In [1]:
```
#Set notebook figure print parameters
options(repr.plot.width=20, repr.plot.height=80, repr.plot.res = 400, repr.plot.quality = 500, repr.plot.pointsize = 12)

# Set working directory
setwd('/Users/leesh/Documents/MS analysis pipeline/Benezra_pilot oxidised lipids/Full set/')

#install.packages("ggplot2",repos='http://cran.us.r-project.org',dependencies = TRUE)
#install.packages("dplyr",repos='http://cran.us.r-project.org',dependencies = TRUE)
#install.packages("reshape",repos='http://cran.us.r-project.org',dependencies = TRUE)
#install.packages("data.table",repos='http://cran.us.r-project.org',dependencies = TRUE)
#install.packages("reshape2",repos='http://cran.us.r-project.org',dependencies = TRUE)
#install.packages("Polychrome",repos='http://cran.us.r-project.org',dependencies = TRUE)
#install.packages("here",repos='http://cran.us.r-project.org',dependencies = TRUE)
```

In [2]:
```
# Load packages

library ('ggplot2')
library ("data.table")
library("dplyr")
library("reshape")
library('reshape2')
library("Polychrome")
```

```
Attaching package: ‘dplyr’


The following objects are masked from ‘package:data.table’:

    between, first, last


The following objects are masked from ‘package:stats’:

    filter, lag


The following objects are masked from ‘package:base’:

    intersect, setdiff, setequal, union


Attaching package: ‘reshape’


The following object is masked from ‘package:dplyr’:

    rename


The following object is masked from ‘package:data.table’:

    melt


Attaching package: ‘reshape2’


The following objects are masked from ‘package:reshape’:

    colsplit, melt, recast


The following objects are masked from ‘package:data.table’:

    dcast, melt
```

## Set color palette

In [3]:
```
options(repr.plot.width=50, repr.plot.height=10, repr.plot.res = 200, repr.plot.quality = 300, repr.plot.pointsize = 12)
# Create a color palette for the features
set.seed(723451) # for reproducibility
ConditionsPalette <- createPalette(50, c("#0ACFF6", "#f6310a"), M=1000)
#swatch (ConditionsPalette)
ConditionsPalette_list <- as.list(ConditionsPalette)
names(ConditionsPalette) <- NULL
```

# Import data and reshape into tidy format (rows are samples, columns are metabolite intensities)

In [4]:
```
# Import data from lipid annotator csv
All <- read.csv('/Users/leesh/Documents/MS analysis pipeline/Benezra_pilot oxidised lipids/Full set/csvs to combine for P
cells_4T1 <- read.csv('/Users/leesh/Documents/MS analysis pipeline/Benezra_pilot oxidised lipids/Full set/csvs to combine
cells_HCT <- read.csv('/Users/leesh/Documents/MS analysis pipeline/Benezra_pilot oxidised lipids/Full set/csvs to combine
cells_TK1 <- read.csv('/Users/leesh/Documents/MS analysis pipeline/Benezra_pilot oxidised lipids/Full set/csvs to combine
```

In [5]:
```
#### Function to add column signifiying PCDL, a unique name (Compound name - RT - ion species) and  remove empty rows fro
Add_PCDL_Column_RemoveEmpty <- function(df, name) {
df <- df %>% mutate (PCDL = name, UniqueName = paste(Compound.Name, RT, Ion.Species     , sep = '-'))
df <- df[!(df$Lipid.Class==""), ]
}
```

In [6]:
```
# Create dfs to be used for pie charts

All <-  Add_PCDL_Column_RemoveEmpty(All, "All")
cells_4T1 <- Add_PCDL_Column_RemoveEmpty (cells_4T1, "cells_4T1")
cells_HCT <- Add_PCDL_Column_RemoveEmpty (cells_HCT, "cells_HCT")
cells_TK1 <- Add_PCDL_Column_RemoveEmpty (cells_TK1, "cells_TK1")
```

In [7]:
```
## create concatenated master PCDL (including duplicates)

combinedPCDL_allrows <- rbind(All, cells_4T1,cells_HCT,cells_TK1)
```

In [8]:
```
#### Function to summarise number of annotated features)
Summarise <- function(df,group_var) {
group_var <- enquo(group_var)
  summary_df <- df %>%
    dplyr::group_by(!!group_var) %>%
    summarise(n = n())
    return (summary_df)
    }
```

In [9]:
```
Summarise(combinedPCDL_allrows, PCDL)
```

A tibble: 4 × 2

| PCDL | n |
|---|---|
| <chr> | <int> |
| All | 449 |
| cells_4T1 | 426 |
| cells_HCT | 504 |
| cells_TK1 | 348 |

## Individual pie charts for each data table

## summarize data

In [10]:
```
####  Function to select relevant columns

ColumnSelect <- function(df) {
selectdf <- df %>%
    select (Feat.ID, Abundance, Lipid.Class, Score)
    return (selectdf)
    }
```

In [11]:
```
PooledQC_all_cells <- ColumnSelect(All) #enter dfs created above
PooledQC_4T1_cells <- ColumnSelect(cells_4T1)
PooledQC_HCT_cells  <- ColumnSelect(cells_HCT)
PooledQC_TK1_cells <- ColumnSelect(cells_TK1)
```

In [12]:
```
####  Function to select create data tables for pie charts

PieDataTable <- function(df) {
Summary <- df %>%
    group_by(Lipid.Class) %>%
    summarize(Counts = n())
#Add percent
    Counts_percent <- Summary %>%
            mutate(Lipid.Class = factor(Lipid.Class,
            levels = Lipid.Class[length(Lipid.Class):1]),
            cumulative = cumsum(Counts),
            midpoint = cumulative - Counts / 2,
```

```
            labels = paste0(round((Counts/ sum(Counts)) * 100, 2), "%"))
     dataframename <- deparse(substitute(df))
     Counts_percent <- Counts_percent %>% mutate (Cell_method = dataframename)
    return (Counts_percent)
    }
```

In [13]:
```
PieData_All <- PieDataTable (PooledQC_all_cells) #enter dfs created above (selected columns)
PieData_cells_4T1 <- PieDataTable (PooledQC_4T1_cells)
PieData_cells_HCT <- PieDataTable (PooledQC_HCT_cells)
PieData_cells_TK1 <- PieDataTable (PooledQC_TK1_cells)
```

In [14]:
```
# Bind all data together for stacked bar plot faceted by group
Counts_all <- rbind(PieData_All,PieData_cells_4T1, PieData_cells_HCT, PieData_cells_TK1)#enter dfs created above
```

## create df in format for base R pie chart

In [15]:
```
#long > wide for pie chart to keep palette consistent across charts
wide_Counts_all <- dcast(Counts_all, Cell_method ~  Lipid.Class, value.var="Counts")

#Replace NAs with zero (as blanks will have many missing values)
wide_Counts_all <- wide_Counts_all %>%
mutate_if(is.numeric, ~replace(., is.na(.), 0))

#Transpose such that each row is a lipid species annotation
wide_Counts_all <- as.data.frame(t(wide_Counts_all))

#Make first row column names
colnames(wide_Counts_all) <- wide_Counts_all[1,]
wide_Counts_all <- wide_Counts_all[-1, ]

#Change column class to numeric
wide_Counts_all <- wide_Counts_all %>%
  mutate_all(~as.numeric(as.character(.)))

#Make row name Lipid.Class column
wide_Counts_all <- setDT(wide_Counts_all, keep.rownames = "Lipid.Class")
```

In [16]:
```
####  Function to split into separate tables for each lipid annotator file (and pie chart) and add percent

Pie <- function(wide_Counts_all, select_df) {
Pie <- wide_Counts_all %>%
    select (Lipid.Class,all_of(select_df))
colnames(Pie) <- c("Lipid.Class", "Counts")
Pie$Counts <- as.numeric(Pie$Counts)
Pie <- Pie  %>%
    mutate(Lipid.Class = factor(Lipid.Class,
    levels = Lipid.Class[length(Lipid.Class):1]),
    cumulative = cumsum(Counts),
    midpoint = cumulative - Counts / 2,
    labels = paste0(round((Counts/ sum(Counts)) * 100, 2), "%"))
    return (Pie)
    }
```

In [17]:
```
names (wide_Counts_all)
```

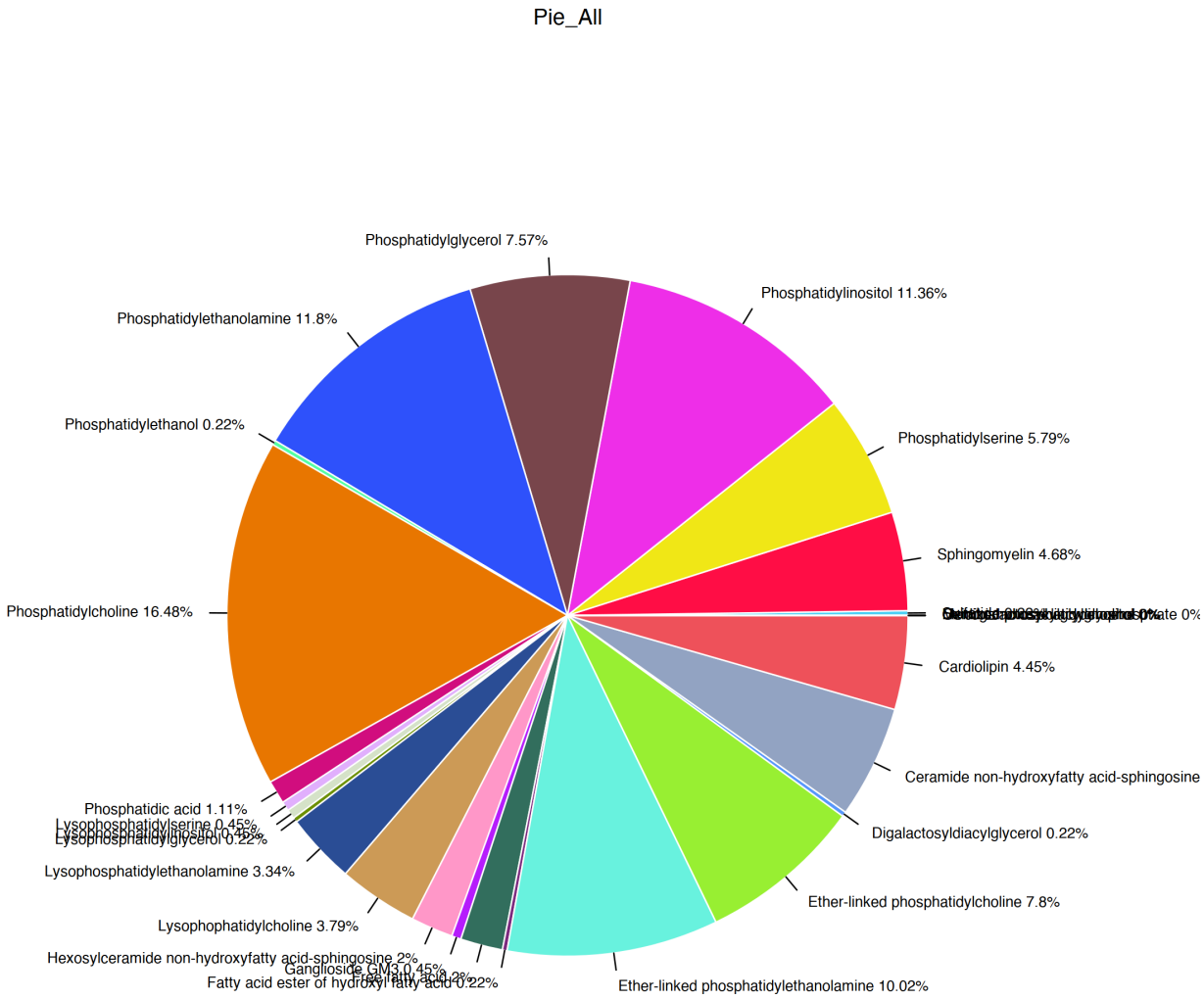
'Lipid.Class' · 'PooledQC_4T1_cells' · 'PooledQC_HCT_cells' · 'PooledQC_TK1_cells' · 'PooledQC_all_cells'

In [18]:
```
Pie_All <- Pie (wide_Counts_all, "PooledQC_all_cells") # df, then column name from df for each lipid annotator file (see
Pie_4T1 <- Pie (wide_Counts_all, "PooledQC_4T1_cells")
Pie_HCT <- Pie (wide_Counts_all, "PooledQC_HCT_cells")
Pie_TK1 <- Pie (wide_Counts_all, "PooledQC_TK1_cells")
```

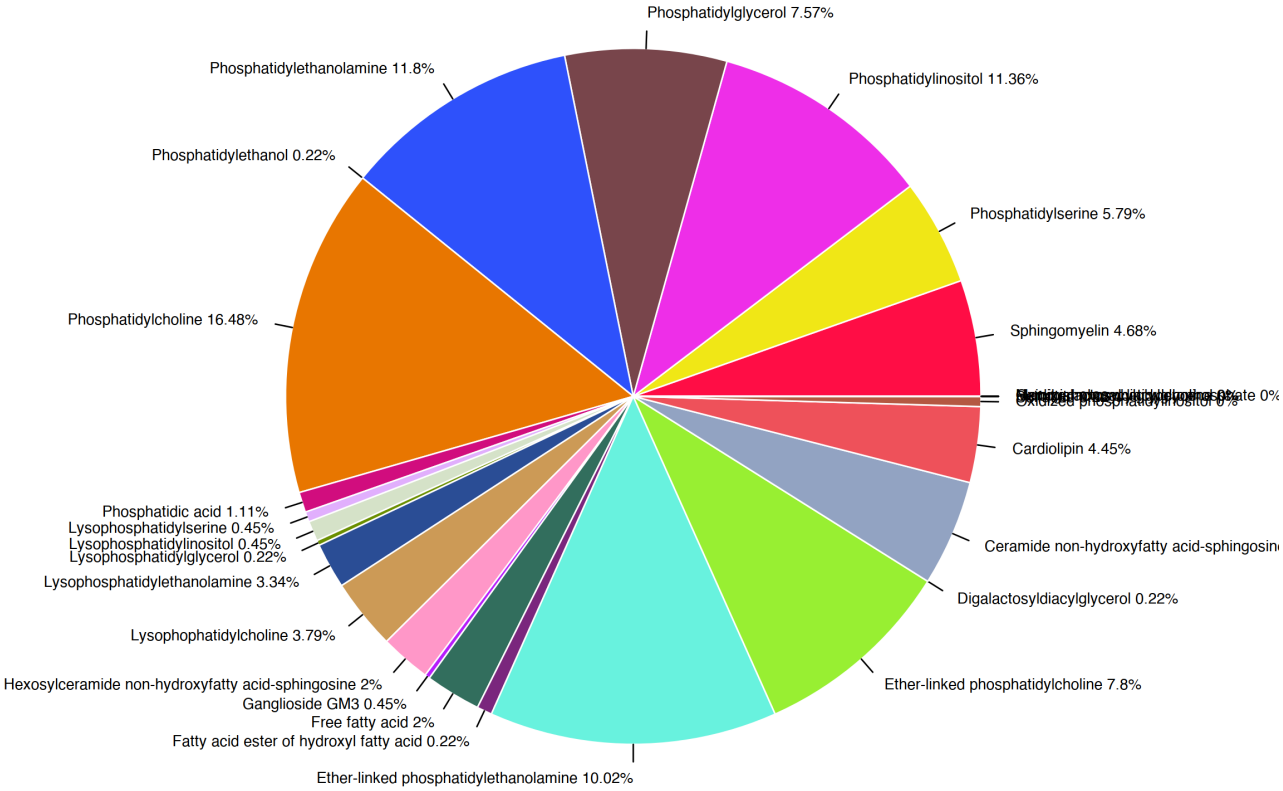
In [19]:
```
####  function to print piechart figures in notebook

Print_Pie <- function(df, na.rm = TRUE, ...) {
  dataframename <- deparse(substitute(df))
pie(df$Counts,
    labels = paste(df$Lipid.Class, sep = " ", Pie_All$labels),
    col = ConditionsPalette,
    border="white",
    main = dataframename,
    radius = 0.7, cex = 0.8)
      }
```

In [20]:
```
options(repr.plot.width=12, repr.plot.height=12, repr.plot.res = 200, repr.plot.quality = 300, repr.plot.pointsize = 12)
```

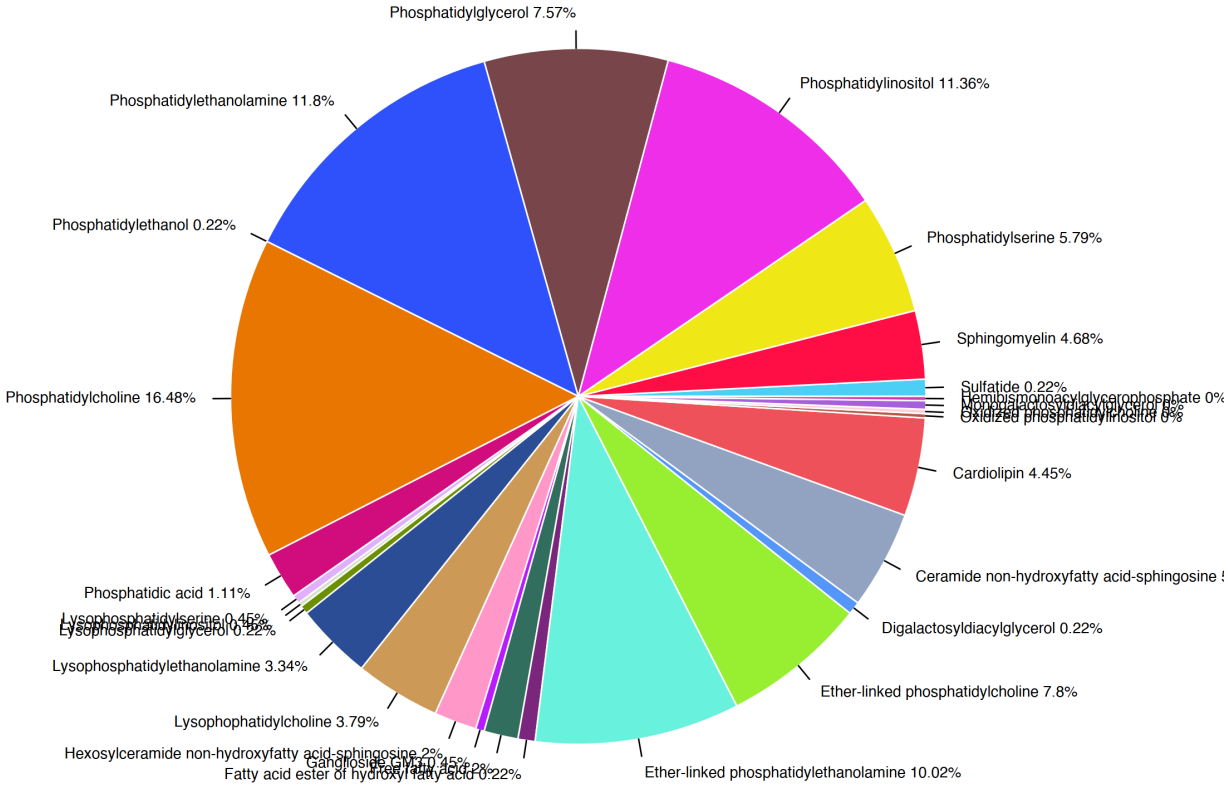
```
Print_Pie(Pie_All)
Print_Pie(Pie_4T1)
Print_Pie(Pie_HCT)
Print_Pie(Pie_TK1)
```

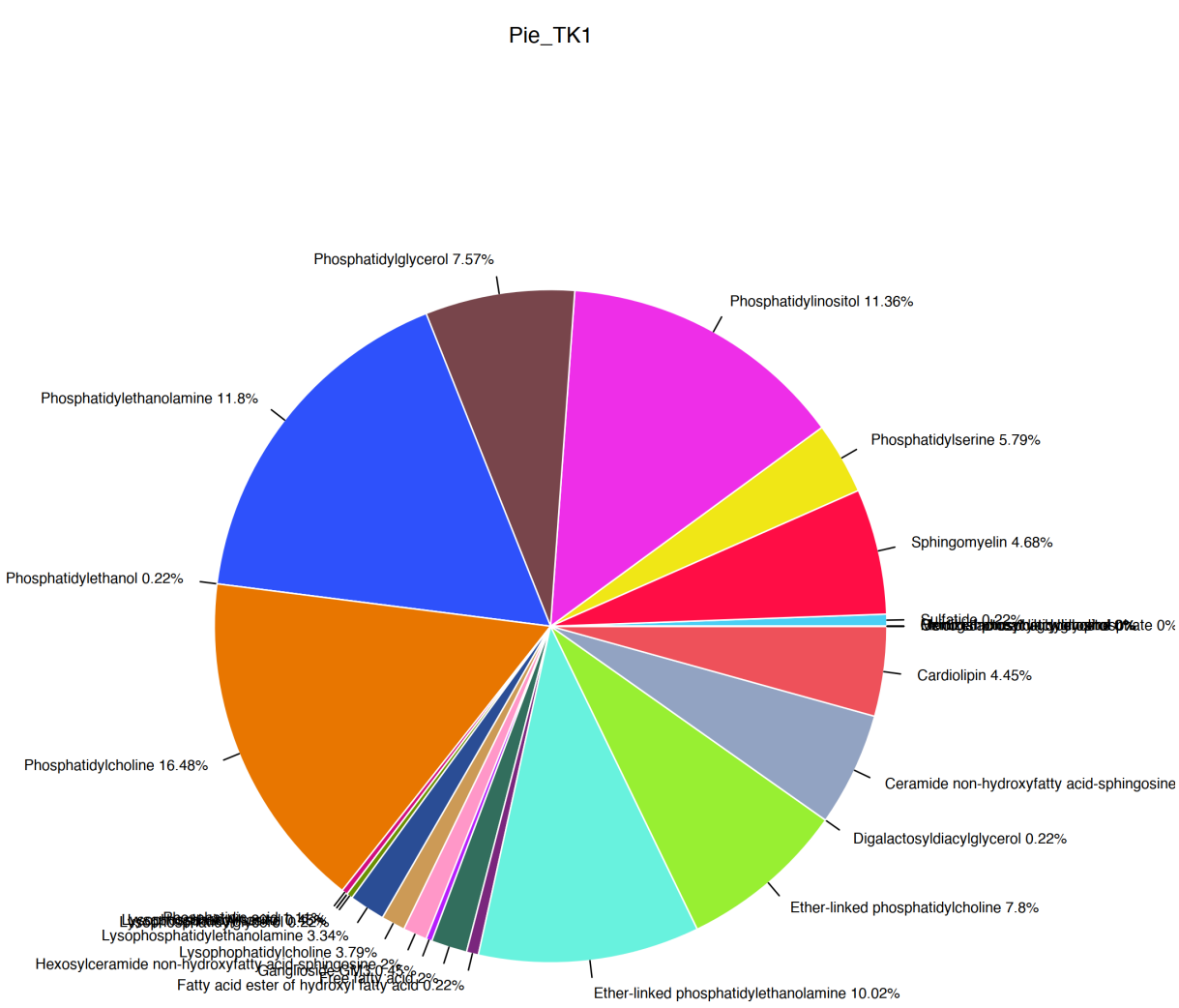


Pie_4T1


Phosphatidylglycerol 7.57%
Phosphatidylinositol 11.36%
Phosphatidylserine 5.79%
Sphingomyelin 4.68%
Cardiolipin 4.45%
Ceramide non-hydroxyfatty acid-sphingosine
Digalactosyldiacylglycerol 0.22%
Ether-linked phosphatidylcholine 7.8%
Ether-linked phosphatidylethanolamine 10.02%
Fatty acid ester of hydroxyl fatty acid 0.22%
Free fatty acid 2%
Ganglioside GM3 0.45%
Hexosylceramide non-hydroxyfatty acid-sphingosine 2%
Lysophosphatidylcholine 3.79%
Lysophosphatidylethanolamine 3.34%
Lysophosphatidylglycerol 0.22%
Lysophosphatidylinositol 0.45%
Lysophosphatidylserine 0.45%
Phosphatidic acid 1.11%
Phosphatidylcholine 16.48%
Phosphatidylethanol 0.22%
Phosphatidylethanolamine 11.8%

Pie_HCT


Phosphatidylglycerol 7.57%
Phosphatidylinositol 11.36%
Phosphatidylethanolamine 11.8%
Phosphatidylethanol 0.22%
Phosphatidylserine 5.79%
Sphingomyelin 4.68%
Sulfatide 0.22%
Phosphatidylcholine 16.48%
Cardiolipin 4.45%
Ceramide non-hydroxyfatty acid-sphingosine
Phosphatidic acid 1.11%
Digalactosyldiacylglycerol 0.22%
Lysophosphatidylglycerol 0.22%
Lysophosphatidylethanolamine 3.34%
Ether-linked phosphatidylcholine 7.8%
Lysophophatidylcholine 3.79%
Hexosylceramide non-hydroxyfatty acid-sphingosine 2%
Ether-linked phosphatidylethanolamine 10.02%
Fatty acid ester of hydroxyl fatty acid 0.22%




Save piecharts as PDF

In [21]:
```
pdf(file = "piecharts of lipid annotations from pooled QCs.pdf", width = 18, height = 12, family = "Helvetica")

Print_Pie(Pie_All)
Print_Pie(Pie_4T1)
Print_Pie(Pie_HCT)
Print_Pie(Pie_TK1)

dev.off()
```

**pdf:** 2

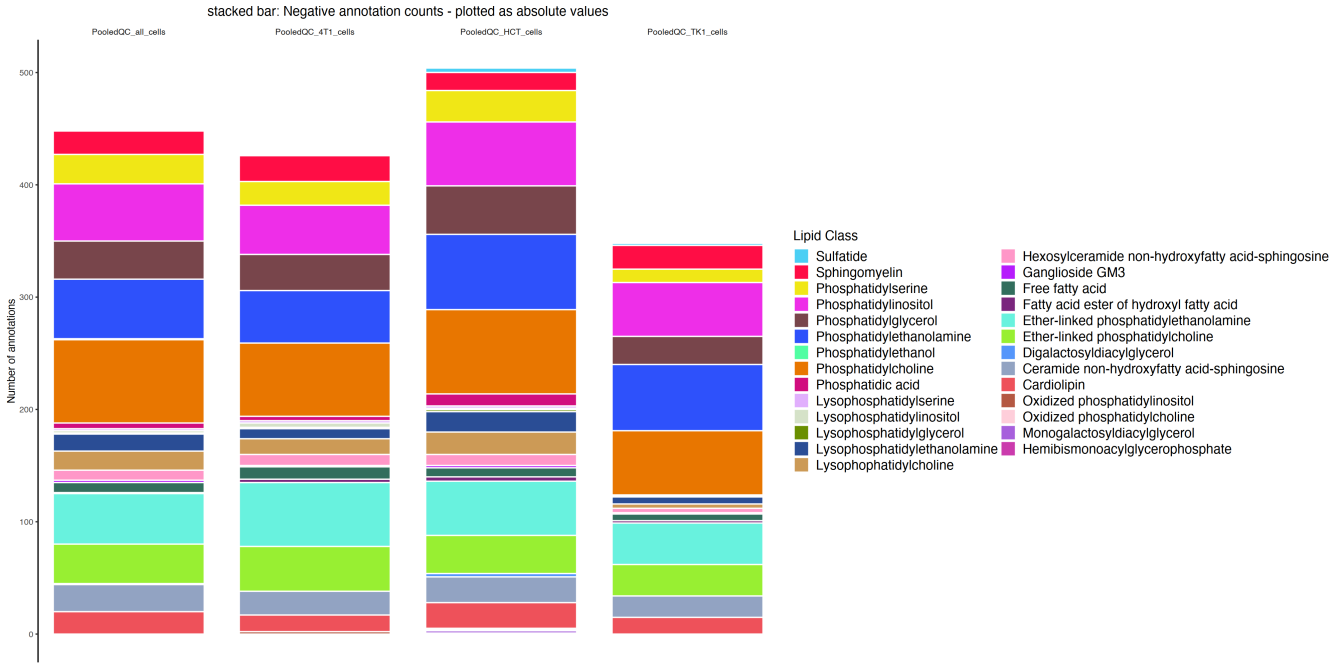
# Create faceted stacked bar charts of absolute annotation counts (rather than relative as in pie charts)

In [22]:
```
unique (Counts_all$Cell_method)
```

'PooledQC_all_cells' · 'PooledQC_4T1_cells' · 'PooledQC_HCT_cells' · 'PooledQC_TK1_cells'

In [23]:
```
Counts_all$Cell_method <- factor(Counts_all$Cell_method, c('PooledQC_all_cells','PooledQC_4T1_cells','PooledQC_HCT_cells'
```

```
options(repr.plot.width=20, repr.plot.height=10, repr.plot.res = 200, repr.plot.quality = 300, repr.plot.pointsize = 12)

ggplot(Counts_all, aes (x="", y = Counts, fill = factor(Lipid.Class))) +
  geom_bar(width = 1, stat = "identity", color = "white") +
  theme_classic() +
 scale_fill_manual(values = ConditionsPalette)+
  theme(plot.title = element_text(hjust=0.5),
        axis.line.x = element_blank(),
        axis.text.x = element_blank(),
        axis.ticks.x = element_blank()) +
  labs(fill = "Lipid Class",
       x = NULL,
       y = "Number of annotations",
       title = "stacked bar: Negative annotation counts - plotted as absolute values") +
facet_wrap(~Cell_method, ncol =4,labeller = label_wrap_gen()) +
  theme(strip.background = element_blank())+
theme(plot.title = element_text(size = 14, face = "bold"),
    legend.title=element_text(size=14),
    legend.text=element_text(size=14))
```




Save stacked barchart as PDF

In [24]:

```
pdf(file = "faceted stacked bar chart absolute annotations_neg.pdf", width = 20, height = 12, family = "Helvetica")

ggplot(Counts_all, aes (x="", y = Counts, fill = factor(Lipid.Class))) +
  geom_bar(width = 1, stat = "identity", color = "white") +
  theme_classic() +
 scale_fill_manual(values = ConditionsPalette)+
  theme(plot.title = element_text(hjust=0.5),
        axis.line.x = element_blank(),
        axis.text.x = element_blank(),
        axis.ticks.x = element_blank()) +
  labs(fill = "Lipid Class",
       x = NULL,
       y = "Number of annotations",
       title = "stacked bar: Negative annotation counts - plotted as absolute values") +
facet_wrap(~Cell_method, ncol =4,labeller = label_wrap_gen()) +
  theme(strip.background = element_blank())+
theme(plot.title = element_text(size = 14, face = "bold"),
    legend.title=element_text(size=14),
    legend.text=element_text(size=14))

dev.off()
```

**pdf:** 2