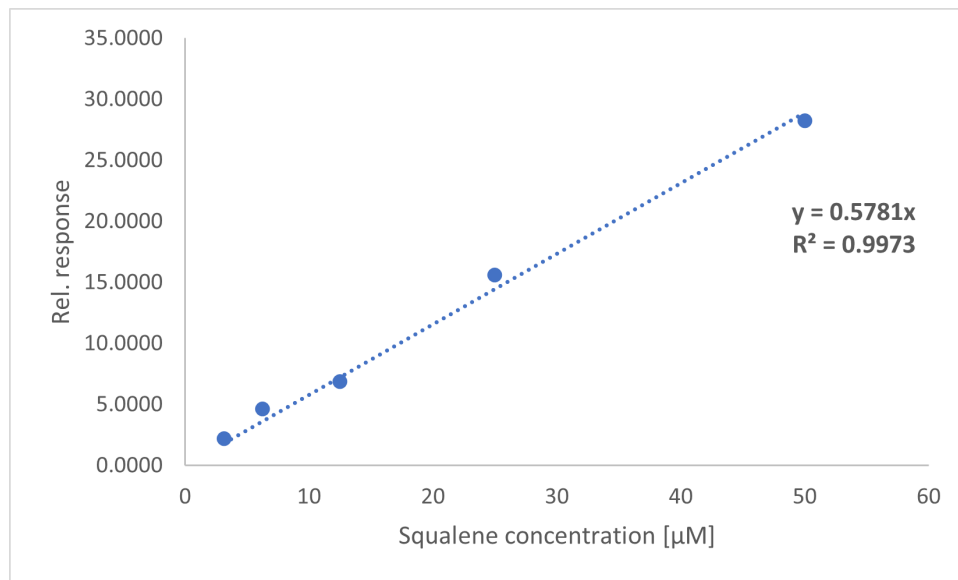
Supplementary Table 2. DNA sequences of primers used in this study and their modifications for cloning purposes.

Gene	P fwd (5'-3')	P rev (5'-3')	Other modifications
<i>idi</i>	TGACATGGCTAGCGATA GCACCCCCCACC GTAA	AGCCTGCAGTTAAGGTT TAGTTAACCTTT	
<i>dxs</i>	TGACATGGCTAGCCACATC AGCGAACTGACCCACCCCAA TGAG	GCTACTGCAGCTAACTAACTC CAGGAGCGACA ACTG	
<i>sqs</i>	TGACATGGCTAGCTCAG GAGTTGATCGCATGAGC	AGCTACTGCAGCTAACTGG CAATAACCCGATTAA	silent mutation in 110L to remove NheI
<i>ispD</i>	TGACATGGCTAGCCATTT ACTAATTCCAGCGGC	GCTACTGCAGTCAGGCGGA TTTTGCCGACC	
<i>ispE</i>	TGACATGGCTAGCCATT CCTACACCTCCATGCCCCG	GCTACTGCAGTCAATTATTC ATAATTTGGATGCCG	
<i>ispF</i>	TGACGCTAGCACTGCTC TACGCATCGGCAACGG	GCTACTGCAGTTACCCTTCT TTGATTAACAAAGCCACG	
<i>ispG</i>	TGACATGGCTAGCGT AACCGCTTCCCTGCCGACC	GCTACTGCAGTTAAGGGTCA ACCCAACGGC	
<i>ispH</i>	TGACATGGCTAGCGATACCA AAGCTTTTAAACGGTCTCTGC	GCTACTGCAGCTATCCCGCA ATTTCTAGGACG	
<i>gap2</i>	TGACATGGCTAGCACTA GAGTAGCAATTAACGG	GCTACTGCAGCTATTTCCAGTT TTTAGCCAC	silent mutation in 192A to remove NheI
<i>pyrK</i>	TGACATGGCTAGCCAAA CGTCTCCCCCTCCCCGTCG	GCTACTGCAGCTATCCTTTGG ACACCGGGGGTAATGC	
<i>tpi</i>	TGACATGGCTAGCGTGC GAAAAATCATTATTGC	GCTACTGCAGTCAGGGCTGA AAATTAACAA	
<i>dxs</i> qPCR	CCCATAACCAGACTAATGGTG ATT	TGCTGAGGCGGACTTTATTT	
<i>sqs</i> qPCR	GCGATCGATGAAGTGGAAGA	CGTCGCACTCTGGAGATTAAAG	
<i>rpoA</i> qPCR	CCATGAGTTCGCCACTATTCT	GGCTGATCGGTGTAGCTTT	
Colony PCR	ATGCGAATTCGCGGCCGCTTC TAGAG	CTGCAGCGGCCGCTACTAGT ATATAAACGCAGAAAGGCC CACCCGAAGG	Colony PCR primers for insert in pEERM4

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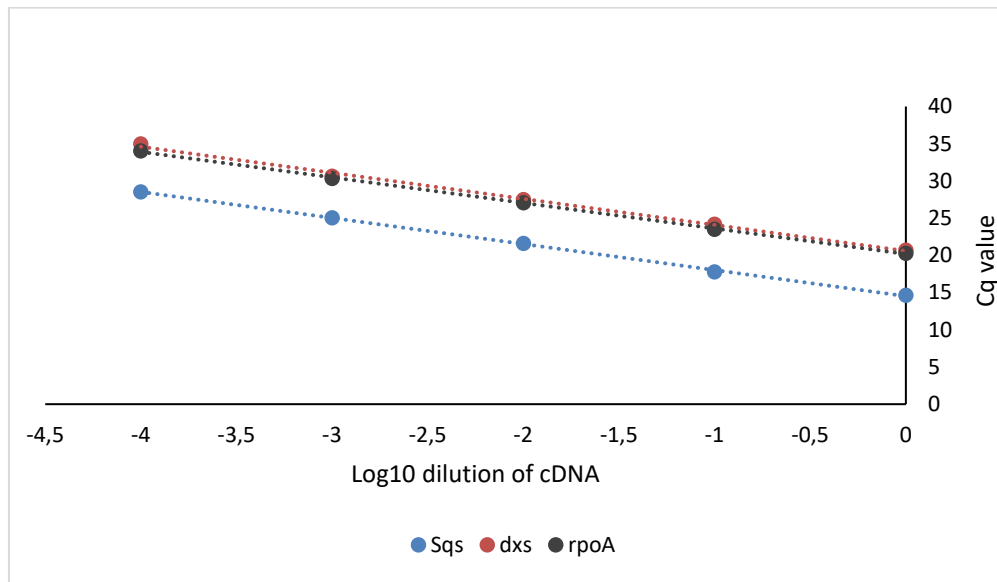


Supplementary Figure 1. Agarose gel electrophoresis of colony PCR products to prove the integration of the respective gene into the genome through heterologous recombination into the neutral site 2 (NS2). Number denote tested colonies, sizes of the expected PCR bands are shown. PCR was carried out with the colony PCR primers shown in Supplementary Table 2 (A) Dxs: 2232 bp, IspD: 1002 bp, IspF: 795 bp (B) Tpi: 2041 bp, gapDH: 1323 bp, IspE: 1257 bp (C) IspG: 1521 bp (D) IspH: 1401 bp, CrtE M87F: 1089 bp (E) PyrK: 2085 bp (F) Sqs: 1119 bp, Ipi: 1359 bp



Supplementary Figure 2. GC-MS calibration curve for squalene after extraction of 50, 25, 12.5, 6.25 and 3.125 μM of squalene using the method for squalene extraction from *Synechocystis* cells. Relative response is in relation to the 25 μM β -sitosterol standard, which was solved in the acetone used for extraction.

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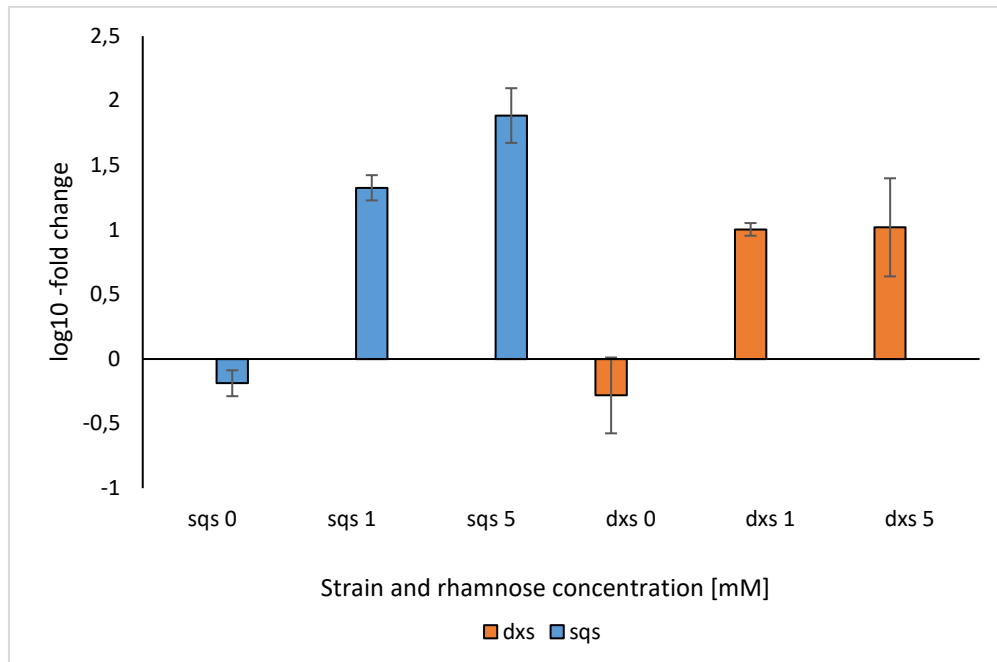


Supplementary Figure 3: Cq values of qRT-PCR primer pairs used with dilution series of cDNA. Primers for *sqs*, *dxs* and *rpoA* were tested with cDNA extracted after 3 days from *Synechocystis* Δshc pEERM P_{rha} *sqs* pSHDY *rhaS*, *Synechocystis* Δshc pEERM P_{rha} *dxs* pSHDY *rhaS* and *Synechocystis* Δshc pSHDY *rhaS*, induced with 5 mM rhamnose respectively. Primer sequences are shown in Suppl. Table 2.

Supplementary Table 3: Primer efficiencies of qRT-PCR primers used with dilution series of cDNA. Primers for *sqs*, *dxs* and *rpoA* were tested with cDNA extracted after 3 days from *Synechocystis* Δshc pEERM P_{rha} *sqs* pSHDY *rhaS*, *Synechocystis* Δshc pEERM P_{rha} *dxs* pSHDY *rhaS* and *Synechocystis* Δshc pSHDY *rhaS*, induced with 5 mM rhamnose respectively. Primer sequences are shown in Suppl. Table 2.

Primer target gene	Efficiency
Sqs	92.96103
Dxs	92.99725
RpoA	95.64115

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Supplementary Figure 4: Results of qRT-PCR for genes *sqs* and *dxs* as log10-fold changes compared to the control strain in the strains *Synechocystis* Δshc pEERM P_{rha} *sqs* pSHDY *rhaS*, *Synechocystis* Δshc pEERM P_{rha} *dxs* pSHDY *rhaS*, respectively. Values were calculated *via* the $2^{-\Delta\Delta C_T}$ method, using *rpoA* as a housekeeping gene and cDNA extracted from *Synechocystis* Δshc pSHDY *rhaS*, treated with the same rhamnose concentration as a control strain. Primer sequences are shown in Suppl. Table 2. The mean and standard deviation of two biological replicates is shown, which were measured in technical triplicates.

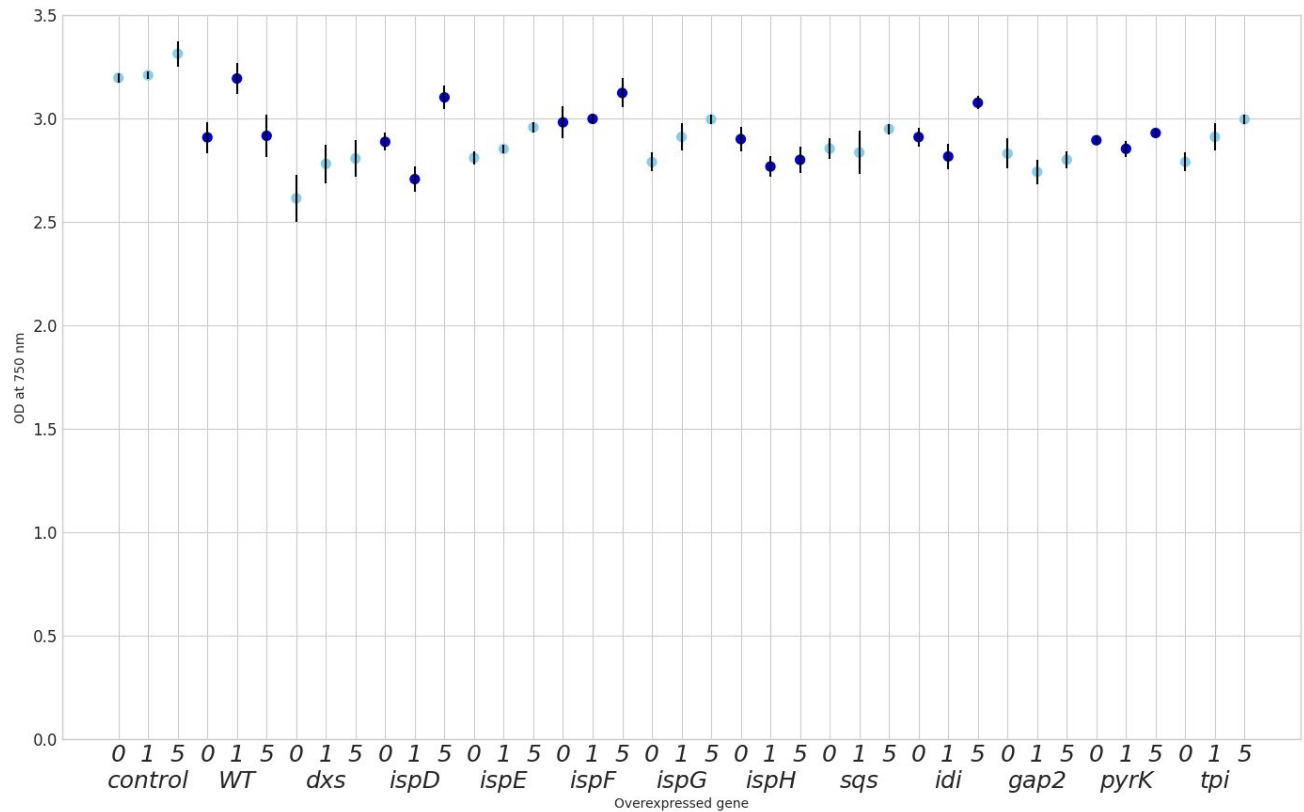
Supplementary Table 4. Squalene yield of all controls and overexpression strains under three different inducer concentrations. WT = Wild type *Synechocystis* sp. PCC 6803, Δshc is the control strain, in which overexpression of the specified genes took place. Mean values and standard deviations of three biological replicates are shown.

Strain	Rhamnose concentration [mM]	Yield [mg L ⁻¹]	Yield [mg OD750 -1 L ⁻¹]	[mg gCDW ⁻¹]
WT	0	0.003 ± 0.101	0.001 ± 0.023	0.002 ± 0.058
	1	0.01 ± 0.03	0.003 ± 0.008	0.006 ± 0.011
	5	0.004 ± 0.06	0.001 ± 0.017	0.003 ± 0.021
Δshc	0	1.5 ± 0.1	0.47 ± 0.03	0.94 ± 0.06
	1	1.41 ± 0.02	0.44 ± 0.01	0.89 ± 0.02
	5	1.39 ± 0.06	0.42 ± 0.01	0.84 ± 0.02
<i>dxs</i>	0	1.47 ± 0.04	0.56 ± 0.04	1.13 ± 0.07
	1	1.64 ± 0.22	0.59 ± 0.06	1.18 ± 0.12
	5	1.76 ± 0.27	0.62 ± 0.08	1.26 ± 0.15
<i>ispD</i>	0	1.85 ± 0.12	0.64 ± 0.04	1.29 ± 0.07
	1	1.91 ± 0.15	0.71 ± 0.04	1.42 ± 0.08
	5	2.45 ± 0.14	0.79 ± 0.04	1.59 ± 0.08
	0	2.53 ± 0.07	0.9 ± 0.02	1.81 ± 0.03

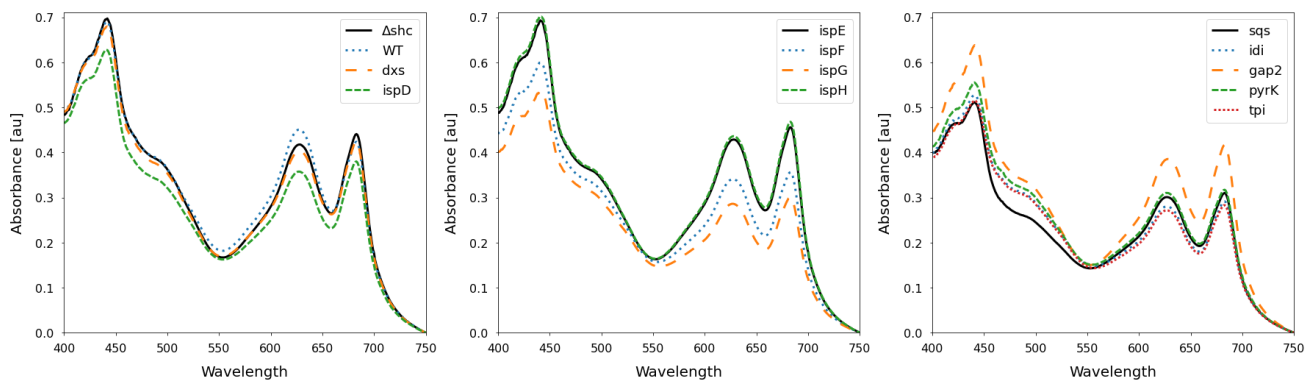
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<i>ispE</i>	1	2.52 ± 0.03	0.89 ± 0	1.78 ± 0.01
	5	2.64 ± 0.03	0.89 ± 0.01	1.79 ± 0.02
<i>ispF</i>	0	2.29 ± 0.02	0.77 ± 0.03	1.55 ± 0.05
	1	2.28 ± 0.06	0.76 ± 0.01	1.53 ± 0.03
	5	2.25 ± 0.08	0.72 ± 0.02	1.45 ± 0.05
<i>ispG</i>	0	1.72 ± 0.19	0.62 ± 0.06	1.24 ± 0.13
	1	1.98 ± 0.03	0.68 ± 0.03	1.37 ± 0.05
	5	2.01 ± 0.2	0.67 ± 0.06	1.35 ± 0.12
<i>ispH</i>	0	2.66 ± 0.05	0.92 ± 0.02	1.85 ± 0.03
	1	2.86 ± 0.04	1.03 ± 0.02	2.08 ± 0.03
	5	2.93 ± 0.17	1.05 ± 0.04	2.1 ± 0.08
<i>sqs</i>	0	2.13 ± 0.03	0.75 ± 0	1.5 ± 0.01
	1	4.96 ± 0.29	1.76 ± 0.16	3.53 ± 0.33
	5	6.23 ± 0.31	2.11 ± 0.11	4.25 ± 0.22
<i>Idi</i>	0	2.09 ± 0.09	0.72 ± 0.02	1.44 ± 0.04
	1	2.19 ± 0.07	0.78 ± 0.01	1.56 ± 0.02
	5	2.52 ± 0.04	0.82 ± 0.02	1.65 ± 0.04
<i>gap2</i>	0	1.66 ± 0.36	0.59 ± 0.13	1.18 ± 0.27
	1	1.85 ± 0.22	0.67 ± 0.07	1.36 ± 0.15
	5	1.98 ± 0.14	0.71 ± 0.05	1.42 ± 0.11
<i>pyrK</i>	0	1.94 ± 0.06	0.67 ± 0.03	1.35 ± 0.05
	1	1.93 ± 0.05	0.68 ± 0.02	1.36 ± 0.05
	5	2.28 ± 0.19	0.78 ± 0.06	1.57 ± 0.13
<i>tpi</i>	0	1.84 ± 0.03	0.66 ± 0.02	1.33 ± 0.05
	1	2.03 ± 0.06	0.7 ± 0.01	1.4 ± 0.02
	5	2.03 ± 0.03	0.68 ± 0.01	1.36 ± 0.03

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Supplementary Figure 5. Effect of overexpressions on growth of the different strains after 3 days of growth with the indicated rhamnose concentration. Control denotes the Δshc strain in which the overexpression strains were constructed, WT denotes the *Synechocystis* sp. PCC 6803 wild type. Average values from three biological replicates, error bars represent the standard deviation.



Supplementary Figure 6: Spectra of *Synechocystis* cells after 3 days' incubation with 5 mM rhamnose, measured in 1 cm cuvettes. OD₇₅₀ values were equalized across all measurements in the cuvettes, then the spectra were baseline corrected by subtracting the OD₇₅₀ value.