

**Supplementary Materials:** Non-significant ( $p > 0.05$ ) serum metabolites at –8 wks and –4 wks before calving

Table S1. Concentration of serum metabolites (MEAN  $\pm$  SEM) in pre-subclinical mastitis cows only (pre-SCM,  $n = 10$ ) and healthy controls (CON,  $n = 15$ ) cows at –8 wks before parturition, as identified by LC-MS/MS.

| Metabolites ( $\mu$ M)   | MEAN $\pm$ SEM         |                        | <i>P</i> value | Fold change | SCM/CON |
|--------------------------|------------------------|------------------------|----------------|-------------|---------|
|                          | Pre-SCM ( $n = 10$ )   | CON ( $n = 15$ )       |                |             |         |
| Acetyl-ornithine         | 4.58 $\pm$ 0.609       | 4.17 $\pm$ 0.535       | 0.4            | 1.1         | up      |
| alpha-Ketoglutaric acid  | 25.3 $\pm$ 3.91        | 24.6 $\pm$ 3.44        | 0.7            | 1.03        | up      |
| Aspartic acid            | 7.57 $\pm$ 0.951       | 7.74 $\pm$ 0.836       | 0.7            | 0.98        | down    |
| beta-Hydroxybutyric acid | 730 $\pm$ 148          | 781 $\pm$ 131          | 0.3            | 0.93        | down    |
| C0                       | 4.23 $\pm$ 0.443       | 3.04 $\pm$ 0.389       | 0.1            | 1.39        | up      |
| C10                      | 0.0481 $\pm$ 0.00471   | 0.0467 $\pm$ 0.00414   | 0.4            | 1.03        | up      |
| C10:1                    | 0.093 $\pm$ 0.00774    | 0.0989 $\pm$ 0.0068    | 0.4            | 0.94        | down    |
| C10:2                    | 0.0246 $\pm$ 0.00258   | 0.0236 $\pm$ 0.00227   | 0.9            | 1.04        | up      |
| C12                      | 0.027 $\pm$ 0.00204    | 0.0216 $\pm$ 0.0018    | 0.1            | 1.25        | up      |
| C12:1                    | 0.0476 $\pm$ 0.003     | 0.053 $\pm$ 0.00264    | 0.3            | 0.9         | down    |
| C12DC                    | 0.018 $\pm$ 0.00181    | 0.0182 $\pm$ 0.00159   | 0.4            | 0.99        | down    |
| C14                      | 0.0252 $\pm$ 0.00314   | 0.0182 $\pm$ 0.00276   | 0.2            | 1.38        | up      |
| C14:1                    | 0.0448 $\pm$ 0.00883   | 0.0677 $\pm$ 0.00777   | 0.1            | 0.66        | down    |
| C14:2                    | 0.00996 $\pm$ 0.000707 | 0.00856 $\pm$ 0.000622 | 0.4            | 1.16        | up      |
| C14:2OH                  | 0.01086 $\pm$ 0.00103  | 0.00964 $\pm$ 0.00091  | 0.7            | 1.13        | up      |
| C16                      | 0.0188 $\pm$ 0.00183   | 0.0168 $\pm$ 0.00161   | 0.7            | 1.12        | up      |
| C16:1                    | 0.0188 $\pm$ 0.000993  | 0.0184 $\pm$ 0.000873  | 0.7            | 1.02        | up      |
| C16:1OH                  | 0.0134 $\pm$ 0.0012    | 0.0141 $\pm$ 0.00105   | 0.3            | 0.95        | down    |
| C16:2                    | 0.00732 $\pm$ 0.000679 | 0.00698 $\pm$ 0.000597 | 0.9            | 1.05        | up      |
| C16:2OH                  | 0.00668 $\pm$ 0.000728 | 0.00731 $\pm$ 0.00064  | 0.1            | 0.91        | down    |
| C16OH                    | 0.00798 $\pm$ 0.000888 | 0.0075 $\pm$ 0.000781  | 0.6            | 1.06        | up      |
| C18                      | 0.0408 $\pm$ 0.00456   | 0.0343 $\pm$ 0.00401   | 0.7            | 1.19        | up      |
| C18:1                    | 0.0151 $\pm$ 0.00139   | 0.0146 $\pm$ 0.00122   | 0.8            | 1.03        | up      |
| C18:1OH                  | 0.00863 $\pm$ 0.000569 | 0.00774 $\pm$ 0.0005   | 0.4            | 1.11        | up      |
| C18:2                    | 0.00685 $\pm$ 0.000629 | 0.00694 $\pm$ 0.000554 | 0.6            | 0.99        | down    |
| C2                       | 1.74 $\pm$ 0.15        | 1.46 $\pm$ 0.132       | 0.8            | 1.19        | up      |
| C3                       | 0.191 $\pm$ 0.0175     | 0.178 $\pm$ 0.0154     | 0.2            | 1.07        | up      |
| C3:1                     | 0.0292 $\pm$ 0.00278   | 0.0301 $\pm$ 0.00245   | 0.6            | 0.97        | down    |
| C3OH                     | 0.0184 $\pm$ 0.00159   | 0.0178 $\pm$ 0.0014    | 0.5            | 1.03        | up      |
| C4                       | 0.093 $\pm$ 0.00793    | 0.0796 $\pm$ 0.00697   | 0.2            | 1.17        | up      |

|                        |                    |                    |      |      |      |
|------------------------|--------------------|--------------------|------|------|------|
| C4:1                   | 0.0155 ± 0.00153   | 0.0164 ± 0.00134   | 0.4  | 0.95 | down |
| C5                     | 0.0617 ± 0.00617   | 0.0648 ± 0.00542   | 0.5  | 0.95 | down |
| C5:1                   | 0.0159 ± 0.00131   | 0.0172 ± 0.00115   | 0.08 | 0.92 | down |
| C5MDC                  | 0.0189 ± 0.000967  | 0.0203 ± 0.000851  | 0.09 | 0.93 | down |
| C5OH                   | 0.065 ± 0.00942    | 0.0629 ± 0.00828   | 0.6  | 1.03 | up   |
| C6                     | 0.0411 ± 0.00578   | 0.0516 ± 0.00509   | 0.3  | 0.8  | down |
| C9                     | 0.00766 ± 0.000669 | 0.00777 ± 0.000589 | 0.2  | 0.99 | down |
| Citrulline             | 91.1 ± 8.57        | 87.6 ± 7.53        | 0.6  | 1.04 | up   |
| Creatine               | 228 ± 12.1         | 241 ± 10.6         | 0.08 | 0.95 | down |
| Creatinine             | 72.2 ± 4.65        | 74.4 ± 4.09        | 0.1  | 0.97 | down |
| Glucose                | 3602 ± 144         | 3462 ± 127         | 0.8  | 1.04 | up   |
| Glutamic acid          | 70 ± 5.43          | 65.3 ± 4.78        | 0.4  | 1.07 | up   |
| Glutamine              | 281 ± 15.4         | 263 ± 13.6         | 0.3  | 1.07 | up   |
| Histidine              | 56.8 ± 4.13        | 51.7 ± 3.63        | 0.1  | 1.1  | up   |
| Indole acetic acid     | 0.455 ± 0.0988     | 0.555 ± 0.0869     | 0.1  | 0.82 | down |
| Isobutyric acid        | 3.41 ± 0.89        | 4.72 ± 0.782       | 0.1  | 0.72 | down |
| Kynurenine             | 7.21 ± 1.108       | 7.79 ± 0.974       | 0.2  | 0.93 | down |
| Lactic acid            | 2270 ± 330         | 1895 ± 290         | 0.1  | 1.2  | up   |
| LYSOC17:0              | 1.58 ± 0.172       | 1.65 ± 0.151       | 0.1  | 0.96 | down |
| LYSOC20:3              | 3.23 ± 0.376       | 3.66 ± 0.331       | 0.1  | 0.88 | down |
| LYSOC20:4              | 2.33 ± 0.262       | 2.6 ± 0.231        | 0.1  | 0.9  | down |
| LYSOC24:0              | 0.0979 ± 0.01118   | 0.1123 ± 0.00983   | 0.1  | 0.87 | down |
| Methionine             | 22.6 ± 1.75        | 20.5 ± 1.54        | 0.2  | 1.1  | up   |
| Methyl histidine       | 8.33 ± 0.892       | 11.32 ± 0.784      | 0.06 | 0.74 | down |
| Methylmalonic acid     | 0.569 ± 0.0736     | 0.547 ± 0.0647     | 0.9  | 1.04 | up   |
| Proline                | 98.7 ± 9.07        | 111.1 ± 8.42       | 0.2  | 0.89 | down |
| Serine                 | 76.2 ± 7.76        | 86.8 ± 6.83        | 0.09 | 0.88 | down |
| Serotonin              | 12.01 ± 2.32       | 7.44 ± 2.04        | 0.1  | 1.61 | up   |
| Spermidine             | 0.354 ± 0.0514     | 0.325 ± 0.0452     | 0.6  | 1.09 | up   |
| Succinic acid          | 1.58 ± 0.148       | 1.62 ± 0.13        | 0.4  | 0.98 | down |
| Taurine                | 82.3 ± 9.43        | 71.7 ± 8.3         | 0.5  | 1.15 | up   |
| Threonine              | 108.2 ± 8.94       | 93.6 ± 7.87        | 0.15 | 1.16 | up   |
| Total dimethylarginine | 1.89 ± 0.174       | 1.85 ± 0.153       | 0.9  | 1.02 | up   |
| Trimethylamine N-oxide | 47.3 ± 15.7        | 38.7 ± 13.8        | 0.9  | 1.22 | up   |
| Tryptophan             | 42.4 ± 2.86        | 44.8 ± 2.52        | 0.2  | 0.95 | down |
| Tyrosine               | 69.8 ± 6.39        | 66.1 ± 5.62        | 0.4  | 1.06 | up   |
| Uric acid              | 30 ± 5.21          | 37.9 ± 4.58        | 0.1  | 0.79 | down |

Table S2 Concentration of serum metabolites (MEAN  $\pm$  SEM) in pre-subclinical mastitis cows only (pre-SCM,  $n = 10$ ) and healthy controls (CON,  $n = 15$ ) cows at -4 wks before parturition, as identified by LC-MS/MS.

| Metabolites ( $\mu$ M)      | MEAN $\pm$ SEM         |                        | $p$ value | Fold change | SCM/ CON |
|-----------------------------|------------------------|------------------------|-----------|-------------|----------|
|                             | Pre-SCM ( $n = 10$ )   | CON ( $n = 15$ )       |           |             |          |
| 14:1SMOH                    | 8.67 $\pm$ 1.042       | 9.55 $\pm$ 0.941       | 0.2       | 0.91        | down     |
| 16:0SM                      | 88 $\pm$ 9.99          | 104 $\pm$ 9.02         | 0.06      | 0.85        | down     |
| 16:1SM                      | 10.2 $\pm$ 1.073       | 11.5 $\pm$ 0.969       | 0.1       | 0.89        | down     |
| 16:1SMOH                    | 9.1 $\pm$ 0.963        | 10.5 $\pm$ 0.87        | 0.08      | 0.87        | down     |
| alpha-Aminoadipic acid      | 2.83 $\pm$ 0.505       | 2.2 $\pm$ 0.456        | 0.4       | 1.29        | up       |
| alpha-Ketoglutaric acid     | 19.5 $\pm$ 1.9         | 16.3 $\pm$ 1.72        | 0.2       | 1.2         | up       |
| Arginine                    | 154 $\pm$ 7.42         | 157 $\pm$ 6.7          | 0.1       | 0.98        | down     |
| Aspartic acid               | 7.05 $\pm$ 1.22        | 7.95 $\pm$ 1.1         | 0.3       | 0.89        | down     |
| Asymmetric dimethylarginine | 0.962 $\pm$ 0.076      | 1.092 $\pm$ 0.0686     | 0.1       | 0.88        | down     |
| beta-Hydroxybutyric acid    | 630 $\pm$ 100.3        | 618 $\pm$ 90.6         | 0.7       | 1.02        | up       |
| Betaine                     | 155 $\pm$ 14.3         | 162 $\pm$ 13           | 0.2       | 0.96        | down     |
| Butyric acid                | 5.42 $\pm$ 0.986       | 6.76 $\pm$ 0.891       | 0.06      | 0.8         | down     |
| C0                          | 5.1 $\pm$ 0.541        | 4.88 $\pm$ 0.488       | 0.5       | 1.05        | up       |
| C10                         | 0.0448 $\pm$ 0.00465   | 0.035 $\pm$ 0.0042     | 0.2       | 1.28        | up       |
| C10:1                       | 0.129 $\pm$ 0.0184     | 0.117 $\pm$ 0.0166     | 0.6       | 1.1         | up       |
| C12:1                       | 0.057 $\pm$ 0.00723    | 0.054 $\pm$ 0.00653    | 0.9       | 1.06        | up       |
| C12DC                       | 0.0103 $\pm$ 0.000992  | 0.0095 $\pm$ 0.000896  | 0.5       | 1.08        | up       |
| C14                         | 0.0129 $\pm$ 0.00117   | 0.0139 $\pm$ 0.00106   | 0.3       | 0.93        | down     |
| C14:1OH                     | 0.00776 $\pm$ 0.000576 | 0.00738 $\pm$ 0.00052  | 0.4       | 1.05        | up       |
| C14:2                       | 0.00849 $\pm$ 0.001064 | 0.0083 $\pm$ 0.000961  | 0.7       | 1.02        | up       |
| C16:1                       | 0.0161 $\pm$ 0.00118   | 0.017 $\pm$ 0.00106    | 0.2       | 0.95        | down     |
| C16:2                       | 0.00611 $\pm$ 0.000575 | 0.00583 $\pm$ 0.00052  | 0.7       | 1.05        | down     |
| C16:2OH                     | 0.00709 $\pm$ 0.000683 | 0.007 $\pm$ 0.000617   | 0.5       | 1.01        | up       |
| C16OH                       | 0.00538 $\pm$ 0.000716 | 0.00662 $\pm$ 0.000647 | 0.1       | 0.81        | down     |
| C18:1OH                     | 0.01052 $\pm$ 0.00142  | 0.00982 $\pm$ 0.00128  | 0.8       | 1.07        | up       |
| C18:2                       | 0.00602 $\pm$ 0.000733 | 0.00548 $\pm$ 0.000662 | 0.8       | 1.1         | up       |
| C2                          | 1.7 $\pm$ 0.223        | 1.99 $\pm$ 0.202       | 0.1       | 0.85        | down     |
| C3                          | 0.203 $\pm$ 0.0173     | 0.191 $\pm$ 0.0157     | 0.6       | 1.06        | up       |
| C3:1                        | 0.0233 $\pm$ 0.0023    | 0.0217 $\pm$ 0.00208   | 0.4       | 1.07        | up       |
| C3OH                        | 0.0254 $\pm$ 0.0026    | 0.0215 $\pm$ 0.00235   | 0.6       | 1.18        | up       |
| C4                          | 0.123 $\pm$ 0.00729    | 0.116 $\pm$ 0.00658    | 0.4       | 1.06        | up       |
| C4OH                        | 0.0317 $\pm$ 0.00354   | 0.0266 $\pm$ 0.0032    | 0.3       | 1.19        | up       |
| C5                          | 0.074 $\pm$ 0.00673    | 0.0756 $\pm$ 0.00608   | 0.4       | 0.98        | down     |

|                    |                  |                  |      |      |      |
|--------------------|------------------|------------------|------|------|------|
| C5MDC              | 0.017 ± 0.00159  | 0.0153 ± 0.00143 | 0.2  | 1.11 | up   |
| C5OH               | 0.0664 ± 0.0117  | 0.0518 ± 0.0105  | 0.2  | 1.28 | up   |
| C6                 | 0.0342 ± 0.00413 | 0.0328 ± 0.00373 | 0.6  | 1.04 | up   |
| C6:1               | 0.0214 ± 0.00297 | 0.0212 ± 0.00268 | 0.8  | 1.01 | up   |
| C8                 | 0.0133 ± 0.00118 | 0.0111 ± 0.00106 | 0.1  | 1.2  | up   |
| Carnosine          | 10.4 ± 1.7       | 13.5 ± 1.53      | 0.08 | 0.77 | down |
| Choline            | 10.6 ± 1.93      | 13.1 ± 1.74      | 0.1  | 0.81 | down |
| Citric acid        | 310 ± 40.2       | 289 ± 36.3       | 0.7  | 1.07 | up   |
| Citrulline         | 84.6 ± 6.83      | 89.7 ± 6.17      | 0.3  | 0.94 | down |
| Creatine           | 233 ± 12.2       | 244 ± 11         | 0.2  | 0.95 | down |
| Creatinine         | 80.3 ± 5.1       | 88.8 ± 4.6       | 0.1  | 0.9  | down |
| Fumaric acid       | 1.137 ± 0.188    | 0.912 ± 0.17     | 0.4  | 1.25 | up   |
| Glutamic acid      | 63.3 ± 6.55      | 67.6 ± 5.91      | 0.5  | 0.94 | down |
| Glutamine          | 324 ± 14.9       | 341 ± 13.5       | 0.1  | 0.95 | down |
| Glycine            | 267 ± 11.6       | 287 ± 10.5       | 0.1  | 0.93 | down |
| Hippuric acid      | 67.7 ± 6.24      | 70 ± 5.64        | 0.6  | 0.97 | down |
| Indole acetic acid | 0.39 ± 0.0578    | 0.382 ± 0.0522   | 0.6  | 1.02 | up   |
| Isobutyric acid    | 4.49 ± 0.642     | 4.84 ± 0.58      | 0.4  | 0.93 | down |
| Kynurenine         | 7.16 ± 0.759     | 6.51 ± 0.686     | 0.5  | 1.1  | up   |
| Leucine            | 241 ± 16.5       | 250 ± 14.9       | 0.1  | 0.96 | down |
| LYSOC14:0          | 0.794 ± 0.0798   | 0.854 ± 0.0721   | 0.2  | 0.93 | down |
| LYSOC16:0          | 16.4 ± 1.74      | 17.5 ± 1.57      | 0.1  | 0.94 | down |
| LYSOC16:1          | 1.08 ± 0.14      | 1.11 ± 0.126     | 0.5  | 0.97 | down |
| LYSOC17:0          | 1.4 ± 0.127      | 1.29 ± 0.115     | 0.9  | 1.09 | up   |
| LYSOC18:0          | 15.8 ± 1.43      | 16.1 ± 1.29      | 0.2  | 0.98 | down |
| LYSOC18:1          | 9.62 ± 1.29      | 10.98 ± 1.16     | 0.1  | 0.88 | down |
| LYSOC18:2          | 19.1 ± 2.17      | 20.5 ± 1.96      | 0.2  | 0.93 | down |
| LYSOC20:4          | 1.93 ± 0.262     | 1.75 ± 0.237     | 0.8  | 1.1  | up   |
| LYSOC24:0          | 0.111 ± 0.00934  | 0.12 ± 0.00843   | 0.4  | 0.93 | down |
| LYSOC26:0          | 0.1028 ± 0.0113  | 0.0938 ± 0.0102  | 0.5  | 1.1  | up   |
| LYSOC26:1          | 0.0387 ± 0.00579 | 0.0369 ± 0.00523 | 0.9  | 1.05 | up   |
| LYSOC28:0          | 0.246 ± 0.0213   | 0.251 ± 0.0192   | 0.3  | 0.98 | down |
| Methyl histidine   | 11.1 ± 1.7       | 14.2 ± 1.53      | 0.07 | 0.78 | down |
| Phenylalanine      | 65.4 ± 2.33      | 66.7 ± 2.1       | 0.06 | 0.98 | down |
| Propionic acid     | 19.4 ± 3.11      | 18.1 ± 2.81      | 0.8  | 1.07 | up   |
| Serotonin          | 5.94 ± 3.21      | 5.95 ± 2.9       | 0.5  | 1    | down |
| Spermidine         | 0.419 ± 0.0553   | 0.351 ± 0.05     | 0.7  | 1.19 | up   |
| Succinic acid      | 1.33 ± 0.0757    | 1.09 ± 0.0684    | 0.1  | 1.22 | up   |

|                        |             |              |      |      |      |
|------------------------|-------------|--------------|------|------|------|
| Taurine                | 66.2 ± 7.6  | 75 ± 6.86    | 0.08 | 0.88 | down |
| Threonine              | 103 ± 7.42  | 102 ± 6.7    | 0.8  | 1.01 | up   |
| Total dimethylarginine | 1.97 ± 0.17 | 2.23 ± 0.154 | 0.3  | 0.88 | down |
| trans-Hydroxyproline   | 12.5 ± 1.33 | 14.4 ± 1.2   | 0.06 | 0.87 | down |
| Trimethylamine N-oxide | 49.8 ± 14.8 | 19.3 ± 13.4  | 0.1  | 2.58 | up   |
| Tryptophan             | 45.7 ± 2.24 | 47.1 ± 2.03  | 0.1  | 0.97 | down |
| Tyrosine               | 67 ± 5.13   | 66.1 ± 4.64  | 0.4  | 1.01 | up   |
| Uric acid              | 26.8 ± 9.88 | 35.2 ± 8.93  | 0.3  | 0.76 | down |

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