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Figures and figure supplements

A bacterial sulfonolipid triggers multicellular development in the closest living relatives of animals

Rosanna A Alegado, et al.

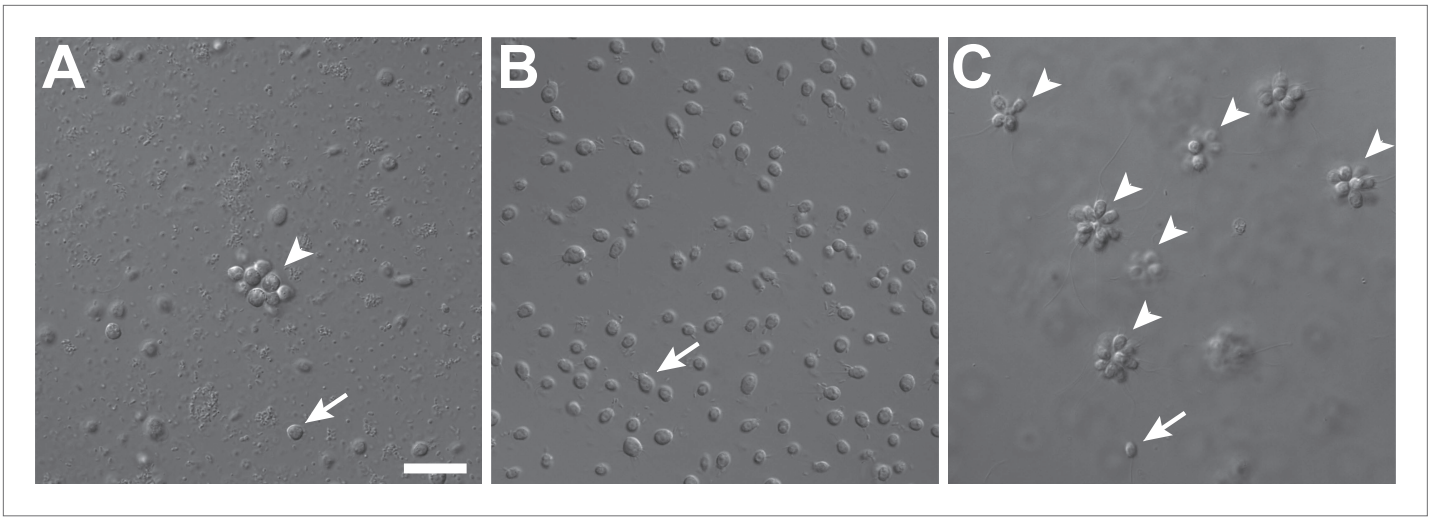

Figure 1. Rosette colony development in *S. rosetta* is regulated by *A. machipongonensis*. (**A**) The original culture of *S. rosetta*, ATCC 50818, contains diverse co-isolated environmental bacteria and forms rosette colonies (arrowheads) rarely. (**B**) Treatment of ATCC50818 with a cocktail of antibiotics reduced the bacterial diversity and yielded an *S. rosetta* culture line, RCA, in which rosette colonies never formed. (Representative single cells indicated by arrows.) (**C**) Addition of *A. machipongonensis* to RCA cultures was sufficient to induce rosette development. Scale bar, 2 μm.

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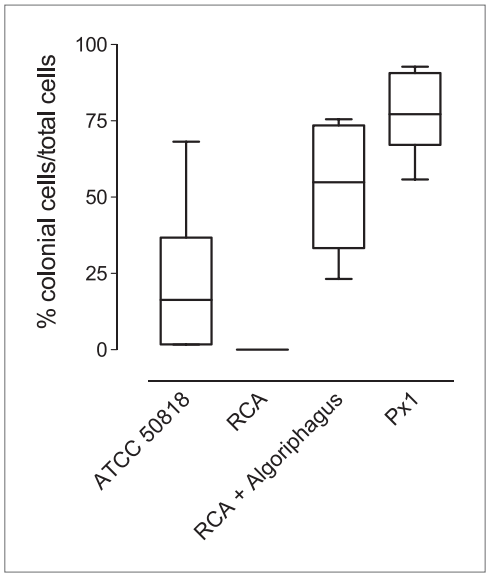

Figure 1—figure supplement 1. Frequency of rosette colonies in *S. rosetta* environmental isolate ATCC 50818, RCA with and without *A. machipongonensis* and a monoxenic line with *A. machipongonensis* feeder bacteria (Px1). Altering bacterial diversity in *S. rosetta* cultures alters the frequency of rosette colonies. Data are the whisker-box plots of the frequency of colonial cells in ATCC 50818 and a monoxenic culture of *S. rosetta* fed only *A. machipongonensis* bacteria (Px1) for three experiments.

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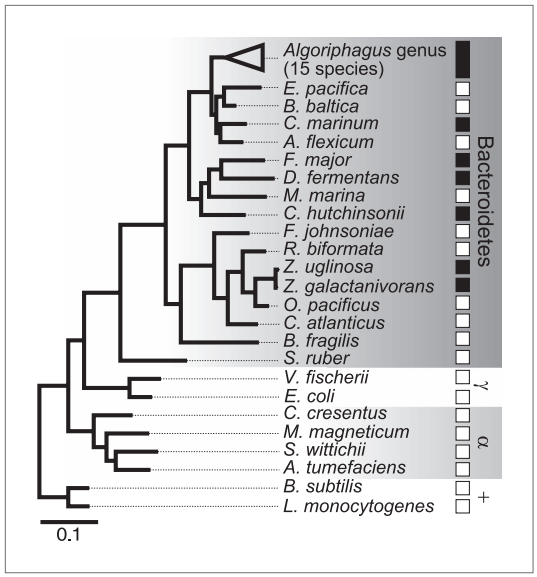

Figure 2. Diverse members of the Bacteroidetes phylum induce rosette colony development. A maximum likelihood phylogeny inferred from 16S rDNA gene sequences reveals the evolutionary relationships among *A. machipongonensis*, other members of the Bacteroidetes phylum, and representative γ-proteobacteria (γ), α-proteobacteria (α), and Gram-positive (+) bacteria. All 15 members of the *Algoriphagus* genus (***Table 1***), as well as six other species in the Bacteroidetes phylum, were competent to induce colony development (filled squares). In contrast, no species outside of Bacteroidetes and most of the non-*Algoriphagus* bacteria tested failed to induce rosette colony development (open squares). Scale bar, 0.1 substitutions per nucleotide position.
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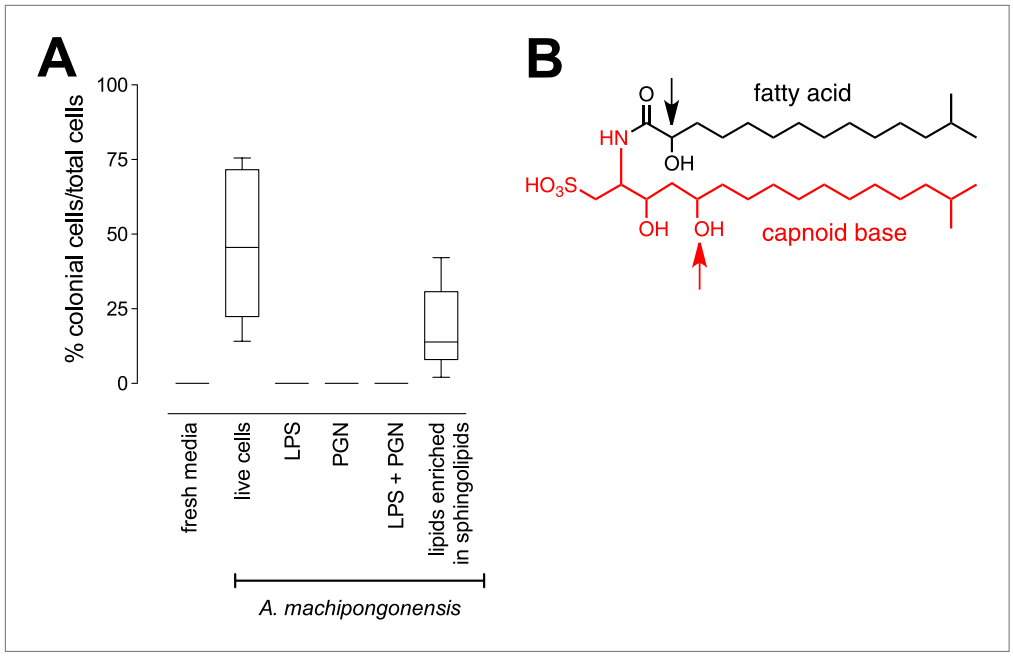

Figure 3. RIF-1, a sulfonolipid that induces rosette colony development. (**A**) Rosette colony development is induced by live *A. machipongonensis* and the sphingolipid-enriched lipid fraction (20 mg mL^{-1}), but not by fresh medium, *A. machipongonensis* LPS (10 mg mL^{-1}), PGN (50 mg mL^{-1}), or LPS+PGN. Shown are the whisker-box plots of the % colonial cells/total cells under each condition in three independent experiments. (**B**) The molecular structure of RIF-1 deduced from MS and 1D- and 2D-NMR data. The RIF-1 structure, 3,5-dihydroxy-2-(2-hydroxy-13-methyltetradecanamido)-15-methylhexadecane-1-sulfonic acid, has two parts: a base (shown in red) that defines the capnine, and a fatty acid (shown in black). Features that distinguish RIF-1 from other known capnoids are shown with colored arrows: the 2-hydroxy on the fatty acid (black) and the 5-hydroxy on the capnine base (red).
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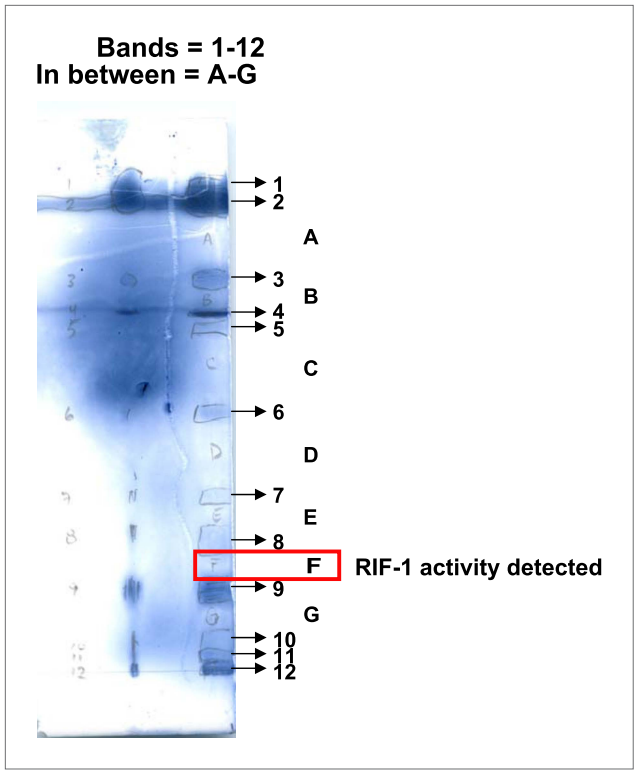

Figure 3—figure supplement 1. Separation of *A. machipongonensis* sphingolipids by thin layer chromatography (TLC). Lipids enriched in sphingolipids were separated by TLC after visualization with ammonium molybdate in 10% H_2SO_4. Bands (1-12) as well as regions between bands (A-F) were tested for morphogenic activity. Region F possessed activity and was further purified.
DOI: 10.7554/eLife.00013.009

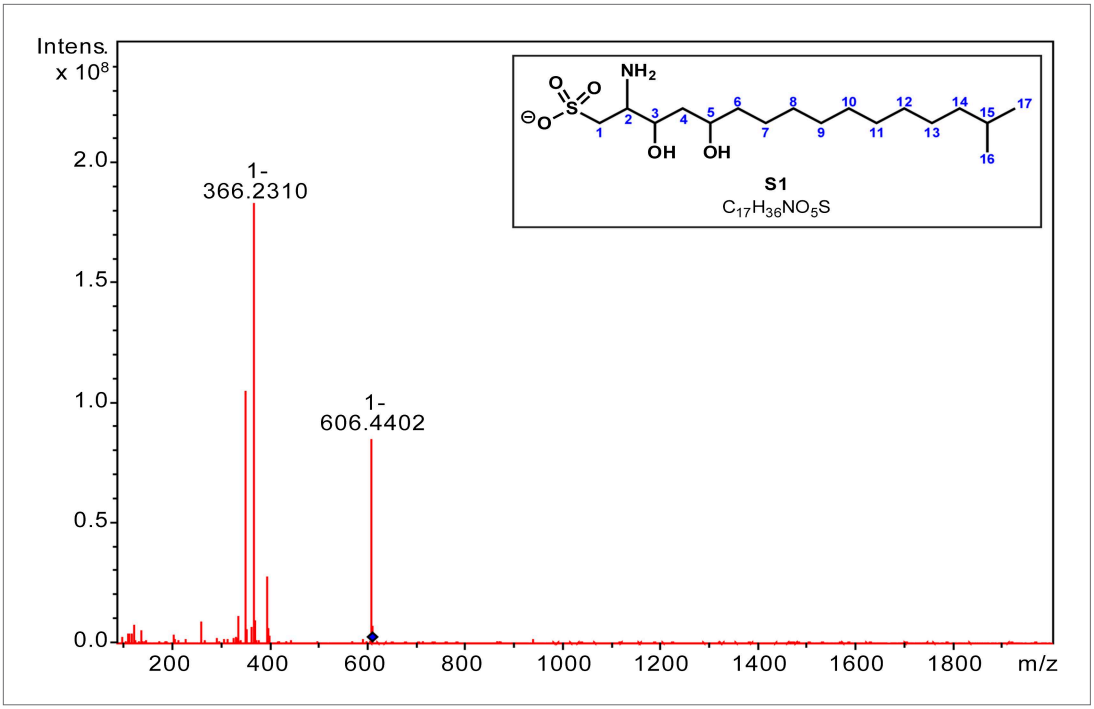

Figure 3—figure supplement 2. MS/MS analysis of RIF-1. A major fragment derived from *m/z* = 606 (M-H) in the MS/MS spectrum of RIF-1 corresponds to amino-sulfonic acid **S1**. **HRMS** *m/z* calcd for $C_{17}H_{36}NO_5S$ (M-H): 366.23142. Found: 366.2310 $(M-H)^-$.

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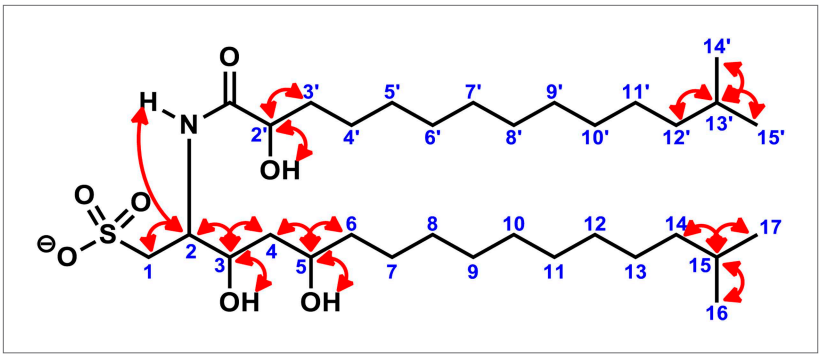

Figure 3—figure supplement 3. Key two-dimensional (2D) correlations of RIF-1: Observed COSY correlations. Red double-head arrows show key ^{3}J or ^{4}J H-H correlations in the head regions (1 to 6 and 2′ to 3′) of the fatty acid and the capnine base and in the tail regions with geminal dimethyl groups (14 to 17 and 12′ to 15′).

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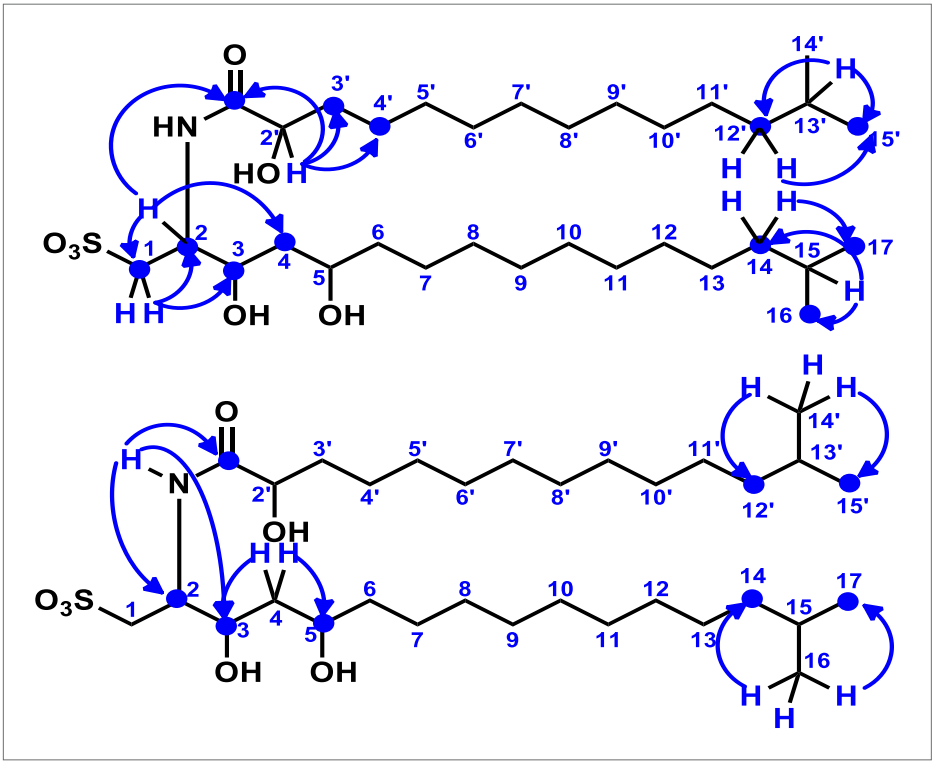

Figure 3—figure supplement 4. Key two-dimensional (2D) correlations of RIF-1: Observed HMBC spin system. Blue single-head arrows show key ^{2}J or ^{3}J H-C correlations in the head and tail regions of the fatty acid and capnine base. The correlations between C-1′ and H-2/N-H demonstrated that the fatty acid and capnine base are joined through an amide bond.

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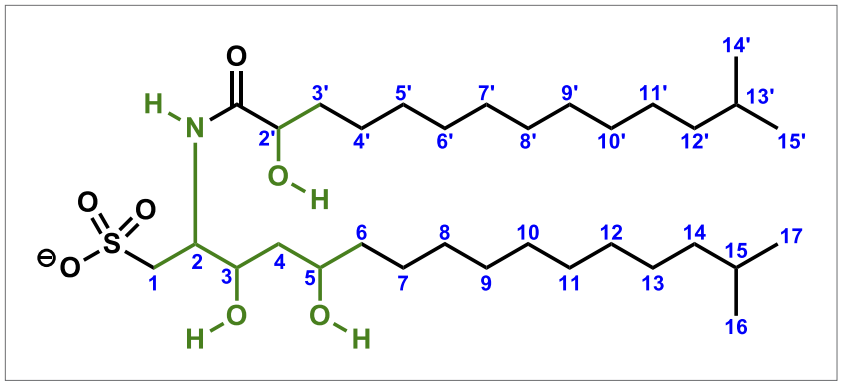

Figure 3—figure supplement 5. Key two-dimensional (2D) correlations of RIF-1: Observed TOCSY spin system. Green bonds show two key spin systems in RIF-1 - HO-CH- in the fatty acid fragment and -CH_2-CH(NH)-CH(OH)-CH_2-CH(OH)-CH_2- in the capnine base fragment.

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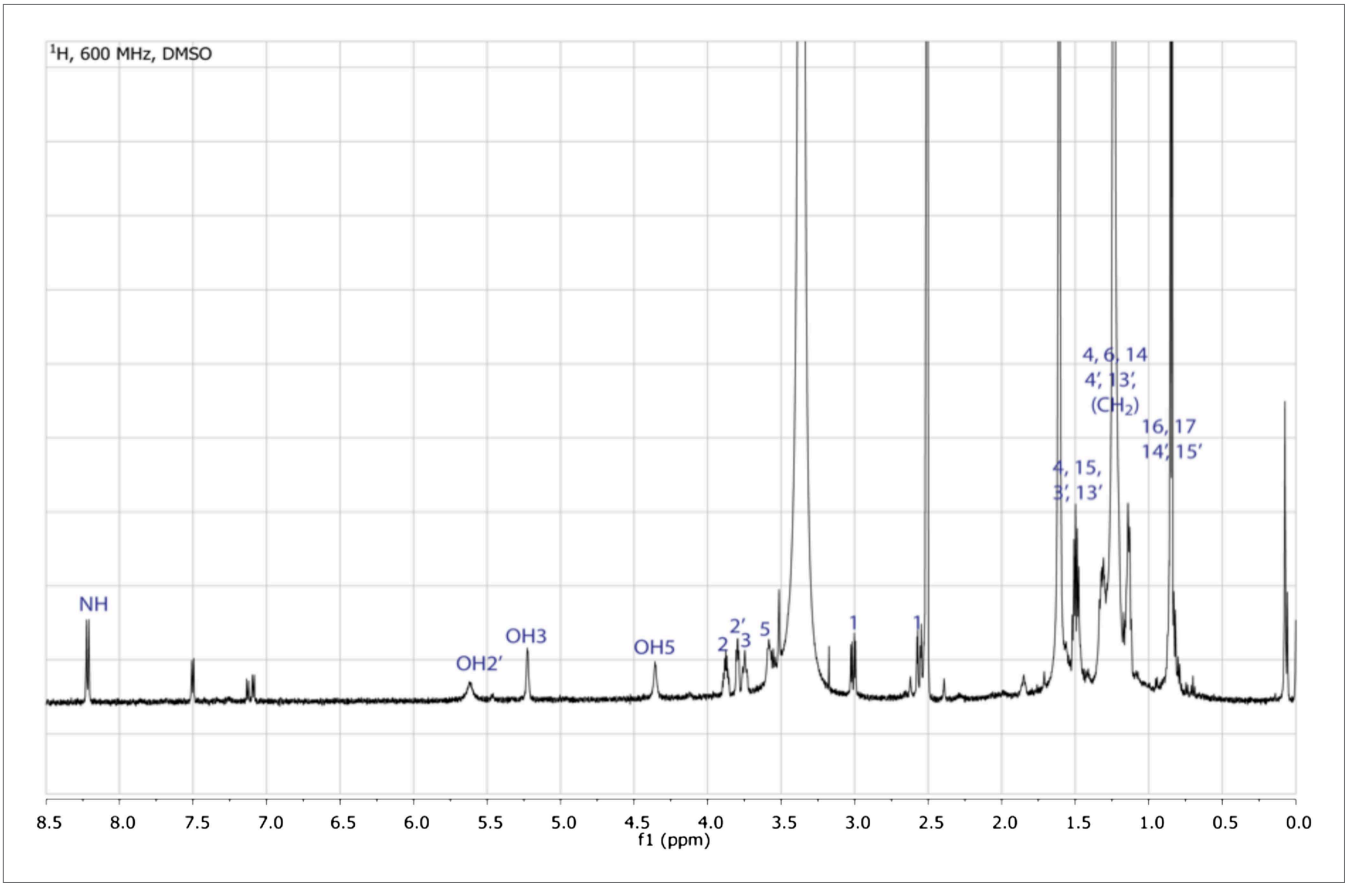

Figure 3—figure supplement 6. ^{1}H NMR spectrum of RIF-1. The spectrum exhibits one NH (δ_H 8.21), three hydroxyl groups (δ_H 5.52, 5.20, and 4.31), five signals from 2.50-4.00 ppm (four methines connected to either nitrogen at $\delta_{H\text{-}2}$ 3.88 or oxygens at $\delta_{H\text{-}2'}$ 3.80/$\delta_{H\text{-}3}$ 3.71-3.78/$\delta_{H\text{-}5}$ 3.53-3.61, and one methylene connected to sulfur at $\delta_{H\text{-}1}$ 2.56 & 3.01), twenty methylenes and four methyls (δ_H d, J = 6.6 Hz, 12H) in the high field region (δ_H 0.75-1.75 ppm).

DOI: 10.7554/eLife.00013.014

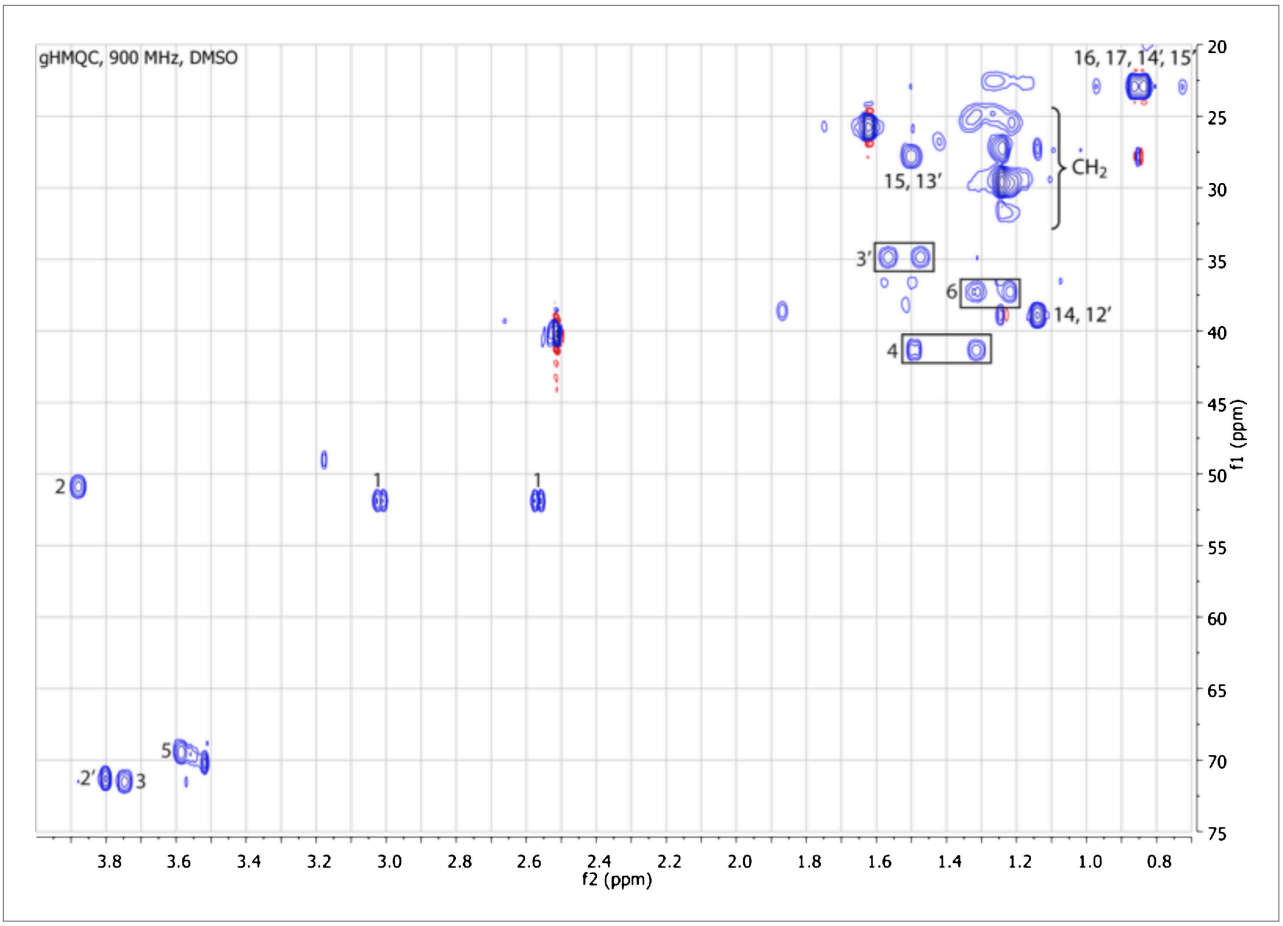

Figure 3—figure supplement 7. gHMQC spectrum of RIF-1. The ^{1}J H-C correlations demonstrate that 2 (δ_H 3.88, δ_C 50.89) is connected to a nitrogen; 2′, 3, and 5 (δ_H 3.80/δ_C 71.29, δ_H 3.71-3.78/δ_C 71.51, and δ_H 3.53-3.61/δ_C 70.20, respectively) are oxygenated; 1 (δ_H 3.01 and 2.56, δ_C 51.87) is adjacent to a sulfonic acid group; and all the other twenty methylenes and four methyls at high filed (δ_H 0.75-1.75/δ_C 22.00-42.00).

DOI: 10.7554/eLife.00013.015

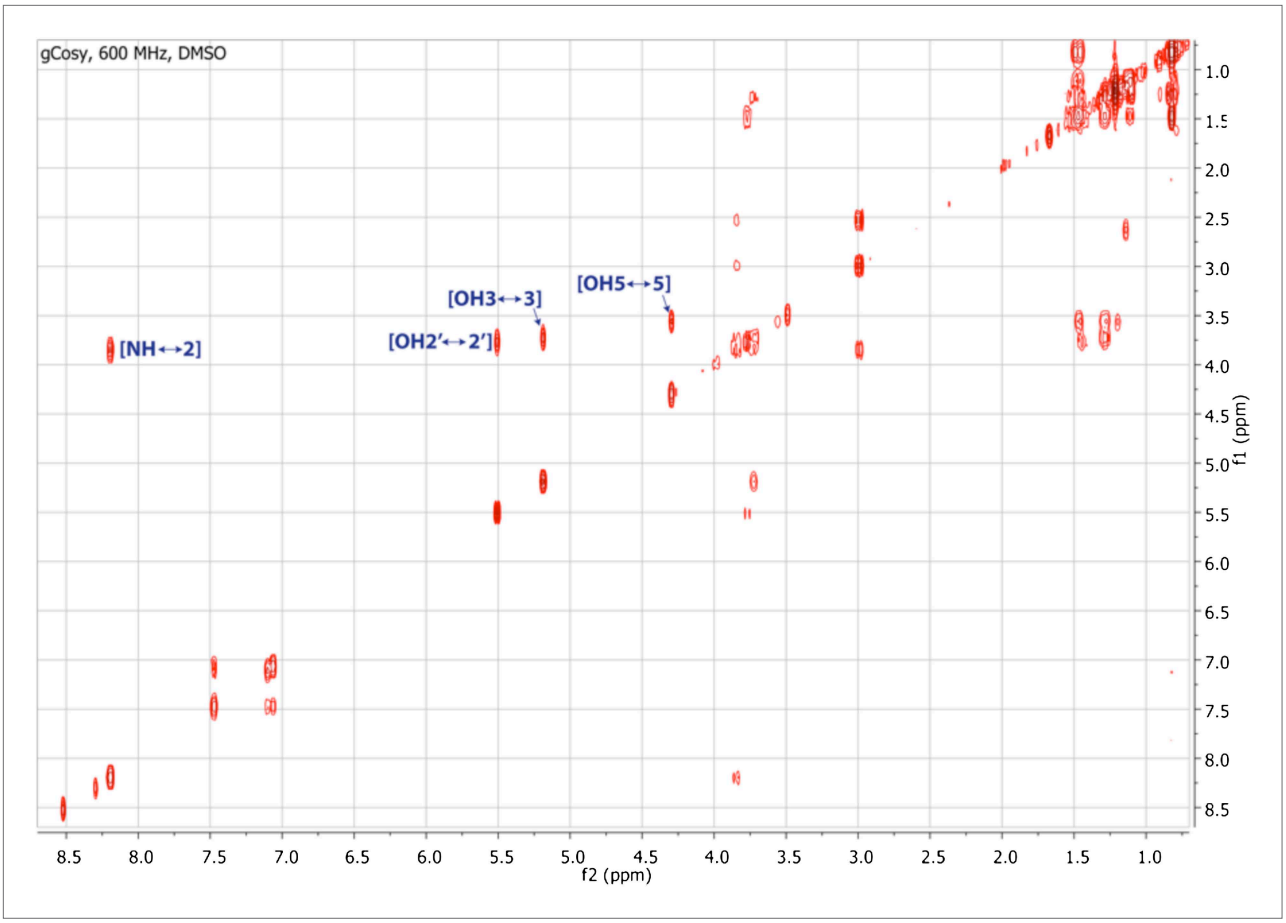

Figure 3—figure supplement 8. gCOSY spectrum of RIF-1. Indicated are important H-H correlations between NH and H-2, 2′-OH and H-2′, 3-OH and H-3, and 5-OH and H-5.

DOI: 10.7554/eLife.00013.016

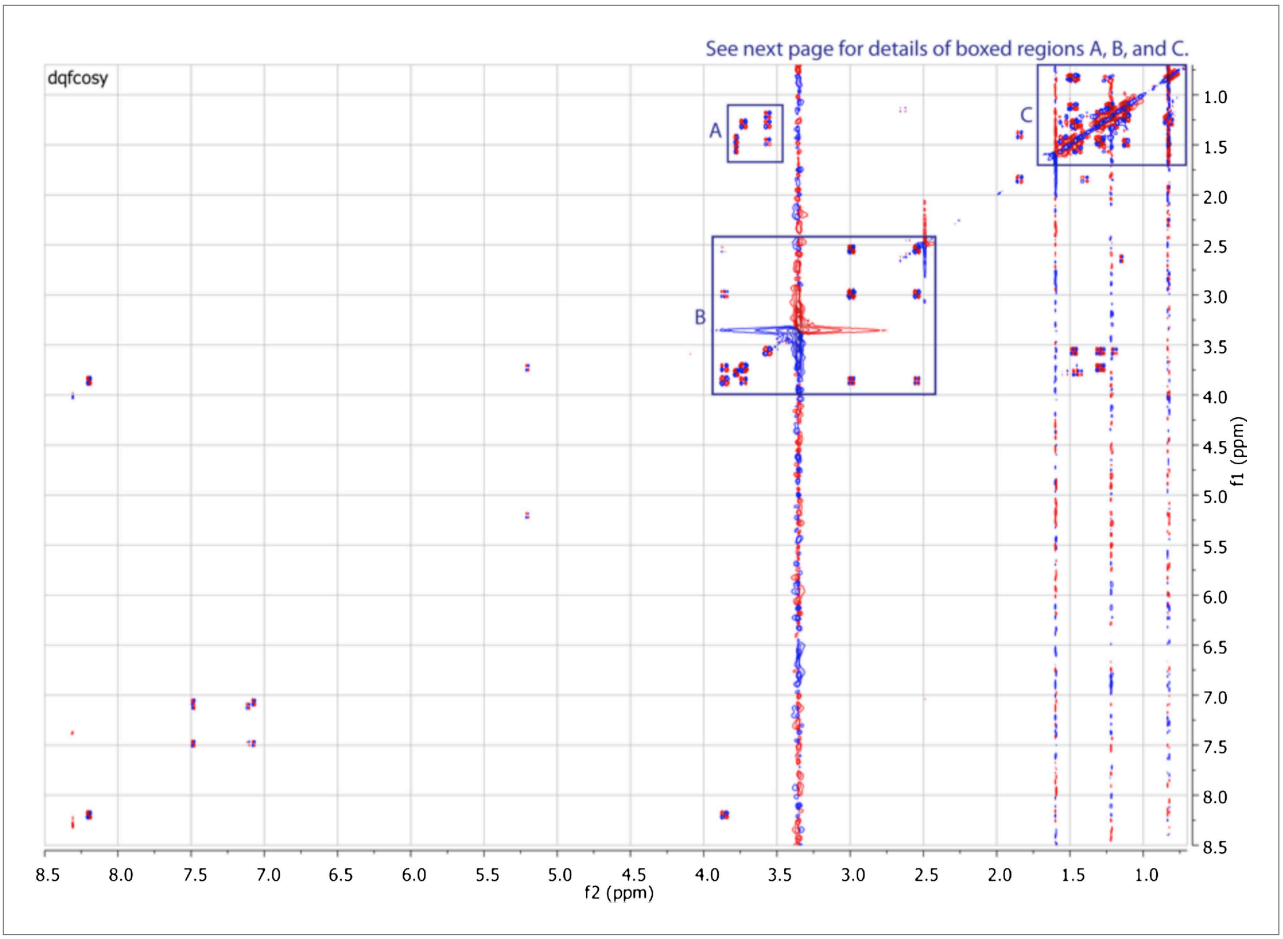

Figure 3—figure supplement 9. Expanded dqfCOSY spectrum of RIF-1. In panel A (δ_H 1.15-1.65 ppm/δ_H 3.52-3.82 ppm), H-3 (δ_H 3.73) shows correlation to H-4a (δ_H 1.30), H-5 (δ_H 3.57) to H-4a/H-4b (δ_H 1.30/1.47), and H-5 to H_2-6 (δ_H 1.20/1.29); H-2′ (~δ_H 3.8) correlates to H_2-3′ (δ_H 1.45 and 1.54). Panel B (δ_H 2.4-4.0 ppm/δ_H 2.4-4.0 ppm) demonstrates the correlations between H_2-1 (δ_H 2.56/~3.0) and H-2 (δ_H 3.88), and between H-2 and H-3. Panel C (δ_H 0.75-1.60 ppm/δ_H 0.75-1.60 ppm) exhibits correlations in the other methylenes and methyl groups.

DOI: 10.7554/eLife.00013.017

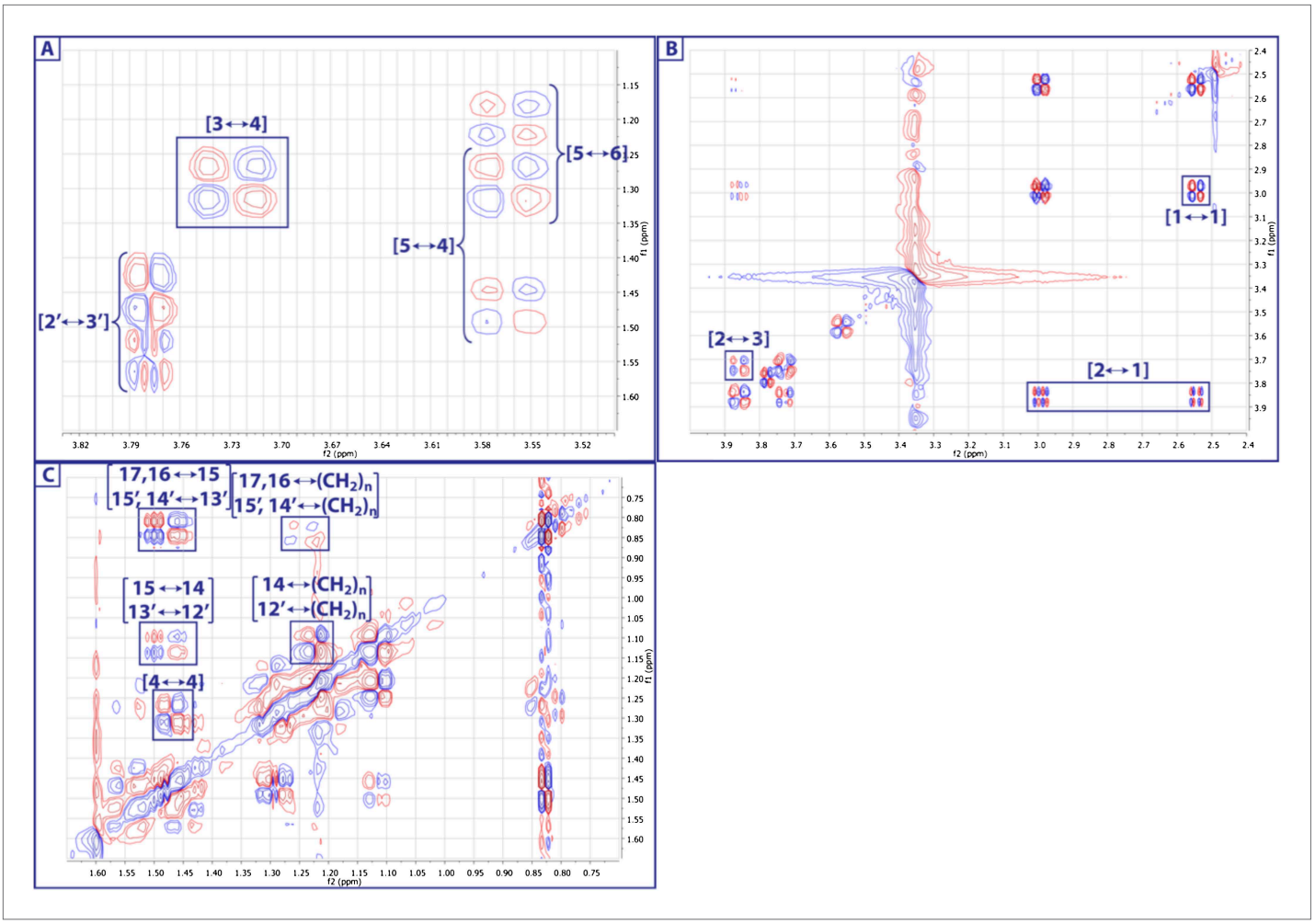

Figure 3—figure supplement 10. Expanded dqfCOSY spectra of RIF-1. A dqfCOSY spectrum was collected in order to get a clear connectivity in the oxygenated region (1-position to 6-position) in the capnoid base fragment.

DOI: 10.7554/eLife.00013.018

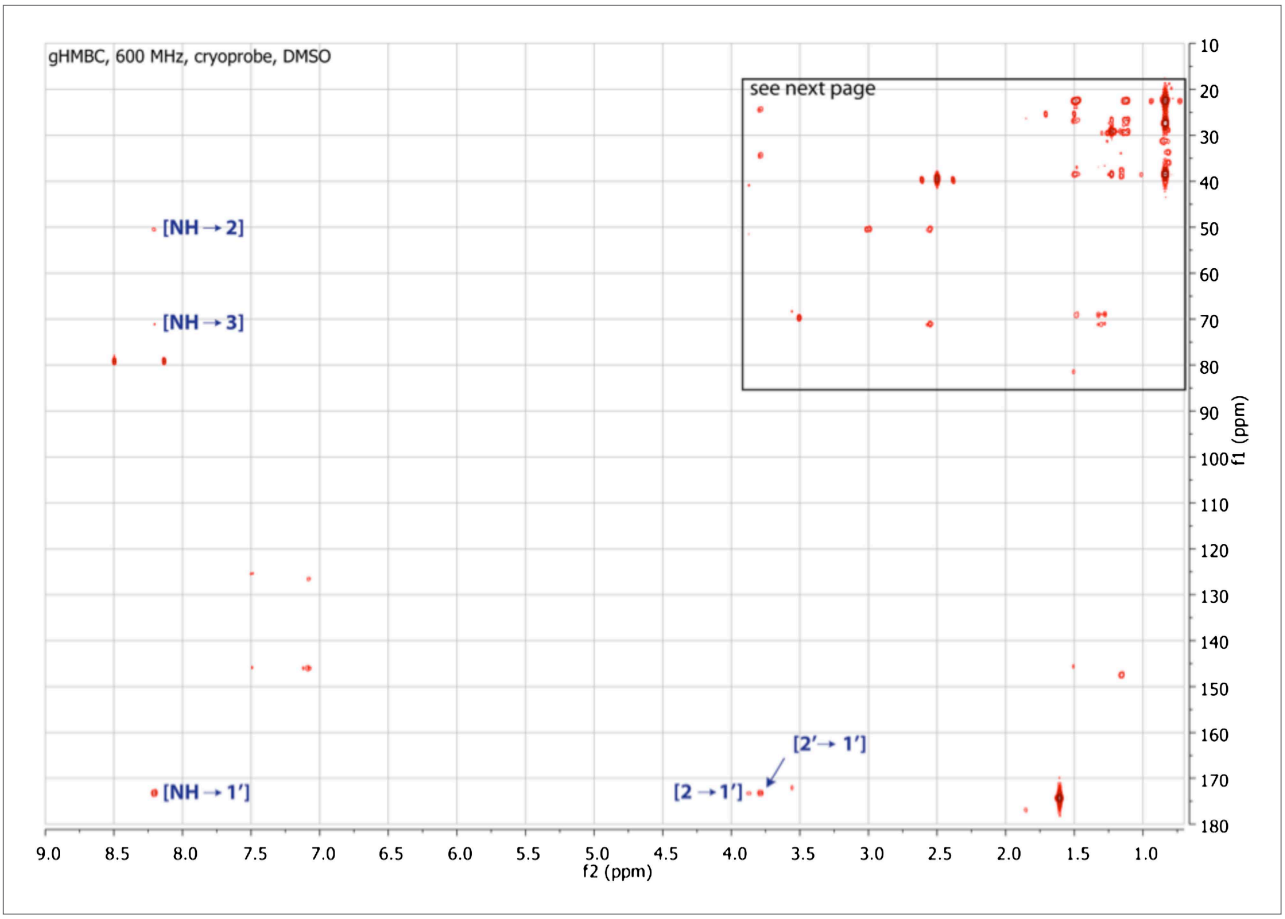

Figure 3—figure supplement 11. gHMBC spectrum of RIF-1. Indicated are important ^{2}J or ^{3}J H-C correlations between NH/H-2/H-2′ and C-1′ (δ_C 173.23), and between NH and C-2/C-3 (δ_C 50.89/71.51). Based on the MS/MS analysis, the fatty acid fragment must be 2-hydroxy-13-methyltetradecanoyl, and the capnine base fragment must be 2-NH-3,5-dihydroxy-15-methylhexadecane-1-sulfonate. Hence, the planar structure of RIF-1 is determined as shown in ***Fig. 3*** and ***Fig. 3 – Figure Supplements 3-5***.

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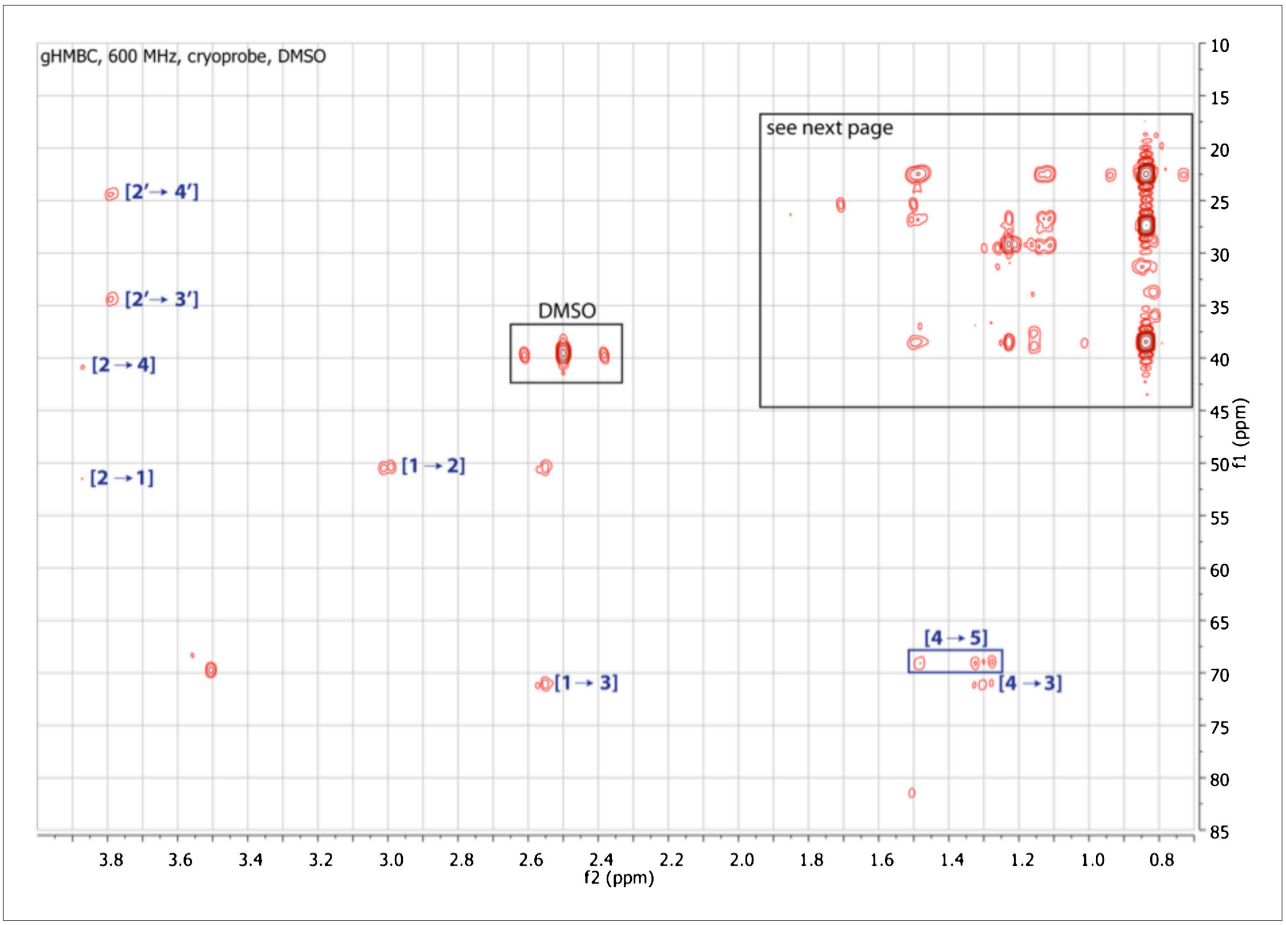

Figure 3—figure supplement 12. Expanded gHMBC spectrum of RIF-1 (δ_H 0-4.00 ppm/δ_C 15.0-85.0 ppm). Indicated are correlations between H-2 to C-4/C-1 (δ_C 41.36/51.87), between H-2′ and C-4′/C-3′ (δ_C 24.99/34.85), between H-1 and C-2/C-3 (δ_C 50.89/71.51), and between H-4 and C-5/C-3 (δ_C 70.20/71.51).

DOI: 10.7554/eLife.00013.020

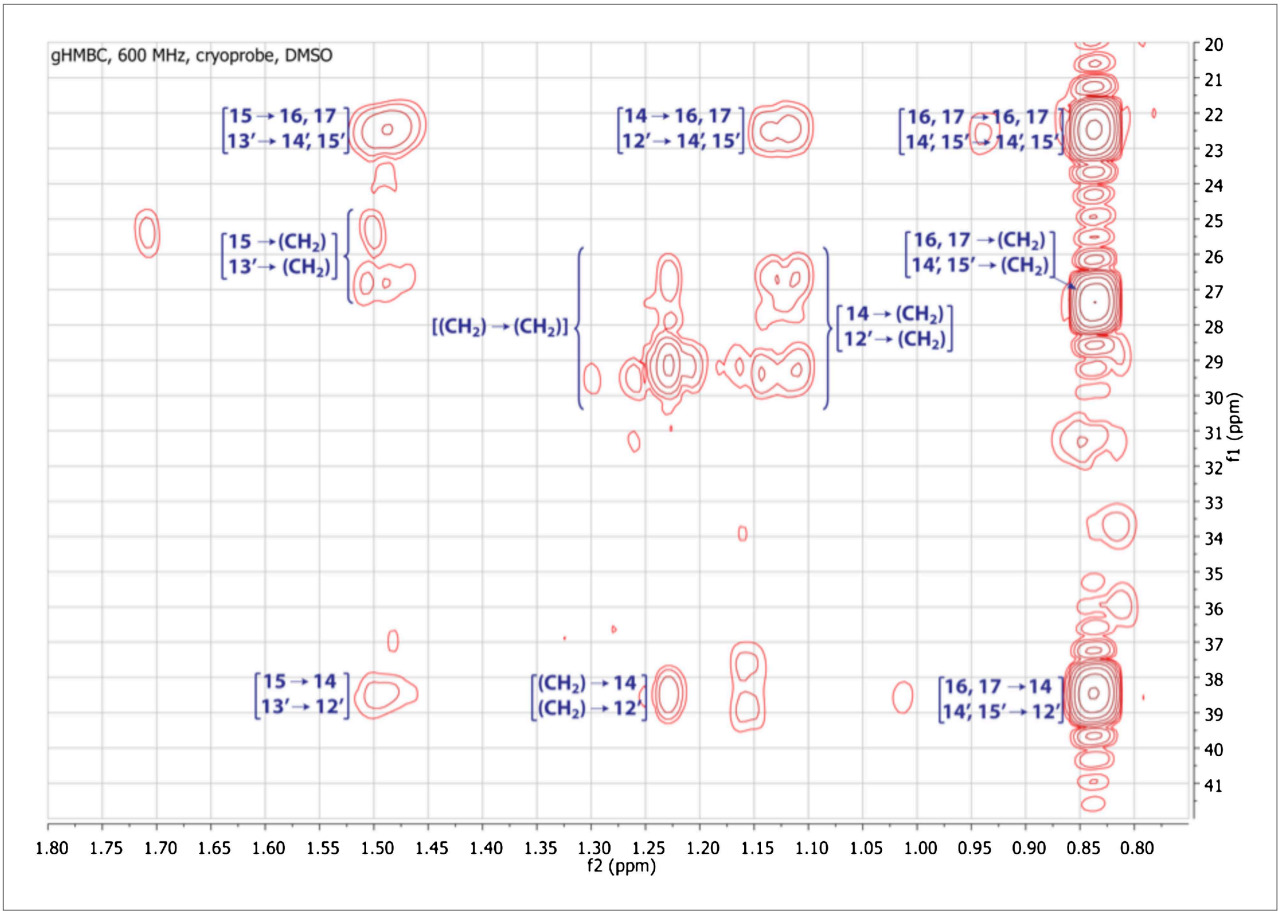

Figure 3—figure supplement 13. Expanded gHMBC spectrum of RIF-1 (δ_H 0.80-1.80 ppm/δ_C 20.0-40.0 ppm). Key correlations are between H-16 and C-14/C-15/C-17 (δ_C 38.91/27.79/22.09), between H-17 and C-14/C-15/C-16 (δ_C 38.91/27.79/22.09), between H-14′ and C-12′/C-13′/C-15′ (δ_C 38.91/27.79/22.09), and between H-15′ and C-12′/C-13′/C-14′ (δ_C 38.91/27.79/22.09).

DOI: 10.7554/eLife.00013.021

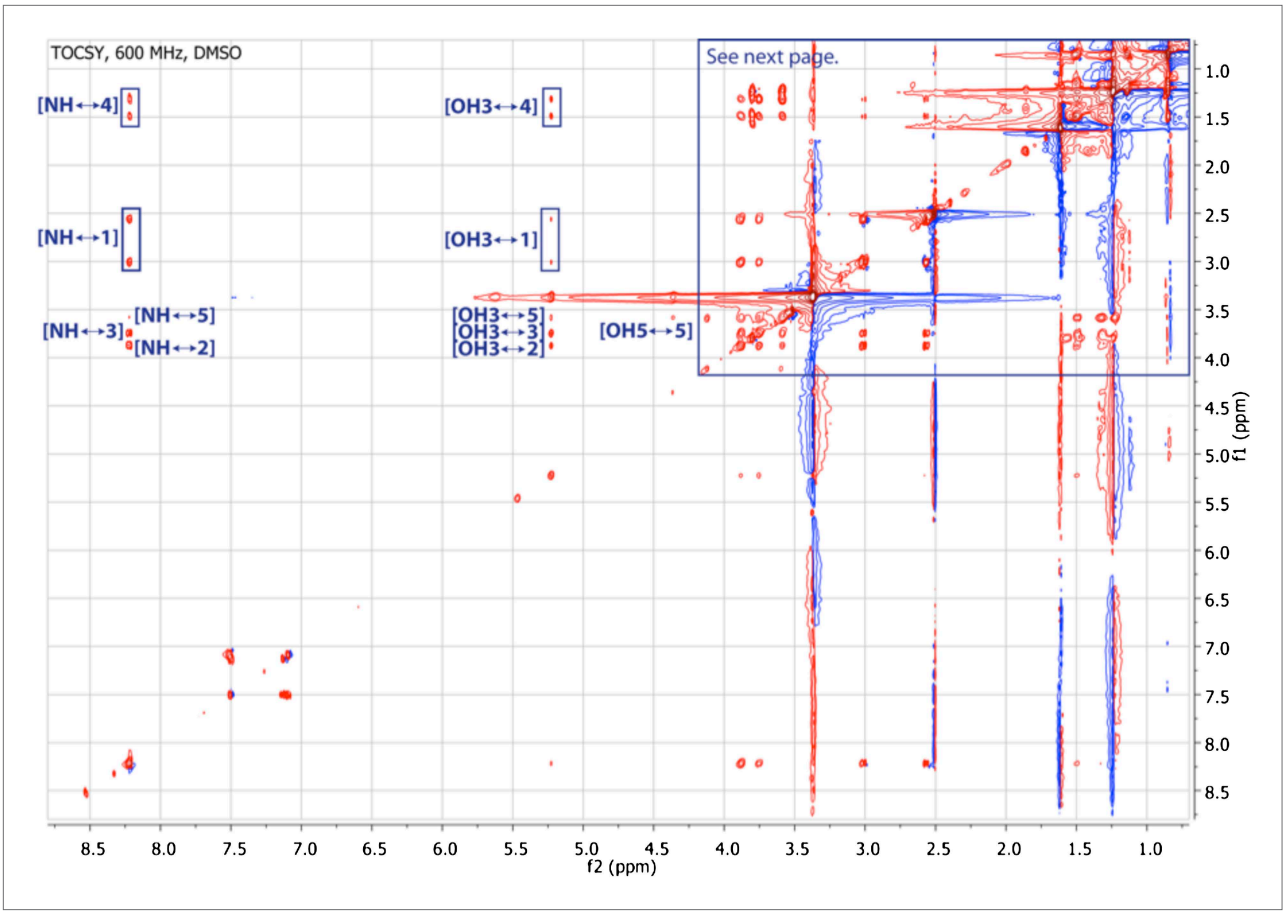

Figure 3—figure supplement 14. TOCSY spectrum of RIF-1. The spectrum shows correlations from NH to H_2-1 and H-5 through H-2, H-3 and H_2-4, and from 3-OH to H-5 via H_2-4 and H_2-1 through H-3 and H-2.

DOI: 10.7554/eLife.00013.022

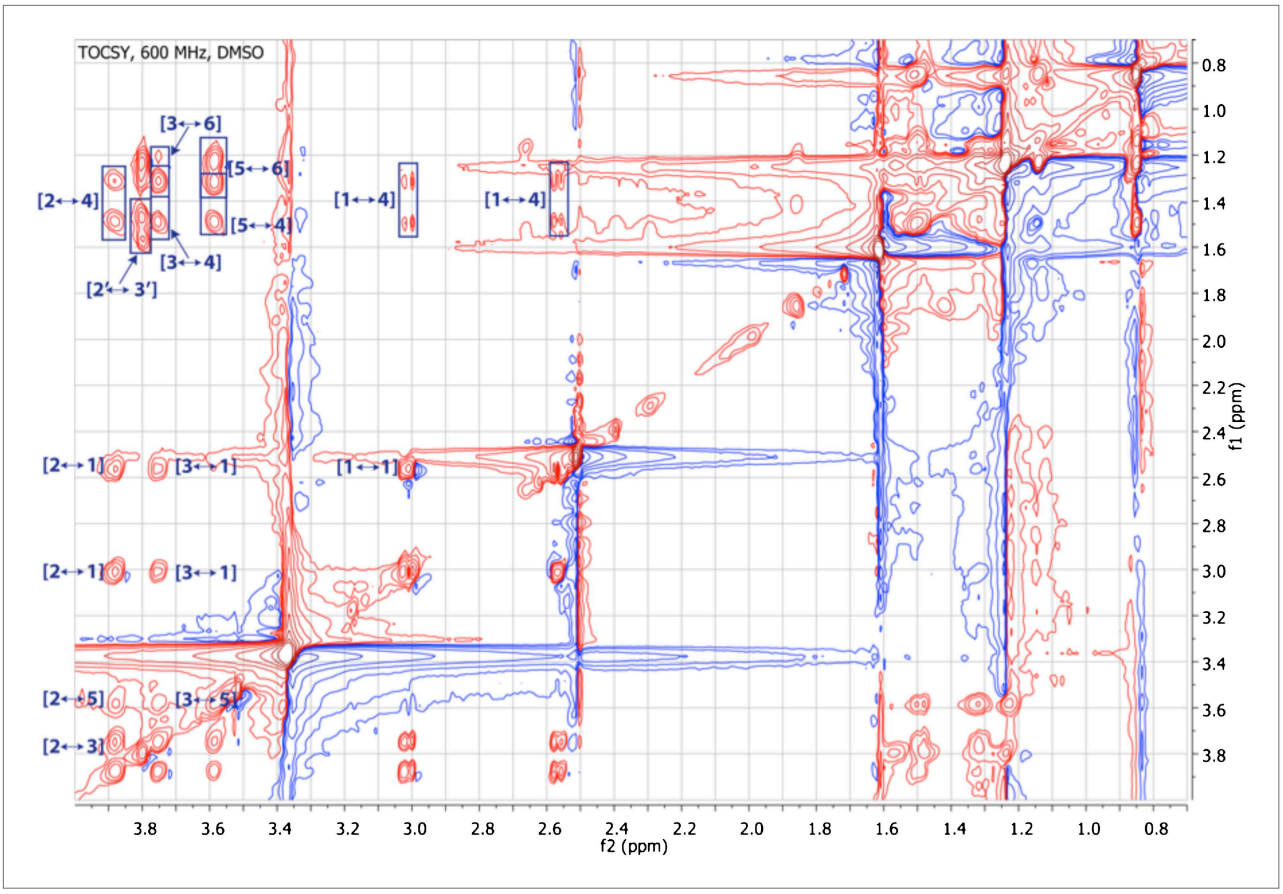

Figure 3—figure supplement 15. Expanded TOCSY spectrum of RIF-1 (δ_H 0.50-4.25 ppm/δ_C 0.50-4.25 ppm). Another important spin system is clearly demonstrated by the TOCSY correlations between H-2′ and H_2-3′/H-4′/H-5′ on the top left of the expanded spectrum.

DOI: 10.7554/eLife.00013.023

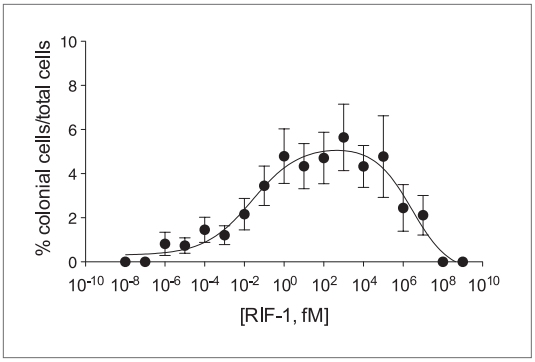

Figure 4. Purified RIF-1 is active at plausible environmental concentrations. RIF-1 concentrations ranging from 10^{-2} to 10^{7} fM induce rosette colony development in RCA cultures. Frequency of rosette colony development was quantified in RCA cultures 2 days after treatment with a dilution series of purified RIF-1. Data are mean ± s.e. from three independent experiments. Line indicates non-linear regression of the RIF-1 activity profile.

DOI: 10.7554/eLife.00013.025

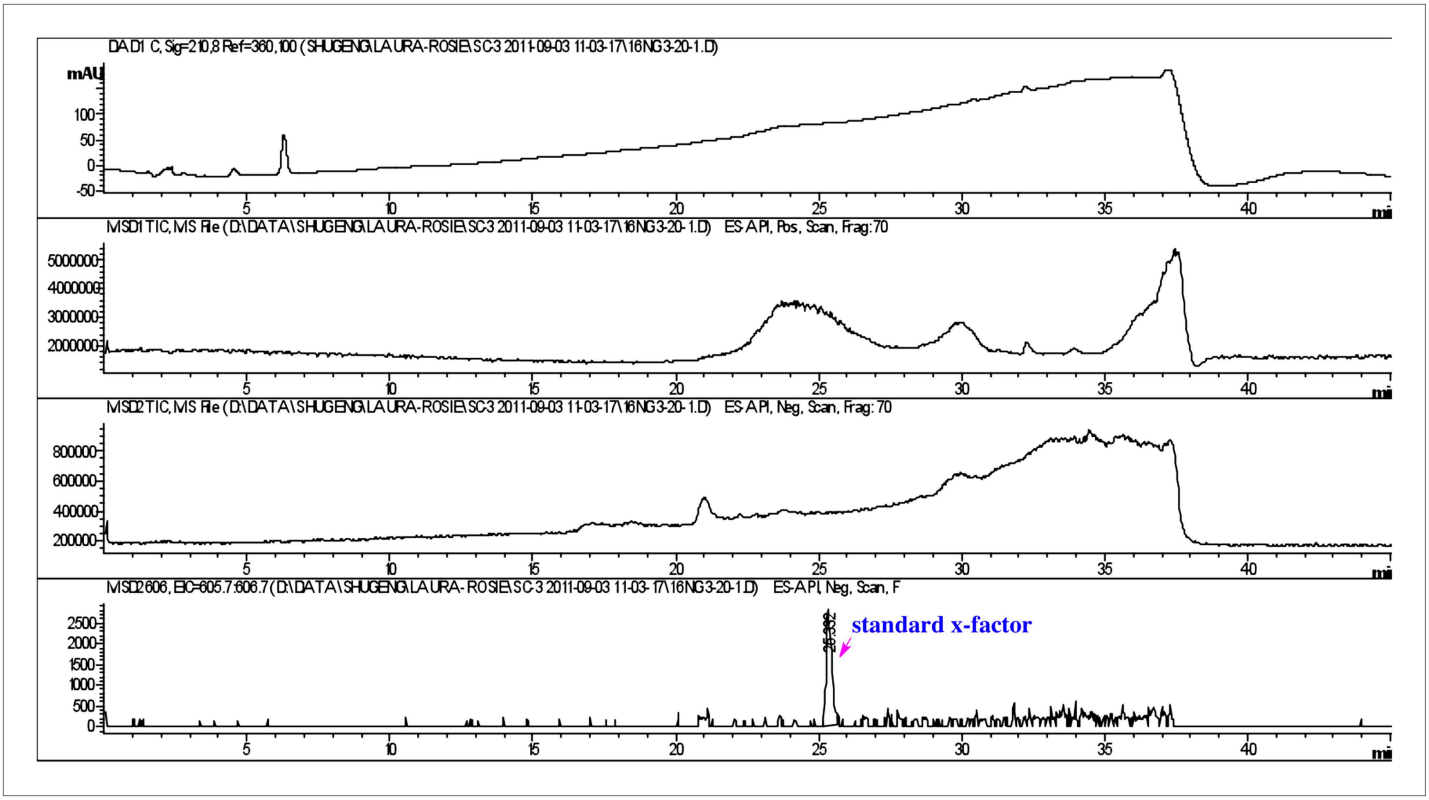

Figure 4—figure supplement 1. Detection of purified RIF-1. RIF-1 did not show absorbance at 210 nm (top panel), but the molecule (606 Da, M-H) was detected between 25 and 26 minutes (bottom panel).

DOI: 10.7554/eLife.00013.026

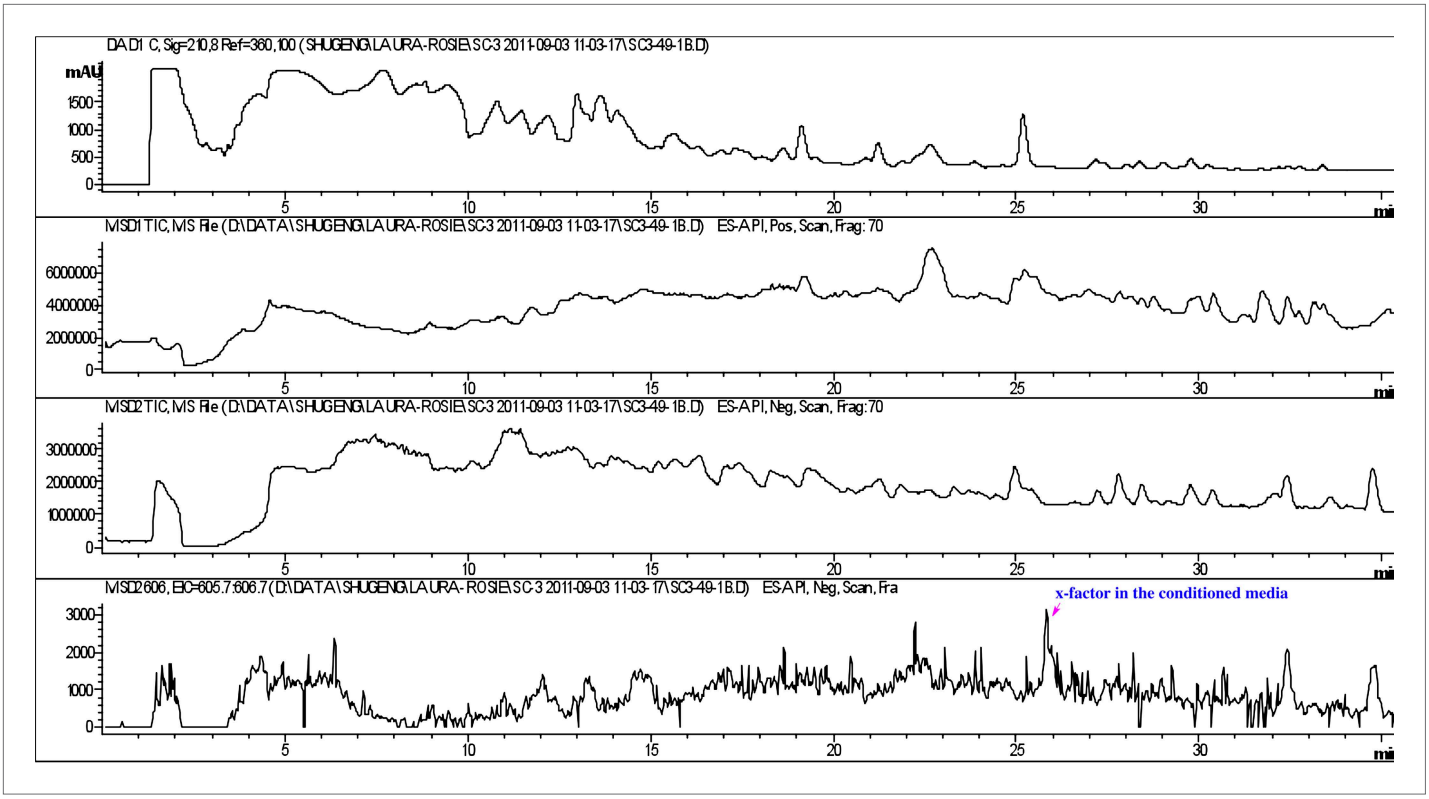

Figure 4—figure supplement 2. Detection of RIF-1 in the conditioned medium of *A. machipongonensis*. RIF-1 (bottom panel) was detected in the conditioned medium after the broth was concentrated 250 times.

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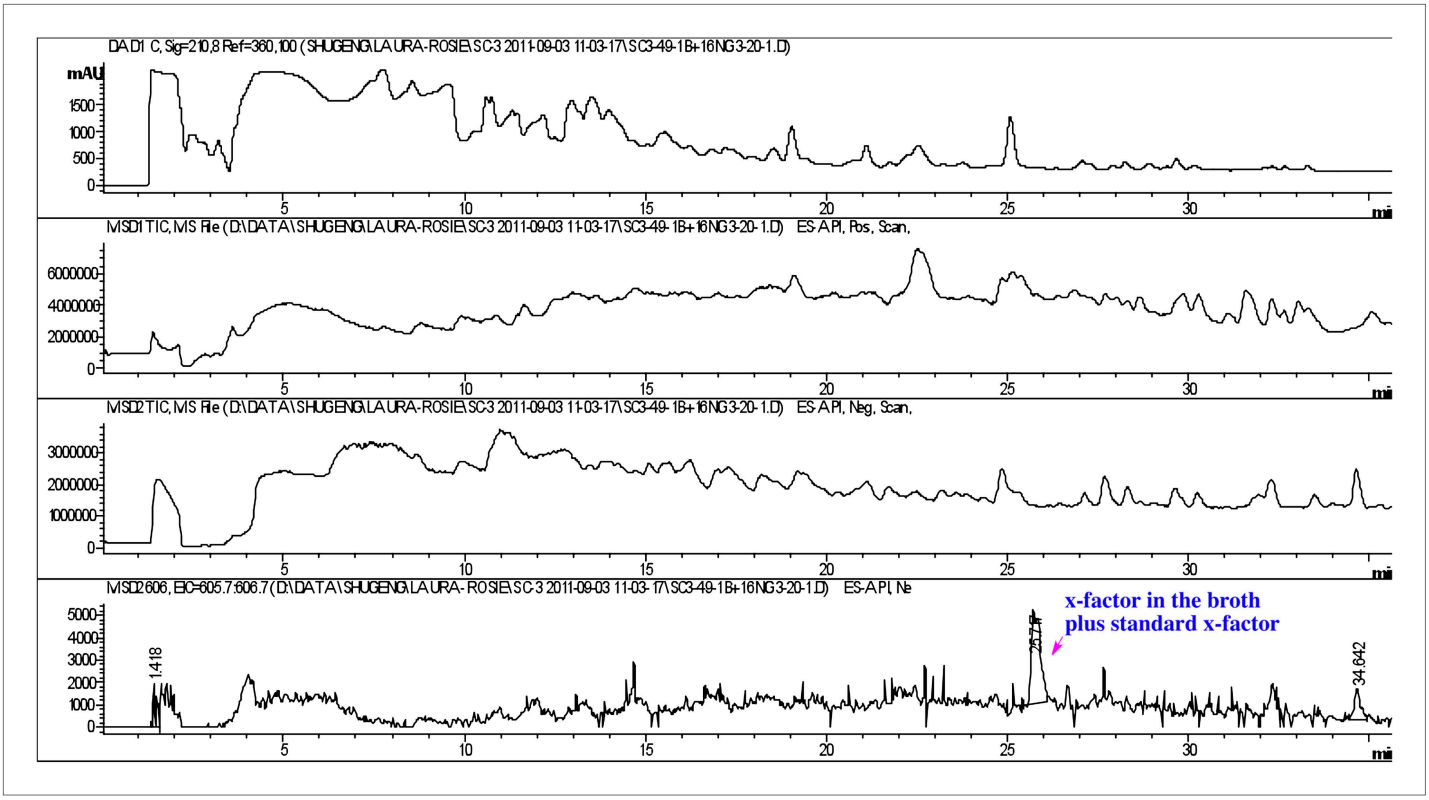

Figure 4—figure supplement 3. Co-injection of concentrated conditioned medium with purified RIF-1. The peak of the RIF-1 in the conditioned medium was enhanced after the sample was spiked with purified RIF-1 (bottom panel).

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