Supplementary Information for

Microbially Guided Discovery and Biosynthesis of Biologically Active Natural Products

Ankur Sarkar[a], Edward Y. Kim[a], Taehwan Jang[b], Akarawin Hongdusit[a], Hyungjun Kim[b], Jeong-Mo Choi[c], and Jerome M. Fox[a*]

[a]Department of Chemical and Biological Engineering, University of Colorado Boulder, 3415 Colorado Avenue, Boulder, CO, 80303
[b]Department of Chemistry, Korea Advanced Institute of Science and Technology, Yuseong-gu, Daejeon 34141, Republic of Korea
[c]Department of Chemistry, Pusan National University, Geumjeong-gu, Busan 46241, Republic of Korea
*To whom correspondence should be addressed. E-mail: jerome.fox@colorado.edu

Note S1: The orthogonality of proteomes. *E. coli* and *S. cerevisiae* are both well-developed platforms for the production of pharmaceutically relevant natural products[1–3]. We chose to use *E. coli* for this study because its machinery for phosphorylating proteins is dissimilar from that of eukaryotic cells and thus less likely to interfere with the function of genetically encoded systems that link the inhibition of PTP1B to cellular growth[4]. By contrast, the overexpression of Src kinase in *S. cerevisiae* is lethal and is mitigated by PTP1B[5]; these effects are inconsistent with our biochemical objective. More broadly, *S. cerevisiae* and humans, despite having evolved from a common ancestor approximately 1 billion years ago[6], share many functionally equivalent proteins; orthologous genes, in fact, account for more than one-third of the yeast genome[7]. Most strikingly, a recent study found that nearly half (47%) of 414 essential genes from *S. cerevisiae* could be replaced with human orthologs without growth defects[8]. This finding suggests that yeast is a particularly restrictive host for genetically encoded systems that link arbitrary changes in the activities of human regulatory enzymes to fitness advantage.

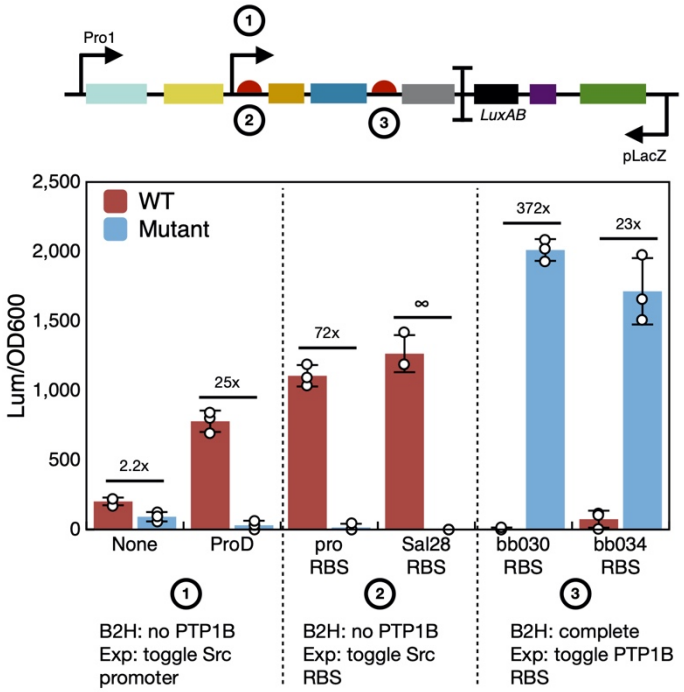

Figure S1. Optimization of the bacterial-two hybrid (B2H) system. We optimized the transcriptional response of the B2H system by adjusting the strength of various genetic elements. In three sequential phases, we changed (1) the promoter for Src/CDC37, (2) the ribosome binding site (RBS) for Src/CDC37, and (3) and the RBS for PTP1B. In phases 1 and 2, we used a PTP1B-deficient system with either a wild-type (WT, EPQ**Y**EEIPYL) or a phosphorylation-deficient (Mut, EPQ**F**EEIPYL) substrate domain. In this figure, "none" indicates the absence of an additional promoter, and the promoter Pro1 controls the transcription of all five genes to its left. In phase 3, we used a complete B2H system with either a wild-type (WT) or catalytically inactive (C215S, Mut) variant of PTP1B. The remaining B2H components of each phase are detailed in Tables S2 and S7. Error bars denote standard error with $n \geq 3$ biological replicates with exact sample sizes reported in Table S8.

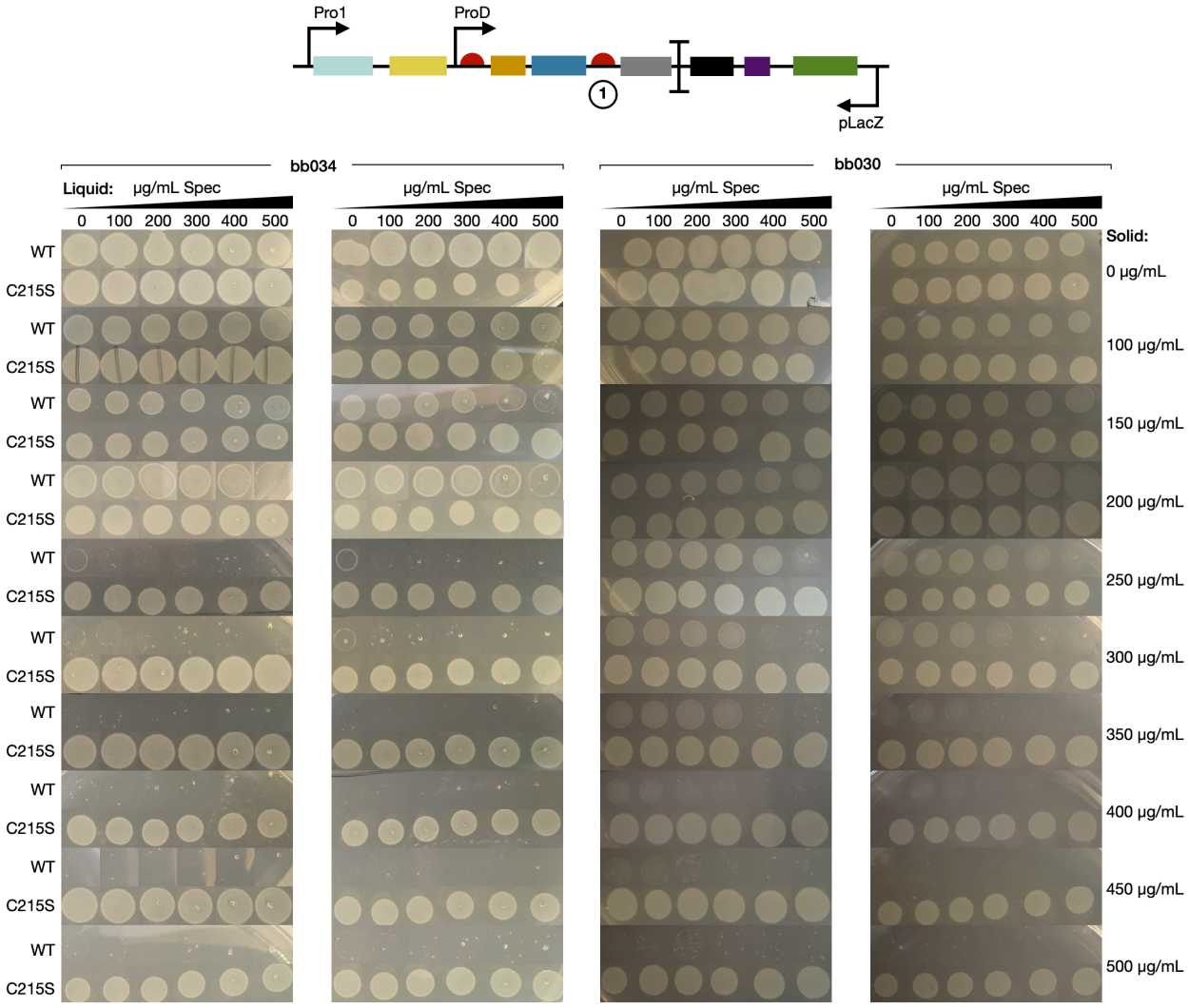

Figure S2. Analysis of different selection conditions. A comparison of the antibiotic resistance conferred by B2H systems with different RBSs for PTP1B (see Tables S2 and S8 for the remaining components of each system). Images show the growth of *E. coli* on agar plates (LB) seeded from drops of liquid culture (10 µL) with two biological replicates for each condition. The RBS bb034 confers a greater sensitivity to spectinomycin on agar plates; concentrations of spectinomycin in the liquid culture, by contrast, do not have a strong influence on bacterial growth. Informed by this analysis, we incorporated bb034 into our "optimized" B2H system and ceased adding spectinomycin to liquid culture.

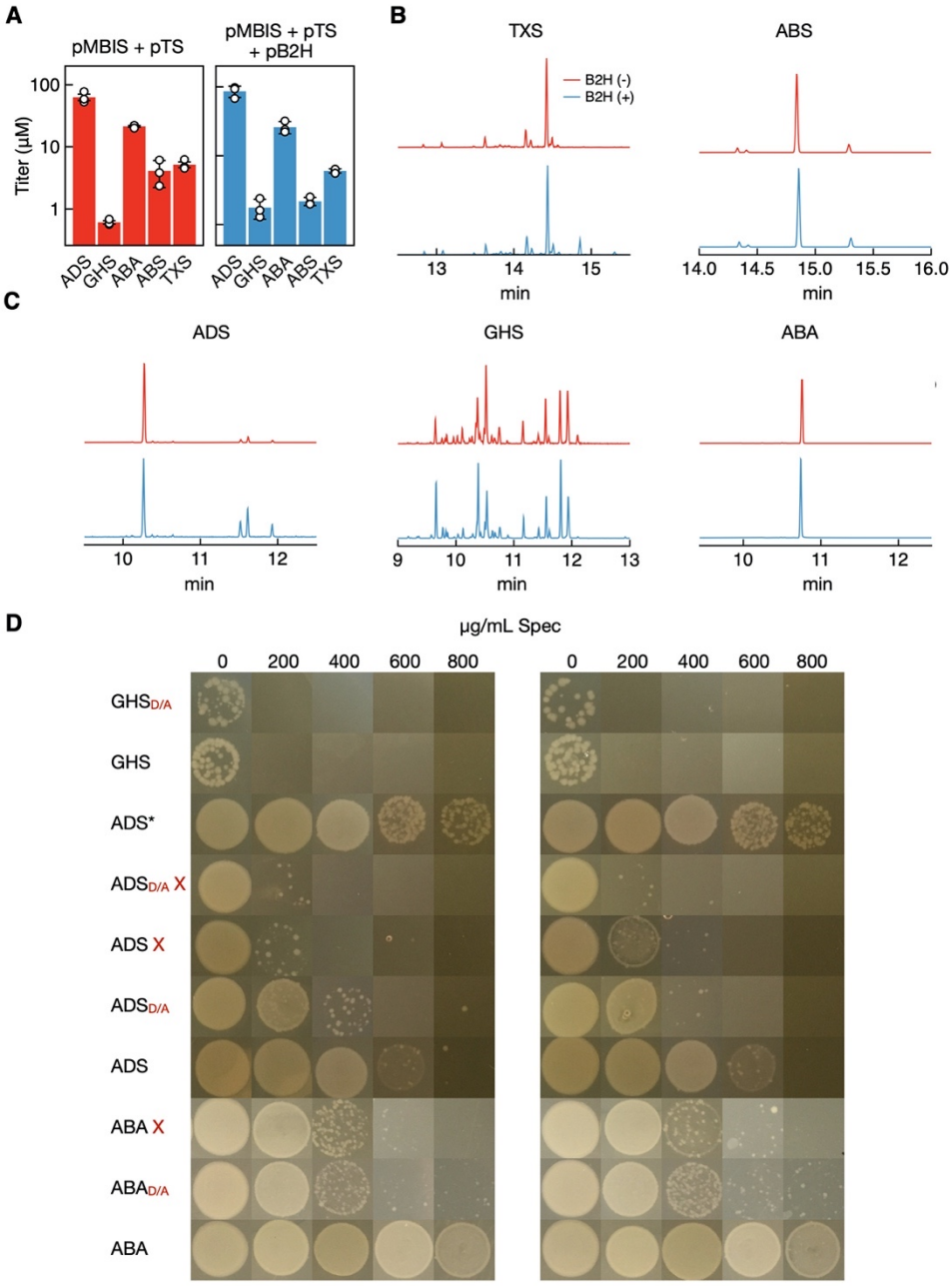

Figure S3. Analysis of the products of different terpene synthases. (A) Total terpene titers generated by each TS-specific strain in the absence (red) and presence (blue) of the B2H system. These results indicate that the B2H system does not disrupt terpenoid biosynthesis. (B) GC/MS chromatograms of the terpenoids generated by the diterpene synthases in the absence (top) and

presence (bottom) of the B2H system (m/z=272). (C) GC/MS chromatograms of the terpenoids generated by the sesquiterpene synthases in the absence (top) and presence (bottom) of the B2H system (m/z=204). Similar profiles in (B) and (C) indicate that the B2H system does not alter product distributions. (D) Analysis of the contributions of either (i) TS activity or (ii) B2H function to the death and survival of GHS, ADS, and ABA strains. Inactivation of GHS does not enhance survival, an indication that this enzyme does not produce growth-inhibiting terpenoids. Inactivation of either ADS, ABA, or the B2H system, by contrast, weakens the antibiotic resistance of the ADS and ABA strains; maximal resistance thus requires both terpenoid production and B2H activation. Labels denote the following controls: D/A, an inactive terpene synthase (contains a D/A mutation at the catalytic aspartic acid, preventing the initial metal-binding step in terpene cyclization) ; *, a constitutively active B2H (contains $PTP1B_{C215S}$, preventing dephosphorylation); X, an inactive B2H (contains a substrate domain with a Y/F mutation, prohibiting phosphorylation and thus binding with the SH2 domain). Images show LB plates seeded with drops of liquid culture (10 μL) from two biological replicates. Supplementary Tables 2 and 8 detail the B2H systems used for these analyses. Error bars in (A) denote standard deviation for $n \geq 3$ biological replicates with exact sample sizes reported in Table S9.

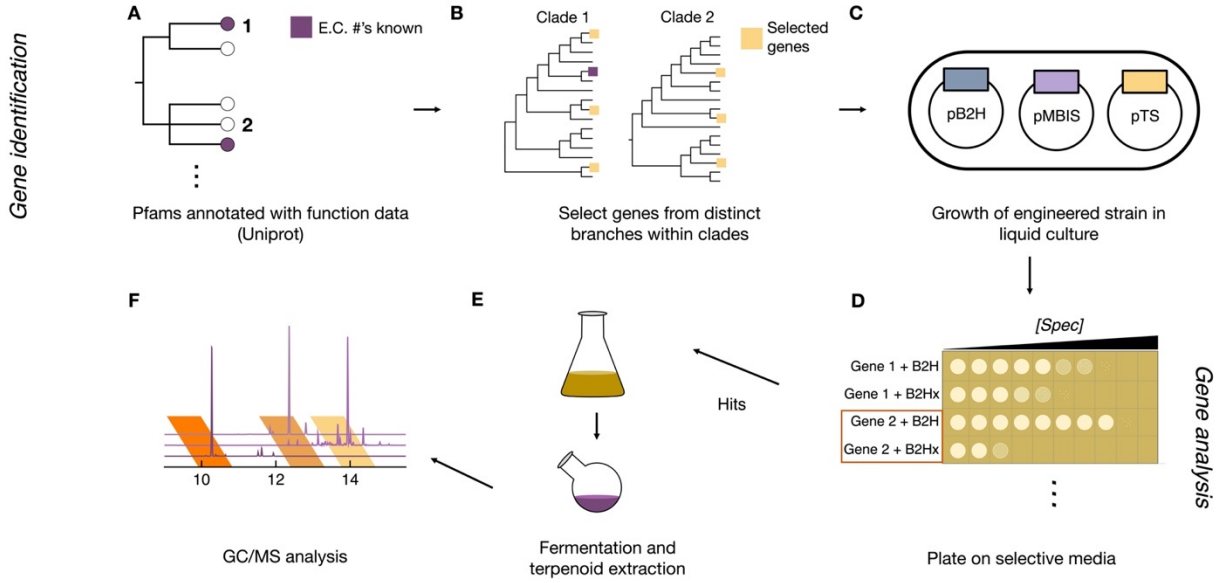

Figure S4. Identification and analysis of uncharacterized terpene synthases. (A) We annotated the PF03936 family with Enzyme Commission (EC) numbers from Uniprot. (B) We created a cladogram of this family and selected uncharacterized genes from different clades. (C) We transformed *E. coli* with plasmids harboring both (i) the B2H system and (ii) terpenoid pathways (i.e., $pMBIS_{CmR}$ + pTS with the selected genes) and grew the transformants in liquid culture. (D) We used drop-based plating (10 µL) of the liquid cultures to evaluate the resistance conferred by each terpene synthase gene. We note: a B2Hx control for each gene allowed us to ensure that enhanced resistance came from B2H activation. We selected strains with antibiotic resistance exceeding 400 µg/ml as hits. (E) We grew up hits in liquid culture, extracted their products with a hexane overlay, and concentrated this overlay by drying it in a rotary evaporator and/or with compressed air. (F) We used GC/MS to identify and quantify the products (Figures S15-S20).

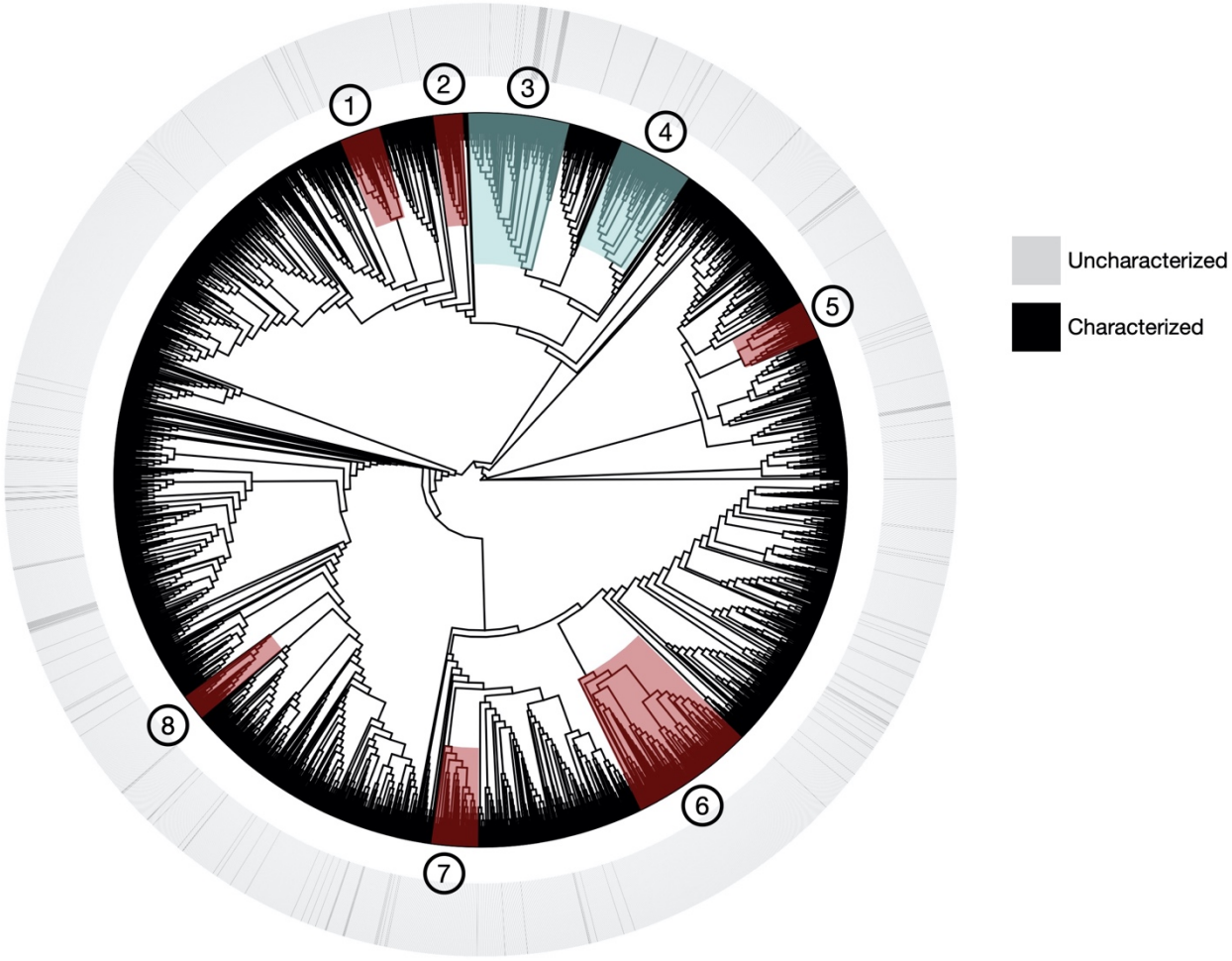

Figure S5. An annotated cladogram of terpene synthases. This cladogram of the PF03936 family is surrounded by a heatmap that shows the presence/absence of known EC numbers of the form 4.2.3.# (which includes terpene cyclization reactions) from the Uniprot database. We selected three genes from each of eight clades: six with no characterized genes (red) and two with characterized genes (blue). Table S1 summarizes the genes.

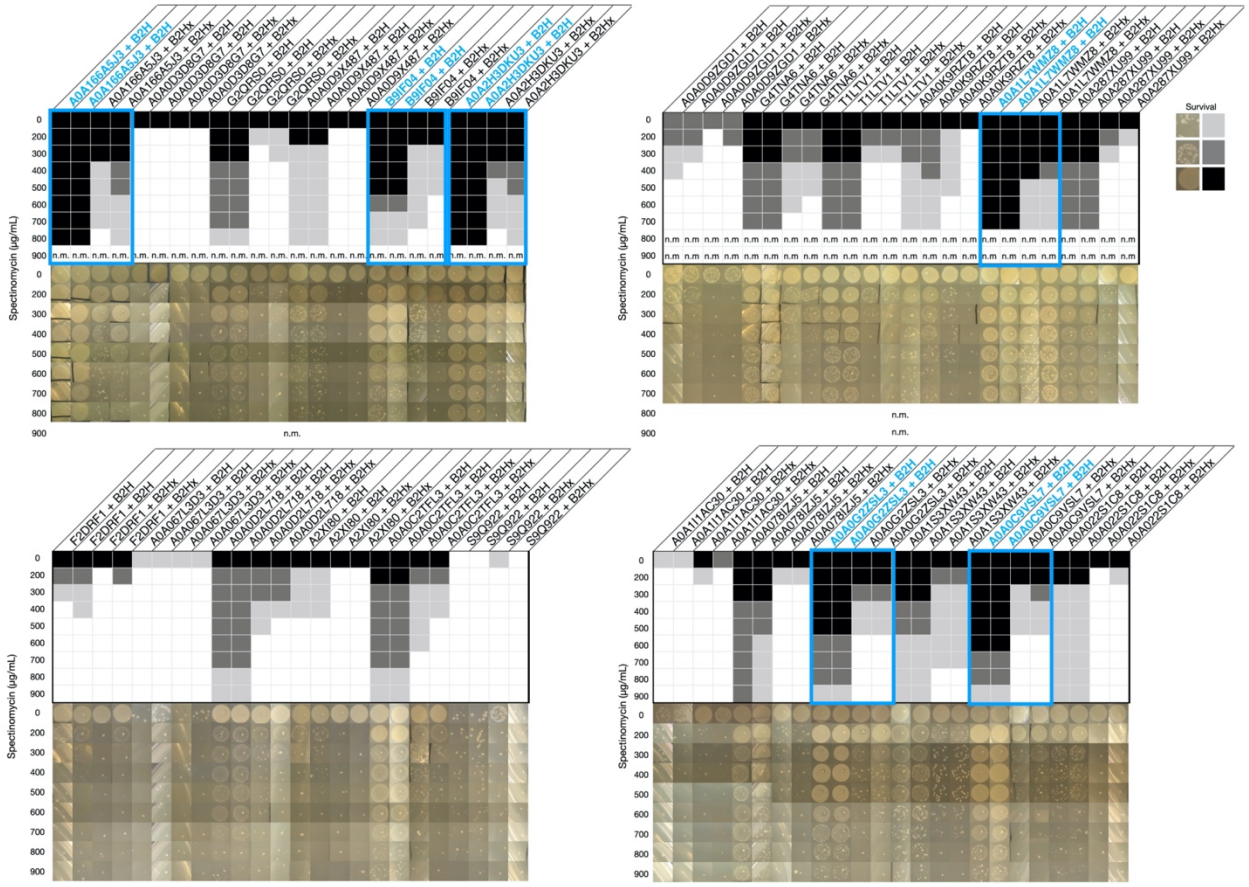

Figure S6. Analysis of selected genes. We searched for sesquiterpene inhibitors of PTP1B by screening each of the 24 uncharacterized genes alongside the FPP pathway (i.e., pMBIS). These pictures show the antibiotic resistance conferred by each gene. We selected strains with antibiotic resistance exceeding 400 μg/ml as hits (blue). Importantly, for these genes, the reduced survival of B2Hx controls indicates that enhanced resistance requires activation of the B2H system. In the top diagrams, n.m. indicates conditions that were not measured. Genes were distributed in the eight selected clades as follows: Clade 1: A0A166A5J3, A0A0D9X487, F2DRF1. Clade 2: A2XI80, A0A0D9ZGD1, A0A0K9RZT8. Clade 3: A0A1I1AC30, A0A1S3XW43, A0A0D3D8G7. Clade 4: A0A078IZJ5, A0A0C9VSL7, G2QRS0. Clade 5: B9IF04, A0A067L3D3, A0A0C2TFL3. Clade 6: A0A022S1C8, G4TNA6, A0A1L7WMZ8.

Clade 7: A0A2H3DKU3, A0A0D2L718, S9Q922. Clade 8: T1LTV1, A0A287XU99, A0A0G2ZSL3.

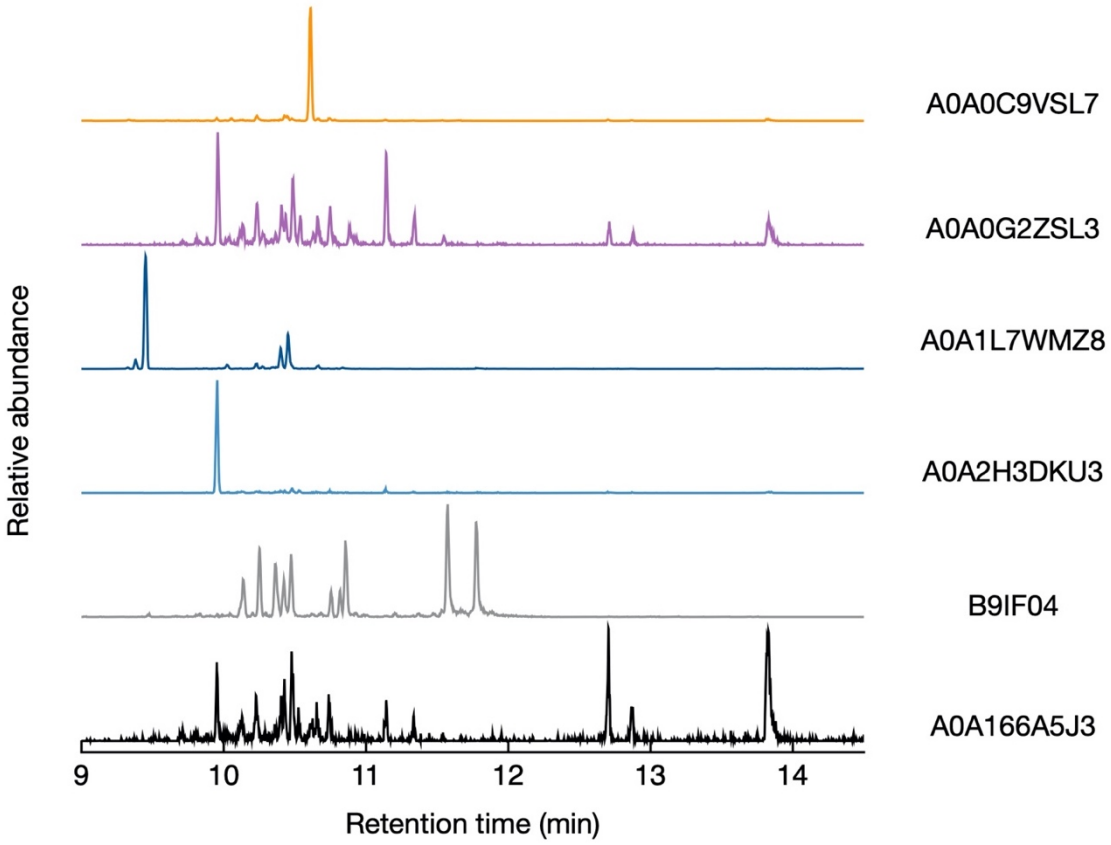

Figure S7. Product profiles of selected hits. The product profiles of selected hits (extracted ion chromatograms, m/z = 204). In brief, we grew up hits (i.e., pB2H$_{opt}$, pMBIS$_{CmR}$, and pTS) in liquid culture for 72 hours. With the exception of A0A0G2ZSL3, all hits were grown in 10 mL of 2% TB; A0A0G2ZSL3 was grown in a 4-mL culture of 2% TB. Notably, both A0A0C9VSL7 and A0A2H3DKU3 generate one dominant product: (+)-δ-cadinene and β-farnesene, respectively. We focused on A0A0C9VSL7 because (+)-δ-cadinene is a structural analog of amorphadiene, an inhibitor identified in our initial screen.

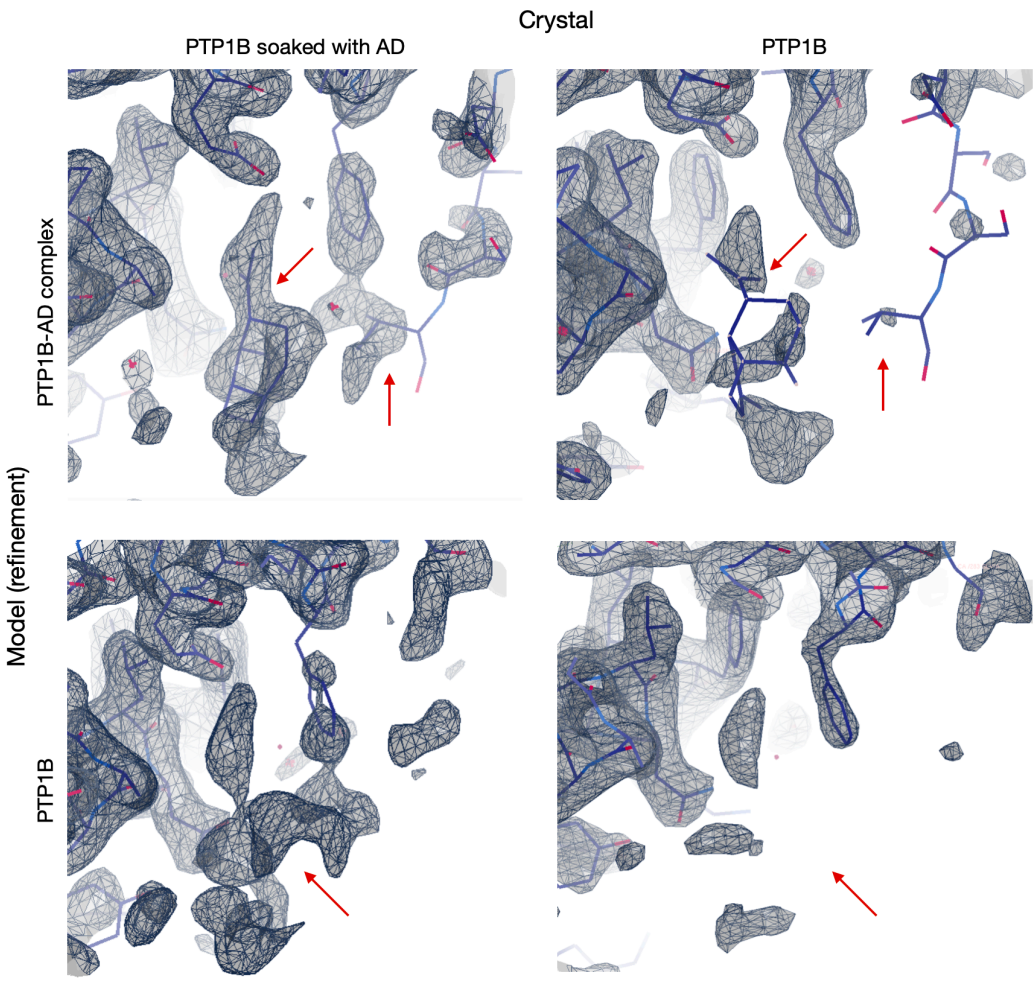

Figure S8. Crystallographic analysis of PTP1B bound to AD. Crystal structures of PTP1B collected in the (left) presence or (right) absence of amorphadiene. Resolutions: 2.10 Å (PTP1B-AD) and 1.94 Å (PTP1B). We refined these structures by modeling (top) the PTP1B-amorphadiene complex or (bottom) the apo form PTP1B. For PTP1B soaked with amorphadiene (left), the 1.0 σ 2Fo-Fc electron density supports the modeled position of amorphadiene (and the presence of V287) but suggests multiple bound conformations; this density appears even when amorphadiene is excluded from the model. By contrast, in the dataset for apo PTP1B (right), the 1.0 σ 2Fo-Fc electron does not support the presence of amorphadiene; small regions of unexplained density may reflect water molecules or partial occupancy of the α7 helix[9].

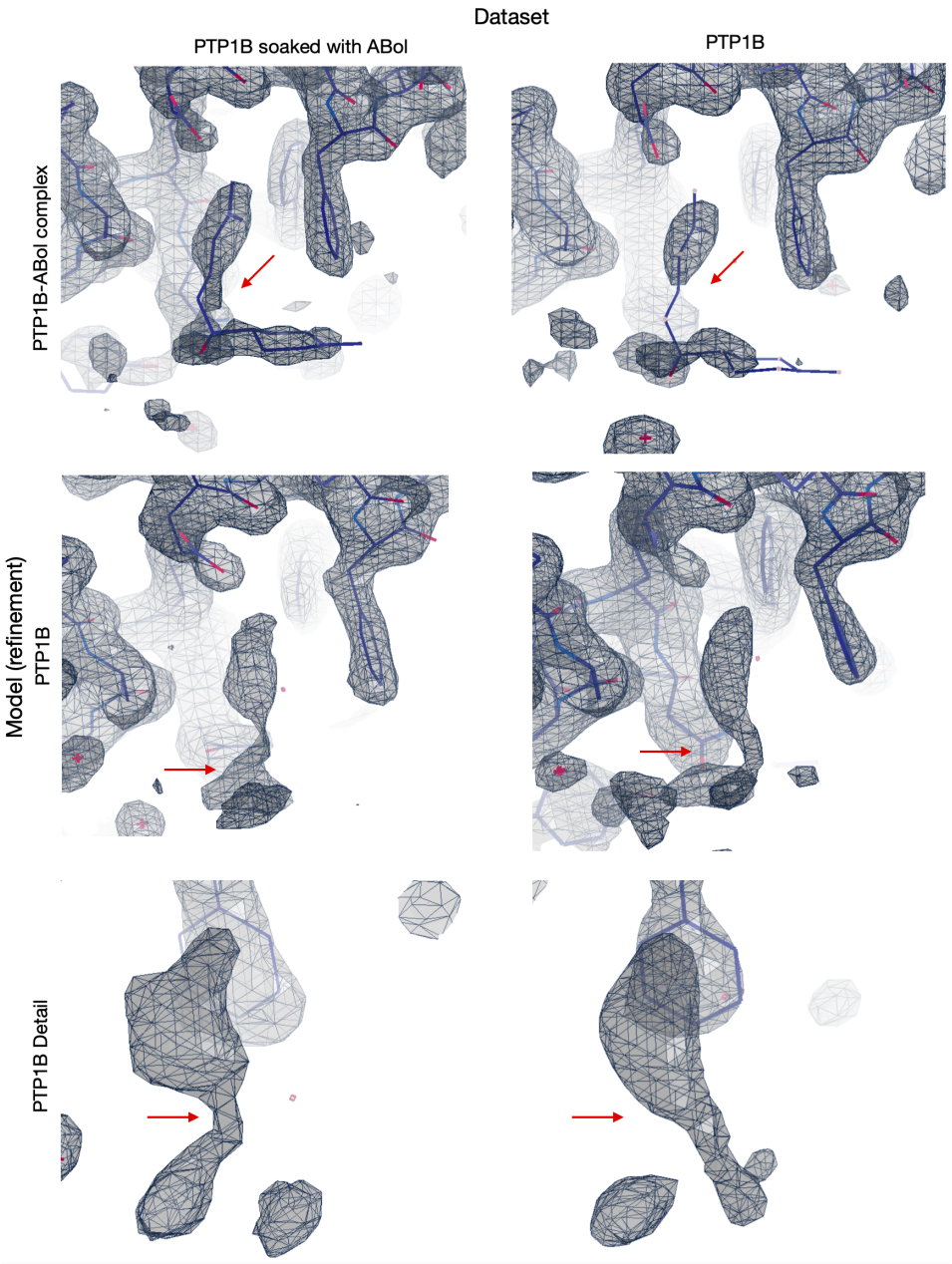

Figure S9. Crystallographic analysis of PTP1B bound to AD. Crystal structures of PTP1B collected in the (left) presence or (right) absence of α-bisabolol (ABol), which is more soluble than α-bisabolene. Resolutions: 2.11 Å (PTP1B-ABol) and 1.94 Å (PTP1B). We refined these structures by modeling (top) the PTP1B-ABol complex or (middle/bottom) the apo form PTP1B. For PTP1B soaked with ABol (left), the 0.90 σ 2Fo-Fc electron density is consistent with the modeled position of ABol, but it becomes less pronounced when ABol is excluded from the

model. The apo form of PTP1B (right) shows similar density for both models; small differences in the shape of the 0.90 σ 2Fo-Fc electron density between datasets suggests that this density may have a different origin (e.g., a ligand vs. partial occupancy of the α7 helix). The unambiguous determination of a binding site for ABol, however, requires additional data.

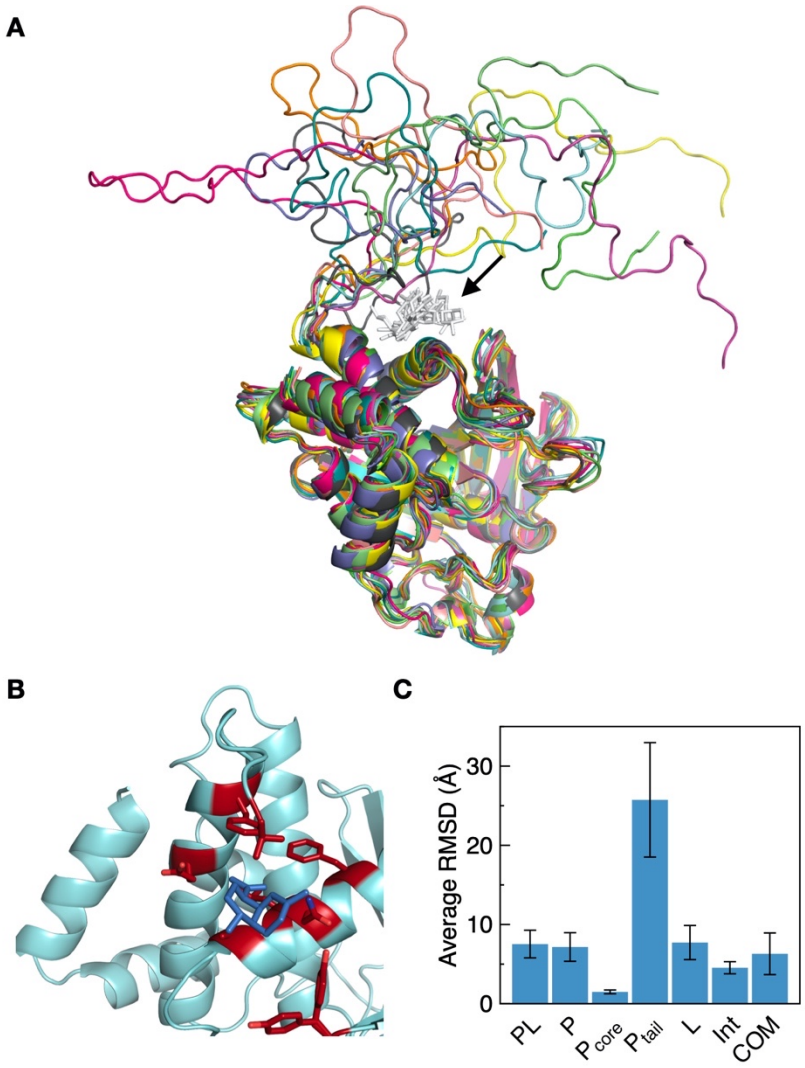

Figure S10. Evidence of multiple bound conformations. (A) Snapshots from molecular dynamics (MD) simulations of PTP1B bound to amorphadiene. Arrows indicate clusters of ligand. (B) A crystal structure of PTP1B bound to amorphadiene highlights residues that undergo high-frequency contacts. Here, contacts have residue-ligand distances < 4 Å, and high frequencies exceed 10% of all snapshots in the MD simulations. (C) Estimates of the average root-mean-square deviation (RMSD) of the complete system (PL), the protein (P), the protein core (P_{core}; residues 1-287), the disordered region of the protein (P_{tail}; residues 288-321), and the ligand (L) over MD simulations indicate that both amorphadiene and the disordered region of the protein are mobile (the latter more so than the former), while the protein core remains fixed. The

average RMSDs of both (i) the re-centered ligand (Int), a metric for rotational and vibrational fluctuations, and (ii) the center of mass (COM) of the ligand, a metric for its positional deviation, are large, an indication that the ligand can adopt multiple bound conformations and/or positions. Despite its flexibility, the ligand localizes to the crystallographically determined binding site. By contrast, the tail undergoes largescale movements around the C-terminus of the protein

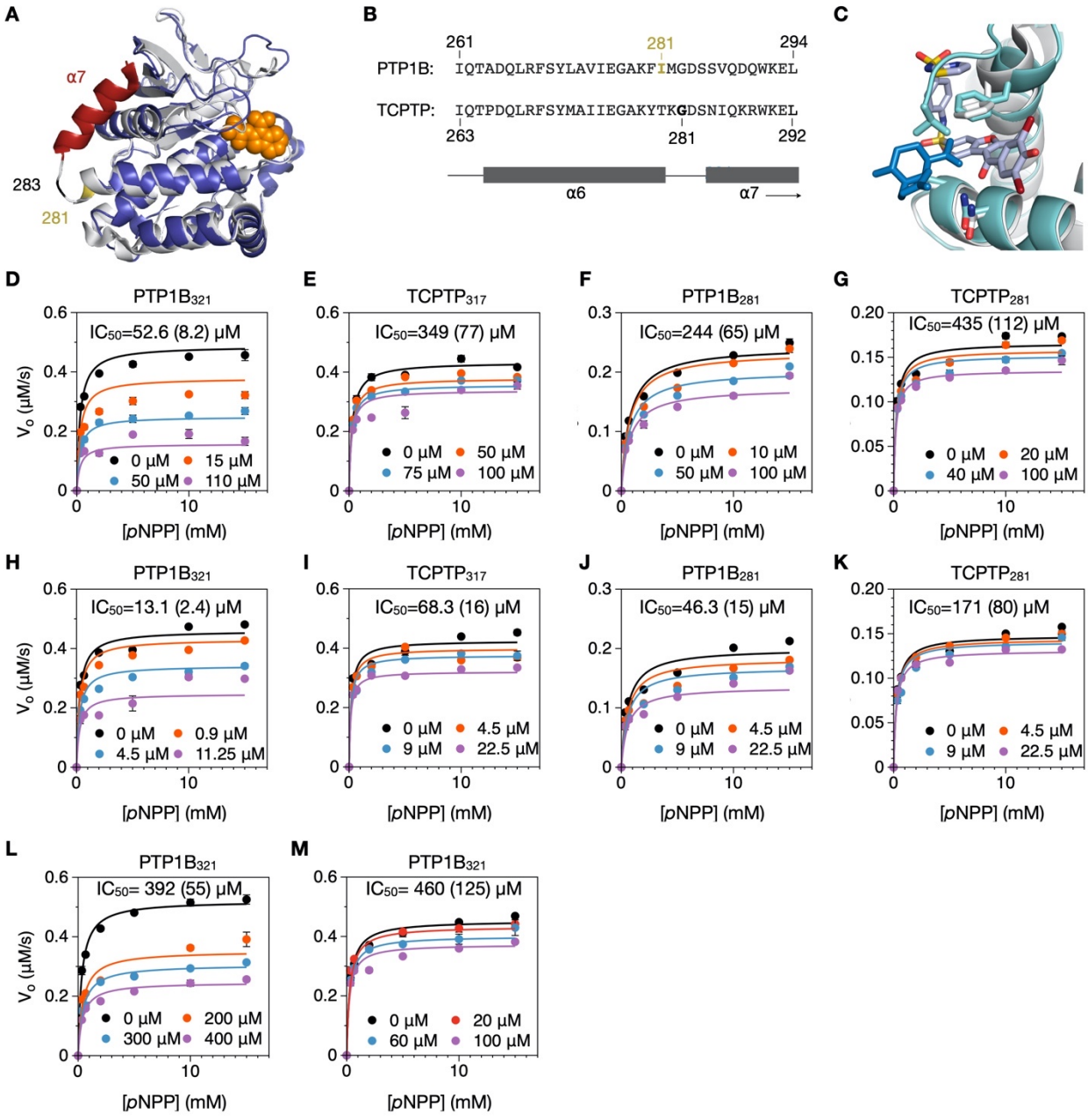

Figure S11 Summary of kinetics analyses. (A) Aligned crystal structures of PTP1B (gray, pdb entry 5k9w) and TC-PTP (blue, pdb entry 1l8k). Highlights on PTP1B: a competitive inhibitor (orange), the α7 helix (red), and truncation points used for kinetic studies (281 and 283, the 281-equivalent of TC-PTP). (B) Sequence alignment of the α6/7 regions of PTP1B and TC-PTP. The truncation points used in our kinetics analysis. (C) Aligned structures of the binding sites of BBR (gray, pdb entry 1t4j) and amorphadiene (blue). (D)-(M), Initial rates of pNPP hydrolysis by

various PTPs in the presence of increasing concentrations of (D)-(G) amorphadiene, (H)-(K) α-bisabolene, (L) dihydroartimesinic acid, and (M) α-bisabolol. In all figures, lines show the best-fit models of inhibition (Table S13). Error bars in (D)-(M) represent standard error of at least 3 measurements (Table S10). Error in IC50s represent 95% confidence intervals determined from fits to models of inhibition (Table S13).

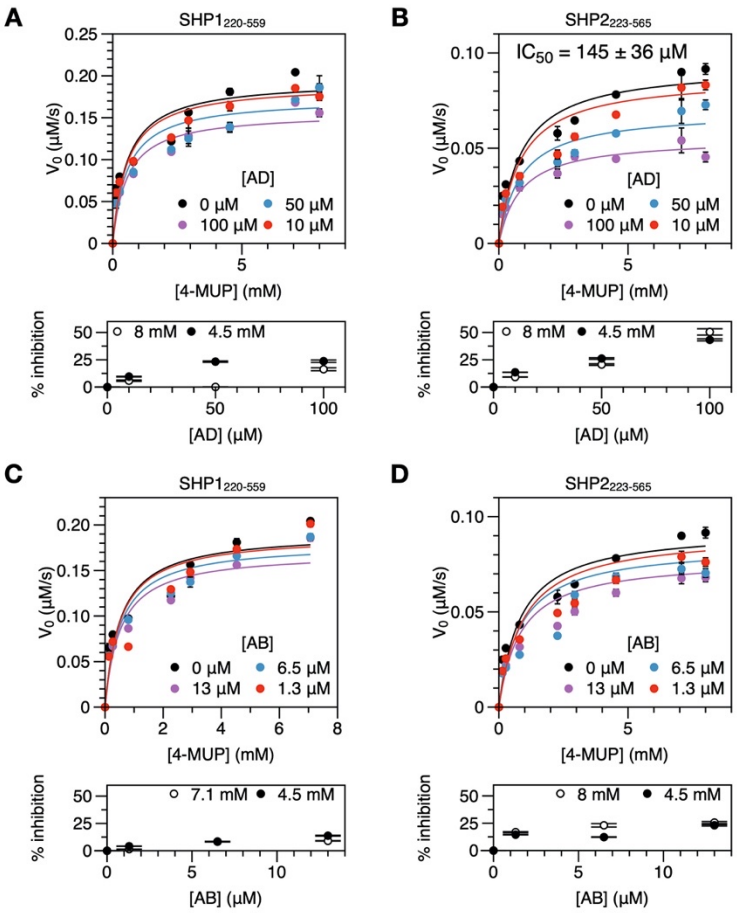

Figure S12. Expanded analysis of selectivity. Initial rates of 4-methylumbelliferyl phosphate (4-MUP) hydrolysis by various PTPs in the presence of increasing concentrations of (A)-(B) amorphadiene (AD), (C)-(D) α-bisabolene (AB). The lower panels show percent inhibition at three inhibitor concentrations (x-axis) and two substrate concentrations (open vs. closed circles). In (A), (C), and (D) our inability to measure inhibition greater than 25% (lower panels) at the solubility limit of our molecules, in combination with the high K_m for 4-MUP, precluded accurate measurements of IC_{50}s. In all panels, error bars denote standard error of n = 3 biological replicates and lines show fit to a noncompetitive inhibition model.

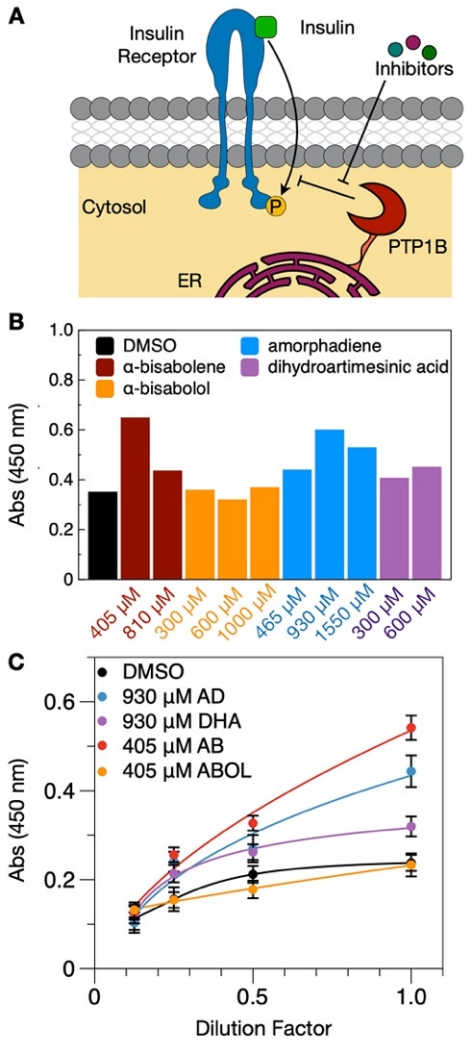

Figure S13. Analysis of PTP1B-mediated IR dephosphorylation. (A) A depiction of insulin signaling in HEK293T/17 cells. Extracellular insulin binds to the transmembrane insulin receptor (IR), triggering phosphorylation of its intracellular domain. PTP1B, which localizes to the endoplasmic reticulum (ER) of mammalian cells, dephosphorylates this domain to regulate downstream signaling pathways. In starved cells, exogenously supplied inhibitors can permeate the cell membrane and inhibit PTP1B-mediated dephosphorylation of the IR. (B) A screen of inhibitor concentrations for enzyme-linked immunosorbent assay (ELISAs). We used this screen to identify biologically active concentrations of amorphadiene and α-bisabolene to study further. (C) ELISA-based measurements of IR phosphorylation in HEK293T/17 cells incubated with

amorphadiene (AD), α-bisabolene (AB), dihydroartimesnic acid (DHA), and α-bisabolol (ABOL)**.** Curves denote fits to the four-parameter logistic equation: y = d+(a-d)/(1+(x/c)^b), where y is absorbance at 450 nm, and x is the sample dilution (e.g., 1 denotes no dilution, 0.5 denotes a 2-fold dilution, and so on; Table S11). These signals indicate that amorphadiene and α-bisabolene can increase IR phosphorylation over a negative control (3% DMSO) and their less inhibitory analogs. Error bars denote standard error with $n \geq 3$ biological replicates.

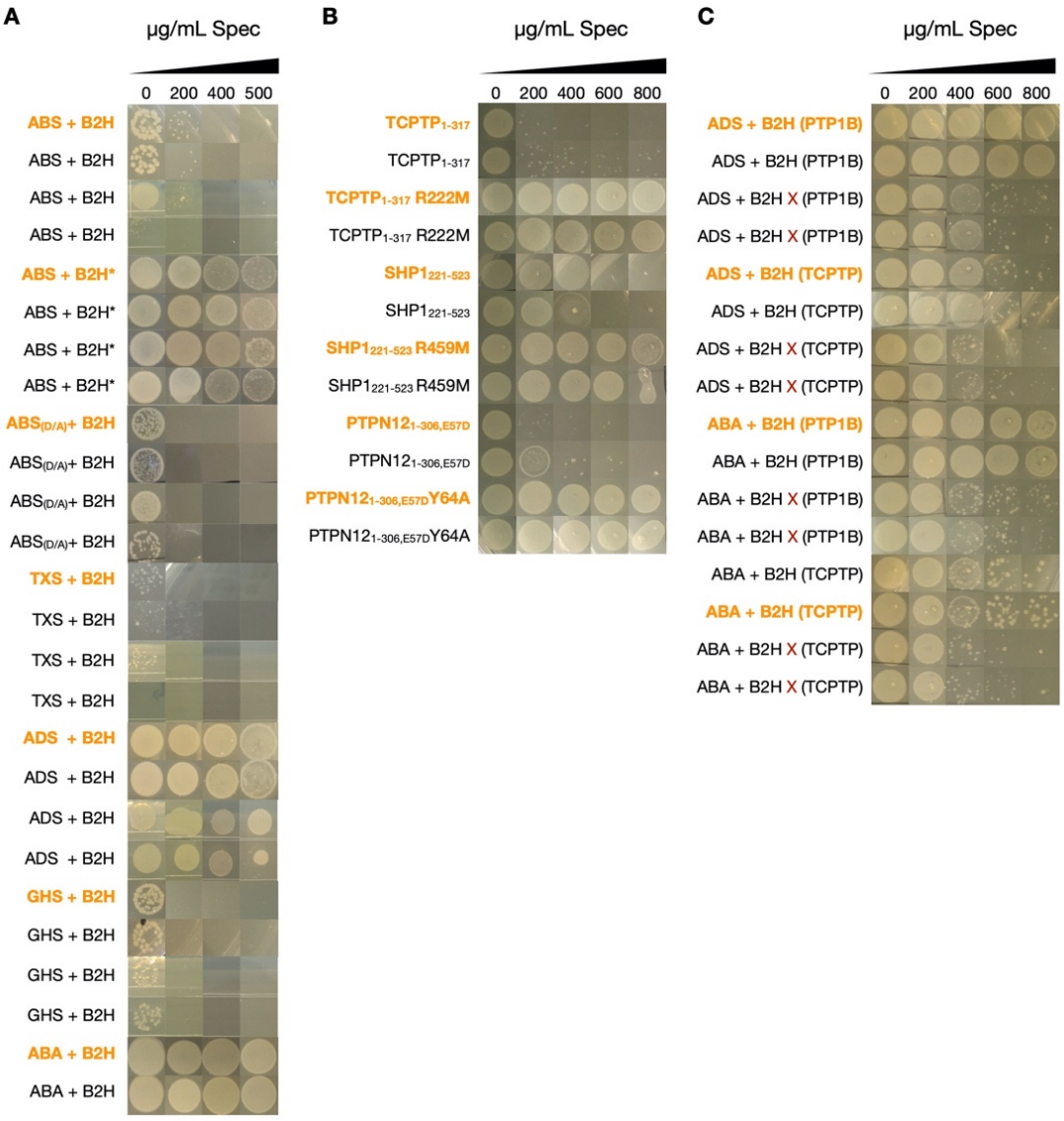

Figure S14. Full datasets for B2H-mediated antibiotic resistance. (A) Biological replicates for Figure 2C. (B) Biological replicates for Figure 5A. (C) Biological replicates and inactive B2H controls for Figure 5B. Orange highlights correspond to the data displayed in Figures 2C and 5A-B.

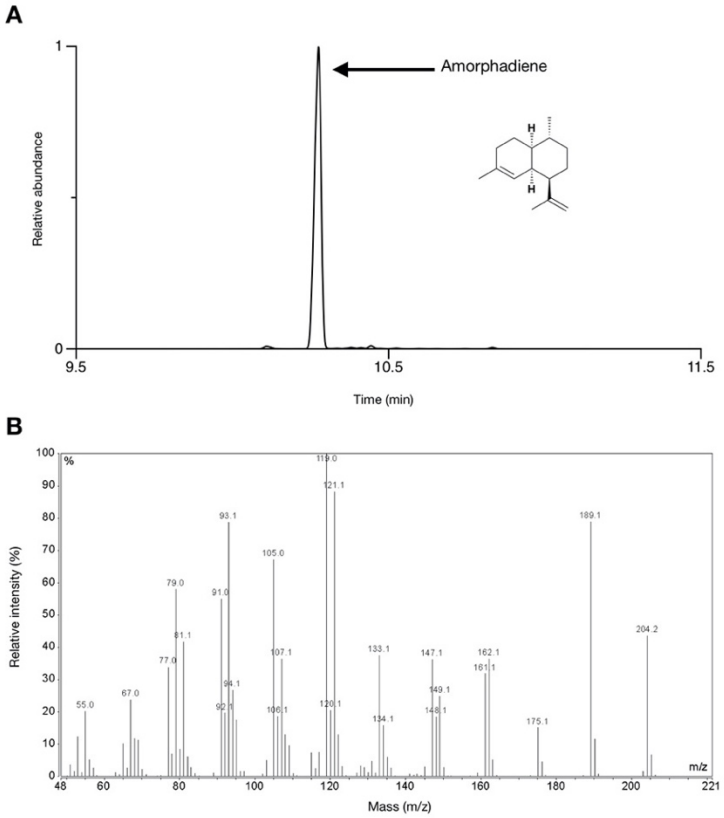

Figure S15. GC/MS analysis of amorphadiene production. (A) A GC/MS chromatogram of amorphadiene (95% purity, manufacturer spec; Ambeed). (B) The mass spectrum of the indicated peak from (A).

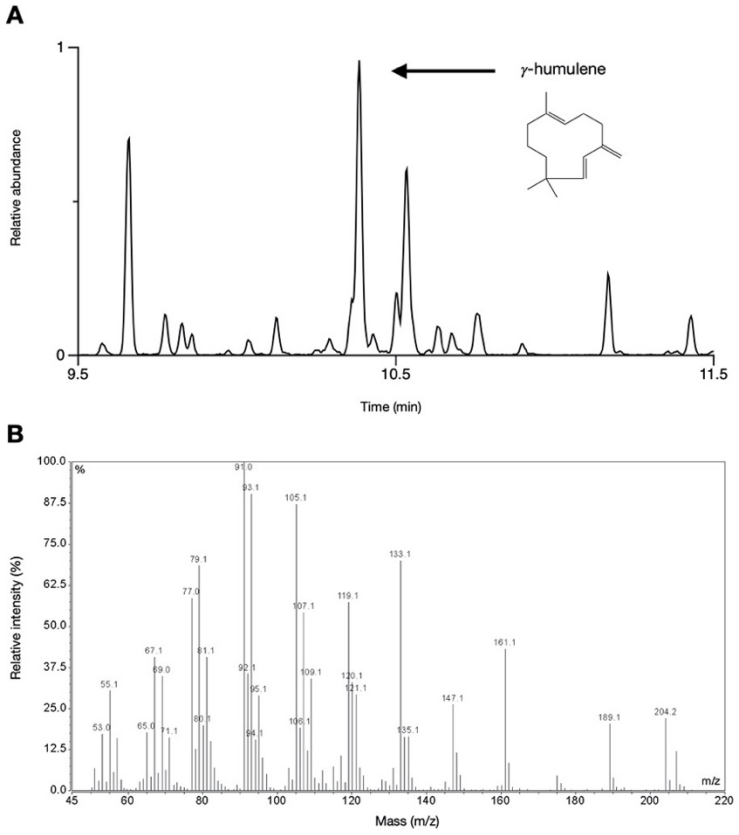

Figure S16. GC/MS analysis of ɣ-humulene production. (A) A GC/MS chromatogram shows the production of ɣ-humulene by a strain of *E. coli* engineered to produce it (i.e., pMBIS + pGHS). (B) The mass spectrum of the indicated peak from (A).

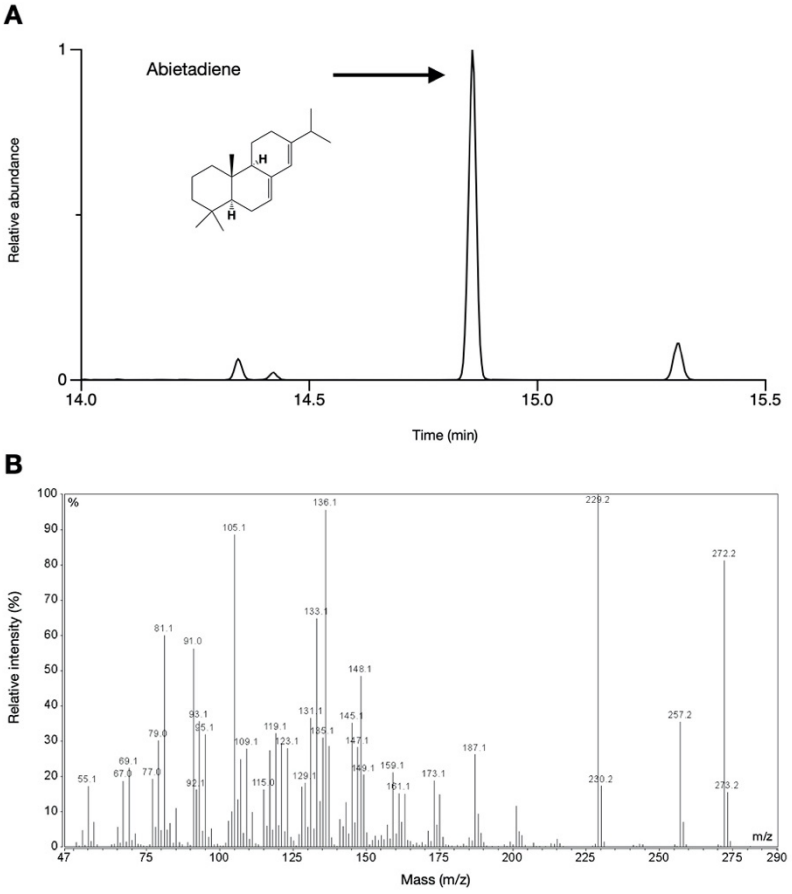

Figure S17. GC/MS analysis of abietadiene production. (A) A GC/MS chromatogram shows the production of abietadiene by a strain of *E. coli* engineered to produce it (i.e., pMBIS + pABS). (B) The mass spectrum of the indicated peak from (A).

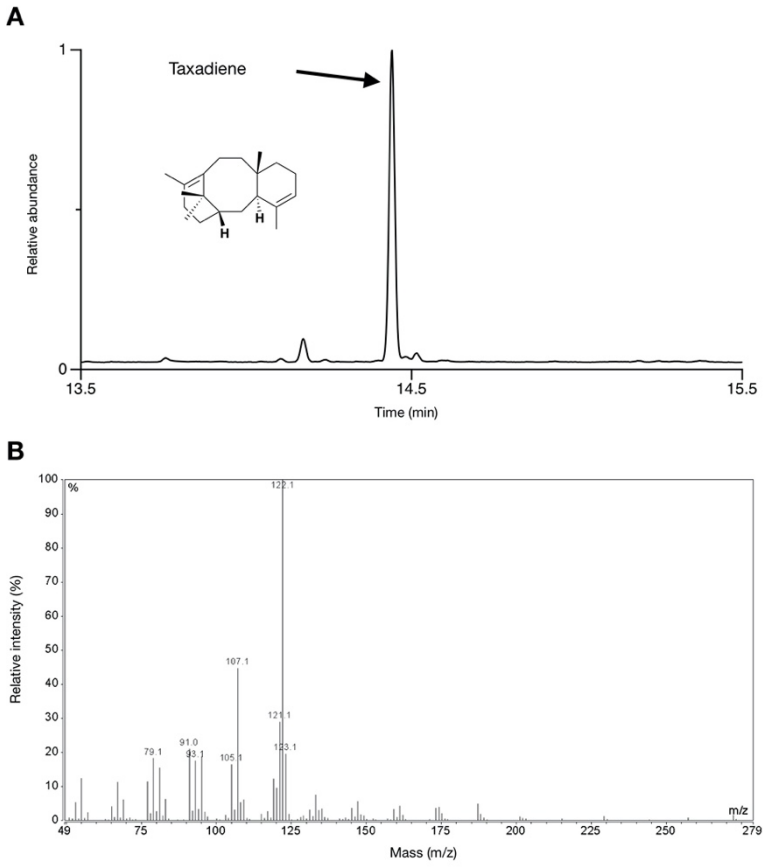

Figure S18. GC/MS analysis of taxadiene production. (A) A GC/MS chromatogram of taxadiene standard (70% pure by GC/MS; a kind gift from Phil Baran). (B) The mass spectrum of the indicated peak from (A).

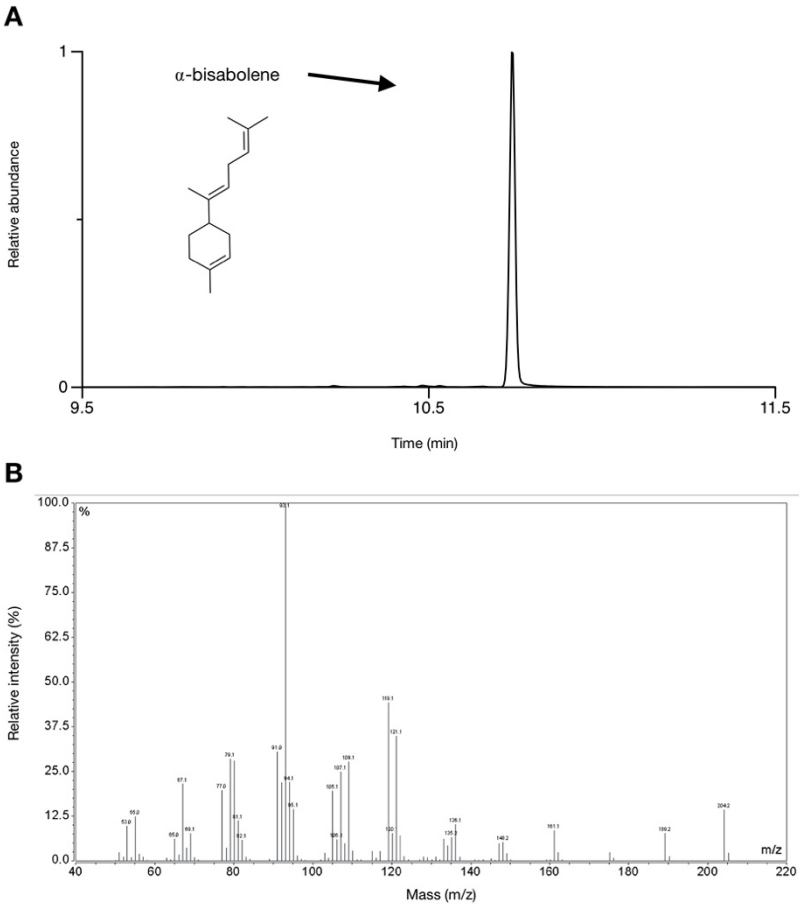

Figure S19. GC/MS analysis of α-bisabolene production. (A) A GC/MS chromatogram shows the production of α-bisabolene by a strain of *E. coli* engineered to produce it (i.e., pMBIS + pABA). (B) The mass spectrum of the indicated peak from (A).

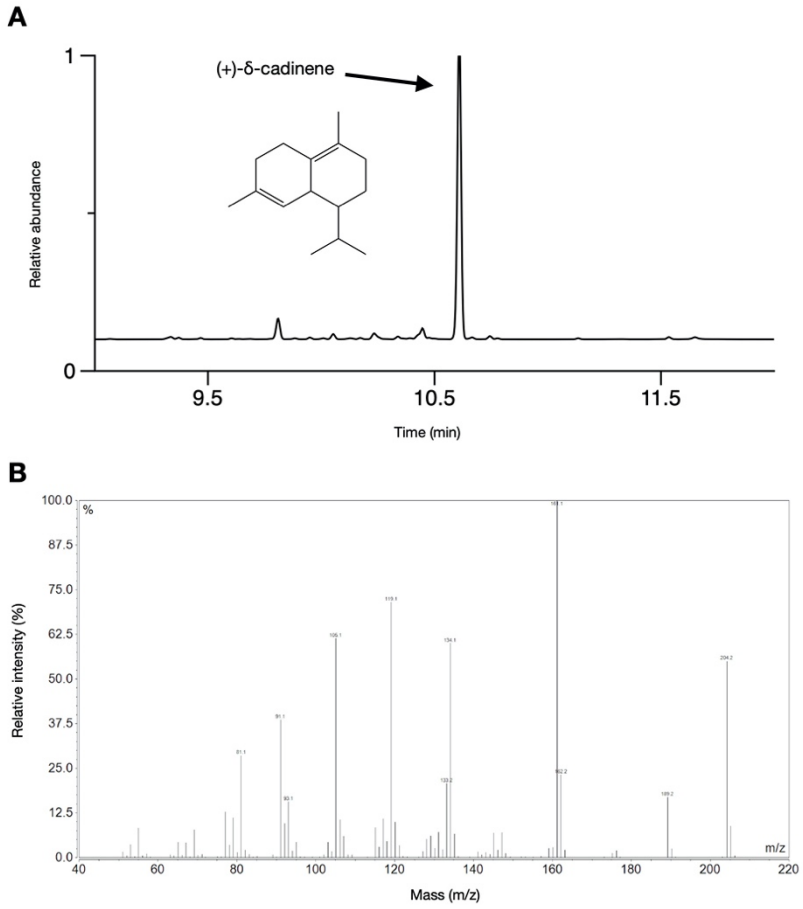

Figure S20. GC/MS analysis of (+)-δ-cadinene. (A) A GC/MS chromatogram shows the production of (+)-δ-cadinene by a strain of *E. coli* engineered to produce it (i.e., pMBIS + pA0A0C9VSL7). (B) The mass spectrum of the indicated peak from (A).

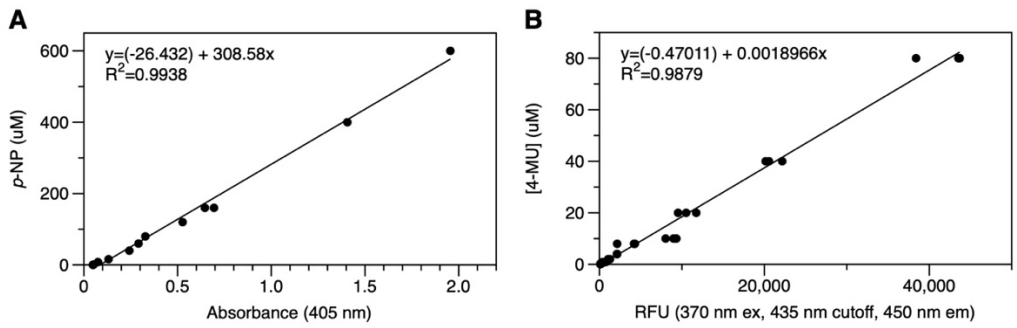

Figure S21. Standard curves for artificial phosphatate substrates. (A) We dissolved different amounts of *p*-nitrophenol (*p*-NP) in 100 µL buffer (50 mM HEPES, pH=7.3) and measured the absorbance of the resulting solutions with a SpectraMax M2 plate reader. A linear fit to this curve allowed us to convert absorbance measurements taken during kinetic assays (*p*NPP) to p-NP concentrations. (B) We dissolved different amounts of 4-methyl umbelliferone (4-MU) in 100 µL buffer (50 mM HEPES, pH=7.3) and measured the flourescence of the resulting solutions with a SpectraMax M2 plate reader. A linear fit to this curve allowed us to convert absorbance measurements taken during kinetic assays (4-MUP) to 4-MU concentrations.

Table S1. Gene Sources

Component	*Organism*	*Plasmid*	*Source*
Src	*H. sapiens*	pDONR223_SRC_WT	Addgene: 82165
CDC37	*H. sapiens*	pBACgus4x/cdc37/RocCOR LRRK2 1867-2176	Addgene: 40398
PTP1B	*H. sapiens*	pET21B_PTP1B	Nicholas Tonks, Cold Spring Harbor
TC-PTP	*H. sapiens*	pBG100-TCPTP	Addgene: 33365
PTPN6	*H. sapiens*	pGEX-2T SHP1 WT	Addgene: 8594
PTPN12	*H. sapiens*	pDONR223_PTPN12_p.E57D	Addgene: 81528
LuxAB		pAB078d8	Addgene: 79206
RpoZ	*Escherichia coli*	pAB094a	Addgene: 79241
cI434	*Escherichia virus Lambda*	pAB078d8	Addgene: 79206
SH2	*Rous sarcoma virus*	Kras-SRC FRET Biosensor	Addgene: 78302
p130cas	*H. sapiens*	Synthetic	Integrated DNA Technologies, Inc.
midT	*H. sapiens*	Synthetic	Integrated DNA Technologies, Inc.
EGFR	*H. sapiens*	Synthetic	Integrated DNA Technologies, Inc.
ShcA	*H. sapiens*	Synthetic	Integrated DNA Technologies, Inc.
MBIS	*S. cerevisiae*	pMBIS	Addgene: 17817
ADS	*Artemisia annua*	pADS	Addgene: 19040
GHS	*Abies grandis*	pTrcHUM	Addgene: 19003
ABS	*Abies grandis*	pSBET/AgAs	Reuben Peters, Iowa State University
TXS	*Taxus brevifola*	M60	David W. Christianson, University of Pennsylvania
ABA	*Abies grandis*	pTrc99a	Addgene: 35153
GGPPS	*Taxus canadensis*	gBlock	Integrated DNA Technologies, Inc.
A0A166A5J3	*S. Suecicum HHB10207 ss-3*	Synthetic	Twist Bioscience
A0A0D9X487	*L. perrieri*	Synthetic	Twist Bioscience
F2DRF1	*H. vulgare*	Synthetic	Twist Bioscience
A2XI80	*O. sativa*	Synthetic	Twist Bioscience
A0A0D9ZGD1	*O. glumipatula*	Synthetic	Twist Bioscience
A0A0K9RZT8	*S. olaracea*	Synthetic	Twist Bioscience
A0A1I1AC30	*A.aquimarinus*	Synthetic	Twist Bioscience
A0A1S3XW43	*N. tabacum*	Synthetic	Twist Bioscience
A0A0D3D8G7	*B. oleracea*	Synthetic	Twist Bioscience
B9IF04	*P. trichocarpa*	Synthetic	Twist Bioscience
A0A067L3D3	*J. curcas*	Synthetic	Twist Bioscience
A0A0C2TFL3	*A.Muscaria Koide BX008*	Synthetic	Twist Bioscience
A0A022S1C8	*E. guttata*	Synthetic	Twist Bioscience
G4TNA6	*S. indica*	Synthetic	Twist Bioscience
A0A1L7WMZ8	*P. subalpine*	Synthetic	Twist Bioscience

A0A078IZJ5	*B. napus*	Synthetic	Twist Bioscience
A0A0C9VSL7	*S. stellatus SS14*	Synthetic	Twist Bioscience
G2QRS0	*T. terrestris ATCC 38088*	Synthetic	Twist Bioscience
A0A2H3DKU3	*A.gallica*	Synthetic	Twist Bioscience
A0A0D2L718	*H. sublateritium FD-334 SS-4*	Synthetic	Twist Bioscience
S9Q922	*C. Fuscus DSM 2262*	Synthetic	Twist Bioscience
T1LTV1	*T. urartu*	Synthetic	Twist Bioscience
A0A287XU99	*H. vulgare*	Synthetic	Twist Bioscience
A0A0G2ZSL3	*A.gephyra*	Synthetic	Twist Bioscience

Table S2. Plasmids

Plasmid	*Description*	*Antibiotic**	*Availabiity*
F-plasmid	The F-plasmid from the S1030 strain of *E. coli*.	T	AG: 105063‡
pB2H1b	An early version of B2H that lacks PTP1B and contains LuxAB as the GOI.	K	Fox Lab
pBAD1b.Src	Enables inducible expression of Src and CDC37	P	Fox Lab
pBAD1b.SH2	Enables inducible expression of the SH2 domain.	P	Fox Lab
pBAD1b.S	Enables inducible expression of the substrate domain.	P	Fox Lab
pBAD1b.All	Enables inducible expression of Src, CDC37, the SH2 domain, and the substrate domain.	P	Fox Lab
pB2H1c.p130cas	An early version of B2H that (i) lacks PTP1B and Src, (ii) contains LuxAB, and (iii) includes a substrate from p130cas.	K	Fox Lab
pB2H1c.midT	An early version of B2H that (i) lacks PTP1B and Src, (ii) contains LuxAB, and (iii) includes a substrate from midT.	K	Fox Lab
pB2H1c.ShcA	An early version of B2H that (i) lacks PTP1B and Src, (ii) contains LuxAB, and (iii) includes a substrate from ShCA.	K	Fox Lab
pB2H1c.EGFR	An early version of B2H that (i) lacks PTP1B and Src, (ii) contains LuxAB, and (iii) includes a substrate from EGFR.	K	Fox Lab
pBAD1d	Enables inducible expression of Src and PTP1B.	P	Fox Lab
pBAD1d.mut	Enables inducible expression of Src and catalytically inactive PTP1B (C215S).	P	Fox Lab
pB2HS1.1Pro1	An early version of B2H that (i) lacks PTP1B, (ii) contains LuxAB, (iii) places expression of Src, CDC37, the SH2 domain, and the substrate domain under control of the same Pro1 promoter, and (iv) uses the BB034 RBS for Src.	K	Fox Lab
pB2HS1.1Pro1.mut	Identical to pB2H$_{S1.1Pro1}$ except for a mutation in the substrate (Y4F)	K	Fox Lab
pB2HS1.1ProD	An early version of B2H that (i) lacks PTP1B, (ii) contains LuxAB, and (iii) includes the ProD promoter and pro RBS for Src.	K	Fox Lab
pB2HS1.1ProD.mut	Identical to pB2H$_{S1.1ProD}$ except for a mutation in the substrate (Y4F)	K	Fox Lab
pB2HS1.2pro	An early version of B2H that (i) lacks PTP1B, (ii) contains LuxAB, and (iii) includes the pro RBS for Src.	K	Fox Lab
pB2HS1.2pro.mut	Identical to pB2H$_{S1.2pro}$ except for a mutation in the substrate (Y4F)	K	Fox Lab
pB2HS1.2Sal28	An early version of B2H that (i) lacks PTP1B, (ii) contains LuxAB, and (iii) includes the Sal28 RBS for Src.	K	Fox Lab
pB2HS1.2Sal28.mut	Identical to pB2H$_{S1.2Sal28}$ except for a mutation in the substrate (Y4F)	K	Fox Lab
pB2HS1.3RBS30	An early version of B2H that (i) contains LuxAB and (ii) includes the bb030 RBS for PTP1B.	K	Fox Lab
pB2HS1.3RBS30.mut	Identical to pB2H$_{S1.3RBS30}$ except for a mutation in the substrate (Y4F)	K	Fox Lab
pB2HS1.3RBS34	An early version of B2H that (i) contains LuxAB and (ii) includes the bb034 RBS for PTP1B.	K	Fox Lab
pB2HS1.3RBS34.mut	Identical to pB2H$_{S1.3RBS34}$ except for a mutation in the substrate (Y4F)	K	Fox Lab
pB2HS2RBS30	An early version of B2H that (i) contains SpecR and (ii) includes the bb030 RBS for PTP1B.	K	Fox Lab
pB2HS2RBS30.mut	Identical to pB2H$_{S2RBS30}$ except for an inactivating mutation in PTP1B (C215S)	K	Fox Lab
pB2Hopt	Final, optimized B2H that (i) contains SpecR and (ii) includes the bb034 RBS for PTP1B.	K	AG: 163830

*pB2H$_{opt}$**	Identical to pB2H$_{opt}$ except for an inactivating mutation in PTP1B (C215S)	K	AG: 163831
pB2H$_{optX}$	Identical to pB2H$_{opt}$ except for a mutation in the substrate domain (Y4F)	K	AG: 163832
pB2H$_2$	Identical to pB2H$_{opt}$ with TC-PTP in place of PTP1B	K	AG: 163833
*pB2H$_2$**	Identical to pB2H$_2$ except for an inactivating mutation in TC-PTP (R222M)	K	AG: 163834
pB2H$_6$	Identical to pB2H$_{opt}$ with SHP1 (catalytic domain) in place of PTP1B	K	AG: 163835
*pB2H$_6$**	Identical to pB2H$_6$ except for an inactivating mutation in SHP1 (R459M)	K	AG: 163836
pB2H$_{12}$	Identical to pB2H$_{opt}$ with PTPN12 in place of PTP1B	K	AG: 163837
*pB2H$_{12}$**	Identical to pB2H$_{12}$ except for an inactivating mutation in PTPN12 (Y64A)	K	AG: 163838
pMBIS	A plasmid that harbors genes for the mevalonate-dependent isoprenoid pathway from *S. cerevisiae* and harbors a tetracycline resistance marker.	T	AG: 17817
pMBIS$_{CmR}$	A plasmid that harbors genes for the mevalonate-dependent isoprenoid pathway from *S. cerevisiae* and harbors a chloramphenicol resistance marker.	P	Fox Lab
pTrc99t	A pTrc99a variant with BsaI removed for use in Golden Gate cloning	C	Fox Lab
pTS$_{ADS}$	A plasmid that harbors ADS.	C	AG:19040
pTS$_{ADS(D299A)}$	A plasmid that harbors ADS (D299A, inactivating).	C	Fox Lab
pTS$_{GHS}$	A plasmid that harbors GHS.	C	AG:19003
pTS$_{GHS(D343A)}$	A plasmid that harbors GHS (D343A, inactivating).	C	Fox Lab
pTS$_{ABA}$	A plasmid that harbors ABA.	C	Fox Lab
pTS$_{ABA(D566A)}$	A plasmid that harbors ABA (D566A, inactivating).	C	Fox Lab
pTS$_{ABS}$	A plasmid that harbors ABS and GGPPS.	C	AG: 163840
pTS$_{ABS(D404A/D621A)}$	A plasmid that harbors ABS (D404A/D621A, inactivating) and GGPPS.	C	Fox Lab
pTS$_{TXS}$	A plasmid that harbors TXS and GGPPS.	C	AG: 163839
pTS$_{A0A166A5J3}$	A plasmid that harbors A0A166A5J3 (Clade 1)	C	Fox Lab
pTS$_{A0A0D9X487}$	A plasmid that harbors A0A0D9X487 (Clade 1)	C	Fox Lab
pTS$_{F2DRF1}$	A plasmid that harbors F2DRF1 (Clade 1)	C	Fox Lab
pTS$_{A2XI80}$	A plasmid that harbors A2XI80 (Clade 2)	C	Fox Lab
pTS$_{A0A0D9ZGD1}$	A plasmid that harbors A0A0D9ZGD1 (Clade 2)	C	Fox Lab
pTS$_{A0A0K9RZT8}$	A plasmid that harbors A0A0K9RZT8 (Clade 2)	C	Fox Lab
pTS$_{A0A1I1AC30}$	A plasmid that harbors A0A1I1AC30 (Clade 3)	C	Fox Lab
pTS$_{A0A1S3XW43}$	A plasmid that harbors A0A1S3XW43 (Clade 3)	C	Fox Lab
pTS$_{A0A0D3D8G7}$	A plasmid that harbors A0A0D3D8G7 (Clade 3)	C	Fox Lab
pTS$_{B9IF04}$	A plasmid that harbors B9IF04 (Clade 4)	C	Fox Lab
pTS$_{A0A067L3D3}$	A plasmid that harbors A0A067L3D3 (Clade 4)	C	Fox Lab
pTS$_{A0A0C2TFL3}$	A plasmid that harbors A0A0C2TFL3 (Clade 4)	C	Fox Lab
pTS$_{A0A022S1C8}$	A plasmid that harbors A0A022S1C8 (Clade 5)	C	Fox Lab
pTS$_{G4TNA6}$	A plasmid that harbors G4TNA6 (Clade 5)	C	Fox Lab
pTS$_{A0A1L7WMZ8}$	A plasmid that harbors A0A1L7WMZ8 (Clade 5)	C	Fox Lab
pTS$_{A0A078IZJ5}$	A plasmid that harbors A0A078IZJ5 (Clade 6)	C	Fox Lab
pTS$_{A0A0C9VSL7}$	A plasmid that harbors A0A0C9VSL7 (Clade 6)	C	AG: 163841
pTS$_{G2QRS0}$	A plasmid that harbors G2QRS0 (Clade 6)	C	Fox Lab
pTS$_{A0A2H3DKU3}$	A plasmid that harbors A0A2H3DKU3 (Clade 7)	C	Fox Lab
pTS$_{A0A0D2L718}$	A plasmid that harbors A0A0D2L718 (Clade 7)	C	Fox Lab
pTS$_{S9Q922}$	A plasmid that harbors S9Q922 (Clade 7)	C	Fox Lab
pTS$_{T1LTV1}$	A plasmid that harbors T1LTV1 (Clade 8)	C	Fox Lab
pTS$_{A0A287XU99}$	A plasmid that harbors A0A287XU99 (Clade 8)	C	Fox Lab
pTS$_{A0A0G2ZSL3}$	A plasmid that harbors A0A0G2ZSL3 (Clade 8)	C	Fox Lab

pET21b$_{ptp1b}$	A plasmid that encodes a His-tagged catalytic domain of PTP1B (for protein expression)	C	N/A[+]
pET16B$_{TCPTP}$	A plasmid that encodes a His-tagged catalytic domain of TCPTP (for protein expression)	C	Fox Lab

[*]Antibiotic resistance: carbenicillin (C, 50 μg/ml), kanamycin (K, 50 μg/ml), tetracycline (T, 10 μg/ml), chloramphenicol (P, 34 μg/ml), and spectinomycin (S, conditional).
[+]This plasmid was a kind gift from Nicholas Tonks of Cold Spring Harbor Laboratory.
[‡]AG=Addgene accession # (Addgene.com).

Table S3. Components of various B2H systems.

Component	*Name*	*DNA*	*Amino Acid*
Kinase	c-Src	ATGGGCTCCAAGCCGCAGACTCAGGGCCTGGC	MGSKPQTQGLAK
		CAAGGATGCCTGGGAGATCCCTCGGGAGTCGC	DAWEIPRESLRLE
		TGCGGCTGGAGGTCAAGCTGGGCCAGGGCTGC	VKLGQGCFGEVW
		TTTGGCGAGGTGTGGATGGGGACCTGGAACGG	MGTWNGTTRVAI
		TACCACCAGGGTGGCCATCAAAACCCTGAAGC	KTLKPGTMSPEAF
		CTGGCACGATGTCTCCAGAGGCCTTCCTGCAG	LQEAQVMKKLRH
		GAGGCCCAGGTCATGAAGAAGCTGAGGCATGA	EKLVQLYAVVSEE
		GAAGCTGGTGCAGTTGTATGCTGTGGTTTCAGA	PIYIVTEYMSKGSL
		GGAGCCCATTTACATCGTCACGGAGTACATGA	LDFLKGETGKYLR
		GCAAGGGGAGTTTGCTGGACTTTCTCAAGGGG	LPQLVDMAAQIAS
		GAGACAGGCAAGTACCTGCGGCTGCCTCAGCT	GMAYVERMNYV
		GGTGGACATGGCTGCTCAGATCGCCTCAGGCA	HRDLRAANILVGE
		TGGCGTACGTGGAGCGGATGAACTACGTCCAC	NLVCKVADFGLA
		CGGGACCTTCGTGCAGCCAACATCCTGGTGGG	RLIEDNEYTARQG
		AGAGAACCTGGTGTGCAAAGTGGCCGACTTTG	AKFPIKWTAPEAA
		GGCTGGCTCGGCTCATTGAAGACAATGAGTAC	LYGRFTIKSDVWS
		ACGGCGCGGCAAGGTGCCAAATTCCCCATCAA	FGILLTELTTKGR
		GTGGACGGCTCCAGAAGCTGCCCTCTATGGCC	VPYPGMVNREVL
		GCTTCACCATCAAGTCGGACGTGTGGTCCTTCG	DQVERGYRMPCP
		GGATCCTGCTGACTGAGCTCACCACAAAGGGA	PECPESLHDLMCQ
		CGGGTGCCCTACCCTGGGATGGTGAACCGCGA	CWRKEPEERPTFE
		GGTGCTGGACCAGGTGGAGCGGGGCTACCGGA	YLQAFLEDYFTST
		TGCCCTGCCCGCCGGAGTGTCCCGAGTCCCTGC	EPQYQPGENL*
		ACGACCTCATGTGCCAGTGCTGGCGGAAGGAG	
		CCTGAGGAGCGGCCCACCTTCGAGTACCTGCA	
		GGCCTTCCTGGAGGACTACTTCACGTCCACCGA	
		GCCCCAGTACCAGCCCGGGGAGAACCTCTAA	
Chaperone	CDC37	ATGGTGGACTACAGCGTGTGGGACCACATTGA	MVDYSVWDHIEV
		GGTGTCTGATGATGAAGACGAGACGCACCCCA	SDDEDETHPNIDT
		ACATCGACACGGCCAGTCTCTTCCGCTGGCGG	ASLFRWRHQARV
		CATCAGGCCCGGGTGGAACGCATGGAGCAGTT	ERMEQFQKEKEEL
		CCAGAAGGAGAAGGAGGAACTGGACAGGGGC	DRGCRECKRKVA
		TGCCGCGAGTGCAAGCGCAAGGTGGCCGAGTG	ECQRKLKELEVAE
		CCAGAGGAAACTGAAGGAGCTGGAGGTGGCC	GGKAELERLQAE
		GAGGGCGGCAAGGCAGAGCTGGAGCGCCTGC	AQQLRKEERSWE
		AGGCCGAGGCACAGCAGCTGCGCAAGGAGGA	QKLEEMRKKEKS
		GCGGAGCTGGGAGCAGAAGCTGGAGGAGATG	MPWNVDTLSKDG
		CGCAAGAAGGAGAAGAGCATGCCCTGGAACGT	FSKSMVNTKPEKT
		GGACACGCTCAGCAAAGACGGCTTCAGCAAGA	EEDSEEVREQKHK
		GCATGGTAAATACCAAGCCCGAGAAGACGGAG	TFVEKYEKQIKHF
		GAGGACTCAGAGGAGGTGAGGGAGCAGAAAC	GMLRRWDDSQKY
		ACAAGACCTTCGTGGAAAAATACGAGAAACAG	LSDNVHLVCEETA
		ATCAAGCACTTTGGCATGCTTCGCCGCTGGGAT	NYLVIWCIDLEVE
		GACAGCCAAAAGTACCTGTCAGACAACGTCCA	EKCALMEQVAHQ
		CCTGGTGTGCGAGGAGACAGCCAATTACCTGG	TIVMQFILELAKSL
		TCATTTGGTGCATTGACCTAGAGGTGGAGGAG	KVDPRACFRQFFT
		AAATGTGCACTCATGGAGCAGGTGGCCCACCA	KIKTADRQYMEG
		GACAATCGTCATGCAATTTATCCTGGAGCTGGC	FNDELEAFKERVR
		CAAGAGCCTAAAGGTGGACCCCCGGGCCTGCT	GRAKLRIEKAMK
		TCCGGCAGTTCTTCACTAAGATTAAGACAGCC	EYEEEERKKRLGP
		GATCGCCAGTACATGGAGGGCTTCAACGACGA	GGLDPVEVYESLP
		GCTGGAAGCCTTCAAGGAGCGTGTGCGGGGCC	EELQKCFDVKDV
		GTGCCAAGCTGCGCATCGAGAAGGCCATGAAG	QMLQDAISKMDP

		GAGTACGAGGAGGAGGAGCGCAAGAAGCGGC	TDAKYHMQRCID
		TCGGCCCCGGCGGCCTGGACCCCGTCGAGGTC	SGLWVPNSKASE
		TACGAGTCCCTCCCTGAGGAACTCCAGAAGTG	AKEGEEAGPGDPL
		CTTCGATGTGAAGGACGTGCAGATGCTGCAGG	LEAVPKTGDEKD
		ACGCCATCAGCAAGATGGACCCCACCGACGCA	VSV*
		AAGTACCACATGCAGCGCTGCATTGACTCTGG	
		CCTCTGGGTCCCCAACTCTAAGGCCAGCGAGG	
		CCAAGGAGGGAGAGGAGGCAGGTCCTGGGGA	
		CCCATTACTGGAAGCTGTTCCCAAGACGGGCG	
		ATGAGAAGGATGTCAGTGTGTAA	
Phospha-tase	PTP1B	ATGGAGATGGAAAAGGAGTTCGAGCAGATCGA	MEMEKEFEQIDKS
		CAAGTCCGGGAGCTGGGCGGCCATTTACCAGG	GSWAAIYQDIRHE
		ATATCCGACATGAAGCCAGTGACTTCCCATGT	ASDFPCRVAKLPK
		AGAGTGGCCAAGCTTCCTAAGAACAAAAACCG	NKNRNRYRDVSP
		AAATAGGTACAGAGACGTCAGTCCCTTTGACC	FDHSRIKLHQEDN
		ATAGTCGGATTAAACTACATCAAGAAGATAAT	DYINASLIKMEEA
		GACTATATCAACGCTAGTTTGATAAAAATGGA	QRSYILTQGPLPN
		AGAAGCCCAAAGGAGTTACATTCTTACCCAGG	TCGHFWEMVWE
		GCCCTTTGCCTAACACATGCGGTCACTTTTGGG	QKSRGVVMLNRV
		AGATGGTGTGGGAGCAGAAAAGCAGGGGTGTC	MEKGSLKCAQYW
		GTCATGCTCAACAGAGTGATGGAGAAAGGTTC	PQKEEKEMIFEDT
		GTTAAAATGCGCACAATACTGGCCACAAAAAG	NLKLTLISEDIKSY
		AAGAAAAAGAGATGATCTTTGAAGACACAAAT	YTVRQLELENLTT
		TTGAAATTAACATTGATCTCTGAAGATATCAAG	QETREILHFHYTT
		TCATATTATACAGTGCGACAGCTAGAATTGGA	WPDFGVPESPASF
		AAACCTTACAACCCAAGAAACTCGAGAGATCT	LNFLFKVRESGSL
		TACATTTCCACTATACCACATGGCCTGACTTTG	SPEHGPVVVHCSA
		GAGTCCCTGAATCACCAGCCTCATTCTTGAACT	GIGRSGTFCLADT
		TTCTTTTCAAAGTCCGAGAGTCAGGGTCACTCA	CLLLMDKRKDPSS
		GCCCGGAGCACGGGCCCGTTGTGGTGCACTGC	VDIKKVLLEMRKF
		AGTGCAGGCATCGGCAGGTCTGGAACCTTCTG	RMGLIQTADQLRF
		TCTGGCTGATACCTGCCTCTTGCTGATGGACAA	SYLAVIEGAKFIM
		GAGGAAAGACCCTTCTTCCGTTGATATCAAGA	GDSSVQDQWKEL
		AAGTGCTGTTAGAAATGAGGAAGTTTCGGATG	SHEDLEPPPEHIPP
		GGGCTGATCCAGACAGCCGACCAGCTGCGCTT	PPRPPKRILEPHN*
		CTCCTACCTGGCTGTGATCGAAGGTGCCAAATT	
		CATCATGGGGGACTCTTCCGTGCAGGATCAGT	
		GGAAGGAGCTTTCCCACGAGGACCTGGAGCCC	
		CCACCCGAGCATATCCCCCCACCTCCCCGGCCA	
		CCCAAACGAATCCTGGAGCCACACAATTGA	
Phospha-tase	TC-PTP	ATGCCCACCACCATCGAGCGGGAGTTCGAAGA	MPTTIEREFEELDT
		GTTGGATACTCAGCGTCGCTGGCAGCCGCTGT	QRRWQPLYLEIRN
		ACTTGGAAATTCGAAATGAGTCCCATGACTAT	ESHDYPHRVAKFP
		CCTCATAGAGTGGCCAAGTTTCCAGAAAACAG	ENRNRNRYRDVS
		AAATCGAAACAGATACAGAGATGTAAGCCCAT	PYDHSRVKLQNA
		ATGATCACAGTCGTGTTAAACTGCAAAATGCT	ENDYINASLVDIE
		GAGAATGATTATATTAATGCCAGTTTAGTTGAC	EAQRSYILTQGPL
		ATAGAAGAGGCACAAAGGAGTTACATCTTAAC	PNTCCHFWLMVW
		ACAGGGTCCACTTCCTAACACATGCTGCCATTT	QQKTKAVVMLNR
		CTGGCTTATGGTTTGGCAGCAGAAGACCAAAG	IVEKESVKCAQY
		CAGTTGTCATGCTGAACCGCATTGTGGAGAAA	WPTDDQEMLFKE
		GAATCGGTTAAATGTGCACAGTACTGGCCAAC	TGFSVKLLSEDVK
		AGATGACCAAGAGATGCTGTTTAAAGAAACAG	SYYTVHLLQLENI
		GATTCAGTGTGAAGCTCTTGTCAGAAGATGTG	NSGETRTISHFHY
		AAGTCGTATTATACAGTACATCTACTACAATTA	TTWPDFGVPESPA
		GAAAATATCAATAGTGGTGAAACCAGAACAAT	SFLNFLFKVRESG

		ATCTCACTTTCATTATACTACCTGGCCAGATTT	SLNPDHGPAVIHC
		TGGAGTCCCTGAATCACCAGCTTCATTTCTCAA	SAGIGRSGTFSLV
		TTTCTTGTTTAAAGTGAGAGAATCTGGCTCCTT	DTCLVLMEKGDDI
		GAACCCTGACCATGGGCCTGCGGTGATCCACT	NIKQVLLNMRKY
		GTAGTGCAGGCATTGGGCGCTCTGGCACCTTCT	RMGLIQTPDQLRF
		CTCTGGTAGACACTTGTCTTGTTTTGATGGAAA	SYMAIIEGAKCIK
		AAGGAGATGATATTAACATAAAACAAGTGTTA	GDSSIQKRWKELS
		CTGAACATGAGAAAATACCGAATGGGTCTTAT	KEDLSPAFDHSPN
		TCAGACCCCAGATCAACTGAGATTCTCATACAT	KIMTEKYNGNR*
		GGCTATAATAGAAGGAGCAAAATGTATAAAGG	
		GAGATTCTAGTATACAGAAACGATGGAAAGAA	
		CTTTCTAAGGAAGACTTATCTCCTGCCTTTGAT	
		CATTCACCAAACAAAATAATGACTGAAAAATA	
		CAATGGGAACAGATAA	
Phospha-tase	SHP1	ATGGCTGACATTGAGAACCGAGTGTTGGAACT	MADIENRVLELNK
		GAACAAGAAGCAGGAGTCCGAGGATACAGCC	KQESEDTAKAGF
		AAGGCTGGCTTCTGGGAGGAGTTTGAGAGTTT	WEEFESLQKQEV
		GCAGAAGCAGGAGGTGAAGAACTTGCACCAGC	KNLHQRLEGQRPE
		GTCTGGAAGGGCAACGGCCAGAGAACAAGGG	NKGKNRYKNILPF
		CAAGAACCGCTACAAGAACATTCTCCCCTTTG	DHSRVILQGRDSN
		ACCACAGCCGAGTGATCCTGCAGGGACGGGAC	IPGSDYINANYIKN
		AGTAACATCCCCGGGTCCGACTACATCAATGC	QLLGPDENAKTYI
		CAACTACATCAAGAACCAGCTGCTAGGCCCTG	ASQGCLEATVNDF
		ATGAGAACGCTAAGACCTACATCGCCAGCCAG	WQMAWQENSRVI
		GGCTGTCTGGAGGCCACGGTCAATGACTTCTG	VMTTREVEKGRN
		GCAGATGGCGTGGCAGGAGAACAGCCGTGTCA	KCVPYWPEVGMQ
		TCGTCATGACCACCCGAGAGGTGGAGAAAGGC	RAYGPYSVTNCG
		CGGAACAAATGCGTCCCATACTGGCCCGAGGT	EHDTTEYKLRTLQ
		GGGCATGCAGCGTGCTTATGGGCCCTACTCTGT	VSPLDNGDLIREI
		GACCAACTGCGGGGAGCATGACACAACCGAAT	WHYQYLSWPDHG
		ACAAACTCCGTACCTTACAGGTCTCCCCGCTGG	VPSEPGGVLSFLD
		ACAATGGAGACCTGATTCGGGAGATCTGGCAT	QINQRQESLPHAG
		TACCAGTACCTGAGCTGGCCCGACCATGGGGT	PIIVHCSAGIGRTG
		CCCCAGTGAGCCTGGGGGTGTCCTCAGCTTCCT	TIIVIDMLMENIST
		GGACCAGATCAACCAGCGGCAGGAAAGTCTGC	KGLDCDIDIQKTIQ
		CTCACGCAGGGCCCATCATCGTGCACTGCAGC	MVRAQRSGMVQT
		GCCGGCATCGGCCGCACAGGCACCATCATTGT	EAQYKFIYVAIAQ
		CATCGACATGCTCATGGAGAACATCTCCACCA	FIETTKKKL*
		AGGGCCTGGACTGTGACATTGACATCCAGAAG	
		ACCATCCAGATGGTGCGGGCGCAGCGCTCGGG	
		CATGGTGCAGACGGAGGCGCAGTACAAGTTCA	
		TCTACGTGGCCATCGCCCAGTTCATTGAAACCA	
		CTAAGAAGAAGCTGTGA	
Phospha-tase	PTPN12	ATGGAGCAAGTGGAGATCCTGAGGAAATTCAT	MEQVEILRKFIQR
		CCAGAGGGTCCAGGCCATGAAGAGTCCTGACC	VQAMKSPDHNGE
		ACAATGGGGAGGACAACTTCGCCCGGGACTTC	DNFARDFMRLRR
		ATGCGGTTAAGAAGATTGTCTACCAAATATAG	LSTKYRTEKIYPT
		AACAGAAAAGATATATCCCACAGCCACTGGAG	ATGEKEDNVKKN
		AAAAAGAAGACAATGTTAAAAAGAACAGATA	RYKDILPFDHSRV
		CAAGGACATACTGCCATTTGATCACAGCCGAG	KLTLKTPSQDSDY
		TTAAATTGACATTAAAGACTCCTTCACAAGATT	INANFIKGVYGPK
		CAGACTATATCAATGCAAATTTTATAAAGGGC	AYVATQGPLANT
		GTCTATGGGCCAAAAGCATATGTAGCAACTCA	VIDFWRMIWEYN
		AGGACCTTTAGCAAATACAGTAATAGATTTTTG	VVIIVMACREFEM
		GAGGATGATATGGGAGTATAATGTTGTGATCA	GRKKCERYWPLY
		TTGTAATGGCCTGCCGAGAATTTGAGATGGGA	GEDPITFAPFKISC

		AGGAAAAAATGTGAGCGCTATTGGCCTTTGTA TGGAGAAGACCCCATAACGTTTGCACCATTTA AAATTTCTTGTGAGGATGAACAAGCAAGAACA GACTACTTCATCAGGACACTCTTACTTGAATTT CAAAATGAATCTCGTAGGCTGTATCAGTTTCAT TATGTGAACTGGCCAGACCATGATGTTCCTTCA TCATTTGATTCTATTCTGGACATGATAAGCTTA ATGAGGAAATATCAAGAACATGAAGATGTTCC TATTTGTATTCATTGCAGTGCAGGCTGTGGAAG AACAGGTGCCATTTGTGCCATAGATTATACGTG GAATTTACTAAAAGCTGGGAAAATACCAGAGG AATTTAATGTATTTAATTTAATACAAGAAATGA GAACACAAAGGCATTCTGCAGTACAAACAAAG GAGCAATATGAACTTGTTCATAGAGCTATTGCC CAACTGTTTGAAAAACAGCTACAACTATATGA AATTCATGGAGCTTAA	EDEQARTDYFIRT LLLEFQNESRRLY QFHYVNWPDHDV PSSFDSILDMISLM RKYQEHEDVPICI HCSAGCGRTGAIC AIDYTWNLLKAG KIPEEFNVFNLIQE MRTQRHSAVQTK EQYELVHRAIAQL FEKQLQLYEIHGA *
Substrate	p130cas	TGGATGGAGGACTATGACTACGTCCACCTACA GGGG	WMED**Y**D**Y**VHLQ G
Substrate	midT	GAACCGCAGTATGAAGAAATTCCGATTTATCT G	EPQ**Y**EEIPIYL
Substrate	ShcA	GATCATCAGTATTATAACGATTTTCCGGGC	DHQ**Y**YNDFPG
Substrate	EGFR	CCGCAGCGCTATCTGGTGATTCAGGGCGAT	PQR**Y**LVIQGD
Substrate	p130cas Y/F	TGGATGGAGGACTTTGACTTCGTCCACCTACAG GGG	WMED**F**D**F**VHLQG
Substrate	midT Y/F	GAACCGCAGTTTGAAGAAATTCCGATTTATCTG	EPQ**F**EEIPIYL
Promoter	pBAD	AGAAACCAATTGTCCATATTGCATCAGACATT GCCGTCACTGCGTCTTTTACTGGCTCTTCTCGC TAACCAAACCGGTAACCCCGCTTATTAAAAGC ATTCTGTAACAAAGCGGGACCAAAGCCATGAC AAAAACGCGTAACAAAAGTGTCTATAATCACG GCAGAAAAGTCCACATTGATTATTTGCACGGC GTCACACTTTGCTATGCCATAGCATTTTTATCC ATAAGATTAGCG	N/A
Promoter	Pro1[10]	TTCTAGAGCACAGCTAACACCACGTCGTCCCTA TCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGA TAACTTTACGGGCATGCATAAGGCTCGGTATCT ATATTCAGGGAGACCACAACGGTTTCCCTCTAC AAATAATTTTGTTTAACTTTTACTAGAG	N/A
Promoter	placZopt[11]	CATTAGGCACCCCGGGCTTTACTCGTAAAGCTT CCGGCGCGTATGTTGTGTCGACCG	N/A
Promoter	ProD[10]	TTCTAGAGCACAGCTAACACCACGTCGTCCCTA TCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGA TAACTTTACGGGCATGCATAAGGCTCGGTATCT ATATTCAGGGAGACCACAACGGTTTCCCTCTAC AAATAATTTTGTTTAACTTTTACTAGAG	N/A
RBS	Pro	GTGCAGTTAAAGAGGAGAAAGGTC	N/A
RBS	Sal28‡	CGAAAAAAAGTAAGGCGGTAATCC	N/A
RBS	BB030	TCTAGAGATTAAAGAGGAGAAATACTAG	N/A
RBS	BB034	TCTAGAAAAGAGGAGAAATACTAG	
GOI	LuxAB	ATGAAATTTGGAAACTTTTTGCTTACATACCAA CCTCCCCAATTTTCCCAAACAGAGGTAATGAA ACGTTTGGTTAAATTAGGTCGCATCTCTGAGGA GTGTGGTTTTGATACCGTATGGTTACTGGAGCA TCATTTCACGGAGTTTGGTTTGCTTGGTAACCC TTATGTCGCTGCTGCATATTTACTTGGCGCGAC TAAAAAATTGAATGTAGGAACTGCCGCTATTG	MKFGNFLLTYQPP QFSQTEVMKRLV KLGRISEECGFDT VWLLEHHFTEFGL LGNPYVAAAYLL GATKKLNVGTAAI VLPTAHPVRQLED

TTCTTCCCACAGCCCATCCAGTACGCCAACTTG	VNLLDQMSKGRF
AAGATGTGAATTTATTGGATCAAATGTCAAAA	RFGICRGLYNKDF
GGACGATTTCGGTTTGGTATTTGCCGAGGGCTT	RVFGTDMNNSRA
TACAACAAGGACTTTCGCGTATTCGGCACAGA	LAECWYGLIKNG
TATGAATAACAGTCGCGCCTTAGCGGAATGCT	MTEGYMEADNEH
GGTACGGGCTGATAAAGAATGGCATGACAGAG	IKFHKVKVNPAAY
GGATATATGGAAGCTGATAATGAACATATCAA	SRGGAPVYVVAE
GTTCCATAAGGTAAAAGTAAACCCCGCGGCGT	SASTTEWAAQFGL
ATAGCAGAGGTGGCGCACCGGTTTATGTGGTG	PMILSWIINTNEKK
GCTGAATCAGCTTCGACGACTGAGTGGGCTGC	AQLELYNEVAQE
TCAATTTGGCCTACCGATGATATTAAGTTGGAT	YGHDIHNIDHCLS
TATAAATACTAACGAAAAGAAAGCACAACTTG	YITSVDHDSIKAK
AGCTTTATAATGAAGTGGCTCAAGAATATGGG	EICRKFLGHWYDS
CACGATATTCATAATATCGACCATTGCTTATCA	YVNATTIFDDSDQ
TATATAACATCTGTAGATCATGACTCAATTAAA	TRGYDFNKGQWR
GCGAAAGAGATTTGCCGGAAATTTCTGGGGCA	DFVLKGHKDTNR
TTGGTATGATTCTTATGTGAATGCTACGACTAT	RIDYSYEINPVGTP
TTTTGATGATTCAGACCAAACAAGAGGTTATG	QECIDIIQKDIDAT
ATTTCAATAAAGGGCAGTGGCGTGACTTTGTAT	GISNICCGFEANGT
TAAAAGGACATAAAGATACTAATCGCCGTATT	VDEIIASMKLFQS
GATTACAGTTACGAAATCAATCCCGTGGGAAC	DVMPFLKEKQRSL
GCCGCAGGAATGTATTGACATAATTCAAAAAG	LYYGGGGSGGGG
ACATTGATGCTACAGGAATATCAAATATTTGTT	SGGGGSGGGGSK
GTGGATTTGAAGCTAATGGAACAGTAGACGAA	FGLFFLNFINSTTV
ATTATTGCTTCCATGAAGCTCTTCCAGTCTGAT	QEQSIVRMQEITE
GTCATGCCATTTCTTAAAGAAAAACAACGTTC	YVDKLNFEQILVY
GCTATTATATTATGGCGGTGGCGGTAGCGGCG	ENHFSDNGVVGA
GTGGCGGTAGCGGCGGTGGCGGTAGCGGCGGT	PLTVSGFLLGLTE
GGCGGTAGCAAATTTGGATTGTTCTTCCTTAAC	KIKIGSLNHIITTH
TTCATCAATTCAACAACTGTTCAAGAACAGAG	HPVRIAEEACLLD
TATAGTTCGCATGCAGGAAATAACGGAGTATG	QLSEGRFILGFSDC
TTGATAAGTTGAATTTTGAACAGATTTTAGTGT	EKKDEMHFFNRP
ATGAAAATCATTTTTCAGATAATGGTGTTGTCG	VEYQQQLFEECYE
GCGCTCCTCTGACTGTTTCTGGTTTTCTGCTCG	IINDALTTGYCNP
GTTTAACAGAGAAAATTAAAATTGGTTCATTA	DNDFYSFPKISVN
AATCACATCATTACAACTCATCATCCTGTCCGC	PHAYTPGGPRKY
ATAGCGGAGGAAGCTTGCTTATTGGATCAGTT	VTATSHHIVEWA
AAGTGAAGGGAGATTTATTTTAGGGTTTAGTG	AKKGIPLIFKWDD
ATTGCGAAAAAAAAGATGAAATGCATTTTTTT	SNDVRYEYAERY
AATCGCCCGGTTGAATATCAACAGCAACTATTT	KAVADKYDVDLS
GAAGAGTGTTATGAAATCATTAACGATGCTTT	EIDHQLMILVNYN
AACAACAGGCTATTGTAATCCAGATAACGATT	EDSNKAKQETRAF
TTTATAGCTTCCCTAAAATATCTGTAAATCCCC	ISDYVLEMHPNEN
ATGCTTATACGCCAGGCGGACCTCGGAAATAT	FENKLEEIIAENAV
GTAACAGCAACCAGTCATCATATTGTTGAGTG	GNYTECITAAKLA
GGCGGCCAAAAAAGGTATTCCTCTCATCTTTAA	IEKCGAKSVLLSF
GTGGGATGATTCTAATGATGTTAGATATGAAT	EPMNDLMSQKNV
ATGCTGAAAGATATAAAGCCGTTGCGGATAAA	INIVDDNIKKYHT
TATGACGTTGACCTATCAGAGATAGACCATCA	EYT*
GTTAATGATATTAGTTAACTATAACGAAGATA	
GTAATAAAGCTAAACAAGAGACGCGTGCATTT	
ATTAGTGATTATGTTCTTGAAATGCACCCTAAT	
GAAAATTTCGAAAATAAACTTGAAGAAATAAT	
TGCAGAAAACGCTGTCGGAAATTATACGGAGT	
GTATAACTGCGGCTAAGTTGGCAATTGAAAAG	
TGTGGTGCGAAAAGTGTATTGCTGTCCTTTGAA	

		CCAATGAATGATTTGATGAGCCAAAAAAATGT AATCAATATTGTTGATGATAATATTAAGAAGT ACCACACGGAATATACCTAA	
GOI	SpecR	ATGAGGGAAGCGGTGATCGCCGAAGTATCGAC TCAACTATCAGAGGTAGTTGGCGTCATCGAGC GCCATCTCGAACCGACGTTGCTGGCCGTACATT TGTACGGCTCCGCAGTGGATGGCGGCCTGAAG CCACACAGTGATATTGATTTGCTGGTTACGGTG ACCGTAAGGCTTGATGAAACAACGCGGCGAGC TTTGATCAACGACCTTTTGGAAACTTCGGCTTC CCCTGGAGAGAGCGAGATTCTCCGCGCTGTAG AAGTCACCATTGTTGTGCACGACGACATCATTC CGTGGCGTTATCCAGCTAAGCGCGAACTGCAA TTTGGAGAATGGCAGCGCAATGACATTCTTGC AGGTATCTTCGAGCCAGCCACGATCGACATTG ATCTGGCTATCTTGCTGACAAAAGCAAGAGAA CATAGCGTTGCCTTGGTAGGTCCAGCGGCGGA GGAACTCTTTGATCCGGTTCCTGAACAGGATCT ATTTGAGGCGCTAAATGAAACCTTAACGCTAT GGAACTCGCCGCCCGACTGGGCTGGCGATGAG CGAAATGTAGTGCTTACGTTGTCCCGCATTTGG TACAGCGCAGTAACCGGCAAAATCGCGCCGAA GGATGTCGCTGCCGACTGGGCAATGGAGCGCC TGCCGGCCCAGTATCAGCCCGTCATACTTGAA GCTAGACAGGCTTATCTTGGACAAGAAGAAGA TCGCTTGGCCTCGCGCGCAGATCAGTTGGAAG AATTTGTCCACTACGTGAAAGGCGAGATCACC AAGGTAGTCGGCAAATGA	MREAVIAEVSTQL SEVVGVIERHLEP TLLAVHLYGSAV DGGLKPHSDIDLL VTVTVRLDETTRR ALINDLLETSASPG ESEILRAVEVTIVV HDDIIPWRYPAKR ELQFGEWQRNDIL AGIFEPATIDIDLAI LLTKAREHSVALV GPAAEELFDPVPE QDLFEALNETLTL WNSPPDWAGDER NVVLTLSRIWYSA VTGKIAPKDVAA DWAMERLPAQYQ PVILEARQAYLGQ EEDRLASRADQLE EFVHYVKGEITKV VGK*

‡RBS designed computationally using the Ribosome Binding Site Calculator.[12]

Table S4. Primers used to assemble the bacterial two-hybrid system.

Component	*F Primer*	*R Primer*
RpoZ/HA4 with pAB078d8 overhangs	GTGCAGTAAGGAGGAAAAAA	GTCAGGGGCGGGGTTTTTTTTTAGG GCCCTACTGACTGTTAGCAGGTGC GGTAATTGA
pAB078d8 with RpoZ/HA4 overhang piece 1	CAGTCAGTAGGGCCCTAAAA	CACAGTTCTCGTCATCAGCTCTCTG GTTGCTTTAGCTAATACACCATAAG CATTTTCC
pAB078d8 with RpoZ/HA4 overhang piece 2	TAGCTAAAGCAACCAGAGAG	CAGTTACGCGTGCCATTTTTTTTTC CTCCTTACTGCACTTAGCGTTTCGG CGCCGGAT
Src/CDC37 into pAB078d8	CAATTCCCCTCTAGAAATAATTTTG	GTCAGGGGCGGGGTTTTTTTTTAGG GCCCTACTGACTG TTACACACTGACATCCTTCTCATCG
*Insulin Receptor Substrate_ RpoZ fusion into pAB078d8**	CGCTGTAGAGAAAATTGGTA	CAGGGGCGGGGTTTTTTTTTAGGGC CCTACTGACTGTTATTAGCCAAGAT CCATCTTCA
*Insulin Receptor SH2 cI fusion into pAB078d8**	GACGCGGAATGGTACTGGGG	GTTACGCGTGCCATTTTTTTTTCCT CCTTACTGCACTTATTACGAAACCG GATACAACA
Src/CDC37 into pBAD33t	ATATGGTCTCACATGTCCAAGCCG CAGACTCAG	ATATGGTCTCATTTACACACTGACA TCCTTCTCATCG
RpoZ/p130cas substrate into pBAD33t	ATATGGTCTCACATGGCACGCGTA ACTGTTC	ATATGGTCTCATTTACCCCTGTAGG TGGACG
cI/SH2 into pBAD33t	ATATGGTCTCACATGAGTATCAGC AGCAGGGTAAAAAG	ATATGGTCTCATTTAGCAGACGTTG GTCAGGC
pB2H$_{1b}$ Gibson piece 1	ATGACTACGTCCACCTACAGGGGT AATAACAATTCCCCTCTAGAAATA ATTTTGTTTAAC	AAGATAAAAAGAATAGATCCCAGC CCTGTGTATAACTCACTACTTTAGT CAGTTCCGCA
pB2H$_{1b}$ Gibson piece 2	TGAGTTATACACAGGGCTGG	CCCCTGTAGGTGGACGTAGTCATA GTCCTCCATCCACGCAGCTGCACG ACGA
pB2H$_{1b}$ Gibson piece 3	GTGCAGTAAGGAGGAAAAAAAAA TGGC	GCCCATGGTATATCTCCTTCTTAAA GT
pB2H$_{1b}$ Gibson piece 4	TAAAATTCGTAGACTACAAGGACG ACGATGACAAGTGGTATTTTGGGA AGATCACTCGT	ACAGTTACGCGTGCCATTTTTTTTT CCTCCTTACTGCACTTAGCAGACGT TGGTCAGGC
B2H ShcA substrate	TAATAACAATTCCCCTCTAGAAAT AATTTTGTTTAACTTTAAG	GGGAATTGTTATTAGCCCGGAAAA TCGTTATAATACTGATGATCCGCAG CTGCACGACG

B2H EGFR Substrate	TAATAACAATTCCCCTCTAGAAAT AATTTTGTTTAACTTTAAG	GGGAATTGTTATTAATCGCCCTGA ATCACCAGATAGCGCTGCGGCGCA GCTGCACGACG
B2H MidT Substrate	TAATAACAATTCCCCTCTAGAAAT AATTTTGTTTAACTTTAAG	GAATTGTTATTACAGATAAATCGG AATTTCTTCATACTGCGGTTCCGCA GCTGCACGACG
BB034 PTP1B$_{1-321}$ into pBAD$_{1c}$	GTCAGTGTGTAAGTGCAGAAAGAG GAGAAATACTAGATGGAGATGGAA AAGGAGTTCGAG	CTCATCCGCCAAAACAGCCTCAAT TGTGTGGCTCCAGGATTCG
BB034 Src/CDC37	TAATCTAGAGAAAGAGGAGAAATA CTAGATGTCCAAGCCGCAGACTC	TTACACACTGACATCCTTCTCATCG
ProD into B2H	CTCTAGTAAAAGTTAAACAAAATT ATTTGTAGAGGG	TTCTAGAGCACAGCTAACACCAC
ProD Overhang ProRBS Src/CDC37	AACTTTTACTAGAGGAATTCGAGC TCTTAAAGAGGAGAAAGGTCATGG GCTCCAAGCCGC	AAGATAAAAAGAATAGATCCCAGC CCTGTGTATAACTCACTACTTTAGT CAGTTCCGCA
Sal28 RBS Src/CDC37	AACTTTTACTAGAG CGAAAAAAAGTAAGGCGGTAATCC ATGGGCTCCAAGCCGC	GAACCAATGAATGATTTGATGAGC
BB030 PTP1B into pB2H$_{S1.2Sal28}$	AGTGTGTAAGTGCAGATTAAAGAG GAGAAATACTAGATGGAGATGGAA AAGGAGTTCGAG	GTTTTTTTTTAGGGCCCTACTGACT GTCAATTGTGTGGCTCCAGGATTC
BB034 PTP1B into pB2H$_{1.2Sal28}$	TCAGTGTGTAAGTGCAGTCACACA GGAAAGTACTAGATGGAGATGGAA AAGGAGTTCGAG	GTTTTTTTTTAGGGCCCTACTGACT GTCAATTGTGTGGCTCCAGGATTC
B2H Swap LuxAB/SpecR	GCGTACATTGGCTCCGTTCA TTTGCCGACTACCTTGGTGATC	GACCTGCAGATTAAAGAGGAGAAA ATG AGGGAAGCGGTGATCG

*Insulin receptor substrate/SH2 domains[13] were used initially, but failed to activate the operon (data not shown)

Table S5. Primers used to assemble pathways for terpenoid biosynthesis.

Component	***F Primer***	***R Primer***
GGPPS into pTrc99t	TATTGAGCTCCACCGCGGAGGAGGAATG	TATTGTCGACTTATTTATTACGCTGGATGATGTAGTC
TXS into pTrc99t	TATTGGTCTCCCATGAGCAGCAGCACTGGCAC	TATTGGTCTCCGTCCTTCCAACGCATTCAACATGTTG
ABS into pTrc99t	ATAAAGGTCTCCCATGGTGAAACGAGAATTTCCTCCAG	TATTAGGTCTCGAGCTCTTAGGCAACTGGTTGGAAGAGGC
pMBIS TetR->CmR	AGATCACTACCGGGCGTATTTTTTGAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCAC	GCCGCCGGCTTCCATTTATTACGCCCCGCCCTG
ABA into pTrc99	AACAATTTCACACAGGAAACAGACCATGGCGGGTGTTTCTGCG	GCCTGCAGGTCGACTCTAGATTACAGCGGCAGCGGTTC

Table S6. Primers used for site-directed mutagenesis.

Mutant	*F Primer*	*R Primer*
PTP1B (C215S)	GTCCAGTACTTTATTGGGGTTCAGGCG GATGGAACTGAGCATGTCCGAGAT	ATCTCGGACATGCTCAGTTCCATCCGCC TGAACCCCAATAAAGTACTGGAC
TCPTP (R222M)	CAGAGAGAAGGTGCCAGACATCCCAA TGCCTGCACTACA	TGTAGTGCAGGCATTGGGATGTCTGGCA CCTTCTCTCTG
SHP1 (R459M)	CAATGATGGTGCCTGTCATGCCGATG CCGGCGCTG	CAGCGCCGGCATCGGCATGACAGGCAC CATCATTG
PTPN12 (Y64A)	GCTGTGATCAAATGGCAGTATGTCCTT CGCTCTGTTCTTTTTAACATTTTCTTCT TTTTC	GAAAAAGAAGAAAATGTTAAAAAGAAC AGAGCGAAGGACATACTGCCATTTGATC ACAGC
ABS (D404A)	GAGAGAGAATCCTGTTCCTGATATTG CGGATACAGCCATGGGCCTTC	GAAGGCCCATGGCTGTATCCGCAATATC AGGAACAGGATTCTCTCTC
ABS (D621A)	ACAAAAACTTCCAATTTCACTGTTATT TTAGCGGATCTTTATGACGCCCATGG	CCATGGGCGTCATAAAGATCCGCTAAA ATAACAGTGAAATTGGAAGTTTTTGT
ADS (D299A)	CGTAAGCATCGTAAGTGTCCGCGATC AGGGTGATAACAGC	GCTGTTATCACCCTGATCGCGGACACTT ACGATGCTTACG
GHS (D343A)	CCCATGCGTGTCGTATAAGTCCGCTA ACATTGTCATCAAGATCG	CGATCTTGATGACAATGTTAGCGGACTT ATACGACACGCATGGG
MidT Substrate (Y/F)	CAGCTGCGGAACCGCAGTTTGAAGAA ATTCCGAT	ATCGGAATTTCTTCAAACTGCGGTTCCG CAGCTG
p130Cas Substrate (Y/F)	TGGATGGAGGACTTTGACTTCGTCCA CCTACAGGGGTAATAACAATTC	GTCAAAGTCCTCCATCCACGCAGCTGCA CGACG
SH2 (Superbinder mutations)	CTCTCCGTTTCTGACTTTGACAACGCC AAGGGGCTCAATGTGCTGCACTACAA GATCCGCAAGCTG	AAGTCAGAAACGGAGAGGGCATAGGCA CCTTTTACCGTCTCGCTCTCCCG
*SH2 (L13K K15L)**	AAACACTACCTGATCCGCAAGCTGGA CAGC	GCTGTCCAGCTTGCGGATCAGGTAGTGT TTCACATTGAGCCCCTTGGC[*]
pTrc99a (remove BsaI sites) piece 1	TATTGGTCTCTCGCGGTATCATTGCAG CAC	TATTGGTCTCAGTGACCCCACACTACCA TCGG
pTrc99a (remove BsaI sites) piece 2	TATTGGTCTCATCACCCCATGCGAGA GTAGG	TATTGGTCTCACGCGTGACCCACGCTCA CCG
ABA D/A	AGGTGTCGTACATGTCCGCCAGAACG GTCTGCAG	CTGCAGACCGTTCTGGCGGACATGTACG ACACCT

[*]The original superbinder primer mutated the incorrect lysine residue (13 vs. 15). This primer corrects that error. The residue numbering system used for this protein matches that of Kaneko et. al.[14]

Table S7. Development of the B2H system. The accompanying Excel file provides measurements of OD-normalized luminescence, including error and sample sizes, for strains containing various B2H systems.

Table S8. Analysis of antibiotic resistance. The accompanying Excel file describes the growth conditions (i.e., antibiotic concentrations in liquid and solid media) and give the experimental replicates for all analyses of antibiotic resistance.

Table S9. Titers of terpenoid-producing pathways. The accompanying Excel file provides measurements of titers, including error and sample sizes, for strains containing various TS-specific pathways for terpenoid biosynthesis.

Table S10. Kinetics of terpenoid-mediated inhibition. The accompanying Excel provides the discrete kinetic measurements made in this study, including standard error and exact sample sizes.

Table S11. The accompanying Excel file provides absorbance measurements made for the completion of ELISAs, including standard error and exact sample sizes.

Table S12A. Scaling factor for amorphadiene/caryophyllene (m/z=204)

Technical Replicate	A_{std} (counts*min)	A_{ref} (counts*min)	C_{std} (µg/mL)	C_{ref} (µg/mL)	*R*
1	74520	88358	20	0.4	0.017
2	71037	142415	20	0.4	0.010
3	75761	49011	20	0.4	0.031
				Avg R	**0.019 (0.006)**

* R was computed using eq. 2. Standard error is shown in parentheses.

Table S12B. Scaling factor for taxadiene/caryophyllene (m/z=93)

Technical Replicate	A_{std} (counts*min)	A_{ref} (counts*min)	C_{std} (µg/mL)	C_{ref} (µg/mL)	*R*
1	1399872	847009	20	10	0.83
2	1247250	605265	20	10	1.0
3	1291028	547740	20	10	1.2
				Avg R	**1.0 (0.10)**

Table S12C. Scaling factor for amorphadiene/methyl abietate (m/z=121)

Technical Replicate	A_{std} (counts*min)	A_{ref} (counts*min)	C_{std} (µg/mL)	C_{ref} (µg/mL)	*R*
1	949492	868168	20	3.162	0.17
2	920694	908257	20	3.162	0.16
3	898594	1106474	20	3.162	0.13
				Avg R	**0.15 (0.01)**

Table S13A. Analysis of the inhibition of $PTP1B_{1-321}$ by amorphadiene.

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM)*
Competitive	0.14	27	Δ_i= 51.2	noncompetitive	K_i=2.85
Uncompetitive*	0.023	27	Δ_i = 1.16	noncompetitive	K_i=46.3
Noncompetitive*	0.023	27			K_i=52.6*
Mixed	0.022	26	F=0.47 p = 0.972	noncompetitive	$K_{i,c}$=86.2 $K_{i,u}$=50.1

Table S13B. Analysis of the inhibition of $PTP1B_{1-321}$ by α-bisabolene.

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM))*
Competitive	0.082	27	Δ_i= 39.1	noncompetitive	K_i=1.05
Uncompetitive*	**0.023**	**27**	**Δ_i= 3.81**	**noncompetitive**	**K_i=11.7**
Noncompetitive*	**0.021**	**27**			**K_i=13.1**
Mixed	0.020	26	F=0.24 p = 1.0	noncompetitive	$K_{i,c}$=9.51 $K_{i,u}$=13.7

Table S13C. Analysis of the inhibition of $PTP1B_{1-321}$ by alpha bisabolol.

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM)*
Competitive	0.039	27	Δ_i= 34.4	uncompetitive	K_i=178
Uncompetitive*	**0.011**	**27**			**K_i=469**
Noncompetitive*	**0.013**	**27**	**Δ_i= 4.65**	**uncompetitive**	**K_i=541**
Mixed	0.011	26	F=0 p = 1.0	uncompetitive	$K_{i,c}$=3.5e^{16} $K_{i,u}$=469

Table S13D. Analysis of the inhibition of $PTP1B_{1-321}$ by dihydroartimesnic acid.

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM)*
Competitive	0.129	27	Δ_i= 60.7	noncompetitive	K_i=178
Uncompetitive	0.025	27	Δ_i= 15.2	noncompetitive	K_i=469
Noncompetitive	0.015	27			K_i=541
Mixed	**0.013**	**26**	**F=2.69** **p = 6.9e^{-3}**	**noncompetitive**	**$K_{i,c}$=3.5e^{16}** **$K_{i,u}$=469**

Table S13E. Analysis of the inhibition of $TCPTP_{1-317}$ by amorphadiene.

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM)*
Competitive	0.053	27	Δ_i= 41.1	uncompetitive	K_i=87.2
Uncompetitive*	**0.012**	**27**			**K_i=356**
Noncompetitive*	**0.013**	**27**	**Δ_i= 2.22**	**uncompetitive**	**K_i=400**
Mixed	0.012	26	F=0 p = 1.0	uncompetitive	$K_{i,c}$=3.7e^{15} $K_{i,u}$=356

*Blue highlights indicate models of best fit.
**When the uncompetitive and noncompetitive models are both highlighted, the SSEs of these models are indistinguishable from one another.

Table S13F. Analysis of the inhibition of $TCPTP_{1-317}$ by α-bisabolene.

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM)*
Competitive	0.046	27	Δ_i= 37.6	uncompetitive	K_i=13.7
Uncompetitive	**0.012**	**27**			**K_i=69.2**
Noncompetitive	**0.012**	**27**	**Δ_i= 1.12**	**uncompetitive**	**K_i=76.2**
Mixed	0.012	26	F=0 p = 1.0	uncompetitive	$K_{i,c}$=3610 $K_{i,u}$=69.3

Table S13G. Analysis of the inhibition of $PTP1B_{1-281}$ by amorphadiene.

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM)*
Competitive	0.010	27	Δ_i= 16.3	noncompetitive	K_i=37.9
Uncompetitive	**0.006**	**27**	**Δ_i= 3.51**	**noncompetitive**	**K_i=210**
Noncompetitive	**0.006**	**27**			**K_i=244**
Mixed	0.006	26	F=0.41 p = 0.99	noncompetitive	$K_{i,c}$=157 $K_{i,u}$=271

Table S13H. Analysis of the inhibition of $PTP1B_{1-281}$ by α-bisabolene.

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM)*
Competitive	0.012	27	Δ_i= 14.4	noncompetitive	K_i=6.51
Uncompetitive	**0.008**	**27**	**Δ_i=1.41**	**noncompetitive**	**K_i=40.0**
Noncompetitive	**0.007**	**27**			**K_i=46.3**
Mixed	0.007	26	F=0 p = 1.0	noncompetitive	$K_{i,c}$=39.0 $K_{i,u}$=47.7

Table S13I. Analysis of the inhibition of $TCPTP_{1-281}$ by amorphadiene.

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM)*
Competitive	0.005	27	Δ_i= 22.9	uncompetitive	K_i=87.2
Uncompetitive	**0.002**	**27**			**K_i=356**
Noncompetitive	**0.002**	**27**	**Δ_i= 0.83**	**uncompetitive**	**K_i=400**
Mixed	0.002	26	F=0.03 p = 1.0	uncompetitive	$K_{i,c}$=3.7e^{15} $K_{i,u}$=356

Table S13J. Analysis of the inhibition of $TCPTP_{1-281}$ by α-bisabolene.

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM)*
Competitive	0.083	27	Δ_i= 39.1	noncompetitive	K_i=13.7
Uncompetitive	**0.023**	**27**	**Δ_i= 3.81**	**noncompetitive**	**K_i=69.2**
Noncompetitive	**0.021**	**27**			**K_i=76.2**
Mixed	0.020	26	F=0 p = 1.0	noncompetitive	$K_{i,c}$=3610 $K_{i,u}$=69.3

*Blue highlights indicate models of best fit.
**When the uncompetitive and noncompetitive models are both highlighted, the SSEs of these models are indistinguishable from one another.

Table S13K. Analysis of the inhibition of $PTP1B_{1-321}$ by (+)-δ-cadinene

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM)*
Competitive	0.115	27	Δ_i= 48.3	uncompetitive	K_i=14.75
Uncompetitive	**0.020**	**27**			**K_i=168.09**
Noncompetitive	**0.022**	**27**	Δ_i= 2.5	**uncompetitive**	**K_i=190.44**
Mixed	0.020	26	F=0 p = 1.0	uncompetitive	$K_{i,c}$=5689.38 $K_{i,u}$=168.78

Table S13L. Analysis of the inhibition of $SHP2_{223-565}$ by Amorphadiene

Model	*SSE ($\mu M^2/s^2$)*	*DF*	*Criteria*	*Reference*	*Fit par. (μM)*
Competitive	.0024	27	Δ_i= 10.6	noncompetitive	K_i=25.1
Uncompetitive	**.0017**	**27**	Δ_i= 0.5	**noncompetitive**	**K_i=116.51**
Noncompetitive	**.0017**	**27**			**K_i=145.69**
Mixed	0.0017	26	F=0.15 p = 1.0	noncompetitive	$K_{i,c}$=236.21 $K_{i,u}$=132.37

*Blue highlights indicate models of best fit.
**When the uncompetitive and noncompetitive models are both highlighted, the SSEs of these models are indistinguishable from one another.

Table S14. Data collection and refinement statistics (molecular replacement)

	PTP1B: amorphadiene (6W30)	PTP1B (7LFO)
Data Collection		
Space group	P 3_1 2 1	P 3_1 2 1
Cell dimensions		
***a*, *b*, *c* (Å)**	89.03, 89.03, 105.56	87.96, 87.96, 104.43
α, β, ɣ (°)	90.00, 90.00, 120.00	90.00, 90.00, 120.00
Resolution (Å)	62.26-2.10 (2.13-2.10)*	61.58-1.94 (1.97-1.94)*
R_{sym} **or** R_{merge}	0.130 (0.442)	0.065 (0.250)
$I/\sigma I$	5.4 (1.0)	5.9 (0.2)
Completeness (%)	99.8 (93.3)	100.0 (100.0)
Redundancy	10.7 (10.8)	11.6 (11.9)
Refinement		
Resolution (Å)	44.52-2.10 (2.17-2.10)	61.58-1.94 (1.94-2.00)
No. reflections	28,654	35,232
R_{work} / R_{free}	0.20 / 0.24	0.20 / 0.24
No. atoms		
Protein	2355	2308
Ligand/ion	22	1
Water	170	149
***B*-factors**		
Protein	37	42
Ligand/ion	90/61	64
Water	47	50
R.m.s. deviations		
Bond lengths (Å)	0.42	0.48
Bond angles (°)	0.56	0.58

*Values in parentheses correspond to the highest-resolution shell.
**Number of crystals used for each structure: 1
***We did not deposit our structure of PTP1B soaked with α-bisabolol into the RCSB Protein Data Bank because our dataset does not permit unambiguous placement of the bound ligand (Figure S9).

Table S15. Details of hypothesis testing

Figure	*Null hypothesis*	*Δμ*	*Test*	*DF*	*t*	*95% confidence intervals*	*P-value*
3H	AD - (-) = 0	0.212	t-test, unequal variance	2	6.61	(0.092,0.332)	0.02
3H	AB - (-) = 0	0.310	t-test, unequal variance	2	13.5	(0.138,0.482)	0.005
3H	AD - DHA = 0	0.124	t-test, unequal variance	3	3.59	(0.069,0.179)	0.04
3H	AB - ABol = 0	0.309	t-test, unequal variance	3	12.6	(0.170,0.447)	0.001

Table S16. Ligand efficiency

Ligand	**IC_{50} (μM)**	**# Heavy Atoms**	**Ligand Efficiency***
Amorphadiene	50	15	0.39
α-bisabolene	13	15	0.44
BBR	8[15]	41	0.17
MSI-1436	0.6[16]	47	0.17

***** Ligand efficiency = (-2.303RT)/HAC*log(IC_{50}), where R is the gas constant, T is the temperature in K, and HAC is the number of heavy atoms. ******

Supplementary References

(1) Luo, X., Reiter, M. A., D'Espaux, L., Wong, J., Denby, C. M., Lechner, A., Zhang, Y., Grzybowski, A. T., Harth, S., Lin, W., et al. (2019) Complete biosynthesis of cannabinoids and their unnatural analogues in yeast. *Nature 567*, 123–126.

(2) Galanie, S., Thodey, K., Trenchard, I. J., Interrante, M. F., and Smolke, C. D. (2015) Complete biosynthesis of opioids in yeast. *Science 349*, 1091–1095.

(3) Paddon, C. J., and Keasling, J. D. (2014) Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. *Nat. Rev. Microbiol. 12*, 355–367.

(4) Grangeasse, C., Nessler, S., and Mijakovic, I. (2012) Bacterial tyrosine kinases: Evolution, biological function and structural insights. *Philos. Trans. R. Soc. B Biol. Sci. 367*, 2640–2655.

(5) Montalibet, J., Skorey, K., McKay, D., Scapin, G., Asante-Appiah, E., and Kennedy, B. P. (2006) Residues distant from the active site influence protein-tyrosine phosphatase 1B inhibitor binding. *J. Biol. Chem. 281*, 5258–5266.

(6) Douzery, E. J. P., Snell, E. A., Bapteste, E., Delsuc, F., and Philippe, H. (2004) The timing of eukaryotic evolution: Does a relaxed molecular clock reconcile proteins and fossils? *Proc. Natl. Acad. Sci. U. S. A. 101*, 15386–15391.

(7) O'Brien, K. P., Remm, M., and Sonnhammer, E. L. L. (2005) Inparanoid: A comprehensive database of eukaryotic orthologs. *Nucleic Acids Res. 33*, D476–D480.

(8) Kachroo, A. H., Laurent, J. M., Yellman, C. M., Meyer, A. G., Wilke, C. O., and Marcotte, E. M. (2015) Systematic humanization of yeast genes reveals conserved functions and genetic modularity. *Science 348*, 921–925.

(9) Keedy, D. A., Hill, Z. B., Biel, J. T., Kang, E., Rettenmaier, T. J., Brandão-Neto, J., Pearce, N. M., von Delft, F., Wells, J. A., and Fraser, J. S. (2018) An expanded allosteric network in

PTP1B by multitemperature crystallography, fragment screening, and covalent tethering. *Elife 7*.

(10) Davis, J. H., Rubin, A. J., and Sauer, R. T. (2011) Design, construction and characterization of a set of insulated bacterial promoters. *Nucleic Acids Res. 39*, 1131–1141.

(11) Badran, A. H., Guzov, V. M., Huai, Q., Kemp, M. M., Vishwanath, P., Kain, W., Nance, A. M., Evdokimov, A., Moshiri, F., Turner, K. H., et al. (2016) Continuous evolution of Bacillus thuringiensis toxins overcomes insect resistance. *Nature 533*, 58–63.

(12) Salis, H. M. (2011) The ribosome binding site calculator. *Methods Enzymol. 498*, 19–42.

(13) Sato, M., Ozawa, T., Inukai, K., Asano, T., and Umezawa, Y. (2002) Fluorescent indicators for imaging protein phosphorylation in single living cells. *Nat Biotechnol 20*, 287–294.

(14) Kaneko, T., Huang, H., Cao, X., Li, X., Li, C., Voss, C., Sidhu, S. S., and Li, S. S. C. (2012) Superbinder SH2 domains act as antagonists of cell signaling. *Sci. Signal. 5*.

(15) Wiesmann, C., Barr, K. J., Kung, J., Zhu, J., Erlanson, D. A., Shen, W., Fahr, B. J., Zhong, M., Taylor, L., Randal, M., et al. (2004) Allosteric inhibition of protein tyrosine phosphatase 1B. *Nat. Struct. Mol. Biol. 11*, 730–737.

(16) Krishnan, N., Koveal, D., Miller, D. H., Xue, B., Akshinthala, S. D., Kragelj, J., Jensen, M. R., Gauss, C.-M., Page, R., Blackledge, M., et al. (2014) Targeting the disordered C terminus of PTP1B with an allosteric inhibitor. *Nat. Chem. Biol. 10*, 558–566.