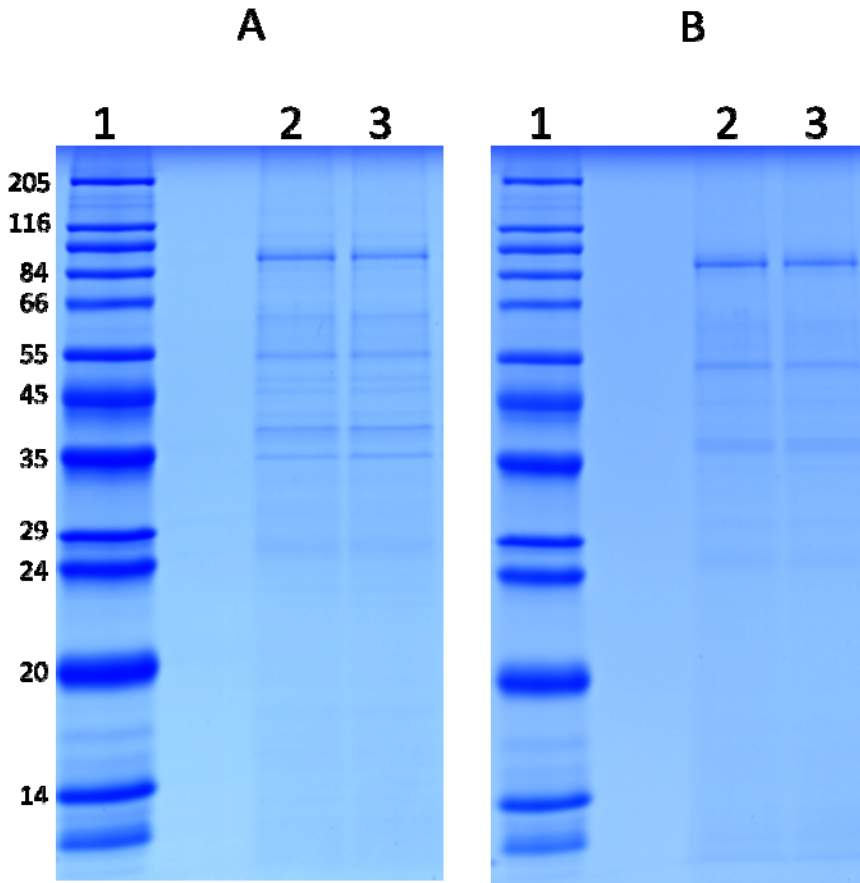


Supplementary figure 1. Intact genomic DNA from the insoluble material recovered during centrifugation of the HFIP lysate prior to addition of MTBE. DNA was isolated using the Qiagen DNeasy Blood and Tissue Kit.

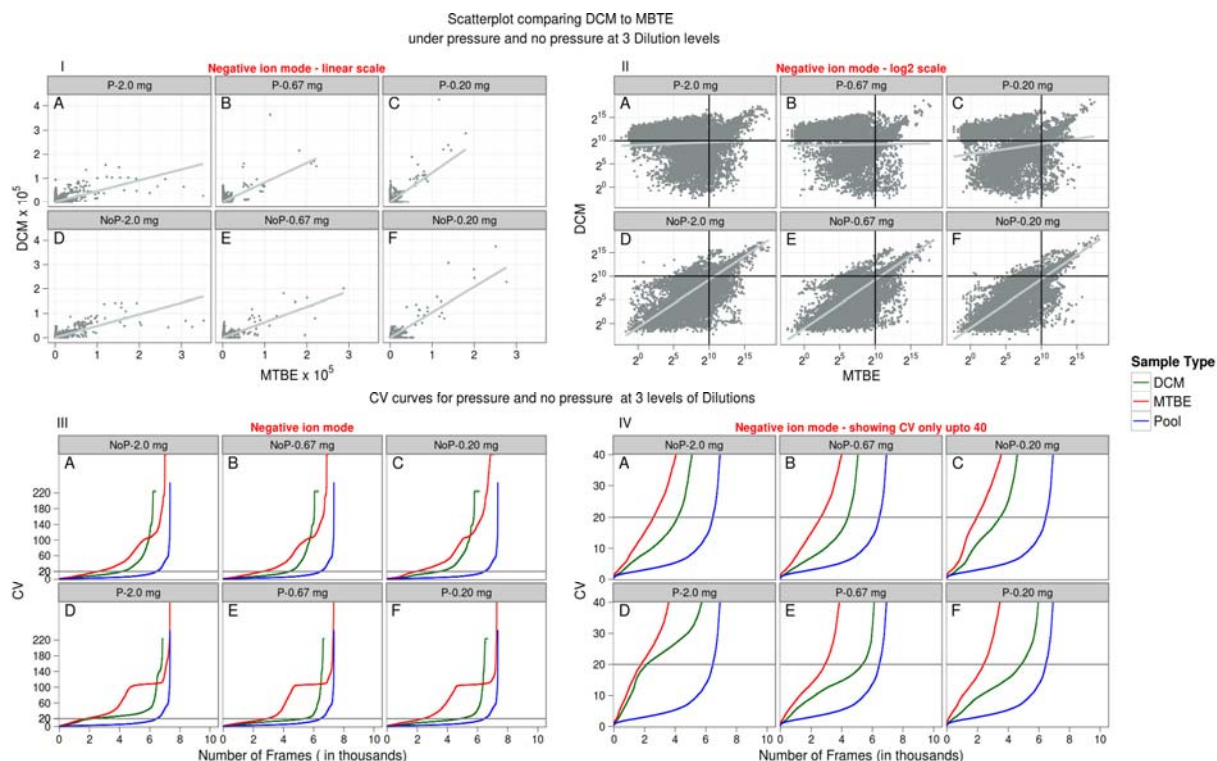


Supplementary figure 2. Examples of proteins recovered from the MTBE/HFIP extraction protocol. Only the 2mg fecal samples were used for protein recovery. In both panels, lane 1- Molec Wgt Std; lane 2- conventional extraction; lane 3- extraction with PCT.

A. Proteins precipitated from HFIP upon addition of MTBE. Pellets were dissolved directly in Laemmli loading buffer with 50mMDTT.

B. Proteins that remained in the HFIP phase after lipid extraction with MTBE were recovered by evaporating the solvent. 0.5 ml of the ~3ml residual HFIP phase was dried in a speedvac. The resulting protein pellets were dissolved directly in Laemmli loading buffer with 50mMDTT.

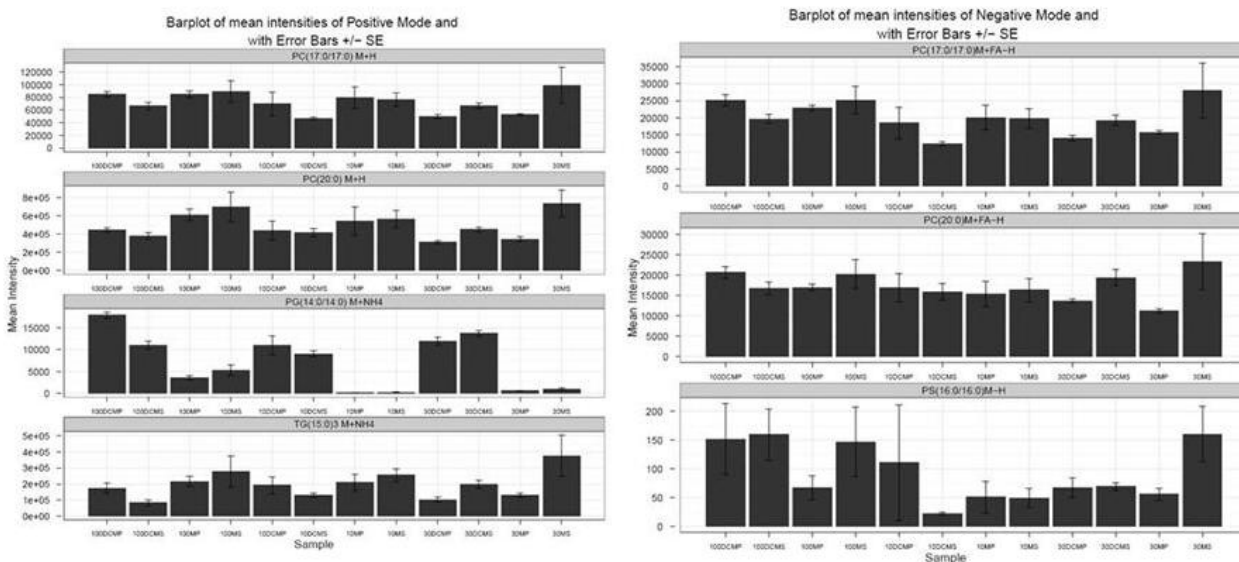
## Supplementary Information:



### Supplementary Figure 3.

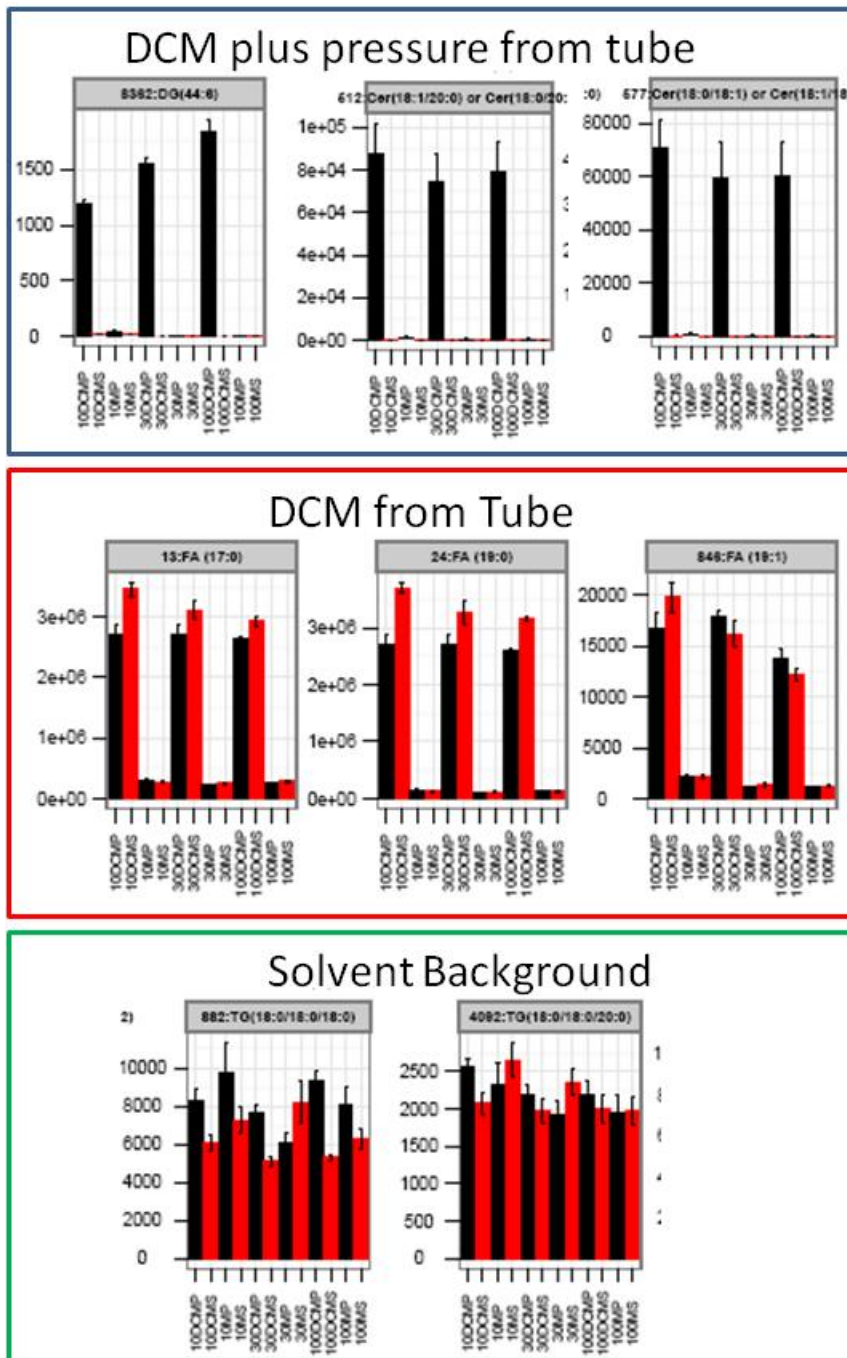
#### Solvent Comparison Negative Ion Analysis:

In this supplemental figure, there are a string of frames which trace both the X and Y axis, suggesting two groups of lipids (or contaminants) which distinctly prefer one solvent system over the other. Additionally, these data make a claim for the use of PCT, showing only advantages to the number of reproducible peaks detected, for both solvent systems. It should be noted that these results only hold true when analyzing the sample at an appropriate dilution. At too high concentrations (ie. the 2.0 mg sample) the extraction reproducibility was negatively affected, consistent with results shown in the main report. Panel I shows qualitatively similar but smaller horizontal and vertical lines, again consistent with solvent specific extraction. Comparison of panels A-C vs. D-F in Figure 5, Panel II shows the advantage of pressure with DCM as a solvent, which is similar to but more obvious than the data in Figure 3 as there is clearly an increase in frames observed at relative abundances which are more readily detected by the LC-MS platform. The CV curves (Figure 5, Panel III and Panel IV) shows the quantitative advantage of DCM as an extraction solvent at the population level, similar to but more obvious than the data in Figure 3 that had also suggested that pressure enhanced the extraction using DCM and negative ionization mode.



#### Supplement Figure 4. Internal Standard analysis:

All samples analyzed were spiked with an IS mixture containing 5 individual lipids, TG (15:0)<sub>3</sub>, PC (17:0/17:0), PG (14:0/14:0), (lysoPC (16:0)) and (PS (16:0/16:0)), each at the concentration of 5 μg/mL. The results in supplement figure 2 clearly show how both pressure and solvent chemistry can affect the extraction. Little variation in the extraction was seen based on sample dilution; however, this may be due to the external spike of these non-endogenous species in contrast to the endogenous compounds. Of the 5 standards, only two PC (17:0/17:0) and PC (20:0) were observed using both positive and negative ionization mode. TG(15:0)<sub>3</sub> and PG (14:0/14:0) were both only observed in positive ion as [M+NH<sub>4</sub>]<sup>+</sup> peaks, and PS(16:0/16:0) was only detected in negative ion and at very low intensities. From supplement figure 2 it becomes clear that changes to the solvent, pressure and dilution do not affect the PC standards. They are quantitatively consistent across the board. PG (14:0/14:0) very specifically prefers the DCM solvent, both with and without pressure, as when MTBE is used it is barely observed. TG (15:0)<sub>3</sub> on the other hand, although also having a glycerol head group, shows a slight preference for the MTBE solvent; however the difference in signal intensity between the methods is negligible and not clearly significant. The signal intensity of PS (14:0/14:0) makes it difficult to interpret. These results further suggest that lipids of different individual chemistries, ie PG versus PC, do have specific needs when it comes to the extraction solvent chemistry and that both solvents tested should be used when a full fecal lipidome assessment is needed. The error bars represent the SEM for all 5 injections.

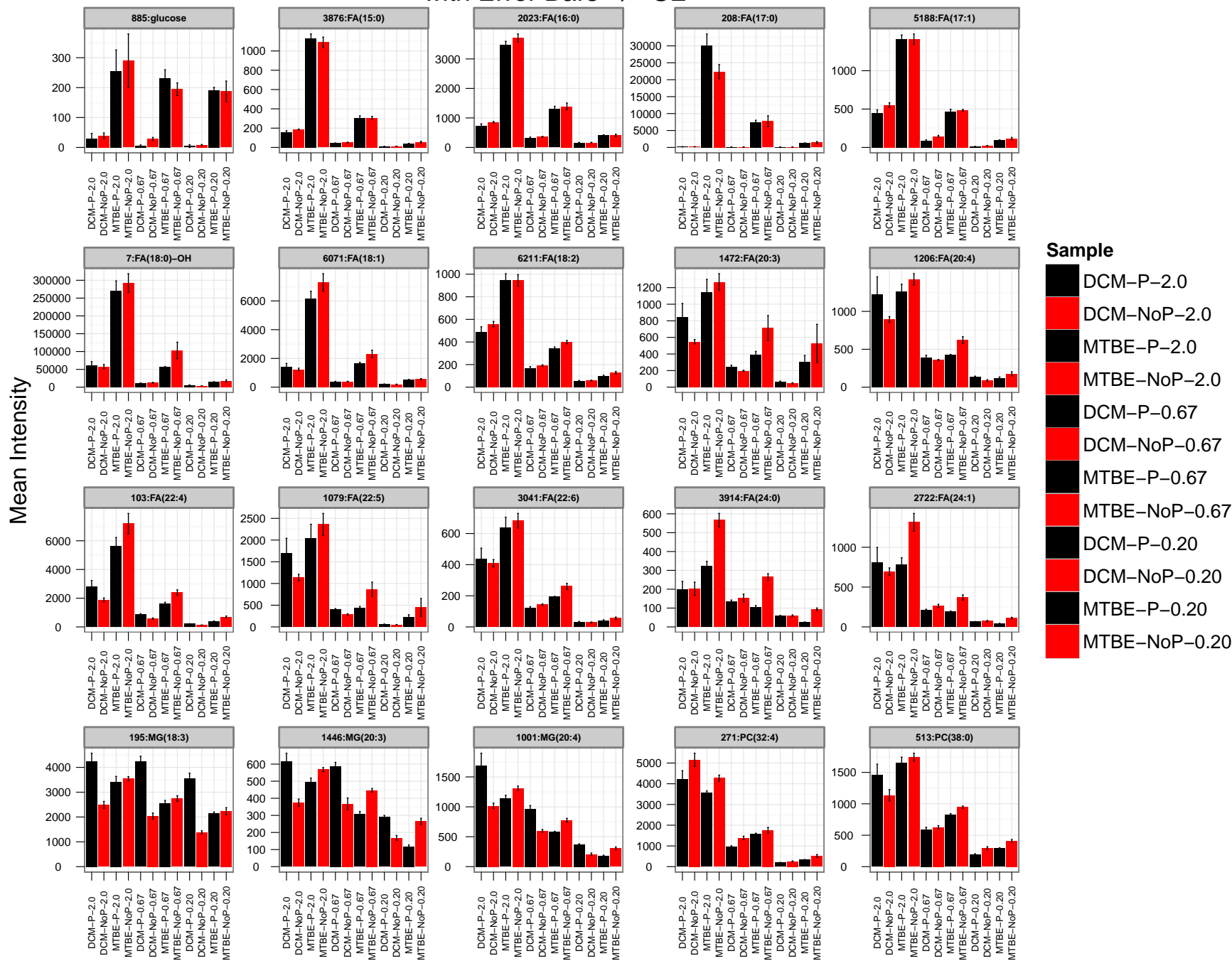


Supplement Figure 5. Background ion analysis:

The first 3 species in panel A, outlined in black, not only extract preferentially with DCM plus pressure, but they did not show any change in the dilution analysis. This pattern indicates that unless unambiguously identified with fragmentation, as was done with the oligosaccharide species, these frames could very well be background ions originating from the extraction tube. We note that, to date, the lipids unique to pressure extraction have not been identified by exact mass and RT matching or HCD fragmentation, suggesting that the advantage of pressure may lie

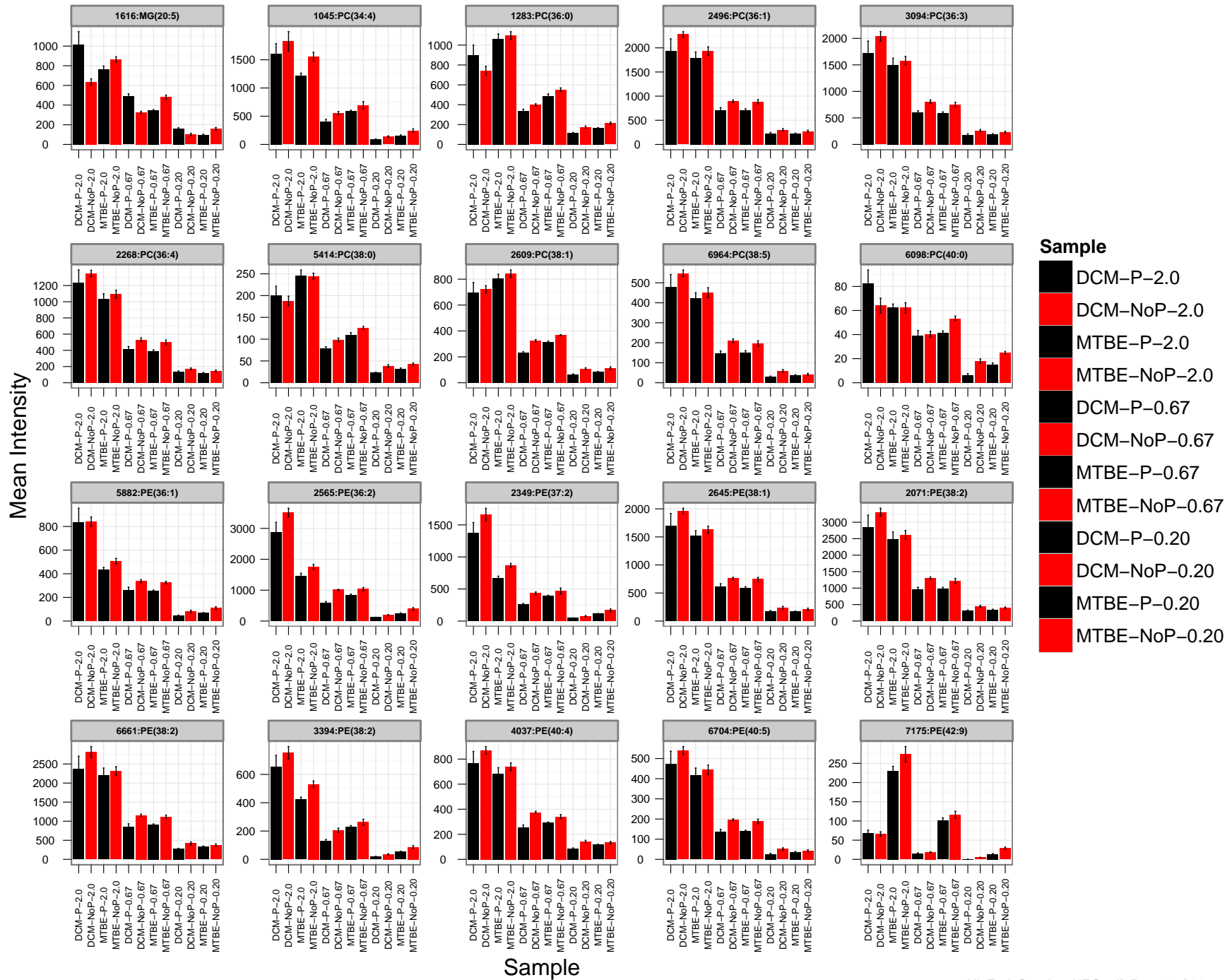
primarily in the increased precision. Additionally, we found other frames that indicated they were coming from DCM both with and without pressure (panel B, outlined in red) and others that were equally extracted across the methods and dilutions suggesting they could have been originating from the tubes regardless of solvent or from the solvents themselves (panel C, outlined in green).

# Barplot of mean intensities of Negative Mode and with Error Bars +/- SE



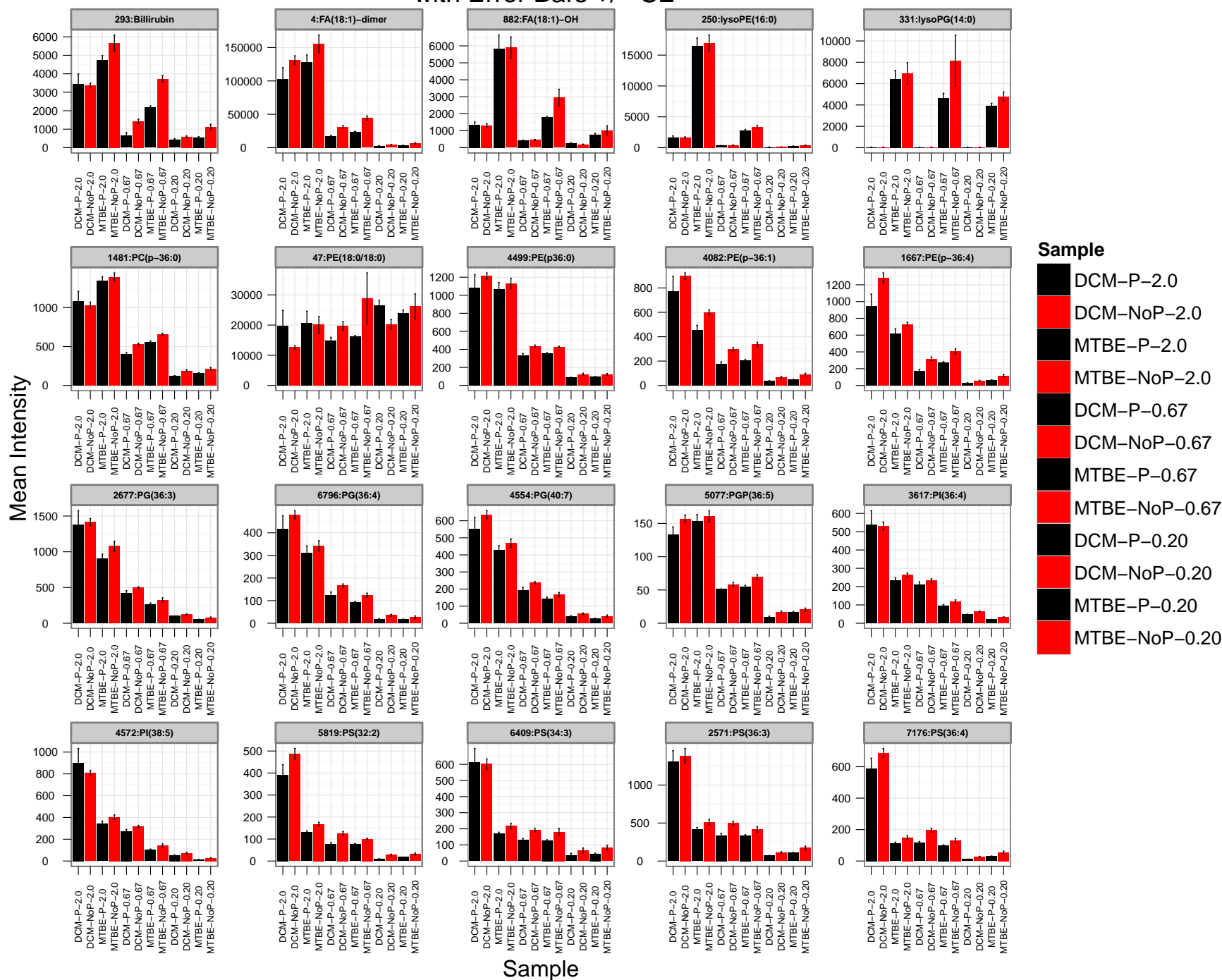
Sample

# Barplot of mean intensities of Negative Mode and with Error Bars +/- SE

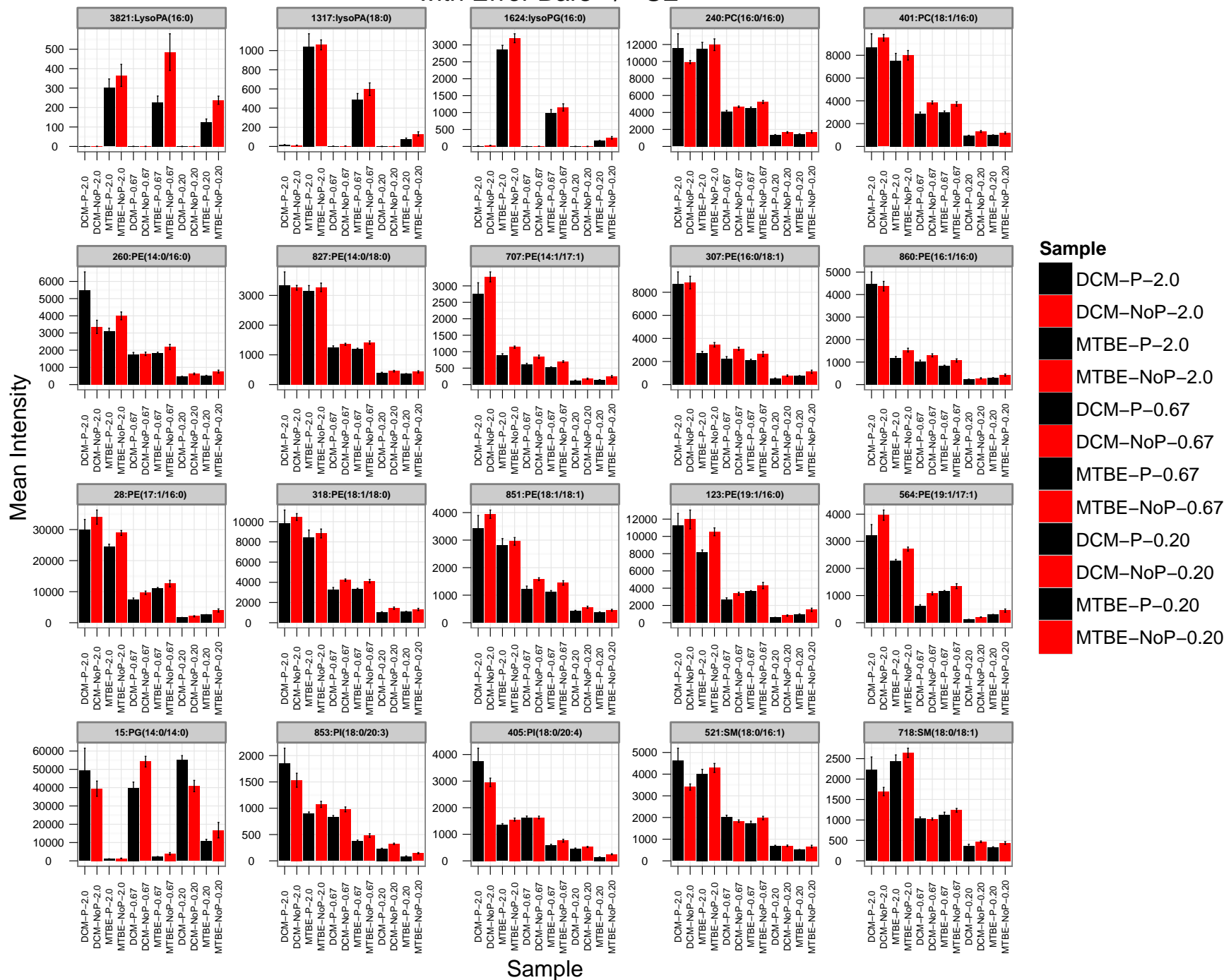




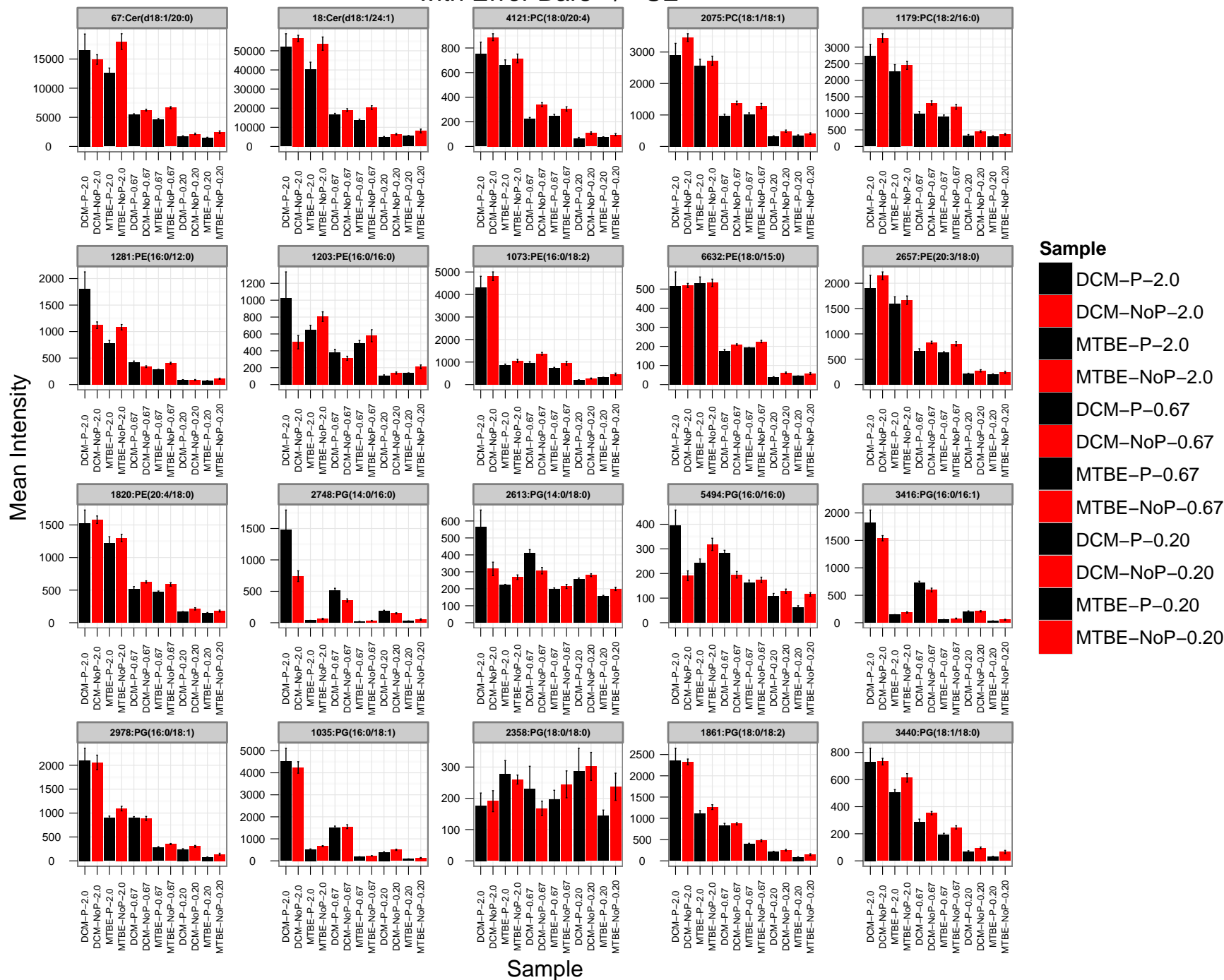
# Barplot of mean intensities of Negative Mode and with Error Bars +/- SE



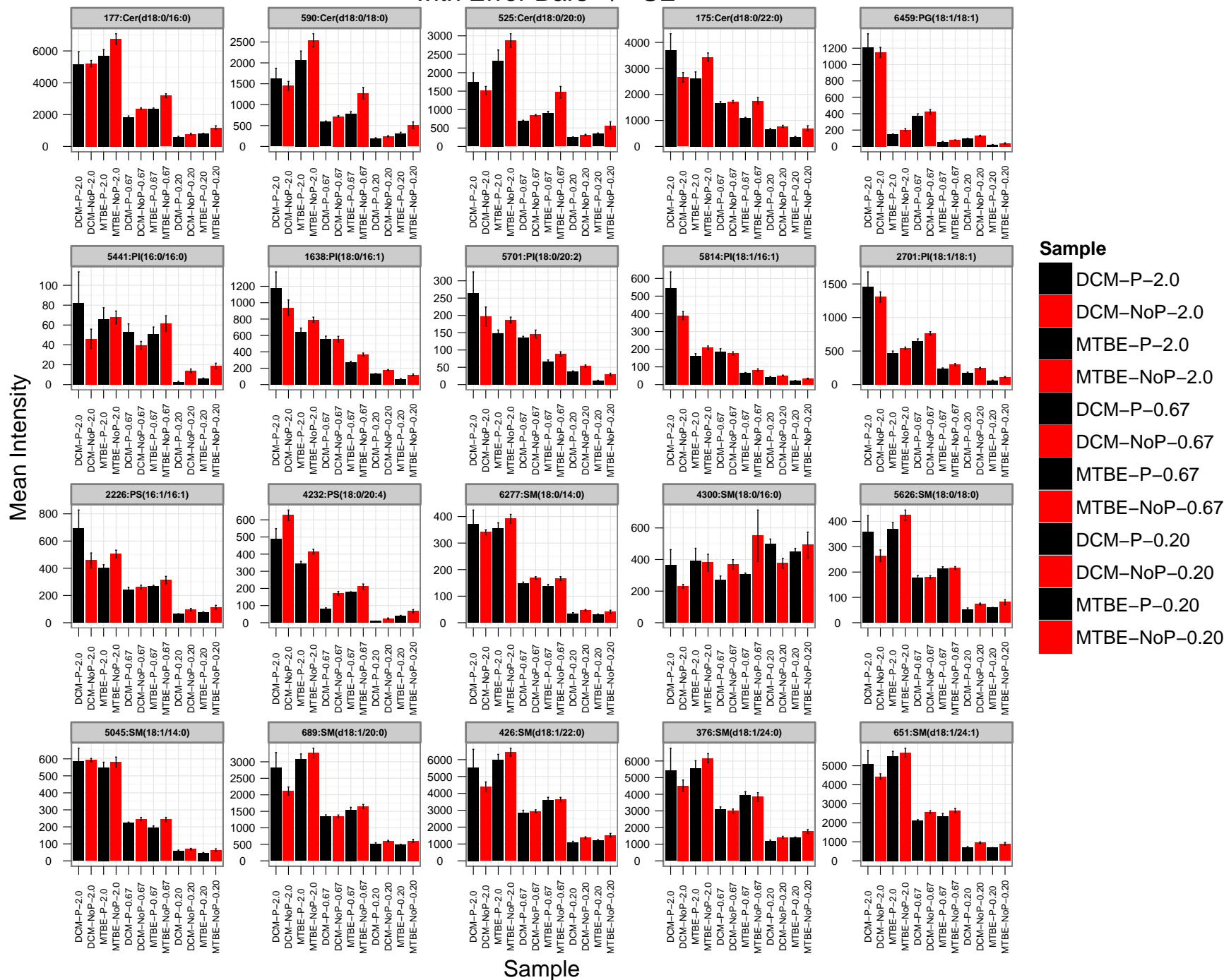
# Barplot of mean intensities of Negative Mode and with Error Bars +/- SE



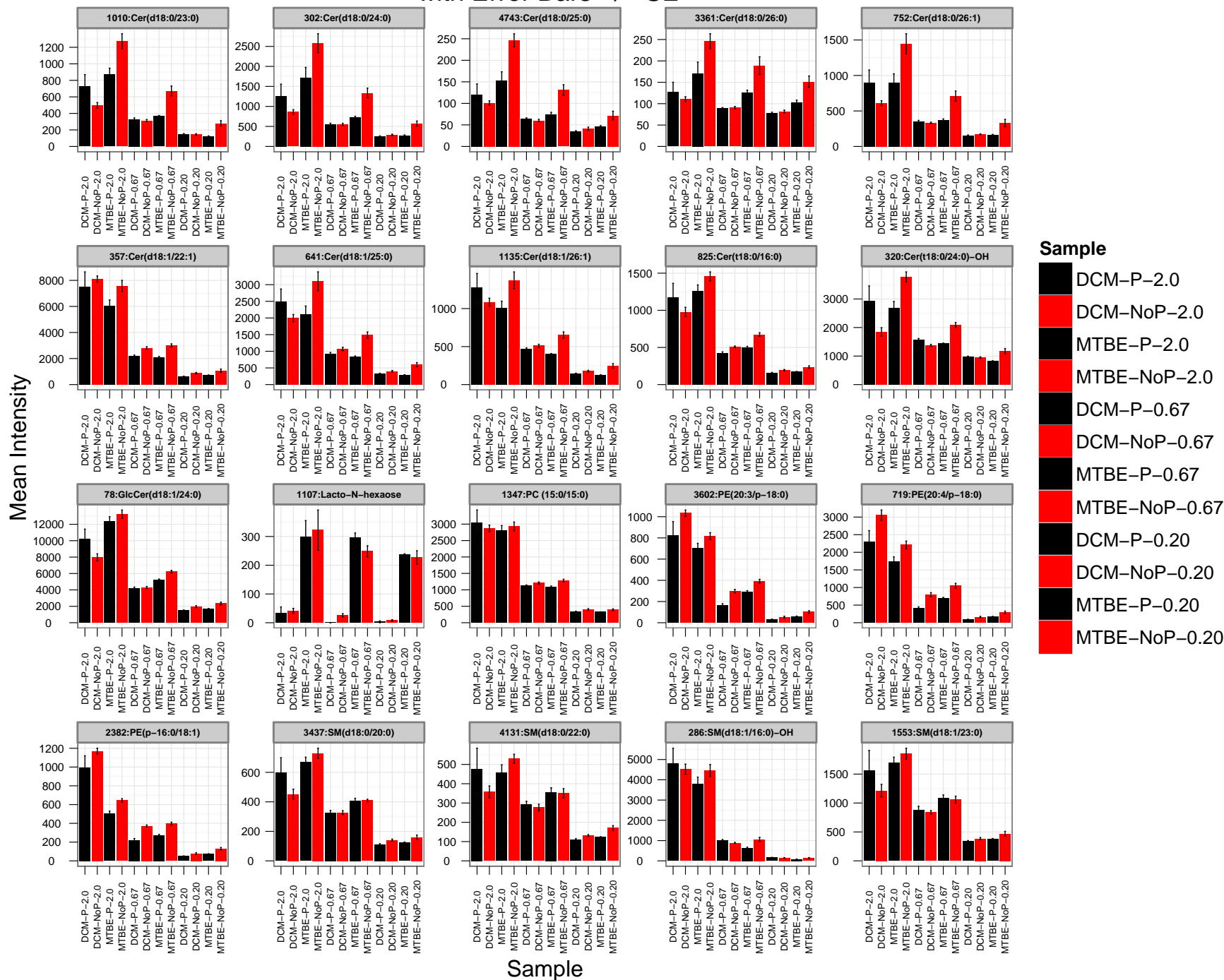
## Barplot of mean intensities of Negative Mode and with Error Bars +/- SE



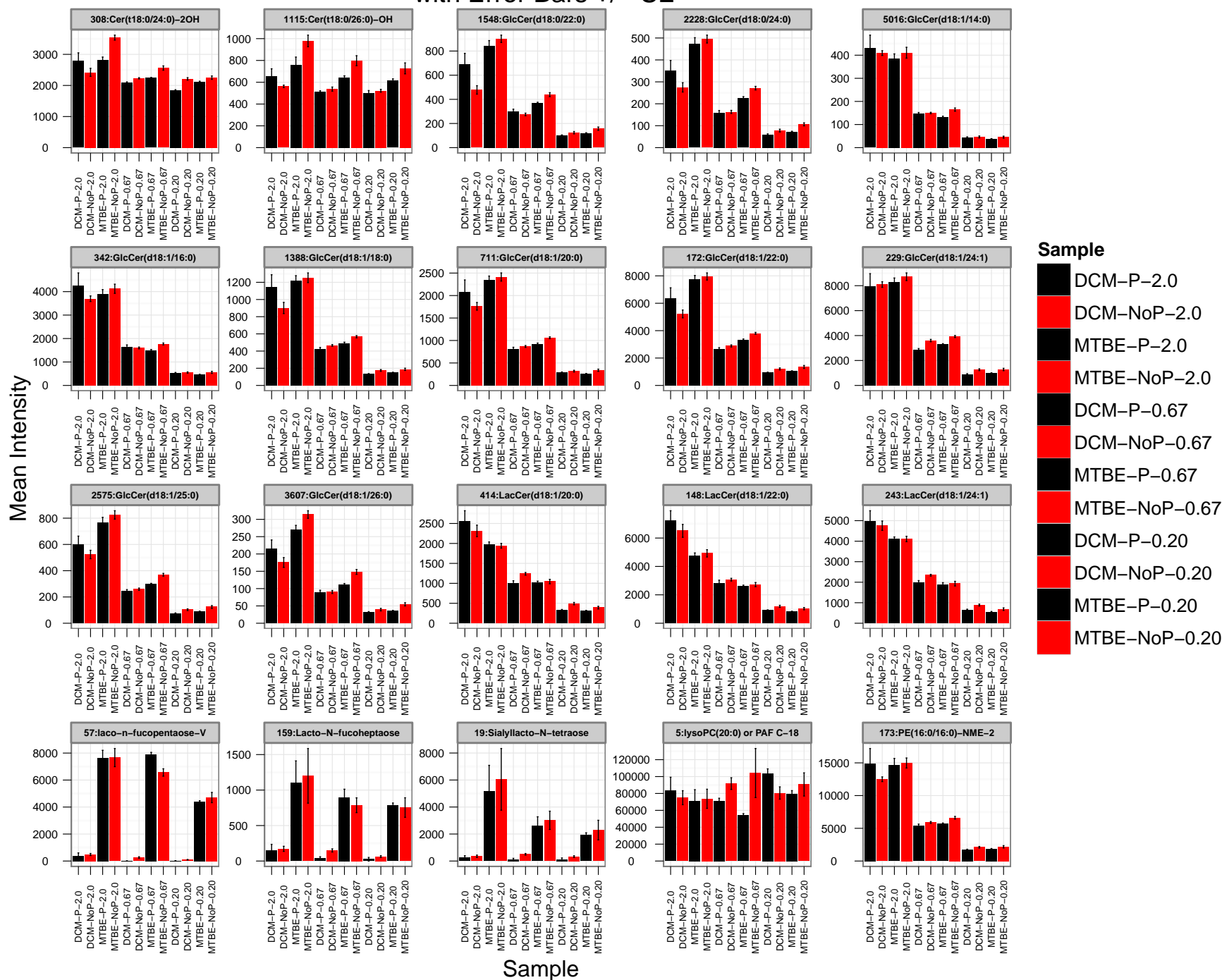
# Barplot of mean intensities of Negative Mode and with Error Bars +/- SE



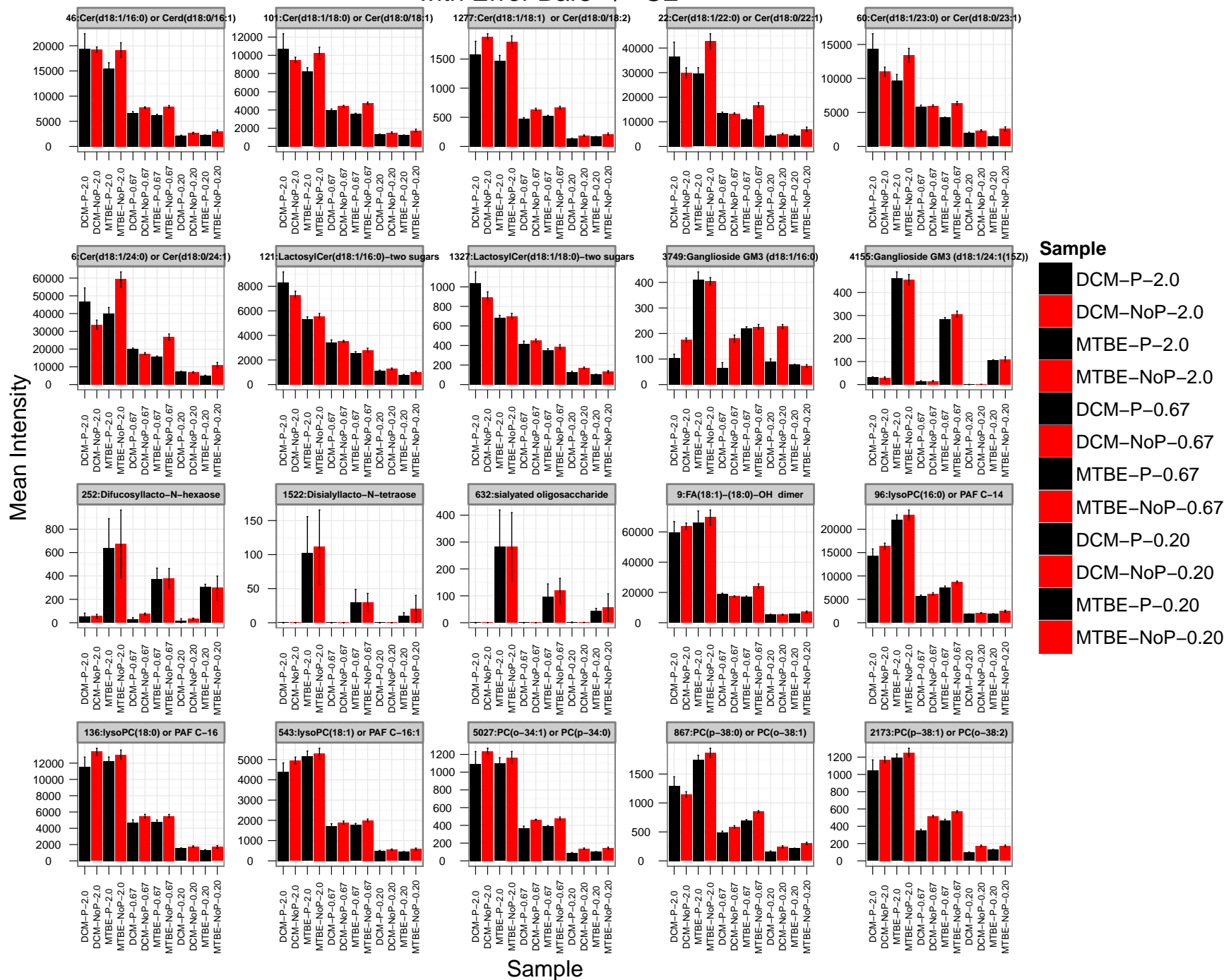
# Barplot of mean intensities of Negative Mode and with Error Bars +/- SE



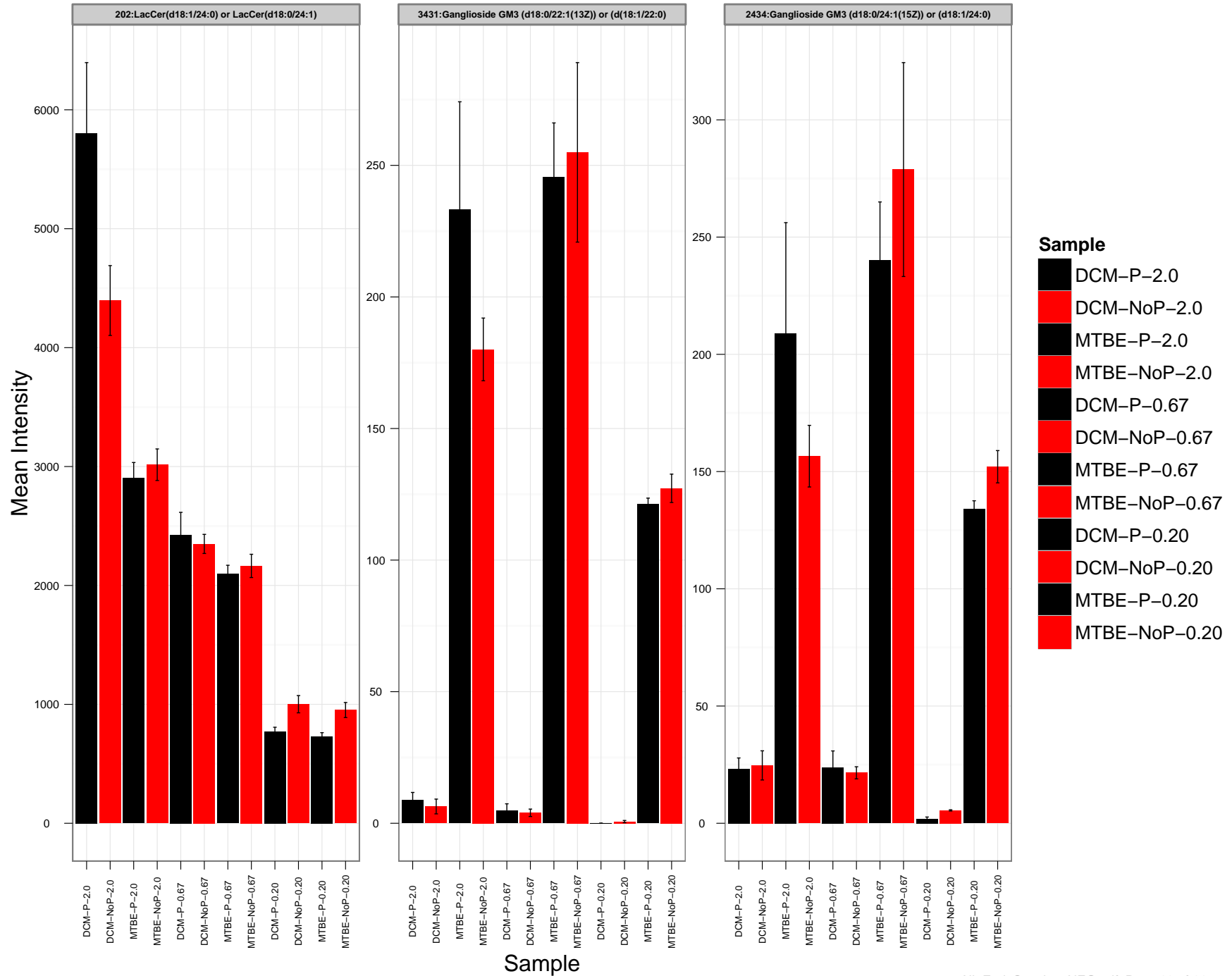
# Barplot of mean intensities of Negative Mode and with Error Bars +/- SE



# Barplot of mean intensities of Negative Mode and with Error Bars +/- SE

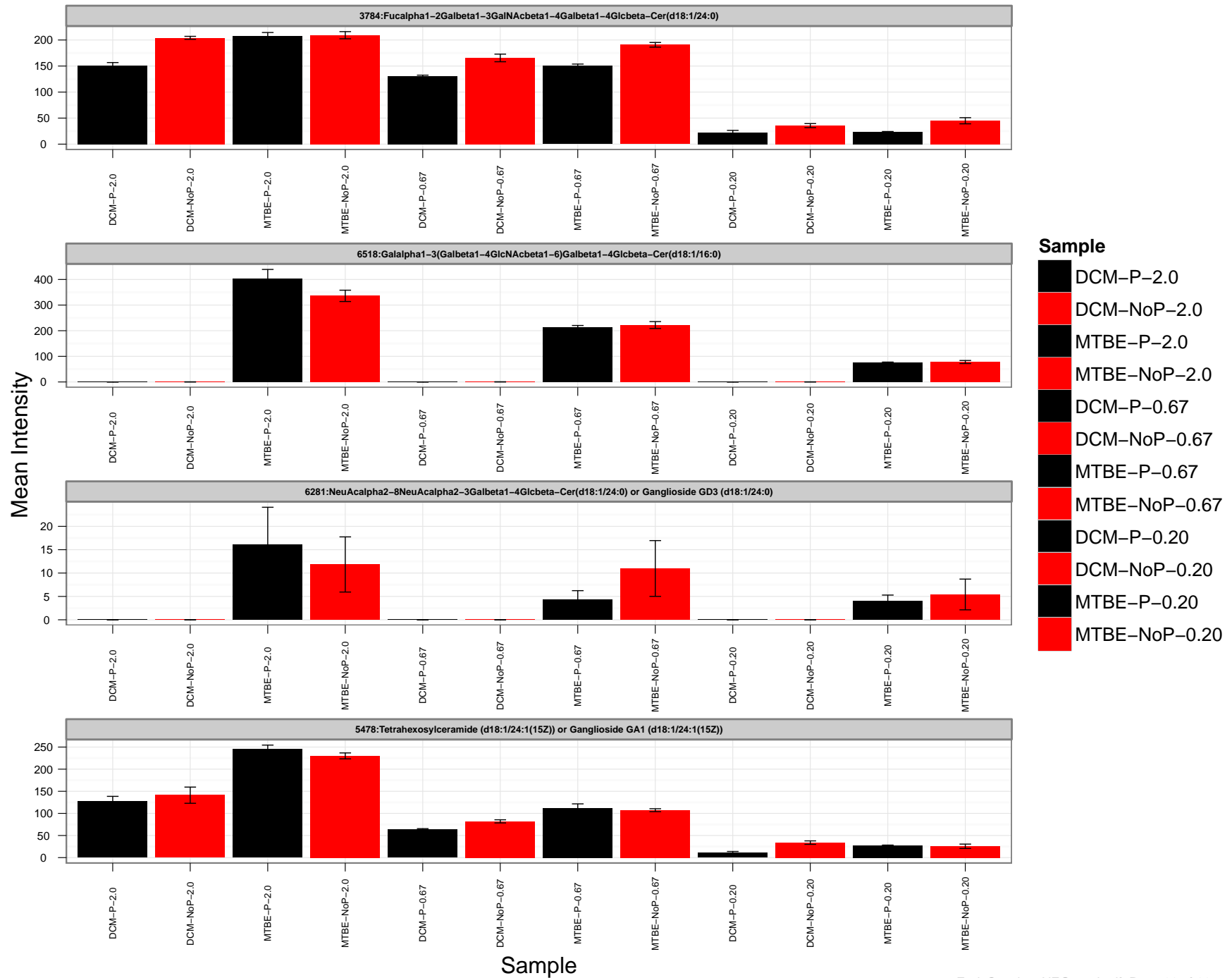


## Barplot of mean intensities of Negative Mode and with Error Bars $\pm$ SE

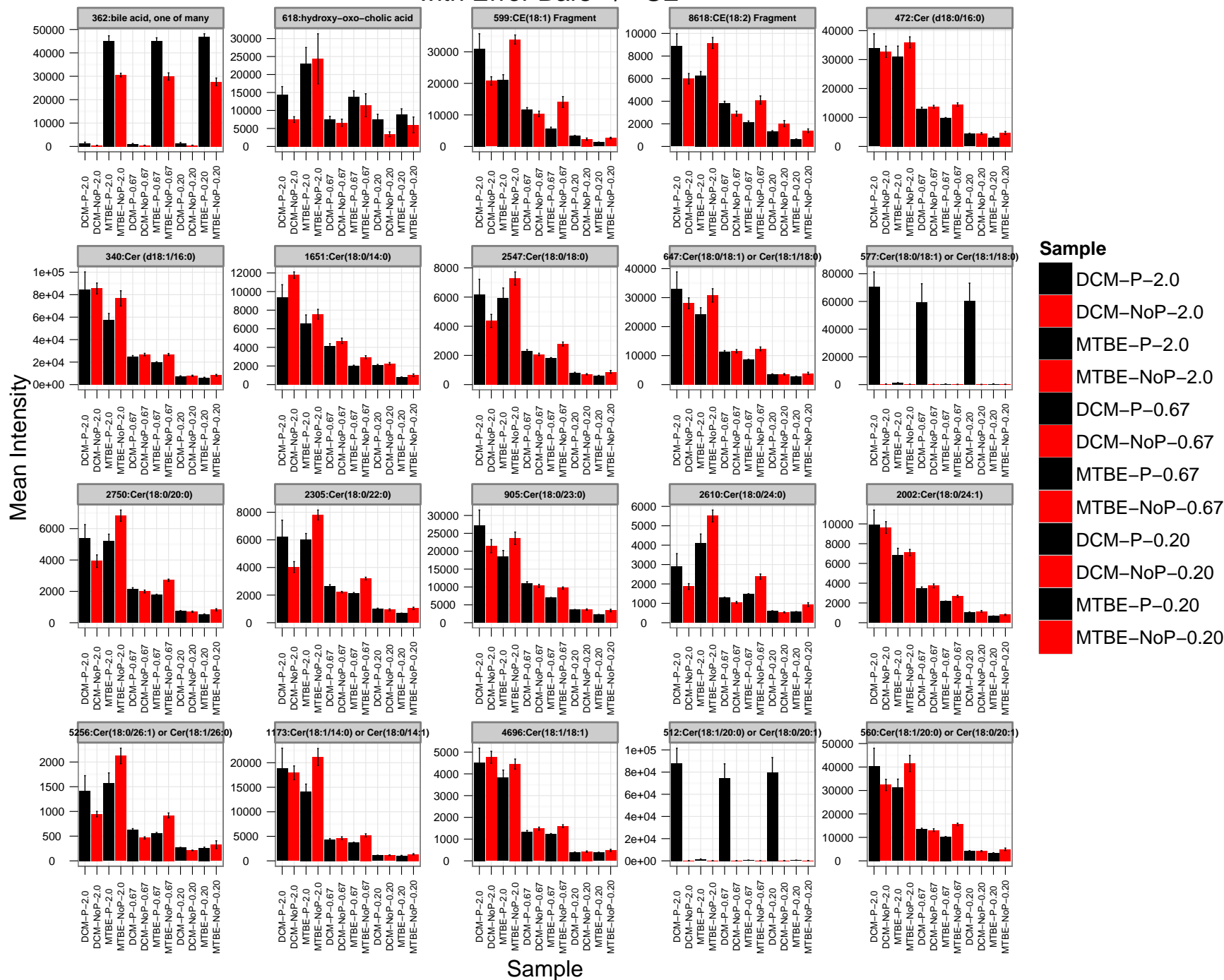




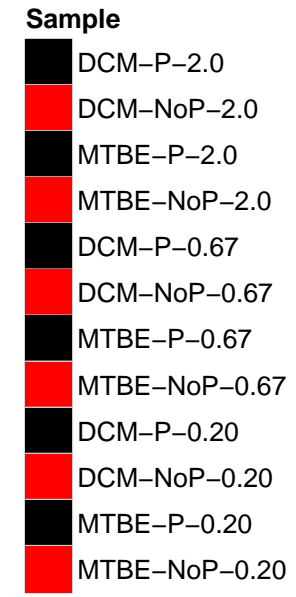
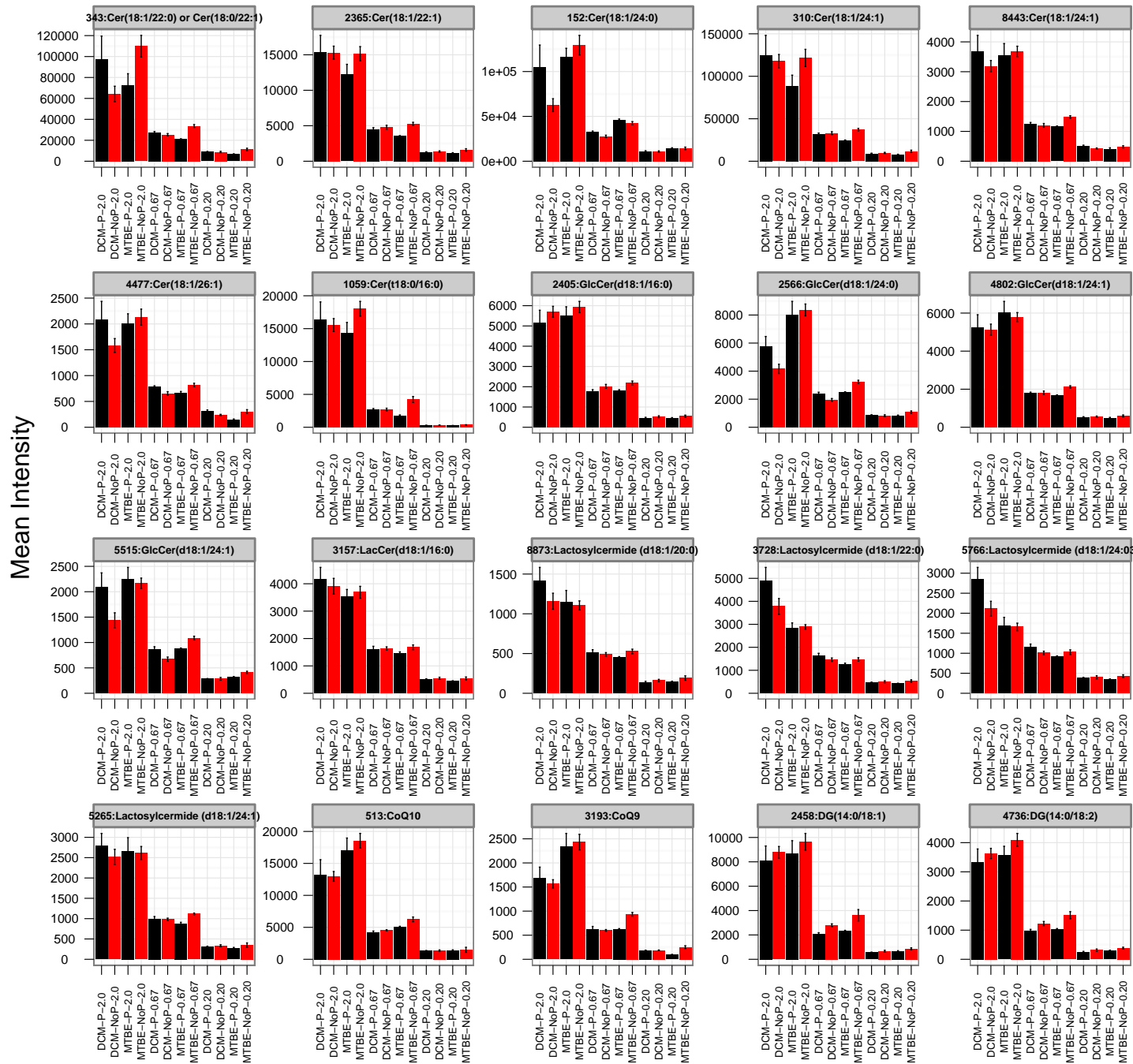
## Barplot of mean intensities of Negative Mode and with Error Bars +/- SE



# Barplot of mean intensities of Positive Mode and with Error Bars +/- SE

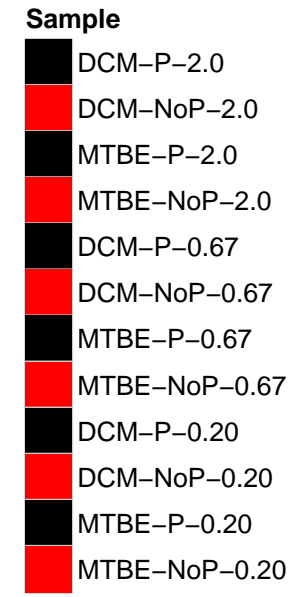
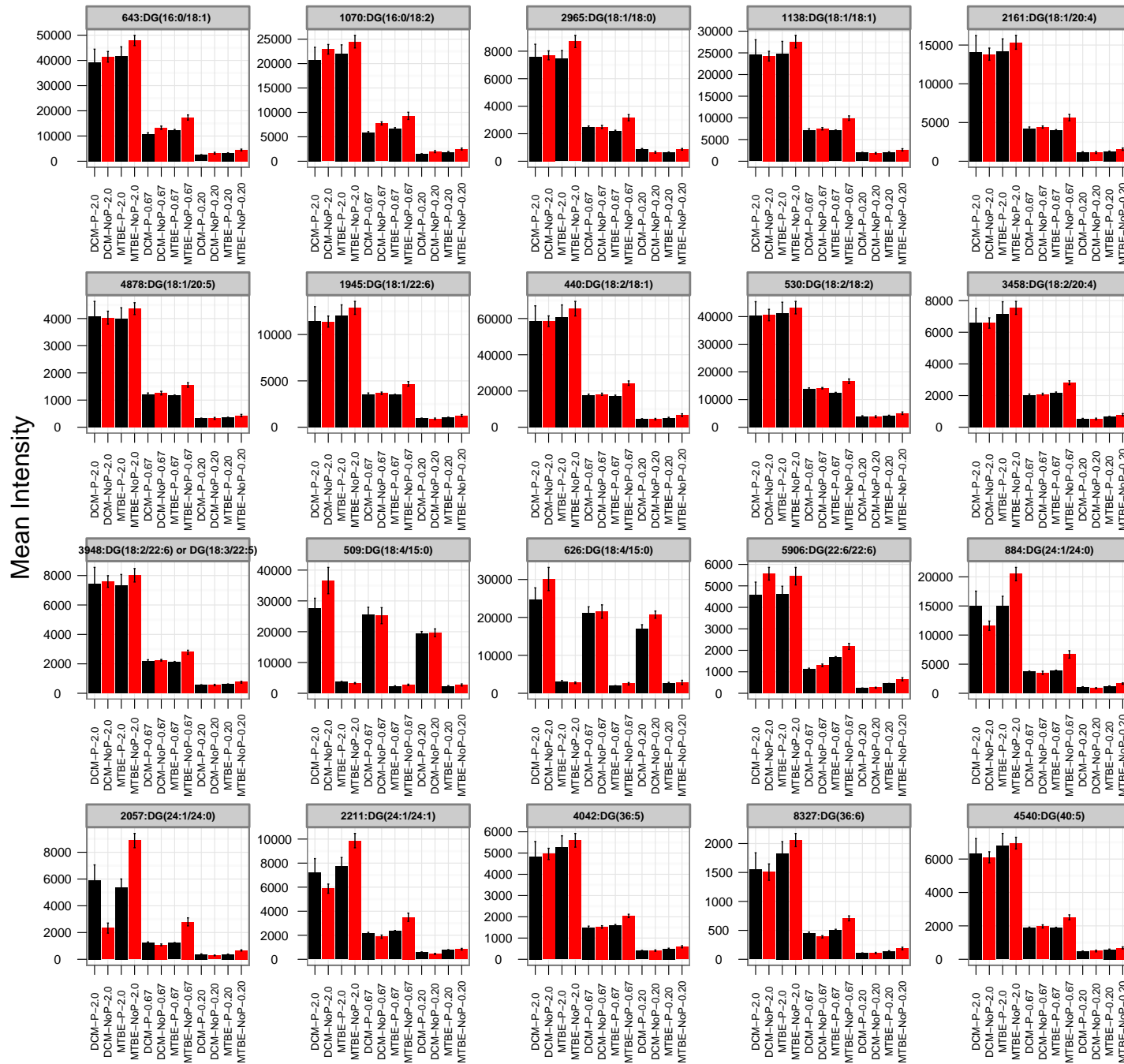


# Barplot of mean intensities of Positive Mode and with Error Bars +/- SE



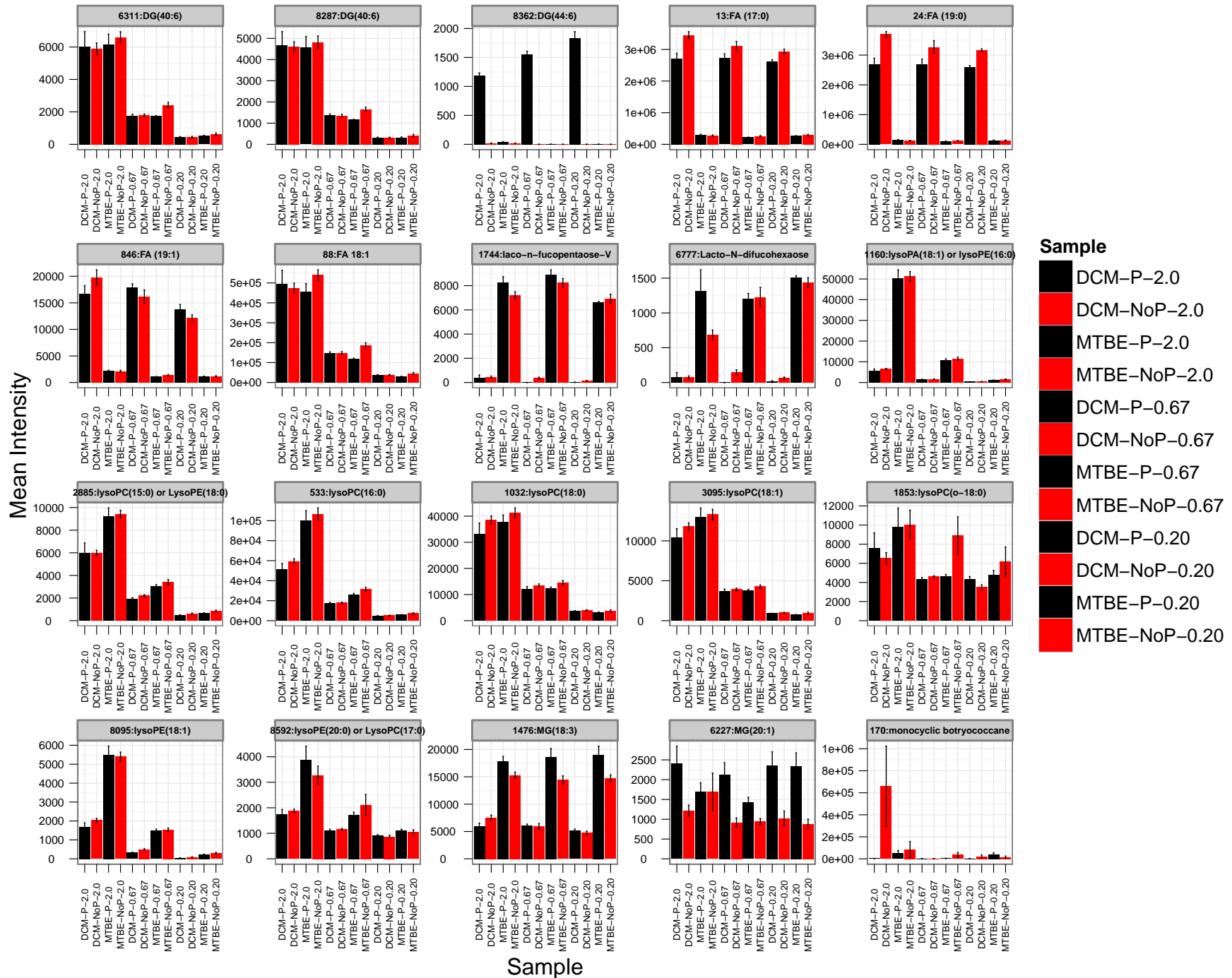
Sample

# Barplot of mean intensities of Positive Mode and with Error Bars +/- SE

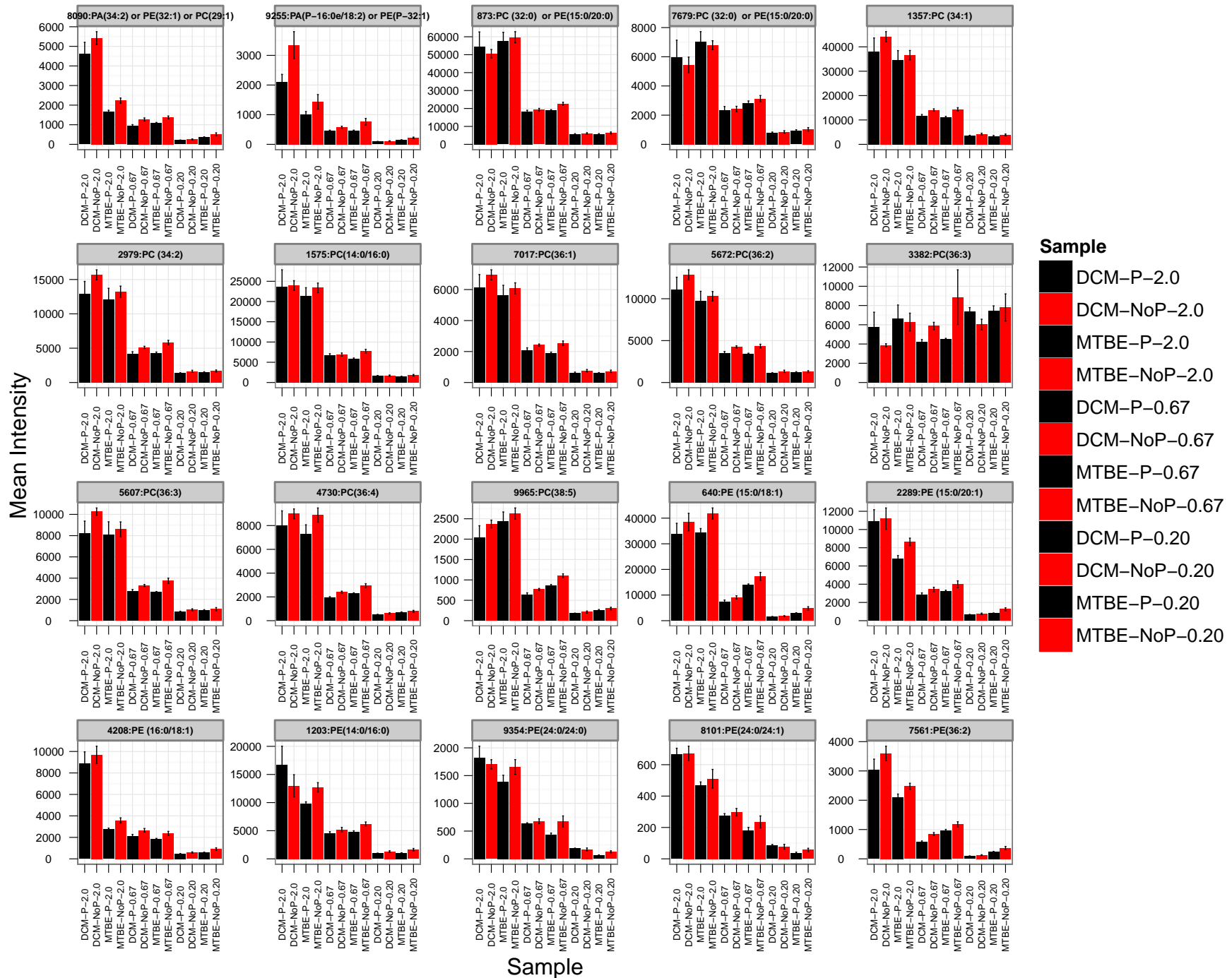


Sample

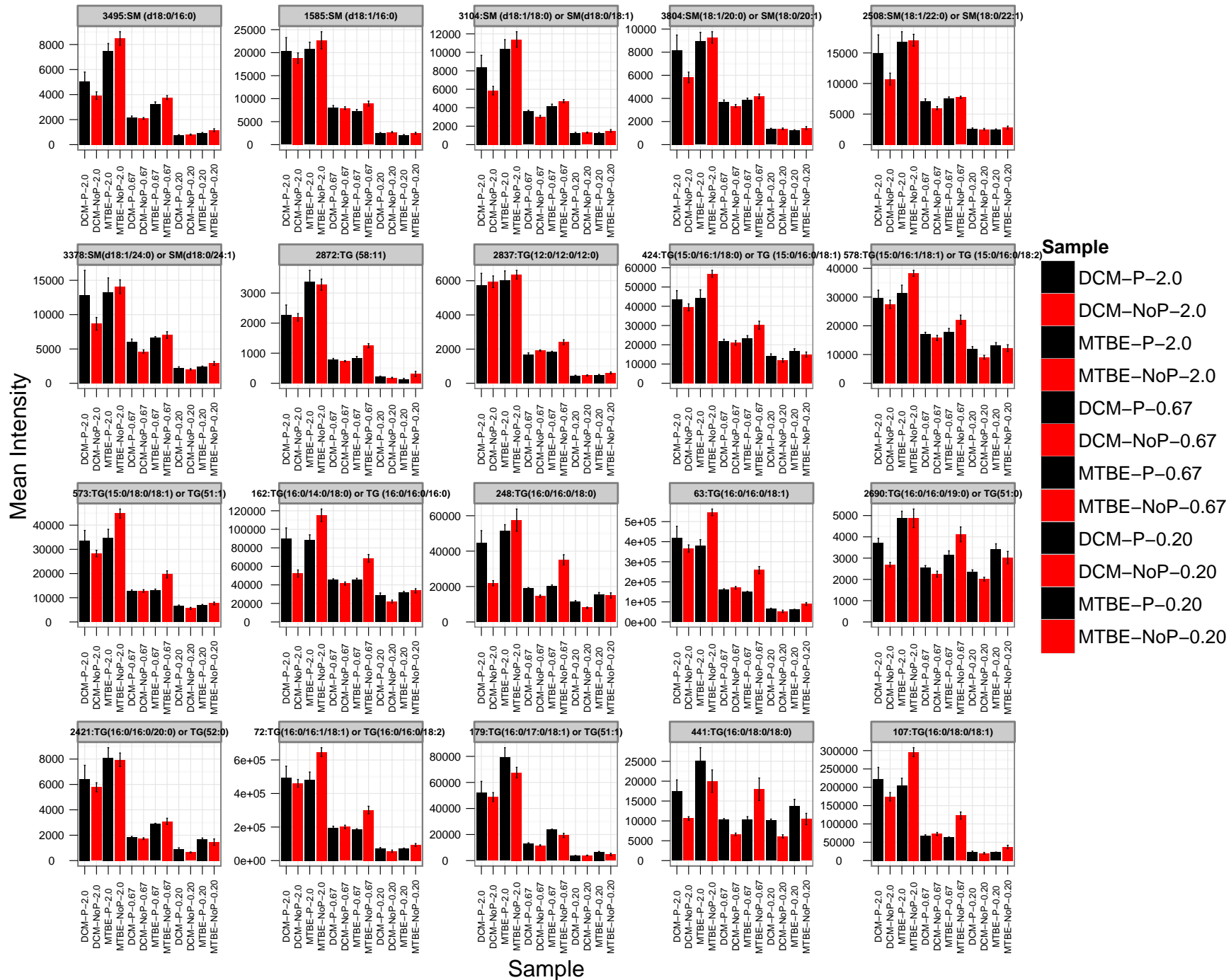
# Barplot of mean intensities of Positive Mode and with Error Bars +/- SE



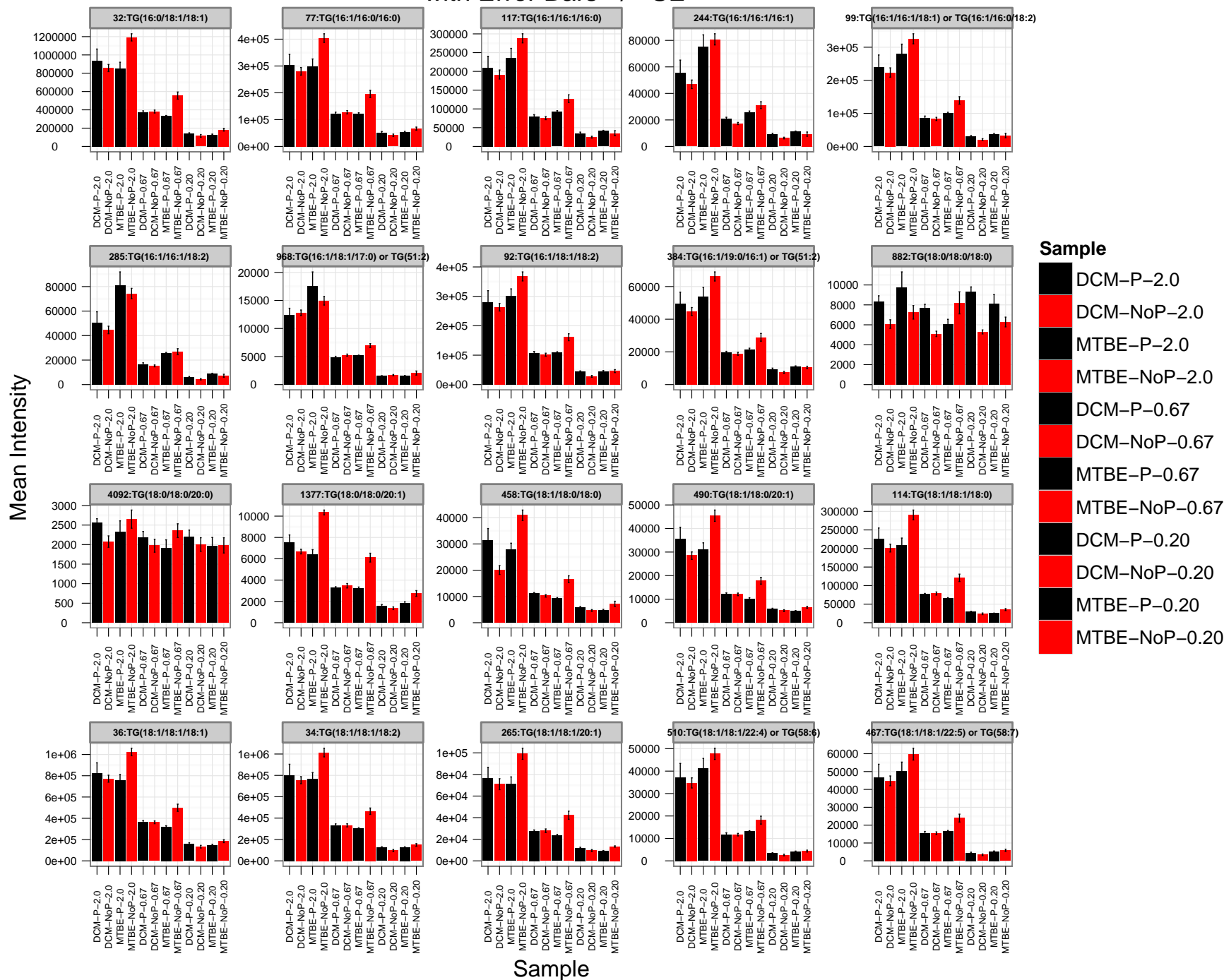
# Barplot of mean intensities of Positive Mode and with Error Bars +/- SE



# Barplot of mean intensities of Positive Mode and with Error Bars +/- SE

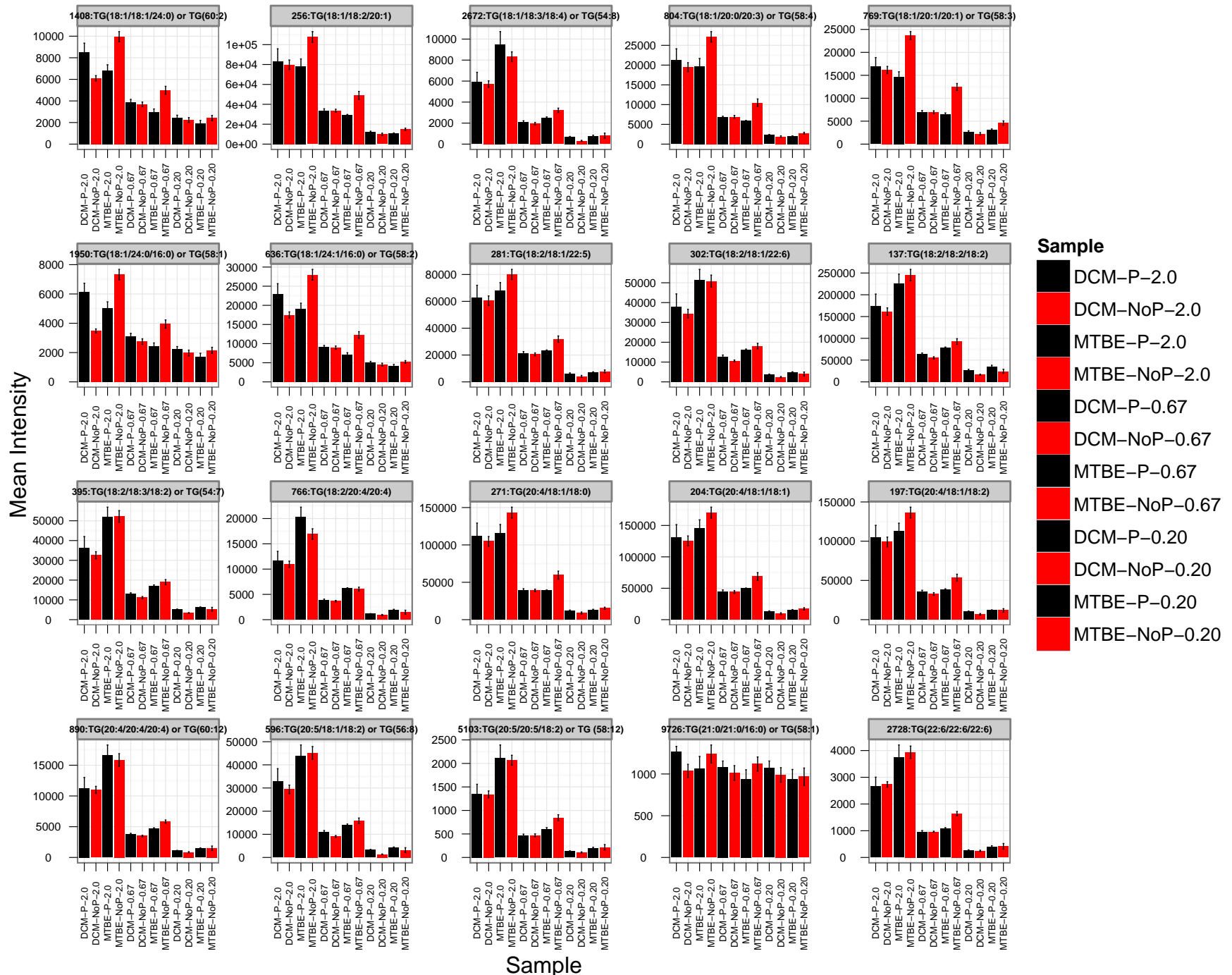


# Barplot of mean intensities of Positive Mode and with Error Bars +/- SE

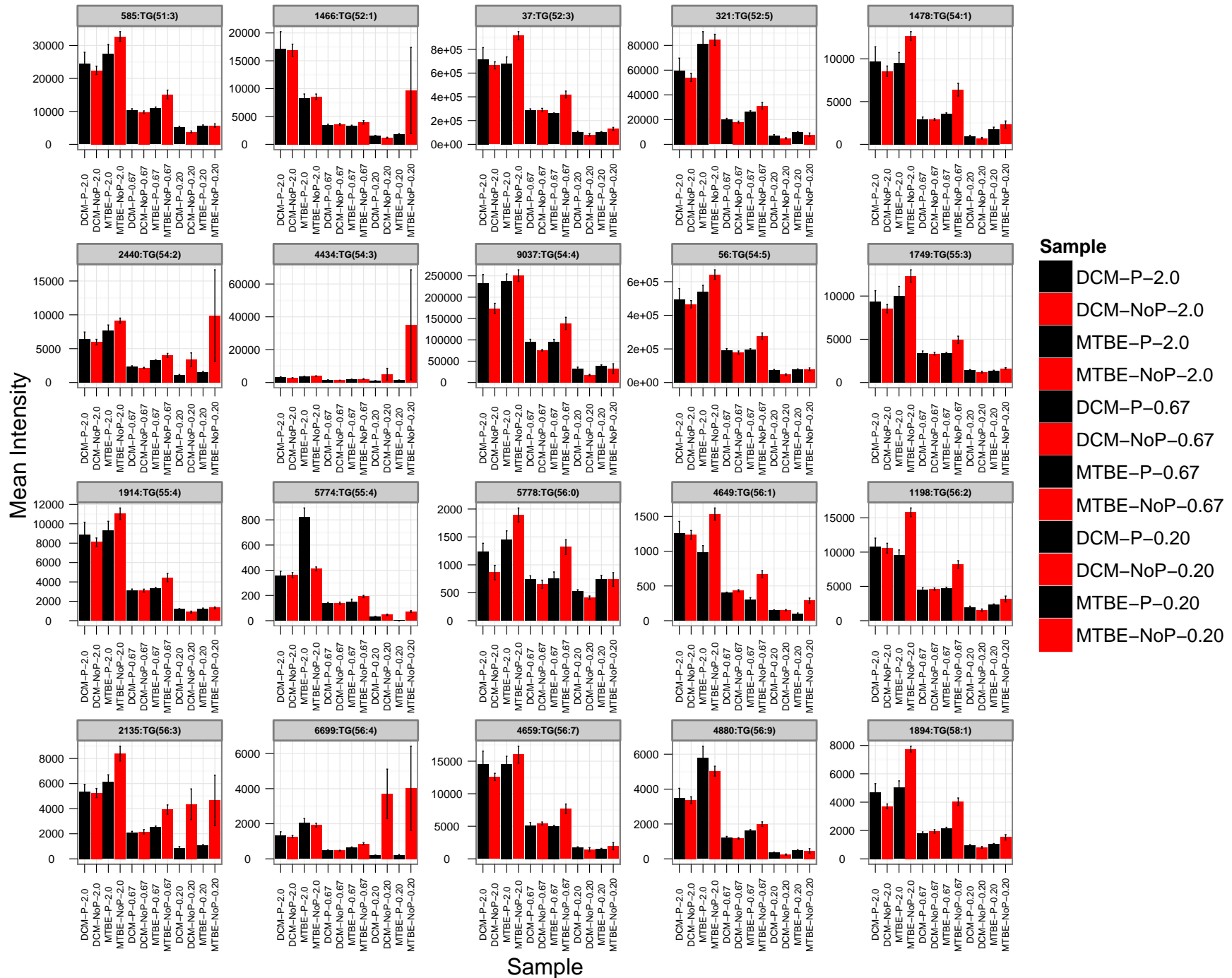




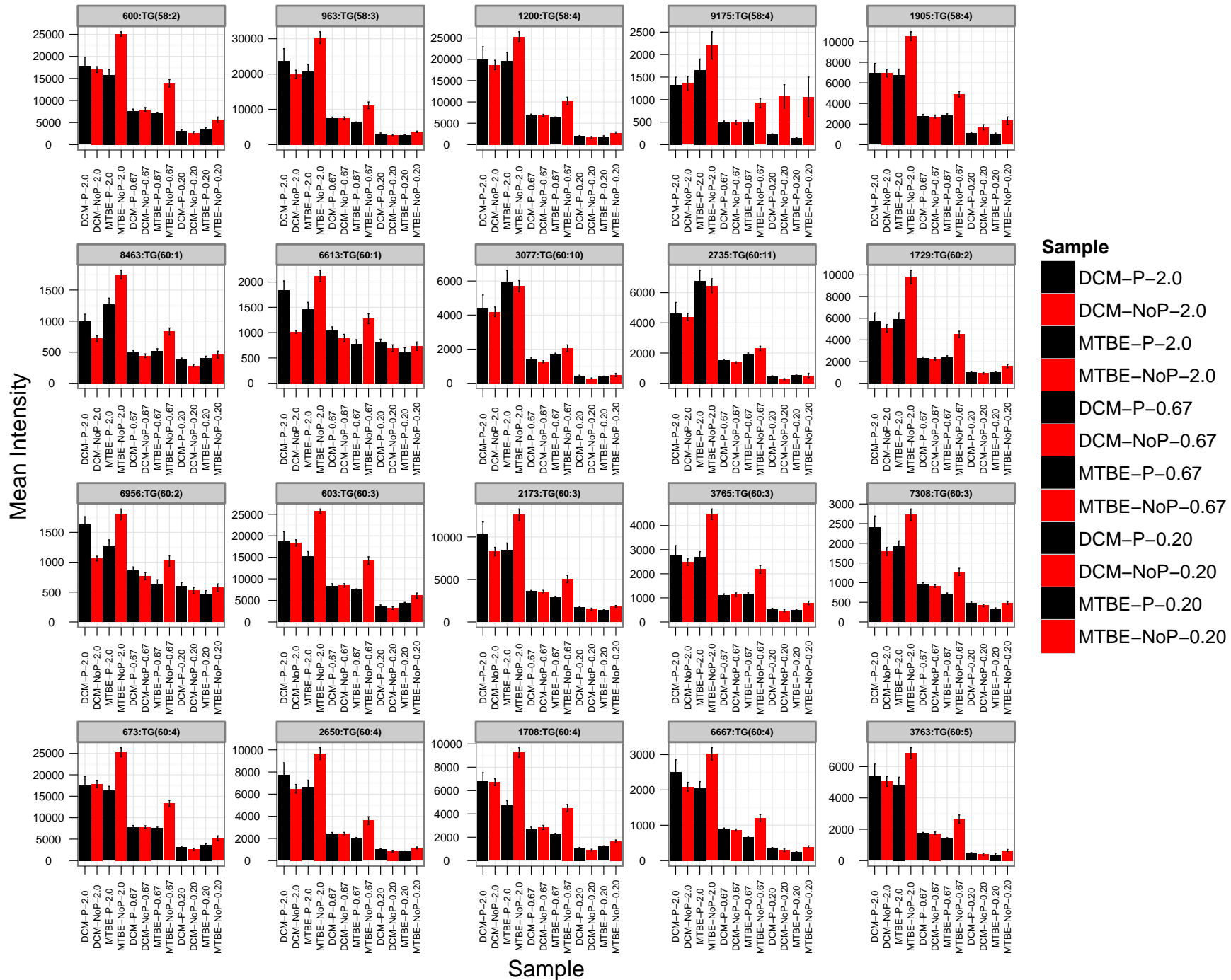
# Barplot of mean intensities of Positive Mode and with Error Bars +/- SE



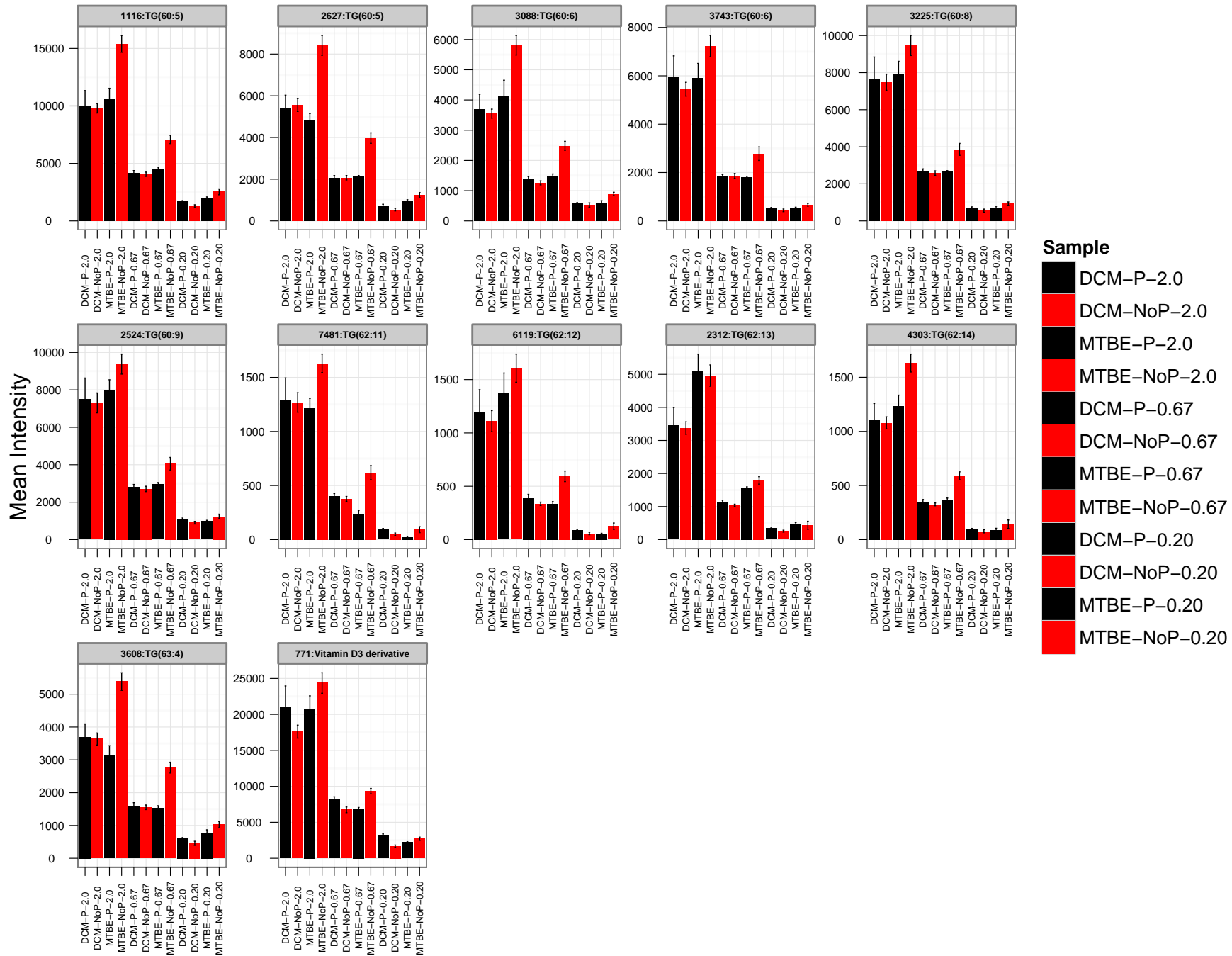
# Barplot of mean intensities of Positive Mode and with Error Bars +/- SE



# Barplot of mean intensities of Positive Mode and with Error Bars +/- SE



# Barplot of mean intensities of Positive Mode and with Error Bars +/- SE



Sample

Supplement Figure 7

Glycerophospholipids (GPs)

Phosphocholine (PC)

PC(14:0/16:0)	+
PC (15:0/15:0)	-
PC(16:0/16:0)	+/-
PC (32:0) or PE(15:0/20:0)	+
PC(18:0/20:4)	-
PC(18:1/16:0)	-
PC(18:1/18:1)	-
PC(18:2/16:0)	-
PC(32:4)	-
PC (34:1)	+
PC (34:2)	+
PC(34:4)	-
PC(36:0)	-
PC(36:1)	+/-
PC(36:2)	+
PC(36:3)	+/-
PC(36:4)	+/-
PC(38:0)	-
PC(38:0)	-
PC(38:1)	-
PC(38:5)	+/-
PC(40:0)	-

plasmalogenphosphocholine (plsPC)

PC(o-34:1) or PC(p-34:0)	-
PC(p-36:0)	-
PC(p-38:0) or PC(o-38:1)	-
PC(p-38:1) or PC(o-38:2)	-

lysoPhosphocholine (LysoPC)

lysoPC(15:0) or LysoPE(18:0)	+
lysoPC(16:0) or PAF C-14	+/-
lysoPC(18:0) or PAF C-16	+/-
lysoPC(18:1) or PAF C-16:1	+/-
lysoPC(o-18:0)	+
lysoPC(20:0) or PAF C-18	-

Phosphoethanolamine (PE)

PE(14:0/16:0)	+/-
PE(16:0/12:0)	-
PE(32:0)	-
PE(16:0/16:0)-NME-2	-
PE(16:0/18:1)	+/-
PE(16:0/18:2)	-
PE(32:1)	-
PE(17:1/16:0)	-
PE(18:0/15:0)	-
PE(18:0/18:0)	-

plasmalogenphosphoethanolamine (plsPE)

PE(p-32:1)	-
PE(p-16:0/18:1)	-
PE(p36:0)	-
PE(p-36:1)	-
PE(p-36:4)	-
PE(20:3/p-18:0)	-
PE(20:4/p-18:0)	-

lysoPhosphoethanolamine (LysoPE)

PE(18:1/18:0)	-	lysoPE(18:1)	+
PE(18:1/18:1)	+/-	lysoPE(20:0) or LysoPC(17:0)	+
PE(19:1/16:0)	-	lysoPE(16:0)	-
PE(19:1/17:1)	-		
PE(20:3/18:0)	-		
PE(20:4/18:0)	-		
PE (15:0/18:1)	+		
PE (15:0/20:1)	+		
PE(36:1)	-		
PE(36:2)	-		
PE(37:2)	-		
PE(38:1)	-		
PE(38:2)	-		
PE(38:2)	-		
PE(38:2)	-		
PE(40:4)	-		
PE(40:5)	-		
PE(24:0/24:0)	+		
PE(24:0/24:1)	+		

### Phosphoserine (PS)

PS(16:1/16:1)	-
PS(18:0/20:4)	-
PS(32:2)	-
PS(34:3)	-
PS(36:3)	-
PS(36:4)	-

### Phosphoinositol (PI)

PI(16:0/16:0)	-
PI(18:0/16:1)	-
PI(18:0/20:2)	-
PI(18:0/20:3)	-
PI(18:0/20:4)	-
PI(18:1/16:1)	-
PI(18:1/18:1)	-
PI(36:4)	-
PI(38:5)	-

### Phosphoglycerol (PG)

PG(14:0/14:0)	-
PG(14:0/16:0)	-
PG(14:0/18:0)	-
PG(16:0/16:0)	-
PG(16:0/16:1)	-
PG(16:0/18:1)	-
PG(16:0/18:1)	-

PG(18:0/18:2)	-
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PG(18:1/18:0)	-
PG(18:1/18:1)	-
PG(36:3)	-
PG(36:4)	-
PG(40:7)	-
PGP(36:5)	-

### lysoPhosphoglycerol (LysoPG)

lysoPG(14:0)	-
lysoPG(16:0)	-

### lysoPhosphatidic Acid (LysoPA)

LysoPA(16:0)	-
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lysoPA(18:0) -

### Fatty Acyls (FAs)

#### Free Fatty Acids (FFA)

FA(15:0)	-
FA(16:0)	-
FA(17:0)	+/-
FA(17:1)	-
FA(18:0)-OH	-
FA(18:1)	-
FA(18:1)-(18:0)-OH dimer	-
FA(18:1)-dimer	-
FA(18:1)-OH	-
FA(18:2)	-
FA (19:0)	+
FA (19:1)	+
FA(20:3)	-
FA(20:4)	-
FA(22:4)	-
FA(22:5)	-
FA(22:6)	-
FA(24:0)	-
FA(24:1)	-

### Sphingolipids (SLs)

#### Sphingomyelins (SM)

SM(18:0/14:0)	-
SM(18:0/16:0)	-
SM(18:0/16:1)	+/-
SM(18:0/18:0)	-
SM(18:0/18:1)	+/-
SM(18:1/14:0)	-
SM(d18:0/20:0)	-
SM(d18:0/22:0)	-
SM(d18:1/20:0)	+/-
SM(d18:1/22:0)	+/-
SM(d18:1/23:0)	-
SM(d18:1/24:1)	-
SM(d18:1/24:0)	+/-

## Ceramides (Cer)

Cer(18:0/14:0)	+
Cer(18:1/14:0) or Cer(18:0/14:1)	+
Cer (d18:0/16:0)	+/-
Cer(18:0/18:0)	+/-
Cer(18:0/20:0)	+/-
Cer(18:0/22:0)	+/-
Cer(18:0/23:0)	+/-
Cer(18:0/24:0)	+/-
Cer(d18:0/25:0)	-
Cer(18:0/26:1) or Cer(18:1/26:0)	+/-
Cer(d18:0/26:1)	-
Cer (d18:1/16:0)	+/-
Cer(18:0/18:1) or Cer(18:1/18:0)	+/-
Cer(18:1/18:1)	+/-
Cer(18:1/20:0) or Cer(18:0/20:1)	+/-
Cer(18:1/22:0) or Cer(18:0/22:1)	+/-
Cer(18:1/22:1)	+/-
Cer(d18:1/23:0) or Cer(d18:0/23:1)	-
Cer(18:1/24:0)	+/-
Cer(18:0/24:1)	-
Cer(18:1/24:1)	+/-
Cer(d18:1/24:1)	-
Cer(18:1/26:1)	+/-
Cer(t18:0/16:0)	+/-
Cer(t18:0/24:0)-OH	-
Cer(t18:0/26:0)-OH	-
GlcCer(d18:0/22:0)	-
GlcCer(d18:0/24:0)	-
GlcCer(d18:1/14:0)	-
GlcCer(d18:1/16:0)	+/-
GlcCer(d18:1/18:0)	-
GlcCer(d18:1/20:0)	-
GlcCer(d18:1/22:0)	-
GlcCer(d18:1/24:0)	+/-
GlcCer(d18:1/24:1)	+/-
GlcCer(d18:1/25:0)	-
GlcCer(d18:1/26:0)	-
LacCer(d18:1/16:0)	+
Lactosylceramide (d18:1/20:0)	+/-
Lactosylceramide (d18:1/22:0)	+/-



Lactosylceramide (d18:1/24:0)	+/-
Lactosylceramide (d18:1/24:1)	+/-
LactosylCer(d18:1/16:0)-two sugars	-
LactosylCer(d18:1/18:0)-two sugars	-
Fucalalpha1-2Galbeta1-3GalNAcbeta1-4Galbeta1-4Glcbeta-Cer(d18:1/24:0)	-
Galalpha1-3(Galbeta1-4GlcNAcbeta1-6)Galbeta1-4Glcbeta-Cer(d18:1/16:0)	-
NeuAcalpha2-8NeuAcalpha2-3Galbeta1-4Glcbeta-Cer(d18:1/24:0) or Ganglioside GD3 (d18:1/24:0)	-
Tetrahexosylceramide (d18:1/24:1(15Z)) or Ganglioside GA1 (d18:1/24:1(15Z))	-

### Gangliosides (Gan)

Ganglioside GM3 (d18:0/22:1(13Z)) or (d(18:1/22:0)	-
Ganglioside GM3 (d18:0/24:1(15Z)) or (d18:1/24:0)	-
Ganglioside GM3 (d18:1/16:0)	-
Ganglioside GM3 (d18:1/24:1(15Z))	-

### Glycerolipids (GLs)

#### Diacylglycerol (DG)

DG(14:0/18:1)	+
DG(14:0/18:2)	+
DG(16:0/18:1)	+
DG(16:0/18:2)	+
DG(18:1/18:0)	+
DG(18:1/18:1)	+
DG(18:1/20:4)	+
DG(18:1/20:5)	+
DG(18:1/22:6)	+
DG(18:2/18:1)	+
DG(18:2/18:2)	+
DG(18:2/20:4)	+
DG(18:2/22:6) or DG(18:3/22:5)	+
DG(22:6/22:6)	+
DG(36:5)	+
DG(36:6)	+
DG(40:5)	+
DG(40:6)	+

DG(40:6) +

### Triacylglycerol (TG)

TG (58:11)	+	TG(51:3)	+
TG(12:0/12:0/12:0)	+	TG(52:3)	+
TG(15:0/16:1/18:0) or TG (15:0/16:0/18:1)	+	TG(52:5)	+
TG(15:0/16:1/18:1) or TG (15:0/16:0/18:2)	+	TG(54:1)	+
TG(15:0/18:0/18:1) or TG(51:1)	+	TG(54:4)	+
TG(16:0/14:0/18:0) or TG (16:0/16:0/16:0)	+	TG(54:5)	+
TG(16:0/16:0/18:0)	+	TG(55:3)	+
TG(16:0/16:0/18:1)	+	TG(55:4)	+
TG(16:0/16:0/20:0) or TG(52:0)	+	TG(56:2)	+
TG(16:0/16:1/18:1) or TG(16:0/16:0/18:2)	+	TG(56:7)	+
TG(16:0/17:0/18:1) or TG(51:1)	+	TG(56:9)	+
TG(16:0/18:0/18:0)	+	TG(58:2)	+
TG(16:0/18:0/18:1)	+	TG(58:3)	+
TG(16:0/18:1/18:1)	+	TG(58:4)	+
TG(16:1/16:0/16:0)	+	TG(58:5)	+
TG(16:1/16:1/16:0)	+	TG(60:1)	+
TG(16:1/16:1/16:1)	+	TG(60:10)	+
TG(16:1/16:1/18:1) or TG(16:1/16:0/18:2)	+	TG(60:11)	+
TG(16:1/16:1/18:2)	+	TG(60:3)	+
TG(16:1/18:1/17:0) or TG(51:2)	+	TG(60:4)	+
TG(16:1/18:1/18:2)	+	TG(60:5)	+
TG(16:1/19:0/16:1) or TG(51:2)	+	TG(60:5)	+
TG(18:0/18:0/18:0)	+	TG(60:6)	+
TG(18:0/18:0/20:0)	+	TG(60:8)	+
TG(18:0/18:0/20:1)	+	TG(60:9)	+
TG(18:1/18:0/18:0)	+	TG(62:11)	+
TG(18:1/18:0/20:1)	+	TG(62:12)	+
TG(18:1/18:1/18:0)	+	TG(62:13)	+
TG(18:1/18:1/18:1)	+	TG(62:14)	+
TG(18:1/18:1/18:2)	+	TG(62:2)	+
TG(18:1/18:1/20:1)	+	TG(62:3)	+
TG(18:1/18:1/22:4) or TG(58:6)	+	TG(62:4)	+
TG(18:1/18:1/22:5) or TG(58:7)	+	TG(63:4)	+
TG(18:1/18:1/24:0) or TG(60:2)	+	TG(o-48:0)	+
TG(18:1/18:2/20:1)	+	TG(o-48:1)	+
TG(18:1/18:3/18:4) or TG(54:8)	+	TG(o-48:2)	+
TG(18:1/20:0/20:3) or TG(58:4)	+		
TG(18:1/20:1/20:1) or TG(58:3)	+		

TG(18:1/24:0/16:0) or TG(58:1)	+
TG(18:1/24:1/16:0) or TG(58:2)	+
TG(18:2/18:1/22:5)	+
TG(18:2/18:1/22:6)	+
TG(18:2/18:2/18:2)	+
TG(18:2/18:3/18:2) or TG(54:7)	+
TG(18:2/20:4/20:4)	+
TG(20:4/18:1/18:0)	+
TG(20:4/18:1/18:1)	+
TG(20:4/18:1/18:2)	+
TG(20:4/20:4/20:4) or TG(60:12)	+
TG(20:5/18:1/18:2) or TG(56:8)	+
TG(20:5/20:5/18:2) or TG (58:12)	+
TG(21:0/21:0/16:0) or TG(58:1)	+
TG(22:6/22:6/22:6)	+

Sterol Lipids (STs)

Bile acids and derivatives

Cholic acid  
hydroxy-oxo-cholic acid

Cholesterol Esters (CE)

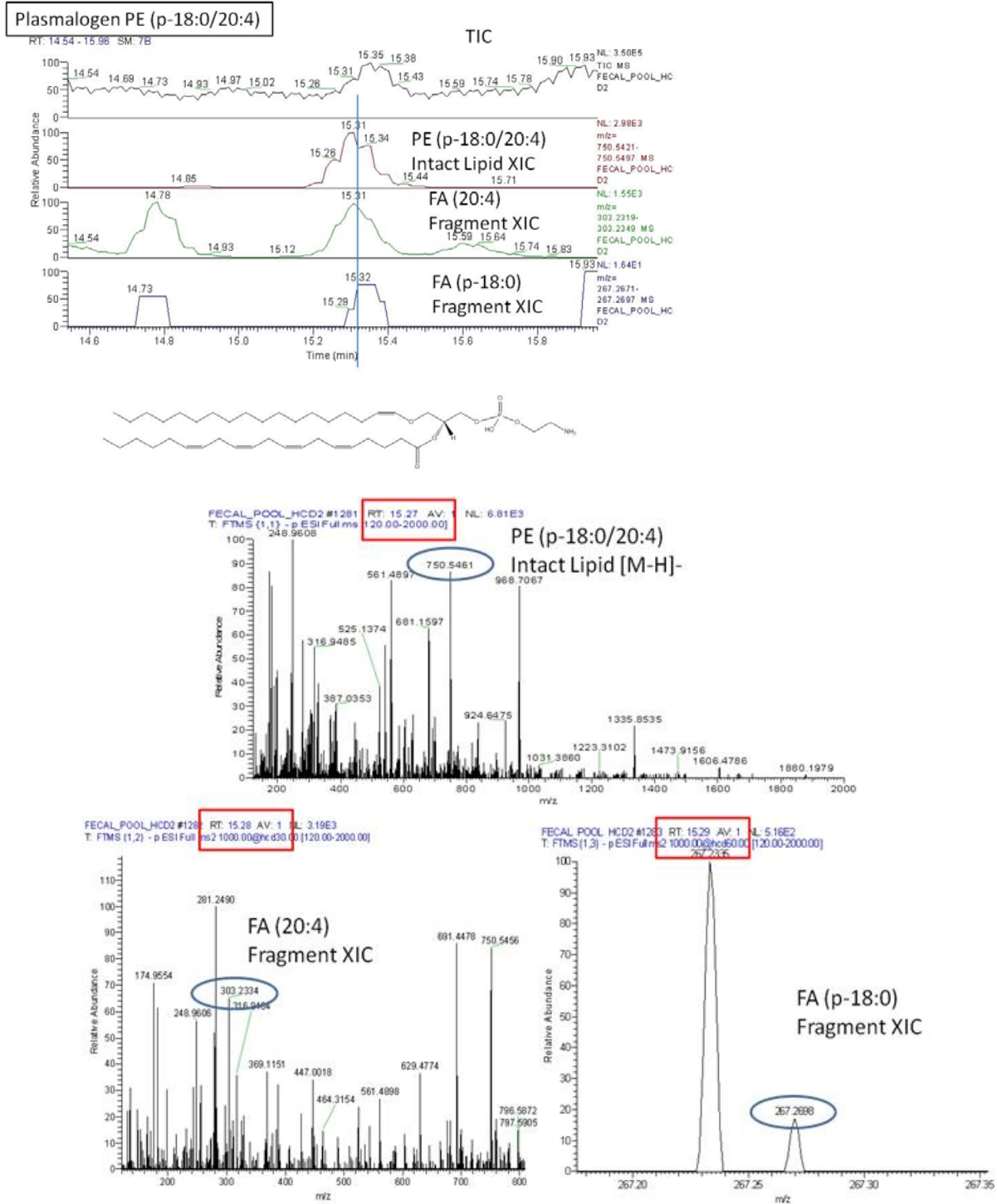
+	CE(18:1)	+
+	CE(18:2)	+

Prenol Lipids (PLs)

coenzyme-Q (CoQ)

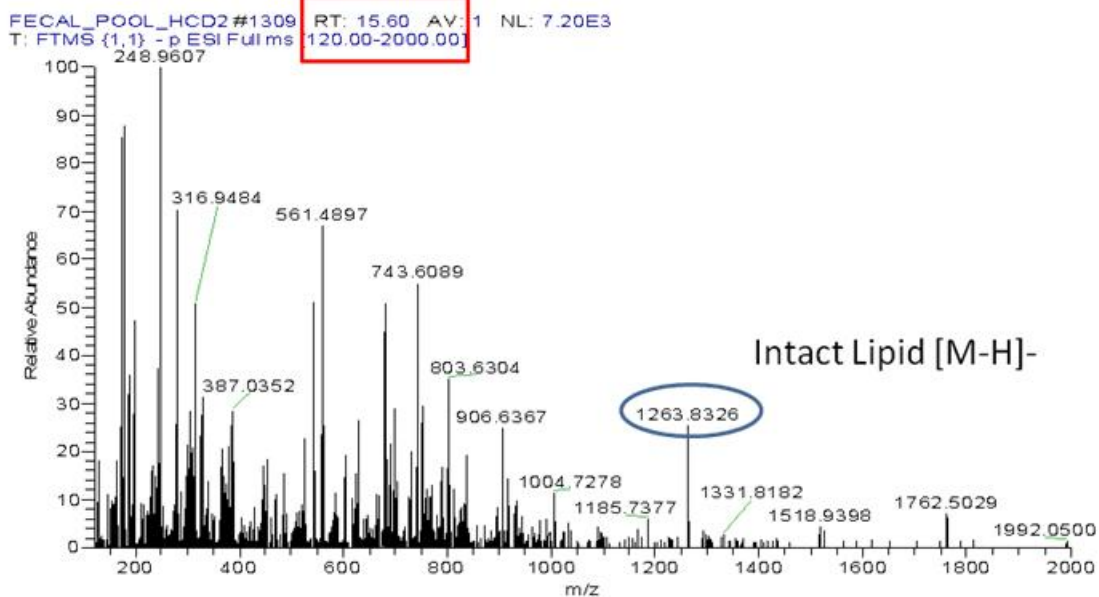
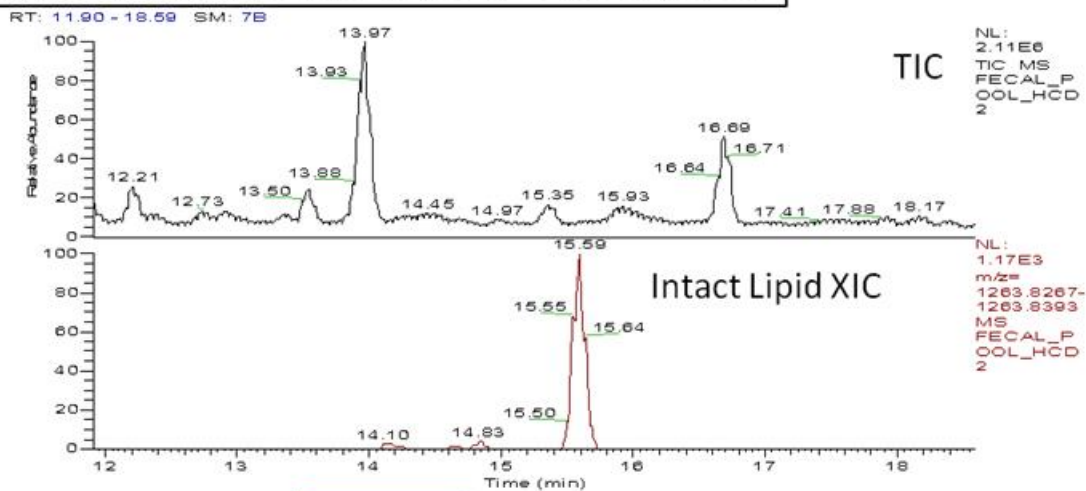
CoQ10	+
CoQ9	+

Supplementary Figure 8 Unique Lipids Fragmentation Analysis

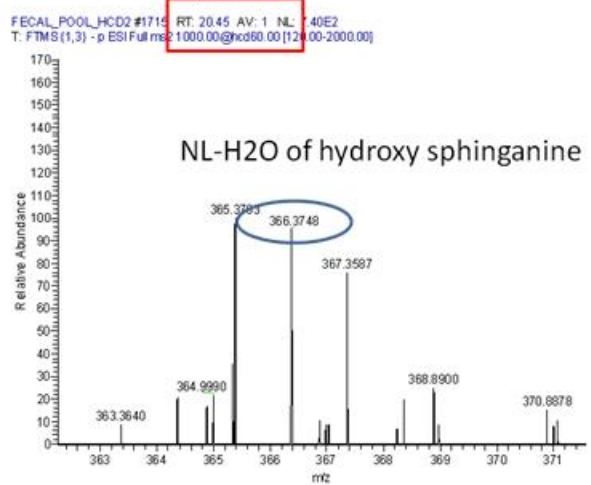
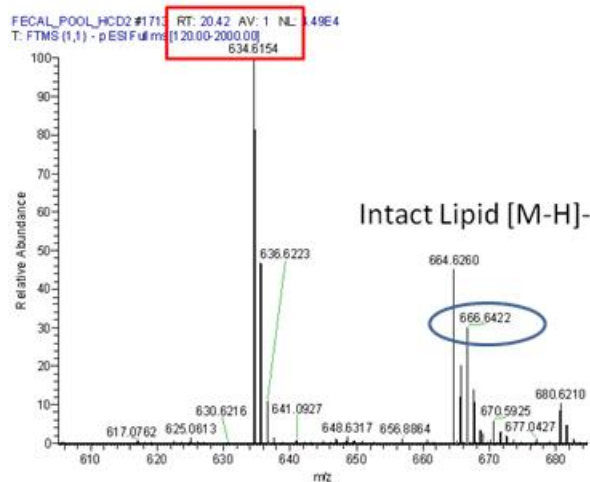
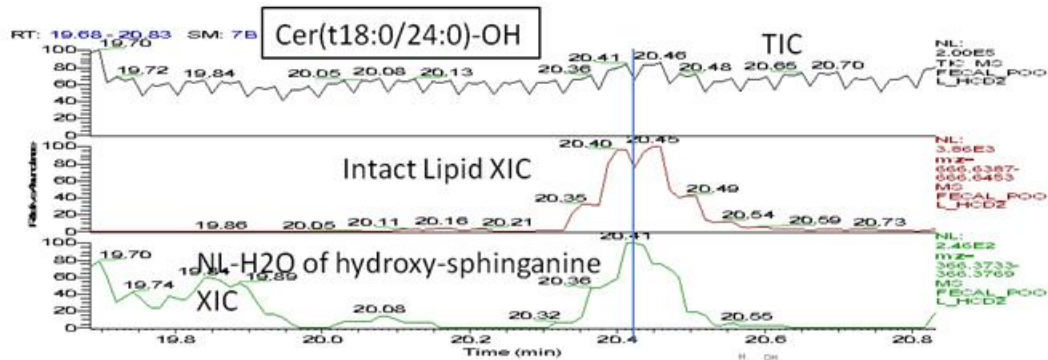


8a. Plasmalogen PE(p18:0/20:4) LC-MS analysis with all ion fragmentation is used to align the three unique masses for the intact lipid at  $m/z$  750.5461, the FA(20:4) fragment at  $m/z$  303.2324 and the FA (p-18:0) at  $m/z$  267.2698 at the RT of 15.3 minutes. We used the RT, exact mass database search and HCD fragmentation to confirm the characterization.

Ganglioside GM3 (d18:0/24:1(15Z)) or (d18:1/24:0)

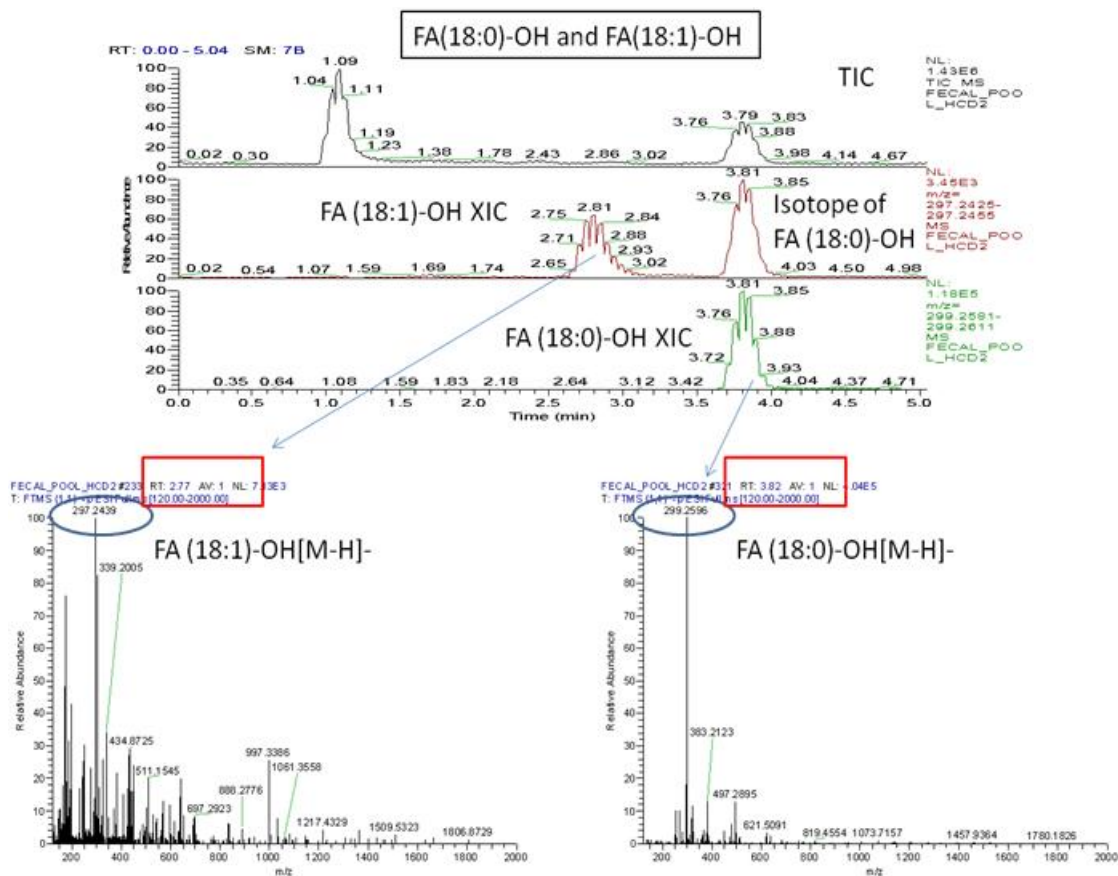


8b. Ganglioside GM3(d18:0/24:1) or (d18:1/24:0) Due to the limited fragmentation of this lower intensity peak, we used the RT, and exact mass database search to provide the provisional identification of this unknown lipid.

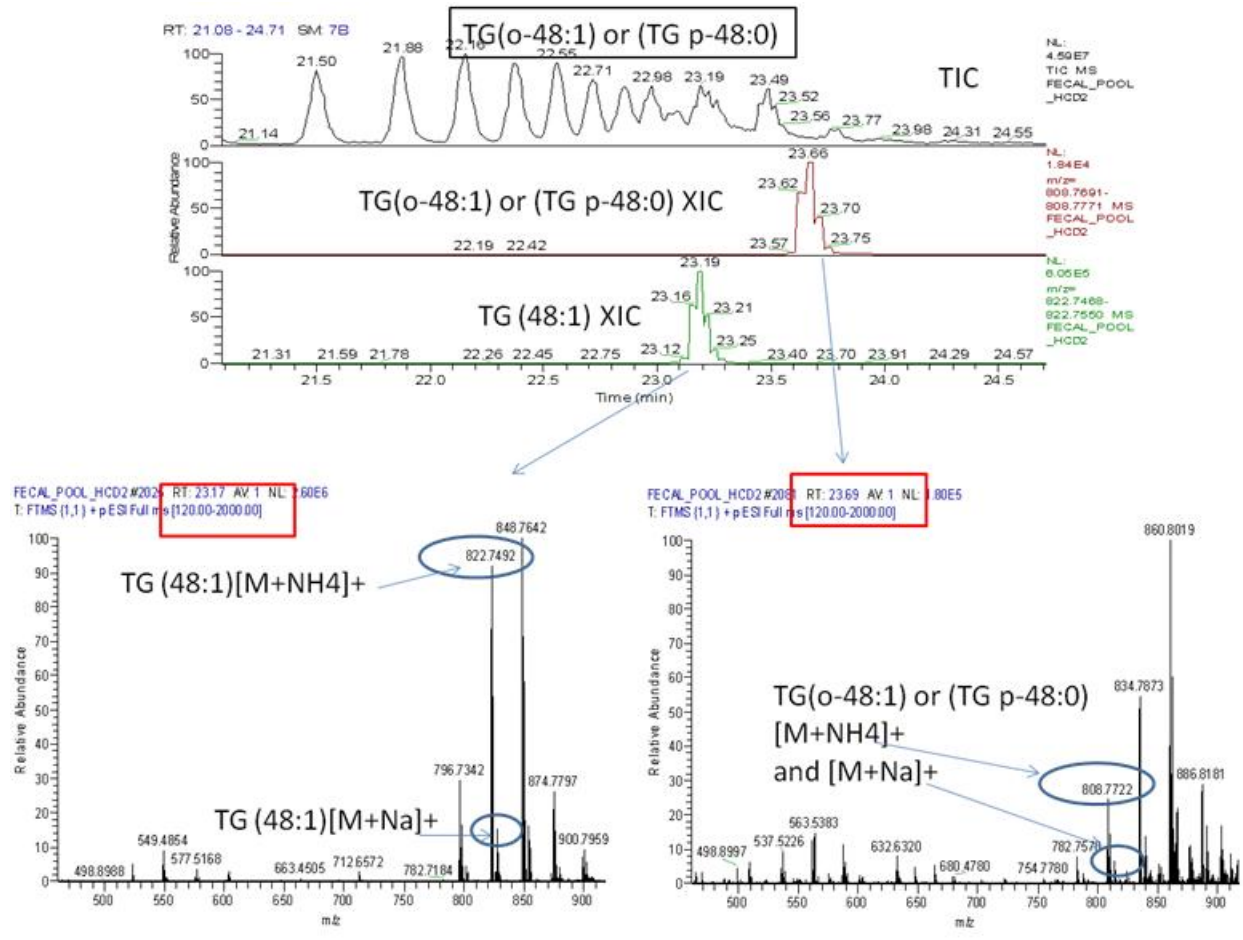


8c. Cer(t18:0/24:0)-OH LC-MS analysis with all ion fragmentation is used to align the intact lipid at  $m/z$  666.6422, with the NL-H<sub>2</sub>O of the hydroxyl sphinganine base at  $m/z$  366.3748 at the RT of 20.4 minutes. We used the RT, exact mass database search and HCD fragmentation to confirm the characterization and compared this fragmentation pattern to what was observed previously in our liver mitochondria ceramide analysis where non-hydroxylated sphinganine Ceramides were characterized<sup>1</sup>.

- (1) Bird, S.; Marur, V.; Stavrovskaya, I.; Kristal, B. *Metabolomics* **2012**, Accepted Online February 21st.



8d. FA (18:0)-OH and FA (18:1)-OH Due to the limited fragmentation of this molecule, we used the RT, and exact mass database searches to provide the provisional identification of these unknown lipids.



8e. TG (o-48:1) or (p-48:0) Due to fragmentation of this molecule which could not be discerned from the other TGs eluting near it, we used the RT, and exact mass database searches to provide the provisional identification of this unknown lipid. We also provide an example of a non-ether or plasmalogen-TG to compare the relative RT and isotope pattern, which further confirms our identification.