library(vegan); library(ape); library(ggpubr); library(ggplot2); library (ggrepel)

design <- read.table("gene.design.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F, quote="")

Gene <- read.table("gene.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

taxa <- read.table("Lib.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F, quote="")

dim(taxa)

identical(rownames(design), colnames(Gene))

###########################################################################################################

library (readr); library(edgeR); library(phyloseq)

Gene <- as.matrix(Gene) #rownames (otu\_filter\_16s); #colnames (otu\_filter\_16s)

dim(Gene)

##### TMM normalize #####

edgeR.gene<- DGEList(counts= Gene,

group=design$Trt )

edgeR.gene <- calcNormFactors(edgeR.gene)

Gene\_norm <- **cpm**(edgeR.gene, normalized.lib.sizes=T, log=F)

pca <- rda( t(Gene\_norm), scale = T)

s.pca = as.data.frame(pca$CA$u[, 1:2])

s.pca$Trt = design$Trt

s.pca$sample = rownames(s.pca)

e.pca= pca$CA$v[, 1:2]

PC1 = round(pca$CA$eig[1]/sum(pca$CA$eig)\*100, 2)

PC2 = round(pca$CA$eig[2]/sum(pca$CA$eig)\*100, 2)

**write.table**(s.pca, file="PCA.Coord.txt", sep="\t",quote=F)

PCA.P <- ggplot(data= s.pca, aes(PC1, PC2, color= Trt))+

geom\_point(size=4, shape=16) +

geom\_text\_repel(data= s.pca, aes(PC1, PC2, label=sample), colour="black", size=5) +

xlab(paste("PCA1 (", PC1,"%)")) +

ylab(paste("PCA2 (", PC2,"%)")) +

**stat\_ellipse**(aes(group=Trt, fill=Trt), type="norm", linewidth=0.5, alpha=0.2, level=0.65, geom="polygon", lty="dashed", color=NA) +

xlim(-0.55, 0.45) +

ylim(-0.75, 0.61) +

geom\_hline(yintercept=0, linetype=3, linewidth=1, color='gray')+

geom\_vline(xintercept=0, linetype=3, linewidth=1, color='gray')+

theme\_bw()+

theme(panel.grid=element\_blank(), plot.title = element\_text(face="bold", hjust = 0.5),

axis.text=element\_text(size=12, color="black"), axis.title=element\_text(size=12), #face="bold"

legend.title=element\_text(size=12), legend.text=element\_text(size=12),

legend.key = element\_blank(), legend.background= element\_blank() )

PCA.P

####

library(vegan); library(ape); library(ggpubr); library(ggplot2); library (ggrepel); library (dplyr); library(DESeq2)

design <- read.table("gene.design.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F, quote="")

Gene <- read.table("gene.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

taxa <- read.table("Lib.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F, quote="")

dim(taxa)

Gene <- as.matrix(Gene) ; dim(Gene)

design.Sel <- **filter**(design, Trt %in% c("DCon", "DBC") )

Gene.Sel <- Gene[, rownames(design.Sel)]

Gene.Sel <- round(Gene.Sel, 0)

Gene.Sel <- Gene.Sel [rowSums(Gene.Sel)>0, ]

CK.rep=4; Trt.rep=4

#############################################################################

data <- as.data.frame(Gene.Sel)

Mydesign <- data.frame(Trt = factor(c(rep(c('D'), Trt.rep), rep(c('A'), CK.rep)), levels = c('D', 'A')))

dds <- DESeqDataSetFromMatrix( countData=data, colData = Mydesign , design= ~Trt )

dds1 <- DESeq(dds, fitType = 'mean', minReplicatesForReplace = 7, parallel = F)

res <- results(dds1, contrast = c('Trt', 'D', 'A'))

res

res1 <- data.frame(res, stringsAsFactors = FALSE, check.names = FALSE)

tt\_enrich <- as.data.frame(res1)

Altered.gene <- tt\_enrich[abs(tt\_enrich$log2FoldChange) > 1, ]

Altered.gene <- Altered.gene [Altered.gene $pvalue < 0.01, ]

Altered.gene <- Altered.gene [Altered.gene $ padj < 0.01, ]

Altered.gene.BC<- Altered.gene

####################################################

design.Sel <- **filter**(design, Trt %in% c("RCMN", "RCon"))

Gene.Sel <- Gene[, rownames(design.Sel)]

Gene.Sel <- round(Gene.Sel, 0)

Gene.Sel <- Gene.Sel [rowSums(Gene.Sel)>0, ]

CK.rep=4; Trt.rep=4

data <- as.data.frame(Gene.Sel)

Mydesign <- data.frame(Trt = factor(c(rep(c('D'), Trt.rep), rep(c('A'), CK.rep)), levels = c('D', 'A')))

dds <- DESeqDataSetFromMatrix( countData=data, colData = Mydesign , design= ~Trt )

dds1 <- DESeq(dds, fitType = 'mean', minReplicatesForReplace = 7, parallel = F)

res <- results(dds1, contrast = c('Trt', 'D', 'A')) # 默认 pAdjustMethod="BH"

res

res1 <- data.frame(res, stringsAsFactors = FALSE, check.names = FALSE)

tt\_enrich <- as.data.frame(res1)

Altered.gene <- tt\_enrich[abs(tt\_enrich$log2FoldChange) > 1, ]

Altered.gene <- Altered.gene [Altered.gene $pvalue < 0.01, ]

Altered.gene <- Altered.gene [Altered.gene $ padj < 0.01, ]

Altered.gene.CMN <- Altered.gene

##########################################

Altered.gene.BC.up <- Altered.gene.BC [Altered.gene.BC $log2FoldChang < 1, ]

Altered.gene.BC.down <- Altered.gene.BC [Altered.gene.BC $log2FoldChang > 1, ]

Altered.gene.CMN.up <- Altered.gene.CMN [Altered.gene.CMN $log2FoldChang > 1, ]

Altered.gene.CMN.down <- Altered.gene.CMN [Altered.gene.CMN $log2FoldChang < 1, ]

length ( intersect(rownames (Altered.gene.CMN.up), rownames (Altered.gene.BC.up)) )

dim (Altered.gene.BC.up)

dim (Altered.gene.CMN.up)

length ( intersect(rownames (Altered.gene.CMN.down), rownames (Altered.gene.BC.down)) )

dim (Altered.gene.BC.down)

dim (Altered.gene.CMN.down)

###Veen plotting###

library(vegan); library(ape); library(ggpubr); library(ggplot2); library (ggrepel); library (dplyr); library(DESeq2)

design <- read.table("gene.design.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F, quote="")

Gene <- read.table("gene.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

taxa <- read.table("Lib.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F, quote="")

dim(taxa)

Gene <- as.matrix(Gene) ; dim(Gene)

design.Sel <- **filter**(design, Trt %in% c("DCon", "DBC") )

Gene.Sel <- Gene[, rownames(design.Sel)]

Gene.Sel <- round(Gene.Sel, 0)

Gene.Sel <- Gene.Sel [rowSums(Gene.Sel)>0, ]

CK.rep=4; Trt.rep=4

#############################################################################

data <- as.data.frame(Gene.Sel)

Mydesign <- data.frame(Trt = factor(c(rep(c('D'), Trt.rep), rep(c('A'), CK.rep)), levels = c('D', 'A')))

dds <- DESeqDataSetFromMatrix( countData=data, colData = Mydesign , design= ~Trt )

dds1 <- DESeq(dds, fitType = 'mean', minReplicatesForReplace = 7, parallel = F)

res <- results(dds1, contrast = c('Trt', 'D', 'A'))

res

res1 <- data.frame(res, stringsAsFactors = FALSE, check.names = FALSE)

tt\_enrich <- as.data.frame(res1)

Altered.gene <- tt\_enrich[abs(tt\_enrich$log2FoldChange) > 1, ]

Altered.gene <- Altered.gene [Altered.gene $pvalue < 0.01, ]

Altered.gene <- Altered.gene [Altered.gene $ padj < 0.01, ]

Altered.gene.BC<- Altered.gene

####################################################

design.Sel <- **filter**(design, Trt %in% c("RCMN", "RCon"))

Gene.Sel <- Gene[, rownames(design.Sel)]

Gene.Sel <- round(Gene.Sel, 0)

Gene.Sel <- Gene.Sel [rowSums(Gene.Sel)>0, ]

CK.rep=4; Trt.rep=4

data <- as.data.frame(Gene.Sel)

Mydesign <- data.frame(Trt = factor(c(rep(c('D'), Trt.rep), rep(c('A'), CK.rep)), levels = c('D', 'A')))

dds <- DESeqDataSetFromMatrix( countData=data, colData = Mydesign , design= ~Trt )

dds1 <- DESeq(dds, fitType = 'mean', minReplicatesForReplace = 7, parallel = F)

res <- results(dds1, contrast = c('Trt', 'D', 'A')) # 默认 pAdjustMethod="BH"

res

res1 <- data.frame(res, stringsAsFactors = FALSE, check.names = FALSE)

tt\_enrich <- as.data.frame(res1)

Altered.gene <- tt\_enrich[abs(tt\_enrich$log2FoldChange) > 1, ]

Altered.gene <- Altered.gene [Altered.gene $pvalue < 0.01, ]

Altered.gene <- Altered.gene [Altered.gene $ padj < 0.01, ]

Altered.gene.CMN <- Altered.gene

##########################################

Altered.gene.BC.up <- Altered.gene.BC [Altered.gene.BC $log2FoldChang < 1, ]

Altered.gene.BC.down <- Altered.gene.BC [Altered.gene.BC $log2FoldChang > 1, ]

Altered.gene.CMN.up <- Altered.gene.CMN [Altered.gene.CMN $log2FoldChang > 1, ]

Altered.gene.CMN.down <- Altered.gene.CMN [Altered.gene.CMN $log2FoldChang < 1, ]

length ( intersect(rownames (Altered.gene.CMN.up), rownames (Altered.gene.BC.up)) )

dim (Altered.gene.BC.up)

dim (Altered.gene.CMN.up)

length ( intersect(rownames (Altered.gene.CMN.down), rownames (Altered.gene.BC.down)) )

dim (Altered.gene.BC.down)

dim (Altered.gene.CMN.down)

#####JA signaling related gene##

library(vegan); library(ape); library(ggpubr); library(ggplot2); library (ggrepel); library (dplyr); library(DESeq2)

design <- read.table("gene.design.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F, quote="")

Gene <- read.table("gene.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

taxa <- read.table("Lib.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F, quote="")

dim(taxa)

Gene <- as.matrix(Gene) ; dim(Gene)

design.Sel <- **filter**(design, Trt %in% c("DCon", "DBC") )

Gene.Sel <- Gene[, rownames(design.Sel)]

Gene.Sel <- round(Gene.Sel, 0)

Gene.Sel <- Gene.Sel [rowSums(Gene.Sel)>0, ]

CK.rep=4; Trt.rep=4

#############################################################################

data <- as.data.frame(Gene.Sel)

Mydesign <- data.frame(Trt = factor(c(rep(c('D'), Trt.rep), rep(c('A'), CK.rep)), levels = c('D', 'A')))

dds <- DESeqDataSetFromMatrix( countData=data, colData = Mydesign , design= ~Trt )

dds1 <- DESeq(dds, fitType = 'mean', minReplicatesForReplace = 7, parallel = F)

res <- results(dds1, contrast = c('Trt', 'D', 'A'))

res

res1 <- data.frame(res, stringsAsFactors = FALSE, check.names = FALSE)

tt\_enrich <- as.data.frame(res1)

Altered.gene <- tt\_enrich[abs(tt\_enrich$log2FoldChange) > 1 & tt\_enrich$baseMean>0 & tt\_enrich$padj < 0.01, ]

Altered.gene <- **mutate**(Altered.gene, sig=ifelse(Altered.gene$log2FoldChange > 1, "down", "up")); dim(Altered.gene)

Altered.gene.BC <- tt\_enrich

####################################################

design.Sel <- **filter**(design, Trt %in% c("RCMN", "RCon"))

Gene.Sel <- Gene[, rownames(design.Sel)]

Gene.Sel <- round(Gene.Sel, 0)

Gene.Sel <- Gene.Sel [rowSums(Gene.Sel)>0, ]

CK.rep=4; Trt.rep=4

data <- as.data.frame(Gene.Sel)

Mydesign <- data.frame(Trt = factor(c(rep(c('D'), Trt.rep), rep(c('A'), CK.rep)), levels = c('D', 'A')))

dds <- DESeqDataSetFromMatrix( countData=data, colData = Mydesign , design= ~Trt )

dds1 <- DESeq(dds, fitType = 'mean', minReplicatesForReplace = 7, parallel = F)

res <- results(dds1, contrast = c('Trt', 'D', 'A'))

res

res1 <- data.frame(res, stringsAsFactors = FALSE, check.names = FALSE)

tt\_enrich <- as.data.frame(res1)

Altered.gene <- tt\_enrich[abs(tt\_enrich$log2FoldChange) > 1 & tt\_enrich$baseMean>0 & tt\_enrich$padj < 0.01, ]

Altered.gene <- **mutate**(Altered.gene, sig=ifelse(Altered.gene$log2FoldChange > 1, "down", "up")); dim(Altered.gene)

Altered.gene.CMN <- tt\_enrich

####################**Plotting**###############################################

JAgene.name <- read.table("JAgene.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F, quote="")

JA.gene.CMN <- Altered.gene.CMN [JAgene.name$GeneID, ]

JA.gene.BC <- Altered.gene.BC [JAgene.name$GeneID, ]

JA.gene.data <- JA.gene.CMN

JA.gene.data $ FC.BC <- JA.gene.BC $ log2FoldChange

JA.gene.data $ FC.CMN <- JA.gene.CMN $ log2FoldChange

JA.gene.data <- JA.gene.data [, c("FC.BC", "FC.CMN") ]

JA.gene.data$ FC.BC <- abs(JA.gene.data$ FC.BC)

range(JA.gene.data)

JA.gene.data $Name <- JAgene.name$Name

JA.gene.data <- rbind(JA.gene.data, c(1, 2.5, 0) )

library(reshape2)

mydata=melt(JA.gene.data, id.vars=c("Name"),

measure.vars=c("FC.BC", "FC.CMN"),

variable.name='Trt', value.name='FC')

range(mydata$FC)

JAgene <- c("AOS1", "AOC", "OPR", "JAZ1", "JAZ2", "JAZ3", "JAZ5", "JAZ9", "JAZ10", "JAZ11", "MTB1", "MTB2", "JRE2", "0")

write.table(mydata, file="JAgeneFC.txt", sep="\t",quote=F)

JA.P <- ggplot(mydata, aes(x=Trt, y= factor(Name, levels= rev(JAgene) ))) +

geom\_point(aes(size= FC), colour = "grey15") +

scale\_size\_continuous(name="log2FoldChange", range = c(2, 8), breaks=c(1.0, 1.5, 2.0, 2.5)) +

theme(axis.text=element\_text(size=10, color="black"), axis.title= element\_blank(),

legend.text=element\_text(size=8), legend.background=element\_rect(fill='NA') )

JA.P

############ RT-qPCR results####

library(ggplot2); library(ggpubr); library (dplyr); library(car); library(lsmeans); library(multcomp)

mydata <- read.table("JAgeneExp.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

AOS1.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= AOS1, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y="AOS1 Relative expression level")+

scale\_y\_continuous (limits=c(0, 13), expand=c(0, 0.0001), breaks = c(0, 3, 6, 9, 12))+

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

AOS1.box

AOC.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= AOC, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y="AOC Relative expression level")+

scale\_y\_continuous (limits=c(0, 25), expand=c(0, 0.0001) )+ #, breaks = c(0, 3, 6, 9, 12)

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

AOC.box

OPR3.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= OPR3, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y=" OPR3 Relative expression level")+

scale\_y\_continuous (limits=c(0, 15), expand=c(0, 0.0001))+ #, breaks = c(0, 3, 6, 9, 12)

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

OPR3.box

JAZ1.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= JAZ1, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y=" JAZ1 Relative expression level")+

scale\_y\_continuous (limits=c(0, 8.5), breaks = c(0, 2, 4, 6, 8), expand=c(0, 0.0001))+ #

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

JAZ1.box

JAZ2.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= JAZ2, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y=" JAZ2 Relative expression level")+

scale\_y\_continuous (limits=c(0, 10), expand=c(0, 0.0001))+ #, breaks = c(0, 3, 6, 9, 12)

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

JAZ2.box

JAZ3.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= JAZ3, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y=" JAZ3 Relative expression level")+

scale\_y\_continuous (limits=c(0, 22), expand=c(0, 0.0001))+ #, breaks = c(0, 3, 6, 9, 12)

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

JAZ3.box

JAZ5.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= JAZ5, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y=" JAZ5 Relative expression level")+

scale\_y\_continuous (limits=c(0, 8), expand=c(0, 0.0001))+ #, breaks = c(0, 3, 6, 9, 12)

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

JAZ5.box

JAZ9.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= JAZ9, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y=" JAZ9 Relative expression level")+

scale\_y\_continuous (limits=c(0, 11), breaks = c(0, 2, 4, 6, 8, 10), expand=c(0, 0.0001))+ #

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

JAZ9.box

JAZ10.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= JAZ10, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y=" JAZ10 Relative expression level")+

scale\_y\_continuous (limits=c(0, 25), expand=c(0, 0.0001))+ #, breaks = c(0, 3, 6, 9, 12)

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

JAZ10.box

JAZ11.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= JAZ11, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y=" JAZ11 Relative expression level")+

scale\_y\_continuous (limits=c(0, 15), expand=c(0, 0.0001))+ #, breaks = c(0, 3, 6, 9, 12)

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

JAZ11.box

MTB1.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= MTB1, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y=" MTB1 Relative expression level")+

scale\_y\_continuous (limits=c(0, 15), expand=c(0, 0.0001))+ #, breaks = c(0, 3, 6, 9, 12)

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

MTB1.box

MTB2.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= MTB2, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y=" MTB2 Relative expression level")+

scale\_y\_continuous (limits=c(0, 20), expand=c(0, 0.0001))+ #, breaks = c(0, 3, 6, 9, 12)

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

MTB2.box

JRE2.box <- **ggplot**(mydata, aes(x= **factor**(Trt, levels=c('CMN+BC-.D', 'CMN+BC+.D', 'CMN-BC+.R', 'CMN+BC+.R' )), y= JRE2, color=Trt) ) +

**stat\_boxplot** (geom="errorbar", width=0.35) +

**geom\_boxplot**(alpha=0, outlier.size=0.75, outlier.shape=1, width=0.5, lwd=1,

outlier.color = "grey30", fill="transparent", show.legend = F) +

**geom\_jitter(**color="grey30", position=position\_jitter(0.22), size=2, alpha=0.70) +

labs(x=NULL, y=" JRE2 Relative expression level")+

scale\_y\_continuous (limits=c(0, 16), expand=c(0, 0.0001))+ #, breaks = c(0, 3, 6, 9, 12)

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=12, color="black"),

axis.title=element\_text(size=12),

axis.text.x = element\_text(angle = 75, hjust = 0.5, vjust = 0.5, color="black"),

legend.position = "none" )

JRE2.box