

SUPPLEMENTARY MATERIALS

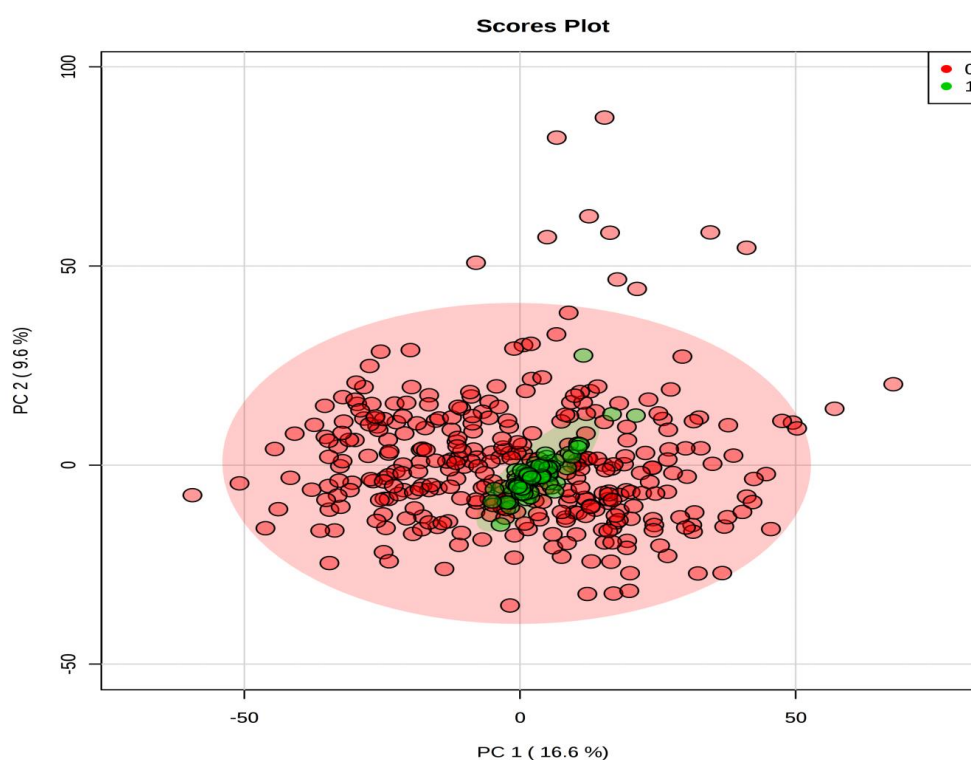


Figure S1: PCA score plot of patients (red) and QC samples (green). QC samples are seen to cluster tightly together indicating that the data is of good quality.

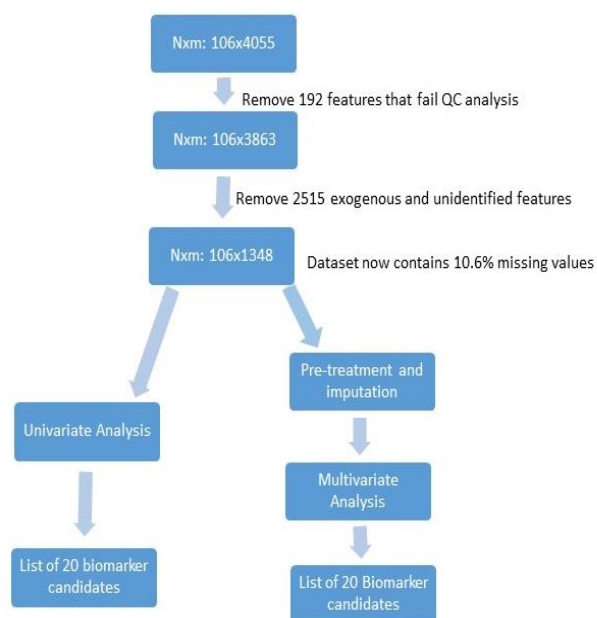


Figure S2. Flow of data from raw data stage through data analysis to biomarker candidate lists: 15 weeks dataset: 106 samples (50 cases and 56 controls) x 4055 features.

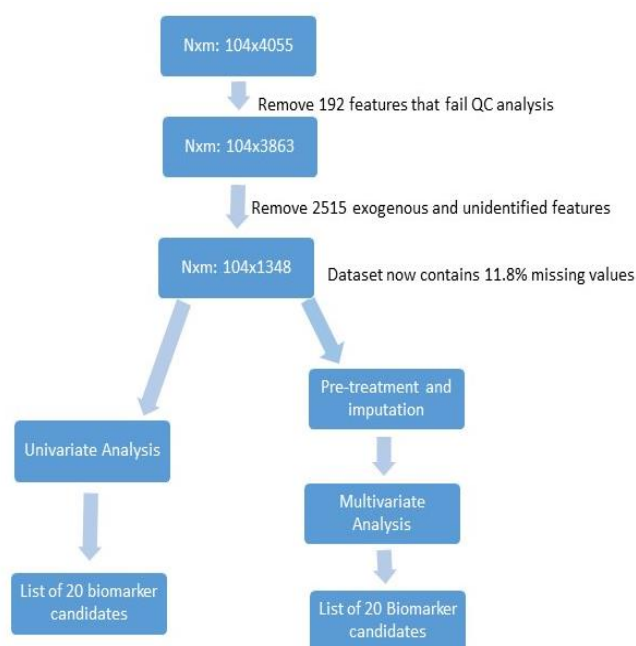


Figure S3. Flow of data from raw data stage through data analysis to biomarker candidate lists: 20 weeks dataset: 104 samples (49 cases and 55 controls) x 4055 features.

Table S1: Ranked results of univariate (Fold Change) analysis on **15** weeks GA dataset. (VIP scores are not reported as the results of the permutation showed that the PLSDA model was invalid.)

| Rank | Feature | Fold Change | Log 2 Fold Change | Direction of Dysregulation in Cases | % missing values cases | % missing values controls | Biological Grouping | Panel C |
|------|---------|-------------|-------------------|-------------------------------------|------------------------|---------------------------|---------------------|---------|
| 1 | neg_18 | 2.440485 | 1.287168 | UP | 39 | 45 | BA | |
| 2 | neg_19 | 2.25624 | 1.173921 | UP | 37 | 46 | BA | |
| 3 | neg_20 | 0.514199 | 0.9596 | DOWN | 2 | 5 | PL | * |
| 4 | neg_21 | 0.563609 | 0.827234 | DOWN | 41 | 50 | FA | |
| 5 | neg_22 | 0.594372 | 0.750562 | DOWN | 6 | 5 | AA | * |
| 6 | neg_23 | 0.603438 | 0.728722 | DOWN | 18 | 20 | PL | |
| 7 | neg_24 | 0.609538 | 0.714212 | DOWN | 41 | 32 | SP/PP/AA/DA | |
| 8 | neg_25 | 0.609667 | 0.713906 | DOWN | 41 | 39 | DIAG | |
| 9 | neg_26 | 1.508704 | 0.59331 | UP | 43 | 30 | C21-STR | |
| 10 | neg_27 | 0.67683 | 0.563134 | DOWN | 35 | 25 | ARA/HA/ALC/PO/CAT | |
| 11 | neg_28 | 0.693309 | 0.52843 | DOWN | 0 | 0 | PL | * |

| | | | | | | | | |
|----|--------|--------------|----------|------|----|----|---------------|---|
| 12 | neg_29 | 1.4403 32 | 0.526402 | UP | 24 | 18 | BA | |
| 13 | neg_30 | 0.6956 96 | 0.523471 | DOWN | 29 | 21 | PL | |
| 14 | neg_31 | 0.6980 78 | 0.51854 | DOWN | 39 | 43 | VITD &d | |
| 15 | neg_32 | 0.7016 71 | 0.511133 | DOWN | 0 | 0 | PL | |
| 16 | neg_33 | 0.7029 33 | 0.50854 | DOWN | 0 | 2 | PL | |
| 17 | neg_34 | 1.4173 47 | 0.503194 | UP | 45 | 23 | FA & c/STR &d | |
| 18 | neg_35 | 0.7060 89 | 0.502078 | DOWN | 0 | 0 | PL | |
| 19 | neg_36 | 1.4159 8 | 0.501801 | UP | 39 | 52 | PTR | |
| 20 | neg_37 | 0.7086 61 | 0.496832 | DOWN | 4 | 7 | MOAG | * |

Table S2: Non-parametric test MWU ranked top 20 features from 15 week dataset

| Feature | P-VALUE | FDR | % mv in cases | %mv in controls | ID/Biological Grouping | Panel D |
|---------|----------|---------|---------------------|--------------------|------------------------|---------|
| neg_38 | 0.000951 | 0.83074 | 0 | 0 | HFA | * |
| neg_39 | 0.002162 | 0.83074 | 0 | 0 | IND | * |
| neg_40 | 0.002619 | 0.83074 | 39 | 59 | PTR | |
| neg_41 | 0.004049 | 0.83074 | 33 | 33 | GLP | |
| neg_42 | 0.004952 | 0.83074 | 16 | 12 | VIT D | |
| neg_43 | 0.007468 | 0.83074 | 4 | 8 | MOAG | * |
| neg_44 | 0.010098 | 0.83074 | 2 | 6 | PL | * |
| neg_45 | 0.010479 | 0.83074 | 24 | 16 | VITD/BA/PP | |
| neg_46 | 0.010674 | 0.83074 | 37 | 59 | PTR | |
| neg_47 | 0.010674 | 0.83074 | 0 | 0 | DIAG | |
| neg_48 | 0.0117 | 0.83074 | 2 | 0 | MOAG/PL | |
| neg_49 | 0.0117 | 0.83074 | 6 | 6 | DIAG | |
| neg_50 | 0.012356 | 0.83074 | 10 | 24 | N_GLSP | |
| neg_51 | 0.013282 | 0.83074 | 0 | 0 | VITD | |
| neg_52 | 0.014016 | 0.83074 | 12 | 6 | PL | |
| neg_53 | 0.014269 | 0.83074 | 0 | 0 | DIAG | |
| neg_54 | 0.014786 | 0.83074 | 0 | 0 | DIAG | |
| neg_55 | 0.014786 | 0.83074 | 0 | 0 | DIAG | |
| neg_56 | 0.014786 | 0.83074 | 2 | 0 | DIAG | |
| neg_57 | 0.015871 | 0.83074 | 0 | 0 | PL | |

Table S3: non-parametric test MWU ranked top 20 features from 20 week dataset

| FEATURE | p-value | FDR | % MV in cases | % mv in controls | ID/Biological Grouping | Panel B |
|---------|----------|---------|------------------|---------------------|---------------------------|---------|
| neg_58 | 0.000726 | 0.71503 | 0 | 0 | HFA | * |

| | | | | | | |
|--------|----------|---------|----|----|------------|---|
| neg_59 | 0.003315 | 0.71503 | 2 | 2 | VITD | * |
| neg_60 | 0.003757 | 0.71503 | 0 | 0 | FALD | * |
| neg_61 | 0.003836 | 0.71503 | 20 | 15 | PL | |
| neg_62 | 0.005877 | 0.71503 | 28 | 29 | PL | |
| neg_63 | 0.005995 | 0.71503 | 26 | 29 | N-ACLA | |
| neg_64 | 0.006237 | 0.71503 | 18 | 25 | PP/IND & d | |
| neg_65 | 0.006237 | 0.71503 | 0 | 2 | PG | * |
| neg_66 | 0.006882 | 0.71503 | 0 | 0 | HFA/FALD | * |
| neg_67 | 0.007018 | 0.71503 | 0 | 0 | PL | * |
| neg_68 | 0.007587 | 0.71503 | 0 | 5 | FA & c | |
| neg_69 | 0.008517 | 0.71503 | 2 | 0 | FA & c | |
| neg_70 | 0.008849 | 0.71503 | 30 | 40 | OCT | |
| neg_71 | 0.008849 | 0.71503 | 44 | 22 | SPM | |
| neg_72 | 0.009192 | 0.71503 | 18 | 11 | FA & c | |
| neg_73 | 0.009369 | 0.71503 | 0 | 0 | FA & c | |
| neg_74 | 0.009548 | 0.71503 | 0 | 0 | PA/A/PO | |
| neg_75 | 0.009548 | 0.71503 | 22 | 29 | GLP | |
| neg_76 | 0.010104 | 0.71685 | 12 | 16 | DIAG | |

15 weeks gestational age dataset: Top features according to Fold Change Ranking

Figure S4: neg_18 at 15 weeks in cases and controls

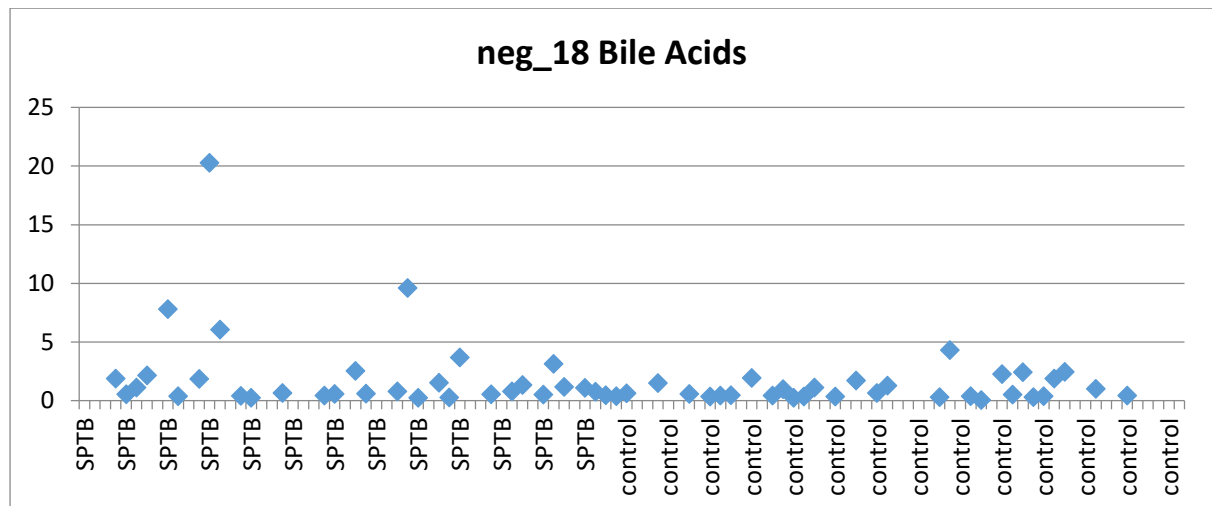


Figure S5: neg_19 at 15 weeks in cases and controls

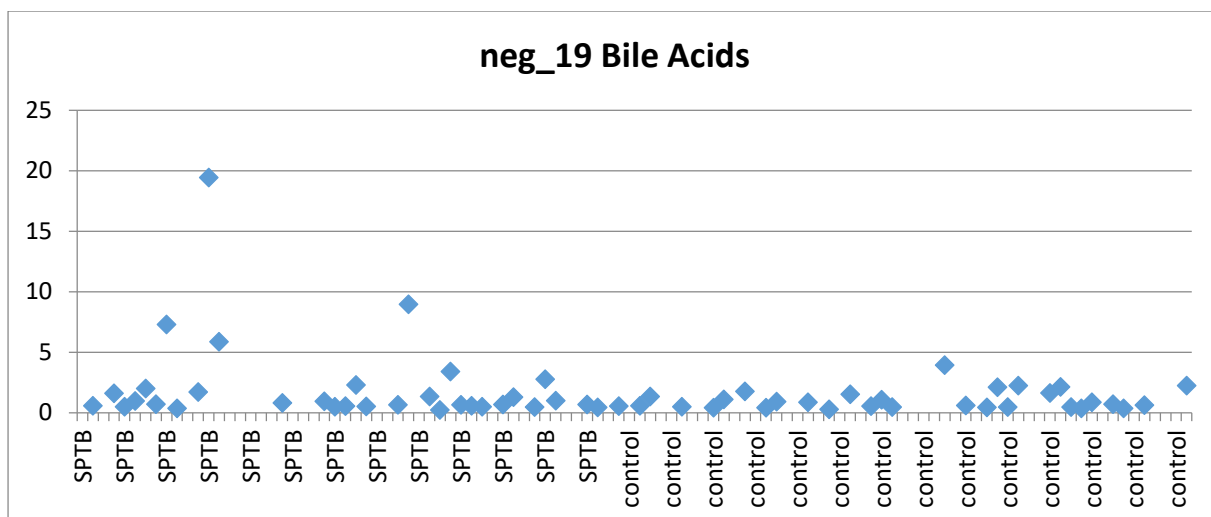


Figure S6: neg_20 at 15 weeks in cases and controls

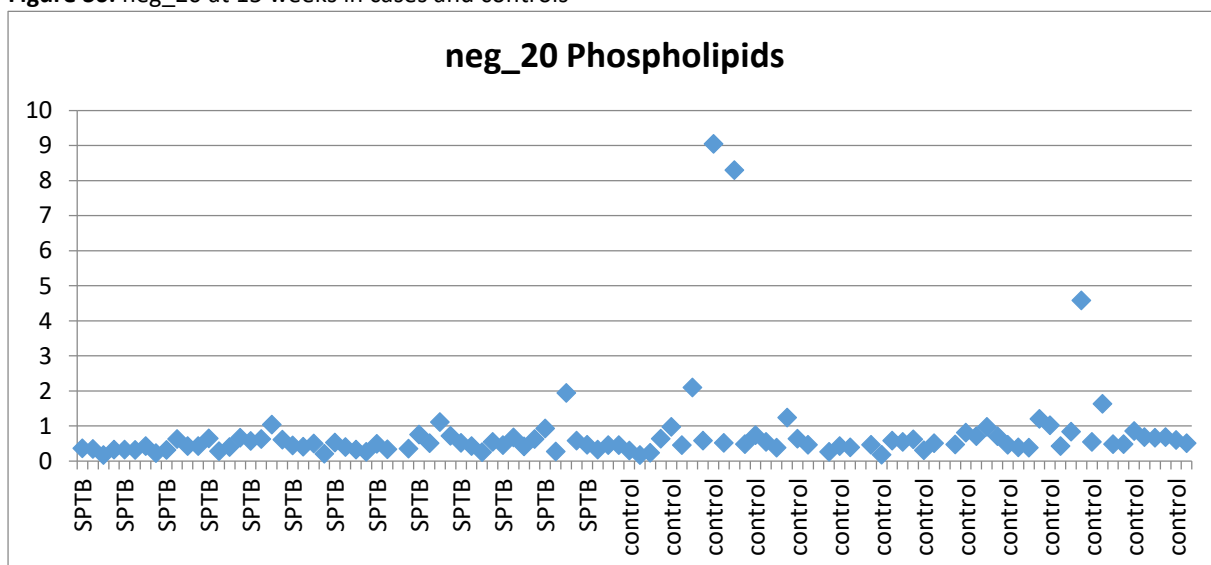


Figure S7: neg_21 at 15 weeks in cases and controls

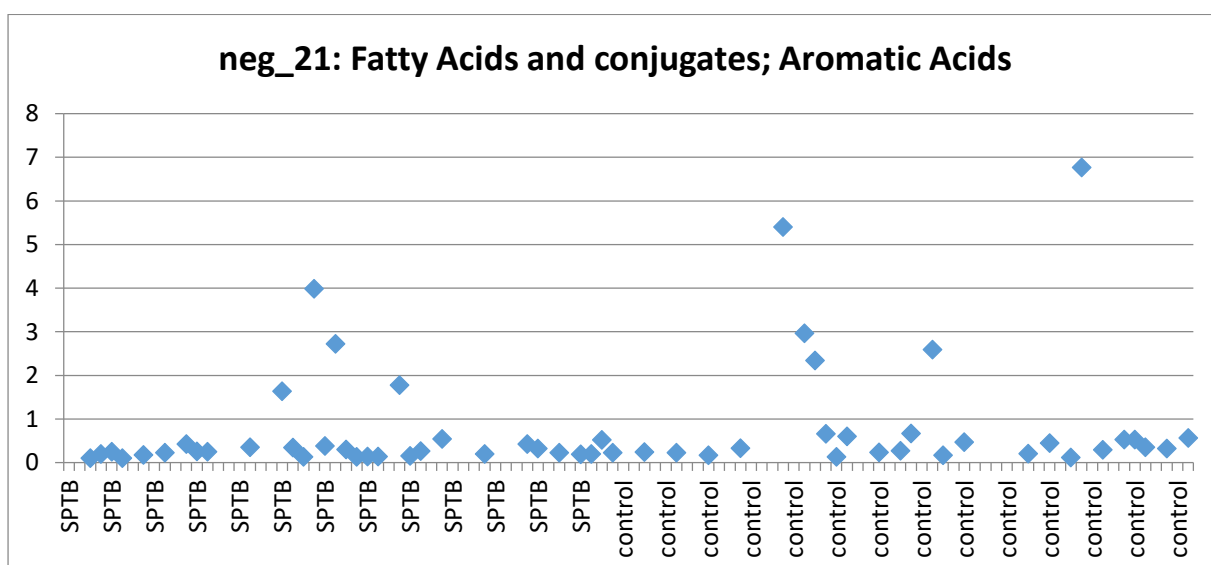


Figure S8: neg_22 at 15 weeks in cases and controls

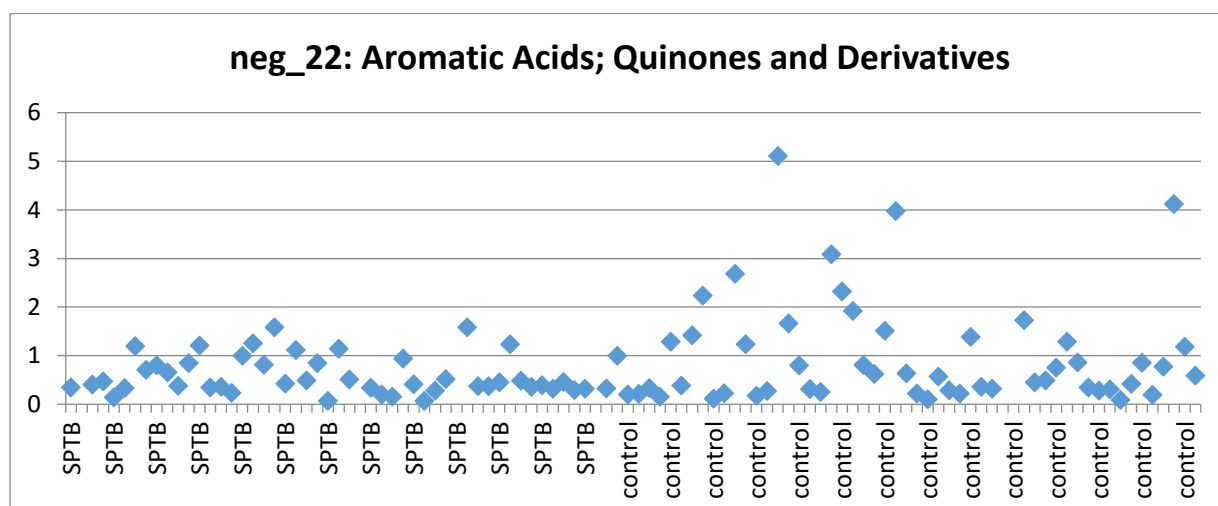


Figure S9: neg_23 at 15 weeks in cases and controls

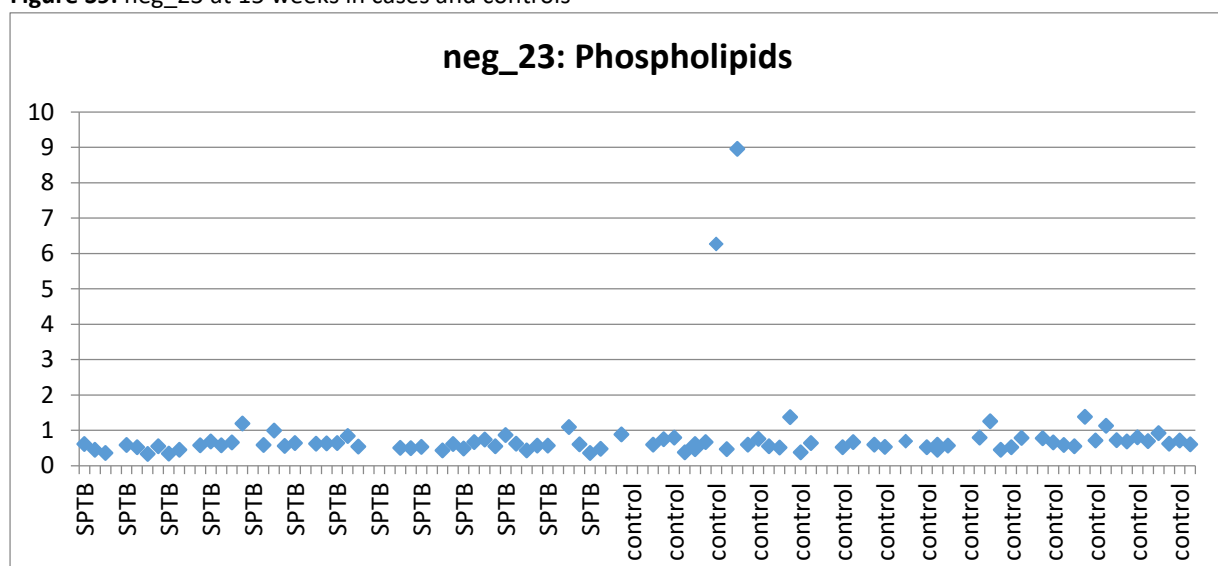


Figure S10: neg_24 at 15 weeks in cases and controls

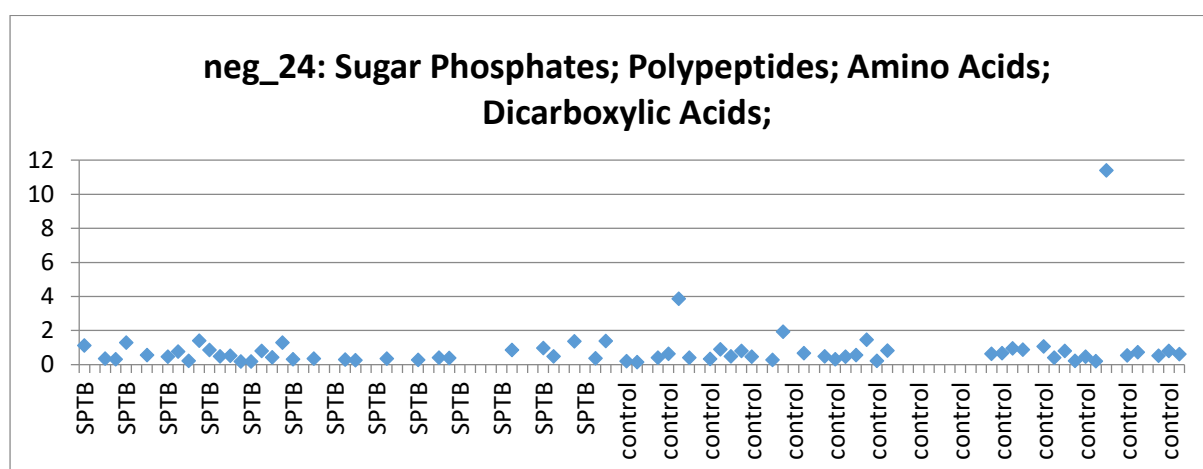


Figure S11: neg_25 at 15 weeks in cases and controls

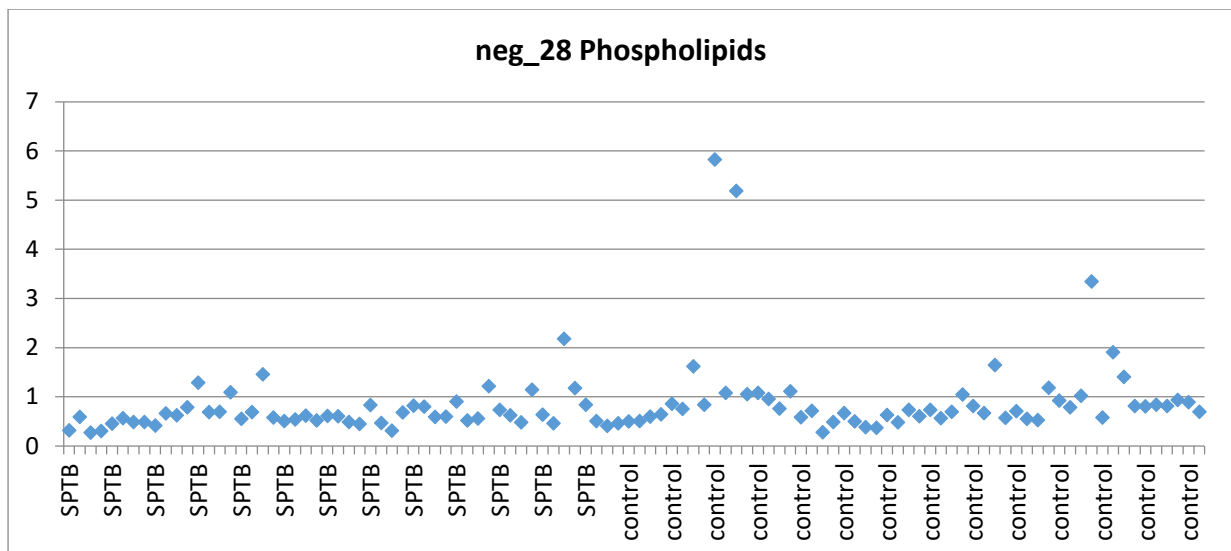


Figure S15: neg_29 at 15 weeks in cases and controls

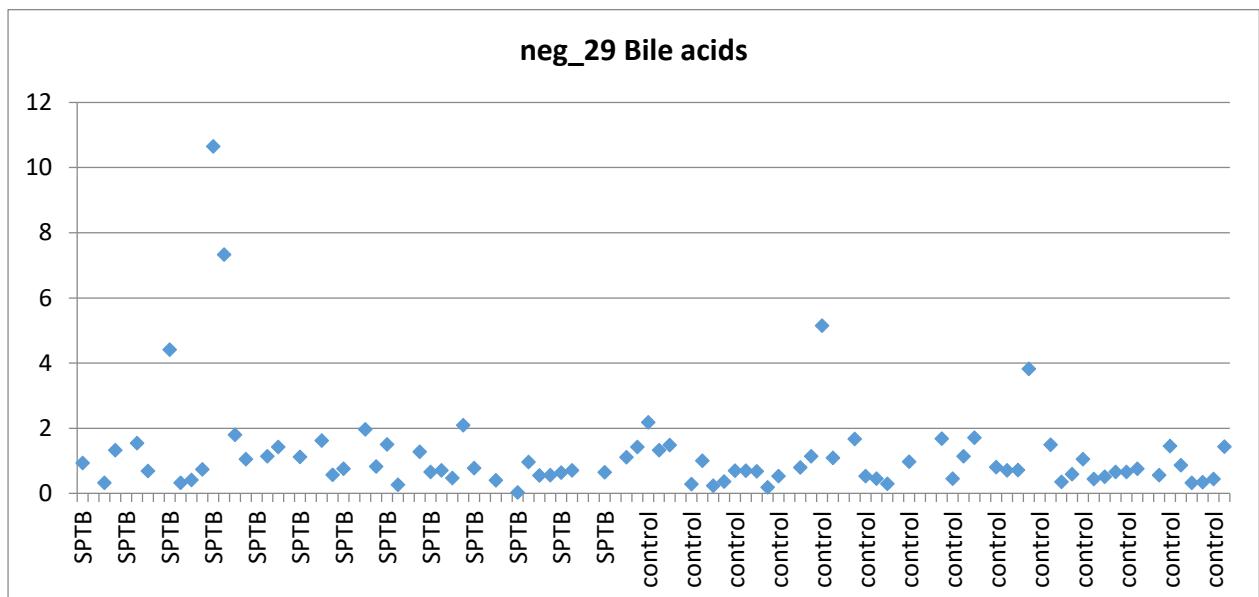


Figure S16: neg_30 at 15 weeks in cases and controls

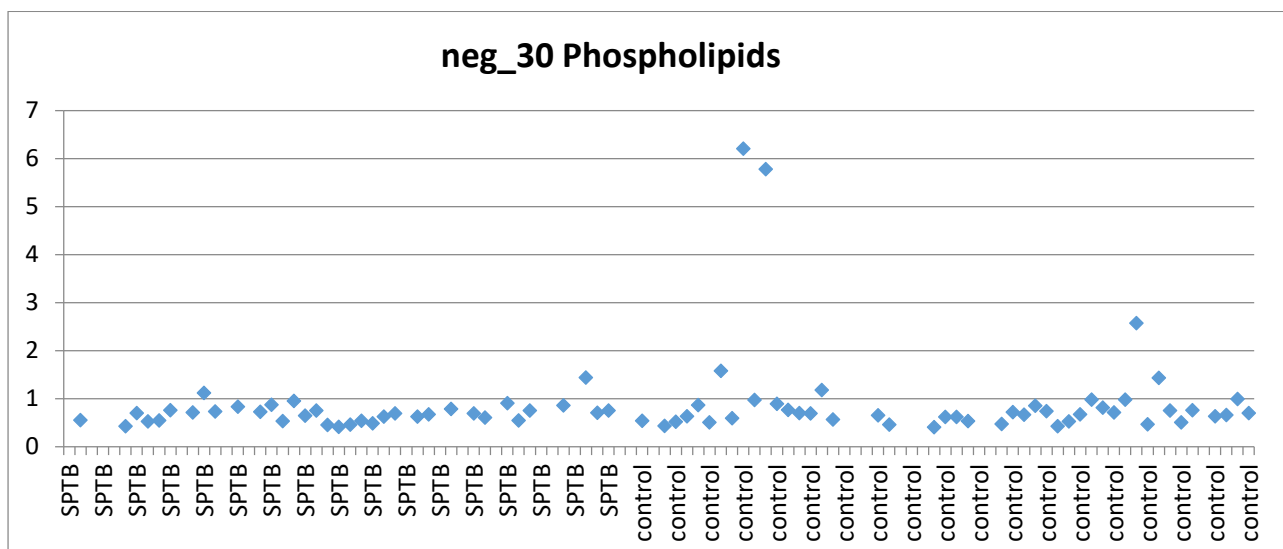


Figure S17: neg_31 at 15 weeks in cases and controls

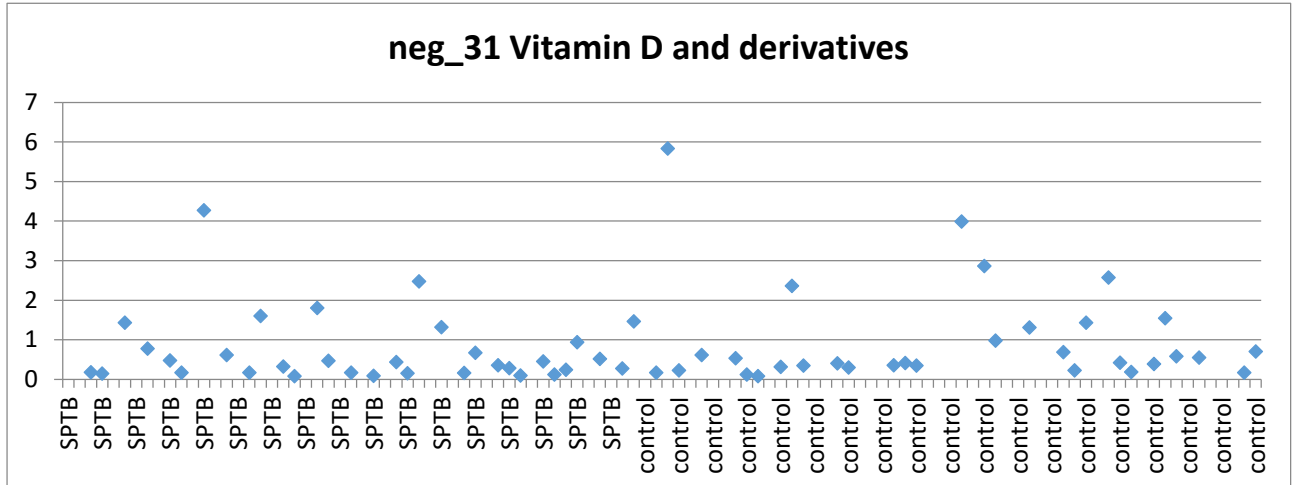


Figure S18: neg_32 at 15 weeks in cases and controls

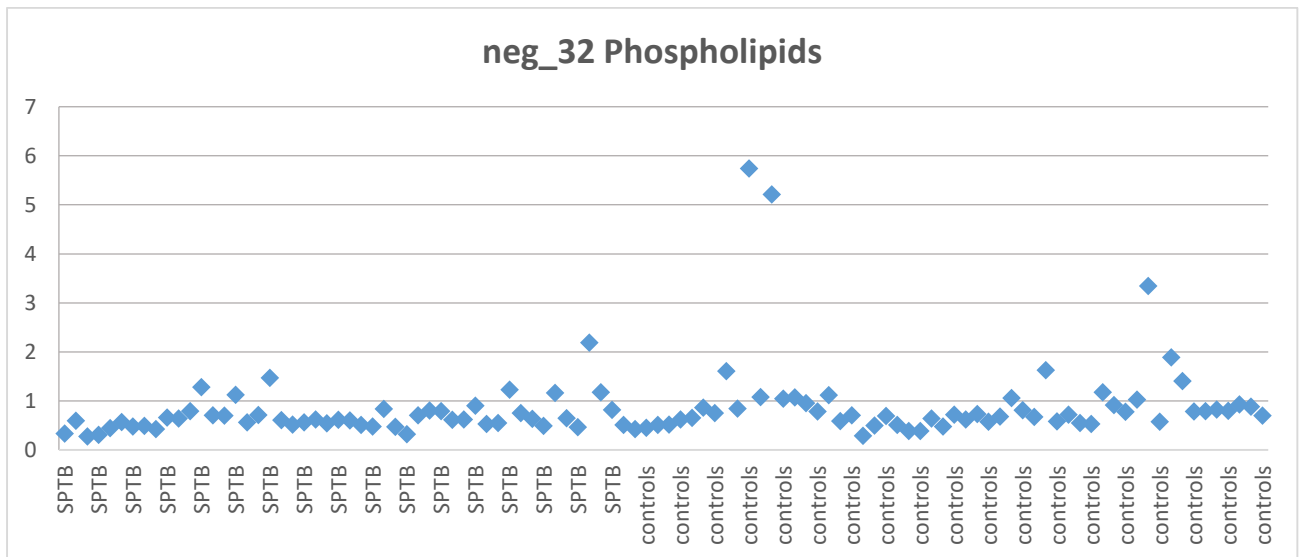


Figure S19: neg_33 at 15 weeks in cases and controls

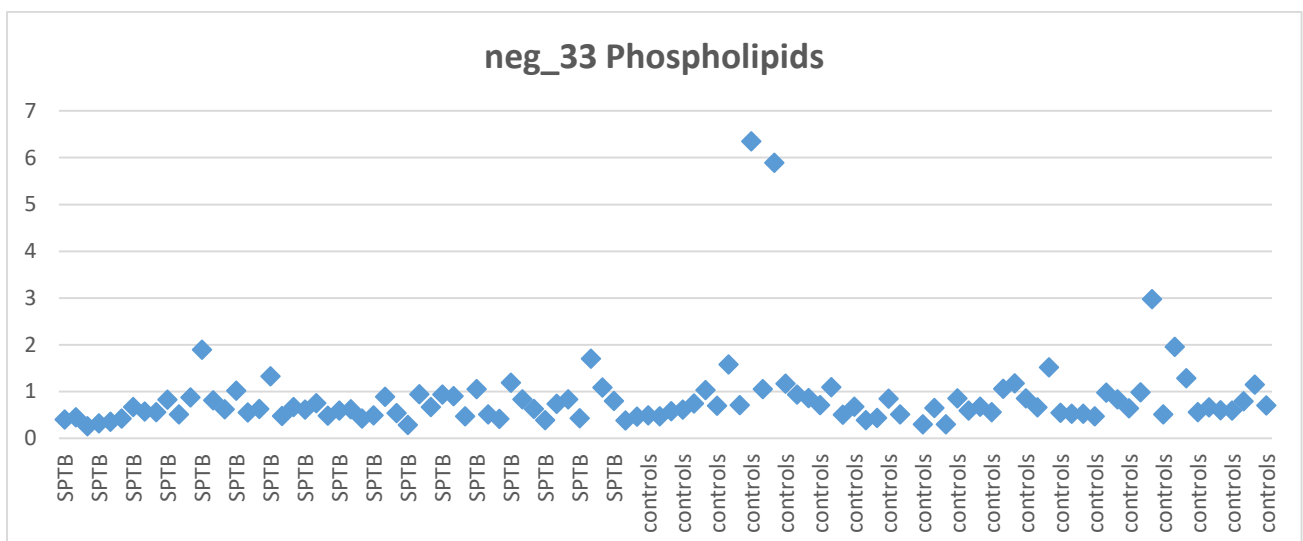


Figure S20: neg_34 at 15 weeks in cases and controls

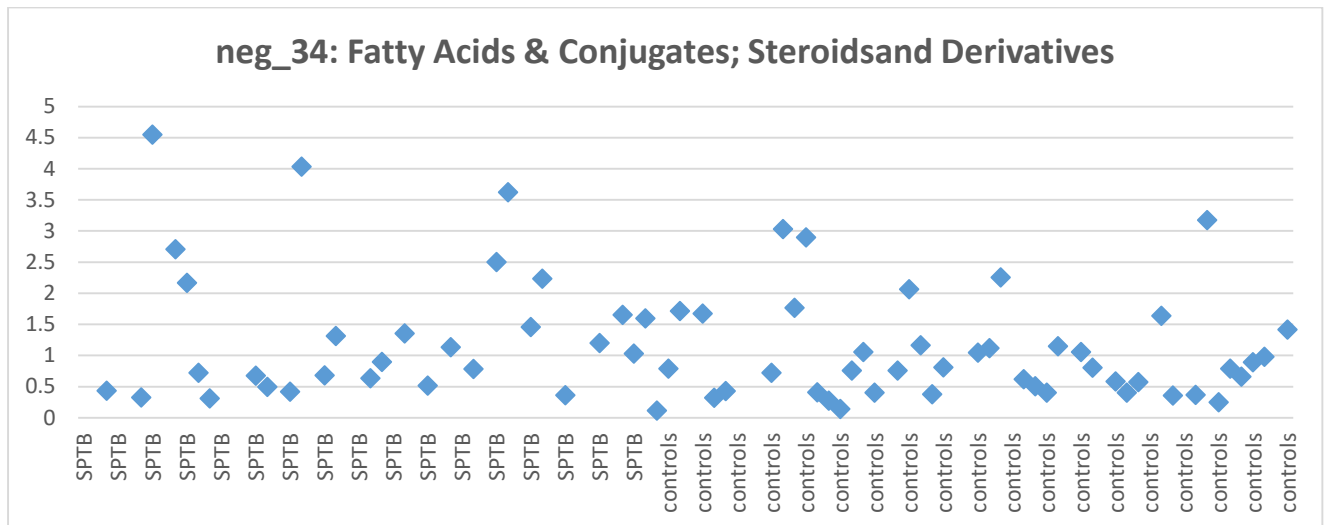


Figure S21: neg_35 at 15 weeks in cases and controls

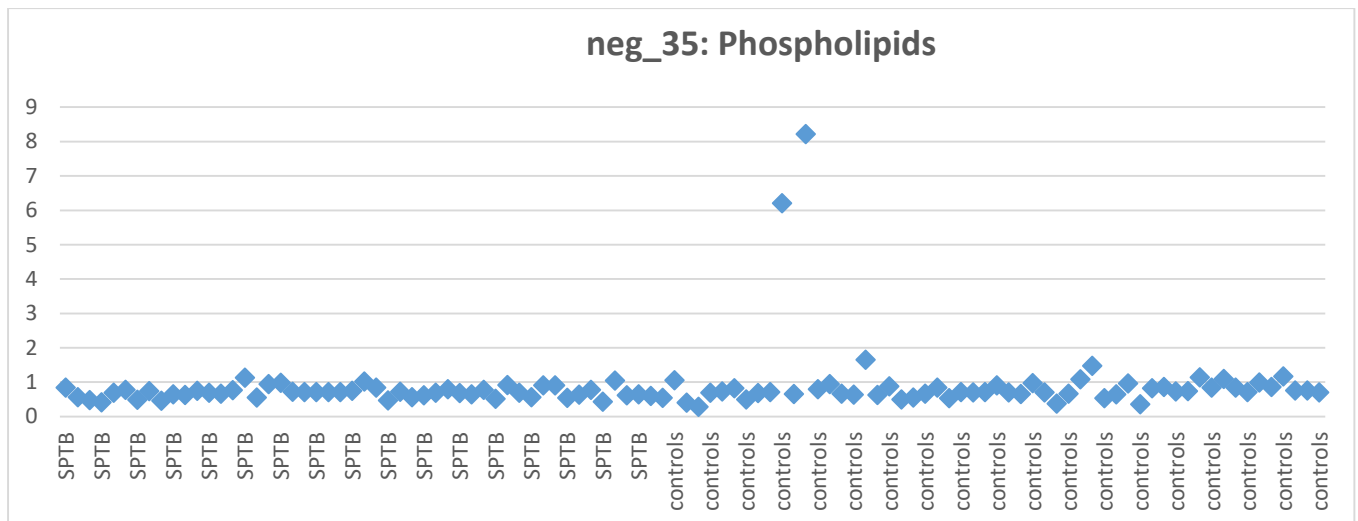


Figure S22: neg_36 at 15 weeks in cases and controls

20 weeks gestational age dataset: Top features according to both Fold Change Ranking and PLSDA and VIP ranking preceded by separate imputation of cases and controls

Figure S24: neg_1 at 20 weeks in cases and controls

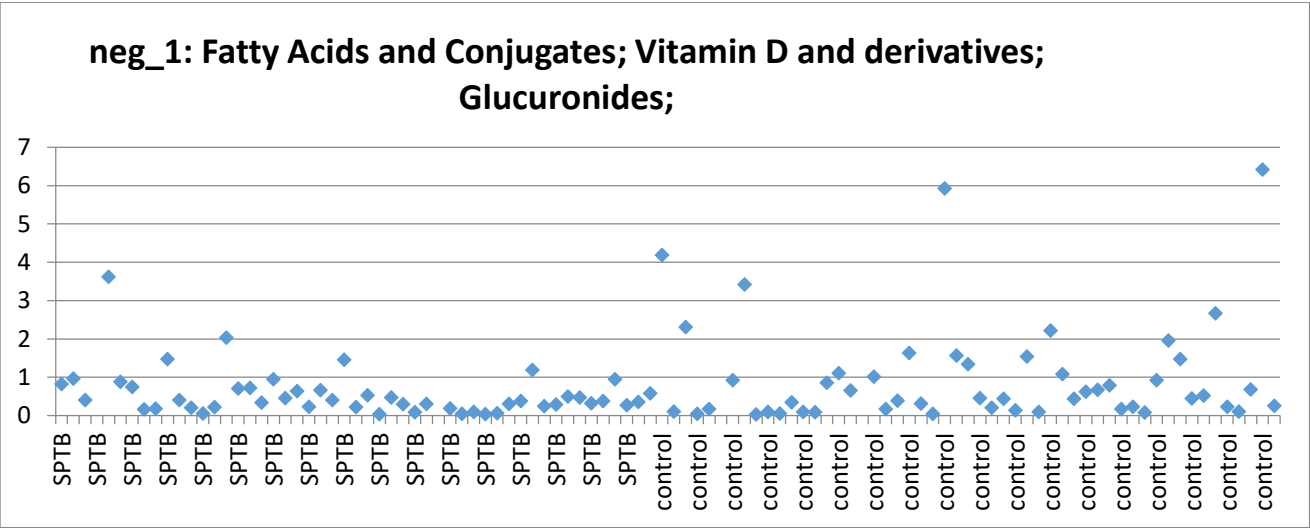


Figure S25: neg_2 at 20 weeks in cases and controls

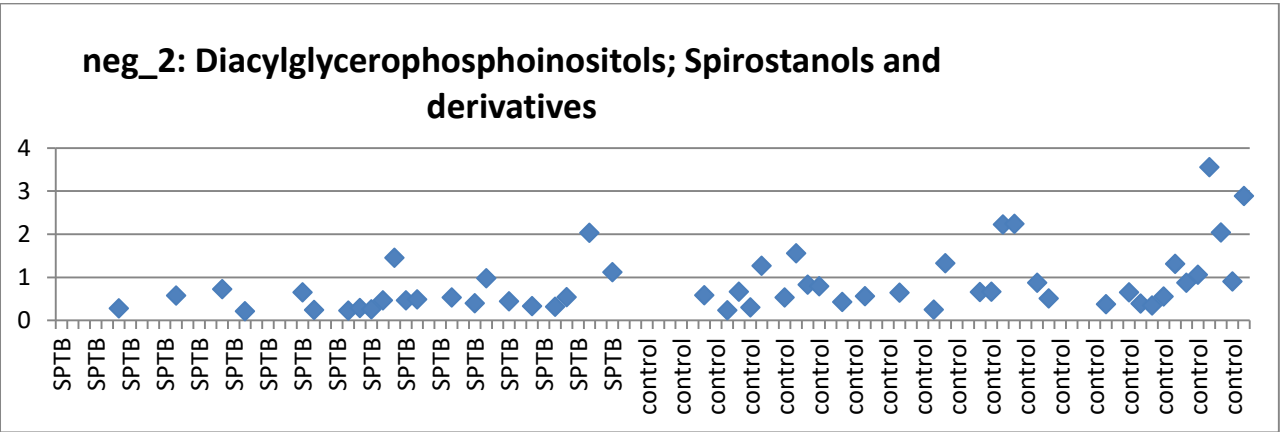
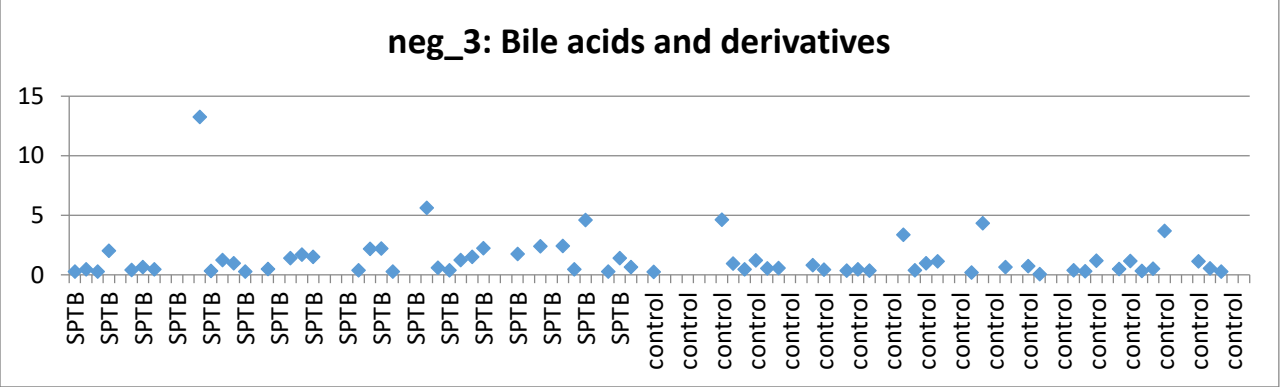


Figure S26: neg_3 at 20 weeks in cases and controls



[illegible]

neg_5: Bile acids and derivatives

| Sample | log2 fold change |
|---------|------------------|
| SPTB | 0.5 |
| SPTB | 2.0 |
| SPTB | 0.8 |
| SPTB | 0.6 |
| SPTB | 13.5 |
| SPTB | 1.5 |
| SPTB | 1.0 |
| SPTB | 0.5 |
| SPTB | 1.8 |
| SPTB | 1.5 |
| SPTB | 0.8 |
| SPTB | 2.2 |
| SPTB | 2.0 |
| SPTB | 0.5 |
| SPTB | 2.2 |
| SPTB | 2.0 |
| SPTB | 0.5 |
| SPTB | 5.5 |
| SPTB | 0.8 |
| SPTB | 1.0 |
| SPTB | 2.2 |
| SPTB | 0.8 |
| SPTB | 1.8 |
| SPTB | 2.5 |
| SPTB | 4.5 |
| SPTB | 0.5 |
| SPTB | 1.5 |
| control | 0.8 |
| control | 0.8 |
| control | 0.5 |
| control | 4.8 |
| control | 1.0 |
| control | 1.5 |
| control | 0.8 |
| control | 0.8 |
| control | 0.5 |
| control | 0.8 |
| control | 3.2 |
| control | 0.5 |
| control | 1.0 |
| control | 1.2 |
| control | 4.5 |
| control | 0.8 |
| control | 0.5 |
| control | 0.8 |
| control | 1.5 |
| control | 0.8 |
| control | 0.5 |
| control | 3.5 |
| control | 0.8 |
| control | 1.0 |
| control | 0.5 |

[illegible]

Figure S30: neg_7 at 20 weeks in cases and controls

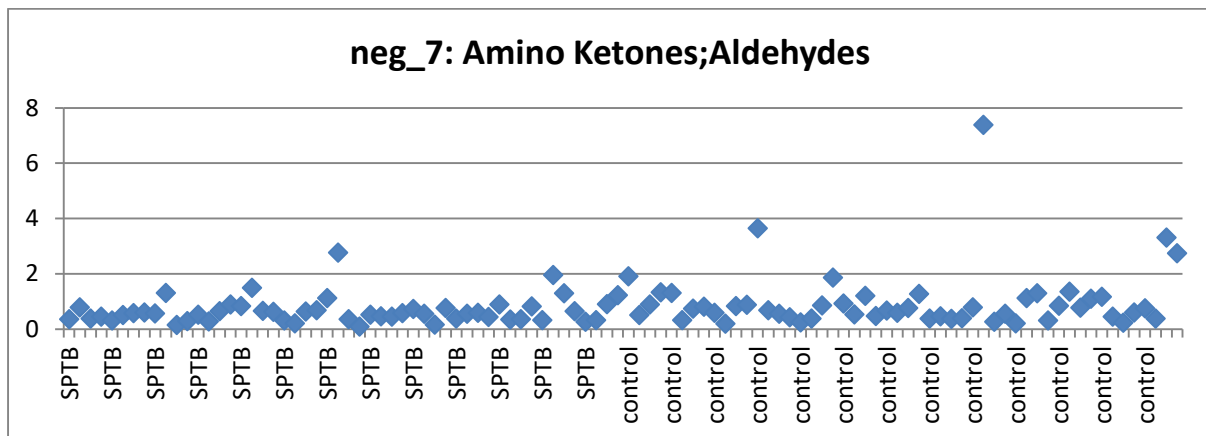


Figure S31: neg_8 at 20 weeks in cases and controls

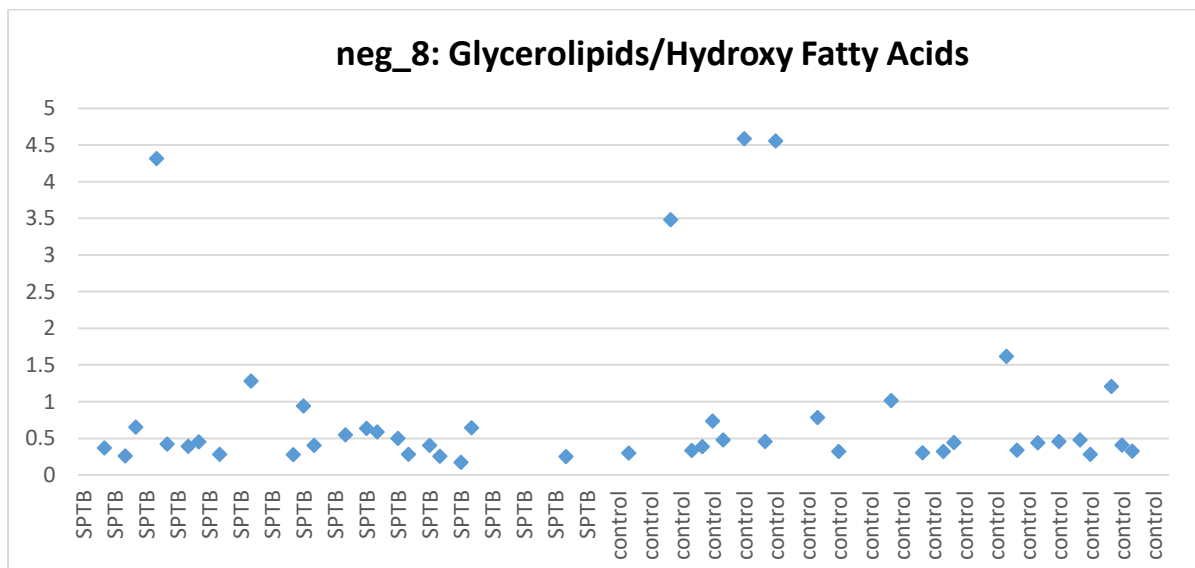
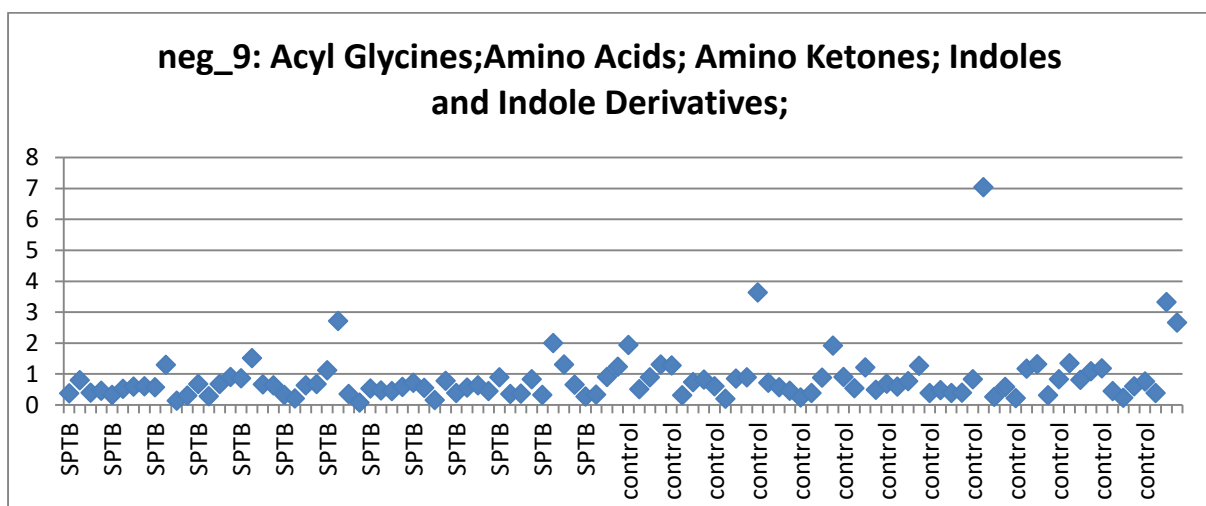


Figure S32: neg_9 at 20 weeks in cases and controls



neg_10: Fatty Acids and Conjugates

| Sample | log2 Fold Change (approx.) |
|---------|----------------------------|
| SPTB | 0.8 |
| SPTB | 0.6 |
| SPTB | 1.2 |
| SPTB | 0.7 |
| SPTB | 1.8 |
| SPTB | 0.8 |
| SPTB | 1.4 |
| SPTB | 0.8 |
| SPTB | 1.4 |
| SPTB | 2.9 |
| SPTB | 1.1 |
| SPTB | 0.9 |
| SPTB | 1.2 |
| SPTB | 0.8 |
| SPTB | 1.8 |
| SPTB | 1.4 |
| SPTB | 0.6 |
| SPTB | 9.0 |
| SPTB | 0.9 |
| SPTB | 1.8 |
| SPTB | 0.5 |
| SPTB | 1.3 |
| SPTB | 1.9 |
| SPTB | 0.4 |
| SPTB | 7.7 |
| SPTB | 0.4 |
| SPTB | 0.8 |
| SPTB | 0.6 |
| SPTB | 1.8 |
| SPTB | 3.8 |
| SPTB | 0.8 |
| SPTB | 1.4 |
| SPTB | 0.9 |
| SPTB | 1.4 |
| control | 0.4 |
| control | 0.8 |
| control | 0.9 |
| control | 0.6 |
| control | 0.8 |
| control | 0.5 |
| control | 0.4 |
| control | 0.6 |
| control | 1.3 |
| control | 1.8 |
| control | 0.5 |
| control | 1.0 |
| control | 1.0 |
| control | 1.0 |
| control | 1.2 |
| control | 0.8 |
| control | 1.0 |
| control | 0.7 |
| control | 1.1 |
| control | 0.6 |
| control | 1.2 |
| control | 0.8 |
| control | 1.1 |
| control | 0.4 |
| control | 1.5 |
| control | 1.0 |
| control | 6.5 |
| control | 0.8 |
| control | 1.9 |
| control | 0.6 |
| control | 0.8 |
| control | 0.5 |
| control | 2.4 |
| control | 0.4 |
| control | 0.8 |
| control | 0.5 |
| control | 0.9 |
| control | 0.8 |
| control | 0.7 |

neg_11: Acyl Glycines; Amino Acids; Amino Ketones

| Category | Metabolite | log2 fold change |
|----------|------------|------------------|
| SPTB | 1 | 0.4 |
| SPTB | 2 | 0.9 |
| SPTB | 3 | 0.3 |
| SPTB | 4 | 0.4 |
| SPTB | 5 | 0.5 |
| SPTB | 6 | 0.6 |
| SPTB | 7 | 0.6 |
| SPTB | 8 | 0.6 |
| SPTB | 9 | 1.2 |
| SPTB | 10 | 0.1 |
| SPTB | 11 | 0.2 |
| SPTB | 12 | 0.5 |
| SPTB | 13 | 0.3 |
| SPTB | 14 | 0.7 |
| SPTB | 15 | 1.0 |
| SPTB | 16 | 1.6 |
| SPTB | 17 | 0.8 |
| SPTB | 18 | 0.7 |
| SPTB | 19 | 0.2 |
| SPTB | 20 | 0.3 |
| SPTB | 21 | 0.7 |
| SPTB | 22 | 1.3 |
| SPTB | 23 | 0.3 |
| SPTB | 24 | 0.4 |
| SPTB | 25 | 0.8 |
| SPTB | 26 | 0.4 |
| SPTB | 27 | 0.7 |
| SPTB | 28 | 0.4 |
| SPTB | 29 | 0.8 |
| SPTB | 30 | 0.4 |
| SPTB | 31 | 0.3 |
| SPTB | 32 | 0.9 |
| SPTB | 33 | 0.4 |
| SPTB | 34 | 0.7 |
| SPTB | 35 | 0.4 |
| SPTB | 36 | 0.3 |
| SPTB | 37 | 0.9 |
| SPTB | 38 | 0.3 |
| SPTB | 39 | 0.7 |
| SPTB | 40 | 0.3 |
| SPTB | 41 | 0.9 |
| SPTB | 42 | 0.3 |
| SPTB | 43 | 0.7 |
| SPTB | 44 | 0.3 |
| SPTB | 45 | 0.9 |
| SPTB | 46 | 0.3 |
| SPTB | 47 | 0.7 |
| SPTB | 48 | 0.3 |
| SPTB | 49 | 0.9 |
| SPTB | 50 | 0.3 |
| SPTB | 51 | 0.7 |
| SPTB | 52 | 0.3 |
| SPTB | 53 | 0.9 |
| SPTB | 54 | 0.3 |
| SPTB | 55 | 0.7 |
| SPTB | 56 | 0.3 |
| SPTB | 57 | 0.9 |
| SPTB | 58 | 0.3 |
| SPTB | 59 | 0.7 |
| SPTB | 60 | 0.3 |
| SPTB | 61 | 0.9 |
| SPTB | 62 | 0.3 |
| SPTB | 63 | 0.7 |
| SPTB | 64 | 0.3 |
| SPTB | 65 | 0.9 |
| SPTB | 66 | 0.3 |
| SPTB | 67 | 0.7 |
| SPTB | 68 | 0.3 |
| SPTB | 69 | 0.9 |
| SPTB | 70 | 0.3 |
| SPTB | 71 | 0.7 |
| SPTB | 72 | 0.3 |
| SPTB | 73 | 0.9 |
| SPTB | 74 | 0.3 |
| SPTB | 75 | 0.7 |
| SPTB | 76 | 0.3 |
| SPTB | 77 | 0.9 |
| SPTB | 78 | 0.3 |
| SPTB | 79 | 0.7 |
| SPTB | 80 | 0.3 |
| SPTB | 81 | 0.9 |
| SPTB | 82 | 0.3 |
| SPTB | 83 | 0.7 |
| SPTB | 84 | 0.3 |
| SPTB | 85 | 0.9 |
| SPTB | 86 | 0.3 |
| SPTB | 87 | 0.7 |
| SPTB | 88 | 0.3 |
| SPTB | 89 | 0.9 |
| SPTB | 90 | 0.3 |
| SPTB | 91 | 0.7 |
| SPTB | 92 | 0.3 |
| SPTB | 93 | 0.9 |
| SPTB | 94 | 0.3 |
| SPTB | 95 | 0.7 |
| SPTB | 96 | 0.3 |
| SPTB | 97 | 0.9 |
| SPTB | 98 | 0.3 |
| SPTB | 99 | 0.7 |
| SPTB | 100 | 0.3 |
| SPTB | 101 | 0.9 |
| SPTB | 102 | 0.3 |
| SPTB | 103 | 0.7 |
| SPTB | 104 | 0.3 |
| SPTB | 105 | 0.9 |
| SPTB | 106 | 0.3 |
| SPTB | 107 | 0.7 |
| SPTB | 108 | 0.3 |
| SPTB | 109 | 0.9 |
| SPTB | 110 | 0.3 |
| SPTB | 111 | 0.7 |
| SPTB | 112 | 0.3 |
| SPTB | 113 | 0.9 |
| SPTB | 114 | 0.3 |
| SPTB | 115 | 0.7 |
| SPTB | 116 | 0.3 |
| SPTB | 117 | 0.9 |
| SPTB | 118 | 0.3 |
| SPTB | 119 | 0.7 |
| SPTB | 120 | 0.3 |
| SPTB | 121 | 0.9 |
| SPTB | 122 | 0.3 |
| SPTB | 123 | 0.7 |
| SPTB | 124 | 0.3 |
| SPTB | 125 | 0.9 |
| SPTB | 126 | 0.3 |
| SPTB | 127 | 0.7 |
| SPTB | 128 | 0.3 |
| SPTB | 129 | 0.9 |
| SPTB | 130 | 0.3 |
| SPTB | 131 | 0.7 |
| SPTB | 132 | 0.3 |
| SPTB | 133 | 0.9 |
| SPTB | 134 | 0.3 |
| SPTB | 135 | 0.7 |
| SPTB | 136 | 0.3 |
| SPTB | 137 | 0.9 |
| SPTB | 138 | 0.3 |
| SPTB | 139 | 0.7 |
| SPTB | 140 | 0.3 |
| SPTB | 141 | 0.9 |
| SPTB | 142 | 0.3 |
| SPTB | 143 | 0.7 |
| SPTB | 144 | 0.3 |
| SPT | | |

[illegible]

Figure S36: neg_13 at 20 weeks in cases and controls

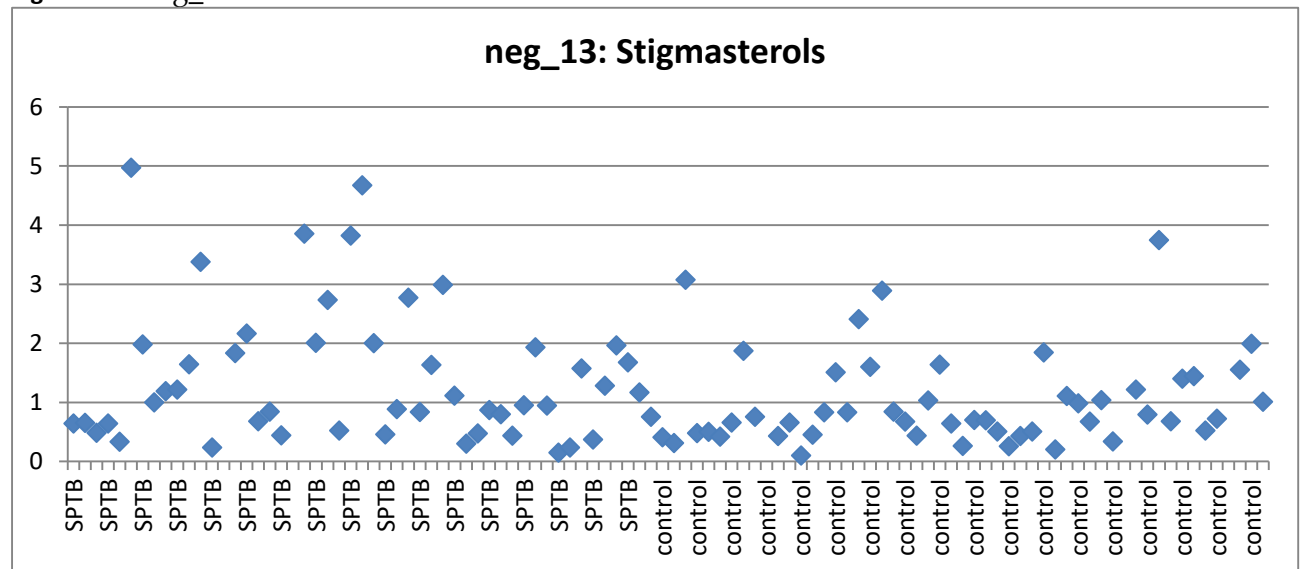


Figure S37: neg_14 at 20 weeks in cases and controls

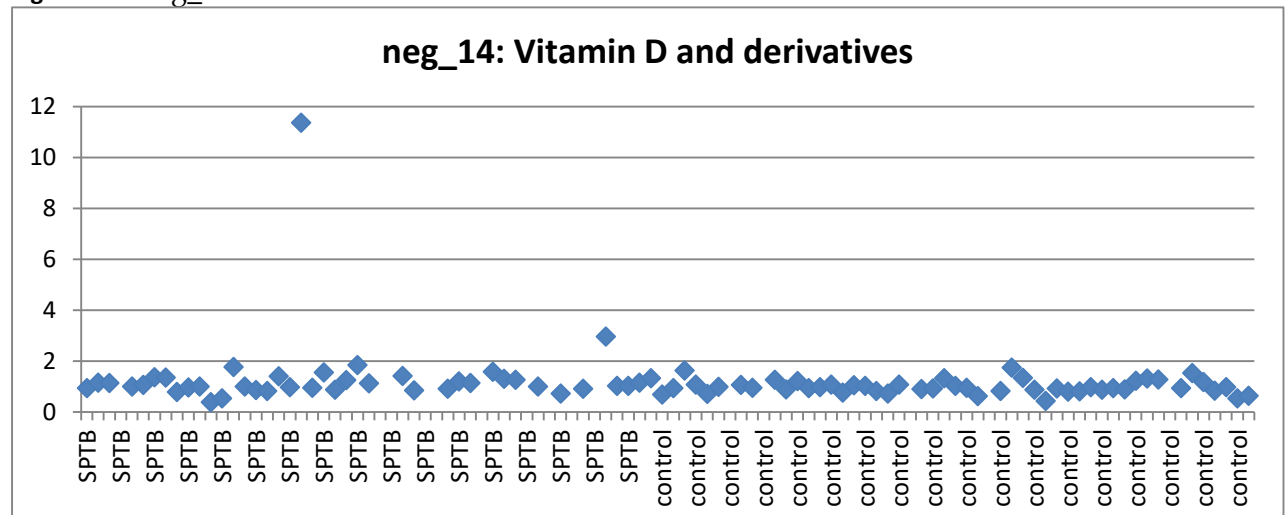


Figure S38: neg_15 at 20 weeks in cases and controls

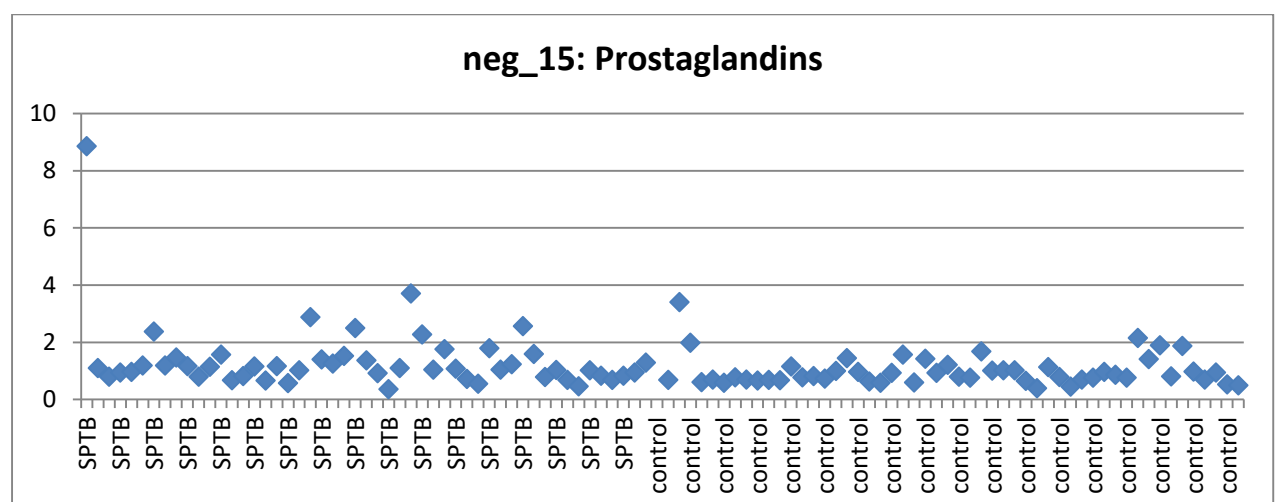


Figure S39: neg_16 at 20 weeks in cases and controls

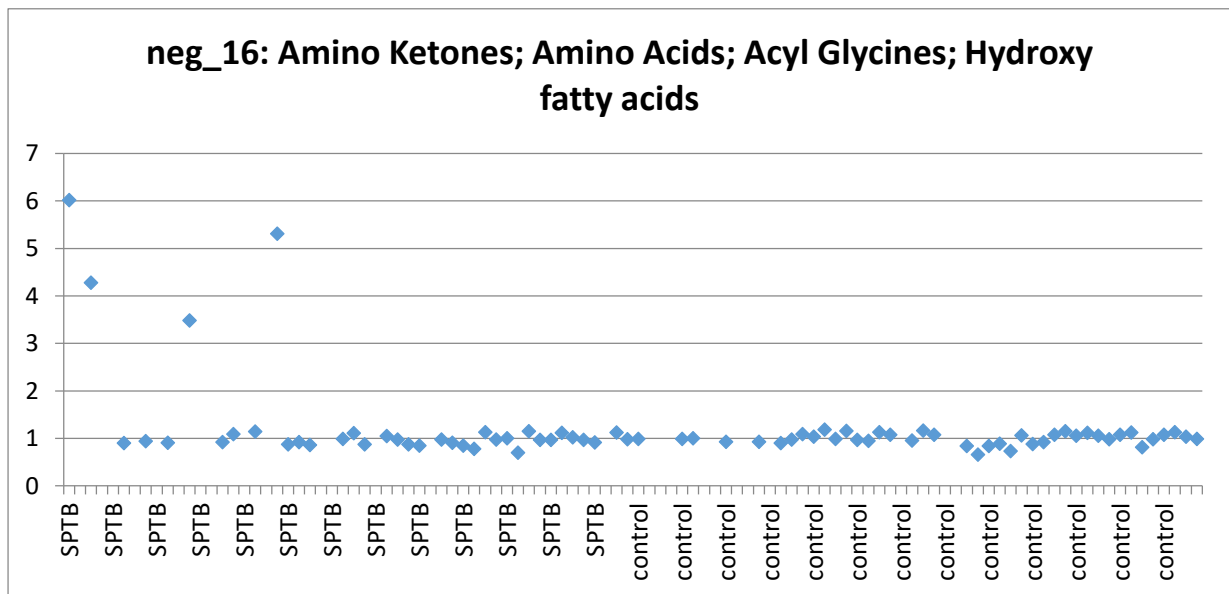


Figure S40: neg_17 at 20 weeks in cases and controls

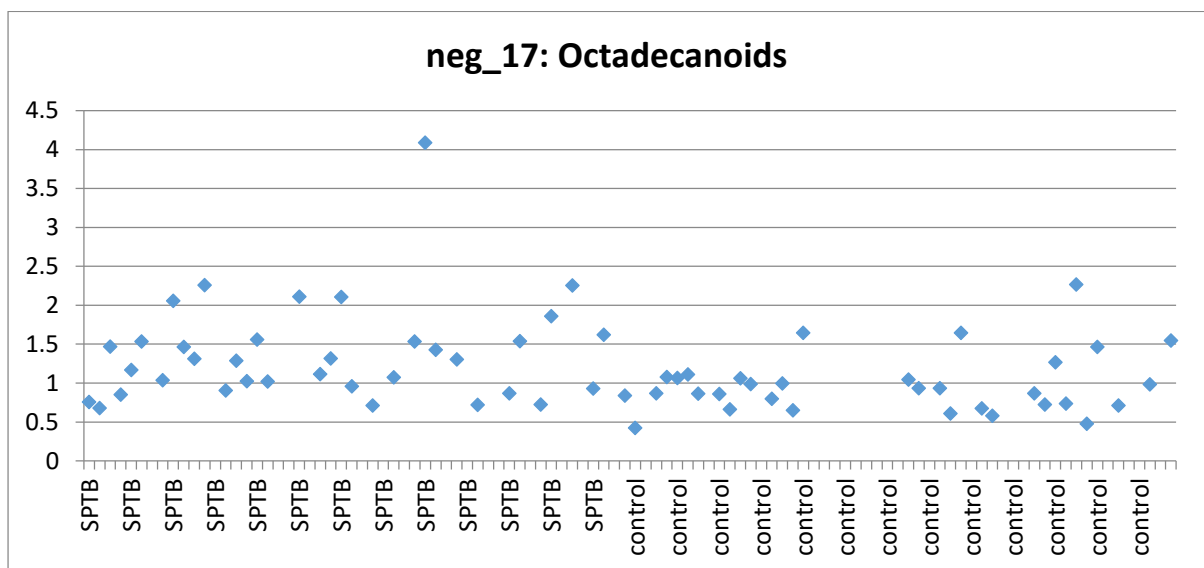


Table S4: Missing value % in 15 week dataset candidate lists and panels assessed by Panelomix

| 15 week dataset | Top 20 rank by FC | Top 20 rank MWU | Panel D | Panel E |
|-----------------|-------------------|-----------------|---------|---------|
| CASES | 24 | 9 | 3 | 2 |
| CONTROLS | 23 | 11 | 4 | 4 |
| OVERALL | 24 | 10 | 4 | 3 |

Table S5: Missing value % in 20 week dataset candidate lists and panels assessed by Panelomix

| 20 week dataset | Top 17 rank FC and PLSDA | Top 20 rank MWU | Panel A FC/PLSDA | Panel B FC/PLSDA | Panel C MWU |
|-----------------|--------------------------|-----------------|------------------|------------------|-------------|
| CASES | 19 | 11 | 6 | 12 | 0.3 |
| CONTROLS | 18 | 11 | 4 | 8 | 0.6 |
| OVERALL | 18 | 11 | 5 | 10 | 0.5 |

Abbreviations:

AA: Amino Acids;
 AG: Acyl Glycines;
 AK: Amino Ketones;
 ALC: Alcohols;
 ALD: Aldehydes;
 ARA: Aromatic Acids;
 BA: Bile Acid;
 CAT & D: Catecholamines and Derivatives;
 C21-STR: C-21 Steroids
 DA: Dicarboxylic Acids;
 DIAG: Diacylglycerophosphoinositols;
 FA: Fatty Acid;
 FALD: Fatty Aldehydes;
 GLU: Gluconorides;
 GLP: Glycerolipids;
 HA: Hydroxy Acids;
 HFA: Hydroxy Fatty Acids;
 IND: Indoles;
 ISO: Isoprenoids
 MOAG: Monoacylglycerophosphoglycerols
 NA: Nucleic Acids;
 N_ACLA: N_Acyl Amines;
 N_GLSP: Neutral glycosphingolipids;
 NUC: Nucleotides;
 OCT: Octadecanoids;
 PA: Polyamines;
 PG: Prostaglandin;
 PL: Phospholipids;
 PO: Polyols
 PP: Polypeptides;
 PTR: Pterins;
 PUR: Purines;
 SP: Sugar Phosphates;
 STG: Stigmasterols;

STR: Steroids;
 SPM: Sphingomyelins;
 VITD: Vitamin D and derivatives;

Table S6. Logistic Regression performed in Panelomix for the panels of biomarkers

| Panel | Dataset (GA in weeks) | Selection method | % missing values | # features in final panel | pAUC | % Specificity (95%CI) | |
|---------|--------------------------|------------------|------------------|---------------------------|-------------------|--------------------------|---------------------|
| Panel A | 20 | FC | 4 | 5 | 9.2 (4.0-15.1) | 70.9 (52.7-78.2) | 79.6 (67.3-89.8) |
| Panel B | 20 | MWU | 0.5 | 5 | 10.9 (6.9–14.9) | 54.5 (41.8–67.3) | 85.7 (75.5–93.9) |
| Panel C | 15 | FC and VIP | 4 | 4 | 8.1 (4.9–11.9) | 41.1 (28.6–53.6) | 85.7 (75.5–93.9) |
| Panel D | 15 | MWU | 3 | 4 | 8.9 (5.1–13.7) | 62.5 (50.0–75.0) | 75.5 (63.3–87.8) |