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RESEARCH ARTICLE

# Figures and figure supplements

Genome mining unearths a hybrid nonribosomal peptide synthetase-like-pteridine synthase biosynthetic gene cluster

**Hyun Bong Park *et al***

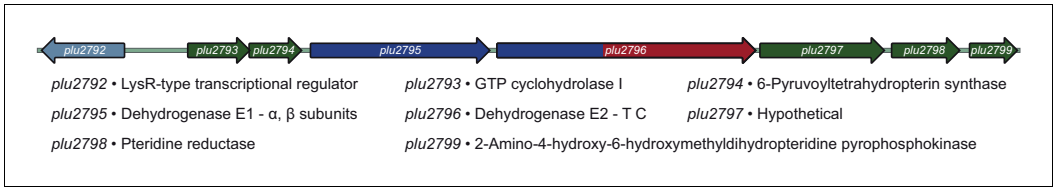


**Figure 1.** The pepteridine biosynthetic locus. Green, pteridine synthesis genes; Blue, pyruvate dehydrogenase-like genes; Red, NRPS-like genes. T, thiolation domain; C, condensation domain.
DOI: 10.7554/eLife.25229.003

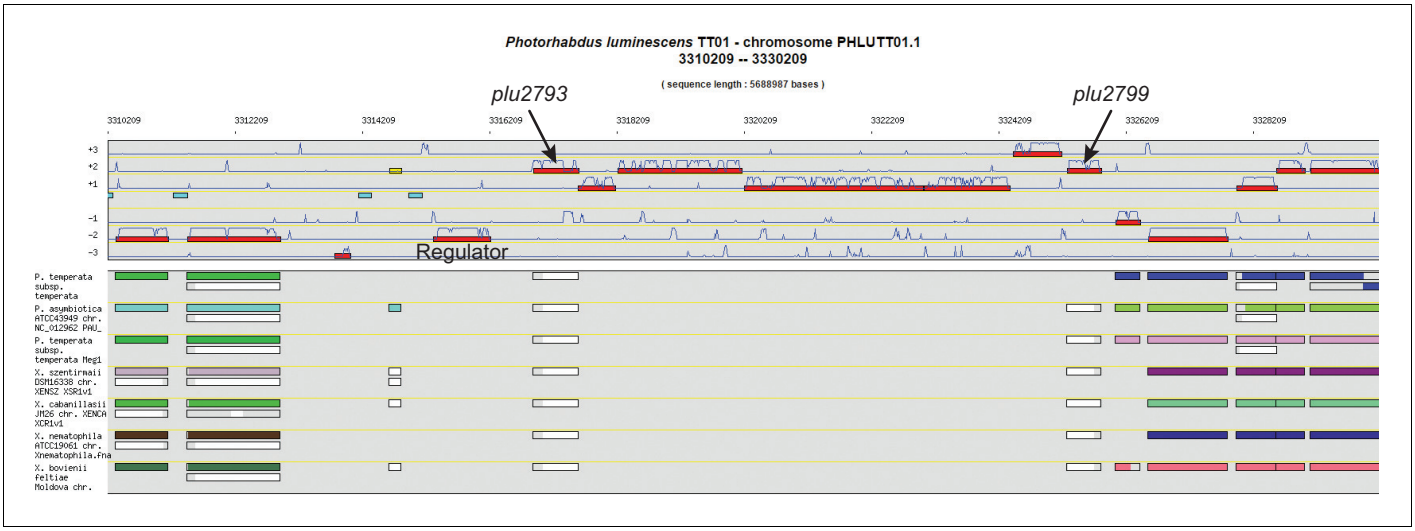


**Figure 1—figure supplement 1.** Genome synteny analysis using MicroScope. Genome synteny analysis revealed a genomic island in *P. luminescens* TT01 (note absence of biosynthetic genes in phylogenetically related species, lower panel) harboring a number of enzymes possessing homology to pteridine and NRPS biosynthetic machineries (+1, +2, and +3 reading frames, upper panel). The regulator in the genomic island was not included in our design, as the pathway was placed under the control of a T7 promoter.

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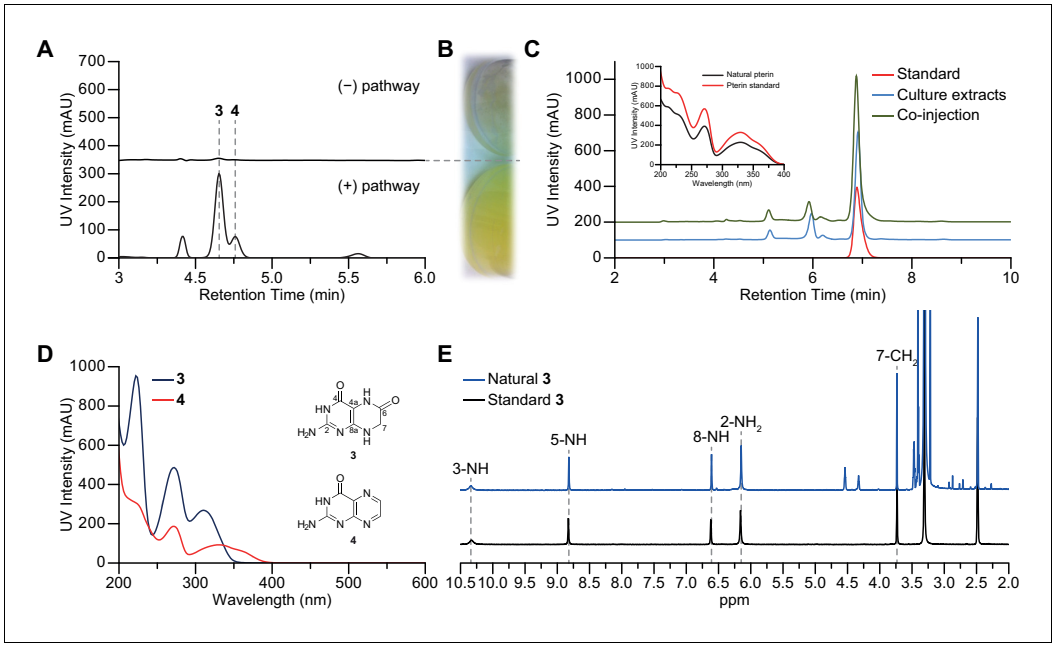


**Figure 2.** Production of 7,8-dihydroxanthopterin (**3**) and pterin (**4**) by heterologous expression. (**A**) HPLC traces (310 nm) of butanol extracts showing compounds **3** and **4** being over-produced in the heterologous expression strain. (**B**) The edge of a culture flask growing *E. coli* BAP1 carrying an empty vector pET28a (top), and a culture flask growing *E. coli* BAP1 carrying the wild-type pepteridine pathway (bottom). (**C**) HPLC traces (310 nm) from HPLC co-injection with authentic pterin (**4**), and UV absorption spectral comparison between natural and authentic pterin (inset). (**D**) UV-vis absorption spectra of compounds **3** and **4**. (**E**) $^{1}$H NMR comparison of natural (blue) and standard (black) **3** in DMSO-$d_6$.

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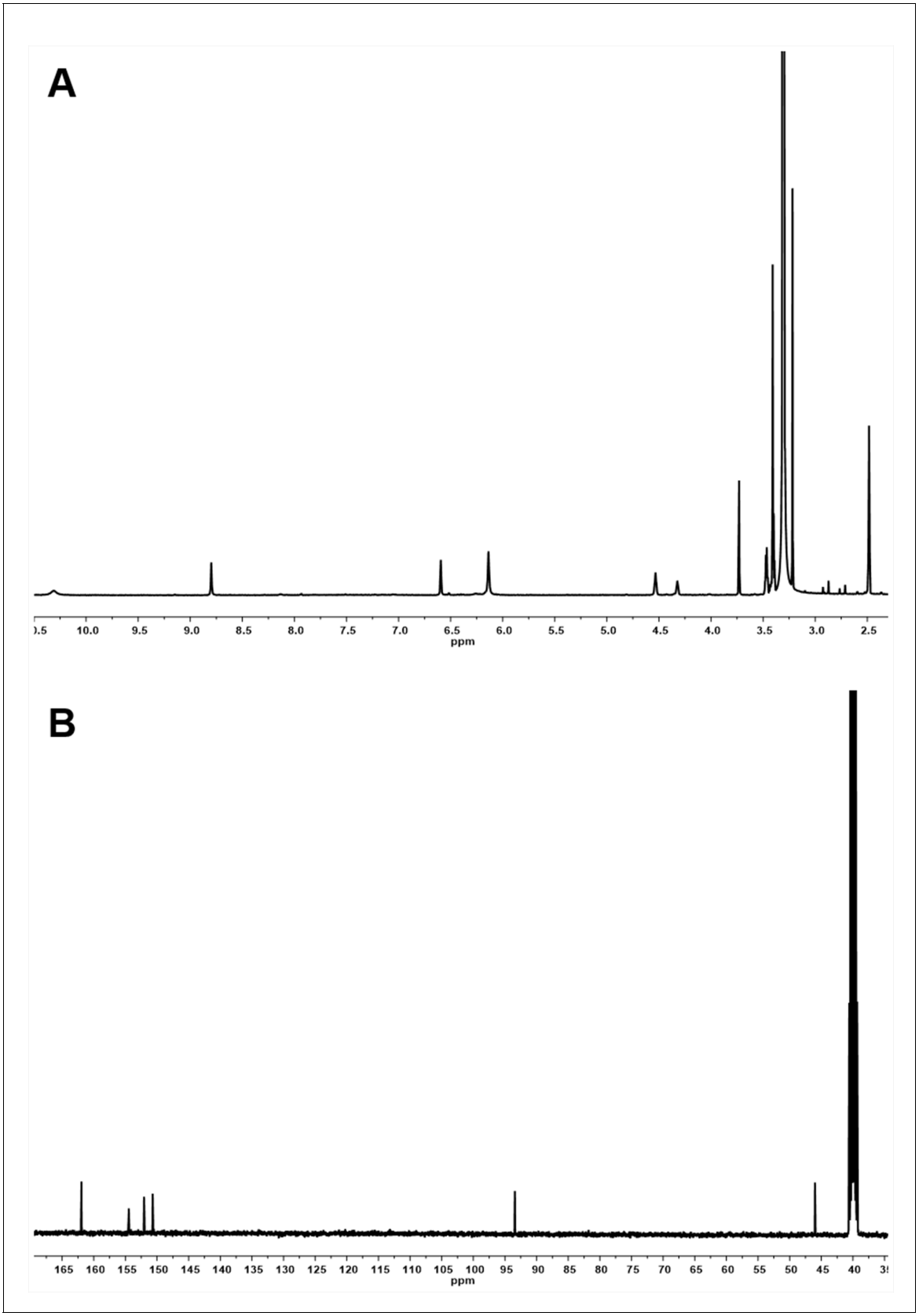


**Figure 2—figure supplement 1.** $^{1}H$ and $^{13}C$ NMR spectra of compound **3**. (**A**) $^{1}H$ NMR spectrum of compound **3** in DMSO-$d_6$. (**B**) $^{13}C$ NMR spectrum of compound **3** in DMSO-$d_6$.

*Figure 2—figure supplement 1 continued on next page*

*Figure 2—figure supplement 1 continued*

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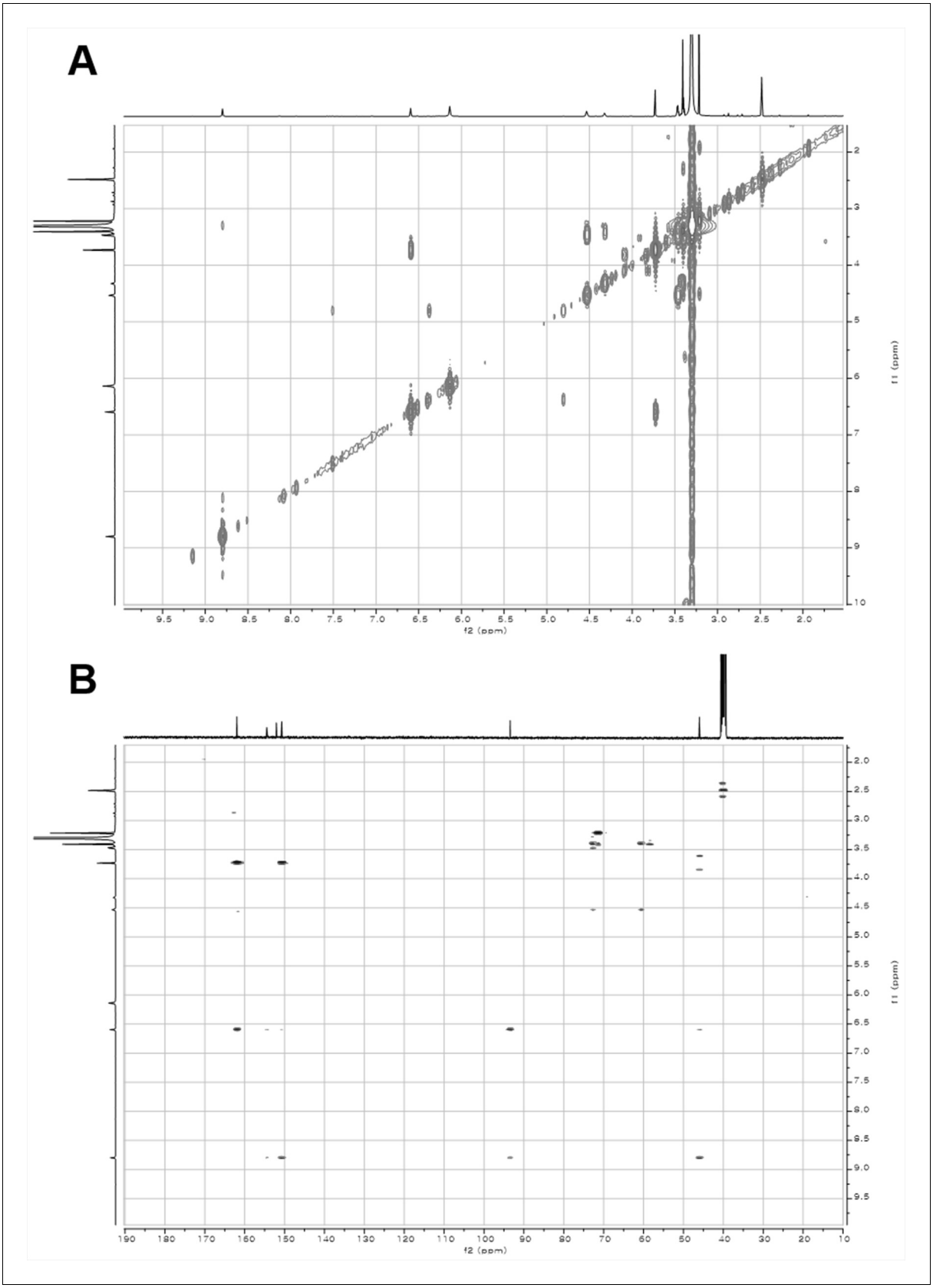
A
f2 (ppm)
f1 (ppm)
B
f2 (ppm)
f1 (ppm)

**Figure 2—figure supplement 2.** 2D- (gCOSY and gHMBCAD) NMR spectra of compound **3**. (**A**) gCOSY NMR spectrum of compound **3** in DMSO-$d_6$. (**B**) gHMBCAD NMR spectrum of compound **3** in DMSO-$d_6$.
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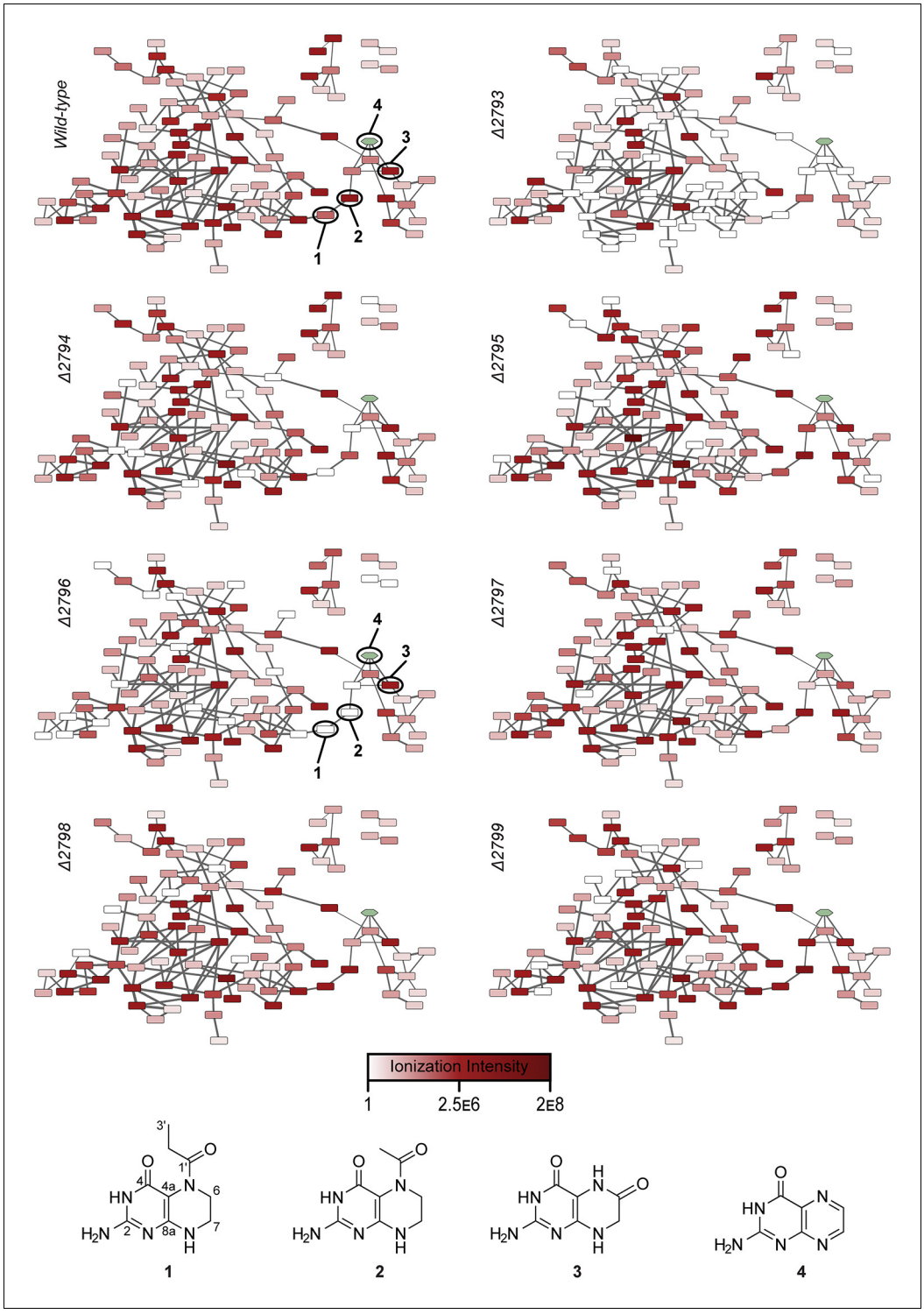



**Figure 3.** Relative abundances of wild-type pathway-dependent metabolites in wild-type and mutant pepteridine pathways. The average ionization intensity is depicted for each molecular feature under a given genetic condition (wild-type and Δ*plu2793* through Δ*plu2799*). Alterations in abundance are correlated with changes in nodal color intensity among genetic constructs, allowing visual assessment of product distributions for a given mutation. See ***Figure 3—figure supplement 2*** for information on mass of pathway-dependent metabolites. Structural characterization of compounds **1** and **2** is shown in ***Figure 3—figure supplements 4–10***, ***Table 2***, and the Materials and methods section.

DOI: 10.7554/eLife.25229.009

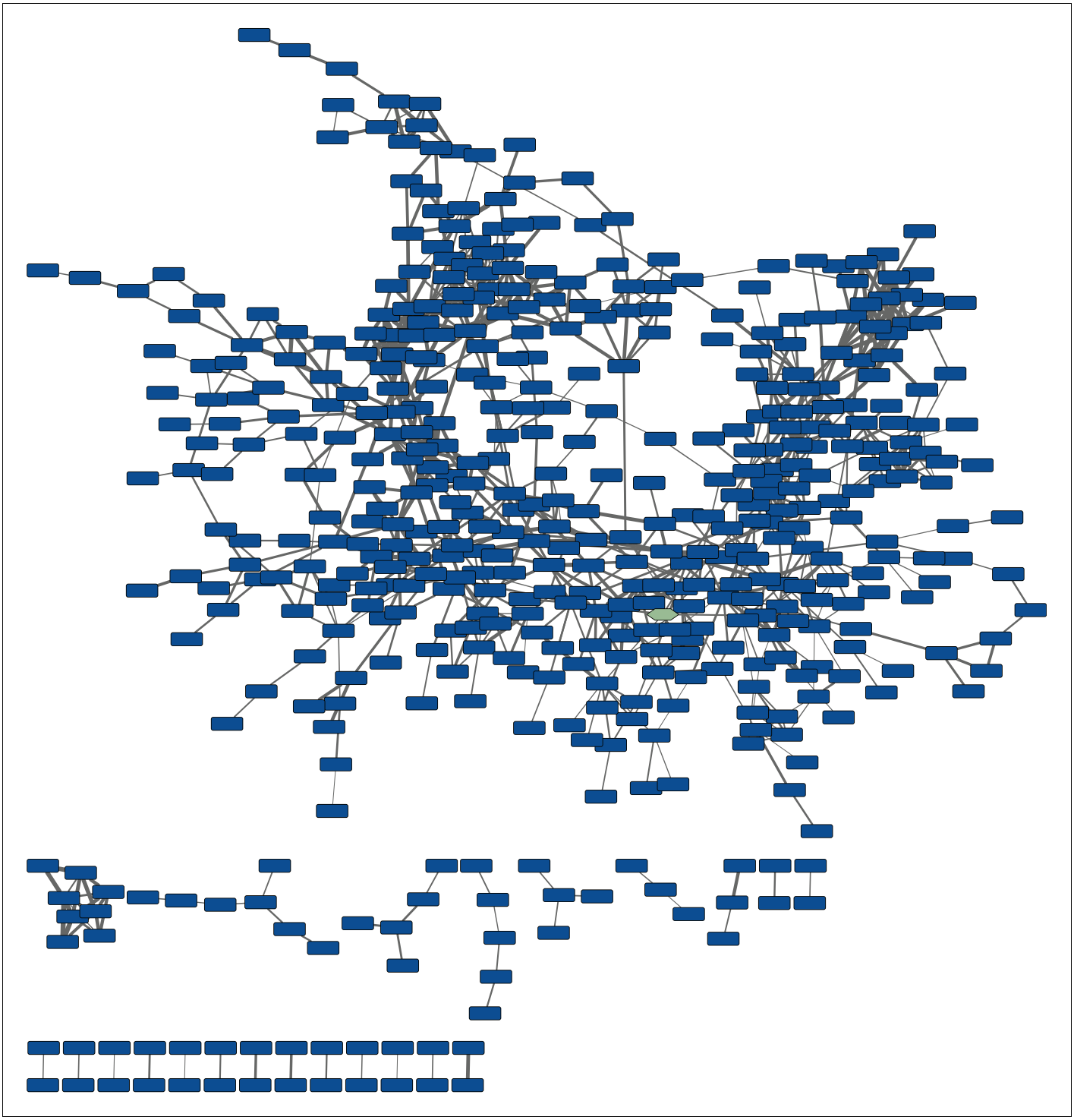


**Figure 3—figure supplement 1.** Untargeted molecular networking of culture extracts from cells harboring the wild-type pepteridine biosynthetic pathway. The node with the green hue represents pterin (**4**) networked into the untargeted wild-type map. This network includes abundant molecules from the pathway in addition to primary metabolites and media components.

DOI: 10.7554/eLife.25229.010

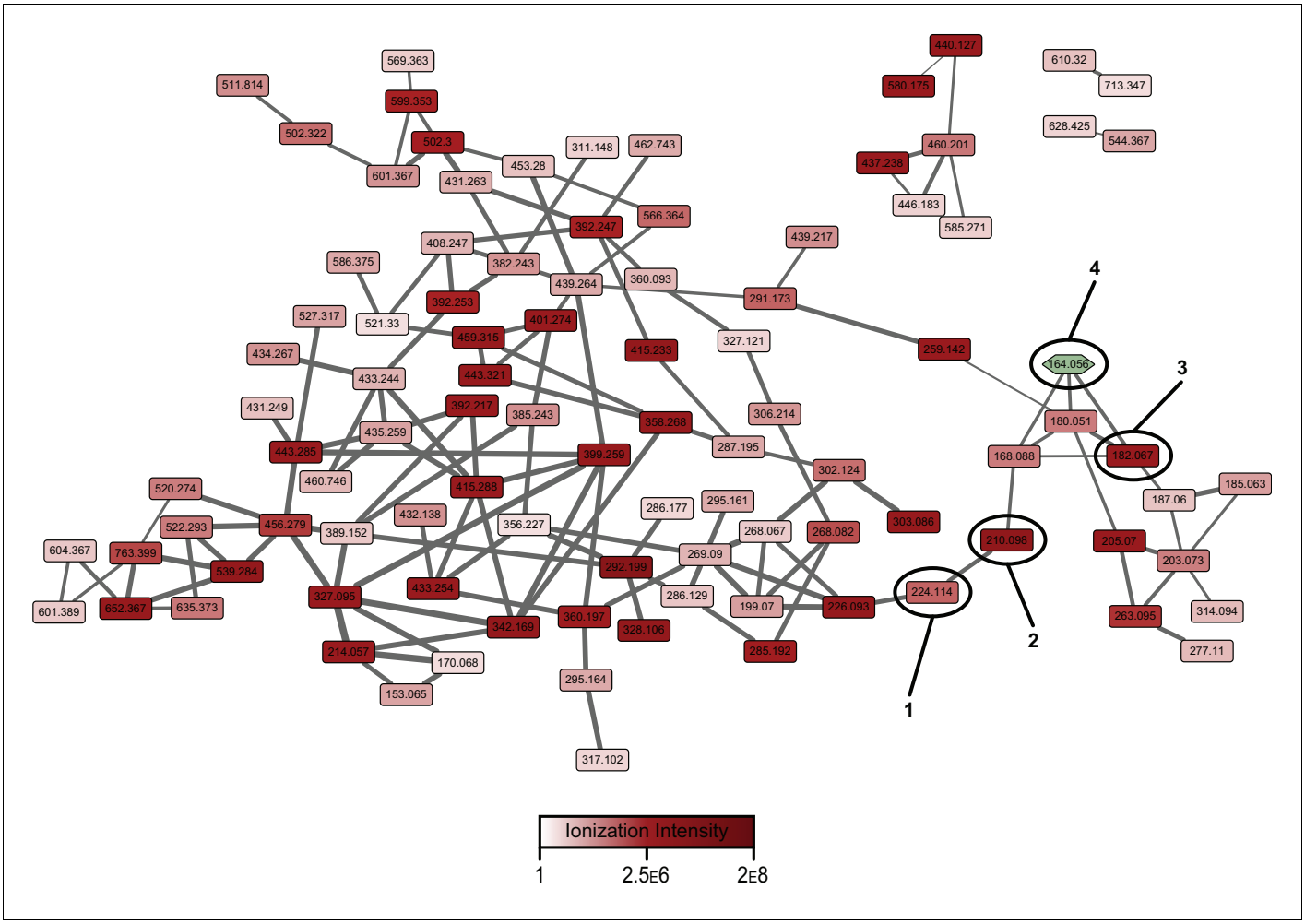



**Figure 3—figure supplement 2.** Pathway-targeted molecular networking of the wild-type pepteridine biosynthetic pathway. Comparative metabolomic analysis between control and experimental culture samples enabled targeting ($MS^2$) of molecular features dependent on the presence of the biosynthetic pathway.

DOI: 10.7554/eLife.25229.011

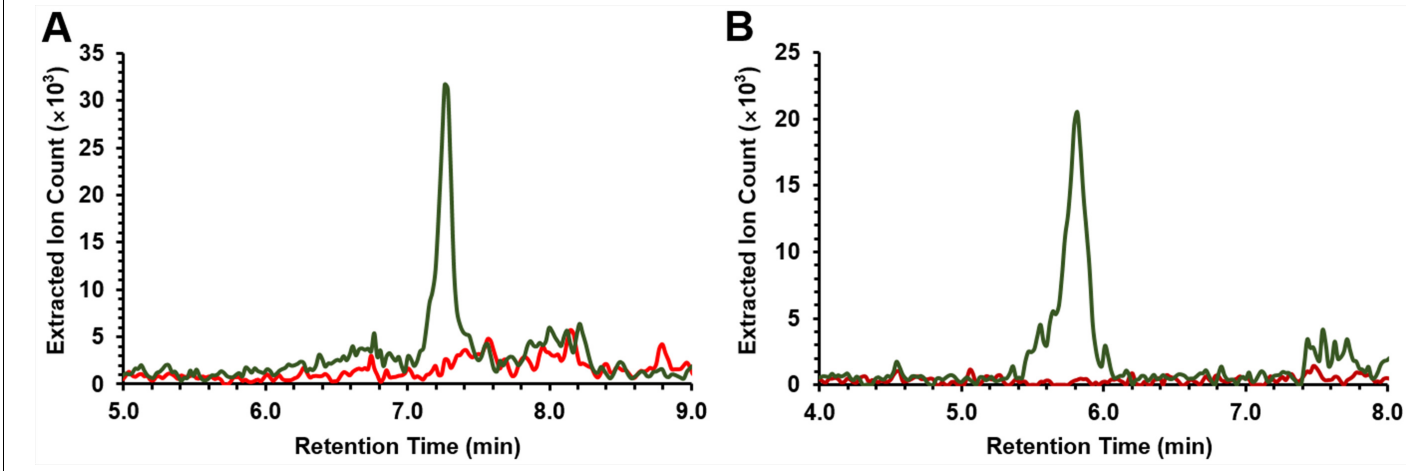



**Figure 3—figure supplement 3.** LC/MS Extracted Ion Count (EIC) chromatograms of compounds **1** (**A**) and **2** (**B**) from butanol extracts of the pepteridine heterologous expression strain. LC/MS traces were extracted with *m/z* 224 corresponding to compound **1** (**A**) and *m/z* 210 corresponding to compound **2** (**B**) from butanol extracts of the *E. coli* BAP1 culture broths carrying the wild-type pepteridine pathway (green) or the empty vector (pET28a, red).

DOI: 10.7554/eLife.25229.012

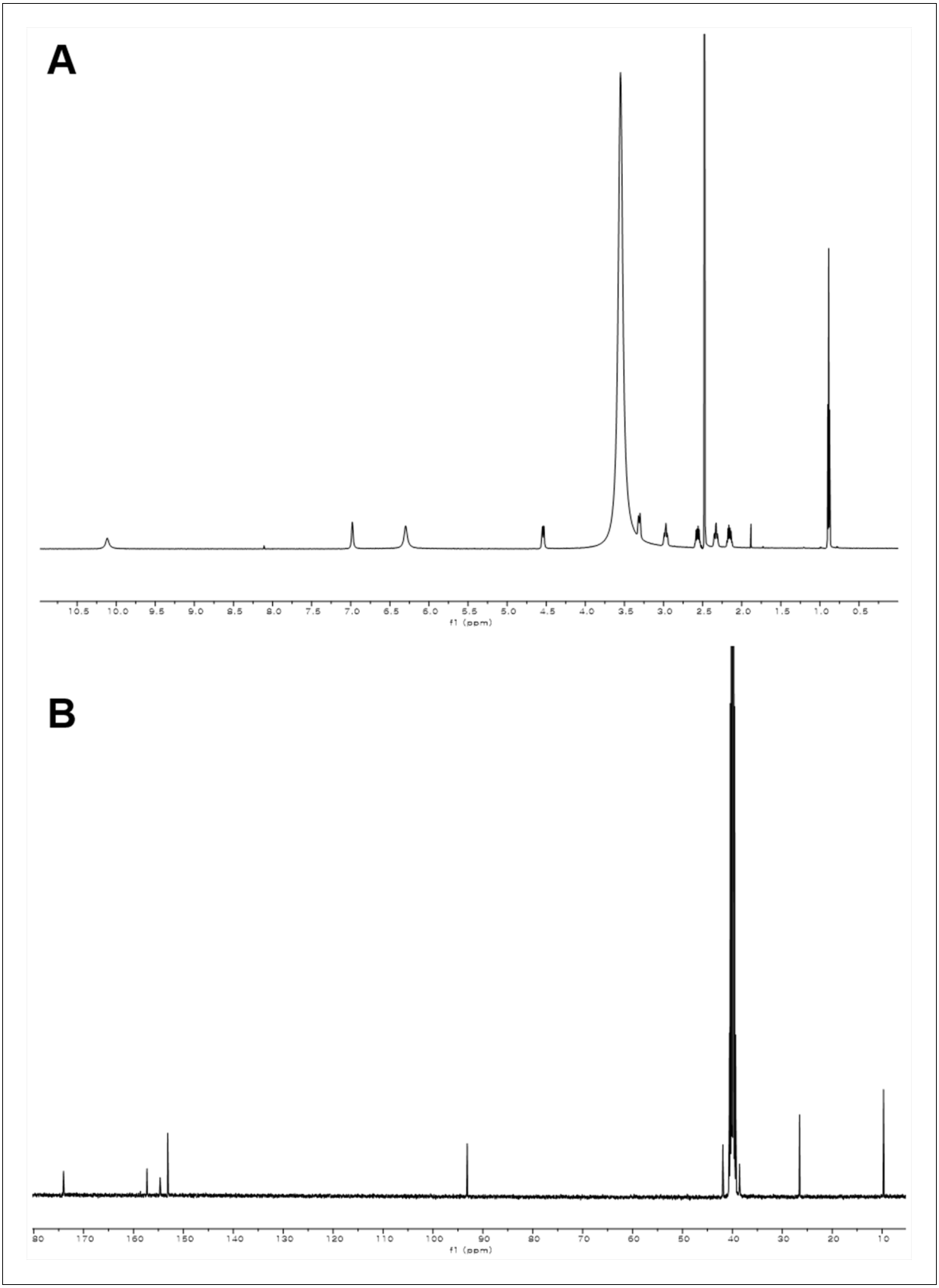

A
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)
B
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

**Figure 3—figure supplement 4.** $^{1}H$ and $^{13}C$ NMR spectra of compound **1**. (**A**) $^{1}H$ NMR spectrum of compound **1** in DMSO-$d_6$. (**B**) $^{13}C$ NMR spectrum of compound **1** in DMSO-$d_6$.
DOI: 10.7554/eLife.25229.013

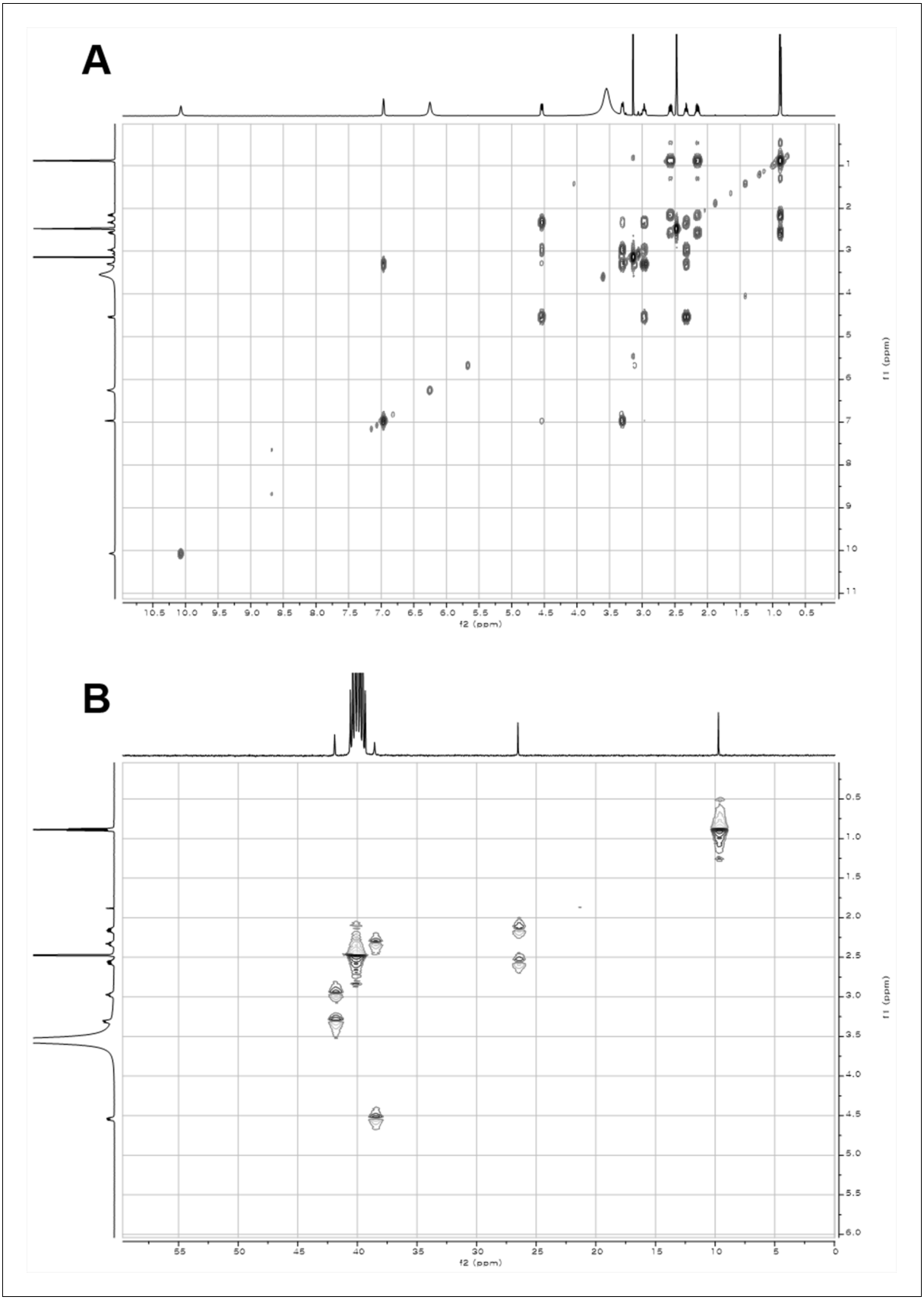

A
f2 (ppm)
f1 (ppm)
B
f2 (ppm)
f1 (ppm)

**Figure 3—figure supplement 5.** 2D- (gCOSY and gHSQCAD) NMR spectra of compound **1**. (**A**) gCOSY NMR spectrum of compound **1** in DMSO-$d_6$. (**B**) gHSQCAD NMR spectrum of compound **1** in DMSO-$d_6$.
DOI: 10.7554/eLife.25229.014

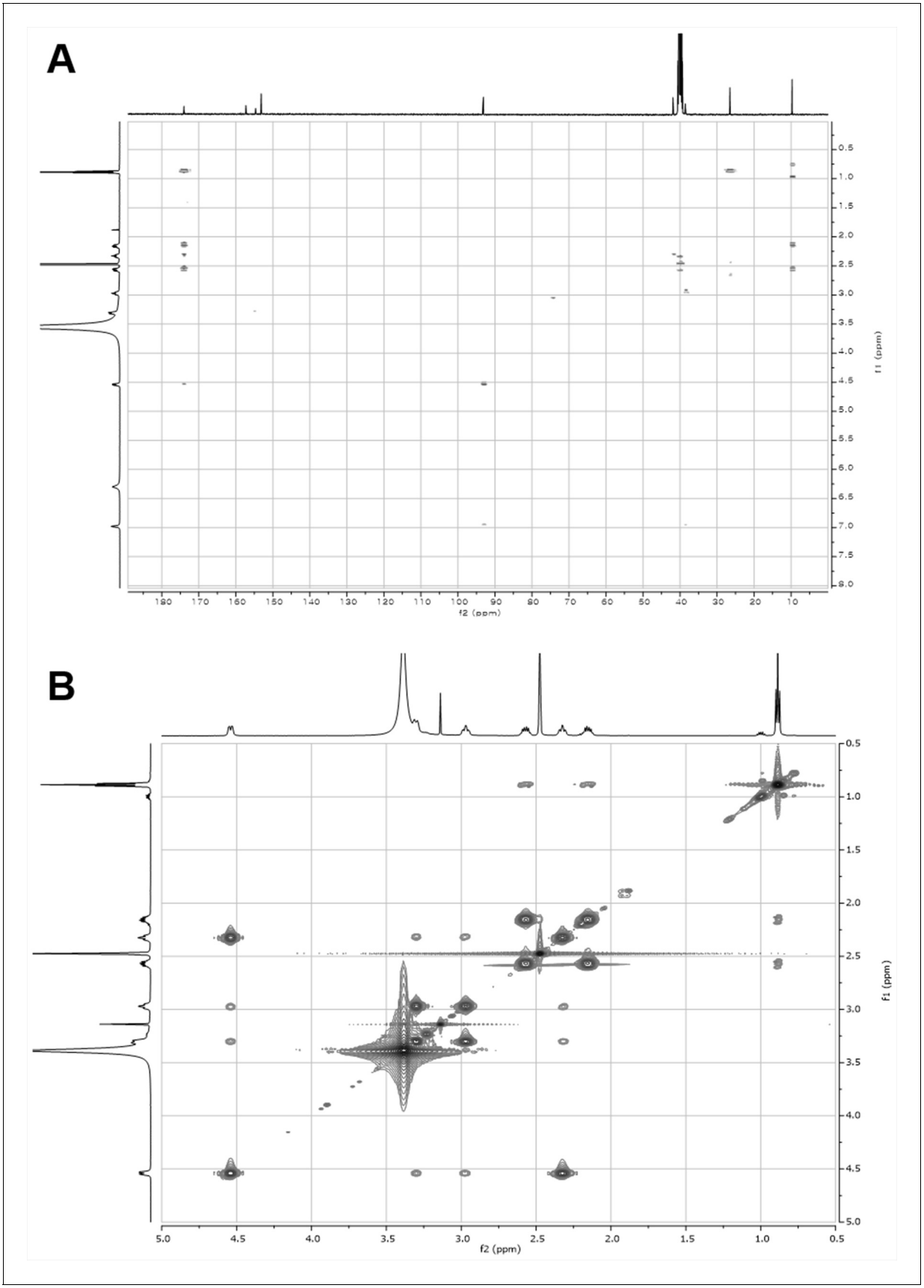

A
f2 (ppm)
f1 (ppm)
B
f2 (ppm)
f1 (ppm)

**Figure 3—figure supplement 6.** 2D- (gHMBCAD and NOESY) NMR spectra of compound **1**. (**A**) gHMBCAD NMR spectrum of compound **1** in DMSO-$d_6$. (**B**) NOESY NMR spectrum of compound **1** in DMSO-$d_6$.
DOI: 10.7554/eLife.25229.015

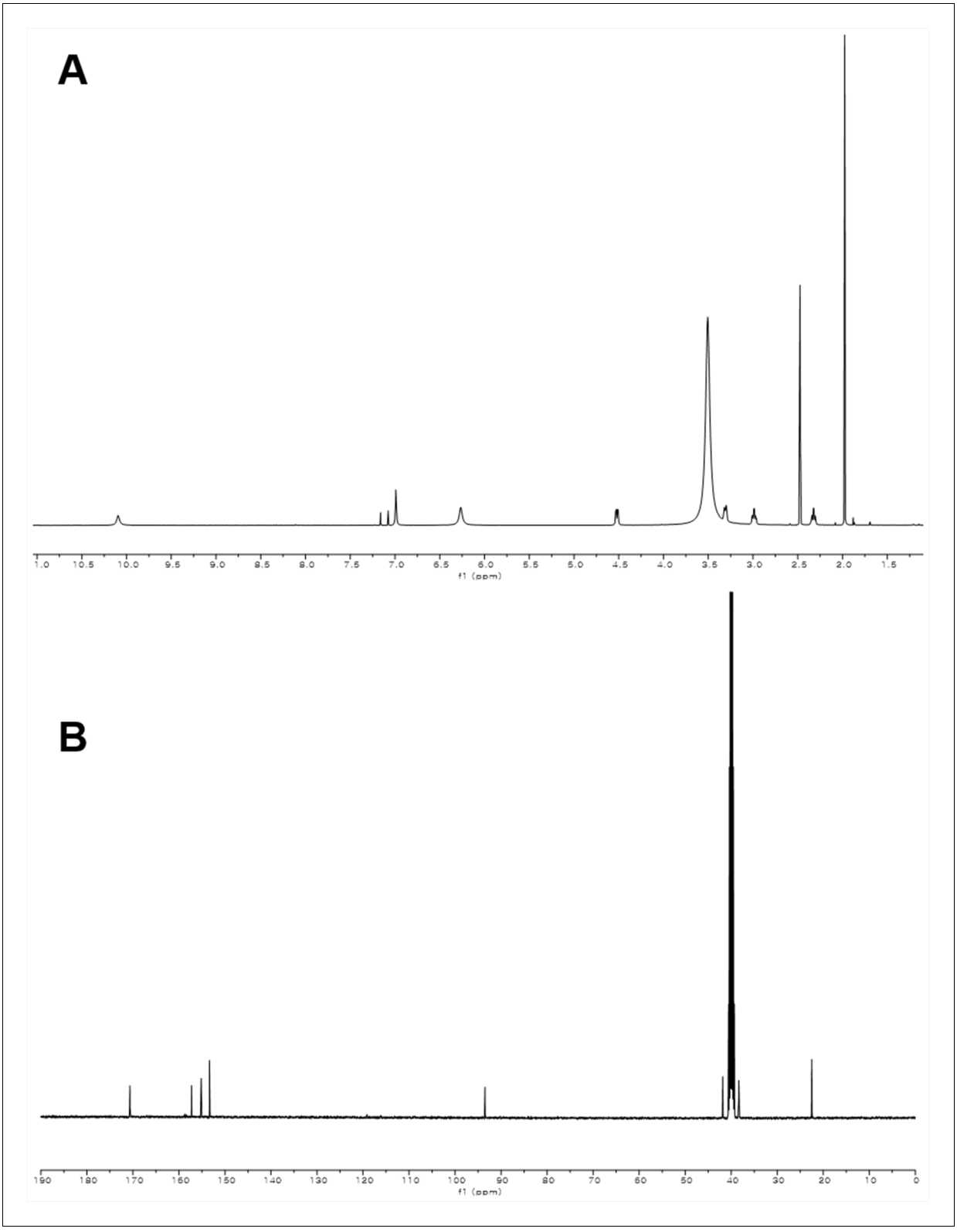
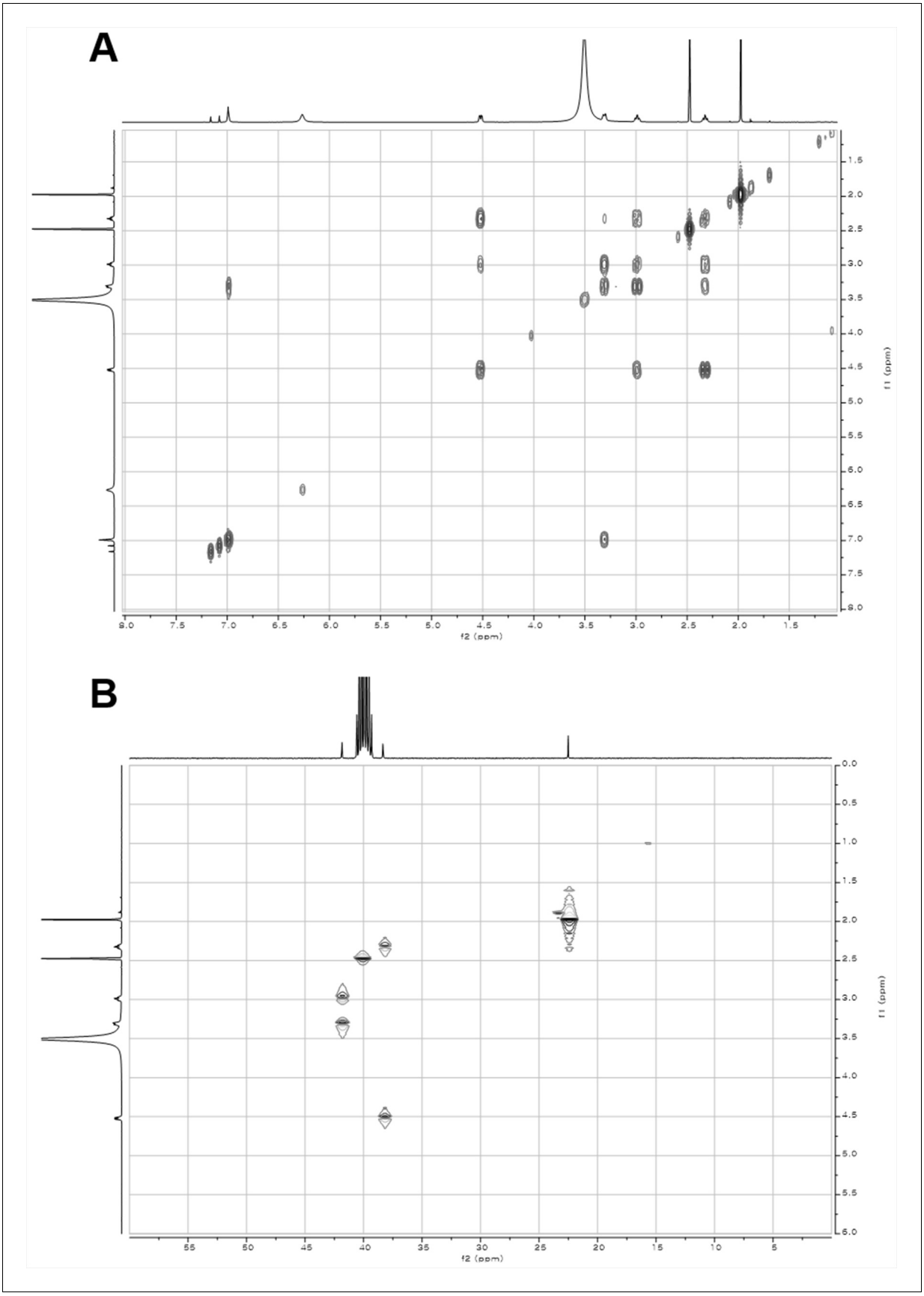



**Figure 3—figure supplement 7.** $^{1}H$ and $^{13}C$ NMR spectra of compound **2**. (**A**) $^{1}H$ NMR spectrum of compound **2** in DMSO-$d_6$. (**B**) $^{13}C$ NMR spectrum of compound **2** in DMSO-$d_6$.
DOI: 10.7554/eLife.25229.016


A
f2 (ppm)
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B
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**Figure 3—figure supplement 8.** 2D- (gCOSY and gHSQCAD) NMR spectra of compound **2**. (**A**) gCOSY NMR spectrum of compound **2** in DMSO-$d_6$. (**B**) gHSQCAD NMR spectrum of compound **2** in DMSO-$d_6$.
DOI: 10.7554/eLife.25229.017

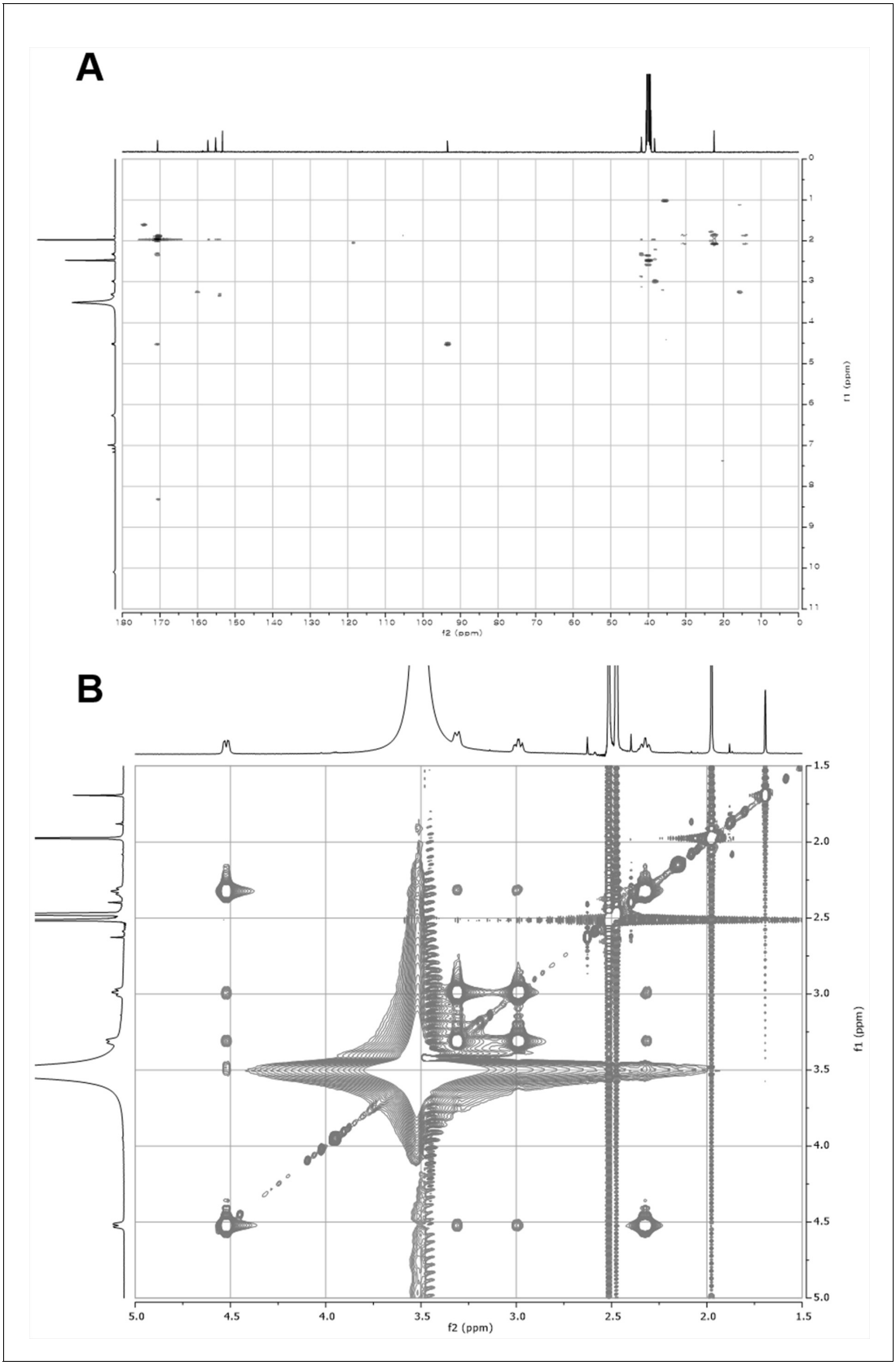


**Figure 3—figure supplement 9.** 2D- (gHMBCAD and NOESY) NMR spectra of compound **2**. (**A**) gHMBCAD NMR spectrum of compound **2** in DMSO-$d_6$. (**B**) NOESY NMR spectrum of compound **2** in DMSO-$d_6$.

*Figure 3—figure supplement 9 continued on next page*

*Figure 3—figure supplement 9 continued*

DOI: 10.7554/eLife.25229.018

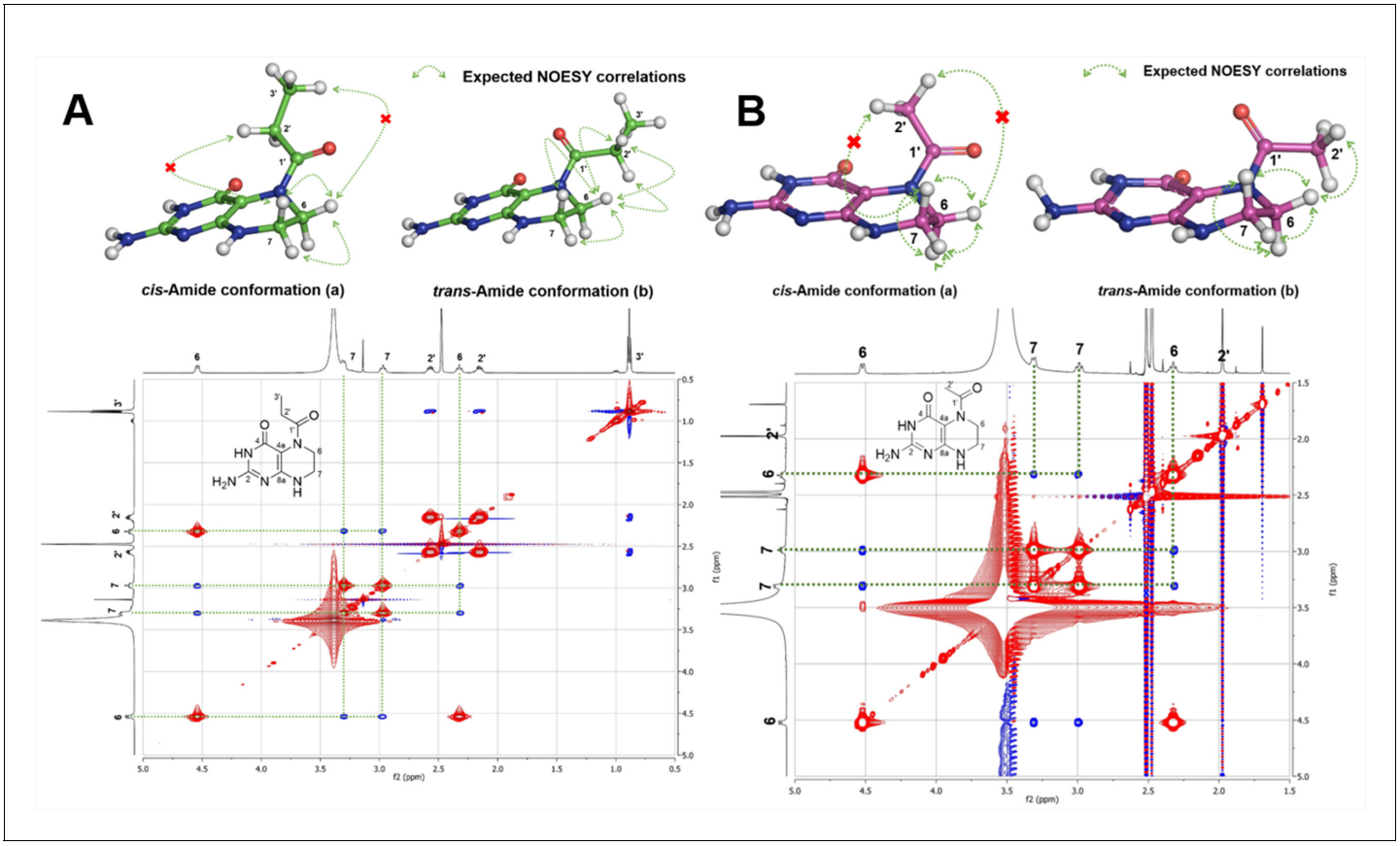


**Figure 3—figure supplement 10.** Amide conformation of compounds **1** and **2**. (**A**) Plausible *cis*- (a) and *trans*- (b) amide conformations of compound **1** (top) and interpretation of 2D NOESY spectral data of compound **1** (bottom), (**B**) Plausible *cis*- (a) and *trans*- (b) amide conformations of compound **2** (top) and interpretation of 2D NOESY spectral data of compound **2** (bottom).

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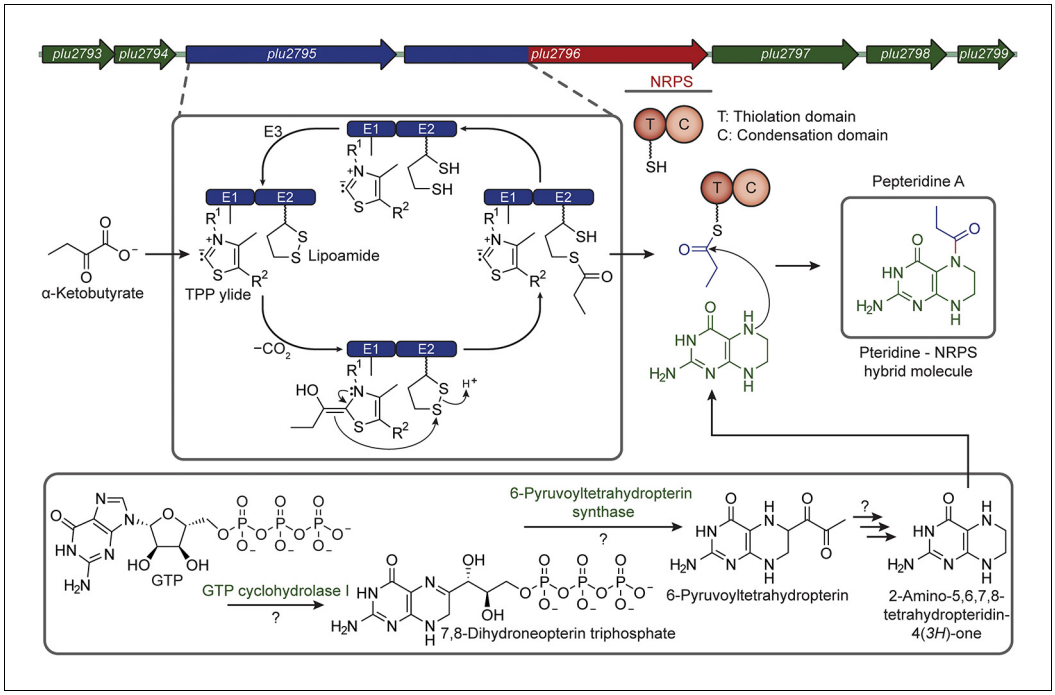


**Figure 4.** Proposed pepteridine biosynthesis. α-Ketobutyrate is processed by the atypical dehydrogenase-NRPS-like complex to generate a propionyl-loaded carrier protein (blue). Pteridine enzymes interact with the metabolism of the host to generate tetrahydropterin (green). The NRPS C domain couples these substrates to form the *cis*-amide bond (red) as illustrated for **1**.

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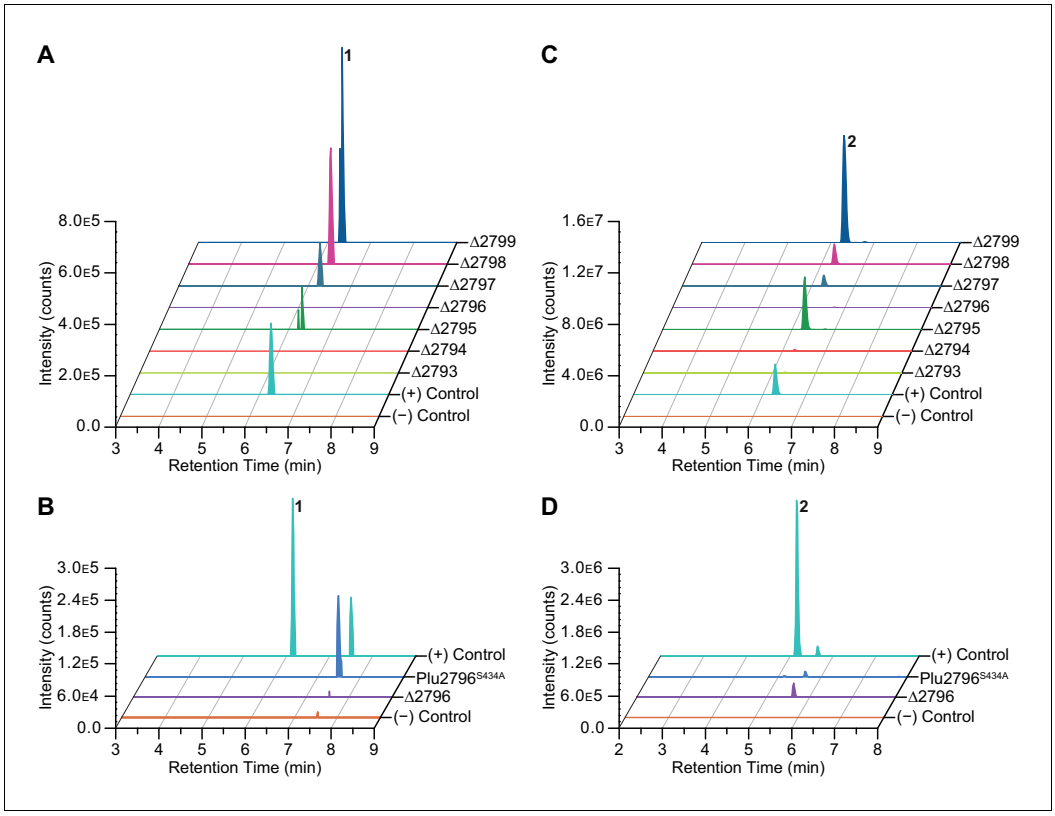


**Figure 5.** Gene deletion and NRPS inactivation analyses on **1** (**A–B**) and **2** (**C–D**). (**A–B**) Extracted ion chromatograms of **1** for wild-type and mutants are shown. (**C–D**) Extracted ion chromatograms of **2** for wild-type and mutants are shown.

DOI: 10.7554/eLife.25229.022

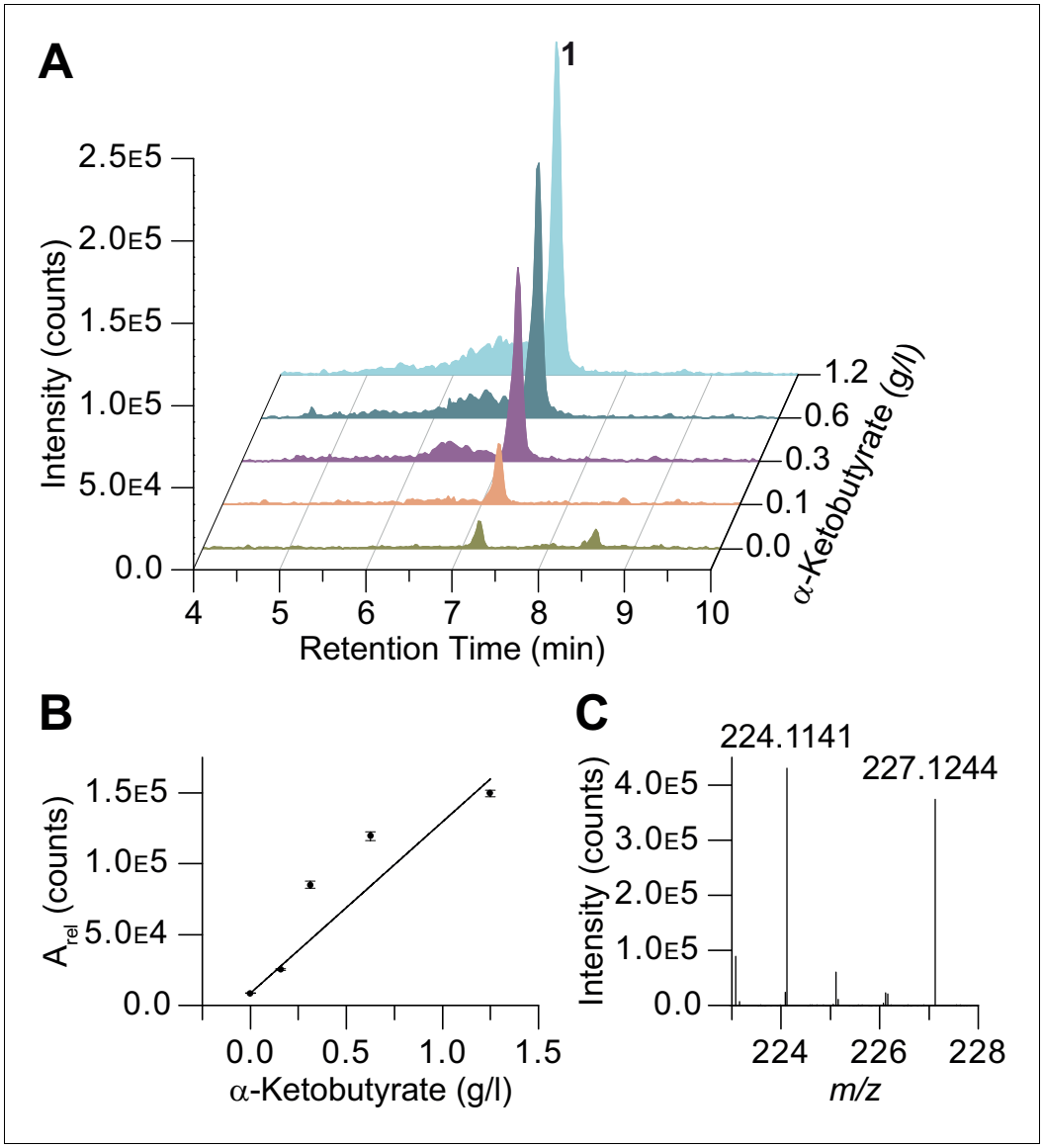


**Figure 6.** α-Ketobutyrate feeding studies on **1**. (**A–B**) Production enhancement of **1** in α-ketobutyrate supplementation studies ($A_{rel}$, relative integration value). (**C**) $^{13}C_4$-α-ketobutyrate supplementation leads to $^{13}C_3$ incorporation in **1** consistent with the proposed biosynthesis.

DOI: 10.7554/eLife.25229.023

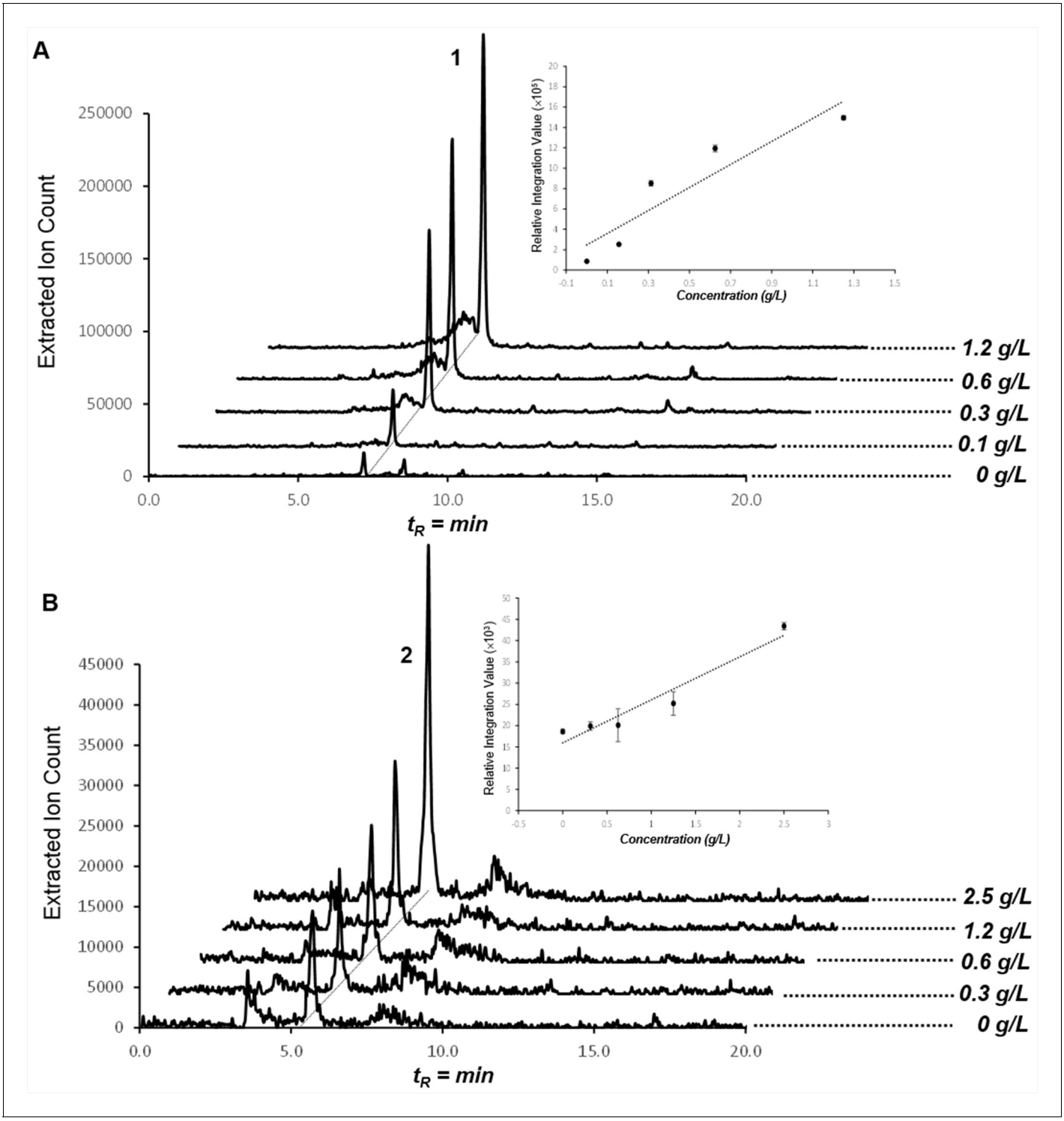


**Figure 6—figure supplement 1.** Production of compounds 1 and 2 in dose-dependent α-ketobutyrate (A) and pyruvate (B) feeding studies. HPLC/MS traces were extracted with *m/z* 224 corresponding to compound 1 (A) and *m/z* 210 corresponding to compound 2 (B) from butanol extracts of the culture broths fed with varying concentrations of α-ketobutyrate (A) and pyruvate (B). Dose-response plots for 1 and 2 were determined using extracted peak integration values.

DOI: 10.7554/eLife.25229.024

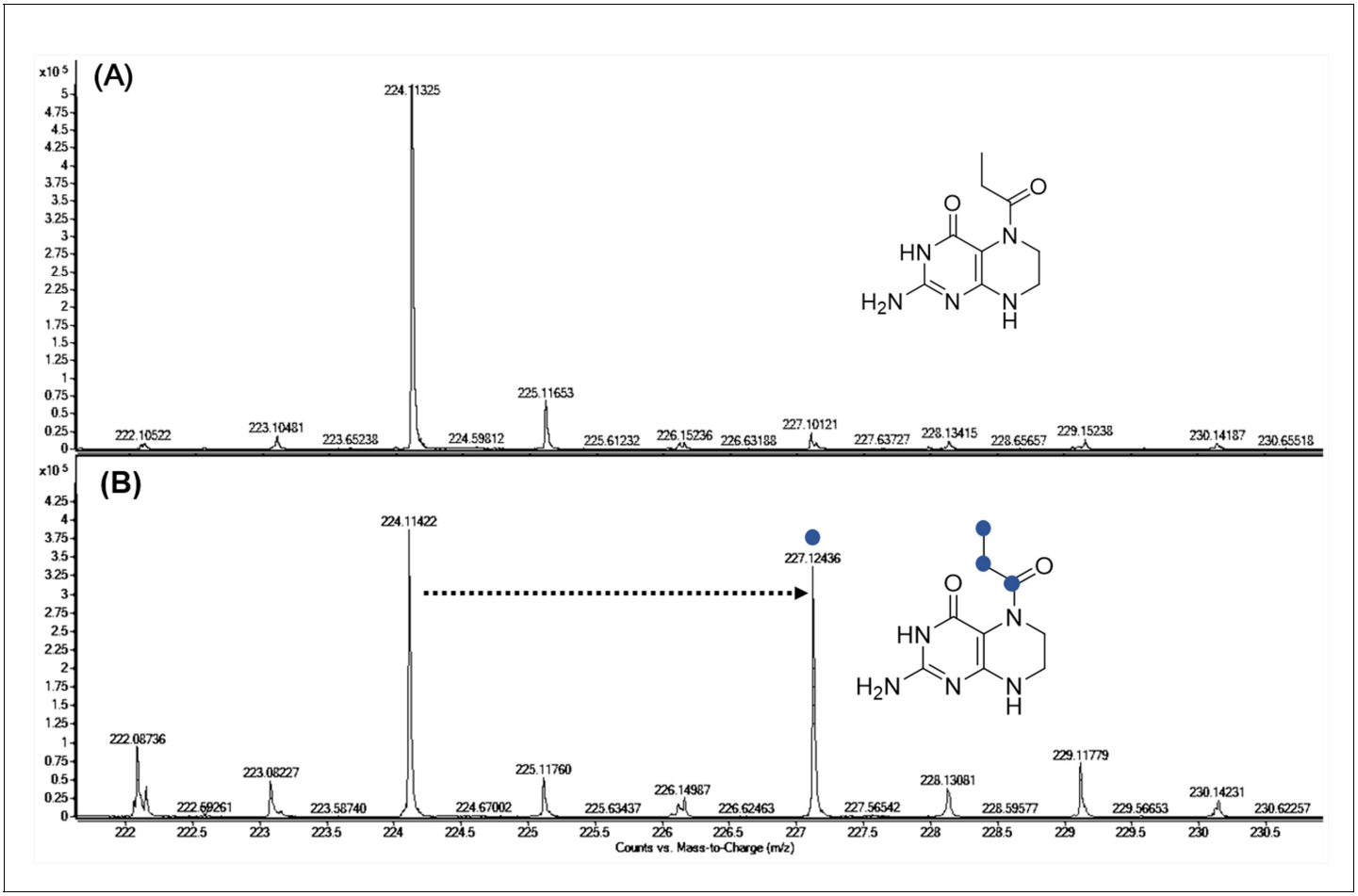


**Figure 6—figure supplement 2.** Characterization of $^{13}C_4$-α-ketobutyrate incorporation in compound **1**. Incorporation of $^{13}C_4$-α-ketobutyrate into compound **1** (**B**) led to a clear 3 Da mass shift of native **1** (**A**) via oxidative decarboxylation of α-ketobutyrate.
DOI: 10.7554/eLife.25229.025

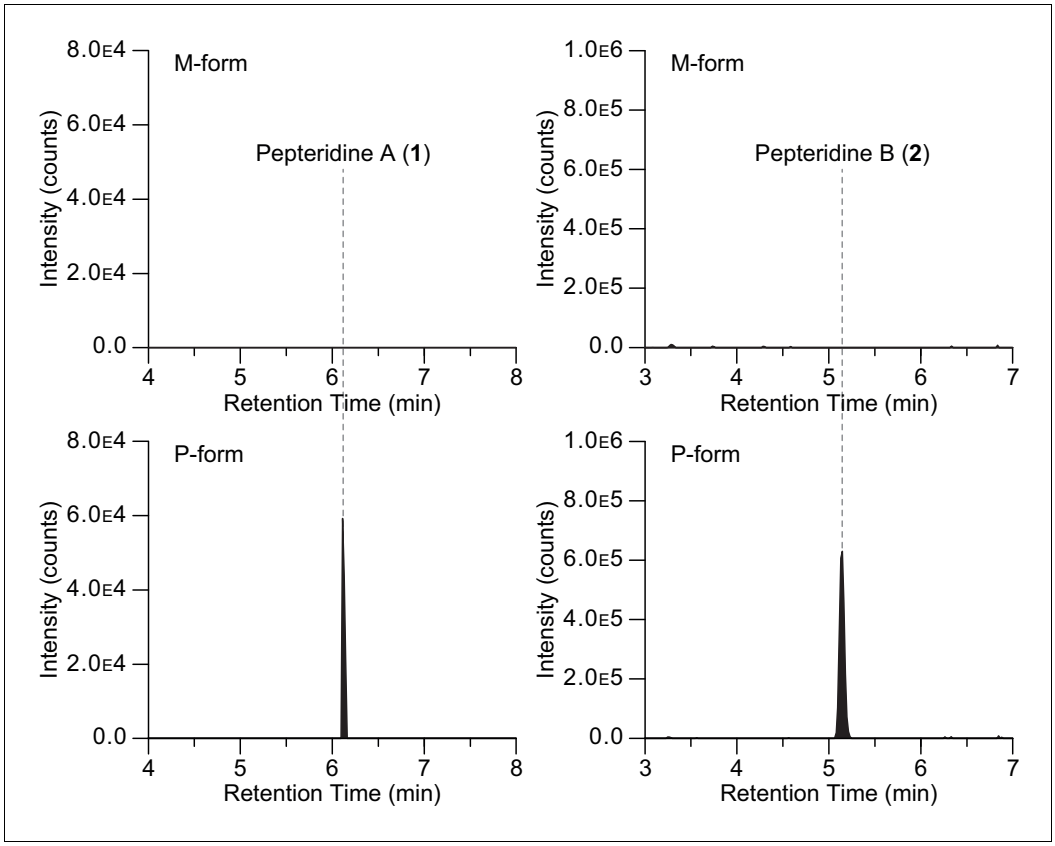


**Figure 7.** Extracted ion counts LC/HR-ESI-QTOF-MS analysis of pepteridines A (left panel) and B (right panel) from two genetically engineered *P. luminescens* strains locked in the phenotypic variants M-form (top) and P-form (bottom).

DOI: 10.7554/eLife.25229.026

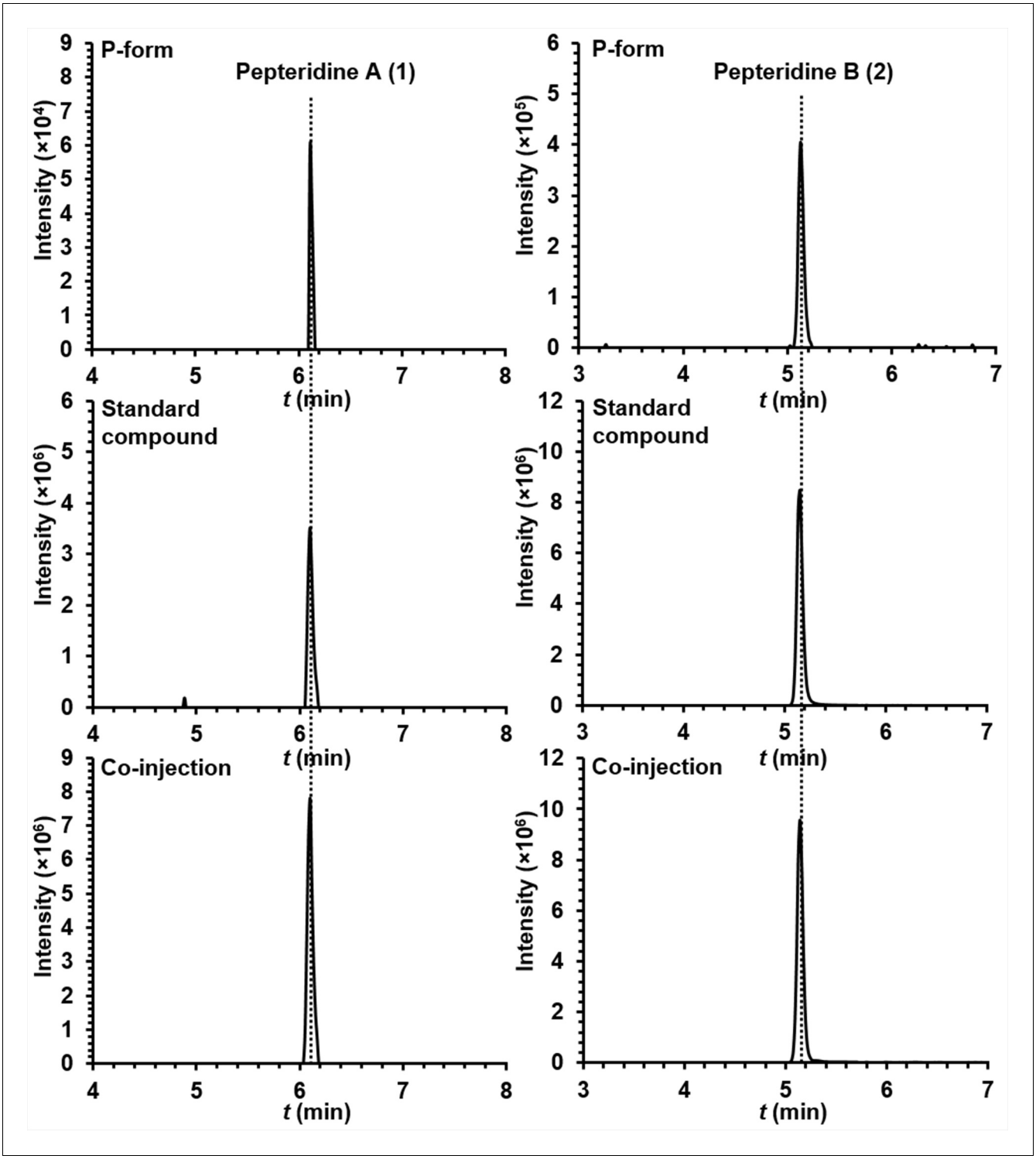


**Figure 7—figure supplement 1.** Extracted ion counts chromatograms from LC/HR-ESI-QTOF-MS analysis of pepteridines A (left panel) and B (right panel) from the butanol extracts of P-form culture broth (top), standard compounds (middle), and co-injection (bottom).
DOI: 10.7554/eLife.25229.027

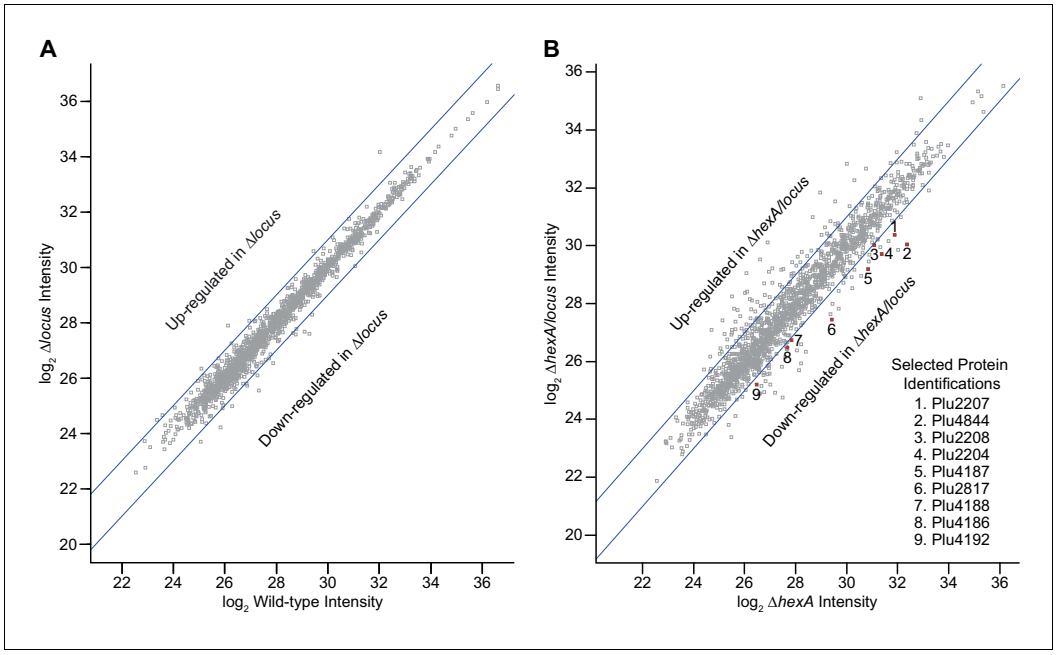


**Figure 8.** Quantitative proteomic analysis of a *Δlocus* strain in a wild-type background (**A**) and *ΔhexA* background (**B**).
DOI: 10.7554/eLife.25229.028
The following source data is available for figure 8:

**Source data 1.** Proteins increased in WT vs. WT*Δlocus* strains by LC-MS/MS.
DOI: 10.7554/eLife.25229.029
**Source data 2.** Proteins increased in WT*Δlocus* vs. WT strains by LC-MS/MS.
DOI: 10.7554/eLife.25229.030
**Source data 3.** Proteins increased in *ΔhexA* vs. *ΔhexAΔlocus* strains by LC-MS/MS.
DOI: 10.7554/eLife.25229.031
**Source data 4.** Proteins increased in *ΔhexAΔlocus* vs. *ΔhexA* strains by LC-MS/MS.
DOI: 10.7554/eLife.25229.032
**Source data 5.** All proteins observed in WT and WT*Δlocus* strains by LC-MS/MS.
DOI: 10.7554/eLife.25229.033
**Source data 6.** All proteins observed in *ΔhexA* and *ΔhexAΔlocus* strains by LC-MS/MS.
DOI: 10.7554/eLife.25229.034