WAT metabolomics overview

- 1286 features in total
- 933 unique features, 353 redundant features, within which 144 features are unique* (including 6 internal standard), were measured with more than one assays (*explained further in next step)
- Metabolomics assays: from 6 sites, 12 platforms

Site	platforms
broad_met	u-hilicpos
Duke	t-ka, t-nuc, t-acoa
Emory	t-etamidpos [one feature: Linoleoyl-EA]
Gtech	u-lrppos, u-lrpneg
Mayo	t-amines, t-tca
umichigan	u-rpneg, u-rppos, u-ionpneg

Note: There are a total of 13 platforms, but t-oxylipneg (Emory) did not contain redundant features, so it is not listed in the above table.

Finding duplicates (#=353/unique#144)

- The following steps were performed using the MAWG merged DEA table [pass1b-06_t70-white-adipose_metab-meta-reg_training-dea-fdr_20211018.txt] to identify redundant features:
- (1) redundant features identified by BIC before meta-regression (dataset == “meta-reg”)
- (2) then, features with the same feature_ID and/or metabolite_refmet were also determined as redundant
- (3) During manual curation and inspection later on, identified three more duplicates that were missed in the first two rounds:
  - CoA (2:0)/acetyl-CoA from duke targeted assay (t-acoa)
  - linoleoyl-EA from Emory targeted assay (etamidpos)
  - 5'-methylthioadenosine (feature ID)/ 5'-Methylthioadenosine (metabolite_refmet) from duke targeted assay (t-nuc)
  - (these were all selected over untargeted counterparts)

De-duplication strategy

- The 933 unique features were kept as is. The goal/focus of this work is to reduce the 353 redundant features to 138 unique features (144 minus the 6 internal standards)
- All 353 features were plotted and inspected. I used the following criteria for removing duplicates:
- (1) Picked targeted assay over untargeted assay (also removed internal standards from Gtech).
- This left redundant features that were repeatedly measured by more than one untargeted assays:
- (2) Lipids that were duplicated in rppos (Umich) and hilicpos (Broad) assays, picked rppos (C18 silica column) over hilic
- (3 & 4) Then, for lipid duplicates within one site (Gtech or Umich) from positive and negative modes:
- (3) Lipid classes PC, PE, LPC, LPE, SM (lipids with positively charged head group): pick positive mode over negative mode
- (4) Lipid classes PI, PS, PA, FFA (lipids with negatively charged head group): pick negative mode over positive mode
- Then, the rest of the redundant features were visually inspected and selection was made case-by-case by discussion within WAT working group (mostly within Emory and Gtech). Features with measurements from multiple sites agreeing with one another (by visual inspection) were put in group (5), features with measurements from sites disagreeing were put in group (6).

Rest of the slides show feature names in the format of:
An index:: metabolite_refmet:: dataset

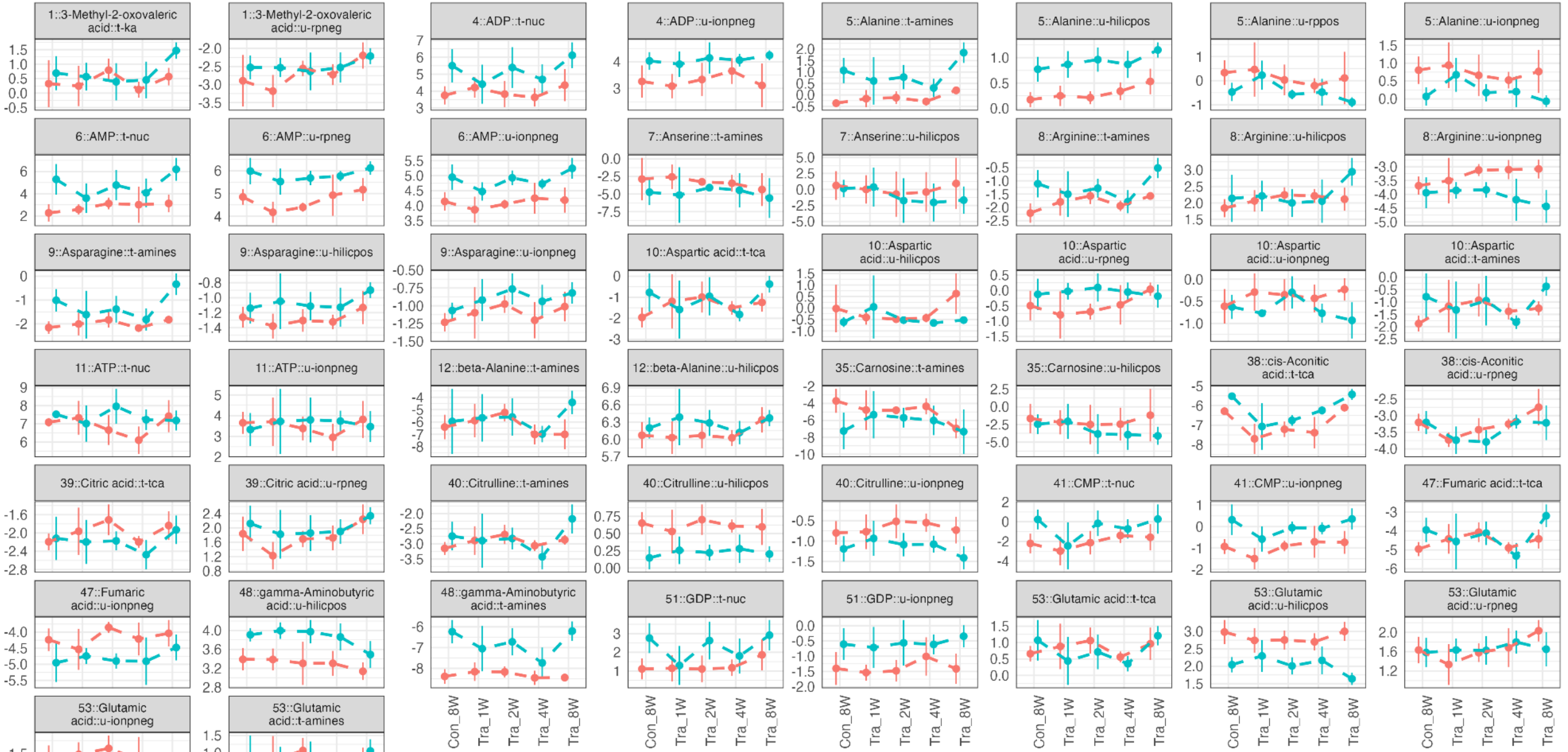
- Index = an ID number given to each of the uniques features
- Metabolite_refmet = ‘standardized’ name given to metabolites by BIC
- Dataset = targeted/untargeted + assay name

Values in the plots were normalized values (from MAWG data freeze); color code: Female=red; Male=green
Note that some metabolites are measured in >2 platforms

1. Choose targeted over untargeted

(# of features: 158 -> 55)

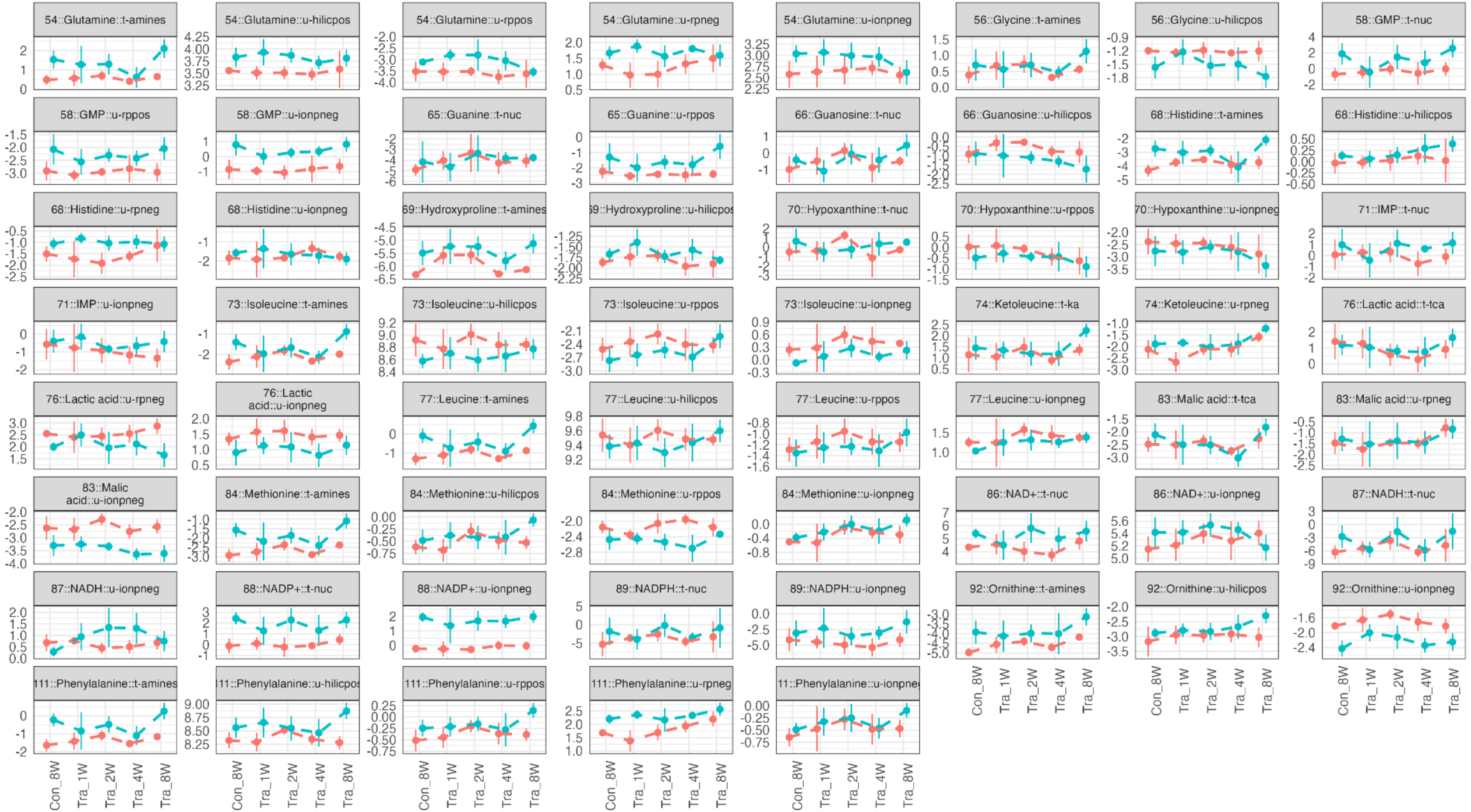
Also removed GT internal standard ((# of features: 12 -> 0)

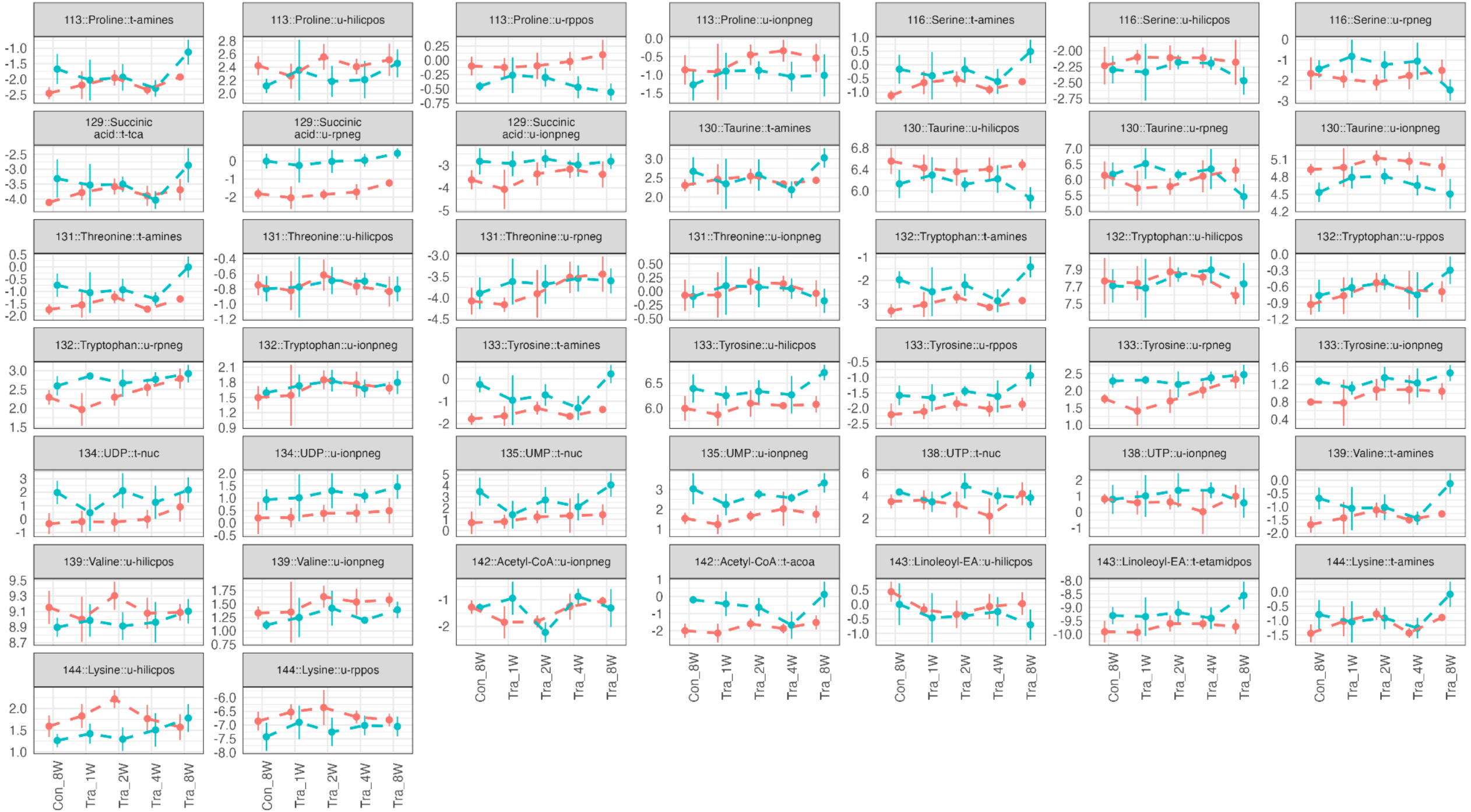


Values in the plots were normalized values (from MAWG data freeze); color code: Female=red; Male=green

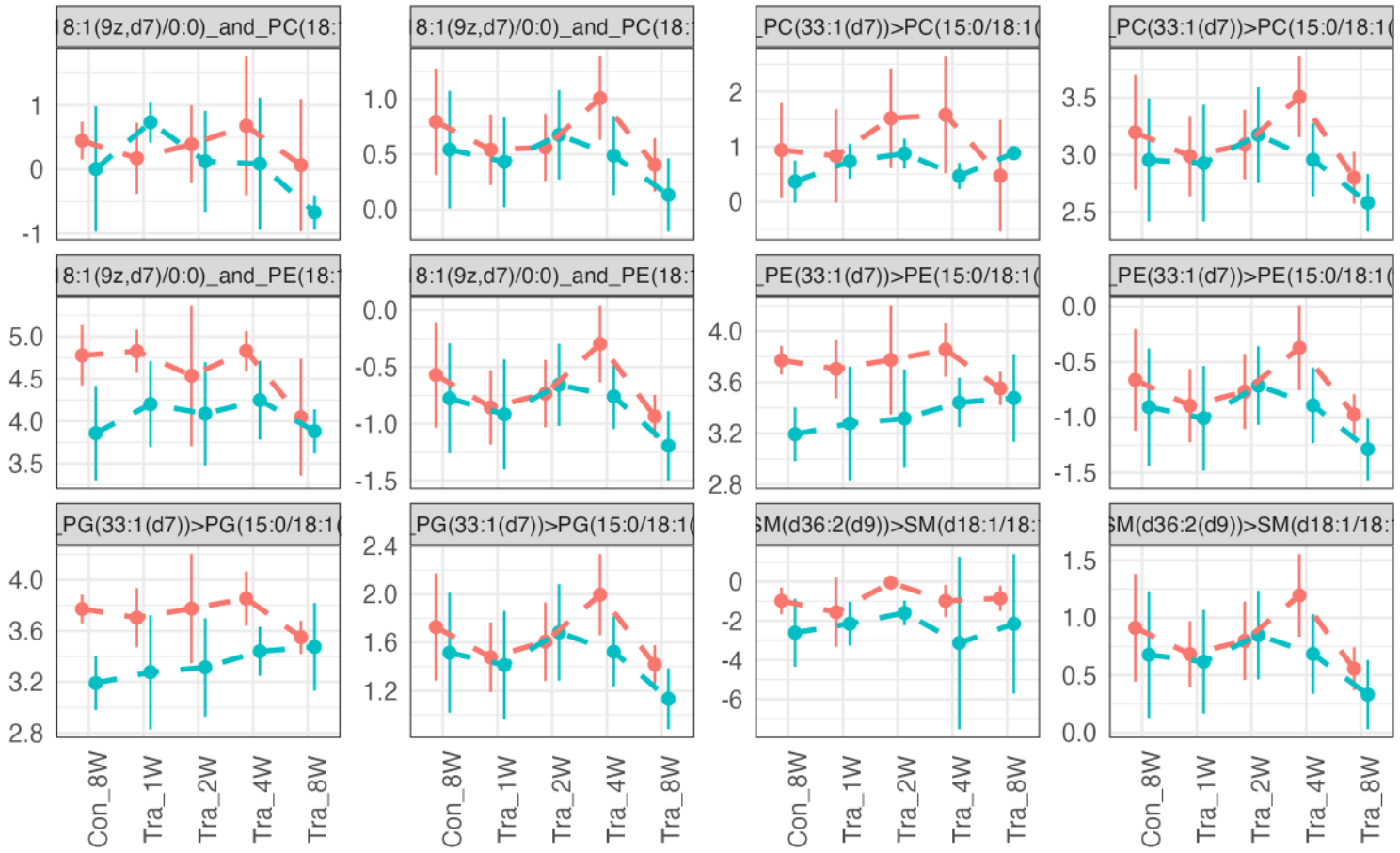
10 – the two from targeted assay look similar. Picked t-amines assay (The ionic form of aspartic acid is known as aspartate)

53 – the two from targeted assay look similar. Picked t-amines assay (ionic form is known as glutamate)


54::Glutamine::t-amines
54::Glutamine::u-hilicpos
54::Glutamine::u-rppos
54::Glutamine::u-rpneg
54::Glutamine::u-ionpneg
56::Glycine::t-amines
56::Glycine::u-hilicpos
58::GMP::t-nuc
58::GMP::u-rppos
58::GMP::u-ionpneg
65::Guanine::t-nuc
65::Guanine::u-rppos
66::Guanosine::t-nuc
66::Guanosine::u-hilicpos
68::Histidine::t-amines
68::Histidine::u-hilicpos
68::Histidine::u-rpneg
68::Histidine::u-ionpneg
69::Hydroxyproline::t-amines
69::Hydroxyproline::u-hilicpos
70::Hypoxanthine::t-nuc
70::Hypoxanthine::u-rppos
70::Hypoxanthine::u-ionpneg
71::IMP::t-nuc
71::IMP::u-ionpneg
73::Isoleucine::t-amines
73::Isoleucine::u-hilicpos
73::Isoleucine::u-rppos
73::Isoleucine::u-ionpneg
74::Ketoleucine::t-ka
74::Ketoleucine::u-rpneg
76::Lactic acid::t-tca
76::Lactic acid::u-rpneg
76::Lactic acid::u-ionpneg
77::Leucine::t-amines
77::Leucine::u-hilicpos
77::Leucine::u-rppos
77::Leucine::u-ionpneg
83::Malic acid::t-tca
83::Malic acid::u-rpneg
83::Malic acid::u-ionpneg
84::Methionine::t-amines
84::Methionine::u-hilicpos
84::Methionine::u-rppos
84::Methionine::u-ionpneg
86::NAD+::t-nuc
86::NAD+::u-ionpneg
87::NADH::t-nuc
87::NADH::u-ionpneg
88::NADP+::t-nuc
88::NADP+::u-ionpneg
89::NADPH::t-nuc
89::NADPH::u-ionpneg
92::Ornithine::t-amines
92::Ornithine::u-hilicpos
92::Ornithine::u-ionpneg
111::Phenylalanine::t-amines
111::Phenylalanine::u-hilicpos
111::Phenylalanine::u-rppos
111::Phenylalanine::u-rpneg
11::Phenylalanine::u-ionpneg
Con_8W
Tra_1W
Tra_2W
Tra_4W
Tra_8W


113::Proline::t-amines
113::Proline::u-hilicpos
113::Proline::u-rppos
113::Proline::u-ionpneg
116::Serine::t-amines
116::Serine::u-hilicpos
116::Serine::u-rpneg
129::Succinic acid::t-tca
129::Succinic acid::u-rpneg
129::Succinic acid::u-ionpneg
130::Taurine::t-amines
130::Taurine::u-hilicpos
130::Taurine::u-rpneg
130::Taurine::u-ionpneg
131::Threonine::t-amines
131::Threonine::u-hilicpos
131::Threonine::u-rpneg
131::Threonine::u-ionpneg
132::Tryptophan::t-amines
132::Tryptophan::u-hilicpos
132::Tryptophan::u-rppos
132::Tryptophan::u-rpneg
132::Tryptophan::u-ionpneg
133::Tyrosine::t-amines
133::Tyrosine::u-hilicpos
133::Tyrosine::u-rppos
133::Tyrosine::u-rpneg
133::Tyrosine::u-ionpneg
134::UDP::t-nuc
134::UDP::u-ionpneg
135::UMP::t-nuc
135::UMP::u-ionpneg
138::UTP::t-nuc
138::UTP::u-ionpneg
139::Valine::t-amines
139::Valine::u-hilicpos
139::Valine::u-ionpneg
142::Acetyl-CoA::u-ionpneg
142::Acetyl-CoA::t-acoa
143::Linoleoyl-EA::u-hilicpos
143::Linoleoyl-EA::t-etamidpos
144::Lysine::t-amines
144::Lysine::u-hilicpos
144::Lysine::u-rppos
Con_8W
Tra_1W
Tra_2W
Tra_4W
Tra_8W

Removed Gtech internal standard (each measured twice: lrppos, lrpneg)

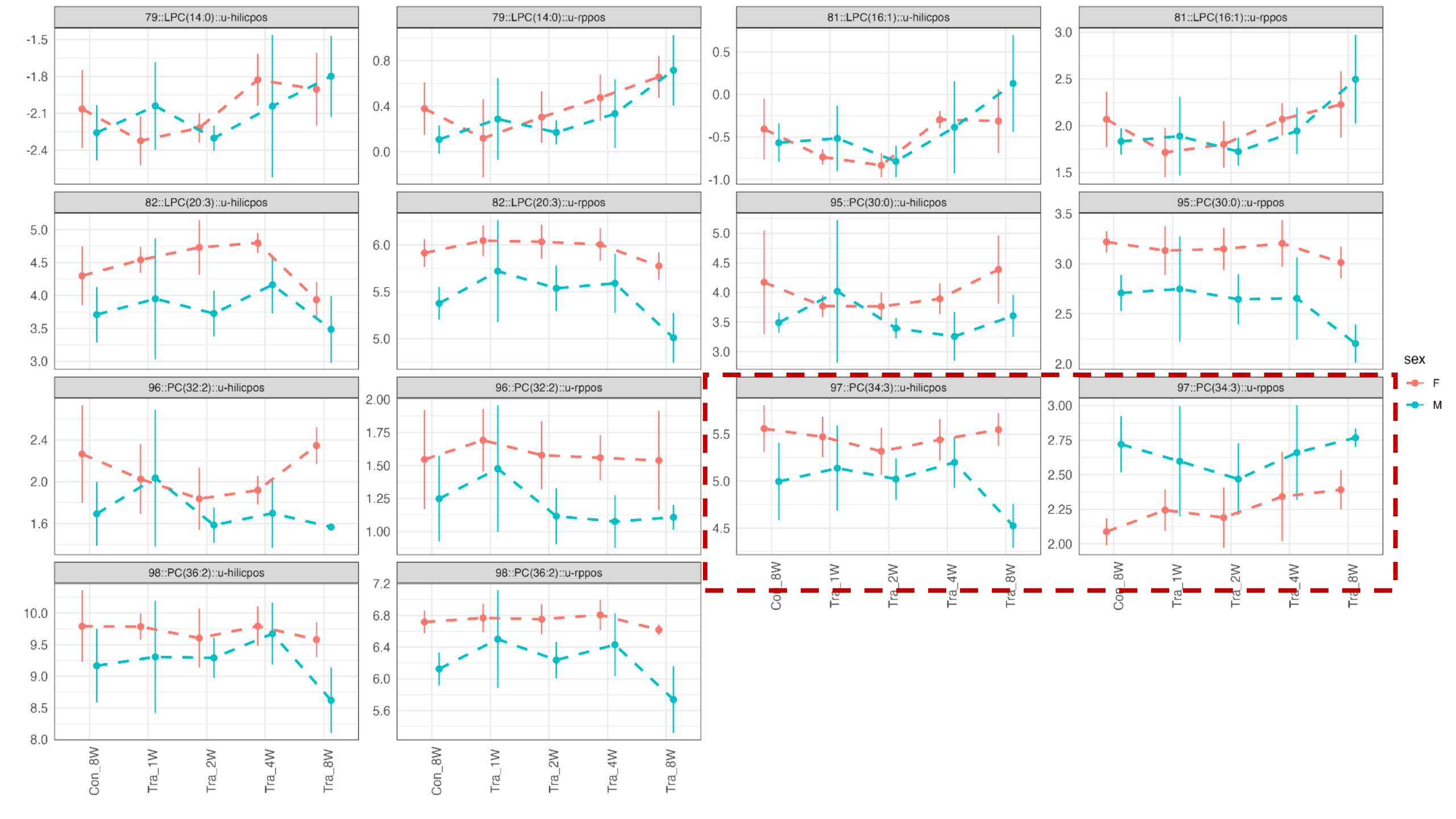


#59-#64

2. For lipids:
reverse phase positive mode > hilic positive mode (umich > broad)

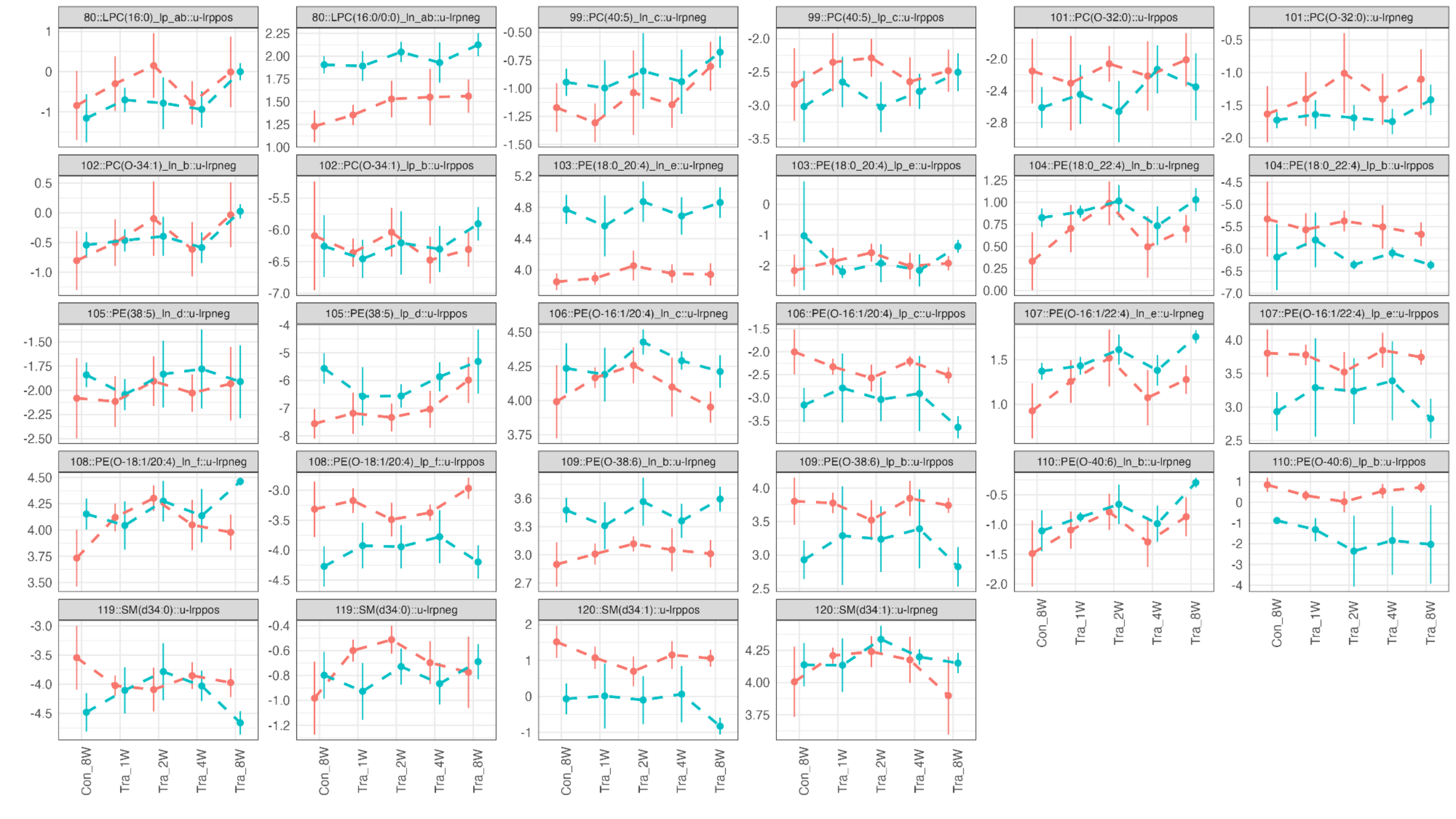
((# of features: 14 -> 7)

6 out of 7 features agree between the two sites


79::LPC(14:0)::u-hilicpos
79::LPC(14:0)::u-rppos
81::LPC(16:1)::u-hilicpos
81::LPC(16:1)::u-rppos
82::LPC(20:3)::u-hilicpos
82::LPC(20:3)::u-rppos
95::PC(30:0)::u-hilicpos
95::PC(30:0)::u-rppos
96::PC(32:2)::u-hilicpos
96::PC(32:2)::u-rppos
97::PC(34:3)::u-hilicpos
97::PC(34:3)::u-rppos
98::PC(36:2)::u-hilicpos
98::PC(36:2)::u-rppos
Con_8W
Tra_1W
Tra_2W
Tra_4W
Tra_8W
sex
F
M

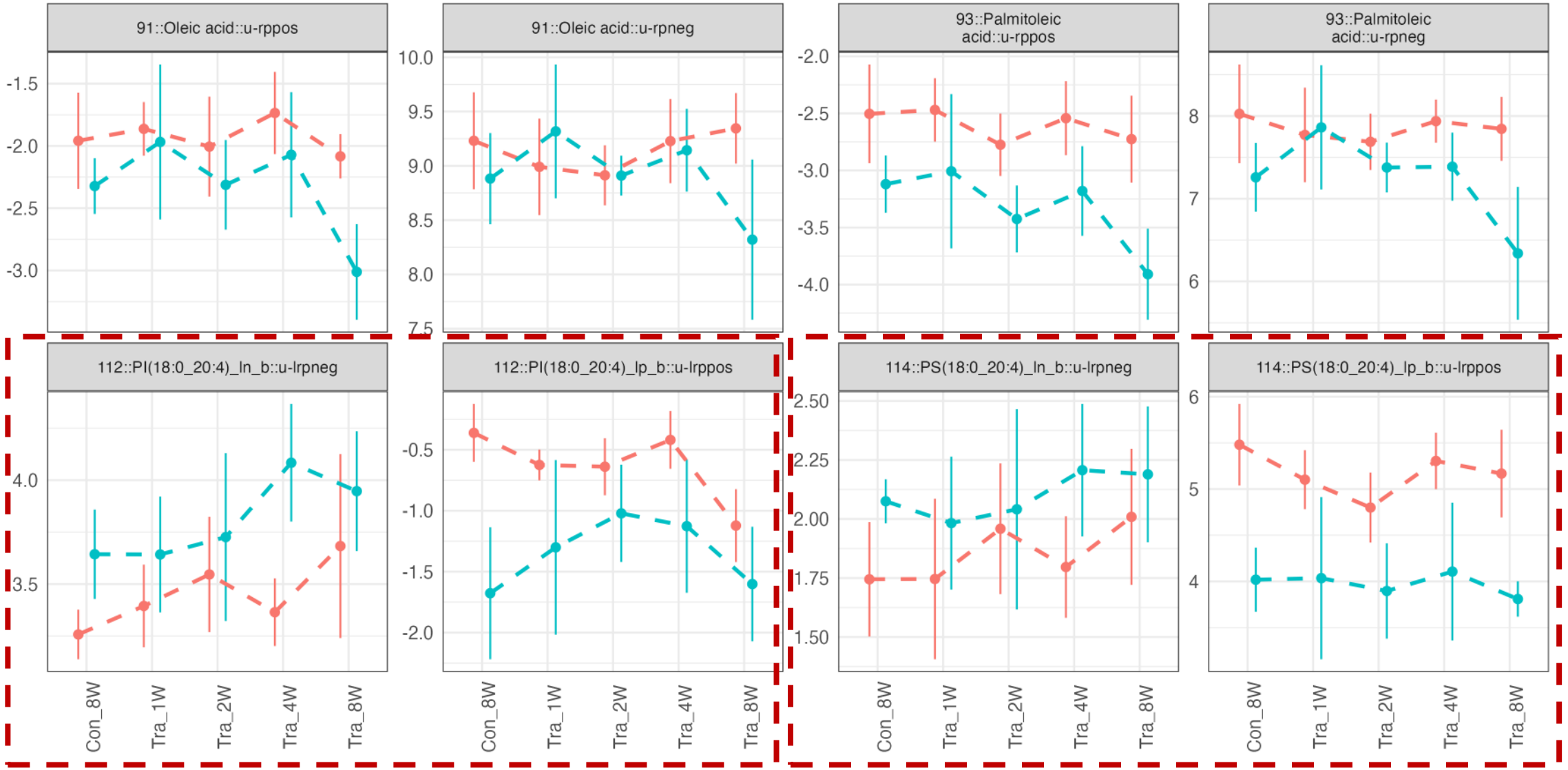
3. For PC, PE, LPC, LPE, SM, TG, DG, duplicates only within Gtech, Positive mode > negative mode

((# of features: 28 -> 14)


80::LPC(16:0)_lp_ab::u-lrppos
80::LPC(16:0/0:0)_ln_ab::u-lrpneg
99::PC(40:5)_ln_c::u-lrpneg
99::PC(40:5)_lp_c::u-lrppos
101::PC(O-32:0)::u-lrppos
101::PC(O-32:0)::u-lrpneg
102::PC(O-34:1)_ln_b::u-lrpneg
102::PC(O-34:1)_lp_b::u-lrppos
103::PE(18:0_20:4)_ln_e::u-lrpneg
103::PE(18:0_20:4)_lp_e::u-lrppos
104::PE(18:0_22:4)_ln_b::u-lrpneg
104::PE(18:0_22:4)_lp_b::u-lrppos
105::PE(38:5)_ln_d::u-lrpneg
105::PE(38:5)_lp_d::u-lrppos
106::PE(O-16:1/20:4)_ln_c::u-lrpneg
106::PE(O-16:1/20:4)_lp_c::u-lrppos
107::PE(O-16:1/22:4)_ln_e::u-lrpneg
107::PE(O-16:1/22:4)_lp_e::u-lrppos
108::PE(O-18:1/20:4)_ln_f::u-lrpneg
108::PE(O-18:1/20:4)_lp_f::u-lrppos
109::PE(O-38:6)_ln_b::u-lrpneg
109::PE(O-38:6)_lp_b::u-lrppos
110::PE(O-40:6)_ln_b::u-lrpneg
110::PE(O-40:6)_lp_b::u-lrppos
119::SM(d34:0)::u-lrppos
119::SM(d34:0)::u-lrpneg
120::SM(d34:1)::u-lrppos
120::SM(d34:1)::u-lrpneg
Con_8W
Tra_1W
Tra_2W
Tra_4W
Tra_8W

4. For PI, PS, PA, PIP, FFA duplicates only within Gtech or within Umich: negative mode > positive mode

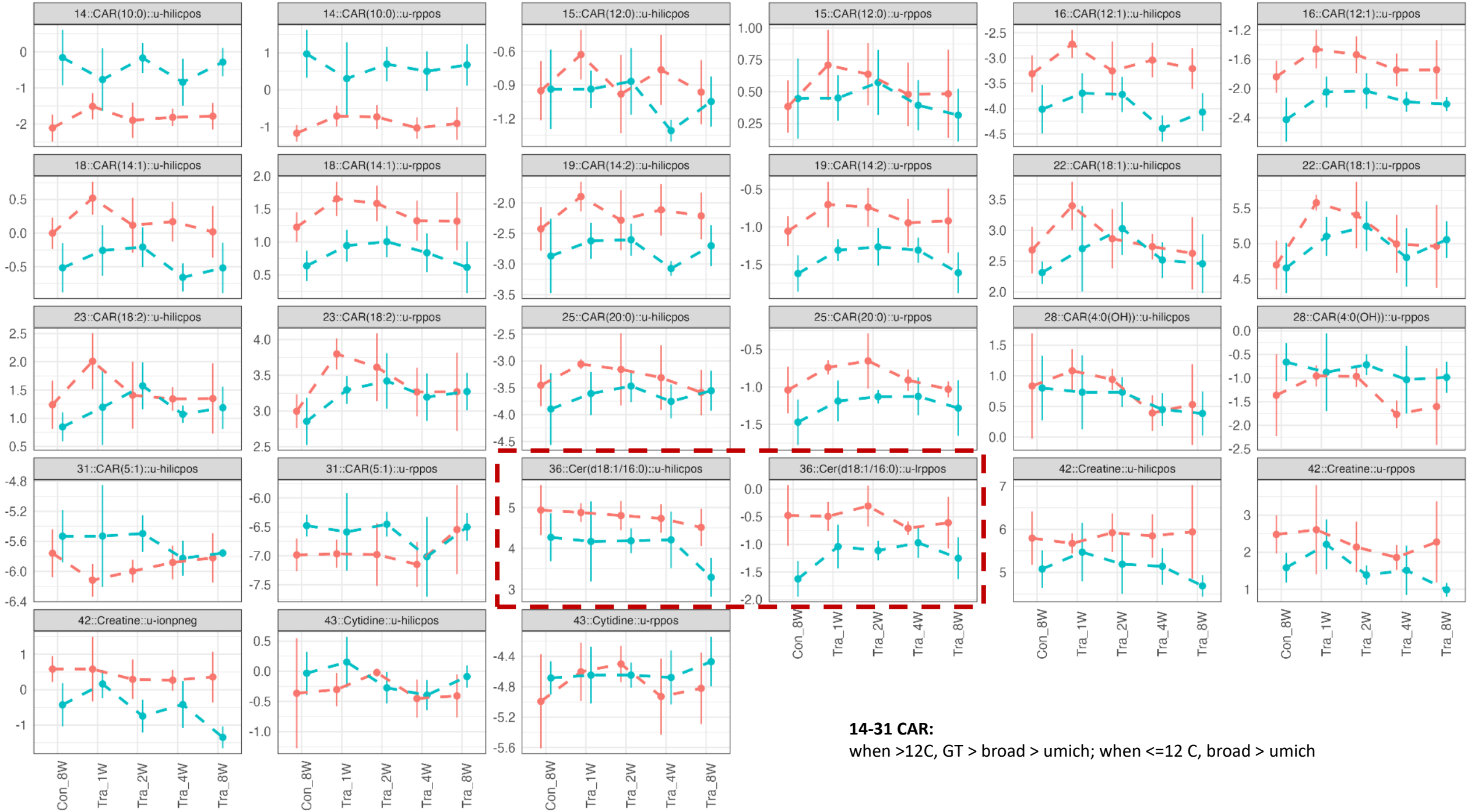
((# of features: 8 -> 4)


91::Oleic acid::u-rppos
91::Oleic acid::u-rpneg
93::Palmitoleic acid::u-rppos
93::Palmitoleic acid::u-rpneg
112::PI(18:0_20:4)_ln_b::u-lrpneg
112::PI(18:0_20:4)_lp_b::u-lrppos
114::PS(18:0_20:4)_ln_b::u-lrpneg
114::PS(18:0_20:4)_lp_b::u-lrppos
Con_8W
Tra_1W
Tra_2W
Tra_4W
Tra_8W

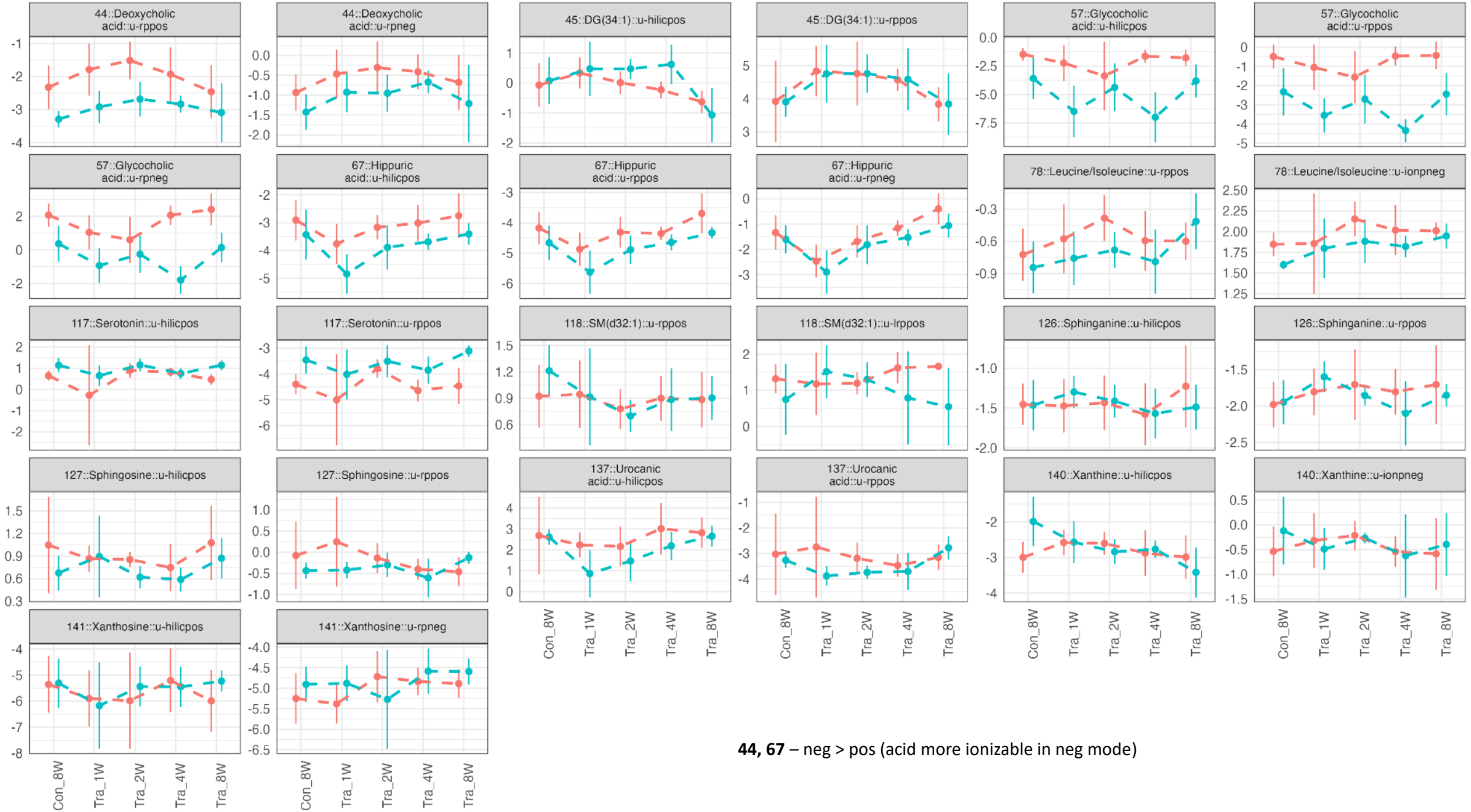
Others: visual inspection and manual selection

Decisions regarding platform were based on property of the metabolites (polarity, solubility, etc) and assay methodology (extraction solvent, elution solvent, column, etc)

5: multiple sites agree

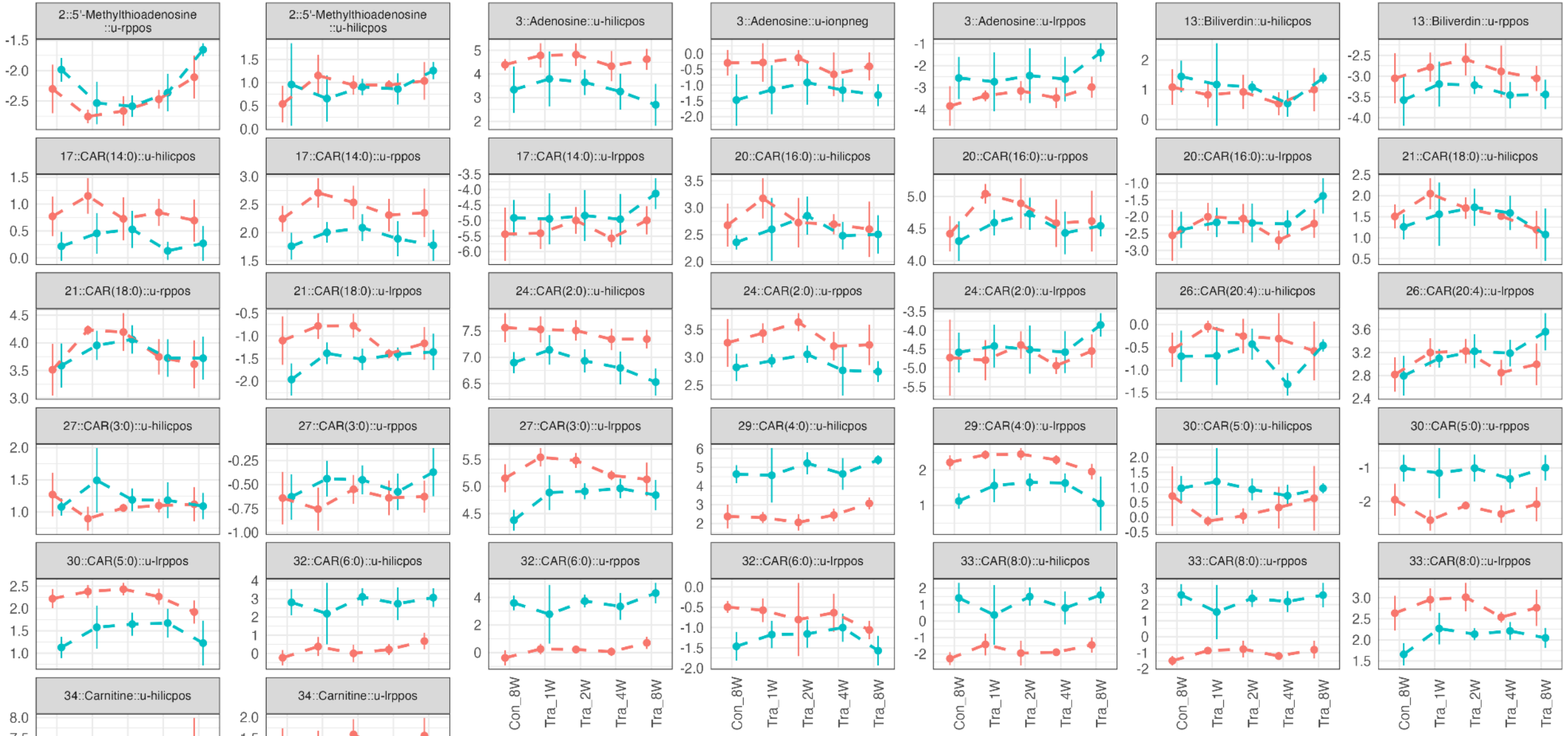


14-31 CAR:
when >12C, GT > broad > umich; when <=12 C, broad > umich



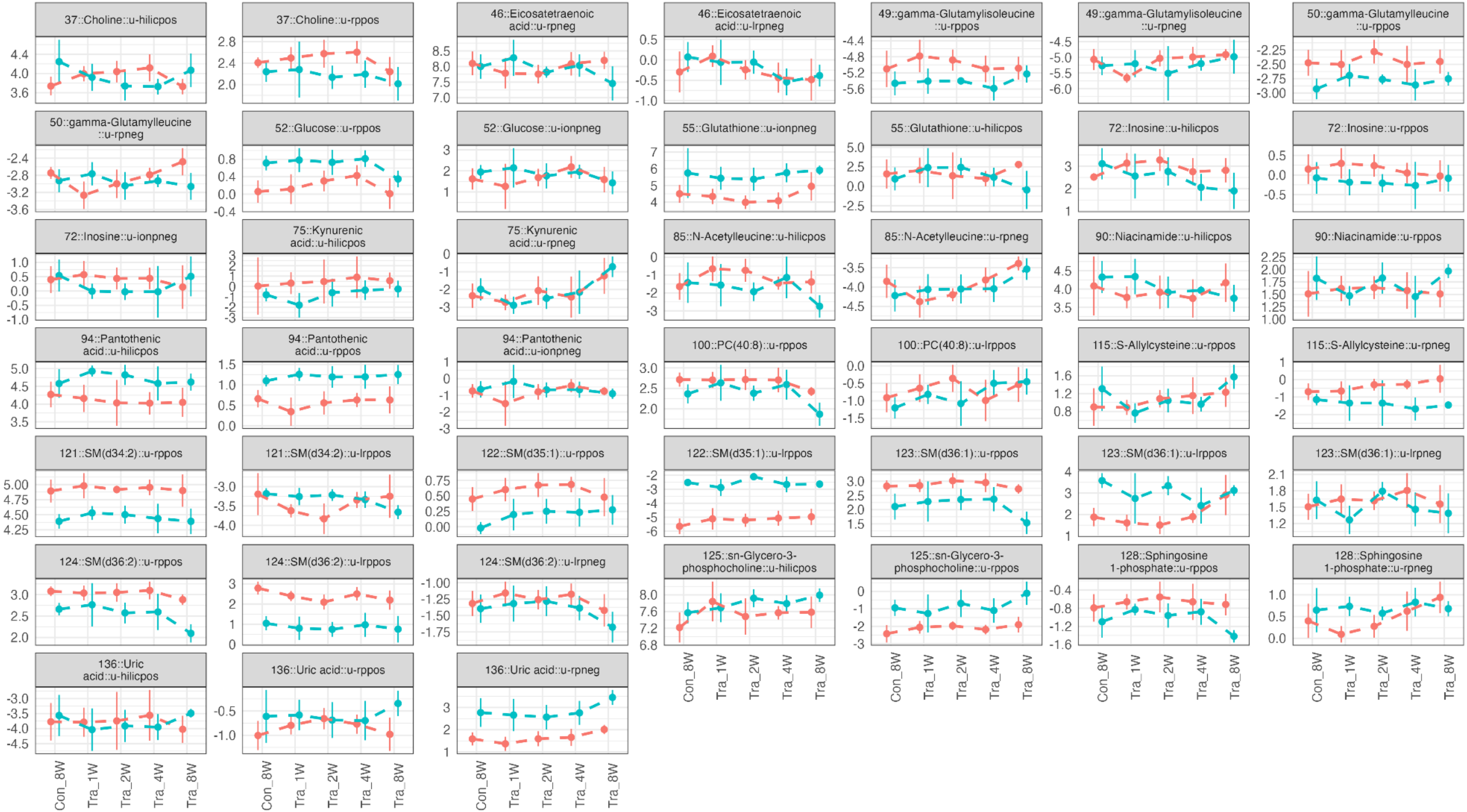
44, 67 – neg > pos (acid more ionizable in neg mode)

6: multiple sites disagree



#2: 5'-Methylthioadenosine found in duke targeted assay (so untargeted measurements removed)

20, 21, 26 – LC-CAR picked lipidomics over metabolomics
24, 27, 29, 30, 32, 33, 34 – CAR, when <=12 C, broad > umich
Exception: 17 - CAR (14:0) – picked broad since GT disagrees with other two


37::Choline::u-hilicpos
37::Choline::u-rppos
46::Eicosatetraenoic acid::u-rpneg
46::Eicosatetraenoic acid::u-lrpneg
49::gamma-Glutamylisoleucine ::u-rppos
49::gamma-Glutamylisoleucine ::u-rpneg
50::gamma-Glutamylleucine ::u-rppos
50::gamma-Glutamylleucine ::u-rpneg
52::Glucose::u-rppos
52::Glucose::u-ionpneg
55::Glutathione::u-ionpneg
55::Glutathione::u-hilicpos
72::Inosine::u-hilicpos
72::Inosine::u-rppos
72::Inosine::u-ionpneg
75::Kynurenic acid::u-hilicpos
75::Kynurenic acid::u-rpneg
85::N-Acetylleucine::u-hilicpos
85::N-Acetylleucine::u-rpneg
90::Niacinamide::u-hilicpos
90::Niacinamide::u-rppos
94::Pantothenic acid::u-hilicpos
94::Pantothenic acid::u-rppos
94::Pantothenic acid::u-ionpneg
100::PC(40:8)::u-rppos
100::PC(40:8)::u-lrppos
115::S-Allylcysteine::u-rppos
115::S-Allylcysteine::u-rpneg
121::SM(d34:2)::u-rppos
121::SM(d34:2)::u-lrppos
122::SM(d35:1)::u-rppos
122::SM(d35:1)::u-lrppos
123::SM(d36:1)::u-rppos
123::SM(d36:1)::u-lrppos
123::SM(d36:1)::u-lrpneg
124::SM(d36:2)::u-rppos
124::SM(d36:2)::u-lrppos
124::SM(d36:2)::u-lrpneg
125::sn-Glycero-3-phosphocholine::u-hilicpos
125::sn-Glycero-3-phosphocholine::u-rppos
128::Sphingosine 1-phosphate::u-rppos
128::Sphingosine 1-phosphate::u-rpneg
136::Uric acid::u-hilicpos
136::Uric acid::u-rppos
136::Uric acid::u-rpneg
Con_8W
Tra_1W
Tra_2W
Tra_4W
Tra_8W

Final: 1063 non-redundant features

Final results: features with unique 'feature_ID', 'metabolite', 'metabolite_refmet' columns in the DEA table

Additionally removed two metabolites: Oleoyl-EA (Emory etamidpos assay), due to too many missing values; TG(36:1)>TG(4:0_16:0_16:1)_and_TG(2:0_16:0_18:1)_feature4 (Gtech lrppos assay) due to inconsistent names (in metabolite_refmet column, ID'ed as an LPC)