

Additional File 1

Table S1. **Plasmids used in this study**

Plasmid name	Description
pLR1	Yep_pGAL1-3Xflag-M60TASYBRE-tCYC1
pLR2	Yep_pSV1-3Xflag-M60TASY-tCYC1
pLR3	Yep_pVAN1-3Xflag-M60TASYBRE-tCYC1
pLR4	Yep_pADH1-3Xflag-M60TASYBRE-tCYC1
pLR5	Yep_pTDH3-3Xflag-M60TASYBRE-tCYC1
pL10	Yep_pGAL1-M60TASYBRE-tCYC1
pLR24	Yep_URA17_pGAL1-3Xflag-M60TASYBRE-tCYC1
pLR25	Yep_Leu2d_pGAL1-3Xflag-M60TASYBRE-tCYC1
pLR33	Yep_pTDH3-M60TASYBACA-tCYC1
pLR34	Yep_pTDH3-M50TTASYBRE-tCYC1
pLR35	Yep_pTDH3-KOZ-M50TTASYBRE-tCYC1
pLR36	Yep_pTDH3--M60TASYBRE-MBP-tADH1 pGAL1-T5aH-tCYC1
pLR37	Yep_pTDH3-CAS9-tADH1
pLR40	Yep_pGAL1-M40TASYBRE-tCYC1
pLR41	Yep_pGAL1-M44TASYBRE-tCYC1
pLR42	Yep_pGAL1-M48TASYBRE-tCYC1
pLR43	Yep_pGAL1-M52TASYBRE-tCYC1
pLR44	Yep_ pGAL1-M54TASYBRE-tCYC1
pLR45	Yep_ pGAL1-M56TASYBRE-tCYC1

pLR46	Yep_ pGAL1-M58TASYBRE-tCYC1
pLR47	Yep_ pGAL1-M62TASYBRE-tCYC1
pLR48	Yep_ pGAL1-M64TASYBRE-tCYC1
pLR49	Yep_ pGAL1-M66TASYBRE-tCYC1
pLR50	Yep_ pGAL1-M68TASYBRE-tCYC1
pLR51	Yep_ pGAL1-M70TASYBRE-tCYC1

Table S2. **List of primers used in the study**

Name	Sequence
M13 F Backbone Ups of Promoter	GTAAAACGACGGCCAGT
M13 R backbone down of Term	CAGGAAACAGCTATGAC
TASY1	GGTAGAGACATGTTGGCCCA
TASY2	AGCGGAGTATTCTGGTTCGA
TASY3	TCCGTCATTGCCTTGTCTGT
TASY4	ACCGATACCCAAATGTTCAATATTGT
beginning of TASY with 3Xflag F	TGACAAATCCTCTTCCACTGGT
213 BP TASY R	ATCACCCAAGGCGTTGAACA
TASY5	TCTGCTGGTCAAACCTCACGT
Backbone R after Term	CAAGCGCGCAATTAACCCTC
(backbone1)_forward	TACAGACACTGGTCTGAACGTGGTATTGGTTGGGG

(backbone1)_reverse	GCGGCCAGCAAAACTAAGTTTCTTAGTATGATCCAATATCAAA GGAAATG
(URA17)_forward	TGGATCATACTAAGAACTTAGTTTTGCTGGCCGCATCTTCTCA AATATGC
(ura17)_reverse	AGACGAACTATATACGCAGGAAACGAAGATAAATCATGTCTGA AA
(bakbone2)_forward	CGACATGATTTATCTTCGTTTCCTGCGTATATAGTTTCGTCTACC CTATGAACA
(Backbone2)_reverse	ATACCACGTTTCAGACCAGTGTCTGTAGACGTAATCCAAAGCAC C
(PAr1TASY)_forward	AAGCTCCCTCGTGCGCTCTCCTGTTCCGACC
(PAr1TASY)_reverse	AGAAATATCTTGACCGCAGTTTATATATATTTCAAGGATATACC ATTCTAA TGT CTG
(part2TASY)_forward	CCTTGAAATATATATAAACTGCGGTCAAGATATTTCTTGAATCA GGCGC
(part2TASY)_reverse	GAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGG
(Backbone1)_forward	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
(Backbone1)_reverse	GACATAACTAATTACATGACTCGAGAAGAGATCACACCTTCCT
(Backbone2)_forward	AGGTGTGATCTCTTCTCGAGTCATGTAATTAGTTATGTCACGCT TACGTTACGCCC
(Backbone2)_(CAN1Y)_reverse	AGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTCCTCCATA GAGAACGTATCAAAGTCCCATTCGCCACCCGAAGG
Gal1p F 3/4	GGTAATTAATCAGCGAAGCGATG
Gal1p F 1/2	CCCACAAACCTTCAAATCAACG
3xflag3	GGTTTGAATGACGTTATGATGCC

3Xflag4	CAAGGCGTTGAACATATCCTTG
DOWNTASY and UPCYC1 R	TCGGTTAGAGCGGATTCAAAC
Backbone after CYC1T R	CGC AAC GCA ATT AAT GTG AGT
(ORICYC1)_(KOZAK- SEQ)_reverse	CCGCAGACATTTTTTTGTTTGTTTATGTGTGTTTATTCGAAAC
GAL1 1/2 F	AGTAACCTGGCCCCACAAAC
Reverse3Xflag	ACCCAATTTAAGGCTTGTGGGA
(ORICYC1)_forward	GGTATATCCTTGAAATATATATAGGAGAAAATACCGCATCAGG AAATTG
BACKBONEtruncatedleu2d) _reverse	CCTGATGCGGTATTTTCTCCTATATATATTTCAAGGATATACCA TTCTAATGTCTGCCCCTAAGAAGATCGTCG
CYC1T F Beginning	ATCCGCTCTAACCGAAAAGGAAGG
(vectorpart1)_reverse CASURA17	GATTTTGTTGATGCCATTATAGTTTTTTCTCCTTGACGTTAAAGT ATAGAGG
(vectorpart1)_forward CAS URA17	GGTGTCTATTTTCTCTTCCATAAAAAAGCCTGACTCCACTTCC CGC
vectorpart2_forward CASURA17	TTAATTTGCGGCCAAGCTTGATATCGAATTCCTGCAGCCCG
vectorpart2_reverse CASURA17	TGGAGTCAGGCTTTTTTTATGGAAGAGAAAATAGACACCAAAG TAGCC
term backbone kozak reverse	GAAAATCCTTGCTTAATCATCACCGAAACGCGCG
term backbone kozak forward	CCAATTCAAGTTTGAATCCGCTCTAACCGAAAAGGAAGG

TASYN50 kozak reverse	CCTTTTCGGTTAGAGCGGATTCAAACCTTGAATTGGATCAATGTA GAC
TASYN50 kozak forward	CTGGTCCTGTCGTAATGTCCTCTTCCACTGGTACCTC
promoter backbone kozak reverse	GGAAGAGGACATTACGACAGGACCAGGACCACCACTTCCTCTC ATAGACATTTTTTTGTTTGTTTATGTGTGTTTATTCGAAAC
promoter backbone kozak forward	CGTTTCGGTGATGATTAAGCAAGGATTTTCTTAACTTCTTCGGC
term backbone reverse	GAAAATCCTTGCTTAATCATCACCGAAACGCGCG
term backbone forward	CCAATTCAAGTTTGAATCCGCTCTAACCGAAAAGGAAGG
TASYN50 reverse	CCTTTTCGGTTAGAGCGGATTCAAACCTTGAATTGGATCAATGTA GAC
TASYN50 forward	CTGGTCCTGTCGTAATGTCCTCTTCCACTGGTACCTC
promoter backbone reverse	GGAAGAGGACATTACGACAGGACCAGGACCACCACTTCCTCTC ATTTTGTTTGTTTATGTGTGTTTATTCGAAAC
promoter backbone forward	CGTTTCGGTGATGATTAAGCAAGGATTTTCTTAACTTCTTCGGC
Beginning- TASY MBP primer F	TCCAGTTCGAGTTTATCATTATCAATACTGCCA
Beginning-TASY MBP primer R	GGTTTCAGAAACGACCTTAGAGGTACCAGTGAAGAGG
End- TASY MBP primer F	GGTACCTCTAAGGTCGTTTCTGAAACCTCCTCTACCATCGTCG
End- TASY MBP primer R	GGCTTCTAATCCGTACTGGAGTTAGCATATCTACAATTGGGTGA AATGGGG
CRISPR Backbone 1 forward	GTTTTAGAGCTAGAAATAGCAAGTTAAAAT

CRISPR Backbone 1 reverse	TGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGAC AAAGATT
CRISPR Backbone 2 forward	CCGATAACAAAATCTTTGTCGCTCTTCGCAATGTCAACAGTACC CTTAGTATATTCTCCA
CRISPR Backbone 2 reverse	AAAGTCCCATTGCGCCACCCGAA
(upstream)_reverse	CTAGCTCTAAAACTCTCCCGGGGGCGAGTCG
(upstream)_forward	CGTTTACAATTTCTGATGCGGTATTTTCTCCTTACGC
F-up1622b GAL1	AACATTTAAGTCACAAGGAGGAATATCAGTT
R-up1622b	GCAGTATTGATAATGATAAACTCGAACTGAACTACTTTTCTTAA ACTGTCAACAGCCA
F-dn1622b	TCACCCAATTGTAGATATGCTAACTCCGTAGATACTCGTCTTAC GAAATTGGATATAGTT
R-dn1622b	ACTTTGGAAAAGAAGGTACGGACTACT
F-up1014a GAL1	TATTGACCAGTAGTCATATTACTGGCATATTATC
R-up1014a	GCAGTATTGATAATGATAAACTCGAACTGAGGATATTAATTTTA GGGTCTCTTGATGCAC
F-dn1014a	ACCCAATTGTAGATATGCTAACTCCCCTGTTTTCATCTAGACG TGGGAC
R-dn1014a	GCAATACCAGAGATGACTGGCC
F-TDH3-TASY-MBP-ADH1	CAGTTCGAGTTTATCATTATCAATACTGCC
R-TDH3-TASY-MBP-ADH1	GGAGTTAGCATATCTACAATTGGGTGAA
ADH1 fwd	CCCAAGACCATAAGCGAATTTCTTATG
ADH1 rev	GGAATTGTGAGCGGATAACGGAGTTAGCATATCTAC

1622bUP 8/10 F	TGCGAGCAGACTTTGTCCAT
1622bDOWN 2/10 R	TCGTTCTACAAGTCCAGCCA
1014a UP 8/10 F	AGGATTTCTATGTTCTCGAGGAGA
1014a DOWN 2/10 R	TCCGCCCTTTGCATCTATAAA
1014 (Scaffold)_forward	TTATGTGCGTATTGCTTTCAGTTTTAGAGCTAGAAATAGCAAGT TAAAATAAGGC
Scaffold_reverse	GCACCGACTCGGTGCCAC
Scaffold forward	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
(1622b)_(Scaffold)_forward	GTCACGTTCTGAGGTTACTGTTTTAGAGCTAGAAATAGCAAGT TAAAATAAGGC

Table S3. **DNA sequences**

Gene	Sequence	Reference
<i>E. coli</i> MBP (Yeast codon-optimised)	TCTAAGATTGAAGAAGGTAAGTTGGTTATCTGGAT TAACGGTGACAAGGGTTACAACGGTTTGGCTGAAG TTGGTAAGAAATTTGAAAAAGATACCGGTATCAAG GTCACTGTTGAACACCCAGACAAGTTGGAAGAAA AGTTTCCACAAGTTGCTGCCACTGGTGATGGTCCA GACATTATCTTCTGGGCTCATGACAGATTCGGTGG TTACGCCCAATCCGGTTTGTTAGCCGAGATCACCC CAGATAAGGCTTTTCAAGATAAGTTGTATCCATTC ACTTGGGATGCCGTCAGATACAACGGTAAGTTAAT CGCCTACCCAATTGCTGTTGAAGCTTTGTCTTTGAT CTACAATAAGGACTTGTTACCTAACCACCAAAGA	[1]

	CCTGGGAAGAAATCCCAGCTTTAGATAAGGAGTTA AAAGCTAAGGGTAAGTCCGCTTTGATGTTTAACTT GCAAGAACCATACTTCACTTGGCCATTGATCGCTG CTGATGGTGGTTACGCTTTTAAGTATGAAAACGGT AAATACGACATTAAGGATGTCGGTGTGACAATGC TGGTGCTAAGGCCGGTTTAACTTTCTTAGTCGATTT GATTAAGAATAAACATATGAATGCTGACACTGATT ACTCTATTGCTGAAGCTGCTTTCAACAAGGGTGAA ACCGCTATGACTATTAACGGTCCATGGGCCTGGTC TAACATTGATACCTCTAAAGTCAACTACGGTGTCA CCGTCTTGCCAACTTTTAAGGGTCAACCATCTAAG CCATTCGTCGGTGTCTTGTCTGCCGGTATTAACGCT GCCTCTCCAAATAAGGAATTGGCCAAGGAATTCTT AGAAACTACTTGTTAACCGATGAAGGTTTAGAGG CCGTTAACAAGGATAAGCCATTAGGTGCTGTTGCT TTGAAGTCTTACGAAGAAGAGTTGGCTAAGGATCC AAGAATTGCTGCTACTATGGAAAACGCTCAAAAG GGTGAAATTATGCCAAACATCCCACAAATGTCTGC TTTCTGGTACGCTGTTCGTACCGCCGTCATTAATGC CGCTTCTGGTCGTCAAACCTGTTGATGAAGCCTTGA AGGACGCTCAAACCAGAATTACTAAG	
Mutant variant of <i>Aequorea victoria GFP</i>	AGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCC AATTCTTGTTGAATTAGATGGTGATGTTAATGGGC ACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGAT GCAACATACGGAAAACCTTACCCTTAAATTTATTG	[2]

	CACTACTGGAAAACCTACCTGTTCCATGGCCAACAC TTGTCACTACTCTCACTTATGGTGTTC AATGCTTTT CAAGATACCCAGATCAcATGAAACaGCATGACTTTT TCAAGAGTGCCATGCCCCGAAGGTTATGTACAGGAA AGAACTATATTTTTCAAAGATGACGGGAACTACA AGACACGTGCTGAAGTCAAGTTTGAAGGTGAT ACCCTTGTTAATAGAATCGAGTTAAAAGGTAT TGATTTTAAAGAAGATGGAAACATTCTTGGAC ACAAATTGGAATACA ACTATAACTCACACAAT GTATACATCATGGCAGACAAACAAAAGAATG GAATCAAAGcTAACTTCAA AATTAGACACAAC ATTGAAGATGGAAGCGTTCAACTAGCAGACCA TTATCAACAAAATACTCCAATTGGCGATGGCC CTGTCCTTTTACCAGACAACCATTACCTGTCCA CACAATCTGCCCTTTCGAAAGATCCCAACGAA AAGAGAGACCACATGGTCCTTCTTGAGTTTGT AACAGCTGCTGGGATTACACATGGCATGGATG AACTATACAAA	
Yeast codon-optimised <i>TASY</i>	ATGTCCTCTTCCACTGGTACCTCTAAGGTCGTTTCT GAAACCTCCTCTACCATCGTCGATGACATTCCAAG ATTGTCTGCTAATTACCACGGTGACTTGTGGCATC ATAACGTCATTCAAACCTTGGA AACTCCATTTAGA GAATCTTCTACTTATCAAGAGAGAGCTGATGAATT GGTTGTCAAGATCAAGGATATGTTCAACGCCTTGG	This Study

	GTGATGGTGATATCTCTCCATCTGCTTATGATACTG CCTGGGTCGCTAGATTGGCTACCATCTCTTCCGAC GGTTCGAAAAGCCAAGATTCCCACAAGCCTTAAA TTGGGTTTTTAACAACCAATTGCAAGACGGTTCTT GGGGTATTGAATCTCATTTCTCTTTGTGTGATAGAT TGTTGAACACCACTAACTCCGTCATTGCCTTGTCTG TTTGGAAGACTGGTCACTCTCAAGTTCAACAAGGT GCCGAATTCATTGCCGAAAACCTTGAGATTATTGAA CGAAGAAGATGAATTGTCTCCAGACTTCCAAATCA TTTTTCCAGCTTTGTTGCAAAAGGCCAAGGCCTTA GGTATCAACTTGCCATACGACTTGCCATTCATCAA GTACTTGTCTACTACCAGAGAAGCTAGATTGACTG ACGTCTCCGCTGCTGCTGACAACATTCCAGCCAAC ATGTTGAATGCCTTGGAAGGTTTAGAAGAAGTCAT TGATTGGAACAAGATTATGAGATTCCAATCTAAAG ACGGTTCTTTTTTGTCTTCCCCTGCTTCTACTGCTTG TGTCTTGATGAACACCGGTGATGAGAAGTGTTTCA CTTTCTTGAATAACTTGTTGGATAAATTCGGTGGTT GTGTTCCATGTATGTATTCCATTGATTTATTGAAA GATTGTCTTTAGTTGACAATATTGAACATTTGGGT ATCGGTAGACACTTCAAGCAAGAAATTAAGGGTG CTTTGGATTACGTCTACAGACACTGGTCTGAACGT GGTATTGGTTGGGGTAGAGATTCTTTGGTTCCAGA TTTAAACACTACTGCCTTGGGTTTGCGTACCTTGAG AATGCACGGTTACAACGTTTCTTCCGACGTTTTGA	
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	ACAAC TTCAAGGATGAAAACGGTAGATTCTTTTCC TCTGCTGGTCAAAC TCACGTCGAATTAAGATCCGT TGTCAACTTGTT CAGAGCTTCTGATTTGGCCTTCCC AGACGAAAGAGCTATGGATGATGCTAGAAAATTC GCTGAACCATATTTGAGAGAGGCCTTGGCCACCAA GATTTCTACCAACACTAAGTTGTTCAAGGAGATTG AATACGTTGT CGAATACCCATGGCACATGTCCATC CCAAGATTGGAAGCTAGATCCTATATTGACTCCTA CGACGATAACTACGTTTGGCAAAGAAAAACCTTGT ACCGTATGCCATCCTTGTCTAACTCCAAGTGT TTGG AGTTAGCTAAATTAGACTTCAATATCGTCCAATCC TTACATCAAGAAGAATTGAAATTGTTGACCAGATG GTGGAAGGAATCTGGTATGGCTGATATCAACTTCA CCAGACACAGAGTCGCCGAAGTTTACTTCTCCTCT GCTACTTTTCGAACCAGAATACTCCGCTACCAGAAT TGCTTTCACTAAGATCGGTTGTTTACAAGTTTTATT CGATGATATGGCTGACATTTTCGCTACTTTGGATG AATTGAAGTCTTTCACTGAAGGTGTTAAGAGATGG GATACTTCTTTGTTGCATGAAATCCCAGAATGTAT GCAAACCTGTTTTAAGGTTTGGTTTAAGTTGATGG AGGAAGTCAACAACGACGTTGTTAAGGTTCAAGGT AGAGACATGTTGGCCACATCAGAAAGCCATGGG AATTGTACTTCAACTGTTACGTCCAAGAAAGAGAA TGGTTGGAAGCTGGTTATATCCCTACCTTCGAAGA ATACTTGAAGACTTACGCTATCTCTGTCGGTTTAG	
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	GTCCTTGTACTTTGCAACCAATTTTGTTGATGGGTG AATTAGTTAAGGACGATGTTGTTGAAAAAGTTCAC TACCCATCCAACATGTTCTGAATTAGTTTCCTTGTCC TGGAGATTAACCAATGACACCAAGACTTACCAAGC CGAAAAGGCTAGAGGTCAACAAGCTTCTGGTATCG CTTGTTACATGAAGGATAATCCAGGTGCCACTGAA GAAGATGCTATTAAGCACATTTGTAGAGTTGTTGA CAGAGCTTTGAAGGAAGCCTCTTTCGAATACTTTA AGCCATCCAACGATATCCCAATGGGTTGCAAGTCT TTCATCTTCAACTTGAGATTGTGTGTCCAAATCTTC TACAAGTTTATCGACGGTTACGGTATTGCTAACGA AGAAATCAAGGATTACATTAGAAAAGTCTACATTG ATCCAATTCAAGTTTGA	
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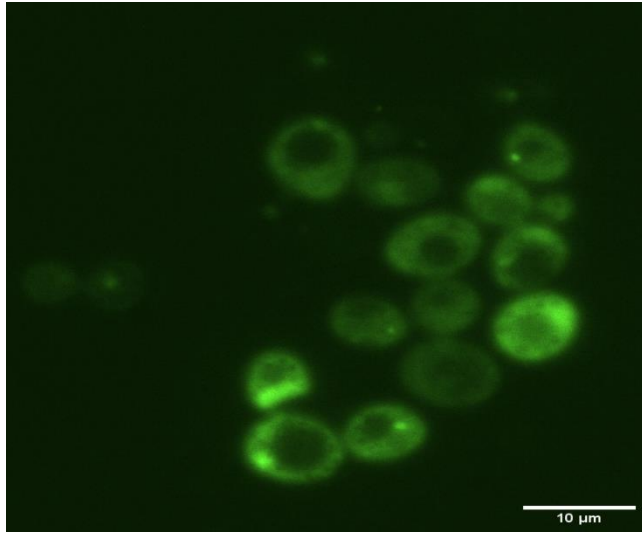


Figure S1. **Fluorescence imaging of GFP-tagged *TASY* showing spotted subcellular localization consistent with poor *TASY* solubility**

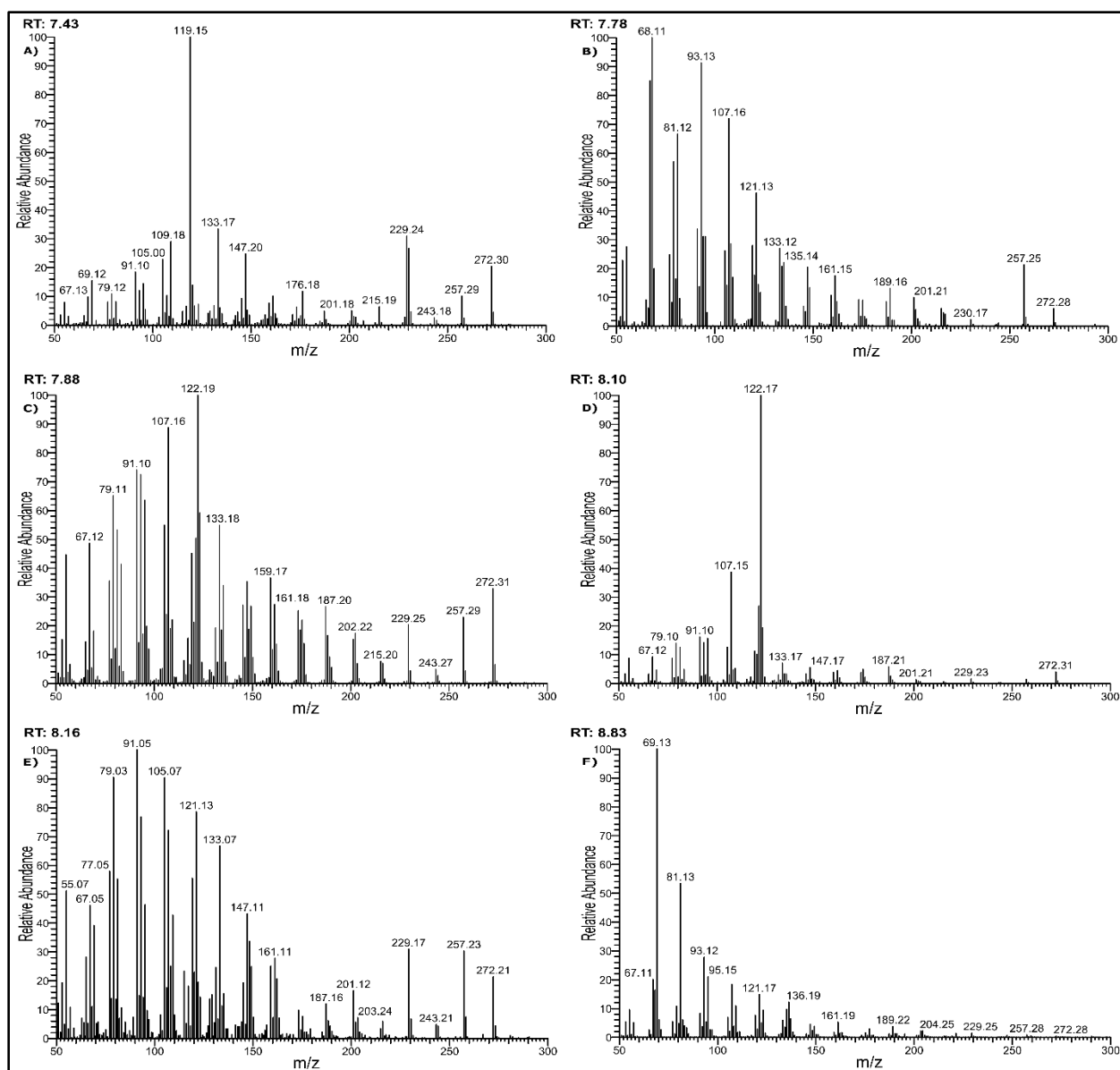


Figure S2. **Mass spectra for compounds produced by LRS5.** A) Verticillene; B) Diterpene 1; C) Iso-taxadiene; D) Taxadiene; E) Diterpene 2; F) GGOH

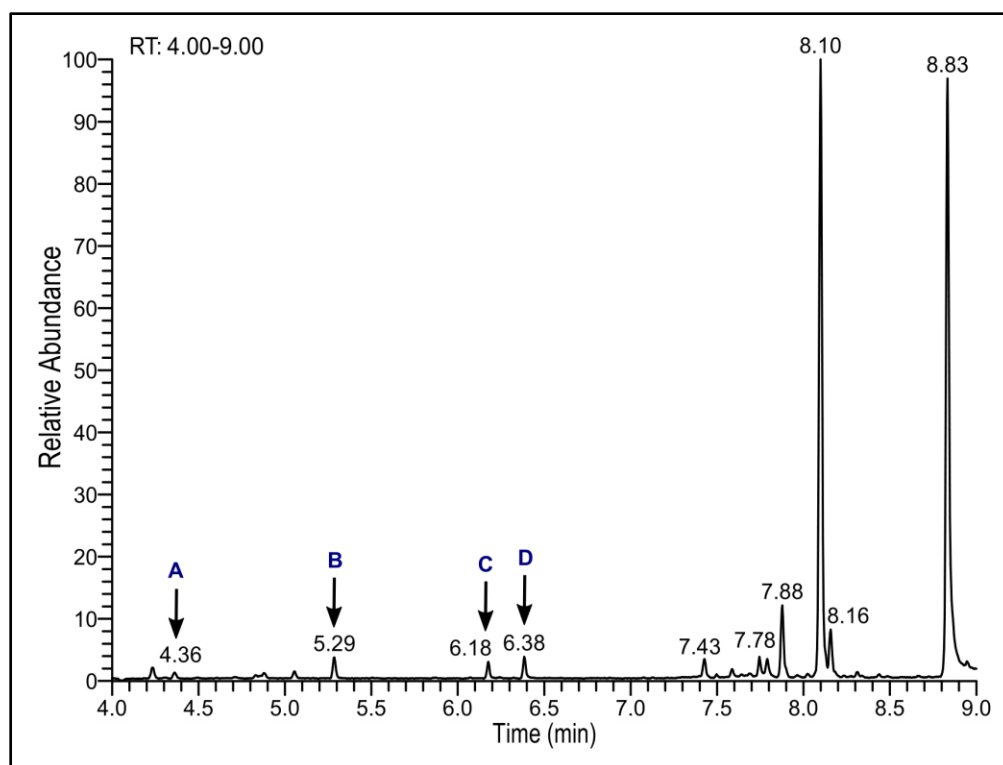


Figure S3. **LRS5 gas chromatogram showing additional potential terpenoids.** Compounds produced during LRS5 shake flask cultivation at 30 °C. The mass spectra of unknown potential terpenoids A, B, C and D are shown in Figure S4.

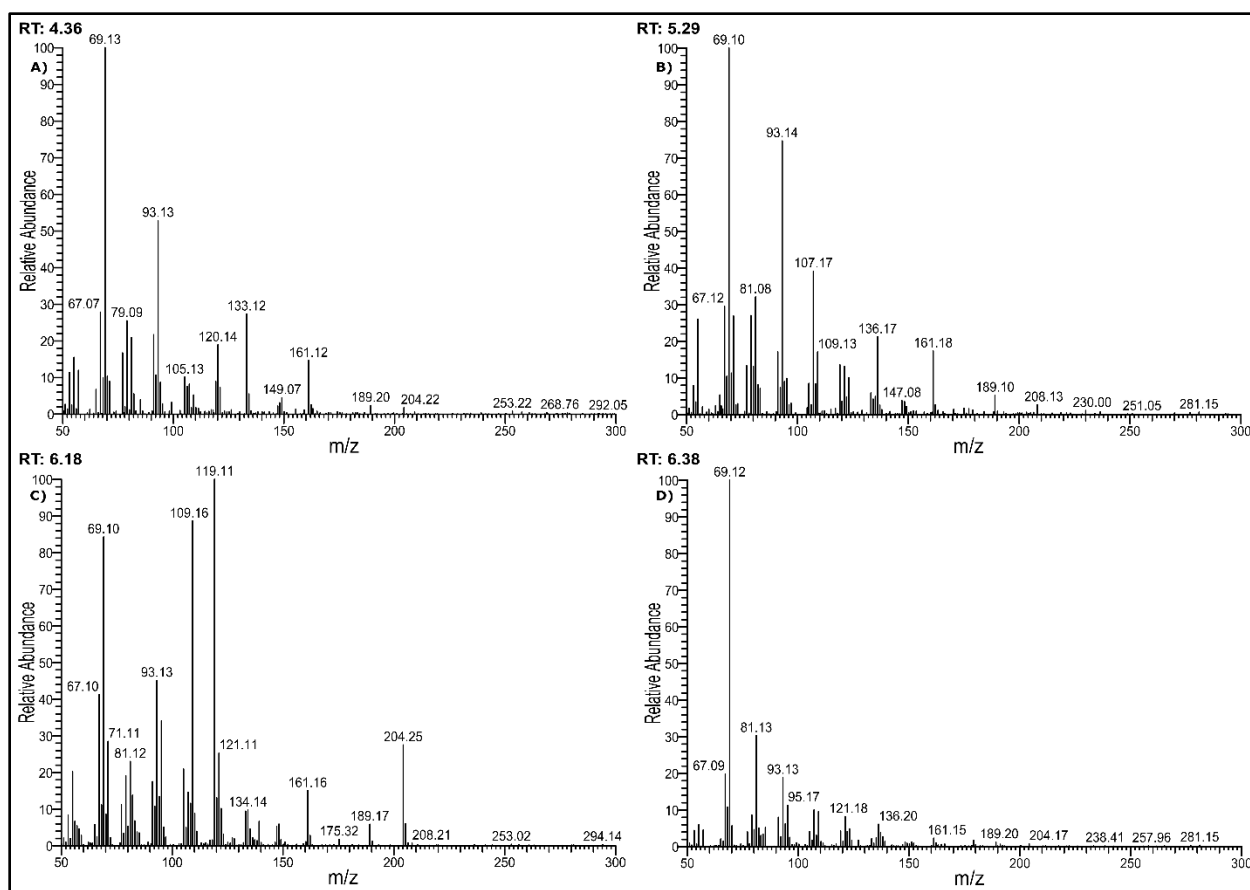


Figure S4. Mass spectra of the LRS5 additional potential terpenoids (A-D) as shown in Figure S3.

References

1. Sun P, Tropea JE, Waugh DS. Enhancing the solubility of recombinant proteins in *Escherichia coli* by using hexahistidine-tagged maltose-binding protein as a fusion partner. *Methods Mol Biol.* 2011;705:259-274.
2. Houser JR, Ford E, Chatterjea SM, Maleri S, Elston TC, Errede B. An improved short-lived fluorescent protein transcriptional reporter for *Saccharomyces cerevisiae*. *Yeast.* 2012;29:519–530.
3. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* 2012;9:671–675.