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Supporting Information

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Supporting Information

for

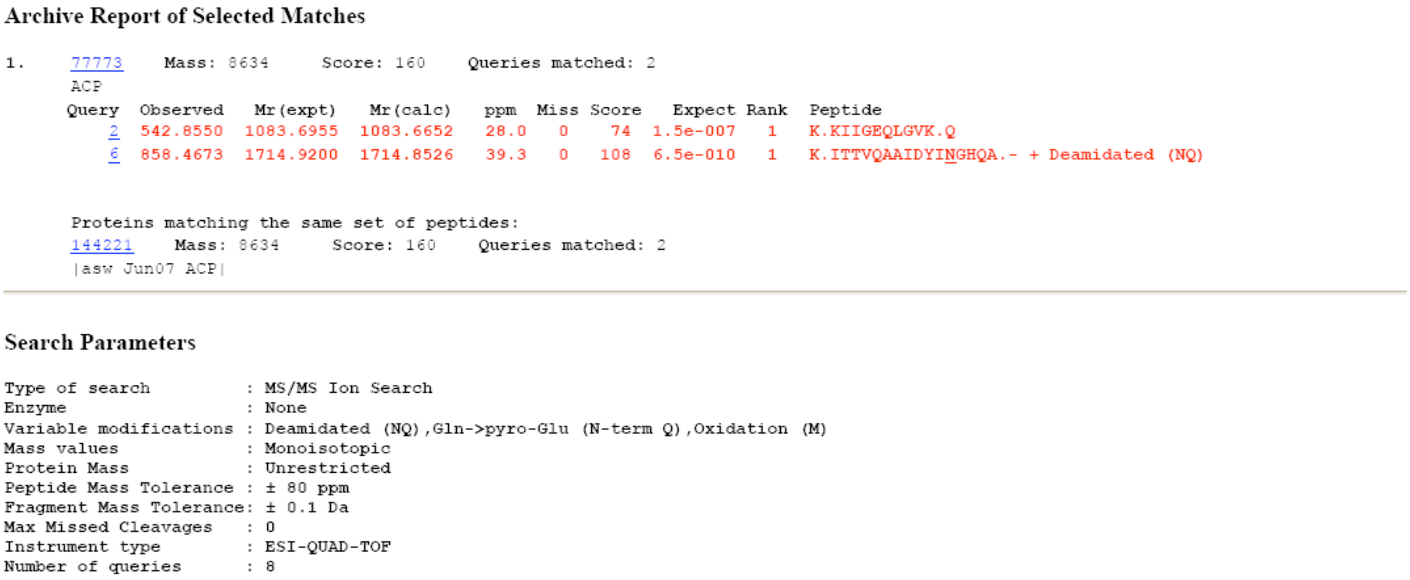
In Vivo Modification of Native Carrier Protein Domains

Andrew C. Mercer, Jordan L. Meier, Justin W. Torpey, and Michael D. Burkart*

Contents

Mascot Reports from MS/MS Analysis

Figure S1. Mascot search report of labeled band from *E. coli* K12 lysate.



Archive Report of Selected Matches

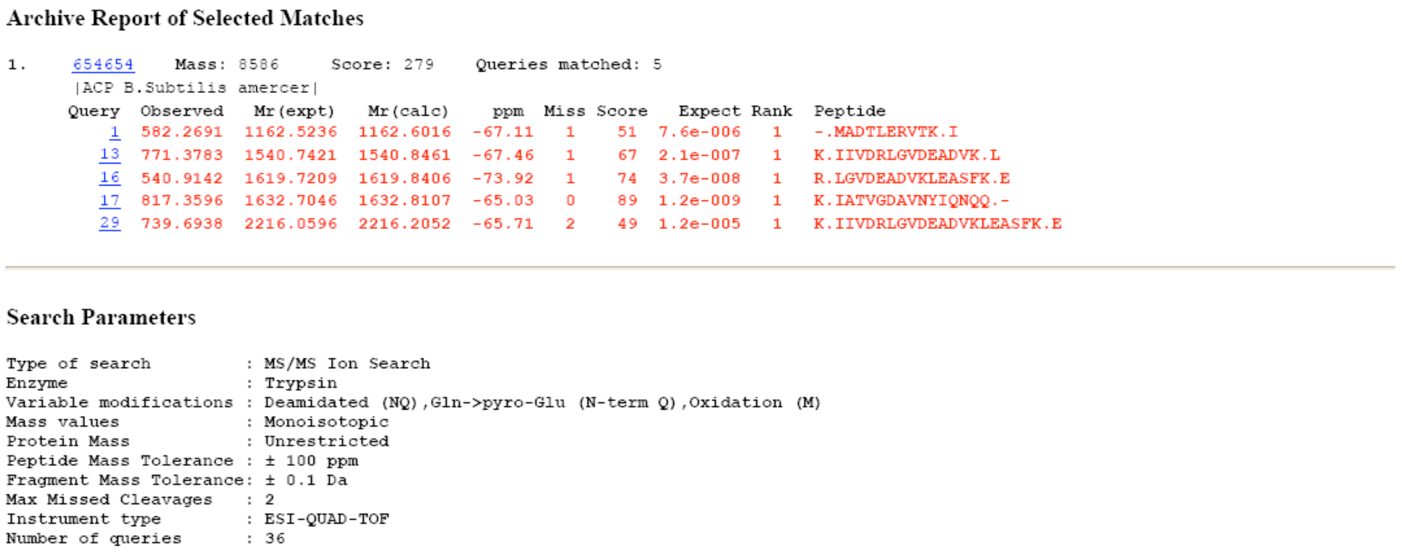
```
1.      77773     Mass: 8634     Score: 160     Queries matched: 2
        ACP
       Query  Observed    Mr(expt)   Mr(calc)    ppm  Miss Score    Expect Rank  Peptide
           2  542.8550  1083.6955  1083.6652   28.0   0     74  1.5e-007   1   K.KIIGEQLGVK.Q
           6  858.4673  1714.9200  1714.8526   39.3   0    108  6.5e-010   1   K.ITTVQAAIDYINGHQA.- + Deamidated (NQ)


        Proteins matching the same set of peptides:
        144221     Mass: 8634     Score: 160     Queries matched: 2
        |asw Jun07 ACP|
```

Search Parameters

```
Type of search         : MS/MS Ion Search
Enzyme                 : None
Variable modifications : Deamidated (NQ),Gln->pyro-Glu (N-term Q),Oxidation (M)
Mass values            : Monoisotopic
Protein Mass           : Unrestricted
Peptide Mass Tolerance : ± 80 ppm
Fragment Mass Tolerance: ± 0.1 Da
Max Missed Cleavages   : 0
Instrument type        : ESI-QUAD-TOF
Number of queries      : 8
```

Figure S2 Mascot search results for labeled band from *B. subtilis* 6051 lysate



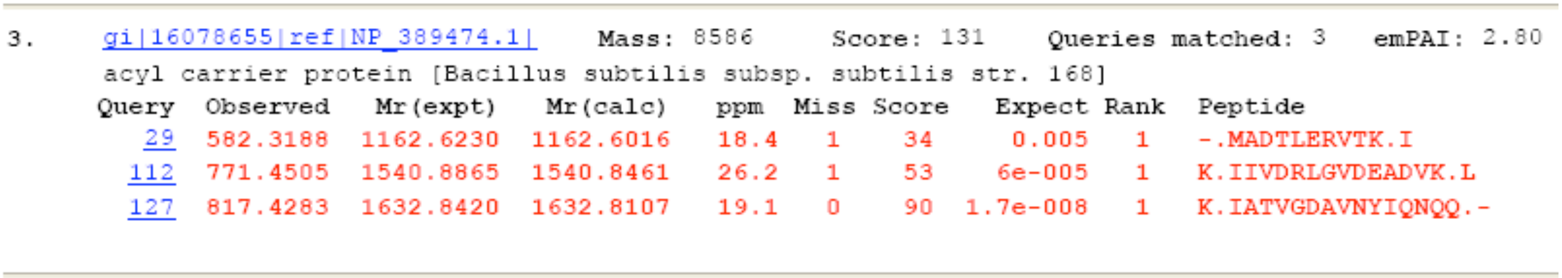
Archive Report of Selected Matches

```
1.    654654     Mass: 8586     Score: 279     Queries matched: 5
      |ACP B.Subtilis amercer|
      Query  Observed   Mr(expt)   Mr(calc)     ppm  Miss  Score   Expect Rank  Peptide
          1  582.2691  1162.5236  1162.6016  -67.11    1     51  7.6e-006    1  -.MADTLERVTK.I
         13  771.3783  1540.7421  1540.8461  -67.46    1     67  2.1e-007    1  K.IIVDRLGVDEADVK.L
         16  540.9142  1619.7209  1619.8406  -73.92    1     74  3.7e-008    1  R.LGVDEADVKLEASFK.E
         17  817.3596  1632.7046  1632.8107  -65.03    0     89  1.2e-009    1  K.IATVGDAVNYIQNQQ.-
         29  739.6938  2216.0596  2216.2052  -65.71    2     49  1.2e-005    1  K.IIVDRLGVDEADVKLEASFK.E
```

Search Parameters

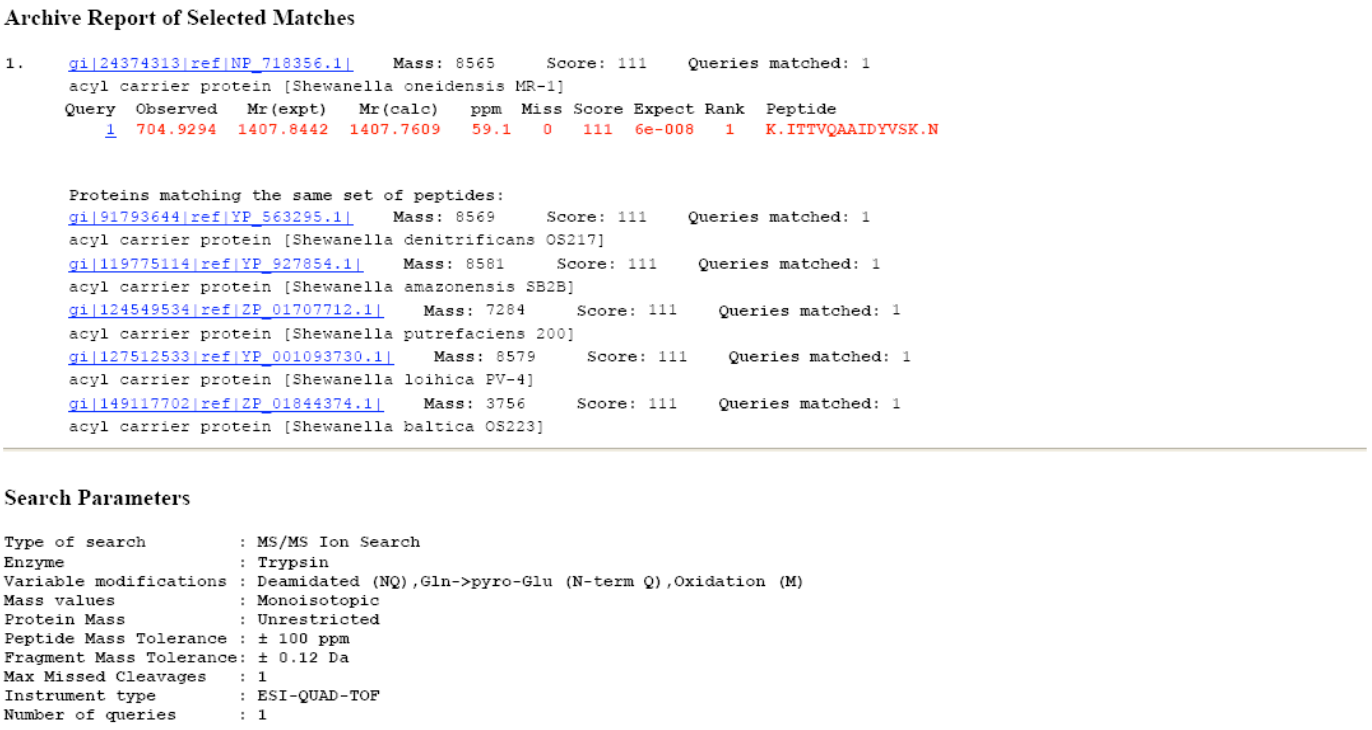
```
Type of search         : MS/MS Ion Search
Enzyme                 : Trypsin
Variable modifications : Deamidated (NQ),Gln->pyro-Glu (N-term Q),Oxidation (M)
Mass values            : Monoisotopic
Protein Mass           : Unrestricted
Peptide Mass Tolerance : ± 100 ppm
Fragment Mass Tolerance: ± 0.1 Da
Max Missed Cleavages   : 2
Instrument type        : ESI-QUAD-TOF
Number of queries      : 36
```

Figure S3 Mascot search results for labeled band from *B. subtilis* 168 lysate



```
3.   gi|16078655|ref|NP_389474.1|     Mass: 8586     Score: 131     Queries matched: 3   emPAI: 2.80
     acyl carrier protein [Bacillus subtilis subsp. subtilis str. 168]
     Query  Observed   Mr(expt)   Mr(calc)     ppm  Miss  Score    Expect Rank  Peptide
        29  582.3188  1162.6230  1162.6016    18.4    1     34     0.005    1  -.MADTLERVTK.I
       112  771.4505  1540.8865  1540.8461    26.2    1     53    6e-005    1  K.IIVDRLGVDEADVK.L
       127  817.4283  1632.8420  1632.8107    19.1    0     90  1.7e-008    1  K.IATVGDAVNYIQNQQ.-
```

Figure S3 Mascot search results for labeled band from *S. oneidensis* MR-1



Archive Report of Selected Matches

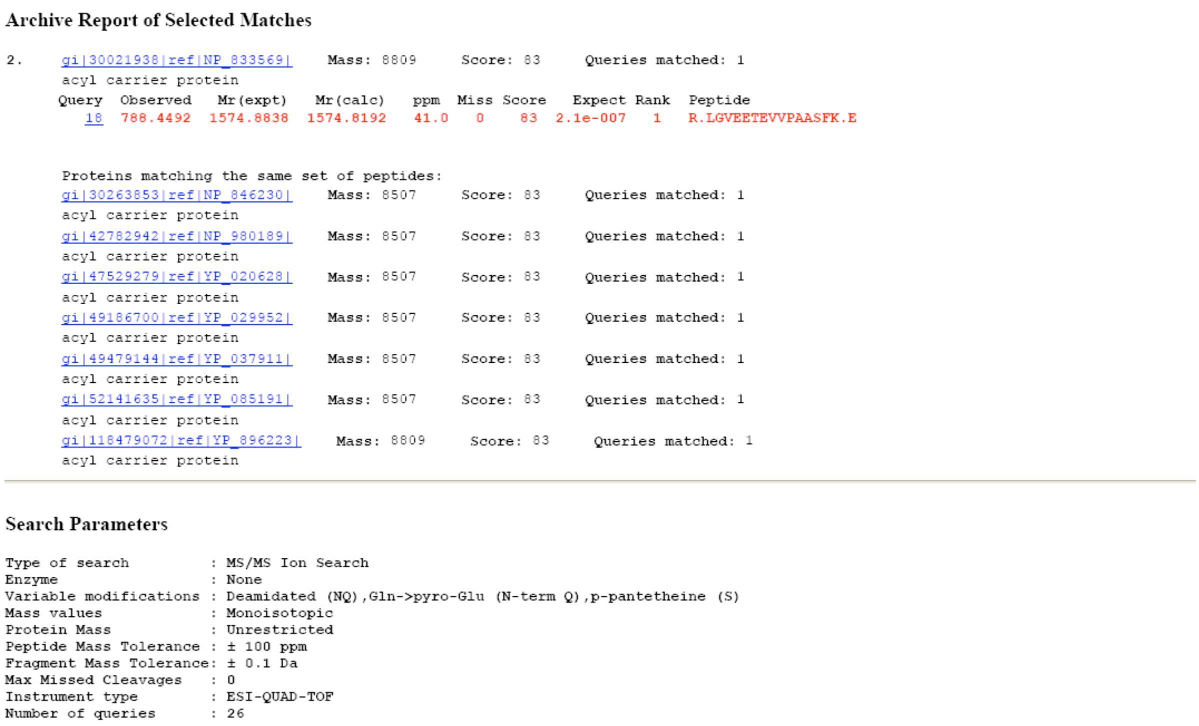
```
1.     gi|24374313|ref|NP_718356.1|    Mass: 8565     Score: 111    Queries matched: 1
       acyl carrier protein [Shewanella oneidensis MR-1]
      Query  Observed   Mr(expt)   Mr(calc)    ppm  Miss Score Expect Rank  Peptide
         1   704.9294  1407.8442  1407.7609   59.1   0   111  6e-008    1   K.ITTVQAAIDYVSK.N


       Proteins matching the same set of peptides:
       gi|91793644|ref|YP_563295.1|    Mass: 8569     Score: 111    Queries matched: 1
       acyl carrier protein [Shewanella denitrificans OS217]
       gi|119775114|ref|YP_927854.1|    Mass: 8581     Score: 111    Queries matched: 1
       acyl carrier protein [Shewanella amazonensis SB2B]
       gi|124549534|ref|ZP_01707712.1|    Mass: 7284     Score: 111    Queries matched: 1
       acyl carrier protein [Shewanella putrefaciens 200]
       gi|127512533|ref|YP_001093730.1|    Mass: 8579     Score: 111    Queries matched: 1
       acyl carrier protein [Shewanella loihica PV-4]
       gi|149117702|ref|ZP_01844374.1|    Mass: 3756     Score: 111    Queries matched: 1
       acyl carrier protein [Shewanella baltica OS223]
```

Search Parameters

```
Type of search         : MS/MS Ion Search
Enzyme                 : Trypsin
Variable modifications : Deamidated (NQ),Gln->pyro-Glu (N-term Q),Oxidation (M)
Mass values            : Monoisotopic
Protein Mass           : Unrestricted
Peptide Mass Tolerance : ± 100 ppm
Fragment Mass Tolerance: ± 0.12 Da
Max Missed Cleavages   : 1
Instrument type        : ESI-QUAD-TOF
Number of queries      : 1
```

Figure S4 Mascot search result for labeled band in *B. brevis* 26A1 lysate.



Archive Report of Selected Matches

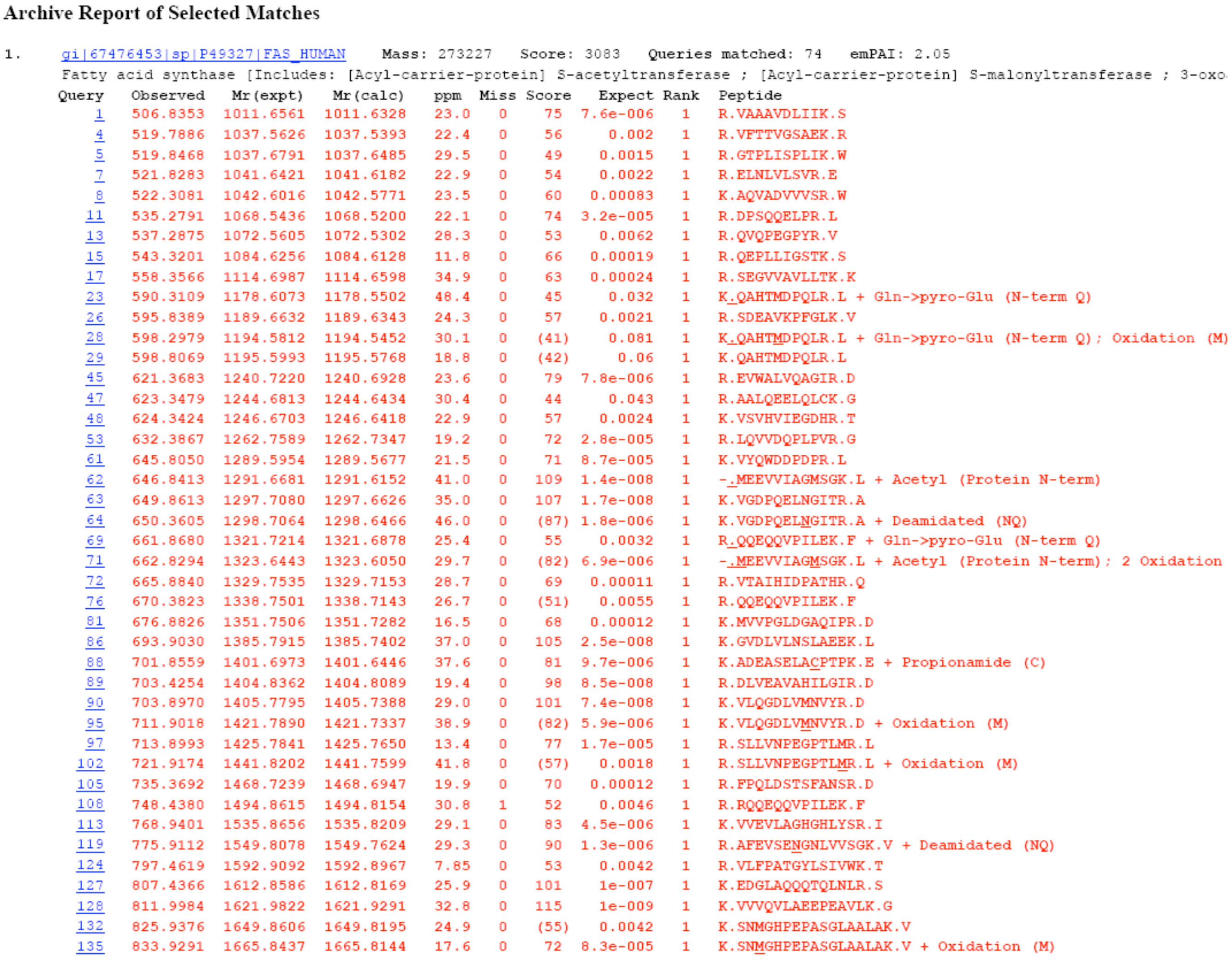
```
2.     gi|30021938|ref|NP_833569|    Mass: 8809     Score: 83     Queries matched: 1
       acyl carrier protein
      Query  Observed   Mr(expt)   Mr(calc)    ppm  Miss Score   Expect Rank  Peptide
        18   788.4492  1574.8838  1574.8192   41.0   0     83  2.1e-007    1   R.LGVEETEVVPAASFK.E


       Proteins matching the same set of peptides:
       gi|30263853|ref|NP_846230|    Mass: 8507     Score: 83     Queries matched: 1
       acyl carrier protein
       gi|42782942|ref|NP_980189|    Mass: 8507     Score: 83     Queries matched: 1
       acyl carrier protein
       gi|47529279|ref|YP_020628|    Mass: 8507     Score: 83     Queries matched: 1
       acyl carrier protein
       gi|49186700|ref|YP_029952|    Mass: 8507     Score: 83     Queries matched: 1
       acyl carrier protein
       gi|49479144|ref|YP_037911|    Mass: 8507     Score: 83     Queries matched: 1
       acyl carrier protein
       gi|52141635|ref|YP_085191|    Mass: 8507     Score: 83     Queries matched: 1
       acyl carrier protein
       gi|118479072|ref|YP_896223|    Mass: 8809     Score: 83     Queries matched: 1
       acyl carrier protein
```

Search Parameters

```
Type of search         : MS/MS Ion Search
Enzyme                 : None
Variable modifications : Deamidated (NQ),Gln->pyro-Glu (N-term Q),p-pantetheine (S)
Mass values            : Monoisotopic
Protein Mass           : Unrestricted
Peptide Mass Tolerance : ± 100 ppm
Fragment Mass Tolerance: ± 0.1 Da
Max Missed Cleavages   : 0
Instrument type        : ESI-QUAD-TOF
Number of queries      : 26
```

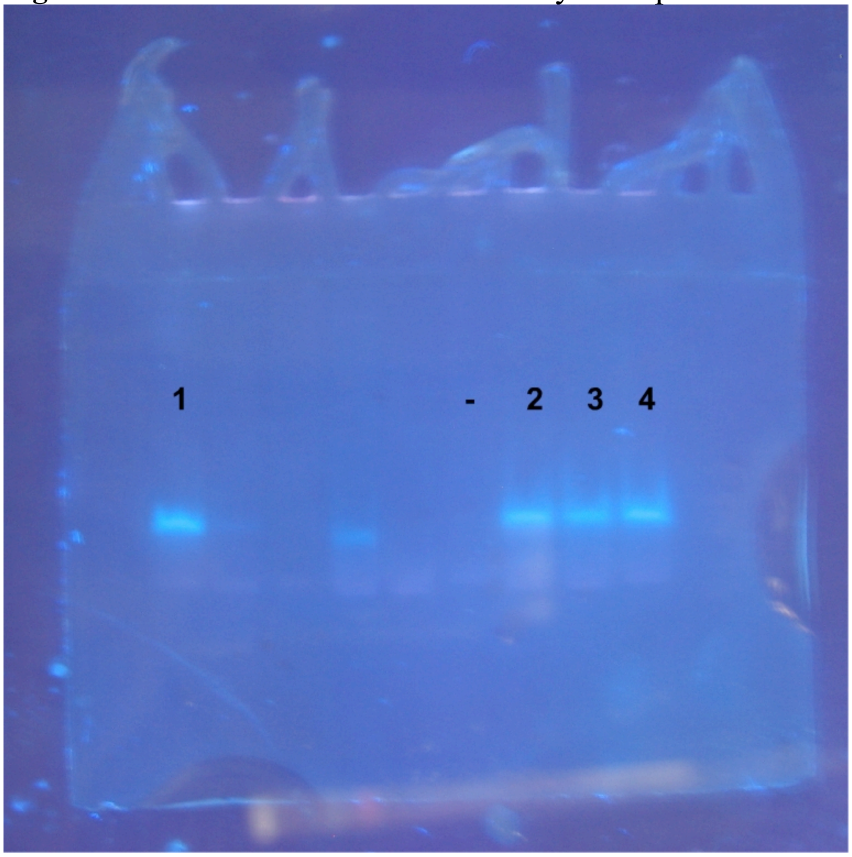
Figure S5 Mascot search results for labeled band in SKBR3 lysate.



Archive Report of Selected Matches

```
1.     gi|67476453|sp|P49327|FAS_HUMAN     Mass: 273227    Score: 3083    Queries matched: 74    emPAI: 2.05
       Fatty acid synthase [Includes: [Acyl-carrier-protein] S-acetyltransferase ; [Acyl-carrier-protein] S-malonyltransferase ; 3-oxo
       Query    Observed   Mr(expt)   Mr(calc)    ppm  Miss Score    Expect Rank  Peptide
           1    506.8353  1011.6561  1011.6328   23.0    0    75   7.6e-006    1   R.VAAAVDLIIK.S
           4    519.7886  1037.5626  1037.5393   22.4    0    56      0.002    1   R.VFTTVGSAEK.R
           5    519.8468  1037.6791  1037.6485   29.5    0    49     0.0015    1   R.GTPLISPLIK.W
           7    521.8283  1041.6421  1041.6182   22.9    0    54     0.0022    1   R.ELNLVLSVR.E
           8    522.3081  1042.6016  1042.5771   23.5    0    60    0.00083    1   K.AQVADVVVSR.W
          11    535.2791  1068.5436  1068.5200   22.1    0    74   3.2e-005    1   R.DPSQQELPR.L
          13    537.2875  1072.5605  1072.5302   28.3    0    53     0.0062    1   R.QVQPEGPYR.V
          15    543.3201  1084.6256  1084.6128   11.8    0    66    0.00019    1   R.QEPLLIGSTK.S
          17    558.3566  1114.6987  1114.6598   34.9    0    63    0.00024    1   R.SEGVVAVLLTK.K
          23    590.3109  1178.6073  1178.5502   48.4    0    45      0.032    1   K.QAHTMDPQLR.L + Gln->pyro-Glu (N-term Q)
          26    595.8389  1189.6632  1189.6343   24.3    0    57     0.0021    1   R.SDEAVKPFGLK.V
          28    598.2979  1194.5812  1194.5452   30.1    0   (41)     0.081    1   K.QAHTMDPQLR.L + Gln->pyro-Glu (N-term Q); Oxidation (M)
          29    598.8069  1195.5993  1195.5768   18.8    0   (42)      0.06    1   K.QAHTMDPQLR.L
          45    621.3683  1240.7220  1240.6928   23.6    0    79   7.8e-006    1   R.EVWALVQAGIR.D
          47    623.3479  1244.6813  1244.6434   30.4    0    44      0.043    1   R.AALQEELQLCK.G
          48    624.3424  1246.6703  1246.6418   22.9    0    57     0.0024    1   K.VSVHVIEGDHR.T
          53    632.3867  1262.7589  1262.7347   19.2    0    72   2.8e-005    1   R.LQVVDQPLPVR.G
          61    645.8050  1289.5954  1289.5677   21.5    0    71   8.7e-005    1   K.VYQWDDPDPR.L
          62    646.8413  1291.6681  1291.6152   41.0    0   109   1.4e-008    1   -.MEEVVIAGMSGK.L + Acetyl (Protein N-term)
          63    649.8613  1297.7080  1297.6626   35.0    0   107   1.7e-008    1   K.VGDPQELNGITR.A
          64    650.3605  1298.7064  1298.6466   46.0    0   (87)  1.8e-006    1   K.VGDPQELNGITR.A + Deamidated (NQ)
          69    661.8680  1321.7214  1321.6878   25.4    0    55     0.0032    1   R.QQEQQVPILEK.F + Gln->pyro-Glu (N-term Q)
          71    662.8294  1323.6443  1323.6050   29.7    0   (82)  6.9e-006    1   -.MEEVVIAGMSGK.L + Acetyl (Protein N-term); 2 Oxidation
          72    665.8840  1329.7535  1329.7153   28.7    0    69    0.00011    1   R.VTAIHIDPATHR.Q
          76    670.3823  1338.7501  1338.7143   26.7    0   (51)    0.0055    1   R.QQEQQVPILEK.F
          81    676.8826  1351.7506  1351.7282   16.5    0    68    0.00012    1   K.MVVPGLDGAQIPR.D
          86    693.9030  1385.7915  1385.7402   37.0    0   105   2.5e-008    1   K.GVDLVLNSLAEEK.L
          88    701.8559  1401.6973  1401.6446   37.6    0    81   9.7e-006    1   K.ADEASELACPTPK.E + Propionamide (C)
          89    703.4254  1404.8362  1404.8089   19.4    0    98   8.5e-008    1   R.DLVEAVAHILGIR.D
          90    703.8970  1405.7795  1405.7388   29.0    0   101   7.4e-008    1   K.VLQGDLVMNVYR.D
          95    711.9018  1421.7890  1421.7337   38.9    0   (82)  5.9e-006    1   K.VLQGDLVMNVYR.D + Oxidation (M)
          97    713.8993  1425.7841  1425.7650   13.4    0    77   1.7e-005    1   R.SLLVNPEGPTLMR.L
         102    721.9174  1441.8202  1441.7599   41.8    0   (57)    0.0018    1   R.SLLVNPEGPTLMR.L + Oxidation (M)
         105    735.3692  1468.7239  1468.6947   19.9    0    70    0.00012    1   R.FPQLDSTSFANSR.D
         108    748.4380  1494.8615  1494.8154   30.8    1    52     0.0046    1   R.RQQEQQVPILEK.F
         113    768.9401  1535.8656  1535.8209   29.1    0    83   4.5e-006    1   K.VVEVLAGHGHLYSR.I
         119    775.9112  1549.8078  1549.7624   29.3    0    90   1.3e-006    1   R.AFEVSENGNLVVSGK.V + Deamidated (NQ)
         124    797.4619  1592.9092  1592.8967   7.85    0    53     0.0042    1   R.VLFPATGYLSIVWK.T
         127    807.4366  1612.8586  1612.8169   25.9    0   101      1e-007    1   K.EDGLAQQQTQLNLR.S
         128    811.9984  1621.9822  1621.9291   32.8    0   115      1e-009    1   K.VVVQVLAEEPEAVLK.G
         132    825.9376  1649.8606  1649.8195   24.9    0   (55)    0.0042    1   K.SNMGHPEPASGLAALAK.V
         135    833.9291  1665.8437  1665.8144   17.6    0    72   8.3e-005    1   K.SNMGHPEPASGLAALAK.V + Oxidation (M)
```

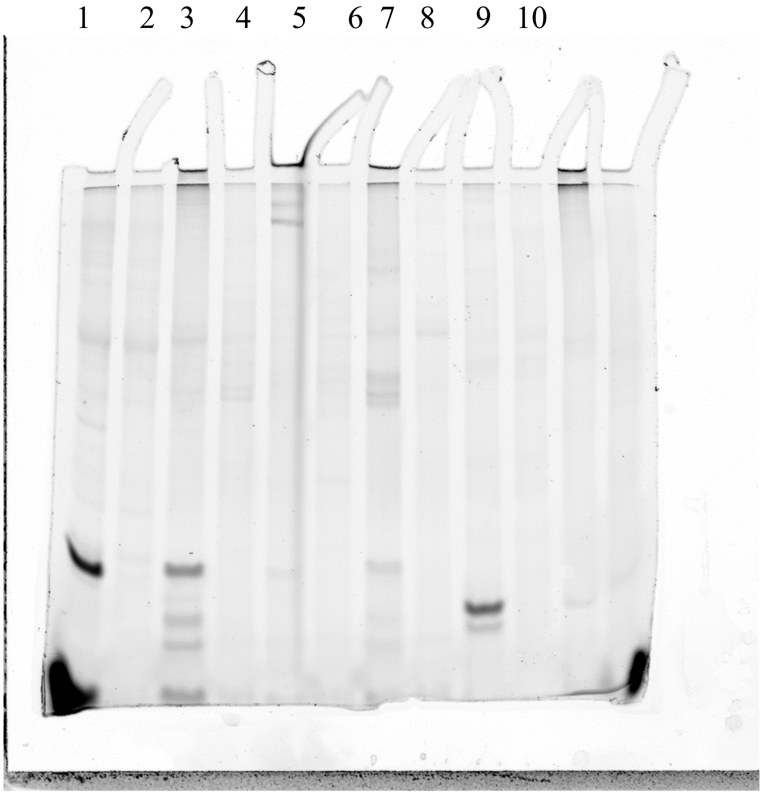
Figure S6 Fluorescent SDS-PAGE analysis of pan KO's



E.coli lacking the genes panF (2), panC (3), and panD (4), were incubated overnight with compound **1**. After lysis, the lysate was subjected to the conjugation reaction with fluorescent alkyne **3**. In each of the knockout strains, fatty acid ACP was labeled similarly as ACP in the native organism (1).

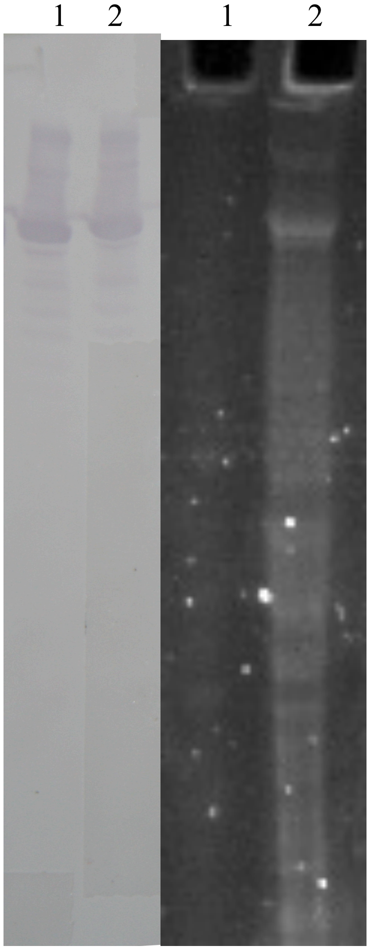
Complete gel images

Figure S7 In vivo ACP labeling in bacteria.



E. coli lysate from treated and untreated cultures (lanes 1&2). *B. subtilis* 6051 lysate from treated and untreated cultures (3&4). *B. brevis* 26A1 lysate from treated and untreated cultures (5&6). *B. subtilis* 168 lysate from treated and untreated cultures (7&8). *S. oneidensis* MR-1 lysate from treated and untreated cultures (9&10).

Figure S8 SKBR3 lysate.



Human FAS detected by western blotting with Anti-FAS (Left). Lysate from treated (2) and untreated cultures (1)

HPLC traces for all compound 2 CoA pathway intermediates

Figure S9 HPLC trace for compound **2**

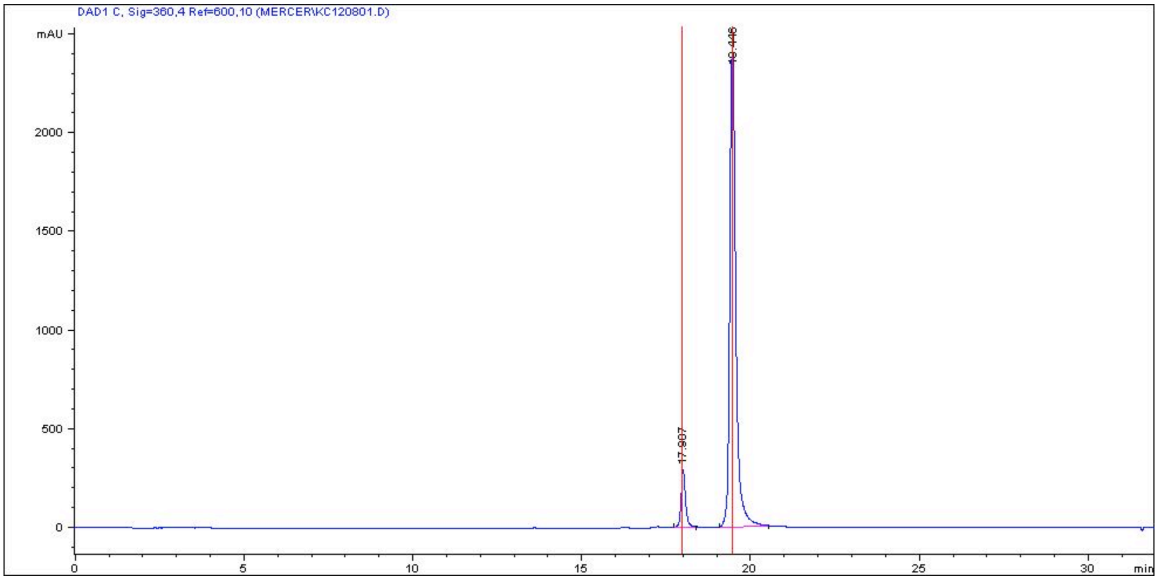


Figure S10 HPLC trace for compound **2** + PanK

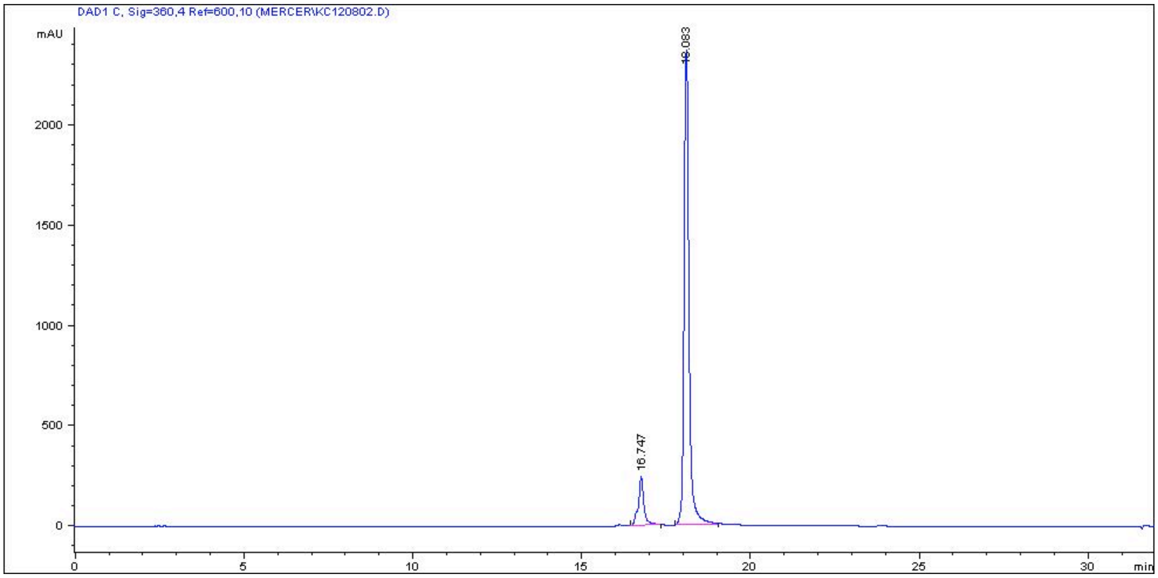


Figure S11 HPLC trace for compound **2** + PanK + PPAT

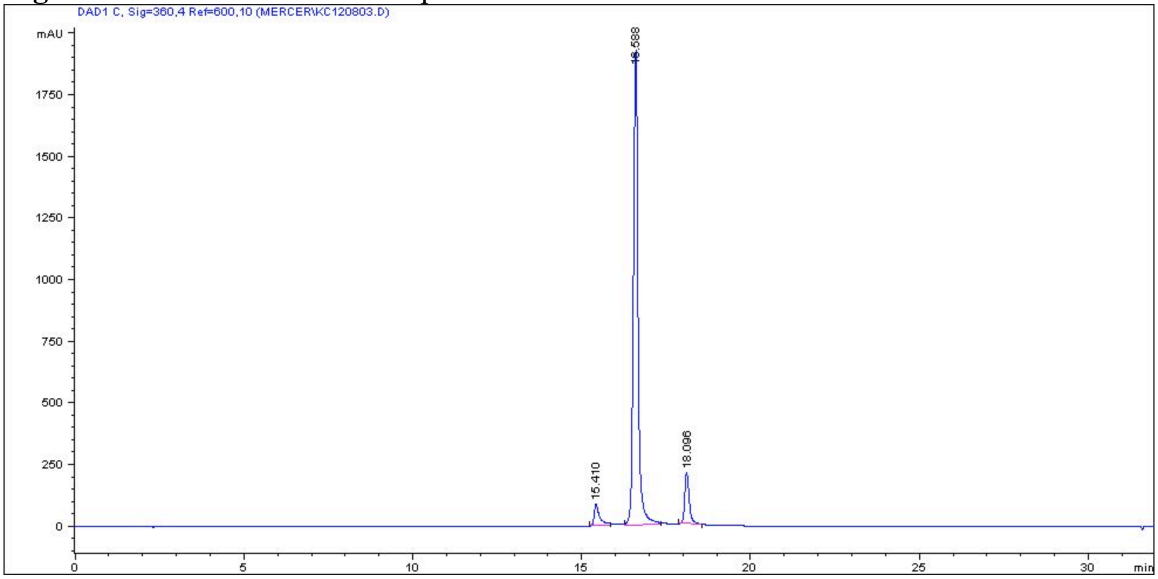


Figure S12 HPLC trace for compound **2** + PanK + PPAT + DPCK

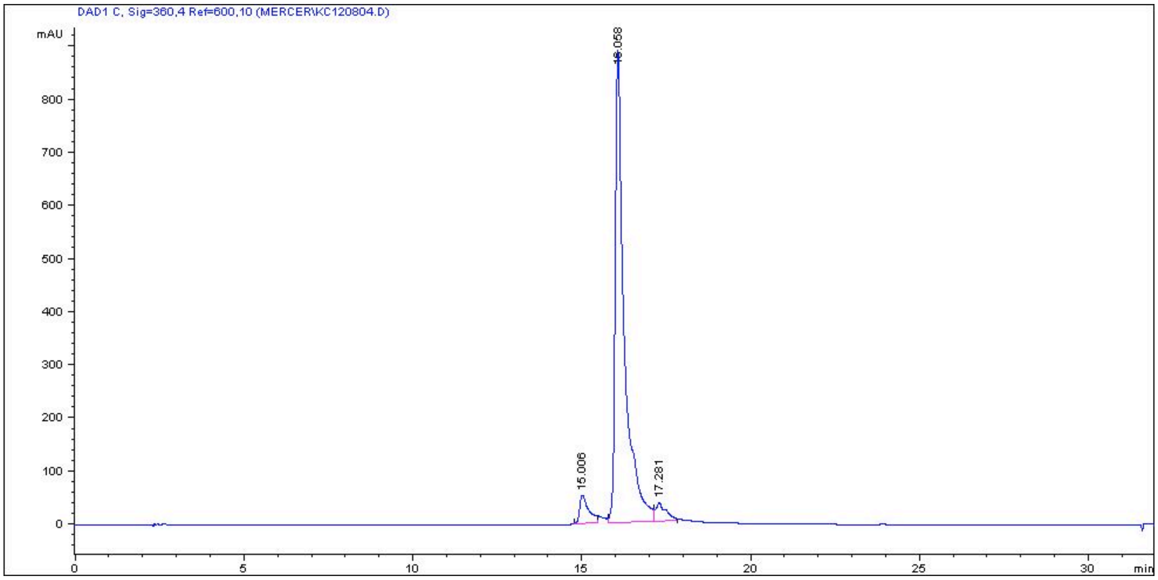


Figure S13 HPLC trace of SKBR3 lysate.

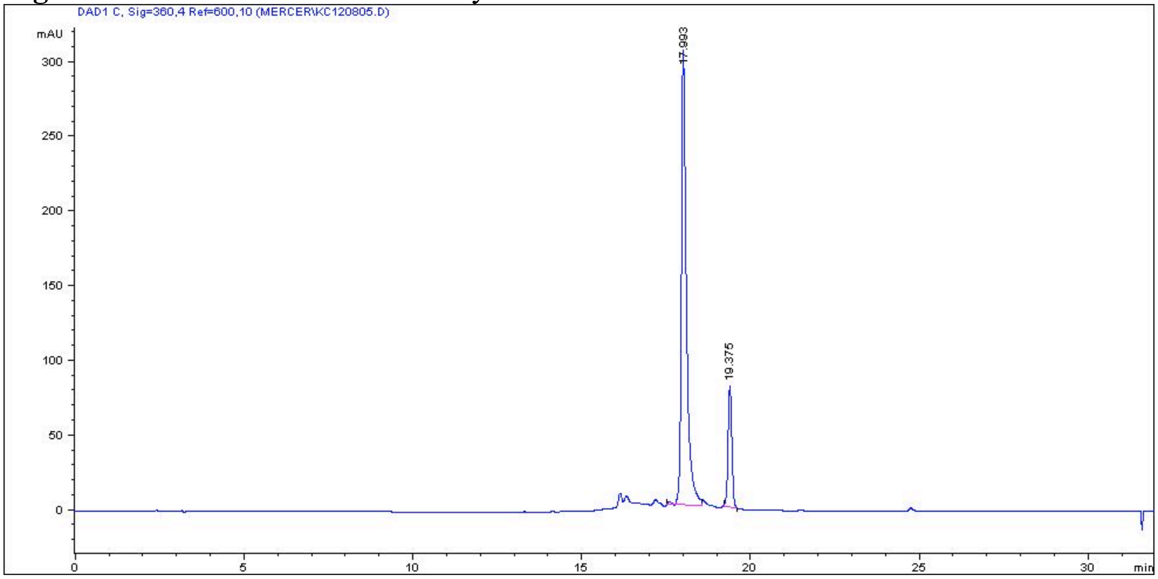
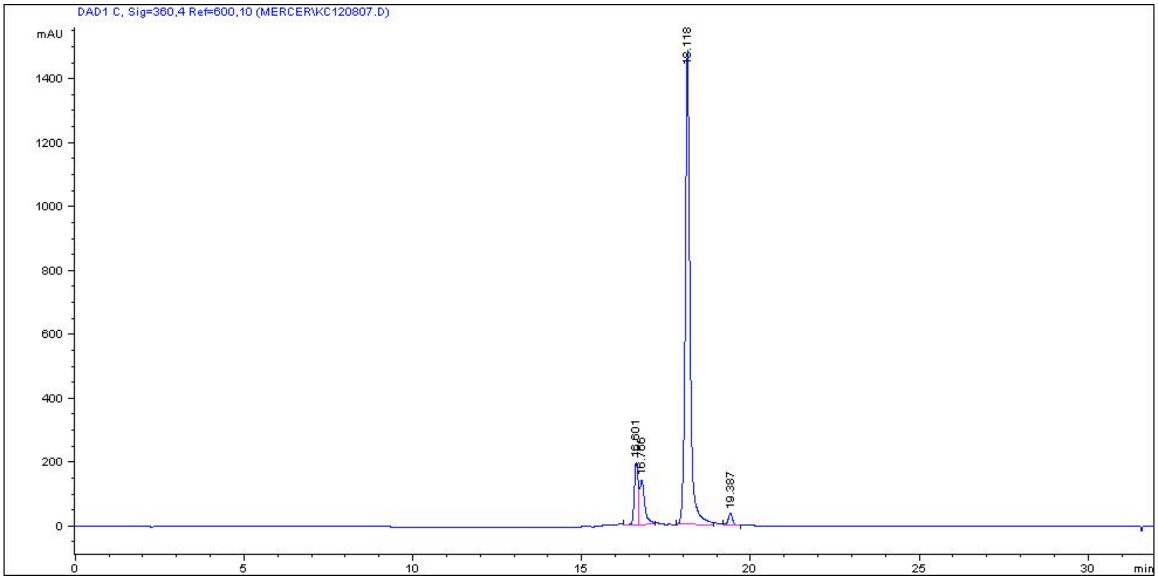


Figure S14 HPLC trace showing co-elution of phospho-**2** and SKBR3 lysate



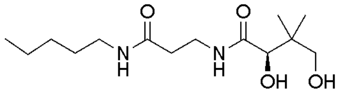
Kinetics for pantetheine analogs

Table S1 Kinetic values of pantetheine analogues with *E. coli* PanK

Compound #	Km	Vmax	Kcat	Kcat/Km
1	36.04±6.05	0.31±0.02	0.77±0.04	21.38±2.1
2	28.40±6.92	0.12±0.01	0.32±0.01	11.25±1.4
N5-Pan	33.98±7.11	0.34±0.02	0.84±0.05	24.59±2.9
Pantothenic acid	28.56±1.76	0.21±0.00	0.52±0.02	18.25±1.9

.

Figure S15 Structure of N5-Pan



N5-Pan

Scheme for synthesis of fluorescent alkynes 3 and 4

Synthesis detailed in main text.