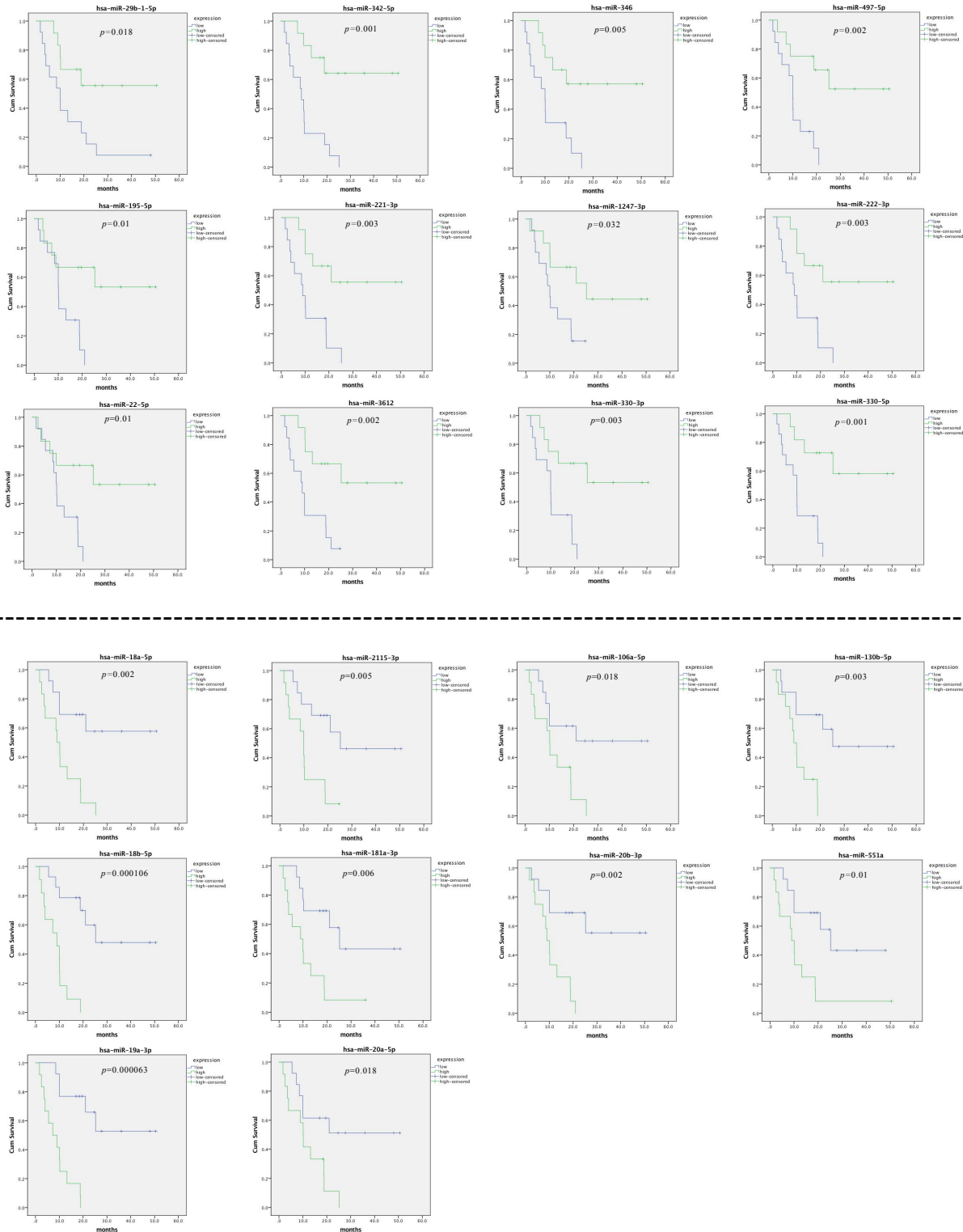


# Identification of grade-associated microRNAs in brainstem gliomas based on microarray data

Chen, et al.

## Data Supplement

### Supplementary Figures



**Figure S1. Survival rate calculated by the Kaplan-Meier survival curve in patients separated according to the expression level of each DE-miRNA. The rest 22 DE-miRNAs are shown ( $p < 0.05$ ). The first 12 miRNAs are lower expression in high grade BSGs, the last 10 miRNAs are higher expression in high grade BSGs.**

## Supplementary Tables

**Table S1. All real-time quantitative PCR primer sequences used in this study.**

hsa-miR-551a-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTggaaa
hsa-miR-551a-F	AGGGCGACCCACTCTTGG
hsa-miR-551a-R	GTGCAGGGTCCGAGGT
hsa-miR-181a-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACggtaca
hsa-miR-181a-3p-F	AGGACCATCGACCGTTGAT
hsa-miR-181a-3p-R	GTGCAGGGTCCGAGGT
hsa-miR-2115-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACctagcc
hsa-miR-2115-3p-F	GCAGGCATCAGAATTCATGGA
hsa-miR-2115-3p-R	GTGCAGGGTCCGAGGT
hsa-miR-130b-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACgtagtg
hsa-miR-130b-5p-F	GCAGGACTCTTCCCTGTTG
hsa-miR-130b-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-19a-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtcagtt
hsa-miR-19a-3p-F	CAGGTGTGCAAATCTATGCAA
hsa-miR-19a-3p-R	GTGCAGGGTCCGAGGT
hsa-miR-18a-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACcctatct
hsa-miR-18a-5p-F	AGGTAAGGTGCATCTAGTGC
hsa-miR-18a-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-18b-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACcctaact
hsa-miR-18b-5p-F	AGGTAAGGTGCATCTAGTGC
hsa-miR-18b-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-18a-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACccagaa
hsa-miR-18a-3p-F	GGAAGTCCCTAAGTCTCC
hsa-miR-18a-3p-R	GTGCAGGGTCCGAGGT
hsa-miR-20b-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACcctggaa
hsa-miR-20b-3p-F	CAGGACTGTAGTATGGGCAC
hsa-miR-20b-3p-R	GTGCAGGGTCCGAGGT
hsa-miR-31-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACagctat
hsa-miR-31-5p-F	AGGAGGCAAGATGCTGGC
hsa-miR-31-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-22-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtaaagc
hsa-miR-22-5p-F	CAGGAGTTCTTCAGTGGCAA
hsa-miR-22-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-221-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACgaaacc
hsa-miR-221-3p-F	AGGAGCTACATTGTCTGCTG
hsa-miR-221-3p-R	GTGCAGGGTCCGAGGT
hsa-miR-330-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACgcctaa
hsa-miR-330-5p-F	AGGTCTCTGGGCCTGTGTC
hsa-miR-330-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-330-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtctctg
hsa-miR-330-3p-F	GCAAAGCACACGGCCTG
hsa-miR-330-3p-R	GTGCAGGGTCCGAGGT
hsa-miR-346-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACagagggc
hsa-miR-346-F	TGTCTGCCCGCATGCCT
hsa-miR-346-R	GTGCAGGGTCCGAGGT

hsa-miR-3612-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtccatt
hsa-miR-3612-F	CAGGAGGAGGCATCTTGAGA
hsa-miR-3612-R	GTGCAGGGTCCGAGGT
hsa-miR-497-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACacaaac
hsa-miR-497-5p-F	GGCAGCAGCACACTGTG
hsa-miR-497-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-1247-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACgctcca
hsa-miR-1247-3p-F	CCCGGGAACGTCGAGAC
hsa-miR-1247-3p-R	GTGCAGGGTCCGAGGT
hsa-miR-29b-1-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtctaaa
hsa-miR-29b-1-5p-F	GGGCTGGTTTCATATGGTGG
hsa-miR-29b-1-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-1290-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtccctg
hsa-miR-1290-F	TGGATTTTTGGATCAGGGAGTCG
hsa-miR-1290-R	GTGCAGGGTCCGAGGT
hsa-miR-130b-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACatgccc
hsa-miR-130b-3p-F	CGCCAGTGC AATGATGAAAG
hsa-miR-130b-3p-R	GTGCAGGGTCCGAGGT
hsa-miR-20a-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACctacct
hsa-miR-20a-5p-F	GCGCTAAAGTGCTTATAGTGC
hsa-miR-20a-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-106a-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACctacct
hsa-miR-106a-5p-F	CGCAAAGTGCTTACAGTGC
hsa-miR-106a-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-34a-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACagggca
hsa-miR-34a-3p-F	CAATCAGCAAGTATACTGCCCT
hsa-miR-34a-3p-R	GTGCAGGGTCCGAGGT
hsa-miR-34a-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACacaacc
hsa-miR-34a-5p-F	CTGGCAGTGTCTTAGCTGG
hsa-miR-34a-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-222-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACaccag
hsa-miR-222-3p-F	AGCTACATCTGGCTACTGGGT
hsa-miR-222-3p-R	GTGCAGGGTCCGAGGT
hsa-miR-342-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtcaatc
hsa-miR-342-5p-F	AGGGGTGCTATCTGTGATTGA
hsa-miR-342-5p-R	GTGCAGGGTCCGAGGT
hsa-miR-195-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACgccaat
hsa-miR-195-5p-F	CGCTAGCAGCACAGAAAT
hsa-miR-195-5p-R	GTGCAGGGTCCGAGGT
U6-F	CTCGCTTCGGCAGCACA
U6-R	AACGCTTCACGAATTTGCGT

hsa-miR-7-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACaacaac
hsa-miR-7-F	CGTCCTGGAAGACTAGTGATTTT
hsa-miR-7-R	GTGCAGGGTCCGAGGT
hsa-miR-21-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTcaaca
hsa-miR-21-F	CCGTCCTAGCTTATCAGACTGA
hsa-miR-21-R	GTGCAGGGTCCGAGGT
hsa-miR-451-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACaactca
hsa-miR-451-F	CGTCCAAACCGTTACCATTAC
hsa-miR-451-R	GTGCAGGGTCCGAGGT
CDK4-F	CCATCAGCACAGTTCGTGAGGT
CDK4-R	TCAGTTCGGGATGTGGCACAGA
CCNE1-F	TGTGTCTGGATGTTGACTGCC
CCNE1-R	CTCTATGTCGCACCACTGATACC
CDC25A-F	TCTGGACAGCTCCTCTCGTCAT
CDC25A-R	ACTTCCAGGTGGAGACTCCTCT
DDX21-F	TGGACTCAGAGGGCAGCAGTTA
DDX21-R	TGTCTCCATGCAAGGACTGAGC
VEGFA-F	TTGCCTTGCTGCTCTACCTCCA
VEGFA-R	GATGGCAGTAGCTGCGCTGATA
c-MYC-F	CCTGGTGCTCCATGAGGAGAC
c-MYC-R	CAGACTCTGACCTTTTGCCAGG
CDC25C-F	AGAAGCCCATCGTCCCTTTGGA
CDC25C-R	GCAGGATACTGGTTCAGAGACC
LDHA-F	GGATCTCCAACATGGCAGCCTT
LDHA-R	AGACGGCTTTCTCCCTCTTGCT
GAPDH-F	GTCTCCTCTGACTTCAACAGCG
GAPDH-R	ACCACCCTGTTGCTGTAGCCAA
NOTCH1-F	GGTGAAGTCTCTGAGGAGATC
NOTCH1-R	GGATTGCAGTCGTCCACGTTGA
FOS-F	GCCTCTCTTACTACCACTCACC
FOS-R	AGATGGCAGTGACCGTGGGAAT
BCL2-F	ATCGCCCTGTGGATGACTGAGT
BCL2-R	GCCAGGAGAAATCAAACAGAGGC
PHLPP2-F	CCTTCCAACACTGGTAGAGCAC
PHLPP2-R	CGGATGGTAAAGACTCCAGACTA
APP-F	CCTTCTCGTTCCTGACAAGTGC
APP-R	GGCAGCAACATGCCGTAGTCAT
RB1-F	CAGAAGGTCTGCCAACACCAAC
RB1-R	TTGAGCACACGGTCGCTGTTAC
SP1-F	ACGCTTCACACGTTCCGGATGAG
SP1-R	TGACAGGTGGTCACTCCTCATG

**Table S2. Identification of grade-associated miRNAs validated in BSG samples.**

Upregulated miRNA ID	p-value	Downregulated miRNA ID	p-value
hsa-miR-1290	0.312	hsa-miR-31-5p	0.051
hsa-miR-130b-3p	0.001	hsa-miR-34a-3p	0.106
hsa-miR-18a-3p	0.583	hsa-miR-34a-5p	0.0008
hsa-miR-20b-3p	0.092	hsa-miR-222-3p	0.127
hsa-miR-18b-5p	0.449	hsa-miR-22-5p	0.415
hsa-miR-18a-5p	0.215	hsa-miR-221-3p	0.308
hsa-miR-19a-3p	0.002	hsa-miR-330-5p	0.07
hsa-miR-130b-5p	0.322	hsa-miR-330-3p	0.084
hsa-miR-2115-3p	0.428	hsa-miR-346	0.128
hsa-miR-181a-3p	0.355	hsa-miR-3612	0.081
hsa-miR-20a-5p	0.001	hsa-miR-497-5p	0.678
hsa-miR-551a	0.175	hsa-miR-342-5p	0.129
hsa-miR-106a-5p	0.001	hsa-miR-195-5p	0.002
		hsa-miR-1247-3p	0.2
		hsa-miR-29b-1-5p	0.067

**Table S3. Enriched functions for the target genes of the 6 validated miRNAs. The top 5 terms are showed.**

Category	Term	Count	P-Value	FDR	Fold Enrichment
BP	GO:0045944-positive regulation of transcription from RNA polymerase II promoter	155	1.1698E-20	2.1958E-17	2.162322929
	GO:0000122-negative regulation of transcription from RNA polymerase II promoter	109	3.3539E-13	6.2957E-10	2.071819252
	GO:0045893-positive regulation of transcription, DNA-templated	85	1.9255E-12	3.6145E-09	2.25875725
	GO:0006366-transcription from RNA polymerase II promoter	74	2.5682E-08	4.8208E-05	1.97411395
	GO:0007411-axon guidance	34	3.6278E-08	6.8096E-05	2.926440211
CC	GO:0005654-nucleoplasm	280	1.2017E-10	1.7954E-07	1.42636075
	GO:0005737-cytoplasm	464	1.516E-09	2.265E-06	1.260148399
	GO:0005769-early endosome	41	8.1061E-08	0.00012111	2.539153484
	GO:0005667-transcription factor complex	36	2.0278E-07	0.00030296	2.645366021
	GO:0005829-cytosol	304	2.722E-07	0.00040667	1.30056071
MF	GO:0005515-protein binding	820	2.7973E-26	4.4945E-23	1.273797983
	GO:0003700-transcription factor activity, sequence-specific DNA binding	128	2.9114E-11	4.6778E-08	1.817670054
	GO:0001077-transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding	46	2.3943E-09	3.8469E-06	2.659955058
	GO:0043565-sequence-specific DNA binding	73	9.3513E-08	0.00015025	1.923187248
	GO:0001047-core promoter binding	19	4.5624E-07	0.00073304	4.051371766
KEGG_PATHWAY	hsa05200:Pathways in cancer	62	1.8642E-07	0.00024187	1.978452317
	hsa04144:Endocytosis	46	3.1021E-07	0.00040248	2.235962802
	hsa04360:Axon guidance	28	1.6933E-06	0.00219694	2.76490847
	hsa05212:Pancreatic cancer	19	1.7979E-06	0.00233267	3.665782493
	hsa05220:Chronic myeloid leukemia	19	8.8205E-06	0.01144355	3.309386973

**Table S4. Key codes and parameters.**

1. Screening for functional DE-miRNAs:

```
library(oligo)
celfiles <- list.files("/",pattern = "CEL",full.names = TRUE)
rawData <- read.celfiles(celfiles[1:25])
pData(rawData)$Type <- rep(c("high","low"), c(15,10))
pData(rawData)
eset.rma <- rma(rawData)
emat.rma.log2 <- exprs(eset.rma)
highMean <- apply(emat.rma.log2[,1:15],1,mean)
lowMean <- apply(emat.rma.log2[,16:25],1,mean)
results.rma <- data.frame(cbind(highMean,lowMean))
results.rma$logFC <- results.rma[,2]-results.rma[,1]
filter <- apply(emat.rma.log2, 2, FUN = function(x){y <- ifelse(x >= quantile(as.numeric(x), 0.75),1,0)})
#ap <- filter[which(rowSums(filter==0)==0),]
AP <- apply(filter,1,function(x)any(x == "1"))
present.probes <- names(AP[AP])
paste(length(present.probes),'/',length(AP))
results.present <- results.rma[present.probes,]
sum(abs(results.present[, 'logFC'])>log2(2))
results.st <- results.present[abs(results.present$logFC)>=log2(2),]
sel.genes <- row.names(results.st)
p.value <- apply(emat.rma.log2[sel.genes,],1,function(x){t.test(x[1:15],x[16:25])$p.value})
results.st$p.value <- p.value
results.st <- results.st[p.value<0.05,]
nrow(results.st)
write.csv(results.st,".csv")
rm(list=ls())
```

2. Volcanoplot:

```
library(ggplot2)
setwd('/')
data =read.csv(".csv",header=T,row.names=1)
r03 = ggplot(data,aes(logFC,lpv))
r03 + geom_point()
r03 + geom_point(color ="red")
r03 +geom_point(aes(color ="red"))
r03 + geom_point(aes(color =yanse))
r03xy = r03 +geom_point(aes(color =yanse)) + xlim(-4,4) + ylim(0,4)
r03xy + labs(title="Volcanoplot",x="log2(FC)")
r03xyp=r03xy + labs(title="Volcanoplot",x="log2(FC)")
r03xyp + scale_color_manual(values =c("red", "green", "black"))
volcano=r03xyp + scale_color_manual(values =c("#619cff", "#00ba38", "#f8766d"))
volcano+geom_hline(yintercept=1.3,linetype=4)+geom_vline(xintercept=c(-0.84,0.84),linetype=4)
rm(list=ls())
```

### 3.GO and KEGG enrichment:

```
biocLite("clusterProfiler")
rm(list=ls())
require(DOSE)
require(clusterProfiler)
setwd("/")
a=read.table("11.txt",header = TRUE)
gene=as.character(a[,1])
egoBP <- enrichGO(gene=gene,'org.Hs.eg.db',ont="BP",pvalueCutoff=0.01,readable=TRUE)
ekk <- enrichKEGG(gene=gene,organism = "hsa",pAdjustMethod = "BH",pvalueCutoff=0.01)
egoCC <- enrichGO(gene=gene,'org.Hs.eg.db',ont="CC",pvalueCutoff=0.01,readable=TRUE)
egoMF <- enrichGO(gene=gene,'org.Hs.eg.db',ont="MF",pvalueCutoff=0.01,readable=TRUE)
write.csv(as.data.frame(egoBP),"GO-enrichBPdown.csv",row.names =F)
write.csv(as.data.frame(ekk),"KEGG-enrichdown.csv",row.names =F)
write.csv(as.data.frame(egoCC),"GO-enrichCCdown.csv",row.names =F)
write.csv(as.data.frame(egoMF),"GO-enrichMFdown.csv",row.names =F)
rm(list=ls())
```

### 4.Heatmap:

```
setwd('/')
data <- read.csv('.csv',row.names = 1)
matrixdata <- as.matrix(data)
#matrixdata[is.na(matrixdata)] <- 0
#heatmap(matrixdata)
library(pheatmap)
bk = unique(c(seq(-2.15,2, length=100)))
#heatmap(matrixdata, Colv=NA, col = topo.colors(50), scale="column", margins=c(5,10))
matrix_heatmap <- pheatmap(matrixdata,breaks = bk,cluster_cols= FALSE,cluster_rows=
TRUE,border_color =
NA,scale="row",treeheight_row=40,treeheight_col=20,color=colorRampPalette(c("purple","white","yellow"
))
(100),fontsize=10,fontsize_row=1,fontsize_col=2)
```