## Supplementary Materials

Branched-chain amino acid metabolism controls membrane phospholipid structure in *Staphylococcus aureus*

Matthew W. Frank, Sarah G. Whaley and Charles O. Rock

Tables S1 to S6  
Figures S1 to S4

### Table S1

**Fatty acid composition of *S. aureus* grown with different branched-chain amino acids**

Strain AH1263 was grown in either complete defined medium or in media lacking either Ile, Leu or Ile plus Leu as described under Experimental Procedures. Cells were extracted and the derived fatty acid methyl esters were resolved on a capillary gas chromatography column equipped with flame ionization detector for quantification. Triplicate biological samples were analyzed. Means ± S.E.M. were calculated using GraphPad/Prism software.

| Fatty Acid | w/ Ile, Leu, Val | w/ Ile, Val | w/ Leu, Val | w/ Val |
|------------|------------------|-------------|-------------|--------|
|            | % Total | SEM | % Total | SEM | % Total | SEM | % Total | SEM |
| i14:0      | ND*       |      | ND    |     | 2.67   | 0.13 | 5.73   | 0.20 |
| 14:0       | ND        |      | ND    |     | 0.20   | 0.01 | 0.58   | 0.01 |
| i15:0      | 3.45      | 0.30 | ND    |     | 28.77  | 0.88 | 4.31   | 0.04 |
| a15:0      | 39.04     | 0.98 | 40.28 | 0.78| 11.67  | 0.29 | 24.51  | 1.01 |
| i16:0      | ND        |      | 0.61  | 0.01| 3.12   | 0.05 | 7.59   | 0.50 |
| 16:0       | 2.46      | 0.20 | 2.04  | 0.33| 2.80   | 0.08 | 2.51   | 0.04 |
| i17:0      | 3.31      | 0.16 | ND    |     | 10.91  | 0.54 | 1.30   | 0.07 |
| a17:0      | 28.35     | 0.95 | 34.35 | 0.41| 2.71   | 0.10 | 6.10   | 0.21 |
| 17:0       | ND        |      | ND    |     | 1.00   | 0.05 | 0.70   | 0.01 |
| i18:0      | ND        |      | 0.59  | 0.03| 1.67   | 0.06 | 4.40   | 0.16 |
| 18:0       | 7.08      | 0.43 | 5.27  | 0.12| 11.53  | 0.26 | 11.62  | 0.44 |
| i19:0      | 1.70      | 0.02 | ND    |     | 5.36   | 0.07 | 1.05   | 0.04 |
| a19:0      | 8.58      | 0.19 | 12.47 | 0.36| 0.74   | 0.05 | 2.35   | 0.06 |
| 19:0       | ND        |      | ND    |     | 4.53   | 0.09 | 3.59   | 0.21 |
| i20:0      | ND        |      | ND    |     | 0.44   | 0.03 | 2.27   | 0.16 |
| 20:0       | 6.02      | 0.33 | 4.39  | 0.08| 11.88  | 0.31 | 21.37  | 0.65 |

* ND means less than 0.2%.
Table S2
Composition of defined growth medium for *S. aureus*.

| Components            | Final Concentration |
|-----------------------|---------------------|
| M9 Salts\(^a\)        | 1X                  |
| MgSO\(_4\)            | 1 mM                |
| CaCl\(_2\)            | 0.1 mM              |
| Vitamin B1            | 0.0005%             |
| Vitamin B3            | 32 \(\mu\)M         |
| Glucose               | 0.4%                |
| Biotin                | 0.1 \(\mu\)g/ml    |
| Pantothenate          | 2 \(\mu\)g/ml      |
| FeCl\(_3\)            | 10 \(\mu\)M        |
| Citrate               | 6 \(\mu\)g/ml      |
| MnCl\(_2\)            | 10 \(\mu\)g/ml     |
| Lipoic Acid           | 0.1 \(\mu\)g/ml    |
| Phenylalanine         | 15 \(\mu\)g/ml     |
| Lysine-HCl            | 40 \(\mu\)g/ml     |
| Arginine              | 200 \(\mu\)g/ml    |
| Glycine               | 10 \(\mu\)g/ml     |
| Serine                | 30 \(\mu\)g/ml     |
| Proline               | 20 \(\mu\)g/ml     |
| Hydroxyproline        | 20 \(\mu\)g/ml     |
| Aspartic Acid         | 20 \(\mu\)g/ml     |
| Threonine             | 20 \(\mu\)g/ml     |
| Histidine             | 15 \(\mu\)g/ml     |
| Cystine-2HCl          | 65 \(\mu\)g/ml     |
| Tyrosine              | 24 \(\mu\)g/ml     |
| Methionine            | 15 \(\mu\)g/ml     |
| Asparagine            | 57 \(\mu\)g/ml     |
| Glutamine             | 50 \(\mu\)g/ml     |
| Valine                | 20 \(\mu\)g/ml     |
| Leucine               | 50 \(\mu\)g/ml     |
| Isoleucine            | 50 \(\mu\)g/ml     |

\(^a\)M9 salts formula from Ref. (48).
Table S3
MRM table for the detection of acyl-ACP by mass spectrometry.

| acyl-ACP | Q1<sup>a</sup> | Q3<sup>b</sup> | DP<sup>c</sup> | CE<sup>d</sup> |
|----------|----------------|----------------|--------------|---------------|
| apo-ACP  | 334.2          | 203.1          | 60           | 15            |
| holo-ACP | 674.2          | 261.1          | 80           | 40            |
| C2-ACP   | 716.3          | 303.2          | 85           | 40            |
| Mal-ACP  | 760.2          | 347.2          | 85           | 45            |
| C3-ACP   | 730.3          | 317.2          | 85           | 40            |
| C4-ACP   | 744.3          | 331.2          | 85           | 40            |
| C5-ACP   | 758.3          | 345.2          | 90           | 45            |
| C6-ACP   | 772.3          | 359.2          | 90           | 45            |
| C7-ACP   | 786.3          | 373.2          | 90           | 45            |
| C8-ACP   | 800.4          | 387.3          | 90           | 45            |
| C9-ACP   | 814.3          | 401.2          | 90           | 45            |
| C10:1-ACP| 826.4          | 413.3          | 90           | 45            |
| C10-ACP  | 828.4          | 415.3          | 90           | 45            |
| C11-ACP  | 842.3          | 429.2          | 90           | 45            |
| C12:1-ACP| 854.4          | 441.3          | 90           | 45            |
| C12-ACP  | 856.4          | 443.3          | 90           | 45            |
| C13-ACP  | 870.3          | 457.2          | 90           | 45            |
| C14:1-ACP| 882.4          | 469.3          | 90           | 45            |
| C14-ACP  | 884.4          | 471.3          | 90           | 45            |
| C15-ACP  | 898.3          | 485.2          | 90           | 45            |
| C16:1-ACP| 910.5          | 497.4          | 90           | 45            |
| C16-ACP  | 912.5          | 499.4          | 90           | 45            |
| C17-ACP  | 926.3          | 513.2          | 90           | 45            |
| C18:1-ACP| 938.5          | 525.4          | 90           | 45            |
| C18-ACP  | 940.5          | 527.4          | 90           | 45            |
| C19-ACP  | 954.3          | 541.2          | 90           | 45            |
| C20:1-ACP| 966.3          | 553.2          | 90           | 45            |
| C20-ACP  | 968.3          | 555.2          | 90           | 45            |
| C21-ACP  | 982.3          | 569.2          | 90           | 45            |

<sup><small>a</small></sup> Q1 is the parent m/z.

<sup><small>b</small></sup> Q3 is the product m/z.

<sup><small>c</small></sup> DP is declustering potential (V).

<sup><small>d</small></sup> CE is collision energy (V).
Table S4
MRM table for the detection of hydroxyacyl-ACP by mass spectrometry.

| hydroxyacyl-ACP | Q1\(^a\) | Q3\(^b\) | DP\(^c\) | CE\(^d\) |
|-----------------|--------|--------|--------|--------|
| hC4-ACP         | 760.3  | 347.2  | 85     | 40     |
| hC5-ACP         | 774.3  | 361.2  | 90     | 45     |
| hC6-ACP         | 788.3  | 375.2  | 90     | 45     |
| hC7-ACP         | 802.3  | 389.2  | 90     | 45     |
| hC8-ACP         | 816.3  | 403.2  | 90     | 45     |
| hC9-ACP         | 830.3  | 417.2  | 90     | 45     |
| hC10:1-ACP      | 842.4  | 429.3  | 90     | 45     |
| hC10-ACP        | 844.4  | 431.3  | 90     | 45     |
| hC11-ACP        | 858.4  | 445.3  | 90     | 45     |
| hC12:1-ACP      | 870.4  | 457.3  | 90     | 45     |
| hC12-ACP        | 872.4  | 459.3  | 90     | 45     |
| hC13-ACP        | 886.4  | 473.3  | 90     | 45     |
| hC14:1-ACP      | 898.4  | 485.3  | 90     | 45     |
| hC14-ACP        | 900.4  | 487.3  | 90     | 45     |
| hC15-ACP        | 914.4  | 501.3  | 90     | 45     |
| hC16:1-ACP      | 926.5  | 513.4  | 90     | 45     |
| hC16-ACP        | 928.5  | 515.4  | 90     | 45     |
| hC17-ACP        | 942.5  | 529.4  | 90     | 45     |
| hC18:1-ACP      | 954.5  | 541.4  | 90     | 45     |
| hC18-ACP        | 956.5  | 543.4  | 90     | 45     |
| hC19-ACP        | 970.5  | 557.4  | 90     | 45     |
| hC20:1-ACP      | 982.5  | 569.4  | 90     | 45     |
| hC20-ACP        | 984.5  | 571.4  | 90     | 45     |
| hC21-ACP        | 998.5  | 585.4  | 90     | 45     |

\(^a\) Q1 is the parent m/z.
\(^b\) Q3 is the product m/z.
\(^c\) DP is declustering potential (V).
\(^d\) CE is collision energy (V).
Table S5
MRM table for the detection of trans-2-acyl-ACP by mass spectrometry.

| trans-ACP    | Q1\(^a\) | Q3\(^b\) | DP\(^c\) | CE\(^d\) |
|--------------|----------|----------|----------|----------|
| tC4-ACP      | 742.3    | 329.2    | 85       | 40       |
| tC5-ACP      | 756.3    | 343.2    | 90       | 45       |
| tC6-ACP      | 770.3    | 357.2    | 90       | 45       |
| tC7-ACP      | 784.3    | 371.2    | 90       | 45       |
| tC8-ACP      | 798.3    | 385.2    | 90       | 45       |
| tC9-ACP      | 812.3    | 399.2    | 90       | 45       |
| tC10:1-ACP   | 824.4    | 411.3    | 90       | 45       |
| tC10-ACP     | 826.4    | 413.3    | 90       | 45       |
| tC11-ACP     | 840.3    | 427.2    | 90       | 45       |
| tC12:1-ACP   | 852.4    | 439.3    | 90       | 45       |
| tC12-ACP     | 854.4    | 441.3    | 90       | 45       |
| tC13-ACP     | 868.4    | 455.3    | 90       | 45       |
| tC14:1-ACP   | 880.4    | 467.3    | 90       | 45       |
| tC14-ACP     | 882.4    | 469.3    | 90       | 45       |
| tC15-ACP     | 896.4    | 483.3    | 90       | 45       |
| tC16:1-ACP   | 908.5    | 495.4    | 90       | 45       |
| tC16-ACP     | 910.5    | 497.4    | 90       | 45       |
| tC17-ACP     | 924.5    | 511.4    | 90       | 45       |
| tC18:1-ACP   | 936.5    | 523.4    | 90       | 45       |
| tC18-ACP     | 938.5    | 525.4    | 90       | 45       |
| tC19-ACP     | 952.5    | 539.4    | 90       | 45       |
| tC20:1-ACP   | 964.5    | 551.4    | 90       | 45       |
| tC20-ACP     | 966.5    | 553.4    | 90       | 45       |
| tC21-ACP     | 980.5    | 567.5    | 90       | 45       |

\(^a\) Q1 is the parent m/z.
\(^b\) Q3 is the product m/z.
\(^c\) DP is declustering potential (V).
\(^d\) CE is collision energy (V).
Table S6
MRM table for the detection of 3-ketoacyl-ACP by mass spectrometry.

| 3-ketoacyl-ACP | Q1<sup>a</sup> | Q3<sup>b</sup> | DP<sup>c</sup> | CE<sup>d</sup> |
|----------------|---------------|---------------|---------------|---------------|
| kC4-ACP        | 758.3         | 345.2         | 90            | 40            |
| kC5-ACP        | 772.3         | 359.2         | 95            | 45            |
| kC6-ACP        | 786.3         | 373.2         | 95            | 45            |
| kC7-ACP        | 800.3         | 387.2         | 95            | 45            |
| kC8-ACP        | 814.3         | 401.2         | 95            | 45            |
| kC9-ACP        | 828.3         | 415.2         | 95            | 45            |
| kC10:1-ACP     | 840.3         | 427.3         | 95            | 45            |
| kC10-ACP       | 842.3         | 429.3         | 95            | 45            |
| kC11-ACP       | 856.4         | 443.3         | 95            | 45            |
| kC12:1-ACP     | 868.3         | 455.3         | 95            | 45            |
| kC12-ACP       | 870.3         | 457.3         | 95            | 45            |
| kC13-ACP       | 884.4         | 471.3         | 95            | 45            |
| kC14:1-ACP     | 896.3         | 483.3         | 95            | 45            |
| kC14-ACP       | 898.3         | 485.3         | 95            | 45            |
| kC15-ACP       | 912.4         | 499.3         | 95            | 45            |
| kC16:1-ACP     | 924.4         | 511.4         | 95            | 45            |
| kC16-ACP       | 926.4         | 513.4         | 95            | 45            |
| kC17-ACP       | 940.5         | 527.4         | 95            | 45            |
| kC18:1-ACP     | 952.5         | 539.4         | 95            | 45            |
| kC18-ACP       | 954.5         | 541.4         | 95            | 45            |
| kC19-ACP       | 968.5         | 555.4         | 95            | 45            |
| kC20:1-ACP     | 980.5         | 567.4         | 95            | 45            |
| kC20-ACP       | 982.5         | 569.4         | 95            | 45            |
| kC21-ACP       | 996.5         | 583.4         | 95            | 45            |

<sup>a</sup> Q1 is the parent m/z.
<sup>b</sup> Q3 is the product m/z.
<sup>c</sup> DP is declustering potential (V).
<sup>d</sup> CE is collision energy (V).
| Carbon Number | m/z  | Acyl Chain Composition |
|---------------|------|------------------------|
|               |      | 1-position  | 2-position |
| 30:0          | 693.5| 15:0        | 15:0       |
| 31:0          | 707.6| 16:0        | 15:0       |
| 32:0          | 721.5| 17:0        | 15:0       |
| 33:0          | 735.6| 18:0        | 15:0       |
| 34:0          | 749.6| 19:0        | 15:0       |
| 35:0          | 763.6| 20:0        | 15:0       |

**Figure S1.** Structures of phosphatidylglycerol (PG) molecular species in *S. aureus* grown in defined medium. The table on the left lists the carbon number and *m/z* masses detected in the mass spectrometry experiments and the composition of the 1- and 2-position fatty acids in these molecular species. The chemical structure on the right shows the chemical structure of the major PG molecular species (32:0) with the 1- and 2-position indicated. In defined medium (with isoleucine present) the most abundant branched-chain fatty acids have anteiso branching.
Figure S2. PG molecular species of *S. aureus* grown with different branched-chain amino acids. Strain AH1263 was grown in defined media as described in Experimental Procedures and representative mass spectra illustrating the PG molecular species composition are shown for each growth condition. *A*, complete defined medium containing Ile, Leu and Val. *B*, medium lacking Leu. *C*, medium lacking Ile. *D*, medium lacking Ile and Leu.
Strain AH1263 was grown in defined medium containing Ile concentrations between 0 and 160 µg/ml. Triplicate biological replicates were harvested at an $A_{600}$ of 0.6, extracted and the PG molecular species distribution determined by LC-MS/MS. The contribution of each PG molecular species to the total was calculated using LipidView software (Sciex) and the contribution of each of the 8 species was plotted as a function of Ile concentration. A, 29:0-PG. B, 30:0-PG. C, 31:0-PG. D, 32:0-PG. E, 33:0-PG. F, 34:0-PG. G, 35:0-PG. H, 36:0-PG. The data are plotted as mean ± S.E.M. with three biological replicates.
Figure S4. Branched-chain amino acid impact on the growth of *S. aureus* in defined medium. The growth rates of strain AH1263 in complete defined medium (black) containing Ile, Leu and Val (Table S2) is compared to the growth rate when one or more of the branched-chain amino acids were removed. Leu removed (red), Ile removed (green); and Ile + Leu removed (blue).