library(dplyr)

library(tidyverse)

library(dplyr)

library(splines)

library(survminer)

library(survival)

library(lubridate)

library(shinythemes)

library(shiny)

library(ggplot2)

library(lazyeval)

library(tibble)

# DIRECTIONS: Only input needed is lines 19 to 26.

setwd("")

Data\_Input\_Sheet<-Excel\_sample\_sheet

Experiment<-"Sample"

Reference\_Group<-"Group1"

x\_axisLabel<-"Hours"

x\_axisScale<-24

time\_interval<-8

groupsexamine<-c("Group1","Group2","Group3","Group4")

Scoring<- subset(Data\_Input\_Sheet, select=c("Image", "Cell", "Time", "Status","ImagingPlate","transfection","DrugPlate","Drug","Class"))

Scoring$Alive<-ifelse(Scoring$Status=="TRUE",1,2)

Scoring$Well\_ROI<-paste(Scoring$Image,Scoring$Cell, sep="\_")

Scoring$Well\_ROI\_ImagingPlate<-paste(Scoring$Well\_ROI,Scoring$ImagingPlate,sep="\_")

Scoring$TimeAdjusted <- Scoring$Time -1

Scoring$TimeAdjusted<-Scoring$TimeAdjusted\*time\_interval

Scoring$NplusOneTimepoint<- append(Scoring$TimeAdjusted[-1], 0, length(Scoring$TimeAdjusted-1))

Scoring$IsFirstImage <-ifelse(Scoring$TimeAdjusted==0,"FIRST","")

Scoring$NplusOneScore<-append(Scoring$Alive[-1], 0, length(Scoring$Alive-1))

d<- append(Scoring$Alive, 0, after=0)

Scoring$NminusOneScore<-d[1:length(d)-1]

e<- append(Scoring$NminusOneScore,0, after=0)

Scoring$NminusTwoScore<-e[1:length(e)-1]

Scoring$Sum3TimepointsforMiddle <- rowSums(cbind(Scoring$Alive,Scoring$NminusOneScore,Scoring$NplusOneScore))

Scoring$Sum2TimepointsforFirst<- rowSums(cbind(Scoring$Alive,Scoring$NplusOneScore))

Scoring$Sum3TimepointsforLast<-rowSums(cbind(Scoring$Alive,Scoring$NminusOneScore,Scoring$NminusTwoScore))

Scoring$CellPositionID\_Last<-ifelse(Scoring$NplusOneTimepoint==0,"LAST","")

Scoring$CellPositionID\_FirstandLast <- paste(Scoring$CellPositionID\_Last,Scoring$IsFirstImage)

Scoring$CellPostion<-ifelse(Scoring$IsFirstImage=="FIRST"|Scoring$CellPositionID\_Last=="LAST",Scoring$CellPositionID\_FirstandLast,"MIDDLE")

Scoring$MiddleStatus<-ifelse(Scoring$CellPostion=="MIDDLE" & Scoring$Sum3TimepointsforMiddle>=5, 1,0)

Scoring$FirstStatus<-ifelse(Scoring$CellPostion==" FIRST" & Scoring$Sum2TimepointsforFirst>=4, 1,0)

Scoring$LastStatus<-ifelse(Scoring$CellPositionID\_Last=="LAST" & Scoring$Alive ==2, 1,0)

Scoring$ScoringALL<-rowSums(cbind(Scoring$FirstStatus,Scoring$MiddleStatus,Scoring$LastStatus))

Scoring$ImageTimeDeadFirst<-ifelse(Scoring$CellPostion==" FIRST"&Scoring$ScoringALL==1,Scoring$Image,"X")

Scoring$CensorImageTimeDeadFirst<-ifelse(Scoring$ImageTimeDeadFirst=="X","",1)

w<-append(Scoring$ScoringALL,0,after=0)

Scoring$ScoringAllNminus1<-w[1:length(w)-1]

Scoring$ScoringAllnonFirst<-ifelse(Scoring$TimeAdjusted>0 & Scoring$ScoringALL==1 & Scoring$ScoringAllNminus1==0,Scoring$Image,"Y")

Scoring$CensorImageDeadExceptFirst<-ifelse(Scoring$ScoringAllnonFirst=="Y","",1)

Scoring$NotDeadLast<-ifelse(Scoring$CellPositionID\_Last=="LAST" & Scoring$ScoringALL==0,Scoring$Image,"Z")

Scoring$CensorNotDeadLast<-ifelse(Scoring$NotDeadLast=="Z","",0)

Scoring$Well<-ifelse(Scoring$CensorImageDeadExceptFirst==1|Scoring$CensorImageTimeDeadFirst=="1"|Scoring$CensorNotDeadLast==0,Scoring$Well\_ROI\_ImagingPlate,"")

Scoring$Timepoint<-ifelse(Scoring$Well=="","",Scoring$TimeAdjusted)

Scoring$TimepointNumeric<-as.numeric(as.character(Scoring$Timepoint))

Scoring$Censorship<-ifelse(Scoring$Timepoint=="","",Scoring$ScoringALL)

Scoring$CensorshipNumeric<-as.numeric(as.character(Scoring$Censorship))

Scoring$ClassAndWellandImagingPlateInfo<-paste(Scoring$Image,Scoring$ImagingPlate,Scoring$Class, sep="\_")

#write.csv(Scoring, paste(Experiment,"\_scoring\_output\_with\_Duplicates.csv",sep=""))

ScoringUnique<- Scoring[!(duplicated(Scoring$Well)|duplicated(Scoring$Well, fromLast = TRUE)),]

#write.csv(ScoringUnique, paste(Experiment,"\_scoring\_output\_No\_Duplicates.csv",sep=""))

ScoringUnique$well\_noTimeZeroDead<-ifelse(ScoringUnique$TimepointNumeric==0,"X",ScoringUnique$Well)

ScoringUnique$Timepoint\_noTimeZeroDead<-ifelse(ScoringUnique$TimepointNumeric==0,"",ScoringUnique$TimepointNumeric)

ScoringUnique$Censorship\_noTimeZeroDead<-ifelse(ScoringUnique$TimepointNumeric==0,"",ScoringUnique$CensorshipNumeric)

ScoringUnique$Timepoint\_noTimeZeroDead\_Numeric<-as.numeric(as.character(ScoringUnique$Timepoint\_noTimeZeroDead))

ScoringUnique$Censorship\_noTimeZeroDead\_Numeric<-as.numeric(as.character(ScoringUnique$Censorship\_noTimeZeroDead))

ScoringUnique\_NoTimeZeroDead\_USE<- ScoringUnique[!(duplicated(ScoringUnique$well\_noTimeZeroDead)|duplicated(ScoringUnique$well\_noTimeZeroDead, fromLast = TRUE)),]

#write.csv(ScoringUnique\_NoTimeZeroDead\_USE, paste(Experiment,"\_scoring\_output\_No\_Duplicates\_NoTimeZero.csv",sep=""))

#ScoringUnique\_No\_TimeZeroDead\_USE: Can then be Read for survival output and HR using code below

ExperimentName<- ScoringUnique\_NoTimeZeroDead\_USE%>% group\_by(Class)

groupsExamine2<-filter(ExperimentName, Class %in% groupsexamine)

ExperimentName2<-groupsExamine2

ExperimentName2$Class = factor(ExperimentName2$Class)

ExperimentName2$Class=relevel(ExperimentName2$Class,ref = Reference\_Group)

newdata <- ExperimentName2

#write.csv(newdata, "newdata.csv")

#use 93 if want to include time zero dead

#sfit<-survfit(Surv(TimepointNumeric,CensorshipNumeric)~Class, data=newdata)

sfit<-survfit(Surv(Timepoint\_noTimeZeroDead\_Numeric,Censorship\_noTimeZeroDead\_Numeric)~Class, data=newdata)

SurvivalPlot<-ggsurvplot(sfit,palette = "grey",break.time.by=x\_axisScale, conf.int=FALSE, pval=FALSE, risk.table=TRUE,legend.title="", main="Kaplan-Meier Curve",font.legend=10,legend=c("bottom"),font.x=15,font.y=15)

SurvivalPlot2<-SurvivalPlot+xlab(x\_axisLabel)

#

jpeg(paste(Experiment,"\_Survival\_Curve.jpg", sep=""))

SurvivalPlot2

graphics.off()

#

CumulativeHazard <- ggsurvplot(sfit,palette = "grey",break.time.by=x\_axisScale, pval=FALSE, fun="cumhaz", censor=FALSE,risk.table=FALSE,risk.table.title="Living Neurons",font.x=15,font.y=15,legend.title="",xlab="Day", font.legend=10,legend=c("top"))

CumulativeHazard2<-CumulativeHazard+xlab(x\_axisLabel)

jpeg(paste(Experiment,"\_Cumulative\_Hazard.jpg", sep=""))

CumulativeHazard2

graphics.off()

#use 106 if with time zero

#fit <- coxph(Surv(TimepointNumeric, CensorshipNumeric)~Class, data=newdata)

fit <- coxph(Surv(Timepoint\_noTimeZeroDead\_Numeric,Censorship\_noTimeZeroDead\_Numeric)~Class, data=newdata)

COXPH\_info<-summary(fit)

COXPH\_HRoutput <- COXPH\_info$coefficients

#write.csv(COXPH\_HRoutput, paste(Experiment, "\_Cumulative\_Hazard\_without\_sfitSummary\_noTime0dead.csv", sep= ""))

aaa<-COXPH\_info$loglik

bbb<-COXPH\_info$waldtest

ccc<-COXPH\_info$rsq

ddd<-COXPH\_info$concordance

eee<-COXPH\_info$sctest

write.csv(eee,paste(Experiment, "\_LogRankTest.csv", sep= "") )

#

sfit\_summary <- summary(sfit)

sfit\_summary\_output<-sfit\_summary$table

#write.csv(sfit\_summary\_output, paste(Experiment,"\_Sfit\_SummaryTable\_Alone\_notime0Dead.csv", sep= ""))

sfit\_summary\_output\_mediary<-as.data.frame(sfit\_summary\_output)

hh<-append(sfit\_summary\_output\_mediary$records[-1], "", length(sfit\_summary\_output\_mediary$records-1))

yy<-hh[1:length(hh)-1]

oo<-append(sfit\_summary\_output\_mediary$n.max[-1], "", length(sfit\_summary\_output\_mediary$n.max-1))

pp<-oo[1:length(oo)-1]

aa<-append(sfit\_summary\_output\_mediary$n.start[-1],"",length(sfit\_summary\_output\_mediary$n.start-1))

bb<-aa[1:length(aa)-1]

cc<-append(sfit\_summary\_output\_mediary$events[-1],"",length(sfit\_summary\_output\_mediary$events-1))

dd<-cc[1:length(cc)-1]

ee<-append(sfit\_summary\_output\_mediary$`\*rmean`[-1],"",length(sfit\_summary\_output\_mediary$`\*rmean`-1))

ff<-ee[1:length(ee)-1]

gg<-append(sfit\_summary\_output\_mediary$`\*se(rmean)`[-1],"",length(sfit\_summary\_output\_mediary$`\*se(rmean)`-1))

hh<-gg[1:length(hh)-1]

ii<-append(sfit\_summary\_output\_mediary$median[-1],"",length(sfit\_summary\_output\_mediary$median-1))

jj<-ii[1:length(ii)-1]

kk<-append(sfit\_summary\_output\_mediary$`0.95LCL`[-1],"",length(sfit\_summary\_output\_mediary$`0.95LCL`-1))

ll<-kk[1:length(kk)-1]

mm<-append(sfit\_summary\_output\_mediary$`0.95UCL`[-1],"",length(sfit\_summary\_output\_mediary$`0.95UCL`-1))

nn<-mm[1:length(mm)-1]

COXPH\_HRoutputWithN<- as.data.frame(COXPH\_HRoutput)

COXPH\_HRoutputWithN$records<- yy

COXPH\_HRoutputWithN$n.max<-pp

COXPH\_HRoutputWithN$n.start<-bb

COXPH\_HRoutputWithN$events<-dd

COXPH\_HRoutputWithN$'\*rmean'<-ff

COXPH\_HRoutputWithN$'\*se(rmean)'<- hh

COXPH\_HRoutputWithN$median<-jj

COXPH\_HRoutputWithN$'0.95LCL'<-ll

COXPH\_HRoutputWithN$'0.95UCL'<-nn

reference<- sfit\_summary\_output\_mediary[1,]

referenceWithPvalue<-add\_column(reference, "Pr(>|z|)"="", .before = 1)

referenceWithZ<-add\_column(referenceWithPvalue, "z"="", .before = 1)

referenceWithSEcof<-add\_column(referenceWithZ, "se(coef)"="", .before = 1)

referenceWithExpCoF<-add\_column(referenceWithSEcof, "exp(coef)"=1, .before = 1)

referenceWithCoF<-add\_column(referenceWithExpCoF, "coef"= "REFERENCE", .before=1)

COXPH\_HRoutputWithN\_USE<-rbind(referenceWithCoF,COXPH\_HRoutputWithN)

write.csv(COXPH\_HRoutputWithN\_USE, paste(Experiment,"\_Cumulative\_Hazard\_with\_sfitSummaryTable.csv", sep = ""))