Modulation of acyl-carnitines, the broad mechanism behind *Wolbachia*-mediated inhibition of medically important flaviviruses in *Aedes aegypti*.

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SI Appendix

Dataset 1. Complete set of median-normalized LCMS data. Names of individual metabolites are listed on top and median normalized raw data is shown. Each sheet includes data from each experimental combination (i.e. *w*Mel/ZIKV African, *w*Mel/ZIKV Asian and *w*Mel/DENV-1).

Dataset 2. Table of significantly modulated metabolites (p-value <0.05). Two-way ANOVA analysis was performed on each set of experimental data from *w*Mel/ZIKV African, *w*Mel/ZIKV Asian and *w*Mel/DENV-1 experiments. Both p-values and BH-adjusted p-values are shown.

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Fig. S1. Confirmation of the presence of *Wolbachia* in *Aag2.w*Mel, but not *Aag2*.TET cells. (A) Bar chart displaying *w*Mel density in *Aag2.w*Mel and *Aag2*.TET cells. *Wolbachia* density in cells was determined by comparing the relative abundance *of Wolbachia TM513 gene* to that of the single-copy *mosquito rps17* gene using qPCR. *Aag2.w*Mel cells had *Wolbachia* density of about 35 and *Aag2*.TET cells had none. All conditions were performed in triplicates; error bars represent SEM. (B) Western blot identifying presence of *Wolbachia* surface protein (WSP) in cells. A thick band correlating to *Wolbachia* surface protein was observed in *Aag2.w*Mel but not *Aag2*.TET cells. (C) Visualization of *w*Mel in *Aag2* cells using a fluorescent in situ hybridisation (FISH) assay. *w*Mel was labelled with Rhodamine/ROX (Red) and cells were visualized by a DAPI (blue) stain. *w*Mel (Red dots) was seen in *Aag2.w*Mel but not *Aag2*.TET cells.

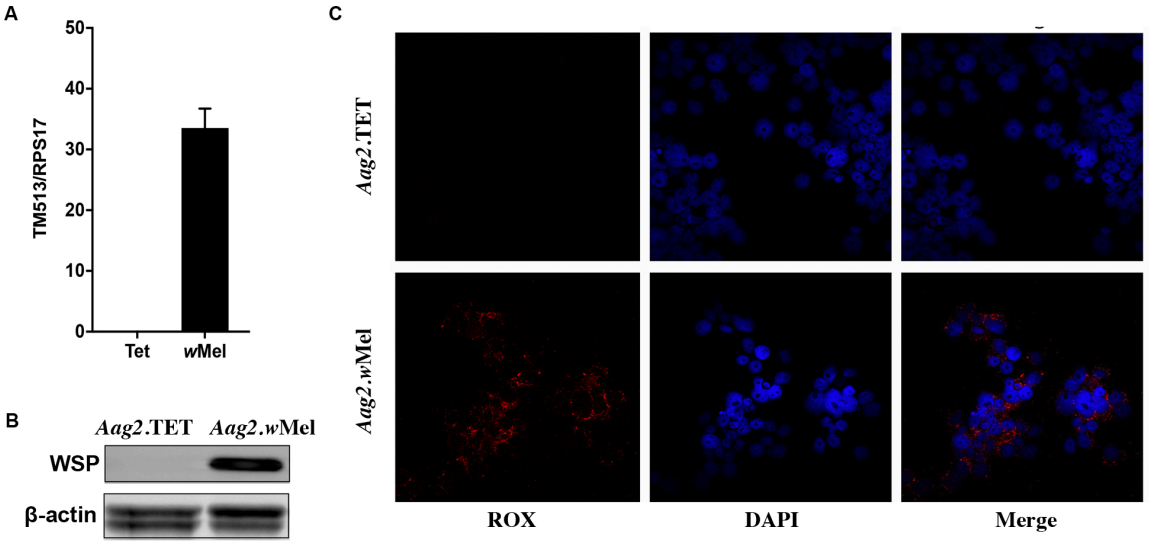


Fig. S2. **Effects of the presence of *Wolbachia* strain *w*Mel on ZIKV and DENV replication in *Ae. aegpyti* cell culture.** *Aag2*.*w*Mel (*w*Mel) and *Aag2*.TET (Tet) cells were infected with ZIKV and DENV-1 at a multiplicity of infection (MOI) of 5 and levels of virus were measured at 24, 48, and 72 h.p.i. (A, D) Plaque assay was performed in BHK-21 cells to quantify the levels of infectious virus produced; A: ZIKV; D: DENV (B, E) Viral genome replication was analyzed by qRT-PCR and relative expression of virus against *rps17*, a mosquito housekeeping gene is shown. Virus-specific primers targeting the NS5 region were used to identify infection; B: ZIKV; E: DENV (C, F) Levels of virus protein were measured by western blotting using virus-specific antibodies against the NS1 protein (ZIKV) (C) and the envelope protein (DENV) (F). b-actin is used as a loading control. N=2 experiments were conducted in triplicates independently of each other to generate data. Data are represented as mean ± SEM; *p < 0.05; **p < 0.01; ***p < 0.001; ***p < 0.0001; n.s. : Not significant, two-tailed Student's *t*-test.

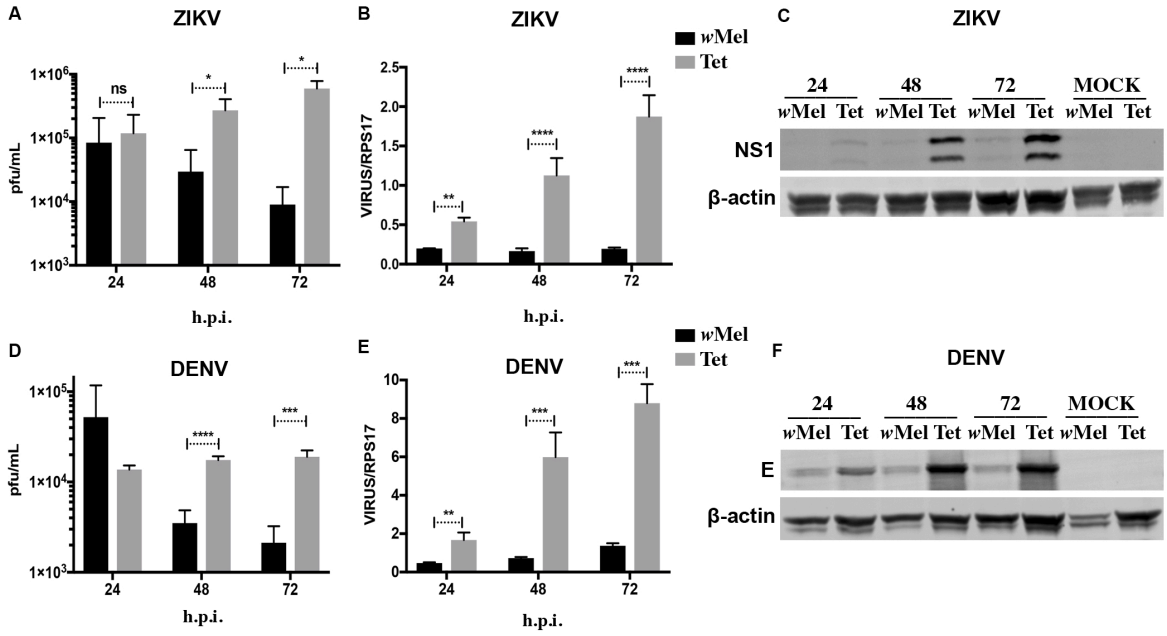
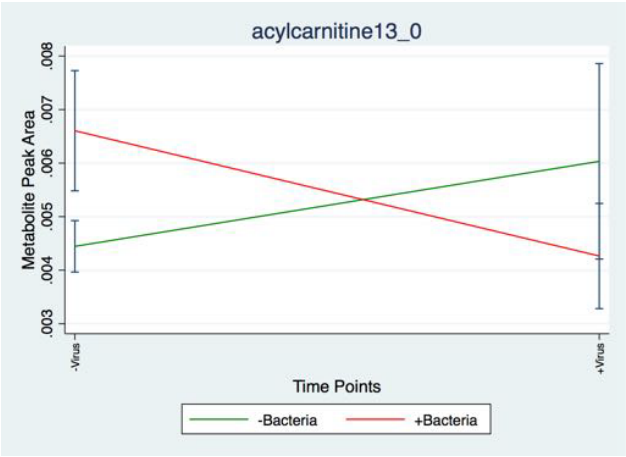
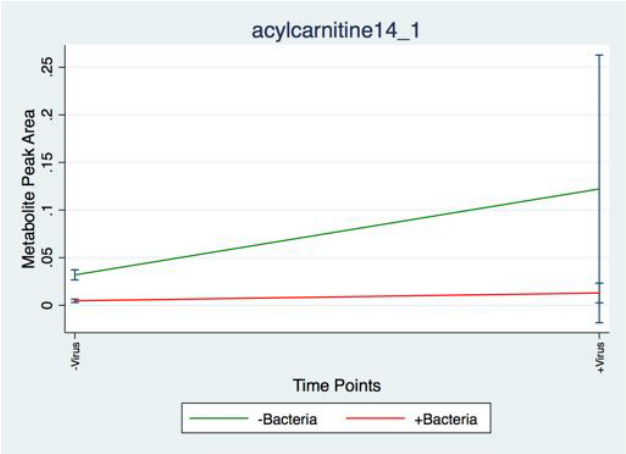
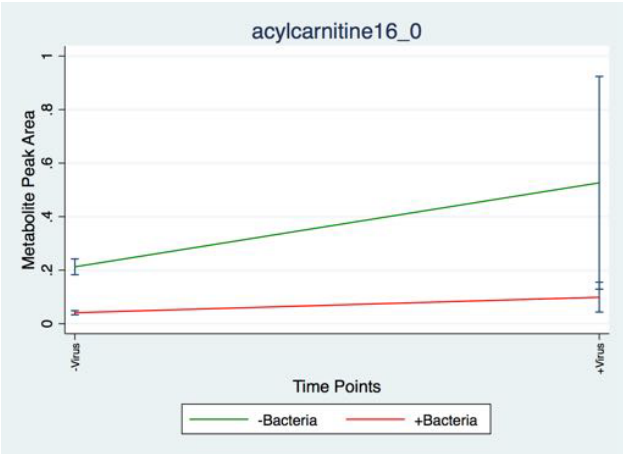


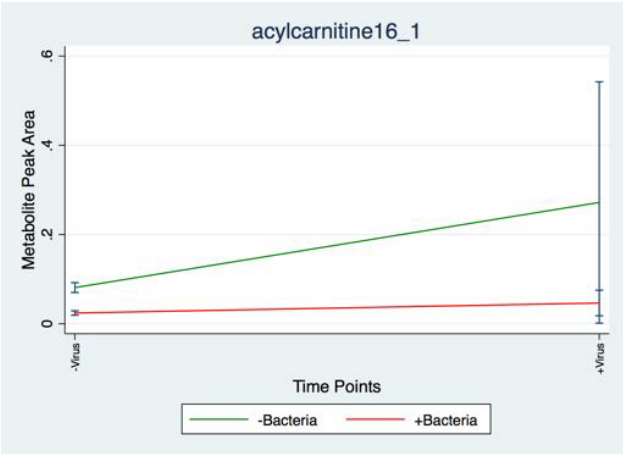
Fig. S3. Two-way ANOVA plots from LCMS analysis. Each metabolite was normalised to the median of the sample, as shown in Dataset 1. The interaction between bacteria and virus (i.e. all four data sets: bacteria +/- and virus +/-) were analysed using a two-way ANOVA test. The two-way ANOVA plot for each significantly modulated metabolite is displayed. Briefly, the red line and green line depict the presence and absence of *w*Mel respectively. The endpoints on the x-axis depict absence of virus and virus infection. The plots, thus, simultaneously demonstrate the pattern of behaviour for each metabolite in the presence of *w*Mel only, virus only and in the presence of both *w*Mel and virus. Hence, three sets of data can be interpreted from each plot. Names of each metabolite are shown above the plots and "_" represents " : ". i.e. dg32_0_16_0_ is DG 32:0 16:0.

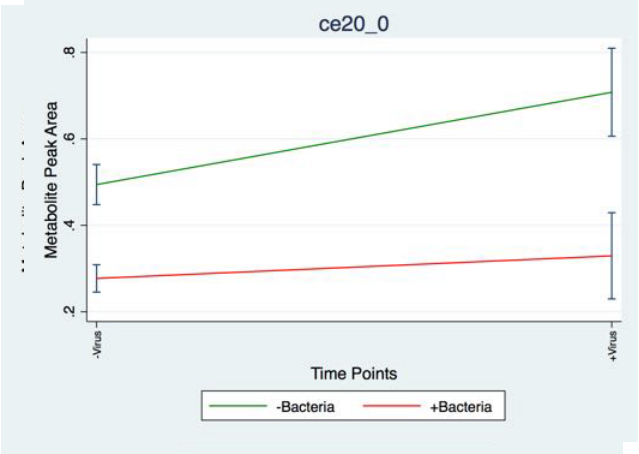
Two-way ANOVA plots – DENV-1









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Metabolite Peak Area
.04
.06
.08
.1
.12
.14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


ce24_0
Metabolite Peak Area
.02
.04
.06
.08
.1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


ce24_1
Metabolite Peak Area
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.2
.3
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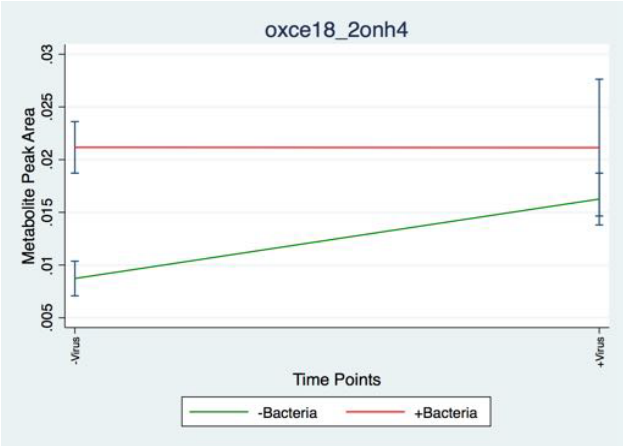
.5
.6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria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Time Points
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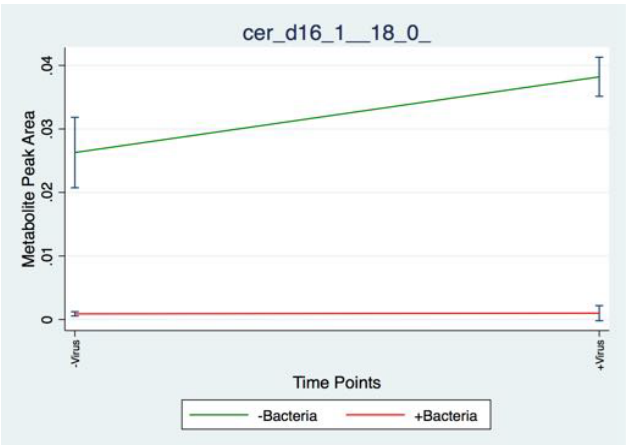
+Bacteria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Time Points
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+Bacteria


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2.5
3
3.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d17_1__24_0_
Metabolite Peak Area
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1
1.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d17_1__24_1_
Metabolite Peak Area
.2
.4
.6
.8
1
-Virus
+Virus
Time Points
-Bacteria
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15
20
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+Virus
Time Points
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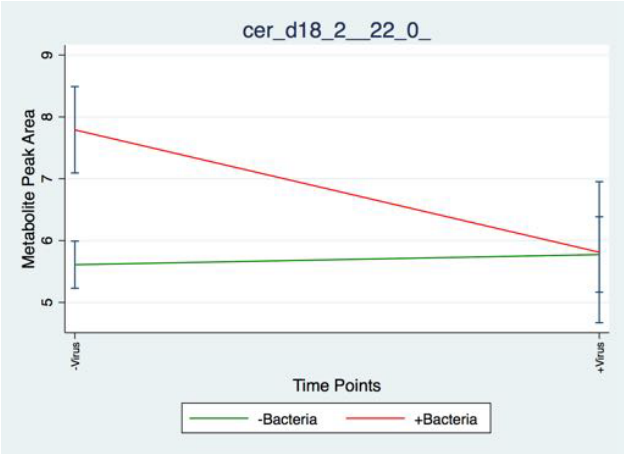
+Bacteria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7
8
9
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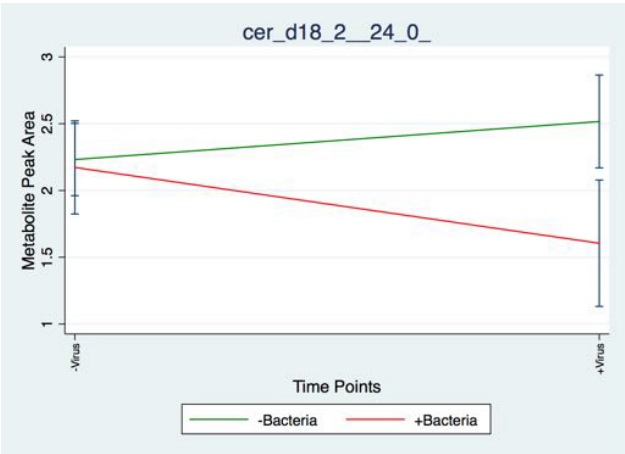
+Virus
Time Points
-Bacteria
+Bacteria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2.5
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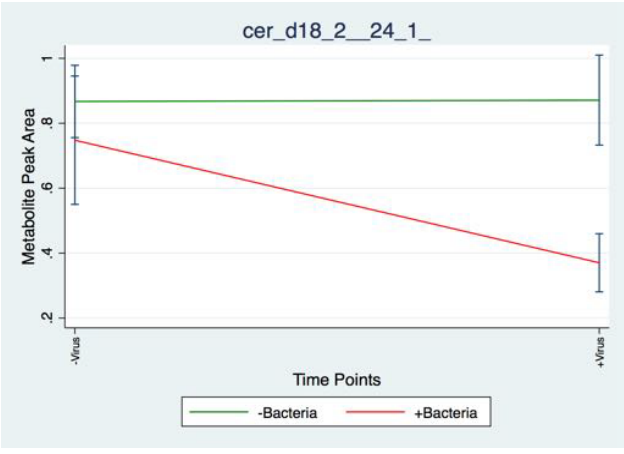
+Virus
Time Points
-Bacteria
+Bacteria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Time Points
-Bacteria
+Bacteria


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Metabolite Peak Area
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.015
.02
.025
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d19_1__22_0_
Metabolite Peak Area
.2
.3
.4
.5
.6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d19_1__24_0_
Metabolite Peak Area
.05
.1
.15
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d19_1__24_1_
Metabolite Peak Area
.05
.1
.15
.2
.25
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d20_1__24_0_
Metabolite Peak Area
.03
.04
.05
.06
.07
.08
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d20_1__24_1_
Metabolite Peak Area
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.05
.1
.15
.2
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


dhcer24_0
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.05
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.15
.2
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+Virus
Time Points
-Bacteria
+Bacteria


dhcer24_1
Metabolite Peak Area
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.1
.2
.3
.4
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+Virus
Time Points
-Bacteria
+Bacteria


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Time Points
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+Bacteria


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.2
.3
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-Virus
+Virus
Time Points
-Bacteria
+Bacteria


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+Virus
Time Points
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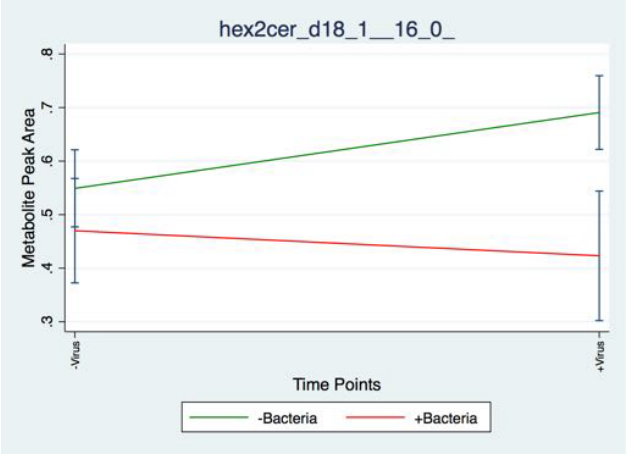
+Bacteria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.8
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+Virus
Time Points
-Bacteria
+Bacteria


hex2cer_d18_1__18_0_
Metabolite Peak Area
0
.2
.4
.6
.8
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


hex2cer_d18_1__24_0_
Metabolite Peak Area
.2
.4
.6
.8
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


hex2cer_d18_1__24_1_
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+Virus
Time Points
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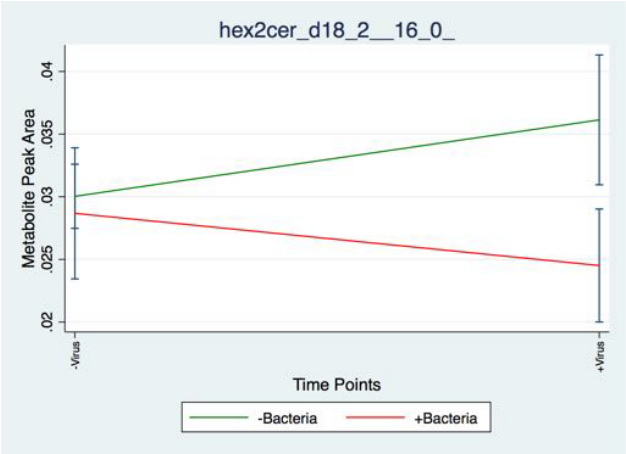
+Bacteria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+Virus
Time Points
-Bacteria
+Bacteria


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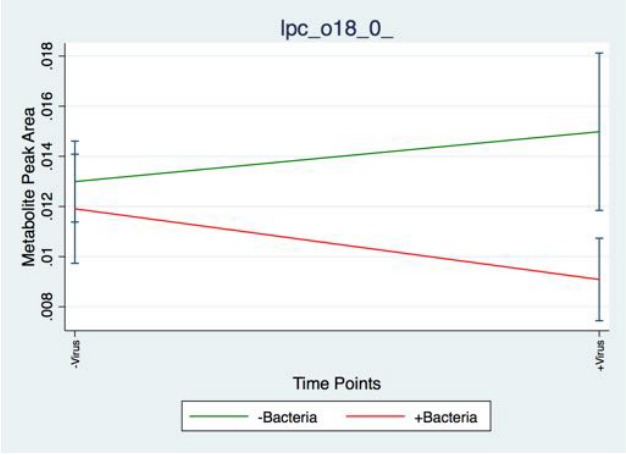
+Virus
Time Points
-Bacteria
+Bacteria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Time Points
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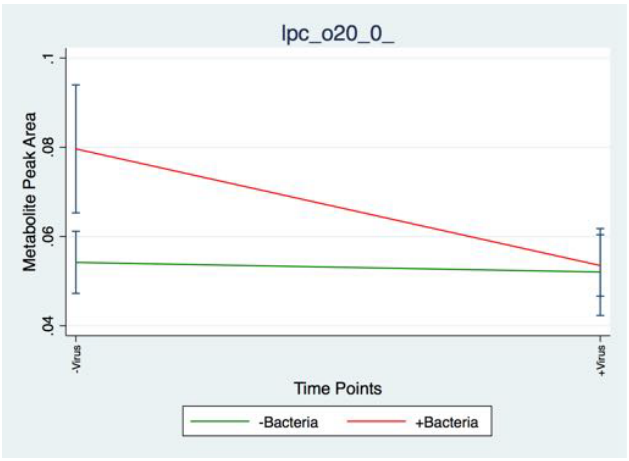
+Bacteria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Time Points
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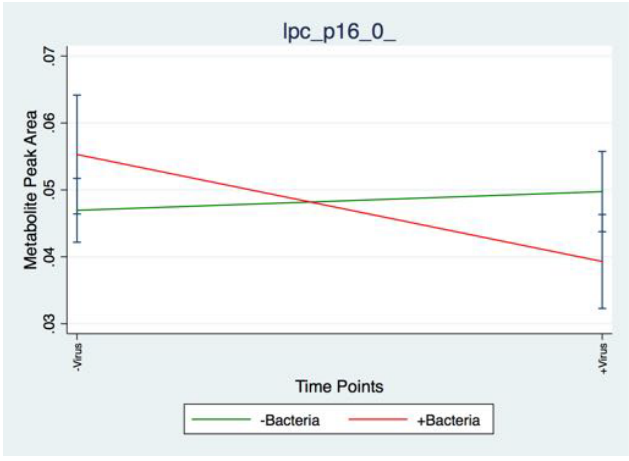
+Bacteria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Time Points
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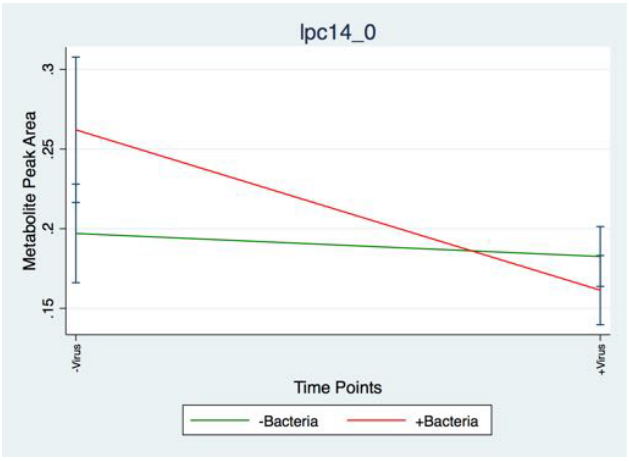
+Bacteria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Time Points
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Time Points
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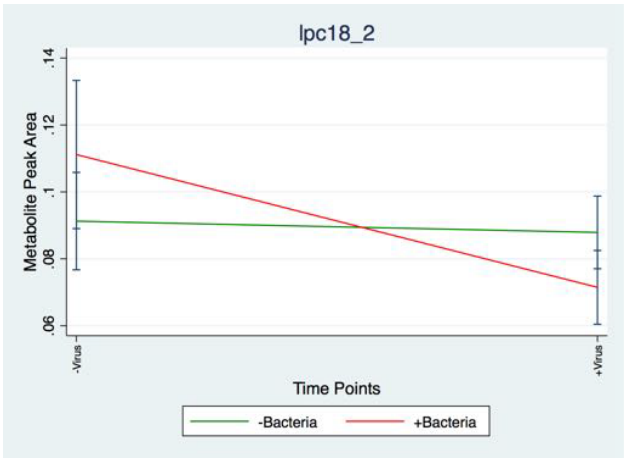
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Time Points
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Metabolite Peak Area
.06
.08
.1
.12
.14
.16
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpc22_1
Metabolite Peak Area
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.035
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+Virus
Time Points
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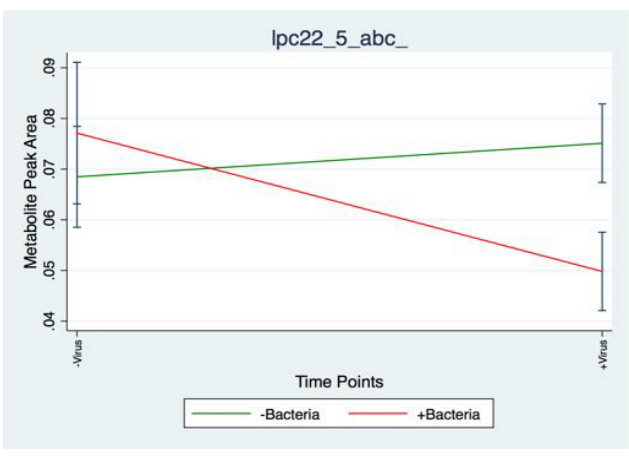
+Bacteria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Time Points
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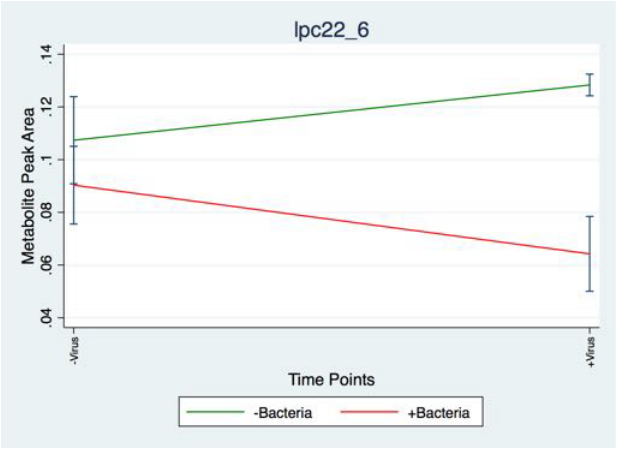
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Time Points
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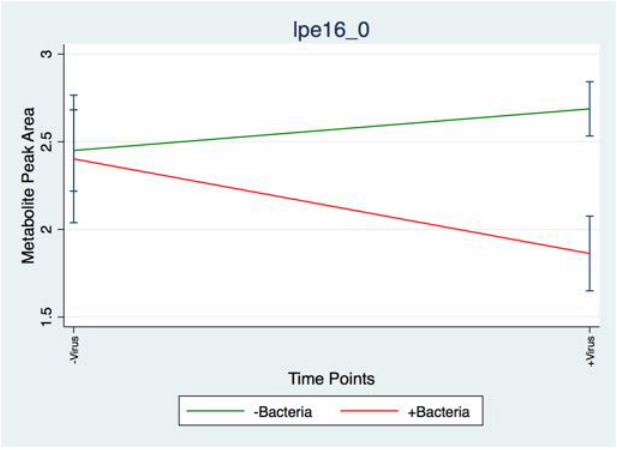
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3
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+Virus
Time Points
-Bacteria
+Bacteria


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Metabolite Peak Area
1.5
2
2.5
3
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpe18_1
Metabolite Peak Area
2.5
3
3.5
4
4.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pc_o35_4_
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6
8
10
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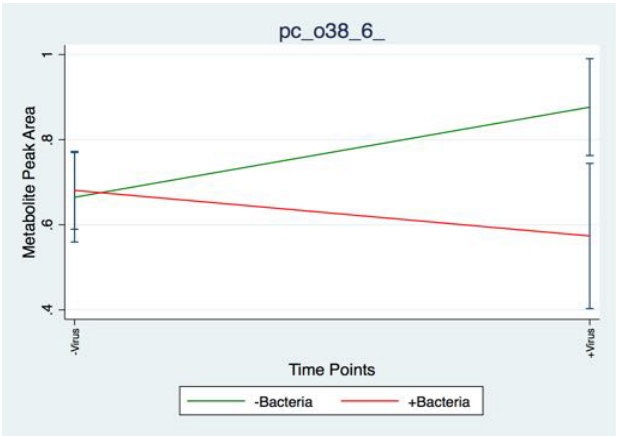
+Virus
Time Points
-Bacteria
+Bacteria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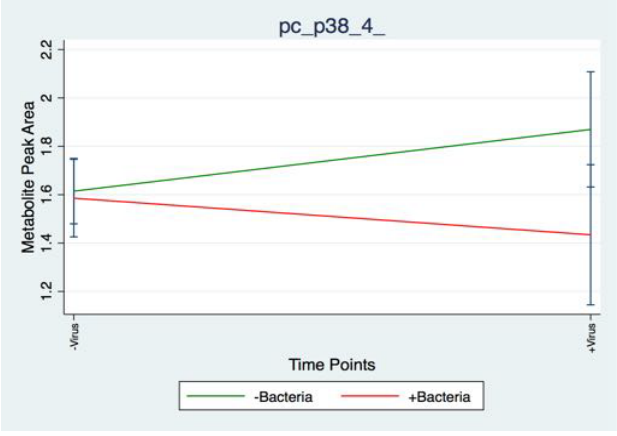
.6
.8
1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria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1.4
1.6
1.8
2
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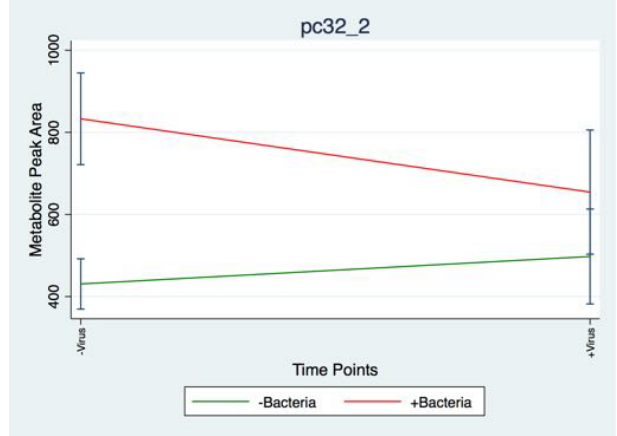
+Virus
Time Points
-Bacteria
+Bacteria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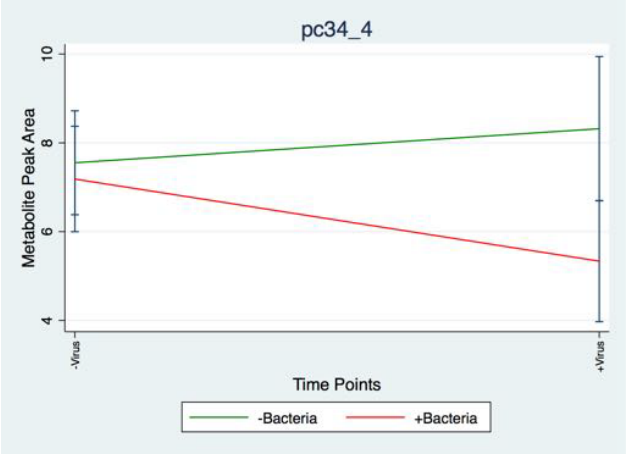
600
800
1000
-Virus
+Virus
Time Points
-Bacteria
+Bacteria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Time Points
-Bacteria
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.6
.8
1
1.2
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+Virus
Time Points
-Bacteria
+Bacteria


pc36_2_ab_
Metabolite Peak Area
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14
16
18
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Time Points
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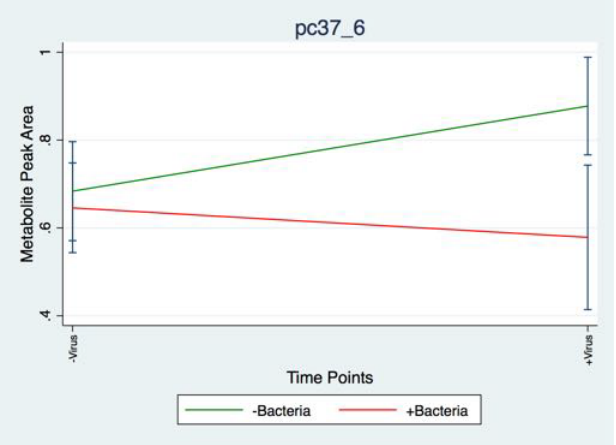
+Bacteria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Metabolite Peak Area
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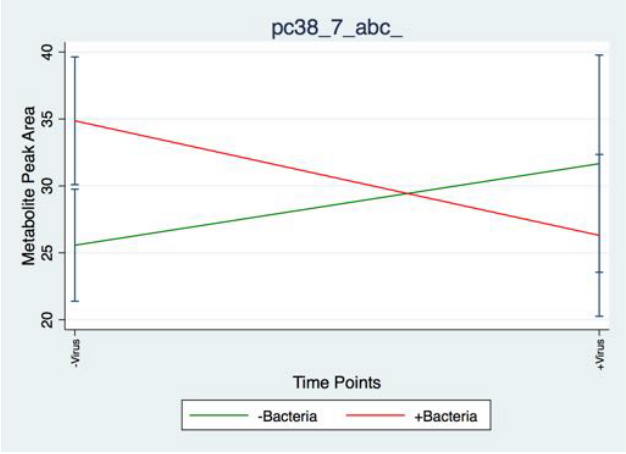
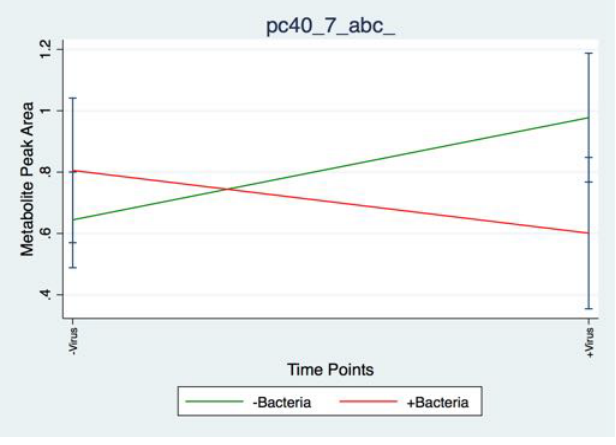
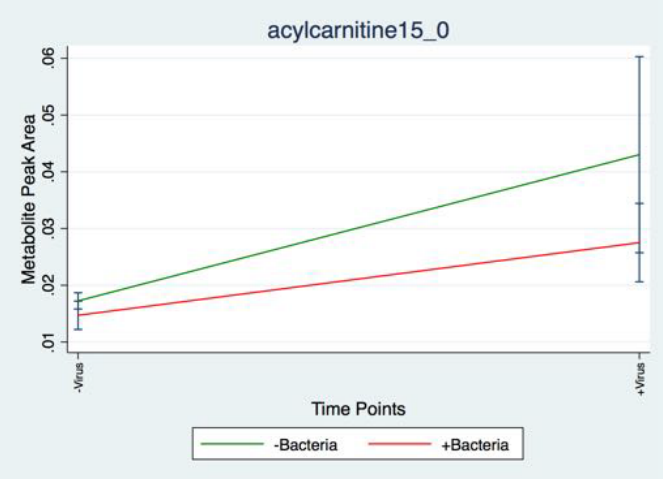
.6
.8
1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pc38_7_abc_
Metabolite Peak Area
20
25
30
35
40
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pc40_7_abc_
Metabolite Peak Area
.4
.6
.8
1
1.2
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pi38_3_ab_
Metabolite Peak Area
0
1
2
3
4
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

Two-way ANOVA plots – ZIKV Asian




acylcarnitine18_0
Metabolite Peak Area
0
.5
1
1.5
2
2.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


acylcarnitine18_1
Metabolite Peak Area
0
1
2
3
4
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


ce20_0
Metabolite Peak Area
.2
.4
.6
.8
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


ce22_0
Metabolite Peak Area
.05
.1
.15
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


ce22_1
Metabolite Peak Area
.06
.08
.1
.12
.14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


ce24_0
Metabolite Peak Area
.02
.04
.06
.08
.1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

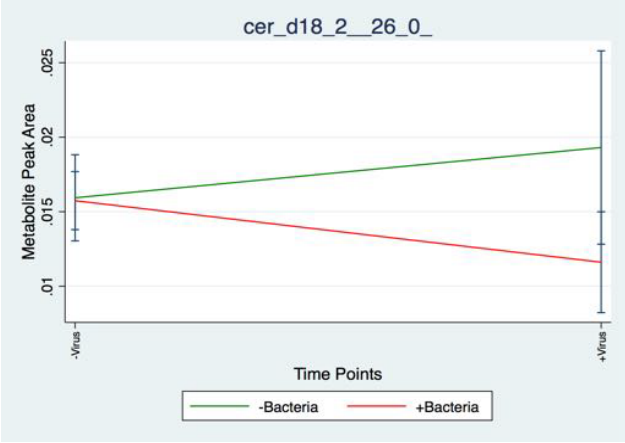

ce24_1
Metabolite Peak Area
.1
.2
.3
.4
.5
.6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

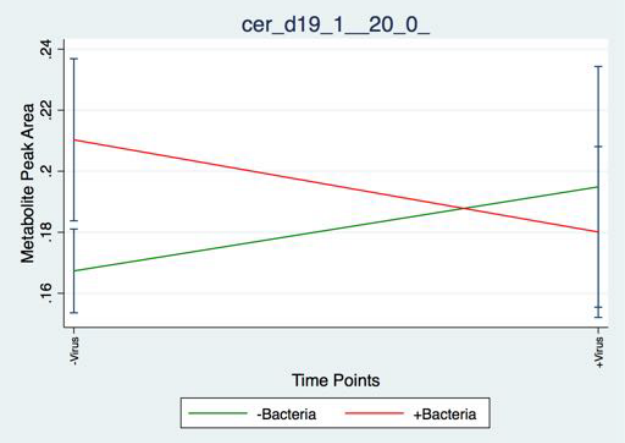

cer_d18_1__24_0_
Metabolite Peak Area
10
15
20
25
30
35
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d18_1__26_0_
Metabolite Peak Area
.08
.1
.12
.14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d18_2__22_0_
Metabolite Peak Area
5
6
7
8
9
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

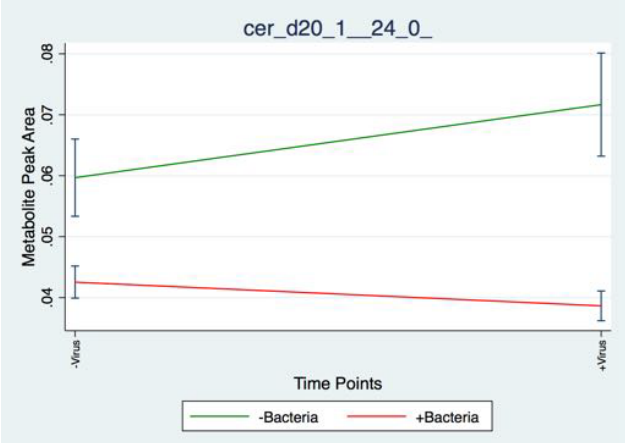

cer_d18_2__24_0_
Metabolite Peak Area
1
1.5
2
2.5
3
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d18_2__26_0_
Metabolite Peak Area
.01
.015
.02
.025
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d19_1__20_0_
Metabolite Peak Area
.16
.18
.2
.22
.24
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d19_1__22_0_
Metabolite Peak Area
.2
.3
.4
.5
.6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d19_1__24_0_
Metabolite Peak Area
.05
.1
.15
.2
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


cer_d20_1__24_0_
Metabolite Peak Area
.04
.05
.06
.07
.08
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


dg32_1_16_1_
Metabolite Peak Area
.5
1
1.5
2
2.5
3
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


dg34_1_18_1_
Metabolite Peak Area
1
2
3
4
5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


dg36_2_18_2_
Metabolite Peak Area
.05
.1
.15
.2
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


dg36_4_20_4_
Metabolite Peak Area
.05
.1
.15
.2
.25
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

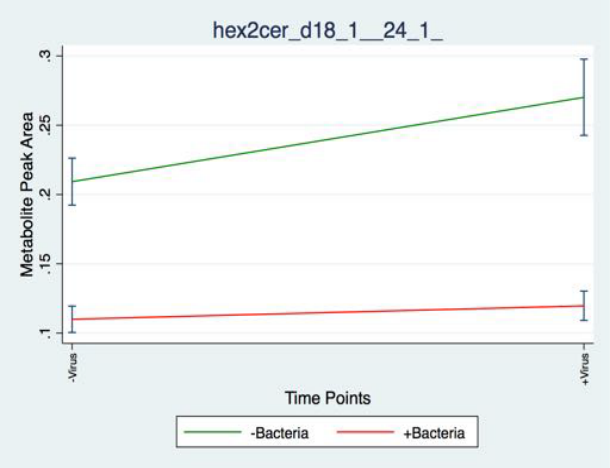

dg38_5_20_4_
Metabolite Peak Area
0
.1
.2
.3
.4
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


dg38_5_22_5_
Metabolite Peak Area
.05
.1
.15
.2
.25
.3
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

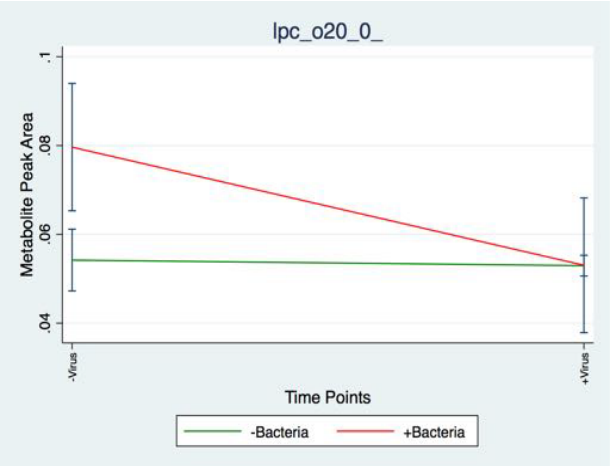

hex1cer_d16_1__18_0_
Metabolite Peak Area
0
.1
.2
.3
.4
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


hex1cer_d18_2__24_0_
Metabolite Peak Area
.03
.04
.05
.06
.07
.08
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

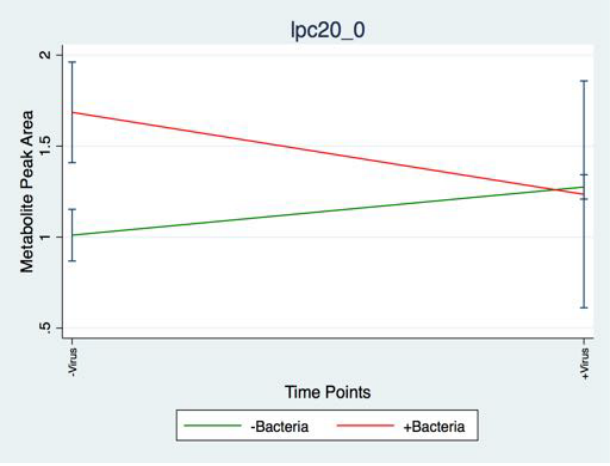

hex2cer_d18_1__24_0_
Metabolite Peak Area
.2
.4
.6
.8
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


hex2cer_d18_1__24_1_
Metabolite Peak Area
.1
.15
.2
.25
.3
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

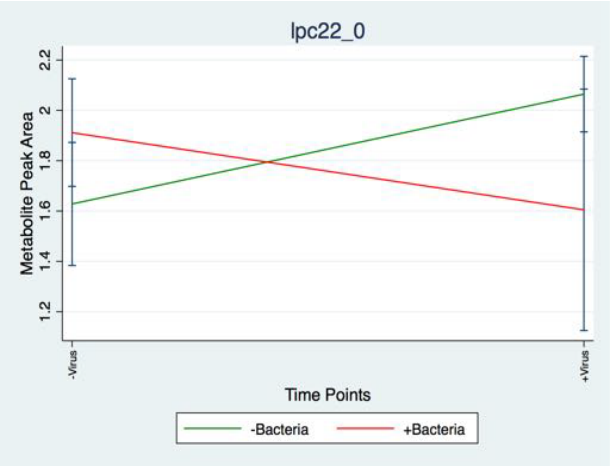

lpc_o18_0_
Metabolite Peak Area
.008
.01
.012
.014
.016
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpc_o20_0_
Metabolite Peak Area
.04
.06
.08
.1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpc_o22_0_
Metabolite Peak Area
.1
.15
.2
.25
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpc20_0
Metabolite Peak Area
.5
1
1.5
2
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

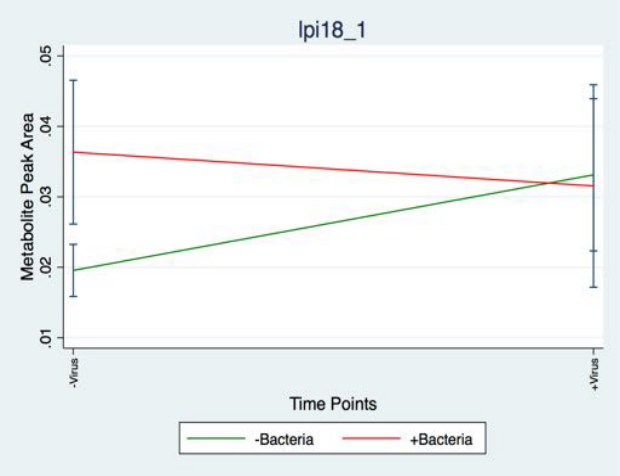

lpc20_1
Metabolite Peak Area
.05
.1
.15
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

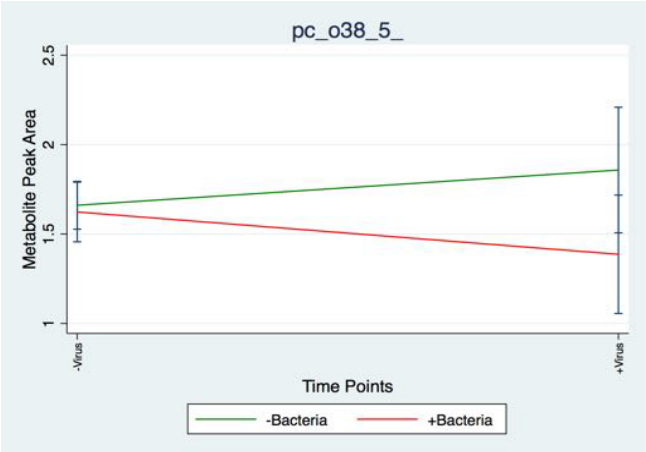

lpc22_0
Metabolite Peak Area
1.2
1.4
1.6
1.8
2
2.2
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpc22_1
Metabolite Peak Area
.01
.02
.03
.04
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpe18_0
Metabolite Peak Area
1
1.5
2
2.5
3
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpe18_1
Metabolite Peak Area
2.5
3
3.5
4
4.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

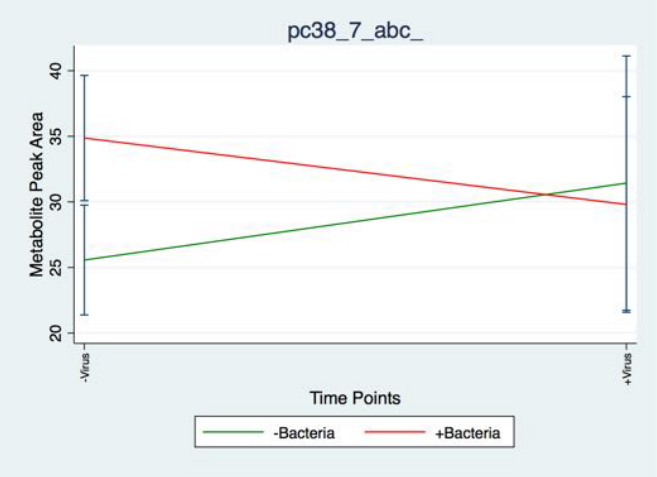

lpi18_1
Metabolite Peak Area
.01
.02
.03
.04
.05
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pc_o38_5_
Metabolite Peak Area
1
1.5
2
2.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

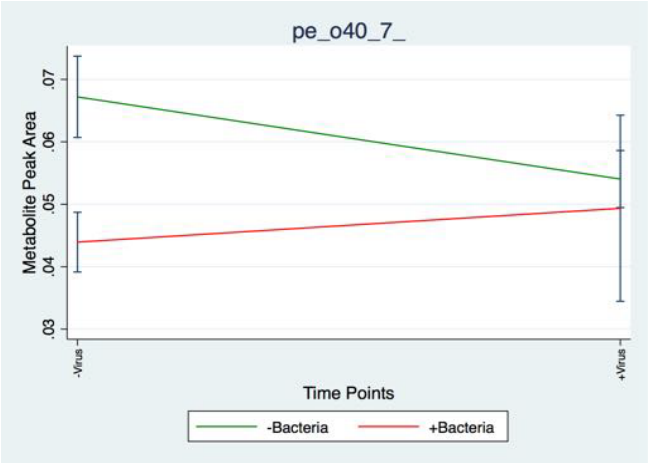

pc_o38_6_
Metabolite Peak Area
.4
.6
.8
1
1.2
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

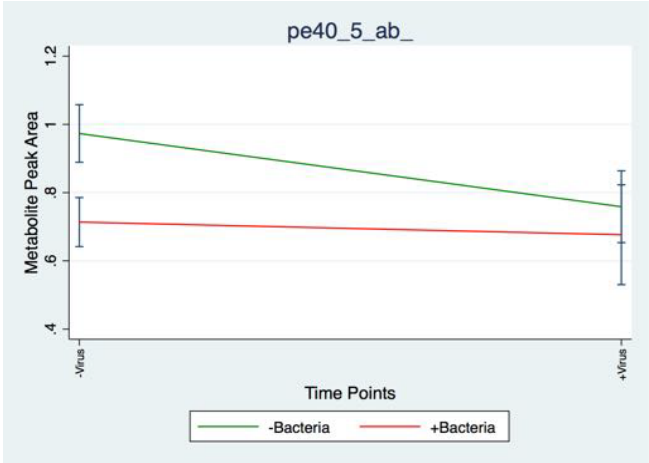

pc_p38_4_
Metabolite Peak Area
1
1.5
2
2.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pc32_2
Metabolite Peak Area
400
600
800
1000
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

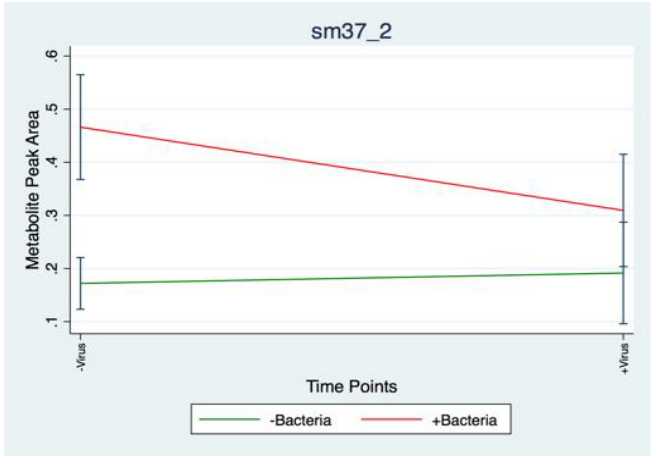

pc38_7_abc_
Metabolite Peak Area
20
25
30
35
40
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pc40_7_abc_
Metabolite Peak Area
.4
.6
.8
1
1.2
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pe_o40_7_
Metabolite Peak Area
.03
.04
.05
.06
.07
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pe40_5_ab_
Metabolite Peak Area
.4
.6
.8
1
1.2
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

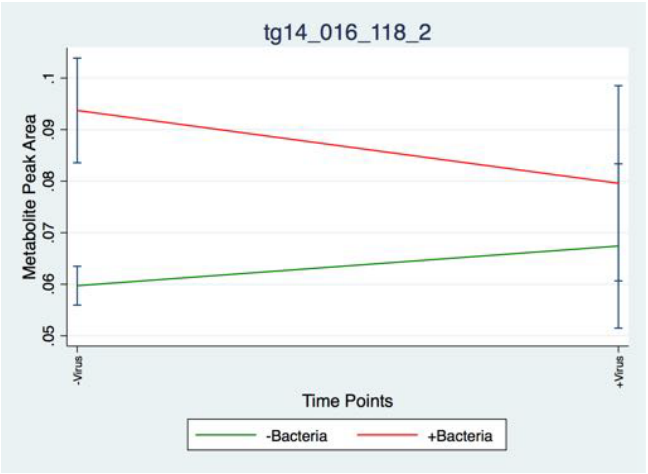

sm36_1
Metabolite Peak Area
200
300
400
500
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


sm37_2
Metabolite Peak Area
.1
.2
.3
.4
.5
.6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

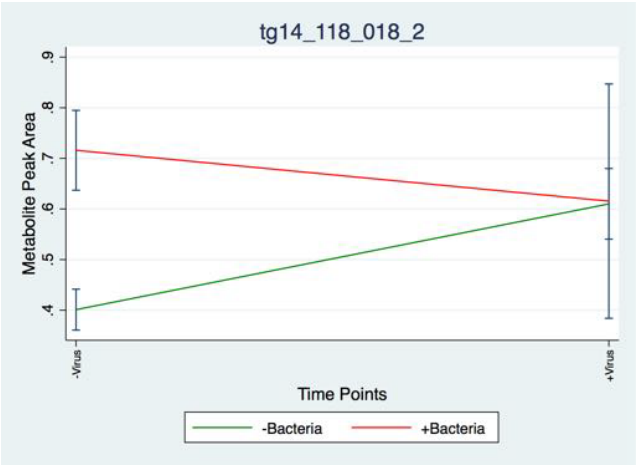

sm40_2_ab_
Metabolite Peak Area
60
80
100
120
140
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

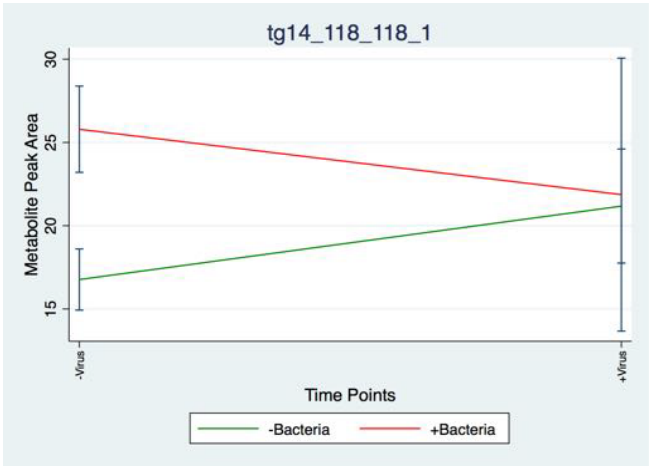

tg14_016_018_2
Metabolite Peak Area
7
8
9
10
11
12
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg14_016_118_1
Metabolite Peak Area
4
5
6
7
8
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg14_016_118_2
Metabolite Peak Area
.05
.06
.07
.08
.09
.1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

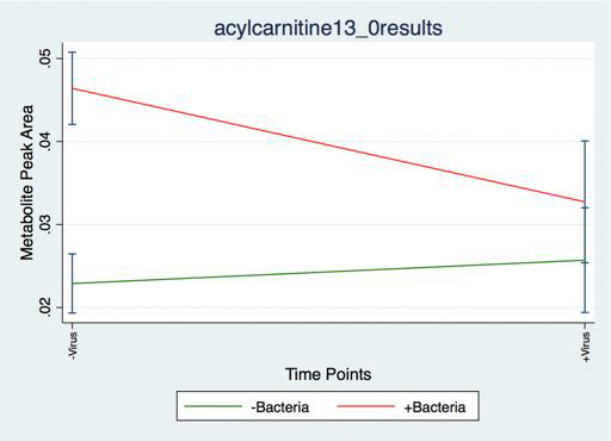

tg14_116_018_1
Metabolite Peak Area
.5
.6
.7
.8
.9
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

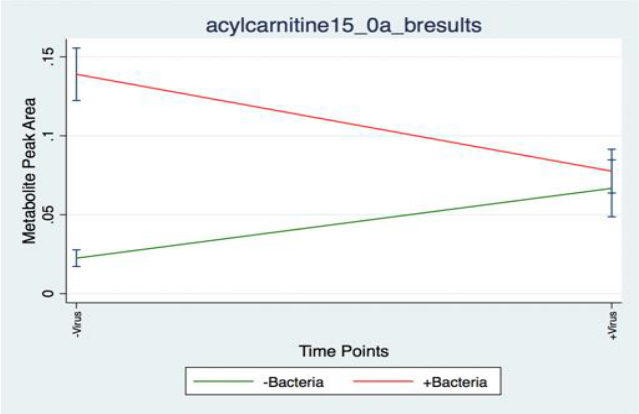

tg14_118_018_2
Metabolite Peak Area
.4
.5
.6
.7
.8
.9
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

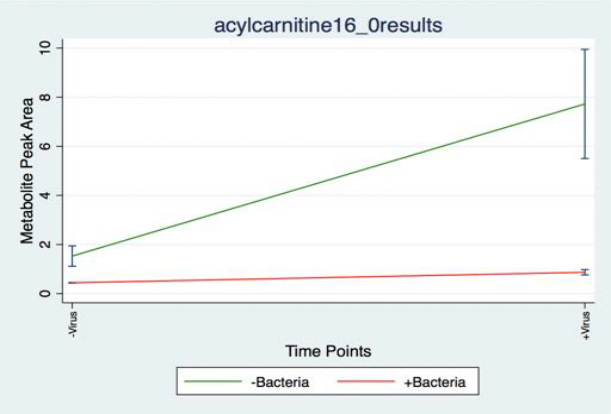

tg14_118_118_1
Metabolite Peak Area
15
20
25
30
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

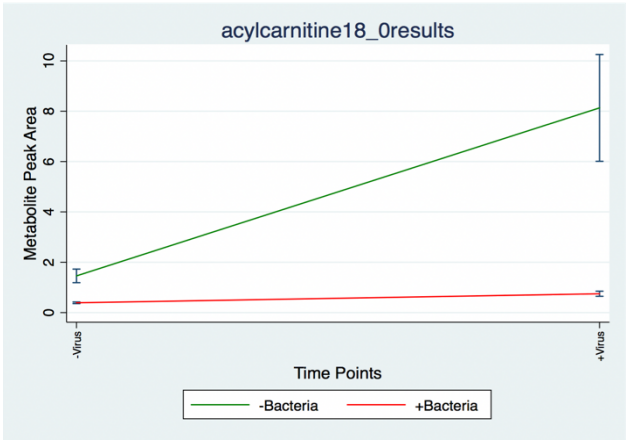

tg16_116_116_1
Metabolite Peak Area
4
5
6
7
8
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

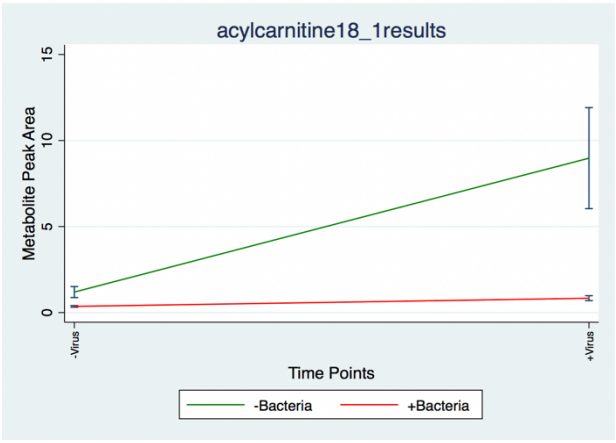
Two-way ANOVA plots – ZIKV African

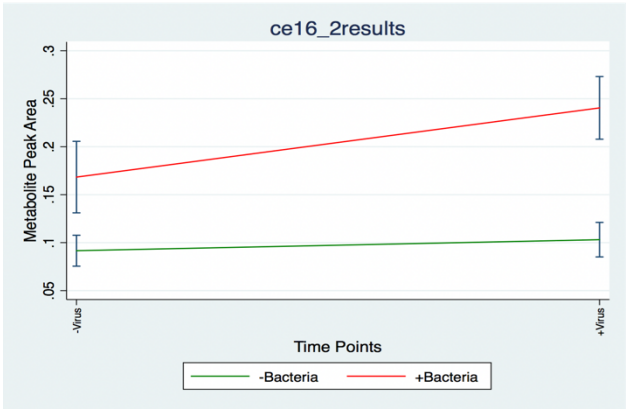


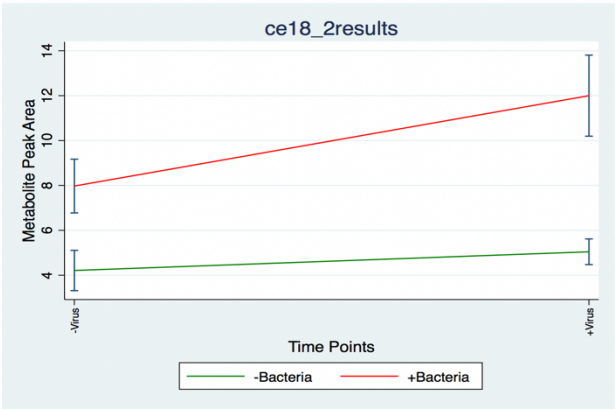


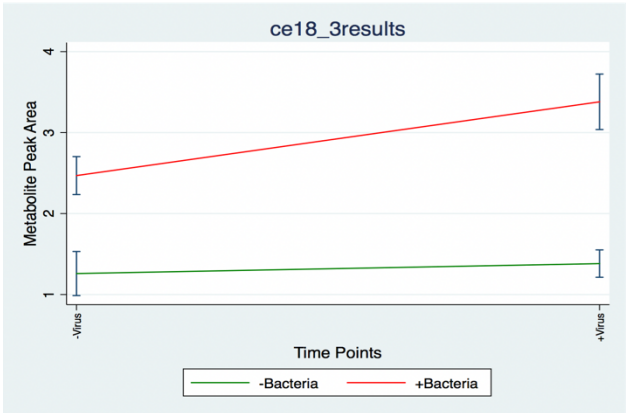


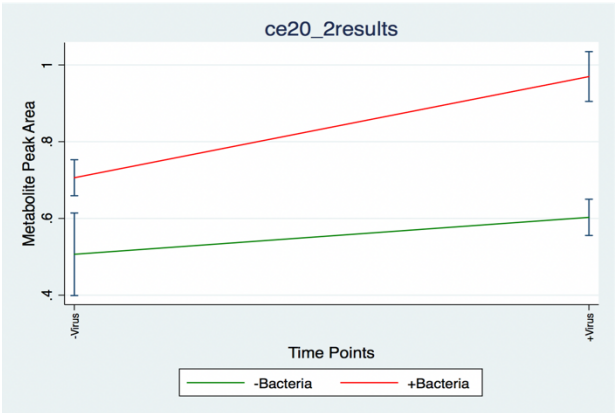

acylcarnitine18_0results
Metabolite Peak Area
0
2
4
6
8
10
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

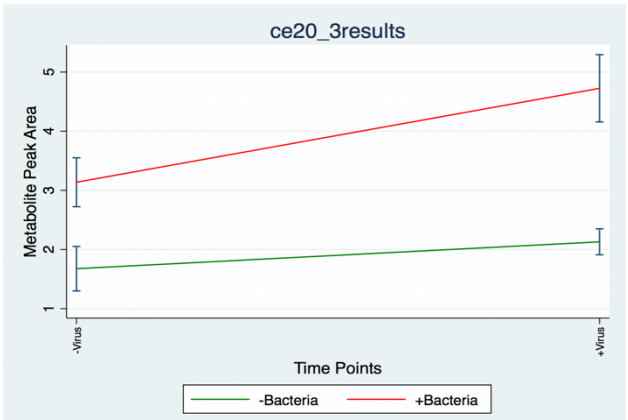

acylcarnitine18_1results
Metabolite Peak Area
0
5
10
15
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

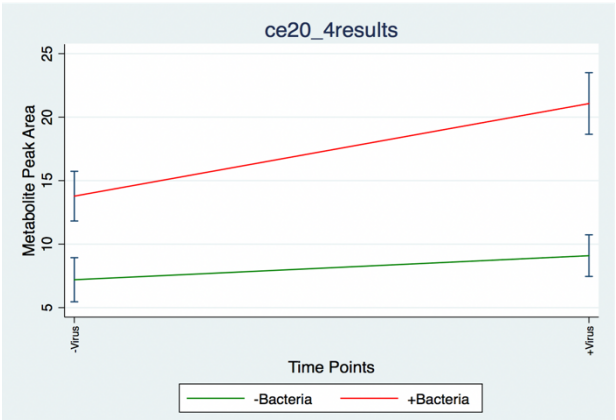

ce16_2results
Metabolite Peak Area
.05
.1
.15
.2
.25
.3
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

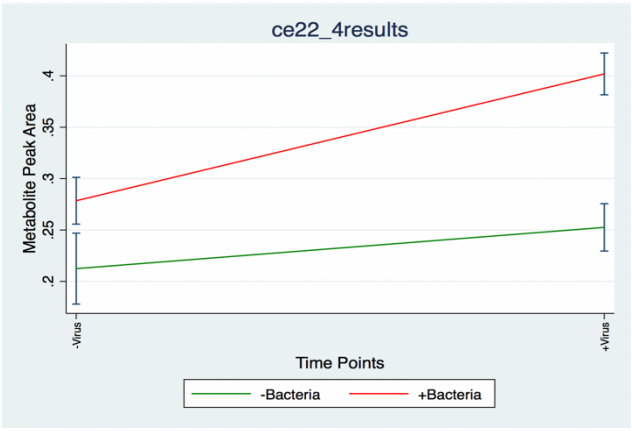

ce18_2results
Metabolite Peak Area
4
6
8
10
12
14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

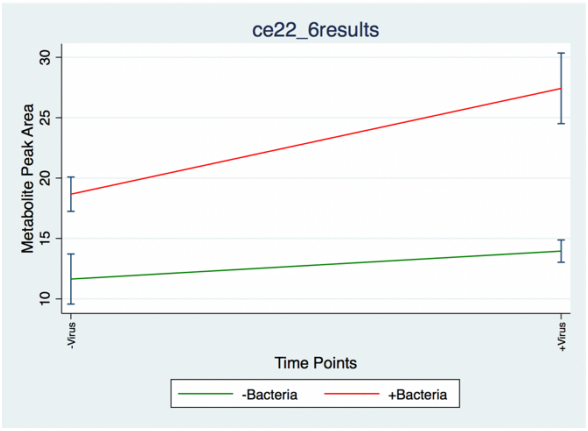

ce18_3results
Metabolite Peak Area
1
2
3
4
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

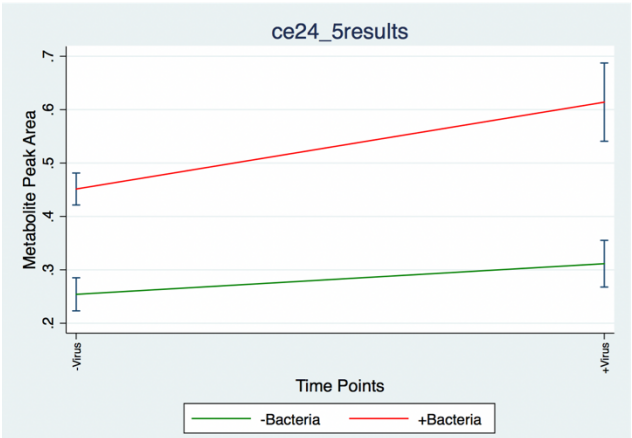

ce20_2results
Metabolite Peak Area
.4
.6
.8
1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

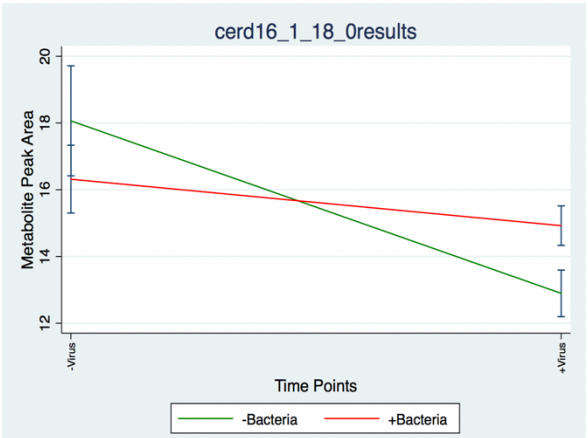

ce20_3results
Metabolite Peak Area
1
2
3
4
5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

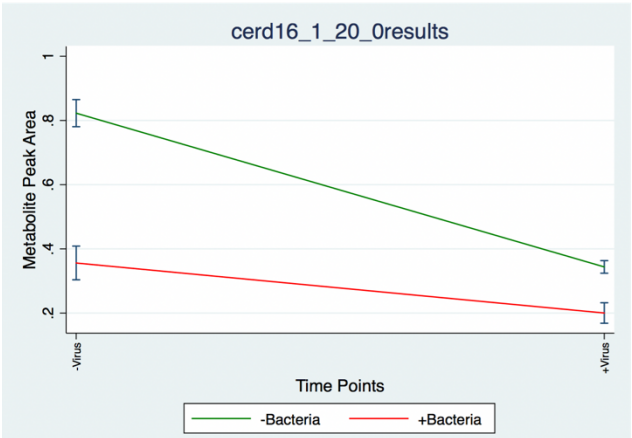

ce20_4results
Metabolite Peak Area
5
10
15
20
25
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


ce22_4results
Metabolite Peak Area
.2
.25
.3
.35
.4
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

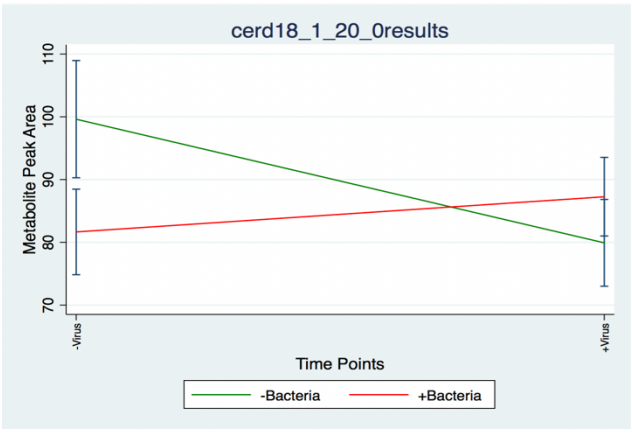

ce22_6results
Metabolite Peak Area
10
15
20
25
30
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

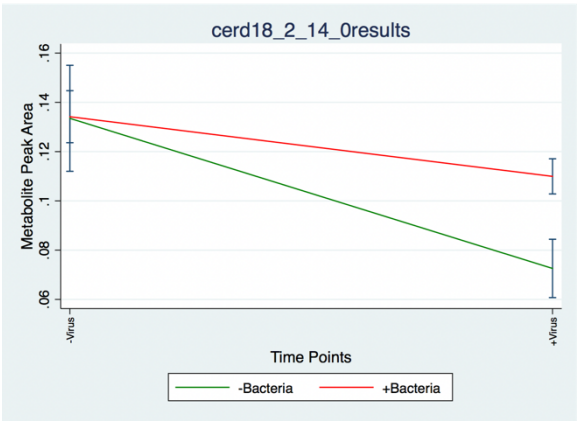

ce24_5results
Metabolite Peak Area
.2
.3
.4
.5
.6
.7
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

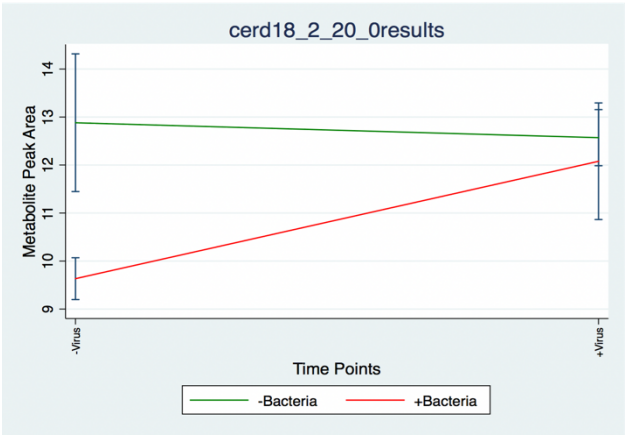

cerd16_1_18_0results
Metabolite Peak Area
12
14
16
18
20
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

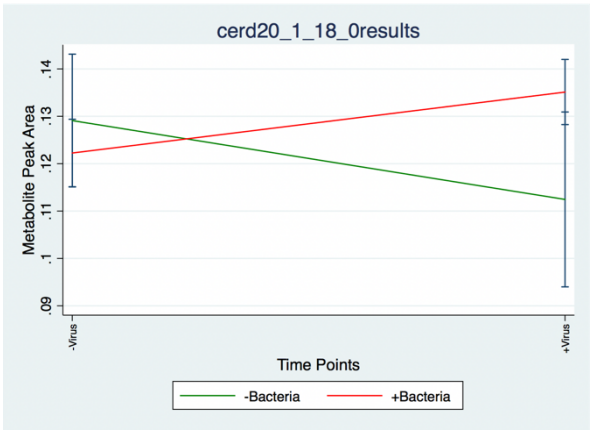

cerd16_1_20_0results
Metabolite Peak Area
.2
.4
.6
.8
1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

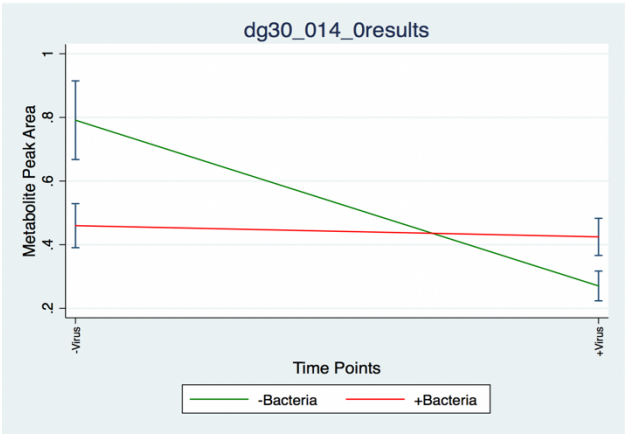

cerd17_1_20_0results
Metabolite Peak Area
3.5
4
4.5
5
5.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

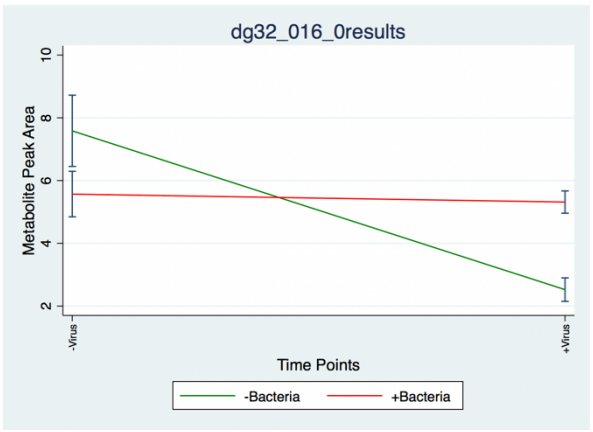

cerd18_1_20_0results
Metabolite Peak Area
70
80
90
100
110
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

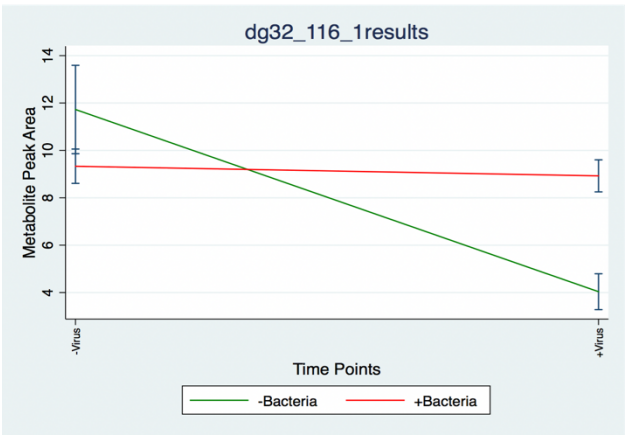

cerd18_2_14_0results
Metabolite Peak Area
.06
.08
.1
.12
.14
.16
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

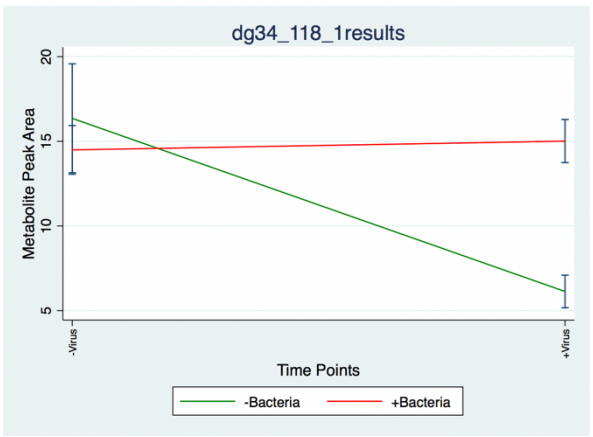

cerd18_2_20_0results
Metabolite Peak Area
9
10
11
12
13
14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

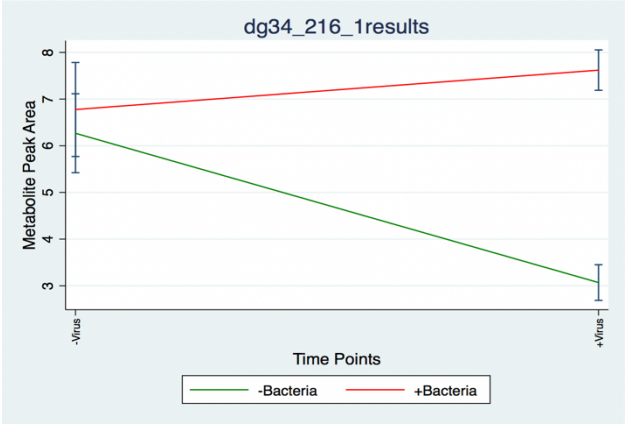

cerd20_1_18_0results
Metabolite Peak Area
.09
.1
.11
.12
.13
.14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

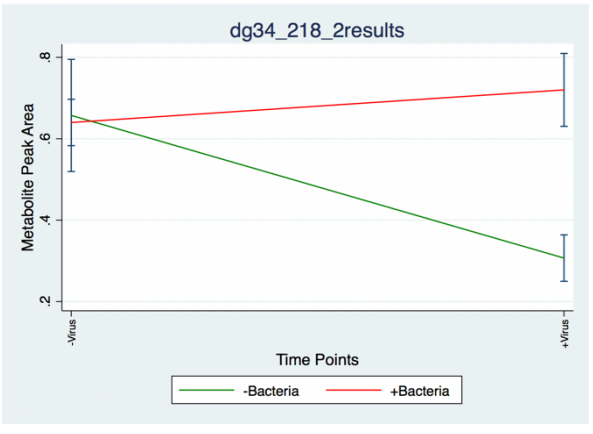

dg30_014_0results
Metabolite Peak Area
.2
.4
.6
.8
1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

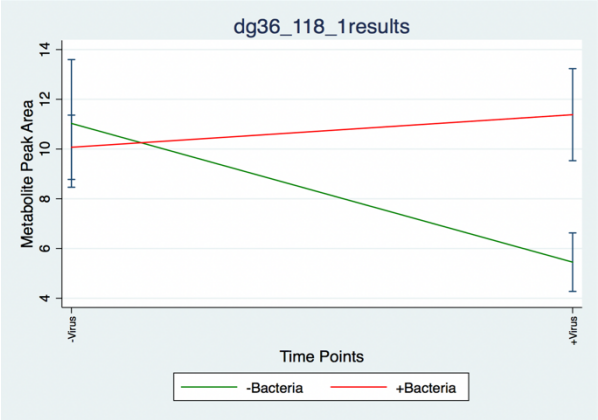

dg32_016_0results
Metabolite Peak Area
2
4
6
8
10
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

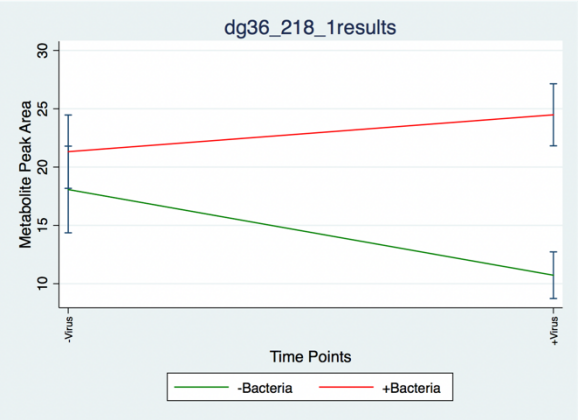

dg32_116_1results
Metabolite Peak Area
4
6
8
10
12
14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

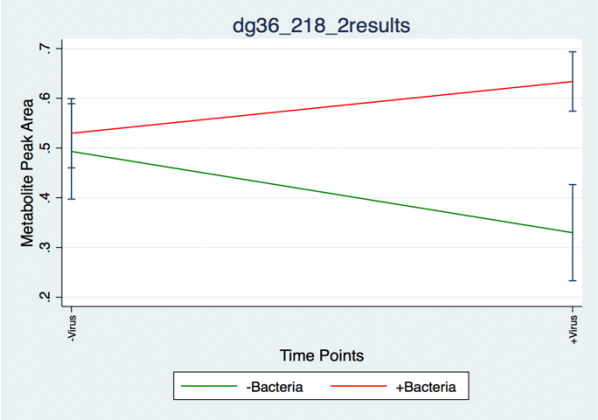

dg34_118_1results
Metabolite Peak Area
5
10
15
20
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

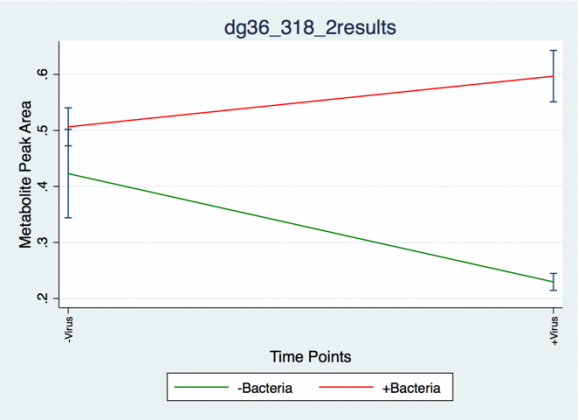

dg34_216_1results
Metabolite Peak Area
3
4
5
6
7
8
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

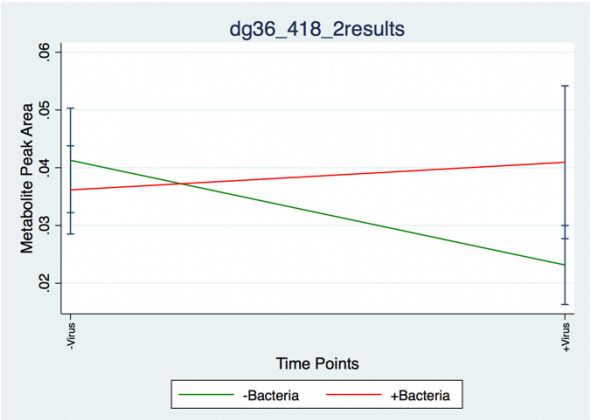

dg34_218_2results
Metabolite Peak Area
.2
.4
.6
.8
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

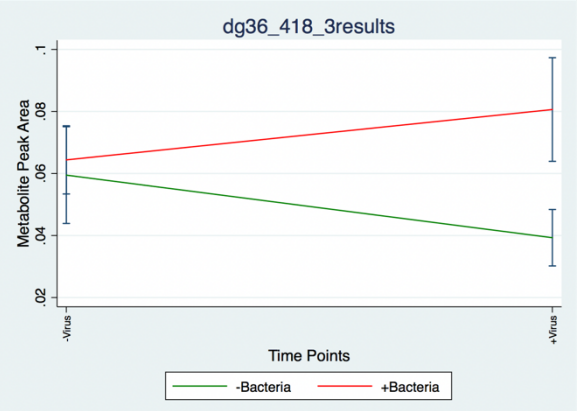

dg36_118_1results
Metabolite Peak Area
4
6
8
10
12
14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

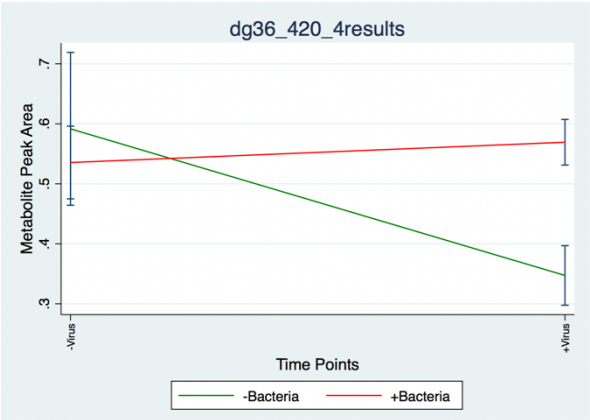

dg36_218_1results
Metabolite Peak Area
10
15
20
25
30
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

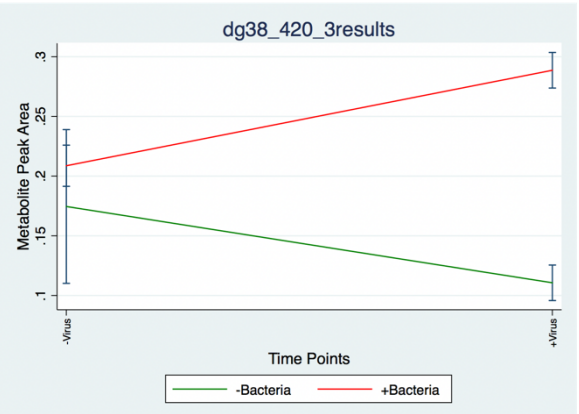

dg36_218_2results
Metabolite Peak Area
.2
.3
.4
.5
.6
.7
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

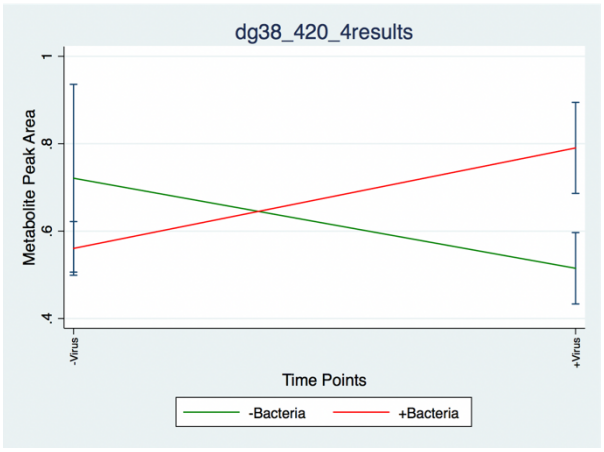

dg36_318_2results
Metabolite Peak Area
.2
.3
.4
.5
.6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

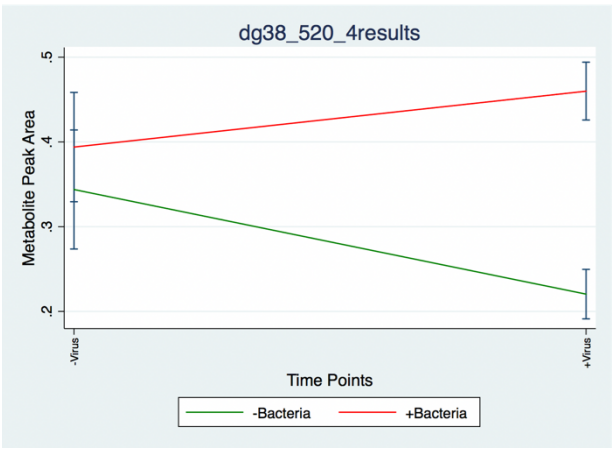

dg36_418_2results
Metabolite Peak Area
.02
.03
.04
.05
.06
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

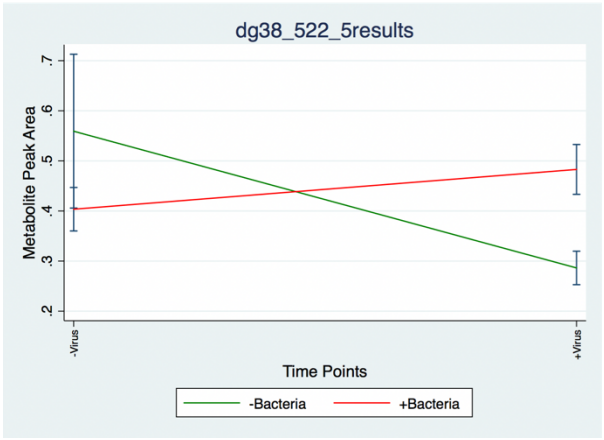

dg36_418_3results
Metabolite Peak Area
.02
.04
.06
.08
.1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

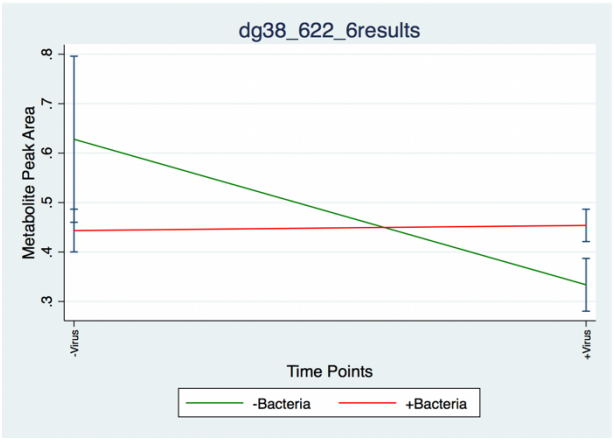

dg36_420_4results
Metabolite Peak Area
.3
.4
.5
.6
.7
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


dg38_420_3results
Metabolite Peak Area
.1
.15
.2
.25
.3
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

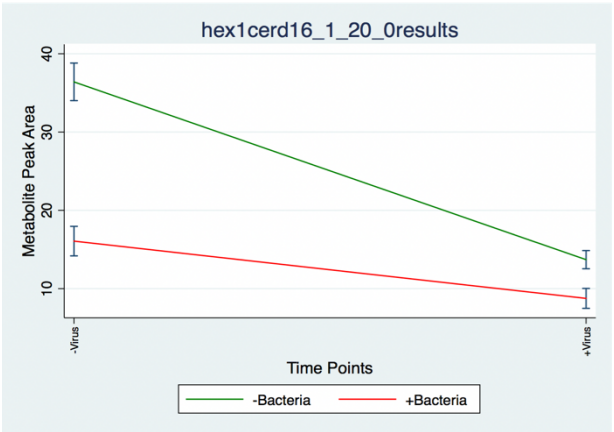

dg38_420_4results
Metabolite Peak Area
.4
.6
.8
1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

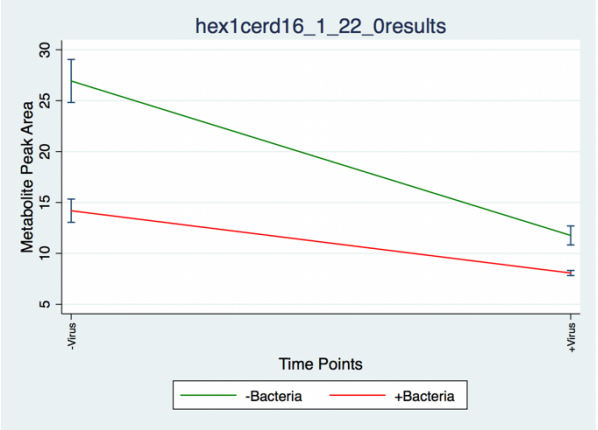

dg38_520_4results
Metabolite Peak Area
.2
.3
.4
.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

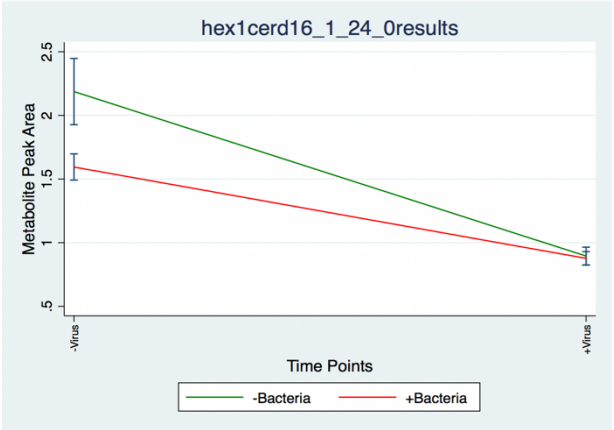

dg38_522_5results
Metabolite Peak Area
.2
.3
.4
.5
.6
.7
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

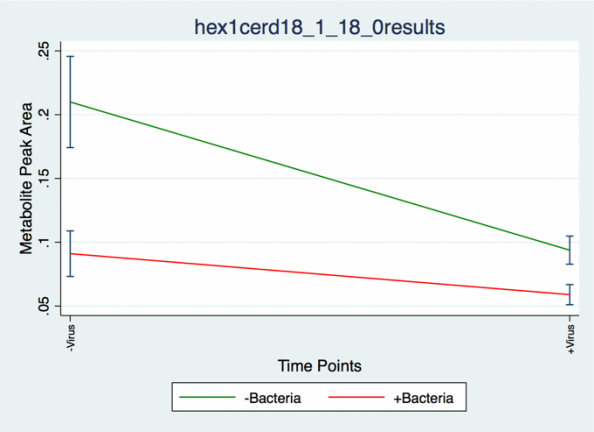

dg38_622_6results
Metabolite Peak Area
.3
.4
.5
.6
.7
.8
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

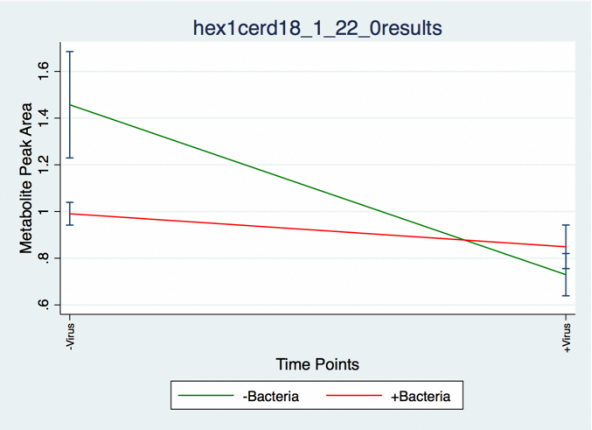

hex1cerd16_1_18_0results
Metabolite Peak Area
.1
.2
.3
.4
.5
.6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

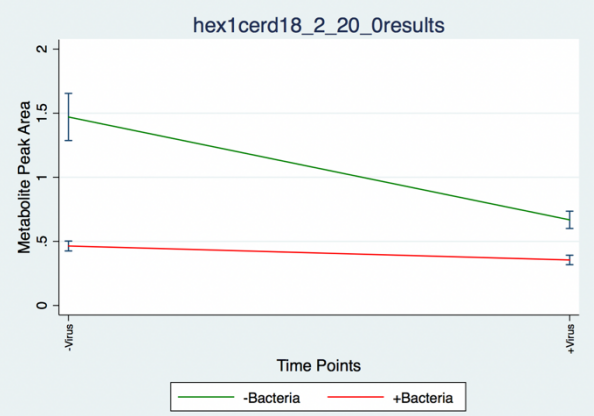

hex1cerd16_1_20_0results
Metabolite Peak Area
10
20
30
40
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

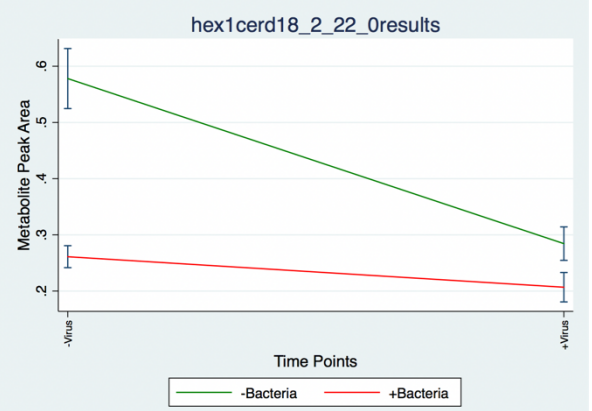

hex1cerd16_1_22_0results
Metabolite Peak Area
5
10
15
20
25
30
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

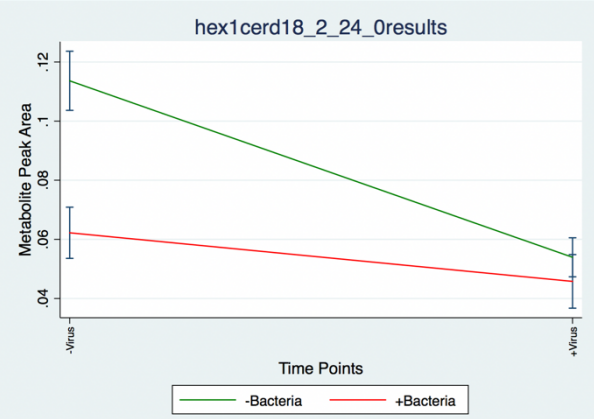

hex1cerd16_1_24_0results
Metabolite Peak Area
.5
1
1.5
2
2.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

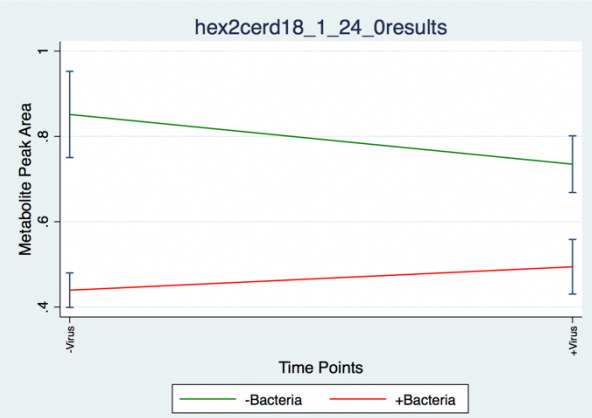

hex1cerd18_1_18_0results
Metabolite Peak Area
.05
.1
.15
.2
.25
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

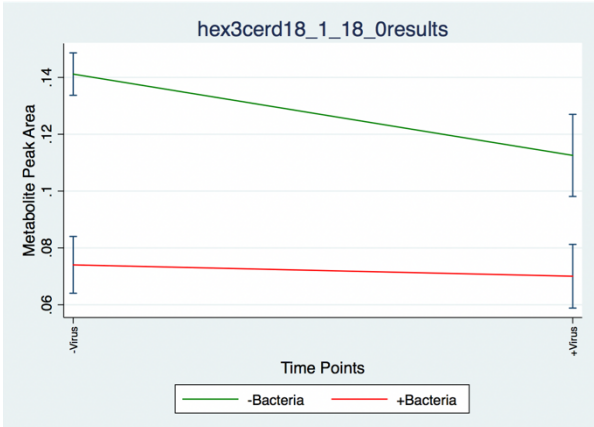

hex1cerd18_1_22_0results
Metabolite Peak Area
.6
.8
1
1.2
1.4
1.6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

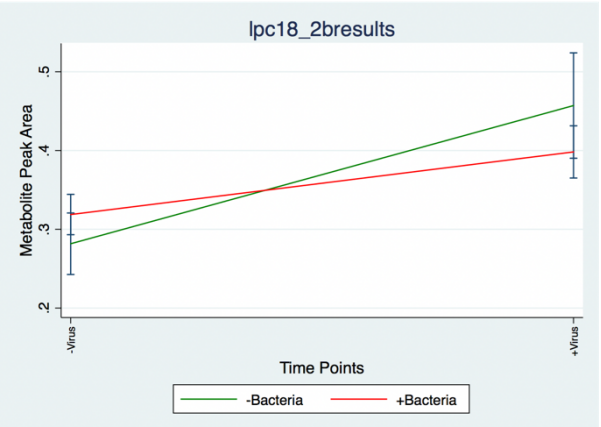

hex1cerd18_2_20_0results
Metabolite Peak Area
0
.5
1
1.5
2
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

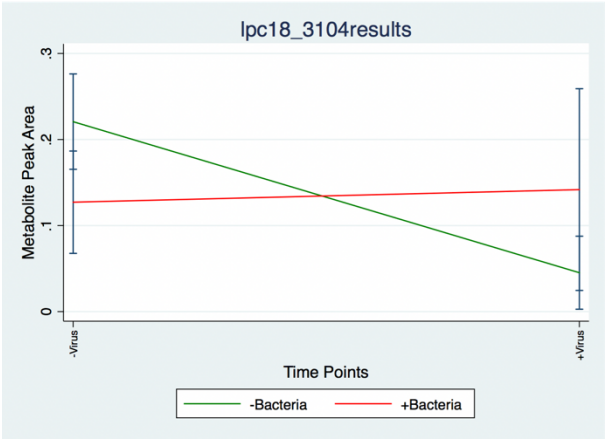

hex1cerd18_2_22_0results
Metabolite Peak Area
.2
.3
.4
.5
.6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

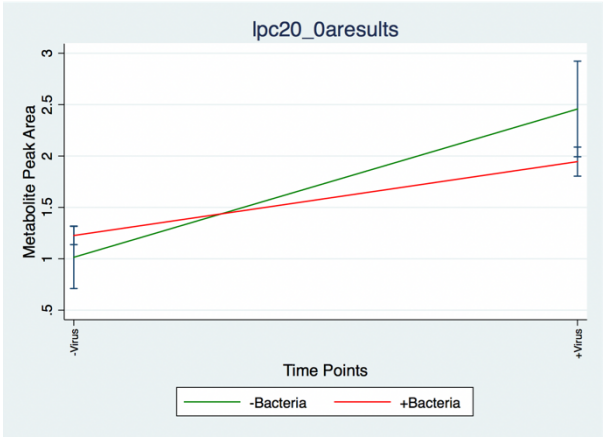

hex1cerd18_2_24_0results
Metabolite Peak Area
.04
.06
.08
.1
.12
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

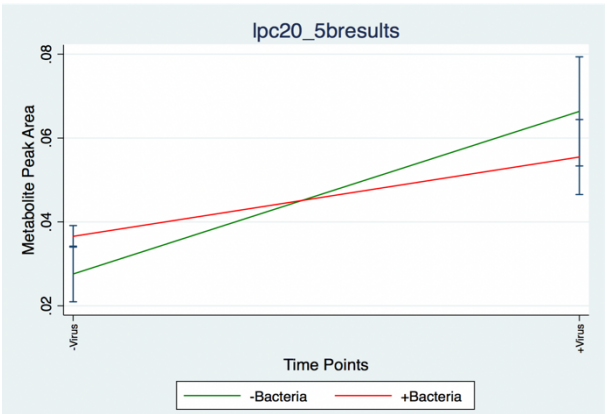

hex2cerd18_1_24_0results
Metabolite Peak Area
.4
.6
.8
1
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

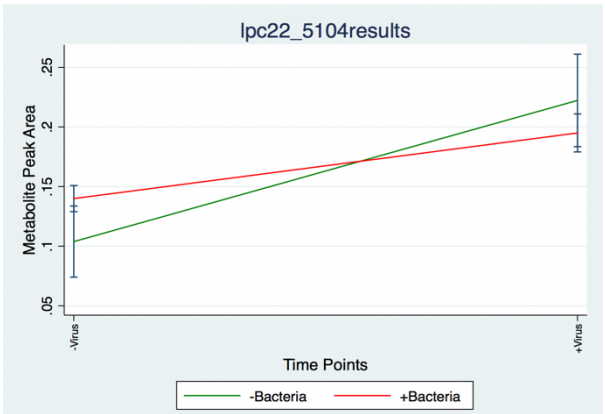

hex3cerd18_1_18_0results
Metabolite Peak Area
.06
.08
.1
.12
.14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

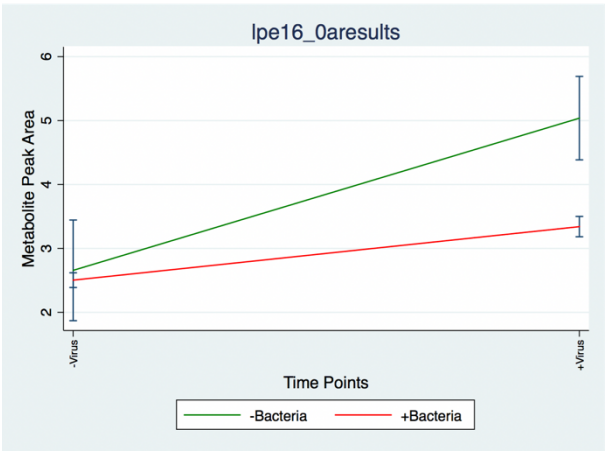

lpc18_2bresults
Metabolite Peak Area
.2
.3
.4
.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

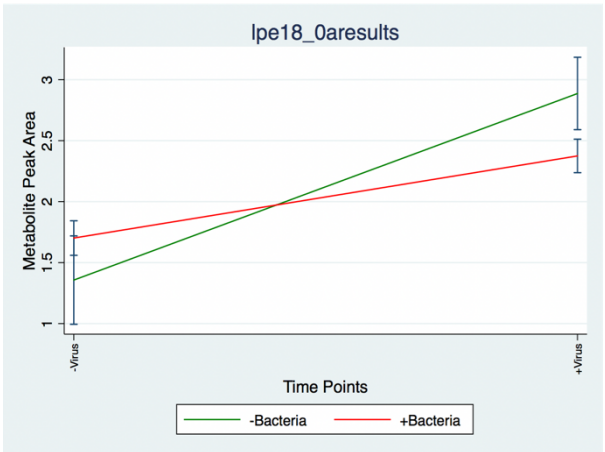

lpc18_3104results
Metabolite Peak Area
0
.1
.2
.3
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

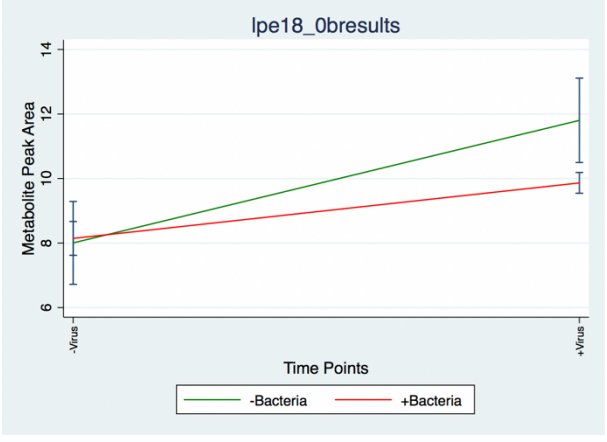

lpc20_0aresults
Metabolite Peak Area
.5
1
1.5
2
2.5
3
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

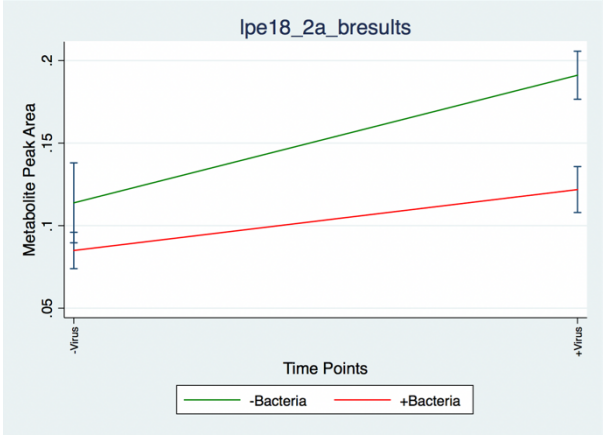

lpc20_5bresults
Metabolite Peak Area
.02
.04
.06
.08
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpc22_5104results
Metabolite Peak Area
.05
.1
.15
.2
.25
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpe16_0aresults
Metabolite Peak Area
2
3
4
5
6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

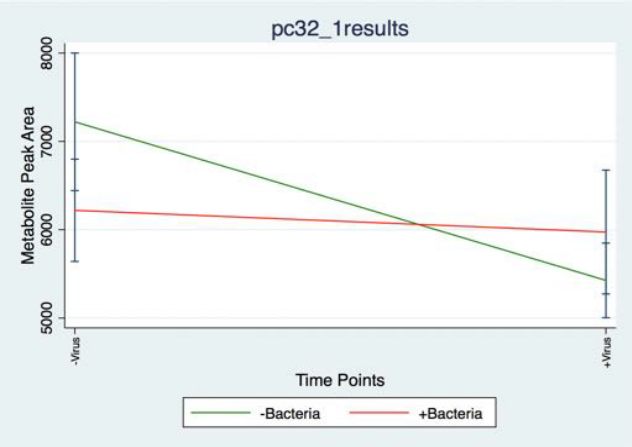

lpe18_0aresults
Metabolite Peak Area
1
1.5
2
2.5
3
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpe18_0bresults
Metabolite Peak Area
6
8
10
12
14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpe18_2a_bresults
Metabolite Peak Area
.05
.1
.15
.2
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


lpe20_4a_bresults
Metabolite Peak Area
.05
.1
.15
.2
.25
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

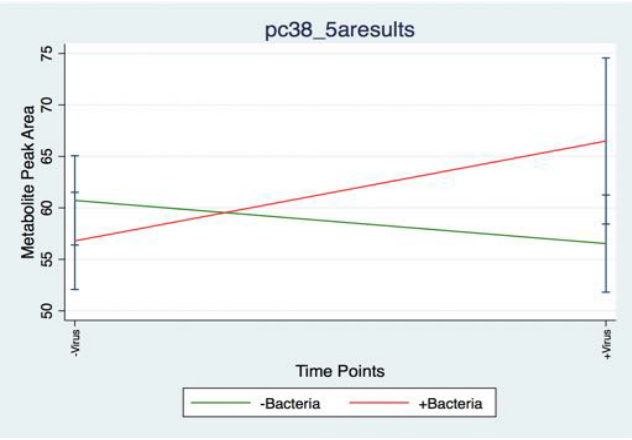

pc31_1results
Metabolite Peak Area
0
20
40
60
80
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pc32_1results
Metabolite Peak Area
5000
6000
7000
8000
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

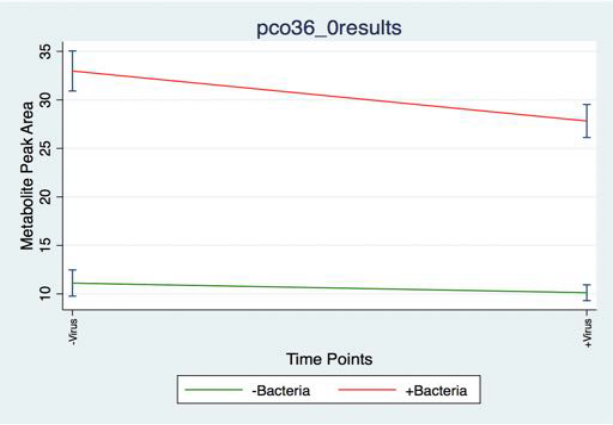

pc34_0results
Metabolite Peak Area
600
700
800
900
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pc34_1results
Metabolite Peak Area
4500
5000
5500
6000
6500
7000
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pc36_2a_bresults
Metabolite Peak Area
3500
4000
4500
5000
5500
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pc38_5aresults
Metabolite Peak Area
50
55
60
65
70
75
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pco32_1results
Metabolite Peak Area
0
20
40
60
80
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pco36_0results
Metabolite Peak Area
10
15
20
25
30
35
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pe32_0results
Metabolite Peak Area
100
150
200
250
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pe32_1results
Metabolite Peak Area
2000
2500
3000
3500
4000
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

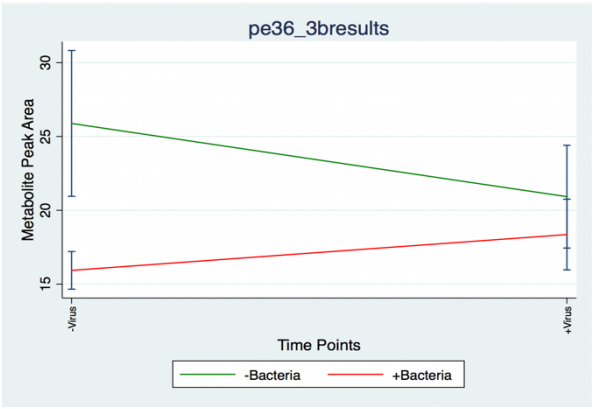

pe34_1results
Metabolite Peak Area
3500
4000
4500
5000
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

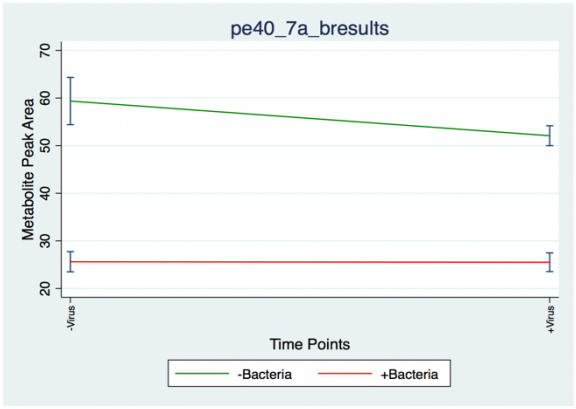

pe34_1results
Metabolite Peak Area
3500
4000
4500
5000
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

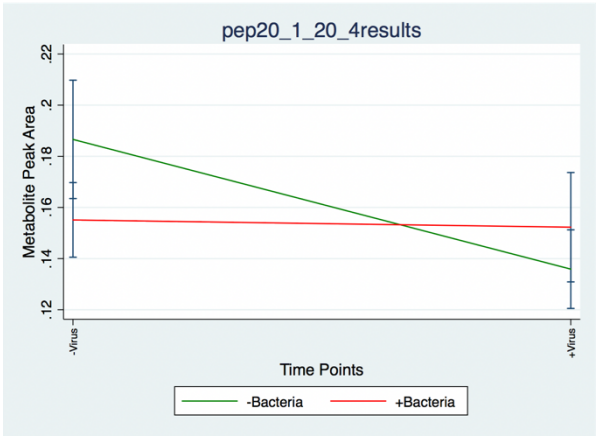

pe36_0results
Metabolite Peak Area
80
100
120
140
160
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

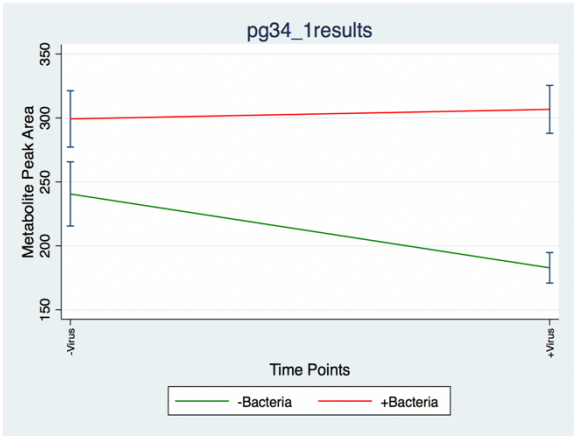

pe36_1results
Metabolite Peak Area
1200
1400
1600
1800
2000
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

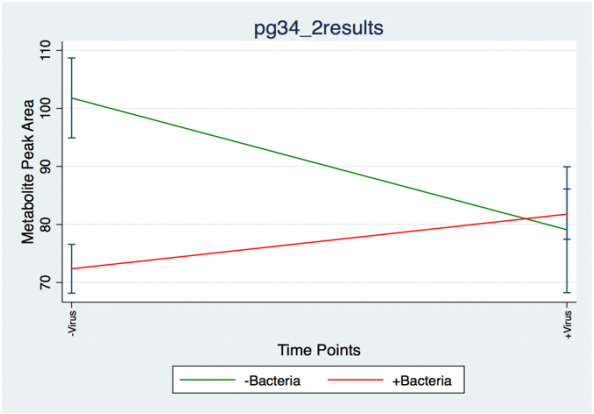

pe36_2a_bresults
Metabolite Peak Area
2000
2500
3000
3500
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

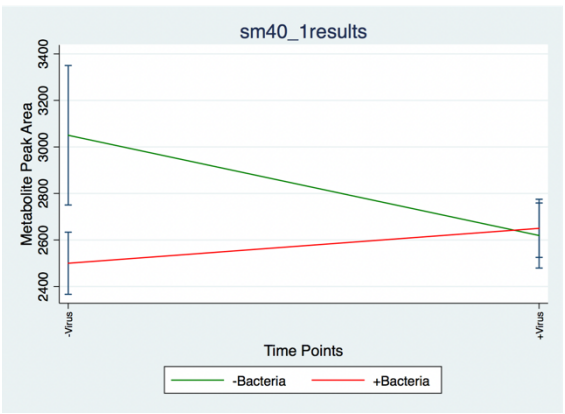

pe36_3bresults
Metabolite Peak Area
15
20
25
30
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

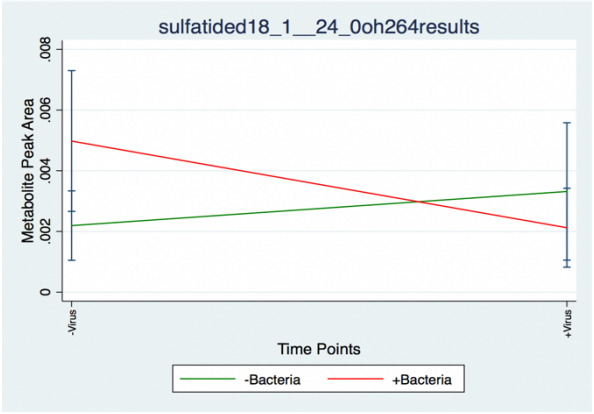

pe40_7a_bresults
Metabolite Peak Area
20
30
40
50
60
70
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

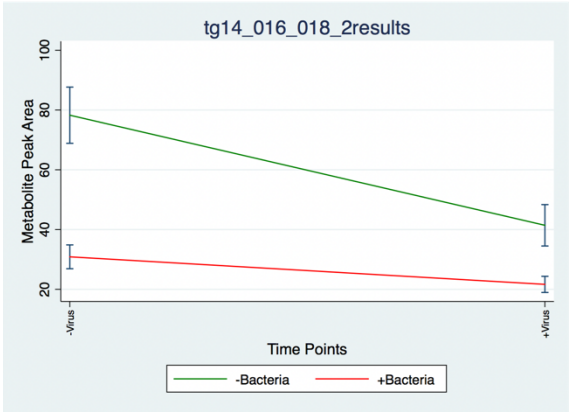

pep20_1_20_4results
Metabolite Peak Area
.12
.14
.16
.18
.2
.22
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pg34_1results
Metabolite Peak Area
150
200
250
300
350
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


pg34_2results
Metabolite Peak Area
70
80
90
100
110
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


sm40_1results
Metabolite Peak Area
2400
2600
2800
3000
3200
3400
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

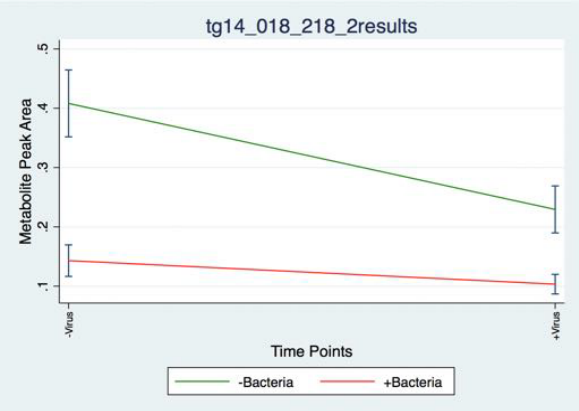

sulfatided18_1__24_0oh264results
Metabolite Peak Area
0
.002
.004
.006
.008
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

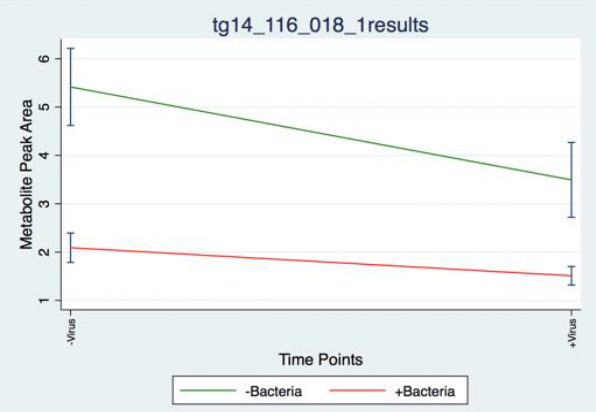

tg14_016_018_2results
Metabolite Peak Area
20
40
60
80
100
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg14_016_118_1results
Metabolite Peak Area
10
20
30
40
50
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

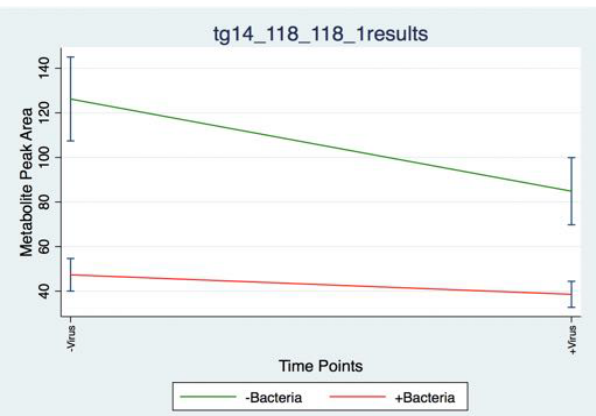

tg14_016_118_2results
Metabolite Peak Area
.1
.2
.3
.4
.5
.6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg14_018_018_1results
Metabolite Peak Area
5
10
15
20
25
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg14_018_218_2results
Metabolite Peak Area
.1
.2
.3
.4
.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

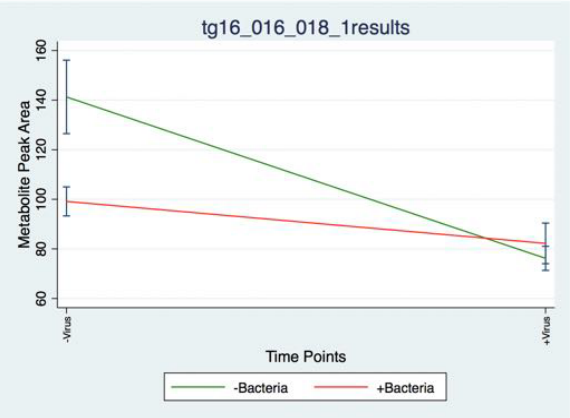

tg14_116_018_1results
Metabolite Peak Area
1
2
3
4
5
6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg14_116_118_0results
Metabolite Peak Area
0
50
100
150
200
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg14_118_118_1results
Metabolite Peak Area
40
60
80
100
120
140
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

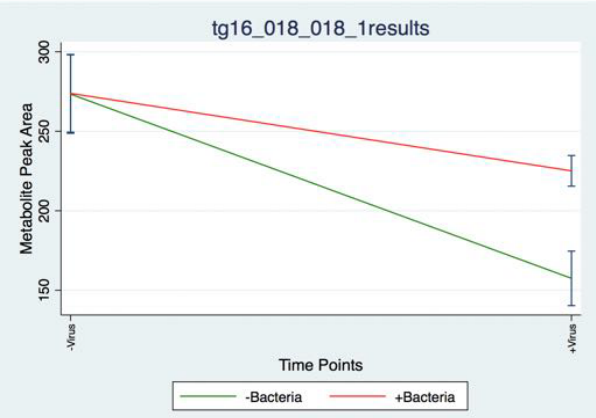

tg16_016_016_0results
Metabolite Peak Area
30
40
50
60
70
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg16_016_018_0results
Metabolite Peak Area
15
20
25
30
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg16_016_018_1results
Metabolite Peak Area
60
80
100
120
140
160
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg16_016_018_2results
Metabolite Peak Area
3
4
5
6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg16_016_118_1results
Metabolite Peak Area
200
250
300
350
400
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

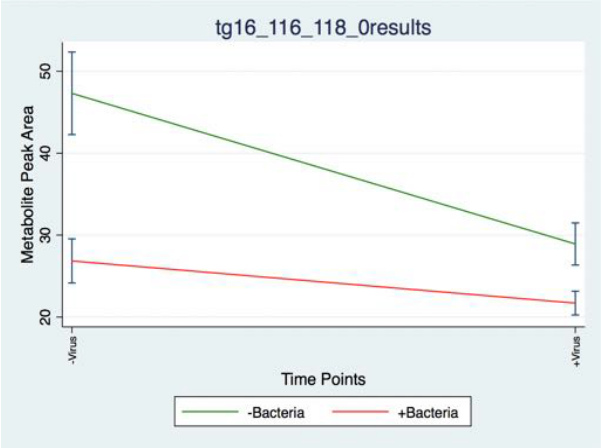

tg16_018_018_1results
Metabolite Peak Area
150
200
250
300
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

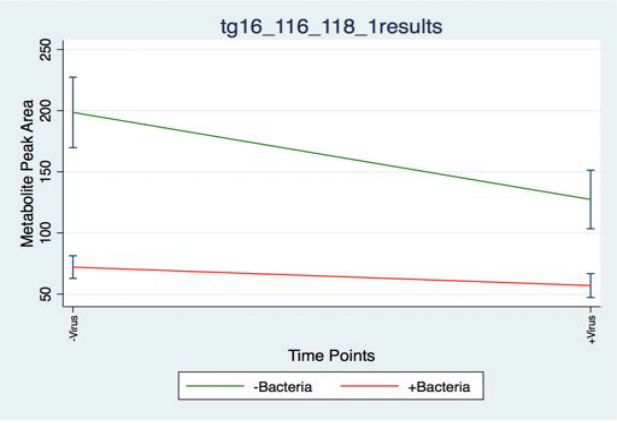

tg16_018_118_1results
Metabolite Peak Area
120
140
160
180
200
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg16_018_118_2results
Metabolite Peak Area
10
12
14
16
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg16_018_218_2results
Metabolite Peak Area
2
3
4
5
6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

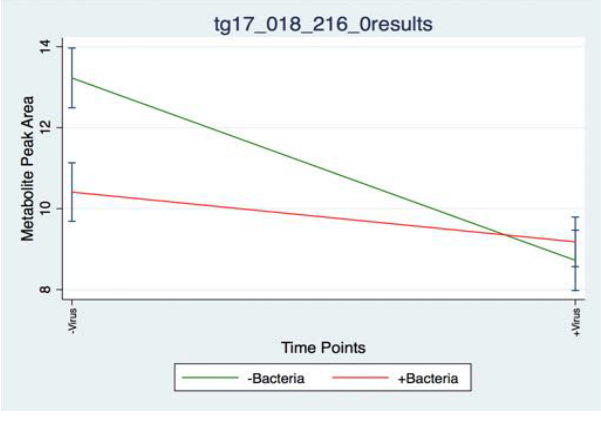

tg16_116_116_1results
Metabolite Peak Area
10
20
30
40
50
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg16_116_118_0results
Metabolite Peak Area
20
30
40
50
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg16_116_118_1results
Metabolite Peak Area
50
100
150
200
250
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg16_118_118_1results
Metabolite Peak Area
100
150
200
250
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

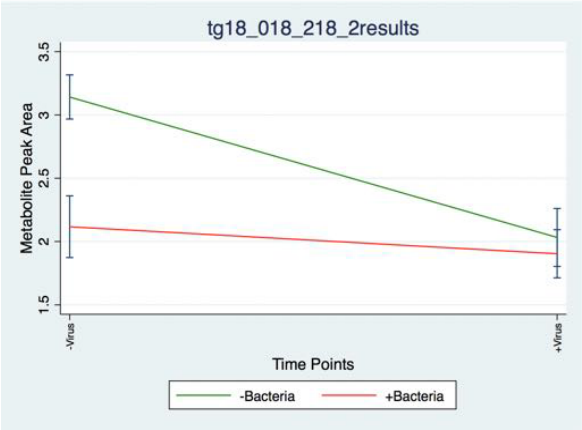

tg16_118_118_2results
Metabolite Peak Area
2
4
6
8
10
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg17_018_216_0results
Metabolite Peak Area
8
10
12
14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg18_018_018_0results
Metabolite Peak Area
5
10
15
20
25
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg18_018_018_1results
Metabolite Peak Area
80
100
120
140
160
180
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg18_018_118_1results
Metabolite Peak Area
80
100
120
140
160
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg18_018_218_2results
Metabolite Peak Area
1.5
2
2.5
3
3.5
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

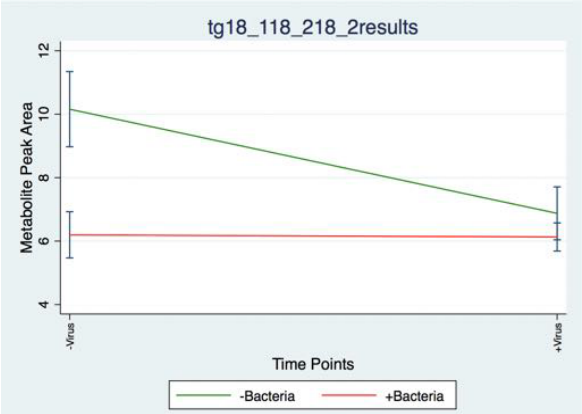

tg18_114_016_0results
Metabolite Peak Area
10
20
30
40
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

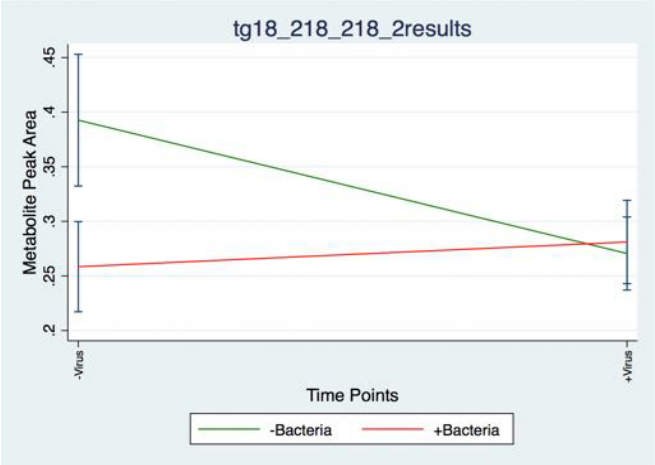

tg18_118_118_1results
Metabolite Peak Area
300
350
400
450
500
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg18_118_118_2results
Metabolite Peak Area
3
4
5
6
7
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg18_118_120_4results
Metabolite Peak Area
2
3
4
5
6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg18_118_122_6results
Metabolite Peak Area
1
2
3
4
5
6
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg18_118_218_2results
Metabolite Peak Area
4
6
8
10
12
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg18_218_218_2results
Metabolite Peak Area
.2
.25
.3
.35
.4
.45
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tg18_218_220_4results
Metabolite Peak Area
0
.05
.1
.15
-Virus
+Virus
Time Points
-Bacteria
+Bacteria


tgo52_2results
Metabolite Peak Area
8
10
12
14
-Virus
+Virus
Time Points
-Bacteria
+Bacteria

Fig. S4. Validation of etomoxir treatment. (A) Drug cytotoxicity profile of HuH7 cells after treatment with Etomoxir or the vehicle control DMSO at various concentrations over 24 H or 48 H. Cytotoxicity was measured using the CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega). LDH+ release was measured by adding the Cytotox reagent and measuring absorbance at 490nm on a plate reader (ClarioStar). Data are represented as mean ± SEM; *p < 0.05; **p < 0.01; ***p < 0.001; ***p < 0.0001; n.s. : Not significant, two-tailed Student's t-test. (b-c) Nile red staining of HuH7 cells after etomoxir treatment for 48 H. Cells were fixed and stained with Nile red, a dye for lipid droplets. (B) As etomoxir treatment inhibits acylcarnitines, there is an upstream accumulation of lipid droplets which can be observed in the treated cells (C) The corrected total cell fluorescence (CTCF) shows that there is significantly higher fluorescence in the etomoxir-treated cells which confirms that the treatment worked.

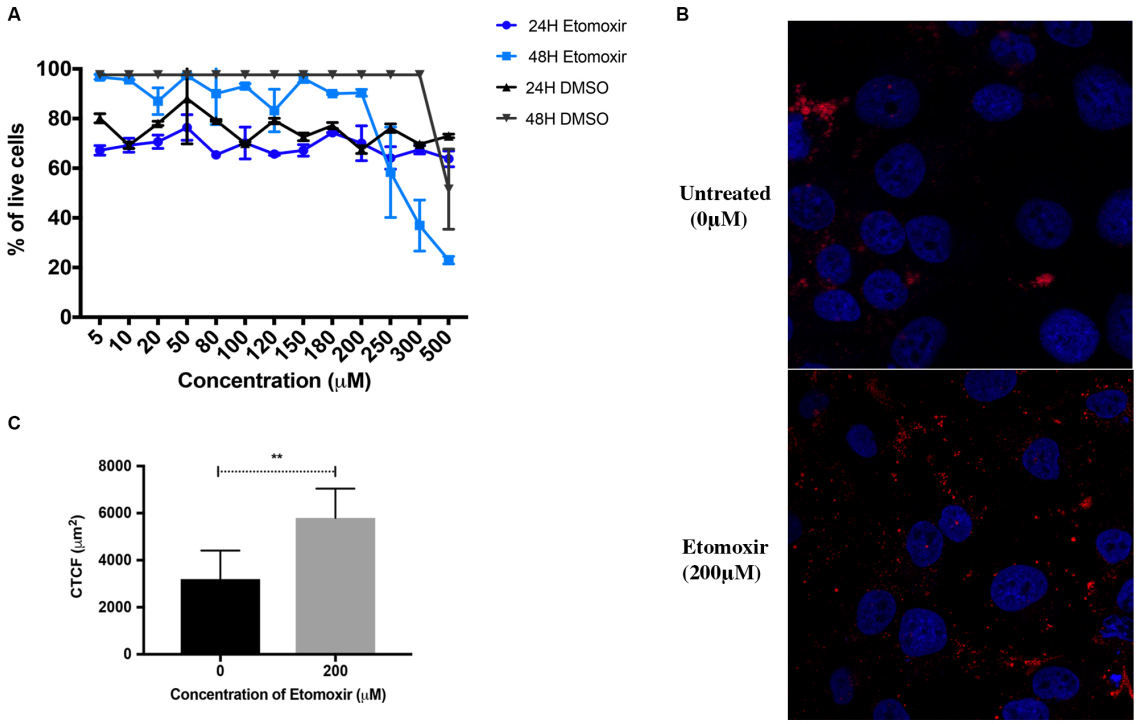
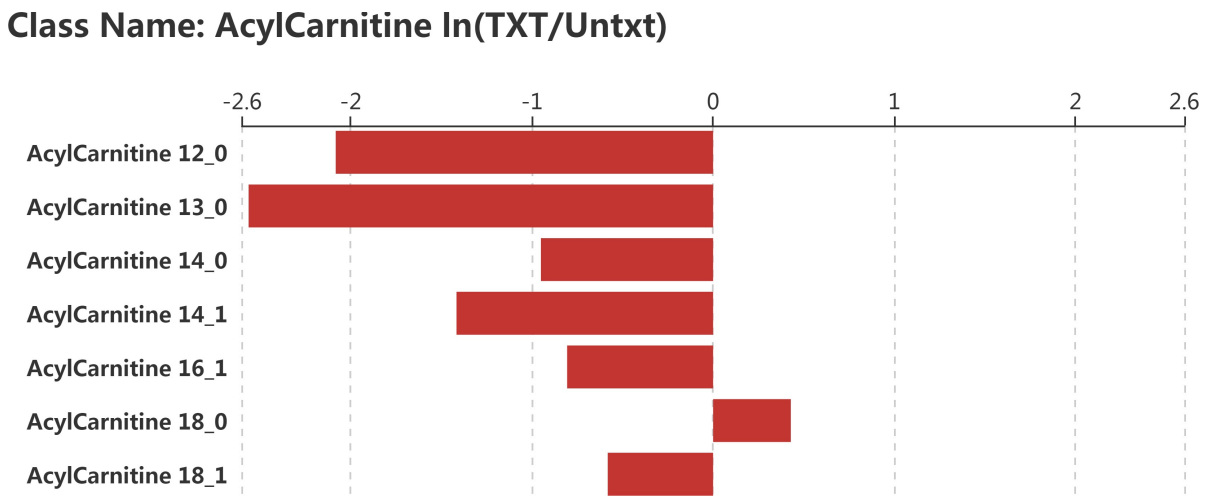


Fig. S5. LCMS results post-etomoxir treatment. Plots showing the fold changes of significantly modulated metabolites after treatment with Etomoxir. Fold changes were calculated as etomoxir-treated cells (TXT) against untreated cells (Untxt) and results were grouped according to lipid species. Briefly, HuH7 cells were treated with 200µM etomoxir or left untreated for 48 H after which cells were lysed for LCMS. Etomoxir treatment results in significantly changed acyl-carnitine levels as compared to untreated cells. 6 out of 7 acylcarnitines (except for acyl-carnitine 18:0) which were significantly modulated, were reduced in treated cells. There were corresponding changes in other lipid species as shown since many lipid pathways are intertwined and interrelated. It is virtually impossible to downregulate one pathway and not expect a co-related change in another. All conditions were performed in replicates of six; error bars represent SEM.



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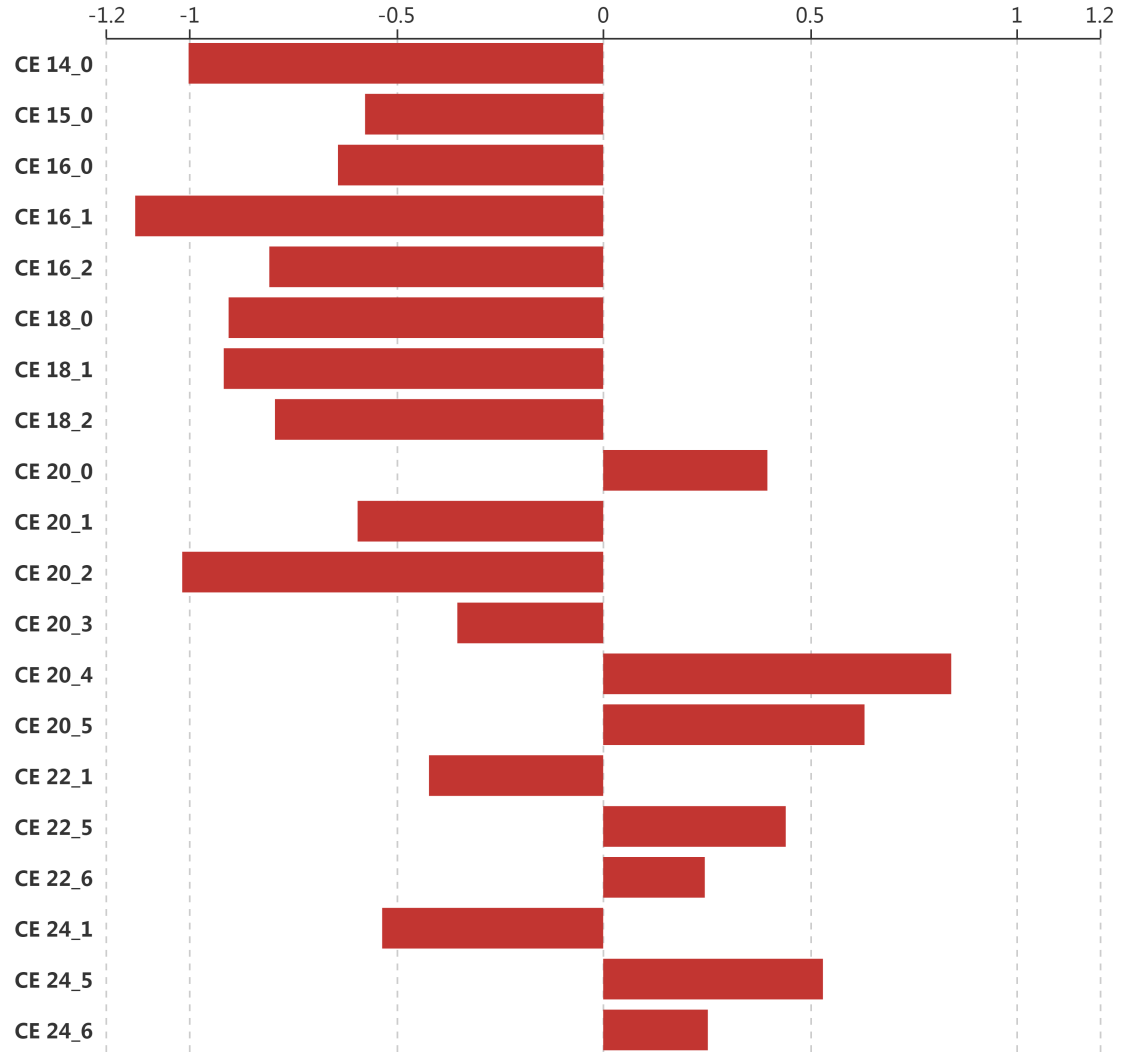

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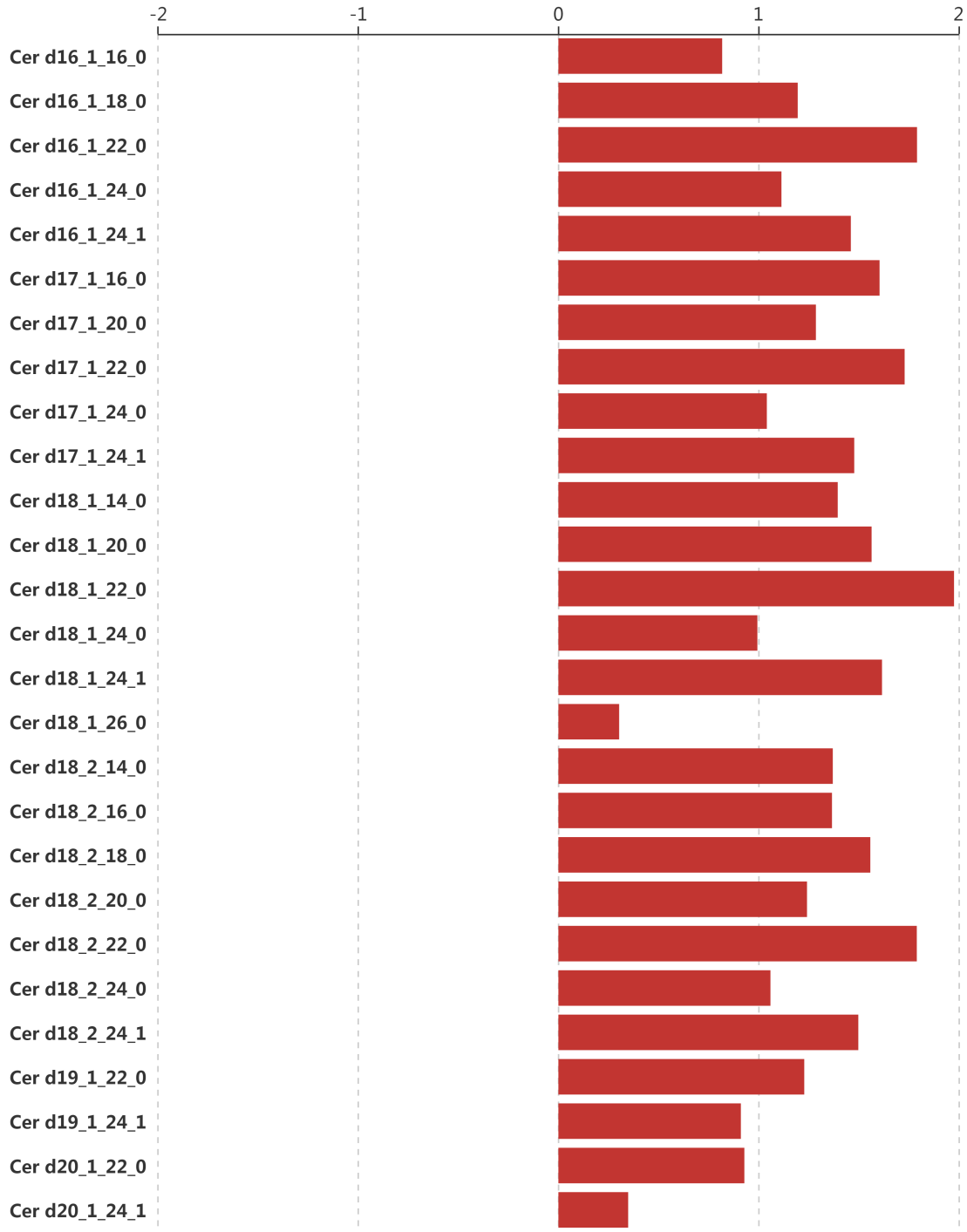

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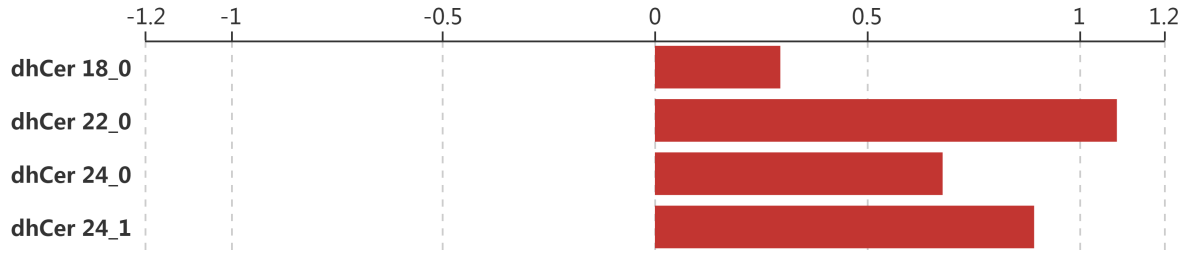

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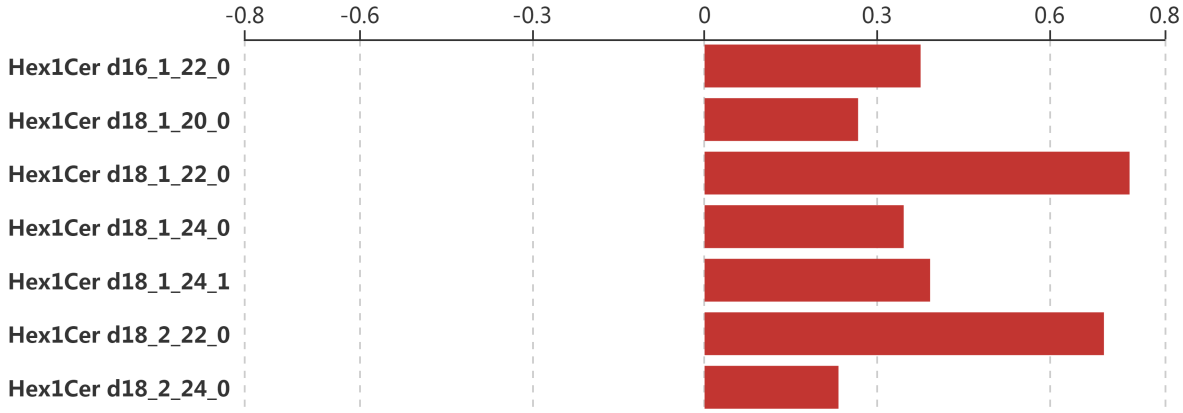

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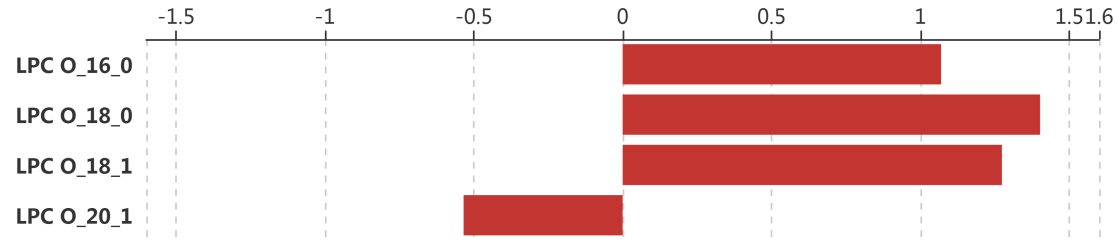

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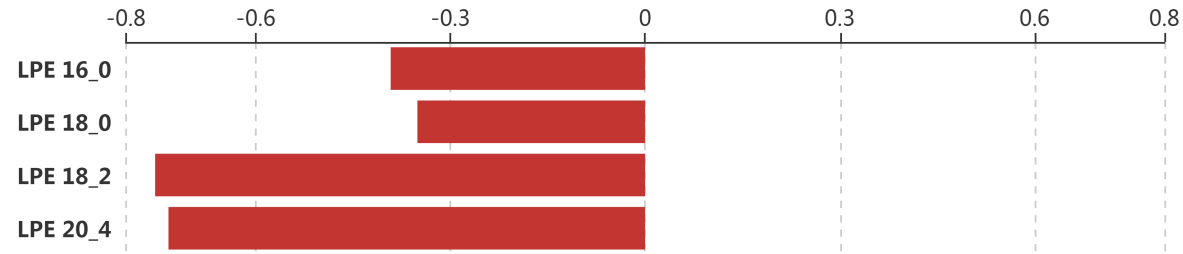

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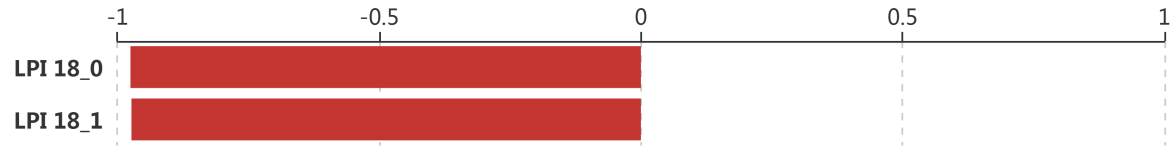

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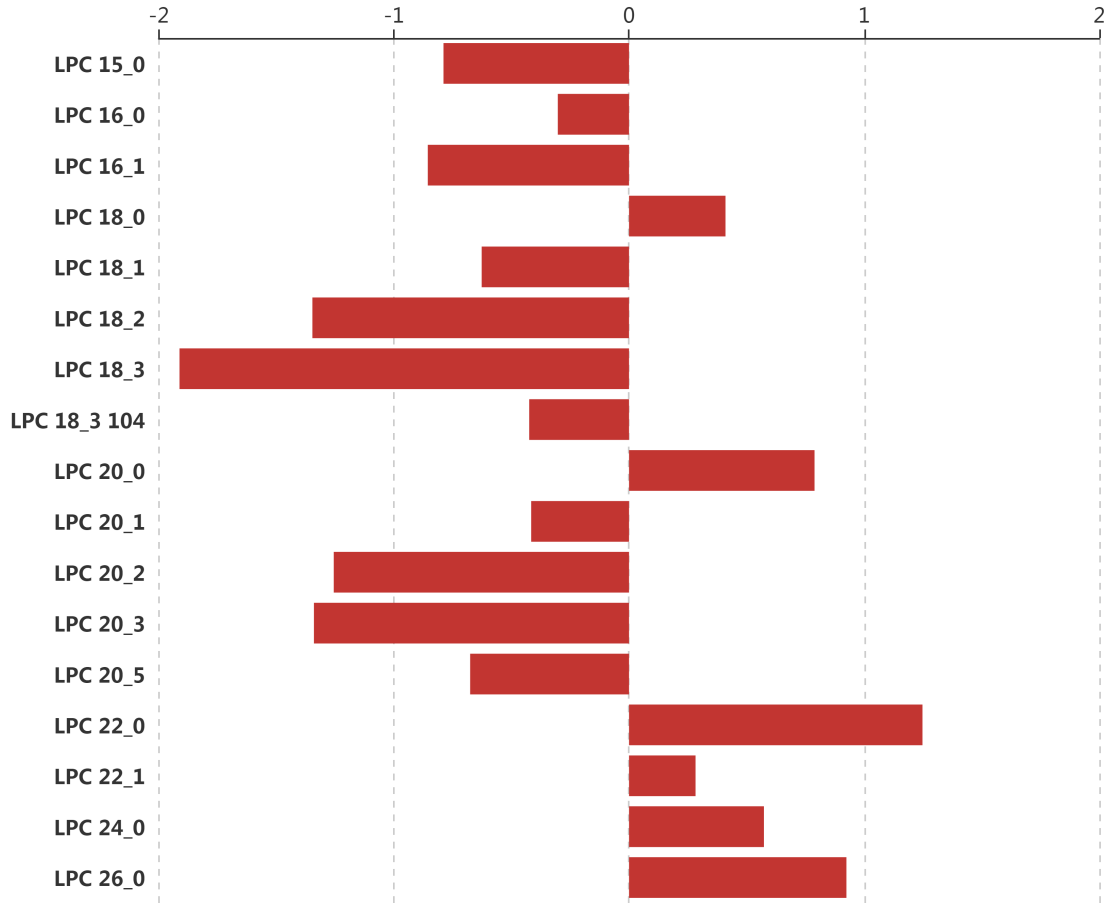

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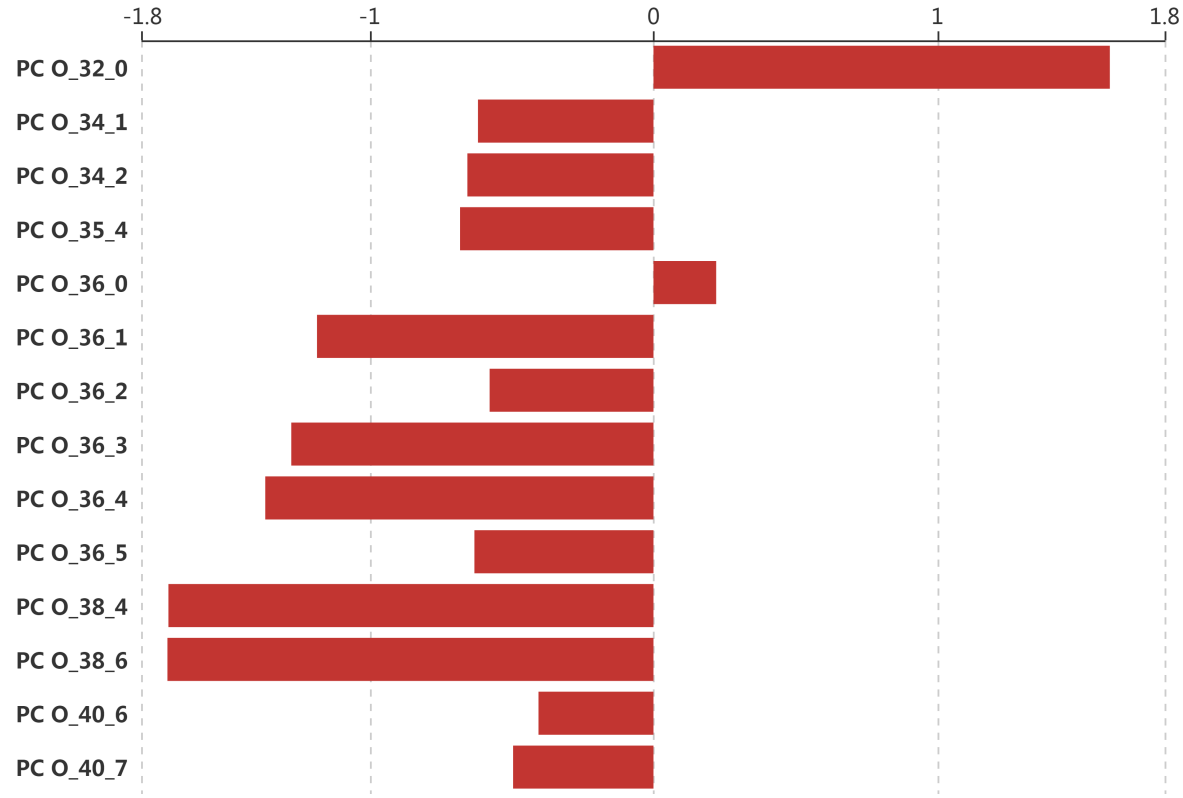

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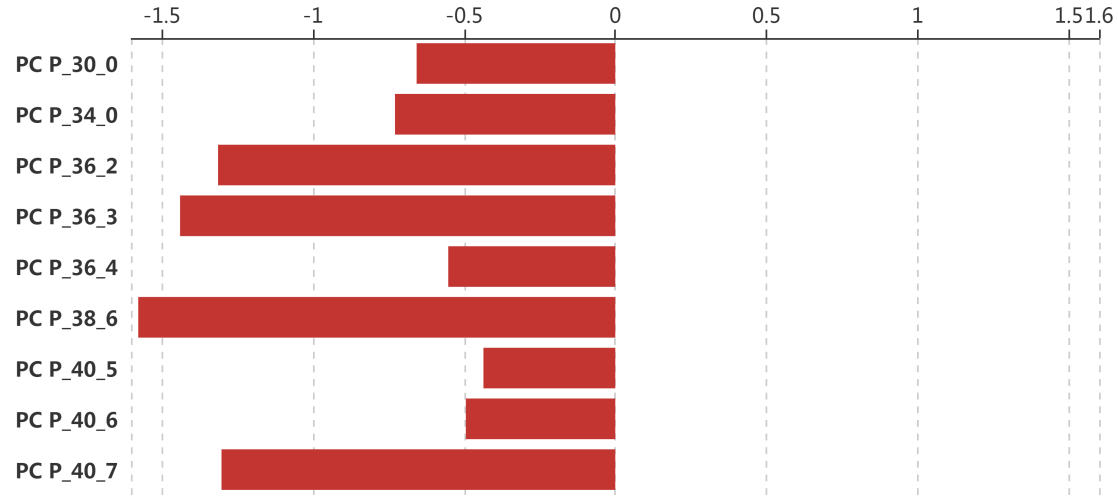
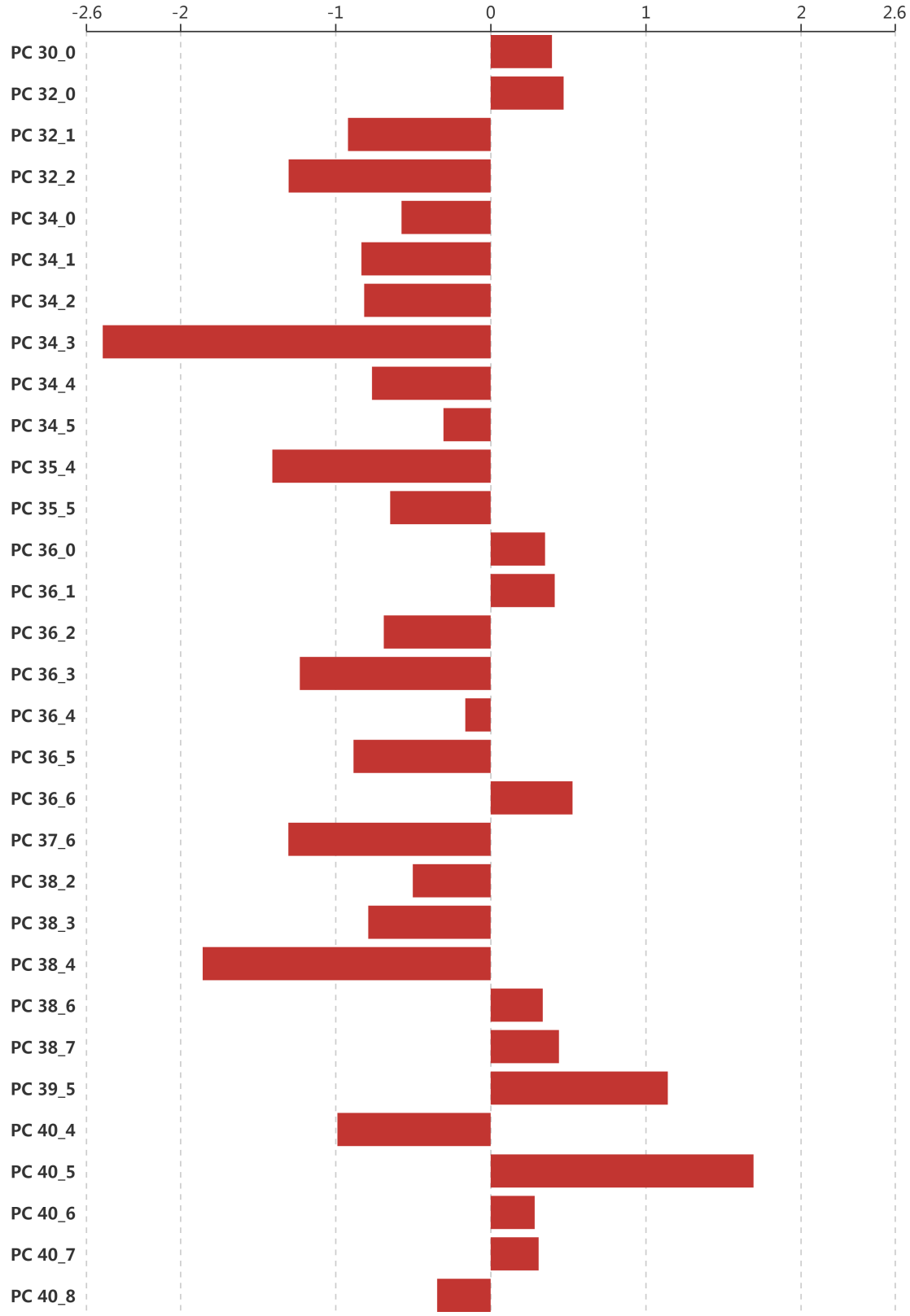


Class Name: PC ln(TXT/Untxt)



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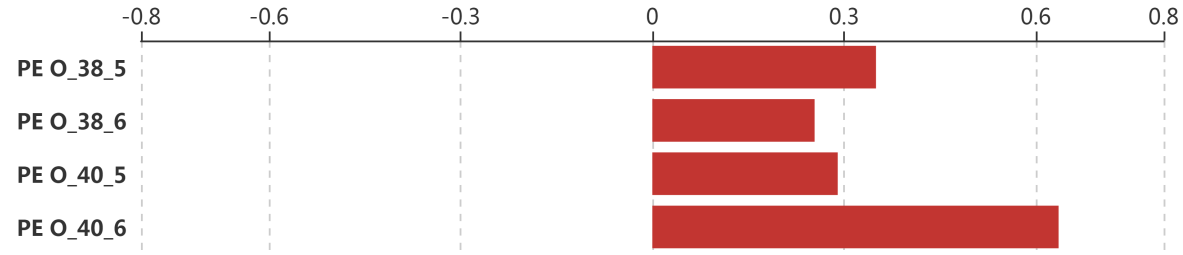

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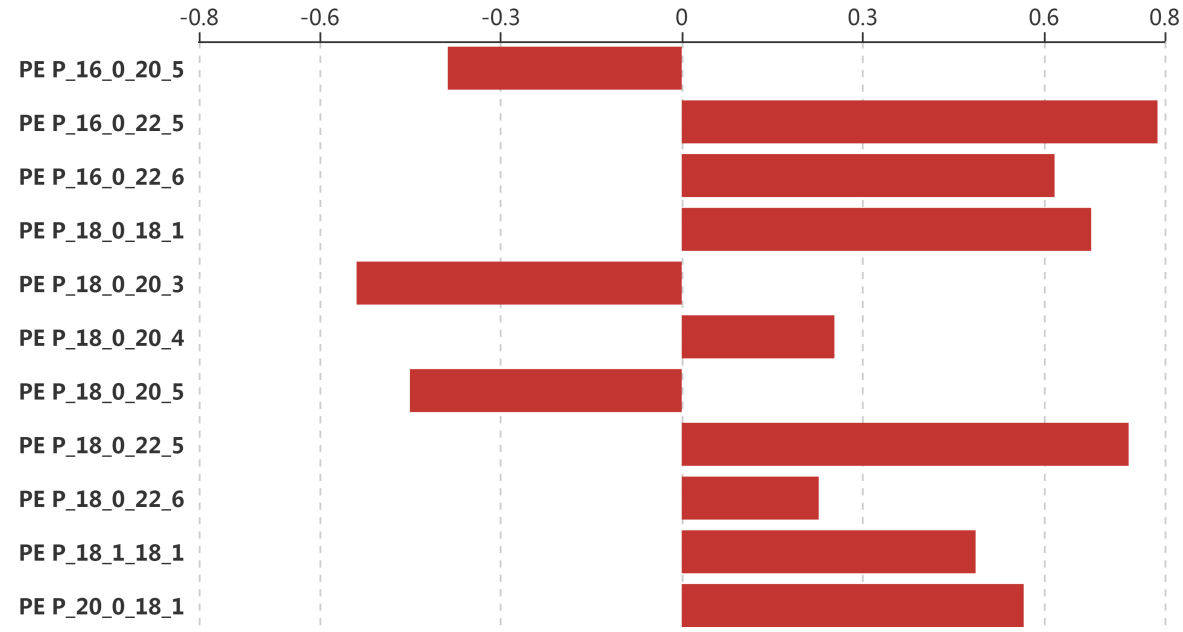
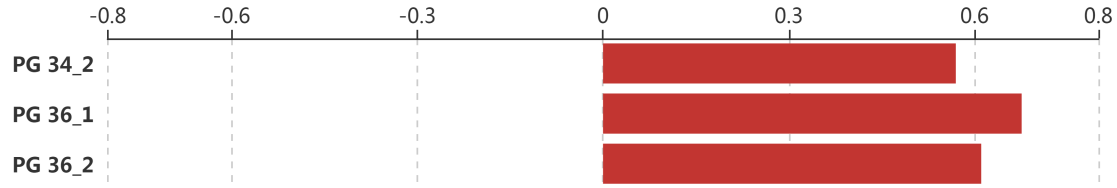


Class Name: PG ln(TXT/Untxt)



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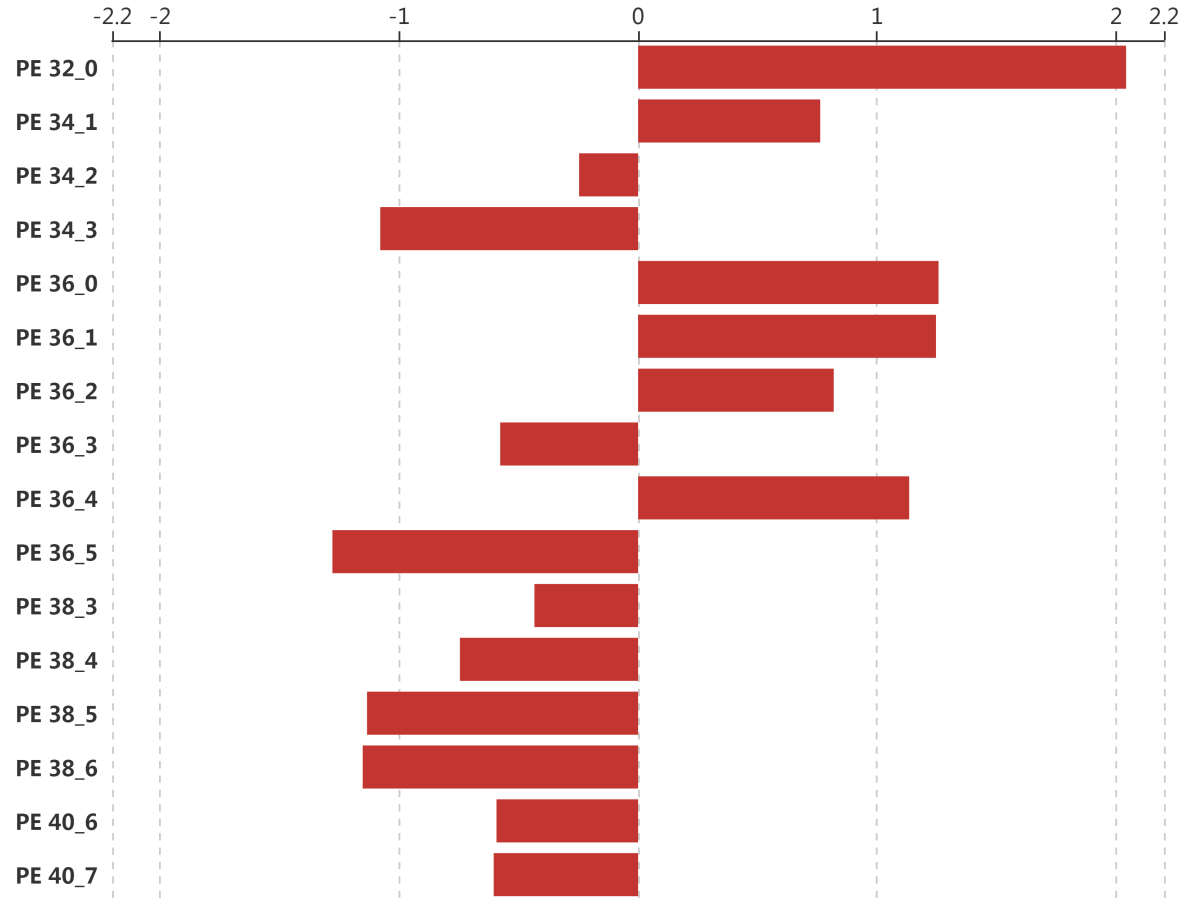

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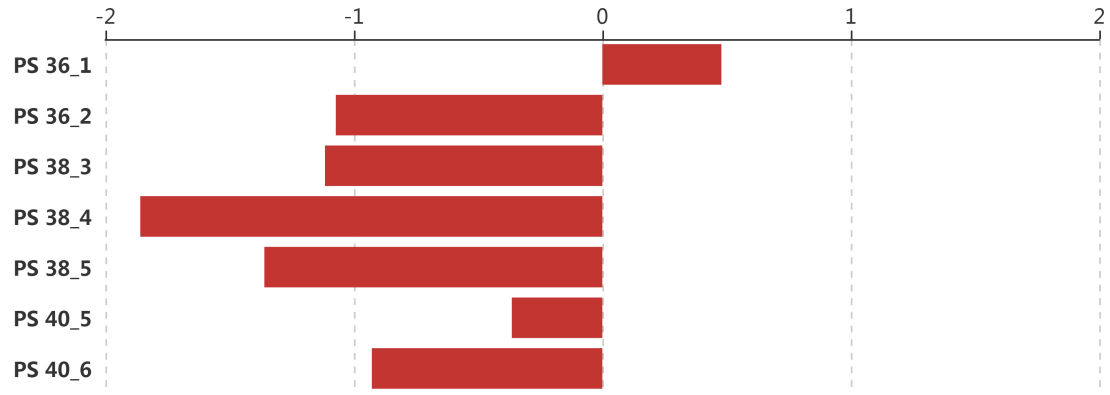
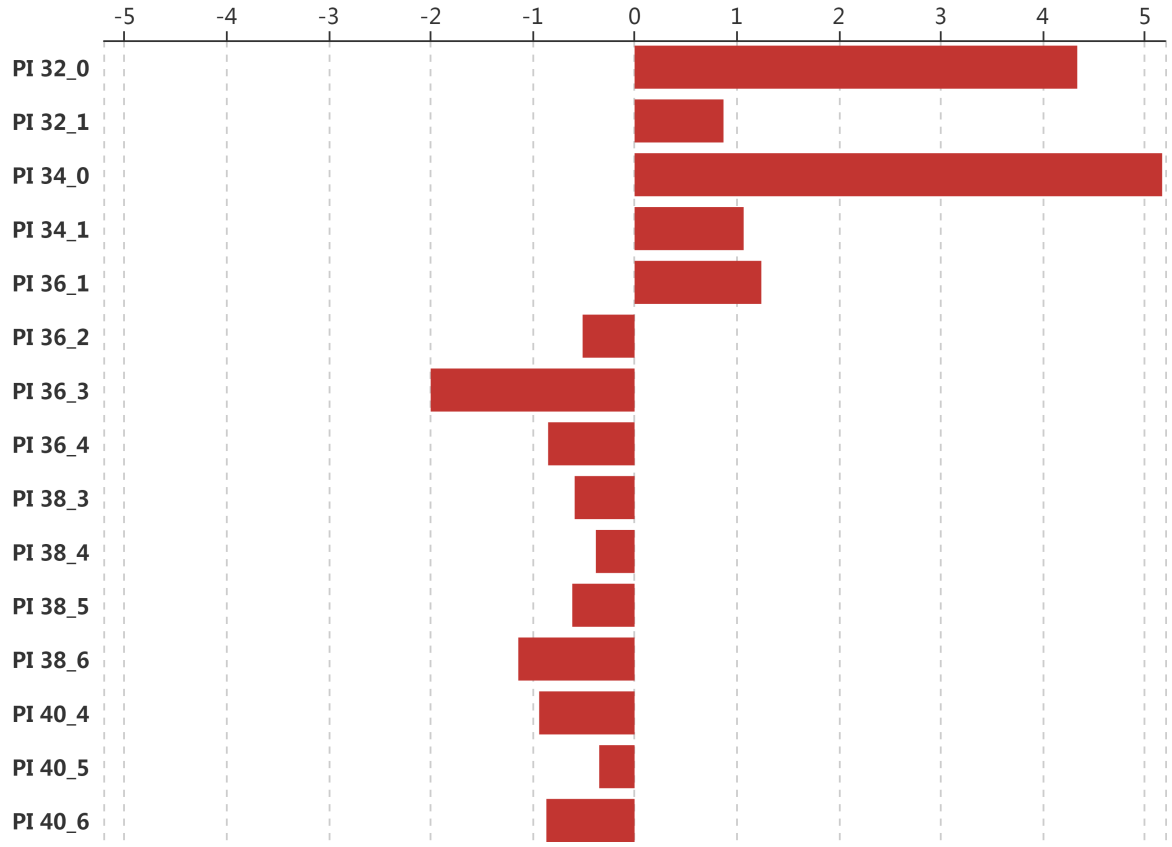


Class Name: PI ln(TXT/Untxt)



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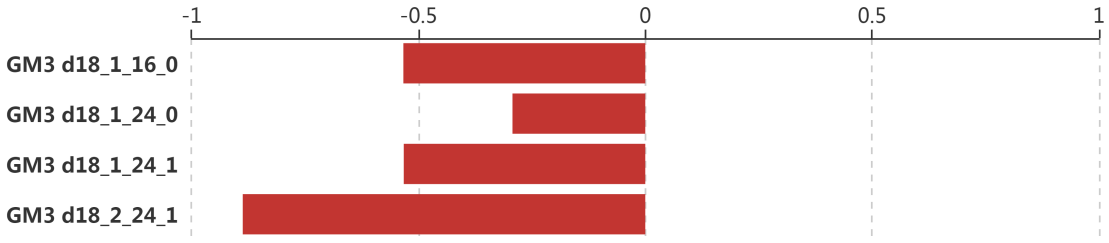
Class Name: TG-O ln(TXT/Untxt)

Class Name: GM3 ln(TXT/Untxt)



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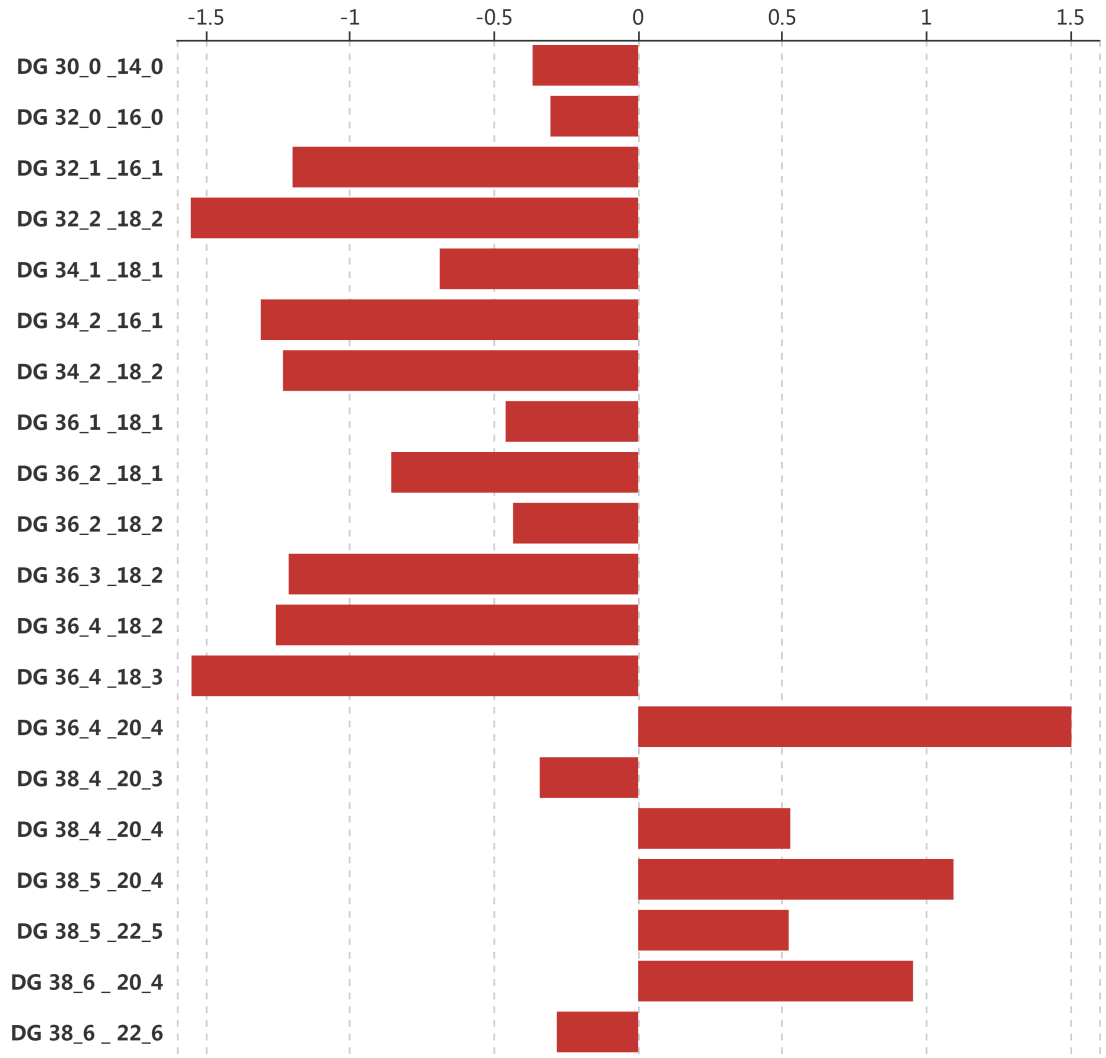

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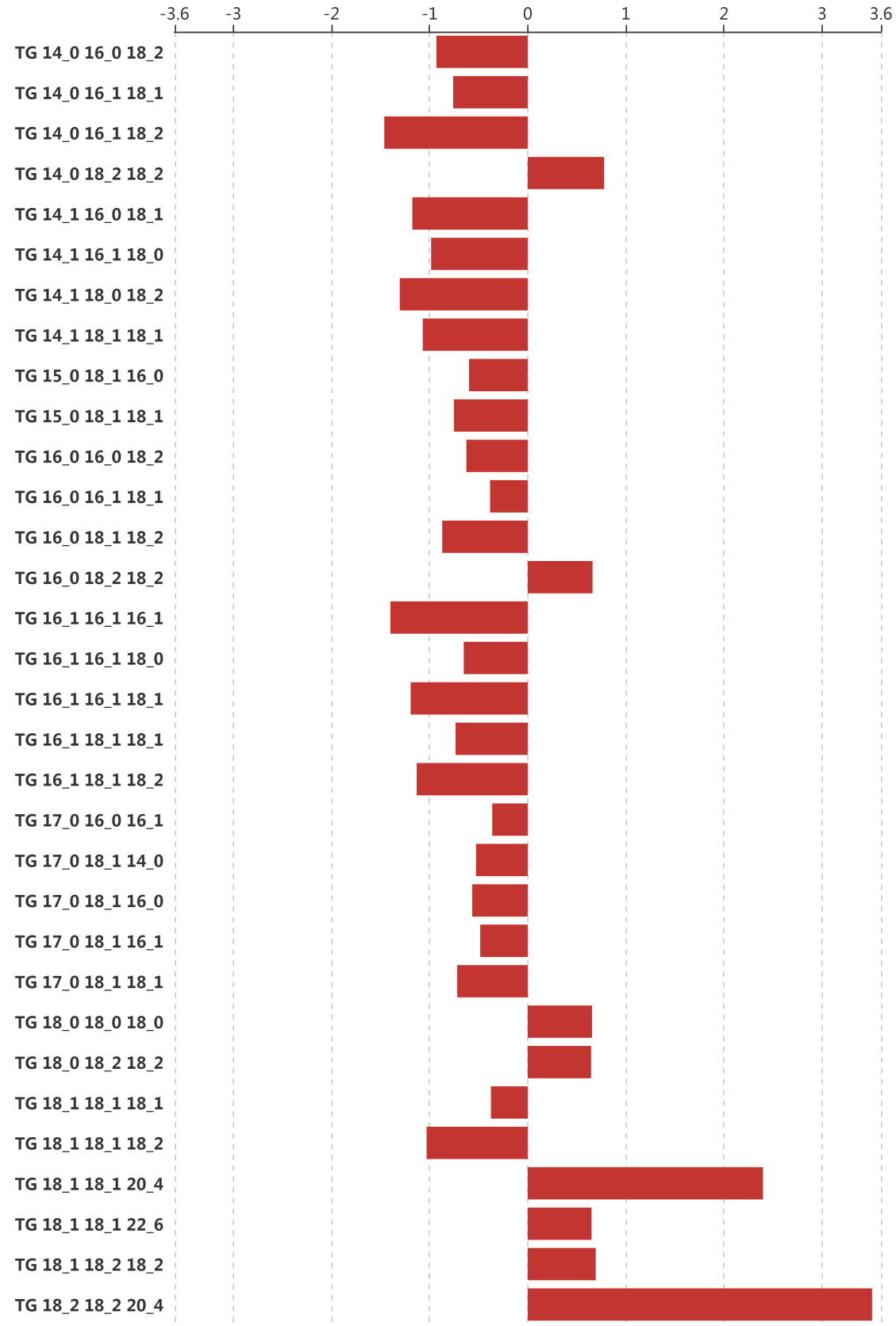

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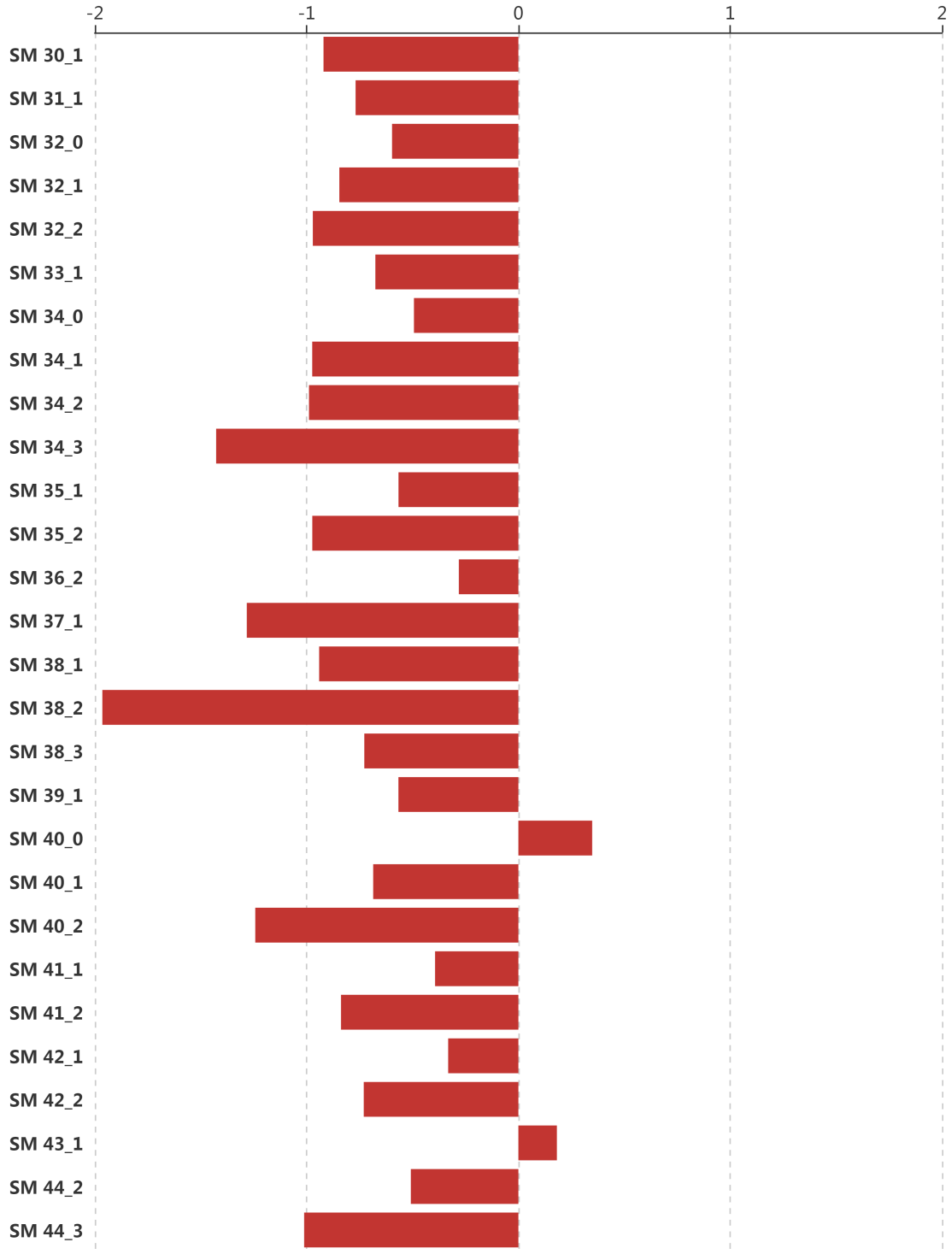

Fig. S6. Cellular ATP assay. Plots showing the cellular ATP levels of *Aag*2.*w*Mel and *Aag*2.TET cells. Cellular ATP levels were plotted against cell counts. Blue: *Aag*2.TET; Red: *Aag*2.*w*Mel. Replicates of four were measured; error bars represent SEM.