Supporting Appendix for

A fluorothreonyl-tRNA deacylase prevents mistranslation in the organofluorine producer *Streptomyces cattleya*

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Materials and Methods

Commercial materials. Amylose resin, Hiscribe T7 transcription kit, Phusion DNA polymerase, Q5 DNA polymerase, deoxynucleotide triphosphates (dNTPs), and restriction enzymes were obtained from New England Biolabs (Ipswich, MA). Asparagine, apramycin, benzoyl chloride, β-mercaptoethanol, β-Nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate, boric acid, Dowex 50WX8, ethyl fluoroacetate, iodoacetamide, inorganic pyrophosphatase, lithium diisopropylamine, myokinase, naladixic acid, *o*-phthaldialdehyde, phthaloyl chloride, protamine sulfate from salmon sperm, pyruvate kinase/lactic acid dehydrogenase from rabbit muscle, sodium borohydride, and (R)-(−)-2-(tert-Butyl)-3-methyl-4-imidazolidinone trifluoroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO). Amicon Ultra 3,000 MWCO and 30,000 MWCO centrifugal concentrators, Milli-Q Gradient water purification system, chloramphenicol, malt extract and yeast extract were purchased from EMD Millipore (Billerica, MA). Adenosine triphosphate disodium salt hydrate, ammonium chloride, carbenicillin, 3 kDa MWCO dialysis tubing, dithiothreitol, ethylenediaminetetraacetic acid disodium salt, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), hydrochloric acid, glucose, kanamycin sulfate, magnesium chloride, magnesium sulfate, phenylmethylsulfonyl chloride (PMSF), potassium phosphate dibasic, sodium acetate, sodium hydroxide, and tris base were purchased from Fisher Scientific (Waltham, MA). Deuterium oxide was purchased from Cambridge Isotopes (Tewksbury, MA). Fluka brand LCMS grade ammonium formate, ammonium bicarbonate, formic acid, methanol and acetonitrile were purchased from Sigma Aldrich (St. Louis, MO). Phenol chloroform (pH 5.2), sodium chloride, and sodium glutamate were purchased from MP Biosciences. Inositol was purchased from the Nutritional Biochemicals Corporation (Cleveland, OH). Tris(2-carboxyethyl)phosphine (TCEP) was purchased from Biosynth Corporation (Itasca, IL). Pierce brand analytical grade 6 N HCl and trypsin were purchased from Thermo Fisher (Waltham, MA). Ni-NTA resin, Quia-quick PCR cleanup kit, and Quia-spin miniprep kit were purchased from Quiagen USA (Valencia, CA). ATP-^{13}C was purchased from Santa Cruz Biotechnology (Dallas, TX). Bacto™ Agar was purchased from BD (Sparks, Maryland). Soy flour was purchased from Berkeley Bowl (Berkeley, CA). Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA), resuspended at a concentration of 100 μM, and stored at 4°C. Ammonium persulfate, acrylamide/bisacrylamide (37.5:1 and 19:1), N,N,N',N'-tetramethyl-ethane-1,2-diamine (TEMED), and sodium dodecyl sulfate (SDS) were purchased from Bio-Rad Laboratories (Hercules, CA). Lysogeny Broth (LB), Lysogeny Broth Agar (LBA), and Terrific Broth (TB) were purchased from VWR International (Radnor, PA). Ultrayield baffled flasks were purchased from Thompson Instrument Company (Oceanside, CA).

Phylogenetic and genomic context analysis. The NCBI RefSeq database was searched with BLAST using SCAT_p0564 (FthB) as the query sequence. Sequences with e-values < 1E-50 (roughly corresponding to >50% sequence identity) were initially selected for phylogenetic analysis, however a subset of sequences from highly populated branches of the tree were removed in order to fit the tree on a single page. Multiple sequence alignment was performed with ClustalW [*1*]. The MEGA6 interface was then used to analyze the alignment by Maximum Likelihood based on the JTT matrix-based model, and uncertainty in the topology of the resulting tree was evaluated with 500 bootstrap replicates [*2*, *3*]. Positions with gaps were discarded, and 142 amino acid positions were used to construct the final tree. Genbank files with the pertinent genomic contexts of were obtained from NCBI RefSeq and visualized using Snapgene viewer (GSL Biotech LLC, Chicago IL). Domain architecture was assigned using the NCBI CDD server [*4*].

Preparation of fluorothreonine. Fluorothreonine was synthesized as previously reported [*5*].

Bacterial strains. Cloning was performed in *E. coli* DH10B-T1^{R}**,** and protein expression was performed in *E. coli* BL21(DE3) harboring the pRARE2 plasmid for the expression of the IRLP tRNAs. *Streptomyces cattleya* NRRL 8057 (ATCC 35852) was purchased from the American Tissue Type Collection (Manassas, VA), and *Streptomyces coelicolor* M1152 was obtained from the John Innes Centre (Norwich, Norfolk, UK). The non-methylating strain *E. coli* GM272 [*6*] harboring the plasmid pUZ8002 was used for conjugative transfer of DNA into *Streptomyces* species.

Construction of plasmids. Gibson assembly was used to carry out plasmid construction with *E. coli* DH10B-T1^{R} as the cloning host [*7*]. PCR amplifications were carried out using Phusion polymerase (New England Biolabs, Ipswich MA) or Platinum Taq High Fidelity polymerase (Thermo Fisher, Waltham MA), and restriction digests were performed using enzymes obtained from New England Biolabs. The plasmid pET16b-His$_{10}$-ThrRS was constructed by amplification of the target gene from *S. cattleya* genomic DNA using the primers J303/J304 (*Table S1*), and insertion into NdeI/BamHI digested pET16b. pSV272.1-His$_{6}$-MBP-Tev-FthB was constructed by amplification from gDNA with the primers J400/J401 and insertion into SfoI/HindII digested pSV272.1. The plasmid pIJ10702-SCAT_tRNA25 was constructed by amplification from gDNA with the primers J062/J063 and insertion into BamHI digested pIJ10702. The plasmid pSET152-ermEp*-FthB was constructed by amplification from gDNA with primers J118/J119 and insertion into NdeI/BamHI digested pSET152-ermEp*. The plasmid pET16b was obtained from Novagen, while PSV272.1 was derived from pET27b, also from Novagen. Plasmids pSET152-ermEp* and pIJ10702 were obtained from the John Innes Center [*8*, *9*]. Descriptions of the plasmids and strains

used in this study, as well as sequences of primers are detailed in Table S1. Constructs were sequenced by Quintara Biosciences (South San Francisco, CA).

Purification of *S. cattleya* threonyl aminoacyl-tRNA synthetase (TRS). For purification of His_{10}-TRS, *E. coli* Bl21 (DE3) were transformed with the expression plasmid pET16b-TRS and pRARE2. Overnight cultures in Terrific Broth (TB) were used to innoculate expression cultures to OD_{600} = 0.05 in 3 × 750 mL TB in UltraYield flasks. Cultures were grown at 37°C with shaking (200 rpm) until the OD_{600} reached 0.6-0.8 before being chilled on ice for 30 min, after which IPTG was then added to 0.2 mM final concentration and growth was continued overnight at 16°C. Cells were harvested by centrifugation and resuspended in 5 mL 50 mM HEPES pH 7.5, 150 mM sodium chloride, 10 mM magnesium chloride, 5 mM BME, 10% glycerol per gram cell paste before storage at -80°C.

Cells were thawed, incubated with 0.5 mg/mL lysozyme on ice for 30 min, and lysed by passage through a French pressure cell (Thermo Scientific; Waltham, MA) at 15,000 psi. DNAse I (Fermentas) was added to 1 u/mL, and the lysate was clarified by centrifuging 2 × 20 min at 15,000 RCF. Lysate was then supplemented with imidazole to 20 mM, applied to 4 mL NiNTA resin and washed with 25 column volumes 25 mM HEPES, 500 mM sodium chloride, 10 mM magnesium chloride, 5 mM BME, 10% (*v/v*) glycerol, 20 mM imidazole, pH 7.5, at which point the A_{280} of the eluent dropped below 0.1. Protein was eluted with 25 mM HEPES, 20 mM sodium chloride, 10 mM magnesium chloride, 5 mM BME, 10% (*v/v*) glycerol, 250 mM imidazole, pH 7.5, and applied directly to a 5 mL HiTrap Q column (GE). Separation was accomplished by a gradient from 0-500 mM sodium chloride over 20 column volumes. Fractions containing His_{10}-TRS were diluted with 25 mM HEPES, 10 mM magnesium chloride, 50% (*v/v*) glycerol, pH 7.5, to obtain a final glycerol concentration of 20%. Protein was then concentrated to ~6 mg/mL using a 30 kDa MWCO spin filter (Amicon) and flash-frozen in liquid nitrogen before storage at -80°C. The resulting protein could be applied to tRNA without reduction in RNA integrity as visualized by urea-PAGE. The specific activity for aminoacylation of Thr was measured to be 0.58 units/mg. Concentration was 6.2 mg/mL as determined using the extinction coefficient calculated by ExPASY ProtParam for His_{10}-TRS ($\varepsilon_{280\ nm}$= 75,750 $M^{-1}cm^{-1}$) [*10*].

Purification of FthB. For the purification of FthB, *E coli* BL21(DE3) were transformed with the expression plasmid pSV272.1-FthB and pRARE2. Overnight cultures in ZY505 media (non-inducing) were used to innoculate expression cultures to OD_{600} = 0.05 in 2 × 1L ZY5052 media (auto-inducing) in UltraYield flasks [*11*]. Cultures were grown for 10 h at 37°C with shaking at 300 rpm, and were harvested by centrifugation for 10 min at 10,000 RCF before storage at -80°C.

Pellets were resuspended at 5 mL/g cell paste in 50 mM HEPES, 300 mM sodium chloride, 5 mM BME, 10% (*v/v*) glycerol, 10 mM magnesium chloride, pH 7.5 and homogenized. Lysozyme was added to 0.5 mg/mL, followed by shaking for 20 min at 30°C. Lysis was accomplished with a Misonix Sonicator 3000 (power = 8, 5 s on, 25 s off, 2.5 min total process time, 1/2" tip). Lysate was clarified by centrifugation at 15,000 RCF for 20 min. DNA was then precipitated from the supernatant by the addition of protamine sulfate to 0.075% (*w/v*) followed by centrifugation at 15,000 RCF for 20 min. The supernatant was removed, and imidazole was added to 20 mM before batch binding with 4 mL Ni-NTA resin for 30 min at 4°C. Resin was washed with 30 column volumes lysis buffer supplemented with 20 mM imidazole, at which point the A_{280} of the eluent had dropped below 0.1. His_6-MBP-TEV-FthB was eluted with lysis buffer supplemented with 250 mM imidazole, and dialyzed against lysis buffer. After 2 buffer changes, TEV protease was added (1:50 mass ratio with respect to substrate) and cleavage was allowed to continue overnight at 4°C. TEV-cleaved protein was separated from MBP by sequential passage over 4 mL Ni-NTA resin and 2 mL amylose resin. Protein was then diluted 1:1 with 50% (*v/v*) glycerol, 5 mM BME, to obtain a final glycerol concentration of 30%. It was then concentrated using a 3 kDa MWCO Amicon spin concentrator and flash-frozen in liquid nitrogen before storage at -80°C. Concentration was 3.4 mg/mL as determined using the extinction coefficient calculated by ExPASY ProtParam for FthB ($\varepsilon_{280\ nm}$= 8,940 $M^{-1}cm^{-1}$) [*10*].

Selection and preparation of transfer RNA. The genome of *S. cattleya* encodes four $tRNA^{Thr}$ isoacceptors, as predicted by tRNAscan SE [*12*]. Two of these (SCAT_tRNA18 and SCAT_tRNA25) are functionally identical, differing only in whether or not the 3′-terminal CCA is encoded in the genome. This sequence is also nearly identical to $tRNA^{Thr}$ encoded by the model organism *S. coelicolor*, and is predicted to recognize the ACC codon, which is the dominant codon used for threonine in *Streptomyces*. Consequently, this tRNA was used for *in vitro* studies.

Preparation of SCAT_tRNA25 was accomplished by transcription using a Hiscribe T7 High Yield RNA Synthesis Kit (NEB). Template was prepared by amplification from plasmid pIJ10702-SCAT_tRNA25 with primers J064 and J071 using Phusion polymerase (*Table S1*). The reaction was then conducted using the manufacturer's recommended conditions for short RNAs, with the exception that inorganic pyrophosphatase (Sigma) was added to 5 U/mL and RNAse-IN (Ambion) to 0.1 U/mL. For the production of ^{13}C-labeled tRNA, ATP-^{13}C (Santa Cruz) was used instead of the supplied ATP. Transcription reactions were quenched with formamide and separated on a 30 cm, 8% urea-PAGE gel with TBE buffer system. The desired band was by visualized by UV-shadow, excised, and extracted overnight at 4 °C with 200 mM sodium chloride, 10 mM Tris, 1 mM EDTA, pH 7.5. RNA was precipitated by addition of 1:10 5 M ammonium acetate (Life

Technologies) and 1:1 IPA, followed by centrifugation for 10 min at 20,817 RCF. At this point it was washed three times with ice cold 70% (*v/v*) ethanol and air dried before resuspension in nuclease-free water.

For concentration measurements, RNA was hydrolyzed by addition of 1 volume 1 N NaOH and incubation at 37°C for > 1 h, and the pH was restored to neutral by addition of 2 volumes 1 M sodium phosphate monobasic. The absorbance at 260 nM was used to calculate concentration based on the extinction coefficients of free NMPs and the nucleotide composition of the RNA ($\varepsilon_{260\ nm}$= 823,780 $M^{-1}cm^{-1}$) [*13*]. The tRNA prepared in this fashion was typically 68-80% active, with aminoacyl-tRNA formation determined by LCMS as described below and total tRNA concentration determined by A_{260}.

Aminoacylation assay. Aminoacylation reactions were performed at 30°C in 50 mM HEPES, 10 mM magnesium chloride, 25 mM potassium chloride, 5 mM DTT, pH 7.5 containing 5 mM ATP, 2 U/mL inorganic pyrophosphatase, 24 µM tRNA, 5 mM amino acid, and 50 nM ThrRS. The deacylating protein FthB was present at 0 nM, 50 nM, or 250 nM (0:1, 1:1, or 5:1 with respect to ThrRS). The reaction was initiated by addition of tRNA. Aliquots (2.5 µL) were removed and quenched by mixing with 5 µL of digestion solution consisting of 200 mM ammonium acetate (pH 5.2), 1.5 U/uL RNAse A, and ^{13}C-labeled aminoacyl tRNA internal standard. Protein was precipitated by addition of 52.5 µL of 5.7% (*w/v*) TCA (final concentration 5% *w/v*), and centrifugation for 1 h at 4,000 RCF.

The resulting 3'-*O*-threonyladenosine adducts were analyzed by UPLC-QQQ. Samples were injected using an Agilent 1290 autosampler, and separated on a Sigma Titan C18 column (1.9 µm, 2.1 × 30 mm; Sigma-Aldrich) connected to an Agilent 1290 UPLC (Agilent Technologies, Santa Clara CA). Column temperature was maintained at 20°C, and a flow rate of 0.7 mL/min was used. The gradient was isocratic 4% mobile phase B until 0.8 min, followed by a linear gradient from 4-30% mobile phase B from 0.8 to 1.55 min, with 5 mM ammonium formate as mobile phase A and acetonitrile as mobile phase B. Mass spectrometry was performed on an Agilent 6460C QQQ with Agilent Jet Stream source. Drying gas flow was 11 L/min at 325°C while sheath gas flow was 12 L/min at 350°C. Quantification was accomplished using the (M+H) → 136.1 transition for analytes, and the (M+H) →141.1 transition for internal standards. Fragmentor voltage was set to 135 V for all analytes. Collision energy was set to 15 V for adenosine and 3'-*O*-threonyladenosine, and 20 V for 3'-*O*-fluorothreonyladenosine. Integration was performed using Masshunter Quantitative Analysis software (Agilent) and fitting of standard curves was performed using Microsoft Excel (Microsoft, Redmond WA).

Deacylation assay. Kinetic analysis of deacylation was performed using a reaction system that couples AMP release to NADH consumption. Reactions were performed in a Helma 105.252-QS 1.5 mm pathlength microcuvette and monitored at 340 nM using an Agilent 8453 spectrophotometer with Agilent 89090A temperature controller set to 30°C (Agilent). Aminoacylation was initiated by the addition of TRS to 2.5 μM in 50 mM HEPES, 10 mM magnesium chloride, 25 mM potassium chloride, 5 mM DTT, pH 7.5 containing 5 mM ATP, 400 μM NADH 2 U/mL inorganic pyrophosphatase, 10 U/mL myokinase, 10 U/mL pyruvate kinase/lactate dehydrogenase, tRNA, and 2 mM amino acid. The aminoacylation reaction was allowed to proceed to completion (1-4 min depending on the concentration of tRNA), and then deacylation was initiated by adding FthB to a final concentration of 10-65 nM (For Fth reactions) or 6-19 μM (For Thr reactions). The rate of FthB-catalyzed hydrolysis was obtained from the rate of NADH consumption as measured by ΔA_{340}. The observed rate scaled with the concentration of deacylating protein added. The change in A_{340} observed after equilibration, but prior to the addition of deacylating protein was not subtracted. The concentration of aminoacyl tRNA was measured at steady state by quenching 2.5 μL aliquots of reaction mixture into 5 μL of digestion solution (200 mM ammonium acetate, pH 5.2, 1.5 U/uL RNAse A, and ^{13}C-labeled aminoacyl tRNA). Sample processing and LCMS was performed as for the aminoacylation reaction, with the exception that particulates were removed by centrifugation for 30 min at 10,000 RCF. Nonlinear curve fitting was performed using Microcal Origin 6.0 (Microcal Software Inc., Northampton MA).

Preparation of aminoacyl-tRNA standards. Aminoacyl-tRNA standards were prepared by aminoacylation of 24 μM tRNA in 50 mM HEPES, 10 mM magnesium chloride, 25 mM potassium chloride, 5 mM DTT, pH 7.5 containing 5 mM ATP, 1 mM amino acid, 10 U/mL inorganic pyrophosphatase, 20 μM tRNA, and 100 nM ThrRS for 30 min. Reactions were quenched by extraction with phenol chloroform, pH 5.2, and an equimolar amount of the amino acid not present in the charging reaction was added to the aqueous phase. The quenched reaction was then diluted with 2 volumes of 20 mM sodium acetate, 10 mM magnesium chloride, pH 5.2 (DEAE-A). The tRNA was bound to 0.5 mL pre-equilibrated DEAE-sepharose and washed with 5 column volumes DEAE-A supplemented with 200 mM sodium chloride to remove free amino acids before elution with 3 column volumes of DEAE-A supplemented with 1 M sodium chloride. Eluent was precipitated by the addition of 0.1 volume 5 M ammonium acetate and 1 volume IPA, then washed with 3 × 70% (*v/v*) ethanol and air dried before resuspension in 10 mM sodium acetate pH 5.2 and storage at -80°C. For quantification of the extent of aminoacylation, aminoacyl tRNAs were diluted 1:4 and 1:16 with 100 mM borate, pH 10.4 and allowed to hydrolyze for 20 min before

quantification via amino acid analysis. Removal of free (non-aminoacylated) amino acid was verified by the absence of the amino acid that was added post-quench.

Construction of cosmids for gene deletion in *S. cattleya*. The *S. cattleya* NRRL 8057 cosmid library described previously was used to prepare cosmids for the deletion of SCAT_p0564 (*fthB*) and SCAT_p0565 (*fthC*) [*14*]. Plates containing the cosmid library in pooled form were screened for the *fthB* coding sequence by colony PCR using primers J144 and J145 (*Table S1*). Wells resulting in initial hits were streaked out to individual colonies, which were re-screened to obtain the desired cosmid (f7.2). The cosmid was isolated using the Qiagene QIAprep Miniprep Kit, and the boundaries of the inserted fragment were identified with sequencing from T7 and T3 promoters. The Am^R/OriT cassette was then amplified using primers J239/240 (for Δ*fthB*::Am^R) and J240/241 (for Δ*fthC*::Am^R). The cassette was inserted into f7.2 using the REDIRECT protocol in BW25113 cells containing the helper plasmid pIJ790 [*15*]. Colonies harboring the Am^R marker were isolated on selective media and the resulting cosmids prepared from these strains, f7.2Δ*FthB* and f7.2Δ*FthC*, were sequenced with primers J235 and 236 to confirm the insertion site.

Conjugative transfer of plasmids from *E. coli* to *S. cattleya* and *S. coelicolor*. Conjugations were performed using a modified version of the standard procedure [*8*]. Chemically competent *E. coli* GM272 cells harboring pUZ8002 were transformed with OriT-containing plasmids or cosmids, and selected on the appropriate antibiotics. Single colonies were inoculated into 5 mL LB supplemented with the appropriate antibiotics and grown overnight at 30ºC with shaking (200 pm). Seed cultures were used to inoculate 50 mL growths to OD_{600} = 0.05 in 250 mL baffled flasks, which were then grown at 37ºC at 200 rpm until the OD_{600} reached 0.3-0.4. The *E. coli* culture was then pelleted by centrifugation for 5 min at 9,000 RCF and supernatant was discarded. The pellet was washed by three successive additions of ice cold, antibiotic-free LB and centrifugation. After the last spin, the pellet was resuspended in 500 µL water, and mixed with *Streptomyces* spores (~1×10^8) that had previously been heat shocked for 5 min at 55ºC. The combined cells were then centrifuged for 5 min at 2,000 RPM in a benchtop microcentrifuge. The majority of the supernatant was discarded, and cells were resuspended and plated on mannitol-soy (MS) flour plates [*8*] supplemented with magnesium chloride (MSMg, 20 g soy flour, 20 g mannitol, 20 g agar, 2.03 g magnesium chloride hexahydrate L^{-1}, 30 mL per plate) which were incubated at 30°C. After 24 h, 750 µg naladixic acid was applied in 500 µL water to repress growth of the *E. coli*. Immediately afterward (for *S. coelicolor M1152*) or after another 24 h (for *S. cattleya*), apramycin (1.5 mg) was applied in 800 µL water. Plates were incubated for another 5-7 d, at which point colonies were evident on successful conjugation plates.

Colonies surviving apramycin selection were restreaked on MSMg, and propagated in 3 mL cultures of GYM (yeast extract, 4 g/L; malt extract, 10 g/L; glucose, 4 g/L; agar, 10 g/L) at 30°C. Half the biomass was plated for spores on MSMg with 1.5 mg apramycin, while the other half was pelleted and stored at -20°C. Genomic DNA was prepared from pellets using the salting out procedure [*8*]. For *S. cattleya* knockout strains, double crossover homologous recombination was confirmed by PCR with primers J146_564middleF/J147_564middleR (for Δ*fthB*::Am^R) and J144_565middleF/J145_565middleR (for Δ*fthC*::Am^R) (*Table S1*). PCR from WT and single crossover gDNA prepared using the salting out method [*8*] gave a short band while the double crossover strains showed a single, longer band. *S. coelicolor* knockins were identified by Am^R phenotype.

***S. cattleya* growths.** Spores of *S. cattleya* were heat shocked at 55°C for 5 min before inoculation into 50 mL seed cultures of GYM media (yeast extract, 4 g/L; malt extract, 10 g/L; glucose, 4 g/L; presterile pH 5.0) in 250 mL baffled flasks with glass beads. After 14 h of growth, cultures were re-inoculated to OD 0.05 in 100 mL GYM media in 500 mL UltraYield flasks with glass beads. Following another 12 h of growth, sodium fluoride was added to a final concentration of 2 mM. Growth was monitored by OD_{600}, and biomass was collected 6 d subsequent to fluoride addition. Culture media was removed, split into 2 mL portions, and centrifuged for 1 min at 18,000 RCF at room temperature to pellet the cells. Supernatant was removed by decanting, and cell pellets were flash frozen in liquid nitrogen and stored at -80°C. Four biological replicates of each strain (WT, Δ*fthB*::Am^R, and Δ*fthC*::Am^R) were grown, harvested, and used for downstream analysis. Statistical comparison of measured physiological parameters was performed in Microsoft Excel (Microsoft, Redmond WA) using single tailed t-tests with Welch's correction for unequal sample variance.

Viability experiments. To assess the viability of strains following organofluorine production, growths were conducted in 50 mL of GYM media in 250 mL baffled flasks with glass beads. Approximately 1×10^8 spores of the WT and *ΔfthB* strains were used to inncoulate seed cultures, which were grown overnight. Secondary cultures were then inoculated to an OD_{600} of 0.05, and grown for a further 8 hrs. Both strains were then inoculated or co-innoculated to an OD_{600} of 0.05 in GYM with 0 and 2 mM sodium fluoride. Growths were continued for 7 days, at which point 1 mL portions of culture were harvested by centrifugation at 20,000 RCF for 1 m. The supernatant was decanted and cells were resuspended in water. Resuspended cells were diluted to the same OD_{600}, agitated briefly in a sonicating waterbath, and then used to prepare a 6-fold serial dilution series before plating 5 µL drops on GYM agar (10 g malt extract, 4 g yeast extract, 4 g dextrose, 10 g agar per liter). Plates were grown for 2 days at 37° C before being photographed.

Competitive growths. For competition experiments, growths were conducted in 50 mL of GYM media in 250 mL baffled flasks with glass beads. Approximately 1×10^8 spores of the WT and *ΔfthB* strains were used to inoculate seed cultures, which were grown overnight. Secondary cultures were then inoculated to an OD_{600} of 0.05, and grown for a further 8 hrs. Both strains were then co-inoculated to an OD_{600} of 0.05 in GYM with 0 and 2 mM sodium fluoride. Growths were continued for 6 days with periodic removal of 1mL samples to harvest genomic DNA. After 6 days, cultures were re-diluted to an OD600 of 0.05 and grown for one day to test their ability to grow after exposure to endogenously produced FThr. Aliquots removed for gDNA preparation were centrifuged for 1 minute at 20,000 RCF and centrifuged to remove culture supernatant. Pellets were resuspended in 500 µL cell lysis solution from the Promega Wizard® gDNA preparation kit and lysed via a 3-minute bead-beater cycle. Lysate was clarified by centrifugation for 10 minutes at 20,000 RCF and the supernatant was processed following the manufacturer's instructions. The resulting genomic DNA was diluted to 5 ng/µL and used as a substrate for qPCR. Primers J639 and J640 were used to detect the AmR/OriT cassette present in the *ΔfthB* strain, while primers J597 and J598 were used to detect the *fthB* gene present in the WT strain. Trials with gDNA prepared from individual strains confirmed that these primer sets did not display any cross-reactivity. The Biorad iTaq™ supermix was used in conjunction with a Biorad iQ5 thermocycler; the program employed 40 cycles of denaturation at 95 C for 30 s, followed by annealing at 60 C for 30s, and extension at 72 C for 30 s. Signal was monitored using the FAM filter set. The ratio of WT to *ΔfthB* strains was calculated from Ct values as:

$$\frac{WT}{\Delta fthB} = 2^{(Ct_{\Delta fthB} - Ct_{WT})}$$

^{19}F NMR analysis of organofluorine production. Lyophilized supernatant was resuspended in ^{19}F NMR buffer (100 mM Tris-HCl pH 7.5 using 20% D_2O/80% H_2O) containing 1 mM 5-fluorouracil interntal standard at a ratio of 1 buffer:10 culture supernatant. NMR spectra were collected on a Bruker AVQ-400 spectrometer at the College of Chemistry NMR Facility at the University of California, Berkeley (64 scans; o1p = -200 ppm; d1 = 15 s). Spectra were referenced to 5-fluorouracil (-168.33 ppm vs. $CFCl_3$). Concentrations of fluorothreonine and fluoroacetate were calculated by normalizing the total integrated area of fluorothreonine, fluoroacetate, and fluoride to 2 mM.

Amino acid analysis. Amino acid analysis was performed by *o*-phthalaldehyde (OPA) derivatization followed by LCMS. Derivatization of samples was accomplished on an Agilent 1290 autosampler using a program that sequentially mixed 0.5 µL of sample, 1.25 µL 400 mM pH

10.4 borate, 0.25 µL 10 mg/mL OPA/mercaptopropionic acid, and 16 µL 0.4 % (*v/v*) acetic acid before injection. OPA adducts were then separated on an Agilent Eclipse Plus RRHD C18 column (1.8 µm, 2.1 × 50 mm; Agilent) connected to an Agilent 1290 UPLC. Column temperature was maintained at 40°C, and the flow rate was 0.6 mL/min. The gradient was isocratic at 2% mobile phase B (45% methanol, 45% acetonitrile, 10% water)/98% mobile phase A (10 mM ammonium acetate pH 8.2) until 0.2 min, followed by a linear gradient from 2-31.45% B from 0.2 to 4.2 min. Detection was performed on an Agilent 6460C QQQ with Agilent Jet Stream source. Drying gas flow was 10 L/min at 325°C while sheath gas flow was 12 L/min at 350°C. Quantification was performed using a (M+H) → (M+H-105.1) transition that was found to be characteristic of OPA adducts. Fragmentor voltage was set to 135 V and collision energy was set to 15 V for all analytes. Integration of peaks was performed using Masshunter Quantitative Analysis software (Agilent) and fitting of standard curves was performed with Microsoft Excel.

Preparation of samples for whole-protein amino acid analysis. Culture media (2 mL) was removed from shake flasks and harvested by centrifugation at 18,000 RCF for one min before supernatant was discarded. The sample was then flash frozen and stored at -80 before analysis. Pellets were thawed and washed 2 times with 1 mL lysis buffer (50 mM Tris, 150 mM sodium chloride, 1 mM EDTA, 1 mM PMSF, pH 7.5). They were then lysed by sonication with Misonix 3000 with 1/8" microtip (power =5, 5 s on 25 s off for 45 s total process time). Lysate was clarified by centrifugation for 10 min at 18,000 RCF. The supernatant was diluted with lysis buffer to 300 µg/mL in 500 µL total volume (as determined by BCA assay) and spiked with 5 µL of 100 mM β-hydroxy norvaline. TCA was added to 10% (*w/v*) and protein was precipitated by centrifugation for 20 min at 20,817 RCF. The supernatant was stored for analysis of free amino acids, while the pellet was washed 3 times with 100 µL 10% (*w/v*) TCA and 2 times with 90% acetone 0.01% HCl. It was then air-dried and stored at -20°C. Pellets were subsequently resuspended in amino acid grade 6 N HCl in anaerobic culture tubes, and the headspace was cleared of oxygen by 3 cycles of vacuum followed by backfilling with argon. Hydrolysis was performed at 100°C for 24-28 h, after which samples were evaporated to dryness by SpeedVac, resuspended, and analyzed by LCMS as described previously. All glassware used for hydrolysis was pre-cleaned by boiling in 1 N HCl and rinsing with MilliQ water. Samples collected at 48 and 72 h indicate that threonine and fluorothreonine are both stable under the conditions employed, while an absence of β-hydroxy norvaline in the hydrolysate indicated that <1% of free amino acids remained after TCA precipitation.

Preparation of whole *S. cattleya* RNA. Pellets were thawed in 200 mM sodium acetate, 1 mM EDTA, 4 M guanidinium hydrochloride, pH 5.2 and lysed by sonication using a Misonix 3000

with 1/8" microtip (5 s on 25 s off, 30 s total process time). Lysate was clarified by 1 min centrifugation at 20,000 RCF, and the supernatant was extracted twice with phenol-chloroform, pH 5.2 and once with chloroform alone. RNA was then precipitated by addition of one volume isopropanol, and pelleted by centrifugation for 30 min at 20,000 RCF and 4°C. The pellet was washed with ice cold 70% (*v/v*) ethanol, then with ethanol, and was dried briefly before storage at -80°C. Pellets were resuspended in 100 mM ammonium acetate, pH 5.2 containing 10 μg/μL RNAse A for analysis. Aminoacylation state was assessed using by LCMS. Samples were separated on an Agilent Eclipse Plus RRHD C18 column (1.8 μm, 2.1 × 50 mm; Agilent) connected to an Agilent 1290 UPLC. Column temperature was maintained at 20°C, with a flow rate of 0.6 mL/min. Mobile phase A was 5 mM ammonium formate, and mobile phase B was acetonitrile. The gradient was isocratic 4% B for 1.5 min, followed by a linear gradient to 30% B over the next 1.5 min. Detection was performed using an Agilent 6460C QQQ, with source and MRM settings as described for in vitro aminoacylation assays. Isotopically labeled internal standards were not used for this analysis.

Preparation of samples for proteomics. Whole-protein samples for proteomics were lysed using a Misonix Sonicator 3000 with 1/8" microtip (power =5, 5 s on 25 s off for 45 s total process time) in 50 mM Tris, 150 mM sodium chloride, 1 mM EDTA, pH 7.5. Lysate was cleared by centrifugation for 10 min at 18,000 RCF at 4°C. The resulting supernatant was precipitated by addition of TCA to 10% (*w/v*) and pelleted by centrifugation for 10 min at 20,817 RCF at 4°C.

Pellets were resuspended in 8 M urea in phosphate buffered saline (PBS), and the concentration was determined using BCA protein detection reagent (Pierce) with lysozyme standards. Samples were then diluted to obtain 80 μg protein in 30 μL 8 M urea. Subsequently, 40 μL of 100 mM ammonium bicarbonate and 30 μL of 0.2% (*w/v*) Rapigest in 100 mM ammonium bicarbonate were added in succession and the sample was vortexed briefly. The sample was then reduced by addition of 10 μL of 110 mM TCEP (Bisynth, Itasca IL) followed by gentle room temperature inversion for 30 min, at which point 2.5 μL of 500 mM iodoacetamide was added and samples were incubated in the dark for 30 min. Unreacted iodoacetamide was quenched by addition of 25 μL 100 mM DTT, and the sample was diluted with 120 μL PBS and 1.2 μL 1% (*w/v*) Rapigest before addition of MS-grade trypsin (Pierce) on a 1:25 mass:mass basis. Digestion was allowed to proceed overnight at 37°C, and was quenched by the addition of formic acid to 5% (*v/v*) final concentration. After centrifugation for 30 min at 20,817 RCF, samples were desalted by application to a pre-conditioned Sep-Pak Vac 1cc (50 mg) tc18 SPE column (Waters, Milford MA), washed with 2 × 1 mL 0.1% (*v/v*) formic acid, and eluted with 2 × 150 μL 80% (*v/v*) acetonitrile 0.5% (*v/v*) formic acid. Desalted samples were dried by Speedvac. For shotgun

proteomics, samples were submitted to the UC Davis Proteomics Core at the UC Davis Genome Center for analysis by LC-Q Exactive. Targeted proteomics analysis was performed in house by LC-QQQ.

Shotgun proteomics data analysis: Raw data files were searched with MaxQuant version 1.5.7.0 [*16*]. The database search was performed against the *S. cattleya* proteome (Uniprot accessions FQ859185 and FQ859184) with reversed protein sequences as decoys. Precursor mass tolerance was set to 5 ppm, and fragment mass tolerance was set to 10 ppm. Up to one missed cleavage was allowed, and carbamidomethylation of Cysteine was set as a fixed modification. Oxidation of methionine, N-terminal acetylation, and Thr to FThr (+17.99058 Da) substitutions were allowed as variable modifications. Peptide identifications were filtered to Posterior Error Probability values < 1% for downstream analysis of fluorothreonine incorporation. For assessment of differential incorporation of fluorothreonine, data for peptides encoding single threonine residues were split into separate datasets corresponding to the FThr-containing and unmodified peptides. Intensities from the Label Free Quantitation routine of MaxQuant were used to tabulate histograms of FThr incorporation, and were also input into the MSStats package to assess differential fluorothreonine incorporation [*17*]. Codon usage statistics were tabulated using custom Python scripts.[*18*] Gene ontology assignments were obtained from MicroScope [*19*]. R and Python scripts used for data analysis are available from: https://github.com/mcmurryj/Fthr-proteomics. Annotated tandem MS spectra were generated using PeptideShaker [*20*].

Targeted proteomics. Aliquots of peptide (2.5 μg) were injected via autosampler and separated on an Agilent AdvanceBio Peptide Mapping C18 column (2.7 μm, 2.1 × 250 mm; Agilent) connected to an Agilent 1290 UPLC. Column temperature was maintained at 60°C, and the flow rate was 0.4 mL/min. The gradient was isocratic at 3% mobile phase B (90% acetonitrile, 10% water, 0.1% formic acid)/ 97% mobile phase A (0.1% formic acid) until 5 min, followed by a linear gradient from 5-50% B from 5 to 65 min. Peptides were detected with an Agilent 6460C QQQ with Jet Stream source for quantification runs, while an Agilent 6530 QTOF with ESI source was used for initial survey runs. For quantification runs, drying gas flow was 10 L/min at 280°C while sheath gas flow was 12 L/min at 325°C. For survey runs, drying gas flow was 10 L/min at 300°C, and data acquisition was performed with 1 MS and 3 MS/MS spectra per second.

Two criteria were applied to select peptides detected in survey runs for downstream analysis. First, peptides from abundant organofluorine biosynthesis and housekeeping proteins were chosen in order to ensure robust detection. Second, the threonine-containing variant of each peptide was

required to be detected with high confidence (Q-value < 0.01). Database searches were conducted using Morpheus [*21*].

The Skyline targeted proteomics package was then used to select an initial set of transitions, which were pared down to those exhibiting the best signal to noise for final analysis. For all fragments associated with a given FThr-containing peptide, the corresponding y- or b-ions were also considered for the equivalent unmodified (threonine-containing) peptide. Masshunter Quantitative Analysis software was used to perform integration of the resulting data. Integration was initially performed using Agile parameter-free integration, with manual correction applied in cases where the software assigned a single peak to multiple integration regions. The FThr incorporation rate p was then estimated as $p = \frac{F}{(F+T)}$ where F is the peak area corresponding to the fluorothreonine-containing peptide and T is the peak area corresponding to the Thr-containing peptide. For peptides with multiple threonine residues, it was sometimes possible to identify a transition that specifically measured incorporation at one site. When this was not possible, incorporation rate was estimated as $p = \frac{F}{(F+T)*n}$ where F is the peak area corresponding to the FThr-containing peptide, T is the peak area corresponding to the Thr-containing peptide, and n is the number of Thr residues encompassed by the quantifier transition. Incorporation rates inferred in this fashion are consistent with those measured using single-threonine peptides. Transitions used for targeted proteomics are detailed in Table S2, with quantifier transitions marked in bold.

Fluorothreonine Growth Inhibition Assays. Spores for FThr growth inhibition assays were prepared by plating from liquid media onto mannitol-soy flour plates supplemented with 10 mM magnesium chloride. Spores were harvested by scraping with a plastic sterile scraper (*S. cattleya*) or cotton swabs (*S. coelicolor*), and were washed 3 times with water and once with 20% (*v/v*) glycerol before resuspension in 20% (*v/v*) glycerol for storage at -20°C. Approximately 2.5×10^7 spores were heat shocked for 5 m at 55°C, plated on Hopwood minimal media (10 g/L agar, 10g/L sugar 0.5 g/L asparagine, 0.2 g/L magnesium sulfate heptahydrate, 0.5 g/L potassium phosphate dibasic, 10 mg/L iron sulfate heptahydrate) and allowed to dry for 1 h at room temperature. Fluorothreonine was applied to a sterilized disc of filter paper which was then placed in the center of the plate. Plates were cultured at 30°C and photographed 5 d after plating with a Nikon Coolpix P300 (Nikon USA, Melville NY).

For growth inhibition assays in liquid media, approximately 1×10^7 spores were heat shocked for 5 min at 55°C, and inoculated into 4 g/L inositol, 10 g/L glycerol, 0.5 g/L potassium phosphate dibasic, 1.5 g/L ammonium chloride, 0.2 g/L magnesium sulfate heptahydrate, 10 mg/L each of zinc sulfate, iron sulfate heptahydrate, manganese chloride, and calcium chloride [*22*].

Supplementary Results

Table S1. (A) Strains and plasmids used for this study. (B) Oligonucleotides used for cloning, sequencing, cosmid screening, and preparation of *in vitro* transcription template.

A. Strains and plasmids

Strain	*Genotype*	*Source*
S. cattleya ATCC 35852 (NRRL 8057)	Wild-type	ATCC
S. cattleya Δ*fthB*	Δ*fthB*::Am^R	This study
S. cattleya Δ*fthC*	Δ*fthC*::Am^R	This study
S. coelicolor M1152	Δ*act* Δ*red* Δ*cpk* Δ*cda rpoB*(C1298T)	John Innes Center [*23*]
S. coelicolor M1152 ermEp*-FthB	Δ*act* Δ*red* Δ*cpk* Δ*cda rpoB*(C1298T), ermEp*-FthB Am^R in ΦC31 *attB*	This study
S. coelicolor M1152 ermEp*-empty	Δ*act* Δ*red* Δ*cpk* Δ*cda rpoB*(C1298T), ermEp*-empty Am^R in ΦC31 *attB*	This study
E. coli BL21(de3)	*F– ompT gal dcm lon hsdSB(rB–mB–) [malB+]*K-12(*λS*)	Novagen
E. coli GM272	*dam-3 dcm-6 hsdS21 metB1 lacY1 or Z4 galK2 galT22 mtl-2 tonA2 or A31 tsx-1 or78 supE44 (thi-1)?*	[*6*]

Name	*Description*	*Source*
pET16b-TRS	His$_{10}$-SCAT_TRS (T7), *lacI,* Cb^R, ColEI	This study
pSV272.1-FthB	His$_6$-MBP-TEV-FthB (T7), *lacI,* Km^R, ColEI	This study
pSET152-ermEp*	ΦC31 int, ermEp*, Am^R, pUC	John Innes Center [*8*]
pSET152-ermEp*-FthB	ΦC31 int, ermEp*-FthB, Am^R, pUC	This study
f7.2	Km^R, Cb^R, pUC, FThr transaldolase gene cluster	This study
f7.2Δ*FthB*	f7.2 Δ*FthB*::Am^R-OriT	This study
f7.2Δ*FthC*	f7.2 Δ*FthC*::Am^R-OriT	This study
pIJ10702-SCAT_tRNA25	SCAT_tRNA25 (T7), Cb^R, OriT, pUC	This study
pUZ8002	tra, Km^R, RP4; derivative of pUZ8	[*24*]
pRare2	CmR, p15a, tRNA^{Ile}(AUA), tRNA^{Ser}(AGG), tRNA^{Ser}(AGA), tRNA^{Asp}(CUA), tRNA^{Gly}(CCC), tRNA^{Gly}(GGA),	Novagen

B. Oligonucleotide sequences

Name	*Sequence*
J303	catcatcatcacagcagcggccatatcgaaggtcgtcatatgtctcagatccgtgtggtcatcaaacgcg
J304	aactcagcttcctttcgggctttgttagcagccggatcctcacacctggacgcggcgctccaccacatca
J400	gcgagttctggctggctagcccgtttgatctcgagtgcggccgcaagctttcagccggtcgcggcgatcggctcg
J401	aataacaacaacctcgggatcgaggaaaacctgtattttcagggcatggaaaccgagagcacatacgacaagctc
J062	gagaattcgcggccgcataatacgactcactatagccccaatagctcagtcggcagagcg
J063	ggccgcaattaaccctcactaaagggatcctggagccccaatacggaatcgaaccgtaga
J064	tggagccccaatacggaatcgaaccg
J071	gcagagcttatcgatgataagcggtcaaacatgag
J118	ggtaggatcgtctagaacaggaggccccatatggaaaccgagagcacatacgacaagctc
J119	caagcttgggctgcaggtcgactctagaggatcctcagccggtcgcggcgatcggctcgg
J144	ggccggaactgatcggtcggtcacgggaggtggcccggtatgccc
J145	gctccgggcggtaccctggttggccacgagcgggaagcgg
J146	cggcccggtccgaccgaccctaggtggaggtggcggtcagg
J147	ccgggccacctcccgtgaccgaccgatcagttccggcc
J235	agaggcgggatgcgaagaatgcgat
J236	cggtatatccatccttttcgca
J239	gttccggcctaccgggacggggtgcccggggacccgcggagccgcgggggtcatgtaggctggagctgcttcgaagttcc
J240	ctcaccgtctccgagcttcccaccgaccgccggcacggcggctgagaggtgttcatgattccggggatccgtcgacctgc
J241	gtgcagatgggcgtcgaagatccgggaaccggtcccggccgtaccgtcggtcatgtaggctggagctgcttcgaagttcc
J242	tgacaccatgggcatggtggtccctccccccacctcatcctccggaggacccttgtgattccggggatccgtcgacctgc
J597	tggcgaacgatacgtaagtg
J598	gtcatggtcaagatcggaaaga
J639	tcaaaaaggatcttcacctagatccttttggttcatgtgcagctccatcagcaaaagggg
J640	tgcccctccaacgtcatctcgttctccgctcatgagctcaggcgccgggggcggtgtccg

Figure S1. Expanded phylogeny of FthB homologues with species of origin and NCBI RefSeq ID. Clades of putative editing proteins that display qualitative conservation of genomic context are denoted with brackets A-E. Example contexts are presented in Figure S2. Sequence WP_040742858 from *Nocardia tenerifensis*, denoted with an asterisk, groups with editing proteins from known organofluorine clusters despite lacking a fluorinase. Bootstrap values are indicated at branch points.

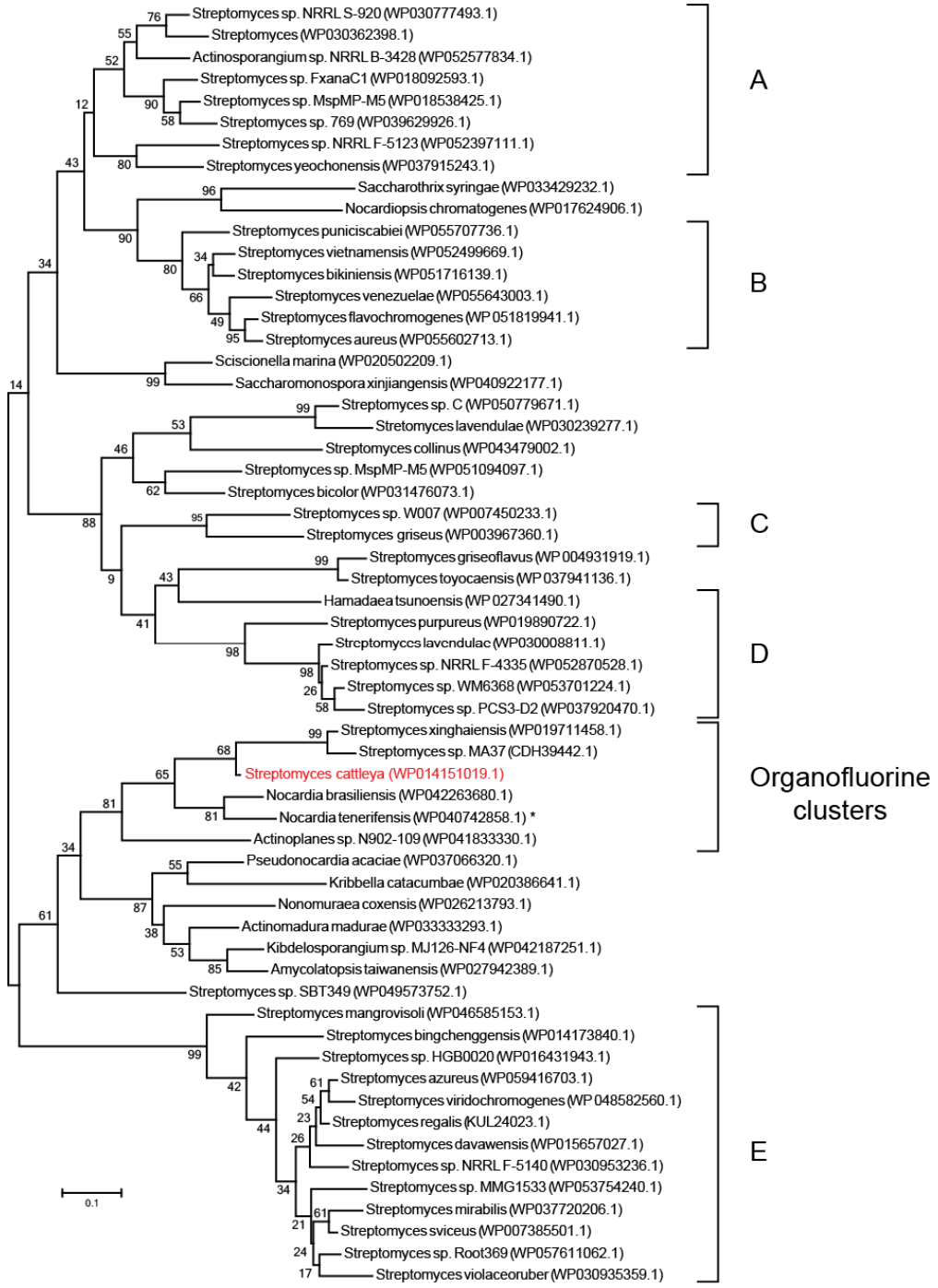


Figure S2. Example genomic contexts of FthB homologs from phylogenetic clades A-D (*Figure S1*). Editing proteins are marked in red. These proteins are found in a diverse set of genomic contexts, suggesting that they have been adapted to target a variety of mischarged aminoacyl-tRNA substrates. Of particular interest are clades C and D. Clade C encodes a putative nonribosomal peptide synthetase module. Clade D encodes several proteins with domain architectures that are commonly associated with amino acid biosynthesis pathways, as well as a truncated paralog of the methionyl aminoacyl-tRNA synthetase.

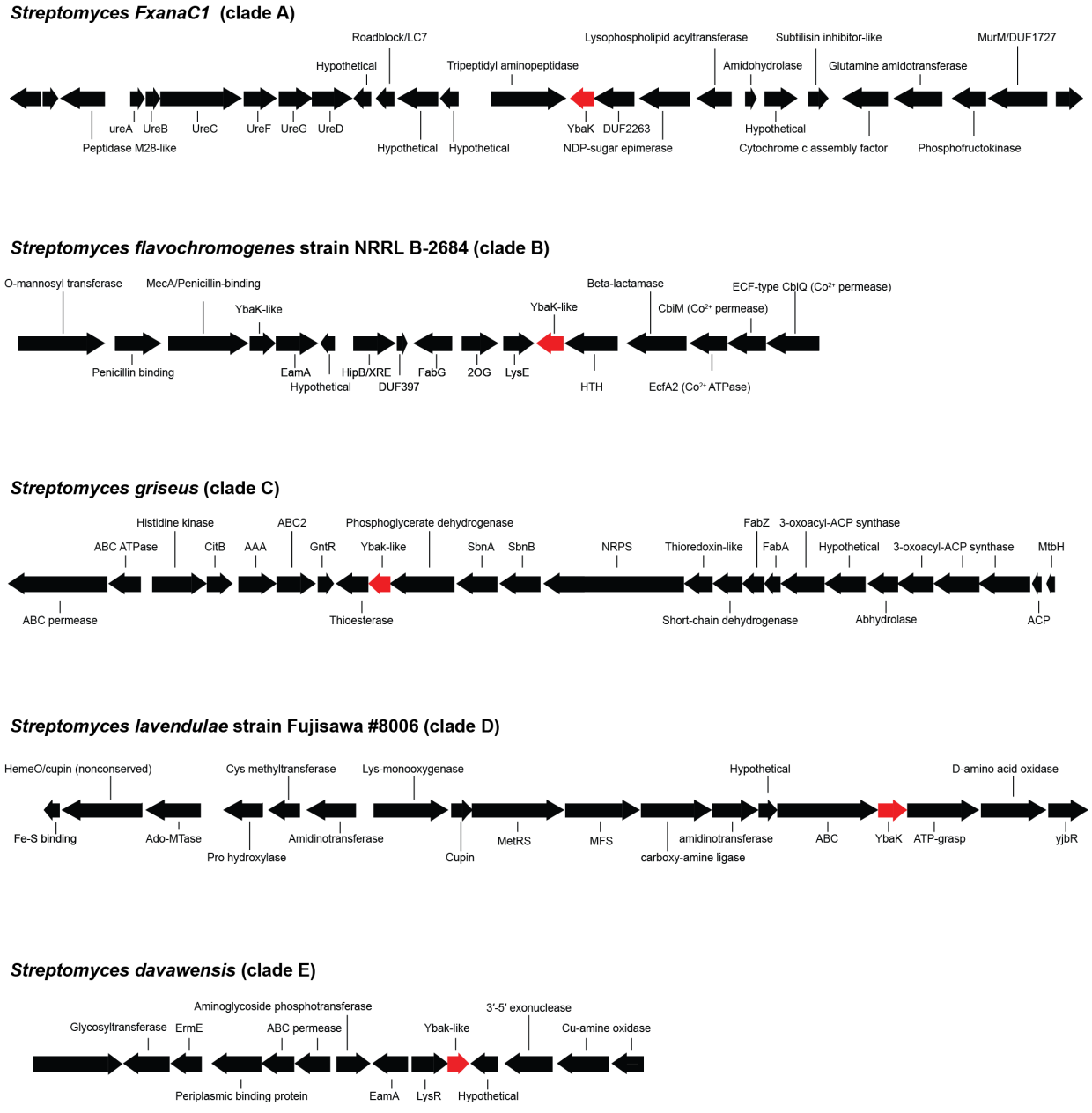


Figure S3. SDS-PAGE gel of purified *S. cattleya* enzymes. Genes were cloned from *S. cattleya* genomic DNA and expressed in *E. coli*. His_{10}-TRS (lane 1) and FthB (lane 2).

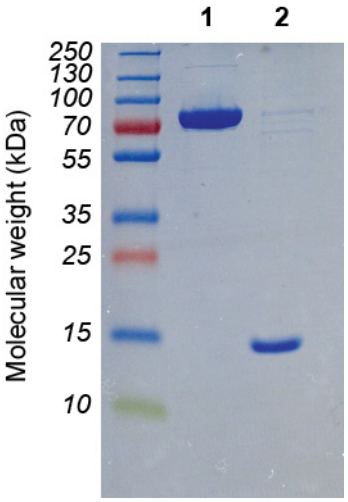


Figure S4. Measuring aminoacylation by LCMS. (A) Chromatograms from LC-MS/MS detection of adenosine and adenosine derivatives resulting from the cleavage of aminoacyl-tRNA by RNAse A. Adenosine, 3′-*O*-threonyladenosine, and 3′-*O*-fluorothreonyladenosine were respectively quantified using the *m/z* 268.1→136.1, 369.1→136.1, and 387.1→136.1 transitions. The use of a rapid UPLC separation permits the analysis of ~17 samples/h. (B) Control reactions for the LC-MS/MS aminoacylation assay. In this assay, aminoacylation is monitored by RNAse A digestion of the aminoacylation reaction followed by LC-MS/MS analysis. Uncharged tRNA is cleaved to release Adenosine from the 3′-terminus, while charged tRNA is digested to produce a 3′-*O*-aminoacyl ester. Production of product and consumption of substrate are measured in arbitrary units (counts). As the reaction progresses, we observe that adenosine is consumed with concomitant formation of the 3′-*O*-aminoacyl ester. The 3′-*O*-aminoacyl esters were ultimately used for quantification, as the signal from adenosine suffered from greater variability due to the poor retention of this compound on reverse phase columns.

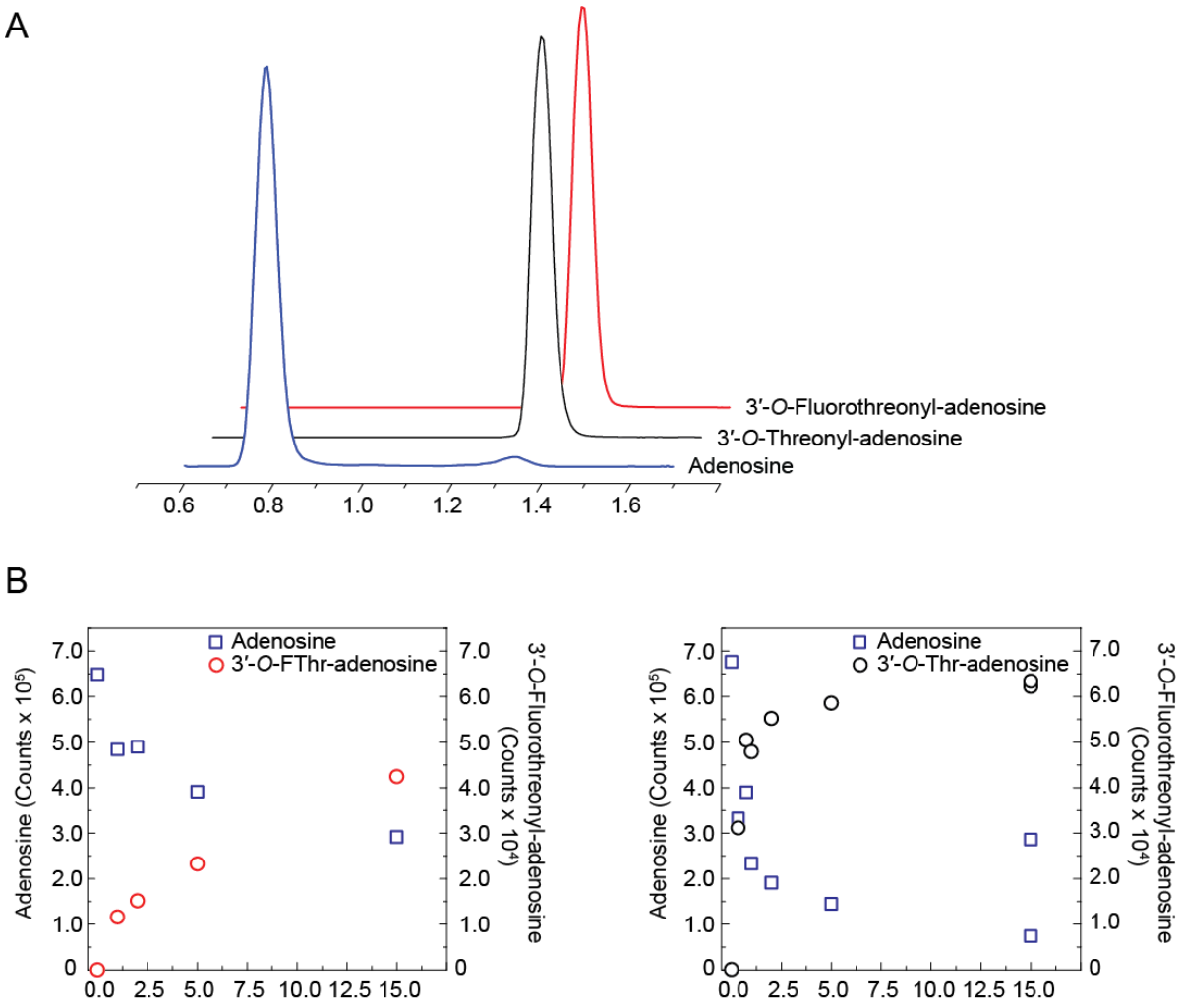


Figure S5. Measuring aminoacyl-tRNA concentration by LC-MS/MS quantification of amino acid content. (A) Aminoacyl-tRNA was prepared, and subjected to phenol-chloroform extraction. The quenched reaction was then spiked with the amino acid that was not charged on the tRNA, and free amino acids were separated from RNA by anion exchange on DEAE resin. Hydrolyzed aminoacyl-tRNA samples were derivatized with OPA and subjected to LC-MS/MS with MRM detection. OPA-derivatized threonine was measured using the *m/z* 324.1→ 219.0 transition and the equivalent fluorothreonine adduct was measured with the *m/z* 342.1→237.0 transition. LC-MS traces are normalized to the highest signal observed within each sample. The absence of Thr from fluorothreonyl-tRNA and vice-versa demonstrates that anion exchange separates aminoacyl-tRNA from free amino acid. (B) Representative standard curves for quantification of amino acids derivatized with OPA. The *m/z* 324.1→ 219.0 and 342.1→237.0 transitions were used for threonine and fluorothreonine, respectively. This method was used for quantification of aminoacyl-tRNA, free amino acids, and hydrolyzed whole protein samples.

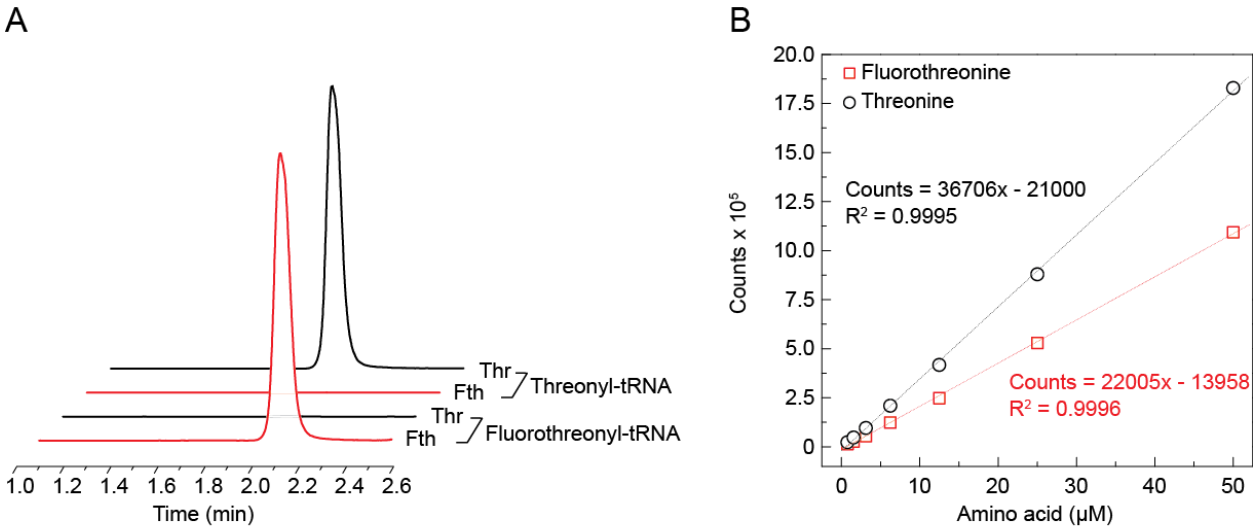


Figure S6. Representative standard curves for the quantification of aminoacyl tRNA by RNAse A digestion followed by LC-MS/MS. Peak areas for analytes are normalized to ^{13}C-labeled internal standards. The absolute concentration of the aminoacyl-tRNA standards was benchmarked by quantification of amino acid content, as described in *Figure S6*. The method is suitable for the quantification of threonyl- and fluorothreonyl-tRNA with a dynamic range of ~3 orders of magnitude.

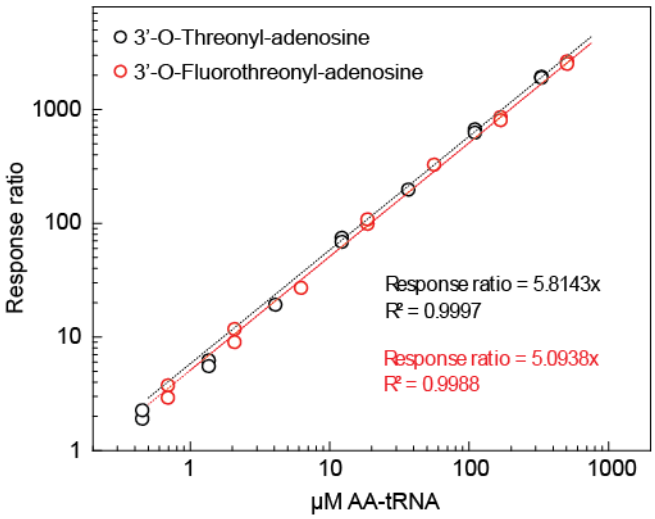


Figure S7. Validation of *S. cattleya* Δ*fthB* and Δ*fthC* strains. Genomic DNA of WT and Δ*fthB* strains were amplified with primers J146/7 to yield either a 766 bp band (WT) or a 1649 bp band (Δ*fthB*). Genomic DNA of WT and Δ*fthC* strains were amplified with primers J144/5 to yield either a 1231 bp band (WT) or a 1613 bp band (Δ*fthC*).

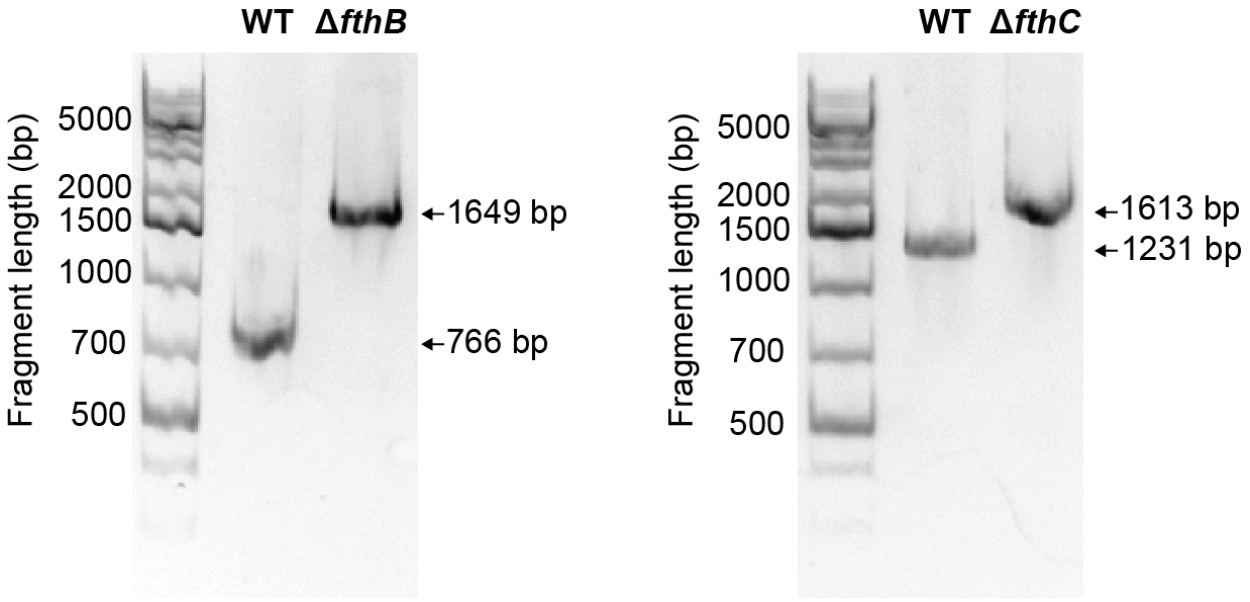


Figure S8. Growth curves of *S. cattleya* strains in GYM with 2 mM fluoride. Fluoride was added at time zero, and samples were collected 6 d (144 h) later. Culture supernatant was analyzed by ^{19}F- NMR, and cell pellets were used for downstream analysis of fluorothreonine physiology. Cell density is plotted as the mean ± s.d. (n = 4).

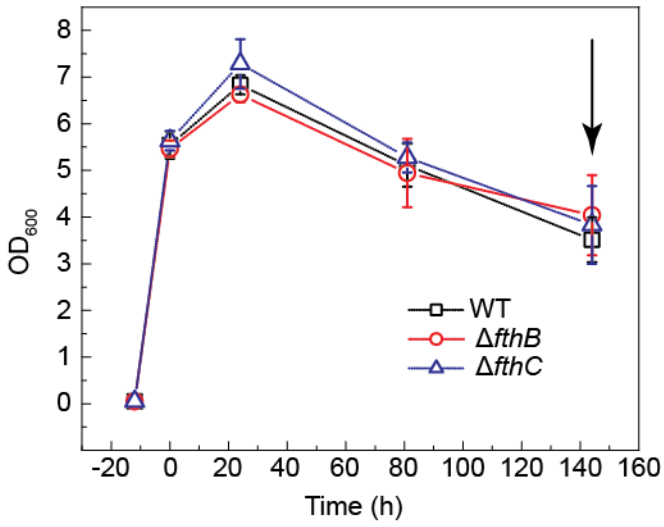


Figure S9. Viability of *S. cattleya* strains grown for 7 d in the presence and absence of fluoride. 6-fold serial dilutions were performed, and cells were spotted on GYM-agar plates before growth at 37°C. All strains remain viable even after growth at high fluoride concentrations.

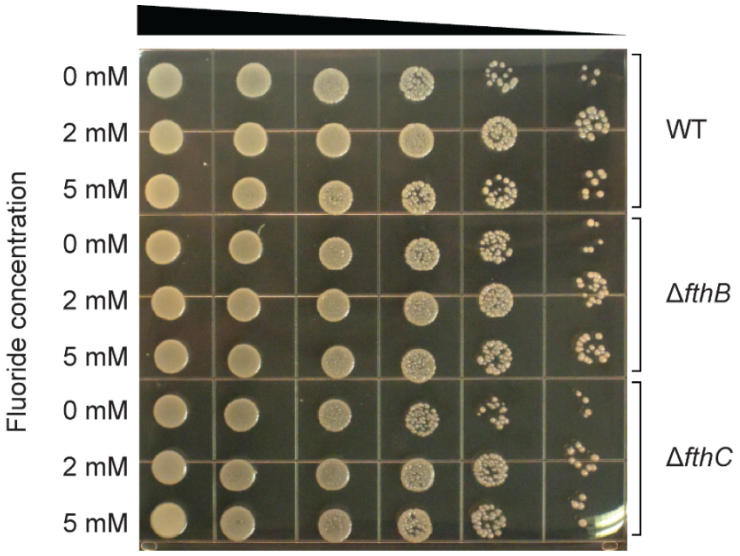


Figure S10. Competition assay with wild-type and Δ*fthB* strains of *S. cattleya* grown in the presence and absence of fluoride. Cultures were grown for 6 days with sampling at days 1 and 6, and were then diluted and grown for one more day to generate the day 6+1 sample. Measurements were performed using qPCR primers for the *fthB* coding sequence and the Am^R marker.

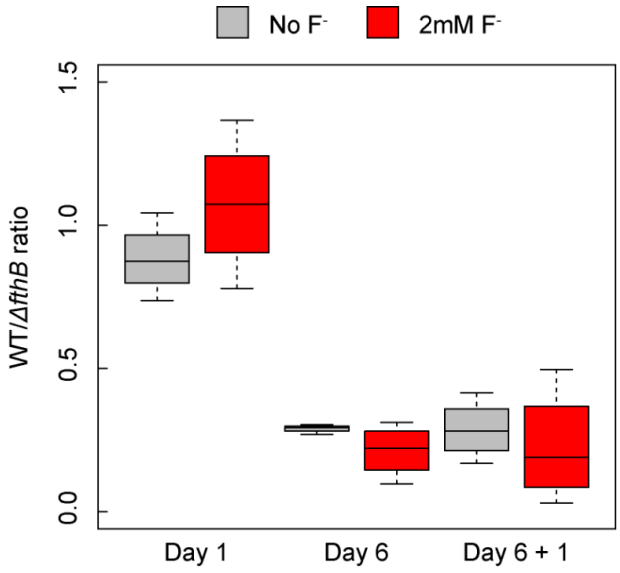


Figure S11. Representative ^{19}F-NMR spectra from *S. cattleya* WT, Δ*fthB,* and Δ*fthC* strains, centered on the regions where fluoroacetate (216 ppm) and fluorothreonine (230.5 ppm) are observed.

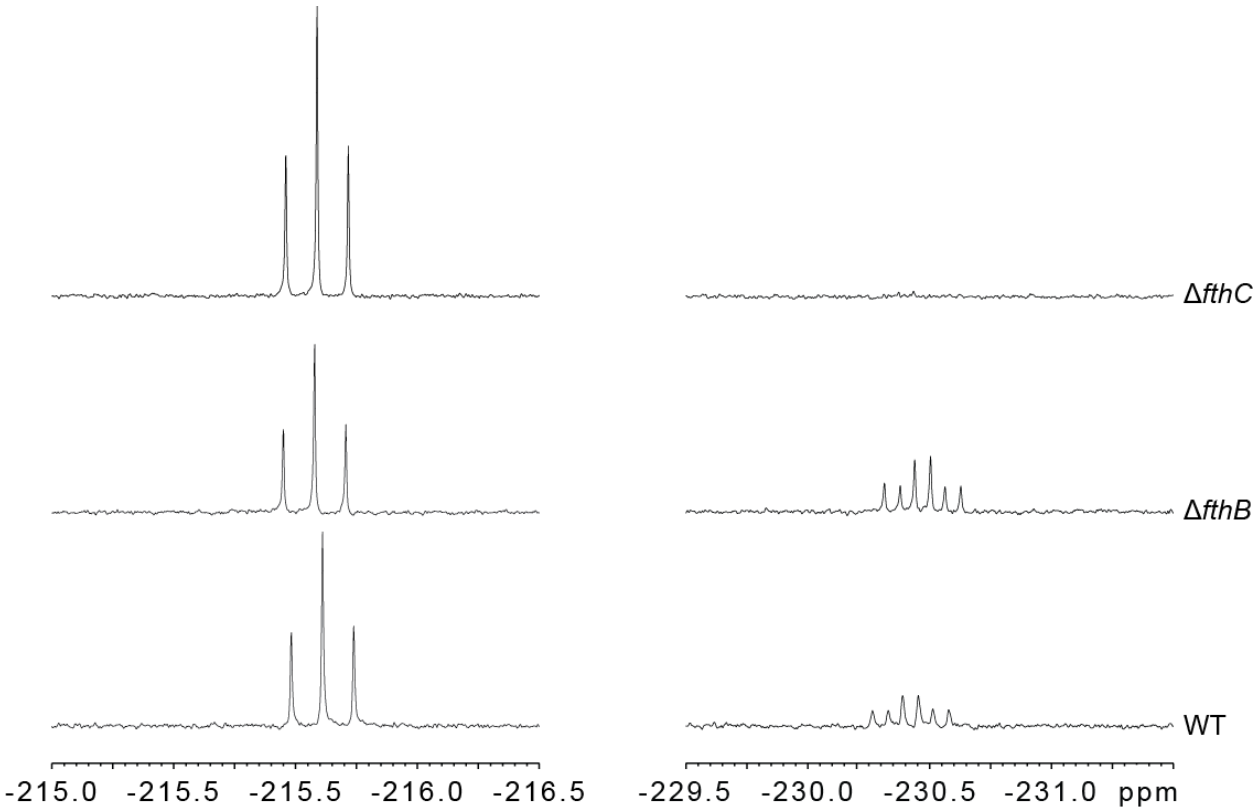


Figure S12. Example MRM chromatograms for threonine- and fluorothreonine-containing peptides from *S. cattleya* WT and Δ*fthB* strains. Chromatograms are normalized such that the plotted intensity equals the measured signal divided by the signal corresponding to the threonine-containing peptide collected for the same sample. (Thr-containing peptide, black; FThr-containing peptide, red)

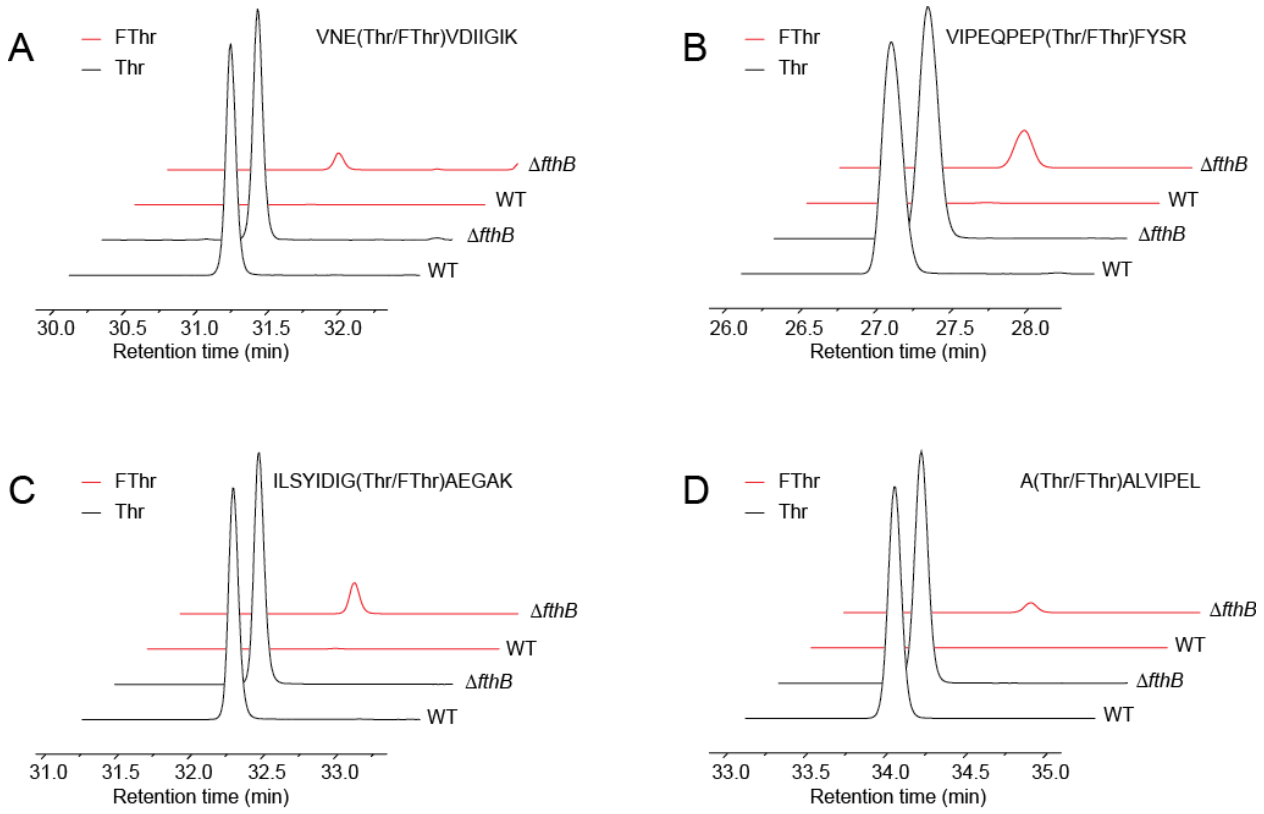


Table S2. Settings used for dMRM acquisition of targeted proteomics data. In every case, the fragmentor voltage was set to 130 V, while the retention time window was 2.5 minutes. Quantifier transitions are marked in bold. [+18] indicates substitution of Thr with FThr.

Protein	*Peptide*	*Precursor ion*	*Product ion*	*t_R (min)*	*Collision energy (V)*
FaDH	VLTGGER	366.2	418.2	9.7	12.4
FAIDH	**VLTGGER**	**366.2**	**519.3**	**9.7**	**12.4**
FAIDH	VLTGGER	366.2	632.3	9.7	12.4
FAIDH	VLT[+18.0]GGER	375.2	418.2	9.8	12.6
FAIDH	**VLT[+18.0]GGER**	**375.2**	**537.2**	**9.8**	**12.6**
FAIDH	VLT[+18.0]GGER	375.2	650.3	9.8	12.6
FAIDH	DLSTAYR	413.2	409.2	17.1	13.8
FAIDH	DLSTAYR	413.2	417.2	17.1	13.8
FAIDH	DLSTAYR	413.2	488.2	17.1	13.8
FAIDH	DLSTAYR	413.2	510.3	17.1	13.8
FAIDH	**DLSTAYR**	**413.2**	**597.3**	**17.1**	**13.8**
FAIDH	DLST[+18.0]AYR	422.2	409.2	17.3	14.1
FAIDH	DLST[+18.0]AYR	422.2	435.2	17.3	14.1
FAIDH	DLST[+18.0]AYR	422.2	506.2	17.3	14.1
FAIDH	DLST[+18.0]AYR	422.2	528.3	17.3	14.1
FAIDH	DLST[+18.0]AYR	422.2	615.3	17.3	14.1
FAIDH	**DLST[+18.0]AYR**	**422.2**	**728.4**	**17.3**	**14.1**
FAIDH	ILSYIDIGTAEGAK	725.9	275.2	33.2	23.5
FAIDH	ILSYIDIGTAEGAK	725.9	746.4	33.2	23.5
FAIDH	**ILSYIDIGTAEGAK**	**725.9**	**861.4**	**33.2**	**23.5**
FAIDH	ILSYIDIGTAEGAK	725.9	974.5	33.2	23.5
FAIDH	ILSYIDIGT[+18.0]AEGAK	734.9	275.2	33.4	23.8
FAIDH	ILSYIDIGT[+18.0]AEGAK	734.9	764.4	33.4	23.8
FAIDH	**ILSYIDIGT[+18.0]AEGAK**	**734.9**	**879.4**	**33.4**	**23.8**
FAIDH	ILSYIDIGT[+18.0]AEGAK	734.9	992.5	33.4	23.8
FAIDH	VDLGGSLSGGYYVAPTIFEGDNR	796.4	461.2	40.8	23.9
FAIDH	VDLGGSLSGGYYVAPTIFEGDNR	796.4	850.4	40.8	23.9
FAIDH	VDLGGSLSGGYYVAPTIFEGDNR	796.4	951.5	40.8	23.9
FAIDH	**VDLGGSLSGGYYVAPTIFEGDNR**	**796.4**	**1048.5**	**40.8**	**23.9**
FAIDH	VDLGGSLSGGYYVAPT[+18.0]IFEGDNR	802.4	461.2	41.0	24.1
FAIDH	VDLGGSLSGGYYVAPT[+18.0]IFEGDNR	802.4	850.4	41.0	24.1
FAIDH	VDLGGSLSGGYYVAPT[+18.0]IFEGDNR	802.4	969.4	41.0	24.1
FAIDH	**VDLGGSLSGGYYVAPT[+18.0]IFEGDNR**	**802.4**	**1066.5**	**41.0**	**24.1**
FAIDH	IFQEEIFGPVVSVTR	861.0	757.5	40.8	27.7
FAIDH	**IFQEEIFGPVVSVTR**	**861.0**	**961.5**	**40.8**	**27.7**
FAIDH	IFQEEIFGPVVSVTR	861.0	1074.6	40.8	27.7
FAIDH	IFQEEIFGPVVSVTR	861.0	1203.7	40.8	27.7

FAIDH	IFQEEIFGPVVSVT[+18.0]R	870.0	775.4	41.1	28.0
FAIDH	**IFQEEIFGPVVSVT[+18.0]R**	**870.0**	**979.5**	**41.1**	**28.0**
FAIDH	IFQEEIFGPVVSVT[+18.0]R	870.0	1092.6	41.1	28.0
FAIDH	IFQEEIFGPVVSVT[+18.0]R	870.0	1221.7	41.1	28.0
flA	**FFPEGTVFATTTYPATGTTTR**	**756.0**	**804.4**	**38.3**	**22.4**
flA	FFPEGTVFATTTYPATGTTTR	756.0	967.5	38.3	22.4
flA	FFPEGTVFATTTYPATGTTTR	756.0	1068.5	38.3	22.4
flA	FFPEGT[+18.0]VFATTTYPATGTTTR	762.0	804.4	38.5	22.6
flA	**FFPEGTVFATTTYPAT[+18.0]GTTTR**	**762.0**	**822.4**	**38.5**	**22.6**
flA	FFPEGTVFATTTYPATGT[+18.0]TTR	762.0	822.4	38.5	22.6
flA	FFPEGT[+18.0]VFATTTYPATGTTTR	762.0	967.5	38.5	22.6
flA	FFPEGTVFATTTYPAT[+18.0]GTTTR	762.0	985.5	38.5	22.6
flA	FFPEGTVFATTTYPATGT[+18.0]TTR	762.0	985.5	38.5	22.6
flA	FFPEGT[+18.0]VFATTTYPATGTTTR	762.0	1068.5	38.5	22.6
flA	FFPEGTVFATTTYPAT[+18.0]GTTTR	762.0	1086.5	38.5	22.6
flA	FFPEGTVFATTTYPATGT[+18.0]TTR	762.0	1086.5	38.5	22.6
flA	VIPEQPEPTFYSR	781.9	770.4	28.0	25.2
flA	VIPEQPEPTFYSR	781.9	899.4	28.0	25.2
flA	**VIPEQPEPTFYSR**	**781.9**	**996.5**	**28.0**	**25.2**
flA	VIPEQPEPTFYSR	781.9	1124.5	28.0	25.2
flA	VIPEQPEPTFYSR	781.9	1350.6	28.0	25.2
flA	VIPEQPEPT[+18.0]FYSR	790.9	788.4	28.2	25.5
flA	**VIPEQPEPT[+18.0]FYSR**	**790.9**	**1014.5**	**28.2**	**25.5**
flA	VIPEQPEPT[+18.0]FYSR	790.9	1142.5	28.2	25.5
flA	VIPEQPEPT[+18.0]FYSR	790.9	1368.6	28.2	25.5
GapA	**ATALVIPELK**	**527.8**	**486.3**	**35.1**	**17.4**
GapA	ATALVIPELK	527.8	599.4	35.1	17.4
GapA	ATALVIPELK	527.8	698.4	35.1	17.4
GapA	ATALVIPELK	527.8	811.5	35.1	17.4
GapA	**AT[+18.0]ALVIPELK**	**536.8**	**486.3**	**35.4**	**17.6**
GapA	AT[+18.0]ALVIPELK	536.8	599.4	35.4	17.6
GapA	AT[+18.0]ALVIPELK	536.8	698.4	35.4	17.6
GapA	AT[+18.0]ALVIPELK	536.8	811.5	35.4	17.6
GapA	LVDLTTFVGGR	589.3	636.3	37.0	19.3
GapA	**LVDLTTFVGGR**	**589.3**	**737.4**	**37.0**	**19.3**
GapA	LVDLTTFVGGR	589.3	850.5	37.0	19.3
GapA	LVDLT[+18.0]TFVGGR	598.3	636.3	36.6	19.5
GapA	LVDLTT[+18.0]FVGGR	598.3	654.3	36.7	19.5
GapA	**LVDLT[+18.0]TFVGGR**	**598.3**	**755.4**	**36.6**	**19.5**
GapA	LVDLT[+18.0]TFVGGR	598.3	868.5	36.6	19.5
GapA	**AAAENIIPTTTGAAK**	**714.9**	**746.4**	**25.1**	**23.2**
GapA	AAAENIIPTTTGAAK	714.9	859.5	25.1	23.2
GapA	AAAENIIPTTTGAAK	714.9	972.6	25.1	23.2
GapA	**AAAENIIPT[+18.0]TTGAAK**	**723.9**	**764.4**	**25.3**	**23.4**

GapA	AAAENIIPT[+18.0]TTGAAK	723.9	877.5	25.3	23.4
GapA	AAAENIIPT[+18.0]TTGAAK	723.9	990.6	25.3	23.4
TufA	TTLTAAITK	460.3	503.3	22.6	15.3
TufA	TTLTAAITK	460.3	604.4	22.6	15.3
TufA	**TTLTAAITK**	**460.3**	**717.5**	**22.6**	**15.3**
TufA	TT[+18.0]LTAAITK	469.3	503.3	22.5	15.5
TufA	TTLT[+18.0]AAITK	469.3	503.3	22.8	15.5
TufA	TTLTAAIT[+18.0]K	469.3	521.3	22.8	15.5
TufA	TT[+18.0]LTAAITK	469.3	604.4	22.5	15.5
TufA	TTLT[+18.0]AAITK	469.3	622.4	22.8	15.5
TufA	TT[+18.0]LTAAITK	469.3	717.5	22.5	15.5
TufA	**TTLT[+18.0]AAITK**	**469.3**	**735.4**	**22.8**	**15.5**
TufA	VNETVDIIGIK	600.8	658.4	32.1	19.6
TufA	VNETVDIIGIK	600.8	757.5	32.1	19.6
TufA	**VNETVDIIGIK**	**600.8**	**858.5**	**32.1**	**19.6**
TufA	VNET[+18.0]VDIIGIK	609.8	658.4	32.3	19.9
TufA	VNET[+18.0]VDIIGIK	609.8	757.5	32.3	19.9
TufA	**VNET[+18.0]VDIIGIK**	**609.8**	**876.5**	**32.3**	**19.9**
TufA	LLGLMHTIDEAIPTPQR	635.7	400.2	39.1	18.1
TufA	**LLGLMHTIDEAIPTPQR**	**635.7**	**598.3**	**39.1**	**18.1**
TufA	LLGLMHTIDEAIPTPQR	635.7	711.4	39.1	18.1
TufA	LLGLMHT[+18.0]IDEAIPTPQR	641.7	400.2	38.7	18.3
TufA	**LLGLMHT[+18.0]IDEAIPTPQR**	**641.7**	**598.3**	**38.7**	**18.3**
TufA	LLGLMHTIDEAIPT[+18.0]PQR	641.7	616.3	38.7	18.3
TufA	LLGLMHT[+18.0]IDEAIPTPQR	641.7	711.4	38.7	18.3
TufA	LLGLMHTIDEAIPT[+18.0]PQR	641.7	729.4	38.7	18.3

Figure S13: High-resolution MS/MS spectra showing fluorothreonine in peptides from *S. cattleya*, as identified by MS/MS database search. Spectra above the axis gave Peptide-Spectrum Matches (PSMs) to sequences with FThr substituted for Thr. The spectra below the axis are from the corresponding Thr-containing peptide. (A) Peptide from the putative cold shock protein (SCAT_3469). (B) Peptide from the fluoroacetaldehyde dehydrogenase (SCAT_0945). (C) Peptide from the putative superoxide dismutase (SCAT_p0640).

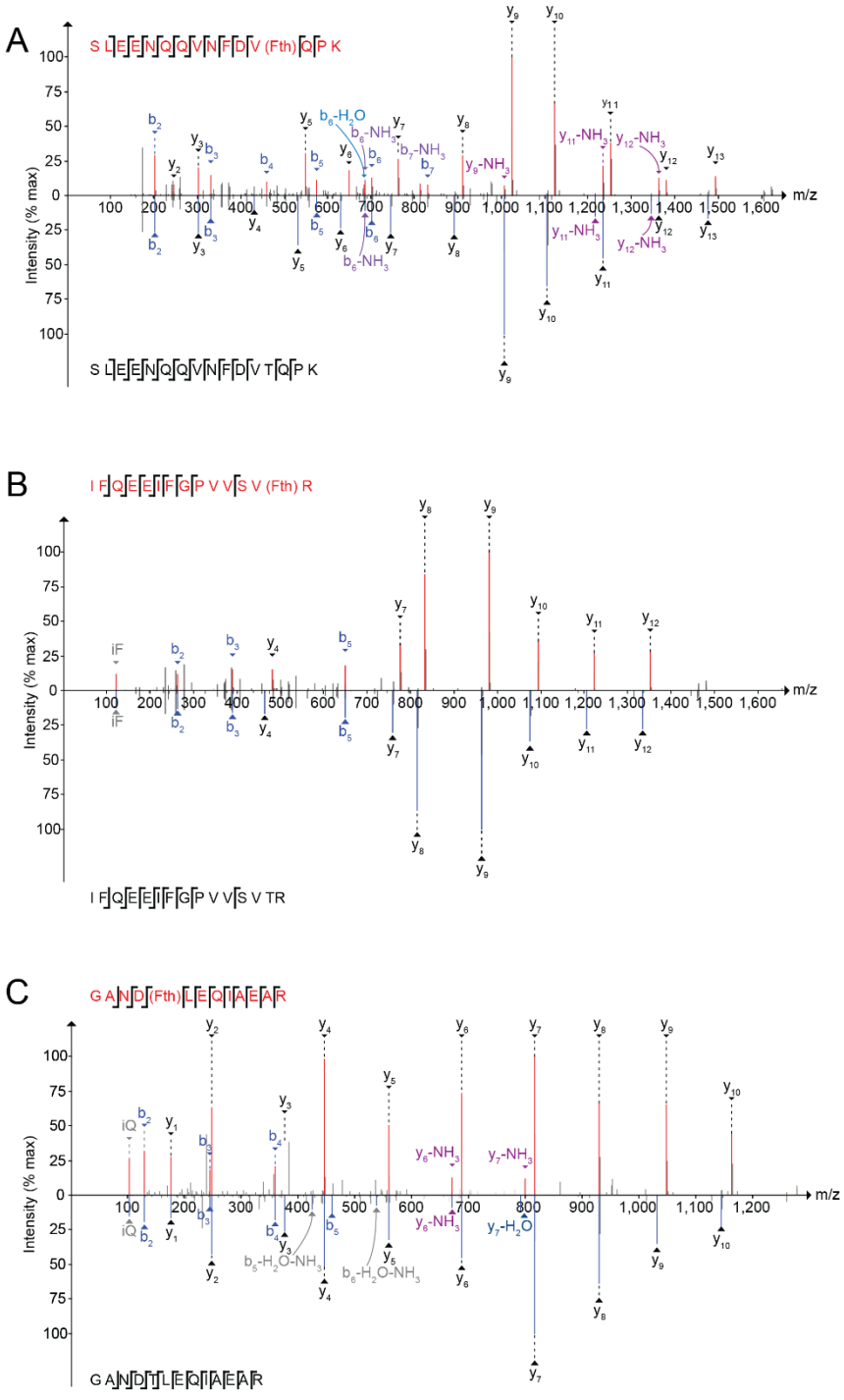


Table S3. Distribution of Peptide Spectrum Matches conditioned by threonine codon usage and presence of threonine or fluorothreonine. Frequency (left) and raw counts (right) are displayed. Only hits to peptides containing a single threonine are tabulated to eliminate ambiguity in codon usage. Peptide-spectrum matches are cumulative over four samples of *S. cattleya* Δ*fthB*; the WT strain did not afford enough FThr-containing PSMs to assemble a meaningful dataset.

Amino acid	ACA		ACC		ACG		ACT	
FThr	1.7%	45	83.6%	2184	14.0%	365	0.7%	17
Thr	1.1%	337	83.3%	25772	15.3%	4725	0.4%	121

Figure S14. Histograms representing estimated incorporation of fluorothreonine into peptides as measured by precursor ion intensity from shotgun proteomics datasets. PSMs corresponding to single-Thr encoding peptides with at least one FThr-containing PSM were analyzed.

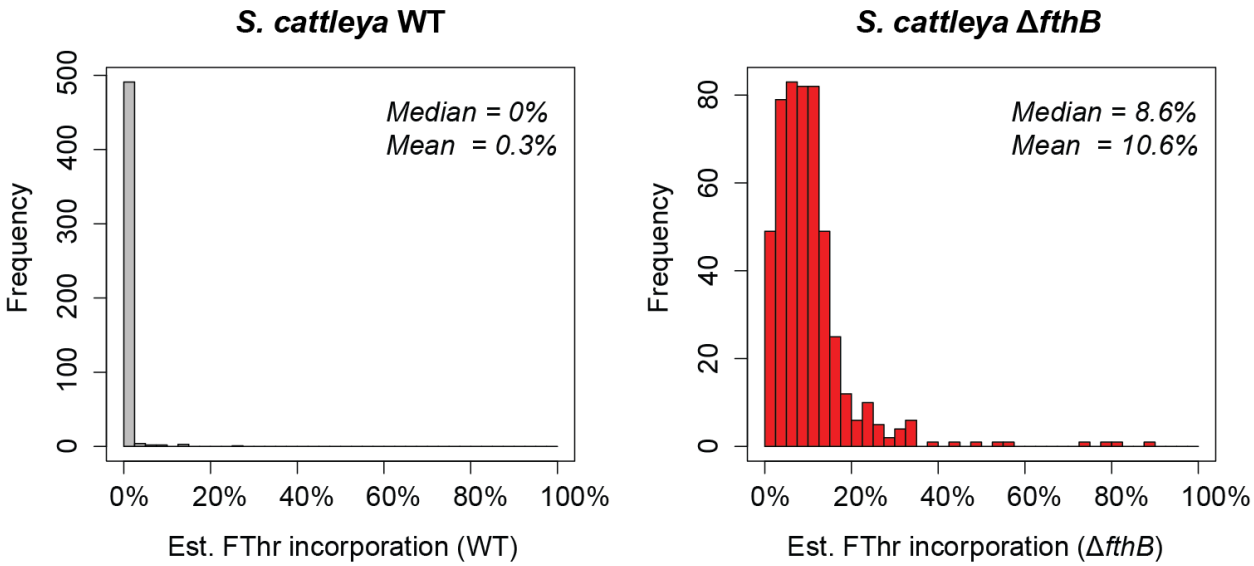


Table S4. Over-and under representation of fluorothreonine in shotgun proteomics data at (A) the protein level and (B) the peptide level, as determined by MSstats processing of intensity data from MaxQuant. Positive log_2FC values indicate greater-than-expected fluorothreonine content, while negative values indicate lower-than-expected fluorothreonine incorporation.

A

ID	log_2FC	p-Value	Adjusted p-value	Description
embl-cds:CCB72001	4.4	3.5E-02	1.8E-01	putative thiol peroxidase
embl-cds:CCB72272	2.3	1.1E-01	2.9E-01	putative D-alanyl-D-alanine carboxypeptidase
embl-cds:CCB76874	2.3	1.2E-01	3.0E-01	transcriptional terminator Rho (fragment)
embl-cds:CCB77194	1.9	5.7E-02	2.3E-01	beta-N-acetylhexosaminidase
embl-cds:CCB73659	1.8	1.3E-02	1.4E-01	putative membrane protease subunit, stomatin/prohibitin homolog
embl-cds:CCB73698	1.7	4.6E-04	5.7E-02	L-alanine dehydrogenase
embl-cds:CCB73807	1.7	6.8E-02	2.4E-01	FeS cluster formation protein
embl-cds:CCB76653	1.5	3.6E-01	5.1E-01	putative succinate dehydrogenase iron-sulfur protein (Complex II)
embl-cds:CCB77531	1.5	3.3E-03	1.1E-01	putative aminotransferase
embl-cds:CCB71186	1.4	4.0E-02	1.8E-01	protein of unknown function
embl-cds:CCB77453	1.3	6.4E-02	2.4E-01	acyl-CoA synthetase family member 2, mitochondrial
embl-cds:CCB78369	1.3	2.7E-02	1.6E-01	3'-hydroxymethylcephem-O-carbamoyltransferase
embl-cds:CCB73801	1.2	4.6E-01	6.1E-01	conserved protein of unknown function
embl-cds:CCB76925	1.1	1.6E-02	1.5E-01	putative isobutyryl-CoA mutase, chain A
embl-cds:CCB78318	1.1	1.2E-01	3.0E-01	cytochrome P450 107B1
embl-cds:CCB74577	1	3.5E-01	5.1E-01	acyl-CoA dehydrogenase, short-chain specific
embl-cds:CCB73553	0.9	3.3E-03	1.1E-01	oxidoreductase
embl-cds:CCB74188	0.9	3.0E-01	4.7E-01	O-methyltransferase mdmC
embl-cds:CCB74213	0.9	6.1E-02	2.3E-01	acyl carrier protein
embl-cds:CCB75156	0.9	3.6E-02	1.8E-01	chaperonin large subunit
embl-cds:CCB75716	0.9	8.8E-03	1.3E-01	Xaa-Pro aminopeptidase 1
embl-cds:CCB75748	0.9	1.9E-02	1.5E-01	conserved protein of unknown function
embl-cds:CCB77988	0.9	9.6E-02	2.6E-01	glutamine amidotransferase for pyridoxal phosphate synthesis
embl-cds:CCB78371	0.9	1.6E-01	3.5E-01	N-(5-amino-5-carboxypentanoyl)-L-cysteinyl-D-valine synthase
embl-cds:CCB77171	0.8	1.5E-03	9.4E-02	aldehyde dehydrogenase
embl-cds:CCB77760	0.8	2.6E-01	4.4E-01	conserved protein of unknown function
embl-cds:CCB71397	0.7	3.7E-02	1.8E-01	putative amidotransferase
embl-cds:CCB71592	0.7	1.4E-01	3.3E-01	conserved protein of unknown function
embl-cds:CCB72069	0.7	6.1E-02	2.3E-01	conserved protein of unknown function
embl-cds:CCB76262	0.7	7.9E-02	2.5E-01	DNA-directed RNA polymerase subunit beta
embl-cds:CCB76489	0.7	9.6E-02	2.6E-01	putative adenosine deaminase 5
embl-cds:CCB76604	0.7	1.6E-01	3.5E-01	putative methyltransferase

embl-cds:CCB76610	0.7	2.9E-02	1.6E-01	putative fructose 1,6-bisphosphatase class II
embl-cds:CCB77173	0.7	6.5E-02	2.4E-01	conserved protein of unknown function
embl-cds:CCB71860	0.6	3.0E-01	4.7E-01	putative oxidoreductase
embl-cds:CCB71908	0.6	1.5E-01	3.4E-01	ferredoxin-2
embl-cds:CCB72073	0.6	2.7E-01	4.4E-01	conserved protein of unknown function
embl-cds:CCB72473	0.6	1.1E-01	3.0E-01	dihydrodipicolinate synthase
embl-cds:CCB74007	0.6	9.1E-03	1.3E-01	conserved protein of unknown function
embl-cds:CCB74113	0.6	3.6E-02	1.8E-01	UfaA2 protein
embl-cds:CCB75168	0.6	3.4E-01	4.9E-01	putative N-acetylglucosamine-6-phosphate deacetylase
embl-cds:CCB72323	0.5	3.1E-01	4.7E-01	biotin synthase
embl-cds:CCB73111	0.5	5.2E-02	2.2E-01	conserved protein of unknown function
embl-cds:CCB73675	0.5	8.8E-02	2.6E-01	putative aldehyde dehydrogenase
embl-cds:CCB75834	0.5	2.2E-01	4.0E-01	thioredoxin reductase
embl-cds:CCB72149	0.4	7.2E-01	7.9E-01	18 kDa antigen 2
embl-cds:CCB73838	0.4	1.1E-01	3.0E-01	tellurium resistance protein TerZ
embl-cds:CCB73850	0.4	2.2E-01	4.1E-01	30S ribosomal protein S1
embl-cds:CCB73923	0.4	2.6E-01	4.4E-01	UDP-N-acetylmuramoylalanine--D-glutamate ligase
embl-cds:CCB74160	0.4	2.7E-01	4.4E-01	DNA-binding protein
embl-cds:CCB75751	0.4	1.2E-01	3.1E-01	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex
embl-cds:CCB76632	0.4	4.6E-01	6.1E-01	conserved protein of unknown function
embl-cds:CCB77049	0.4	7.0E-02	2.4E-01	putative delta-1-pyrroline-5-carboxylate dehydrogenase
embl-cds:CCB77555	0.4	2.0E-01	3.9E-01	conserved protein of unknown function
embl-cds:CCB77791	0.4	2.9E-01	4.6E-01	putative fatty acid oxidation complex alpha-subunit
embl-cds:CCB77865	0.4	9.1E-02	2.6E-01	conserved protein of unknown function
embl-cds:CCB72967	0.3	5.1E-03	1.2E-01	conserved exported protein of unknown function
embl-cds:CCB74298	0.3	5.3E-01	6.7E-01	extracellular metalloprotease
embl-cds:CCB74582	0.3	2.7E-01	4.4E-01	oligoribonuclease
embl-cds:CCB74741	0.3	5.0E-01	6.4E-01	adenosylhomocysteinase
embl-cds:CCB76631	0.3	2.0E-01	3.9E-01	aminoglycoside phosphotransferase
embl-cds:CCB76857	0.3	1.5E-01	3.3E-01	adenosylhomocysteine hydrolase
embl-cds:CCB77092	0.3	3.1E-01	4.7E-01	conserved protein of unknown function
embl-cds:CCB77214	0.3	2.0E-01	3.9E-01	regulatory protein
embl-cds:CCB77233	0.3	4.2E-01	5.7E-01	multifunctional S
embl-cds:CCB77532	0.3	1.7E-01	3.6E-01	D-hydantoinase
embl-cds:CCB77884	0.3	2.9E-02	1.6E-01	betaine aldehyde dehydrogenase
embl-cds:CCB71707	0.2	7.2E-01	7.9E-01	protein of unknown function
embl-cds:CCB73420	0.2	5.0E-01	6.4E-01	3-oxoacyl-[acyl-carrier-protein] reductase 1
embl-cds:CCB73502	0.2	6.6E-01	7.6E-01	PTS-dependent dihydroxyacetone kinase, dihydroxyacetone-binding subunit dhaK
embl-cds:CCB73674	0.2	4.9E-01	6.4E-01	conserved protein of unknown function
embl-cds:CCB74112	0.2	1.6E-01	3.5E-01	putative zinc-binding dehydrogenase

embl-cds:CCB74676	0.2	3.0E-01	4.7E-01	4-hydroxyphenylpyruvate dioxygenase
embl-cds:CCB75548	0.2	3.3E-01	4.9E-01	protein ArsC
embl-cds:CCB76011	0.2	2.0E-01	3.9E-01	protein GrpE
embl-cds:CCB76186	0.2	7.2E-02	2.4E-01	NADH-quinone oxidoreductase subunit G
embl-cds:CCB76654	0.2	1.3E-01	3.1E-01	succinate dehydrogenase flavoprotein subunit
embl-cds:CCB76674	0.2	1.3E-01	3.1E-01	putative peptide ABC transporter solute-binding protein
embl-cds:CCB76816	0.2	5.5E-01	6.8E-01	conserved protein of unknown function
embl-cds:CCB77212	0.2	6.1E-01	7.3E-01	hydrolase of the metallo-beta-lactamase superfamily
embl-cds:CCB77423	0.2	4.2E-01	5.7E-01	protein AidB
embl-cds:CCB77621	0.2	6.3E-01	7.4E-01	nucleotide sugar-1-phosphate transferase
embl-cds:CCB78036	0.2	5.4E-01	6.7E-01	S-adenosylmethionine synthetase
embl-cds:CCB78368	0.2	8.0E-01	8.5E-01	deacetoxycephalosporin C hydroxylase
embl-cds:CCB71862	0.1	7.7E-01	8.3E-01	oxygenase
embl-cds:CCB73453	0.1	7.3E-01	8.0E-01	putative mannose-1-phosphate guanyltransferase
embl-cds:CCB73911	0.1	7.3E-01	8.0E-01	putative DNA-binding protein
embl-cds:CCB74462	0.1	5.8E-01	7.0E-01	trigger factor
embl-cds:CCB74718	0.1	5.0E-01	6.4E-01	putative multiple sugar ABC transporter solute-binding protein
embl-cds:CCB76165	0.1	5.6E-01	6.8E-01	cold-shock protein
embl-cds:CCB76263	0.1	6.2E-01	7.4E-01	RNA polymerase (beta' subunit)
embl-cds:CCB76311	0.1	7.6E-01	8.3E-01	initiation factor IF-I
embl-cds:CCB76315	0.1	6.5E-01	7.5E-01	RNA polymerase (alpha subunit)
embl-cds:CCB76322	0.1	7.2E-01	7.9E-01	30S ribosomal protein S9
embl-cds:CCB76567	0.1	6.7E-01	7.7E-01	conserved protein of unknown function
embl-cds:CCB76815	0.1	8.2E-01	8.5E-01	conserved protein of unknown function
embl-cds:CCB77858	0.1	8.2E-01	8.5E-01	conserved protein of unknown function
embl-cds:CCB73758	0	9.0E-01	9.1E-01	putative transcriptional regulator
embl-cds:CCB75653	0	8.6E-01	8.8E-01	DNA binding protein
embl-cds:CCB76257	0	9.5E-01	9.6E-01	ribosomal protein L11 (BL11)
embl-cds:CCB76309	0	9.9E-01	9.9E-01	adenylate kinase
embl-cds:CCB76861	0	7.0E-01	7.9E-01	5'-fluoro-5'-deoxyadenosine synthase (fluorinase)
embl-cds:CCB76862	0	8.7E-01	8.8E-01	5'-fluoro-5'-deoxy-adenosine phosphorylase (PNPase)
embl-cds:CCB77813	0	8.6E-01	8.8E-01	conserved protein of unknown function
embl-cds:CCB78391	0	8.0E-01	8.5E-01	short-chain alcohol dehydrogenase
embl-cds:CCB72072	-0.1	7.0E-01	7.9E-01	ArpA protein
embl-cds:CCB73414	-0.1	8.7E-01	8.8E-01	mini-circle uncharacterized 19.1 kDa protein
embl-cds:CCB73482	-0.1	8.8E-01	8.9E-01	conserved protein of unknown function
embl-cds:CCB74150	-0.1	7.8E-01	8.4E-01	conserved protein of unknown function
embl-cds:CCB74268	-0.1	7.9E-01	8.4E-01	conserved protein of unknown function
embl-cds:CCB74799	-0.1	8.2E-01	8.5E-01	urocanase
embl-cds:CCB75075	-0.1	8.2E-01	8.5E-01	OsmC-like protein

embl-cds:CCB75190	-0.1	8.3E-01	8.6E-01	multiple sugar-binding transporter ATP-binding protein
embl-cds:CCB75769	-0.1	5.8E-01	7.0E-01	dehydrogenase
embl-cds:CCB76345	-0.1	4.3E-01	5.8E-01	chaperonin large subunit
embl-cds:CCB76909	-0.1	7.0E-01	7.9E-01	cellulose-binding protein
embl-cds:CCB77885	-0.1	8.1E-01	8.5E-01	3-oxoacyl-[acyl-carrier-protein] reductase
embl-cds:CCB77983	-0.1	7.1E-01	7.9E-01	elongation factor G-like protein
embl-cds:CCB71405	-0.2	9.3E-02	2.6E-01	putative phage tail sheath protein
embl-cds:CCB71588	-0.2	3.9E-01	5.4E-01	4-fluorothreonine transaldolase
embl-cds:CCB71733	-0.2	6.8E-01	7.8E-01	conserved protein of unknown function
embl-cds:CCB72047	-0.2	4.2E-01	5.7E-01	alpha-mannosidase
embl-cds:CCB74469	-0.2	3.1E-01	4.7E-01	ribose-5-phosphate isomerase B
embl-cds:CCB74747	-0.2	2.0E-02	1.5E-01	phosphomannomutase
embl-cds:CCB75578	-0.2	5.4E-01	6.7E-01	acetyl-coenzyme A synthetase
embl-cds:CCB75911	-0.2	5.7E-01	7.0E-01	conserved exported protein of unknown function
embl-cds:CCB76293	-0.2	7.1E-01	7.9E-01	ribosomal protein L22 (BL17)
embl-cds:CCB76687	-0.2	4.1E-01	5.7E-01	ferredoxin
embl-cds:CCB76819	-0.2	2.0E-01	3.9E-01	ATP/GTP binding protein
embl-cds:CCB77061	-0.2	2.6E-01	4.4E-01	citrate lyase beta subunit
embl-cds:CCB77306	-0.2	6.6E-01	7.6E-01	phosphocarrier protein HPr
embl-cds:CCB77533	-0.2	2.9E-01	4.6E-01	N5,N10-methylene
embl-cds:CCB78000	-0.2	4.8E-01	6.3E-01	conserved protein of unknown function
embl-cds:CCB71452	-0.3	1.7E-01	3.6E-01	catalase-peroxidase
embl-cds:CCB72320	-0.3	3.1E-01	4.7E-01	thioesterase superfamily protein
embl-cds:CCB73177	-0.3	2.6E-01	4.4E-01	amidohydrolase 2
embl-cds:CCB73628	-0.3	5.0E-01	6.4E-01	quinone oxidoreductase-like protein 2 homolog
embl-cds:CCB74199	-0.3	4.8E-01	6.3E-01	putative Peroxiredoxin
embl-cds:CCB74201	-0.3	2.1E-01	3.9E-01	pyruvate dehydrogenase E1 component
embl-cds:CCB74457	-0.3	7.4E-01	8.1E-01	valyl-tRNA synthetase
embl-cds:CCB74766	-0.3	1.8E-01	3.7E-01	carboxyvinyl-carboxyphosphonate phosphorylmutase
embl-cds:CCB76266	-0.3	4.4E-01	5.9E-01	elongation factor G
embl-cds:CCB76307	-0.3	2.3E-01	4.3E-01	ribosomal protein L15
embl-cds:CCB76697	-0.3	2.4E-01	4.3E-01	putative enoyl-CoA hydratase
embl-cds:CCB76891	-0.3	3.5E-02	1.8E-01	ATP synthase (subunit beta, component F1)
embl-cds:CCB76961	-0.3	8.3E-02	2.6E-01	putative enoyl-CoA hydratase echA8
embl-cds:CCB77035	-0.3	5.3E-01	6.6E-01	acetohydroxy-acid isomeroreductase
embl-cds:CCB77148	-0.3	3.3E-01	4.9E-01	ribosome recycling factor
embl-cds:CCB77344	-0.3	4.1E-01	5.7E-01	putative hydrolase
embl-cds:CCB77481	-0.3	3.6E-01	5.1E-01	putative glutathione peroxidase
embl-cds:CCB77530	-0.3	6.3E-02	2.4E-01	N-carbamoylputrescine amidase
embl-cds:CCB77883	-0.3	5.4E-01	6.7E-01	putative glutamine synthetase
embl-cds:CCB73862	-0.4	6.4E-01	7.5E-01	nucleotidase

embl-cds:CCB74023	-0.4	2.7E-01	4.4E-01	branched-chain alpha-keto acid dehydrogenase E3 subunit (dihydrolipoamide dehydrogenase)
embl-cds:CCB75045	-0.4	4.0E-01	5.5E-01	DNA-binding protein
embl-cds:CCB75259	-0.4	6.3E-01	7.5E-01	putative thiosulfate sulfurtransferase
embl-cds:CCB75804	-0.4	6.5E-01	7.5E-01	putative nosiheptide resistance regulatory protein
embl-cds:CCB76010	-0.4	4.0E-01	5.5E-01	molecular chaperone
embl-cds:CCB76267	-0.4	7.4E-02	2.4E-01	elongation factor Tu
embl-cds:CCB76399	-0.4	2.5E-01	4.4E-01	succinyl-CoA synthetase (alpha subunit)
embl-cds:CCB76448	-0.4	2.8E-02	1.6E-01	ornithine aminotransferase
embl-cds:CCB76889	-0.4	3.3E-01	4.9E-01	ATP synthase (subunit alpha, component F1)
embl-cds:CCB78023	-0.4	2.1E-01	3.9E-01	putative transcriptional regulator
embl-cds:CCB73264	-0.5	3.5E-01	5.1E-01	conserved protein of unknown function
embl-cds:CCB73825	-0.5	1.9E-01	3.8E-01	glyceraldehyde-3-phosphate dehydrogenase
embl-cds:CCB74675	-0.5	2.6E-01	4.4E-01	glycolate oxidase subunit glcD
embl-cds:CCB75205	-0.5	8.9E-02	2.6E-01	putative lipoprotein
embl-cds:CCB76258	-0.5	3.1E-02	1.7E-01	ribosomal protein L1 (BL1)
embl-cds:CCB76288	-0.5	3.9E-02	1.8E-01	ribosomal protein L3 (BL3)
embl-cds:CCB76406	-0.5	3.1E-01	4.7E-01	bifunctional protein folD [Includes: methylenetetrahydrofolate dehydrogenase ; methenyltetrahydrofolate cyclohydrolase]
embl-cds:CCB77168	-0.5	6.1E-01	7.3E-01	4-aminobutyrate aminotransferase
embl-cds:CCB71406	-0.6	6.9E-02	2.4E-01	conserved protein of unknown function
embl-cds:CCB71666	-0.6	2.1E-02	1.5E-01	superoxide dismutase [Fe-Zn] 1
embl-cds:CCB71865	-0.6	1.1E-02	1.3E-01	hydrolase
embl-cds:CCB72474	-0.6	3.1E-01	4.7E-01	conserved protein of unknown function
embl-cds:CCB73714	-0.6	4.2E-02	1.8E-01	putative UPF0098 class protein
embl-cds:CCB73816	-0.6	2.3E-01	4.2E-01	transketolase
embl-cds:CCB74474	-0.6	3.4E-03	1.1E-01	conserved protein of unknown function
embl-cds:CCB75372	-0.6	2.6E-01	4.4E-01	protein of unknown function
embl-cds:CCB77215	-0.6	3.0E-01	4.7E-01	sensor protein
embl-cds:CCB77595	-0.6	9.4E-03	1.3E-01	exported protein of unknown function
embl-cds:CCB77861	-0.6	8.4E-02	2.6E-01	20S proteasome beta-subunit
embl-cds:CCB78370	-0.6	2.9E-01	4.6E-01	isopenicillin N synthetase
embl-cds:CCB78452	-0.6	1.3E-01	3.2E-01	3-demethylubiquinone-9 3-methyltransferase
embl-cds:CCB72210	-0.7	6.9E-02	2.4E-01	non-hemolytic phospholipase C
embl-cds:CCB72264	-0.7	1.5E-01	3.4E-01	glycogen operon protein glgX homolog
embl-cds:CCB74224	-0.7	4.1E-02	1.8E-01	organic hydroperoxide resistance reductase B
embl-cds:CCB75365	-0.7	1.5E-01	3.4E-01	conserved protein of unknown function
embl-cds:CCB75802	-0.7	1.0E-02	1.3E-01	peptidyl-prolyl cis-trans isomerase B
embl-cds:CCB75849	-0.7	2.6E-01	4.4E-01	30S ribosomal protein S6
embl-cds:CCB76025	-0.7	2.2E-02	1.5E-01	fructose-bisphosphate aldolase
embl-cds:CCB76291	-0.7	7.2E-02	2.4E-01	ribosomal protein L2 (BL2)
embl-cds:CCB76316	-0.7	4.3E-02	1.8E-01	50S ribosomal protein L17

embl-cds:CCB76519	-0.7	1.6E-01	3.5E-01	putative enoyl-CoA dehydratase
embl-cds:CCB71775	-0.8	8.5E-02	2.6E-01	conserved protein of unknown function
embl-cds:CCB73009	-0.8	8.7E-02	2.6E-01	putative secreted protein
embl-cds:CCB75307	-0.8	4.5E-02	1.9E-01	dihydroxy-acid dehydratase
embl-cds:CCB76913	-0.8	2.6E-01	4.4E-01	degradative acetoacetyl-CoA thiolase
embl-cds:CCB77525	-0.8	6.7E-02	2.4E-01	putative gamma-glutamyltranspeptidase
embl-cds:CCB78341	-0.8	1.4E-01	3.3E-01	putative ThiJ/PfpI family protein
embl-cds:CCB71078	-0.9	9.1E-02	2.6E-01	NAD-dependent epimerase/dehydratase
embl-cds:CCB72071	-0.9	1.2E-01	3.1E-01	protein of unknown function
embl-cds:CCB72162	-0.9	2.0E-01	3.9E-01	putative polyketide oxygenase/hydroxylase
embl-cds:CCB73408	-0.9	3.5E-01	5.0E-01	beta-ketoadipyl CoA thiolase
embl-cds:CCB73547	-0.9	2.1E-02	1.5E-01	electron transfer flavoprotein (alpha subunit)
embl-cds:CCB73649	-0.9	1.3E-02	1.4E-01	homogentisate 1,2-dioxygenase
embl-cds:CCB74824	-0.9	2.0E-02	1.5E-01	enolase
embl-cds:CCB75589	-0.9	2.6E-01	4.4E-01	conserved protein of unknown function
embl-cds:CCB76477	-0.9	2.6E-01	4.4E-01	lipoprotein
embl-cds:CCB77159	-0.9	1.7E-01	3.6E-01	putative adenosine deaminase 1
embl-cds:CCB77204	-0.9	5.6E-02	2.3E-01	polynucleotide phosphorylase (PNPase)
embl-cds:CCB74164	-1	1.5E-01	3.3E-01	putative cytochrome P450 125
embl-cds:CCB76820	-1	1.3E-01	3.1E-01	2-oxoglutarate decarboxylase
embl-cds:CCB77051	-1	1.9E-01	3.8E-01	putative branched-chain-amino-acid aminotransferase
embl-cds:CCB77457	-1	9.1E-02	2.6E-01	acetyl-coenzyme A synthetase
embl-cds:CCB77809	-1	5.0E-01	6.4E-01	conserved protein of unknown function
embl-cds:CCB77898	-1	2.6E-02	1.6E-01	phenylalanyl-tRNA synthetase beta chain
embl-cds:CCB73477	-1.1	2.6E-02	1.6E-01	conserved exported protein of unknown function
embl-cds:CCB74477	-1.1	2.3E-02	1.5E-01	aminopeptidase N
embl-cds:CCB76410	-1.1	7.2E-02	2.4E-01	malate dehydrogenase
embl-cds:CCB77359	-1.1	1.4E-03	9.4E-02	aconitate hydratase (aconitase)
embl-cds:CCB77913	-1.1	1.7E-01	3.6E-01	glyceraldehyde-3-phosphate dehydrogenase
embl-cds:CCB71752	-1.2	1.1E-04	2.6E-02	protein KorA
embl-cds:CCB72125	-1.2	7.3E-02	2.4E-01	isocitrate dehydrogenase [NADP]
embl-cds:CCB72579	-1.2	4.2E-02	1.8E-01	YciI-related
embl-cds:CCB72036	-1.3	1.3E-01	3.1E-01	putative peptidase inhibitor
embl-cds:CCB72139	-1.3	1.1E-02	1.3E-01	conserved exported protein of unknown function
embl-cds:CCB75954	-1.3	2.1E-02	1.5E-01	exported protein of unknown function
embl-cds:CCB71784	-1.4	1.6E-02	1.5E-01	putative hydrolase
embl-cds:CCB73823	-1.4	1.1E-02	1.3E-01	triose phosphate isomerase
embl-cds:CCB74454	-1.4	1.9E-02	1.5E-01	nucleoside diphosphate kinase
embl-cds:CCB75819	-1.4	6.5E-03	1.2E-01	conserved protein of unknown function
embl-cds:CCB72337	-1.5	2.6E-01	4.4E-01	conserved exported protein of unknown function
embl-cds:CCB73800	-1.5	2.0E-01	3.9E-01	2,3,4,5-tetrahydropyridine-2

embl-cds:CCB77145	-1.5	3.0E-01	4.7E-01	ribosomal protein S2
embl-cds:CCB72250	-1.6	1.5E-02	1.5E-01	nocardamine synthetase
embl-cds:CCB75567	-1.6	4.8E-03	1.2E-01	anti-sigma-B factor antagonist (fragment)
embl-cds:CCB76511	-1.6	1.0E-01	2.8E-01	acetyl-/propionyl-coenzyme A carboxylase alpha chain
embl-cds:CCB76855	-1.6	5.7E-03	1.2E-01	fluoroacetyl coenzyme A thioesterase
embl-cds:CCB77845	-1.6	5.8E-02	2.3E-01	solute binding transport lipoprotein
embl-cds:CCB74055	-1.7	2.6E-02	1.6E-01	extracellular solute-binding protein family 1
embl-cds:CCB71663	-1.8	2.9E-03	1.1E-01	exported protein of unknown function
embl-cds:CCB77005	-1.8	1.9E-02	1.5E-01	putative metallopeptidase, secreted
embl-cds:CCB78006	-2	9.2E-02	2.6E-01	conserved protein of unknown function
embl-cds:CCB74619	-2.3	2.3E-02	1.5E-01	putative metallopeptidase, secreted
embl-cds:CCB77364	-2.3	4.0E-02	1.8E-01	solute-binding protein
embl-cds:CCB71463	-2.4	1.3E-01	3.2E-01	conserved protein of unknown function
embl-cds:CCB75283	-2.7	1.1E-02	1.3E-01	phosphate-binding protein pstS 3
embl-cds:CCB75991	-2.8	6.3E-03	1.2E-01	putative transcriptional regulator, TetR family
embl-cds:CCB76720	-3.1	2.8E-02	1.6E-01	ATP-binding protein

B

Peptide	Protein	log_2FC	p-Value	Adjusted p-value
VVNIFPSVDTGVCAASVR	embl-cds:CCB72001	4.4	3.5E-02	1.7E-01
EDLITGLIEAR	embl-cds:CCB78318	2.8	4.5E-02	1.9E-01
LVELGHDVVVLLDSITR	embl-cds:CCB76874	2.3	1.2E-01	3.0E-01
VAITPAGVHELVR	embl-cds:CCB73698	2.3	1.4E-03	9.4E-02
VGELTAGGGMGEVR	embl-cds:CCB72272	2.3	1.1E-01	2.9E-01
PAGLFHGVQTLR	embl-cds:CCB77194	1.9	5.7E-02	2.1E-01
SLAQTLVPAER	embl-cds:CCB77525	1.8	2.1E-01	4.0E-01
FAVLQVAQTSLR	embl-cds:CCB73659	1.8	1.3E-02	1.4E-01
INTENMGQFER	embl-cds:CCB73807	1.7	6.8E-02	2.3E-01
AHSVDSSDAAFQTAGALALR	embl-cds:CCB77983	1.6	2.4E-01	4.3E-01
APIELTHGEGR	embl-cds:CCB77531	1.5	3.3E-03	9.4E-02
DLPDSLVATPDSK	embl-cds:CCB76674	1.5	1.4E-02	1.4E-01
DLVTDVSFNYAK	embl-cds:CCB76653	1.5	3.5E-01	5.1E-01
AWLEQTQSEVR	embl-cds:CCB73850	1.4	3.1E-03	9.4E-02
TEGADEAMPVPGK	embl-cds:CCB71186	1.4	4.0E-02	1.8E-01
VEAALESGQDVPTK	embl-cds:CCB76262	1.3	2.3E-03	9.4E-02
ELLAAGETVPADR	embl-cds:CCB77453	1.3	6.4E-02	2.2E-01
SLLAEPFGAATR	embl-cds:CCB78369	1.3	2.7E-02	1.7E-01
SNDTDYPFR	embl-cds:CCB75716	1.2	9.3E-03	1.3E-01
NPEGPALIFTPAEWR	embl-cds:CCB73482	1.2	5.3E-01	6.7E-01

SATEGIVEDLK	embl-cds:CCB73801	1.2	4.6E-01	6.1E-01
TVVSNAFELEK	embl-cds:CCB73698	1.2	3.6E-02	1.7E-01
AQADTVDELGR	embl-cds:CCB77092	1.2	9.9E-02	2.7E-01
AAHHVTVEGHGR	embl-cds:CCB78371	1.1	1.7E-02	1.5E-01
STGAVVGQQPFGGGR	embl-cds:CCB77049	1.1	5.5E-03	1.0E-01
VLSTDDLVR	embl-cds:CCB76610	1.1	3.7E-02	1.7E-01
QFAGFGNAEQTNER	embl-cds:CCB76925	1.1	1.6E-02	1.5E-01
IFQEEIFGPVVSVTR	embl-cds:CCB73675	1.0	5.2E-02	2.0E-01
ILEIGEGTSEVQR	embl-cds:CCB74577	1.0	3.5E-01	5.1E-01
SNMGIPEGDGTINAK	embl-cds:CCB71733	1.0	2.7E-02	1.7E-01
ALDAPGNLDSPGAHLTR	embl-cds:CCB75748	0.9	1.9E-02	1.6E-01
LVAEDPTMR	embl-cds:CCB77983	0.9	2.8E-02	1.7E-01
ETASYVLESGGAR	embl-cds:CCB74676	0.9	1.6E-02	1.5E-01
GQVADPAATGAAAAVR	embl-cds:CCB74188	0.9	3.0E-01	4.7E-01
LPVVLFSAGGVATPADAALMR	embl-cds:CCB77988	0.9	9.6E-02	2.7E-01
TVGDAVDYILK	embl-cds:CCB74213	0.9	6.1E-02	2.2E-01
TVYDAHHEQSLPGDK	embl-cds:CCB74298	0.9	2.8E-02	1.7E-01
YLYDPPGSTLDK	embl-cds:CCB71733	0.9	3.3E-02	1.7E-01
AGAATEVELK	embl-cds:CCB75156	0.9	3.6E-02	1.7E-01
IIIDTYGGMAR	embl-cds:CCB78036	0.8	6.5E-03	1.0E-01
AHFAEVAAAGLPVVAYNNPVDTK	embl-cds:CCB72473	0.8	6.4E-02	2.2E-01
GHLSFYDTVAR	embl-cds:CCB77760	0.8	2.6E-01	4.5E-01
ALVIGHTDEER	embl-cds:CCB77858	0.8	3.7E-03	9.6E-02
FVIEPLEPGFGYTLGNSLR	embl-cds:CCB76315	0.8	2.7E-01	4.6E-01
WVNATYGNVNGYVR	embl-cds:CCB71733	0.8	5.9E-02	2.2E-01
MIEAGTVMR	embl-cds:CCB77865	0.7	9.0E-03	1.3E-01
GDLDVPATLR	embl-cds:CCB71592	0.7	1.4E-01	3.2E-01
YDETGVPR	embl-cds:CCB76604	0.7	1.6E-01	3.4E-01
TEDDDLLDVVAAAFR	embl-cds:CCB72323	0.7	3.3E-01	4.9E-01
AEPLAEPGAYALTGHK	embl-cds:CCB77423	0.7	4.3E-02	1.8E-01
VDGTDEAFAAVNDSK	embl-cds:CCB77171	0.7	5.9E-03	1.0E-01
PAVEQDGEALVGVVSAIDHPFGNVWTNIHR	embl-cds:CCB76861	0.7	4.0E-01	5.5E-01
AGVAVPGGTSEVLR	embl-cds:CCB72069	0.7	6.1E-02	2.2E-01
GEAQLAGYTR	embl-cds:CCB74799	0.7	4.1E-02	1.8E-01
EFVGDDIATEYSALMSK	embl-cds:CCB74676	0.7	2.3E-01	4.1E-01
YASTGGLEDR	embl-cds:CCB71397	0.7	3.7E-02	1.7E-01
LTLALIEAK	embl-cds:CCB77212	0.7	3.0E-01	4.7E-01
GFETADSLAAR	embl-cds:CCB77173	0.7	6.5E-02	2.2E-01
LLAGTLR	embl-cds:CCB76857	0.6	1.4E-01	3.2E-01
EIADHLPDDQVPTR	embl-cds:CCB75716	0.6	3.2E-02	1.7E-01
VVLPTGVAEVPLAIEGGR	embl-cds:CCB75168	0.6	3.4E-01	5.0E-01

TEQPPPEQHDAAR	embl-cds:CCB71908	0.6	1.5E-01	3.3E-01
EVNDGTFIHYDDINR	embl-cds:CCB72073	0.6	2.7E-01	4.5E-01
PASAAAEGVAPQATAADHPER	embl-cds:CCB74298	0.6	1.7E-01	3.5E-01
MDAVTQVPAPVNEPVHSYAPGSPER	embl-cds:CCB77049	0.6	7.8E-02	2.4E-01
FAGVVLPGETLR	embl-cds:CCB74113	0.6	3.6E-02	1.7E-01
TAVSDGIILSDAAAGK	embl-cds:CCB74007	0.6	9.1E-03	1.3E-01
TLVELFGLAR	embl-cds:CCB76315	0.6	2.3E-01	4.1E-01
GIETVAADLAR	embl-cds:CCB71860	0.6	3.0E-01	4.7E-01
IILNMTWAGER	embl-cds:CCB72072	0.6	7.8E-02	2.4E-01
TDEACAAQVAR	embl-cds:CCB71862	0.5	4.4E-01	6.0E-01
FGATEAVEPDGLADAK	embl-cds:CCB74112	0.5	6.2E-02	2.2E-01
VAEVTGQPQGK	embl-cds:CCB77858	0.5	1.0E-01	2.7E-01
VTNGADSFDLDR	embl-cds:CCB71733	0.5	4.9E-02	2.0E-01
PIPMPSAHVPTIDISPLSGSDADAK	embl-cds:CCB78370	0.5	5.3E-01	6.7E-01
LTSGLAVHEAGEGLR	embl-cds:CCB73111	0.5	1.2E-01	3.0E-01
EEHLAGLGLPQITADHLR	embl-cds:CCB76632	0.5	2.3E-01	4.2E-01
ITGASAAVPQQSGPALDEPSPHG	embl-cds:CCB74298	0.5	5.2E-01	6.6E-01
THQIGQVVPGK	embl-cds:CCB73850	0.5	3.3E-01	4.9E-01
YTGDPASAAGPPAR	embl-cds:CCB71588	0.5	2.8E-02	1.7E-01
AGGESGPGAAATIVVVK	embl-cds:CCB73553	0.5	5.1E-01	6.6E-01
VWTNCYHAYPAHAAFGGYK	embl-cds:CCB73675	0.5	2.3E-01	4.2E-01
LGLPNEDTFSGR	embl-cds:CCB75834	0.5	2.2E-01	4.0E-01
IGAPHSVVFGGFSADALATR	embl-cds:CCB75578	0.4	5.9E-01	7.2E-01
LSNVFGDHPVTLGPVYAGVR	embl-cds:CCB73009	0.4	6.0E-01	7.2E-01
EAATLMALNK	embl-cds:CCB76632	0.4	3.2E-01	4.9E-01
ADLTVDHDAYAEHVR	embl-cds:CCB72473	0.4	2.8E-01	4.6E-01
VTVVDGGDGER	embl-cds:CCB73923	0.4	2.6E-01	4.5E-01
MPDVGEGLTEAEILK	embl-cds:CCB75751	0.4	1.2E-01	3.0E-01
WINDPATDPAYIK	embl-cds:CCB76631	0.4	8.2E-02	2.5E-01
LTEAAGVAAK	embl-cds:CCB73111	0.4	3.7E-02	1.7E-01
VTEAAAMAAGR	embl-cds:CCB76610	0.4	2.8E-01	4.6E-01
VASVIEHGLTPAEAGDAEVPR	embl-cds:CCB77555	0.4	2.0E-01	3.9E-01
VLASGLSVSQLVSTAWASASSFR	embl-cds:CCB71452	0.4	6.5E-01	7.5E-01
NVTIQVMPLGR	embl-cds:CCB74160	0.4	2.7E-01	4.6E-01
LVDETNGQELAR	embl-cds:CCB73838	0.4	1.1E-01	2.9E-01
LQDGDEALAATR	embl-cds:CCB76186	0.4	2.7E-02	1.7E-01
AAVLQETGQEK	embl-cds:CCB74112	0.4	3.4E-01	5.1E-01
DVTAEAAER	embl-cds:CCB77791	0.4	2.9E-01	4.7E-01
IAITGGGSPK	embl-cds:CCB72149	0.4	7.2E-01	7.9E-01
EEVGTVSVAGDGIAK	embl-cds:CCB76889	0.4	3.9E-01	5.5E-01
TVHLEAADGVGVIR	embl-cds:CCB76961	0.4	3.8E-01	5.4E-01

QAADYTAPR	embl-cds:CCB75804	0.4	1.1E-01	2.8E-01
MAYGEPAWLATR	embl-cds:CCB78318	0.4	3.0E-01	4.7E-01
GLWAGIDISPGHGTGR	embl-cds:CCB76448	0.4	1.2E-01	3.0E-01
APGTAYDDPK	embl-cds:CCB74298	0.3	3.0E-01	4.7E-01
TPAQDPSPADR	embl-cds:CCB77214	0.3	2.0E-01	3.9E-01
NNTDSAGNSYGCHENYLVAR	embl-cds:CCB77865	0.3	2.3E-01	4.1E-01
GPQAENVTPV	embl-cds:CCB76165	0.3	2.8E-01	4.6E-01
EAVFVPAPGPDSETAR	embl-cds:CCB74582	0.3	2.7E-01	4.6E-01
LEAGTQVQNSVK	embl-cds:CCB76266	0.3	1.7E-01	3.5E-01
AGAAPDPSTADSDEFR	embl-cds:CCB74741	0.3	4.3E-02	1.8E-01
AGAVPDPATAGSEEEAVILR	embl-cds:CCB76857	0.3	2.9E-02	1.7E-01
AVTAGNLSVNSHSAVR	embl-cds:CCB77884	0.3	2.9E-02	1.7E-01
IDTCSWNR	embl-cds:CCB72967	0.3	5.1E-03	1.0E-01
TEAPGGEPGPDAAK	embl-cds:CCB77233	0.3	4.2E-01	5.7E-01
TAPSLPVLDQDR	embl-cds:CCB76631	0.3	1.9E-01	3.9E-01
FATSDLNDLYR	embl-cds:CCB76263	0.3	4.0E-01	5.5E-01
ALLEAEATHR	embl-cds:CCB77532	0.3	1.7E-01	3.5E-01
ELSENPVDLPMTIGGVK	embl-cds:CCB77049	0.3	5.7E-01	7.0E-01
TEVEAAVADLK	embl-cds:CCB76010	0.2	7.8E-01	8.3E-01
VTNLSQALNAAYDGK	embl-cds:CCB71707	0.2	7.2E-01	7.9E-01
ANISQGTALGK	embl-cds:CCB76309	0.2	4.0E-01	5.5E-01
LLTPDQIDALR	embl-cds:CCB71862	0.2	6.2E-01	7.4E-01
VVEALGAPTILVNNAGVLR	embl-cds:CCB73420	0.2	7.0E-01	7.8E-01
NGAGAGTVPGAHDPSER	embl-cds:CCB71452	0.2	1.5E-01	3.3E-01
TIFDSGLAVFPIYQDNAR	embl-cds:CCB71733	0.2	8.4E-01	8.8E-01
GILVTESVR	embl-cds:CCB76654	0.2	6.6E-01	7.5E-01
IEDDILVTEDGNR	embl-cds:CCB75716	0.2	8.4E-01	8.8E-01
VVTADGQGVR	embl-cds:CCB77621	0.2	6.3E-01	7.4E-01
TQIAQLQR	embl-cds:CCB75190	0.2	2.2E-01	4.1E-01
EITLAEHEMPGLMAMR	embl-cds:CCB76857	0.2	5.4E-01	6.7E-01
ATNGDNHLGGDDWDQR	embl-cds:CCB76010	0.2	3.6E-02	1.7E-01
LGPLGTALLPAR	embl-cds:CCB76815	0.2	6.0E-01	7.2E-01
MLINVPETVVADGLR	embl-cds:CCB73502	0.2	6.6E-01	7.5E-01
SVGESLETVVAK	embl-cds:CCB76011	0.2	2.0E-01	3.9E-01
VGVTEEAAR	embl-cds:CCB73674	0.2	4.9E-01	6.4E-01
AADVSAAELSAAQLTQR	embl-cds:CCB75769	0.2	7.6E-01	8.2E-01
TLVEGLIEEIAPEK	embl-cds:CCB75548	0.2	3.3E-01	5.0E-01
HILLATGSVPK	embl-cds:CCB74023	0.2	7.2E-01	7.9E-01
LEEVNDALTQMR	embl-cds:CCB74112	0.2	2.1E-01	4.0E-01
TASAGISAGAK	embl-cds:CCB72323	0.2	5.1E-01	6.6E-01
LLTDEELAALGEER	embl-cds:CCB73674	0.2	4.6E-01	6.1E-01

TAAASQIPVQR	embl-cds:CCB73420	0.2	2.8E-02	1.7E-01
FAEVWTGYFDR	embl-cds:CCB78368	0.2	8.0E-01	8.5E-01
IGLATADDIR	embl-cds:CCB76263	0.2	6.4E-01	7.5E-01
MDEWVTFYNK	embl-cds:CCB74676	0.2	2.8E-01	4.6E-01
SAGYALAEPAEIAAMVAEVVAGGDTGR	embl-cds:CCB78391	0.2	2.2E-01	4.0E-01
VGATDLVLR	embl-cds:CCB77865	0.2	7.9E-01	8.4E-01
AVVMAGGEGTR	embl-cds:CCB73453	0.1	7.3E-01	7.9E-01
IADQISDTILDALLR	embl-cds:CCB78036	0.1	8.0E-01	8.4E-01
AVLASPLTSR	embl-cds:CCB71588	0.1	6.1E-01	7.3E-01
DSGDGAGDDQADVGTK	embl-cds:CCB73911	0.1	7.3E-01	7.9E-01
MMLDHYQQTK	embl-cds:CCB73675	0.1	4.3E-01	5.8E-01
YEDGSPVTAQDIK	embl-cds:CCB76674	0.1	2.3E-01	4.2E-01
MDMSDQAPAAFGGTK	embl-cds:CCB74718	0.1	2.8E-01	4.6E-01
GAADAEDPATAAR	embl-cds:CCB76816	0.1	5.1E-01	6.6E-01
GIAGGLPLAAVTGR	embl-cds:CCB77168	0.1	6.4E-01	7.5E-01
SVLGTFR	embl-cds:CCB77049	0.1	7.8E-01	8.3E-01
VVVELSPYDLTR	embl-cds:CCB76311	0.1	7.6E-01	8.2E-01
DMDAMTLAVVR	embl-cds:CCB77809	0.1	5.5E-01	6.7E-01
GESLEEYWWCTR	embl-cds:CCB76857	0.1	7.2E-01	7.9E-01
PHPTDEAVCR	embl-cds:CCB77885	0.1	5.5E-01	6.7E-01
VLGTEPAGAGR	embl-cds:CCB77983	0.1	7.3E-01	7.9E-01
GTDVLIGGK	embl-cds:CCB76857	0.1	6.3E-01	7.4E-01
VYLTDPGSFEEFNR	embl-cds:CCB76567	0.1	6.7E-01	7.6E-01
THGAGPADHVGPDPEAAGLEEQGLGWR	embl-cds:CCB71452	0.1	9.7E-02	2.7E-01
VDVTAVQAR	embl-cds:CCB74462	0.1	5.8E-01	7.0E-01
SLDPSVYPDEVTR	embl-cds:CCB74718	0.1	8.6E-01	8.8E-01
FGEPQPAAGTGR	embl-cds:CCB76322	0.1	7.2E-01	7.9E-01
TVQLAVVSHCEQMGDR	embl-cds:CCB71405	0.1	7.7E-01	8.2E-01
SPGVYFDSTIDK	embl-cds:CCB76262	0.1	8.5E-01	8.8E-01
DYTIADPQR	embl-cds:CCB77813	0.0	8.6E-01	8.8E-01
YDLHPVSTGPYK	embl-cds:CCB76674	0.0	6.5E-01	7.5E-01
MLSESGVTLR	embl-cds:CCB73758	0.0	9.0E-01	9.1E-01
TQQGLSLHGVEEK	embl-cds:CCB78023	0.0	9.9E-01	9.9E-01
VIPEQPEPTFYSR	embl-cds:CCB76861	0.0	3.1E-01	4.8E-01
TAAGLEYSSGFGNEHVSEAVPGALPVGR	embl-cds:CCB73649	0.0	1.0E+00	1.0E+00
WAEPVPDGPTVR	embl-cds:CCB77530	0.0	9.7E-01	9.7E-01
IGAAGADGTEVER	embl-cds:CCB76186	0.0	8.5E-01	8.8E-01
VQQVDGITR	embl-cds:CCB77092	0.0	9.1E-01	9.2E-01
ATVSGAGELVGLVIDPK	embl-cds:CCB75653	0.0	8.6E-01	8.8E-01
GMVVPVEITVYEDR	embl-cds:CCB76257	0.0	9.5E-01	9.6E-01
AAQAAADFETNLAK	embl-cds:CCB76909	-0.1	7.0E-01	7.8E-01

TGVIEQVLDLWGR	embl-cds:CCB77865	-0.1	9.1E-01	9.2E-01
FQAPTHVSYR	embl-cds:CCB77525	-0.1	8.8E-01	9.0E-01
GYLSPYFVTDQER	embl-cds:CCB76345	-0.1	4.3E-01	5.9E-01
SANSGGIGGVPGPHNGLTDVPGVR	embl-cds:CCB71865	-0.1	8.0E-01	8.4E-01
VVFPELTEER	embl-cds:CCB77148	-0.1	8.6E-01	8.8E-01
AYGDSGTGQGPAEGK	embl-cds:CCB75769	-0.1	7.8E-01	8.3E-01
LTGSSDLYQNDGR	embl-cds:CCB72264	-0.1	2.8E-01	4.6E-01
GTQAQYLAK	embl-cds:CCB76309	-0.1	8.8E-01	9.0E-01
ADPTAPAEGVANEGISGNR	embl-cds:CCB73009	-0.1	8.6E-01	8.8E-01
TADADGEAGDIQWNFEK	embl-cds:CCB77481	-0.1	7.6E-01	8.2E-01
VAADEVTDQAASHG	embl-cds:CCB75075	-0.1	8.2E-01	8.6E-01
STAADIDLALDAAHAAAPAWGR	embl-cds:CCB73675	-0.1	4.2E-01	5.8E-01
MAVVTPFDK	embl-cds:CCB77148	-0.1	5.3E-01	6.7E-01
DTDEDVFR	embl-cds:CCB76262	-0.1	5.8E-01	7.1E-01
TVIVLEGVNDIK	embl-cds:CCB73009	-0.1	8.9E-01	9.1E-01
FESNTWLVADWELVK	embl-cds:CCB76632	-0.1	6.6E-01	7.6E-01
IAAAGGSVTELAK	embl-cds:CCB76307	-0.1	7.0E-01	7.8E-01
SLEENQQVNFDVTQGPK	embl-cds:CCB76165	-0.1	8.9E-02	2.6E-01
DAGFEVQVPFTPGR	embl-cds:CCB71452	-0.1	5.4E-01	6.7E-01
TGDQFWQMADQLAR	embl-cds:CCB74201	-0.1	6.5E-01	7.5E-01
EAAVAPSELTLLGLVR	embl-cds:CCB73414	-0.1	8.7E-01	8.9E-01
FYGATGIQPFDQR	embl-cds:CCB74268	-0.1	7.9E-01	8.4E-01
FFLIEAPAVVLATGGIGK	embl-cds:CCB76654	-0.2	6.5E-01	7.5E-01
VLGEEAPLTVQDR	embl-cds:CCB76819	-0.2	3.9E-01	5.5E-01
AGAAPVGQDTGLAQIR	embl-cds:CCB74747	-0.2	2.0E-02	1.6E-01
VESAATVHAVER	embl-cds:CCB77061	-0.2	2.6E-01	4.5E-01
QDIDAGHDDDVTK	embl-cds:CCB76820	-0.2	4.6E-01	6.1E-01
FSGLTSIVVDPGR	embl-cds:CCB73177	-0.2	6.1E-01	7.3E-01
ADFDEMTEICR	embl-cds:CCB77983	-0.2	5.2E-01	6.6E-01
TEVSGGPALPQER	embl-cds:CCB77168	-0.2	1.5E-01	3.3E-01
PAAPAPGGCAATAR	embl-cds:CCB72047	-0.2	4.2E-01	5.7E-01
QAAAGTGVASGSEAPR	embl-cds:CCB76489	-0.2	6.0E-01	7.2E-01
AAVPSGASTGAFEALELR	embl-cds:CCB74824	-0.2	3.8E-01	5.4E-01
DAQTAIEHLR	embl-cds:CCB78000	-0.2	4.8E-01	6.3E-01
TDALDGAVIAVAGAGGPAGR	embl-cds:CCB73553	-0.2	3.5E-01	5.1E-01
AGNPDTDFVPDAVVDR	embl-cds:CCB77533	-0.2	2.9E-01	4.7E-01
TYVIAQPCVDVK	embl-cds:CCB76687	-0.2	4.1E-01	5.7E-01
CGLTPQYEGLER	embl-cds:CCB77481	-0.2	1.3E-01	3.2E-01
TPGEYFYSQNFDR	embl-cds:CCB73477	-0.2	4.9E-01	6.4E-01
GLDATEAQAVLR	embl-cds:CCB76293	-0.2	7.1E-01	7.9E-01
GLNVHDGQIVYPEVGEAHGLPVTDLNSLLG	embl-cds:CCB73698	-0.2	7.3E-01	7.9E-01

VTVGWAEGLHAR	embl-cds:CCB77306	-0.2	6.6E-01	7.5E-01
TALNLVLNGVIGR	embl-cds:CCB73177	-0.2	4.3E-01	5.9E-01
FVEAFLATPFSGDER	embl-cds:CCB74469	-0.2	3.1E-01	4.7E-01
FSLATLAAR	embl-cds:CCB75911	-0.2	5.7E-01	7.0E-01
WESLDTDAAGGCIR	embl-cds:CCB75307	-0.2	1.5E-01	3.3E-01
VTPLGGLGEIGR	embl-cds:CCB77212	-0.3	1.3E-01	3.1E-01
ATQEDPWQQFAR	embl-cds:CCB73850	-0.3	4.2E-02	1.8E-01
AESTIGQPVADQK	embl-cds:CCB73482	-0.3	3.1E-01	4.7E-01
AAGATVIGVAGGPEK	embl-cds:CCB73628	-0.3	5.0E-01	6.5E-01
AISMQPTDGLVR	embl-cds:CCB76891	-0.3	1.4E-01	3.2E-01
VTEGMSYVNPK	embl-cds:CCB77344	-0.3	4.1E-01	5.7E-01
NLGDLFDTFER	embl-cds:CCB74150	-0.3	3.5E-01	5.1E-01
VLGEIQDGTFAR	embl-cds:CCB77035	-0.3	5.3E-01	6.7E-01
TVFAAGGEGAER	embl-cds:CCB74457	-0.3	7.4E-01	8.1E-01
AAAAHTMEVLR	embl-cds:CCB77049	-0.3	1.6E-01	3.4E-01
AALAATAGEDR	embl-cds:CCB72320	-0.3	3.1E-01	4.8E-01
VIVQGMTGSEGQK	embl-cds:CCB76399	-0.3	1.4E-01	3.3E-01
FSGVEYTADGVK	embl-cds:CCB74023	-0.3	1.2E-01	3.0E-01
LVTGLVPLSEMFGYVGDLR	embl-cds:CCB76266	-0.3	6.0E-01	7.2E-01
FLIAPDGTVAAR	embl-cds:CCB77481	-0.3	1.7E-01	3.5E-01
VNALCPGPVDTPLLR	embl-cds:CCB77885	-0.3	6.7E-01	7.6E-01
WTVVNGLPDAR	embl-cds:CCB74199	-0.3	4.8E-01	6.3E-01
GECNLGQHEITFR	embl-cds:CCB77883	-0.3	5.4E-01	6.7E-01
IFSYDVTGGR	embl-cds:CCB77861	-0.3	4.5E-01	6.0E-01
ATLDQALQAIR	embl-cds:CCB75769	-0.3	4.6E-02	1.9E-01
TGEVLSVPVGEGYLGR	embl-cds:CCB76889	-0.3	6.7E-02	2.2E-01
FEIGGPMGDAGLTGR	embl-cds:CCB78036	-0.3	2.4E-01	4.3E-01
ILDLTEYLDR	embl-cds:CCB75190	-0.3	7.0E-01	7.8E-01
EVGTEGQLGGQAR	embl-cds:CCB77215	-0.4	1.1E-01	2.9E-01
AAVDAGWYPHSNQVGQTGK	embl-cds:CCB73547	-0.4	9.1E-02	2.6E-01
QTVDQADWLIGANLLAPGVHGQALR	embl-cds:CCB74298	-0.4	6.7E-01	7.6E-01
IVELQTNLAGVSAQNER	embl-cds:CCB77858	-0.4	3.7E-01	5.3E-01
NANTVAEDPHVAR	embl-cds:CCB73862	-0.4	6.4E-01	7.5E-01
DTAAHYADEAR	embl-cds:CCB75804	-0.4	6.1E-01	7.3E-01
FGVQEITR	embl-cds:CCB71588	-0.4	1.4E-01	3.3E-01
AQALSAEQTR	embl-cds:CCB75045	-0.4	4.0E-01	5.5E-01
VSTELLEK	embl-cds:CCB74766	-0.4	1.1E-01	2.9E-01
GIYPAVDPLDSTSR	embl-cds:CCB76891	-0.4	1.2E-01	3.0E-01
AQPQDTSIR	embl-cds:CCB75259	-0.4	6.3E-01	7.4E-01
VNETVDIIGIK	embl-cds:CCB76267	-0.4	7.4E-02	2.3E-01
VTMNPHSAGAESVVEK	embl-cds:CCB75205	-0.5	8.9E-02	2.6E-01

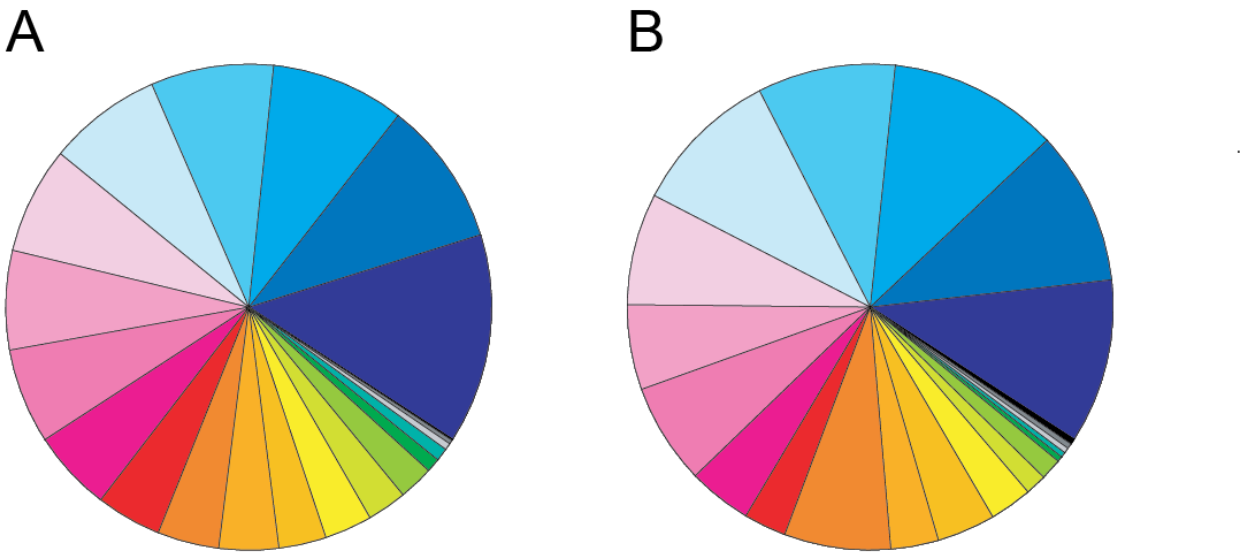
VSTDSAPGEADNVHR	embl-cds:CCB77359	-0.5	2.0E-01	3.9E-01
EALAQTPESHR	embl-cds:CCB76816	-0.5	6.3E-01	7.4E-01
LIMQYASQNLIPVTLELGGK	embl-cds:CCB73675	-0.5	3.1E-01	4.7E-01
TAEAVGLGEK	embl-cds:CCB76025	-0.5	5.3E-02	2.0E-01
SEEHGYAATGSGSVFAR	embl-cds:CCB77861	-0.5	2.1E-01	4.0E-01
EDVTDLILNIK	embl-cds:CCB76315	-0.5	6.1E-01	7.3E-01
EGVLLFPAINVNDAVTK	embl-cds:CCB74741	-0.5	3.3E-01	4.9E-01
DGGTLPDAQVYAK	embl-cds:CCB73714	-0.5	1.5E-01	3.3E-01
TAVVEPGVVNAVLSR	embl-cds:CCB74675	-0.5	2.6E-01	4.5E-01
VLVFATGDR	embl-cds:CCB76258	-0.5	3.1E-02	1.7E-01
SPGSIGGCATPGR	embl-cds:CCB76288	-0.5	3.9E-02	1.8E-01
EADDLDGVAAAVVLHGTR	embl-cds:CCB76631	-0.5	3.7E-01	5.3E-01
ELPASATQEEIEAVVR	embl-cds:CCB76406	-0.5	3.1E-01	4.7E-01
EAAQTLLENLYFNPK	embl-cds:CCB76262	-0.5	2.9E-01	4.7E-01
LAEGPTVAYAALK	embl-cds:CCB76697	-0.6	1.7E-03	9.4E-02
AEQLYATLK	embl-cds:CCB75802	-0.6	3.8E-03	9.6E-02
LAGTAHDGLAR	embl-cds:CCB71865	-0.6	1.4E-01	3.2E-01
WPVVPTAQALAAPFR	embl-cds:CCB73649	-0.6	2.8E-01	4.6E-01
EISNLTVDQDVVEYK	embl-cds:CCB71406	-0.6	1.7E-01	3.5E-01
SSMSPTIVLR	embl-cds:CCB77525	-0.6	5.0E-01	6.5E-01
EALQAEGVPTR	embl-cds:CCB73816	-0.6	1.9E-01	3.7E-01
AVESFCYLNPAFTDDHPMIK	embl-cds:CCB78370	-0.6	4.6E-01	6.1E-01
DFVNESFIGTR	embl-cds:CCB72474	-0.6	3.1E-01	4.8E-01
SITYAELQEEVAR	embl-cds:CCB75578	-0.6	3.6E-01	5.2E-01
LQVEHPVTEEVSGIDLVR	embl-cds:CCB76511	-0.6	3.0E-01	4.7E-01
ALTAVEAHIAR	embl-cds:CCB75372	-0.6	2.6E-01	4.5E-01
LNIPTVFVSGGPMESGR	embl-cds:CCB75307	-0.6	2.0E-01	3.9E-01
EHVATLHGDGR	embl-cds:CCB76697	-0.6	6.9E-02	2.3E-01
LATDAELELLR	embl-cds:CCB71752	-0.6	5.2E-03	1.0E-01
GGLTAENWALGWHR	embl-cds:CCB76631	-0.6	2.2E-01	4.0E-01
ELIDGATGHQLAAPGR	embl-cds:CCB77595	-0.6	9.4E-03	1.3E-01
ILSYIDIGTAEGAK	embl-cds:CCB73675	-0.6	2.9E-01	4.7E-01
TVAEVMAANVIAAR	embl-cds:CCB76862	-0.6	8.4E-04	9.4E-02
GANDTLEQIAEAR	embl-cds:CCB71666	-0.6	2.1E-02	1.6E-01
SADQLLNAIYLTQQAR	embl-cds:CCB76815	-0.6	4.8E-01	6.3E-01
HYTSVFDDSR	embl-cds:CCB78452	-0.6	1.3E-01	3.2E-01
IAFFGPVVTPAPK	embl-cds:CCB74474	-0.6	1.8E-03	9.4E-02
LTEAEMSEIAR	embl-cds:CCB77861	-0.6	7.1E-02	2.3E-01
LVIANSGPGTVR	embl-cds:CCB72210	-0.7	6.9E-02	2.3E-01
ATVGEVGNAEQSNINWGK	embl-cds:CCB76291	-0.7	7.2E-02	2.3E-01
VTVAAQNLR	embl-cds:CCB76025	-0.7	3.1E-02	1.7E-01

YDTILGR	embl-cds:CCB73825	-0.7	9.0E-02	2.6E-01
AVSPLIETFLAVVR	embl-cds:CCB75849	-0.7	2.6E-01	4.5E-01
YAATIQSQR	embl-cds:CCB78023	-0.7	4.6E-01	6.1E-01
SVLVTGAASGIGAAVAR	embl-cds:CCB78391	-0.7	9.6E-02	2.7E-01
NMVEGMTDDQILHLGR	embl-cds:CCB74201	-0.7	2.2E-01	4.0E-01
AVVLSSTHDR	embl-cds:CCB76519	-0.7	1.6E-01	3.4E-01
LSDTIMQNDVPAVPFAYDK	embl-cds:CCB76674	-0.7	5.3E-02	2.0E-01
ADEEFLITR	embl-cds:CCB72072	-0.7	8.8E-02	2.6E-01
LVLEVITDK	embl-cds:CCB76316	-0.7	4.3E-02	1.8E-01
VIGEVFGLTVR	embl-cds:CCB75365	-0.7	1.5E-01	3.3E-01
ALGLAGAQLVYNPSATSR	embl-cds:CCB77530	-0.8	2.0E-02	1.6E-01
DQLDFFPLTVDVEER	embl-cds:CCB77204	-0.8	1.5E-01	3.3E-01
GIDSNTANALLVK	embl-cds:CCB74824	-0.8	5.3E-02	2.0E-01
YGAVEMLDAMAYDGLTDAFEK	embl-cds:CCB76913	-0.8	2.6E-01	4.5E-01
HAAELVAPYAGTAK	embl-cds:CCB75804	-0.8	8.4E-02	2.5E-01
QAVAQATHHAAYR	embl-cds:CCB77423	-0.8	1.6E-01	3.3E-01
TVVYDVLEEVIAEHPVLLNR	embl-cds:CCB76263	-0.8	7.5E-02	2.3E-01
AEGDPLSTHIFGVQLGGYNVESVK	embl-cds:CCB71406	-0.8	7.6E-02	2.3E-01
DHADTALLVAEHPGAVR	embl-cds:CCB78341	-0.8	1.4E-01	3.2E-01
VVAGQVLADGPSTER	embl-cds:CCB76262	-0.8	7.1E-02	2.3E-01
IAAIEEGLTESR	embl-cds:CCB71775	-0.8	8.5E-02	2.5E-01
NDYGTQDFGGAAPPPGDGPHR	embl-cds:CCB73714	-0.8	3.5E-02	1.7E-01
VGAEVTAEEAK	embl-cds:CCB75589	-0.9	2.6E-01	4.5E-01
LAALYPEGGEVTVEDLVAK	embl-cds:CCB76307	-0.9	2.8E-02	1.7E-01
TELGLLAAGPGEVALIPR	embl-cds:CCB73649	-0.9	8.9E-02	2.6E-01
AHQVCPYSNATR	embl-cds:CCB74224	-0.9	1.2E-02	1.4E-01
IINQYQPFVDMLNINTK	embl-cds:CCB76674	-0.9	3.8E-01	5.4E-01
ISTEDFAVALVDEIESGDHVGER	embl-cds:CCB71078	-0.9	9.1E-02	2.6E-01
LDQTQYLHHFIEASR	embl-cds:CCB77525	-0.9	1.6E-01	3.4E-01
PIVGYGHNNVGDYVCTDGANGGVHCGVR	embl-cds:CCB72139	-0.9	3.8E-01	5.4E-01
IDTSNYGDHSGPGER	embl-cds:CCB77809	-0.9	5.3E-01	6.7E-01
IGHGTSAVQDPR	embl-cds:CCB77159	-0.9	1.7E-01	3.5E-01
YDAEVVPVDGTGLQR	embl-cds:CCB73408	-0.9	3.5E-01	5.1E-01
WVVLPDGTR	embl-cds:CCB73264	-0.9	1.5E-01	3.3E-01
NFVELAEGTR	embl-cds:CCB75802	-0.9	1.2E-01	3.0E-01
AAGLGVTIR	embl-cds:CCB72162	-0.9	2.0E-01	3.9E-01
LGQPEILLGLIPGAGGTQR	embl-cds:CCB76961	-0.9	2.9E-02	1.7E-01
YLPFYITGMAVK	embl-cds:CCB72071	-0.9	1.2E-01	3.0E-01
TGHVGFIGGVDNALIK	embl-cds:CCB76477	-0.9	2.6E-01	4.5E-01
LADILIPFLVTR	embl-cds:CCB77865	-0.9	3.0E-01	4.7E-01
FASVPGDYTR	embl-cds:CCB77457	-1.0	2.3E-01	4.2E-01

TPAGGFGLK	embl-cds:CCB74224	-1.0	7.7E-03	1.2E-01
LGPDASATPAVVGFNLR	embl-cds:CCB73714	-1.0	2.0E-02	1.6E-01
IVITPDDGSDEIAYGVSK	embl-cds:CCB76263	-1.0	6.2E-02	2.2E-01
WVGDPAFENVPTK	embl-cds:CCB77525	-1.0	1.4E-02	1.4E-01
FLAPYTGSAIGQHWMYQGK	embl-cds:CCB76889	-1.0	8.7E-02	2.6E-01
SIGIEHEGFLADPDAWYTEAMYR	embl-cds:CCB73477	-1.0	6.6E-02	2.2E-01
GTDPQAASAAAQR	embl-cds:CCB77898	-1.0	2.6E-02	1.7E-01
MLASGTNIVGGVNPR	embl-cds:CCB76399	-1.0	1.0E-01	2.8E-01
ALIPVLPTR	embl-cds:CCB77204	-1.0	6.4E-02	2.2E-01
WATPVVSFQR	embl-cds:CCB74164	-1.0	1.5E-01	3.3E-01
SLLTIAEDLGYEVSEGR	embl-cds:CCB77051	-1.0	1.9E-01	3.7E-01
TVSCPAQYNK	embl-cds:CCB72139	-1.0	1.1E-02	1.4E-01
SFAGALITR	embl-cds:CCB72250	-1.0	3.7E-02	1.7E-01
VIDTLELLAAR	embl-cds:CCB77913	-1.1	2.0E-01	3.9E-01
TDEQLDELAGLLDGSR	embl-cds:CCB74477	-1.1	2.3E-02	1.6E-01
WLAEEFIPTVAK	embl-cds:CCB76410	-1.1	7.2E-02	2.3E-01
VAISASIPNVDAATAK	embl-cds:CCB74224	-1.1	4.6E-02	1.9E-01
LATGYGFNR	embl-cds:CCB71733	-1.1	5.2E-01	6.6E-01
GMITALAR	embl-cds:CCB72139	-1.1	1.8E-05	7.8E-03
AAALAETDADVVLVDGVR	embl-cds:CCB74766	-1.2	9.9E-02	2.7E-01
TDDPSVVEDFAR	embl-cds:CCB72579	-1.2	4.2E-02	1.8E-01
YAPEQHLDGGLTLEQVVEAVNDGFR	embl-cds:CCB76489	-1.2	2.6E-01	4.5E-01
ADFGPYTPGFDVVPYGDIAALR	embl-cds:CCB76448	-1.2	3.3E-03	9.4E-02
EYFLSTNFR	embl-cds:CCB72036	-1.3	1.3E-01	3.2E-01
LYDGTVFHR	embl-cds:CCB75802	-1.3	2.2E-02	1.6E-01
AVLYGLTSK	embl-cds:CCB75954	-1.3	3.5E-02	1.7E-01
LHASDVVTVR	embl-cds:CCB75954	-1.3	3.5E-02	1.7E-01
TPWCLAGVYYVPISK	embl-cds:CCB72139	-1.3	1.4E-01	3.2E-01
PVTADGDALVVDGR	embl-cds:CCB77913	-1.3	1.3E-01	3.1E-01
VLAADTPEFAEYLVVPK	embl-cds:CCB73547	-1.4	1.2E-02	1.4E-01
ALAGPTDPIAAAPGSIR	embl-cds:CCB74454	-1.4	1.9E-02	1.6E-01
GLSTGLGDEGGFAPDLESNR	embl-cds:CCB74824	-1.4	3.2E-03	9.4E-02
QYHGETDEQVNAK	embl-cds:CCB73823	-1.4	1.1E-02	1.4E-01
NQFGGHAVEAAGSSDTK	embl-cds:CCB75819	-1.4	6.5E-03	1.0E-01
AVLAVIEQIGPELVGYDATEQR	embl-cds:CCB74824	-1.4	1.2E-01	3.0E-01
LVTDAEDSPHR	embl-cds:CCB71784	-1.4	1.6E-02	1.5E-01
EIVIESEQSGEEIEQAVAAALTGSAK	embl-cds:CCB76720	-1.4	6.0E-02	2.2E-01
AQVLADTLDR	embl-cds:CCB72125	-1.5	1.6E-02	1.5E-01
MTDYVAPAGVR	embl-cds:CCB73800	-1.5	2.0E-01	3.9E-01
FLSQNTHAAK	embl-cds:CCB76891	-1.5	2.4E-02	1.7E-01
GFAHLENGATGLER	embl-cds:CCB72337	-1.5	2.4E-01	4.3E-01

AVAFNHLDTIR	embl-cds:CCB71588	-1.5	1.1E-02	1.4E-01
VPSAVWIVDTK	embl-cds:CCB77145	-1.5	3.0E-01	4.7E-01
VVPPGTGIVHQVNIEHLAR	embl-cds:CCB77359	-1.5	1.1E-03	9.4E-02
LVEEAPAPFLTDGQVAELYR	embl-cds:CCB76511	-1.6	3.7E-01	5.3E-01
VSAHDGVDEIGSGTHER	embl-cds:CCB76855	-1.6	5.7E-03	1.0E-01
AFIIQYFSDPSTLK	embl-cds:CCB77845	-1.6	5.8E-02	2.2E-01
VFPIHDSVEAAVSATD	embl-cds:CCB75567	-1.6	4.8E-03	1.0E-01
LAYVEIGEPTK	embl-cds:CCB76720	-1.6	4.2E-01	5.7E-01
FMTSAQSQR	embl-cds:CCB74055	-1.7	2.6E-02	1.7E-01
VAVLDTPPGLSAQQVR	embl-cds:CCB71405	-1.7	1.3E-02	1.4E-01
LIDNDQVLASTR	embl-cds:CCB72250	-1.7	1.6E-01	3.4E-01
PIATPEIYNEMLDR	embl-cds:CCB76025	-1.7	2.9E-03	9.4E-02
SSAADVNTGFR	embl-cds:CCB77364	-1.8	3.5E-02	1.7E-01
VAQICTPQYAAACAK	embl-cds:CCB77364	-1.8	1.8E-01	3.7E-01
AELGDGTLAR	embl-cds:CCB72250	-1.8	2.2E-02	1.6E-01
GFLLNNELTDFDFAPANPALPDPNLPGPGK	embl-cds:CCB77525	-1.8	1.3E-01	3.2E-01
GPLAPGATAVYR	embl-cds:CCB71663	-1.8	2.9E-03	9.4E-02
VDLTPGLLAALHGDGGVVAVK	embl-cds:CCB72473	-1.8	5.1E-02	2.0E-01
YGNAQTSDFVR	embl-cds:CCB77005	-1.8	1.9E-02	1.6E-01
GNLLASGSTAQK	embl-cds:CCB75283	-1.9	4.5E-02	1.9E-01
GLAVAGLDANSYPSGEFLTAEPR	embl-cds:CCB73264	-1.9	2.0E-02	1.6E-01
VLATAYEVYAYLFR	embl-cds:CCB76632	-1.9	1.0E-01	2.7E-01
VMDQAPHDFLVTAGLDDVAR	embl-cds:CCB73264	-1.9	5.4E-03	1.0E-01
YSANPAPHGLALTR	embl-cds:CCB73816	-2.0	2.4E-01	4.3E-01
DGGVILATAPDR	embl-cds:CCB78006	-2.0	9.2E-02	2.6E-01
YGEVLASAGLTPNDGLGGIFK	embl-cds:CCB72125	-2.0	1.7E-02	1.5E-01
AIAGDVPLVFDASHTLGLIAGGR	embl-cds:CCB71588	-2.1	6.6E-02	2.2E-01
TGEVIDWESSIFGR	embl-cds:CCB74619	-2.3	2.3E-02	1.6E-01
LAAAYDADTEHER	embl-cds:CCB77423	-2.4	3.2E-03	9.4E-02
TGAPDDGDGPDAR	embl-cds:CCB71463	-2.4	1.3E-01	3.2E-01
ATALVIPELK	embl-cds:CCB73825	-2.7	1.5E-02	1.5E-01
GLSGQGYAPLPAEIATK	embl-cds:CCB75283	-2.7	6.3E-02	2.2E-01
TPAVLAAIVR	embl-cds:CCB75991	-2.8	6.3E-03	1.0E-01
IATGAAAPVEASSDAASK	embl-cds:CCB75283	-3.0	1.9E-03	9.4E-02
INLLDTPGYADFVGELR	embl-cds:CCB77983	-3.3	3.3E-02	1.7E-01
VDLGGSLSGGYYVAPTIFEGDNR	embl-cds:CCB73675	-3.6	3.4E-02	1.7E-01

Figure S15. Gene ontology assignments for proteins with PSMs corresponding to peptides that encode a single threonine residue. Gene ontology assignments were obtained from the Microscope platform (http://www.genoscope.cns.fr/agc/microscope). (A) Thr-containing peptides (B) FThr-containing peptides.



	Thr-containing	FThr-containing
General function prediction only	212 (14.0)	31 (10.95)
Amino acid transport and metabolism	146 (9.6)	29 (10.25)
Energy production and conversion	135 (8.9)	32 (11.31)
Carbohydrate transport and metabolism	124 (8.2)	26 (9.2)
Translation, ribosomal structure and biogenesis	115 (7.6)	28 (9.9)
Lipid transport and metabolism	108 (7.1)	21 (7.4)
Secondary metabolites biosynthesis, transport, and catabolism	100 (6.6)	16 (5.7)
Transcription	96 (6.3)	19 (6.7)
Function unknown	82 (5.4)	12 (4.2)
Signal transduction mechanisms	66 (4.4)	8 (2.8)
Post-translational modification, protein turnover, and chaperones	62 (4.1)	20 (7.1)
Coenzyme transport and metabolism	60 (4.0)	9 (3.2)
Cell wall/membrane/envelope biogenesis	48 (3.2)	11 (3.9)
Nucleotide transport and metabolism	48 (3.2)	8 (2.8)
Replication, recombination and repair	40 (2.6)	4 (1.4)
Inorganic ion transport and metabolism	35 (2.3)	4 (1.4)
Defense mechanisms	14 (0.9)	1 (0.4)
Intracellular trafficking, secretion, and vesicular transport	13 (0.9)	1 (0.4)
Cell cycle control, cell division, chromosome partitioning	7 (0.5)	1 (0.4)
Cell motility	4(0.3)	1 (0.4)
RNA processing and modification	1 (2.16)	1 (0.4)
Total	**1516**	**283**

Figure S16. Growth inhibition of *S. coelicolor* M1152 with and without *fthB* by fluorothreonine. Spores of *S. coelicolor* M1152, *S. coelicolor* M1152 ermEp*-empty, and *S. coelicolor* M1152 ermEp*-FthB were plated on Hopwood media, and 200 µg FThr was applied via filter paper. Plates were incubated 5 d at 30°C prior to observation.

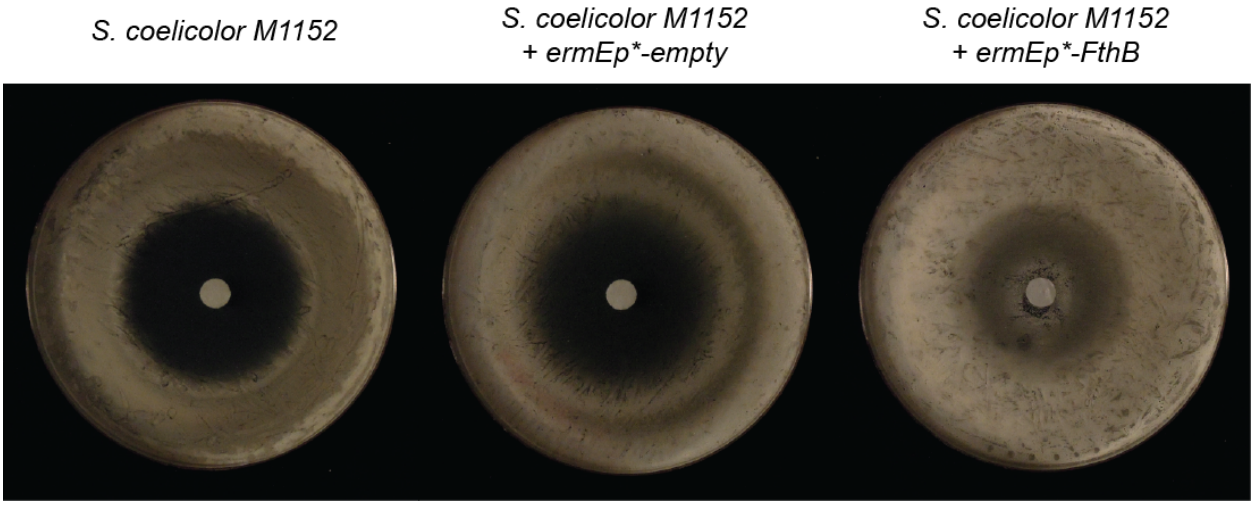


Figure S17. Photographs of representative *S. coelicolor* M1152 liquid cultures from fluorothreonine inhibitions assays. (A, B) *S. coelicolor* M1152 in the absence and presence of FThr. (C, D) *S. coelicolor* M1152 ermEp*-blank in the absence and presence of FThr. (E, F) *S. coelicolor* M1152 ermEp*-FthB in the absence and presence of FThr.

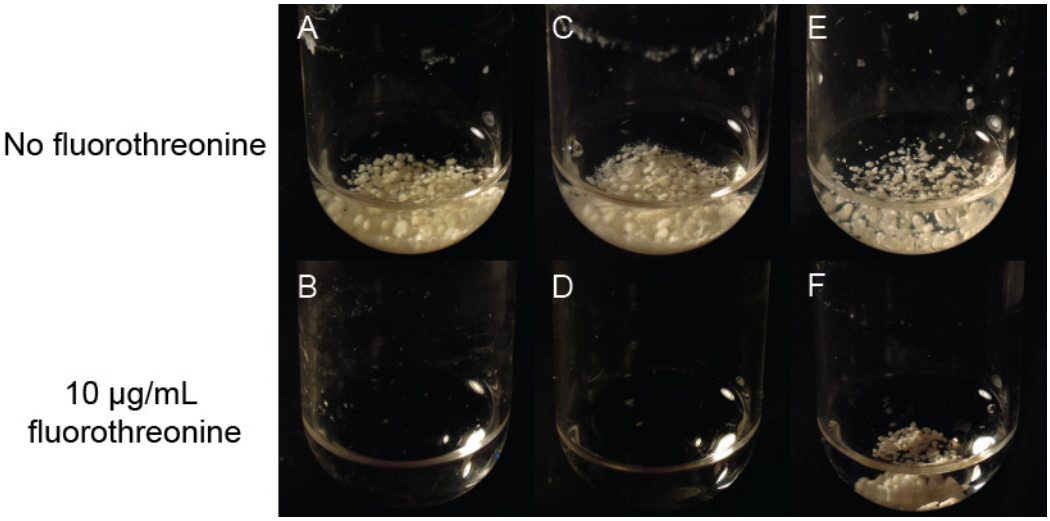
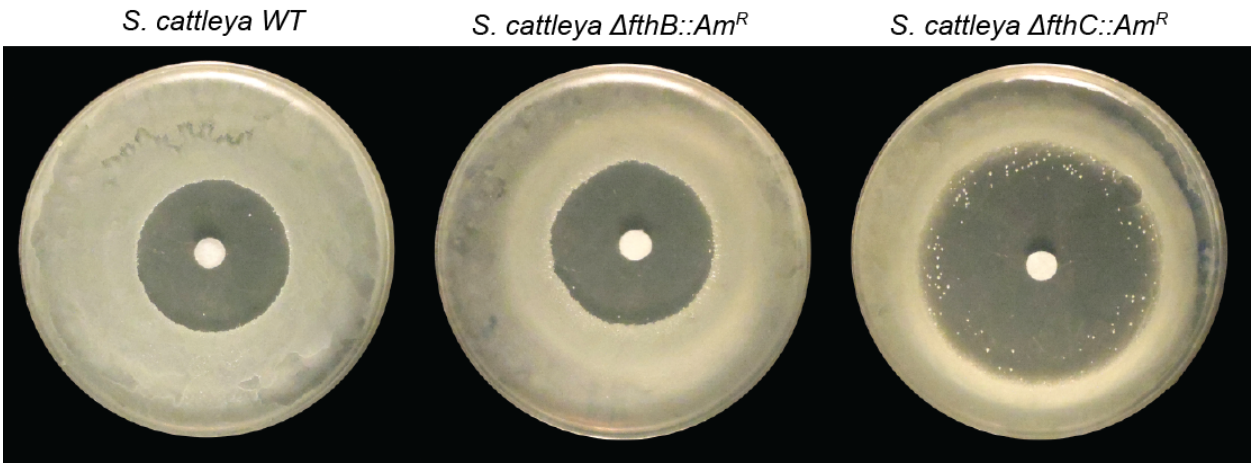


Figure S18. Growth inhibition of *S. cattleya* strains by fluorothreonine. Spores of *S. cattleya* WT, Δ*fthB*, and Δ*fthC* were plated on Hopwood media, and 200 μg Fthr was applied via filter paper. Plates were incubated 5 d at 30°C prior to observation.



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