面试题

要求：

以中文报告形式呈现分析结果（说明文字——图标），另外需要附代码

思路

平时做的是植物的，对应动物的数据接触的比较少。 首先，有个大概的认识

> TCGA系列5-UCSC Xena网站的简单使用和数据下载-哔哩哔哩

- 如何确定TCGA-DD-AA3A-01编码含义
  - 官方网站查询

> 简而言之，编码机构BRS（Biospeciman Core Resource）根据来源机构（Tissue Source Site，TSS）和捐献者（Participation），给予编号TCGA-02 和 TCGA-02-0001，根据组织类型（Sample）如癌组织、正常组织等，编为TCGA-02-0001-01（01-09为癌组织，10-14为正常组织，组织类型编码详见 https://gdc.cancer.gov/resources-tcga-users/tcga-code-tables/sample-type-codes）。同一种组织的标本又会被分装进不同容器（Vial），同一容器内又可分为多个小份（Portion），进一步编为 TCGA-02-0001-01B 和TCGA-02-0001-01B-02。样品送至检测机构后，制备成不同的分析物（Analyte）检测，用不同字母编码，例如D表示DNA，R表示RNA。同一份分析物在检测过程中被加到检测板的某一加样孔中，分别编号 TCGA-02-0001-01B-02D-0182和TCGA-02-0001-01B-02D-0182-06。

> 组织类型编码 TCGA条码

- 临床数据如何确定可用信息 其他的大概涉及到梳理统计，选择什么工具
- 生产环境 实验室设备 Linux 4.10.0-38-generic #42~16.04.1-Ubuntu 8 cpu 125G mem

> online R clod

- 数据下载来源

> dataset: gene expression RNAseq - IlluminaHiSeq phenotype

1.从UCSC Xena数据库TCGA 肝癌转录组数据及临床信息

1. clinical information

```
1  wget -c  https://tcga-xena-hub.s3.us-east-
1.amazonaws.com/download/TCGA.LIHC.sampleMap%2FLIHC_clinicalMatrix
2  wget -c https://tcga-xena-hub.s3.us-east-
1.amazonaws.com/download/TCGA.LIHC.sampleMap%2FHiSeqV2.gz
3 wget -c https://xenabrowser.net/datapages/?dataset=TCGA-
LIHC.survival.tsv&host=https%3A%2F%2Fgdc.xenahubs.net&removeHub=https%3A%2F%2Fxena.treehouse.
gi.ucsc.edu%3A443
4  wget -c https://tcga-xena-hub.s3.us-east-
1.amazonaws.com/download/probeMap%2Fhugo_gencode_good_hg19_V24lift37_probemap
```

2. 从TCGA下载临床数据，并查看 clinical.project-TCGA-LIHC.2023-04-02.tar.gz

数据的其他下载方式

通过R包UCSCXenaTools连接UCSC的XENA浏览器来探索TCGA等公共浏览器

> 生信专栏 | TCGA数据下载友好型——利用UCSC xena下载 TCGA数据库的初次了解TCGA-LIHC

> 一文讲清TCGA数据库中样本编码信息

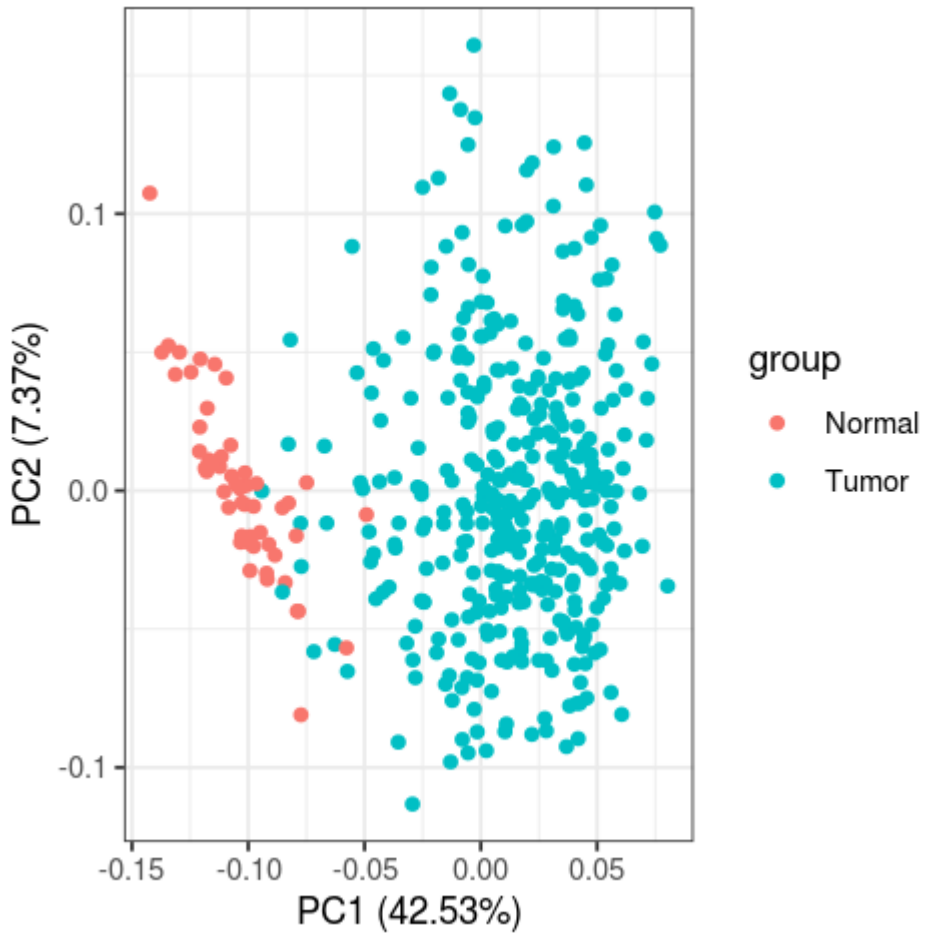
2.对疾病和正常样本进行差异分析筛选出基因并进行差异分析

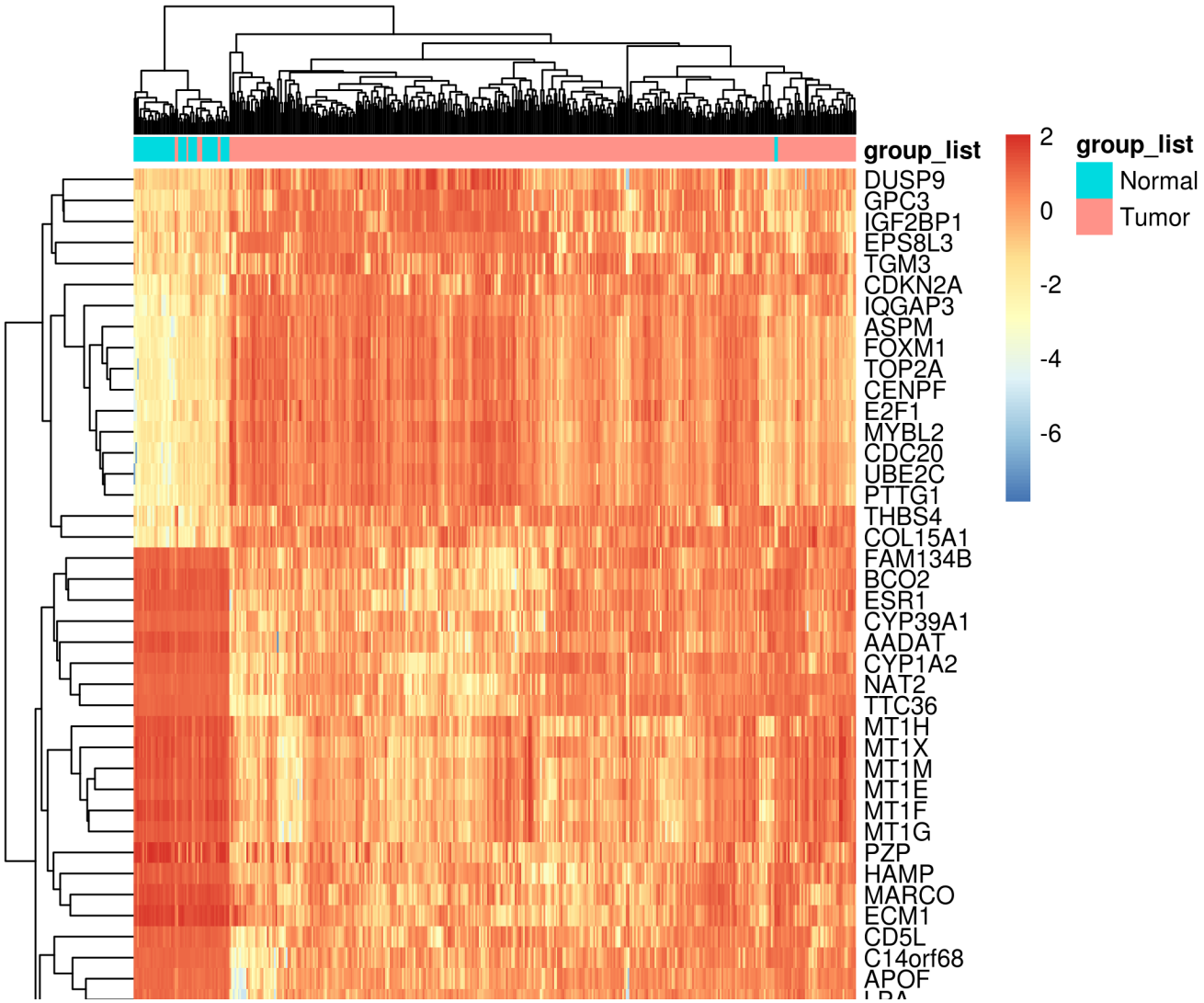
```r
# version 03
rm(list = ls())
options(stringsAsFactors = F)

# 安装并载入所需的包
if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

# BiocManager::install("edgeR")
library(edgeR)
# install.packages("SummarizedExperiment")
# BiocManager::install("locfit")
# BiocManager::install("DESeq2")
# install.packages("DESeq2")
# BiocManager::install("SummarizedExperiment",lib="/cloud/lib")
# library(DESeq2)


# BiocManager::install("DESeq2")

# 1. 数据预处理
data <- read.table("/fafu/jiangxiaojiao/data/TCGA.LIHC.sampleMap%2FHiSeqV2.gz",
                   header = TRUE, sep = "\t", row.names = 1)
data_clean <- na.omit(data) # 删除缺失值或空值

data_clinal <-
```

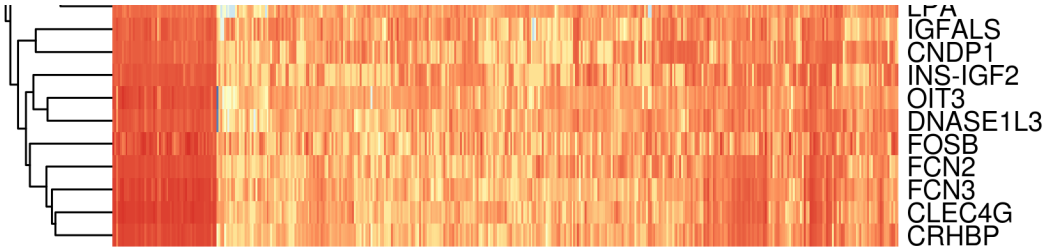
```
read.table("/fafu/jiangxiaojiao/data/TCGA.LIHC.sampleMap%2FLIHC_clinicalMatrix",
                                header = TRUE, sep = "\t", row.names = 1)
# data_clean_clinal <- na.omit(data_clinal)

# 获取患者的癌症状态
sample_id <- colnames(data_clean)
num <- as.numeric(substring(sample_id, 14, 15))  #截取字符串后转为数字
group_list = ifelse(num%in%1:9,"Tumor","Normal")  #ifelse实现分组

# 将 group_list 转换为只有一列的数据框
group_df <- as.data.frame(group_list)
group_df_clean <- na.omit(group_df)

# 在合并后的数据框中添加分组信息
df <- as.data.frame(sample_id)
df$Group <- ""
# 使用 substr 截取 A 列的最后 2 个字符
last_two_chars <- substr(df$A, nchar(df$A) - 1, nchar(df$A))

# 使用 ifelse 根据截取结果设置 B 列的值
df$Group <- ifelse(num%in%1:9,"Tumor","Normal")  #ifelse实现分组
# merged_data$Group <- ifelse(merged_data$Sample_Type == "Tumor", "disease", "normal")
# 将两个数据框按照 ID 列进行合并
# merged_df <- merge(sample_id, group_list)
# # 使用 ifelse 设置 B 中值的条件
# merged_df$y <- ifelse(df$A>0, TRUE, FALSE)

# 按照样品名以字母顺序排序
pheno <- pheno[order(rownames(pheno)), ]

# 将表型信息添加到矩阵中，并为每组分配分组信息
group <- factor(df$Group)
design <- model.matrix(~group)



# 查看 group_list 的类型
class(group_list)
class(data_clean)

# 创建 edgeR 对象
y <- DGEList(counts = data_clean, group = group)

# 进行基因过滤，按照要求选择出需要进行差异分析的基因
keep <- filterByExpr(y, design)
y <- y[keep,,keep.lib.sizes=FALSE]

# 规范化矩阵
y <- calcNormFactors(y)

# 估计离散度
y <- estimateDisp(y, design)

# 对规范化后的表达矩阵进行差异分析
fit <- glmQLFit(y, design)
qlf <- glmQLFTest(fit, coef=2)
```

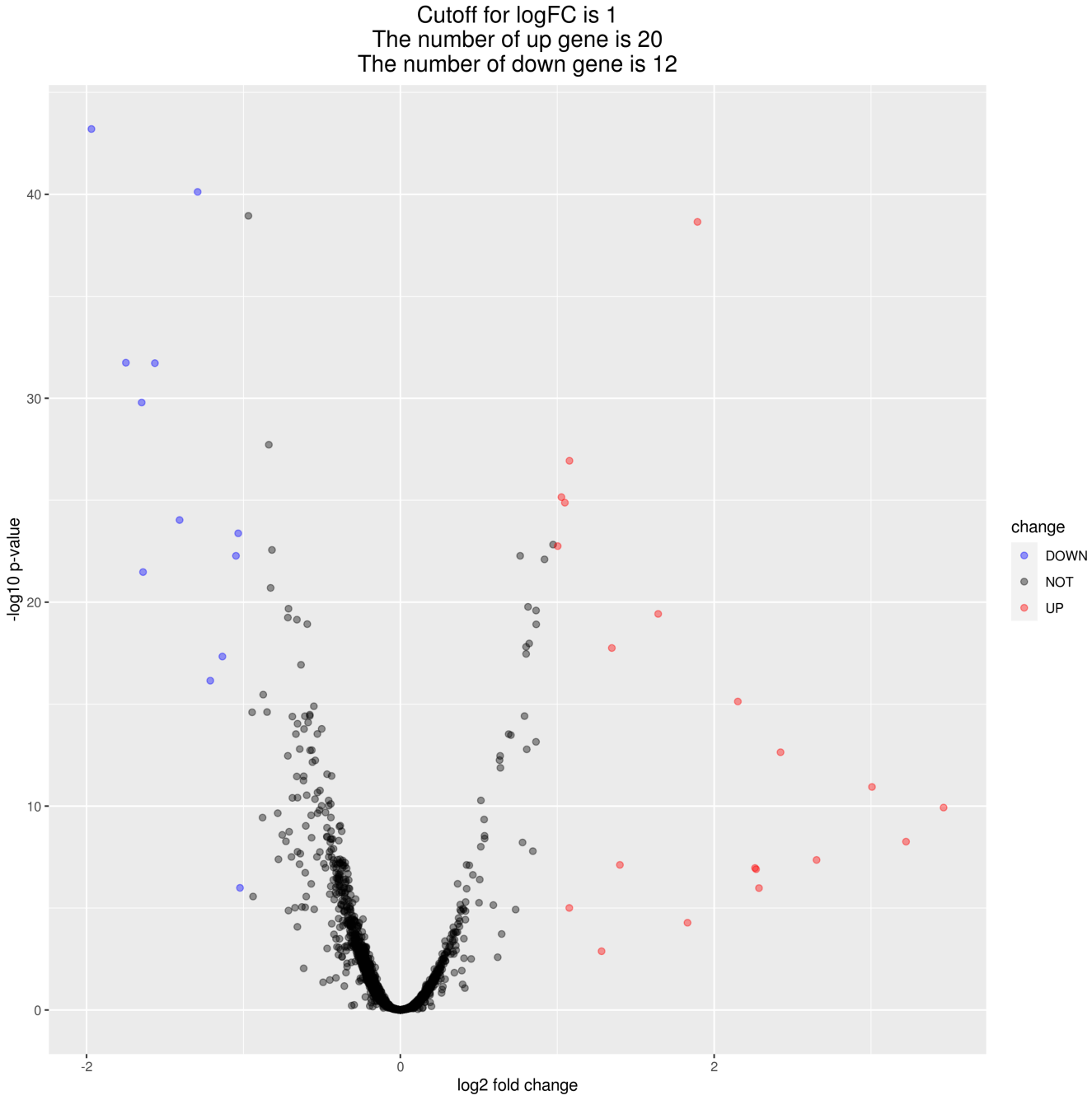
```
# 筛选差异表达显著的基因
differentially_expressed_genes <- topTags(qlf, adjust.method = "BH", sort.by = "PValue", n =
Inf)

differentially_expressed_genes <-
differentially_expressed_genes[differentially_expressed_genes$table$PValue < 0.05, ]
nrDEG=as.data.frame(differentially_expressed_genes)

head(nrDEG)
edgeR_DEG =nrDEG
nrDEG=edgeR_DEG[,c(1,5)]
colnames(nrDEG) = c("log2FoldChange", "pvalue")

draw_h_v <- function(exprSet,need_DEG,n='DEseq2',group_list,logFC_cutoff){
  ## we only need two columns of DEG, which are log2FoldChange and pvalue
  ## heatmap

  library(pheatmap)
  choose_gene=head(rownames(need_DEG),50) ## 50 maybe better
  choose_matrix=exprSet[choose_gene,]
  choose_matrix[1:4,1:4]
  choose_matrix=t(scale(t(log2(choose_matrix+1))))
  ## http://www.bio-info-trainee.com/1980.html
  annotation_col = data.frame( group_list=group_list  )
  rownames(annotation_col)=colnames(exprSet)
  pheatmap(choose_matrix,show_colnames = F,annotation_col = annotation_col,
           filename = paste0(n,'_need_DEG_top50_heatmap.png'))


  library(ggfortify)
  df=as.data.frame(t(choose_matrix))
  df$group=group_list
  png(paste0(n,'_DEG_top50_pca.png'),res=120)
  p=autoplot(prcomp( df[,1:(ncol(df)-1)] ), data=df,colour = 'group')+theme_bw()
  print(p)
  dev.off()


  if(! logFC_cutoff){
    logFC_cutoff <- with(need_DEG,mean(abs( log2FoldChange)) + 2*sd(abs( log2FoldChange)) )

  }
  # logFC_cutoff=1

  need_DEG$change = as.factor(ifelse(need_DEG$pvalue < 0.05 & abs(need_DEG$log2FoldChange) >
logFC_cutoff,
                                     ifelse(need_DEG$log2FoldChange > logFC_cutoff
,'UP','DOWN'),'NOT')
  )
  this_tile <- paste0('Cutoff for logFC is ',round(logFC_cutoff,3),
                      '\nThe number of up gene is ',nrow(need_DEG[need_DEG$change =='UP',]) ,
                      '\nThe number of down gene is ',nrow(need_DEG[need_DEG$change
=='DOWN',])
  )
  library(ggplot2)
  g = ggplot(data=need_DEG,
             aes(x=log2FoldChange, y=-log10(pvalue),
```

```
                    color=change)) +
    geom_point(alpha=0.4, size=1.75) +
    theme_set(theme_set(theme_bw(base_size=20)))+
    xlab("log2 fold change") + ylab("-log10 p-value") +
    ggtitle( this_tile ) + theme(plot.title = element_text(size=15,hjust = 0.5))+
    scale_colour_manual(values = c('blue','black','red')) ## corresponding to the
levels(res$change)
  print(g)
  ggsave(g,filename = paste0(n,'_volcano.png'))
  dev.off()
}
draw_h_v(data_clean, nrDEG, "edgeR", group_list, 1)
percentage <- nrow(differentially_expressed_genes) / nrow(data_clean)

# 取交集
# 将differentially_expressed_genes转换为数据框对象
differentially_expressed_genes_name <- row.names(differentially_expressed_genes)
# differentially_expressed_genes_name <- as.data.frame()
autophagy_genes <- read.table("/fafu/jiangxiaojiao/methy_array/gene_temp.txt", header =
FALSE)
# write.table(differentially_expressed_genes_name, file = "output.txt", sep = ",", row.names
= FALSE)
vec2 <- as.vector(autophagy_genes[,])
result <- intersect(differentially_expressed_genes_name, vec2)

# 使用unique函数去除重复行
unique_result <- unique(result)

# 输出结果
unique_result
```


PC2 (7.37%)
0.1
0.0
-0.1
-0.15
-0.10
-0.05
0.00
0.05
PC1 (42.53%)
group
Normal
Tumor


group_list
DUSP9
GPC3
IGF2BP1
EPS8L3
TGM3
CDKN2A
IQGAP3
ASPM
FOXM1
TOP2A
CENPF
E2F1
MYBL2
CDC20
UBE2C
PTTG1
THBS4
COL15A1
FAM134B
BCO2
ESR1
CYP39A1
AADAT
CYP1A2
NAT2
TTC36
MT1H
MT1X
MT1M
MT1E
MT1F
MT1G
PZP
HAMP
MARCO
ECM1
CD5L
C14orf68
APOF
2
0
-2
-4
-6
group_list
Normal
Tumor





3.将差异基因与自噬基因取(需要自己去找自噬基因集)交集

自噬,先搜集资料

- Autophagy（自噬）来自于希腊语，是auto=self和phagy=phagein=to eat的结合。
- 您可以通过在公共数据库（如HADb Human Autophagy Database、KEGG、GO等）中查找与自噬相关的基因集，然后将其与差异表达基因进行比较以找出它们的交集。

获取自噬基因

```
# /usr/bin/python3
# -- encoding: utf-8 -

import requests
import functools
from bs4 import BeautifulSoup
import bs4

# 发送HTTP请求
url = "http://www.autophagy.lu/clustering/index.html"
response = requests.get(url)

# 解析HTML页面
soup = BeautifulSoup(response.content, 'html.parser')
# print(soup)
# 从第四个table开始
global flag
flag = True

def extract_table_data(table):
    """
    提取单个表格的数据

    table like this:


    <table width="100%">
        <tr>
            <td width="30%">
                <a href="http://www.lih.lu" style="border-color:white">
                    <img alt="LIH" height="" src="/Supplements/Pictures/public-research-
center.png" style="border-color:white" title="LIH" width=""/>
                </a>
            </td>
            <td width="40%"></td>
            <td id="update_in_progress" style="border-color:red;text-align:right;font-
size:12px" width="30%">
                <!--- marquee -->
                Update in progress
                <!---/marquee-->
            </td>
        </tr>
    </table>
    """


    soup = BeautifulSoup(str(table), 'html.parser')
    table = soup.table
    rows = table.find_all('tr')

    for row in rows:
        cells = row.find_all('td')
        row_data = [cell.text.strip() for cell in cells]
```

```
            print(row_data)
            load_into_file(row_data)

    def load_into_file(line):
        # 去重不必要的字符
        line = [element.replace("\n", "").replace("\t","") for element in line]
        # line =  ','.join(str(line).split())

        # 由于tables是GeneId Name    Symbol的结构（三个字符）
        # 起始字符GeneId是数字
        # 只需要判断line长度起码在3
        length = len(line)
        if length >=3:
            line_start = line[0]
            # 起始字符GeneId不是数字，舍弃
            if not line_start[:1].isdigit():
                return
            with open("./gene_temp.csv", mode="a+", encoding="utf-8") as f:
                f.write(",".join(line))
                f.write("\n")
            print("**** genes list ****")
            print(line[-1], file=open("./gene_temp.txt", mode="a+", encoding="utf-8"))
        # for element in line:
        #     print(element.strip(), file=open("./gene_temp.txt", mode="a+", encoding="utf-8"))
        #     print("\n")
    # functools模块中的lru_cache装饰器来优化函数的性能。
    # lru_cache装饰器会缓存函数的结果，并且在后续调用相同参数的函数时，直接返回缓存的结果，避免重复计算
    @functools.lru_cache(maxsize=None)
    def extract_all_tables(soup):
        """
        递归地提取所有表格的数据
        """
        all_data = []
        # if all_data == []:
        #     tables = soup.find_all('table')[:4]
        #     flag = False
        # else:
        tables = soup.find_all('table')
        valid_tables = []  # 保存有效的HTML标签对象
        for table in tables:
            if isinstance(table, bs4.element.Tag):  # 判断是否为HTML标签对象
                valid_tables.append(table)
        if valid_tables:
            # 第四个table
            for table in tables:
                # data = extract_table_data(table)
                # all_data.append(data)
                if len(table.find_all('table')) > 0:
                    sub_soup = BeautifulSoup(str(table), 'html.parser')
                    sub_data = extract_all_tables(sub_soup)
                    # all_data += sub_data
                else:
                    yield table
        # return all_data

    # 解析HTML代码
    # soup = BeautifulSoup(html, 'html.parser')
```

```
# 提取所有表格的数据
# tables = soup.find_all('table')[:3]
# tables = BeautifulSoup(str(tables), 'html.parser')
soup = BeautifulSoup(response.content, 'html.parser')
all_data = extract_all_tables(soup)

# print(all_data)
for table in all_data:
    # print(table)
    extract_table_data(table)
```

取交集

```
percentage <- nrow(differentially_expressed_genes) / nrow(data_clean)

# 取交集
# 将differentially_expressed_genes转换为数据框对象
differentially_expressed_genes_name <- row.names(differentially_expressed_genes)
# differentially_expressed_genes_name <- as.data.frame()
autophagy_genes <- read.table("/fafu/jiangxiaojiao/methy_array/gene_temp.txt", header =
FALSE)
# write.table(differentially_expressed_genes_name, file = "output.txt", sep = ",", row.names
= FALSE)
vec2 <- as.vector(autophagy_genes[,])
result <- intersect(differentially_expressed_genes_name, vec2)

# 使用unique函数去除重复行
unique_result <- unique(result)

# 输出结果
unique_result
# [145] "ARNT"      "SESN2"     "DNAJB1"    "RAF1"      "EIF4EBP1"  "SIRT2"
```

4.利用交集基因构建多因素预后模型，画模型的ROC曲线

在R中，可以使用多种方法构建多因素预后模型，并使用ROC曲线评估模型的性能。以下是一种基于逻辑回归模型的方法：

1. 准备数据

首先，需要准备数据，包括基因表达数据和生存数据。基因表达数据可以是一个包含基因表达矩阵的数据框，生存数据可以是一个包含生存时间、生存状态（0表示死亡，1表示存活）和其他相关信息的数据框。需要将两个数据框按照样本编号进行合并，得到一个包含基因表达数据和生存数据的完整数据框。

2. 特征选择

然后，可以使用交集基因作为特征，或者使用其他方法进行特征选择，例如LASSO、Elastic Net等。这些方法可以帮助筛选出与生存相关的基因，并排除噪音。

3. 构建逻辑回归模型

接下来，可以使用逻辑回归模型构建多因素预后模型。逻辑回归模型可以通过glm函数进行拟合，其中dependent变量为生存状态（0或1），independent变量为基因表达数据。在拟合模型时，需要使用交叉验证等方法进行参数选择和模型评估。

4. 评估模型性能

最后，可以使用ROC曲线等指标评估模型的性能。ROC曲线可以使用pROC包中的roc函数绘制，其中需要提供真阳性率（TPR）和假阳性率（FPR）两个参数。可以使用predict函数预测样本的生存概率，并根据生存概率和实际生存状态计算TPR和FPR。通过改变逻辑回归模型的阈值，可以得到不同的TPR和FPR，从而绘制ROC曲线。

要利用交集基因构建多因素预后模型并绘制模型的ROC曲线，您可以按照以下步骤操作：

筛选交集基因：从多个研究中筛选出在所有研究中都具有显著差异表达的基因，作为交集基因。

构建预后模型：使用多因素Cox回归分析，以交集基因的表达水平和临床协变量（如年龄、性别、分期等）作为自变量，构建预后模型。

验证模型：使用独立数据集验证预后模型的准确性和可靠性。可以计算模型的一些评估指标，如一致性指数（C-index）和生存曲线比较的P值等。

绘制ROC曲线：根据预后模型的预测结果和实际观察结果，绘制接收者操作特征曲线（ROC曲线）。ROC曲线展示了模型在不同阈值下的真阳性率和假阳性率。您可以计算曲线下面积（AUC），以评估模型的整体预测能力。

这些步骤并不是固定不变的，具体的流程可能会根据实际情况和研究目的而有所不同。此外，构建多因素预后模型并绘制ROC曲线通常需要使用专业的统计软件和生物信息学工具。

script

```
rm(list = ls())
options(stringsAsFactors = F)

# 安装并载入所需的包
if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")


library(dplyr)
library(edgeR)
library(survival)
```

```r
library(pROC)


# 1. 数据预处理
data <- read.table("/fafu/jiangxiaojiao/data/TCGA.LIHC.sampleMap%2FHiSeqV2.gz",
                   header = TRUE, sep = "\t", row.names = 1)
data_clean <- na.omit(data) # 删除缺失值或空值

data_clinal <-
read.table("/fafu/jiangxiaojiao/data/TCGA.LIHC.sampleMap%2FLIHC_clinicalMatrix",
                                header = TRUE, sep = "\t", row.names = 1)



# 获取患者的癌症状态
sample_id <- colnames(data_clean)
num <- as.numeric(substring(sample_id, 14, 15))  #截取字符串后转为数字
group_list = ifelse(num%in%1:9,"Tumor","Normal")  #ifelse实现分组

# 将 group_list 转换为只有一列的数据框
group_df <- as.data.frame(group_list)
group_df_clean <- na.omit(group_df)

# 在合并后的数据框中添加分组信息
df <- as.data.frame(sample_id)
df$Group <- ""
# 使用 substr 截取 A 列的最后 2 个字符
last_two_chars <- substr(df$A, nchar(df$A) - 1, nchar(df$A))

# 使用 ifelse 根据截取结果设置 B 列的值
df$Group <- ifelse(num%in%1:9,"Tumor","Normal")  #ifelse实现分组

# 将表型信息添加到矩阵中，并为每组分配分组信息
group <- factor(df$Group)
design <- model.matrix(~group)



# 查看 group_list 的类型
class(group_list)
class(data_clean)

# 创建 edgeR 对象
y <- DGEList(counts = data_clean, group = group)

# 进行基因过滤，按照要求选择出需要进行差异分析的基因
keep <- filterByExpr(y, design)
y <- y[keep,,keep.lib.sizes=FALSE]

# 规范化矩阵
y <- calcNormFactors(y)

# 估计离散度
y <- estimateDisp(y, design)

# 对规范化后的表达矩阵进行差异分析
fit <- glmQLFit(y, design)
```

```
qlf <- glmQLFTest(fit, coef=2)

# 筛选差异表达显著的基因
differentially_expressed_genes <- topTags(qlf, adjust.method = "BH", sort.by = "PValue", n =
Inf)

differentially_expressed_genes <-
differentially_expressed_genes[differentially_expressed_genes$table$PValue < 0.05, ]
nrDEG=as.data.frame(differentially_expressed_genes)

head(nrDEG)
edgeR_DEG =nrDEG
nrDEG=edgeR_DEG[,c(1,5)]
colnames(nrDEG) = c("log2FoldChange", "pvalue")

draw_h_v <- function(exprSet,need_DEG,n='DEseq2',group_list,logFC_cutoff){
  ## we only need two columns of DEG, which are log2FoldChange and pvalue
  ## heatmap

  library(pheatmap)
  choose_gene=head(rownames(need_DEG),50) ## 50 maybe better
  choose_matrix=exprSet[choose_gene,]
  choose_matrix[1:4,1:4]
  choose_matrix=t(scale(t(log2(choose_matrix+1))))
  ## http://www.bio-info-trainee.com/1980.html
  annotation_col = data.frame( group_list=group_list  )
  rownames(annotation_col)=colnames(exprSet)
  pheatmap(choose_matrix,show_colnames = F,annotation_col = annotation_col,
           filename = paste0(n,'_need_DEG_top50_heatmap.png'))


  library(ggfortify)
  df=as.data.frame(t(choose_matrix))
  df$group=group_list
  png(paste0(n,'_DEG_top50_pca.png'),res=120)
  p=autoplot(prcomp( df[,1:(ncol(df)-1)] ), data=df,colour = 'group')+theme_bw()
  print(p)
  dev.off()


  if(! logFC_cutoff){
    logFC_cutoff <- with(need_DEG,mean(abs( log2FoldChange)) + 2*sd(abs( log2FoldChange)) )

  }
  # logFC_cutoff=1

  need_DEG$change = as.factor(ifelse(need_DEG$pvalue < 0.05 & abs(need_DEG$log2FoldChange) >
logFC_cutoff,
                                     ifelse(need_DEG$log2FoldChange > logFC_cutoff
,'UP','DOWN'),'NOT')
  )
  this_tile <- paste0('Cutoff for logFC is ',round(logFC_cutoff,3),
                      '\nThe number of up gene is ',nrow(need_DEG[need_DEG$change =='UP',]) ,
                      '\nThe number of down gene is ',nrow(need_DEG[need_DEG$change
=='DOWN',])
  )
  library(ggplot2)
```

```
  g = ggplot(data=need_DEG,
             aes(x=log2FoldChange, y=-log10(pvalue),
                 color=change)) +
    geom_point(alpha=0.4, size=1.75) +
    theme_set(theme_set(theme_bw(base_size=20)))+
    xlab("log2 fold change") + ylab("-log10 p-value") +
    ggtitle( this_tile ) + theme(plot.title = element_text(size=15,hjust = 0.5))+
    scale_colour_manual(values = c('blue','black','red')) ## corresponding to the
levels(res$change)
  print(g)
  ggsave(g,filename = paste0(n,'_volcano.png'))
  dev.off()
}
draw_h_v(data_clean, nrDEG, "edgeR", group_list, 1)

# differentially_expressed_genes$PValue
# 检查两个数据框的行数
nrow(data_clean)
nrow(group_df)
nrow(differentially_expressed_genes)

percentage <- nrow(differentially_expressed_genes) / nrow(data_clean)

# 取交集
# 将differentially_expressed_genes转换为数据框对象
differentially_expressed_genes_name <- row.names(differentially_expressed_genes)
# differentially_expressed_genes_name <- as.data.frame()
autophagy_genes <- read.table("/fafu/jiangxiaojiao/methy_array/gene_temp.txt", header =
FALSE)
# write.table(differentially_expressed_genes_name, file = "output.txt", sep = ",", row.names
= FALSE)
vec2 <- as.vector(autophagy_genes[,])
result <- intersect(differentially_expressed_genes_name, vec2)

# 使用unique函数去除重复行
unique_genes <- unique(result)

# 输出结果
unique_genes


################### 2023/03/31 ##########################
BRCA <- data_clean[unique_genes,]
survival_data<- read.table("/fafu/jiangxiaojiao/methy_array/TCGA-LIHC.survival.tsv", header =
T)


################### 2023/04/03 ##########################
# 获取Expr的rownames
sample_id_with_lasfix <- survival_data$sample
sample_id_without_lasfix <- substring(sample_id_with_lasfix, 1, 15) # 截取字符串后转为数字
survival_data$sample_id_without_lasfix <- sample_id_without_lasfix

meta = left_join(survival_data,data_clinal,by = c("sample"= "bcr_sample_barcode"))
# 去掉表达矩阵里没有的样本
library(stringr)
exprSet <- data_clean
```

```
# 将列名中的点号替换为下划线
tmp <- t(exprSet)
colnames(exprSet) <- gsub("\\.", "-", colnames(exprSet))



k = meta$sample_id_without_lasfix %in% colnames(exprSet);table(k)
meta = meta[k,]

# 去掉生存信息不全或者生存时间小于30天的样本，样本纳排标准不唯一，且差别很大
# 去掉没有性别的样本
k1 = meta$OS.time >= 30;table(k1)
k2 = !(is.na(meta$OS.time)|is.na(meta$OS));table(k2)
k3 = !(is.na(meta$gender)|is.na(meta$gender));table(k3)
meta = meta[k1 & k2, ]
meta = meta[k3,]

# 选择有用的列
tmp = data.frame(colnames(meta))
meta = meta[, c(
    "sample",
    "sample_id_without_lasfix",
    "OS",
    "OS.time",
    "histological_type",
    "age_at_initial_pathologic_diagnosis",
    "gender",
    "pathologic_T"
)]


dim(meta)

meta[1:4,1:4]

#简化meta的列名
colnames(meta)=c('sample','sample_id','event', 'time','type','age','gender','stage')

# 空着的值、not reported改为NA
meta[meta == "" | meta == "not reported"] = NA
meta <- na.omit(meta)
rownames(meta) <- meta$sample_id
survival_data[1:4, 1:4]
dim(survival_data)

########## 3.实现表达矩阵与临床信息的匹配 ############
# clinical_data_preparation <- merge(meta_filed_with_data_clinal, survival_data)
# dim(clinical_data_preparation)
# dim(meta_filed_with_data_clinal)
dim(survival_data)
# 将列名中的点号替换为下划线
colnames(BRCA) <- gsub("\\.", "-", colnames(BRCA))
# tmp <- t(BRCA)

# 输出修改后的列名
# 将矩阵转换为数据框
```

```
tmp_df <- t(as.data.frame(BRCA))
# tmp_df$X_PATIENT <- rownames(tmp_df)
# 将矩阵转换为数据框
# survival_data <- as.data.frame(survival_data)

# 调整meta行名与exprSet列名一一对应
s = intersect(rownames(tmp_df), meta$sample_id);table(s)
exprSet <- tmp_df[s,]
meta = meta[s,]
identical(rownames(meta), rownames(exprSet))


#### 4. 整理生存分析的输入数据
#### 生存分析的输入数据里，要求结局事件必须用0和1表示，0表示活着，1表示死了；生存时间的单位（月）；
table(meta$event)
range(meta$time)
meta$time = meta$time/30
range(meta$time)

# 去除stage里的冗余信息
head(meta$stage)

meta$stage = meta$stage %>%
  str_remove("stage ") %>%
  str_to_upper()

table(meta$stage,useNA = "always")

# 不需要ABC可以去掉，需要的话就保留，不运行下面这句
meta$stage = str_remove(meta$stage,"A|B|C")

head(meta)

# 二.生存分析 ##################################
ls()
exprSet[1:4,1:4]
meta[1:4, 1:4]
library(survival)
library(survminer)

sfit <- survfit(Surv(time, event)~gender, data=meta)
ggsurvplot(sfit,pval=TRUE)
ggsurvplot(sfit,
           palette = "jco",
           risk.table =TRUE,
           pval =TRUE,
           conf.int =TRUE)
# save(meta,exprSet,proj,file = paste0(proj,"_sur_model.Rdata"))

# 年龄
group = ifelse(meta$age>median(meta$age,na.rm = T),"older","younger")
table(group)
sfit=survfit(Surv(time, event)~group, data=meta)
ggsurvplot(sfit,pval =TRUE, data = meta, risk.table = TRUE)

# 基因
g = colnames(exprSet)[1]
```

```
g
# 将数据框中所有列转换为数值型
# exprSet <- apply(exprSet, 2, as.numeric)
meta$gene = ifelse(exprSet[,g]> median(exprSet[,g],na.rm = T),'high','low')
sfit=survfit(Surv(time, event)~gene, data=meta)
ggsurvplot(sfit,pval =TRUE, data = meta, risk.table = TRUE)

save(meta,exprSet,file = paste0("step_01","_sur_model.Rdata"))
# 如何保存图片来着?

# logrankfile = paste0("step_01", "_sur_model.Rdata")
# 3.log-rank test ############################
logrankfile = paste0("step_01","_log_rank_p.Rdata")
load("step_01_sur_model.Rdata")
if(!file.exists(logrankfile)){
  log_rank_p <- apply(t(exprSet) , 1 , function(gene){
    meta$group=ifelse(gene>median(gene),'high','low')
    data.survdiff=survdiff(Surv(time, event)~group,data=meta)
    p.val = 1 - pchisq(data.survdiff$chisq, length(data.survdiff$n) - 1)
    return(p.val)
  })
  log_rank_p=sort(log_rank_p)
  save(log_rank_p,file = logrankfile)
}
load(logrankfile)
table(log_rank_p<0.01)
table(log_rank_p < 0.05)

# 4.批量单因素cox ##############################
proj <- "step_01"
coxfile = paste0(proj,"_cox.Rdata")
if(!file.exists(coxfile)){
  cox_results <- apply(t(exprSet), 1 , function(gene){
  meta$gene = gene
  #可直接使用连续型变量
  m = coxph(Surv(time, event) ~ gene, data =  meta)
  #也可使用二分类变量
  #meta$group=ifelse(gene>median(gene),'high','low')
  #meta$group = factor(meta$group,levels = c("low","high"))
  #m=coxph(Surv(time, event) ~ group, data =  meta)

  beta <- coef(m)
  se <- sqrt(diag(vcov(m)))
  HR <- exp(beta)
  HRse <- HR * se

  #summary(m)
  tmp <- round(cbind(coef = beta,
                     se = se, z = beta/se,
                     p = 1 - pchisq((beta/se)^2, 1),
                     HR = HR, HRse = HRse,
                     HRz = (HR - 1) / HRse,
                     HRp = 1 - pchisq(((HR - 1)/HRse)^2, 1),
                     HRCILL = exp(beta - qnorm(.975, 0, 1) * se),
                     HRCIUL = exp(beta + qnorm(.975, 0, 1) * se)), 3)

  return(tmp['gene',])
```

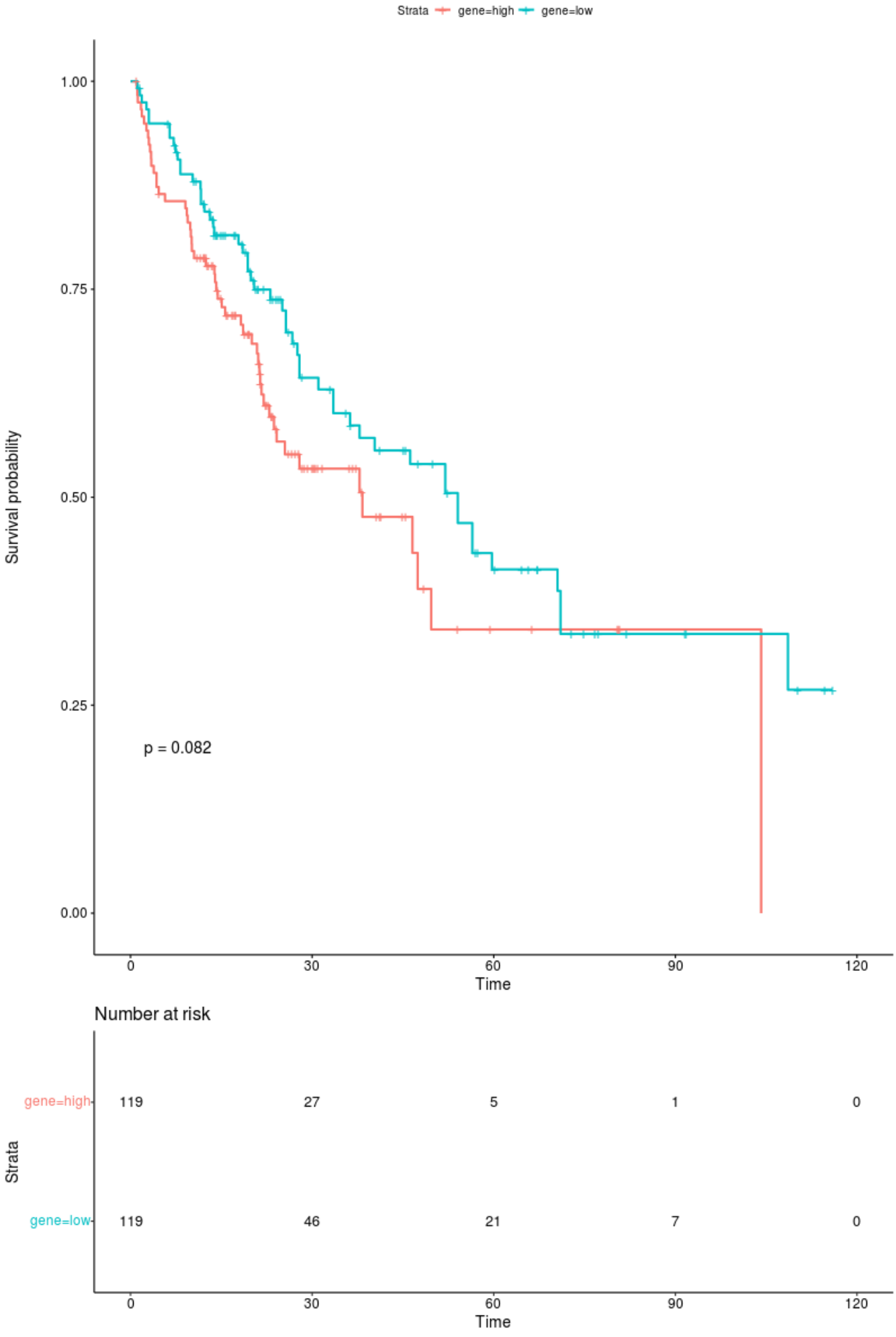
```
  #return(tmp['grouphigh',])#二分类变量
})
  cox_results=as.data.frame(t(cox_results))
  save(cox_results,file = coxfile)
}
load(coxfile)
table(cox_results$p<0.01)
table(cox_results$p<0.05)

lr = names(log_rank_p)[log_rank_p<0.01];length(lr)
cox = rownames(cox_results)[cox_results$p<0.01];length(cox)
length(intersect(lr,cox))
save(lr,cox,file = paste0(proj,"_logrank_cox_gene.Rdata"))


# 5.lasso回归
# 1.准备输入数据
rm(list = ls())
# proj = "TCGA-KIRC"
proj <- "step_01"
load(paste0(proj,"_sur_model.Rdata"))
ls()
exprSet[1:4,1:4]
meta[1:4,1:4]
load(paste0(proj,"_logrank_cox_gene.Rdata"))
exprSet = t(exprSet)[cox, ]

# 2.构建lasso回归模型
x=t(exprSet)  # x行名为样本，列名为基因
y=meta$event
library(glmnet)
library(ggplot2)
## 2.1挑选合适的λ值
#调优参数
set.seed(1006) # 选取不同的数，画出来的效果不同
cv_fit <- cv.glmnet(x = x, y = y)
# png("myplot.png", width = 1000, height = 1000)

plot(cv_fit)
# dev.off()
# ggsave("myplot.png", plot = myplot, device = "png", width = 6, height = 4, dpi = 300)
# #系数图
# fit <- glmnet(x=x, y=y)
# plot(fit, xvar = "lambda")

# 2.2 用这两个λ值重新建模
model_lasso_min <- glmnet(x=x, y=y,lambda=cv_fit$lambda.min)
model_lasso_1se <- glmnet(x = x, y = y, lambda = cv_fit$lambda.1se)

head(model_lasso_min$beta,20)
choose_gene_min=rownames(model_lasso_min$beta)[as.numeric(model_lasso_min$beta)!=0]
choose_gene_1se=rownames(model_lasso_1se$beta)[as.numeric(model_lasso_1se$beta)!=0]
length(choose_gene_min)
length(choose_gene_1se)
save(choose_gene_min,file = paste0(proj,"_lasso_choose_gene_min.Rdata"))
save(choose_gene_1se, file = paste0(proj, "_lasso_choose_gene_1se.Rdata"))
```

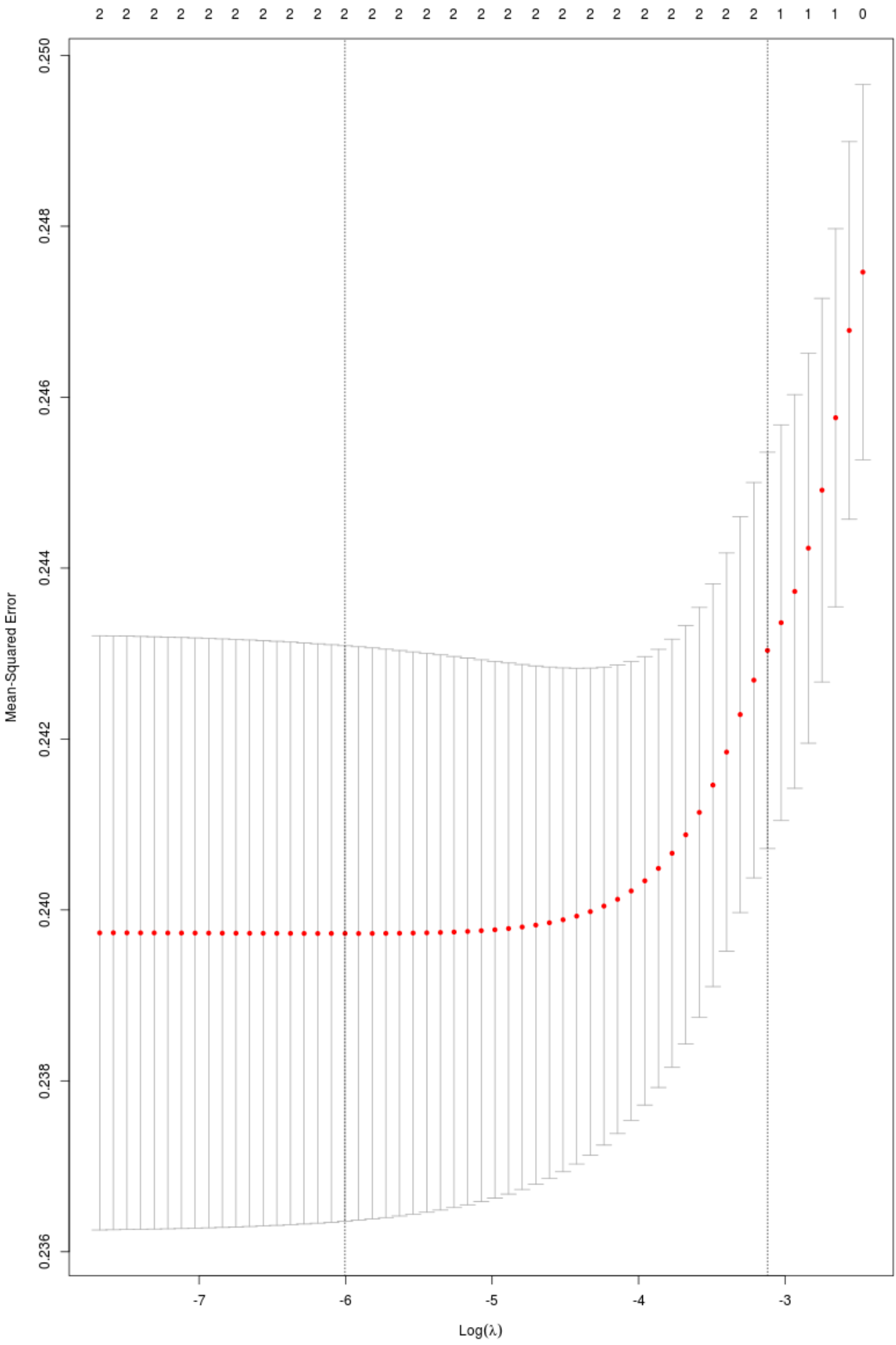
```
# 3.模型预测和评估
lasso.prob <- predict(cv_fit, newx=x , s=c(cv_fit$lambda.min,cv_fit$lambda.1se) )
re=cbind(y ,lasso.prob)
head(re)
re=as.data.frame(re)
colnames(re)=c('event','prob_min','prob_1se')
re$event=as.factor(re$event)
# ROC曲线
library(pROC)
library(ggplot2)
m <- roc(meta$event, re$prob_min)
g <- ggroc(m,legacy.axes = T,size = 1,color = "#2fa1dd")
auc(m)  # Area under the curve: 0.9953

g + theme_minimal() +
  geom_segment(aes(x = 0, xend = 1, y = 0, yend = 1),
               colour = "grey", linetype = "dashed")+
  annotate("text",x = .75, y = .25,
           label = paste("AUC of min = ",format(round(as.numeric(auc(m)),2),nsmall =
2)),color = "#2fa1dd")

# 计算AUC取值范围在0.5-1之间，越接近于1越好。可以根据预测结果绘制ROC曲线。
# 两个模型的曲线画在一起
m2 <- roc(meta$event, re$prob_1se)
auc(m2) # Area under the curve: 0.9136
g <- ggroc(list(min = m,se = m2),legacy.axes = T,size = 1)

g + theme_minimal() +
  scale_color_manual(values = c("#2fa1dd", "#f87669"))+
  geom_segment(aes(x = 0, xend = 1, y = 0, yend = 1),
               colour = "grey", linetype = "dashed")+
  annotate("text",x = .75, y = .25,
           label = paste("AUC of min = ",format(round(as.numeric(auc(m)),2),nsmall =
2)),color = "#2fa1dd")+
  annotate("text",x = .75, y = .15,
           label = paste("AUC of 1se = ",format(round(as.numeric(auc(m2)),2),nsmall =
2)),color = "#f87669")

# 5.切割数据构建模型并预测
## 5.1 切割数据
library(caret)
set.seed(12345679)
sam<- createDataPartition(meta$event, p = .5,list = FALSE)
head(sam)
train <- exprSet[,sam]
test <- exprSet[,-sam]
train_meta <- meta[sam,]
test_meta <- meta[-sam,]

prop.table(table(train_meta$stage))
prop.table(table(test_meta$stage))
prop.table(table(test_meta$gender))
prop.table(table(train_meta$gender))

# 5.2 切割后的train数据集建模
#计算lambda
x = t(train)
```

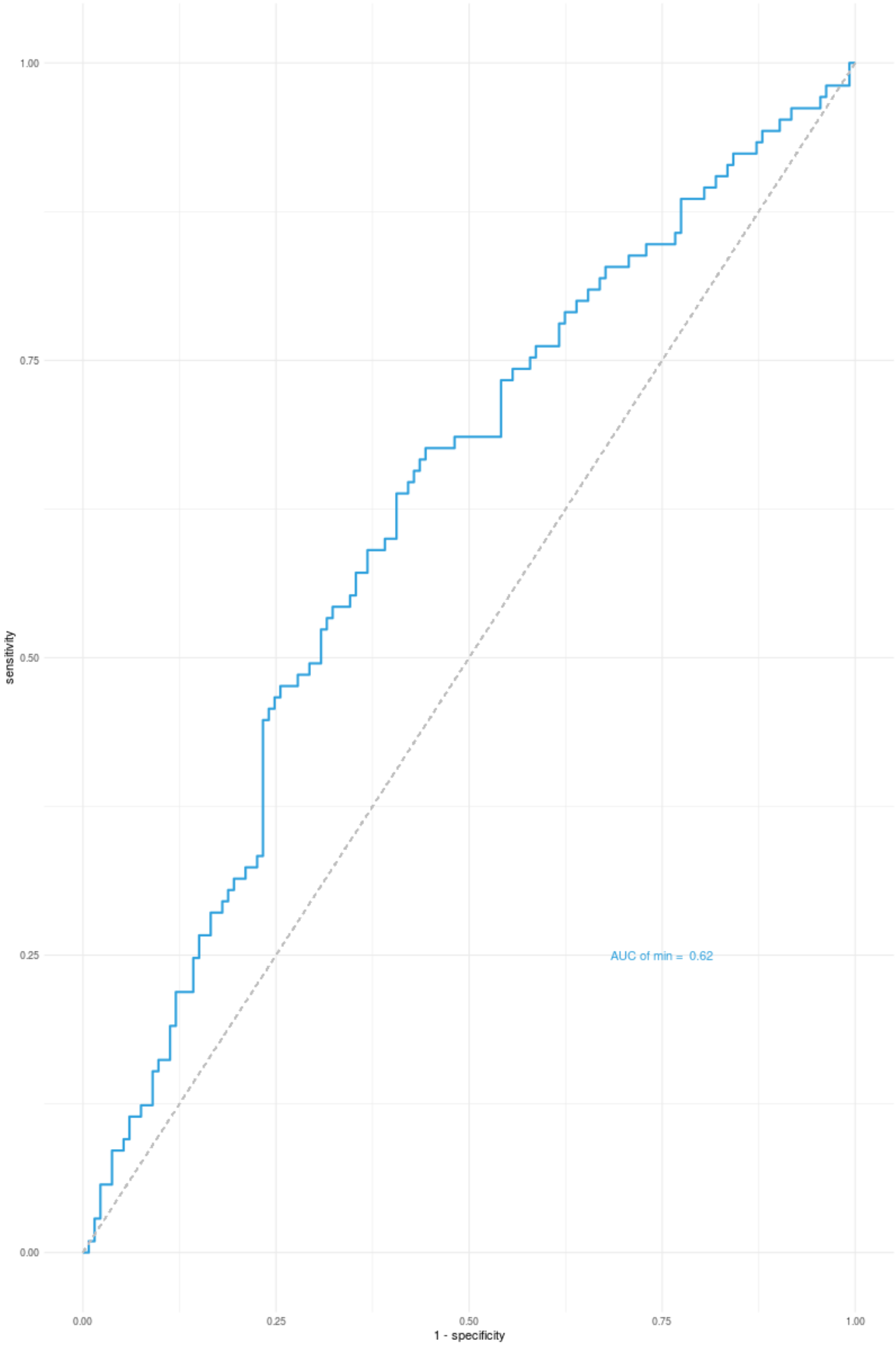
```
y = train_meta$event
cv_fit <- cv.glmnet(x=x, y=y)
plot(cv_fit)

#构建模型
model_lasso_min <- glmnet(x=x, y=y,lambda=cv_fit$lambda.min)
model_lasso_1se <- glmnet(x=x, y=y,lambda=cv_fit$lambda.1se)
#挑出基因
head(model_lasso_min$beta)
choose_gene_min=rownames(model_lasso_min$beta)[as.numeric(model_lasso_min$beta)!=0]
choose_gene_1se=rownames(model_lasso_1se$beta)[as.numeric(model_lasso_1se$beta)!=0]
length(choose_gene_min)
length(choose_gene_1se)

# 4.模型预测
lasso.prob <- predict(cv_fit, newx=t(test), s=c(cv_fit$lambda.min,cv_fit$lambda.1se) )
re=cbind(event = test_meta$event ,lasso.prob)
re=as.data.frame(re)
colnames(re)=c('event','prob_min','prob_1se')
re$event=as.factor(re$event)
head(re)

# 再画ROC曲线
library(pROC)
library(ggplot2)
m <- roc(test_meta$event, re$prob_min)
g <- ggroc(m,legacy.axes = T,size = 1,color = "#2fa1dd")
auc(m) #Area under the curve: 0.7752

g + theme_minimal() +
  geom_segment(aes(x = 0, xend = 1, y = 0, yend = 1),
               colour = "grey", linetype = "dashed")+
  annotate("text",x = .75, y = .25,
           label = paste("AUC of min = ",format(round(as.numeric(auc(m)),2),nsmall =
2)),color = "#2fa1dd")


# 计算AUC取值范围在0.5-1之间，越接近于1越好。可以根据预测结果绘制ROC曲线。
# 两个模型的曲线画在一起
m2 <- roc(test_meta$event, re$prob_1se)
auc(m2)  # Area under the curve: 0.7426
g <- ggroc(list(min = m,se = m2),legacy.axes = T,size = 1)

g + theme_minimal() +
  scale_color_manual(values = c("#2fa1dd", "#f87669"))+
  geom_segment(aes(x = 0, xend = 1, y = 0, yend = 1),
               colour = "grey", linetype = "dashed")+
  annotate("text",x = .75, y = .25,
           label = paste("AUC of min = ",format(round(as.numeric(auc(m)),2),nsmall =
2)),color = "#2fa1dd")+
  annotate("text",x = .75, y = .15,
           label = paste("AUC of 1se = ",format(round(as.numeric(auc(m2)),2),nsmall =
2)),color = "#f87669")

# 6.cox-forest
# 输入数据
if(!require(My.stepwise))install.packages("My.stepwise")
```

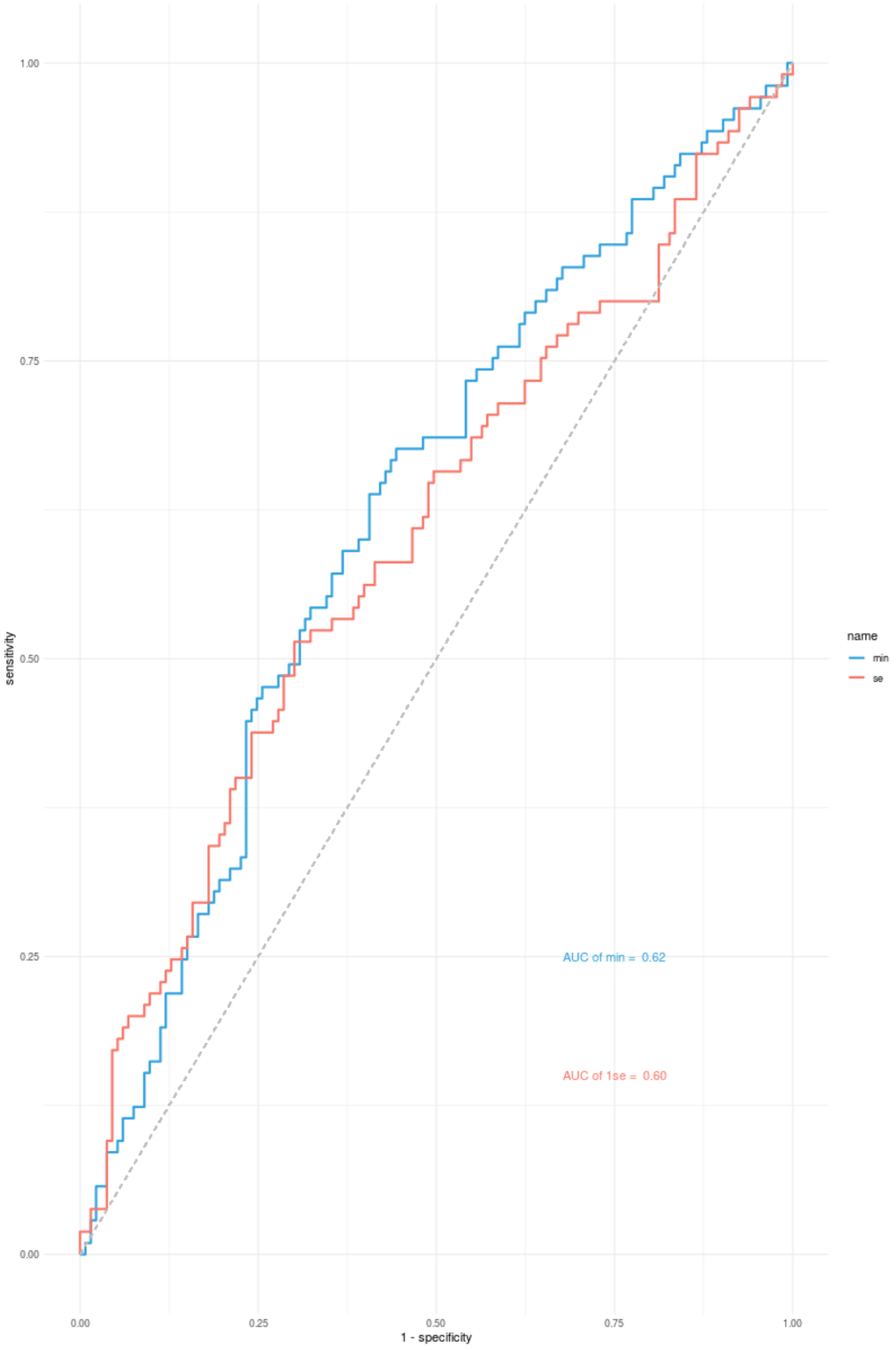
```
load(paste0(proj,"_sur_model.Rdata"))
load(paste0(proj,"_lasso_choose_gene_1se.Rdata"))
g = choose_gene_1se
# 2.构建coxph模型
library(stringr)
e=t(exprSet[,g])
colnames(e)= str_replace_all(colnames(e),"-","_")
dat = cbind(meta, t(e))

dat$gender=as.numeric(factor(dat$gender))
dat$stage=as.numeric(factor(dat$stage))
colnames(dat)

# 逐步回归法构建最优模型
library(survival)
library(survminer)
# 不能允许缺失值
dat2 = na.omit(dat)
library(My.stepwise)
vl <- colnames(dat)[c(5:ncol(dat))]
# My.stepwise.coxph(Time = "time",
#                   Status = "event",
#                   variable.list = vl,
#                   data = dat2)
# 使用输出结果里的最后一个模型
model = coxph(formula = Surv(time, event) ~ stage + age  +
    PPP1R15A + GABARAPL1, data = dat2)

# 3.模型可视化-森林图
ggforest(model,data = dat2)

fp <- predict(model,newdata = dat2)
library(Hmisc)
options(scipen=200)
with(dat2,rcorr.cens(fp,Surv(time, event)))
# > with(dat2,rcorr.cens(fp,Surv(time, event)))
#        C Index            Dxy           S.D.              n        missing
#     0.33787188    -0.32425625     0.05842713   238.00000000     0.00000000
#     uncensored Relevant Pairs     Concordant      Uncertain
#   105.00000000 29378.00000000  9926.00000000 26986.00000000

# 7.切割数据构建模型并预测
# 7.1 用R包caret切割数据，生成的结果是一组代表列数的数字

library(caret)
set.seed(12345679)
sam <- createDataPartition(meta$event, p = .5, list = FALSE)
exprSet <- t(exprSet)
train <- exprSet[,sam]
test <- exprSet[,-sam]
train_meta <- meta[sam,]
test_meta <- meta[-sam,]
# 7.2 切割后的train数据集建模
e=t(train[g,])
colnames(e)= str_replace_all(colnames(e),"-","_")
dat=cbind(train_meta,e)
```

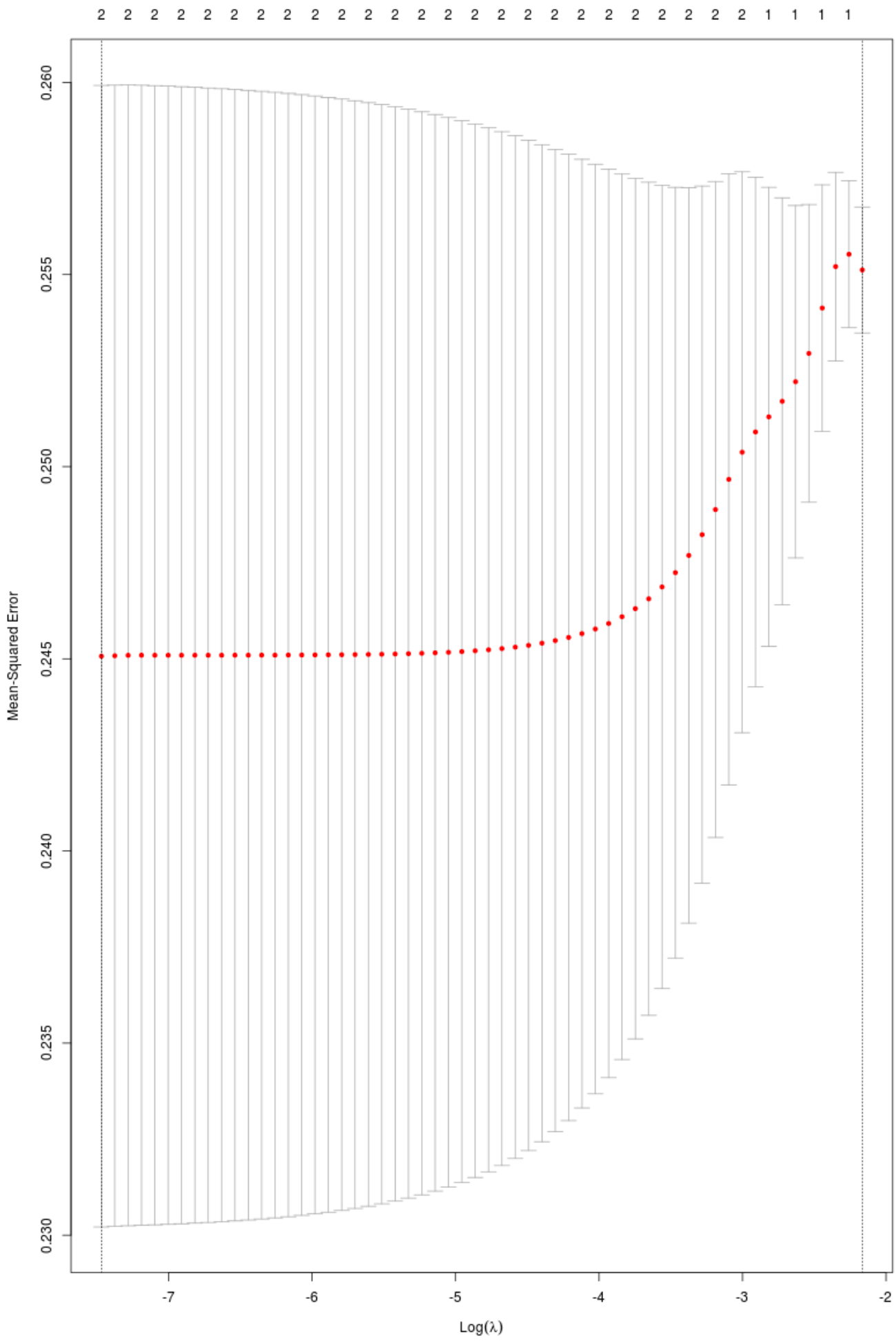
```r
dat$gender=as.numeric(factor(dat$gender))
dat$stage=as.numeric(factor(dat$stage))
colnames(dat)
library(My.stepwise)
dat2 = na.omit(dat)
vl <- colnames(dat2)[c(5:ncol(dat2))]
# My.stepwise.coxph(Time = "time",
#                   Status = "event",
#                   variable.list = vl,
#                   data = dat2)
model = coxph(formula = Surv(time, event) ~ stage + age  +
    PPP1R15A + GABARAPL1, data = dat2)

# 7.3 模型可视化
ggforest(model, data =dat2)
# 7.4 用切割后的数据test数据集验证模型
e=t(test[g,])
colnames(e)= str_replace_all(colnames(e),"-","_")
test_dat=cbind(test_meta,e)
test_dat$gender=as.numeric(factor(test_dat$gender))
test_dat$stage=as.numeric(factor(test_dat$stage))
fp <- predict(model,newdata = test_dat)
library(Hmisc)
with(test_dat,rcorr.cens(fp,Surv(time, event)))
#  > with(test_dat,rcorr.cens(fp,Surv(time, event)))
#        C Index            Dxy           S.D.              n        missing
#     0.37171655    -0.25656689     0.09203007   119.00000000     0.00000000
#     uncensored Relevant Pairs     Concordant      Uncertain
#    47.00000000  6548.00000000  2434.00000000  7488.00000000
```

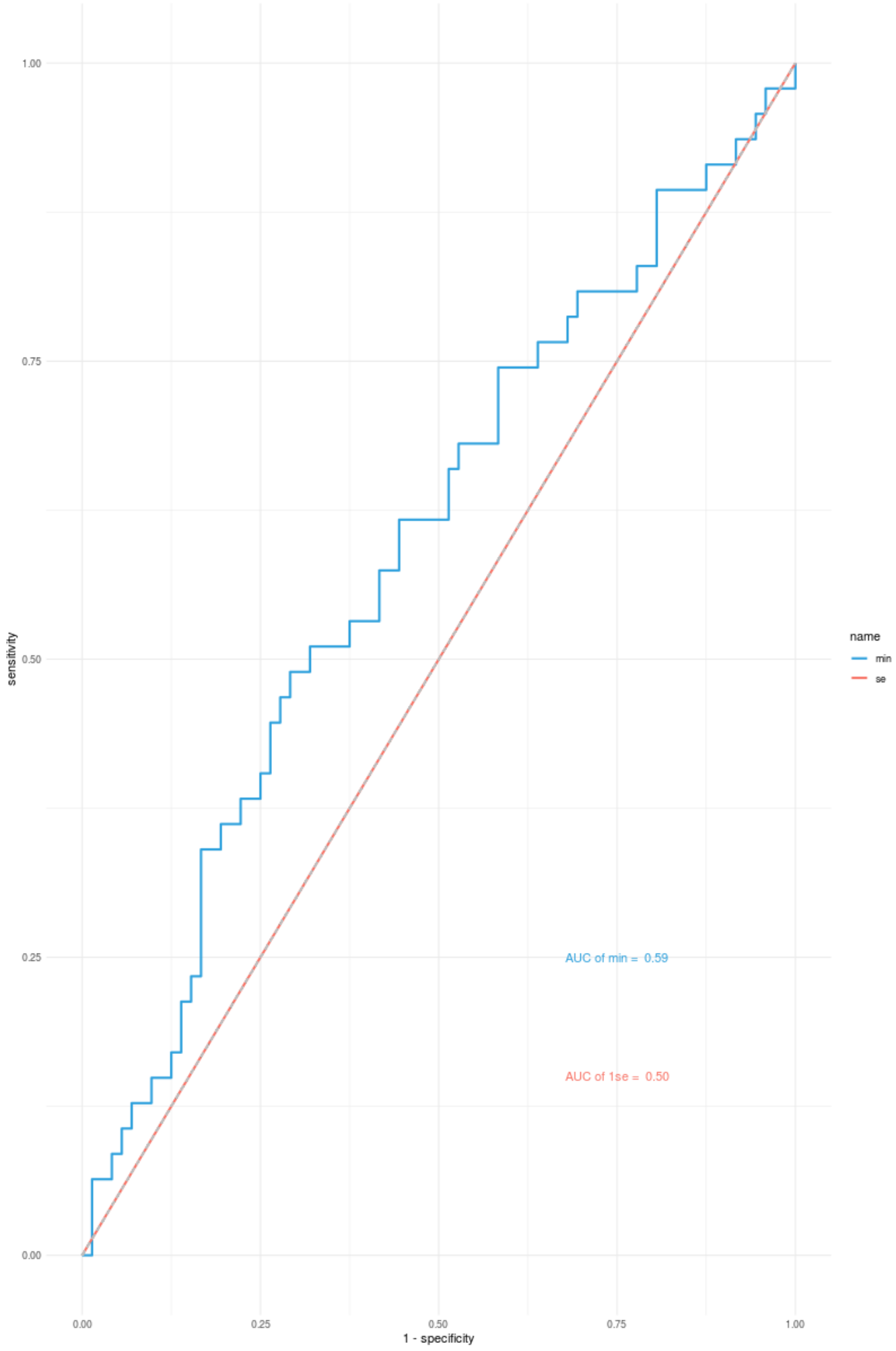

Strata gene=high gene=low
1.00
0.75
0.50
0.25
0.00
Survival probability
p = 0.082
0
30
60
90
120
Time
Number at risk
gene=high
gene=low
Strata
119
27
5
1
0
119
46
21
7
0
0
30
60
90
120
Time

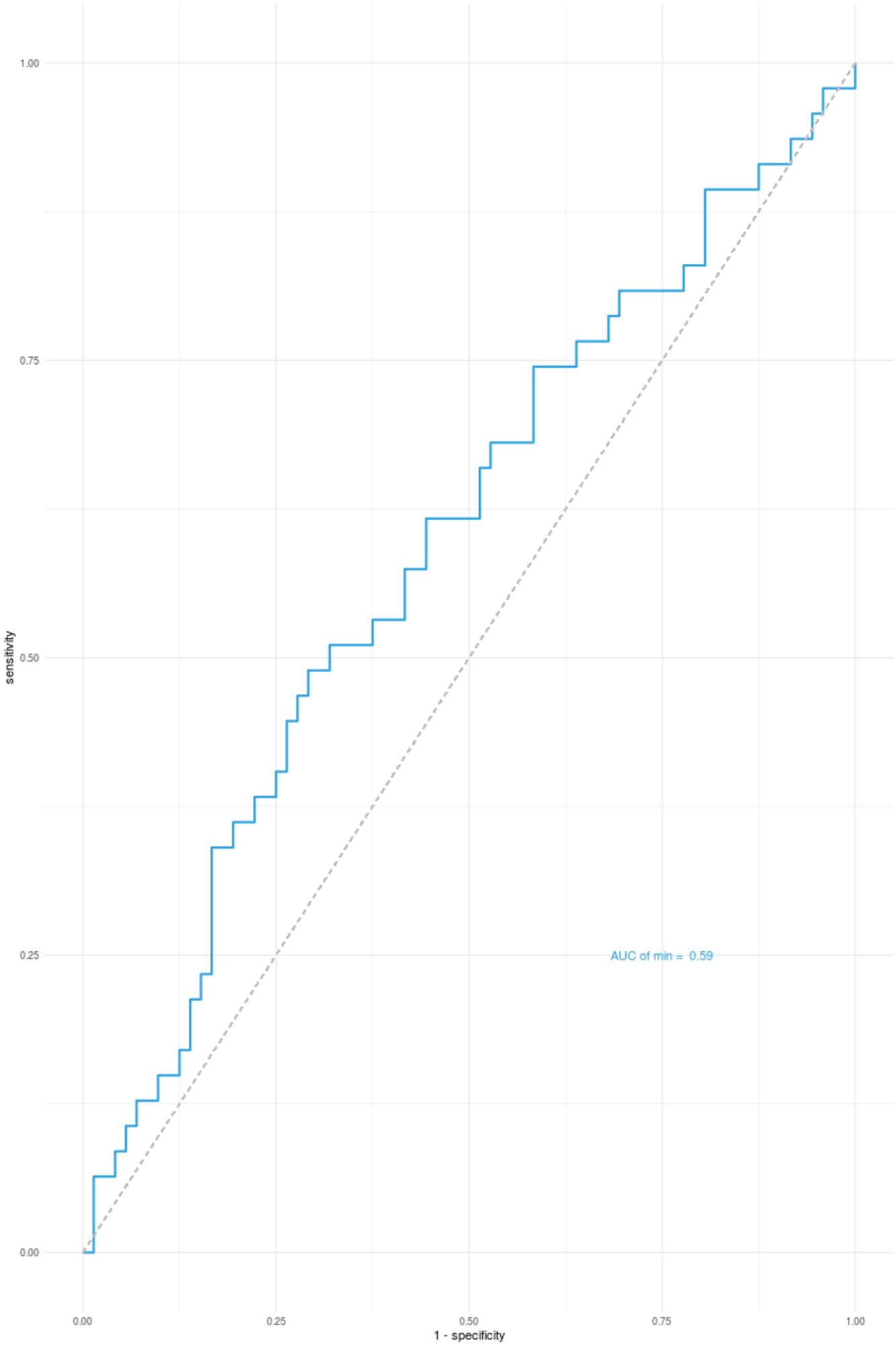

2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 1 1 0
0.250
0.248
0.246
0.244
0.242
0.240
0.238
0.236
Mean-Squared Error
-7
-6
-5
-4
-3
Log(λ)

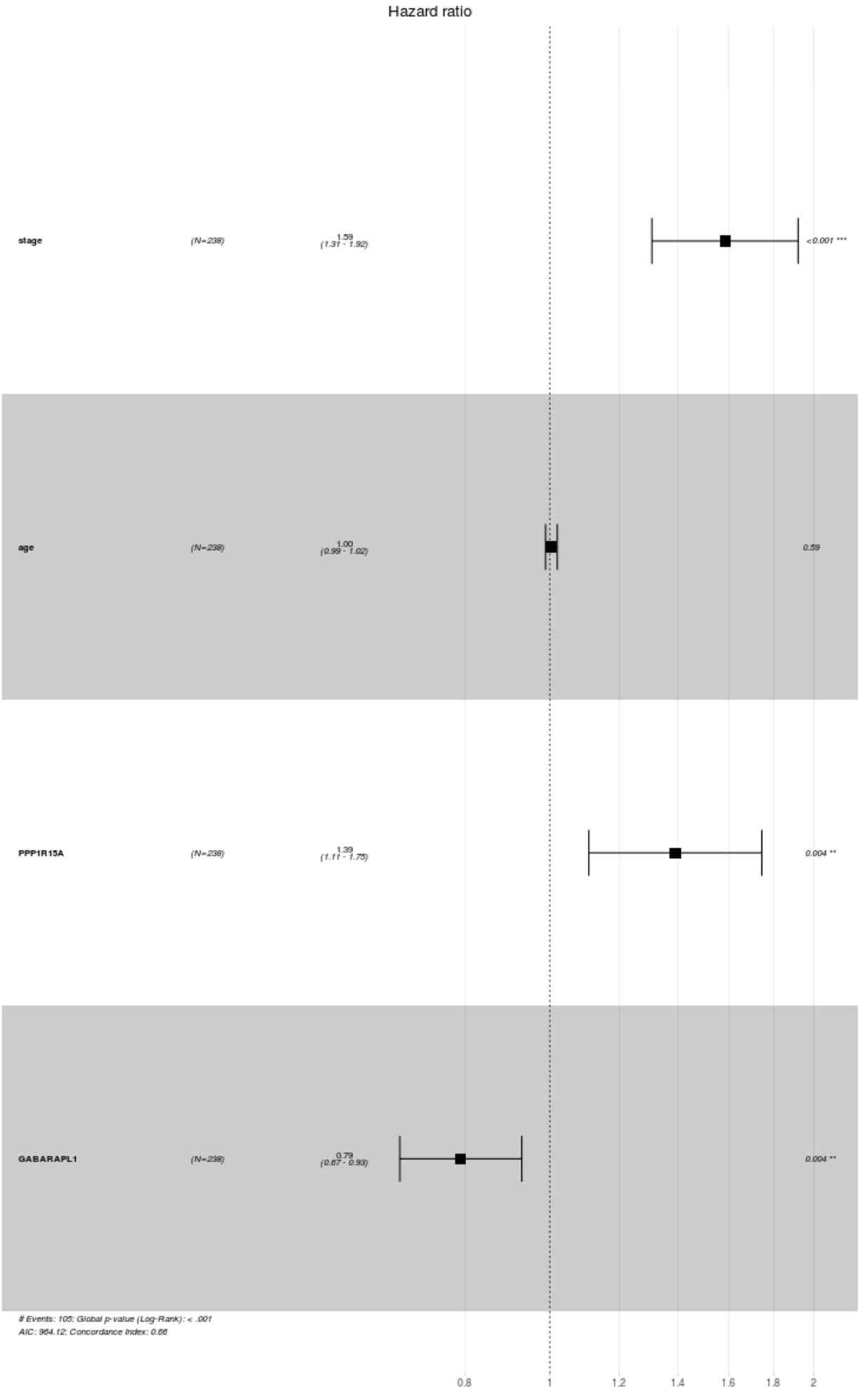

1.00
0.75
0.50
0.25
0.00
sensitivity
AUC of min = 0.62
0.00
0.25
0.50
0.75
1.00
1 - specificity

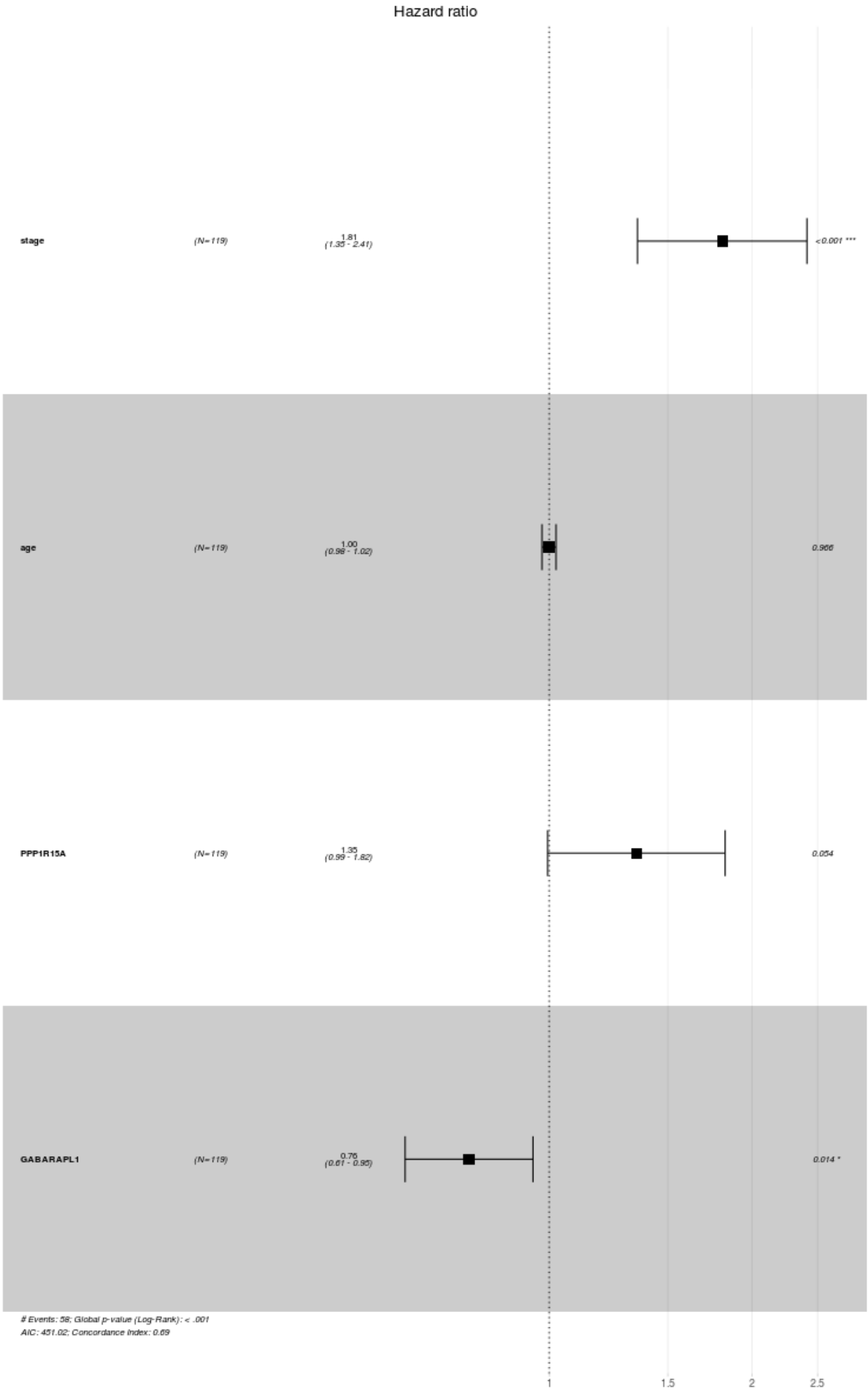

1.00
0.75
0.50
0.25
0.00
sensitivity
0.00
0.25
0.50
0.75
1.00
1 - specificity
name
min
se
AUC of min = 0.62
AUC of 1se = 0.60


2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 1 1 1
0.260
0.255
0.250
0.245
0.240
0.235
0.230
Mean-Squared Error
-7
-6
-5
-4
-3
-2
Log(λ)


1.00
0.75
0.50
0.25
0.00
sensitivity
0.00
0.25
0.50
0.75
1.00
1 - specificity
name
min
se
AUC of min = 0.59
AUC of 1se = 0.50


1.00
0.75
0.50
0.25
0.00
sensitivity
AUC of min = 0.59
0.00
0.25
0.50
0.75
1.00
1 - specificity


Hazard ratio
stage (N=238) 1.59 (1.31 - 1.92) <0.001 ***
age (N=238) 1.00 (0.99 - 1.02) 0.59
PPP1R15A (N=238) 1.39 (1.11 - 1.75) 0.004 **
GABARAPL1 (N=238) 0.79 (0.67 - 0.93) 0.004 **
Events: 105; Global p-value (Log-Rank): < .001
AIC: 964.12; Concordance Index: 0.66
0.8
1
1.2
1.4
1.6
1.8
2


Hazard ratio
stage
(N=119)
1.81
(1.35 - 2.41)
<0.001 ***
age
(N=119)
1.00
(0.98 - 1.02)
0.966
PPP1R15A
(N=119)
1.35
(0.99 - 1.82)
0.054
GABARAPL1
(N=119)
0.76
(0.61 - 0.95)
0.014 *
Events: 58; Global p-value (Log-Rank): < .001
AIC: 451.02; Concordance Index: 0.69
1
1.5
2
2.5

参考文献

> UCSC xena如何下载TCGA临床数据

> TCGA_BRCA数据挖掘测试

> An Integrated TCGA Pan-Cancer Clinical Data Resource (TCGA-CDR) to drive high quality survival outcome analytics

> 利用R代码从UCSC XENA下载mRNA, lncRNA, miRNA表达数据并匹配临床信息

> 自噬相关基因集合

> HADb Human Autophagy Database

> 生新技能树 TCGA知识图谱

> 预后模型构建”思路攻略

> 【生信常用图表】一：数据挖掘之预后模型的构建

> 生信技能树 TCGA癌症数据挖掘之预后模型建立和评价