

Supplementary Material

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

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Figure S1: ISPS abundance in clarified *E. coli* cell lysates

Figure S2: Uncropped gel image of SpyTag-GFP-His₆ conjugation reactions

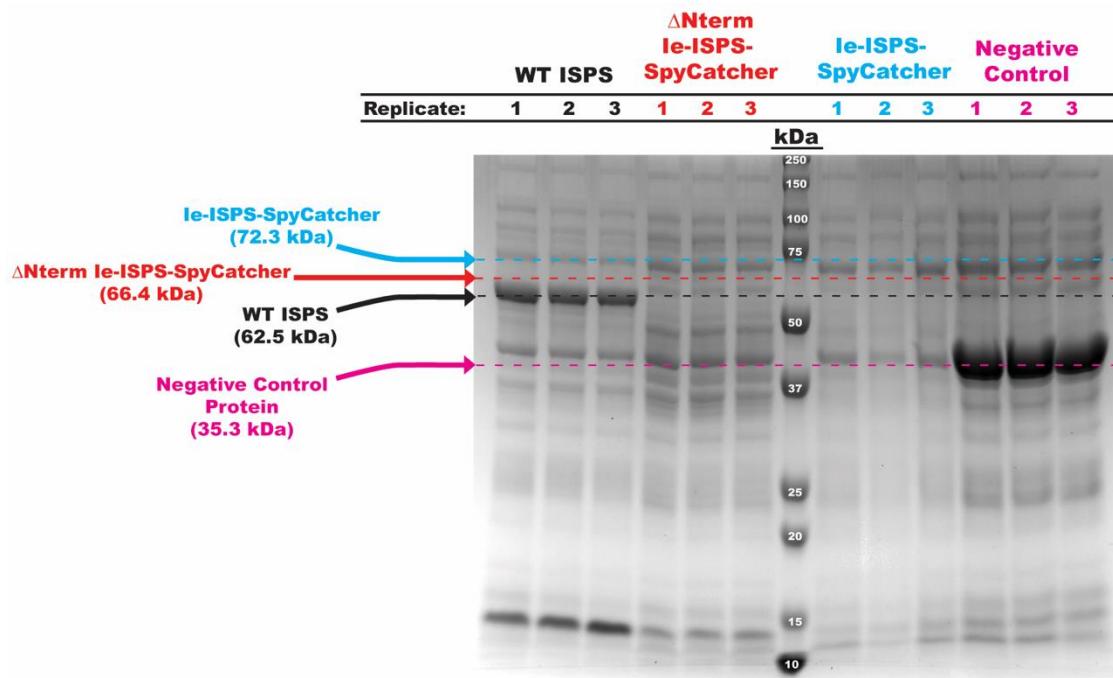
Figure S3: The plot of appearance of SpyTag-GFP-His₆ + le-ISPS-SpyCatcher fusion protein

Figure S4: Response of le-ISPS-GFP to Na⁺ and K⁺ concentrations, and Mn²⁺ and Mg²⁺

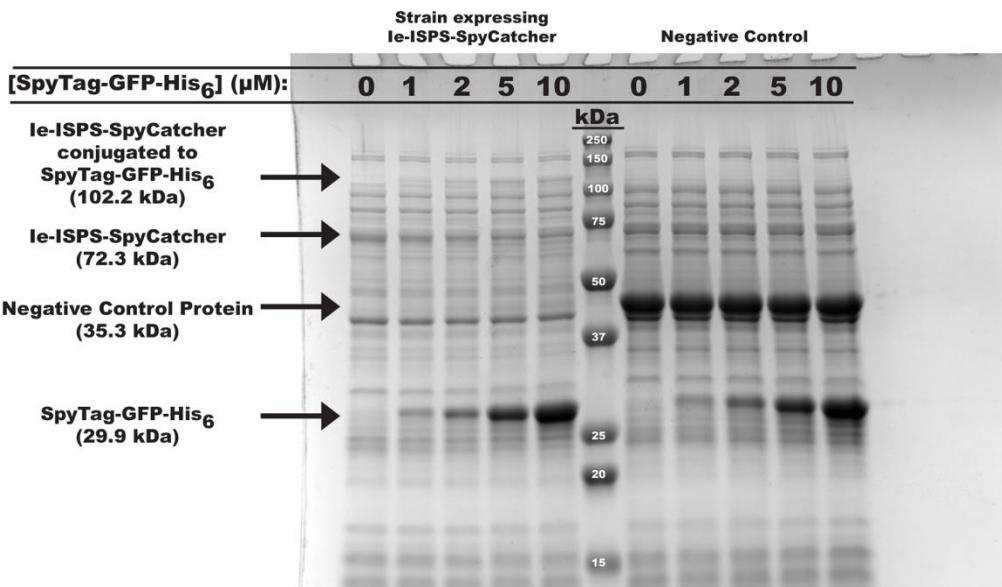
Figure S5: Isoprene synthase activity of le-ISPS-SpyCatcher

Table S1: Amino acid sequences for proteins used in this study;

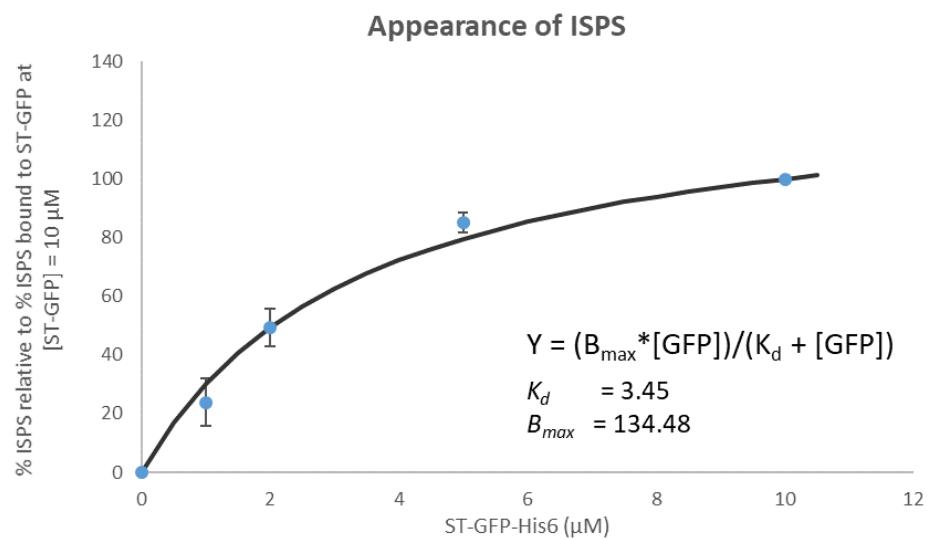
Sequences S1-5: Plasmids sequences used in this study



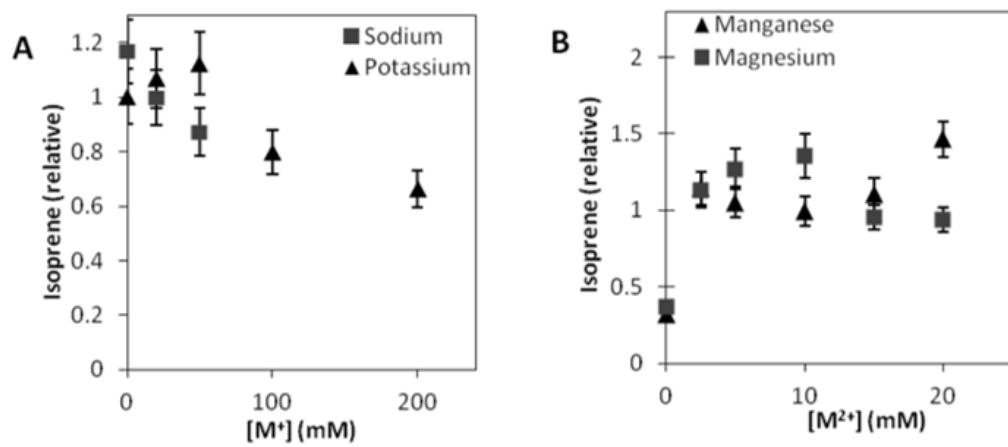
Supplementary Figure S1. ISPS abundance in clarified *E. coli* cell lysates. SDS-PAGE gel of whole cell lysates from *E. coli* strains expressing different ISPS variants. Coomassie Blue stained gel of whole cell lysates from strains expressing WT ISPS (black), Δ Nterm Ie-ISPS-SpyCatcher (red), Ie-ISPS-SpyCatcher (cyan), or a negative control (magenta). 4 μ g 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), Δ Nterm 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.



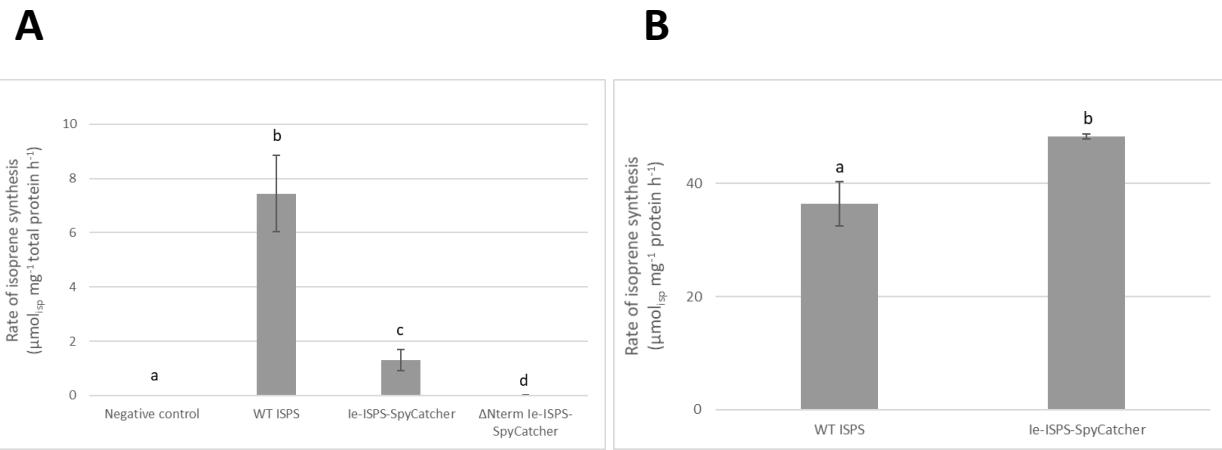
Supplementary Figure S2. Uncropped gel image of SpyTag-GFP-His₆ conjugation reactions. Uncropped image of the Coomassie Blue stained gel used in Figure 4. Predicted molecular weights for individual and conjugated proteins: le-ISPS-SpyCatcher (72.3 kDa), SpyTag-GFP-His₆ (29.9 kDa), le-ISPS-SpyCatcher conjugated with SpyTag-GFP-His₆ (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 + le-ISPS-SpyCatcher fusion protein. The % of SpyTag-GFP-His6 + le-ISPS-SpyCatcher fusion protein (% ISPS) expressed as a % of the SpyTag-GFP-His6 + le-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 \pm SE of three estimates of % SpyTag-GFP-His6 + le-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 le-ISPS-SpyCatcher) and K_d (dissociation constant at equilibrium).



Supplementary Figure S4. Response of le-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 le-ISPS-SpyCatcher. (A) Isoprene synthase activity, measured as μmol of isoprene produced per mg of total protein per h, in lysates from cells expressing le-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 le-ISPS-SpyCatcher lacking the N-terminal alpha helix preceding the γ -site ($\Delta\text{Nterm le-ISPS-SpyCatcher}$). (B) Isoprene synthase activity measured as μmol of isoprene produced per mg of either the WT ISPS or le-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 KKLEAEVREINNEKAELTLELIDNVQRLGLG YRFESDIRGALDRFVSSGGFDAVTKTSIHGTALS FRLLRQHGFEVSQEAFSGFKDQNGNFLLENLKEDI KAILSPLYEASFLALEGENILDEAKVFAISHLKEL SEEKIGKELAEQVNHAELPLHRRRTQRLEAVWSI EAYRKEDANQVLLELAILDYNMIQSVDYQRDLRE TSRWWRRVGLATKLHFARDRLIESFYWAVGVAFE PQYSDCRNSVAKMFSFVTIIDDIYDVYGTLDLE LFTDAVERWDVNAINDLPDYMKLCFLALYNTINE IAYDNLKDKGENILPYLTAKADLCNAFLQEA LYNKSTPTFDDYFGNAWKSSSGPLQLVFAYFAVV QNIKKEEIENLQKYHDTISRPISHIFRLCNDLASA SAEIA TARGETANSVSCYMRTKGISEELATESVMNL IDETWKKMNKEKLGGSLFAKPFVETAINLARQSH CTYHNGDAHTSPDELTRKRVLSVITEPILP FER	
Ie-ISPS-GFP	MARRSANYEPNSWDYDYLLSSDTDESIEVYKDKA KKLEAEVREINNGSGSGSGGS SKGEELFTGVV PILVELGDVNGHKFVS SGEGEGDATYGKLT LKFICTTGKLPVPWP TLVTTLYGVQCFARYPDHM KQHDFFKSAMPEGYVQERTIFFKDDGNYKTRA EVKFEGDTLVNRIELKGIDFKEDGNILGHKLEY NNSHKVYITADKQKNGIKVNFKTRHNIEDGSVQL ADHYQQNTPIGDGPVLLPD NHYLSTQSALSKD PNEKRDHMV LLEFVTAA GITALGMDELYKGSGSGSRSEK AEFLTLELIDNVQRLGLGYRF ESDIRGALDRFVSSGGFDAV TKTSIHGTALSFRLLRQH GFEVSQEAFSGFKDQNG NFLLENLKEDIKAIL SPLYEASFLALEGEN ILDEAKVFAISHL KELSEEKIGKELAEQVN HAALEPLHRRRTQR LEAVWSIEAYRK EDANQVLLELA ILDYNMIQSVDYQR DLRETSRWWRRVGL ATKLHFARDRLIES FYWAVGVAFE PQYSDCRNSVAKM FSFVTIIDDIYDV YGTLDLELFTDA VERWDVNAINDL PDYMKLCFLALY NTINEIAYDNL KDKGENILPYLT AKADLCNAFLQEA KLYNKSTPTFDDY FGNAWKSSSGPLQL VFAYFAVVQNI KKEEIENLQKYH DTISRPISHIFRL CNDLASASAEIA TARGETANSVSC YMRTKGISEELATE SVMLIDETWKKMN KEKLGGSLFAKPF VETAINLARQSHCT YHNGDAHTSPDEL TRKRVLSVITEPI LPER	GFP sequence in bold.

SUMO-Ie-ISPS-GFP	MGSWSHPQFEKGSGLVPRGSASMSDSEVNQEAK <u>PEVKPEVKPETHINLKVDGSSEIFFKIKTTPL</u> <u>RRLMEAFAKRQGKEMDSLRFLYDGIRIQAQTP</u> <u>DLDMEDNDIIEAHREQIGGMTEARRSANYEPNSW</u> DYDYLSSDTDESIEVYDKAKKLEAEVRREINN GSGSGSGSGS SKGEELFTGVVPILVELGDVN GHS KFSVS GEGEGLDATYGKLT LKFICTTGKLPV PWPT LVTTLTYGVQCFARYPDHMKQHDFFKSAMPEGYV QERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKG IDFKEDGNILGHKLEYNNSHKVYITADKQKNGI KVNFKTRHNIEDGSVQLADHYQONTPIGDGPVLL PDNHYLSTQSALS KDPNEKRDHMV LLEFVT AAGI T LGMD EYL KGSGSGSRSEKAFLTLEIDNV QRGLGYRFESDIRGALDRFVSSGGFDAVTKTS HGTALSFRLLRQHGFEVSQEA FG KDQNGN NLKEDIKAILSLYEASFLALEGENILDEAKVFA SHLKELSEEKIGKELAEQVNHAELPLHRR EAVWSIEAYRK KEDANQVL LELAILDYN MIQS VY QRDLRET SRWW RVGLAT KLHFARD RLIES FYWA VGVA FEPQY SDCRNS VAKMFS FVTI IIDD YDVY G TLDE LELF TDA VER WDV NA IND LPD YMKLC FLAL YNT INE IAY DNL KDK GEN ILP YLT KAW ADLC NAF LQE AKW LYNK STPT FDDY FGNA WKS SSG PLQL VF AY FAVV QNI KKE EIEN LQKY HDT ISRP SHIF RLC NDL ASASAE IAR GET ANS VSC YMRT KG ISEEL LAT ESVM NLID ETWK KM KE LGG SLFA KPF VET AIN LAR QSH CTY HNG DAHT SPDEL TRK RVL SVITE PI LP FER	SUMO-Smt3 underlined and GFP sequence in bold.
SpyTag-GFP-His ₆	MGE AHIVMVDAYKPTK GSMRKGEELFTGVVPIL VELGDVN GHKF SVRG EGER GEGD ATNG KLT LKF ICT TGK LPV PW PTL VTT LTYGV QCF ARYP DHMK QHDF FKS AMPEG YV QERT ISF KDD GTY KTRA EVKF EGD TLV NRI ELKG IDF KED GNIL GHK LEYN FNS HN VY ITAD KQK NGI KAN FKIR HN VED GSV QLAD HYQ QN TP IGD GPV LLP DNH YLST QS VLS KD PNE KR HM V LLE F V TA A G I T H G M D E L Y K L E HHHHHH	N-terminal SpyTag and C-terminal His ₆ sequences are in bold.
ΔNterm Ie-ISPS- SpyCatcher	MDSATH I FKFSKRDED G KELAGAT M ELRDSSGKT I STW ISDGQVKDFYLYPGKYTFVETAAPDG YEVAT A ITFTVNEQGQVTVN <u>GGSG</u> SEKAFLTLEID NVQR LGL GYRF ESDI RGAL DRF VSS GGF DAV TKT SLHG TALS FRLL RQHG FEVS QEA FSG FKD QNG NF LEN LKEDI KAIL SLYE ASFL ALE GEN ILDE AKV F AI SHLK ELSE EKIG KEL AEQ VN HAEL PLH RR TQ RLE AVWS IEAY RK KED ANQ VL LE LAI LDY N MIQS VY QRDL RET SRWW RVGL AT KLH FARD RLIES FY	SpyCatcher is in bold, GlySer linker is underlined.

	WAVGVAFEPQYSDCRNSVAKMFSFVTIIDDIYDV YGTLDELELFTDAVERWDVNAINDLPDYMKLCFL ALYNTINEIAYDNLKDKGENILPYLTAKAWADLCN AFLQEAKWLYNKSTPTFDDYFGNAWKSSSGPLQL VFAYFAVVQNIKKEEIEENLQKYHDTISRPSHIFR LCNDLASASAEIARGETANSVSCYMRKGISEEL ATESVMNLIDETWKMNKEKLGGSLFAKPFVETA INLARQSHCTYHNGDAHTSPDELTRKRVLSVITE PILPFER	
Ie-ISPS-SpyCatcher	MARRSANYEPNSWDYDYLLSSDTDESIEVYKDKA KKLEAEVRREIN <u>NGGSGGS</u> DSATHIKFSKRDEDG KELAGATMELRDSSGKTISTWISDGQVKDFYLYP GKYTFVETAAAPDGYEVATAITFTVNEQGQVTVNG <u>GSGGSEKA</u> EFLTLLELIDNVQRLGLGYRFESDIR GALDRFVSSGGFDAVTKTSLHGTALSFRLLRQHG FEVSQEAFSGFKDQGNFLENLKEDIKAILSlyE ASFЛАLEGЕNILDEAKVFAISHLKELSEEKIGKE LAEQVNHAELPLHRRRTQRLEAVWSIEAYRKED ANQVLLELAILDYNMIQSVYQRDLRETSRWWRRV GLATKLHFARDRLIESFYWAVGVAFEPQYSDCRN SVAKMFSFVTIIDDIYDVYGTLD ELELFTDAVER WDVNAINDLPDYMKLCFLALYNTINEIAYDNLKD KGENILPYLTAKAWADLCNAFLQEAKWLYNKSTPT FDDYFGNAWKSSSGPLQLVFAYFAVVQNIKKEEI ENLQKYHDTISRPSHIFRLCNDSLASASAEIARGE TANSVSCYMRKGISEELATESVMNLIDETWKKM NKEKLGGSLFAKPFVETAINLARQSHCTYHNGDA HTSPDELTRKRVLSVITEPILPFER	SpyCatcher is in bold, insulating GlySer linkers underlined.

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

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

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CACCCGAAGGTGAGCCAGGTGATTACATTGGGCCCTCATTAGAAAACCTCATCGAGC

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-His₆. Coding sequence for SpyTag-GFP-His₆ is indicated in bold.

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

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