Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Material

Validation of an insertion-engineered isoprene synthase as a strategy to functionalize terpene synthases

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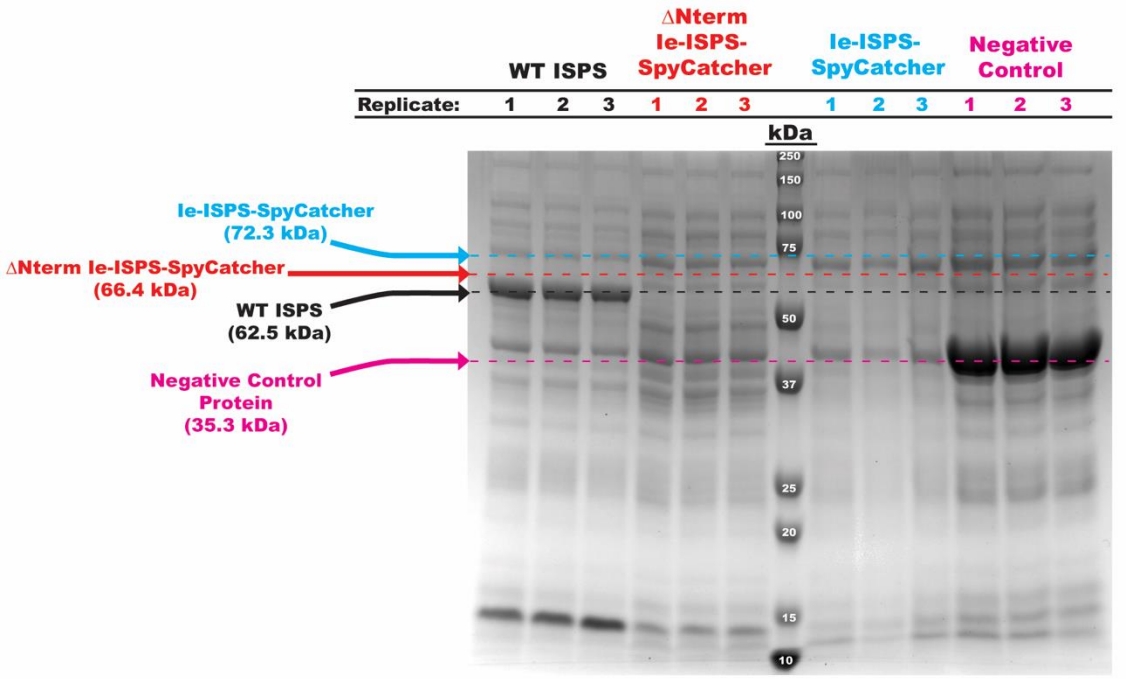
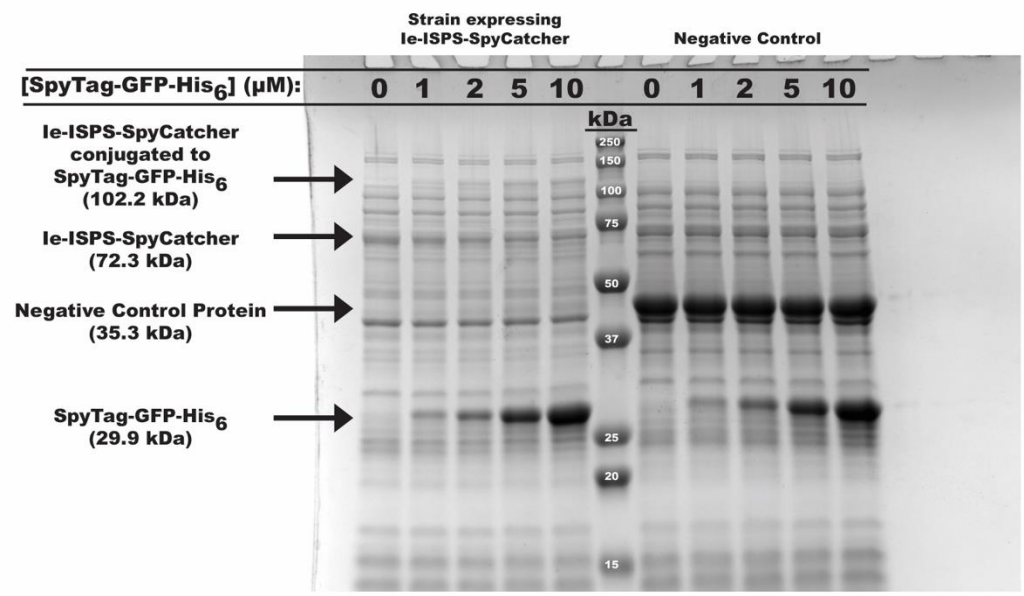
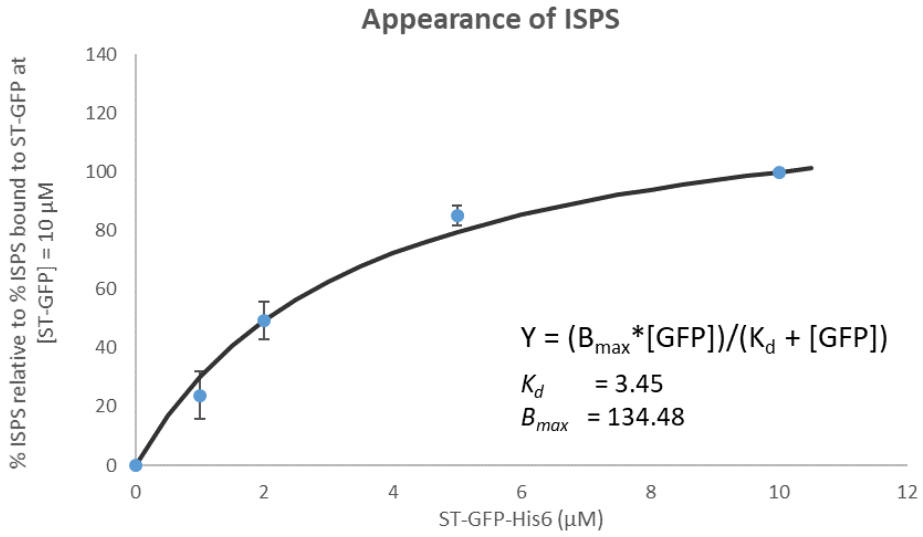


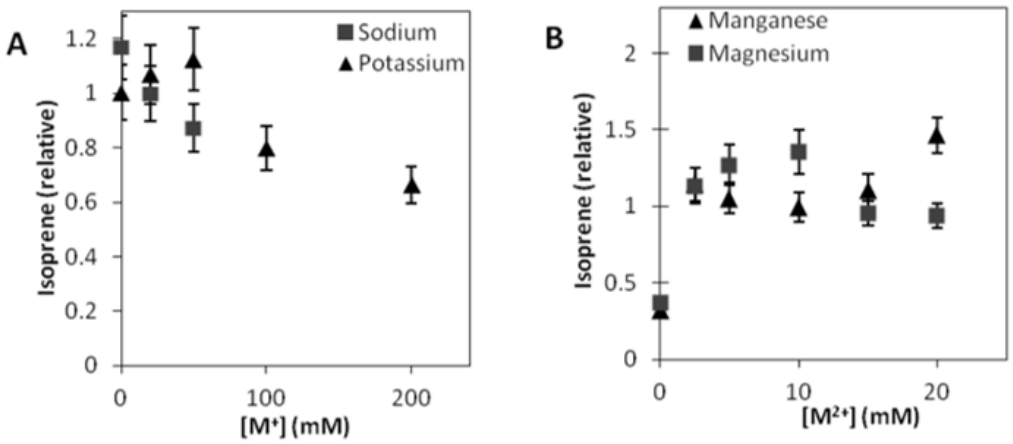
Supplementary Figure S1. ISPS abundance in clarified *E. coli* cell lysates. SDS-PAGE gel of whole cell lysates from *E. coli* strains expressing different ISPS variants. Coomassie Blue stained gel of whole cell lysates from strains expressing WT ISPS (black), ΔNterm Ie-ISPS-SpyCatcher (red), Ie-ISPS-SpyCatcher (cyan), or a negative control (magenta). 4 µg of total protein was loaded per well for three replicates of each strain. Predicted molecular weights for each ISPS variant and the negative control protein are: WT ISPS (62.5 kDa), ΔNterm Ie-ISPS-SpyCatcher (66.4 kDa), Ie-ISPS-SpyCatcher (72.3 kDa), and the negative control protein (35.3 kDa). Molecular weights were calculated using Benchling (https://benchling.com). Bio-Rad Precision Plus Dual Color Standards were used to estimate molecular weights in the gel.



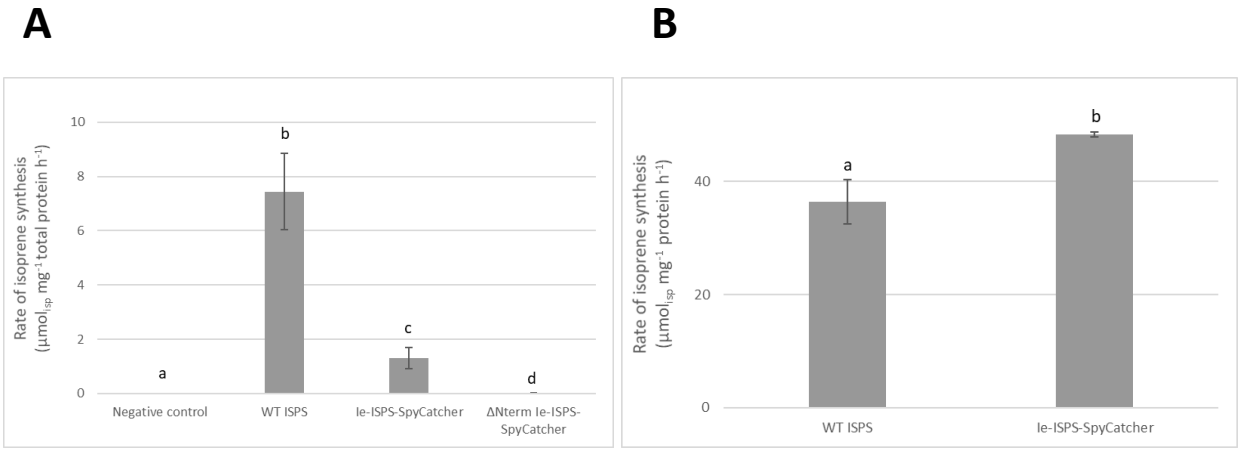
Supplementary Figure S2. Uncropped gel image of SpyTag-GFP-His$_6$ conjugation reactions. Uncropped image of the Coomassie Blue stained gel used in Figure 4. Predicted molecular weights for individual and conjugated proteins: Ie-ISPS-SpyCatcher (72.3 kDa), SpyTag-GFP-His$_6$ (29.9 kDa), Ie-ISPS-SpyCatcher conjugated with SpyTag-GFP-His$_6$ (102.2 kDa), and a negative control protein (35.3 kDa). Molecular weights were calculated using Benchling (https://benchling.com). Bio-Rad Precision Plus Dual Color Standards were used to estimate molecular weights in the gel.



Supplementary Figure S3. The plot of appearance of SpyTag-GFP-His6 + Ie-ISPS-SpyCatcher fusion protein. The % of SpyTag-GFP-His6 + Ie-ISPS-SpyCatcher fusion protein (% ISPS) expressed as a % of the SpyTag-GFP-His6 + Ie-ISPS-SpyCatcher fusion protein detected with 10 µM of SpyTag-GFP-His6, is plotted against the concentration of SpyTag-GFP-His6 added to each corresponding conjugation mixture. The values presented in the graph represent mean±SE of three estimates of % SpyTag-GFP-His6 + Ie-ISPS-SpyCatcher fusion protein obtained from three independent conjugation reactions carried out using three independent lysates. The data was fitted with a rectangular hyperbola (Y = B_{max}*[GFP]/(K_d + [GFP]) to estimate B_{max} (maximum number of binding sites of Ie-ISPS-SpyCatcher) and K_d (dissociation constant at equilibrium).



Supplementary Figure S4. Response of Ie-ISPS-GFP to sodium and potassium ion concentrations (A) and manganese and magnesium (B). In A, data is presented relative to potassium at 0 mM and in B, relative to 1 mM. Averages with standard error shown (n=3).



Supplementary Figure S5. Isoprene synthase activity of Ie-ISPS-SpyCatcher. (A) Isoprene synthase activity, measured as µmol of isoprene produced per mg of total protein per h, in lysates from cells expressing Ie-ISPS-SpyCatcher is compared with a negative control protein expressed from the same vector as all ISPS variants, WT ISPS, and a truncated form of Ie-ISPS-SpyCatcher lacking the N-terminal alpha helix preceding the γ-site (ΔNterm Ie-ISPS-SpyCatcher). (B) Isoprene synthase activity measured as µmol of isoprene produced per mg of either the WT ISPS or Ie-ISPS-SpyCatcher protein per h, respectively. 1 mM DMADP was used in the assay. Averages with standard errors are shown (n=3). Different lower case letters signify statistically significant differences between means at $\alpha = 0.05$.

Supplementary Table S1. Amino acid sequences for proteins used in this study

Protein	Amino Acid Sequence	Comments
WT ISPS	MARRSANYEPNSWDYDYLLSSDTDESIEVYKDKA KKLEAEVRREINNEKAEFLTLLELIDNVQRLGLG YRFESDIRGALDRFVSSGGFDAVTKTSLHGTALS FRLLRQHGFEVSQEAFSGFKDQNGNFLENLKEDI KAILSLYEASFLALEGENILDEAKVFAISHLKEL SEEKIGKELAEQVNHALELPLHRRTQRLEAVWSI EAYRKKEDANQVLLELAILDYNMIQSVYQRDLRE TSRWWRRVGLATKLHFARDRLIESFYWAVGVAFE PQYSDCRNSVAKMFSFVTIIDDIYDVYGTLDELE LFTDAVERWDVNAINDLPDYMKLCFLALYNTINE IAYDNLKDKGENILPYLTKAWADLCNAFLQEAKW LYNKSTPTFDDYFGNAWKSSSGPLQLVFAYFAVV QNIKKEEIENLQKYHDTISRPSHIFRLCNDLASA SAEIARGETANSVSCYMRTKGISEELATESVMNL IDETWKKMNKEKLGGSLFAKPFVETAINLARQSH CTYHNGDAHTSPDELTRKRVLSVITEPILPFER	
Ie-ISPS-GFP	MARRSANYEPNSWDYDYLLSSDTDESIEVYKDKA KKLEAEVRREINNGSGSGSGSGS**SKGEELFTGVV** **PILVELDGDVNGHKFSVSGEGEGDATYGKLTLKF** **ICTTGKLPVPWPTLVTTLTYGVQCFARYPDHMKQ** **HDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKF** **EGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSH** **KVYITADKQKNGIKVNFKTRHNIEDGSVQLADHY** **QQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRD** **HMVLLEFVTAAGITLGMDELYK**GSGSGSGSRSEK AEFLTLLELIDNVQRLGLGYRFESDIRGALDRFV SSGGFDAVTKTSLHGTALSFRLLRQHGFEVSQEA FSGFKDQNGNFLENLKEDIKAILSLYEASFLALE GENILDEAKVFAISHLKELSEEKIGKELAEQVNH ALELPLHRRTQRLEAVWSIEAYRKKEDANQVLLE LAILDYNMIQSVYQRDLRETSRWWRRVGLATKLH FARDRLIESFYWAVGVAFEPQYSDCRNSVAKMFS FVTIIDDIYDVYGTLDELELFTDAVERWDVNAIN DLPDYMKLCFLALYNTINEIAYDNLKDKGENILP YLTKAWADLCNAFLQEAKWLYNKSTPTFDDYFGN AWKSSSGPLQLVFAYFAVVQNIKKEEIENLQKYH DTISRPSHIFRLCNDLASASAEIARGETANSVSC YMRTKGISEELATESVMNLIDETWKKMNKEKLGG SLFAKPFVETAINLARQSHCTYHNGDAHTSPDEL TRKRVLSVITEPILPFER	GFP sequence in bold.

SUMO-Ie-ISPS-GFP	MGSSWSHPQFEKGSGLVPRGSASMSDSEVNQEAK PEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPL RRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPE DLDMEDNDIIEAHREQIGGMTEARRSANYEPNSW DYDYLLSSDTDESIEVYKDKAKKLEAEVRREINN GSGSGSGSGS**SKGEELFTGVVPILVELDGDVNGH** **KFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPT** **LVTTLTYGVQCFARYPDHMKQHDFFKSAMPEGYV** **QERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKG** **IDFKEDGNILGHKLEYNYNSHKVYITADKQKNGI** **KVNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLL** **PDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGI** **TLGMDELYK**GSGSGSGSRSEKAEFLTLLELIDNV QRLGLGYRFESDIRGALDRFVSSGGFDAVTKTSL HGTALSFRLLRQHGFEVSQEAFSGFKDQNGNFLE NLKEDIKAILSLYEASFLALEGENILDEAKVFAI SHLKELSEEKIGKELAEQVNHALELPLHRRTQRL EAVWSIEAYRKKEDANQVLLELAILDYNMIQSVY QRDLRETSRWWRRVGLATKLHFARDRLIESFYWA VGVAFEPQYSDCRNSVAKMFSFVTIIDDIYDVYG TLDELELFTDAVERWDVNAINDLPDYMKLCFLAL YNTINEIAYDNLKDKGENILPYLTKAWADLCNAF LQEAKWLYNKSTPTFDDYFGNAWKSSSGPLQLVF AYFAVVQNIKKEEIENLQKYHDTISRPSHIFRLC NDLASASAEIARGETANSVSCYMRTKGISEELAT ESVMNLIDETWKKMNKEKLGGSLFAKPFVETAIN LARQSHCTYHNGDAHTSPDELTRKRVLSVITEPI LPFER	SUMO-Smt3 underlined and GFP sequence in bold.
SpyTag-GFP-His_6	MGEL**AHIVMVDAYKPTK**GSMRKGEELFTGVVPIL VELDGDVNGHKFSVRGEGEGDATNGKLTLKFICT TGKLPVPWPTLVTTLTYGVQCFARYPDHMKQHDF FKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGD TLVNRIELKGIDFKEDGNILGHKLEYNFNSHNVY ITADKQKNGIKANFKIRHNVEDGSVQLADHYQQN TPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMV LLEFVTAAGITHGMDELYKLE**HHHHHH**	N-terminal SpyTag and C-terminal His_6 sequences are in bold.
ΔNterm Ie-ISPS-SpyCatcher	M**DSATHIKFSKRDEDGKELAGATMELRDSSGKTI** **STWISDGQVKDFYLYPGKYTFVETAAPDGYEVAT** **AITFTVNEQGQVTVN**GGSGGSEKAEFLTLLELID NVQRLGLGYRFESDIRGALDRFVSSGGFDAVTKT SLHGTALSFRLLRQHGFEVSQEAFSGFKDQNGNF LENLKEDIKAILSLYEASFLALEGENILDEAKVF AISHLKELSEEKIGKELAEQVNHALELPLHRRTQ RLEAVWSIEAYRKKEDANQVLLELAILDYNMIQS VYQRDLRETSRWWRRVGLATKLHFARDRLIESFY	SpyCatcher is in bold, GlySer linker is underlined.

	WAVGVAFEPQYSDCRNSVAKMFSFVTIIDDIYDV YGTLDELELFTDAVERWDVNAINDLPDYMKLCFL ALYNTINEIAYDNLKDKGENILPYLTKAWADLCN AFLQEAKWLYNKSTPTFDDYFGNAWKSSSGPLQL VFAYFAVVQNIKKEEIENLQKYHDTISRPSHIFR LCNDLASASAEIARGETANSVSCYMRTKGISEEL ATESVMNLIDETWKKMNKEKLGGSLFAKPFVETA INLARQSHCTYHNGDAHTSPDELTRKRVLSVITE PILPFER	
Ie-ISPS-SpyCatcher	MARRSANYEPNSWDYDYLLSSDTDESIEVYKDKA KKLEAEVRREINNGGSGGS**DSATHIKFSKRDEDG KELAGATMELRDSSGKTISTWISDGQVKDFYLYP GKYTFVETAAPDGYEVATAITFTVNEQGQVTVN**G GSGGSEKAEFLTLLELIDNVQRLGLGYRFESDIR GALDRFVSSGGFDAVTKTSLHGTALSFRLLRQHG FEVSQEAFSGFKDQNGNFLENLKEDIKAILSLYE ASFLALEGENILDEAKVFAISHLKELSEEKIGKE LAEQVNHALELPLHRRTQRLEAVWSIEAYRKKED ANQVLLELAILDYNMIQSVYQRDLRETSRWWRRV GLATKLHFARDRLIESFYWAVGVAFEPQYSDCRN SVAKMFSFVTIIDDIYDVYGTLDELELFTDAVER WDVNAINDLPDYMKLCFLALYNTINEIAYDNLKD KGENILPYLTKAWADLCNAFLQEAKWLYNKSTPT FDDYFGNAWKSSSGPLQLVFAYFAVVQNIKKEEI ENLQKYHDTISRPSHIFRLCNDLASASAEIARGE TANSVSCYMRTKGISEELATESVMNLIDETWKKM NKEKLGGSLFAKPFVETAINLARQSHCTYHNGDA HTSPDELTRKRVLSVITEPILPFER	SpyCatcher is in bold, insulating GlySer linkers underlined.

Supplementary Sequence S1. DNA Sequence of the pD881/Ie-ISPS-GFP plasmid.

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Supplementary Sequence S2. DNA Sequence of the pD881/SUMO-Ie-ISPS-GFP plasmid.

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GCACTTCACTGACACCCTCATCAGTGCCAACATAGTAAGCCAGTATACACTCCGCTAGCGCAGAAAGGCC
CACCCGAAGGTGAGCCAGGTGATTACATTTGGGCCCTCATTAGAAAAACTCATCGAGC

Supplementary Sequence S3. DNA sequence for the pBbE2K-Ie-ISPS-SpyCatcher

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Supplementary Sequence S4. DNA sequence for the pBbE2K-SpyTag-GFP-His$_6$. Coding sequence for SpyTag-GFP-His$_6$ is indicated in bold.

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A

Supplementary Sequence S5. DNA sequence for the pBbE2K-ΔNterm Ie-ISPS-SpyCatcher

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